Orthogonal or simultaneous use of disulfide and hydrazone exchange in dynamic covalent chemistry in aqueous solution†

Zaida Rodriguez-Docampo[a] and Sijbren Otto*[ab]

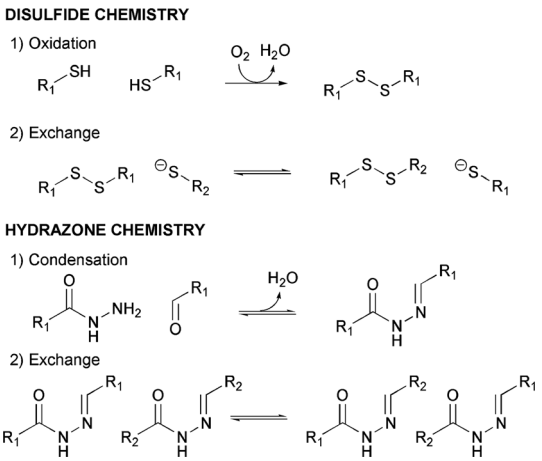
Received (in Cambridge, UK) 22nd May 2008, Accepted 16th July 2008
First published as an Advance Article on the web 11th September 2008
DOI: 10.1039/b808725c

Hydrazone and disulfide exchange have been combined in a single system, but can be addressed independently: by adjusting the pH of the solution from acidic to mildly basic it is possible to switch from exclusively hydrazone exchange to exclusively disulfide exchange, while at intermediate pH both reactions occur simultaneously.

Dynamic covalent chemistry combines the robustness of covalent bonds with the proof-reading ability of noncovalent self-assembly.[1] This makes it a powerful tool for the synthesis of impressively complex structures.[2] Reversible covalent reactions also form the core of the topical field of dynamic combinatorial chemistry[3] and provide an exciting entry into the emerging field of systems chemistry.[4]

With the number of reversible covalent chemistries at our disposal increasing steadily, studies are starting to appear which combine two or more reversible chemistries in a single system.[5] Some of the most striking complex molecular architectures such as interlocked structures[2a] or supramolecular assemblies[2b,c] are the result of the successful combination of covalent exchange reactions and more labile metal coordination[2a,c] or hydrogen bonds.[2b,5c] If two or more reversible chemistries could be switched on and off independently this would offer enhanced control and create new opportunities for evolving the system by alternating use of the different chemistries. We now report the first example of two dynamic covalent chemistries in a single system that can be either operated fully orthogonally‡ or occur simultaneously (albeit slowly), depending on the pH of the solution.

We focussed our efforts on combining disulfide and hydrazone exchange, which are currently two of the most popular reversible covalent exchange reactions. In general, the optimum pH for disulfide exchange in water is 7–9 (Scheme 1),[3] while in the same solvent hydrazone exchange requires acid catalysis (pH 2.5 to 5; Scheme 1).[3] Outside these ranges the efficiencies of the reactions generally decrease. However, exceptions to these rules have been reported where hydrazone exchange occurs at higher pH[6a] and disulfide exchange at lower pH.[6b] The goal of the present investigation was to establish the structural requirements and experimental conditions that would enable the orthogonal use of the two chemistries.



Scheme 1 Reactions involved in disulfide and hydrazone chemistry.

Compound **1** (Fig. 1) is a suitable model system containing two hydrazide termini that can be reacted with two equivalents of aldehyde to give the desired hydrazone product, which can subsequently undergo hydrazone exchange upon introducing a second aldehyde. Reaction of the central disulfide bond with a thiol would allow us to probe disulfide exchange. In the presence of air irreversible thiol oxidation will also occur.

Through a combination of these four different reactions (Scheme 1), a mixture of hydrazide/disulfide **1**, aldehydes **2** and **4** and thiol **3** can potentially produce products **5–13** depicted in Fig. 2. With four simple experiments (A–D) we show how the

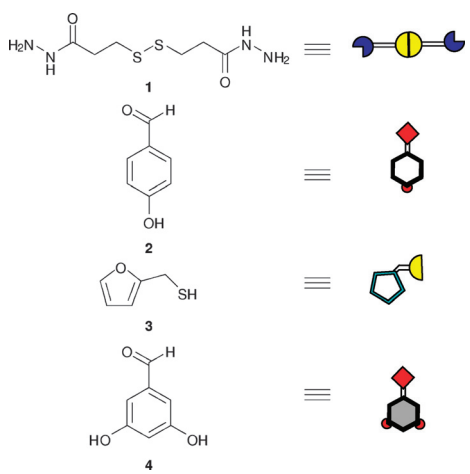


Fig. 1 Building blocks and their schematic representations.

[a] *Department of Chemistry, University of Chemistry, Lensfield Road, Cambridge, UK CB2 1EW. Fax: +44 1223 336017; Tel: +44 1223 336509*
[b] *Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands. E-mail: s.otto@rug.nl*

† Electronic supplementary information (ESI) available: Materials and experimental procedures; LC-MS characterisation data; data for disulfide exchange with various thiols at pH 4.5–2.5. See DOI: 10.1039/b808725c

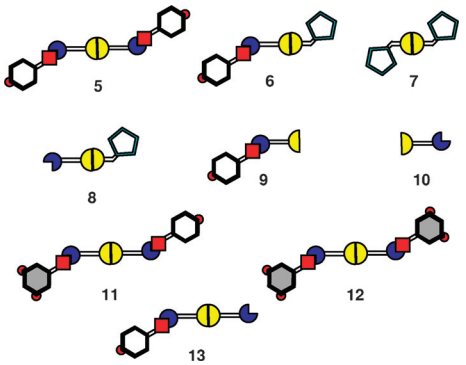


Fig. 2 Schematic representations of possible products.

formation of these products can be controlled by activating or deactivating the disulfide and hydrazone formation and exchange processes. We first set out to establish how thiol oxidation and disulfide exchange depend on the pH of the solution.

In experiment A we mixed disulfide **1**, aldehyde **2** and thiol **3** at pH 8.5. We analysed the mixture after 3 days by HPLC, using two different wavelengths: $\lambda = 290$ nm corresponding to the maximum UV absorbance of the hydrazone moiety and $\lambda = 245$ nm which allows monitoring of furanmethanethiol **3**.§ We were able to directly observe thiol oxidation giving rise to disulfide **7** (Fig. 3a). Disulfide exchange could only be confirmed indirectly, as we were unable to detect any free hydrazides including **8** and **10** (and parent compound **1**) with our chromatographic methods. Thus, within 48 h after acidification to pH 2.5 we observed the formation of **6** and **9**, which are the expected hydrazone derivatives of disulfide exchange product **8** and thiol **10**, respectively. No change in the concentrations of **3** and **7** was observed after addition of acid, suggesting disulfide oxidation and exchange no longer occurred at pH 2.5.

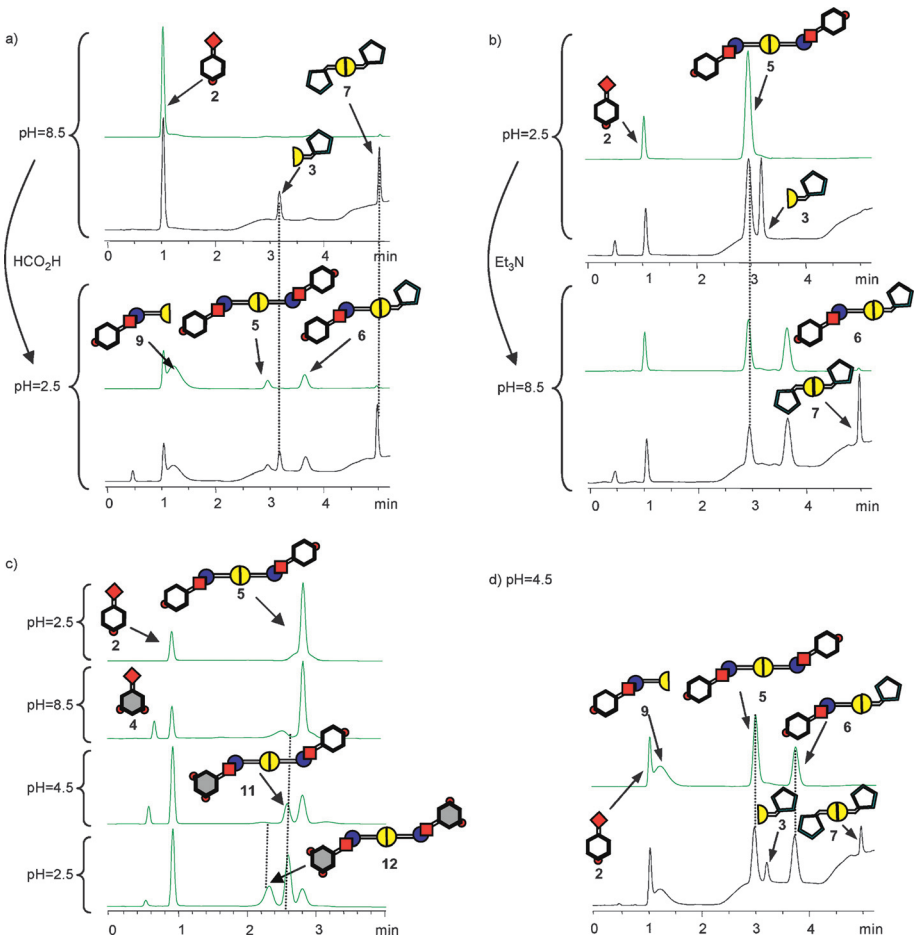


Fig. 3 HPLC analyses recorded at $\lambda = 290$ nm (green traces) and $\lambda = 245$ nm (black traces) for the mixtures prepared from hydrazide **1** (5.0 mM); aldehyde **2** (10 mM) and thiol **3** (10 mM) in (a) 1 : 1 CH_3CN–ammonium acetate buffer at pH 8.5 after 3 days (top two chromatograms) and 2 days after the addition of formic acid to reach pH 2.5 (bottom two chromatograms); (b) 1 : 1 CH_3CN–ammonium formate buffer at pH 2.5 after 3 days (top two chromatograms) and 2 days after the addition of Et_3N to reach pH 8.5 (bottom two chromatograms); (c) HPLC traces recorded at $\lambda = 290$ nm for the mixtures containing hydrazide **1** (5.0 mM) and aldehyde **2** (10 mM) (top chromatogram) and 3 days after the addition of 1 equiv. (5.0 mM) of aldehyde **4** in 1 : 1 CH_3CN–ammonium formate buffer at pH 2.5 and in 1 : 1 CH_3CN–ammonium acetate buffer at pH 4.5 and 8.5; (d) HPLC analyses of the mixture prepared from hydrazide **1** (5.0 mM); aldehyde **2** (10 mM) and thiol **3** (10 mM) in 1 : 1 CH_3CN–ammonium acetate buffer at pH 4.5 after 5 days. All samples, except those at pH 4.5, had reached stable product distributions well within the specified reaction times.

In experiment B the same three starting materials (**1–3**) were mixed at pH 2.5 and analysed after 3 days. Only thiol **3** and hydrazone **5** are observed (Fig. 3b). The absence of disulfide **6** and thiol **9** proves that disulfide exchange does not take place and the absence of disulfide **7** indicates also that disulfide oxidation does not happen at this low pH. However, upon raising the pH to 8.5 disulfide oxidation and exchange were activated: after 48 h thiol **3** was completely consumed and disulfide exchange product **6** had appeared.

The same two experiments also provide information about the pH dependence of hydrazone *formation*. Experiment A demonstrates that with aldehyde **2** no significant hydrazone formation occurs at pH 8.5, while it is rapid at pH 2.5 (Fig. 3a). The results of experiment B confirm this conclusion (Fig. 3b). At pH 2.5 building blocks **1** and **2** react rapidly to give dihydrazone **5**, although some free aldehyde **2** can still be observed. After raising the pH to 8.5, the amount of **5** decreased solely as a result of disulfide exchange. The fact that the peak for aldehyde **2** remained constant indicates that at pH 8.5 no additional hydrazone is produced.

In experiment C we probed the pH dependence of the hydrazone *exchange* reaction (Fig. 3c). We mixed building block **1** with aldehyde **2** at pH 2.5, giving the dihydrazone **5**. The pH of the mixture was then raised to 8.5, followed by addition of 1 equiv. of 3,5-dihydroxybenzaldehyde **4**. Comparison of the HPLC traces before and 3 days after the addition of **4** showed that the peaks corresponding to compounds **2** and **5** were not altered, indicating that no hydrazone exchange took place at this pH. We did detect a small amount of hydrazone **11**, which therefore can only have formed through reaction of aldehyde **4** with monohydrazide **13** (itself not detectable using our HPLC method). We then reduced the pH back to 2.5 and observed rapid hydrazone exchange to give the new hydrazone **12** together with more hydrazone **11**, at the expense of **5**.

While experiments A–C demonstrate that hydrazone exchange and disulfide exchange are orthogonal when operating at either pH 2.5 or 8.5, respectively, it is also possible to operate both chemistries simultaneously. Mixing building blocks **1**, **2** and **3** at pH 4.5 generated all the possible products expected from the simultaneous occurrence of thiol oxidation, disulfide exchange and hydrazone formation: **5**, **6**, **7** and **9** (experiment D; Fig. 3d). Hydrazone exchange at this pH was studied in an independent experiment. The addition of aldehyde **4** to a pre-formed mixture of **1** and **2** at pH 4.5 resulted in reaction with dihydrazone **5** displacing aldehyde **2** to produce **11** and **12**. Hydrazone exchange at pH 4.5 was much slower than at pH 2.5, as noticed previously,[3] so that after 3 days the mixture had not yet reached equilibrium (third trace in Fig. 3c). However, the rate of exchange can be increased by the addition of aniline,[7] producing within 4 h the same product distribution as that obtained at pH 2.5 (see fourth trace in Fig. 3c and ESI† Fig. S14).

Finally, we briefly investigated the generality of the experimental protocol using different building blocks. We noticed that aromatic aldehydes activated by electron withdrawing groups form hydrazones more readily than those with electron donating groups, as apparent from the different tendency for hydrazone formation exhibited by aldehydes **2** and **4** in experiments A and C.¶ Also the aliphatic nature of thiol **3** turned out to be crucial for orthogonality. Aromatic thiols exhibited a significant rate of exchange even at pH 2.5 (see ESI†). This difference reflects the higher pK_a of aliphatic thiols (*ca.* 9) as compared to that of their aromatic counterparts (*ca.* 6.5).[8]

In conclusion, we have identified conditions under which two different covalent exchange reactions can be used orthogonally or simultaneously (albeit slowly) in the same system. Depending on the pH, hydrazone or disulfide exchange can be selectively activated or deactivated. Different product distributions are obtained depending on the order in which the two exchange processes are activated, indicating that the equilibrium of the system can be shifted in directions different from the global energy minimum attained when both reactions occur simultaneously.‖ These findings pave the way to future construction of more complex molecular architectures and chemical systems[2] capable of being evolved by alternating use of the two reversible covalent chemistries.

This work was supported by the EU (Marie Curie EST 8303; ChemBioCam); the EPSRC and the Royal Society. We thank Dr Ana Belenguer for help with the HPLC/ESI-MS analysis.

Notes and references

‡ With orthogonal we imply the ability to selectively activate one exchange process while the other occurs slowly enough to be negligible on the same timescale.

§ The maximum of UV absorbance for furanmethanethiol **3** (λ = 239 nm) is close to the acetonitrile absorbance (λ = 230 nm). However, monitoring at 245 nm allowed the analysis of the mixtures, albeit with a considerable drift in the baseline reflecting the increasing amount of acetonitrile during gradient elution.

¶ Aldehyde **2** is deactivated by the *para*-OH group (electron donation through resonance; Hammett $\sigma_p = -0.38$), while aldehyde **4** is activated by the two *meta*-OH groups (electron withdrawal by induction, Hammett $\sigma_m = +0.13$).

‖ This difference most likely results from the different environments experienced by the different exchange processes, with respect to both pH and the chemical nature of the non-exchanging end of the molecule.

1 (*a*) S. J. Rowan, S. J. Cantrill, G. R. L. Cousins, J. K. M. Sanders and J. F. Stoddart, *Angew. Chem., Int. Ed.*, 2002, **41**, 898; (*b*) J.-M. Lehn, *Chem. Soc. Rev.*, 2007, **36**, 151.

2 (*a*) C. D. Meyer, C. S. Joiner and J. F. Stoddart, *Chem. Soc. Rev.*, 2007, **36**, 1705; (*b*) N. Sreenivasachary and J.-M. Lehn, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 5938; (*c*) J. R. Nitschke and J.-M. Lehn, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 11970.

3 P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders and S. Otto, *Chem. Rev.*, 2006, **106**, 3652.

4 (*a*) R. F. Ludlow and S. Otto, *Chem. Soc. Rev.*, 2008, **37**, 101; (*b*) R. F. Ludlow, R. J. Liu, H. Li, S. L. Roberts, J. K. M. Sanders and S. Otto, *Angew. Chem., Int. Ed.*, 2007, **46**, 5762; (*c*) P. T. Corbett, J. K. M. Sanders and S. Otto, *Angew. Chem., Int. Ed.*, 2007, **46**, 8858.

5 (*a*) V. Goral, M. I. Nelen, A. V. Eliseev and J.-M. Lehn, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 1347; (*b*) J. Leclaire, L. Vial, S. Otto and J. K. M. Sanders, *Chem. Commun.*, 2005, 1959; (*c*) A. T. ten Cate, P. Y. W. Dankers, R. P. Sijbesma and E. W. Meijer, *J. Org. Chem.*, 2005, **70**, 5799; (*d*) D. Schultz and J. R. Nitschke, *Angew. Chem., Int. Ed.*, 2006, **45**, 2453; (*e*) R. J. Sarma, S. Otto and J. R. Nitschke, *Chem.–Eur. J.*, 2007, **13**, 9542; (*f*) N. Christinat, R. Scopelliti and K. Severin, *Angew. Chem., Int. Ed.*, 2008, **47**, 1848.

6 (*a*) R. Nguyen and I. Huc, *Chem. Commun.*, 2003, 942; (*b*) S. Otto, R. L. E. Furlan and J. K. M. Sanders, *J. Am. Chem. Soc.*, 2000, **122**, 12063.

7 (*a*) A. Dirksen, S. Dirksen, T. M. Hackeng and P. E. Dawson, *J. Am. Chem. Soc.*, 2006, **128**, 15602; (*b*) E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, 1962, **84**, 826.

8 (*a*) F. G. Bordwell and D. L. Hughes, *J. Org. Chem.*, 1982, **47**, 3224; (*b*) D. M. E. Reuben and T. C. Bruice, *J. Am. Chem. Soc.*, 1976, **98**, 114.