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Novel route in the synthesis of $\psi[CH_2NH]$ amide bond surrogate

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Abstract

An alternative method for the synthesis of pseudopeptides containing a $\psi[CH_2NH]$ amide bond surrogate is reported. The synthetic approach is based on a nucleophilic displacement of the chiral N-protected β-iodoamines with conveniently protected amino acid esters. The compatibility of this method with both conventional and microwave-assisted peptide synthesis should increase the potentiality of the $\psi[CH_2NH]$ peptide bond isostere in peptide chemistry.
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Peptides are involved in many important biological processes but only very few are used today as pharmaceutical agents.[1] The amide bond is extremely polar and it is a natural target of many enzymes causing a rapid degradation of peptides. To transform a bioactive peptide into a useful molecule with potential pharmacological properties, with enhanced bioavailability and improved transfer rate across cell membranes, modifications of the peptide backbone are applied. Among the modifications necessary for rendering peptides more stable are described cyclization,[2] unnatural amino acids insertion,[3] and backbone replacements by amide bond surrogates.[4] In particular, the substitution of amide bond by an aminomethylene group, [–CONH–→–CH_2NH–] produces a reduction of polarity and strengthens resistance against protease degradation when compared to natural peptides. Pseudopeptides containing $\psi[CH_2NH]$ amide bond surrogates have been used in design of enzyme inhibitors,[5] in the development of antagonists against several receptors,[6] and recently as potential agents for gene delivery.[7]

Currently, the reductive alkylation of an α-amino group by a protected amino acid aldehyde is the main methodology to get a $\psi[CH_2NH]$ surrogate in both solution and solid-phase synthesis.[8,9] This methodology is limited for some negative features relative to the preparation of conveniently protected amino aldehydes.[10–13] Racemization through keto–enol tautomerism,[14] and undesirable side reaction as the double alkylation[9,15] are often been observed. An alternative approach, recently described by Kirillova et al. consists in the formation of an aminomethylene pseudodipeptide via Mitsunobu condensation of an alcohol with an acidic component. The reaction proceeds under a mild condition without racemization.[16]

We present herein an alternative method for the synthesis of $\psi[CH_2NH]$ amide bond surrogate based on a synthetic approach involving a nucleophilic displacement of the chiral N-protected β-iodoamines (**1**) with conveniently protected amino acid esters. The N-protected β-iodoamines represent a potential source of molecular diversity, recently reported in several communications. In fact, *N*-Fmoc-β-iodoamines have been recently used in solid-phase synthesis of $\psi[CH_2S]$ pseudopeptides,[17] in the synthesis of *S*-Glycoamino acids through their reaction with anchored thioglycosides,[18] while *N*-Boc-protected analogues were used in total synthesis of efrapeptin C.[19]

The $\psi[CH_2NH]$ pseudodipetides of general formula **2** were synthesized according to the synthetic pathway showed in Scheme 1. Initially, the enantiomeric pure N-protected-β-iodoamines used in this work were obtained from the corresponding N-protected β-amino alcohols by

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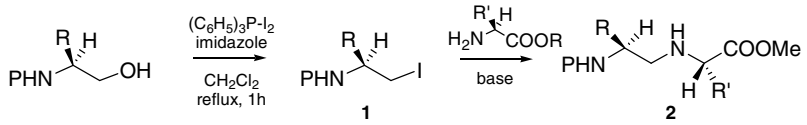


Scheme 1. General procedure for the synthesis of $\psi[CH_2NH]$ pseudo-dipetides **2**.

means of polymer bound triphenylphosphine-iodine complex, or by soluble triphenylphosphine, according to the previously described method.[17,20]

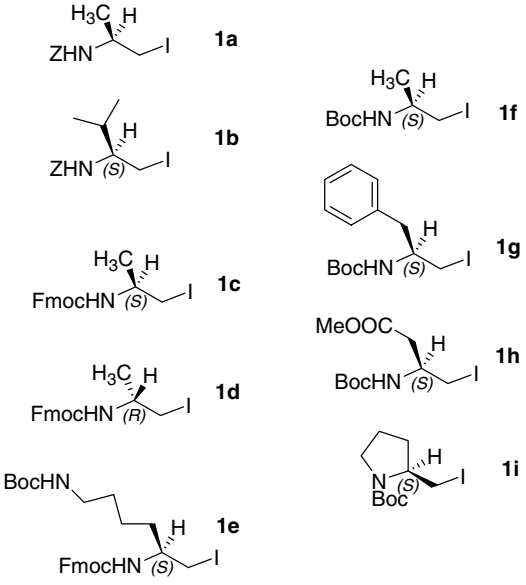
Following this synthetic procedure, a series of Fmoc, Boc and *Z*N-protected β-iodoamines derived from Ala, Val, Phe and α,β-diaminopropionic acid were synthesized (Table 1, **1a**–**i**). The nature of side chains was chosen as a representative example of aliphatic, aromatic and polar amino acid residues to address their reactivity.

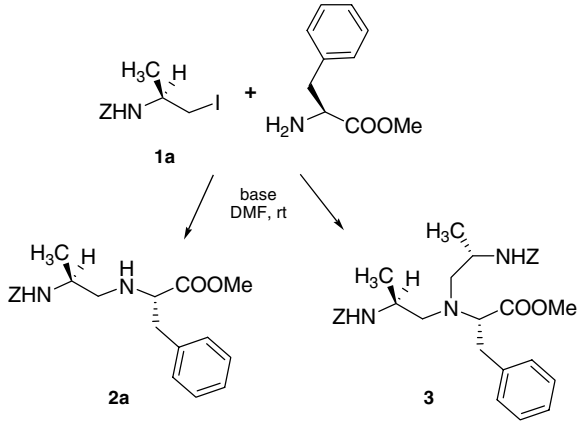
A preliminary study of the influence of different bases (Cs_2CO_3, K_2CO_3, triethylamine, and DBU) and solvents (DMF, NMP, DMSO, CH_2Cl_2, THF, and acetone) on nucleophilic displacement of the iodine group by α-amino acid esters was performed using the iodo-derivative **1a** and H-Phe-OMe (Scheme 2).

As showed in Table 2, after 16 h Cs_2CO_3 gave the highest yields and better selectivity in the formation of pseudodipeptide **2a** using dimethylformamide as the solvent, and no byproducts of dialkylation **3** were detected.[21] K_2CO_3 and triethylamine gave low yields with a little percentile of dialkylation product, while DBU was ineffective in degrading the starting iodo-derivates (Table 2).

When various solvents were examinated (Table 3), *N*,*N*-dimethylformamide was found to be the better solvent for the reaction. *N*-Methylpyrrolidinone and dimethylsulfoxide gave lower yields while other solvents like dichloromethane, tetrahydrofuran or acetone were demonstrated unsuitable for this type of reaction.

Table 1
N-Protected β-iodoamine derivatives used in this study





Scheme 2. Reaction of **1a** with H-Phe-OMe.

Table 2
N-Alkylation of H-Phe-OMe with **1a** utilizing different bases

Compound	Yield (%)			
	Cs_2CO_3	K_2CO_3	TEA	DBU
2a	85	35	15	>5
3	Not detected	15	8	0

Table 3
Use of various solvents in cesium base promoted N-alkylation of H-Phe-OMe

Compound	Yield (%)			
	DMF	NMP	DMSO	CH_2Cl_2, THF, acetone
2a	80	70	60	5
3	0	6	0	0

According to these results the reaction of **1a** with H-Phe-OMe in DMF at room temperature using Cs_2CO_3 as the base[22] generated the Z-Ala-$\psi[CH_2NH]$Phe-OMe (**2a**) in 80% of yield after a flash chromatography purification. Product of dialkylation of amine (**3**) or pseudopeptide diastereomers owing to racemization were not detected by HPLC and ^1H NMR analysis of crude product. Based on these preliminary experiments, which optimized the synthetic parameters, various N-protected β-iodoamines (**1a**–**i**) and amino acid esters (H-Phe-OMe, H-Pro-OBz, and H-Pro-OMe) were subjected to N-alkylation to validate this new synthetic route. As shown in Table 4 the new method developed was compatible with all β-iodoamines used in this study. The corresponding $\psi[CH_2NH]$ pseudodipetides (**2aa**–**2i**) were obtained in yields in the range of 65–86%. Variations of reactivity were not detected using different N-protecting groups, such as Fmoc, Boc, and *Z* (entries 1, 4, and 8).

Table 4
Reactivity of β-iodoamines with different amino acid esters

Entry	β-Iodoamines	Amine	2		Yield (%)
1	**1a**	H-Phe-OMe	**2a**	*Z*-Ala-ψ[CH_2NH]Phe-OMe	80
2		H-Pro-OBz	**2aa**	*Z*-Ala-ψ[CH_2NH]Pro-OBz	75
3	**1b**	H-Phe-OMe	**2b**	*Z*-Val-ψ[CH_2NH]Phe-OMe	75
4	**1c**	H-Phe-OMe	**2c**	Fmoc-Ala-ψ[CH_2NH]Phe-OMe	86
5		H-Pro-OBz	**2cc**	Fmoc-Ala-ψ[CH_2NH]Pro-OBz	70
6	**1d**	H-Phe-OMe	**2d**	Fmoc-DAla-ψ[CH_2NH]Phe-OMe	83
7	**1e**	H-Phe-OMe	**2e**	Fmoc-Lys(Boc)ψ[CH_2NH]Phe-OMe	70
8	**1f**	H-Phe-OMe	**2f**	Boc-Ala-ψ[CH_2NH]Phe-OMe	75
9	**1g**	H-Pro-OMe	**2g**	Boc-Phe-ψ[CH_2NH]Pro-OMe	75
10	**1h**	H-Phe-OMe	**2h**	Boc-Asp(OMe)ψ[CH_2NH]Pre-OMe	83
11	**1i**	H-Pro-OBz	**2i**	Boc-Proψ[CH_2NH]Pro-OMe	70

The use of β-iodoamine derived from the hindered Val (**1b**) afforded the corresponding ψ[CH_2NH] analogue **2b** in good yield (75%, entry 3). Since we were also interested in demonstrating the compatibility of the optimized ψ[CH_2NH] procedure with different side chain-functionalized building blocks, we performed the N-alkylation using as substrates, H-Pro-OBz and H-Pro-OMe. It has been shown that the introduction of a reduced amide linkage between Phe and Pro residues, by reductive alkylation of the Pro nitrogen with Boc-Phe-H using $NaBH_3CN$ under acidic conditions leads to epimerization of the Phe residue.[23] Under the same conditions described above, the pseudodipeptides Fmoc-Ala-ψ[CH_2NH]Pro-OBz (2cc), Boc-Pheψ[CH_2NH]Pro-OMe (**2g**), and Boc-Proψ[CH_2NH]Pro-OMe (**2i**) were obtained in 70%, 75%, and 70% yields, respectively (entries 5, 9, and 11).

Finally, the chirality of starting β-iodoamine derivatives had no influence on N-alkylation reaction (entries 4 and 6). No racemization was observed under the conditions shown as evidenced by analytical HPLC (Fig. 1) for compounds **2c** and **2d**.[24] The physicochemical properties and purities of the final compounds were assessed by TLC, FAB-MS, analytical RP-HPLC (Table 5), and ^1H NMR.[25] Subsequently, aiming both to reduce the reaction times and to

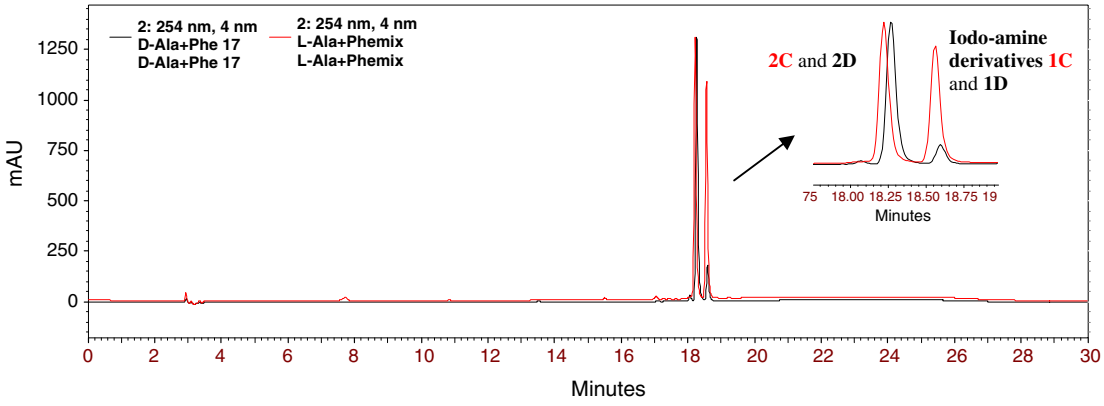


Fig. 1. Superimposition of chromatograms relate of crude products Fmoc-LAla-ψ[CH_2NH]Phe-OMe **2c** and Fmoc-DAla-ψ[CH_2NH]Phe-OMe **2d**. In red is showed compound Fmoc-LAla-ψ[CH_2NH]Phe-OMe and in black compound Fmoc-DAla-ψ[CH_2NH]Phe-OMe.[24]

Table 5
Analytical data of the P-Xaa ψ[CH_2NH] Yaa-OR derivatives **2**

Compound	P	Xaa	Yaa	R	Formula	FAB-MS found	$[\alpha]_D^{20}$	RP HPLC[a] t_R (min)
2a	*Z*	Ala	Phe	Me	$C_{22}H_{28}N_2O_4$	384.32	−1.8 (*c* 0.1, MeOH)	14.87
2aa	*Z*	Ala	Pro	Bz	$C_{24}H_{28}N_2O_5$	424.39	−15.1 (*c* 0.1, MeOH)	11.38
2b	*Z*	Val	Phe	Me	$C_{24}H_{32}N_2O_4$	412.37	+3.7 (*c* 0.2, MeOH)	14.68
2c	Fmoc	Ala	Phe	Me	$C_{29}H_{32}N_2O_4$	472.72	−6.8 (*c* 0.2, MeOH)	11.91
2cc	Fmoc	Ala	Pro	Bz	$C_{31}H_{32}N_2O_5$	512.43	−15.1 (*c* 0.1, MeOH)	12.32
2d	Fmoc	D-Ala	Phe	Me	$C_{29}H_{32}N_2O_4$	472.69	+24.6 (*c* 0.1, MeOH)	12.26
2e	Fmoc	Lys(Boc)	Phe	Me	$C_{37}H_{47}N_3O_6$	629.52	−12.9 (*c* 0.3, MeOH)	15.89
2f	Boc	Ala	Phe	Me	$C_{19}H_{30}N_2O_4$	350.43	−20.0 (*c* 0.1, MeOH)	13.67
2g	Boc	Phe	Pro	Me	$C_{21}H_{32}N_2O_4$	376.49	−32.9 (*c* 0.2, MeOH)	13.65
2h	Boc	Glu(OMe)	Phe	Me	$C_{22}H_{34}N_2O_6$	422.41	−22.6 (*c* 0.2, MeOH)	14.69
2i	Boc	Pro	Pro	Me	$C_{16}H_{28}N_2O_4$	312.38	−62.16 (*c* 0.1, MeOH)	12.26

[a] Analytical RP HPLC of final products was performed on a C18 (Vydac 218TP54) column using the gradient 10–90% acetonitrile/0.05% TFA in H_2O at 1 mL/min.

improve the yields we performed the syntheses in a microwave dedicated oven (Milestone, CombiChem) and compared the results with the conventional procedure.[26] This further study demonstrated that the microwave-assisted reaction resulted in a clear advantage only in reduction on reaction time, being reduced to 1 h from 16 h, without appreciable variation in yield.

A simple and convenient optimized procedure is described for the preparation of $\psi[CH_2NH]$ surrogate in solution. We have demonstrated the feasibility of our strategy by conventional and microwave synthesis of the aminomethylene surrogate bond. Every step of the procedure was optimized with respect to time and economy.

The compatibility of this method with conventional solid phase synthesis is currently under investigation in our laboratory. Achieving this objective will facilitate the routine introduction of a $\psi[CH_2NH]$ peptide bond surrogate into various biologically active peptides leading to the synthesis of many important compounds and interesting structure activity relationship studies.

Acknowledgments

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22. *Representative experimental procedure*: To the activated powdered 4 Å molecular sieves (500 mg) in anhydrous *N*,*N*-dimethylformamide (10 mL) was added cesium carbonate (326 mg, 1.0 mmol), and the suspension was stirred for 10 min. Then phenylalanine methyl ester hydrochloride (108 mg, 0.5 mmol) was added and followed by additional 30 min of stirring, β-iodoamine derivative **1a** (160 mg, 0.5 mmol) was added. The reaction was stirred for 16 h, filtered to remove the molecular sieves and undissolved inorganic salts, and rinsed several times with EtOAc. Then the filtrate was concentrated and the residue was taken up in 10% $NaHCO_3$ solution and extracted with CH_2Cl_2 (4 × 25 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. Flash column chromatography (*n*-hexane–EtOAc, 3:2 v/v) afforded the aminomethylene analogue **2a** (128 mg, 80%) as a colorless oil.
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24. No racemization was confirmed by the HPLC analysis of the crude products Fmoc-Ala-$\psi[CH_2NH]$Phe-OMe **2c** and Fmoc-DAla-$\psi[CH_2NH]$Phe-OMe **2d**. This analysis showed the signal corresponding to one single isomer (Fig. 1). In addition the ^1H NMR spectra of the crude products **2c** and **2d** did not show significant differences of chemical shifts. Thus, the resonance values of α-H Phe residues were 4.32 for **2c** and 4.39 for **2d**, while the values for α-H Ala residues were 3.85 and 3.84, respectively.
25. As example, significant ^1H NMR analytical data of **2g**: ^1H NMR (500 MHz, CD_3OD): δ, 1.42 (s, 9H, Boc); 1.94–2.00 (m, 3H, H-γ, H-β Pro); 2.26–2.29 (m, 1H, H-β Pro); 2.72–2.80 (m, 2H, CH–CH_2–Ph); 3.47–3.50 (m, 2H, H-δ Pro); 3.74 (s, 3H, OCH_3); 4.00–4.02 (m, 1H, H-α Pro); 4.07 (m, 2H, CH_2N); 4.37 (m, 1H, CH-CH_2-Ph); 4.81 (br s, 1H, NH-Boc); 7.33–7.22 (m, 5H, aryl).
26. *General procedure*: All experiments were carried out in a Milestone CombiChem Microwave Synthesizer with vessels of 4-mL volume, using DMF as the solvent. In all irradiation experiments, rotation of the rotor, irradiation time, temperature, and power were monitored with the 'easyWAVE' software package. Temperature was monitored with the aid of an optical fiber inserted into one of the reaction containers. Once 50 °C was reached the reaction mixture was held at this temperature for 10 min and then cooled rapidly to room temperature.