(m, 1 H, H-4′), 3.55 (m, 3 H, H-5′). MS (FAB; glycerol): m/e 288 ($M^+ + H$), 112 (cytosine$^+ + H$). Anal. ($C_9H_{10}FN_3O_3$) C, H, N.

1-(3-Azido-2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)-thymine (44). *O*-2,3′-Anhydro-1-[5-*O*-(monomethoxytrityl)-2-deoxy-2-fluoro-β-D-lyxofuranosyl]thymine (**36**) (0.85 mg, 1.65 mmol) was dissolved in dry DMF (25 mL) and stirred with lithium azide (0.98 g, 20 mmol) for 62 h at 105 °C. A few crystals of potassium carbonate were added, and the mixture was partitioned between water and ethyl acetate. Unreacted starting material (0.14 g) crystallized out upon trituration with diethyl ether–methylene chloride. The mother liquor (crude **43**, 0.7 g) was dissolved in 80% acetic acid (5 mL) and stirred for 6 h at 35 °C. After removal of the volatiles in vacuo, the crude product was purified on silica gel column (50% ethyl acetate in dichloromethane) to give **44** (0.15 g, 31.9% overall). ^{1}H NMR (360 MHz; DMSO-d_6): δ 11.46 (br s, 1 H, NH), 7.60 (s, 1 H, H-6), 6.14 (dd, $J_{1',F}$ = 10.9 Hz, $J_{1',2'}$ = 5.4 Hz, 1 H, H-1′), 5.37 (dt, $J_{2',F}$ = 54.0 Hz, $J_{2',1'} = J_{2',3'}$ = 5.4 Hz, 1 H, H-2′), 5.34 (br s, 1 H, OH), 4.51 (ddd, $J_{3',F}$ = 22.4 Hz, $J_{3',4'}$ = 7.5 Hz, $J_{3',2'}$ = 5.3 Hz, 1 H, H-3′), 3.82 (m, 1 H, H-4′), 3.68 (m, 2 H, H-5′), 1.77 (br s, 3 H, CH_3). ^{13}C NMR (50.3 MHz; acetone-d_6): δ 137.02 (C-6), 110.23 (C-5), 95.11 (d, $J_{2',F}$ = 195.0 Hz, C-2′), 83.21 (d, $J_{1',F}$ = 16.8 Hz, C-1′), 81.35 (d, J = 0.4 Hz, C-4′), 64.21 (d, $J_{3',F}$ = 25.0 Hz, C-3′), 60.95 (C-5′), 12.51 (CH_3). Anal. ($C_{10}H_{12}FN_5O_4$) C, H, N.

1-[5-*O*-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]uracil (21). Method B. 1-[5-*O*-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl]uracil (**39**) (0.45 g, 0.9 mmol) was dissolved in ethanol (60 mL) and hydrogenated over 10% palladium on carbon catalyst (85 mg) for 2 h. Filtration and evaporation of the solvent in vacuo yielded the crude product **21**, which was subsequently purified on a silica gel column (15–25% ethyl acetate in dichloromethane). Yield: 300 mg (66.4%). MS (EI): m/e 502 (M^+). This compound is identical with **21** obtained through the deoxygenation method described earlier.

Antiviral Assays. Antiviral activity against HIV activity was measured as described earlier.[10] The assay for the granulocyte-macrophage CFU inhibition was also previously described.[3] The CEM/LAV assay (Table III) uses a T cell line, CEM-SS, infected with HIV-1, LAV strain. The antiviral endpoint was p24 (viral antigen) and was measured 6 days postinfection; the endpoint for cellular toxicity was inhibition of cellular DNA synthesis. The U937/LAV test used a monocytic cell line; the antiviral endpoint was again p24 viral antigen measured 6 days postinfection; the cellular toxicity endpoint was inhibition of cellular DNA synthesis. The T cell/HTLV$_{IIIB}$ assay used freshly isolated T cells, stimulated with the mitogen PHA, and subsequently infected with HIV (HTLV III$_B$ strain). The antiviral endpoint was measured with p24 viral antigen at 10 days postinfection. Cell viability was taken as a measure of the toxicity endpoint. The macrophage monocyte (Mø.MO/PDS assay used freshly isolated monocyte/macrophage cells infected with a monocytotropic strain of HIV (Pan Data Strain, PDS). The antiviral endpoint was p24 viral antigen measured three (3) weeks postinfection, the toxicity endpoint was cell viability. The PBL/HTLV$_{IIIB}$ test used freshly isolated peripheral blood lymphocytes, stimulated with PHA and infected with HTLV III$_B$ strain. The antiviral endpoint was viral RNA and p24 viral antigen; the toxicity endpoint was cell viability.

Acknowledgment. The authors would like to thank Christopher Maupin for technical assistance in the scale up of the 2′-*threo*-FddC synthesis. Dr. Alan Crosswell and Dr. Joan Fung-Tomc are acknowledged for arranging the antitumor and antibacterial tests. Finally, we wish to thank Dr. Elaine Kinney-Thomas for the initial anti-HIV data.

2-(4-Amino-4-carboxybutyl)aziridine-2-carboxylic Acid. A Potent Irreversible Inhibitor of Diaminopimelic Acid Epimerase. Spontaneous Formation from α-(Halomethyl)diaminopimelic Acids

Fritz Gerhart, William Higgins,* Chantal Tardif, and Jean-Bernard Ducep

Merrell Dow Research Institute, Strasbourg Center, 16, rue d'Ankara, 67084 Strasbourg Cedex, France.
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2-(4-Amino-4-carboxybutyl)aziridine-2-carboxylic acid (**3**) (aziridino-DAP) was identified as the product of spontaneous hydrolysis of α-(halomethyl)diaminopimelic acids (α-halomethyl-DAPs) **2a–c**. Under physiological conditions, **3** is an extremely potent irreversible inhibitor of the bacterial enzyme diaminopimelic acid epimerase (DAP-epimerase; EC 5.1.1.7). This unusual mode of action of an α-halomethyl amino acid with a *non*-pyridoxal enzyme is investigated. Synthesis and characterization of **2a–c** and **3**, kinetics of spontaneous formation of **3** from α-halomethyl-DAPs, and kinetics of enzyme inhibition by both **3** and by α-halomethyl-DAPs are reported.

The unusual amino acids D-alanine, D-glutamic acid, and D,L-diaminopimelic acid (**1**, D,L- or *meso*-DAP) are important components of bacterial cell wall.[1] Their biosynthesis is restricted to bacteria in general (and Gram negatives in particular *for most meso*-DAP incorporating bacteria[2]). Therefore, specific inhibition of the biosynthesis of these crucial cell-wall components could lead to a new generation of antibacterials.

Studies on alanine racemase (EC 5.1.1.1), which converts L-alanine to D-alanine, are numerous;[3-7] little work, however, has been reported on diaminopimelate epimerase (EC 5.1.1.7),[8] the enzyme which converts L-DAP to *meso*-DAP. As part of our program on the design of enzyme-activated irreversible inhibitors aimed at specific bacterial pathways, we prepared α-(halomethyl)diaminopimelic acids **2** (see

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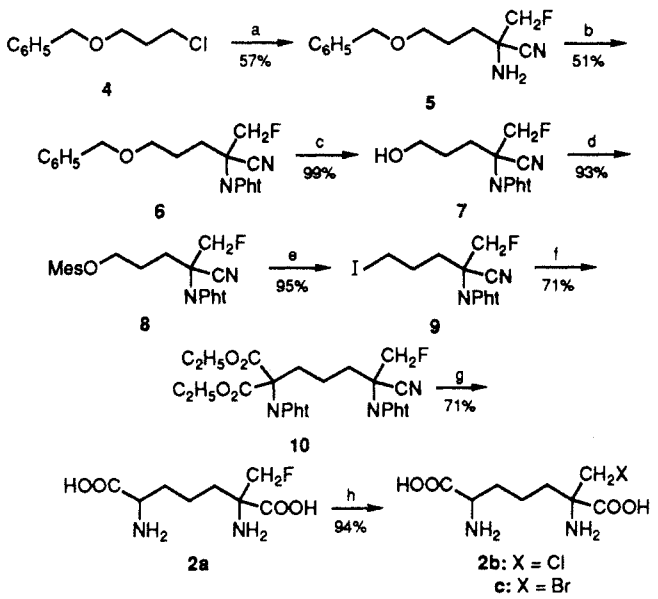
Scheme I[a]



[a] (a) Mg, ether; CH_2FCN, ether; NH_4Cl, NaCN, H_2O; (b) ClCO-C_6H_4COCl (1 equiv), Et_3N, CH_2Cl_2, room temperature overnight; (c) $(CH_3)_3SiI$ (1.1 equiv), CH_2Cl_2; (d) CH_3SO_2Cl (1 equiv), Et_3N (3.5 equiv), CH_2Cl_2, −10–0 °C; (e) NaI (2 equiv), acetone, reflux 15 h; (f) diethyl phthalimidomalonate (1 equiv), t-BuOK (1 equiv), DMF, 60 °C, 48 h; (g) concentrated HCl, reflux, 24 h; (h) NaOH, 100 °C, 2 h; HX, 0 °C.

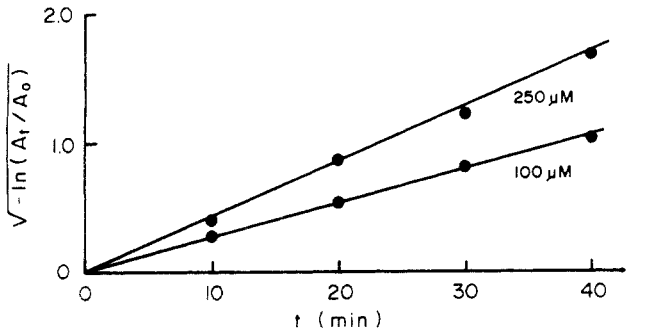


Figure 1. Secondary kinetic plot of the inhibition of DAP-epimerase by **2a**.

Scheme I) as potential inhibitor of diaminopimelate decarboxylase (EC 4.1.1.20), the enzyme which converts *meso*-DAP to lysine (a constituent of cell-wall in Gram positives). This enzyme is pyridoxal based whose mode of action is presumed to follow the general mechanism and "enzyme-based inactivation" by α-fluoromethyl analogues.[9] The enzyme from *Bacillus* was previously studied as the target of substrate analogues of DAP.[10] Although **2a** was a poor inhibitor of the target enzyme in vitro, it nevertheless was found to inhibit the growth of log phase cultures of *Escherichia coli* to a significant extent. This result was atypical of other DAP-decarboxylase inhibitors (W. Higgins, unpublished result), and interference with another enzyme in the DAP-lysine pathway, DAP-epimerase (EC 5.1.1.7), was suspected. Although this is a *non*-pyridoxal enzyme, it was believed to be more crucial in Gram-negative bacteria. Previously, the facile formation of aziridine carboxylic acids from α-halomethyl amino acids (mono-

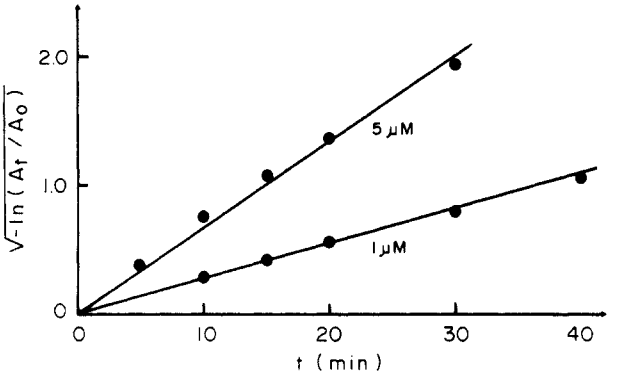
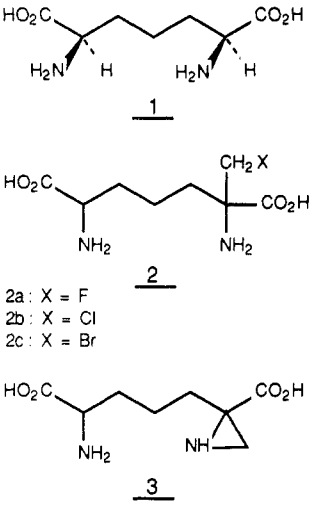


Figure 2. Secondary kinetic plot of the inhibition of DAP-epimerase by **2b**.

fluoromethyl, but not difluoromethyl derivatives) has been observed.[11] Therefore spontaneously formed aziridino-DAP (**3**) was suspected to be the actual inhibitor.



Later we discovered that this kind of inhibition had been proposed for the action of aziridine-2-carboxylate on proline racemase. Thus, on the basis of our hypothesis that non-pyridoxal phosphate racemases/epimerases derive from a common evolutionary origin,[13] a common mechanism, and therefore inhibition by aziridine-2-carboxylate analogues, is not unreasonable.

Results

1. Enzyme Kinetics. When **2a** was incubated with partially purified DAP-epimerase from *E. coli*, time-dependent and *irreversible* inhibition (as judged by dilution and dialysis experiments) was observed. Even faster inhibition was found with the chloro analogue **2b** and bromo analogue **2c** (unpublished result). Kinetics of inactivation indicated second order: plots of ln (A_t/A_0) (A_t and A_0 representing enzyme activities at times t and 0, respectively) versus t give nonlinear curves whereas plots of $\sqrt{-\ln A_t/A_0}$ versus t are linear (Figures 1 and 2). This supported our hypothesis of a spontaneous (or enzymatic) conversion of α-halomethyl-DAPs (S in eqs 1 and 4) into an intermediate species aziridino-DAP (I in eqs 1–4); this

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(11) α-(Fluoromethyl)tyrosine and α-(fluoromethyl)histidine give the corresponding aziridine carboxylic acids in quantitative yield when heated in basic medium at 100 °C for about 30 min: F. Gerhart, unpublished.

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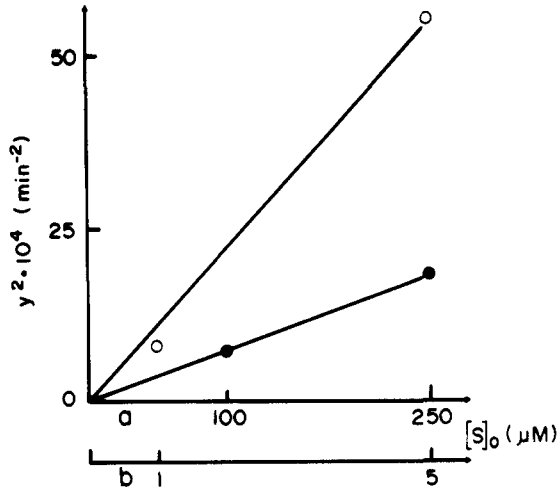

Figure 3. Tertiary plots of inhibition by **2a**, open circles, abscissa *a*; and by **2b**, closed circles, abscissa *b*.

being the actual irreversible inhibitor.

Enzyme catalysis of the conversion of S to I was ruled out as follows: A linear plot of percent activity remaining after 10.0 min of incubation of (1–10) × 10^{-7} M **3** with DAP-epimerase was first obtained. Next, freshly diluted solutions of **2a** were permitted to cyclize one sample in the presence and one sample in the absence of enzyme; aliquots were removed, suitably diluted, and incubated with DAP-epimerase as described above for exactly 10.0 min. From the percent activity remaining the concentrations of **3** were determined. No difference in the rate of formation of **3** was observed. For the case of spontaneous conversion of α-halomethyl-DAPs(S) into aziridino-DAP (**3**) (I), the kinetic situation should be as described by eqs 1–3, where E represents enzyme, I represents inhibitor, S represents substrate, [E] represents enzyme concn, $[E]_0$ represents enzyme concn at time $t = 0$, $[E]_t$ represents enzyme concn at time t, [I] represents inhibitor concn, $[I]_0$ represents inhibitor at time $t = 0$, [S] represents substrate concn, $[S]_0$ represents concn at time $t = 0$:

$$S \xrightarrow{k_0} I \tag{1}$$

$$E + I \underset{}{\overset{K_i}{\rightleftharpoons}} E{\cdot}I \xrightarrow{k_i} \text{inhibited enzyme} \tag{2}$$

with

$$[E] + [EI] = [E]_0 \tag{3}$$

Equation 1 integrates to

$$[I]_t = [S]_0\,[1 - \exp(-k_0 t)] \tag{4}$$

Thus, residual enzyme activity is given by

$$\ln (A_t/A_0) = \ln ([E]_t/[E]_0) = -\int_0^t \frac{k_i}{1 + K_i/[I]_t'}\,dt' \tag{5}$$

with $[I]_t'$ given by eq 4.

For small values of t fulfilling both the conditions $t \ll 1/k_0$ and $t \ll K_i/([S]_0 k_0)$, eq 5 simplifies to

$$-\ln (A_t/A_0) = \frac{k_i k_0 [S]_0 t^2}{2K_i} \tag{6}$$

Thus, plots $\sqrt{-\ln (A_t/A_0)}$ versus t should be linear, as found (Figures 1 and 2), with a slope y given by

$$y = -\sqrt{\frac{k_0 k_i [S]_0}{2K_i}} \tag{7}$$

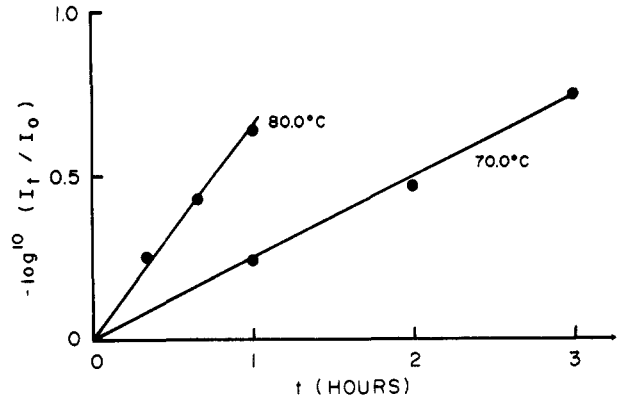

Figure 4. Liberation of fluoride ion from **2a** at 80 and 70 °C. I_t and I_0 indicate ^{19}F NMR intensities of **2a** at times t and 0, respectively.

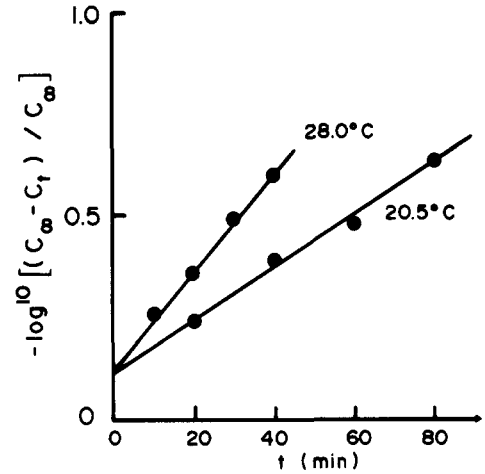

Figure 5. Liberation of chloride ion from **2b** at 28 and 20.5 °C. C_t and C_∞ indicate concentration of chloride ion at time t and after completion of reaction, respectively. The curves do not cut the ordinate at zero since this sample of **2b** contained an excess of chloride ion at time zero. This, however, does not affect the kinetic data.

A plot y^2 versus $[S]_0$ (Figure 3: **2a**, open circles, abscissa *a*; **2b**, closed circles, abscissa *b*) gives values for $k_i k_0/K_i$ of 1.45 × 10^{-5} and 2 × 10^{-3} μM^{-1} min^{-2} for **2a** and **2b**, respectively. Thus, spontaneous conversion to the putative irreversible inhibitor **3** is about 140 times faster for **2b** than for **2a**.

These conclusions drawn from enzyme kinetics obviously needed confirmation by both kinetics of conversion in the absence of enzyme and by preparation of the putative inhibitor, confirmation of its structure, and determination of K_i and k_i (or their ratio K_i/k_i).

2. Rate of Halide Liberation from α-(Halomethyl)diaminopimelic Acids. The rate of halide liberation under conditions of complete deprotonation of the NH_2 functions (aqueous alkali) was measured at two different temperatures for both **2a** (70.0 and 80.0 °C) and **2b** (20.5 and 28.0 °C). For **2a**, ^{19}F NMR was used; liberation of Cl^- from **2b** was monitored with a chloride-specific electrode. Results are shown in Figures 4 and 5. Halide liberation is first order; free enthalpies and entropies of activation are $\Delta H^\ddagger$ = 23.5 kcal/mol, $\Delta S^\ddagger$ = −8.2 eu and $\Delta H^\ddagger$ = 15.5 kcal/mol, $\Delta S^\ddagger$ = −22.8 eu for **2a** and **2b**, respectively. With these parameters, the following values of k_0 are calculated for 37 °C: 2.6 × 10^{-4} min^{-1} (**2a**) and 6.3 × 10^{-2} min^{-1} (**2b**); corresponding $t_{1/2}$ values are 45 h and 11 min, respectively. With the values for $k_i k_0/K_i$ obtained from enzymes kinetics, the ratio K_i/k_i for the

tentative common inhibitory intermediate is calculated to be 18 μM min for **2a** and 31 μM min for **2b**. It is obvious that these values cannot be very accurate since k_0 and k_0k_i/K_i were determined at different pH and thus at different degree of deprotonation of the amine functions. Nevertheless, the closeness of the values (ideally they should be identical) supports the assumption of a common inhibitory intermediate and the validity of the kinetic scheme.

3. Preparation of Aziridino-DAP (3) and Kinetics of Inhibition of DAP-epimerase. When **2a** (or **2b**, **2c**) are heated (or, for **2b**, **2c**, kept at room temperature for a sufficient time) in aqueous solution in the presence of excess alkali, **3** is formed in quantitative yield. A sample of **3**, containing a defined amount of NaCl, obtained by alkali treatment of **2b**, neutralization (HCl), and lyophilization, was characterized by ^{13}C NMR and FAB-MS (Xe) (see Experimental Section). **3** is remarkably stable;[14] nevertheless, its isolation in salt-free form is difficult. Thus, for enzyme studies, solutions of **3** obtained by heating of **2a** with alkali, were neutralized (HCl) to pH = 7 and used as such.[15]

Results of inhibition of DAP-epimerase by **3** have recently been published.[13] First-order inactivation was observed at concentrations ranging from 2×10^{-7} to 2×10^{-6} M. Kitz and Wilson plots of half life of inactivation versus reciprocal inhibitor **3** concentration were linear but cut the ordinate very close to the origin. Thus K_i and k_i could not be determined separately, but the value 5.5 μM was calculated for K_i/k_i.

Discussion

Aziridino-DAP (**3**) is an extremely potent inhibitor of DAP-epimerase; inhibition is irreversible, and in this respect **3** is the only compound known exhibiting this property. The formation of **3** from α-(halomethyl)diaminopimelic acids under physiological conditions is spontaneous with values for K_ik_i = 5.5 μM min (for **3**) and k_ik_0/K_i values of 1.45×10^{-5} and 2×10^{-3} μM^{-1} min^{-2} for **2a** and **2b**, respectively. Rate constants k_0 for aziridine formation under physiological conditions (37 °C, pH = 7.2) of 7.9×10^{-5} and 1.1×10^{-2} min^{-1} are calculated for **2a** and **2b**, respectively. Activation energy parameters (ΔH, ΔS) indicate that the rate of aziridine formation depends very much on the nature of the halogen. Steric requirements are obviously much higher for the chloro compound **2b** than for the fluoro derivative **2a** ($\Delta S^{\ddagger}$ = −22.8 and −8.2 eu, respectively). The opposite is, of course, true for $\Delta H^{\ddagger}$. With the data available, it is not possible to determine K_i and k_i separately; it may only be concluded that $K_i > 25$ μM and that $^1/_{k_i} < 10$ s, i.e. conversion of the enzyme–inhibitor complex to the inactivated enzyme must be extremely fast. Further studies would require fast kinetics techniques (e.g., stopped flow).

Although previously proposed as an inhibitor of proline racemase,[12] no data on aziridine-2-carboxylate has been published. Nevertheless we feel that these two examples of aziridine-2-carboxylate and its analogue aziridino-DAP may constitute a new kind of inhibition of non-pyridoxal enzymes, of which three, proline racemase, 4-hydroxyproline epimerase (previous nomenclature 2-hydroxyproline epimerase) and DAP-epimerase, are believed to be derived from a common evolutionary origin.[13]

Aziridino-DAP does not seem to be a reactive species per se. Chemically, it is remarkably stable,[14] and it does not apparently exhibit random alkylating properties that would result in general toxicity upon chronic administration to animals.[16] Aziridinecarboxylic acids do not always inhibit enzymes whose natural substrate is the parent amino acid to which the aziridinecarboxylic acid corresponds. For example, "aziridino-histidine" prepared by alkali treatment of α-(fluoromethyl)histidine,[11] does *not* inhibit histidine decarboxylase.[17] Although this is only one example where an aziridino analogue does *not* inhibit a pyridoxal enzyme, we feel sure that on mechanistic grounds this is entirely reasonable.

The inactivation of DAP-epimerase by **3** has been shown to involve reaction with a SH group of a cysteine residue.[13] A model involving a composite active site from two identical subunits was proposed[13] and may be applicable to the inactivation of proline racemase and 4-hydroxyproline epimerase.

Experimental Section

1. Chemistry and Chemical Kinetics. ^1H NMR spectra were obtained at 60, 90, or 360 MHz. ^{13}C NMR spectra were obtained at 90.5 MHz, and ^{19}F NMR spectra were obtained at 338.8 MHz. For ^{13}C NMR, the assignment was done by 2D spectrum and DEPT.

2-Amino-5-(benzyloxy)-2-(fluoromethyl)pentanenitrile (5). To magnesium turnings (8.93 g) was added 3-(benzyloxy)-1-chloropropane (**4**) (67.8 g, 0.367 mol) in ether (800 mL) while a gentle reflux was maintained. The mixture was stirred overnight. The mixture was cooled to −20 °C, and fluoroacetonitrile (21.32 g, 0.362 mol) dissolved in ether (100 mL) was added. The mixture was stirred for 30 min at −20 °C, and a mixture of sodium cyanide (27 g) and ammonium chloride (39 g) in water (400 mL) was added. The mixture was stirred for 1 h at room temperature. The reaction mixture was decanted and the aqueous layer was extracted twice with ether. The organic layer was extracted with 1 N aqueous hydrochloric acid. The aqueous layer was neutralized with sodium bicarbonate and extracted three times with ether. The combined organic phase was dried over magnesium sulfate, filtered, and concentrated under reduced pressure to afford the expected amino nitrile **5** (50 g, 57%) which was used without purification.

5-(Benzyloxy)-2-(fluoromethyl)-2-phthalimidopentanenitrile (6). The amino nitrile **5** (50 g, 0.211 mol) was dissolved in methylene chloride (400 mL), and triethylamine (41 g) was added followed by phthaloyl dichloride (42.8 g, 0.211 mol). The mixture was stirred overnight at room temperature. The mixture was washed with normal aqueous hydrochloric acid and twice with water. The organic layer was dried with sodium sulfate, filtered, and concentrated under reduced pressure to afford a brown oil. Flash chromatography over silica gel and elution with a 8:2 mixture of petroleum ether and hexane afforded the expected nitrile **6** (39.5 g, 51%). NMR ($CDCl_3$): δ 1.66–2.93 (m, 4 H), 3.53 (t, J_{HH} = 6 Hz, 2 H), 4.45 (s, 2 H), 5.06 (d AB, J_{HF} = 46 Hz, J_{AB} = 9 Hz, 2 H), 7.32 (s, 5 H), 7.8 (m, 4 H).

5-Hydroxy-2-(fluoromethyl)-2-phthalimidopentanenitrile (7). The nitrile **6** (39.5 g, 0.107 mol) was dissolved in dry methylene chloride (400 mL), and trimethylsilyl iodide (23.9 g, 0.118 mol) was added dropwise. The mixture was stirred overnight at room temperature. Methylene chloride was distilled off and replaced by chloroform (350 mL) and triethylamine (40 mL). The reaction mixture was refluxed for 1 h. The reaction mixture was washed with normal aqueous hydrochloric acid and water and separated; the organic phase was dried over sodium sulfate, filtered, and concentrated under reduced pressure to afford the expected alcohol **7** (29.6 g, 99%), which was used without further

(14) Upon heating to 110 °C in 10% aqueous NaOH for 1 h **3** was perfectly stable; after 1 h at 22 °C in 6 N HCl ca. 50% decomposition was observed.

(15) Purity and stability of these solutions were checked by HPLC; 10 mM stock solutions showed no significant decomposition after 1 year at 4 °C.

(16) Upon chronic treatment in mice (100 mg/kg per day/po for 7 days) no visible signs of toxicity were seen. Weight gain and food intake were identical with control group. W. Higgins, unpublished results.

(17) F. Gerhard and M. Jung, unpublished results.

purification. NMR ($CDCl_3$): δ 1.56–2.83 (m, 4 H), 2.33 (s, 1 H), 3.66 (t, J_{HH} = 6 Hz, 2 H), 5 (d, AB q, J_{HF} = 46 Hz, J_{AB} = 9 Hz, 2 H), 7.78 (m, 4 H).

2-(Fluoromethyl)-5-(mesyloxy)-2-phthalimidopentanenitrile (8). The alcohol 7 (15 g, 0.054 mol) was dissolved in a mixture of methylene chloride (50 mL) and pyridine (20 mL). Methanesulfonyl chloride (6.2 g, 54 mmol) was added, and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with methylene chloride and washed twice with normal aqueous hydrochloric acid and twice with saturated brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure to afford the expected mesylate 8 (18 g, 93%) which was used without purification. NMR ($CDCl_3$): δ 1.66–2.8 (m, 4 H), 3 (s, 3 H), 4.26 (d, J_{HH} = 6 Hz, 2 H), 5.05 (d, AB q, J_{HF} = 45 Hz, J_{AB} = 9 Hz, 2 H), 7.84 (m, 4 H).

2-(Fluoromethyl)-5-iodo-2-phthalimidopentanenitrile (9). The mesylate 8 (18 g, 50 mmol) was dissolved in dry acetone (100 mL), sodium iodide (15 g, 0.1 mol) was added, and the mixture was refluxed under vigorous stirring during 15 h. The salt was filtered off, and acetone was evaporated under reduced pressure. The residue was dissolved in methylene chloride and was successively washed with aqueous sodium hydrogen sulfite and saturated brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure to afford the expected iodide 9 as an oil (19 g, 95%). NMR ($CDCl_3$): δ 1.76–2 (m, 4 H), 3.25 (t, J_{HH} = 7 Hz, 2 H), 5.33 (d, AB q, J_{HF} = 46.5 Hz, J_{AB} = 9 Hz, 2 H), 7.86 (m, 4 H).

Ethyl 2-(Carbethoxy)-6-cyano-2,6-diphthalimido-7-fluoroheptanoate (10). A mixture of diethyl phthalimidomalonate (16.6 g, 54 mmol) and potassium *tert*-butanoate (6.7 g, 54 mmol) in anhydrous dimethylformamide (50 mL) was stirred at room temperature during 30 min. Then the iodide 9 (21 g, 54 mmol) dissolved in dimethylformamide (50 mL) was added, and the mixture was stirred at 60 °C during 48 h. Dimethylformamide was evaporated under reduced pressure. The residue was taken up in methylene chloride and washed successively with water and saturated brine. The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. Flash chromatography over silica gel and elution with a 1:1 mixture of hexane and ethyl acetate afforded the expected nitrile 10 (21.6 g, 71%). NMR ($CDCl_3$): δ 1.23 (t, J_{HH} = 7, 6 H), 1.53–2.83 (m, 6 H), 4.26 (q, J_{HH} = 7 Hz, 4 H), 5.03 (d, AB q, J_{HF} = 46.5 Hz, J_{AB} = 9 Hz, 2 H), 7.73 (m, 8 H).

2,6-Diamino-2-(fluoromethyl)heptanedioic Acid (2a). The nitrile 10 (21.6 g, 38.4 mmol) was heated under reflux in concentrated hydrochloric acid (200 mL) during 48 h. The reaction mixture was cooled at 0 °C and filtered. The solvents were evaporated under reduced pressure. The residue was dissolved in water and extracted twice with ether. The aqueous phase was evaporated under reduced pressure. The residue was dissolved in ethanol. The mixture was filtered, and the amino acid was precipitated with propylene oxide to give white crystals (7 g, 79%), mp = 204 °C. NMR (D_2O): δ 1.46–2.26 (m, 6 H), 4.16 (t, J_{HH} = 6 Hz, 1 H), 4.9 (d, AB q, J_{HF} = 47 Hz, J_{AB} = 10.5 Hz, 2 H). Anal. ($C_8H_{15}FNO_4 \cdot 0.1HCl \cdot {}^1/_2H_2O$) C, H, N.

2,6-Diamino-2-(chloromethyl)heptanedioic Acid (2b). 2,6-Diamino-2-(fluoromethyl)heptanedioic acid monohydrochloride (3.5 g, 13.5 mmol) was heated with aqueous NaOH (2.9 g, 72 mmol, 50 mL of water) at 100 °C for 2 h. With cooling (ice), concentrated HCl (100 mL) was added. Evaporation to dryness followed by stripping twice with water gave a residue which was triturated with 2-propanol. Filtration and evaporation gave a residue which was dissolved in 2-propanol/ethanol (8:2, 100 mL) and treated with an excess of propylene oxide. Recrystallization of the crude precipitated material (3.2 g) from water/2-propanol gave the pure compound **2b**, mp 230 °C dec. ^1H NMR (D_2O): δ 3.96 (AB, ΔAB = 0.14, J_{AB} = 12 Hz, CH_2Cl). ^{13}C NMR (D_2O, ref *t*-BuOH): δ 173.61, 174.26 (C1, C7), 66.61 (C2), 35.07 (C3), 20.96 (C4), 31.56 (C5), 54.76 nC6), 48.54 (C_2') (see structure). Anal. ($C_8H_{15}ClN_2O \cdot 0.75H_2O$) C, H, N.

2,6-Diamino-2-(bromomethyl)heptanedioic Acid (2c). 2,6-Diamino-2-(bromomethyl)heptanedioic acid (**2c**) was obtained as described above for **2b** from **2a** but with replacement of concentrated HCl by concentrated HBr.

2-(4-Amino-4-carboxybutyl)-2-aziridinecarboxylic Acid (3) Disodium Salt. 3 (276 mg, 109 mmol) was dissolved in aqueous NaOH (175 mg, 4.37 mmol; water, 4 mL) and kept at room temperature for 6 h. Filtration through a membrane filter (Millipore) followed by lyophilization gave the title compound as a mixture with sodium chloride. ^1H NMR (D_2O): δ 1.63, 1.94 (2s, aziridine CH_2), 3.24 (m, $HCCO_2H$). ^{13}C NMR (D_2O, ref *t*-BuOH): δ 181.50, 185.28 (C_1, C_7), 42.28 (C_2), 36.57 (C_3)8 23.89 (C_4), 32.90 (C_5), 57.67 (C_6), 34.19 (C_2') (see structure **3**). FAB-MS (Xe): MH^+ = 247.

Liberation of Fluoride Ion from 2a. 2a (100 mg) was dissolved in 1 N NaOH (2 mL) containing sodium trifluoroacetate as an internal standard. Samples (0.4 mL) were heated in NMR tubes in a thermostat at 70.0 ± 0.05 °C or 80.0 ± 0.05 °C. ^{19}F NMR spectra were recorded at time zero and at 20, 40 and 60 min (80 °C) or 1, 2 and 3 h (70 °C). Peak heights (central line of triplet of **2a**), averaged over five scans, were used for the determination of I_t/I_0.

Liberation of Chloride Ion from 2b. 2b (50 mg) was dissolved in 1 N NaOH (2.8 mL), and the solution was heated in a stoppered flask at 20.5 ± 0.05 °C or 28.0 ± 0.05 °C. Aliquots (0.4 mL) were taken at 10, 20, 30, and 40 min (28 °C) and 20, 40, 60, and 80 min (20.5 °C), respectively. They were quenched with acetic acid (0.5 mL) and diluted with water to 10 mL before measuring chloride ion concentration with a Cl^--specific electrode.

2. Biochemistry and Enzyme Kinetics. Growth of Bacteria and Isolation of DAP-epimerase. Bacterial strains: *E. coli* K12 (PR7) was a kind gift of Dr. J. E. Davis (University of Wisconsin). Strains were inoculated into 5 mL of nutrient broth and grown overnight for 16 h at 37 °C. Small amounts of cells were obtained in shake flask cultures (50–1000 mL) obtained by inoculating Igarashi medium[18] with 2% of the overnight culture. Cells were harvested at late-log phase (OD_{540nm} 0.7–0.8) and rinsed before storage at −20 °C. Larger amounts of cells from strain (PR7) were obtained by using an overnight culture (Igarashi medium) of 2.5 L as inoculum for 50 L of Igarashi medium in a Biolafitte Fermentor. Cells were harvested in late-log phase (OD_{540nm} 2.0) with use of a filtration system for cell concentration (40-fold), followed by centrifugation.

Cells from *E. coli* (PR7), usually in batches of 40 g, were suspended in 100 mL of buffer A (20 mM potassium phosphate, 1 mM EDTA, 1 mM DTT, pH 7.0) by using the Ultra-turrax apparatus (10 min). Passage through the French press (20 000 lb/in.^{2}) and centrifugration 30000*g* for 20 min gave the S30 crude extract. The supernatent was fractionated by adding solid ammonium sulfate to 30%; centrifugration was followed by further ammonium sulfate to 45%. The precipitated protein was dissolved in buffered A to give a final volume of 10 mL which was dialyzed overnight against 2 L of buffer A.

The dialyzed crude enzyme preparation was applied to a DEAE-cellulose column (2.3 × 49 cm) DE52, equilibrated with buffer A. The column was washed with 250 mL of buffer A and eluted with a linear gradient formed by adding 250 mL of buffer A containing 0.22 M KCl to 250 mL of buffer A. The flow rate was 25 mL h^{-1}, and 10-mL fractions were collected. DAP-epimerase eluted near the end of the gradient at approximately 0.20 M KCl. Pooled fractions containing DAP-epimerase activity were dialyzed overnight against 2 L buffer A. Enzyme was concentrated by applying the dialyzate to a small DE52 column (0.9 × 10 cm), rinsing with 50 mL of buffer A and eluting with buffer A containing 0.50 M KCl. The flow rate was 20 mL h^{-1} and 2.5-mL fractions were collected. Enzyme activity eluted immediately and pooled fractions were dialyzed against 1 L buffer A. We estimate that purified thus far DAP-epimerase was ~20% pure—sufficient for kinetic purposes. Enzyme, concentrated in this fashion, was stable at 4 °C for many months but lost activity upon freezing.

Enzyme Activity. DAP-epimerase activity was routinely measured at 25 °C essentially as described by Richaud et al.[19] Reaction mixtures contained 0.1 M Tris-HCl (pH 7.8), 1 mM EDTA, 1 mM DTT, and 0.5 μCi of (G-^3H)DAP in a total volume of 0.10 mL. After incubation for 40 min, reaction mixtures were quenched with 0.5 mL of 10% TCA and applied to 1 mL of AG50

(18) Igarashi, K.; Kashiwazi, K.; Kishida, K.; Watanabe, Y.; Kogo, A.; Hirosa, S. *Eur. J. Biochem.* **1979**, *93*, 345.
(19) Richaud, C.; Higgins, W.; Mengin-Lecreulx, D.; Stragier, P. *J. Bacteriol.* **1987**, *169*, 1547.

× 4 ion-exchange resin (H^+ form) contained in a column (0.4 × 2 cm) fashioned from a blue pipet cone. The column was washed with 3 × 0.5 mL of water, and the combined eluants were counted for radioactivity with use of Aquasol-2 (15 mL).

Inhibition Studies. DAP-epimerase, partially purified as described above, was incubated in 50 mM TrisHCl (pH 7.8) with various concentrations of **3** and **2a–c** at 25 °C. At the time intervals indicated in the various figures (Figures 1, 2, and 8), aliquots were removed and diluted 5–10-fold into the assay reaction mix, and the remaining enzyme activity was determined.

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Synthesis and Antiviral Activity of Some Acyclic and C-Acyclic Pyrrolo[2,3-*d*]pyrimidine Nucleoside Analogues

Sharon M. Bennett, Nghe Nguyen-Ba, and Kelvin K. Ogilvie*,†

Department of Chemistry, McGill University, Montreal, Canada, H3A 2K6. *Received August 16, 1989*

A series of acyclic and C-acyclic 7-deazapurine nucleosides have been synthesized and tested for antiviral activity. Reaction of the sodium salt of 2-amino-3,4-bis(aminocarbonyl)-5-(methylthio)pyrrole (**6**) with an appropriate electrophile gave pyrrole nucleosides which served as common intermediates to both the 7-deazaadenosine and the 7-deazaguanosine series. Several of these 5- and 5,6-substituted pyrrolo[2,3-*d*]pyrimidine nucleosides have shown activity against HIV virus in preliminary in vitro screens.

Pyrrolo[2,3-*d*]pyrimidine nucleosides have been the subject of a number of scientific studies since the report of the pronounced biological activity of some of these nucleosides.[1] In fact, the antiviral activity of several of the naturally occurring pyrrolo[2,3-*d*]pyrimidine nucleosides, and of some of their analogues, has been well documented.[2] These natural products are commonly referred to as the 7-deazapurines and some representative examples of this class of compounds are illustrated in Chart I.[3]

As part of our continuing efforts in the synthesis of acyclic nucleosides as potential antiviral agents,[4] we were interested in exploring the possibility that some acyclic 7-deazapurine analogues bearing C-5, or C-5 and C-6 substituents, may also have antiviral activity. We,[5] along with others,[6] found that replacing the furanose ring of guanosine with an acyclic carbohydrate [i.e. (1,3-dihydroxy-2-propoxy)methyl] resulted in a modified nucleoside **5** which possesses pronounced activity against the human herpes simplex viruses 1 and 2. We observed that varying the nature of the heterocyclic base has a pronounced effect on the antiviral activity of the acyclic nucleoside[7] and were interested in determining whether antiviral activity is retained when the (1,3-dihydroxy-2-propoxy)methyl group is employed in combination with the 7-deazapurine skeleton.[8,9] While none of the compounds of this series are active against herpes viruses, we were pleased to discover that compounds **55** and **67** in the (1,3-dihydroxy-2-propoxy)methyl series (Tables IV and V) show activity against the HIV virus. These results encouraged us to extend our synthetic efforts towards other acyclic and C-acyclic pyrrolo[2,3-*d*]pyrimidine analogues. We report here the synthesis and preliminary antiviral (HIV) screening results of a series of new 7-deazapurine acyclonucleoside analogues.

Chart I. Nucleoside Analogues

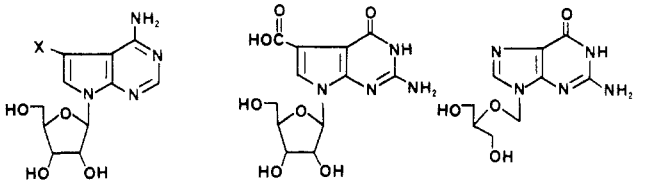
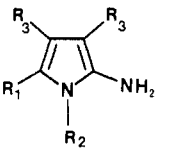
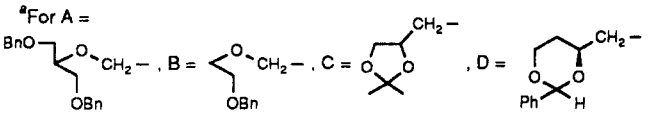


Table I. Substituted Pyrroles



compd	R_2[a]	R_1	R_3	yield, %
11	A	SCH_3	$CONH_2$	32
12	B	SCH_3	$CONH_2$	32
13	C	SCH_3	$CONH_2$	62
14	D	SCH_3	$CONH_2$	51
15	A	H	$CONH_2$	75
16	B	H	$CONH_2$	76
17	C	H	$CONH_2$	73
18	D	H	$CONH_2$	54
19	A	SCH_3	CN	51
20	B	SCH_3	CN	53
21	C	SCH_3	CN	60
22	D	SCH_3	CN	59
23	A	H	CN	50
24	B	H	CN	52
25	C	H	CN	51
26	D	H	CN	42

[a] For A = 

Results and Discussion

A number of syntheses of pyrrolo[2,3-*d*]pyrimidine nucleosides and their analogues have been published over the past 20 years. Some of the synthetic approaches to the natural products themselves have relied on the construction of the pyrrolo[2,3-*d*]pyrimidine ring as a first stage, followed by subsequent attachment of the carbohydrate,[10] while others have involved pyrrole *N*-nucleosides as a key

† Address for correspondence: Vice President Academic, Acadia University, Wolfville, Nova Scotia, Canada, B0P 1X0.

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