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Protein β-Turn Mimetics II: Design, Synthesis, and Evaluation in the Cyclic Peptide Gramicidin S

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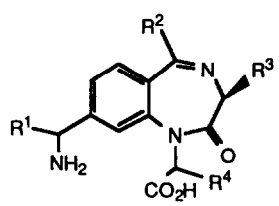
As discussed in the previous paper[1] an important structural feature of many biologically active peptides and proteins is the beta-turn motif. Substantial evidence now exists to suggest that small, biologically active peptide possess beta turns in their active conformations and the resulting compact structures have clustered side chains available for interactions with receptors. A popular approach towards more active peptides which recognizes this fact is one in which conformationally constrained analogs are synthesized to retain or improve the biological activity of the native, linear peptides. The benzodiazepine (BZD) structure has been shown to satisfy many of the requirements of a useful beta turn mimic by closely maintaining the overall geometric requirements of the beta turn while projecting the sidechains of residues i through i+3 in their proper directions[1]. Additionally, the benzodiazepines have the important advantage that they are readily synthetically available. To establish the validity of the BZD group as a beta turn mimic we have been interested in incorporating it into cyclic peptides containing known beta-turns[1]. The concept is to replace the four amino acids that make up one of the beta turns in these cyclic peptides with an appropriate BZD peptidomimetic. If the peptidomimic is a good one then it should stabilize the resulting BZD containing cyclic peptide in a similar conformation as the all peptide model. Gramicidin S appears to be a particularly attractive cyclic peptide to further test this hypothesis.

Gramicidin S (GS) has been examined by NMR, CD, and x-ray[2] analysis and shown to exist in a C_2 symmetric, very stable, double beta-turn conformation with a beta sheet connecting the two turns. Gramicidin S has the additional advantage of being a biologically active (anti-bacterial) peptide and its biological activity has been shown to be related to its conformation. Thus, this molecule can serve not only as a physical model but also as a biological model system.

Gramicidin S Model System: GS Based Cyclic Octapeptide

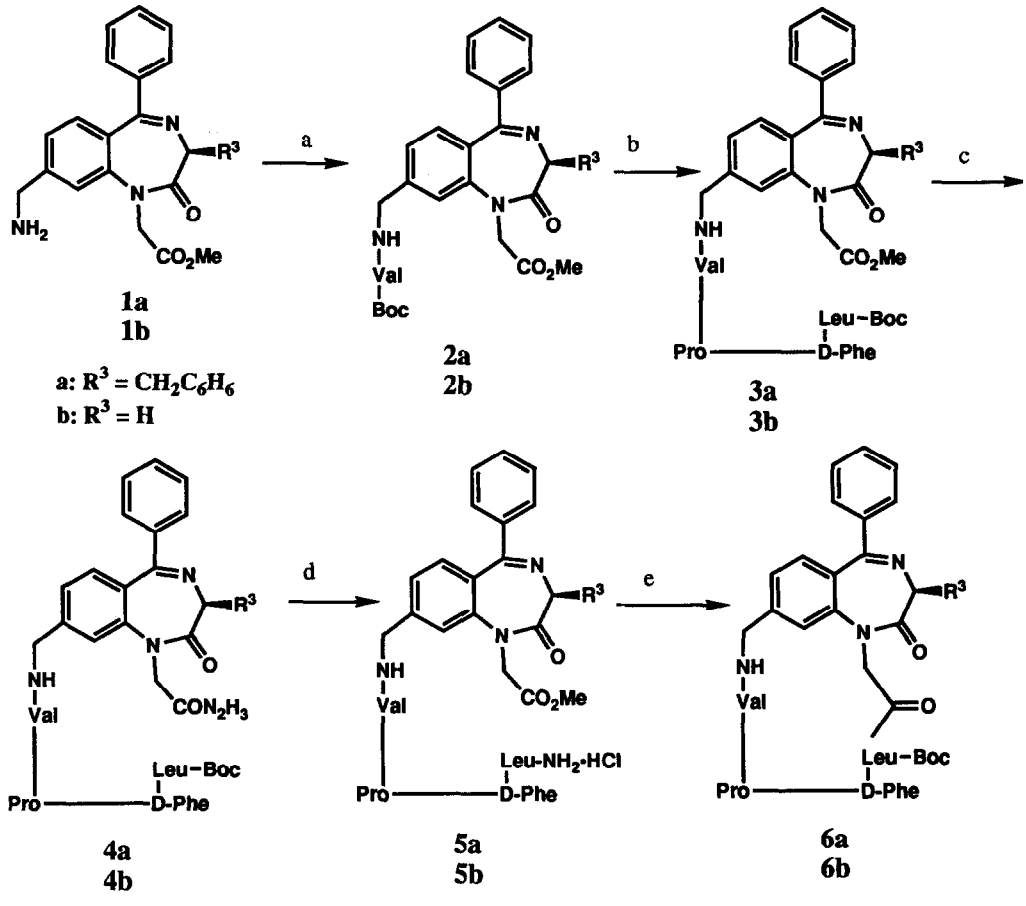
Encouraged by the results of the cyclic octapeptide model system (compound **20**, ref. 1) we were interested in using the BZD peptidomimetic to substitute for other types of beta turns and to incorporate it into biologically active peptides. Gramicidin S (Leu-D-Phe-Pro-Val-Orn)$_2$ is a very stable, C_2 symmetric, cyclic decapeptide anti-bacterial agent. Its conformation consists of two type II' beta turns at Leu-D-Phe-Pro-Val and a beta sheet, connecting the two turns, at Val-Orn-Leu (Figure 1). For GS it is possible to examine both the effect of an incorporated beta turn mimic on the conformation as well as on its biological activity. In addition, GS also offers the opportunity to examine a Type II' turn. Based on molecular modeling studies and experimental work in other cyclic octapeptides[1] the BZD moiety was expected be a good mimetic for various types of turns and therefore peptidomimetic I was expected to be a Type II' beta turn mimic as well.



I

The use of Gramicidin S as model system represents a significant increase in complexity over the earlier model cyclic octapeptides[1]. In a

Scheme 1



i. isobutylchloroformate/N-methylmorpholine/Boc-Val/THF/0°C

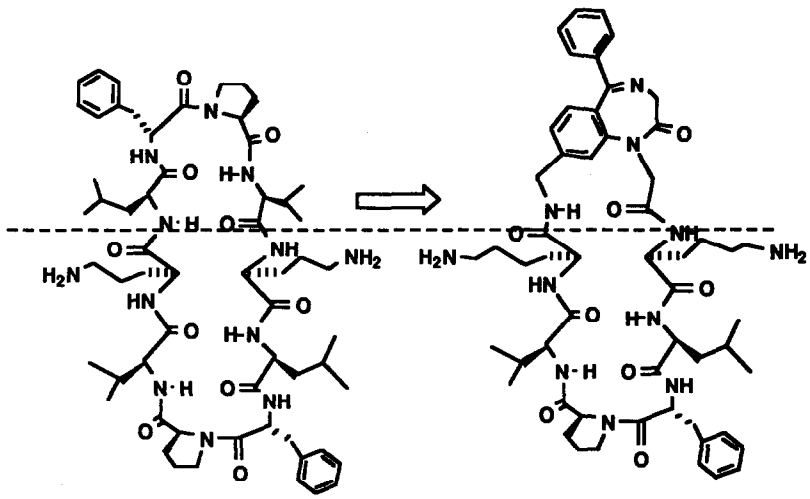
j. 1. $HCl_{(g)}$, 0°C, 1 hr. 2. N-methylmorpholine/THF 3. couple with next amino acid 4. repeat steps

k. N2H4/MeOH/rt/2 days

l. $HCl_{(g)}$/0°C/60 min

m. 1. DMF/-20°C/HONO 2. Dilute with DMF/DIEA

preliminary study we first tested the prediction that our peptidomimetic could mimic the Type II' turn present in GS. BZD **1** (Scheme 1) was incorporated into a cyclic "octapeptide" **6** containing the GS beta



GRAMICIDIN S
c-(O-L-dF-P-V)2

BZD/GS analog
c-(K-L-dF-P-V-BZD)

Figure 1. Gramicidin S Model

turn sequence (Leu-D-Phe-Pro-Val). Since we could not employ a proline side chain at the 3-position of the BZD we examined the effect on the conformation of having no substituent (Gly side chain **6b**) or a large substituent (Phe side chain **6a**) at this position. The synthesis of cyclic "octapeptides" **6a/b** is analogous to that described earlier (see the synthesis of compound **20**, ref. 1) and is outlined in **Scheme 1**.

Conformational Analysis Of *c*-(L-dF-P-V-BZD) **6ab**.

The assignment of **6b** was made in DMSO and $CDCl_3$ (data not shown). It became clear that in solution **6b** consisted of a mixture of two conformations. This is due to the fact that the benzodiazepine seven-membered ring can flip between the two chair forms when it is not substituted at the three position. When a 3-substituent is present, the conformation is frozen into a single chair form with the substituent in the equatorial position. Because of this conformational flexibility of the BZD ring no further conformational analysis was attempted with **6b** and instead we concentrated our analysis on **6a**.

The assignment of **6a** was made in DMSO and $CDCl_3$ (Table 1). During the analysis of the coupling constant data from **6a** in $CDCl_3$, it became clear that the solution conformation was changing in a temperature and concentration dependent manner (data not shown). All further analysis was based on data collected in DMSO.

Amide temperature dependence experiments (Table 2) indicate the 8-benzyl, Leu^2 and $D\text{-}Phe^3$ amide protons are exposed to bulk solvent while the Val^5 amide proton is sequestered from bulk solvent. In a molecule the size of **6a**, the most likely cause for the sequestering of an amide proton is participation in an intramolecular hydrogen bond. These data strongly suggest the Val^5 amide proton is intramolecularly hydrogen bonded. There is no independent confirmation for this hydrogen bond.

A similarly located intramolecular hydrogen bond is observed in GS. However, the intramolecular hydrogen which involves the Leu amide proton in GS is not observed in **6a**.

Table 1. Assignment of 6a in DMSO/CDCl3

Residue	Atom	DMSO[1]	$CDCl_3$[2]
BZD[1]	H9	7.10	6.96
8-benzyl	NH	8.16	7.96
	H^{α}	4.27,4.14	4.62,4.11
3-benzyl	H^{α}	3.75	3.95
	H^{β}	3.40	3.67, 3.53
C10	H^{α}	4.83, 3.62	4.97, 3.70
Leu[2]	NH	8.10	5.58
	H^{α}	4.57	4.43
	H^{β}	1.40	1.76, 1.49
	H^{γ}	1.57	1.58
	H^{δ}	0.88	0.92
D-Phe[3]	NH	9.08	6.93
	H^{α}	4.36	4.51
	H^{β}	3.03,2.88	3.14,3.00
Pro[4]	H^{α}	4.24	4.25
	H^{β}	1.87	2.14, 1.69
	H^{γ}	1.71	1.67
	H^{δ}	3.69, 3.23	3.88, 2.78
Val[5]	NH	7.15	6.63
	H^{α}	3.98	4.24
	H^{β}	1.99	2.29
	H^{γ}	0.82, 0.70	0.93

[1]Concentration: 6.3 mM. [2]Concentration: 9.8 mM

Table 3. NOE Intensity Data Of 6a

Proton	Proton	Intensity
Bzd[1] NH	Bzd[1] H^{α}	M
	Bzd[1] H^{α}	M
	BZD[1] H9	W
	Val[5] NH	M
	Val[5] H^{α}	M
	Val[5] H^{β}	W
Bzd[1] H9	Bzd[1] H^{α}	W
	C10 H1	M
Leu[2] NH	Leu[2] H^{α}	M
	Leu[2] H^{β}	M
	C10 H1	S
	C10 H2	S
D-Phe[3] NH	D-Phe[3] H^{α}	M
	D-Phe[3] $H^{\beta 1}$	M
	D-Phe[3] $H^{\beta 2}$	M
	Leu[2] H^{α}	M
D-Phe[3] H^{α}	D-Phe[3] $H^{\beta 1}$	M
	D-Phe[3] $H^{\beta 2}$	M
	Pro[4] H^{δ}	M
	Pro[4] H^{δ}	M
Val[5] NH	Val[5] H^{α}	M

Table 2. Amide Proton Data For 6a And GS In DMSO.

	6			Gramicidin S		
Residue	$\Delta\delta/\Delta T$	$J^{N\alpha}$	Possible ϕ	$\Delta\delta/\Delta T$	$J^{N\alpha}$	Possible ϕ
BZD	-3.5	Σ=11.5	-130, -60, 60, 130	-	-	-
Leu[2]	-4.8	7.4	-160, -85, 40, 85	-2.8	9.2	-145, -95,
D-Phe[3]	-5.6	5.5	-25, -100, 170, 75	-7.4	2.6	-175, -5, 55, 115
Val[5]	-1.1	8.7	-150, -95, 60	-1.8	9.7	-140, -95,

Table 4. Dihedral Angles Of 6a

Turn	D-Phe[3]		Pro[4]	
	i+1ϕ	i+1ψ	i+2ϕ	i+2ψ
Ideal	60	-120	-80	0
GS	60	-123	-65	-38
low E	75	-159	-68	-34
high E	59	-136	-82	61

Table 5. Antibacterial Activity of GS Analogs
MIC (minimum inhibitory conc) $\mu g/ml$

Organism	GS	26	11c	11a
E. coli (3552)	16	128	>128	>128
S. aureus	4	8	32	128
B. subtilis	2	4	8	64

The four $J^{N\alpha}$s in **6a** (Table 2) suggest possible constrained conformations of the backbone using a Karplus type equation. The $J^{N\alpha}$s are similar to those observed in GS; relatively large values for the Leu^2 and Val^5 constants and a smaller value for D-Phe^3. These similarities suggest similar average conformations in **6a** and GS. The small $J^{N\alpha}$ for D-Phe^3 is diagnostic of a turn as is seen in native GS. The larger $J^{N\alpha}$ values observed for Leu^2 and Val^5 are characteristic of anti parallel β sheets. The most likely φ values based on the similarity to the GS crystal structure are underlined in Table 2.

NOE data for **6a** contained enough information to make sequential assignments (see Table 3). There were no transannular NOEs observed. Conclusive information about a turn in the D-Phe^3-Pro^4 region is also present in the data. The NOEs observable in a particular turn depend on turn type. A Type I or I' turn will have a strong NOE between the i+1 amide proton and the i+2 amide proton. A Type II or II' turn will have a strong NOE between the i+1 H^{α} proton and the i+2 amide proton (see Figure 2). An NOE is observed between the D-Phe^3 H^{α} proton and each of the Pro^4 H^{δ} protons. The H^{δ} protons correspond to the amide proton in Figure 4, indicating a Type II' turn.

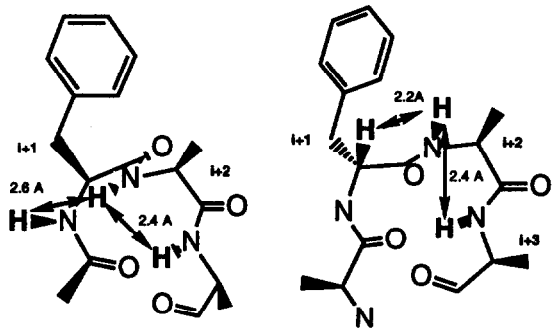
The 21 NOEs listed in Reference 1,Table 3 and the intramolecular hydrogen bond present between the Val^5 NH and the Leu^2 carbonyl were used as input constraints for the DGEOM[3] program. One hundred structures were generated, and a manual sorting of the D-Phe^3 and Pro^4 φ and ψ values for those within 30° of an ideal β turn found 32 structures consistent with all the available data. These 32 structures clustered into 17 families with similar backbone structure. Energy minimization, 5 ps of molecular dynamics and a second minimization of these structures gave a new clustering into 14 families. The lowest energy structure had an energy of 34.6 Kcal/mol; the highest 54.2 Kcal/mol. The low-energy structure (Figure 3), had dihedral angles for D-Phe^3 and Pro^4 that are similar to those found in the GS crystal structure (Table 4). These angles are consistent with a Type II' β turn conformation.

From NMR data and distance geometry calculations, it appears that **6a** adopts a Type II' β turn in the peptide portion of the compound. In solution, the Leu^2-D-Phe^3-Pro^4-Val^5 portion of **6a** forms a Type II' β turn. The presence of a β turn in the peptide portion of **6a** strongly suggests the BZD group is topologically mimicking a β turn and maintaining the overall native conformation of this cyclic peptide.

Gramicidin S Model

Cyclic octapeptide **6a** demonstrated that the BZD beta turn mimic could presumably mimic the Type II' beta turn found in GS. In designing our initial target for the GS model system two assumptions were made to make the molecules more easily synthesized. First, Lys was substituted for Orn in our target cyclic decapeptide. Based on the known structural activity relationship (SAR) of GS this change is not thought to result in conformational changes for this analog and has only a small effect on the biological activity (1/2 as active). Second, the Leu and Val side chains were not incorporated in the beta turn mimic and are instead substituted with Gly equivalents (R1 = R4 = H). Although the Leu and Val side chains are important for biological activity the presence of a rigid beta turn mimic in the proposed analog may obviate the need for these residues to stabilize the native conformation That is, if the peptidomimetic maintains the beta turn geometry then these side chains may be less critical for structural stability in the BZD containing GS analog. Based on these considerations, we substituted one of the beta turns in GS (Leu-D-Phe-Pro-Val) with the BZD **1** to give the target cyclic decapeptide shown in Figure 1.

The synthesis of the desired benzodiazepine containing cyclic decapeptide GS analogs is outlined in Scheme **2.** The chemistry to synthesize **1** has previously been described[1]. Starting with the appropriate BZD amino acid ester **1a or 1c** the linear sequence was constructed and then cyclized using the azide method as shown in Scheme 2. Finally the Cbz protecting groups are removed using HF/anisole to give the desired GS analogs **11ac.**



Type I β turn **Type II' β turn**

Figure 2. Ideal β turns

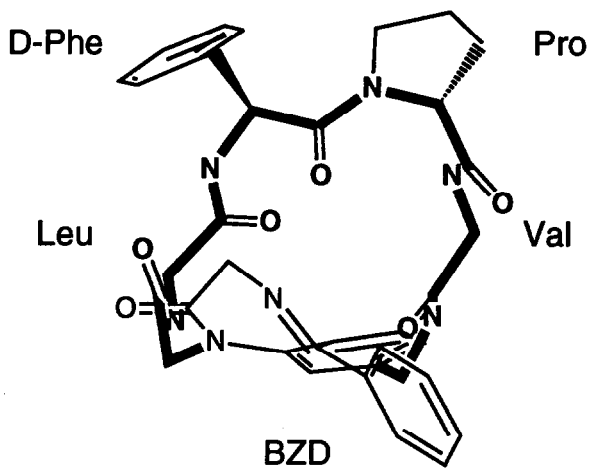
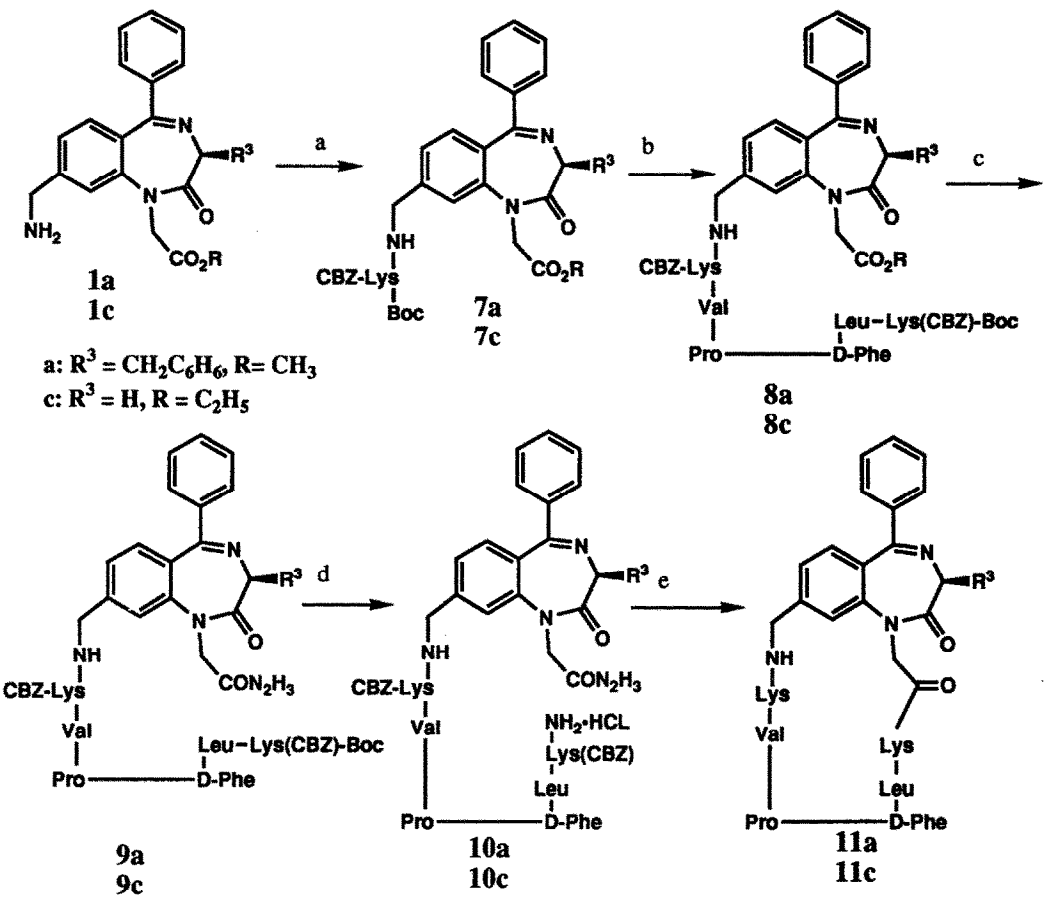


Figure 3. Lowest energy structure of **6**, showing the β turn in the peptide portion. The peptide backbone has been darkened and the Leu, Val, and BZD-3-benzyl side chains have been removed for clarity.

Scheme 2



i. isobutylchloroformate/N-methylmorpholine/Boc-CBZ-Lys/THF/0°C

j. 1. $HCl_{(g)}$, 0°C, 1 hr. 2. N-methylmorpholine/THF 3. couple with next amino acid 4. repeat steps

k. N2H4/MeOH/rt/2 days

l. $HCl_{(g)}$/0°C/60 min

m. 1. DMF/-20°C/HONO 2. Dilute with DMF/DIEA 3. HF/anisole

Conformational Analysis

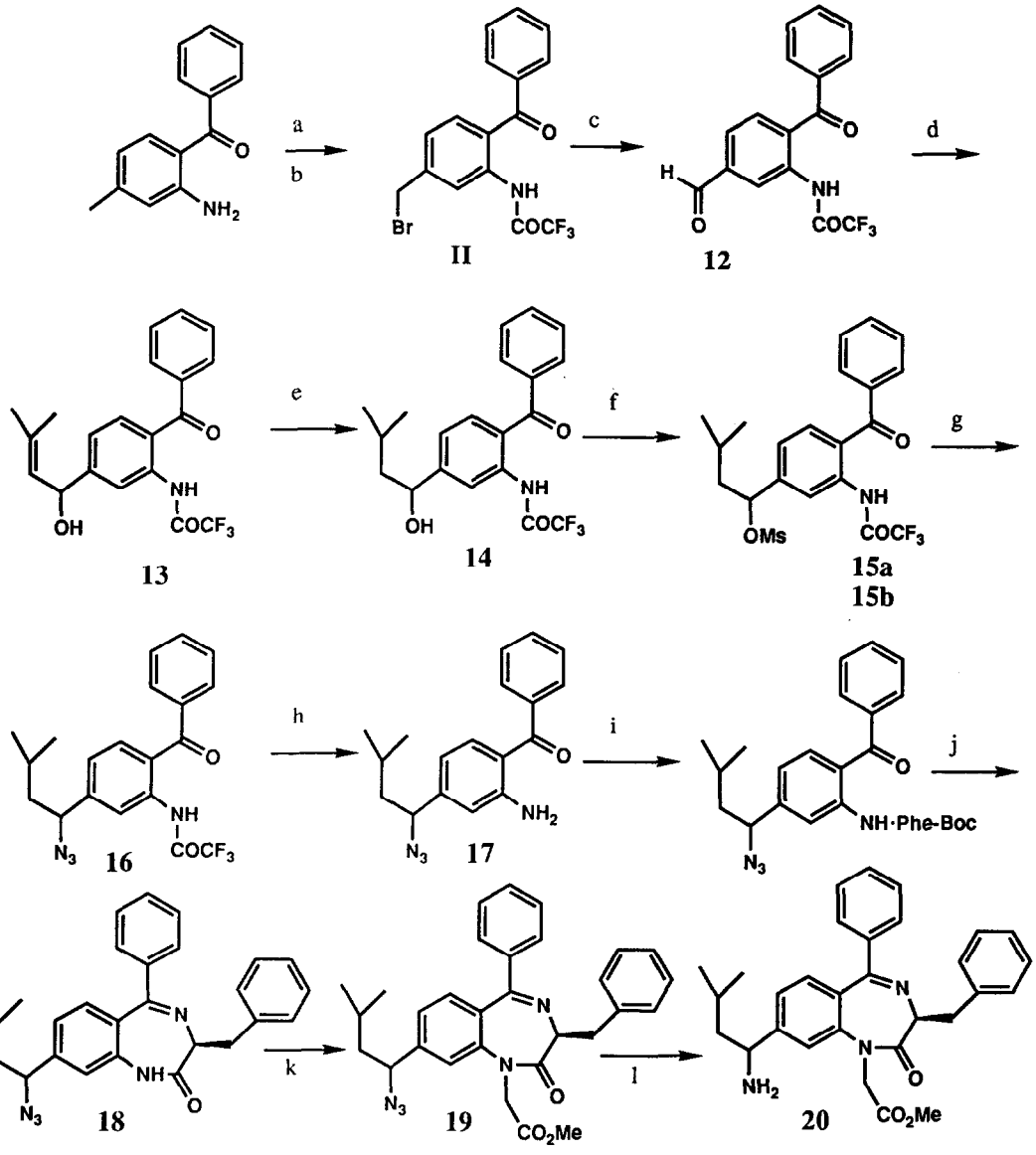
Unfortunately, conformational analysis of the cyclic decapeptides containing the BZD beta turn mimic could not be determined by NMR. Using a variety of different solvents and temperature variations spectra could not be obtained that suggested secondary structure. In all cases the spectra were broad and featureless, possibly indicative of conformational averaging or aggregation. It was determined, however, that **11c** was a mixture of two interconverting conformations (two sets of resonances were observed) whereas **11a** showed only a single conformation (one set of resonances). The two conformations can be attributed to the puckering of the seven-membered BZD ring which could be frozen into one conformation by substitution of a benzyl group at the 3 position of the BZD ring system. This was also observed in the cyclic octapeptide model system.

In an effort to understand the difference in behavior between the cyclic decapeptide GS system and the cyclic octapeptide model systems studied earlier[1] the assumptions made in the design of the initial target **11a** were tested using a molecular mechanics and dynamics approach. Models of GS and of **11a** were subjected to 100-200 ps of molecular dynamic simulations at 600° Kto determine possible conformational differences for the two analogs. The starting structures were energy minimized using molecular mechanics calculations (Amber)[4]. While the BZD/GS **11a** molecule maintained a similar conformation to GS in the molecular mechanics minimizations it was clear after a short time of molecular dynamics (10-20 ps), under conditions that GS remained conformationally fixed in a very deep energy well, **11a** quickly adopted a different conformation. Since the original mimic **11a** did not incorporate the Leu and Val side chains found in GS the effect of these side chains on conformational stability was next examined. Molecular dynamics simulations on Gly-Gly GS suggested that the native GS conformation remained as the lowest energy state in this analog. Molecular dynamics simulations were then carried out on a peptidomimetic analog which incorporated both the Leu and Val side chains in the BZD unit. With these side chains incorporated into the peptidomimetic the conformation of native GS was retained as the lowest energy state. In contrast to our original assumptions these results suggested that the side chains Leu/Val are more important to maintain the desired conformation in the BZD beta turn mimic analog than in the all peptide analog. To determine which, if either, side chain was more important for conformational stability in the beta turn mimetic the simulation was also run in which only the Leu or only the Val side chains were incorporated into the mimic. The results indicated the Leu side chain was more important for maintaining the GS conformation and this suggested the synthesis of an analog of GS which incorporates the Leu side chain in the peptidomimetic as in **26**.

Synthesis Of c(K-L-dF-P-V-BZD) **26**

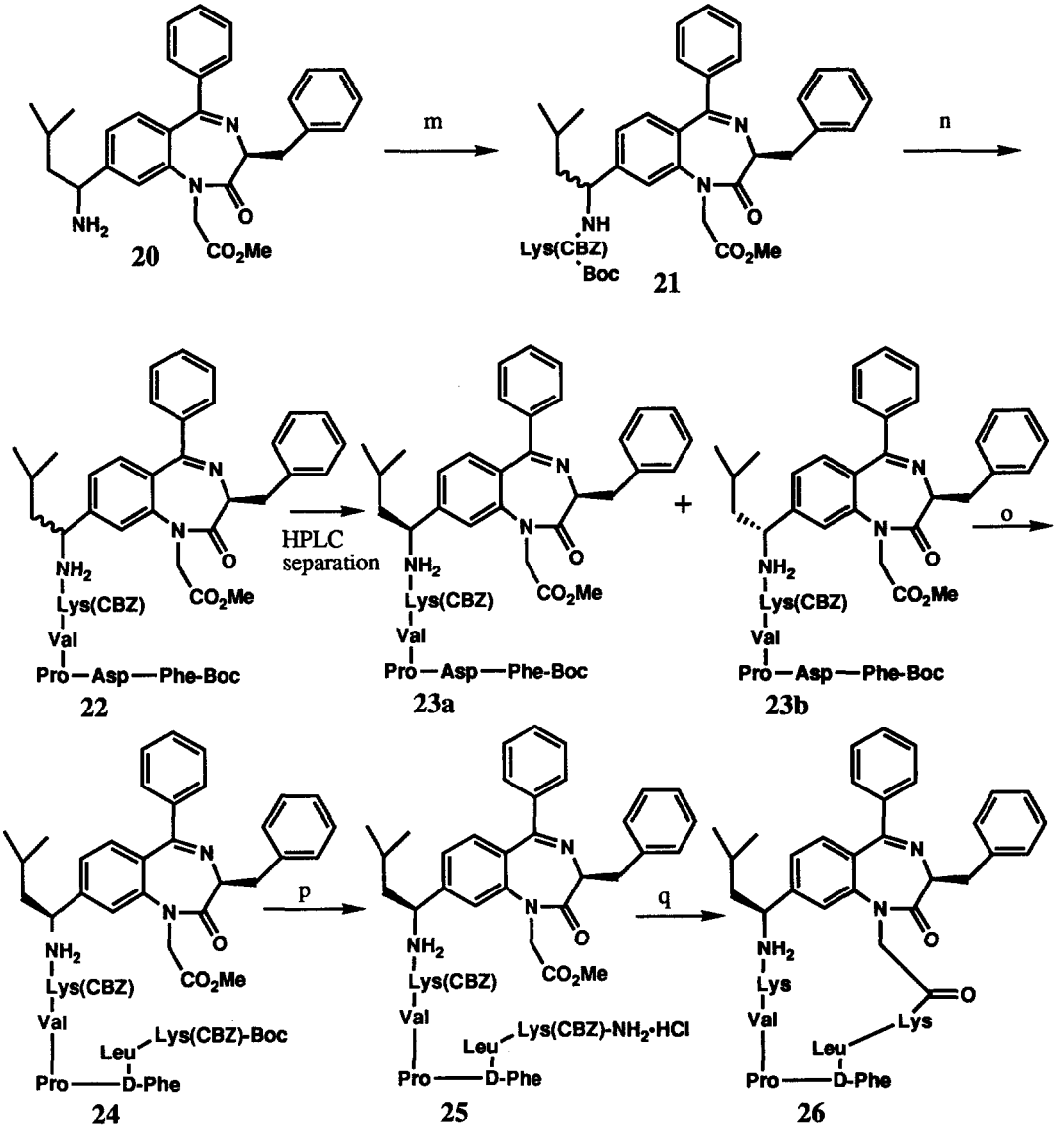
The synthesis of the Leu side chain containing peptidomimetic is outlined in Schemes **3** and **4**. The benzyl bromide **II**[1] is oxidized with DMSO to the aldehyde **12** in about 60% yield. It was necessary to use the vinyl Grignard to incorporate the needed isobutyl side chain since the saturated Grignard gave only reduction of the aldehyde to the alcohol. The lithium reagent did not selectively add to the aldehyde and gave a mixture of products derived from attack of the ketone. Thus, addition of 2,2-dimethylvinyl magnesium bromide to the aldehyde **12** gave the allylic alcohol **13**. The hydrogenation of the double bond without hydrogenolysis was best accomplished with Pt catalyst to give the alcohol **14**. This alcohol is converted to the mesylate **15** and treated with sodium azide to give the azide **16**. The rest of the synthesis is the same as that previously described for **1**[1]. The amino acid ester **20** is a mixture of 2 diastereomers which could be separated by HPLC but the separation was not convenient on a large scale at this point. The mixture of diastereomeric amines **20** was used to construct the linear sequence. As the linear sequence was built up the separation of the diastereomers by HPLC became gradually more convenient and reached a maximum after the coupling with D-Phe. At this point the two diastereomers could easily

Scheme 3



a. $(CF_3CO)_2/CH_2Cl_2/0^oC$. b. NBS/CCl_4/reflux c. DMSO/AgOTs d. 2,2-Dimethylvinyl-MgBr/THF
e. PtO_2/H_2 f. MsCl/Et_3N/CH_2Cl_2 g. NaN_3/DMF/RT h. K_2CO_3/Aq. dioxane/reflux/2 hrs
i. N-methylmorpholine/isobutylchloroformate/Boc-Phe/rt/18 hrs
j. 1. $HCl_{(g)}$/0oC/30 min. 2. MeOH/1N NaOH/r.t. k. 1. NaH/DMF/rt. 2. $BrCH_2CO_2Me$
l. 10% Pd/C/EtOH

Scheme 4



m. isobutylchloroformate/N-methylmorpholine/Boc-CBZ-Lys/THF/0°C

n. 1. $HCl_{(g)}$, 0°C, 1 hr. 2. N-methylmorpholine/THF 3. couple with next amino acid 4. repeat steps

o. 1. couple Leu and Lys 2. N2H4/MeOH/rt/2 days

p. $HCl_{(g)}$/0°C/90 min

q. 1. DMF/-20°C/HONO 2. Dilute with DMF/DIEA 3. HPLC 4. HF/anisole

be separated by HPLC to give **23a** and **23b**. The linear sequence was completed for each of the two diastereomers and the standard cyclization conditions were attempted. One of the diastereomers does not cyclize at all and gives a mixture of products that could not be identified. The other diastereomer, presumably the one with the correct stereochemistry, cyclized very cleanly to give the desired GS analog **26**.

Analysis And Biological Activity Of GS Analogs

Structural analysis by NMR of **26** proved as difficult as earlier analogs. With the analogs investigated to date GS does not appear to be a straight-foward physical model system for the analysis of beta turn mimetics. Fortunately, GS is biologically active and offers the opportunity to compare activities of appropriate analogs. If the BZD mimic is functioning as a beta turn in GS, as it appears to be in other systems we have investigated, these compounds should possess some of the antibacterial biological activity of the parent GS. To test this we have measured the antibacterial activity of **11a**, **11c** and **26** in various assays and the data is summarized in Table 8. The analog containing the simplest mimic **11a**, which we know from NMR analysis exists in two interconverting conformations, is only very weakly active. Considering that it does not contain the Leu and Val side chains that are known to be important for biological activity its weak activity is not too surprising. The more conformationally well defined analog, **11c** shows significantly more activity. Finally, analog **26**, the one that more closely resembles GS by having incorporated the Leu side chain found in GS, has about 50% the activity of GS. Since the Lys for Orn substitution is known to reduce the activity of GS in half, it can be concluded that **26** is an excellent mimic of GS.

Conclusion

We have shown that the benzodiazepine (BZD) scaffold is a synthetically accessible, general mimic of protein and peptide beta turns and effectively reproduces both the geometry of the peptide turn and the positioning of the side chain functionality. Evaluation of this system in cyclic octapeptide systems shows that it is able to function as a beta turn mimic and maintain the conformation of the native cyclic peptide. We have now demonstrated that when incorporated into a biologically active peptide with known beta turns the BZD beta turn mimic results in a peptidomimetic with the equivalent biological activity of a close analog of the native peptide. Physical and biological models of BZD peptidomimetics support the role of this molecular unit as an effective beta turn replacement.

Acknowledgements

The authors would like to thank Shaun Knapp, Francis Farrari, David Eustice and Kathleen Reilly for technical assistance. Dr. David Spellmeyer provided valuable consultation on the use of SPASMS.

Experimental Section

NMR methods and Molecular Dynamics

All NMR experiments for conformational analysis were performed at 499.8 MHz on a Varian VXR-500S spectrometer. NMR samples were prepared under dry nitrogen gas in 0.8 ml of DMSO-*d6* or $CDCl_3$ (Merck). TMS was used as an internal reference. A 1.5 second relaxation delay was used in all experiments.

One dimensional FT experiments were collected with 16384 complex points. For variable temperature experiments, the sample was allowed to equilibrate in the probe for 30 minutes before data collection.

DQF-COSY [5, 6], TOCSY [7], NOESY [8] and ROESY [9, 10] two-dimensional spectra were recorded. TOCSY data sets were recorded using the MLEV-17 sequence to generate spin locking times of 35 to 70 ms with 1 ms trim pulses. NOESY and ROESY spectra were recorded at 100 and 200 ms.

ROESY spectra were recorded with a 4.0 KHz spin locking field using the method of Kessler [11]

All two-dimensional experiments were obtained with spectral widths of at least 6500 Hz in both dimensions. 2048 complex points were collected in t_2 and 256 complex FIDs were collected in t_1. 32 or 64 transients were co-added for each FID. All spectra were acquired in the phase-sensitive absorption mode with quadrature detection in both dimensions [12, 13].

Two-dimensional data sets were processed using the FTNMR program (Hare Research Inc.). Submatrix files were 1024 by 1024 points in size. All t_2 time domain transforms were weighted with a sine bell, shifted 45 degrees, apodization function. NOESY, ROESY and TOCSY t_1 interferograms were apodized with a sine bell, shifted 60 degrees, function and then zero-filled to 1024 points. DQF-COSY t_1 interferograms were weighted by a sine bell function before zero-filling to 1024 points. After the t_2 Fourier transform, the first point in each t_1 transform was multiplied by 0.5 to eliminate t_1 ridge artifacts [14].

NOE distances from NOESY spectra were estimated by comparing the number of contour levels displayed for crosspeaks to those observed in the crosspeaks between the Pro H^{δ} protons. These methylene protons are separated by a distance of 1.8 Å. Intensities were ranked as strong, medium, or weak using this comparison. For distance geometry calculations upper bounds were assigned as follows: **strong**- 2.5 Å, **medium**- 3.0 Å, **weak**- 4 Å. Lower bounds were set to van der Waals radii.

Distance geometry calculations were performed using the DGEOM program[17]. Subsequent energy minimization and molecular dynamics were performed using the SPASMS program[15]. One hundred structures were generated during the initial distance geometry calculations. The structures were manually sorted so that the D-Phe[3] and Pro[4] ϕ and ψ values were within 30° of an ideal β turn. The sorted structures were clustered by backbone backbone and the parent BZD group atoms. One structure from each cluster was minimized, then subjected to 5 ps of molecular dynamics and finally minimized again. Temperatures used in the dynamics runs were 300°K with a dielectric constant of 1.00.

General Methods.

All reactions were conducted under a dry nitrogen atmosphere except when noted otherwise. Commercial dry Tetrahydrofuran (THF), dimethylsulfoxide (DMSO), and dimethylformamide (DMF) were obtained from Aldrich in Sure Seal bottles. All reactions were followed by thin layer chromatography (TLC) using silica gel 60 /F_{254} glass-backed plates (E. Merck). Unless otherwise noted, all chromatography refers to medium pressure liquid chromatography (MPLC) using columns packed with EM Reagents silica gel 60 (0.040-0.063 mm particle size, 230-400 mesh). Melting points (Thomas Hoover apparatus, open capillary) are uncorrected. All ^{1}H NMR spectra were determined at 300 MHz in $CDCl_3$ and chemical shifts are reported in parts per million down field from Me_4Si. Significant ^{1}H NMR data are tabulated in order: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; b, broad), number of protons, and coupling constant(s). IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer.

Cyclo-[Leu-D-Phe-Pro-Val-BZD] 6a: A solution of N-Boc-Val (1.62g , 0.0074 mol) in THF is cooled in a salt-ice bath to -15° C and treated with 0.76g (0.0074 mol) of N-Methylmorpholine. Isobutyl chloroformate (1.02 g, 0.0074 mol) is slowly added to the mixture. The solution is stirred for 5 min. before 1.6 g (0.0037 mol) of the amino-BZD **1a** is added. The solution is stirred at -15° C for 20 min., diluted with 100 mL of cold water and extracted into methylene chloride (2 X 100 mL). The extracts are washed with water, 0.1 N NaOH, brine, and finally dried. The solvent is evaporated to give a white solid which is dissolved in a minimum amount of CH_2Cl_2 and chromatographed (ethyl acetate elution) to give 2.1 g (0.0034 mol) of Boc-Val-BZD **2a** as a white foam.

The Boc-Val-BZD **2a** obtained above (2.1g, 0.0034 mol) was dissolved in 50 mL of methylene chloride cooled to 0°C and treated with $HCl_{(g)}$ for 1 hour. The solvents were evaporated under reduced pressure at room temperature to give a solid. The solid was dissolved in THF and made slightly basic with

N-methylmorpholine. This solution was then added to a solution of activated Boc-Pro (1.44 g, 0.0067 mol). The activation and work-up procedure is the same as that describe above for **2a**. The Boc-Pro-Val-BZD product is used without further purification.

Starting with the crude Boc-Pro-Val-BZD obtained above and 1.78 g (0.0067 mol) of Boc-D-Phe the same deprotection, coupling, and work-up procedure described above is followed to give Boc-D-Phe-Pro-Val-BZD as a white foam.

Starting with the crude Boc-D-Phe-Pro-Val-BZD obtained above and 1.66 (0.0067 mol) of Boc-Leu-H_2O the same deprotection, coupling, and work-up procedure described above is repeated. Purification by chromatography (ethyl acetate) gives 2.4 g (0.0024 mol) of Boc-Leu-D-Phe-Pro-Val-BZD**3a** as a white foam: mp 125-127°C, $[\alpha]^{25}_D$ = -9.70° (c = 0.608, MeOH), MS (DCI - NH_3) $(M+H)^+$ 984. FAB-MS $(M+H)^+$ 984.82.

A solution of **3a** in 20 mL of MeOH is treated with 0.64g of anhydrous hydrazine and stirred at room temperature for 30 hr.. The solvent is removed under vacuum to give 1.35 g of hydrazide **4a** as a white solid. This is chromatographed (2 % MeOH/$CHCl_3$) to give 0.75 g of white solid. mp 118-9°C MS (DCI - NH_3) $(M+H)^+$ 984

The solid (0.26 g) is dissolved in ethyl acetate (75 mL), cooled to 0° C and treated with $HCl_{(g)}$ for 1 hr. Evaporation at room temperature under vacuum gives a white solid which is dissolved in 12 mL of DMF, transferred to a 1 L flask and cooled to - 20 ° C. To this solution is added 0.2 mL of conc. aq. HCl and 0.1 mL of 5 M aq $NaNO_2$. The solution is stirred at - 20 °C for 20 min. and then diluted with 500 mL of cold DMF (- 20 °C) and made basic with diisopropylethylamine (1 mL). The solution is allowed to warm to room temperature and is stirred overnight. The solvent is removed under vacuum (pump) on a rotary evaporator while warming at 50 °C. The residue is dissolved in chloroform and chromatographed (5% MeOH/$CHCl_3$) to give 0.10 g (0.00012 mol) of **6a** as a white foam: mp >240°C, $[\alpha]^{25}_D$ =+38.31° (c = 0.402, MeOH), MS (DCI - NH_3) $(M+H)^+$ 852, FAB-MS $(M+H)^+$ 852.44.

Cyclo-{Leu-D-Phe-Pro-Val-BZD(3-Benzyl)} ***6b****:* A solution of N-Boc-Val (1.3g , 0.0059 mol) in THF is cooled in a salt-ice bath to -15° C and treated with 0.6g (0.006 mol) of N-Methylmorpholine. Isobutyl chloroformate (1.3 g, 0.0059 mol) is slowly added to the mixture. The solution is stirred for 5 min. before 1.0 g (0.0029 mol) of the amino-BZD **1b** is added. The solution is stirred at -15° C for 20 min., diluted with 100 mL of cold water and extracted into methylene chloride (2 X 100 mL). The extracts are washed with water, 0.1 N NaOH, brine, and finally dried. The solvent is evaporated to give a white solid which is dissolved in a minimum amount of CH_2Cl_2 and chromatographed (50% EtOAc/Hexane-70% EtOAc/Hexane elution) to give 0.7 g (0.0013 mol) of Boc-Val-BZD **2b** as a white foam.

The Boc-Val-BZD **2b** obtained above (0.7g, 0.0013 mol) was dissolved in 50 mL of methylene chloride cooled to 0°C and treated with $HCl_{(g)}$ for 1 hour. The solvents were evaporated under reduced pressure at room temperature to give a solid. The solid was dissolved in THF and made slightly basic with N-methylmorpholine. This solution was then added to a solution of activated Boc-Pro (0.56 g, 0.0026 mol). The activation and work-up procedure is the same as that describe above for **2b**. The Boc-Pro-Val-BZD product is used without further purification.

Starting with the crude Boc-Pro-Val-BZD obtained above and 0.69g (0.0026 mol) of Boc-D-Phe the same deprotection, coupling, and work-up procedure described above is followed to give Boc-D-Phe-Pro-Val-BZD as a white foam.

Starting with the crude Boc-D-Phe-Pro-Val-BZD obtained above and 0.65 (0.0026 mol) of Boc-Leu-H_2O the same deprotection, coupling, and work-up procedure described above is repeated to give Boc-Leu-D-Phe-Pro-Val-BZD as a white foam. This was used without further purification.

A solution of **3b** in 20 mL of MeOH is treated with 0.64g of anhydrous hydrazine and stirred at room temperature for 30 hr.. The solvent is removed under vacuum to give a white solid. This is chromatographed (0-5 % MeOH/$CHCl_3$ gradient) to give 0.46 g of white solid.

The solid (0.46 g, 0.5 mmol) is dissolved in methylene chloride (200 mL), cooled to 0° C and treated with $HCl_{(g)}$ for 1 hr. Evaporation at room temperature under vacuum gives a white solid which is dissolved in 12 mL of DMF, transferred to a 1 L flask and cooled to - 20 ° C. To this solution is added 0.2 mL of conc. aq. HCl and 0.1 mL of 5 M aq $NaNO_2$. The solution is stirred at - 20 °C for 20 min. and then diluted with 300 mL of cold DMF (- 20 °C) and made basic with diisopropylethylamine (1 mL). The solution is allowed to warm to room temperature and is stirred overnight. The solvent is removed under vacuum (pump) on a rotary evaporator while warming at 50 °C. The residue is dissolved in chloroform and chromatographed (1% MeOH/$CHCl_3$) to give 0.20 g (0.00026 mol) of **6b** as a white foam: mp 208-210°C, $[\alpha]^{25}_D$ = -14.53° (c = 0.592, MeOH), MS (DCI - NH_3) $(M+H)^+$ 762.5, FAB-MS $(M+H)^+$ 762.40, HRMS calc for $C_{43}H_{51}N_7O_6$ 761.3901, found 761.3927.

Synthesis of **12**: Silver tosylate (25 g, 0.090 mol) is dissolved in 90 mL of DMSO in a flame-dried RB flask under nitrogen. Then 20 g (0.052 mol) of the benzyl bromide **II** is added . A precipitate forms immediately and the resulting mixture is stirred at room temperature for 1 hour. Excess triethylamine (50 mL) is added while cooling in a water bath, stirred for 1 hour, and is filtered through a pad of Celite. The filtrate is diluted with water and brine and extracted several times into ethyl acetate (2 L total volume). The extracts are dried and the solvent evaporated to give a dark residue. This is chromatographed (30% ethyl acetate/hexane to 50% ethyl acetate/hexane) to give 11 g (0.034 mol) of aldehyde **31** as a yellow solid:. mp 106-108 °C, IR ($CDCl_3$ cm^{-1}) ; NMR ($CDCl_3$) δ 11.84 (bs, 1 H), 10.14 (s, 1 H), 9.12 (d, 1 H, 1.1 Hz), 7.8-7.6 (m, 5 H), 7.55 (t, 2 H, 7.7 Hz); MS (DCI CH_4) $(M+H)^+$ 322 (3%), $(M+NH_4)^+$ 339.0 (100%), HRMS calc for $C_{16}H_{10}NO_3F_3$ 321.0613, found M^+ 321.0612.

Synthesis of **13**: A solution of aldehyde **12** (11 g, 0.034 mol) in THF is cooled to 0 °C. To this is added 40 mL (0.080 mol) of 2 M solution of the Grignard prepared from 27 g (0.20 mol) of 1-bromo-2-methyl-propene and 5 g (0.21 mol) of Mg in 100 mL of THF. The mixture is stirred at 0 C for 30 min.. The solution is then diluted with 200 mL of water, neutralized with 1 N HCl and extracted into ethyl acetate. The extracts are washed with water and brine, dried and evaporated to give a yellow residue. This is chromatographed (25% ethyl acetate/hexane) to give 6.8 g (0.018 mol) of the allylic alcohol **13** as a solid: mp 118-119 °C, IR ($CDCl_3$ cm^{-1}) ; NMR ($CDCl_3$) δ 12.20 (bs, 1 H), 8.62 (d, 1 H, 1.5 Hz), 7.71-7.60 (m, 4 H), 7.51 (t, 2 H, 7.3 Hz), 7.32 (dd, 1 H, 1.5, 7.3 Hz), 5.57 (dd, 1 H, 8.8, 3.5 Hz), 5.36 (dd, 1 H, 9.8, 1.4 Hz), 1.90 (d, 1 H, 1.4 Hz), 1.88 (d, 3 H, 1.4 Hz), 1.78 (d, 3 H, 1.1 Hz); MS (DCI NH_3) $(M+NH_4)^+$ 395, $(M+NH_4-H_2O)^+$ 377,. $(M+H-H_2O)^+$ 360.

Synthesis of **14**: A solution of 3.0 g (0.0079 mol) of allylic alcohol **13** in ethanol was hydrogenated with Pt catalyst (from 0.8 g of PtO_2 that has been recovered from previous hydrogenations -note: previously used PtO_2 gives best results) at 45 psi for 1 hour. The solution is decanted and the catalyst is rinsed with ethanol. The solvent is evaporated to give 3.0 g (0.079 mol) of the benzyl alcohol **14** as a light yellow gum that is be used without further purification. NMR ($CDCl_3$) δ 12.20 (bs, 1 H), 8.60 (d, 1 H, 1.5 Hz), 7.71-7.58 (m, 4 H), 7.50 (t, 2 H, 7.5 Hz), 7.28 (dd, 1 H, 7.5, 1.5 Hz), 4.86 (m, 1 H), 2.22 (d, 1 H, OH, 1.5 Hz), 1.80 (m 2 H), 1.5 (m, 1 H), 1.96 (m, 6 H); MS (DCI CH_4) $(M+H)^+$ = 380, HRMS (M+) Calc for $C_{20}H_{20}F_3NO_3$: 379.1395, found: 379.1395

Synthesis of **16**: A solution of alcohol **14** (10.0 g, 0.026 mol) in methylene chloride is cooled to 0 °C in an ice bath and treated with triethylamine (5.9 g, 0.058 mol) and methanesulfonyl chloride (6.6 g, 0.058 mol) and stirred at 0 °C for 3 hours. The mixture is washed with water, sat'd $NaHCO_3$, and brine, dried and evaporated at room temperature to give an light yellow residue. The residue is dissolved in DMF and treated with 3.8 g (0.058 mol) of NaN_3 and stirred overnight at room temperature. The solution is diluted with water and extracted into ethyl acetate. The extracts are washed with water and brine, dried and evaporated to give 10.5 g (0.026 mol) of azide **16** as light yellow oil: IR (thin film cm^{-1}) 3582, 2959, 2933, 2872, 2102, 1735, 1639, 1614, 1579, 1535, 1465, 1446, 1426, 1319, 1267, 1202, 1154, NMR

($CDCl_3$) δ 12.20 (bs, 1 H), 8.63 (d, 1 H, 1.5 Hz), 7.71-7.58 (m, 4 H), 7.55 (t, 2 H, 7.5 Hz), 7.28 (dd, 1 H, 7.5, 1.2 Hz), 4.60 (m, 1 H), , 1.80 (m 2 H), 1.58 (m, 1 H), 1.96 (m, 6 H); MS (DCI CH_4) $(M+H)^+$ 405

Synthesis of **17**: To a solution of azide **16** (10.5 g, 0.026 mol) in 300 mL of dioxane is added an aqueous solution of K_2CO_3 (35 g in 75 mL of water) and heated to reflux for 4 hours. The solution is cooled to room temperature, diluted with 500 mL of water and extracted into ethyl acetate. The extracted is washed with water, and brine, dried and evaporated to give 10 g of dark residue. This is chromatographed (10% ethyl acetate/hexane) to give 5.5 g (0.018 mol) of azide amine **17** as a yellow oil: NMR ($CDCl_3$) δ 7.63 (d, 2 H, 7.5 Hz), 7.48 (m, 4 H), 6.66 (d, 1 H, 1.5 Hz), 6.51 (dd, 1 H, 7.5 , 1.5 Hz), 6.2 (bs, 2 H), 4.38 (m, 1 H), 1.70 (m, 2 H), 1.55 (m, 1 H), 0.92 (m, 6 H)

Benzodiazepine **18**: A solution of N-Boc-Phe (7.95 g , 0.03 mol) in THF is cooled in a salt-ice bath to -15° C and treated with 3 g (0.03 mol) of N-Methylmorpholine. Isobutyl chloroformate (4.0 g, 0.03 mol) is then slowly added to the mixture. The solution is stirred for 5 mins and then 5.0g (0.016 mol) of the azide-aminobenzophenone **17** is added. The mixture is allowed to warm to room temperature and stirred overnight. The solution is diluted with water and extracted into methylene chloride. The organic extract is washed with water, 0.1 N NaOH, brine, and dried over $MgSO_4$. The solvent is evaporated to give 8.9 g of a thick yellow oil. The oil is dissolved in ethyl acetate (500 mL) and cooled to 0° C and $HCl_{(g)}$ is bubbled into the solution for a period of 1 hour. The solvent is evaporated under vacuum at room temperature to give a solid. The solid is dissolved in MeOH (100 mL) and made slightly basic (pH 8) by the addition of 1 N NaOH and stirred overnight at room temperature. Most of the solvent is evaporated and the residue is extracted into methylene chloride. The extract is washed with brine and dried. The solvent is evaporated to give 6.5 g (0.016 mol) of the benzodiazepine **18** as a foam. This was used without further purification. A small amount was chromatographed on silica gel (15% EtOAc/Hex - 50% EtOAc/Hex gradient) to give a light yellow solid: mp 154-155 °C, $[\alpha]^{25}_D$ = +36.26 ° (c = 0.604, $CHCl_3$), NMR ($CDCl_3$) gives two sets of resonances: δ 8.23 (s, 1 H), 7.48-7.22 (m, 10 H), 7.06 (m, 2 H), 4.52 (m, 1 H), 3.81 (t, 1 H, 7 Hz), 3.59 (d, 2 H, 7 Hz), 1.72 (m, 2 H), 1.56 (m, 1 H), 0.98 (m, 6 H), MS (DCI-NH3) $(M+H)^+$ 438.2 , HRMS calc for $C_{27}H_{27}N_5O_1$ 437.2216, found M^+ 437.2229

Synthesis of **19**: Under a blanket of nitrogen, sodium hydride (0.46 g, 0.012 mol of a 60% dispersion in oil) is washed several times with hexane and decanted. To a slurry of the washed NaH in dry DMF (25 mL) is added in small portions 4.37g (0.01 mol) of BZD **18** and stirred at room temperature for 30 min.. Then 1.85 g (0.012 mol) of methyl bromoacetate is added dropwise and allowed to stir at room temperature for 1 hour. The solution is diluted with 200 mL of water and extracted into methylene chloride. The organic extract is washed with water, and brine and then dried. The solvent was evaporated at room temp to give 4.0 g (0.0078 mol) of azido-methyl ester **19** as a light yellow foam which was used without further purification. A small amount was chromatographed on silica gel (50% EtOAc/Hex) to give a solid: mp 57-58 °C, $[\alpha]^{25}_D$ = +64.03 ° (c = 0.620, $CHCl_3$), NMR ($CDCl_3$) δ 7.57 (d, 2 H, 7.1 Hz), 7.48-7.08 (m, 11 H), 4.61 (m, 2 H), 3.88 (m, 1 H), 3.68 (s 3 H), 3.60 (m, 2 H), 1.75 (m, 2 H), 1.51 (m, 1 H), 0.99-0.95 (m, 6 H), MS (DCI-NH3) $(M+H)^+$ 510.2 , HRMS calc for $C_{30}H_{31}N_5O_3$ 509.2427, found M^+ 509.2438

Synthesis of **20**: A solution of crude azido-BZD **19** (2.6 g, 0.0057 mol) obtained above in THF (75 mL) is hydrogenated at 45 psi in a Parr Hydrogenator for 5 hr with 1 g of 10% Pd/C. The solution is filtered through Celite and the filtrate is concentrated at room temp. The resulting solid is dissolved in 1 N aq HCl (150 mL) and washed with ethyl acetate. The acid solution is cooled in an ice bath and made basic with the dropwise addition of 50% aq. NaOH. The resulting solid is extracted into ethyl acetate (2 X 200 mL) and washed with water and brine. After drying the solvent is evaporated to give 3 g (0.0062 mol) of the amino ester **20** as a white foam: mp 74-75 °C, $[\alpha]^{25}_D$ = +62.67 ° (c = 0.602,

$CHCl_3$), NMR ($CDCl_3$) d 7.58 (d, 7.5 Hz), 7.50-7.10 (m, 11 H), 4.62 (m, 2 H, 2 overlapping AB quartets), 4.05 (m, 1 H), 3.88 (m, 1 H), 3.62 (ds, 3 H), 3.60 (m, 2 H), 1.70-1.40 (m, 3 H), 0.95 (m, 6 H), MS (DCI-NH3) $(M+H)^+$ 484 , HRMS calc for $C_{30}H_{33}N_3O_3$ 483.2522, found M^+ 483.2524

GRAMICIDIN S ANALOGS 11a, 11c, and 26.

Gramicidin S analog 11a: A solution of α-N-Boc-ε–N-Cbz-Lys-DCHA (4.2g , 0.0075 mol) in THF is cooled in a salt-ice bath to -15° C and treated with 0.76g (0.0075 mol) of N-Methylmorpholine. Isobutyl chloroformate (1.02 g, 0.0075 mol) is slowly added to the mixture. The solution is stirred for 5 min. before 1.6 g (0.0037 mol) of the amino-BZD **1a** is added. The solution is stirred at -15° C for 20 min., diluted with 100 mL of cold water and extracted into methylene chloride (2 X 100 mL). The extracts are washed with water, 0.1 N NaOH, 1 N HCl, brine, and finally dried. The solvent is evaporated to give a white solid which is dissolved in a minimum amount of CH_2Cl_2 and chromatographed (75% EtOAc/ Hexane) to give 2.1 g (0.0026 mol) of α-N-Boc-ε–N-Cbz-Lys-(3-benzyl-BZD) **7a** as a white foam.

The α-N-Boc-ε–N-Cbz-Lys-(3-benzyl-BZD) **7a** obtained above (1.87g, 0.0024 mol) was dissolved in 50 mL of methylene chloride cooled to 0°C and treated with $HCl_{(g)}$ for 1 hour. The solvents were evaporated under reduced pressure at room temperature to give a solid. The solid was dissolved in THF and made slightly basic with N-methylmorpholine.

A solution of N-Boc-Val (1.03g , 0.0047 mol) in THF is cooled in a salt-ice bath to -15° C and treated with 0.5g (0.005 mol) of N-Methylmorpholine. Isobutyl chloroformate (0.64 g, 0.0047 mol) is slowly added to the mixture. The solution is stirred for 5 min. before the NH_2-ε-N-Cbz-Lys-(3-benzyl-BZD) solution prepared above is added. The solution is stirred at -15° C for 20 min., diluted with 100 mL of cold water and extracted into methylene chloride (2 X 100 mL). The extracts are washed with water, 0.1 N NaOH, brine, and finally dried. The solvent is evaporated to give N-Boc-Val-ε-N-Cbz-Lys-(3-benzyl-BZD) as a white solid which is used without further purification.

The crude N-Boc-Val-ε-N-Cbz-Lys-(3-benzyl-BZD) obtained above was dissolved in 50 mL of methylene chloride cooled to 0°C and treated with $HCl_{(g)}$ for 1 hour. The solvents were evaporated under reduced pressure at room temperature to give a solid. The solid was dissolved in THF and made slightly basic with N-methylmorpholine. This solution was then added to a solution of activated Boc-Pro (1.01 g, 0.0047 mol). The activation and work-up procedure is the same as that describe above. The Boc-Pro-Val-ε-N-Cbz-Lys-(3-benzyl-BZD) product is used without further purification.

Starting with the crude Boc-Pro-Val-ε-N-Cbz-Lys-(3-benzyl-BZD) obtained above and 1.27g (0.0047 mol) of Boc-D-Phe, the same deprotection, coupling, and work-up procedure described above is followed to give a white solid. This was chromatographed on silica gel (EtOAc elution) to give 1.7 g (0.0015 mol) of Boc-D-Phe-Pro-Val-ε-N-Cbz-Lys-(3-benzyl-BZD) as a white foam.

Starting with the Boc-D-Phe-Pro-Val-ε-N-Cbz-Lys-(3-benzyl-BZD) obtained above and 0.76 (0.003 mol) of Boc-Leu-H_2O the same deprotection, coupling, and work-up procedure described above is repeated to give Boc-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-(3-benzyl-BZD) as a white foam. This was used without further purification.

Starting with the Boc-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-(3-benzyl-BZD) obtained above and 1.68 (0.003 mol) of α-N-Boc-ε–N-Cbz-Lys-DCHA, the same deprotection, coupling, and work-up procedure described above is repeated to give α-N-Boc-ε–N-Cbz-Lys-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-(3-benzyl-BZD) as a white foam. This was used without further purification.

A solution of the above α-N-Boc-ε–N-Cbz-Lys-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-(3-benzyl-BZD) in 20 mL of MeOH is treated with 0.8 g of anhydrous hydrazine and stirred at room temperature for 30 hr.. The solvent is removed under vacuum to give a white solid. This is chromatographed (5 % MeOH/$CHCl_3$) to give 2.0 g of white solid. The solid (1.1 g, 0.7 mmol) is dissolved in methylene chloride (200 mL), cooled to 0° C and treated with $HCl_{(g)}$ for 1 hr. Evaporation at room temperature

under vacuum gives a white solid which is dissolved in 12 mL of DMF, transferred to a 1 L flask and cooled to - 20 ° C. To this solution is added 0.3 mL of conc. aq. HCl and 1.4 mL of 1 M aq $NaNO_2$. The solution is stirred at - 20 °C for 20 min. and then diluted with 300 mL of cold DMF (- 20 °C) and made basic with diisopropylethylamine (1 mL). The solution is allowed to warm to room temperature and is stirred overnight. The solvent is removed under vacuum (pump) on a rotary evaporator while warming at 50 °C. The residue is dissolved in chloroform and chromatographed (1% MeOH/$CHCl_3$) to give 0.30 g (0.0002 mol) of *c*-(N-Cbz-Lys-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-(3-benzyl-BZD)) as a white foam: FAB-MS $(M+H)^+$ 1376.7.

The above cyclic peptide is treated at 0 °C with 1 mL of anisole and 20 mL of HF for 30 min. The solution is evaporated under high vacuum and the residual solid is washed on with ether on a filter funnel to give 55 mg of *cyclo*-(Lys-Leu-D-Phe-Pro-Val-Lys-(3-benzyl-BZD)) **11a** as an off-white solid. FAB MS $(M+H)^+$ = 1108.95

Gramicidin S analog 11c: A solution of α-N-Boc-ε–N-Cbz-Lys-DCHA (7.9g , 0.014 mol) in THF is cooled in a salt-ice bath to -15° C and treated with 1.4g (0.014 mol) of N-Methylmorpholine. Isobutyl chloroformate (1.9 g, 0.014 mol) is slowly added to the mixture. The solution is stirred for 5 min. before 1.6 g (0.0037 mol) of the amino-BZD **1c** is added. The solution is stirred at -15° C for 20 min., diluted with 100 mL of cold water and extracted into methylene chloride (2 X 100 mL). The extracts are washed with water, 0.1 N NaOH, 1 N HCl, brine, and finally dried. The solvent is evaporated to give a white solid which is dissolved in a minimum amount of CH_2Cl_2 and chromatographed (50% EtOAc/ Hexane) to give 1.5 g (0.002 mol) of α-N-Boc-ε–N-Cbz-Lys-BZD **7c** as a white foam.

The α-N-Boc-ε–N-Cbz-Lys-BZD **7c** obtained above (1.5g, 0.002 mol) was dissolved in 50 mL of methylene chloride cooled to 0°C and treated with $HCl_{(g)}$ for 1 hour. The solvents were evaporated under reduced pressure at room temperature to give a solid. The solid was dissolved in THF and made slightly basic with N-methylmorpholine.

A solution of N-Boc-Val (0.87g , 0.0040 mol) in THF is cooled in a salt-ice bath to -15° C and treated with 0.8g (0.008 mol) of N-Methylmorpholine. Isobutyl chloroformate (0.54 g, 0.004 mol) is slowly added to the mixture. The solution is stirred for 5 min. before the NH_2-ε-N-Cbz-Lys-BZD solution prepared above is added. The solution is stirred at -15° C for 20 min., diluted with 100 mL of cold water and extracted into methylene chloride (2 X 100 mL). The extracts are washed with water, 0.1 N NaOH, brine, and finally dried. The solvent is evaporated to give a solid which is chromatographed (EtOAc elution) to give 0.8 g (0.001 mol) of N-Boc-Val-ε-N-Cbz-Lys-BZD as a white solid.

N-Boc-Val-ε-N-Cbz-Lys-BZD (1.9 g, 0.0023 mol) obtained as above was dissolved in 50 mL of methylene chloride cooled to 0°C and treated with $HCl_{(g)}$ for 1 hour. The solvents were evaporated under reduced pressure at room temperature to give a solid. The solid was dissolved in THF and made slightly basic with N-methylmorpholine. This solution was then added to a solution of activated Boc-Pro (1.01 g, 0.0047 mol). The activation and work-up procedure is the same as that describe above. The Boc-Pro-Val-ε-N-Cbz-Lys-BZD product is purified by chromatography (EtOAc elution) to give 1.64 g of a white solid.

Starting with 1.64 g (0.0018 mol) of the Boc-Pro-Val-ε-N-Cbz-Lys-BZD obtained above and 1.06g (0.004 mol) of Boc-D-Phe the same deprotection, coupling, and work-up procedure described above is followed to give a white solid. This was chromatographed (5% MeOH/$CHCl_3$ elution) to give 1.6 g (0.0015 mol) of Boc-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD as a white foam.

Starting with the Boc-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD obtained above and 1.0 (0.004 mol) of Boc-Leu-H_2O the same deprotection, coupling, and work-up procedure described above is repeated to give Boc-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD as a white foam. This is purified by chromatography (75% EtOAc/hexane elution) to give 1.2 g (1.0 mmol) of a white solid.

Starting with the Boc-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD obtained above and 1.68 (0.003 mol) of α-N-Boc-ε–N-Cbz-Lys-DCHA, the same deprotection, coupling, and work-up procedure described above is repeated to give α-N-Boc-ε–N-Cbz-Lys-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD as a white foam. This is purified by chromatography (100% EtOAc elution) to give 1.2 g (0.84 mmol) of a white solid.

A solution of the above α-N-Boc-ε–N-Cbz-Lys-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD (0.7 g, 0.4 mmol) in 20 mL of MeOH is treated with 0.8 g of anhydrous hydrazine and stirred at room temperature for 30 hr.. The solvent is removed under vacuum to give a white solid. This is chromatographed (5 % $MeOH/CHCl_3$) to give 0.55 g of α-N-Boc-ε–N-Cbz-Lys-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD hydrazide as a white solid.

The hydrazide (0.55 g, 0.38 mmol) is dissolved in ethyl acetate (30 mL), cooled to 0° C and treated with $HCl_{(g)}$ for 1 hr. Evaporation at room temperature under vacuum gives a white solid which is dissolved in 12 mL of DMF, transferred to a 1 L flask and cooled to - 20 ° C. To this solution is added 0.1 mL of conc. aq. HCl and 1 mL of 1 M aq $NaNO_2$. The solution is stirred at - 20 °C for 20 min. and then diluted with 300 mL of cold DMF (- 20 °C) and made basic with diisopropylethylamine (1 mL). The solution is allowed to warm to room temperature and is stirred overnight. The solvent is removed under vacuum (pump) on a rotary evaporator while warming at 50 °C. The residue is dissolved in chloroform and chromatographed (1% $MeOH/CHCl_3$) to give 0.10 g (0.08 mmol) of *c*-(N-Cbz-Lys-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD) as a white foam: FAB-MS $(M+H)^+$ 1287.6.

The above cyclic peptide is treated at 0 °C with 1 mL of anisole and 20 mL of HF for 30 min. The solution is evaporated under high vacuum and the residual solid is washed on with ether on a filter funnel to give 55 mg of *cyclo*-(Lys-Leu-D-Phe-Pro-Val-Lys-BZD) **11c** as an off-white solid. FAB MS $(M+H)^+$ = 1018.44

Gramicidin S analog 26: A solution of α-N-Boc-ε–N-Cbz-Lys-DCHA (5.6 g , 0.010 mol) in THF is cooled in a salt-ice bath to -15° C and treated with 1.0 g (0.010 mol) of N-Methylmorpholine. Isobutyl chloroformate (1.36 g, 0.010 mol) is slowly added to the mixture. The solution is stirred for 5 min. before 2.7 g (0.0056 mol) of the amino-BZD **20** is added. The solution is stirred at -15° C for 20 min., diluted with 100 mL of cold water and extracted into methylene chloride (2 X 100 mL). The extracts are washed with water, 0.1 N NaOH, 1 N HCl, brine, and finally dried. The solvent is evaporated to give a white solid which is dissolved in a minimum amount of CH_2Cl_2 and chromatographed (50% EtOAc/ Hexane) to give 2.3 g (0.0028 mol) of α-N-Boc-ε–N-Cbz-Lys-BZD **21** as a white foam.

The α-N-Boc-ε–N-Cbz-Lys-BZD **21** obtained above (1.2g, 0.0014 mol) was dissolved in 50 mL of methylene chloride cooled to 0°C and treated with $HCl_{(g)}$ for 1 hour. The solvents were evaporated under reduced pressure at room temperature to give a solid. The solid was dissolved in THF and made slightly basic with N-methylmorpholine.

A solution of N-Boc-Val (0.43 g , 0.0020 mol) in THF is cooled in a salt-ice bath to -15° C and treated with 0.2g (0.002 mol) of N-Methylmorpholine. Isobutyl chloroformate (0.27 g, 0.002 mol) is slowly added to the mixture. The solution is stirred for 5 min. before the NH_2-ε-N-Cbz-Lys-BZD solution prepared above is added. The solution is stirred at -15° C for 20 min., diluted with 100 mL of cold water and extracted into methylene chloride (2 X 100 mL). The extracts are washed with water, 0.1 N NaOH, brine, and finally dried. The solvent is evaporated to give a solid which is chromatographed (30%-60% EtOAc/hex elution) to give 0.5 g (0.0005 mol) of N-Boc-Val-ε-N-Cbz-Lys-BZD as a white solid.

N-Boc-Val-ε-N-Cbz-Lys-BZD (0.5 g, 0.0005 mol) obtained as above was dissolved in 50 mL of methylene chloride cooled to 0°C and treated with $HCl_{(g)}$ for 1 hour. The solvents were evaporated

under reduced pressure at room temperature to give a solid. The solid was dissolved in THF and made slightly basic with N-methylmorpholine. This solution was then added to a solution of activated Boc-Pro (0.22 g, 0.001 mol). The activation and work-up procedure is the same as that describe above. The Boc-Pro-Val-ε-N-Cbz-Lys-BZD product obtained is used without further purification.

Starting with the crude Boc-Pro-Val-ε-N-Cbz-Lys-BZD obtained above and 0.27 g (0.001 mol) of Boc-D-Phe the same deprotection, coupling, and work-up procedure described above is followed to give a white solid. This was HPLC chromatographed on silica gel (65% EtOAc/hexane elution) to give the two diastereomers of Boc-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD. The first diastereomer **23b** that eluted from column gave 250 mg of a white foam: MS (DCI-NH_3) (M+H)+ 1189.
The second diastereomer **23a** gave 300 mg of a white foam: MS (DCI-NH_3) (M+H)+ 1189.

Starting with 0.6 g(0.5 mmol) of Boc-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD **23a** obtained above and 0.25 (1.0 mmol) of Boc-Leu-H_2O the same deprotection, coupling, and work-up procedure described above is repeated to give Boc-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD as a white foam. This is used without further purification.

Starting with the Boc-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD obtained above and 0.56 (1.0 mmol) of α-N-Boc-ε–N-Cbz-Lys-DCHA, the same deprotection, coupling, and work-up procedure described above is repeated to give α-N-Boc-ε–N-Cbz-Lys-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD as a white foam. This is purified by silica gel chromatography (50% - 100% EtOAc elution) to give 0.4 g (0.25 mmol) of a white solid.

A solution of the above α-N-Boc-ε–N-Cbz-Lys-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD (0.2 g, 0.25 mmol) in 20 mL of MeOH is treated with 0.4 g of anhydrous hydrazine and stirred at room temperature for 30 hr.. The solvent is removed under vacuum to give a white solid. This is chromatographed on a HPLC silica gel (100% EtOAc) to give 0.10 g of α-N-Boc-ε–N-Cbz-Lys-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD hydrazide as a white solid.

The hydrazide obtained above is dissolved in ethyl acetate (30 mL), cooled to 0° C and treated with $HCl_{(g)}$ for 1 hr. Evaporation at room temperature under vacuum gives a white solid which is dissolved in 12 mL of DMF, transferred to a 1 L flask and cooled to - 20 ° C. To this solution is added 0.2 mL of conc. aq. HCl and 0.3 mL of 1 M aq $NaNO_2$. The solution is stirred at - 20 °C for 20 min. and then diluted with 300 mL of cold DMF (- 20 °C) and made basic with diisopropylethylamine (1 mL). The solution is allowed to warm to room temperature and is stirred overnight. The solvent is removed under vacuum (pump) on a rotary evaporator while warming at 50 °C. The residue is dissolved in chloroform and chromatographed (100% EtOAc) to give 95 mg (0.08 mmol) of *c*-(N-Cbz-Lys-Leu-D-Phe-Pro-Val-ε-N-Cbz-Lys-BZD) as a white foam: MS (DCI NH3) $(M+H)^+$ 1432-1434, $(M+H-OBn)^+$1324 , $(M+H-2x\ OBn)^+$ = 1216; FAB-MS $(M+H)^+$ = 1431-1436

The above cyclic peptide is treated at 0 °C with 1 mL of anisole and 20 mL of HF for 30 min. The solution is evaporated under high vacuum and the residual solid is washed with ether on a filter funnel to give the *cyclo*-(Lys-Leu-D-Phe-Pro-Val-Lys-BZD) **26** as an off-white solid:
FAB-MS $(M+H)^+$ = 1164.99 $(M+Na)^+$ = 1186.97

References

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