Differential Azobenzene Solubility Increases Equilibrium *Cis/Trans* Ratio in Water

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Accompanying text (200 x 1328 pixels)

Differential aqueous solubility of azobenzene shifts equilibrium to less stable *cis* isomer

Graphical Abstract (538 x 1328 pixels):

$$K = \frac{S_w(cis)}{S_w(trans)}$$

$$S_w(cis) = 5-40X S_w(trans)$$

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ABSTRACT

We have determined aqueous solubility (S_w) for purified *cis* and *trans* isomers of azobenzene (AB) and AB_n derivatives. S_w for *trans*-AB_n is 0.05-0.15 mM and the *cis* isomer is 6-40x more soluble. The effect of differential solubility is important for aqueous sample concentration $> S_w$ (*trans*) where *cis/trans* ratios >1 were observed via ¹H and ¹⁹F NMR. This phenomenon is general for AB_n(aq) that included semifluorinated AB_n and peptide conjugates. The van't-Hoff-Dimroth relationship predicts the equilibrium *cis/trans* ratio is proportional to the ratio of S_w values. Comparing NMR data to S_w values confirms the trend observed in the *cis/trans* ratios, but the magnitude of the ratio is lower. This phenomenon is observed in μmol concentration and may be a factor in photopharmacology. We have also studied the thermal *cis*-to-*trans* isomerization as a function of temperature for eight AB_n compounds. E_a values AB_n(aq) are ~ 25 kJ mol⁻¹ lower compared to E_a measured in nonpolar solvents and $\Delta S^\ddagger$ values in water are more negative so that aqueous rates are 2-5 times greater in water. This class of AB_n compounds generally show little solvent effect and this change in E_a is small compared to the behavior of donor-acceptor ABs. The inversion mechanism for this isomerization is supported by density functional theory.

1. Introduction

Azobenzene (AB) was first reported in 1834 by Mitscherlich [1], studied by Nobel in 1856 [2], and its photoisomerization reported by Hartley in 1937 [3]. AB is arguably the most studied organic chromophore in chemistry. AB exists as two geometric isomers with the *E* (*trans*) conformation 50 kJ/mol more stable than the *Z* (*cis*) isomer. The reader is referred to current reviews on AB [4,5] and potential applications in photopharmacology [6], photomechanics [7], biomolecule photoswitches [8] and photomodulation of macromolecules [9]. Structural classes of substituted AB based on spectral characteristics have been classified [4]. The compounds studied in this report are within “AB_n” group. Oft-cited property differences between *cis* and *trans* isomers include: dipole moment, molecular shape and end-to-end distance. One property that is implied but not specified is the differential solubility of the geometric isomers. Nonpolar AB_n compounds exhibit μmol aqueous solubility. This concentration regime is relevant for hybrid systems [10] and may be especially relevant in assessing AB-based drug candidates that are dosed at this concentration. One impetus for this study was the report by Chambers and Haworth [11] that aqueous solutions of amino acid conjugates **8** and **10** contain >30% *cis* under ambient laboratory conditions. They hypothesized that the *cis* isomer was stabilized by intramolecular interaction with the adjacent Pro residue. In this article we offer evidence for a solubility-controlled equilibrium that favors the more soluble *cis* isomer. We also report kinetic data and density functional theory results for the thermal *cis*-to-*trans* isomerization.

2. Results

2.1. Solubility

Compounds used in this study are shown in Scheme 1. The synthesis of **4-6** has been reported [12], **1**, **2**, **8**, **10** are commercially available and **3** was prepared in a conventional manner by the Mills reaction [5]. Compounds **10** and **8** are reagents in a collagenase assay [13]. The syntheses of **7** and **9** are described in supplemental material. Studies were performed using distilled water for **1-7** and amino acid conjugates were studied in 10 mM K₂HPO₄. Table 1 lists spectroscopic parameters and water solubility

(S_w , mM L⁻¹) for compounds **1**, **4-6** and **10**. The *cis* and *trans* isomers were studied separately following flash chromatography separation and isomeric purity was established by NMR. Beer-Lambert graphs (Figures 1, S4-S6) were made for sample concentrations < S_w . UV-vis characterization of saturated aqueous solutions afforded S_w .

Scheme 1. Compounds used in this study.

We acknowledge the compromise between expeditious study of *cis* isomer to prevent significant isomerization and establishing equilibrium solution concentrations. *trans*(aq) solutions remained stable while significant isomerization was observed for *cis*(aq) within a day. Our method of S_w measurements is time-consuming and we have concerns about achieving equilibrium concentrations for metastable solutions of the *cis* isomer. We explored two alternative methods that measure $\log P$ (P = water:*n*-octanol partition coefficient). There is a linear relationship between $\log S_w$ and $\log P$ [14]. The $\log P$ methods studied were 1) an ¹⁹F NMR analysis of *n*-octanol and water phases in classic partition experiment, and 2) reverse-phase HPLC (RP-HPLC). Linclau and co-workers [15] recently described a technique to determine $\log P$ for sparingly soluble, fluorinated compounds. ¹⁹F NMR of samples are drawn from a separated 2-phase mixture that also contains a $\log P$ reference fluorinated compound. We attempted this method several times and concluded that our semi-fluorinated compounds of interest (**3-6**) were too insoluble to achieve significant partitioning into water phase.

Because $\log P$ of a putative drug is one of the key molecular attributes for pharmacological activity, the pharmaceutical industry has developed models using linear free energy relationships and

retention times (t_R) from RP-HPLC [16-18]. Log P determination is also used in ground water contamination studies [19-21]. Log P values for **1** have been reported but most values are based on computed solubility from a linear free energy relationship. Log P values of 3.82 [17] and 4.11 [20] have been reported for **1** and log P = 3.4 for dipeptide **8** [17]. Using the method of Abraham [21] we obtained $S_w(trans)$ values of 0.07 and 0.03 mM for AB **1**, similar to our results in Table 1. The calculated log P of **8** corresponds to $S_w(trans)$ = 0.26 mM. Literature studies do not distinguish geometrical isomers. Yet, it is common for chemists to analyze or purify *cis/trans* AB_n mixtures via HPLC and the isomers exhibit significantly different elution times. To properly assess RP-HPLC for S_w measurements of AB_n, we established a log P correlation using a ‘training’ set of 20 reference compounds and subsequently analyzed 16 AB_n compounds from our laboratory. See supplemental for t_R values of all compounds (Table S1). The t_R values for the *trans* isomers fell within a small range, as did values for *cis* isomers; $t_R(trans)$ = 40 ± 4 and $t_R(cis)$ = 30.0 ± 4 min. There is no or little correlation between t_R and either μ or S_w . Compound **3** was specifically studied to assess a correlation with μ because it is one of the few AB_n compounds where the computed $\mu(trans) > \mu(cis)$ (Table 4). For all compounds studied, the *cis* isomer elutes first; the lowest $\Delta t_R(cis-trans)$ was observed for **3**, but no consistent trend was observed for **1,2,4-10**. We have concluded that RP-HPLC is not a viable technique to obtain log P values for this group of compounds.

Figure 1. UV-vis spectra for (a) *trans*-1 and (b) *cis*-1 in water at 23°C.

2.2. NMR Studies of ABn(aq)

The μmol aqueous concentrations of samples of **1-7** require long NMR acquisition times and suppression of water resonance. Amino-acid conjugates **8-10** could be prepared at higher concentrations which facilitated NMR studies. We replicated the results of Chambers and Haworth [11] for samples of **8** and **10** in buffered D_2O (Figure 2). If no measures were taken to exclude ambient lighting from samples, we observed a *cis/trans* ratio of 0.4 after 1 day. However, if freshly prepared solutions were handled in the dark, the initial *cis/trans* ratio (~ 0.05) remained unchanged over 2 weeks. Once the NMR samples are handled and stored under ambient laboratory lighting, the value of the *cis/trans* ratio increased to 0.4 within 24 h. Compound **9** has the reverse order of amino acids relative to **8** and behaved similarly (Figure S7). This observation that AB_n(aq) contains stable and moderately high amounts of *cis* isomer is a *general* phenomenon and was also observed for aqueous solutions of **1,4** and **6**. ^1H NMR analysis of parent AB_n **1** displays a *cis/trans* ratio of 2.0; based on ^{19}F NMR analysis, the ratios for aqueous solutions of **4** and **6** (Figure S8) were 1.2 and 0.7. Peaks in the ^{19}F NMR spectrum were assigned based on prior work.⁷ UV irradiation of NMR samples were recorded to observe changes in peak intensity for *cis* and *trans* (see Figure S8).

Figure 2. 500.13 MHz ^1H NMR in D_2O with 10 mM KH_2PO_4 buffer 23°C: (a) 10 mM AB-Pro-Leu dipeptide (**8**), (b) 10 mM AB-Pro-Leu-Gly-Pro-D-Arg (**10**). Spectral identification: Lower-as prepared, Middle-14 days in dark, and Upper-1 day under ambient lighting.

2.3. Temperature-dependence of thermal *cis*-to-*trans* isomerization

To better understand the behavior of $\text{AB}_n(\text{aq})$, we measured the temperature dependence of the rate for thermal *cis*-to-*trans* isomerization. Experiments were run using saturated aqueous solutions prepared from purified *trans* isomer. Rates are the average of at least two independent runs. The data in Table 2 was obtained from Arrhenius (not shown) and Eyring plots (Figures 4, S9); error values are the uncertainty estimates of linear least squares fitting. Concentration changes in either the *cis* or *trans* isomer followed first order kinetics. We did not study the kinetics of compound **3** or **4**.

To validate our experimental technique, we measured the isomerization kinetics of AB_n **1** and **2** in toluene for comparison to literature values reported for benzene solutions. For **1** and **2**, E_a values of 96 ± 4 and 92 ± 4 kJ mol^{-1} have been reported [25,26]; which are nearly identical to our results. The activation enthalpies are similar in magnitude and entropies more negative than the values reported by Otruba and Weiss [27] for isomerization performed in benzene. The decrease in E_a is balanced by a decrease in entropy so that $\Delta G^\ddagger$ is similar for the compounds in Table 2. For the three amino acid conjugates, neither the order of amino acids (**8** vs. **9**) nor length (di- versus penta-peptide) affected activation parameters.

Values for aqueous E_a are similar with average $E_a = 68 \pm 5.9 \text{ kJ mol}^{-1}$; similarly, close values are also observed for $\Delta H^\ddagger$ and $\Delta S^\ddagger$.

Figure 3. (a) 400.13 MHz ^1H NMR spectrum of 0.05 mM **1** in D_2O . (b) 376.45 MHz $\{^1\text{H}\}^{19}\text{F}$ NMR spectrum of compound 0.05 mM **4** in D_2O ; 23°C.

Figure 4. Eyring plots for the thermal cis-to-trans isomerization of **1** in (a) water and (b) toluene; rates based on increase in absorbance at 320 nm.

2.4. Density Functional Theory (DFT)

DFT was used to calculate dipole moments of *cis* and *trans* isomers. For AB_n **2**, **7**, and **8**, the transition structure was determined. Computational methodology was partially based on Hecht, Saalfrank and coworkers [28]. One of the conclusions of their study is that the inversion pathway is preferred for AB_n compounds. Table 3 summarizes computations performed at B3LYP/6-311+G(d) level of theory [29] using the Gaussian 09 program package [30]. Dipole moment results were obtained both in gas phase and in water using a polarizable continuum model [31]. Comparison of Tables 2 and 3 shows no correlation between μ and 1) isomerization rate, 2) S_w or 3) HPLC t_R . The dipole moments of **8-10** containing amino acid substituents are arguably more reflective of the amino acid side-chain dipole moment rather than the *cis/trans* geometry of the AB. In this analysis, we have not included back polarization of the solute on its own reaction field or the effect of the solute's quadrupole moment.

Similar to the transition state geometries reported by Hecht, Saalfrank, *et al.* [27], DFT was also the transition state structures of **2**, **7** and **8** (see Figure 5) indicate an inversion mechanism with a 180° bond angle for N=N-C and 90° dihedral angle for C-N=N-C. Transition state structures were the same for gas phase and water. The computed barriers for thermal *cis*-to-*trans* isomerization (Table 4) are 79-96 kJ mol⁻¹ which compare reasonably well with barriers calculated from $\Delta H^\ddagger$ and $\Delta S^\ddagger$ in Table 2. One notable feature of Table 4 data is the *higher* transition state μ relative to $\mu(cis)$ which argues that polar media should lower the activation barrier and increase rates.

Figure 5. *Ab initio* structure of the dipeptide **8** transition state for the thermal *cis-to-trans* isomerization, 298.15°K in the gas phase (B3LYP/6-311+G(d)).

3. Discussion

The aggregate of our results for AB_n under aqueous conditions can be summarized: 1) *cis*AB_n is 5-40x more soluble than the *trans* isomer, 2) for sample concentrations > *S_w* (*trans*), differential solubility can shift the *cis-trans* equilibrium in favor of *cis*, 3) amino acid conjugates of AB_n are similar in reactivity and mechanism to other AB_n, and 4) rates in water are slightly higher due to lowered *E_a* and higher negative entropies.

Given the number of literature reports on AB-peptide conjugates, it is important to fully understand model systems like **8-10**. Chambers and Haworth [21] observed ~30% *cis* for NMR samples of **8** and **10** in buffered D₂O; the authors postulated intramolecular stabilization of the *cis* isomer. Our results do not support this hypothesis; instead we propose a solubility-driven isomerization. We have measured the magnitude of differential *cis/trans* solubility in water for 4 AB_n compounds. To the best of our knowledge, this is the first report of *S_w* values for both *cis* and *trans* isomers of AB_n systems since

1938. Only two previous reports are known and they only reported the parent AB **1** [23,24]. Our values for S_w are similar to Hartley's data that was obtained with a photometric null instrument [23].

Solubility-driven isomerization is a *general* phenomenon and was also observed in **1,4** and **6** with *cis/trans* equilibrium ratios of 0.7 – 2.0. The *trans-to-cis* isomerization is a photochemical process that only requires several hours in ambient laboratory lighting to occur. This phenomenon can be represented by the following equilibria for sample concentrations below $S_w(cis)$:

The influence of solvent on equilibria based on differential solubility is known [32]. The van't-Hoff-Dimroth relationship states that two interconverting isomers are in equilibrium when the ratio of their concentrations is proportional with that of their solubilities (Eqn. 1) [33]. From Eqn 1, the predicted equilibrium *cis/trans* ratios based on S_w can be compared to NMR data reported here (Table 5). The observed ratios increase in the same order as predicted values from S_w but are 20x lower than predicted which could be caused by NMR samples not reaching equilibrium. NMR analyses of $\text{AB}_n(\text{aq})$ was performed within 24 h.

The differential solubility may be less pronounced for AB conjugates where AB is only a small part of the structure; but there will be micro-environmental effects that may still be important or polarity changes that are not linear. For AB_n systems **1,4-6**, $S_w(\text{trans}) \sim 50\text{-}150 \mu\text{mol}$ which lies within a concentration range sometimes used in photopharmacology.

The thermodynamics of the thermal *cis-to-trans* isomerization indicate that E_a (water) is 25-33 kJ mol⁻¹ lower compared to nonpolar solvents. E_a values were similar for the compounds studied in water with an average $E_a = 68 \pm 5.9 \text{ kJ mol}^{-1}$. The values for $\Delta S^\ddagger$ are negative and the magnitude is higher than typical values for AB_n and indicate a higher loss of entropy in for isomerization in water which may be

attributable to organized water molecules around a polar transition state. The lower E_a (water) is counterbalanced energetically by the observed entropy decrease so that the net effect is that isomerization rates are slightly higher in water. The methoxy AB_n **2** exhibits the fastest rate at 298.15 K. These results are opposite to the rate effect reported by two groups. De Maria and co-worker [34] reported lower aqueous rates by a factor of 0.1-0.5 x compared to benzene; 100% water rate values were obtained by extrapolation of rates in methanol:water mixtures. Halpern, *et al* [35] reported 6x lower rates in water for AB **1** but water was the one solvent that did not correlate properly with internal solvent pressure. The authors suggested anomalies in water may be attributable to hydrogen ions. Arguing against that effect in this study are similar kinetics obtained in distilled water (**1,2,4-7**) versus the conjugates in 10 mM phosphate buffer. Our data for E_a in water is nearly identical to the value reported by Borisenko and Woolley [36]. They reported $E_a = 74.1 \pm 5.9 \text{ kJ mol}^{-1}$ (10 mM phosphate buffer) for a model cross-linker (azobenzene core is doubly substituted with amide groups) used in peptide conjugation. The cross-linker structure contains a 4,4'-bisamidoazobenzene unit which has similar reactivity to AB_n compounds studied here.²⁸

Supported by *ab initio* calculations, the mechanism of thermal *cis*-to-*trans* isomerization follows the inversion pathway. In 2 of the 3 systems studied by DFT, the transition state structures had higher μ than the *cis* isomer which argues for faster rates in water. There is a good correspondence between experimental $\Delta G^\ddagger$ and *ab initio* values modeled in the gas phase. The computed values using a CPM water model are several kJ mol^{-1} higher than experimental values.

4. Conclusion

The physical organic chemistry of sparingly soluble AB_n compounds has been studied in water. The *cis* isomers are 5-40 times more soluble which can manifest itself in shifting the *cis/trans* equilibrium toward *cis*. This is a general phenomenon that was observed in semifluorinated compounds and amino acid conjugates. AB_n solubility for the *trans* isomers is 50-100 μmol , which is a concentration range near

or slightly above many photopharmacological studies. The *cis/trans* differential solubility should be a consideration in those studies.

5. Experimental Section

5.1. Kinetics. Photochemical kinetic experiments were performed with an Ocean Optics component system and an Oriel 66901 50-200W Research Arc Lamp mounted to irradiate sample perpendicular to monitoring direction. The Oriel lamp output was cooled with a water filter and sample temperature was verified by thermocouple immersion into cuvette. Filters used for UV and visible were obtained from Newport Corporation; Figure S10 displays the transmission characteristics of these two filters. Kinetic samples were aqueous solutions saturated with the *trans* isomer and were used immediately after filtration through 0.45 μ nylon syringe filters. Samples were 1) temperature equilibrated for 10 min prior to UV irradiation, 2) irradiation was continued until the photostationary state (PSS) was reached, and 3) after irradiation, data was collected by time evolution of the intensity at one wavelength while excluding stray laboratory light. The wavelength chosen was sample dependent but generally the $\lambda_{\text{max}}(\text{trans})$ was monitored. At least two and usually three separate runs were taken at one temperature. The averaged rate values were plotted as function of temperature using either Arrhenius plot ($\ln k$ vs. $1/T$) or Eyring plot ($\ln(k/T)$ vs. $1/T$).

4.2. Computational Details. Density functional theory (DFT) calculations were performed with the 6-311+G(d) basis set and the restricted B3YLP functional [29] implemented in Gaussian 09 [30]. Calculations were done without solvent (gas phase) and with water using the polarizable continuum model [31]. Ground and transition states energies are zero point energies (Sum of Electronic and Thermal Free Energies in G09 output). Vibrational analysis was used to confirm ground state (0 imaginary frequencies) and transition state (1 imaginary frequency) structures. Transition state structures were identified and confirmed by multistep process: 1) potential energy scans about CCNN dihedrals, 2)

geometry optimization while holding dihedral angle at fixed value until desired level of theory converges with Berny algorithm [37], 3) unconstrained geometry optimization, and 4) frequency analysis.

4.3. Reverse-Phase High Pressure Liquid Chromatography (RP-HPLC). Chromatography was performed with an Agilent Prostar Purification System equipped with a Waters Delta-Pak C18 300Å (39 x 300 mm). A gradient elution methanol:water was used; over 50 min, the composition was linearly changed from 1:9 methanol:water to 100% methanol. Samples were prepared in 9:1 methanol:water containing ~1% compound and were prepared by adding solvent to dried film (sample administered as chloroform solution followed by solvent evaporation). After 30 min sonication, the samples were filtered through a 25 µ GFP syringe filter. Literature log *P* values were obtained from standard databases [38]. The training set of reference compounds (**Table S1**) and data for unknown AB_n compounds (**Table S2**) are provided in supplementary materials.

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Notes

The authors declare no competing financial interests.

7. References

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Table 1. Solubility and Spectroscopic Parameters of AB_n Derivatives in Water at 23°C

Compound	<i>trans</i>			<i>Cis</i>		
	π - π^* λ_{max} (nm)	ϵ^a (mM ⁻¹ cm ⁻¹)	S_w , mM	n- π^* λ_{max} (nm)	ϵ^a (mM ⁻¹ cm ⁻¹)	S_w , mM
1	319	10.7 ± 0.9	0.05 (0.02, 0.035) ^b	427	1.5 ± 0.1	2.0 (0.65, 1.80) ^a
4	350	5.5 ± 0.1	0.06	428	1.47 ± 0.02	1.2
5	328	12.0 ± 0.3	0.15	428	0.8 ± 0.2	0.4
6	343	5.0 ± 0.8	0.07	426	1.5 ± 0.2	0.7
8	324	19.0 ± 1.0	[0.27] ^c	422	1.1 ± 0.3	[14] ^d

^a The reported error is from linear regression uncertainty estimates. ^b S_w literature values, see Ref. 23,24.

^c Ref. 17, estimated S_w . ^d Maximum conc. under active UV irradiation that remained clear, estimated S_w .

Table 2. Temperature dependence of the rate of thermal *cis*-to-*trans* isomerization for azobenzene derivatives in water (except as noted).

Compound	E_a , [kJ mol ⁻¹]	$\Delta H^\ddagger$ [kJ mol ⁻¹]	$\Delta S^\ddagger$ [J mol ⁻¹ K ⁻¹]	k [s ⁻¹] x 10 ⁶ , 298.15°K	$\tau_{1/2}$ [h], 298.15°K
1	65.3 ± 3.3	62.3 ± 3.3	-138 ± 12	4.3	45
2	62.8 ± 4.2	58.6 ± 8.4	-134 ± 13	34.2	6
5	70.7 ± 4.2	67.4 ± 1.7	-117 ± 14	6.3	31
6	77.8 ± 1.7	74.9 ± 3.3	-109 ± 8	1.0	200
7	67.0 ± 2.9	62.3 ± 1.3	-130 ± 4	9.8	20
8^a	68.2 ± 3.3	65.7 ± 2.5	-126 ± 4	7.5	26
9^a	63.2 ± 3.3	62.8 ± 4.2	-138 ± 13	9.3	21
10^a	74.9 ± 2.5	71.5 ± 3.8	-105 ± 8	4.5	43
1 (toluene)	96.2 ± 4.2	92.0 ± 4.1	-59 ± 4	0.3	550
2 (toluene)	90.8 ± 2.5	87.9 ± 4.0	-54 ± 8	3.9	50

^a 10 mM phosphate buffer.

Table 3. Computed dipole moments of *cis* and *trans* AB_n in water at 298.15°K and RP-HPLC retention times.^a

Compound	<i>Trans</i>		<i>Cis</i>	
	μ , D	HPLC t_R , min	μ , D	HPLC t_R , min
1	0.0	42.8	4.6	30.0
2	2.8	43.0	5.4	30.9
3	4.8	48.0	3.7	44.3
4	3.4	38.0	8.0	25.6
5	3.4	42.6	7.6	30.6
6	2.2	40.7	6.8	32.1
7	3.0	31.7	5.6	18.7
8	4.4	32.1	6.0	22.0
9	2.3	49.1	11.7	42.5
10	9.3	40.7	6.5	31.5

^a B3LYP/6-311+G(d), PCM in water.

Table 4. Transition state dipole moments and energies for *cis*-AB_n thermal isomerization in water and gas phase at 298.15°K.^a

Compound	<i>Trans</i>		<i>Cis</i>		<i>Transition state</i>		
	μ , D	Energy, J mol ⁻¹	μ , D	Energy, J mol ⁻¹	μ , D	Energy, J mol ⁻¹	$\Delta G^\ddagger$ ^b J mol ⁻¹
Gas phase							
2	2.3	0.0	4.3	71.1	4.9	151.5	80.3
7	2.3	0.0	4.3	63.6	5.3	149.4	85.8
8	3.6	0.0	4.7	65.7	6.5	154.8	89.1
Water							
2	2.8	0.0	5.4	60.7	6.9	156.5	95.8 (96.2)
7	3.0	0.0	5.6	51.5	6.8	149.0	97.5 (104.6)
8	4.6	0.0	7.2	59.8	6.4	150.6	90.8 (140.6)

^a B3LYP/6-311+G(d), PCM in water. ^b $\Delta G^\ddagger = E^\ddagger - E(\text{cis})$, barrier for thermal *cis*-to-*trans* isomerization; experimental values calculated from Table 2 data shown in parentheses.

Table 5. Predicted and Observed Equilibrium [cis]/[trans] Ratios in Water

AB _n	Equilibrium <i>cis</i> aq/ <i>trans</i> aq	
	$S_w(\text{cis})/S_w(\text{trans})$	NMR [cis]/[trans]
1	40	2
4	20	1.2
6	10	0.7