Thermodynamic Description of a Contact and Solvent-Separated Ion Pair as a Function of Solvation: A Model for Salt Bridges and Proton-Transfer Reactions in Biology

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Abstract: Solvation free energies for 1α-amino-8aβ-methylbicyclo[4.4.0]decane-7α-carboxylic acid (**1**), a *cis*-decalin amino acid that populates two conformers in which either an intramolecular contact or solvent-separated ion pair is formed, have been determined in a wide range of solvents ($CDCl_3$ to D_2O). The nature of the ion pairs, and the range of conditions evaluated, mimicked ion pairs found in biomolecules ("salt bridges"). Conformational and proton-transfer equilibria were evaluated from changes in ^1H NMR coupling constants and chemical shifts, respectively, and the ion pair ΔGs could be extracted directly. Correlations of ion pair ΔGs with solvent polarity scales ($E_T(30)$ values) and solvent hydrogen bond acidities demonstrated the importance of stabilizing the carboxylate ion in low-polarity solvents. Comparisons of ion pair stability for contact and separated ion pairs revealed that the electrostatic attraction is secondary to relative solvent dielectric and hydrogen bond acidity at stabilizing the interaction; conversely, solvent hydrogen bond basicity did not contribute to the stabilization. The failure of bulk solvent properties, such as the Kirkwood–Onsager dielectric ϵ_K, to adequately correlate ion pair energetics (and previously, hydrogen bond energetics: Beeson, C.; Pham, N.; Shipps, G.; Dix, T. A. *J. Am. Chem. Soc.* **1993**, *115*, 6803) indicated the limited applicability of macroscopic electrostatic models; rather, the correlations between electrostatic ΔGs and empirical solvent parameters amplified the need for molecular solvation models. Ultimately, the sensitivity of electrostatics to solvent donor–acceptor properties argues that a successful treatment of protein structure must be done on the molecular level, by evaluating local interactions and solvation. The results thus have significance for a description of electrostatic interactions in biological structure and function.

Introduction

The role of electrostatic interactions in protein architecture, ligand–receptor binding phenomena, and enzyme catalysis has been well documented.[1] A particularly relevant interaction is the "salt bridge" that can form between ammonium and carboxylate ions. Salt bridges have clearly defined roles in the organization of multimeric proteins and complexes; classic examples include the charge networks that mediate the differential oxygen affinity of hemoglobin[2] and that assure the proper alignment of hemes in cytochrome complexes.[3] Electrostatic fields, as generated by individual charged residues or intact salt bridges, are intimately involved in substrate binding and transition-state stabilization by enzymes.[4,5] The free energy available from these interactions is considerable but quite variable: contributions of salt bridges to the thermal stabilities of proteins, for example, range from 0.2 to 3 kcal mol^{-1} for those with substantial access to solvent (the most common environment),[6] while the range extends to 5 kcal mol^{-1} for a rare example of a solvent-restricted salt bridge in which "solvation" is provided exclusively by the protein's local, largely hydrophobic, structure.[7] The study of biomimetic molecular models has contributed to the understanding of these interactions; for example, the structural roles of salt bridges have been demonstrated in model peptide turns[8] and helices.[9] In an extensive study of salt bridge energetics, the intrinsic free energy (ΔG_i) loss for binding of carboxylate, phosphate, sulfate, and phenoxide ions with various ammonium ions in water was estimated to be about 1.2 kcal mol^{-1}.[10] A catalytic model also has been reported in which the electrostatic field of a salt bridge was used for transition-state stabilization.[11] While a significant body of data exists, a clear link between these specific observations and general treatments for protein ion pair energetics has yet to be made, although purely theoretical approaches have proven to be successful.[5c]

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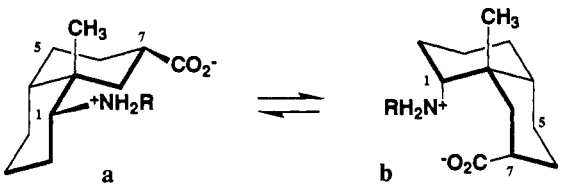

Figure 1. Chemical structure of the *cis*-decalin amino acids **1** (R = H) and **2** (R = CH_3) illustrated as the diequatorial (a) and diaxial (b) zwitterion species. Carbons 1, 5, and 7 are labeled for text reference.

At a first level of approximation, the well-developed theories[12] for the solvation of inorganic ion pairs could be applied to biological salt bridges. However, these studies do not consider explicitly the local environment of the ions and ammonium–carboxylate ion pairs have an added level of complexity: proton-transfer tautomerization. Indeed, proton transfer within an ion pair is a significant biological reaction, with roles in, for example, photochemical signal transduction, receptor activation, and proteolysis.[13] A variety of solvent studies of inter- and intramolecular proton transfers between amines and acids have correlated the equilibrium constants (K_{PTS}) with electronic, steric, and solvation parameters.[14-23] These studies have focused primarily on contact ion pairs (which predominate in nonaqueous solution);[12] evaluations of separated ion pairs (*i.e.*, those without a hydrogen bond) have been limited. The lack of such studies reflects the relative instability of separated ion pairs; in the few cases in which an intramolecular, separated ion pair was evaluated, aggregates stabilized by intermolecular contact ion pairs were observed.[15-23]

A better understanding of the role of solvation in controlling the free energies of salt bridges would thus greatly enhance our understanding of biological structure and function. The *cis*-bicyclo[4.4.0]decane (*cis*-decalin) amino acid **1**, in which the ammonium and carboxylate ions alternate between a solvent-separated and contact ion pair (**1a** and **1b**, respectively, Figure 1), was conceptualized as a conformation-based probe for the analysis of ion pair energetics. The design and synthesis of **1**, a specific example of a general approach to the analysis of electrostatic interactions,[24] have been described previously.[24d] The *N*-methyl derivative of **1** (**2**, Figure 1) also was prepared to probe the energetics due to perturbation of the ion pair solvation sphere.

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Table I. Molecular Mechanics Estimated Relative Energies for the *cis*-Decalin Amino Acids **1** and **2**

species	$\Delta E_{(g)}$[a] 1	2
classical (gas phase)	5.8	6.2
zwitterion (gas phase)	−28.1	−25.7
zwitterion (aqueous)[b]	−0.5	−0.8

[a] Relative energy (kcal mol^{-1}) for the conformational equilibrium illustrated in Figure 1. [b] Calculated with the semianalytical solvation algorithm[26] incorporated into Macromodel.[25]

In this paper, ^1H NMR analyses of **1** and **2** in a wide range of solvents are described, conformational and proton-transfer equilibria have been calculated, and the corresponding ΔGs have been correlated with solvent characteristics. The ΔGs calculated for **1** and **2** directly calibrate the relative energetics of contact and separated ion pairs and address the relevance of micro- versus macroscopic treatments of solvation. The results have implications for the description of ion pairs in biology.

Results

Molecular Modeling. As previously described,[24d] placement of the charged groups on the *cis*-decalin skeleton of **1** was designed to counterbalance steric relief in one low-energy conformer with the anticipated free energy loss in forming a contact ion pair in the other low-energy conformer. Accordingly, determining the magnitude of salt bridge ΔGs is dependent on estimating the steric bias between conformers. Relative energies for **1** and **2**, estimated from molecular mechanics[25] calculations, are listed in Table I. Minimizations for the classical (nonionized) forms were done with a gas-phase, distance dependent, dielectric; minimizations of the zwitterion structures were done in the same manner and with the semianalytical aqueous solvation algorithm[26] recently incorporated into Macromodel.[25] Calculated structures of the zwitterions minimized in the gas phase were torsionally distorted to maximize the ion pair interaction; in "solution" the ring structures were relaxed. Of interest is that the relative geometry of the ammonium and carboxylate in the zwitterion differed between minimizations; a single ammonium to carboxylate ion hydrogen bond was formed for each oxygen in the gas phase, while in "solution", the carboxylate was rotated 90° such that one oxygen bifurcates two ammonium hydrogens. The relative ammonium–carboxylate geometry in the minimized structure for the classical form was that of the "solution" structure of the zwitterion. The calculated N^+O^- distances for **1** and **2** ranged from 2.9 to 3.0 Å and from 5.4 to 5.7 Å in the contact (diaxial) and separated (diequatorial) ion pairs, respectively.[27]

Synthesis. The synthesis of amino acid **1** has been described.[24d] In the preparation of **2**, lactam[24d] **3** (Figure 2) was methylated to give *N*-methyl lactam **4** (Figure 2). Vigorous acidic hydrolysis of **4** provided the *N*-methyl amino acid **2** (80–90% yield). Ion-exchange chromatography of the hydrochloride salts of **1** and **2** induced a small amount of reversion to the corresponding lactams. Presumably, exposure of the zwitterion to nonpolar media (*i.e.*,

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(27) The shorter distances correspond to the zwitterions minimized in the gas phase, and the larger distances correspond to the zwitterion minimized in "water" and the classical species in the gas phase. The existence of a salt bridge has been previously defined by the distance between charged atoms; investigators have used either the explicit N^+O^- distance or the distance between nitrogen and a bisector of the two carboxylate oxygens ($N^+\langle O^-$)). The maxima in normalized plots of the frequency of occurrence of oppositely charged residues relative to distance obtained from statistical analyses of protein X-ray structures generated maxima at 4–5 Å, and thus, a minimum N^+O^- or $N^+\langle O^-$ distance of 4 Å has been used commonly as a criterion to identify a salt bridge.[6]

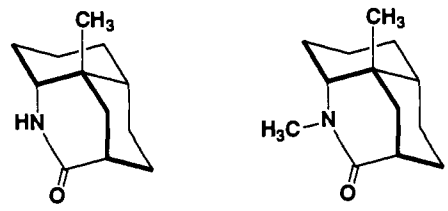

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Figure 2. Chemical structures of the *cis*-decalin lactams **3** and **4**.

the exchange resin) induced proton transfer and subsequent amidation. Indeed, the free base of the corresponding amino esters of **1** and **2**, which would have been useful for structural comparisons, could not be isolated due to spontaneous lactamization.

Conformational and Proton Transfer Equilibria. *cis*-Decalin amino acids **1** and **2** potentially exist as an ensemble of four species: classical and zwitterionic forms of the two chair–chair conformations (as illustrated for **1** in Figure 3). The diaxial conformation of the classical species would not be significantly populated due to its high steric energy (Table I); thus, three species must be evaluated: the diequatorial zwitterion **A**, the diequatorial classical species **B**, and the diaxial zwitterion **C** (Figure 3). A thermodynamic cycle was generated between all species in which water was chosen as the reference state to take advantage of available data for aqueous amino acid solvation. The estimated relative energies, 5.8 and 6.2 kcal mol^{-1} for **1** and **2**, respectively (Table I), were taken for the ΔG between the diequatorial and diaxial conformations of the classical species (ΔG_{ab}, Figure 3).[28] The ΔGs for transfer of the classical species from organic solvent to water (ΔG_{4a} and ΔG_{4b}, Figure 3) are not accessible from either theory or experiment. At a first level of approximation, it was assumed that ΔG_{4a} and ΔG_{4b} were equal due to the similar volume and solvent accessible hydrophobic/hydrophilic surface areas for the two conformations;[25] this is an implicit advantage in the use of conformational equilibria to evaluate solvation effects.[29] Conceivably, solvation of the two conformations of **1** for each solvent employed could be calculated from the surface areas; however, such treatments[26,30] as yet do not encompass a wide range of solvents. Thus, ΔG_{4a} and ΔG_{4b} were set equal to 0 with the reasonable assumption that ΔG of transfer for the classical species was much smaller than for the zwitterions.[7] Clearly, these values will not be 0; the corresponding error, however, was anticipated to be much smaller than the ΔGs of interest (*vide infra*).[31]

The aqueous ΔGs for ionization of the diequatorial and diaxial conformations, ΔG_{1a} and ΔG_{1b} (Figure 3), respectively, were estimated with the relation[7]

$$\Delta G_1(r) = \Delta\Delta G(r) + \Delta^{+-}$$

in which ΔG of ionization for an amine and carboxylic acid at infinite separation Δ^{+-} (–9 kcal mol^{-1}) has been calculated from ionization equilibria.[7] Similarly, $\Delta\Delta G(r)$, the difference in ΔG for an ammonium–carboxylate ion pair at a separation r relative to infinite separation, has been calculated from the pK_as of amino acids with differing chain lengths in water ($\Delta\Delta G(r) = -1$ and –3 kcal mol^{-1} for $r = 5$ and 3 Å, respectively).[7] The ΔGs for proton transfer within amino acids **1** and **2** in organic solvents were calculated from experimentally determined relative populations of **A**, **B**, and **C** (Figure 3) with the relations

$$\Delta G_{3a} = -RT \ln [B][A]^{-1}$$

$$\Delta G_{3b} = \Delta G_{ab} - RT \ln [B][C]^{-1}$$

The corresponding ΔGs for transfer of the diequatorial and diaxial zwitterions from water to the organic solvent, ΔG_{2a} and ΔG_{2b}, respectively, were calculated from the thermodynamic cycle; thus, the $\Delta\Delta G$s between the two zwitterions in the organic solvent were calculated from the differences between ΔG_{2a} and ΔG_{2b}. The conformational and proton-transfer equilibria for **1** and **2** were evaluated from ^{1}H NMR spin coupling and chemical shifts, respectively. Spectral data for **A** (Figure 3) were obtained directly from spectra obtained in D_2O;[32] however, spectral information was also required for the diaxial conformation **C**. Spectra of lactams **3** and **4** were thus used to estimate spectral data for the diaxial conformation of amino acids **1** and **2**, respectively. The two most useful resonances were those corresponding to the H-1 and H-7 protons (Figure 1), which directly reported on the ionization state of the amine and carboxylic acid, respectively, and underwent substantial changes in coupling between the diequatorial and diaxial conformations.

Calculations of the proton-transfer equilibria directly from the H-1 and H-7 chemical shifts were complicated by their intrinsic solvent dependence. In ^{1}H NMR evaluations of conformational equilibria in different solvents, it has been demonstrated that changes in chemical shifts ($\Delta\delta$s) observed in different solvents could be dissected into two components: one intrinsic to the solvent and one due to the conformational equilibrium.[33] For example, the resonance for a proton whose magnetic environment was not altered by the conformational transition would produce only an intrinsic solvent dependent $\Delta\delta$ in each solvent. The observed $\Delta\delta$ for a second proton, whose magnetic environment was affected by the conformational transition, could be corrected with the intrinsic solvent $\Delta\delta$ from the first proton to reveal the conformational $\Delta\delta$. Clearly, the validity of this method depends on the assumption that the intrinsic solvent dependent $\Delta\delta$ is identical for the two protons that were compared; although this is unlikely to be universally true, this method has been employed quite successfully in a number of studies.[33] (A structural model incapable of the conformational transition could also have been used to estimate the intrinsic solvent $\Delta\delta$.) To calculate proton-transfer equilibria for **1**, a proton resonance unaffected by the ionization state was sought. The ^{1}H NMR chemical shift for the H(5) resonance of **1**, partly shielded by the angular methyl from the proton transfer, shifted little during a complete NaOH titration of the amino acid hydrochloride. Thus, differences between δH-(5) and the resonances of interest ($\Delta\delta^{H(5)}$) were used to evaluate proton-transfer equilibria. The $\Delta\delta^{H(5)}$ values for the classical form of **1** were estimated from chemical shifts of the sodium and hydrochloride salts in D_2O, respectively;[34] observed $\Delta\delta^{H(5)}$ values in polar solvents (*i.e.*, alcohols or DMSO) were used to calculate

(28) The use of molecular mechanics energies introduces an error in the absolute value of ΔG that is canceled in the comparison of relative ΔGs. The inferred equality of ΔG_{ab} in aqueous and organic solutions (Figure 3) is only an approximation partly justified by similar volumes and surface areas for the two conformations (see text).

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(31) The magnitude of the error could be estimated with a comparison of the deviation of estimated ΔGs from linear correlations (see text) between a highly polar and nonpolar solvent (*i.e.*, CD_3OD and $CDCl_3$), in which ΔGs of transfer would be anticipated to differ the most. As seen in Figure 4, such deviations are small.

(32) The steric bias (Table I) assured complete population of the diequatorial conformation for the zwitterion (*i.e.*, **1a**, Figure 1) in the absence of a significant ion-pairing interaction, as anticipated for D_2O solutions.

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(34) During a NaOH titration of the hydrochloride salt of **1** in D_2O, the $\Delta\delta^{H(5)}$ value for the H-7 resonance was constant as the ammonium ion was titrated; thus, $\Delta\delta^{H(5)}$ for H-7 is unaffected by the ionization state of the amine. The $\Delta\delta^{H(5)}$ value for the H-1 resonance of the sodium salt of **1**, however, required a +0.14 ppm correction to account for the change in the H-1 $\Delta\delta^{H(5)}$ value induced by titration of the carboxylic acid.

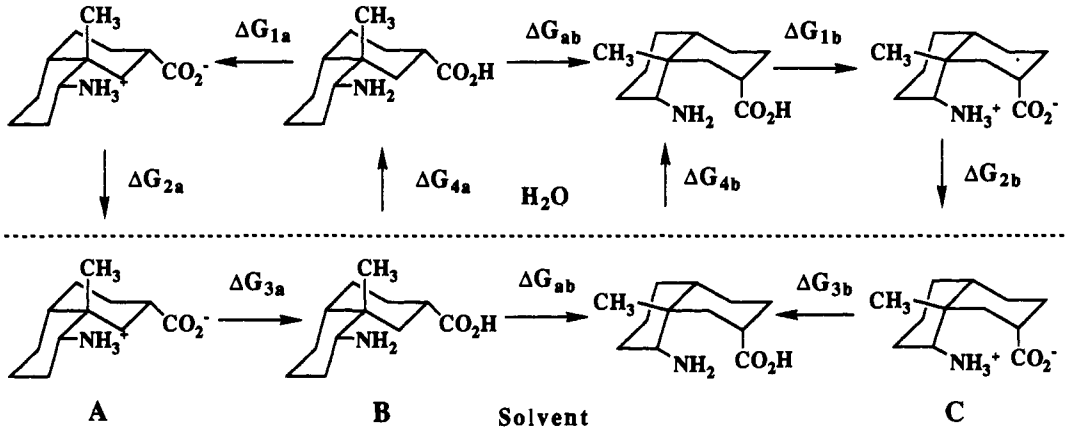

Figure 3. Thermodynamic cycle for the evaluation of the proton-transfer and conformational equilibria for the *cis*-decalin amino acids **1** and **2**.

K_{PT}s. However, in less polar solvents (*i.e.*, ethers or acetone), observed $\Delta\delta^{H(5)}$ values gave anomalous K_{PT}s and, thus, spectra of the sodium and hydrochloride salts of **1** in these solvents were used to estimate $\Delta\delta^{H(5)}$ values for the classical species. Chemical shift differences for the diaxial zwitterion (species **C**, Figure 3) were estimated from chemical shift differences observed in the ^{1}H NMR spectra of lactam **3**.[35] Use of **3** to estimate chemical shifts for **C** was unlikely to introduce significant errors as the latter was sparsely populated, typically less than 10 mol %.

The ^{1}H–^{1}H NMR coupling constants (^3J values) that were used to calculate the conformational equilibria were obtained directly from spectra of the amino acids and lactams. Typically, intrinsic solvent-induced changes in ^3J values are small and of approximately the same order as the reproducibility of multiple measurements (±0.1 Hz).[24d,37] Further, ^3J values for the hydrochlorides of **1** and its corresponding methyl ester[24d] were identical and invariant in many different solvents ($CDCl_3$ to D_2O). The H-1 and H-7 ^3J values of the hydrochloride salt and zwitterion of **1** in D_2O were identical, which confirmed the absence of the diaxial conformation in these solutions. However, the ^3J values did change slightly (0.1–0.3 Hz) during ionization, which was anticipated from the dependence of ^3J values on the electronegativity of attached substituents[38] and from similar examples in the literature.[39] The H-1 and H-7 ^3J values estimated for the classical form of the diequatorial conformation (species **B** in Figure 3) were taken from aqueous spectra of the amino acid sodium and hydrochloride salts, respectively. The ^3J values for the diaxial conformation were taken from high-resolution decoupling of the H-1 and H-7 resonances of lactam **3**.[40] The sum of observed ^3J values (Σ^3J) for the H-1 and H-7 resonances was used to calculate conformational equilibria to minimize absolute errors in spectral measurements. Representative $\Delta\delta^{H(5)}$ (in D_2O) and Σ^3J values for the three species **A**-**C** of **1** are given in Table II. Spectral data for the three forms of **2** were estimated in a manner analogous to that for **1** (data not shown).

In a typical set of calculations, relative populations of zwitterionic and classical diequatorial conformations (**A** and **B**, Figure 3), determined from the $\Delta\delta^{H(5)}$ values, were used to calculate a weighted value for the H-1 and H-7 Σ^3J values (diequatorial conformation); observed Σ^3J values were subsequently used to calculate populations of the diaxial zwitterion **C** (Figure 3). The H-7 Σ^3J value changed very little during proton transfer in the diequatorial conformation (**A** and **B**, Table II) and served as an internal check against small changes in the H-1 Σ^3J value that might have been attributed to either proton-transfer or conformational equilibria (*i.e.*, compare ^3J H-7 in **B** and **C**). The rather large differences in the H-1 and H(5) Σ^3J values between **A**/**B** and **C** also facilitated calculation of conformational K_{eq}s for low concentrations of **C**. The ΔGs for the equilibria in Figure 3, estimated from the ^{1}H NMR determined populations of **A**-**C** for the amino acid **1** in various solvents, are listed in Table III. Aggregation of the amino acids was evaluated with spectra at different concentrations: at low concentrations (0.2–2 mM) spectra of **1** were concentration-independent in relatively polar solvents (alcohols, d_6-DMSO). In less polar solvents, poor solubility prevented concentration evaluations; maximal solubilities ranged from 0.2 to 0.3 mM. Although aggregation of amino acids at these concentrations has been observed,[12,14–23] it was deemed unlikely for **1** and **2**, as both reverted predominantly to the classical form (species **B**, Figure 3) in nonpolar solvents; for example, the population of **B** was 93 mol % in d_8-1,4-dioxane. Clearly, in these solvents, proton transfer to generate the classical species was favored over aggregation of the zwitterion. The steric

Table II. Representative Spectral Data for **A** (the Diequatorial Zwitterion), **B** (the Diequatorial Classical Form), and **C** (the Diaxial Zwitterion) of *cis*-Decalin Amino Acid **1** in D_2O

		H-1	H-7
A[a]	$\Delta\delta^{H(5)}$	1.13 ppm	0.49 ppm
	Σ^3J	16.70 Hz	33.60 Hz
B[b]	$\Delta\delta^{H(5)}$	0.54 ppm	0.64 ppm
	Σ^3J	15.6 Hz	33.8 Hz
C[b]	$\Delta\delta^{H(5)}$	1.54 ppm	0.72 ppm
	Σ^3J	6 Hz	14 Hz

[a] Observed values. [b] Estimated values.

Table III. Free Energies (kcal mol^{-1}), as Defined in Figure 3, for the Proton-Transfer Reactions and Solvation of the *cis*-Decalin Amino Acid **1** in Different Solvents

entry	solvent	ΔG_{21}	ΔG_{2b}	ΔG_{3a}	ΔG_{3b}	$\Delta\Delta G_2$
1	CD_3OD	5.5	6.0	4.5	6.0	0.5
2	d_6-EtOH	8.5	7.0	1.5	5.0	–1.5
3	CD_3CN	9.4	7.0	0.6	5.0	–2.4
4	d_6-DMSO	10.8	7.3	–0.8	4.7	–3.5
5	CD_3COCD_3	10.5	7.8	–0.5	4.2	–2.7
6	d_7-DMF	10.4	8.2	–0.4	3.8	–2.1
7	$CDCl_3$	10.1	6.4	–0.1	5.6	–3.7
8	d_8-THF	12.0	7.5	–2.0	4.5	–4.4
9	d_8-1,4-dioxane	12.3	7.8	–2.3	4.2	–4.5

(35) It was assumed that $\Delta\delta^{H(5)} = \Delta\delta_{anis} + \Delta\delta_{ion}$, where $\Delta\delta_{anis}$ and $\Delta\delta_{ion}$ are perturbations in chemical shift due to the diequatorial–diaxial conformational anisotropy and ionization, respectively. The chemical shifts should be similar for the lactam and classical form of the diaxial conformation, and ionization changes for H-1 and H-7 were taken from the sodium and hydrochloride salts of **1** (see above). A correction of –0.43 ppm, the $\Delta\delta$ for protons α to the nitrogen of 2-piperidone relative to piperidine,[36] was applied to the lactam H-1 resonance to account for the change in nitrogen hybridization.

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(39) Abraham, R. J.; Hudson, B. D.; Thomas, W. A. *J. Chem. Soc., Perkin Trans. 2* **1986**, 1635.

(40) The computer[25,38] predicted ^3J values for the lactam and the amino acid in a diaxial conformation were nearly identical to each other and, also, very similar to those observed (deviation in $\Sigma^3J < 0.2$ Hz).

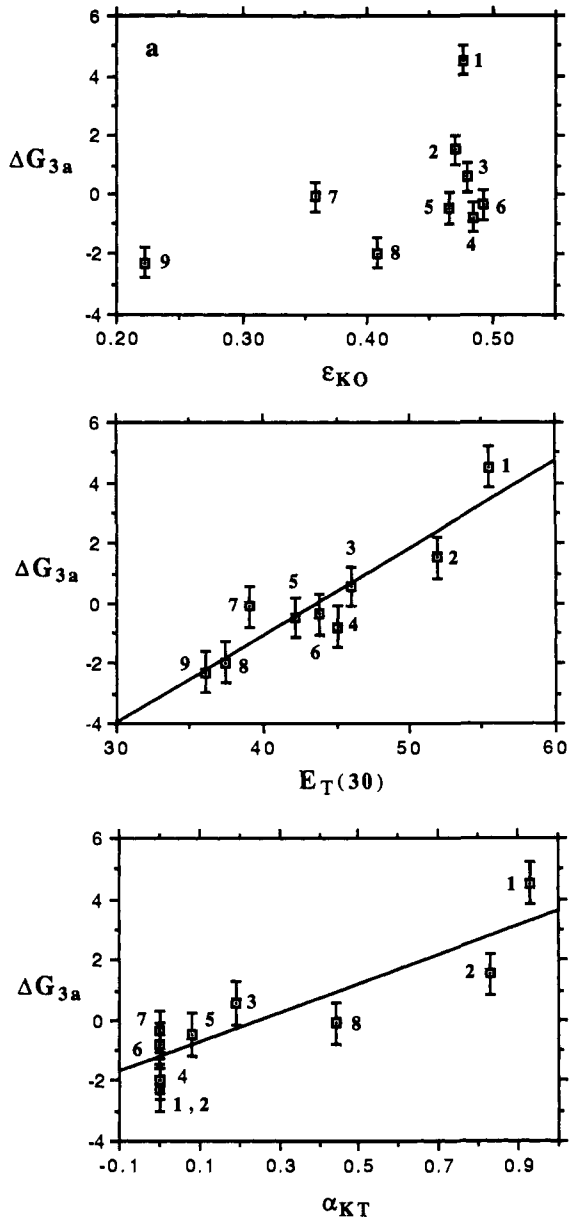

Figure 4. Graphs of ΔG_{3a} (kcal mol^{-1}) for *cis*-decalin amino acid **1** plotted against (a) dielectric ϵ_K, (b) $E_T(30)$, and (c) α_{KT}. Solvents are numbered as in Table III.

inhibition of aggregation and consequent proton transfer observed for **1** is in stark contrast to the findings of previous studies[14-23] in which formation of contact ion pairs allowed for more substantial ionization in low-polarity solvents. Further, the observation of small populations of diaxial zwitterions (species **C**, Figure 3) in this study suggests that the approximately 6 kcal mol^{-1} (Table I) of energy required to overcome the steric bias to form a contact ion pair is similar to the energy difference between the separated ion pair and classical species.

Correlations with Solvent Parameters. The solvation effect on the proton-transfer equilibria for amino acid **1** was evaluated with comparisons of ΔG_3 values and solvent parameters.[41] The Kirkwood–Onsager dielectric ϵ_K [$\epsilon_K = (\epsilon + 1)(2\epsilon - 1)^{-1}$], the measure of solvent polarity in reaction field theories,[42] did not correlate with ΔG_{3a} (Figure 4a); comparisons with the empirical

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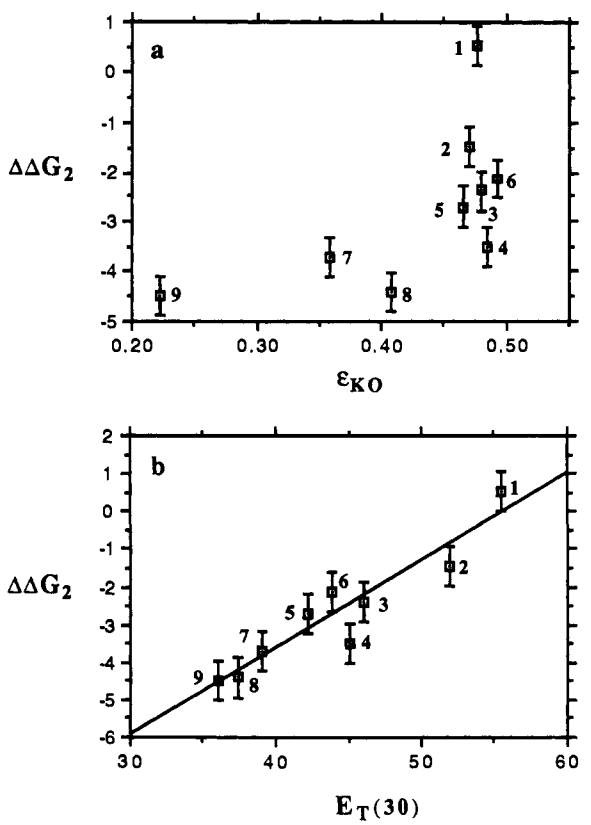


Figure 5. Graphs of the difference in electrostatic ΔG between the contact and separated ion pairs ($\Delta\Delta G_2$ in kcal mol^{-1}) of *cis*-decalin amino acid **1** plotted against (a) dielectric ϵ_K and (b) $E_T(30)$. Solvents are numbered as in Table III.

solvent polarity π^* and hydrogen bond acceptor β_{KT} scales were similarly scattered (data not shown). However, a graph of ΔG_{3a} plotted against the empirical solvent polarity scale $E_T(30)$ and the hydrogen bond donor scale α_{KT} produced approximately linear relations (Figure 5, parts b and c, respectively). The number of solvents assayed does not allow for rigorous multiparameter correlations; however, preliminary screens (*i.e.*, direct sums of polarity and acidity/basicity scales[38]) gave no significant correlations. The difference in ΔG between the contact and solvent-separated ion pairs in the diaxial and diequatorial zwitterions ($\Delta\Delta G_2$) of **1**, respectively, were determined by subtraction of ΔG_{2a} from ΔG_{2b} (Table III). The $\Delta\Delta G_2$ is a measure of the *electrostatic* ΔG between the two conformations. A graph of $\Delta\Delta G_2$ plotted against ϵ_K (Figure 5a) was scattered, while a graph against $E_T(30)$ (Figure 5b) was approximately linear; $\Delta\Delta G_2$ also was approximately linear with α_{KT}, although the correlation was not as significant as seen with ΔG_3 values (data not shown). The *N*-methyl amino acid **2** also was evaluated in a limited number of solvents to explore the consequence of restricted solvent access. Both ΔG_3 and $\Delta\Delta G_2$ values demonstrated correlations similar to those seen with **1**; a representative graph of ΔG_{3a} plotted against $E_T(30)$ is illustrated in Figure 6.

The *cis*-decalin amino acid **5** (Figure 7), which has a much smaller steric bias[24d] than **1** and **2**, was designed to further evaluate ion pair energetics in highly polar solvents. Methylation of the *N*-methyl lactam **4** gave the *C,N*-dimethyl lactam **6** (Figure 7); however, hydrolysis under a variety of conditions rarely gave more than a 1–2% yield of amino acid **5**. Apparently, the steric bias in **5** was such that reversion to the lactam was unavoidable; indeed, subsequent attempts to purify the small quantities of **5** obtained from acidic hydrolyses resulted in almost complete reversion to lactam. However, the H-1 ^3J values in ^{1}H NMR spectra of the crude HCl salt and deionized (zwitterion) in D_2O were identical ($\Sigma^3J = 16.6$ Hz), which demonstrated the complete absence of the diaxial conformation in these solutions. Thus, the

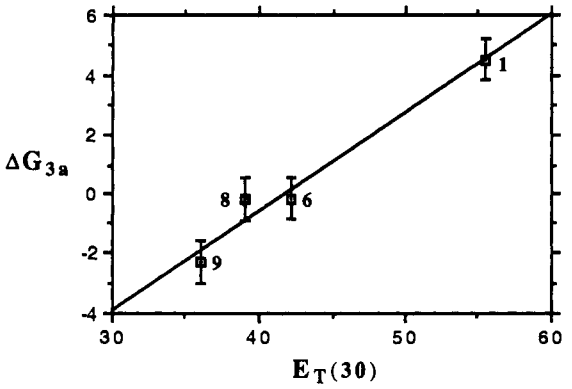
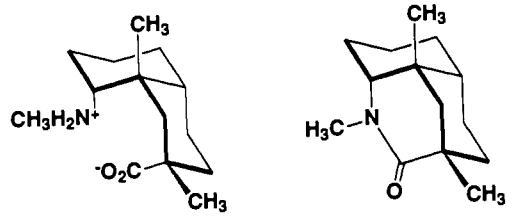


Figure 6. Graph of ΔG_{3a} (kcal mol^{-1}) for the *N*-methyl *cis*-decalin amino acid **2** plotted against $E_T(30)$. Solvents are numbered as in Table III.



5 6

Figure 7. Chemical structure of the *C,N*-dimethyl *cis*-decalin amino acid **5** and lactam **6**.

energetic difference between the contact and separated ion pairs in water is much less than the approximately 2 kcal mol^{-1} steric barrier. These results are consistent with the calculations of Warshel.[5]

Discussion

Solvent reaction field[42] stabilization of zwitterion dipoles has been demonstrated previously for inter- and intramolecular hydrogen bonds between weak acids and amines in nonpolar, aprotic solvents.[14-23] In more associated solvents, however, proton-transfer ΔGs and ϵ_K were not correlated; it was suggested that changes in solute–solvent and solvent–solvent interactions produced variable ΔS terms not accounted for in reaction field theories.[42] The lack of correlation between proton transfer ΔGs and ϵ_K for the *cis*-decalin amino acid **1** (Figure 4a) does not address the issue of solvent reaction field polarity; with the exception of 1,4-dioxane and chloroform, only high-dielectric solvents were evaluated, and thus the range of ϵ_K was not fully explored (see the abscissa of Figure 4a). The extent of proton transfer (K_{PT}) for **1** in nonpolar solvents was much smaller than that observed for contact ion pairs in the same solvents;[14-23] presumably, the difference reflects the relative stability of contact versus separated ion pairs; for **1**, the former was inhibited with a large steric barrier (Figure 1 and Table I). The predominance of the classical form of **1** in $CDCl_3$ and d_8-1,4-dioxane argues that ΔG of the separated ion pair is larger than the steric barrier in these solvents; thus, reaction field and Coulombic stabilization of the ion pair must prevail in nonpolar, aprotic solvents. Aggregation to form intermolecular contact ion pairs, a problem that had plagued previous attempts to evaluate separated ion pairs in nonpolar solvents, was presumably inhibited with sterics, as evidenced by the predominance of the classical form of **1**.

Table IV. Solvent Hydrogen Bond Donor (α_{KT}), Acceptor (β_{KT}), and Bulk Polarity (ϵ_{KO}) Parameters for Selected Solvents and Relative Free Energies of Transfer (in kcal mol^{-1}) between Solvents for the Diequatorial ($\Delta\Delta G_{2a}$) and Diaxial ($\Delta\Delta G_{2b}$) Zwitterions of **1** as Defined in Figure 3

solvent	α_{KT}[a]	β_{KT}[a]	ϵ_{KO}[a]
CD_3CN	0.19	0.37	0.48
d_6-DMSO	0	0.76	0.48
$CDCl_3$	0.44	0	0.36
d_8-1,4-dioxane	0	0.37	0.22
d_8-THF	0	0.55	0.41

solvent transfer	$\Delta\Delta G_{2a}$	$\Delta\Delta G_{2b}$
$CD_3CN \rightarrow d_6$-DMSO	1.4	0.3
$CDCl_3 \rightarrow d_8$-1,4-dioxane	2.2	1.4
d_8-THF $\rightarrow d_8$-1,4-dioxane	0.3	0.3

[a] Parameters are for the protic solvents.

The linear correlations between the proton-transfer ΔGs and $E_T(30)$ or α_{KT} observed for **1** (Figure 4, parts b and c, respectively) demonstrate specific solvation of ion pairs. It is apparent that solvent hydrogen bond acidity is the dominant stabilizing factor as the correlation with the $E_T(30)$ scale, which has been alternately treated as a measure of bulk solvent polarity[46] and of solvent hydrogen bond acidity,[47] was not significantly improved relative to α_{KT}. The extreme sensitivity of carboxylic acids to solvent hydrogen bond acidity, as compared to bulk polarity, has been amply demonstrated.[48] For example, in a related study,[16] as the water content of aqueous DMSO solutions was lowered, the pK_a of carboxylic acids increased while ammonium ion pK_as remained unchanged. Solvent hydrogen bond basicity, the basis of ammonium ion stabilization, has not been similarly evaluated. Ideally, a study of proton-transfer equilibria, such as reported for **1**, in a wide range of solvents and subsequent multiparameter linear regressions with different solvent parameters is needed. Unfortunately, the poor solubility of **1** in many solvents precluded more extensive studies; the number of solvents evaluated would not have justified fully linear regression with two parameters. However, comparisons of the ΔGs for **1** in a few different solvents offer a qualitative view of the relative importance of polarity and hydrogen bonding to the solvation of the two respective ions. Relevant solvent parameters and ΔGs of transfer for a few selected solvents are given in Table IV.

The difference in ΔG for transfer of the zwitterion of **1** from water to an organic solvent (ΔG_2 in Figure 3) for two different solvents evaluates the relative stability of the ion pair. For example, the difference in solvation upon transferring the diequatorial zwitterion from CD_3CN to d_6-DMSO ($\Delta\Delta G_{2a}$ = 1.4 kcal mol^{-1}, Table IV) reflects the importance of solvent hydrogen bond acidity to ion pair stabilization; both solvents have similar polarities (ϵ_K) and hydrogen bond basicities (β_{KT}), while d_6-DMSO lacks hydrogen bond acidity. This is confirmed by the much smaller ΔG of transfer for the diaxial zwitterion ($\Delta\Delta G_{2b}$ = 0.3 kcal mol^{-1}, Table IV) in the same two solvents; in the diaxial conformation the carboxylate is internally hydrogen bonded with the ammonium ion. A similar energy difference between solvation of the two conformations of **1** is observed for transfer from $CDCl_3$ and d_8-1,4-dioxane (Table IV); however, in this case, the $CDCl_3$ is not a hydrogen bond acceptor, which allows inference that hydrogen bond stabilization of the ammonium ion is of secondary importance. The difference in energy for transfer from $CDCl_3$ to d_8-1,4-dioxane does not reflect entirely

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(43) The proton-transfer equilibria for the diaxial zwitterion, characterized by ΔG_{3b} (Figure 3), demonstrated correlations equivalent to those for ΔG_{3a} (data not shown).

(44) The molecular mechanics estimated energy for the conformational equilibria of **5** ($\Delta E_{(g)}$ = 2.1 kcal mol^{-1} for the classical species) was substantially reduced relative to **1** (Table I).

(45) Assuming the 2 kcal mol^{-1} steric barrier to be correct, a 0.5 kcal mol^{-1} difference in ΔG between the contact and separated ion pair would have produced an easily detected 10% change in the ^3J values.

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Coulombic stabilization of the diaxial conformation; in transferring from d_8-THF to d_8-1,4-dioxane, in which the hydrogen bond basicity is similar but polarity (ϵ_K) differs, there is no apparent energy difference between the two conformations of **1** (Table IV). The large, unfavorable, ΔGs for transferring the zwitterions from water to low-polarity solvents (10–12 kcal mol^{-1} for ϵ° =2–4) confirm the previously demonstrated sensitivity to bulk polarity;[14-23] the observed ΔGs are also consistent with theoretical calculations[48] for transfer of ion pairs (ΔG = 10–16 kcal mol^{-1} for ϵ° =2–4). In conclusion, there is a strong interplay between solvent polarity and hydrogen bond stabilization of the carboxylate ion; thus, an ion pair could be easily stabilized by a few specific interactions within a protein interior. This concept has been elegantly illustrated with theoretical calculations.[7,49]

Obstruction of solvent access to an ion pair due to a proximal alkyl group—steric hindrance to solvation—was demonstrated previously in polar solvents.[14-23,24b] The slopes obtained from linear regression of ΔG correlations with $E_T(30)$ for the *N*-methyl amino acid **2** are about 20% higher than for **1**; thus, it is inferred that steric hindrance of solvation due to the methyl group does occur. However, the difference is small and, thus, steric hindrance to solvation may be most important in highly coordinating solvents such as water.[16] An interesting aspect in the proton-transfer reactions for the *cis*-decalin amino acids is the mechanistic consequence of a lack of intramolecular hydrogen bonding. It has been well established in the gas phase that proton transfer occurs through an approximately linear hydrogen bond,[14,50] which cannot exist in either of the two chair–chair conformations for **1** and **2**. However, an approximately linear hydrogen bond in a twist–boat conformational intermediate was identified with molecular mechanics. It appears possible that the proton transfer occurs in a conformational intermediate rather than in the diaxial conformation. This may be the source of the high propensity for lactamization of the amino acids; proton transfer to generate the classical species in an intermediate conformation provides a nucleophilic amine which, upon conversion to the diaxial conformation, would have a high chance for carbonyl addition. Further, the rate of interconversion coupled with solvent reorientation could produce transiently desolvated ions in close proximity[51] and, thus, stabilize the conformational intermediate. Such a process may explain the fast conformational rate (NMR time scale) that was not anticipated. This argument presumes that the requirement for linearity in proton transfers in the gas phase also holds in the solution phase; no data are available to address this point.

Summary

The *cis*-decalin amino acid proved to be a useful, although not perfect, probe for analysis of the interplay between solvent and structure in promoting ion-pairing in competition with proton transfers. The range of solvents evaluated was mitigated somewhat by the large conformational bias against the contact ion pair, which forced the molecule under certain conditions to stabilize by an intramolecular proton transfer from the zwitterionic to classical species; this resulted in somewhat limited solubility in many solvents of interest. Nevertheless, the failure of bulk solvent properties, such as the Kirkwood–Onsager dielectric ϵ_K, to adequately correlate ion pair energetics indicated the limited applicability of macroscopic electrostatic models; rather, the correlations between electrostatic ΔGs and empirical solvent parameters amplified the need for molecular solvation models. A qualitatively identical conclusion was reached previously in the study of a related electrostatic interaction of biological relevance, the hydrogen bond.[24e] Ultimately, the sensitivity of electrostatics to solvent donor–acceptor properties argues that a successful treatment of protein structure must be done on the molecular level, by evaluating the stabilization of electrostatic interactions by the local environment, which is defined by the protein's tertiary structure and whether the interaction is buried in the protein or exposed to solvent.

Experimental Section

8a,*N*-Dimethyl-1α-aminodecalin-7-carboxylic acid (2). Excess CH_3I and KH were added to a stirred solution of lactam[24] **3** (0.10 mmol) in 25 mL of ether at ambient temperature; after 4 h, the solution was quenched with NH_4Cl(aq), and standard workup (CH_2Cl_2) and chromatography (2% CH_3OH/CH_2Cl_2) gave the *N*-methyl lactam **4** as a clear oil in a nearly quantitative yield. Hydrolysis of **4** in a sealed tube (6 M HCl, 140 °C) for 1 week and subsequent evaporation provided the hydrochloride salt of amino acid **2**. The amino acid hydrochloride was deionized with ion-exchange chromatography on Dowex 50(H^+); elution with 1 M NH_3(aq) gave 12 mg (63% yield from **3**) of amino acid **2**: ^{1}H NMR (500 MHz, D_2O) δ 2.80 (dd, 1H, *J* = 4.0, 12.6 Hz), 2.67 (s, 3H), 2.38 (tt, 1H, *J* = 12.9, 4.0 Hz), 1.95 (m, 1H), 1.86 (tt, 1H, *J* = 12.7, 4.0 Hz), 1.74 (t, 1H, *J* = 12.8 Hz), 1.7–1.3 (m, 8H), 1.20 (s, 3H), 1.16 (dd, 1H, *J* = 4.0, 12.9 Hz); ^{13}C NMR (125 MHz, D_2O) δ 169.8, 69.5, 41.8, 39.5, 32.0, 27.7, 25.9, 23.6, 23.6, 22.8, 22.6; HRMS (CI) calcd for $C_{13}H_{23}NO_2H^+$ 226.1807, found 226.1819.

1,9,12-Trimethyl-10-oxo-9-azatricyclo[6.2.2.0^{4,12}]dodecane (6). Bu^{t}Li (0.12 nmol) was added to a stirred solution of the *N*-methyl lactam **4** (0.10 mmol) in 7 mL of THF at –70°C; after 5 min, CH_3I (0.2 mmol) was added, and the solution was quenched 5 min later with NH_4Cl(aq). Standard workup (CH_2Cl_2) and chromatography (2% MeOH/CH_2Cl_2) gave 16 mg (78% yield) of **6** as a viscous oil, which solidified upon standing: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.08 (t, 1H, *J* = 2.9 Hz), 2.91 (s, 3H), 1.98 (m, 1H), 1.87 (tt. 1H, *J* = 4.6, 13.9 Hz), 1.84 (m, 1H), 1.74–1.57 (m, 4H), 1.47–1.30 (m, 4H), 1.32 (d, 1H, *J* = 12.9 Hz), 1.22 (dt, 1H, *J* = 4.8, 13.5 Hz), 1.15 (s, 3H), 1.07 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.2, 63.6, 47.7, 39.6, 38.3, 37.7, 32.7, 30.8, 27.5, 26.3, 26.2, 25.7, 25.0, 15.2; HRMS (CI) calcd for $C_{13}H_{21}NO_2^+$ 222.1858, found 222.1846. Optimal conditions for hydrolysis (1–2% yield of amino acid **5**) were 1–2 mM lactam in 3 M HCl(aq) at 160 °C (sealed under Ar) for 5 days. Extraction of the lactam (CH_2Cl_2) and evaporation gave the crude amino acid hydrochloride; subsequent attempts at purification of amino acid **5** invariably resulted in lactamization to **6**.

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