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Structure-stability correlations for imine formation in aqueous solution

C. Godoy-Alcántar,[1,2] Anatoly K. Yatsimirsky[3] and J.-M. Lehn[2]*

[1]Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, 62210 Cuernavaca Morelos, México
[2]Institut de Science et d'Ingénierie Supramoléculaires, Université Louis Pasteur, 8 Allée Gaspard Monge, BP 70028, F-67083 Strasbourg, France
[3]Facultad de Química, Universidad Nacional Autónoma de México, 04510 México D.F., México

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ABSTRACT: Imine formation between 25 aldehydes and 13 amines in aqueous solution in the pH range 7–11 was studied by ^{1}H NMR spectroscopy. A three-parameter linear equation correlating logarithms of imine formation constants with pK_a and HOMO energies of amines and LUMO energies of aldehydes is proposed. In view of the widespread occurrence of imine-forming processes in both chemistry and biology, the data presented are of significance for physical organic chemistry and of particular interest for dynamic combinatorial chemistry. Copyright © 2005 John Wiley & Sons, Ltd.

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KEYWORDS: amine; aldehyde; imine formation; structure–stability correlations

INTRODUCTION

One of the fundamental characteristics required for the creation of dynamic combinatorial libraries is the operation of one or several reversible processes that involve covalent connections, non-covalent interactions or reversible intramolecular changes (see Fig. 2 in Ref. 1a).[1] Among the various reversible reactions, imine formation by condensation of carbonyl groups with amines is of special interest, in view of its role in both chemical and biological processes.

The condensations of carbonyl compounds with amines have been broadly used reactions since they were discovered by Schiff in 1864[2] and have been implemented in numerous processes, such as the synthesis of salen-type ligands for metal ion coordination[3,4] and the synthesis of macrocycles of diverse structures often via reductive amination for which imine formation serves as an intermediate step.[5–9] Mostly these reactions have been carried out in different organic solvents in order to shift reversible imine formation towards the condensation product. The creation of libraries of constitutionally dynamic compounds requires, however, reversibility, and therefore, in the field of dynamic combinatorial chemistry, in addition to organic solvents,[10] aqueous organic mixtures[11,12] and aqueous solutions[13–17] have been employed as reaction media.

Although general features of imine formation reactions are well understood,[18] results on imine formation in water are rather limited[19–22] and insufficient to establish the factors which determine the stability of imines. The biological importance of Schiff bases formed between amino acids and pyridoxal stimulated intensive studies in this area,[23] but the complex nature of the acid–base and tautomeric equilibria in which this coenzyme participates make the determination of equilibrium constants for individual forms a very difficult task.[24–27] There are a small number of equilibrium constants available for Schiff base formation between simple aldehyde and primary amine molecules in water, which involve condensation of aniline with benzaldehyde[28] and *p*-chlorobenzaldehyde,[29] and aliphatic amines with pyridine-2-aldehyde and salicylaldehyde.[30] From these results, one may conclude that equilibrium constants with, e.g., benzaldehyde generally are low, increasing from $<10\,\text{M}^{-1}$ for aromatic amines to ca $10^3\,\text{M}^{-1}$ for more basic aliphatic amines, but no quantitative interrelations between amine and aldehyde structures and imine stability have been proposed so far because of a lack of sufficiently extensive experimental data. In this paper, we present the results for imine formation between 25 aldehydes and 13 primary amines of different structures studied in aqueous solution by ^{1}H NMR spectroscopy, aimed initially at optimizing the use of this reaction in the design of dynamic combinatorial libraries, but also providing a basis for the search for structure–stability correlations for imine formation in aqueous solution.

**Correspondence to:* J.-M. Lehn, Institut de Science et d'Ingénierie Supramoléculaires, Université Louis Pasteur, 8 Allée Gaspard Monge, BP 70028, F-67083 Strasbourg, France.
E-mail: lehn@isis.u-strasbg.fr

EXPERIMENTAL

All amines and the majority of aldehydes and components of buffer solutions were obtained from commercial suppliers and used without further purification. Aldehydes **5**, **17–20**, and **22** (Scheme 1) were synthesized by using published procedures.[31–34] ^{1}H NMR spectra were recorded on Bruker 200 MHz, Varian Unity Inova 400 MHz and Bruker Avance 400 MHz spectrometers.

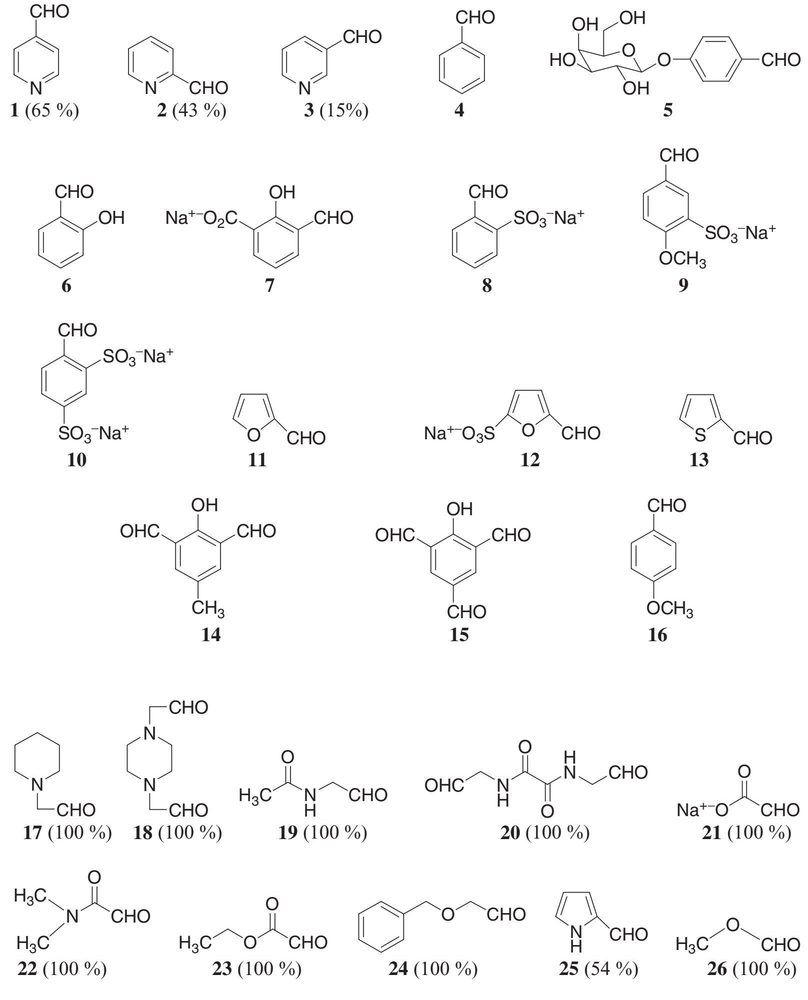
The buffer concentration was 160 mM in all cases and buffer solutions at different pDs were prepared according to pD 7.5 ($D_3PO_4 + NaOD$), pD 8.5 ($ND_4OD + DCl$) and pD 11 ($NaHCO_3 + NaOD$). The pD was calculated employing $pD = pH + 0.4$.[35] Typically 16.5 mM aldehyde and variable amine concentrations were employed.

The course of condensation reactions was followed by appearance of the CH signal of the imine bond in the ^{1}H NMR spectra shifted downfield with respect to the peak of the parent aldehyde and the yield of imine was determined by integration of aldehyde and imine peaks. The time required for equilibration was about 5 min in neutral solutions and 30 min in alkaline media. The degree of aldehyde hydration was determined by integration of aldehyde and hydrate forms in the absence of added amine. Chemical shifts of parent aldehydes, their hydrated forms and imines are given in Table 1S (Supplementary material).

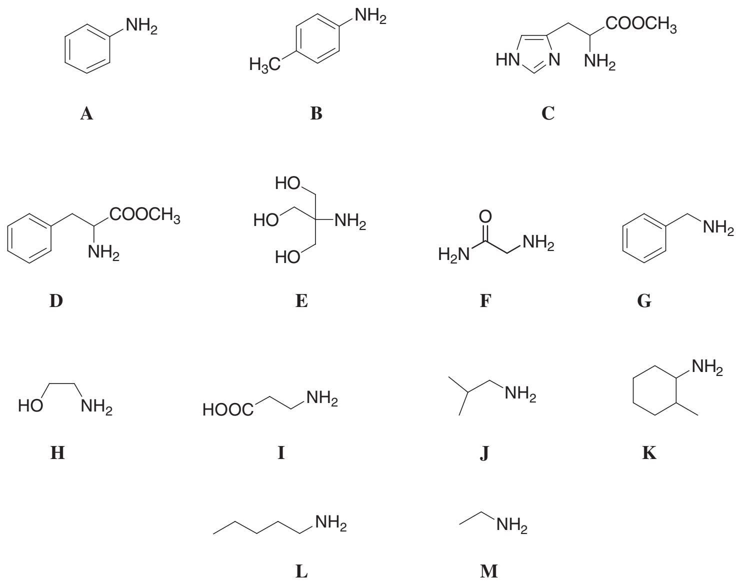
All calculations were carried out in the gas phase using the Gaussian 98 program[36] running on a Compaq-Alpha Unix workstation. The structures of all compounds were energy minimized at the *ab initio* DFT B3LYP/6–31G(d,p) level. Charges, HOMO and LUMO energies and other properties for the energy-minimized structures were obtained from the Gaussian output file. The Fukui indices *f*+ and *f*− were calculated from the functions expressed in terms of the frontier orbital density through the frozen core approximation.[37] Tables 2S and 3S (Supplementary material) show the HOMO and LUMO energies and Fukui indices for some of the amines and aldehydes studied.

RESULTS AND DISCUSSION

Schemes 1 and 2 show the structures of the aldehydes and amines employed, respectively. The aldehyde series



Scheme 1. Structures of aldehydes tested for imine formation. The fraction of hydrated aldehyde is given in parentheses when it was detectable by NMR. Aldehydes **17**–**26** did not form detectable amounts of imines with amine **A**



Scheme 2. Structures of amines tested for imine formation

includes isomeric pyridine aldehydes (**1**–**3**), substituted benzaldehydes (**4**–**10**, **14**–**16**), furanaldehydes (**11**–**13**) and some aliphatic aldehydes (**17**–**26**). The amines tested were aliphatic (**G**, **J**–**M**) and aromatic (**A**, **B**) amines, free and C-protected amino acids (**C**, **D**, **F**, **I**) and amino alcohols (**E**, **H**). All compounds were chosen in order to provide structural diversity, while remaining fairly simple.

Figure 1 shows a typical NMR experiment for determination of the yield of imine in the reaction between aldehyde **8** and increasing amounts of amine **A** at pH 7.6. With weakly basic amines such as **A**, the measurements can be easily performed at pH well above the pK_a of the amine, when practically all amine exists as a free base. These results allow one to calculate the equilibrium constant for the imine formation K directly in accordance with Eqns (1) and (2).

$$\mathrm{RCHO} + \mathrm{R'NH_2} \rightleftharpoons \mathrm{RCH{=}NR'} \qquad (1)$$

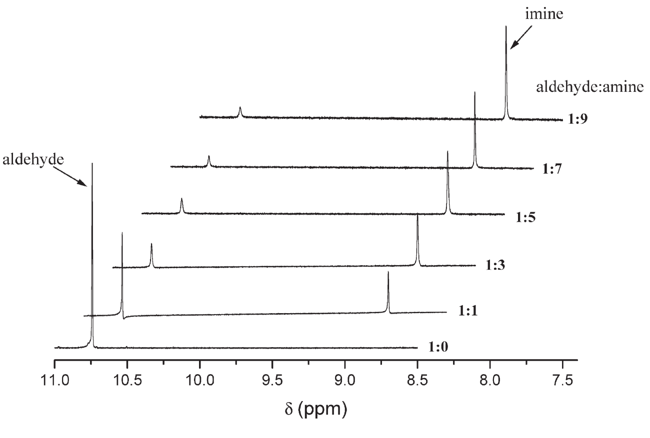


Figure 1. ^{1}H NMR spectra for the aldehyde **8** in the presence of increasing amounts of amine **A** at pH 7.6

$$\begin{aligned} K &= [\mathrm{RCH{=}NR'}]/[\mathrm{RCHO}][\mathrm{R'NH_2}] \\ &= Y/(100 - Y)([\mathrm{R'NH_2}]_\mathrm{T} - [\mathrm{RCHO}]_\mathrm{T}Y/100) \end{aligned} \qquad (2)$$

where Y (%) is the yield of imine at equilibrium calculated as

$$Y = 100[\mathrm{RCH{=}NR'}]/([\mathrm{RCH{=}NR'}] + [\mathrm{RCHO}]) \qquad (3)$$

and $[\mathrm{R'NH_2}]_\mathrm{T}$ and $[\mathrm{RCHO}]_\mathrm{T}$ are the total concentrations of amine and aldehyde.

With highly basic amines such as **J**–**M**, the measurements were performed at pH values below the pK_a and, assuming that only the neutral form of the amine participates in the reaction, one obtains the following expression for the imine formation equilibrium constant:

$$\begin{aligned} K = Y(1 + [\mathrm{H^+}]/K_\mathrm{a})/\{(100 - Y)([\mathrm{R'NH_2}]_\mathrm{T} \\ - [\mathrm{RCHO}]_\mathrm{T}Y/100)\} \end{aligned} \qquad (4)$$

where K_a is the acid dissociation constant for the protonated amine.

Since measurements were performed in D_2O and there is a significant solvent isotope effect on pK_a values,[38] other experiments were performed for the simultaneous determination of K and pK_a values. They consisted in varying the pH at a fixed amine concentration taken in a high excess over also fixed aldehyde concentrations, as illustrated in Fig. 2. The results were fitted to Eqn (4) rearranged, assuming that $[\mathrm{R'NH_2}]_\mathrm{T} \gg [\mathrm{RCHO}]_\mathrm{T}$, as

$$Y = 100K[\mathrm{R'NH_2}]_\mathrm{T}/(1 + [\mathrm{H^+}]/K_\mathrm{a} + K[\mathrm{R'NH_2}]_\mathrm{T}) \qquad (5)$$

In addition, plotting the chemical shifts of the free amines measured in these experiments as a function of pH allowed us to determine the pK_a values of the amines under the conditions employed. They were used to calculate K and in the correlation analysis discussed below.

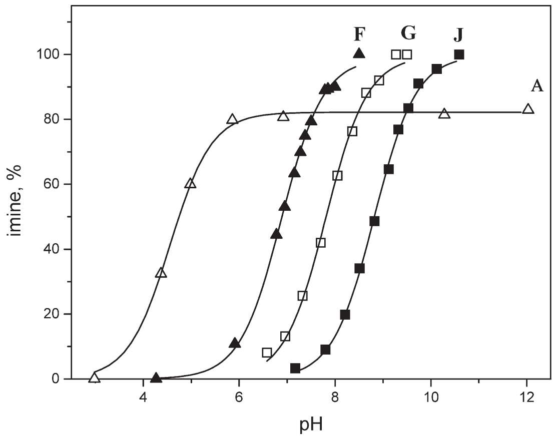


Figure 2. Typical plots of the yield of imine vs pH at fixed concentrations of different amines (0.1 M) and aldehyde **8** (0.01 M)

In preliminary experiments, the ability of aldehydes to form imines was tested by adding $\geq$1 equiv. of aniline (**A**) to neutral solutions of aldehydes. No imine formation was detected with the group of compounds **17–26**, which are completely hydrated in water. Less hydrated pyridine aldehydes **1–3** reacted with aniline and the highest yields of imine were observed with the sulfonated aldehydes **8**, **10** and **12**. On the basis of these results, the aldehyde **8** was chosen for testing imine formation with different amines.

Hydration, of course, competes with imine formation, but on the other hand the same structural factors which favor hydration of aldehydes should also be favorable for imine formation. It has been shown that equilibrium constants for nucleophilic addition of semicarbazide and hydroxylamine to carbonyl compounds correlate very well with those for hydration.[39] In the case of imine formation, the nucleophilic addition of a primary amine is the first step of the overall reaction and probably it also correlates with hydration, but the subsequent dehydration step may show an opposite trend.

Table 1. Imine formation constants K for aldehyde **8** and different amines

Amine	K M^{-1}	pK_a	E(HOMO) (eV)
A	45.0	5.30	−5.393
C	63.4	7.15	−5.835
D	58.6	7.39	−5.965
E	12.2	8.42	−6.621
F	38	8.45	−6.312
G	2610	10.23	−6.198
H	1630	10.54	−6.317
J	4450	11.47	−6.191

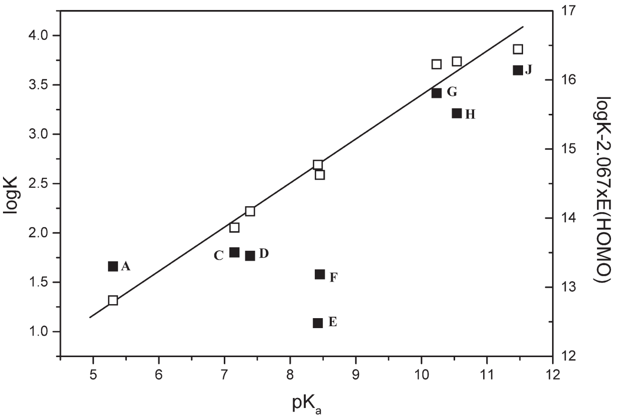


Figure 3. Correlation of imine formation constants for aldehyde **8** and different primary amines with pK_a of protonated amines (closed squares) and in terms of two-parameter Eqn (6) (open squares)

Table 1 gives imine formation constants for aldehyde **8** and a series of amines of increased basicity. As expected, the values of K are higher for more basic amines, but the correlation between log K and pK_a of protonated amines is rather poor, as can be seen in Fig. 3 (closed squares). A possible reason for this is that pK_a values do not reflect adequately the ability of amines to form a covalent bond. An often used measure of the potential strength of covalent bonding is the difference between the HOMO and LUMO of donor and acceptor molecules, respectively.[40] Since the level of the LUMO for a given aldehyde is constant, we attempted to correlate the results in Table 1 with a two-parameter equation, which takes the form of Eqn (6), where E_{HOMO} is the energy of the HOMO (eV) for amine molecules.

$$\log K = (9.364 \pm 1.507) + (2.067 \pm 0.291)E_{\mathrm{HOMO}} + (0.641 \pm 0.053)\mathrm{p}K_a \quad (6)$$

The quality of the correlation is good ($R^2 = 0.967$, SD $= 0.211$, Prob $> |t|$ below 0.0016 for all coefficients and Prob $> F$ below 0.0002), as illustrated graphically in Fig. 3 (open squares). Other parameters tested for correlations were the difference between HOMO and LUMO for amines and Fukui parameters (see Supplementary material for the set of these parameters), both by themselves and in combination with pK_a, but they did not give better results.

The variation in log K between the least and most basic amines **A** and **J** is just 2 units whereas the pK_a values for these amines differ by 6 units (Table 1). This may be attributed to the fact that weakly basic aromatic amines also possess higher HOMO energies and, at least in part, in accordance with Eqn (6), this improves the binding. In general, higher values of E_{HOMO} for amines favor the covalent contribution and higher pK_a values favor the electrostatic contribution to the imine bond formation, a situation reminiscent of the interpretation of nucleophilic reactivity in terms of a hard/soft approach.[40]

Table 2 shows the results for imine formation between amine **A** and a series of aldehydes. Different empirical and theoretically calculated characteristics of aldehyde molecules, such as chemical shifts of aldehyde protons,

Table 2. Imine formation constants K for amine **A** and different aldehydes

Aldehyde	K M^{-1}	E(LUMO) (eV)
1	23	−2.309
3	19.8	−2.09
4	2.29	−1.726
5	4.95	−1.562
6	6.65	−1.588
7	7.41	−2.129
8	45.0	−2.407
9	4.0	−2.067
10	154	−3.099
11	11.4	−1.677
12	99.2	−2.561
13	34.0	−1.926

vibration frequency of carbonyl group, effective charges on the carbonyl carbon, energies of HOMO and LUMO, their difference and Fukui parameters were tested for single- and multi-parameter correlations of log K values. The best, although still rather scattered, correlation was obtained with E_{LUMO} values in terms of Eqn (7) with $R^2 = 0.89216$ and SD = 0.27458 (Fig. 4).

$$\log K = -(1.30 \pm 0.40) - (1.12 \pm 0.18)E_{\mathrm{LUMO}} \qquad (7)$$

Apparently, results for furan aldehydes form a separate line with the same slope, but shifted upwards by ca 0.55.

Equations (6) and (7) can be combined to afford Eqn (8), parameters for which were obtained by multiple regression on all data given in Tables 1 and 2 together, with $R^2 = 0.91206$ and SD = 0.29522 (Prob > |t| were below 0.001 for all coefficients, including the intercept, and Prob > F was below 0.0001, indicating significance of all three parameters of the multiple regression).

$$\log K = 6.40 \pm 1.62 - (1.04 \pm 0.19)E^{\mathrm{aldehyde}}_{\mathrm{LUMO}} + (2.00 \pm 0.36)E^{\mathrm{amine}}_{\mathrm{HOMO}} + (0.65 \pm 0.07)\mathrm{p}K^{\mathrm{amine}}_{\mathrm{a}} \qquad (8)$$

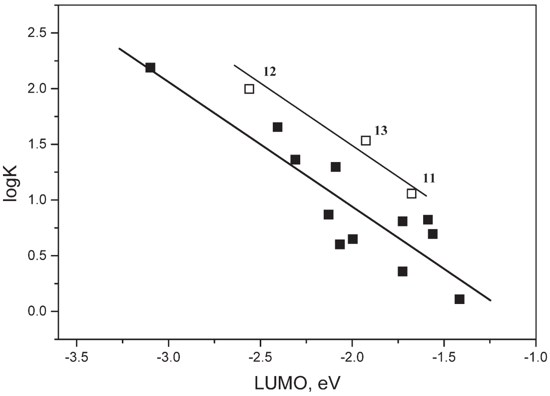


Figure 4. Correlation of imine formation constants for aniline and different aldehydes (Table 2). solid squares, substituted benzaldehydes [the solid line is a regression line in accordance with Eqn (7)]; open squares, aldehydes of the furan series

Table 3. Additional imine formation constants in water at 25 °C

Aldehyde	Amine	Log K_{obs}[a]	Aldehyde	Amine	Log K_{obs}[a]
1	**L**	3.57	**7**	**L**	3.77
	K	3.17		**I**	2.60
	I	2.96	**8**	**M**	3.66
2	^{n}BuNH$_2$	3.4[b]		**L**	3.67
	^{t}BuNH$_2$	2.21[b]		**K**	3.80
4	**A**	0.78[c]		**B**	1.45
5	**M**	3.57		**I**	1.78
	L	3.19	**12**	**M**	3.57
	K	3.60		**L**	3.80
6	**I**	2.97		**K**	4.51
	C	0.48		**I**	2.52
	^{n}BuNH$_2$	4.74[b]	**14** anion	**L**	1.90
	^{t}BuNH$_2$	3.97[b]		**I**	2.26
	M	4.44[b]	**15** anion	**L**	1.78
	H	3.70[b]		**I**	1.00
6 anion	^{n}BuNH$_2$	1.0[b]	**16**[d]	**A**	0.11
	^{t}BuNH$_2$	−0.70[b]	*p*-Cl-**4**[e]	**A**	0.65

[a] Calculated by using data from Table 1S (Supplementary material) and Eqn (4).
[b] Ref. 30.
[c] Ref. 28.
[d] Measured spectrophotometrically.
[e] Ref. 29.

In addition to the results given in Tables 1 and 2, about 20 additional K values were estimated from single measurements for different pairs of amines and aldehydes (Table 1S, Supplementary material). These values, together with several formation constants reported in the literature, are given in Table 3. Equation (8) was used to calculate K values for all pairs of aldehydes and amines reported and the respective K_{calc} values are given in Table 4S in (Supplementary material) together with the data on each amine and aldehyde employed in the calculation of Eqn (8).

Figure 5 shows the plot of experimental log K values vs log K_{calc}, from which one can see that with the exclusion of a few points the correlation is reasonably good. The

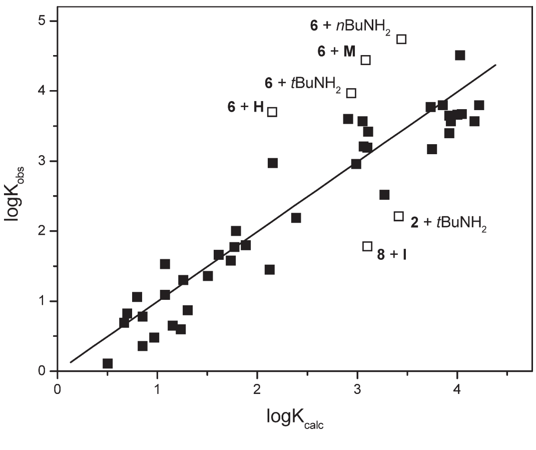


Figure 5. Experimental vs calculated [Eqn (8)] formation constants

regression line for points shown as solid squares follows Eqn (9) with $R^2 = 0.957$ and $SD = 0.375$.

$$\log K = -0.03 \pm 0.13 + (0.96 \pm 0.05) \log K_{\text{calc}} \quad (9)$$

Hence Eqn (8) allows one to predict the imine formation constant for a given pair of amine and aldehyde within limits of a twofold variation. Significant positive deviations are observed with aldehyde **6** bearing an *o*-hydroxyl group and can be attributed to intramolecular hydrogen bonding with the imine nitrogen. Also, significant but negative deviations for $\mathbf{8} + \mathbf{I}$ and for $\mathbf{2} + {}^{t}BuNH_2$ can be explained by electrostatic repulsion between negatively charged reactants for the former case and by steric effects for the latter.

It is worth noting that Eqn (6) is closely related to the two-parameter Edwards equation[40] and the final Eqn (8) proposed in this paper for equilibrium constants of imine formation may be considered as a simplified empirical version of the Klopman equation derived on the basis of generalized polyelectronic perturbation theory[41] for nucleophilic reactivity.

Finally, imine exchange (transimination) experiments involving the addition of another amine or aldehyde to the equilibrium solution of a given amine–aldehyde pair indicated that exchange and re-equilibration occurred instantaneously, i.e. within the time needed to perform the measurement. This fast exchange is of particular significance for dynamic combinatorial chemistry.[1] Imine formation and exchange are well suited for such purposes, provided that components and conditions can be found where formation is high while exchange remains fast.

In conclusion, the present results provide basic information for the implementation of imine formation processes in constitutional dynamic chemistry[42] (for a recent review on dynamic covalent chemistry, see Ref. 43) towards the generation of dynamic libraries of constituents for both drug discovery[44] and development of dynamic materials.[45]

Supplementary material

The following tables are available in Wiley Interscience: Table 1S, selected data for imine formation in D_2O at 25 °C together with chemical shifts for aldehyde and imine CH protons; Tables 2S and 3S, HOMO and LUMO energies and Fukui indices for some amines and aldehydes studied; Table 4S, observed and calculated imine formation constants in water at 25 °C and parameters of Eqn (8).

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