Synthesis of novel bicarbocyclic nucleosides

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Abstract

The stereospecific syntheses of new bicarbocyclic nucleosides are described. The regioisomers were differentiated by selective INEPT NMR studies and the stereochemistry of these compounds was confirmed by differential NOE NMR experiments. Antiviral results are mentioned.

Keywords: Carbocyclic nucleosides, synthesis, NOE, stereochemistry, antiviral

Introduction

There is increasing interest in nucleosides where the oxygen of the furan ring is replaced by a methylene group. Due to the absence of the glycosidic bond, these compounds referred to as carbocyclic nucleosides are more stable with respect to degradation by enzymes such as phosphorylases.[1] Isomeric nucleosides where the base is transposed from the 1'- to the 2'-position can also be viewed as carbocyclic nucleosides because the nucleobase is not attached at the glycosidic position.[2,3] Many carbocyclic nucleosides are known including those where the ribose unit has been replaced by cyclopropane,[4] cyclobutane,[5] cyclopentane,[6] and cyclohexane.[7] Carbocyclic nucleosides have shown potential as antiviral agents against HIV, HSV and other viruses.[8-10] Modification of the sugar moiety further to include fused ring systems[11] has expanded the area of carbocyclic nucleosides. However, bicyclo[2.2.1]carbocyclic nucleosides have not been synthesized or investigated and this is the subject of this communication. Our targeted nucleosides have the secondary hydroxyl group whereas standard nucleosides have the primary hydroxyl group. It has been well known that some biologically active compounds, such as 5′-noraristeromycin,[12] have a secondary hydroxyl group instead of hydroxymethyl group. We wish

to report on the stereospecific synthesis of novel type of bicyclo[2.2.1] carbocyclic nucleoside analogues.

Results and Discussion

The synthesis commenced with commercially available 5-norbornen-2-ol (**1**) which was a mixture of *exo* and *endo* isomers. Treatment of **1** with tosyl chloride in pyridine gave a mixture of *exo-* and *endo*-5-norbornen-2-tosylate (**2**) in 80% yield (Scheme 1). The coupling reactions with nucleoside bases, adenine, thymine, and uracil in the presence of K_2CO_3 and 18-crown-6 in DMF at 100-110 °C for 3 days gave only one stereoisomeric product, 2(*S*)-(nucleobase)-5-norbornene (**3a-c**) in approximately 30% yield. The relatively low yield of the desired compound can be explained by the formation of the elimination product, 2,5-norbornadiene. The stereochemistry of compound **3** was confirmed by differential NOE experiments. For example, in case of **3a**, irradiation of H-8 of the adenine base resulted in the enhancement of the signal for H-7′. The chemical shift of the latter was assigned by homodecoupling experiments. The NOE data confirmed the proximity of the adenine base to the bridgehead position and that the nucleophilic displacement of the tosyl group by the adenine base had occurred from the *exo* face.
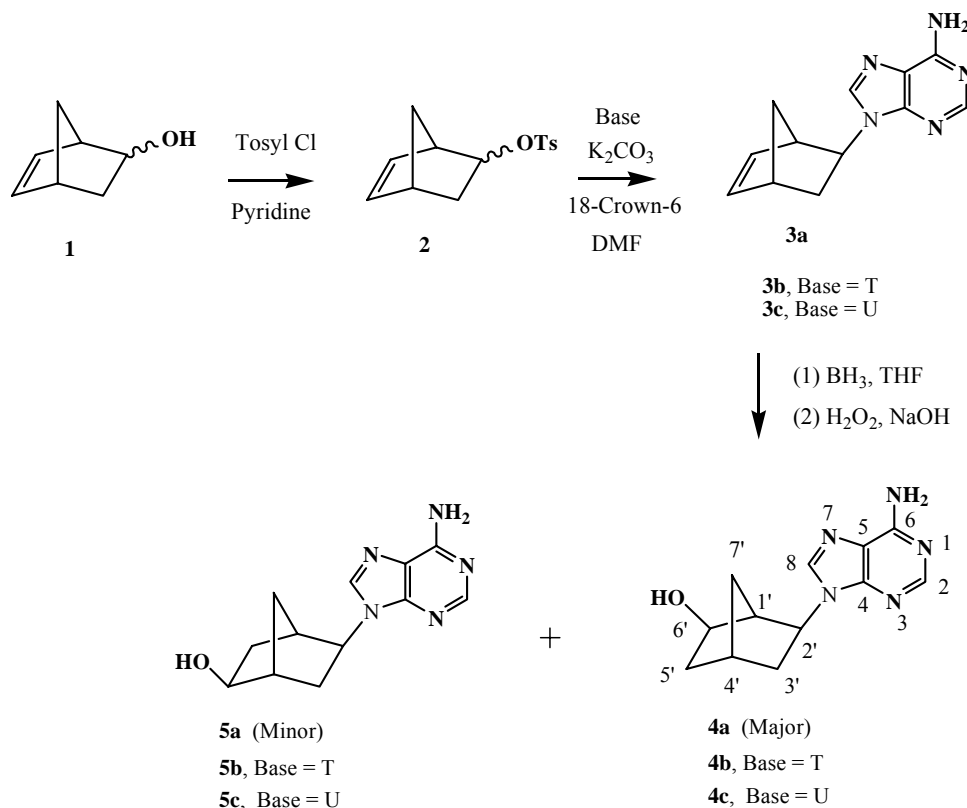
Scheme 1

Hydroboration of **3** was accomplished by using 4 equivalents of BH_3-THF in THF, followed by oxidation with 30% H_2O_2 and 2N NaOH(aq) to afford two regioisomers in 80% yield: norbornan-6(R)-ol (**4**) as a major product (45%) and norbornan-5(S)-ol (**5**) as a minor product (35%) (Scheme 1). These regioisomers were separated by reversed-phase HPLC on Amberlite XAD-4 resin as the stationary phase with ethanol/water as the eluting solvent.

The cytidine derivative (**4d**) was synthesized from uridine **3c** which was treated first with a solution of phosphorus oxychloride and 1,2,4-triazole in pyridine, followed by treatment with NH_4OH to afford 2(S)-[4-amino-2-oxo-1(2H)-pyrimidinyl]-5-norbornene (**3d**). The cytidine derivative **3d** was treated with 4 mol equivalents of BH_3-THF followed by 30% H_2O_2 and NaOH to afford 2(S)-[4-amino-2-oxo-1(2H)-pyrimidinyl]-norbornan-6 (R)-ol (**4d**) in 40% yield and 2(S)-[4-amino-2-oxo-1(2H)-pyrimidinyl]-norbornan-5(S)-ol (**5d**) in 30% (Scheme 2).
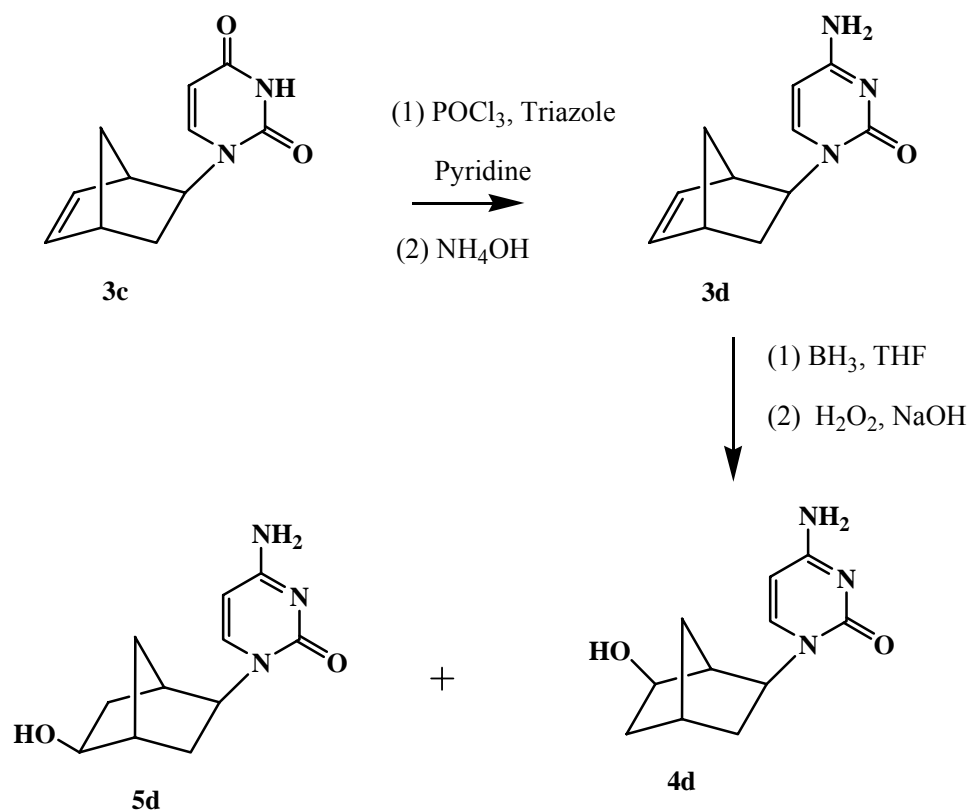
Scheme 2

The structures of **4** and **5** were confirmed by ^{1}H and ^{13}C NMR data and additional NMR experiments such as homodecoupling, DEPT, and NOE. The two regioisomers were differentiated by selective INEPT [1]H-^{13}C correlations.[13] For example, in the case of **4a**, selective irradiation of the *endo*-H-2′ resulted in the enhancement of the resonances at 33.1 (C-7′), 36.1 (C-4′), 38.70 (C-3′), 51.2 (C-1′), 73.0 (C-6′), 139.6 (C-8), and 150.2 (C-4). No enhancement was observed at 40.9 ppm (C-5′). Enhancement of the resonance at 72.7 ppm indicates that the

hydroxyl group is attached at the C-6′ position. The *exo*-addition of the hydroxyl group to the double bond was confirmed by NOE experiments. For example, in the case of **4a**, irradiation of H-6′ resulted in enhancement of H-2′ by 15% (Figure 1).
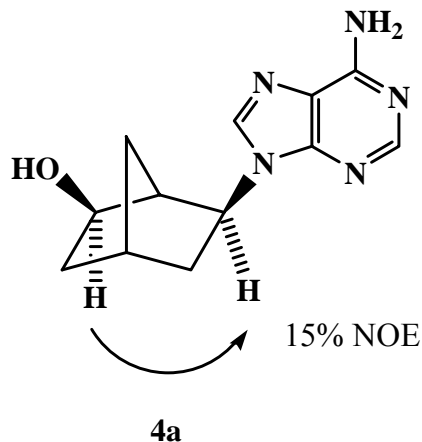

Figure 1. Differential NOE Experiments with **4a**.

In summary, the hitherto unknown bicyclo [2.2.1] carbocyclic nucleosides have been prepared. These compounds maintain the 1,3-*cis*- relationship between the nucloside base and the hydroxyl group. *In vitro* anti-HIV studies showed that the target compounds were inactive. Further antiviral studies are in progress and will be reported elsewhere.

Experimental Section

General Procedures. Melting points reported were uncorrected and were determined on an Electrothermal Engineering Ltd. melting point apparatus. Ultraviolet (UV) spectra were recorded on a Cary 3 UV-Visible spectrophotometer. ^{1}H NMR and ^{13}C NMR were recorded on a AC-300 and WM-360 instruments. Chemical shifts are referenced to an internal TMS standard for 1H-NMR spectra and to solvent (CDCl$_3$, DMSO-d$_6$, Acetone-d$_6$ or CD$_3$OD) for ^{13}C NMR spectra. Column chromatographic separations were carried out using 230-400 mesh silica gel. HPLC separations were carried out at 80 psi on Altex columns packed with Amberlite XAD-4 resin. Fractions were monitored by a Pharmacia UV-2 ultraviolet monitor and were collected on a Gilson FC-100 fraction collector. Purities of intermediates and final products were determined by a combination of ^{1}H and ^{13}C NMR spectra, quantitative UV data and HPLC analysis.

2-(*S*)-[6-Amino-9(*H*)-purin-9-yl]]-5-norbornene (3a). To a solution of **2** (5.2g, 19.67 mmole) in DMF (100 mL) were added adenine (4.0 g, 29.5 mmole), K$_2$CO$_3$ (4.35 g, 31.47 mmole) and 18-crown-6 (6.76 g, 25.57 mmole). The resulting solution was warmed to 100-105 °C for 48 h.

The solvent was removed in under high vacuum and the residue was purified on silica gel with 10-15% methanol/chloroform to give only one isomer **3a** (1.34g, 5.9 mmol, 30% yield, 39% conversion). Starting material (1.2g, 4.54 mmol, 30%) was recovered together with 2,5-norbornadiene. ^{1}H NMR (CDCl$_3$) δ 1.26 (s, 1H, H-7′), 1.77 (s, 1H, H-7′), 1.94 (dt, 1H, H-3′, J=3.68, 7.35 Hz), 2.03 (m, 1H, H-3′), 3.11 (s, 1H, H-4′), 3.19 (s, 1H, H-1′), 4.51 (dd, 1H, H-2′, J=3.88, 7.97), 6.26 (dd, 1H, H-6′, J=3.17, 5.69 Hz), 6.32 (dd, 1H, H-5′, J=2.91, 5.89 Hz), 6.34 (s, 2H, NH$_2$, exchangeable), 7.97 (s, 1H, H-8), 8.37 (s, 1H, H-2); ^{13}C NMR δ 33.3 (C-7′), 41.4 (C-4′), 46.4 (C-3′), 47.7 (C-1′), 54.9 (C-2′), 119.9 (C-5), 134.4 (C-6′), 137.9 (C-8), 139.7 (C-5′), 150.5 (C-4), 152.7 (C-2), 155.7 (C-6); UV λ$_{max}$ (MeOH) 260 nm.

2(*S*)-[6-Amino-9(*H*)-purin-9-yl-norbornan-6(*R*)-ol (4a) and 2(*S*)-[6-amino-9(*H*)-purin-9-yl]-norbornan-5(*S*)-ol (5a). To a solution of **3a** (0.55g, 2.4 mmole) in THF (25 mL) was added 1.0M BH$_3$·THF (9.7 mL, 9.7 mmole) at 0 °C. The resulting solution was warmed to room temperature and stirred for 5 h. It was quenched with water, followed by treatment with 30% H$_2$O$_2$ and 2N NaOH (aq). It was neutralized with dil. HCl. The solvent was removed under reduced pressure and then the residue was purified on silica gel with 15-20% methanol/chloroform to give a mixture of two isomers, **4a** and **5a** (0.5 g, 2.039 mmol, 80%). The two regioisomers were separated by reversed-phase HPLC with water-ethanol as the eluting solvent. Spectral data for **4a** (45%): mp 199-201 °C, ^{1}H NMR (CD$_3$OD) δ 1.46 (m, 1H, H-5′), 1.69 (d, 1H, H-5′, J=10.57), 1.85 (m, 3H, H-7′, H-3′), 2.01 (m, 1H, H-3′), 2.50 (br s, 1H, H-4′), 2.54 (br s, 1H, H-1′), 4.04 (d, 1H, H-6′, J=6.59 Hz), 4.40 (dd, 1H, H-2′, J=4.79, 8.24 Hz), 8.21 (s, 1H, H-8), 8.22 (s, 1H, H-2); ^{13}C NMR δ 33,1 (C-7′), 36.1 (C-4′), 38.7 (C-3′), 40.9 (C-5′), 51.2 (C-1′), 55.0 (C-2′), 73.0 (C-6′), 120.1 (C-5), 139.6 (C-8), 150.2 (C-4), 153.2 (C-2), 157.0 (C-6; UV λ$_{max}$ (H$_2$O) 260 nm (ε 13,500): Spectral data for **5a** (35%): mp 125-126 ºC, ^{1}H NMR (CD$_3$OD) δ 1.53 (m, 1H, H-6′), 1.62 (d, 1H, H-6′, J=11.92 HZ), 1.89 (m, 4H, H-7′, H-3′), 2.42 (d, 1H, H-4′, J=4.14 Hz), 2.63 (d, 1H, H-1′, J=4.65 Hz), 3.89 (d, 1H, H-5′, J=6.38 Hz), 4.39 (m, 1H, H-2′), 8.04 (s, 1H, H-8), 8.25 (s, 1H, H-2); ^{13}C NMR δ 32.8 (C-7′), 33.9 (C-4′), 39.7 (C-3′), 42.1 (C-6′), 44.6 (C-1′), 57.6 (C-2′), 73.1 (C-5′), 119.9 (C-5), 138.4 (C-8), 149.9 (C-4), 152.7 (C-2), 156.1 (C-6); UV λ$_{max}$ (H$_2$O), 260 nm (ε11,400).

2(*S*-)-[3,4-Dihydro-2,4-dioxo-5-methyl-1(2*H*)-pyrimidinyl]-5-norbornene (3b). To a solution of **2** (2.94g, 11.12 mmole) in DMF (100 mL) were added thymine (2.10g, 16.68 mmole), K$_2$CO$_3$(2.46g, 17.79 mmol), and 18-crown-6 (3.82g, 14.46 mmol). The resulting solution was warmed to 110 ºC for 48 h. The solvent was removed under vacuum and the residue was purified on silica gel with 5-10% methanol/chloroform to give an *O*-coupled isomer (0.2g, 0.916 mmol, 8.3%) and the desired *N*-coupled isomer **3b** (0.26g, 1.19 mmol, 10.7%). Spectral data for **3b**: ^{1}H NMR (CDCl$_3$) δ 1.46 (m, 1H, H-7′), 1.58 (m, 1H, H-7′), 1.73 (m, 1H, H-3′), 1.95 (m, 4H, H-3′, CH$_3$), 3.00 (m, 1H, H-4′), 4.22 (dd, 1H, H-1′, J=3.07, 7.07), 6.16 (dd, 1H, H-6′, J=3.29, 5.53 Hz), 6.28 (dd, 1H, H-5′, J=2.80, 4.64 Hz), 7.23 (s, 1H, H-6), 9.97 (br s, 1H, NH, exchangeable); ^{13}C NMR δ 12.5 (CH$_3$), 33.6 (C-7′), 40.9 (C-4′), 45.3 (C-1′), 46.1 (C-3′), 56.8 (C-2′), 106.6 (C-5), 134.3 (C-6′), 136.2 (C-6), 140.1 (C-5′), 151.8 (C-2), 164.2 (C-4); UV λ$_{max}$ (MeOH) 271 nm.

2(*S*)-[3, 4-Dihydro-2, 4-dioxo-5-methyl-1(2*H*)-pyrimidinyl]-norbornan-6 (*R*) – ol (4b) and 2(*S*)-[3,4-dihydro-2,4-dioxo-5-methyl-1(2*H*)-pyrimidinyl]-norbornan-5(*S*)-ol (5b). To a solution of **3b** (0.25g, 1.15 mmole) in THF (20 mL) was added 1.0M BH₃·THF (2.86 ml, 2.86 mmol) at 0°C.. The resulting solution was warmed to room temperature and stirred for 5 h and worked up as described for **4a** and **5a** to give the regioisomers, **4b** and **5b** (70 mg, 0.296 mmol, 26%). The two isomers were separated by reversed-phase HPLC with water-ethanol as the eluting solvent. Data for **4b:** mp 98-100 °C (lyophilized powder); 1H NMR (CDCl₃) δ 1.41 (m, 2H, H-5′), 1.75 (m, 3H, H-7′, H-3′), 1.90 (m, 4H, H-3′, CH₃), 2.33 (s, 1H, H-4′), 2.46 (s, 1H, H-1′), 3.99 (d, 1H, H-6′, J=16.33 Hz), 4.20 (m, 1H, H-2′), 7.15 (s, 1H, H-6), 8.77 (br s, 1H, NH, exchangeable); ¹³C NMR δ 13.0 (CH₃), 33.2 (C-7′), 35.0 (C-4′), 38.4 (C-3′), 40.4 (C-5′), 48.9 (C-1′), 55.0 (C-2′), 73.1 (C-6′), 111.0 (C-5), 136.2 (C-6), 151.4 (C-2), 163.5 (C-4); UV λ_max (H₂O) 272 nm (ε 5,600). Data for **5b:** mp 115-117 °C (lyophilized powder); ¹H NMR (CDCl₃) δ 1.46 (m, 3H, H-6′, H-7′), 1.89 (m, 6H, H-3′, H-7′, CH₃), 2.33 (d, 1H, H-4′, J=4.57 Hz), 2.47 (d, 1H, H-1′, J=4.89 Hz), 3.91 (m, 1H, H-5′), 4.12 (m, 1H, H-2′), 7.18 (s, 1H, H-6), 8.31 (br s, 1H, NH, exchangeable); ¹³C NMR δ 12.7 (CH₃), 32.9 (C-7′), 34.7 (C-4′), 39.7 (C-3′), 40.2 (C-6′), 44.0 (C-1′), 58.3 (C-2′), 73.2 (C-5′), 110.1 (C-5), 136.1 (C-6), 151.1 (C-2), 163.0 (C-4).

2(*S*)-[3,4-Dihydro2,-4-dioxo-1 (2*H*)-pyrimidinyl]-norbornan-6 (*R*)-ol (4c) and 2(*S*)-[3,4-dihydro-2, 4-dioxo-1(2*H*)-pyrimidinyl]-norbornan-5(*S*)-ol (5c). Intermediate **3c** was prepared as described for **3b** (yield: 56%): ¹H NMR (CDCl₃) δ 1.45 (dt, 1H, H-3′, J=3.62 Hz), 1.57 (d, 1H, H-7′, J=9.09 Hz), 1.74 (d, 1H, H-7′, J=8.04 Hz), 2.00 (m, 1H, H-3′), 3.00 (m, 2H, H-1′, H-4′), 4.22 (m, 1H, H-2′), 5.73 (d, 1H, H-5, J=8.11 Hz), 6.17 (dd, 1H, H-6′, J=3.36, 5.58 Hz), 6.30 (dd, 1H, H-5′, J=2.84, 5.63 Hz), 7.42 (d, 1H, H-6, J=7.94 Hz), 9.76 (br s, 1H, NH, exchangeable); ¹³C NMR δ 34.1 (C-7′), 41.2 (C-4′), 45.3 (C-1′), 46.2 (C-3′), 52.3 (C-2′), 101.4 (C-5), 134.2 (C-6′), 140.5 (C-6), 141.6 (C-5′), 151.7 (C-2), 163.6 (C-4). Compound **3c** was converted to **4c** and **5c** (25%) as described for **4b** and **5b**. Data for **4c:** ¹H NMR CD₃OD) δ 1.42 (m, 3H, H-5′, H-7′), 1.78 (m, 2H, H-7′, H-3′), 1.91 (m, 1H, H-3′), 2.31 (s, 1H, H-4′), 2.45 (s, 1H, H-1′), 3.80 (m, 1H, H-6'), 4.14 (dd, 1H, H-2′, J=4.79, 5.11), 5.69 (d, 1H, H-5, J=8.07 Hz), 7.45 (d, 1H, H-6, J=8.12 Hz); ¹³C NMR (CD₃OD) δ 33.4 (C-7′), 35.4 (C-4′), 39.0 (C-3′), 43.8 (C-5′), 56.0 (C-2′), 72.5 (C-6′), 101.8 (C-5), 141.0 (C-6), 151.5 (C-2), 164.3 (C-4).

2(*S*)-[4-Amino-2-oxo-1(2*H*)-pyrimidinyl]-5-norbornene (3d). To a solution of **3c** (0.18 g, 0.88 mmole) in pyridine (7 mL) was added phosphorus oxychloride (0.16 ml, 1.76 mmol) and 1,2,4-triazole (0.24 g, 3.52 mol) in pyridine (7 mL) at 0 °C. The resulting solution was warmed to room temperature and stirred for 26 h, followed by treatment with NH₄OH (2 ml, 17.6 mmole) and further stirring for 12 h. The solvent was removed and the residue was purified on silica gel with 10-15% methanol/chloroform to give **3d** (60 mg, 0.295 mmol, 34% yield). ¹H NMR (CD₃OD) δ 1.44 (dt, 1H, H-3′, J=3.59), 1.64 (m, 2H, H-7′), 1.94 (m, 1H, H-3′), 2.96 (s, 1H, H-4′), 3.01 (s, 1H, H-1′), 4.14 (m, 1H, H-2′), 5.87 (d. 1H, H-5, J=7.39 Hz), 6.18 (dd, 1H, H-6′, J=3.33, 5.62 Hz), 6.32 (dd, 1H, H-5′, J=2.85, 5.55 Hz), 7.65 (d, 1H, H-6, J=7.47); ¹³C NMR (CD₃OD) δ 35.3 (C-7′), 42.6 (C-4′), 46.6 (C-1′), 46.9 (C-3′), 59.3 (C-2′), 95.3 (C-5), 135.7 (C-6′), 141.5 (C-5′), 143.3 (C-6), 157.3 (C-2), 164.3 (C-4); UV λ_max (H₂O) 276.5 nm.

2(*S*)-[4-Amino-2-oxo-1(2*H*)-pyrimidinyl]-norbornan-6(*R*)-ol (4d) and 2(*S*)-[4-Amino-2-oxo-1(2*H*)-pyrimidinyl]-norbornan-5(*S*)-ol (5d). Compounds **4d** (31%) and **5d** (6%) were prepared by hydroboration followed by oxidation as described for **4a** and **5a.** Data for **4d**: 1H NMR (CD$_3$OD) 1.34 (m, 3H, H-5′, H-7′), 1.64 (m, 2H, H-7′, H-3′), 1.78 (m, 1H, H-3′), 2.20 (s, 1H, H-4′), 2.32 (s, 1H, H-1′), 3.77 (d, 1H, H-6′, J=6.34 Hz), 4.08 (m, 1H, H-2′), 5.79 (d, 1H, H-5, J=7.42 Hz), 7.62 (d, 1H, H-6, J=7.49 Hz), ^{13}C NMR (CD$_3$OD) 30.7 (C-7′), 37.1 (C-4′), 36.8 (C-3′), 38.4 (C-5′), 46.8 (C-1′), 53.8 (C-2′), 70.2 (C-6′), 91.9 (C-5), 140.2 (C-6), 152.7 (C-2), 163.1 (C-4); UV λ$_{max}$ (H$_2$O) 275 nm (ε 6,900).

Acknowledgements

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