Controlling a recognition-mediated reaction using a pH switch†

Simon M. Turega and Douglas Philp*

Received (in Cambridge, UK) 30th May 2006, Accepted 7th July 2006
First published as an Advance Article on the web 28th July 2006
DOI: 10.1039/b607551g

The selective recognition-mediated reaction between a nitrone bearing a urea recognition site and a maleimide bearing a proton switchable recognition site can be turned 'on' and 'off' by the addition of base and acid respectively.

The development and deployment of self-assembling and self-replicating molecular and supramolecular architectures is a challenging current goal for the fabrication of complex structures at the nanometre scale. The emergence of protocols that can harness assembly[1] and replication[2] directed by molecular recognition would deliver synthetic machinery that is capable of directing its own synthesis and cooperating[3] with other similar systems to create an organised hierarchy. In order to achieve this ambitious goal, a means of sequencing individual events within the overall assembly process must be developed, allowing stages of synthesis and assembly to be separated in time. In this respect, a clear understanding of the basic requirements for a recognition-mediated reaction that can be turned 'on' and 'off' is important. Reactions in which both partners bear mutually complementary recognition sites are an interesting target in this respect. We envisaged a system (Fig. 1a) that, in the 'off' state, bears a recognition site on **B′** that is blocked. In these circumstances, **A** and **B′** can only react through bimolecular collision and, therefore, the reaction will be slow and unselective. Transformation of **B′** into **B**, by chemical or other means, facilitates the association of **A** and **B**, through their mutually complementary recognition sites, forming the complex [**A**·**B**]. Within this complex, the association of the reagents pays some of the entropic cost of organising the transition state of the reaction, resulting in enhanced reaction rates. Since the approach of the two reagents, **A** and **B**, is now controlled to some extent by the nature of the non-covalent tether that associates them, reaction within [**A**·**B**] should proceed selectively, forming $\mathbf{T_2}$ in preference to $\mathbf{T_1}$.

We identified the recognition (Fig. 1b) between a urea and a carboxylate salt[4] as a suitable platform on which to construct a model system to test this hypothesis. At high pH, the carboxylate is the dominant species in solution and this anion associates strongly with the urea—recognition is turned 'on'. Lowering the pH protonates[5] the carboxylate forming the carboxylic acid. This species associates only weakly with the urea—recognition is turned 'off'.

EaStCHEM and Centre for Biomolecular Sciences, School of Chemistry, University of St Andrews, North Haugh, St Andrews, Fife, UK KY16 9ST. E-mail: d.philp@st-andrews.ac.uk; Fax: +44 (0)1334 463808; Tel: +44 (0)1334 467264

† Electronic supplementary information (ESI) available: Details of electronic structure calculations and coordinates of the transition state leading to ***cis*-3** and the product ***cis*-3** itself. Spectroscopic data for compounds **1**, **2a**, **2b**, **2d** and ***cis*-3**. See DOI: 10.1039/b607551g

Building on our previous work[6] in the area of recognition-mediated cycloaddition reactions, we designed the system shown in Scheme 1 as a test bed for these ideas. Nitrone **1** bears a urea recognition site. In acetone, this nitrone associates only weakly with either the maleimide ester **2a** or the maleimide acid **2b**. In this state, with recognition 'off', reaction of the nitrone with either maleimide will be slow and unselective, forming a mixture of the two diastereoisomeric‡ products, ***trans*-3** and ***cis*-3**. Deprotonation of the maleimide acid **2b** by Et_3N or tetra-*n*-butylammonium hydroxide (TBAOH) will form the corresponding carboxylate salts **2c** and **2d**, respectively. Both of these salts are capable of recognising and binding nitrone **1**, rendering the dipolar cycloaddition reaction between them pseudointramolecular. In this state, with recognition 'on', reaction of the nitrone with either maleimide will be fast and selective, forming ***cis*-3** selectively.

Compounds **1** and **2a** to **2d** were synthesised and characterised by standard methods. Full details are given in the ESI.†

In order to verify that the maleimides in which the recognition was switched 'on' were indeed capable of recognising the urea **1**, we measured the association constant for the complex between urea **1** and tetra-*n*-butylammonium acetate. Using ^{1}H NMR titration methodology, we were able to determine that the complex between **1** and the acetate anion has a K_a of 6000 M^{-1} in d_6-acetone at 10 °C. Using similar methodology, we demonstrated that **1** did form a weak complex with acetic acid, but that this

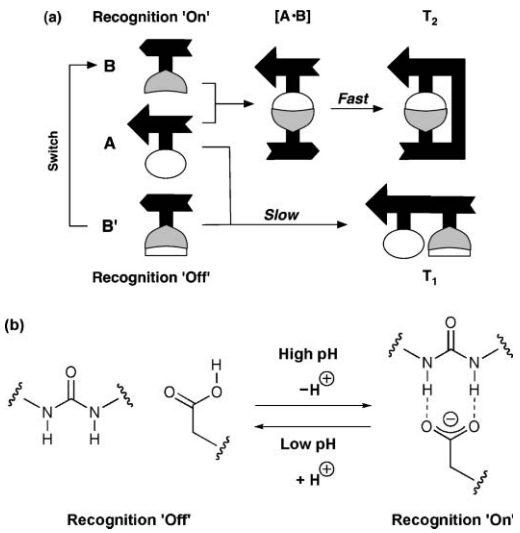
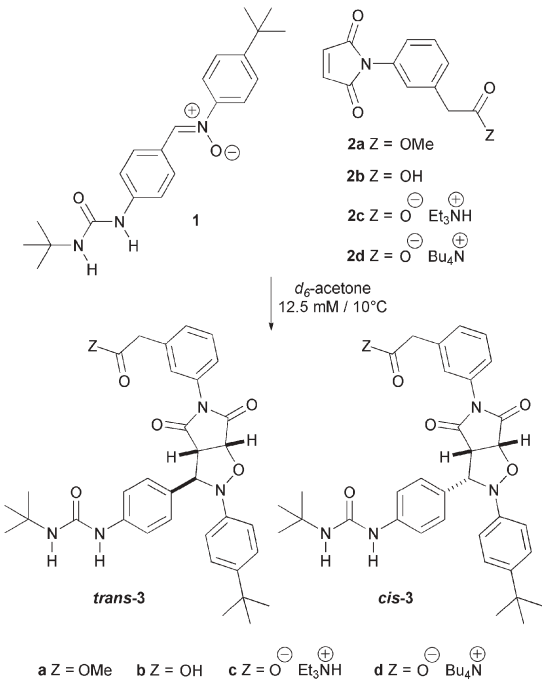


Fig. 1 (a) Schematic representation of a switchable recognition-mediated reaction. **B′** cannot associate with **A** and so undergoes reaction slowly with **A** through a non-recognition mediated pathway. Conversion of **B′** to **B** allows reaction through the fast recognition-mediated pathway. (b) Recognition between a urea and a carboxylate can be turned off and on by means of a pH change.



Scheme 1

complex has a $K_a \ll 100\ M^{-1}$ under the same conditions. These results indicate that there is a significant difference in the ability of **1** to recognise and bind maleimides in the 'on' and 'off' states.

Initially, we wished to establish that the recognition 'off' state and the recognition 'on' state gave significantly different results in terms of the rates of reaction and the ratios of the two products, ***trans*-3** and ***cis*-3**, formed. We therefore conducted a series of reactions in which **1** was allowed to react with maleimides **2a** to **2d** in d_6-acetone solution at 10 °C ([**1**] = [**2**] = 12.5 mM). The results of these experiments are summarised in Fig. 2.

It is clear from Fig. 2 that there is indeed a significant difference between the recognition 'off' state and the recognition 'on' states. For maleimides **2a** and **2b**, the overall conversions are similar and the ratios of the two diastereoisomeric products are essentially 1 : 1 (20% ***cis*-3a**, 4% de and 21% ***cis*-3b**, 0% de). After 36 h, the overall conversion to the two cycloadducts is 40% in the reaction between **1** and **2a** and 41% in the reaction between **1** and **2b**. When these maleimides are replaced by their recognition-enabled counterparts, namely **2c** and **2d**, the diastereoisomeric ratios observed in both reactions are over 4 : 1 in favour of the respective *cis* products. Overall conversion after 36 h increases to 67% (54% ***cis*-3c**, 62% de) in the case of the reaction between **1** and **2c**, and to 70% (58% ***cis*-3d**, 64% de) in the case of the reaction between **1** and **2d**. These data demonstrate that the introduction of recognition in **2c** and **2d** ('on' state) increases the rate of formation of the *cis* cycloadduct by almost three-fold when compared with **2a** and **2b** ('off' state).

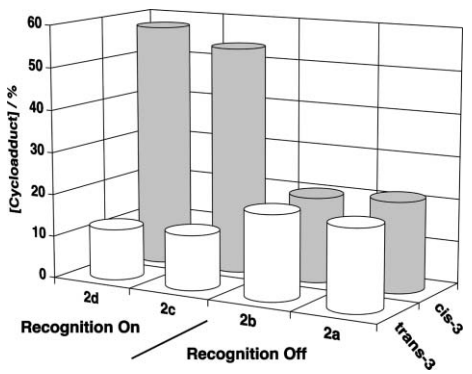


Fig. 2 Formation of ***cis*-3** and ***trans*-3** in the reactions between nitrone **1** and maleimides **2a** to **2d**. All reactions were conducted at 12.5 mM concentration of reagents at 10 °C in d_6-acetone. The concentrations of the respective cycloadducts are measured after 36 h and are expressed as % conversion (100% = 12.5 mM).

Having demonstrated that there is indeed a significant difference between the recognition 'off' state and the recognition 'on' state, we wished to demonstrate that the selective reaction between the nitrone and the maleimide could be switched 'on' and 'off' *in situ*. To this end, we performed a reaction in which **1** and **2d** were allowed to react in d_6-acetone at 10 °C ([**1**] = [**2d**] = 12.5 mM). Initially, this reaction is selective (Fig. 3a,) for ***cis*-3d** (62% de). After 200 min, 1 equiv. of trifluoroacetic acid is added to turn recognition 'off'. Immediately, the selectivity of the reaction is eroded and it continues to decline throughout the rest of the reaction. This effect on the diastereoselectivity of the reaction is accompanied by a concomitant reduction in the reaction rate to the level of the bimolecular reaction between **1** and **2a**. The reduction in the selectivity is readily explained by recognising that reaction does not stop on addition of acid, it merely slows to the level of the bimolecular reaction—a reaction which has no selectivity for either cycloadduct. Therefore, although ***cis*-3d** is

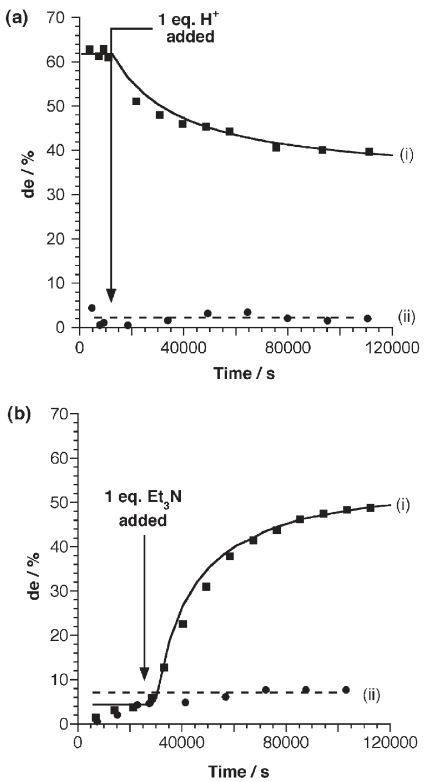


Fig. 3 (a) Reactions between **1** and (i) **2d** or (ii) **2a** in d_6-acetone at 10 °C (starting concentrations 12.5 mM). 1 equiv. of trifluoroacetic acid is added to both of the reaction mixtures at the point indicated. (b) Reactions between **1** and (i) **2b** or (ii) **2a** in d_6-acetone at 10 °C (starting concentrations 12.5 mM). 1 equiv. of triethylamine is added to both of the reaction mixtures at the point indicated. Lines represent the fits of the data to the appropriate kinetic models.

formed with a 62% de until t = 200 min, when the acid is added, the *cis* cycloadduct formed after that time (***cis*-3d**) is formed with 0% de. The addition of acid to a reaction (Fig. 3a, ●), performed under identical conditions as before, between nitrone **1** and the ester maleimide **2a**, which is incapable of recognition, has no effect on the rate or the selectivity. This observation confirms that the effects observed after the addition of acid to the reaction between **1** and **2d** are the result of switching the state of the recognition within the maleimide from 'on' to 'off'.

Next, we performed a reaction in which **1** and **2b** were allowed to react in d_6-acetone at 10 °C ([**1**] = [**2b**] = 12.5 mM). Initially, this reaction is unselective (Fig. 3b,) for ***cis*-3b** (6% de). After 400 min, 1 equiv. of triethylamine is added to turn recognition 'on'. Immediately after the addition of the base, the selectivity of the reaction is enhanced dramatically and it continues to increase throughout the rest of the reaction. This effect on the diastereoselectivity of the reaction is accompanied by a significant increase in the reaction rate to a level similar to that recorded for the reaction between **1** and **2c**. The increase in the selectivity never reaches that observed in the reaction between **1** and **2c**. This observation is readily explained by recognising that, in the period before the addition of Et_3N, the reaction between **1** and **2b** proceeds with very low selectivity for ***cis*-3b** (8% de). Since some of the starting material has already been consumed by the time the Et_3N is added, the final selectivity can never reach the same level as that observed when selective production of ***cis*-3c** occurs from t = 0. Once again, the addition of base to a reaction (Fig. 3b, ●), performed under identical conditions as before, between nitrone **1** and the ester maleimide **2a**, has no effect whatsoever on the rate or the selectivity of the reaction. This observation provides further confirmation that the effects observed are the result of switching the state of the recognition within the maleimide—in this case, from 'off' to 'on'.

Reasonable fits of the data, for the experiments involving the addition of acid and base, to an appropriate kinetic model (Fig. 3, solid and dashed lines) are obtained by simulation and fitting. The kinetic parameters extracted in this manner allow the calculation of the effective molarity (EM) generated within the [**1**·**2**] complex. This complex is not efficient at accelerating the reaction between the nitrone and the maleimide, *i.e.* 12.5 mM < effective molarity < 1 M, irrespective of the counterion.

In order to understand the reasons behind this observation, we turned to electronic structure calculations. The structure of the transition state (HF/6-31G(d)) leading to ***cis*-3** reveals that only one of the carboxylate oxygen atoms is hydrogen bonded strongly to one of the urea NH protons and this situation is mirrored in the product. The [**1**·**2**] complex must therefore make a significant distortion away from the ideal $R_2^2(8)$ hydrogen bonded array that normally characterises urea–carboxylate interactions in order to reach the transition state leading to ***cis*-3**. Thus, the transition state does not make best use of the recognition potential available to it, since this distortion must destabilise the assembly, raising the energy of the transition state.

In this communication, we have demonstrated that it is possible to use a simple pH switch to turn selective, recognition-mediated synthesis 'on' and 'off' within the kinetic framework of a binary reactive complex. Although the design is not optimal, we have demonstrated that this system will respond to the addition of either acid or base to switch recognition 'off' or 'on', respectively. While the results presented here represent a proof of principle, to be of practical use, we must achieve three further goals. Firstly, the stimulus used should be external to the system to prevent interference from chemical changes in the reaction medium. Photochemical methods would seem to be most appropriate for this task. Secondly, we must incorporate the switching methodology into a kinetic framework capable of amplification in order to achieve a more rapid deployment of the target structure. This goal can be accomplished by use of minimal or reciprocal replication strategies. Finally, we must also develop recognition systems that can be removed readily from the final product if required. All of these issues are currently being addressed in our laboratory.

We thank the University of St Andrews and BBSRC (Grant B20402) for financial support. One of us (DP) gratefully acknowledges the use of facilities at the Department of Chemistry and Biochemistry, University of California, Los Angeles during the preparation of this manuscript.

Notes and references

‡ The two diastereoisomers formed by the reaction of the nitrone and a maleimide are given the designators *trans* or *cis* based on the relative configurations of the proton derived from the nitrone with respect to those derived from the alkene on the isoxazolidine ring.

1 (*a*) A. Mulder, J. Huskens and D. N. Reinhoudt, *Org. Biomol. Chem.*, 2004, **2**, 3409; (*b*) M. Mammen, S. K. Choi and G. W. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2755; (*c*) D. Philp and J. F. Stoddart, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1154.

2 (*a*) N. Paul and G. F. Joyce, *Curr. Opin. Chem. Biol.*, 2004, **8**, 634; (*b*) A. Robertson, A. J. Sinclair and D. Philp, *Chem. Soc. Rev.*, 2000, **29**, 141; (*c*) E. A. Wintner and J. Rebek, Jr., *Acta Chem. Scand.*, 1996, **50**, 469; (*d*) G. von Kiedrowski, *Bioorg. Chem. Front.*, 1993, **3**, 113.

3 (*a*) M. Kindermann, I. Stahl, M. Reimold, W. M. Pankau and G. von Kiedrowski, *Angew. Chem., Int. Ed.*, 2005, **44**, 6750; (*b*) G. Ashkenasy, R. Jegasia, M. Yadav and M. R. Ghadiri, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 10872; (*c*) G. Ashkenasy and M. R. Ghadiri, *J. Am. Chem. Soc.*, 2004, **126**, 11140.

4 (*a*) S. J. Brooks, P. R. Edwards, P. A. Gale and M. E. Light, *New J. Chem.*, 2006, **30**, 65; (*b*) S. J. Brooks, P. A. Gale and M. E. Light, *Chem. Commun.*, 2005, 4696; (*c*) K. H. Choi and A. D. Hamilton, *Coord. Chem. Rev.*, 2003, **240**, 101; (*d*) T. Gunnlaugsson, A. P. Davis, J. E. O'Brien and M. Glynn, *Org. Biomol. Chem.*, 2005, **3**, 48; (*e*) T. Haino, M. Nakamura, N. Kato, M. Hiraoka and Y. Fukazawa, *Tetrahedron Lett.*, 2004, **45**, 2281; (*f*) G. V. Oshovsky, W. Verboom and D. N. Reinhoudt, *Collect. Czech. Chem. Commun.*, 2004, **69**, 1137.

5 For some recent examples of systems which respond to pH changes, see: (*a*) J. D. Badjic, C. M. Ronconi, J. F. Stoddart, V. Balzani, S. Silvi and A. Credi, *J. Am. Chem. Soc.*, 2006, **128**, 1489; (*b*) S. G. Tajc and B. L. Miller, *J. Am. Chem. Soc.*, 2006, **128**, 2532; (*c*) A. M. Costero, S. Peransi and K. Gloe, *Tetrahedron*, 2006, **62**, 2671; (*d*) S. Faulkner and B. P. Burton-Pye, *Chem. Commun.*, 2005, 259; (*e*) K. Nakabayashi, M. Kawano and M. Fujita, *Angew. Chem., Int. Ed.*, 2005, **44**, 5322; (*f*) N. A. Schnarr and A. J. Kennan, *Org. Lett.*, 2005, **7**, 395; (*g*) S. G. Telfer, X. J. Yang and A. F. Williams, *Dalton Trans.*, 2004, 699; (*h*) F. Auer, G. Nelles and B. Sellergren, *Chem.–Eur. J.*, 2004, **10**, 3232; (*i*) J. F. Callan, A. P. de Silva and N. D. McClenaghan, *Chem. Commun.*, 2004, 2048; (*j*) H. Xu, S. P. Stampp and D. M. Rudkevich, *Org. Lett.*, 2003, **5**, 4583; (*k*) A. M. Costero, J. P. Villarroya, S. Gil, P. Gavina and M. C. R. De Arellano, *Supramol. Chem.*, 2003, **15**, 403; (*l*) M. G. Gorbunova, P. V. Bonnesen, N. L. Engle, E. Bazelaire, L. H. Delmau and B. A. Moyer, *Tetrahedron Lett.*, 2003, **44**, 5397; (*m*) T. Gunnlaugsson, D. A. Mac Dónaill and D. Parker, *J. Am. Chem. Soc.*, 2001, **123**, 12866.

6 (*a*) R. J. Pearson, E. Kassianidis, A. M. Z. Slawin and D. Philp, *Chem.–Eur. J.*, 2006, DOI: 10.1002/chem.200501189; (*b*) E. Kassianidis, R. J. Pearson and D. Philp, *Org. Lett.*, 2005, **7**, 3833; (*c*) R. J. Pearson, E. Kassianidis, A. M. Z. Slawin and D. Philp, *Org. Biomol. Chem.*, 2004, **2**, 3434; (*d*) J. M. Quayle, A. M. Z. Slawin and D. Philp, *Tetrahedron Lett.*, 2002, **43**, 7229; (*e*) S. J. Howell, D. Philp and N. Spencer, *Tetrahedron*, 2001, **57**, 4945.

