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# Synthesis and properties of (2′*S*)- and (2′*R*)-2′-deoxy-2′-*C*-methyl oligonucleotides

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**Abstract**—The synthesis of (2′*S*)- and (2′*R*)-2′-deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl) uridine, (2′*S*)-2′-deoxy-2′-*C*-methyluridine and (2′*S*)-2′-deoxy-2′-*C*-methyl-$N^4$-isobutyryl cytidine building blocks are here described. The preparation of oligonucleotides carrying these monomers in all positions but 3′-end is presented and the binding affinity between these new fragments and the complementary DNA and RNA sequences is also assessed. (2′*R*) substituted oligonucleotides did not hybridize with either the complementary DNA or RNA sequences. However, the first derivative of melting curves of hybrids containing (2′*S*) modified oligonucleotides indicated melting transitions. © 2001 Elsevier Science Ltd. All rights reserved.

## 1. Introduction

The synthesis of modified oligonucleotides has received a great deal of attention during the last years, due to the very important applications that this class of compounds has displayed as antisense fragments. Several modifications have been proposed in order to obtain molecules resistant to the action of nucleases and that can recognize complementary sequences.[1] Oligonucleotides based on a nuclease resistant phosphorothioate backbone have proved to be very potent and selective inhibitors of protein expression in vitro as well as in vivo.[2,3] Despite this therapeutic potential, the detailed analysis of their whole chemical, biochemical and biophysical characteristics still indicates that this class of compounds may not represent the final solution to the problem of designing functional antisense inhibitors.[4] 2′-Modifications have also proved to have interesting hybridization properties.[5] 2′-*O*-alkyloligoribonucleotides[6,7] which have shown to be resistant to nucleases and to form stable hybrids are widely used both alone and in combination with modified phosphate backbones.[8,9] The improved hybridization of 2′-*F*- and 2′-*O*-alkyloligonucleotides to complementary RNA has been attributed to the tendency of these electronegative substituents to shift the conformational equilibrium in the sugar moiety towards C3′-*endo* pucker consistent with the A-form geometry of RNA duplexes. Therefore, the sugar ring puckering seems to constitute an important structural variable to take into account in the design of antisense oligonucleotides.[5,10,11] We have previously determined the influence of 2′-*C*-alkyl groups on the sugar conformation of nucleosides in solution based on NMR analysis.[12] The results showed that the preferred sugar conformation in the case of (2′*S*)-2′-deoxy-2′-*C*-alkylnucleosides is C-3′ *endo* while the (2′*R*) isomers exist predominantly in the C-2′ *endo* conformation. These findings could be explained since 2′-*C* alkyl substituents tend to attain a pseudoequatorial location for steric reasons while an oxygenated subtituent on C-2′ tends to occupy a pseudoaxial site because of the stabilizing O-4′/O-2′ *gauche* effect.[13]

As far as we know, there is only one report regarding to the synthesis and properties of chimeric sequences containing 2′-deoxy and 2′-*C*-alkyldeoxynucleotides which exhibited a reduced binding affinity for complementary DNA and RNA.[14] Despite these results, (2′*R*)-2′-*C*-alkylnucleotides have proved to be useful in the design of modified hammerhead ribozymes.[15,16] In order to further investigate the properties of oligonucleotides carrying these modifications we carried out preliminary attempts to explore the influence of the 2′-methyl group on the orientation of the base as well on vicinal nucleotides. In view of the results afforded by these preliminary studies, and since differences between the hybridization affinity of oligodeoxynucleotides carrying 2′-modified moieties in certain positions and completely modified sequences has been formerly observed[10], we consider interesting to further investigate the synthesis and hybridization properties of oligonucleotides carrying

*Keywords*: antisense; modified oligonucleotides; 2′-deoxy-2′-*C*-alkylnucleotides; hybridization properties.

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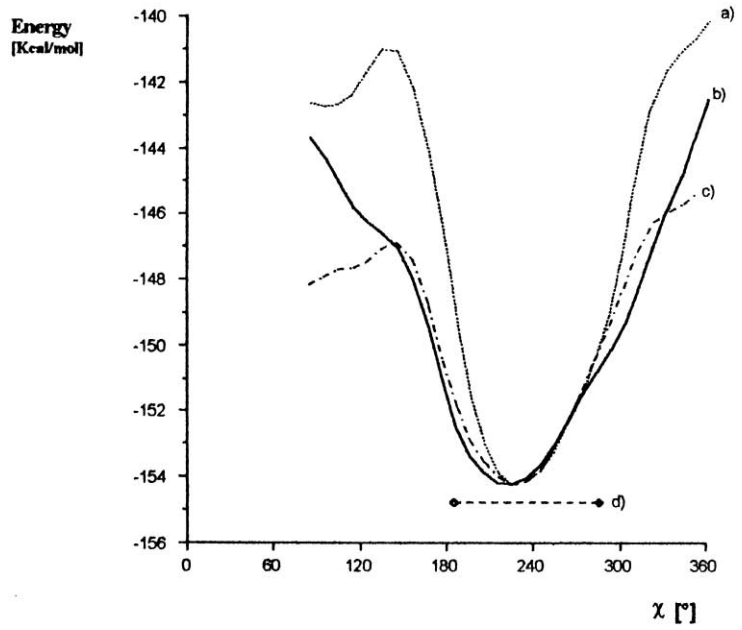


**Figure 1.** Potential energy as a function of the glycosidic angle *c* for: (a) $U_{(2'S)Me}$, (b) dU and (c) rU. (d) Represents the range of *c* usually found in natural DNA duplexes.

2′-*C*-methyl substituents with both *arabino* and *ribo* configurations in all but 3′-positions.

## 2. Results and discussion

Preliminary studies were done in order to explore the effect of the methyl group on the orientation of the base which could produce structural distortions in the case of the (2′*S*)-isomer. The energy of different rotamers around the glycosidic bond was analyzed by means of molecular mechanics calculations. Curves of Fig. 1 show the calculated energy as a function of the glycosidic angle *c*, using a grid step of 10°; the curves for natural and 2′-*C*-alkyl-modified uridine nucleosides present similar shapes in the range 180–280°. These results suggest that the introduction of the (2′*S*)-methyl group does not impair the orientation of the base within the range of glycosidic angles found in natural duplexes.[11]

A further attempt to predict the hybridization properties of this kind of modified oligonucleotides was made by exploring the interaction of the 2′-methyl group with vicinal nucleotides. The pyrimidine–DNA strand from a DNA–RNA hybrid constructed using the Builder module of the program Insight,[17] was modified by direct replacement of the 2′-β-hydrogen by a methyl group. An analysis of bumping interactions showed that the 2′-methyl group most likely sterically interferes with the 3′-nucleotide in the oligomer, though these minor interactions would not justify an absence of hybridization. On the other hand, the substitution of the 2′-α-hydrogen produced strong destabilizing interactions with vicinal nucleotides. To verify experimentally this preliminary conclusions we decided to synthesize oligonucleotides carrying (2′*S*)- and (2′*R*)-*C*-methylnucleotides in all positions but 3′-end.

Fig. 2 shows the synthesis of the uridine building blocks **5a** and **5b** starting from the mixture of (2′*S*)- and (2′*R*)-2′-deoxy-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-*C*-methyluridine (**1a** and **1b**).[18]

Reaction of the mixture of **1a** and **1b** with 4-*t*-butylbenzoyl chloride yielded compounds **2a** and **2b**. The introduction of the lipophilic acyl group made chromatographic separation of both diasteromers possible, and also conferred additional protection for the lactam function during the longer coupling times required for 2′-substituted nucleotides.[19] The disiloxane bridge of the isolated isomers was removed with tetrabutylammonium fluoride to afford compounds **3a** (97 % yield) and **3b** (69 % yield). The configuration at *C*-2′ was assigned by comparing the NMR data of **3a** and **3b** with those of (2′*S*)- and (2′*R*)-2′-deoxy-2′-*C*-2′-methyluridine[18] (data not shown).

5′-*O*-Dimethoxytrityl derivatives (**4a** and **4b**), as well as 3′-*O*-phosphoramidites, **5a** and **5b**, were prepared following standard procedures.

In order to assess the hybridization properties of completely modified oligonucleotides carrying 2′-modified building blocks in all but 3′-position the following fragments were synthesized: $(U_{(2'R)Me})_{10}T$ (**I**), and $(U_{(2'S)Me})_{10}T$ (**II**), (where $U_{(2'R)Me}$=(2′*R*)-2′-deoxy-2′-*C*-methyluridine; $U_{(2'S)Me}$=(2′*S*)-2′-deoxy-2′-*C*-methyluridine and T=thymidine). Sugar-modified phosphoramidites **5a** and **5b** were used as building blocks in an automated DNA synthesizer. Due to the steric interference caused by the bulky 2′-*C*-alkyl substituent, the normal coupling cycle was modified to ensure high coupling yield of the protected monomers (7 min). Deprotection and purification were performed as described in Section 3.

On the other hand, with the goal of analyzing the hybridization behavior of a mixed cytidine–uridine sequence carrying (2′*S*)-2′-deoxy-2′-*C*-methylnucleotides, the synthesis of the cytidine building block was performed (Fig. 3). The

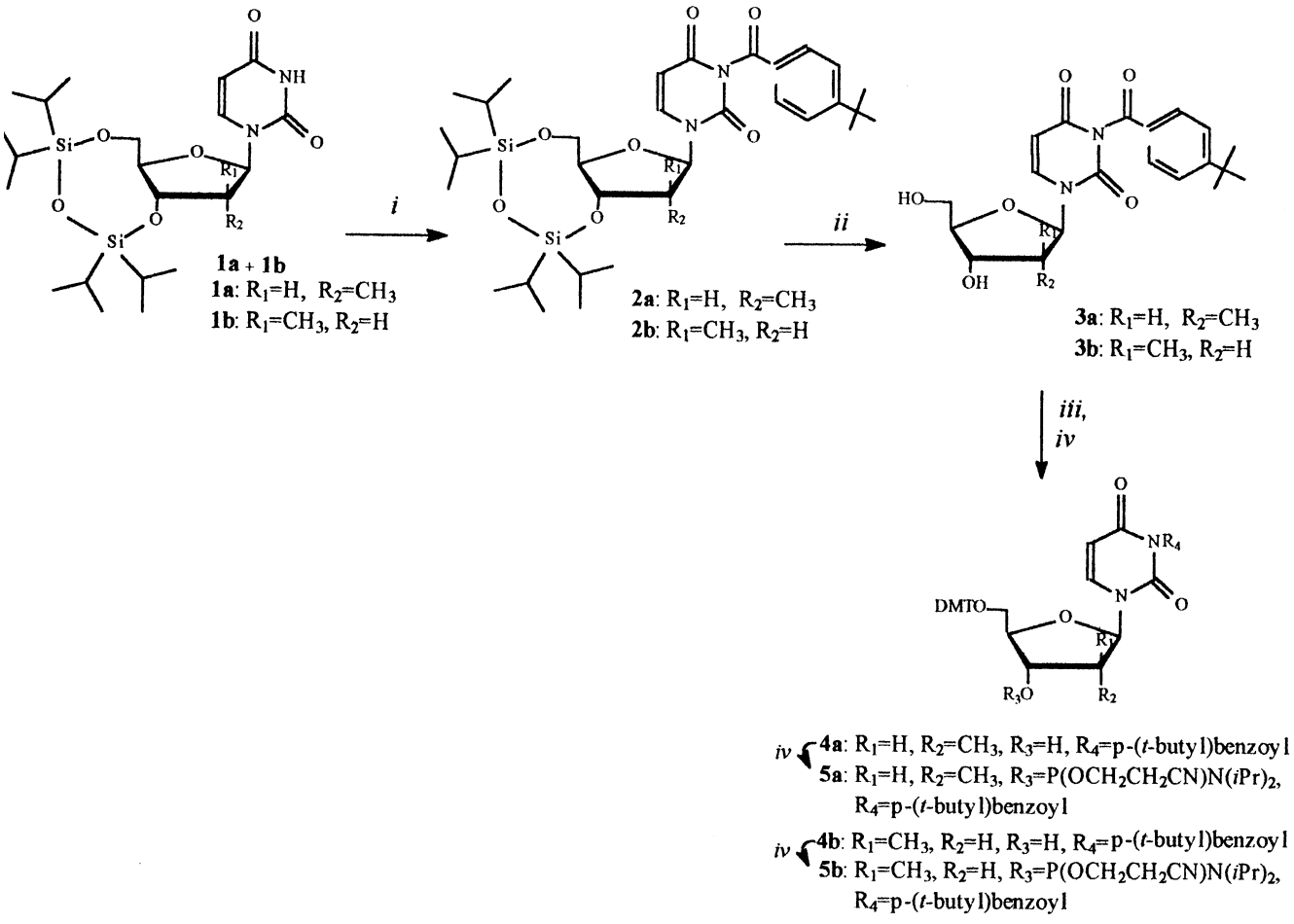


**Figure 2.** Reaction scheme for the preparation of (2′*S*)- and (2′*R*)-2′-deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl)uridine building blocks. *Reagents*: (i) tetrabutylammonium bromide, $Na_2CO_3$ and 4-*t*-butylbenzoyl chloride in dichloromethane/$H_2O$ (1:1, v/v); (ii) tetrabutylammonium fluoride in tetrahydrofuran; (iii) 4,4′-dimethoxytrityl chloride and triethylamine in pyridine; (iv) 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine and *N,N*-diisopropylethylamine in 1,2-dichloroethane.

mixture of compounds **1a** and **1b** was treated with 2-nitrophenol and diazabicyclo[2,2,2]octane in 1,2-dichloroethane/triethylamine to afford the two diasteromers of the corresponding $O^4$-nitrophenylnucleosides. This mixture could be resolved by silica gel column chromatography to afford pure compound **6** which was the common intermediate for the synthesis of the uridine (**9**) and cytidine phosphoramidites (**12**). Using the same conditions described above for the preparation of the 2′-modified homo-oligomers, the synthesis of (UCCUCCCUCUUCCU-CUCC)$_{(2'S)Me}$dC (**III**) (where $C_{(2'S)Me}$=(2′*S*)-2′-deoxy-2′-*C*-methylcytidine; $U_{(2'S)Me}$=(2′*S*)-2′-deoxy-2′-*C*-methyluridine and dC=deoxycytidine) was performed. No difference in the yield of the oligomers between the preparation using lactam protected uridine building block **5b**, and the lactam unprotected **9** were observed.

The thermal stability of the hybrids formed between these oligonucleotides and the complementary sequences was tested. Oligonucleotide **I** did not hybridize with $dA_{11}$ or -$rA_{11}$ sequences. This result is in agreement with that reported by Schmidt et al. for the case of chimeric oligonucloetides carrying (2′*R*)-2′-*C*-alkyloligonucleotide.[14] On the other hand, fragment **II** showed a thermal transition with both $dA_{11}$ and $rA_{11}$. In both cases, the observed difference in absorbance is much smaller than those obtained for the hybrids $T_{11}/dA_{11}$ and $T_{11}/rA_{11}$, but transitions were evidenced by the first derivatives of the corresponding melting curves. These results are not due to particular single strand structures, since the melting curves of the single oligonucleotides did not show any transition.

According to the first derivatives, the melting points measured were the following: $T_{11}/dA_{11}$: 24.0°C; **II**/$dA_{11}$: 25.9°C; $T_{11}/rA_{11}$: 16.5°C; **II**/$rA_{11}$: 26.2°C. These results show that only the modified oligonucleotides carrying (2′*S*)-alkyl groups presented good hybridization properties, with melting points higher than those measured for the natural DNA–DNA and DNA–RNA hybrids. It also suggests that although the stacking interactions seem to be disturbed by the presence of the methyl group, the hybrids containing modified uridines are thermally more stable than the corresponding hybrids carrying thymidines.

In order to assess the hybridization behavior of a mixed cytidine–uridine sequence, the hybridization behavior of the mixed sequence **III** with the complementary RNA olilgonucleotide was performed. In the case of the hybrid formed between oligonucleotide **III** and the corresponding complementary RNA sequence the melting temperature

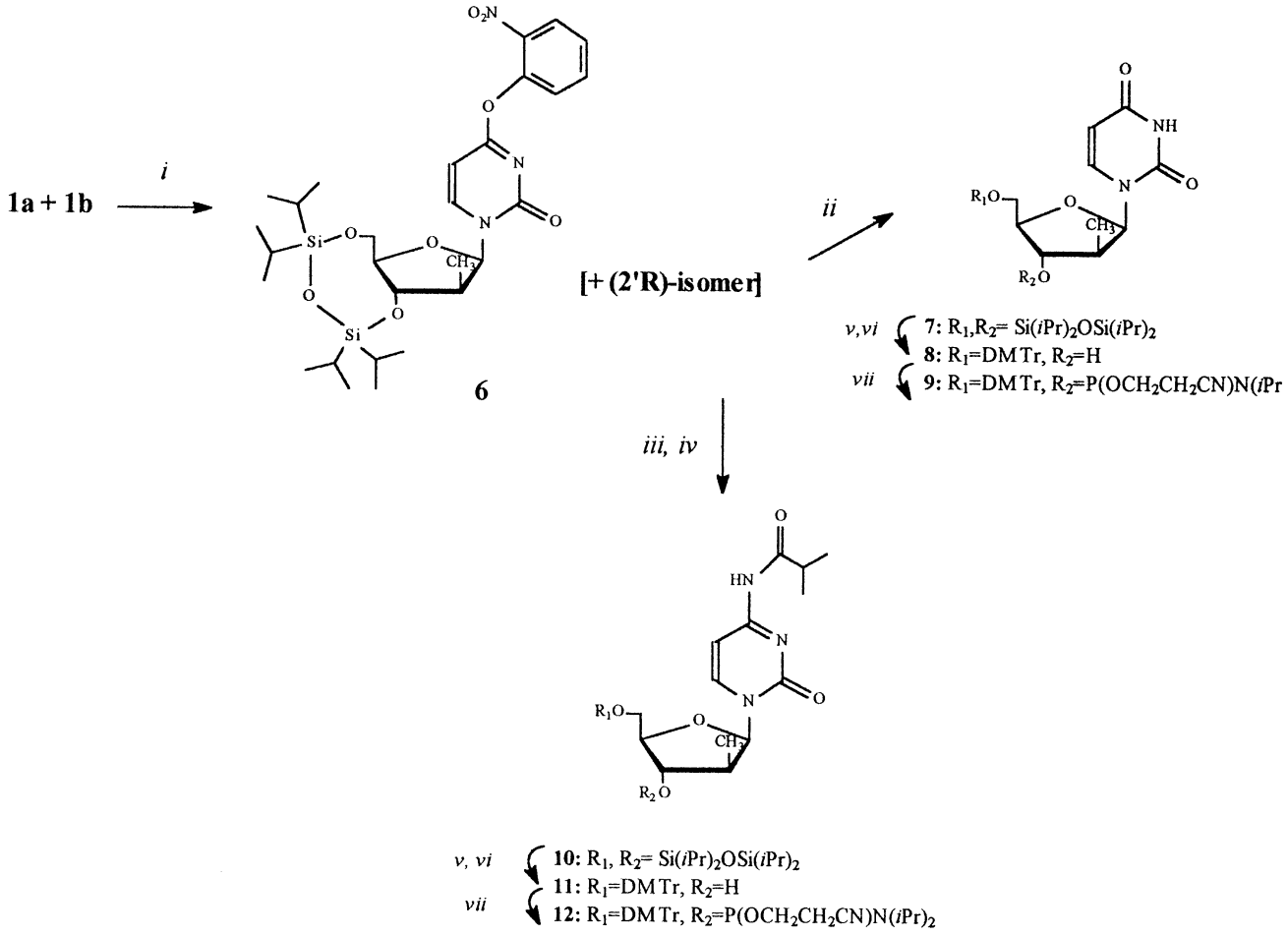


**Figure 3.** Reaction scheme for the synthesis of (2′*S*)-2′-deoxy-2′-*C*-methyluridine and (2′*S*)-2′-deoxy-2′-*C*-methyl-$N^4$-isobutyrylcytidine building blocks. *Reagents*: (i) mesitylenesulfonyl chloride, dimethylaminopyridine, triethylamine, diazabicyclo[2.2.2] octane and 2-nitrophenol in 1,2-dichloroethane; (ii) 2-nitro-benzaldoxime and tetramethylguanidine in $CH_3CN$; (iii) ammonia in tetrahydrofuran; (iv) isobutyryl chloride in pyridine; (v) tetrabutylammonium fluoride in tetrahydrofuran; (vi) 4,4′-dimethoxytrityl chloride and triethylamine in pyridine; (vii) 2-cyanoethoxy-*N*,*N*-diisopropylaminochlorophosphine and *N*,*N*-diisopropylethylamine in 1,2-dichloroethane.

obtained is lower than that measured for the natural DNA–RNA duplex of the same sequence, the respective melting points being 38.8 and 43.0°C. As observed earlier, the difference in absorbance between the hybrid and the melted strands is smaller in the case of the sugar-modified oligonucleotide. The different melting behavior of **II** and **III** is not surprising since previous reports showed similar variations depending on the sequence and the length of the modified oligonucleotides.[5]

The reason why the (2′*S*)-methyloligodeoxynucleotides hybridize better than the (2′*R*) analogues could be attributed to the fact that the (2′*S*)-methyloligodeoxynucleotides have the right preorganization for fitting with the complementary RNA strand,[20] since the sugar moieties are locked in the 3′-*endo* conformation.

The flatter transitions suggest that the presence of the β-methyl group influences the hybrid structures as observed in our preliminary analysis. The differences between our findings and those reported by Schmidt et al.[14] could be explained, as previously reported for related modifications, by the fact that the binding affinity depends on the geometry of the heteroduplex and therefore on the context where the modification is located, whether it is located in a background of deoxynucleotides or modified nucleotides.[9]

The results regarding to (2′*S*)-2′-deoxy-2′-*C*-alkyloligonucleotides, challenge us to investigate more exhaustively the properties and stability characteristics of this class of sugar modified oligonucleotides. We are undertaking the synthesis of mixed sequences which include purine nucleotides, and further structural studies.

## 3. Experimental

### 3.1. General

2′-Deoxy-2′-*C*-alkylnucleosides were synthesized as previously described.[19] Column chromatography was performed on silica gel 60, particle size 20–45 mm (Amicon). All the reagents used were of the highest available purity.

$^1H$, $^{13}C$ and $^{31}P$ NMR spectra were recorded on a Bruker AC200 or Bruker AM500 using tetramethyl-silane and phosphoric acid as the respective references.

Oligonucleotides were synthesized on a 380B Applied Biosystems synthesizer using β-cyanoethyl phosphoramidite chemistry. Commercially available (Millipore) derivatized CPG-linked to either 2′-deoxy or ribonucleosides were used. Coupling efficiency was in all the cases higher than 98%. Deprotection of oligonucleotides was achieved by treatment with concentrated aqueous ammonia at 55°C, overnight. Oligonucleotides (trityl-on), which showed a major peak, were purified by reverse phase HPLC on a Nova-Pak C-18 column (8×10 Radial-Pak Cartridge, 4 mm) using a gradient of 16–60% of acetonitrile in 0.1 M triethylammonium acetate buffer pH 7. Cleavage of DMTr group was accomplished by treatment with 80% acetic acid for 30 min. The solution was diluted with an equal volume of water, washed three times with two volumes of diethyl ether, and the aqueous layer was lyophilized.

Melting curves were measured on a Cary 3E (Varian) spectrophotometer with temperature controller. Hybrids were previously annealed by heating the solution of the oligonucleotides 3 mM of each strand in100 mM NaCl, 10 mM Tris buffer (pH=7.1) at 80°C for 2 min and subsequent cooling for 40 min to a final temperature of 5°C. The melting curves were obtained by measuring the absorbance at 260 nm, using a heating rate of 0.5°C/min. Melting temperatures ($T_m$) were determined as the maximum of the first derivative of the plot of absorbance vs. temperature.

Microanalysis of previously recrystallized samples was done using an automatic analyzer (Fisons Instruments). Molecular mechanics calculations were performed using the program Discover (Biosym). The energy minimizations were performed in vacuo, using the AMBER force field. Curves of Fig. 1 were obtained using a grid step of 10°. At each value of the glycosidic angle, the structure was minimized to an rms energy gradient of 0.001 kcal/mol A, using a 10 kcal/mol harmonic restraint to maintain the appropriate value of *c*.

**3.1.1. 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-(2′*R*)- and -(2′*S*)-2′-deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl) uridine (2a and 2b).** To a vigorously stirred mixture of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-(2′*R*)- and (2′*S*)-2′-deoxy-2′-*C*-methyluridine (**1a**, **1b**) (30 mg, 0.06 mmol), tetrabutylammonium bromide (2 mg), $Na_2CO_3$ (50 mg, 0.47 mmol) and dichloromethane/$H_2O$ (2 mL, 1:1, v/v), was added dropwise 4-*t*-butylbenzoyl chloride (20 mL, 0.10 mmol). The reaction mixture was stirred overnight at room temperature. The organic phase was separated, washed with $H_2O$ (5 mL) and left with stirring overnight (in order to obtain the thermodynamically more stable $N^3$-acyl derivative[21]). The solvent was evaporated in vacuo and the residue chromatographied on silica gel column using as eluant a gradient of petroleum ether/ethyl acetate 9:1 to 7:1 (v/v). Pure compounds **2a** (12 mg, 0.018 mmol) and **2b** (19 mg, 0.029 mmol) were obtained. $^{13}$C NMR spectrum of **2a** ($CDCl_3$): (δ, ppm) 168.8 (*t*-butylbenzoyl); 162.0 (C-4); 154.6 (*t*-butylbenzoyl); 149.2 (C-2); 138.8 (C-6); 131.7, 128.8, 124.4 (*t*-butylbenzoyl); 102.1 (C-5); 90.4 (C-1′); 82.7 (C-4′); 68.3 (C-3′); 59.5 (C-5′); 43.4 (C-2′); 34.8 and 31.4 (*t*-butylbenzoyl); 17.3–12.3 (isopropyls), 10.5 (2′-methyl). $^1$H NMR spectrum of **2a** ($CDCl_3$): (δ, ppm) 0.81–1.23 (m, 28H, –CHs, $-CH_3$s, disiloxane); 0.87 (d, 3H, $-CH_3$, $J_{CH3,2'}$=7 Hz); 1.32 (s, 9H, $-CH_3$s, *t*-butyl); 2.23 (m, 1H, H-2′); 3.87 (dd, 1H, H-5′, $J_{5',5''}$=13 Hz; $J_{4',5'}$=1 Hz); 4.00 (dd, 1H, H-5″, $J_{5',5''}$=13 Hz; $J_{4',5''}$=1 Hz); 4.15 (m, 1H, H-4′); 4.42 (m, 1H, H-3′); 5.72 (d, 1H, H-5, $J_{6,5}$=8 Hz); 5.95 (d, 1H, H-1′, $J_{1,2}$=9 Hz); 7.40 (d, 2H, *t*-butylbenzoyl, *J*=9 Hz); 7.81 (d, 1H, H-6, $J_{6,5}$=8 Hz); 8.02 (d, 2H, *t*-butylbenzoyl, *J*=9 Hz). $^{13}$C NMR spectrum of **2b** ($CDCl_3$): (δ, ppm) 168. 9 (*t*-butylbenzoyl); 161.6 (C-4); 154.8 (*t*-butylbenzoyl); 149.0 (C-2); 138.5 (C-6); 131.7, 128.8 and 124.2 (*t*-butylbenzoyl); 102.4 (C-5); 85.8, 84.3 (C-1′ and C-4′); 72.1 (C-3′); 60.3 (C-5′); 44.1 (C-2′); 34.9, 31.5 (*t*-butylbenzoyl); 17.4–12.3 (isopropyls), 11.1 (2′-methyl). $^1$H NMR spectrum of **2b** ($CDCl_3$): (δ, ppm) 0.80–1.22 (m, 28H, –CHs, $-CH_3$s, disiloxane); 0.92 (d, 3H, $-CH_3$, $J_{CH3,2'}$=7 Hz); 1.34 (s, 9H, $-CH_3$s, *t*-butyl); 2.73 (m, 1H, H-2′); 3.75 (dd, 1H, H-5′, $J_{5',5''}$=12 Hz; $J_{4',5'}$=1 Hz); 3.96 (dd, 1H, H-5″, $J_{5',5''}$=13 Hz; $J_{4',5''}$=1 Hz); 4.01 (m, 1H, H-4′); 4.22 (m, 1H, H-3′); 5.81 (d, 1H, H-5, $J_{6,5}$=8 Hz); 6.25 (d, 1H, H-1′, $J_{1,2}$=8 Hz); 7.38 (d, 2H, *t*-butylbenzoyl, *J*=9 Hz); 7.76 (d, 1H, H-6, $J_{6,5}$=8 Hz); 8.11 (d, 2H, *t*-butylbenzoyl, *J*=9 Hz). Anal. calcd for $C_{33}H_{52}O_7N_2Si_2$ (**2a** and **2b**): C, 61.46; H, 8.13; N, 4.34. Found for **2a**: C, 61.50; H, 8.15; N, 4.40; **2b**: C, 61.50; H, 8.10; N, 4.45.

**3.1.2. (2′*R*)-2′-Deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl) uridine (3a).** 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-(2′*R*)-2′-deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl) uridine (**2a**) (300 mg, 0.46 mmol) was dissolved in dry tetrahydrofuran (1.4 mL) and tetrabutylammonium fluoride (1 mmol) was added with stirring and exclusion of moisture. After 5 min the reaction mixture was quenched with MeOH/pyridine/$H_2O$ (2.8 mL, 3:3:1 v/v/v), and stirred for 5 min. Pyridinium form Dowex 50×2–200 resin (15 g) was added to the solution and the mixture was stirred for 15 min. The resin was filtered off and washed with MeOH (2×2.8 mL). Combined filtrate and washings were evaporated in vacuo and the crude product was purified by silica gel column, eluting with a gradient of MeOH from 2 to 5% in dichloromethane. Pure title compound was obtained (180 mg) in 97% yield. $^{13}$C NMR spectrum of **3a** ($CDCl_3$): (δ, ppm) 168.8 (*t*-butylbenzoyl); 162.0 (C-4); 154.7 (*t*-butylbenzoyl); 148.9 (C-2); 139.0 (C-6); 131.7, 128.8, 124.4 (*t*-butylbenzoyl); 102.4 (C-5); 89.2, 87.8 (C-1′ and C-4′); 72.9 (C-3′); 61.0 (C-5′); 43.9 (C-2′); 35.0, 31.5 (*t*-butylbenzoyl), 10.3 (2′-methyl). $^1$H NMR spectrum of **3a** ($CDCl_3$): (δ, ppm) 0.91 (d, 3H, $CH_3$, $J_{CH3,2'}$=7 Hz); 1.32 (s, 9H, $-CH_3$s, *t*-butyl); 2.21 (m, 1H, H-2′); 3.70 (m, 2H, H-5′and H-5″); 3.83 (ddd, 1H, H-4′, $J_{5''-4'}$=$J_{5'-4'}$=4 Hz, $J_{3'-4'}$=2 Hz); 4.04 (dd, 1H, H-3′, $J_{2',3'}$=6 Hz, $J_{3'-4'}$=2 Hz); 5.02 (brs, 1H, –OH); 5.21 (brs, 1H, –OH); 5.65 (d, 1H, H-5, $J_{6,5}$=8 Hz); 5.75 (d, 1H, H-1′, $J_{1,2}$=9 Hz); 7.39 (d, 2H, *t*-butylbenzoyl, *J*=9 Hz); 7.83 (d, 1H, H-6, $J_{6,5}$=8 Hz); 8.05 (d, 2H, *t*-butylbenzoyl, *J*=9 Hz).

**3.1.3. (2′*S*)-2′-Deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl) uridine (3b).** 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-(2′*S*)-2′-deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl)uridine (**2b**) (510 mg, 0.79 mmol) was desilylated and purified according to the procedure used to prepare compound **3a** above to yield pure title compound (220 mg, 69%). $^{13}$C

NMR spectrum of **3b** ($CDCl_3$): ($\delta$, ppm) 168.9 (*t*-butylbenzoyl); 161.5 (C-4); 154.9 (*t*-butylbenzoyl); 148.9 (C-2); 139.3 (C-6); 131.7, 128.5, 124.3 (*t*-butylbenzoyl); 102.6 (C-5); 86.0 (C-1′); 83.7 (C-4′); 73.5 (C-3′); 61.9 (C-5′); 45.1 (C-2′); 34.8, 31.3 (*t*-butylbenzoyl), 11.1 (2′-methyl). $^1$H NMR spectrum of **3b** ($CDCl_3$): ($\delta$, ppm) 0.85 (d, 3H, $CH_3$, $J_{CH3,2'}$=7 Hz); 1.34 (s, 9H, $-CH_3$s, *t*-butyl); 2.48 (m, 1H, H-2′); 3.65 (m, 2H, H-5′ and H-5″); 3.75 (m, 1H, H-4′); 3.80 (m, 1H, H-3′); 5.14 (brs, 1H, –OH); 5.35 (brs, 1H, –OH); 5.63 (d, 1H, H-5, $J_{6,5}$=8 Hz); 6.18 (d, 1H, H-1′, $J_{1,2}$=8 Hz); 7.41 (d, 2H, *t*-butylbenzoyl, *J*=9 Hz); 8.00 (d, 1H, H-6, $J_{6,5}$=8 Hz); 8.15 (d, 2H, *t*-butylbenzoyl, *J*=9 Hz). Anal. calcd for $C_{21}H_{26}O_6N_2$ (**3a** and **3b**): C, 62.67; H, 6.51; N, 6.96. Found for **3a**: C, 62.71; H, 6.38; N, 7.01; **3b**: C, 62.75; H, 6.44; N, 6.94.

**3.1.4. 5′-*O*-Dimethoxytrityl-(2′*R*)-2′-deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl)uridine (4a).** (2′*R*)-2′-Deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl) uridine **3a** (2 g, 5 mmol) was dried by evaporation of pyridine in vacuo. Anhydrous pyridine (20 mL), triethylamine (10 mmol) and 4,4′-dimethoxytrityl chloride (6 mmol) were added with stirring and exclusion of moisture. The reaction was checked by TLC and after completion quenched by addition of MeOH and evaporated to dryness. The residue was dissolved in (50 mL) and washed with 1 M aqueous $NaHCO_3$ (2×50 mL). The organic layer was dried ($Na_2SO_4$), filtered and solvent was removed in vacuo. The residue was purified by column cromatography on silica gel using 50% ethyl acetate in petroleum ether containing 1% triethylamine as eluant (63%). $^{13}$C NMR spectrum of **4a** ($CDCl_3$): ($\delta$, ppm) 168.9 (*t*-butylbenzoyl); 161.8 (C-4); 158.5 ($C_{Ar}$–$OCH_3$, DMTr); 154.7 (*t*-butylbenzoyl); 149.3 (C-2); 144.2 (Ar, DMTr); 140.2 (C-6); 135.3, 130.0, 128.6, 128.0, 127.3 (Ar, DMTr+butylbenzoyl); 131.7, 124.4 (*t*-butylbenzoyl); 113.1 (Ar, DMTr); 102.3 (C-5); 86.6 (C–$Ar_3$, DMTr); 89.3 (C-1′); 82.4 (C-4′); 70.0 (C-3′); 60.2 (C-5′); 55.1 (–$OCH_3$, DMTr); 44.5 (C-2′); 34.7, 31.3 (*t*-butylbenzoyl), 10.4 (2′-methyl). $^1$H NMR spectrum of **4a** ($CDCl_3$): ($\delta$, ppm) 0.95 (d, 3H, $-CH_3$, $J_{CH3,2'}$=7 Hz); 1.30 (s, 9H, $-CH_3$s, *t*-butylbenzoyl); 2.45 (brs, 1H, OH-3′); 2.20 (m, 1H, H-2′); 3.25 (m, 1H, H-5′); 3.45 (m, 1H, H-5″); 3.81 (s, 6H, $-OCH_3$); 4.00 (m, 1H, H-4′); 4.31 (m, 1H, H-3′); 5.45 (d, 1H, H-5, $J_{5,6}$=8 Hz); 6.15 (d, 1H, H-1′, $J_{1',2'}$=8 Hz); 6.80 (d, 4H, Ar–$OCH_3$, DMT, *J*=9 Hz); 7.21–7.35 (m, 11H, Ar+Ar–$OCH_3$, DMT and Ar, *t*-butylbenzoyl); 8.03 (d, 1H, H-6, $J_{5,6}$= 8 Hz), 8.11 (d, 2H, *t*-butylbenzoyl, *J*=9 Hz). Anal. calcd for $C_{42}H_{44}O_8N_2$: C, 71.57; H, 6.29; N, 3.98. Found: C, 71.52; H, 6.33; N, 4.01.

**3.1.5. 5′-*O*-Dimethoxytrityl-(2′*S*)-2′-deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl)uridine (4b).** Compound **3b** (2 g, 5 mmol) was dimethoxytritylated and worked up as described for the synthesis of compound **4a** above. Pure compound **4b** was obtained as a foam (73%). $^{13}$C NMR spectrum of **4b** ($CDCl_3$): ($\delta$, ppm) 169.3 (*t*-butylbenzoyl); 161.3 (C-4); 158.5 ($C_{Ar}$–$OCH_3$, DMTr); 155.0 (*t*-butylbenzoyl); 149.1 (C-2); 143.9 (Ar, DMTr); 141.2 (C-6); 135.1, 129.8, 128.5, 128.0, 127.3 (Ar, DMTr+butylbenzoyl); 131.6, 124.4 (*t*-butylbenzoyl); 113.2 (Ar, DMTr); 101.8 (C-5); 86.3 (C–$Ar_3$, DMTr); 86.1 (C-1′); 83.7 (C-4′); 72.8 (C-3′); 60.9 (C-5′); 54.9 (–$OCH_3$, DMTr); 45.2 (C-2′); 34.6, 31.5 (*t*-butylbenzoyl), 10.9 (2′-methyl). $^1$H NMR spectrum of **4b** ($CDCl_3$): ($\delta$, ppm) 0.94 (d, 3H, $-CH_3$, $J_{CH3,2'}$=8 Hz); 1.32 (s, 9H, $-CH_3$s, *t*-butylbenzoyl); 2.41 (brs, 1H, OH-3′); 2.58 (m, 1H, H-2′); 3.20 (dd, 1H, H-5′, $J_{5',5''}$=12 Hz; $J_{4',5''}$=1 Hz); 3.42 (dd, 1H, H-5″, $J_{5',5''}$=12 Hz; $J_{4',5''}$=1 Hz); 3.80, (s, 3H, $-OCH_3$); 3.82, (s, 3H, $-OCH_3$); 4.05 (m, 1H, H-4′); 4.11 (m, 1H, H-3′); 5.32 (d, 1H, H-5, $J_{5,6}$=8 Hz); 6.28 (d, 1H, H-1′, $J_{1',2'}$=8 Hz); 6.82 (d, 4 H, Ar–$OCH_3$, DMT, *J*=9 Hz); 7.20–7.35 (m, 11 H, Ar+Ar–$OCH_3$, DMT and Ar, *t*-butylbenzoyl); 8.05 (d, 1H, H-6, $J_{5,6}$=8 Hz), 8.09 (d, 2H, *t*-butylbenzoyl, *J*=9 Hz). Anal. calcd for $C_{42}H_{44}O_8N_2$: C, 71.57; H, 6.29; N, 3.98. Found: C, 71.50; H, 6.25; N, 3.95.

**3.1.6. 5′-*O*-Dimethoxytrityl-(2′*R*)-2′-deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl)uridine-3′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (5a).** 5′-*O*-Dimethoxytrityl-(2′*R*)-2′-deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl)uridine (**4a**) (3.23 g, 5 mmol) was dried by evaporation of $CH_3CN$ and dissolved in dry 1,2-dichloroethane (20 mL) containing *N*,*N*-diisopropylethylamine (10 mmol) under argon. 2-Cyanoethoxy-*N*,*N*-diisopropylaminochlorophosphine (6 mmol) was added drop wise with stirring. When TLC showed complete reaction, the mixture was quenched by addition of MeOH. After 3 min, dichloromethane (50 mL) was added and the solution was washed with 1 M aqueous $Na_2CO_3$ (70 mL) followed by saturated brine (50 mL). The organic phase was dried ($Na_2SO_4$), filtered and evaporated to dryness. The residue was purified by silica gel column, eluting with 33% ethyl acetate in petrol containing 1% triethylamine to yield pure title compound (78%). $^{13}$C NMR spectrum of **5a** ($CDCl_3$): ($\delta$, ppm) 168.9/168.7 (*t*-butylbenzoyl); 161.9 (C-4); 158.7/158.6 ($C_{Ar}$–$OCH_3$, DMTr); 154.2 (*t*-butylbenzoyl); 149.3 (C-2); 144.3/144.2 (Ar, DMTr); 140.2 (C-6); 135.3/135.2, 130.5/130.4/130.3, 128.6/128.4/128.2, 127.9/127.4/127.3 (Ar, DMTr+butylbenzoyl); 131.5, 124.4 (*t*-butylbenzoyl); 117.4 (CN, phops);113.2/113.1 (Ar, DMTr); 102.1 (C-5); 87.0 (C–$Ar_3$, DMTr); 89.2/89.4 (C-1′); 82.4 (C-4′); 72.0/71.8 (C-3′); 60.5 (C-5′); 58.0/57.9 (–$OCH_2$, phosph); 55.2/55.1 (–$OCH_3$, DMTr); 44.5/44.4 (C-2′); 43.7/43.4 (CHs, phosp); 34.7, 31.3 (*t*-butylbenzoyl); 24.6 ($CH_3$, phosp); 18.9/18.8 ($CH_2$–CN, phosp); 10.4/10.5 (2′-methyl). $^1$H NMR spectrum of **5a** ($CDCl_3$): $\delta$ (ppm) 0.95/0.99 (d, 3H, $-CH_3$, $J_{CH3,2'}$=7 Hz); 1.08, 1.15, 1.16, 1.17 (d, 12H, $-CH_3$, phosp, $J_{CH3,CH}$=7 Hz); 1.30/1.33 (s, 9H, $-CH_3$s, *t*-butylbenzoyl); 2.28/2.30, (m, 3H, $CH_2$–CN and H-2′); 3.40–3.45 (m, 2H, –CHs, phosp); 3.44–3.66 (m, 2H, H-5′and H-5″; 3.85 (m, 1H, H-3′); 3.79/3.80 (s, 3H, $-OCH_3$); 3.81/3.82 (s, 3H, $-OCH_3$); 3.83/3.86 (m, 1H, $-CH_2O$, phosp); 3.91/3.95 (m, 1H, $-CH_2O$, phosp); 4.40/4.42 (m, 1H, H-4′); 5.20/5.25 (d, 1H, H-5, $J_{5,6}$=8 Hz); 6.20/6.23 (s, 1H, H-1′); 6.81–6.88, 7.23–7.30, 7.35–7.45 (m, 17H, Ar+Ar–$OCH_3$, DMT and Ar, *t*-butylbenzoyl); 7.98/8.02 (d, 1H, H-6, $J_{5,6}$=8 Hz). $^{31}$P NMR spectrum of **5a** ($CDCl_3$): ($\delta$, ppm) 147.20; 149.34. Anal. calcd for $C_{51}H_{61}O_9N_4P$: C, 67.68; H, 6.79; N, 6.19. Found: C, 67.56; H, 7.23; N, 6.25.

**3.1.7. 5′-*O*-Dimethoxytrityl-(2′*S*)-2′-deoxy-2′-*C*-methyl-$N^3$-(4-*t*-butylbenzoyl) uridine-3′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (5b).** Compound **4b** (3.23 g, 5 mmol) was phosphitylated, worked up and purified according to the procedure used to prepare **5a** above to

yield the title compound **5b** pure (80%). $^{13}$C NMR spectrum of **5b** ($CDCl_3$): ($\delta$, ppm) 169.0/168.9 (*t*-butylbenzoyl); 161.5 (C-4); 158.7/158.6 ($C_{Ar}$–$OCH_3$, DMTr); 154.9 (*t*-butylbenzoyl); 149.2 (C-2); 144.4/144.2 (Ar, DMTr); 141.1 (C-6); 135.3/135.1, 130.5/130.3/130.2, 128.7/128.6/128.4, 127.7/127.4/127.3 (Ar, DMTr+butylbenzoyl); 131.4, 124.5 (*t*-butylbenzoyl); 117.3 (CN, phops); 113.3/113.2 (Ar, DMTr); 102.0 (C-5); 86.5 (C–$Ar_3$, DMTr); 86.3/86.2 (C-1′); 83.9 (C-4′); 72.8/72.5 (C-3′); 60.8 (C-5′); 57.9/57.6 (–$OCH_2$, phosph); 55.3/55.2 (–$OCH_3$, DMTr); 45.4/45.2 (C-2′); 43.8/43.5 (CHs, phosp); 34.6, 31.5 (*t*-butylbenzoyl); 24.5 ($CH_3$, phosp); 18.9/18.6 ($CH_2$–CN, phosp); 10.9/10.8 (2′-methyl). $^1$H NMR spectrum of **5b** ($CDCl_3$): $\delta$ (ppm) 0.99/1.02 (d, 3H, –$CH_3$, $J_{CH3,2'}$=7 Hz); 1.09, 1.16, 1.17, 1.18 (d, 12H, –$CH_3$, phosp, $J_{CH3,CH}$=7 Hz); 1.31/1.32 (s, 9H, –$CH_3$s, *t*-butylbenzoyl); 2.29/2.31, (m, 2H, $CH_2$–CN) 2.55/2.60 (m, 1H, H-2′); 3.39–3.44 (m, 2H, –CHs, phosp); 3.45–3.65 (m, 2H, H-5′and H-5–); 3.75 (m, 1H, H-3′); 3.80 (s, 3H, –$OCH_3$); 3.81/3.83 (s, 3H, –$OCH_3$); 3.83/3.85 (m, 1H, $CH_2O$, phosp); 3.90/3.92 (m, 1H, –$CH_2O$, phosp); 4.35/4.40 (m, 1H, H-4′); 5.12/5.15 (d, 1H, H-5, $J_{5,6}$=8 Hz); 6.25/6.29 (s, 1H, H-1′); 6.80–6.85, 7.25–7.30, 7.37–7.45 (m, 17H, Ar+Ar–$OCH_3$, DMT and Ar, *t*-butylbenzoyl); 8.05/8.09 (d, 1H, H-6, $J_{5,6}$=8 Hz). $^{31}$P NMR spectrum of **5b** ($CDCl_3$): ($\delta$, ppm) 145.94; 148.55. Anal. calcd for $C_{51}H_{61}O_9N_4P$: C, 67.68; H, 6.79; N, 6.19. Found: C, 67.60; H, 7.83; N, 6.23.

**3.1.8. 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-(2′*S*)-2′-deoxy-2′-*C*-methyl-4-*O*-(2-nitrophenyl) uridine (6).** A mixture of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-(2′*R*)- and -(2′*S*)-2′-deoxy-2′-*C*-methyluridine (**1a** and **1b**) (~1:3,as determined by NMR, 10.1 g, 20.8 mmol) in 1,2-dichloroethane (50 mL) was treated with mesitylenesulfonyl chloride (6.2 g, 28.5 mmol), dimethylaminopyridine (930 mg, 7.6 mmol) and triethylamine (13.4 mL, 96 mmol). After stirring for 1 h, diazabicyclo [2.2.2] octane (DABCO) (424 mg, 2.8 mmol), 2-nitrophenol (7.92 g, 47 mmol) and triethylamine (13.4 mL, 96 mmol) were added and stirring was continued for three more hours. The resulting solution was diluted with dichloromethane (100 mL) and poured in 1 M aqueous $NaHCO_3$ (500 mL) with vigorous stirring. The organic layer was washed with $H_2O$ (2×150 mL), dried ($Na_2SO_4$), filtered and evaporated in vacuo leaving a yellow foam that was purified by column chromatography on silica gel eluting with petroleum ether/ethyl acetate/dichloromethane (10:1:10, v/v/v) to afford the 2′*S*-isomer **6** (5.17 g, 8.5 mmol) and the 2′*R*-isomer (1.3 g, 2.1 mmol). $^{13}$C NMR spectrum of **6** ($CDCl_3$): ($\delta$, ppm) 170.2 (C-4); 154.8 (C-2); 145.0 (C-6); 144.7, 141.3, 134.6, 126.3, 125.6, 125.2 (2-nitrophenyl); 86.7 (C-1′); 83.8 (C-4′); 72.1 (C-3′); 59.6 (C-5′); 43.9 (C-2′); 17.1–12.2 (isopropyls), 11.0 (2′-methyl). $^1$H NMR spectrum of **6** ($CDCl_3$): ($\delta$, ppm) 0.80–1.10 (m, 28H, –CHs and –$CH_3$s, disiloxane); 0.95 (d, 3H, –$CH_3$, $J_{CH3,2'}$=7 Hz); 2.70 (m, 1H, H-2′); 3.80–4.25 (m, 4H, H-3′, H-4′, H-5′ and H-5″); 6.19 (d, 1H, H-5, $J_{5,6}$=8 Hz); 6.32 (d, 1H, H-1′, $J_{1',2'}$=6 Hz); 7.27 (dd, 1H, H-*6*, nitrophenyl, $J_{6,5}$=9 Hz, $J_{6,4}$=1 Hz); 7.4 (ddd, 1H, H-*4*, nitrophenyl, $J_{4,5}$=9 Hz, $J_{4,3}$=9 Hz, $J_{6,4}$=1 Hz); 7.65 (ddd, 1H, H-*5*, nitrophenyl, $J_{4,5}$=9 Hz, $J_{5,6}$=9 Hz, $J_{5,3}$=1 Hz); 8.13 (dd, 1H, H-*3*, nitrophenyl, $J_{4,3}$=9 Hz, $J_{5,3}$=1 Hz); 8.25 (d, 1H, H-6, $J_{5,6}$=8 Hz). Anal. calcd for $C_{28}H_{43}O_8N_3Si_2$: C, 55.51; H, 7.15; N, 6.94. Found: C, 55.63; H, 7.25; N, 6.84.

**3.1.9. 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-(2′*S*)-2′-deoxy-2′-*C*-methyluridine (7).** A mixture of 2-nitrobenzaldoxime (880 mg, 5.31 mmol) and tetramethylguanidine (420 ml, 3.32 mmol) in dry $CH_3CN$ (10 mL) was poured over the solid compound **6** (2.02 g, 3.32 mmol) under anhydrous conditions and stirred at room temperature for 4 h. The solvent was removed in vacuo to produce a syrup that was redissolved in dichloromethane (200 mL) and washed with $H_2O$ (3×200 mL). The organic layer was dried ($Na_2SO_4$), filtered and evaporated in vacuo to give an oil which was purified by column chromatography on silica gel using dichloromethane/ethyl acetate (10:1 v/v) as eluent. The title compound was obtained in 93 % yield (1.50 g). The spectroscopic data of this compound were in agreement with those previously reported.[18]. Anal. calcd for $C_{22}H_{40}O_6N_2Si_2$: C, 54.51; H, 8.32; N, 5.78. Found: C, 54.61; H, 8.35; N, 5.84.

**3.1.10. 5′-*O*-Dimethoxytrityl-(2′*S*)-2′-Deoxy-2′-*C*-methyluridine (8).** 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-(2′*S*)-2′-deoxy-2′-*C*-methyluridine (**7**) (0.95 g, 1.8 mmol) was desylilated as described above for the synthesis of compound **3a**. The crude product was dimethoxytritylated, worked up and purified as described for the synthesis of compound **4a** above. Pure compound **8** was obtained as a foam (96%).$^{13}$C NMR spectrum of **8** ($CDCl_3$): ($\delta$, ppm) 163.0 (C-4); 158.4 ($C_{Ar}$–$OCH_3$, DMTr); 150.2 (C-2); 144.2 (Ar, DMTr); 141.2 (C-6); 135.2, 129.6, 128.5, 128.0, 127.4 (Ar, DMTr); 113.0 (Ar, DMTr); 101.4 (C-5); 86.4 (C–$Ar_3$, DMTr); 86.1 (C-1′); 82.9 (C-4′); 72.7 (C-3′); 60.4 (C-5′); 54.9 (–$OCH_3$, DMTr); 45.1 (C-2′); 10.9 (2′-methyl). $^1$H NMR spectrum of **8** ($CDCl_3$): ($\delta$, ppm) 1.00 (d, 3H, –$CH_3$, $J_{CH3,2'}$=7 Hz); 2.40 (brs, 1H, OH-3′); 2.55 (m, 1H, H-2′); 3.18 (dd, 1H, H-5′, $J_{5',5''}$=12 Hz, $J_{5'',4'}$= 2 Hz); 3.50 (dd, 1H, H-5″, $J_{5',5''}$=12 Hz, $J_{5',4'}$=2 Hz); 3.83 (s, 6H, –$OCH_3$); 4.00 (m, 1H, H-4′); 4.12 (dd, 1H, H-3′, $J_{3',2'}$=$J_{3',4'}$=6 Hz); 5.30 (d, 1H, H-5, $J_{5,6}$=8 Hz); 6.29 (d, 1H, H-1′, $J_{1',2'}$=8 Hz); 6.82 (d, 4H, Ar–$OCH_3$, DMT, $J$=9 Hz); 7.20–7.35 (m, 9H, Ar+Ar–$OCH_3$, DMT); 8.05 (d, 1H, H-6, $J_{5,6}$=8 Hz); 9.35 (brs, 1H, NH). Anal. calcd for $C_{31}H_{32}O_7N_2$: C, 68.37; H, 5.92; N, 5.14. Found: C, 68.45; H, 6.00; N, 5.23.

**3.1.11. 5′-*O*-Dimethoxytrityl-(2′*S*)-2′-deoxy-2′-*C*-methyluridine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (9).** Compound **8** (2.26, 5 mmol) was phosphitylated, worked up and purified according to the procedure used to prepare **5a** above to yield the title compound **9** pure (73%). $^{13}$C NMR spectrum of **9** ($CDCl_3$): ($\delta$, ppm) 162.9 (C-4); 158.8/158.7 ($C_{Ar}$–$OCH_3$, DMTr); 150.3 (C-2); 144.3/144.2 (Ar, DMTr); 141.1 (C-6); 135.3/135.1, 130.5/130.4/130.3, 128.9/128.6/128.4/128.2, 127.9/127.4/127.3 (Ar, DMTr); 117.4/117.3 (CN, phops);113.2 (Ar, DMTr); 101.8 (C-5); 87.0 (C–$Ar_3$, DMTr); 86.4/86.2 (C-1′); 83.3 (C-4′); 76.2/76.0 (C-3′); 60.7 (C-5′); 58.0/57.8 (–$OCH_2$, phosph); 55.3/55.2 (–$OCH_3$, DMTr); 44.8/44.7 (C-2′); 43.9/43.3/43.2 (CHs, phosp); 24.6/24.5 ($CH_3$, phosp); 17.4/17.3 ($CH_2$–CN, phosp); 11.6/11.4 (2′-methyl). $^1$H NMR spectrum of **9** ($CDCl_3$): $\delta$ (ppm) 1.00/1.05 (d, 3H, –$CH_3$, $J_{CH3,2'}$=7 Hz); 1.06, 1.13, 1.17, 1.18 (d, 12H, –$CH_3$, phosp, $J_{CH3,CH}$=7 Hz); 2.28/2.29 (m, 2H, $CH_2$–CN); 2.60/2.63 (m, 1H, H-2′); 3.39–3.46 (m, 2H, –CHs, phosp); 3.48–3.61 (m, 2H, H-5′

and H-5″); 3.70 (m, 1H, H-4′); 3.81 (s, 3H, –$OCH_3$); 3.80/3.79 (s, 3H, –$OCH_3$); 3.80/3.85 (m, 1H, $CH_2O$, phosp); 3.91/3.96 (m, 1H, $CH_2CO$, phosp); 4.33/4.41 (m, 1H, H-3′); 5.10/5.16 (d, 1H, H-5, $J_{5,6}$=8 Hz); 6.29/6.30 (s, 1H, H-1′); 6.82–6.87, 7.22–7.30, 7.35–7.42 (m, 13H, DMT); 8.03/8.07 (d, 1H, H-6, $J_{5,6}$=8 Hz); 8.50 (brs, 1H, NH). $^{31}$P NMR spectrum of **9** ($CDCl_3$): ($\delta$, ppm) 145.87; 148.51. Anal. calcd for $C_{40}H_{49}O_8N_4P$: C, 64.50; H, 6.63; N, 7.52. Found: C, 64.55; H, 6.57; N, 7.58.

**3.1.12. 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-(2′*S*)-2′-deoxy-2′-*C*-methyl-$N^4$-isobutyryl cytidine (10).** 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-(2′*S*)-2′-deoxy-2′-*C*-methyl-4-*O*-(2-nitrophenyl) uridine (**6**) (2,04 g, 3.36 mmol) in dry tetrahydrofuran (120 mL) was treated at room temperature with ammonia (10 bar) in a stainless steel bomb for four days. The solvent was evaporated in vacuo, the residue was dissolved in dry pyridine (30 mL) and the solution cooled in an ice bath. Isobutyrylchloride (1.05 mL, 10.12 mmol) was added with stirring and exclusion of moisture and after two hours the reaction was completed. The reaction mixture was evaporated to dryness, redissolved in dichloromethane (100 mL) and washed with 10% aqueous $NaHCO_3$ (2×100 mL) and $H_2O$ (2×100 mL). The organic phase was dried ($Na_2SO_4$), filtered and evaporated to dryness. The residue was purified by column chromatography using dichloromethane/MeOH (99:1, v/v) as eluant to afford pure compound **10** in 88% yield (1.62 g). $^{13}$C NMR spectrum of **10** ($CDCl_3$): ($\delta$, ppm)177.5 (CO isobutyryl); 162.7 (C-4); 155.4 (C-2); 144.9 (C-6); 96.4 (C-5); 86.7 (C-1′); 84.1 (C-4′); 72.3 (C-3′); 59.7 (C-5′); 44.1 (C-2′); 36.1, 19.0 (isobutyryl); 17.3–12.5 (isopropyls), 11.3 (2′-methyl). $^1$H NMR spectrum of **10** ($CDCl_3$): ($\delta$, ppm) 0.80–1.20 (m, 28H, –CHs and –$CH_3$s, disiloxane); 0.95 (d, 3H, –$CH_3$, $J_{CH3,2'}$=7 Hz); 1.28 (d, 6H, –$CH_3$, isobutyryl, $J_{CH,CH3}$=7 Hz); 2.50 (m, 1H, H-2′); 2.65 (q, 1H, –CH, isobutyryl, $J_{CH,CH3}$=7 Hz); 3.65–4.20 (m, 3H, H-4′, H-5′ and H-5″); 4.25 (m, 1H, H-3′); 6.18 (s, 1H, H-1′); 6.95 (d, 1H, H-5, $J_{5,6}$=8 Hz); 8.00 (d, 1H, H-6, $J_{5,6}$=8 Hz); 9.58 (brs, 1H, NH). Anal. calcd for $C_{26}H_{47}O_6N_3Si_2$: C, 56.39; H, 8.55; N, 7.59. Found: C, 56.44; H, 8.51; N, 7.49.

**3.1.13. 5′-*O*-Dimethoxytrityl- (2′*S*)-2′-Deoxy-2′-*C*-methyl-$N^4$-isobutyrylcytidine (11).** 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-(2′*S*)-2′-deoxy-2′-*C*-methyl-$N^4$-isobutyryl cytidine (**10**) was desilylated and worked up according to procedures used to prepare **3a** and the crude product were dimethoxytritylated according to procedures given for the synthesis of **4a**. The residue was purified by column chromatography which was first washed with 50% ethyl acetate in petrol and then pure compound **11** was eluted by using 2% MeOH in dichloromethane (79%).

$^{13}$C NMR spectrum of **11** ($CDCl_3$): ($\delta$, ppm) 177.1 (CO isobutyryl); 162.3 (C-4); 158.5 ($C_{Ar}$–$OCH_3$, DMTr); 155.6 (C-2); 143.2 (Ar, DMTr); 145.7 (C-6); 135.1, 129.7, 128., 128.0, 127.4 (Ar, DMTr); 113.0 (Ar, DMTr); 96.4 (C-5); 87.1 (C-1′); 86.4 (C–$Ar_3$, DMTr); 83.6 (C-4′); 73.7 (C-3′); 61.3 (C-5′); 54.9 (–$OCH_3$, DMTr); 45.9 (C-2′); 35.9, 18.8 (isobutyryl); 10.9 (2′-methyl). $^1$H NMR spectrum of **11** ($CDCl_3$): ($\delta$, ppm) 0.94 (d, 3H, –$CH_3$, $J_{CH3,2'}$=7 Hz); 1.25 (d, 6H, –$CH_3$, isobutyryl, $J_{CH,CH3}$=7 Hz); 2.29 (m, 1H, H-2′); 2.65 (q, 1H, –CH, isobutyryl, $J_{CH,CH3}$=7 Hz); 2.67 (brs, 1H, OH-3′); 3.54 (m, 2H, H-5′ and H-5″); 3.75 (s, 6H, –$OCH_3$); 3.81 (m, 1H, H-4′); 4.27 (m, 1H, H-3′); 6.21 (s, 1H, H-1′); 6.84 (d, 4H, Ar–$OCH_3$, DMT, $J$=9 Hz); 6.98 (d, 1H, H-5, $J_{5,6}$=8 Hz); 7.18–7.40 (m, 9H, Ar+Ar–$OCH_3$, DMT); 8.02 (d, 1H, H-6, $J_{5,6}$=8 Hz); 8.30 (brs, 1H, NH). Anal. calcd for $C_{35}H_{39}O_7N_3$: C, 64.50; H, 6.41; N, 6.85. Found: C, 64.57; H, 6.46; N, 6.89.

**3.1.14. 5′-*O*-Dimethoxytrityl-(2′*S*)-2′-deoxy-2′-*C*-methyl-$N^4$-isobutyrylcytidine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (12).** Compound **11** (3.01 g, 5 mmol) was phosphitylated, worked up according to the procedure used to prepare **5a** above. The crude product was purified by column chromatography on silica gel eluting with 30% ethyl acetate in dichloromethane containing 1% of triethylamine to yield the title compound **12** pure (72%). $^{13}$C NMR spectrum of **12** ($CDCl_3$): ($\delta$, ppm) 177.0 (CO, isobutyryl); 162.1 (C-4); 158.8/158.7 ($C_{Ar}$–$OCH_3$, DMTr); 155.3 (C-2); 145.6 (Ar, DMTr); 144.0 (C-6); 135.5/135.4, 130.4/130.4/130.3/130.2, 128.6/128.5, 127.9/127.3/127.1 (Ar, DMTr); 117.3 (CN, phops);113.2 (Ar, DMTr); 95.9 (C-5); 87.6/87.3 (C–$Ar_3$, DMTr); 86.9 (C-1′); 83.3 (C-4′); 76.9 (C-3′); 61.3/61.1 (C-5′); 58.1/58.0 (–$OCH_2$, phosph); 55.2 (–$OCH_3$, DMTr); 44.6/44.5 (C-2′); 43.3/43.2/43.1 (CHs, phosp); 36.7 (CH, isobutyryl); 24.6/24.5/24.4 ($CH_3$, phosp); 20.3/20.0 ($CH_3$, isobutyryl); 19.0 ($CH_2$–CN, phosp); 11.8/11.6 (2′-methyl). $^1$H NMR spectrum of **12** ($CDCl_3$): ($\delta$, ppm) 0.93/0.94 (d, 3H, –$CH_3$, $J_{CH3,2'}$=7 Hz); 1.00, 1.13 1.16 (d, 12H, –$CH_3$, phosp, $J_{CH3,CH}$=7 Hz); 1.20 (m, 6H, –$CH_3$, isobutyryl); 2.29/2.30 (m, 2H, $CH_2$–CN); 2.58 (m, 1H, H-2′); 2.79 (m, 1H, –CH, isobutyryl); 3.41–3.47 (m, 2H, –CHs, phosp); 3.48–3.58 (m, 2H, H-5′ and H-5″); 3.61/3.63 (m, 1H, H-4′); 3.80 (s, 3H, –$OCH_3$); 3.82 (s, 3H, –$OCH_3$); 3.75/3.87 (m, 1H, $CH_2O$, phops); 3.97/4.04 (m, 1H, $CH_2O$, phosp); 4.21/4.30 (m, 1H, H-3′); 6.39/6.41 (d, 1H, H-1′, $J_{1'-2'}$=7 Hz); 6.97/7.04 (d, 1H, H-5, $J_{5,6}$=7 Hz); 6.82–6.88, 7.25–7.35, 7.38–7.42 (m, 13H, DMT); 8.29 (brs, 1H, NH); 8.34/8.40 (d, 1H, H-6, $J_{5,6}$=7 Hz).

$^{31}$P NMR spectrum of **12** ($CDCl_3$): ($\delta$, ppm) 145.88; 148.39. Anal. calcd for $C_{44}H_{56}O_8N_5P$: C, 64.93; H, 6.94; N, 8.60. Found: C, 64.98; H, 7.02; N, 8.66.

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