Higher levels of drug were required in the cotton pellet granuloma test than in the direct application to skin procedure. This could possibly be due to rapid metabolism of compound 1.

Significance of the differences between test group (T) and control group (C) was determined as above.

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3-N-Substituted Aminomethyl Derivatives of Rifamycin SV. A Convenient Method of Synthesis, Cyclization of Certain Derivatives, and Anticellular and Antiviral Activities of Several Derivatives

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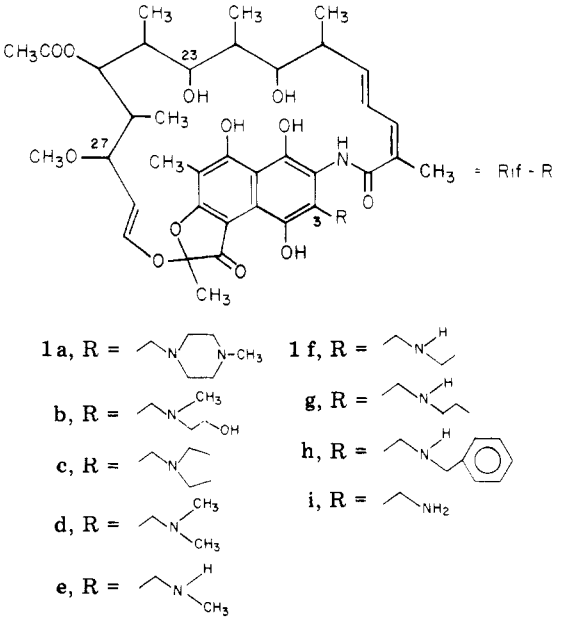
A new synthesis of Mannich bases of rifamycin SV using the Borch[2] procedure with rifaldehyde is described. This new synthesis offers two advantages over the previously published method.[3] It provides a route to monoalkylaminomethylrifamycins (**1e–h**) and to unsubstituted aminomethylrifamycins that were not accessible by the old procedure. The new method also offers a preparative route to Mannich bases **1a** and **1b** which were needed in multigram quantities for biological testing. In addition, the cyclization of certain of the monoalkylaminomethylrifamycins to the novel *N*,15-didehydro-15-deoxo-3,15-epi[methano(alkylimino)]rifamycin SV derivatives (**2**) is described. The anticellular and antiviral effects of representatives of both series of compounds against cultured mouse cells and murine oncornavirus are discussed.

3-Dialkylaminomethylrifamycin SV derivatives are active antibacterial agents[3] and certain of these derivatives (**1a–c**) interrupt oncornavirus replication in infected 3T3 cells.[4] Because of the antiviral activity of rifamycins **1a–c** larger quantities of these materials and compound **1d** were needed for in vivo testing.[5] The route available to these compounds[3] uses rifamycin S (quinone form of **1** where R = H), which is treated under classical Mannich reaction conditions with an excess of the appropriate secondary amine and aqueous formaldehyde. Unfortunately, the reported[3] yields for both **1a** and **1b** are 5%. It was also of interest to us to prepare monoalkylaminomethylrifamycins (e.g., **1e–h**) and the unsubstituted Mannich base **1i**. However, there was no published method available for obtaining these types of rifamycins. Therefore, it was imperative for us to develop a new more general procedure for the preparation of Mannich base rifamycins.

Chemistry. The synthesis of the desired 3-N-substituted aminomethyl derivatives of rifamycin SV (**1a–i**) (Chart I) was achieved by using the reductive amination procedure of Borch.[2] Rifaldehyde (**1**, R = CH=O) was treated with a mixture of the desired amine, its hydrochloride (which can be prepared in situ), and Borch's reagent[2] (sodium cyanoborohydride). This results in a mildly exothermic reaction which proceeds to completion within a few hours. For the two rifamycins (**1a** and **1b**) that we needed in multigram quantities and were difficult to obtain in even small quantities, the new procedure provided the compounds in a much improved yield. Mannich base **1a** was isolated as an orange crystalline solid in 47% yield. Likewise, rifamycin **1b** was isolated as a crystalline solid from acetonitrile–ether in 38% yield. The

Chart I. 3-Alkylaminomethylrifamycin SV Derivatives



other two rifamycins that were needed, the dimethyl (**1d**) and diethyl (**1c**) derivatives, could be prepared by the Borch procedure in good and comparable yields to those reported by the Mannich base procedure.[3] In addition, the previously unavailable monoalkylaminomethylrifamycins **1e–h** and the unsubstituted aminomethylrifamycin **1i** were readily prepared by the new procedure.

Table I. 3-Alkylaminomethylrifamycin SV Derivatives

Compd	Yield, %	Dec pt, °C	Recrystn solvent	Formula[a]	UV (MeOH) λ_{max} (ϵ)
1a[b]	47	200-205	EtOAc	$C_{43}H_{59}N_3O_{12}$	314 (21 050)[b] 450 (14 110)
1b[b]	38	188-193	CH_3CN, Et_2O	$C_{41}H_{56}N_2O_{13}$	314 (18 980)[b] 447 (14 430)
1e	31	Undefined	EtOAc	$C_{39}H_{52}N_2O_{12}\cdot CH_3CO_2C_2H_5$	314 (17 400) 447 (13 400)
1f	24	200-210	MeOH	$C_{40}H_{54}N_2O_{12}\cdot 3CH_3OH$	313 (16 500) 445 (13 500)
1g	60	195-205	EtOAc	$C_{41}H_{56}N_2O_{12}\cdot H_2O$	315 (16 300) 448 (12 800)
1h	42	180-190	MeOH	$C_{45}H_{56}N_2O_{12}\cdot 2H_2O$	313 (17 700) 446 (14 300)
1i	14	>200 slow	EtOAc	$C_{38}H_{50}N_2O_{12}\cdot H_2O$	313 (17 200) 445 (13 400)

[a] All new compounds analyzed correctly for C, H, and N. [b] Reference 3; UV spectra were run in phosphate buffer pH 7.38.

It should be noted that in our hands treatment of rifamycin S with methylamine and aqueous formaldehyde led to unreacted starting material. The same unsatisfactory result was obtained with ammonia and ethylamine (Table I).

We recently reported[6] that 3-methylaminomethylrifamycin SV (**1e**) undergoes intramolecular dehydration to the novel dihydropyrimidine, *N*,15-didehydro-15-deoxo-3,15-epi[methano(methylimino)]rifamycin SV (**2e**) in chloroform after standing several days at room temperature (Scheme I). The reaction is acid catalyzed as shown by the fact that the transformation is complete in 15 h when a drop of glacial acetic acid is added to the chloroform solution. The dihydropyrimidine **2e** was also found in the reaction used to prepare the Mannich base **1e** and was the major product if the reaction was allowed to proceed for 16 h. The intramolecular dehydration was much less facile for 3-aminomethylrifamycin SV (**1i**) and required refluxing in 1,2-dichloroethane with a catalytic amount of glacial acetic acid to obtain **2i**. Rifamycin **2i** was difficult to crystallize and was oxidized to the quinone form **3** with basic potassium ferricyanide. For the corresponding ethylamino compound (**1f**) cyclization could not be effected because the vigorous reaction conditions required caused extensive decomposition. However, the cyclic *n*-propyl analogue **2g** was isolated in low yield when rifaldehyde was treated with *n*-propylamine, hydrogen chloride, and Borch's reagent for 25 days at room temperature. It was readily identified by its characteristic UV–visible spectrum (see the Experimental Section).

Treatment of dihydropyrimidine **2i** or the quinone form **3** with activated manganese dioxide gave the crystalline pyrimidine **4**. Initial attempts to isolate the compound in the hydroquinone form using a mildly reductive ascorbic acid work-up yielded a crystalline compound that gave an elemental analysis consistent with dihydrohydroxypyrimidine **5**. Additional support for this proposed structure was obtained from the ^1H NMR spectrum which lacked an aromatic pyrimidine proton in the region of δ 9. This peak appeared as a sharp singlet at δ 9.50 in the ^1H NMR spectrum ($CDCl_3$) of the quinone form (**4**), which was prepared by oxidizing **5** with basic potassium ferricyanide.

Biological Results. Quantitative focal transformation of 3T3 mouse cells by murine sarcoma virus (MSV) is inhibited by compounds **1a**, **1b**, and **1d**, and replication of MSV by newly infected cells is interrupted by concentrations of approximately 10^{-5} M of those compounds.[4] Compounds **1c** and **1f** were reported as being noninhibitory to focal transformation of 3T3FL cells by MSV. Of the newer derivatives discussed above, compounds **1e**, **1g**, **1i**, **2e**, and **2i** were made available for biological testing by the Drug Evaluation Branch, National Cancer Institute. None of these cyclized and noncyclized 3-substituted derivatives of rifamycin SV synthesized as described above significantly inhibited quantitative focal transformation of 3T3 cells by MSV (Table II). In addition, inhibition of growth of uninfected 3T3 cells by the derivatives shown in Table II occurred at concentrations five- to tenfold lower than previously reported[4] for compounds **1a**, **1b**, and **1d**.

Subsequent experimentation has indicated that interruption of progeny virus replication also occurs when 3T3 cells newly infected with murine leukemia virus (MuLV) are incubated with compound **1d** at 1.3×10^{-5} M (Figure 1). The sevenfold decrease in progeny MuLV production 24 h after infection and exposure to drug, followed by an increase in virus production to levels approximating those of untreated control cells, parallels the results previously reported for MSV. Decreased production of MuLV by newly infected 3T3 cells exposed to compound **1d** (Figure

Scheme I

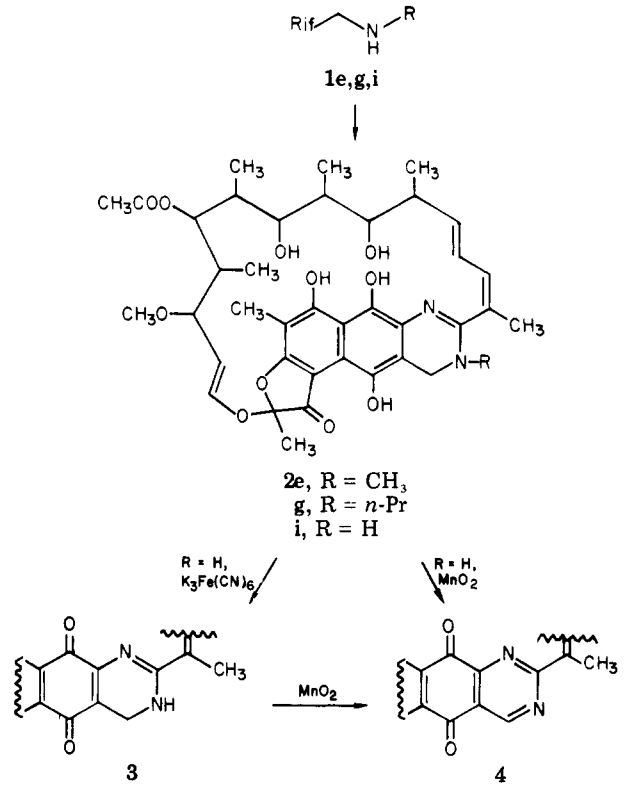


Table II. Inhibition of Focus Formation by MSV

Compd	Highest nontoxic concn,[a] M	% inhibn of focus formation by MSV at highest nontoxic concn[b,c]
1e	1.4×10^{-6}	14
1g	1.3×10^{-6}	10
1i	1.4×10^{-6}	0
2e	1.4×10^{-6}	0
2i	1.4×10^{-6}	26

[a] Defined as the highest concentration of derivative at which the logarithmic growth rate of treated cells was ≥75% of the growth rate of untreated cells and total cell growth after 5 days' exposure was ≥75% of control growth. [b] Relative to number of foci in parallel control cultures. [c] $p > 0.05$ for compounds 1e, 1g, and 2i.

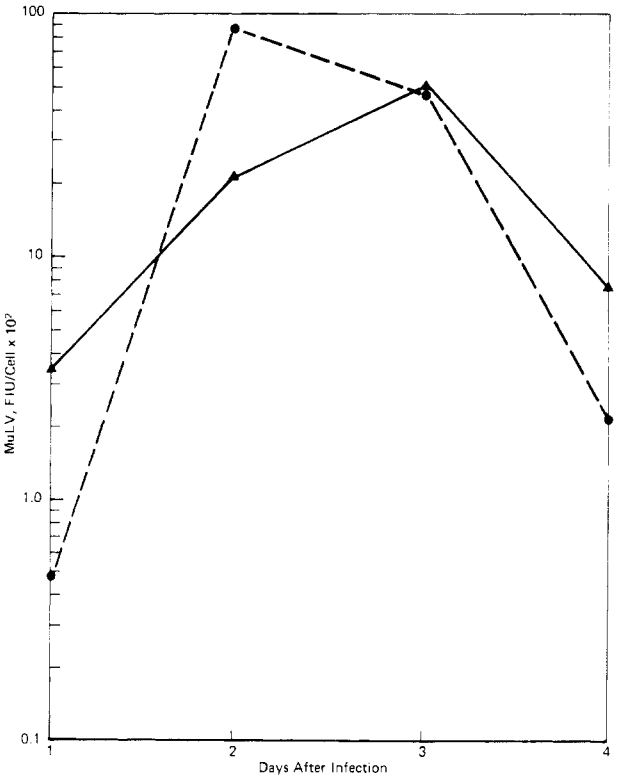


Figure 1. Transient inhibition of MuLV production from freshly infected 3T3 cells by compound **1d**: (▲-▲) control; (●--●) 1.3×10^{-5} M compound **1d**.

1) at 24 h after infection and exposure to drug was not seen when 3T3 cells chronically infected with MuLV were exposed to the same drug concentrations. No difference between control and drug-treated cells in the amount of virus capable of inducing foci in S^+L^- mouse cells was seen at any time during a 4-day exposure of chronically infected cells to compound **1d** (data not shown). This result parallels our previously reported findings with MSV.[4] From these observations we have concluded that certain 3′-substituted derivatives interfere with an undefined early process in oncornavirus infection. A number of 3-N-substituted derivatives of rifamycin SV, including compound **1d**, were previously reported[4] to exert no significant inhibition of oncornavirus reverse transcriptase or cellular nucleic acid polymerases. Consequently, we do not believe that inhibition of reverse transcriptase is the mechanism whereby this group of rifamycin derivatives exerts antiviral and antitransforming effects.

A degree of selective anticellular activity by compound **1d** against a clonal line of MSV-transformed 3T3 cells was suggested by our earlier results.[4] This selective cytostatic

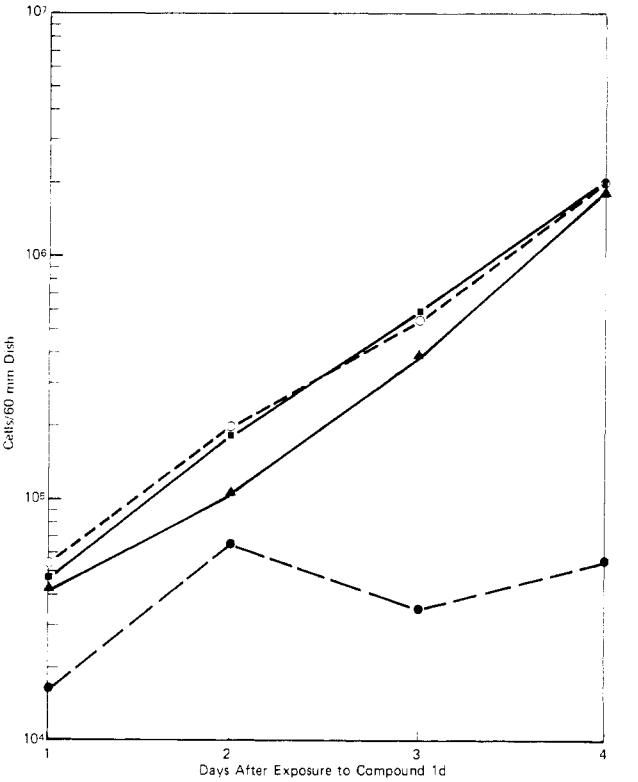


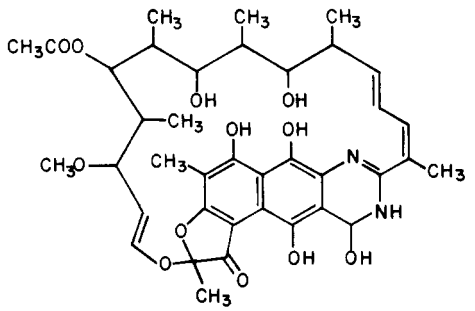
Figure 2. Cytostatic effect of compound **1d** on 3T3 cells chronically infected with MuLV. Uninfected 3T3 cells and 3T3 cells chronically infected with MuLV were seeded at 2.5×10^4 cells per plate in drug-free medium. After overnight incubation medium containing compound **1d** at 1.3×10^{-5} M was placed on half the infected and uninfected cell cultures. Three plates of each cell type with and without drug were counted at 24, 48, 72, and 96 h after exposure to drug-containing or control media: (●-●) uninfected 3T3, control; (▲-▲) uninfected 3T3, 1.3×10^{-5} M compound **1d**; (○--○) chronically infected 3T3, control; (●--●) chronically infected 3T3, 1.3×10^{-5} M compound **1d**. The cell population after 4 days exposure to compound **1d** was significantly below the control level ($p < 0.01$).

effect was also exerted by compound **1d** against 3T3 cells chronically infected with MuLV (Figure 2). This suggests that some alteration(s) which results from oncornavirus infection, with or without those changes in morphology and growth pattern by which we define "transformation", render the infected cells sensitive to cytostatic effects of that derivative. Compound **1d** is the only member of the group of 3′-substituted derivatives of rifamycin SV for which this selective effect has been observed in our experiments.

In summary, the anticellular and antiviral effects of an "active" rifamycin derivative reported here indicate that those effects are not specific for a virus which causes infected cells to undergo morphological transformation or for morphologically transformed cells. Our results further substantiate that antiviral and antitransforming effects are not a general property of 3-N-substituted aminomethyl derivatives of rifamycin SV.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. UV spectra were obtained with a Beckman Model 25 spectrophotometer. NMR spectra were run on either a Varian T-60 or a Perkin-Elmer R-32. Thin-layer chromatography (TLC) was run on glass plates coated with silica gel (Quantum Industries, Q1F). All chromatograms were run using the following solvent systems (SS): (1) chloroform–methanol (9:1), (2) chloroform–methanol (8:2), (3) chloroform–methanol (98:2). Migrated spots were



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observed under UV light or by visible means when the compounds were colored. All evaporations were accomplished under reduced pressure using a Büchler rotating evaporator unless otherwise specified.

Procedure for Preparing Mannich Bases. 3-(*N*-Methylpiperazinomethyl)rifamycin SV (1a). To a solution of 6.0 g (60 mmol) of *N*-methylpiperazine in 25 mL of methanol was added 4 mL (20 mmol) of 5 N methanolic hydrogen chloride. The solution was cooled to room temperature and rifaldehyde (7.25 g, 10 mmol) was added followed by 450 mg (7 mmol) of sodium cyanoborohydride. A mildly exothermic reaction ensued. After 3 h at room temperature protected from moisture, the reaction mixture was poured into 50 mL of 10% ascorbic acid. The mixture was extracted with ethyl acetate (2 × 75 mL). The combined organic layer was washed with brine, dried (Na_2SO_4), and evaporated to dryness. The resulting fluffy solid was dissolved in a small volume of chloroform–methanol (9:1) and applied to a column of silica gel (350 g, 50 × 5 cm) packed with the same solvent pair. The column was eluted with chloroform–methanol (9:1) and 75-mL fractions were collected as soon as colored material started to elute from the column. Fractions 1–4 were pure by TLC (SS 1) and were combined, evaporated to dryness, and crystallized from ethyl acetate. The orange crystals (3.8 g, 47%) of **1a** were collected by filtration: mp 200–205 °C dec; ^{1}H NMR ($CDCl_3$) δ 0.2 (d, 3, J = 7 Hz, 34-CH_3), 1.75 (s, 3, 13-CH_3), 2.33 (s, 3, N-CH_3), and 3.03 (s, 3, 37-OCH_3); TLC (SS 1) R_f 0.75.

***N*,15-Didehydro-15-deoxo-3,15-epi[methano(methylimino)]rifamycin SV (2e). Method I.** Reductive amination of 3-formylrifamycin SV (7.25 g, 10 mmol) with methylamine was accomplished as in the preparation of **1a**, except the reaction time was increased to 16 h. TLC (SS 1) showed two products (both orange), R_f 0.50 (minor component) and R_f 0.55 (major component). The crude product was chromatographed on 600 g of silica gel (Merck 60, 5 × 67 cm) using SS 1. The eluent was collected in 50-mL fractions and examined by TLC (SS 1). Fractions which contained only the main component were combined and concentrated to give 1.0 g of red-orange solid. The product was crystallized from reagent methanol to give 562 mg (0.78 mmol) of **2e** as bright orange crystals: IR (Nujol) 3400, 1715, 1700, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.08 (s, 6 H, OCH_3 and NCH_3), 2.08 (s, 6 H, 14-CH_3 and acetate CH_3), 1.97 (s, 3 H, 30-CH_3); UV (MeOH) λ_{max} 271 nm (ε 12 700), 282 (12 590), 317 (16 600), 374 (9400), 457 (16 200). Anal. ($C_{39}H_{50}N_2O_{11}$) C, H, N.

Method II. Cyclization of 1e to 2e. After standing for several days, a $CDCl_3$ solution of **1e** examined by TLC (SS 1) showed one orange spot (R_f 0.55) which was identical with **2e**. Likewise a chloroform solution of **1e** which remained tightly capped in a vial for several days was converted to **2e**, according to TLC.

***N*,15-Didehydro-15-deoxo-3,15-epi[methano(imino)]rifamycin SV (2i).** To a dry, ice-cooled 250-mL three-neck round-bottom flask under a nitrogen atmosphere was added 60 mL of methanolic ammonia (3.4 g of NH_3) (200 mmol) and 33 mL of 3 N methanolic hydrogen chloride (100 mmol). The mixture was treated with 14.5 g (20 mmol) of rifaldehyde and 900 mg (14 mmol) of sodium cyanoborohydride. The reaction was stirred overnight at room temperature, poured into 100 mL of 10% aqueous ascorbic acid, and extracted with ethyl acetate (2 × 150 mL). The combined organic layer was dried (Na_2SO_4) and evaporated to dryness. The resulting solid was chromatographed on 600 g of silica gel (Merck 60, 5 × 65 cm) using chloroform–methanol (9:1). Once colored material started to elute from the column, fractions were collected as follows: fraction 1 (250 mL), 2 (250 mL), 3 (150 mL), 4–8 (1200 mL), and 9–11 (900 mL).

Fraction 2 was homogeneous by TLC (SS 2), R_f 0.91, and was concentrated in vacuo yielding 0.55 g of a orange red solid **2i**. This material has the same characteristic UV absorption spectrum as **2e**: UV (MeOH) λ_{max} 267, 277, 316, 371, and 453 nm; IR (Nujol) 3400, 3150, 1735 (shoulder), 1720, 1650, 1640, and 1620 cm^{-1}; NMR (Me_2SO-d_6–$CDCl_3$) δ 9.7 (br s, 1, amidine NH), 4.57 (s, 2, CH_2NH), 3.0 (s, 3, OCH_3), 2.08 (s, 3, 14-CH_3), 2.0 (s, 3, acetate), 1.95 (s, 3, 30-CH_3), 1.65 (s, 3, 13-CH_3), 0.95, 0.85, and 0.67 (3 doublets, 9, each d has J = 7 Hz, 3 ansa CH_3).

3-Aminomethylrifamycin SV (1i). Fractions 4–8 obtained from the column used in the preparation of **2i** contained a single component as observed by TLC (SS 2), R_f 0.87, and were combined and evaporated to dryness. The resulting solid was crystallized from 50 mL of ethyl acetate yielding 2 g of **1i**. A small sample was recrystallized by dissolving in chloroform–methanol (1:1), adding an equal volume of ethyl acetate, concentrating the solution at reflux, and gradually adding additional ethyl acetate. The analytically pure sample was dried under high vacuum for 24 h at 40 °C: mp >200 °C dec slowly; IR (Nujol) 3590, 3450, 3400, 3125, 1720, 1640, 1630, and 1620 cm^{-1} (shoulder); ^{1}H NMR (Me_2SO-d_6–$CDCl_3$) δ 9.25 (s, 1, amide NH); (Me_2SO-d_6–$CDCl_3$–D_2O) δ 3.0 (s, 3, OCH_3), 2.0 (s, 9, 14, 30, and acetate methyl groups), 1.71 (s, 3, 13-CH_3), 1.0, 0.85, and 0.63 (3 methyl doublets, J = 7 Hz, 3 ansa methyl groups).

***N*,15-Didehydro-15-deoxo-3,15-epi[methano(imino)]rifamycin S (3).** A 1.20-g (1.69 mmol) quantity of the hydroquinone **2i** was dissolved in 100 mL of reagent chloroform and was washed thoroughly with aqueous $K_3Fe(CN)_6$ (3.0 g in 150 mL). The chloroform layer was dried (Na_2SO_4), filtered, and concentrated to dryness. A concentrate of the residue in chloroform–methanol (98:2) was passed through 30 g of silica gel (Woelm, 70–230 mesh) using the same solvent pair. The filtrate was concentrated to dryness and the residue was crystallized from anhydrous ether. The crystals were filtered, washed with a small volume of ether, and dried (vacuum) to give 413 mg (34%) of the quinone as fine green crystals, homogeneous by TLC: R_f 0.75 (SS 1); IR (Nujol) 3400, 3375, 3300, 1735, 1720, 1640, and 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 12.3 (v br s, 1 H, exchanges with D_2O, phenolic OH), 5.7–6.7 (m, 4 H), 5.10 (dd, $J_{27,28}$ = 8 Hz, $J_{28,29}$ = 12.5 Hz, 1 H, 28-H), 4.90 (d, J = 19 Hz, 1 H, ArCH_2NH–), 4.80 (d, J = 10 Hz, 25-H), 4.33 (d, J = 19 Hz, 1 H, ArCH_2NH), 3.10 (s, 3 H, OCH_3), 2.30 (s, 3 H, 14-CH_3), 2.04 (s, 6 H, 30-CH_3 and 36-CH_3), 1.70 (s, 3 H, 13-CH_3), 0.50–1.0 (3 d, J = 7 Hz, each 3 H, 31-CH_3, 32-CH_3, 33-CH_3), 0.22 (d, J = 7 Hz, 3 H, 34-CH_3); UV (CH_3OH) λ_{max} 272 nm (ε 17 400), sh 303 (14 100), 375 (7300), 439 (8000). Anal. ($C_{38}H_{46}N_2O_{11}$) C, H, N.

***N*,15-Didehydro-15-deoxo-1′,6′-dihydro-6′-hydroxypyrimidino[4,5-*b*]rifamycin SV (5).** A 2.5-g (3.5 mmol) quantity of the dihydropyrimidine **2i** or the quinone form **3** was dissolved in 50 mL of reagent methanol. To this solution was added 5 g of activated manganese dioxide[9] and the resulting suspension was refluxed. At 1-h intervals, aliquots of the reaction were added to aqueous ascorbic acid, extracted with ethyl acetate, and examined by TLC (SS 1). After 3 h, the reaction was complete. The reaction was allowed to cool and was vacuum filtered, and the residue was thoroughly washed with methanol. The filtrate was added to 100 mL of 5% aqueous ascorbic acid, diluted with 40 mL of brine, and extracted with chloroform (3 × 60 mL). The extracts were dried (Na_2SO_4), filtered, and concentrated to give 2.3 g of dark residue. The residue was dissolved in chloroform–methanol (90:10) and filtered through 40 g of silica gel (Merck 60, 2.5 × 18 cm) using the same solvent pair. The filtrate was treated briefly with crystalline citric acid monohydrate, washed with brine, dried (Na_2SO_4), filtered, and concentrated to give 2.3 g of dark residue. The residue was crystallized from ether to give 1.555 g (61%) of **5** as dark crystals, homogeneous by TLC: R_f 0.43 (SS 1) (purple spot); ^{1}H NMR ($CDCl_3$) showed the absence of a singlet into region of δ 9 indicative of a pyrimidine proton; δ 6.5–5.5 (m, 4), 5.3–4.6 (m, 2), 3.45 (m, 2), 3.06 (s, 3, OCH_3), 2.37 (s, 3, 14-CH_3), 2.03 (s, 6); UV (CH_3OH) λ_{max} 225 nm (ε 37 400), 2.68 (18 900), 312 (16 700), 370 (8000), 449 (11 600). Anal. ($C_{38}H_{48}N_2O_{12}$) C, H, N.

***N*,15-Dihydro-15-deoxopyrimidino[4,5-*b*]rifamycin S (4).** The hydroxydihydropyrimidine **5** (0.7 g, 1 mmol) dissolved in 50 mL of reagent chloroform was oxidized by thorough washing with

aqueous $K_3Fe(CN)_6$ solution (0.5 g in 50 mL). The chloroform layer was dried (Na_2SO_4), filtered, and concentrated to dryness. A concentrate of the residue was applied to a 80-g column of silica gel (Woelm, 70–230 mesh, 35 × 2.4 cm) packed and eluted with chloroform–methanol (98:2). The eluent was collected in 50-mL fractions and examined by TLC. The first four fractions were combined and concentrated to give 0.5 g of residue which was crystallized from ether to give 133 mg of pyrimidino derivative **4**, homogeneous by TLC: R_f 0.57 (SS 3); IR (Nujol) 3475, 1735, 1710, 1675, 1645, 1600, 1565, and 1540 cm^{-1}; UV (CH_3OH) λ_{max} 265 nm (ϵ 20 600), 280 (20 700), 410 (7400); ^1H NMR ($CDCl_3$) δ 12.95 (s, 1 H, exchanges with D_2O, phenolic OH), 9.50 (s, 1 H, ArH), 5.9–6.7 (m, 4 H), 5.15 (dd, $J_{27,28}$ = 12.5 Hz, 1 H, 28-H), 5.00 (d, J = 10 Hz, 1 H, 25-H), 3.07 (s, 3 H, OCH_3), 2.37 (s, 6 H, 14-CH_3 and 30-CH_3), 2.04 (s, 3 H, 36-CH_3), 1.78 (s, 3 H, 13-CH_3), 0.5–1.1 (3 d, J = 7 Hz, each 3 H, methyl groups). Anal. ($C_{38}H_{48}N_2O_{12}$) C, H, N.

***N*,15-Didehydro-15-deoxo-3,15-epi[methano(*n*-propylimino)]rifamycin SV (2g).** To a solution of 3.54 g (60 mmol) of dry *n*-propylamine (distilled from CaH_2), 25 mL of dry methanol, and 4 mL of 5 N methanolic hydrogen chloride (20 mmol) were added 7.25 g (10 mmol) of rifaldehyde and 450 mg (7 mmol) of sodium cyanoborohydride. The reaction was allowed to run 25 days at room temperature under a N_2 atmosphere and then poured into 50 mL of 10% aqueous ascorbic acid and extracted with ethyl acetate (2 × 75 mL). The combined organic layer was washed with brine (50 mL), dried (Na_2SO_4), and evaporated to dryness in vacuo. The concentrate was chromatographed on silica gel (750 g, 80 × 5 cm) using $CHCl_3$–MeOH (19:1). Fractions were collected as follows once colored material started to elute from the column: fraction 1 (200 mL), 2 (200 mL), 3 (200 mL), 4–6 (1000 mL), 7 (500 mL), 8–10 (1000 mL), and 11 (300 mL). Fractions 2, 4–6, and 8–10 contained three different components and were homogeneous by TLC. Each set of fractions was combined and evaporated to dryness. Fraction 4–6 was triturated with ether and the resulting solid (6 g) of 3-propylaminomethylrifamycin SV (**1g**) was collected by filtration. Fraction 2 (450 mg) is as yet unidentified. Fraction 8–10 (1.2 g) was crystallized from ~10 mL of ethyl acetate to yield 300 mg of desired crystalline product **2g**, mp 184–187 °C dec. A small sample of **2g** was recrystallized from ethyl acetate for analysis: UV (MeOH) λ_{max} 268 nm (ϵ 13 800), 281 (13 650), 316 (17 700), 375 (8600), 454 (17 000). Anal. ($C_{41}H_{54}N_2O_{11}\cdot H_2O$) C, H, N.

Cells and Viruses. All experiments involving quantitative focal transformation by MSV were performed in the 3T3FL subline[7] of the Swiss mouse embryo-derived 3T3 cell line. Cells to be used for focal transformation assays and virus yield experiments were seeded at 1 × 10^5 cells/dish in 60-mm plastic culture dishes (Falcon Plastics) in 4 mL of McCoy's medium 5A (Grand Island Biologicals) containing 10% fetal calf serum (Reheis), 100 units/mL of penicillin, and 100 μg/mL of streptomycin. Cultures in plastic dishes were incubated in a humidified atmosphere of 5% CO_2 in air.

The Moloney strains of MuLV and MSV were used for all experiments.

Focal Transformation Assays and Toxicity Evaluation. Focus formation by standardized MSV inocula was assayed as described by O'Connor et al.[4] Briefly, after overnight incubation of 3T3FL cells (seeded as described above), the medium was removed and the cells were treated with 25 μg/mL of DEAE–dextran in serum-free medium for 20 min at room temperature. DEAE–dextran-containing medium was removed and the cells were washed with sterile phosphate-buffered saline. Treated cells were infected with 0.3 mL/dish of MSV inoculum containing approximately 100 focus-forming units (FFU), and virus was allowed to adsorb at 37 °C for 1 h with frequent agitation of the plates. Drug-containing or control medium (4 mL) was added to each plate. Infected cultures were incubated for 5 days at 37 °C without change of medium, and foci of transformed cells were counted microscopically.

All transformation assays were paralleled by evaluation of toxicity of derivatives for uninfected 3T3 cells. Three plates of control cells and three plates incubated with each drug concentration were counted at 24, 48, 96, and 120 h after exposure to the appropriate medium and growth curves constructed.

All drugs were dissolved in 100% dimethyl sulfoxide (Me_2SO), and Me_2SO at all drug concentrations and in control medium was adjusted to 0.25% (v/v).

Measurement of MuLV Production. 3T3 cells seeded at 1 × 10^5 cells/60-mm dish were infected with MuLV exactly as described above for MSV at a multiplicity of infection of 0.4 focus-inducing units (FIU) per cell. Medium was collected from treated and control plates at 24, 48, 72, and 96 h after infection and application of drug. The medium was filtered, diluted, and assayed for focus-inducing ability in D-56 S^+L^- mouse cells as described by Bassin et al.[8] MuLV-induced foci of transformation in the cells carrying the MSV genome were counted 5 days after infection. Cells in the MuLV-infected plates were counted after collection of the medium, and virus yields were calculated as FIU/cell. Significance of all results showing differences between control and treated groups was analyzed by the t test.

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