Tetrahedron Vol. 49, No. 17, pp. 3593-3608, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Protein β-Turn Mimetics I. Design, Synthesis, and Evaluation in Model Cyclic Peptides.

W.C. Ripka*[a], G.V. De Lucca*, A.C. Bach II, R. S. Pottorf [b] and J.M. Blaney [c]
Du Pont Merck Pharmaceutical Co.
Experimental Station, Wilmington, DE 19880-0353

(*Received in USA* 16 *March* 1993)

Peptides and proteins play a central role in the control and modulation of virtually all biological processes regulating biological functions by acting as hormones, enzymes, receptors, and inhibitors. The explosive growth in the fields of molecular biology, peptide synthesis, structure elucidation (x-ray and NMR), and molecular modeling has dramatically increased understanding of the relationship between protein and peptide structure and their biological function. This and the recent progress in the synthesis and screening of huge peptide libraries has focused attention on small peptides as important lead structures for the development of potential therapeutic agents. Problems of metabolic instability and lack of oral bioavailability, however, severely restrict the use of peptides themselves as therapeutic candidates and emphasizes the importance of establishing general synthetic strategies for converting these potent peptides into peptidomimetics that may overcome the pharmacokinetic shortcomings of the peptides themselves.

In the design of peptidomimetics one approach attempts to determine or, more likely, hypothesize a biologically relevant conformation of the peptide of interest and then to construct a non-peptide scaffolding that places previously determined critical functional groups in their proper three-dimensional orientation. This approach requires the design of a completely new scaffolding for every peptide of interest.

It is known, however, from the growing number of three-dimensional structures of proteins that there are preferred structural elements that are involved in packing and folding - these include beta sheets, helices, and turns or loops. A peptide ligand binding to a receptor site or an active site of a larger protein results in a larger, packed protein complex that should still obey the rules of protein packing. It would be expected that the bound peptide would adopt similar conformations as required of other local structure of typical proteins. Recent structural evidence suggests that this is indeed the case.

A number of x-ray structures of proteases with their corresponding peptide inhibitors show that local regions of the peptides bound to the active sites adopt an extended conformation[1] very similar to a protein beta sheet or beta strand. This same extended conformation has recently been shown to be important in recognition of viral peptides by MHC (Major Histocompatibility Complex) proteins as shown by x-ray analysis of the complex[2]. Alternatively, peptide-calmodulin complexes show a helix conformation imposed on the peptide on binding to the protein.[3] Finally, antibody-antigen complexes[4] appear to frequently involve binding of the peptide antigens in a beta turn conformation. The implication of this for drug design is that the design of peptidomimetics may be based on a particular protein structural motif. Of the three types of structural motifs the beta turn offers the significant advantage that it is compact and of such a size that it can readily be mimicked by a small organic molecule. To successfully mimic even a small helix will require molecules significantly larger than steroids. In this paper we wish to report our work on the design, synthesis, and conformational validation of appropriately substituted benzodiazepines as beta turn peptidomimetics that reproduce both the geometric constraints of a beta-turn as well as the positions of the functionality of the four side chains in their proper three-dimensional orientation.

An important structural feature of many biologically active peptides and proteins is the beta-turn motif. Beta turns in proteins are segments between secondary structural elements that reverse the direction of the chains. These turns are often situated on the protein surface and usually consist of polar residues that offer the opportunity of intermolecular interactions with other protein surfaces and hence provide sites for intermolecular recognition[5]. Substantial evidence exists to suggest that smaller peptides also possess beta turns in their biologically active conformations and the resulting compact structures have clustered side chains available for interactions with receptors[6]. 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[7]. It has been suggested[8] that backbone conformations of beta bends are not always critical for activity provided that optimal side chain conformations are preserved. This may explain why several types of beta turns, existing in known proteins, differ only in their backbone configurations with the overall geometry and clustering of side chains being relatively similar. In turn, this implies many different backbone conformations may be consistent with an active peptide pharmacophore and it is the spatial disposition of the side chains that determines activity.

The importance of beta turns in peptides or proteins may well be crucial in receptor interactions that ultimately lead to biological activity. In recognition of this there have been several efforts to 'lock' peptides into beta turn configurations[9] and to synthesize organic molecules that might mimic a beta turn[10] in an otherwise 'normal' peptide. In the latter cases, except for a few exceptions[11], molecules were usually tailored for a specific problem with the general problem of converting peptide structure to smaller, less peptidic molecules being unsolved.

The possibility of small, organic molecules acting as peptide mimics has already been demonstrated although they were found by serendipity. Opiate analgesics and antagonists for the endogenous enkephalins/β-endorphins[12], benzodiazepines as cholecystokinin (CCK) antagonists[13], and substituted imidazoles as angiotensin II receptor antagonists[14] are examples of small, organic molecules able to mimic peptides. Interestingly, in each case a beta turn is believed to be involved in the biologically active conformation of the natural peptides and there are reasons to suspect that the organic structures are mimicking parts of these bends[15]. The general problem of converting many active peptides (or short segments from proteins) into small organic molecules may in fact be reduced to finding a general mimic of beta turns. While these examples show the feasibility of beta turn mimetics, the structures are so different that the relationship of the mimic to the peptide is not obvious. A systematic and rational approach to this problem is needed.

Design of Peptidomimetics

In the design of beta turn peptidomimetics, it would be advantageous to have a rigid molecule that could mimic any beta turn structure and position the four side chains (for the i, i+1, i+2, i+3 residues) in their proper orientation as determined by x-ray crystallography of beta turn sequences in known proteins. With the few exceptions noted above, attempts to accomplish this have been either specific to a particular sequence or have ignored the side chains of the turn residues altogether. An ideal case would possess both the proper turn geometry and position the side chains in their proper orientations. An important consideration is the compounds also be synthetically accessible.

From x-ray structures of a number of proteins several types of turns have been identified[16]. A number of these have different backbone conformations but position substituents in similar areas of space. In our initial molecular modeling search for an appropriate mimetic of a type I bend (**1** in Figure 1) the hydrogen bond unit between residues i and i + 3 was replaced first with one methylene then two and the amide bonds with two trans and one cis double bond (**2**). In a second case one amide bond was retained (**3**). An initial fit of these structures to the original peptide turn (**1**) looked satisfactory. Distance

geometry[17] was used to generate several conformational possibilities for each of these macrocyclic cases and molecular mechanics was used to find the low energy conformations. When superimposed on **1**, low energy conformations of **2b** and **3b** gave reasonable fits but the expected synthetic difficulties for these macrocycles caused us to look for other simplifications.

A second analysis led to the bicyclic ring system **4**. Potential stability and synthetic problems with **4** suggested expanding one ring to give **5**, **6** and **7** as shown in Figure 1. Interestingly, benzodiazepines **5**, **6** and **7** show a remarkable fit to each one of the beta turn examples from the basis set[16], not just the type I turns. Importantly, side chains (R^1 to R^4) in **5** and **6** can be positioned in close proximity to those present in the peptide. One of the peptide units (between residue 3 and 4) is preserved in **6** and the stereochemistry of R3, which is expected to be important, can easily be incorporated by the use of the appropriate amino acid. The side chain R^2 can be on one of two positions (**5** or **6**) and still reach similar positions in space. Note this substituent is also attached to an sp^2 center which agrees with the finding that D-residues as well as prolines are often found at this position. Since proline is found almost 30% of the time in position 2 of beta turns, this suggests that in most cases either no substituent or a

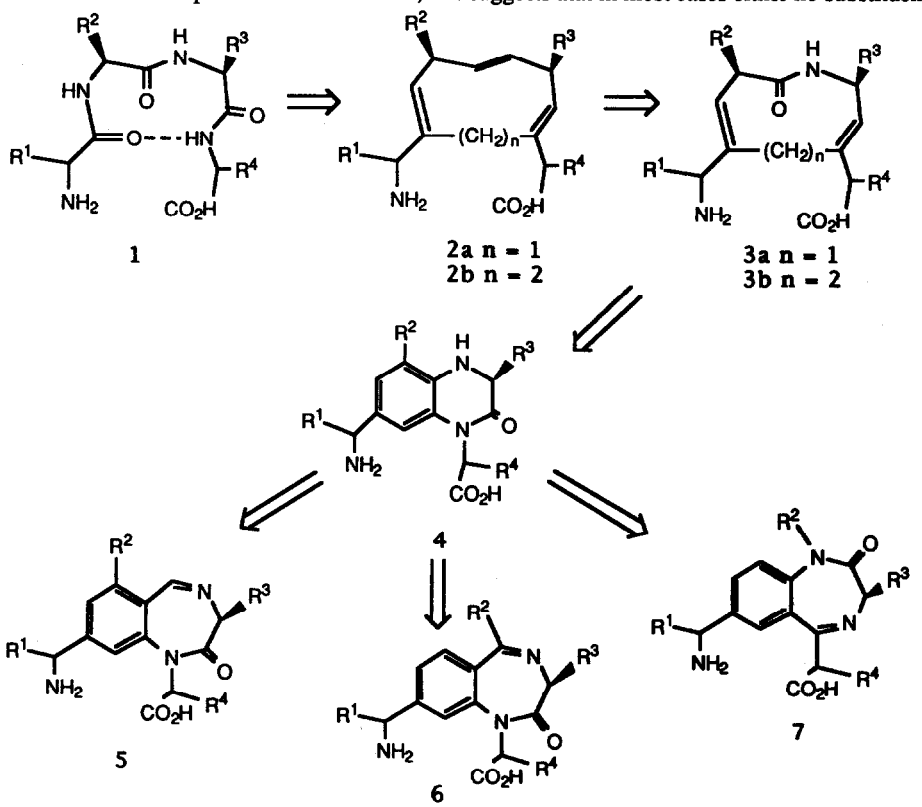
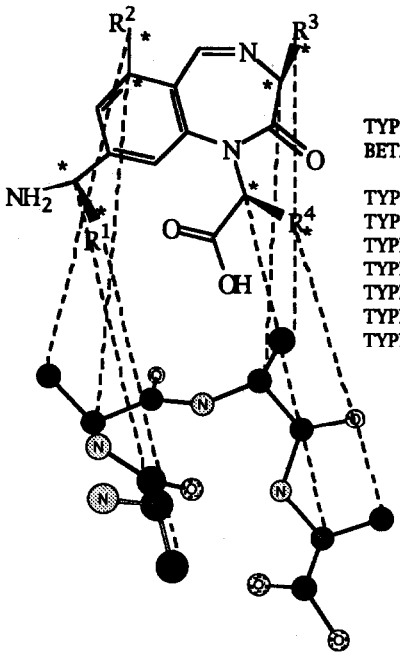


Figure 1. Design of beta turn peptidomimetics

hydrophobic group (e.g. phenyl) might be acceptable at this position. The benzodiazepine structures also closely maintain the overall geometric requirements of the beta turn and project residues 1 and 4 in directions that correspond with the requirements of the actual beta turns I-VI. When a least squares fit

is made of the starred atoms of **5** to the corresponding atoms in each of the turn types as shown in Figure 2, the root mean square (RMS) fits were less than 1 Å. The RMS fits for **6** and **7** to the beta turns I-VI are comparable to those shown in Figure 2. This clearly establishes the benzodiazepine (BZD) nucleus as an excellent potential mimic of each of the beta turn types.



TYPE OF BETA TURN	RMS (Å)
TYPE I	0.56
TYPE I'	0.65
TYPE II	0.59
TYPE II'	0.47
TYPE III	0.79
TYPE IV	0.54
TYPE VI	0.26

Figure 2. BZD comparison to beta turns

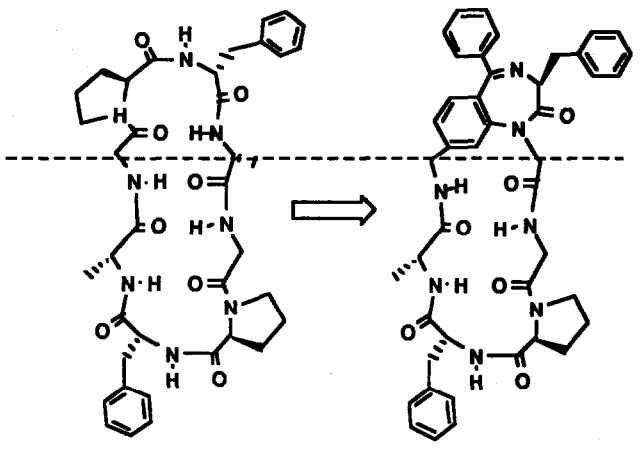
Although derived from an independent analysis of the requirements of a peptide beta turn, the idea of the benzodiazepine nucleus mimicking these turns might explain a number of reports in the literature concerning several different biological activities for these compounds (see below). Importantly it suggests a general approach to reduced peptides, and peptide fragments from proteins, into rigid organic structures. Benzodiazepine analogs have been implicated, in addition to anxiolytic activity[18], in analgesia[19], phospholipase A_2 inhibition[20], cholecystokinin antagonism, PAF antagonist[21], cholesterol lowering activity[22], anti-fungal/anti-tumor/antibiotic activity[23], anti-hypertension[24], anti-HIV activity[25], etc. This appears to support a more general role for this structural class; this role may be as a general beta turn mimic as described above.

Model Cyclic Peptides

While computer models and molecular mechanics calculations suggested that the BZD nucleus would be an excellent mimic of various beta turns we felt it was important to verify this with experimental results that would directly assess the ability of the benzodiazepine nucleus to substitute for a known peptide beta turn. Two possible options were available to validate this hypothesis: a biological model and a physical model. The first would involve the synthesis of a mimetic of a known biologically active peptide that is believed to exist in a beta turn conformation and to test the biological activity of the mimic via appropriate *in vivo* or *in vitro* assays. This is clearly important since our ultimate purpose was to design mimetics for biologically active peptides which retain or enhance their activity while improving their pharmacokinetic properties. The uncertainty in this approach is that the role of the beta turn in biologically active linear peptides has only been surmised and not proven. A second option would be to incorporate the beta turn mimetic into a model in which the effectiveness of the peptidomimetic could be determined by physical methods (x-ray, NMR). We chose to do both concurrently and in this paper we wish to report our findings.

Model cyclic peptides have been used to identify the rules governing stability and turn structure

preferences of various amino acids[26]. Considerable work has been reported for model cyclic penta-, hexa-, octapeptides and for the naturally occurring cyclic decapeptide antibiotic Gramicidin S (GS)[27]. The concept was *to replace the four amino acids that makes up one of the beta turns in these cyclic peptides with the BZD peptidomimetic as shown in Figure 3. If the mimic was a good one then it should stabilize the resulting BZD containing cyclic peptide in a similar conformation as the all peptide model.* While there have been examples of turn mimics incorporated into cyclic peptides[28], most notably the BTD mimetic of Nagai and Sato[10cd] , the end points of biological activity for assessing the degree of mimickry has been primarily biological activity with only a few examples studied rigorously by physical means[29]. The cyclic pentapeptides and cyclic hexapeptides did not seem appropriate to study a four residue mimic since that would leave too few amino acids, and thus observable NMR parameters, to understand the effects of the peptidomimetic substitution. Larger cyclic peptides are more suitable and we were especially interested in the cyclic octapeptide work of Kopple[30] and in the work done with Gramicidin S (GS), a cyclic decapeptide. In both cyclic peptide systems direct structural studies (both x-ray and NMR) and studies looking at the effects of substitution with other amino acid sequences have been reported. Kopple[28a] has shown by NMR and X-ray analysis that the cyclic octapeptide (Gly-Pro-D-Phe-D-Ala)$_2$ exist in a C_2 symmetric, stable, double beta-turn conformation. Substitution of one of the beta turns with a random four amino acid sequence destabilizes the structure of the resulting cyclic octapeptide (Gly-Pro-D-Phe-D-Ala-Asn-Ala-Val-Ser). For the cyclic octapeptide (Gly-Pro-D-Phe-D-Ala)$_2$ we have substituted one of the type II beta turn sequences (Gly^1-Pro^2-D-Phe^3-D-Ala^4) with the BZD peptidomimetic **15** as shown in Figure 3. Incorporation of the Gly^1 (R^1 = H) and Phe^3 (R^3 = $CH_2C_6H_6$) residues into the BZD nucleus is synthetically straightforward. Although Pro^2 can not be directly accommodated by the BZD peptidomimetic the use of a hydrophobic group at this position (R^2 = C_6H_6) appeared to be a reasonable compromise. Substitution of D-Ala^4 residue with a Gly equivalent (R^4 = H) was done for synthetic simplification. Thus, the target BZD containing cyclic octapeptide (with G-P-F-G equivalents incorporated in the peptidomimetic) is shown in Figure 3.



Cyclic-octapeptide
c-(Gly-Pro-D-Phe-D-Ala)2

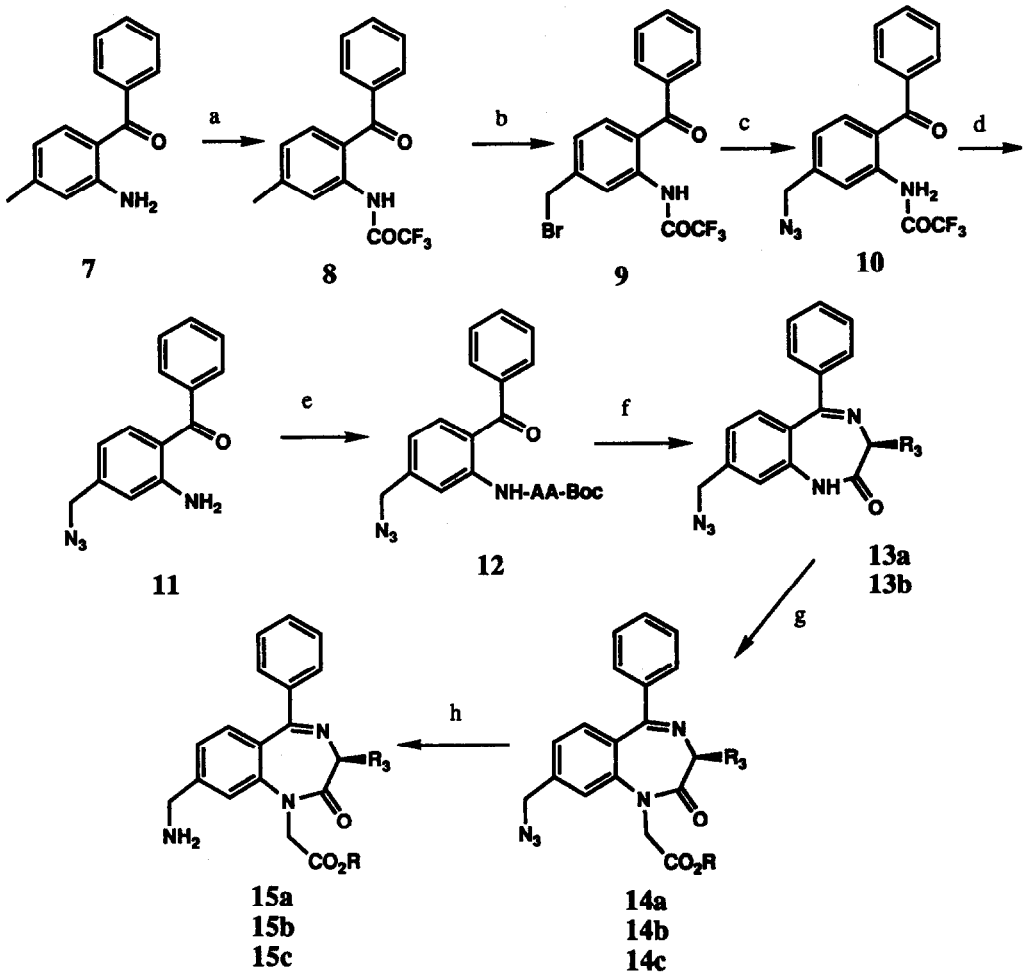
Peptidomimetic Cyclic peptide
c-(Gly-Pro-D-Phe-D-Ala-BZD)

Figure 3. Model System

Synthesis of Beta Turn Peptidomimetics: Model Cyclic Octapeptides

The synthesis of the desired benzodiazepine containing cyclic octapeptide analogs is outlined in **Scheme 1** and **2.** The key intermediate for the benzodiazepine containing cyclic octapeptide model peptides is the benzodiazepine amino ester **15.** This is obtained in 9 steps from the commercially

Scheme 1



a: $R^3 = CH_2C_6H_6$, R= CH_3
b: R^3 = H, R = CH_3
c: R^3 = H, R = C_2H_5
AA-Boc = N-Boc-Amino Acid

a. $(CF_3CO)_2/CH_2Cl_2/0^\circ$ C. b. NBS/CCl_4/reflux. c. NaN_3/DMF/RT
d. K_2CO_3/Aq. dioxane/ reflux/ 2 hrs.
e. N-methylmorpholine/isobutylchloroformate/Boc-amino acid/ rt/18 hrs.
f. 1. $HCl_{(g)}/0^\circ C$/30 min. 2. MeOH/1N NaOH/rt. g. 1. NaH/DMF/rt. 2. $BrCH_2CO_2R$ h. 10% Pd/C/EtOH

available aminobenzophenone **7** as shown in **Scheme 1.** The synthetic details are given in the Experimental Section.

The synthesis of the desired cyclic analog is outlined in **Scheme 2.** The linear peptide sequence is first constructed from amino ester **15** by sequential coupling of the suitably blocked amino acids using solution phase mixed anhydride coupling methods. The linear peptide is then cyclized using the azide cyclization procedure. The benzodiazepine methyl ester **17** containing the protected linear peptide is converted to the hydrazide **18** by treatment with excess hydrazine in methanol at room temperature over a period of 2 days. After removal of the Boc protecting group hydrazide **19** is converted to the azide using nitrous acid and subsequently cyclized under dilute conditions to give the BZD containing cyclic analog **20** in 50% yield. The fact that the cyclization proceeds readily suggests that the peptidomimetic does stabilize turn conformations.

Conformational Analysis of *c*(G-P-dF-dA-BZD)

The assignments of the model cyclic octapeptide were done in both DMSO and $CDCl_3$ (Table 1) by the use of 1D and 2D NMR techniques. In $CDCl_3$ only one conformation is observed. In DMSO two conformations were apparent with the major conformation about 85% populated. Since the conformational analysis of the all peptide cyclic octapeptide was reported in DMSO, the analysis of the peptidomimetic containing model cyclic "octapeptide" **20** is also determined in DMSO to make direct comparison possible. All amino acid residues of the major conformation in DMSO were identified in the DQF-COSY spectrum according to their characteristic pattern of chemical shifts (Table 1).

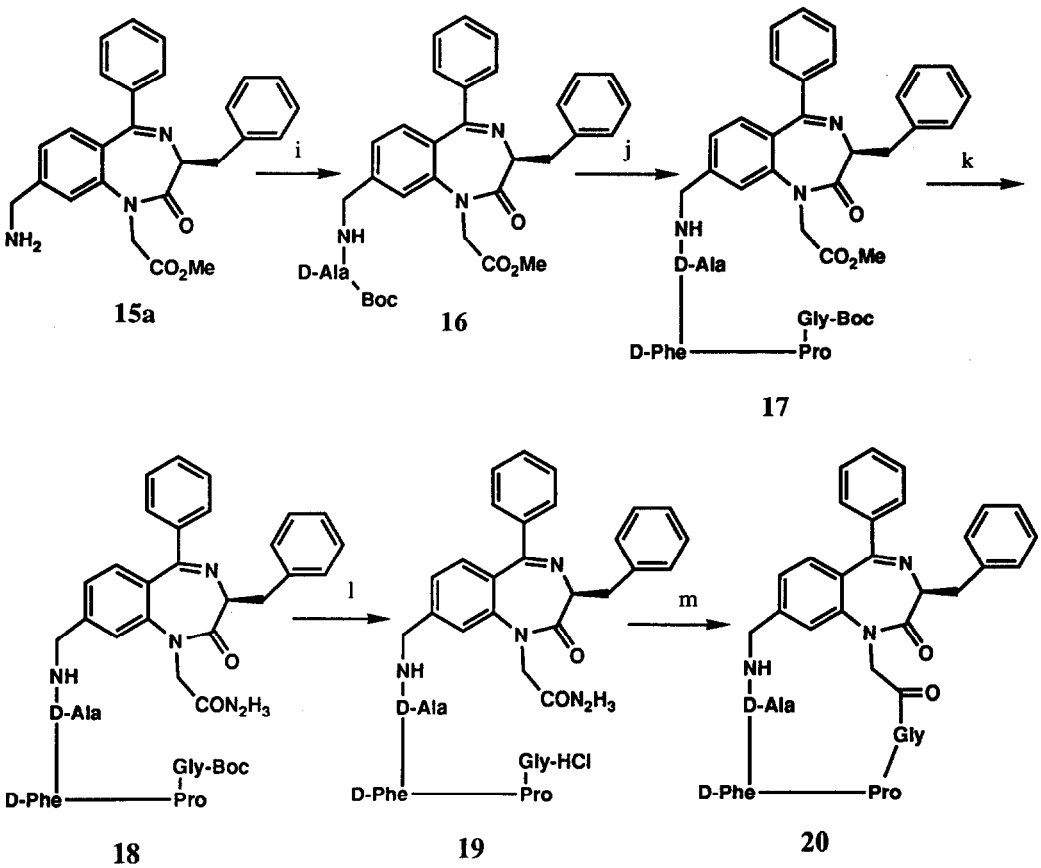
Conformational analysis of cyclic peptides using NMR techniques are well established. Important parameters for conformational analysis include scalar coupling constants of vicinal protons from which dihedral angles can be derived. Distances between protons can be derived from dipolar proton-proton coupling using 2D NOE techniques. Besides these parameters, temperature dependencies of amide proton chemical shifts indicate the solvent accessibility of these protons.

There are several indications that the major conformation of the model cyclic octapeptide is adopting a well defined structure in DMSO. The large spread of the various amide protons (~ 1 ppm) as well as the large chemical shift difference of the geminal H^{α} of the Gly (and pseudo "Gly"s in BZD) residue is an indication that they are in different environments.

In a beta turn there is a hydrogen bond between the carbonyl of the ith residue and the amide proton of the i+3 residue. This proton would be sequestered from solvent and show a chemical shift that has a small dependency on temperature (0 to -3 ppb) or solvent ($CDCl_3$ vs. DMSO). The D-Ala H^N (Table 2) shows the typically small temperature dependency for amino acids in position i + 3 of beta turns. The small difference in the chemical shift of the D-Ala H^N in DMSO and $CDCl_3$ ($\Delta\delta = 0.24$) is also indicative of a sequestered proton. The results (Table 2) indicate that the BZD H^N, D-Phe H^N, and the Gly H^N amide protons are all exposed to bulk solvent. In a type II beta turn the $H^{\alpha}i+1$ to $H^N i+2$ proton-proton distance is short (about 2.1 Å). In the NOESY spectra of the BZD containing octapeptide an intense cross-peak is observed between the D-Phe H^N and the Pro H^{α}. This is consistent with the major conformation in DMSO adopting a type II beta turn with Gly in position i of the turn.

The size of the coupling constant between the Cα proton and the amide proton ($J^{N\alpha}$) is a function of the dihedral angle ϕ in the peptide residue. Based on the $J^{N\alpha}$ measured (Table 2) one can derive possible ϕ angle consistent with the data. These possible ϕ values are listed in Table 3 with the most likely values underlined based on the similarity with the all peptide cyclic octapeptide. Also included in the table are the corresponding values for the solution conformation of $c(\text{G-P-dF-dA})_2$ in DMSO and the average values of ϕ obtained from the x-ray structure of $c(\text{G-P-dF-dA})_2$ for comparison. Based on this data the backbone conformation of the beta turn mimic cyclic octapeptide and the all peptide analog are similar

Scheme 2



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

j. 1. TFA/CH_2Cl_2, rt, 1 hr. 2. N-methylmorpholine/THF 3. couple with next amino acid 4. repeat steps

k. N_2H_4/MeOH/rt/2 days.

l. $HCl_{(g)}$/0°C/60 min.

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

TABLE 1. Assignments Of 20 In DMSO And $CDCl_3$.

Residue	Atom	DMSO	$CDCl_3$
D-Phe	NH	8.698	6.249
	Hα	4.490	4.585
	Hβ	2.760 - 3.260	3.280
Gly	NH	8.095	6.356
	Hα	4.190 - 3.509	4.300 - 3.410
D-Ala	NH	7.875	7.632
	Hα	4.220	4.570
	Hβ	1.550	1.620
N-BZD	NH	7.698	6.803
	Hα	4.475 - 4.110	4.80 - 4.06
Pro	Hα	4.120	4.00
	Hδ	3.450 - 3.350	3.385 - 3.595
3-Phe	Hα	3.76	3.87
	Hβ	3.25	3.59
BZD-CH_2CO-	Hα	5.025, 4.310	5.190, 4.120

TABLE 2. Amide Proton Data For 20 And *c*(G-P-dF-dA)$_2$ In DMSO.

	c(G-P-dF-dA-BZD) 20	*c*(G-P-dF-dA)$_2$
	DMSO	DMSO
D-Phe		
δ NH	8.698	8.77
Δδ/ΔT	5.3	4.3
^{J}HNCH	8.5	7.8
Δδ	1.45	
Gly		
δ NH	8.095	6.84
Δδ/ΔT	4.7	0.3
^{J}HNCH	Σ = 10.0	7.7 7.6
Δδ	1.74	
D-Ala		
δ NH	7.875	7.68
Δδ/ΔT	2.5	1.2
^{J}HNCH	7.6	8.4
Δδ	0.24	
HN-BZD		
δ NH	7.698	
Δδ/ΔT	3.7	
^{J}HNCH	Σ = 12.2	
Δδ	0.895	

Table 3. Possible ϕ Angles For 20 And Structure Comparison.

c(G-P-dF-dA-BZD) 20				*c*(G-P-dF-dA)$_2$ in DMSO			x-ray	ideal β Turn
RES	Δδ/ΔT	^{J}Nα	ϕ	Δδ/ΔT	^{J}Nα	ϕ	ϕ	ϕ
BZD	-3.5	Σ=12.2	+120, -120 +60, - 60	-	-			
D-Ala	-2.5	7.6	+160, ±85 -40, -85	-1.2	8.4	+100	+74	
D-Phe	-5.3	8.5	+155, ±85	-4.3	7.8	+95	+69	+80
Gly	-4.7	Σ=10.0	+145, -145 +50, -50	+0.3	7.6 6.7	-80	-73	
Pro	-----	-----	-60	-----	---	-60	-64	-60

Conclusion

The data presented here demonstrate that a four amino acid sequence, which is known to adopt a beta turn conformation, can be substituted by the benzodiazepine beta turn peptidomimetic **6** in the model cyclic octapeptide (*c*(G-P-dF-dA)$_2$) and still retain the double beta turn conformation in the resulting "cyclic octapeptide" (*c*(G-P-dF-dA-BZD)) **20**. Given that the peptide portion of **20** maintains a similar beta turn conformation as present in the all amino acid cyclic peptide the benzodiazepine peptidomimetic **6** is presumably effectively mimicking the beta turn opposite in the ring. A complete structure from NMR was not possible because of the lack of sufficient NOEs .

Experimental Section

NMR methods

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 [31, 32], TOCSY [33], NOESY [34] and ROESY [35, 36] 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 [37]

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 [38, 39].

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 [40].

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.

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 ^1H 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 ^1H 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.

3-(S)-benzyl-8-azidomethyl-1,4-Benzodiazepine-2-one (**13a**). A solution of 100 g (0.0473 mol) of 2-amino-4-methylbenzophenone (**7**) and 39.3 g (0.497 mol) of pyridine in 400 mL of methylene chloride is cooled in an ice bath under nitrogen. To this solution is added 104.4g (0.497 mol) of trifluoracetic anhydride slowly from an addition funnel over a period of 40 min. while cooling at 0° C. The mixture is stirred an additional hour and then washed with water (3 x 200 mL), sat'd aq. sodium bicarbonate (100 ml), and brine (100 mL), and then dried, filtered and the solvent removed on a rotary evaporator to give 145 g (0.47 mol) of the trifluoracetamide **8** as an off-white solid: NMR ($CDCl_3$) δ 12.30 (bs, 1 H), 8.51 (s, 1 H), 7.75-7.41 (m, 7 H), 7.00 (d, 1 H, 11 Hz), 2.45 (s, 3 H).

A solution of **8** (145 g, 0.47 mol), N-bromosuccinimide (104 g, 0.59 mol) and benzoyl peroxide (5.2 g) in CCl_4 (400 mL) is heated to reflux for 3 hr.. After cooling to room temperature the solid was filtered off and the filtrate is washed with 600 mL of 0.1 N NaOH, water (200 mL), and brine (2 x 200 mL). After drying, the solution is evaporated to give a dark orange oil. This was titurated with 500 mL of ether, cooled to -60° C and filtered. The solid is washed with cold ether (-60° C) to give 85 g (0.22 mol) of **9** as an off-white solid: mp 120 °C; NMR ($CDCl_3$) δ 12.15 (bs, 1 H), 8.71 (s, 1 H), 7.75-7.65(m, 4 H), 7.52 (t, 2 H, 7 Hz), 7.28 (d, 1 H, 9 Hz), 4.51 (s, 2 H); MS (DCI CH_4) $(M+H)^+$ 386.0 (100 %), 388.0 (95.5%), , HRMS calc. for $C_{16}H_{11}F_3BrNO_2$ 384.9925; found: (M^+) 384.9935.

A solution of the benzyl bromide **9** (69.7 g , 0.180 mol.) and sodium azide (69.7g, 0.189 mol) in DMF (180 mL) is stirred at room temperature for 4 hours. The solution was diluted with water (1 L) and extracted into ethyl acetate (3 X 200 mL). The organic extract is washed with water (3 X 100 mL), brine (2 X 100 mL) and then dried. Evaporation of solvent under vacuum gives 62.7 g (0.18 mol) of the azide **10** as a yellow oil. A small amount was chromatography by HPLC on silica gel (20% ethyl acetate/ hexane) to give a white solid: mp 47-48 °C, IR ($CDCl_3$ cm^{-1}) 3286, 2105, 1733, 1639, 1616, 1580, 1537, 1265, 1154; NMR ($CDCl_3$) δ 12.15 (bs, 1 H), 8.63 (s, 1 H), 7.75-7.62 (m, 4 H), 7.52 (t, 2 H, 7 Hz), 7.25 (d, 1 H, 9 Hz), 4.50 (s, 2 H); MS (DCI CH_4) $(M+H)^+$ = 349, $(M+H-N_2)^+$ 321, $(M+H-HN_3)^+$ 306.

A solution of 500 mL of dioxane, 500 mL of sat'd K_2CO_3 and azide **10** (62.7 g, 0.18 mol) is stirred and heated to reflux for 3 hr.. The solution is cooled to room temperature, diluted with 600 mL of water and extracted into ethyl acetate. The organic extract is washed with water (2 x 100 mL), brine (2 x 100 mL) and dried. The solvent is evaporated to give 49 g of a yellow/orange oil. This was chromatographed (eluted with 10% ethyl acetate/hexane) to give 35.7 g (0.15 mol) of the azide-aminobenzophenone **11** as an yellow oil. IR (neat film cm^{-1}) 3462, 3345, 2099, 1622, 1584, 1541, 1319, 1247; NMR ($CDCl_3$) δ 7.64-7.43 (m, 6 H), 6.69 (s, 1 H), 6.52 (d, 1 H, 8 Hz), 6.18 (bs, 2 H), 4.29 (s, 2 H), MS (DCI CH_4) $(M+H)^+$ 253.1

A solution of N-Boc-Phe (19.1 g , 0.072 mol) in THF is cooled in a salt-ice bath to -15° C and treated with 7.3 g (0.073 mol) of N-Methylmorpholine. Isobutyl chloroformate (9.7 g, 0.072 mol) is then slowly added to the mixture. The solution is stirred for 5 min. and then 15.13g (0.060 mol) of the azide-aminobenzophenone **11** 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 36 g of **12** as 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 and the residue is chromatographed (30% ethyl acetate/hexane elution) to give 22.0 g (0.058 mol) of the benzodiazepine **13a** as a solid: mp 84-86 °C, $[\alpha]^{25}_D$ = +82.22 ° (c = 0.602, MeOH), NMR ($CDCl_3$) δ 8.97 (s, 1 H), 7.48-7.20 (m, 11 H), 7.08 (M, 2 H), 4.45 (s , 2 H), 3.80 (m, 1 H), 3.61 (m, 2 H). MS (DCI CH_4) $(M+H)^+$ = 382.2, HRMS calc for $C_{23}H_{19}N_5O$ 381.1589, found 381.1589

Synthesis of **13b**: A solution of N-Boc-Gly (41.7 g , 0.24 mol) in THF is cooled in a salt-ice bath to -15° C and treated with 24.0 g (0.24 mol) of N-Methylmorpholine. Isobutyl chloroformate (32.3 g, 0.24 mol) is then slowly added to the mixture. The solution is stirred for 5 min. and then 30.0 g (0.118 mol) of the azide aminobenzophenone **11** is added. The mixture is allowed to warm to room temperature and stirred overnight, 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 50 g of a thick yellow oily residue. The residue is chromatographed (30% ethyl acetate/hexane) to give 44.0 g (0.108 mol) of yellow solid. This is dissolved in chloroform (500 mL) and cooled to 0° C and treated with $HCl_{(g)}$ for a period of 1.5 hours. 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 and the residue is chromatographed (30% ethyl acetate/hexane elution) to give 30.0 g (0.103 mol) of the benzodiazepine **13b** as a solid: mp 173-174 ° C, NMR ($CDCl_3$) δ 8.92 (s, 1 H), 7.53-7.33 (m, 6 H), 7.12 (m, 2 H), 4.46 (s, 2 H), 4.35 (bs, 2 H), MS (DCI-CH_4) $(M+H)^+$ 292.1 (65%), $(M+H-N_2)^+$ 264.1 (100%), HRMS calc for $C_{16}H_{13}N_5O_1$ 291.1120, found $(M)^+$ = 291.1119

Methyl-[3-(S)-benzyl-8-azidomethyl-1,4-Benzodiazepine-2-one]-acetate (**14a**). Under a blanket of nitrogen, sodium hydride (0.42 g, 0.01 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 3.8 g (0.01 mol) of BZD **13a** and stirred at room temperature for 30 min.. Then 1.6 g (0.011 mol) of methyl bromoacetate is added drop-wise 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 and the residue chromatographed (eluting with 50% ethyl acetate/hexane) to give 3.0 g (0.007 mol) of azido-methyl ester **14a** as a white foam: mp 62-65° C, $[\alpha]^{25}_D$ = +44.85 ° (c = 0.602, MeOH), NMR ($CDCl_3$) δ 7.56 (d, 2 H, 6.6 Hz), 7.48-7.11 (m, 11 H), 4.61 (s , 2 H), 4.45 (s , 2 H), 3.88 (t, 1 H, 6.8 Hz), 3.68 (s, 3 H), 3.59 (m, 2 H), MS (DCI - NH_3) $(M+H)^+$ 454.5, HRMS calc for $C_{26}H_{23}N_5O_3$ 453.1801, found: (M^+) 453.1811.

Synthesis of **14b**: Under a blanket of nitrogen, sodium hydride (2.2 g, 0.055 mol of a 60% dispersion in oil) was washed several times with hexane and decanted. To a slurry of the washed NaH in dry DMF (80 mL) is added in small portions 16 g (0.055 mol) of BZD **13b** and stirred at room temperature for 30 min.. Then 8.4 g (0.055 mol) of methyl bromoacetate is added dropwise and allowed to stir at room temperature for 1 hour. The solution is then diluted with 500 mL of water and extracted into ethyl acetate. The organic extract is washed with water, and brine and dried The solvent is evaporated at room temp and the residue chromatographed (50% ethyl acetate/hexane) to give 17.0 g (0.046 mol) of azido-methyl ester **14b** as a thick oil: NMR ($CDCl_3$) δ 7.61 (d, 2 H, 7 Hz), 7.5-7.29 (m, 4 H), 7.26 (s, 1 H), 7.15 (d, 1 H, 8.1 Hz), 4.84 (d, 1 H, 10.6 Hz), 4.62 (d, 1H, 17.2 Hz), 4.45 (s, 2 H), 4.51 (d, 1 H, 17.2 Hz), 3.88 (d, 1 H, 10.6 Hz), 3.71 (s, 3 H) MS (DCI-CH_4) $(M+H)^+$ 364.2 (86%), $(M+H-N_2)^+$ 336 (100%)

Synthesis of **14c**: Under a blanket of nitrogen, sodium hydride (2.0 g, 0.05 mol of a 60% dispersion in oil) was washed several times with hexane and decanted. To a slurry of the washed NaH in dry DMF (120 mL) is added in small portions 14.6 g (0.05 mol) of BZD **13b** and stirred at room temperature for 30 min.. Then 8.35 g (0.05 mol) of ethyl bromoacetate is added dropwise and allowed to stir at room temperature for 1 hour. The solution is then diluted with 500 mL of water and extracted

into ethyl acetate. The organic extract is washed with water, and brine and dried The solvent is evaporated at room temp and the residue chromatographed (50% ethyl acetate/hexane) to give 13.0 g (0.034 mol) of azido-ethyl ester **14c** as a thick oil: NMR ($CDCl_3$) δ 7.61 (d, 2 H, 7 Hz), 7.5-7.29 (m, 4 H), 7.26 (s, 1 H), 7.16 (d, 1 H, 8.1 Hz), 4.84 (d, 1 H, 10.6 Hz), 4.63 (d, 1H, 17.2 Hz), 4.47 (s, 2 H), 4.47 (d, 1 H, 17.2 Hz), 4.21 (m, 2 H), 3.88 (d, 1 H, 10.6 Hz), 1.23 (t, 3 H, 7.1 Hz) MS (DCI-CH_4) $(M+H)^+$ 378.2 (86%), $(M+H-N_2)^+$ 350 (100%), HRMS calc for $C_{20}H_{19}N_5O_3$ 377.1488, found $M^+ = 377.1490$

Methyl -[3-(S)-benzyl-8-aminomethyl-1,4-Benzodiazepine-2-one]-acetate (**15a**). A solution of azido-BZD **14a** (2.6 g, 0.0057 mol) in ethyl acetate (150 mL) is hydrogenated at 45 psi in a Parr Hydrogenator for 1 hr with 1 g of 10% Pd/C. The solution is filtered through Celite and the filtrate is extracted with 1 N aq HCl (2 X 200 mL). The acid extract is cooled in an ice bath and made basic with the drop-wise 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 1.4 g (0.0033 mol) of the amino ester **15a** as a white foam. mp 76-80 °C, $[\alpha]^{25}_D = +42.95°$ (c = 0.610, MeOH), NMR ($CDCl_3$) δ 7.57 (d, 1 H, 7 Hz), 7.48-7.15 (m, 11 H), 4.62 (AB q, 2 H), 3.95 (s, 3 H), 3.89 (t, 1 H, 7 Hz), 3.59 (d, 2 H, 7 Hz), 1.6 (bs, 2 H), MS (DCI - NH_3) $(M+H)^+ = 428.5$, HRMS calc for $C_{26}H_{25}N_3O_3$ 427.1896, found (M^+) 427.1895

Synthesis of **15b**: A solution of azide **14b** in ethanol is treated with 10% Pd/C and hydrogenated in a Parr Hydrogenator at 50 psi for 1 hour. The solution is filtered through Celite and evaporated to give the amine as a white solid. This is used without further purification. mp 85-90 °C, NMR ($CDCl_3$) δ 7.61 (d, 2 H, 7 Hz), 7.5-7.20 (m, 5 H), 7.15 (d, 1 H, 8. Hz), 4.84 (d, 1 H, 10.6 Hz), 4.62 (d, 1H, 17.2 Hz), 4.56 (d, 1 H, 17.2 Hz), 3.96 (s, 2 H), 3.88 (d, 1 H, 10.6 Hz), 3.71 (s, 3 H), 1.5 (bs, 2 H) MS (DCI-CH_4) $(M+H)^+$ 338.2 HRMS calc for $C_{19}H_{19}N_3O_3$ 337.1427 found $(M)^+$ 337.1428

Synthesis of **15c**: A solution of azide **14c** in ethanol is treated with 10% Pd/C and hydrogenated in a Parr Hydrogenator at 50 psi for 1 hour. The solution is filtered through Celite and evaporated to give the amine as a white solid: mp 76-80°C, NMR ($CDCl_3$) δ 7.61 (d, 2 H, 7 Hz), 7.5-7.29 (m, 4 H), 7.26 (s, 1 H), 7.16 (d, 1 H, 8.1 Hz), 4.82 (d, 1 H, 10.6 Hz), 4.63 (d, 1H, 17.2 Hz), 4.47 (d, 1 H, 17.2 Hz), 4.21 (m, 2 H), 3.97 (s, 2 H), 3.86 (d, 1 H, 10.6 Hz), 1.21 (t, 3 H, 7.1 Hz) MS (DCI-CH_4) $(M+H)^+$ 352.2, HRMS calc for $C_{20}H_{21}N_3O_3$ 351.1583, found $M^+ = 351.1583$

Cyclo-[D-Ala-D-Phe-Pro-Gly-BZD] 20: A solution of N-Boc-D-Ala (1.15 g , 0.0061 mol) in THF is cooled in a salt-ice bath to -15° C and treated with 0.62 g (0.0062 mol) of N-Methylmorpholine. Isobutyl chloroformate (0.83 g, 0.0061 mol) is slowly added to the mixture. The solution is stirred for 5 min. before 1.3 g (0.003.5 mol) of the amino-BZD **15a** 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 1.6 g (0.0027 mol) of Boc-D-Ala-BZD **16** as a white foam: mp 108-114 °C, $[\alpha]^{25}_D = +13.79°$ (c = 0.602, MeOH), MS (DCI - NH_3) $(M+NH_4-H_2O)^+$ 598, (DCI CH4) $(M+H)^+$ 599, HRMS calc for $C_{34}H_{38}N_4O_6$ 598.2791, found (M^+) 598.2798, FAB MS $(M+H)^+$ 599.37.

The Boc-D-Ala-BZD **16** obtained above (1.44 g, 0.0024 mol) was dissolved in 5 mL of methylene chloride and treated with 20 mL of trifluoroacetic acid at room temperature for 1 hour. The solvents were evaporated under reduced pressure at room temperature and the residue titurated with ether 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-D-Phe (1.27 g, 0.0049 mol). The activation and work-up procedure is the same as that described above for **16**. The crude product is chromatographed (ethyl acetate elution) to give 1.5 g (0.0020 mol) of Boc-D-Phe-D-Ala-BZD as a white foam: mp 115-119 °C, $[\alpha]^{25}_D = +6.00°$ (c = 0.600, MeOH), MS (DCI - NH_3) $(M+H)^+$ 746, HRMS calc for $C_{43}H_{47}N_5O_7$ 745.3476, found (M^+) 745.3483, FAB MS $(M+H)^+$ 746.44.

Starting with 1.30 g (0.0017 mol) of Boc-D-Phe-D-Ala-BZD and 0.75 g (0.0035 mol) of Boc-Pro the same deprotection, coupling, and work-up procedure described above is followed. Chromatography (ethyl acetate elution) gives 1.1 g (0.0013 mol) of Boc-Pro-D-Phe-D-Ala-BZD as a white foam: mp 160-161 °C, $[\alpha]^{25}_D$ = -5.74° (c = 0.610, MeOH), MS (DCI - NH3) $(M+H)^+$ 843, HRMS calc for $C_{48}H_{54}N_6O_8$ 842.4003, found (M^+) = 842.4012 FAB MS $(M+H)^+$ = 843.40.

Starting with 1.00 g (0.00118 mol) of Boc-Pro-D-Phe-D-Ala-BZD and 0.42 g (0.0023 mol) of Boc-Gly the same deprotection, coupling, and work-up procedure described above is repeated. Purification by chromatography (ethyl acetate) gives 0.83 g (0.0092 mol) of Boc-Gly-Pro-D-Phe-D-Ala-BZD **17** as a white foam: mp 126-132 °C, $[\alpha]^{25}_D$ = -8.80 ° (c = 0.602, MeOH), MS (DCI - NH3) $(M+H)^+$ 900, HRMS calc for $C_{45}H_{49}N_7O_7$ 799.3693, found $(M-C_5H_8O_2)^+$ = 799.3735, FAB-MS $(M+H)^+$ 900.57.

A solution of **17** 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. The solid 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 0.3 mL of conc. aq. HCl and 2 mL of 1 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 (3 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 0.30 g (0.00039 mol) of **20** as a white foam: mp 216-220 °C (decomp.), $[\alpha]^{25}_D$ = +101.48 ° (c = 0.608, MeOH), MS (DCI - NH_3) $(M+H)^+$ = 768, HRMS calc for $C_{44}H_{45}N_7O_6$ 767.3431, found(M^+) 767.3486, FAB-MS $(M+H)^+$ 768.33.

References

[a] Current address: Corvas International 3030 Science Park Road, San Diego, CA 92121.

[b] Current address: Marion Merrell Dow Pharmaceuticals, 2110 East Galbraith Rd., Cincinnati, Ohio 45215

[c] Current address: Chiron Corp., 4560 Horton St., Emeryville, California 94608

[1] For HIV protease: a). Miller, M.; Schneider, J.; Sathyanarayana, B.K.; Toth, M.V.; Marshall, G.R.; Clawson, L.; Selk, L.M.; Kent, S.B.H.; Wlodawer, A. *Science*, **1989**, *245*, 1149. b). Erickson, J.; Neidhart, D.J.; VanDrie, J.; Kempf, D.J.; Wang, X.C.; Norbeck, D.W.; Plattner, J.J.; Rittenhouse, J.W.; Turon, M.; Wideburg, N; Kohlbrenner, W.E.; Simmer, R.; Helfrich, R.; Paul, D.A.; Knigge, M.; *Science*, **1990**, *249*, 527. c). Swain, A.L.; Miller, M.; Green, J.; Rich, D.H.; Schneider, J.; Kent, S.B.H.; Wlodawer, A.; *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 8805. d). Jaskolski, M.; Tomasselli, A.G.; Sawyer, T.K.; Staples, D.G.; Heinrikson, R.L.; Schneider, J.; Kent, S.B.H.; Wlodawer, A.; *Biochemistry* **1991**, *30*, 1600. e). Bone, R.; Vacca, J.P.; Anderson, P.S.; Holloway, M.K.; *J. Am. Chem. Soc.* **1991**, *113*, 9382.
For Renin: Rahuel, J.; Priestle, J.P.; Grutter, M.G. *J. Struct. Bio.* **1991**, *107*, 227.

[2] a). Fremont, D.H.; Matsumura, M.; Stura, E.A.; Peterson, P.A.; Wilson, I.A.; *Science*, **1992**, *257*, 919. b). Matsumura, M.; Fremont, D.H.; Peterson, P.A.; Wilson, I.A.; *Science*, **1992**, *257*, 927.

[3] a). Meador, W.E.; Means, A.R.; Quiocho, F.A.; *Science* **1992**, *257*, 1251. b). Ikura, M.; Clore, G.M.; Gronenborn, A.M.; Zhu, G.; Klee, C.B.; Bax, A.; *Science*, **1992**, *256*, 632.

[4] Rini, J.M.; Schulze-Gahmen, U.; Wilson, I.A.; *Science* **1992**, *255*, 959.

[5] Ref 4. Also see: a). Kuntz, I.D. *J. Am. Chem. Soc.* **1972**, *94*, 4009. b) For recent review see: Rose, G.D.; Gierasch, L.M.; Smith, J.A. *Adv. Protein Chem.* **1985**, *37*, 1-109. c). Venkatachalam, C.M. *Biopolymers* **1968**, *6*, 1425. d) Lewis, P.N.; Monamy, F.A.; Scheraga, H.A. *BioChim. Biophys. Acta.* **1973**, *303*, 211. e) Chou, P.Y.; Fasman, G.D. *J. Mol. Biol.* **1977**, *115*, 135.

[6] a) Oka, M.; Montelione, G.T.; Scheraga, H.A. *J. Am. Chem. Soc.* **1984**, *106*, 7959; b) Smith, J.A., Pease, L.G. *CRC Crit. Rev. Biochem* **1980**, *8*, 315.

[7] a) Hruby, V.J. *Trends Pharmacol. Sci.* **1985**, *6*, 259. b)Veber, D.F.; Freidinger, R.M.; Perlow, D.S.; Paleveda, W.J., Jr.; Holly, F.W.; Strachan, R.G.; Nutt, R.F.; Arison, B.H.; Homnick, C.; Randell, W.C.; Slitzer, M.S.; Saperstein, R.; Hirschmann, R. *Nature* **1981**, *292*, 55. c) Sawyer, T.K.; Hruby, V.J.; Darman, P.S.; Hadley, M.E. *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 1751.; d)London, R.E.; Stewart, J.M.; Williams, R.; Cann, J.R.,; Mataiyoff, N.A. *J. Am. Chem. Soc.* **1979**, *101*, 2455.

e) Freidinger, R.M.; Veber, D.F.; Perlow, D.S.; Brooks, J.R.; Saperstein, R. *Science* **1980**, *210*, 656. f)
[8] Freidinger, R.M.; Colton, C.D.; Perlow, D.S.; Whitten, W.L.; Paleveda, W.J.; Veber, D.F.; Arison, B.H.; Saperstein, R. in *Peptides, Structure and Function*; Hruby, V.J. and Rich, D.H. Eds.; Pierce Chem. Co.; Rockford, Illinois; 1983 pp 349-352.
[9] a) Prasad, B.V.V.; Ravi, A.; Balaram, P. Biochem. Biophys. Res. Commun. 1981, 103, 1138. b) Rao, B.N.N.; Kumar, A.; Balaram, H.; Ravi, A.; Balaram, P. *J. Am. Chem. Soc.* **1983**, *105*, 7423; c) Mosberg, H.I.; Omnaas, J.R. *J. Am. Chem. Soc.* **1985**, *107*, 2986; d) Elseviers, M.; Van Der Auwera, L; Pepermans, H.; Tourwe, D.; Van Binst, G; *Biochem. Biophys. Res. Commun.* **1988**, *154*, 515.
[10] a) Brandmeier, V. Feigel, M. ; *Tetrahedron* **45**, *1365*, 1989; b) Feigel, M.; *J. Am. Chem. Soc.* **1986**, *108*, 181; c) Sato, K.; Nagai, U.; *J. Chem. Soc. Perkin Trans I.* **1986**, 1231; d) Nagai, U.; Sato, K.; *Tetrahedron Lett.* **1985**, 647; e) . Kemp, D.S ; McNamara, P.E.; *J. Org. Chem.* **1984**, *49*, 2268.; f) D.S. Kemp and W.E. Stites; *Tetrahedron Lett.* **1988**, *29*, 5057; g)Krstenansky,J.L.; Baranowski, R.L.; Currie, B.L.; *Biochem. Biophys. Res. Commun.*, **1982**, *109*, 1368 ; h) Kahn, M.; Bertenshaw, S.; *Tetrahedron Lett.* **1989**, *30*, 2317; i) Ernest, I, Kalvoda, J.; Rihns, G.; Muter, M.; *Tetrahedron Lett.* **1990**, *31*, 4011; j)Hinds,M.G.; Richards,N.G.J; Robinson, J.A.; *J. Chem. Soc. Chem. Commun.* **1988**, 1447.; k). Morgan, B.A.; Gainor, J.A. in *Annual Reports in Medicinal Chemistry* Allen, R.C Ed.; Academic; New York; 1989; Vol 24; pp 243-252.
[11] a) Kahn,M.; Wilke,S.; Chen,B; K. Fuijita,K; *J. Molecular Recognition* **1988**, *1*, 75; b)Kahn,M.; Wilke,S.; Chen, B.; Fujita,K.; *J. Am. Chem. Soc.* **1988**, *110*, 1638; c) Kahn, M; Chen, B.; *Tetrahedron Lett.* **1987**, 1623; d) Olson, G.L.; Voss, M.E.; Hill, D.E.; Kahn, M.; Madison, V.S.; Cook, C.M.; *J. Am. Chem. Soc.* **1990**, *112*, 323.; e) Farmer, P.S. in *Drug Design*, Ariens, E.J., Ed.; Academic: New York, 1980; Vol X, pp 119-143; f) Farmer, P.S.; Ariens, E.J.; *Trends Pharmacol. Sci.* **1982**, *9*, 362.
[12] Johnson, M.R.; Milne, G.M. In *Burger's Medicinal Chemistry*, 4th ed.; Wolf, M.E. Ed.; Wiley, New York, 1980; Part III, pp 699-758.
[13] a) Evans, B.E.; Bock, M.G.; Rittle, K.E.; Dipardo, R.M.; Whitter, W.L.; Veber, D.F.; Anderson, P.S.; Freidinger, R.M.; *Proced. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4918. b). Bock, M.G.; Dipardo, R.M.; Rittle, K.E.; Evans, B.E.; Freidinger, R.M.; Veber, D.F.; Chang, R.S.L.; Chen, T.B.; Keegan, M.E.; Lotti, V.J. *J. Med. Chem.* **1986**, *29*, 1941. c) Evans, B.E.; Rittle, K.E.; Bock, M.G.; Dipardo, R.M.; Freidinger, R.M.; Whitter, W.L.; Gould, N.P.; Lundell, G.F.; Homnick, C.F.; Veber, D.F.; Anderson, P.S.; Chang, R.S.L.; Lotti, V.J.; Cerino, D.J.; Chen, T.B.; King, P.J.; Kunkel, K.A.; Springer, J.P.; Hirschfield, J. *J. Med. Chem.* **1987**, *30*, 1229.
[14] a) Wong, P.C.; Chiu, A.T.; Price, W.A.; Thoolen, M.J.M.C.; Carini, D.J.; Johnson, A.L.; Taber, R.I.; Timmermans, P.B.M.W.M. *J. Pharm. Expt. Ther.* **1988**, *247*, 1. b). Furukawa, Y.; Kishimoto, S.; Nishikawa, K.; U.S. Patent 4,355,040 (1982) c). Carini, D.J.; Duncia, J.V.; Johnson, A.L.; Chiu, A.T.; Price, W.A.; Wong, P.C.; Timmermans, P.B.M.W.M. *J. Med. Chem.* **1990**, *33*, 1330. d). Duncia, J.V.; Chiu, A.T.; Carini, D.J.; Gregory, G.B.; Johnson, A.L.; Price, W.A.; Wells, G.J.; Wong, P.C.; Calabrese, J.C.; Timmermans, P.B.M.W.M. *J. Med. Chem.* **1990**, *33*, 1312.
[15] a) See ref 8 and 10 for some possibilities. b).For a possible explanation of how the benzodiazepine is mimicking CCK see: Pincus, M.R.; Carty, R.P.; Chen, J.; Lubowsky, J.; Avitable, M.; Shah, D.; Scheraga, H.A.; Murphy, R.B. *Proc. Natl. Acad. Sci. USA*, **1987**, *84*, 4821-4825.
[16] From the x-ray structures of several proteins available from the Protein Data Bank a basis set of various types of turns was established to use in our study. Type I: Subtilisin BPN', Gly^{23}-Val^{26}; Type I': Staphlococcal Nuclease, Ala^{94}-Lys^{97}; Type II: Hen Egg White Lysozyme, Cys^{115}-Thr^{118}; Type II': Carboxypeptidase A, Tyr^{277}-Leu^{280}; Type III: Sperm Whale Myoglobin, Phe^{46}-Leu^{49}; Type III': Pro^{210}-Lys^{213}; Type IV: α-Chymotrypsin, Ile^{99}-Asp^{102}; Type V: Horse Ferricytochrome c, Ala^{43}-Phe^{46}; Type VI: Bovine Ribonuclease S, Lys^{91}-Asn^{94}; Type VII: α-Chymotrypsin, Val^{67}-Glu^{70}.
[17] Blaney, J. M.; Crippen, G; Dearing, A; Dixon, S; DGEOM, program #590, Quantum Chemistry Program Exchange, Indiana University, Bloomington, Indiana (1990)

[18] Childress, J.S. In *Burger's Medicinal Chemistry*, 4th ed.; Wolf, M.E. Ed.; Wiley, New York, 1980; Part III, pp 981-996.
[19] Romer, D.; Buscher, H.H.; Hill, R.C.; Maurer, R.; Petcher, T.J.; Zeugner, H.; Bensen, W.; Finner, E.; Milkowski, W.; Theis, P.W. *Nature* **1982**, *298*, 760.
[20] Wallach, D.P. U.S. Patent 4,590,187 1984.
[21] a) Heuer, H.; Birke, F.; Brandt, K.; Muacevic, G.; Weber, K.H. *Prostaglandins* **1988**, *35*, 847. b) Heuer, H.; Casals-Stenzel, J.; Muacevic, G.; Sransky, W.; Weber, K.H. *Clin. Exp. Pharmacol. Physiol. Suppl.*. **1988**, *13*, 7.
[22] Balasubramaniam, S.; Simons, L.A.; Chang, S. *Atherosclerosis* **1986**, *60*, 263.
[23] a)Thakar, K.A.; Padhyl, A.M. *J Ind. Chem. Soc.* **1985**, *62*, 465. b) Hertberg, R.P.; Hecht, S.M.; Reynolds, V.L.; Molineux, I.J.; Hurley, L.H. *Biochemistry* **1986**, *25*, 1249. c) Barkley, M.D.; Cheatham, S.; Thurston, D.E.; Hurley, L.H. *Biochemistry* **1986**, *25*, 3021. d) Kaneko, T.; Wong, H.; Doyle, T.W.; Rose, W.C.; Bradner, W.T. *J. Med. Chem.* **1985**, *28*, 388
[24] a) Kim, D.H.; Baum, T. *J. Med. Chem.* **1977**, *20*, 209. b) Pozenel, H.; Buckert, A.; Amrein, R. *Int. J. Pharmacol. Biopharm.* **1977**, *15*, 31. c) Nasipwi, D.; Mukherjee, S. *Ind. J. Chem. Sect. B* **1984**, *23*, 1184. d) Stanton, J.L.; Whatthey, J.W.H.; Desai, M.N.; Finn, B.M.; Bariarz, J.E. *J. Med. Chem.* **1985**, *28*, 1603.

[25] Pauwels, R.; Andries,K.; Desmyter,J.; Schols,D.; Kukla,M.J.; Breslin,H.J.; Raeymaeckers,A.; Van Gelder,J.; Woestenborghs,R.; Heykants,J.; Schellekens, K.; Janssen, M.A.C.; De Clerq, E.; Janssen, P.A.J. *Nature*. **1990**, *343*, 470.
[26] a) see reference 1b. b) Gierasch, L.M.; Deber, C.M.; Madison, V.; Niu, C-H.; Blout, E.R. *Biochemistry* **1981**, *20*, 4730.
[27] a) For a good overview see Izumiya, N.; Kato, T.; Aoyagi, H.; Waki, M.; Kondo, M. in *Synthetic Aspects of Biologically Active Cyclic Peptides - Gramicidin S and Tyrocidines*; Wiley and Sons; New York, 1979.
[28] For examples see ref 6a and 6b and references therein Also: Ernest, I.; Vuilleumier, S.; Fritz, H.; Mutter, M. *Tetrahedron Lett.* **1990**, *31*, 4015.

[29] Bach, A. C.; Markwalder, J. A.; Ripka, W. C.; *Int. J. Pept. Protein Res* **1991** *38*, 314
[30] a) Kopple, K.D.; Parameswarean, K.N.; Yonan, J. *J. Am. Chem. Soc.* **1984**, *106*, 7212. b) Kopple, K.D.; Kartha, G.; Bhandary, K.K.; Romanowska, K. *J. Am. Chem. Soc.* **1985**, *107*, 4893. c) Kopple, K.D.; Bhandary, K.K.; Kartha, G.; Wang, Y-S.; Parameswarean, K.N. *J. Am. Chem. Soc.* **1986**, *108*, 4637. d) Vishwanath, C.K.; Go, A.; Parameswarean, K.N.; Wang, Y-S.; Kopple, K.D. *Int. J. Peptide Protein Res.* **1986**, *28*, 428. e) Kopple, K.D.; Wang, Y-S.; Cheng, A.G. Bhandary, K.K. *J. Am. Chem. Soc.* **1988**, *110*, 4168.
[31] Piantini, U.; Sorensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800- 6801.
[32] Shaka, A. J.; Freeman, R. *J. Magn. Reson.* **1983**, *51*, 169-173.
[33] Davis, D. G.; Bax, A. *J. Am. Chem. Soc.* **1985**, *107*, 7197-7198.
[34] Macura, S.; Ernst, R. R. *Mol. Phys.* **1980**, *41*, 95-117.
[35] Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811-813.
[36] Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207-213.
[37] Kessler, H.; Griesinger, C.; Kerssebaum, R.; Wagner, K.; Ernst, R. R. *J. Am. Chem. Soc.* **1987**, *109*, 607-609.
[38] Mueller, L.; Ernst, R. R. *Mol. Phys.* **1979**, *38*, 963-992.
[39] States, J. D.; Harberkorn, R. A.; Reuben, D. J. *J. Magn. Reson.* **1982**, *48*, 286-292.
[40] Otting, G.; Widmer, H.; Wagner, G.; Wuethrich, K. *J. Magn. Reson.* **1986**, *66*, 187-193.