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An intramolecular ionic hydrogen bond stabilizes a *cis* amide bond rotamer of a ring-opened rapamycin-degradation product

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Rapamycin (1), a macrolide immunosuppressant, undergoes degradation into ring-opened acid products 2 and 3 under physiologically relevant conditions. The unsaturated product (3) was isolated and studied in this work. Unlike 1, which has its amide primarily in a *trans* conformation in solution, 3 has both *cis* and *trans* conformations in approximately a 1:1 ratio in dimethyl sulfoxide (DMSO). The amount of *cis* rotamer was increased dramatically in the presence of an organic base such as triethylamine. The detailed NMR results indicate that the *cis* rotamer is stabilized through an intramolecular ionic hydrogen bond of the carboxylate anion with the tertiary alcohol as part of a nine-membered ring system. This hydrogen bond was characterized further in organic media and the *trans–cis* rotamer equilibria were used to estimate the relative bond strengths in several solvents. The additional stabilization arising from this ionic hydrogen bond in the *cis* rotamer was determined to be 1.4 kcal mol^{-1} in DMSO-d_6, 2.0 kcal mol^{-1} in CD_3CN and 1.1 kcal mol^{-1} in CD_3OD. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; amide bond rotamers; ionic hydrogen bond; rapamycin degradation product

INTRODUCTION

A majority of molecules containing amide bonds show *trans* and *cis* rotamers in solution due to a partial double-bond character of the C–N bond. Secondary amides typically exist mainly as the *trans* rotamer to avoid steric repulsion between the nitrogen atom and the substituent on the carbonyl carbon. However, there are several reports in the literature that show the *cis* rotamer can be stabilized through non-covalent interactions such as hydrogen bonding,[1] hydrophobic effects,[2] C–H $\ldots\pi$ interactions[3] and other factors.[4] In contrast, the stability of the *trans* and *cis* rotamers is hard to predict for tertiary cyclic amides. For acyclic tertiary amides, the *trans* and *cis* rotamers are expected to be populated to similar extents in the absence of driving forces such as hydrogen bonding, steric and hydrophobic effects.[5]

Rapamycin is a tertiary cyclic amide that exhibits significant immunosuppressant activities.[6,7] In solution, two amide bond rotamers are observed.[8] The predominant rotamer is the *trans* conformation, which is consistent with the X-ray crystal structure of free or bound rapamycin.[9–11] The ring-opened acid form of rapamycin (**2** and **3**) is an acyclic tertiary amide, which has been identified as the major metabolite *in vitro* as shown in Scheme 1.[12–14] There is less information about the *trans* and *cis* rotamers of the acyclic tertiary amide.

In this paper, compound **3** was isolated and studied in organic solvents by NMR. In comparison to rapamycin, there is a large difference in the ratio of *trans* to *cis* rotamers in **3**. Interestingly, the ratio changes dramatically in the presence of organic bases such as triethylamine (TEA) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The detailed NMR analysis indicates that an ionic hydrogen bond stabilizes the *cis* rotamer. This is a good model system for assessing the relative strengths of ionic hydrogen bonds through analysis of the *trans–cis* rotamer equilibria in various solvents. Factors that potentially contribute to the hydrogen bond strength, such as the hydrogen bond angle and the pK_a difference of the hydrogen bond donor and acceptor, will be discussed.

EXPERIMENTAL

Rapamycin easily degrades in the presence of acid or base in organic solvents.[15–18] The reaction of rapamycin with DBU efficiently provides the degradation product **3**.[16] Rapamycin (1.0 g, 1.09 mM) was dissolved in tetrahydrofuran (THF) at 0 °C in an ice bath, DBU (0.15 g, 1 mM) was added and the reaction mixture was stirred at 0–5 °C for 6 h. The reaction mixture was diluted with ethyl acetate (25 ml), washed with 1 N hydrochloric acid (2 × 25 ml) and water (2 × 25 ml),

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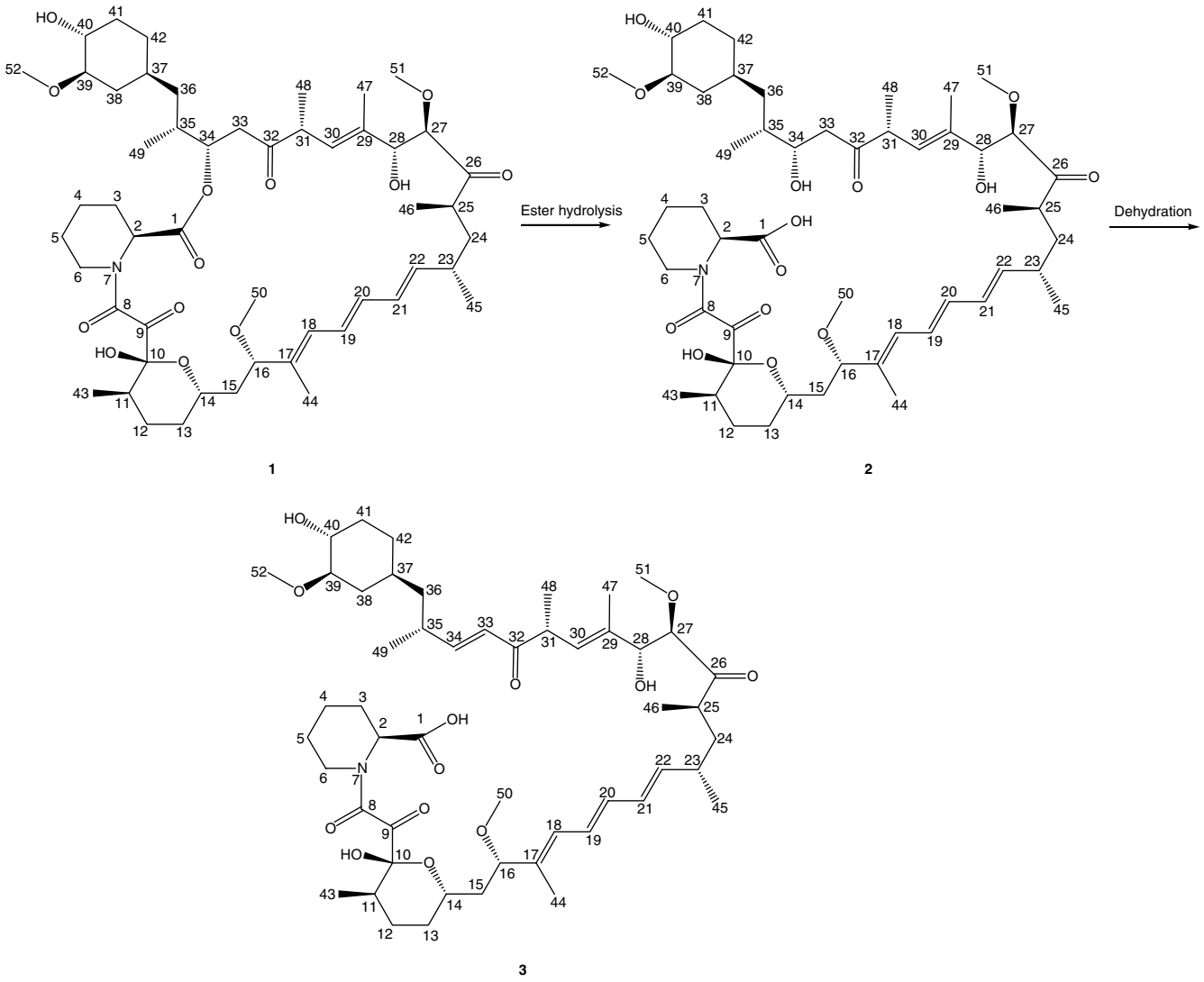

Scheme 1

dried with sodium sulfate and concentrated. The product was dried under vacuum for 1 h to give a foamy solid. The yield was 0.95 g (95%) and the purity of **3** was typically >85%. A minor impurity (identified as an epimer of **3**) also was observed, but its presence did not influence the conformational analysis presented here.

The NMR spectra were recorded on a Varian Unity Plus 500 MHz spectrometer equipped with a 5 mm inverse $^{1}H-^{13}C-^{15}N$ gradient probe and a broadband gradient probe. All data were recorded at 25 °C unless specified. Proton chemical shifts were referenced relative to the residual ^{1}H signal of DMSO-d_6 at 2.50 ppm and carbon chemical shifts were referenced to the solvent signal of DMSO-d_6 at 39.5 ppm.

The standard ^{1}H, ^{13}C and ^{2}H spectra were collected with a 45° pulse flip angle. A series of two-dimensional spectra were acquired to make the full ^{1}H and ^{13}C assignments for **3**. The pulse programs of the gDQCOSY, gHSQC and gHMBC experiments were taken from the Varian software library.

The gDQCOSY experiments were performed using eight scans with 256 increments in F1, 1024 data points in F2 and a relaxation delay of 1.0 s. The data were processed with Gaussian weighting functions.

Phase-sensitive gHSQC was used for single-bond $^{1}H,^{13}C$ chemical shift correlation, using the BIRD sequence, and WURST ^{13}C decoupling. Two sets of 256 time increments were acquired with 8–32 scans per increment and a 0.75 s relaxation delay. The data were zero filled to 2048 × 2048 and processed with Gaussian weighting functions.

The gHMBC spectra were used to assign multiple-bond $^{1}H,^{13}C$ chemical shift correlations with a long-range coupling constant of 8 Hz. Data were collected with 16–512 scans, using 256 increments and a relaxation delay of 1.0 s. The data were zero filled to 2048 × 2048 and processed with sine-bell weighting functions.

RESULTS AND DISCUSSION

The *trans* and *cis* rotamers

Compound **3** showed two sets of peaks in all the organic solvents that we examined. These two sets of peaks are due to isomerization between *cis* and *trans* amide rotamers, which interconvert at room temperature at a slow rate relative to the NMR time scale. Almost equal populations of *trans* and *cis* rotamers were observed, as would be expected for an acyclic tertiary amide. The assignments of the *trans* and *cis* rotamers

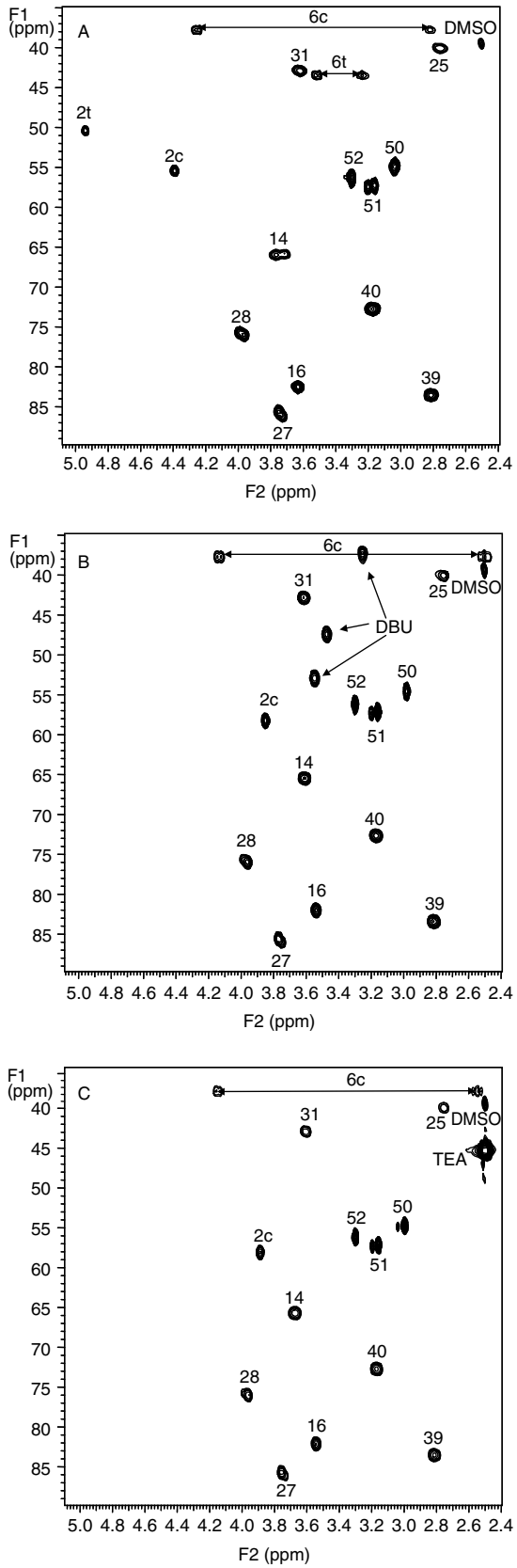

Figure 1. The gHSQC spectra of compound **3** in DMSO-d_6 (A), of rapamycin with 1.5 μl of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), added to produce compound **3** (B) and of compound **3** with 25 μl of triethylamine TEA added (C). Letters 't' and 'c' are abbreviations of the *trans* and *cis* rotamer, respectively.

were derived by analogy with the previous detailed NMR and molecular-dynamics investigation of rapamycin[8] and FK506.[19] The ^{1}H and ^{13}C chemical shifts of positions 2 and 6 in the pipecolinyl ring have unique patterns for the *trans* and *cis* rotamers, as shown in the gHSQC spectra (Fig. 1A). However, when NMR was used to monitor the reaction of rapamycin and DBU to produce **3**, only one set of peaks was observed (as shown in Fig. 1B). The pattern of NMR signals at positions 2 and 6 are similar to the *cis* rotamer of **3**, although position 2 is significantly shifted in both the proton and carbon dimensions. DBU is not only a catalyst in the reaction but also serves as an organic base. In the presence of DBU, **3** is observed to be in the deprotonated state. This was demonstrated by the addition of the organic base TEA to the DMSO-d_6 solution of **3**, which yields an identical gHSQC spectrum (Fig. 1C). The addition of sodium hydroxide also gave similar results, but unfortunately led to degradation of **3**.

Ratios of *trans* and *cis* rotamers of **3** were measured under different conditions and the results of these studies are listed in Table 1. Three solvents DMSO-d_6, CD_3CN and CD_3OD were chosen. Compound **3** does not dissolve well in D_2O or $CDCl_3$. Ratios of *trans* and *cis* rotamers of **3** were observed to be independent of concentration and moderately influenced by the solvent. However, the ratio of *trans*–*cis* rotamer dramatically changed upon addition of TEA.

The intramolecular ionic hydrogen bond

All the correlations in two-dimensional spectra (gDQCOSY, gHSQC and gHMBC) are consistent with the structure of **3** both with and without base. The chemical shifts of the pipecolinyl ring, dicarbonyl and the hemiketal ring regions are listed in Table 2. The chemical shift differences are minimal for the rest of the molecule. The large shift of the hydroxyl group at position 10 from ~6.4 ppm without TEA to ~9.2 ppm with TEA indicates that this hydroxyl group is involved in a hydrogen bond in the presence of TEA. This scenario is similar to several diacids that form low-barrier hydrogen bonds in the presence of base,[20,21] therefore the hydroxyl group at position 10 forms an intramolecular hydrogen bond with the carboxylate anion. This ionic hydrogen bond appears to stabilize the *cis* rotamer by forming a nine-membered ring between the pipecolinyl ring and the hemiketal ring, as shown in Fig. 2, structure **f**.

The *trans* rotamer of **3** was always observed as a minor component in the presence of TEA. Full assignments for the deprotonated *trans* rotamer could not be obtained due to weak and overlapping signals with the *cis* rotamer. At equilibrium, the ratio of *trans*–*cis* rotamers is different in the three solvents. The ionic hydrogen bond is more stable in less polar solvents, so the *cis* isomer population is higher in CD_3CN than in DMSO-d_6. Because CD_3OD can serve as a hydrogen bond donor to the carboxylate anion, the intramolecular ionic hydrogen bond should be weakened by the intermolecular hydrogen bond. Therefore, as observed in the NMR studies, the *cis* isomer population is lower in CD_3OD.

The relative strengths of normal and low-barrier hydrogen bonds in apolar organic media have been studied using diacid and monoanion equilibria. The additional

Table 1. Ratios of *trans–cis* rotamers of compound **3** under various conditions

Solvent (ε)[a]	Addition (μl)		Ratio of *trans–cis* rotamer[b] 2 mg	5 mg	10 mg	20 mg
DMSO-d_6 (47.24)	None		46.0 : 54.0	46.0 : 54.0	46.3 : 53.7	46.8 : 53.2
	Formic acid	(2)		46.3 : 53.7		
		(7)		46.3 : 53.7		
	TEA	(1)			23.5 : 76.5	
		(2)			11.9 : 88.1	
		(3)			7.9 : 92.1	
		(4)			8.0 : 92.0	
		(25)				7.9 : 92.1
CD_3CN (36.64)	None				56.1 : 43.9	
	TEA	(2)			5.5 : 94.5	
		(6)			4.8 : 95.2	
		(10)			4.3 : 95.7	
CD_3OD (33.0)	None					56.6 : 43.4
	TEA	(25)				17.4 : 82.6

[a] Dielectric constant, value reported at 293.2 K and without deuterium correction from David R. Lide, *Handbook of Chemistry and Physics* (1998–1999), vol. 6, page 139.

[b] The ratio of *trans–cis* rotamers was calculated from the intensity of the *trans* proton 2 (2t) to the *cis* equatorial proton 6 (6c, downfield).The signals of the *cis* proton 2 and the *trans* proton 6 were overlapped with other signals.

Table 2. Partial ^{1}H and ^{13}C assignments of compound **3** in DMSO-d_6 with and without TEA

No.	Group	^{1}H chemical shifts (ppm)[a,c] No TEA	With TEA	^{13}C chemical shifts (ppm)[b,c] No TEA	With TEA
1	C=O	—	—	171.3 (171.6)	172.0
1	OH	13.01	[d]	—	—
2	CH	4.93 (4.38)	3.88	50.7 (55.7)	58.5
3	CH_2	1.56, 2.14 (1.65, 2.11)	1.29, 2.15	26.2 (27.0)	26.9
4	CH_2	1.36, 1.69 (1.31, 1.65)	1.55	20.9 (20.9)	21.2
5	CH_2	1.27, 1.67 (1.36, 1.61)	1.25, 1.59	24.2 (24.6)	24.5
6	CH_2	3.22, 3.51 (2.81, 4.25)	2.55, 4.14	43.8 (38.1)	38.2
8	C=O	—	—	167.0 (166.1)	166.9
9	C=O	—	—	199.4 (199.8)	205.4
10	C	—	—	99.0 (99.3)	99.2
10	OH	6.41 (6.46)	9.25	—	—
11	CH	2.07 (2.01)	1.80	34.6 (34.8)	34.9
12	CH_2	1.47	1.46	26.4 (26.5)	26.8
13	CH_2	0.82, 1.60	0.82, 1.59	30.7	30.1
14	CH	3.76	3.66	66.2 (66.2)	66.1

[a] Relative to the residual signal of DMSO-d_6 assigned to 2.50 ppm.

[b] Relative to DMSO-d_6 assigned to 39.5 ppm.

[c] The values in parentheses are assignments for the *cis* rotamers, where these can be distinguished. Some of the *cis/trans* assignments for **3** (no TEA) may be switched due to the similar concentrations of *trans* and *cis* rotamers in solution.

[d] This peak disappeared when TEA was added.

stabilization arising from the low-barrier hydrogen bonds was determined from these studies to be in the range of 1.4–5.0 kcal mol^{-1}.[20–23] The low-barrier hydrogen bond is an ionic hydrogen bond in which the proton is equally or almost equally shared between the two heteroatoms. A widely accepted feature associated with low-barrier hydrogen bonds is an unusual downfield proton NMR resonance of >16 ppm.[21,24] The chemical shift of the hydroxyl group in **3** is 9.2 ppm when forming an intramolecular ionic hydrogen bond. This ionic hydrogen bond is not in the low barrier category.

A deuterium isotope shift experiment can be used to identify the hydrogen bond type. As shown in previous studies, near-zero, positive and negative values of $\Delta[\delta(^1H) - \delta(^2H)]$ can be related to the three categories of hydrogen bond: weak, strong and very strong.[25] The values of

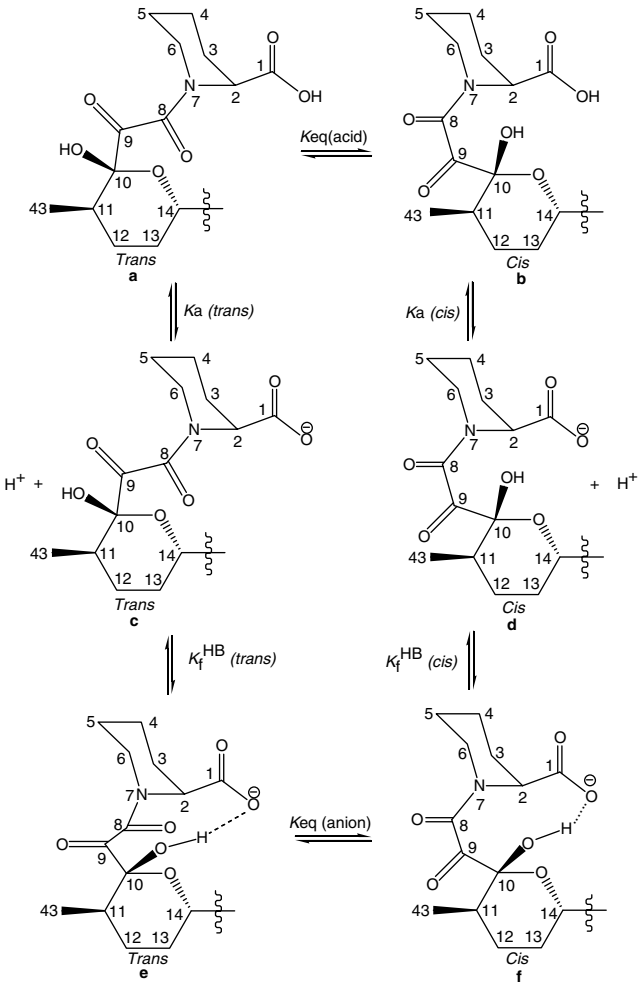

Figure 2. Equilibria of compound **3** in solution.

$\Delta[\delta(^1H) - \delta(^2H)]$ have been reported for intramolecular hydrogen bonding of hemi-salts in CH_2Cl_2, such as maleate (−0.03 ppm), phthalate (−0.15 ppm) and 3,4-furoic diacid (0.11 ppm).[25] These intramolecular hydrogen bonds are believed to be strong hydrogen bonds with small $\Delta[\delta(^1H) - \delta(^2H)]$ values. These examples are exceptions to the general trend observed with weak, strong and very strong hydrogen bonds, as outlined above. The deuterium NMR spectrum of the hydroxyl group of **3** was obtained in CD_3CN in the presence of TEA. The $\Delta[\delta(^1H) - \delta(^2H)]$ value was observed to be 0.09 ppm for **3**, which is close to the previously reported values for the intramolecular hydrogen bonds in carboxylates.

Compound **3** provides a model system for assessing the relative strength of intramolecular ionic hydrogen bonds. The equilibria involved in the formation of **3** in solution are shown in Fig. 2. The relative strength of the ionic hydrogen bond can be calculated from Eqn (1)

$$K_f^{HB}(cis)/K_f^{HB}(trans) = K_{eq}(\text{anion})/K_{eq}(\text{acid}) \qquad (1)$$

Isomers **a, c** and **e** are in fast exchange in solution, giving rise to just one set of peaks for the *trans* rotamer. The same is true for isomers **b, d** and **f**, giving rise to just one set of peaks for the *cis* rotamer. There are two extreme cases that can be used to generate the $K_f^{HB}(cis)/K_f^{HB}(trans)$ ratio. Without addition of organic base, the *cis/trans* ratio of compound **3** is close to **[b]**/**[a]**, i.e. K_{eq}(acid). With the addition of excess TEA, the *cis/trans* ratio of compound **3** can be approximated as **[f]**/**[e]**, i.e. K_{eq}(anion).

Both K_{eq}(acid) and K_{eq}(anion) are known from the rotamer ratios and the relative hydrogen bond energy difference can be calculated as listed in Table 3. The extra strength of the ionic hydrogen bond in the *cis* rotamer of **3** is at the lower end of the range found for other short ionic hydrogen bonds, i.e. 1.4–5.0 kcal mol^{-1} of additional energy compared to ordinary hydrogen bonds. Previously reported values of low-barrier hydrogen bonds were derived from studies of compounds in which the pK_a difference of hydrogen bond donors and acceptors was zero or close to zero. In the case of compound **3**, pK_a of the donor alcohol is predicted to be ∼11 and that of the acceptor carboxylate is predicted to be ∼4 (Advanced Chemistry Development ILAB software, v.3.6, www.acdlabs.com). Therefore, the pK_a difference is ∼7 and is much larger than the difference reported for molecules exhibiting low-barrier hydrogen bonds. Thus, ΔpK_a is not expected to be a critical factor for the hydrogen bond strength.

Models of truncated analogs (Fig. 3; CFF force field, unsolvated, Insight II software, v.2000, Accelrys, San Diego, CA) indicate that an intramolecular ionic hydrogen bond in both a *cis* and *trans* amide conformation is possible for compound **3**. The *cis* form was determined to be 5 kcal mol^{-1} lower in energy than the *trans* form in an *in vacuo* calculation. Solvation would be expected to diminish this energy difference but still favor the *cis* form, in agreement with experimental observations. Closer inspection of the modeled structures indicates that the *cis* rotamer exhibits a more ideal hydrogen bond configuration of length (2.7 Å) and angle (171°) than the corresponding values of the *trans* rotamer (2.8 Å, 165°). Because studies of hydrogen bond geometry and bond strength showed that bond strength dramatically decreases with decreasing O–H–O angle,[26,27] we propose that the more optimal hydrogen bond geometry in the

Table 3. Determination of the intramolecular ionic hydrogen bond energy difference in the *cis* and *trans* rotamer of compound **3** in various solvents

Solvent	Ratio of *cis*–*trans* rotamers[a]		$\frac{K_f^{HB}(cis)}{K_f^{HB}(trans)}$[b]	$-\Delta\Delta G_f^{HB}$[c] (kcal mol^{-1})
	K_{eq} (acid)	K_{eq} (anion)		
DMSO-d_6	1.2 : 1	12 : 1	10	1.4
CD_3CN	0.78 : 1	22 : 1	29	2.0
CD_3OD	0.77 : 1	4.8 : 1	6.2	1.1

[a] The values were calculated from Table 2. The concentration for **3** was 10 mg in 0.7 ml of DMSO-d_6 and 0.7 ml of CD_3CN and was 20 mg in 0.7 ml of CD_3OD at 25 °C.

[b] $K_f^{HB}(cis)/K_f^{HB}(trans)$ was calculated from Eqn (1).

[c] Values of $-\Delta\Delta G_f^{HB}$ were calculated from $\Delta\Delta G_f^{HB} = -RT \ln [K_f^{HB}(cis)/K_f^{HB}(trans)]$, where R is the gas constant (1.987 cal mol^{-1} K^{-1}) and T is the temperature in Kelvin (298 K).

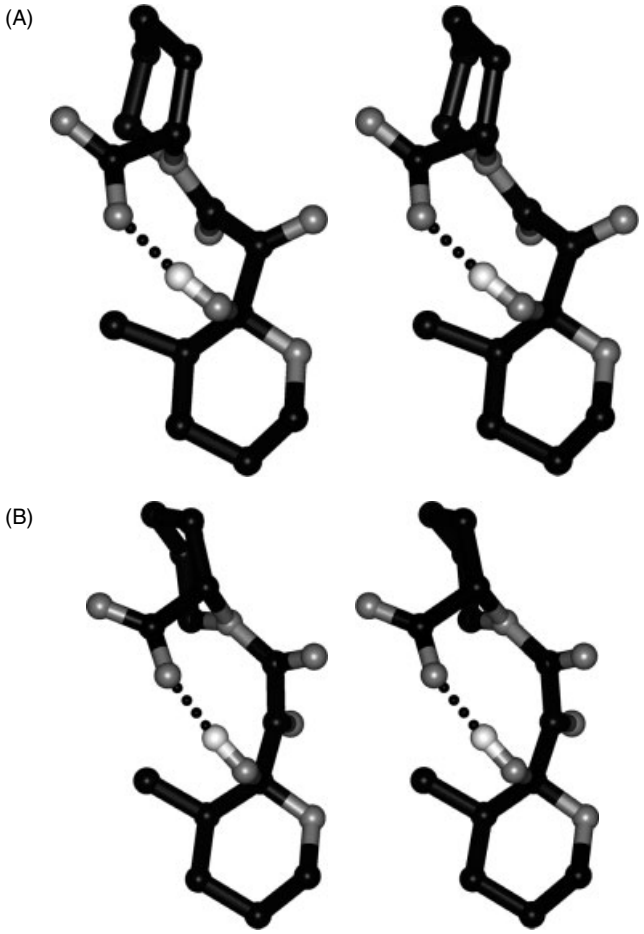

Figure 3. Stereoviews of energy-minimized models of truncated analogs of compound **3** in the *cis* (A) and *trans* (B) amide conformations. The O–H–O hydrogen bond lengths and angles are 2.7 Å and 171° for the *cis* isomer and 2.8 Å and 165° for the *trans* isomer. The *cis* isomer is calculated to be 5 kcal mol^{-1} lower in energy than the *trans* isomer.

cis rotomer plays a role in controlling the conformational equilibrium reported here.

CONCLUSIONS

Compound **3**, a derivative of rapamycin, exhibits an intramolecular ionic hydrogen bond between the tertiary alcohol and the carboxylate group. The resulting nine-membered ring exists in two conformational states resulting from *cis/trans* isomerism around the amide bond. An NMR-based spectral method was developed to evaluate the energetics of the conformational equilibrium under a variety of conditions. The predominance of the *cis* conformation was attributed to more idealized hydrogen bond geometry relative to the *trans* conformation. Extension of this analytical strategy to other chemical systems can be envisaged and this method may prove generally useful.

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