Report

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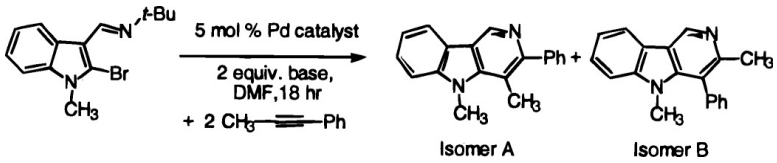

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Combinatorial Screening of Homogeneous Catalysis and Reaction Optimization Based on Multiplexed Capillary Electrophoresis

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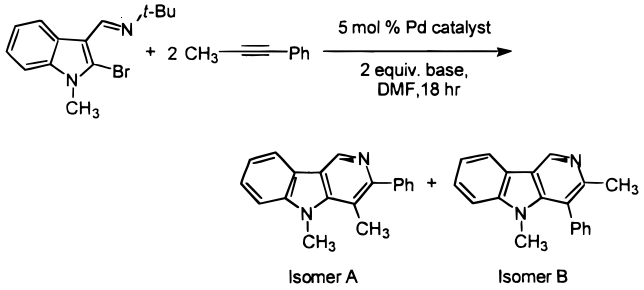
Combinatorial chemistry is revolutionizing the discovery of new drugs,[1] novel materials, and efficient catalysts[2] by scanning and testing vast numbers of possibilities. To fully realize the potential of combinatorial chemistry, general and powerful schemes for high-throughput screening (HTS) are essential.[3] Capillary array electrophoresis (CAE), a high-throughput technique driven by the Human Genome Project, has taken a key role in genomic analysis[4] and potentially will contribute to proteomics as well.[5] We report here the use of CAE for the rapid screening of a homogeneous catalytic reaction in a combinatorial manner. This approach has allowed the effective optimization of a homogeneously catalyzed synthetic organic reaction and the discovery of conditions that produce yields superior to those obtained previously by a less systematic approach.

So far there are several parallel assays for screening homogeneous catalysts. Modifications in UV absorption,[6] fluorescence,[7] color,[8] or temperature[9] induced by the catalytic reactions are indicators of catalytic activity. In these approaches, although the relative activity of the catalyst is determined quickly, quantitative information about the overall yield or the regioselectivity and stereoselectivity of the process has been difficult to obtain. It is also necessary that the products exhibit very different measurable properties compared to the solvent or the reagents. Most of the time, secondary screening is necessary. Mass spectrometry (MS),[10−12] which has also been widely used to screen catalysts, can provide selective detection. However, to address stereoselectivity, these procedures still tend to be laborious.[13] So far, MS is still a serial, rather than a parallel, approach although the analysis time is reasonably short. Application to the optimization of synthetic organic reactions will require the development of a high-throughput interface.

Separation-based techniques can solve the above problems. Serial methods, which include fast high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), have been used to analyze asymmetric catalysis[14] and alkylation reactions.[15] The throughput that can be achieved with these serial separation schemes is low even with special techniques, such as sequential sample injection[16] and sample multiplexing.[17] Multiplex HPLC is another interesting approach,[18] but achieving a high degree of multiplexing, such as 96 capillaries in capillary array electrophoresis (CAE), is not trivial. Thin-layer chromatography and gel electrophoresis, on the other hand, are difficult to completely automate.

Scheme 1



The uniqueness of CAE with absorption detection[19] is the easy adaptation to large numbers of samples and near-universal UV detection. Also, the injection volume is minimal (10−100 nL), and thus microreactors can be used to save reagent cost.

The model reaction we have used for demonstration of the unique capabilities of multiplexed CAE is a new palladium-catalyzed annulation reaction,[20] which readily affords γ-carbolines, noteworthy for their biological activity (Scheme 1). The optimal reaction conditions and the regiochemistry for this type of annulation are generally highly dependent on the nature of the palladium catalyst and the base employed. Previous efforts to optimize this process employed 5% $Pd(OAc)_2$, 10% PPh_3, and Na_2CO_3 as base and afforded a 1:1 ratio of isomers A/B in essentially a quantitative yield.

The nature of this and other catalytic reactions is that a lot of parameters can affect the yield, and "optimum" conditions are often found by trial and error. The general scale on which the above reaction has been run is 0.25 mmol in 5 mL of DMF. We have reduced the volume to 120 μL by using 6 mm o.d. glass tubes sealed at one end arranged in a 96-well format. The individual components were added as a DMF solution or as a slurry by pipetting. Septums were used to cap the reaction tubes to prevent evaporation. All reactions were thus run on a 5 μmol scale. Heating was provided by a dry heat bath kept at 110 °C. As an internal standard, 1 μmol of norharman was added to the reaction mixture. We did not observe any catalytic effect on the system from the addition of the norharman in control experiments. The CAE experiment is similar to what we have reported before for peptide mapping and for studying enzyme kinetics.[5] Here, organic-based buffers,[21] which are more appropriate for organic synthesis, must be used because of the low solubility of the products in water. They also make it possible to sample the reaction mixture without additional purification steps. Figure 1 shows the separation of the two isomeric forms of the product from the reagents and the internal standard using two different buffers. EtOH and pure DMF were also tested, but the separation was not acceptable. No bubbles were found in CAE, even when we used a low boiling point solvent, such as MeOH.

One important feature of the experimental protocol is that we injected the reaction mixture into CAE without diluting

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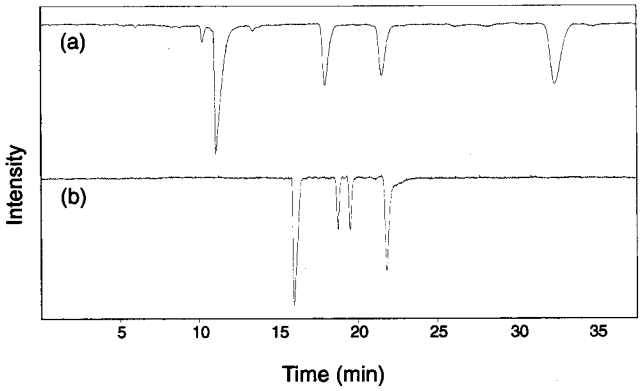

Figure 1. Separations of (in order) reactant, products (isomer A and B), and internal standard by two different solvents. Buffer, 40 mM NH_4OAc, 0.75% formic acid in (a) MeOH, (b) 80% DMF with 20% H_2O. Applied electric field, 140 V/cm. Column, bare fused-silica capillary with effective/total length of 50/75 cm and 50 μm i.d. Hydrodynamic injection, 15 s at 8 cm height.

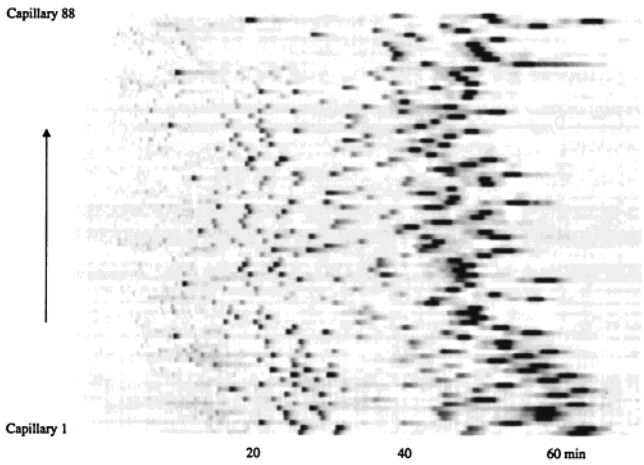

Figure 2. Result of CE separation of reaction mixtures in the 96-capillary array. Separation conditions are as listed in Figure 1b. Hydrodynamic injection, 1 min. The horizontal direction spans 88 capillaries and the vertical direction represents time. Another eight capillaries contain solvent only and are not plotted.

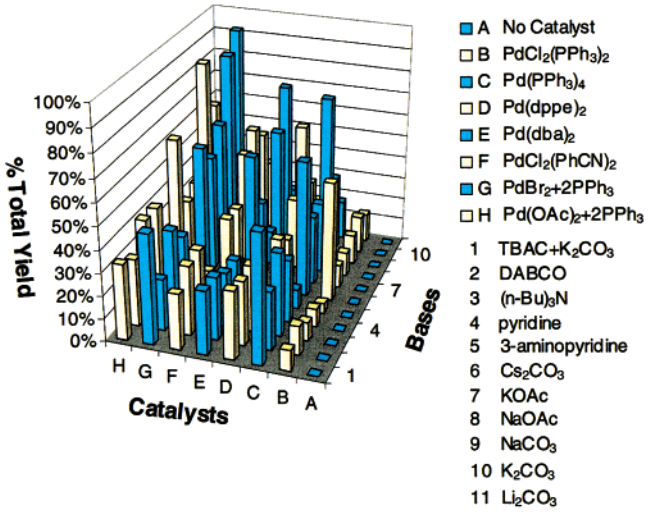

Figure 3. Total yield of the reaction after 17 h at 110 °C. dppe = bis(diphenylphosphino)ethane, TBAC = tetra-*n*-butylammonium chloride, DABCO = 1,4-diazabicyclo[2.2.2]octane. dba = *trans,trans*-dibenzylideneacetone.

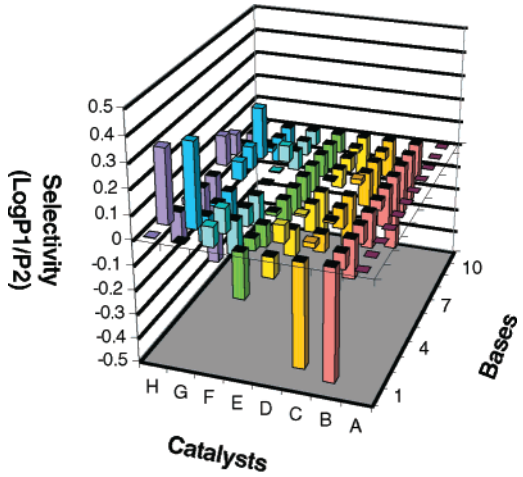

Figure 4. Selectivity plot of the two isomers produced in the reactions. P1/P2 is the ratio of the two isomers A and B, respectively.

or quenching before analysis. At predetermined times during the reaction, the reaction block was removed from the heating platform, quickly cooled, and put under the injection ends of the capillary array. No deleterious effect on the catalytic system was observed by this operation. By avoiding additional liquid manipulation (e.g., by first pipetting out of the reaction vials), we can reduce errors associated with transfer and contamination and reduce the reaction volume needed. We also noted that the CAE running buffer should be compatible with the reaction buffer for hydrodynamic injection. When using methanol as the buffer, injection was not uniform. Only about half of the 96 capillaries had adequate signal. It was not possible to increase the injection time, because some capillaries then became overloaded. When DMF-based buffer was used, all 96 channels had uniform signal over three consecutive runs. This buffer compatibility issue for CAE may be attributed to the differences in solution properties, such as viscosity and surface tension, and was not observed in single-capillary experiments. The total analysis time is typically 60 min, plus 30 min for capillary cleaning. Judging from the resolution in Figure 1, the capillaries could have been shortened to 25% of the effective length to provide analysis times of 15 min.

By choosing 8 different Pd catalysts and 11 different bases, 88 different combinations have been tested. Figure 2 shows such a 96-capillary separation. We can get information on the total yield (Figure 3), selectivity (Figure 4), and reaction kinetics (Figure 5) from the electropherograms. Some of the conditions have been tested previously.[20] Our results agree well with those. One example is that by using $Pd(OAc)_2$ with the ligand PPh_3 as catalyst and Na_2CO_3 as the base, a total yield of 84% was achieved with virtually no regioselectivity in the microreactor, compared with a quantitative conversion (90% after 17 h) with no selectivity under the protection of N_2 in a 5 mL reaction. Among all of the bases, inorganic bases proved to be more effective in promoting the reaction. When pyridine or other organic bases were used, the yield was low and some side products appeared. The ability to detect side products is clearly an advantage of CAE. Our preliminary results also reveal several new conditions which are quite effective in this annulation

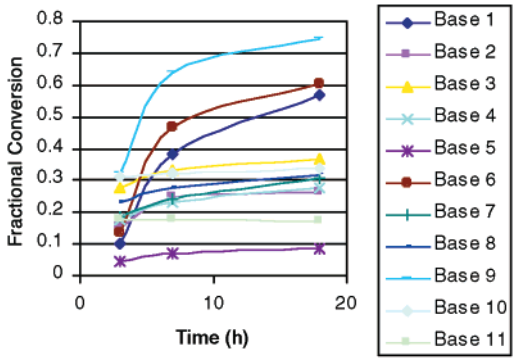

Figure 5. Kinetics of reactions using $Pd(PPh_3)_4$ as the catalyst.

reaction. They are $Pd(PPh_3)_4$ with Na_2CO_3 (C9, 74%), $Pd(dba)_2$ with K_2CO_3 (E10, 72%), $PdBr_2$ plus $2PPh_3$ with Na_2CO_3 (G9, 88%), and $PdBr_2$ plus $2PPh_3$ with K_2CO_3 (G10, 96%). The latter two are in fact superior to our previous best catalytic condition.[20] Complete regioselectivity is not observed in any of the test conditions (Figure 4), even though some prove to be better than other systems.[22] The conditions G2, H2, and B1 have some selectivity, but unfortunately their yields are low. Figure 5 shows one of the kinetics plots using $Pd(PPh_3)_4$ as the catalyst with various bases. There are significant differences in the rates and the shapes of the plots. This illustrates the need to monitor the reactions at several points in time. However, no attempt was made to correlate the reaction mechanism with the kinetics in this work.

In summary, a new methodology, nonaqueous capillary array electrophoresis coupled with microreaction, is developed to address the throughput needs of combinatorial approaches to homogeneous catalysis and reaction optimization. Catalytic activity, selectivity, and kinetics of the various combinations are determined quickly. Other combinatorial applications that can be envisioned based on this method are screening for asymmetric catalysts and drugs, as well as combinatorial library synthesis. Although we used a home-built prototype in this study, commercial 96-capillary fluorescence instruments for DNA sequencing are readily adaptable to provide similar analyses, as long as the products fluoresce. The anticipated commercialization of our 96-capillary absorption instrument[18] will provide nearly universal detection if far UV wavelengths (e.g., 214 nm) are used.

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