Pseudopeptide Synthesis *via* Fmoc Solid-Phase Synthetic Methodology

Predrag Cudic* and Maciej Stawikowski

Florida Atlantic University, Department of Chemistry and Biochemistry, 777 Glades Road, Boca Raton, Florida 33431, USA

Abstract: Peptidomimetic modifications are frequently used as attractive methods to provide more conformationally constrained and thus more stable and bioactive peptides. Among peptidomimetic approaches, particularly attractive are pseudopeptides, or peptide bond surrogates in which peptide bonds have been replaced with other chemical groups. Since these peptidomimetics can be constructed in a modular way from orthogonally protected monomeric building blocks, solid-phase Fmoc methodology, used routinely for peptide synthesis, emerged as a particularly attractive synthetic approach.

Keywords: Peptidomimetics, pseudopeptides, isosteres, Fmoc solid-phase synthesis.

I. INTRODUCTION

In recent years, peptides have gained momentum as therapeutic agents. According to Frost and Sullivan business consulting firm, around 720 peptide drugs and drug candidates were reported in 2004, among which 5% are already marketed worldwide, 1% are in registration, 38% are in clinical trials and 56% are in advanced preclinical phases [1]. Peptides potential for high efficacy combined with minimal side effects has allowed them to be widely considered as lead compounds in drug development, and at present, peptide based therapeutics exist for a wide variety of human diseases including osteoporosis (Calcitonin), diabetes (Insulin), infertility (Gonadorelin), carcinoid tumors and acromegaly (Octreotide), hypothyroidism (THR-Thyrotropin releasing hormone) and bacterial infections (Daptomycin) [2]. However, despite the high potentials, there are still some limitations for peptides as drugs *per se*. Major disadvantages are short half-life, rapid metabolism and poor oral bioavailability. Nevertheless, pharmacokinetic properties of peptides can be improved by different types of modifications [2,3]. Peptidomimetic modifications or cyclization of linear peptides are frequently used as attractive methods to provide more conformationally constrained and thus more stable bioactive peptides [4-11]. Taking into consideration various peptidomimetic approaches used for the design and synthesis of peptide analogs with improved pharmacological properties pseudopeptides or peptide bond surrogates, in which peptide bonds have been replaced with other chemical groups, are especially attractive. This is mainly because such approaches create an amide bond surrogate with defined three dimensional structures similar to those of natural peptides, yet with significant differences in polarity, hydrogen bonding capability and acid-base character. Also important, the structural and stereochemical integrities of the adjacent pair of α-carbon atoms in these pseudopeptides are unchanged. The psi-bracket (ψ[ ]) nomenclature, introduced by A. Spatola, is used for this type of modification [12]. The introduction of such modifications to the peptide sequence is expected to completely prevent protease cleavage of amide bond and significantly improve the peptides metabolic stability. However, such modifications may also have some negative effects on peptides biophysical and biochemical properties, in particular their conformation, flexibility and hydrophobicity. Therefore, the choice of an amide bond surrogate is a compromise between positive effects on pharmacokinetics and bioavailability and potential negative effects on activity and specificity. In particular, the conservation of the stereochemistry of the parent peptide should be an important criterion in surrogate selection. The ability of the surrogate to mimic the steric, electronic and solvation properties of the amide bond is certainly the most important characteristic in determining the potency of pseudopeptide analogs.

From the synthetic point of view, the methods for assembly of peptidosulfonamides, phosphonopeptides, oligoureas, depsides, depsipeptides, peptoids and azapeptides parallel those for standard solid-phase peptide synthesis, although different reagents and different coupling and protecting strategies need to be employed. Since these peptidomimetics can be constructed in a modular way from orthogonally protected monomeric building blocks and are therefore suitable for potential combinatorial chemistry diversity, the solid-phase methodology is the method of choice for their synthesis. Particularly attractive is Fmoc solid-phase methodology since it is now a standard approach for the routine peptide synthesis.

In this review we consider Fmoc solid phase synthesis of peptide analogs containing the amide surrogate that tend to be isosteric with the natural amide. This includes synthesis of peptidosulfonamides, phosphonopeptides, oligoureas, depsides, depsipeptides, peptoids and azapeptides (Fig. **1**). Given the importance of the peptides as lead compounds for drug discovery and development, it is not surprising that the above mentioned peptidomimetic strategies used to enhance peptide stability have attracted a great deal of attention in the last few decades [2,11].

II. PEPTIDOSULFONAMIDE SYNTHESIS

The tetrahedral achiral sulfur atom bonded to the two oxygen atoms possesses geometry similar to the high energy

*Address correspondence to this author at the Florida Atlantic University, Department of Chemistry and Biochemistry, 777 Glades Road, Boca Raton, Florida 33431, USA; E-mail: pcudic@fau.edu

1570-193X/07 $50.00+.00

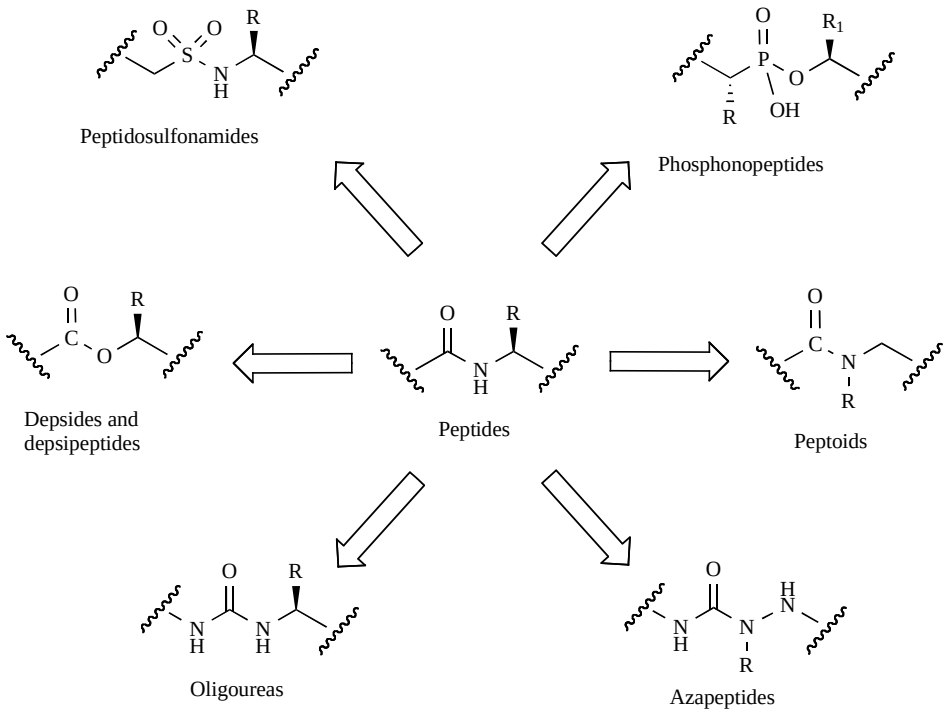

Fig. (1). Schematic representation of peptidomimetics containing the amide surrogates that are isosteric with the natural peptidic amide bonds.

intermediate formed during the amide-bond hydrolysis or amide-bond formation [13,14]. Therefore, the peptidosulfonamides are at the same time stable to proteolytic hydrolysis and are capable of significantly altering the polarity and H-bonding patterns of native peptide. Because of the relative acidic N-H in a sulfonamide moiety, it can be expected that H-bonds involving this amide surrogate will be stronger as compared to the amide analogs. These sulfonamides' properties made the peptidosulfonamides attractive building blocks for the synthesis of peptidomimetics with enhanced metabolic stability and potentially potent enzyme inhibitory activities. Due to the intrinsic chemical instability of α-peptidosulfonamides, most of the peptidomimetics containing sulfonamide bond have been limited to more stable β-peptidosulfonamides **2** (Fig. **2**) [15]. However, this peptidomimetic approach is not without disadvantages. The most significant drawback of this approach is the capability of the sulfonamide moiety to disrupt any defined secondary structure of the parent peptide in solution, even if present at the *N*-terminus [16,17]. Following structural features of the sulfonamide moiety may contribute to this phenomenon:

a) Sulfonamide N-H is more acidic than amide N-H and is therefore a better H-bond donor but a poorer H-bond acceptor.

b) Presence of two sulfonamide oxygens as H-bond acceptors may also impair an H-bonding network, which holds together a secondary structure.

c) The sulfonamide oxygens can assume varying positions due to less energy demanding rotation about sulfonamide bond. This may also prevent a proper alignment of the H-bonds necessary for a particular secondary structure.

Despite the disadvantages associated with the use of sulfonamide peptidomimetics, as shown in the literature, the pharmacological properties and biological activities of peptides may be improved by introducing the sulfonamide residue at a specific position within the peptide sequence [18,19]. Conversion of an amino acid into corresponding activated sulfonic acid derivatives such as sulfinyl- and sulfonylchlorides represent the first step in the synthesis of peptidosulfonamides [16,20]. Typical Synthesis of Fmoc-protected β-substituted-β-aminoalkylsulfonyl chlorides **1** include reduction of Fmoc-protected amino acid to the corresponding alcohol, conversion of the alcohol to sulfonic acid and in the final step, chlorination with thionyl chloride [21,22], phosgene [17,23-25], or triphosgene [18,26]. Another interesting approach for the synthesis of β-amino-sulfonamides requires the synthesis of sulfinylchlorides, followed by coupling of an amino acid or peptide *via* the amino group and subsequent oxidation using OsO_4/*N*-methylmorpholine-*N*-oxide mixture [27]. For the synthesis of peptidosulfonamides, especially long and complex ones, a solid-phase methodology is indispensable (Fig. **2**). Liskamp *et al.* developed methods for solid-phase synthesis of peptidosulfonamides **2** that include both sulfonyl- [17,18] and sulfinylchloride amino acid analogs couplings [20,27-29]. However, the sulfinylchloride method turned out to be inferior mainly because the yield of peptidosulfinamide oxidation to the corresponding peptidosulfonamide strongly depends on the peptidomimetic sequence and the type of resin used in the synthesis. Potential effect of pseudopeptide conformers on the peptidosulfinamide oxidation was not discussed.

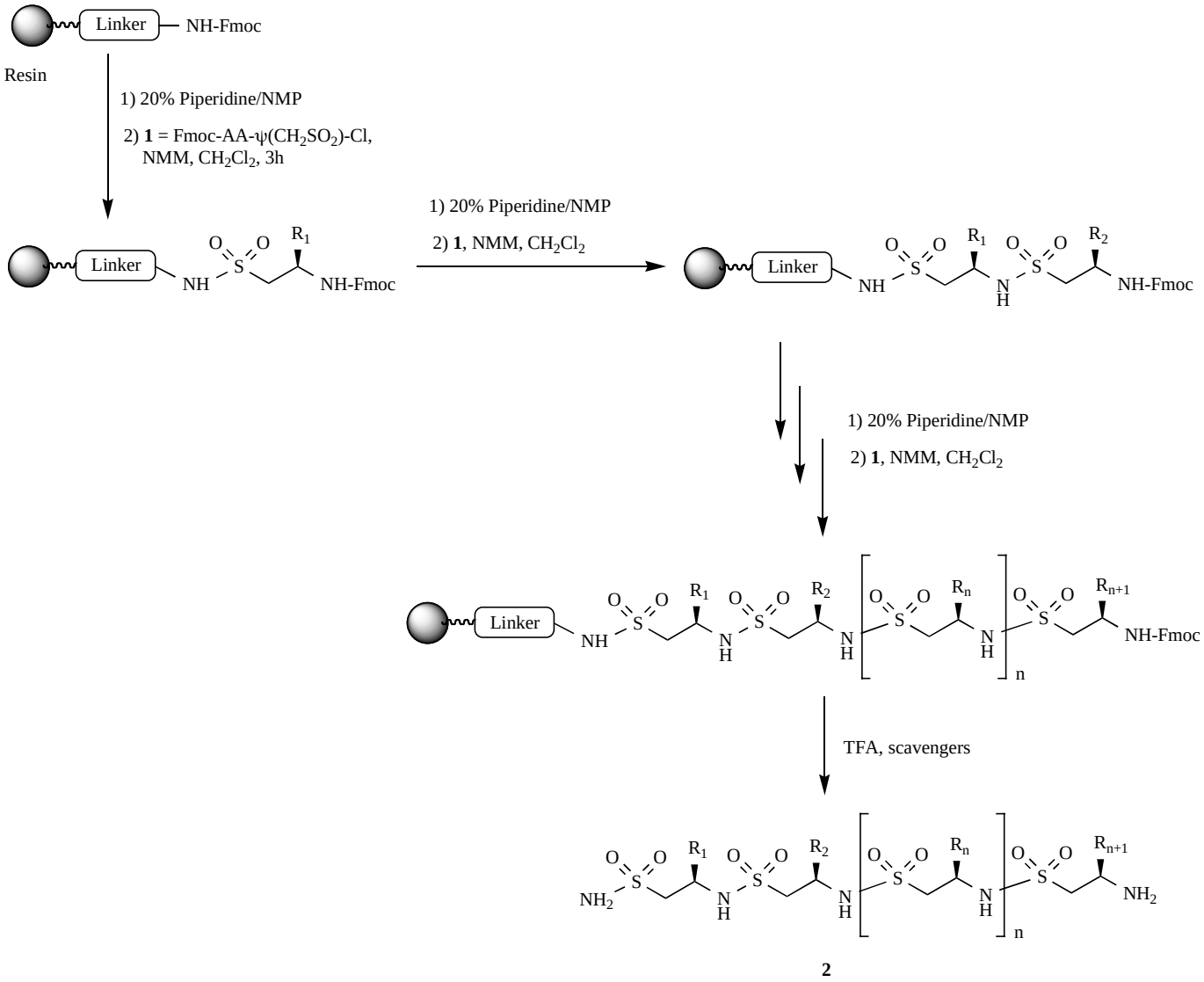

Fig. (2). Solid-phase peptidosulfonamide synthesis using sulfonyl chloride methodology.

III. PHOSPHONOPEPTIDE SYNTHESIS

Phosphonopeptides containing a transition state analog of the hydrolysis of the amide bond represent another attractive approach for the preparation of proteolytically stable peptides [10,30,31]. In addition to increased stability, incorporation of a phosphonate moiety into the peptide sequence also provides access to additional binding interactions within the transition-state conformation of the enzyme/substrate complex [13]. This peptidomimetic approach is used to design very effective protease inhibitors [31-34]. As in the case of peptidosulfonamides, preparation of phosphorous amino acid analog suitable for incorporation into peptidic backbone represents the first step toward preparation of phosphonopeptide peptidomimetics.

Methods for obtaining α-aminoalkylphosphonic acid analogs of amino acids in stereochemically pure form have been recently summarized in the literature [30,40-42]. Moreover, some of these analogs are also commercially available. Typical methods for solid-phase incorporation of α-aminoalkylphosphonic acid into the peptide main chain include a modified Mitsunobu condensation, which proceeds with an inversion of configuration of a alcohol-bearing carbon [35-37] (Fig. **3**). In order to apply the Mitsunobu coupling methodology to phosphonopeptide solid phase-synthesis, the hydroxyl group in α-hydroxy acid needs to be protected with the Fmoc-protecting group and aminoalkyl-phosphonate **3** activated with 4-nitrophenethyloxycarbonyl (NPEOC) group. This method was successfully applied by Campbell *et al.* for the synthesis of a series of peptidyl-phosphonates **4** as potential metalloprotease inhibitors [35]. The phosphonopeptides were elongated at the *N*-terminus using standard Fmoc chemistry and the final product was obtained after selective hydrolysis of the phosphonate protection group followed by cleavage from the resin.

Another interesting approach includes solution synthesis of aminophosphonate dipeptide analogs by benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) or benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) promoted reaction between appropriate phosphonic acid monoesters and hydroxyl acid

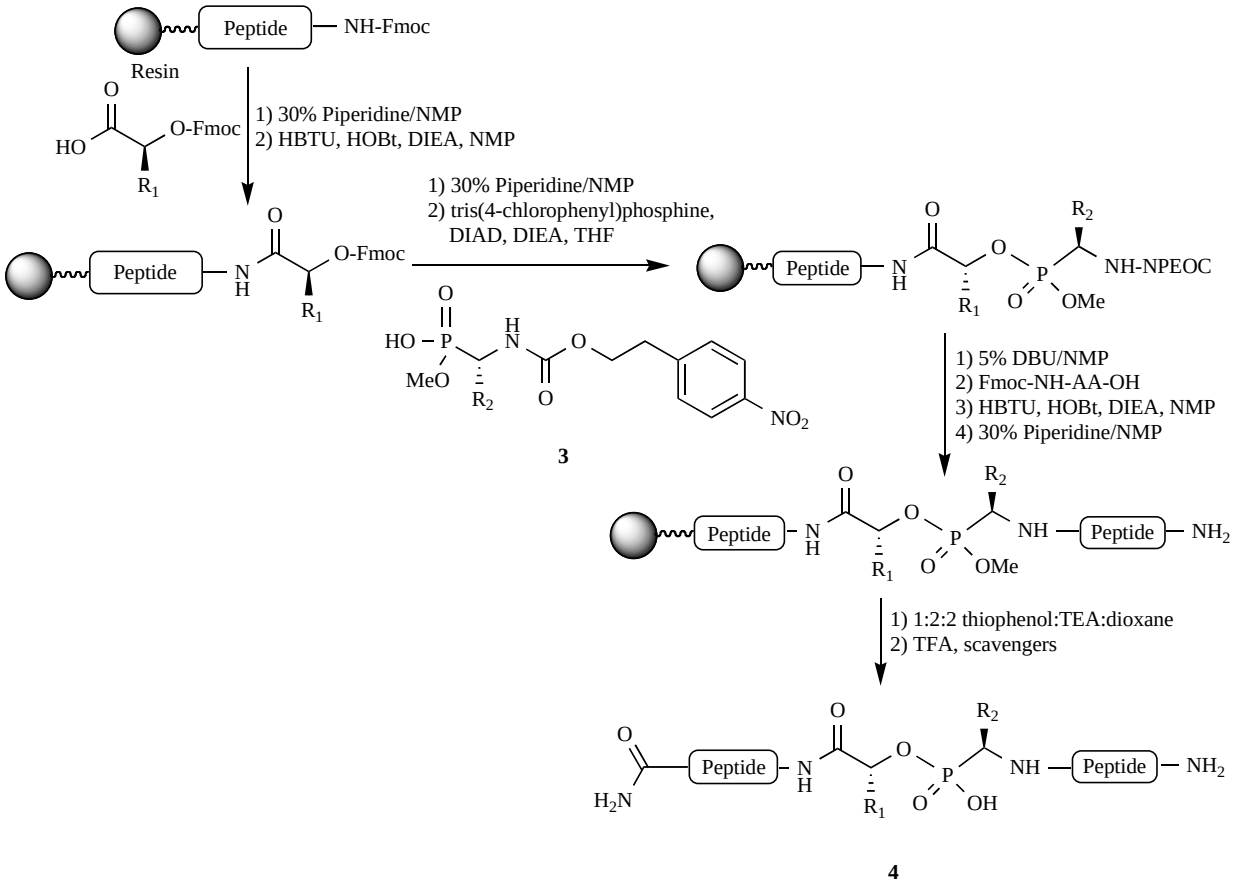

Fig. (3). Solid-phase phosphonopeptide synthesis using modified Mitsunobu coupling procedure.

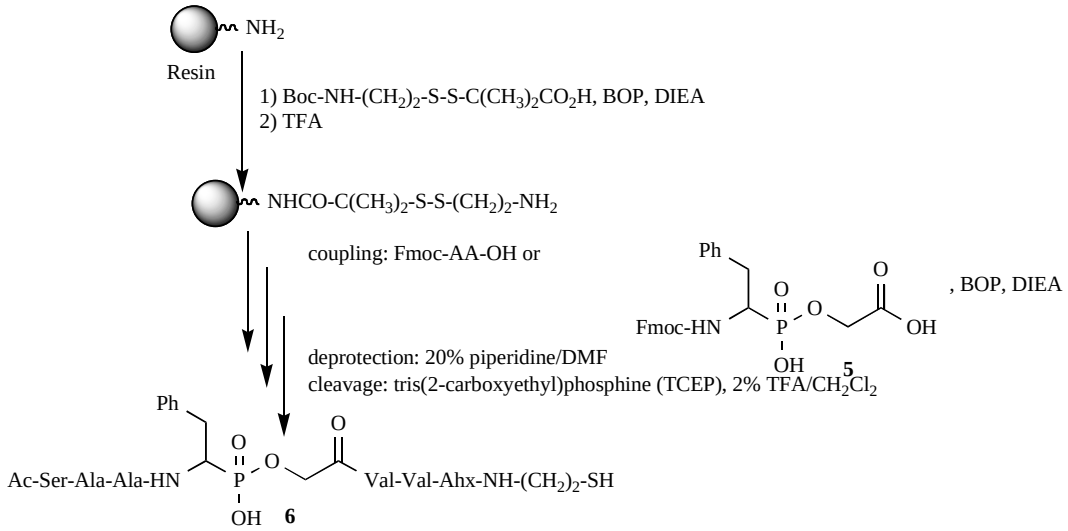

Fig. (4). Solid-phase phosphonopeptide synthesis using aminophosphonate dipeptide building block.

followed by their coupling using standard Fmoc chemistry. Using this synthetic strategy, Coste *et al.* prepared phosphonopeptide **6** in 32% overall yield starting from aminophosphonate **5** unprotected on the phosphorus moiety, avoiding selective deprotection of the phosphonate group in the final step, and therefore simplifying the synthetic procedure (Fig. **4**) [38,39]. The findings that the acidity of the α-proton depends on the character of C^{α}-substituent and that benzotriazole esters of phosphonic acid monoester, formed during BOP or PyBOP promoted reactions, are poorly reactive with amines, allowed efficient use of **5** in phosphonopeptide solid-phase synthesis [38]. On the other hand, these esters are very reactive with alcohols, especially

in water. Quite importantly, this synthesis proceeded with neither formation of a *P-N* derivative nor epimerization.

IV. OLIGOUREA SYNTHESIS

Considering a planar conformation of urea moiety, urea replacement of the amide linkage in native peptides represents conformationally more conservative type of peptidomimetics [43-50]. Several solid-phase synthetic approaches for the preparation of oligoureas employing different monomeric building blocks were described in the literature. Methods that utilize phthalimide protected isocyanate [51], and Boc- or Teoc-protected azido-4-nitrophenyl carbamate [52] monomeric building blocks, were among the first ones reported in the literature.

However, none of these methods are compatible with Fmoc-chemistry, which is currently a standard method for peptide preparation. At present, *N,N*'-linked oligoureas are readily accessible by Fmoc solid-phase synthetic methodology with a variety of appropriate building blocks [53,54]. Guichard *et al.* developed solid-phase methodology based on the use of *O*-succinimidyl-(Fmoc-amino)ethyl-carbamate monomers derived from selected *N*-Fmoc protected β-amino acids [53]. Solid-phase synthesis of a series of oligoureas was performed on Rink amide resin by coupling *O*-succinimidyl carbamates with *N,N*-diisopropyl-ethylamine (DIEA) in *N,N*-dimethylformamide (DMF). However, when using this synthetic methodology, the amount of impurities reported was significant due to incomplete coupling, incomplete Fmoc deprotection, and/or double insertion of certain monomeric building blocks. Significant improvement was achieved by replacing DIEA with *N*-methylmorpholine (NMM), or by performing the coupling without the presence of any base. Another interesting approach for the solid-phase synthesis of oligoureas, fully compatible with Fmoc-chemistry, was developed by Liskamp *et al.* (Fig. **5**). In this case, the monomeric building blocks for oligourea solid-phase synthesis were prepared from *N*-Fmoc protected α-amino acids [54]. This synthetic strategy includes the reduction of *N*-Fmoc protected α-amino acids into the corresponding alcohol, then conversion into an azide by a Mitsunobu reaction, followed by reduction of the azide to an amine using catalytic hydrogenation. In the final step, obtained Fmoc protected monomeric amino building blocks were then activated by conversion to the corresponding carbamate with 4-nitrophenyl chloroformate. The use of the Fmoc-protected 4-nitrophenyl carbamate building blocks **7** and resins with acid-labile linkers allowed for the synthesis of the final oligourea products with *C*-terminal amide groups **8** or carboxylic acid **9**, (Fig. **6**).

Unfortunately, Fmoc solid-phase synthesis of oligourea peptidomimetics with *C*-terminal carboxylic acids also leads to the formation of the corresponding hydantoin byproducts **10** [54-57] (Fig. **6**). In this case, hydantoin formation arises as a result of an acid catalyzed intramolecular cyclization

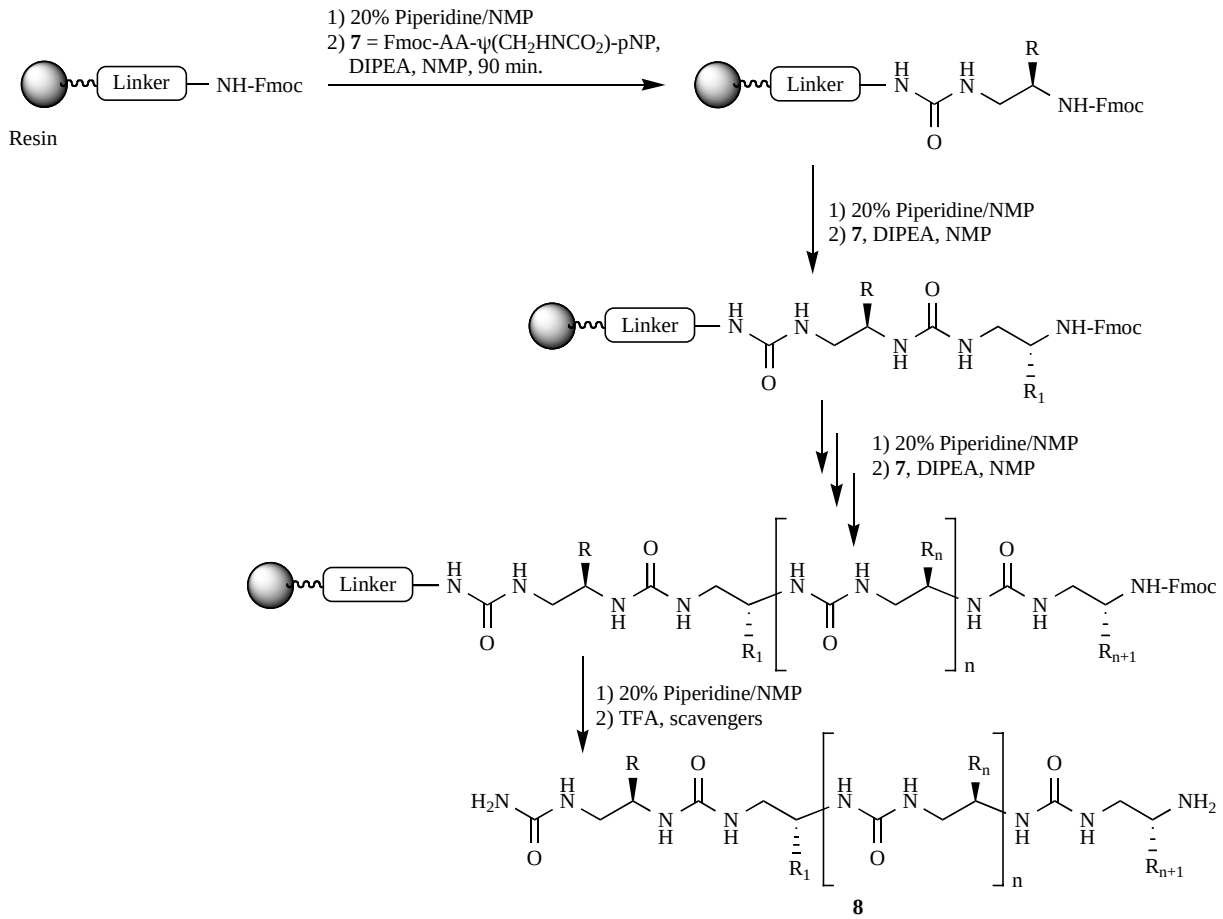

Fig. (5). Solid-phase synthesis of oligourea amide peptidomimetics.

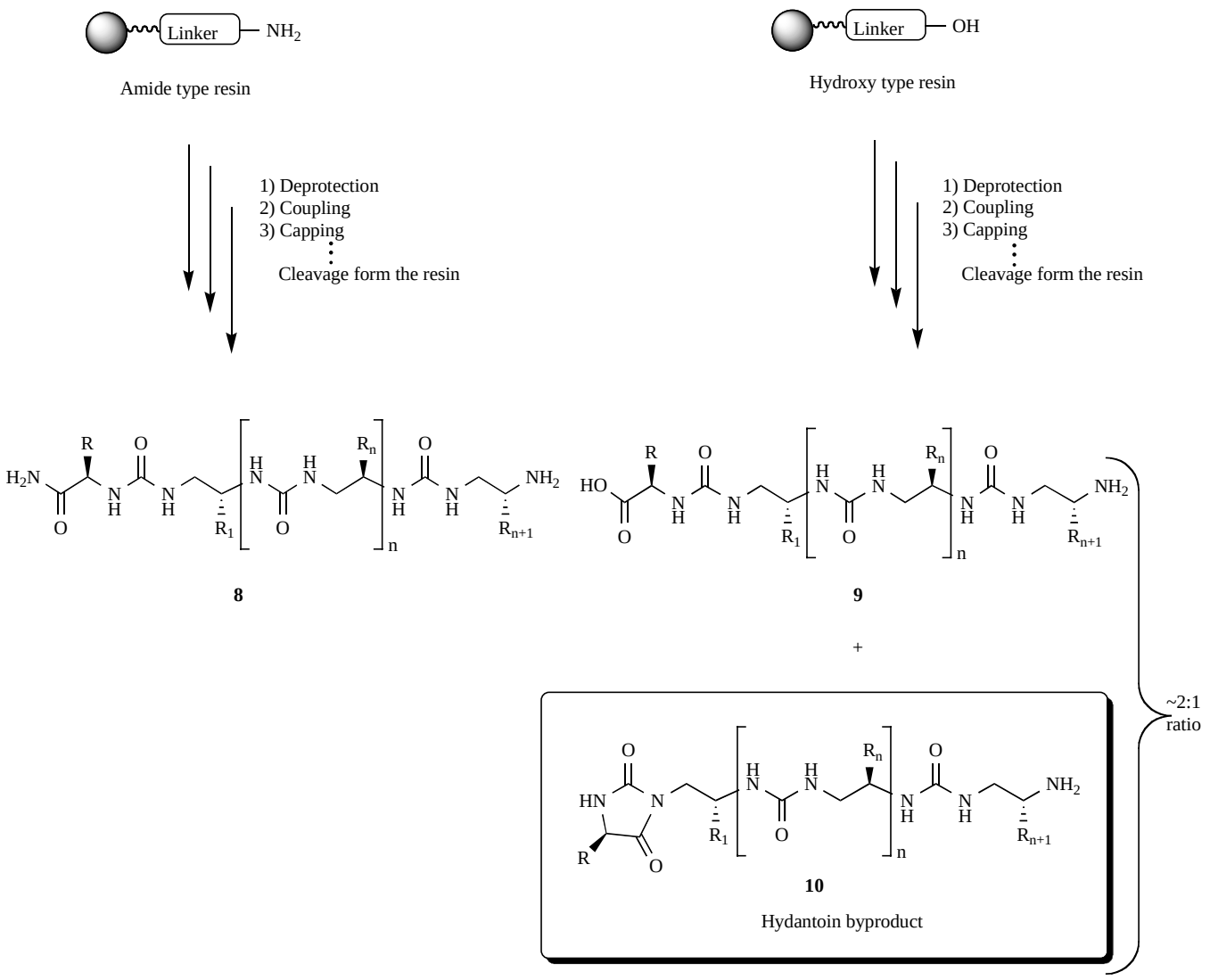

Fig. (6). Solid-phase synthesis of oligourea peptidomimetics on amide and hydroxy type resins.

reaction. It has been reported that the ratio of desired oligourea peptidomimetic acid product and hydantoin byproduct is approximately 2:1 [54]. However, these two compounds were separable by preparative HPLC.

Due to the synthetic efficiency and stability of final products as mentioned above, Fmoc methods resulted in the preparation of oligourea peptidomimetics in which each urea repeating unit was extended by one additional carbon atom in comparison with the amino acid counterpart. This extra carbon atom may increase the lipophilicity and flexibility of the main chain, which could therefore make these peptidomimetics easier to cross the cell wall or the blood–brain barrier. On the other hand, the hydrogen-bond forming urea units may also increase their water solubility and provide additional binding sites for interaction with their biological targets [43].

V. DEPSIPEPTIDE AND DEPSIDE SYNTHESIS

Replacement of the amide groups that undergo proteolytic hydrolysis with ester groups may also lead to longer acting compounds not so prone to proteolysis [58-61]. Naturally occurring depsipeptides that contain one or more ester bonds in addition to the amide bonds, have been found in many natural organisms, such as fungi, bacteria, and marine organisms [62,63]. It is very well known that these natural products and their derivatives exhibit a diverse spectrum of biological activities including insecticidal, antiviral, antimicrobial, anti-tumor, tumor-promotive, anti-inflammatory and immunosuppressive actions. On the other hand, depsides are peptide analogs entirely built up by hydroxyl acids mutually connected through ester bonds. A representative example of depsides is naturally occurring macrotetralide antibiotic nonactin [64]. Nonactin has been shown to possess activity against the P170-glycoprotein efflux pump associated with multiple drug resistant cancer cells.

Among many approaches described in the literature for depsipeptide and depside synthesis, carbodiimide/4-dimethylaminopyridine (DMAP) coupling method developed by Riguera *et al*. showed to be the most efficient, and also importantly, it is fully compatible with Fmoc solid-phase synthetic methodology [65-67]. Carbodiimide reagents have

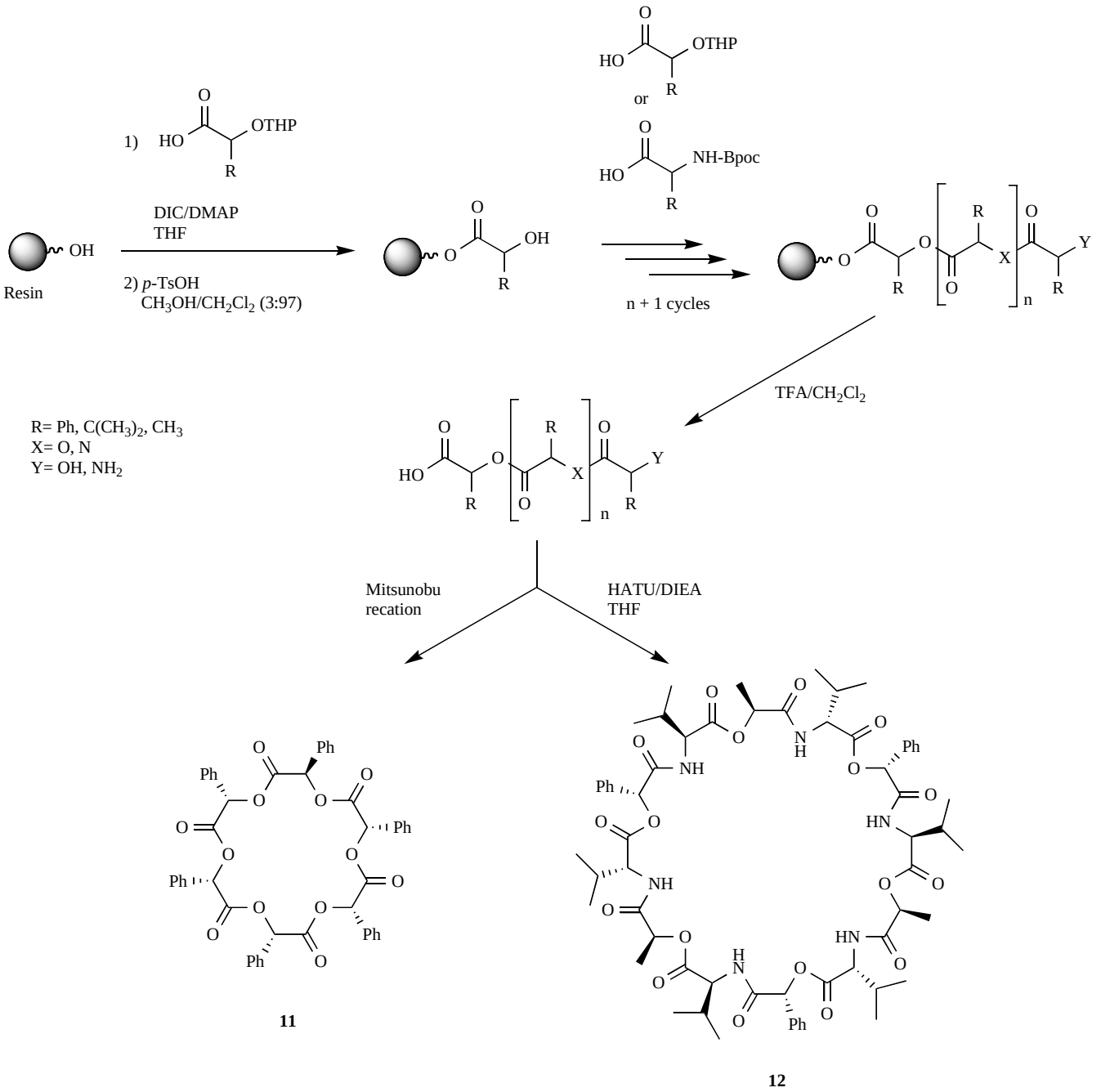

Fig. (7). Depside and depsipeptide synthesis using DIC/DMAP method.

been widely used in peptide synthesis because of their moderate activity and low cost [68]. They are used as coupling reagents and esterification reagents during loading of the first amino acid on the resin. The most commonly used carbodiimide reagent is 1,3-diisopropylcarbodiimide (DIC). By using 2:1 molar ratio of amino acid to DIC, the symmetrical anhydride is formed, which in turn reacts with the free hydroxyl group and the ester bond is formed. When carbodiimide is used in 1:1 molar ratio with amino acid, the reaction proceeds *via O*-acylisourea mechanism [69]. The reaction is catalyzed by the presence of DMAP, which increases the nucleophilicity of the hydroxyl group [70]. Using this synthetic methodology, Riguera *et al.* prepared linear precursors for depside **11** and depsipeptide **12**, (Fig. **7**) [67]. Final cyclic products were obtained after cleavage of linear precursors from the resin and cyclization in solution under high dilution conditions.

Cudic *et al.* demonstrated that cyclic depsipeptides, in particular cyclic lipodepsipeptides, can be synthesized entirely on the solid support [71]. This synthetic approach includes resin attachment of the first amino acid *via* side chain, use of combination of four quasi-orthogonal removable protecting groups, stepwise solid-phase synthesis of linear peptide analog, lipid tail attachment followed by depsipeptide bond formation and on-resin head-to-tail cyclization (Fig. **8**). An analog of naturally occurring cyclic lipodepsipeptide antibiotic fusaricidin A **13** was synthesized using this strategy. The best results for amino acid coupling

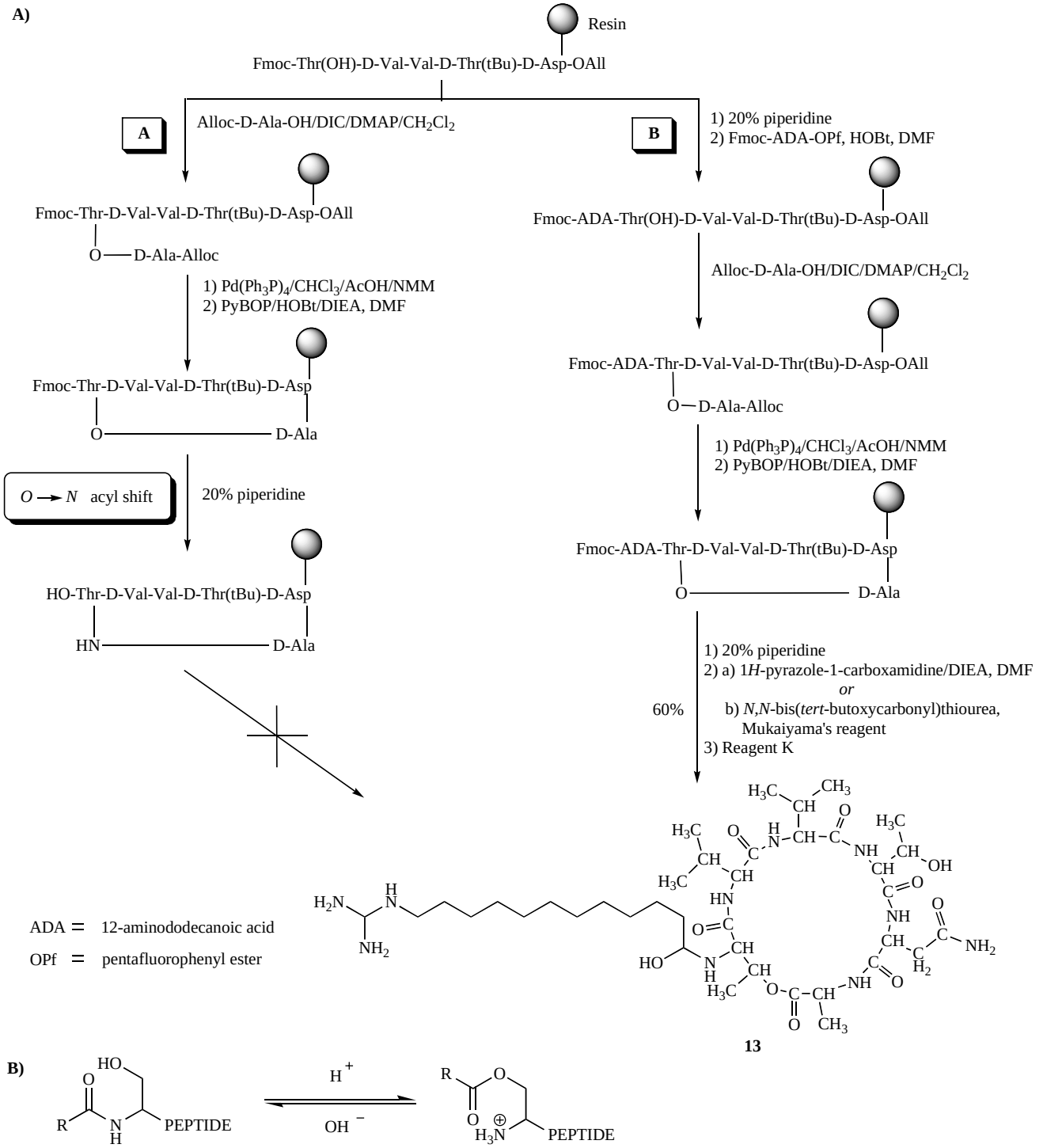

Fig. (8). Solid-phase synthesis of cyclic lipodepsipeptide (A); General mechanism for reversible intramolecular *O→N* or *N→O* acyl shift (B).

via an ester bond were obtained using polyethylene glycol (PEG) based resins such as TentaGel S RAM and CH_2Cl_2 as a solvent [71]. The use of polar DMF, a typical solvent for SPPS, leads to no ester bond formation. Poor coupling yields were obtained on polystyrene (PS) based Rink-MBHA resins regardless of the solvent used. These results could be attributed to a better swelling of PEG based resins [72], rapid DIC activation of the carboxylic group [73] and significant suppression of *N*-acylurea byproduct formation [74] in a non-polar solvent such as CH_2Cl_2. Another obstacle in depsipeptide synthesis is represented as the intramolecular *O→N* acyl shift that may occur if basic conditions were to be used [71,75]. Reversible intramolecular *O→N* or *N→O* acyl shifts are well known side reactions that may occur during peptide synthesis [75]. Peptides containing Ser or Thr residues undergo *N→O* acyl shift under acidic conditions, while exposure of corresponding depsipeptides to basic

conditions leads to opposite *O*→*N* acyl shift. This undesired acyl shift can be avoided by incorporation of an amino acid with an acid labile N^{α}-protecting group into the peptide chain, or by permanently blocking peptide's *N*-terminus prior depsipeptide's ester bond formation [71].

VI. PEPTOIDS AND PEPTIDE-PEPTOID HYBRID SYNTHESIS

The oligomeric peptidomimetics such as peptoids are particularly interesting compounds since they provide access to an enormous molecular diversity by variation of the building blocks. Peptoids represent a class of polymers that are not found in nature. They differ from the peptides in the manner of side chain attachment and thus can be considered as peptide mimetics in which the side chain has been shifted from the chiral α-carbon atom in a peptide to the achiral nitrogen [76]. Peptoids lack the hydrogen of the peptide secondary amides and thus are incapable of forming the same type of hydrogen bond networks that stabilize peptide helices and β-sheets. In addition, the peptoid polymer backbone is achiral. However, chiral center can be introduced in the side chains in order to obtain preferred secondary structures. Among many peptoid properties, an improved bioavailability [77,78] and protease resistance [79,80] are especially attractive for peptidomimetic design.

Two methods for the synthesis of peptoids are described in the literature: *monomeric* (Fig. **9A**) and *submonomeric* (Fig. **9B**), both developed by Zuckermann *et al*. The monomeric method is analogous to the standard solid-phase peptide synthesis [81-83]. In general, this method includes activation of the N^{α}-Fmoc protected *N*-substituted glycine using standard reagents for the Fmoc solid-phase chemistry, followed by its coupling to the secondary amino group of the resin bound peptoid chain. This step is repeated until the desired peptoid sequence is synthesized. However, a major disadvantage of this method is the requirement of the separate synthesis of suitably protected *N*-substituted glycine monomeric building blocks. On the other hand, the submonomeric method represents a more practical approach since this method does not require the use of Fmoc protected *N*-substituted glycines. Instead, any amine (side chain protected, if necessary) can be used. In the submonomeric method, each cycle of monomeric building blocks addition consists of an acylation step using haloacetic acid (bromoacetic acid) and a nucleophilic displacement step. This method is particularly useful for the synthesis of peptoid based combinatorial libraries [84]. Protection of carboxyl, thiol, amino and other reactive side chain functionalities is required to minimize undesired side reactions. However, the mild reactivity of some side-chain moieties toward displacement or acylation may allow their use without protection (e.g. indole, imidazole and phenol).

Since the monomeric method for peptoid synthesis is in principle identical to the standard Fmoc solid-phase peptide synthesis and the methods for preparation of fully protected monomeric building blocks are described in the literature [81,82], it will not be further described in this chapter. It is noteworthy that coupling of monomeric units using this method is more difficult to perform in comparison to the peptides due to the secondary amine's low reactivity if electron withdrawing groups are attached, and also due to the sterical hindrance around this atom. Therefore for these difficult couplings, reagents such as bromo-tri-pyrrolidino-phosphonium hexafluorophosphate (PyBroP), PyBOP or *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) are recommended.

Since the standard solid-phase peptide synthesis starts from the *C*-terminus and finishes at the *N*-terminus, the solid-phase peptoid and peptide synthesis could be combined, giving peptide–peptoid hybrid polymers. Ostergaard and Holm named these hybrids peptomers [85]. This approach may also be used in the conversion of biologically active peptide ligands, such as peptide hormones or protease inhibitors, into an active peptomeric version by ensuring that the essential amino acids comprising the lead motif are included in the synthesis [80]. Both peptoids and peptomers can be easily sequenced using modified Edman degradation conditions [86].

VII. AZAPEPTIDE SYNTHESIS

Azapeptides are peptide analogs in which one or more α-carbon atoms are replaced with nitrogen atoms [2,87]. It has been reported that the incorporation of azaaminoacid into peptide sequences induces turn conformations [87] and reduce the flexibility of the parent linear peptide due to the replacement of the rotatable C^{α}-CO by a more rigid urea N^{α}-CO bond [88,89]. In addition, this modification may also increase the number and strength of potential H-bonds. The NH-group attached to N^{α}-atom is more acidic than in non-

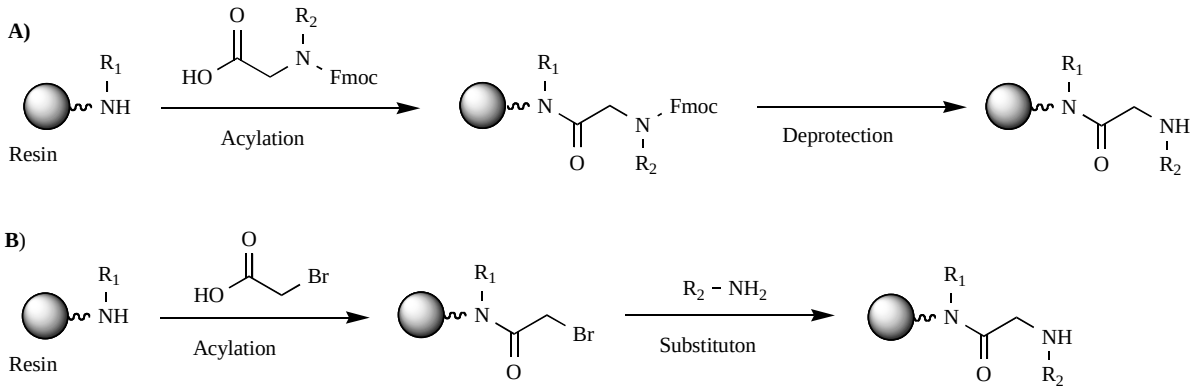

Fig. (9). Two methods of peptoids synthesis: monomeric (A) and submonomeric (B).

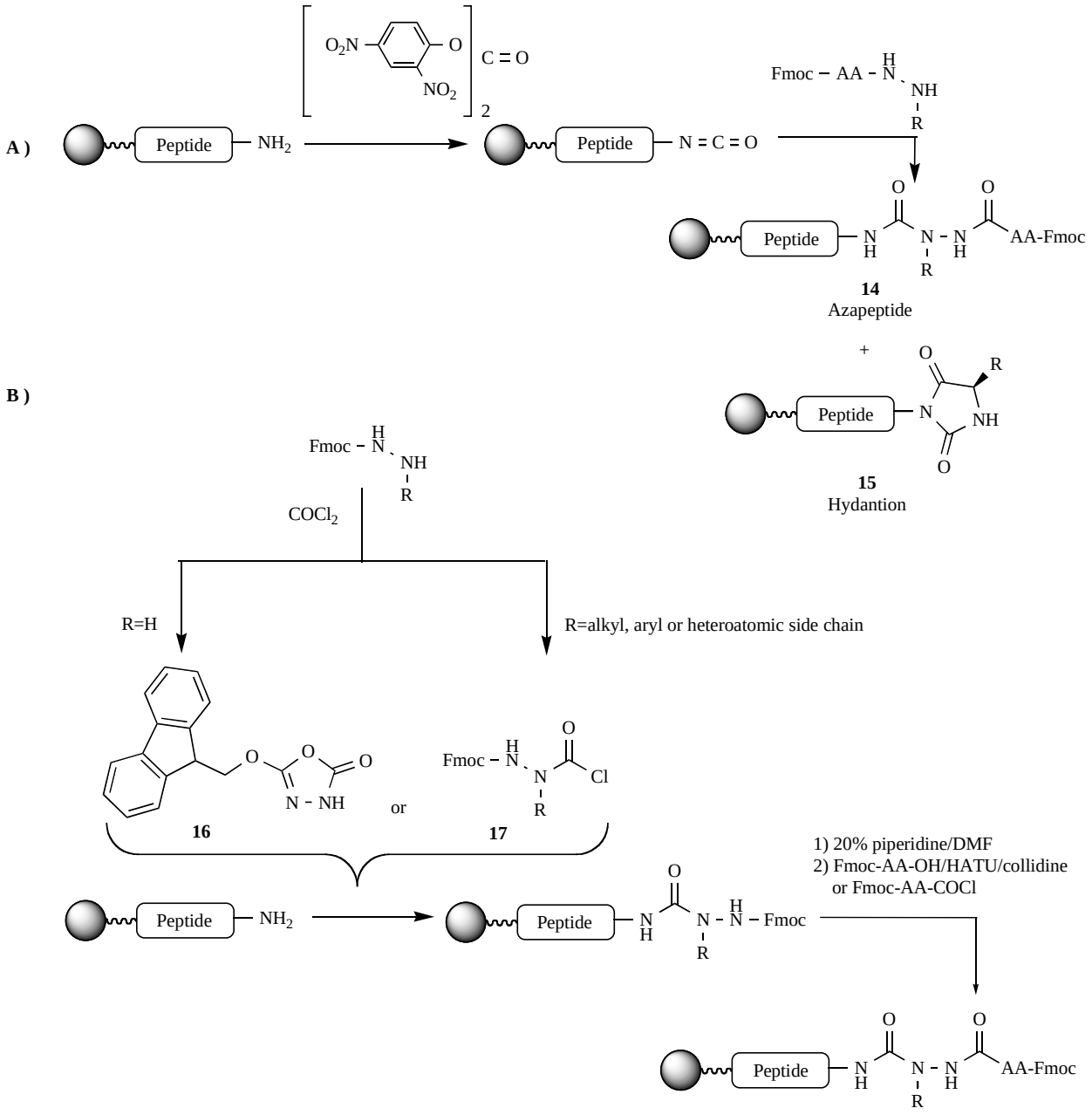

Fig. (10). Solid-phase synthesis of azapeptides.

aza peptides, therefore favoring stronger H-bonding [90]. All these characteristics make azapeptides particularly attractive leading structures for drug design. Numerous aza-peptides with improved pharmacological properties compared to their parent peptides have been reported. Such examples are aza-analogs of angiotensin [91], enkephalin [92], oxytocin [93], eledoisin [94], lubilerin [95], thyrotropin-releasing hormone (TRH) [96] and somastatin [97].

A variety of methods have been developed for azapeptide synthesis. However, only a few strategies have been reported for azapeptide solid-phase synthesis using Fmoc-chemistry. These methods are based on either activation of the peptide *N*-terminus or activation of the Fmoc protected hydrazine building blocks. Quibell *et al.* prepared azapeptides **14** on a solid-support *via* activation of the *N*-terminal amino group of a growing peptide chain with *bis*-2,4-dinitrophenyl carbonate in the presence of a base, followed by a reaction with Fmoc-protected amino acid carbazates and hydrazides (Fig. **10A**), [98]. Unfortunately, this methodology also led to the formation of resin-bound hydantoin byproducts **15** and therefore reduced azapeptides purity and yields. Hydantoin formation arises as a result of intramolecular nucleophilic attack on the activated intermediate by the secondary nitrogen from the preceding *C*-terminal peptide backbone chain. However, undesired hydantoin formation could be prevented by omission of the base from the activation procedure or, as shown by Johnson *et al.* [99], by using the *N*-2-hydroxy-4-methoxybenzyl (Hmb) reversible amide bond protecting group. Kessler *et al.* developed a method for the solid-phase synthesis of aza-RGD-mimetics that utilizes activated *N*-protected carbazoic acid or carbazic acid chloride building blocks (Fig. **10B**) [100]. Treatment of

Fmoc-hydrazine with phosgene in toluene resulted in the formation of corresponding carbazoic acid **16**, while Fmoc-methylhydrazines under slightly modified reaction conditions yielded carbazic acid chlorides **17**. RGD-mimetics were assembled on a solid support using Fmoc-chemistry and individually optimized reaction conditions for activated aza-building blocks coupling. In all prepared RGD-mimetics, glycine was replaced with aza-building blocks and the effect of aza-substituents on binding to $\alpha v\beta 3$- and αIIbβ3 integrins was examined.

A more general Fmoc-method for azapeptide solid-phase synthesis was described by Lubell *et al.* [101]. In this method, *N*'-substituted Fmoc-carbazates were activated with phosgene in toluene and coupled to the resin-bound peptide in the presence of DIEA (Fig. **10B**). In the next step, the Fmoc protecting group was removed using standard deprotection protocol and the resulting free aza-amino acid residue was acylated with the next Fmoc-amino acid. However, due to low reactivity of aza-amino acid residues [100-102], acylation was not observed using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate (HBTU) coupling reagents. An efficient acylation was achieved using only Fmoc-amino acid chlorides, which are in this case generated *in situ* with *bis*-(trichloromethyl)-carbonate (BTC) and 2,4,6-collidine. This method showed to be very effective for introducing aza-amino acid residues with aliphatic, aromatic and heteroatomic side chains.

Other interesting azapeptide analogs are hydrazino-peptides (Fig. **11**), which can be viewed as azaanalogs of β-peptides [103]. Due to interesting biological (proteolytic stability) [103] and conformational properties (induced β-turn type conformation) [104], hydrazinopeptides also represent an attractive peptidomimetic approach for drug design applications. However, despite these interesting properties, hydrazinopeptides have not been widely studied mainly because of the difficulties associated with their synthesis. The regioselective acylation of hydrazinoacids is particularly difficult without orthogonal protection of their two nitrogen atoms [105,106], whereas coupling of unprotected hydrazinoacids can give rise to diketopiperazine or oligomeric byproducts [107].

Recently, Jamart-Grégoire *et al.* described the first Fmoc solid-phase synthesis of some hydrazinopeptides [108]. Two

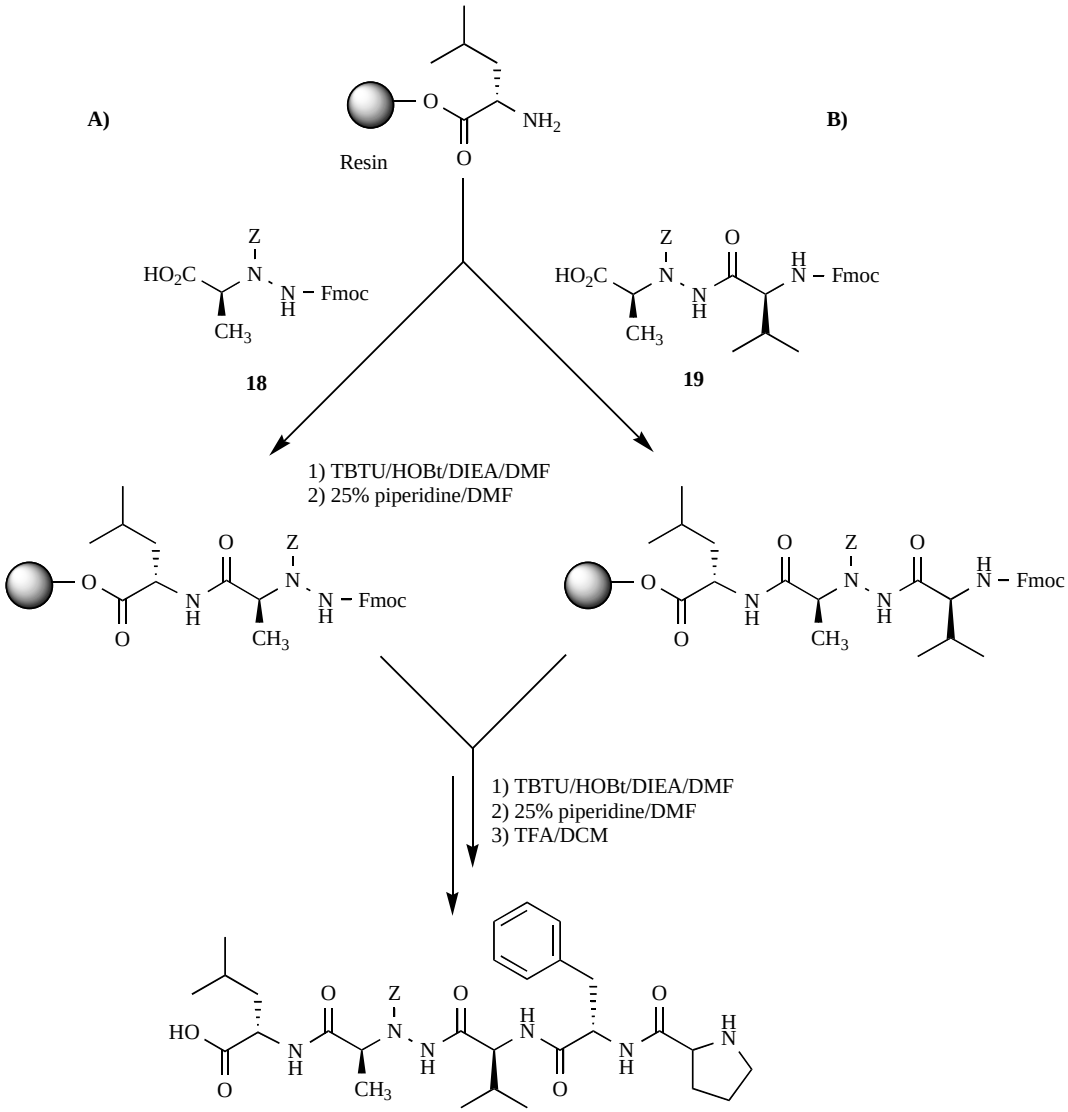

Fig. (11). Solid-phase synthesis of PFVh(Z)AL *via* step-by-step (A) and semi-convergent (B) strategies.

strategies were employed for the solid-phase synthesis of PFVh(Z)AL and PFh(Z)AVL as model hydrazinopeptides: a step-by-step synthesis, consisting of the coupling of N^{α},N^{β}-orthogonally protected hydrazinoacid **18**, (Fig. **11A**) and semi-convergent synthesis that involved direct incorporation of a hydrazinodipeptide **19**, (Fig. **11B**). In both cases, Fmoc chemistry was used throughout the synthesis. N^{β}-Fmoc-N^{α}-Z-hydrazino acids **18** and building block **19** were prepared over six steps starting from commercially available α-amino acids [109]. The efficiency of every coupling and deprotection step was monitored by HR-MAS NMR (High-Resolution Magic Angle Spinning) analysis. Coupling of the Fmoc hydrazinoacids **18** was found to be difficult and strongly dependent on both amino acid side chain bulkiness and solvent. Using standard Fmoc synthetic protocol, only 13% yield of the final product was obtained. The low yield was explained by a decreased accessibility of the reactants due to the structuration of the growing pseudopeptide on the solid support [104]. However, a slight increase in the yield (27%) was obtained by using a solvent that has a destructuring effect on peptides, such as DMF/DCM/NMP (33/33/33 v/v/v) [110] and piperidine/DMF/NMP/toluene (25/25/25/25 v/v/v/v) for the deprotection of the Fmoc group. The effect of steric hindrance on the coupling reactions was demonstrated by the synthesis of less sterically hindered PFh(Z)AVL hydrazinopeptide in 36% yield. The second synthetic approach that requires coupling of hydrazinodipeptide building block **19** was more efficient. When DMF was used as a solvent, an overall 35% yield was obtained, whereas the use of DMF/DCM/NMP mixture led to 46% of the final pure product.

ACKNOWLEDGMENTS

We wish to acknowledge the support of cyclic lipodepsipeptide work described in this article by the NIH (1S06-GM073621-01) and AHA (0630175N) Grant to P.C.

REFERENCES

[1] Marx, V. *C&EN*, **2005**, *83*, 17.
[2] Adessi, C.; Soto, C. *Curr. Med. Chem.*, **2002**, *9*, 963.
[3] Sawyer, T.K. In *Peptide and Protein Drug Analysis*; Reid, R.E. Ed.; Marcel Dekker, Inc.: New York, **2000**; Vol. *101*, pp. 81-115.
[4] Davies, J.S. *J. Pept. Sci*., **2003**, *9*, 471.
[5] Lambert, J.N.; Mitchell, J.P.; Roberts, K.D. *J. Chem. Soc. Perkin Trans*., **2001**, *1*, 471.
[6] Li, P.; Roller, P.P. *Curr. Top. Med. Chem.*, **2002**, *2*, 325.
[7] Blackburn, C.; Kates, S.A. *Methods Enzymol.*, 1**997**, *289*, 175.
[8] Hruby, V.J.; Bonner, G. G. *Methods Mol. Biol*., **1994**, *35*, 201.
[9] Kates, S.A.; Sole, N.A.; Albericio, F.; Barany, G. In *Peptides: Design, Synthesis, and Biological Activity*, Brikhauser: Boston: **1994**; pp. 39-59.
[10] Ahn, J.M.; Boyle, N.A.; MacDonald, M.T.; Janda, K.D. *Mini Rev. Med. Chem*., **2002**, *2*, 463.
[11] *Synthesis of Peptidomimetics, Synthesis of Peptides and Peptidomimetics. Houben-Weyl*, Goodman, M.; Felix, A.; Moroder, L.; Toniolo, C., Ed.; Thieme: Stuttgard, New York, **2004**; Vol. *E 22c*.
[12] Spatola, A.F. In *Peptides and Proteins*, Weinstein, B., Ed.; Marcel Dekker Inc.: New York, **1983**; Vol. *3*, pp. 267-357.
[13] Radkiewicz, J.; McAllister, M.A.; Goldstein, E.; Houk, K.N. *J. Org. Chem.*, **1998**, *63*, 1419.
[14] Moree, W.J.; Schouten, A.; Kroon, J.; Liskamp, R.M.J. *Int. J. Pept. Protien Res.*, **1995**, *45*, 501.
[15] Paik, S.; White, E.H. *Tetrahedron*, **1996**, *52*, 503.
[16] Liskamp, R.M.J.; Kruijtzer A.W. *Mole. Divers.*, **2004**, *8*, 79.
[17] De Jong, R.; Rijkers, D.T.S.; Liskamp, R.M.J. *Helv. Chim. Acta*, **2002**, *85*, 4230.
[18] De Bont, D.B.A.; Dijkstra, G.D.H.; Den Hartog, J.A.J.; Liskamp, R.M.J. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 3035.
[19] De Bont, D.B.A.; Sliedregt, K.M.; Hofmeyer, L.J.F.; Liskamp, R.M.J. *Bioorg. Med. Chem.*, **1999**, *7*, 1043.
[20] Moree, W.J.; Vand der Marel, G.A.; Liskamp, R.M.J. *J. Org. Chem.*, **1995**, *60*, 5157.
[21] Humljan, J.; Kotnik, M.; Boniface, A.; Solmajer. T.; Urleb, U.; Blanot, D.; Gobec, S. *Tetrahedron*, **2006**, *62*, 10980.
[22] Humljan, J.; Gobec, S. *Tetrahedron Lett.* **2005**, *46*, 4069.
[23] Brouwer, A.J.; Monnee, M.C.F.; Liskamp, R.M.J. *Synthesis*, **2000**, 1579.
[24] Monnee, M.C.F.; Marijne, M.F.; Brouwer, A.J.; Liskamp, R.M.J. *Tetrahedron Lett*., **2000**, *41*, 7991.
[25] Van Ameijde, J.; Liskamp, R.M.J. *Tetrahedron Lett.*, **2000**, *41*, 1103.
[26] Gennari, C.; Gude, M.; Potenza, D.; Piarulli, U. *Chem. Eur. J.*, **1998**, *4*, 1924.
[27] De Bont, D.B.A.; Moree, W.J.; Liskamp, R.M.J. *Bioorg. Med. Chem.*, **1996**, *4*, 667.
[28] Rodriguez, M.; Llinares, M.; Doulut, S.; Heitz, A.; Martinez, J. *Tetrahedron Lett*., **1991**, *32*, 923.
[29] Krchnak, V.; Vagner, J.; Lebl, M. *Int. J. Pept. Protein Res.*, **1988**, *32*, 415.
[30] Bartlett, P.A.; Hanson, J.E.; Morgan, B.P.; Ellsworth, B.A. In *Synthesis of Peptides and Peptidomimetics.* Houben-Weyl, Goodman, M.; Felix, A.; Moroder, L.; Toniolo, C. Ed.; Thieme: Stuttgard, New York, **2004**, Vol. *E 22c*. pp. 492-528.
[31] Palacios, F.; Alonso, C.; de los Santos, J. *Curr. Org. Chem*., **2004**, *8*, 1481.
[32] Bird, J.; De Mello, R.C.; Harper, G.P.; Hunter, D.J.; Karran, E.H.; Markwell, R.E.; Miles-Williams, A.J.; Rahman, S.S.; Ward, R.W. *J. Med. Chem.*, **1994**, *37*, 158.
[33] De Lombeart, S.; Erion, M.D.; Tan, J.; Blanchard, L.; El-Chehabi, L.; Ghai, R.D.; Sakane, Y.; Berry, C.; Trapani, A.J. *J. Med. Chem*., **1994**, *37*, 1498.
[34] Barlett, P.A.; Hanson, J.E.; Giannousis, P.P. *J. Org. Chem.*, **1990**, *55*, 6268.
[35] Campbell, D.A.; Bermak, J.C. *J. Am. Chem. Soc*., **1994**, *116*, 6039.
[36] Campbell, D.A.; Bermak, J.C. *J. Org. Chem.*, **1994**, *59*, 658.
[37] Campbell, D.A. *J. Org. Chem.*, **1992**, *57*, 6331.
[38] Champagne, J.–M.; Coste, J.; Jouin, P. *Tetrahedron Lett.*, **1995**, *36*, 2079.
[39] Champagne, J.–M.; Coste, J.; Jouin, P. *Tetrahedron Lett.*, **1993**, *34*, 6743.
[40] Seebach, D.; Charczuk, R.; Berber, C.; Renaud, P.; Bener, H.; Schneider, H. *Helv. Chim. Acta*, **1989**, *72*, 401.
[41] Nicolau, K.C.; Winssinger, N.; Pastor, J.; DeRoose, F. *J. Am. Chem. Soc*., **1997**, *119*, 449.
[42] Gademann, K.; Seebach, D. *Helv. Chim. Acta*, **2001**, *84*, 2924.
[43] Tamilarasu, N.; Hoq, I.; Rana, T.M. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 505.
[44] Appella, D.H.; Christianson, L.A., Karle, I.L., Powell, D.R.; Gellman, S.H. *J. Am. Chem. Soc*., **1996**, *118*, 13071.
[45] Wang, X.; Espinosa, J.F.; Gellman, S.H. *J. Am. Chem. Soc.*, **2000**, *122*, 4821.
[46] Hanessian, S.; Luo, R.; Schaum, S.; Michcnik, S. *J. Am. Chem. Soc*., **1998**, *120*, 8569.
[47] Hanessian, S.; Luo, X.; Schaum, R. *Tetrahedron Lett*., **1999**, *40*, 4925.
[48] Seebach, D.; Ciceri, P.; Overhand, M.; Juan, B.; Rigo, D.; Oberer, L.; Hommel, U.; Amstutz, R.; Widmer, H. *Helv. Chim. Acta*, **1996**, *79*, 2043.
[49] Seebach, D.; Sifferlen, T.; Mathieu, P.A.; Hane, A.M.; Krell, C.M.; Bierbaum, D.J.; Abele, S. *Helv. Chim. Acta*, **2000**, *83*, 2849.
[50] Skurski, P.; Simons, J. *J. Chem. Phys*., **2001**, *115*, 8373.
[51] Burgess, K.; Ibarzo, J.; Linthicum, D.S.; Russell, D.H.; Shin, H.; Shitangkoon, A.; Totani, R.; Zhang, A.J. *J. Am. Chem. Soc.*, **1997**, *119*, 1556.
[52] Kim, J.–M.; Bi, Y.; Paikoff, S.J.; Scultz, P.G. *Tetrahedron Lett.*, **1996**, *37*, 5305.
[53] Guichard, G.; Semetey, V.; Rodriguez, M.; Briand, J.-P. *Tetrahedron Lett*., **2000**, *41*, 1553.
[54] Boeijen, A.; van Ameijde, J.; Liskamp, R.M. *J. Org. Chem.*, **2001**, *66*, 8454.
[55] Boeijen, A.; Liskamp, R.M.J. *Eur. J. Org. Chem*., **1999**, 2127.

[56] Alsina, J.; Scott, W. L.; O'Donell, M.J. *Tetrahedron Lett.*, **2005**, *46*, 3131.
[57] Nefzi, A.; Giulianotti, M.; Truong, L.; Rattan, S.; Ostresh, J.M.; Houghten, R.A. *J. Comb. Chem.*, **2002**, *4*, 175.
[58] Shemyakin, M.M.; Shchukina, L.A.; Vinogradova, E.I.; Ravidel, G.A.; Ovchinnikov, Y.A. *Experimentia*, **1966**, *22*, 535.
[59] Bramson, H.N.; Thomas, N.E.; Kaiser, E.T. *J. Biol. Chem.*, **1985**, *260*, 15452.
[60] Arad, O.; Goodman, M. *Biopolymers*, **1990**, *29*, 1633.
[61] Coombs, G.S.; Rao, M.S.; Olson, A.J.; Dawson, P.E.; Madison, E.L. *J. Biol. Chem.*, **1999**, *274*, 24074.
[62] Davidson, B.S. *Chem. Rev.*, **1993**, *93*, 1771.
[63] Fusetani, N.; Matsunaga, S. *Chem. Rev.*, **1993**, *93*, 1793.
[64] Woo, A.J.; Strohl, W.R.; Priestley, N.D. *Antimicrob. Agents Chemother.*, **1999**, *43*, 1662.
[65] Kuisle, O.; Quinoa, E.; Riguera, R. *Tetrahedron Lett.*, **1999**, *40*, 1203.
[66] Kuisle, O.; Lolo, M.; Quinoa, E.; Riguera, R. *Tetrahedron*, **1999**, *55*, 14807.
[67] Kuisle, O.; Quinoa, E.; Riguera, R. *J. Org. Chem.*, **1999**, *64*, 8063.
[68] Marder, O.; Albericio, F. *Chim. Oggi*, **2003**, *6*, 35.
[69] Stawikowski, M.; Cudic, P. In *Methods in Molecular Biology*, Fields, G.B. Ed., Humana Press, Inc.: Totowa, **2006**; Vol. *386*, pp. 321-339.
[70] Berry, J.D.; Digiovanna, V.C.; Metrick, S.S.; Murugan, R. *Arkivoc*, **2001**, 201.
[71] Stawikowski, M.; Cudic, P. *Tetrahedron Lett.*, **2006**, *47*, 8587.
[72] Park, B.–D.; Lee, Y.–S. *React. Funct. Polymers*, **2000**, *44*, 41.
[73] Hudson, D. *J. Org. Chem*. **1988**, *53*, 617.
[74] Podlech, J. In *Synthesis of Peptides and Peptidomimetics*, Houben-Weyl, Goodman, M.; Felix, A.; Moroder, L.; Toniolo, C. Ed.; Thieme: Stuttgart, New York, **2001**; Vol. *22a*, pp. 517 - 533.
[75] Mouls, L.; Subra, G.; Enjabal. C.; Martinez, J.; Aubagnac, J.L. *Tetrahedron Lett*., **2004**, *45*, 1173.
[76] Patch, J.A.; Kirshenbaum, K.; Seurynck, S.; Zuckermann, R.; Barron, A.E. In *Pseudo-peptides in Drug Development*. Neilsen, P.E., Ed.; Wiley-VCH Verlag: Weinheim, **2004**; pp. 1-31.
[77] Kwon, Y.U.; Kodadek, T. *J. Am. Chem. Soc*., **2007**, *129*, 1508.
[78] Schröder, T.; Schmitz, K.; Niemeier, N.; Balaban, T.S.; Krug, H.F.; Schepers, U.; Bräse, S. *Bioconjug. Chem*. **2007**, ASAP Article; DOI: 10.1021/bc0602073.
[79] Miller, S.M.; Simmon, R.J.; Ng, S.; Zuckermann, R.N.; Kerr, J.M.; Moos, W.H. *Drug Dev. Res*., **1995**, *35*, 20.
[80] Stawikowski, M.; Stawikowska, R.; Jaskiewicz, A.; Zablotna, E.; Rolka, K. *Chembiochem*, **2005**, *6*, 1057.
[81] Simon, R.J.; Kania, R.S.; Zuckermann, R.N.; Huebner, V.D.; Jewell, D.A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C.K. *Proc. Natl. Acad. Sci. USA*, **1992**, *89*, 9367.
[82] Kruijtzer, J.A.W.; Hofmeyer, L.J.F.; Heerma, W.; Versluis, C.; Liskamp, R.M.J. *Chem. Eur. J.*, **1998**, *4*, 1570.
[83] Uno, T.; Beausoleil, E.; Goldsmith, R.A.; Levine, B.H.; Zuckermann, R.N. *Tetrahedron Lett.*, **1999**, *40*, 1475.
[84] Zuckermann, R.N.; Kerr, J.M.; Kent, S.B.H.; Moos, W.H. *J. Am. Chem. Soc*., **1992**, *114*, 10646.
[85] Ostergaard, S.; Holm, A. *Mol. Divers.*, **1997**, *3*, 17.
[86] Boeijen, A.; Liskamp, R.M.J. *Tetrahedron Lett.*, **1998**, *39*, 3589.
[87] Zega, A.; Urleb, U. *Acta Chim. Slov*., **2002**, *49*, 649.
[88] Raynolds, C.H.; Horman, R.E. *J. Am. Chem. Soc*., **1996**, *118*, 9395.
[89] Lee, H.J.; Lee, M.H.; Choi, Y.S.; Park, H.M.; Lee, K.B. *J. Mol. Struct*., **2003**, *631*, 101.
[90] Gante, J. *Synthesis*, **1989**, 405.
[91] Hess, H.-J.; Moreland, W.T.; Laubach, G.D. *J. Am. Chem. Soc*., **1963**, *85*, 4041.
[92] Niedrich, H. *J. Prakt. Chem*., **1972**, *314*, 749.
[93] Oehme, P.; Bergmann, J.; Niedrich, H.; Jung, F.; Menzel, G.; Eichenstadt, M. *Acta Biol. Med. Ger*., **1970**, *25*, 613.
[94] Han, H.; Yoon, J.; Janda, K.D. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 117.
[95] Dutta, A.S.; Furr, B.J.A. *Ann. Rep. Med. Chem*., **1985**, *20*, 203.
[96] Zhang, W.J.; Berglund, A.; Kao, J.L.F.; Couty, J.P.; Gershengorn, M.C.; Marshall, J.R. *J. Am. Chem. Soc*., **2003**, *125*, 1221.
[97] Vedel, M.; Diaz, J.; Cazaubon, C. European patent 2523126, **1983**.
[98] Quibell, M.; Turnell, W.G.; Johnson, T. *J. Chem. Soc. Perkin Trans. 1*, **1993**, 284.
[99] Liley, M.; Johnson, T. *Tetrahedron Lett*., **2000**, *41*, 3983.
[100] Gibson, C.; Goodman, S.L.; Hahn, D.; Holzemann, G.; Kessler, H. *J. Org. Chem.*, **1999**, *64*, 7388.
[101] Boeglin, D.; Lubell, W.D. *J. Comb. Chem.*, **2005**, *7*, 864.
[102] Gray, C.J.; Quibell, M.; Bagget, N.; Hammerle, T. *Int. J. Pept. Protein Res*., **1992**, *40*, 351.
[103] Lelais, G.; Seebach, D. *Helv. Chim. Acta*, **2003**, *86*, 4152.
[104] Aubry, A.; Mangeot, J.P.; Vidal, J.; Collet, A.; Zerkout, S.; Marraud, M. *Int. J. Pept. Protein Res.*, **1994**, *43*, 305.
[105] Viret, J.; Collet, A.; Pichon-Pesme, V.; Aubry, A. *New J. Chem.*, **1988**, *12*, 253.
[106] Lecoq, A.; Marraud, M. *Tetrahedron Lett*., **1991**, *24*, 2765.
[107] Guy, L.; Vidal, J.; Collet, A. *J. Med. Chem.*, **1998**, *41*, 4833.
[108] Bouillon, I.; Vanderesse, R.; Brosse, N.; Fabre, O.; Jamart-Grégoire, B. *Tetrahedron*, **2007**, *63*, 9635.
[109] Bouillon, I.; Brosse, N.; Vanderesse, R.; Jamart-Grégoire, B. *Tetrahedron*, **2007**, *63*, 2223.
[110] Zhang, L.; Goldammer, C.; Henkel, B.; Zuhl, F.; Panhaus, G.; Jungs, G.; Bayer, E. In *Innovation and Perspectives in Solid Phase Synthesis*; Epton, R., Ed.; Mayflower: Birmingham, **1994**; pp. 711-716.

Received: August 06, 2007 **Revised: September 07, 2007** **Accepted: September 15, 2007**