Synthesis and Anti-Trypanosomal Activity of Various 8-Aza-7-deaza-5′-noraristeromycin Derivatives

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A recent observation that (+)-7-deaza-5′-noraristeromycin (**1**), as an L-like analogue of aristeromycin, possessed meaningful anti-trypanosomal properties has prompted a search of other 7-deazapurines with similar or improved anti-trypanosomal responses. In that direction a series of pyrazolo[3,4-*d*]pyrimidines (that is, 8-aza-7-deaza-5′-noraristeromycin derivatives, **2**−**11**) related to **1** have been prepared. These derivatives were evaluated against bloodstream forms of *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense* grown *in vitro*. Of these compounds, the parent L-like derivative **2** was less potent (IC_{50} 40−70 μM) than **1** (IC_{50} 0.165−5.3 μM) whereas the D-like analogue **3** was inactive, which is the same trend observed previously with 7-deaza-5′-noraristeromycin. Interestingly, some moderate activity (IC_{50} 12.2−16.8 μM) was seen in the D-like 4′-methyl derivative **7** while its L-like partner was inactive.

As part of our ongoing efforts into the investigation of the biological properties of 5′-nor carbocyclic nucleosides,[1] we recently reported[2] that (+)-7-deaza-5′-noraristeromycin (**1**, Chart 1) possessed significant anti-trypanosomal properties. In view of the undesirable side effects associated with currently used drugs to treat African trypanosomiasis and the development of drug resistance,[3] this observation stimulated further scrutiny of other 7-deazapurine analogs of **1** with sights set on uncovering additional anti-trypanosomal agents. In that direction, the pyrazolo[3,4-*d*]pyrimidine (8-aza-7-deazapurine) derivative (**2**) was established as a target compound. To extend the analogues of this series further, compounds with variations at C-4′ (**4**, **6**, and **8**) and C-2′/C-3′ (**10**) have also been studied. Since it was also desirable to determine if the anti-trypanosomal properties of **1** were unique for the L-like configuration in the 8-aza-7-deaza series, the D-like compounds (**3**, **5**, **7**, **9**, and **11**) were evaluated as well.

At the conception of this project, expectations that an analysis of the 8-aza-7-deazapurines (**2**−**11**) would result in new anti-trypanosomal candidates were high since similar properties were inherent to allopurinol riboside.[4]

Chemistry

The plan to the parent compounds **2** and **3** was to follow a procedure used in preparing **1**[2] and its enantiomer[5] by reacting 4-chloropyrazolo[3,4-*d*]pyrimidine[6] (**12**) with the synthetically nontrivial allylic acetates[7] **13** and **14** in the presence of a palladium catalyst. However, due to the instability of **12**, the 4-methoxy derivative **15**[6] was used in the palladium coupling reaction with **13** and **14**. The products from this reaction (**16** and **17**) were treated with osmium tetroxide followed by ammonia to provide **2** and **3**.

The coupling reactions between **13** and **14** with **15** produced **16** and **17**, respectively, as the major products, with only a trace of a second material as noted in both cases by TLC. Literature precedence[8] indicated these two products were the N-1 and N-2 (N-9 and N-8, respectively, by purine numbering) substituted isomers. The major product was assigned as the desired N-1 adduct by ^{13}C NMR analysis,[9] its greater TLC mobility by analogy to the ribofuranosides,[9] and the fact that a MOPAC analysis[10] confirmed N-1 as the preferred site of alkylation. These MOPAC calculations showed not only that N-1 was the preferred site of alkylation due to its greater anionic character but also that the heat of formation for the N-1 adduct was substantially lower than for the N-2 adduct.

Target compounds **8** and **9**, which were to serve as precursors to **4** and **5**, were prepared via a Mitsunobu reaction[11] of **18**[12] and **19**[12] with **15**. The products from this latter reaction (**20** and **21**) were subjected to ammonolysis followed by removal of the ketal protecting group to yield **8** and **9**. Catalytic hydrogenation[13] of **8** and **9** gave **4** and **5**.

Coupling **22**[14] and **23**[12] in the Mitsunobu reaction with **15** yielded **24** and **25**, respectively. As before, ammonolysis and deprotection of the latter two products provided **6** and **7**. Finally, ammonolysis of **16** and **17** produced target compounds **10** and **11**.

Anti-trypanosomal Results

Table 1 details results of growth inhibition studies with compounds **2**−**11**. The data[2] for compound **1** is included as reference. Of the other analogues, compound **7** showed activity, with IC_{50} values of 12.2−16.8 μM with three test strains. The other compounds showing activity, although at reduced levels, were **2** (44−72 μM in four strains) and **4**, which was 23−37% at 100 μM inhibitory to growth in two strains. All other analogues were inactive, including the D-like enantiomer of **2** (**3**), which is similar to the enantiomeric trends observed with **1** and its D-like partner. The L-preference trend, however, is not followed with the 4′-methyl systems, where the D-like derivative **7** is more potent than the inactive L-analogue **6**.

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Chart 1

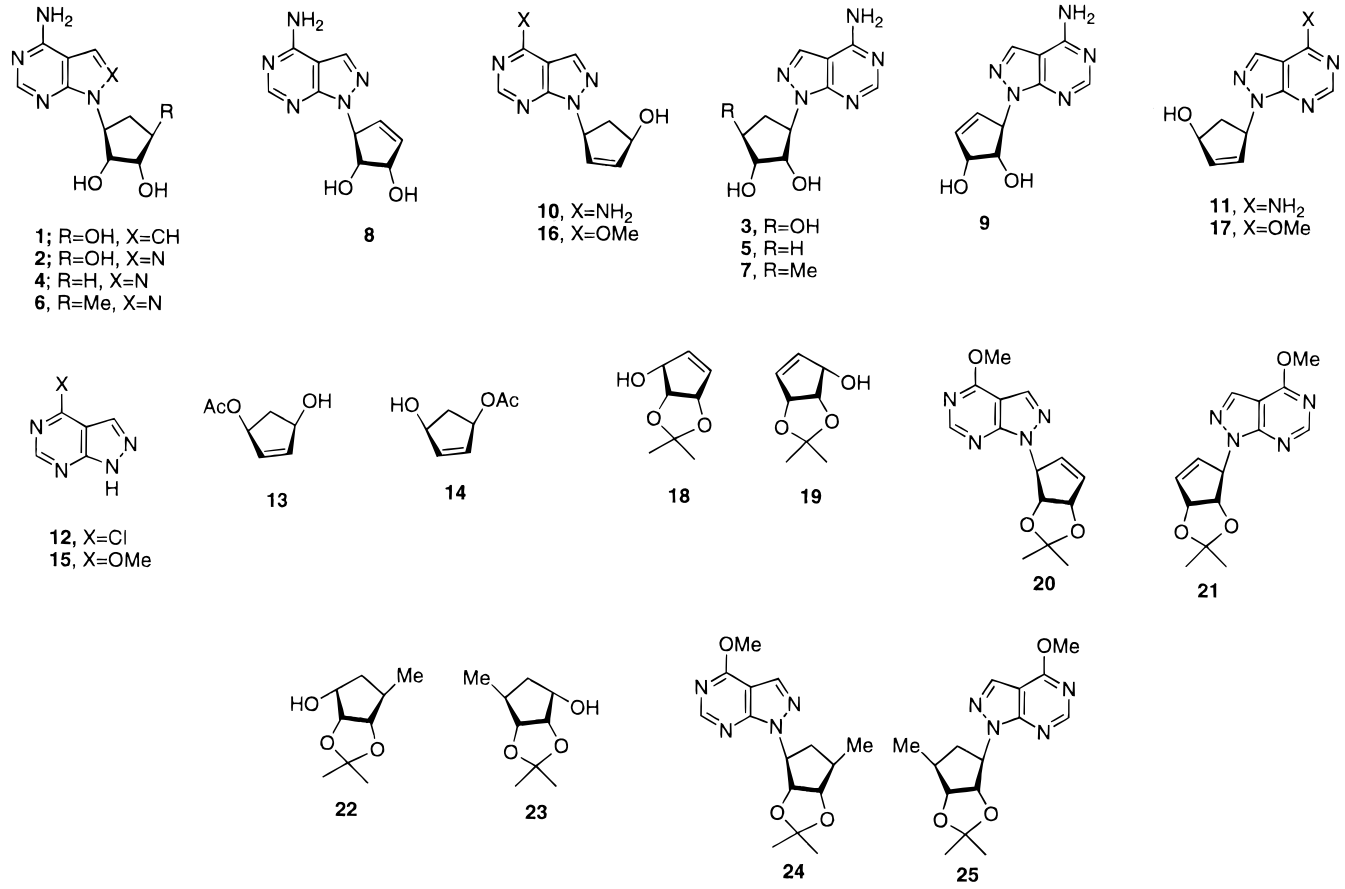

Table 1. IC_{50} Values for 8-Aza-7-deaza-5′-noraristeromycin Analogues *in Vitro* vs Bloodstream Form Trypanosomes[a]

	IC_{50} (μM)			
compound	EATRO 110	KETRI 243	KETRI 269	KETRI 243-As-10-3
1	0.85	0.165	1.55	5.3
2	53	44	67	72
3	>100	>100		
4	23%	37%		
5	>100	>100	>100	>100
6	>100	>100	>100	>100
7	12.2	16.8	14.0	
8	>100	>100		
9	>100	>100	>100	>100
10	>100	>100		
11	>100	>100	>100	>100

[a] Percent values are inhibition of growth at 100 μM; >100 indicates no inhibition of growth at 100 μM.

In addition, all compounds were tested[15] for cytotoxicity against HEL, E_6SM, HeLa, and Vero cell lines with no toxicity being noted.

Experimental Section

Melting points were recorded on a Meltemp II melting point apparatus and are uncorrected. Combustion analyses were performed by M-H-W Laboratories, Phoenix, AZ. ^{1}H and ^{13}C spectra were recorded on a Bruker AC 250 spectrometer (operated at 250 and 62.5 MHz, respectively) all referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) and br (broad). Optical rotations were measured on a JASCO DIP-370 polarimeter. Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm E. Merck silica gel 60-F_{254} precoated silica gel plates with visualization by irradiation with a Mineralight UVGL-25 lamp or exposure to iodine vapor. Column chromatography was performed on Aldrich silica gel (average particle size 5−25 μm, 60 Å) and elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

(1′*R*,4′*S*)-4′-(4-Methoxypyrazolo[3,4-*d*]pyrimidin-1-yl)-cyclopent-2′-en-1′-ol (16). To a solution of 4-methoxypyrazolo[3,4-*d*]pyrimidine[6] (**15**) (1.95 g, 12.99 mmol) in dry DMSO (30 mL) was added NaH (0.33 g, 12.99 mmol). The mixture was stirred at room temperature under an argon atmosphere for 30 min. Tetrakis(triphenylphosphine)palladium (1.0 g, 0.87 mmol), Ph_3P (0.50 g, 1.91 mmol), and a solution of **13**[7] (2.0 g, 14.08 mmol) in dry THF (30 mL) was added.[16] The mixture was stirred at 55 °C for 2 days. The volatiles evaporated under reduced pressure, and the residue was slurried in CH_2Cl_2 and filtered. The filtrate was washed with brine and evaporated. The resultant brown residue was purified via column chromatography eluting with hexane/EtOAc (8:1, followed by 5:1, then 3:1). Fractions containing product were combined and evaporated. The resultant solid was recrystallized from EtOAc to afford 0.98 g (30%) of **16** as white crystals: mp 119−120 °C; ^{1}H NMR (DMSO-d_6) δ 1.90−1.98 (p, 1H), 2.80−2.91 (dt, 1H), 4.11 (s, 3H), 4.76 (br, 1H), 5.19−5.21 (d, 1H), 5.75−5.81 (t, 1H), 5.87−5.91 (dt, 1H), 6.06−6.10 (dt, 1H), 8.26 (s, 1H), 8.60−8.61 (d, 1H); ^{13}C NMR (DMSO-d_6) δ 40.44, 54.12, 59.98, 73.49, 102.01, 130.71, 131.07, 138.64, 153.72, 154.88, 163.30. Anal. ($C_{11}H_{12}N_4O_2$·0.5MeOH) C, H, N.

(1′*R*,4′*S*)-4′-(4-Aminopyrazolo[3,4-*d*]pyrimidin-1-yl)cyclopent-2′-en-1′-ol ((−)-10). A solution of **16** (0.95 g, 4.09 mmol) in saturated methanolic NH_3 (50 mL) was sealed in a steel vessel and heated at 120 °C for 2 days. The vessel was cooled to 0 °C and the solvent removed. The residue was then purified via column chromatography, eluting with EtOAc/hexane (5:1). Fractions containing product were combined and evaporated. The resultant solid was recrystallized with EtOAc to afford 0.85 g (79%) of **10** as a white crystalline solid: mp 167−168 °C; $[\alpha]^{24}_D$ −14.48° (*c* 1.12, MeOH); ^{1}H NMR (DMSO-

d_6) δ 1.86−1.96 (m, 1H), 2.73−2.85 (dt, 1H), 4.68−4.75 (q, 1H), 5.25−5.28 (d, 1H), 5.61−5.68 (t, 1H), 5.83−5.85 (d, 1H), 6.04−6.07 (dt, 1H), 7.68 (br s, 2H), 8.09 (s, 1H), 8.17 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 40.12, 59.59, 73.56, 100.17, 131.09, 131.86, 138.23, 152.41, 155.53, 158.00. Anal. ($C_{10}H_{11}N_5O$) C, H, N.

(1′*R*,2′*S*,3′*R*,4′*S*)-4′-(4-Aminopyrazolo[3,4-*d*]pyrimidin-1-yl)-cyclopentane-1′,2′,3′-triol ((+)-2). To a solution of **10** (0.50 g, 2.30 mmol) in THF/H_2O (60 mL, 10:1) were added OsO_4 (0.015 g) and NMO (1 mL).[17] The mixture was stirred at room temperature for 6 h until TLC (CH_2Cl_2/MeOH, 3:1) showed no remaining starting material. The solvent was evaporated, and the residue was purified via column chromatography, eluting first with CH_2Cl_2/MeOH (9:1, followed by 5:1, then 3:1). Fractions containing product were combined and evaporated. The resultant solid was recrystallized twice from MeOH to afford 0.26 g (45%) of **2** as white crystals: mp 236−237 °C; $[\alpha]^{24}_D$ +24.62° (*c* 1.15, DMF) ^{1}H NMR (DMSO-d_6) δ 1.71−1.82 (dq, 1H), 2.37−2.49 (m, 1H), 3.75 (br s, 1H), 3.89 (br s, 1H), 4.39−4.45 (q, 1H), 4.76 (br s, 1H), 4.92−4.98 (q, 1H), 5.05 (br s, 1H), 7.66 (br s, 2H), 8.11 (s, 1H), 8.14 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 35.82, 59.72, 73.28, 74.61, 76.86, 100.20, 131.77, 153.22, 155.45, 157.95. Anal. ($C_{10}H_{13}N_5O_3$) C, H, N.

(1′*S*,4′*R*)-4′-(4-Methoxypyrazolo[3,4-*d*]pyrimidin-1-yl)-cyclopent-2′-en-1′-ol (17). Employing the same procedure used to obtain **16**, compound **15**[6] (2.16 g, 14.41 mmol) and **14**[7] (2.22 g, 15.62 mmol) were coupled to afford 1.41 g (39%) of **17** as white crystals: mp 135.5−136 °C; ^{1}H NMR (DMSO-d_6) δ 1.88−1.98 (p, 1H), 2.80−2.91 (dt, 1H), 4.11 (s, 3H), 4.74−4.77 (q, 1H), 5.20−5.23 (d, 1H), 5.76−5.81 (t, 1H), 5.88−5.90 (dd, 1H), 6.07−6.10 (dd, 1H), 8.26 (s, 1H), 8.60−8.61 (d, 1H); ^{13}C NMR (DMSO-d_6) δ 40.50, 54.17, 60.05, 73.56, 102.07, 130.76, 131.12, 138.71, 153.79, 154.93, 163.36. Anal. ($C_{11}H_{12}N_4O_2$·0.5MeOH) C, H, N.

(1′*S*,4′R)-4′-(4-Aminopyrazolo[3,4-*d*]pyrimidin-1-yl)cyclopent-2′-en-1′-ol ((+)-11). Following the same ammonolysis conditions used to obtain **10**, compound **17** (0.4 g, 1.72 mmol) afforded 0.26 g (69.5%) of **11** as white crystals: mp 140−141 °C; $[\alpha]^{24}_D$ +168.14° (*c* 1.12, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.86−1.96 (p, 1H), 2.74−2.85 (dt, 1H), 4.70−4.73 (q, 1H), 5.28−5.31 (d, 1H), 5.63−5.68 (t, 1H), 5.84−5.86 (d, 1H), 6.04−6.08 (dt, 1H), 7.71 (br s, 2H), 8.10 (s, 1H), 8.17 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 40.33, 59.59, 73.61, 100.21, 131.16, 131.93, 138.30, 152.45, 155.59, 158.03. Anal. ($C_{10}H_{11}N_5O$) C, H, N.

(1′*S*,2′*R*,3′*S*,4′*R*)-4′-(4-Aminopyrazolo[3,4-*d*]pyrimidin-1-yl)cyclopentane-1′,2′,3′-triol ((−)-3). Using the procedure analogous to that for **2**, compound **11** (0.36g, 1.66 mmol) in THF/H_2O (10:1) was treated with 0.01 g of OsO_4 and 1 mL of NMO[17] to provide 0.14 g (33%) of **3** as white crystals, following recrystallization from EtOAc: mp 226.5−227 °C; $[\alpha]^{24}_D$ −39.90° (*c* 0.91, DMF); ^{1}H NMR (DMSO-d_6) δ 1.70−1.81 (dq, 1H), 2.37−2.46 (m, 1H), 3.72−3.76 (q, 1H), 3.85−3.91 (m, 1H), 4.37−4.43 (q, 1H), 4.77 (s, 1H), 4.79−4.80 (d, 1H), 4.92−4.80 (q, 1H), 5.05−5.07 (d, 1H), 7.67 (br s, 2H), 8.11 (s, 1H), 8.14 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 35.77, 59.67, 73.24, 74.51, 76.81, 100.16, 131.71, 153.17, 155.41, 157.92. Anal. ($C_{10}H_{13}N_5O_3$) C, H, N.

(1′*R*,2′*S*,3′*R*)-1-[2′,3′-(Isopropylidenedioxy)cyclopent-4′-en-1′-yl]-4-methoxypyrazolo[3,4-*d*]pyrimidine (21). To a suspension of **15**[6] (2.89 g, 19.25 mmol) and Ph_3P (5.05 g, 19.25 mmol) in dry THF (100 mL) was added DEAD (3.35 g, 19.25 mmol). The mixture was stirred vigorously under an argon atmosphere at room temperature for 5 min, and a solution of (−)-2,3-(isopropylidenedioxy)-4-cyclopenten-1-ol[7] (**19**) (3.0 g, 19.21 mmol) in dry THF (25 mL) was added. The reaction mixture stirred at 55 °C for 2 days.[11] The solvent was removed and the residue was purified via column chromatography, eluting with hexane and then hexane/EtOAc (9:1 followed by 5:1). Fractions containing product were evaporated to afford 2.16 g (67%) of **21** as a colorless syrup, which crystallized upon cooling: mp 108−110 °C; ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 3H), 1.51 (s, 3H), 4.15 (s, 3H), 4.80−4.82 (d, 1H), 5.53−5.55 (d, 1H), 5.93-5.95 (dd, 1H), 6.04 (br, 1H), 6.25−6.27 (d, 1H), 8.03 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.81, 27.45, 54.22, 67.82, 83.72, 84.99, 103.04, 112.16, 131.00, 131.82, 136.58, 154.73, 155.42, 164.04. Anal. ($C_{14}H_{16}N_4O_3$) C, H, N.

(1′*R*,2′*S*,3′*R*)-1′-(4-Aminopyrazolo[3,4-*d*]pyrimidin-1-yl)cyclopent-4′-ene-2′,3′-diol ((−)-9). A solution of **21** (3.52 g, 12.21 mmol) in saturated methanolic NH_3 (100 mL) was sealed in a steel vessel and heated at 120 °C for 2 days. The vessel was cooled. The solvent was removed, and the residue was then stirred at room temperature with 1 N HCl (75 mL) until TLC (CH_2Cl_2/MeOH, 9:1) showed no remaining starting material. The mixture was concentrated and the residue loaded onto a Dowex 50 × 8 resin column. The product was eluted with 50% concentrated NH_4OH solution. Fractions containing product were combined and evaporated. The residue was purified via column chromatography eluting with CH_2Cl_2/MeOH (9:1). Fractions containing product were combined and evaporated to afford 2.3 g (81%) of **9** as an off-white foam: mp 178.5−179 °C; $[\alpha]^{25}_D$ −245.13° (*c* 1.03, MeOH); ^{1}H NMR (DMSO-d_6) δ 4.27−4.34 (q, 1H), 4.54 (br s, 1H), 4.75 (s, 1H), 4.91 (br, 2H), 5.64−5.66 (d, 1H), 5.83−5.86 (dd, 1H), 6.06−6.10 (dt, 1H), 7.73 (br, 2H), 8.12 (s, 1H), 8.17 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 66.33, 72.59, 76.64, 100.12, 132.09, 133.01, 135.32, 153.35, 155.60, 157.90. Anal. ($C_{12}H_{16}N_4O_2$·0.5MeOH) C, H, N.

(1′*S*,2′*R*,3′*S*)-1-[2′,3′-(Isopropylidenedioxy)cyclopent-4′-en-1′-yl]-4-methoxypyrazolo[3,4-*d*]pyrimidine (20). Pursuing the same procedure used to obtain **21**, compound **15**[6] (1.83 g, 12.2 mmol) was coupled with (+)-2,3-(isopropylidenedioxy)-4-cyclopenten-1-ol[7] (**18**) (1.90 g, 12.2 mmol) to afford 2.35 g (67%) of **20** as a colorless syrup, which crystallized upon cooling: mp 103−104 °C; ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 3H), 1.51 (s, 3H), 4.16 (s, 3H), 4.80−4.82 (d, 1H), 5.53−5.55 (d, 1H), 5.91−5.95 (dd, 1H), 6.04 (br, 1H), 6.25−6.27 (d, 1H), 8.02 (s, 1H), 8.59 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.80, 27.44, 54.21, 67.81, 83.71, 84.97, 103.04, 112.15, 130.99, 131.81, 136.57, 154.73, 155.42, 164.04. Anal. ($C_{14}H_{16}N_4O_3$) C, H, N.

(1′*S*,2′*R*,3′*S*)-1′-(4-Aminopyrazolo[3,4-*d*]pyrimidin-1-yl)-cyclopent-4′-ene-2′,3′-diol ((+)-8). Employing the same ammonolysis and deprotection steps that afforded **9**, compound **20** (2.16 g, 7.49 mmol) gave 1.10 g (63%) of **8** as an off-white solid: mp 97−98 °C; $[\alpha]^{25}_D$ +272.67° (*c* 1.02, MeOH); ^{1}H NMR (DMSO-d_6) δ 4.27−4.34 (q, 1H), 4.53 (br s, 1H), 4.90 (br, 1H), 5.63−5.65 (d, 1H), 5.83−5.87 (dd, 1H), 6.06−6.10 (dt, 1H), 7.68 (br, 2H), 8.09 (s, 1H), 8.17 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 66.30, 72.57, 75.63, 100.11, 132.00, 133.02, 135.29, 153.35, 155.61, 157.90. Anal. ($C_{10}H_{11}N_5O_2$) C, H, N.

(1′*R*,2′*S*,3′*R*)-1′-(4-Aminopyrazolo[3,4-*d*]pyrimidin-1-yl)cyclopentane-2′,3′-diol ((−)-5). A solution of **9** (1.3 g, 5.57 mmol) in MeOH (100 mL) was treated with PtO_2 (125 mg), placed under H_2 at 25 psi, and shaken overnight.[13] The mixture was filtered through a Celite pad and the solvent evaporated to yield a white solid, which was recrystallized from MeOH to afford 0.73 g (57%) of **5** as white crystals: mp 198.5−199 °C; $[\alpha]^{24}_D$ −65.194° (*c* 0.80, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.59−1.88 (m, 2H), 1.98−2.23 (m, 2H), 3.16−3.18 (d, 1H), 4.01−4.05 (p, 1H), 4.08−4.14 (q, 1H), 4.22−4.30 (qd, 1H), 4.63−4.65 (d, 1H), 4.79−4.81 (d, 1H), 4.98-5.08 (q, 1H), 7.66 (br, 2H), 8.10 (s, 1H), 8.15 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 25.87, 28.98, 60.78, 70.91, 76.85, 100.17, 131.66, 153.29, 155.45, 157.92. Anal. ($C_{11}H_{14}N_4O_2$·0.75MeOH) C, H, N.

(1′*S*,2′*R*,3′*S*)-1′-(4-Aminopyrazolo[3,4-*d*]pyrimidin-1-yl)-cyclopentane-2′, 3′-diol ((+)-4). Using the identical hydrogenation procedure that produced **5**, compound **8** (0.65 g, 2.78 mmol) afforded 0.62 g (95%) **4** as white crystals: mp 201−201.5 °C; $[\alpha]^{25}_D$ +68.194° (*c* 1.00, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.59−1.88 (m, 2H), 1.98−2.23 (m, 2H), 3.16−3.18 (d, 1H), 4.01−4.05 (p, 1H), 4.08−4.14 (q, 1H), 4.22−4.30 (qd, 1H), 4.63−4.65 (d, 1H), 4.78−4.80 (d, 1H), 4.98−5.08 (q, 1H), 7.65 (br, 2H), 8.10 (s, 1H), 8.15 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 25.90, 29.01, 60.81, 70.93, 76.88, 100.20, 131.68, 153.22, 155.48, 157.96. Anal. ($C_{11}H_{14}N_4O_2$·0.75MeOH) C, H, N.

(1′*R*,2′*S*,3′*R*,4′*S*)-1-[4′-Methyl-2′,3′-(isopropylidenedioxy)-cyclopent-1′-yl]-4-methoxypyrazolo[3,4-*d*]pyrimidine (25). To a suspension of **15**[6] (3.24 g, 21.58 mmol) and Ph_3P (5.78 g, 22.04 mmol) in dry THF (150 mL) was added DEAD (4.09 g, 22.04 mmol). The mixture was stirred vigorously for 5 min under an argon atmosphere. To this mixture was added a solution of (1*R*,2*R*,3*S*,4*R*)-4-methyl-2,3-(isopropylidenedioxy)-cyclopentan-1-ol[12] ((−)-**23**) (3.38 g, 19.63 mmol) in dry THF (100 mL), and the reaction mixture was stirred at 55 °C for 2 days.[11] The solvent was removed, and the residue was purified

via column chromatography eluting first with hexane and then with hexane/EtOAc (10:1) to yield 1.48 g (25%) of **25** as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 1.20−1.25 (d, 3H), 1.30 (s, 3H), 1.58 (s, 3H), 2.08−2.17 (t, 1H), 2.21−2.36 (m, 1H), 2.38−2.48 (dt, 1H), 4.15 (s, 3H), 4.36−4.41 (dd, 1H), 5.06−5.11 (dd, 1H), 5.26−5.35 (qd, 1H), 8.05 (s, 1H), 8.57 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 18.01, 25.11, 27.49, 38.94, 39.21, 54.16, 62.51, 84.45, 86.02, 103.03, 113.45, 131.44, 154.78, 155.21, 164.01. Anal. ($C_{15}H_{20}N_4O_3$) C, H, N.

(1′*R*,2′*S*,3′*R*,4′*S*)-4′-Methyl-1′-(4-aminopyrazolo[3,4-*d*]-pyrimidin-1-yl)cyclopentane-2′,3′-diol ((−)-7). A solution of **25** (1.45 g, 4.76 mmol) in saturated methanolic NH_3 (50 mL) was sealed in a steel vessel and heated at 120 °C for 2 days. The vessel was cooled to 0 °C, the solvents were removed, and the residue was refluxed with Dowex 50 × 8 resin beads and dilute HCl (75 mL) until TLC (CH_2Cl_2/MeOH, 9:1) showed no remaining starting material. The mixture was concentrated and loaded onto a Dowex 50 × 8 resin column. The product was eluted with concentrated NH_4OH. All product-containing fractions were combined and evaporated and the residue purified via column chromatography eluting with CH_2Cl_2/MeOH (12:1, then 9:1). Fractions containing product were combined and evaporated. The resultant white solid was recrystallized from MeOH to afford 0.36 g (30%) of **7** as white crystals: mp 178−179 °C; $[\alpha]^{24}_D$ −31.64° (*c* 1.29, MeOH) ^{1}H NMR (DMSO-d_6) δ 1.07−1.10 (d, 3H), 1.42−1.55 (dt, 1H), 1.93−2.04 (m, 1H), 2.19−2.26 (dt, 1H), 3.16−3.18 (d, 1H), 3.59−3.66 (q, 1H), 4.11−4.20 (m, 2H), 4.64−4.66 (d, 1H), 4.78−4.80 (d, 2H), 4.92−5.02 (dt, 1H), 7.66 (br s, 2H), 8.11 (s, 1H), 8.16 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 18.61, 34.50, 36.98, 61.19, 75.32, 76.94, 100.06, 131.78, 152.98, 155.52, 157.92. Anal. ($C_{12}H_{16}N_4O_2$·0.5MeOH) C, H, N.

(1′*S*,2′*R*,3′*S*,4′*R*)-1-[4′-Methyl-2′,3′-(isopropylidenedioxy)-cyclopent-1′-yl]-4-methoxypyrazolo[3,4-*d*]pyrimidine (24). Employing the same Mitsunobu[10] procedure used to produce **25**, compound **15**[6] and (1*S*,2*S*,3*R*,4*S*)-4-methyl-2,3-(isopropylidenedioxy)cyclopentan-1-ol[7] ((+)-**22**) (2.72 g, 15.91 mmol) were coupled to give 1.17 g (24%) of **24** as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 1.19−1.22 (d, 3H), 1.30 (s, 3H), 1.58 (s, 3H), 2.04−2.22 (p, 1H), 2.24−2.38 (m, 1H), 2.41−2.48 (m, 1H), 4.15 (s, 3H), 4.36−4.41 (dd, 1H), 5.06−5.11 (dd, 1H), 5.26−5.35 (qd, 1H), 8.06 (s, 1H), 8.57 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 18.02, 25.12, 27.50, 38.95, 39.21, 54.17, 62.52, 84.47, 86.03, 103.03, 113.44, 131.44, 154.78, 155.21, 164.00. Anal. ($C_{15}H_{20}N_4O_3$) C, H, N.

(1′*S*,2′*R*,3′*S*,4′*R*)-4′-Methyl-1′-(4-aminopyrazolo[3,4-*d*]-pyrimidin-1-yl)cyclopentane-2′,3′-diol ((+)-6). As in the preparation of (−)-**7**, ammonolysis followed by deprotection of the ketal saw compound **24** (1.17 g, 3.84 mmol) give 0.65 g (68%) of **6** as white crystals: mp 102−102.5 °C; $[\alpha]^{24}_D$ +47.166° (*c* 0.35, DMF); ^{1}H NMR (DMSO-d_6/D_2O) δ 1.08−1.11 (d, 3H), 1.43−1.55 (dt, 1H), 2.00−2.04 (m, 1H), 2.21−2.30 (dt, 1H), 3.63−3.67 (t, 1H), 4.16−4.21 (t, 1H), 4.93−5.03 (q, 1H), 8.16 (s, 1H), 8.20 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 18.60, 34.50, 36.96, 61.20, 75.32, 76.94, 100.06, 132.17, 152.93, 155.36, 157.82. Anal. ($C_{12}H_{16}N_4O_2$·MeOH) C, H, N.

Anti-Trypanosomal. Trypanosomes were grown in modified IMDM+ 20% horse serum in 24-well microplates at 37 °C in 5% CO_2, 95% air.[18] The wells were inoculated with 1 × 10^5 trypanosomes, and test compounds were solubilized in medium, with half of the volume of each well changed daily. Cell counts (Coulter Counter) were made at 24 and 48 h. Control cells grew to 5 × 10^6/mL. IC_{50} values were determined from semi-log plots. Strains used were *Trypanosoma brucei brucei* LAB 110 EATRO, a laboratory-passaged strain, and *Trypanosoma brucei rhodesiense* strains KETRI 243 and 269, clinical isolates showing resistance to arsenical drugs and/or diamidines.[19] Also used was KETRI 243-As-10−3, which is a clone of KETRI 243 highly resistant to pentamidine and melarsen oxide.

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Supporting Information Available: MOPAC data used to confirm N-1 as the preferred site of alkylation in the pyrazolo[3,4-*d*]pyrimidines studied in this manuscript (1 page). Ordering information is given on any current masthead page.

References

(1) See, for example: (a) Siddiqi, S. M.; Chen, X.; Schneller, S. W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. Antiviral Enantiomeric Preference for 5′-Norаristeromycin. *J. Med. Chem.* **1994**, *37*, 551−554. (b) Siddiqi, S. M.; Chen, X.; Schneller, S. W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. An Epimer of 5′-Noraristeromycin and Its Antiviral Properties. *J. Med. Chem.* **1994**, *37*, 1382−1384.

(2) Seley, K. L.; Schneller, S. W.; Rattendi, D.; Bacchi, C. J. (+)-7-Deaza-5′-noraristeromycin as an Anti-trypanosomal Agent. *J. Med. Chem.* **1997**, *40*, 622−624.

(3) (a) *Tropical Disease Research. Progress, 1975−1994*. Twelfth Programme Report, World Health Organization, Geneva 1995, pp 115−123. (b) Bacchi, C. J.; McCann, P. P. Parasitic Protazoa and Polyamines. In *Inhibition of Polyamine Metabolism: Biological Significance and Basis for New Therapies*; McCann, P. P., Pegg, A. E., Sjoerdsma, A., Eds.; Academic Press. Orlando, 1987; pp 317−344.

(4) (a) Cottam, H. B.; Petri, C. R.; McKernan, P. A.; Goebel, R. J.; Dalley, N. K.; Davidson, R. B.; Robins, R. K.; Revankar, G. R. Synthesis and Biological Activity of Certain 3,4-Disubstituted Pyrazolo[3,4-*d*]pyrimidine Nucleosides. *J. Med. Chem.* **1984**, *27*, 1119−1127. (b) Berens, R. L.; Marr, J. J.; Brun, R. Pyrazolopyrimidine Metabolism in African Trypansomes: Metabolic Similarities to *Trypanosoma cruzi* and *Leishmania*. *Mol. Biochem. Parasitol.* **1980**, 1, 67−73.

(5) Siddiqi, S. M.; Chen, X.; Schneller, S. W. Enantiospecific Synthesis of 5′-Noraristeromycin and its 7-Deaza Derivative and a Formal Synthesis of (−)-5′-Homoaristeromycin. *Nucleosides Nucleotides* **1993**, *12*, 267−278.

(6) Robins, R. K. Potential Purine Antagonists. I. Synthesis of Some 4,6-Substituted Pyrazolo[3,4-*d*]pyrimidines. *J. Am. Chem. Soc.* **1956**, *78*, 784−790.

(7) (a) Laumen, K.; Schneider, M. Enzymatic Hydrolysis of Prochiral *Cis*-1,4-Diacyl-2-cyclopentenediols: Preparation of (1*S*,4*R*)- and (1*R*,4*S*)-4-Hydroxy-2-cyclopentenyl Derivatives, Versatile Building Blocks for Cyclopentanoid Natural Products. *Tetrahedron Lett.* **1984**, *25*, 5875−5878. (b) Laumen, K.; Reimerdes, E. H.; Schneider, M. Immobilized Porcine Liver Esterase: A Convenient Reagent for the Preparation of Chiral Building Blocks. *Tetrahedron Lett.* **1985**, *26*, 407−410.

(8) (a) Davoll, J. A.; Kerridge, K. A. The Preparation of Some 1- and 2-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidines. *J. Chem. Soc.* **1961**, 2589−2591. (b) Montgomery, J. A.; Clayton, S. J.; Fitzgibbon, W. E., Jr. The Ribonucleosides of 4-Aminopyrazolo-[3,4-*d*]pyrimidine. *J. Heterocycl. Chem.* **1964**, *1*, 215−216. (c) Revenkar, G. R.; Townsend, L. B. Pyrazolopyrimidine Nucleosides. Part 1. Synthesis of Pyrazolopyrimidine Nucleosides Related to the Nucleoside Antibiotic Tubercidin. *J. Chem. Soc.* **1971**, 2440−2442.

(9) Seela, F.; Steker, H. 168. Synthesis of 2′-Deoxyribofuranosides of 8-Aza-7-deazaguanine and Related Pyrazolo[3,4-*d*]pyrimidines. *Helv. Chem. Acta* **1986**, *69*, 1602−1613.

(10) (a) Stewart, J. J. P. MOPAC. A Semi-Emperical Molecular Orbital Program. *J. Comput.-Aided Mol. Des.* **1990**, *4*, 1−105. (b) All anions were first calculated with MM2, and that structure was used as a starting point for the MOPAC program. MOPAC calculations were on singlet state molecules using PM3 parameters. (c) See, URL http://www.forestry.auburn.edu/people/faculty/products/elder/drk2.html or Supporting Information.

(11) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1981**, 1−28.

(12) Siddiqi, S. M.; Schneller, S. W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. S-Adenosylhomocysteine Hydrolase Inhibitors as Antiviral Agents: 5′-Deoxyaristeromycin. *Nucleosides Nucleotides* **1993**, *12*, 185−198.

(13) Wolfe, M. S.; Lee, Y.; Bartlett, W. J.; Borcherding, D. R.; Borchardt, R. T. 4′-Modified Analogues of Aristeromycin and Neplanocin A: Synthesis and Inhibitory Activity towards *S*-Adenosyl-L-homocysteine Hydrolase. *J. Med. Chem.* **1992**, *35*, 1782−1791.
(14) Seley, K. L.; Schneller, S. W.; Rattendi, D.; Lane, S.; Bacchi, C. J. Synthesis and Anti-trypanosomal Activity of a Series of 7-Deaza-5′-noraristeromycin Derivatives Varied in the Cyclopentyl Ring. *Antimicrob. Agents Chemother.*, submitted.
(15) De Clercq, E.; Balzarini, J.; Torrence, P. F.; Mertes, M. P.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. Thymidylate Synthetase as a Target Enzyme for the Inhibitory Activity of 5-Substituted 2′-Deoxyuridine on Mouse Leukemia L-1210 Cell Growth. *Mol. Pharmacol.* **1981**, *19*, 321−330.
(16) (a) Deardorff, D. R.; Linde, R. G. II; Martin, A. M.; Shulman, M. J. Enantioselective Preparation of Functionalized Cyclopentanoids via a Common Chiral (π-Allyl)palladium Complex. *J. Org. Chem.* **1989**, *54*, 2759−2762. (b) Deardorff, D. R.; Shulman, M. J.; Sheppeck, J. E. II. Palladium-Assisted Route to Carbocyclic Nucleosides: A Formal Synthesis of (±)-Aristeromycin. *Tetrahedron Lett.* **1989**, *30*, 6625−6628.
(17) (a) Trost, B. M.; Kuo, G.-H.; Benneche, T. A. Transition-Metal-Controlled Synthesis of (±)-Aristeromycin and (±)-2′,3′-*diepi*-Aristeromycin. An Unusual Directive Effect in Hydroxylations. *J. Am. Chem. Soc.* **1988**, *110*, 621−622. (b) VanReheenen, V.; Kelly, R. C.; Cha, D. Y. An Improved Catalytic OsO_4 Oxidation of Olefins to *Cis*-1,2-glycols Using Tertiary Amine Oxides as the Oxidant. *Tetrahedron Lett.* **1976**, *23*, 1973−1976.
(18) Hirumi, H.; Hirumi, K. Continuous Cultivation of *Trypanosoma brucei* Bloodstream Forms in a Medium Containing a Low Concentration of Serum Protein Without Feeder Layer Cells. *J. Parasitol.* **1989**, *75*, 985−989.
(19) Bacchi, C. J.; Brun, R.; Croft, S. L.; Alicea, K.; Bühler, Y. *In Vivo* Activities of New S-Adenosylmethionine Decarboxylase Inhibitors. *Antimicrob. Agents Chemother.* **1996**, *40*, 1448−1453.

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