Notes

(+)-7-Deaza-5′-noraristeromycin as an Anti-Trypanosomal Agent

Katherine L. Seley and Stewart W. Schneller*

Department of Chemistry, Auburn University, Auburn, Alabama 36849-5312

Donna Rattendi and Cyrus J. Bacchi

Haskins Laboratories, Pace University, New York, New York 10038

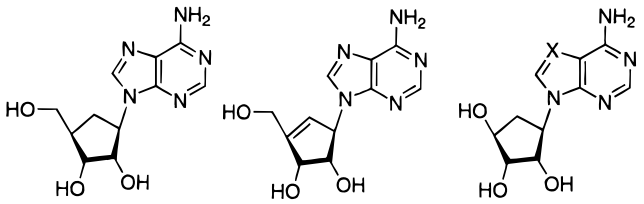
Received July 11, 1996[⊗]

The (+)-enantiomer of 7-deaza-5′-noraristeromycin (**4**) has been found to show IC_{50} values ranging from 0.16 to 5.3 μM against four strains of African trypanosomes, one *Trypanosoma brucei brucei* isolate, and several clinical isolates of *Trypanosoma brucei rhodesiense* (agent of east African sleeping sickness), including a multidrug resistant clone of one isolate. While this compound was originally designed to inhibit *S*-adenosyl-L-homocysteine hydrolase, it has been found to have no effect on this enzyme.

Many of the people living in sub-Saharan Africa confront African trypanosomiasis, or sleeping sickness, on a continual basis. This disease is caused by two subspecies of the protozoan parasite *Trypanosoma brucei* (*gambiense* and *rhodesiense*) and is transmitted by the bite of an infected tsetse fly vector.[1] Chemotherapeutic treatment of trypanosomiasis has been accomplished using suramin, pentamidine, melarsoprol, and, recently, D,L-α-difluoromethylornithine (DFMO).[2] However, the undesirable side effects associated with these drugs point to an urgent need for the development of new agents.[1,3,4]

For this purpose, the attention of the Auburn group was drawn to the spliced leader at the 5′-end of mRNA of *Trypanosoma brucei*, which begins with the customary N-7 methylguanosine.[5] This unit is linked, via a 5′−5′ triphosphate bridge, to four methylated nucleotides[5] (referred to as the cap 4-structure and composed of N^6,N^6,2′-*O*-trimethyladenosine, 2′-*O*-methyladenosine, 2′-*O*-methylcytidine, and 3,2′-*O*-dimethyluridine).[6] These four modified nucleotides arise via reactions promoted by methyltransferases that utilize *S*-adenosyl-L-methionine (AdoMet) as the methyl donor.[5,6] Inhibition of any or all of these methylations would provide an incomplete mRNA and, in turn, a mRNA not likely capable of proper functioning for trypanosomal development.

The product following methyl transfer from AdoMet is *S*-adenosyl-L-homocysteine (AdoHcy), which functions as a feedback inhibitor of many biomethylations.[7] As a result of this effect, AdoHcy offers a potentially attractive agent for use in treating trypanosomal infections. However, since administration of AdoHcy would also inhibit numerous other beneficial methylations, this is not feasible. Two possible approaches to circumvent this problem would be (i) to develop more selective analogues of AdoHcy and (ii) to consider inhibitors of the enzyme responsible for AdoHcy breakdown (that is, AdoHcy hydrolase). AdoHcy hydrolase regulates the level of AdoHcy (and, in turn, biomethylations) by affecting the conversion of AdoHcy to adenosine (Ado) and homocysteine (Hcy).[8] Thus, inhibition of this enzyme would cause an accumulation of AdoHcy and prevent the requisite methylations. Carbocyclic adenosine (aristeromycin, **1**)[9] and some of its derivatives (neplanocin A, **2**,[10] and 5′-noraristeromycin, **3**[11]) have been proven to have chemotherapeutic potential due to inhibition of AdoHcy hydrolase.[12] The patent literature[13] suggests such derivatives may be of value in antitrypanosomal drug development.



1 **2** **3**, X=N (-)-**4**, X=CH

In planning an exploration of the potential of AdoHcy hydrolase inhibitors as anti-trypanosomal drugs, we were guided by the report that replacing the N-7 of Ado in AdoHcy with a CH (hence, 7-deaza AdoHcy) led to a significant inhibitor of the hydrolase[14] and to selective inhibition of a (nucleoside-2′)methyltransferase.[15] Thus, by drawing upon the observations that the biological properties of 5′-noraristeromycin (**3**)[11] are due to its inhibition of AdoHcy hydrolase, as well as the ability of the 7-deazaadenosine ring to inhibit O-2′ methylation,[15] 7-deaza-5′-noraristeromycin emerged as a meaningful chemotherapeutic target. Further support for the 7-deaza-5′-noraristeromycin ring system as the basis for design of potentially useful agents is the report that the parent ring carbocyclic 7-deazaadenosine[16] is a good inhibitor of AdoHcy hydrolase. This paper reports the results of this plan.

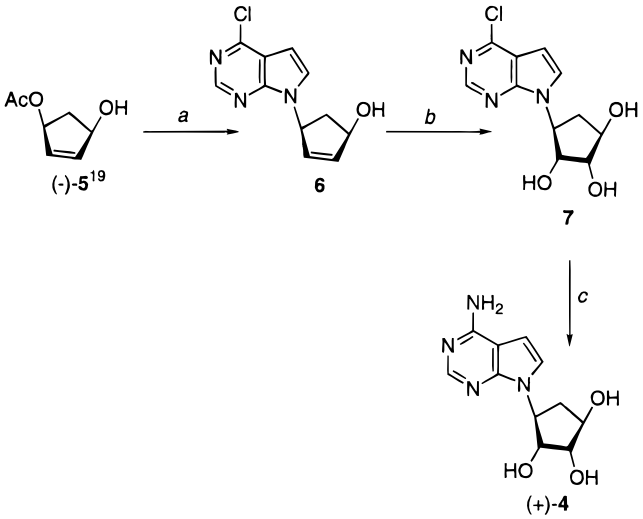
Chemistry

Due to its structural resemblance to aristeromycin whose biological activity resides in the enantiomer with the D-like configuration,[12] it was initially expected that any possible anti-trypanosomal properties for 7-deaza-

[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1997.

S0022-2623(96)00503-1 CCC: $14.00 © 1997 American Chemical Society

Scheme 1[a]



[a] Reaction conditions: (a) (i) 4-chloropyrrolo[2,3-*d*]pyrimidine[20] and NaH in DMSO and then add (−)-**5**/$(Ph_3P)_4Pd/PPh_3$ in THF at room temperature and then 55 °C; (b) OsO_4/60% aqueous 4-methylmorpholine *N*-oxide in THF; (c) NH_3 in MeOH, 120 °C, 2 days.

5′-noraristeromycin would be a property of its D-like enantiomer. However, because recent studies have shown meaningful chemotherapeutic properties for L-nucleosides,[17] both enantiomers were part of this study. Synthesis of (−)-**4** has been previously described.[18]

The preparation of (+)-**4** began with the coupling of (−)-(1*S*,4*R*)-4-hydroxy-2-cyclopenten-1-yl acetate (**5**)[19] with 4-chloropyrrolo[2,3-*d*]pyrimidine[20] in the presence of tetrakis(triphenylphosphine)palladium[18] to yield **6** (Scheme 1). Using standard vicinal glycolization conditions of osmium tetroxide and 4-methylmorpholine *N*-oxide afforded **7**, which, upon treatment with methanolic ammonia at 55 °C for 2 days, gave (+)-**4**.

Biological Results

Both (+)-**4** and (−)-**4** were evaluated *in vitro* against four *Trypanosoma brucei* test strains. Only (+)-**4** showed activity with IC_{50} values of 0.165−5.3 μM with KETRI 243 being the most sensitive and KETRI 243-As 103 least effected, indicating potential differences in uptake and/or target vulnerability. The IC_{50} values of (+)-**4** were within the range observed for other active nucleoside analogues.[21]

Compound (+)-**4** was then tested in a *T. b. brucei* model infection in mice. Dosing was begun 24 h post-inoculation and mice were given 50−100 mg/kg/day via ip or iv routes for 3 or 5 days. Alternately, Alza mini-osmotic pumps were used for dosing at 100 mg/kg/day for 7 days. None of these regimens produced cures, or average survival beyond 24 h of control deaths. Also, no visible signs of toxicity were observed.

Compound (+)-**4** was evaluated as an inhibitor of AdoHcy hydrolase and found to have an IC_{50} >500 μM. A similar result was reported[22] for (−)-**4**. Further mechanistic work on (+)-**4** will be the subject of future detailed biochemical studies.

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), 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.

Preparation of (1*R*,4*S*)-4-(4-Chloropyrrolo[2,3-*d*]pyrimidin-7-yl)cyclopent-2-en-1-ol (6). To a solution of 4-chloropyrrolo[2,3-*d*]pyrimidine[20] (3.9 g, 25.4 mmol) in anhydrous DMSO (50 mL) was added NaH (95%, 0.65 g, 25.4 mmol) and the mixture stirred for 30 min under an argon atmosphere. To this mixture was added tetrakis(triphenylphosphine)-palladium (1.95 g, 1.69 mmol), triphenylphosphine (0.98 g, 3.74 mmol), and a solution of **5**[19] (3.9 g, 27.44 mmol) in anhydrous THF (50 mL) and the new mixture stirred at 55 °C for 2 days. The volatiles were evaporated under reduced pressure, the residue was slurried in CH_2Cl_2, followed by filtration, and the filtrate was washed with brine (3 × 100 mL), dried ($MgSO_4$), and evaporated *in vacuo*. The residue was triturated with Et_2O, which was followed by filtration, and the filtrate was evaporated to a residue that was purified via column chromatography, eluting with hexane followed by hexane−EtOAc (1:1). Fractions containing product were combined and evaporated, and the resultant solid was recrystallized in EtOAc to afford 3.44 g (53%) of **6** as white crystals: mp 146.5−147.5 °C; ^{1}H NMR (DMSO-d_6) δ 1.57−1.67 (dt, 1H), 2.85−2.97 (dt, 1H), 4.74 (br, 1H), 5.33−5.35 (d, 1H), 5.78 (br, 1H), 5.93−5.95 (d, 1H), 6.18−6.22 (dt, 1H), 6.79−6.82 (d, 1H), 7.69−7.01 (d, 1H), 8.66 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 41.29, 57.47, 73.38, 73.48, 99.10, 116.84, 128.58, 130.96, 139.49, 150.18, 150.56. Anal. ($C_{11}H_{10}ClN_3O$) C, H, N.

Preparation of (1*R*,2*S*,3*R*,4*S*)-4-(4-Chloropyrrolo[2,3-*d*]pyrimidin-7-yl)cyclopentane-1,2,3-triol (7). To a solution of **6** (1.8 g, 7.66 mmol) and 4-methylmorpholine *N*-oxide (60%, 2.5 g, 7.68 mmol) in THF (100 mL) was added OsO_4 (100 mg), and the resultant mixture stirred at room temperature for 4 h. To this was added sodium bisulfite (2 g), and the stirring was continued for 15 min. The solvent was removed and the residue purified by column chromatography eluting with EtOAc, followed by EtOAc/acetone (9:1). Fractions containing product were combined and evaporated, and the resultant solid was recrystallized in EtOAc to afford 1.3 g (57%) of **7** as a white solid: mp 156−158 °C; ^{1}H NMR (DMSO-d_6) δ 1.60−1.70 (qd, 1H), 2.50−2.65 (qd, 1H), 3.76−3.78 (d, 1H), 3.93 (br, 1H), 4.38−4.43 (dd, 1H), 4.91 (br s, 2H), 5.02−5.12 (q, 1H), 5.21 (br s, 1H), 6.71−6.73 (d, 1H), 7.84−7.86 (d, 1H), 8.62 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 37.02, 58.49, 73.44, 76.19, 76.66, 99.01, 116.82, 128.96, 150.11, 150.44, 151.00. Anal. ($C_{11}H_{12}ClN_3O_3$) C, H, N.

Preparation of (1*R*,2*S*,3*R*,4*S*)-4-(4-Aminopyrrolo[2,3-*d*]pyrimidin-7-yl)cyclopentane-1,2,3-triol ((+)-4). A solution of **7** (0.70 g, 2.6 mmol) in 25 mL of MeOH saturated with NH_3 was heated at 120 °C for 2 days in a Parr stainless steel, sealed reaction vessel. The solvent was evaporated under reduced pressure and the residue purified by column chromatography eluting with CH_2Cl_2/MeOH (9:1) followed by CH_2Cl_2/MeOH (3:2). The fractions containing product were combined and evaporated *in vacuo*, and the resultant solid was recrystallized from MeOH/EtOAc to afford 0.31 g (48%) of (+)-**4** as a white solid: mp 252−254 °C; $[\alpha]^{24}_D$ +26.29° (*c* 0.78, DMF); ^{1}H NMR (DMSO-d_6) δ 1.63−1.66 (m, 1H), 2.51−2.62 (m, 1H), 3.77 (s, 1H), 3.90 (s, 1H), 4.39 (m, 1H), 4.83−4.88 (m, 2 OH + 1H), 5.45 (br s, 1H), 6.60−6.61 (d, 1H), 7.05 (br s, 2H), 7.24−7.25 (d, 1H), 8.04 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 37.20, 58.16, 73.62, 76.05, 76.96, 98.91, 102.68, 122.36, 149.53, 150.84, 157.23. Anal. ($C_{11}H_{14}N_4O_3$) C, H, N.

Anti-trypanosomal Assays. Trypanosomes were grown in modified IMDM+ 20% horse serum in 24-well microplates at 37 °C in 5% CO_2, 95% air.[23] 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 *T. b. brucei* LAB 110 EATRO, a laboratory-passaged strain, and *T. b. rhodesiense* strains KETRI 243 and 269, clinical isolates showing resistance to arsenical drugs and/or diamidines.[21] Also used was KETRI 243-As-103, which is a clone of KETRI 243 highly resistant to pentamidine and melarsen oxide. Using this procedure, IC_{50} values of 0.165−5.3 μM were observed.

Compound (+)-**4** was tested *in vivo* against *T. b. brucei* LAB 110 EATRO mouse model infection. Animals (groups of five) were infected with 2.5×10^5 trypanosomes, and the infection was allowed to develop for 24 h before treatment was begun. Compound (+)-**4** was dissolved in distilled H_2O and given in single daily doses of 50 or 100 mg/kg/day ip or iv for 3 or 5 days. In one experiment, (+)-**4** was administered in Alza minisosmotic pumps, which dispense drug continuously (1 μL/h) for 7 days. In this model, untreated controls die within 4 days and cures are taken as survival >30 days beyond control death. Compound (+)-**4** was found to have no effect.

Inhibition of AdoHcy Hydrolase Activity. The L929 cell AdoHcy hydrolase inhibitory activity for (+)-**4** was determined as described by Cools and co-workers.[24]

Acknowledgment. The assistance of Dr. C. K. Chu and his graduate students of the College of Pharmacy, University of Georgia, in obtaining the optical rotation data is much appreciated. This investigation received financial support from UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDK) Grant 930041 (S.W.S.) and Grant 950594 (C.J.B.). This assistance is gratefully acknowledged. We also thank Dr. Erik De Clercq, Katholieke Universiteit Leuven, for determining the effect of compound (+)-**4** on *S*-adenosyl-L-homocysteine hydrolase.

References

(1) *Tropical Disease Research. Progress, 1975−1994*; Twelfth Programme Report; World Health Organization: Geneva, 1995; pp 115−123.
(2) Bacchi, C. J.; McCann, P. P. In *Inhibition of Polyamine Metabolism: Biological Significance and Basis for New Therapies*; McCann, P. P., Pegg, A. E., Sjoerdsma, A., Eds.; Academic Press: Orlando, FL, 1987; pp 317−344.
(3) Van Nieuwenhove, S. Advances in Sleeping Sickness Therapy. *Ann. R. Soc. Med. Trop. Bel.* **1992**, *72*, Suppl. 1, 39−51.
(4) Bacchi, C. J.; Sufrin, J. R.; Nathan, H. C.; Spiess, A. J.; Hannan, T.; Garofalo, J.; Alecia, K.; Katz, L.; Yarlett, N. 5'-Alkyl-substituted Analogs of 5′-Methylthioadenosine as Trypanocides. *Antimicrob. Agents Chemother.* **1991**, *35*, 1315−1320.
(5) Ullu, E.; Tschudi, C. Trans Splicing in Trypanosomes Requires Methylation of the 5′ End of the Spliced Leader RNA. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10074−10078.
(6) Bangs, J. D.; Crain, P. F.; Hashizume, T.; McCloskey, J. A.; Boothroyd, J. C. Mass Spectrometry of mRNA Cap 4 from Trypanosomatids Reveals Two Novel Nucleosides. *J. Biol. Chem.* **1992**, *267*, 9805−9815.
(7) (a) Borchardt, R. T. *S*-Adenosyl-L-methionine-Dependent Macromolecule Methyltransferases: Potential Targets for the Design of Chemotherapeutic Agents. *J. Med. Chem.* **1980**, *23*, 347−357. (b) Ueland, P. M. Pharmacological and Biochemical Aspects of *S*-Adenosylhomocysteine and *S*-Adenosylhomocysteine Hydrolase. *Pharmacol. Rev.* **1982**, *34*, 223−253. (c) Wolfe, M. S.; Borchardt, R. T. *S*-Adenosyl-L-homocysteine Hydrolase as a Target for Antiviral Chemotherapy. *J. Med. Chem.* **1991**, *34*, 1521−1530.
(8) Palmer, J. L.; Abeles, R. H. The Mechanism of Action of *S*-Adenosylhomocysteine. *J. Biol. Chem.* **1979**, *254*, 1217−1226.
(9) Marquez, V. E.; Lim, M.-I. Carbocyclic Nucleosides. *Med. Res. Rev.* **1986**, *6*, 1−40.
(10) De Clercq, E. Antiviral and Antimetabolic Activities of Neplanocins. *Antimicrob. Agents Chemother.* **1985**, *28*, 84−89.
(11) Siddiqi, S. M.; Chen, X.; Schneller, S. W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. Antiviral Enantiomeric Preference for 5′-Noraristeromycin. *J. Med. Chem.* **1994**, *37*, 551−554.
(12) De Clercq, E. *S*-Adenosylhomocysteine Hydrolase Inhibitors as Broad-Spectrum Antiviral Agents. *Biochem. Pharmacol.* **1987**, *36*, 2567−2575.
(13) Daluge, S. M. European Patent 0 368 640, May 16, 1990.
(14) (a) Borchardt, R. T.; Huber, J. A.; Wu, Y. S. Potential Inhibitors of *S*-Adenosylmethionine-Dependent Methyltransferases. 4. Further Modifications of the Amino Acid and Base Portions of *S*-Adenosyl-L-homocysteine. *J. Med. Chem.* **1976**, *19*, 1094−1099. (b) Coward, J. K.; Motola, N. C.; Moyer, J. D. Polyamine Biosynthesis in Rat Prostrate. Substrate and Inhibitor Properties of 7-deaza Analogues of Decarboxylated *S*-Adenosylmethionine and 5′-Methylthioadenosine. *J. Med. Chem.* **1977**, *20*, 500−505. (c) Wertheimer, A. M.; Chen, S.-Y.; Borchardt, R. T.; Furuichi, Y. *S*-Adenosylmethionine and Its Analogs. Structural Features Correlated with Synthesis and Methylation of mRNAs of Cytoplasmic Polyhedrosis Virus. *J. Biol. Chem.* **1980**, *255*, 5924−5930.
(15) Pugh, C. S. G.; Borchardt, R. T. Effects of *S*-Adenosylhomocysteine Analogues on Vaccinia Viral Messenger Ribonucleic Acid Synthesis and Methylation. *Biochemistry* **1982**, *21*, 1535−1541.
(16) Secrist, J. A., III; Clayton, S. J.; Montgomery, J. A. (±)-3-(4-Amino-1*H*-pyrrolo[2,3-*d*]pyrimidin-1-yl)-5-(hydroxymethyl)-(1α,2α,3*β*,5*β*)-1,2-cyclopentane-diol, the Carbocyclic Analogue of Tubercidin. *J. Med. Chem.* **1984**, *27*, 534−536.
(17) Liang, C.; Lee, D. W.; Newton, M. G.; Chu, C. K. Synthesis of L-Dioxolane Nucleosides and Related Chemistry. *J. Org. Chem.* **1995**, *60*, 1546−1553.
(18) 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.
(19) (a) 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. (b) Laumen, K.; Schneider, M. Enzymatic Hydrolysis of Prochiral *cis*-1,4-Diacyl-2-cyclopentene-diols: 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.
(20) Davoll, J. 26. Pyrrolo[2,3-*d*]pyrimidines. *J. Chem. Soc.* **1960**, 131−138.
(21) Bacchi, C. J.; Nathan, H. C.; Livingston, T.; Valladares, G.; Saric, M.; Sayer, P. D.; Njogu, A. R.; Clarkson, A. B., Jr. Differential Susceptibility to DL-α-difluoromethylornithine in Clinical Isolates of *Trypanosoma brucei rhodesiense*. *Antimicrob. Agents Chemother.* **1990**, *34*, 1183−1188.
(22) Siddiqi, S. M.; Chen, X.; Rao, J.; Schneller, S. W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. 3-Deaza- and 7-Deaza-5′-noraristeromycin and Their Antiviral Properties. *J. Med. Chem.* **1995**, *38*, 1035−1038.
(23) 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.
(24) Cools, M.; Balzarini, J.; De Clercq, E. Mechanism of Antiviral and Cytotoxic Action of (±)-6′-*β*-fluoroaristeromycin, a Potent Inhibitor of *S*-Adenosylhomocysteine Hydrolase. *Mol. Pharmacol.* **1991**, *39*, 718−724.

JM9605039