Covalent double level dynamic combinatorial libraries: selectively addressable exchange processes†

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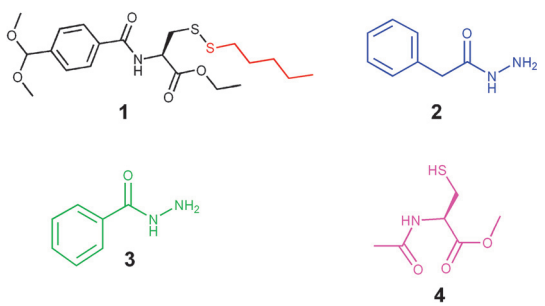
Hydrazones and disulfides have been combined in one dynamic system: hydrazones were exchanged by acid catalysis in the presence of disulfide and a thiol group without interference; neutralization of the reaction medium turns off the exchange of hydrazones and, at the same time, activates thiolate–disulfide exchange.

Dynamic combinatorial libraries (DCLs)[1] are mixtures of compounds that interconvert as part of an equilibrium that can be shifted by molecular recognition of a specific template molecule "ideally" toward the library member that is best bound. These libraries have become a useful source of new receptors, ligands for biomacromolecules, and catalysts.[2]

A key issue in designing dynamic combinatorial libraries is the choice of a reversible chemical reaction to interconvert the library components.[3] Ideally, a rapid reversible reaction is required that proceeds under mild conditions and that is tolerant of a wide range of functional groups and recognition events. Furthermore, it should be possible to turn the reaction off in order to isolate and handle selected members of the library individually. Within the various reactions tested to date by different research groups, two of the most frequently used are thiol/disulfide exchange and hydrazide/hydrazone exchange.

The combination of different reversible reactions in one dynamic system can enhance the level of diversity achieved. Two types of multilevel dynamic libraries have been described to date: "orthogonal", when the reversible processes operate independently, and "communicating", when the processes cross over. Eliseev, Lehn *et al.* have reported the use of double-level "orthogonal" libraries where hydrazone exchange and ligand exchange around a cobalt ion can be addressed independently.[4] Otto, Sanders *et al.* reported that structural diversity within a DCL can be expanded by maintaining two exchange processes simultaneously generating a double-level "communicating" library based on exchange of disulfide and thioester linkages.[5] In this work, we report the first example of the combination of two selectively addressable reversible reactions that can be alternated for the preparation of covalently assembled double level dynamic combinatorial libraries.



Scheme 1 Mono- and bifunctionalized building blocks used to evaluate the exchange processes.

It has been reported that the exchange of hydrazones in chlorinated solvents can be achieved by acid catalysis with trifluoroacetic acid (TFA)[6] whereas disulfide exchange proceeds smoothly in the presence of organic bases like triethylamine (TEA).[7,8]

In order to evaluate the compatibility of both exchange processes several building blocks were prepared incorporating either one disulfide bond and one protected aldehyde group (**1**), one hydrazide group (**2** and **3**), or one thiol group (**4**) (Scheme 1).

Initially we studied the effect of increasing amounts of TEA on the exchange of hydrazones in chloroform solutions acidified with 15 equivalents of TFA with respect to the concentration of hydrazones. The general procedure for the exchange experiments entailed dissolution of the building blocks **1** and **2** (5 mM) in $CHCl_3$ containing TFA (75 mM). The reaction was stirred at room temperature for 24 h. TEA (an appropriate amount) followed by one equivalent of building block **3** were added and the reaction was kept stirring at room temperature. The exchange of hydrazide **3** and hydrazone **5** can be detected by HPLC‡ after 24 h of reaction only if there is an excess of TFA (Fig. 1). When the amount of TEA equals or surpasses the amount of TFA, formation of the hydrazone **6** is not detected by HPLC.

The effect of increasing amounts of TEA on the exchange of disulfide **5** and thiol **4** was also studied with a similar experimental setup. The general procedure involved dissolution of the building blocks **1** and **2** (5 mM) in $CHCl_3$ containing TFA (75 mM). The reaction was stirred at room temperature for 24 h. TEA (an appropriate amount) followed by one equivalent of building block **4** were added and the reaction was kept stirring at room temperature. In this case the formation of

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† Electronic supplementary information (ESI) available: Materials and experimental procedures; LC data for preparation of libraries starting with disulfide exchange followed by hydrazone exchange and *vice versa*. See DOI: 10.1039/b808565j

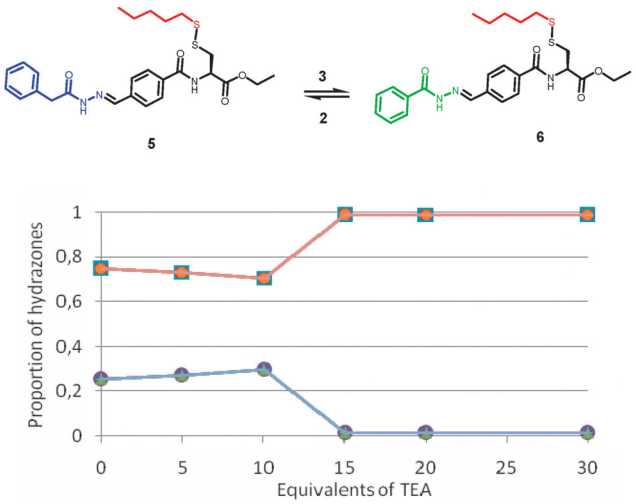

Fig. 1 Proportion of **5** (■) and **6** (●) after 24 h of reaction in chloroform in the presence of 15 equivalents of TFA and different amounts of TEA.

products resulting from the exchange is observed after 24 h either when TEA is in excess or when the amount of TEA equals the amount of TFA (Fig. 2).

The results suggest that each of these exchange processes can be activated whereas the other is inactive by changing the relative amounts of TFA and TEA. This was proved by adding hydrazide **3** and thiol **4** to a series of chloroform solutions of **5** containing 15 equivalents of TFA plus different amounts of TEA. The general procedure involved dissolution of the building blocks **1** and **2** (5 mM) in $CHCl_3$ containing TFA (75 mM, 15 equivalents). The reaction was stirred at room

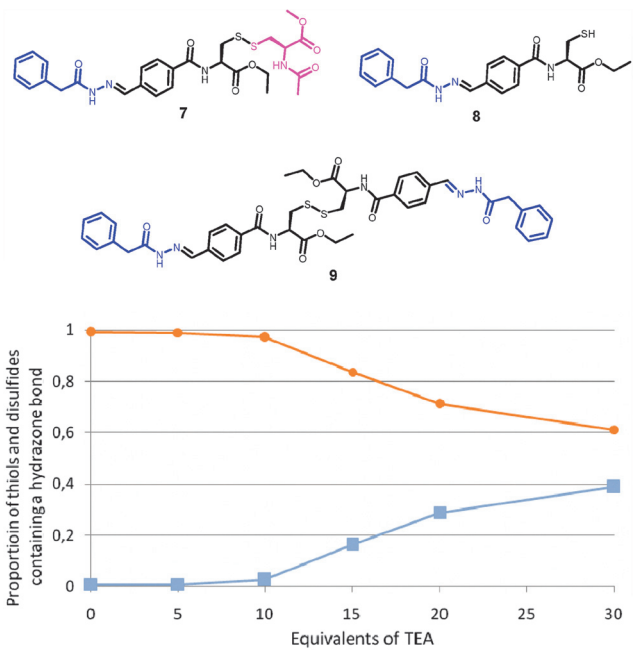

Fig. 2 Proportion of **5** (●), and proportion of the sum of products of disulfide exchange **7–9** containing hydrazones (■) after 24 h of reaction in chloroform in the presence of 15 equivalents of TFA and different amounts of TEA. Compound **4** and its dimer are not detected by the analytical system.

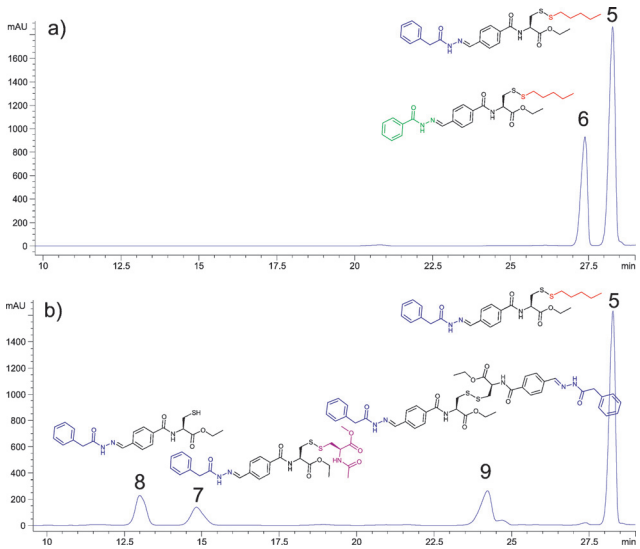

Fig. 3 HPLC traces recorded at $\lambda = 290$ nm for the mixture prepared from **5**, **3** and **4** (a) in the presence of 15 equivalents of TFA and 5 equivalents of TEA, and (b) in the presence of 15 equivalents of TFA and 20 equivalents of TEA.

temperature for 24 h. TEA (an appropriate amount) followed by one equivalent of building blocks **3** and **4** were added and the reaction was kept stirring at room temperature. As expected, in the presence of an excess of 10 or more equivalents of TFA only products of hydrazone exchange are observed after 24 h of reaction (Fig. 3a), whereas in the presence of excess of TEA, only the products of disulfide exchange are observed (Fig. 3b).

When the ratio TFA : TEA is in the range 10 : 15 to 15 : 15, both exchange processes are activated, however they are so slow that the amount of library members that are products of both exchange processes cannot be detected by HPLC after 24 h of reaction. Four days after the reaction was started they still represent less than 1.5% of the library. Consequently, in practice the exchange processes are not completely independent since the conditions that are necessary to produce hydrazones at a convenient rate (high proportion of TFA) affect the thiol/disulfide exchange, whereas under the conditions at which disulfide exchange is fast enough, hydrazones do not exchange. These results show that two selectively addressable dynamic chemistries can be combined for the preparation of fully covalent double level dynamic combinatorial libraries from building blocks appropriately functionalized to participate in both exchange processes. This process can be carried out in both directions: disulfide exchange followed by hydrazone exchange and *vice versa* (see Fig. S1 in ESI†).

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Notes and references

‡ HPLC analysis was carried out using a Hewlett-Packard 1050 instrument, coupled to a HP 1050 DAD; data were analysed using

HP ChemStation. Reverse phase HPLC separations were carried out using a 25 cm, 4.6 mm i.d. 5 μm particle size, symmetry column using acetonitrile and water gradients.

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