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From static to dynamic: escaping kinetic traps in hydrazone-based dynamic combinatorial libraries†

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Thermodynamic control over kinetically-trapped mixtures of hydrazone-based macrocycles is achieved by addition of an aromatic monohydrazide to generate dynamic combinatorial libraries (DCLs) of linear and macrocyclic oligomers.

We address here one of the fundamental requirements for dynamic combinatorial chemistry—how to ensure the formation of a truly dynamic system operating under thermodynamic control.[1] Dynamic combinatorial libraries (DCLs) are established when building blocks are combined *via* reversible reactions to generate mixtures of oligomers at equilibrium; receptors may be identified amongst the library members by subsequent addition of a guest that will alter the distribution of library members to amplify, and thus identify, effective hosts. The power of dynamic combinatorial chemistry to reveal host–guest interactions using a systems-based approach has been demonstrated by the discovery of many elegant and effective receptors.[2] Nevertheless, there remain some limitations which must be addressed. Here we show how libraries may become trapped in an irreversible regime when library members have too great a stability, and we describe a strategy to resolve such situations.

In an ideal DCL, all library members would be located in energetically shallow potential wells so that their interconversion in the absence of a template should be rapid and unbiased.[1c] However, this is often not the case; macrocycles, for example, are often located in deep potential wells. The exchange of building blocks between such hydrazone-based macrocycles[3] may generally be disfavoured because, when one hydrazone linkage is cleaved and the macrocycle opens, the linear intermediate will quickly snap shut because the high effective molarity of the intramolecular reaction will lead to ring closure being faster than any intermolecular reactions with other species in the library.[4] In extreme cases the macrocycles may be additionally stabilised by the presence of multiple reversible links in parallel,[5] or by self-templating that is either intramolecular[6] or intermolecular (aggregation).[7] Therefore, where DCL members are located in too deep potential wells they may be kinetically-trapped so that even though the covalent linkages are of an intrinsically reversible nature, the DCL may never reach thermodynamic equilibrium within the timescale of the experiment (hours, days, weeks).[8] We have discovered that this situation may be avoided, in the case of a DCL of hydrazone-based macrocycles, by the addition to the reaction mixture of an excess of an aromatic monohydrazide to form a library of cyclic and linear oligomers (Fig. 1). We apply this strategy to release two different kinetically-trapped DCLs, one in organic and one in aqueous solution.

A library may be assumed to be under thermodynamic control if the same distribution of macrocycles can be reached from different starting points.[1b] For example, a DCL of hydrazone macrocycles should show the same distribution of library members regardless of whether it is formed by dissolving the individual building blocks in the solvent/acid mixture, or by dissolving a pre-formed macrocycle in the same solvent/acid mixture. The most rigorous testing requires that the most stable macrocycle in the mixture (the most abundant, in a DCL at equilibrium) be chosen to test the re-equilibration, since the thermodynamic equilibrium distribution must be accessible from all points—including local minima—on the energy surface.

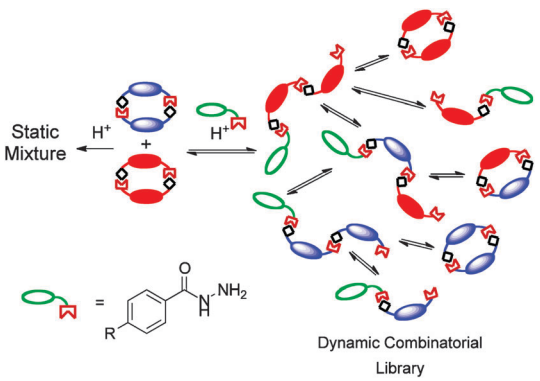

Fig. 1 When hydrazone macrocycles are reacted together under conditions expected to facilitate hydrolysis and transimination they may fail to mix and exchange building blocks because the macrocycles are too stable (kinetically trapped). By addition of an aromatic monohydrazide a DCL of cyclic and linear oligomers can be obtained.

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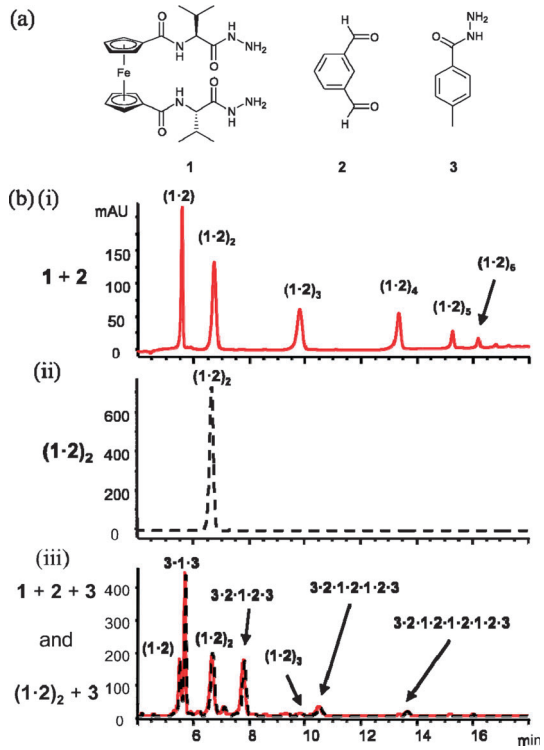

Fig. 2 (a) Organic soluble building blocks Fc-[CO-Val-NHNH$_2$]$_2$ (**1**), isophthalaldehyde (**2**) and 4-methylbenzhydrazide (**3**); (b) HPLC chromatograms (290 nm) for the reaction after 5 days of (i) **1** (0.5 mM) and **2** (0.5 mM); (ii) **(1·2)$_2$** (0.25 mM) and (iii) after 11 days (overlaid) **1** (0.5 mM), **2** (0.5 mM) and **3** (5 mM) (red) and **(1·2)$_2$** (0.25 mM) and **3** (5 mM) (black dashed) in 0.5% acetic acid in $CHCl_3$. Note in (iii) unreacted **3** elutes after 2.5 mins.

We have previously reported the kinetically-controlled synthesis of a series of ferrocene-based macrocycles by means of hydrazone formation from dihydrazide and dialdehyde building blocks: Fc-[CO-Val-NHNH$_2$]$_2$ (**1**) and isophthalaldehyde (**2**) (Fig. 2a).[9] The two building blocks (each 0.5 mM) were reacted in 0.5% acetic acid in $CHCl_3$. The reaction mixture was analysed by LC/MS after 5 days and found to contain macrocycles **(1·2)**, **(1·2)$_2$**, **(1·2)$_3$**, **(1·2)$_4$**. . .**(1·2)$_8$** (Fig. 2b(i)). It had been expected that the library of hydrazone macrocycles would form under thermodynamic control, as had been reported on numerous occasions for acid-catalysed hydrazone formation in DCLs.[3]§ Nevertheless, a test for thermodynamic control was carried out. The most abundant library member, **(1·2)$_2$** was isolated[9] and dissolved at 0.25 mM in 0.5% acetic acid in $CHCl_3$. After two weeks, no exchange of building blocks had taken place and **(1·2)$_2$** was the only species observed in the solution (Fig. 2b(ii)).

The first step required in order for **(1·2)$_2$** to convert to a mixture of macrocycles is the hydrolysis, or partial hydrolysis of a fraction of **(1·2)$_2$** to form a linear tetramer with a free hydrazide. This may then react with the aldehyde of another linear tetramer, or directly attack a hydrazone linkage, either in another macrocycle or intramolecularly to give a smaller macrocycle. It seemed likely that, under the reaction conditions employed, ring-opening hydrolysis would be slow compared to ring-closing hydrazone formation. Free hydrazides would be scarce and unable to compete with the high effective molarity of the ring-closing reaction. We reasoned that the addition of excess of a monohydrazide would bypass the need for hydrolysis, enabling the direct nucleophilic attack on the hydrazones to form new hydrazone linkages *via* an aminal intermediate. Excess mono-hydrazides would also provide competition for the intramolecular ring-closing reactions, shifting the equilibria towards capped, and therefore stable linear oligomers, and ultimately providing an alternative route for the exchange of building blocks and interconversion of macrocycles.

To test this theory, two libraries were prepared: one containing building blocks **1** and **2** (each 0.5 mM) with 4-methylbenzhydrazide (**3**) (5 mM), and the other containing macrocycle **(1·2)$_2$** (0.25 mM) with monohydrazide **3** (5 mM), both in 0.5% acetic acid in $CHCl_3$. Gratifyingly, after 11 days, analysis by LC/MS revealed that the two libraries contained an identical distribution of cyclic and linear oligomers formed from **1**, **2** and **3**, showing that the mixture was now at thermodynamic equilibrium (Fig. 2b(iii)). The molecular recognition potential of the library members could now be explored.[9a] Not only were the libraries under thermodynamic control, but by adjusting the distribution of the library members away from one particularly stabilised species, the difficulty associated with the detection of small template-induced amplifications of species present in large proportions—a limitation which has been addressed elsewhere in the context of disulfide DCLs[10]—was avoided. The addition of a mono-functionalised building block to a DCL of macrocycles introduced linear species, but an exploration of their properties alongside their cyclic counterparts could reveal new and unexpected receptors.[9a]

The use of aniline as a nucleophilc catalyst to facilitate hydrazone and oxime formation and transimination has recently been implemented in biomolecular labelling[11] and in DCLs.[12] We found, however, that if 4-methylbenzhydrazide was replaced with aniline, at least 50 equiv. were required before the libraries began to approach equilibrium within 11 days. With 50 equiv. aniline the reaction medium was so altered that the library members began to precipitate. Imines formed from aniline are easily hydrolysed so in order to be present in sufficient quantities to allow intermolecular hydrazone exchange to compete with intramolecular ring-closing hydrazone formation a large excess of aniline is required.

Our strategy of adding excess monohydrazide to kinetically-trapped libraries can also be applied successfully to DCLs in aqueous solution. We had attempted to form a DCL from two bifunctional hydrazide/protected aldehyde building blocks: a tetrahydroisoquinoline building block **4**,[3c] and a new water-soluble pseudotripeptide building block **5** (see ESI† for synthesis) (Fig. 3a). The building blocks were reacted together (each 0.5 mM) in pH 3 sodium formate buffer (18 mM), and analysed by LC/MS. After 14 days the reaction mixture contained the two homodimers **(4)$_2$** and **(5)$_2$** and heterodimer **(4·5)** (Fig. 3b(i)). When however, building blocks **4** and **5** (each 1 mM) were individually dissolved in buffer and left overnight to cyclise before combining the two in a 1 : 1 ratio, a very different distribution was observed. After 14 days only the homodimers were found in the solution, which indicated that once formed,

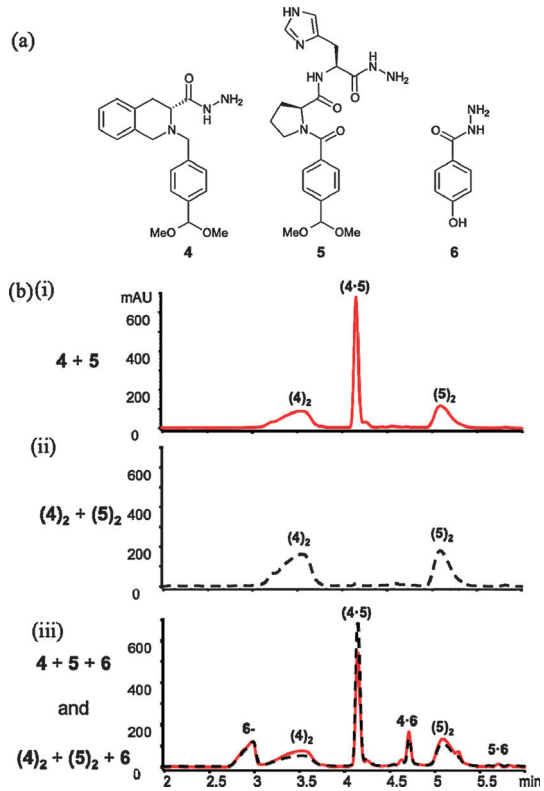

Fig. 3 (a) Aqueous hydrazide/protected aldehyde building blocks **4**, **5** and 4-hydroxybenzhydrazide (**6**); (b) HPLC chromatograms (290 nm) for the reaction after 14 days of (i) **4** (0.5 mM) with **5** (0.5 mM); (ii) preformed macrocycles $\mathbf{(4)_2}$ (0.25 mM) and $\mathbf{(5)_2}$ (0.25 mM) and (iii) (overlaid) **4** (0.5 mM), **5** (0.5 mM) and **6** (8 mM) (red), and $\mathbf{(4)_2}$ (0.25 mM), $\mathbf{(5)_2}$ (0.25 mM) and **6** (8 mM) (black dashed) in pH 3 NaHCOO buffer (18 mM).

the homodimers were kinetically-trapped (Fig. 3b(ii)). We found, however, that by adding 4-hydroxybenzhydrazide (**6**) (8 mM), the mixtures could be brought to equilibrate under thermodynamic control and after 14 days the two DCLs showed the same distributions of macrocycles $\mathbf{(4)_2}$, $\mathbf{(5)_2}$, **(4·5)** and linears **4·6** and **5·6**.

Different monohydrazides were tested to investigate the general applicability of this approach. We found that if the *para*-substituent on a benzhydrazide was varied from hydroxy to nitro, dimethylamino or methoxy there was no significant change in equilibration time. However, if **6** was replaced by an aliphatic hydrazide, Girard T reagent ((carboxymethyl)-trimethyl-ammonium chloride hydrazide) the equilibration of $\mathbf{(4)_2}$ and $\mathbf{(5)_2}$ was significantly slower. The ability of aniline to facilitate equilibration in these libraries was also tested but even with a 25-fold excess of aniline the mixtures failed to equilibrate.

In conclusion, we have demonstrated that the addition of a small excess of an aromatic monohydrazide is an effective means to promote the exchange of building blocks between kinetically-trapped hydrazone-based macrocycles that are stabilised by intramolecular or intermolecular self-templating. The strategy is applicable to DCLs in organic or aqueous solution and to different building blocks, although the concentration of monohydrazide required to facilitate exchange will be dependent upon the reactivity of the specific monohydrazide[13] and the relative stabilities of, and energy barriers between, library members, and therefore should be assessed on a case-by-case basis. Here we have considered only hydrazone macrocycles, but the lessons learned with regards to the introduction of monofunctionalised building blocks will be relevant to any kinetically-trapped DCL.

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Notes and references

§ In previously reported DCLs in which rapid exchange of hydrazone macrocycles has been observed,[3] more than 150 equiv. of TFA were used to facilitate exchange. These reaction conditions limit the types of building blocks that may be explored. The ferrocene-based building block **1** is not stable under these conditions.

1 (*a*) *Dynamic Combinatorial Chemistry*, ed. J. N. H. Reek and S. Otto, Wiley-VCH, Weinheim, Germany, 2010; (*b*) P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders and S. Otto, *Chem. Rev.*, 2006, **106**, 3652–3711; (*c*) S. Ladame, *Org. Biomol. Chem.*, 2008, **6**, 219–226.
2 (*a*) S. Otto, R. L. E. Furlan and J. K. M. Sanders, *Science*, 2002, **297**, 590–593; (*b*) T. Nishinaga, A. Tanatani, K. C. Oh and J. S. Moore, *J. Am. Chem. Soc.*, 2002, **124**, 5934–5935; (*c*) R. T. S. Lam, A. M. Belenguer, S. L. Roberts, C. Naumann, T. Jarrosson, S. Otto and J. K. M. Sanders, *Science*, 2005, **308**, 667–669; (*d*) K. R. West, R. F. Ludlow, P. T. Corbett, P. Besenius, F. M. Mansfeld, P. A. G. Cormack, D. C. Sherrington, J. M. Goodman, M. C. A. Stuart and S. Otto, *J. Am. Chem. Soc.*, 2008, **130**, 10834–10835; (*e*) R. Cacciapaglia, S. Di Stefano and L. Mandolini, *J. Am. Chem. Soc.*, 2005, **127**, 13666–13671.
3 (*a*) S. M Voshell, S. J. Lee and M. R. Gagné, *J. Am. Chem. Soc.*, 2006, **128**, 12422–12423; (*b*) L. A. Ingerman and M. L. Waters, *J. Org. Chem.*, 2009, **74**, 111–117; (*c*) M. G. Simpson, M. Pittelkow, S. P. Watson and J. K. M. Sanders, *Org. Biomol. Chem.*, 2010, **8**, 1181–1187; (*d*) J. M. Klein, V. Saggiomo, L. Reck, M. McPartlin, G. D Pantoş, U. Lüning and J. K. M. Sanders, *Chem. Commun.*, 2011, **47**, 3371–3373; (*e*) M. K. Chung, K. Severin, S. J. Lee, M. L. Waters and M. R. Gagné, *Chem. Sci.*, 2011, **2**, 744–747.
4 (*a*) E. V. Anslyn and D. A. Dougherty, *Modern Physical Organic Chemistry*, University Science Books, Sausalito, California, 2006; (*b*) C. A. Hunter and H. L. Anderson, *Angew. Chem., Int. Ed.*, 2009, **48**, 7488–7499.
5 E. L. Elliott, C. S. Hartley and J. S. Moore, *Chem. Commun.*, 2011, **47**, 5028–5030.
6 K. Oh, K. S. Jeong and J. S. Moore, *Nature*, 2001, **414**, 889–893.
7 (*a*) R. A. R. Hunt, R. F. Ludlow and S. Otto, *Org. Lett.*, 2009, **11**, 5110–5113; (*b*) S. Xu and N. Giuseppone, *J. Am. Chem. Soc.*, 2008, **130**, 1826–1827.
8 F. B. L. Cougnon, H. Y. Au-Yeung, G. D. Pantoş and J. K. M. Sanders, *J. Am. Chem. Soc.*, 2011, **133**, 3198–3207.
9 (*a*) S. R. Beeren and J. K. M. Sanders, *J. Am. Chem. Soc.*, 2011, **133**, 3804–3807; (*b*) S. R. Beeren and J. K. M. Sanders, *Chem. Sci.*, 2011, DOI: 10.1039/c1sc00168j.
10 S. Ladame, A. M. Whitney and S. Balasubramanian, *Angew. Chem., Int. Ed.*, 2005, **44**, 5736–5739.
11 (*a*) A. Dirksen, T. M. Hackeng and P. E. Dawson, *Angew. Chem., Int. Ed.*, 2006, **45**, 7581–7584; (*b*) A. Dirksen, S. Dirksen, T. M. Hackeng and P. E. Dawson, *J. Am. Chem. Soc.*, 2006, **128**, 15602–15603; (*c*) A. Dirksen, S. Yegneswaran and P. E. Dawson, *Angew. Chem. Int. Ed.*, 2010, **49**, 2023–2027.
12 V. T. Bhat, A. M. Caniard, T. Luksch, R. Brenk, D. J. Campopiano and M. F. Greaney, *Nat. Chem.*, 2010, **2**, 490–497.
13 R. Nguyen and I. Huc, *Chem. Commun.*, 2003, 942–943.