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A Hierarchical Approach to Peptidomimetic Design

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Introduction

Peptides are the primary means of intercellular communication in many diverse biological systems, but lack appropriate physical chemical properties and metabolic stability to be ideally suited as therapeutics. This has led to the concept of peptidomimetics, compounds which have different chemical structures, but still maintain the ability to interact with a specific peptide receptor. In other words, a compound which abandons the peptide backbone, but retains those essential chemical functionalities and the ability to display them in a characteristic three-dimensional pattern which is complimentary to the peptide receptor.

Because of the combinatorial possibilities of amino acid sequences, even a short peptide offers considerable potential as a unique message. The information content of peptides is not linearly encoded into the sequence, but depends on a three-dimensional, complimentary interaction with a receptor. This increases the difficulty in deciphering their unique messages which could then be exploited in the design of novel therapeutics. What has evolved over the last two decades is a hierarchical approach to the design of peptidomimetics, which still is undergoing improvements. By probing the recognition requirements of the receptor by chemical modification of sidechains and the conformational requirements necessary to orient those chemical functionalities in the correct three-dimensional arrangement, a paradigm for determining the receptor-bound conformation of peptides as a prelude to peptidomimetic design is available.

The hierarchical approach (Fig. 1) is based on probing the conformational and sidechain-functionality requirements of the receptor by single amino acid modifications. The information derived provides the basis for incorporation of β–turn mimetics and chimeric amino acids as cyclization points. The third phase is analysis of the pharmacophoric implications of the set of active compounds to determine the three-dimensional arrangement of critical sidechain and backbone functionality. The final phase is design and synthesis of non-peptide mimetics in which the peptide scaffold has been replaced by other organic moieties which position the crucial recognition elements correctly.

Conformational implications of individual amino acid residues.

The first systematic investigation of the influence of the structure of an individual amino acid residue on the conformational ensemble was that of Ramachandran. The plot of the two torsional variables, Φ (NH-Cα) and Ψ (Cα–CO), indicating energetically allowed combinations of the two backbone torsional angles adjacent to the α–carbon has become known as a Ramachandran plot. In order to accurately represent the experimental data when using the rigid geometry (fixed bond angles and lengths) approximation, the VDW radii of the atoms in the peptide bond must be modified by reducing their radii slightly in order to simulate valence angle flexibility(1). The rigid

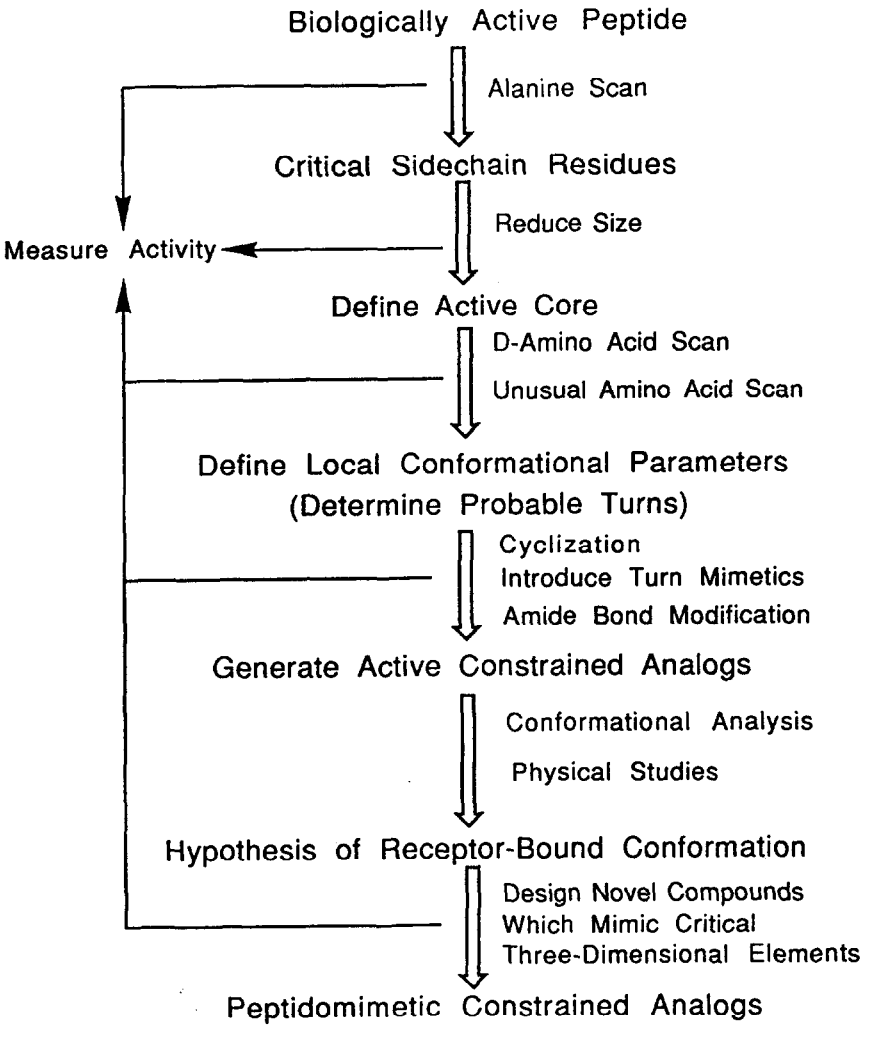

Fig. 1. A hierarchical approach to peptidomimetic design.

geometry approximation allows for more efficient exploration of the conformational space available to peptides and assessment of the conformational effects of chemical modification. Based on simple examination of the Ramachandran plots, amino acids fall into three basic categories; glycine which is the most flexible with most of the torsional area of the Ramachandran plot available, proline which has a cyclic constraint on the values of the torsional angle Φ and which is the restricted naturally occurring amino acid residue in proteins, and all of the other residues whose Ramachandran plots are nearly identical to that of alanine which has only 50% of the torsional space available to glycine.

While the areas outlined in Ramachandran plot indicate the most probable torsional values available to an individual residue, incorporation of that residue into a longer peptide allows for additional interactions which can modify the potential surface for the residue. A simple, but useful example, is the replacement of hydrogens on the peptide backbone by methyl groups(2). The effect can be quite dramatic; for example, the replacement of the carbon-α–proton by a methyl group to give the class of α–methyl amino acids. A simple way (Fig. 2) of looking at the restriction imposed is to consider that the α–methyl amino acid combines the steric effects of both L-alanine and D-alanine (a chimeric amino acid) and, thereby, reduces the area of a Ramachandran plot to approximately 1% centered on torsional values associated with turns and helices. This modification can be quite useful to impose a turn in a peptide and incorporation of several of these residues leads to helical peptides(3).

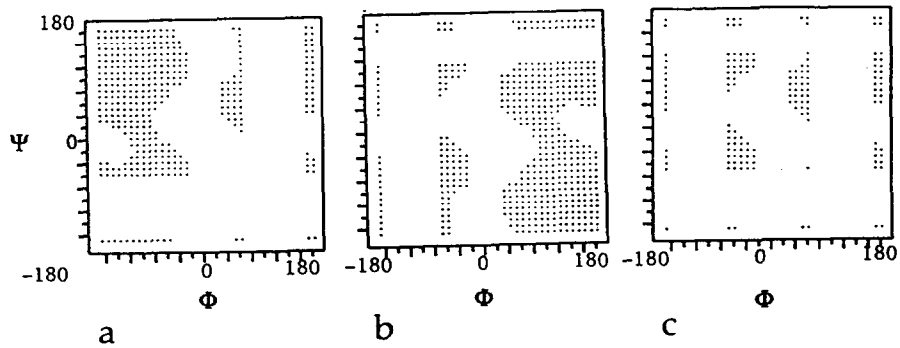

Fig. 2. Ramachandran plots for L-alanine (a), D-alanine (b) and α,α-methyl-alanine (c). Dotted areas indicate sterically allowed regions.

During the last two decades, conformationally constrained peptides have been synthesized and tested(4,5) in an effort to deduce the receptor-bound conformation. Other classes of amino acids which have proven useful in probing the conformational requirements of receptor recognition are N-methyl-amino acids, and dehydroamino acids in which the bond between the α– and β–carbons is unsaturated. In the latter case, the delocalized system introduces considerable rigidity(6) in the peptide backbone. The proclivity of dehydroamino acids to induce β–turns has been well documented by experimental studies(7-9). N-Methyl amino acids restrict torsional space(2) due to the increased steric bulk of methyl group and preclude participation of the amide as a hydrogen bond donor.

As the peptide chain is extended, it becomes feasible to hydrogen bond internally rather than with solvent and turns (β and γ) become probable(10). If these turns continue in a regular way, then regular structures result such as the α–helix. As these turns have been found to be common recognition motifs in biological systems(11), the design and synthesis of β–turn mimetics in which the sidechain functionalities of the amino acids in the second and third positions has become of increasing importance(12).

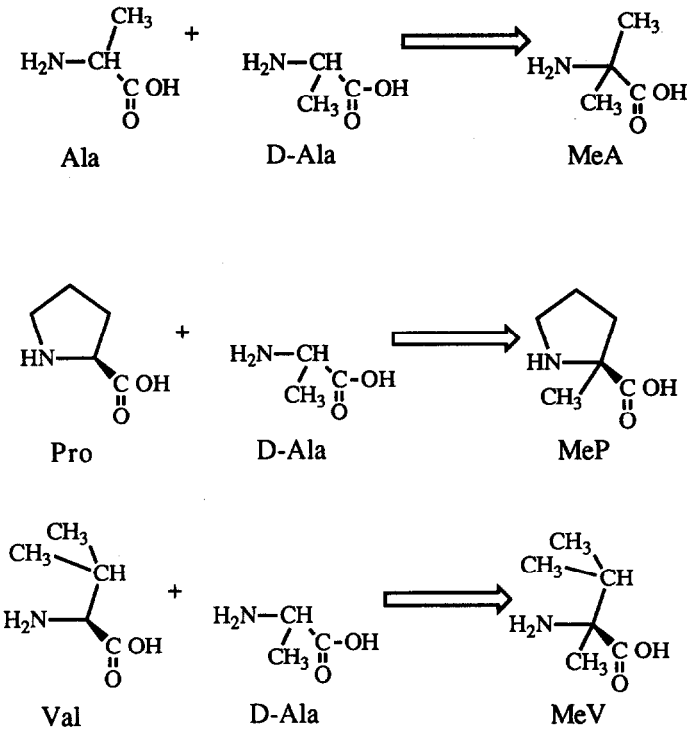

Fig. 3. Chimeric amino acids. Combination of L- amino acid and D-amino acid gives corresponding α-methylamino acid.

One strategy to assess both conformational and sidechain requirements is to systematically scan the peptide by incorporation of the same amino acid. Alanine scans help determine the relative importance of individual sidechains in recognition. A proline scan of sequence positions which tolerate Ala will determine if restricting the Φ torsional value at that position is acceptable. For those active proline analogs, the torsional angle Ψ can be probed by substitution of MeA which allows conformations which overlap proline allowed areas only at values associated with right-handed helices. The requirement for appropriate sidechain functionality in order to preserve recognition and allow activation of the receptor has led to the development of chimeric amino acids in which conformational properties are combined with sidechain requirements for recognition. One

example is α,α–dialkylamino acids such as MeF, MeP, MeY, and MeA (Aib) where D-Ala has been hybridized (Fig. 3) with Phe, Pro, Tyr, and Ala, respectively. The combined steric requirements of both a D- and an L-residue restrict the conformational preference of the backbone to torsional values associated with turns which form helices if continued by multiple substitution(3).

Replacement of each residue by its optical isomer provides useful information regarding possible turn positions as only certain turn types can accommodate both L- and D-residues and still place the amino acid side chain in the same relative position to the peptide. Once probable turn positions are located, then cyclization can be used to confirm the turn location and constrain the peptide backbone to a particular turn type.

Conformational effects of sidechain and backbone cyclization

Cyclization of peptides has been shown to possess utility(13-15) as a constraint (see review(16) by Toniolo of short-range cyclizations). One example is the stabilization of β–turns, which are well known secondary structures in peptides and proteins(10), and are common conformations for biologically active cyclic peptides(17). The biologically relevant conformation of somatostatin, for example, contains a β–turn with the two essential residues for biological activity occupying positions $i+1$ and $i+2$ of the turn.

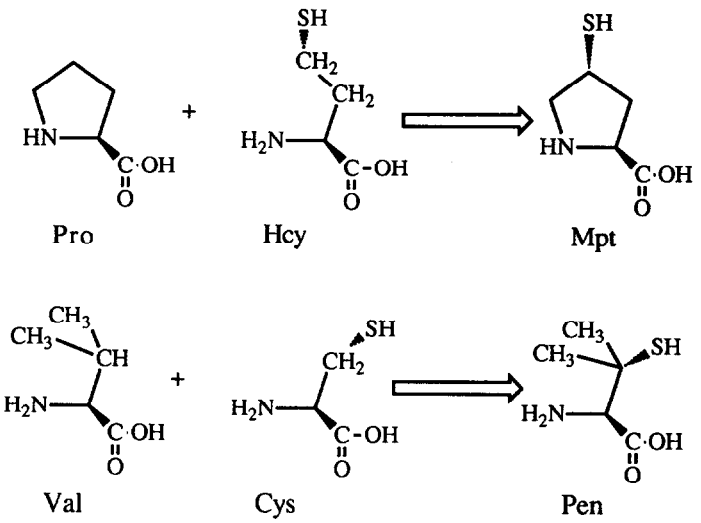

Fig. 4. Chimeric amino acids. Combination (top) of proline and homocysteine (Hcy) leading to *trans*-4-mercaptoproline (Mpt). Combination (bottom) of valine and cysteine leading to penicillamine (Pen).

In order to retain biological activity, cyclic constraints must influence the backbone conformation without compromising a crucial sidechain interaction with the receptor(18). This requirement has led to the development of chimeric, or hybrid, amino acids which add a functional group to the sidechain to allow cyclization while preserving the essential recognition or

conformational effects. Examples would be the use of penicillamine (Pen), a hybrid of Cys and Val, in enkephalin analogs(19) and the use of 4-mercaptoproline, a hybrid of homocysteine and proline, in the case (fig. 4) of angiotensin and bradykinin analogs(20).

Chimeric Proline Derivatives

Sidechain cyclization has been a popular approach to reduce conformational freedom; most examples do little to limit the backbone conformation to a discernible few because of the large ring size which normally results. In order to further constrain such systems, incorporation of a chimeric amino acid based on proline was conceived. One such chimeric proline derivative which has proven exceptionally useful in exploring(20,21) the receptor-bound conformations of angiotensin II and bradykinin has been 4-mercaptoproline (Fig. 4, Mpt, *trans* and Mpc, *cis*) which combines the conformational restrictions of Pro with the sulfhydryl function for introduction of sidechain cyclization. Other examples of proline chimeras which we are utilizing in our work for sidechain cyclization include 4-carboxy-Pro, 4-amino-Pro, and 3-mercapto-Pro. Analyses on the impact of these and other modifications on sterically allowed conformations offers a rationale for the choice of peptide modification to probe the conformational preference of the receptor-bound peptide.

Conformational analysis of cyclic peptides

One can understand intuitively that the introduction of a cyclic constraint restricts the torsional mobility of the peptide. It is not necessarily clear, however, what exact influence a cyclic constraint exerts on the conformation of the peptide backbone. Clarifying the effect of various cyclizations provides a rational basis for their use in determining the receptor-bound conformation of peptides by conformational structure-activity analysis.

Determination of all sterically allowed conformations of cyclic peptides in order to determine the set of candidate conformations(22,23) is still computationally daunting. In the conformational analysis of peptides, various techniques have been used: most commonly distance geometry(24), molecular dynamics(25) and systematic, or grid, search(26). Although distance geometry and molecular dynamics are widely used in the elucidation of solution conformations(24,25), questions about the adequacy of their sampling properties persist. Because all sterically allowed conformations are generated at the selected torsional grid parameters, systematic search methods should not have this limitation, assuming that the resolution of the sampling grid is sufficient. Kataoka *et al.*(27) have examined the conformational effects of sidechain cyclization and shown that introduction of a methylene group into c[Cys-Ala-Cys] by replacement of one Cys with homocysteine will increase the conformational freedom of the ring by a factor of twenty. Use of mercaptoproline analogs and the formation of a bicyclic ring system can severely restrict the conformational freedom of the system and lead to unique conformers observed in solution for the tripeptide portion. These calculations are relevant to the central tripeptide segment of angiotensin II, Val^3-Tyr^4-Val^5, which can be substituted with Cys^3-Tyr^4-Cys^5 with retention of significant activity(28,29). Mercaptoproline can successively be substituted for either of the Cys residues(20,21).

Peptidomimetics

Once turn positions are determined by the ability to successfully incorporate D-amino acids, α-methylamino acids or dehydroamino acids, then more elaborate turn mimetics(12) can be introduced. These are generally bicyclic structures which force the peptide to maintain a turn structure. The initial effort in this area was the lactam of Freidinger which has been successfully incorporated into a number of biologically active peptides(12). Unfortunately, one of the

sidechains involved in the turn is used to introduce the conformational constraint. Other β–turn mimetics are under intense investigation as witnessed by several articles in this issue and have recently been reviewed(12).

The role of each amide bond in a peptide in recognition also needs investigation. In particular, the *cis*-conformer of the amide bond cannot be ignored as a possible candidate for the receptor-bound conformation. When bombolitin I is bound to SDS micelles, an α-helix is induced which extends from residue 3 to 15(30). Bombolitin I shows an NOE between the alpha protons of residues Ile^1 and Lys^2, indicating a *cis*-amide bond between the two when bound to the micelle. This implies that the placement of the α-amino of Ile-1 and ε-amino of Lys^2 on the same hydrophilic face is energetically favored and overcomes the 2-3 kcal/mole *cis-trans* amide bond isomerization energy(30). Another example determined by crystallography of a peptide complex is a human Bence-Jones dimer(31) where a fragment of the chemotactic peptide, formyl-Met-Trp, actually binds with the amide bond in the *cis*-conformation, clearly not favored in solution. Dipeptide analogs can be introduced to stabilize a particular isomer of the peptide amide bond(32). The 1,5-disubstituted tetrazole ring system replacement for the amide fixes the amino acids in a *cis*-conformation (Fig. 5) and has been incorporated into several different peptide systems by Zabrocki and colleagues(33).In the case of three bradykinin analogs, [L-Pro^2ψ[CN4]-L-Ala^3]-BK, [L-Ala^6ψ[CN4]-L-Ala^7]-BK and [L-Ala^6ψ[CN4]-D-Ala^7]-BK, in which the peptide bond of a proline residue was replaced with the tetrazole surrogate significantly reduced the activity. In the

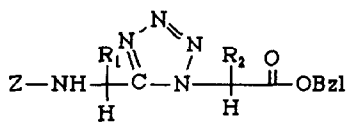

Fig. 5. A blocked dipeptide tetrazole analog in which amide bond is replaced (32).

case of an analog of the cyclic hexapeptide of somatostatin, significant biological activity was retained in accord with the proposed bioactive conformation(34). Alternatively, the amide bond can be replaced with a *trans*-double bond to test for recognition of that conformation. In both cases, negative results may be due to perturbation of the amide bond itself which may be crucially involved in recognition rather than to the conformational constraint imposed.

Receptor-Bound Conformation

In order to determine the receptor-bound conformation of peptides, one must reduce the degrees of freedom. Incorporation of unnatural amino acids allows one to probe the local conformational requirements for recognition and deduce the probable position of turns. These can be confirmed by stabilizing the turn with additional constraints such as disulfide bonds and incorporation of other peptidomimetic subunits as described above. In order to proceed further, one often assumes that the analogs bind to the receptor with a common conformation of the peptide backbone. In the case of enzymes, this may be a more tenable assumption than in the case of receptors which are more likely to recognize the three-dimensional arrangement of sidechains(11).

In the case of smaller biologically active peptides, such as thyroliberin (TRH), Glp-His-Pro-NH_2, or morphiceptin, Tyr-Pro-Phe-Pro-NH_2, the active analog approach(35) may be used with a series of analogs and the assumption of a pharmacophore. Nelson *et al.*(36) deduced a pharmacophore for morphiceptin at the opioid μ-receptor based on a series of morphiceptin analogs

containing constrained amino acids. Olson *et al.*(37) has used a pharmacophore model(38) of TRH to generate a non-peptide analog (Fig. 6) which is active in behavioral models. With the exception of the binding site analysis(39) on ACE inhibitors which are dipeptide analogs, the additional variables introduced by this approach would only complicate the difficult task with larger peptides.

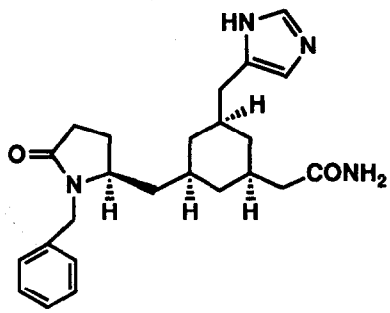

Fig. 6. Peptidomimetic analog of thyrotropin releasing hormone, Glp-His-Pro-NH_2.

Solution Conformation

The solution conformation of proteins can be determined experimentally by modern NMR techniques. In a few cases, such as somatostatin, insights from NMR proved useful in guiding the medicinal chemist in simplifying the structure. Rotameric averaging of sidechains in flexible small peptides minimize the informational content of NOE's and when combined with conformer averaging make determination of solution conformations of small peptides (under 20 residues) problematic. In the final analysis, the relevance of the solution conformations for systems where the conformation is sensitive to environment to the receptor-bound conformation is questionable, and its is generally advisable to wait until a more conformational constrained analog which retains activity is found before attempting to utilize NMR solution structures in the design process. As a case in point with regard to environmental effects on the conformation of what was felt to be a relatively rigid cyclic peptide, the recent history of the immunosuppressant cyclosporin A is instructive. The conformation of cyclosporin in the crystal and in various organic solvents has been determined. Recently, the conformation of cyclosporin complexed with its putative receptor, cyclophilin, a peptidylprolyl cis-trans isomerase or rotamase, has been determined(40) by transfer NOE NMR(41) and by a combination of NMR and crystallography(42,43). In solution in $CDCl_3$ or deuterated tetrahydrofuran, or during molecular dynamics simulations, or as determined in the crystal, this hydrophobic peptide maximizes its internal hydrogen bonding capability by forming a twisted β–sheet with one of the 7 N-methyl amide bonds in the *cis*-conformation. As the solvent becomes more polar (methanol or DMSO), several other conformers due to amide bond isomerism become populated. When bound to cyclophilin, all of the amide bonds assume the *trans*-conformation and the internal hydrogen bonds are broken in favor of a polar surface of amide hydrogens and carbonyls, some of which bind to cyclophilin. In effect, the structure has turned itself inside out in response to the more polar environment (Fig. 7).

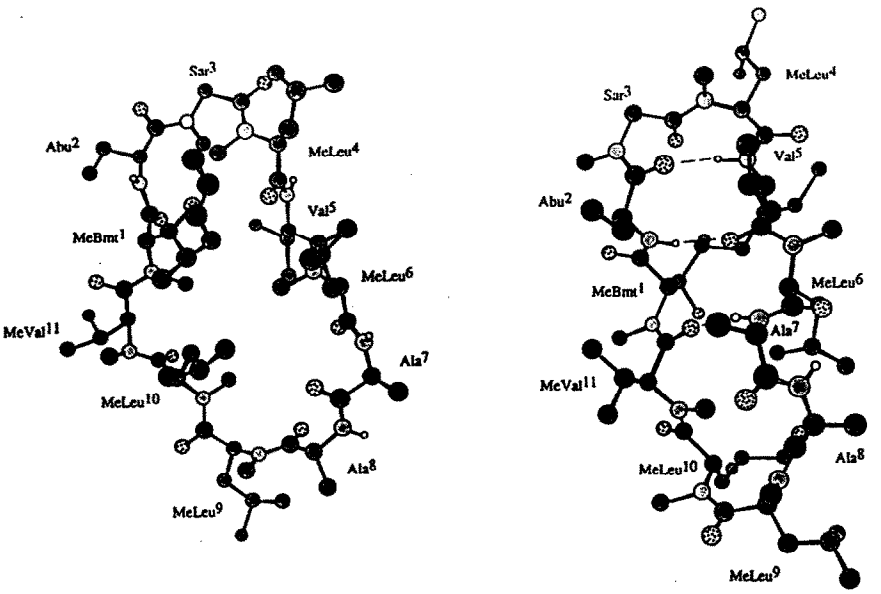

Fig. 7. Conformation of cyclosporin when complexed to cyclophilin (at the left), and conformation of cyclosporin as determined in crystal (at the right).

Examples of Applications

Several examples exist where the process of determining the receptor-bound conformation has been sufficiently demonstrated that a paradigm can be assumed. Angiotensin showed a pattern of substitution which clearly was indicative of a turn centered on residue 4(2,44). This was confirmed by the synthesis of 3-5 disulfide-bridged analogs(28,29). Incorporation of mercaptoproline into positions 3 or 5 further rigidified the peptide while maintaining the activity(20). A similar situation occurred with bradykinin where hydrogens along a stretch of backbone, residues 4 and 5, were not able to be substituted by methyl groups without serious loss of activity. When combined with the potent analog in which dehydrophenylalanine replaced Phe in position 5, a turn was strongly indicated which was confirmed by cyclization(20).

The work of the Merck group(15) in reducing the size of somatostatin, a tetradecapeptide, to a cyclic hexapeptide has been a model. SAR indicated that two sidechains were primarily involved in recognition and recent work by Hirschmann *et al.*(45) has shown that the cyclic peptide backbone can be replaced by a sugar ring (Fig. 8) with retention of the full spectrum of activity.

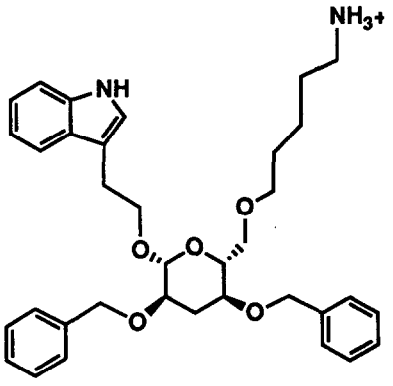

Fig. 8. Peptidomimetic analog of hexapeptide inhibitor of somatostatin (45).

Conclusions

While it remains non-trivial to convert a peptide into a steroid or terpenoid with retention of specific biological activity, a hierarchical approach based on the use of unusual amino acids and incorporation of cyclic constraints followed by pharmacophoric, or active site analysis, as a prelude to peptidomimetic design provides a useful, systematic paradigm for such problems. After this overview was completed, relevant reviews by Schiller (46), Huffman (47) and Kemp (48) have appeared.

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