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Synthesis of Differentially Protected *N*-Acylated Reduced Pseudodipeptides as Building Units for Backbone Cyclic Peptides

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Abstract: Backbone cyclization has become an important method for generating or stabilizing the bioactive conformation of peptides without affecting the amino acid side-chains. Up to now, backbone cyclic peptides were mostly synthesized with bridges between *N*-amino- and *N*-carboxy-functionalized peptide bonds. To study the influence of a more flexible backbone on the biological activity, we have developed a new type of backbone cyclization which is achieved via the *N*-functionalized moieties of acylated reduced peptide bonds. As described in our previous publications, the formation of *N*-functionalized dipeptide units facilitates the peptide assembly compared with the incorporation of *N*-alkyl amino acids. Besides the racemization-free synthesis of Fmoc-protected pseudodipeptide esters with reduced peptide bonds, the new type of backbone modification allows the use of a great variety of ω-amino- and α,ω-dicarboxylic acids differing in chain length and chemical properties. Best results for the coupling of the ω-amino- and α,ω-dicarboxylic acids to the reduced peptide bond were obtained by the formation of mixed anhydrides with alkyl chloroformates. Whereas the protecting group combination of Z/OBzl in the dipeptide unit and Boc/O*t*Bu for the *N*-functionalized moiety leads to the formation of 2-ketopiperazine during hydrogenation, the combination of Fmoc/O*t*Bu and Alloc/OAll is very suitable for the synthesis of backbone cyclic peptides on solid support.

Keywords: *N*-acylated reduced peptide bonds; backbone cyclization; pseudodipeptides; synthesis

INTRODUCTION

A wide range of synthetic methods is available for the imposition of conformational constraints on peptides. Besides the classical ways of peptide cyclization, via the carboxyl and amino termini or the side-chains of naturally occurring amino acids, forming lactam bridges (Lys, Glu) or disulfide bonds (Cys), interest has been focused on the method of backbone cyclization developed by Gilon *et al.* [1] a few years ago. Here, cyclization is achieved through bridging *N*-carboxyalkyl and *N*-aminoalkyl amino acids, called building units. Several groups have already described the preparation of a variety of *N*-functionalized amino acids employing different synthetic strategies and different protecting group combinations [2–7]. Recently, we have reported the

Abbreviations: Alloc, allyloxycarbonyl; All, allyl; Boc, *tert*-butyloxycarbonyl; *t*Bu, *tert*-butyl; Fmoc, 9-fluorenylmethoxycarbonyl; DIEA, diisopropylethylamine; DCC, dicyclohexylcarbodiimide; DCM, methylene chloride; HATU, 1-hydroxy-7azabenzotriazole so-called uronium salt; PyBrop, Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate; TEA, triethylamine; THF, tetrahydrofuran; TFA, trifluoroacetic acid; TLC, thin layer chromatography; TMS, tetramethylsilane; HPLC, high-performance liquid chromatography; CI MS, chemical ionization mass spectrometry; FAB MS, fast atom bombardment mass spectrometry; NMR, nuclear magnetic resonance.

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synthesis of *N*-functionalized dipeptide building units with a pseudopeptide bond of the general structure Ψ[CON((CH$_2$)$_n$–X)] that contain a peptide bond modified by alkylation of the N^{α}-atom and which were prepared in solution [8]. From our studies on the solid phase synthesis of backbone cyclic peptides [3,12] we have decided to use dipeptide units since they can be applied in the same manner as protected amino acids for the condensation to the resin bound peptide, as well as for the coupling of the next amino acid to the dipeptide building unit. The smooth assembly of backbone cyclic peptides with the help of *N*-functionalized dipeptide units is the reason why we have also studied strategies for the synthesis of another type of *N*-modified dipeptide building units.

Alterations of peptide bonds in a given sequence have the potential to change their pharmacokinetic behavior. Conformationally restricted, backbone cyclic peptides containing *N*-functionalized peptide bonds were synthesized to improve bioactivity, receptor subtype selectivity and to increase the resistance against proteolytic degradation [1,3]. The replacement of the peptide bond by a reduced amide bond may also enhance metabolic stability and probably produces interesting biological properties in peptide analogs [9]. Although a reduced amide bond in a peptide chain is expected to cause increased backbone flexibility and a loss of hydrogen bond acceptor properties, a comparison of peptide sequences with different backbone modifications would give an insight into the influences of the backbone structure on the conformational flexibility, conformation and consequently on the biological activity [10,11]. To form backbone cyclic peptides with a reduced amide bond in the peptidic backbone Ψ[CH$_2$NH], pseudopeptide bonds have to be modified either by alkylation or acylation of the N^{α}-atom.

The formation of building units with alkylated reduced peptide bonds Ψ[CH$_2$N((CH$_2$)$_n$–X)] was described by Kaljuste and Unden [4]. In contrast with their synthesis on the solid phase we were not able to succeed with the formation of this type of building unit in solution. We have therefore focused our attention on the formation of building units with an acylated reduced peptide bond Ψ[CH$_2$N(CO(CH$_2$)$_n$–X)]. Contrary to the commonly used building units of the structure Ψ[CON((CH$_2$)$_n$–X)], the reduced peptide bond is located in the backbone.

For the assembly of these kind of backbone cyclic peptides, a new type of dipeptide unit with the structure Fmoc-AA$_1$Ψ[CH$_2$N(CO(CH$_2$)$_n$–X)] is required. These dipeptide units can be formed by acylation of the reduced peptide bond in the corresponding pseudodipeptides with semiprotected ω-amino and α,ω-dicarboxylic acids. Here, an optimized strategy for the synthesis of these dipeptide units and their chemical properties such as optical purity and stability in comparison with the known *N*-functionalized dipeptide units [8] is described. The presented building units were designed for the assembly of backbone cyclic somatostatin octapeptides.

MATERIALS AND METHODS

General

Purification by column chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). The compounds were characterized by TLC on precoated plates of silica gel 60 F$_{254}$ (Merck, Darmstadt, Germany). HPLC was performed on a Shimadzu chromatograph equipped with a Vydac 218TP54 column (5 μm particle size, 80 Å pore size, 4 × 250 mm), detection at 220 nm. The elution gradient was 20–80% B in 60 min with a flow set at 1 ml/min, where A was 0.1% TFA in water and B 0.1% TFA in acetonitrile. Retention times (t_R) are reported in minutes. Molecular weights were determined by CI or FAB mass spectrometry (Trio 2000, Fisons, UK). ^{1}H-NMR spectra were recorded on a Bruker instrument at 250 MHz using TMS as internal standard. Optical rotation was measured on a Polamat (Carl Zeiss Jena, Germany).

Syntheses

The *Z*- and Fmoc-protected amino aldehydes were prepared according to the literature [16,17].

Z-ValΨ(CH$_2$NH)Phe-OBzl (1). General Procedure for Ψ(CH$_2$NH) Pseudo-Dipeptides (Method A)

To a solution of Phe-OBzl × Tos (4.27 g, 10.0 mmol) in 100 ml of dry methanol, TEA (1.40 ml, 10.0 mmol) was added. *Z*-Val-H (2.58 g, 11.0 mmol) was dissolved in dry methanol, 2 g of molecular sieve were added and the reaction mixture was stirred at room temperature for 1 h. To the cooled solution (0°C) sodium cyanoborohydride (0.69 g, 11.0 mmol) was added in small portions. Stirring was continued for 12 h at room temperature. The molecular sieve was filtered off and the solvent evaporated *in vacuo*.

The residue was taken up with ethyl acetate, washed with 5% $KHSO_4$, saturated $NaHCO_3$ and saturated NaCl solution. The organic phase was dried over Na_2SO_4 and the solvent was evaporated off *in vacuo*. The product was obtained as a white solid compound in 69% yield. t_R 34.9 min. R_f 0.47 (27:10:0.5 benzene:aceton:AcOH). ^{1}H-NMR δ 0.79 (m, 6H), 1.73 (m, 2H), 1.88 (s, 1H), 2.87 (m, 1H), 3.35 (m, 2H), 3.61 (m, 2H), 5.0–5.15 (m, 4H), 6.18 (1H), 7.18–7.32 (m, 15H). CI MS $[MH]^+$ 475.

Z-D-PheΨ(CH_2NH)Phe-OBzl (2), According to Method A

Compound **2** was produced in 65% yield. t_R 37.8 min. R_f 0.55 (27:10:0.5 benzene:aceton:AcOH). ^{1}H-NMR δ 2.09 (s, 1H), 2.34 (m, 2H), 2.78 (m, 2H), 3.02 (m, 2H), 3.75 (m, 2H), 4.87 (m, 1H), 5.05–5.21 (m, 4H), 7.21–7.40 (m, 20H). CI MS $[MH]^+$ 523.

Z-ValΨ(CH_2N($COCH_2CH_2NHBoc$))Phe-OBzl (3). General Procedure for the Acylation of Ψ(CH_2NH) Pseudodipeptide Bond (Method B)

The acylation of the reduced peptide bond was performed according to the mixed anhydride method of Bodanszky and Bodanszky [22]. Boc-β-Ala (0.38 g, 2 mmol) was dissolved in 5 ml of dry THF and cooled to -15°C. *N*-methylmorpholine (0.22 ml, 2 mmol) and iso-butyl chloroformate (0.27 ml, 2 mmol) were added. After 10 min a solution of **1** (0.95 g, 2 mmol) and *N*-methylmorpholine (0.22 ml, 2 mmol) in 5 ml of THF were added. The mixture was allowed to warm up to room temperature and stirring was continued overnight. The solvent was evaporated off *in vacuo*. The residue was dissolved in ethyl acetate and washed with 5% $KHSO_4$, saturated $NaHCO_3$ and saturated NaCl. The organic phase was dried over Na_2SO_4 and the solvent evaporated off *in vacuo*. For analytical data of compound **3** see Table 2.

H-ValΨ(CH_2N($COCH_2CH_2NHBoc$))Phe-OH (4). General Procedure for the Hydrogenation of Z/OBzl-Protecting Groups (Method C)

Compound **3** (5 g) was dissolved in 500 ml of 90% aqueous acetic acid. After adding the catalyst, Pd(acetate), the mixture was hydrogenated at room temperature overnight. The catalyst was removed by filtration and washed with acetic acid. The combined filtrates were evaporated *in vacuo*, the residue washed with benzene and concentrated *in vacuo*. The product was then washed with DCM before purification by column chromatography using 9:1 chloroform:methanol as solvent system to yield 30–45% of a white solid substance. Analytical data for compound **4** are listed in Table 2.

Fmoc-ValΨ(CH_2NH)Phe-O*t*Bu (5), According to Method A

Phe-O*t*Bu × HAc (2.81 g, 10.0 mmol) and 3.23 g of Fmoc-Val-H (10.0 mmol) were used to yield 83% of compound **5**. t_R 42.0 min. R_f 0.40 (27:10:0.5 benzene:aceton:AcOH). ^{1}H-NMR δ 0.86 (m, 6H), 1.35 (s, 9H), 1.70 (s, 1H), 2.18 (m, 1H), 2.82 (m, 2H), 3.02 (m, 2H), 3.28 (m, 1H), 3.60 (m, 1H), 4.48 (m, 1H), 4.70 (m, 2H), 6.05 (s, 1H), 7.17–7.38 (m, 13H). CI MS $[MH]^+$ 529.

Fmoc-D-PheΨ(CH_2NH)Phe-O*t*Bu (6), According to Method A

Fmoc-D-Phe-H (3.55 g, 10.0 mmol) were used for the reaction with Phe-O*t*Bu × HAc (2.81 g, 10.0 mmol) to yield 78% of compound **6**. t_R 43.8 min. R_f 0.49 (27:10:0.5 benzene:aceton:AcOH). ^{1}H-NMR δ 1.40 (s, 9H), 2.78 (m, 4H), 3.02 (m, 2H), 3.72 (m, 1H), 4.05 (m, 1H), 4.20 (m, 1H), 4.62 (m, 2H), 5.82 (s, 1H), 7.12-7.45 (m, 18H). CI MS $[MH]^+$ 577.

Fmoc-ValΨ(CH_2N($COCH_2CH_2NHAlloc$))Phe-O*t*Bu (7), According to Method B

Alloc-β-Ala (0.35 g, 2.0 mmol) and compound **5** (1.06 g, 2.0 mmol) were used to produce compound **7**. t_R 55.1 min. R_f 0.42 (27:10:0.5 benzene:aceton:AcOH). FAB MS $[MH]^+$ 685.

Fmoc-D-PheΨ(CH_2N($COCH_2CH_2NHAlloc$))Phe-O*t*Bu (8), According to Method B

Alloc-β-Ala (0.35 g, 2.0 mmol) and compound **6** (1.15 g, 2.0 mmol) were used to produce compound **8**. t_R 55.5 min. R_f 0.44 (27:10:0.5 benzene:aceton:AcOH). FAB MS $[MH]^+$ 732.

Succinic Acid Monoallyl Ester (9) (Method D)

The formation of succinic acid monoallyl ester from succinic anhydride and allyl alcohol has been described by Casimir *et al.* [23]. Succinic anhydride (20.0 g, 0.2 mol) was treated with allyl alcohol (12.30 ml, 0.6 mol) in toluene and DMAP was added. The mixture was refluxed for 4 h. The pure compound was obtained by fractionated distillation as a colorless oil in 84% yield. Kp: 101°C at 0.07 mbar. ^{1}H-NMR δ 2.62 (m, 4H), 4.56 (m, 2H), 5.23

(m, 2H), 5.89 (m, 1H), 11.29 (s, 1H). EI MS $[MH]^+$ 159.

Fmoc-ValΨ(CH_2N($COCH_2CH_2$COOAll))Phe-O*t*Bu (10), According to Method B

Compound **10** was produced using 0.32 g of compound **9** (2.0 mmol) and 1.06 g of compound **5** (2.0 mmol). t_R 58.2 min. R_f 0.46 (27:10:0.5 benzene:aceton:AcOH). FAB MS $[MH]^+$ 660.

Fmoc-D-PheΨ(CH_2N($COCH_2CH_2$COOAll))Phe-O*t*Bu (11), According to Method B

Compound **11** was produced using 0.32 g of compound **9** (2.0 mmol) and 1.15 g of compound **6** (2.0 mmol). t_R 58.7 min. R_f 0.50 (27:10:0.5 benzene:aceton:AcOH) FAB MS $[MH]^+$ 717.

Fmoc-ValΨ(CH_2N($COCH_2CH_2$NHAlloc))Phe-OH (12), General Procedure for *tert*-Butyl Cleavage (Method E)

The cleavage of the *tert*-butyl ester of compound **7** was carried out with 90% TFA/water for 1 h. Then, TFA and water were removed by evaporation and the residue was washed several times with diethyl ether. The crude product was obtained as a yellow oil and purified by column chromatography on silica gel eluted with 9:1 chloroform:methanol.

Compounds **13**–**15** were prepared according to method E. Analytical data of these compounds are summarized in Table 2.

RESULTS AND DISCUSSION

The synthesis of dipeptide units containing an acylated reduced amide bond (Figure 1) is reported. These building units are suitable for solid phase synthesis of backbone cyclic somatostatin octapeptides, the preparation of which will be presented elsewhere.

Synthesis of Pseudodipeptides with a Reduced Peptide Bond

For the preparation of Ψ[CH_2NH] pseudodipeptides different synthetic methods have been applied—either by reductive alkylation of an α-amino group by the reaction of a N^{α}-protected amino aldehyde using sodium cyanoborohydride as reducing agent directly on the solid phase [14,15] and in solution [7], or via reduction of a peptide thioamide bond Ψ[CSNH] by employing $NiCl_2$ [11]. From our experience in the field of ACE and renin inhibitors [13], we prefer to synthesize Ψ[CH_2NH] pseudodipeptides by the reductive alkylation reaction.

Several pseudodipeptides containing a reduced peptide bond have been prepared. Sasaki and Coy [14] as well as Geyer *et al.* [11] used the Boc-group for *N*-terminal protection of the concerned pseudodipeptide. Our dipeptide units were intended to be used in solid phase synthesis under the conditions of the Fmoc chemistry. Their preparation is thus possible with different protecting group strategies as shown in Figure 2.

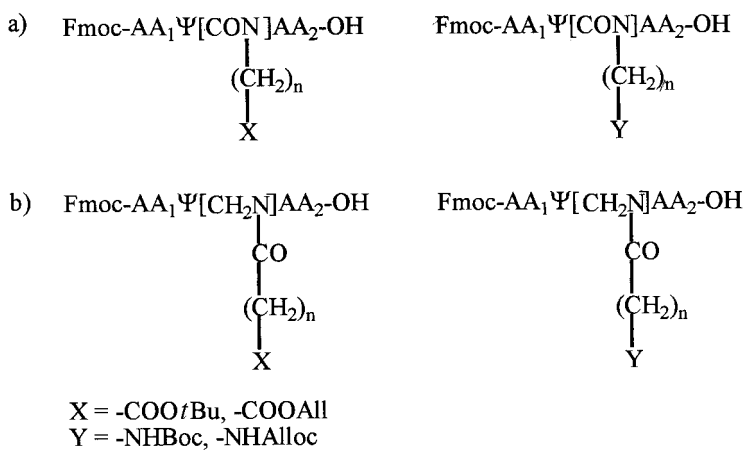

Figure 1 Different types of dipeptide building units: (a) *N*-functionalized dipeptides, (b) *N*-acylated reduced pseudodipeptides.

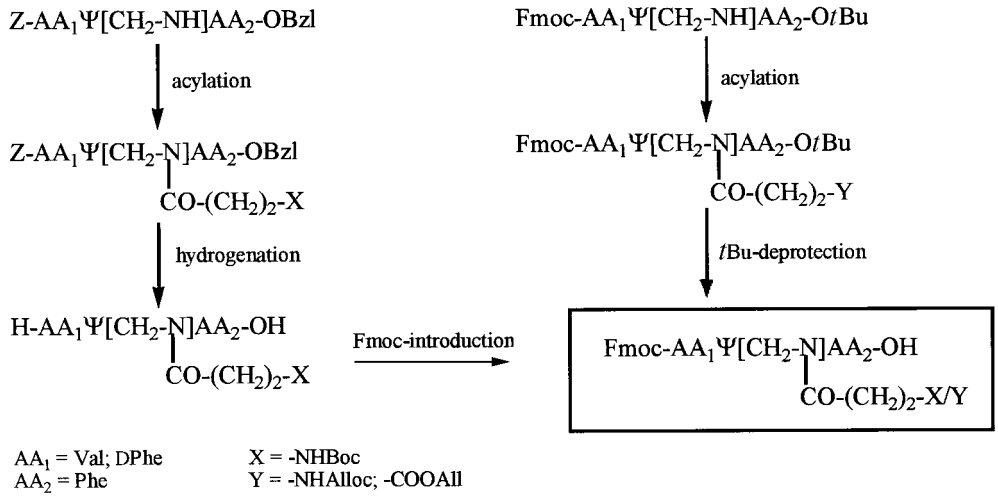

Figure 2 Different protecting group strategies for the synthesis of *N*-acylated reduced pseudodipeptides.

Both strategies start from the synthesis of N^{α}-protected amino aldehydes which in the case of the Fmoc-protection have been described for both the Fehrentz and Castro method [16,17] as well as starting from *S*-benzylthioesters [18].

As is known, racemization of protected amino aldehydes may occur during their preparation, especially purification on silica gel and storage, and further processing [25,26]. We tried to circumvent these potential causes by the immediate use of the prepared aldehydes for the further reaction. No detectable racemization was observed after the following condensation to generate pseudopeptide bonds.

Acylation of the Pseudodipeptides with Semiprotected ω-Amino and α,ω-Dicarboxylic Acids

Whereas the preparation of the $\Psi[CH_2NH]$ pseudodipeptide analogs proceeds without difficulties and could be performed with good yields, first attempts to acylate the reduced amide bond failed. Problems found for peptide or amino acid coupling to secondary amino acids have already been reported and it is known that special activating reagents are required [19]. The DCC-mediated acylation of the $\Psi[CH_2NH]$ bond with various Boc-amino acids were already studied by Sasaki and Coy [14]. They found a very poor reactivity of the secondary amine function and could therefore use the pseudodipeptides unprotected during the carbodiimide couplings in solid phase synthesis. More efficient coupling reagents like HATU were tested by us, but only poor results were obtained. We also tried PyBrop, a coupling reagent which has been recommended for coupling to secondary amino acids [27], but its use was not efficient. The same findings were made by Byk and Scherman for the preparation of peptide coupling to *N*-farnesyl amino acids [20]. They could successfully apply the mixed anhydride activation method with isobutyl chloroformate to obtain *N*-farnesyl dipeptides. Independently we had used this coupling method originally described by Vaughan [21] for our study of the acylation of $\Psi[CH_2NH]$ pseudodipeptide bonds to yield Fmoc-protected dipeptide units.

For the acylation, various derivatives of semiprotected ω-amino and α,ω-dicarboxylic acids (Figure 3) can be used in order to introduce different chain lengths and thus generate different sized cycles in a peptide.

Different Protecting Group Strategies for the Formation of *N*-Acylated Reduced Pseudodipeptides

We first used the temporary Z/OBzl-protection for the pseudodipeptide formation. This strategy had to be given up because of the 2-ketopiperazine formation occurring during hydrogenation, which led to a loss of the desired product (Figure 4).

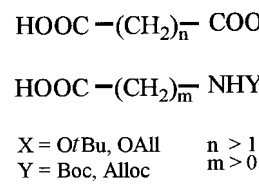

Figure 3 Monoprotected ω-amino- and α,ω-dicarboxylic acids for the acylation of reduced amide bonds.

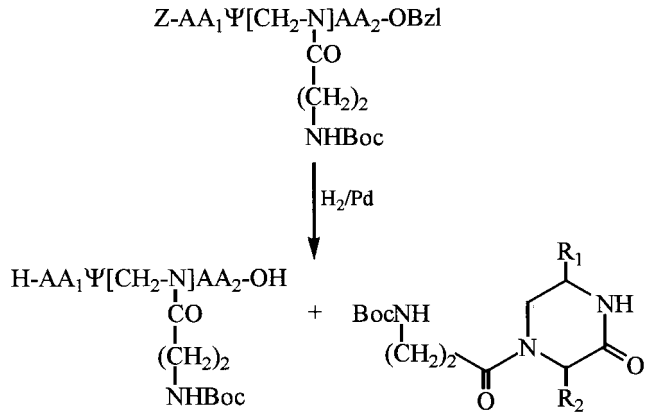

Figure 4 2-Ketopiperazine formation during the hydrogenation of Z/Obzl-protected dipeptide building units.

Bravo *et al.* have also reported this cyclization for *Z*-protected pseudodipeptide methylesters [24]. The reason for that easy lactam formation seems to be the faster removal of the *Z* group compared with the benzyl ester group. Attempts to prevent this cyclization by the addition of Fmoc-OSu during hydrogenation to block the *N*-terminus or by carrying out the reaction in the presence of weak acids not interfering with the Boc/O*t*Bu-protection were not effective, especially regarding yield and purity of the product. The second route via the Fmoc-protection of the starting aldehyde seemed to be advantageous, not only because the side reaction is prevented but also because the final Fmoc-introduction can be omitted. Due to the very convenient preparation of pseudodipeptides using Fmoc-protected amino aldehydes combined with *tert*-butyl ester protection for the *C*-terminal amino acid we stopped all further attempts to form pseudodipeptides through temporary Z/OBzl-protection.

The herein synthesized dipeptide units are shown in Table 1.

Optical Purity of the Synthesized Dipeptide Units

As described above the pseudodipeptides containing a reduced peptide bond were prepared in an optically pure state. At first, the optical purity of the starting pseudodipeptides was ascertained by HPLC analysis after derivatization of the *N*-terminal unprotected pseudodipeptide-*tert*-butylesters with Marfey's reagent [28].

After acylation of the pseudodipeptides, the dipeptide units formed are optically pure, too. This is, in contrast to the *N*-functionalized dipeptide building units [8], due to the fact that the acylation of the pseudopeptide bond required only an activation of the nonchiral acids β-alanine and succinic acid avoiding racemization during this synthetic step. The HPLC analysis of the Fmoc-protected dipeptide units shows single sharp peaks, whereas a mixture of diastereomeric units, especially synthesized for that purpose, gives two peaks under the same conditions (Figure 5). Our attempts to analyze the optical purity of both types of dipeptide units and the synthesized peptides will be published separately.

Table 1 Structures of the Dipeptide Building Units Synthesized

Compound	Structure
3	Z-ValΨ[CH_2N]Phe-OBzl CO-$(CH_2)_2$-NHBoc
4	H-ValΨ[CH_2N]Phe-OH CO-$(CH_2)_2$-NHBoc
12	Fmoc-ValΨ[CH_2N]Phe-OH CO-$(CH_2)_2$-NHAlloc
13	Fmoc-ValΨ[CH_2N]Phe-OH CO-$(CH_2)_2$-COOAll
14	Fmoc-d-PheΨ[CH_2N]Phe-OH CO-$(CH_2)_2$-NHAlloc
15	Fmoc-d-PheΨ[CH_2N]Phe-OH CO-$(CH_2)_2$-COOAll

Comparison of Syntheses and Chemical Properties of Different Types of Dipeptide Units

The synthetic procedures for the two types of dipeptide building units differ to a certain degree. But, neither in the number of synthetic steps nor in the yield and purity estimated after column chromatography, are there remarkable differences. Regarding the preparation, the use of the Ψ[CH_2N(CO-$(CH_2)_n$–X)] units has some advantages. For the acylation of pseudodipeptides, a large variety of ω-amino and α,ω-dicarboxylic acids can be applied differing in length, flexibility and also steric and electronic properties of the generated moiety. Using the mixed anhydride method for the activation of the nonchiral acyl component, good yields could be achieved without the risk of racemization. In contrast to the Ψ[CON($(CH_2)_n$–X)] units, the insertion of acylated reduced peptide bonds in the peptidic backbone is supposed to increase the

Table 2 Analytical Data of the Dipeptide Building Units Synthesized

No.	Yield (%)	HPLC t_R (min)	R_{f1}^a	R_{f2}^b	MS $[M+H]^+$	$[\alpha]_D^{25}$ ($c = 1$; MeOH)	CHN (%)
3	71	51.04	0.60[d]	0.64	646 (calcd. 645.7)	−2.7	n.d.[e]
4	30–45[c]	23.37	0	0.28	422 (calcd. 421.5)	+12.9	n.d.
12	34[f]	42.46	0.37	0.13	628 (calcd. 627.2)	−51.6	Calcd.: C 68.88; H 6.54; N 6.69 Found: C 68.34; H 6.11; N 6.72
13	36	45.25	0.50	0.18	613 (calcd. 612.5)	−49.9	Calcd.: C 70.53; H 6.53; N 4.57 Found: C 70.22; H 6.32; N 4.61
14	38	43.25	0.48	0.25	676 (calcd. 675.4)	−32.6	Calcd.: C 71.07; H 6.07; N 6.22 Found: C 71.10; H 5.97; N 6.32
15	36	46.00	0.55	0.27	661 (calcd. 660.3)	−23.5	Calcd.: C 72.69; H 6.06; N 4.24 Found: C 72.24; H 6.46; N 4.15

[a] System 1: 9:1 chloroform:methanol.
[b] System 2: 27:10:0.5 benzene:aceton:acetic acid.
[c] Yield dependent on 2-ketopiperazine formation.
[d] System: 8:2 hexane:ethyl acetate.
[e] Not determined.
[f] Overall yields after purification.

conformational flexibility and the stability against proteolytic cleavage. The effect of both types of backbone modifications on the chemical and biological properties has been studied on somatostatin and bradykinin analogs and will be published elsewhere.

CONCLUSIONS

Backbone cyclization via *N*-acylated reduced pseudodipeptides is a new method to form constrained peptides, with the same potential as commonly used *N*-functionalized building units, in order to stabilize the bioactive conformation without affecting the amino acid side-chains. This modification is supposed to enhance the flexibility of the peptidic backbone compared with the alkylated peptide bonds containing dipeptide units. The assembly of backbone cyclic peptides on the solid support requires the use of the preformed dipeptide units which are synthesized from pseudodipeptides with a reduced peptide bond by acylation using ω-amino or α,ω-dicarboxylic acid derivatives. Best results for the coupling of the acyl residues to the reduced peptide bond were obtained by the formation of mixed anhydrides with alkyl chloroformates. The method allows the use of a variety of acyl moieties

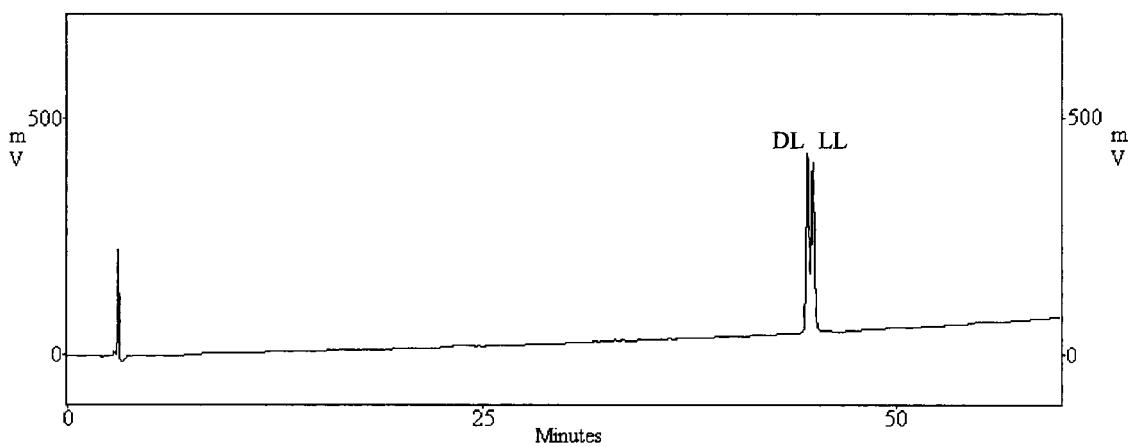

Figure 5 HPLC-analysis of a diastereomeric mixture of Fmoc-L/D-ValΨ[$CH_2N(CO(CH_2)_2COOAll)$]Phe-OH.

differing in, e.g. length and chemical properties. With the developed route for the synthesis of these dipeptide units good yields and products of high chemical and optical purity could be obtained. It has been proven that the allyl type protection of the *N*-functionalized moiety is very suitable for peptide synthesis on resins carrying acid labile linkers.

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