Solid-Phase Synthesis and Structural Characterization of Highly Substituted Hydroxyproline-Based 2,5-Diketopiperazines

Alberto Bianco,*[,†] Carsten P. Sonksen,[‡] Peter Roepstorff,[‡] and Jean-Paul Briand[†]

Laboratoire de Chimie Immunologique, UPR 9021 CNRS, Institut de Biologie Moléculaire et Cellulaire, 15 rue René Descartes, 67000 Strasbourg, France, and Department of Molecular Biology, Odense University, Campusvej 55, DK-5230 Odense M, Denmark

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Two general solid-phase methods for the synthesis of a new class of 2,5-diketopiperazines (DKPs) containing the *trans*-4-hydroxy-L-proline amino acid residue (Hyp) have been developed. An N-protected hydroxyproline methyl ester was linked through the hydroxyl function to the Ellman resin. The synthesis procedures were conceived to enable a sequence of Hyp alkylation, Hyp N-acylation, cyclization, and amide bond alkylation. Up to three different centers of molecular diversity were introduced around the DKP scaffold. Highly functionalized bicyclic compounds were obtained in good yield and purity. The alkylation of hydroxyproline $^{\alpha}$CH was performed without control of the diastereoselectivity. During the final alkylation of the backbone, amide bond epimerization at the α-carbon atoms of the two amino acid residues was observed. The structures of representative DKPs were elucidated with multidimensional NMR experiments. The described reaction pathways can be applied to the identification of heterocyclic molecule inhibitors to diverse enzyme targets.

Introduction

Increasingly, heterocyclic compounds are considered the most promising molecules as lead structures for the design of new drugs.[1] These small molecules are generally characterized by a relatively rigid scaffold on which potential pharmacophoric groups can be assembled.[2] Solid-phase organic synthesis (SPOS) and combinatorial techniques allow then to introduce randomization and diversity in the spatial and positional arrangement of the functionalities around the cyclic template.[3,4] The application of conformationally constrained molecules in drug discovery, including also those defined peptidomimetics, is often focused to gain a higher affinity between a particular ligand and its receptor.[2,5]

Among several heterocycles, diketopiperazines (DKPs) have attracted considerable attentions in the recent years.[6] They can be considered as the smallest cyclic peptides, derived from the folding head-to-tail of a linear unprotected dipeptide.[7] Diketopiperazine scaffolds characterize the core of many natural products which present potential therapeutic properties. The different biological DKP activities comprise (i) inhibition of the mammalian cell cycle and of several enzymes;[8] (ii) modulation of the activity of human plasminogen activator inhibitor-1, which is considered a risk factor in thrombotic disease when it is present in high concentration, and control of dopamine receptor activity;[9] (iii) inhibition of mammalian DNA topoisomerase I and suppression of tumor cells growth;[10] and (iv) selectivity in the recognition of opioid receptors.[11] Moreover, combinatorial chemistry has been applied to identify new DKP-based compounds with a wide range of biological applications, such as highly selective collagenase-1 inhibitors and bradykinin antagonists.[12]

Several methods for the synthesis of 2,5-diketopiperazines on solid support have been already proposed and they are based mainly on the cleavage-induced cyclization of linear dipeptides.[6] Recently, two new solid-phase

† Institut de Biologie Moléculaire et Cellulaire.
‡ Department of Molecular Biology, Odense University.

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syntheses of DKPs, in which a resin-bound linear dipeptide is cyclized before the final cleavage from the solid support, have been reported.[13]

The present paper describes a general strategy for the synthesis of a novel class of highly substituted hydroxyproline-based 2,5-diketopiperazines, which exploits the peculiar feature of proline amino acid residue to adopt a *cis* conformation about the -Xaa-Pro- tertiary amide bond and, therefore, to favor the cyclization of a N-free Pro-containing dipeptide methyl ester.[14] Indeed, many diketopiperazines with important biological implications contain the proline moiety.[8a,8b,15] Very recently, the hydroxyproline scaffold has been also inserted in a series of peptidomimetics which inhibits the activity of farnesyltransferase.[16]

Two different solid-phase synthetic pathways will be presented, and the stereochemical assignment and the structural characterization of the generated compounds will be discussed.

Chemistry. The synthesis of DKPs **1**−**30** (Table 1) has been accomplished starting from an N-protected *trans*-4-hydroxy-L-proline methyl ester linked through the hydroxyl function to the Ellman resin.[17] We have developed two complementary reaction pathways that permit us to introduce up to three different centers of molecular diversity around the bicyclic template.

Method A. Fmoc-N-protected hydroxyproline methyl ester was attached to the Ellman resin through the hydroxyl function (Scheme 1).[17] The Fmoc (fluorenylmethyloxycarbonyl) group was removed and replaced by Teoc (trimethylsilylethoxycarbonyl), which had been previously activated as a benzotriazole ester (Bt).[18] This protecting group allows the alkylation of the $^{\alpha}$CH atom of hydroxyproline using LHMDS [lithium bis(trimethylsilyl)amide] in THF as a base.[19] After cleavage of Teoc with TBAF (tetrabutylammonium fluoride),[20] the pyrrolidine nitrogen was acylated with an Fmoc-N-protected amino acid fluoride in the presence of BSA [*N*,*O*-bis-(trimethylsilyl)acetamide].[21] The coupling was generally repeated two or three times, until the chloranil test gave a negative result.[22] The formation of the 2,5-diketopiperazine was initiated immediately during the cleavage of the Fmoc group. In case of incomplete cyclization after the treatment with piperidine, the resin was heated in DMF, adding a catalytic amount of potassium cyanide. The backbone amide function was finally alkylated with the suitable electrophile using a suspension of sodium hydride in DMF. The DKP was removed from the resin with a mixture of trifluoroacetic acid and water.

Method B. The second reaction route differs from that of method A in the acylation step of the pyrrolidine amino function (Scheme 2). After Teoc deprotection, the nitrogen of hydroxyproline was coupled with an azido acid activated as a chloride.[23] The azide reduction was subsequently accomplished using a suspension of tin chloride, triethylamine, and thiophenol in THF.[24] The time necessary for the transformation of the azide into the amino function was not sufficient for the complete cyclization to 2,5-diketopiperazine. Therefore, the resin was again heated in DMF in the presence of potassium cyanide as catalyst. The final DKP amide alkylation and cleavage from the resin were performed as described in method A.

Results and Discussion

The molecular structures of DKPs, which have been prepared following the two different reaction pathways described above, are displayed in Table 1. We initially synthesized the series of compounds **1**−**13** to investigate the high propensity to cyclization of a N-free dipeptide methyl ester containing the hydroxyproline residue linked to the resin through the alcoholic group. These molecules are not alkylated at the $^{\alpha}$CH atom of the pyrrolidine ring and, therefore, less sterically demanding. For this reason, the acylation of the ring nitrogen was performed immediately after the hydroxyproline Fmoc cleavage, using a standard peptide synthesis procedure (HOBt/DIC, 1-hydroxybenzotriazole/diisopropylcarbodiimide),[25] instead of the fluoride activation (Scheme 1). We have used several amino acid residues to form the DKP linear dipeptide precursor, including aliphatic, aromatic and trifunctional derivatives, such as Thr, Tyr and Trp (Table 1, R^2). After the cyclization, the DKP amide group was alkylated by treating the resin with a suspension of sodium hydride in DMF, followed by the addition of different electrophiles (Table 1, R^3). The heterocyclic compounds were cleaved from the resin and characterized by RP-HPLC, MALDI-TOF, and ESI high-resolution mass spectrometry (ESI−HRMS) (Table 1). Some representative compounds were also submitted to ^{1}H and ^{13}C NMR analysis (see the Experimental Section). These DKPs were obtained in high yield (>70%) at a purity comprised between 55% and 90%.

For the series of compounds **1**−**13**, two main peaks were systematically observed on each HPLC chromatogram. As the amide alkylation was performed without elimination of the base, epimerization occurred at $^{\alpha}$CH atoms of the DKP amino acid residues.[26] The *trans*-4-hydroxy-L-proline building block contains already two chiral centers, namely the $^{\alpha}$CH and the carbon atom bearing the hydroxyl function, which is in configuration R and remained intact during the treatment with the base. In the case of compound **1**, constituted of a Hyp and a Gly, and which has been alkylated at the amide with 4-methoxybenzyl chloride, two diastereoisomers were formed in proportion 1:0.4. Concerning DKPs **2**−**13**, which carry a second chiral amino acid, four diastereo-

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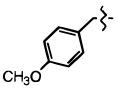
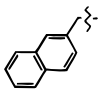
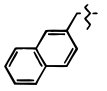
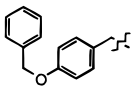
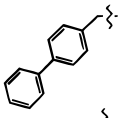
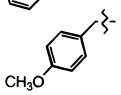
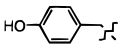
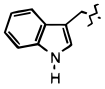
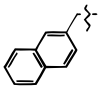
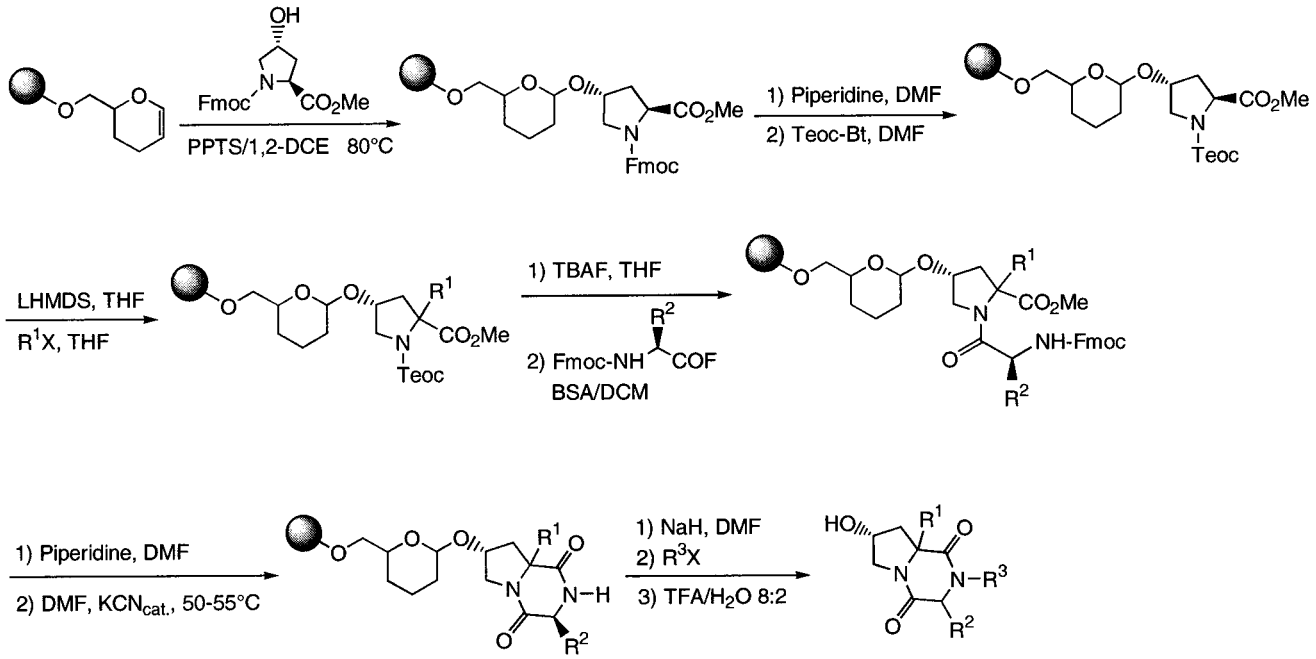
Table 1

Entry	R^1	R^2 (chirality)	R^3	HPLC purity[d]	Mass $[MH]^+$ (calcd.)	Entry	R^1	R^2 (chirality)	R^3	HPLC purity[d]	Mass $[MH]^+$ (calcd.)
1[a]	H	H		83%	291.1331 (291.1339)	**16**[b]		 (D)	H	77%	367.1982 (367.2016)
2[a]	H	CH_3 (L)		78%	325.1554 (325.1547)	**17**[b]		 (D)	H	66%	323.2323 (323.2329)
3[a]	H	 (L)		88%	317.1845 (317.1860)	**18**[b]		 (D)	H	69%	423.2290 (423.2278)
4[a]	H	 (L)		90%	287.1380 (287.1390)	**19**[b]		 (D)	H	74%	393.2179 (393.2173)
5[a]	H	 (L)		86%	357.2159 (357.2173)	**20**[c]		 (L)	H	86%	381.1818 (381.1809)
6[a]	H	 (L)		80%	317.1481 (317.1496)	**21**[c]		 (L)	H	65%	351.1693 (351.1703)
7[a]	H	 (L)		55%	340.1667 (340.1656)	**22**[c]		 (L)	H	88%	301.1559 (301.1547)
8[a]	H	 (L)		69%	355.1634 (355.1652)	**23**[c]	CH_3	 (L)	H	92%	275.1380 (275.1390)
9[a]	H	 (L)		84%	301.1524 (301.1547)	**24**[c]		 (L)		83%	341.1858 (341.1860)
10[a]	H	 (D)		87%	301.1526 (301.1547)	**25**[c]	CH_3	 (L)		90%	315.1705 (315.1703)
11[a]	H	 (L)	CH_3	85%	275.1382 (275.1390)	**26**[c]		(L)		51%	391.2043 (391.2016)
12[a]	H	(D)	CH_3	77%	275.1386 (275.1390)	**27**[b]		(D)		65%	433.2516 (433.2486)
13[a]	H	(L)		83%	381.1836 (381.1809)	**28**[b]		(D)		57%[e]	437.2429 (437.2435)
14[b]		(D)	H	66%	267.1715 (267.1703)	**29**[b]		(D)	CH_3	40%[f]	381.2162 (381.2173)
15[b]		(D)	H	67%	347.1966 (347.1965)	**30**[b]		(D)		56%[g]	463.2948 (463.2955)

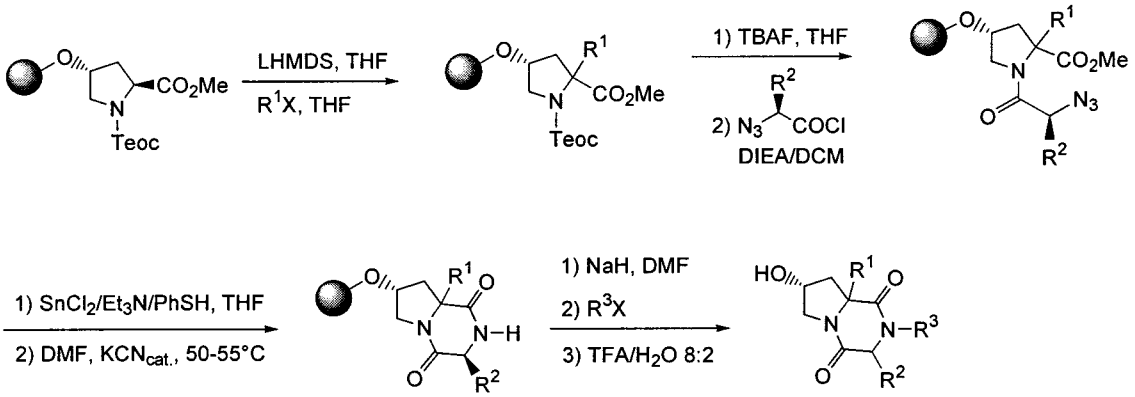
[a] Method A using standard peptide coupling conditions (HOBt/DIC), instead of fluoride activation, immediately after the hydroxyproline Fmoc cleavage. [b] Method B. [c] Method A. [d] Sum of diastereoisomers. [e] 18% of starting material **15** was present. [f] 35% of compound was not cyclized after reduction of azide and bismethylation at αNH of Ile occurred. [g] 24% of a DKP without cyclohexylmethyl group was found.

isomers were expected after the alkylation step, however only two of them could be detected on the HPLC chromatogram of the crude compounds after cleavage from the resin. We have verified that the two diastereoisomers are the same either when using a L amino acid or a D residue to form the DKP scaffold (compounds **9**−**12**). DKPs **11** and **12** were also synthesized in an independent way. After cleavage of the pyrrolidine Fmoc (Scheme 1), the acylation of nitrogen was done using Fmoc-L-MePhe-OH and Fmoc-D-MePhe-OH, respectively, activated with HOBt/DIC in DMF. After the second Fmoc removal, the cyclization afforded the single chirally intact products **31** and **32** (see molecular structures of compound **11** and **12**, respectively). Figure 1 compares the HPLC chro-

Scheme 1



Scheme 2



matograms of crude compounds **11** (panel A) and **12** (panel B) with those deriving from the synthesis employing Fmoc-L-MePhe-OH (**31**) (panel C) and Fmoc-D-MePhe-OH (**32**) (panel D). Two identical diastereoisomers were formed almost in the same ratio, during the alkylation of both cyclo-L-Hyp-L-Phe (**11a**, **11b**, panel A) and cyclo-L-Hyp-D-Phe (**12a**, **12b**, panel B) with methyl iodide.

The configurational assignment of the two diastereoisomers of DKP **11** and **12** were done by 2D NMR COSY and NOESY experiments, directly on the crude compound. The main isomer (**11b** and/or **12b**) corresponds to compound **32** and it is characterized by the $^{\alpha}$CH of the hydroxyproline in configuration *S* and that of phenylalanine in configuration *R* (Figure 2). Behind the clear correlations between $^{\gamma}$CH and $^{\beta 1}$CH$_2$, and $^{\beta 2}$CH$_2$ and $^{\alpha}$CH of hydroxyproline ring, a NOE contact between the aromatic protons of Phe and $^{\delta 2}$CH$_2$ of Hyp is observed and indicative of the same spatial orientation of both the phenyl group and the Hyp hydrogen, which point downward the plane of the bicyclic scaffold (Figure 2). The smaller peak (**11a** and/or **12a**), which has the same HPLC retention time of compound **31**, was instead assigned to the diastereoisomer cyclo-D-Hyp-L-MePhe. Again, the spatial arrangement of the $^{\alpha}$CH proton of hydroxyproline, in configuration R, was clearly attributed from the NOE cross-peaks with the neighboring hydrogens of the ring. A weak NOE contact between the Hyp $^{\alpha}$CH and the aromatic moiety of phenylalanine indicated that the configuration of Phe $^{\alpha}$CH was S.

In both diastereoisomers the aromatic group induces a strong ring current shift on the hydroxyproline $^{\alpha}$CH proton. Indeed, this proton should have a chemical shift about 4.0−4.5 ppm for an homochiral cyclic backbone, as shown in the case of cyclo-L-Hyp-L-MePhe (**31**), while the effect of the aromatic shield moves the NMR signal toward higher fields (around 3.0 ppm). The same structural evidences, relative to the spatial arrangement of the functional groups and protons, have been found for the two diastereoisomers of DKP **9** and **10**. This has permitted to determine that again two heterochiral compounds, namely cyclo-L-Hyp-D-AllylPhe (**9b** and/or **10b**) and cyclo-D-Hyp-L-AllylPhe (**9a** and/or **10a**), were obtained. These results are in agreement with previous conformational studies by NMR spectroscopy on cyclic

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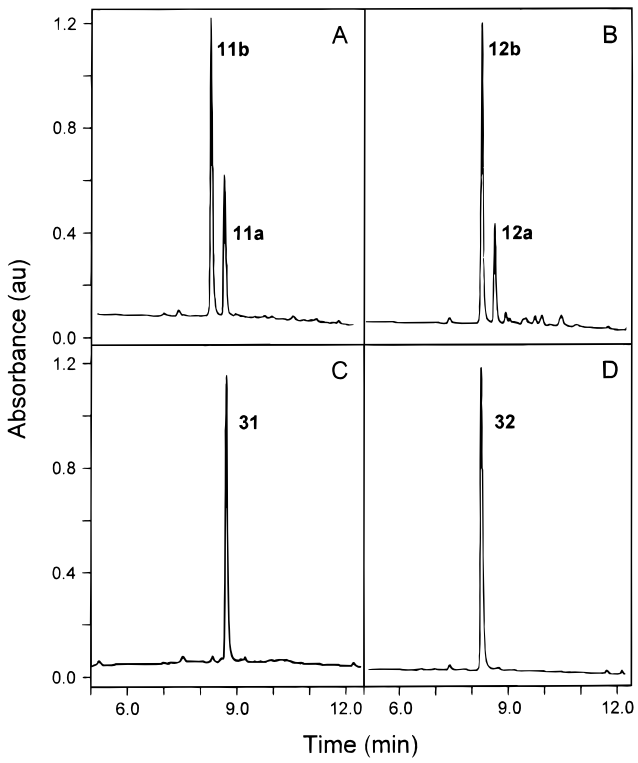

Figure 1. RP-HPLC profiles of the compounds **11** (A), **12** (B), **31** (C), and **32** (D) analyzed immediately after the cleavage from the resin. Linear gradient of A, 0.1% TFA in water, and B, 0.08% TFA in acetonitrile, 0−100% B in 20 min at 1.2 mL/min flow rate.

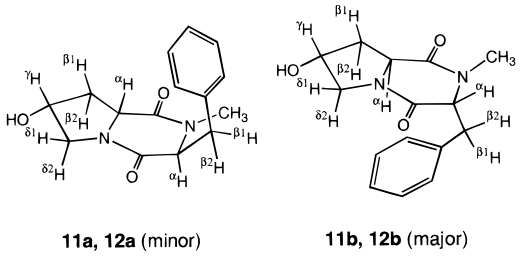

Figure 2. Molecular structures of the two diastereoisomers of DKPs **11** and **12**, as determined by 2D NMR spectroscopy.

dipeptides containing arylmethyl side chains, in which the aromatic ring is in close proximity, or better, faces the 2,5-diketopiperidine ring.[27] Such a spatial arrangement shifts by 1 ppm or more the resonance of the $^{\alpha}$CH proton of the second amino acid, when it is positioned at the same side of the aryl group (*cis*-proton). This shift has been shown also for bicyclic 2,5-piperazinediones containing a phenylalanine residue and 4-, 5-, and six-membered ring amino acids as a second residue.[7,28,29] Additional information about the conformations of the side chains of the DKP residues can be obtained from the values of the coupling constants between the $^{\alpha}$CH and $^{\beta}$CH$_2$ protons of a given residue.[27,28] For the diastereoisomers of compounds **9**−**12** we have found that the $\alpha-\beta$ coupling constants of phenylalanine are in the range 5.1−5.9 Hz, which indicates a certain predominance of the folded conformation. It has been calculated that for a completely folded conformation, in which the aromatic ring is located above the diketopiperazine backbone, $J^{\alpha\beta}$ should be around 3 Hz, while for equally populated unfolded structures a $J^{\alpha\beta}$ of 8 Hz is expected.[27]

The detailed structural analysis of the two diastereoisomers obtained from the amide alkylation of the DKPs enabled to deduce that, for representative compounds **9**−**12**, the epimerization favors the formation of cyclic compounds with heterochiral amino acid residues. We could extend this trend for all the DKPs of series **2**−**13**. Such generalization is supported by the studies done by Eguchi and Kakuta on the *cis*−*trans* isomerization of the side chains in 2,5-diketopiperazines.[30] The isomerization of both homochiral and heterochiral (LL/DD and/or LD/DL sequences) cyclic dipeptides was analyzed after treating the compounds with alkali solutions. The epimerization proceeds through a $^{\alpha}$CH carbanion formation and evolves toward a predominance of the heterochiral diastereoisomer.[30] The equilibrium is more shifted toward the *trans* orientation of the amino acid side chains for DKPs containing *N*-alkyl residues. The extreme equilibrium in favor of cyclo-L/D-Pro-D/L-Phe isomer (more than 90% of population is in *trans* form) during the epimerization of DKP results from the high tendency of the aromatic group to stack over the DKP cyclic backbone.[7,27] In our case the isomerization occurs in different conditions and was not optimized to reach the equilibrium. Moreover, as the molecules are still linked to the resin, the spatial orientation of the protons and of the functional groups, and consequently the percentage of the diastereoisomers, can be influenced by the vicinity of the solid support.

The second series of compounds **14**−**30** is characterized by the presence of a $C^{\alpha,\alpha}$-tetrasubstituted hydroxyproline residue within the bicyclic template. Eight different electrophiles have been used as alkylating agents of the Hyp $^{\alpha}$CH, namely allyl bromide, methyl iodide, benzyl bromide, 4-methoxybenzyl chloride, 2-(bromomethyl)-naphthalene, 4-phenylbenzyl chloride, 4-benzyloxybenzyl chloride and (bromomethyl)cyclohexane. DKPs **14**−**30** were obtained in fairly good purity ranging from 40% to 92%. Table 1 reports the synthetic routes that have been used to obtain the different compounds. The pathway of Scheme 1 was more convenient when less sterical hindered electrophiles (R^1 = methyl and/or allyl) were used to alkylate the $^{\alpha}$CH atom of hydroxyproline (compounds **22**−**25**). The difficult step was the acylation of the pyrrolidine nitrogen even though Fmoc-N-protected amino acid fluorides were used. The silylation of the extremely hindered pyrrolidine amino function with BSA, before starting its acylation with a Fmoc-protected amino acid fluoride, increased the rate of formation of the linear dipeptide.[21b] However, when benzyl and/or 4-methoxybenzyl moieties were introduced (compounds **20**, **21**, and **26**), the formation (via method A) of the linear dipeptide, precursor of DKP, still required very long reaction times and repetition of the coupling three times. The reaction route illustrated in the Scheme 2 is more advantageous and faster: (i) it requires shorter reaction time; (ii) it is not necessary to repeat the coupling.

The azido acids can be easily prepared from natural amino acids by replacement of the amino group with a bromine and its subsequent nucleophilic substitution with sodium azide.[31] Their transformation into the active chloride derivatives renders these synthons very useful for the preparation of encumbered peptides.[23] In the case

(28) Vičar, J.; Buděšinský, M.; Bláha, K. *Collec. Czech. Chem. Commun.* **1973**, *38*, 1940−1956.
(29) Siemion, I. Z. *Org. Magn. Reson.* **1971**, 545−550.
(30) Eguchi, C.; Kakuta, A. *J. Am. Chem. Soc.* **1974**, *96*, 3985−3989.

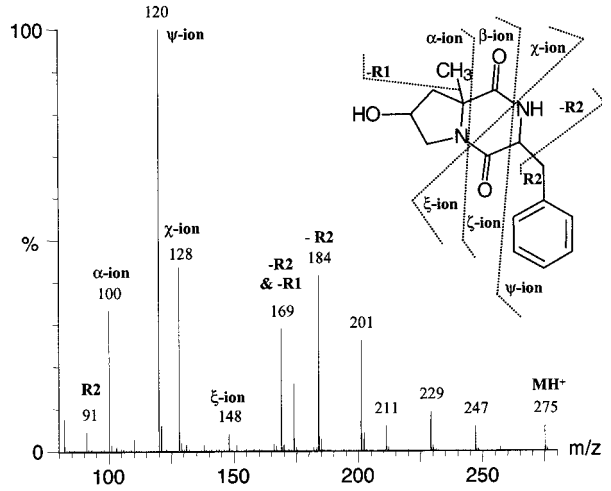

Figure 3. MS/MS spectrum of compound **23** together with the applied fragment ion nomenclature. The expected identity of the major peaks are reported: α-ion $m/z = 100$, χ-ion $m/z = 128$, ξ-ion $m/z = 148$, ψ-ion $m/z = 120$, R^2-ion $m/z = 91$, loss of R^2 ion $m/z = 184$, loss of R^2 and R^1 ion $m/z = 169$ (R^1 and R^2 correspond to the functional group of Table 1).

of trifunctional residues, the conversion into azido acids can be made using directly the free amino acids by reaction with TfN_3 (triflyl azide).[32]

Again RP-HPLC, MALDI-TOF, and ESI−HRMS have permitted a detailed characterization of compounds **14**−**30** (see also the Experimental Section for ^1H and ^{13}C NMR analysis of representative compounds). Moreover, low energy collision dissociation (CID) MS/MS analysis provided further information on the DKP compounds, confirming the expected substitution on each molecule. Figure 3 shows, as example, the MS/MS spectrum of compound **23** as well as the applied fragment ion nomenclature with the assignment of the main fragments.

During the hydroxyproline $^{\alpha}$CH alkylation two diastereisomers were generated. The reaction was carried out at room temperature by adding the electrophile and the base in tetrahydrofuran.[19] A certain degree of diastereoselectivity was induced by the R chiral center at position four of the hydroxyproline ring, which corresponds to the hydroxyl function linked to the resin. For compounds **14**−**23**, the two diastereoisomers were formed in a 1:0.45 to 1:0.85 ratio. The configurational geometry of the two diastereoisomers of bicyclic scaffold **23** was determined using standard bidimensional experiments. The two isomers were present in a ratio of 1:0.6 as shown in the ^1H NMR spectrum of the crude compound (Figure 4). The nuclear Overhauser effects allowed then to understand the relative spatial relationship of the hydrogens and functional groups around the cyclic backbone (Figure 4, inset).

In Figure 5 the most important NOEs cross-peaks used for the configurational assignment of the two diastereoisomers of **23** are labeled. In the case of the major isomer **23b** a strong NOE signal is due to the spatial proximity between the α-methyl group and the $^{\alpha}$CH proton of L-phenylalanine. NOE contacts of the methyl with $^{\beta 2}$H and $^{\delta 2}$H of pyrrolidine ring are also visible. For the minor diastereoisomer **23a** NOE contacts between $^{\beta 1}$H,$^{\gamma}$H and $^{\delta 1}$H protons of the hydroxyproline and the methyl group at the Hyp α-carbon atom are indicative of a R configuration of the latter (Figure 4, inset). In this isomer (**23a**) the folding of the aromatic group over the 2,5-diketopiperazine plane creates a strong ring current shift on the α-methyl, which appears at higher fields (0.61 ppm). The corresponding signal for the isomer **23b** is located around 1.5 ppm, as expected (Figure 4).

Finally, the amide alkylation of the cyclic dipeptides containing the $C^{\alpha,\alpha}$-tetrasubstituted hydroxyproline gave the compounds **24**−**30**, which present the highest molecular diversity, in terms of functionalities and number of diastereoisomers. All four possible combinations deriving from the two amino acid α-chiral centers could be detected. We successfully determined the stereochemistry of four isomers of DKP **24**, which were present in the proportion 0.6:0.7:1:0.15. Two preparative HPLC fractions were collected: one contained the two isomers cyclo-D-(αAllyl)Hyp-D-AllylPhe (**24a**) and cyclo-L-(αAllyl)Hyp-L-AllylPhe (**24b**) and the other corresponding to the couple of isomers cyclo-D-(αAllyl)Hyp-L-AllylPhe (**24c**) and cyclo-L-(αAllyl)Hyp-D-AllylPhe (**24d**). The two mixtures were submitted to COSY and NOESY experiments and the configurations of the chiral carbon atoms were unambiguously assigned.

In the case of compounds **28**−**30**, we were also able to characterize some byproducts: (i) 18% of compound **15**, precursor of the DKP **28** did not react, and was collected as starting material; (ii) bismethylation occurred at $^{\alpha}$NH of Ile during the alkylation of the DKP **29** amide bond with methyl iodide; indeed 35% of the linear dipeptide, alkylated at the $^{\alpha}$CH of the hydroxyproline with the naphthylmethyl group, did not cyclize after reduction of the azide protecting group; (iii) the final DKP **30** contained 24% of a bicyclic compound without cyclohexylmethyl group. These charaterizations are important in view of a further optimization of the synthesis of more sterically hindered substituted 2,5-diketopiperazines.

Conclusions

In summary, we have reported an efficient methodology, based on two different synthetic pathways, for the preparation on solid support of a new class of 2,5-diketopiperazines containing the *trans*-4-hydroxyproline residue. Using a parallel solid-phase synthesis, we have generated bicyclic DKP scaffolds which hold up to three different functionalities. Although epimerization occurred during the alkylation steps, we were able to characterize all the diastereoisomers by means of multidimensional NMR spectroscopy. The racemization is not a completely undesired side reaction, since it increases the molecular diversity of the compounds synthesized. This can be of particular interest during the generation of combinatorial libraries and their application to enzyme inhibitors drug discovery. We are currently modifying the conditions of alkylation reactions in order to induce a higher diastereoselectivity and to limit the epimerization on the final compounds. In due course, we will describe also the application of our approach to the synthesis of bicyclic DKP libraries.

Experimental Section

General. All reagents and solvents were obtained from commercial suppliers and used without further purification.

(31) (a) Saito, K.; Harada, K. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 2562−2566. (b) Briggs, M.; Morley, J. S. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2138−2143. (c) Hoffman, R. V.; Kim, H.-O. *Tetrahedron* **1992**, *48*, 3007−3020.

(32) Zaloom, J.; Shiner, V. J. *J. Org. Chem.* **1981**, *46*, 5173−5176.

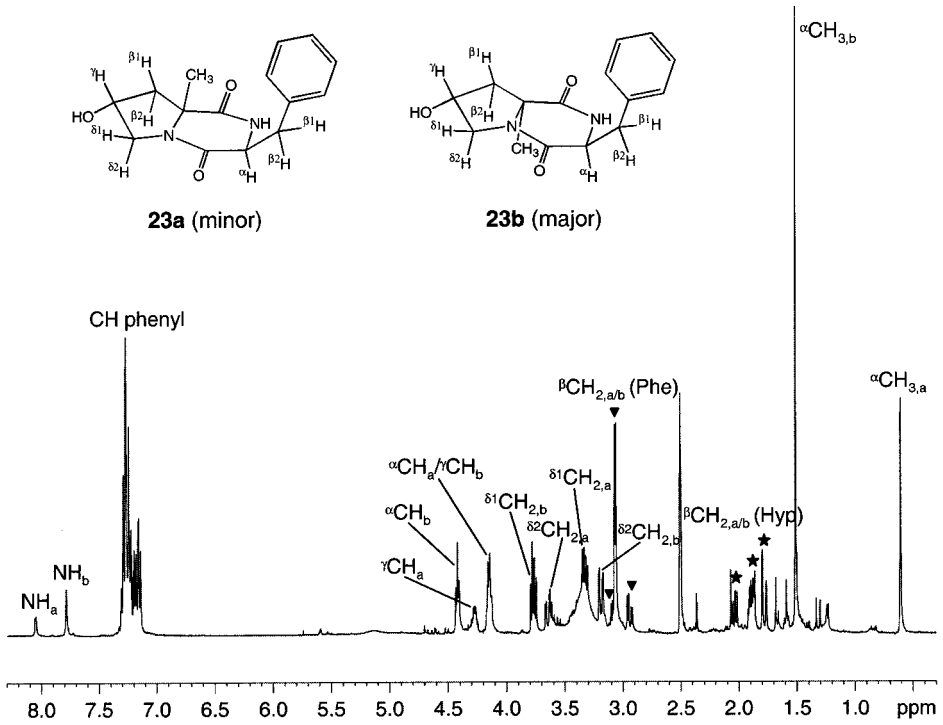

Figure 4. ^{1}H NMR spectrum of the crude compound **23** in DMSO-d_6. Subscript letters a and b on the assigned protons indicate the minor isomer **23a** and the major isomer **23b**, respectively. Inset: molecular structures of the two diastereoisomers formed during the synthesis.

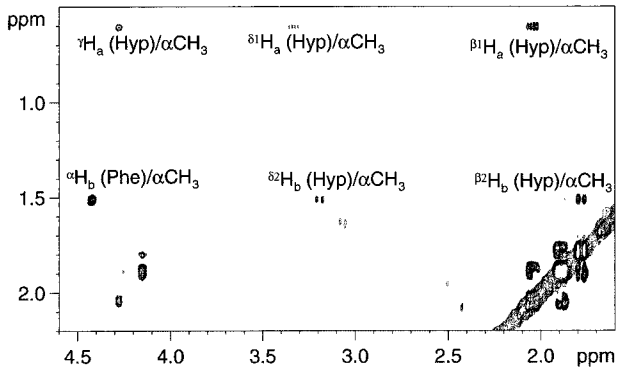

Figure 5. Partial 400 MHz NOESY spectrum of the two diastereoisomers of compound **23** in DMSO-d_6. The NOE cross-peaks used for the configurational assignment are labeled. Subscript letters a and b on the assigned protons indicate the minor isomer **23a** and the major isomer **23b**, respectively.

Tetrahydrofuran (THF), dichloromethane (DCM), and 1,2-dichloroethane (1,2-DCE) were carefully distilled prior to use. 3,4-Dihydro-2*H*-pyran-2-ylmethoxymethyl polystyrene (DHP-HM resin, Ellman's dihydropyran resin) was purchased from Novabiochem (Läufelfingen, Switzerland) and loaded with Fmoc-L-Hyp-OMe as reported.[17] The alkylations of the hydroxyproline CH$^\alpha$ atom were done under nitrogen on a glass tube and the cyclization of DKPs were then continued manually in small plastic syringes fitted with a frit. Teoc-OBt,[18] Fmoc-protected amino acid fluorides[33] and azido acids[23,31] were synthesized as described in the literature. RP-HPLC analysis was done on a C_{18} column (5 μm, 150 × 4.6 mm) using a linear gradient of A: 0.1% TFA in water and B: 0.08% TFA in acetonitrile, 0−100% B in 20 min at 1.2 mL/min flow rate. Chromatograms were recorded at 210 nm wavelength. MALDI-TOF mass analysis was performed on a linear MALDI-TOF instrument using α-cyano-4-hydroxycinnamic acid and/or 2,5-dihydroxybenzoic acid as matrixes. Precise mass determination of the mono isotopic DKP ions was performed by using two internal references and electrospray ionization on a Q-TOF mass spectrometer. The MS/MS data of the fragment ions were obtained on the same instrument. 1D and 2D NMR spectra were recorded on a 400 MHz spectrometer. The samples were dissolved in $CDCl_3$-*d* and/or DMSO-d_6.

Abbreviations. Symbols and abbreviations for amino acids and peptides are in accord with the recommendations of the IUPAC−IUB Commission on Nomenclature (*J. Biol. Chem.* **1972**, *247*, 977). Other abbreviations used are as follows: DKP, 2,5-diketopiperazine; SPOS, solid-phase organic synthesis; Fmoc, fluorenylmethyloxycarbonyl; Teoc, trimethylsilylethoxycarbonyl; LHMDS, lithium bis(trimethylsilyl)amide; Bt, benzotriazole; TBAF, tetrabutylammonium fluoride; BSA; N,O-bis(trimethylsilyl)acetamide; HOBt, 1-hydroxybenzotriazole; DIC, diisopropylcarbodiimide; DMSO, dimethyl sulfoxide; NOE, nuclear Overhauser effect; 1,2-DCE, 1,2-dichloroethane; DCM, dichloromethane; DMF, dimethylformamide; THF, tetrahydrofuran; -OMe, methoxy; PPTS, pyridinium *p*-toluenesulfonate; TFA, trifluoroacetic acid.

General Procedures for Preparation of 2,5-Diketopiperazines Using Method A. DHP−HM resin (50 mg, 0.049 mmol) loaded with Fmoc-L-Hyp-OMe was treated with 800 μL of 25% piperidine in DMF (2 × 15 min). After washings with DMF and DCM, a solution of Teoc-OBt (41 mg, 3 equiv) in DMF (350 μL) was added to the resin, and the mixture was stirred for 24 h at rt. The solution was eliminated and the resin washed with DMF, DCM, Et_2O and dried under vacuum. The resin was suspended, under nitrogen, in dry THF (1 mL) and after 5 min a solution of an electrophile (12 equiv) in dry THF (250 μL) was added (Table 1, entries 20−26). The mixture was stirred for 5 min, and a 1 M solution of LHMDS in THF (590 μL, 12 equiv) was added at room temperature. After 6 h, the excess of base was hydrolyzed with a saturated solution of NH_4Cl, and the resin was extensively washed with water, methanol, DMF, and DCM. Teoc was removed using a fresh 1 M solution of TBAF (735 μL, 15 equiv) in THF along 1 h. After washing, the resin was treated with BSA (60 μL, 5 equiv) in dry DCM (400 μL) for 5 h, followed by the addition of a Fmoc-N-protected amino acid fluoride (5 equiv) dissolved in dry DCM

(33) Carpino, L. A.; Sadat-Aalaee, D.; Chao, H. G.; DeSelms, R. M. *J. Am. Chem. Soc.* **1990**, *112*, 9651−9652.

(400 μL). The mixture was shaken 24 h at rt, and the acylation was repeated a second time in the same conditions. When the chloranil test was not completely negative, a further treatment with the fluoride was performed. Following several washings with DCM and DMF, the Fmoc group was removed with 25% piperidine in DMF (800 μL, 2 × 15 min), which immediately started the cyclization to DKP. This step was monitored by Kaiser test. After elimination of the base, the resin was swollen in DMF and heated at 50−55 °C for 18 h in the presence of a catalytic amount of KCN. After washing, the resin was treated with a suspension of NaH (37 mg, 60% in oil, 20 equiv) in dry DMF (2 mL) for 7 h followed by the addition of an electrophile (20 equiv) (Table 1). The mixture was stirred for 16 h at rt and the excess of NaH hydrolyzed with a saturated solution of NH_4Cl. After filtration, the resin was washed with water, methanol, DMF and DCM. The DKP was finally cleaved from the resin using a 8:2 mixture of TFA/H_2O (500 μL, 30 min) and recovered as an oily compound after evaporation of the acidic solution under reduced pressure.

General Procedures for Preparation of 2,5-Diketopiperazines Using Method B. DHP−HM resin (50 mg, 0.049 mmol) loaded with Fmoc-L-Hyp-OMe underwent the same reaction steps of method A up to the cleavage of Teoc (Table 1, entries 14−19 and 27−30). After deprotection, the resin was swollen in dry DCM (200 μL), and DIEA (170 μL, 20 equiv) and a solution of an azido acid chloride (15 equiv) in dry DCM (500 μL) were added successively. The resin was shaken for 2 h at rt. After several washings with DCM and DMF, the azide was reduced by adding a suspension of 0.2 M $SnCl_2$, 1 M Et_3N, and 0.8 M PhSH in THF (2 mL) for 4 h. Following washings with methanol, water, DMF, and DCM, the cyclization to DKP appeared incomplete (Kaiser test positive). The resin was then swollen in DMF and heated at 50−55 °C for 18 h in the presence of a catalytic amount of KCN. Again, the alkylation of the amide group was performed as reported in method A. The DKP was finally cleaved from the resin using a 8:2 mixture of TFA/H_2O (500 μL, 30 min) and recovered as an oily compound after evaporation of the acidic solution under reduced pressure.

All crude compounds were analyzed by RP-HPLC, MALDI-TOF, ESI high-resolution MS, and MS/MS. Compounds **9**, **10**, **11**, **12**, **23**, **24**, cyclo-L-Hyp-L-MePhe (**31**), and cyclo-L-Hyp-D-MePhe (**32**) were further characterized by 1D and 2D NMR spectroscopy. The NOE contacts used for the configurational assignment of such compounds are also reported (see Figure 2 and Figure 4, inset, for the spatial orientation of the hydrogens and the functional groups around the bicyclic DKP scaffold).

Compound 9 (mixture of two diastereoisomers, ratio **9b**/**9a** 1:0.7): ^{1}H NMR (400 MHz, DMSO) δ 7.29 (m, CH phenyl), 7.15 (m, CH phenyl), 5.72 (m, CH allyl), 5.15 (m, CH_2 allyl), 4.39 (m, NCH_2 allyl), 4.19 (t, γCH Hyp, **b**), 4.12 (m, γCH Hyp, **a**), 4.06 (m, αCH Phe), 3.59 (dd, δCH_2 Hyp, **b**), 3.48 (dd, δCH_2 Hyp, **a**), 3.34 (dd, NCH_2 allyl, **b**), 3.28 (m, NCH_2 allyl, **a**), 3.26 (m, αCH Hyp, **b**), 3.22 (m, αCH Hyp, **a**), 3.18 (m, δCH_2 Hyp, **a**), 3.10 (m, δCH_2 Hyp, **b**, βCH_2 Phe), 2.14 (qd, βCH_2 Hyp, **a**), 1.95 (m, βCH_2 Hyp, **a**), 1.90 (dd, βCH_2 Hyp, **b**), 1.71 (qd, βCH_2 Hyp, **b**); ^{13}C NMR (100 MHz, DMSO) **9a** δ 167.23, 165.04, 136.51, 133.14, 130.16, 128.87, 127.58, 117.83, 67.22, 62.97, 55.99, 53.33, 46.59, 37.52, 36.92; **9b** δ 167.63, 164.59, 136.29, 133.27, 130.13, 128.87, 127.58, 118.35, 66.73, 62.84, 56.10, 54.47, 46.26, 38.68, 36.88. NOE contacts, **9a**: H($^{\gamma}$CH-Hyp):H($^{\beta1}CH_2$-Hyp); H($^{\beta1}CH_2$-Hyp):H($^{\alpha}$CH-Hyp). **9b**: H($^{\gamma}$CH-Hyp):H($^{\beta1}CH_2$-Hyp); H($^{\beta2}CH_2$-Hyp):H($^{\alpha}$CH-Hyp); H(^{Ph}CH-Phe):H($^{\delta2}CH_2$-Hyp); H(^{Ph}CH-Phe):H($^{\alpha}$CH-Hyp); MALDI-MS [M + H]$^+$ calcd for $C_{17}H_{20}N_2O_3$ 301.36, found: 301.28;. MS/MS α-ion m/z = 86, ψ-ion m/z = 160, β-ion m/z = 141, ξ-ion m/z = 188, loss of R^2 ion m/z = 210.

Compound 10 (mixture of two diastereoisomers, ratio **10b**/**10a** 1:0.33): ^{1}H NMR (400 MHz, DMSO) δ 7.29 (m, CH phenyl), 7.15 (m, CH phenyl), 5.72 (m, CH allyl), 5.15 (m, CH_2 allyl), 4.39 (m, NCH_2 allyl), 4.19 (t, γCH Hyp, **b**), 4.12 (m, γCH Hyp, **a**), 4.06 (m, αCH Phe), 3.59 (dd, δCH_2 Hyp, **b**), 3.48 (dd, δCH_2 Hyp, **a**), 3.34 (dd, NCH_2 allyl, **b**), 3.28 (m, NCH_2 allyl, **a**), 3.26 (m, αCH Hyp, **b**), 3.22 (m, αCH Hyp, **a**), 3.18 (m, δCH_2 Hyp, **a**), 3.10 (m, δCH_2 Hyp, **b**, βCH_2 Phe), 2.14 (m, βCH_2 Hyp, **a**), 1.95 (m, βCH_2 Hyp, **a**), 1.90 (dd, βCH_2 Hyp, **b**), 1.71 (qd, βCH_2 Hyp, **b**); ^{13}C NMR (100 MHz, DMSO) **10a** δ 167.21, 165.03, 136.51, 133.14, 130.15, 128.86, 127.57, 117.82, 67.21, 62.96, 55.98, 53.33, 46.58, 37.50; **10b** δ 167.62, 164.58, 136.29, 133.27, 130.12, 128.86, 127.57, 118.34, 66.71, 62.84, 56.09, 54.46, 46.25, 38.67, 36.88; MALDI-MS [M + H]$^+$ calcd for $C_{17}H_{20}N_2O_3$ 301.36, found 301.05; MS/MS α-ion m/z = 86, ψ-ion, m/z = 160, β-ion m/z = 141, ξ-ion m/z = 188, loss of R^2 ion m/z = 210.

Compound 11 (mixture of two diastereoisomers, ratio **11b**/**11a** 1:0.4): ^{1}H NMR (400 MHz, DMSO) δ 7.27 (m, CH phenyl), 7.13 (m, CH phenyl), 4.19 (m, γCH Hyp, **b**, αCH Phe), 4.09 (m, γCH Hyp, **a**), 3.57 (dd, δCH_2 Hyp, **b**), 3.42 (dd, δCH_2 Hyp, **a**), 3.15 (m, δCH_2 Hyp, **a**), 3.08 (m, δCH_2 Hyp, **b**, αCH Hyp, **b**, βCH_2 Phe), 2.92 (t, αCH Hyp, **a**), 2.81 (s, NCH_3, **b**), 2.80 (s, NCH_3, **a**), 2.12 (qd, βCH_2 Hyp, **a**), 1.86 (dd, βCH_2 Hyp, **b**), 1.81 (m, βCH_2 Hyp, **a**), 1.67 (qd, βCH_2 Hyp, **b**); ^{13}C NMR (100 MHz, DMSO) **11a** δ 136.44, 130.13, 128.84, 127.60, 67.16, 65.29, 55.92, 53.17, 37.70, 32.61; **11b** δ 167.59, 164.63, 136.28, 130.07, 128.84, 127.60, 66.66, 65.41, 56.01, 54.46, 38.70, 36.67, 32.29; NOE contacts, **11a** H($^{\gamma}$CH-Hyp):H($^{\beta1}CH_2$-Hyp); H($^{\beta1}CH_2$-Hyp):H($^{\alpha}$CH-Hyp); H(^{Ph}CH-Phe):H($^{\alpha}$CH-Hyp). **11b**: H($^{\gamma}$CH-Hyp):H($^{\beta1}CH_2$-Hyp); H($^{\beta2}CH_2$-Hyp):H($^{\alpha}$CH-Hyp); H(^{Ph}CH-Phe):H($^{\delta2}CH_2$-Hyp); H(^{Ph}CH-Phe):H($^{\alpha}$CH-Hyp); MALDI-MS [M + H]$^+$ calcd for $C_{15}H_{18}N_2O_3$ 275.32, found 275.20; MS/MS α-ion m/z = 86, ψ-ion m/z =134, χ-ion m/z = 114, loss of R^2 ion m/z = 184.

Compound 12 (mixture of two diastereoisomers, ratio **12b**/**12a** 1:0.32): ^{1}H NMR (400 MHz, DMSO) δ 7.27 (m, CH phenyl), 7.13 (m, CH phenyl), 4.18 (m, γCH Hyp, **b**, αCH Phe), 4.09 (m, γCH Hyp, **a**), 3.57 (dd, δCH_2 Hyp, **b**), 3.42 (dd, δCH_2 Hyp, **a**), 3.15 (m, δCH_2 Hyp, **a**), 3.08 (m, δCH_2 Hyp, **b**, αCH Hyp, **b**, βCH_2 Phe), 2.91 (m, αCH Hyp, **a**), 2.81 (s, NCH_3, **b**), 2.80 (s, NCH_3, **a**), 2.12 (qd, βCH_2 Hyp, **a**), 1.85 (dd, βCH_2 Hyp, **b**), 1.80 (m, βCH_2 Hyp, **a**), 1.66 (qd, βCH_2 Hyp, **b**); ^{13}C NMR (100 MHz, DMSO) **12a** δ 167.08, 164.88, 136.43, 130.11, 128.83, 127.59, 67.15, 65.29, 55.92, 53.15, 37.69, 32.60; **12b** δ 167.58, 164.61, 136.27, 130.05, 128.83, 127.59, 66.65, 65.41, 56.00, 54.44, 38.69, 36.67, 32.29; MALDI-MS [M + H]$^+$ calcd for $C_{15}H_{18}N_2O_3$ 275.32, found 275.10; MS/MS α-ion m/z = 86, ψ-ion m/z =134, χ-ion m/z = 114, loss of R^2 ion m/z = 184.

Compound 23 (mixture of two diastereoisomers, ratio **23b**/**23a** 1:0.6): ^{1}H NMR (400 MHz, DMSO) δ 8.05 (d, αNH, **a**), 7.78 (s, αNH, **b**), 7.27 (m, CH phenyl), 4.42 (m, αCH Phe, **b**), 4.28 (m, γCH Hyp, **a**), 4.15 (m, αCH Phe, **a**, γCH Hyp, **b**), 3.77 (dd, δCH_2 Hyp, **b**), 3.65 (dd, δCH_2 Hyp, **a**), 3.34 (dd, δCH_2 Hyp, **a**), 3.19 (dd, δCH_2 Hyp, **b**), 3.09 (dd, βCH_2 Phe, **a**), 3.07 (d, βCH_2 Phe, **b**), 2.94 (dd, βCH_2 Phe, **a**), 2.04 (dd, βCH_2 Hyp, **a**), 1.88 (m, βCH_2 Hyp), 1.77 (d, βCH_2 Hyp, **b**), 1.51 (s, αCH_3, **b**), 0.61 (s, αCH_3, **a**); ^{13}C NMR (100 MHz, DMSO) **23a** δ 170.36, 164.16, 136.84, 130.49, 128.75, 127.25, 66.57, 63.28, 58.05, 52.88, 46.63, 26.12; **23b** δ 172.17, 165.15, 137.56, 130.37, 128.39, 126.76, 66.79, 64.30, 55.48, 54.33, 44.58, 35.99, 24.67; NOE contacts, **23a** H($^{\gamma}$CH-Hyp):H($^{\beta1}CH_2$-Hyp); H($^{\beta1}CH_2$-Hyp):H($^{\alpha}CH_3$); H($^{\gamma}$CH-Hyp):H($^{\alpha}CH_3$); H($^{\delta1}CH_2$-Hyp):H($^{\alpha}CH_3$); H(^{Ph}CH-Phe):H($^{\alpha}CH_3$); **23b** H($^{\gamma}$CH-Hyp):H($^{\beta1}CH_2$-Hyp); H($^{\beta2}CH_2$-Hyp):H($^{\alpha}CH_3$); H($^{\delta2}CH_2$-Hyp):H($^{\alpha}CH_3$); H($^{\alpha}CH_3$-Hyp):H($^{\alpha}$CH-Phe); MALDI-MS [M + H]$^+$ calcd for $C_{15}H_{18}N_2O_3$ 275.32, found 275.14; MS/MS α-ion m/z = 100, χ-ion m/z = 128, ξ-ion m/z = 148, ψ-ion m/z = 120, R^2-ion m/z = 91, loss of R^2 ion m/z = 184, loss of R^2 and R^1 ion m/z = 169.

Compound 24 (mixture of four diastereoisomers, ratio **24a**/**24b**/**24c**/**24d** 0.6:0.7:1:0.15): ^{1}H NMR (400 MHz, $CDCl_3$) **24a**/**24b** δ 7.30 (m CH phenyl), 7.17 (m CH phenyl), 7.06 (m CH phenyl), 5.80 (m, CH allyl(N)), 5.58 (m, CH allyl(α)), 5.38 (m, CH_2 allyl(N)), 5.14 (m, CH_2 allyl(α)), 5.05 (m, NCH_2 allyl(N)), 4.44 (t, αCH Phe, **a**), 4.38 (t, αCH Phe, **b**), 4.33 (m, γCH Hyp, **a**), 4.20 (dd, δCH_2 Hyp, **b**), 4.00 (m, γCH Hyp, **b**), 3.69 (dd, δCH_2 Hyp, **a**), 3.59 (2dd, NCH_2 allyl(N)), 3.50 (qd, δCH_2 Hyp, **a**), 3.39 (dd, βCH_2 Phe, **a**), 3.34 (dd, βCH_2 Phe, **b**), 3.24 (d, βCH_2 Phe, **a**), 3.16 (m, βCH_2 Phe, **b**, δCH_2 Hyp, **b**), 2.77 (dd, αCH_2 allyl(α), **b**), 2.60 (dd, αCH_2 allyl(α), **b**), 2.52 (dd, αCH_2 allyl(α), **a**), 2.26 (dd, βCH_2 Hyp, **a**), 2.18 (dd, αCH_2 allyl(α), **a**), 1.71 (d, βCH_2 Hyp, **b**), 0.92 (m, βCH_2 Hyp, **a**), 0.88 (m, βCH_2

Hyp, **b**); ^{13}C NMR (100 MHz, $CDCl_3$) **24a** δ 167.43, 163.54, 135.43, 131.76, 130.71, 130.36, 128.69, 127.42, 120.99, 120.25, 68.24, 60.03, 52.89, 46.06, 45.33, 42.80, 35.90, 29.74; **24b** δ 167.43, 163.54, 134.94, 131.88, 131.33, 130.04, 128.47, 127.31, 120.42, 120.17, 67.47, 59.83, 52.72, 45.97, 44.80, 42.57, 36.30, 29.74; ^{1}H NMR (400 MHz, $CDCl_3$) **24c**/**24d** δ 7.32 (m CH phenyl), 5.76 (m, CH allyl(α), **d**), 5.66 (m, CH allyl(α), **c**, CH allyl(N)), 5.18 (m, CH_2 allyl(N) and CH_2 allyl(α), **c**), 5.08 (m, CH_2 allyl(N) and CH_2 allyl(α), **c**), 5.02 (m, CH_2 allyl(N), **d**), 4.63 (m, NCH_2 allyl(N), **c**), 4.59 (m, NCH_2 allyl(N), **d**), 4.51 (m, γCH Hyp, **d**), 4.49 (m, γCH Hyp, **c**), 4.36 (dd, δCH_2 Hyp, **d**), 4.33 (dd, αCH Phe, **c**), 4.25 (dd, αCH Phe, **d**), 4.11 (m, δCH_2 Hyp, **c**), 3.40 (dd, δCH_2 Hyp, **c**), 3.36 (m, δCH_2 Hyp, **d**), 3.30 (m, βCH_2 Phe), 3.11 (dd, NCH_2 allyl(N), **c**), 3.09 (m, NCH_2 allyl-(N), **d**), 2.61 (m, αCH_2 allyl(α), **d**), 2.42 (m, βCH_2 Hyp, **c**), 2.36 (m, βCH_2 Hyp, **d**), 2.20 (m, βCH_2 Hyp, **d**), 1.95 (m, αCH_2 allyl-(α), **c**), 1.85 (m, αCH_2 allyl(α), **d**); ^{13}C NMR (100 MHz, $CDCl_3$) **24c** δ 168.72, 165.74, 136.39, 131.68, 131.37, 129.69, 129.02, 127.55, 120.19, 118.53, 68.34, 62.53, 54.83, 47.12, 44.30, 44.14, 38.88, 31.98; **24d** not detected; NOE contacts, **24a** H($^{\gamma}$CH-Hyp):H($^{\beta 1}CH_2$-Hyp); H($^{\beta 1}CH_2$-Hyp):H($^{\alpha}CH_2$-allyl(α)); H($^{\gamma}$CH-Hyp):H($^{\alpha}CH_2$-allyl(α)); H($^{\delta 1}CH_2$-Hyp):H($^{\alpha}CH_2$-allyl(α)); H($^{\alpha}$CH-Phe):H(CH-allyl(α)); **24b** H($^{\gamma}$CH-Hyp):H($^{\beta 1}CH_2$-Hyp); H($^{\beta 2}CH_2$-Hyp):H($^{\alpha}CH_2$-allyl(α)); H($^{\delta 2}CH_2$-Hyp):H($^{\alpha}CH_2$-allyl(α)); H($^{\alpha}$CH-Phe):H($^{\alpha}CH_2$-allyl(α)); H(^{Ph}CH-Phe):H($^{\delta 1}CH_2$-Hyp), H($^{\alpha}$CH-Phe):H(CH-allyl(α)); **24c** H($^{\gamma}$CH-Hyp):H($^{\beta 1}CH_2$-Hyp); H($^{\beta 1}CH_2$-Hyp):H($^{\alpha}CH_2$-allyl(α)); H($^{\gamma}$CH-Hyp):H($^{\alpha}CH_2$-allyl(α)); H($^{\delta 1}CH_2$-Hyp):H($^{\alpha}CH_2$-allyl(α)); H($^{\beta}CH_2$-Phe):H($^{\alpha}CH_2$-allyl(α)); **24d** H($^{\gamma}$CH-Hyp):H($^{\beta 1}CH_2$-Hyp); H($^{\beta 2}CH_2$-Hyp):H($^{\alpha}CH_2$-allyl(α)); MALDI-MS: $[M + H]^+$ calcd for $C_{20}H_{24}N_2O_3$ 341.42, found 341.30; MS/MS α-ion $m/z = 126$, ψ-ion $m/z = 160$, loss of R^3 ion $m/z = 299$, loss of R^3 and part of R^1 ion $m/z = 271$.

Cyclo-L-Hyp-L-MePhe 31: ^{1}H NMR (400 MHz, DMSO) δ 7.26 (m, 3H, phenyl), 6.99 (m, 2H, phenyl), 4.41 (t, 1H, αCH Phe), 4.03 (dd, 1H, αCH Hyp), 3.89 (m, 1H, γCH Hyp), 3.74 (dd, 1H, δCH_2 Hyp), 3.17 (d, 2H, βCH_2 Phe), 2.98 (s, 3H, CH_3), 2.91 (d, 1H, δCH_2 Hyp), 1.59 (dd, 1H, βCH_2 Hyp), 0.16 (td,1H, βCH_2 Hyp); ^{13}C NMR (100 MHz, DMSO) δ 165.85, 163.84, 136.12, 130.08, 128.70, 127.37, 66.54, 62.82, 56.86, 54.51, 39.18, 36.15, 32.17; NOE contacts H($^{\gamma}$CH-Hyp):H($^{\beta 1}CH_2$-Hyp); H($^{\beta 2}CH_2$-Hyp):H($^{\alpha}$CH-Hyp); H($^{\alpha}$CH-Hyp):H($^{\alpha}$CH-Phe); MALDI-MS $[M + H]^+$ calcd.for $C_{15}H_{18}N_2O_3$ 275.32, found 275.15; HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{18}N_2O_3$ 275.1390, found 275.1378; MS/MS α-ion $m/z = 86$, ψ-ion $m/z = 134$, χ-ion $m/z = 114$, loss of R^2 ion $m/z = 184$.

Cyclo-L-Hyp-D-MePhe 32: ^{1}H NMR (400 MHz, DMSO) δ 7.27 (m, 3H, phenyl), 7.12 (m, 2H, phenyl), 4.19 (m, 2H, αCH Phe, γCH Hyp), 3.57 (dd, 1H, δCH_2 Hyp), 3.09 (m, 4H, δCH_2 Hyp, βCH_2 Phe, αCH Hyp), 2.81 (s, 3H, CH_3), 1.86 (dd, 1H, αCH Hyp), 1.66 (td, 1H, αCH Hyp); ^{13}C NMR (100 MHz, DMSO) δ 167.58, 164.62, 136.27, 130.06, 128.83, 127.59, 66.66, 65.42, 56.00, 54.46, 38.71, 36.68, 32.29; MALDI-MS $[M + H]^+$ calcd for $C_{15}H_{18}N_2O_3$ 275.32, found 275.16; HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{18}N_2O_3$ 275.1390, found 275.1388; MS/MS α-ion $m/z = 86$, ψ-ion $m/z = 134$, χ-ion $m/z = 114$, loss of R^2 ion $m/z = 184$.

Supporting Information Available: 400 MHz ^{1}H NMR and ^{13}C NMR, HR-MS, and MS/MS spectra of compounds **9**−**12**, **23**, **24**, **31**, and **32**; 400 MHz COSY and NOESY spectra of compounds **9**, **11**, **23**, **24**, and **31**. This material is available free of charge via the Internet at http://pubs.acs.org.

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