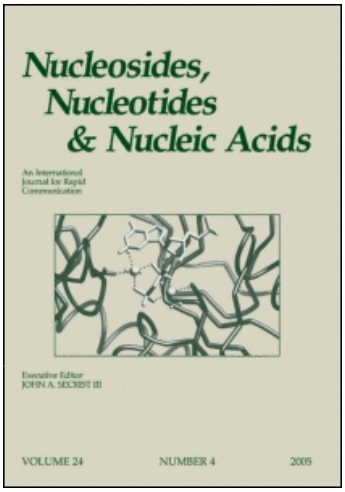

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Ping Ge[a]; Gregory O. Voronin[a]; Thomas I. Kalman[a]
[a] Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Ny, Amherst

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SYNTHESIS AND STRUCTURE DETERMINATION OF A NUCLEOSIDE-DERIVED NEW HETEROCYCLIC SYSTEM: 8*H*,10*H*,15b(*S*)-2,3,6,7-TETRAHYDRO-1,5,3-DIOXAZEPINO[3,2-*c*]INDOLO[3,2-*g*]PTERIDINE-7-ONE

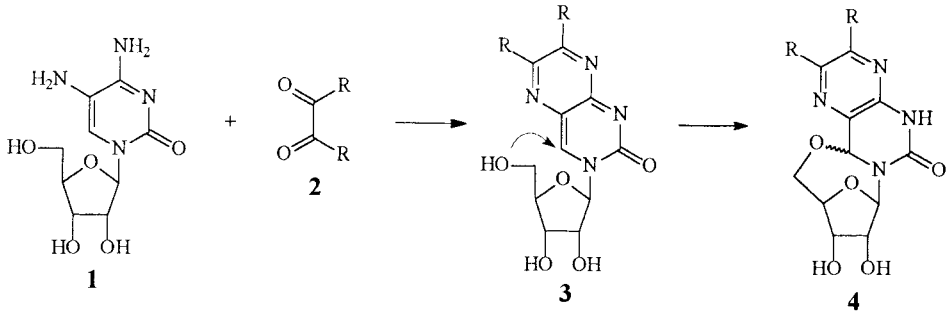
Ping Ge, Gregory O. Voronin and Thomas I. Kalman*

Department of Medicinal Chemistry, School of Pharmacy,
State University of New York at Buffalo, Amherst, NY 14260

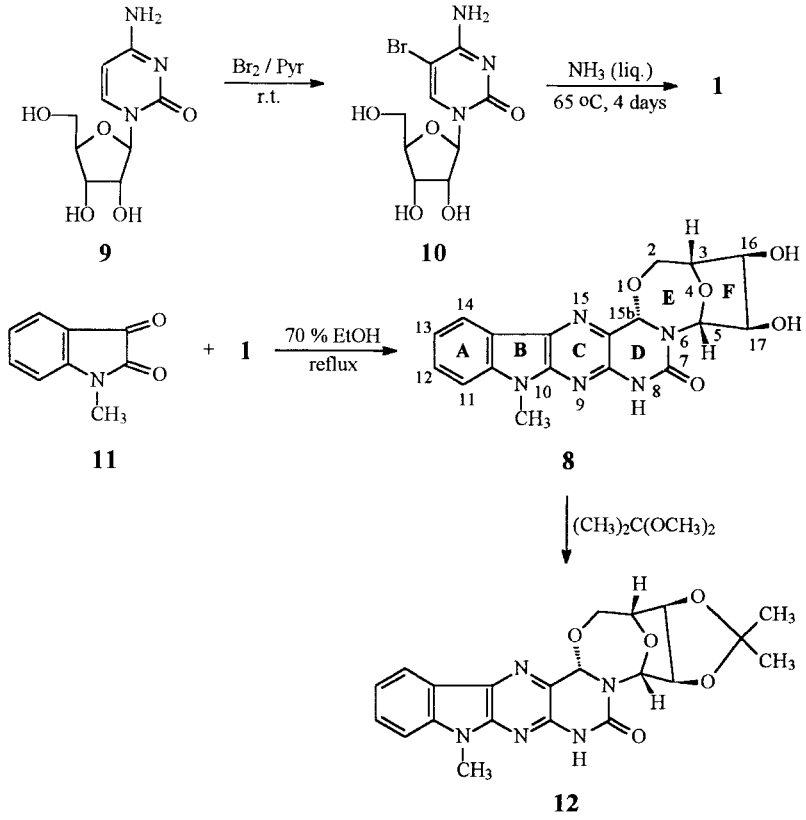
Abstract. Condensation of 5-aminocytidine with *N*-methylisatin yielded a new heterocyclic system, 8*H*,10*H*,15b(*S*)-2,3,6,7-tetrahydro-1,5,3-dioxazepino[3,2-*c*]indolo[3,2-*g*]pteridine-7-one. The assignment of *S* configuration at position 15b, which is generated as a result of the nucleophilic attack of the 5'-hydroxy of the ribosyl moiety at the position 6 of the cytosine base, is discussed.

INTRODUCTION

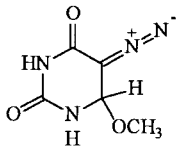
In our continuing effort to discover novel anticancer agents derived from nucleosides, we became interested in the condensation of 5-aminocytidine (**1**, see Scheme 1 and 2) with 1,2-dicarbonyl compounds (**2**). We found that formation of a fused pyrazine ring (**3**) activates the 6-position of cytidine to accept a nucleophile.[1] In 5'-unsubstituted cytidines an intramolecular attack by the 5'-hydroxy at the position 6 of the pyrimidine ring can result in the formation of cyclonucleoside derivatives (**4**). This nucleophilic addition can produce two possible stereoisomers. In their study of the analogous structures of 5-diazo-6-methoxy-1,6-dihydropyrimidin-2,4(1*H*,3*H*,6*H*)dione (**5**), 1-(β-D-ribofuranosyl)-$O^{5'}$-6(*S*)cyclo-5-diazo-1,6-dihydro-pyrimidin-2,4(3*H*,6*H*)dione (**6**) and 1-(2-deoxy-β-D-ribofuranosyl)-$O^{5'}$-6(*S*)cyclo-5-diazo-1,6-dihydropyrimidin-2,4(3*H*,6*H*)-dione (**7**), Thurber and Townsend[2] suggested that



SCHEME 1



SCHEME 2



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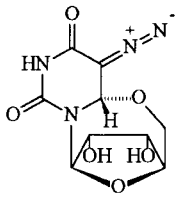

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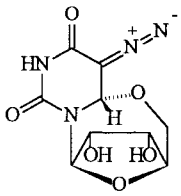

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the new chiral center has the *S* configuration. It was observed that the C-6 proton (at 6.15 ppm) of the two cyclonucleosides (**6**, **7**) had a 0.43 ppm downfield shift compared with the chemical shift of the C-6 proton (at 5.72 ppm) of the methoxy adduct **5**. Electron withdrawing by the carbohydrate moiety was considered unlikely to be solely responsible for this effect. The additional deshielding may be due to the furanosyl ring oxygen, which is consistent with their assignment.[2] We now report that by using a combination of NOE techniques and molecular modeling studies, the stereochemistry at this newly formed chiral center can be established when isatins are used as dicarbonyl compounds to form cyclonucleosides from 5-aminocytidines.

SYNTHESIS

Condensation of 5-aminocytidine with isatins yielded a new heterocyclic system (**8**), 8*H*,10*H*,15b(*S*)-2,3,6,7-tetrahydro-1,5,3-dioxazepino[3,2-*c*]indolo[3,2-*g*]pteridine-7-one, as illustrated in Scheme 2.

Starting from cytidine (**9**), 5-bromocytidine (**10**) was obtained in 58% yield. Amination of 5-bromocytidine in liquid ammonia gave 5-aminocytidine (**1**).[3] 8*H*,10*H*-3(*R*),5(*R*),15b(*S*),16(*R*),17(*R*)-10- Methyl-16,17- dihydroxy-3,5-ethano-2,3,6,7-tetra-hydro-1,5,3-dioxazepino[3,2-*c*]indolo[3,2-*g*]pteridine-7-one (**8**), as a representative of this class of compounds, was obtained by condensation of 5-aminocytidine with *N*-methylisatin (**11**) in 70% ethanol. Treatment with dimethoxypropane gave its 16,17-*O*-isopropylidene derivative (**12**). Determination of the regiochemistry of **8** by ^{13}C NMR will be published elsewhere.[4]

STEREOCHEMISTRY

Some interesting features of the new hexacyclic system (**8**) are worth noting. The chiral centers at positions 3, 5, 16 and 17 are known since the condensation started from a cytidine derivative. However, the newly formed chiral center at 15b can be either *R* or *S*. The

conformation of the relatively rigid ring system is mainly determined by the 7-membered ring E which can adopt a pseudo chair or boat conformation, as indicated by molecular modeling. Two pairs of possible isomers, 15b(*S*) / E-ring chair, 15b(*S*) / E-ring boat, 15b(*R*) / E-ring chair and 15b(*R*) / E-ring boat may be considered (see Figure 1. A). The lowest energy state is the chair form for the pair of 15b(*S*) conformers, whereas the boat form is preferred for the 15b(*R*) conformers (Table 1).

Table 2 summarizes the distances from the 15b-proton to the other protons of the four possible isomers within the [4.2.1] bicyclic ring system. We found that in the *S* configuration 15b-H is very close to the *cis* 2-H if the E-ring adopts the chair conformation. In the *R* configuration, 15b-H is close to 17-H if the E-ring is in the chair conformation, whereas it is close to *cis* 2-H, 16-H and 17-H if the E-ring is in the boat conformation. In order to assign the stereochemistry at 15b, NOE experiments were perfomed.

For the spectroscopic study, the ketal **12** was synthesized. This modification removed the coupling between the hydroxy groups and the ring protons and increased the solublility in *d*-chloroform. Molecular modeling confirmed that ketal formation did not alter the overall conformation (see Figure 1. B).

The 15b-H is the most isolated proton, so it is logical to do the NOE difference experiment by observing its enhancement. However, we did not know which proton is close to 15b-H. Alternetively, one can irradiate 15b-H and once the resulting enhancement is identified, do a reverse NOE difference experiment to verify the interaction.

When 15b-H was irradiated, only one enhancement was identified corresponding to one of the protons at the 2-position (Figure 2. A). The other signals were clearly canceled. This result suggested that the configuration of 15b is *S*, since only one proton (2-H) within the [4.2.1] bicyclic system is close enough to 15b-H to give a NOE (see Table 2). To verify this relationship, the enhanced 2-H was irradiated and a strong enhancement at the15b proton was observed (Figure 2. B). The NOESY spectrum confirmed that only one proton, 2-H (cis to 15b-H), showed a NOE with 15b-H within the [4.2.1] bicyclic system (Figure 3). Considering the results from the molecular modeling and the NOE experiments, we concluded that the evidence strongly supports the *S* configuration at position 15b.

EXPERIMENTAL

Melting points were determined in open-end capillary tubes on a Mel-Temp apparatus and are uncorrected. Proton NMR and NOE were recorded on Varian Gemini 300 spectrometer

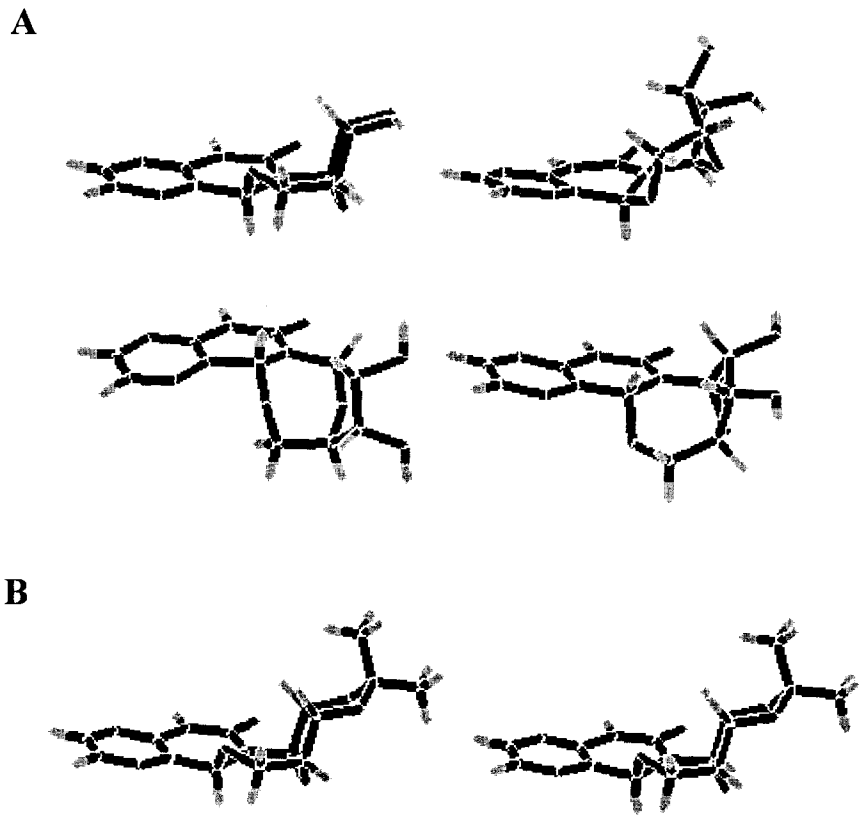


FIGURE 1. A. Energy minimized structures of **8** (without the A and B rings). Top: 15b(S) / E-ring chair (left); 15b(S) / E-ring boat (right). Bottom: 15b(R) / E-ring chair (left); 15b(R) / E-ring boat (right).
B. Stereoview of the energy minimized form of 15b(S) / E-ring chair of ketal **12** (without the A and B rings).

Table 1. Energy difference compared to the isomer 15b(S) / E-Ring chair.

15b	E-Ring Conformation	ΔE (Kcal / mol)
S	chair	-
S	boat	3.0
R	chair	7.0
R	boat	4.4

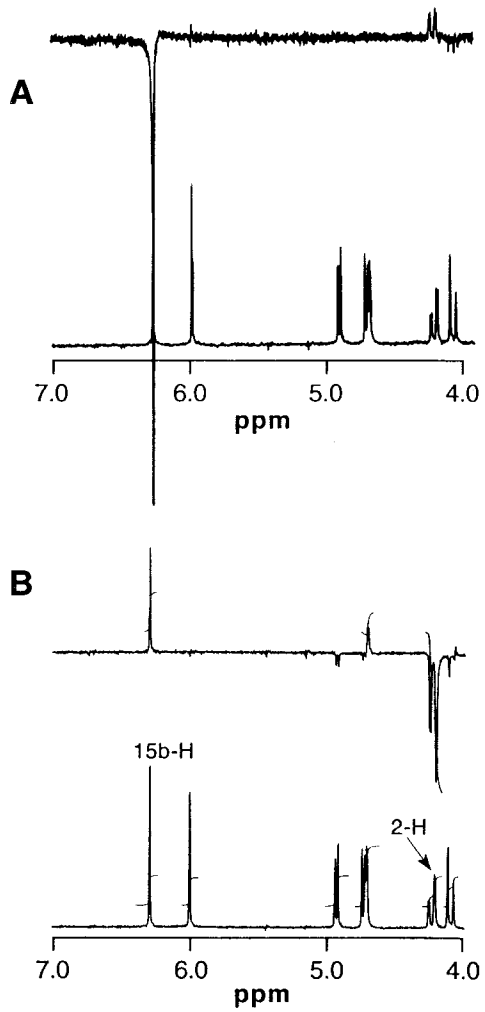
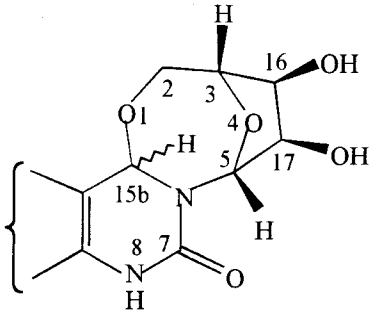


Figure 2. NOE spectra of **12**. **A.** Irradiation of 15b-H. **B.** Irradiation of 2-H.

and chemical shifts are reported in ppm relative to tetramethylsilane. TLC analyses were performed on Uniplate GHLF silica gel (Analtech). Solvents and reagents were purchased from Aldrich, Fluka and Sigma. Chromatographic separations were performed by flash column chromatography on silica gel. Chromatographic spots on TLC were visualized by UV. Elemental analyses were carried out by Atlantic Microlab, Inc.

Table 2. Distances from 15b-H to other protons in the four possible isomers of the [4.2.1.] bicyclic system.[a]



Isomer	*cis*-2H	*trans*-2H	3H	5H	16H	17H
S-chair	**2.4**	3.6	3.9	3.2	4.1	4.7
S-boat	4.0	3.6	4.7	3.3	4.9	4.8
R-chair	4.1	3.7	5.0	3.3	4.0	**2.4**
R-boat	**2.6**	3.6	4.0	3.8	**2.4**	**2.4**

[a] distance in angstrom.

5-Bromocytidine (10).

To a suspension of cytidine **9** (88.1 g, 0.36 mole) in pyridine (350 ml), a solution of bromine (27 ml, 0.52 mole) in carbon tetrachloride (200 ml) was added at room temperature. The resulting solution was allowed to stir overnight. During this period, the product partially crystallized out of the solution. The crystals were separated by vacuum filtration, washed with ice-cold water and dried to give **10** as pale yellow crystals (35.2 g). The filtrate was evaporated to remove most of the solvent. The residual pyridine was removed by co-evaporation with water. The residue was then dissolved in water, the pH was adjusted to 8 with saturated sodium carbonate solution. After 14.5 g of additional precipitated product was collected, the solution was evaporated to dryness. A resin was used to separate the rest of product from the inorganic salts. The final portion of the product (18.3 g) was purified on a Dowex-50 H^+ column eluted with 1N ammonium hydroxide. Total yield of **10**: 68 g (58 %), mp 182 - 184 °C (lit.[5] 182 - 183 °). ^{1}H NMR (DMSO-d_6): δ ppm: 3.64 (m, 2H, 5'-H), 3.85 (m, 1H, 3'-H), 3.95 (m, 2H, 2',4'-H), 5.02 (d, 1H, J = 6.5 Hz, 3'-OH), 5.25 (t, 1H, J = 6.2 Hz, 5'-OH), 5.38 (d,

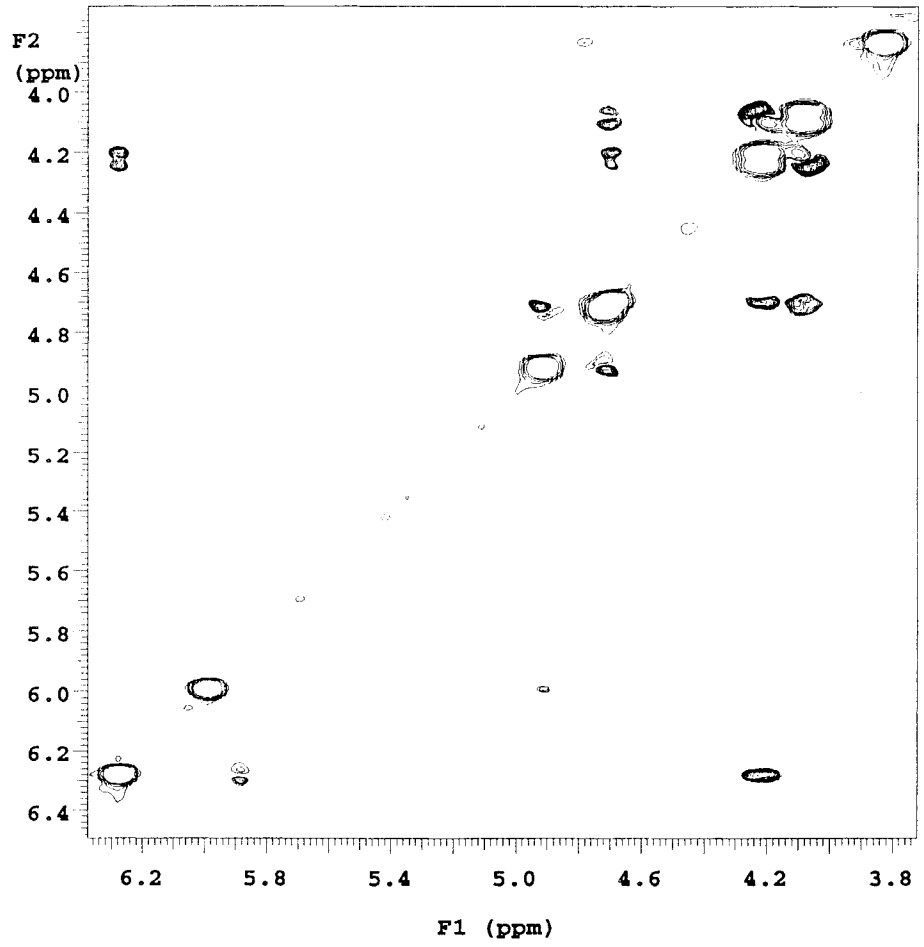


Figure 3. NOESY spectrum of **12**.

1H, J = 4.8 Hz, 2'-OH), 5.71 (d, 1H, 1'-H), 6.98 (s, 1H, NH), 7.85 (s. 1H, NH), 8.40 (s, 1H, 6-H).

Anal. Calcd. for $C_9H_{12}N_3O_5Br \cdot 1/4\ H_2O$: C, 33.10; H, 3.86; N, 12.87. Found: C, 33.26; H, 3.84; N, 12.66.

5-Aminocytidine (1).

5-Bromocytidine (**10**, 46.5 g, 0.144 mole) was placed in a 100-ml steel bomb which was pre-cooled with dry ice, then liquid ammonia (70 ml) was added. The bomb was tightly sealed and allowed gradually to warm up to 65 °C (oil bath temperature) and stirred for four days. The

excess ammonia was then evaporated in the hood. A minimum amount of water was added to give a deep brown solution. The product partially precipitated on standing as a gray solid, which was recrystallized from aqueous ethanol to afford **1** as pale yellow solid 14.9 g (40 %), mp 220-222 °C (lit.[3] 220 °C). ^{1}H NMR (DMSO-d_6) δ ppm: 3.55 ~ 3.64 (m, 2H, 5'-H), 3.75 (m, 1H, 3'-H), 3.85 ~ 3.95 (m, 2H, 2',4'-H), 4.95 (m, 2H, 3',5'-OH), 5.20 (d, 1H, J = 4.6 Hz, 2'-OH), 5.78 (d, 1H, J = 3.6 Hz, 1'-H), 6.65 (br s, 1H, NH), 7.01 (s, 1H, 6-H), 7.30 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 62.34 (C5'), 70.97 (C2'), 75.29 (C3'), 85.42 (C4'), 91.07 (C1'), 117.22 (C5), 127.86 (C6), 157.51 (C2), 163.27 (C4).

8*H*,10*H*-3(*R*),5(*R*),15b(*S*),16(*R*),17*(R)*-10-Methyl-16,17-dihydroxy-3,5-ethano-2,3,6,7-tetrahydro-1,5,3-dioxazepino[3,2-*c*]indolo[3,2-*g*]pteridine-7-one (8).

To a solution of 5-aminocytidine **1** (100 mg, 0.38 mmole) in 70 % ethanol (1.5 ml) at 90 °C, *N*-methylisatin (65 mg, 0.38 mmole) was added to give a red solution which was stirred under argon overnight. The mixture was cooled to room temperature and the solid formed was separated, washed with water and ethanol to afford **11** as a pale yellow solid (125 mg, 80%), mp 220 - 221 °C (dec.). ^{1}H NMR (DMSO-d_6) δ ppm: 3.84 (s, 3H, 10-N-CH_3), 3.95 (s, 2H, 2-H), 4.17 (m, 1H, 16-H), 4.25 (m, 1H, 17-H), 4.35 (s, 1H, 3-H), 5.05 (d, 1H, J = 6.5 Hz, 16-OH), 5.26 (d, 1H, J = 5.4 Hz, 17-OH), 5.53 (s, 1H, 5-H), 6.16 (s, 1H, 15b-H), 7.34 (t, 1H, J = 7.6 Hz, 13-H), 7.59 (t, 1H, J = 7.3 Hz, 12-H), 7.70 (d, 1H, J = 7.8 Hz, 11-H), 8.12 (d, 1H, J = 7.6 Hz, 14-H), 10.74 (s, 1H, 8-NH).

Anal. Calcd. for $C_{18}H_{17}N_5O_5 \cdot H_2O$: C, 53.86; H, 4.77; N, 17.45. Found: C, 53.83; H, 4.69; N, 17.47.

8*H*,10*H*-3(*R*),5(*R*),15b(*S*),16(*R*),17*(R)*-10-Methyl-16,17-*O*-isopropylidene-3,5-ethano-2,3,6,7-tetrahydro-1,5,3-dioxazepino[3,2-*c*]indolo[3,2-*g*]pteridine-7-one (12).

To a solution of compound **8** (100 mg, 0.26 mmole) in DMF, 5 ml 2,2-dimethoxypropane was added, followd by p-toluenesulfonic acid monohydrate (5 mg). The resulting yellow solution was stirred at room temperature for 4 hours. The solution was slowly evaporated to a small volume (2 ml). Pale yellow crystals formed and were separated. Column chromatography on silica gel (5 % methanol in dichloromethane) efforded **12** as light yellow crystals (100 mg, 90 %), mp 230 °C (dec.). ^{1}H NMR ($CDCl_3$) δ ppm: 1.35 (s, 3H, -CH_3), 1.56 (s, 3H, -CH_3), 3.85 (s, 3H, N-CH_3), 4.06 (d, 1H, J = 13.0 Hz, 2-H), 4.22 (dd, 1H, J = 2.3 and 13 Hz, 2-H), 4.68 (d, 1H, J = 2.3 Hz, 3-H), 4.70 (d, 1H, J = 6.1 Hz, 16-H), 4.90 (d, 1H, J = 6.1Hz, 17-H), 5.97 (s, 1H, 5-H), 6.28 (s, 1H, 15b-H), 7.34 (t, 1H, J = 7.6 Hz, 13-H), 7.42 (d, 1H, J = 8.1 Hz,

11-H), 7.57 (t, 1H, J = 7.9 Hz, 12-H), 8.25 (d, 1H, J = 8.1Hz, 14-H), 7.38 (s, 1H, 8-NH). Anal. Calcd. for $C_{21}H_{21}N_5O_5 \cdot 0.5H_2O$: C,58.33; H,5.13; N,16.20. Found: C,58.14; H,5.18; N,16.14.

Molecular modeling and NOE experiments. Molecular modeling was performed using Alchemy III V2.0 (Tripos Associates, Inc.). Standard parameters and force fields were applied. For energy minimization, the maximun iterations were 32000 and the gradient cutoff was 0.0100; PI and charge calculations were included.

For NOE experiments, a 5 mg sample was dissolved in $CDCl_3$ (0.6 ml) and degassed. NOESY spectra were collected using the following parameters: mixing times = 100 ms and 300 ms; pw90 = 26 μs; np = 1024; sw = 2466.3; ni = 350; gf = 0.096; fn = 2048; gf1 = 0.06; and fn1 = 1024.

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