2.01 (q, 2 H, $OCH_2CH_2CH_2N^+$), 2.09 (s, 3 H, CH_3CO), 2.91 (s, 6 H, $^+N(CH_3)_2$), 3.24 (t, 2 H, $OCH_2CH_2CH_2N^+$), 3.46 (m, 2 H, OCH_2C_{17}), 3.55 (d, 2 H, CH_2OC_{18}), 3.62 (m, 4 H, $CH_2O + CH_2N^+$), 5.17 (q, 1 H, CHOCO); mass spectrum, m/z 472 ($MH^+ - HCl$). Anal. ($C_{28}H_{58}NO_4Cl \cdot 2H_2O$) C, H, N, Cl.

[3-(1-*O*-Octadecyl-2-*O*-benzylglycero-3)propyl]trimethylammonium Iodide (5). Compound **2** (1.37 mmol) and MeI (13.7 mmol) in 10 mL of dry acetone were stirred overnight at room temperature. Acetone and the excess of MeI were evaporated, and the residue was purified on a silica gel column using $CHCl_3$/MeOH (90:10, v/v) as eluent to yield **5** (48%): R_f 0.29 ($CHCl_3$/MeOH/H_2O, 80:20:2, v/v/v); ^1H NMR (80 MHz, CD_3OD) δ 0.82 (t, 3 H, CH_3), 1.22 (large s, 32 H, $(CH_2)_{16}$), 2.00 (m, 2 H, $OCH_2CH_2CH_2N^+$), 3.05 (s, 9 H, $^+N(CH_3)_3$), 3.27–3.75 (m, 11 H, $CH_2O + CHO + CH_2N^+$), 4.61 (s, 2 H, $CH_2C_6H_5$), 7.27 (large s, 5 H, C_6H_5).

[3-(1-*O*-Octadecylglycero-3)propyl]trimethylammonium Chloride (6). A solution of 0.1–0.2 mol of HCl in 40 mL of MeOH was added to **5** (1.5 mmol). MeOH and MeI were distillated under normal pressure. This operation was repeated twice. The remaining residue was chromatographed on a silica gel column using $CHCl_3$/MeOH (80:20, v/v) as eluent. This purification yielded pure **6** (40%): R_f 0.12 ($CHCl_3$/MeOH/H_2O, 80:20:2, v/v/v).

[3-(1-*O*-Octadecyl-2-*O*-acetylglycero-3)propyl]trimethylammonium Chloride (7). Compound **6** was acetylated in the same manner as described above for **4** and gave **7** in 100% yield: R_f 0.13 ($CHCl_3$/MeOH/H_2O, 80:20:2, v/v/v); IR (film) 3400, 1640 (H_2O), 1730 (C=O), 1260 (COC ester), 1020 (COC ether) cm^{-1}; ^1H NMR (CD_3OD) δ 0.81 (t, 3 H, CH_3), 1.21 (large s, 30 H, $(CH_2)_{15}$), 1.45 (m, 2 H, $OCH_2CH_2C_{16}$), 1.95 (m + s, 5 H, $OCH_2CH_2CH_2N^+ + CH_3CO$), 3.05 (s, 9 H, $^+N(CH_3)_3$), 3.26–3.56 (m, 10 H, $CH_2O + CH_2N^+$), 5.04 (q, 1 H, CHOCO); mass spectrum, m/z 472 ($MH^+ - CH_3Cl$). Anal. ($C_{29}H_{60}NO_4Cl \cdot 2H_2O$) C, H, N, Cl.

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Improved Synthesis of 2′-Deoxyformycin A and Studies of Its in Vitro Activity against Mouse Lymphoma of T-Cell Origin

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7-Amino-3-(2′-deoxy-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (2′-deoxyformycin A) was synthesized from formycin A by a sequence consisting of (i) 3′,5′-cyclosilylation with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, (ii) 2′-acylation with phenoxythiocarbonyl chloride and 4-(*N*,*N*-dimethylamino)pyridine, (iii) N-trimethylsilylation with hexamethyldisilazane, (iv) reduction of the 2′-*O*-phenoxythiocarbonyl group with tri-*n*-butyltin hydride, and (v) desilylation with tetra-*n*-butylammonium fluoride. 2′-Deoxyformycin A was a potent inhibitor of the in vitro growth of S49 lymphoma, a murine tumor of T-cell origin. The IC_{50} of 2′-deoxyformycin A against S49 cells was 10–15 μM, whereas that of 2′-deoxyadenosine (dAdo) under the same conditions (72-h incubation in medium containing heat-inactivated horse serum) was 180 μM. In the presence of 10 μM *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) to block intracellular adenosine deaminase (ADA) activity, 2′-deoxyformycin A and dAdo both gave IC_{50}'s of 5–10 μM. When assayed against a mutant S49 subline lacking adenosine kinase (AK) or a subline with a combined deletion of AK and deoxycytidine kinase (dCK), 2′-deoxyformycin A in combination with 10 μM EHNA was inactive at concentrations of up to 50 μM. Similar lack of activity against kinase-deficient cells was shown by formycin A. Thus, phosphorylation of 2′-deoxyformycin A appears to be required for biological activity and is probably catalyzed by AK rather than dCK. 2′-Deoxyformycin A and related 2′-deoxyribo-C-nucleoside analogues of the purine type may be of interest as potential T-cell specific cytotoxic agents.

Formycin A (7-amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine (1) is a C-nucleoside antibiotic with biochemical and biological properties that reflect its ability to mimic adenosine in being incorporated into RNA.[1] The resultant production of altered RNA species is lethal to cells. For a number of years, clinical enthusiasm for **1** was dampened by the fact that it is hydrolyzed readily by adenosine deaminase (ADA) to the less active 7(6*H*)-oxo derivative, formycin B.[2] Recently, interest in the potential of **1** as an anticancer agent has been rekindled by the finding that, in combination with an ADA inhibitor, this compound is superior to adenine arabinoside (*ara*-A) in prolonging the life of mice with L1210 leukemia.[3] Moderate antiviral activity is also observed.[4] Moreover, it has been demonstrated in mammalian cells[5,6] and in *Leishmania*[7,8] that the adenylosuccinate synthetase/lyase system provides a pathway for the metabolic regeneration of

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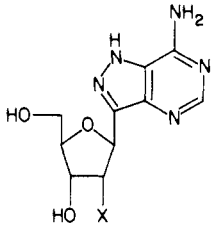
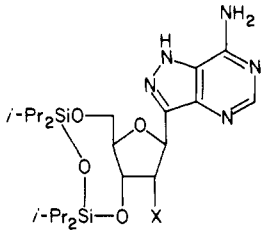
1 from formycin B. These findings, together with the improved availability of 1 via the elegant route of Buchanan and co-workers,[9] make this compound and its structural analogues attractive candidates for further investigation.

While much has been done to delineate the mode of action of 1,[1] little is known concerning the 2′-deoxyribonucleoside 2, which may be viewed as a 2′-deoxyadenosine (dAdo) analogue. A suggestion that this compound may indeed be of chemotherapeutic interest has come recently from the report[10] that partial incorporation of tritium occurs in the DNA of cultured human colon carcinoma cells upon incubation with [^{3}H]-1. Similarly, when mouse fibroblasts (L cells) are treated with [^{3}H]formycin B to generate [^{3}H]-1 and its phosphorylated metabolites in situ, there is partial recovery of the label in DNA, implying intracellular nucleotide to deoxynucleotide reduction.[6]

Moffatt and co-workers[11] reported in 1973 a synthesis of 2 that involved reaction of 1 with acetoxyisobutyryl bromide followed by ammonolysis and reductive dehalogenation. The approach produced a mixture of 2 and the 3′-deoxy isomer, with the latter predominating by a ratio of more than 2:1. Since we desired to evaluate the biological activity of 2 in connection with a proposed larger study of dAdo analogues as antileukemic agents with T-cell specificity,[12] an improved route to 3 was sought. In this paper we describe a new synthesis of 2 that is superior to the older one in that it avoids formation of the 3′-deoxy isomer. We also report the in vitro antitumor activity of 2 in the presence and absence of the ADA inhibitor *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA)[13] and compare the activity of 2 with that of 1 and dAdo. Finally, we present evidence that 2, like 1, requires phosphorylation in order to inhibit cell growth and that the enzyme responsible for this reaction is probably adenosine kinase (AK) rather than deoxycytidine kinase (dCK).



1. X = OH (formycin A)
2. X = H (2′-deoxyformycin A)



3. X = OH
4. X = OC(=S)OPh

Chemistry. An advantageous method of 3′,5′-protection of ribonucleosides uses the reagent 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane,[14,15] which in the presence of a proton acceptor affords a cyclic disiloxy derivative. Reaction of 1 with 1.1 mol equiv of the dichlorodisiloxane in dry pyridine gave a single major product, from which was isolated after silica gel chromatography a 46% yield of the crystalline compound 3. As expected, and in contrast to 1, this compound was soluble in nonpolar solvents such as acetonitrile, from which it could be recrystallized. The NMR spectrum of 3 showed the four isopropyl groups as a multiplet at δ 1.04. Also prominent were the anomer $C_{1'}$-H (δ 5.36) and heterocyclic ring C_5-H (δ 8.2). It may be noted that, despite several attempts to improve the final yield of 3, this did not exceed 50%. Thus, 3′,5′-protection of 1 with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane is less efficient than the reaction of the more common purine and pyrimidine ribonucleosides.[15] We found that 1, which is available as a hemihydrate, remains tenaciously solvated despite strenuous drying under reduced pressure. It is therefore very likely that some decomposition of the reagent occurs via hydrolysis and that the resultant monochloro compound reacts with 1 to give 5′- and/or 3′-monosilyl derivatives. Polar by-products of this type were indeed observed in the reaction of 1, but not in that of adenosine. Use of a greater than 10% excess of reagent failed to increase the yield of 3, and we assume this reflects increased disilylation of the 3′- and 5′-hydroxyls to give various noncyclic products including some in which two or more molecules of 1 are cross-linked. A further complication is the presence of a reactive site at N-1, which is lacking in adenosine and could consume some of the reagent.

Acylation of the 2′-hydroxyl in 3 with phenoxythiocarbonyl chloride was performed at room temperature (24 h) in acetonitrile containing 7 mol equiv of 4-(*N*,*N*-dimethylamino)pyridine. A 66% yield of the crystalline thiono ester 4 was obtained after silica gel chromatography. The product was readily distinguishable from 3 on the basis of its higher R_f on TLC and its spectral characteristics, which included strong UV absorption at 258 nm corresponding to the phenoxy group, a C=S stretching peak at 1210 cm^{-1} in the IR, and an aromatic proton signal at δ 7.3 in the NMR. In addition, the mass spectrum of 4 showed prominent peaks at *m*/*e* 510, 492, and 376 representing the sequential loss of $C_6H_5C(=S)$, H_2O, and 7-aminopyrazolo[4,3-*d*]pyrimidine fragments from the parent ion (*m*/*e* 646).

Initial efforts to reductively cleave the phenoxythiocarbonyl group in 4 with tri-*n*-butyltin hydride in toluene containing α,α'-azobisisobutyronitrile (AIBN)[15] were only partly successful, as evidenced by the fact that subsequent desilylation with fluoride ion yielded a mixture of 2 and unchanged 1. Since this occurred even with excess tri-*n*-butyltin hydride added to compensate for possible moisture, we reasoned that formation of a complex via N_1-stannylation was preventing smooth reaction. To overcome this problem, we treated 4 with hexamethyldisilazane and ammonium sulfate under reflux for 1 h and then added the reducing agent. When this was done, the reduction proceeded nearly to completion (75 °C, 4 h) and only a minor amount of 1 was formed upon desilylation.[16] The desilylation step was accomplished in situ by adding 1 M tetra-*n*-butylammonium fluoride in THF directly to the reaction mixture and heating again (75 °C, 2 h). Purification of the product was achieved by ion-exchange

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Table I. Inhibitory Effect of 2′-Deoxyformycin A (2) on the Growth of S49 Mouse Lymphoma T-Cells in Culture

drug treatment[a]	IC_{50}, μM (72-h culture)[b]	drug treatment[a]	IC_{50}, μM (72-h culture)[b]
2	13.5 (14.5, 12.5)	dAdo + 10 μM EHNA	4.5 (6.5, 2.5)
2 + 10 μM EHNA	9.3 (8.5, 10.0)	1	2.3 (1.5, 3.0)
dAdo	180	1 + 10 μM EHNA	0.03 (0.03, 0.03)

[a] EHNA was added to prevent intracellular deamination of the nucleosides by ADA. In control experiments with 10 μM EHNA alone, cell growth at 72 h was unaffected. [b] Values given are the averages of two separate experiments, and numbers in parentheses are actual IC_{50}'s from which means were obtained. The dAdo assay was performed once. In a previous paper the IC_{50}'s against S49 cells for dAdo alone and for dAdo + 10 μM EHNA were reported to be 100 and 4 μM, respectively.[17]

chromatography on Dowex 50W-X8 (H^+) followed by preparative TLC on silica gel. The yield of TLC-purified 2 was 53%, and the overall four-step yield starting from 1 was 16%. This may be compared with the 3% overall yield reported by Moffatt and co-workers.[11]

Bioassay. A cell growth assay using cultured S49 mouse lymphoma was employed to evaluate the biological activity of 2 in comparison with 1 and dAdo. The S49 tumor is of T-cell origin and has been studied previously, along with a panel of mutant sublines lacking one or more enzymes of the purine pathway, as a model system for inherited immunodeficiency disease.[17-19] As shown in Table I, 2 at 13.5 μM caused a 50% reduction of cell growth during 72 h of continuous drug exposure, whereas with dAdo the same effect was achieved only at 180 μM. Since heat-inactivated horse serum was used in the growth medium, it is unlikely that these results reflect differences in extracellular ADA activity. When 10 μM EHNA was added to prevent intracellular deamination, the IC_{50} for dAdo decreased 40-fold, in agreement with previous results,[17] whereas the IC_{50} for 2 decreased less than 2-fold. EHNA alone at 10 μM caused no inhibition of cell growth. The finding that EHNA causes only slight potentiation of 2 is unusual, since the activity of 1 has been shown to increase nearly 100-fold in a cell growth assay using Yoshida sarcoma and the ADA inhibitor coformycin.[20] The activity of 1 also increases markedly against S49 cells in the presence of EHNA (Table I). It should be noted, however, that 2 was less toxic than 1 in both the presence and absence of EHNA.

In order to address the question of whether inhibition of the growth of S49 cells by 2 requires intracellular phosphorylation, we tested the compound against two mutant sublines of this tumor that were shown earlier by Ullman and co-workers[17] to be kinase deficient. One of them (S49/tub-5-4) was derived by selection for tubercidin resistance and was shown to lack adenosine kinase (AK); the other (S49/*ara*-C-15-tub-1-1) was selected for resistance to both tubercidin and cytosine arabinoside (*ara*-C) and was shown to lack deoxycytidine kinase (dCK) as well as AK. At concentrations of up to 50 μM, 2 in combination with 10 μM EHNA was devoid of activity against both sublines. Similarly, when the mutants were treated with 10 μM 1 or with 0.5 μM 1 in combination with 10 μM EHNA, there was no growth inhibition. This suggests that 2, like 1, requires phosphorylation in order to be active. Furthermore, the enzyme responsible for this phosphorylation is probably AK rather than dCK, because if 2 were phosphorylated by dCK, activity should be retained in the S49/tub-5-4 cells. The apparent lack of activation of 2 by dCK is noteworthy in view of the fact that dAdo is known to be a substrate for this enzyme as well as for AK.[21] It thus appears that replacement of the imidazole ring in dAdo by a pyrazole ring, as in 2, alters the ability of the molecule to interact with the active site of dCK, but not AK. This contrasts with the introduction of a 2-halo substituent in dAdo, which results in exclusive phosphorylation by dCK.[21]

There is at present no direct evidence concerning the biochemical site of action of 2. Since 2 is a 2′-deoxy derivative and is unlikely to be converted to a riboside, it is reasonable to assume that, in contrast to 1,[1] the toxicity of 2 relates to pathways of DNA rather than RNA synthesis. Among the possibilities that have to be considered are (i) that 2 may be converted to a 5′-triphosphate and that the latter can compete with dATP for DNA polymerase, (ii) that 2 may be incorporated into DNA and may cause faulty base pairing or premature chain termination, and (iii) that the 5′-triphosphate of 2 may be a feedback inhibitor of ribonucleotide reductase and may thereby interfere with the synthesis of DNA precursors. The recent finding by Spector and co-workers[6] that a metabolite of 1 is incorporated into DNA would be consistent with the second possibility listed above. The relatively small increase in cytotoxicity of 2 in the presence of an ADA inhibitor is reminiscent of 2-halogenated dAdo analogues, which are inherently resistant to ADA hydrolysis. On the other hand, the fact that 2 is activated by AK, rather than dCK, sets this compound apart from the 2-halo-2′-deoxyadenosines and adds to its interest as a potential T-cell specific antileukemic agent.

Experimental Section

Melting points were determined in Pyrex capillary tubes in a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) and are not corrected. IR spectra were obtained on a Perkin-Elmer Model 781 double-beam spectrophotometer, and UV spectra were recorded on a Varian Cary Model 210 instrument. NMR spectra were determined on Varian T60A and CFT-20 instruments with Me_4Si as the reference. TLC was performed on Whatman MK6F silica gel and Baker 250F silica gel plates containing a fluorescent indicator. Column chromatography was carried out on Baker 3405 silica gel (60–200 mesh). Preparatory TLC was performed on Analtech GF plates (1000-μm thickness). Mass spectral data were obtained on a Finnigan MAT-312 instrument using reverse geometry, with the magnetic sector preceding the electric sector. A Finnigan MAT-200 control system was used to process the mass spectral data. Microchemical analyses were performed by Galbraith Laboratories, Knoxville, TN, and Multi Chemical Laboratories, Lowell, MA, and were within ±0.4% of the theoretical values. Reagent grade solvents were redistilled and stored over Davison 4A molecular sieves (Fisher Scientific, Boston, MA). Formycin A was purchased from Calbiochem-Behring, San Diego, CA. Other chemicals were obtained from Sigma Chemical Co., St. Louis, MO, and Aldrich Chemical Co., Milwaukee, WI.

7-Amino-3-[3′,5′-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-β-D-ribofuranosyl]pyrazolo[4,3-*d*]pyrimidine (3). 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (1.74 g, 5.5 mmol) was added to a suspension of formycin A hemihydrate (1.38 g, 5.0 mmol) in anhydrous pyridine (50 mL), and the reaction was stirred at room temperature for 20 h. The solvent was evaporated under reduced pressure at 35–40 °C (bath temperature), and the

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residue was partitioned between EtOAc and H_2O. The EtOAc layer was washed successively with ice-cold 1 N HCl (2 × 50 mL), H_2O, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl. Drying (Na_2SO_4) and solvent evaporation left a gum (3.3 g), which was chromatographed on a silica gel column (40 × 2.5 cm) with 2.5% MeOH in $CHCl_3$ followed by 5% MeOH in $CHCl_3$ as the eluents. TLC homogeneous fractions (R_f 0.82, silica gel, 4:1 $CHCl_3$–MeOH) were pooled and evaporated, and the residue was recrystallized from MeCN: yield 1.04 g (46%); mp 123–126 °C; IR (KBr) ν 3420, 2950, 1650 cm^{-1}; NMR ($CDCl_3$) δ 1.03 [s, 28 H, 2 $(Me_2CH)_2Si$], 4.03 (m, 2 H, C_3-H and C_4-H), 4.7 (m, 1 H, C_2-H), 5.36 (m, 1 H, C_1-H), 8.2 (s, 1 H, C_5-H). Anal. ($C_{22}H_{39}N_5Si_2O_5$) C, H, N, Si.

A more polar fraction (R_f 0.48, silica gel, 4:1 $CHCl_3$–MeOH) was recovered from the column. Solvent evaporation left a gum (0.8 g) that, on treatment with 1 M tetra-*n*-butylammonium fluoride in THF and with ion-exchange chromatography on Dowex 50W-X8 (H^+) as described below, gave unchanged 1 (0.34 g, 25% recovery). The yield of **2** after correction for this recovery was 61%. The chromatographic behavior of the silylation by-product and its subsequent conversion to formycin A in the presence of fluoride ion suggest that it is a noncyclic 5′- and/or 3′-monosilyl derivative.

7-Amino-3-[3′,5′-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-2′-(phenoxythiocarbonyl)-β-D-ribofuranosyl]pyrazolo[4,3-*d*]pyrimidine (4). Phenoxythiocarbonyl chloride (400 μL, 2 mmol) was added from a dry syringe to a solution of **3** (968 mg, 1.9 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (1.85 g, 15.2 mmol) in anhydrous MeCN (50 mL). Stirring was continued at room temperature for 24 h, the solvent was removed by rotary evaporation, and the crude product was partitioned between EtOAc and H_2O. The EtOAc layer was washed as in the preceding experiment, the solvent was evaporated, and the crude product (1.38 g) was chromatographed on silica gel with 1:1 $CHCl_3$–MeCN as the eluent. Pooled TLC homogeneous fractions (R_f 0.46, silica gel, 9:1 $CHCl_3$–MeOH) were evaporated, and the residue was recrystallized from MeCN: yield 0.8 g (66%); mp 142–145 °C; IR (KBr) ν 3420, 3200, 1650 (C=N), 1210 (C=S) cm^{-1}; NMR ($CDCl_3$) δ 1.1 [s, 28 H, 2 $(Me_2CH)_2Si$], 4.15 (m, 2 H, C_3-H and C_4-H), 4.93 (m, 1 H, C_2-H), 5.7 (m, 1 H, C_1-H), 7.3 (m, 5 H, aromatic), 8.3 (s, 1 H, C_5-H); mass spectrum 646 (M + 1), 510 (M − C(=S)OC_6H_5), 492 (510 − H_2O), 376 (510 − base). Anal. ($C_{29}H_{43}N_5Si_2O_6S$) C, H, N, S.

7-Amino-3-(2′-deoxy-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (2). Hexamethyldisilazane (50 mL) and $(NH_4)_2SO_4$ (25 mg) were added to **4** (484 mg, 0.75 mmol), and the reaction mixture was heated to reflux for 1 h. After evaporation under reduced pressure, the crude trimethylsilyl derivative was dissolved in dry redistilled toluene (50 mL) and α,α′-azobisisobutyronitrile (60 mg, 0.75 mmol) and tri-*n*-butyltin hydride (3 mL, 16.9 mmol) were added. The solution was purged with O_2-free N_2 for 0.5 h and then heated at 75 °C for 4 h, with TLC monitoring to ensure that reduction was complete. Deprotection was performed directly by adding 2 mol equiv of tetra-*n*-butylammonium fluoride in THF (1.5 mL of a 1 M solution) and heating at 75 °C for another 2 h. The solvent was evaporated under reduced pressure, and the residue was partitioned between Et_2O and H_2O. The H_2O layer was concentrated to a small volume and applied onto a column of Dowex 50W-X8 (H^+) resin. The column was washed first with H_2O (2 L) and then 15% NH_4OH. Fractions containing the product (TLC) were combined and purified further by preparative TLC on silica gel using 4:1 $CHCl_3$–MeOH as the developing solvent: yield 100 mg (53%); mp 163–166 °C; R_f 0.3 (silica gel, 4:1 $CHCl_3$–MeOH); IR (KBr) ν 3420, 2930, 1650, 1520, 1400 cm^{-1}; UV (MeOH) λ_{max} 229 nm (ϵ 5932), 293 (8898), 304 (6471); NMR (CD_3OD) δ 2.3–2.6 (m, 1 H, C_2-H), 3.57 (d, 1 H, C_5-H_a), 3.8 (m, 1 H, C_5-H_b), 4.15 (m, 1 H, C_4-H), 4.5 (d, 1 H, C_3-H), 5.6 (dd, 1 H, C_1-H), 8.2 (s, 1 H, C_5-H); mass spectrum 252 (M + 1). Anal. ($C_{10}H_{13}N_5O_3 \cdot 0.4CH_3OH \cdot 1.5H_2O$) C, H, N.

Cytotoxicity Assays. The properties and growth characteristics of S49 murine lymphoma cells and of their kinase-deficient mutants have been described previously.[19,22] Cell growth experiments were performed with suspensions of log phase phase cells (initial density 10^5/mL) in multiwell microtiter plates, using Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated horse serum and 2 mM L-glutamine. Drugs were dissolved in H_2O at pH 7.5. Cells were counted after 72 h with a Coulter Counter Model ZB1, and cell growth was expressed as a percent of the growth in untreated control cultures. The drug concentration giving a 50% reduction in growth (IC_{50}) was determined from the dose–response curve. Duplicate experiments showed good reproducibility (see Table I).

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Correlation of Structure and Activity in Ansamycins: Structure, Conformation, and Interactions of Antibiotic Rifamycin S

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The crystal and molecular structure of the DNA-dependent RNA polymerase inhibiting antibiotic rifamycin S ($C_{37}H_{45}O_{12}N$) as a dihydrate has been determined, and the conformation necessary for activity has been correlated with those of other active rifamycins. The orthorhombic unit cell, space group $P2_12_12_1$ with dimensions of a = 13.010 (2), b = 14.236 (2), c = 20.571 (4) Å, contains 4 molecules. The structure was solved by a combination of vector search and direct methods and refined anisotropically to an R factor of 0.048 for 2855 reflections. The conformation of the *ansa* chain differs from those of rifampicin and rifamycin B but resembles that of rifamycin SV at the joining points, C(2) and C(12), of the *ansa* chain to the naphthoquinone chromophore. The middle part of the *ansa* chain, which is essential for its activity against the enzyme, has the same conformation as other active rifamycins. The effect of the 3-substitution on the *ansa* chain conformation is that the carboxyl (C(15)=O) group wings around the N–C(16) direction, depending upon the electronegativity of the 3-substituent. The hydrogen bonding involves O(1), O(2), O(8), O(9), O(10), and the water molecules. A possible four-stage model for the interaction of the rifamycins with the enzyme DNA-dependent RNA polymerase has been speculated.

Rifamycins are naphthalenic ansamycins, which are produced from *Streptomyces mediterranei*.[1] These antibiotics are active against a large variety of organisms, including bacteria, eukaryotes, and viruses. They have

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