Hybrids of Antibiotics Inhibiting Protein Synthesis. Synthesis and Biological Activity

Jiri Zemlicka,*,†,‡ M. C. Fernandez-Moyano,§ Mario Ariatti,⊥ Gary E. Zurenko,∥ Joseph E. Grady,∥ and Juan P. G. Ballesta§

Department of Chemistry, Michigan Cancer Foundation, Detroit, Michigan 48201, Departments of Internal Medicine and Biochemistry, Wayne State University School of Medicine, Detroit, Michigan 48201, Centro de Biologia Molecular Severo Ochoa (CSIC-UAM), Canto Blanco, Madrid 28049, Spain, Department of Biochemistry, University of Durban-Westville, Durban, 4000 South Africa, and The Upjohn Company, Kalamazoo, Michigan 49001

Received December 7, 1992

Four hybrid antibiotics combining structural features of chloramphenicol (**1a**), sparsomycin (**2b**), lincomycin (**5c**), and puromycin (**6d**)—lincophenicol (**1c**), chloramlincomycin (**5a**), sparsolincomycin (**5b**), and sparsopuromycin (**6b**)—were synthesized. They were investigated as inhibitors of several partial reactions of procaryotic and eucaryotic protein synthesis as well as potential antimicrobial agents. Lincophenicol (**1c**) was active as inhibitor of *Escherichia coli* ribosomal peptidyltransferase-catalyzed puromycin reaction. Both lincophenicol (**1c**) and sparsophenicol (**1b**) inhibited the binding of the iodophenol analogue of sparsomycin to *E. coli* ribosomes. The results are discussed in terms of a retro-inverso hypothesis advanced earlier for interpretation of biological activity of chloramphenicol (**1a**) and sparsophenicol (**1b**). Chloramlincomycin (**5a**) suppressed the growth of *Streptococcus pyogenes* with MIC 6.25 μg/mL.

Antibiotics inhibiting ribosomal protein synthesis such as chloramphenicol (**1a**), sparsomycin (**2b**), and lincomycin (**5c**) are effective antibacterial agents.[1] Other inhibitors of this group, such as puromycin[2] (**6d**) and sparsomycin[3] (**2b**), were once considered as antitumor agents, but later studies revealed the toxicity of both agents.[4,5] More recently, several lipophilic analogues of sparsomycin (**2b**) were found to inhibit protein synthesis as well as the growth of various bacteria and tumor cell lines,[6,7] and some of them have apparently progressed to the stage of clinical trials.[8] These compounds, including the most potent derivative ethyldeshydroxysparsomycin[9] (**3b**), are also able to potentiate the antileukemic effect of cisplatin in mice. Last but not least, phenol–alanine sparsomycin (**4b**) is a more effective inhibitor of ribosomal binding than sparsomycin (**2b**).[8]

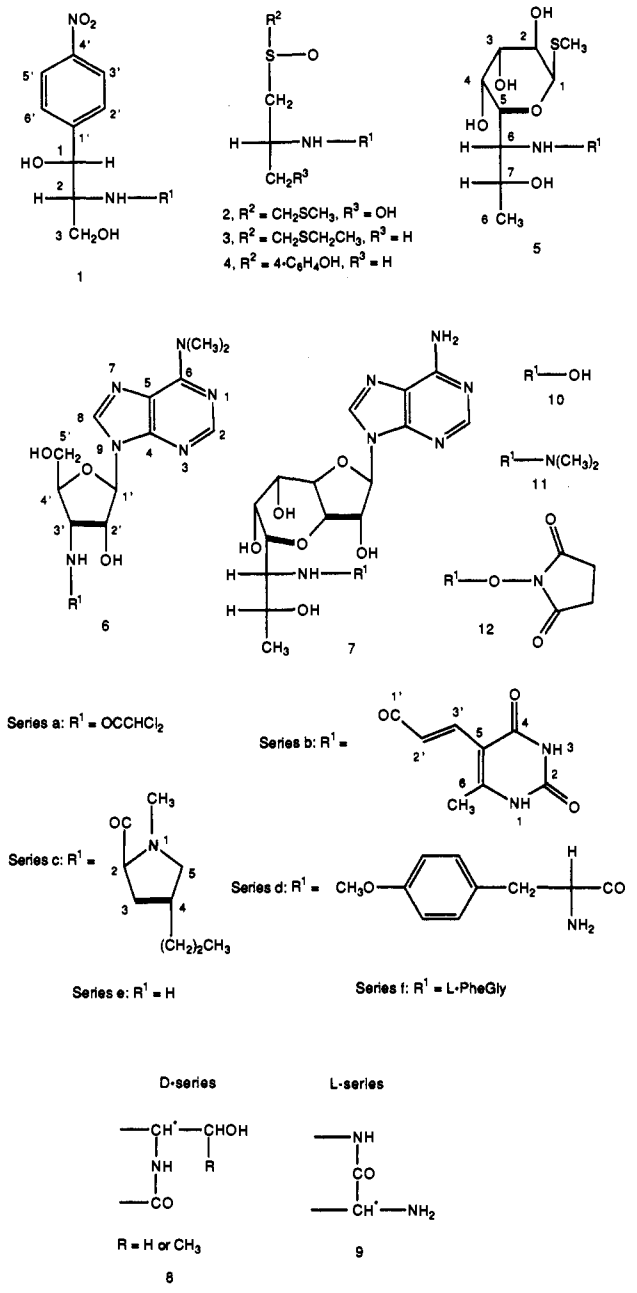
Despite extensive studies of these antibiotics, details of their mechanism of action are still far from being elucidated. This is especially apparent in case of the structurally simplest antibiotic of this group, chloramphenicol (**1a**). Over the years, several different concepts[10–12] were invoked to interpret its inhibitory properties in molecular terms. Attempts to find common links related to the biological function in this group of antibiotics led to investigation of "hybrids" comprising structural features of two antibiotics, e.g. puromycin (**6d**) and chloramphenicol (**1a**). Thus, *N*-(dichloroacetyl)puromycin (**6a**) and, conversely, 4-methoxy-L-phenylalanine derivative of chloramphenicol base **1d** were found to exhibit moderate inhibition of polyphenylalanine synthesis in *Escherichia coli* cell free system.[13] Sparsophenicol (**1b**), an inhibitor of puromycin reaction catalyzed by *E. coli* ribosomes,[14] combines the structural features of chloramphenicol (**1a**) and sparsomycin (**2b**). Quantamycin (**7c**), a hybrid of lincomycin (**5c**) and the 3′ terminal adenosine unit of peptidyl tRNA designed by computer-assisted molecular modeling and obtained by ingenious synthesis,[15] displays some inhibition of lincomycin (**5c**) binding to ribosomes, but it lacks antibacterial activity.

The design of sparsophenicol (**1b**) was based on a hypothesis[10,14] stipulating that antibiotics inhibiting protein synthesis and carrying an acylamido function attached to a D-aminopropanol moiety (structure **8**), such as chloramphenicol (**1a**), sparsomycin (**2b**), and lincomycin (**5c**), can be regarded as retro-inverso analogues of L-amino acid residue of puromycin (**6d**, see structure **9**) or the terminal 3′-*O*-(aminoacyl)adenosine unit of aminoacyl-tRNA. The scope and limitations of this hypothesis can be studied by interchange of acyl residues of **1a**, **2b**, and **5c** and inhibition of protein synthesis by the resultant hybrids. This contribution describes synthesis and biological testing of four new hybrids, lincophenicol (**1c**), chloramlincomycin (**5a**), sparsolincomycin (**5b**), and sparsopuromycin (**6b**). The latter compound which contains an acylamido function found in sparsomycin (**2b**) is not a retro-inverso analogue in terms of structures **8** and **9**. Rather, compound **6b** belongs to a group of hybrids such as *N*-(dichloroacetyl)puromycin aminonucleoside[13] (**6a**) or quantamycin[15] (**7c**) combining the relevant features of antibiotics or peptidyl tRNA according to a different rationale.

Synthesis. The synthesis of hybrid **5b** followed the procedure described previously[14] for sparsophenicol (**1b**). The corresponding acid[16] **10b** was activated with isobutyl chloroformate and triethylamine in acetonitrile. The resultant active ester was reacted in situ with methyl thiolincosaminide[17] (**5e**) in aqueous acetonitrile to give hybrid **5b** in only 10% yield. The yield increased considerably (to 49%) when both activation and reaction were carried out in dimethylformamide[18] (DMF). Therefore, the syntheses of compounds **1c**, **6b**, and dimethylamide **11b** were all performed in DMF as a solvent. Thus, hybrid **1c** was obtained from *trans*-L-4-propylhygric acid[19] (**10c**) and chloramphenicol base **1e** in 44% yield. In a similar fashion, the reaction of acid **10b** with puromycin aminonucleoside (**6e**) afforded compound **6b** (64%) where-

† Michigan Cancer Foundation.
‡ Wayne State University School of Medicine.
§ Centro de Biologia Molecular Severo Ochoa (CSIC-UAM).
⊥ University of Durban-Westville.
∥ The Upjohn Company.

0022-2623/93/1836-1239$04.00/0



as with dimethylamine compound **11b** resulted in 54% yield. Hybrid **5a** was prepared by a prolonged reflux (40 h) of methyl thiolincosaminide (**5e**) with methyl dichloroacetate in acetonitrile in 22% yield. An alternate method was sought for the synthesis of sparsophenicol (**1b**). The reaction of chloramphenicol base (**1e**) with acid **10b**, *N*-hydroxysuccinimide (HSI), and dicyclohexylcarbodiimide (DCC) in DMF[20] resulted in formation of three products which were separated by column chromatography on silica gel: *N*-hydroxysuccinimide ester **12b** (44%), dimethylamide **11b** (17%) and sparsophenicol (**1b**, 7%). Apparently, the reactivity of **12b** is significantly lower than that of active ester generated from isobutyl chloroformate.

Inhibition of Ribosomal Protein Synthesis. The following testing systems were employed to determine activity of hybrid antibiotics in ribosome- (peptidyltransferase-) catalyzed protein synthesis: (i) inhibition of the puromycin reaction (formation of a single peptide bond) catalyzed by ribosomes from *E. coli*[6] and rat liver;[21] (ii) suppression of the polyphenylalanine synthesis (model of polypeptide formation) catalyzed by ribosomes from *E. coli* and *Saccharomyces cerevisiae*;[6] (iii) inhibition of binding of iodo analogue of phenol–alanine sparsomycin (**4b**) to ribosomes[8] from *E. coli*. Sparsomycin (**2b**) was used as a positive control in assays (i) and (ii). The results are summarized in Tables I–IV and Figure 1.

Table I. Inhibition of the Puromycin Reaction in *E. coli* Ribosome System

inhibitor[a]	concentration (M)				
	1×10^{-7}	1×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
sparsolincomycin (**5b**)	101.1	98.1	97.3	105.1	82.6
lincophenicol (**1c**)	101.8	93.4	91.3	28.8	7.3
chloramlincomycin (**5a**)	100.7	105.4	107.3	103.8	71.1
sparsopuromycin (**6b**)	100.0	100.3	101.7	107.0	87.8
sparsophenicol (**1b**)	103.1	104.4	92.7	32.3	13.2
sparsomycin (**2b**)	50.6	13.7	2.8	1.1	1.8

[a] The results are expressed as percent of controls without inhibitors. For details see the Experimental Section.

Table II. Inhibition of the Puromycin Reaction with Hybrid Antibiotics in a Rat Liver Ribosome System

inhibitor[a,b]	puromycin concentration (M)	
	2×10^{-4}	1×10^{-3}
sparsopuromycin (**6b**)	69	76
sparsolincomycin (**5b**)	90	91
sparsophenicol (**1b**)	83	86
lincophenicol (**1c**)	100	98.5

[a] See Table I. [b] Inhibitor concentration was 5×10^{-4} M.

It is clear that the only active hybrids are sparsophenicol (**1b**) and lincophenicol (**1c**), both derived from chloramphenicol base (**1e**). Compounds **1b** and **1c** have similar activity profiles in inhibition of the puromycin reaction (Table I) and binding of the iodo analogue of phenol–alanine sparsomycin (**4b**) to *E. coli* ribosomes (Figure 1). Compounds **1b**, **1c**, and **5b** did not inhibit the puromycin reaction catalyzed by rat liver ribosomes (Table II). Sparsopuromycin (**6b**) exhibited only a low activity in the latter system, and it was a noncompetitive inhibitor of puromycin (data not shown). All hybrids were inactive as inhibitors of the polyphenylalanine synthesis in procaryotic and eucaryotic systems (Table III and IV). These results indicated that analogues **1b** and **1c** comprising one CONH (amide) grouping are capable of inhibiting the synthesis of only a single peptide bond in procaryotic system (*E. coli*).

Regardless of rationale, it seemed unlikely that chloramlincomycin (**5a**) and lincophenicol (**1c**) whose parent antibiotics are selective inhibitors of procaryotic protein synthesis could effectively participate in any inhibition of eucaryotic peptide bond formation. Also, active hybrids resulted only from a combination of chloramphenicol base (**1e**) with acylamido functions found in sparsomycin (**2b**) or lincomycin (**5c**). Other analogues, such as compounds **5a**, **5b**, and **6b**, were inactive. It is then obvious that the validity of the retro-inverso hypothesis within the group of tested hybrids is restricted to analogues of chloramphenicol (**1a**). Although binding of CONH moiety is, among other factors, important for biological activity of puromycin[22] (**6d**) and lincomycin[23] (**5c**) the relative contributions of different functions to the overall binding capacity of a given antibiotic cannot be assessed. Affinity of such groups for appropriate binding sites on peptidyltransferase may well surpass that of CONH moiety. It was argued[11] that inhibitory activity of aminoacyl derivatives of chloramphenicol base is at variance with retro-inverso hypothesis. However, configuration of an aminoacyl residue does not substantially affect the magnitude

Table III. Inhibition of the Polyphenylalanine Synthesis with Hybrid Antibiotics in *E. coli* Ribosome System

inhibitor[a]	concentration (M)						
	1×10^{-6}	5×10^{-6}	1×10^{-5}	5×10^{-5}	1×10^{-4}	5×10^{-4}	1×10^{-3}
sparsolincomycin (**5b**)	103.1	102.2	111.2	106.4	103.1	97.4	84.5
lincophenicol (**1c**)	95.5	97.6	92.0	96.4	107.6	94.4	87.4
chloramlincomycin (**5a**)	95.1	93.7	91.0	99.8	121.7	108.6	113.9
sparsopuromycin (**6b**)	94.9	92.4	87.0	94.5	97.8	96.8	94.4
sparsophenicol (**1b**)	94.6		97.1		100.3		79.4
sparsomycin (**2b**)	77.3	52.8	41.3		39.4		20.2
lincomycin (**5c**)	85.8		66.5		45.3		28.0

[a] See Table I.

Table IV. Inhibition of the Polyphenylalanine Synthesis in *S. cerevisiae* Ribosome System

inhibitor[a]	concentration (M)		
	1×10^{-5}	1×10^{-4}	1×10^{-3}
sparsolincomycin (**5b**)	100.9	97.7	93.4
lincophenicol (**1c**)	101.8	102.0	99.5
chloramlincomycin (**5a**)	97.3	90.9	96.8
sparsopuromycin (**6b**)	106.3	95.8	95.7
sparsophenicol (**1b**)	115.2	98.9	79.0
sparsomycin (**2b**)	44.1	30.3	15.5

[a] See Table I.

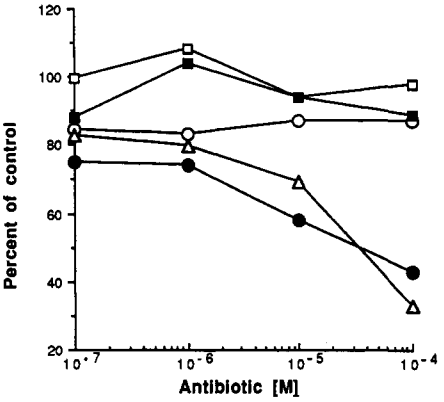


Figure 1. Inhibition of binding of [^{125}I]iodophenol analogue of sparsomycin to *E. coli* ribosomes with hybrid antibiotics: (□) chloramlincomycin (**5a**), (○) sparsopuromycin (**6b**), (△) sparsophenicol (**1b**), (■) sparsolincomycin (**5b**), (●) lincophenicol (**1c**).

of inhibition[13] of the respective analogues of chloramphenicol (**1a**), and it is not clear whether such a factor will influence activity of dipeptide analogue **1f**. Also, as indicated in structures **8** and **9**, the configuration of amino acid moiety of puromycin (**6d**) must be related to the chloramphenicol base (**1e**) and not to the *N*-aminoacyl residue.[11]

Antibacterial Activity. Analogues **1b**, **1c**, **5a**, **5b**, **6b**, and **11b** were screened for antimicrobial activity against 23 microorganisms (supplementary material, Table 1). Compounds which showed any antimicrobial activity were quantitatively tested against 11 microorganisms (Table V). Lincomycin (**5c**) and chloramphenicol (**1a**) were used as positive controls. It is interesting that chloramlincomycin (**5a**), a hybrid antibiotic completely inert in all cell free systems investigated, inhibited the growth of *Streptococcus pyogenes* with minimal inhibitory concentration (MIC) of 6.25 μg/mL (Table V). Some activity of **5a** was also observed in *Staphylococcus aureus* (MIC 50 μg/mL) and *Streptococcus pneumoniae* (25 μg/mL). The MIC of sparsophenicol (**1b**) in *S. pyogenes* was only 50 μg/mL. It is noteworthy that activity of **5a** against *S. pyogenes* retained 50% of inhibitory potency of chloramphenicol (**1a**, MIC 3.12 μg/mL). It would then seem that target receptor of **5a** in *S. pyogenes* is different from ribosomal peptidyltransferase or that the ribosomal function in the latter organism is modified relative to that of *E. coli*. The rest of hybrid analogues were inactive in most of the microbial cultures employed. This is hardly surprising in view of the results of inhibition of partial reactions of protein synthesis in cell free systems (vide infra) and the fact that in preliminary assays[14] sparsophenicol (**1b**) did not exhibit any antibacterial activity. Thus, hybrid antibiotics **1b** and **1c**, active in some partial reactions of procaryotic protein synthesis, are either unable to penetrate the cell membrane using active transport systems of parent antibiotics and/or to inhibit the protein synthesis in the intact cells.

Experimental Section

General Procedures. Acetonitrile and dimethylformamide (DMF) were stored over Linde molecular sieves 4A. Thin-layer chromatography (TLC) was performed as described[14] on aluminum sheets of silica gel in the following solvents: S_1, CH_2Cl_2–MeOH (9:1); S_2, CH_2Cl_2–MeOH (95:5); S_3, CH_2Cl_2–MeOH (4:1); and S_4, 2-propanol–NH_4OH–H_2O (7:1:2). For preparative TLC loose layers[24] and column chromatography Kiesel Gel 60 was employed. Paper electrophoresis was performed as described[25] at 15 °C and 40 V/cm for 1 h in the following buffers: 0.02 M Na_2HPO_4 (pH 7), 0.02 M $Na_2B_4O_7$ (pH 9), and 0.05 M sodium citrate (pH 3.5). The ^{1}H NMR spectra were determined at 100 MHz with an FX-100 Fourier transform NMR spectrometer JEOL (100 MHz) or at 300 MHz with QE 300 instrument. The ^{13}C NMR spectra were measured at 75.48 MHz with QE 300 instrument. The Me_4Si was used as a reference. Chemical ionization (CI-MS) and fast atom bombardment (FAB-MS) mass spectra were determined with a Kratos MS80 RFA high-resolution instrument. 2-Methylpropane was used as an ionization gas in CI-MS and 1-thioglycerol (*m/z* 108) as a matrix for FAB-MS. UV spectra were determined in 0.01 M Na_2HPO_4 (pH 7) unless specified otherwise. Circular dichroism (CD) spectra were obtained at pH 7 as described.[14,25]

Starting Materials. Chloramphenicol base (**1e**) and puromycin aminonucleoside (**6e**) were products of Sigma Chemical Co., St. Louis, MO. Lincomycin hydrochloride (Lincocin, **5c**) and *trans*-L-4-propylhygric acid (**10c**) were gifts of the Upjohn Co., Kalamazoo, MI. Compound **10c** was also obtained by acid hydrolysis[19] of lincomycin (**5c**) or hydrazide[17] of **10c** as described. Methyl thiolincosaminide[17] (**5e**) and acid[16] (**10b**) were obtained according to the literature.

Reaction of Acid 10b with Chloramphenicol Base (1a) Using *N*-Hydroxysuccinimide/Dicyclohexylcarbodiimide Reagent. A mixture of chloramphenicol base (**1e**, 0.21 g, 1 mmol), acid **10b** (0.2 g, 1.01 mmol), and *N*-hydroxysuccinimide (HSI, 0.12 g, 1.03 mmol) was dissolved in warm DMF, and it was made anhydrous by evaporation in vacuo (oil pump). The residue was redissolved in DMF (6 mL), the solution was cooled in an ice bath, and dicyclohexylcarbodiimide (DCC, 0.22 g, 1.06 mmol) was added. The mixture was stirred at 0 °C for 2 h, at room temperature for 24 h, and at 60–65 °C (bath temperature) for another 26 h. The reaction was monitored by TLC (S_1). After cooling in an ice bath, new portions of reagents (HSI, 1.03 mmol and DCC, 1.06 mmol) were added. The stirring at room temperature was then continued for a total of 5 days. The mixture was filtered, the filtrate was evaporated, and silica gel (2 g) was

Table V. Inhibition of Microbial Growth with Hybrid Antibiotics

		minimal inhibitory concentration (MIC, μg/mL)					
organism[a]	UC no.[b]	chloramlinco-mycin (5a)	sparsolinco-mycin (5b)	sparso-phenicol (1b)	linco-phenicol (1c)	linco-mycin (5c)	chloramphen-icol (1a)
Staphylococcus aureus	76	50	>400	>400	800	0.78	6.25
	6685	>400	>400	>400	800	>400	6.25
	6690	50	>400	>400	>800	0.78	50
Enterococcus faecalis	694	>400	>400	>400		50	6.25
Streptococcus pyogenes	152	6.25	>400	50	200	0.20	3.12
Streptococcus pneumoniae	41	25	>400	>400	100	0.78	3.12
Escherichia coli	45	>400	>400	>400	>800	>400	3.12
Klebsiella pneumoniae	58	>400	>400	>400	800	400	3.12
Salmonella schottmuelleri	126	>400	>400	>400	>800	>800	6.25
Pseudomonas aeruginosa	95	>400	>400	>400	>800	>400	100
	6676	>400	>400	>400		>400	200

[a] For details, see the Experimental Section and supplementary material. [b] UC = Upjohn culture.

added to the residue along with CH_2Cl_2–MeOH. The solvents were evaporated, and the solids were put on the top of silica gel column (50 × 1.5 cm) which was eluted with CH_2Cl_2 (0.5 L) and solvent S_2 (2.4 L). Compound **12b** was eluted first (elution volume ca. 600 mL), and the appropriate fractions were evaporated to give a solid which was washed with CH_2Cl_2 (90 mg in two crops, 44%), homogeneous on TLC (S_1): mp 273–274 °C dec; UV (ethanol) max 316 nm (ε 14 000), sh 266 (ε 4400); ^{1}H NMR (CD_3SOCD_3) δ 7.66 and 7.16 (2d, 2, *E*-CH=CH, J = 15.5 Hz), 2.82 (s, 4, CH_2), 2.36 (s, 3, Me). Anal. ($C_{12}H_{11}N_3O_6 \cdot 0.25H_2O$) C, H, N.

Amide **11b** was eluted at an elution volume of 1–1.4 L, and the product was rechromatographed on two 2-mm-thick 20 × 20 cm loose layers of silica gel which were developed in solvent S_2 and then several times in S_1. Evaporation of the eluate afforded amide **11b** which was filtered off after addition of methanol, 40 mg (17%), mp 320–323 °C dec, identical with an authentic sample (vide supra).

A continuing elution of the column with solvent S_1 (0.4 L) and S_3 (0.5 L) gave sparsophenicol (**1b**) which was rechromatographed on a loose layer of silica gel in solvent S_1 (double development) and then in S_3, 30 mg (7%), identical with an authentic specimen: [14] ^{1}H NMR (300 MHz, CD_3SOCD_3) δ 11.18 (s, 2, NH-uracil), 8.11 and 7.56 (2 dd, 4, 4-nitrophenyl), 7.77 (d, 1, NH, amide,[26] $J_{NH,H-2}$ = 9 Hz), 7.02 and 7.01 (2 s, 2, CH=CH), 5.78 and 4.84 (poorly resolved d and broad s, 2, OH), 5.01 (apparent s, 1, H_1), 4.09 (dd, 1, H_2), 3.55 and 3.29 (2 m, 2, H_3), 2.16 (Me); ^{13}C NMR (chloramphenicol base portion) 151.87 ($C_{4'}$), 146.16 ($C_{1'}$), 127.16 ($C_{3'}$, $C_{5'}$), 122.67 ($C_{2'}$, $C_{6'}$), 69.16 (C_1), 60.29 (C_2), 56.15 (C_3); (3-(6-methyluracil-5-yl)acryloyl residue) 165.85 ($C_{1'}$), 162.64 (C_2), 153.62 (C_4), 149.77 (C_5), 129.98 (C_6), 121.53 ($C_{3'}$), 104.49 ($C_{2'}$), 16.62 (Me). FAB-MS 499 (21.8, M + H + 108), 391 (31.1, M + H), 148 (100.0).

Sparsolincomycin (5b). A. In MeCN. Isobutyl chloroformate (0.13 mL, 1 mmol) was added to a stirred mixture of acid **10b** (0.2 g, 1 mmol), triethylamine (0.14 mL, 1 mmol), and MeCN (10 mL) cooled in an ice bath. After 15 min a solution of methyl thiolincosaminide (**5e**, 0.25 g, 1 mmol) in 40% aqueous MeCN (10 mL) was added dropwise. The mixture was stirred at 0 °C for 30 min and at room temperature for 1 h. The precipitated product **5b** was filtered off, 70 mg (16%), mp 260–262 °C dec, and it was recrystallized from water (10 mL), 47 mg (10%), mp 290–292 °C dec, uniform on TLC (S_4) and paper electrophoresis at pH 7 (mobility 0.14 of **5e**) and pH 9 (mobility −0.36 of **5e**): UV max 303 nm (ε 22 800), sh 268 (ε 13 900); CD max (pH 7) 305 nm (Θ 4000); ^{1}H NMR (CD_3SOCD_3 + D_2O) δ 7.28 and 7.07 (2 d, 2, J = 15.4 Hz, *E*-CH=CH), 5.18 (d, 1, H_1, $J_{1,2}$ = 5.4 Hz), 2.27 (s, 3, Me uracil), 1.99 (s, 3, SMe), 1.07 (d, 1, 8-Me, $J_{8,7}$ = 6.1 Hz). Anal. ($C_{17}H_{25}N_3O_8S \cdot 2H_2O$) C, H, N, S.

B. In DMF. The reaction was performed as in method A on a 0.87-mmol scale in DMF (4 mL) instead of MeCN. Addition of isobutyl chloroformate was followed by an immediate precipitation of NEt_3·HCl. Base **5e** in DMF (15 mL) was added after 2 min. The mixture was stirred at 0 °C for 20 min and at room temperature for 3 h. The solution was evaporated in vacuo (oil pump), water (10 mL) was added, the pH was adjusted to 10 with NH_4OH, the product **5b** was filtered off and dried, 0.23 g (57%), mp 285–287 °C dec, uniform on TLC (S_4) and paper electrophoresis (pH 7). Recrystallization from water (35 mL) with the aid of Norit A and Celite gave 0.2 g (49%), mp 298–299 °C dec, $[\alpha]_D$ 184.8° (*c* 0.5, DMF), and identical with a sample prepared by method A.

Lincophenicol (1c). The experiment was performed on a 1-mmol scale as in case of sparsolincomycin (**5b**, method B) with chloramphenicol base (**1e**), acid **10c** (hydrochloride, Upjohn Co.), and triethylamine (0.28 mL, 0.2 mmol). After filtration of NEt_3·HCl the mixture was evaporated and the crude product was partitioned between CH_2Cl_2 (25 mL) and water (15 mL). The aqueous phase was extracted with CH_2Cl_2 (15 mL). The combined organic layers were washed with water (15 mL), dried ($MgSO_4$), and evaporated to give lincophenicol (**1c**), 0.19 g (52%). Crystallization from benzene (3 mL) afforded 0.16 g (44%), mp 137–139 °C, ninhydrin-positive (yellow on Whatman No. 1 paper, violet on silica gel[27]). Recrystallization from 50% aqueous ethanol (3 mL, Norit A) gave 90 mg (25%), uniform on TLC (S_1, S_2 and MeCN–H_2O, 1:1) and paper electrophoresis at pH 7 (mobility 0.68 of **5e**) and pH 3.5 (mobility 0.79 of **5e**): mp 140–142 °C; $[\alpha]_D$ −63.6° (*c* 0.5, ethanol); UV max 277 nm (ε 9600); CD max (pH 7) 330 nm ([Θ] −500), 280 ([Θ] −500), 246 ([Θ] 2000); ^{1}H NMR (CD_3COCD_3 + D_2O) δ 8.20 and 7.72 (2 d, 4, 4-nitrophenyl), 5.33 (bs, 1, H_1), 4.19 (poorly resolved t, 1, H_2), 3.72 (m, 2, H_3), 2.29 (s, 3, NMe), 1.22 (m, 4, CH_2 of 1-propyl), 0.86 (t, 3, Me of 1-propyl); pyrrolidine ring multiplets at δ 3.13, 2.75, 2.00 and 1.66; ^{13}C NMR (chloramphenicol base portion) 149.09 ($C_{4'}$), 147.33 ($C_{1'}$), 126.69 ($C_{3'}$, $C_{5'}$), 123.36 ($C_{2'}$, $C_{6'}$), 68.43 (C_1), 62.68 (C_2), 55.70 (C_3); (4-propylhygric acid portion) 176.00 (CO), 72.67, 63.15, 41.64, 37.89, 37.48, 35.36, 21.51, 14.12; CI-MS 366 (22.3, M + H), 126 (100.0). Anal. ($C_{18}H_{27}N_3O_5$) C, H, N.

Compound **1c** was also obtained by reaction of acid **10c** prepared by acid hydrolysis[17,19] of lincomycin (**5c**) or hydrazide of **10c**.

Chloramlincomycin (5a). A stirred mixture of methyl thiolincosaminide (**5e**, 0.5 g, 2 mmol) and methyl dichloroacetate (4 mL) was refluxed in MeCN (40 mL) for 40 h. After cooling, a small amount of tan solid was filtered off (cotton plug), and the filtrate was evaporated. The residue was crystallized from MeCN (7 mL) with the aid of Norit A (0.1 g) to give product **5a** which was washed with ether (10 mL) and dried, 0.39 g (49%), uniform on TLC (S_1, detection by charring with 10% $HClO_4$), mp 169–171 °C dec. For analysis, it was recrystallized twice from MeCN, 0.16 g (22%): mp 179–181 °C dec; $[\alpha]_D$ 198.6° (*c* 0.5, ethanol); ^{1}H NMR (D_2O) δ 6.27 (s, 1, Cl_2CH), 5.29 (d, 1, H_1, $J_{1,2}$ = 5.6 Hz), 2.06 (s, 3, SMe), 1.11 (d, 3, 8-Me, $J_{8,7}$ = 6.1 Hz). Anal. ($C_{11}H_{19}Cl_2NO_6S$) C, H, Cl, N, S.

Sparsopuromycin (6b). The reaction was run on a 0.5-mmol scale as described for sparsolincomycin (**5b**, method B), only base **5e** was replaced with puromycin aminonucleoside (**6e**). A foamy residue obtained after evaporation of DMF was converted to a solid by addition of water (5 mL). The product **6b** was filtered off, washed successively with water (2 mL), dilute NH_4OH (pH 10, 3 mL), and water (2 × 3 mL), and dried, 0.2 g (85%), mp 207–209 °C. Crystallization from 50% ethanol (40 mL) afforded 0.15 g (64%) of **6b**, mp 212–214 °C, uniform on TLC (S_1 and S_3), and paper electrophoresis (pH 7, mobility 0.0) showed absence of **6e** and **10b**: $[\alpha]_D$ −75.5° (*c* 0.5, DMF); UV max 279 nm (ε 34 100), sh 306 (ε 22 500); CD max (pH 7) 290 nm ([Θ]

-21 100), 262 ([θ] 12 000); ^{1}H NMR (CD_3SOCD_3 + D_2O) δ 8.38 (s, 1, H_8), 8.17 (s, 1, H_2), 7.27 and 7.08 (2 d, 2, *E*-CH=CH, J = 15.4 Hz), 5.97 (d, 1, $H_{1'}$, $J_{1',2'}$ = 2.2 Hz), 4.5 (m, 2, $H_{2'}$ + $H_{3'}$), 3.40 (s, 6, NMe_2), 2.30 (s, 3, Me uracil); FAB-MS 581 (7.9, M + H + 108), 473 (9.0, M + H), 164 (100.0). Anal. ($C_{20}H_{24}N_8O_6 \cdot 1.5H_2O$) C, H, N.

***N,N*-Dimethyl-β-(*E*)-1,2,3,4-tetrahydro-6-methyl-2,4-dioxo-5-pyrimidineacrylamide (11b).** The reaction was performed on a 1-mmol scale according to the procedure described above for sparsolincomycin (**5b**, method B). Base **5e** was replaced with dimethylamine hydrochloride, and therefore, 2 mmol of triethylamine was used. The crude product was dissolved in NH_4OH (15%, 50 mL), and the solution was evaporated to dryness. The residue was washed with water (4 mL) and it was dried to give 0.16 g (72%) of dimethylamide **11b**, uniform on TLC (S_1). This material was crystallized from 50% ethanol, furnishing 0.12 g (54%): mp 320–323 °C; UV (ethanol) max 306 nm (ε 21 200), sh 268 (ε 11 300); ^{1}H NMR (CD_3SOCD_3 + D_2O) δ 7.47 and 7.20 (2 d, 2, *E*-CH=CH, J = 15 Hz), 3.00 and 2.86 (2 s, 6, NMe_2), 2.22 (s, 3, Me). Anal. ($C_{10}H_{13}N_3O_3 \cdot 0.5H_2O$) C, H, N.

Biological Assays. Puromycin Reaction. A. *E. coli* Ribosomes. Ribosomes were prepared from MRE600 cells grown in rich medium at mid-log exponential phase.[6] Cells were ground with alumina and ribosomes were separated by centrifugation following standard methods.[28] The ribosome solutions had ϵ_{260} 4.8 × 10^7. The estimated "molecular weight" of 70S ribosome was 2.5 × 10^6. The S-100 fraction corresponds to the supernatant after centrifuging the bacterial cell extracts using a Sorvall T 860 rotor at 48 K for 2 h. The reaction mixture contained in 25 μL: *E. coli* ribosomes (10 pmol), poly U (100 μg/mL), *N*-acetyl-[^{3}H]-Phe-tRNA (15 pmol), $MgCl_2$ (20 mM), KCl (30 mM), NH_4Cl (50 mM), and Tris-HCl (30 mM, pH 7.8). The mixtures were kept at 37 °C for 30 min, puromycin (**6d**, 20 mM, 1 μL) was added, and the incubation was continued for additional 5 min. After addition of Na_2CO_3 (0.1 M, 0.25 mL) and ethyl acetate (0.7 mL), the mixtures were shaken for 1 min and centrifuged to separate the phases, and aliquots (0.5 mL) of the organic phase were taken for radioactivity counting. In control experiments without inhibitors, an average of 0.65 pmol of *N*-acetyl-[^{3}H]-Phe-puromycin was obtained. For results, see Table I.

B. Rat Liver Ribosomes. Rat liver polyribosomes were prepared as described.[21] Puromycin (**6d**) and hybrid antibiotics stocks were in dimethyl sulfoxide (DMSO), so that final DMSO concentration was 8%. The assay mixtures contained in 0.125 mL: Tris-HCl (50 mM, pH 7.6), NH_4Cl (20 mM), KCl (60 mM), $MgCl_2$ (20 mM), 2-mercaptoethanol (1 mM), GTP (potassium salt, 0.5 mM), poly U (15 μg), *N*-acetyl-[^{3}H]-L-phenylalanyl-tRNA (16 μg, 4200 cpm), G-factor (200 μg protein), NH_4Cl-washed rat liver ribosomes (170 μg), and puromycin or hybrid antibiotics as indicated in Table II. The mixtures were incubated at 37 °C for 15 min; Tris-HCl (100 mM, pH 7.6, 0.5 mL) and ethyl acetate (1.5 mL) were added. After shaking (0.5 min) and centrifugation a 1-mL portion of the clear supernatant was counted in instagel. In control experiments (spontaneous hydrolysis of *N*-acetyl-[^{3}H]-L-phenylalanyl-tRNA) the incubation mixtures lacked puromycin and hybrid antibiotics.

Inhibition of Polyphenylalanine Synthesis.[6] A. *E. coli* Ribosomes. The ribosomes were prepared as described for the puromycin reaction assay, method A. The reaction mixtures contained in 50 μL: ribosomes (0.3 μM), tRNA (1.25 mg/mL), poly U (2.5 mg/mL), phosphoenol pyruvate (2.5 mg/mL), pyruvate kinase (20 μg/mL), S-100 fraction (5–10 μL), GTP (1 mM), ATP (10 mM), 2-mercaptoethanol (5 mM), $MgCl_2$ (15 mM), NH_4Cl (90 mM), and Tris-HCl (50 mM, pH 7.4). The reaction was initiated by the addition of [^{3}H]phenylalanine (30 μM), and the mixture was incubated at 37 °C for 30 min. After quenching with trichloroacetic acid (5%, 1 mL), the mixtures were placed in boiling water for 3 min and filtered through a glass fiber filter. In control experiments without inhibitors ca. 80 000 cpm of phenylalanine (300 cpm/pmol) was polymerized which corresponded to 17 pmol/ribosome. The results are summarized in Table III.

B. *S. cerevisiae*. The ribosomes from *S. cerevisiae* were prepared[6] as described for *E. coli* ribosomes. The assays were performed as in method A, but phosphoenol pyruvate and pyruvate kinase were replaced with creatine phosphate (20 mM) and creatine phosphokinase (50 μg/mL). The *E. coli* tRNA was substituted with yeast tRNA (0.5 mg/mL). In control experiments 8–9 pmol of phenylalanine/pmol ribosomes was polymerized. For results, see Table IV.

Inhibition of Binding of [^{125}I]Iodophenol Analogue of Sparsomycin to Ribosomes. The binding was performed[8] under the conditions of polyphenylalanine synthesis as described above. Concentration of ribosomes and labeled sparsomycin derivative were 0.3 and 0.1 μM, respectively. About 600 cpm of analogue per picomole of ribosomes were usually bound in the control experiments which corresponded to ca. 0.2 molecules/ribosome. For results, see Figure 1.

Antimicrobial Activity. Disk-Diffusion Assay. For each test organism an appropriate agar medium was prepared and sterilized. The agar was cooled to 40–50 °C, and then it was inoculated with a suspension of organism. The seeded agar was poured into a plastic assay tray (22 × 53 cm), and it was spread to form a uniform layer. The agar was allowed to solidify at room temperature. This procedure was repeated for each of 23 test organisms. Compounds were dissolved in DMF, and water was added to yield a final concentration of 1 mg/mL drug in 40% DMF. Absorbent paper disks (12.7 mm) were loaded with drug solution (80 μL/compound), and they were air-dried. The disks were then applied to the surface of the organism-seeded assay trays. The trays were incubated overnight under conditions appropriate for the growth of each organism. The diameter of the inhibition zone was measured to the nearest millimeter. For results, see Table 1 in the supplementary material.

Minimal Inhibitory Concentration (MIC) Assay. The MIC values were determined by an agar dilution method. Serial 2-fold dilutions of solution of tested compound were prepared in 1.0-mL volumes of sterile water. Molten (47 °C) brain heart infusion agar medium (BHIA, 9.0 mL) was added to each 1.0-mL dilution. The drug-supplemented agar was mixed, it was poured into 15 × 100-mm Petri dishes, and it was allowed to solidify and dry at room temperature prior to inoculation. The test organisms were grown overnight on BHIA medium at 35 °C under aerobic conditions. Colonies were harvested with a sterile swab, and cell suspensions were prepared in Trypticase Soy Broth (TSB) to equal the turbidity of a 0.5 McFarland standard. A 1:20 dilution of the suspension was prepared in TSB, and the drug-supplemented agar plates were inoculated with a 1-μL drop of the diluted cell suspension. The final concentration of inoculum was approximately 10^4 colony-forming units per drop. The plates were incubated for 18 h at 35 °C. The MIC was read at the lowest concentration of compound that inhibited a visible growth of organism. For results, see Table V.

Acknowledgment. The 100-MHz ^{1}H NMR spectra were determined by S. Grunfeld whereas 300-MHz ^{1}H and ^{13}C NMR spectra were provided, along with mass spectra, by the Central Instrumentation Facility (Department of Chemistry, Wayne State University, Dr. Robin J. Hood, Director). Our thanks are also due to Drs. L. A. G. M. van den Broek and H. C. J. Ottenheijm for a generous gift of phenol-alanine sparsomycin. The work at the Michigan Cancer Foundation was supported by an institutional grant from the United Way of Southeastern Michigan. The support for investigation at the CSIC-UAM was provided by a grant PB87-0450 from Direccion General de Investigacion Cientifica y Tecnica and an institutional grant from Fundacion Ramon Areces.

Supplementary Material Available: Table 1, disk-diffusion assay of hybrid antibiotics (1 page). Ordering information is given on any current masthead page.

References

(1) Vazquez, D. *Inhibitors of Protein Synthesis*; Springer: New York, 1979; pp 108 and 127.
(2) Suhadolnik, R. J. *Nucleoside Antibiotics*; John Wiley & Sons: New York, 1970; pp 1–50.
(3) Owen, S. P.; Dietz, A.; Camiener, G. W. Sparsomycin, a New Antitumor Antibiotic. I. Discovery and Biological Properties. *Antimicrob. Agents Chemother.* **1962**, 772–779.

(4) Suhadolnik, R. J. *Nucleosides as Biological Probes*; John Wiley & Sons: New York, 1979; p 195.
(5) Close, H. P.; McFarlane, J. R. Ocular Toxicity with Sparsomycin (NSC-59729) in a Phase I Study: A Preliminary Report. *Cancer Chemother. Rep. No. 43*, 1964, 29–31.
(6) van den Broek, L. A. G. M.; Liskamp, R. M. J.; Colstee, J. H.; Lelieveld, P.; Remacha, M.; Vazquez, D.; Ballesta, J. P. G.; Ottenheim, H. C. J. Structure–Activity Relationships of Sparsomycin and Its Analogues. Inhibition of Peptide Bond Formation in Cell-Free Systems and of L1210 and Bacterial Cell Growth. *J. Med. Chem.* 1987, *30*, 325–333.
(7) van den Broek, L. A. G. M.; Lazaro, E.; Zylicz, Z.; Fennis, P. J.; Missler, F. A. N.; Lelieveld, P.; Garzoto, M.; Wagener, D. J. T.; Ballesta, J. P. G.; Ottenheijm, H. C. J. Lipophilic Analogues of Sparsomycin as Strong Inhibitors of Protein Synthesis and Tumor Growth. *J. Med. Chem.* 1989, *32*, 2002–2015.
(8) Lazaro, E.; San Felix, A.; van den Broek, L. A. G. M.; Ottenheijm, H. C. J.; Ballesta, J. P. G. Interaction of the Antibiotic Sparsomycin with the Ribosome. *Antimicrob. Agents Chemother.* 1991, *35*, 10–13.
(9) Hofs, H. P.; Wagener, D. J. T.; de Valk-Bakker, V.; van Rennes, H.; van Zeist, A. J.; van den Broek, L. A. G. M.; Ottenheijm, H. C. J. Potentiation of Cisplatin Antitumour Activity by Ethyldeshydroxy–Sparsomycin in L1210 Leukemia. *Anticancer Res.* 1992, *12*, 167–170.
(10) Bhuta, P.; Chung, H. L.; Hwang, J.-S.; Zemlicka, J. Analogues of Chloramphenicol: Circular Dichroism Spectra, Inhibition of Ribosomal Peptidyltransferase, and Possible Mechanism of Action. *J. Med. Chem.* 1980, *23*, 1299–1305. See also refs 4, 7, 9–13, and 16–18 cited therein.
(11) McFarlan, S. C.; Vince, R. Inhibition of Peptidyltransferase and Possible Mode of Action of a Dipeptidyl Chloramphenicol Analogue. *Biochem. Biophys. Res. Commun.* 1984, *122*, 748–754.
(12) Vester, B.; Garrett, R. A. The Importance of Highly Conserved Nucleotides in the Binding Region of Chloramphenicol at the Peptidyl Transfer Centre of Escherichia Coli 23S Ribosomal RNA. *EMBO J.* 1988, *7*, 3577–3587.
(13) Vince, R.; Almquist, R. G.; Ritter, C. L.; Daluge, S. Chloramphenicol Binding Site with Analogues of Chloramphenicol and Puromycin. *Antimicrob. Agents Chemother.* 1975, *8*, 439–443.
(14) Bhuta, A.; Zemlicka, J. Sparsophenicol: A New Synthetic Hybrid Antibiotic Inhibiting Ribosomal Peptide Synthesis. *J. Med. Chem.* 1982, *25*, 1123–1125.
(15) Hanessian, S.; Sato, K.; Liak, T. J.; Danh, N.; Dixit, D.; Cheney, B. V. "Quantamycin": A Computer-Simulated New-Generation Inhibitor of Bacterial Ribosomal Binding. *J. Am. Chem. Soc.* 1984, *106*, 6114–6115.
(16) Wiley, P. F.; MacKellar, F. A. Sparsomycin, Structure and Chemistry. *J. Org. Chem.* 1976, *41*, 1858–1862.
(17) Schroeder, W.; Bannister, B.; Hoeksema, H. Lincomycin. III. The Structure and Stereochemistry of the Carbohydrate Moiety. *J. Am. Chem. Soc.* 1967, *89*, 2448–2453.
(18) van Zon, A.; Beyerman, H. C. Repetitive Excess Mixed Anhydride (REMA) Synthesis of Peptides. The Protected C-terminal Hexapeptide of Secretin. *Helv. Chim. Acta* 1973, *56*, 1729–1740.
(19) Herr, R. R.; Slomp, G. Lincomycin. II. Characterization and Gross Structure. *J. Am. Chem. Soc.* 1967, *89*, 2444–2447.
(20) Daluge, S.; Vince, R. Synthesis and Antimicrobial Activity of a Carbocyclic Puromycin Analog. 6-Dimethylamino-9-{R-[2R-hydroxy-3R-(p-methoxyphenyl-L-alanylamino)]cyclopentyl}-purine. *J. Med. Chem.* 1972, *15*, 171–177.
(21) Ariatti, M.; Hawtrey, A. O. Synthesis of Cyclohexylpuromycin and its Reaction with N-Acetylphenylalanyl-Transfer Ribonucleic Acid on Rat Liver Ribosomes. *Biochem. J.* 1975, *145*, 169–176.
(22) Kato, T.; Zemlicka, J. "Reduced" Analogues of Puromycin. Synthesis of 3′-O-(L-2-amino-3-phenylpropyl)-N^6,N^6-dimethyladenosine and the Corresponding 2′ Isomer. *J. Org. Chem.* 1980, *45*, 4006–4010.
(23) Campbell, J. M.; Reusser, F.; Caskey, C. T. Specificity of Lincomycin Action on Peptidyl Transferase Activity. *Biochem. Biophys. Res. Commun.* 1979, *90*, 1032–1038.
(24) Zadrazil, S. Chromatography of Nucleic Acid Components. In *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W., Tipson, R. S., Eds.; Wiley-Interscience: New York, 1973; Vol. 2, pp 533–660, loc. cit. p 632.
(25) Zemlicka, J.; Owens, J. Simple Models of Nucleic Acid Interactions. I. Base-Base Interactions in 1,2-Di(adenosin-N^6-yl)ethane and 1,4-Di(adenosin-N^6-yl)butane. *J. Org. Chem.* 1977, *42*, 517–523.
(26) After addition of D_2O only 37% exchange of amidic proton was observed. Heating at 65 °C for 7 h raised the deuterium content to 74%.
(27) In contrast to previous observations,[19] free acid **10c** reacted similarly with ninhydrin.
(28) Staehelin, T.; Maglott, D. R. [47] Preparation of *Escherichia coli* Ribosomal Subunits Active in Polypeptide Synthesis. In *Methods of Enzymology*; Moldave, K., Grossman, L., Eds.; Academic Press: New York, 1971; Vol. XX, pp 449–456.