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C-Backbone branched peptides via reductive amination of cyanomethyleneamino pseudopeptides

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Abstract—The synthesis of branched peptides from cyanomethylenamino pseudopeptides, via catalytic hydrogenation in the presence of amino acid derivatives, is described. When the inserted amino acid was a glycine derivative, the resulting branched peptide lactamized in situ to 2-oxopiperazine derivatives. This new *C*-backbone peptide branching approach is compatible with the diversity of amino acid side chains.

Branched peptides have been extensively used in the preparation of multiple antigen peptides (MAP) for immunology applications,[1] in the synthesis of template-assembled synthetic proteins (TASP)[2,3] and dendrimers,[4,5] or for the introduction of conformational constrains into peptides through side-chain cyclizations.[6] In most of these applications, the branching points are amino acids containing reactive side chains, such as Asp, Glu, Lys or Cys. In general, this fact does not limit the scope of these applications. However, when the aim is to introduce conformational constrains by cyclization, the replacement of some amino acids by those above mentioned is permissible in biological irrelevant regions, but it may lead to inactive peptides when applied to their active regions. To avoid this drawback, diverse strategies of *N*-backbone cyclization, involving introduction of a branching point into the peptide bond, by *N*-alkylation with a functionalized carbon chain linker have been proposed.[7–10]

Based on the hypothesis that the [CH(CN)NH] group could be a good peptide bond surrogate, we developed a versatile method for the synthesis of cyanomethyleneamino pseudopeptides,[11] which was applied to the synthesis of aminopeptidase inhibitors,[12] and neurotensin[13] and CCK-4 analogues,[14] respectively. On the other hand, due to its chemical reactivity, the cyano group is a good tool for *C*-backbone modification, such as branching or cyclization reactions. Thus, we have previously reported the synthesis of branched peptides and conformationally constrained analogues from cyanomethyleneamino pseudopeptides, via catalytic hydrogenation, followed by peptide coupling.[15] Now, we have studied and communicate herein a new type of *C*-backbone peptide branching, involving insertion of amino acid derivatives via reductive amination of the cyano group of Ψ[CH(CN)NH] pseudopeptides, by catalytic hydrogenation in the presence of amino acid derivatives (Scheme 1).

Catalytic hydrogenation of nitriles may give rise to a number of products including primary, secondary and tertiary amines, imines, hydrocarbons, aldehydes, amides, and alcohols. Despite the complexity of the reaction, its selectivity, mainly towards primary or secondary amines, can be controlled with the reaction conditions, such as catalyst, temperature, solvent, and addition of amines.[16–18] With respect to this last factor, when the hydrogenation is carried out in the presence of primary amines, the formation of asymmetrical secondary amines competes with that of the symmetrical one. In our case, we carried out an initial study on the hydrogenation of pseudodipeptides Boc-PheΨ[CH-(CN)NH]Leu-OMe [Scheme 1, (*R*,*S*)-**1**, (1:1) epimeric mixture at the stereogenic center of the peptide bond surrogate] in the presence of H-Ala-OMe·HCl, to determine the optimal reaction conditions for the preparation of the asymmetrical secondary amines (*R*)- and (*S*)-**9**. As indicated in Table 1, these branched pseudotripeptides were obtained along with the 2-oxopiperazine derivatives (*R*)- and (*S*)-**30**, resulting from lactamization of the corresponding primary amines **A**, in different ratios, depending on the hydrogenation conditions. The corresponding symmetrical secondary amines were not detected in the crude reaction mixture. Both epimeric mixtures **9** and **30** were

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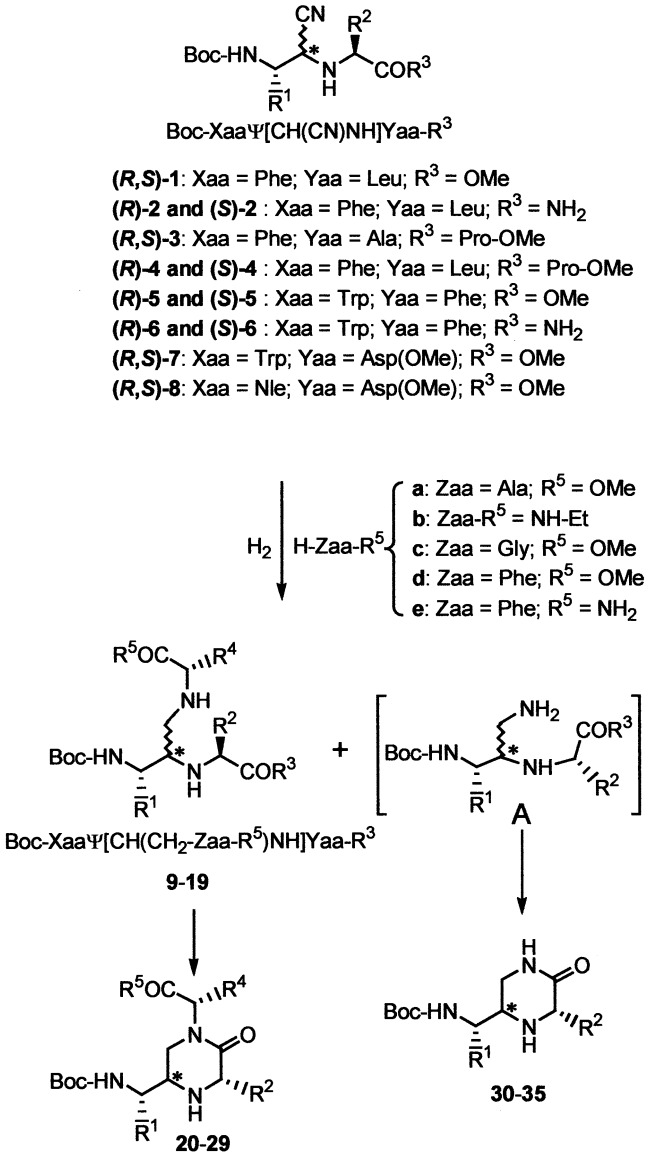
resolved by flash chromatography. To favor the formation of the secondary amines, hydrogenations were carried out at low pressure (1 atm).[16,17] Amino acid/nitrile ratios higher than 2 (entry 2), as well as the increase in the temperature[18] (entry 3) did not lead to significant increases in the ratio of branched peptides **9**. The most significant improvement in the yield of these secondary amines was achieved when the amino acid hydrochloride was neutralized in situ, by adding one equivalent amount of Et_3N to the hydrogenation medium, (compare entries 1 and 6).† With respect to the catalyst, the use of 10% Pd(C) led to the highest yield of branched peptides **9**, while Raney Ni gave the highest ratio of 2-oxopiperazines **30** (entry 4). Although, it has been described that the use of rhodium generally favors the formation of secondary amines,[16–18] in our case (entries 8 and 9), only the primary amine derived 2-oxopiperazines **30** were obtained, and in low yield. The use of EtOAc as solvent (entry 7) led to a decrease in reduction products, and dirtier reaction mixtures. No racemization at the Ala residue nor epimerization at the stereogenic centers of the starting pesudodipeptides **1** were detected in any case.

Branched pseudotripeptides (*R*)- and (*S*)-**9** could not be obtained by alkylation of the corresponding primary amines **A** [isolated as acetates from the hydrogenation of (*R*,*S*)-**1** in the presence of HOAc[15]] with methyl (*S*)-2-bromopropanoate in the presence of Ag_2O,[19,20] or with the triflate of (*R*)-methyl lactate.[21]

After having determined the optimal reaction conditions for the preparation of branched pseudopeptides (*R*)- and (*S*)-**9**, we studied the versatility of the methodology for different starting amino acids and cyanomethyleneamino pseudodipeptides (Table 2). In general, it was necessary to increase the reaction time from 8 h for the reductive coupling of pseudodipeptides (*R*,*S*)-**1** or their amides (*R*)- and (*S*)-**2** with H-Ala-OMe, ethylamine or H-Gly-OMe (entries 1–5) to 1 day for pseudotripeptides (*R*,*S*)-**3** or (*S*)-**4** (entries 6 and 8), and 5 days for the rest of the couplings. In most of the reactions, a variable percentage of the starting Ψ[CH(CN)NH] pseudopeptide was recovered unchanged, which ranged from 3% in the coupling of pseudodipeptides (*R*,*S*)-**7** with H-Phe-NH_2 (entry 14) to 100% in the case of the reduction of (*R*)-**4** (entry 7).



Scheme 1.

The significant different results obtained for the reaction of pseudotripeptides **3** and **4** with H-Ala-OMe (entries 6–8) could be attributed to a higher steric hindrance in the Leu-containing pseudotripeptides **4**, which hampered the coupling in the (*R*)-epimer.

When the inserted amino acid was H-Gly-OMe (entries 3 and 9–12), or when it was replaced by ethylamine (entry 2), the resulting branched pseudopeptides lactamized in situ to the corresponding 1-substituted-2-oxopiperazine derivatives **21**, **22** and **26**.

Finally, it is worth noting that the presence of free carboxy groups, either in the Ψ[CH(CN)NH] pseudopeptide or in the amino acid that is inserted, hampered the coupling reaction of reductive amination, recovering the starting materials unchanged.

In conclusion, the reductive amination of cyanomethileneamino pseudopeptides, by catalytic hydrogenation in

† **Representative procedure**: Et_3N (0.14 mL, 1 mmol) was added to a suspension of the amino acid derivative hydrochloride H-Zaa-R^5·HCl (1 mmol) in MeOH (10 mL), and the mixture was stirred at room temperature for 15 min. Then, the corresponding Ψ[CH(CN)NH] pseudopeptide **1**–**10** (0.5 mmol) and 10% Pd(C) (300 mg) were added, and the mixture was hydrogenated at 1 atm of H_2 and room temperature for 8 h to 5 days, depending on the starting materials. Afterwards, the catalyst was filtered off and washed with MeOH (3×5 mL), the solvent was evaporated, and the reaction crude was dissolved in EtOAc (20 mL). This solution was washed successively with H_2O (2×10 mL), brine (10 mL), dried over Na_2SO_4, and evaporated to dryness. The residue were purified by flash chromatography using 5–30% EtOAc in hexane or 0–5% MeOH in CH_2Cl_2 gradients as mobile phases.

Table 1. Influence of hydrogenation conditions on the product ratio in the reductive amination of (*R*,*S*)-**1**[a]

Entry	Amino acid derivative	H-Ala-OMe equivalents[b]	Catalyst	Solvent	*T* (°C)	[(*R*)+(*S*)]-**9** (%)[c]	[(*R*)+(*S*)]-**30** (%)[c]
1	H-Ala-OMe·HCl	2	10% Pd(C)	MeOH	20	30	64
2	H-Ala-OMe·HCl	4	10% Pd(C)	MeOH	20	32	60
3	H-Ala-OMe·HCl	2	10% Pd(C)	MeOH	50	28	66
4	H-Ala-OMe·HCl	2	Raney Ni	MeOH	20	7	81
5	H-Ala-OMe·HCl	2	PtO_2	MeOH	20	0	40
6	H-Ala-OMe[d]	2	10% Pd(C)	MeOH	20	66	20
7	H-Ala-OMe[d]	2	10% Pd(C)	EtOAc	20	52	15
8	H-Ala-OMe[d]	2	5% Rh(C)	MeOH	20	0	39
9	H-Ala-OMe[d]	2	5% Rh(Al_2O_3)	MeOH	20	0	39

[a] Hydrogenation at 1 atm of H_2 pressure for 8 h.
[b] Equivalents of the amino acid derivative **a** with respect to the pseudopeptide (*R*,*S*)-**1**.
[c] Yield determined by RP-HPLC of the crude reaction mixture [μBondapapk C_{18} (3.9×300 mm), 40% of CH_3CN and 60% of 0.05% solution of TFA as mobile phase].
[d] From the hydrochloride by addition of the equivalent amount of Et_3N.

Table 2. Results of the reductive amination of Ψ[CH(CN)NH] pseudopeptides[a]

			Reaction products					
					1-Substituted		1-Unsubstituted	
Entry	Boc-XaaΨ[CH(CN)NH]Yaa-R^3	H-Zaa-R^5	Branched peptide	(%)[b]	2-Oxopiperazine	(%)[b]	2-Oxopiperazine	(%)[c]
1	(*R*,*S*)-**1**[d]	H-Ala-OMe	(*R*)- and (*S*)-**9**	66	(*R*,*S*)-**20**	0	(*R*)- and (*S*)-**30**	20
2	(*R*,*S*)-**1**[d]	H_2N-Et	(*R*,*S*)-**10**	0	(*R*)- and (*S*)-**21**	68	(*R*)- and (*S*)-**30**	30
3	(*R*,*S*)-**1**[d]	H-Gly-OMe	(*R*,*S*)-**11**	0	(*R*)- and (*S*)-**22**	62	(*R*)- and (*S*)-**30**	35
4	(*R*)-**2**	H-Ala-OMe	(*S*)-**12**	53	(*S*)-**23**	0	(*S*)-**30**	28
5	(*S*)-**2**	H-Ala-OMe	(*R*)-**12**	56	(*R*)-**23**	0	(*R*)-**30**	27
6	(*R*,*S*)-**3**[d]	H-Ala-OMe	(*R*,*S*)-**13**	55	(*R*,*S*)-**24**	0	(*R*,*S*)-**31**	0
7	(*R*)-**4**	H-Ala-OMe	(*S*)-**14**	0	(*S*)-**25**	0	(*S*)-**32**	0
8	(*S*)-**4**	H-Ala-OMe	(*R*)-**14**	44	(*R*)-**25**	0	(*R*)-**32**	0
9	(*R*)-**5**	H-Gly-OMe	(*S*)-**15**	0	(*S*)-**26**	59	(*S*)-**33**	31
10	(*S*)-**5**	H-Gly-OMe	(*R*)-**15**	0	(*R*)-**26**	62	(*R*)-**33**	23
11	(*R*)-**6**	H-Gly-OMe	(*S*)-**16**	0	(*S*)-**26**	57	(*S*)-**33**	26
12	(*S*)-**6**	H-Gly-OMe	(*R*)-**16**	0	(*R*)-**26**	55	(*R*)-**33**	20
13	(*R*,*S*)-**7**[e]	H-Phe-OMe	(*R*)- and (*S*)-**17**	55	(*R*)- and (*S*)-**27**	0	(*R*)- and (*S*)-**34**	30
14	(*R*,*S*)-**7**[e]	H-Phe-NH_2	(*R*)- and (*S*)-**18**	68	(*R*)- and (*S*)-**28**	0	(*R*)- and (*S*)-**34**	22
15	(*R*,*S*)-**8**[f]	H-Phe-NH_2	(*R*,*S*)-**19**	71	(*R*,*S*)-**29**	0	(*R*,*S*)-**35**	23

[a] (*R*)- and (*S*)- refers to the absolute configuration at the peptide bond surrogate. In the specification of this chirality, the ligand preference in products resulting from hydrogenation of Ψ[CH(CN)NH] pseudopeptides changes. Thus, the configuration of compounds **9**–**35** resulting from the reduction of the (*R*)-epimers of **1**–**8** is denoted as (*S*).
[b] Yield of isolate product.
[c] Yield determined in the reaction crude by RP-HPLC.
[d] (1:1) (*R*)/(*S*) epimeric ratio. The epimeric mixtures **10**, **11**, **21**, **22**, and **30** were resolved by flash chromatography.
[e] (1:2) (*R*)/(*S*) epimeric ratio. The epimeric mixtures resulting from the reduction of (*R*,*S*)-**7** were resolved by flash chromatography.
[f] (1:3) (*R*)/(*S*) epimeric ratio.

the presence of amino acid derivatives, is a versatile method for amino acid insertion as *C*-backbone branching points, compatible with the presence of diversity of amino acid side chains. These branched peptides can lactamize to give 2-oxopiperazine derivatives. This cyclization opens an easy access to conformationally restricted peptide derivatives.

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