Synthesis and Properties of Spiro Nucleosides Containing the Barbituric Acid Moiety

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The two chiral spiro nucleosides **4** and **5** containing the barbituric acid moiety were efficiently synthesized from optically pure precursors, and their properties were studied. The carbocyclic nucleoside **5** is considerably more stable against ring opening than the deoxyribosyl derivative **4**. Both compounds present enhanced hydrogen bonding capacity with diacetyladenosine.

Introduction

Synthesis of conformationally constrained nucleosides has been shown to be a useful strategy to design new enzyme inhibitors showing enhanced affinity for the nucleoside binding site.[1] Such "locked" nucleosides have also been explored in antisense and antigene approaches.[2] In this context we recently reported synthesis of 2′-deoxyhydantocidin **1** and its epimer **2**.[3] These compounds are deoxy analogues of (+)-hydantocidin **3** (Figure 1) which is the first natural spiro nucleoside isolated from the culture broth of *Streptomyces hygroscopicus* SANK 63584,[4] Tu-2474,[5] and A1491.[6] The (+)-hydantocidin **3** possesses an interesting profile of herbicidal and plant growth regulatory activity that has been related to the inhibitory activity of its 5′-phosphorylated metabolite against adenylsuccinate synthase.[7]

In our previous study, we showed that the two epimers **1** and **2** are interchanging in basic medium. This observation prompted us to design the new spiro nucleosides **4** and **5** in which the hydantoin ring is replaced by the barbiturate ring so that isomerization around the C-1′ carbon cannot occur. Similar to the hydantoin ring, the barbiturate ring system is known to possess thymine-like hydrogen bonding capacity against adenine derivatives[8] and is found in many pharmaceutically important molecules.[9] In this paper, we describe the synthesis of the spiro barbituric deoxyribofuranose **4** and its carbocyclic analogue **5** and evaluate their interest as building blocks for oligonucleotide synthesis. We show that the carbocyclic analogue **5** is considerably more stable against nucleophilic ring opening in aqueous medium compared to the deoxyribonucleoside **4**. Both compounds exhibit enhanced hydrogen bonding capacity with diacetyldeoxyadenosine as compared to the reference diacetylthymidine.

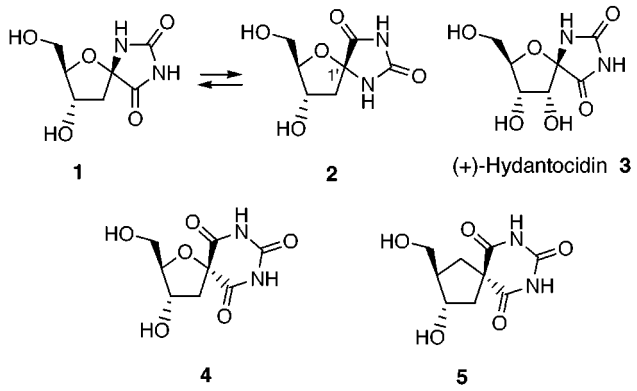

Figure 1. Spiro nucleosides containing the hydantoin moiety and the barbituric acid moiety.

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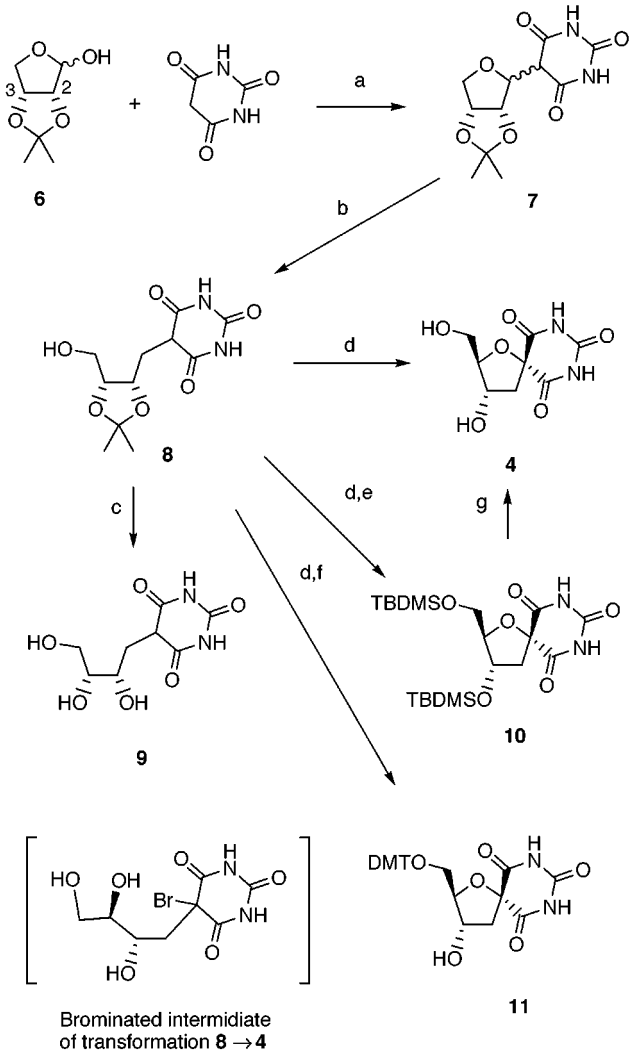
Scheme 1[a]



[a] Reagents and conditions: (a) Na_2CO_3; (b) H_2, Pd−C; (c) TFA/H_2O; (d) Br_2; (e) TBDMSCl, imidazole; (f) DMTCl, pyridine; (g) TMSCl, MeOH.

Results and Discussion

Synthesis of the Spiro Barbituric Deoxyribonucleoside 4. We reported previously the interest of erythrolactol **6** as a chiral synthon to build up the sugar part of spiro deoxyribonucleosides as exemplified by the straightforward synthesis of the spiro hydantoins **1** and **2**.[3] This four-carbon unit is easily accessible from the commercially available erythronolactone by DIBAL reduction, and it possesses the correct configuration (2*R*,3*R*) corresponding to the C-3′ and C-4′ chiral centers of the deoxyribose unit.[10]

The lactol **6** was thus condensed with barbituric acid in the presence of sodium carbonate to give the erythrosyl barbituric acid derivative **7** in 59% yield (Scheme 1). This compound was fully characterized by NMR, IR, and MS spectra and by elementary analysis. However the ^1H and ^{13}C NMR spectra in DMSO-d_6 of **7** were complex. This is partly due to the presence of two epimers around the C-1′ carbon. Another factor contributing to the complexity of the NMR spectra is the instability of the acetonide group. In D_2O solution, the acetal function of **7** hydrolyzes spontaneously due to the acidity of the barbituric part of the molecule. Upon hydrogenolysis (H_2/Pd−C in MeOH, r.t., 1.5 h), **7** was converted to the alcohol **8** in 91% yield. The ^1H and ^{13}C NMR spectra of **8** in DMSO-d_6 are quite simple and well resolved while in D_2O solution, the acetal function undergoes spontaneous hydrolysis to triol **9**. The triol **9** was also obtained quantitatively and conveniently from **8** by TFA/H_2O treatment. When **8** was treated with bromine followed by neutralization with $NaHCO_3$, simultaneous deprotection and spiro cyclization through the C-5 brominated intermediate occurred to afford the desired spiro deoxybarbituric acid nucleoside **4**. The analysis of the crude reaction product by ^1H NMR spectroscopy showed that the spiro furan **4** is the unique product formed in these conditions (Figure 2). The furanosyl structure was determined by ^1H and ^{13}C NMR (Figure 3 and Figure 4). In DMSO-d_6, the 5′-OH resonance was observed at 4.70 ppm as a broad triplet indicating the presence of the neighboring CH_2 group.

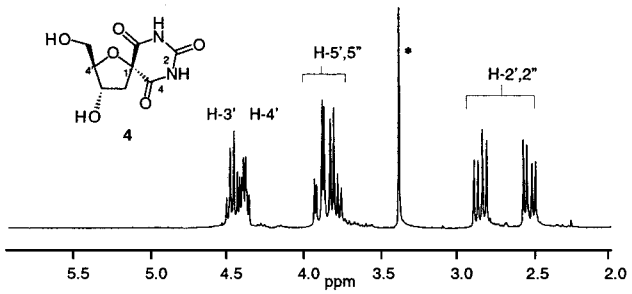


Figure 2. 200 MHz ^1H NMR spectrum of **4** (crude reaction product from **8**) measured in D_2O, * MeOH (internal reference).

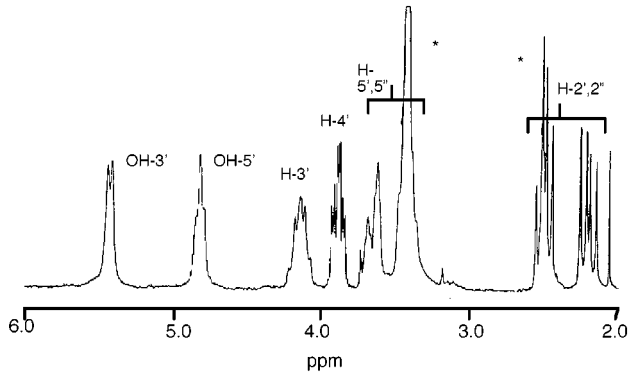


Figure 3. ^1H NMR spectrum of **4** (purified product) in DMSO-d_6, * residual proton of solvent.

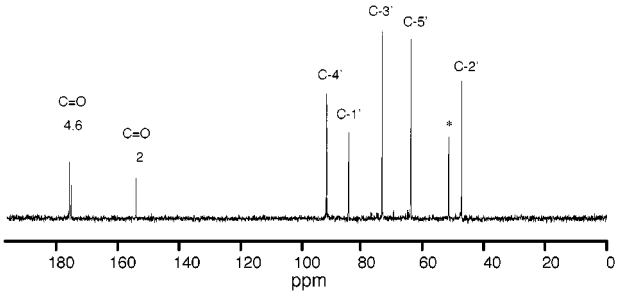


Figure 4. ^{13}C NMR spectrum of **4** (crude reaction product from **8**) in D_2O, * MeOH (internal reference).

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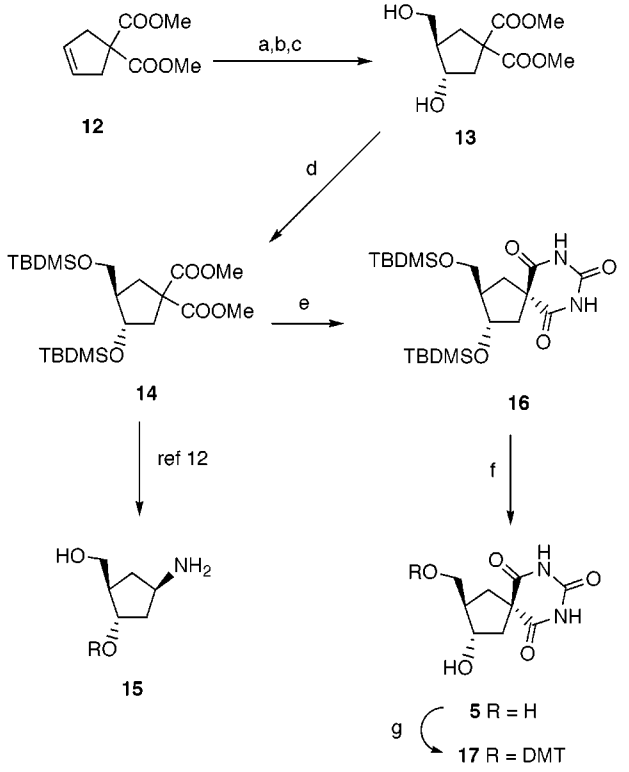
Scheme 2[a]



[a] Reagents and conditions: (a) $(CH_2O)_n$, AcOH, H_2SO_4, 60 °C, 24 h; (b) TMSCl, MeOH; (c) resolution; (d) TBDMSCl, imidazole; (e) urea, *t*-BuOK; (f) TMSCl, MeOH; (g) DMTCl, pyridine.

The chemical shift value for the C-4′ resonance at 92 ppm in the ^{13}C NMR spectrum confirmed the deoxyribofuranose structure of the compound.

However, **4** turned out to hydrolyze partly during isolation. A more convenient two-step isolation procedure was developed involving first silylation of the crude bromine reaction mixture to the bis *tert*-butyldimethylsilyl derivative **10** that could be purified by column chromatography (63%). Subsequent removal of the silyl protections was accomplished using TMSCl/MeOH to yield **4** in 95% yield. The DMT derivative **11** could also be prepared directly from **8** (80% yield) for direct use in subsequent oligonucleotide synthesis.

Synthesis of the Carbocyclic Analogue 5. As the barbituric ring in **4** appeared to be unstable through ring opening in aqueous neutral medium (vide infra), we were prompted to design the spiro nucleoside analogue **5**, replacing the oxygen atom by a methylene unit. While many synthetic routes to carbocyclic analogues of nucleosides have been described,[11] none of them could be used directly for the synthesis of the spiro carbocyclic nucleoside **5**. We thus developed a new synthetic route to the required chiral precursor (−)-**13**.[12]

Diol (±)-**13** was prepared from the cyclopentene diester **12** in 60% yield (Scheme 2) by Prins reaction[13] (paraformaldehyde, AcOH, H_2SO_4, 60 °C, 24 h; then TMSCl, MeOH). Pancreatin-catalyzed resolution of (±)-**13** gave optically pure (−)-**13** in 25% yield (ee > 98%). The disilylated derivative (−)-**14** was obtained in 90% yield by TBDMSCl/imidazole treatment of (−)-**13**. The absolute configuration of (−)-**14** was determined by chemical correlation to the known (+)-(1*R*,3*S*,4*R*)-3-hydroxy-4-hydroxymethylcyclopentylamine **15**.[14] From this chiral diester (−)-**14**, synthesis of the target spiro barbituric acid **5** appeared straightforward.

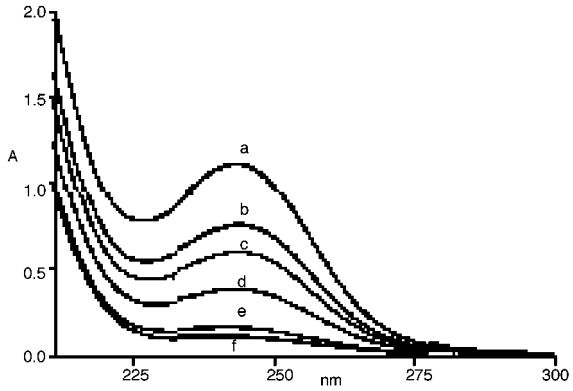


Figure 5. UV spectra of **4** at pH 8 (a) $t = 3$ min; (b) 14 min; (c) 18 min; (d) 30 min; (e) 60 min; (f) 127 min.

(−)-**14** was successfully condensed with urea in the presence of potassium *tert*-butoxide to give **16** in 68% yield. Final deprotection of the silyl groups gave the spiro barbituric acid **5** in 99% yield. This new stable barbiturate derivative was fully characterized. This compound was also obtained as crystals, and its structure in the solid state was determined by X-ray diffraction analysis.[15] The DMT derivative **17** was prepared from **5** (80% yield).

Properties of the Spiro Barbituric Acid Nucleosides 4 and 5. The two spiro barbituric acid nucleosides **4** and **5** were studied in order to evaluate their possible use as building blocks in modified oligonucleotide synthesis in view of applications such as antisense and antigene strategies. We first studied their chemical stability in aqueous medium and then determined their hydrogen bond forming capacity with deoxyadenosine derivatives in organic solvent.

Ring opening of barbiturates **4** and **5** was studied in aqueous solution.[16] 5,5-Disubstituted barbituric acid derivatives, when deprotonated above pH 7, present a UV absorption at $\lambda_{max} = 240$ nm owing to negative charge delocalization. The ring-opening reaction could thus be monitored by measuring at different time intervals the UV variation at this wavelength (Figure 5).

At pH = 7, rapid ring-opening was observed for the furanosyl barbiturate **4** while the carbocyclic compound **5** was quite stable. At higher pH however (pH > 9), the rate of the ring-opening reaction became significant for compound **5**. The results of the pseudo first-order kinetic measurement are shown in Table 1. By comparison with the data described for simple spiro barbiturates,[16a] the ring-opening reaction rate is enhanced for these spirobarbituric acids **4** and **5**. These results indicate that the

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Table 1. Rate of Ring Opening Reaction of Spiro Nucleosides 4 and 5[a]

4			5		
pH	10^3k [s^{-1}]	$t_{1/2}$ [min]	pH	10^3k [s^{-1}]	$t_{1/2}$ [min]
7.0	0.15	76	10.0	0.44	26
8.0	0.77	15	10.5	1.1	10
8.5	2.4	4.8	11.0	2.8	4.2[c]
9.0	7.5	1.5[b]			
9.5	12	1.0			

[a] ±10% (b) and (c) values to be compared to those reported for spirocyclopentanebarbituric acid in the same conditions. [b] 17 h. [c] 40 min; see ref 16a.

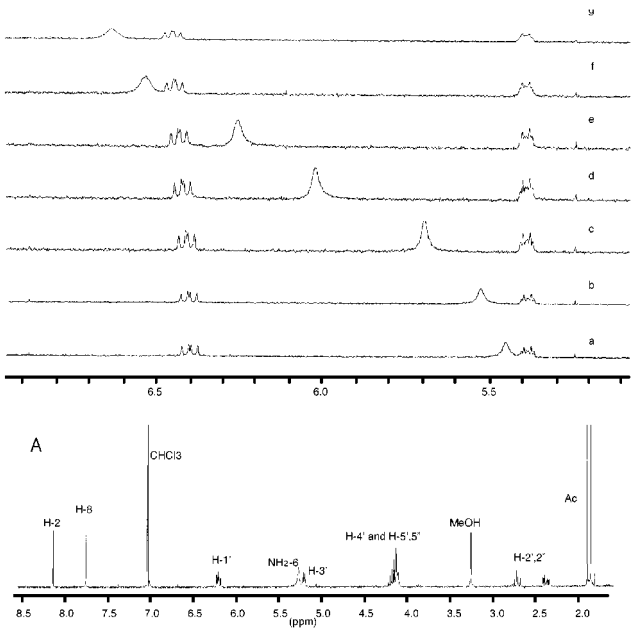

Figure 6. (A) ^{1}H NMR spectrum of diacetyldeoxyadenine **19** in $CDCl_3$ (1.6−8.8 ppm). (B) Same spectra (5.1−6.9 ppm) upon addition of **16** (a) 0 eqmol; (b) 0.1 eqmol; (c) 0.5 eqmol; (d) 1.3 eqmol; (e) 2.1 eqmol; (f) 3.8 eqmol; (g) 5.3 eqmol.

nucleophilic attack on the barbituric ring is accelerated by the presence of the oxygen atom in the five-membered ring. The presence of the two hydroxyl substituents also increases the rate (15-fold increase as compared to the unsubstituted analog).

These observations are of interest to establish the scope and the limitations for using these spiro barbituric moieties as building units of oligonucleotides. As basic conditions (conc NH_3, 55 °C) are required in the final deprotecting step in the conventional phosphoramidite based oligonucleotide synthesis, incorporation of these units necessitates some modification of the chemistry.

The hydrogen bonding capacity of the silylated spiro barbituric acid nucleosides **10**, **16** and of the spiro hydantoin nucleoside **18** against diacetyldeoxyadenine **19** was evaluated by ^{1}H NMR spectroscopy. Association constants were obtained by NMR titration protocols involving addition of the spiro nucleosides **10**, **16**, and **18** to a solution of **19** in $CDCl_3$. Complexation results in downfield shifts of the NH_2-6 resonance of **19** (from 5.5 to 6.6 ppm in the case of complexation between **16** and **19**, Figure 6). Compared to the association constant between diacetyl thymidine **20** and **19**, the association constants were significantly enhanced for all three spiro nucleosides **10**, **16**, and **18** (Figure 7).

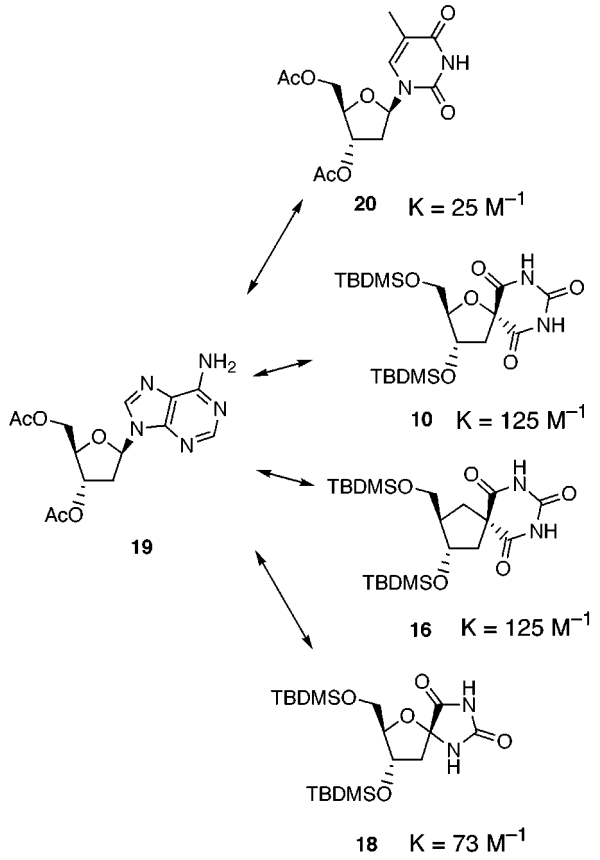

Figure 7. Association constants of the spiro nucleosides with diacetyldeoxyadenosine **19** ($CDCl_3$, 25 °C).

Conclusion

The barbituric acid containing spiro nucleosides **4** and **5** could be prepared efficiently from simple precursors. It appears that the deoxyribonucleoside **4** exhibits too low stability for use as building block in conventional oligonucleotide synthesis. However, the carbocyclic analogue **5** shows both enough stability and hydrogen bonding capacity with the complementary deoxyadenosine derivative to be used as a conformationally locked unit in the construction of modified oligonucleotides.

Experimental Section

General. All chemicals and solvents were purchased from Fluka, Aldrich, Merck, SDS, Carlo-Elba; they were of analytical or HPLC grade or otherwise distilled before use. TLC: Merck Kieselgel 60 F_{254}, layer thickness 0.25 mm. Visualization by UV light (254 nm), H_2SO_4 solution, and/or phosphomolybdic acid solution. Preparative column chromatographies: Merck Kieselgel, 230−400 mesh. Melting point: Reichert Thermovar (uncorrected). Optical rotations: Perkin-Elmer polarimeter 341. IR: Nicolet Impact 400. UV/Vis: Perkin-Elmer lambda 5. NMR: Bruker WP 80, AM 200 WP 250 AM 300 and Varian U+500 spectrometers. NMR spectra were referenced to the residual solvent peak; chemical shifts δ in ppm; apparent scalar coupling constants J in Hz. MS: Delsi-Nermag R10−10. Elemental analysis were performed by "Service central de microanalyse du CNRS".

5-(2′,3′-*O*-Isopropylidene-D-erythrosyl)barbituric Acid (7). A mixture of Na_2CO_3 (6.73 g, 23.5 mmol), barbituric acid (6.03 g, 47.0 mmol), and lactol **6** (7.53 g, 47.0 mmol) in H_2O (55 mL) was stirred at 80 °C for 5 h. After cooling at 4 °C, a HCl solution (2 N, 23.5 mL) was added dropwise with stirring. The resulting suspension was further kept at 4 °C for 1 h, and

the precipitate was filtered and dried to give **7** (7.46 g, 59%). mp 166−171 °C. IR (KBr): 1698, 1652, 1606, 1561 cm^{-1}. NMR Data for the major isomer of **7**: ^1H NMR (200 MHz, DMSO-d_6): δ = 1.26 (s, 3 H), 1.38 (s, 3 H), 3.77 (m, 2 H), 3.83 (m, 1 H), 4.46 (m, 1 H), 4.72 (m, 1 H), 5.22 (m, 1 H), 11.38 (br. s, 1 H), 11.40 (br. s, 1 H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 24.8, 26.7, 51.2, 74.4, 80.9 83.2 and 85.5, 111.9, 150.7, 169.2. MS (DCI, NH_3/isobutane): m/z = 271 $[M + H]^+$. $C_{11}H_{14}N_2O_6 \cdot 0.25\ H_2O$ (274.7): calcd C 48.09, H 5.32, N 10.20; found C 48.03, H 5.63, N 10.19.

(2′*S*,3′*R*)-5-(4′-Hydroxy-2′,3′-isopropylidenedioxybutyl-)barbituric Acid (8). A mixture of **7** (6.5 g, 24 mmol) and Pd−C 10% (500 mg) in MeOH (200 mL) was stirred under H_2 atmosphere at r.t. for 1.5 h. The suspension was then filtered on Celite, and the filtrate was concentrated under reduced pressure. The resulting foam was triturated into Et_2O to afford **8** as a white powder (5.9 g, 91%). mp 136−139 °C. TLC (MeOH/CH_2Cl_2 1:4): R_f = 0.34. IR (KBr): 1711, 1591 cm^{-1}. ^1H NMR (250 MHz, DMSO-d_6): δ = 1.19 (s, 3 H), 1.27 (s, 3 H), 1.90−2.28 (m, 2 H), 3.45 (m, 2 H), 3.59 (m, 1 H), 4.17 (m, 1 H), 4.23 (m, 1 H), 4.82 (br. s, 1 H), 11.08 (br. s, 1 H), 11.16 (br. s, 1 H). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 25.6, 27.5, 28.2, 44.5, 59.3, 73.7, 77.4, 107.6, 150.9, 170.4, 170.8. MS (DCI, NH_3/isobutane): m/z = 273 $[M + H]^+$.

(2′,3′,4′-Trihydroxybutyl)barbituric Acid (9). A solution of **8** (100 mg, 0.368 mmol) in TFA:H_2O 1:1 (2 mL) was stirred at r.t. for 15 min. After removal of solvent under reduced pressure, the resulting foam was triturated in Et_2O to afford **9** as a white solid. ^1H NMR (200 MHz, D_2O): δ = 2.23 (m, 2 H), 3.30−3.69 (m, 5 H). ^{13}C NMR (50 MHz, D_2O): δ = 34.0, 47.2, 64.9, 70.8, 77.2, 154.2, 174.6, 175.2.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)spirodeoxyribosylbarbituric Acid (10). To a solution of **8** (810 mg, 3.0 mmol) in H_2O (13 mL) was added dropwise a saturated aqueous solution of Br_2 (13.4 mL, 3.2 mmol) at r.t. The reaction mixture was stirred for 30 min and then neutralized by addition of solid $NaHCO_3$ (6.4 mmol). Solvents were removed under reduced pressure. This crude material (1.71 g) containing the compound **4** and NaBr was directly silylated. After coevaporation (three times) with a small amount of dry DMF, the residue was suspended in dry DMF (3 mL). To this suspension were added imidazole (1.63 g, 24.0 mmol) and TBDMSCl (1.81 g, 12.1 mmol). The mixture was stirred at r.t. for 14 h. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel (AcOEt/cyclohexane 1:2) to give **10** as a white powder (850 mg; 63%). mp 162−167 °C. TLC (AcOEt/pentane 1:2): R_f 0.29. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.04−0.06 (4s, 12 H), 0.88 and 0.89 (2s, 18 H), 2.43 (dd, J = 5 and 8 Hz, 1H), 2.52 (dd, J = 5 and 8 Hz, 1 H); 3.75 (dd, J = 3 and 8 Hz, 1H), 3.82 (dd, J = 2 and 8 Hz, 1H), 4.14 (m, 1 H), 4.49 (m, 1 H), 8.3 (br. s, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = −5.5, −5.3, −4.9, −4.7, 17.8, 18.4, 25.6, 25.9, 43.5, 62.09, 71.3, 79.9, 88.4, 149.2, 169.0, 169.6. MS (FAB+, NBA/NaI): m/z: = 459 $[M + H]^+$. $C_{20}H_{38}N_2O_6Si_2$ (458,7) calcd C 52.37, H 8.35, N 6.11; found C 52.33, H 8.40, N 6.03.

5′-*O*-Dimethoxytritylspirodeoxyribosylbarbituric Acid (11). The crude mixture containing the compound **4** and NaBr was obtained from **8** (1.088 g, 4.0 mmol) as above and used directly for tritylation. After coevaporation (three times) with a small amount of dry pyridine, the residue was suspended in dry pyridine (15 mL). To this suspension was added DMTCl (2.18 g, 6.43 mmol). The mixture was stirred at r.t. for 3 h. MeOH (2 mL) was added, and the mixture was stirred for 15 min at r.t. The solvent was removed under reduced pressure, and the residue was partitioned between dichloromethane (250 mL) and water (100 mL). The aqueous phase was extracted three times, and the combined organic phase was dried on $MgSO_4$ and evaporated. The oily residue was chromatographed on silica gel (acetone/CH_2Cl_2 1:4). The resulting foam was dissolved in CH_2Cl_2 and then precipitated by addition of hexane to give **11** as a white powder (1.32 g; 62%). TLC (acetone/CH_2Cl_2 1:4): R_f 0.24. ^1H NMR (200 MHz, acetone-d_6): δ = 2.47 (dd, J = 6 and 13 Hz, 1H, H-2′), 2.72 (dd, J = 7 and 13 Hz, 1H, H-2″), 3.27 (m, 2H, H-5′), 3.75 (s, 3H, CH_3O), 4.26 (m, 1 H, H-3′), 4.46 (m, 1 H, H-4′), 6.75−6.95 (m, 4H, H arom.), 7.15−7.55 (m, 9H, H arom), 8.5 (m, 2H, NH). ^{13}C NMR (50 MHz, acetone-d_6): δ = 41.6 (C-2′), 64.0 (C-5′), 72.7 (C-3′), 87.9 (C-4′), 80.0 (CAr_3), 85.9 (C-1′), 112.9, 126.3, 127.6, 128.2, 130.1, 136.1, 145.4, 149.2 (C arom), 158.7 (C-2), 169.5, 171.1 (C-4 and C-6). MS (FAB, NBA/NaI): m/z: = 531 $[M - H]$. $C_{29}H_{28}N_2O_8$+1.25H_2O (555.04) calcd C 62.75, H 5.54, N 5.05; found C 62.41, H 5.64, N 5.38.

Spirodeoxyribosylbarbituric Acid (4). To a solution of the compound **10** (401 mg; 0.87 mmol) in MeOH (3 mL) was added TMSCl (100 μL, 0.79 mmol). This solution was stirred at r.t. for 15 min. Solvents were removed under reduced pressure, and the resulting residue was then triturated in Et_2O to afford **4** as a white powder (190 mg, 95%). mp 186−187 °C. TLC (MeOH/CH_2Cl_2 1:2): R_f 0.43. $[\alpha]^{25}_D$ = +32.0 (c =1.13, H_2O). IR (KBr): 1778, 1735 cm^{-1}. ^1H NMR (250 MHz, D_2O): δ = 2.53 (dd, J = 6 and 14 Hz, 1 H), 2.85 (dd, J = 6 and 14 Hz, 1 H), 3.72 (dd, J = 6 and 13 Hz, 1 H), 3.84 (dd, J = 3 and 13 Hz, 1 H), 4.43 (m, 1 H), 4.51 (m, 1 H). ^1H NMR (200 MHz, DMSO-d_6): δ = 2.18 (dd, J = 8 and 13 Hz, 1 H); 2.47 (dd, J = 8 and 13 Hz, 1 H); 3.30−3.68 (m, 2 H), 3.80 (m, 1 H), 4.03 (m, 1 H), 4.70 (m, 1 H), 5.27 (m, 1 H); 11.26 (br. s, 2 H). ^{13}C NMR (75 MHz, D_2O): δ = 47.7, 64.3, 73.6, 84.5, 91.8, 153.8, 175.0, 175.6. MS (DCI, NH_3/isobutane): m/z: = 231$[M+H]^+$. $C_8H_{10}N_2O_6$ (230.18) calcd C 41.75, H 4.38, N 12.17; found C 41.52, H 4.39, 11.76.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)spirocyclopentylbarbituric Acid (16). To a solution of diester **14**[12] (2.8 g, 6.1 mmol) in DMSO (6 mL) were added urea (2.2 g, 36 mmol) and *t*BuOK (1.5 g, 13.4 mmol). The mixture was stirred at r.t. for 15 min, diluted with AcOEt (200 mL), and washed with a HCl solution (0.1 N, 130 mL). The aqueous phase was extracted three times with AcOEt, and the combined organic phase was washed with brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure, and the crude product was chromatographed on silica gel (AcOEt/pentane 1:2) to furnish **16** as a white powder (1.9 g, 4.2 mmol, 68%). mp 187.5−188.5 °C. TLC (AcOEt/pentane 5:95): R_f 0.30. $[\alpha]^{25}_D$ = +41.0 (c = 2.12, $CHCl_3$). IR (KBr): 1754, 1723, 1682 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ = 0.01, 0.02, 0.03, 0.05 (4s, 12 H), 0.86 (s, 18 H), 2.05−2.42 (m, 5 H), 3.60 (dd, J = 5 and 10 Hz, 1 H), 3.69 (dd, J = 2 and 10 Hz, 1 H), 4.23 (q, J = 8 Hz, 1 H), 8.5 (br.s, 1 H), 8.6 (br.s, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = −5.6, −5.4, −4.9, −4.5, 17.9, 18.2, 25.7, 25.9, 37.3, 43.0, 49.2, 52.3, 61.4, 72.9, 150.0, 173.6. MS (FAB$^-$, NBA): m/z: 455 $[M - H]^+$. $C_{21}H_{40}N_2O_5Si_2$ (456.73) calcd C 55.22, H 8.82, N 6.13; found C 55.17, H 8.98, N 5.97.

Spirocyclopentylbarbituric Acid (5). To a solution of **16** (2.0 g; 4.4 mmol) in MeOH (10 mL) was added TMSCl (500 μL). This solution was stirred at r.t. for 15 min while the deprotected **5** precipitated in the medium. The volatile material was removed under reduced pressure, and the resulting residue was triturated into Et_2O to afford **5** as a white powder (1.0 g, 4.3 mmol, 99%). mp 214−216 °C. TLC (MeOH/CH_2Cl_2 20:80): R_f 0.28. $[\alpha]^{25}_D$ = +34.8 (c = 0.91, MeOH). IR (KBr): 1754, 1740, 1689 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ = 1.65−2.30 (m, 5 H), 3.31 (m, 1 H), 3.56 (m, 1 H), 3.82 (m, 1 H), 4.42 (m, 1 H), 4.84 (m, 1 H), 11.0 (br.s, 2 H). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 37.9, 42.5, 49.0, 51.9, 61.3, 72.5, 150.5, 174.6. MS (FAB$^-$, glycerol): m/z: 227 $[M - H]$. $C_9H_{12}N_2O_5$ (228.20) calcd C 47.37, H 5.30, N 12.28; found C 47.46, H 5.46, N 12.27.

5′-*O*-Dimethoxytritylspirocyclopentylbarbituric Acid (17). To a solution of **5** (280 mg, 1.20 mmol) in dry pyridine (5 mL) was added DMTCl (575 mg, 1.70 mmol) at r.t. The reaction mixture was stirred for 30 min, and then MeOH (1 mL) was added and stirred for 15 min. Solvents were removed under reduced pressure. The resulting residue was coevaporated three times with toluene and then chromatographed on silica gel (acetone/CH_2Cl_2 1:4).The resulting foam was dissolved in CH_2Cl_2 and then precipitated by addition of pentane to furnish **17** as a white powder (505 mg, 80%). mp 199−220 °C. TLC (acetone/CH_2Cl_2 1:3): R_f 0.32. IR (KBr): 1717, 1692, 1603 cm^{-1}. ^1H NMR (250 MHz, DMSO-d_6): δ =

1.75−2.00 (m, 2H), 2.05−2.40 (m, 3H), 2.82 (t, J = 9 Hz, 1H); 3.20 (dd, J = 3, 9 Hz, 1 H), 3.72 (s, 3H), 3.82 (m, 1H), 4.91 (d, 1 H), 6.85−6.90 (m, 4 H), 7.20−7.40 (m, 9 H), 11.1 (br. s, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 37.7, 43.1, 46.6, 51.5, 54.8, 63.8, 72.6, 84.9, 112.9, 127.5, 129.4, 135.7, 145.0, 150.3, 157.8, 174.4, 174.5. MS (FAB$^-$, glycerol): m/z: 529 [M − H]. $C_{30}H_{30}N_2O_7$ (530.57) calcd C 67.91, H 5.70, N 5.28; found C 67.92, H 5.76, N 5.41.

Kinetic of Barbiturate Ring Opening in 4 and 5 Determined by UV Spectrophotometry. A solution of barbituric derivative **4** or **5** (0.02 M, 20 μL) was added in a 3 mL UV cell (1 cm optical length) containing 2.5 mL of a 10 mM buffer solution (phosphate buffer for pH 7 to 8 and borate buffer for pH 8.5 to 11). The ionic strength of this solution was adjusted with NaCl to μ = 0.1. The decrease of A_{243} was monitored at regular time intervals at 25 °C.

Complexation Study by ^{1}H NMR Spectroscopy. To a solution of diacetylated adenine **19** in $CDCl_3$ (800 μL in a 5 mm NMR tube, [**19**] = 40 mM for the titration experiments with **20** and **18** or [**19**] = 4.5 mM with spiro barbiturates **10** and **16**) were added increasing volumes of a solution of the nucleoside in $CDCl_3$ (concentrations: [**20**] = 200 mM, [**18**] = 200 mM, [**10**] = 46.5 mM, [**16**] = 45 mM). For each new addition, 1D ^{1}H NMR spectra were recorded. The association constants K_d were determined from the plot of $\Delta\delta$ for the adenine amino proton (NH-6) as a function of the amount of added ligand (eqmol).

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