A recognition-mediated reaction drives amplification within a dynamic library†

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A single, appropriately designed, recognition event targets and transforms one of two reactive members of an exchanging pool of compounds through a recognition-mediated irreversible cycloaddition reaction, altering dramatically the final composition and kinetic behaviour of the dynamic library.

Introduction

Classically, dynamic covalent chemistry‡ has created and exploited networks of interconverting compounds, so-called dynamic combinatorial libraries (DCLs), which operate under thermodynamic control. Within a DCL, the relative abundances of library members are therefore governed by the total free energy of the system. Recognition events that are capable of altering the free energy relationships§ within a DCL can alter the final distribution of compounds in the library. Selection strategies for DCLs based on thermodynamic control often have limited effectiveness, since selectivity is based on the differences in the strengths of interactions between library members. Employing kinetic effects†‡,11 in DCLs can improve selectivity, but this approach is currently underexploited. We have become interested in exploiting recognition-mediated reactions‡ to accelerate the selection of target molecules within DCLs. Previously, we described12 the application of autocatalysis, mediated by a self-replicating template, to successfully amplify a single product from an exchanging pool of reagents. This approach, we exploited an irreversible chemical reaction in a constructive manner, using recognition to direct reaction selectively to one library member, and, hence, influence the composition of the library. The design13 of autocatalytic templates can be problematic and we wished to study a system in which a simpler recognition-mediated reaction, operating through a binary reactive complex,12 could operate in a constructive manner in order to influence the distribution of products within a DCL. Additionally, we wished to compare the efficiency of this simpler kinetic selection strategy with more complex autocatalytic scenarios. Here, we report the design of an extremely simple exemplar system, based on a recognition-mediated reaction that operates through a reactive binary complex, that demonstrates the power of this approach whilst simultaneously permitting the detailed real-time tracking of the system-wide effects of the single recognition event used to direct the system.

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Results and discussion

In order to allow direct comparison with our previous work, we employed the same dynamic library (Fig. 1), constructed from two aldehydes, one of which bears an amidopyridine recognition site, as used previously. The library contains two nucleophiles – 4-fluoroaniline and 4-fluorophenylhydroxylamine. The aniline reacts to form imines 1 and 3 and the hydroxylamine reacts to form nitrones 2 and 4. The DCL will therefore contain all of the compounds within the area marked Exchange Pool in Fig. 1, namely the two imines, 1 and 3, and the two nitrones, 2 and 4, together with the components necessary to synthesise all of these compounds. Transfer of material from the Exchange Pool to the Product Pool occurs irreversibly through the 1,3-dipolar cycloaddition reactions of either nitrone 2 or nitrone 4 with a maleimide, either 5a or 5b. The cycloaddition reactions between 2 and 5 afford the diastereoisomeric trans- and cis-6 and the cycloaddition reaction between 2 and 5 affords the diastereoisomeric trans- and cis-7. Critically, within the Exchange Pool, only one compound, nitrone 4, bears a carboxylic acid recognition site and is reactive towards maleimides. This compound is, therefore, the target for the recognition-mediated irreversible chemical reaction that will ultimately resolve the DCL.

Previously, we have demonstrated\textsuperscript{12} that it is possible to accelerate cycloaddition reactions significantly through the formation of a reactive binary complex between the 4π (diene or 1,3-dipole) and the 2π components. Hence, we needed to identify the most appropriate structure for a dipolarophile that could recognise 4 and, subsequently, participate in a 1,3-dipolar cycloaddition reaction through a recognition-mediated pathway. We used electronic structure calculations to design maleimide 5b. Our calculations indicated (Fig. 2a) that 5b was capable of binding to nitrone 4 and this complex was capable of accessing the transition state leading to cis-7b. This expectation was verified experimentally (Fig. 2b) – the reaction between 4 and 5b proceeds 125× faster that the corresponding reaction between 4 and maleimide 5a, in which recognition has been disabled. Further, the reaction between 4 and 5b is highly diastereoselective – trans-7b : cis-7b = 1 : 35 – compared to the control reaction between 4 and 5a – trans-7a : cis-7a = 3 : 1 (see ESI†).

Fig. 1 A solution of imine 1 and nitrone 2 in CD\textsubscript{2}Cl\textsubscript{2} saturated with p-toluenesulfonic acid monohydrate at 273 K can exchange freely to form all compounds in the Exchange Pool. The introduction of a maleimide, 5a or 5b, converts nitrone 2 or nitrone 4 irreversibly into the corresponding cycloadducts 6 or 7 and material transferred in this way into the Product Pool cannot be interconverted within the Product Pool or returned to the Exchange Pool. Note that both pools of products are present simultaneously in the same solution.
These experiments demonstrate that the mixture of imines and nitrones is, indeed, dynamic and that all four condensation products are present in similar amounts at equilibrium. We were now in a position to couple this exchange process to our recognition-mediated cycloaddition reaction. Initially, we wished to perform a control experiment to determine the effect of providing an exit route through irreversible reaction to the nitrones within the Exchange Pool. Methyl ester 5a is incapable of recognising the amidopyridines present in 1 and 4 and, hence, reactions involving 5a cannot proceed through any recognition-mediated pathway. A CD$_2$Cl$_2$ solution of imine 1 and nitrone 2 ([I] = [2] = 20 mM) was saturated with PTSA and maleimide 5a was added immediately as the dipolarophile ([5a] = 20 mM). The composition of this mixture was monitored by $^1$H and $^{19}$F NMR spectroscopy at 273 K every 30 minutes for 16 hours. These spectra were used to determine the concentrations of all of the compounds present in the mixture as a function of time (see ESI†). The results of this experiment are summarised in Fig. 3.

After 16 h, the Product Pool contains all four possible cycloadducts at relatively low concentrations. The cycloadducts formed between nitroene 2 and maleimide 5a – trans-6a and cis-6a – are present in the Product Pool at a total concentration of around 3 mM and the diastereoselectivity (trans-6a : cis-6a) is 2:1. Similarly, the cycloadducts formed between nitroene 4 and maleimide 5a – trans-7a and cis-7a – are present at a lower concentration ~1.4 mM. Once again the diastereoselectivity is very modest (trans-7a : cis-7a = 2.5 : 1). After 16 h, all cycloaddition reactions within the library have converted only 22% of maleimide 5a. Compounds 1 to 4 are present (Fig. 3a) in similar amounts ([I] = 8.1 mM, [2] = 7.1 mM, [3] = 7.6 mM, [4] = 8.5 mM). The composition of the Exchange Pool after 16 h is similar (1 : 2 : 3 : 4 = 1.1 : 1.0 : 1.1 : 1.2) to that observed at equilibrium when maleimide 5a is absent from the reaction mixture. The main compositional difference is the depletion of nitrones 2 and 4 as a consequence of their reaction with maleimide 5a. Since the rates of the cycloaddition reactions between 2 or 4 and 5a are significantly slower than the rates of the exchange processes, these irreversible reactions have little influence on the Exchange Pool and, since the diastereoselectivity of both cycloaddition reactions is poor, all four cycloadducts are present in the Product Pool (Fig. 3b and c).

We were now in a position to establish the effect of this rapid and selective reaction on the set of equilibria present within the Exchange Pool. However, in the first instance, it is important to establish the dynamic behaviour of the Exchange Pool itself and determine its equilibrium composition. A CD$_2$Cl$_2$ solution of imine 1 and nitroene 2 ([I] = [2] = 20 mM), saturated with p-toluene sulfonic acid monohydrate (PTSA), was incubated at 273 K. As expected, analysis of this mixture after 16 hours showed the presence of all four condensation products, 1 to 4, with some selectivity for the nitrones 2 and 4 as a result of their higher hydrolytic stability. At equilibrium, some 4-fluoroaniline and benzaldehyde can still be detected in the mixture by $^1$H and $^{19}$F NMR spectroscopy and the distribution of compounds 1 to 4 is 1 : 2 : 3 : 4 = 1.0 : 1.4 : 1.0 : 1.7. Reassuringly, if the equilibration process is started from imine 3 and nitroene 4 ([3] = [4] = 20 mM, CD$_2$Cl$_2$/sat. PTSA, 273 K, 16 h), the same mixture of compounds 1 to 4 is formed establishing this composition as the equilibrium position of this small library.
After 16 h, the Product Pool contains both trans-6b and cis-6b—the products of reaction between nitrene 2 and maleimide 5b—at a total concentration of 1.1 mM and exhibiting the same modest diastereoselectivity as the experiment with maleimide 5a (trans-6b : cis-6b = 2 : 1). Although, as expected, the diastereoselectivity is the same as in the control experiment involving maleimide 5a, the overall concentration of products has decreased significantly (3 mM → 1.1 mM). By contrast, trans-7b and cis-7b—the products of reaction between nitrene 4 and maleimide 5b—are present (Fig. 4b) at a total concentration of 13.3 mM ([trans-7b] : [cis-7b] = 1 : 43). There has been a dramatic increase in the overall conversion. Cycloadduct cis-7b now constitutes more than 90% of the total cycloadduct present in the Product Pool (Fig. 4c) and the conversion of maleimide 5b is 72%. The effects of the introduction of the recognition process into the system are equally significant (Fig. 4a) in the Exchange Pool. After 16 h, the relative concentrations of compounds 1 to 4 present in the Exchange Pool are significantly different compared to the same exchange process in the presence of the control maleimide 5a (Fig. 3). Given the large increase in overall conversion to cycloadducts, it is unsurprising that the concentrations of both of the nitrones, 2 and 4, are depressed significantly ([2] = 3.8 mM, [4] = 1.9 mM). Whilst the rate of the cycloaddition reaction between 2 and 5b is still slower than the rate of exchange, the cycloaddition reaction between nitrene 4 and 5b is comparable in rate to the exchange processes. Thus, once the exchange processes generate a concentration of nitrene 4 that is close to the K_d (~3 mM) of the [4·5b] complex, rapid reaction to form cis-7b occurs through this binary complex, thereby removing nitrene 4 from the Exchange Pool. This rapid depletion of one component of the Exchange Pool drives the equilibration of compounds 1–4 to regenerate the depleted species. Since 4-fluorophenyl hydroxylamine is required to form both 2 and 4, the concentration of 2 is suppressed indirectly by the consumption of nitrene 4 in the recognition-mediated reaction. The results presented in Fig. 4 establish unambiguously the irreversible recognition-mediated cycloaddition reaction, when coupled to exchange, generates significant selectivity in both the Exchange Pool and in the Product Pool.

These comparisons give us insight into the endpoints of these processes. However, during these experiments, concentration-time data was acquired at 30 min resolution. Therefore, we are in a position to examine in detail the rates of the processes that take place in both the Exchange Pool and the Product Pool during these experiments. Therefore, we analysed...
the time course NMR data collected during these experiments and extracted the rates of reaction \( \frac{d[X]}{dt} \) for each species in the system by fitting polynomial expressions to the concentration-time data and generating the rate-time data for each species by differentiation of these best-fit polynomials.

For the recognition-inactive experiment, i.e. that performed in the presence of maleimide 5a, the results are shown in Fig. 5. In this case, the cycloaddition reactions are effectively decoupled from the exchange processes – no cycloaddition has a maximum rate greater than 0.08 \( \mu M \) s\(^{-1}\) (Fig. 5, right side). It is clear that any selectivity for cis- and trans-6a in the Product Pool is only generated as a result of the initial presence of nitrone 2 in the reaction mixture. The formation of cis- and trans-7a in the Product Pool relies on the formation of nitrone 4 through exchange. Consequently, the rate maximum for the formation of cis- and trans-7a occurs after around 30 000 s and the peak rate for the formation of cis- and trans-7a in the Product Pool (0.03 \( \mu M \) s\(^{-1}\)) is lower than that for the formation of cis- and trans-6a because the concentration of nitrone 4 never reaches 20 mM – the starting concentration of nitrone 2.

By contrast, the exchange processes that interconvert compounds 1 through 4 have maximum rates of around \( \pm 0.40 \mu M \) s\(^{-1}\) (Fig. 5, left side). The rate-time profiles for compounds 1 through 4 are essentially mirror images of each other indicating that the progress of the exchange process towards equilibrium is unperturbed by the very slow bimolecular transformations of the two nitrones to the corresponding cycloadducts.

The results of the same analysis for the recognition-active experiment, i.e. that performed in the presence of maleimide 5b, are shown in Fig. 6. In this case, the exchange reactions are now being driven at significantly faster rates by the rapid recognition-mediated cycloaddition between 4 and 5b. This cycloaddition requires nitrone 4, however, at the start of the process the concentration of this compound is zero. Nitrone 4 is formed by exchange (Fig. 6, bottom left, solid line), but as soon as it forms, it interacts with maleimide 5b, forming the reactive binary complex \([4\cdot5b]\).

Cycloadduct cis-7b is formed rapidly (Fig. 6, bottom right, dashed line) within this complex, removing nitrone 4 from the Exchange Pool. The effect of this rapid removal of 4 from the Exchange Pool can be seen clearly in the rate-time profiles for the exchange processes that interconvert compounds 1 to 4. These processes now have maximum rates of around \( \pm 1 \mu M \) s\(^{-1}\) (Fig. 6, left side) – more than twice as fast as in the case of the recognition-inactive experiment (Fig. 5, left side). The accelerated formation of cis-7b also distorts the shapes of the rate-time profiles in the Exchange Pool – in particular, that of the nitrone 4 (Fig. 6, bottom left). Until 10 000 s, the concentration of nitrone 4 is increasing (rate > 0), however, its maximum rate of formation (\( +0.9 \mu M \) s\(^{-1}\)) is passed after only 5000 s. After 10 000 s, the concentration of nitrone 4 is always falling (rate < 0) – this point corresponds to the rate maximum (\( +0.45 \mu M \) s\(^{-1}\)) for the formation of cis-7b. After this point, the rate of reaction of 4 with 5b, mediated by the reactive binary complex \([4\cdot5b]\) is limited by the availability of 4, hence, exchange has become rate limiting.
In order to understand the broader effect of this single, recognition-mediated reaction process within the pool of exchanging species, we can make an overall comparison (Fig. 7) between the compositions of the exchange and Product Pools after 16 h in the absence and in the presence of recognition. This data can in turn be compared with our data.13

Fig. 5 Extracted rate vs. time plots (d[X]/dt in µM s⁻¹) for the formation and consumption of each species present in the recognition-disabled experiment (starting conditions: CD₂Cl₂/saturated with PTSA/[I] = [2] = [5a] = 20 mM). Species bearing a recognition site are coloured blue and those having no recognition site are coloured red. The identities of the dashed and solid lines in each graph are denoted by the assignment shown in the centre of the figure.
obtained previously when exploiting an autocatalytic reaction in order to resolve the library.

For the purposes of comparison, we define the enhancement factor, EF, for a given species as being $\log_{10}$ of the ratio of the concentration of that species after 16 h in the experiment where recognition is active (data in Fig. 4) to that of the experiment where recognition is inactive (data in Fig. 3). Within the Exchange Pool (Fig. 7), only imine 3 is enhanced...
The work presented here demonstrates that the fate of an exchanging pool of compounds can be influenced profoundly by a single, appropriately designed, recognition event that operates on a single chemical reaction. In the system presented here, one of two reactive members of an exchanging pool of compounds can be targeted and transformed rapidly by a recognition-mediated irreversible cycloaddition reaction, altering dramatically the final composition and kinetic behaviour of the entire system. We have been able to demonstrate, through close to real time monitoring that the fate of the library is determined not only by the direct acceleration of the cycloaddition reaction, but also by the effect that this acceleration has on the rest of the system. Comparison with data from resolution of the same library using an autocatalytic process reveals that the different kinetic profile of an autocatalytic reaction leads to significantly poorer resolution of the library. These results indicate that the construction of more complex systems that integrate both minimal\textsuperscript{7\textsuperscript{a},15\textsuperscript{b}} and reciprocal\textsuperscript{17} replication within the type of framework presented here will

Conclusions

The work presented here demonstrates that the fate of an exchanging pool of compounds can be influenced profoundly by a single, appropriately designed, recognition event that operates on a single chemical reaction. In the system presented here, one of two reactive members of an exchanging pool of compounds can be targeted and transformed rapidly by a recognition-mediated irreversible cycloaddition reaction, altering dramatically the final composition and kinetic behaviour of the entire system. We have been able to demonstrate, through close to real time monitoring that the fate of the library is determined not only by the direct acceleration of the cycloaddition reaction, but also by the effect that this acceleration has on the rest of the system. Comparison with data from resolution of the same library using an autocatalytic process reveals that the different kinetic profile of an autocatalytic reaction leads to significantly poorer resolution of the library. These results indicate that the construction of more complex systems that integrate both minimal\textsuperscript{7\textsuperscript{b},16} and reciprocal\textsuperscript{17} replication within the type of framework presented here will
require careful design to achieve optimum library resolution allowing the generation of systems that can express more complex behaviour, e.g. act as primitive models of metabolism through template-directed synthesis of specific products in response to chemical inputs. These strategies are currently under development in our laboratory.

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Notes and references


