Rifamycin Derivatives as Inhibitors of a Ribonucleic Acid Instructed Deoxyribonucleic Acid Polymerase Function. Effect of Lipophilicity

Allan N. Tischler, Frances M. Thompson, Louis J. Libertini, and Melvin Calvin*

Laboratory of Chemical Biodynamics, University of California, Berkeley, California 94720. *Received January 28, 1974*

Several new rifamycin derivatives have been synthesized and tested as inhibitors of an RNA-instructed DNA polymerase (RDP) function in an effort to determine the effect of rifamycin structure on RDP inhibition. It was found, in general, that RDP inhibition is favored by lipophilic "tails" bound to the 3 position of rifamycin SV. Relative lipophilicities were measured by a reversed phase thin-layer chromatographic technique.

The discovery of RNA-instructed DNA polymerase (RDP)[1,2] associated with RNA tumor viruses has provided an explanation for the ability of these viruses to express themselves genetically in host cells. According to the provirus theory, the virus upon entering the host cell, utilizing its RDP, can produce its corresponding DNA copy (provirus), which can become integrated into the host DNA. This integration, or later acts as a consequence of integration, could then lead to the transformation of the host cell.[3] Indeed, evidence for the existence of the provirus associated with the DNA of transformed human cells has been presented utilizing hybridization techniques.[4-6] The viral genetic information is usually found in transformed human cells and not in normal cells.[5] Spiegelman reports that for two different sets of identical twins, in which one twin from each set is leukemic, the viral genetic information is found only in the leukemic twins.[6] These findings can be taken as evidence for horizontal transmission of cancer in accordance with the provirus theory.

It has, therefore, been of great interest to obtain inhibitors of RDP. To date many compounds have been found capable of RDP inhibition to varying degrees.[7] Some of these inhibitors have been shown to inhibit the transformation of tissue culture cells inoculated with appropriate RNA tumor viruses.[7-10] Certain derivatives of the rifamycins, which are among these, are of particular interest.

In a preceding article[11] we described the synthesis of new rifamycin inhibitors of RDP and presented evidence establishing size and lipophilicity of the "tail" bound to rifamycin SV as desirable properties for maximizing inhibition. We found that the derivative rifazacyclo-16 (RC-16) was especially potent. Since that time we have devoted ourselves to further investigating the effect of size and lipophilicity of rifamycin "tails." Toward this end, three synthetic approaches have been taken: first, the synthesis of a series of rifamycin derivatives with cyclic aliphatic tails (rifazacyclo series) to investigate the effect of lipophilicity in an analogous series; second, the synthesis of an acyclic analogous series (rifazone series) to investigate the difference between cyclic and acyclic derivatives; and third, the synthesis of a quaternary derivative of dimethylbenzyldesmethylrifampicin (DMB), DMB methyl iodide (DMB-MI), and an analog of DMB in which the 4′ nitrogen is replaced with a carbon atom (DMB analog). Since the DMB-MI has a fixed charge on nitrogen and the DMB analog has no charge, we can investigate the effect of lipophilicity in a series of compounds each having approximately the same configuration. The structures of all these derivatives are given in Figure 1.

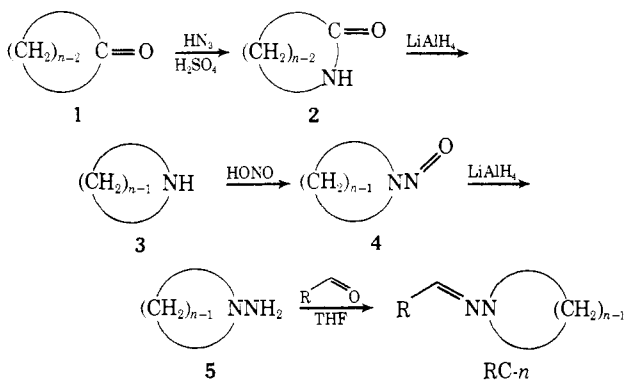
The technique of reversed phase thin-layer chromatography was used as an indication of the relative lipophilicity of all the derivatives in the present study. From the R_f values thus obtained the free-energy related parameter, ΔR_M, could be calculated.[12,13] This parameter, which is analogous to the π parameter developed by Hansch,[14] is especially well suited for lipophilic molecules with very low water solubility. The ΔR_M parameter has been shown to correlate well with the actions of penicillins, rifamycins, and other classes of compounds.[12,13,15]

Finally, each derivative was tested as an inhibitor of RDP. The inhibition constant, K_I, is defined as the molar concentration of derivative corresponding to 50% inhibition of the control RDP activity. The RDP was derived from UC1-B cells[16] and was used after partial purification.[17] Triton DN-65 (Rohm & Haas Co.) was the detergent used in the RDP assay since inhibition by rifamycins is constant over a large range of detergent[18] concentrations with this detergent. While the enzyme used was isolated from a cellular source, it has been shown to have the same template responses as the mouse leukemia virus polymerase. The enzymes show a preference for a template comprised of poly(rA):oligo(dT) over poly(rA):poly(dT). Moreover, the enzyme is capable of utilizing poly(rC):oligo(dG) as template but not poly(dA):oligo(dT). It should be noted that the K_I reported may represent submaximal inhibition due to the interaction of the derivatives with the extraneous protein present as impurities. As the enzyme is more highly purified, the measured K_I may thus become smaller.

Results and Discussion

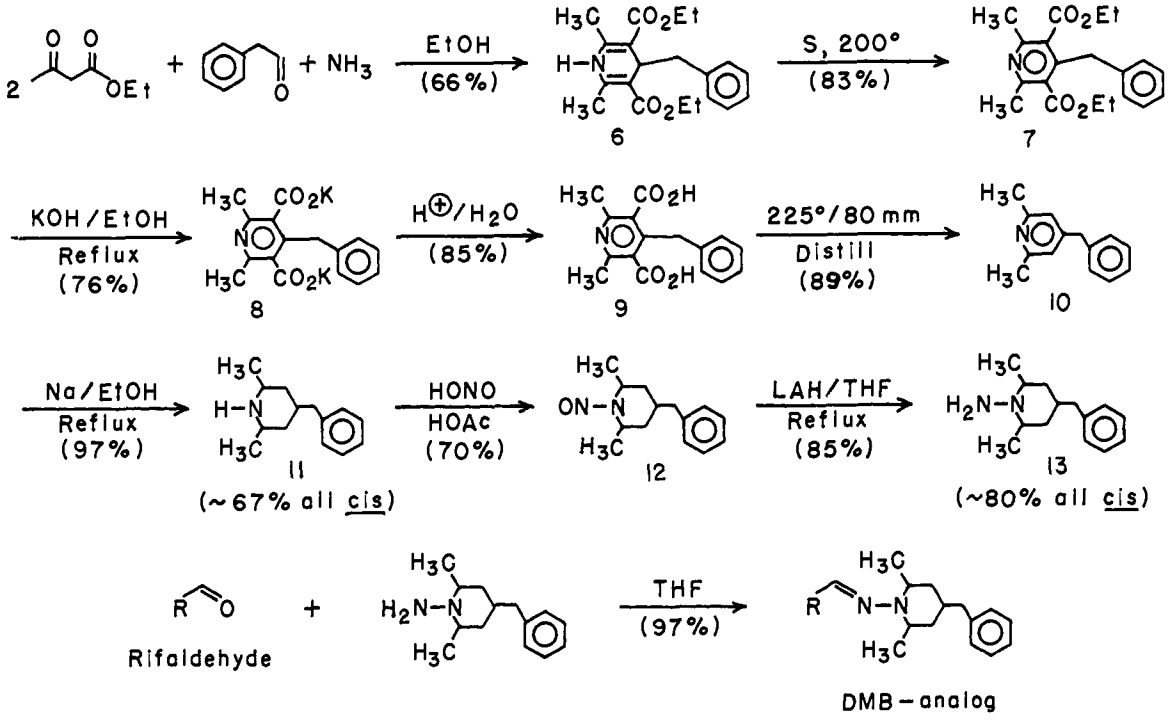
The general synthesis of the derivatives of the rifazacyclo series is outlined in Scheme I. The position in Scheme I with which synthesis began depended on the availability of the starting materials. For RC-6 and RC-7, which have been synthesized before, the hydrazine **5** (n = 6 and 7) was available, and the rifamycin derivatives were readily prepared by condensation with rifaldehyde. For RC-8, RC-9, and RC-13 the synthesis began with the lactams **2** (n = 8, 9, and 13), and for RC-11 and RC-16[11] we started with the cyclic ketones **1** (n = 11 and 16).

Scheme I



The starting material for each of the derivatives in the rifazone series was the appropriate disubstituted amine which was converted to the respective hydrazine by nitrosation followed by lithium aluminum hydride ($LiAlH_4$) reduction.

Scheme II. Synthesis of a Lipophilic Analog of DMB



Overall yield ≈18%

DMB-MI was obtained by the reaction of DMB in methyl iodide. The synthesis of the DMB analog is given in Scheme II. The first three steps, *i.e.*, Hansch condensation, aromatization, and saponification, were performed as described.[19] Several attempts at the saponification of the dihydropyridine **6** were unsuccessful, usually resulting only in the partial decomposition of the starting material. The saponification of the pyridine **7** was quite facile, as described.[19] The diacid **9** was found to readily decarboxylate above 200° to give the desired compound **10**. Several efforts were made to reduce **10** to **11** by hydrogenation over Adam's catalyst. In each case reduction of the phenyl ring was at least as fast as the reduction of the pyridine ring. In simpler molecules containing both phenyl and pyridine rings it is generally found that the pyridine ring is preferentially reduced.[20] We suspect that the lack of preference for the reduction of the pyridine ring in **10** is due to the steric interaction of the two methyl groups. The desired reduction was readily affected by a modified Birch reduction.[21] Nitrosation and subsequent $LiAlH_4$ reduction of piperidine **11** to hydrazine **13** was relatively straightforward.

Table I. RDP Inhibition and Tail Lipophilicity

Derivative	ΔR_M	K_I
Rifazacyclo-6	0.56	58
Rifazacyclo-7	0.68	33
Rifazacyclo-8	0.80	17
Rifazacyclo-9	0.92	9.0
Rifazacyclo-11	1.20	4.9
Rifazacyclo-13	1.49	3.0
Rifazacyclo-16	2.06	2.0
Rifazone-4_2	1.11	8.8
Rifazone-5_2	1.43	4.1
Rifazone-6_2	1.80	2.5
Rifazone-8_2	2.61	3.5
DMB-MI	~0	>100
DMB	0.85	19
DMB analog	1.15	5.9
Rifamazine	0	16
Dirifampin	0.15	25

In order to compare the lipophilicity within each series and between series, a quantitative measure was needed. A useful parameter of lipophilicity is the substituent coefficient, π, which is defined by the free-energy relationship[14]

$$\pi = \log (P_X/P_H)$$

where P_X and P_H are the experimentally determined partition coefficients for the substituted and unsubstituted compounds, respectively. This parameter, developed by Hansch and coworkers, is thus a measure of the lipophilicity contributed by the substituent.

Because many of the rifamycins presented here have extremely low solubilities in water, we resorted to the technique of reversed phase thin-layer chromatography (tlc).[13,15] The R_f values thus obtained have been shown to be related to the partition coefficients, P, by the expression

$$P = \text{const } (1/R_f - 1)$$

We can therefore express π as a function of R_f by the relation

$$\pi = R_M(\text{derivative}) - R_M(\text{rifamycin SV}) = \Delta R_M$$

where ΔR_M has been defined as $\log (1/R_f - 1)$.[22] For our purposes the unsubstituted compound is rifamycin SV.

The R_M and the K_I with RDP are given for each derivative in Table I. Figure 2 shows that there exists a near linear relationship between ΔR_M and the number of carbon atoms in the tail for both the rifazacyclo and the rifazone series. The graph also shows that carbon for carbon the acyclic rifazone series is more lipophilic than the cyclic rifazacyclo series.

A plot of inhibition ($\log K_I$) as a function of lipophilicity (ΔR_M) is given in Figure 3 for both the rifazacyclo and rifazone series. In addition, the points for other rifamycin derivatives from Table I are plotted. In general, it can be concluded that RDP inhibition is favored by tail lipophilicity. There appears to be an optimal lipophilicity

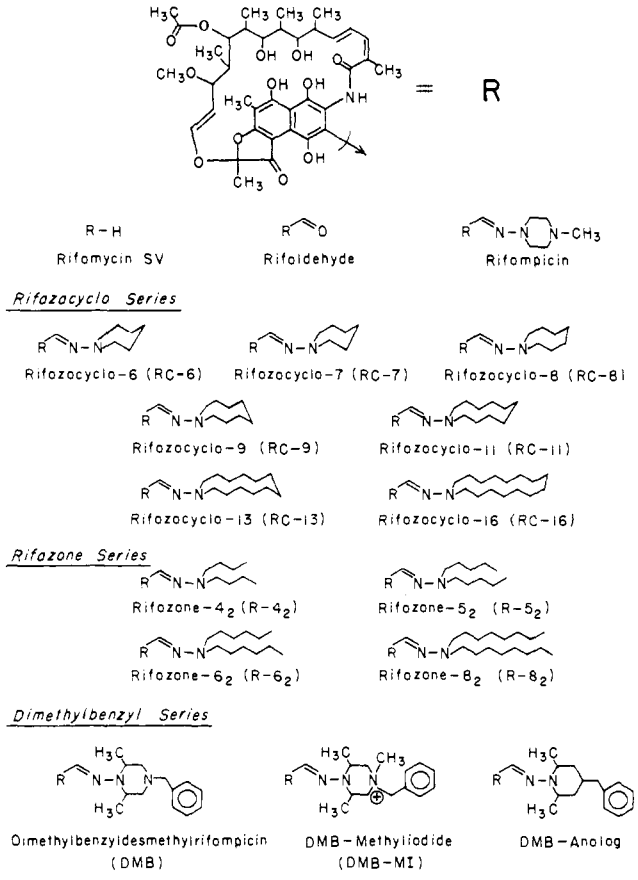
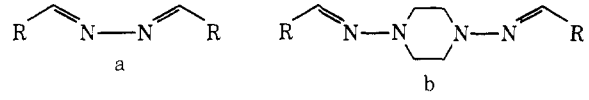


Figure 1. Rifamycin derivatives.

for both the rifazacyclo and rifazone series at approximately $\Delta R_M = 2.1$. Accordingly, R-8_2, the only derivative with $\Delta R_M > 2.1$, is less effective an inhibitor than the less lipophilic R-6_2. It is of interest to note that while there is no large difference between the cyclic and acyclic series, the more lipophilic rifazone series is less effective than the rifazacyclo series at inhibiting RDP. This can be taken as evidence for the existence of factors other than lipophilicity for optimal RDP inhibition.

The derivatives of the dimethylbenzyl series, DMB and DMB analog, fall in the same area of Figure 3 as the cyclic and acyclic series. This suggests to us that their inhibition is due primarily to their lipophilicity and not to their particular configuration. Also, the fact that the uncharged DMB analog is over three times more effective than DMB, while the charged DMB-MI is ineffective, suggests that the inhibition exhibited by DMB itself, which should be largely charged at the assay pH (7.8), is due to the unprotonated form only.

There is one anomalous group of derivatives to the generalized inhibition as a function of lipophilicity. These are the rifamycin dimers which we have earlier described.[11] As can be seen from the positions of rifamazine (a) and dirifampin (b)



on Figure 3, they are both reasonably good inhibitors in spite of the fact that they are relatively hydrophilic. Either these dimers inhibit RDP through a different mechanism of interaction or they possess a desirable tail property not yet recognized.

It has been suggested that lipophilic rifamycins as a class, possibly due to their detergent-like nature, act as nonspecific inhibitors of enzymes in general.[23] If this is so, then increasing inhibition of RDP with increasing lipophilicity would also result in increased inhibition of other polymerases, not to mention enzymes in general. We have made a study of this possibility with many of the derivatives discussed here[24] and have found that the rifazacyclo series is rather nonspecific when RDP inhibition is compared to *Escherichia coli* DNA-dependent DNA polymerase (DDP) inhibition. However, the rifazone series appears to be rather specific. R-8_2, for example, is approximately 33 times more inhibitory against RDP than DDP while RC-16 is nearly equally inhibitory toward both enzymes. That data indicate to us that larger rifazone derivatives yet to be synthesized may be even more specific for RDP, even though the K_I may increase.

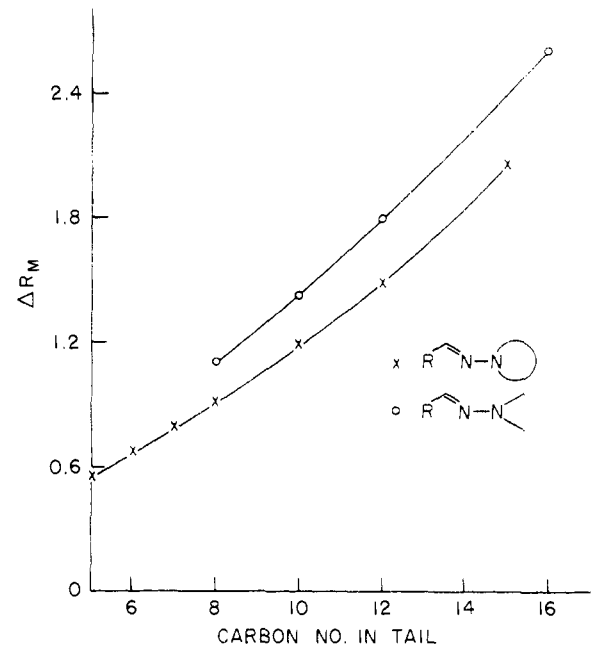


Figure 2. Lipophilicity *vs.* tail size in the rifazacyclo and rifazone series.

We have also investigated the possibility of detergents with lipophilicities similar to the rifamycins being inhibitory toward RDP and have found no correlation. For example, the R_M for the detergent Triton X-100 is nearly identical with R-6_2 and yet no inhibition of RDP is observed, even at concentrations as high as 0.1%.

In vitro specificity may or may not be significant in *in vivo* systems. It is possible for a nonspecific inhibitor of polymerases to act specifically within a cell because of cellular restrictions on the transport and accumulation of the compound. The compound may be so compartmentalized as to be able to inhibit only one polymerase. Work is currently in progress to determine the ability of some of these new rifamycins to inhibit transformation in tissue culture. In one study, RC-16 in the presence of amphotericin B, a compound often used to facilitate cell transport, was found to very effectively inhibit transformation in UC1-B cells infected with mouse leukemia virus without any sign of toxicity in the normal cells.[9] In another study, currently in progress, R-8_2 alone has been found to be an effective inhibitor of transformation in chick fibroblast monolayers infected with Rous sarcoma virus, again with negligible toxicity to the untransformed cells.[25]

Experimental Section

Ir spectra were taken on either a Perkin-Elmer Model 137 or a Beckman Model 5A grating infrared spectrometer. Nmr of all compounds except the rifamycin derivatives were recorded on a

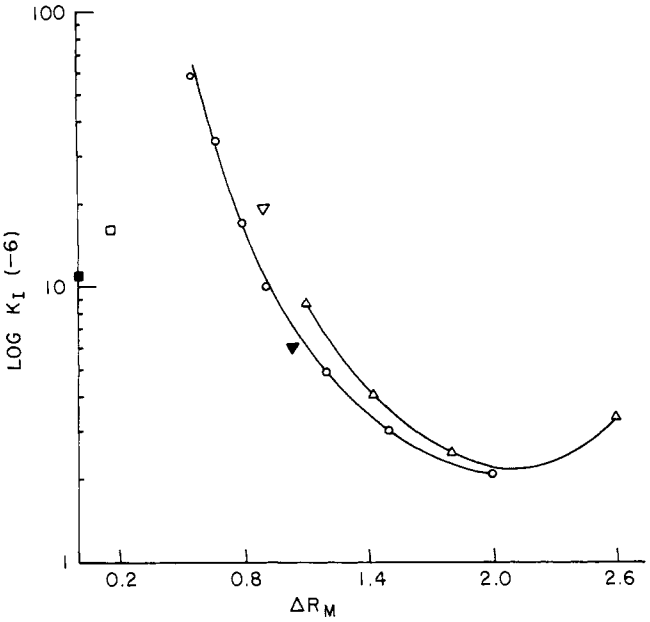


Figure 3. Plot of RDP inhibition *vs.* lipophilicity: ○, rifazacyclo series; △, rifazone series; ▽, DMB; ▼, DMB analog; ■, rifamazine; □, dirifampin.

Varian Associates Model T-60 instrument, and the rifamycin derivatives were recorded on a Varian Associates Model HR-220 instrument. Rifamycin derivatives used as precursors were kindly supplied by Gruppo Lepetit S.p.A., Milan, Italy. Where analyses are indicated only by symbols of elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

Reversed Phase Tlc. Tlc plates were prepared with silica gel G (E. Merck AG, Darmstadt) spread at a thickness of 0.25 mm (50 g to 100 ml of water). The plates were activated and developed in a 5% solution of Dow-Corning 200 fluid (10 cc) in ether overnight. The ether was allowed to evaporate and then the rifamycin derivatives, dissolved in acetone (10 mg/ml), were spotted (~6 μg). The plates were developed in an acetone–water solution (2:3 v/v) saturated with the Dow-Corning fluid. No means of visualizing was required as the spots are distinctly colored. In all cases the reported ΔR_M is the average of at least five determinations.

2-Azacycloundecanone. The cyclic ketone (5.00 g, 0.032 mol) is dissolved in chloroform (100 ml) in a flask fitted with a magnetic stirring bar and a thermometer. The solution is cooled in an ice bath to 5–10° and sulfuric acid (25 ml) is added. NaN_3 (2.23 g, 0.034 mol) is added approximately 10% at a time over a period of 1 hr. The ice bath is removed and the reaction is stirred for another hour. The contents of the reaction flask are then poured into ice and water (125 ml) and the resulting chloroform layer is separated. The water layer is washed with additional chloroform and the chloroform solutions are combined, washed with 1 *N* KOH (25 ml), dried over Na_2SO_4, and evaporated under vacuum to yield the product. The product is recrystallized from acetone: yield, 4.7 g (91%); mp 162–165°.

Nitrosations. Method 1. For Water-Soluble Amine Hydrochlorides. The amine (*n* moles) and water (~250 *n* ml) are added to a flask fitted with a thermometer, magnetic stirring bar, and addition funnel. The flask is placed in an ice bath and concentrated HCl (1.25 *n* mol) is added dropwise maintaining the temperature below 10°. After the addition the ice bath is removed and the solution is heated to 65°. A solution of $NaNO_2$ (1.25 *n* mol) in water (250 *n* ml) is added dropwise at a rate maintaining the temperature between 65 and 70°. After the addition the reaction is stirred at temperature for another 10 min. The flask is cooled to 25° and the contents are transferred to a separatory funnel where the product is extracted with benzene. The benzene solution is dried over Na_2SO_4 and evaporated under vacuum. For **dibutylnitrosamine**, *n* = 0.100, yield 99%; **di-*n*-pentylnitrosamine**, *n* = 0.100, yield 98%; **di-*n*-hexylnitrosamine**, *n* = 0.125, yield 85%; ***N*-nitrosoazacyclooctane**, *n* = 0.0945, yield 57%, bp 96–98° (6.5 mm), structure by nmr and ir; ***N*-nitrosoazacyclononane**, *n* = 0.0865, yield 65%, bp 118° (10 mm), structure by nmr and ir, mp 42–47°; ***N*-nitrosoazacycloundecanone**, *n* = 0.0535, yield 86%, structure by nmr and ir; ***N*-nitrosoazacyclotridecanone**, *n* = 0.0490, yield 95%, structure by nmr and ir.

Method 2.[26] For Water-Insoluble Amine Hydrochlorides. The amine (*n* mol) and acetic acid (1000 *n* ml) are added to a flask fitted with a thermometer, magnetic stirring bar, and addition funnel. The flask is placed in an ice bath and concentrated HCl (1.25 *n* mol) is added dropwise, keeping the temperature below 10°. The solution is then heated to 25° and a solution of KNO_2 (3 *n* mol) in water (~150 *n* ml) is added dropwise over a period of 20 min. The reaction is stirred for another 20 min after the addition. Water (~1500 *n* ml) is then added; the product is extracted with ether, which, in turn, is washed with 5% Na_2CO_3, dried over Na_2SO_4, and evaporated under vacuum. For **di-*n*-octylnitrosamine**, *n* = 0.0249, yield 92%; ***N*-nitroso-2,6-dimethyl-4-benzylpiperidine**, *n* = 0.0345, yield 68%, structure by nmr and ir.

Lithium Aluminum Hydride ($LiAlH_4$) Reductions. $LiAlH_4$ was used for the reduction of amides to amines and of *N*-nitroso compounds to hydrazines. It was generally noted that the reaction time required for high conversions was quite sensitive to the freshness of the $LiAlH_4$. The use of fresh $LiAlH_4$ resulted, in every case, in shorter reaction times, *i.e.*, less than 4 hr of reflux in THF or ether.

The compound to be reduced (*n* mol), either neat or dissolved in THF, ether, or benzene, is added dropwise to a stirred refluxing mixture of $LiAlH_4$ (*n*–2*n* mol) in THF or ether over a period of approximately 30 min. The reaction is maintained at reflux until the conversion is complete. (This is determined by the work-up of 3-ml aliquots. Amide reduction is monitored by the loss of the carbonyl by ir. Nitrosamine reduction is monitored by the loss of the characteristic downfield α protons by nmr.) The reaction is then cooled in a cold water bath. For *x* g of $LiAlH_4$ used, a sequence of water (*x* ml), 15% NaOH (*x* ml), and water (3*x* ml) is added dropwise very slowly with vigorous stirring. The resulting crystalline mixed hydroxides are removed by vacuum filtration. The hydroxides are washed with additional solvent. The combined filtrates are evaporated under vacuum to yield the crude product.

Amide Reduction. For **azacyclooctane amide**, *n* = 0.118 in THF (25 ml), $LiAlH_4$ (0.20 mol) in THF (125 ml), yield 99%; **azacyclononane amide**, *n* = 0.106 in THF (50 ml), $LiAlH_4$ (0.21 mol) in THF (150 ml), yield 88%; **azacycloundecane amide**, *n* = 0.0622 in THF (50 ml),† $LiAlH_4$ (0.13 mol) in THF (100 ml), yield 88%; **azacyclotridecane amide**, *n* = 0.0507 in THF (100 ml),† $LiAlH_4$ (0.108 mol) in THF (75 ml), yield 99%.

Nitrosamine Reduction. For *N*-aminoazacyclooctane, *n* = 0.0423 in THF (15 ml), $LiAlH_4$ (0.0810 mol) in THF (60 ml), yield 80%, bp 67–73° (21 mm); ***N*-aminoazacyclononane**, *n* = 0.0513 in THF (50 ml), $LiAlH_4$ (0.103 mol) in THF (75 ml), yield 43%, bp 87° (19 mm); ***N*-aminoazacycloundecane**, *n* = 0.0407 in THF (25 ml), $LiAlH_4$ (0.092 mol) in THF (75 ml), yield 61%, bp 113° (10 mm); ***N*-aminoazacyclotridecane**, *n* = 0.0400 in THF (50 ml), $LiAlH_4$ (0.090 mol) in THF (50 ml), yield 64%, bp 95–99° (60 mm); ***N,N*-dibutylhydrazine**, *n* = 0.0633 in THF (25 ml), $LiAlH_4$ (0.132 mol) in THF (150 ml), yield 86%, bp 109–113° (80 mm); ***N,N*-di-*n*-pentylhydrazine**, *n* = 0.0633 in THF (25 ml), $LiAlH_4$ (0.132 mol) in THF (150 ml), yield 85%, bp 124–130° (51 mm); ***N,N*-di-*n*-hexylhydrazine**, *n* = 0.084 neat, $LiAlH_4$ (0.168 mol) in THF (125 ml), yield (dist) 96% (74%), bp 124–128° (15 mm); ***N,N*-di-*n*-octylhydrazine**, *n* = 0.0204 in THF (25 ml), $LiAlH_4$ (0.0254 mol) in THF (30 ml), yield 97%; ***N*-amino-2,6-dimethyl-4-benzylpiperidine**, *n* = 0.0216 in THF (25 ml), $LiAlH_4$ (0.0526 mol) in THF (50 ml), yield 85%, bp 118–123° (0.4 mm).

Rifaldehyde Condensations. Rifaldehyde (*n* mol) and the appropriate hydrazine (1.05 *n* mol) are dissolved in THF (~500 ml per 10 g of rifaldehyde) and stirred at room temperature until the reaction is complete by silica gel tlc in THF (1–24 hr). The solvent is then evaporated under vacuum, and the product is pulverized, washed with *n*-hexane, and dried again. The products can be further purified either by recrystallization from hexane or hexane–toluene or on a silica gel column eluted with ethyl acetate. Yields are all above 90%. For RC-8, *n* = 0.0140; RC-9, *n* = 0.0167; RC-11, *n* = 0.0132; RC-13, *n* = 0.0126; $R\text{-}4_2$, *n* = 0.0165; $R\text{-}5_2$, *n* = 0.0165; $R\text{-}6_2$, *n* = 0.0140; $R\text{-}8_2$, *n* = 0.0108; DMB analog, *n* = 0.00917.

The reaction has been found to be near quantitative when both the rifaldehyde and the hydrazine are of high purity. The hydrazines were usually distilled prior to use.

2,6-Dimethyl-4-benzylpyridine. 2,6-Dimethyl-3,5-dicarboxy-4-benzylpyridine (23.0 g, 0.081 mol) was placed in a flask connected to a Vigreux vacuum distillation apparatus. The system

†Solution must be kept warm (~40°).

was evacuated to 85 mm by means of an air bleed valve. The flask was heated until all the distillate, which came over at 200–204°, was collected as a ruby red liquid. The red color was found to be due to a highly colored impurity which was removed by dissolving the product in n-hexane (250 ml), cooling to 0° for 1 hr, filtering out the red precipitate, and evaporating the filtrate: yield 14.1 g (89%).

2,6-Dimethyl-4-benzylpiperidine. 2,6-Dimethyl-4-benzylpyridine (3.24 g, 0.0164 mol) was dissolved in 100% EtOH (30 ml) in a flask fitted with a reflux condenser. Na metal (4.5 g, 0.196 mol) was added gradually in small pieces to maintain a very gentle reflux. After the Na addition was complete (5 min), EtOH (15 ml) was added and the reaction was refluxed until all the Na reacted. (Note: when the Na was first added, the solution turned yellow; during the final reflux, the yellow color abruptly disappeared.) Water (20 ml) was then added dropwise. The EtOH was evaporated under vacuum, resulting in two phases which were transferred into a separatory funnel containing water (15 ml). The product was obtained by extraction with ether, drying over Na_2SO_4, and evaporation under vacuum: yield 3.12 g (94%).

DMP-MI. DMB (0.200 g) was dissolved in methyl iodide (20 ml) and the solution was stirred at 25° overnight. Evaporation of the methyl iodide afforded the product in quantitative yield.

RDP Assay. All assays were run in a total volume of 100 μl which was 94 m*M* Tris-HCl (pH 7.8), 100 m*M* KCl, 0.4 m*M* dithiothreitol, 0.02 m*M* ^{3}H-dTTP (1 Ci/mmol), 0.1 m*M* $MnCl_2$, 4% glycerol, 10 μg/ml of poly(rA):oligo(dT) (approximately 0.015 OD_{260}/100 μl assay), 0.01% Triton DN-65, 1.0% DMSO, and 43 units of enzyme activity (0.46 μg of protein). The rifamycin derivatives tested were initially dissolved in DMSO (at 10 mg/ml) and were diluted as necessary before adding to the reaction mixture.

All assays were initiated by the addition of the enzyme extract and were incubated for 30 min at 37°. A carrier RNA (0.5 ml of 5 mg/ml of Torula RNA, 10 m*M* sodium pyrophosphate, and 20 m*M* EDTA) was added and the material insoluble in 6.7% trichloroacetic acid was collected on 0.45-μ Millipore filters (presoaked in 0.2 *M* $Na_4P_2O_7$) and washed extensively with 5% trichloroacetic acid. After they were dried, the filters were dissolved in scintillation fluid containing ethanol and dioxane. Fumed colloidal silica was added to form a stable gel. The gel was counted in a scintillation counter and dpm was calculated from the cpm by comparison with an automatic external standard.

Approximate inhibition constants were first measured based on the authors estimated potency. Once the approximate values were known for each derivative, they were measured more accurately. In these final assays at least six concentrations, each in duplicate, were tested for each derivative. Dimethylbenzyldesmethylrifampicin was always included as a standard.

All final inhibition values are reported as an inhibition constant, K_I. The K_I for each derivative is obtained from a plot of derivative concentration vs. per cent control activity, and it is defined as the concentration of the rifamycin derivative corresponding to 50% inhibition of the control RDP activity. The accuracy of the K_I's measured under these conditions is estimated to be ±5%.

Acknowledgment. This work was supported, in part, by the U. S. Atomic Energy Commission and, in part, by the Dow Chemical Co. Two of us (A. N. T. and F. M. T.) were supported by the Elsa U. Pardee Foundation for Cancer Research. One of us (L. J. L.) was a Fellow of the Damon Runyon Cancer Fund, 1971-1973.

References

(1) H. M. Temin and S. Mizutani, *Nature (London)*, **226**, 1211 (1970).
(2) D. Baltimore, *Nature (London)*, **226**, 1209 (1970).
(3) H. M. Temin, *Sci. Amer.*, **226** (1), 25 (1972).
(4) J. Schlom and S. Spiegelman, *Science*, **174**, 840 (1971).
(5) W. Cuatico, J.-R. Cho, and S. Spiegelman, *Proc. Nat. Acad. Sci. U. S.*, **70**, 2789 (1973).
(6) W. Baxt, J. W. Yates, H. J. Wallace, Jr., J. F. Holland, and S. Spiegelman, *Proc. Nat. Acad. Sci. U. S.*, **70**, 2629 (1973).
(7) M. A. Apple, *Annu. Rep. Med. Chem.*, **8**, 251 (1973).
(8) M. Calvin, U. R. Joss, A. J. Hackett, and R. B. Owens, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1441 (1971).
(9) A. J. Hackett, S. S. Sylvester, U. R. Joss, and M. Calvin, *Proc. Nat. Acad. Sci. U. S.*, **69**, 3653 (1972).
(10) R. C. Ting, S. S. Yang, and R. C. Gallo, *Nature, New Biol.*, **236**, 163 (1972).
(11) A. N. Tischler, U. R. Joss, F. M. Thompson, and M. Calvin, *J. Med. Chem.*, **16**, 1071 (1973).
(12) G. L. Biagi, A. M. Barbaro, M. F. Gamba, and M. C. Guerra, *J. Chromatogr.*, **41**, 371 (1969).
(13) C. B. C. Boyce and B. V. Milborrow, *Nature (London)*, **208**, 537 (1965).
(14) A. Leo, C. Hansch, and D. Elkens, *Chem. Rev.*, **71**, 525 (1971).
(15) G. Pelizza, G. C. Lancini, G. C. Allievi, and G. G. Gallo, *Farmaco, Ed. Sci.*, **28**, 298 (1973).
(16) A. J. Hackett and S. Sylvester, *Nature, New Biol.*, **239**, 164 (1972).
(17) F. M. Thompson, A. J. Hackett, and M. Calvin, *Cancer Biochem. Biophys.*, submitted for publication.
(18) F. M. Thompson, L. J. Libertini, U. R. Joss, and M. Calvin, *Science*, **178**, 505 (1972).
(19) E. H. Huntress and E. N. Shaw, *J. Chem. Soc.*, 674 (1948).
(20) R. L. Augustine, "Catalytic Hydrogenation," Marcel Dekker, New York, N. Y., 1965, p 104.
(21) C. S. Marvel and W. A. Lazier, "Organic Syntheses," Collect. Vol. I, Wiley, New York, N. Y., 1944, p 99.
(22) E. C. Bate-Smith and R. G. Westall, *Biochim. Biophys. Acta*, **4**, 427 (1950).
(23) S. Riva, S. Fietta, and L. G. Silvestri, *Biochem. Biophys. Res. Commun.*, **49**, 1263 (1972).
(24) F. M. Thompson, A. N. Tischler, J. Adams, and M. Calvin, *Proc. Nat. Acad. Sci. U. S.*, **71**, 107 (1974).
(25) M. J. Bissell, C. Hatie, A. N. Tischler, and M. Calvin, *Proc. Nat. Acad. Sci. U. S.*, in press.
(26) K. K. Carroll and G. F. Wright, *Can. J. Res., Sect. B*, **26**, 276 (1948).