Dynamic Chemistry

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# Dynamic Combinatorial Resolution: Direct Asymmetric Lipase-Mediated Screening of a Dynamic Nitroaldol Library**

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Dynamic combinatorial chemistry (DCC) has become established as a powerful approach to efficiently identify ligands and inhibitors for receptors and enzymes, as well as hosts for various ligands.[1] The concept is based on reversible interconnections, either covalent or noncovalent, between different library components, resulting in compound libraries with dynamic composition. The process allows for the spontaneous generation of all possible combinations of the components and thus enables efficient one-step generation of extended dynamic combinatorial libraries (DCLs) under thermodynamic control.

The adaptive nature of DCC systems, whereby the DCLs reconstitute themselves to adopt the best overall arrangement,[2,3] make them especially interesting for coupled secondary processes. Until now, most DCLs have been subjected to subsequent affinity events, in which specific library constituents are selected and their relative concentrations amplified. However, we have been more interested in kinetically controlled secondary processes.[4,5] If, for example, the bound species is transformed in an irreversible process and expelled from the binding site, the site is free to host more of the DCL constituent, and the reactions of the dynamic system are thus forced to proceed to completion. An additional advantage with this approach is that in principle only catalytic amounts of the selector need to be present. The combined events amount to a situation in which the selector directs the kinetic resolution of the library in a dynamic combinatorial resolution (DCR) process (Figure 1).

An important challenge with DCC is also the development of efficient reversible reactions. Until now, transimination, transacylation, alkene metathesis, and thiol–disulfide exchange have mainly been used to form DCLs, as these processes have proven the most efficient in the systems studied.[1] New methods are needed, however, to advance the technique, and new reaction types are necessary for the rapid generation and screening of sufficiently stable DCLs. For example, with the exception of the powerful alkene-metathesis reaction, C–C bond formation has only been explored in a few systems.[6–8] Carbon–carbon bond-forming reactions are essential in synthetic organic chemistry, and particularly methods for the synthesis of optically pure compounds are of fundamental importance.

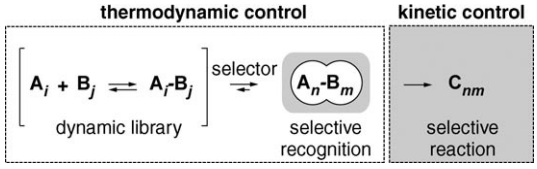


***Figure 1.*** The concept of DCR. A dynamic library is formed from *i* components **A** and *j* components **B**. Selective recognition of a specific constituent $\mathbf{A}_n$–$\mathbf{B}_m$ by a selector (e.g., an enzyme) enables the formation of a specific product $\mathbf{C}_{nm}$ under kinetic control.

These challenges prompted us to explore the potential of DCLs generated from C–C bond-forming reactions under mild conditions that are both compatible with selector entities and applicable to a DCR process. During these studies, we came across the nitroaldol (Henry) reaction,[9–12] in which β-nitroalcohols are formed by the addition of nitroalkanes to carbonyl compounds. This powerful C–C bond-forming reaction is a useful synthetic tool for the construction of complex molecules. Herein, we describe the generation of dynamic combinatorial libraries based on the nitroaldol reaction carried out under mild conditions. The stereogenic carbon center created in the reaction could also be resolved through a coupled process in the form of a kinetically controlled lipase-mediated acylation. Asymmetric amplification of specific nitroaldol adducts could in this case be efficiently achieved in a one-pot process.

The conditions of the nitroaldol reaction had first to be optimized for its use in a DCR process. Several bases were screened initially to find suitable conditions for DCL generation. In preliminary studies, the reactions were conducted in the presence of 1 equivalent each of the nitroalkane, the aldehyde, and the base, and the reactions were monitored by $^1$H NMR spectroscopy by comparing the signals of the aldehyde with those of the nitroalcohol adduct. Triethylamine proved optimal for use in this system as a result of the rapid and stable equilibration observed and, very importantly, the high compatibility of this base with the subsequent enzymatic reaction.

Next, DCL generation was addressed (Scheme 1). A dynamic set of ten nitroaldol adducts (each enantiomer of **1**–**6** to **5**–**6**) was generated from equimolar amounts of 4-trifluoromethylbenzaldehyde (**1**), 2-fluorobenzaldehyde (**2**), 3-nitrobenzaldehyde (**3**), 2-chlorobenzaldehyde (**4**), and 2,4-dichlorobenzaldehyde (**5**), together with 1 equivalent of 2-nitropropane (**6**).

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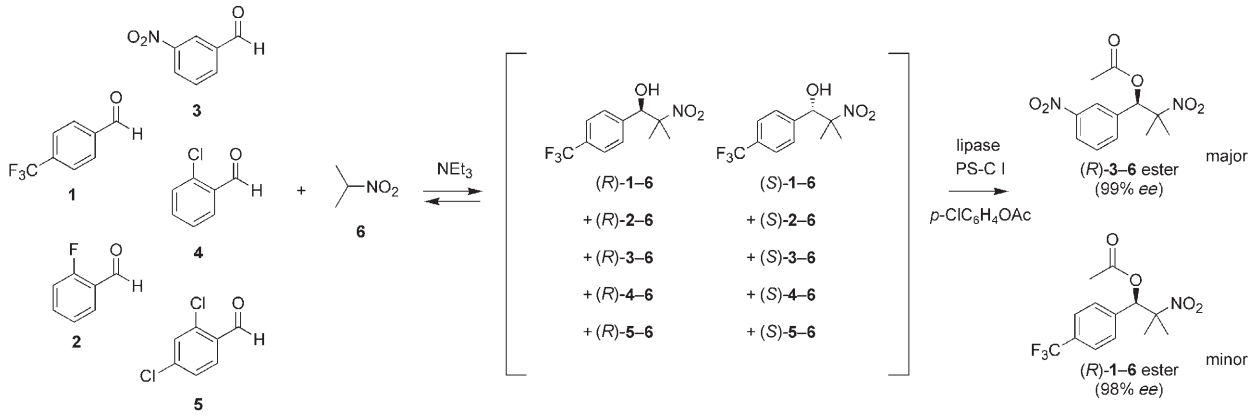
Supporting information for this article, including experimental methods, is available on the WWW under http://www.angewandte.org or from the author.




***Scheme 1.*** Generation of a dynamic nitroaldol library and lipase-mediated asymmetric resolution.

Owing to the slightly different molar ratio of the aldehydes to the nitroalkane (5:1) in the Henry reaction, the quantity of the base was increased to 10 equivalents to attain a reasonable equilibration. These benzaldehydes were chosen in view of their similar individual reactivity in the nitroaldol reaction, with the result of close to isoenergetic behavior in the DCL produced. Figure 2 shows the $^1$H NMR spectroscopic analysis of the creation and further reaction of β-nitroalcohol DCLs. The $^1$H NMR spectrum of the initial components before library generation is displayed in Figure 2a. The generation of the nitroaldol DCLs was initiated subsequently by the addition of triethylamine, and equilibration between the initial components and all ten chiral nitroaldol adducts were followed. In the absence of a selector, the library thus reached equilibrium within hours (Figure 2b).

One of the most efficient methods for the preparation of enantiomerically pure compounds is the kinetic resolution of racemic mixtures by enzymes, such as lipases.[13–15] In addition to their hydrolytic activity towards triglycerides, lipases catalyze (trans)esterification reactions and recognize a broad range of nonnatural substrates in both aqueous and non-aqueous media. Moreover, lipases are readily available commercially, do not require expensive cofactors, and are easily recoverable. These properties make this family of enzymes potentially very useful in a DCR system, and these enzymes were therefore tested in the present study. However, the lipase-catalyzed transesterification of β-nitroalcohol substrates has not been reported. The reaction conditions were optimized by initial screening of a series of enzymes followed by a series of acyl donors. On the basis of these results, the lipase PS-C I from *Pseudomonas cepacia* and *p*-chlorophenyl acetate[16] were selected as the lipase and the acyl donor, respectively, in the DCR system.

The lipase PS-C I was added together with *p*-chlorophenyl acetate (5 equiv) to the nitroaldol libraries at 40 °C without stirring. The transesterification of selected β-nitroalcohols resulted in the corresponding acetylated products, as shown by the $^1$H NMR spectra of the mixtures formed (Figure 2c,d). As can be seen from the spectra, two products from the DCL were resolved by the process.

The major product was found to be the **3**–**6** ester, produced from 3-nitrobenzaldehyde (**3**) and 2-nitropropane

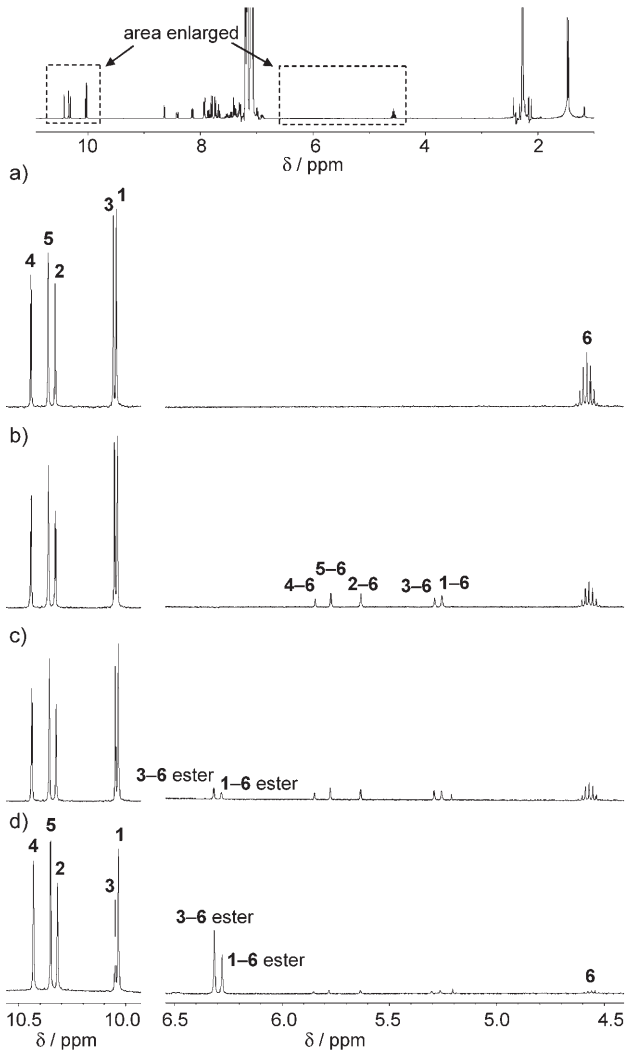



***Figure 2.*** $^1$H NMR spectra of the reaction mixture at various stages of the DCR process: a) before library generation; b) DCL in the absence of PS-C I and *p*-chlorophenyl acetate; c) DCL in the presence of PS-C I and *p*-chlorophenyl acetate ($t$ = 24 h); d) DCL in the presence of PS-C I and *p*-chlorophenyl acetate ($t$ = 14 days). Compound numbering as in Scheme 1.

(**6**). However, the relative concentration of the **3**–**6** nitroaldol adduct, the corresponding substrate for the lipase reaction, was among the lowest in the DCL in the absence of the enzyme. Nevertheless, **3**–**6** was the main compound selected as a substrate for the transesterification. A minor amount of the **1**–**6** ester, derived from 4-trifluoromethylbenzaldehyde (**1**) and 2-nitropropane (**6**), was also formed in the reaction. The two final products were obtained in a combined overall yield of 24% after 24 h, although the resolution of the two products could be identified earlier in the reaction. Better yields were observed after longer reaction times; the products were obtained in over 80% yield (**3**–**6** ester: 52%, **1**–**6** ester: 33%) after 14 days (Figure 2d). The reaction proceeded almost to completion (95% yield) in 20 days. The amplification was slightly improved when the reactions were performed at ambient temperature and when less enzyme was used; however, the time required also increased (65% yield after 14 days).

The nitroaldol–lipase DCR process does not only amplify specific β-nitroalcohol derivatives, but also leads to their asymmetric discrimination. HPLC analysis showed that the enantioselectivity of the process is very high: The *R* enantiomer of the **3**–**6** ester was resolved to 99% *ee*, and the *R* enantiomer of the **1**–**6** ester to 98% *ee*. The Mosher method was used to determine the absolute configuration of the products.[17,18]

In conclusion, we have identified the nitroaldol (Henry) reaction as a new and efficient C–C bond-forming route to DCL formation. Furthermore, we have demonstrated that primary DCLs under thermodynamic control can be coupled successfully to a secondary synthetic process mediated by a lipase under kinetic control. This dynamic combinatorial resolution process was used to generate a collection of potential enzyme substrates and to identify the best substrates for the lipase PS-C I from *Pseudomonas cepacia*. When the formation of a nitroaldol DCL was combined with lipase-mediated transesterification in a one-pot process, complete asymmetric resolution of the library occurred to produce enantiomerically pure β-nitroacetates in high yield.

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