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Synthesis of Some Rifamycin Derivatives as Inhibitors of an RNA-Instructed DNA Polymerase Function

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Several new derivatives of the antibiotic rifamycin SV have been prepared in the search for potent inhibitors of an RNA-instructed DNA polymerase function. It was observed that derivatives containing large, hydrophobic substituents bound to the 3 position of the rifamycin molecule are particularly potent inhibitors. Derivatives containing nitroxyl and dansyl functionalities were synthesized as potentially useful labeled rifamycins.

With the discovery of RNA-instructed DNA polymerase (RIDP)[1] came the attractive hypothesis proposing the DNA provirus of the tumor virus RNA as the genetic material ultimately responsible for cell transformation by tumor viruses. If this is so, then inhibition of the RIDP, which would be required for the synthesis of the DNA provirus, would be an effective means of preventing transformation of cells inoculated with tumor viruses. A similar comment holds for the more recently formulated protovirus modification.[2] Certain derivatives of rifamycin SV have been shown to be promising inhibitors of RIDP in *in vitro* studies.[3] Moreover, some of these derivatives have been shown to reduce the incidence of transformation in both BALB/3T3 cells[4] and normal rat kidney (NRK) cells[5] infected with murine sarcoma virus (MSV).

In this paper we present the synthesis of several new derivatives of rifamycin SV, some of which (especially **17**) are more potent inhibitors of RIDP than the best derivatives previously synthesized and studied.[3,6] Based on the results of the derivatives presented here,[7] and elsewhere,[3] we propose that *in vitro* inhibition of RIDP is favored by large and hydrophobic substituents attached to the 3 position of rifamycin SV. Derivatives with 3 substituents meeting both criteria are especially active. The test results of the rifamycin derivatives expressed as 50% inhibition concentrations are presented in Table I.

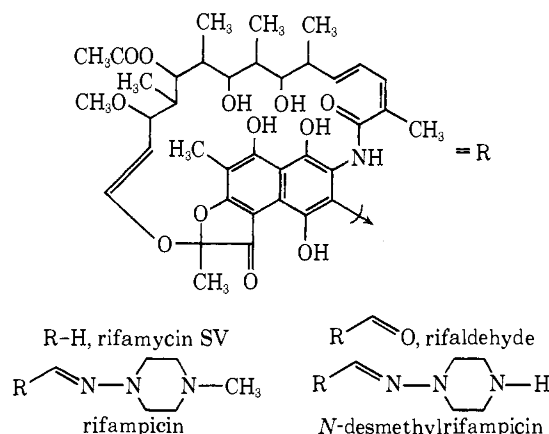

Figure 1. Structure of rifamycin and derivatives.

The drugs whose syntheses are described in this paper were either prepared by the condensation of rifaldehyde with the appropriate hydrazine or by acylation of *N*-desmethylrifampicin (**6**) in the 4 position of the piperazine ring. Figure 1 presents the basic rifamycin structure and the structures of some common rifamycin derivatives. The structures of all of the products and intermediates were assigned on the basis of ir and nmr. Uv and esr were also taken when appropriate.

Discussion and Results

Because of its potential use in RIDP purification and as a tracer in biological systems, we undertook the synthesis of two spin-labeled derivatives **3** and **20** and a fluorescent derivative **21** of rifamycin SV. The synthesis of **3** is outlined in Scheme I.

When *N*-oxyl-2,2,6,6-tetramethylpiperidin-4-one (**1**) is treated with a large excess of hydrazine in methanol, two reactions occur simultaneously: formation of the hydrazone of the keto group and reduction of the oxyl group to the hydroxylamine. The reaction proceeds with the evolution of nitrogen as a result of the latter reaction. Removal of the excess hydrazine followed by air oxidation in ethanol results in the oxidation of the hydroxylamine, yielding the oxyl hydrazone **2**. Condensation of this hydrazone with rifaldehyde affords the spin-labeled drug. Both **2** and **3** give the expected nitroxyl triplet in the esr. The hydrazone **2** was never isolated in completely pure form. It slowly disproportionates to the azine dimer **4**. This proved to be no hindrance as **4** does not react with rifaldehyde.

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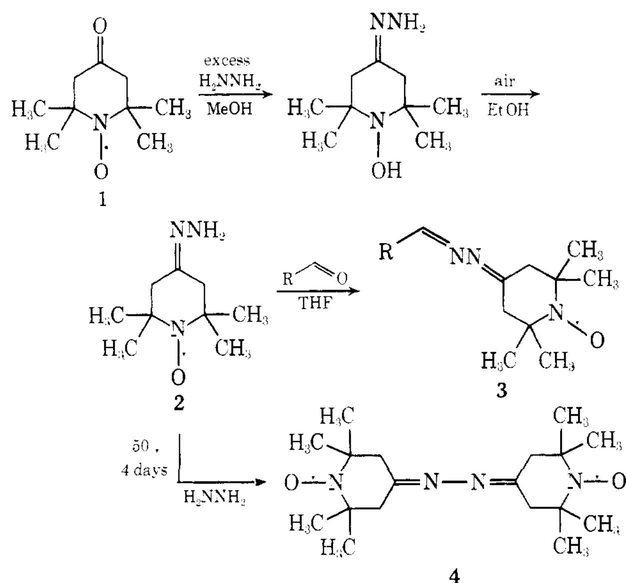
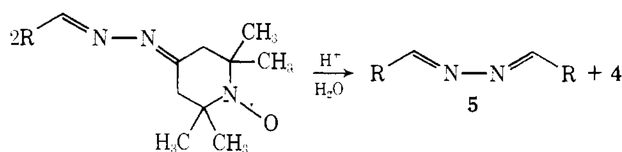
Scheme I



When 2 is evacuated at 50° for 4 days, the azine dimer, which gives an esr consistent with a biradical,[8] is quantitatively formed. Spin-label compound 3 was found to be unsatisfactory as it shows little inhibition of RIDP and as it slowly decomposes with loss of its esr signal. Presumably, an oxidation-reduction takes place between the oxyl group and the hydroquinone of the rifamycin chromophore.

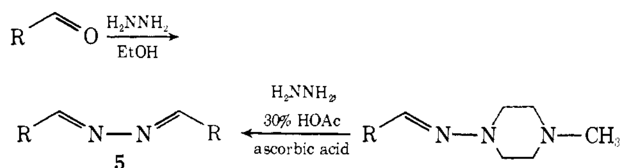
Compound 3 was found to readily disproportionate in aqueous acid to give a bright red compound identified as the azine of rifaldehyde (rifamazine) on the basis of its ir and nmr, Rast molecular weight determination, and uv. In the latter, one sees a bathochromic shift due to extension of conjugation across two rifamycin chromophores (Scheme II). The rifaldehyde azine 5 was found to be a potent inhibitor of RIDP.

Scheme II



Compound 5 can be more conveniently prepared either by the reaction of rifaldehyde with hydrazine, or by the reaction of rifampicin with hydrazine, under hydrolyzing conditions (Scheme III). Both routes afford the azine dimer in near quantitative yield.

Scheme III



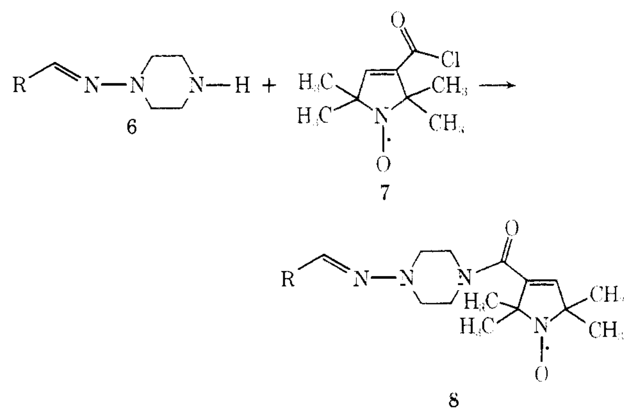
The second spin-labeled rifamycin derivative 8 was synthesized by acylation of 6 with the acid chloride 7 of 1-oxyl-2,2,5,5-tetramethyl-3-carboxypyrroline (Scheme IV). It is stable in pure form and like 3 it shows a three-line esr signal.

Table I. Inhibitors of RIDP

Derivative	50% inhibition concn,[a] μg/ml Triton X-100[b]	Triton DN-65[c]
Rifampicin[d]	>100	>100
Dimethylbenzyl-desmethylrifampicin[d]	22	18
Rifamazine (**5**)	21	16
Rifurea (**10**)	27	
Dirifampin (**11**)	18	25
Rifazabicyclo-**9** (**21**)	12	
Rifazacyclo-**16** (**17**)	4	2
Dansyldesmethylrifampicin (**9**)	11	

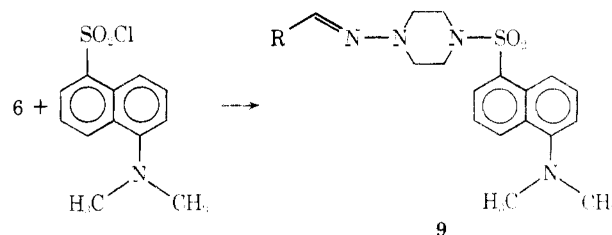
[a] Values were taken from an inhibition curve which was determined for each derivative. Measurements were originally made in Triton X-100. Since then we have found that Triton DN-65, a detergent with a higher critical micelle concentration, is a better detergent for these assays. [b] 0.005%. [c] 0.010%. [d] Included for reference.

Scheme IV



The dansyl derivative 9 was prepared by acylation of 6 with dansyl chloride (Scheme V). Compound 9 shows only approximately 1% of the expected fluorescence in organic solvents at around 520 nm, presumably due to quenching by the naphthalene system of the rifamycin chromophore. 8 was found to be noninhibitory, while 9 was found to be a potent inhibitor at low detergent levels.

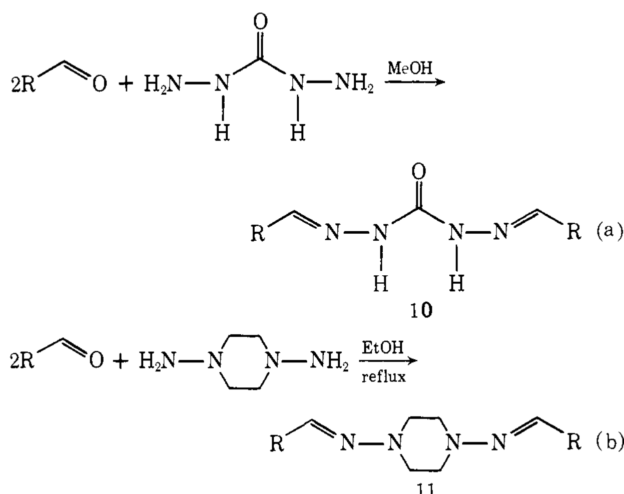
Scheme V



The high degree of RIDP inhibition shown by 5 encouraged us to look further at rifamycin dimers. Two additional dimers were thus prepared from readily available bifunctional compounds (Scheme VI). Urea derivative 10 (Rifurea) was prepared by the condensation of rifaldehyde with carbohydrazide, and the piperazine derivative 11 (dirifampin) was prepared by the condensation of rifaldehyde with N,N'-diaminopiperazine. Both of these dimers were also found to be potent inhibitors of RIDP.

The question arose as to whether the activity of these dimers is due to two chromophores in one molecule or one chromophore with one very bulky group bound to it. A test of the latter possibility, which seemed more reason-

Scheme VI



able to us, would involve the synthesis and evaluation of derivatives with large (preferably cyclic) groups attached to the 3 position. In addition, the work of Green[3a-c] and Gallo[3d] suggested to us that, substituent size being approximately equal, hydrophobic "tails" are more effective than hydrophilic. For example, Figure 2 gives sets of compounds listed in increasing order of activity. Desmethylrifampicin and rifampicin are known to be zwitterionic at pH 7.8, the pH at which RIDP activity is measured, with the amine being protonated (pK_a ≈ 10) and the juglone system existing as the anion (pK_a ≈ 2.6).

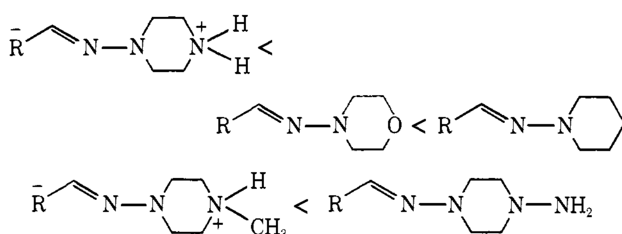
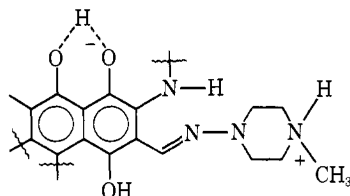


Figure 2.

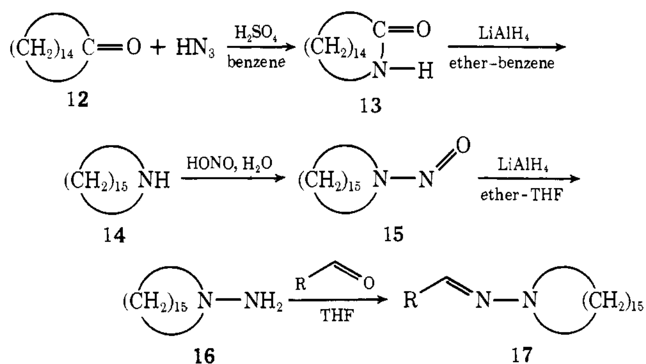


The analogous nitrogen in *N*-aminodesmethylrifampicin would not be expected to be largely protonated since it is a hydrazine (pK_a ≈ 6–7).

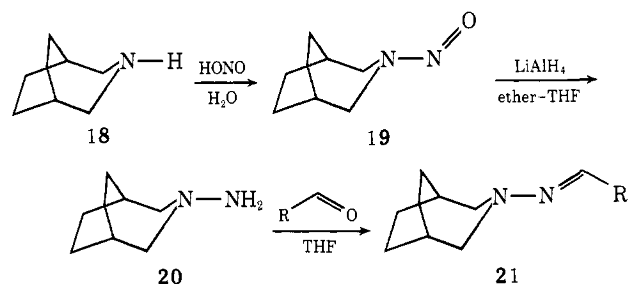
From the above it was inferred that, in addition to being large, hydrophobicity might also be desirable. As a result, two new drugs were prepared following this reasoning. Their preparations are outlined in Schemes VII and VIII.

Cyclopentadecanone (**12**) undergoes the Schmidt reaction to give the cyclic amide **13**, which is readily reduced by lithium aluminum hydride to azacyclohexadecane (**14**).[9] This secondary amine is converted to *N*-aminoazacyclohexadecane (**15**) by nitrosation to *N*-nitrosoazacyclohexadecane (**15**) followed by $LiAlH_4$ reduction. Analogously, 3-azabicyclo[3.2.2]nonane (**18**) is converted to *N*-

Scheme VII



Scheme VIII



amino-3-azabicyclo[3.3.2]nonane (**20**) by nitrosation to *N*-nitroso-3-azabicyclo[3.2.2]nonane (**19**) followed by $LiAlH_4$ reduction. These two hydrazines **16** and **20** were condensed with rifaldehyde in THF to yield the corresponding hydrazones **17** (rifazacyclo-16) and **21** (rifazabicyclo-9), respectively. Both drugs were found to be very potent inhibitors of RIDP. Rifazacyclo-16 is the most potent drug tested by this laboratory to date.[7]

Experimental Section

Ir spectra were taken on Perkin-Elmer Models 137 and 257 grating infrared spectrometers. Nmr spectra were recorded on a Varian Associates Model HR-220 instrument. Chemical shifts are reported in δ (parts per million downfield). Epr spectra were taken on a Varian Associates spectrometer Model E-3. Uv spectra were recorded on a Cary Model 14 spectrophotometer. Tlc was done on Eastman Chromagram 6060 silica gel sheets. 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.

***N*-Oxyl-2,2,6,6-tetramethylpiperidin-4-one Hydrazone.** *N*-Oxyl-2,2,6,6-tetramethylpiperidin-4-one (1.00 g, 0.0058 mol) was dissolved in methanol (3 ml). The solution was cooled to 0° and hydrazine hydrate (2.90 g, 0.058 mol) was added to it dropwise. The flask was fitted with an air lock and then allowed to stand at room temperature for 5 days. All volatile material was removed under vacuum. The resultant pale yellow oil, which gave no esr signal and one spot by the thin-layer chromatography (tlc) (silica gel in both dioxane and chloroform), was dissolved in 95% ethanol (15 ml) and stirred vigorously open to the air for 24 hr. Removal of the solvent afforded a bright yellow liquid (0.90 g, 84% of theory) which gave both esr and ir consistent with the proposed structure. The hydrazone was used without further purification.

Di(*N*-oxyl-2,2,6,6-tetramethylpiperidin-4-one)azine. *N*-Oxyl-2,2,6,6-tetramethylpiperidin-4-one hydrazone (0.50 g, 0.0027 mol) was heated at 50° under vacuum for 4 days, affording a yellow crystalline product (0.45 g), mp 173–177°. A degassed THF solution gave a typical binitroxyl spectrum. Ir was consistent with the proposed structure. *Anal.* C, H, N.

Spin-Label 3. Hydrazone **2** (0.17 g, 0.0010 mol) dissolved in 95% ethanol (3.5 ml) was added to a solution of rifaldehyde (0.500

g, 0.00069 mol) in 95% ethanol (10.5 ml) and the resultant mixture was stirred at room temperature for 15 hr.

A column of alumina (activity 1) was prepared with a bed volume of 100 ml in 3:1 ethyl acetate-ethanol (by volume). The above reaction mixture was added to the column and then eluted with 3:1 ethyl acetate-ethanol until the wash gave no signal by esr. The contents of the column were then placed in a flask and extracted with three portions of 100% ethanol (100 ml each). Removal of the solvents under vacuum afforded 0.43 g of the spin-labeled drug 3. Esr, ir, and uv are consistent with the assigned structure. Tlc showed R_f 0.43 (dioxane).

Spin-Label 8. Equal weights of 6 and acid chloride 7 were mixed as 10% solutions in chloroform. After completion of the reaction, which was checked by tlc, the reaction mixture was directly chromatographed on silica gel (BioSil-A, BioRad Laboratories, Richmond, Calif.) with benzene-chloroform: yield 90% (with respect to 6); tlc R_f 0.50 (ether-ethanol-ethyl acetate, 1:1:1); uv (in DMSO) 342 nm (ϵ 26,400), 485 (13,200); epr g value, 2.0051.

Dansyl Derivative 9. To a mixture of 200 mg of 6, 1 ml of benzene, and 5 ml of 1 M bicarbonate buffer (pH 8.7), a solution of 70 mg of dansyl chloride in 1 ml of benzene was added. The mixture was heated to 40° and stirred at this temperature until completion of the acylation, which was checked by tlc. The organic phase was dried with sodium sulfate and chromatographed on SiO_2 (BioSil-2) with chloroform-ether: yield 80% (with respect to 6); tlc R_f 0.56 (ether-ethanol-ethyl acetate, 1:1:1): uv (in DMSO) 341 nm (ϵ 25,700), 483 (11,900).

Rifamazine. Method A. Rifaldehyde (0.100 g, 0.000138 mol) was dissolved in 95% ethanol (17 ml). To it was added 0.100 M hydrazine in 95% ethanol (2.80 ml, 0.00028 mol). The pH of the resultant solution was adjusted to 6.0 with 0.10 M HCl. Within 5 min a red precipitate was observed. The solution was then stirred for an additional hour, after which time water (20 ml) was added. The solution was filtered, and the red precipitate was washed with 50% aqueous ethanol and dried under vacuum: yield 0.098 g (100%); tlc R_f 0.28 (ether-ethanol-ethyl acetate, 1:1:1); uv (in ethanol) 228 nm (ϵ 54,200), 358 (33,500), 505 (ϵ 20,000).

Method B. Rifampicin (0.100 g, 0.000122 mol), 0.20 M aqueous hydrazine (3.0 ml, 0.00060 mol), and ascorbic acid (0.025 g) were dissolved in 30% aqueous acetic acid (25 ml). The solution was stirred in the dark for 5 days at room temperature. The resultant red precipitate was collected by filtration, washed with ethanol, and dried under vacuum: yield 0.085 g (97.5%).

Rifurea. Rifaldehyde (0.100 g, 0.000138 mol) and carbohydrazide (0.00585 g, 0.000065 mol) were dissolved in methanol (10 ml). After stirring 4 hr at room temperature, water (10 ml) was added dropwise to affect crystallization. The orange precipitate was collected and washed twice (50% ethanol) by centrifugation. The product was dried under vacuum: yield 0.068 g (67%); tlc R_f 0.33 (ether-ethanol-ethyl acetate, 1:1:1); uv (in ethanol) 232 nm (ϵ 51,500), 337 (38,800), 475 (20,000).

Dirifampin. Rifaldehyde (0.100 g, 0.000138 mol) and N,N'-diaminopiperazine dihydrate (0.00988 g, 0.000065 mol) were dissolved in ethanol (12 ml). The reaction vessel was fitted with a condenser and then heated to reflux for 2.5 hr. An orange precipitate was observed soon after reaching reflux. The solution was then cooled to 0° to complete precipitation. The precipitate was collected and washed (100% ethanol) by centrifugation and then dried under vacuum: yield 0.085 g (84%); tlc R_f 0.75 (ethanol); uv (in ethanol) 234 nm (ϵ 32,700), 348 (29,900), 476 (17,700).

2-Azacyclohexadecanone. Cyclopentadecanone (4.50 g, 0.0201 mol) and hydrazoic acid (16.9 ml of 1.25 M HN_3 in benzene) in benzene (30 ml) was added dropwise to an ice-cold mixture of sulfuric acid (15.5 ml) and benzene (47 ml) with stirring. The temperature was maintained below 10°. After the addition, the ice bath was removed and the reaction mixture was stirred for another 20 min. Ice water (100 ml) was then added. The benzene layer was separated and the aqueous layer was washed once with benzene (15 ml). The two benzene solutions were combined and washed once with 1.0 N KOH (50 ml) and dried over Na_2SO_4. The benzene was removed under vacuum, affording a white crystalline solid which was recrystallized from 50% aqueous acetone: yield 4.30 g (89%); mp 131-134°.

Azacyclohexadecane. 2-Azacyclohexadecanone (4.30 g, 0.0179 mol) dissolved in benzene (12 ml) was added dropwise to a stirred mixture of $LiAlH_4$ (0.70 g, 0.018 mol) in ether (12 ml) at a rate to maintain a gentle reflux. Reflux was then maintained by heating for 15 hr. The sequence water (0.70 ml), 15% NaOH (0.70 ml), and water (2.1 ml) was added dropwise to the reaction mixture. Benzene (10 ml) was then added and the reaction mixture was filtered and washed with additional benzene. The filtrate was dried over Na_2SO_4 and evaporated under vacuum affording the product as a waxy solid: mp 45-47.5°; yield 3.90 g (96%).

N-Nitrosoazacyclohexadecane. Concentrated HCl (1.35 ml, 0.0167 mol) was slowly added to a mixture of azacyclohexadecane (3.00 g, 0.0133 mol) and water (3.0 ml) at 0°. The reaction flask was then fitted with a thermometer and heated to 65°. A solution of $NaNO_2$ (1.02 g, 0.0167 mol) in water (3.0 ml) was then added dropwise at a rate which maintained the temperature between 65 and 70°. This temperature was maintained by heating for an additional 5 min after the addition. The reaction mixture was then cooled to 25° and titrated with 15% NaOH to pH 7.0. The organic layer was removed by extraction with three portions of benzene (15 ml each). The benzene solutions were combined, treated with Na_2SO_4 and decolorizing carbon, filtered, and evaporated under vacuum. The N-nitroso compound resulted as a pale yellow, low-melting solid: mp 32-34°; yield 3.02 g (90%). *Anal.* C, H, N.

N-Aminoazacyclohexadecane. N-Nitrosoazacyclohexadecane (2.95 g, 0.0116 mol) in ether (12 ml) was added dropwise to a stirred mixture of $LiAlH_4$ (0.50 g, 0.013 mol) in ether (6 ml) at a rate to maintain a gentle reflux. Reflux was then maintained by heating for 15 hr, after which time the sequence water (0.50 ml), 15% NaOH (0.50 ml), and water (1.50 ml) was slowly added dropwise. Benzene (10 ml) was then added and the reaction mixture was filtered and washed with additional benzene. The filtrate was dried over Na_2SO_4 and evaporated under vacuum affording the product as a waxy solid: mp 39-41°; yield 2.58 g (93%); hydrochloride salt, recrystallized from cyclohexane, mp 145-147°. *Anal.* C, H, N (as the hydrochloride).

Rifazacyclo-16. Rifaldehyde (0.190 g, 0.000262 mol) and N-aminoazacyclohexadecane (0.0630 g, 0.000262 mol) were dissolved in THF (12 ml) from which oxygen had been removed by bubbling in nitrogen. The solution was stirred at 25° for 48 hr, after which time the solvent was removed under vacuum. The resulting orange solid was recrystallized from petroleum ether (40 ml): yield 0.205 g (81%); tlc R_f 0.087 (tetrahydrofuran); uv (in ethanol) 227 nm (ϵ 24,100), 279 (22,300), 350 (20,500), 479 (12,900).

Acetone Derivative of N-Aminoazacyclohexadecane. N-Aminocyclohexadecane (0.10 g, 0.00042 mol) was dissolved in acetone (10 ml) and stirred at 25° for 48 hr. Removal, under vacuum, of the excess acetone yielded a light yellow oil which gave the expected nmr and ir for the acetone hydrazone derivative: yield 0.12 g (100%). *Anal.* C, H, N.

N-Nitroso-3-azabicyclo[3.2.2]nonane. 3-Azabicyclo[3.2.2]nonane (10.0 g, 0.080 mol) was slowly added to ice-cold concentrated HCl (8.12 ml, 0.100 mol). The flask was then fitted with a thermometer and heated to 65°. A solution of $NaNO_2$ (6.0 g, 0.10 mol) in water (18 ml) was added dropwise at a rate which maintained the temperature between 65 and 70°. This temperature was maintained by heating for 10 min after the addition. The reaction mixture was cooled to 25°. The yellow precipitate that resulted was collected by filtration, dissolved in ether (100 ml), and treated with Na_2SO_4 and decolorizing carbon. The filtered solution was evaporated under vacuum affording the N-nitroso compound as a pale yellow solid, which readily sublimes above 150°: yield 6.0 g (49%). *Anal.* C, H, N.

N-Amino-3-azabicyclo[3.2.2]nonane. N-Nitroso-3-azabicyclo-[3.2.2]nonane (3.00 g, 0.0195 mol) in ether (10 ml) was added dropwise to a stirred mixture of $LiAlH_4$ (0.75 g, 0.020 mol) in ether (10 ml) and THF (10 ml) at 25°. Ten minutes after the addition, the reaction was refluxed for 15 hr, after which time the sequence water (0.75 ml), 15% NaOH (0.75 ml), and water (2.25 ml) was added dropwise. The reaction mixture was then filtered, and the precipitated hydroxides were washed twice with ether (10 ml each). The filtrate was dried over Na_2SO_4 and evaporated under vacuum. The bicyclic hydrazine (2.5 g, 90%) was obtained as a low-melting, very hydroscopic white solid. *Anal.* C, H, N.

Rifazabicyclo-9. Rifaldehyde (0.100 g, 0.000138 mol) and N-amino-3-azabicyclo[3.2.2]nonane (0.0193 g, 0.000138 mol) were dissolved in THF (12 ml). The solution was stirred for 24 hr at 25°. The solvent was then removed under vacuum. The product, which resulted as an orange solid, was recrystallized from ethyl acetate: yield 0.071 g (61%); tlc R_f 0.54 (tetrahydrofuran).

N-Amino-N-desmethylrifampicin. Rifaldehyde (1.00 g, 0.00138 mol) in THF (50 ml) was added dropwise to a stirred so-

lution of *N,N'*-diaminopiperazine dihydrate (3.80 g, 0.028 mol) in water (50 ml). The addition was made over the period of 1 hr. The reaction was stirred for an additional 1 hr after the addition. Half of the reaction volume was evaporated under vacuum. The remaining portion was extracted once with chloroform (100 ml). The chloroform solution was evaporated under vacuum affording the product as an orange powder: yield 0.98 g (83%); tlc R_f 0.45 (ethanol); uv (in ethanol) 237 nm (ϵ 25,500), 340 (21,200), 479 (11,500).

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Synthesis and Biological Activity of Certain Carbamoyl and Alkoxycarbonyl Derivatives of Adenosine 3′,5′-Cyclic Phosphate

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N-Methyl, -*n*-propyl, and -phenyl N^6,2′-*O*-bis(carbamoyl)adenosine 3′,5′-cyclic phosphates were prepared from adenosine 3′,5′-cyclic phosphate (cAMP) and the corresponding isocyanates. Selective basic hydrolysis of the N^6,2′-*O*-bis(carbamoyl)-cAMP derivatives gave N^6-(*N*-methylcarbamoyl)-cAMP, N^6-(*N*-*n*-propylcarbamoyl)-cAMP, and N^6-(*N*-phenylcarbamoyl)-cAMP, respectively. N^6,2′-*O*-Bis(*N*-methylcarbamoyl)-8-bromo-cAMP and 8-benzylthio-N^6-(*N*-phenylcarbamoyl)-cAMP were prepared similarly from the respective isocyanate and the 8-substituted cAMP derivative. Treatment of N^6,2′-*O*-bis(*N*-methylcarbamoyl)-8-bromo-cAMP with thiourea gave N^6,2′-*O*-bis(*N*-methylcarbamoyl)-8-thio-cAMP. cAMP and ethyl chloroformate gave N^6-ethoxycarbonyl-cAMP. The protein kinase and phosphodiesterase structure-activity relationships of these derivatives are discussed. The K_a for activation of protein kinase for N^6-(*N*-phenylcarbamoyl)-cAMP was one-fifth the K_a for cAMP. Other derivatives had K_a values either similar to the K_a of cAMP or considerably higher where a 2′-O substituent was present. The N^6-ethoxycarbonyl and carbamoyl derivatives were poor substrates (<10%) for rabbit kidney high K_m phosphodiesterase. Varying degrees of inhibition of the beef heart and rabbit lung low K_m phosphodiesterases by the derivatives were observed. No unusual cytotoxicity of the derivatives to KB cells was observed, with the possible exception of N^6,2′-*O*-bis(*N*-phenylcarbamoyl)-cAMP.

The ureidopurines, *N*-(purin-6-ylcarbamoyl)threonine, *N*-(purin-6-ylmethylcarbamoyl)threonine,[1] and *N*-(purin-6-ylcarbamoyl)glycine, have been found to occur naturally as the β-D-ribofuranosyl derivative in tRNA.[2-5] *N*-(Purin-6-ylcarbamoyl)threonine has also been identified in human urine.[6] Several ureidopurine derivatives related to *N*-(purin-6-ylcarbamoyl)threonine have been synthesized and found to have cytokinin-like growth-promoting properties.[7,8] Other ureido compounds, such as *N*-phenyl-*N′*-allylurea and phenylurea analogs, induce cell division.[9] Adenosine 3′,5′-cyclic phosphate (cAMP, 1) has been well established as a mediator of many hormonal effects. cAMP and some 8-substituted cAMP derivatives have also been found to control cell growth in tumor cell lines, as well as to effect differentiation of tumor cell lines.[10] It was of interest, therefore, to incorporate the ureido moiety into cAMP analogs and examine their biological activity.

As a measure of biological activity, the carbamoyl and ethoxycarbonyl cAMP derivatives were examined for their ability to activate a purified cAMP-dependent protein kinase isolated from bovine brain. Kuo and Greengard[11] have postulated the activation of this protein kinase may indeed explain the mechanism of action of cAMP in most biological systems. It has been shown that several 6- and 8-substituted derivatives of cAMP inhibit cAMP phosphodiesterase while exhibiting resistance to hydrolysis by the enzyme.[12,13] Examination of the present ureido derivatives has been made for their stability toward enzymatic hydrolysis and for inhibition of two different cAMP phosphodiesterases. All of these compounds were also examined for their toxicity to tumor cells (KB cells) in tissue culture.

Chemistry. The *N*-methyl, -*n*-propyl, and -phenyl N^6,2′-*O*-bis(carbamoyl)adenosine 3′,5′-cyclic phosphates **2**, **3**, and **4** were prepared by treatment of adenosine 3′,5′-cyclic phosphate (cAMP, 1) with the respective isocyanates at 60 to 80°. These N^6,2′-*O*-bis(carbamoyl)adenosine 3′,5′cyclic phosphates **2**, **3**, and **4** were then selectively deblocked with either refluxing $NaOCH_3$ or aqueous NaOH at room temperature to give the respective N^6-carbamoyladenosine 3′,5′-cyclic phosphates **6**, **5**, and **7**. Similarly, treatment of cAMP (1) with ethyl chloroformate and then 2 *N* NaOH gave N^6-ethoxycarbonyl-cAMP (**8**) (see Scheme I).

Muneyama, *et al.*,[12] and Bauer, *et al.*,[14] have shown that 8 substituents, *e.g.*, bromo and benzylthio, of cAMP increase the relative potency of cAMP to activate protein kinases and to stimulate glycogenolysis. Attempts to