Dynamic Combinatorial Chemistry

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Adsorption-Driven Self-Sorting of Dynamic Imine Libraries**

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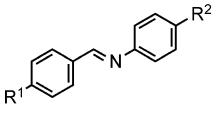
Abstract: *Differences in adsorption among the components of complex mixtures play a role in separations, surface-based sensing, and heterogeneous catalysis, and have been implicated in theories of the origin of life. Herein, we consider mixtures of imines and we show that if such complex mixtures are also dynamic—that is, if their components equilibrate among themselves—then they can dramatically simplify in composition during the course of column chromatography. As they travel down the column, imines continuously trade their aldehyde and amine constituents, favoring the formation of molecules with extremes of polarity at the expense of species with intermediate polarities. Iterative application of this principle leads to simplification of imine libraries containing up to 16 members into 4 major products.*

The differential adsorption of compounds from a liquid or gaseous mixture on solid materials is of critical importance in the fields of heterogeneous catalysis, surface sensing, separations, and even in theories of the origin of life.[1] Adsorption of components of dynamic combinatorial libraries (DCLs)[2] should additionally result in substantial redistribution of the material among the equilibrating species so that the best-adsorbed species are amplified at the expense of their less-well-adsorbed counterparts. Further benefits can come from the use of open systems which do not establish a thermodynamic equilibrium and can amplify even small differences in equilibrium partition coefficients to yield synthetically useful product distributions. Herein, we present such a case: simplification of dynamic libraries containing as many as 16 components into just 4 final products during the course of column chromatography on silica gel.

Dynamic combinatorial chemistry (DCC) studies equilibrating compound mixtures which can respond to external stimuli by increasing the proportion of (i.e. amplifying) those library components that best adopt to the disturbing stimulus, at the expense of those that do not. This error-correcting mechanism allows production of thermodynamically stabilized species in yields that are often quantitative, as the material can be continuously recycled across shallow potential-energy surfaces until the thermodynamic minimum is reached. This key benefit of DCC is also its key drawback as working in a closed system at equilibrium does not allow the production of kinetically favored products.

By switching instead to an open system with a continuous flow of material, kinetic and thermodynamic factors can work together, so that kinetically controlled products can be produced in high yields enabled by continuous recycling of precursors before they enter an irreversible removal process.[3] We and others have explored this behavior to achieve self-sorting,[3,4] dynamic kinetic resolution,[5] and self-replication.[6] Our work demonstrated that iterative application of an irreversible kinetic stimulus in an open system results in the cascade of disproportionation processes, simplifying a library containing n^2 members into just n products. The stimulus for these self-sorting processes can be a chemical reaction[7] or a physical transformation, such as distillation,[8] precipitation,[9] or transfer of materials between two liquid phases.[10] Herein, we show that DCL members can be amplified based on the difference in their partition coefficients between the mobile and the stationary phase during the course of column chromatography.[11]

We hypothesized that slow elution of a dynamic combinatorial library constructed from some of the imines **A1**–**E5** (Figure 1) would amplify the least polar component that



R1 \ R2	Br	Me	OMe	COOH	H
Br	**A1**	**A2**	**A3**	**A4**	–
Me	**B1**	**B2**	**B3**	**B4**	–
OMe	**C1**	**C2**	**C3**	**C4**	–
COOH	**D1**	**D2**	**D3**	**D4**	**D5**
H	–	–	–	**E4**	**E5**

Figure 1. Structures of imines employed in this study.

elutes first. As this occurred, its more polar counterparts that share either the same aldehyde or amine starting components as this least polar imine, would disproportionate to replenish the least polar imine and to amplify the most polar imine at the expense of species of intermediate polarity (illustrated on a mixture of imines **B2**–**C3** in Scheme 1).[12]

Each of the examined imine libraries was formed by mixing equimolar amounts of the requisite aldehydes and anilines in toluene as the solvent, followed by heating the mixture at reflux for 12 hours. During the heating, H_2O was removed using a Dean–Stark trap. Once dehydration was complete, the toluene was evaporated and the crude imine library mixture was pre-adsorbed onto oven-dried silica with an approximate mass 2.5 times that of the crude reaction mixture. This solid mixture was placed on top of a chroma-

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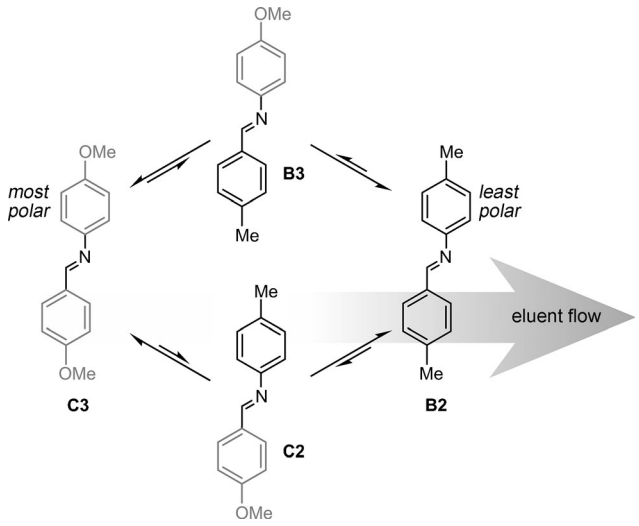


Scheme 1. Simplification of a [2×2] imine library during the course of column chromatography. The least polar imine **B2** combines the two black components and travels fastest through the column. As it is being depleted, the two crossover components (**B3** and **C2**) react to produce more of **B2**, while simultaneously amplifying the most polar fraction **C3**, which combines the two gray "sticky" components.

tography column (inner diameter 150 mm) which was preloaded with a layer of oven-dried silica gel (10–20 cm high). Finally, the part of the column containing the silica gel was wrapped in a heating tape, which was used to keep the column at a temperature of approximately 50 °C.

In the first experiment, a mixture of imines **B2**, **B3**, **C2**, and **C3** was prepared and analyzed by ^{1}H NMR spectroscopy. The relative ratios of the four imines were calculated to be 1.00:0.65:0.66:0.99 (Figure 2a, white vertical bars), respectively. Column chromatography of this mixture was initially performed with a 100:1 v/v mixture and then with a 20:1 v/v mixture of hexane and EtOAc. Two major fractions were isolated. In the first fraction, the dominant product was **B2** (85 ± 1%), as determined by integration of the resonance signals in the ^{1}H NMR spectrum of this fraction by comparison with an internal standard 1,3,5-trimethoxybenzene. Similar analysis of the second fraction revealed **C3** as the major component (89 ± 1%) and a small amount of **C2** (8 ± 0.2%). In essence, fast elution of the least polar imine **B2** disturbed the equilibrium distribution, forcing **B3** and **C2** to react and replenish the removed **B2**—until their constituents were completely consumed in the process. The most polar imine **C3**—which did not share either of the components of **B2**—was also amplified in the mixture at the expense of its counterparts of intermediate polarity (Figure 2a, black bars).

As this experiment required the addition of a significant amount of silica gel to the imine mixture, we next confirmed that this addition does not significantly change the equilibrium. The initial mixture was combined with an approximately 2.5 times greater mass of silica gel and then heated at reflux in toluene overnight. After the removal of silica gel by filtration, integration of the resonance signals in the ^{1}H NMR spectrum of the resultant solution revealed a **B2**:**B3**:**C2**:**C3** ratio of 1:0.96:0.88:0.80, suggesting that silica gel does not dramatically change the ratio of the library members (Fig-

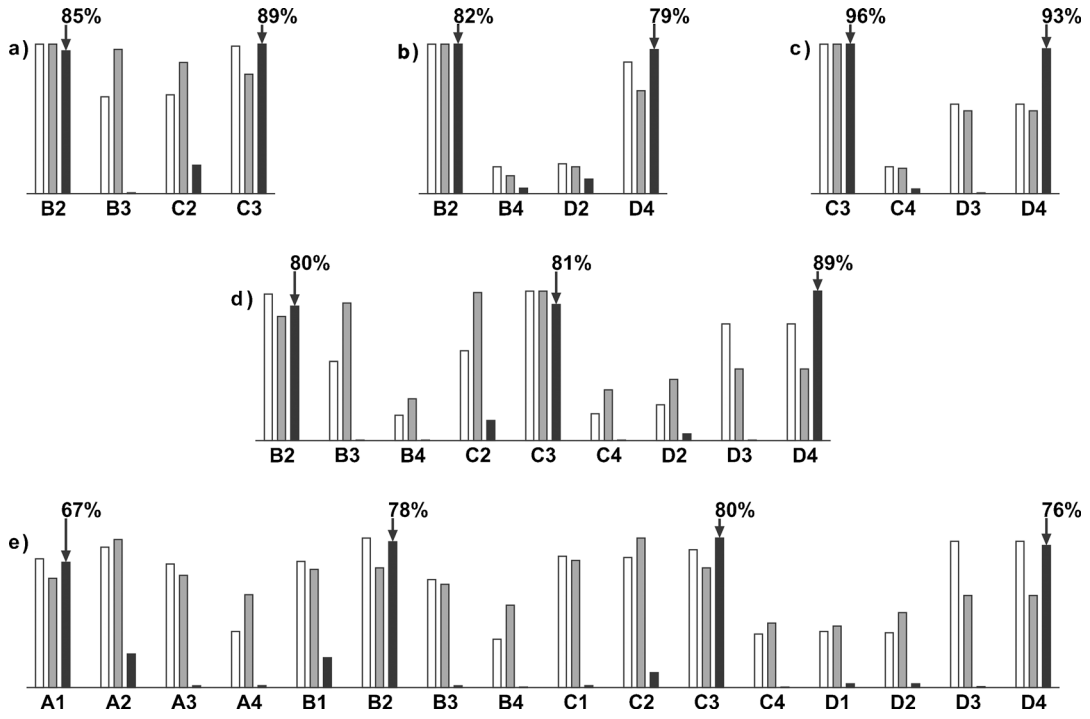


Figure 2. Relative normalized distributions of imine components in dynamic libraries before (white bars) and after (gray bars) addition of silica gel. The black bars show the total yields of individual imines from the isolated fractions, which were determined using the integrals of resonance signals in the ^{1}H NMR spectra with an internal standard.

ure 2a, gray bars). As only the solution composition was monitored, the measured changes in library composition can be interpreted as a consequence of differences in adsorption on silica gel among the various imines in the mixture.

Two additional [2×2] experiments were performed. In the first (Figure 2b), imines **B2**, **B4**, **D2**, and **D4** were similarly analyzed with and without silica gel, before being subjected to column chromatography. This mixture was biased from the outset: the equilibrated library shows a high preference for the formation of the least polar imine **B2** (relative abundance 1.00) and the most polar **D4** (0.88), relative to their counterparts of intermediate polarity (**B4**: 0.18, **D2**: 0.20). This ratio changed minimally upon addition of silica gel. Ultimately, chromatography—eluting first with a mixture of hexane/EtOAc (20:1 v/v) and then with pure THF—led to the isolation of **B2** (82±1%) as the dominant component of the first fraction, followed by **D4** (79±3%) as the major component in the second fraction. Small amounts of **B4** (3±0.1%) and **D2** (8±0.3%) were also detected.

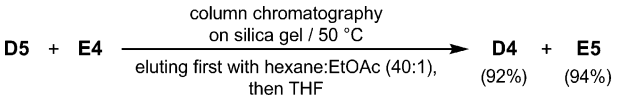
In the final [2×2] system (Figure 2c), the initial distribution was between the first two experiments—biased towards the least polar imine **C3** and the most polar imine **D4**, but not as dramatically as in the previous experiment. Column chromatography significantly amplified this bias, producing **C3** in 96±1% yield and **D4** in 93±3% yield.

In a more complex [3×3] system, three aldehyde and three amine starting materials were reacted together to yield a mixture of nine imines, the distribution of which is also represented by white vertical bars (Figure 2d). The proportion of the most abundant imine in the mixture, imine **C3**, is approximately six times greater than that of the least abundant member of the library, imine **B4**. Upon addition of silica, this distribution equalizes somewhat, with the **C3**/**B4** ratio decreasing to approximately 3.5. Column chromatography of the system initially employed pure hexane as the eluent. The polarity of the eluent was then increased to hexane/EtOAc 100:1 v/v and then to a 10:1 ratio, and was finally changed to pure THF. Imine **B2** was isolated in the first fraction in 80±1% yield, essentially consuming (almost) all of benzaldehyde and aniline constituents in the process. The second small fraction contained 12±0.2% of **C2**, while the third fraction carried **C3** (81±1%). In the final fraction, imine **D4** dominated (89±3%), and small amounts of **D2** (4±0.1%) were also detected.

The final [4×4] experiment started with a mixture of 16 imines in which the most abundant imine **B2** was present in approximately threefold excess relative to the least abundant imine **B4** (Figure 2e, white bars). Upon addition of silica gel the relative abundances changed, leaving **C2** as the imine with the highest proportion and **D1** as the least concentrated member of the library ([**C2**]/[**D1**] = 2.44). Column chromatography—eluting first with hexane/toluene (10:1 v/v, then 8:1), then with hexane/EtOAc (40:1, then 10:1, then 2:1), and finally with pure THF—enabled us to isolate six distinct fractions: a) the first fraction composed mostly of **A1** (64±1%); b) the second fraction with small amounts of **A2** (1±0.1%) and **B1** (3±0.1%); c) the third fraction, which was a mixture of **B2** (78±1%), **A2** (17±0.1%), **B1** (13±0.1%), and an additional amount of **A1** (3±0.02%); d) the fourth fraction composed of **C1** (5±0.1%) and **C2** (8±0.1%); e) the fifth fraction made up mostly of **C3** (80±1%) and some **C1** (5±0.1%); and f) a final fraction as a mixture of **D4** (76±3%), **D2** (2±0.1%), **D1** (2±0.1%), and **A4** (1±0.1%). Conceptually, rapid elution of **A1** forced imines **A2**–**A4** to react with **B1**, **C1**, and **D1** to replenish **A1**. After elution of **A1** is completed, the original 16-imine library lost these seven members and was reduced in size to form a [3×3] library which now had **B2** as the least polar member. In the next step, its elution consumed **B3**, **B4**, **C2**, and **D2**, decreasing the size of the leftover library to just 4 members, and so on.

This procedure is limited in resolution. Each of the isolated imines is eluted in several fractions and that is a necessary feature of this procedure. For example, in the experiment shown in Figure 2d, imine **B2** is eluted as three separate fractions, although they may not be collected as such. The amount of **B2** that is present in the initial mixture is eluted quickly. The remainder of **B2** has to be produced on the column by disproportionation of partners that a) contain its constituents and b) elute at approximately the same rate. Thus, **B3** and **C2**—which are of comparable polarity—produce the second batch of **B2**, while **B4** and **D2** elute later and are responsible for the production of the third batch of **B2**. With larger libraries, it is likely that there will be overlap of multiple fractions, and thus the amplification loses fidelity as the library increases in complexity.

Based on the same logic, an even more curious transmutation experiment was performed (Scheme 2). Equimolar amounts of pure **D5** and **E4** (which both contain one highly



Scheme 2. Transmutation of imines during column chromatography.

polar and one highly nonpolar component) were loaded onto a silica gel column, and eluted first with hexane/EtOAc (40:1 v/v) and subsequently with pure THF. The first isolated fraction contained **D4** (92%) and the second **E5** (94%). This is a particularly unusual column chromatography experiment, given that two compounds eluted from the column are different to the two compounds loaded onto it.

In conclusion, we have shown that complex libraries of equilibrating compounds can simplify in composition during the course of column chromatography on silica gel. This process could easily be combined with chemical reactions—by, for example, performing a chromatographic separation on a column impregnated with a catalyst—resulting in multidimensional simplification of complex libraries.

In the future, we will attempt to automate and monitor this procedure using an HPLC instrument. Such an extension would allow the use of this dynamic procedure as a physical chemistry tool to, for example, determine polarity indicators[13] in a direct competition experiment. This method will also be expanded to other adsorbents and dynamic compound classes.[14]

Experimental Section

Representative self-sorting experiment (Figure 2a): Equimolar amounts of 4-methylbenzaldehyde (110 μL, 0.933 mmol), 4-methoxybenzaldehyde (114 μL, 0.933 mmol), 4-methylaniline (100 mg, 0.933 mmol), and 4-methoxyaniline (115 mg, 0.933 mmol) were added into a 50 mL round-bottom flask, along with toluene (20 mL). The mixture was heated at reflux for 12 h, and a Dean–Stark trap was used to remove H_2O. Toluene was then removed under vacuum to afford a mixture of four imines as a light brown solid. This mixture was preadsorbed onto silica gel (approx. 1 g) and then added on top of a chromatography column, which was pre-loaded with oven-dried silica gel (approximately a 10 cm high layer). The column was then wrapped with a heating tape and heated to approximately 50 °C. The elution was performed first with hexane (300 mL), followed by a 100:1 hexane/EtOAc (v/v, 200 mL), and 10:1 hexane/EtOAc (v/v, 110 mL) mixtures, and finally with pure EtOAc (100 mL). Three fractions were isolated. In the first fraction, imine **B2** was the dominant product (85% yield, based on an internal standard calculation with 171 mg of the crude product and 22.3 mg of the internal standard, see Figure S1). The second fraction contained imine **C2** as the major component (8% yield, based on an internal standard calculation with 27 mg of the crude compound and 9.7 mg of the internal standard, see Figure S2). The final fraction contained imine **C3** (89% yield, based on an internal standard calculation with 224 mg of the crude compound and 21 mg of the internal standard, see Figure S3).

Full experimental data, including the procedures for the calculations of yields using an internal standard, can be found in the Supporting Information.

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[12] Strictly speaking, column chromatography sorts compounds not based on their polarity, but on their affinity for silica gel. Although the two concepts are not the same, there is a strong correlation between them and—in the jargon of preparative organic chemistry—the two terms are used interchangeably with polar compounds being defined as having a large affinity for silica gel. For the sake of brevity, we will continue this tradition and use the term "polar" to signify compounds which we experimentally found to elute later.

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