Synthesis of Novel 2′,3′-Linked Bicyclic Thymine Ribonucleosides

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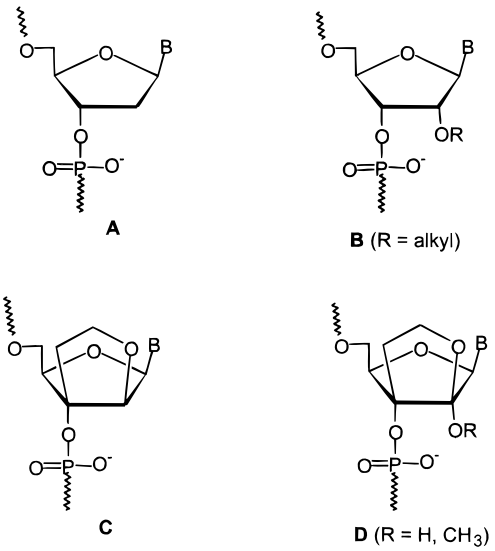
With the aim of efficient nucleic acid recognition, a variety of oligonucleotide mimics have been synthesized in recent years.[1] Though a number of promising analogues have been reported, e.g., PNA,[2] phosphoramidates,[3] and anhydrohexitol nucleic acid,[4] no synthetic oligonucleotide analogue has so far exhibited the desired combination of high-affinity, straightforward oligomerization and DNA/RNA-like structure. In this context, we believe that oligonucleotides containing bicyclic pentofuranose building blocks and a natural 5′-O- to 3′-O-linked phosphodiester backbone are attractive novel candidates.[5,6] Thus, we have recently reported strong binding of an oligonucleotide of type C (2′,3′-BcNA, Figure, B = nucleobase) toward complementary RNA.[5] To evaluate the possibility of further improving the nucleic acid recognition properties of this class of compounds, we have developed a synthetic route to the nucleoside building blocks suitable for preparation of novel oligonucleotides of type **D**. Our interest in this type of structural modification was further stimulated by the improved properties of 2′-*O*-alkyl oligonucleotides of type **B** compared to those of the parent type **A**, e.g., enhanced RNA-binding because of N-type conformational preference and improved 3′-exonucleolytic stability.[1e,7] In addition, the novel nucleosides **5a**,**b** and **10**, and derivatives thereof, are direct analogues of the ribonucleoside constituents of RNA and may exhibit interesting biological activities.

For stereoselective synthesis of the novel bicyclic nucleosides analogues **5a**,**b** and **10**, we chose β-D-ribo configured thymine derivatives **1a**,**b** (Scheme 1) as key intermediates. They were prepared from 1,2-di-*O*-isopropylidine-α-D-xylofuranose as previously described.[5] Selective silylation of the primary hydroxy groups of compounds **1a** and **1b** using *tert*-butyldimethylsilyl chloride (TBDMSCl) in anhydrous pyridine afforded nucleosides **2a** and **2b** in 89% and 92% yield, respectively, after column chromatographic purification. During oxidation of **2a**, precautions had to be taken to avoid overoxidation. Thus, when cooling was omitted the major product (yield ∼60%) was assigned (MS, ^{1}H NMR, ^{13}C NMR) as the 3′,5′-di-*O*-benzoylated nucleoside corresponding to 2′-ulose **3a**. Oxidation using pyridinium dichromate (PDC) yielded 2′-uloses **3a** and **3b** in yields of 84% and 91%, respectively, after column chromatographic purification. Compounds **3a** and **3b** were converted to the bicyclic nucleosides **4a** and **4b** simply by removing the silyl protection group from the 3′-*C*-hydroxyethyl or 3′-*C*-hydroxypropyl substituents. Both acid- and fluoride-mediated desilylation proved effective, allowing the preparation of compounds **4a**,**b** in 94% yield. As clearly evidenced by ^{13}C NMR, compounds **4a**,**b** existed exclusively as the bicyclic hemiacetals. Palladium hydroxide-assisted catalytic removal of the benzyl protection groups of **4a** and **4b** afforded the targeted bicyclic thymidine derivatives **5a** and **5b** in yields of 82% and 79%, respectively, after column chromatographic purification (Scheme 1).

Theoretically, two possibilities exist for hemiacetal formation in compounds **5a**,**b**, namely attack on the 2′-keto function from the 3′-*C*-hydroxyalkyl or the 5′-hydroxy groups. To prove the existence of free 5′-hydroxy groups, we selectively acetylated the free primary hydroxy groups of compounds **5a** and **5b** using acetic anhydride in anhydrous pyridine, affording derivatives **6a** and **6b** in yields of 52% and 75%, respectively. The structures of nucleosides **6a**,**b** were confirmed by ^{1}H and ^{1}H−^{1}H COSY NMR experiments. By the process of acetylation, the resonance signals of the 5′-protons were shifted downfield by approximately 0.5 ppm, proving that the acetylation took place at the 5′-hydroxy groups of **5a** and **5b**. No traces of acetylation at the 3′-*C*-hydroxyalkyl branches were detected even after 24 h reaction.



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(1) (a) Wagner, R. W. *Nature* **1994**, *372*, 333. (b) De Mesmaeker, A.; Häner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995**, *28*, 366. (c) Akhtar, S.; Agrawal, S. *Trends Pharmacol. Sci.* **1997**, *18*, 12. (d) Herdewijn, P. *Liebigs Ann.* **1996**, 1337. (e) Freier, S. M.; Altmann, K.-H. *Nucleic Acids Res.* **1997**, *25*, 4429.

(2) (a) Hyrup, B.; Nielsen, P. E. *Bioorg. Med. Chem.* **1996**, *4*, 5. (b) Nielsen, P. E.; Haiima, G. *Chem. Soc. Rev.* **1997**, 73.

(3) Schultz, D. G., Gryaznov, S. M. *Nucleic Acids Res.* **1996**, *24*, 2966.

(4) (a) Van Aershot, A.; Verheggen, I.; Hendrix, C.; Herdewijn, P. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1338. (b) Hendrix, C.; Rosemeyer, H.; Verheggen, I.; Seela, F.; Van Aershot, A.; Herdewijn, P. *Chem. Eur. J.* **1997**, *3*, 110.

(5) (a) Nielsen, P.; Pfundheller, H. M.; Wengel, J. *Chem. Commun.* **1997**, *9*, 825. (b) Nielsen, P.; Pfundheller, H. M.; Olsen, C. E.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3423.

(6) A number of bicyclic nucleosides have been incorporated into oligonucleotides. See, e.g.: (a) Bolli, M.; Litten, J. C.; Schültz, R.; Leumann, C. J. *Chem. Biol.* **1996**, *3*, 197. (b) Altmann, K.-H.; Kesselring, R.; Francotte, E.; Rihs, G. *Tetrahedron Lett.* **1994**, *35*, 2331. (c) Altmann, K.-H.; Imwinkelried, R.; Kesselring, R.; Rihs, *G. Tetrahedron Lett.* **1994**, *35*, 7625. (d) Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Russ, P.; Wang, J.; Wagner, R. W.; Matteucci, M. D. *J. Med. Chem.* **1996**, *39*, 3739. (e) Zou, R.; Matteucci, M. *Tetrahedron Lett.* **1996**, *37*, 941. (f) Wang, J.; Matteucci, M. D. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 229.

(7) Cummins, L. L.; Owens, S. R.; Risen, L. M.; Lesnik, E. A.; Freier, S. M.; McGee, D.; Guinosso, C. J.; Cook, P. D. *Nucleic Acids Res.* **1995**, *23*, 2019.

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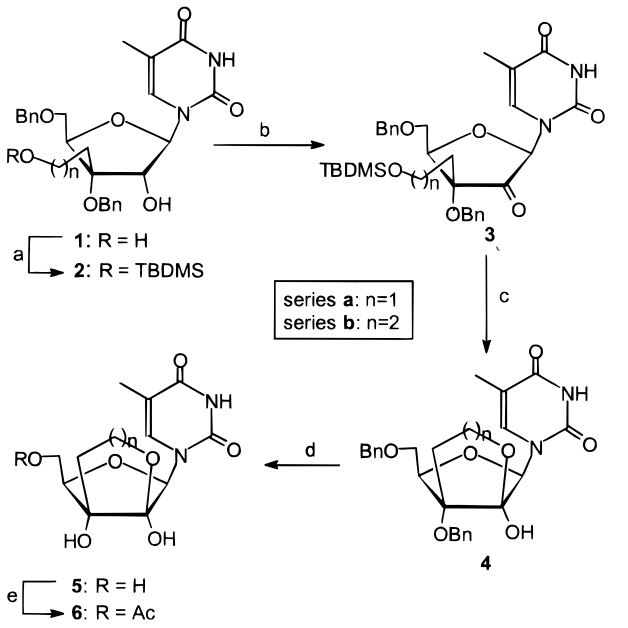
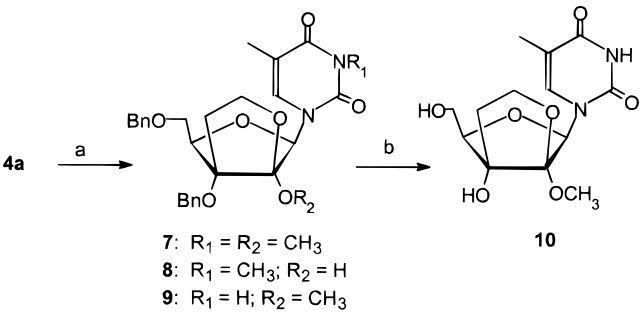
Scheme 1[a]



[a] Reagents and conditions: (a) 1.2 equiv of TBDMSCl, pyridine, 2 h, rt, 92% (**2a**); 1 equiv of TBDMSCl, pyridine, 2 h, rt, 90% (**2b**); (b) 1.1 equiv of PDC, Ac_2O, 3 Å molecular sieve powder, 1.5 h, rt, CH_2Cl_2 , 84% (**3a**); 1.7 equiv of PDC, Ac_2O, 3 Å molecular sieve powder, CH_2Cl_2, 1.5 h, rt, 91% (**3b**); (c) 0.5% HCl in methanol, 30 min, rt, 94% (**4a**); 3.1 equiv of triethylamine trihydrofluoride, THF, 12 h, rt, 94% (**4b**); (d) 20% $Pd(OH)_2$, H_2, methanol, 12 h, rt, 82%, **5a**; 20% $Pd(OH)_2$, H_2, methanol, 24 h, rt, 79%, **5b**; (e) 1.4 equiv of Ac_2O, pyridine, 16 h, 7 °C, 52%, **6a**; 1.5 equiv of Ac_2O, pyridine, 4 h, rt, 75%, **6b**.

Scheme 2[a]



[a] Reagents and conditions: (a) 2 equiv of NaH, 7.4 equiv of CH_3I, CH_2Cl_2, 23 h, 36 °C, 4% (**7**), 9% (**8**), 62% (**9**); (b) **9**, 20% $Pd(OH)_2$, H_2, methanol, rt, 12 h, 79%.

Compound **4a** was additionally utilized as an intermediate for synthesis of the 2′-*O*-alkylated bicyclic nucleoside **10** (Scheme 2). The key reaction was alkylation to give the bicyclic acetal **9**. A wide variety of *O*-glycosylation methods have been described,[8] but all attempts to prepare derivative **9** by the use of reactions involving carbocation intermediates were unsuccessful. Thus, we failed to produce the desired product using the method of Ficher−Helferich (up to 5% HCl in anhydrous methanol was used), *p*-toluenesulfonic acid/2,2-dimethoxypropane,[9] Noyori's acetalization under aprotic conditions (methoxytrimethylsilane in dichloromethane catalyzed by trimethylsilyl triflate),[10] and acetalization in the presence of chlorotrimethylsilane.[11] Eventually, compound **9** was synthesized in 62% yield by direct alkylation of the 2′-hydroxy group of **4a** by reaction with methyl iodide in anhydrous dichloromethane in the presence of sodium hydride. This approach was, however, complicated by 3-*N*-alkylation, resulting in the formation of byproducts **7** and **8** in yields of 4% and 9%, respectively. The structural assignment of compounds **7**−**9** was based on ^{13}C NMR spectra, especially the appearance of signals corresponding to *N*- and/or *O*-methyl derivatives (at approximately 28 and 51 ppm, respectively). The deprotected bicyclic nucleoside analogue **10** was finally obtained in 79% yield by catalytic hydrogenation of nucleoside **9** (Scheme 2).

In summary, synthesis of three novel 2′,3′-linked bicyclic nucleoside analogues **5a**,**b** and **10** has been accomplished by ring closure of 2′-ketonucleosides. A similar strategy should prove viable for synthesis of other bicyclic nucleosides. Efforts are underway transforming **5a** and **10** into building blocks for automated oligomerization and evaluating the biological activity of this novel class of ribonucleoside analogues.

Experimental Section

General Methods. Chemicals and solvents were purchased from commercial suppliers and used as such. Silica gel 60 (0.040−0.063 mm) was used for chromatography. Silica gel HPLC was performed by use of PrepPAK-500/silica cartridges (flow rate 60 mL/min). NMR spectra were recorded at 400 MHz (^1H spectra) or 100 MHz (^{13}C spectra) using tetramethylsilane as internal reference. Chemical shifts δ are reported in parts per million (ppm) and coupling constants J in Hz. $^1H-^1H$ COSY NMR spectra were recorded for compounds **6a**,**b**. Fast-atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode.

1-[3-*C*-[2-*O*-[(*tert*-Butyldimethylsilyl)oxy]ethyl]-3,5-di-*O*-benzyl-*β*-D-ribofuranosyl]thymine (2a). A mixture of nucleoside **1a**[5] (1.80 g, 3.4 mmol) and TBDMSCl (0.585 g, 3.9 mmol) was dissolved in anhydrous pyridine (20 mL). After being stirred for 2 h at room temperature, the reaction mixture was evaporated, coevaporated with toluene (2 × 10 mL), and redissolved in dichloromethane (150 mL). The solution was washed with a saturated aqueous solution of sodium hydrogen carbonate (2 × 50 mL), and the separated organic phase was evaporated to give a foam. This material was purified by preparative silica gel HPLC (0−3% methanol in dichloromethane, v/v) to give nucleoside **2a** as a white solid material (1.86 g, 92%): ^1H NMR ($CDCl_3$) 7.61 (1H, d, J = 1.1), 7.35−7.20 (10H, m), 6.27 (1H, d, J = 7.9), 4.65−4.40 (4H, m), 4.37 (1H, s), 4.28 (1H, t, J = 7.9), 4.10−3.55 (4H, m), 2.30−2.05 (2H, m), 1.46 (3H, s), 0.90 (9H, m), 0.08 (6H, m); ^{13}C ($CDCl_3$) 163.6, 151.0, 137.5, 136.6, 135.8, 128.3, 128.1, 127.8, 127.2, 127.1, 126.8, 126.7, 110.7, 86.8, 82.5, 81.2, 78.3, 73.3, 69.8, 64.5, 58.2, 32.9, 25.6, 25.4, 17.9, 11.6, −3.9, −5.7; FAB-MS m/z 597 $[M + H]^+$, 619 $[M + Na]^+$. Anal. Calcd for $C_{32}H_{44}O_7N_2Si$: C, 64.4; H, 7.4; N, 4.7. Found: C, 64.2; H, 7.4; N, 4.2.

1-[3-*C*-[2-*O*-[(*tert*-Butyldimethylsilyl)oxy]propyl]-3,5-di-*O*-benzyl-*β*-D-ribofuranosyl]thymine (2b). The same procedure as decribed above for **2a** was used: nucleoside **1b**[5] (3.64 g, 7.34 mmol), anhydrous pyridine (25 mL), TBDMSCl (1.12 g, 7.42 mmol), reaction time 2 h at room temperature, toluene (2 × 50 mL), dichloromethane (300 mL), a saturated aqueous solution of sodium hydrogen carbonate (2 × 150 mL). The residue obtained after evaporation of the organic phase was purified by preparative silica gel HPLC (0−5% methanol in dichloromethane, v/v) to give nucleoside **2b** as a white solid material (4.01 g, 90%): ^1H NMR ($CDCl_3$) 7.60 (1H, d, J = 1.2), 7.38−7.25 (10H,

(8) (a) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212. (b) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503.

(9) Rupprecht, K. M.; Boger, J.; Hoogsteen, K.; Nachbar, R. B.; Springer, J. P. *J. Org. Chem.* **1991**, *56*, 6180.

(10) Tsunoda, T.; Suzuki, M.; Noyori, R. *Tetrahedron Lett.* **1980**, *21*, 1357.

(11) Chan, T. H.; Brook, M. A.; Chaly, T. *Synthesis* **1983**, 203.

m), 6.14 (1H, d, J = 7.9), 4.62−4.54 (4H, m), 4.37 (1H, d, J = 1.3), 4.18 (1H, m), 3.84 (1H, m), 3.75−3.57 (2H, m), 3.55 (1H, m), 2.20−1.65 (4H, m), 1.47 (3H, d, J = 1.1), 0.90 (9H, m), 0.06 (6H, m); ^{13}C NMR ($CDCl_3$) 163.5, 151.1, 137.3, 136.8, 136.1, 128.7, 128.5, 128.3, 127.9, 127.7, 127.5, 127.4, 111.2, 87.5, 82.7, 81.3, 79.3, 73.7, 70.0, 64.3, 63.0, 26.6, 26.1, 26.0, 18.3, 11.9, −5.3, −5.3; FAB-MS m/z 611 $[M + H]^+$. Anal. Calcd for $C_{33}H_{46}O_7N_2Si$: C, 64.9; H, 7.6; N, 4.6. Found: C, 64.9; H, 7.5; N, 4.5.

1-[3-*C*-[2-*O*-[(*tert*-Butyldimethylsilyl)oxy]ethyl)-3,5-di-*O*-benzyl-*β*-D-*erythro*-pentofuran-2-ulosyl]thymine (3a). Nucleoside **2a** (2.14 g, 3.59 mmol), pyridinium dichromate (1.48 g, 3.95 mmol), and activated 3A molecular sieve powder (4 g) was suspended in anhydrous dichloromethane (80 mL). After the mixture was cooled to −10 °C, acetic anhydride (10 mL, 98 mmol) was added dropwise under vigorous stirring. The mixture was allowed to warm to room temperature, and stirring was continued for 1.5 h, whereupon triethylamine (20 mL) was added. The mixture was diluted with dichloromethane to 300 mL and was washed with water (2 × 200 mL). The organic phase was evaporated, and the residue was purified by flash silica gel chromatography (2.5 × 20 cm column) in a steplike gradient of 1.0, 1.2, 1.3, 1.4, and 1.5% methanol in dichloromethane (v/v, 250 mL each) to give nucleoside **3a** (1.89 g, 84%) as a white solid material: ^{1}H NMR ($CDCl_3$) 9.21 (1H, br s), 7.35−7.20 (11H, m), 6.40 (1H, s), 4.57 (1H, s), 4.52 (1H, s), 4.46 (1H, d, J = 11.0), 4.29 (1H, d, J = 11.0), 4.07 (1H, dd, J = 10.5, 2.2), 3.95−3.70 (4H, m), 2.42 (1H, m), 2.05 (1H, m), 1.42 (3H, d, J = 1.1), 0.86 (9H, s), 0.01 (6H, s); ^{13}C NMR ($CDCl_3$) 202.6, 163.7, 151.2, 137.7, 136.6, 136.5, 128.7, 128.5, 128.2, 128.1, 127.7, 126.4, 126.3, 110.9, 84.5, 81.3, 80.2, 73.6, 70.4, 66.0, 57.6, 27.3, 25.9, 25.7, 18.2, 11.7, −5.8, −5.9; FAB-MS m/z 595 $[M + H]^+$. Anal. Calcd for $C_{32}H_{42}O_7N_2Si$: C, 64.6; H, 7.1; N, 4.7. Found: C, 64.1; H, 6.9; N, 4.5.

1-[3-*C*-[2-*O*-[(*tert*-Butyldimethylsilyl)oxy]propyl]-3,5-di-*O*-benzyl-*β*-D-*erythro*-pentofuran-2-ulosyl]thymine (3b). To a suspension of 3A molecular sieve powder (360 mg) and pyridimium dichromate (275 mg, 0.73 mmol) in anhydrous dichloromethane (5 mL) was added a solution of nucleoside **2b** (280 mg, 0.46 mmol, in 2 mL of dichloromethane). Acetic anhydride (0.12 mL, 1.17 mmol) was added dropwise at room temperature under vigorous stirring. After 1.5 h at room temperature, the reaction mixture was subjected to column chromatographic purification (2 × 15 cm column, silica gel, 0−2% methanol in dichloromethane, v/v), affording nucleoside **3b** as a white solid material (254 mg, 91%): ^{1}H NMR ($CDCl_3$) 9.43 (1H, br s), 7.41−7.22 (11 H, m), 6.26 (1H, s), 4.58−4.48 (4H, m), 4.29 (1H, d, J = 10.8), 3.87 (1H, dd, J = 10.9, 2.7), 3.73 (1H, dd, J = 10.8, 2.9), 3.68−3.58 (2H, m), 2.33−2.26 (1H, m), 1.87−1.73 (2H, m), 1.61−1.54 (1H, m), 1.47 (3H, d, J = 1.0), 0.88 (9H, m), 0.04 (6H, m); ^{13}C NMR ($CDCl_3$) 202.4, 163.6, 151.0, 137.8, 136.7, 136.6, 128.6, 128.5, 128.1, 128.0, 127.8, 127.7, 127.6, 127.4, 111.2, 83.7, 82.2, 80.5, 73.6, 69.6, 65.7, 62.7, 25.9, 25.7, 21.4, 18.3, 11.7, −5.3; FAB-MS m/z 609 $[M + H]^+$. Anal. Calcd for $C_{33}H_{44}O_7N_2Si$: C, 65.1; H, 7.3; N, 4.6. Found: C, 64.8; H, 7.2; N, 4.6.

(1*S*,5*R*,6*R*,8*R*)-5-(Benzyloxy)-6-(benzyloxymethyl)-1-hydroxy-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (4a). Compound **3a** (1.80 g, 3.03 mmol) was dissolved in 0.5% HCl in methanol (20 mL, w/w) and the mixture was stirred for 30 min at room temperature. After evaporation, the residue was dissolved in dichloromethane (100 mL) and washed with a saturated aqueous solution of sodium hydrogen carbonate (2 × 40 mL). The organic phase was evaporated, and the residue was purified by flash silica gel chromatography (2.5 × 20 cm column), eluting with 2% methanol in dichloromethane (v/v) to yield nucleoside **4a** (1.35 g, 94%) as a white solid material: ^{1}H NMR ($CDCl_3$) 9.54 (1H, br s), 7.37−7.27 (11H, m), 5.87 (1H, s), 4.71 (2H, s), 4.64 (1H, d, J = 12.0), 4.56 (1H, d, J = 12.0), 4.36 (1H, t, J = 5.7), 4.16 (1H, m), 3.96 (1H, m), 3.74 (2H, m), 2.35−2.15 (2H, m), 1.88 (3H, s, J = 1.1); ^{13}C NMR ($CDCl_3$) 163.7, 151.4, 137.8, 137.3, 136.7, 128.5, 128.4, 128.0, 127.8, 127.5, 109.9, 108.6, 88.8, 87.1, 80.9, 73.6, 68.5, 68.1, 67.9, 30.9, 12.6; FAB-MS m/z 481 $[M + H]^+$, 503 $[M + Na]^+$. Anal. Calcd for $C_{26}H_{28}O_7N_2$: C, 65.0; H, 5.9; N, 5.8. Found: C, 64.6; H, 5.8; N, 5.7.

(1*S*,6*R*,7*R*,9*R*)-6-(Benzyloxy)-7-(benzyloxymethyl)-1-hydroxy-9-(thymin-1-yl)-2,8-dioxabicyclo[4.3.0]nonane (4b). To a solution of nucleoside **3b** (1.2 g, 1.97 mmol) in anhydrous THF (20 mL) was added triethylamine trihydrofluoride (1 mL, 6.2 mmol), and the mixture was stirred for 12 h at room temperature. Dichloromethane (100 mL) was added, and the mixture was washed with a saturated aqueous solution of sodium hydrogen carbonate (2 × 100 mL) and water (100 mL). The organic phase was concentrated, and the residue was purified by silica gel HPLC (eluent: 0−8% methanol in dichloromethane (v/v) during 60 min) to yield compound **4b** (0.91 g, 94%) as a white solid material: ^{1}H NMR ($CDCl_3$) 9.79 (1H, br s), 7.38−7.25 (11H, m), 6.13 (1H, s), 4.68−4.54 (5H, m), 4.03−3.88 (1H, m), 3.83−3.74 (2H, m), 3.72−3.60 (1H, m), 2.30−2.18 (1H, m), 2.07−1.90 (1H, m), 1.87 (3H, d, J = 1.0), 1.72−1.54 (2H, m); ^{13}C NMR ($CDCl_3$) 164.3, 151.9, 138.0, 137.5, 137.0, 128.6, 128.4, 128.0, 127.6, 127.2, 109.4, 99.5, 89.0, 83.6, 80.2, 73.6, 70.5, 64.9, 58.9, 23.4, 21.2, 12.7; FAB-MS m/z 495 $[M + H]^+$. Anal. Calcd for $C_{27}H_{30}O_7N_2$: C, 65.6; H, 6.1; N, 5.7. Found: C, 64.9; H, 6.0; N, 5.5.

(1*S*,5*R*,6*R*,8*R*)-1,5-Dihydroxy-6-(hydroxymethyl)-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (5a). A mixture of nucleoside **4a** (192 mg, 0.40 mmol) and 20% palladium hydroxide on carbon (40 mg) was suspended in methanol (5 mL). The mixture was degassed under reduced pressure and placed in a hydrogen atmosphere with a balloon. After being stirred for 12 h at room temperature, the reaction mixture was evaporated. The residue was purified by silica gel chromatography (2 × 5 cm column) using methanol in dichloromethane (6−14%, v/v) as eluent to give a glass after evaporation of the solvents. A solution of this glass in 5% methanol in benzene (5 mL, v/v) was frozen and lyophilized to give compound **5a** (98 mg, 82%) as a white solid material: ^{1}H NMR (CD_3OD) 7.44 (1H, d, J = 1.2), 5.83 (1H, s), 4.10−3.80 (5H, m), 2.39−2.25 (1H, m), 2.00−1.90 (1H, m), 1.87 (3H, d, J = 1.2); ^{13}C NMR (CD_3OD) 166.2, 152.6, 139.7, 109.9, 109.6, 87.8, 84.6, 84.6, 68.8, 61.6, 35.6, 12.4; FAB-MS m/z 301 $[M + H]^+$.

(1*S*,6*R*,7*R*,9*R*)-1,6-Dihydroxy-7-(hydroxymethyl)-9-(thymin-1-yl)-2,8-dioxabicyclo[4.3.0]nonane (5b). The same procedure as described above for **5a** was used: nucleoside **4b** (650 mg, 1.31 mmol), 20% palladium hydroxide on carbon (100 mg), methanol (15 mL), reaction time 24 h at room temperature. After evaporation, the residue was purified by silica gel chromatography (1.5 × 10 cm column) using 3−12% methanol in dichloromethane as eluent to give a glass after evaporation of the solvents. A solution of this glass in 5% methanol in benzene (21 mL, v/v) was frozen and lyophilized to give compound **5b** (325 mg, 79%) as a white solid material: ^{1}H NMR (CD_3OD) 7.58 (1H, d, J = 1.3), 6.11 (1H, s), 4.14−4.11 (1H, m), 3.92−3.82 (3H, m), 3.66−3.62 (1H, m), 2.03−2.00 (1H, m), 1.88 (3H, d, J = 1.2), 1.77−1.54 (3H, m); ^{13}C NMR (CD_3OD) 166.4, 153.4, 139.6, 109.8, 100.5, 90.5, 89.6, 75.9, 63.7, 59.7, 27.8, 22.2, 12.5; FAB-MS m/z 315 $[M + H]^+$.

(1*S*,5*R*,6*R*,8*R*)-6-[(Acetyloxy)methyl]-1,5-dihydroxy-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (6a). Acetic anhydride (0.026 mL, 0.27 mmol) was added dropwise at room temperature to a stirred solution of nucleoside **5a** (55 mg, 0.18 mmol) in anhydrous pyridine (5 mL). After 16 h at 7 °C, the reaction mixture was evaporated. The residue was coevaporated with toluene (2 × 5 mL), and extraction was performed in a 1:1(v/v) mixture of dichloromethane and saturated aqueous sodium hydrogen carbonate (60 mL). The separated organic phase was concentrated, and the residue was purified by silica gel flash chromatography (1 × 25 cm column) using 2−4% methanol in dichloromethane as eluent. The monoacetylated product **6a** (32 mg, 52%) was isolated as a white solid material: ^{1}H NMR (CD_3OD) 7.40 (1H, d, J = 1.1, 6-H), 5.84 (1H, s, 1′-H), 4.43−4.35 (2H, m, 5′-H), 4.14−3.98 (3H, m, 2″-H, 4′-H), 2.34−2.26 (1H, m, 1″-H), 2.08 (3H, s, acetyl), 2.06−1.96 (1H, m, 1″-H), 1.87 (3H, d, J = 1.1, 5-CH_3); ^{13}C NMR (CD_3OD) 172.4, 166.2, 152.6, 139.6, 110.1, 109.5, 87.8, 84.7, 81.4, 68.8, 63.7, 35.8, 20.7, 12.4.

(1*S*,6*R*,7*R*,9*R*)-7-[(Acetyloxy)methyl]-1,6-dihydroxy-9-(thymin-1-yl)-2,8-dioxabicyclo[4.3.0]nonane (6b). The same procedure as described above for **6a** was used: acetic anhydride (0.082 mL, 0.81 mmol), nucleoside **5b** (170 mg, 0.54 mmol), anhydrous pyridine (5 mL), reaction time 4 h at room temperature, toluene (2 × 5 mL), a 1:1 (v/v) mixture of dichloromethane and H_2O (100 mL). The residue obtained after evaporation of the organic phase was purified by silica gel flash chromatography (1.5 × 30 cm column) using methanol in dichloromethane (2−4%) as eluent, affording monoacetylated compound **6b** (145

mg, 75%) as a white solid material: ^{1}H NMR (CD_3OD) 7.54 (1H, d, J = 1.1, 6-H), 6.16 (1H, s, 1′-H), 4.68 (1H, dd, J = 11.9, 10.1, 4′-H), 4.26−4.22 (2H, m, 5′-H), 3.94−3.66 (2H, m, 3″-H), 2.10 (3H, s, acetyl), 1.87 (3H, s, 5-CH_3), 2.07−1.55 (4H, m, 1″-H, 2″-H); ^{13}C NMR (CD_3OD) 172.5, 166.3, 153.3, 139.7, 109.9, 100.3, 89.1, 87.3, 76.1, 65.7, 59.6, 27.9, 22.5, 20.8, 12.5.

(1*S*,5*R*,6*R*,8*R*)-5-(Benzyloxy)-6-(benzyloxymethyl)-1-methoxy-8-(3-*N*-methylthymin-1-yl)-2,7-dioxabicyclo[3.3.0]-octane (7), (1*S*,5*R*,6*R*,8*R*)-5-(Benzyloxy)-6-(benzyloxymethyl)-1-hydroxy-8-(3-*N*-methylthymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (8), and (1*S*,5*R*,6*R*,8*R*)-5-(Benzyloxy)-6-(benzyloxymethyl)-1-methoxy-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (9). A mixture of nucleoside **4a** (1.035 g, 2.16 mmol) and a 60% suspension of sodium hydride (171 mg, 4.30 mmol) in mineral oil was dissolved in anhydrous dichloromethane (4 mL). Methyl iodide (1 mL, 16 mmol) was added, and the reaction mixture was stirred for 23 h at 36 °C. After evaporation of the solvents in vacuo, the residue was purified by silica gel chromatography (4 × 35 cm column) using 0.4−2.4% methanol in dichloromethane (v/v) as eluent to give products **7**−**9** and starting material **4a** (212 mg, 21%). Compound **7**: yield 47 mg (4%); ^{1}H NMR ($CDCl_3$) 7.25−7.37 (11H, m), 6.15 (1H, s), 4.74 (1H, d, J = 11.5), 4.67 (1H, d, J = 11.3), 4.62 (1H, d, J = 12.1), 4.55 (1H, d, J = 11.9), 4.34 (1H, t, J = 5.6), 4.22 (1H, m), 3.99, (1H, m), 3.72 (2H, m), 3.41 (3H, s), 3.35 (3H, s), 2.27 (1H, m), 2.41 (1H, m), 1.93 (3H, s); ^{13}C NMR ($CDCl_3$) 163.3, 151.0, 138.2, 137.3, 135.7, 128.3, 128.2, 127.8, 127.6, 127.4, 126.9, 110.8, 108.5, 89.1, 84.8, 79.5, 73.5, 68.4, 68.2, 67.3, 50.8, 32.6, 27.9, 13.2; FAB-MS m/z 509 $[M + H]^+$. Compound **8**: yield 97 mg (9%); ^{1}H NMR ($CDCl_3$) 7.37−7.28 (11H, m), 5.86 (1H, s), 4.72 (2H, s), 4.64 (1H, d, J = 11.9), 4.58 (1H, d, J = 11.9), 4.37 (1H, t, J = 5.6), 4.13 (1H, m), 3.93 (1H, m), 3.75 (2H, m), 3.34 (3H, s), 2.32−2.16 (2H, m), 1.93 (3H, s); ^{13}C NMR ($CDCl_3$) 163.2, 151.9, 137.5, 137.1, 134.0, 128.4, 128.3, 128.1, 127.9 127.7, 127.6, 127.3, 108.8, 108.5, 88.7, 88.0, 81.0, 73.5, 68.3, 67.9, 67.7, 30.6, 27.8, 13.2; FAB-MS m/z 495 $[M + H]^+$, 517 $[M + Na]^+$. Compound **9**: yield 665 mg (62%); ^{1}H NMR ($CDCl_3$) 8.71 (1H, br s), 7.35−7.25 (11H, m), 6.06 (1H, s), 4.73 (1H, d, J = 11.5), 4.66 (1H, d, J = 11.3), 4.61 (1H, d, J = 11.9), 4.55 (1H, d, J = 12.0), 4.34 (1H, t, J = 5.6), 4.20 (1H, m), 3.98 (1H, m), 3.72 (2H, m), 3.40 (3H, s), 2.45−2.35 (1H, m), 2.30−2.20 (1H, m), 1.90 (3H, d, J = 1.1); ^{13}C NMR ($CDCl_3$) 163.2, 150.1, 138.2, 137.9, 137.3, 128.4, 128.2, 127.8, 127.6 127.4, 127.1, 110.8, 109.3, 89.2, 84.2, 79.6, 73.6, 68.5, 68.3, 67.4, 50.8, 32.6, 12.5; FAB-MS m/z 495 $[M + H]^+$, 517 $[M + Na]^+$.

(1*S*,5*R*,6*R*,8*R*)-5-Hydroxy-6-(hydroxymethyl)-1-methoxy-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (10). To a solution of nucleoside **9** (1.20 g, 2.43 mmol) in methanol (10 mL) was added 20% palladium hydroxide over charcoal (250 mg), and the mixture was degassed under reduced pressure. An atmosphere of hydrogen was applied, and stirring was continued for 12 h at room temperature. The catalyst was removed by filtration through a glass column (1 × 8 cm) packed with silica gel in methanol. The column was additionally washed with methanol (20 mL). All fractions were collected, evaporated, and coevaporated with petroleum ether to yield a glasslike solid. This residue was purified by silica gel chromatography (5 × 15 cm column), eluting with a gradient of 5−10% methanol in dichloromethane (v/v). The fractions containing the product were collected, combined, and evaporated. The residue was dissolved in anhydrous methanol (5 mL), and anhydrous benzene (100 mL) was added. The solution was frozen and lyophilized under reduced pressure to give nucleoside **10** (0.61 g, 79%) as a white solid material: ^{1}H NMR (CD_3OD) 7.45 (1H, s), 5.93 (1H, s), 4.15−3.81 (5H, m), 3.43 (3H, s), 2.47−2.40 (1H, m), 2.03−1.93 (1H, m), 1.92 (3H, s); ^{13}C NMR (CD_3OD) 166.0, 152.0, 140.2, 111.5, 86.3, 86.0, 84.3, 70.0, 61.4, 51.5, 36.0, 12.4; FAB-MS m/z 315 $[M + H]^+$, 337 $[M + Na]^+$. Anal. Calcd for $C_{13}H_{18}O_7N_2$: C, 49.7; H, 5.8; N, 8.9. Found: C, 49.9; H, 5.7; N, 8.3.

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Supporting Information Available: Copies of ^{13}C NMR spectra for compounds **5a**,**b**, **6a**,**b**, **7**−**9** (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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