Peptidomimetics and Peptide Backbone Modifications

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Abstract: The replacement of the amide bond in a peptide backbone is a widely used form of peptide mimicry. Several of the most common amide bond surrogates, including peptidomimetic work done in this laboratory, and their biological applications are presented in this review.

INTRODUCTION

Peptides and proteins play a crucial role in the transmission of information in biological systems. However, their utility as therapeutic agents is limited due to their susceptibility to protease degradation and their poor absorption through cell membranes. Efforts to alleviate these problems have fueled the growth of the now well-developed field of peptidomimetics. Different classes of peptidomimetics range from simple substitution of amino acids with unnatural residues [1, 2]. To the replacement of a peptide segment with a scaffold [3]. Some mimetics hardly resemble peptides, with the exception of side chain-like moieties attached to rigid core [4]. The purpose of these modifications is to 1) increase the selectivity and potency at the target site, 2) increase the longevity of the compound by reducing its likelihood of degradation, and 3) increase the lipophilicity so the compound will be more easily absorbed into biological systems. A common use of rigid scaffolds is to lock or strongly influence the conformation of the peptide pharmacophore in an effort to enhance binding to a specific target [3]. Some scaffolds are amenable for synthesis on a solid support, thus allowing for the creation of combinatorial libraries [5, 6]. Such peptidomimetic libraries have been useful in the elucidation of the spatial requirements of the binding sites for target receptors [7].

An important subset of peptide mimicry is the replacement of the amide with a bioisosteric group that resembles an amide without the drawbacks listed above. Amide bond surrogates range from simple olefinic groups to more sophisticated heterocycles. This review focuses on the various types of amide bond replacements and their biological targets. In addition, the peptidomimetic work performed in this laboratory is also reviewed. This section covers the investigation of α-aza-amino acids; β-amino acids; α-aminophosphinic acids; phosphonamidates, and their respective applications.

MODIFICATION OF THE PEPTIDE BACKBONE

Backbone modifications generally relate to the isosteric or isoelectronic replacement of amide functionality in the peptide chain and/or introduction of additional groups, as reviewed by Spatola [8]. Although there are quite a number of amide bond replacements reported in the literature, this review will focus on the most widely used surrogates, namely aminomethylene, oxomethylene, thiomethylene, ketomethylene, ester, sulfoxide, sulfonamide, thioamide, (*E*)-alkene, tetrazole, other heterocycles, and retro-inverso surrogates, as well as β-amino acids, α-aza-amino acids, α-aminophosphinic acids, and phosphonamidates, which will be discussed in detail in the later section of this review (Fig. (**1**)). It should be noted that each of these surrogates has its own unique physicochemical properties that need to be considered before incorporation into a peptide chain.

1. Aminomethylene, Ψ[CH_2NH]

The Ψ[CH_2NH] surrogate is one of the simplest isosteres of the amide bond. The Ψ[CH_2NH] moiety can be introduced by reductive alkylation with a preformed *N*-protected amino acid aldehyde in the presence of reducing agent such as $NaBH_3CN$ [9, 10]. Reduction of a peptide bond introduces a new basic center, as a form of a secondary amine group. This unnatural positive charge decreases the hydrophobicity of pseudopeptides [9]. In addition, the reduction of a carbonyl group to a methylene group enhanced the flexibility of pseudopeptides, and the acquired flexibility of pseudopeptides resulted in more rapid synthesis of cyclic pseudopeptides [9]. Interestingly, peptides containing the Ψ[CH_2NH] replacement demonstrated an increased cell permeability in addition to a heightened stability toward destructive enzymes [11].

On the other hand, conversion of the secondary amine of the Ψ[CH_2NH] moiety to the tertiary amine Ψ[CH_2NR] was performed by a second round of reductive alkylation, and significantly increased the receptor affinity of the alkylated pseudopeptide analogs of neurokinin [12].

2. Oxomethylene, Ψ[CH_2O] and Thiomethylene, Ψ[CH_2S]

Other simple amide bond replacements consist of a methylene group in place of a carbonyl group and the substitution of the amine functionality with other heteroatoms, such as oxygen and sulfur. Modification with oxomethylene offers a polar, flexible, proteolytically

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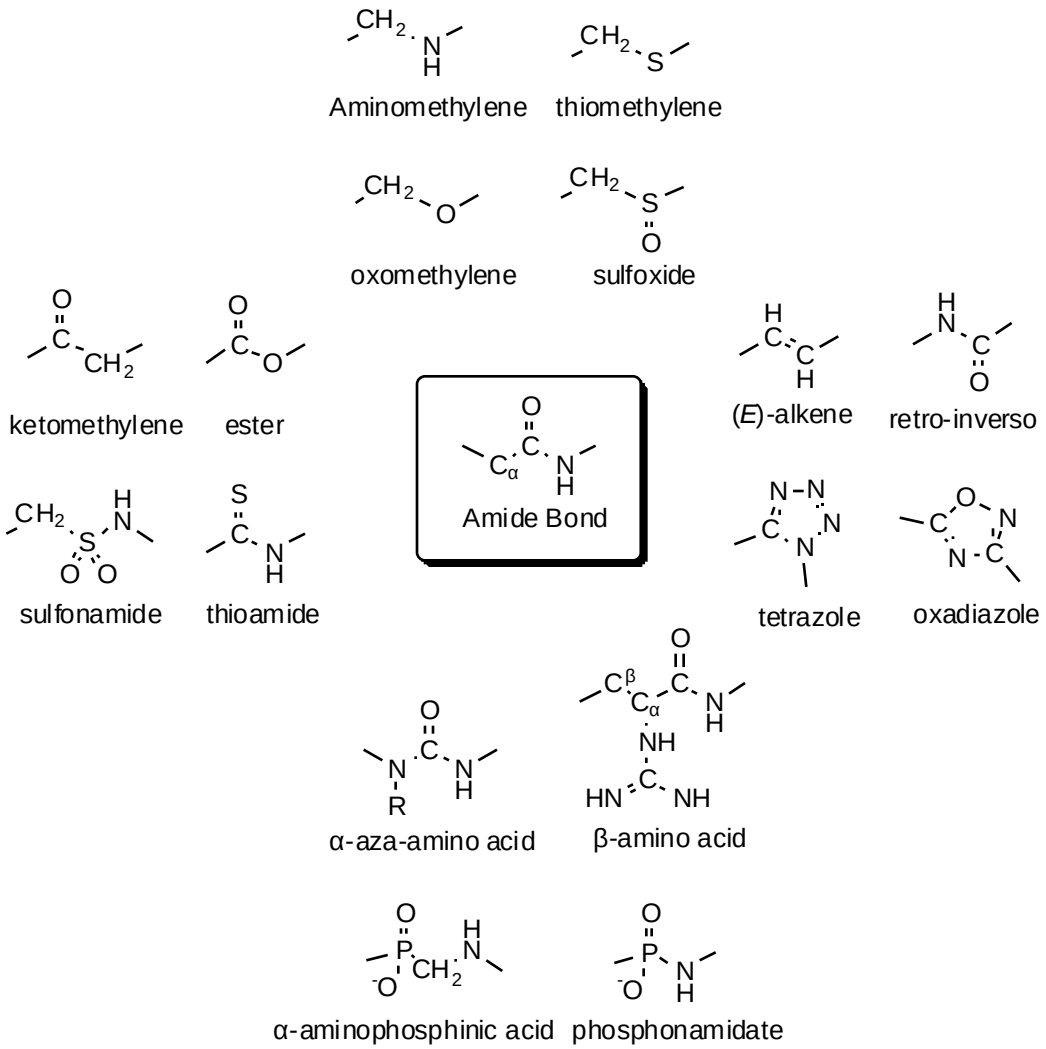


Fig. (1). Structures of peptide backbone replacements discussed in this review.

resistant surrogate to the amide bond. The calculated $C^{i}\alpha$-$C^{i+1}\alpha$ distances in the $\Psi[CH_2O]$ pseudodipeptide units are very close to those found in unmodified dipeptides, whereas these distances are longer for the thioether $\Psi[CH_2S]$ units, thus causing a greater distortion in the latter modification [13]. Furthermore, due to the reduced electronegativity and larger size of the sulfur atom, the $\Psi[CH_2S]$ modification is less polar and acts as a poor hydrogen bond acceptor. Conformational studies of model pseudopeptides containing $\Psi[CH_2O]$ using X-ray crystallography and 2D-NMR spectroscopy showed that the oxomethylene surrogate adopts a preferred orientation similar to the trans amide bond [14]. The synthesis of oxomethylene and thiomethylene groups usually consists of S_N2 displacement of α-bromo acid ester with the corresponding β-amino alcohol or β-amino thiol [13, 15].

3. Sulfoxide, $\Psi[CH_2SO]$

The simple oxidation of the thiomethylene ether to the (*R*)- and (*S*)-sulfoxides generates a new amide surrogate which contains a new chiral center. The sulfoxide provides a strong hydrogen bond acceptor and retains enzymatic stability [16]. Based on a conformational study by X-ray crystallography and NMR spectroscopy, it is believed that a sulfoxide-containing pseudopeptide induces conformational changes that are distinctly different from its sulfide $\Psi[CH_2S]$ precursor. Spatola *et al*.. have synthesized a cyclic peptide incorporating both $\Psi[CH_2NH]$ and $\Psi[CH_2S]$ to behave as a hydrogen bond donor and acceptor, respectively, as a means of stabilizing the conformation through hydrogen bonding between those functionalities [17].

4. Ketomethylene, $\Psi[COCH_2]$

Replacement of the amide nitrogen with a methylene group yields the ketomethylene surrogate. Nucleophilic attack at the carbonyl carbon by an active site serine hydroxyl group forms the tetrahedral intermediate which resembles a transition state intermediate. Also, the ketomethylene group is not prone to proteolytic degradation. A series of peptidomimetics containing amide bond replacements were synthesized and assayed for their ability to inhibit thrombin. Those with the ketomethylene component demonstrated the best inhibitory activity [18].

5. Ester, Ψ[COO]

The exchange of the amide functionality with an ester results in a depsipeptide. An ester has many structural similarities to amide bonds in that they are planar, possess similar bond angles and lengths, and strongly favor the trans conformation. The lack of hydrogen bond donating properties and the decreased ability of the carbonyl oxygen to act as a hydrogen bond acceptor are the most significant distinctions between amides and esters. Recently, amide bonds have been replaced by ester bonds in order to evaluate the role of the backbone hydrogen bonding in an α-helix [19, 20]. Similarly, the importance of backbone amide groups in proteins forming ion channels was examined through ester bond replacement. This substitution perturbed the subunit folding and dimer assembly in addition to any effects on ion permeation [21].

6. Sulfonamide, Ψ[CH_2SO_2NH] and Thioamide, Ψ[CSNH]

The sulfonamide group was also investigated. A positional scan of Leu-enkephalin in which each peptide bond was systematically replaced with a sulfonamide moiety [22], demonstrated an increased stability toward protease-catalized degradation [23]. Thioamides Ψ[CSNH] have also been used as amide bond surrogates in a number of systems, including cyclosporin [24], Leu-enkephalin [25], and inhibitors of angiotensin-converting enzyme inhibitor [26], and HIV-1 protease inhibitor [27]. The thioamide replacement also increases proteolytic stability and restricts the allowable ϕ and ψ angles in the vicinity of the thioamide linkage.

7. Trans-Carbon-Carbon Double Bond Isostere, Ψ[(E)-CH=CH]

Amide bonds play an important role in determining peptide conformation through restricted rotation resulting from partial double bond character and potential hydrogen bonding interactions. Among the many amide bond surrogates studied, the *trans*-carbon-carbon double bond best mimics the transoid nature of the amide bond in terms of rigidity, bond angle and bond length [28]. Therefore, Ψ[(*E*)-CH=CH] analogs provide valuable information concerning the role of each amide bond in a peptide. The synthesis of trans double bond analogs is often carried out by using Wittig chemistry [29]. A novel polymeric Horner-Wadsworth-Emmons reagent was used for the synthesis of alkenylaziridines, which were subsequently treated with organocopper reagents on solid support to produce (*E*)-alkene amide bond surrogates [30]. The (*E*)-alkene amide bond replacement was utilized for the synthesis of cholecystokinin analogs and several pseudopeptides retained most of the binding potency and functional activity [31].

While the (*E*)-alkene surrogate is an accurate mimic of the steric demand of the amide bond, it does not have the polarized nature of the amide bond. Therefore, a fluoroolefin isostere Ψ[(*E*)-CF=CH] retains these attributes of the (*E*)-alkene surrogate, but accurately mimics the electronic nature of the amide bond to include dipole moment, charge distribution, and electrostatic potential [32-34]. The synthesis of the fluoroolefin isostere is well summerized in a review article by Welch and Allmendinger [35]. This vinyl fluoride surrogate Ψ[(*E*)-CF=CH] was applied for the synthesis of an analgesic dipeptide [36], however, it appeared that the amide bond of the dipeptide is critical for opioid activity.

8. 1,5-Disubstituted Tetrazole, Ψ[CN_4]

Proline often has a special role among many amino acids incorporated into peptides as it is the only residue leading to an *N*-alkylamide bond when incorporated into a peptide. The presence of proline in naturally occurring peptides causes cis-trans isomerization of amide bonds leading to conformational changes which may be important for their biological activity [37]. Therefore, the 1,5-disubstituted tetrazole ring, Ψ[CN_4], has been proposed as a surrogate for cis amide bonds, making it a valuable tool in the design of conformationally constrained peptide receptor probes [38]. The X-ray crystal structure of diketopiperazine containing a tetrazole as a cis amide bond surrogate revealed that the tetrazole ring system, which constrains the peptide bond to a cis conformation, is almost planar [39]. The tetrazole analog is an excellent conformational mimic of the cis amide bond, but it does not have the capacity to be a hydrogen bond donor or acceptor. The steric bulk of the tetrazole ring could impede the peptide in the active site of the receptor. The synthesis of the 1,5-disubstituted tetrazole ring is well described in a recent review article by Zabrocki and Marshall [40], and the typical synthesis of tetrazole is accomplished by treating a dipeptide with PCl_5 and hydrazoic acid to convert the amide bond to the tetrazole via an imidoyl chloride. The tetrazole surrogate has been used for many biologically active peptides. In particular, cyclic somatostatin analog with tetrazole was synthesized in order to constrain the putative cis amide bond, and the pseudopeptide was found to retain 83% of the biological activity [41]. The conformation of this pseudopeptide was found to be similar to those previously reported for cyclic peptide analogs when studied by 2D-NMR spectroscopy. This confirmed that the tetrazole moiety is an excellent geometric mimic of the cis amide bond in solution [41]. Similarly, bradykinin analogs containing a tetrazole ring were synthesized to evaluate the possibility of cis-trans isomerization because of the high percentage of proline residues in the nonapeptide bradykinin [42]. Unfortunately, significantly reduced binding affinity by all of the pseudopeptides with tetrazole suggests that the cis amide conformer is disfavored in peptides.

9. Azoles

1,2,4-oxadiazole, 1,3,4-oxadiazole, and 1,2,4-triazole have been used for the isosteric replacement of peptide backbones [43]. These ring systems are similar in size and shape but show variation in aromatic, electrostatic, and hydrogen bonding properties [44]. In order to examine and improve physical properties such as gut transport and plasma stability, oxazole- and thiazole-containing endothelin

pseudopeptides were synthesized. Despite the formal isosteric replacement of the peptide backbone, a markedly different structure-activity profile was observed [45]. On the other hand, in order to enforce a reverse turn on a peptide chain, 3-(1-aminoalkyl)isoxazole was used. The isoxazole amino acid moiety may be used as a cis amide bond replacement [46].

10. Retro-inverso, Ψ[NHCO]

The retro-inverso transformation reverses the order of carbonyl and amine functional groups in an amide bond resulting in a more closely related isosteric replacement for the original peptide bond [47]. The reversed order of an amide bond in a partially modified retro-inverso (PMRI) pseudopeptide enhances protection over hydrolysis and enzymatic attack, which, in turn, can extend the *in vivo* half-life. For synthesis of PMRI analogs, two different compounds are used, *gem*-diaminoalkyl and alkylmalonyl residues. The *gem*-diaminoalkyl residue can be prepared by Hofmann-type rearrangement of *N*-protected peptidyl or aminoacyl carboxamide derivatives using a mild oxidizing reagent [48]. However, the synthesis of the optically pure pseudopeptides containing alkylmalonamide residues requires the separation of diastereomers by reverse phase HPLC and assignment of the absolute configuration at the malonyl center by using 2D-NMR techniques [49]. Recently, a PMRI analog has been used successfully to modulate the cellular immune response, suggesting the potential use of PMRI derivatives as an immunotherapeutic approach for the treatment of allergies, autoimmune diseases, or cancers [50].

BIOLOGICAL APPLICATIONS

Various amide bond surrogates and their applications are summarized in Table 1. Each type has been designed to resist enzymatic degradation with concomitant improvement in peptide bioavailability and enhance ligand binding affinity to its receptor. Some of the surrogates have been used as an aid in determining the mechanism of action of peptide ligands. The effects of an aminomethylene group, perhaps the simplest of the amide bond isosteres, have been examined in several different targets. The uses of the methyleneoxy group have not been as widespread as its aminomethylene congener. This replacement has, however, shown better activity when incorporated into gastrin releasing peptide antagonists. The methylene sulfoxide derivative has been used to stabilize reverse turns in a peptide chain. When placed in an opioid derivative, it participated in a γ-turn. Peptides containing a thiomethylene replacement occupy similar conformations as the all-amide precursor. The ketomethylene group has shown promising results when inserted into peptide-based thrombin inhibitors and neurotensin analogs. The thioamide group has been successfully used in a series of different applications. A simple trans carbon-carbon double bond resembles an amide in the trans conformation. Placement of olefin groups in the peptide backbone has been the basis of structure activity relationship (SAR) studies directed toward elucidating which amide bonds are necessary for biological activity. The fluoroolefin isostere has also been useful in similar applications. A tetrazole moiety has been incorporated into a peptide backbone in the design of bradykinin and somatostatin analogs. Other five-membered heterocycles such as oxazoles, thiazoles, and imidazoles have been utilized in the design of endothelin antagonists. The retro-inverso amide bond is another commonly used replacement which has been examined in several biological targets. When the hydroxyethylamine group was synthesized into a pseudodipeptide substrate, the substrate demonstrated an enhanced ability to cross lipid membranes by way of membrane transporters. Other examples of amide bond isosteres include a rigid, substituted cyclopropane dicarboxylate derivative, an α-ketoamide, the hydroxymethylcarbonyl, a cyanomethylamine, and the sulfonamide. In summary, positive results from SAR studies have indicated that amide bond substitution is a beneficial tool in peptide mimicry.

RECENT DEVELOPMENT OF PEPTIDE BACKBONE REPLACEMENT

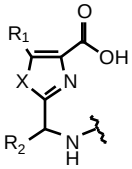
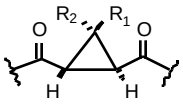
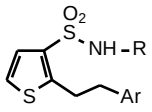
Over the last 20 years, peptidomimetic research has focused on the chemical modification of the peptide backbone. More recently, a focus has been the rational design of peptidomimetics with well-defined secondary structures and/or specific biological functions. The expedient and efficient syntheses of these molecules on solid supports are essential to facilitate rapid screening of potentially bioactive oligomers (the solid supported synthesis of peptidomimetics has been reviewed elsewhere [51, 52]. Our laboratory has investigated peptidomimetics containing moieties such as β-amino acids; α-aza-amino acids; *N*-phosphonomethylene derivatives; and phosphonamidates. In the preparation of novel peptidomimetic compounds we exploited the convenience and efficiency of solid supported chemistry and new methodologies have been developed.

1. β-Amino Acids

Oligomers of β-amino acids (β-peptides) can fold into well-defined bioactive conformations containing secondary structures such as helices, sheets and turns that are analogous to those of proteins [53-55]. Some β-peptides, containing as few as six amino acids, adopt a stable helical conformation designated as the L + 2, 3_1 or 14 helix which is characterized by a perfect three-residue geometric repeat with a 5 Å pitch [56]. This regular repeat should make it possible to design pseudopeptides in which selected side chains lie on a straight line parallel to the helix axis. Such molecules might act as templates for the ligation of 'complimentary' β-peptides as do some α-peptides [57].

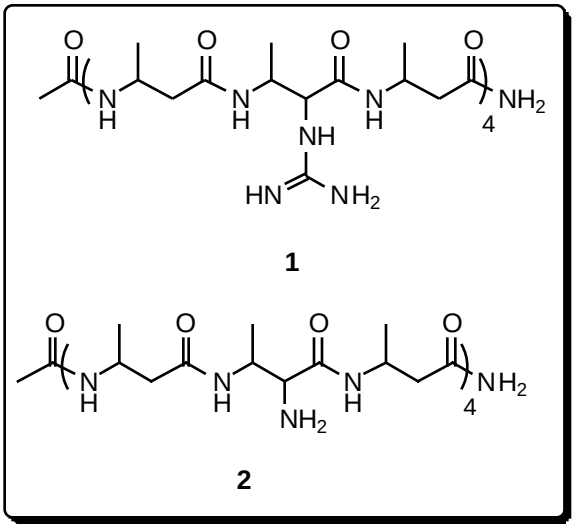
The guanidine group plays an important role in biologically active proteins, peptides and peptidomimetics [58], particularly in arginine-containing peptides and proteins. Guanidine and its simple analogs are strongly basic (guanidinium $pK_a \approx 13.5$) and are fully protonated under physiological conditions. The positive charge of guanidine groups and their ability to form hydrogen bonds permits

Table 1. Amide Bond Replacement and Heir Applications

Type	Application	Reference
Ψ[CH_2NH]	HIV-1 protease inhibitor, dermorphin analog, neurokinin antagonist, thrombin inhibitor, plasma kallikrein inhibitor, corticotropin releasing factor agonist	[12, 94-99]
Ψ[CH_2O]	gastrin releasing peptide antagonist	[100]
Ψ[CH_2S] and Ψ[CH_2SO]	reverse turn stabilizers	[16]
Ψ[$COCH_2$]	thrombin inhibitor, neurotensin analog	[18, 101]
Ψ[CSNH]	HIV-1 protease inhibitor	[24-27]
Ψ[(*E*)-CH=CH]	determination of bioactive conformation of cholecystokinin terminal hexapeptide and mechanism of peptide-based enantioselective catalysts, HIV-1 protease inhibitor	[31, 102-104]
Ψ[(*E*)-CF=CH]	opioid agonist	[36]
Ψ[CN_4]	somatostatin and bradykinin analogs	[41, 42]
 X = O, S, NH	endothelin receptor antagonist	[45]
Ψ[NHCO]	initiate or modulate cellular immune response	[47, 50]
Ψ[$CH(OH)CH_2NH$]	examined in membrane transport assays	[105]
	ras farnesyltransferase inhibitors	[106]
Ψ[COCONH]	HIV-1 protease inhibitors	[107]
Ψ[CH(OH)CONH]	HIV-1 protease inhibitors	[108]
Ψ[CH(CN)NH]	neurotensin analog	[101]
	endothelin receptor antagonist	[109]

specific interactions between biological molecules. On this basis, we prepared a positively charged peptide **1** which could, in principle, facilitate the ligation of complementary negatively charged β-peptides, Fig. (**2**) [59]. The circular dichroism spectra of **1** was recorded to probe its conformation. The spectrum in water has a minimum at 203 nm and a maximum at 186 nm, indicating the presence of a well-defined secondary structure. The shape of the curve suggests that the peptide forms a 12/10/12 helix [60], or a half-pin structure [55]. NMR studies are under way to reveal detailed structural information.

Peptide **1** was obtained from peptide **2** by converting its amino moieties to guanidine groups using *N*, *N'*-di-Boc-*N*"-triflylguanidine in methanol, in the presence of diisopropylethylamine, at 45° for 6 days. Dialysis and RP-HPLC purification provided peptide **1** in 31% yield. Synthesis of β-peptide **2** was carried out using Fmoc solid-phase peptide synthesis on the Applied Biosystems' AM_{NH2} resin.



The methods described should make accessible a variety of β-peptides containing 2-guanidino-3-aminobutanoic acid

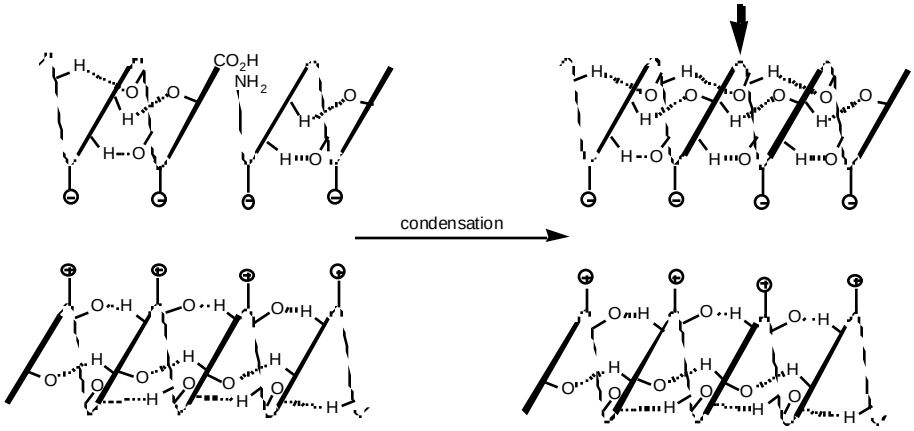


Fig. (2) Schematic representation of proposed template-directed ligation of peptides. The positive charged β-peptide serves as a template by aligning complimentary negative charges on substrate peptides. The arrow indicates the position of the new peptide bond.

residues. These compounds should prove useful in template-directed synthesis and possibly at as high affinity optomers for proteins and other biomolecules.

2. α-Aza-amino Acids

An important class of backbone-modified peptides in the pharmaceutical industry are azapeptides [61, 62]. Replacements of the α-hydrogen of the common amino acids by a methyl group or any other substituent ($NH_2CRR'CO_2H$) are both examples of α-alkyl modification [15]. Azapeptides, however, are peptides in which the α-carbon of one or more amino acid residues in a peptide chain is replaced isoelectronically with a trivalent nitrogen atom, Fig. (**3**). This transformation results in a loss of asymmetry associated with the α-carbon and yields a structure that can be considered intermediate in configuration between *D*- and *L*-amino acids [8, 63]. Interest in this α-carbonreplacement unit stems from its ability to provide resistance to enzymatic cleavage and its capacity to act as a selective inhibitor of cysteine [64] and serine proteases [65-69].

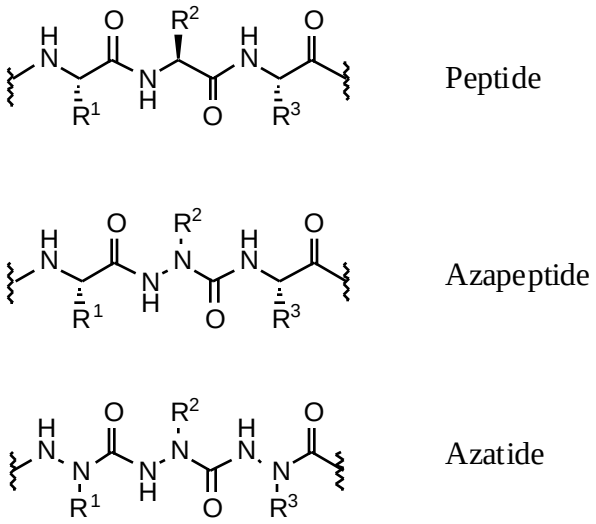
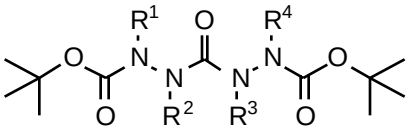


Fig. (3) Peptide analogs containing azide groups.

We developed a strategy utilizing solution or liquid phase synthetic methodologies for the coupling of monomeric α-aza-amino acids in a linear, stepwise, chain-lengthening fashion to construct what we term azatides, or 'pure azapeptides', Fig. (**3**) [70]. Our investigations addressed three key stages: 1) the development of general synthetic procedures that allowed the synthesis of a wide variety of Boc-protected aza-amino acid monomers; 2) optimization of solution phase procedures for the coupling of aza-amino acids in a repetitive manner; and 3) design and synthesis of a linker that would support azatide synthesis using a soluble polymer that we term a liquid phase format.

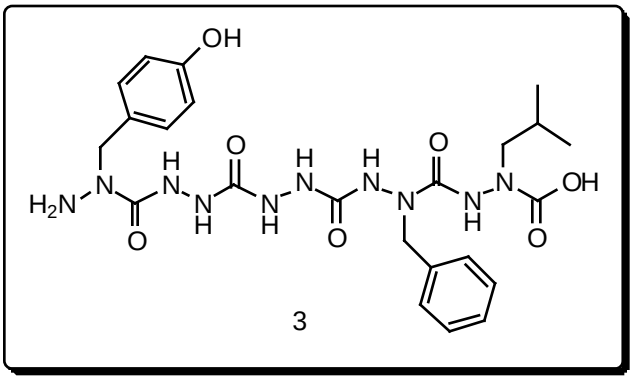
Table 2. Synthesis of Diazatides Starting from 1-R'-Hydrazinecarboxylic Acid, 1,1-Dimethylethyl Ester



R^1	R^2	R^3	R^4	Yield (%)
H	H	H	H	92
Methyl	H	H	Methyl	91
H	Methyl	H	Methyl	90
H	Methyl	H	Benzyl	85
H	Methyl	H	Isobutyl	84
H	Isobutyl	H	Isobutyl	82
H	Isopropyl	H	Isopropyl	84

Using the soluble linear homopolymer, poly(ethylene glycol) monomethyl ether (MeO-PEG), with a *p*-substituted benzyl ester linker, azatides were prepared by sequential coupling of Boc-protected aza-amino acids. Coupling of aza-amino acids was facilitated by using bis(pentafluorophenyl) carbonate as the carbonyl activating agent [71]. Some of the

results from this work, Table (**2**), show that azatides can readily be prepared in good yields, even with sterically demanding substrates.



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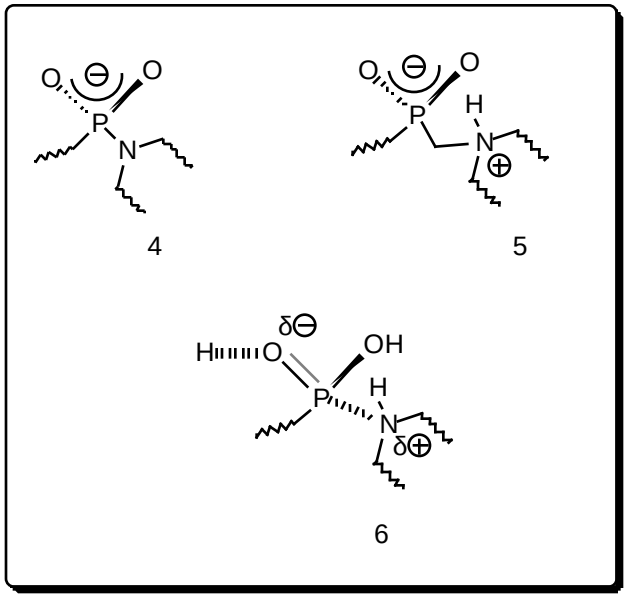
Leu- and Met-enkephalin are endogenous opioid peptides with morphine-like activity. Most natural opioid peptides have the *N*-terminal sequence Tyr-Gly-Gly-Phe (YGGF) recognized by the δ–opioid receptor [72]. Therefore, azatide pentamer **3** ($Y^aG^aG^aF^aL^a$) was prepared and evaluated for its binding affinity to monoclonal antibody 3-E7, a hybridoma raised against the antigen β-endorphin which recognizes the *N*-terminal portion of the protein. At 1 mM, azatide **3** showed no propensity to compete with the natural peptide for antibody 3-E7 by competition ELISA. Despite this result, we believe that azatides will be of considerable interest as a readily accessible new material with potential for novel biological properties and as a source of new peptidomimetic libraries. Furthermore, the structural and pharmacological properties of these azatides have potential to provide important leads for the drug industry and may prove useful as probes for elucidating receptor-ligand interactions.

Recently, azapeptides have been investigated as novel growth hormone secretagogues [73]; inhibitors of hepatitis A virus 3C proteinase [74]; inhibitors of cysteine [75] and serine [76] proteases; and as antagonists for the α-IIb, β-3 and α v β-3 integrins [77].

3. N-Phosphonomethylene Derivatives (Ψ[$PO_2^-CH_2N^+$])

The discovery that the human immunodeficiency virus encodes an aspartic protease (HIV PR) vital for its propagation has brought this protein under intense scrutiny [78, 79]. Accordingly, the development of compounds which inhibit the HIV PR has been particularly rapid [80]. It seemed rational that an effective modification of the phosphonamidate structure **4**, a functionality well known in protease inhibition [81-84], would be to include additional features along the reaction coordinate for amide hydrolysis. The insertion of a methylene spacer between phosphorus and nitrogen produces the nonhydrolyzable moiety **5**, which is likely a zwitterion near physiological pH. This construct could be representative of a late transition state/early product formation for amide bond cleavage in mimicking both the tetrahedral hydrate and the departing amine, **6**. Its incorporation into a peptide backbone affords what we term an "exploding transition-state analog".



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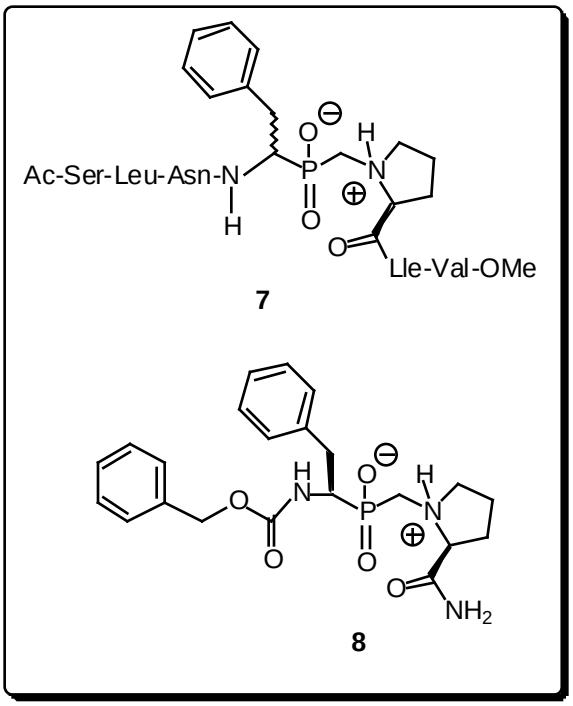
Phosphonate compounds **7** and **8** were prepared and evaluated for their ability to inhibit HIV PR [85]. Compound **7** demonstrated potent inhibition in the nanomolar concentration range (K_i = 82 nM ± 8) indicating that this novel amide bond replacement is a suitable substitute for phosphonamidate based inhibitors of HIV PR.



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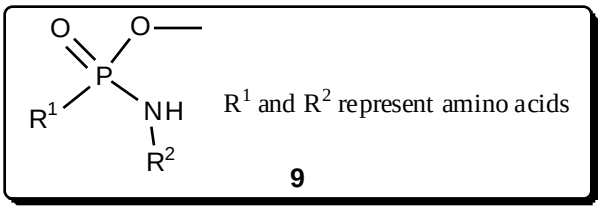
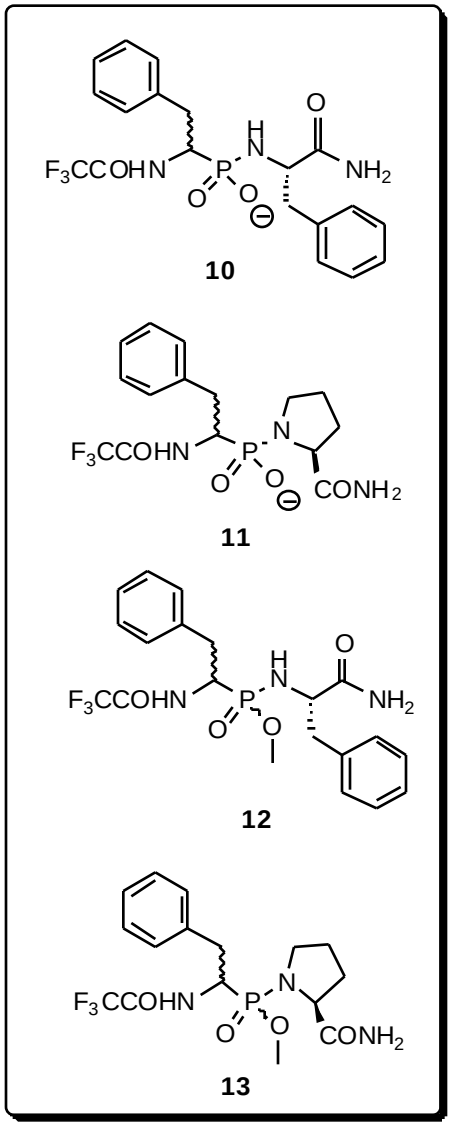
4. Phosphonamidates

Phosphonamidates have been very effective inhibitors of metalloproteases (carboxypeptidase A, thermolysin) [81, 83, 86], however, because of their instability under acidic conditions, the more stable tetrahedral phosphinates/ phosphonates have been utilized as protease inhibitors [87-90]. Because of the importance of hydrogen bonding in enzyme-inhibitor interactions [82, 91, 92], it might be



9

advantageous to substitute a stable form of the phosphonamidate for the scissile amide moiety to be cleaved. Consequently, our strategy was to synthesize what we term a "capped" phosphonamidate (phosphonamidate ester, **9**) to bridge the gap between simple phosphinates and the more sophisticated, yet pH sensitive, phosphonamidates [93]. The desirable features of this scissile bond replacement are, that 1) it retains the nitrogen as an important recognition element of the scissile amide bond and depending on the R' amino acid, provides an additional hydrogen bond, 2) the methylated phosphonamidate is not susceptible to acid hydrolysis, and 3) there is no overall charge carried within this isosteric replacement, which may facilitate entry into the cell.



Phosphonamidates **10** and **11**, and their phosphonamidate ester counterparts **12** and **13** were prepared and evaluated for their ability to inhibit HIV PR. These compounds possess inhibition constants in the micromolar range demonstrating that these compounds are good inhibitors of HIV-1 PR and that the phosphonamidate need not be in the form [PO_2^- NH] to be a protease inhibitor. Hence, while the tetrahedral geometry at the phosphorus atom is still likely of significance, its anionic charge may be of lesser importance.

FINAL REMARKS

We have presented here a brief up-to-date review of several, but not all, peptide mimetics. In general, peptide backbone modifications make peptide analogs more resistant to proteolytic enzymes, increase permeability through biological membranes, and assume an alternative conformation. The investigation of several amide bond replacements has resulted in the realization of these goals and new analogs will further elaborate the scope for this field of research. Peptide backbone modifications have been applied to numerous fields including HIV protease inhibitors and receptor mediators.

With the recent elucidation of the human genomic DNA sequence, it can be expected that a multitude of biologically important peptides and proteins, previously undiscovered, will be revealed. Consequently, as new pharmaceutical targets are identified, we believe that peptidomimetics research will have a crucial role in the development of future drugs and biochemical tools.

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