for 59 days. At intervals during the reaction, samples were withdrawn and analyzed by tlc, glc, and mass spectrometry. The following times and percentages (determined by glc) of starting material, monoethoxy, diethoxy, triethoxy, and tetraethoxy derivatives, were obtained: 1 hr, 2, 95, 3, 0, 0; 4 hr, 0, 50, 50, 0, 0; 6 hr, 0, 25, 75, 0, 0; 43 hr, 0, 0, 65, 30, 5; 117 hr, 0, 0, 23, 60, 17; 208 hr, 0, 0, 4, 73, 23; 384 hr, 0, 0, 0, 76, 24. After 59 days (1416 hr) the reaction mixture was cooled, absorbed on silica gel, and chromatographed. CCl_4 eluted two bands from which were obtained 0.192 g (28%) of triethoxyheptachloroferrocene as an oil and 0.480 g (72%) of tetraethoxyhexachloroferrocene as an oily solid. Glc of the triethoxy derivative showed two isomers of which the relative percentages in order of increasing retention time were 83 and 17%. Glc of the tetraethoxy derivative showed three isomers of which the relative percentages in order of increasing retention time were 59, 36, and 5%.

A second reaction was carried out under the same conditions as described above, using 0.10 mol of sodium ethoxide. The reaction mixture was worked up after 6 hr to give 0.81 g (67%) of 1,1′-diethoxyoctachloroferrocene (Im) and 0.27 g (22%) of triethoxyheptachloroferrocene. The Im was recrystallized from a small volume of hexane to give mp 107–108°. *Anal.* Calcd for $C_{14}H_{10}O_2Cl_8Fe$: C, 30.69; H, 1.83; Cl, 51.59; Fe, 10.16. Found: C, 30.60, 30.46; H, 2.21, 2.29; Cl, 51.30; Fe, 10.13.

Attempted Preparations of Decafluoroferrocene. Decachloroferrocene was heated with excess (~50:1) cesium fluoride in sulfolane at 100° for 24 hr to give no reaction. When the temperature was raised to 145°, gradual decomposition took place. At 190°, decomposition was very rapid.

1,1′-Diiodooctachloroferrocene was heated with excess cesium fluoride in sulfolane at 155–160° for 20 min. Extensive decomposition occurred, and only a small amount of material was obtained from column chromatography. Mass spectrometric analysis indicated the eluate to consist of octachloroferrocene, iodooctachloroferrocene, and butyliodooctachloroferrocene, the last named possibly being formed by breakdown of the sulfolane.

Attempted Preparation of Decafluororuthenocene. Decachlororuthenocene and excess cesium fluoride were heated in sulfolane at 240° for 1 hr to give a 50% recovery of the starting material after column chromatography. Gradual decomposition also occurred upon heating 1,1′-diiodooctachlororuthenocene and excess cesium fluoride in sulfolane at 190°.

1,2-Dichloroferrocene (IIb). A solution of chloroferrocene (4.42 g, 0.0200 mol) in dry THF (125 ml) was stirred at 0° while a solution of *n*-butyllithium in hexane (30 ml, 0.045 mol) was added. The reaction mixture was stirred at 0° for 1.5 hr and then cooled at −78°. A solution of hexachloroethane (14.2 g, 0.060 mol) in hexane (50 ml) was added, and the reaction mixture was then allowed to warm to 0° over a 30-min period. Subsequent work-up consisted of dry-column chromatography on alumina with hexane as eluent. The excess hexachloroethane eluted first, followed by a yellow band from which, after solvent removal, there was obtained 3.48 g of material consisting (by glc analysis) of 1,2-dichloroferrocene (81%) and a material (19%) determined (by mass spectral analysis) to be a trichloroferrocene. Recrystallization from methanol afforded 1,2-dichloroferrocene of mp 88–90° (lit.[1] mp 88.5–90°). *Anal.* Calcd for $C_{10}H_6Cl_2Fe$: C, 47.11; H, 3.16; mol wt 253.9351. Found: C, 47.39, 47.22, H, 3.14, 3.05; mol wt 253.9355.

1,2,3-Trichloroferrocene (IIc). The experimental procedure used was the same as described above for 1,2-dichloroferrocene. From 4.50 g of 1,2-dichloroferrocene, there was obtained 5.12 g of material which consisted (by glc analysis) of 1,2-dichloroferrocene (3%), 1,2,3-trichloroferrocene (86%), and tetrachloroferrocene (11%). Recrystallization from methanol afforded 1,2,3-trichloroferrocene of mp 103.5–104.5°. *Anal.* Calcd for $C_{10}H_7Cl_3Fe$: C, 41.51; H, 2.44; mol wt 287.8961. Found: C, 41.57, 41.46, H, 2.47, 2.42; mol wt 287.8982.

1,2,3,4-Tetrachloroferrocene (IId). The experimental procedure used was the same as described above for 1,2-dichloroferrocene. From 0.70 g of 1,2,3-trichloroferrocene, there was obtained 0.62 g of material which consisted (by glc analysis) of 1,2,3-trichloroferrocene (10%), 1,2,3,4-tetrachloroferrocene (80%) and pentachloroferrocene (10%). Preparative glc afforded 1,2,3,4-tetrachloroferrocene of mp 81–82°. *Anal.* Calcd for $C_{10}H_6Cl_4Fe$: C, 37.09; H, 1.87; mol wt ($^{35}Cl_3{}^{37}Cl$ isotopes), 323.8543. Found: C, 37.38, 37.12; H, 1.86, 1.98; mol wt, 323.8559.

1,2,3,4,5-Pentachloroferrocene (IIe). A mixture of 1,2-dichloroferrocene (25%) and 1,2,3-trichloroferrocene (75%) was subjected to the reaction procedure described above for 1,2-dichloroferrocene to give a material consisting of 1,2,3-trichloroferrocene (42%), 1,2,3,4-tetrachloroferrocene (29%), and pentachloroferrocene (29%). Repetition of the reaction with this mixture afforded a material consisting of 1,2,3,4-tetrachloroferrocene (42%) and 1,2,3,4,5-pentachloroferrocene (58%) together with a small amount of hexachloroferrocene. This mixture was reacted in dry ether with excess *n*-butyllithium–hexane solution, followed by addition of hexachloroethane at 0°. Dry-column chromatography afforded a mixture of tetrachloroferrocene (20%), 1,2,3,4,5-pentachloroferrocene (70%), and hexachloroferrocene (10%). Recrystallization from methanol afforded 1,2,3,4,5-pentachloroferrocene of mp 143–144°. *Anal.* Calcd for $C_{10}H_5Cl_5Fe$: C, 33.52; H, 1.41; mol wt ($^{35}Cl_4{}^{37}Cl$ isotopes) 357.8154. Found: C, 33.25, 33.53; H, 1.41, 1.45; mol wt 357.8144.

Total Synthesis of a Monocyclic Peptide Lactone Antibiotic, Etamycin

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Abstract: The antibiotic etamycin **1** has been synthesized by joining two peptide fragments, 3-benzyloxypicolinyl-L-threonyl-D-leucine *tert*-butyl ester (**2**) and *tert*-butoxycarbonyl-(*O*-*tert*-butyl)-D-allohydroxyprolylsarcosyl-L-*N*,β-dimethylleucyl-L-alanyl-L-phenylsarcosine (**3**), through an ester linkage, **6**. Deblocking, cyclization, and hydrogenation gave etamycin. A new synthesis of threo-L-*N*,β-dimethylleucine is also reported.

The antibiotic etamycin **1** has been shown to be a macrocyclic peptide lactone. Of the natural peptide lactones, actinomycin C[1] and actinomycin D[1-3] have been synthesized, both of which have double lactone structures. Two analogs of monocyclic peptide lactones, 6-proline-staphylomycin S[4] and 6-(4-hydroxy-

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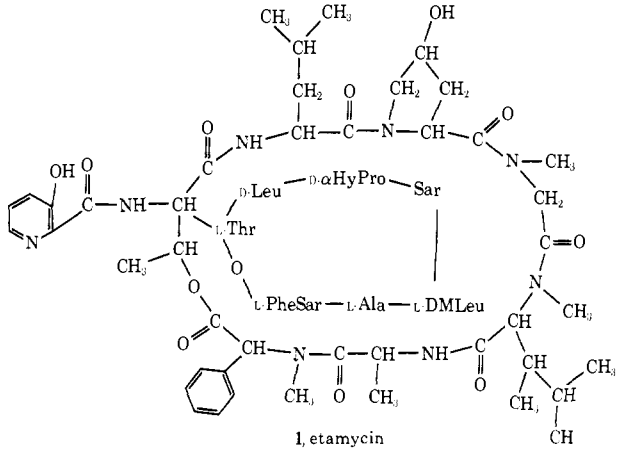
(3) J. Meienhofer, *Experientia*, **24**, 776 (1968).

(4) M. A. Ondetti and P. L. Thomas, *J. Amer. Chem. Soc.*, **87**, 4373 (1965).

proline)ostreogrycin B,[5] have been prepared. A number of lactone antibiotics[6] and antibiotic analogs[7] containing depsipeptide segments have also been synthesized.

This paper reports the synthesis of a natural monocyclic peptide lactone antibiotic. The structure of etamycin **1** was determined by degradation studies[8] and



1, etamycin

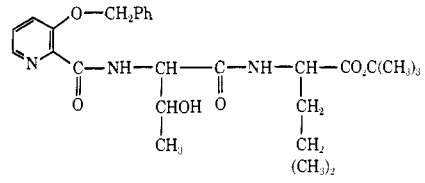
has since been confirmed by physical methods.[9,10] The structure has many similarities to that of the previously elucidated actinomycins[11] and to the antibiotics staphylomycin S[12] and ostreogrycin B.[13]

Synthesis of Etamycin. In order to avoid protecting group incompatability etamycin was prepared by joining two fragments. A threonine containing peptide and a phenylsarcosine containing peptide were linked through an ester to give a complete linear peptide **4**. Cyclization then gave etamycin. This method was chosen since previous syntheses of peptide lactones showed that cyclization *via* peptide bond formation is preferable to ring closure by lactone formation.

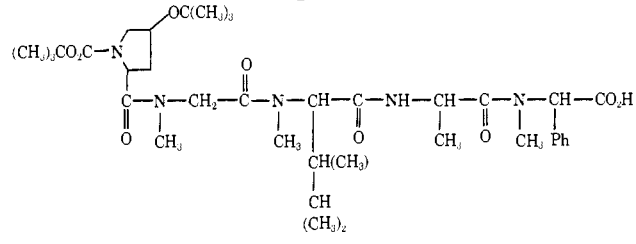
In our first synthesis we attempted to cyclize between D-allohydroxyproline and sarcosine. This route is preferable to cyclization between D-leucine and D-allohydroxyproline because racemization of the C-terminal amino acid in the presence of carbodiimide is minimized. However, cyclization attempts gave only the internal lactone of D-allohydroxyproline. Amination of this lactone has been observed[14] but did not occur in the presence of carbodiimide or 1,2,4-triazole.[15]

The most promising methods of activation for the preparation of *O*-threonine esters are mixed anhydride, imidazolide, and carbodiimide. In work with model peptides carbonyldiimidazole seemed to be the only activating reagent to effect acylation.

The threonine-containing segment **2** is 3-benzyloxy-



2



3

picolinyl-L-threonyl-D-leucine *tert*-butyl ester. Coupling of carbobenzoxy-L-threonine to D-leucine *tert*-butyl ester gave a 78% yield of carbobenzoxy-L-threonyl-D-leucine *tert*-butyl ester (**5**). Cleavage of the carbobenzoxy group by hydrogenation gave a dipeptide ester which was condensed with 3-benzyloxypicolinic acid to give the desired protected peptide in 59% yield.

The phenylsarcosine-containing segment **3** is *tert*-butoxycarbonyl-(*O*-*tert*-butyl)-D-allohydroxyprolylsarcosyl-L-*N*,β-dimethylleucyl-L-alanyl-L-phenylsarcosine. A linear stepwise synthesis failed at the second coupling. Therefore, a less desirable route was used; carbobenzoxy-L-*N*,β-dimethylleucine was coupled to L-alanine methyl ester to give the dipeptide **6** in 45% yield. Hydrogenation and coupling to carbobenzoxysarcosine gave 50% of the tripeptide **7**. Hydrogenation and coupling to *tert*-butoxycarbonyl-(*O*-*tert*-butyl)-D-allohydroxyproline gave 51% of tetrapeptide **8**. Saponification and condensation with L-phenylsarcosine *p*-nitrobenzyl ester gave 42% of pentapeptide. The *p*-nitrobenzyl group was removed by hydrogenation to give 92% of the desired pentapeptide acid **3**.

Coupling of the dipeptide with L-phenylsarcosine *p*-nitrobenzyl ester using carbodiimide introduced the danger of racemization of L-alanine. However, other coupling reagents examined with model peptides were not promising.

The formation of the ester bond between phenylsarcosine and L-threonine was mediated by carbonyldiimidazole. The coupled material was separated from starting material by gel filtration on Sephadex LH-20. The coupled material was found to be three closely related compounds which were separated by column chromatography. Fraction one of the coupled products was lacking the nmr peak corresponding to the *O*-*tert*-butyl group of hydroxyproline; fraction two had an altered pattern in the *N*-methyl region in the nmr (δ 2.7–3.1). A molecular weight determination by uv absorption of fraction three gave the theoretical molecular weight, whereas the compound of fraction two

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(9) R. B. Arnold, A. W. Johnson, and A. B. Mauger, *J. Chem. Soc.*, 4466 (1958).

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(11) H. Brockmann, *et al.*, *Angew. Chem.*, **68**, 70 (1956).

(12) H. Vanderhaeghe and G. Parmentier, *J. Amer. Chem. Soc.*, **82**, 4414 (1960).

(13) F. W. Eastwood, R. K. Snell, and A. Todd, *J. Chem. Soc.*, 2286 (1960).

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was somewhat higher than theoretical. Therefore, fraction three was taken as the desired coupled product.

Most of the peptide intermediates in this synthesis are oils or glassy solids. This is commonly encountered with *N*-methyl peptides,[16] However, these peptides are very soluble in organic solvents, making nmr spectroscopy simple and useful even for large peptides.

The blocking groups of the desired peptide were cleaved with trifluoroacetic acid and the free peptide was cyclized in very dilute solution with an excess of carbodiimide. Hydrogenation gave crude etamycin which was purified by chromatography. The physical constants of synthetic and natural etamycin are compared in Table I and can be seen to be essentially identical. Acid hydrolyses of synthetic and natural etamycins gave identical amino acid patterns.

Table I. Physical Properties of Synthetic and Natural Etamycin

	Natural	Synthetic
Mp, °C	168–170	165–169, mmp 165–169
$[\alpha]_D$, deg	62 ($[\alpha]^{25}$) (*c* 5, $CHCl_3$)	59.7 ($[\alpha]^{22}$) (*c* 1, $CHCl_3$)
λ_{max}, mμ (log ε)	304.5 (3.91)	304 (3.89)
Tlc	R_f 0.62, water–acetone, 4:1	R_f 0.62, water–acetone, 4:1
	R_f 0.83, methanol	R_f 0.83, methanol
	R_f 0.92, BuOH–AcOH–H_2O, 4:1:5	R_f 0.92
Ir, cm^{-1}	3300, 1745, 1650, 1510, 1095, 1060, 745	
Nmr ($CDCl_3$), δ	1.1 (m), 1.8, 2.2, 3.0 (m), 3.5–7.0 (complex pattern), 7.5 (s) with a shoulder at 7.4	

Amino Acid Syntheses. A. *threo*-L-*N*,β-Dimethylleucine has been synthesized previously by Sheehan and Howell[17a] and Kotake, *et al.*[17b] An intermediate of the former method is 2,3-dimethylbutyraldehyde which can only be obtained in poor yield. Therefore, a new route was investigated as shown in Scheme I. Unfortunately only 25% of the isolated product is the threo isomer. Even with this poor ratio, however, this synthesis seems to be preferable to that of Kotake, *et al.*,[17b] where a better ratio was found. Resolution of the carbobenzoxy derivative and L-ephedrine salt has been described.[17b]

B. Direct resolution of D,L-phenylsarcosine with L-10-camphorsulfonic acid[18] was not satisfactory. Therefore, the methylation of L-phenylglycine was investigated. Resolution of D,L-phenylglycine with D-10-camphorsulfonic acid gave L-phenylglycine of about 80% optical purity. Methylation by the method of Quitt, *et al.*,[19] gave L-phenylsarcosine (80% optical purity).

For our purposes a L-phenylsarcosine ester was required. However, phenylsarcosine is known to be racemized under the usual esterification procedures.[18]

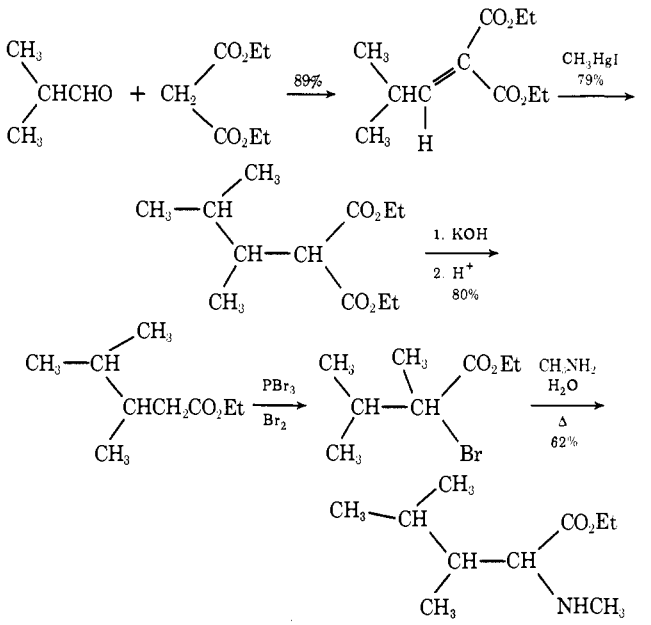
(16) E. Schroeder and K. Lubke, "The Peptides," Vol. 1, Academic Press, New York, N. Y., 1965, p 144.

(17) (a) J. C. Sheehan and M. G. Howell, *J. Org. Chem.*, **28**, 2279 (1963); (b) H. Kotake, T. Saito, and K. Okubo, *Bull. Chem. Soc. Jap.*, **42**, 1367 (1969).

(18) R. L. Cargill, Ph.D. Thesis, Massachusetts Institute of Technology, 1960.

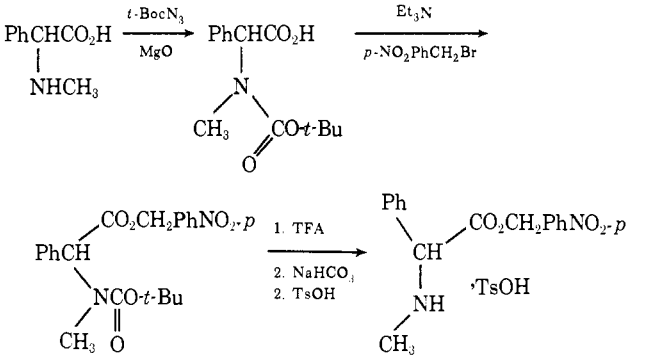
(19) P. Quitt, J. Hellerbach, and K. Vogler, *Helv. Chim. Acta*, **46**, 327 (1963).

Scheme I



Preparation of L-phenylsarcosine *p*-nitrobenzyl ester was carried out without racemization by the following route (Scheme II). The *p*-toluenesulfonic acid salt of

Scheme II



L-phenylsarcosine *p*-nitrobenzyl ester was recrystallized to remove the racemic ester and yield optically pure ester.

Experimental Section

Carbobenzoxy-L-threonyl-D-leucine *tert*-Butyl Ester (5). To a mixture of 3.16 g (12.5 mmol) of carbobenzoxy-L-threonine and 2.73 g (12.2 mmol) of D-leucine *tert*-butyl ester hydrochloride in 40 ml of methylene chloride was added 1.27 g (12.5 mmol) of triethylamine and 2.46 g (12.8 mmol) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) hydrochloride.[20] After stirring at 0° for 1.0 hr and at 24° for 16 hr the solution was evaporated and the residue partitioned between ethyl acetate and water. The ethyl acetate layer was further washed with water, 5% citric acid, water, saturated sodium bicarbonate, and water. Drying over sodium sulfate and evaporation gave an oil which solidified by trituration with petroleum ether at 0°. The yield was 4.01 g (78%), mp 89–92°, $[\alpha]^{22}D$ +13.9° (*c* 3, MeOH).

Anal. Calcd for $C_{22}H_{34}N_2O_6$: C, 62.54; H, 8.11; N, 6.63. Found: C, 62.73; H, 8.12; N, 6.69.

3-Benzyloxypicolinic Acid. According to a known method[21] pyridol (Aldrich) was converted to 3-hydroxy-2-hydroxymethylpyridine hydrochloride which was benzylated and oxidized to 3-benzyloxypicolinic acid hydrochloride sesquihydrate by a published route,[22] mp 110–115°, lit.[22] 108–112°.

(20) J. C. Sheehan, J. Preston, and P. A. Cruickshank, *J. Amer. Chem. Soc.*, **87**, 2492 (1965).

(21) D. Heinert and A. E. Martell, *Tetrahedron*, **3**, 49 (1958).

(22) J. T. Sheehan, *J. Org. Chem.*, **31**, 636 (1966).

3-Benzyloxypicolinyl-L-threonyl-D-leucine *tert*-Butyl Ester (2). A solution of 4.01 g (9.63 mmol) of carbobenzoxy-L-threonyl-D-leucine *tert*-butyl ester in 50 ml of methanol containing 2 drops of acetic acid was added to 1.5 g of 10% palladium on carbon moistened with ethyl acetate. The solution was hydrogenated at atmospheric pressure for 4.5 hr followed by filtration and evaporation of the filtrate. The residue in ethyl acetate was washed with saturated sodium bicarbonate and water, dried over sodium sulfate, and evaporated to give an oil. To 0.721 g (2.48 mmol) of this oil, 0.767 g (2.62 mmol) of 3-benzyloxypicolinic acid hydrochloride sesquihydrate and 0.263 g (2.60 mmol) of triethylamine in 25 ml of methylene chloride was added 0.498 g (2.60 mmol) of EDCI. The mixture was stirred at 24° for 16 hr, evaporated, and partitioned between ethyl acetate and water. The organic layer was washed with water, 10% citric acid, water, saturated sodium bicarbonate, and water. The solution was dried over sodium sulfate and evaporated to give 0.726 g (59%) of a glassy solid, **2**: mp 44–47°; $[\alpha]^{22}D$ +24.0° (*c* 5, MeOH).

Anal. Calcd for $C_{27}H_{37}N_3O_6$: C, 64.91; H, 7.47; N, 8.41. Found: C, 64.70; H, 7.57; N, 8.24.

***N*,β-Dimethylleucine.** Using the method of Cope,[23] a mixture of 160 g (1.0 mol) of diethyl malonate, 80 g (1.1 mol) of isobutyraldehyde, 3.4 g (0.04 mol) of piperidine, and 12 g (0.20 mol) of acetic acid was refluxed in 100 ml of benzene with a water separator overnight. The cool mixture was washed with water, dried, and evaporated. The residue was distilled to give 190 g (89.0%) of ethyl isobutylidenemalonate: bp 135–137° (25 mm); $n^{25}D$ 1.4373 (lit.[23] bp 135–137° (27 mm); $n^{25}D$ 1.4398).

A Grignard reagent prepared from 24.3 g (1.0 mol) of magnesium and 142.0 g (1.0 mol) of methyl iodide in 300 ml of ether was cooled to 0°. The mixture was treated with 5.0 g (0.026 mol) of cuprous iodide followed by the dropwise addition of 190.0 g (0.885 mol) of ethyl isobutylidenemalonate. The solution was stirred 2 hr at 25° and poured into 500 g of ice and 100 ml of 37% hydrochloric acid. The organic layer was washed with water, saturated sodium bicarbonate, and water, dried, and evaporated. Distillation of the crude product gave 161.0 g (79.0%) of ethyl 3-methyl-2-butylmalonate as a colorless liquid: bp 131–134° (25 mm); $n^{27}D$ 1.4271.

Ethyl 3-methyl-2-butylmalonate (156.0 g, 0.687 mol) was refluxed in 300 ml of water containing 112 g (2.0 mol) of potassium hydroxide for 12 hr. The mixture was distilled until 200 ml remained, cooled in a water bath and diluted to 400 ml. Concentrated sulfuric acid (111 ml, 4.0 mol) was added slowly. The resulting solution was refluxed for 16 hr and cooled and the organic layer separated and distilled to give 70.4 g (79.8%) of 3,4-dimethylpentanoic acid: bp 115–118° (20 mm); $n^{25}D$ 1.4232.

Anal. Calcd for $C_7H_{14}O_2$: C, 64.57; H, 10.84. Found: C, 64.34; H, 11.16.

To 87.5 g (0.670 mol) of 3,4-dimethylpentanoic acid and 37.5 ml (0.729 mol) of bromine was added 1.5 ml of phosphorus tribromide. The mixture was heated at 80° until evolution of hydrogen bromide ceased (6 hr). Heating was continued at 110° until the color of bromine faded (1 hr) The cooled mixture was shaken with water and the organic layer was added to 360 ml of 40% aqueous methylamine. The mixture was refluxed for 10 hr, cooled, and filtered. The collected solid (53.1 g) was nearly pure erythro isomer (ir 1325 cm^{-1}) of *N*,β-dimethylleucine. The filtrate was concentrated and filtered in several crops to give mixtures of diastereomers, pure threo isomer (ir 1350 cm^{-1}), and threo isomer mixed with methylamine hydrobromide. Fractional crystallization of the mixed isomers and impure threo isomer from water–acetone gave pure threo isomer. The total yields were 14.7 g of threo and 51.4 g of erythro (62% overall).

Anal. Calcd for $C_7H_{14}O_2N$: C, 60.35; H, 10.76; N, 8.80. Found: C, 60.34; H, 11.04; N, 8.99.

The carbobenzoxy derivative was prepared with carbobenzoxy chloride and sodium hydroxide and resolved with L-ephedrine by a published procedure.[17] Carbobenzoxy-L-*N*,β-dimethylleucine was obtained in 39% yield: mp 95.0–95.5°; $[\alpha]^{22}D$ −68.8° (*c* 0.6, EtOH) [lit.[17] mp 99°; $[\alpha]^{26}D$ −75.9° (*c* 1, EtOH)].

Anal. Calcd for $C_{16}H_{23}NO_4$: C, 65.50; H, 7.90; N, 4.77. Found: C, 65.10; H, 7.97; N, 4.77.

Cbz-L-allo-*N*,β-DM-leucyl-L-alanine Methyl Ester (6). To a mixture of carbobenzoxy-L-*N*,β-dimethylleucine (5.18 g, 17.7 mmol), L-alanine methyl ester hydrochloride (2.09 g, 15.0 mmol), and triethylamine (1.52 g, 15.1 mmol) in 15 ml of methylene chloride at 0° was added EDCI hydrochloride (3.46 g, 18.1 mmol). The mixture was stirred at 0° for 4 hr and at 25° for 16 hr. The solvent was evaporated and the residue partitioned between ethyl acetate and water. The organic layer was washed with 10% citric acid, saturated sodium bicarbonate, and water. The solution was dried over sodium sulfate and evaporated to give 2.54 g (45%) of dipeptide: $[\alpha]^{22}D$ −71.8° (*c* 5, CH_3OH).

Anal. Calcd for $C_{26}H_{30}N_2O_5$: C, 63.45; H, 8.02; N, 7.41. Found: C, 63.16; H, 8.09; N, 7.60

Carbobenzoxysarcosyl-L-allo-*N*,β-DM-leucyl-L-alanine Methyl Ester (7). Compound **6** (2.38 g, 6.28 mmol) was hydrogenated in 0.24 *N* HCl in methanol over 0.5 g of 10% palladium on carbon. The crude product (foam) was treated with triethylamine and condensed with carbobenzoxy sarcosine (1.82 g, 8.17 mmol) in methylene chloride containing EDCI (1.72 g, 9.00 mmol) using the same method as already described to give 1.42 g (50%) of a yellow oil: $[\alpha]^{22}D$ −66.2° (*c* 3, MeOH). The nmr confirmed the assigned structure and the material was used directly for the next step.

6-Butyloxycarbonyl-(*O*-*tert*-butyl)-D-allohydroxyprolysarcosyl-L-allo-*N*,β-dimethylleucyl-L-alanine Methyl Ester (8). Compound **7** (1.42 g, 3.16 mmol) was hydrogenated in 0.2 *N* methanolic HCl over 0.4 g of 10% palladium on carbon. The resulting peptide ester hydrochloride was treated with triethylamine, dissolved in methylene chloride, and condensed with 0.95 g (3.31 mmol) of *tert*-butoxycarbonyl-(*O*-*tert*-butyl)-D-allohydroxyproline using EDCI as described previously. The product was obtained in 51% yield (0.93 g), mp 73–75°, $[\alpha]^{22}D$ −16.0° (*c* 2, CH_3OH), and used directly for the next step since the nmr corresponded to that expected for the above peptide.

L-Phenylsarcosine *p*-Nitrobenzyl Ester. D,L-Phenylglycine was resolved with D-10-camphorsulfonic acid according to Marvel[24] to give L-phenylglycine of about 80% optical purity: $[\alpha]^{25}D$ +134.2° (*c* 1, 5 *N* HCl) [lit.[25] $[\alpha]^{25}D$ 125° (20% HCl)].

To a solution of 5.8 g (0.038 mmol) of L-phenylglycine (80% optical purity) in 19 ml of 2 *N* sodium hydroxide at 0° was added 4.0 ml of benzaldehyde (40 mmol) with stirring. After 20 min 0.45 g (12 mmol) of sodium borohydride was added in portions. The mixture was stirred 30 min and the additions of benzaldehyde and borohydride were repeated. The mixture was stirred for 1.0 hr and extracted with ether. The aqueous layer was acidified to pH 6 with 6 *N* HCl and filtered. The solid was washed with water and acetone and dried to give 9.0 g. The product was methylated in 4.0 ml of 40% aqueous formaldehyde, 6.0 ml of formic acid, and 5.0 ml of water. The solution was heated to reflux until all the solid had dissolved and then concentrated and taken up in 50 ml of acetic acid. The solution was added to 0.50 g of 10% palladium on carbon and hydrogenated at atmospheric pressure for 4.0 hr, followed by filtration and evaporation of the filtrate. Addition of acetone to the resulting oil gave 2.4 g of a colorless powder: $[\alpha]^{25}D$ +139.4° (*c* 1, 5 *N* HCl); optical purity 77%.

This product was acylated with *tert*-butoxycarbonyl azide to give 4.2 g (15.8 mmol) of *tert*-butoxycarbonyl-L-phenylsarcosine (77% optical purity) and esterified with 3.68 g (17.0 mmol) of *p*-nitrobenzyl bromide and 2.01 g (20.0 mmol) of triethylamine. The nitrogen protecting group was removed with trifluoroacetic acid and the amino acid ester isolated as the *p*-toluenesulfonate salt. Recrystallization from methanol–ether gave a racemic first crop. Condensation of the filtrate gave a second crop (2.3 g, mp 130–135°, $[\alpha]^{25}D$ −36.8° (*c* 0.6, MeOH)) which was determined to be 96% optically pure by removal of the *p*-nitrobenzyl ester by hydrogenation, and comparison of the optical rotation of this material, $[\alpha]^{25}D$ 164.7° (*c* 1, 1 *N* HCl), with that of L-phenylsarcosine,[18] $[\alpha]^{27}D$ 172° (*c* 1.4, 1 *N* HCl).

***tert*-Butoxycarbonyl-(*O*-*tert*-butyl)-D-allohydroxyproline.** L-Hydroxyproline (50 g, 0.38 mol, Nutritional Biochemical Corp.) was epimerized according to a published method[26] to give 19.9 g (40%) of D-allohydroxyproline, $[\alpha]^{22}D$ 51.5° (*c* 6.6, H_2O) (lit.[26] $[\alpha]D$ 58.6° (*c* 1, H_2O)). The D-allohydroxyproline was protected using carbobenzoxy chloride and *p*-nitrobenzyl bromide. The hydroxyl group was protected using isobutylene. Subsequent hydrogenation and reaction with *tert*-butyloxycarbonyl azide gave the desired compound: mp 142–144°; $[\alpha]^{25}D$ +56.3° (*c* 0.5, EtOAc).

Anal. Calcd for $C_{14}H_{25}NO_5$: C, 58.51; H, 8.77; N, 4.87. Found: C, 58.46; H, 8.83; N, 4.62.

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tert-**Butoxycarbonyl-(*O-tert*-butyl)-D-allohydroxyprolylsarcosyl-L-allo-*N*,β-dimethylleucyl-L-alanyl-L-phenylsarcosine (3).** Saponification of **8** in 0.4 *N* NaOH in acetone–water (5:2) gave 0.91 g (99%) of a foam, $[\alpha]^{22}D$ −13.1° (*c* 2, MeOH). L-Phenylsarcosine *p*-nitrobenzyl ester *p*-toluenesulfonate (0.810 g, 1.71 mmol) was treated with bicarbonate and subsequently condensed with the above peptide in methylene chloride using EDCI (0.33 g, 1.72 mmol). The resulting oil was purified by chromatography on SilicAR cc-7, 200–325 mesh (Mallinckrodt), to give 0.573 g (42.4%) of **3** *p*-nitrobenzyl ester. This material was hydrogenated directly over 0.2 g of 10% palladium on carbon moistened with ethyl acetate to give a colorless glass, **3**: 0.346 g (71.7%); mp 122–123°; $[\alpha]^{22}D$ −2.7° (*c* 1, MeOH).

Anal. Calcd for $C_{37}H_{59}N_5O_9$: C, 61.88; H, 8.31; N, 9.75. Found: C, 61.59; H, 8.34; N, 10.10.

Synthesis of 3-Benzyloxypicolinyl-*O*-(*tert*-butoxycarbonyl-(*O-tert*-butyl)-D-allohydroxyprolylsarcosyl-L-*N*,β-dimethylleucyl-L-alanyl-L-phenylsarcosyl)-L-threonyl-D-leucine *tert*-Butyl Ester (4). A solution of 1.07 g (1.49 mmol) of **3** in 5.0 ml of dry ethanol-free chloroform was treated with 0.271 g (1.68 mmol) of *N*,*N*′-carbonyldiimidazole (Aldrich Chemical Co.). After stirring for 5 min the mixture was treated with a solution of 1.90 g (3.77 mmol) of **2** in 8 ml of dry, ethanol-free chloroform. The mixture was refluxed for 20 hr and evaporated, and the residue taken up in ethyl acetate. The solution was washed with saturated sodium bicarbonate and water, dried, and evaporated to give 2.79 g of a yellow foam. The products were separated from starting material by chromatography on Sephadex LH-20 in ethyl acetate. The desired product was separated from contaminants by chromatography on SilicAR cc-7, 200–235 mesh (Malinckrodt), using chloroform–methanol (96:4). The desired peptide was identified by nmr, molecular weight, and amino acid content. The yield of **4** was 0.238 g (13.3%).

Synthesis of Etamycin 1. Compound **4** (0.238 g, 0.198 mmol) was dissolved in 3.0 ml of trifluoroacetic acid and stirred for 2 hr. Excess trifluoroacetic acid was removed by washing with petroleum ether and drying over sodium hydroxide under reduced pressure. The residue was dissolved in 15 ml of methylene chloride and treated with 1.0 ml of triethylamine followed by evaporation and drying under reduced pressure. The residue was taken up in 350 ml of methylene chloride and cooled to 0°. EDCI[20] (1.91 g, 9.93 mmol) was added with stirring and the mixture allowed to stir at 5° for 5 days. The solution was washed twice with 100 ml of water and evaporated. The residue was taken up in ethyl acetate and washed with 10% citric acid, saturated sodium bicarbonate, and water, dried, and evaporated. This crude product was taken up in 50 ml of methanol and hydrogenated for 5.5 hr over 10% palladium on carbon (0.5 g). The solution was filtered and evaporated to give 0.123 g of crude product. Chromatography of SilicAR cc-7, 200–325 mesh, with chloroform–methanol (95:5) gave 0.076 g (45%) of synthetic etamycin: mp 165–169°; $[\alpha]^{22}D$ +59.7° (*c* 1, $CHCl_3$); λ_{max} 304 mμ (log ε 3.89). Purified natural etamycin[8] has mp 168–170°, $[\alpha]^{22}D$ 62° (*c* 1, $CHCl_3$), λ_{max} 304.5 mμ (log ε 3.91). Tlc values for natural and synthetic etamycin are identical in water–acetone (4:1), R_f 0.62, methanol, R_f 0.83, and butanol–acetic acid–water (4:1:5), R_f 0.92. The ir spectra are identical with absorptions at 3300, 1745, 1650, 1510, 1095, 1060, and 745 cm^{-1}. The nmr ($CDCl_3$) spectra are identical with peaks at δ 1.1 (m), 1.8 (s), 2.2 (s), 3.0 (m), 3.5–7.0 (complex weak pattern), 7.5 (s).

A bioassay was conducted by the standard twofold dilution method in Nutrient Broth. The minimum inhibitory concentrations (micrograms/milligram) for natural and synthetic etamycin, respectively, are: *S. pneumonia*, 0.3, 0.3; *St. pyogenes*, 0.3, 0.3; *Staph. aureus*, 1.0, 1.0; *Sal. enteritidis*, 32, 32; *Sar. marcesens*, 125, 125; *E. coli*, 250, 250.

Hydrolysis of Etamycin. Samples (5 mg) of synthetic or natural etamycin were treated with 0.3 ml of 6 *N* hydrochloric acid and the mixture was heated in a sealed vial for 20 hr at 120°. Thin layer chromatography of the hydrolysates using phenol–water (3:1 w/w) gave the following results. Ninhydrin development gave identical patterns for synthetic and natural etamycin: leucine, R_f 0.55 (red); sarcosine, R_f 0.48 (purple); hydroxyproline, R_f 0.40 (yellow); alanine, R_f 0.36 (red); and threonine, R_f 0.32 (red). Development using *p*-nitrobenzoyl chloride in petroleum ether followed by pyridine in petroleum ether[8] gave a diffuse red spot at R_f 0.83 (phenylsarcosine and *N*,β-dimethylleucine) for all hydrolysates.

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