Reversible Michael addition of thiols as a new tool for dynamic combinatorial chemistry†

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The Michael addition of thiols to enones is reported as a new method for dynamic combinatorial library synthesis.

Dynamic combinatorial chemistry (DCC) offers a conceptually new approach to the investigation of host–guest interactions.[1] A dynamic combinatorial library (DCL) consists of a series of molecules assembled by means of a reversible reaction, each member of the library being in dynamic exchange under thermodynamic control. Addition of a suitable template molecule will then alter the equilibrium distribution, amplifying those components that bind the template strongly, at the expense of the poor-binding library members. The reversible connection is then switched off, and the best binders identified and isolated. The concept has been recently applied to the study of catalysis,[2] oligonucleotide[3] and peptide assembly,[4] metal-templated macrocyclisation,[5] and drug discovery.[6]

The reversible reaction used to construct a DCL is fundamental to the design, assembly and analysis of the library. For pharmaceutical applications of DCC, where a protein is used to template the synthesis of a potential ligand, the number of reactions currently reported for DCL construction is small; transthioesterification,[7] C=N bond formation,[6,8] disulfide exchange[9] and the enzyme-mediated aldol reaction.[10] This narrow diversity of chemistry is likely due to the stringent criteria necessary for successful construction of a DCL that may be used in concert with a biological macromolecule. The minimum requirement is a reversible reaction that operates under essentially physiological conditions. Clearly, such parameters are quite different to those governing most organic synthesis transformations. If the potential of DCC as a method for interrogating protein–ligand interactions is to be realised, an increase in the number of reversible reactions available for DCL construction is desirable. With this in mind, we have been studying carbon–sulfur bond forming reactions with a view to introducing a new class of bond construction to DCC.

Our work is based upon a consideration of the Michael-addition chemistry of glutathione (GSH, **1**), the most abundant non-protein thiol in eukaryotic cells. GSH plays a critical role in protecting the cell from cytotoxic, heavy metal and oxidative stress.[11] The nucleophilic thiol effectively scavenges peroxides, alkylating agents and other toxic electrophiles, making them more water soluble and easily eliminated from the cell; a process mediated by the glutathione-*S*-transferase (GST) family of enzymes. In many cases, the conjugation reaction is thought to be reversible, and this has been unequivocally demonstrated for the Michael addition of GSH to certain anti-inflammatory sesquiterpene lactones.[12] Schmidt has reported thermodynamic and kinetic data for the conjugation of GSH with helenalin, **2**, (Scheme 1) revealing a reaction that is potentially tailor-made for DCC for the following reasons:

1) An equimolar mixture of GSH and **2** reaches equilibrium in approx. 30 min at pH 8 at room temperature. This relatively fast rate of reaction permits rapid DCL assembly and screening.

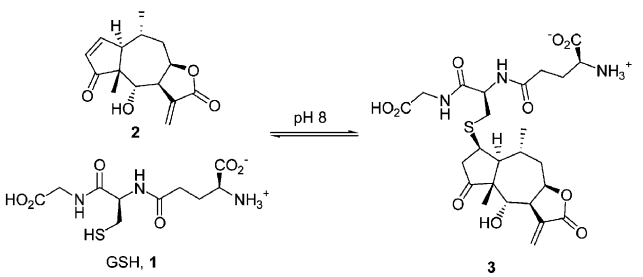
2) An association constant of 0.93 μmol^{-1} at pH 8 indicates a fairly even distribution of starting materials and adducts at near physiological concentrations, enabling the synthesis of DCLs with maximal structural diversity.

3) The reaction can be switched on or off. The reaction proceeds rapidly at basic pH, but at low pH it becomes extremely slow and irreversible.

4) The reaction takes place in water at room temperature. The mild reaction conditions and absence of any external reagents should permit the successful interfacing of proteins with the DCL, one of the most challenging aspects of DCC.

In order to establish whether the Michael addition of GSH could be applied to DCC, we elected to study the reaction using derivatives of ethacrynic acid (EA), **4a**. EA is one of the best characterized inhibitors of GSTs,[13] (both on its own and as a GSH conjugate) and contains a reactive α-methylene ketone that would be expected to serve as an excellent Michael acceptor. We derivatised EA through amide bond formation at the carboxylic acid functionality, preparing a small collection of amides **4b–4f** as components for the DCL (Scheme 2).[14]

GSH (250 μmol) was mixed with 50 μmol each of the five EA derivatives **4a–4e** in water–DMSO mixtures at pH 8. No special precautions were taken to prevent GSH dimerisation, as disulfide exchange is reversible under these reaction conditions. Analysis of the reaction mixture by LC-MS was performed at regular intervals, and revealed an equilibrium distribution of all five of the possible Michael adducts **5a–5e**, plus each of the starting EA derivatives,



Scheme 1 Reversible conjugation of GSH to helenalin.

† Electronic Supplementary Information (ESI) available: characterisation data for compounds **4b–f** and **5a**. See http://www.rsc.org/suppdata/cc/b4/b414300k/

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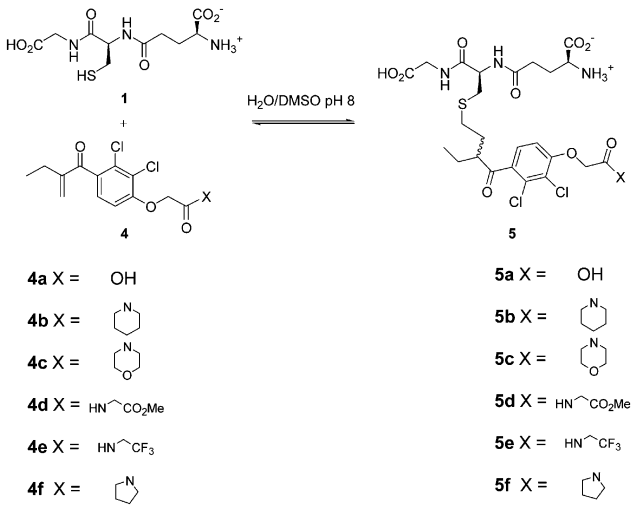

Scheme 2 Dynamic Combinatorial Library of GSH-EA derivatives.

being attained in three hours (Fig. 1, DCL A).[15] The DCL remained unchanged for several days, and was notably clean of side products that could attend the base-mediated Michael addition. A qualitative analysis of the LC data reveals an approximately equitable distribution of adducts, which is to be expected given that the variation in functionality of the enone starting materials occurs relatively far from the reactive site. This is a deliberate strategy aimed at producing an isoenergetic library that avoids large differences in reaction rate between the various library components, ensuring that the maximum number of structures can be represented in the DCL without having to bias reaction stoichiometry.

Crucially, we could demonstrate that the DCL was both under thermodynamic control and responsive to changes in pH. Addition of 50 μmol of the pyrrolidine derivative **4f** to the equilibrated DCL A at pH 8 led to the smooth incorporation of the corresponding adduct **5f** into the equilibrium distribution (DCL B), indicating that the system is in dynamic equilibrium. A further addition of ten equivalents of **4f** shifted the equilibrium such that the adduct distribution was dominated by adduct **5f** (DCL C). When DCL A was acidified to pH 4 the Michael addition was effectively switched off, proven by the subsequent addition of ten equivalents of **4f** which failed to produce any of the corresponding adduct **5f** (DCL D).

In conclusion, we have established that the Michael addition of thiols to enones is an effective chemical reaction for the synthesis of DCLs. The libraries are simple to prepare, quick to equilibrate and may be switched on or off by adjusting the pH. Additionally, the mild conditions and aqueous reaction medium should be compatible with GSH-related protein targets. Future work will examine the application of GSH-EA libraries to the discovery of new inhibitors of the GST family of enzymes.

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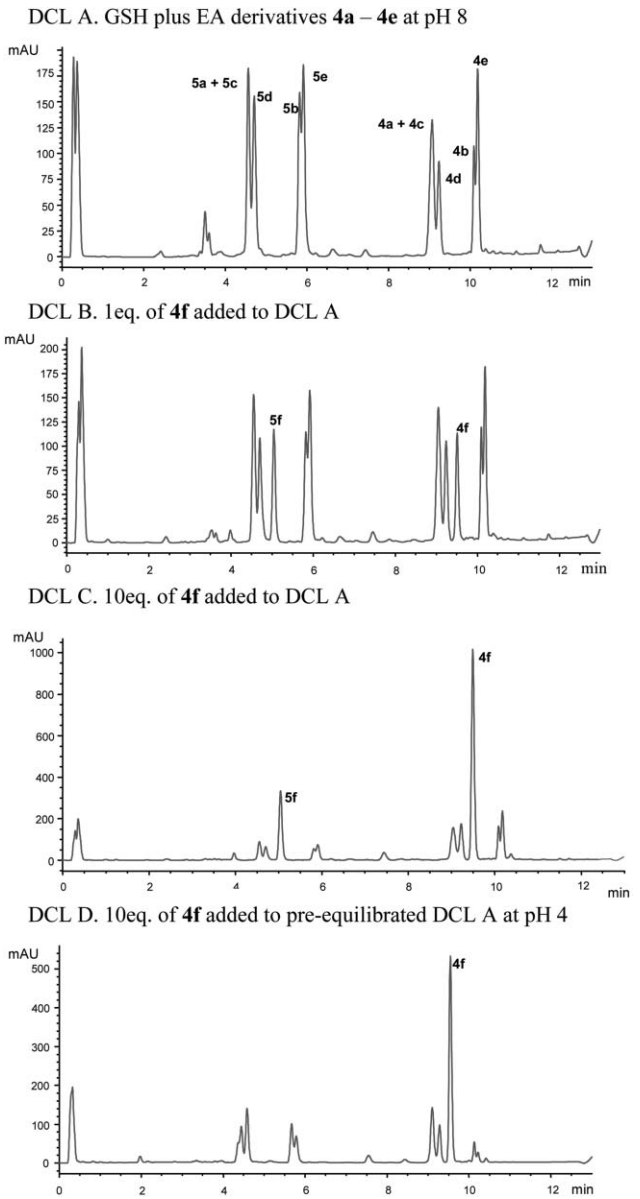

Fig. 1 HPLC traces of DCLs.

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14 The amides **5a**–**9a** were synthesised through EDCI coupling of commercially available EA to the appropriate amine.
15 Each adduct is a mixture of diastereoisomers due to the newly formed stereocentre α to the ketone in the Michael acceptors. Adduct **5a** was synthesised separately and shown to be a 1 : 1 diastereomeric mixture (see ESI†). Resolution of each diastereoisomer could not be achieved on the LC-column.

