Tetrahedron Vol. 49, No. 17, pp. 3467-3478, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

β-Turn Topography

Jonathan B. Ball*

CSIRO McMaster Laboratory, Private Bag No.1, PO Glebe, NSW 2037, Australia

Richard A. Hughes

Max-Planck-Institut for Psychiatry, Department of Neurochemistry,
Am Klopferspitz 18a, 8033 Planegg-Martinsried, Federal Republic of Germany

Paul F. Alewood, Peter R. Andrews*

Centre for Drug Design and Development, University of Queensland, Queensland 4072, Australia

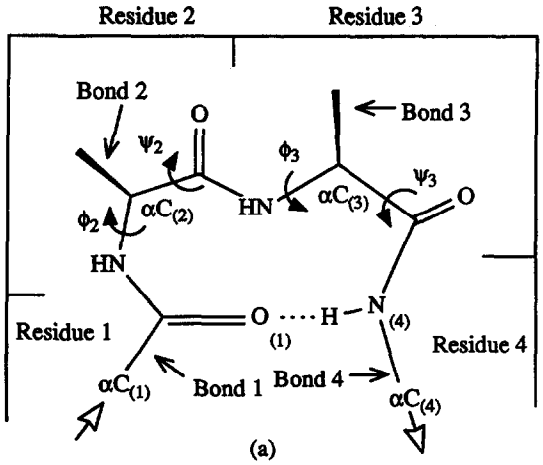
(*Received in USA* 22 *February* 1993)

Abstract: The topography of the β-turn was investigated using 146 examples of β-turns reported in the literature. Common topographical features were observed across a wide variety of β-turn types. Based on this, it was proposed that β-turns could be described in terms of a single dihedral angle, which was defined as β, which provides a complete description of the spatial relationship between the entry and exit peptide bonds as well as the relative orientations of the intervening sidechains for any β-turn. This simplification was made possible by the reduction of the β-turn structure into two conformationally invariant units, the median geometries of which are reported herein. This description should prove particularly useful in the development and application of novel peptide mimetic drugs, compounds for which a classification based on a peptide backbone geometry may be entirely irrelevant.

INTRODUCTION

The β-turn constitutes a well studied subset of the reverse turn and is a common feature in biologically active peptides and globular proteins where it is widely thought to act as a molecular recognition site for many biological processes[1,2]. Unlike the α-helix and the β-sheet, the backbone conformation of the β-turn (Figure 1) is highly variable. This is partly a result of the selection criteria generally adopted for β-turns which state that any tetrapeptide sequence in which the $\alpha C_{(1)}$-$\alpha C_{(4)}$ distance is ≤ 7 Å and which occurs in a non-helical region is a β-turn. The specific type of β-turn is then classified according to the geometry of the peptide backbone, as described by the backbone torsion angles in residues 2 and 3 (Figure 1). Although there are slight variations in the classification of β-turns based on the φ and ψ peptide backbone torsion angles, all workers[3-7] have found similar distributions of β-turn conformers, as well as large numbers (30-50%) of non-ideal or distorted β-turns (generally defined as those β-turns which have a single torsion angle differing by more than 45-50° from the ideal) and one or more ill-defined categories (types IV and VII in Figure 1(b)).

There is overwhelming evidence that sidechains are often extremely important in peptide-receptor interactions[8]. From a molecular recognition perspective, therefore, it can be argued the most important features of the β-turn are the relative dispositions of bonds 1, 2, 3 and 4. Bonds 2 and 3 are important because they govern the placement of the sidechains of residues 2 and 3, whose exposed nature make a logical recognition site. Bonds 1 and 4 are important because they determine the position of any binding groups which might occur before and after the β-turn. None of these features is clearly defined by the current classification and this is a hindrance to the construction of conformationally constrained organic molecules designed to be topographical mimics of the β-turn. There is increasing interest in such molecules[9-45] since conformational constriction can help render a biologically active peptide more potent (or alternatively, render it an antagonist), longer lasting, more specific and orally active[46-50] and in these ways can circumnavigate the multitude of problems which beset the therapeutic use of peptides[51] while also providing information on the



Turn type	ϕ_2	ψ_2	ϕ_3	ψ_3
	degrees			
I	-60	-30	-90	0
I′	60	30	90	0
II	-60	120	80	0
II′	60	-120	-80	0
III	-60	-30	-60	-30
III′	60	30	60	30
IV	see caption			
V	-80	80	80	-80
V′	80	-80	-80	80
VI	see caption			
VII	see caption			

(b)

Figure 1. The structure (a) and classification[3-5] (b) of the β-turn. The hydrogen bond shown from $N_{(4)}$ to $O_{(1)}$ in (a) is not present in all β-turns. In (b), type IV β-turns are defined as those having 2 or more angles which differ by at least 40° from the definitions of β-turn types I, I′, II, II′, III and III′. Type VI β-turns are those turns which have a *cis*-Pro at position 3, while type VII β-turns form a kink in the protein chain created by $\psi_2 \approx 180°$ and $|\phi_3| < 60°$ or $|\psi_2| < 60°$ and $\phi_3 \approx 180°$.

receptor-bound conformation. Peptide secondary structure mimics might also be of use in studying protein structure and function[1,6,11,15,52-55]. For example, it has been suggested[5,55] that protein folding - a process about which there is much yet to be discovered[53] - is directed by β-turns. Consequently, there is interest in employing β-turn mimics in studies of protein folding[54] but such studies are likely to be hampered by the lack of knowledge of the topography of the β-turn. It is clear that the geometrical relationship between bond 1 and bond 4, which represents the position at which the peptide chain would respectively enter and exit the β-turn, could be fundamental to this process for which the peptide backbone of the β-turn may serve merely as a scaffold.

A classification based on the peptide backbone conformation may be entirely irrelevant for these molecules. We report here the results of an investigation into the topography of the β-turn and propose a description which should facilitate the construction of nonpeptide β-turn mimics.

RESULTS AND DISCUSSION

Preliminary observations based on Dreiding models

From Dreiding models with backbones set to the ideal β-turn types as defined in Figure 1(b) (except IV, VI and VII, which are ill-defined), it was observed that despite large changes in the geometry of the peptide backbone, the relative positions of bond 1, bond 2 and atom $\alpha C_{(3)}$ remained similar (Figure 2(a)). In other words, these three components appeared to comprise a single conformational unit across all the β-turn types in the dataset. Likewise, it was observed that the relative positions of atom $\alpha C_{(2)}$, bond 3 and bond 4 varied only slightly between the different types of β-turn and these therefore also constitute a single conformational unit. In fact, the only significant difference between the various conformations of the β-turns, with respect to bonds 1, 2, 3 and 4, appeared to be in the dihedral angle between these two conformationally invariant units. This conformational simplification is directly attributable to the planar, *trans* nature of the intervening peptide bonds. For instance, when $\phi_2 = \psi_2 = 0°$, the angle defined by $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$ is 61° and when $\phi_2 = \psi_2 = 180°$, this angle is 158° (Figure 2(b,c)). Even though this angular difference is large, the *projection* of bond 1 relative to bond 2 does not vary at all and the distances $C_{(1)}$-$\alpha C_{(2)}$, $\alpha C_{(1)}$-$\alpha C_{(2)}$ and $\alpha C_{(2)}$-$\alpha C_{(3)}$ also remain

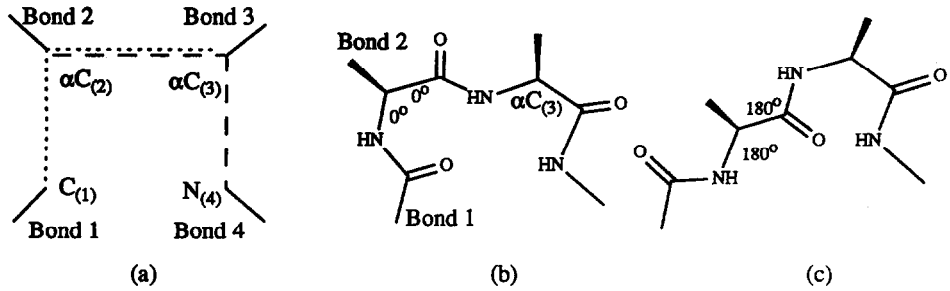


Figure 2. Topographical simplification of the β-turn. We observed from Dreiding models that the β-turn could be reduced to 2 conformational units with respect to bonds 1, 2, 3 and 4. As shown in (a), one unit is defined by bonds 1 and 2 and atom $\alpha C_{(3)}$ and the second by bonds 3 and 4 and atom $\alpha C_{(2)}$. The basis for this is shown in (b) and (c). Even though in (c) the chain is fully extended between bond 1 and $\alpha C_{(3)}$ while in (b) it is fully contracted, the projection of bond 1 relative to bond 2, and the $C_{(1)}$ to $\alpha C_{(2)}$ and $\alpha C_{(3)}$ to $\alpha C_{(4)}$ interatomic distances do not change.

invariant at 2.43 Å, 3.80 Å and 3.80 Å respectively for any combination of ϕ_2, ψ_2 and ϕ_3. Moreover, the allowed variation in this angle is much less in β-turns because of the primary selection criterion that the $\alpha C_{(1)}$-$\alpha C_{(4)}$ distance ≤ 7 Å. The standard definitions for the β-turns in Figure 1(b), for example, restrict the angular variation of $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$ to 89 ± 7°, the minimum value of which is occupied by β-turn types I, I′, III and III′, and the maximum value of which is occupied by types II and II′.

Similar reasoning can be applied to the second conformational unit, which is close to a geometrical mirror image of the first conformational unit. The theoretical extremes in the angle $\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ are 65° for $\phi_3 = \psi_3 = 0°$, and 154° for $\phi_3 = \psi_3 = 180°$. This variation is restricted to 85 ± 7° by the definitions in Figure 1(b), the minimum value of which is occupied by β-turn types II and II′ and the maximum value of which is occupied by β-turn types V and V′. Likewise, the *projection* of bond 4 relative to bond 3 does not vary at all and the distances $N_{(4)}$-$\alpha C_{(3)}$, $\alpha C_{(4)}$-$\alpha C_{(3)}$ and $\alpha C_{(2)}$-$\alpha C_{(3)}$ remain invariant at 2.51 Å, 3.80 Å and 3.80 Å respectively for any combination of ψ_2, ϕ_3 and ψ_3.

To see whether the above observations - which are based only on ideal β-turns of types I, I′, II, II′, III, III′, V and V′ - could be applied to β-turn types IV, VI and VII and to non-ideal (distorted) β-turns of all types, we extracted and studied 146 examples of β-turns from the literature.

Computer-aided superimposition of 146 examples of β-turns extracted from the literature

The program CRYS-X[56] was used to generate a database of tetrapeptides (L-alanine was used for the sake of simplicity) in 146 different β-turn conformations using data listed in the literature[3]. The first 20 sequential examples of each type of β-turn were selected, except for those types for which data were lacking (namely I′ (13 examples), III′ (13 examples), V (3 examples), V′ (4 examples), VI (6 examples) and VII (7 examples)).

Through the use of computer graphics, all except for the *cis*-proline-containing type VI β-turns were superimposed, as shown in stereo in Figure 3. All superimpositions involved a rigid, least-squares fit and were performed using the program A-LOOK (CRYS-X and A-LOOK are programs within the MORPHEUS[56] software package). For the sake of clarity, only bonds 1, 2, 3 and 4 are illustrated. In (a), the template of superimposition is defined by atoms $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$ using the average angle for all examples of 93°. Immediately obvious are the relatively tight conical clusters formed by bonds 1 and 2 which result from slight variations in the shape of the first conformational unit. The variation in the *projection* of bonds 3 and 4, which are part of the second conformational unit, relative to bonds 1 and 2, is clearly very large. The fact that bond 4 is similarly projected and positioned relative to bond 3 in all β-turns is illustrated in (b). The

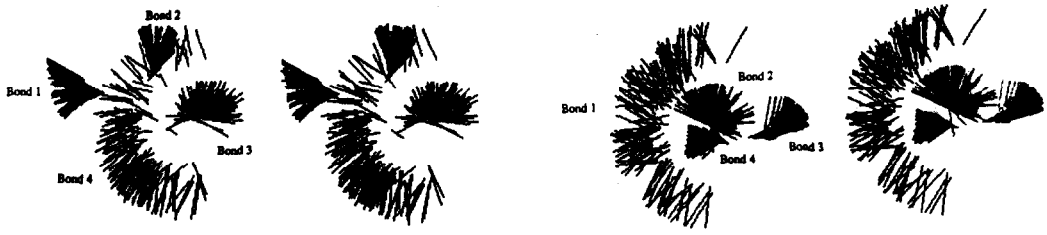


Figure 3. Stereoview of the superimposition of 146 β-turns. In (a), the template for superimposition is defined by $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$ set in the average angle of 93°. In (b), the template for superimposition is defined by $\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ set in the average angle of 90°. For the sake of clarity, only bonds 1, 2, 3 and 4 are shown.

template of superimposition here is defined by atoms $\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ using the average angle for all examples of 90°. The tight conical clusters of bonds 3 and 4 illustrate the similar shape of the second conformational unit across all the β-turns. Obvious again is the great variation in the dispositions of both bonds 1 and 2 relative to bonds 3 and 4.

Based on these observations, the variable dihedral angle $\underline{\beta}$ ($C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$) was then defined[57] which describes the twist of one conformational unit relative to the other, and therefore the topographical footprint of any β-turn. The relationship between $\underline{\beta}$ and the standard β-turn types is shown in Figure 4. There

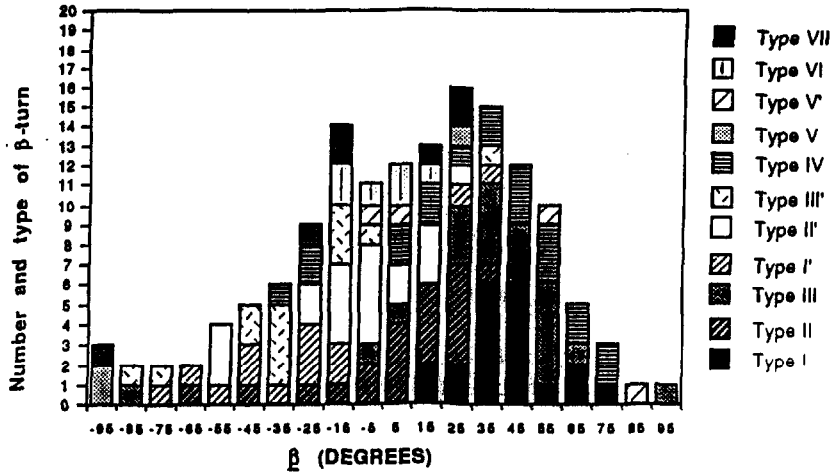


Figure 4. Correlation of the dihedral angle $\underline{\beta}$ ($C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$) with the β-turn type for a series of 146 β-turns. The relative conformation of bonds 1, 2, 3 and 4 are different for type VI β-turns because they contain a *cis*-peptide bond, but the variable $\underline{\beta}$ is still useful to describe the degree of planarity of the turn and so these β-turn types are included in this figure. Each column spans 10° such that, for example, the column at -5° represents the range from $\underline{\beta}$ = -9° to 0°.

is clearly significant topographical overlap between all of the β-turn types and the inadequacy of the peptide backbone classification to provide a description of the twist that a β-turn imparts to the peptide chain is thus clearly brought out. Type III β-turns, for example, span the large range $\underline{\beta}$ = -85° to 95°. A general preference for a small, positive value of $\underline{\beta}$ can be seen in Figure 4. In a randomly chosen database of β-turn examples,

this bias would be accentuated, since this domain is dominated by types I, II, III and IV β-turns, all which are underrepresented in the database of 146 β-turns. For the purposes here, β-turn examples were deliberately extracted according to their type. In a randomly chosen database of 146 β-turns from a pool of 421, one would statistically expect to extract only one example of the relatively rare type V β-turn, for example, but 61 examples of type I β-turns. This is clearly inappropriate when it is information about the conformational behaviour within each type as well between different types of β-turn, that is needed.

As a further illustration of the simplification brought about by the use of $\underline{\beta}$, the eight sub-parts of Figure 5 show the superimpositions of the β-turns which comprise each column of Figure 4 for $\underline{\beta}$ = -15 to 55°. In

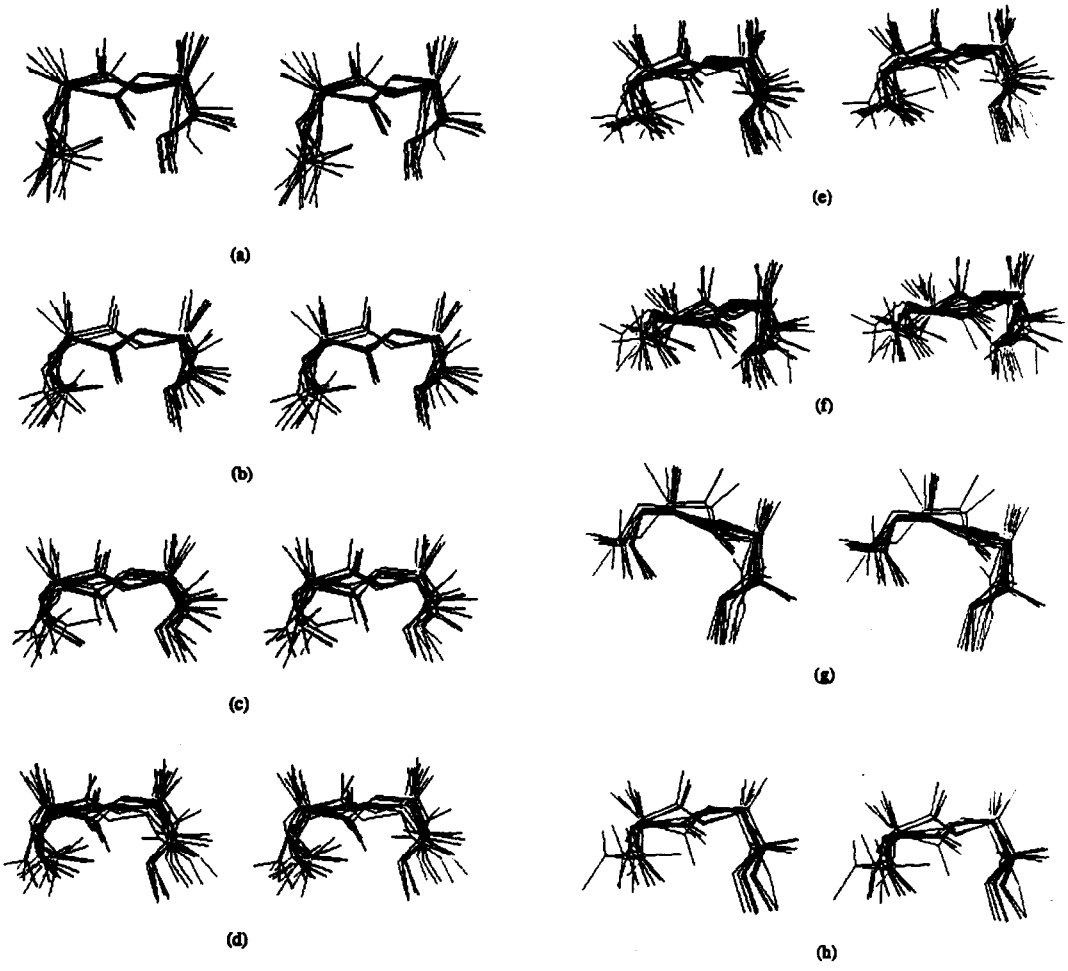


Figure 5. Stereoview of the superimposition of β-turns which comprise the columns in Figure 4 for which $\underline{\beta}$ = -15 to +55°, each of which contain 10 or more examples. The template for superimposition is defined by $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ set in the appropriate $\underline{\beta}$ value for each column, and with angles $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$ and $\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ set in the calculated averages for each column. For the sake of clarity, all hydrogen atoms have been deleted. The orientation is such that bond 1 lies on the bottom left. Bond 1 is not to be confused with $C_{(1)}$-$O_{(1)}$, in which case its conformational variation will appear artificially great. To aid interpretation, bonds 1 to 4 are just discernably lighter in shade than the connecting bonds.

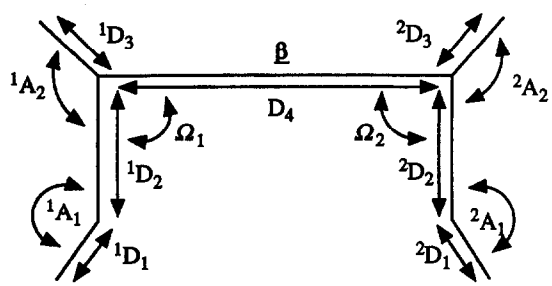
each case, the template for superimposition is defined by $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ set in the appropriate value of $\underline{\beta}$ for that column, with the angles $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$ and $\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ set to the average value for that particular column. These columns were chosen because each contains 10 or more examples or individual β-turns and thus are more meaningful in displaying any trends. All type VI β-turns have been omitted from the calculations and figures. A brief discussion of Figure 5(e), which constitutes the greatest number of examples as well as types of β-turn, is given in the next paragraph.

There are sixteen examples of β-turns in which $\underline{\beta}$ is 21-30° (Figure 5(e)). The template for superimposition is defined by $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ with $\underline{\beta}$ set to 25° and with angles $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$ and $\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ set to the averages of 96° and 90°, respectively. The large variation in the geometry of the peptide backbone is witness to the fact that eight different classical types of β-turn are present (I, II, III, I′, II′, IV, V and VII), yet the relative dispositions of bonds 1, 2, 3 and 4 are very similar indeed. The untidy problems of ill-defined β-turn types, of which there are two in this sample (IV and VII), and of non-ideal β-turns, of which there are three examples here (one a type V, one a type I′, and the other a type II′), are eliminated when $\underline{\beta}$ is used as a topographical descriptor. Analogous remarks apply to the other parts of Figure 5.

The type VI β-turns are included in Figure 4 because $\underline{\beta}$ is still useful to describe the planarity of the β-turn and it can be seen that these β-turns span the meagre range of $\underline{\beta}$ = -19° to +20°. These turns have not been included in any of the superimpositional figures so far, however, by virtue of other geometrical differences induced by the presence of the *cis*-peptide bond. For example, the $\alpha C_{(2)}$-$\alpha C_{(3)}$ distance, although invariant, is 3.01 Å and significantly shorter than the corresponding distance of 3.80 Å present in other β-turns. As will be seen in the next section, sidechain disposition is also quite different.

Geometrical relationship between bonds 1, 2, 3 and 4

The distances (D_x), angles (A_x and Ω_x) and dihedral angles (T_x) needed to fully describe the geometry of the two "invariant" conformational units (bond 1-bond 2-$\alpha C_{(3)}$ and $\alpha C_{(2)}$-bond 3-bond 4) are defined Figure 6, and their values given in Table 1. From Table 1 it can be seen that there are major geometrical differences



$^1T_1 = \alpha C_{(1)}$-$C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$
$^2T_1 = \alpha C_{(4)}$-$N_{(4)}$-$\alpha C_{(3)}$-$\alpha C_{(2)}$
$^1T_2 = \beta C_{(2)}$-$\alpha C_{(2)}$-$C_{(1)}$-$\alpha C_{(3)}$
$^2T_2 = \beta C_{(3)}$-$\alpha C_{(3)}$-$N_{(4)}$-$\alpha C_{(2)}$
$\underline{\beta} = C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$
$\underline{\epsilon} = \alpha C_{(1)}$-$C_{(1)}$-$N_{(4)}$-$\alpha C_{(4)}$

Figure 6. Definitions of the distances (D), angles (A, Ω), and dihedral angles (T) needed to fully describe the geometry of the two conformational units shown in Figure 2(a). Whether the symbol is part of the description for conformational unit 1 or 2, is denoted by superscript 1 or 2 respectively. D_4 is common to both and thus has no superscript. A common subscript numeral is assigned to symmetrical counterparts between the two units. Also defined are $\underline{\beta}$ (see Figure 4) and $\underline{\epsilon}$. The latter value describes the dihedral angle between bonds 1 and 4. See Figures 1(a) and 2(b) for atom assignment. Bond lengths $^1D_1$, $^2D_1$, $^1D_3$ and $^2D_3$ are defined only for the sake of completion and are 1.51, 1.46, 1.54 and 1.54Å respectively.

between type VI β-turns and all other β-turns. For instance, the shape of the triangular template defined by $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$ for unit 1, from which bonds 1 and 2 project, is quite different between the two groups of β-turns because of D_4. In addition, bond 2 projects quite differently from this same triangular template between the two groups of β-turns as witnessed by large differences in the two $^1T_2$ values. Similar remarks apply to the second conformational unit, where the respective $^2T_2$ values (which describes bond 3 projection)

Table 1. Dimensions of β-Turns and some β-Turn Mimics[a].

Structure(s)[b]	$^1D_2$	$^1A_1$	$^1A_2$[c]	Ω_1	$^1T_1$	$^1T_2$[c]	$^2D_2$	$^2A_1$	$^2A_2$	Ω_2	$^2T_1$	$^2T_2$	D_4	$\underline{\beta}$	$\underline{\epsilon}$	rms[d]
General β-turns	2.43	147	109±29	101±35	0-360	233±41	2.43	156	113±27	108±35	0-360	128±37	3.80	-2±96	5±108	-
Type VI β-turns	2.43	147	122±15	121±14	136±31	164±23	2.51	156	115±14	85±11	31±100	172±6	3.01	2±16	28±25	-
Trans1	2.67	101	90(140)	86	124	263(145)	2.68	139	140	67	168	138	3.78	18	95	0.50
Trans2	2.61	139	142(98)	74	192	225(93)	2.64	138	148	80	197	102	3.80	-12	-21	0.45
Trans3	2.64	138	137(109)	76	178	269(126)	2.62	140	143	79	182	143	3.81	3	0	0.40
Trans4	2.67	98	93(141)	85	141	229(107)	2.61	131	149	72	172	107	3.77	0	84	0.43
Cis1	2.60	140	139(90)	75	190	181(68)	2.63	141	137	92	211	197	3.10	-11	-29	0.63
Cis2	2.63	142	116(81)	89	118	171(70)	2.68	140	121	85	250	191	3.05	-1	-5	0.35
Type VI mimic	2.46	151	129	107	181	170	2.53	152	137	104	176	191	2.52	7	1	0.54

[a]See Figure 6 for key to symbols. Distances (D) are in angstroms, angles (A, Ω) and dihedral angles (T, $\underline{\beta}$, $\underline{\epsilon}$) in degrees. Median values (with variation) are given for variable dimensions.

[b]"General β-turns" are all types but VI, while "Trans1" up to "Cis2" are all conformers of a single β-turn mimic studied by Olson *et al.*[59], using their terminology. For our purposes, a C3 hydrogen atom was replaced with a carbon, such that C3 became an R chiral centre. The "Type VI mimic" is a spirocyclic compound designed by Paul *et al.*[24]. For our purposes, we we replaced the pyrrolidone methine hydrogen atom with a carbon.

[c]Parenthetic values correspond to the epimer[59] with respect to C3 ("bond 2"), generated by passive modification of a C3 hydrogen atom to a carbon such that C3 became chirally S.

[d]Root mean square value of bonds 1 to 4 of mimic superimposed (least squares fit) on corresponding bonds in template. For "Trans" conformers the general β-turns template was used, whilst for "Cis2" and "Type VI mimic", the type VI template was used. The rms value for the "Cis1" conformer was the same for both templates. For the general β-turns template, the median values as listed were used, except for $^1T_1$, $^2T_1$ and $\underline{\beta}$, which were set to the values found for the particular conformer being superimposed. Also, Ω_1 and Ω_2 were set at 85° and 80° respectively rather than the respective median values of 101° and 108° in order to obtain a better compromise between goodness of fit and proportion of β-turns mimicked (see Figure 7). Similar remarks apply to the use of the type VI β-turns template, with Ω_1 and Ω_2 set to 108° and 78° respectively for "Cis1" and "Cis2" comparisons, and 108° and 85° respectively for the "Type VI mimic" comparison.

are also mutually exclusive.

Another point of interest is the relative lack of variation not just between the two conformational units ($\underline{\beta}$ = 2 ± 16°) but also within each conformational unit for the type VI β-turns with respect to $^1A_2$, Ω_1 and $^1T_2$ for unit 1 and $^2A_2$, Ω_2 and $^2T_2$ for unit 2. This is despite the fact that, for example, Ω_1 can hypothetically vary from approximately 21° (for $\phi_2 = \psi_2 = 0$) to 198° (for $\phi_2 = \psi_2 = 180°$) (c.f. Figure 2(b,c)) and Ω_2 from approximately 80° (for $\psi_3 = 60°$) to 136° (for $\psi_3 = -120°$) for these β-turns. It would thus appear that in reality the presence of the *cis*-Pro greatly restricts the number of energetically-allowed conformations that obey the $\alpha C_{(1)}$-$\alpha C_{(4)} \leq 7$Å rule. Despite the observed variation in $^2T_1$ for these β-turns, relatively little conformationally space is explored by bond 4 because of the obtuseness of $^2A_1$.

The geometrical variation within each conformational unit for the rest of the β-turns - the *trans*-peptide bond-containing β-turns - is generally greater than that for the *cis*-proline-containing (type VI) β-turns. There is a not unexpected difference between the theoretical variation in the angles Ω_1 and Ω_2 as discussed earlier (see Figure 2), which were found to be 89 ± 7° and 82 ± 7° respectively, based on the standard β-turn definition, with those actually found (108 ± 35° and 101 ± 35° respectively). However, rather than being evenly distributed, we found that 89% of the β-turns had $\Omega_1 = 90 \pm 20°$ (Figure 7(a)) and 88% had $\Omega_2 = 90 \pm 15°$ (Figure 7(b)). Since β-turn types I, II, III and IV dominate both of these regions, it would be expected

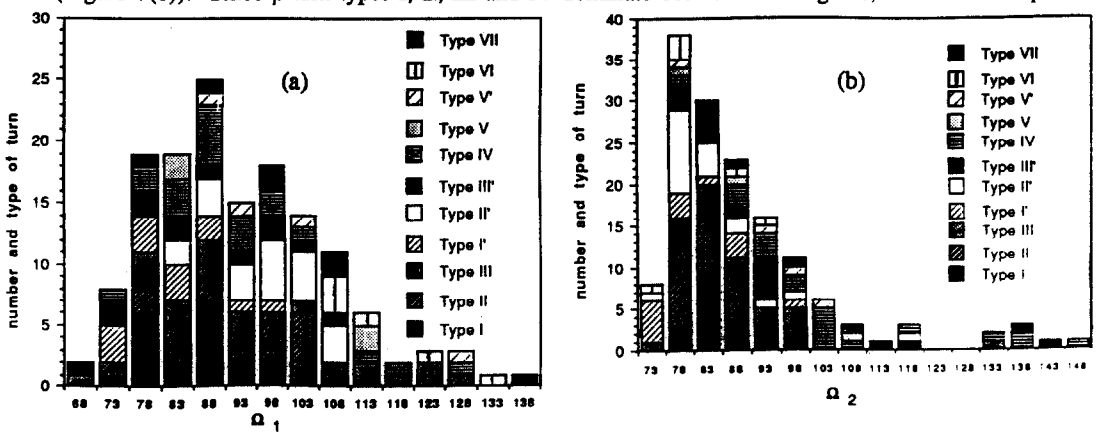
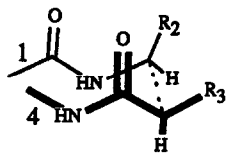


Figure 7. Distribution of (a) the angle $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$, defined here as Ω_1, and (b) the angle $\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$, defined here as Ω_2, amongst 146 β-turns extracted from the literature[3].

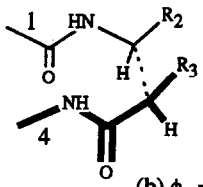
that in a randomly chosen database, as explained earlier, this uneven distribution would be even further exaggerated.

The dihedral angles $^1T_1$ and $^2T_1$ vary greatly, in this case 360° (Table 1), but relatively tight clusters result in Figure 3 for bonds 1 and 4, because, as for the type VI β-turns, the angles $^1A_1$ and $^2A_2$ are so obtuse. This geometrical leniency nevertheless allows for an observable difference between a given value of $\underline{\beta}$ and the dihedral angle formed between bonds 1 and 4. The source of this difference lies in the ability of the ψ_2 and ϕ_3 torsion angles to counteract the effect of changes in the ϕ_2 and ψ_3 torsion angles, so that in two β-turn conformers, the $\underline{\beta}$ value could be the same, but the dihedral angle between bonds 1 and 4 could be different. For instance, consider the two cases in Figure 8 in which (a) $\phi_2 = \psi_3 = 90°$ and (b) $\phi_2 = \psi_3 = -90°$. In (a), the dihedral angle between the entry (bond 1) and exit (bond 4) peptide bonds, which has been termed $\underline{\epsilon}$ by Hughes[58], is approximately -30°, whereas in (b), the value is approximately 30°, even though $\underline{\beta} = 0°$ in each case. A comparison of $\underline{\beta}$ and $\underline{\epsilon}$ in the 146 β-turns in Figure 9 shows the expected loosely proportional relationship between these two continuous variables, with the value of $\underline{\epsilon}$ increasing with $\underline{\beta}$.

The dimensions (including $\underline{\epsilon}$) of two particularly interesting β-turn mimics reported in the literature[24,59] are also contained in Table 1. Four (Trans1 to Trans4) of the six (Trans1 to Cis2) low energy conformers of the mimic reported by Olson *et al.*[59] superimpose very nicely on the general β-turns template, with rms

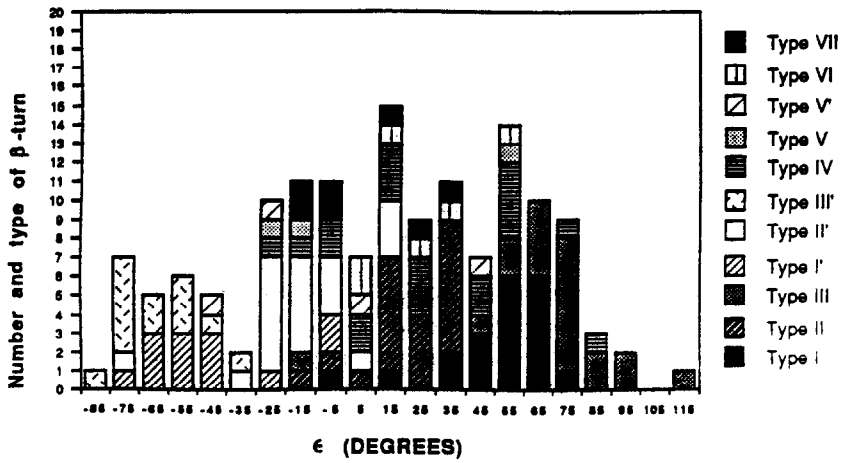


(a) $\phi_2 = \psi_3 = 90°$, $\epsilon \sim -30°$

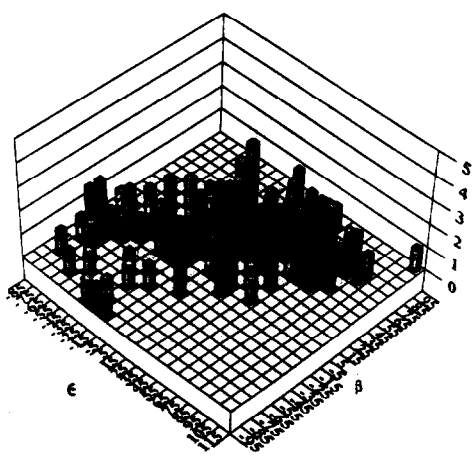


(b) $\phi_2 = \psi_3 = -90°$, $\epsilon \sim +30°$

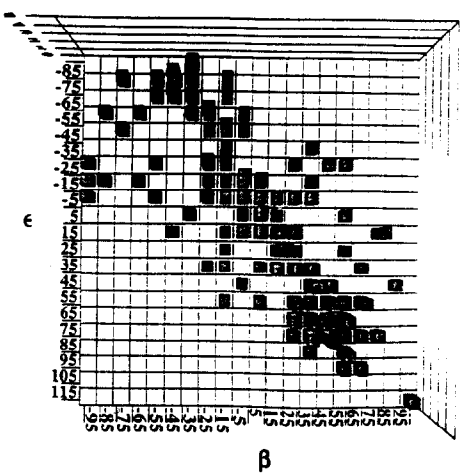
Figure 8. Schematic illustration of how two β-turns, each with a $\underline{\beta}$ value of 0°, can differ significantly in the dihedral angle formed by bonds 1 and 4, which has been termed[58] $\underline{\epsilon}$. The backbone atoms joining each segment have been omitted, for the sake of clarity.



(a)



(b)



(c)

Figure 9. Comparison of $\underline{\epsilon}$ with (a) the standard β-turn types, and for (b) and (c), with $\underline{\beta}$ (from two different perspectives). In (b) and (c), $\underline{\epsilon}$ and $\underline{\beta}$ are in degrees and the vertical axes represent the number of β-turns.

values ranging from 0.43 to 0.50 for an eight atom fit. They are all fairly planar with respect to $\underline{\beta}$, but as a

family explore different extremes of $^1A_2$ and $^1T_2$ (which describe bond 2 projection) and $^2A_1$ and $^2T_2$ (which describe bond 3 projection). Since all conformers are within 2.5 kcal/mol of each other[59] (approximately the equivalent of an "average" hydrogen bond[60]), this molecule should be able to mimic a large proportion of β-turns, the appropriate conformer being filtered out by receptor requirements. Correlation of activity with relative conformer stability of subtly altered analogues would give further insight into active site topography. On the other hand, complete inactivity of an analogue containing this molecule would direct attention to the values of $\underline{\beta}$, Ω_1 or Ω_2 in the mimic, which are clearly quite different to those observed in some β-turns (see Figures 4 and 7). Although substantial conformational flexibility of receptors is known[61,62], Ω_1 and Ω_2 values could clearly still have a great bearing on activity if small sidechains on residues 1 and 4 (e.g. methyl, hydroxymethyl) are crucial for receptor interaction.

Interestingly, the Cis2 conformer superimposes very nicely on the type VI β-turn template (rms 0.35 over eight atoms). When combined with the the type VI β-turn mimic reported by Paul *et al.*[24], these two molecules should be powerful probes for type VI β-turns. Note that even though the median value of $\underline{\epsilon}$ is 28°, three (50%) of the type VI β-turns have $\underline{\epsilon}$ in the 3-12° range (see Figure 9), which matches nicely with the corresponding values for the Cis2 conformer (-5°) and type VI β-turn mimic (1°).

As a final note, correlation studies of $\underline{\beta}$ with the tetrapeptide sequence might prove useful in simplifying the design of a constraint library.

SUMMARY

Dissatisfaction has been expressed[1] about the ability of the unwieldy traditional β-turn classification to reveal aspects important for molecular recognition, knowledge about which is vital for efficient construction of mimetic compounds. Recent works[63-65], in which novel approaches to the description of backbone conformation are discussed, give promise of new insights into peptide and protein secondary (and even tertiary[65]) structure, but β-turn sidechain disposition in these approaches remains obscure. In our investigation it is assumed that a greater role is played by the sidechains than the peptide backbone in receptor-ligand interactions[66]. We propose that all β-turns can be topographically described by the continuous variable $\underline{\beta}$ with respect to bonds 1, 2, 3 and 4, which defines the dihedral angle in the atom sequence $C_{(1)}$-$\alpha C_{(2)}$-$\alpha C_{(3)}$-$N_{(4)}$ and which has been found to vary from approximately -100° through 0° to +100° in a database of 146 β-turns extracted from the literature. This topographical simplification was made possible by the fact that the conformational relationship between bond 1, bond 2, and atom $\alpha C_{(3)}$ remains similar for any *trans*-peptide-containing β-turn, as does that between $\alpha C_{(2)}$, bond 3, and bond 4. The continuous variable $\underline{\beta}$ merely relates the dihedral angle between these two conformationally invariant units, the median geometries of which are given. In the case of the *cis*-proline-containing type VI β-turns, which were segregated from the other turns by virtue of distinct geometry, the dihedral angle $\underline{\beta}$ also completely describes the conformational relationship between bonds 1, 2, 3 and 4, in this case only varying from approximately -19° to 20°. The median geometries of the two conformationally invariant units in type VI β-turns were also given and were clearly different in geometry to the *trans*-peptide-containing β-turns.

It is anticipated that future use of $\underline{\beta}$ will facilitate the understanding of the physicochemical properties of β-turns and, when combined with the known relative geometry of bonds 1 to 4, will also provide a rapid means of designing or selecting appropriate conformationally constrained analogues for any peptide or protein β-turn. We are currently designing constraint libraries based on the principles outlined in this paper.

ACKNOWLEDGEMENTS

Many thanks go to Dr Mark Smythe for use of his software in the calculation of angles and distances and to Ms Tracie Ramsdale for help in generating some of the β-turn conformers. Thanks go also to our librarian Ms Jill Franklin for her time spent on online computer searching.

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