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(E) AND (Z) 5'-FLUORO OLEFIN CARBOCYCLIC NUCLEOSIDES: EFFECT OF OLEFIN GEOMETRY ON INHIBITION OF S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE

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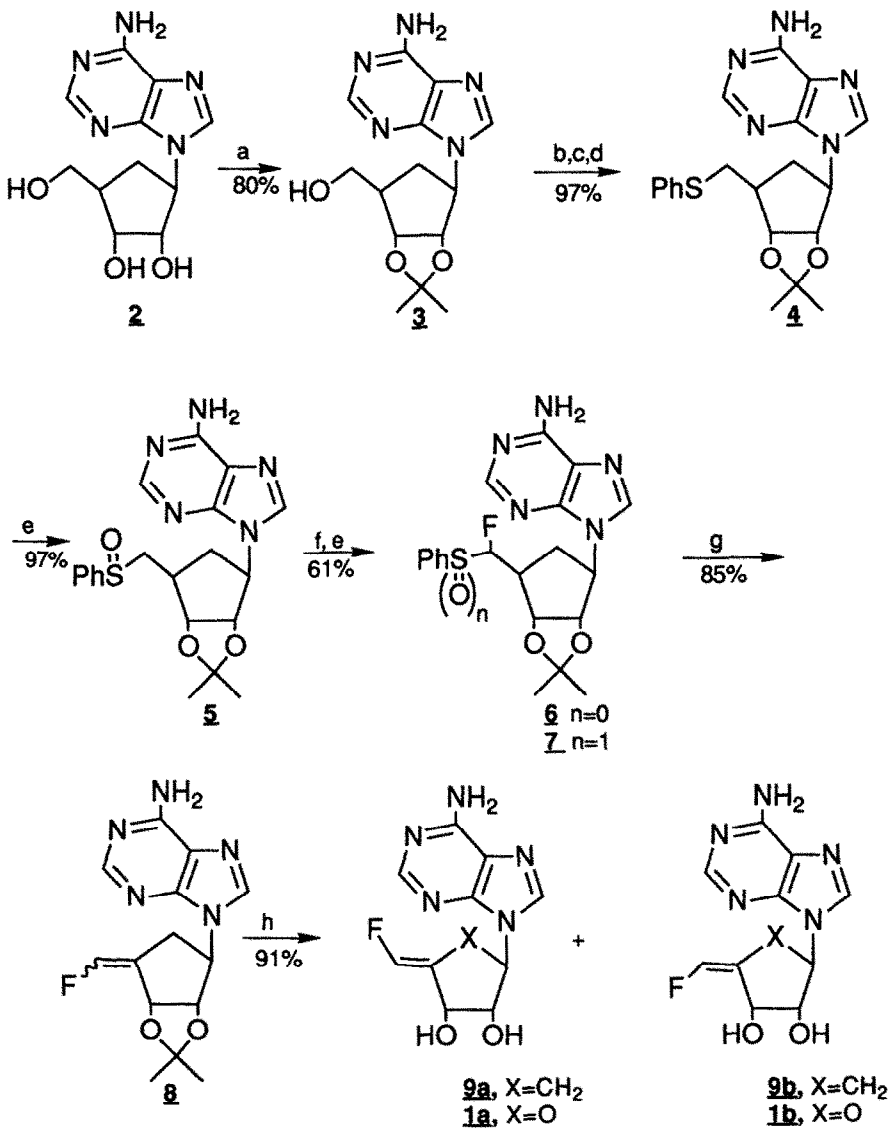
Abstract: (E) and (Z) 4',5'-Didehydro-5'-deoxy-5'-fluoroaristeromycin (**9a** and **9b**) were synthesized utilizing the fluoro-Pummerer reaction. Fluoro olefin **9a** was a time-dependent inhibitor of S-adenosyl-L-homocysteine hydrolase whereas **9b** was a competitive inhibitor. The effects of **9a** and **9b** on T cell proliferation are presented.

The design and synthesis of mechanism-based inhibitors of S-adenosyl-L-homocysteine (SAH) hydrolase (EC 3.3.1.1) have received considerable attention.[1] Recently, we reported a new class of mechanism-based inhibitors of this enzyme represented by (Z) and (E) 4',5'-didehydro-5'-deoxy-5'-fluoroadenosine (**1a** and **1b**).[2-4] The design of these compounds was based on the chemical mechanism for the catalytic process. The inhibitors demonstrated moderate antiviral activity,[5] but more importantly **1a** and **1b** were shown to be potent inhibitors of T cell proliferation and to prevent collagen-induced arthritis in mice.[6,7] Although the exact mechanism for these observed biological activities is unclear, the relative potencies of **1a** and **1b** correlated with k_{inact}/K_I values. Herein, we report the synthesis of the carbocyclic analogs (E) and (Z) 4',5'-didehydro-5'-deoxy-5'-fluoroaristeromycin (**9a** and **9b**)[8] and the surprising difference in the apparent mode of inhibition of SAH hydrolase by these fluoro olefins.

Aristeromycin[9] (**2**) was treated with acetone, triethyl orthoformate and p-toluenesulfonic acid to provide the acetonide **3** in 80% yield (mp 217-218°C from EtOAc/MeOH). The conversion of **3** to the 5'-S-phenylthio ether **4** by treatment with diphenyl disulfide and tributylphosphine, as in the synthesis of **1a** and **1b**,[4] proceeded in poor yield (5-15%) with concomitant formation of N3,5'-cyclonucleoside. Protection of the N6 amino group as the N,N-dimethylamidine by reaction of **3** with dimethylformamide dimethyl acetal followed by treatment with diphenyl disulfide and tributylphosphine in pyridine and subsequent removal of the protecting group with methanolic ammonia gave **4** (mp 167-169°C from EtOAc) in an overall yield of 97% from **3**.[10] Oxidation of **4** to the sulfoxide **5** (white foam) with meta-chloroperbenzoic acid (MCPBA) proceeded in 97% yield. Fluorine was introduced on C5' via the fluoro-Pummerer reaction,[11] using antimony trichloride as a catalyst;[12] the intermediate fluorosulfide **6** was oxidized to the fluorosulfoxide **7** (white powder) with MCPBA in 61% yield from **5**. Pyrolysis of **7** in diglyme at 120°C in the presence of Hünig's base gave a 1:1 mixture of two geometric isomers of fluoro olefin **8** (foam) as determined by ^{19}F NMR of the crude reaction mixture. Removal of the acetonide group on **8** with 99% trifluoroacetic acid and 1% water at room temperature (5 h) and separation of the geometric isomers on a Dekker column[13] (MeOH/H_2O,1/1) gave **9a** (mp 200-202°C, dec, from EtOAc) and **9b** (mp 207-208°C, dec, from acetone/EtOAc) in a combined yield of 91%. NOESY experiments established the configuration of each isomer.[10]

Scheme. Synthesis of **9a** and **9b**



a) $HC(OEt)_3$, TsOH, acetone; b) $(CH_3O)_2CN(CH_3)_2$ c) PhSSPh, $(Bu)_3P$; d) NH_3;
e)MCPBA; f) DAST, $SbCl_3$; g) $(i\text{-}Pr)_2NEt$,heat; h) TFA, Dekker resin.

SAH hydrolase from rat liver was purified and the enzyme activity was assayed in the lytic direction spectrophotometrically.[3] Pseudo-first-order loss of activity was observed with varying concentrations of **9a**. The inhibition parameters k_{inact} = 0.18 min^{-1} and K_I = 6.9 μM were estimated from progress curves as previously described.[3] However, **9b** was found to be a competitive inhibitor within the time scale of the assay (30 min) with a K_i = 1.1 μM. This result was unexpected since both **1a** and **1b** are potent time-dependent inhibitors of the enzyme. The presumed oxidation of **9a** to the 3' ketone by the enzyme during the inactivation process was also indicated by a time-dependent increase in the absorbance at 320 nm (complete within 20 min) due to the formation of the putative enzyme NADH complex. With **9b**, however, a very slow increase in absorbance at 320 nm (complete in 3 to 5 hours) was observed, suggesting that **9b** may be a very slow time-dependent inhibitor which appears to be a competitive inhibitor in the normal assay time course.[14]

A possible explanation for the apparent difference in the mode of inhibition of SAH hydrolase by **9a** and **9b** is that the conformation of **9a** in the active site of the enzyme is favorable for hydride transfer to the nicotinamide of NAD from the 3' position but is not favorable for **9b**. There may be a less significant difference in the bound conformations of the isomers **1a** and **1b**.

The time-dependent inhibitor **9a** was a potent inhibitor of T cell proliferation (IC_{50} = 0.8 μM) whereas the competitive inhibitor **9b** inhibited T cells less effectively with an IC_{50} of 6.7 μM.[15,16] Aristeromycin (**2**), a potent competitive inhibitor of the enzyme[17] was compared with **9a** in a separate study and was toxic to the murine cells.

The different modes of inhibition of SAH hydrolase by the structurally similar **9a** and **9b** have important implications in understanding the active site of the enzyme and provide a starting point for the design of new inhibitors.

References and Notes

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6. Wolos, J.A.; Frondorf, K.A.; Davis, G.G.; Jarvi, E.T.; McCarthy, J.R.; Bowlin, T.L. *J. Immunology*, in press.
7. Wolos, J.A.; Akeson, A.K.; Frondorf, K.A.; *J. Cell Biochem.* **1991**, Suppl. 15E, 187.
8. The E and Z notations for **1b** and **1a**, reverse for **9a** and **9b** because of the priority change of O4 to CH_2.
9. Aristeromycin was obtained by fermentation from Dr.Luciano Gastaldo, Marion Merrell Dow Research Institute, Gerenzano, Italy.
10. All new compounds gave satisfactory elemental analysis and/or high resolution mass spectra and other spectral data consistent with the assigned structure; **3**: ^{1}H NMR (DMSO-d_6) δ 1.23 (s, 3), 1.48 (s, 3), 2.16-2.30 (m, 3), 3.48-3.53 (m, 2), 4.55 (m, 1), 4.75-4.83 (m, 2), 5.02 (t, 1, J=6.8 Hz), 7.24 (br s, 2), 8.14 (s, 1), 8.27 (s, 1); **4**: ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 3), 1.54 (s, 3), 2.39-2.58 (m, 3), 3.04 (m, 1), 3.32 (m, 1), 4.63-4.75 (m, 2), 5.09 (m, 1), 5.88 (br s, 2), 7.17-7.41 (m, 5), 7.83 (s, 1), 8.33 (s, 1); **5**: ^{1}H NMR ($CDCl_3$) mixture of

diastereomers δ 1.27, 1.32, 1.51, 1.58 (s, 6), 2.50-2.78 (m, 2), 2.87-3.14 (m, 2), 3.34 (dd, 1, J=5.2 and 13.0 Hz), 4.49-4.65 (m, 2), 5.15 (m, 1), 5.76 (br d, 2) 7.48-7.70 (m, 5), 7.81 (s, 1), 8.30, 8.32 (s, 1); **6**: (not isolated) ^{19} F NMR ($CDCl_3$) δ -149.89 (dd, J=13.8 and 55.0 Hz) (60%), -154.62 (dd, J=17.5 and 55.5 Hz) 40%; **7**: (mixture of 4 diastereomers) ^{1}H NMR ($CDCl_3$) δ 1.26, 1.29, 1.32, 1.35, 1.51, 1.54, 1.57, 1.62 (s, 6), 2.45-3.64 (m, 3), 4.60-5.16 (m, 4), 5.62-5.70 (m, 2), 7.53-7.78 (m, 5), 7.79, 7.82, 7.84 (s, 1), 8.30, 8.31, 8.32, 8.35 (s, 1); ^{19}F NMR ($CDCl_3$) δ -183.56 (dd, J=23.7 and 48.2 Hz) (major diastereomer - 53%), -188.86 (dd, J=16.9 and 48.2 Hz) (10%), -190.96 (dd, J=30.2 and 48.8 Hz) (25%), -195.12 (dd, J=20.6 and 48.2 Hz) (12%); **8**: (E isomer) ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3), 1.55 (s, 3), 3.00-3.26 (m, 2), 4.90 (m, 1), 5.03 (m, 1), 5.14 (m, 1), 5.90 (br s, 2), 6.94 (d, 1, J=82.0 Hz), 7.69 (s, 1), 8.38 (s, 1); ^{19}F NMR ($CDCl_3$) δ -124.55 (d, J=82.4 Hz) **8**: (mixture 2/3 E/Z, only data for Z isomer shown ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 3), 1.55 (s, 3), 3.03-3.32 (m, 2), 4.91 (m, 1), 5.02 (m, 1), 5.06 (m, 1), 5.14 (d, 1, J=5.7 Hz), 5.67 (br s, 1), 6.69 (d, 1, J=81.5 Hz), 7.70 (s, 1), 8.37 (s, 1); ^{19}F NMR ($CDCl_3$) δ -125.72 (d, J = 81.8 Hz; **9a**: (E isomer) ^{1}H NMR (DMSO-d_6) δ 2.85-2.95 (m, 2), 4.30 (t, 1, J=4.0 Hz), 4.44 (ddd, 1, J = 9.7, 7.0, 4.5 Hz), 4.85 (q, 1, J=9.7 Hz), 5.10 (d, 1, J=3.5 Hz), 5.20 (d, 1, J=6.9 Hz), 7.04 (d, 1, J=84.1 Hz), 7.20 (br s, 2), 8.11 (s, 1), 8.19 (s, 1); ^{19}F NMR (DMSO-d_6) δ -128.85 (d, J=84.3 Hz); **9b**: (Z isomer); ^{1}H NMR (DMSO-d_6) δ 2.76-2.84 (m, 2), 4.43 (m, 1), 4.55 (t, 1, J=4.7 Hz), 4.79 (q, 1, J=9.7 Hz), 5.12-5.17 (m, 2), 6.82 (d, 1, J=84.8 Hz), 7.20 (br s, 2), 8.11 (s, 1), 8.19 (s, 1); ^{19}F NMR (DMSO-d_6) δ -129.35 (d, J=84.6 Hz). A 2D NOE experiment (NOESY) with **9a** (DMSO-d_6) showed a NOE correlation between the 5'-vinyl proton (δ 7.04) and the 3'-proton (δ 4.30) and none with the 6'-protons; in contrast, a NOESY with **9b** showed a NOE correlation between the vinyl proton (δ 6.82) and the 6'-protons (δ 2.76-2.84) and none with the 3'-proton.

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14. Liu et al. (S. Liu, M.S. Wolfe, C. Yuan, S.M ALi and R.T. Borchardt, manuscript submitted to this journal; personal communication by R.T. Borchardt) have found that **9a** and **9b** are approximately equipotent time-dependent inhibitors of recombinant rat liver SAH hydrolase. Theoretically, there should be no difference in the kinetics obtained by either group. Two possible explanantions for discrepancies are the differences in the assay method and the different source of the enzyme. We are currently seeking an explanation for the discrepancy between our results and those of Liu et al. The difference we report here between the inhibition constants for **9a** and **9b** appears to be corroborated by (a) a difference in the rate of absorbance increase with Enz + **9a** and Enz + **9b**, and (b) a difference in the extent of inhibition by the two compounds of T-cell proliferation.
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16. Murine splenic mononuclear cells (5 x 10^5) from 12 mice were stimulated individually in vitro with Concanavalin A (10 μg/ml), a potent T cell mitogen, in the absence or presence of **9a** or **9b**. Cells were cultured for 48 h at 37°C in 5% CO_2 in a final volume of 0.2 ml. T cell proliferation was determined by the incorporation of [^{3}H]-thymidine into the DNA of dividing cells over the last 6 h of culture, expressed as counts per minute (cpm) recorded in a beta counter.
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