Solvation-enhanced salt bridges.

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ABSTRACT: Salt bridges formed by amidines and carboxylic acids represent an important class of non-covalent interaction in biomolecular and supramolecular systems. Isothermal titration calorimetry was used to study the relationships between the strength of the interaction, the chemical structures of the components and the nature of the solvent. The stability of the 1:1 complex formed in chloroform changed by two orders of magnitude depending on the basicity of the amidine and the acidity of the acid, which is consistent with proton transfer in the complex. Polar solvents reduce the stabilities of salt bridges formed with *N,N'*-dialkylamidines by up to three orders of magnitude, but this dependence on solvent polarity can be eliminated if the alkyl groups are replaced by protons in the parent amidine. The enhanced stability of the complex formed by benzamidine is due to solvation of the NH sites not directly involved in salt bridge formation, which become significantly more polar when proton transfer takes place, leading to more favourable interactions with polar solvents in the bound state. Calculation of H-bond parameters using density functional theory were used to predict solvent effects on the stabilities of salt bridges to within 1 kJ mol^{-1}. While H-bonding interactions are strong in non-polar solvents, and solvophobic interactions are strong in polar protic solvents, these interactions are weak in polar aprotic solvents. In contrast, amidinium-carboxylate salt bridges are stable in both polar and non-polar aprotic solvents, which is attractive for the design of supramolecular systems that operate in different solvent environments.

INTRODUCTION

Salt bridges represent an important class of non-covalent interactions that involve both H-bonding and ion-pairing when a cationic H-bond donor interacts with an anionic H-bond acceptor. These interactions play a pivotal role in biological systems, particularly in protein folding, protein-nucleic acid recognition and medicinal chemistry.[1–4] The amidinium-carboxylate salt bridge has been widely used in synthetic supramolecular systems due to the large association constants found in non-polar solvents and the well-defined geometry dictated by two cooperative H-bonds (Figure 1). Applications include sensing,[5,6] crystal engineering,[7,8] catalysis,[9] H-bonded organic frameworks,[10] polymer chemistry,[11,12] self-replicating systems,[13] self-assembly of duplexes and capsules,[14–17] and template synthesis.[18,19] In contrast to other non-covalent interactions that have been the subject of quantitative systematic studies,[20–23] salt bridges have received relatively little attention. Reliable implementation of non-covalent chemistry in molecular design requires an understanding of the relationships between the strength of the interaction, the chemical structures of the components and the nature of the solvent.[24] Here we use the formation of salt bridges between a series of carboxylic acid and benzamidine derivatives in a range of organic solvents to establish these principles.

The combination of H-bonding and ion-pairing involved in the formation of a salt bridge means that multiple equilibria may be involved, as illustrated in Figure 1. Proton transfer may take place to different extents before and after formation of the H-bonds in the salt bridge, so there is a complex interplay of acid-base chemistry and solvation, as well as the effects of charge on the strength of the H-bonds.[25] Here we show that, starting from the neutral species (top left in Figure 1), the stability of the salt bridge interaction in chloroform can be modulated by two orders of magnitude depending on the X and Y substituents. Polar solvents reduce the stabilities of salt bridges formed with *N,N'*-dialkylamidines by up to three orders of magnitude, but this dependence on solvent polarity can be practically eliminated if the alkyl groups are replaced by protons in the parent amidine (R = H in Figure 1). We show that this unusual property of salt bridges formed by amidines and carboxylic acids is due to changes in the solvation shell associated with the proton transfer that takes place within the salt bridge.

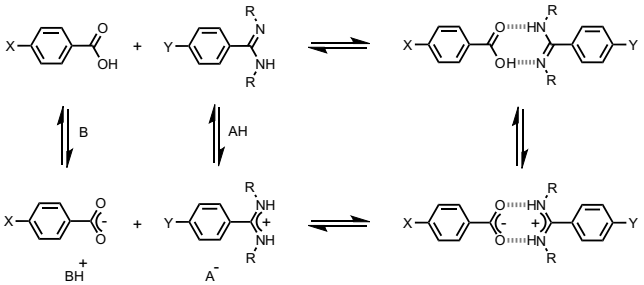


Figure 1. Salt bridge interaction between a benzoic acid and benzamidine derivative. AH and B represent an acid and base respectively, and X, Y and R are substituents.

RESULTS AND DISCUSSION

Synthesis

All of the benzoic acids investigated were commercially available (X = H, NMe_2, OMe, CF_3, NO_2 in Figure 1). The synthesis of 4-substituted benzamidines was carried out by the routes shown in Scheme 1. 4-Hydroxybenzonitrile was first alkylated with 2-ethylhexyl bromide, and subsequent treatment with acetyl chloride in methanol followed by methanolic ammonia solution gave the 4-alkoxybenzamidine **1**. 4-Mercaptobenzonitrile was similarly alkylated and then oxidised with 3-chloroperbenzoic acid (*m*CPBA) to

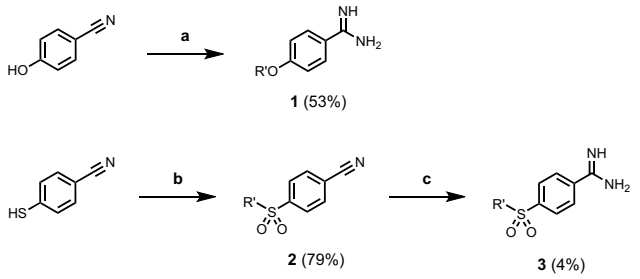
give the 4-sulfonylbenzonitrile **2**. Conversion of **2** to the corresponding amidine gave the 4-sulfonylbenzamidine **3**.

N,N'-Dialkylbenzamidines **4**-**7** were synthesised by the routes shown in Scheme 2. The *N,N'*-dimethyl and *N,N'*-diethyl derivatives **4** and **5** were prepared by alkylation of benzonitrile with the relevant alkyl triflate followed by reaction with the corresponding primary amine. The *N,N'*-di-*i*-propyl and *N,N'*-di-*t*-butyl derivatives **6** and **7** were obtained by reaction of the corresponding carbodiimide with phenyl magnesium bromide.

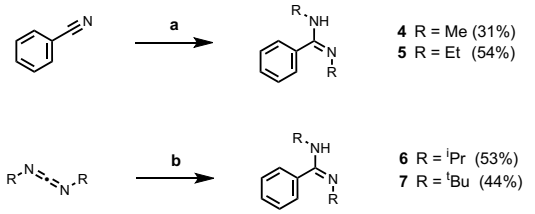
Effect of aromatic substituents

The interaction of pairwise combinations of benzoic acid and benzamidine derivatives was investigated in chloroform solution using isothermal titration calorimetry (ITC). In each case, the titration data fit well to a 1:1 binding isotherm (see SI for details), and the resulting thermodynamic parameters are summarised in Table 1.

The nature of the aromatic substituents X and Y have a large impact on the stability of the 1:1 complex formed in chloroform, and the association constants span almost two orders of magnitude. The stability of the complex increases for electron-withdrawing groups on the carboxylic acid and electron-donating groups on the amidine. Figure 2 shows that the association constants correlate well with the Hammett substituent parameter, σ, which measures the effect of substituents on the acidity of the corresponding benzoic acid. The results show that the strength of the interaction between a neutral carboxylic acid and a neutral amidine can be directly and predictably tuned by changing the acidity and/or basicity of the interacting partners.



Scheme 1. Synthesis of 4-substituted benzamidines (R' = 2-ethylhexyl). Conditions: (a) 1. 2-Ethylhexyl bromide; 2. AcCl, MeOH then NH_3; (b) 1. 2-Ethylhexyl bromide; 2. *m*CPBA; (c) AcCl, MeOH then NH_3.



Scheme 2. Synthesis of *N,N'*-dialkylbenzamidines. Conditions: (a) 1. ROTf; 2. RNH_2; (b) phenyl magnesium bromide.

Table 1. Substituent effects on the thermodynamic parameters for salt bridge formation between benzoic acid and benzamidine derivatives determined by ITC in $CHCl_3$ at 298 K.[a]

X	Y[b]	R	Log(K/ M^{-1})	$\Delta G°$ / kJ mol^{-1}	$\Delta H°$[c]/ kJ mol^{-1}	$\Delta S°$[c]/ J K^{-1} mol^{-1}	N
H	H	H	6.01 ± 0.05	-34.3 ± 0.3	-70.0 ± 3.0	-120 ± 10	1.07 ± 0.06[d]
NMe_2	H	H	5.10 ± 0.10	-29.1 ± 0.6	-60.0 ± 0.8	-104 ± 5	1.08 ± 0.06[d]
OMe	H	H	5.69 ± 0.03	-32.5 ± 0.2	-66.0 ± 3.0	-110 ± 10	1.10 ± 0.01[d]
CF_3	H	H	6.51 ± 0.02	-37.1 ± 0.1	-76.0 ± 1.0	-130 ± 4	0.80 ± 0.01[d]
NO_2	H	H	6.84 ± 0.09	-39.0 ± 0.5	-75.4 ± 0.6	-122 ± 4	0.90 ± 0.10[d]
H	OR'	H	6.55 ± 0.09	-37.4 ± 0.5	-70.0 ± 10.0	-110 ± 30	1.20 ± 0.20[d]
H	SO_2R'	H	4.92 ± 0.03	-28.1 ± 0.2	-61.0 ± 1.0	-111 ± 4	0.90 ± 0.01[e]
H	H	Me	6.37 ± 0.05	-36.3 ± 0.3	-73.0 ± 9.0	-120 ± 30	1.20 ± 0.1[e]
H	H	Et	6.52 ± 0.09	-37.2 ± 0.5	-74.0 ± 3.0	-120 ± 10	1.20 ± 0.1[d]
H	H	iPr	6.40 ± 0.20	-37.0 ± 1.0	-75.0 ± 1.0	-128 ± 7	1.00 ± 0.1[e]
H	H	tBu	6.36 ± 0.08	-36.3 ± 0.5	-80.0 ± 3.0	-150 ± 10	1.17 ± 0.03[e]

[a] Errors are twice the standard deviation of at least two repeat measurements. [b] R' = 2-ethylhexyl. [c] Errors in $\Delta H°$ and $\Delta S°$ do not take into account the uncertainty in N. [d] N is the number of amidine molecules bound to one carboxylic acid in the complex. [e] N is the number of carboxylic acid molecules bound to one amidine in the complex.

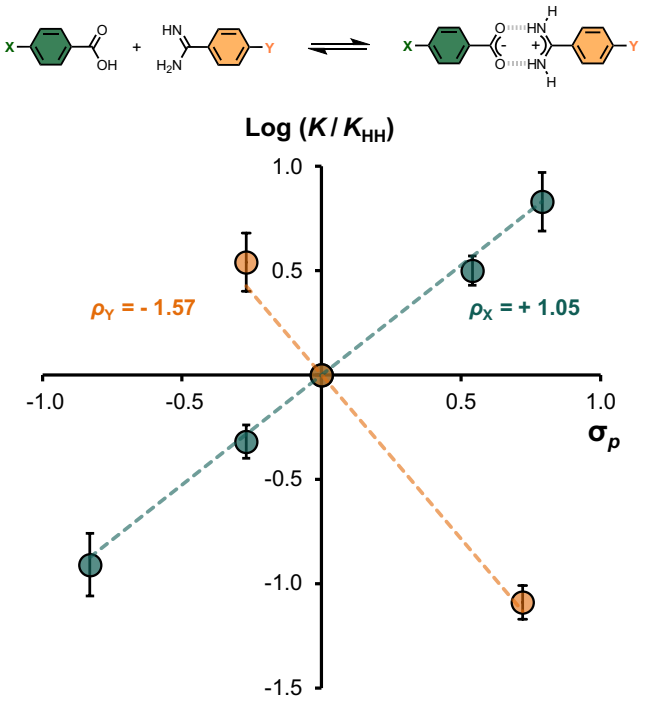


Figure 2. Hammett plots showing the relationship between the association constant for salt bridge formation measured in chloroform at 298 K and substituents on the benzoic acid (X, green) or the benzamidine (Y, orange). K_{HH} is the association constant for X = Y = H. The lines of best fit were fixed to pass through the origin and correspond to Log(K_X/K_{HH}) = 1.05 σ_X and Log(K_Y/K_{HH}) = -1.57 σ_Y.

The slopes of the Hammett plots in Figure 2, ρ, quantify the complexation-induced changes in charge on the carboxylic acid and amidine groups. The values of ρ are large and positive for X and large and negative for Y, which indicates a substantial change in charge for both partners when the salt bridge is formed. These observations suggest that a proton is transferred from the carboxylic acid to the amidine in the salt bridge, which exists in the zwitterionic form even in non-polar solvents like chloroform. These results might be interpreted as a simple proton transfer reaction between the acid and amidine, generating the ionised species without forming an intermolecular complex. However, ^{1}H NMR DOSY experiments in acetonitrile, a more polar solvent that would stabilise the separated ions better than chloroform, show that the complex is fully assembled in a 1:1 mixture at millimolar concentrations (see SI for details).

Effect of *N*-alkyl amidine substituents

N,N'-Dialkylamidines have been commonly used in supramolecular systems, because they can easily be made from *N,N'*-dialkylcarbodiimides and show increased solubility in organic solvents compared with the parent amidines.[26] Figure 3 shows how the stability of the salt bridge formed with benzoic acid is affected by *N*-alkyl substituents (R) of increasing steric bulk in chloroform. Alkylation of the amidine increases the stability of the complex relative to the parent amidine (R = H), but the association constants measured for all four *N,N'*-dialkylamidines are the same within experimental error. The steric bulk of the alkyl groups does not play a role in determining salt bridge stability. By analogy with the results for substituent effects described above, the increase in stability observed for the *N,N'*-dialkylamidines is most likely due to the higher basicity of more substituted nitrogen atoms.

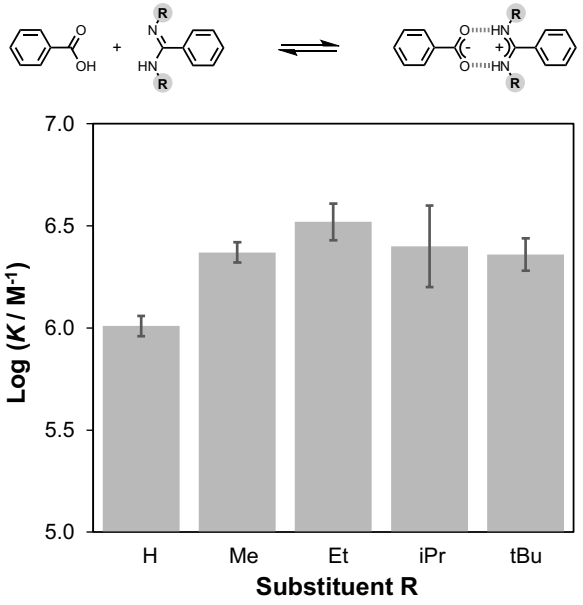


Figure 3. Effect of benzamidine *N,N'*-dialkyl substituents (R) on the association constant for salt bridge formation with benzoic acid measured in chloroform at 298 K.

Solvent effects

Although association constants for amidinium-carboxylate salt bridges have previously been measured in different solvents and in solvent mixtures,[16,27–31] no systematic study has been attempted. To investigate the role of the solvent, ITC titrations were used to measure the interaction of formic acid with benzamidine (Y = H, R = H) and with *N,N'*-dimethylbenzamidine (Y = H, R = Me) in eight organic solvents with a wide range of polarities. Polar protic solvents were not included in these experiments, because of the increased probability of proton transfer between solute and solvent, which would change the nature of the free species on the left-hand side of the equilibrium (for details see Equation 7 and associated discussion later in the text). Table 2 summarizes the thermodynamic parameters obtained from the ITC experiments, and Figure 4 compares the stabilities of the complexes formed with the two different amidines. The association constant decreases with increasing solvent polarity for both benzamidine (red) and *N,N'*-dimethylbenzamidine (blue), but the behaviour of the two systems is quite different. The benzamidinium-formate complex is much less sensitive to the solvent polarity than the *N,N'*-dimethylbenzamidinium-formate complex. Benzamidine forms a slightly less stable salt bridge than *N,N'*-dimethylbenzamidine in chloroform, but *N*-alkylation leads to a decrease the association constant by two orders of magnitude in THF and DMF compared with the parent benzamidine.

No correlation was found between the association constants and common descriptors of solvent polarity. For example, Figure 5a shows the relationship between the association constants and the solvent dielectric constant, ε_r, which indicates that the ionising power of the solvent is not an important factor governing the observed solvent effects. Figure 5b shows the relationship with the solvent polarity parameter $E_T(30)$, which highlights the failure of bulk solvent descriptors to account for the observed solvent effects. Similar results were found for the Hansen solubility parameters (see SI).

However, a correlation was found between the solvent hydrogen bond acceptor parameter, β_s, and the difference between the free energy changes for formation of the benzamidine and *N,N'*-dimethylbenzamidine complexes ($\Delta\Delta G^\circ$, Figure 5c). The enhanced stability of the benzamidine complex in polar solvents is therefore related to interactions between solvent H-bond acceptors and H-bond donor sites in the benzamidine complex that are not present in the *N,N'*-dimethyl complex. Since the difference between the two complexes is simply the replacement of two NH H-bond donor sites with non-polar methyl groups, this observation suggests that a more explicit analysis of the details of the solvation shell may shed light on the effect of solvent on salt bridge stability.

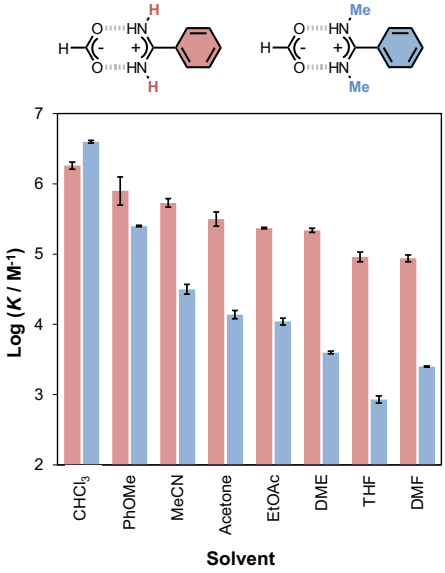


Figure 4. Solvent effects on the association constant for salt bridge formation between formic acid and benzamidine (red) or *N,N'*-dimethylbenzamidine (blue).

Solution-phase complexation can be described by a solvent competition model that uses the parameters α and β to quantify the non-covalent interaction properties of solute and solvent functional groups.[24,33–35] These parameters can be determined by experiment or calculated using density functional theory (DFT) molecular electrostatic potential surfaces in conjunction with a footprinting algorithm described previously.[36] The free energy contribution due to an intermolecular interaction between two functional groups is given by Equation 1.

$$\Delta\Delta G^\circ \text{ / kJ mol}^{-1} = -\alpha\beta \qquad \text{Eq. 1}$$

This approach can be used to build up a quantitative picture of the solvent-solute interactions that govern the behaviour of the salt bridges. Figure 6 compares the primary interactions in the solvation shells of the free and bound species involved in formation of the two different salt bridges. The major difference between the two complexes relates to solvation of the two peripheral NH sites that are not directly involved in the solute-solute H-bonding interactions (the solvation interactions highlighted in blue in Figure 6). These NH groups become significantly more polar in the zwitterionic complex compared with the neutral free state, which will lead to stronger interactions with the solvent in the bound state. It is the change in solvation of these peripheral sites that accounts for the difference in behaviour between benzamidine and *N,N'*-dimethylbenzamidine. Stronger solvation of the peripheral NH protons upon formation of the salt bridge enhances the stability of the benzamidine complex by almost two orders of magnitude in DMF compared with the *N,N'*-dimethylbenzamidine complex.

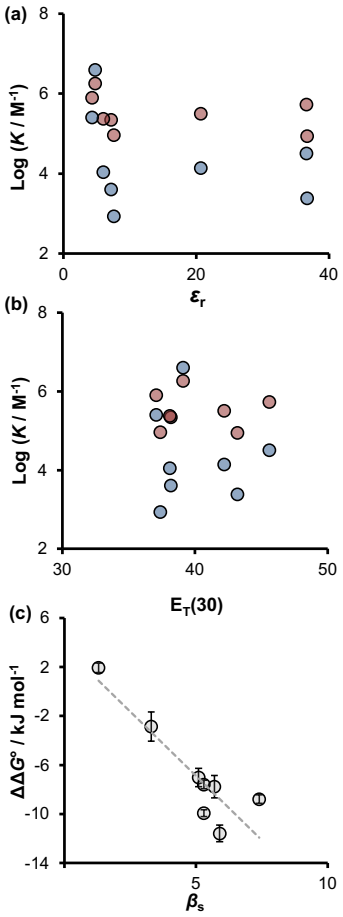


Figure 5. Comparison of the association constants for salt bridge formation between formic acid and benzamidine (red) or *N,N'*-dimethylbenzamidine (blue) with **(a)** solvent dielectric constant, ε_r, and **(b)** solvent polarity, $E_T(30)$.[37] **(c)** Comparison of the difference between the free energy changes for formation of the benzamidine and *N,N'*-dimethylbenzamidine complexes ($\Delta\Delta G^\circ$) with the solvent hydrogen bond acceptor parameter (β_s); $R^2 = 0.81$. (See SI for details).

The change in solvation free energy between free and bound species on formation of the salt bridge ($\Delta\Delta G^\circ_{solv}$) can be calculated in terms the H-bond parameters for the solvent and solute by summing the free energy contributions of each pairwise interaction shown in Figure 6 (Equation 2).

$$\Delta\Delta G^\circ_{solv} \text{ / kJ mol}^{-1} = \alpha_S \sum \beta_f + \beta_S \sum \alpha_f - \alpha_S \sum \beta_b - \beta_S \sum \alpha_b - 2\alpha_S\beta_S \qquad \text{Eq. 2}$$

where α_f, β_f, α_b and β_b are the H-bond parameters of the sites on the free and bound solutes, and α_S and β_S are the H-bond parameters of the solvent.

Table 2. Solvent effects on the thermodynamic parameters for salt bridge formation between formic acid and benzamidine derivatives determined by ITC at 298 K.[a]

Solvent	R	Y	Log (K/ M^{-1})	$\Delta G°$ kJ mol^{-1}	$\Delta H°$ [b] kJ mol^{-1}	$\Delta S°$ [b] J K^{-1} mol^{-1}	N[c]
$CHCl_3$	H	H	6.26 ± 0.05	-35.7 ± 0.3	-58.0 ± 1.0	-75 ± 4	1.01 ± 0.01
PhOMe	H	H	5.90 ± 0.20	-34.0 ± 1.0	-67.8 ± 0.1	-113 ± 4	0.80 ± 0.30
MeCN	H	H	5.73 ± 0.06	-32.7 ± 0.3	-63.0 ± 5.0	-102 ± 18	0.90 ± 0.20
Acetone	H	H	5.50 ± 0.10	-31.4 ± 0.6	-64.0 ± 2.0	-109 ± 9	0.89 ± 0.03
EtOAc	H	H	5.37 ± 0.01	-30.6 ± 0.1	-65.0 ± 4.0	-120 ± 10	0.89 ± 0.03
DME	H	H	5.34 ± 0.03	-30.5 ± 0.2	-68.0 ± 2.0	-126 ± 7	0.96 ± 0.04
THF	H	H	4.96 ± 0.07	-28.3 ± 0.4	-61.0 ± 0.4	-110 ± 3	0.79 ± 0.06
DMF	H	H	4.94 ± 0.05	-28.2 ± 0.3	-54.9 ± 0.3	-90 ± 2	0.97 ± 0.01
$CHCl_3$	Me	H	6.60 ± 0.05	-37.7 ± 0.3	-60.0 ± 10	-75 ± 30	0.93 ± 0.03
PhOMe	Me	H	5.40 ± 0.01	-30.8 ± 0.1	-51.0 ± 0.1	-68 ± 1	0.88 ± 0.01
MeCN	Me	H	4.50 ± 0.07	-25.7 ± 0.4	-53.0 ± 5.0	-90 ± 20	0.95 ± 0.07
Acetone	Me	H	4.14 ± 0.06	-23.6 ± 0.3	-37.0 ± 2.0	-45 ± 8	1.03 ± 0.01
EtOAc	Me	H	4.04 ± 0.05	-23.0 ± 0.3	-44.0 ± 0.7	-70 ± 3	0.93 ± 0.04
DME	Me	H	3.60 ± 0.02	-20.5 ± 0.1	-46.0 ± 4.0	-90 ± 10	1.00 [d]
THF	Me	H	2.93 ± 0.05	-16.7 ± 0.3	-23.0 ± 2.0	-21 ± 8	1.00 [d]
DMF	Me	H	3.34 ± 0.07	-19.1 ± 0.4	-50.0 ± 10.0	-100 ± 30	1.00 [d]

[a] Errors are twice the standard deviation of at least two repeat measurements. [b] Errors do not take into account the uncertainty in N. [c] N is the number of carboxylic acid molecules bound to one amidine in the complex. [d] Titrations carried out in the low c-value regime were analysed with N fixed at 1.00.[32]

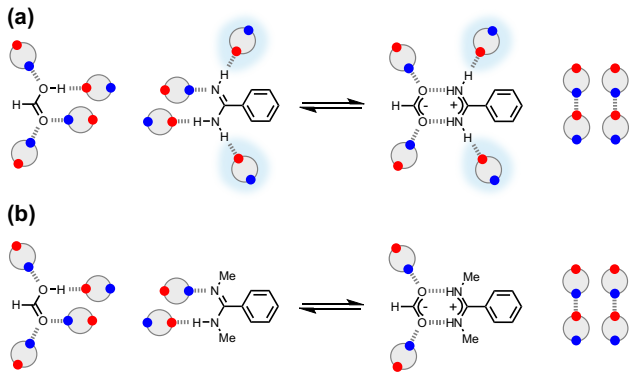


Figure 6. Primary solute-solvent interactions in the solvation shells of the free and bound species involved in the formation of (a) the benzamidinium-formate salt bridge, and (b) the *N,N'*- benzamidinium-formate salt bridge. The major difference is due to the solvation interactions highlighted in blue.

H-bond parameters for all of the sites on the free and bound solutes were calculated or obtained from experimental data (Figure 7). The calculated H-bond parameters confirm that the peripheral NH protons and oxygen lone pairs that are not directly involved in the salt bridge H-bonds become significantly better hydrogen bond donors and acceptors respectively when the proton is transferred in the salt bridge. Using these H-bond parameters in Equation 2, it is possible to estimate how differences in solvation energy affect the relative stability of the two different salt bridges (Equations 3 and 4).

$$\Delta\Delta G°_{solv}(\mathrm{H}) \,/\, \mathrm{kJ\ mol^{-1}} = 1.4\alpha_S + 3.4\beta_S - 2\alpha_S\beta_S \quad \text{Eq. 3}$$

$$\Delta\Delta G°_{solv}(\mathrm{Me}) \,/\, \mathrm{kJ\ mol^{-1}} = 1.9\alpha_S + 5.7\beta_S - 2\alpha_S\beta_S \quad \text{Eq. 4}$$

The coefficients of the solvent H-bond parameters in Equations 3 and 4 predict that the *N,N'*-dimethyl complex should be significantly more sensitive to solvent polarity than the complex formed with the parent benzamidine, particularly with respect the H-bond acceptor properties of the solvent, which is consistent with the experimental observations described above. Figure 8 compares the change in solvation energy predicted by Equations 3 and 4 (see SI for solvent H-bond parameters) with the experimental values of the free energy change for formation of the salt bridge in different solvents. There is an excellent correlation for both the benzamidine (R = H, red) and *N,N'*-dimethylbenzamidine (R = Me, blue) complexes, and the slope of line of best fit is 1.0 in both cases. In other words, the

experimentally observed solvent effects on the stability of the salt bridge interactions are almost perfectly described by the primary solvation model in Figure 6.

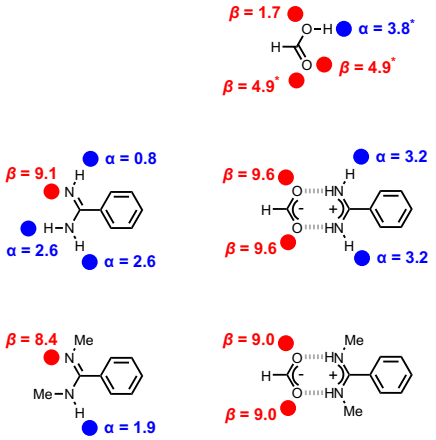


Figure 7: Free and bound solute H-bond parameters calculated using DFT (the carboxylic acid parameters labelled with an asterisk were obtained from experimental data).[35,36]

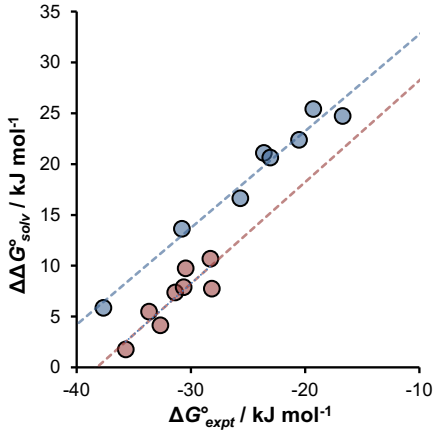


Figure 8. Comparison of the experimental free energy changes for formation of salt bridges in different solvents with the associated change in solvation free energy calculated using Equations 3 and 4 ($\Delta\Delta G^{\circ}_{solv}$). The lines of best fit are ΔG°_{expt} = 38.2 + 1.00 $\Delta\Delta G^{\circ}_{solv}$ (R=H, red, RMSE = 1 kJ mol^{-1}) and ΔG°_{expt} = 42.3 + 0.95 $\Delta\Delta G^{\circ}_{solv}$ (R=Me, blue, RMSE = 1 kJ mol^{-1}).

The intercepts on the ΔG°_{expt} axis in Figure 8 represent the intrinsic stabilities of the salt bridges in a completely non-polar solvent (i.e. $\Delta\Delta G^{\circ}_{solv}$ = 0) and give -38 kJ mol^{-1} for the benzamidinium-formate complex and -44 kJ mol^{-1} for the *N,N'*-dimethylbenzamidinium-formate complex. Using the change in solvation energy from Equations 3 and 4 together with these values allows prediction of the stability of the salt bridge relative to the neutral carboxylic acid and amidine in any solvent for which the H-bond parameters are available (Equations 5 and 6). The only caveat is that the solutes should not be significantly ionised in the free state, otherwise the competing equilibria shown in Figure 1 would complicate the analysis. Figure 9 compares the associations constants calculated using Equations 5 and 6 with those obtained experimentally.

$$\Delta G^{\circ}(\mathrm{H})\ /\ \mathrm{kJ\ mol^{-1}} = -38.3 + 1.4\alpha_S + 3.4\beta_S - 2\alpha_S\beta_S \qquad \text{Eq. 5}$$

$$\Delta G^{\circ}(\mathrm{Me})\ /\ \mathrm{kJ\ mol^{-1}} = -44.5 + 1.9\alpha_S + 5.7\beta_S - 2\alpha_S\beta_S \qquad \text{Eq. 6}$$

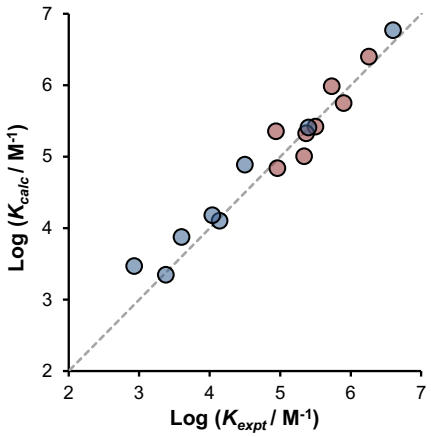


Figure 9. Comparison of experimentally determined association constants for formation of salt bridges with formic acid in different solvents (K_{expt}) with the corresponding values calculated using Equations 5 and 6 (K_{calc}): benzamidine complex in red, and *N,N'*-dimethylbenzamidine complex in blue. The dashed line is $y = x$ (RMSE = 0.25).

Equation 5 predicts that the association constant for the benzamidinium-formate salt bridge should be 6 × 10^7 M^{-1} in water (α_s = 2.8, β_s = 4.5). However, experimental measurements for similar systems, e.g. the guanidinium-acetate salt bridge, show that salt bridges are much less stable in water (K < 1 M^{-1}).[38,39] The discrepancy comes from differences in the nature of the free species. The experiments described here were all carried out in organic solvents in which ionisation of the free carboxylic acid and free amidine is negligible. In water, the ionised species are substantially populated in the free state (Figure 1), and these competing equilibria must be considered in estimation of the overall association constant for formation of the salt bridge. The association constant for formation of a salt bridge (K) can be expressed in terms of the equilibrium constants for protonation of the amidine (K_A), deprotonation of the acid (K_C) and formation of the salt bridge starting from the neutral species (K_N) (Equation 7).

$$K = \frac{K_N}{(1+K_A)(1+K_C)} \qquad \text{Eq. 7}$$

The equilibrium constants for ionisation of the free species in water can be calculated from the acidity constants of benzamidinium (pK_a = 11.6)[40] and formic acid (pK_a = 3.8)[41] giving K_A = 4.0 × 10^4 and K_C = 1.6 × 10^3 at pH 7. Using these values in Equation 7 together with the association constant predicted using Equation 5 (K_N) gives an association constant of K = 0.9 M^{-1} for the benzamidinium-formate salt bridge in water at pH 7, which is consistent with the experimental observations. The difference between the behaviour in water and in organic solvents lies in the ability of water to strongly solvate both anions and cations in the free state, whereas polar aprotic solvents solvate anions poorly.

Analogous behaviour is observed in aprotic solvents if salts of the acid and amidine are used as the starting materials instead of the neutral species. For example, the association constant for the salt bridge formed on mixing tetrabutylammonium benzoate and

benzamidinium chloride in dimethyl sulfoxide (DMSO) is 2,500 M^{-1}.[7] Although we have not measured association constants in DMSO, it is possible to predict the value with Equation 5 using $\alpha_s = 1.4$ and $\beta_s = 8.6$. The calculated association constant for formation of the benzamidinium-formate complex from the neutral species is 3×10^5 M^{-1}. The experimental value reported above is two orders of magnitude lower due to the two competing ion-pair equilibria in the free state, analogous to the competing ionisation equilibria described by Equation 7.

CONCLUSIONS

A systematic investigation of factors affecting the strength of the amidinium-carboxylate salt bridge has been carried out by measuring association constants for 27 different systems using isothermal titration calorimetry. Aromatic substituent effects show that electron-rich amidines and electron-poor carboxylic acids form the most stable complexes, suggesting that there is extensive proton transfer on salt bridge formation. The steric size of alkyl substituents on the nitrogen atoms of the amidine does not affect the stability of the salt bridge.

The results show that solvent plays an important role in determining the stability of salt bridges, and the effects cannot be explained with bulk solvent descriptors. The complex of formic acid with *N,N'*-dimethylbenzamidine shows surprisingly different behaviour to the corresponding complex formed with benzamidine. In chloroform, the presence of the methyl groups increases the stability of the salt bridge slightly. In more polar solvents, there is a decrease of three orders of magnitude in stability of the *N,N'*-dimethylbenzamidine complex, whereas the association constant measured for formation of the benzamidinium-formate salt bridge is between 10^5 and 10^6 M^{-1} in eight different solvents, ranging in polarity from chloroform to dimethylformamide.

The increase in stability of the benzamidine complex relative to the *N,N'*-dimethyl analogue correlates with the solvent H-bond acceptor parameter β_s, which indicates that the stabilisation is due to interactions with the two additional NH H-bond donor sites that are present in the benzamidine complex. There is a substantial increase in the polarity of these two peripheral NH groups when the proton transfer takes place in the benzamidine salt bridge, and the associated increase in free energy contributions due H-bonding interactions with polar solvents stabilises the complex. In very polar solvents (THF and DMF), these solvation effects enhance the stability of the benzamidine-formate complex by two orders of magnitude.

These conclusions are supported by density functional theory calculations of the H-bond parameters for all of the H-bonding sites in the free and bound species. The H-bond parameters were used to calculate the free energy contributions due to solvent-solute interactions in the primary solvation shell, and the calculations quantitatively predict the experimentally observed solvent effects to within 1 kJ mol^{-1}. The approach provides a simple method that accurately predicts the stability of amidinium-carboxylate salt bridges in any solvent. The model also provides a quantitative explanation for the low stability of salt bridges in water, if the equilibria between neutral and ionised species in the free state are taken into account. The solvent effects observed here represent an extreme example of what might be expected to be a more general phenomenon: if formation of a complex increases the polarity of peripheral functional groups exposed to the solvent, then more favourable interactions in the solvation shell will give rise to an unexpected stabilisation of the complex in polar solvents.[42,43]

While H-bonding interactions are strong in non-polar solvents, such as chloroform, and solvophobic interactions are strong in polar protic solvents, such as water, both types of interaction are weak in polar aprotic solvents, such as DMF. The exceptionally large association constants reported here in polar aprotic solvents highlight the unique properties of the amidinium-carboxylate salt bridge, making this interaction a very attractive option for the design of supramolecular systems that operate in a largely unexplored area of solvent space.

ASSOCIATED CONTENT

Supporting Information

Materials and methods, detailed synthetic procedures, characterisation including ^1H and ^{13}C NMR spectra of all compounds, ITC titration data, DOSY NMR experiments, and solvent parameters.

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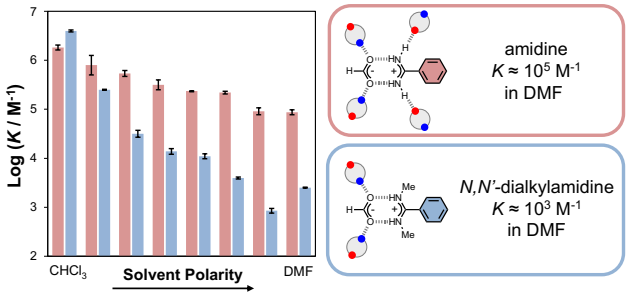
Log (K / M-1)
7
6
5
4
3
2
CHCl3
Solvent Polarity
DMF
amidine
K ≈ 10^5 M^-1
in DMF
N,N'-dialkylamidine
K ≈ 10^3 M^-1
in DMF