ATH8 Cells. The HIV-I cytopathic effect inhibition assay was performed as previously described.[2,3] Briefly, susceptible ATH8 cells (2×10^5) were pelleted, exposed to HIV-I (HTLV-III_B: 2,000 virus particles per cell) in the form of cell-free virions, resuspended in interleukin-2-containing medium, and cultured in the presence or absence of various concentrations of compounds. Control cells were similarly treated but not exposed to the virus. On day 7 in culture, the total viable target cells were counted in a hemocytometer under the microscope by the trypan blue dye exclusion method. Control-infected cultures untreated with drug were almost completely destroyed by the cytopathic effect of the virus. **3a** was tested in the range 0.25 to 25 μg/mL.

Acknowledgment. We warmly thank Drs. M. Kinns and D. V. Bowen and Mr. M. J. Newman (Pfizer, U.K.) for spectroscopic support, especially the NOE studies, Dr. D. J. Rance and his group (Pfizer, U.K.) for expert HPLC analyses, and Mr. S. A. Smith (Pfizer, U.K.) and Ms. A. Newell (Clinical Research Centre, U.K.) for antiviral testing.

Registry No. **3a**, 116195-58-5; **3b**, 118629-53-1; α-**6**, 118597-64-1; β-**6**, 118597-65-2; α-**7**, 118597-66-3; β-**7**, 118597-67-4; α-**8**, 118597-68-5; β-**8**, 118597-69-6; **9**, 116195-59-6; thymine, 65-71-4; (1*S*)-(−)-camphanoyl chloride, 39637-74-6.

Supplementary Material Available: X-ray data (coordinates, anisotropic temperature factors, distance, and angles) for compounds **3b** and **9** (16 pages). Ordering information is given on any current masthead page.

A Dihydropyridine Carrier System for Sustained Delivery of 2′,3′-Dideoxynucleosides to the Brain

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The present study evaluates the utility of the dihydropyridine ⇌ pyridinium salt redox system for the specific delivery and sustained release of a model 2′,3′-dideoxynucleoside to the brain of mice as the initial effort in a search for agents that may prove effective in reversing the complicating neurological disorders of AIDS. The unsaturated nucleoside 2′,3′-didehydro-2′,3′-dideoxythymidine (**1**), which is effective in protecting ATH8 cells against the cytopathogenicity of HIV-1, was converted to the corresponding *N*-methyl-1,4-dihydronicotinate derivative, **4**, in three steps. The 5′-*O*-nicotinate ester, **2**, obtained by reaction of **1** with nicotinyl chloride, was converted in quantitative yield to the *N*-methylpyridinium salt **3** on treatment with MeI in acetone. Reduction of the latter with $Na_2S_2O_4$ gave **4** in 50% yield. Pseudo-first-order rate constants for the oxidation of **4** to **3** were observed in plasma ($k = 3.54 \times 10^{-5}$ s^{-1}) and in homogenates of mouse liver ($k = 9.2 \times 10^{-5}$ s^{-1}) and brain ($k = 8.85 \times 10^{-5}$ s^{-1}). None of the chemical delivery system **4** could be detected in the brain of female BDF/1 mice at 1 h postinjection. The peak level of **3** in the brain occurred at 3 h with a half-life of 25 h. Both **1** and *N*-methylnicotinic acid (trigonelline, **5**) were readily identified by HPLC in a brain homogenate derived from mice injected (25 mg/kg) with **4**. TLC showed a low level penetration of mouse brain by **1** (0.44 μg/g wet tissue) following injection of the corresponding labeled [*methyl*-^{3}H]-2′,3′-unsaturated nucleoside (25 mg/kg). The data indicate that **4** crosses the blood–brain barrier to be oxidized by cerebral tissue to the ionic structure **3**, which is "locked therein". The sustained local release of a 2′,3′-dideoxynucleoside, such as **1**, from a chemical delivery system (**4**) represents a potentially useful approach to the treatment of AIDS dementia complex.

A common and important cause of morbidity in patients with advanced stages of infection with human immunodeficiency virus type 1 (HIV-1) is AIDS dementia complex, a complicating neurological syndrome characterized by abnormalities in cognition, motor performance, and behavior.[1] There is evidence that AIDS dementia complex is caused either partially or wholly by direct HIV-1 brain infection and, further, that virus frequently invades the central nervous system (CNS) early in the course of systematic infection, even in the absence of symptoms.[2] Indeed, there is the possibility that the CNS serves as the major reservoir for HIV in the body.[3] Clearly a rationale exists for seeking antiviral drugs that can penetrate the blood–brain (BB) and blood–cerebrospinal fluid barriers (BCSFB). In this connection it may be noted that 3′-azido-2′,3′-dideoxythymidine (AZT) penetrates the BCSFB[4,5] and can, at least partly, reverse the neurological dysfunction due to HIV-1 in some patients.[6]

Balzarin et al.[7] have reported that AZT and the 2′,3′-unsaturated nucleoside 2′,3′-didehydro-2′,3′-dideoxythymidine (**1**) were equally effective in protecting ATH8 cells against the cytopathogenicity of HIV-1, but **1** had a higher in vitro chemotherapeutic index; i.e., it was less cytostatic and cytotoxic against ATH8 cells than AZT. Moreover, when evaluated for their inhibiting effects on the cytopathogenicity of HIV-1 in MT-4 cells, **1** was about 5 times more potent than 2′,3′-dideoxycytidine,[8] which is currently being evaluated for its therapeutic potential in

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Table I. Kinetics of in Vitro Oxidation of the Dihydropyridine Ester 4 to Quaternary Structure 3 in Biological Fluids

medium	K, $s^{-1} \times 10^{-5}$	$t_{1/2}$, min
hydrogen peroxide	55 ± 3.2	21 ± 1.3
human plasma	3.54 ± 0.11	325 ± 10
mouse brain homogenate	8.85 ± 0.34	130 ± 5
mouse liver homogenate	9.20 ± 0.37	125 ± 5

the treatment of AIDS.[9] Other data clearly indicate that 1 is a potent inhibitor of HIV-1 in human peripheral blood mononuclear cells in vitro. These findings prompted the suggestion that an evaluation of the in vivo pharmacological properties of 1 is now warranted.[7,10]

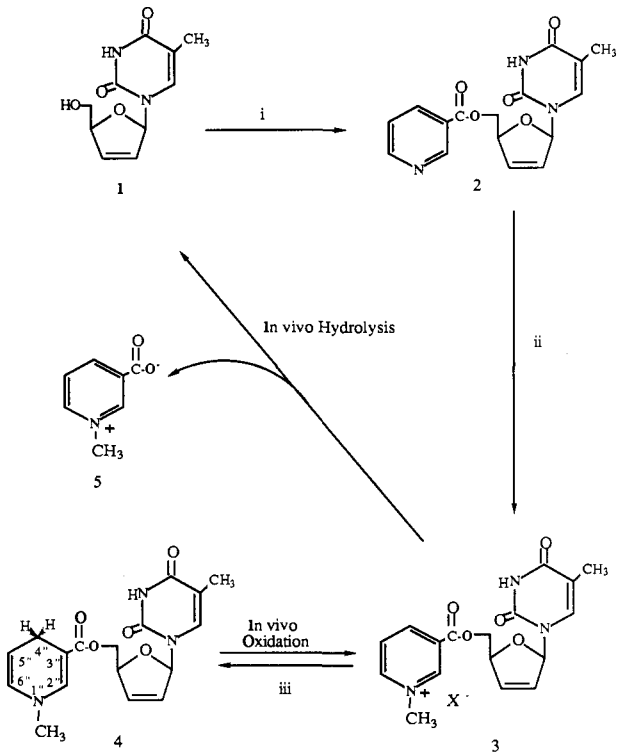
It appears that AZT is highly unusual among nucleoside analogues in that it traverses the cell membrane chiefly by nonfacilitated diffusion and not via a nucleoside transport system.[11] The unusual ability of AZT to diffuse across cell membranes has been attributed to the considerable lipophilicity imparted to this molecule by replacement of the 3'-hydroxyl group of thymidine (dThd) by an azido substituent.[11] Thus, the octanol–water partition coefficient, which essentially is a measure of lipophilicity, was determined to be 1.26 for AZT compared to a value of 0.064 for dThd.[11] The significantly greater lipophilicity of AZT correlates with its capacity to permeate the BCSFB. In contrast, the considerably reduced lipophilicity of 1 (vide infra) coupled with the presumed absence of a carrier with affinity for 2',3'-unsaturated pyrimidine nucleosides would seem to preclude the possibility of achieving an effective in vivo level of this agent via passive influx to the CNS.

Bodor and co-workers demonstrated[12,13] in a series of studies that drugs can be specifically delivered to the CNS with use of a dihydropyridine ⇌ pyridinium salt redox system. Application of this chemical delivery system to drugs that either do not readily cross the BBB, e.g., simple amines, catecholamines, or to larger molecules such as steroid hormones,[14] which readily pass into and out of the brain due to their high lipophilicity, has led to selective and sustained delivery of diverse structures to the brain. Recently, Torrence et al.[15] described the application of this principle to AZT, which included preliminary in vivo experiments to determine the specific delivery of the redox carrier system to the brain of rats. This paper has prompted us to report the evaluation of the same system to the sustained, brain-specific delivery of 1.

Chemistry

The synthesis of the drug carrier system 4 (Scheme I) proceeded from 1,[16] which was first esterified with nicotinyl

Scheme I[a]



[a] Reagents: i, nicotinyl chloride, pyridine; ii, MeI/acetone; iii, $Na_2S_2O_4$, aqueous methanol.

chloride to give 2 in 63% yield. Quaternization of 2 with methyl iodide in acetone provided the trigonellinate ester 3 in near-quantitative yield. Reduction of 3 with $Na_2S_2O_4$ provided the desired 1,4-dihydropyridine derivative (4, 50% yield), which showed no signs of oxidation (to 3) or deterioration (TLC) after storage at room temperature for 1 month. With low concentrations of the dihydro derivative 4 (5×10^{-6} M) and higher concentrations of hydrogen peroxide (0.2 M), the oxidation was observed to occur according to first-order kinetics (see Table I). The UV spectrum of 4 exhibited absorption maxima (358 and 262 nm) in accord with other 1-substituted 1,4-dihydropyridines.[12,13] The NCH_3 group and its two C-4'' hydrogens appeared in the ^1H NMR spectrum (DMSO) of 4 as a broad singlet (5 H). In addition the C-2' (6.42 ppm) and C-3' (5.98 ppm) olefinic protons of the unsaturated sugar moiety comprising 4 were readily identifiable.

The partition coefficient of 4 (1-octanol–water) was determined to be 7.05, which is approximately 31-fold greater than that of 1 (0.23). Accordingly it was expected that 4, by virtue of its significantly enhanced lipid solubility, would readily cross the BBB. Biological oxidation of the latter to the quaternary structure 3 would result in the unique cerebral trapping of this ionic, hydrophilic product. Subsequent slow hydrolysis of 3 should then provide the desired site-specific, elevated, and prolonged delivery of 1. It is important to note that 1-methylnicotinic acid (trigonelline, 5), generated in the hydrolysis of 3, is nontoxic and is readily cleared from the brain.[15]

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Pseudo-first-order rate constants and half-lives for the process of oxidation of the 1,4-dihydropyridine ester 4 in

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Table II. Distribution of the Quaternary Salt 3, 2′,3′-Didehydro-2′,3′-dideoxythymidine (1), and Trigonelline (5) in Liver and Brain after Administration of Nucleoside-Carrier System 4 to Mice[a]

time, h	liver: 3	brain 3	brain 1	brain 5
1	3.3 ± 0.4	4.7 ± 0.7	2.7 ± 0.5	2.2 ± 0.6
2	2.5 ± 0.3	4.9 ± 0.6	2.5 ± 0.4	2.1 ± 0.3
3	2.0 ± 0.2	5.3 ± 0.6	2.6 ± 0.5	2.4 ± 0.4
6	1.3 ± 0.3	5.1 ± 0.7	2.3 ± 0.4	1.9 ± 0.4
24	b	3.0 ± 0.4	1.2 ± 0.3	0.9 ± 0.2

[a] Compound 4 was injected into the tail vein in BDF/1 female mice at a dose of 25 mg/kg in a vehicle of 5% Tween 80 containing 5% ethanol. Animals were sacrificed at the indicated intervals; the organs were taken and homogenized in 50% methanol in preparation for HPLC studies. Three mice were used to establish each time point. Units equal μg/g of wet tissue. [b] Undetected.

different biological media are shown in Table I. The rates of oxidation in mouse liver ($k = 9.20 \times 10^{-5}$ s^{-1}) and brain ($k = 8.85 \times 10^{-5}$ s^{-1}) tissues were 2.6- and 2.5-fold higher, respectively, than in (human) plasma ($k = 3.54 \times 10^{-5}$ s^{-1}). There was no evidence of significant hydrolysis of 4 to 1 under the imposed conditions. The latter observation, together with the higher oxidation rate in the brain than in plasma, predicts for an accumulation of the charged pyridinium derivative (3) in the brain following intravenous administration of the drug delivery system (2).

Compound 4 was injected in BDF/1 female mice at a dose of 25 mg/kg, and following sacrifice, blood, brain, and liver samples were obtained 1, 2, 3, 6, and 24 h later. The distribution of the quaternary pyridinium salt 3 in the two tissues was determined with the aid of HPLC (see Table II).

None of the carrier delivery system (4) could be detected in the brain within the sensitivity limits of the method. However, the concentration of 3 rose from a level of 4.7 μg/g (wet tissue) at 1 h to a peak value of 5.3 μg/g at 3 h and then slowly declined to 3.0 μg/g at the last sampling time (24 h) with a calculated half-life of 25 h.

Trigonelline (5) and 1, which showed retention times of 3.4 and 4.0 min, respectively, on C-18 reverse-phase HPLC (linear gradient 50–70% acetonitrile–water), were readily identified in brain as well as in liver tissue homogenates, prepared at the same intervals (see Table II) after injection of 4. By contrast, HPLC failed to indicate the penetration of mouse brain by 1 following the injection (10 mg/kg) of [*methyl*-^{3}H]-1 (limit of detection: 0.1 μg/g wet tissue). The detection of [*methyl*-^{3}H]-1 at a level of 0.44 ± 0.12 μg/g wet tissue was only achieved via scintillation counting of the radioactivity contained in an aliquot of a 50% aqueous methanol homogenate of brains of mice injected with 25 mg/kg of the labeled unsaturated nucleoside. That the radioactivity, indeed, represented unchanged 1 was indicated following TLC (RP-18 plates) of the homogenate in 0.1 M NH_4OAc–CH_3CN (80:20) whereupon >90% of the total radioactivity was localized in a spot with an R_f identical with that produced by an authentic sample of 1.

The limited capacity of 1 to penetrate mouse brain is consistent with its poor lipid solubility (vide supra). For the same reason, it would be expected that the charged pyridinium ester 3 would manifest only minimal (mouse) brain penetration. The unavailability of labeled 3 precluded any attempt to determine the level of the latter in the brain.

Whereas 3 could not be detected in the blood at any of the sampling intervals, the levels of the quaternary salt in liver declined from 3.3 μg/g (wet tissue) at 1 h to 1.3 μg/g at 6 h and was undetectable after 24 h. The calculated half-life of 3 in liver was 5 h.

These data indicate that the 1,4-dihydropyridine derivative 4 penetrates the BBB where it is oxidized to the quaternary derivative 3. The latter is "locked in", providing thereby the basis for a long half-life of 3 in the brain relative to its rapid clearance from tissue such as liver. This cerebral trapping of 3 and the sustained local release of 1, which is very effective in protecting ATH8 cells against the cytopathogenicity of HIV-1, may be useful clinically in the treatment of the neurological disorders associated with AIDS.

Experimental Section

General Methods. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. ^{1}H NMR spectra were obtained with a Nicolet QE 300 FT spectrometer. Electron-impact mass spectra (EI-MS) were run with a Kratos MS80 RFA high-resolution instrument. UV spectra were obtained with a Perkin-Elmer Lambda 5 spectrophotometer. HPLC was performed with a Bondapak C_{18} reverse-phase column (5 × 100 mm) with either a mobile phase of CH_3CN (60%)–50 mM phosphate buffer, pH 6.2 (40%), or a linear gradient of 50–70% CH_3CN. With the latter system, retention times were as follows: 1, 4.0 min, 3, 5.7 min, 4, 5.2 min, and 5, 3.4 min. Partition coefficients were determined in 1-octanol–water according to the method described by Kessel.[17] [*methyl*-^{3}H]-2′,3′-Didehydro-2′,3′-dideoxythymidine (15–20 Ci/mmol) was obtained from Moravek Biochemicals, Brea, CA 92621-4890.

5′-*O*-(3′-Pyridinylcarbonyl)-2′,3′-dideoxythymidine (2). To a pyridine (20 mL) solution of nicotinyl chloride, prepared by treatment of nicotinic acid (0.57 g, 4.6 mmol) with excess thionyl chloride, and 1 (1 g, 4.5 mmol) was added 0.08 g (0.65 mmol) of DMAP, and the mixture was stirred at 40 °C for 2 days. To the cooled reaction mixture was added an equal volume (30 mL) of CH_2Cl_2, and the undissolved solids were removed by filtration. The filtrate was evaporated to dryness in a rotary evaporator, and the residue was then coevaporated with ethanol (3 × 5 mL). The residual solid was dissolved in a mixture of ethyl acetate–ethanol (8:2), the solution was passed through a short column (50 × 30 mm) of silica gel, and the filtrate was evaporated to dryness in vacuo. The residue crystallized from ethyl acetate–ethanol (8.2) to give 0.92 (63% yield) of a solid: mp 174–176 °C; ^{1}H NMR (DMSO-d_6) δ 1.390 (s, 3 H, CH_3), 4.55 (m, 2 H, $H_{5'}$), 5.11 (m, $H_{4'}$), 6.03 (m, $H_{3'}$), 6.51 (m, $H_{2'}$), 6.80 (d, 1 H, $H_{1'}$), 7.114 (s, 1 H, 3-NH), 7.56 (m, 1 H, $H_{5''}$-pyridine), 8.25 (m, 1 H, $H_{4''}$-pyridine), 8.80 (d, 1 H, $H_{6''}$-pyridine), 9.045 (s, 1 H, $H_{2''}$-pyridine). Anal. ($C_{16}H_{15}N_3O_5$) C, H, N.

5′-*O*-(1″-Methyl-3″-pyridiniocarbonyl)-2′,3′-didehydro-2′,3′-dideoxythymidine (3). To a solution of 2 (0.5 g, 1.52 mmol) in 200 mL of dried (molecular sieves, 4 Å) acetone was added 2.0 mL of methyl iodide (31.4 mmol), and the solution was refluxed gently until TLC (ethyl acetate–ethanol, 8:2) indicated complete quaternization (18 h). The mixture was evaporated to dryness in vacuo and the residue, after trituration with acetone, was dried in vacuo to give 0.715 g (near-quantitative yield) of product: mp 90 (sinters)–170 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.606 (s, 3 H), 9.18 (d, 1 H, C_5-CH_3), 4.428 (s, 3 H, NCH_3), 4.65 (m, 2 H, $H_{5'}$), 5.12 (br s, 1 H, $H_{4'}$), 6.07 (d, 1 H, $H_{3'}$), 6.54 (d, 1 H, $H_{2'}$), 6.817 (br s, 1 H, $H_{1'}$), 7.231 (s, 1 H, H_6), 8.25 (t, 1 H, $H_{5''}$), 8.94 (d, 1 H, $H_{4''}$), 9.18 (d, 1 H, $H_{6''}$), 9.534 (s, 1 H, $H_{2''}$). Anal. ($C_{17}H_{18}N_3O_5I$) C, H, N, I.

5′-*O*-[(1,4-Dihydro-1-methyl-3-pyridinyl)carbonyl]-2′,3′-didehydro-2′,3′-dideoxythymidine (4). To a solution of 0.715 g (1.52 mmol) of 3 in 10% aqueous methanol, through which a stream of N_2 had previously been passed for 5 min, were added sequentially and in the course of 10 min under N_2 0.12 g of sodium bicarbonate (5 mmol) and 1.4 g of sodium dithionite (8 mmol). After 20 min, the collected and the dried solid, dissolved in a mixture of ethyl acetate–ethanol (8:2), was passed through a short column (50 × 30 mm) of silica gel. The eluate was evaporated to dryness to give a yellow solid (0.25 g, 50% yield): mp 145–147 °C; UV λ_{max} (EtOH) 358.3 nm (ε 6008), 262.4 (8580); ^{1}H NMR

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1.747 (s, 3 H, C_5-CH_3), 2.902 (s, 5 H, NCH_3 + $H_{4''}$), 4.15 (m, 1 H, $H_{5'}$), 4.26 (m, 1 H, $H_{5'}$), 4.70 (m, 1 H, $H_{5''}$), 4.94 (m, 1 H, $H_{4'}$), 5.82 (d, 1 H, $H_{6''}$), 5.98 (d, 1 H, $H_{3'}$), 6.42 (d, 1 H, $H_{2'}$), 6.77 (br s, 1 H, $H_{1'}$), 7.00 (s, 1 H, $H_{2''}$), 7.139 (s, 1 H, H_6); exact mass calcd 345.1324, found 345.1330. Anal. ($C_{17}H_{19}N_3O_5$) C, H, N.

Oxidation of 4 by Hydrogen Peroxide. To a solution of H_2O_2 (0.2 M) contained in a UV cuvette equilibrated at 22 °C was added a solution of 4 to a final concentration of 5×10^{-6} M. The mixture was thoroughly mixed and followed for the disappearance of the dihydronicotinate absorbance max at 360 nm.

In Vitro Oxidation of 4 in Biological Media. A solution of 4 (5×10^{-5} M) in freshly prepared human plasma diluted with an equal volume of 50 mM phosphate buffer, pH 7.4, was maintained at 37 °C in a UV cuvette and the rate of disappearance of the (dihydronicotinate) absorbance peak at 360 nm was determined.

The rates of disappearance of 4 (5×10^{-5} M) in a mouse liver (20 mg/mL) and mouse brain (40 mg/mL) homogenates in 50 mM phosphate buffer, pH 7.4, was determined similarly at 37 °C. The rate constant for the oxidation of 4 to 3 in both plasma and tissues was determined from the slope of the log of the disappearance curve.

In Vivo Administration of 4. Female BDF/1 mice (15) were injected via the tail vein with 4 (25 mg/kg), which was first dissolved in ethanol and then homogenized with 5% Tween 80 to yield a final mixture which contained 5% ethanol. Blood, brain, and liver tissues were obtained at 1, 2, 3, 6, and 24 h postinjection. Tissue homogenizations and HPLC studies were carried out as described above. The half-lives of compound 4 in brain, liver, and plasma were determined by calculating the negative slope of the log of the disappearance of 3 following the intravenous administration of 4.

In Vivo Administration of 1. The same strain of female mice (3) received, via tail-vein injection, 25 mg/kg of 1 containing 100 μCi of [*Me*-^{3}H]-1. After 1 h, the animals were sacrificed and the weighed brains were homogenized in 5 volumes of 50% MeOH. The supernatant layer, obtained after centrifugation of the homogenate (10000*g*) for 20 min, was evaporated to dryness and the residue dissolved in MeOH. Aliquots (900 μL) of the latter solution were dissolved in 10 mL of ACS (Amersham Corp.) liquid phosphor, and the level of radioactivity was monitored in a scintillation spectrometer. It was determined from the data that the brain contained 0.44 + 0.12 μg of 1/g of wet tissue.

TLC of homogenate on RP-18 plates (0.1 M NH_4OAc–CH_3CN, 80/20) gave a spot, containing >90% of the radioactivity, with an R_f identical with that of an authentic sample of unlabeled 1.

Acknowledgment. This investigation was supported, in part, by a grant from the American Foundation for AIDS Research (Award No. 000280) and an institutional grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit.

A Novel Prodrug of an Impermeant Inhibitor of 3-Deoxy-D-*manno*-2-octulosonate Cytidylyltransferase Has Antibacterial Activity

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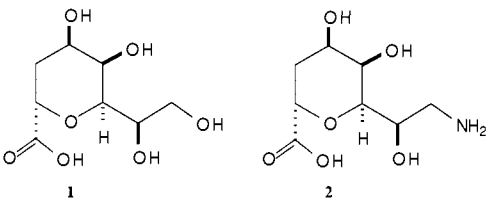
Although 8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonic acid (2) is a potent inhibitor of 3-deoxy-D-*manno*-octulosonate cytidylyltransferase (CMP-KDO synthetase), it is unable to reach its cytoplasmic target and is therefore inactive as an antibacterial agent. However, esterification of 2 with 8-(hydroxymethyl)-1-naphthyl methyl disulfide (8) generates a prodrug (12), which gains entry into bacterial cells. Intracellular reduction of the disulfide leads to a rapid, intramolecular, displacement of the acid 2, which then inhibits the growth of Gram-negative bacteria by interfering with the biosynthesis of lipopolysaccharide.

The molecular properties required for the effective delivery of an agent to its biological target are frequently incompatible with the structural features necessary for optimal activity at that target. This dichotomy can usually be bridged by the development of a prodrug.[1] Regardless of the goal—improved oral absorption, passage through the blood–brain barrier, or intraocular delivery, for example—the limiting factor is often the ability of a molecule to penetrate lipid bilayers. Even apart from the human host, this problem arises at a fundamental level in the development of antibacterial agents that act at a cytoplasmic target.[2]

Compounds 1[3] and 2,[3] for instance, are both potent (K_i = 12 μM and 4 μM, respectively) in vitro inhibitors of



1 2

CMP-KDO synthetase (3-deoxy-D-*manno*-octulosonate cytidylyltransferase), a key enzyme in the biosynthesis of

(1) For a review of prodrugs, see: *Bioreversible Carriers in Drug Design: Theory and Application*; Roche, E. E., Ed.; Pergamon Press: New York, 1987.

(2) For a discussion of the transport of antibiotics into bacteria, see: Chopra, I.; Ball, P. *Adv. Microb. Physiol.* **1982**, *23*, 183–240.

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