Reciprocal template effects in a simple synthetic system†

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Two mutually-complementary templates are capable of catalysing the formation of each other, creating a framework for their reciprocal replication.

The emergence of synthetic machinery that is capable of directing its own synthesis and cooperating[1] with other similar systems to create an organised hierarchy is an important and challenging target for facilitating the fabrication of molecular architectures at the nanometre scale. A fundamental understanding of recognition-mediated processes that allow molecules to function as specific and efficient templates for the formation of themselves (autocatalysis) and other templates (crosscatalysis) should permit the development of efficient protocols[2] that allow us to establish and manage replication, organization and evolution within synthetic supramolecular assemblies. This approach to predetermined dynamic behaviour has been termed[3] "systems chemistry". This programmed systems behaviour can ultimately be exploited in the construction, selection[4] and amplification[5] of large molecular and supramolecular assemblies.

The development of complex replication networks relies on our ability to design and implement synthetic reciprocal replicating systems. In contrast to minimal replicating systems reported previously by us[6] and others,[7] in which a single autocatalytic cycle drives the replication of a template, reciprocal replicating systems rely (Fig. 1) on two interlinked crosscatalytic cycles in which two templates catalyse the formation of each other. Thus, in order to implement a reciprocal replicating system, we must design four reactive partners (**A** to **D**, Fig. 1) that bear appropriate recognition sites. Compounds **A** and **B** can react to form the template $\mathbf{T_{AB}}$ and, similarly, compounds **C** and **D** can react to form template $\mathbf{T_{CD}}$. Since $\mathbf{T_{AB}}$ and $\mathbf{T_{CD}}$ are mutually complementary, we might expect $\mathbf{T_{AB}}$ to assemble **C** and **D** into the ternary complex $[\mathbf{C{\cdot}D{\cdot}T_{AB}}]$ and, hence, catalyse the formation of $\mathbf{T_{CD}}$. Likewise, we might expect $\mathbf{T_{CD}}$ to assemble **A** and **B** into the ternary complex $[\mathbf{A{\cdot}B{\cdot}T_{CD}}]$ and, hence, catalyse the formation of $\mathbf{T_{AB}}$. These two interlinked crosscatalytic cycles formally represent a reciprocal replication[8] cycle.

In order to implement the reciprocal replication framework outlined in Fig. 1, it is necessary to identify suitable compounds to fulfil the roles of the building blocks **A** to **D** and, hence, determine the identities of $\mathbf{T_{AB}}$ and $\mathbf{T_{CD}}$. Using molecular mechanics calculations, we designed the compounds shown in Fig. 2a. These designs exploit our experience[6,9] in using the recognition between amidopyridines and carboxylic acids to associate reagents with templates and with each other. In arriving at the molecular structures of **A** to **D**, we paid careful attention to removing the possibility of binary reactive complexes forming between **A** and **D** or **B** and **C**, since we have demonstrated previously[9] that such reactions are often fast and very selective. Each of the two templates, $\mathbf{T_{AB}}$ and $\mathbf{T_{CD}}$, can be formed as two diastereoisomers – *endo* and *exo* in the case of $\mathbf{T_{AB}}$ and *trans* and *cis* in the case of $\mathbf{T_{CD}}$. The presence of these diastereoisomers allows us to probe information transfer between the templates by measuring the differences in diastereoselectivity of the bimolecular and template-directed reactions. Electronic structure calculations suggested (Fig. 2b) that the principal reciprocal template effects in this system should be between ***exo*-$\mathbf{T_{AB}}$** and ***trans*-$\mathbf{T_{CD}}$**. These two templates form a reasonably well-matched duplex (Fig. 2b) and since the Diels–Alder reaction leading to ***exo*-$\mathbf{T_{AB}}$** and the 1,3-dipolar cycloaddition leading to ***trans*-$\mathbf{T_{CD}}$** have late transition states, we reasoned that one template would show significant complementarity to the transition state leading to its partner.

In order to probe for the presence of the expected reciprocal template effects, we first synthesised compounds **A** to **D** and all four of the templates – ***exo*-$\mathbf{T_{AB}}$**, ***endo*-$\mathbf{T_{AB}}$**, ***trans*-$\mathbf{T_{CD}}$** and ***cis*-$\mathbf{T_{CD}}$**. Next, we performed the reactions between **A** and **B** and that between **C** and **D** in order to establish the rates of these reactions and their diastereoselectivities in the absence of any template effects. All reactions were carried out in $CDCl_3$ at 35 °C from starting concentrations of the appropriate reagents of 25 mM. The reactions were monitored by 500 MHz ^{1}H NMR spectroscopy and deconvolution of the appropriate resonances arising from starting

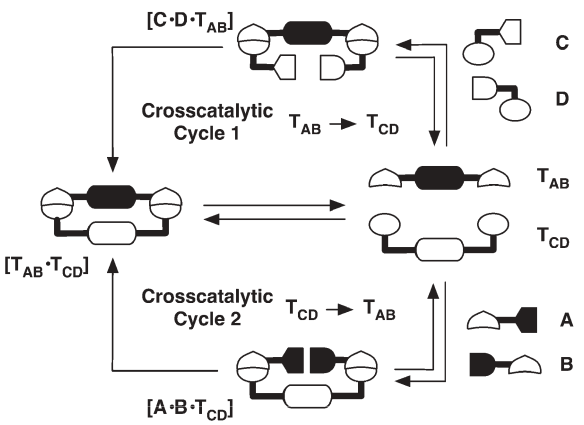

Fig. 1 Schematic representation of a reciprocal replicating system. $\mathbf{T_{AB}}$ can assemble **C** and **D** into the ternary complex $[\mathbf{C{\cdot}D{\cdot}T_{AB}}]$ and, hence, catalyse the formation of $\mathbf{T_{CD}}$. $\mathbf{T_{CD}}$ can assemble **A** and **B** into the ternary complex $[\mathbf{A{\cdot}B{\cdot}T_{CD}}]$ and, hence, catalyse the formation of $\mathbf{T_{AB}}$.

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† Electronic supplementary information (ESI) available: Spectroscopic data for compounds **A**, **B**, **C** and **D** and all four templates and descriptions of molecular mechanics and electronic structure calculations. See DOI: 10.1039/b608148g

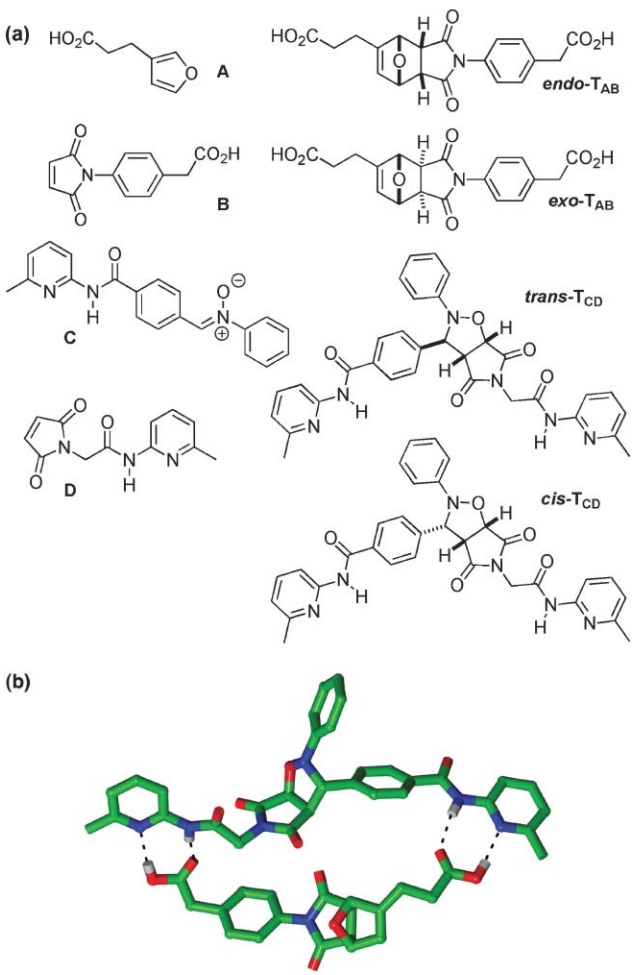

Fig. 2 (a) Diene **A** and maleimide **B** react to form two diastereoisomeric templates – ***exo*-$\mathbf{T_{AB}}$** and ***endo*-$\mathbf{T_{AB}}$**. Nitrone **C** and maleimide **D** react to form two diastereoisomeric templates – ***trans*-$\mathbf{T_{CD}}$** and ***cis*-$\mathbf{T_{CD}}$**. (b) Stick representation of the calculated (HF/6-31G(d)) structure of the [***exo*-$\mathbf{T_{AB}}$·*trans*-$\mathbf{T_{CD}}$**] complex. Carbon atoms are green, nitrogen atoms are blue, oxygen atoms are red and hydrogen atoms are white. Dashed black lines show hydrogen bonds. Most hydrogen atoms are omitted for clarity.

materials and products were used to construct concentration–time profiles for the reactions. These reaction profiles were then used to compute initial rates of reaction for each diastereoisomer. The results of these experiments are shown in Fig. 3.

In the case of the Diels–Alder reaction between **A** and **B** (Fig. 3a, solid line), the reaction is slow, reaching a total conversion of 50% after 16 hours (57600 s). The diastereoselectivity is also poor – the ***endo*-$\mathbf{T_{AB}}$** : ***exo*-$\mathbf{T_{AB}}$** ratio is only 1 : 1.3 after this time. In the case of the 1,3-dipolar cycloaddition reaction between **C** and **D** (Fig. 3b, solid line) the reaction is faster, reaching a total conversion of 85% after 16 hours. The diastereoselectivity is also relatively poor; the ***trans*-$\mathbf{T_{CD}}$** : ***cis*-$\mathbf{T_{CD}}$** ratio is 3 : 1 after the same time.

Next, we conducted experiments in which a fixed amount of one of the preformed templates was added to a mixture of reagents at the start of the reaction. Initially, we focused on the combinations of reagents and templates which were predicted by our calculations to be the most likely to exhibit the desired reciprocal template effects. Accordingly, the addition of 60 mol% of template ***trans*-$\mathbf{T_{CD}}$** to a reaction between **A** and **B** in $CDCl_3$ at

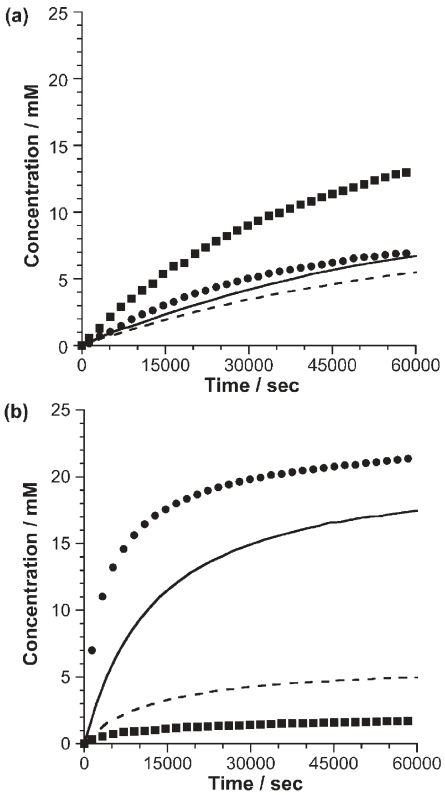

Fig. 3 Concentration–time profiles for the formation of (a) ***exo*-** (solid line) and ***endo*-$\mathbf{T_{AB}}$** (dashed line) from **A** and **B**; (b) ***trans*-** (solid line) and ***cis*-$\mathbf{T_{CD}}$** (dashed line) from **C** and **D**. In both cases, the reactions were performed in the absence of the complementary template. Filled circles (***endo*-$\mathbf{T_{AB}}$** and ***trans*-$\mathbf{T_{CD}}$**) and filled squares (***exo*-$\mathbf{T_{AB}}$** and ***cis*-$\mathbf{T_{CD}}$**) represent the reactions where (a) 60 mol% ***trans*-$\mathbf{T_{CD}}$** or (b) 35 mol% ***exo*-$\mathbf{T_{AB}}$** was added at the start of the appropriate reaction. All reactions were performed at 35 °C in $CDCl_3$ with starting reagent concentrations of 25 mM.

35 °C ([**A**] = [**B**] = 25 mM) results (Fig. 3a, filled circles) in an increase in the rate of formation of ***exo*-$\mathbf{T_{AB}}$** by 2.3-fold and a smaller increase in the rate of formation of ***endo*-$\mathbf{T_{AB}}$** of 1.5-fold. These changes in reaction rates result in an increase in conversion to 81% after 16 hours and a change in diastereoselectivity – the reaction is now 2 : 1 selective in favour of ***exo*-$\mathbf{T_{AB}}$**. Similarly, the addition of 35 mol% of template ***exo*-$\mathbf{T_{AB}}$** to a reaction between **C** and **D** in $CDCl_3$ at 35 °C ([**C**] = [**D**] = 25 mM) results (Fig. 3b, filled circles) in an increase in the rate of formation of ***trans*-$\mathbf{T_{CD}}$** of 3.6-fold and a two-fold reduction in the rate of formation of ***cis*-$\mathbf{T_{AB}}$**. These changes in the rates of reaction translate into a dramatic change in diastereoselectivity – the reaction is now 14 : 1 selective in favour of ***trans*-$\mathbf{T_{CD}}$**. These results clearly establish that our expectations, based on our calculations, are correct – ***exo*-$\mathbf{T_{AB}}$** and ***trans*-$\mathbf{T_{CD}}$** show significant reciprocal template effects.

In order to probe the nature of these template effects further, we next performed a series of reactions in which the amount of the template added at the start of each reaction is increased steadily from 0.1 equivalents up to a maximum of two equivalents. If the binding sites present in the template operate independently, then the amount of the catalytically active complex – either [**A**·**B**·$\mathbf{T_{CD}}$] or [**C**·**D**·$\mathbf{T_{AB}}$] – can be computed[8] readily based on a knowledge of the binary association constant, K_a, between the individual

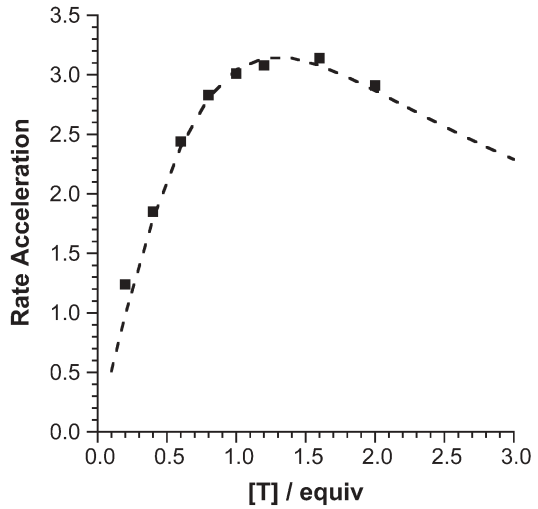

Fig. 4 Rate acceleration observed in the formation of ***exo*-T_{AB}** as a function of the amount of the complementary template ***trans*-T_{CD}** added. Experimental points are the filled squares and the dashed line is a fit of the experimental data to an independent binding site model. See Ref. 8*a* and Supplementary Information for details of this model.

recognition sites. Since the observed rate acceleration should be directly proportional to the concentration of the active ternary complex, we should be able to fit the observed rate accelerations to the function describing the variation of the concentration of the ternary complex with added template using only K_a and a scaling factor as variable parameters. Solubility problems prevented us performing experiments where more than 0.4 equivalents of the diacid template ***exo*-T_{AB}** were added to the reaction between **C** and **D**. This result is somewhat disappointing, as this small amount of ***exo*-T_{AB}** accelerates the rate of formation of ***trans*-T_{CD}** by more than a factor of three. It was, however, possible to add up to 2.0 equivalents of ***trans*-T_{CD}** to reactions between **A** and **B**. The rate acceleration observed for the formation of ***exo*-T_{AB}** rose (Fig. 4) from around 1.2 at 0.2 equivalents of added ***trans*-T_{CD}** to around a factor of three at between 1.0 and 1.5 equivalents of added ***trans*-T_{CD}**. Pleasingly, there is an excellent fit (Fig. 4, dashed line) of the experimental data to the independent binding site model. The best fit of this model to the experimental data is achieved when the K_a for the [***exo*-T_{AB}·*trans*-T_{CD}**] complex is 4500 M^{-1}. This calculated value compares well with the value of 5000 $\pm$ 600 M^{-1} determined using the ^{1}H NMR dilution method in $CDCl_3$ at 35 °C. The individual association between the amidopyridine **D** and phenylacetic acid has an association constant of 200 M^{-1} under the same conditions. This observation suggests that the interactions in the [***exo*-T_{AB}·*trans*-T_{CD}**] complex exhibit significant negative cooperativity and, therefore, the two templates ***exo*-T_{AB}** and ***trans*-T_{CD}** are not a perfect fit for each other. This limited complementarity may well impair the catalytic abilities of these two templates.

We also established the other relationships between templates in this system. We have already shown that the formation of ***trans*-T_{CD}** is accelerated by ***exo*-T_{AB}**, but not ***endo*-T_{AB}**. Template ***trans*-T_{CD}** accelerates the formation of ***exo*-T_{AB}**, and to a much lesser extent ***endo*-T_{AB}**. In addition, neither ***exo*-T_{AB}** nor ***endo*-T_{AB}** can accelerate the formation of ***cis*-T_{CD}**. Template ***cis*-T_{CD}** accelerates the formation of both ***exo*-T_{AB}** and ***endo*-T_{AB}** only slightly (< 1.5-fold).

One of our longer term goals is to probe the relative efficiencies of minimal and reciprocal replicating systems. Alternative combinations of building blocks in Fig. 2 (specifically, **A** with **D** and **B** with **C**) give rise to structures which are expected to be minimal self-replicating systems. In order to focus on reciprocal template effects, we have therefore tailored the manner in which we performed the analysis of T_{AB} and T_{CD} reported here to remove the opportunity for these minimal replication pathways to be expressed here. Studies in which the full complexity of this system is expressed are currently in progress in our laboratory. Having established the nature of the reciprocal template effects which operate in this system, we are now developing strategies for optimising the rate enhancements achieved by these systems and incorporating these crosscatalytic templates into more complex replication networks.

Notes and references

1 (*a*) G. Ashkenasy, R. Jegasia, M. Yadav and M. R. Ghadiri, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 10872; (*b*) G. Ashkenasy and M. R. Ghadiri, *J. Am. Chem. Soc.*, 2004, **126**, 11140.

2 (*a*) P. Szabó, I. Scheuring, T. Czárán and E. Szathmáry, *Nature*, 2002, **420**, 340; (*b*) S. H. Strogatz, *Nature*, 2001, **410**, 2686; (*c*) H. Jeong, B. Tombor, R. Albert, Z. N. Oltval and A. L. Barabasi, *Nature*, 2000, **407**, 651; (*d*) E. Ravasz, A. L. Somera, D. A. Mongru, Z. N. Oltvai and A. L. Barabasi, *Science*, 2002, **297**, 1551; (*e*) M. Girvan and M. E. J. Newman, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 7821; (*f*) R. Guimerà and L. A. N. Amaral, *Nature*, 2005, **433**, 895; (*g*) J. J. Perez, *Chem. Soc. Rev.*, 2005, **34**, 143; (*h*) K. Ding, H. Du, Y. Yuan and J. Long, *Chem.–Eur. J.*, 2004, **10**, 2872; (*i*) R. R. Breaker, *Nature*, 2004, **432**, 838; (*j*) C. M. Dobson, *Nature*, 2004, **432**, 824; (*k*) J. J. Lavigne and E. V. Anslyn, *Angew. Chem., Int. Ed.*, 2001, **40**, 3118.

3 M. Kindermann, I. Stahl, M. Reimold, W. M. Pankau and G. von Kiedrowski, *Angew. Chem., Int. Ed.*, 2005, **44**, 6750.

4 (*a*) I. Saur, R. Scopelliti and K. Severin, *Chem.–Eur. J.*, 2006, **12**, 1058; (*b*) A. Buryak and K. Severin, *Angew. Chem., Int. Ed.*, 2005, **44**, 7935; (*c*) L. Vial, J. K. M. Sanders and S. Otto, *New J. Chem.*, 2005, **29**, 1001; (*d*) P. T. Corbett, J. K. M. Sanders and S. Otto, *J. Am. Chem. Soc.*, 2005, **127**, 9390; (*e*) P. T. Corbett, L. H. Tong, J. K. M. Sanders and S. Otto, *J. Am. Chem. Soc.*, 2005, **127**, 8902.

5 (*a*) E. Kassianidis and D. Philp, *Angew. Chem., Int. Ed.*, 2006 (DOI: 10.1002/anie.200601845); (*b*) J. D. Cheeseman, A. D. Corbett, J. L. Gleason and R. J. Kazlauskas, *Chem.–Eur. J.*, 2005, **11**, 1708; (*c*) J. D. Cheeseman, A. D. Corbett, R. Shu, J. Croteau, J. L. Gleason and R. J. Kazlauskas, *J. Am. Chem. Soc.*, 2002, **124**, 5692.

6 (*a*) E. Kassianidis, R. J. Pearson and D. Philp, *Chem.–Eur. J.*, 2006 (DOI: 10.1002/chem.200600460); (*b*) R. J. Pearson, E. Kassianidis, A. M. Z. Slawin and D. Philp, *Chem.–Eur. J.*, 2006, **12**, 6829; (*c*) R. J. Pearson, E. Kassianidis and D. Philp, *Org. Lett.*, 2005, **7**, 3833; (*d*) R. J. Pearson, E. Kassianidis, A. M. Z. Slawin and D. Philp, *Org. Biomol. Chem.*, 2004, **2**, 3434; (*e*) V. C. Allen, D. Philp and N. Spencer, *Org. Lett.*, 2001, **3**, 777.

7 For reviews, see (*a*) N. Paul and G. F. Joyce, *Curr. Opin. Chem. Biol.*, 2004, **8**, 634; (*b*) X. Li and J. Chmielewski, *Org. Biomol. Chem.*, 2003, **1**, 901; (*c*) R. Isaac, Y.-W. Ham and J. Chmielewski, *Curr. Opin. Struct. Biol.*, 2001, **11**, 458; (*d*) A. Robertson, A. J. Sinclair and D. Philp, *Chem. Soc. Rev.*, 2000, **29**, 141; (*e*) D. H. Lee, K. Severin and M. R. Ghadiri, *Curr. Opin. Chem. Biol.*, 1997, **1**, 491; (*f*) E. A. Wintner and J. Rebek, Jr., *Acta Chem. Scand.*, 1996, **50**, 469; (*g*) G. von Kiedrowski, *Bioorg. Chem. Front.*, 1993, **3**, 113.

8 (*a*) R. J. Pieters, I. Huc and J. Rebek, Jr., *Tetrahedron*, 1995, **51**, 485; (*b*) R. J. Pieters, I. Huc and J. Rebek, Jr., *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1579.

9 (*a*) R. J. Pearson, E. Kassianidis and D. Philp, *Tetrahedron Lett.*, 2004, **45**, 4777; (*b*) S. J. Howell, D. Philp and N. Spencer, *Tetrahedron*, 2001, **57**, 4945; (*c*) A. Robertson, D. Philp and N. Spencer, *Tetrahedron*, 1999, **55**, 11365; (*d*) R. M. Bennes, B. M. Kariuki, K. D. M. Harris, D. Philp and N. Spencer, *Org. Lett.*, 1999, **1**, 1087.