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Carbohydrate Research 331 (2001) 83–90

CARBOHYDRATE RESEARCH

Note

Synthesis of isotopically labeled D-[1′-^{13}C]ribonucleoside phosphoramidites

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Received 27 October 2000; accepted 20 December 2000

Abstract

The preparation of fully protected labeled diisopropylamino-β-cyanoethyl-[1′-^{13}C]ribonucleoside phosphoramidites with regioisomeric purity is described. We demonstrated in this paper that a regioselective 2′-O-silylation, through a 3′,5′-*O*-di-*tert*-butylsilanediyl protection, has been applied for the synthesis of [1′-^{13}C]ribonucleoside phosphoramidite units. This method allowed us to obtain only the desired 2′-*O*-silyl-3′-*O*-phosphoramidites avoiding the undesired 3′-*O*-silyl-2′-*O*-phosphoramidite nucleosides isolated by standard procedures. This is a suitable procedure to RNA precursors with respect to the isotope-containing precursors. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: [1-^{13}C]Ribose; Ribonucleosides; Phosphoramidites; Labeled nucleosides

The availability of ^{13}C-labeled ribonucleosides are of special interest since new NMR techniques have been established which allow us to assign overlapping ^{1}H NMR signals in spectra of large biomolecules such as RNA fragments.[1,2] The chemical synthesis of RNA oligoribonucleotides involves the use of protected ribonucleoside phosphoramidites.[3–5] Standard procedures described by Ogilvie et al.[6–8] for preparation of ribonucleoside phosphoramidite units used in solid-phase synthesis of RNA oligonucleotides include protection of the amino groups on the nucleobases, protection of the 5′- and 2′-hydroxyl groups as dimethoxytrityl (DMTr) and TBDMS ether, respectively, and phosphitylation of the 3′-oxygen to the β-cyanoethyl *N*,*N*-diisopropylphosphoramidite. Nevertheless, the need to protect this additional 2′-hydroxyl group in RNA (compared to DNA synthesis) has hindered the development of a practical method. In fact, the principal consideration in the chemical synthesis of the ribonucleoside phosphoramidites is contamination of the desired 2′-*O*-silyl-3′-*O*-phosphoramidites with undesired 3′-*O*-silyl-2′-*O*-phosphoramidite nucleosides which can yield undesired 5′,2′-internucleotidic bonds, consuming valuable labeled ribonucleosides and lowering the yield of desired phosphoramidite units. Moreover, one of the major problems in the chemical synthesis of ^{13}C enriched nucleoside derivatives is the price of the starting compound that requires optimized reactions. Our efforts aimed at reproducible experimental procedures giving high-yield products with respect to the iso-

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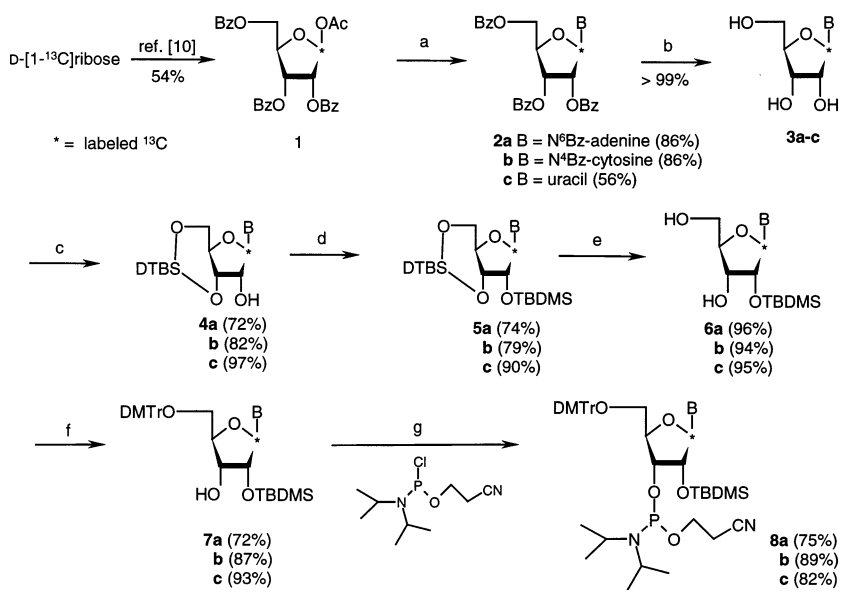
PII: S0008-6215(00)00327-X

tope-containing precursors. We demonstrate in this paper that a regioselective 2′-O-silylation, through the use of di-*tert*-butyldichlorosilane, has been applied for the synthesis of [1′-^{13}C]ribonucleoside phosphoramidite units.

In the course of this work, another approach to this problem has been reported by Song et al.[9] through H-phosphonate derivatives, but through an undesired but separable mixture of 2′- and 3′-silyl derivatives. The D-[1-^{13}C]ribose was converted into [1-^{13}C]-1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**1**) (Scheme 1) according to a procedure used for the synthesis of unlabeled D isomer.[10] Nucleoside derivatives **2a**–**c** were obtained from ribosylation of **1** with persilylated nucleobases (N^6-benzoyladenine, uracil, and N^4-benzoylcytosine) under *Vorbrüggen* conditions.[11] In case of the synthesis of protected uridine **2c**, the temperatures had to be kept under 45 °C to avoid undesired side product. The selective deprotection of *O*-acyl groups from **2a**–**c** was achieved with aqueous 1 M NaOH–ethanol–pyridine (for **3a**,**b**) or NH_3–MeOH (for **3c**). In all cases, de-acylated nucleosides **3a**–**c** were directly used in the next reaction.

To circumvent the problem of undesired 3′-*O*-silyl-2′-*O*-phosphoramidite obtained during the silylation of 2′-hydroxyl by the largely known procedures, we applied a three step procedure involving the initial and regioselective protection of 3′,5′-hydroxyl using di-*tert*-butyldichlorosilane.[12] Thus treatment of D-[1′-^{13}C]ribonucleosides **3a**–**c** with di-*tert*-butyldichlorosilane in the presence of $AgNO_3$[14] in DMF afforded the corresponding 3′,5′-*O*-(di-*tert*-butylsilanediyl) derivatives **4a** (72%), **4b** (82%), and **4c** (97%), respectively. Treatment with *tert*-butyldimethylsilyl chloride (TBDMSiCl) and silver nitrate in DMF led 2′-*O*-TBDMS ether **5a**–**c** in fairly good yields. The di-*tert*-butylsilylene group was then removed by treatment with pyridinium poly(hydrogen fluoride) at −20 °C to afford **6a** (96%), **6b** (94%), and **6c** (95%) yield, respectively. Under these conditions, no migration of the 2′-*O*-TBDMS group to the 3′-oxygen was observed.[12,13] After dimethoxytritylation of **6a**–**c** **to** **7a**–**c**, isomerization free synthesis of the phosphoramidites **8a**–**c** was accomplished using (*N*,*N*-diisopropylamino)(cyanoethyl)phosphonamidic chloride as the phosphitylating agent, in the presence of 2,4,6-collidine and *N*-methylimidazole.[5] As a result, the desired RNA phosphoramidite units **8a**–**c** were obtained avoiding byproducts as found by the classical approach, and with an overall yield for the sequential transformation of D-[1′-



Scheme 1. Reagents and conditions: (a) silylated heterocycle, 1,2-dichloroethane, TMSOTf; (b) (for **3a** and **3b**) pyridine–EtOH–1 M NaOH then Dowex-H^+ or (for **3c**) MeOH–NH_3; (c) $DTBSiCl_2$, $AgNO_3$, DMF; (d) TBDMSiCl, DMAP, pyridine; (e) HF-Py, THF, −20°C; (f) DMTrCl, pyridine; (g) 2,4,6-collidine, CH_2Cl_2, *N*-methylimidazole.

^{13}C]ribonucleosides **3a**–**c** to **8a**–**c** of 28% (for **8a**), 47% (for **8b**) and 63% (for **8c**).[1]

1. Experimental

General methods.—Commercially available chemicals and solvents were reagent grade and used as received. Dry THF, pyridine, CH_2Cl_2, and 1,2-dichloroethane were obtained by distillation from CaH_2, *N*,*N*-dimethylformamide from BaO. The reactions were monitored by thin-layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60 F_{254}, E. Merck). Compounds were visualized by UV irradiation and/or spraying with 20% H_2SO_4 in EtOH, followed by charring at 150 °C. Column chromatography was performed on Silica Gel 60 M (0.040–0.063 mm, E. Merck). NMR spectra were accumulated with a Bruker AVANCE DPX 250 Fourier Transform 250 spectrometer, with Me_4Si as the internal standard, unless otherwise stated. Chemical shifts are given in ppm (δ). Low-resolution mass spectra were obtained on a Perkin–Elmer SCIEX API-300 (heated nebullizer) spectrometer operating in the ion-spray (IS) mode. Elemental analyses were performed by the Service Central de Microanalyse du CNRS (Vernaison, France).

1-O-*Acetyl-2,3,5-tri*-O-*benzoyl-β*-D-*ribofuranoside* (**1**).—Compound **1** was prepared from D-[1-^{13}C]ribose in 54% total yield according to a procedure for the synthesis of the unlabeled D isomer.[10] ^{1}H NMR ($CDCl_3$): δ 2.01 (s, 3 H, OAc), 4.52 (dd, 1 H, $J_{5a,4}$ 4.0, $J_{5a,5b}$ 13 Hz, H-5a), 4.77 (dd, 1 H, H-5b), 4.78 (m, 1 H, H-4), 5.78 (dd, 1 H, $J_{2,1}$ 1.0, $J_{2,3}$ 5 Hz, H-2), 5.91 (ddd, 1 H, $J_{3,4}$ 6.9, $J_{3,2}$ 5.0, $J_{3,C1}$ 1.5 Hz, H-3), 6.43 (dd, 1 H, $J_{1,C1}$ 183, H-1), 7.38–8.15 (m, 15 H, 3 × Bz).

[1′-^{13}C]-2′,3′,5′-Tri-O-*benzoyl*-N^6-*benzoyladenosine* (**2a**).—A mixture of N^6-benzoyladenine (1.30 g, 5.78 mmol) and $(NH_4)_2SO_4$ (catalytic amount) in hexamethyldisilazane (14 mL) was refluxed for 14 h under N_2. The clear solution obtained was concentrated in vacuo and the residue was dissolved in dry 1,2-dichloroethane (DCE, 5 mL) followed by a solution of acetate **1** (1.46 g, 2.89 mmol) in dry DCE (10 mL) and TMSOTf (1.38 mL, 2.89 mmol) at rt. After stirring the reaction mixture for 30 min at rt, it was heated at 45 °C for 5 h under N_2. The reaction solution was poured into an ice-cold mixture of CH_2Cl_2 (50 mL) and satd $NaHCO_3$ solution (50 mL). The aqueous phase was separated and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layer was washed with brine and dried ($MgSO_4$). The solvents were removed under reduced pressure and the residue was separated by silica gel column chromatography (50:1 CH_2Cl_2–CH_3OH) to give **2a** as a colorless oil (1.70 g, 86%). ^{1}H NMR ($CDCl_3$) δ 4.70 (dd, 1 H, $J_{5'a,4'}$ 3.0, $J_{5'a,5'b}$ 12.9 Hz, H-5′a), 4.84 (m, 1 H, H-4′), 4.92 (dd, 1 H, $J_{5'b,4'}$ 3.3 Hz, H-5′b), 6.25 (dd, 1 H, $J_{2',1'}$ 4.6, $J_{2',3'}$ 5.5 Hz, H-2′), 6.41 (dd, 1 H, $J_{3',4'}$ 4.3 Hz, H-3′), 6.50 (dd, 1 H, H-1′, $J_{1',C?'}$166 Hz), 7.36–8.18 (m, 20 H, 4 × Bz), 8.70 (s, 1 H, H-2), 8.98 (s, 1 H, H-8). MS: *m*/*z* 685 $(M+H)^+$.

Compounds **2b** and **2c** were synthesized by a similar methodology as described for the preparation of compound **2a** from compound **1**.

[1′-^{13}C]-2′,3′,5′-Tri-O-*benzoyl*-N^4-*benzoylcytidine* (**2b**).—Isolated as a colorless oil (86%, 2.66 g). $[\alpha]_D^{22}$ −42° (*c*2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.73–4.87 (m, 4 H, H-5, H-4′, H-5′a, H-5′b), 5.87 (dd, 1 H, $J_{2',1'}$ 2.8, $J_{2',3'}$ 5.7 Hz, H-2′), 5.93 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-3′), 6.50 (dd, 1 H, $J_{1',C1'}$ 169 Hz, H-1′), 7.36–8.18 (m, 21 H, H-6, 4 × Bz), 8.70 (s, 1 H, H-2), 8.98 (s, 1 H, H-8). MS: *m*/*z* 661 $(M+H)^+$.

[1′-^{13}C]-2′,3′,5′-Tri-O-*benzoyluridine* (**2c**).—Isolated as a colorless oil (56%, 0.92 g). $[\alpha]_D^{20}$ −37° (*c*2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.69 (dd, 1 H, $J_{5'a,4'}$ 3.7, $J_{5'a,5'b}$ 11.7 Hz, H-5′a), 4.71 (m, 1 H, H-4′), 4.85 (dd, 1 H, $J_{5'b,4'}$ 2.4 Hz, H-5′b), 5.62 (dd, 1 H, $J_{5,NH}$ 2.2, $J_{5,6}$ 8.1 Hz, H-5), 5.76 (dd, 1 H, $J_{2',1'}$ 5.6, $J_{2',3'}$ 6.0 Hz, H-2′), 5.89 (dd, 1 H, $J_{3',4'}$ 4.5 Hz, H-3′), 6.33 (dd, 1 H, $J_{1',C1'}$ 169 Hz, H-1′), 7.34–8.13 (m, 16 H, H-6, 3 × Bz), 8.79 (bs, 1 H, NH). MS: *m*/*z* 558 $(M+H)^+$.

[1′-^{13}C]-N^6-*benzoyladenosine* (**3a**).—To a solution of **2a** (1.41 g, 2.05 mmol) in pyridine (12 mL) and EtOH (6 mL) at 0 °C was added

[1] See Section 2.

dropwise 1 M NaOH (9.1 mL). The mixture was stirred at 0 °C for 30 min and neutralized with Dowex-50WX2-200 (H^+). The solution was filtered and the resin was washed with 1:4 pyridine–water (60 mL). The filtrate was evaporated and co-evaporated with toluene. The remaining residue was triturated with Et_2O, leaving a colorless powder of **3a** (0.80 g, > 99%). $[\alpha]_D^{20}$ − 50° (*c* 1.3, dimethylsulfoxide); ^1H NMR (DMSO-d_6) δ 3.59 (m, 1 H, H-5′a), 3.70 (m, 1 H, H-5′b), 3.91 (m, 1 H, H-4′), 4.21 (m, 1 H, H-3′), 4.66 (m, 1 H, H-2′), 5.11 (dd, 1 H, $C_{5'}OH$), 5.22 (d, 1 H, $C_{3'}OH$), 5.54 (d, 1 H, $C_{2'}OH$), 6.05 (dd, 1 H, $J_{1',C1'}$ 166 Hz, H-1′), 7.55–8.05 (m, 5 H, Bz), 8.71 (s, 1 H, H-2) 8.76 (s, 1 H, H-8), 11.20 (s, 1 H, NH). MS: *m*/*z* 373 $(M+H)^+$. Anal. Calcd for $C_{16}^{13}C_1H_{17}N_5O_5$: C, 55.10; H, 4.60. Found: C, 55.14; H, 4.62.

Compounds **3b** were synthesized by a similar methodology as described for the preparation of compound **3a** from compounds **2b**.

[1′-^{13}C]-N^4-benzoylcytidine (**3b**).—Isolated as a colorless powder of **3b** (0.12 g, > 99%). $[\alpha]_D^{23}$ + 29° (*c* 0.26, dimethylsulfoxide); ^1H NMR (DMSO-d_6) δ 3.62 (m, 1 H, H-5′a), 3.76 (m, 1 H, H-5′b), 3.92 (m, 1 H, H-4′), 3.98 (m, 1 H, H-3′), 4.02 (m, 1 H, H-2′), 5.02 (d, 1 H, $J_{3',OH}$ 5.5 Hz, $C_{3'}OH$), 5.15 (dd, 1 H, $J_{5'a,OH}$ 5.0, $J_{5'b,OH}$ 5.0, $C_{5'}OH$), 5.48 (d, 1 H, $J_{2',OH}$ 5.0 Hz, $C_{2'}OH$), 5.82 (dd, 1 H, $J_{1',C1'}$ 174.5 Hz, H-1′), 7.32 (dd, 1 H, $J_{5,NH}$ 1.5, $J_{5,6}$ 8.0 Hz, H-5), 7.52–7.62 (m, 5 H, Bz), 8.48 (dd, 1 H, $J_{6,C1'}$ 2.5, H-6), 11.20 (s, 1 H, NH). MS: *m*/*z* 349 $(M+H)^+$. Anal. Calcd for $C_{15}^{13}C_1H_{17}N_3O_6$: C, 55.46; H, 4.92. Found: C, 55.51; H, 4.96.

[1′-^{13}C]-Uridine (**3c**).—A solution of **2c** (0.91 g, 1.63 mmol) in satd methanolic ammonia was stirred at rt for 14 h. The solvent was then removed under reduced pressure and the remaining residue triturated with Et_2O, leaving a colorless powder of **3c** (> 98%, 0.39 g). $[\alpha]_D^{23}$ + 8° (*c* 2, water); ^1H NMR (D_2O) δ 3.81 (m, 1 H, H-5′a), 3.92 (m, 1 H, H-5′b), 4.14 (m, 1 H, H-4′), 4.23 (m, 1 H, H-3′), 4.35 (m, 1 H, H-2′), 5.90 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 5.92 (m, 1 H, H-1′), 7.88 (d, 1 H, H-6). MS: *m*/*z* 246 $(M+H)^+$. Anal. Calcd for $C_8^{13}C_1H_{12}N_2O_6$: C, 44.49; H, 4.93. Found: C, 44.52; H, 4.96.

*[1′-^{13}C]-3′,5′-*O*-(Di-*tert*-butylsilanediyl)-N^6-benzoyladenosine* (**4a**).—To a solution of **3a** (0.58 g, 1.54 mmol) and $AgNO_3$ (0.78 g, 4.6 mmol) in anhyd DMF (10 mL) at 0 °C was added dropwise di-*tert*-butyldichlorosilane (0.5 mL, 2.37 mmol) with vigorous stirring. The mixture was warmed to rt and subsequently stirred for 15 min Et_3N (0.65 mL, 4.46 mmol) was added, and the mixture was stirred for an additional 5 min. After addition of water (100 mL), the resulting solution was extracted with EtOAc (2 × 50 mL). The organic layer was washed with brine and dried ($MgSO_4$). The solvents were evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (25:1 CH_2Cl_2–CH_3OH) to give **4a** (72%, 0.58 g) as a colorless waxy solid. ^1H NMR ($CDCl_3$) δ 1.08 (s, 9 H, ^{t}Bu), 1.15 (s, 9 H, ^{t}Bu), 4.06–4.17 (m, 2 H, H-5′a, H-5′b), 4.48 (m, 1 H, H-4′), 4.76 (dd, 1 H, $J_{2',1'}$ 1.0, $J_{2',3'}$ 5.0 Hz, H-2′), 4.90 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-3′), 6.05 (dd, 1 H, $J_{1',C1'}$ 167 Hz, H-1′), 7.50–8.02 (m, 5 H, Bz), 8.06 (s, 1 H, H-2), 8.78 (s, 1 H, H-8). MS: *m*/*z* 513 $(M+H)^+$.

Compounds **4b** and **4c** were synthesized by a similar methodology as described for the preparation of compound **4a** from respective compounds **3b** and **3c**.

*[1′-^{13}C]-3′,5′-*O*-(Di-*tert*-butylsilanediyl)-N^4-benzoylcytidine* (**4b**).—Isolated as a colorless waxy solid (82%, 0.12 mg). ^1H NMR ($CDCl_3$) δ 1.03 (s, 9 H, ^{t}Bu), 1.04 (s, 9 H, ^{t}Bu), 3.98–4.23 (m, 4 H, H-3′, H-4′, H-5′a, H-5), 4.39 (dd, 1 H, $J_{2',1'}$ 1.0, $J_{2',3'}$ 4.7 Hz, H-2′), 4.55 (dd, 1 H, $J_{5'b,4'}$ 4.7, $J_{5'b,5'a}$ 9.1 Hz, H-5′b), 5.77 (dd, 1 H, $J_{1',C1'}$ 175 Hz, H-1′), 7.49–7.93 (m, 6 H, H-6, Bz). MS: *m*/*z* 489 $(M+H)^+$.

[1′-^{13}C]-3′,5′-O-(Di-tert-butylsilanediyl)-uridine (**4c**).—Isolated as a colorless waxy solid (97%, 0.54 g). ^1H NMR ($CDCl_3$) δ 1.03 (s, 9 H, ^{t}Bu), 1.04 (s, 9 H, ^{t}Bu), 4.00–4.07 (m, 2 H, H-5′a, H-5′b), 4.18 (m, 1 H, H-4′), 4.39 (dd, 1 H, $J_{3',2'}$ 4.2, $J_{3',4'}$ 5.3 Hz, H-3′), 4.47 (dd, 1 H, $J_{2',1'}$ 1.0 Hz, H-2′), 5.61 (dd, 1 H, $J_{1',C1'}$ 170 Hz, H-1′), 5.75 (d, 1 H, $J_{5,6}$ 8.3 Hz, H-5), 7.22 (dd, 1 H, $J_{6,NH}$ 3.2 Hz, H-6), 8.70 (bs, 1 H, NH). MS: *m*/*z* 386 $(M+H)^+$.

*[1′-^{13}C]-2′-*O*-tert-Butyldimethylsilyl-3′,5′-O-(di-tert-butylsilanediyl)-N^6-benzoyladenosine* (**5a**).—To a solution of **4a** (0.41 g, 0.81 mmol) and DMAP (catalytic amount) in anhyd pyridine (10 mL) was added dropwise *tert*-

butyldimethylsilyl chloride (0.12 g, 0.78 mmol), and the mixture was refluxed overnight. After evaporation under reduced pressure, the resulting mixture was diluted with CH_2Cl_2 (40 mL), and the organic phase washed with brine (40 mL) and dried ($MgSO_4$). After a column chromatography on silica gel (50:1 CH_2Cl_2–CH_3OH), the desired product **5a** (74%, 0.38 g) was isolated as a colorless waxy solid. ^{1}H NMR ($CDCl_3$) δ 0.18 (s, 3 H, Me), 0.20 (s, 3 H, Me), 0.96 (s, 9 H, ^{t}Bu), 1.07 (s, 9 H, ^{t}Bu), 1.10 (s, 9 H, ^{t}Bu), 4.06 (m, 1 H, $J_{5'a,4'}$ 9.2, $J_{5'a,5'b}$ 10.3 Hz, H-5′a), 4.28 (m, 1 H, H-4′), 4.47–4.56 (m, 2 H, H-3′, H-5′b), 4.65 (d, 1 H, $J_{2',1'}$ 1.0, $J_{2',3'}$ 4.0 Hz, H-2′), 6.11 (dd, 1 H, $J_{1',C1'}$ 170 Hz, H-1′), 7.51–8.02 (m, 5 H, Bz), 8.05 (s, 1 H, H-2), 8.79 (s, 1 H, H-8). MS: m/z 627 $(M+H)^+$.

Compounds **5b** and **5c** were synthesized by a similar methodology as described for the preparation of compound **5a** from respective compounds **4b** and **4c**.

[1′-^{13}C]-2′-O-tert-*Butyldimethylsilyl-3′,5′*-O-*(di* - tert - *butylsilanediyl)* - N^4 - *benzoylcytidine* (**5b**).—Isolated as a colorless waxy solid (79%, 0.12 g). ^{1}H NMR ($CDCl_3$) δ 0.18 (s, 3 H, Me), 0.27 (s, 3 H, Me), 0.96 (s, 9 H, ^{t}Bu), 1.03 (s, 9 H, ^{t}Bu), 1.04 (s, 9 H, ^{t}Bu), 3.81 (dd, 1 H, $J_{5'a,4'}$ 4.4, $J_{5'a,5'b}$ 9.5 Hz, H-5′a), 4.03 (m, 1 H, H-4′), 4.26–4.32 (m, 2 H, H-3′, H-5), 4.35 (dd, 1 H, $J_{2',1'}$ 1.0, $J_{2',3'}$ 4.4 Hz, H-2′), 4.59 (dd, 1 H, $J_{5'b,4'}$ 4.9, H-5′b), 5.76 (dd, 1 H, $J_{1',C1'}$ 178 Hz, H-1′), 7.48–7.96 (m, 6 H, H-6, Bz), 8.75 (bs, 1 H, NH). MS: m/z 603 $(M+H)^+$.

[1′-^{13}C]-2′-O-tert-*Butyldimethylsilyl-3′,5′*-O-*(di*-tert-*butylsilanediyl)uridine* (**5c**).—Isolated as a colorless waxy solid (90%, 0.62 g). ^{1}H NMR ($CDCl_3$) δ 0.14 (s, 3 H, Me), 0.18 (s, 3 H, Me), 0.93 (s, 9 H, ^{t}Bu), 1.02 (s, 9 H, ^{t}Bu), 1.05 (s, 9 H, ^{t}Bu), 3.86 (dd, 1 H, $J_{5'a,4'}$ 4.7, $J_{5'a,5b'}$ 9.5 Hz, H-5′a), 3.97 (dd, 1 H, $J_{3',2'}$ 4.7, $J_{3',4'}$ 9.1 Hz, H-3′), 4.17 (m, 1 H, H-4′), 4.28 (dd, 1 H, $J_{2',1'}$ 1.0 Hz, H-2′), 4.51 (dd, 1 H, $J_{5'b,4'}$ 4.9 Hz, H-5′b), 5.66 (dd, 1 H, $J_{1',C1'}$ 175 Hz, H-1′), 5.74 (dd, 1 H, $J_{5,NH}$ 2.2, $J_{5,6}$ 8.1 Hz, H-5), 7.25 (dd, 1 H, $J_{6,C1'}$ 2.5 Hz, H-6), 8.40 (bs, 1 H, NH). MS: m/z 500 $(M+H)^+$.

[1′-^{13}C]-2′-O-tert-*Butyldimethylsilyl*-N^6-*benzoyladenosine* (**6a**).—HF·pyridine (6 mL, 2.34 mmol) was carefully diluted with anhyd pyridine (0.31 mmol) and then added dropwise to a solution of **5a** (0.36 g, 0.58 mmol) in THF (3 mL) at 0 °C. The mixture was warmed to rt, stirred for 5 min and then diluted with pyridine (0.5 mL). Water (5 mL) was added and the aqueous phase extracted with CH_2Cl_2 (3 × 40 mL). The organic layers were collected, washed with 5% $NaHCO_3$ (50 mL) and dried ($MgSO_4$). After evaporation under reduced pressure, the residue was purified by silica gel column chromatography (20:1 CH_2Cl_2–CH_3OH) to yield the desired compound **6a** (96%, 0.28 g) as a colorless waxy solid. ^{1}H NMR ($CDCl_3$) δ −0.36 (s, 3 H, Me), −0.13 (s, 3 H, Me), 0.83 (s, 9 H, ^{t}Bu), 3.79 (dd, 1 H, $J_{5'a,4'}$ 1.0, $J_{5'a,5'b}$ 13.2 Hz, H-5′a), 4.03 (dd, 1 H, $J_{5'b,4'}$ 1.6 Hz, H-5′b), 4.37–4.42 (m, 2 H, H-3′, H-4′), 5.17 (dd, 1 H, $J_{2',3'}$ 4.7, $J_{2',1'}$ 7.5 Hz, H-2′), 5.86 (dd, 1 H, $J_{1',C1'}$ 163 Hz, H-1′), 7.50–8.02 (m, 5 H, Bz), 8.08 (s, 1 H, H-2), 8.86 (s, 1 H, H-8). MS: m/z 487 $(M+H)^+$.

Compounds **6b** and **6c** were synthesized by a similar methodology as described for the preparation of compound **6a** from respective compounds **5b** and **5c**.

[1′-^{13}C]-2′-O-tert-*Butyldimethylsilyl*-N^4-*benzoylcytidine* (**6b**).—Isolated as a colorless waxy solid (94%, 0.08 g). ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 3 H, Me), 0.12 (s, 3 H, Me), 0.92 (s, 9 H, ^{t}Bu), 3.64–3.84 (m, 2 H, H-5′a, H-5′b), 4.02 (m, 1 H, H-3′), 4.20 (d, 1 H, $J_{5,6}$ 2.1 Hz, H-5), 4.27 (m, 1 H, H-4′), 4.83 (dd, 1 H, $J_{2',3'}$ 4.4, $J_{2',1'}$ 4.7 Hz, H-2′), 5.54 (dd, 1 H, $J_{1',C1'}$ 169 Hz, H-1′), 7.49–8.06 (m, 6 H, H-6, Bz), 8.82 (bs, 1 H, NH). MS: m/z 463 $(M+H)^+$.

[1′-^{13}C]-2′-O-tert-*Butyldimethylsilyluridine* (**6c**).—Isolated as a colorless waxy solid (95%, 0.42 g). ^{1}H NMR ($CDCl_3$) δ 0.09 (s, 3 H, Me), 0.11 (s, 3 H, Me), 0.91 (s, 9 H, ^{t}Bu), 3.79 (dd, 1 H, $J_{5'a,4'}$ 6.1, $J_{5'a,5'b}$ 12.2 Hz, H-5′a), 3.95 (dd, 1 H, $J_{5'b,4'}$ 2.9 Hz, H-5′b), 4.15–4.24 (m, 2 H, H-2′, H-3′), 4.62 (m, 1 H, H-4′), 5.57 (dd, 1 H, H-1′, $J_{1',2'}$ 5.4, $J_{1',C1'}$ 167 Hz, H-1′), 5.75 (d, 1 H, $J_{5,6}$ 8.5 Hz, H-5), 7.55 (dd, 1 H, $J_{6,C1'}$ 3.2, $J_{5,6}$ 8.5 Hz, H-6), 8.20 (bs, 1 H, NH). MS: m/z 360 $(M+H)^+$.

[1′-^{13}C]-5′-O-*Dimethoxytrityl-2′*-O-tert-*butyldimethylsilyl*-N^6-*benzoyladenosine* (**7a**).—To a solution of **6a** (0.65 g, 1.32 mmol) in THF (15 mL) under N_2 were added anhyd pyridine (0.53 mL, 6.60 mmol), $AgNO_3$ (0.27 g, 1.32 mmol) and 4,4′-dimethoxytrityl chlo-

ride (0.54 g, 1.58 mmol) successively with vigorous stirring and exclusion of moisture. The resultant pale-yellow solution was stirred for 1 h and filtered into a 5% $NaHCO_3$ solution. The filtrate was extracted with CH_2Cl_2 (50 mL), dried ($MgSO_4$). After evaporation of the solvent under reduced pressure, the residue was subjected to a silica gel column chromatography (19:1 CH_2Cl_2–CH_3OH) to give **7a** (72%, 0.75 g) as a colorless foam. ^1H NMR ($CDCl_3$) δ −0.15 (s, 3 H, Me), 0.01 (s, 3 H, Me), 0.83 (s, 9 H, ^{t}Bu), 3.38 (dd, 1 H, $J_{5'a,4'}$ 3.4, $J_{5'a,5'b}$ 10.5 Hz, H-5′a), 3.54 (dd, 1 H, $J_{5'b,4'}$ 3.1, H-5′b), 3.77 (s, 6 H, 2 × OMe), 4.28 (m, 1 H, H-4′), 4.35 (dd, 1 H, $J_{3',2'}$ 4.7, $J_{3',4'}$ 3.5 Hz, H-3′), 5.01 (dd, 1 H, $J_{2',1'}$ 5.3 Hz, H-2′), 6.09 (dd, 1 H, $J_{1',C1'}$ 166 Hz, H-1′), 6.75–7.65 (m, 18 H, Bz, DMTr), 8.22 (s, 1 H, H-2), 8.73 (s, 1 H, H-8). MS: m/z 487 $(M+H)^+$. Anal. Calcd for $C^{13}_{43}C_1H_{48}N_5O_7Si$: C, 67.11; H, 6.13. Found: C, 67.23; H, 6.15.

Compounds **7b** and **7c** were synthesized by a similar methodology as described for the preparation of compound **7a** from respective compounds **6b** and **6c**.

[1′-^{13}C]-5′-O-Dimethoxytrityl-2′-O-tert-butyldimethylsilyl-N^4-benzoylcytidine (**7b**).—Isolated as a colorless foam (87%, 0.10 g). ^1H NMR ($CDCl_3$) δ 0.19 (s, 3 H, Me), 0.31 (s, 3 H, Me), 0.97 (s, 9 H, ^{t}Bu), 3.56–3.59 (m, 2 H, H-5′a, H-5′b), 3.83 (s, 6 H, 2 × OMe), 4.11 (m, 1 H, H-4′), 4.29–4.43 (m, 3 H, H-2′, H-3′, H-5), 5.28 (dd, 1 H, $J_{5,NH}$ 1.5, $J_{5,6}$ 7.1 Hz, H-5), 5.96 (dd, 1 H, $J_{1',2'}$ 1.0, $J_{1',C1'}$ 176 Hz, H-1′), 6.80–7.72 (m, 18 H, DMTr, Bz), 7.95 (d, 1 H, H-6), 8.62 (bs, 1 H, NH). MS: m/z 765 $(M+H)^+$. Anal. Calcd for $C^{13}_{42}C_1H_{49}N_3O_8Si$: C, 67.65; H, 6.45. Found: C, 67.72; H, 6.47.

[1′-^{13}C]-5′-O-Dimethoxytrityl-2′-O-tert-butyldimethylsilyluridine (**7c**).—Isolated as a colorless foam (93%, 0.70 g). ^1H NMR ($CDCl_3$) δ 0.08 (s, 3 H, Me), 0.11 (s, 3 H, Me), 0.91 (s, 9 H, ^{t}Bu), 3.49–3.51 (m, 2 H, H-5′a, H-5′b), 3.80 (s, 6 H, 2 × OMe), 4.12 (m, 1 H, H-4′), 4.34–4.36 (m, 2 H, H-2′, H-3′), 5.28 (dd, 1 H, $J_{5,NH}$ 1.5, $J_{5,6}$ 8.1 Hz, H-5), 5.94 (dd, 1 H, $J_{1',2'}$ 2.9, $J_{1',C1'}$ 173 Hz, H-1′), 6.83–7.34 (m, 13 H, DMTr), 7.94 (dd, 1 H, J 2.2 Hz, H-6), 8.26 (bs, 1 H, NH). MS: m/z 663 $(M+H)^+$. Anal. Calcd for $C^{13}_{35}C_1H_{44}N_2O_8Si$: C, 65.48; H, 6.70. Found: C, 65.53; H, 6.74.

[1′-^{13}C]-5′-O-Dimethoxytrityl-2′-O-tert-butyldimethylsilyl-N^6-benzoyladenosine 3′-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (**8a**).—A solution of protected nucleoside **7a** (0.75 g, 0.95 mmol) in dry THF (3 mL), 2,4,6-collidine (0.94 mL, 7.10 mmol) and *N*-methylimidazole (37.7 µl, 0.437 mmol) was stirred under N_2. *N*,*N*-(Diisopropylamino)-(cyanoethyl)phosphoramidic chloride (0.527 mL, 2.37 mmol) was then added dropwise over 5 min at rt. The reaction was completed after 1 h as determined by TLC. The reaction mixture was diluted with EtOAc (100 mL), washed with 5% $NaHCO_3$ (150 mL), then with brine (150 mL). The aqueous layers were back extracted with EtOAc (2 × 50 mL) and combined organic phase were dried ($MgSO_4$). The solvent was removed under reduced pressure yielding a viscous oil. Co-evaporation with toluene afforded the crude phosphoramidite as a white foam. The ribonucleoside phosphoramidites were further purified by silica gel column chromatography (5:4:1 hexane–EtOAc–Et_3N) yielding **8a** (75%, 0.71 g) as a colorless foam and as a mixture of two diastereoisomers. ^1H NMR ($CDCl_3$) δ 0.02 (s, 3 H, Me), 0.04 (s, 3 H, Me), 0.75 (s, 9 H, ^{t}Bu), 1.19 (m, 12 H, ^{i}Pr), 2.32 (t, 1 H, J 7.5 Hz, CH_2–CN), 2.63 (t, 1 H, $-CH_2$–CN), 3.29 (m, 1 H, H-5′a), 3.54–3.78 (m, 5 H, H-5′b, P(O)CH_2–, 2 × CH(^{i}Pr)), 3.78 (s, 6 H, OMe), 4.36 (m, 1 H, H-4′), 4.41 (m, 1 H, H-3′), 5.07 (m, 1 H, H-2′), 6.11 (dd, 1 H, $J_{1',2'}$ 1.0, $J_{1',C1'}$ 166 Hz, H-1′), 6.90–8.10 (m, 18 H, DMTr, Bz), 8.23 (s, 1 H, H-2), 8.70 (s, 1 H, H-8), 9.02 (bs, 1 H, NH) and δ 0.02 (s, 3 H, Me), 0.04 (s, 3 H, Me), 0.75 (s, 9 H, ^{t}Bu), 1.19 (m, 12 H, ^{i}Pr), 2.32 (t, 1 H, J 7.5 Hz, –CH_2–CN), 2.63 (t, 1 H, $-CH_2$–CN), 3.29 (m, 1 H, H-5′a), 3.54–3.75 (m, 5 H, H-5′b, P(O)CH_2–, 2 × CH(^{i}Pr)), 3.78 (s, 6 H, OMe), 4.36 (m, 1 H, H-4′), 4.41 (m, 1 H, H-3′), 5.07 (m, 1 H, H-2′), 6.06 (dd, 1 H, $J_{1',2'}$ 1.0, $J_{1',C1'}$ 166 Hz, H-1′), 6.90–8.10 (m, 18 H, DMTr, Bz), 8.26 (s, 1 H, H-2), 8.68 (s, 1 H, H-8), 9.02 (bs, 1 H, NH) in agreement with Lit.[5] MS: m/z 990 $(M+H)^+$; $C^{13}_{52}C_1H_{66}N_7O_8PSi$ (989.1). Due to the unstable nature of this compound, an acceptable elemental analysis could not be obtained.

[1′-^{13}C]-5′-O-Dimethoxytrityl-2′-O-tert-butyldimethylsilyl-N^4-benzoylcytidine 3′-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (**8b**).—The cytidine phosphoramidite was obtained as described for **8a**. From **7b** (0.04 g, 0.05 mmol), phosphoramidite **8b**[5] (89%, 0.05 g) was obtained after silica gel column chromatography (55:45:3 cyclohexane–EtOAc–Et_3N). Isolated as a colorless foam (89%, 0.05 g). ^{1}H NMR ($CDCl_3$) δ 0.15 (s, 3 H, Me), 0.24 (s, 3 H, Me), 0.93 (s, 9 H, ^{t}Bu), 1.02 (d, 6 H, J 6.7 Hz, 2 × Me), 1.16 (d, 6 H, J 6.8 Hz, 2 × Me), 2.58 (t, 2 H, J 5.9 Hz, $-CH_2-$CN), 3.81 (m, 2 H, $-CH_2-$O–P), 3.61–3.67 (m, 4 H, H-5′a, H-5′b, 2 × C*H*(^{i}Pr)), 4.23–4.46 (m, 4 H, H-5, H-2′, H-3′, H-4′), 5.99 (dd, 1 H, $J_{1',2'}$ 1.0, $J_{1',C1'}$ 176 Hz, H-1′), 6.85–7.90 (m, 18 H, DMTr, Bz), 8.58 (d, 1 H, $J_{5,6}$ 6.1 Hz, H-6) and δ 0.15 (s, 3 H, Me), 0.24 (s, 3 H, Me), 0.91 (s, 9 H, ^{t}Bu), 1.01 (d, 6 H, J 6.4 Hz, 2 × Me), 1.12 (d, 6 H, J 6.8 Hz, 2 × Me), 2.38 (t, 2 H, J 6.1 Hz, $-CH_2-$CN), 3.80 (m, 2 H, CH_2-O–P), 3.49–3.58 (m, 4 H, H-5′a, H-5′b, 2 × C*H*(^{i}Pr)), 4.23–4.46 (m, 4 H, H-5, H-2′, H-3′, H-4′), 5.89 (dd, 1 H, $J_{1',2'}$ 1.0, $J_{1',C1'}$ 177 Hz, H-1′), 6.85–7.90 (m, 18 H, DMTr, Bz), 7.85 (d, 1 H, $J_{5,6}$ 7.3 Hz, H-6) in agreement with Lit.[5] MS: m/z 965 $(M)^+$; $C^{13}_{51}C_1H_{66}N_5O_9PSi$ (965.5). Due to the unstable nature of this compound, an acceptable elemental analysis could not be obtained.

[1′-^{13}C]-5′-O-Dimethoxytrityl-2′-O-tert-butyldimethylsilyl-uridine 3′-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (**8c**).—The uridine phosphoramidite was obtained as described for **8a**. From **7c** (0.06 g, 0.09 mmol), phosphoramidite **8c**[5] (82%, 0.06 g) was obtained after silica gel column chromatography (60:40:3 cyclohexane–EtOAc–Et_3N). ^{1}H NMR ($CDCl_3$) δ 0.12 (s, 3 H, Me), 0.14 (s, 3 H, Me), 0.91 (s, 9 H, ^{t}Bu), 1.02 (d, 6 H, J 6.7 Hz, 2 × Me), 1.16 (d, 6 H, J 6.8 Hz, 2 × Me), 2.64 (t, 2 H, J 6.1 Hz, $-CH_2-$CN), 3.36–3.73 (m, 4 H, H-5′a, H-5′b, 2 × C*H*(^{i}Pr)), 3.94 (m, 2 H, $-CH_2-$O–P), 4.22 (m, 1 H, H-4′), 4.28–4.45 (m, 2 H, H-2′, H-3′), 5.30 (d, 1 H, $J_{5,6}$ 8.1 Hz, H-5), 5.98 (dd, 1 H, $J_{1',2'}$ 4.2, $J_{1',C1'}$ 175 Hz, H-1′), 6.81–6.87 (m, 4 H, DMTr), 7.24–7.42 (m, 9 H, DMTr), 7.98 (d, 1 H, H-6) and δ 0.13 (s, 3 H, Me), 0.14 (s, 3 H, Me), 0.89 (s, 9 H, ^{t}Bu), 1.02 (d, 6 H, J 6.7 Hz, 2 × Me), 1.16 (d, 6 H, J 6.8 Hz, Me), 2.64 (t, 2 H, J 6.1 Hz, $-CH_2-$CN), 3.36–3.73 (m, 4 H, H-5′a, H-5′b, 2 × C*H*(^{i}Pr)), 3.79 (s, 6 H, 2 × OMe), 3.92 (m, 2 H, $-CH_2-$O–P), 4.22 (m, 1 H, H-4′), 4.28–4.45 (m, 2 H, H-2′, H-3′), 5.27 (d, 1 H, $J_{5,6}$ 8.1 Hz, H-5), 5.89 (dd, 1 H, $J_{1',2'}$ 4.9, $J_{1',C1'}$ 173 Hz, H-1′), 6.81–6.87 (m, 4 H, DMTr), 7.24–7.42 (m, 9 H, DMTr), 7.92 (d, 1 H, H-6) in agreement with Lit.[5] MS: m/z 862 $(M+H)^+$; $C^{13}_{44}C_1H_{61}N_4O_9PSi$ (861.2). Due to the unstable nature of this compound, an acceptable elemental analysis could not be obtained.

2. Supplementary material

All new compounds gave satisfactory spectral and analytical data. Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC nos. 140767–140771. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

Acknowledgements

This work was supported by a research grant from the CNRS (Grant ACC Physique Chimie du Vivant). We gratefully acknowledge the Region Centre and the Studium for a postdoctoral fellowship.

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