Influence of Chirality of the Preceding Acyl Moiety on the *cis*/*trans* Ratio of the Proline Peptide Bond

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We report that the *cis*/*trans* ratio of the proline peptide bond can be strongly influenced by the chirality of the acyl residue preceding proline. Acyl moieties derived from (2*S*)-2,6-dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylic acid (**8**) and (2*R*)-3-methoxy-2-methyl-2-(4-methyl-2-nitrophenoxy)-3-oxopropanoic acid (**5**) in acyl-Pro molecules influence isomerization of the proline peptide bond constraining the ω dihedral angle to the *trans* orientation. Structures of benzyl (2*S*)-1-{[(2*S*)-2,6-dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]carbonyl}-2-pyrrolidinecarboxylate (**3**) derived from 2D ^{1}H NMR conformational analysis and crystallographic data exhibit only the *trans* conformation of proline peptide bond. On the other hand the diastereomer **4**, which contains an (*R*) acyl moiety, exhibits two sets of signals in ^{1}H NMR spectra. The signals were assigned to *trans* (72%) and *cis* (28%) conformers. Crystallographic analysis of **4** showed that only the *cis* conformation is present in the crystalline state. The ^{1}H NMR chemical shift pattern of three sets of signals observed in **2** was observed also in benzyl (2*S*)-1-[(2*R*/*S*)-3-methoxy-2-methyl-2-(4-methyl-2-nitrophenoxy)-3-oxopropanoyl]-2-pyrrolidinecarboxylate. (*R*)-Carboxylic acid **5**, after coupling with (*S*)-ProOBn, yielded benzyl (2*S*)-1-[(2*R*)-3-methoxy-2-methyl-2-(4-methyl-2-nitrophenoxy)-3-oxopropanoyl]-2-pyrrolidinecarboxylate (**6**), which in DMSO-d_6 exhibited only the *trans* conformation of the proline peptide bond. These results suggest that in these particular cases acyl-Pro peptide bond isomerization is strongly influenced by the stereochemistry of the acyl residue preceding proline. (2*S*)-2,6-Dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylic acid (**8**) and (2*R*)-3-methoxy-2-methyl-2-(4-methyl-2-nitrophenoxy)-3-oxopropanoic acid (**5**) are promising chiral peptidomimetic building blocks that can be used as acyl moieties to force the proline peptide bond into the *trans* conformation in a variety of acyl-Pro molecules.

Introduction

Naturally occurring amino acids form predominantly *trans* peptide bonds, with the exception of proline whose ability to form *cis* peptide bonds[1] and undergo *cis*/*trans* isomerization is well-known.[2] The isomerization barrier of the acyl-Pro bond in proline-containing molecules is lowered from ca. 20 to ca. 13 kcal/mol as a result of concomitant pyrrolidine puckering changes;[3] additionally, the *trans* bond, involving a nitrogen atom of the pyrrolidine ring, is favored only slightly (ca. 2 kcal/mol) over the *cis* bond.[4] Because of these unique properties proline plays an important role in biological processes[5] and in naturally occurring biologically active and pharmaceutically important peptides and peptide mimetics.[6]

Determination of bioactive conformations of biologically active compounds, followed by optimization of structural and spatial arrangement of groups important for biological activity with the aim to enhance the affinity and the selectivity of a molecule toward the sites of action, is a crucial step in the development of potent therapeutics.[7] Conformational freedom and thus the number of conformations accessible to the molecule can be reduced by introducing constraints that may directly influence the pharmacological effect of the molecule.[8] Proline incorpo-

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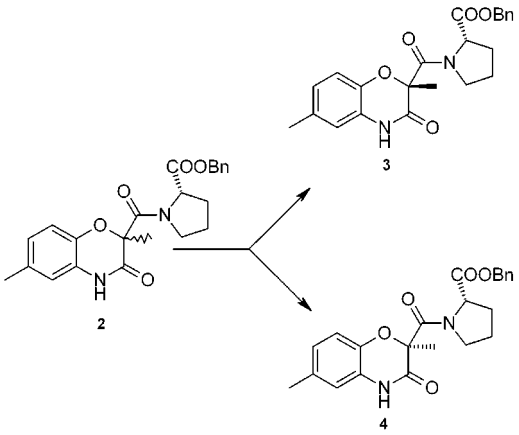
rated into a biologically active compound restricts the local conformational freedom,[9] which leads to changes in activity due to altered molecular flexibility and the number of possible conformers. An example demonstrating the importance of the *cis*/*trans* isomerization of the proline peptide bond is provided by the tripeptidomimetic thrombin inhibitors[10] derived from the peptide sequence D-Phe-Pro-Arg, in which one or more amino acids are replaced by a mimetic or another amino acid. The majority of potent thrombin inhibitors containing proline possess the *trans* conformation of the proline amide bond in order to form a hydrogen bond to Gly 216, which is one of residues defining the S_1 specificity pocket of the thrombin active site.[10,11]

In small linear peptides the *cis*/*trans* ratio of the acyl-Pro bond favors the *trans* conformation[12] but depends considerably on the amino acid adjacent to proline.[12,13] The probability of the *cis* conformation is higher when an aromatic residue such as phenylalanine precedes proline, probably as a result of interactions between the aromatic and pyrrolidine rings.[1b,12a]

Numerous surrogates, mimetics, and analogues of proline have been developed and used in the synthesis of biologically active compounds, with the aim of altering the *cis*/*trans* ratio of acyl-Pro bonds,[14] constraining the conformation of the peptide bond[10a,15] and producing proline-like turns.[16] Because of their ability to enforce the *trans* conformation of the amide bond, some have been used as peptidomimetic scaffolds and introduced into thrombin inhibitors. (*R*)-Thiazolidine-4-carboxylic acid[6b,17] and (3*R*)-6-amino-5-oxohexahydro-5*H*-[1,3]thiazolo[3,2-*a*]pyridine-3-carboxylic acid[11a] were incorporated in a series of thrombin inhibitors that showed high affinity and selectivity.

Scheme 1



Here we report the synthesis, X-ray structure analysis, and NMR conformational study of benzyl (2*S*)-1-{[(2*S*)-2,6-dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]-carbonyl}-2-pyrrolidinecarboxylate (**3**) and the corresponding (*S*,*R*)-diastereomer **4**, providing evidence that the (2*S*)-2,6-dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-2-carbonyl moiety constrains the acyl-Pro bond of **3** to *trans*. Additionally, we report an NMR conformational analysis of benzyl (2*S*)-1-[(2*R*)-3-methoxy-2-methyl-2-(4-methyl-2-nitrophenoxy)-3-oxopropanoyl]-2-pyrrolidinecarboxylate (**6**), an intermediate in the stereoselective synthesis of **3**, which also possesses the *trans* conformation of the proline peptide bond. These results demonstrate that the *cis*/*trans* ratio of the proline peptide bond in acyl-Pro molecules is strongly influenced by the chirality of the acyl moiety derived from **3**, **4**, and **6** and can be fixed to *trans*.

Results and Discussion

Synthesis. The carboxylic acid **1**, providing the acyl residues of **3** and **4**, was synthesized as described previously.[18] Alkylation and concomitant cyclization to benzoxazinone proceeded in a nonstereoselective manner, providing racemic **1** which, after coupling with *S*-ProOBn, afforded **2** as a mixture of diastereomers. Diastereomers **3** and **4** of (2*S*)-1-[(2,6-dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)carbonyl]-2-pyrrolidinecarboxylate (**2**) were obtained after separation by column chromatography (Scheme 1).

The diastereomer **3** was also prepared by a stereoselective synthesis via PLE-catalyzed hydrolysis of dimethyl 2-methyl-2-(4-methyl-2-nitrophenoxy)malonate, affording (2*R*)-monomethyl 2-methyl-2-(4-methyl-2-nitrophenoxy)malonate (**5**) as the key intermediate[19] (Scheme 2). Palladium-catalyzed reduction of **5**, in the presence of an equivalent amount of NaOH in water and following in situ cyclization, yielded **8**,[19] which was easily converted to **3** via EDC-catalyzed coupling with *S*-ProOBn. EDC-catalyzed coupling of **5** with *S*-ProOBn yielded **6**, which because of its lability was not purified but was used immediately to obtain **7**, which was used directly without purification to obtain **3** after esterification with benzyl

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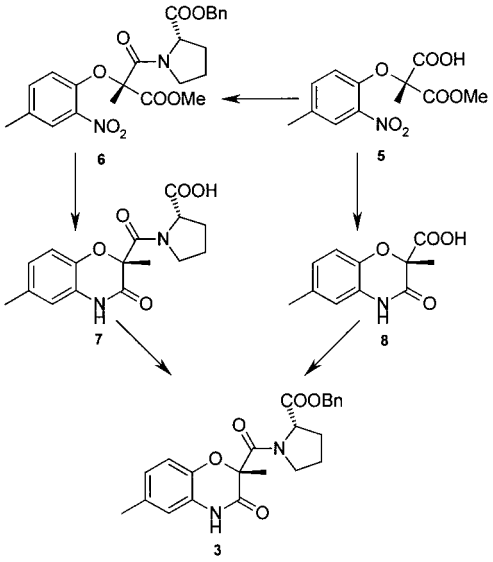
Scheme 2



bromide. The diastereomeric purity of **3**−**7** was checked by ^{1}H NMR spectroscopy.

NMR Spectroscopy. The proton chemical shifts of **3**, **4**, **6**, and **7** were assigned following standard procedures using homonuclear COSY and TOCSY experiments in combination with ^{1}H^{13}C-HMQC experiments. For **3**, **6**, and **7** only one set of resonances was observed in ^{1}H NMR spectra, demonstrating the existence of a single conformation of the proline peptide bond in DMSO-d_6 solution. NOE peaks were observed between δCH$_2$Pro/C^2-CH$_3$ and δCH$_2$Pro/HetC^{8}H (marked with arrow in left spectrum in Figure 1) characterizing the *trans* conformation of the peptide bond of the species. In **6** NOEs between δCH$_2$-Pro/C^2-CH$_3$ and ArC^{6}H/C^2-CH$_3$ were used as relevant restraints for the structure elucidation. Two distinct sets of signals were observed in the one-dimensional ^{1}H spectrum of **4** with relative populations of 28% and 72%, indicating the existence of two conformations of proline peptide bond in DMSO-d_6 solution at 302 K. For the more abundant species, NOE peaks were observed between δCH$_2$Pro/C^2-CH$_3$ and δCH$_2$Pro/HetC^{8}H (marked with arrow in right spectrum in Figure 1) characterizing the *trans* conformation.

For the less abundant species, the aromatic ring and C^2-CH$_3$ exhibited no NOEs with δ protons of proline (Figure 1), indicating that the proline peptide bond of the species in solution adopts the *cis* conformation. Interconversion between these two compounds was observed by exchange cross-peaks between αCHPro resonances of the *cis* and *trans* isomers in the NOESY experiments measured at 302 K (Figure 1). Chemical exchange cross-peaks were identified on the basis of their phases, which were in phase with the diagonal peaks but out of phase with NOE cross-peaks.

The same phenomenon could be observed when a less conformationally constrained acyl residue, i.e., 3-methoxy-2-methyl-2-(4-methyl-2-nitrophenoxy)-3-oxopropanoic acid was used. The ^{1}H NMR chemical shift pattern was similar to that of **2**. When the (2*R*)-isomer (acid (**5**)) was introduced into the Acyl-Pro molecule, the corresponding amide (**6**) (Scheme 2) possessed only the *trans* conformation according to the structure determined in solution by 2D ^{1}H NMR. Cyclization of **6** and concomitant cleavage of the benzyl ester yielded **7**, whose ^{1}H NMR spectra exhibited only one set of resonances; conformational analysis revealed the *trans* conformation of the proline peptide bond.

The proton−proton distances (Table 1) were calculated from the NOESY spectra measured at 150 ms using the two-spin approximation and the integrated intensity of a geminal pair of protons assumed to have a distance of 1.8 Å. All compounds were in the positive NOE regime when studied in DMSO at 302 K.

Molecular Dynamics. The experimentally derived distances were incorporated as conformational restraints

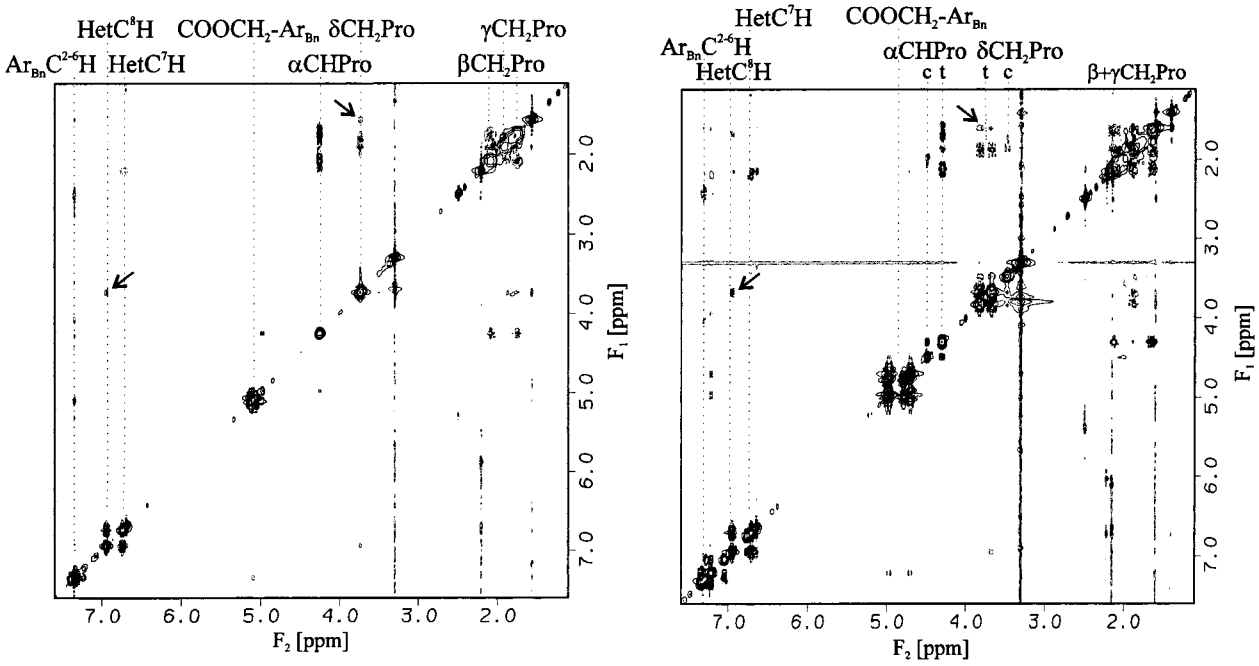


Figure 1. Expanded regions of NOESY spectra of **3** (left) and **4** (right). NOEs that are consistent with the *trans* orientation of the proline peptide bond are marked with arrows. NOE cross-peaks have the opposite sign to the diagonal peaks and are shown by dotted contours. The resonances of the *cis* and *trans* conformers of **4** overlap in the aromatic region.

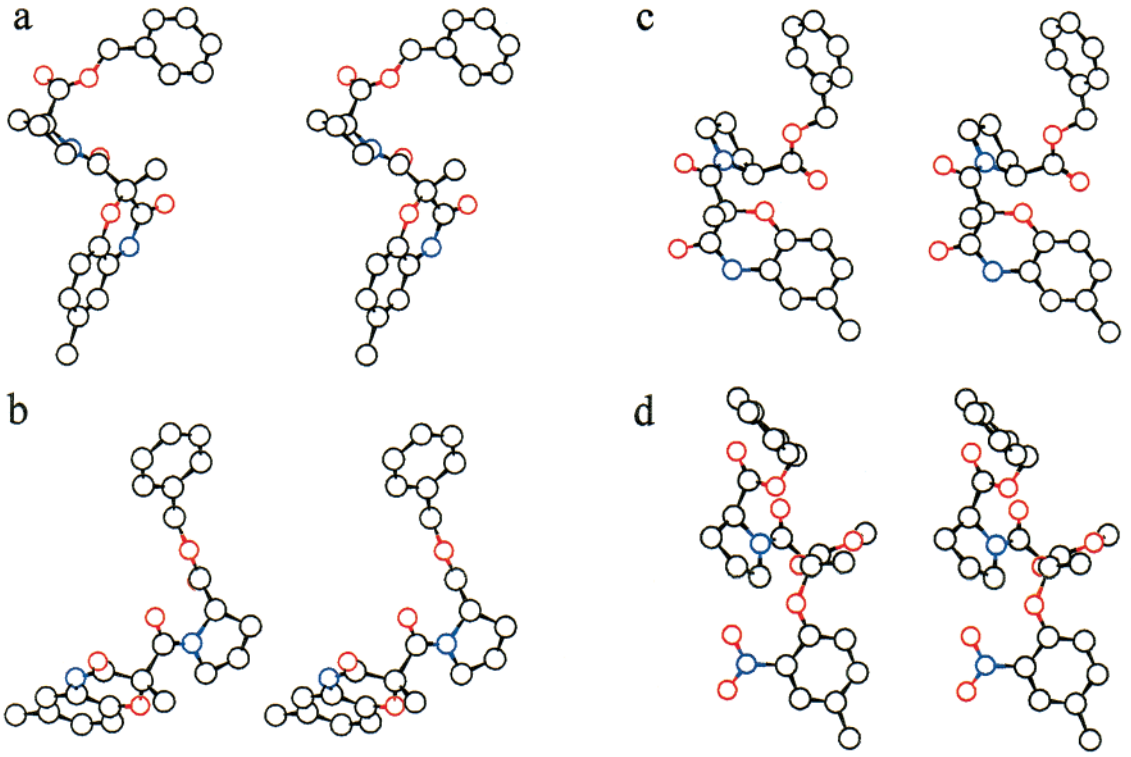


Figure 2. Stereoviews of the solution structures of **3** (a), **4**-*trans* (b), **4**-*cis* (c), and **6** (d), observed in DMSO.

Table 1. NOE Restraints Used To Determine Structures 3, 4, 6, and 7 in DMSO and Distance Violations from Restrained Simulations [Å]

	3	**4**-*trans*	**4**-*cis*	**6**	**7**
$\delta CH_2Pro/C^2\text{-}CH_3$	3.2	3.0	no NOE	3.4	3.0
$\alpha CHPro/\beta CH_2Pro$	1.8	2.1	2.1	2.1	2.2
$\delta CH_2Pro/\gamma CH_2Pro$	2.4	2.6	2.5	2.3	2.4
$HetC^8H/\delta CH_2Pro$	2.9	2.7	no NOE		2.7
$\beta CH_2Pro/\gamma CH_2Pro$	2.6				
$\alpha CH_2Pro/\delta CH_2Pro$	3.6				
$ArC^6H/C^2\text{-}CH_3$				2.8	
largest distance violation	0.3	0.3	0.1	0.1	0.3
average distance violation	0.1	0.1	0.1	0.1	0.1

in molecular dynamics simulations carried out in explicit DMSO.[28] Important energetic features of the molecule are included by application of the force field used in the molecular dynamics simulations. Moreover, the inclusion of explicit solvent molecules improves the energetic refinement given the large surface area of the molecules. The resulting conformations of **3**, **4**-*trans*, **4**-*cis*, and **6** are represented in Figure 2.

The part of these molecules that consists of acyl and proline residues adopts one conformation in DMSO. The average NOE-distance violation from the restrained simulation of each compound is less than 0.2 Å, and the largest NOE-distance violation is less than 0.3 Å (Table 1). In addition, the dihedral angle variations of this part of the molecules are less than 15°.

The conformation of the proline peptide bond of resulting structures is consistent with the observed NOEs. The benzyl part of the molecules is flexible during simulation, not being restrained experimentally by the aromatic part of benzoxazinone ring and proline ring, and the dihedral angles of the methylene group that connects the benzyl ring to the rest of the molecule vary greatly in consequence.

Crystallography. Figure 3 was produced using ORTEPII[29] and Figure 4 using PLATON.[30] Molecule **3** is shown to contain a *trans* peptide bond [torsion angle C2−C10−N21−C22 = 179.0(2)°], whereas the bond in molecule **4** is *cis* [torsion angle C2−C10−N21−C22 = 0.53(3)°] (Figure 3). There is also a difference in the chirality of the C2 atom, the *S* configuration being present in **3**, but the *R* configuration in compound **4**. A stereoscopic view of the molecular packing is presented in Figure 4. The bond lengths and angles are normal and in agreement with the values for the related compounds. L-Proline pyrrolidine ring puckering exists close to C^β-exo/C^γ-endo in both structures. The packing of the molecules in both structures is dictated by an intermolecular, nearly linear hydrogen bond N2−H4···O3 [O3 ($x-1,y,z$), 2.917(2) Å] in **3** and [O3 ($1-x, -1/2+y, 3/2-z$), 2.788(2) Å] in **4**.

Only *trans* conformers of the peptide bond were found in compounds **3**, **6**, and **7**, showing that in these cases

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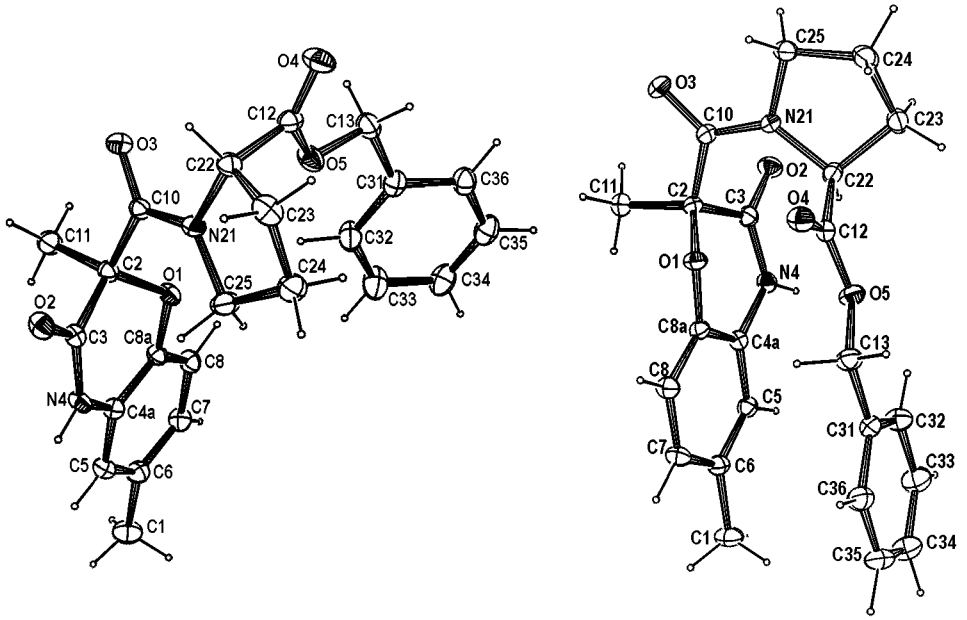

Figure 3. Structures of **3** (left) and **4** (right), showing the atomic numbering scheme. Ellipsoids are drawn at 30% probability level.

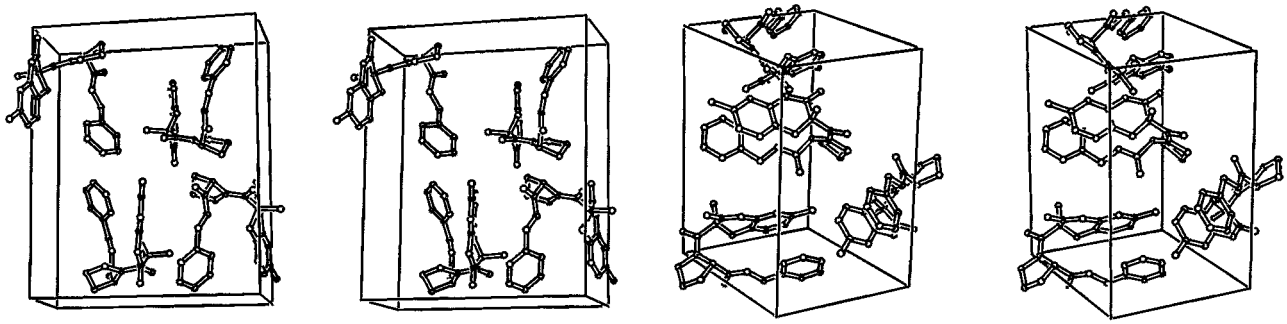

Figure 4. Stereoviews of the molecular packing for **3** (left) and **4** (right). Hydrogens are omitted for clarity.

the *S* conformation of the acyl residue in **3** and **7** and the *R* conformation of the acyl residue in **6** constrain the proline amide bond angle and consequently the conformation of the proline peptide bond to *trans*. This might be due to the steric effect of the methyl group on the stereogenic center. We anticipate that both acids **5** and **8** can be used as peptidomimetic building blocks that lock the proline peptide bond to *trans* in different types of acyl-Pro molecules. Conformational analysis of benzyl (2*S*)-1-{[(2*R*)-2,6-dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]carbonyl}-2-pyrrolidinecarboxylate (**4**) (Figures 1, 2b, 2c) revealed 78% *trans* and 28% *cis* conformer of proline peptide bond, showing that the *cis*/*trans* ratio is only slightly affected in this case.

Crystallographic data of **4** showed only the *cis* conformation of the proline peptide bond, indicating that, in the solid state, the *cis* conformation is energetically favored over *trans*. The pyrrolidine ring puckering of all of the structures of diastereomeres obtained in solution and solid state was C^{β}-exo/C^{γ}-endo, indicating that this most favorable conformation is not affected by any structural changes. The results of NMR conformational analysis suggest that the benzyl part of the molecule is loose, allowing the aromatic ring to move around freely. No NOE peaks were found between the protons of the aromatic ring and the benzyl group in **3**, **4**, and **6**, confirming that enhancement of the *trans* conformation is due to the stereoisomerism and not to hydrophobic collapse[31] of the molecule.

Experimental Section

Melting points were taken on a Reichert hot stage microscope and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrometer as KBr disks. Optical rotations were measured on a Perkin-Elmer 1241 MC polarimeter. The reported specific rotations are average values of 10 successive measurements using an integration time of 5 s. Elemental analyses were performed at the Faculty of Chemistry and Chemical Technology, University of Ljubljana on a Perkin-Elmer C,H,N Analyzer 240 C. Mass spectra were obtained on a Autospec Q, VG-analytical mass spectrometer using ionizing voltage of 70 eV by electron impact.

Preparation of Benzyl (2*S*)-1-[(2*R*/*S*)-(2,6-Dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)carbonyl]-2-pyrrolidinecarboxylate (2). To a stirred solution of 2,6-dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylic acid (**1**) (663 mg, 3.0 mmol), synthesized as described previously,[18] and benzyl (2*S*)-2-pyrrolidinecarboxylate hydrochloride (725 mg, 3.0 mmol) in dry DMF (12 mL) at 0−2 °C were added DPPA (0.81 mL, 3.78 mmol) and triethylamine (1.05 mL, 7.56 mmol). The mixture was stirred for 1 h at 0−2 °C and then for 60 h at room temperature. Ethyl acetate (60 mL) was added, and the mixture was extracted successively with 10% (w/w) citric

(31) Rich, D. H. *Perspectives in Medicinal Chemistry*; VCH: Basel, 1993; pp 15−25.

acid (3 × 5 mL), H_2O (3 × 5 mL), saturated $NaHCO_3$ solution (3 × 5 mL), H_2O (3 × 5 mL), and saturated NaCl solution (3 × 5 mL). The combined organic extracts were dried over $MgSO_4$ and filtered, and the solvent was evaporated to give a crude product **2** (992 mg, 81%) as a white solid foam. The diastereomeres were separated by column chromatography on silica gel using diethyl ether as eluant to give compounds **3** and **4**.

Benzyl (2*S*)-1-{[(2*S*)-2,6-dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]carbonyl}-2-pyrrolidinecarboxylate (3) (282 mg, 28%), colorless crystals: mp 144−148 °C; $[\alpha]^{22}_D = +14.5$ (*c* 0.29, MeOH); IR (KBr) 3252, 2983, 1737, 1704, 1643, 1519, 1449, 1100, 995, 824, 736, 584 cm^{-1}; ^1H NMR [ppm] δ 1.57 (s, 3H, C^2-CH_3), 1.71−2.00 (m, 3H, βCH^APro, γCH_2Pro), 2.04−2.13 (m, 1H, βCH^BPro), 2.22 (s, 3H, Het-CH_3), 3.69−3.81 (m, 2H, δCH_2Pro), 4.27 (dd, 1H, J = 8.7 Hz, J = 4.9 Hz, αCHPro), 5.08{5.14} (AB_{system}, 2H, J = 12.8 Hz, OCH_2−Ar_{Bn}), 6.70 (d, 1H, J = 1.5 Hz, $HetC^5H$), 6.75 (dd, 1H, J = 8.3 Hz, J = 1.5 Hz, $HetC^7H$), 6.95 (d, 1H, J = 8.3 Hz, $HetC^8H$), 7.30−7.42 (m, 5H, $Ar_{Bn}C^{2-6}H$), 10.81 (s, 1H, HetCONH); MS [FAB] *m*/*z* 408 (MH^+, 16%), 176 (100%). Anal. Calcd for $C_{23}H_{24}N_2O_5$: C, 67.63; H, 5.92; N, 6.86. Found: C, 67.38; H, 5.78; N, 6.89.

Benzyl (2*S*)-1-{[(2*R*)-2,6-dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]carbonyl}-2-pyrrolidinecarboxylate (4) (135 mg, 14%), colorless crystals: mp 166−169 °C; $[\alpha]^{22}_D = -65.5$ (*c* 0.33, MeOH); IR (KBr) 3190, 2978, 1755, 1690, 1643, 1519, 1455, 1108, 985, 885, 732, 591 cm^{-1}; ^1H NMR [ppm] δ 1.41*/1.61 (s, 3H, C^2-CH_3), 1.72−2.14 (m, 4H, βCH^APro, βCH^BPro, γCH_2Pro), 2.16/2.22* (s, 3H, Het-CH_3), 3.38−3.51* (m, δCH_2Pro)/3.64−3.88 (m, 2H, δCH^APro, δCH^BPro), 4.27 (dd, J = 8.7 Hz, J = 5.2 Hz)/4.48−4.52* (m) (1H, αCHPro), 4.72{4.92} (AB_{system}, J = 12.8 Hz)/4.80*{5.01*} (AB_{system}, J = 12.4 Hz) (2H, OCH_2−Ar_{Bn}), 6.65−6.97 (m, 3H, $HetC^{5,7-8}H$), 7.04−7.35(m, 5H, $Ar_{Bn}C^{2-6}H$), 10.73(10.92)* (s, 1H, HetCONH)*; MS [FAB] *m*/*z* 408 (MH^+, 11%), 176 (100%). Anal. Calcd for $C_{23}H_{24}N_2O_5$: C, 67.63; H, 5.92; N, 6.86. Found: C, 67.24; H, 5.83; N, 6.78. *Signals of protons of the cis isomer.

General Procedure for EDC-Catalyzed Coupling Used To Prepare Amides 6 and 3. To a stirred solution of the acid **5** or **8** (1 mmol), benzyl (2*S*)-2-pyrrolidinecarboxylate hydrochloride (242 mg, 1.0 mmol), *N*-methylmorpholine (0.36 mL, 3.3 mmol), and HOBT × H_2O (176 mg, 1.3 mmol) in dry DMF (10 mL) was added EDC hydrochloride (249 mg, 1.3 mmol) at −10 °C. The mixture was stirred for 1 h at −10 °C and then for 24 h at room temperature. Ethyl acetate (70 mL) was added, and the mixture was extracted successively with 10% (w/w) citric acid (3 × 5 mL), H_2O (3 × 5 mL), saturated $NaHCO_3$ solution (3 × 5 mL), H_2O (3 × 5 mL), and saturated NaCl solution (3 × 5 mL). The combined organic extracts were dried over $MgSO_4$ and filtered, and the solvent was evaporated in vacuo.

Benzyl (2*S*)-1-[(2*R*)-3-Methoxy-2-methyl-2-(4-methyl-2-nitrophenoxy)-3-oxopropanoyl]-2-pyrrolidinecarboxylate (6). (2*R*)-3-Methoxy-2-methyl-2-(4-methyl-2-nitrophenoxy)-3-oxopropanoic acid (**5**) (283 mg, 1 mmol), synthesized as previously described,[19] yielded **6** (409 mg, 87%) as a colorless oil after EDC-catalyzed coupling with *S*-proline benzyl ester hydrochloride. Product was purified by column chromatography on silica gel using diethyl ether/MeOH/$CHCl_3$ 200/10/1 as eluant: $[\alpha]^{22}_D = -18.9$ (*c* 0.27, MeOH); IR (NaCl) 1738, 1649, 1530, 1411, 1257, 1120, 801 cm^{-1}; ^1H NMR [ppm] δ 1.60 (s, 3H, C^2-CH_3), 1.79−1.87 (m, 3H, βCH^APro, γCH_2Pro), 2.25−2.31 (m, 1H, βCH^BPro), 2.28 (s, 3H, Ar−CH_3), 3.53−3.67 (m, 2H, δCH_2Pro), 3.76 (s, 3H, $COOCH_3$) 4.47 (dd, 1H, J = 8.6 Hz, J = 5.5 Hz, αCHPro), 5.17 (s, 2H, OCH_2−Ar_{Bn}), 7.12 (d, 1H, J = 7.1 Hz, ArC^6H), 7.22 (dd, 1H, J = 7.1 Hz, J = 1.8 Hz, ArC^5H), 7.33−7.39 (m, 5H, $Ar_{Bn}C^{2-6}H$), 7.68 (d, 1H, J = 1.8 Hz, ArC^3H); MS [EI] *m*/*z* 470 (M^+, 2.5%), 335 (100%); MS [FAB] *m*/*z* 471 (MH^+, 30%), 91 (100%); HRMS calcd for $C_{24}H_{26}N_2O_8$ 470.169900, found 470.168916.

Preparation of (2*S*)-1-{[(2*S*)-2,6-Dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]carbonyl}-2-pyrrolidinecarboxylic acid (7). Benzyl (2*S*)-1-[(2*R*)-3-methoxy-2-methyl-2-(4-methyl-2-nitrophenoxy)-3-oxopropanoyl]-2-pyrrolidinecarboxylate (**6**) (470 mg, 1 mmol) (because of rapid decarboxylation **6** was not purified but used immediately to obtain **7**) was dissolved in MeOH and hydrogenated over 10% Pd−C (47 mg) under normal pressure for 24 h at room temperature using 99.9% hydrogen [Messer hydrogen 3.0]. The catalyst was filtered off, and the filtrate was evaporated in vacuo yielding crude **7** (292 mg, 92%) as brownish crystals. Crude product **7** was purified by preparative thin-layer chromatography on 2 mm Merck TLC plates coated with silica gel 60 F254 using BuOH/AcOH/H_2O 50/10/40 as eluant: $[\alpha]^{22}_D = +19.6$ (*c* 0.22, MeOH); IR (KBr) 3452, 2976, 1695, 1613, 1518, 1450, 1382, 1233, 1133, 813, 532 cm^{-1}; ^1H NMR [ppm] δ 1.59 (s, 3H, C^2−CH_3), 1.66−1.73 (m, 3H, βCHPro, γCH_2Pro), 1.83−1.87 (m, 1H, βCHPro), 2.21 (s, 3H, Het-CH_3), 3.62−3.70 (m, 2H, δCH_2Pro), 3.98 (dd, 1H, J = 7.9 Hz, J = 3.9 Hz, αCHPro), 6.64 (s, 1H, $HetC^5H$), 6.68−6.74 (m, 1H, $HetC^7H$), 6.94 (d, 1H, J = 8.1 Hz, $HetC^8H$), 10.57 (s, 1H, HetCONH); MS [EI] *m*/*z* 318 (M^+, 6.5%), 177 (100%); MS [FAB] *m*/*z* 319 (MH^+, 30%), 341 [MNa^+] (100%); HRMS calcd for $C_{16}H_{18}N_2O_5$ 318.122800, found 318.121572.

Preparation of Benzyl (2*S*)-1-{[(2*S*)-2,6-Dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]carbonyl}-2-pyrrolidinecarboxylate (3) via 7. (2*S*)-1-{[(2*S*)-2,6-Dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]carbonyl}-2-pyrrolidinecarboxylic acid (**7**) (318 mg, 1 mmol) (due to rapid decarboxylation, **7** was used immediately for the synthesis of **3**) and KF (145 mg, 2.5 mmol) were stirred and heated to 60 °C, at which point (bromomethyl)benzene (188 mg, 1.1 mmol) was added. The mixture stirred for 6 h at 60 °C, poured on crushed ice, and extracted with diethyl ether (3 × 30 mL). The combined organic extracts were extracted successively with 10% (w/w) citric acid (3 × 5 mL), H_2O (3 × 5 mL), saturated $NaHCO_3$ solution (3 × 5 mL), H_2O (3 × 5 mL), and saturated NaCl solution (3 × 5 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated in vacuo. The crude product was crystallized from MeOH to give **3** (237 mg, 57%) as colorless crystals. The product was in all respects identical to **3** obtained by separation of diastereomers of **2**.

Preparation of Benzyl (2*S*)-1-{[(2*S*)-2,6-Dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]carbonyl}-2-pyrrolidinecarboxylate (3) via 8. (2*S*)-2,6-Dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylic acid (**8**) (221 mg, 1 mmol), synthesized as previously described,[19] yielded **3** (277 mg, 68%) as colorless crystals after EDC-catalyzed coupling with (2S)-2-pyrrolidinecarboxylate hydrochloride (242 mg, 1 mmol) and crystallization from MeOH. The product was in all respects identical to **3** obtained by separation of diastereomers of **2**.

NMR Spectroscopy. 1D and 2D ^1H NMR spectra were obtained on a Bruker Avance DPX 300 instrument operating at 300.15 MHz. All spectra were recorded in DMSO at 302 K, and chemical shifts were calibrated using tetramethylsilane as internal standard. Sample concentrations varied among different experiments but were generally between 10 and 25 mM. Typically, COSY,[20] TOCSY,[21] and HMQC[22] experiments were acquired with 8−16 transients of 1024−2048 complex points per transient for each of 512 *d*1 increments. The data matrix of 512 *d*1 points was zero-filled to 1024 points. NOESY[23] spectra of **3**,**4**, **6** and **7** were recorded using TPPI procedure using mixing time of 150 ms. In *d*1 dimension 512 real data points were collected using 8 acquisitions per FID for **3**, **4**, and **7**, and 16 acquisitions per FID for **6**, with 2.5 s relaxation delay; 2048 real points were obtained in *d*2 dimension. All spectra were processed with XWINNMR on a Silicon Graphics Indy workstation and WINNMR on a personal computer. Distances were determined from the integral intensities of NOE cross-peaks, which were measured by the integration routine within the WINNMR software. The pseudo-atom corrections were added for the methylene and the methyl groups, and the ± 10% were applied to the distances, to produce the upper and lower limit constraints.

Molecular Modeling. Molecular Dynamics. MD simulations were performed with DISCOVER (consistent valence force field) and INSIGHT II from Biosym Technologies. The

molecule with all atoms treated explicitly was centered in a box ($x = y = z = 40$ Å) using three-dimensional periodic boundary conditions. The dielectric constant was set to 1.0. Neighbor lists for calculation of nonbonded interactions were updated every 10 fs within a radius of 14 Å. The actual calculation of nonbonded interactions was carried out up to a radius of 12 Å without use of switching functions. A time step of 1 fs was employed for the MD simulation. The simulation protocol consisted of two minimization cycles (steepest descent and conjugate gradients), first with the solute fixed and then with all of the atoms allowed to move freely. The convergence criterion was 1 kcal $Å^{-1}$. The initial MD phase of the calculation involved a gradual heating, starting from 100 K and then increasing to 150, 200, 250, and finally to 300 K in steps of 0.5, 0.5, 5, 1, and 5 ps, respectively, each by direct scaling of the velocities. The NMR-derived distance restraints with a force constant of 10 kcal mol^{-1} $Å^{-1}$ were applied during the complete simulation. Configurations were saved every 1 ps for another 200 ps of dynamics. The last 100 ps of the trajectory were used for analysis of NOE distance violations and dihedral angles.

X-ray Crystallography. Compounds **3** and **4** crystallize from methanol. Colorless prismatic crystals were cut, fixed on a glass fiber with silicon grease, and mounted in a nitrogen stream (Oxford Cryosystem cooler 700) at 200 K on a Nonius Kappa CCD area-detector diffractometer. Data collection was performed using Mo Kα monochromated radiation ($\lambda = 0.71073$ Å, 55 kV, 32 mA) with the CCD detector at 30 mm from the sample. A selection of φ and ω scans of 1° at different ψ and κ settings using the program COLLECT[24] was used in several sets. The raw data were processed to produce conventional data using the program DENZO-SMN.[25] Both structures were solved by standard automatic direct methods using SHELXS-97[26] and were refined by full-matrix least squares refinement (on F^2) using SHELXL-97.[27] Non-hydrogens were refined with anisotropic displacement parameters, hydrogens were refined with isotropic displacement parameters. In the absence of suitable anomalous scatterers for MoKα radiation, determination of the absolute configuration was not possible. It was assigned, therefore to agree with the known chirality of the L-proline moiety (C22 or Cα in *S* configuration) and the Friedel diffraction data were merged accordingly.

Conclusion

For the reasons stated in the Introduction, it is desirable to have the proline peptide bond constrained in the *trans* conformation. It has been demonstrated that both acids **5** and **8** can be used as peptidomimetic building blocks that achieve this aim in different types of acyl-Pro molecules. Both compounds are shown to be easily accessible and can be coupled with proline in good yield.

The solution structures of compounds **3**, **4**, **6**, and **7** and crystal structures of **3** and **4** clearly show that the absolute configuration of the chiral carbon at position 2 affects the *cis*/*trans* ratio of acyl-Pro bond. Conformational analysis of compounds **3**, **6**, and **7**, together with crystallographic analysis of **3**, proves that these compounds exhibit only *trans* conformation of the proline peptide bond. On the other hand the solution structure of diastereomer **4** revealed a mixture of 72% *trans* and 28% *cis* conformer. In these cases, it is evident that the conformation of the proline peptide bond strongly depends on the stereochemistry of the asymmetric center in the acyl residue and that the *cis*/*trans* ratio of the peptide bond in these types of molecules can be tailored without modification of proline.

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Supporting Information Available: X-ray crystallographic data for compounds **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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