pipette affording ca. 1 mg of 11, α_D +0.030 ± 0.003° ($CHCl_3$); coeluting with synthetic methyl (2S,4S)-dimethylhexanoate on GLC; ^1H NMR identical with that of synthetic methyl (2S,4S)-dimethylhexanoate.

Acknowledgment. We gratefully acknowledge A. Crispino, S. Pacifico, and A. Trabucco for collecting *B. striata* and D. Ricciardi for technical assistance. Mass spectral data were provided by "Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli". The assistance of the staff is gratefully acknowledged.

Synthesis of a Novel Class of Peptides: Dilactam-Bridged Tetrapeptides

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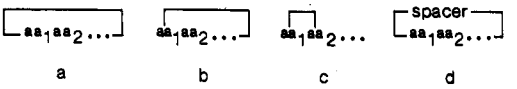
As model compounds for the study of constrained peptides, the following lactam-bridged derivatives were synthesized: Ac-L-Lys-L-Glu-D-Lys-D-Glu-NHMe (I), Ac-L-Lys-L-Glu-L-Lys-L-Glu-NHMe (II), Ac-L-Lys-L-Glu-NHMe (III), Ac-L-Glu-L-Lys-D-Glu-D-Lys-NHMe (IV), Ac-L-Glu-L-Lys-L-Glu-L-Lys-NHMe (V), and Ac-L-Glu-L-Lys-NHMe (VI). Benzyloxycarbonyl and *tert*-butyloxycarbonyl groups were employed for amine protection and the benzyl and methyl esters for carboxyl protection. Coupling reactions were carried out by the use of active esters or through azide activation. Cyclization reactions were carried out by adding the active ester hydrochlorides into large volumes of pyridine at elevated temperatures. The cyclic intermediates were obtained in yields of 45–50%. Fragment condensation of the cyclic dipeptides yielded the corresponding dilactam-bridged tetrapeptides.

Introduction

The investigation of the secondary structures of proteins and polypeptides has been met with extensive interest in recent years.[1-5] However, because of the complexity of many naturally occurring molecules it has been necessary to study synthetic model compounds.[6-8] Such model compounds are usually designed to limit the degree of conformational freedom about the peptide backbone.[9,10] This limited flexibility, thus, facilitates the interpretation of spectroscopic data because of a reduced number of conformers.[11]

Model compounds have usually been constrained by either incorporating sterically hindered residues or via cyclization. Examples of the former type include N- and α-alkylated residues such as those found in N-methylated enkephalin analogues[12,13] and α-aminoisobutyric acid containing bradykinin[14] analogues, respectively. The other method for limiting conformational mobility of peptides is through cyclization. Intramolecular cyclizations can be carried out by coupling (a) backbone to backbone, (b) side chain to backbone, (c) side chain to side chain, or (d) by incorporating a "spacer" group within the ring:



The literature contains an extensive number of compounds belonging to classes a, b, and d. Examples of backbone to backbone, side chain to backbone, and spacer group containing peptides include gramicidin S[15] and valinomycin[16] analogues, enkephalin[17-19] analogues, and the AlaGly cyclic dipeptide[20] containing ϵ-aminocaproic acid, respectively. To date, very few synthetic compounds[21,22] containing side chain to side chain bridging have been reported.

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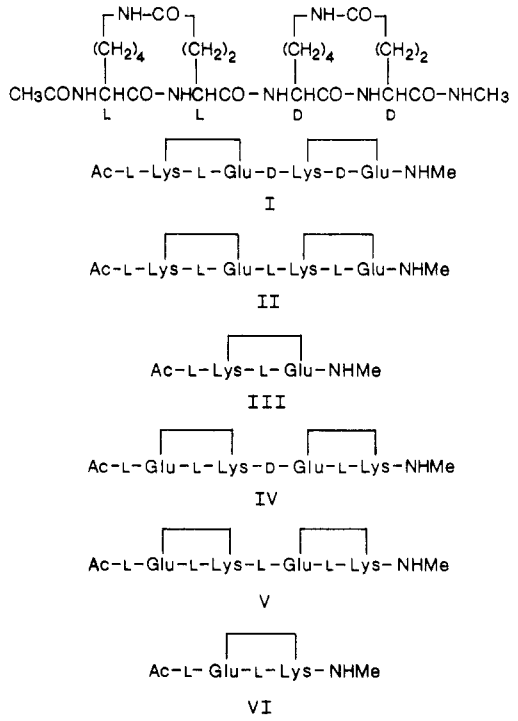
Our approach has been to couple side chains on adjacent residues in order to limit mobility of the peptide backbone. This novel class of compounds is important in mimicking constrained regions in proteins and polypeptides. As model compounds for the study of these constrained regions, the following lactam-bridged peptide derivatives, incorporating lysine and glutamic acid residues, have been synthesized:



Based on energy minimization calculations and molecular dynamics simulations for a series of dilactam-bridged tetrapeptides,[23] it was determined that lysine and glutamic acid containing peptides were the best candidates for the model compounds. Lysine and glutamic acid residues were incorporated because they yielded the optimum size for the dilactam rings and provided the desired rigidity in the peptide backbone. The conformational analysis of these peptides is presented in another paper.[24] The results from these studies are consistent with the presence of a β-turn in compound I.

Results and Discussion

LysGlu Series. The synthetic routes for the preparation of compounds I–III are shown in Schemes I–III, respectively. The strategy involved first coupling the side chains of adjacent amino acids to give the desired dipeptide fragments. Cyclization was then carried out by coupling the α-amino and α-carboxyl groups yielding the protected cyclic dipeptides. In preparing compounds I and II, the corresponding dipeptide fragments were coupled to give the desired dilactam-bridged tetrapeptides.

The advantage of using fragment condensation over the stepwise approach is that the chemistry of each amino acid/peptide derivative (L or D) is the same in the synthesis of the protected cyclic dipeptide (**9a, 9b**). In other words, reactions involving the syntheses of compounds **1a–9a** were completely analogous to those for compounds **1b–9b**.

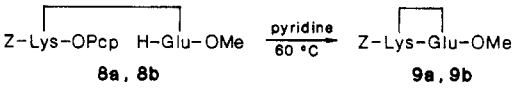
Benzyloxycarbonyl (Z) and *tert*-butyloxycarbonyl (Boc) groups were employed for amine protection and the benzyl (OBzl) and methyl (OMe) esters for carboxyl protection. Coupling reactions were carried out by the use of active esters or through azide activation.

The free acids (**2a, 2b**) were converted to the corresponding methyl esters (**3a, 3b**) by treatment with ethereal solutions of diazomethane in ethanol at 0 °C. The best results were obtained when just a slight excess of diazomethane was used. The formation of a slower moving spot was observed by thin-layer chromatography (TLC) when a large excess of the reagent was used. In this latter case, purification of the product necessitated an aqueous workup. The benzyl group of compounds **3a** and **3b** was cleaved via hydrogenolysis in methanol using palladium (10%) adsorbed on carbon as the catalyst. The free acids could not be crystallized from a variety of solvent systems so were, therefore, converted to their dicyclohexylamine (DCHA) salts (**4a, 4b**), rendering them as nicely crystalline solids.

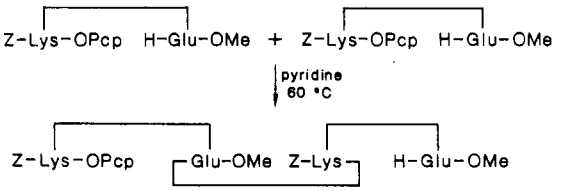
The free acids were liberated from their DCHA salts by washing a suspension of the solid in ethyl acetate with aqueous sodium bisulfate solution. The resulting side chain carboxyl groups of glutamic acid were then activated (**5a, 5b**) via the succinimide ester by reaction of the free acids with *N*-hydroxysuccinimide (HOSu) and the condensing reagent *N,N'*-dicyclohexylcarbodiimide (DCC) in tetrahydrofuran/dichloromethane. Salt couplings were then carried out involving Z-protected lysine (**1a, 1b**) and the active esters (**5a, 5b**) in the presence of sodium bicarbonate in tetrahydrofuran/water. Acidifying with hyrochloric acid and treatment with DCHA yielded the corresponding dipeptides (**6a, 6b**).

After liberation from their salts, the dipeptide free acids were activated via the pentachlorophenyl ester (**7a, 7b**) by treatment with pentachlorophenol (HOPcp) and DCC using tetrahydrofuran and dichloromethane as the solvents. It was found that the best yield of product was obtained by carrying out the reaction at relatively high concentration of reagents. Treatment of the protected dipeptide active esters (**7a, 7b**) with 4 N hydrochloric acid in dioxane cleaved the Boc groups, giving the corresponding hydrochloride salts (**8a, 8b**) in quantitative yields.

Dropwise addition of the active esters into a large volume of pyridine at elevated temperature yielded the cyclic intermediates (**9a, 9b**) in reproducible yields of 45–50%:



There was no detectable level of racemization in the product. To favor intramolecular cyclization over the formation of linear dimers and higher molecular weight polymers:



The reactions were carried out at relatively high dilution. The maximal concentration of the cyclic precursors in the reaction mixtures was 3 mM. Because the starting material was added dropwise, the actual effective concentration of cyclic precursor (assuming all previously added starting material had reacted) was considerably lower (ca. 1.5×10^{-3} mM). The formation of cyclic dimers, trimers, etc., is also theoretically possible:

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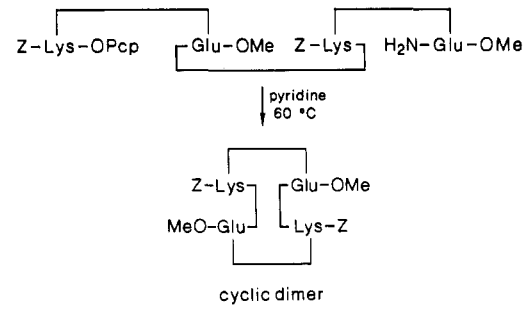


The absence of these species was verified by fast atom bombardment (FAB) mass spectrometry.

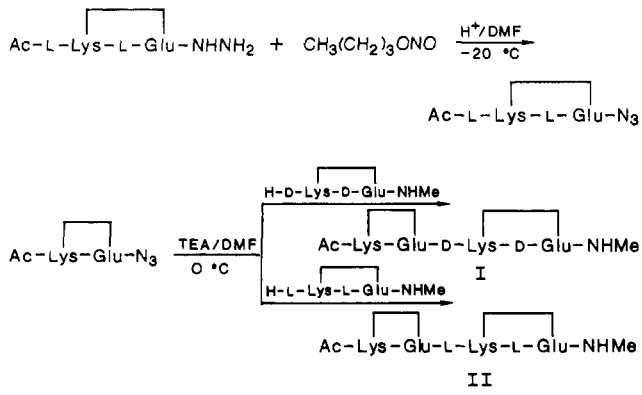
Detection of compounds by TLC became difficult after the cyclization step. More rigorous conditions were employed as compared to those used for the detection of compounds **1a,b–8a,b** (see Experimental Section).

The Z protecting group was removed by catalytic hydrogenolysis in the presence of acid (**10**). Acetylation of the dipeptide salt was carried out by treatment with acetic anhydride in acetic acid (**12**). The acetylated dipeptide was converted to the corresponding hydrazide **14** by treatment with hydrazine hydrate in methanol. Best results were obtained when the volume of the reaction mixture was kept to a minimum.

Aminolysis of the dipeptide methyl esters (**9a**, **9b**, **12**) was achieved by bubbling *N*-methylamine gas into a solution of the starting materials in methanol. The corresponding N-methylamides (**11a**, **11b**, III) were obtained in high yield. The dipeptides (**11a**, **11b**) were deprotected by catalytic hydrogenation giving the free amines (**13a**, **13b**). These were dissolved in a minimum amount of *N*,*N*-dimethylformamide and carried onto the final coupling step.

The coupling of the dipeptide fragments were achieved via an azide intermediate by employing a modified Honzl–Rudinger procedure.[25] The N-terminal hydrazide **14** was converted to the azide by treatment with hydrochloric acid and the organic nitrosating agent, *n*-butyl nitrite (*n*-BuONO), in *N*,*N*-dimethylformamide. To decrease the formation of primary amides[26] and isocyanates[27] as side products, the reactions were carried out at −20 to 0 °C. Azide formation seemed complete within 30 min as evidenced by the formation of an infrared absorption band at 2142 cm^{-1} (see Figure 1).

The hydrochloric acid was neutralized by addition of triethylamine (TEA). Coupling of the azide intermediate with the amino components (**13a**, **13b**) resulted in the desired dilactam-bridged tetrapeptides (I, II):



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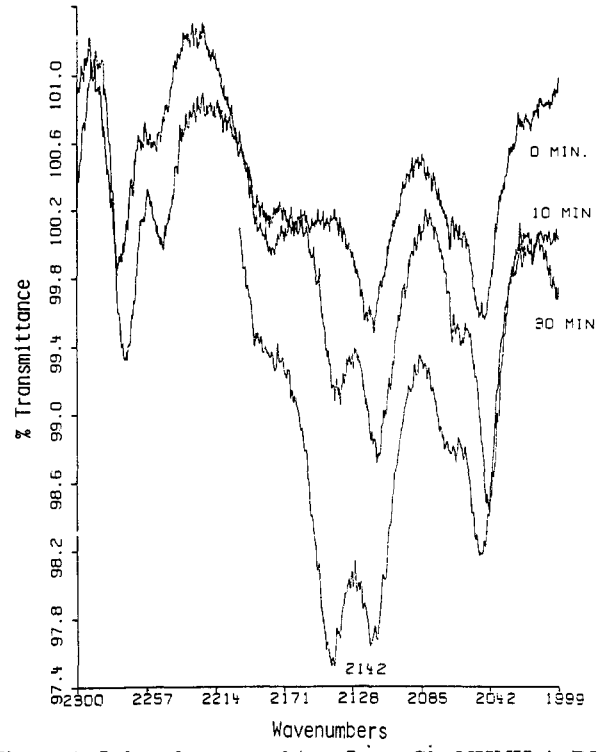


Figure 1. Infrared spectra of Ac-L-Lys-L-Glu-$NHNH_2$ in DMF at various times after addition of HCl/*n*-butyl nitrite.

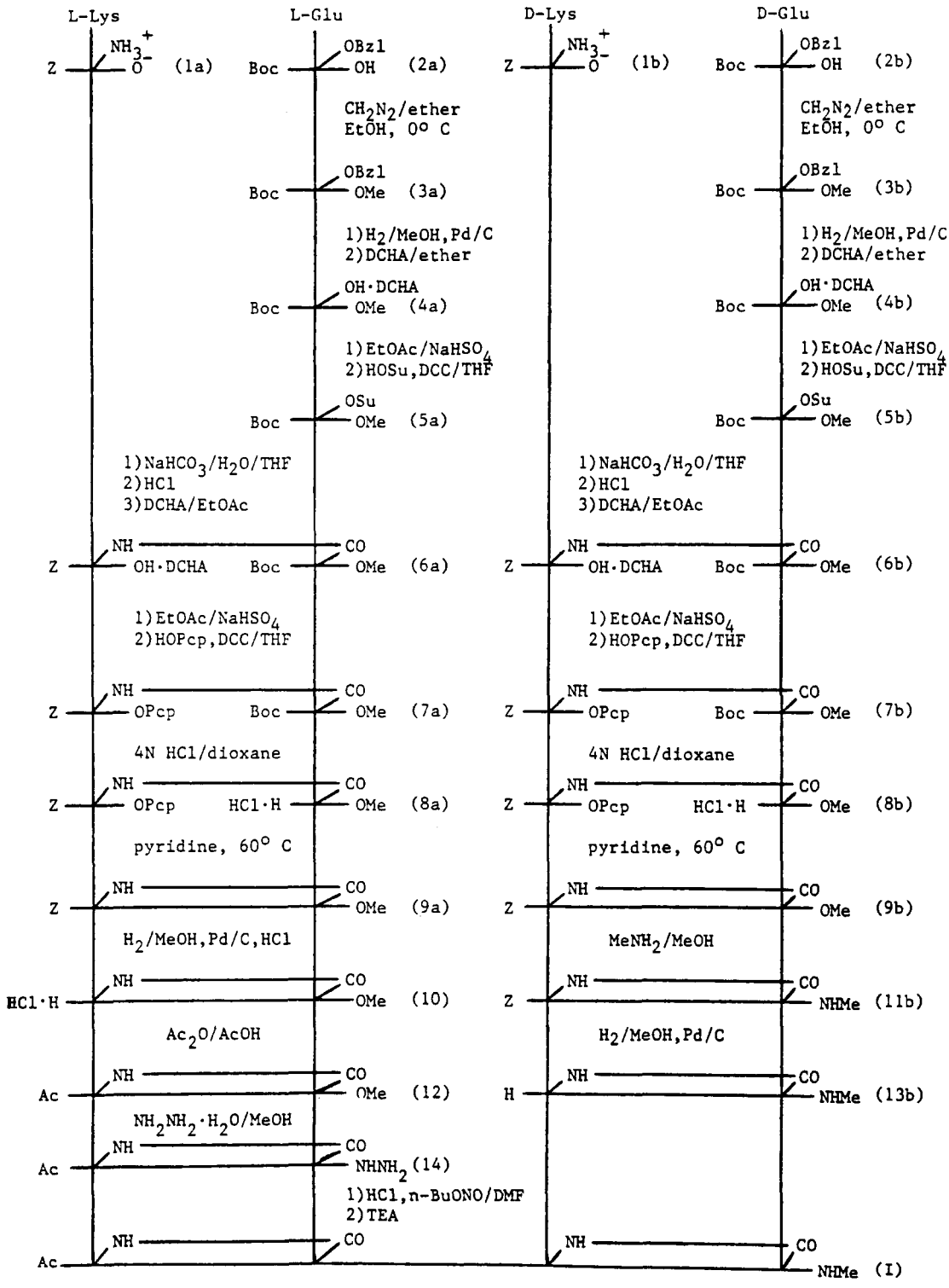
The proton NMR assignments for all isolated compounds are given in Table I.

GluLys Series. The synthetic routes for the preparation of compounds IV–VI are shown in Schemes IV–VI, respectively. As in the LysGlu series, the tetrapeptides were synthesized by the fragment condensation of the appropriate dipeptides via an azide intermediate. The major difference between the LysGlu and GluLys syntheses was that the LysGlu compounds were cyclized by coupling the α-functional groups, whereas in the GluLys series the compounds were cyclized via their side chains. In the GluLys series, the synthetic scheme was designed initially to allow for cyclization of the dipeptides via their α-functional groups. In other words, this scheme was completely analogous to the scheme for the synthesis of the LysGlu compounds. However, results obtained from FAB mass spectrometry and ^1H NMR showed the products to be contaminated with cyclic dimer and in one case, with cyclic trimer. In particular, it was shown that, depending on the reaction conditions, the ratio of monomer/dimer ranged from 10/1 to 1/2. By changing the initial scheme to the strategy actually used, the purest product isolated contained 5% cyclic dimer. This impurity was later removed by recrystallization.

Special mention should be made about the solubility of the GluLys intermediates as compared to the analogous LysGlu compounds. In all cases, reactions involving the GluLys compounds **23a–28** required more rigorous conditions (i.e., temperature and solvent composition) because of poor solubility.

The Z-protected diacids (**15a**, **15b**) were converted to their cyclic anhydrides by heating the starting materials *slowly* to 55 °C with the dehydrating agent, acetic anhydride. After removal of acetic anhydride and acetic acid (formed as a byproduct in the reaction), trituration of the remaining oil with isopropyl ether/petroleum ether gave the desired crystalline products (**17a**, **17b**) in quantitative yields. When the rate of heating of the reaction mixtures was relatively faster and/or the temperature raised higher,

Scheme I. Synthesis of Ac-L-Lys-L-Glu-D-Lys-D-Glu-NHMe



the reaction mixtures turned yellow, more spots were present on TLC, and crystalline products were not isolated.

The diprotected free acids (**16a**, **16b**) were converted to their corresponding methyl esters (**18a**, **18b**) by treatment with ethereal solutions of diazomethane in ethanol at 0 °C. The products were obtained in 97% yield. The Z group in the fully protected lysine derivatives (**18a**, **18b**) was cleaved via hydrogenolysis in methanol using palladium (10%) adsorbed on carbon as the catalyst. This generated the free amines (**19a**, **19b**).

The diprotected free amines (**19a**, **19b**) were subsequently reacted with the Z-protected cyclic anhydrides (**17a**, **17b**) in tetrahydrofuran to give the dipeptide free acids (**20a**, **20b**). Nucleophilic attack of the amines on the anhydride C=O groups generated both the α- and γ-substituted isomers. The isomers were separated by using column chromatography. After isolation, the ratio of the α- to the γ-isomers was approximately 2:3. The R_f values (on TLC) for each fraction were compared with that obtained for the dipeptide free acid **20a** as synthesized by an entirely different, unambiguous route. The detailed experimental procedure (not given here) clearly gave the α-substituted dipeptide. Therefore, by comparing R_f values, the desired isomers were isolated from the reaction

Scheme II. Synthesis of Ac-L-Lys-L-Glu-L-Lys-L-Glu-NHMe

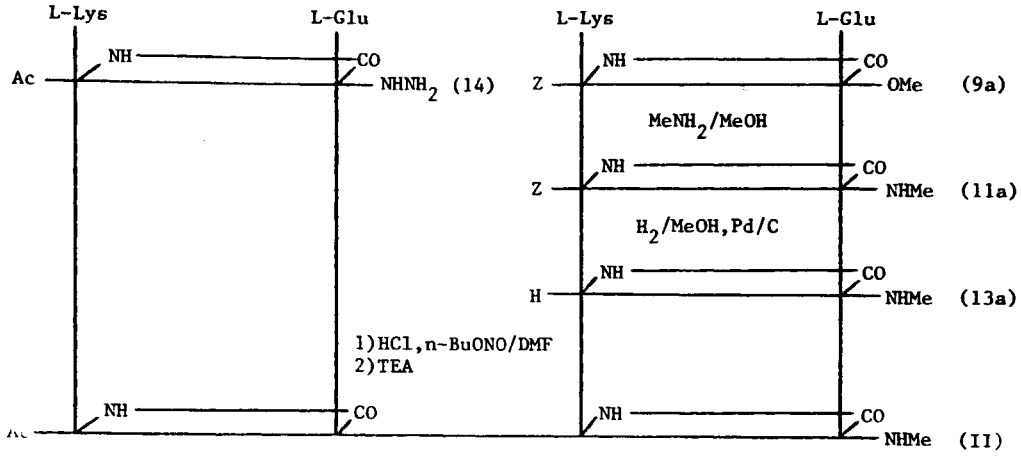
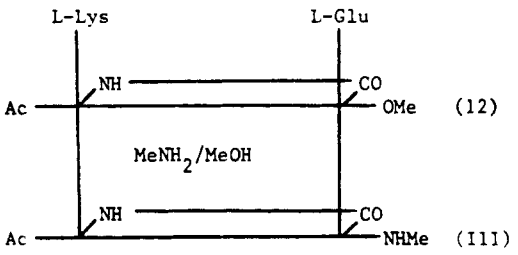


Table I. Proton NMR Assignments of Isolated Compounds in the Synthesis of the LysGlu Series

	Lys							Glu				Z		Boc	ester	acetyl	*N*-methylamide		
compd[a]	NH	$C_\alpha H$	$C_\beta H$	$C_\gamma H$	$C_\delta H$	$C_\epsilon H$	$C_\epsilon NH$	NH	$C_\alpha H$	$C_\beta H$	$C_\gamma H$	Ph	CH_2	CH_3	CH_3	CH_3	NH	NCH_3	other
3a, 3b								7.31 d (7.92)	4.01 m	1.96 m 1.81 m	2.44 m			1.37 s	3.61 s				7.36 s[b] 5.09 s[c]
4a, 4b								7.55 d (6.84)	3.97 m	1.86 m	2.69 m			1.37 s	3.61 s				1.10–1.25 m[d]
5a, 5b								7.38 d (7.89)	4.08 m	2.02 m 1.92 m	2.73 m			1.38 s	3.64 s				2.81 s[e]
6a, 6b	7.32 d (7.79)	3.69 m	2.97 m 2.88 m	1.56 m	1.31 m		7.80 t (5.68)	7.28 d (11.3)	3.92 m	1.91 m	2.13 t (7.13)	7.34 s	5.00 s	1.37 s	3.61 s				1.10–1.25 m[d]
7a, 7b	8.11 d (7.66)	4.42 m	3.04 m	1.43 m	1.32 m		7.83 t (5.30)	7.26 d (7.82)	3.91 m	1.91 m	2.12 t (7.47)	7.37 s	5.08 d (5.43)	1.37 s	3.61 s				
8a, 8b	8.13 d (7.67)	4.42 m	3.03 m	1.43 m	1.26 m		7.37[f]		4.00 m	1.99 m 1.87 m	2.28 m	7.37 s	5.09 d (6.85)		3.78 s				
9a, 9b	7.71 d (8.58)	3.83 m	2.85 m 1.58 m	1.58 m	1.37 m	3.60[f]	7.67 d[f] (8.47)	7.17 d (7.61)	4.23 m	2.07 m	2.20 m	7.35 s	5.01 s		3.59 s				
11a, 11b	7.71 d (9.26)	3.78 m	2.70 m 1.56 m	1.56 m	1.32 m	3.71 m	7.75 d (5.32)	7.00 d (8.02)	4.10 m	2.06 m	2.21 m	7.35 s	5.02 s				7.50 d (3.78)	2.53 d (4.25)	
12	7.71 d (6.45)	4.05 m	2.88 m 1.56 m	1.56 m	1.31 m	3.57[f]	8.11 d (6.75)	7.07 d (7.76)	4.18 m	2.07 m	2.20 m				3.59 s	1.79 s			
14	7.72 d (9.04)	2.82 m	2.70 m 1.56 m	1.56 m	1.30 m	2.61 m	8.21 d (6.00)	6.81 d (8.45)	4.15 m	2.08 m	2.23 m					1.82 s			
I	7.68 d (9.82) 7.57 d[g] (10.1)	3.85 m 3.82 m	2.65 m 1.60 m	1.35 m	1.35 m	3.58 m 3.51 m	8.34 s 7.46 s[g]	7.22 d[g] (9.31) 7.05 d (9.41)	4.22 m	2.04 m	2.20 m					2.10 s	6.88 d (4.79)	2.55 d (4.32)	
II	7.71 d (7.20) 7.65 d (7.56)	4.06 m 3.98 m	2.70 m 1.51 m	1.51 m	1.26 m	3.64 m	8.46 d (3.24) 7.68 d (5.76)	7.32 d (8.64) 7.12 d (8.28)	3.80 m	1.89 m	2.25 m					2.07 s	7.07 d (4.32)	2.59 d (3.96)	
III	7.72 d (9.57)	3.77 m	2.71 m 1.56 m	1.56 m	1.32 m	3.77 m	8.20 d (5.1)	6.89 d (8.39)	4.10 m	2.02 m	2.20 m					1.85 s	7.45 d (4.57)	2.55 d (4.50)	

[a] Compound numbers correspond to those used in Schemes I–III; all spectra were taken at concentrations of 1 mg/0.5 mL DMSO-d_6 at 21 °C except compound II; coupling constants (J in hertz) are given in parentheses; see Experimental Section for further details. [b] Phenyl H's, benzyl ester. [c] Benzylic CH_2. [d] Dicyclohexyl H's. [e] Succinimide H's. [f] Overlapping resonances. [g] d isomer. [h] COOH.

Scheme III. Synthesis of Ac-L-Lys-L-Glu-NHMe



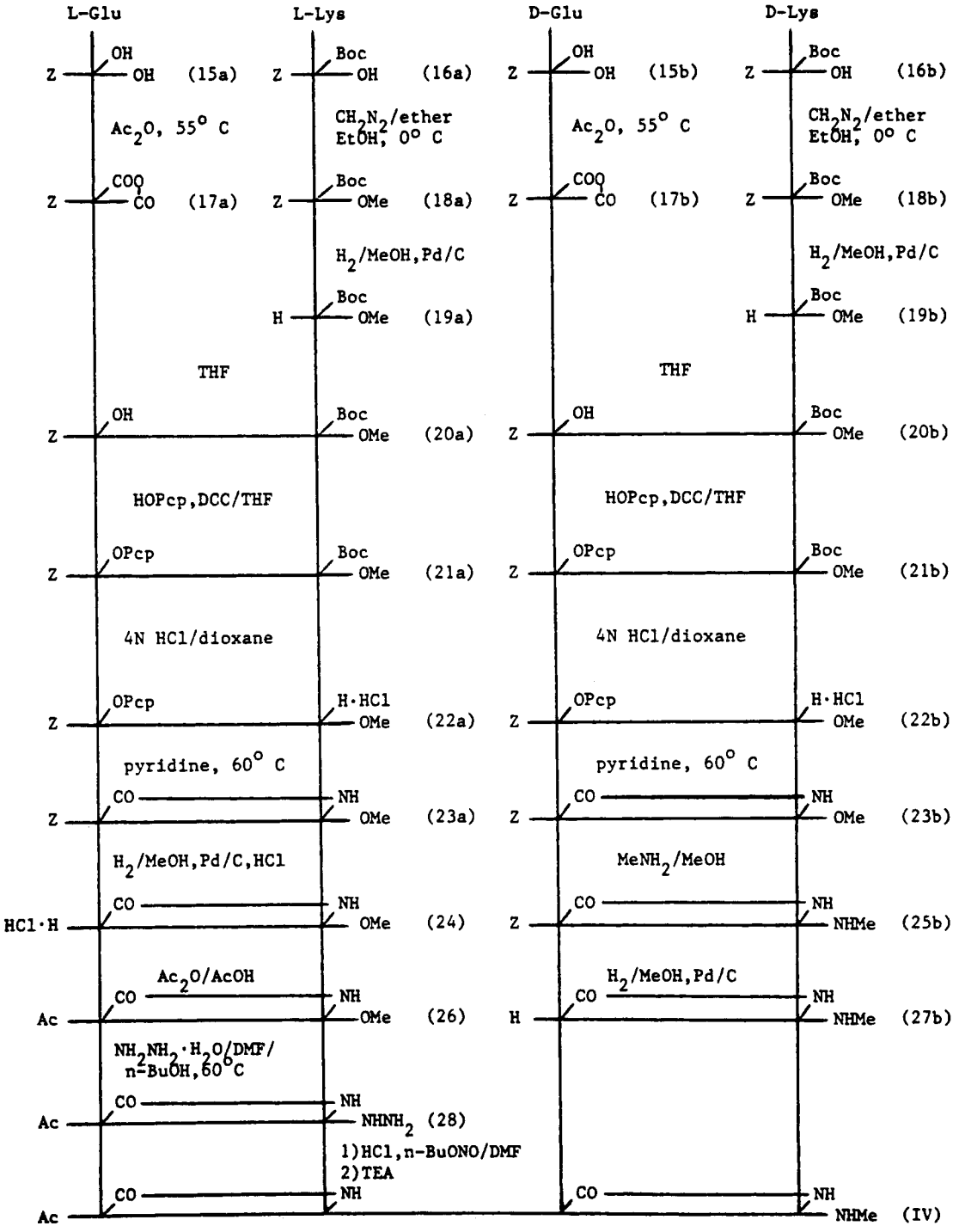
mixtures. On TLC, it was shown that the α-substituted isomer moved faster than the γ-isomer.

The dipeptide free acids (**20a, 20b**) were activated via the pentachlorophenyl ester (**21a, 21b**) by treatment with HOPcp and DCC in tetrahydrofuran. Treatment of the protected dipeptide active esters (**21a, 21b**) with 4 N hydrochloric acid in dioxane cleaved the Boc groups, giving the hydrochloride salts (**22a, 22b**) in quantitative yields.

Dropwise addition of the pentachlorophenyl ester hydrochloride salts (**22a, 22b**) into a large volume of pyridine at 60 °C yielded the cyclic intermediates (**23a, 23b**) in yields of approximately 45%. The final concentration of the cyclic precursors in the reaction mixture was approximately 1 mM. Despite this low concentration of starting material, cyclic dimer contaminated the product to the extent of 5% as evidenced by FAB mass spectrometry and ^{1}H NMR.

As with the LysGlu series, more rigorous conditions had to be employed for the detection of compounds after the

Scheme IV. Synthesis of Ac-L-Glu-L-Lys-NHMe



Scheme V. Synthesis of Ac-L-Glu-L-Lys-D-Glu-D-Lys-NHMe

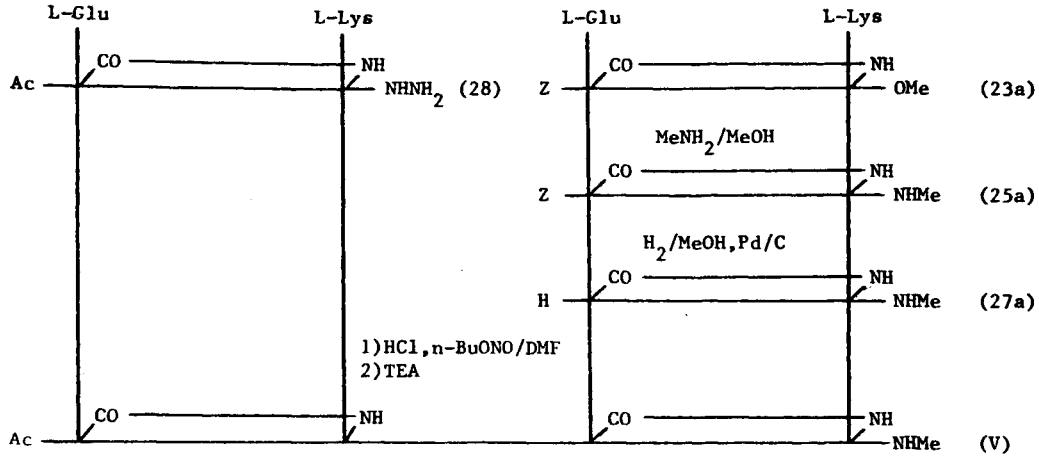
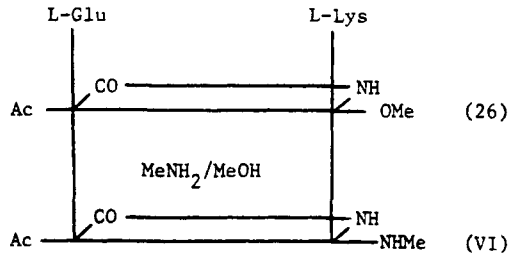


Table II. Proton NMR Assignments of Isolated Compounds in the Synthesis of the GluLys Series

	Glu				Lys							Z		Boc	ester	acetyl	*N*-methylamide		
compd[a]	NH	$C_\alpha H$	$C_\beta H$	$C_\gamma H$	NH	$C_\alpha H$	$C_\beta H$	$C_\gamma H$	$C_\delta H$	$C_\epsilon H$	$C_\epsilon NH$	Ph	CH_2	CH_3	CH_3	CH_3	NH	NCH_3	other
17a, 17b	7.93 d (8.24)	4.62 m	3.00 m 2.08 m	2.84 m 1.96 m										7.3u s	5.08 s				
18a, 18b					7.71 d (7.95)	3.99 m	1.63 m	1.33[c]	1.33[c]	2.87 m	6.78 t (5.09)	7.36 m	5.03 m	1.3l m	3.62 m				
20a, 20b	8.30 d (7.21)	4.07 d	1.89 m	2.28 t (5.50)	7.45 d (7.93)	4.20 m	1.7l m	1.29[c]	1.29 [c]	2.90 m	6.78 t (6.12)	7.35 m	5.02 m	1.36 m	3.61 m				12.11[b]
21a, 21h	8.30 d (7.21)	4.20[c]	1.95 m	2.05 m	7.61 d (7.57)	4.20[c]	1.70 m	1.36[c]	1.36[c]	2.90 m	6.79 t (5.04)	7.36 s	5.03 s	1.36 m	3.62 s				
22a, 22b	8.49 d (7.18)	4.20[c]	2.09 m 2.01 m	2.76 m	7.62 d (7.87)	4.20[c]	1.73[c] 1.66[c]	1.40[c]	1.56[c]	2.88 m	7.86 m	7.36 s	5.03 d (4.63)		3.63 s				
23a, 23b	7.80 d (7.91)	4.19 m	2.37 m 1.75 m	2.25 m 2.00 m	7.65 d (8.77)	4.44 m	1.66 m	1.2l[c]	1.26[c]	3.26 m 2.75 m	7.54 m	7.41 s	5.09 s		3.63 s				
25a, 25b	7.75 d (8.65)	4.11 m	2.37[c] 1.78 m	2.26[c] 2.04 m	7.48 d (8.29)	4.34 m	1.61 m 1.51 m	1.3j[c]	1.34[c]	3.24 m 2.81 m	7.37[c]	7.40 s	5.09 s				7.82 d (3.60)	2.57 d (3.65)	
26	8.31 d (9.37)	4.41[c]	2.34[c] 1.68[c]	2.34[c]	7.78 d (8.65)	4.41[c]	1.68 m 1.48 m	1.28[c]	1.28[c]	2.76 m 3.30[c]	7.84[c]				3.60 s	1.97 s			
28	8.20 d (7.93)	4.23 m	2.31[c] 1.75 m	2.31[c] 2.04 m	7.41 d (8.65)	4.34 m	1.58 m	1.32[c]	1.32[c]	3.20 m 2.84 m	7.57 t (5.76)					1.96 s			
IV	8.29 d (5.86) 7.87 d (7.51)	4.26 m 4.17 m	2.30[c] 1.76 m	2.30[c] 2.08[c]	7.76 d (6.76) 7.40 d (8.71)	4.34[c]	1.65[c] 1.55[c]	1.39 m	1.27 m	3.17 m 2.35 m 2.85 m	7.59 t (5.34) 7.47[c]					1.9m s	7.50 d (4.69)	2.54 d (3.97)	
V	8.29 d (5.83) 7.91 d (7.02)	4.16[c]	2.21[c] 1.81[c]	2.31[c] 2.09[c]	7.62[c] 7.14 d (8.51)	4.29[c]	1.68[c] 1.51[c]	1.33 m	1.25 m	3.13 m 2.87 m	7.60[c] 7.54 t (5.63)					1.98 s	7.49 m	2.57 d (4.16e	
VI	8.11 d (6.90)	4.16 m	2.2,[c] 1.77 m	2.28[c] 2.06 m	7.48 d (8.61)	4.30 m	1.65 m 1.57 m	1.3i[c]	1.32[c]	3.14 m 2.89 m	7.42 t (6.11)					1.95 s	7.66 d (4.62)	2.56 d (4.22)	

[a] Compound numbers correspond to those used in Schemes IV–VI; all spectra were taken at concentrations of 1 mg/0.5 mL DMSO-d_6 at 21 °C except compound V; coupling constants (J in hertz) are given in parentheses; see Experimental Section for further details. [b] COOH. [c] Overlapping resonances.

Scheme VI. Synthesis of Ac-L-Glu-L-Lys-L-Glu-L-Lys-NHMe



cyclization step (see Experimental Section). The Z group was removed from the cyclic dipeptide methyl ester **23a** by catalytic hydrogenation in the presence of acid **24**. The acetylated dipeptide methyl ester **26** was generated by treating the hydrochloride salt **24** with acetic anhydride in acetic acid. Reaction of the acetylated dipeptide methyl ester **26** with hydrazine hydrate in *N,N*-dimethylformamide/1-butanol at 60 °C yielded the corresponding hydrazide **28**. The conditions necessary for the hydrazinolysis of the methyl ester were much more rigorous than those used in the synthesis of the LysGlu analogue **14**. This was due to the GluLys compound **26** being relatively insoluble in the reaction medium.

Aminolysis of the dipeptide methyl esters (**23a, 23b, 26**) was achieved by bubbling *N*-methylamine gas into solutions of the starting materials in methanol. The corresponding Z-protected *N*-methylamides (**25a, 25b**) and acetylated N-methylamide VI were obtained.

The Z-protected dipeptides (**25a, 25b**) were deprotected by catalytic hydrogenation giving the free amines (**27a, 27b**). These were dissolved in a minimum amount of *N,N*-dimethylformamide and carried onto the final coupling step.

The coupling of the dipeptide fragments was achieved via an azide intermediate. The procedure was the same as that used in the LysGlu series. The dipeptide hydrazide **28** was converted to the azide by treatment with hydrochloric acid and *n*-BuONO in *N,N*-dimethylformamide. The reaction was carried out at −20 °C. After 1 h, the hydrochloric acid was neutralized by addition of triethylamine. Coupling of the azide intermediate with the amino components (**27a, 27b**) resulted in the desired dilactam-bridged tetrapeptides (IV, V). The proton NMR assignments for all isolated compounds are shown in Table II.

Experimental Section

Materials. The starting protected amino acids were obtained from Bachem, Inc. *N*-Hydroxysuccinimide was purchased from Sigma Chemical Company and subsequently purified via recrystallization from ethanol/ethyl acetate. *N,N'*-Dicyclohexylcarbodiimide was obtained from Fluka Chemical Company. Pentachlorophenol (Gold Label), dicyclohexylamine, and 2,2,2-trifluoroethanol were obtained from Aldrich Chemical Company. The deprotecting reagent, 4 N hydrochloric acid/dioxane (Sequanal grade), was purchased from Pierce Chemical Company. Hydrazine hydrate was obtained from MCB, Inc. *N,N*-Dimethylformamide was purified by refluxing the stock over ninhyrin for 3 h and distilling, with mild heating, under vacuum. The first 100 mL of solvent were discarded and the remainder was stored over molecular sieves (MCB, type 4a). Pyridine was purified by refluxing the stock over sodium hydroxide (99.1%) for 2 h, distilling, and collecting the fraction boiling at 115 °C (ca. 750 mm). The solvent was stored over molecular sieves (MCB, type 4a). *n*-Butyl nitrite was synthesized by the method of Noyes[28] and stored under nitrogen in the refrigerator. All other solvents were used without purification. Column chromatography was carried out by using silica gel (Kieselgel 60, 0.040–0.063 mm, 230–400 mesh ASTM) obtained from Merck, Inc.

Analytical TLC plates were purchased from E. Merck: silica gel 60 F-254, 0.2 mm thickness, aluminum-backed. All isolated products were run in at least two solvent systems. The following chromatographic systems (by volume) were used: (1) ethyl acetate/acetic acid, 50:1; (2) chloroform/methanol, 10:1; (3) chloroform/methanol/acetic acid, 85:10:5; (4) 2,2,2-trifluoroethanol/acetic acid, 10:1; (5) 1-butanol/acetic acid/water, 3:1:1; (6) chloroform/methanol/acetic acid, 2:1:1. The TLC plates were developed with the following visualizing reagents: (a) ninhydrin, (b) *tert*-butyl hypochlorite/tolidine, (c) bromocresol green, and/or (d) ammonium sulfate/sulfuric acid/water as well as with (e) UV light (254 nm).

Instrumentation. Proton NMR spectra were obtained on a 360-MHz NMR (in the Fourier-transform mode) spectrometer built in-house from a continuous-wave Varian console equipped with an Oxford superconducting magnet and a Nicolet 1280 computer. The spectrometer is equipped with an extra frequency synthesizer for double irradiation (decoupling) experiments.

Peak positions are reported in parts per million (ppm) downfield from internal tetramethylsilane (TMS; Aldrich Chemical Co.). Coupling constants are expressed in hertz (Hz) and splitting patterns are abbreviated as s, singlet; d, doublet; t, triplet; m, unresolved multiplet. Spectra were taken using 99.9% dimethyl sulfoxide-d_6 (DMSO-d_6; MSD Isotopes) as the solvent.

Assignments were made on the basis of one-dimensional decoupling and on two-dimensional homonuclear shift correlation[29] (COSY) and relayed coherence transfer[30,31] (RELCO) spectroscopy. Decoupling experiments were simply carried out by comparing splitting patterns of proton resonances in the normal, undecoupled spectrum with those present in the irradiated spectrum. Typical COSY and RELCO spectra were taken at concentrations of approximately 3–10 mM using 1K data points per spectrum for 128 spectra. The number of acquisitions were routinely 64 or 128 over a 1500-Hz spectral width. This corresponded to acquisition times of approximately 12 h.

Infrared spectra were taken on a Nicolet 7199 FTIR spectrometer using one wavenumber resolution. Absorption bands are reported in inverse centimeters (cm^{-1}). During activation of the N-terminal dipeptide **14**, the formation of the azide was monitored by observing the intensity of the absorption band (N_3 stretch, 2142 cm^{-1}) as a function of time. The sample was prepared by depositing 5 μL of the reaction mixture between two sodium chloride plates. Optical rotations were determined on a Perkin-Elmer 141 polarimeter using a 10-cm water-jacketed cell. Mass spectra were taken on a VG Analytical ZAB-1FHF (magnetic sector) high resolution mass spectrometer located at the University of California at Riverside using the fast atom bombardment method. Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. Microanalyses were performed by MicAnal, Inc., Tucson, AZ, and by Galbraith Laboratories, Inc., Knoxville, TN.

LysGlu Series. *N*-(*tert*-Butyloxycarbonyl)-γ-benzylglutamic Acid Methyl Ester (3a, 3b). To a solution of *N*-(*tert*-butyloxycarbonyl)-γ-benzylglutamate (**2a, 2b**; 5.19 g, 15.4 mmol) dissolved in 75 mL of absolute ethanol at 0 °C was added diazomethane[32] in ether until the yellow color persisted. After 1 h acetic acid was added to destroy the excess diazomethane. The solvents were removed under reduced pressure to give an oil. A solid was obtained after triturating the oil with petroleum ether. The product was obtained by recrystallizing this solid from ether/petroleum ether. The pure product (**3a, 3b**) was collected and washed with cold petroleum ether: 4.81 g (89%); mp 40–41 °C; R_f (**1a,b,e**) 0.66; $[\alpha]^{20}_D$ (*c* 1.0, MeOH), **3a** −16.0°, **3b** +16.0°.

***N*-(*tert*-Butyloxycarbonyl)glutamic Acid Methyl Ester, Dicyclohexylamine Salt (4a, 4b).** Nitrogen was bubbled

(28) Noyes, W. A. (1943) *Organic Syntheses*; Blatt, A. H., Ed.; John Wiley & Sons, Inc.: New York, 1943; Collect. Vol. II, pp 108–109.

(29) Bax, A. In *Two-Dimensional Nuclear Magnetic Resonance in Liquids*; Delft University Press: Dordrecht, 1981; pp 69–78.

(30) Eich, G.; Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 3731–3732.

(31) King, G.; Wright, P. *J. Magn. Reson.* **1983**, *54*, 328–332.

(32) Arndt, F. *Organic Syntheses*; Blatt, A. H.; Ed.; John Wiley & Sons, Inc.: New York, 1943; Collect. Vol. II, pp 165–167.

through a solution of the methyl ester (**3a, 3b**; 3.08 g, 8.77 mmol) in 50 mL of methanol for 15 min. Palladium (10%) on carbon (0.3 g) was added and hydrogen was introduced above the reaction mixture at atmospheric pressure. After 4 h the mixture was filtered through Celite and the filtrate concentrated under reduced pressure to give an oil (lit.[33] oil; R_f(**1a,b,c**) 0.51).

The oil was dissolved in 20 mL of anhydrous ether and cooled to 0 °C. Dicyclohexylamine (1.92 mL, 9.64 mmol) was added, with stirring. After 1 h the crude solid was filtered and washed with cold ether. The product was recrystallized from chloroform/ether. The pure product (**4a, 4b**) was collected and washed with cold ether: 3.80 g (98%); mp 167–168 °C (lit.[34] mp 167–168 °C); R_f(**3a,b,c**)[35] 0.53; $[\alpha]^{20}_D$ (*c* 1.0, MeOH), **4a** −10.2° (lit.[34] $[\alpha]^{25}_D$ (*c* 1.0, MeOH) −13.0°), **4b** +10.2°.

***N*-(*tert*-Butyloxycarbonyl)-γ-succinimidylglutamic Acid Methyl Ester (5a, 5b).** The dicyclohexylamine salt (**4a, 4b**; 9.10 g, 20.6 mmol), 100 mL of ethyl acetate, and 40 mL of 2 M aqueous sodium bisulfate solution were added to a separatory funnel. The solid dissolved upon mixing. The aqueous layer was discarded and the ethyl acetate solution was, once again, extracted with 2 M sodium bisulfate solution. The organic layer was washed 3× with saturated sodium chloride solution and dried over magnesium sulfate. The drying agent was removed and the filtrate concentrated under reduced pressure to give an oil.

The oil and *N*-hydroxysuccinimide (2.37 g, 20.6 mmol) were dissolved in 35 mL of tetrahydrofuran. *N,N'*-Dicyclohexylcarbodiimide (4.25 g, 20.6 mmol) and 25 mL of dichloromethane were then added. After 18 h the reaction mixture was cooled to 0 °C, the *N,N'*-dicyclohexylurea removed by filtration, and the solid washed with 40 mL of cold tetrahydrofuran/dichloromethane (1/1, v/v). The solvents were evaporated under reduced pressure to give the crude product. The product was obtained by recrystallization from ethyl acetate/hexanes. The pure product (**5a, 5b**) was collected and washed with hexanes: 6.20 g (84%); mp 118–119 °C; R_f(**1a,b,e**)[35] 0.54; $[\alpha]^{20}_D$ (*c* 1.0, MeOH), **5a** −15.6°, **5b** +15.6°.

***N*-(Benzyloxycarbonyl)lysyl-*N'*-(*tert*-butyloxycarbonyl)glutamic Acid Methyl Ester, Dicyclohexylamine Salt (6a, 6b).** A solution of the active ester (**5a, 5b**; 6.20 g, 17.3 mmol) in 70 mL of tetrahydrofuran was added to a solution of *N*-(benzyloxycarbonyl)lysine (**1a, 1b**; 7.28 g, 26.0 mmol) in 70 mL of aqueous sodium bicarbonate (2.18 g, 26.0 mmol) solution. After 11 h tetrahydrofuran was removed under reduced pressure. The remaining solution was cooled to 0 °C and acidified to pH 1 with concentrated hydrochloric acid. The resulting oily solution was extracted three times with ethyl acetate. The ethyl acetate solution was washed three times with 2 M aqueous sodium bisulfate and four times with saturated sodium chloride solution and then dried over magnesium sufate. The drying agent was removed and the filtrate was concentrated under reduced pressure to give an oil (R_f(**3a,b,c,e**) 0.32).

The oil was dissolved in 15 mL of ethyl acetate/ether (1/2, v/v) and cooled to 0 °C. With stirring, dicyclohexylamine (3.79 mL, 19.0 mmol) was added. Five minutes later 25 mL of ether were added and the mixture was placed in the freezer overnight. The pure product (**6a, 6b**) was collected and washed with cold ether: 11.0 g (90%); mp 122–123 °C; R_f(**3a,b,c,e**)[33] 0.48; $[\alpha]^{20}_D$ (*c* 1.0, MeOH), **6a** −1.3°, **6b** +1.4°.

***N*-(Benzyloxycarbonyl)-α-(pentachlorophenyl)lysyl-*N'*-(*tert*-butyloxycarbonyl)glutamic Acid Methyl Ester (7a, 7b).** The dicyclohexylamine salt (**6a, 6b**; 10.88 g, 15.43 mmol) was liberated in the same manner as compound **4a**/**4b** given in the preparation of compound **5a**/**5b**. The resulting free acid (oil) was then dissolved in 25 mL of tetrahydrofuran. Pentachlorophenol (4.11 g, 15.4 mmol) was added, followed by 25 mL of dichloromethane and *N,N'*-dicyclohexylcarbodiimide (3.18 g, 15.4 mmol). After 19 h the reaction mixture was cooled to 0 °C, the *N,N'*-dicyclohexylurea filtered, and the solid washed with 60 mL of cold tetrahydrofuran/dichloromethane (1/1, v/v). The solvents were removed under reduced pressure to give a solid. The solid was then recrystallized from ethyl acetate. The pure product (**7a, 7b**) was collected and washed with cold ethyl acetate: 9.18 g (86%); mp 154–155 °C; R_f(**3a,b,c,e**) 0.59; $[\alpha]^{20}_D$ (*c* 1.0, MeOH), **7a** −20.6°, **7b** +20.7°. Anal. Calcd for $C_{31}H_{36}N_3Cl_5O_9$: C, 48.2; H, 4.7; N, 5.4; Cl, 23.0. Found: (**7a**) C, 48.1; H, 4.5; N, 5.2; Cl, 22.9; (**7b**) C, 48.2; H, 4.6; N, 5.2; Cl, 22.9.

***N*-(Benzyloxycarbonyl)-α-(pentachlorophenyl)lysylglutamic Acid Methyl Ester, Hydrochloride Salt (8a, 8b).** The active ester (**7a, 7b**; 6.97 g, 9.03 mmol) was dissolved in 40 mL of 4 N hydrochloric acid/dioxane. After 4 h the solvent was removed under reduced pressure to give an oil. Ethyl acetate was added and subsequently removed. After repeating this procedure, a solid remained. Ethyl acetate was added and the mixture was placed in the freezer overnight. The pure product (**8a, 8b**) was collected and washed with cold ethyl acetate: 6.37 g (100%); mp 123–125 °C; R_f(**5a,b,e**) 0.70; $[\alpha]^{20}D$ (*c* 1.0, MeOH), **8a** −4.9°, **8b** +4.9°.

***N*-(Benzyloxycarbonyl)-*cyclo*(lysylglutamyl) Methyl Ester (9a, 9b).** The hydrochloride salt of the active ester (**8a, 8b**; 3.19 g, 4.50 mmol) was dissolved in 100 mL of *N,N*-dimethylformamide and added, over 2 h with extensive stirring, to 1.5 L of pyridine maintained at 60 °C. After 12 h the yellow solution was concentrated, under reduced pressure, to a volume of 50 mL. Ethyl acetate (750 mL) was added and the resulting solution washed three times with 2 M aqueous sodium bisulfate solution. During the first extraction with aqueous sodium bisulfate, a crystalline solid developed. This was discarded by either filtering the material or by removing it with the aqueous layer. The organic layer was then washed three times with saturated sodium bicarbonate, three times with saturated sodium chloride solution, and finally dried over magnesium sulfate. Filtering of the drying agent and removal of the solvent under reduced pressure gave a solid. Ethyl acetate (50 mL) was added and the mixture cooled in the freezer overnight. The pure product (**9a, 9b**) was collected and washed with ether: 0.84 g (46%); mp 232–232.5 °C dec; R_f (**4d**) 0.54; $[\alpha]^{20}_D$ (*c* 1.0, TFE), **9a** −50.5°, **9b** +50.6°; mass spectrum, *m/e* 406 (M + 1), no dimer observed. Anal. Calcd for $C_{20}H_{27}N_3O_6$: C, 59.2; H, 6.7; N, 10.4. Found: (**9a**) C 58.9; H, 6.7; N, 10.3; (**9b**) C, 59.0; H, 6.7; N, 10.3.

***cyclo*(Lysylglutamyl) Methyl Ester, Hydrochloride Salt (10).** Nitrogen was bubbled through a solution of the cyclic dipeptide (**9a**; 0.090 g, 0.22 mmol) in 50 mL of methanol containing concentrated hydrochloric acid (21 μL, 0.24 mmol) for 15 min. Palladium on carbon (10% by mass, 0.02 g) was added and hydrogen introduced above the reaction mixture at atmospheric pressure. After 21 h the mixture was filtered through Celite and the solvent removed under reduced pressure to give a yellow solid. After drying, in vacuo, the product **10** was carried on to the next reaction step.

***N*-(Benzyloxycarbonyl)-*cyclo*(lysylglutamyl) *N'*-Methylamide (11a, 11b).** The cyclic dipeptide (**9a, 9b**; 0.098 g, 0.24 mmol) was dissolved in 50 mL of methanol, cooled to 0 °C, and *N*-methylamine gas bubbled in. After condensation of the gas ceased the reaction mixture remained at 0 °C for 30 min before being raised to room temperature. After 12 h the solvents were removed under reduced pressure, leaving a white solid. Ether was added and the mixture cooled in the freezer. The pure product (**11a, 11b**) was collected and washed with cold ether: 0.092 g (95%); mp 271–273 °C dec; R_f(**6d**) 0.84; $[\alpha]^{20}_D$ (*c* 1.0, TFE), **11a** −54.6°, **11b** +54.4°.

***N*-Acetyl-*cyclo*(lysylglutamyl) Methyl Ester (12).** The hydrochloride salt of the cyclic dipeptide (**10**; 0.068 g, 0.22 mmol) was dissolved, with heating, in 15 mL of acetic acid. Acetic anhydride (15 mL) was added and the reaction allowed to proceed for 30 h.[36] The solvents were removed under reduced pressure, leaving an oil. After addition and removal of ether, a solid remained. Product was obtained by recrystallizing this solid from methanol/isopropyl ether. The pure product (**12**) was collected and washed with isopropyl ether: 0.059 g (86%); mp 291–292 °C dec; R_f(**5d**) 0.49; $[\alpha]^{20}_D$ (*c* 1.0, TFE), −92.0°.

***cyclo*(Lysylglutamyl) *N*-Methylamide (13a, 13b).** Nitrogen was bubbled through a suspension of the dipeptide (**11a, 11b**; 0.052 g, 0.013 mmol) in 100 mL of methanol for 15 min. Palladium

(33) Anderson, J. C.; Barton, M. A.; Hardy, P. M.; Kenner, G. W.; Preston, J.; Sheppard, R. C. *J. Chem. Soc.* **1967**, 108–113.
(34) Schroder, E.; Klieger, E. *Liebigs Ann. Chem.* **1964**, *673*, 196–207.
(35) The compound decomposes on TLC plate; only the R_f of major spot is reported.
(36) The basic procedure was obtained from Cash, W. D. *J. Org. Chem.* **1962**, *27*, 3329–3331.

(10%) on carbon (0.010 g) was added and hydrogen introduced above the reaction mixture at atmospheric pressure. After 12 h the mixture was filtered through Celite and the solvent removed under reduced pressure to give a white solid (R_f(**5a,b,d**) 0.48). After drying, in vacuo, for 3 h the solid was dissolved, with heating, in 5 mL of *N,N*-dimethylformamide and the solution placed in the freezer. The product (**13a**, **13b**) was carried on to the next reaction step.

***N*-Acetyl-*cyclo*(lysylglutamyl) Hydrazide (14).** The methyl ester (**12**; 0.091 g, 0.29 mmol) was dissolved, with extensive heating, in 15 mL of methanol. Hydrazine hydrate (28.2 μL, 0.580 mmol) was added and the reaction allowed to proceed for 20 h at room temperature. The solvent was removed under reduced pressure and the resulting white solid was recrystallized from methanol/isopropyl ether. The pure product **14** was collected and washed with ether. The compound showed evidence of decomposition (i.e., black color) at room temperature so was, therefore, stored in the freezer. Physical data: 0.078 g (86%); mp 276–278 °C dec; R_f(**6b,d**) 0.34; $[\alpha]^{20}_D$ (*c* 1.0, TFE), −77.5°.

***N*-Acetyl-*cyclo*(L-lysyl-L-glutamyl)-*cyclo*(D-lysyl-D-glutamyl) *N'*-Methylamide (I).** The hydrazide (**14**; 0.075 g, 0.24 mmol) was dissolved, with extensive heating, in 10 mL of *N,N*-dimethylformamide and subsequently cooled to −20 °C by means of an ice/salt bath. Hydrochloric acid in dioxane (4 N, 179 μL, 0.718 mmol) was added followed by *n*-butyl nitrite[28] (30.5 μL, 0.287 mmol).

Azide formation seemed complete after 30 min as evidenced by FTIR and TLC[35] (R_f(**3a,b,d**) 0.41). After 30 min triethylamine (110 μL, 0.790 mmol) was added. The amino component (**13b**; 0.071 g, 0.26 mmol) dissolved in 5 mL of *N,N*-dimethylformamide was precooled to −20 °C and then added to the azide solution. After 1 h at −20 °C, the reaction mixture was warmed to 0 °C and maintained for 24 h. The solvents were then removed, in vacuo, leaving a solid. After drying, in vacuo, for 2 h 25 mL of absolute ethanol were added and the mixture was heated by means of a heat gun. The pure product (I) was collected and washed with absolute ethanol: 0.090 g (68%); mp >320 °C; R_f(**5d**) 0.37; $[\alpha]^{20}_D$ (*c* 0.5, TFE), −21.8°; mass spectrum, *m/e* 552 (M + 1). Anal. Calcd for $C_{25}H_{41}N_7O_7$: C, 54.4; H, 7.5; N, 17.8. Found: C, 54.2; H, 7.4; N, 17.7.

***N*-Acetyl-*cyclo*(L-lysyl-L-glutamyl)-*cyclo*(L-lysyl-L-glutamyl) *N'*-Methylamide (II).** The compound II was obtained according to the above method: 0.077 g (58%); mp 312–315 °C dec; R_f(**6d**) 0.60; $[\alpha]^{20}_D$ (*c* 0.5, TFE), −73.0°; mass spectrum, *m/e* 552 (M + 1). Anal. Calcd for $C_{25}H_{41}N_7O_7$: C, 54.4; H, 7.5; N, 17.8. Found: C, 54.1; H, 7.3; N, 17.7.

***N*-Acetyl-*cyclo*(L-lysyl-L-glutamyl) *N'*-Methylamide (III).** The methyl ester (**12**; 0.055 g, 0.18 mmol) was dissolved, with heating, in 25 mL of methanol and cooled to 0 °C. *N*-Methylamine gas was bubbled in until the solution became saturated. The reaction mixture remained at 0 °C for 30 min before being raised to room temperature. After 12 h the solvents were removed under reduced pressure, leaving a white solid. The solid was recrystallized from methanol/isopropyl ether. The pure product (III) was collected and washed with isopropyl ether: 0.050 g (91%); mp 317–318 °C dec; R_f(**4d**) 0.28; $[\alpha]^{20}_D$ (*c* 0.5, TFE), −92.8°; mass spectrum, *m/e* 313 (M + 1). Anal. Calcd for $C_{14}H_{24}N_4O_4$: C, 53.8; H, 7.8; N, 17.9. Found: C, 53.7; H, 7.7; N, 17.8.

GluLys Series. *N*-(Benzyloxycarbonyl)glutamic Acid Anhydride (17a, 17b). The starting material *N*-(benzyloxycarbonyl)glutamic acid (**15a**, **15b**; 10.39 g, 36.94 mmol) was added, with extensive stirring, to 75 mL of acetic anhydride (Ac_2O).[37] The temperature of the suspension was *slowly* raised to 55 °C by means of a water bath, which after 10 min caused complete dissolution of the solid. After an additional 20 min the solvents were removed under reduced pressure to give an oil. The oil was dried, in vacuo, over sodium hydroxide pellets for 2 h. At this time 200 mL of isopropyl ether/petroleum ether (1/1, v/v) were added. The mixture was cooled to 0 °C and the oil triturated with a stirring bar. After 1 h, with continuous stirring, the mixture was brought to room temperature. After several hours, the pure product (**17a**, **17b**) was collected and washed with cold petroleum ether: 9.63 g (99%); mp 85–86 °C (lit. mp 86–88 °C[38], 93–94 °C[39]); R_f(**3b,e**) 0.23; $[\alpha]^{20}_D$ (*c* 1.0, MeOH), **17a** −46.9°, **17b** +47.0° (lit. mp[39] $[\alpha]^{25}_D$ (*c* 1.0, acetic acid) −45.1° for *l* isomer).

***N*-(Benzyloxycarbonyl)-*N'*-(*tert*-butyloxycarbonyl)lysine Methyl Ester (18a, 18b).** To a solution of *N*-(benzyloxycarbonyl)-*N'*-(*tert*-butyloxycarbonyl)lysine (**16a**, **16b**; 5.36 g, 14.1 mmol) dissolved in 50 mL of absolute ethanol at 0 °C was added diazomethane[32] in ether until the yellow color persisted. After 30 min acetic acid was added to destroy the excess diazomethane. The solvents were removed under reduced pressure to give an oil. A solid formed after triturating the oil with petroleum ether. The pure product (**18a**, **18b**) was collected and washed with petroleum ether: 5.41 g (97%); mp 61–62 °C; R_f(**3a,e**) 0.72; $[\alpha]^{20}_D$ (*c* 1.0, MeOH), **18a** −14.7°, **18b** +14.7°.

***N*-(*tert*-Butyloxycarbonyl)lysine Methyl Ester (19a, 19b).** Nitrogen was bubbled through a solution of the methyl ester (**18a**, **18b**; 5.38 g, 13.6 mmol) in 100 mL of methanol for 15 min. Palladium (10%) on carbon (0.75 g) was added and hydrogen introduced above the reaction mixture at atmospheric pressure. After 4 h the mixture was filtered through Celite and the filtrate concentrated under reduced pressure to give an oil (R_f(**3a**) 0.15). The oil (**19a**, **19b**; 3.54 g, 100%) was carried on to the next reaction step.

***N*-(Benzyloxycarbonyl)glutamyl-*N'*-(*tert*-butyloxycarbonyl)lysine Methyl Ester (20a, 20b).** To a solution of the free amine (**19a**, **19b**; 3.54 g, 13.6 mmol) in 70 mL of tetrahydrofuran was added *N*-(benzyloxycarbonyl)glutamic acid anhydride (**17a**, **17b**; 3.26 g, 12.4 mmol). After 22 h the solvent was removed under reduced pressure to give an oil. The oil was then dried, in vacuo, for 2 h. Column chromatography was subsequently carried out in order to separate the desired isomer (α) from the mixture. The mixture was loaded onto a silica gel column (*d* = 5 cm, *h* = 25 cm) and eluted with ethyl acetate/acetic acid (100/1, v/v) at a flow rate of 2.5 cm/min. The compound corresponding to the faster moving spot (on TLC) was collected and the solvents were removed under reduced pressure to give an oil. Ether was added to the oil and, after swirling, a solid developed. The pure product (**20a**, **20b**) was collected and washed with ether: 2.71 g (42%); mp 134–136 °C; R_f(**1a,e**) 0.37; $[\alpha]^{20}_D$ (*c* 1.0, MeOH), **20a** −14.0°, **20b** +14.0°.

***N*-(Benzyloxycarbonyl)-γ-(pentachlorophenyl)-glutamyl-*N'*-(*tert*-butyloxycarbonyl)lysine Methyl Ester (21a, 21b).** To a solution of the dipeptide free acid (**20a**, **20b**; 2.68 g, 5.12 mmol) in 50 mL of tetrahydrofuran/dichloromethane (1/1, v/v) was added pentachlorophenol (1.50 g, 5.63 mmol) and *N,N'*-dicyclohexylcarbodiimide (1.16 g, 5.63 mmol). After 22 h the reaction mixture was cooled to 0 °C, the *N,N'*-dicyclohexylurea filtered, and the solid washed with 50 mL of cold tetrahydrofuran/dichloromethane (1/1, v/v). The solvents were removed under reduced pressure to give a solid. The solid was recrystallized from ethyl acetate/ether. The pure product (**21a**, **21b**) was collected and washed with cold ether: 3.59 g (91%); mp 138–139 °C; R_f(**1a,e**) 0.66; $[\alpha]^{20}_D$ (*c* 1.0, MeOH), **21a** −10.9°, **21b** +10.9°. Anal. Calcd for $C_{31}H_{36}N_3Cl_5O_9$: C, 48.2; H, 4.7; N, 5.4; Cl, 23.0. Found: (**21a**) C, 48.2; H, 4.6; N, 5.4; Cl, 22.9; (**21b**) C, 48.2; H, 4.7; N, 5.2; Cl, 22.9.

***N*-(Benzyloxycarbonyl)-γ-(pentachlorophenyl)-glutamyl-*N'*-(*tert*-butyloxycarbonyl)lysine Methyl Ester, Hydrochloride Salt (22a, 22b).** The dipeptide active ester (**21a**, **21b**; 3.55 g, 4.60 mmol) was dissolved in 30 mL of 4 N hydrochloric acid/dioxane. After 4 h the solvent was removed under vacuum to give an oil. Ethyl acetate was added and subsequently removed. After repeating this procedure, a solid remained. The pure product (**22a**, **22b**) was collected and washed with cold ethyl acetate: 3.26 g (100%); mp 138–140 °C; R_f(**4a,e**) 0.58; $[\alpha]^{20}_D$ (*c* 1.0, MeOH), **22a** −19.2°, **22b** +19.2°.

***N*-(Benzyloxycarbonyl)-*cyclo*(glutamyllysyl) Methyl Ester (23a, 23b).** The hydrochloride salt of the active ester (**22a**, **22b**; 0.904g, 1.28 mmol) was dissolved in 500 mL of *N,N*-dimethylformamide. This solution (125 mL) was added over 3 h, with extensive stirring, to 850 mL of pyridine maintained at 60 °C. Ten hours after the addition, another 125 mL of the *N,N*-

(37) The basic procedure was obtained from Vogel, A. I. *A Textbook of* Practical *Organic Chemistry*; Longmans, Green, and Co.: London, 1956; p 371.

(38) Gibian, H.; Klieger, E. *Liebigs Ann. Chem.* **1961**, *640*, 145–156.

(39) Le Quesne, W. J.; Young, G. I. *J. Chem. Soc. (London)* **1950**, 1954–1959.

dimethylformamide solution was added over a 3-h period. Ten hours later, the entire procedure was repeated. Ten hours after the last addition of starting material, the solvents were removed under vacuum, leaving a solution volume of approximately 10 mL. Methanol (50 mL) was added and the resulting mixture placed in the freezer overnight. The product (**23a, 23b**) was collected and washed with cold methanol: 0.243 g (47%); mp 268–270 °C dec; R_f(**3d**) 0.52; $[\alpha]^{20}_D$ (*c* 1.0, TFE), **23a** −4.0°, **23b** +4.0°; mass spectrum, *m/e* 406 (M + 1), 811 (2M + 1), ratio 406/811 = 100/5. Anal. Calcd for $C_{20}H_{27}N_3O_6$: C, 59.2; H, 6.7; N, 10.4. Found: (**23a**) C, 59.0; H, 6.5; N, 10.3; (**23b**) C, 59.1; H, 6.5; N, 10.3.

***cyclo*(Glutamyllysyl) Methyl Ester, Hydrochloride Salt (24).** Nitrogen was bubbled through a suspension of the cyclic dipeptide (**23a**; 0.199 g, 0.500 mmol) in 60 mL of 2,2,2-trifluoroethanol/methanol (1/1, v/v) containing concentrated hydrochloric acid (50 μL, 0.55 mmol). Palladium (10%) on carbon (0.2 g) was added and hydrogen introduced above the reaction mixture at atmospheric pressure. After 17 h the mixture was filtered through Celite and the solvents were removed under reduced pressure to yield a solid. After drying, in vacuo, the product (**24**; 0.154 g, 100%) was carried on to the next reaction step.

***N*-(Benzyloxycarbonyl)-*cyclo*(glutamyllysyl) *N'*-Methylamide (25a, 25b).** The cyclic dipeptide methyl ester (**23a, 23b**; 0.150 g, 0.370 mmol) was suspended in 150 mL of methanol and cooled to 0 °C, and *N*-methylamine gas was bubbled in. After condensation of the gas ceased, the reaction mixture remained at 0 °C for 30 min before being raised to room temperature. After 12 h the solvents were removed under reduced pressure, leaving a white solid. The solid was recrystallized from methanol/ether. The pure product (**25a, 25b**) was collected and washed with cold ether: 0.105 g (70%); mp 248–250 °C dec; R_f(**3d**) 0.29; $[\alpha]^{20}_D$ (*c* 0.5, TFE), **25a** −2.8°, **25b** +2.8°.

***N*-Acetyl-*cyclo*(glutamyllysyl) Methyl Ester (26).** The hydrochloride salt of the cyclic dipeptide (**36**; 0.154 g, 0.500 mmol) was dissolved, with heating, in 40 mL of acetic acid. Acetic anhydride (70 mL) was added and the reaction allowed to proceed for 30 h.[24] The solvents were removed under reduced pressure to give a solid. The product was subsequently recrystallized from methanol. The pure product **26** was collected and washed with ether: 0.124 g (79%); mp 319–320 °C dec; R_f(**3d**) 0.19; $[\alpha]^{20}_D$ (*c* 1.0, TFE) −49.6°.

***cyclo*(Glutamyllysyl) *N*-Methylamide (27a, 27b).** Nitrogen was bubbled through a solution of the dipeptide *N*-methylamide (**25a, 25b**; 0.081 g, 0.20 mmol) in 50 mL of methanol for 15 min. Palladium (10%) on carbon (0.03 g) was added and hydrogen introduced above the reaction mixture at atmospheric pressure. After 17 h the mixture was filtered through Celite and the solvent removed under reduced pressure to give a white solid (R_f(**5a,d**) 0.13). After drying, in vacuo, for 3 h the product was dissolved, with heating, in 5 mL of *N,N*-dimethylformamide and the solution placed in the freezer. The product (**27a, 27b**) was carried on to the next reaction step.

***N*-Acetyl-*cyclo*(lysylglutamyl) Hydrazide (28).** The acetylated dipeptide methyl ester (**26**; 0.095 g, 0.30 mmol) was dissolved, with extensive heating, in 5 mL of *N,N*-dimethylformamide/1-butanol (3/2, v/v). After heating the solution to 60 °C, hydrazine hydrate (74 μL, 1.5 mmol) was added. After 14 h the solvents were removed under vacuum, leaving a solid. Methanol (25 mL) was added and the mixture placed in the freezer overnight. The pure product **28** was collected and washed with cold methanol: 0.067 g (71%); mp 277–279 °C dec; R_f(**5d**) 0.19; $[\alpha]^{20}_D$ (*c* 0.1, TFE) −27.0°.

***N*-Acetyl-*cyclo*(L-glutamyl-L-lysyl)-*cyclo*(D-glutamyl-D-lysyl) *N*-Methylamide (IV).** The dipeptide hydrazide (**28**; 0.057 g, 0.18 mmol) was dissolved, with extensive heating, in 9 mL of N,N-dimethylformamide and subsequently cooled to −20 °C by means of an ice/salt bath. Hydrochloric acid in dioxane (4 N; 137 μL, 0.546 mmol) was added followed by *n*-butyl nitrite[28] (21.2 μL, 0.200 mmol). Azide formation seemed complete after 30 min as evidenced by FTIR and TLC[35] (R_f(**3a,d**) 0.19). To ensure complete reaction, 1 h elapsed before the addition of triethylamine (83.6 μL, 0.600 mmol). The amino component (**27b**; 0.200 mmol) dissolved in 5 mL of *N,N*-dimethylformamide was precooled to −20 °C and then added to the azide solution. After 1 h at −20 °C the reaction mixture was warmed to 0 °C and maintained for 40 h. The solvent was then removed under vacuum leaving a solid. After drying, in vacuo, for 2 h 25 mL of absolute ethanol were added and the mixture was stirred for 2 h. The pure product IV was collected and washed with 50 mL of absolute ethanol: 0.064 g (64%); mp 310–312 °C dec; R_f(**6d**) 0.31; $[\alpha]^{20}_D$ (*c* 0.1, TFE) −54.0°; mass spectrum, *m/e* 552 (M + 1), no dimer observed. Anal. Calcd for $C_{25}H_{41}N_7O_7$: C, 54.4; H, 7.5; N, 17.8. Found: C, 54.4; H, 7.4; N, 17.7.

***N*-Acetyl-*cyclo*(L-glutamyl-L-lysyl)-*cyclo*(L-glutamyl-L-lysyl) *N'*-Methylamide (V).** The compound V was obtained according to the above method: 0.060 g (60%); mp >320 °C; R_f(**6d**) 0.27; $[\alpha]^{20}_D$ (*c* 0.1, TFE) −33.0°; mass spectrum, *m/e* 552 (M + 1), no dimer observed. Anal. Calcd for $C_{25}H_{41}N_7O_7$: C, 54.4; H, 7.5; N, 17.8. Found: C, 54.1; H, 7.3, N, 17.6.

***N*-Acetyl-*cyclo*(L-glutamyl-L-lysyl) *N'*-Methylamide (VI).** The acetylated dipeptide methyl ester (**26**; 0.057 g, 0.18 mmol) was suspended in 100 mL of methanol and cooled to 0 °C. *N*-Methylamine gas was bubbled in until the solution became saturated. The reaction mixture remained at 0 °C for 30 min before being raised to room temperature. After 12 h the solvents were removed under reduced pressure to give a white solid. The solid was recrystallized from 25 mL of methanol. The pure product VI was collected and washed with cold methanol: 0.050 g (88%); mp 317–318 °C dec; R_f(**6d**) 0.46; $[\alpha]^{20}_D$ (*c* 0.1, TFE) −42.0°; mass spectrum, *m/e* 313 (M + 1), no dimer observed. Anal. Calcd for $C_{14}H_{24}N_4O_4$: C, 53.8; H, 7.8; N, 17.9. Found: C, 53.8; H, 7.7; N, 17.9.

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Registry No. **1a**, 2212-75-1; **1b**, 70671-54-4; **2a**, 13574-13-5; **2b**, 35793-73-8; **3a**, 59279-58-2; **3b**, 110473-10-4; **4a**, 82152-24-7; **4a** (free acid), 72086-72-7; **4b**, 55227-01-5; **4b** (free acid), 55227-00-4; **5a**, 110473-11-5; **5b**, 110473-12-6; **6a**, 110473-14-8; **6a** (free acid), 110473-13-7; **6b**, 110473-16-0; **6b** (free acid), 110473-15-9; **7a**, 110473-17-1; **7b**, 110473-18-2; **8a**, 110473-19-3; **8b**, 110473-20-6; **9a**, 110473-21-7; **9b**, 110547-95-0; **10**, 110473-22-8; **11a**, 110473-23-9; **11b**, 110547-96-1; **12**, 110473-24-0; **13a**, 110473-25-1; **13b**, 110547-97-2; **14**, 110473-26-2; **15a**, 1155-62-0; **15b**, 63648-73-7; **16a**, 2389-60-8; **16b**, 66845-42-9; **17a**, 4124-76-9; **17b**, 71869-80-2; **18a**, 2389-49-3; **18b**, 84559-78-4; **19a**, 3017-32-1; **19b**, 63328-49-4; **20a**, 110473-27-3; **20b**, 110473-28-4; **21a**, 110473-29-5; **21b**, 110473-30-8; **22a**, 110473-31-9; **22b**, 110473-32-0; **23a**, 110473-33-1; **23b**, 110547-98-3; **24**, 110473-34-2; **25a**, 110473-35-3; **25b**, 110548-84-0; **26**, 110473-36-4; **27a**, 110473-37-5; **27b**, 110547-99-4; **28**, 110473-38-6; I, 110473-39-7; II, 110548-00-0; III, 110473-40-0; IV, 110473-41-1; V, 110548-01-1; VI, 110473-42-2.