Table VI. Mass Spectra of Deuterated 2h[a]

deuteration reagents	relative intensities at m/e			
	158	159	160	161
$NaBH_4$-D_2O	27.0	100	28.5	5.5
$NaBD_4$-H_2O	30.8	100	56.6	16.8
$NaBD_4$-D_2O	11.3	40.3	100	70.2

[a] Mass spectrum of **2h**: *m/e* 156 (7.5), 157 (15.7), 158 (100), 159 (12.2).

The scan speed was 0.2 V s^{-1}, and temperature was kept at 23 ± 0.1 °C. Details were described separately.[17]

Spectral grade acetonitrile was used without further purification. All the arenes, *p*- and *m*-dicyanobenzenes, $NaBH_4$, $NaBD_4$, and $NaBH_3CN$ were used as received (Tokyo Kasei and Nakarai Chemicals). $NaBH(OCH_3)_3$ was prepared according to the known method.[18]

Photoreduction of Arenes. General Procedure. To 10 mL of an aqueous solution of $NaBH_4$ (100 mmol) in a Pyrex vessel was added 90 mL of an acetonitrile solution containing an arene (10 mmol) and *m*- or *p*-DCNB (2.5 mmol). The solution which was not homogeneous was bubbled with N_2 for 20 min and then irradiated with an Eikosha PIH-300 high-pressure mercury lamp under cooling with water. The progress of the reaction was followed by GLC. After the arenes had been completely consumed, 50 mL of brine and then 500 mL of diethyl ether were added to the irradiated solution. After vigorous shaking, the ether layer was separated, washed three times with water, dried ($MgSO_4$), and then evaporated. The residue was distilled under reduced pressure or chromatographed on silica gel (Merk, Art 7734, 70–230 mesh) by using hexane or 10–30% benzene in hexane as the eluents; the products were thus isolated. Irradiation time, yields, and product ratios are listed in Table I, and ^1H NMR spectra are summarized in Table V.

2-Methoxy-4-(4-cyanophenyl)-1,4-dihydronaphthalene (3k): mp 76–77 °C; IR (CCl_4) 2220 cm^{-1} (C≡N); mass spectrum, *m/e* 261 (M^+); NMR (CCl_4) δ 3.45–3.53 (br s, 2 H), 3.50 (s, 3 H), 3.66 (br s, 1 H), 6.76 (br s, 1 H), 6.7–7.0 (m, 4 H), 7.0–7.4 (AB q, 4 H).

Anal. Calcd for $C_{18}H_{15}NO$: C, 82.73; H, 5.79; N, 5.36. Found: C, 82.81; H, 5.56; N, 5.48.

Isomerization of 3k to 2-Methoxy-4-(4-cyanophenyl)-3,4-dihydronaphthalene (3k′). To a carbon tetrachloride solution of **3k** (20 mg/0.3 mL) in a NMR tube was added 1 drop of a carbon tetrachloride solution saturated with dry hydrogen chloride. After a while, the NMR spectrum was completely changed to that of **3k′**: NMR (CCl_4) 2.64 (m, 2 H), 3.66 (s, 3 H), 4.16 (t, 1 H), 5.52 (s, 1 H), 6.6–7.15 (m, 4 H), 7.16–7.6 (AB q, 4 H).

Deuteration Experiments with $NaBH_4$–D_2O, $NaBD_4$–H_2O, and $NaBD_4$–D_2O. A 9:1 acetonitrile–water or deuterium oxide solution (6 mL) containing 0.4 mmol of **1h**, 0.1 mmol of *m*-DCNB, and 2 mmol of $NaBH_4$ or $NaBD_4$ was irradiated for 5 h. After the complete disappearance of **1h**, the product was isolated by vacuum distillation and purified by recrystalization from methanol. Each pure product was subjected to mass spectral analyses; relative intensities at *m/e* 158, 159, 160, and 161 are listed in Table VI.

Acknowledgment. This work was supported by a Scientific Research Grant-in-Aid (No. 447067) from the Ministry of Education of Japan.

Registry No. **1a**, 85-01-8; **1b**, 120-12-7; **1c**, 91-20-3; **1d**, 90-12-0; **1e**, 91-57-6; **1f**, 69470-12-8; **1g**, 581-40-8; **1h**, 581-42-0; **1i**, 83-32-9; **1j**, 2216-69-5; **1k**, 93-04-9; **2a**, 776-35-2; **2b**, 613-31-0; **2c**, 612-17-9; **2d**, 21564-70-5; **2d′**, 4373-24-4; **2e**, 2717-43-3; **2e′**, 2717-46-6; **2f**, 75896-19-4; **2f′**, 75896-20-7; **2g**, 21564-72-7; **2g′**, 21564-73-8; **2h**, 21564-74-9; **2h**-d_1, 75908-20-2; **2h**-d_2, 75908-21-3; **2h**-d_3, 75908-22-4; **2i**, 75896-21-8; **2j**, 75896-22-9; **2j′**, 75896-23-0; **2k**, 40815-22-3; **3k**, 74232-83-0; **3k′**, 74232-84-1; $NaBH_4$, 16940-66-2; *m*-DCNB, 626-17-5; *p*-DCNB, 623-26-7.

(17) Majima, T.; Pac, C.; Sakurai, H. *J. Am. Chem. Soc.* **1980**, *102*, 5265.
(18) Brown, H. C.; Schlesinger, H. I.; Sheft, I.; Ritter, D. M. *J. Am. Chem. Soc.* **1953**, *75*, 192.

(β-Lysyloxy)myoinositol Guanidino Glycoside Antibiotics

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A *Nocardia* sp. was found to produce three broad-spectrum antibiotics, two of which exhibit potent antitubercular activity. They are related to the known antibiotic myomycin. The culture also produces the individual β-lysyl oligopeptide side chains as well as the pseudodisaccharide common to all three antibiotics. As a result of the characterization work done, mostly by using ^{13}C NMR spectral data on degradation fragments, a modification of the published structure of myomycin is presented. An unusual sulfated sugar methanolysis fragment is identified by X-ray crystallography.

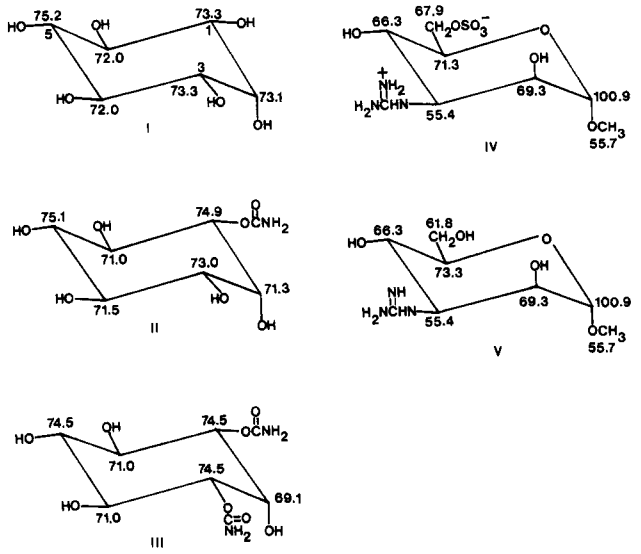
A *Nocardia* sp. (Lederle culture BM782) yielded crude fermentation extracts which showed broad-spectrum activity against Gram-negative infections in mice. Initial characterization work indicated that the antimicrobial material present was similar to the known antibiotic myomycin.[1] The wild strain of BM782 first selected produced antibiotic material at very low levels in the fermentation broth. Later a mutant[2] was obtained which elaborated three antibiotics, LL-BM782α_{1a}, -α_1, and -α_2, and four related metabolites, LL-BM782β, -γ, -δ, and -ε, in isolable quantities.[3] Compounds LL-BM782α_1 and -α_2 were shown to be very effective in protecting mice infected with *Mycobacterium tuberculosis*.[4]

In common with most basic, water-soluble antibiotics, the LL-BM782 complex can be recovered from fermentation filtrates by extraction on a weak carboxylic acid cation exchanger in the basic cycle. Considerable purification was

(1) (a) U.S. Patent 3 795 688, 1974. (b) French, J. C.; Bartz, Q. R.; Dion, H. W. *J. Antibiot.* **1973**, *26*, 272.
(2) The mutant labeled BM782Ce82 was prepared by Dr. A. Fantini of the Medical Research Division at Pearl River, NY.
(3) The natural products are named in order of their polarity with respect to elution off a weak dextran cation exchanger. LL-BM782ε is the least polar compound.
(4) A detailed account of the biological activities of these materials will be published elsewhere.

0022-3263/81/1946-0792$01.00/0

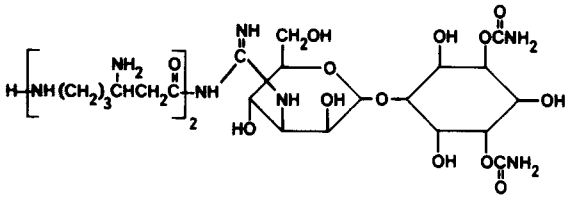
Chart I



achieved by washing the loaded resin with acetate buffer at pH 3.5. The antibiotic complex can then be eluted off with dilute sulfuric acid and after neutralization recovered by lyophilization. These antibiotics are extraordinarily unstable in very dilute alkali but are resonably stable in acid.

The complex could be resolved completely by using a suitable salt gradient across a dextran cation exchanger. The success of this resolution is very dependent on precise loading and pH control as detailed in the Experimental Section. Since the components all lack a useful chromophore, chromatography was monitored by a combination of detection reagents such as Mazur, Sakaguchi, and ninhydrin.

Largely on the basis of hydrolytic studies, French et al. proposed the structure shown below for myomycin, including the *S* configuration for the β-lysine and the D configuration for the 3-deoxy-3-guanidinomannose.



We present in this paper detailed ^{13}C NMR analyses of the spectra of each member of this family of antibiotics as well as of key degradation fragments. These studies indicate not only a different placement of the carbamoyl groups relative to the glycosidic bond but also that the β-lysyl side chain is attached by an ester linkage to the axial hydroyl group of myoinositol. Chart II gives the structures of the new antibiotics LL-BM782α_2 (IX), LL-BM782α_1 (X), and LL-BM782α_{1a} (XI) in addition to that of myomycin.

Acidic hydrolysis of X yielded myoinositol (I, Chart I), shown with appropriate ^{13}C chemical shifts in parts per million. Acidic resin methanolysis of the same antibiotic yielded crystalline II which spectral data showed to be myoinositol bearing a single carbamoyl group. The location of the carbamoyl group was readily determined from the proton NMR spectrum where two sharp doublets centered at δ 4.72 ($J_{1,6}$ = 11 Hz, $J_{1,2}$ = 2 Hz) indicated acylation was at a position adjacent to the equatorial proton δ 4.34.

Methanolysis of a mixture of LL-BM782 antibiotics in the hydrochloride salt form yielded the anticipated fragments III and V. The ^{13}C NMR spectrum of III had in addition to the carbamoyl signal at 159.0 ppm an intense signal at 74.5 ppm, a signal at 71.0 ppm with about two-thirds intensity, and a signal about one-third that size at 69.1 ppm. Only the symmetrical structure III could answer these requirements and also the constraints of the proton spectrum which indicated acylation adjacent to the axial hydroxyl at C_2.

The ^{13}C NMR assignments for the methyl glycoside V were readily made. Persuasive evidence for the α configuration at C_1 in this compound is based on the chemical shift of C_5 at 73.3 ppm which is in excellent agreement with that of 73.2 ppm for the C_5 of α-methyl mannopyranoside.[5] A C_5 resonance of 77.5 ppm is reported for the corresponding β anomer. This argument assumes that the equatorial C_3 guanidino function has no significant influence on the chemical shift of C_5 in V or, indeed, in the other compounds studied. It is well-known that the α and β anomeric ^{13}C shifts for mannose are not of diagnostic value for assigning the stereochemistry of the glycosidic linkages. Field-desorption (FD) mass spectroscopy on V indicated a strong pseudomolecular ion $(M + H)^+$ at m/e 236.[6]

Unexpectedly, methanolysis of the sulfate salts of a mixture of the LL-BM782 antibiotics yielded the sulfated sugar IV in addition to III and V. After careful chromatography, IV was obtained in crystalline form suitable for X-ray analysis. The X-ray work on IV proves the structure of the 3-deoxy-3-guanidino-D-mannose moiety in the intact antibiotics.[7] This zwitterionic compound behaves as a neutral entity during chromatography, and hence it was very difficult to separate it from III. X-ray data further indicates possible hydrogen bonding between the terminal amino and imino nitrogens of one molecule with two oxygens of the sulfate group of a single other molecule. The origin of IV probably can be explained by the sulfation of the hydroxylmethyl group of the mannose by the sulfate of the guanidino function in the presence of hydrogen chloride.

Mild base treatment of the LL-BM782 antibiotics gave pseudodisaccharide fragments, the ^{13}C NMR assignments of which provided evidence for the glycosidic attachment of the 3-deoxy-3-guanidinomannose to the dicarbamoylated myoinositol unit. Treatment of LL-BM782α_1 at room temperature for 3 min with approximately 0.25 M $Ba(OH)_2$

(5) (a) Perlin, A. S.; Casu, B.; Koch, H. J. *Can. J. Chem.* **1970**, *48*, 2596. (b) Dorman, D. E.; Roberts, J. D. *J. Am. Chem. Soc.* **1970**, *92*, 1355. (c) Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972; p 461. (d) Kasai, R.; Okihara, J.; Asakawa, K.; Mitzutani, K.; Tanaka, O. *Tetrahedron* **1979**, *35*, 1427. (e) **Note added in proof**: The ^1J ($^{13}CH_1$) for the guanidinomannose was recently found to be 161 Hz for LL-BM782α_1. This suggests a β anomeric configuration based on the studies of Bock and Pederson (Bock, K.; Pederson, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293). An interchange of the C_5 and $C_{5'}$ chemical-shift assignments would accommodate a β-glycosidic linkage. However, Kasai et al.[5d] have measured ^1J ($^{13}CH_1$) values for a number of mannose and rhamnose α and β glycosides and observed that the couplings for α anomers were 164–166 Hz and 153–156 Hz for the β-glycosides. Thus, there remains uncertainty about the stereochemistry of the glycosidic linkage in the LL-BM782 antibiotics.

(6) Field-desorption mass spectroscopy was carried out courtesy of Professor Gordon Wood, University of Windsor, Ontario, Canada.

(7) When the structure was refined in the configuration corresponding to D-mannose, the reliability index *R* was found to be 0.071 while for the opposite configuration *R* was 0.077. These values are significantly different (Hamilton, W. C. *Acta Crystallogr.* **1965**, *18*, 502) so there is a strong indication that the molecule is derived from D-mannose. The result is substantiated by the chemical data in ref 1b. With regard to the X-ray data, however, the relatively high reliability index values indicate poor crystal quality. Furthermore, systematic error may exist in the calculated structure factors because the occupancy factors of four of the six hydration molecules could not be calculated with great accuracy.

Table I. ^{13}C Chemical Shifts[a] of Carbohydrate Moieties of LL-BM782 Antibiotics

carbon	compd I	II	III	VI	VII	VIII	IX	X	XI	myomycin
C_1	73.3	74.9	74.5	74.5	73.5	73.0	72.5	72.6	72.5	72.6
C_2	73.1	71.3	69.1	69.2	71.6	72.1	71.0	71.1	71.0	71.0
C_3	73.3	73.0	74.5	74.0	72.5	73.5	72.1	72.1	72.1	72.1
C_4	72.0	71.5	71.0	80.4	82.6	82.6	80.0	80.1	80.0	80.0
C_5	75.2	75.1	74.5	77.8	77.8	78.2	77.8	77.9	77.8	77.8
C_6	72.0	71.0	71.0	70.9	71.9	71.9	71.0	71.1	71.0	71.0
C_1'				101.3	101.3	101.6	101.2	101.2	101.2	101.2
C_2'				70.0	69.7	70.5	69.9	70.0	69.9	69.9
C_3'				57.8	57.8	56.1	57.7	57.8	57.7	57.7
C_4'				66.5	66.4	66.2	66.5	66.5	66.5	66.5
C_5'				72.9	73.0	73.0	72.5	72.6	72.5	72.6
C_6'				61.7	61.7	61.7	61.8	61.8	61.8	61.8

[a] In parts per million relative to $Si(CH_3)_4$. Spectra taken in D_2O.

solution yielded impure VI (Chart II).[1b] The impurity manifested itself in the ^{13}C NMR spectrum as an extra signal at 82.5 ppm and the presence of ghost signals accompanying at least two of the inositol carbon signals. If the dilute $Ba(OH)_2$ treatment of LL-BM782α_1 were allowed to continue at room temperature for 16 h, compound VII could be isolated.

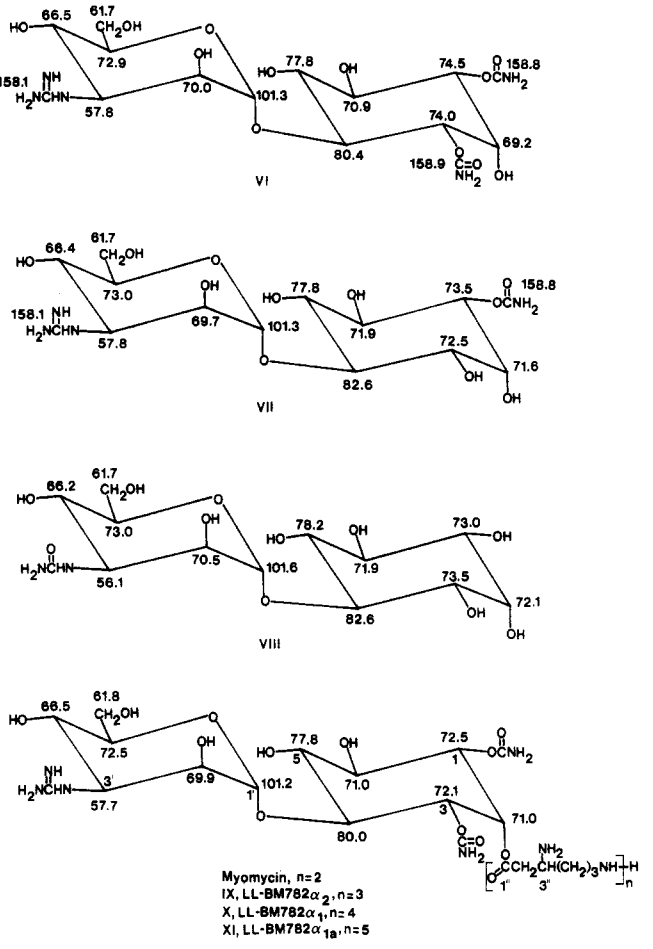
When LL-BM782α_1 was refluxed with 0.25 N $Ba(OH)_2$ for 15 h, compound VIII was recovered. Pure VI was prepared by very mild alkaline treatment of a crude antibiotic mixture (stirring at room temperature for 45 min at pH 8.5). The pure material gave a clean ^{13}C NMR spectrum which matched exactly that of the natural product LL-BM782ϵ. Pure VI, in low yield, could also be isolated by hydrolyzing LL-BM782α_1 in cold 12 N H_2SO_4.

The one other product of barium hydroxide hydrolysis of LL-BM782α_1 was a tetra-β-lysyl oligopeptide. Similar hydrolysis of LL-BM782α_2 and -α_{1a} yielded VI in each instance. The side chain from LL-782α_2 turned out to be the tri-β-lysyl peptide while from α_{1a} the penta-β-lysyl compound was recovered. These oligopeptide compounds will be discussed more fully below.

The structures VI–VIII are the key to the structures of the intact antibiotics. Most of the ^{13}C NMR assignments in these compounds are straightforward, following comparisons with those in III and V and taking into account general ^{13}C NMR assignments.[8] The guanidinomannose is joined glycosidically to the myoinositol moiety through one of the enantiotopic, paired hydroxyls (C_4 or C_6) of the inositol.[5e] This is based on the relative constancy of the C_1–C_3 and C_5 ^{13}C NMR chemical shifts of the inositol carbons in the degradation fragments III–VIII. There is one less C_4–C_6 resonance at ca. 71 ppm in the spectra of VI–VIII compared to that of III. Instead, a new signal at 80.0–82.6 ppm appears in the pseudodisaccharide fragments, consistent with the anticipated downfield shift resulting from glycosidation. Despite some effort, we were unable to obtain any of the relevant compounds in the crystalline form for X-ray work.

The single carbamate missing from VII must be from the C_3 hydroxyl since the 2-ppm β shift at C_4 due to carbamoylation is absent in the spectra of both VII and VIII.[9]

Chart II



In passing, it should be noted that replacing the guanidino function with the ureido group in VIII causes a upfield shift of about 2 ppm of the $C_{3'}$ signal of the 3-deoxy-3-ureidomannose.

An (acylamino)guanidine moiety linking the pseudodisaccharide and the oligopeptide fragments was originally suggested by French et al. although the possibility of an ester linkage was not ruled out partly because of the base lability of these antibiotics.[10] On the basis of comparison ^{13}C NMR spectral studies of the pseudodisaccharide VI and the antibiotics IX–XI, we conclude that these two

(8) Johnson, L. F.; Jankowski, W. C. "Carbon-13 NMR Spectroscopy"; Wiley-Interscience: New York, 1972.

(9) Nakayama, Y.; Kunishima, M.; Omoto, S.; Takita, T.; Umezawa, H. *J. Antibiot.* **1973**, *26*, 400. We did isolate some LL-BM782α_2 with the 3-carbamate missing by using slightly more alkaline conditions in the workup process. This material had only about one-third the activity of the intact antibiotics by in vitro and in vivo testing against a number of Gram-negative organisms.

(10) Muramatsu, N. *Bull. Chem. Soc. Jpn.* **1966**, *66*, 1273.

Table II. Chemical Shift and Integration Data for Carbons of β-Lysine Methyl Ester and Side-Chain Carbons of Myomycin Antibiotic Family (Sulfate Salts)

	integration intensity											
	$C_{4''}$		$C_{5''}$		$C_{2''}$		$C_{6''}$		$C_{3''}$		$C_{1''}$	
	23.6[a]	25.0[a]	29.8[a]	30.2[a]	36.6[a]	37.7[a]	39.7[a]	39.5[a]	48.5[a]	49.4[a]	172.0[a]	172.6[a]
β-lysine OCH_3	1	0	1	0	1	0	1	0	1	0	1	0
myomycin	1	1	1	1	1	1	1	1	1	1	1	1
IX	1	2	1	2	1	2	1	2	1	2	1	2
X	1	3	1	3	1	3	1	3	1	3	1	3
XI	1	4	1	4	1	4	1	4	1	4	1	4

[a] Chemical shift in parts per million.

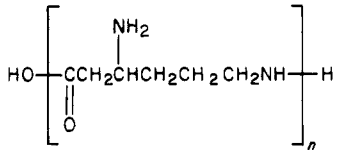
segments are attached through an ester linkage involving the myoinositol C_2 axial hydroxyl. Examination of the data in tabular form (Table I) and in the drawings clearly shows a significant upfield β shift of about 2 ppm for the myoinositol C_1 and C_3 signals and a downfield α shift of 1.9 ppm of the C_2 signal in the spectra of IX–XI and myomycin, which are explainable by acylation at C_2 in the intact antibiotics.[11] In contrast to these changes, the chemical shifts of the other pseudodisaccharide carbons are virtually invariant in the spectra of VI and all the members of this antibiotic family.

Confirmation of the ester linkage was obtained from 270-MHz proton NMR spectroscopy in D_2O at 80 °C. In the spectrum of VI under these conditions the anomeric proton signal of the sugar moiety is observed at δ 4.85 as a broad singlet while that of the equatorial proton of myoinositol is a triplet at δ 4.31 (J = 2.5 Hz). The C_1 and C_3 myoinositol proton signals are also clearly observed on the upfield side of the anomeric signal as two sharp doublets of doublets at δ 4.64 and 4.82 (J = 9.5 and 2.5 Hz). Under identical conditions the spectrum of LL-BM782α_2 (IX) now shows the myoinositol equatorial proton resonance to be shifted downfield by 1.3 ppm to δ 5.69 because of the well-known acylation effect.[12] The C_1 and C_3 myoinositol proton signals undergo a slight downfield shift to δ 4.77 and 4.94 where they straddle the anomeric proton signal which remains constant at δ 4.85. These data and the ^{13}C NMR data discussed above rule out the attachment of the oligopeptide chain to the pseudodisaccharide through the axial hydroxyl of the $C_{2'}$ of the sugar subunit.

IR studies (KBr disks) were of no aid in discerning the presence of an ester function in the antibiotics as only one relatively strong band at 1725 cm^{-1} is observed on the side of the more intense amide I band at 1650 cm^{-1} in these spectra. The former is a combination of the ester and carbamoyl carbonyls since the urethane carbonyl bands appear at 1725 cm^{-1} in the spectra of VI and VII while the ester carbonyl band of the methyl ester of β-lysine is also observed at this frequency.

The antibiotics IX–XI were isolated as hydrated sulfate salts by freeze–drying aqueous solutions in the pH range 5–6. Plasma-desorption mass spectroscopy on LL-BM782α_1 (courtesy of Professor R. MacFarlane of Texas A&M) gave the quasi-molecular ion $(N + Na)^+$ value of m/e 1006 ± 2 which agrees well with the calculated value of m/e 981 of the antibiotic itself.

We now turn to the β-lysine signals in the spectra of the four known members of the antibiotic family. As we proceed from myomycin where n = 2 to LL-BM782α_{1a} where n = 5 (XI, Chart II), the enhancement of the intensities of these signals is immediately obvious. By the use of the data in Table II, it is possible to compare the ^{13}C chemical shifts for all six β-lysine methyl ester carbons with those of the known members of this family of antibiotics. In the case of each antibiotic there is a set of signals which corresponds to the set for β-lysine methyl ester. Myomycin has a di-β-lysyl side chain, and in this spectrum a second set of less intense signals occurs. French et al. have presented proton NMR integration data which indicates head to tail or N^{ϵ} linkage as opposed to the alternate N^{β} linkage in myomycin. The new set of less intense signals is assigned to the inner β-lysyl residue. As the peptide chain lengthens, this set integrates for more and more carbons so that in the case of XI their intensities dwarf those of the first set assigned to the terminal β-lysine. In many instances there is a hint of resolution of each of the 2-, 3-, or 4-carbons in any particular integrated clump. On a more powerful machine they could all doubtless be fully resolved. This integration pattern strongly indicates that the N^{ϵ} linkage is continued throughout the lengthening peptide side chain. Further if the N^{β} linkage were present one would expect $C_{5''}$ and $C_{6''}$ to be single signals which would be well removed from the center of acylations. The peptide isolated following $Ba(OH)_2$ hydrolysis of IX was shown to be the same as LL-BM782γ or XII by TLC and ^{13}C NMR spectroscopy. XII was acetylated and then esterified using diazomethane



XII (LL-BM782γ), n = 3
XIII (LL-BM782δ), n = 4
XIV (LL-BM782β), n = 5

to give a white solid which was a single spot by TLC. The ^{13}C chemical shifts contained all the signals for the tetraacetyl derivative of the methyl ester of tri-β-lysyl peptide. FD mass spectroscopy indicated pseudomolecular ions $(M + H)^+$ at 585 ± 2 and $(M + Na)^+$ at m/e 607 ± 2 in good agreement with the calculated molecular weight of 584.

Similarly, hydrolysis of X yielded a product which by TLC and ^{13}C NMR spectroscopy was identical with LL-BM782δ or XIII. Following acetylation and esterification with diazomethane, the recovered chromatographically pure product was identified as the pentaacetyl derivative of the methyl ester of tetra-β-lysyl peptide. FD mass spectroscopy showed pseudomolecular ions $(M + H)^+$ at m/e 756 ± 2 and $(M + Na)^+$ at m/e 777 ± 2 in good agreement with a molecular weight of 754. Hydrolysis of XI yielded a fragment which by TLC and ^{13}C NMR spectroscopy was identical with natural product LL-BM782β (XIV). Because of its insolubility, we were unable to acetylate and methylate XIV in the manner of the other

(11) Christl, M.; Reich, H. J.; Roberts, J. D. *J. Am. Chem. Soc.* **1971**, *93*, 3463.
(12) Jackman, L. M.; Sternhell, S. "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry"; Pergamon Press: New York, 1969; p 176.

two smaller oligopeptides. However, careful integration studies by ^{13}C NMR spectroscopy indicated the material to be the head to tail pentapeptide of β-lysine.

Biogenesis

Antibiotics BM782α_2, -α_1, and -α_{1a} are readily cleaved to a single pseudodisaccharide and three β-lysyl oligopeptide chains. In fermentation beers, all three oligopeptides are found in reasonable quantities; the pseudodisccharide VI is also present but only in trace quantities. One might infer from this that the formation of this entity is the rate controlling step in the production of the antibiotics. We examined one 300-L fermentation of Lederle culture B01219 known to produce myomycin. We detected small quantities of LL-BM782ϵ, -α_1, α_2, $H_2N(\beta\text{-lys})_2OH$, and a substantial amount of myomycin. In addition, we obtained evidence of a material present consisting of a single β-lysyl residue attached to the pseudodisaccharide. In examination of dozens of LL-BM782 fermentations, we surprisingly never once obtained any trace of myomycin.

Experimental Section

^1H NMR curves were recorded on a Varian HA-100 spectrometer, and ^{13}C spectra were obtained on a Varian XL-100 instrument. ^{13}C chemical shifts were referenced to internal dioxane and reported in parts per million downfield from $Si(CH_3)_4$ (δ_C for dioxane 67.4).

Useful thin-layer systems for compounds mentioned in this report are as follows: (a) 7% NH_4HCO_3 (100 mL), acetone (100 mL), concentrated NH_4OH (1 mL); (b) 10 g of NaOAc and 1 mL of CH_3COOH in 100 mL of H_2O; (c) X mL of 0.4–2 M NaOAc and 100 – X mL of MeOH. For the more polar compounds such as the antibiotics and the oligopeptides, X = 70, and 2 M NaOAc solution was used. For compounds such as I–III, X = 30 and 0.4 M NaOAc solution was useful. These systems were used to develop silica gel thin layers.[13a] About 25–50 μg of sample was loaded at each spot, and detection was by ninhydrin or Mazur reagents. Silica gel thin layers loaded with 5–10 μg of pure antibiotics and developed in system b could be used for bioautography against *Klebsiella pneumoniae*.

The Mazur detection system was used in the following fashion. The developed, dried thin layer was placed in a chamber containing a few drops of *t*-BuOCl for 10 min. It was removed and placed in the hood air stream for 10–15 min and then sprayed lightly with a solution which was 1% in soluble starch and 1% in KI. The ninhydrin reagent consisted of 250 mg of ninhydrin in a solution consisting of 80 mL of acetone with 20 mL of pyridine added. After the dried, developed thin layer was sprayed with ninhydrin solution, hot air was blown on the plate to bring up the purple color.

The antibiotic could also be chromatographed on plastic-backed cellulose sheets.[13a] For bioautography the loading was in the range of 1–5 μg while for detection by Sakaguchi reagent usually 10 μg of sample was applied. The system used varied from 4% to 7% NH_4HCO_3 solution.

The Sakaguchi reagent is applied to cellulose plates as follows. To a solution of 40 mg of α-naphthol in 20 mL of EtOH was added a solution of 16 g of urea in 100 mL of H_2O. This solution is kept refrigerated and is good for about 1–3 weeks. The plate is sprayed with this reagent, allowed to air dry for 5–10 min, and then sprayed with the second Sakaguchi reagent which consists of 1.65 mL of Br_2 in 250 mL of 5% NaOH solution. All monosubstituted guanidino compounds show up instantly as red spots.

Table III

no.	component	Fr. no.	yield, mg	% of crude by wt
XII	LL-BM782γ	52-59	270	2.7
XIII	LL-BM782δ	62-75	630	6.3
XIV	LL-BM782β	80-86	200	2.0
IX	LL-BM782α_2	88-94	360	3.6
X	LL-BM782α_1	98-112	1100	11.0
IX	LL-BM782α_{1a}	113-118	130	1.3

The dextran weak cation-exchange resin was prepared as follows.[13b] The fresh resin was suspended in H_2O overnight, the pH was adjusted to 11.5, and the resin washed with distilled H_2O. It was then suspended in dilute sulfuric acid at pH 2.0 and stirred for 0.5 h. Again the resin was washed and filtered. The resin was resuspended in distilled H_2O, and dilute NaOH solution was added until the pH held constant at pH 6.4 for 30 min. The resin was then filtered and resuspended in 0.5% Na_2SO_4 solution. Unless this regeneration was fairly rigorously adhered to, components IX and XIV were poorly resolved. Since all of the compounds mentioned in this report are water soluble, they were mostly recovered by lyophilization. Karl Fisher testing of freeze–dried preparations indicated moisture contents of between 5% and 7.5%. Some of the preparations would gum up if not stored in desiccators. Finally, it was found very early in our work that the sulfate salts were more stable and much less likely to be troublesome than the hydrochloride salts. Consequently, we almost invariably worked with the sulfate salts.

For many of the compounds described in this report, microanalytical data were often unreliable. The best values obtained are cited even though they frequently fall outside acceptable limits.

Isolation of XII–XIV and IX–XI. Lederle culture BM782-Ce82 was grown in a medium consisting of 3% corn starch, 2% molasses, 1.5% soy flour, and 1% $CaCO_3$ under standard conditions of aeration and agitation. The pH was not allowed to rise beyond 7.5. Upon harvest, the mash was treated with 2% NaF and filtered. The filtrate was stirred with about 3% of a weak cation-exchange resin in the Na^+ cycle.[13c] The resin was washed to pH 3.5 with acetate buffer. After this washing, the complex was eluted by using 0.3 N H_2SO_4, and the eluate was neutralized with $Ba(OH)_2$ solution. After filtration and lypohilization the crude dry complex was obtained.

Approximately 10 g of this crude was dissolved in 50 mL of H_2O and charged to 1.5 L of the dextran weak cation-exchanger prepared as described.[13b] The column (bed depth usually 36–42 in.) was eluted with a gradient of 1–7% Na_2SO_4 solution (4 L vs. 4 L). Fraction volumes were in the 65–75-mL range. The components were detected by ninhydrin or Mazur spot spraying. TLC of the fractions could also be used. Bioautography was not a useful technique at this stage. The components were usually recovered from the salt eluates by carbon absorption.[13d] From a typical column, the combinations and yields of components shown in Table III were observed.

LL-BM782γ (XII): $[\alpha]^{25}_D$ +17 ± 1° (*c* 1.055, H_2O). Anal. Calcd for $C_{18}H_{38}N_6O_4 \cdot 1.5H_2SO_4 \cdot 4H_2O$: C, 34.77; H, 7.94; N, 13.52; S, 7.74. Found C, 34.53; H, 7.46, N, 13.43; S, 7.90. ^{13}C NMR (D_2O) 23.6 (1 C), 25.0 (2 C), 29.8 and 30.3 (3 C), 37.7 and 37.9 (2 C), 39.6 and 39.7 (4 C), 49.2, 49.4, and 49.8 (3 C), 172.5 (2 C), 178.5 ppm (1 C). Hydrolysis of XII using 4 N HCl gave upon workup the dihydrochloride of L-β-lysine as a freeze–dried gum; $[\alpha]^{25}_D$ +15.4° (c 0.9, H_2O) (lit.[14] $[\alpha]^{25}_D$ +19°). Approximately 1.8 g of XII was taken up in 100 mL of H_2O and passed over a strong anion-exchange resin in the OH^- form.[13e] The column was washed with H_2O and eluted with 100 mL of 0.4 M HOAc. The eluate was freeze–dried to 16 g of solid material, and 10 g of this was stirred

(13) (a) Silica gel thin layers of type F-254 by Merck of Germany; cellulose thin layers of type CEL 300 UV_{254} by Macherey-Nagel of Germany (U.S. distributor Brinkmann). (b) Dextran weak cation-exchange resin, CM-Sephadex C25 from Pharmacia Fine Chemicals. (c) Weak cation-exchange resin, Amberlite IRC-50 from Rohm and Haas. (d) The granular carbon used was Darco 20–40 mesh purchased from McKesson Chemical Co. The amount used in any particular experiment was determined largely by trial and error, keeping in mind that 2–3% adsorption by carbon of this kind is the normal loading range. The pH of solutions before carbon adsorption was normally in the range 5–6. The eluting solution was commonly 60% distilled H_2O and 40% acetone. The pH's of solutions which were subjected to freeze–drying were frequently in the range 4–6. (e) Strong anion-exchange resin, Dowex 1-X2 from Bio-Rad Laboratories. (f) Strong cation-exchange resin, Dowex 50W-X2 from Bio-Rad Laboratories. (g) Weak anion-exchange resin, Amberlite IR-4B from Rohm and Haas. (h) Macroreticular strong cation-exchange resin, Amberlyst 15 from Rohm and Haas.

(14) Taniyama, H.; Sawada, Y.; Kitagawa, T. *Chem. Pharm. Bull.* 1971, *19*, 2631.

in 28 mL of pyridine and 14 mL of Ac_2O for 44 h. The reaction mixture was evaporated to dryness several times by using toluene. The residue was taken up in 5 mL of H_2O and passed over 10 mL of strong cation-exchange resin.[13f] The effluent plus about 10 mL of wash was collected and freeze–dried to 470 mg of white solid. Portions of this material were dissolved in MeOH/H_2O and treated with excess CH_2N_2 in THF for 30 min. The solvent was evaporated to dryness, and the residue was redissolved in H_2O and freeze–dried to a white powder. This preparation was the fully acetylated methyl ester of XII: $[\alpha]^{25}_D$ −5 ± 2° (c 0.66, H_2O); FD mass spectrum, m/e 585 $(M + H)^+$, calcd for M^+ m/e 584. The material moved as a single spot on a silica gel thin layer with a system of 70 mL of MeOH and 30 mL of 0.4 M NaOAc and the Mazur method of detection: ^{13}C NMR (D_2O) 22.7 (1 C), 22.8 (3 C), 25.9 (3 C), 32.0 and 32.1 (3 C), 39.8 (3 C), 40.2 (1 C), 42.2 (2 C), 47.4 (1 C), 48.0 (2 C), 53.0 (1 C), 173.9 (2 C), 174.0 (2 C), 174.1 (1 C), 174.6 (1 C), 174.9 ppm (1 C).

LL-BM782δ (XIII): $[\alpha]^{25}_D$ +19 ± 1° (c 1.313, H_2O); ^{13}C NMR (D_2O) 22.6 (1 C), 25.3 (3 C), 29.8 (1 C), 30.2 (3 C), 37.7 and 37.9 (3 C), 39.1, 39.6, and 39.8 (5 C), 49.2, 49.4, and 49.7 (4 C), 172.6 (3 C), 178.2 ppm (1 C). Anal. Calcd for $C_{24}H_{50}N_8O_5 \cdot 2H_2SO_4 \cdot 5H_2O$: C, 35.29; H, 7.84; N, 13.72; S, 8.14. Found: C, 34.77; H, 7.35; N, 13.64; S, 7.97.

The fully acetylated methyl ester of XIII was prepared as described under the previous entry and was obtained as a white powder. The FD mass spectrum on this white powder gave $(M + H)^+$ at m/e 755 and $(M + Na)^+$ at m/e 777 (calcd value for $C_{35}H_{62}N_8O_{10}$ m/e 754).

LL-BM782β (XIV): $[\alpha]^{25}_D$ +21 ± 1° (c 3.636, H_2O); ^{13}C NMR (D_2O) 23.6 (1 C), 25.0 (4 C), 29.8 (1 C), 30.1 (4 C), 37.4 and 37.6 (4 C), 39.5 and 39.7 (6 C), 49.2 (1 C), 49.4 (3 C), 49.8 (1 C) and 172.4 (4 C), peak at about 178 barely visible (1 C). In our hands this material did not lend itself to full acetylation and esterification because of solubility problems.

LL-BM782α_2 (IX): $[\alpha]^{25}_D$ 0 ± 1° (c 0.68, H_2O). Anal. Calcd for $C_{33}H_{63}N_{11}O_{15} \cdot 2.5H_2SO_4 \cdot 5H_2O$ (mol wt 1188): C, 33.90; H, 6.68; N, 13.18; S, 6.85. Found: C, 32.47; H, 5.94; N, 11.98; S, 6.85.

LL-BM782α_1 (X): $[\alpha]^{25}_D$ 0 ± 1° (c 0.4, H_2O). Anal. Calcd for $C_{39}H_{75}N_{13}O_{16} \cdot 3H_2SO_4 \cdot 6H_2O$ (mol wt 1383): C, 33.50; H, 6.72; N, 13.16; S, 6.94. Found: C, 31.79; H, 6.18; N, 12.48; S, 7.40. Californium plasma-desorption mass spectroscopy on X gave $(M + H)^+$ at m/e 984 ± 2 and $(M + Na)^+$ at m/e 1006 ± 2 (calcd for M^+ m/e 981).

LL-BM782α_{1a} (XI): $[\alpha]^{25}_D$ +4 ± 2° (c 0.635, H_2O). Anal. Calcd for $C_{45}H_{87}N_{15}O_{17} \cdot 3.5H_2SO_4 \cdot 7H_2O$: C, 34.35; H, 6.49; N, 13.36, S, 7.12. Found: C, 33.97; H, 6.16; N, 11.76; S, 8.61.

Isolation of LL-BM782ε (VI). Approximately 50 g of crude freeze–dried material recovered by weak cation-exchange chromatography from fermentation filtrates of culture LL-BM782 was charged to 1.5 L of dextran weak cation exchanger equilibrated as described. The column was developed by using a gradient of 0.5–4% of Na_2SO_4 solution. Fraction volumes in the range of 70–75 mL were collected. The desired product was detected by spotting 5 μL of fractions on a cellulose thin-layer plate and spraying in the prescribed manner with Sakaguchi reagents. The presence of VI was indicated by a positive red color. Fractions 45 through 55 were combined and the solution was desalted over granular carbon to yield after lyophilization 109 mg of VI: $[\alpha]^{25}_D$ −20 ± 1° (c 1.6, H_2O). Anal. Calcd $C_{15}H_{27}N_5O_{11} \cdot 0.5H_2SO_4 \cdot 3H_2O$: C, 32.37; H, 6.11; N, 12.51; S, 2.28. Found: C, 31.56; N, 11.69, S, 3.28.

Chemical Preparation of VI from Crude Antibiotic Complex. About 50 g of crude recovered ion-exchange preparation from LL-BM782-Ce82 fermentations was dissolved in 150 mL of H_2O, and the pH was adjusted to 8.5 by using warm $Ba(OH)_2$ solution. The resulting mixture was stirred at room temperature for 45 min. At the end of this period, the pH was readjusted to 6.5 with 4 N H_2SO_4 solution and the mixture filtered. The filtrate was applied to 1.5 L of dextran weak cation exchanger equilibrated as described, and the column was developed by using a gradient of 0.5% to 4% Na_2SO_4 solution. By use of Sakaguchi reagents, VI was detected in fractions 48–62. These fractions of approximately 70-mL volume were combined and desalted over granular carbon. The yield after freeze–drying the concentrated 40% acetone in H_2O eluate was 1.79 g of VI as a white solid. The material had $[\alpha]^{25}_D$ −20°, was a single spot by TLC, and exhibited an absolutely clean ^{13}C NMR spectrum as did natural product described in the previous entry.

Acidic Hydrolysis of a Mixture of IX and X. Approximately 2 g of a mixture of IX and X was refluxed for 20 h in 150 mL of 3 N HCl. The solution was evaporated to dryness. The residue was taken up in H_2O and the pH adjusted to 4.5 with a weak anion-exchange resin.[13g] The solution was then passed over a strong cation resin.[13f] The effluent plus the column wash was neutralized with a weak anion resin (OH^-) and freeze–dried. The solid was redissolved in H_2O and treated with charcoal. The filtrate was again passed over a strong cation exchanger. The effluent was adjusted to neutrality with a resin, concentrated, and allowed to sit overnight to yield 85 mg of white crystals which were shown by melting point, IR, ^1H NMR, and rotation to be myoinositol; $[\alpha]^{25}_D$ 0° (c 0.66, H_2O).

Recovery of II. A macroreticular strong cation exchange resin was treated several times with refluxing MeOH.[13h] Then 1 g of XI and 15 mL of this resin were refluxed for 20 h. The resin was filtered off, and the filtrate was treated with charcoal, filtered, concentrated, and stored overnight to yield 26 mg of white crystals (mp 243–245 °C) which spectral data showed to be II. Anal. Calcd for $C_7H_{13}NO_7$: C, 37.67; H, 5.85; N, 6.28. Found: C, 37.42; H, 5.84; N, 5.75.

Preparation of III. A weak cation exchanger loaded with crude BM782 complex was eluted with 0.5 N HCl and the eluate neutralized with NaOH solution. The neutralized solution was desalted by using granular carbon, and the desalted solution was freeze–dried. Approximately 1.5 g of this freeze–dried material was stirred for 1 week in MeOH saturated with dry HCl gas. The solvent was evaporated, the solid was taken up in H_2O, and the pH was adjusted to 4.5 with a few drops of NaOH solution. The adjusted solution was charged to 350 mL of dextran weak cation-exchange resin and the column was eluted with H_2O. Fractions 5–16 (50–55 mL) were combined and processed batchwise with granular carbon. Elution of the carbon yielded 31 mg of white solid which spectral data showed to be III. This material gave a remarkably clean ^{13}C NMR spectrum in D_2O. Three peaks in an abundance ratio of 1:2:3 were observed as 69.1, 71.0, and 74.5 ppm, and there was a single peak at 158.0 ppm.

In the ^1H NMR spectrum, the protons of C_1 and C_3 are observed as a two-proton doublet of doublets centered at δ 4.67 ($J_{1,2}$ and $J_{2,3}$ = 2.5 Hz; $J_{1,6}$ and $J_{3,4}$ = 9.5 Hz). The equatorial proton of C_2 is observed as a triplet at δ 4.35 (J = 2.5 Hz). The remaining three protons are multiplets at δ 3.49 and 4.35.

Recovery of IV. Approximately 20 g of crude BM782 complex recovered by dilute HCl elution of a weak cation-exchange resin was dissolved in 2 L of H_2O and passed over 600 mL of granular carbon. The carbon column was washed well with H_2O and then eluted with 1 L of 40% acetone in H_2O. The eluate was concentrated and freeze–dried to 8 g which was refluxed for 5 h in 400 mL of MeOH saturated with dry HCl. Nearly all the material went into solution, the MeOH was evaporated to dryness, and the residue was taken up in H_2O, neutralized with NaOH, and charged into 600 mL of dextran weak cation exchanger.

Elution was carried out with distilled H_2O, and fractions 12–26 were combined and desalted by using granular carbon. The 40% acetone in H_2O eluate was concentrated to small volume, and crystals of IV were isolated: mp 280 °C; $[\alpha]^{25}_D$ +55 ± 3° (c 0.31, H_2O). X-ray crystallography data was obtained on one of these crystals. Anal. Calcd for $C_8H_{17}N_3O_8S \cdot 2H_2O$: C, 27.35; H, 6.03; N, 11.96; S, 9.13. Found: C, 28.02; H, 4.91; N, 11.46; S, 8.40.

Methanolysis of Crude LL-BM782 Complex to Obtain V and Other Compounds. Approximately 5 g of crude LL-BM782 from a BM782-Ce82 fermentation was refluxed for 4 h in 400 mL of MeOH which has been saturated with dry HCl gas. The solvent was evaporated, and the residue was dissolved in 40 mL of 1% Na_2SO_4 solution and charged into 500 mL of dextran resin. The column was developed by using a gradient of 1–5% Na_2SO_4 solution. Components were detected by using Mazur's reagent. Fractions of 60–70-mL volumes were collected. The combined fractions for the various components were desalted over an appropriate amount of granular carbon. The yields are given in Table IV.

Preparation A. FD mass spectroscopy showed a large $(M + H)^+$ at 257 and an $(M + Na)^+$ peak at m/e 289. The ^{13}C NMR spectrum showed strong peaks at 74.5, 71.0, and 69.1, but it also

Table IV

fraction no.	label	wt, mg
5-7	A	227
11-14	B	240
20-26	C	96
28-34	D	270
35-43	E	490
45-55	F	680
61-66	G	170

exhibited the peaks shown in the test for IV. TLC indicated a single spot; $[\alpha]^{25}_D$ +29 ± 2° (c 0.67, H_2O). Later work showed this to be a mixture of III and IV.

Preparation B (V): FD mass spectrum for fraction B, ((M + H)$^+$); $[\alpha]^{25}_D$ +41 ± 2° (c 0.620, H_2O). Anal. Calcd for $C_8H_{17}N_3O_5 \cdot 0.5H_2SO_4 \cdot 2H_2O$ (mol wt 320): C, 30.47; H, 6.92; N, 13.12; S, 5.00. Found: C, 30.50; H, 6.33; N, 13.12; S, 5.85.

Preparation C: $[\alpha]^{25}_D$ +11 ± 2° (c 0.317, H_2O); FD mass spectrum, m/e 161 ((M + H)$^+$); ^{13}C NMR (D_2O) 23.6 (1 C), 29.7 (1 C), 36.6 (1 C), 39.7 (1 C), 48.5 (1 C), 53.5 (1 C), 172.5 ppm (1 C). The data clearly showed that this material is the methyl ester of β-lysine as the sulfate salt.

Preparation D: $[\alpha]^{25}_D$ +14 ± 2° (c 0.552, H_2O); ^{13}C NMR (D_2O) 23.6 (1 C), 25.0 (1 C), 29.8 and 30.1 (2 C), 36.8 and 37.8 (2 C), 39.5 and 39.7 (2 C), 48.7 and 49.3 (2 C), 53.5 (1 C), 172.5 (1 C), 173.6 ppm (1 C). The evidence indicates that this compound is the methyl ester of the β-lysyl dipeptide. Anal. Calcd for $C_{13}H_{28}N_4O_3 \cdot 1.5H_2SO_4 \cdot 2H_2O$ (mol wt 471): C, 33.12; H, 7.43; N, 11.89; S, 10.19. Found: C, 33.66; H, 6.45; N, 11.90; S, 9.61.

Preparation E: $[\alpha]^{25}_D$ +15 ± 2° (c 0.637, H_2O); ^{13}C NMR (D_2O) 23.6 (1 C), 25.0 (2 C), 29.8 (1 C), 30.2 (2 C), 36.8 (1 C), 37.6 (1 C), 37.8 (1 C), 39.5 and 39.7 (3 C), 48.7, 49.3, and 49.4 (3 C), 53.5 (1 C), 172.5 (1 C), 172.6 (2 C). Anal. Calcd for $C_{19}H_{40}N_6O_4 \cdot 2H_2SO_4$ (mol wt 648): C, 35.18; H, 7.40; N, 12.69; S, 9.88. Found: C, 35.09; H, 6.82; N, 12.90; S, 9.61. The above data indicate that this material is the methyl ester of XII.

Preparation F: $[\alpha]^{25}_D$ +22 ± 2° (c 0.496, H_2O); ^{13}C NMR (D_2O) 23.6 (1 C), 25.0 (3 C), 29.8 (1 C), 30.2 (3 C), 36.8 (1 C), 37.6 and 37.8 (3 C), 39.5 and 39.7 (4 C), 48.7, 49.2, and 49.4 (4 C), 53.5 (1 C), 171.9 (1 C), 172.6 (3 C). Anal. Calcd for $C_{25}H_{52}N_8O_5 \cdot 2.5H_2SO_4 \cdot 3H_2O$ (mol wt 843): C, 35.58, H, 7.47; N, 13.29; S, 9.69. Found: C, 36.01; H, 6.94; N, 13.40; S, 9.04. This compound is the methyl ester of XIII.

Preparation G: $[\alpha]^{25}_D$ +13 ± 2° (c 0.580, H_2O); ^{13}C NMR (D_2O) 23.6 (1 C), 25.0 (4 C), 29.5 and 30.2 (5 C), 36.5 (1 C), 37.6 and 37.8 (4 C), 39.5 and 39.7 (5 C), 48.5 and 49.5 (5 C) 53.5 (1 C), 172.5 and 172.6 (4 C), 173.6 (1 C). This spectrum was not as clean as observed on the other components. Anal. Calcd for $C_{31}H_{64}N_{10}O_6 \cdot 3H_2SO_4 \cdot 4H_2O$ (mol wt 1038): C, 35.84; H, 7.51; N, 13.49; S, 9.25. Found: C, 35.66; H, 6.51; N, 13.40; S, 8.66. Material G is an impure sample of the methyl ester of XIV.

Recovery of Impure VI. Approximately 0.5 g of X was dissolved in 50 mL of 0.25 N $Ba(OH)_2$ and the mixture stirred for 12 min. The pH was then adjusted to 4.2 with 1 N H_2SO_4 and the suspension filtered. The filtrate was chromatographed over 150 mL of dextran exchanger by using a gradient of 0.5–5% NaCl solution. Fractions of 50–55 mL were collected, and these were checked by spotting on cellulose plates and spraying with Sakaguchi reagent. Fractions 29–33 were combined and desalted with granular carbon to yield following lyophilization 155 mg of impure VI, $[\alpha]^{25}_D$ −18 ± 2° (c 0.414, H_2O). The ^{13}C NMR spectrum in D_2O showed all the peaks of VI and a peak at 82.0 ppm as well as ghost peaks for some of the inositol carbons.

Reaction of X with Cold H_2SO_4 Solution. Approximately 3 g of LL-BM782α_1 was dissolved in 75 mL of cold 12 N H_2SO_4 and left in the cold room for 72 h. The workup, including dextran exchanger chromatography, yielded 70 mg of VI.

Recovery of VII. About 2 g of X was dissolved in 75 mL of 0.25 N $Ba(OH)_2$ and the mixture left to stand at room temperature for 16 h. The pH was then adjusted to 5.0 with 4 N H_2SO_4 solution, and the suspension was filtered. The filtrate was freeze–dried to 1.7 g of white solid which was chromatographed over 300 mL of dextran weak cation resin by using a 0.5–5% Na_2SO_4 gradient. Fraction volumes of 40 mL were collected, and, on the basis of observations made from the use of Mazur reagent, fractions 41–49 were combined. Desalting was carried out with granular carbon. After lyophilization, 130 mg of white powder was obtained. Spectral data showed that this product is VII, $[\alpha]^{25}_D$ −16 ± 2° (c 0.486, H_2O). Anal. Calcd for $C_{14}H_{28}N_4O_{11} \cdot 0.5H_2SO_4 \cdot 2H_2O$: C, 32.94; H, 5.88; N, 10.98; S, 3.13. Found: C, 32.49; H, 5.64; N, 10.09; S, 3.40.

Table V

fraction no.	label	amt recovd, mg
25-31	A	92
45-51	B	85
62-69	C	160
74-88	D	1030
99-108	E	100
118-130	F	145

Table VI. Crystal Data on IV

space group	trigonal, P_3 21
a	11.281 (2) Å
b	11.281 (2) Å
c	21.230 (5) Å
γ	120.0°
cell vol	2339 Å^3
Z	6
ρ(obsd)[a]	1.526 g cm^3
method of collection	$\theta/2\theta$ scan
rflctns measd	1412 ($3° < \theta < 60°$)
unobsd	175 [$I < 2\sigma(I)$]
crystal size	370 × 180 × 90 μm
instrument	Enraf-Nonius CAD-3
absorption	not applied
radiation	Ni-filtered Cu Kα

[a] By flotation in a CCl_4–hexane mixture.

Preparation of VIII. About 2 g of X was refluxed for 16 h in 300 mL of 0.25 N $Ba(OH)_2$. The pH was then adjusted to 5.0 by using 4 N H_2SO_4 and the suspension filtered. The filtrate was freeze–dried to 1.6 g of white solid which was chromatographed over 300 mL of dextran exchanger by using a gradient of 0.5–5% Na_2SO_4 solution. Fraction volumes were about 40 mL. On the basis of observations made by using Mazur's reagent, fractions 6–10 were combined and desalted with granular carbon to yield by freeze–drying 54 mg of white, Sakaguchi-negative solid. Spectral data showed that this product is VIII. Anal. Calcd for $C_{13}H_{24}N_2O_{11} \cdot 2.5H_2O$: C, 35.36; H, 6.76; N, 6.53. Found: C, 36.63; H, 5.95; N, 7.27.

Profile of Metabolites Produced by Culture B01219. A 300-L fermentation of culture B01219 was processed in the regular way used for BM782 culture complex to yield by weak cation-resin extraction 17 g of gray solid. Approximately 8 g of this solid was subjected to dextran resin chromatography with a gradient of 1–7% Na_2SO_4 solution; 70–75 mL fractions were collected (see Table V). Combinations were made as indicated and desalting was carried out with granular carbon. TLC and ^{13}C NMR data indicated that A was VI, B was the dipeptide of β-lysine, C was a mixture of β-lysine dipeptide and a compound which consisted of the pseudodisaccharide VI with one β-lysyl group attached, D was myomycin, E was impure IX, and F was X.

X-ray Crystallography. Crystals of IV grown from aqueous solution were suitable for X-ray work which yielded the data shown in Table VI. Since initially the structure was not known, the Multan calculations were made by assuming that the asymmetric unit contained about 25 nonhydrogen atoms and a chloride ion (based on the method of preparation and spectral data).[15] A trial structure was obtained, but it was clear that this was not consistent with the original assumptions. After further electron-density refinement, the correct structure was established as the unexpected compound IV, $C_8H_{17}N_3O_5S$ (Figure 1).

A complex hydration scheme exists in the crystal which involves six water molecules of which four occupy special positions in the unit cell. Two of the special-position water molecules and the

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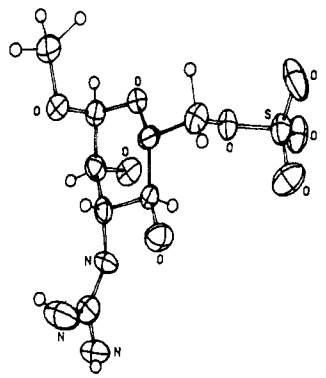

Figure 1. ORTEP diagram of IV.

two in general positions were found to have fractional occupancy factors (~30%). When the hydration was taken into account, reasonable agreement was found between observed and calculated density values.

Final anisotropic refinement of the nonhydrogen atoms to give $R = 0.071$ was made with 12 hydrogens (refined isotropically), and anomalous dispersion effects of sulfur were included.[16] All calculations were made by using the XRAY 76 progam system.[17]

An ORTEP diagram of the structure is shown in Figure 1. The hydrogen atoms depicted in this drawing correspond to those actually located in electron density difference maps; hydrogens of the two hydroxyl groups were not observed.

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Registry No. I, 87-89-8; II, 75919-38-9; III, 75919-39-0; IV, 75919-40-3; V·5H_2SO_4, 75919-42-5; VI·5H_2SO_4, 75947-46-5; VII·5H_2SO_4, 75919-44-7; VIII·5H_2SO_4, 75919-44-7; VIII, 75919-45-8; IX·2.5H_2SO_4, 75919-47-0; X·3H_2SO_4, 75933-28-7; XI·3.5H_2SO_4, 75933-30-1; XII·1.5H_2SO_4, 75919-49-2; XII methyl ester 2H_2SO_4, 75919-51-6; XII acetylated methyl ester, 75919-52-7; XIII·2H_2SO_4, 75919-54-9; XIII methyl ester 2.5H_2SO_4, 75919-56-1; XIII acetylated methyl ester, 75919-57-2; XIV, 75919-58-3; XIV methyl ester 3H_2SO_4, 75919-60-7; myomycin, 75919-61-8; L-β-lysine 2HCl, 35761-15-0; β-lysine methyl ester sulfate salt, 75919-63-0; β-lysine dipeptide methyl ester 1.5H_2SO_4, 75919-64-1.

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Synthesis of Substituted 2,6-Dioxabicyclo[3.1.1]heptanes. 1,3-Anhydro-2,4,6-tri-*O*-benzyl- and 1,3-Anhydro-2,4,6-tri-*O*-(*p*-bromobenzyl)-β-D-mannopyranose

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The title compounds 1,3-anhydro-2,4,6-tri-*O*-benzyl- and 1,3-anhydro-2,4,6-tri-*O*-(*p*-bromobenzyl)-β-D-mannopyranose were synthesized by a reaction sequence involving blocking the C-3 hydroxyl with an allyl group by first forming a dibutylstannylene complex between the C-2 and C-3 hydroxyls of methyl 6-*O*-trityl-α-D-mannopyranoside. The product was then detritylated, fully acetylated, carefully purified, and then benzylated. Acid hydrolysis removed the C-1 methoxy group, while the C-3 allyl was removed by conventional methods. Reaction with hydrogen chloride in ether led to the mannopyranosyl chlorides, which in the presence of strongs bases like NaH and *t*-BuOK yielded the desired anhydro sugars. These compounds are the required precursors for the synthesis of 1,3-mannopyranans by ring-opening polymerizations.

The preparation and polymerization of bicyclic acetals to produce stereoregular polysaccharides or related polyacetals is a goal of studies in a number of laboratories,[1-6] since the polymers obtained have proven to be useful model systems for immunological and other biochemical investigations.[7-9] Examples of stereoregular polymerization have been reported for a 1,2-anhydro-,[10] a 1,4-anhydro-,[11] and a number of 1,6-anhydroglycopyranose

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