Conformational Analysis of Dehydrodidemnin B (Aplidine) by NMR Spectroscopy and Molecular Mechanics/Dynamics Calculations

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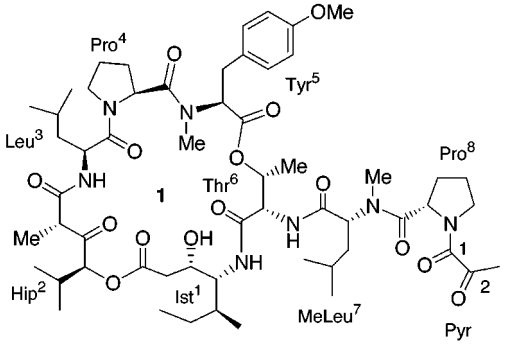
Dehydrodidemnin B (DDB or aplidine), a potent antitumoral natural product currently in phase II clinical trials, exists as an approximately 1:1 mixture of two slowly interconverting conformations. These are sufficiently long-lived so as to allow their resolution by HPLC. NMR spectroscopy shows that this phenomenon is a consequence of restricted rotation about the Pyr-Pro[8] terminal amide bond of the molecule's side chain. The same technique also indicates that the overall three-dimensional structures of both the cis and trans isomers of DDB are similar despite the conformational change. Molecular dynamics simulations with different implicit and explicit solvent models show that the ensembles of three-dimensional structures produced are indeed similar for both the cis and trans isomers. These studies also show that hydrogen bonding patterns in both isomers are alike and that each one is stabilized by a hydrogen bond between the pyruvyl unit at the terminus of the molecule's side chain and the Thr[6] residue situated at the junction betwen the macrocycle and the molecule's side chain. Nevertheless, each conformational isomer forms this hydrogen bond using a different pyruvyl carbonyl group: CO^2 in the case of the cis isomer and CO^1 in the case of the trans isomer.

Introduction

The didemnins are a class of naturally ocurring macrocyclic marine depsipeptides[1−3] that show much promise as therapeutic agents. These natural products have, consequently, been the subject of quite extensive synthetic and structural investigations, and new work continues to appear regularly.[4−8] Of the didemnin family of compounds, dehydrodidemnin B (DDB, also known as aplidine)[5,9−11] **1**, whose total synthesis we have previously reported,[12] stands out on account of its potent antitumoral activity and is currently undergoing multicenter phase II clinical trials.



NMR spectroscopic analysis of both natural and synthetic DDB, together with high performance liquid chromatography (HPLC) data, indicated that the molecule exists as two slowly interconverting conformational isomers,[12] in proportions of 45:55. Although interconversion of conformational isomers is often observed in NMR studies of peptides,[13] cases in which it is sufficiently slow to be observed in chromatography are much less common.[14−16]

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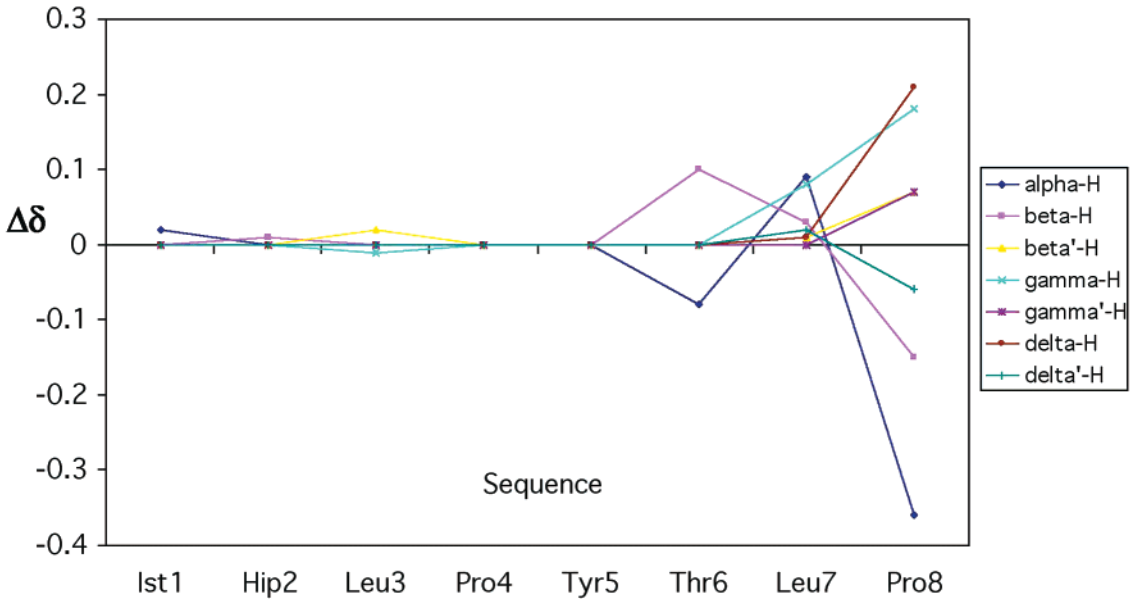

Figure 1. ^{1}H NMR chemical shift differences between DDB conformers.

The impressive biological activity exhibited by DDB made a more in-depth study of its conformational behavior desirable. Here we report on further analysis of the molecule by NMR spectroscopy that establishes that it is rotation about the terminal Pyr-Pro[8] amide bond that is unusually slow and is responsible for the observed conformational equilibrium.[5,9,10] We also report on molecular mechanics/dynamics calculations of the cis and trans isomers of DDB that provide further information on the three-dimensional structure of DDB. The information available from both these techniques indicates that the overall three-dimensional structures of the cis and trans isomers are similar and that furthermore these structures are similar to that previously reported for didemnin B.

Results and Discussion

NMR Spectroscopy. NMR spectra of DDB are complex on account of the presence of two sets of signals of approximately equal intensity, corresponding to each conformational isomer. Nevertheless, assignment of all proton resonances of both conformational isomers of the molecule was carried out on the basis of homonuclear 3D TOCSY−NOESY experiments[17] at 500 MHz by the combined analysis of TOCSY correlations and sequential NOEs. (^{1}H chemical shifts of all protons of both the minor and major isomers of DDB are reported in Tables 1 and 2 of the Supporting Information.)

These measurements indicated that significant differences in proton chemical shifts between conformational isomers were to be found in the side chain of the macrocycle, especially at the Pyr-Pro[8] terminus, where they were greatest, as shown in Figure 1.

Similar observations were noted in ^{13}C HSQC NMR experiments carried out on DDB. (^{13}C chemical shifts of all carbons of both the minor and major isomers of DDB are reported in Tables 3 and 4 of the Supporting Information.) Here again, the most marked differences

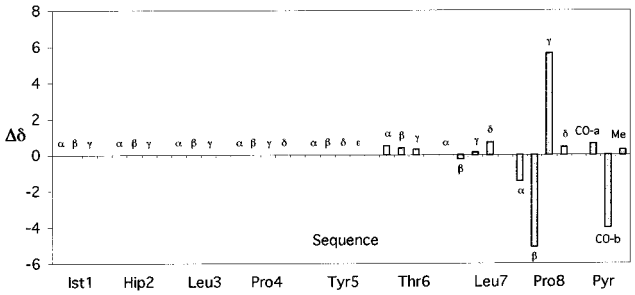

Figure 2. ^{13}C NMR chemical shift differences between DDB conformers.

in chemical shifts were found at the terminus of the side chain of the macrocycle as shown in Figure 2.

^{1}H and ^{13}C NMR spectroscopy of Pyr-Pro-OBn, used as an intermediate in the total synthesis of DDB, in $CDCl_3$ at 500 MHz and 300 K showed that it also exists as a 40:60 mixture of conformational isomers. The ^{13}C HSQC NMR spectra of DDB and Pyr-Pro-OBn showed that the conformational equilibrium was due to cis/trans isomerism about the Pyr-Pro[8] tertiary amide bond. One of the characteristic features of cis X-Pro systems is the large difference in chemical shift between the β and γ carbon atoms (>8 ppm) compared to the corresponding trans X-Pro isomers where the difference is less (<6 ppm).[13,18] As can be seen from Figure 3, the relevant signals due to Pro[4] in the cis and trans conformational isomers of DDB overlap and show a chemical shift difference of 2.8 ppm, characteristic of a *trans*-amide bond. On the other hand, two sets of signals corresponding to the β and γ carbons of Pro[8] are clearly visible, one of which shows a difference in chemical shift ($\Delta\delta^{\beta\gamma}$) of 1.8 ppm, indicating the trans conformation, while the other set has a value of $\Delta\delta^{\beta\gamma}$ of 8.9 ppm, characteristic of the cis isomer.

Both for Pyr-Pro-OBn and for DDB itself, coalescence of the signals corresponding to the pyruvyl methyl groups for each conformational isomer was observed by ^{1}H NMR

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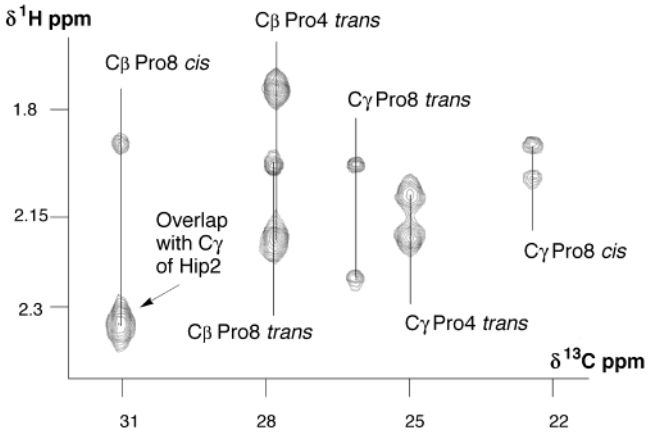

Figure 3. Detail of ^{13}C HSQC NMR spectrum showing Pro^4 and Pro^8 β and γ carbons.

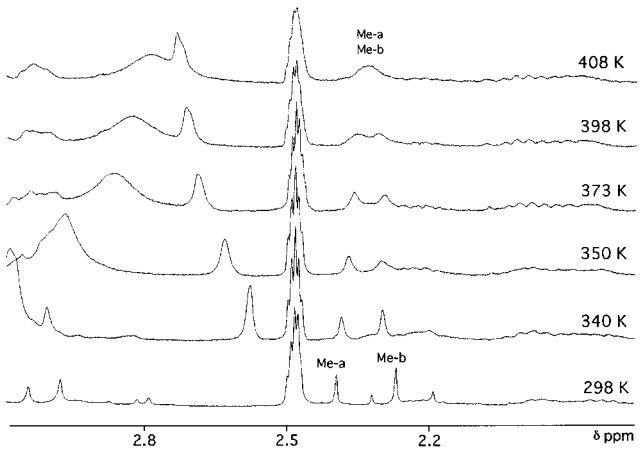

Figure 4. Coalescence of the signals corresponding to the pyruvyl methyl group of the DDB conformers.

spectroscopy at 250 MHz in DMSO. In the latter case (see Figure 4), coalescence was observed at 408 K, corresponding to a value of $\Delta G^{\ddagger} = 86.9 \pm 0.8$ kJ mol^{-1} for the free energy of activation for cis/trans isomerization. This value is between 4 and 8 kJ mol^{-1} higher than the value corresponding to a normal[19] peptide tertiary amide bond involving Pro and leads to a rate constant for cis/trans isomerization in DDB of 3.4×10^{-3} s^{-1} at 298 K and a value of $t_{1/2}$ of approximately 3.4 min for each isomer. Such a value would, in principle, allow both conformational isomers to be resolved by chromatographic analysis of DDB under suitable conditions. Furthermore, the rate of isomerization might be expected to be retarded somewhat in polar solvents[20] such as those used in the reversed-phase HPLC analysis of the didemnins (gradient elutions with mixtures of 0.045% TFA in H_2O and 0.036% TFA in MeCN) leading to an even greater value for $t_{1/2}$.

Analysis of mid- and long-range NOEs showed that the macrocycles of both conformational isomers had similar three-dimensional structures, suggesting that the observed conformational equilibrium did not substantially affect the overall structure of the molecule. A qualitative treatment of the NOE data gave rise to the interproton distances listed in Table 1. These values clearly indicate

Table 1. Proton−Proton Distance Measurements from NOE Buildup Curves for DDB

^{1}H involved	^{1}H involved	trans isomer distance (Å)	cis isomer distance (Å)
NH Thr^6	α Leu^7	2.7	2.8
γ Pro^8	Me N(Me)Leu^7	2.8	2.8
NH Thr^6	β Thr^6	2.7	2.6
Me Tyr^5	β Pro^4	2.6	2.6
NH Thr^6	γ Thr^6	2.1	1.7
γ Leu^3	δ Pro^4	2.9	2.9
α Leu^3	δ Pro^4	2.1	2.1
α Leu^3	δ Pro^4	2.3	2.3
β_{pro-R} Leu^3	δ′ Pro^4	2.1	2.1
ϵ Tyr^5	β_{pro-R} Pro^4	2.1	2.1
NH Leu^3	α Hip^2	2.0	2.1
α Pro^8	Me Leu^7	1.8	1.9
Me Leu^7	β Leu^7	1.9	2.1
Me Pyruvyl	γ Thr^6	2.9	3.1
α Thr^6	β Leu^3	2.2	2.5
NH Leu^3	α Thr^6	2.7	2.5
NH Leu^3	β Ist^1	2.0	2.5
NH Ist^1	α Thr^6	1.9	1.9
ϵ Tyr^5	γ Pro^4	2.3	2.3
Me Tyr^7	δ Pro^4	1.9	1.9

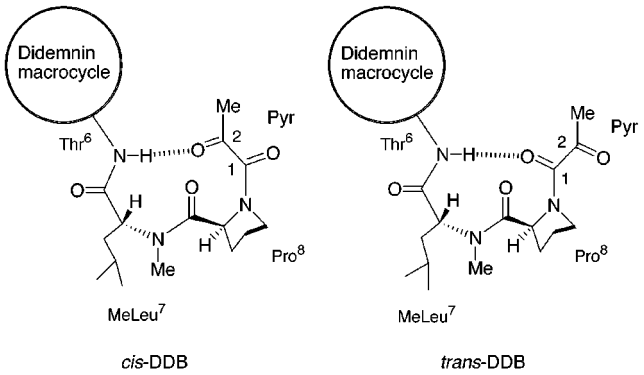

Figure 5. Graphical representations of the hydrogen bonds between Thr^6-NH and the pyruvyl unit in *cis*- and *trans*-DDB.

that there are only relatively minor changes in geometry between the conformational isomers. Moreover, the observed NOEs for both isomers of DDB were in good agreement with similar data previously reported[21−23] for didemnin B, indicating that the three-dimensional structure of the macrocycle was similar in all cases. Didemnin B has a lactyl unit instead of a pyruvyl unit at the terminus of the molecule's side chain but exists as only one major conformer in solution.

Molecular Mechanics/Dynamics Calculations. On account of the inherent lack of precision associated with the determination of interproton distances in molecules of the size[24] of DDB, in addition to the limited number (2.5) of interatomic distances per residue accessible experimentally, molecular mechanics/dynamics calculations were carried out in order to provide more information on the three-dimensional structures of both conformational isomers of the molecule.

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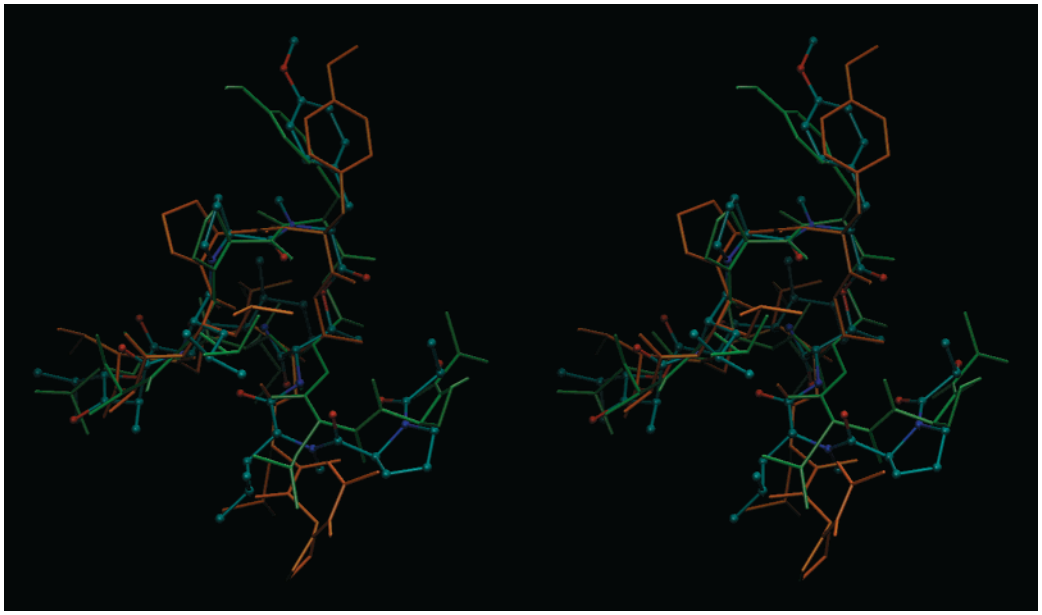

Figure 6. Superposition of the X-ray crystal structure of didemnin-B (CPK) and representative snapshots from the explicit DMSO trajectories for *cis*-DDB (orange) and *trans*-DDB (green).

All calculations were performed using the well-established CHARMm force field[25,26] for reasonably long periods of time (see the Experimental Section) and without distance restraints in order to allow the conformational space of the molecule to be more fully explored. Three implicit solvent descriptions ($\epsilon = 1$, 4.8, and 80) and two explicit solvent models (H_2O and DMSO) were applied for both isomers.

In all simulations, nearly all of the atomic distances that corresponded to the measured NOE distances are within those values that would be expected to produce an NOE, demonstrating good agreement between the unrestrained molecular dynamics simulations and experimental NMR data. A direct comparison of calculated atomic distances with the experimentally observed NMR distances is not possible on account of the united atom representation used in the calculations.

Analysis of the most relevant torsion angles found in the simulations (summarized in Tables 5 and 6 in the Supporting Information) provides further evidence for the similarity of the overall three-dimensional structures of the cis and trans isomers. Furthermore, comparison with reported results for didemnin B by NMR studies [27] and by X-ray diffraction[28] indicates that the three-dimensional structures of the macrocycle both in *cis*-and *trans*-DDB and in didemnin B are similar, in each case adopting a twisted figure of eight conformation.

Analysis of the hydrogen bonds produced in the simulations (see Tables 7 and 8 in the Supporting Information) showed that for both the cis and trans isomers of DDB the transannular hydrogen bond between Ist[1]-NH and Leu[3]-CO was present in all calculations as was that between Leu[3]-NH and MeLeu[7]-CO. The formation of both these hydrogen bonds is compatible with the low $\Delta\delta/\Delta T$ values of 2.0 and 2.2 ppb/K observed by ^{1}H NMR for the Ist[1]-NH and Leu[3]-NH protons, respectively. Both of these hydrogen bonds have previously been reported in didemnin B and are responsible for the abovementioned figure of eight disposition of the macrocycle. In these simulations, another transannular hydrogen bond between Leu[3]-NH and Ist[1]-CO was also detected in all calculations except that for the explicit DMSO solvent model in the case of the trans isomer.

For both *cis*- and *trans*-DDB, a hydrogen bond between Thr[6]-NH and Pro[8]-CO was detected in all calculations except for the explicit DMSO solvent model in the case of the cis isomer. However, in the case of *trans*-DDB, the hydrogen bond between Thr[6]-NH and Pyr-CO[1], analogous to the reported hydrogen bond in didemnin B between Thr[6]-NH and Lac-CO, was found in all calculations whereas this hydrogen bond was lacking in all calculations on the cis isomer. For the cis isomer, on the other hand, a hydrogen bond between Thr[6]-NH and Pyr-CO[2] was detected that was not found in any of the calculations on the trans isomer (see Figure 5).

These latter data provide an explanation for the more or less equal distribution of the two conformational isomers of DDB. The trans isomer would be stabilized by the Thr[6]-NH to Pyr-CO[1] hydrogen bond in a manner similar to the dominant conformer of didemnin B, whereas the cis isomer is stabilized by the hydrogen bond between Thr[6]-NH and Pyr-CO[2]. These hydrogen bonds appear to stabilize both conformational isomers to a similar degree, consistent with the observed NMR values for $\Delta\delta/\Delta T$ of 3.51 and 4.8 ppb/K for the Thr[6]-NH proton in the cis and trans rotamers, respectively. The presence of a weak NOE between the γH of Thr[6] and the methyl group of the pyruvyl unit for both isomers provides further experimental support.

The similarity of the three-dimensional structures of these molecules is illustrated by the superposition of representative snapshots of both the DDB cis and trans isomers from the trajectories of the DMSO explicit-solvent simulations with the reported X-ray crystal structure of didemnin B as shown in Figure 6.

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Only those atoms pertaining to the macrocycle in each case were aligned, minimizing the root-mean-square differences (RMSD). RMSD values of 0.63 and 0.67 Å, respectively, were obtained between *cis*-DDB and didemnin B on one hand and between *trans*-DDB and didemnin B on the other. The corresponding difference between the *cis*- and *trans*-DDB isomers was 0.90 Å. A second graphic showing the superposition of representative snapshots of the DDB *cis*- and *trans*-isomers from the trajectories of the H_2O explicit-solvent simulations, indicating the hydrogen bonds formed between Thr[6]-NH and the pyruvyl carbonyl groups in each case, can be found in the Supporting Information.

Conclusions

The origin of the observed conformational isomerism in dehydrodidemnin B (aplidine) is slow rotation about the Pyr-Pro[8] tertiary amide bond. Despite this conformational equilibrium, which leads to the appearance of two separate peaks in HPLC chromatography, the overall three-dimensional structures of *cis*- and *trans*-DDB are broadly similar, and both are similar to the structure reported for didemnin B. The more or less equal distribution of the cis and trans isomers is associated with each isomer being stabilized by a hydrogen bond between the Thr[6] amino group and a different carbonyl group of the terminal pyruvyl unit. These results suggest that the higher biological activity observed for DDB cannot have its origin in a significant change in its three-dimensional structure as a consequence of the presence of a different conformational isomer to that present in didemnin B.

Experimental Section

Nuclear Magnetic Resonance Spectroscopy. A 15 mM degassed solution of synthetic[12] DDB in $CDCl_3$ (>99.98% deuterium incorporation) was used for all NMR experiments described in this study. Homonuclear 3D NOESY−TOCSY ^{1}H NMR spectra were recorded at 27 °C on a Varian VXR 500 MHz spectrometer. Mixing times for the TOCSY and NOESY spectra were 70 and 300 ms, respectively. The data were collected in phase using the hypercomplex method, and the spectral width used was 4393 Hz in all three dimensions. Eight accumulations and 95 increments were carried out in the NOESY period and eight accumulations and 44 increments in the TOCSY. A final matrix of 2048 × 512 × 256 in F1, F2, and F3, respectively, was obtained by zero filling and linear prediction. A digital resolution of 4.5, 17, and 34 Hz was obtained in the abovementioned axes. The duration of the experiment was 60 h. The spectrum was processed on a Sun SPARC computer using Varian VNMRS 5.1 software, applying the normal processing functions for NOESY and TOCSY experiments. The NOE buildup curves were generated from five NOESY experiments (mixing times 50 ms, 150 ms, 300, 600, and 900 ms) using the distance between the δ-H of Pro[8] for calibration purposes.

Molecular Mechanics/Dynamics Simulations. Molecular mechanics/dynamics calculations were performed on Silicon Graphics 02 and Octane computers using the CHARMM 24b2 program package[29] and the CHARMm 23.1 force field.[25,26] The reported X-ray crystal structure of didemnin B was edited accordingly to provide the *trans*-Pyr-Pro[8] isomer of DDB. After minimization, this structure served as the starting point for molecular mechanics/dynamics calculations on the trans isomer using the CHARMm united atom representation with explicit polar hydrogens. Charges were assigned to the atoms using the method described by Gasteiger and Marsili,[30] after first confirming their suitability by comparison with ESP charges derived from AM1-optimized small substructures for pyruvylproline, dimethyltyrosine, and *N*-methylamides employing MOPAC6. The GAUSSIAN94 program using the Hartree−Fock 6-31G* basis set was used to check that the force field parametrization for the pyruvamide function was adequate. The starting structure for the calculations on the cis isomer was obtained by changing the torsion angle of the trans Pyr-Pro[8] amide bond from 0° to 180°. For both cis and trans isomers implicit solvent descriptions (ϵ = 1, 4.8, and 80) and explicit solvent models for H_2O and DMSO were used. NVT calculations at 300 K were performed using cubic boxes of 30 Å side length and 1000 TIP3P water molecules and of 31 Å side length and 216 DMSO molecules, respectively, applying periodic boundary conditions. After adequate heating and equilibration of the system, evolution times were 20 ns for all implicit solvent models, 2 ns for the DMSO explicit solvent model, and 1 ns for the H_2O explicit solvent model. Structures were saved periodically from each trajectory for further analyses. Trajectories were analyzed for bond distances, torsion angles, and hydrogen bonds.

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Supporting Information Available: A graphic showing the superposition of representative snapshots from the explicit H_2O trajectories for *cis*-DDB and *trans*-DDB indicating the hydrogen bonds between Thr[6]-NH and the different pyruvyl carbonyl groups in each case. Tables of ^{1}H and ^{13}C NMR chemical shifts of *cis*- and *trans*-DDB. Tables of the torsion angles found in the implicit and explicit solvent modeling studies for *cis*-and *trans*-DDB. Tables of H-bonds found in implicit and explicit solvent modeling studies for *cis*- and *trans*-DDB. A graphic showing the numbering scheme for DDB used in this work. This material is available free of charge via the Internet at http://http://pubs.acs.org.

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