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Synthesis and Conformational Studies of Peptidomimetics Containing a New Bifunctional Diketopiperazine Scaffold Acting as a β-Hairpin Inducer

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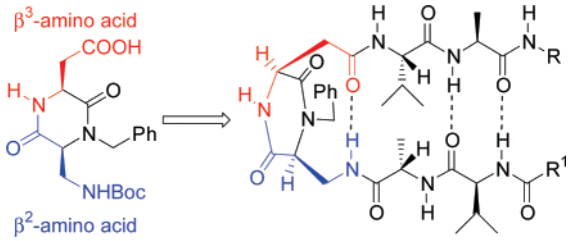

A practical synthesis of a new bifunctional diketopiperazine (DKP) scaffold **1**, formally derived from the cyclization of L-aspartic acid and (*S*)-2,3-diaminopropionic acid, is reported. DKP-**1** bears a carboxylic acid and an amino functionalities in a cis relationship, which have been used to grow peptide sequences. Tetra-, penta-, and hexapeptidomimetic sequences were prepared by solution-phase peptide synthesis (Boc strategy). Conformational analysis of these derivatives was carried out by a combination of ^{1}H NMR spectroscopy, IR spectroscopy, CD spectroscopy, and computer modeling, and reveals the formation of β-hairpin mimics involving 10-membered and 18-membered H-bonded rings and a reverse turn of the growing peptide chain.

Introduction

In the field of peptidomimetics much effort has been focused on the design and synthesis of conformationally constrained compounds that mimic, or induce, specific secondary structural features of peptides and proteins.[1] In fact, short linear peptides are inherently flexible molecules, especially in aqueous solution, and so are often poor mimics of the secondary structures (turns, α-helices, β-strands) found on the surfaces of folded proteins. A common motif in protein structure is the reverse-turn, which is defined as a site where the peptide backbone reverses the direction of propagation by adopting a U-shaped conformation.[2] Reverse-turn mimics are generally cyclic or bicyclic dipeptide analogues which, as a result of their constrained structure, force a peptide chain to fold back upon itself.[3] Some of us have recently prepared several azabicycloalkane amino acid scaffolds containing a bicyclic lactam unit, and studied their conformational properties as reverse-turn inducing dipeptide mimics, where the nature and stereochemistry of the bicyclic lactam strongly influence their turn-inducing abilities.[4]

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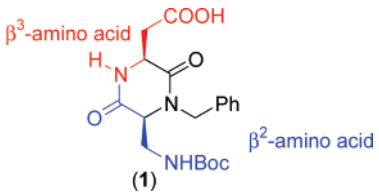

FIGURE 1. Structure of the bifunctional diketopiperazine scaffold **1** (DKP-**1**) highlighting the conformationally constrained $\beta^2-\beta^3$ dipeptide sequence.

Diketopiperazines (DKP), the smallest cyclic peptides, are a common motif found in several natural products with therapeutic properties.[5] In addition, DKP have been used as organic catalysts in the hydrocyanation of imines,[6] and have been shown to be useful scaffolds for the rational design of drugs and peptidomimetics.[7] In these cases, advantage can be taken from the synthesis of symmetrical and unsymmetrical DKP bearing reactive functionalities in the lateral chains of the amino acids. For instance, Wennemers and co-workers have prepared a few symmetrical diketopiperazine two-armed receptors derived from 4-aminoproline where the two amino groups (with a cis disposition) were functionalized with two tripeptide side chains.[8] The resulting two-armed receptors were screened toward a tripeptide library and showed highly selective binding properties which were attributed to the specific turn geometry of the receptor. Alternatively, two different functionalities can be created in the lateral chains of the two amino acids forming the DKP core, such as an amine (e.g., derived from Lys, Orn, or diaminobutyric acid) and a carboxylic acid (e.g., derived from Asp or Glu). In this case, a new peptidomimetic structure is formed, possessing a fixed conformation (due to the cyclic DKP core and the configuration of the two amino acids), and which can now be inserted into oligopeptide sequences.[9] For instance, Royo, Albericio, et al. reported the synthesis of cyclic peptidomimetics containing a bifunctional DKP (cyclo-Lys-Glu) and a RGD sequence and measured their binding affinity to the $\alpha_V\beta_3$ integrin receptor.[10] Robinson and co-workers have synthesized a novel bicyclic template, comprising a diketopiperazine derived from L-aspartic acid and (2*S*,3*R*,4*R*)-diaminoproline, which in the context of a cyclic peptide mimic can stabilize β-hairpin conformations.[11]

In this paper we report the synthesis of a new bifunctional DKP scaffold **1** (DKP-**1**, Figure 1), formally derived from L-aspartic acid and (*S*)-2,3-diaminopropionic acid, bearing a carboxylic acid and an amino functionalities. As a consequence of the absolute configuration of the two α-amino acids forming the cyclic dipeptide unit, the two reactive functionalities (amino and carboxylic acid) are locked in a cis-configuration. When inserted into an oligopeptide sequence, the DKP-scaffold acts as a reverse-turn inducer. In addition, the DKP scaffold **1**, while being derived from α-amino acids [L-aspartic acid and (*S*)-2,3-diaminopropionic acid], can be seen as a conformationally constrained dipeptide formed by two β-amino acids (see Figure 1),[12] and in particular a β^2 and a β^3-amino acids (following Seebach's nomenclature).[13] A few sequences incorporating the DKP-scaffold **1** were synthesized (tetrapeptides AA^1-DKP-AA^2, pentapeptides AA^1-AA^2-DKP-AA^3, and hexapeptides AA^1-AA^2-DKP-AA^3-AA^4), and their conformations were studied by NMR, IR, CD spectroscopy, and molecular modeling showing the formation of a β-hairpin mimic.

Results and Discussion

The synthesis of DKP-**1** was conveniently obtained according to Scheme 1, starting from suitably protected *N*-(*tert*-butoxycarbonyl)-(2*S*)-aspartic acid β-allyl ester[14] and (*S*)-*N*-benzylserine methyl ester,[15] which were coupled to form dipeptide **2**. Dipeptide **2** was then deprotected and its trifluoroacetate salt cyclized, in good yields, to the diketopiperazine **3**, in a basic biphasic system (EtOAc/$NaHCO_{3\,aq}$).[16] These conditions were selected to minimize the epimerization of the serine methyl ester and the formation of the diastereomeric *trans*-DKP (<10%), which could, however, be separated by a chromatographic purification. Other conditions, such as the use of tertiary amines (Et_3N or iPr_2EtN) as base or of other solvents (e.g., dichloromethane), gave increased proportions of the epimeric *trans*-DKP. The stereochemistry of the *cis*-DKP **3** was unequivocally established by X-ray diffraction.

The introduction of the nitrogen functionality was then realized through a Mitsunobu-type reaction, using HN_3·Tol in a toluene/dichloromethane solution, thus obtaining azide **4** in a moderate yield (48%). This procedure had been reported for the successful synthesis of 2,3-diaminopropionic acid starting from serine derivatives.[17] Other methodologies, involving the activation of the hydroxyl group of serine, were hampered by the concurrent elimination reaction leading to the dehydroalanine derivative as the major reaction product. The same Mitsunobu-HN_3 reaction run on dipeptide **2** gave a higher yield of the azide derivative, but, unfortunately, all attempts to cyclize this derivative met with no success. Finally, a one-pot Staudinger−Boc protection[18] yielded the DKP scaffold allyl ester **5**, which was de-allylated[19] to give the amino acid derivative **1** in quantitative yield.

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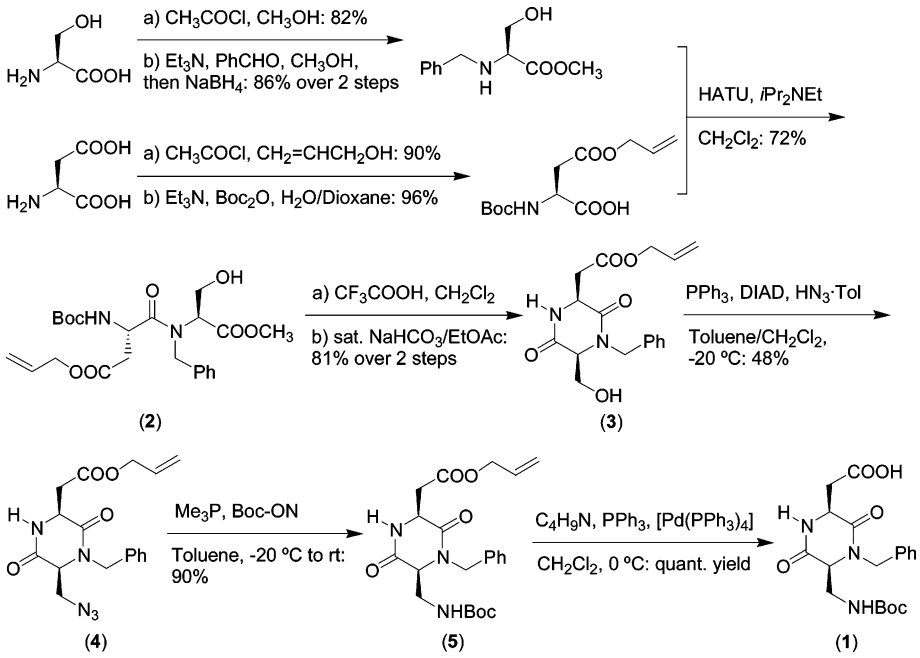
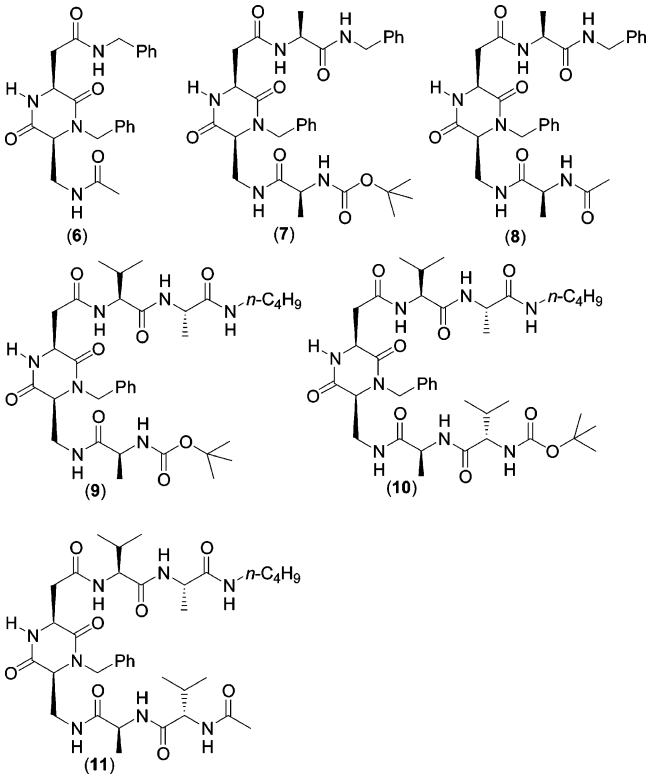
SCHEME 1. Synthesis of the Diketopiperazine Scaffold DKP-1



As we anticipated in the introduction, the diketopiperazine scaffold **1** can be seen as a conformationally constrained dipeptide formed by β^2 and β^3-amino acids (see Figure 1). Extensive investigation on β-peptides indicated that these are able to adopt stable secondary structures such as helices and sheets. The stabilization of β-peptide-hairpins sequences was also studied,[12] and in particular Seebach and co-workers described the formation of turn-like secondary structures in oligo-β-peptides containing the dipeptide sequence formed by a β^2-amino acid (C^2-substituted) followed by a β^3-amino acid (C^3-substituted).[20] Gellman and co-workers reported the formation of a hairpin conformation when a heterochiral dinipecotic acid β-peptide unit was introduced in a tetrapeptide.[21] The intramolecular hydrogen-bonding pattern in these two cases is different: in the first case a 10-membered H-bonded ring is formed involving the C═O of the β^3-amino acid and the NH of the β^2-amino acid, while in the second case a 12-membered H-bonded ring can be identified, which is a two-term homologue of the β-turn structure formed by α-amino acids. β-Hairpins containing both α- and β-amino acids have also been reported to be very stable.[22]

In view of these potential properties, we decided to study the ability of DKP-**1** to form well-defined folded structures, when introduced in peptide sequences. We realized the synthesis of several peptidomimetics (**6**−**11**, Scheme 2) by solution-phase peptide synthesis (Boc strategy) starting from the C-terminus.[23] Good yields were obtained in the coupling of the amino acids

SCHEME 2. Peptidomimetics Containing DKP-1[a]



[a] Synthetic schemes, experimental procedures and characterization of compounds **6**−**11** are reported in the Supporting Information.

to the amino terminus of DKP-**1**, using EDC (*N*-ethyl,*N*′-[3′-(dimethylamino)propyl]carbodiimide)/HOAt (7-aza-1-hydroxy-1,2,3-benzotriazole) or HATU {[(dimethylamino)([1,2,3]triazolo-[4,5*b*]pyridin-3-yloxy)methylene]dimethylammonium-hexafluorophosphate},[24] in a methylene chloride or DMF

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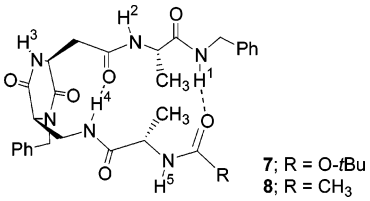
TABLE 1. ^{1}H NMR Data for the Amide Protons in Compounds 7 and 8



	7[a]		**8**[b]			
	δ[c] (ppm)	$\Delta\delta/\Delta T$[d] (ppb/K)	δ[c] (ppm)	$\Delta\delta/\Delta T$[d] (ppb/K)	$\Delta\delta$[e] (added CH_3OH)	NH/ND exchange[f] (min)
NH^1	7.81	−7.6	8.21	−4.6	−0.07	300
NH^2	7.03	−8.2	7.21	−5.3	0.53	160
NH^3	6.28	−9.1	6.61	−15.5	1.25	<10
NH^4	8.10	−4.1	8.18	−2.7	0.03	960
NH^5	5.41	−1.8	6.40	−2.3	≥0.7	*g*

[a] Concentration 0.5 mM in $CDCl_3$. [b] Concentration 2.0 mM in $CDCl_3$. [c] At 298 K. [d] Determined between 238 and 288 K. [e] Measured in $CDCl_3$/CH_3OH 4/1. [f] Measured in $CDCl_3$/CD_3OD 4/1. [g] Not determined due to overlap with other resonances.

solution, and in the presence of a tertiary amine (*N*-methylmorpholine or *N*,*N*-diisopropylethylamine).

The tendency of the DKP-**1**-containing peptidomimetics **6**−**11** to adopt a β-hairpin conformation was then evaluated. Characteristic differences in the NMR spectral parameters for unstructured peptides and peptides in extended and intramolecularly hydrogen bonded conformations have been reported in organic solvents.[4c,25] Chemical shifts and coupling constants for the C_α hydrogens reflect the average conformations of individual amino acid residues, while the chemical shifts of the NH hydrogens and their temperature dependence reveal whether they are solvent exposed or hydrogen bonded intramolecularly.

The dipeptide mimic **6**, in $CDCl_3$, showed some degree of concentration dependence and a strong temperature dependence of the chemical shifts of all the NH's (>20 ppb/K) at a 2 mM concentration, while the chemical shift values were only slightly deshielded (6.25−7.02 ppm) with respect to the average values for non-hydrogen-bonded NH protons (ca. 6.0 ppm). These data are in agreement with an equilibrium between a non-hydrogen-bonded and an intermolecularly H-bonded status (aggregation).

We next turned our attention to the tetrapeptide mimics **7** and **8** (Scheme 2). The NMR studies for these compounds were performed in $CDCl_3$ (see Table 1). Dilution studies indicated that in both cases no aggregates are formed in the concentration range 0.5−10 mM. From the NMR data summarized in Table 1 it appears that the amide protons NH^4 are in an intramolecularly hydrogen-bonded status. In fact, (a) their resonance is shifted substantially downfield (8.10 and 8.18 for **7** and **8**, respectively), (b) the temperature dependence of the NH^4 chemical shift falls within the typical values for intramolecularly hydrogen-bonded protons (−2.7 ppb/K for **8** and slightly higher (−4.1 ppb/K) for **7**), (c) the $\Delta\delta$ (NH^4) upon addition of CH_3OH (obtained measuring the spectrum in a $CDCl_3$/CH_3OH, 4/1 mixture) is small (0.03 in the case of **8**), and (d) the rate of exchange of H^4/D upon addition of CD_3OD is quite slow (ca. 960 min). In the case of proton NH^1, the same parameters (i.e., values of chemical shift, temperature dependence, $\Delta\delta$ upon addition of CH_3OH, and rate of exchange of H^1/D upon addition of CD_3OD) are indicative of an equilibrium between an intramolecularly hydrogen-bonded and a non-hydrogen-bonded status for both **7** and **8**. A similar equilibrium is also partially displayed by proton NH^2, although in this case the NMR parameters reflect a looser intramolecular hydrogen bond.

NOE contacts can be highly indicative of the formation of a β-hairpin mimic when interstrand contacts are visible. Unfortunately, in the case of the tetrapeptide mimics **7** and **8** we could not detect this kind of contacts, and only strong intrastrand contacts were observed, which are indicative of an extended conformation for the amino acid residues. The FT-IR spectrum of the tetrapeptide mimic **7** (2 mM solution in $CHCl_3$) is characterized by two bands at 3427 and 3395 cm^{-1} (free NH groups) and two prominent bands at 3321 and 3291 cm^{-1} (H-bonded NH groups), respectively.[26] In the case of **8**, only two bands can be recognized, one in the free NH region (3410 cm^{-1}) and one at 3291 cm^{-1} indicative of H-bonded NH's. These data support the formation of a β-hairpin mimic involving 10-membered and 18-membered H-bonded rings and a reverse turn of the growing peptide chain. The weak hydrogen-bonded character of NH^2 might indicate that a different β-hairpin mimic involving 12-membered and 16-membered H-bonded rings (vide infra the molecular modeling discussion) is present as a minor conformer at the equilibrium.

Computational studies designed to investigate the ability of the DKP-**1** scaffold to induce β-hairpin conformations were performed on the tetrapeptide mimic **8**. The molecule was subjected to an extensive, unconstrained Monte Carlo/Energy Minimization (MC/EM) conformational search[27] by molecular mechanics methods, using the AMBER* force field[28] and the implicit $CHCl_3$ GB/SA solvent model.[29]

Only two types of conformations, both featuring a β-hairpin-like arrangement, are predominant among the structures found within 3 kcal/mol from the global minimum. The lowest energy conformer features an intramolecular hydrogen-bonding pattern involving the formation of 10-membered and 18-membered H-bonded rings (Figure 2, **8a**). The 10-membered ring of this conformer features a *gauche* orientation of the NH and C═O groups around the C^2−C^3 bond (β-amino acid numbering),[13] with the β^2 and β^3 amino acid torsion angles (θ) of −87° and 81°, respectively.[30] This cross-strand hydrogen-bonding pattern is in agreement with the structure proposed on the basis of the NMR experiments (see Table 1 and the discussion above).

A second β-hairpin-like conformer was found at 1.09 kcal/mol from the global minimum, involving the formation of 12-membered and 16-membered H-bonded rings (Figure 2, **8b**). The 12-membered ring requires *anti* C^2−C^3 torsion angles, with θ values of −171° and −177° for the corresponding β^2 and β^3 amino acids. As also suggested by the NMR data reported in

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(30) This is in agreement with the β-peptide sheetlike conformations proposed by Gellman and co-workers (see ref 12d).

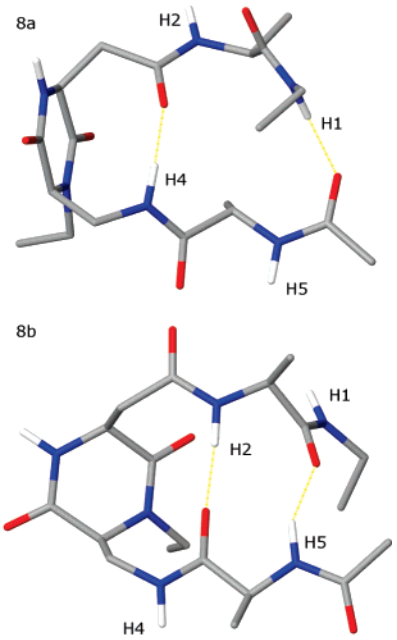
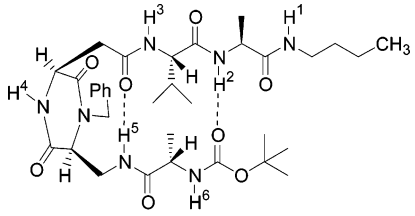


FIGURE 2. Structures of low-energy conformers (MC/EM, AMBER*, $CHCl_3$ GB/SA) calculated for compound **8**: upper row, Global minimum (**8a**); lower row, conformer with relative energy of 1.09 kcal/mol (**8b**). Hydrogen bonds are indicated with dotted lines and for clarity all nonpolar hydrogen atoms and phenyl groups have been omitted.

SCHEME 3. Proposed H-Bonded Structure for Pentapeptide 9



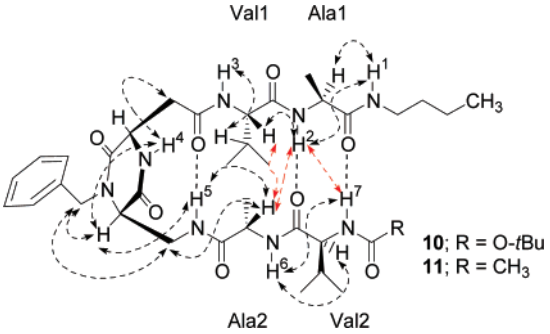
9

Table 1, this second type of β-hairpin structure, or at least its 12-membered H-bonded ring portion, might participate as a minor conformer to the conformational equilibrium.

The solution structure of the pentapeptide mimic **9** was also studied by determining the temperature dependence of the NH chemical shifts at 2 mM in $CDCl_3$ (no aggregation was detected at this concentration). An equilibrium between an intramolecularly hydrogen-bonded and a non-hydrogen-bonded status is suggested by the various parameters for protons NH^2 and NH^5 (δ = 7.71 and 7.77; $\Delta\delta/\Delta T$ = −8.3 and −6.8 ppb/K, respectively; see the Supporting Information for the complete set of parameters), which might be indicative of the presence of a β-hairpin conformation with 10-membered and 18-membered H-bonded rings, in analogy to the tetrapeptide-mimics **7** and **8** (Scheme 3).

The hexapeptides **10** and **11** (Scheme 2) were then prepared; the first dramatic difference with respect to the shorter homologues was the insolubility of these products, in particular **11**. In fact **11** was only soluble in DMSO and in hot methanol, while compound **10** was also sparingly soluble in $CHCl_3$ and soluble in methanol. For this reason the NMR studies of these compounds were performed in DMSO-d_6 and, in the case of **10**, also in 5% CD_3OH−$CDCl_3$. All the proton resonances could be assigned by means of COSY and ROESY spectra. The 2D-

SCHEME 4. Selected Intrastrand (Dashed Black Arrows) and Interstrand (Dashed Red Arrows) NOE Contacts for Hexapeptides 10 and 11



NMR analysis in DMSO-d_6 suggests that both compounds **10** and **11** adopt a β-hairpin-type conformation with a 10-membered H-bonded ring similar to that observed in the previous structures. In fact, the ROESY spectra show a set of NOE cross-peaks that support this conclusion (Scheme 4).

In particular, in the case of **10**, several interstrand NOE contacts were observed: a strong contact between the $C_\alpha H$ of the Val1 and $C_\alpha H$ of the Ala2 residue (see also Figure 3a), a contact between NH^2 and the $C_\alpha H$ of the Ala2 residue (see also Figure 3b), and a weak contact between NH^2 and NH^7 (see also Figure 3c). The same contacts were also observed when the ROESY spectrum of **10** was collected in 5% CD_3OH−$CDCl_3$ (see the Supporting Information). A similar pattern is also shown by the spectrum of **11** in DMSO-d_6, with the exception of the cross-peak between NH^2 and NH^7, which is too weak to be detected in this case.

The analysis of the ^1H NMR spectrum of hexapeptide mimic **10** in 5% CD_3OH−$CDCl_3$ (Table 2) showed that protons NH^2 and NH^5 are notably shifted downfield with respect to the other NH protons. The temperature dependency is indicative of a hydrogen-bonded status for both NH^2 and NH^5, and of an equilibrium between an intramolecularly hydrogen-bonded and a non-hydrogen-bonded status for NH^7. The ^3J values for the NH−$C_\alpha H$ in DMSO-d_6 for both compounds **10** and **11** (Table 2) are in agreement with the typical values for peptides showing a β-hairpin secondary structure (7.5−8.5 Hz).[25a] Similar values were also found for the spectrum of **10** in 5% CD_3OH−$CDCl_3$.

The ability of peptidomimetics **7**, **8**, **10**, and **11** to adopt an ordered secondary structure in solution was also evaluated by CD spectroscopy (Figure 4). The spectra were measured in methanol (0.5 mM) and showed a similar behavior: two negative minima, one at 200−205 nm (201 nm for compound **11**) and a second one at about 220 nm (220 nm for compound **11**), and a negative maximum at 209−215 nm (209 nm for **11**), were displayed by all these compounds. Unfortunately, while several CD studies have been reported for β-peptides adopting helical conformations (and in particular 12- and 14-helices),[12d,31] no conclusive data on β-peptides assuming hairpin-type conformations have appeared in the literature. A hexapeptide consisting of β^3-homo-amino, β^2-homo-amino, and α-amino acids with a central β^2−β^3 segment was recently reported by Seebach and co-workers to adopt a turn-like conformation with a 10-membered H-bonded ring induced by the β^2−β^3 unit.[22] Its CD spectrum (0.2 μM in CH_3OH) displayed a similar

(31) (a) Hart, S. A.; Bahadoor, A. B. F.; Matthews, E. E.; Qiu, X. J.; Schepartz, A. *J. Am. Chem. Soc.* **2003**, *125*, 4022−4023. (b) Ahmed, S.; Beleid, R.; Sprules, T.; Kaur, K. *Org. Lett.* **2007**, *9*, 25−28.

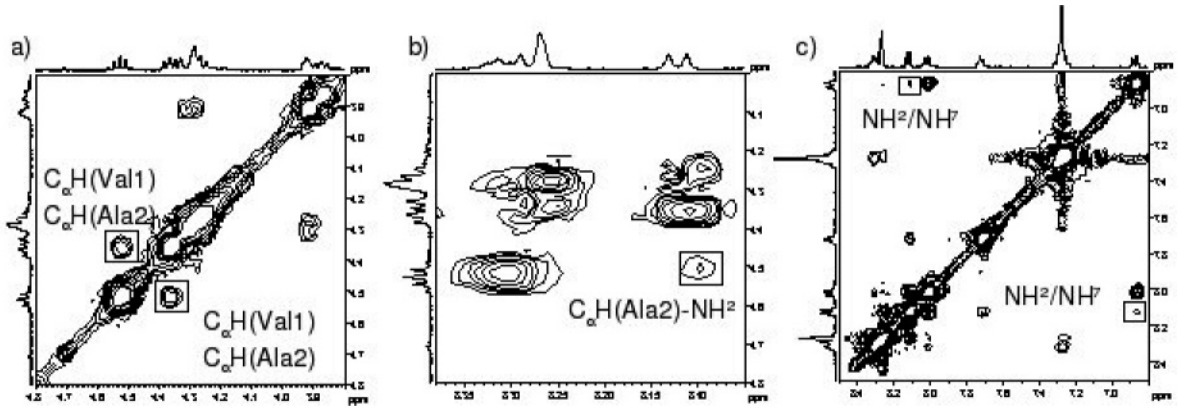
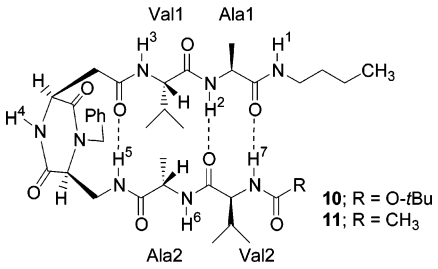


FIGURE 3. Sections of the ROESY spectrum of **10** (2 mM in DMSO-d_6) showing (a) interstrand NOE's in the $C_\alpha H$ region, (b) interstrand NOE's for $C_\alpha H$ and NH, and (c) interstrand NOE's for the NH.

TABLE 2. ^{1}H NMR Data for the Amide Protons in Compounds 10 and 11



	10				**11**	
	$\delta^{a,b}$ (ppm)	$\Delta\delta/\Delta T^{a,c}$ (ppb/K)	$^3J_{NHC\alpha H}{}^{a,d}$	$^3J_{NHC\alpha H}{}^{e,f}$	δ (ppm)^{e,f}	$^3J_{NHC\alpha H}{}^{e,f}$
NH^1	6.99	−8.0	5.7	5.6	7.83	5.6
NH^2	8.24	−2.5	7.7	7.4	8.19	7.9
NH^3	7.61	−15.5	8.0	8.1	8.29	7.2
NH^4	7.68	−16.5	*g*	*g*	8.28	*g*
NH^5	7.97	−1.3	*g*	*g*	8.25	*g*
NH^6	7.42	−8.5	8.0	7.8	8.18	7.8
NH^7	5.76	−6.0	6.8	9.1	7.96	8.9

[a] Concentration 2.0 mM in 5% $CD_3OH-CDCl_3$. [b] At 288 K. [c] Determined between 248 and 288 K. [d] At 278 K. [e] Concentration 2.0 mM in DMSO-d_6. [f] At 298 K. [g] Broad signal.

behavior with respect to our derivatives, with a minimum at 197 nm, a shoulder at 205 nm, a negative maximum at about 215 nm, and a less pronounced minimum at ca. 220 nm. In addition, both minima and the maximum showed negative molar ellipticities of comparable intensity to those of our compounds.

Molecular mechanics calculations were performed on the hexapeptide mimic **11,** similarly to the tetrapeptide mimic **8**, to investigate the ability of the DKP-**1** scaffold to induce β-hairpin conformations. The molecule was subjected to an unconstrained Monte Carlo/Energy Minimization (MC/EM) conformational search[27] in vacuo (the implicit DMSO solvation model is not available in the software employed) with a distance dependent dielectric constant of 4*r* to generate a suitable starting conformation for the following restrained simulation in explicit DMSO solvent (see below). Two different, almost energetically equivalent, β-hairpin conformations were found within 3 kcal/mol from the global minimum (Figure 5). The lowest energy conformer is characterized by the presence of hydrogen bonds involving the amide protons NH^3, NH^6, and NH^1 and forming respectively

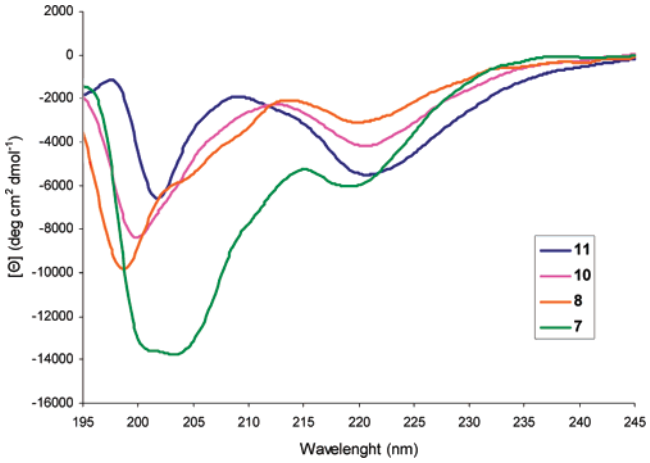


FIGURE 4. CD spectra of peptidomimetics **7**, **8**, **10**, and **11** (0.5 mM in methanol). The data are normalized for peptide concentration and for the number of residues.

TABLE 3. Relevant Proton Distances of the Low-Energy Conformers (MC/EM, AMBER*, in vacuo) Calculated for Compound 11

conformer	ΔE (kcal/mol)	proton distance (Å)	
		$C_\alpha H(Val1)-C_\alpha H(Ala2)$	$C_\alpha H(Ala2)-NH^2$
11a	0.0	7.56	7.92
11b	0.26	2.49	3.95

12-membered, 16-membered, and 24-membered rings (Figure5, **11a**). However, no experimental evidence is provided by the NMR data in solution (Scheme 4, Table 2) for such an intramolecular hydrogen-bonding pattern (resembling conformer **8b** of the tetrapeptide mimic **8**, see the discussion above).

The second conformer (Figure 5, **11b**) shows a β-hairpin conformation in agreement with the structure proposed on the basis of the spectroscopic data (Scheme 4, Table 2). This conformer features 10-membered and 18-membered H-bonded rings that resemble the hydrogen-bonding pattern observed in conformer **8a** of the tetrapeptide mimic **8**, and an additional 22-membered H-bonded ring involving the NH^7 amide proton and the Ala1 carbonyl group.

Furthermore, comparing the calculated interstrand distances in conformers **11a** and **11b** between protons $C_\alpha H$ of the Val1 and $C_\alpha H$ of the Ala2 residues and between the proton $C_\alpha H$ of

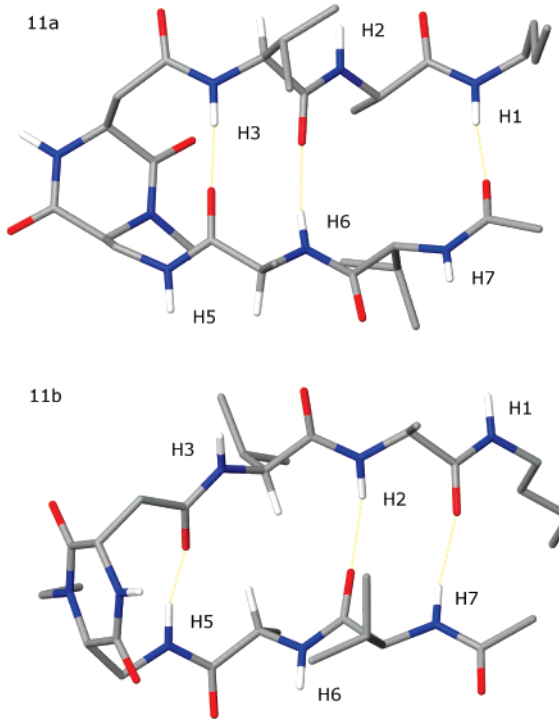


FIGURE 5. Structures of the lowest energy conformers (MC/EM, AMBER*, in vacuo) calculated for compound **11**: upper row, Global minimum (**11a**); lower row, conformer with relative energy of 0.26 kcal/mol (**11b**). Hydrogen bonds are indicated with dotted lines and for clarity all nonpolar hydrogen atoms, except $C_\alpha H$ of Val1 and Ala2 residues, and the phenyl residues, have been omitted.

the Ala2 residue and the NH^2 amide proton (Table 3), only the **11b** conformer shows distance values consistent with the NOE contacts observed in DMSO solution (Scheme 4).

Finally, a simulated annealing protocol in explicit DMSO solvent[32] with the NMR restraints derived from the NOE contacts (see the Supporting Information for computational details) was performed starting from conformer **11b**. The simulation converged to a unique β-hairpin structure (Figure 6). Consistent with the NMR analysis, this β-hairpin arrangement features 10-membered and 18-membered H-bonded rings involving the NH^5 and NH^2 amide protons, respectively, while the NH^7 amide proton does not form any intramolecular hydrogen bonds.

Conclusions

In this paper, we reported the synthesis of a new bifunctional diketopiperazine (DKP) scaffold **1**, derived from L-aspartic acid and (*S*)-2,3-diaminopropionic acid. DKP-**1** bears amino and carboxylic acid functionalities in a cis relationship. As a consequence, DKP scaffold **1** can be seen as a conformationally constrained mimic of a dipeptide formed by two β-amino acids (namely β^2 and β^3 amino acids). When inserted into a peptidic sequence, involving α-amino acids, DKP-**1** is accommodated into the turn position of a β-hairpin. IR, NMR, and CD experiments provide strong support to this conclusion, strengthened by molecular modeling and molecular dynamics calculations.

(32) Fox, T.; Kollman, P. A. *J. Phys. Chem. B* **1998**, *102*, 8070−8079.

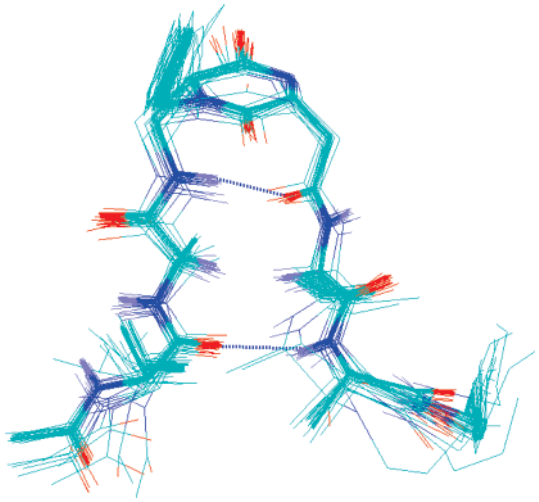


FIGURE 6. Simulated annealing superimposed solutions. Hydrogen bonds are indicated with dotted lines and all nonpolar hydrogen atoms, except $C_\alpha H$ of Val1 and Ala2 residues, have been omitted for clarity.

Experimental Section

(*S*)-*N*-Benzyl-3-*tert*-butoxycarbonylamino-*N*-[(*S*)-2-hydroxy-1-methoxycarbonylethyl]succinamic Acid Allyl Ester (2). To a solution of β-allyl (2*S*)-*N*-(*tert*-butoxycarbonyl)aspartate ester (329 mg, 1.2 mmol) in CH_2Cl_2 (8 mL), under a nitrogen atmosphere and at 0 °C, was added HATU (510 mg, 1.3 mmol, 1.1 equiv) and DIPEA (417 μL, 2.4 mmol, 2 equiv). After 30 min, a solution of (*S*)-*N*-benzylserine methyl ester (251 mg, 1.2 mmol, 1 equiv) in CH_2Cl_2 (1.6 mL) was added and the reaction was stirred at 0 °C for 1 h and at rt for 24 h. The mixture was then diluted with EtOAc (100 mL) and the organic phase was washed in order with 1 M $KHSO_4$ (2 × 20 mL), aqueous $NaHCO_3$ (2 × 20 mL), and brine (2 × 20 mL) and dried over Na_2SO_4, then volatiles were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 75:25) to afford the desired product as a yellow oil (401 mg, 72%). $[\alpha]^{21}_D$ −2.65 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.24−7.34 (m, 5H), 5.83−5.93 (m, 1H), 5.48 (d, 1H, J = 8.2 Hz), 5.30 (d, 1H, J = 17.2 Hz), 5.23 (d, 1H, J = 10.4 Hz), 4.51−4.61 (m, 3H), 4.32−4.48 (m, 2H), 3.88 (d, 1H, J = 13.1 Hz), 3.75 (s, 3H), 3.73 (d, 1H, J = 13.1 Hz), 3.55 (t, 1H, J = 4.7 Hz), 2.99 (dd, 1H, J_1 = 17.0 Hz, J_2 = 4.3 Hz), 2.85 (dd, 1H, J_1 = 17.0 Hz, J_2 = 4.7 Hz), 2.21 (br s, 1H), 1.45 (s, 9H). Two sets of signals were observed in the ^{13}C spectrum due to the presence of two rotational isomers A:B (20:1 ratio): ^{13}C NMR ($CDCl_3$) δ 172.8 (A), 172.1 (B), 171.0 (A), 170.3 (B), 155.7 (A), 155.0 (B), 139.6 (A), 138.9 (B), 132.1 (A), 131.4 (B), 128.9 (A), 128.7 (A), 128.1 (B), 127.9 (B), 127.6 (A), 126.9 (B), 119.1 (A), 118.4 (B), 80.6 (A), 79.9 (B), 66.2 (A), 66.1 (A), 65.5 (B), 65.4 (B), 59.5 (A), 58.8 (B), 52.7 (A), 52.2 (A), 51.9 (B), 51.4 (B), 50.3 (A), 49.6 (B), 37.1 (A), 36.4 (B), 28.7 (A), 27.9 (B); IR ($CHCl_3$) ν_{max} 3438, 3338, 3026, 2983, 2953, 2857, 1739, 1500, 1453, 1378, 1341, 1279, 1247, 1176; HRMS (ESI) *m/z* calcd for $[C_{23}H_{33}N_2O_8]^+$ 465.22314 $[M + H]^+$, found 465.22326. Anal. Calcd for $C_{23}H_{32}N_2O_8$: C 59.47, H 6.94, N 6.03. Found: C 59.07, H 7.01, N 5.91.

[(2*S*,5*S*)-4-Benzyl-5-hydroxymethyl-3,6-dioxopiperazin-2-yl]-acetic Acid Allyl Ester (3). Dipeptide **2** (1.95 g, 4.2 mmol) was dissolved in TFA (32 mL) and stirred for 3 h at rt. The solvent was evaporated, methanol (3 × 50 mL) was added followed by evaporation, and then Et_2O (35 mL) was added and evaporated to give the TFA salt of the dipeptide **2** as a white solid. This salt was dissolved in a mixture of saturated aqueous $NaHCO_3$/EtOAc (0.1 M, 1:1 v/v) and stirred at room temperature for 24−48 h. Subsequently, the layers were separated and the aqueous layer was

extracted with EtOAc (4×). The combined organic layers were washed with brine and dried over Na_2SO_4, then volatiles were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH, 97:3) to afford the desired product as a white solid (1.13 g, 81%). Mp 119−120 °C; $[\alpha]^{25}_D$ −72.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.25−7.37 (m, 5H), 7.05 (br s, 1H), 5.84−5.94 (m, 1H), 5.25−5.34 (m, 3H), 4.55−4.66 (m, 2H), 4.49−4.51 (m, 1H), 4.07 (d, 1H, J = 15.0 Hz), 3.98 (d, 1H, J = 11.1 Hz), 3.85−3.89 (m, 2H), 3.21 (dd, 1H, J_1 = 17.5 Hz, J_2 = 2.8 Hz), 3.16 (br s, 1H), 3.13 (dd, 1H, J_1 = 17.5 Hz, J_2 = 10.4 Hz); ^{13}C NMR ($CDCl_3$) δ 171.9, 167.0, 166.3, 135.6, 131.9, 129.5, 128.6, 119.5, 66.4, 61.3, 60.5, 52.8, 47.6, 40.7; IR ($CHCl_3$) ν_{max} 3388, 3275, 3031, 3017, 2945, 1728, 1680, 1452, 1379, 1336, 1276, 1183, 1124; MS (FAB$^+$) *m*/*z* 333 ([M + 1]$^+$, 80%), 275 (11%), 154 (57%), 136 (48%), 91 (100%). Anal. Calcd for $C_{17}H_{20}N_2O_5$: C 61.44, H 6.07, N 8.43. Found: C 61.23, H 5.97, N 8.24.

X-ray crystallographic data of 3: $C_{17}H_{20}N_2O_5$; MW = 332.35 g mol^{-1}; T = 293 K; λ(Mo Kα) = 0.71073 Å, monoclinic, space group $P2_1$, a = 7.394(4) Å, b = 10.764(19) Å, c = 10.800(5) Å, β = 99.71(4)°, V = 847(2) Å,^{3} ρ_{calc} = 1.303 g cm^{-3}, Z = 2; μ(Mo Kα) = 1.0 cm^{-1}. *R* and *wR*2 0.086 and 0.155, respectively, for 1230 unique data collected in the 3−25.3° 2θ range.

[(2*S*,5*S*)-5-Azidomethyl-4-benzyl-3,6-dioxopiperazin-2-yl]acetic Acid Allyl Ester (4). To a solution of **3** (565 mg, 1.7 mmol) in CH_2Cl_2/toluene (6.6 mL/12.2 mL), under nitrogen atmosphere and at −20 °C, was added PPh_3 (530 mg, 2.0 mmol, 1.2 equiv) and the mixture was stirred until a solution was obtained. Hydrazoic acid (0.45 M in toluene,[33] 7.6 mL, 3.4 mmol, 2 equiv) was added followed by a dropwise addition of DIAD (0.41 mL, 2.0 mmol, 1.2 equiv) and the reaction was stirred at −20 °C for 3.5 h. After evaporation of the solvent under reduced pressure, a quick chromatographic purification (petroleum ether/EtOAc, 6:4) was performed to remove the hydrazo-derivative and the resulting crude residue was then purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH, 99:1) to afford the desired product as a colorless oil (291 mg, 48%). $[\alpha]^{23}_D$ −72.7 (*c* 1.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.26−7.39 (m, 5H), 6.91 (br s, 1H), 5.89−5.99 (m, 1H), 5.36 (d, 1H, J = 17.2 Hz), 5.30 (d, 1H, J = 10.4 Hz), 5.18 (d, 1H, J = 15.0 Hz), 4.62−4.71 (m, 2H), 4.51−4.54 (m, 1H), 4.20 (d, 1H, J = 15.0 Hz), 3.95 (br s, 1H), 3.89 (dd, 1H, J_1 = 12.7 Hz, J_2 = 1.7 Hz), 3.68 (dd, 1H, J_1 = 12.7 Hz, J_2 = 3.4 Hz), 3.31 (dd, 1H, J_1 = 17.7 Hz, J_2 = 2.2 Hz), 3.08 (dd, 1H, J_1 = 17.7 Hz, J_2 = 11.2 Hz); ^{13}C NMR ($CDCl_3$) δ 171.6, 165.7, 165.1, 135.3, 131.8, 129.6, 128.8, 128.6, 119.6, 66.5, 58.8, 52.6, 51.1, 48.0, 40.7; IR (thin film) ν_{max} 2984, 2929, 2853, 2119, 1734, 1686, 1667, 1451, 1336, 1274, 1181; MS (FAB$^+$) *m*/*z* 358 ([M + 1]$^+$, 12%), 330 (2%), 149 (16%), 109 (27%), 91 (100%). Anal. Calcd for $C_{17}H_{19}N_5O_4$: C 57.14, H 5.36, N 19.60. Found: C 57.39, H 5.28, N 19.25.

[(2*S*,5*S*)-4-Benzyl-5-(*tert*-butoxycarbonylaminomethyl)-3,6-dioxopiperazin-2-yl]acetic Acid Allyl Ester (5). To a solution of azide **4** (268 mg, 0.75 mmol) in THF (2.5 mL), under nitrogen atmosphere and at −20 °C, was added Me_3P (830 μL of a 1 M solution in THF, 0.83 mmol, 1.1 equiv) and 2-(*t*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON, 206 mg, 0.83 mmol, 1.1 equiv). After the mixture was stirred for 5 h at rt, CH_2Cl_2 (60 mL) was added and the solution was washed with H_2O (3 × 30 mL) and brine. The organic phase was dried over Na_2SO_4 and volatiles were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH, 99:1) to afford the desired product as a white solid (253 mg, 78%). $[\alpha]^{28}_D$ −123.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.28−7.36 (m, 5H), 7.06 (br s, 1H), 5.86−5.96 (m, 1H), 5.56 (d, 1H, J = 15.1 Hz), 5.25−5.36 (m, 3H), 4.60−4.69 (m, 2H), 4.48−4.51 (m, 1H), 4.09 (d, 1H, J = 15.1 Hz), 3.80−3.86 (m, 2H), 3.45−3.49 (m, 1H), 3.27 (dd, 1H, J_1 = 17.6 Hz, J_2 = 1.7 Hz), 2.85 (dd, 1H, J_1 = 17.6 Hz, J_2 = 11.1 Hz), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.5, 166.7, 164.9, 156.2, 135.6, 131.8, 129.4, 128.9, 128.5, 119.3, 80.8, 66.4, 59.2, 52.4, 47.2, 40.8, 40.6, 28.7; IR (Nujol) ν_{max} 3323, 3308, 1716, 1684, 1658, 1339, 1272, 1167, 1127; MS (FAB$^+$) *m*/*z* 432 ([M + 1]$^+$, 12%), 376 (49%), 332 (41%), 302 (16%), 91 (100%). Anal. Calcd for $C_{22}H_{29}N_3O_6$: C 61.24, H 6.77, N 9.74. Found: C 61.47, H 6.93, N 9.56.

[(2*S*,5*S*)-4-Benzyl-5-(*tert*-butoxycarbonylaminomethyl)-3,6-dioxopiperazin-2-yl]acetic Acid, DKP-1 (1). To a solution of **5** (242 mg, 0.56 mmol) in CH_2Cl_2 (3.0 mL), under nitrogen atmosphere and at 0 °C, was added pyrrolidine (56 μL, 0.67 mmol, 1.2 equiv), PPh_3 (26 mg, 0.10 mmol, 0.18 equiv), and then [Pd(PPh_3)$_4$] (24 mg, 0.02 mmol, 0.04 equiv). After the mixture was stirred for 1 h at 0 °C, EtOAc (25 mL) was added and the solution was extracted with aqueous $NaHCO_3$ (4 × 10 mL). The combined aqueous phases were acidified to pH 2 with a 1 M $KHSO_4$ solution and then extracted with CH_2Cl_2. The resulting organic phase was dried over Na_2SO_4 and the solvent was evaporated to afford the desired product as a fluffy white solid (209 mg, 95%). $[\alpha]^{26}_D$ −69.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 50 °C) δ 10.02 (br s, 1H), 8.05 (br s, 1H), 7.25−7.37 (m, 5H), 5.59 (d, 1H, J = 14.2 Hz), 5.36 (br s, 1H), 4.52 (d, 1H, J = 11.4 Hz), 4.03 (br s, 1H), 3.88 (s, 1H), 3.79−3.85 (m, 1H), 3.49−3.54 (m, 1H), 3.28 (dd, 1H, J_1 = 17.7 Hz, J_2 = 2.3 Hz), 2.74 (dd, 1H, J_1 = 17.7 Hz, J_2 = 11.4 Hz), 1.50 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 °C) δ 175.1, 168.1, 164.9, 157.0, 135.4, 129.4, 128.8, 128.6, 81.4, 59.5, 52.4, 47.3, 40.9, 40.6, 28.7; IR (Nujol) ν_{max} 3382, 3325, 3227, 1715, 1659, 1647, 1272, 1162, 1125; HRMS (ESI) *m*/*z* calcd for [$C_{19}H_{25}N_3NaO_6$]$^+$ 414.16356 [M + Na]$^+$, found 414.16367.

Solution-Phase Synthesis of Peptidomimetics. Representative Procedure for the Coupling with HOBT/EDC: Boc-(*S*,*S*)-DKP-1-(*S*)-Ala-NH-CH_2-Ph. To a solution of Boc-(*S*)-Ala-NH-CH_2-Ph (67 mg, 0.24 mmol) in CH_2Cl_2 (1.85 mL; 0.13 M) was added an equal volume of TFA and the reaction was stirred at rt for 3 h. The solvent was evaporated, methanol (3 × 2 mL) was added followed by evaporation, and then ether was added and evaporated to afford the corresponding TFA salt. This was dissolved in DMF (2.4 mL, 0.1 M), and **1** (98 mg, 0.25 mmol, 1.05 equiv) was added followed by HOBt (36 mg, 0.26 mmol, 1.1 equiv) and DIPEA (84 μL, 0.48 mmol, 2 equiv). The solution was cooled in an ice bath and treated with EDC (40 mg, 0.26 mmol, 1.1 equiv). The reaction was stirred at 0 °C for 1 h and at rt overnight. The mixture was diluted with EtOAc (15 mL) and consecutively extracted with 1 M $KHSO_4$ (2 × 3 mL), aqueous $NaHCO_3$ (2 × 3 mL), and brine (2 × 3 mL) and dried over Na_2SO_4, then the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/CH_3OH, 95/5) to afford the product (122 mg, 92%) as a white solid. Mp 112−113 °C; $[\alpha]^{28}_D$ −98.4 (*c* 0.50, $CDCl_3$); ^{1}H NMR ($CDCl_3$, 40 °C) δ 7.48 (br s, 1H), 7.18−7.34 (m, 12H), 5.83 (br s, 1H), 5.41 (d, 1H, J = 15.0 Hz), 4.51−4.59 (m, 1H), 4.35−4.44 (m, 3H), 3.99 (d, 1H, J = 15.0 Hz), 3.78 (br s, 1H), 3.61−3.72 (m, 1H), 3.49−3.59 (m, 1H), 3.05 (dd, 1H, J_1 = 15.1 Hz, J_2 = 3.9 Hz), 2.74 (dd, 1H, J_1 = 15.1 Hz, J_2 = 8.8 Hz), 1.42 (s, 9H), 1.36 (d, 3H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 40 °C) δ 172.5, 170.4, 166.4, 166.1, 156.4, 138.6, 135.6, 129.3, 128.9, 128.8, 128.5, 128.0, 127.7, 80.6, 59.2, 53.2, 49.6, 47.5, 43.9, 41.7, 41.4, 28.8, 18.5; IR (Nujol) ν_{max} 3354, 3320, 3240, 1717, 1658, 1639, 1552, 1532, 1249, 1173, 1076; MS (FAB$^+$) *m*/*z* 552 ([M + 1]$^+$, 4%), 452 (18%), 369 (6%), 147 (31%), 109 (54%), 91 (100%). Anal. Calcd for $C_{29}H_{37}N_5O_6$: C 63.14, H 6.76, N 12.70. Found: C 62.84, H 6.75, N 12.53.

Solution-Phase Synthesis of Peptidomimetics. Representative Procedure for the Coupling with HATU: Boc-(*S*)-Ala-(*S*,*S*)-DKP-1-(*S*)-Ala-NH-CH_2-Ph (7). To a solution of Boc-(*S*,*S*)-DKP-1-(*S*)-Ala-NH-CH_2-Ph (61 mg, 0.11 mmol) in CH_2Cl_2 (0.85 mL) was added an equal volume of TFA and the reaction was stirred at rt for 3 h. The solvent was evaporated, methanol (3 × 2 mL) was added followed by evaporation, and then ether (3 mL) was added and evaporated to afford the corresponding TFA salt. To a solution

(33) Equi, A. M.; Brown, A. M.; Cooper, A.; Ner, S. K.; Watson, A. B.; Robins, D. J. *Tetrahedron* **1991**, *47*, 507−518.

of Boc-(*S*)-Ala-OH (21 mg, 0.11 mmol, 1 equiv), in CH_2Cl_2 (0.55 mL), under nitrogen atmosphere and at 0 °C, was added HATU (46 mg, 0.12 mmol, 1.1 equiv) and DIPEA (38 μL, 0.22 mmol, 2 equiv). After 30 min, a solution of the TFA salt of the peptide in CH_2Cl_2 (0.55 mL) and DIPEA (19 μL, 0.11 mmol, 1 equiv) was added and the reaction mixture was stirred at 0 °C for 1 h and at rt overnight. The mixture was diluted with EtOAc (10 mL) and consecutively extracted with 1 M $KHSO_4$ (2 × 3 mL), aqueous $NaHCO_3$ (2 × 3 mL), and brine (2 × 3 mL) and dried over Na_2SO_4, then the solvent evaporated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/CH_3OH, 95/5) to afford **7** (54 mg, 82%) as a white solid. Mp 127−128 °C; $[\alpha]^{25}_D$ −42.5 (*c* 0.41, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 8.20 (br s, 1H), 8.06 (br s, 1H), 7.75 (d, 1H, J = 6.9 Hz), 7.55 (br s, 1H), 7.26−7.35 (m, 7H), 7.15−7.21 (m, 3H), 5.52 (d, 1H, J = 7.5 Hz), 5.39 (d, 1H, J = 15.0 Hz), 4.67 (t, 1H, J = 6.0 Hz), 4.47 (t, 1H, J = 6.7 Hz), 4.41 (br s, 2H), 4.08 (br s, 1H), 3.99 (br s, 1H), 3.96 (d, 1H, J = 15.0 Hz), 3.75−3.82 (m, 2H), 3.16 (d, 1H, J = 15.2 Hz), 2.80 (d, 1H, J = 15.2 Hz), 1.42 (d, 3H, J = 6.0 Hz), 1.29 (br s, 12H); ^{13}C NMR ($CDCl_3$) δ 173.9, 173.7, 170.3, 166.1, 165.7, 155.9, 138.4, 135.5, 129.4, 129.0, 128.7, 128.5, 127.6, 127.2, 80.1, 57.1, 52.4, 49.8, 46.9, 43.6, 39.5, 38.3, 28.7, 20.8, 19.4; IR ($CHCl_3$) ν_{max} 3429, 3395, 3330, 3295, 2930, 1689, 1657, 1556, 1506, 1449, 1368, 1332, 1255, 1166; HRMS (ESI) *m*/*z* calcd for $[C_{32}H_{42}N_6NaO_7]^+$ 645.30072 $[M + Na]^+$, found 645.29916. Anal. Calcd for $C_{32}H_{42}N_6O_7$: C 61.72, H 6.80, N 13.50. Found: C 61.42, H 6.78, N 13.35.

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Supporting Information Available: Synthetic schemes, experimental procedures, computational methods, and characterization of compounds **6**−**11**, ^{1}H and ^{13}C NMR spectra of all reported compounds, and conformational studies of compounds **7**−**11**, X-ray crystallographic data for compound **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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