Sensors

High-Throughput Screening by Using a Blue-Fluorescent Antibody Sensor**

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Phase-transfer catalysis (PTC) has received increasing attention in recent years, particularly with regard to asymmetric synthesis, because of its simplicity, mild reaction conditions, and moderate-to-high product yields.[1,2] *Cinchona* alkaloid-derived quaternary ammonium salts have been the most frequently employed chiral catalysts given their efficiency, low cost, ease of preparation, and suitability for introducing structural diversity.[2] Notably, a number of important natural and nonnatural amino-acid derivatives have been synthesized by α-C–C bond formation by quaternary *Cinchona*-based catalysts.[2,3] Yet, despite their successful application to catalytic asymmetric synthesis, dramatic effects of the catalyst structure, solvent, temperature, and metal-ion base on the enantioselectivity have often been problematic with regard to PTC reaction optimization.[2–5]

Combinatorial synthesis can afford large libraries of molecules as a source of new and improved catalysts.[6] However, considerable effort is often entailed in the screen-

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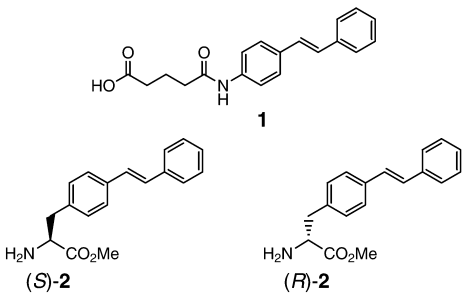
ing of these libraries, particularly with regard to enantioselective reactions. In high-throughput screening (HTS) for the evaluation of catalyst libraries, the determination of the enantiomeric excess (*ee*) of the products is generally the rate-limiting procedure.[6c] Hence, the application of HTS to enantioselective transformations has not been straightforward. In 1997, Reetz and coworkers developed the first HTS of reaction *ee* values by using UV/Vis spectroscopy.[7] Following their pioneering work, other methods that use, for example, IR-thermography,[8] circular dichroism,[9] capillary electrophoresis,[10] fluorescence,[11] mass spectrometry,[12] chemosensing,[13] competitive immunoassay,[14] and enzymatic methods[15] have emerged for particular applications. Most of these techniques have been used to screen the *ee* values of asymmetric reduction or hydrolysis reactions, but, to our knowledge, HTS of the *ee* values of products from an asymmetric C–C bond forming reaction has not been reported.

Recently, we described a series of monoclonal antibodies (mAbs), for example, mAb 19G2, prepared against the *trans*-stilbene hapten **1** (Scheme 1), in which the 19G2-**1** complex produced a blue fluorescence in high quantum yield (λ_{ex} = 327 nm, λ_{em} = 410 nm, Φ_f = 0.78).[16] During subsequent studies to find alternative ligands for these mAbs, we discovered that each of the chiral *trans*-stilbene amino acid esters (*S*)-**2** and (*R*)-**2** (Scheme 1) could bind to 19G2, but only the 19G2-(*S*)-**2** complex afforded a blue fluorescence. Hence, it occurred to us that 19G2 could act as a sensor in the HTS of chiral catalysts used for the synthesis of (*S*)-**2** and (*R*)-**2**. Herein, the aim was to evaluate a panel of derivatized *Cinchona* alkaloids in the PTC of an asymmetric α-alkylation reaction by using a HTS fluorescence plate-reader format for the rapid estimation of *ee* values of products.

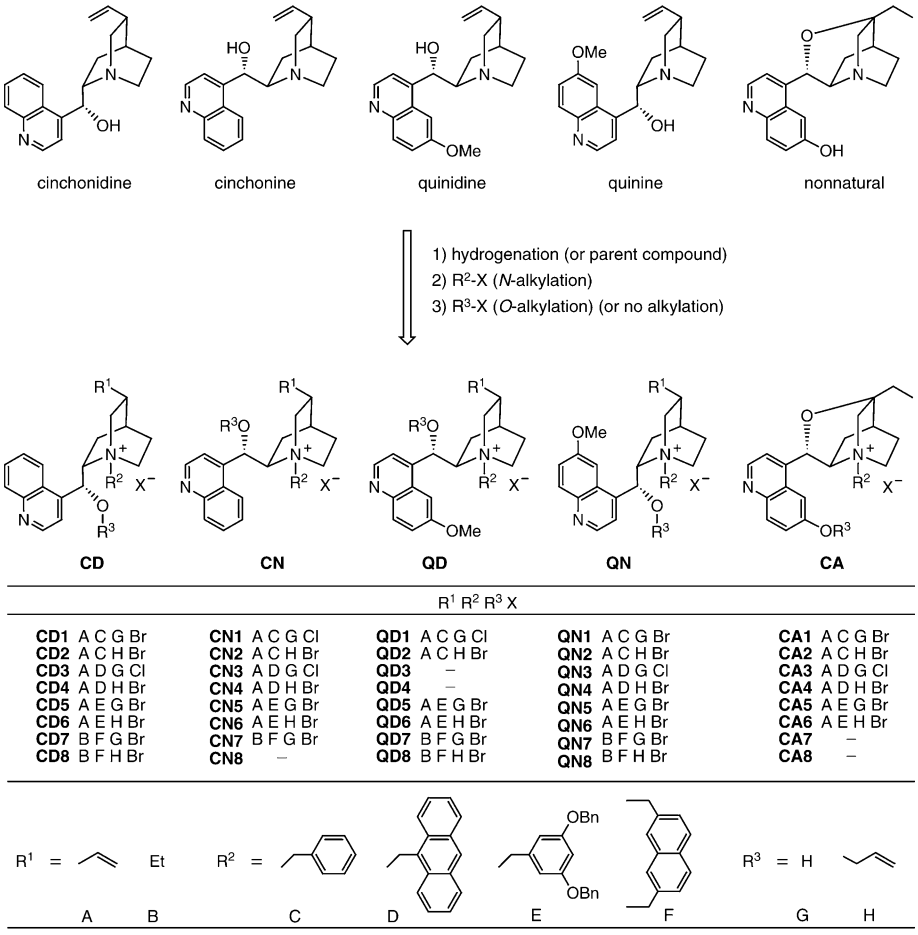
A catalyst library derived from *Cinchona* alkaloids was constructed by individual compound synthesis. Four natural *Cinchona* alkaloids (cinchonidine, cinchonine, quinidine, quinine) and one nonnatural *Cinchona*-type alkaloid[17] were hydrogenated, or left as the parent compound, then derivatized with four different *N*-alkyl substituents, and finally *O*-alkylated, or the hydroxyl was kept unmodified (Scheme 2). Out of a possible 40 *Cinchona* alkaloid ammonium salts, 35 were obtained in high yield and purity. Among these 35 catalysts, 11 had been previously reported[3,4,18] and 24 were new compounds. Each catalyst was then employed in PTC for the



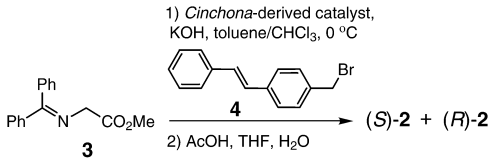
Scheme 1. Blue-fluorescent mAb 19G2 hapten and chiral ligands for sensor applications.

alkylation of *N*-(diphenylmethylene)glycine methyl ester **3** with 4-bromomethyl-*trans*-stilbene **4** (Scheme 3). After the alkylations were complete, the benzophenone Schiff base group was hydrolyzed and the product mixture of (*S*)-**2** and (*R*)-**2** was purified. The simplicity of the sequence allowed us to carry out 35 parallel reactions in two days. Yields of the isolated product mixture ranged from 47% to 83%.

To determine the *ee* values of the 35 product mixtures, we constructed a calibration curve by using independently synthesized (*S*)-**2** and (*R*)-**2**. To this end, (*S*)- and (*R*)-*N*-Boc-iodoalanine methyl ester (Fluka) were each reacted with zinc dust, followed by Pd-catalyzed cross-coupling to 4-



Scheme 2. Preparation of a *Cinchona* alkaloid-derived catalyst library.



Scheme 3. Synthesis of chiral ligands for mAb 19G2 by using *Cinchona*-based PTC.

bromo-*trans*-stilbene in analogy with reported procedures.[19] Both (*S*)-**2** and (*R*)-**2** were obtained in $>99\%$ *ee* as analyzed by chiral HPLC. Precise mixtures of these isomers were then prepared (0, 25, 50, 75, 100 % (*S*)-**2**, corresponding to 100 % *ee* (*R*)-**2**, 50 % *ee* (*R*)-**2**, 0 % *ee*, 50 % *ee* (*S*)-**2**, 100 % *ee* (*S*)-**2**) as solutions in DMF. Each calibration standard (50 μM) was mixed with 19G2 (25 μM; 50 μM binding sites) in PBS (10 mM sodium phosphate, 150 mM NaCl, pH 7.4) with 5 % DMF cosolvent, and the fluorescence intensities ($\lambda_{ex}=327$ nm, $\lambda_{em}=416$ nm) were measured by using a 96-well plate reader. The data fitted well to a hyperbolic, as required by the specific binding of (*S*)-**2** and (*R*)-**2** by the mAb 19G2. The fluorescence values for each of the 35 product mixtures were then obtained in the same way as above, and the corresponding *ee* values were then calculated (Figure 1). The process of sample preparation, plate reading, and catalyst evaluation took less than one hour.

To confirm the validity of the method, 10 samples were randomly chosen across the range of *ee* values of (*S*)-**2** and (*R*)-**2** and reanalyzed by chiral HPLC. The fluorescence sensor and HPLC measurements varied, on the average, $\approx 10\%$ and afforded an excellent linear correlation (slope $=0.92$, $r^2=0.99$). We note that the *tert*-butyl ester analogue of **3** generally gave higher enantioselectivities in asymmetric PTC alkylations.[3] However, it was necessary to use **3** as the alkylation substrate, as 19G2 did not show selective fluorescence with the *tert*-butyl ester derivatives corresponding to (*S*)-**2** and (*R*)-**2**.

Significantly, the blue-fluorescent mAb sensor identified high-performance catalysts that were the same as those found by others to give excellent enantioselectivity, as well as one previously unknown catalyst. If we choose $\approx$ 70 % *ee* as a preliminary cut-off point for catalyst efficiency, then, based on our test panel, such a value would give a reasonable $\approx$ 5–10 % member selection from large libraries for further analysis. The catalyst **CD2** (68 % *ee*, fluorescence; 78 % *ee*, HPLC) was reported by O'Donnell et al. in their seminal work on *Cinchona*-based PTC.[18b] **CD8** (75 % *ee*, fluorescence; 85 % *ee*, HPLC) was also observed by Park et al. to exhibit the highest enantioselectivity among this class of catalysts.[18a] Notably, **QN4** (63 % *ee*, fluorescence; 68 % *ee*, HPLC) is a new quinine-based structure for PTC. Lygo et al.[4b] described a catalyst of this type that afforded a $>80\%$ *ee* in reactions by using the *tert*-butyl ester analogue of **3**.

We have presented the foundation for a novel HTS method that uses a blue-fluorescent mAb to assay the enantionselectivity of products from, for example, a catalytic asymmetric alkylation. The method is: 1) sensitive, as expected for fluorescence detection, with only 10 nmol of sample required for each measurement, 2) rapid, hundreds of catalysts could potentially be screened and ranked in less than a day, and 3) accurate, and with a wide dynamic range, as demonstrated by the comparison to HPLC determinations from 100 % *ee* (*S*)-**2** to 100 % *ee* (*R*)-**2**. However, since the method is based on the catalysis of a reference reaction, the intent is to provide a HTS to obtain a subset of catalysts that produce the highest *ee* values. Then, these candidates can be tested and rigorously analyzed, in this case, for efficiency in preparing other amino acids of interest. Importantly, blue-fluorescent HTS has broad applicability. By using the preparation of the same reference compounds, catalysts of other types could be evaluated in both alkylation and nonalkylation strategies for amino acid syntheses. For example, the latter could include Jacobsen-type catalysts for the Strecker reaction.[20] Finally, we are also currently extending the diversity of the *trans*-stilbene moiety, which will allow the application of the blue-fluorescent mAb sensor in screening asymmetric catalysis for other C–C bond-forming reactions, as well as other classes of reactions, such as oxidations, reductions, and hydrolyses.

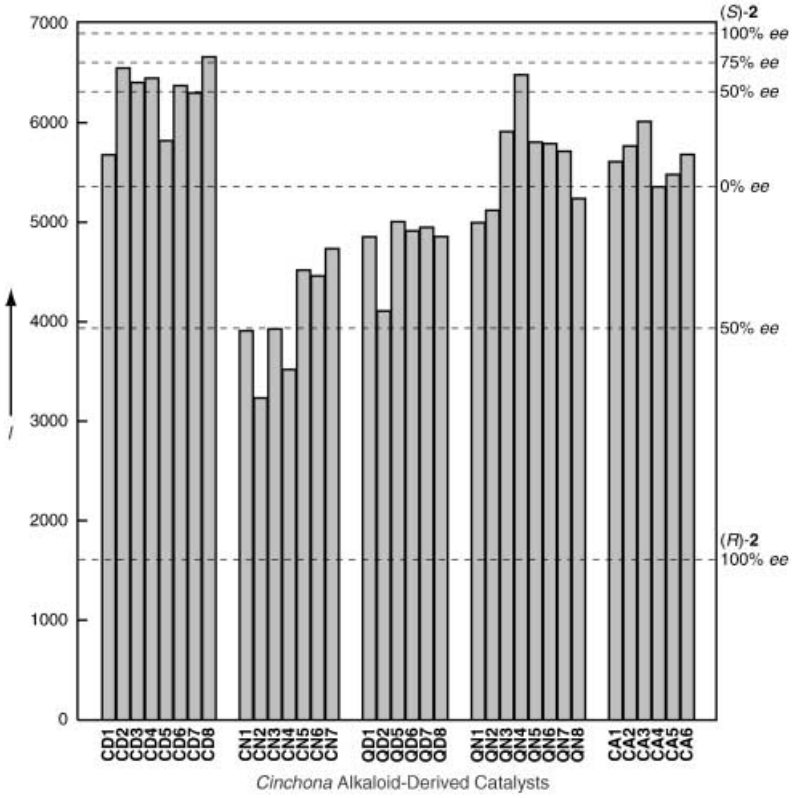


Figure 1. The *ee* values from *Cinchona*-based PTC measured with the mAb 19G2 sensor.

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