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COMMUNICATION

Precipitation-driven self-sorting of imines†

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Judicious choice of precipitation conditions can lead to self-sorting of equilibrating mixtures of aromatic aldehydes and substituted anilines into a handful of imine products. The selectivity of this process is caused by the solubility differences among possible imines in the EtOH–H_2O solvent mixtures used in precipitation.

Outcomes of chemical reactions can be profoundly influenced by precipitation and other phase changes. Selective removal of individual species from the solution can drive their production to completion, and sequestration within a crystal can also create a kinetic barrier to undesirable side reactions. Precipitation-driven selectivity shifts are widely used in academic and industrial settings, and—on account of their simplicity and robustness—they may have played a role in the prebiotic peptide synthesis[1] and enantiomeric amplification[2] processes.

Notable recent work in the area of dynamic combinatorial chemistry (DCC)[3] of imine complexes relied on selective crystallization to achieve diastereomeric amplification of copper–imine helicates,[4] as well as the preparation of interlocked Solomon knots[5] and receptors for CO_2.[6] A perhaps neglected aspect of such selectivity-through-precipitation protocols is that precipitation of a certain pure species from a dynamic combinatorial library (DCL) also simplifies the composition of the solution—often to a single compound that can be isolated in high yield. With our interest in self-sorting processes,[7] we speculated that parallel synthesis of multiple imines could be achieved under precipitative conditions, provided that their low solubilities favor them over the alternative crossover products. In this Communication, we report that mixtures of anilines and aromatic aldehydes give rise to ordered imine combinations, if their synthesis is carried out under selective precipitation conditions.

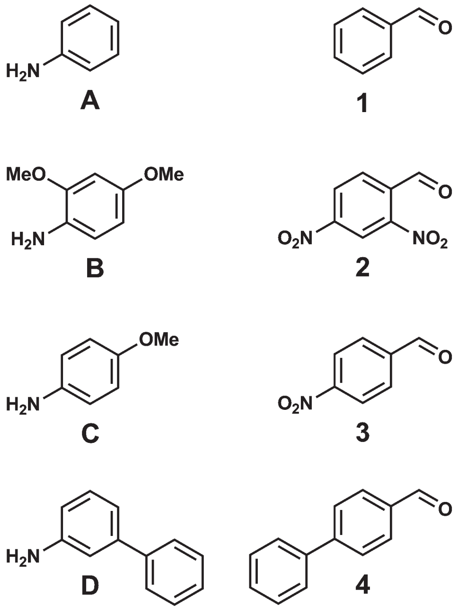

Fig. 1 Anilines and aldehydes discussed in this study. In the text, as well as in the remaining Schemes, imines formed from these compounds will be designated by combining the aniline letter with the aldehyde number—*e.g.* imine **B3** is formed by a dehydrative condensation of aniline **B** with aldehyde **3**.

As our previous work demonstrated, freely equilibrating dynamic imine libraries[3] spontaneously simplify (*i.e.* self-sort) when exposed to an irreversible chemical (oxidation)[8] or physical (distillation) stimulus.[9] These methods require substrates that can be easily oxidized or evaporated, respectively. Precipitation should present a much broader route to self-sorting, as virtually any imine can be precipitated—or dissolved—provided that conditions are appropriately adjusted. We thus set out to explore the self-sorting behaviors of imines constructed from anilines **A–D** and aldehydes **1–4** (Fig. 1). These compounds were chosen because of their widely different electronic and steric characteristics, which suggested that the formed imines may have solubilities different enough to allow selective self-sorting behavior. In our first experiment (Scheme 1, left), an equimolar mixture of aniline (**A** in Fig. 1), 2,4-dimethoxyaniline (**B**), benzaldehyde (**1**), and 2,4-dinitrobenzaldehyde (**2**) was dissolved in EtOH, yielding a 25.5 mM solution with respect to each component. Initially clear solution quickly turned cloudy, indicating the onset of precipitation of an imine product. Two crops of precipitate were collected and combined, and their analysis revealed virtually pure imine **B2**, in 77% total yield. The residual solution contained imine **A1** as the dominant component (82% yield),

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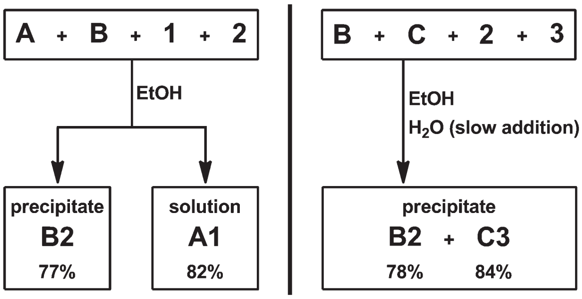
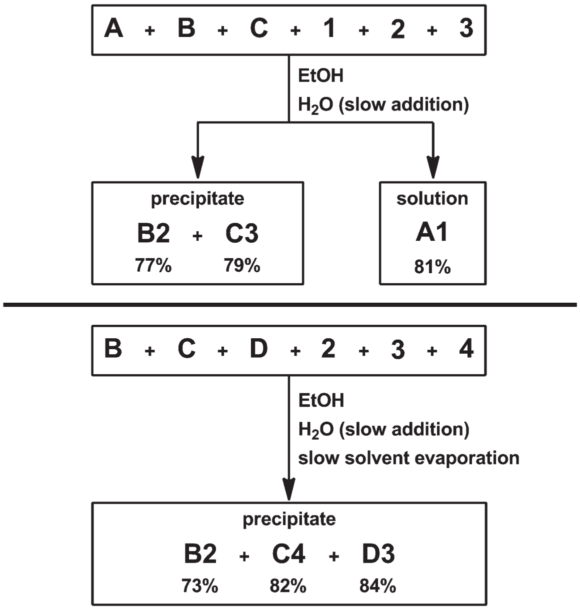


Scheme 1 Self-sorting of two [2 × 2] aniline–aldehyde mixtures. Only the yields of major component imines are given; for the yields of other imines, see the text and the ESI.†

with minor contamination by **B1** (11%), **A2** (7%), and **B2** (2%).[10] The mixture was effectively self-sorted, as products **A2** and **B1** were strongly disfavored in both solution and the precipitate—although they are in principle competitive with the dominant **B2** and **A1**.

This initial result encouraged us to target a mixture of anilines and aldehydes with smaller structural differences (Scheme 1, right). Upon dissolution of compounds **B**, **2**, 4-methoxyaniline (**C**), and 4-nitrobenzaldehyde (**3**) in EtOH, precipitation begun almost instantaneously. Preliminary analysis of the precipitate revealed a combination of **B2** and **C3**; while the two imines co-precipitated, they were curiously uncontaminated by the crossover products **B3** or **C2**. However, analysis of the remaining solution after 24 h indicated significant presence of all four possible imines, suggesting that the precipitation was not complete. To circumvent this problem, we devised a protocol in which H_2O was slowly injected into the EtOH solution during the course of the reaction. The purpose of this modification was to turn EtOH into a poor solvent for imines, but to do so gradually in order to avoid precipitating starting anilines or aldehydes. At the end of H_2O addition, the final H_2O–EtOH volume ratio was 1.33 : 1. Using this new strategy, overnight precipitation resulted in the solid that contained **B2** (78%) and **C3** (84%) as major components, with minor amounts of crossover imines **B3** (10%) and **C2** (11%). Among three independent trials, yields of **B2** and **C3** in this precipitation protocol varied by less than 2%. Importantly, the addition of H_2O did not lower imine yields; once precipitated, imines examined in this study appeared stable to hydrolysis on the time scale of the performed experiments.

High selectivities observed in these two [2 × 2] experiments suggested that self-sorting of imines was possible during their precipitation and that it was not limited to a strict fractionation between a solution and a precipitate—*i.e.* selectivity can be achieved even during co-precipitation. Low solubility of precipitated imines appears to control the selectivities of these reactions. This notion was confirmed by the transmutation experiment in which pure imines **B1** and **A2** were dissolved in EtOH and subjected to the slow addition of H_2O. After overnight stirring, imine **B2** precipitated as a virtually pure compound (90% yield), while the solution contained chiefly **A1**, which could be isolated in 75% yield.



Scheme 2 Self-sorting of two [3 × 3] aniline–aldehyde mixtures. Only the yields of major component imines are given; for the yields of other imines, see the text and the ESI.†

After demonstrating successful self-sorting of [2 × 2] mixtures of anilines and aldehydes, we analysed the behavior of more complex [3 × 3] mixtures. First, we combined anilines **A–C** with aldehydes **1–3** in EtOH (Scheme 2, top), and subjected the mixture to slow addition of H_2O. An orange solid was isolated after overnight precipitation. Its spectroscopic analysis revealed a mixture dominated by imines **B2** (77%) and **C3** (79%). Small amounts (1–15%) of five additional imines could be identified from the crude ^1H NMR spectrum of the mixture. The filtrate contained **A1** as the main component (81%) and low amounts (0.2–9%) of the eight remaining possible imines.

Next [3 × 3] experiment saw the aldehyde **1** replaced with 4-phenylbenzaldehyde (**4**), and aniline **A** with 3-phenylaniline (**D**), while keeping **B**, **C**, **2**, and **3** as the components of the mixture. After a slightly modified procedure, in which H_2O addition to EtOH solution was followed by slow (10 d) evaporation of mixture to dryness, the analysis of the thus formed orange solid revealed exclusive presence of imines **B2** (73%), **C4** (82%), and **D3** (84%). Surprisingly—in light of our previous experiments—no significant amounts of either **C3** or **D4** were detected. This precipitation protocol was also repeated three times to ensure reproducibility, and only minor variations in yields were observed (up to 6% for **B2**, 4% for **C4** and 2% for **D3**).

Successful self-sorting observed in experiments presented in Schemes 1 and 2 is readily explained after examination of solubility data for all possible imines (Table 1). In the first experiment (Scheme 1, left), imine **B2** is clearly the compound with the lowest molar solubility: ∼18 times less soluble than **A2**, and >40 times less soluble than either **A1** or **B2**. Thus, its precipitation is “uncontested” and essentially complete in pure EtOH, removing all **B** and **2** from the solution. With these two components sequestered in the precipitate, remaining **A** and **1** have no choice but to form **A1** as the solution-phase second product.

Examination of the relative solubilities of possible imines in the second [2 × 2] self-sorting experiment (Scheme 1, right) again suggests **B2** as the least soluble compound. However, the

Table 1 Solubilities of imines discussed in this study

Imine	Solubility[a] [mmol L^{-1}] in: EtOH	EtOH–H_2O (1 : 2.4, v/v)
A1	>36.8	13.3
A2	11.1	1.5
A3	29.5	3.5
B1[b]	>27.6	>16.6
B2	0.6	<0.1
B3	>23.3	2.8
B4	8.7	1.6
C1	>31.6	8.5
C2	5.2	0.3
C3	10.9	0.2
C4	2.3	1.7
D2	8.6	2.9
D3	12.1	1.0
D4	7.4	0.3

[a] All solubilities were measured at 22 °C. [b] Compound **B1** was the only liquid among the studied imines.

differences are not as dramatic as in the previous case: **B2** is ∼9 times less soluble than **C2**, ∼18 times less than **C3**, and >35 less than **B3**. These smaller differences (along with the lower absolute solubility of **C3**, *vide infra*) explain why **B2** could not be precipitated alone, leaving the more soluble imines in the solution. The formation of **C3** as the second product is rationalized by the fact that it does not share either the aldehyde or the aniline component with the favored **B2**. Thus, precipitative self-sorting can express the more soluble **C3** (which does not compete with **B2**), rather than the less soluble **C2**—which must compete with **B2** for a resource, aldehyde **2**.

Sorting of the more complex [3 × 3] aniline–aldehyde mixtures could have potentially generated nine imines, but resulted in the high-yield formation of only three. These findings can also be rationalized using the solubility data. In the first [3 × 3] study (Scheme 2, top), possible products included imines **A1–3**, **B1–3**, and **C1–3**. Among these, imine **B2** is ∼9 times less soluble than next most-insoluble compound, **C2**. Precipitation of **B2** will thus be favored, sequestering **B** and **2** from all other imines that contain them. Within the reduced pool of imines that do not share a component with **B2**—that is, compounds **A1**, **A3**, **C1**, and **C3**—the only imine that can be precipitated under the examined conditions is **C3**. The absolute solubility of **A1**, **A3**, and **C1** is too high for them to effectively compete during the precipitation process. Exclusive precipitation of **C3** removes all **C** and **3** from the solution, and the soluble **A1** must form as the third product.

Similar reasoning explains the outcome of the last [3 × 3] experiment. Introduction of the new aldehyde component **4** allows the generation of a highly insoluble imine **C4**, which is now the second least-soluble compound (after **B2**). Since **B2** and **C4** do not share constituents, their precipitation can proceed orthogonally and simultaneously, depleting the solution's supply of aldehydes **2** and **4**, as well as anilines **B** and **C**. This leaves **D3** as the only possible imine in the mother liquor; addition of H_2O ensures its complete precipitation, completing the self-sorting event.

Lastly, we explored the effects of concentration and solvent on the compositions of final imine libraries. If the reaction between **B**, **C**, **2**, and **3** (Scheme 1, right) is performed in EtOH at 10 times lower component concentrations (4.17 mM) than initially described, all four imines remain soluble throughout the course of the reaction. In this case, no self-sorting is observed at equilibrium: relative molar ratios of **B2** (1.00), **B3** (1.15), **C2** (1.08), and **C3** (1.25) are close to random distribution.[11] When a solvent was changed to MeCN—in which all imines are soluble—similarly random mixtures of four or nine components were obtained in all [2 × 2] and [3 × 3] experiments. These two control experiments virtually exclude the possibility that self-sorting was caused by electronic factors in solution.[12] On the other hand, concentrations of all components can be increased up to 125 mM (3×) without a large loss in the fidelity of self-sorting (83% **B2**, 74% **C3**). However, at component concentrations of 209 mM (5×), dramatic deterioration in fidelity is observed: **B2** is formed in 51% yield, **C2** in 42%, and **C3** in 34%.[13]

Experiments performed in this study suggest that self-sorting of DCLs can proceed under precipitative conditions. During precipitation, some imine libraries sorted into segregated solution/solid ensembles, while others produced co-precipitated mixtures—but all reactions proceeded with high selectivities. These four experiments illustrate an important concept in the study of self-sorting systems, namely that collective properties take precedence over the properties of individual components studied in isolation. This switch is not absolute: dominant members of the potential imine pool—such as **B2** in all our examples—will invariably occur in the self-sorted state because of their extreme individual insolubility. On the other hand, library members with higher individual solubilities are forced to compete with each other for resources (aldehyde and aniline components). In those cases, the influence of the remainder of the mixture can be crucial, as a library member (*e.g.* **C2**) can be strongly disfavored if it competes for a resource with a dominant member (**B2**). Conversely, a library member can be favored beyond its individual "merit", if its composition is orthogonal to the dominant member, while that of its less soluble competitors is not. This primitive form of internal regulation of a synthetic process appears to be a general feature of self-sorting systems and the one that we are currently intensively exploring.

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10 Yields of individual imines were calculated from the overall mass recovery of the mixture and ^{1}H NMR spectra integration data. Isolation of individual imines would have likely altered the distribution of the products and was not performed.

11 When H_2O was slowly added to this diluted mixture of imines, slow and incomplete precipitation occurred, yielding mixtures of imines in both the precipitate and the mother liquor.

12 Imine mixtures precipitated from EtOH–H_2O exhibit a certain degree of kinetic stability after re-dissolution in MeCN: mixture obtained in experiment shown in Scheme 2 (bottom) did not change in composition even after 7 days in MeCN solution at 25 °C. Equilibration into the random mixture of products was achieved only after heating in MeCN at reflux for 24 h. This kinetic stability is perhaps unsurprising, as rapid imine exchange often requires catalysis by free anilines. See, for example: (*a*) A. Dirksen, T. M. Hackeng and P. E. Dawson, *Angew. Chem., Int. Ed.*, 2006, **45**, 7581–7584; (*b*) A. Dirksen, S. Dirksen, T. M. Hackeng and P. E. Dawson, *J. Am. Chem. Soc.*, 2006, **138**, 15602–15603.

13 Concentrations higher than 209 mM were not studied because they led to the formation of undesired non-imine products. Most significant of these (29%) was the acetal formed between aldehyde **C** and EtOH which was used as the solvent.