Target-driven selection in a dynamic nitrone library†

Simon M. Turega, Christiane Lorenz, Jan W. Sadownik and Douglas Philp*

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Nitrones undergo dynamic exchange in chloroform at room temperature through two mechanisms—hydrolysis and recombination or hydroxylamine addition/elimination; this dynamic exchange is harnessed to select a nitrone-based bis(amidopyridine) receptor for diacids from a group of four nitrones through its binding to a glutaric acid-based target.

The chemistry of reversible covalent bond formation—dynamic covalent chemistry[1]—allows for the generation of a network of interconverting compounds, where the distribution of library members is governed by their relative free energies and any process that is capable of altering this relationship will affect the distribution of the compounds in the network. This approach has been used for development of synthetic receptors and sensors,[2] creation of supramolecular assemblies[3] and in the search for ligands[4] for biomacromolecules. We have become interested in exploiting recognition-mediated reactions[5] as a tool for selection and amplification of target molecules within dynamic combinatorial libraries (DCLs). Therefore, a need exists for the development of reversible covalent reactions which occur in non-polar organic solvents, where our recognition-mediated reactions are effective, and, at the same time, possess functionality that can be utilised for further chemical transformations. Thus, the bond that is made or broken must in itself be reactive, making it possible to couple the DCL to kinetic amplification strategies that rely on irreversible covalent bond formation.

The reversible formation of imine bonds[6] is one of the most popular exchange reactions used in DCLs. Diversity in the library can be introduced through substituents on either the amine or the aldehyde component of the imine. The amine, however, is a poor nucleophile and imine exchange will only proceed through a hydrolytic mechanism which is catalyzed by Lewis acids, organic acids or in aqueous buffers. These conditions, while not overly harsh, do present limitations to the utility of this reaction, particularly in respect of molecular recognition associated with hydrogen bonds. Hydrazones have also been shown to exchange readily under similar conditions to amines. However, the synthetic opportunities presented by the hydrazone itself are still limited. A logical extension of this family of dynamic covalent reactions based on nitrogen nucleophiles is the use of hydroxylamines. Hydroxylamines are excellent nucleophiles and react rapidly with aldehydes in non-polar solvents to form the corresponding nitrone. Unlike hydrazones, diaryl nitrones are capable of participating in a number of irreversible synthetic transformations directly, the most attractive of which are dipolar cycloadditions. Although a description of nitrone exchange in DCC has appeared[7] in the patent literature, it is restricted, once again, to aqueous buffers and the generation of unreactive nitrones. Here, we report that nitrones are, indeed, capable of undergoing exchange under mild conditions in chloroform. This dynamic exchange is demonstrated and exploited within the context of the selection of a receptor for a dicarboxylic acid from a mixture of nitrones.

Nitrone exchange can occur through two possible pathways (Fig. 1). Any water present in solution can partially hydrolyse the nitrones to afford a pool (Fig. 1(a)) of aldehydes and hydroxylamines, which can then recombine, with loss of water, to form either the original nitrones or the crossover products. At equilibrium, therefore, this exchange leads to a pool of four different nitrones in solution.

Additionally, free hydroxylamine can participate directly (Fig. 1(b)) in exchange through nucleophilic attack on the nitrone. This process leads to exchange and is catalytic in hydroxylamine. In order to establish that nitrone exchange is viable in non-polar organic solvents, where the amount of water present is limited, we designed (Fig. 2) a small library of nitrones. Nitrones **1** and **2** both contain a single amidopyridine recognition site. Upon exchange, nitrones **3** and **4** are formed. While nitrone **3** bears no amidopyridine recognition sites, nitrone **4** bears two such sites. Thus, although three of the nitrones are capable of binding a single carboxylic acid, only one, nitrone **4**, is capable of binding a dicarboxylic acid. We would therefore expect that the addition of an appropriate dicarboxylic acid target to the exchanging library

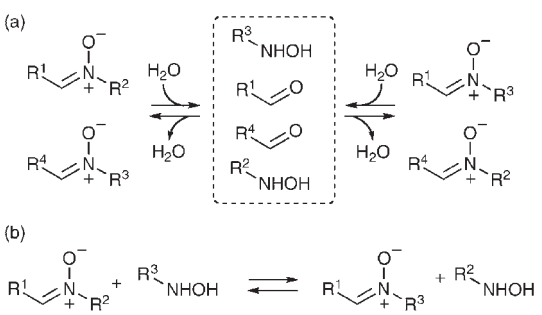

Fig. 1 Nitrone exchange can occur through (a) hydrolysis and recombination or (b) direct attack of a hydroxylamine on a nitrone.

EaStCHEM and Centre for Biomolecular Sciences, School of Chemistry, University of St Andrews, St Andrews, Fife, UK KY16 9ST. E-mail: d.philp@st-andrews.ac.uk; Fax: +44 1334 463808 Tel: +44 1334 467264

† Electronic supplementary information (ESI) available: Spectroscopic data for compounds **1**, **2**, **3** and **4**. Procedure for drying $CDCl_3$. Coordinates for calculated structures for **4** and [**4**·glutaric acid]. Details of association constant determinations and analysis of exchange experiments. See DOI: 10.1039/b805945d

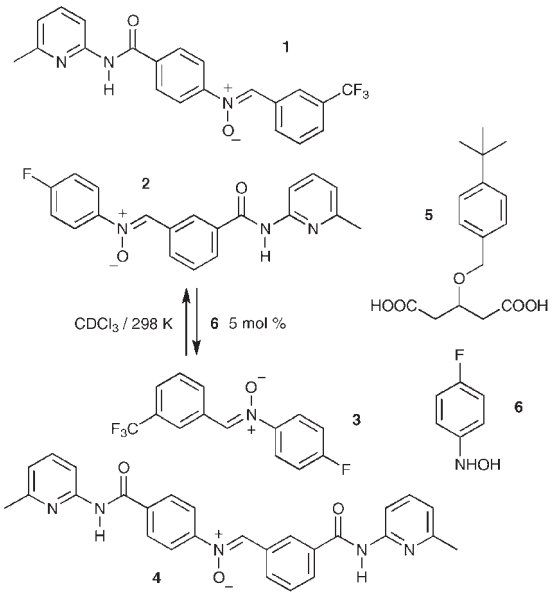

Fig. 2 The exchange between nitrones **1**, **2**, **3** and **4** can be biased by dicarboxylic acid **5** and is catalysed by hydroxylamine **6**.

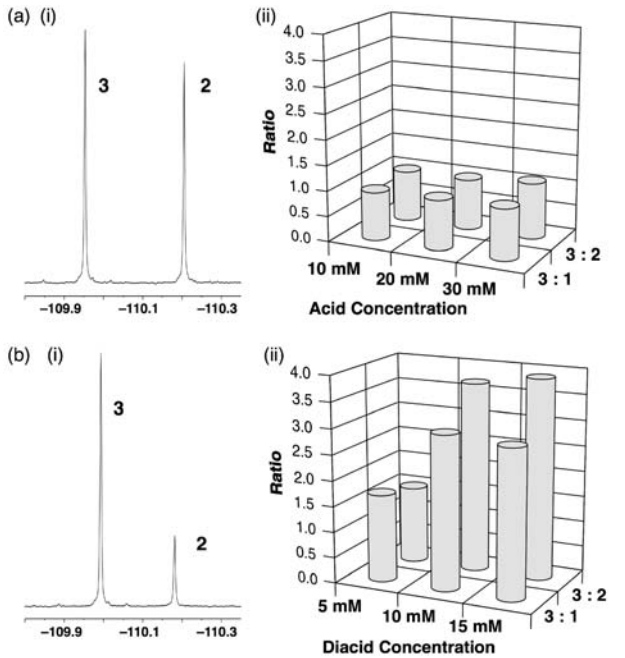

Fig. 3 Nitrone exchange reaction (a) in the absence of target **5** after 48 h. (i) Partial 282.3 MHz ^{19}F NMR spectrum ($CDCl_3$/298 K; initial conditions: [**1**] = [**2**] = 10 mM; [**6**] = 500 μM; [4-bromophenylacetic acid] = 20 mM) showing resonances arising from nitrones **2** and **3**. (ii) Ratio of nitrones **3** : **1** and **3** : **2** as a function of acid concentration. (b) in the presence of target **5** after 48 h. (i) Partial 282.3 MHz ^{19}F NMR spectrum ($CDCl_3$/298 K; initial conditions: [**1**] = [**2**] = 10 mM; [**6**] = 500 μM; [**5**] = 10 mM) showing resonances arising from nitrones **2** and **3**. (ii) Ratio of nitrones **3** : **1** and **3** : **2** as a function of diacid **5** concentration.

would bias the equilibrium in favour of nitrone **4**, since this ditopic receptor might be expected to form the most stable complex with the dicarboxylic acid. Previously, we have described[8] the use of the glutaric acid derivative **5** in recognition-mediated reactions and, therefore, we selected this diacid for this work. In chloroform, where the amount of water present is limited, the direct nitrone attack pathway (Fig. 1(b)) will be a significant contributor to the exchange rate. Therefore, we envisaged that exchange within our small library could be facilitated by the addition of a small amount of hydroxylamine **6**.

Compounds **1** through **4** were synthesised and characterised by standard methods (see ESI† for details); the synthesis of **5** has been described by us previously. Initially, it was important to optimise the conditions for nitrone exchange in the absence of the target diacid **5**. Accordingly, we prepared solutions of nitrones **1** and **2** ([**1**] = [**2**] = 10 mM) in $CDCl_3$ and added a catalytic amount (5 mol%) of hydroxylamine **6** and variable amounts‡ of 4-bromophenylacetic acid. These mixtures were assayed by ^{1}H and ^{19}F NMR spectroscopy after 48 h at room temperature and the concentrations of each of the nitrones **1** through **4** determined. The results of these experiments are summarised in Fig. 3(a). After 48 h, the nitrone exchange process has reached equilibrium and the position of this equilibrium is essentially a 1 : 1 : 1 : 1 mixture (Fig. 3(a)). When the same experiment is repeated but, this time starting with nitrones **3** and **4**, the same equilibrium position is reached. These results demonstrate clearly that, despite the fact that the solution contains§ little water, exchange between the nitrones does, indeed, occur and that the equilibrium position reached is the same, irrespective of which starting point is chosen. Additionally, the same equilibrium point is reached irrespective of the concentration of 4-bromophenylacetic acid added.

We then repeated these exchange experiements in the presence of the dicarboxylic acid target **5**. Accordingly, nitrones **1** and **2** ([**1**] = [**2**] = 10 mM) were dissolved in $CDCl_3$ together with diacid **5** at a concentration‡ of 5, 10 or 15 mM. As before, a small amount (5 mol%) of hydroxylamine **6** was also added to these solutions. These mixtures were assayed by ^{1}H and ^{19}F NMR spectroscopy after 48 h at room temperature and the concentrations of each of the nitrones **1** through **4** determined (see ESI† for details). The results of these three experiments are summarised in Fig. 3(b). In all these cases, there is now significant selectivity, up to 3.9 : 1, for nitrones **3** and **4** over nitrones **1** and **2**. It therefore appears that the equilibrium position of the nitrone exchange process has, indeed, been altered by the formation of the [**4**·**5**] complex. As expected, increasing the concentration of the target **5** increases the selectivity for nitrones **3** and **4**—from a **3** : **2** ratio of 1.5 : 1 at [**5**] = 5 mM to a **3** : **2** ratio of 3.9 : 1 at [**5**] = 15 mM. The **3** : **1** and **3** : **2** ratios are not identical because of differences in the hydrolytic stabilities of **1** and **2** under exchange conditions.

In order to assess the relative strengths of [**1**·**5**], [**2**·**5**] and [**4**·**5**] complexes, ^{1}H NMR dilution experiments were performed in $CDCl_3$ at 298 K. A 100 mM solution of a 1 : 1 mixture of **4** and **5** was diluted successively and the resulting changes in chemical shifts in probe protons were used to extract an association constant (K_a) for the [**4**·**5**] complex of 29 000 M^{-1}. In order to estimate the strength of the 1 : 1 complexes [**1**·**5**] and [**2**·**5**] complexes, we employed a model system—the complex formed between phenylacetic acid and 4-chloro-*N*-(6-methylpyridin-2-yl)benzamide. In this case, the ^{1}H NMR dilution experiment in $CDCl_3$ allowed us to extract an association constant (K_a) for the model complex of 690 M^{-1}. Converting the K_a values for

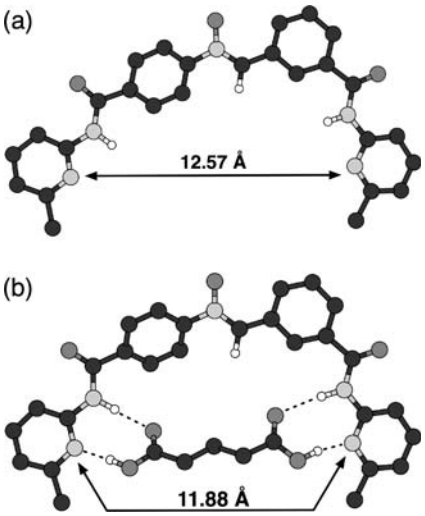

Fig. 4 Ball and stick representations of the calculated structures (B3LYP/6-31G(d,p)) of (a) free receptor **4** and (b) the complex between receptor **4** and glutaric acid. H atoms are white, N atoms are shaded light grey, O atoms are shaded dark grey and C atoms are shaded black. Hydrogen bonds are indicated by dotted lines. In both cases, the distance between the pyridine nitrogen atoms in the receptor is indicated above the double-headed arrow. Most H atoms have been removed for clarity.

[**4**·**5**] and our estimate for the [**1**·**5**] and [**2**·**5**] complexes into free energies reveals that the formation of the [**4**·**5**] complex is associated with a free energy of connection (ΔG^{S})[9] of +1.86 kcal mol^{-1} indicating that the binding within [**4**·**5**] is strongly negatively cooperative. In order to rationalise this negative cooperativity, we calculated¶ (Fig. 4) the structure of free receptor **4** and its complex with glutaric acid using density functional theory. The calculated structures demonstrate clearly that, although receptor **4** can associate with both carboxylic acids simultaneously, there is a clear mismatch between the length of the guest and the receptor. The distance between the pyridine nitrogen atoms in the receptor is shortened by 0.70 Å upon binding glutaric acid. This shortening, in turn, leads to a distortion of some of the bond angles around the receptor—in particular, the two C–N–C angles associated with the amides—thus introducing strain into the receptor framework.

The simple dynamic system reported here demonstrates that diaryl nitrones can undergo dynamic exchange in non-polar organic solvents, such as $CDCl_3$, opening up the possibility of bringing a more diverse array of recognition processes to bear on dynamic covalent systems. In addition, diaryl nitrones are reactive species, capable of participating in dipolar cycloaddition reactions. Thus, the development of this exchange process will facilitate its exploitation in recognition-mediated kinetic amplification strategies which exploit the recognition features of one library component to direct an irreversible covalent bond forming event. These strategies are currently under development in our laboratory.

Notes and references

‡ The exchange process is, unsurprisingly, catalysed by Brønsted acids. Since the target is a dicarboxylic acid, it was appropriate to screen the effect of the acid concentration for any effect on the position of the exchange equilibrium. We used double the concentration of 4-bromophenylacetic acid compared to target **5** in order to take account of the fact that the target is a dicarboxylic acid.

§ Although the chloroform used to prepare the solutions used in the exchange experiment was dried carefully before use (see ESI† for details), we did not take special precautions to exclude water introduced through the compounds used in the experiments existing as water solvates.

¶ Electronic structure calculations were carried out using GAMESS[10] running on a Linux cluster. The 64-bit Linux version dated 24 Mar 2007 (Rev 3) was used in all calculations.

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