Simultaneous and Orthogonal Covalent Exchange Processes in Dynamic Combinatorial Libraries

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Dynamic combinatorial libraries (DCLs) are constituted by members that are reversibly assembled from a set of building blocks.[1,2] The functional groups selected to generate the reversible connections between building blocks will affect the conditions required for activation as well as the formation kinetics of the library members (i.e., the velocity at which the DCL responds to external perturbations). Some of the most successful reversible covalent reactions used for the preparation of DCLs are hydrazone,[3] disulfide,[4] and thioester exchange.[5]

Although most of the DCLs reported to date have been prepared using one reversible exchange process, there are some examples where two exchange reactions are combined in the same system. Depending on the relative functional state of the exchange processes (activated/inactivated), the system can be simultaneous or orthogonal. In simultaneous DCLs,[6,7] both exchange reactions occur at the same time, as observed in the dynamic system based in thioesters and disulfides reported by Otto et al.[6] In orthogonal DCLs,[8] each reversible reaction used can be activated under different conditions. Such setup allows the manipulation of the reactions in a sequential order as described for covalent dynamic systems wherein hydrazone exchange and disulfide exchange were combined in aqueous[9] or in organic media.[10] When orthogonal exchange processes are employed, each reversible reaction can be used to explore independently its own dimension in structural space opening the possibility for evolutionary approaches.[1a]

Each of these strategies increase the DCLs potential molecular diversity in a different manner. In a simultaneous approach, all possible molecular combinations resulting from the different exchange processes are produced in a single step. The whole library molecular space is covered simultaneously. Differently, molecular generation in each step of an orthogonal approach covers a reduced molecular space, allowing optimization of the search in the vicinity of known good targets.

Thus, the selection of one strategy (simultaneous/orthogonal) is a trade-off between exploration and optimization: exploration of a vast space can lead to inefficient search,[11] whereas focusing the search in a limited molecular space can lead to an important loss in the explored diversity, trapping the composition in a local optimum.

In this work, we report the first example of a mixed strategy involving simultaneous and orthogonal covalent reactions in one single system through the combination of the exchange of disulfides, thioesters, and hydrazones. As a consequence of altering the activation order of the sequentially addressable processes, biased DCLs are obtained where the history of each DCL is manifested as distinct distribution of products.

It has been reported that the exchange of hydrazones in chlorinated solvents can be achieved by acid catalysis with trifluoroacetic acid (TFA),[12] whereas disulfide exchange and thioester exchange proceed in the presence of organic bases such as triethylamine (TEA).[13,14]

The compatibility of these three functional groups and their corresponding exchange processes were evaluated with building blocks incorporating either one disulfide bond and one protected aldehyde group (**1**), one hydrazide group (**2** and **3**), one thioester (**4**), and one thiol group (**5**) (Figure 1).

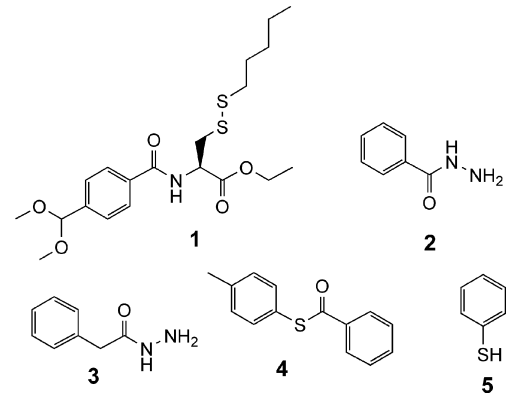


Figure 1

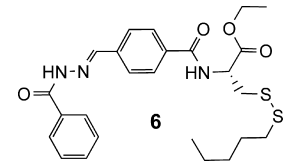
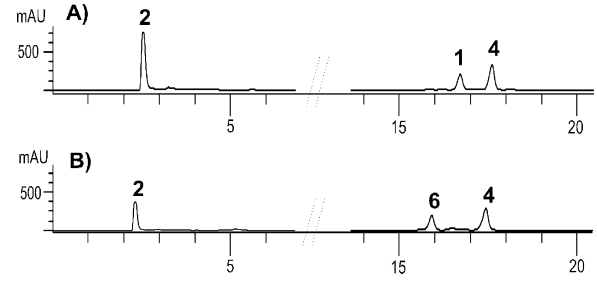


Figure 2

Scheme 1[a]



[a] HPLC traces recorded at λ =250 nm of solutions of **1**, **2**, and **4** stirred for 24 h: (A) without acid and (B) with 20 equiv of TFA with respect to **1**.

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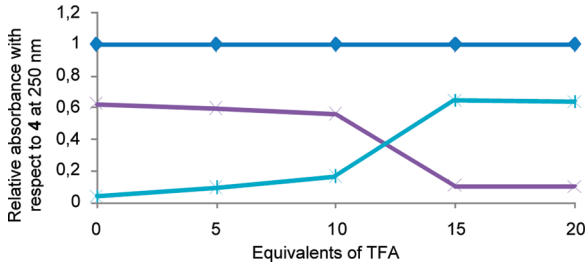
Scheme 2[a]



[a] Proportion of **1** (×), **4** (♦), and **6** (∗) with respect to **4** after 24 h of reaction in chloroform in the range of 0−20 equiv of TFA.

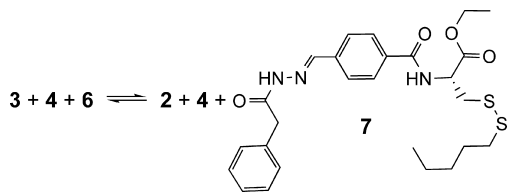
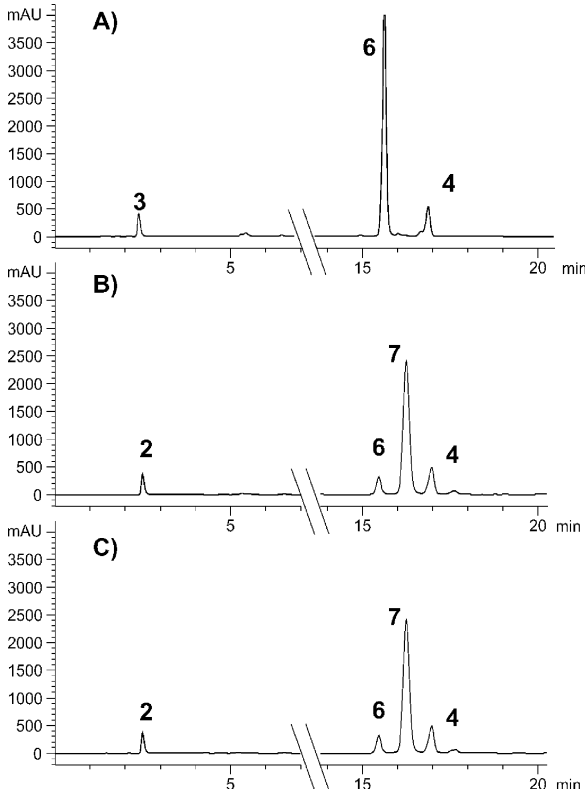


Figure 3

Since hydrazine can react with disulfide bonds[15] and thioesters,[16] we first studied the stability of thioesters and disulfides toward the reagents and conditions required for hydrazone formation: protected aldehyde and hydrazide in acid medium. A series of chloroform solutions of **1** (5 mM), **2** (10 mM), and **4** (5 mM) were mixed with increasing

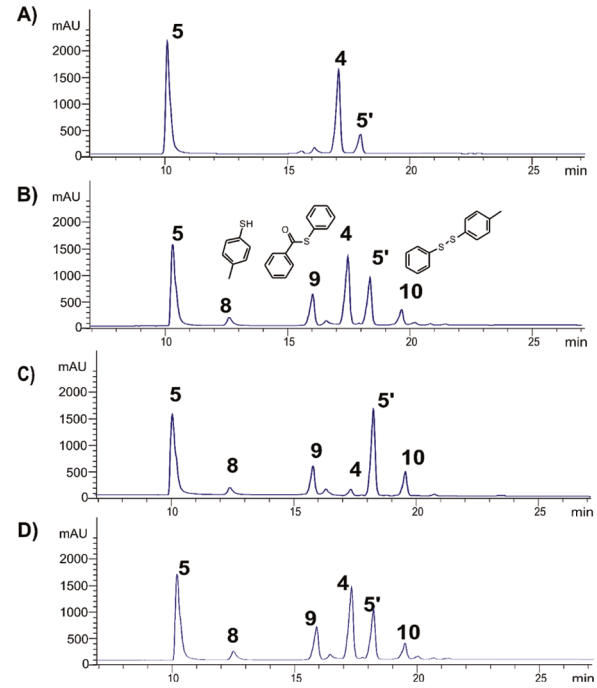
Scheme 3[a]



[a] Exchange reaction of hydrazones. HPLC traces recorded at λ =290 nm for the exchange of **6** and **7**, in the presence of **4** at 0 h (A), 24 h (B), and 48 h (C).

amounts of TFA (0−150 mM) and the formation of hydrazone **6** (Figure 2) was followed by HPLC-UV. In all cases,

Scheme 4[a]



[a] HPLC traces recorded after 24 h of reaction at λ =250 nm for the mixture prepared from **4**, **5**, and **5′** in chloroform without base (A) and with 5 equiv of piperidine (B) and TEA (C). HPLC trace recorded 48 h after the addition of piperidine (D).

the only product detected after 24 h of reaction was the expected hydrazone (Scheme 1).

The bystander thioester **4** and the disulfide bond in compounds **1** and **6** remained unaffected by either the different TFA concentrations or by the presence of starting material and reaction products (Scheme 2). Addition of 15 equiv of TFA was enough to complete the reaction in 24 h.

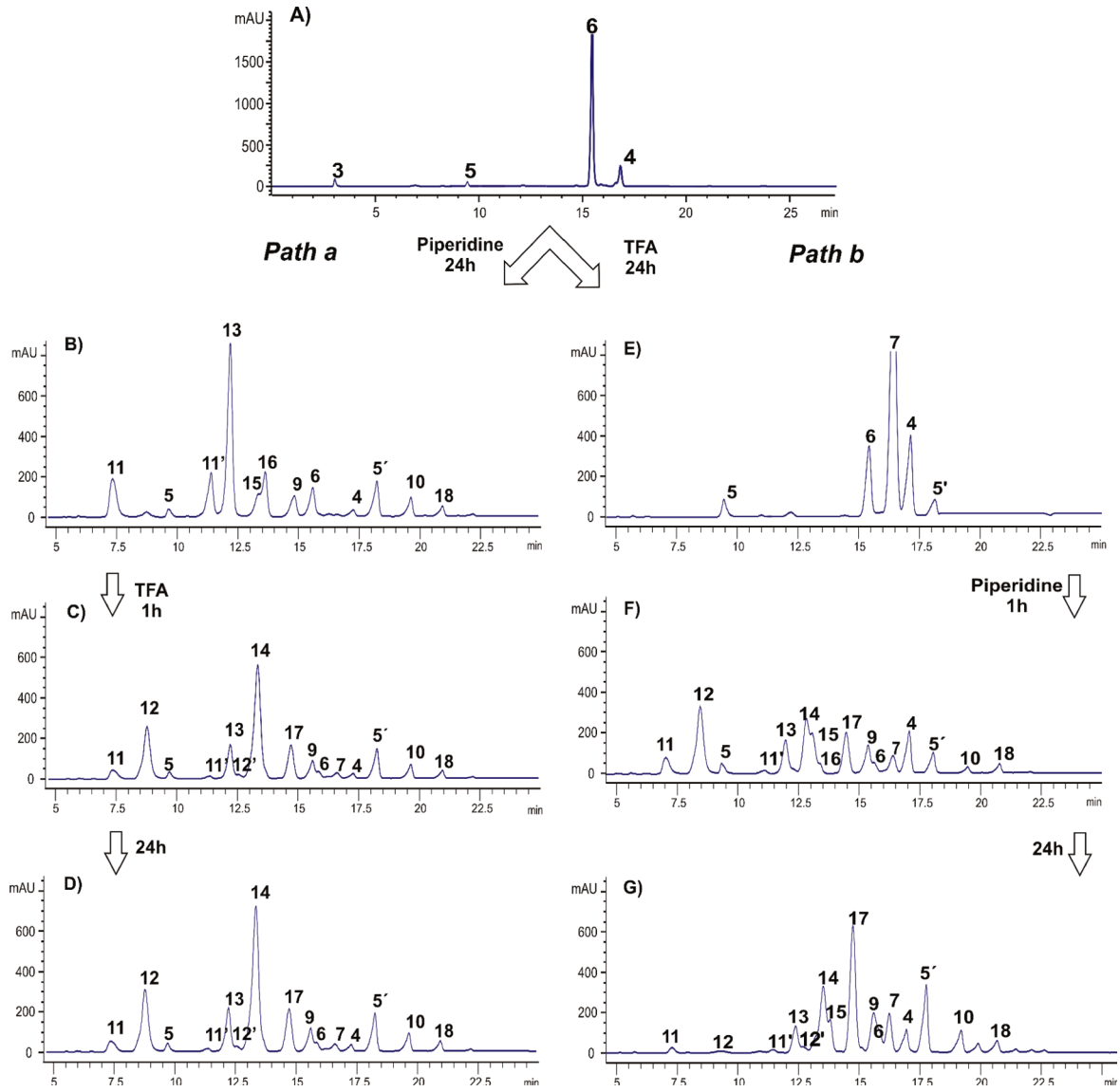
Hydrazone exchange in the presence of disulfide and thioester groups was studied with mixtures of compounds **3**, **4**, and **6** and 5 equiv of TFA in chloroform (Figure 3). The only new product observed by HPLC analysis at 24 h was compound **7** (Scheme 3A and B). The products distribution did not change between 24 and 48 h (Scheme 3B and C).[17]

These results show that the hydrazones can be efficiently generated and exchanged with TFA without affecting the integrity of disulfides and thioesters.

To study the exchange of disulfide and thioester linkages in chloroform, we mixed thioester **4** and thiophenol **5** (containing a small amount of its oxidation product, diphenyl disulfide **5′**) (see Scheme 4A), with 5 equiv of pyridine, piperidine, or triethylamine (TEA) under N_2 atmosphere.[18] Only piperidine and TEA generated the appropriate medium for the simultaneous exchange of thiol/disulfide and thiol/thioester. At 24 h of reaction the new library members **8**, **9**, and **10** were clearly detected in the HPLC-UV trace apart from the initially added **4**, **5**, and **5′** (Scheme 4B). Only the library prepared with piperidine reached a composition that stayed constant at this time (compare Scheme 4B and D).[19]

According to these results, hydrazones can be exchanged in acid medium (5 equiv of TFA) in presence of thioesters and disulfides without interferences. In addition, both thiol/

Scheme 5[a]



[a] HPLC traces recorded at 290 nm of a mixture of **3**, **4**, **5**, and **6** in chloroform (A). Path a: thiol/disulfide/thioester exchange based DCL at 24 h after the addition of piperidine (B), orthogonal hydrazone exchange based DCL at 1 h (C) or 24 h (D) of the addition of an excess of TFA. Path b: hydrazone exchange based DCL at 24 h of the addition of TFA (E), orthogonal activation of thiol/disulfide/thioester exchange at 1 h (F) or 24 h (G) of the addition of an excess of piperidine. The structure of compounds **8**−**18** is shown in Figure S2, Supporting Information.

disulfide exchange and thiol/thioester exchange can be active in the presence of 5 equiv of piperidine. Under such conditions, hydrazones do not exchange.

The alternate use of these two sets of conditions was applied to produce a dynamic system that combines the three exchange processes. One chloroform solution of hydrazide **3**, thioester **4**, thiophenol **5**, and hydrazone **6** (1:1:4:1)[20] was stirred under N_2 atmosphere (Scheme 5A) with 5 equiv of piperidine for 24 h. Subsequently, the mixture was neutralized with TFA (5 equiv), acidified with additional TFA (5 equiv), and the solution was stirred for another 24 h (Scheme 5, path a). Alternatively, the same starting solution was first stirred during 24 h in the presence of 5 equiv of TFA and another 24 h in the presence of 5 equiv of excess of piperidine (Scheme 5, path b).

As expected, only reaction products of the communicating disulfide and thioester exchange processes were present at 24 h following path a (Scheme 5B). At this time, both reversible reactions were stopped with TFA, initiating in that way the exchange of hydrazones as an orthogonal covalent process. At least six new products, resulting from this second level of exchange, could be detected in the chromatograms after 1 h of reaction (Scheme 5C). The system reached a constant composition after 24 h (Scheme 5D).

When the order of addition of reagents (TFA/piperidine) was inverted (path b), only hydrazone exchange products were detected after 24 h of stirring with 5 equiv of TFA (Scheme 5E), and a full assortment of products of hydrazone exchange, disulfide exchange, and thioester exchange were detected at the end of the process (24 h, 5 equiv TFA plus 24 h with an excess of 5 equiv of piperidine; Scheme 5G). Interestingly, the final composition of the DCLs constructed by following each chemical path is different (Schemes 5D and G), opening a gate to evolutionary research in equilibrium state systems, wherein thermodynamically, not kinetically, favored entities prevail.[21]

Different final composition of the DCLs prepared following path a or path b could be expected when (i) the stability of interconverting hydrazones is affected by the disulfide or the thioester partner, (ii) the stability of the disulfides and thioesters is affected by the hydrazone partner, (iii) or when differences in the environment are introduced along the path. In our experiments, the concentration of piperidinium trifluoroacetate increases along both paths. Since the exchange processes are activated in different order, the amount of salt present when thiol/thioester/disulfide exchange is active in path a is different than the amount of salt present when the same exchange process is active in path b, and the same occurs for hydrazone exchange. Such environmental change could be responsible, at least in part, for the differences observed in the final library composition.

In general, the order of the sequence that produces the best result will depend on each system and on each function assigned to that system. At a glimpse, it seems convenient to generate first a small but diverse portion of the molecular space in a controlled manner.[22] This molecular space portion could be accessible with an orthogonal approach, wherein the molecular diversity generated in each step could be regulated through the use of one or more simultaneous exchange processes.

These results show that three covalent reversible connections can be combined for the preparation of fully covalent dynamic combinatorial libraries from building blocks appropriately functionalized to participate in the three exchange reactions.

Although the history of a DCL has been employed in the detection of analytes in systems with one exchange reaction,[23] standard DCLs are not proper systems for evolutionary studies because of the lack of unidirectional reactions that ensure the persistence of structures that are to be involved in change, adaptation, and reproduction/amplification. Such evolutionary approaches can be investigated using multiple orthogonal exchange processes where each exchange process allows the exploration of its own dimension in structural space. In that way, changes in structure are constrained since a minimum of the original target is preserved. In addition, as it is shown here for a particular orthogonal layer of diversity, different communicating chemistries can be combined to increase the number of accessible topologies, connectivities, and as a consequence, structures.

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Supporting Information Available. Detailed experimental procedures and spectroscopic data of unreported compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) In all the experiments the equilibrium was assumed through the confirmation that the library composition stayed constant during 24 h.

(18) Although the reactions were carried under N_2 atmosphere, some oxygen was initially present in the system leading to partial oxidation of thiols.

(19) Apparent partial decomposition of thioester **4** was observed at 24 h of reaction in the experiments with TEA. No precipitation was observed in this experiment.

(20) Two equivalents of thiophenol (**5**) were added for each equivalent of disulfide **6**, and another two equivalents were added for each equivalent of thioester **4**. This relative amount was used to ensure the presence of nucleophile during the entire process despite the partial oxidation of thiophenol in previous experiments.

(21) Since natural evolution is a process operating far from the equilibrium state, most of chemical evolutionary research has been conducted in kinetics-driven systems. Examples of chemical "irreversible" evolution: (a) Robertson, A.; Sinclair, A. J.; Philp, D. *Chem. Soc. Rev.* **2000**, *29*, 141–152. (b) Patzke, V.; von Kiedrowski, G. *ARKIVOC* **2007**, *338*, 293–310. The implementation of adaptation-selection cycles in DCLs would allow unveiling the evolution of chemical entities in an unexplored direction.

(22) In an analogy with the diversity oriented synthesis (DOS) strategy. In this sense, a compromise between target and diversity oriented synthesis is needed to be considered in DCLs. For a review about DOS, see: Burke, M. D.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 46–58.

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