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.

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

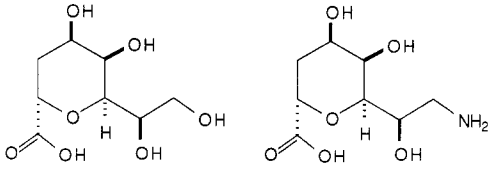
Daniel W. Norbeck,* William Rosenbrook, James B. Kramer, David J. Grampovnik, and Paul A. Lartey

Anti-infective Research Division, Abbott Laboratories, Abbott Park, Illinois 60064. *Received July 22, 1988*

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.

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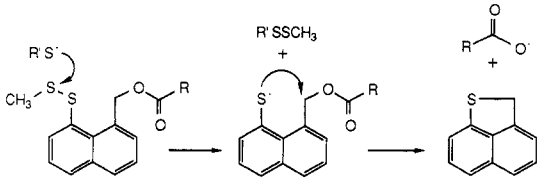
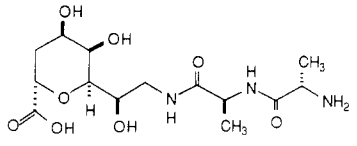
Scheme I



4 5 6

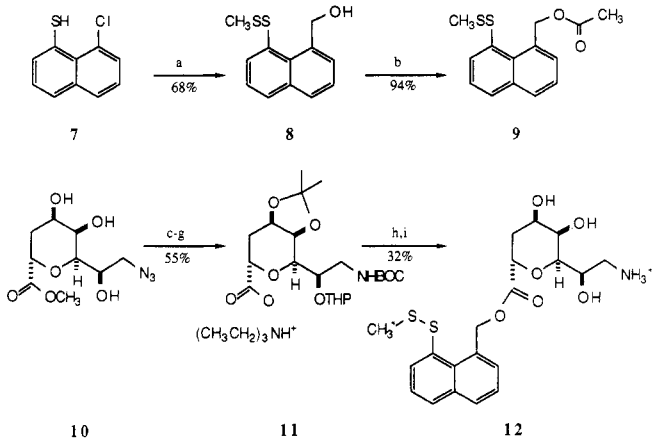
the lipopolysaccharide (LPS) of Gram-negative bacteria.[4,5] Since temperature-induced inhibition of this enzyme in temperature-sensitive mutants stops bacterial growth,[6] compounds 1 and 2 might be expected to act as bacteriostatic agents. In fact, their antibacterial activity is negligible. CMP-KDO synthetase is an intracellular enzyme, but the passage of radiolabeled 1 through the cytoplasmic membrane is insignificant.[7] To overcome this problem, we[3,8] and others[9] have linked the amino acid 2 to various dipeptides to generate prodrugs such as 3 which



3

are delivered to their target by a bacterial oligopeptide permease. Intracellular cleavage of the prodrug by aminopeptidases then releases the inhibitor. In contrast to the free acid, entry of the radiolabeled methyl ester of 1 into the cytoplasm *is* significant.[7] This observation suggested an alternative to the peptide portage strategy[10] for the uptake of impermeant molecules into bacterial cells. Because esters of 1 or 2 do not inhibit CMP-KDO synthetase, the success of this approach would hinge on

Scheme II. Synthesis of a Novel Ester Prodrug[a]



7 8 9 10 11 12

[a] (a) *n*-BuLi, THF; $(CH_2O)_n$; $MeSS(O)_2Me$; (b) Ac_2O, DMAP, CH_2Cl_2; (c) $(CH_3)_2CO$, H^+-Resin; (d) H_2, Pd–C, MeOH; (e) $(BOC)_2O$, TEA, MeOH; (f) dihydropyran, PPTS, CH_2Cl_2; (g) LiOH, H_2O; H_3O^+; TEA; (h) 8, BOP-Cl, TEA; (i) TFA, THF, H_2O.

the development of esters that would deliver and then release the warhead in the cytoplasm.

Initially, a series of simple esters of compound 1 was evaluated in a toluene-permeabilized cell assay[11] in which both compounds 1 and 2 can be shown to inhibit the incorporation of radiolabeled KDO into LPS. Similar inhibition by the esters of 1 and 2 would thus serve as an indirect assay for the hydrolysis of these esters to the corresponding acid. However, of the esters of compound 1 evaluated (methyl, (methylthio)methyl, (pivaloyloxy)methyl, allyl, propargyl, butyl, benzyl, 2-(dimethylamino)ethyl, [(*tert*-butyloxy)carbonyl]methyl, carboxymethyl, and the carboxamide and *N*-hydroxycarboxamide), none were appreciably cleaved by the test organism *Salmonella typhimurium* during a 60-min assay. This disapppointing result prompted us to conceive compounds of the general formula 4 (Scheme I) as esters with a novel, nonhydrolytic cleavage mechanism.

The design of this ester was predicated on both biological and chemical considerations. First, Gram-negative bacteria are known to contain significant quantities of free sulfhdyryl compounds,[12] notably glutathione.[13] Second, disulfide exchange reactions are known to be facile, even in the absence of enzymatic catalysis.[14,15] We anticipated that these circumstances would lead to the rapid generation of the thiolate anion 5. Furthermore, as a consequence of the well known "peri" effect in 1,8-disubstituted naph-

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thalenes,[16] the disulfide exchange was expected to be rendered irreversible by esterolytic dealkylation[17] and at a rate several orders of magnitude greater than that observed in the intermolecular version of this reaction.[18]

The synthesis of the required alcohol **8** from the easily accessible 8-chloro-1-naphthalenethiol (**7**)[19] was straightforward (Scheme II). Metalation of **7** with 2.0 equiv of *n*-butyllithium in THF as described by Meinwald[20] generated a dianion, which was sequentially quenched with dry paraformaldehyde and then methyl methanethiosulfonate to produce **8** in 68% yield. Condensation of **8** with acetic anhydride afforded the model ester **9**.

The reaction of a 0.1 M solution of **9** in $CDCl_3$ with 2.0 equiv of *n*-propanethiol and triethylamine at room temperature was monitored by NMR and TLC. Although the ester was unchanged by the presence of triethylamine alone, addition of *n*-propanethiol caused the gradual disappearance of the methylene resonance of **9** at 5.90 ppm and the emergence of a new singlet at 4.76 ppm due to the thioether **6**.[21] This conversion was 50% complete in 25 min. During the course of this transformation, two transient intermediates appeared on the TLC (silica gel, 1:1 ether/hexane) of the reaction mixture. The major intermediate (R_f = 0.39) was approximately equimolar to the starting ester **9** (R_f = 0.34) after 20 min and is probably the propyl naphthyl disulfide. On the basis of its greater polarity (R_f = 0.07), we believe that the minor intermediate is the triethylammonium thiolate of **5** (R = CH_3). Since this compound never constituted more than 5–10% of the reaction mixture, its generation must be the rate-limiting step in the ester cleavage. Formation of the thioether **6** (R_f = 0.59) appears to be quantitative by TLC and NMR, and the compound could be isolated by chromatography as a white solid in 90% yield.

Having established the mechanism and remarkable facility of this ester cleavage, we turned our attention to the synthesis of the prodrug **12**, in which the 8-amino function was chosen to enhance inhibitory potency and water solubility. As summarized in Scheme II, a series of standard functional group manipulations on the azide **10**[3,9b] set the stage for the BOP-Cl[22] mediated esterification of the carboxylate **11** with the alcohol **8**. The stability of the disulfide ester **12** to aqueous trifluoroacetic acid is suggestive of circumstances (e.g., oligopeptide and oligonucleotide synthesis) in which it could serve as a readily removable protecting group.

Addition of the acetate salt of ester **12** to a suspension of *S. typhimurium* caused a UV-active component identical by TLC with the thioether **6** to appear in the supernatant within 10 min. Release of the acid **2** from **12** was verified by inhibition of the incorporation of radioactive KDO into the LPS of the toluene-permeabilized[11] *S. typhimurium*. Although the rapidity of the cleavage made quantitative data difficult to obtain, analysis of the time dependence of this inhibition showed that the inhibitor was released from the disulfide ester *at least* several times more rapidly than from the dipeptide derivative **3** (33 pmol/min/mg protein).[8a] The antibacterial activity of **12**, however, was somewhat less than that of **3**. In an agar diffusion assay against *S. typhimurium* grown in a MOPS[23] medium, 1 μmol of the dipeptide prodrug applied to a 6.0-mm disk gave rise to a 29-mm zone of inhibition;[8a] 2 μmol of the disulfide prodrug under the same conditions produced a 17-mm zone of inhibition. Similarly, while addition of 100 μg/mL of **3** to a growing suspension of *S. typhimurium* induced bacteriostasis as measured by the absorbance at 420 nm,[8a] 200 μg/mL of **12** was required to induce a comparable effect. The underivatized amino acid **2** exhibited no antibacterial activity in either assay.[8a]

Several experiments were conducted to confirm the disulfide prodrug's mode of action. First, nonspecific effects were ruled out by the lack of antibacterial activity of the acetate **9** or the thioether **6**, alone or in combination with the amino acid **2**. More conclusively, the disulfide prodrug was shown to induce the accumulation of the same lipid A precursors and side products observed in mutant bacteria (kdsB) with a temperature-sensitive CMP-KDO synthetase at the nonpermissive temperature.[8a,11,24] Since the dipeptide **3** also induces these precursors, it is clear that both prodrugs deliver the acid **2** to the cytoplasm and then release it to inhibit CMP-KDO synthetase.

Although the peptide-based delivery system benefits from active transport into the cytoplasm, these systems are prone to mutation and the consequent emergence of resistance.[10,25] The disulfide ester, on the other hand, presumably enters the cytoplasm via passive diffusion, a relatively slow but difficult process to select against. Further modification of the ester prodrug, particularly in the naphthalene portion, should lead to a structure that strikes the optimum compromise between the requirements for passage through the aqueous porin channels of the outer membrane[26] and the lipid cytoplasmic membrane.

Given the ubiquitous nature of free sulfhydryls in living systems,[13] potential applications of the disulfide ester **4** to the development of other prodrugs should not be overlooked.[27] A single intraperitoneal injection of either the ester **9** or its metabolite **6** caused minimal cute toxicity in mice. While the LD_{50} of **6** was ~640 mg/kg, the LD_{50} of **9** was >1000 mg/kg.

Experimental Section

^1H NMR spectra were recorded at 300 MHz. Chemical shifts are reported as values relative to tetramethylsilane (δ = 0.0) as an internal standard. Data are reported as follows: chemical shift

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(27) Prosultiamine and disulfiram are two commonly used disulfide prodrugs. *The Merck Index*, 10th Ed.; Windholz, M., Ed.; Merck: Rahway, NJ, 1983.

(multiplicity, integrated intensity, coupling constants, assignment). Optical rotations were measured in 1-dm cells of 1-mL capacity on a digitial polarimeter. Analytical thin layer chromatography was conducted on precoated glass plates: silica gel 60 F-254, layer thickness 0.25 mm, E. Merck and Co. Silica gel chromatography utilized E. Merck silica gel 60 (70–230-mesh ASTM). Reaction solvents were dried and distilled before use, and all reactions were conducted under an inert atmosphere.

8-(Hydroxymethyl)-1-naphthyl Methyl Disulfide (8). To a stirred solution of 4.00 g (20.5 mmol) of 8-chloro-1-naphthalenethiol[19] in 100 mL of THF under an argon atmosphere at −34 °C was added 17.5 mL (45.5 mmol) of 2.60 M *n*-butyllithium in hexanes over 1.5 min. After 10 min, the temperature had risen to −27 °C, and 1.25 g (41.6 mmol) of dry, solid paraformaldehyde was added in one portion. The temperature was allowed to gradually rise to −1 °C over 60 min and then to 2 °C over the next 50 min. The reaction mixture was then recooled to −60 °C, and 2.30 mL (22.3 mmol) of methyl methanethiolsulfonate was added over 30 s. The cooling bath was then removed, and when the temperature had reached 0 °C (30 min), the reaction mixture was acidified with 100 mL of 2% (v/v) aqueous HOAc, extracted into 500 mL of Et_2O, washed with 150 mL of saturated aqueous NaCl, 150 mL of 5% aqueous $NaHCO_3$, and 150 mL of saturated aqueous NaCl, dried over $MgSO_4$, and then evaporated under reduced pressure. Chromatography of the residue on 300 g of silica gel with 1:1 Et_2O/hexane afforded 3.30 g (68%) of the title compound (**8**) as an oil, judged to be at least 95% pure by TLC and ^1H NMR: R_f = 0.37 (silica gel, 1:1 Et_2O/hexane): IR ($CDCl_3$) 3605, 3530, 3065, 2960, 2920, 2900, 1595, 1570, 1500, 1390, 1170, 1000 cm^{-1}; ^1H NMR ($CDCl_3$) 2.46 (s, 3 H, CH_3S), 5.36 (s, 2 H, CH_2O), 7.46, 7.49 (2 dd, 2 H, J = 7.5 Hz, J' = 4.5 Hz, H-3 and H-6), 7.66 (dd, 1 H, J = 7.5, J' = 1.5 Hz), 7.85 (dd, 1 H, J = 4.5 Hz, J' = 1.5 Hz), 7.87 (dd, 1 H, J = 4.5 Hz, J' = 1.5 Hz), 8.08 (dd, 1 H, J = 7.5 Hz, J' = 1.5 Hz); EI MS, m/z 236 M^+, 188 ($M-CH_3S-H)^+$, 171 ($M-CH_3S-H_2O)^+$; exact mass calcd for $C_{12}H_{12}OS_2$ 236.0330, found 236.0331.

8-(Acetoxymethyl)-1-naphthyl Methyl Disulfide (9). To a stirred solution of 2.00 g (8.46 mmol) of the above alcohol (**8**) in 50 mL of dry CH_2Cl_2 at 0 °C were added 1.04 g (8.51 mmol) of 4-(dimethylamino)pyridine and then 0.96 mL (10.2 mmol) of acetic anhydride. The reaction mixture was then allowed to warm to room temperature, and after 1 h was diluted with 100 mL of CH_2Cl_2, washed with 2 × 25 mL of saturated aqueous NaCl, dried over $MgSO_4$, and concentrated under reduced pressure. Chromatography of the residue on 150 g of silica gel with toluene afforded 2.21 g (94%) of the title compound (**9**) as an oil, judged to be at least 95% pure by TLC and ^1H NMR: R_f = 0.34 (silica gel, 1:1 Et_2O/hexane); IR ($CDCl_3$) 3065, 2920, 1740, 1595, 1570, 1500, 1430, 1385, 1365, 1245, 1235, 1025 cm^{-1}; ^1H NMR ($CDCl_3$) 2.15 (s, 3 H, CH_3CO), 2.41 (s, 3 H, CH_3S), 5.91 (s, 2 H, CH_2O), 7.47 (2 dd, 2 H, J = J' = 7.5 Hz, H-3 and H-6), 7.61 (dd, 1 H, J = 7.5 Hz, J' = 1.5 Hz), 7.86, 7.88 (2 dd, 2 H, J = 7.5 Hz, J' = 1.5 Hz), 8.09 (dd, 1 H, J = 7.5 Hz, J' = 1.5 Hz); EI MS, m/z 278 M^+, 188 ($M-CH_3S-COCH_3)^+$, 171 ($M-CH_3S-HOAc)^+$; exact mass calcd for $C_{14}H_{14}O_2S_2$ 278.0435, found, 278.0427.

***2H*-Naphtho[1,8-*bc*]thiophene (6).** To a stirred solution of 604 mg (2.17 mmol) of the above acetate (**9**) in 20 mL of dry CH_2Cl_2 at room temperature were added 1.00 mL (13.5 mmol) of ethanethiol and 0.70 mL (5.1 mmol) of Et_3N. After 30 min, the reaction mixture was concentrated under reduced pressure. Chromatography of the residue on 60 g of silica gel with hexane afforded 336 mg (90%) of the title compound (**6**) as an amorphous white solid, judged to be greater than 95% pure by TLC and ^1H NMR: R_f = 0.59 (silica gel, 1:1 Et_2O/hexane); IR ($CDCl_3$) 3060, 2920, 1580, 1490, 1360, 1200, 1070 cm^{-1}; ^1H NMR ($CDCl_3$) 4.78 (s, 2 H, CH_2), 7.20 (d, 1 H, J = 7.5 Hz), 7.33 (dd, 1 H, J = J' = 7.5 Hz), 7.35 (dd, 1 H, J = 7.5 Hz, J' = 1 Hz), 7.42 (d, 1 H, J = 7.5 Hz), 7.44 (dd, 1 H, J = J' = 7.5 Hz), 7.60 (dd, 1 H, J = 7.5 Hz, J' = 1.0 Hz); EI MS, m/z 172 M^+, exact mass calcd for $C_{11}H_8S$ 172.0347, found 172.0324.

Methyl *N*-[(1,1-Dimethylethoxy)carbonyl]-8-amino-2,6-anhydro-3,8-dideoxy-4,5-*O*-(1-methylethylidene)-D-*glycero*-D-*talo*-octonate. To a stirred solution of 1.69 g (6.47 mmol) of methyl 2,6-anhydro-8-azido-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (**10**) (R_f = 0.28, silica gel, 9:1 CH_2Cl_2/MeOH) in 100 mL of dry acetone at room temperature was added 3.4 g of dry Dowex HCR-S (H^+) resin. After 4 h, the reaction mixture was filtered into 50 mL of 5% aqueous $NaHCO_3$. The acetone was then removed under reduced pressure, and the remaining aqueous phase was extracted with 2 × 150 mL of CH_2Cl_2. The combined organic phases was dried over $MgSO_4$ and concentrated under reduced pressure to afford 1.55 g (80%) of the acetonide as a syrup homogeneous by TLC (R_f = 0.70, silica gel, 9:1 CH_2Cl_2/MeOH). Then 1.50 g (4.98 mmol) of the acetonide was dissolved in 75 mL of dry MeOH at room temperature and stirred under a hydrogen atmosphere (balloon) over 320 mg of 5% Pd on carbon. After 3 h, the reaction was degassed with nitrogen, and then the catalyst was removed with a 0.5-μm filter. Concentration of the resulting solution under reduced pressure afforded 1.37 g (100%) of the amine (R_f = 0.20, silica gel, 80:20:1 CH_2Cl_2/MeOH/15M NH_4OH) as a white foam. To a solution of 522 mg (1.89 mmol) of this amine in 40 mL of dry MeOH at room temperature were added 0.26 mL (1.9 mmol) of Et_3N and then 465 mg (2.13 mmol) of di-*tert*-butyl dicarbonate. After 1 h, the reaction mixture was concentrated under reduced pressure. Chromatography of the residue on 80 g silica gel with 1:1 EtOAc/hexane afforded 638 mg (90%) of the title compound as a white foam: R_f = 0.14 (silica gel, 1:1 EtOAc/hexane); $[\alpha]^{22}_D$ +23.8° (*c* 3.10, $CHCl_3$); IR ($CDCl_3$) 3445, 2980, 2935, 1740, 1680, 1505, 1450, 1435, 1375, 1365, 1275, 1250, 1200, 1160, 1120, 1065 cm^{-1}; ^1H NMR ($CDCl_3$) 1.37, 1.48 (2s, 6 H, $(CH_3)_2C$), 1.46 (s, 9 H, $(CH_3)_3C$), 1.84 (ddd, 1 H, J = 14 Hz, J' = 11 Hz, J'' = 3 Hz, H-3), 2.33 (ddd, 1 H, J = 14 Hz, J' = 6 Hz, J'' = 3 Hz, H-3), 3.33 (ddd, 1 H, J = 15 Hz, J' = J'' = 5 Hz, H-8), 3.50 (dd, 1 H, J = 9 Hz, J' = 1.5 Hz, H-6), 3.68 (ddd, 1 H, J = 15 Hz, J' = 8 Hz, J'' = 2 Hz, H-8), 3.72 (d, 1 H, J = 5 Hz, OH), 3.77 (s, 3 H, OCH_3), 3.87 (dddd, 1 H, J = 9 Hz, J' = J'' = 5 Hz, J''' = 2 Hz, H-7), 4.45 (dd, 1 H, J = 8 Hz, J' = 1.5 Hz, H-5), 4.56 (dd, 1 H, J = 11 Hz, J' = 6 Hz, H-2), 4.59 (ddd, J = 8 Hz, J' = J'' = 3 Hz, H-4), 5.71 (bdd, 1 H, J = 8 Hz, J' = 5 Hz, NH); EI MS, m/z 376 ($M+H)^+$, 360 ($M-CH_3)^+$.

[8-(Methyldithio)-1-naphthyl]methyl 8-Amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (12). To a stirred solution of 408 mg (1.08 mmol) of the above alcohol in 5.0 mL of CH_2Cl_2 at room temperature were added 0.49 mL (5.4 mmol) of dihydropyran and 27 mg (0.11 mmol) of pyridinium *p*-toluenesulfonate. After 3 h, the reaction mixture was diluted with 50 mL of Et_2O, washed with 2 × 25 mL of 50% saturated aqueous NaCl, dried over $MgSO_4$, and concentrated under reduced pressure. Chromatography of the residue on 40 g of silica gel with 3:7 EtOAc/hexane afforded 407 mg (82%) of the THP ether as an approximately 1:1 mixture of diastereomers (R_f = 0.14, 0.09; silica gel, 1:3 EtOAc/hexane). To a solution of 395 mg (0.859 mmol) of this ester in 5.0 mL of MeOH at room temperature was added 1.89 mL (0.945 mmol) of 0.5 N aqueous LiOH. After 1 h, the reaction mixture was diluted with 100 mL of cold CH_2Cl_2 and then washed with 50 mL of saturated aqueous NaCl maintained at pH 3 with dilute H_2SO_4. The organic phase was separated, and the aqueous phase was quickly extracted with an additional 50 mL of CH_2Cl_2. The combined organic extracts were briefly dried over $MgSO_4$, filtered, treated with 0.395 mL (2.83 mmol) of Et_3N, and then concentrated under reduced pressure to afford 443 mg (94%) of the triethylammonium carboxylate salt **11** as a white solid.

To a solution of 385 mg (0.705 mmol) of this salt and 200 mg (0.846 mmol) of the alcohol (**8**) in 7.0 mL of THF at room temperature was added 0.197 mL (1.41 mmol) of Et_3N and 269 mg (1.06 mmol) of *N,N*-bis(2-oxo-3-oxazolidinyl)phosphordiamidic chloride in one portion. After 24 h, the reaction was diluted with 75 mL of EtOAc and washed with 2 × 20 mL of 90% saturated aqueous NaCl. The combined aqueous phases were extracted with 50 mL of EtOAc, and the combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. Chromatography of the residue on 50 g of silica gel with 15:85 EtOAc/hexane afforded 372 mg (79%) of the ester (**12**) as a syrup, which appeared to be homogeneous by TLC: R_f = 0.13 (silica gel, 1:3 EtOAc/hexane); IR ($CDCl_3$) 3365, 2980, 2940, 1735, 1700, 1515, 1365, 1245, 1080, 1060 cm^{-1}; DCI-NH_3 MS, m/z 664 ($M+H)^+$, 580 ($M-C_5H_8O)^+$. To a stirred solution of 372 mg (0.560 mmol) of the above ester in 1.0 mL of THF was added 20 mL of 2 N aqueous trifluoroacetic acid. After 3 days at room temperature, the reaction mixture was frozen and the solvent removed by lyophilization. Chromatography of the residue on 30 g of silica

gel with 25:70:30:0.2 MeOH/$CHCl_3$/H_2O/HOAc afforded 112 mg (40%) of the amino ester, judged to be 90% pure by TLC and NMR. Rechromatography of a portion of this material as before raised the purity to greater than 95%, and these samples were used for biological testing: R_f = 0.14 (silica gel, 25:70:3.0:0.1 MeOH/$CHCl_3$/H_2O/HOAc); IR (KBR pellet) 3420, 2920, 1735, 1685, 1570, 1420, 1205, 1185, 1130, 1060, 1010, 820, 765 cm^{-1}. ^1H NMR (500 MHz, D_2O, HOD = 4.80 ppm) 2.08 (ddd, 1 H, $J = J'$ = 13 Hz, J'' = 7 Hz, H-3ax), 2.19 (dd, 1 H, J = 13 Hz, J' = 5 Hz, H-3eq), 2.43 (s, 3 H, SCH_3), 2.46 (dd, 1 H, J = 13 Hz, J' = 8 Hz, H-8), 2.97 (dd, 1 H, J = 13 Hz, J' = 4 Hz, H-8), 3.49 (d, 1 H, J = 8 Hz, H-6), 3.78 (ddd, 1 H, J = 13 Hz, J' = 5 Hz J'' = 2.5 Hz, H-4), 3.90 (ddd, 1 H, $J = J'$ = 8 Hz, J'' = 4 Hz, H-7), 3.96 (d, 1 H, J = 2.5 Hz, H-5), 4.74 (d, 1 H, J = 7 Hz, H-2), 5.96, 6.04 (2 d, 2 H, J = 13 Hz, $ArCH_2$), 7.61, 7.615 (2 t, 2 H, $J = J'$ = 7.5 Hz, Ar-H-3 and Ar-H-6), 7.73, 8.07, 8.09, 8.17 (4 d, 4 H, J = 7.5 Hz); positive FAB MS (NBA), m/z 440 $(M + H)^+$; exact mass calcd for $C_{20}H_{26}NO_6S_2$ 440.1202, found 440.1208.

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Antitumor and Antiviral Activity of Synthetic α- and β-Ribonucleosides of Certain Substituted Pyrimido[5,4-*d*]pyrimidines: A New Synthetic Strategy for Exocyclic Aminonucleosides

Yogesh S. Sanghvi,* Steven B. Larson, Steven S. Matsumoto, L. Dee Nord, Donald F. Smee, Randall C. Willis, Thomas L. Avery, Roland K. Robins, and Ganapathi R. Revankar

Nucleic Acid Research Institute, 3300 Hyland Avenue, Costa Mesa, California 92626. *Received May 16, 1988*

A novel and direct synthesis of the antiviral and antitumor agent 4-amino-8-(β-D-ribofuranosylamino)pyrimido-[5,4-*d*]pyrimidine (ARPP, **8**) and its α-anomer (**11**) has been developed. Treatment of 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine (**1**) with 2,3-*O*-isopropylidene-D-ribofuranosylamine gave an anomeric mixture of 2,4,6-trichloro-8-(2,3-*O*-isopropylidene-β- and -α-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidines (**3** and **4**) in a ratio of 1.0:0.7. A nucleophilic displacement of the 4-chloro group of **3** and **4** with NH_3 furnished 4-amino-2,6-dichloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**6**) and its α-anomer (**9**), respectively. Catalytic hydrogenation of **6** and **9**, followed by deisopropylidenation gave ARPP (**8**) and the α-anomer **11**, respectively. Similarly, **3** and **4** have been transformed to 4-methoxy-8-(β-D-ribofuranosylamino)pyrimido-[5,4-*d*]pyrimidine (MRPP, **14**) and its α-anomer (**17**). Application of this procedure to **3** with NH_2Me or $NHMe_2$ resulted in the synthesis of 4-(methylamino)- and 4-(dimethylamino)-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]-pyrimidine (**24** and **27**, respectively). A synthesis of 8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidin-4(3*H*)-one (**21**) has also been accomplished from **3** in three steps. Selective hydrogenation of **6** furnished 4-amino-6-chloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**36**), the structure of which was established by single-crystal X-ray diffraction analysis. Deisopropylidenation of **36** gave 6-chloro-ARPP (**37**). Extended treatment of **36** with NH_3 furnished 4,6-diamino-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**34**), which on deisopropylidenation gave 6-amino-ARPP (**35**). An unambiguous synthesis of **34** and **36** has also been accomplished by the reaction of 4,6,8-trichloropyrimido[5,4-*d*]pyrimidine (**28**) with **2**, followed by the treatment with NH_3. Nucleophilic displacement studies with **1**, **6**, and **28** indicated the reactivity of the halogens in these compounds is in the order of 8 > 4 > 6 > 2. The structures of **3** and **9** have been assigned on the basis of ^1H NMR data and further confirmed by single-crystal X-ray diffraction analysis. The exocyclic aminonucleosides synthesized during this study were tested for their activity against several RNA and DNA viruses in vitro and against L1210, WI-L2, and LoVo/L in cell culture. The effect of these compounds on the de novo nucleic acid biosynthesis has been studied. Compound 14 (MRPP) exhibited enhanced activity against L1210 in vivo, when compared to ARPP (**8**).

The recent molecular biology and biochemistry of purine and purine nucleoside analogues showing potent antiviral and antitumor activity has uncovered a number of new potential targets.[1-4] The pyrimido[5,4-*d*]pyrimidine ring system has attracted considerable attention in recent years as the deaza analogue of the naturally occurring antibiotics toxoflavin and fervenulin.[5] Dipyridamole, a pyrimido-[5,4-*d*]pyrimidine derivative, has shown coronary vasodilator properties.[6] The synthesis of the naturally occurring exocyclic aminonucleoside clitocine has recently been reported from our laboratory.[7] The synthesis[8] and the biological properties[9] of an unusual exocyclic aminonucleoside, 4-amino-8-(β-D-ribofuranosylamino)pyrimido-[5,4-*d*]pyrimidine (ARPP, **8**) has also been reported from our laboratory. ARPP has shown broad-spectrum antiviral activity against both DNA and RNA viruses in cell culture by inhibiting viral protein synthesis.[10] ARPP exhibited immunosuppressive activity and inhibited the growth of L1210 leukemia in mice.[2] Molecular mechanics calculations of ARPP and certain related nucleosides[11] showed that their conformational behavior is very similar even when groups like chloro or amino are introduced at pos-

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