50 ml of H_2O) while stirring and keeping the temperature between 1 and 3°. After stirring for an additional 16 hr and allowing the mixture to reach room temperature, the pH was adjusted to 7.5 by addition of 4 *N* HCl. After filtration the filtrate was acidified with 4 *N* HCl. The resulting amorphous precipitate was crystallized by trituration with MeOH (200 ml), recrystallized from aqueous EtOH, washed thoroughly with MeOH (200 ml), and dried *in vacuo* at 80° to yield **58** (24%), mp 221°. *Anal.* ($C_{14}H_{10}Cl_2N_2O_8S_4$) C, H, N.

4-Chloro-3-mercapto-5-sulfamoylbenzoic Acid (59). To a stirred solution of **58** (1.6 g, 3 mmol) in 1 *N* $NaHCO_3$ (50 ml), $Na_2S_2O_4$ (6 g, 34 mmol) was added in portions followed by heating on a steam bath for 30 min. Cooling, acidification with 4 *N* HCl, and recrystallization of the resulting precipitate from Me_2CO–petroleum ether yielded crude **59** (68%), mp 268–269°, which was used without further purification. For analysis a sample was recrystallized several times from EtOH–H_2O and MeOH–H_2O, mp 277.5–278°. *Anal.* ($C_7H_6ClNO_4S_2$) C, H, N.

Ethyl 2-Chloro-4-phenoxy-5-sulfamoylbenzoate (60). 2-Chloro-4-phenoxy-5-sulfamoylbenzoic acid[4] was esterified in EtOH using concentrated H_2SO_4 as catalyst. Concentration *in vacuo* and addition of H_2O precipitated crude **60**. It was recrystallized from aqueous EtOH and dried *in vacuo* to yield **60** (72%), mp 143–145°. *Anal.* ($C_{15}H_{14}ClNO_4S$) C, H, Cl, N, S.

Ethyl 2-Chloro-4-phenylthio-5-sulfamoylbenzoate (61). 2-Chloro-4-phenylthio-5-sulfamoylbenzoic acid[4] was esterified as described for **60**. Crude **61** precipitated on concentration. It was recrystallized from EtOH to yield **61** (76%), mp 162–164°. *Anal.* ($C_{15}H_{14}ClNO_3S_2$) C, H, Cl, N, S.

Ethyl R_2S-4-R_1-5-Sulfamoylthiosalicylates 62–70 and R_2S-4-R_1-5-Sulfamoylthiosalicylic Acids 71–79 (Table IV). Method Y. To a solution of NaOEt (prepared from 11 mmol of Na) in dry EtOH (10–18 ml), **60** or **61** (5 mmol) was added followed by the appropriate R_2SH (5.5 mmol), and the mixture was refluxed for 4–6 hr. After addition of concentrated HCl (1.0 ml) or AcOH (1.0 ml) and cooling, the crude reaction product crystallized, eventually after dilution with H_2O. The material was washed with H_2O and dried in air, prior to recrystallization.

Method Z. The appropriate Et ester **62–70** was saponified with an excess of 2 *N* NaOH by heating on a steam bath for 15 min. After cooling, the crude reaction product was precipitated by acidification with an excess of 4 *N* HCl or 4 *N* AcOH.

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Puromycin Analogs.[1] Studies on Ribosomal Binding with Diastereomeric Carbocyclic Puromycin Analogs†

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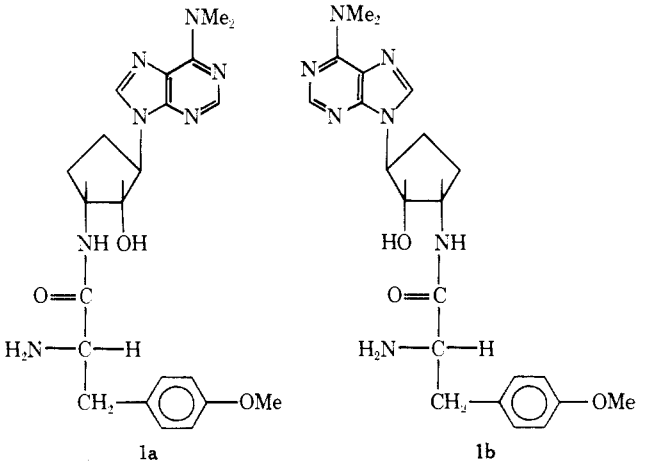
A direct and convenient route to the antimicrobial carbocyclic puromycin analog, 6-dimethylamino-9-[(*R*)-[(2*R*)-hydroxy-(3*R*)-(*p*-methoxyphenyl-L-alanylamino)]cyclopentyl]purine (1a), is described. Epoxidation of 3-acetamidocyclopentene (**3**) gave exclusively *cis*-3-acetamido-1,2-epoxycyclopentane (**4**). Opening of the epoxide with NaN_3, followed by reduction of the resulting azido alcohol **5**, gave a high yield of 2α-acetamido-5β-aminocyclopentan-1α-ol (**6**). This amine was easily resolved *via* tartrate formation. Introduction of the purine moiety by standard methods gave the enantiomeric carbocyclic aminonucleosides (−)- and (+)-2α-acetamido-5β-(6-dimethylamino-9-purinyl)-cyclopentan-1α-ol (**10a** and **10b**). Resolution at an early point allows for the conversion of **10a** and **10b** to a wide variety of diastereomeric aminoacyl derivatives. Studies on protein synthesis inhibition with diastereomeric carbocyclic puromycin analogs indicate that two distinct types of protein synthesis inhibitors may have been developed—series **a** which are peptidyl transferase substrates, and series **b** which are peptidyl transferase inhibitors.

The carbocyclic puromycin analog **1a** exhibits potent antimicrobial activity[2] and is effective against three tumor lines tested in tissue culture[3] while the diastereomer **1b** was only slightly active. *In vitro* testing demonstrated that **1a** inhibits the formation of polyphenylalanine in the *Escherichia coli* cell-free system[3] and that it is an effective competitive inhibitor of puromycin for peptidylpuromycin synthesis.[4] The inhibition is stereospecific with the diastereomer **1b** being much less active than **1a**. The carbocyclic puromycin analog has only slightly less affinity for ribosomes than does puromycin itself.[4] In addition, **1a**, but not **1b**, was shown to accept acetylphenylalanine from acetylphenylalanyl-tRNA.[4] These results firmly establish that **1a** has a mechanism of action identical with that of puromycin and that structural manipulation to obtain various active analogs may be extremely useful in elucidating various aspects of protein biosynthesis.



1a 1b

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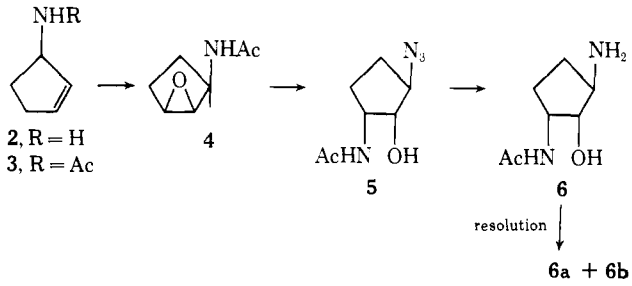
The previous synthetic route leading to **1a** was not desirable for the preparation of compounds having different aminoacyl groups at the 3′ position since the separation of diastereomers in the last step, even when possible, would not ensure that these derivatives would have the same absolute stereochemistry as **1a**.[2] Thus, a shorter and more convenient route to the carbocyclic aminonucleoside precursor of **1a** is now described which has the added advantage of utilizing an intermediate amine **6** (Scheme I) which is easily resolved before introduction of the purine moiety. Also, due to the stereoselectivity of the reactions of this new route, the separation of contaminating stereoisomers is avoided, in contrast to the previously described synthesis in which reduction of an oxime gave a mixture of *cis*- and *trans*-amino alcohols which were separated chromatographically.[2]

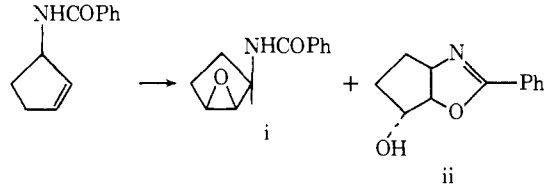
Scheme I[a]



[a] Structures **2**–**6** depict only one enantiomer of the racemic form actually obtained.

3-Aminocyclopentene (**2**) was prepared by a modification of previously reported methods[5,6] in which freshly prepared 3-chlorocyclopentene[7] was added to liquid ammonia in methanol. Previous workers have lost considerable amine due to codistillation with methanol[5] or ethanol.[6] As contaminating methanol did not interfere with acylation, all fractions containing amine (as determined by pmr specta) were combined. The yield, calculated by pmr integration, was 41%. An attempt to react 3-chlorocyclopentene with NH_3, without methanol, resulted in considerable polymerization and only 10% of **2**, apparently due to the low solubility of 3-chlorocyclopentene in liquid ammonia. A 50% yield of **2** was obtained by reaction of 3-bromocyclopentene with ammonia, without methanol. However, the instability and comparative difficulty and low yield (*ca.* 30%) of the large-scale preparation of 3-bromocyclopentene, *via* monobromination of cyclopentene with NBS,[8] made the use of 3-chlorocyclopentene more desirable for our purpose.

Acetylation of **2** gave 3-acetamidocyclopentene (**3**), a stable, crystalline solid (Scheme I). Epoxidation of **3** with *m*-chloroperbenzoic acid gave a 70% yield of *cis*-3-acetamido-1,2-epoxycyclopentane (**4**), with loss occurring due to solubility of **4** in the aqueous extractions necessary for work-up. Epoxidation of 3-benzamidocyclopentene by the same method gave a 90% yield of the *cis*-epoxide (i), along with 3% of the *trans*-hydroxyoxazoline (ii) resulting from back-side benzamido group intervention during epoxidation.‡ Although the yield of epoxide isolated was greater when the benzamido protecting group was used, the acetamido group was chosen for reasons to be discussed.



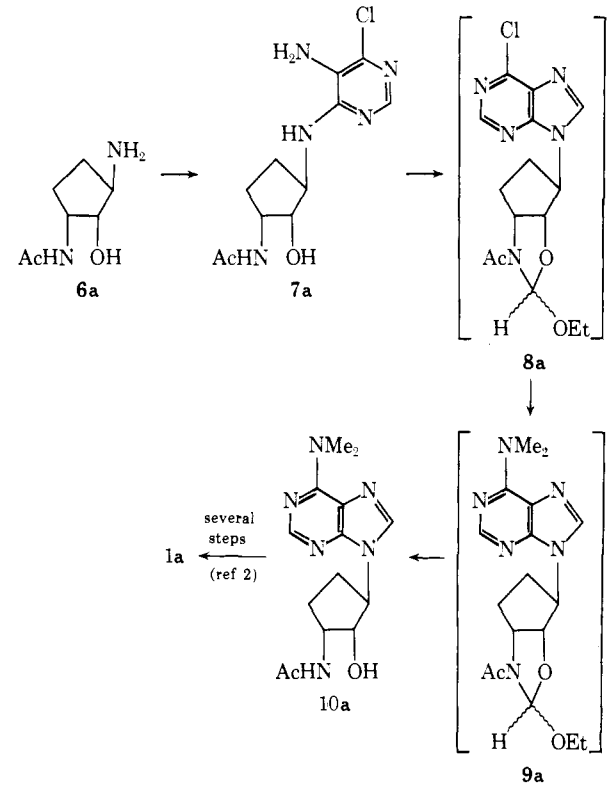
The stereoselectivity noted for these epoxidations is expected since the peracid oxidation of 3-benzamidocyclohexene gives 81% of the *cis*-epoxide, with no *trans*-hydroxyoxazoline detected.[9] This cis-directing effect is well known in the epoxidation of cyclic allylic alcohols[10a] and is attributed to association of the epoxidation reagent with the hydroxyl group prior to attack of the double bond. The predominant cis-directing effect of the allylic amido group has also been noted in the epoxidation of 3-amidocyclopentenes containing allylic and homoallylic hydroxyl groups.[10b]

Epoxide **4** was opened with sodium azide in aqueous methoxyethanol buffered by ammonium chloride, giving azido alcohol **5** (86%). The azide ion attacked only at the position farther from the amide group, as expected.[11] Catalytic reduction of **5** resulted in an almost quantitative yield of the corresponding amine **6**.

The racemic amine **6** was resolved *via* tartrate formation. The (+)-amine **6a**§ formed a tartrate with (−)-tartaric acid which was separated (88% yield) from the more soluble diastereomeric salt by fractional crystallization. Similarly, the (−)-amine **6b** formed a tartrate with (+)-tartaric acid which was purified by crystallization. The free amines, **6a** and **6b**, were obtained by treatment of the enantiomeric tartrates with basic resin. The configuration assigned to **6a** (see Scheme II) is based on its ultimate conversion to **1a**.

The purine moiety was formed *via* a standard method[12] involving condensation of racemic amine **6** or resolved

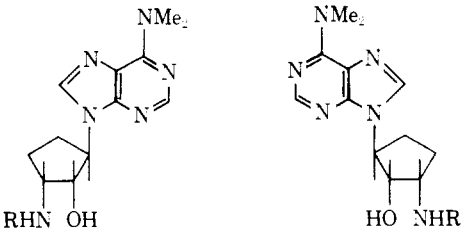
Scheme II



‡ An analytically pure sample of i (white needles from $CHCl_3$–hexane, mp 131–132°) had ir and pmr analogous to those of **4**. Chromatography of the mother liquors gave analytically pure ii (white needles from $CHCl_3$–hexane, mp 138.5–139.5°); ir and pmr as expected.

§ Compound numbers followed by **a** denote enantiomers derived from the (+)-amine, **6a**, and numbers followed by **b** denote enantiomers derived drom the (−)-amine, **6b**. Numbers not followed by **a** or **b** denote racemic mixtures.

Table I



series a (from 10a) series b (from 10b)

Compd	R[a]	Yield, %[b,c]	Mp, °C[b]	Solvent[d]	Formula[e]	Specific rotation[f] $[\alpha]_{589}$	$[\alpha]_{436}$	C	Solvent
11a	Z-L-Phe	90	162–163.5	EtOAc	$C_{29}H_{33}N_7O_4$	−20.7	−45.3	0.497	$CHCl_3$
11b	Z-L-Phe	88	146–148	EtOAc	$C_{29}H_{33}N_7O_4$	+30.4	+68.4	0.467	$CHCl_3$
12a	L-Phe	70	Solid foam	$CHCl_3$	$C_{21}H_{27}N_7O_2$	−73.2	−163.5	0.245	$CHCl_3$
12b	L-Phe	70	174–175	EtOH (abs)	$C_{21}H_{27}N_7O_2$	+32.9	+74.6	0.949	MeOH
13a	Z-D-Phe	(97)							
13b	Z-D-Phe	(95)							
14a	D-Phe	76	174–175	EtOH (abs)	$C_{21}H_{27}N_7O_2$	−32.5	−74.4	0.957	MeOH
14b	D-Phe	77	Solid foam	$CHCl_3$	$C_{21}H_{27}N_7O_2$	+73.1	+163.6	0.371	$CHCl_3$
15a	Z-S-By-L-Cys	82	124–132	EtOAc	$C_{30}H_{35}N_7O_4S$	−31.1	−66.9	0.483	$CHCl_3$
16a	S-By-L-Cys	50[g]	Solid foam	$CHCl_3$	$C_{22}H_{29}N_7O_2S$	−44.9	−100.9	0.495	$CHCl_3$
17a	Z-L-Leu	88	Solid foam	$CHCl_3$	$C_{26}H_{35}N_7O_4$	−32.3	−70.0	0.417	$CHCl_3$
17b	Z-L-Leu	(90)							
18a	L-Leu	69	Solid foam	$CHCl_3$	$C_{18}H_{29}N_7O_2$	−29.8	−62.8	4.40	$CHCl_3$
18b	L-Leu	70	Solid foam	$CHCl_3$	$C_{18}H_{29}N_7O_2$	+8.34	+9.66	0.228	$CHCl_3$

[a] Z = *N*-benzyloxycarbonyl; By = benzyl. [b] Yield and melting point values are for analytical samples except values in parentheses which refer to homogeneous samples suitable for the next reaction. All yields are calculated from **10a** or **10b** [*i.e.*, include $Ba(OH)_2$ hydrolyses of acetamide]. All analytical samples were purified by preparative tlc on silica gel plates developed in 5–15% MeOH–$CHCl_3$ and were chromatographically homogeneous; ir spectra as expected. [c] The diastereomeric pairs **12a** and **12b**, **14b** and **14a**, and **18a** and **18b** (lower R_f compound listed first for each pair) could be separated chromatographically if racemic **10** is used as the starting material, as was originally done in the preparation of **1a** and **1b** (see ref 2). Certain diastereomeric pairs, *e.g.*, the S-By-L-Cys derivatives, could not be separated chromatographically. [d] Crystallization solvent or solvent from which the noncrystallizable samples were foamed (0.1 mm at 56°). [e] Analyzed for C, H, and N. [f] Specific rotation values are in degrees followed by concentration (%) and solvent. [g] The *N*-benzyloxycarbonyl group of **15a** was partially removed by 30% HBr in AcOH (25° for 18 hr); **16a** was then separated from unreacted **15a** chromatographically as described above.

amines **6a** or **6b** with 5-amino-4,6-dichloropyrimidine. (Scheme II depicts only the reaction of **6a**.) Ring closure of the resulting pyrimidines **7**, **7a**, and **7b** with triethyl orthoformate gave the corresponding 6-chloropurine ethoxyoxazolidines **8**, **8a**, and **8b**. Refluxing aqueous dimethylamine converted the chloropurine compounds to the corresponding 6-dimethylaminopurine ethoxyoxazolidines **9**, **9a**, and **9b**. Mild acidic hydrolysis of the ethoxyoxazolidine moiety gave the corresponding acetamido alcohols **10**, **10a**, and **10b**. The overall yield from **7** to **10** was 72%. Racemic **10** prepared by this route is identical with an authentic sample prepared by the previously described route.[2] This provides further confirmation of the assignment of structure of epoxide **4** and azido alcohol **5**.

The benzamido epoxide (i) was also converted to the 6-dimethylaminopurinyl benzamido alcohol analog of **10**. However, difficulty was encountered due to low solubility of the pyrimidinyl compound in triethyl orthoformate. Also, hydrolysis of the benzamide was incomplete (*ca.* 72%) after 18 hr in refluxing saturated barium hydroxide. In contrast, hydrolysis of the acetamide was complete in 3 hr.[2] Thus, although the yield for the epoxidation is significantly greater with 3-benzamidocyclopentene (90% *vs.* 70%), the overall yield is best and the reactions are cleaner for the acetamido-blocked compounds.

The conversion of **10a** to **1a** was carried out by barium hydroxide hydrolysis of the acetamide and then coupling of the resulting carbocyclic aminoucleoside analog with *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine by the dicyclohexylcarbodiimide–*N*-hydroxysuccinimide method, followed by hydrogenolysis of the blocking group, exactly as described previously.[2] The sample of **1a** prepared in this way had identical R_f, ir, and optical rotation to the diastereomer which has been assumed to have the stereochemistry of puromycin on the basis of its activity.[=] Similarly, **10b** was converted to diastereomer **1b** described previously.[2] Other aminoacyl derivatives of **10a** and **10b** (see Table I) were prepared in the same manner.

Results and Discussion

Various aminoacyl analogs of puromycin have been used to study the binding requirements for the formation of a puromycin–ribosome complex. Nathans and Neidle[14] demonstrated that only the amino acids L-phenylalanine and L-tyrosine could replace the *p*-methoxyphenylalanine moiety without significant loss in activity. The requirement for an aromatic amino acid seems to be essential for activity but not absolute for phenylalanine since the *S*-benzyl-L-cysteine analog was also inhibitory.[15] The existence of a hydrophobic ribosomal binding site for these aromatic aminoacyl R groups has been suggested.[16,17]

The high cost of puromycin and the difficulties encountered in preparing 3-aminoribosyl nucleosides have severely limited the availability of compounds to further explore the ribosomal binding site. Previous results with **1a**[2-4] have demonstrated the utility of carbocyclic analogs for this purpose. In addition, the preparation of aminoacyl

[=] Conformational information derived from X-ray diffraction patterns of a crystalline 50:50 mixture of diastereomers **1a** and **1b** has been published.[13] Determination of the absolute configuration of **1a** (as a solid dihydrochloride–dihydrate, mp 217–220°) or of other suitable crystalline analogs of **1a** is being carried out by M. Sundaralingam.

analogs of **1b** allows us to study the binding of the diastereomeric forms which would correspond to puromycin containing an L-ribosyl sugar.

The effect of the carbocyclic analogs of puromycin on the rate of poly-UC-directed polyphenylalanine formation in an *E. coli* cell-free system with washed ribosomes is shown in Table II.** The results obtained with the carbocyclic analogs are consistent with those previously obtained with the corresponding aminoacyl puromycin analogs.[14,15] For example, the replacement of the amino acid moiety of **1a** with L-phenylalanine (**12a**) resulted in only a slight decrease in activity. The 4.5-fold decrease in activity of **14a** compared with **12a** demonstrates a requirement for an L-amino acid, an observation also reported for puromycin containing L- or D-phenylalanine.[14,15] It is also interesting to note that the *S*-benzylcysteine derivative **16a** gave only a slight decrease in activity over **12a** as predicted from the reported activities of the corresponding puromycin analogs prepared by Symons, *et al.*[15] The increased distance between the benzene ring and the free amino group caused by the extra carbon and sulfur atoms may be responsible for the slightly reduced inhibition of **16a** as compared with the phenylalanine analog.

Since the phenyl ring of these compounds seems to extend into a nonpolar region of the ribosome, the L-leucyl analog should provide an isopropyl moiety which can extend into the same area. This would account for the moderate activity of the L-leucine analog **18a** as indicated in Table II. A longer alkyl chain may be required for more effective binding by projecting deeper into the nonpolar region. An extension of this study is being pursued.

Some interesting observations were made when the diastereoisomers (series **b**) were tested. As anticipated, **1b** was inactive at 10^{-4} *M*. When the concentration was increased to 10^{-3} *M*, a 30% inhibition was obtained (data not included in Table II). Although **1b** is able to produce inhibition of the puromycin reaction, unlike **1a** it was not able to act as an acceptor to form the acetylphenylalanyl derivative.[4] A possible explanation for the relatively weak binding of **1b** to the ribosome may be realized by examination of molecular models of both diastereoisomers. When the corresponding hydroxyl groups and the acylamino moieties of **1a** and **1b** are superimposed, the purine moieties of both isomers can reach a common site. However, the cyclopentane rings of the two molecules are projected in different directions.

Unexpectedly, the removal of the *p*-methoxy group resulted in a great increase in activity of **12b** over **1b**. This large change in binding is surprising since the *p*-methoxy group contributes only slightly to the activity of **1a** over **12a** and, in fact, exhibits the opposite effect in the **a** series. These effects may be due to positioning of the aromatic rings in the hydrophobic region such that the para positions extend in different directions. It is interesting to note that a preference for the L configuration of the amino acid moiety also exists in the **b** series as can be seen by comparing **12b** with **14b**. Finally, the ratio of activities of the L-leucyl analogs, **18a** and **18b**, is exactly the same as the L-phenylalanyl disastereoisomers, **12a** and **12b**, as expected if a common binding site is assumed for both series of compounds.

A comparison of the data in Table II with the activities of aminoacyl puromycin analogs clearly established the utility of the carbocyclic compounds for exploring the ribosomal binding site. The facile synthesis of these analogs allows for a great degree of structural modification and also provides a series of stereoisomers which are not available from puromycin. The present data, in conjunction with the mechanism studies on **1a** and **1b**,[4] indicate that two distinct types of inhibitors may have been developed—series **a** which are peptide acceptors with mechanisms of action identical with that of puromycin and series **b** which are competitive inhibitors of the peptidyl transferase reaction. The large increase in activity of **12b** and **18b** over the corresponding *p*-methoxyphenylalanine analog **1b** demonstrates the potential for further development of this unique series of diastereomeric puromycin analogs into more potent peptidyl transferase inhibitors. Kinetic studies on the binding and substrate activities of these and other derivatives in a ribosomal peptidyl transferase system are being pursued and will be presented at a later date.

Table II. Inhibition of Poly-UC-Directed L-[^{14}C]Polyphenylalanine Formation[a]

Compd	R	% inhibition[b] 10^{-4} *M*	% inhibition[b] 10^{-5} *M*
1a	*p*-MeO-L-Phe	100	83.6
1b	*p*-MeO-L-Phe	0	0
12a	L-Phe	96.6	75.2
12b	L-Phe	41.8	25.3
14a	D-Phe	21.2	17.0
14b	D-Phe	9.5	5.1
16a	S-By-Cys	84.8	45.8
18a	L-Leu	48.4	15.1
18b	L-Leu	24.9	0

[a] Assay conditions are those previously described (ref 3) using poly-UC (1:1) mRNA. All counts were corrected by blanks and all values represent an average of triplicate determinations. The average deviation of such replicates is $< \pm 4\%$. [b] The per cent inhibitions by puromycin in this assay were 100 and 93.4 at 10^{-4} and 10^{-5} *M*, respectively.

Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected. Optical rotations were measured at ambient temperature with a Perkin-Elmer 141 automatic polarimeter; ir, in KBr disks, with a Perkin-Elmer 237B spectrophotometer; pmr, with a Varian A-60D spectrometer using TMS as an internal standard. Analytical tlc was run on silica gel (Eastman chromagram sheets with fluorescent indicator); preparative tlc was done on 20 × 20 cm glass plates coated with 2 mm of silica gel F254 (E. Merck, Darmstadt). All compounds were chromatographically homogeneous on tlc developed in 5% MeOH-$CHCl_3$ and visualized by uv light or iodine. Evaporations were carried out *in vacuo* with a bath temperature of less than 50°. Samples were dried *in vacuo* (<1 mm) at 56° before analysis.

3-Aminocyclopentene (2). 3-Chlorocyclopentene prepared from cyclopentadiene (156 g, 2.36 mol) and dry HCl (86 g, 2.4 mol)by the procedure of Moffett[7] was added, without purification, to a mechanically stirred, cooled (Dry Ice-acetone) solution of liquid NH_3 (700 g) in dry MeOH (750 ml) over a period of 20 min. After addition was complete, the bath was removed. Stirring was continued for 5 hr, by which time the temperature had risen to 10° and solid had started to precipitate. The mixture was then allowed to stand overnight, protected by a drying tube. A small amount of gummy white polymer and some NH_4Cl were then filtered off and washed with MeOH (200 ml). The combined filtrate and wash were concentrated to a syrup (<35°, 40 mm), which was dissolved in 6 *N* NaOH (400 ml). This solution was saturated with NaCl and then extracted with Et_2O (3 × 250 ml). The Et_2O extracts were dried (K_2CO_3) and the Et_2O was removed *in vacuo*. The brown liquid remaining was distilled through a 6-in. Widmer column. The fractions boiling in the range 77–108° were combined (94.6 g, determined by integration of a pmr spectrum to be contaminated by *ca.* 15% MeOH). Most of the MeOH could be re-

** It is interesting to note that these analogs, as well as puromycin, exhibit greater inhibitory activities in the presence of poly-UC than when poly-U is used as mRNA; similar observations have been reported for puromycin and chloramphenicol.[18,19] The poly-UC data are recorded here because the values obtained are consistent with polyribosome systems and reflect the effect of these inhibitors on protein synthesis in intact cells.[4]

moved by redistillation of such a sample through a 12-in. Widmer column, giving a 41% yield (from cyclopentadiene) of 3-aminocyclopentene (**2**), bp 105–108° (lit. 25%, bp 108–109°[5]; 30%, bp 110–111°[6]). However, since the MeOH did not interfere with acylation, such samples could be used without redistillation.

3-Acetamidocyclopentene (3). Acetic anhydride (125 ml) was added over 5 min to a stirred, cooled (ice bath) solution of **2** (53.0 g, 0.638 mol) in 6 *N* NaOH (500 ml). Stirring was continued for 5 min with cooling and then for 5 min without cooling. The resulting mixture was extracted with PhH (3 × 250 ml). The PhH solution was dried ($CaSO_4$), concentrated to 100 ml, and diluted with hexane (200 ml), precipitating **3** as white needles (72.97 g, 91%): mp 73–74°; ir identical with that of an analytical sample. An analytical sample of **3** was prepared by crystallization from Et_2O–hexane: mp 73–74°; ir (cm^{-1}) 3260, 3060 (NH), 1637, 1550 (amide). *Anal.* ($C_7H_{11}NO$) C, H, N.

***cis*-3-Acetamido-1,2-epoxycyclopentane (4).** A solution of *m*-chloroperbenzoic acid (85%, 123 g, 0.607 mol) in $CHCl_3$ (900 ml) was added dropwise with stirring to a solution of **3** (69.1 g, 0.552 mmol) in $CHCl_3$ (100 ml) over a period of 20 min. The resulting solution was refluxed for 4 hr and then stirred at ambient temperature overnight. The resulting mixture was cooled to 0° and the *m*-chlorobenzoic acid filtered off (70.5 g, 82%) and washed with additional cold $CHCl_3$ (50 ml). The combined filtrate and wash were stirred with 20% $NaHSO_3$ (100 ml) for 30 min. The $CHCl_3$ layer was separated and extracted with 3 *N* NaOH (3 × 100 ml), then saturated NaCl (100 ml), and dried ($CaSO_4$). Evaporation left a white solid (59.1 g) which crystallized from PhH–hexane, giving **4** as white needles (54.3 g, 70%): mp 92–93.5°. One recrystallization gave an analytical sample of **4**: mp 93–94°; ir (cm^{-1}) 3250, 3060 (NH), 1625, 1550 (amide), 845 (epoxide); pmr ($CDCl_3$) δ 2.02 (s) overlapped by 0.9–2.2 (m, 7, $CH_3C{=}O$ and $2CH_2$), 3.4–3.6 (m, 2, H-1 and H-2), 4.1–4.7 (m, 1, H-3), 6.3–6.8 (br s, 1, NHC=O). *Anal.* ($C_7H_{11}NO_2$) C, H, N.

2α-Acetamido-5β-azidocyclopentan-1α-ol (5). A mixture of **4** (52.2 g, 0.370 mol), NaN_3 (96.2 g, 1.48 equiv), NH_4Cl (21.2 g, 0.396 equiv), 2-methoxyethanol (1 l.), and H_2O (150 ml) was stirred in a bath maintained at 80° for 16 hr. The resulting solution was evaporated to dryness, and the residue was dissolved in H_2O (250 ml). This solution was saturated with NaCl and then extracted with $CHCl_3$ (4 × 200 ml). The $CHCl_3$ solution was dried ($CaSO_4$) and then evaporated, leaving a glass which was dissolved in hot PhH. As the solution cooled, **5** precipitated as a fine white powder (58.4 g, 86%): mp 76–80°; tlc showed lower R_f than **4**. An analytical sample was prepared by crystallization from PhH: mp 75–79°; ir (cm^{-1}) 3350, 3300, 3080 (OH, NH), 2110 (N_3), 1612, 1550 (amide); pmr (DMSO-d_6) δ 1.87 (s) overlapped by 0.9–2.3 (m, 7, $CH_3C{=}O$ and $2CH_2$), 3.4–4.4 (m, 3, 3CH), 5.31 (d, J = 3.8 Hz, 1, OH), 7.4–7.8 (m, 1, NHC=O). *Anal.* ($C_7H_{12}N_4O_2$) C, H, N.

2α-Acetamido-5β-aminocyclopentan-1α-ol (6). A solution of **5** (47.9 g, 0.260 mol) in EtOH (absolute, 260 ml) was shaken with prereduced platinum oxide (500 mg) under H_2 (50 psi) in a Parr apparatus for 18 hr. (The gas was exchanged with fresh H_2 every 15 min for the first hour and then once an hour for 5 hr.) The resulting mixture was warmed to dissolve white solid which had precipitated, and the catalyst was filtered off and washed with additional hot EtOH (200 ml). The combined filtrate and wash were concentrated to 300 ml. On cooling, white crystals of **6** formed (38.8 g, 94%): mp 168–170° dec. Recrystallization gave an analytical sample: mp 167–170° dec; ir (cm^{-1}) 3300, 3260, 3080, 2900–2570 diffuse (NH_2, OH, NH), 1645, 1560 (amide), 1600 sh (NH_2). *Anal.* ($C_7H_{14}N_2O_2$) C, H, N.

Resolution of (±)-2α-Acetamido-5β-aminocyclopentan-1α-ol (6a and 6b). A solution of (+)-tartaric acid (40.2 g, 0.268 mol) in hot EtOH (absolute, 200 ml) was added to a solution of racemic amine **6** (42.4 g, 0.268 mol) in hot EtOH (300 ml)–H_2O (50 ml). The hot solution was filtered and warm Me_2CO was added until solid started to form. After cooling, the solution was decanted from the gummy solid. The solid was dissolved in hot H_2O (300 ml). Warm EtOH (500 ml) and then warm Me_2CO (about 700 ml, to cloud point) were added, and the solution was seeded with a crystal of analytical sample. White needles formed as the solution cooled slowly (34.2 g): mp 198.5–200° dec. One recrystallization gave the (+)-tartrate of **6b** as white needles (31.2 g), mp 199–200° dec, having the same optical purity as an analytical sample. A second crop (19.3 g) was recrystallized twice, giving an additional 4.39 g (total yield 86%) of the (+)-tartrate of **6b**. The analytical sample was originally prepared by six recrystallizations of the (+)-tartrate, at which point the optical rotation no longer changed on recrystallization: mp 198–199° dec; $[\alpha]_{589}$ −14.5°, $[\alpha]_{578}$ −15.2°, $[\alpha]_{546}$ −18.0°, $[\alpha]_{436}$ −38.4°, $[\alpha]_{365}$ −76.6° (*c* 1.7, H_2O). *Anal.* ($C_7H_{14}N_2O_2 \cdot C_4H_6O_6$) C, H, N.

A sample of the (+)-tartrate of **6b** (35.6 g, 0.116 mol) was dissolved in H_2O (100 ml) and passed through a column of IRA-400 (OH^-) resin (300 ml) packed in 95% EtOH. The column was eluted with 95% EtOH (1800 ml). Evaporation, followed by azeotropic drying with absolute EtOH, gave **6b**, after crystallization from absolute EtOH, as white needles (16.8 g, 92%): mp 155–157° dec; $[\alpha]_{589}$ −34.4°, $[\alpha]_{578}$ −36.2°, $[\alpha]_{546}$ −41.9°, $[\alpha]_{436}$ −78.8°, $[\alpha]_{365}$ −138.6° (*c* 1.0, MeOH). *Anal.* ($C_7H_{14}N_2O_2$) C, H, N.

The contents of the mother liquors from crystallization of the (+)-tartrate of **6b** were treated with IRA-400 (OH^-) resin and a sample of the free amine obtained (16.5 g, 0.104 mol) was dissolved in hot EtOH (200 ml)–H_2O (50 ml). A solution of (−)-tartaric acid (15.8 g, 0.104 mol) in EtOH (150 ml) was added. The hot solution was filtered and warm Me_2CO added to the cloud point. The white needles which formed (27.12 g) were recrystallized, giving the (−)-tartrate of **6a** (24.8 g, 88%): same optical purity as an analytical sample prepared by a third crystallization of such a sample; mp 198–199° dec; $[\alpha]_{589}$ +14.5°, $[\alpha]_{578}$ +15.3°, $[\alpha]_{546}$ +18.0°, $[\alpha]_{436}$ +38.5°, $[\alpha]_{365}$ +76.5° (*c* 1.8, H_2O). *Anal.* ($C_7H_{14}N_2O_2 \cdot C_4H_6O_6$) C, H, N.

The free (+)-amine **6a** was obtained in almost quantitative yield by IRA-400 (OH^-) treatment of the (−)-tartrate, exactly as for **6b**. One crystallization from absolute EtOH gave an analytical sample of **6a** as white needles: mp 155–157° dec; $[\alpha]_{589}$ +34.2°, $[\alpha]_{578}$ +36.0°, $[\alpha]_{546}$ +41.9°, $[\alpha]_{436}$ +78.8°, $[\alpha]_{365}$ +138.3° (*c* 1.0, MeOH). *Anal.* ($C_7H_{14}N_2O_2$) C, H, N.

(+)-2α-Acetamido-5β-(5-amino-6-chloro-4-pyrimidinylamino)cyclopentan-1α-ol (7a). Also 7 and 7b. A solution of **6a** (4.70 g, 29.7 mmol), 5-amino-4,6-dichloropyrimidine (7.31 g, 44.5 mmol), and triethylamine (12.5 ml, 89 mmol) in 1-BuOH (85 ml) was refluxed under N_2 for 18 hr. The solid which formed was filtered off, washed with $CHCl_3$ (50 ml) and then H_2O (50 ml), and dried, giving **7a** as pale yellow powder (6.62 g, 78%): mp 236–238° dec; chromatographically homogeneous. Crystallization from MeOH gave an analytical sample as cream-colored needles: mp 234–235° dec; $[\alpha]_{589}$ +10.9°, $[\alpha]_{578}$ +11.6°, $[\alpha]_{546}$ +12.5°, $[\alpha]_{436}$ +12.2° (*c* 0.7, 0.1 *N* HCl); ir (cm^{-1}) 3400–3050 (OH, NH_2), 1630, 1560 (amide), 1590 (pyrimidine). *Anal.* ($C_{11}H_{16}ClN_5O_2$) C, H, N.

The BuOH filtrate and H_2O–$CHCl_3$ washes were combined and evaporated to a gum. The gum was dissolved in $CHCl_3$ and extracted with H_2O. The aqueous layer yielded another 6% of **7a** on concentration. The unreacted 5-amino-4,6-dichloropyrimidine could be recovered from the $CHCl_3$ layer.

The enantiomer **7b** was prepared from **6b** in the same manner, giving cream-colored needles: mp 234–235° dec; $[\alpha]_{589}$ −10.8°, $[\alpha]_{578}$ −11.6°, $[\alpha]_{546}$ −12.5°, $[\alpha]_{436}$ −12.3° (*c* 0.6, 0.1 *N* HCl); ir identical with that of **7a**. *Anal.* C, H, N.

The racemate **7** was prepared, starting with **6**. Crystallization from MeOH gave a white powder: mp 233.5–234.5° dec; ir identical with those of **7a** and **7b**. *Anal.* C, H, N.

(−)-2α-Acetamido-5β-(6-dimethylamino-9-purinyl)cyclopentan-1α-ol (10a). Also 10 and 10b. A mixture of **7a** (6.44 g, 22.5 mmol), ethanesulfonic acid (2.48 g, 22.5 mmol), and triethyl orthoformate (175 ml) was stirred for 18 hr. Evaporation left a yellow gum which was triturated with $CHCl_3$ (150 ml). Unreacted **7a** (652 mg, 10%) was filtered off. The $CHCl_3$ filtrate was extracted with half-saturated $NaHCO_3$ (3 × 25 ml), dried ($CaSO_4$), and evaporated, leaving **8a** as a yellow glass (7.05 g); R_f identical with that of racemate **8**.

A sample of the enantiomer **8b** was prepared from **7b**. A sample of the racemate **8** was prepared from **7**. An analytical sample of the racemate **8** was prepared by chromatography of a portion of the crude glass on silica gel preparative tlc plates developed in 5% MeOH–$CHCl_3$. Crystallization from EtOAc gave **8** as white granules: mp 150–154°; ir (cm^{-1}) 1670 (C=O), 1605, 1560 (purine). *Anal.* ($C_{15}H_{18}ClN_5O_3$) C, H, N.

The yellow glass **8a** was refluxed with 40% aqueous dimethylamine (200 ml) for 3 hr. Evaporation left **9a** as a yellow glass. In the same manner **9b** was prepared from crude **8b** and the racemate **9** from **8**. An analytical sample of the racemate **9** was prepared by chromatography of a portion of the material on silica gel preparative tlc plates developed in 5% MeOH–$CHCl_3$. Crystallization from EtOAc gave **9** as white needles: mp 160–170°; ir (cm^{-1}) 1660 (C=O), 1595, 1560, 1525 (purine); pmr (DMSO-d_6) δ 1.27 (t, J = 7 Hz, 3, OCH_2CH_3), 2.10 (s) overlapped by 1.5–2.4 (m, 7, $CH_3C{=}O$ and $2CH_2$), 3.53 (s) overlapped by 3.4–4.1 (m, 8,

NMe_2 and OCH_2CH_3), 4.5–5.3 (m, 3, 3CH), 6.34 (s, 1, CHOEt), 8.40 and 9.38 (both s, 2, 2 purine CH). *Anal.* ($C_{17}H_{24}N_6O_3$) C, H, N.

The yellow glass **9a** was dissolved in 2 *N* HCl (50 ml) and the solution stirred for 10 min. The pH was then adjusted to 9 by addition of 6 *N* NaOH (*ca.* 23 ml). The resulting solution was saturated with NaCl and then extracted with $CHCl_3$ (3 × 100 ml). The $CHCl_3$ solution was dried ($CaSO_4$) and then evaporated, leaving **10a** as a white solid foam (4.27 g, 70% from **7a**), sufficiently pure for use. Chromatography on a preparative tlc plate developed in 10% MeOH–$CHCl_3$ gave a sample which crystallized with difficulty from EtOAc: mp 85–88°; $[\alpha]_{589}$ −18.0°, $[\alpha]_{578}$ −18.9°, $[\alpha]_{546}$ −21.8°, $[\alpha]_{436}$ −40.4°, $[\alpha]_{365}$ −78.8° (*c* 1.3, MeOH); ir similar to that of racemate **10**, identical with that of enantiomer **10b**. *Anal.* ($C_{14}H_{20}N_6O_2$) C, H, N.

A sample of the enantiomer **10b** was prepared in the same way from **9b**. An analytical sample was prepared by chromatography, giving **10b** as a white crystals: mp 85–88°; $[\alpha]_{589}$ +17.9°, $[\alpha]_{578}$ +18.9°, $[\alpha]_{546}$ +21.8°, $[\alpha]_{436}$ +40.4°, $[\alpha]_{365}$ +78.8° (*c* 1.0, MeOH). *Anal.* C, H, N.

A sample of the racemate **10** was prepared in the same way from **9**. Crystallization from EtOAc gave **10** as white granules (72% from **7**): mp 147–150°. Recrystallization from EtOAc gave white granules: mp 150–151°; mixture melting point with an authentic sample prepared by an alternate route[2] undepressed; and ir identical with that of the authentic sample.

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Ab Initio Calculations on Large Molecules Using Molecular Fragments. Lincomycin Model Studies†

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Ab initio Hartree–Fock SCF calculations have been carried out using the molecular fragment approach for a series of molecular species, chosen to model the pyrrolidine and amide portions of the antibiotic lincomycin as well as portions of the carbohydrate moiety in several analogs. The effects of various chemical modifications on the electronic structure and preferred conformations are studied and related to available experimental data. It is found that protonation of the nitrogen atom of the pyrrolidine ring modifies the electronic structure of the ring substantially, and the likely effect of protonation on antibacterial activity is discussed. In addition, modifications of the sugar side chain can cause interactions with the amide and pyrrolidine moieties, and the effect of these interactions on conformational stability and antibacterial activity is discussed.

Lincomycin (see Figure 1) is an antibiotic produced by *Streptomyces lincolnensis* which has been shown to be effective against gram-positive bacteria.[1-4] Currently available evidence indicates that lincomycin inhibits protein synthesis by acting at the 50S ribosomal subunit.[5-13] However, the precise mode of action has not been clearly established by the studies conducted thus far. A theoretical model which rationalizes many structure–activity relationships of lincomycin-related antibiotics is presented in the companion paper.[14]

Chemical modification of the lincomycin molecule has produced a series of analogs with significantly different potency than lincomycin.[15] Among the most interesting analogs are those differing only in the nature and/or configuration of the C(7) substituents. For example, lincomycin, which possesses an *R* configuration at C(7), is twice as effective in the standard plate assay with *Sarcina lutea* as the *S* stereoisomer, 7-epilincomycin. Furthermore, 7(*S*)-chloro-7-deoxylincomycin (clindamycin) and 7(*R*)-chloro-7-deoxylincomycin (7-epiclindamycin) are both more effective than lincomycin in the *S. lutea* assay; the activity of clindamycin shows a fourfold enhancement while 7-epiclindamycin exhibits a twofold improvement. As yet, no satisfactory rationale for these differences has been suggested.

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