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# On double-level orthogonal dynamic combinatorial libraries: Compatibility of hydrazone and disulfide bridge exchange

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## Introduction

Dynamic Combinatorial Chemistry (DCQ) (Lehn and Eliseev, 2001) is a novel strategy with potential applications for the discovery of interesting new molecules such as catalysts (Brady *et al.*, 1997), receptors (Timmerman and Reinhoudt, 1999) or drugs (Otto *et al.*, 2002). Unlike traditional combinatorial libraries, whose composition is static, in a dynamic combinatorial library library constituents are constantly interconverting because they are assembled through reversible reactions. Under some conditions, the mixture composition is under thermodynamic control, so that the concentration of each library constituent depends on its free energy. As a consequence, such libraries have the ability to respond to influences that affect the relative constituent stability.

A key aspect in the preparation of Dynamic Combinatorial Libraries (DCLs) is the choice of a suitable reversible reaction to assemble library constituents from *building blocks*. Several reversible reactions have been studied with this aim, being two of the most popular, the exchange of disulfide bridges (Otto *et al.*, 2000; Ramstrom and Lehn, 2000), and the exchange of hydrazones (Cousins *et al.*, 1999; Bunyapaiboonsri *et al.*, 2001).

Mostly, dynamic libraries prepared so far have been assembled by a single reversible reaction. There is an example of the use of two not orthogonal covalent reactions for a library preparation (Leclaire *et al.*, 2005).

On the other hand, there is a report of a library preparation using two orthogonal reactions, but one of them was not covalent with the limitations that this implied (Goral *et al.*, 2001). In the present work, preliminary results are described aimed to studying the compatibility between the exchange of hydrazones, and the exchange of disulfide bridges for the preparation of double-level dynamic libraries.

## Methodology

For library preparation of macrocyclic hydrazones, a bifunctional *building block* containing a hydrazide group and a protected aldehyde as dimethoxyacetal was used. The formation of the hydrazones leads to the formation of macrocycles, while the exchange of hydrazones produces their interconversion. The *building block* was synthesized by coupling CBZ-*L*-proline to phenylalanine-methyl-ester using DDC and DMAP. After deprotection (hydrogenation) and a second coupling with 4-carboxybenzaldehyde dimethoxyacetal (pDMA), the hydrazynolysis of methyl ester group was carried out in order to introduce the hydrazide group and generate ***pPFm*** bifunctional monomer (Fig. 1).

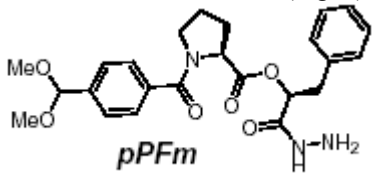


**Figure 1.**

Libraries were prepared from it by activating initially the reaction of formation and exchange of hydrazones in the presence of thiophenol and *N*-acetylcysteine-methyl-ester. This reaction was carried out in chlorinated solvents using TFA as a catalyst (Simpson *et al.*, 2000). Deactivation of this reaction was achieved by TEA addition, thus giving rise to the formation of a disulfide bridge (Kieran *et al.*, 2003). The mixture of formed macrocycles and other compounds was analysed by reversed-phase HPLC using a UV detector (Furlán *et al.*, 2000).

## Results

The acid-catalyzed cyclization primarily gave rise to mixtures of oligomeric macrocycles composed of a different number

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of monomers (dimer, trimer, tetramer, etc.), whose retention times ($t_R$) were very different from that of thiophenol, as shown in Fig. 2. Both control library (macrocycles made by ***pPFm***), and the library that also contained thiophenol and *N*-acetylcysteine-methyl-ester showed that the distribution as the macrocycle concentration was the same in both libraries,

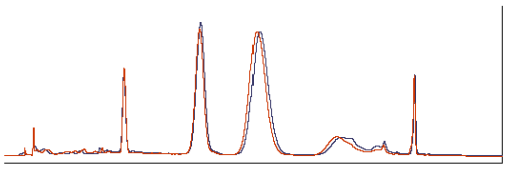

**Figure 2**

indicating that the presence of thiols affects neither the formation nor the exchange of hydrazones. Since the exchange of hydrazones only occurs under acidic conditions, neutralizing the reaction medium by treatment with a base was able to turn dynamic library in a static mixture of stable compounds (Furlán *et al.*, 2000).

Under neutral or slightly basic conditions, the oxidation of thiols is produced, leading to the formation of dimers containing disulfide bridges (Kieran *et al.*, 2003). After TEA addition, a new peak was observed in the library chromatogram that contained thiophenol and *N*-acetylcysteine-methyl-ester, with a consequent decrease in thiophenol concentration, without being modified neither the distribution nor the concentration of the other library constituents (Fig. 3).

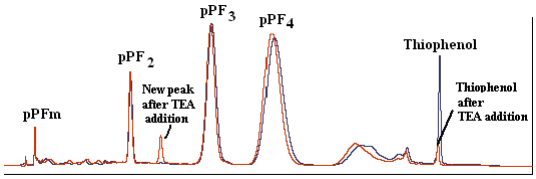


**Figure 3.**

**Conclusions**

According to these results we can draw the following conclusions: ♦ the formation and exchange of hydrazones are compatible with the presence of thiols ♦ the formation of disulfide bridges, and their exchange would take place without modifying the hydrazones ♦ the exchange of hydrazones can be activated in acid medium, and stopped in neutral medium in the presence of thiols ♦ the exchange of disulfide bridges can be activated in a slightly alkaline medium in the presence of hydrazones, and can be stopped by oxidation with environmental oxygen.

This suggests that the preparation of dynamic libraries is possible from *building blocks* that are properly functionalized in order to take part in the exchange of disulfide bridges as well as hydrazones. Therefore, the library would acquire two orthogonal exchange levels. On one hand the adaptability could be measured by interchanging constituents by exchange of hydrazones in an acid medium, which would keep "off" the exchange of disulfide bridges. In a later step, hydrazone interchange could be deactivated by neutralization of the reaction medium, which would in turn trigger the formation and the exchange of disulfide bridges. This exchange could in turn: (a) be blocked by complete thiol groups oxidation to disulfide bridges, keeping blocked the exchange of hydrazones, or (b) be blocked, and reactivate the exchange of hydrazones by addition of an acid medium.

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