New Fluorogenic Probes for Oxygen and Carbene Transfer: A Sensitive Assay for Single Bead-Supported Catalysts

Rayane Moreira, Miroslav Havranek, and Dalibor Sames*

Contribution from the Department of Chemistry, Columbia University, New York, New York 10027

Received October 26, 2000

Abstract: A high-throughput screening assay for atom transfer catalysis has been developed. This assay is based on two probes, developed herein, which generate highly fluorescent products upon carbene or oxygen atom transfer. The emission wavelength of probes **1** and **5** shift significantly (up to 90 nm) upon epoxidation, allowing detection of product at 3% conversion. Probe **7** is not fluorescent, while fluorescence emission by carbene insertion/rearrangement product **8** allows detection at less than 1% conversion. Such sensitivity allows for examination of single-bead reactions in a high throughput array format (1536 wells per plate), and provides a broad detection window ranging from single to high turnover numbers. Thousands of metal complexes are evaluated in a single screening experiment. Preliminary screening of a diverse ligand library with probe **7** in the presence of Rh(II) uncovered new catalysts capable of cyclopropanation and C−H insertion.

Introduction

Realization of combinatorial concepts unlocks new opportunities in the field of catalyst development. Laboratory molecular evolution, originally confined to biological systems, is becoming an active area in the chemical sciences as well. A common limitation to combinatorial endeavors is a functional assay enabling evaluation of all library members.[1] While standard analytical techniques (HPLC, GC) have recently been upgraded for parallel or serial screening of relatively small synthetic libraries ($<10^3$ members),[2] the discovery of entirely new catalytic motifs may require screening of large arrays ($>10^4$ members). Although astronomical numbers of variations are readily available via combination of covalent[3] and noncovalent chemical assembly,[4] the exploitation of such synthetic capabilities requires dependable *en masse* screening assays. In this context, IR thermography[5] and colorimetry/fluorimetry[6] assays have hitherto proven most promising. Fluorimetry is particularly attractive due to its sensitivity and applicability to high throughput formats. In addition to catalysis screening, the detection of chemical reactions with high sensitivity is of broader interest, including the study of chemical processes in vivo,[7] and on a single-molecule level.[8]

We wish to report on the development of new fluorogenic probes for atom group transfer (oxygen, nitrogen, carbene). These probes, designed to change their fluorescent profile during the course of a reaction such as epoxidation or carbene insertion, allow for the detection of product in the presence of a great excess of reactant (<3% conversion). Such sensitivity permits the examination of single-bead reactions in a high throughput array format (1536 wells per plate) and provides a broad detection window ranging from single to high turnover numbers (Figure 1).

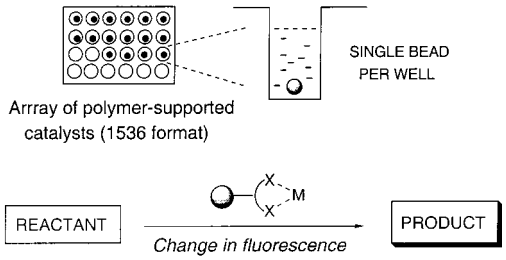


Figure 1. A high throughput assay for atom-transfer catalysis is based on new fluorogenic probes. Arrays of single-bead-supported catalysts are rapidly evaluated.

Results and Discussion

Our probe design was based on either shortening or lengthening the extent of π-conjugation in terphenyl systems associated with the reaction in question. The presence of multiple functional

(1) Jandeleit, B.; Schaefer, D. J.; Powers, T. S.; Turner, H. W.; Weinberg, W. H. *Angew. Chem., Int. Ed.* **1999**, *38*, 2494−2532.

(2) (a) Burgess, K.; Lim, H.-J.; Porte, A. M.; Sulikowski, G. A. *Angew. Chem., Int. Ed.* **1996**, *35*, 220−222. (b) Sigman, M. S.; Jacobsen, E. N. J. *Am. Chem. Soc.* **1998**, *120*, 4901−4902. (c) Porter, J. R.; Wirschun, W. G.; Kuntz, K. W.; Snapper, M. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2000**, *122*, 2657−2658. (d) Havranek, M.; Singh, A.; Sames, D. *J. Am. Chem. Soc.* **1999**, *121*, 8965−8966 and references therein.

(3) (a) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555−600. (b) Balkenhohl, F.; Bussche-Hünnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2288−2337.

(4) Hill, C. L.; Zhang, X. *Nature* **1995**, *373*, 324−326.

(5) (a) Taylor, S. J.; Morken, J. P. *Science* **1998**, *280*, 267−270. (b) Holzwarth, A.; Schmidt, H.-W.; Maier, W. F. *Angew. Chem., Int. Ed.* **1998**, *37*, 2644. (c) Reetz, M. T.; Becker, M. H.; Kuhling, K. M.; Holzwarth, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 2647. (d) Connolly, A. R.; Sutherland, J. D. *Angew. Chem., Int. Ed.* **2000**, *39*, 4268−4271.

(6) (a) Crabtree, R. H. *Chem. Commun.* **1999**, 1611−1616. (b) Shaughnessy, K. H.; Kim, P.; Hartwig, J. F. *J. Am. Chem. Soc.* **1999**, *121*, 2123-2132. (c) Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 4306−4307. (d) Joo, H.; Arisawa, A.; Lin, Z.; Arnold, F. *Chem. Biol.* **1999**, *6*, 699−706. (e) Su, H.; Yeung, E. *J. Am. Chem. Soc.* **2000**, *122*, 7422−7423. (f) Harris, R. F.; Nation, A. J.; Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **2000**, *122*, 11270−11271. (g) Olsen, M. J.; Stephens, D.; Griffiths, D.; Daugherty, P.; Georgiou, G.; Iverson, B. L. *Nature Biotechnol.* **2000**, *18*, 1071−1074. (h) Screening of enantioselective catalysts: Reetz, M. T.; Kühling, K. M.; Deege, A.; Hinrichs, H.; Belder, D. *Angew. Chem., Int. Ed.* **2000**, *39*, 3891−3893 and Korbel, G. A.; Lalic, G.; Shair, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 361−362.

(7) (a) *Handbook of Fluorescent Probes and Research Chemicals, Molecular Probes*, 6th ed.; Haugland, R. P., Ed.; 1996. (b) Franz, K. J.; Singh, N.; Lippard, S. J. *Angew. Chem., Int. Ed.* **2000**, *39*, 2120−2122.

(8) Xie, X. S.; Lu, H. P. *J. Biol. Chem.* **1999**, *274*, 15967−15970.

10.1021/ja003786+ CCC: $20.00
Published on Web 03/29/2001

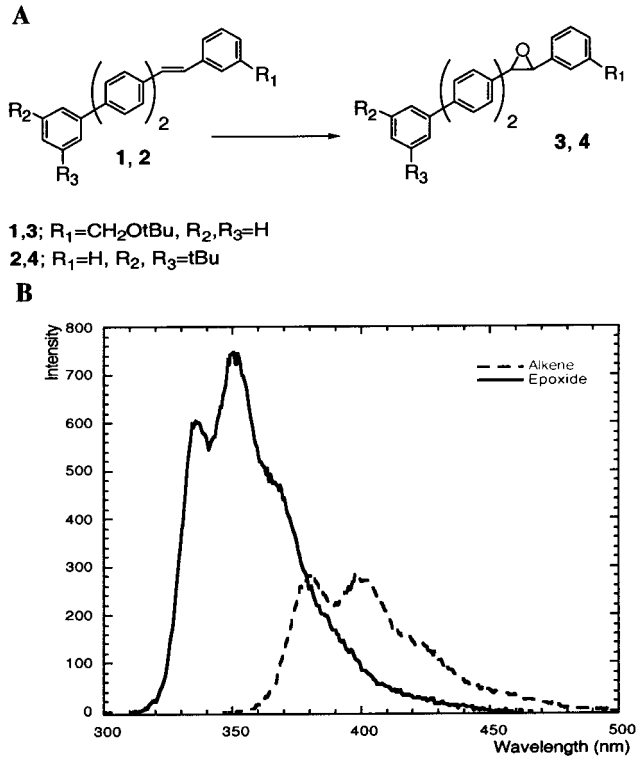


Figure 2. (A) Fluorogenic probe for oxygen transfer. (B) Emission spectra of alkene **1** and epoxide **3** (irradiated at 285 nm).

groups, found in common fluorophores, was avoided to ensure compatibility of these probes with a wide range of reaction conditions.

The first type of probe was based on a biphenyl-stilbene system (Figure 2). Reaction at the double bond (epoxidation, aziridination, cyclopropanation, or saturation reactions), shortens the π-conjugation network, which has two important consequences: first, the emission of the product is blue-shifted in relation to the starting material, and second, the fluorescence intensity of the product is greater relative to that of reactant. We have explored various polycyclic arenes in this context (e.g., pyrene, anthracene, and phenanthrene); however, multiple degradation products were generated under oxidative conditions. The terphenyl-based systems proved robust, furnishing epoxides as major products. Following our initial explorations we synthesized alkenes **1** and **2**, where substituents R_1, R_2, and R_3 were introduced to improve the solubility of these substrates in organic solvents (Figure 2). A number of known epoxidation reagents or catalysts (MCPBA, manganese-based catalysts) afforded corresponding epoxides **3** and **4**, respectively. Excitation and emission maxima were blue-shifted by 50 nm, and the overall fluorescence intensity was increased by a factor of 2.5. As a result, the application of standard absorption and emission filters allowed for isolation of the product emission and, hence, the detection of a low conversion of reactants (2−3% conversion) as determined by a fluorescent plate reader.[9] To extend the applicability of our probes to the visible region, we synthesized system **5** (Figure 3). The pyridinium ring in **5**, previously utilized by Crabtree in the design of related reactive dyes,[10] increased π-delocalization throughout the entire system,

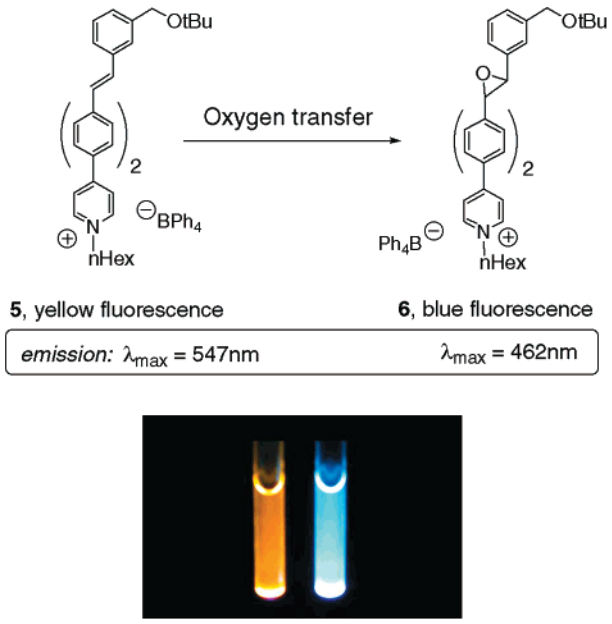


Figure 3. Visible emission shift associated with atom-transfer reactions. Epoxide **6** (blue) shows greater emission intensity in comparison to alkene **5** (yellow). A 1 mM solution in dichloroethane was irradiated with UV lamp (365 nm).

Table 1. Fluorescence Intensity after "One-Bead" Epoxidation of Probe **1**; Irradiated at 280 nm, Read at 340 nm; Average Intensity from 96 Experiments (Standard Deviation in Parentheses)

	Fe(II) catalyst[a]	resin-NH_2 Fe(II)	no bead
130 μm beads (300 pmol)[b]	138 (32)	43 (8)	35 (6)
160 μm beads (860 pmol)[c]	222 (56)	60 (11)	37 (7)

[a] Resin-Ser-Ser-NHCO-2-pyridine + $FeCl_2$.[12] [b] 2.5 nmol substrate, 2 equiv of H_2O_2, 1 μL of 9:1 1,2-dichloroethane/*tert*-butyl alcohol, 5.5 h, 1536-well plate in vapor chamber. [c] 10 nmol substrate, 2 equiv of H_2O_2, 2 μL of 4:1 1,2-dichloroethane/*tert*-butyl alcohol, 5.5 h, 1536-well plate in vapor chamber.

placing the emission maximum well within the visible region. Thus, yellow fluorescent alkene **5** provided intensely blue fluorescent epoxide **6**, a result of a large emission shift (547 → 462 nm) and a large increase in fluorescence intensity. These new fluorogenic probes produce highly fluorescent products and therefore offer superior detection limits in comparison to reactive dyes that are based on bleaching of color or fluorescence (sensitive to >40% conversion).[10]

Both probes **1** and **5** proved suitable for screening single bead-supported catalysts. The beads were distributed into microwell plates (1536 format, one bead per well), followed by addition of the solution of the probe and other reagents. The plates were placed in a vapor chamber for the required time and then scanned in the fluorescent plate reader (5 min per 1536-well plate). Although we now perform the assays manually, as described in the Experimental Section, each step is amenable to automation, and the resulting fully automated assay will offer tremendous throughput power.[11]

Although the detection limit of this assay depended on the concentration of the reactant (probe) and other reagents used,

(9) Although most fluorescent plate readers operate in the visible region, there are commercially available instruments designed for UV region measurements (>275 nm; HTS 7000 Plus, Perkin-Elmer; Spectramax Gemini XS, Molecular Devices).

(10) Cooper, A. C.; McAlexander, L. H.; Lee, D.-H.; Torres, M. T.; Crabtree, R. H. *J. Am. Chem. Soc*. **1998**, *120*, 9971−9972.

(11) There is a commercially available bead-handling instrument (Cartesian Technologies) capable of high-speed bead distribution (one bead per well). We are currently testing this instrument. Also, FACS instrumentation has been adapted to sort polymeric and glass beads: Needels, M. C.; Jones, D. G.; Tate, E. H.; Heinkel, G. L.; Kochersperger, L. M.; Dower, W. J.; Barrett, R. W.; Gallop, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10700−10704. However, there is a need for a low-cost, "low-tech" instrument of this capability.

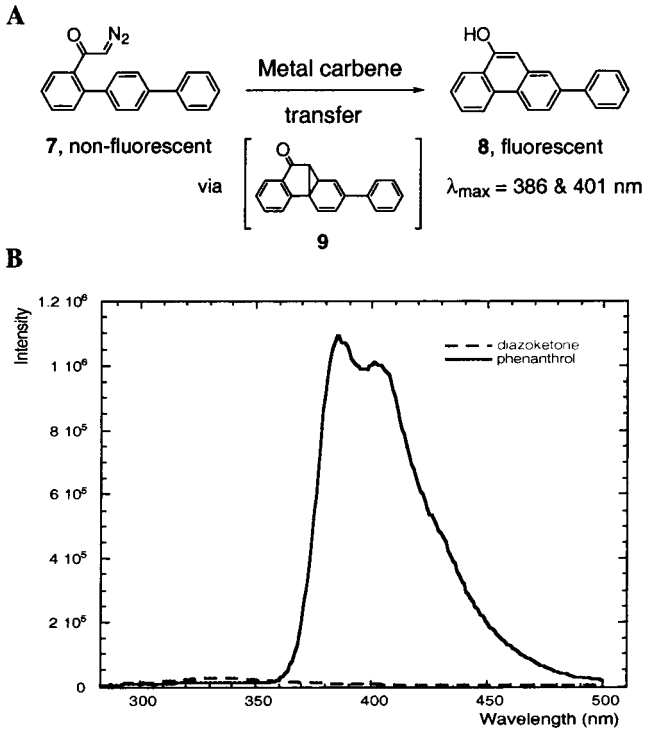


Figure 4. (A) Fluorogenic probe for carbene transfer. A nonfluorescent probe yields a highly fluorescent product. (B) Emission spectra of diazoketone **7** and phenol **8** (excitation at 275 nm).

Table 2. Intensity of Fluorescence after "One-Bead" Cyclization of Probe **7**; Irradiated at 280 nm, Read at 380 nm; Average Intensity from 200 Experiments (Standard Deviation in Parentheses)

	Cu(I) catalyst[a]	resin-ligand	no bead
intensity[b]	344 (215)	48 (12)	51 (7)

[a] 130 μm bead-N=CH−(2-pyridine) + $CuPF_6(MeCN)_4$. [b] 13 nmol substrate, 10 μL of THF, 10 h, sealed 384-well plate.

single catalytic turnovers of a single bead were routinely detected under described conditions. We employed an active catalyst, reported by Jacobsen (Resin-Ser-Ser-NHCO-2-pyridine + $FeCl_2$), for the epoxidation of alkenes in the presence of H_2O_2 as the standard positive.[12] Positive 160 μm beads (0.86 nmol of material per bead, Rapp Polymere TentaGel) were readily identified under the following reaction conditions: 2.0 μL of solvent, 10 nmol of **1** (c = 5 mM) and 20 nmol of H_2O_2 per well (Table 1). This assay, based on the rapid and direct evaluation of all members of an ensemble, represents an alternative to stepwise, parallel approaches.[12]

The second class of probes was designed to "report" on carbene transfer activity. Terphenyl diazoketone **7**, itself nonfluorescent, yields highly fluorescent phenanthrol **8** upon treatment with a suitable metal catalyst (Figure 4). The overall reaction, in this case catalyzed by rhodium(II) acetate presumably proceeds via the cyclopropane intermediate **9**, followed by rearrangement to form a new aromatic ring.[13] Probe **7** therefore represents a nonfluorescent substrate which yields a fluorescent product, providing the basis for a highly sensitive catalysis assay (<1% conversion). A one-bead experiment, using a bead-bound copper(I) catalyst as a standard, successfully identified wells containing active beads (Table 2). Under the screening conditions, the response of the fluorescent plate reader

(12) Francis, M. B.; Jacobsen, E. N. *Angew. Chem., Int. Ed.* **1999**, *38*, 937−941.

(13) Duddeck, H.; Kennedy, M.; McKervey, M. A.; Twohig, F. M. *J. Chem. Soc., Chem. Commun.* **1988**, 1586−1588.

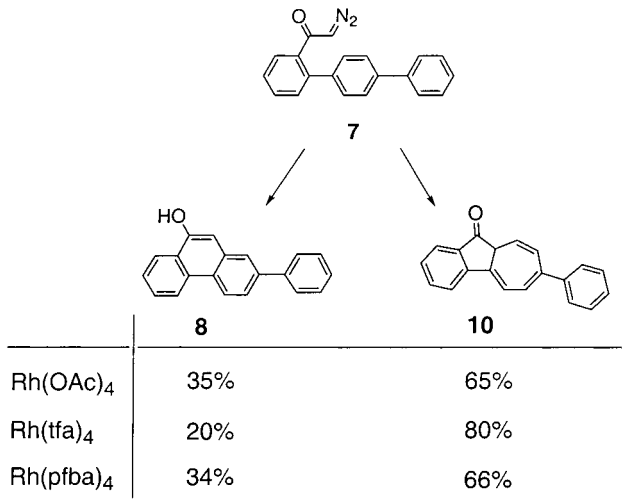


Figure 5. Yields of phenanthrol and cycloheptatriene in $CDCl_3$ with various catalysts as determined by ^1H NMR. Reactions were run in the NMR tube at 1.3 mM with a few grains of catalyst. Once reaction was complete, ratio of **8** to **10** remained constant until decomposition of **10** began (see Supporting Information).

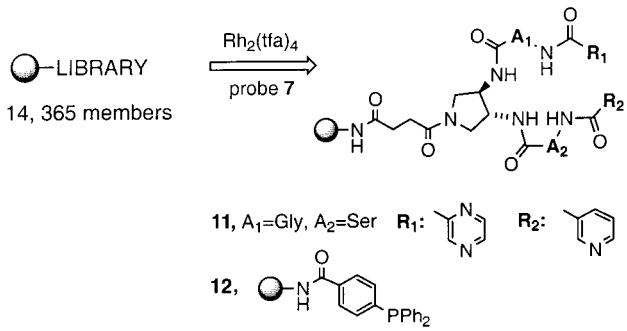


Figure 6. Preliminary screening of a diverse ligand library with probe **7**; 5% of library (~700 complexes) was evaluated.

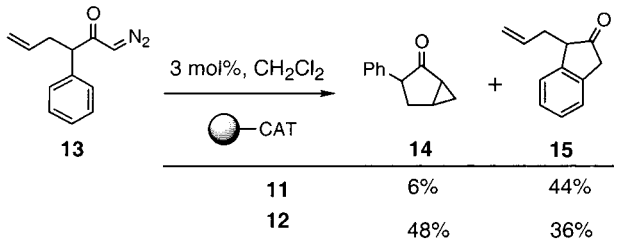
to increasing amounts of phenanthrol **8** is near linear, providing a broad detection window ranging from 1 (2% conversion) to 43 turnover numbers (100% conversion). For detection of higher turnover numbers, an increase of probe concentration will desensitize the assay on the lower end and in the same time expand the upper dynamic range of the assay.

In addition to phenanthrol **8**, cyclization of compound **7** produced significant amounts of cycloheptatriene **10**, the amount of which depended on the catalyst and conditions (Figure 5). This product resulted from 3,3-sigmatropic rearrangement of intermediate **9** and has been previously described in related systems.[13] It was the only byproduct and, importantly, did not affect the detection of fluorescence from compound **8**. Yields for conversion of **7** to **8** in $CDCl_3$, determined by NMR, were within 5% of those determined by fluorimetric measurements.

Probe **7** may also discern between metal-bound and free carbenes. Thus, reaction of **7** with silver benzoate led to the Wolff rearrangement product. Although fluorescent, the emission wavelength of this compound is shorter than that of phenanthrol **8** (see Supporting Information), and with use of appropriate filters it may also be selectively detected by the fluorescent plate reader.

The efficiency of this assay was demonstrated in the preliminary screening of a diverse ligand library in the presence of $Rh_2(O_2CCF_3)_4$ as the metal source (Figure 6). One milligram of resin (5% of library, ~700 metal complexes) was screened, affording a number of positives; approximately 10% of the screened population was found active, showing varying degrees

Scheme 1



of conversion. A high incidence of a pyrazine side chain present in active catalysts was revealed via chemical decoding. Triphenylphosphine ligands also provided active species. Subsequently, the two systems **11** and **12** were synthesized and evaluated in preparative scale experiments. Both complexes catalyzed the cyclization of probe **7** efficiently in CH_2Cl_2 (45% for **11**, 53% for **12**), thus confirming the assay results. Furthermore, systems **11** and **12** catalyzed carbene insertion of related substrate **13**, affording cyclopropanation product **14** and arene C−H insertion product **15** (Scheme 1).[14] Noteworthy is the fact that two distinct catalysts, demonstrating different chemoselectivities, were found through the use of probe **7**. These results suggest that probe **7** may be a general reporter for carbene-transfer chemistry of diazocarbonyl compounds.

Conclusions

In summary, we have developed new fluorogenic probes for atom-transfer reactions. These probes allow for examination of single-bead reactions in a high throughput array format (1536 wells per plate), with a broad dynamic range. This assay is therefore suitable for optimization studies seeking highly efficient catalysts (high-turnover numbers) as well as the discovery of leads in the search for entirely novel systems (low turnovers). Currently, the manually performed assay permits evaluation of thousands of metal complexes in a single screening. Furthermore, each step of the described assay is amenable to automation, which will provide enormous screening capacity and efficiency. Screening of diverse metal complex libraries is underway in our laboratory.

Experimental Section

General. Screenings were analyzed on a Perkin-Elmer HTS 7000 Plus Bio Assay Reader. Fluorescence spectra were recorded on a Perkin-Elmer LS 50B Luminescence Spectrometer. HPLC analysis was performed on a Hewlett-Packard Series 1100 HPLC. Rapp Polymere TentaGel macrobeads (0.45 mmol/g, 160 μm beads) and Novabiochem NovaSynTG 130 μm beads (0.31 mmol/g) were used for bead-supported catalysis.

Preparative Epoxidation of Probe 1 Using Jacobsen Catalyst (TentaGel Resin-Ser-Ser-CONH-2-pyridine + $FeCl_2$)[12] and Hydrogen Peroxide. Compound **1** (12.5 mg, 0.03 mmol), catalyst (10 mol %, 6 mg), and a solution of hydrogen peroxide (0.1 M, 10 equiv, 3 mL) in 1,2-dichloroethane−*tert*-butyl alcohol 4:1 were mixed and shaken for 20 h. The solvent was evaporated and the residue chromatographed on a TLC plate (pentane−dichloromethane 3:7) to yield 2.3 mg (18%) of epoxide and 8.3 mg (66%) of starting material. For analytical data see Supporting Information.

Single-Bead Experiment with Probe 1. Single-polymer beads were distributed into 1536-well plates manually via the use of a capillary tube. For 160 μm beads, compound **1** (4.21 mg, 0.01 mmol) and 2.0 μL of 30% H_2O_2 (2 equiv) were dissolved in 2 mL of 1,2-dichloroethane/*tert*-butyl alcohol (4:1) and 2.0 μL of this freshly prepared stock solution was added to each well. For 130 μm beads, a similar procedure was followed except that compound **1** and 30% H_2O_2 were dissolved in 4 mL of 1,2-dichloroethane/*tert*-butyl alcohol (9:1), and 1.0 μL was added to each well. After incubation for 5.5 h in a vapor chamber (1,2-dichloroethane), the solvent was removed in vacuo (in desiccator), followed by redissolution with 10 μL of dioxane per well and analysis in the plate reader (excitation filter 280 nm, emission filter 340 nm).

Preparative Cyclization of 7. General Procedure. Carbene-transfer catalyst (1 mol %) was added to a 1.3 mM solution of compound **7,** and the solution was stirred for 10 h. Yield of these reactions was determined by fluorimetric measurements, NMR spectroscopy, and HPLC analysis. Compound **8** was purified by crystallization from dichloromethane or by column chromatography (10% ethyl acetate in hexanes). For analytical data of compound **7** and **8**, see Supporting Information.

One-Bead Experiment with Probe 7. This procedure was conducted in a glovebox. Bead-bound catalyst (Table 2) was suspended in dry THF, and the beads were allowed to settle into a 384-well plate such that most wells contained 0−3 beads. After evaporation of solvent, 10.0 μL of a 1.3 mM solution of **7** in THF was added to each well. The plate was sealed with Easy Peel Heat Sealing Foil from Marsh Biomedical Products, Inc. After 10 h at room temperature, the plate was removed from the glovebox, the THF was evaporated, and the contents redissolved in ethanol. The plate was analyzed in the fluorescent plate reader (excitation filter 280 nm, emission filter 380 nm).

Preliminary Screening of Ligand Library with Rh(II) Using Probe 7. A portion of a bead-bound ligand library (25 mg) was shaken overnight with 1 equiv of $Rh_2(tfa)_4$ (2.6 mM solution in $CHCl_3$). The beads were then thoroughly rinsed with chloroform and dried in vacuo. About 1 mg of beads (approximately 700 compounds) was distributed into three 384-well plates by agitating a suspension of beads in THF over each plate and allowing the beads to settle into the wells. The beads were then manually redistributed such that there were 0−2 beads per well. The plate was air-dried, and 10 μL of a 1.3 mM solution of probe **7** in THF was dispensed into each well using a multisyringe pipet. The plate was incubated for 12 h in a vapor chamber, dried in open air, and refilled with ethanol (10 μL per well) prior to evaluation by the fluorescent plate reader (excitation filter 280 nm, emission filter 380 nm). Fifteen positive beads were picked, carefully washed, and decoded. Of these, seven contained a pyrazinecarboxamide side chain; 10 contained carboxylate side chains. Several ligands were resynthesized on a larger scale by standard solid-phase techniques (see Supporting Information).

Preparative-Scale Testing of Positive Catalysts. After resynthesis, ligands were complexed with $Rh_2(tfa)_4$, as described above, and dried in vacuo overnight. One milligram of probe **7** (0.0034 mmol) and 3 mg of bead-bound catalyst (2.3 mol %) were then stirred for 12 h in 1 mL of THF. Yields of phenanthrol **8** were determined by fluorimetric analysis and comparison to a standard curve or by HPLC analysis using naphthalene as a standard.

(14) (a) Padwa, A.; Austin, D. J.; Prose, A. T.; Semones, M. A.; Doyle, M. P.; Protopopova, M. N.; Winchester, W. R.; Tran, A. *J. Am. Chem. Soc*. **1993**, *115*, 8669−8680. (b) Estevan, F.; Lahuerta, P.; Pérez-Prieto, J.; Stiriba, S.-E.; Ubeda, M. A. *Synlett* **1995**, 1121−1122.

Reaction of Resin-Bound Catalysts with 13. Compound **13** (0.17 mmol) was stirred overnight with 3 mg of bead-bound catalyst (0.5 mol %) in 1 mL of dry CH_2Cl_2. The product yields and distributions were analyzed by gas chromatography using naphthalene as a standard.

Acknowledgment. Columbia University and Research Corporation are gratefully acknowledged for support of this work. D.S. is a recipient of the Camille and Henry Dreyfus New Faculty Award. We also thank Dr. J. B. Schwarz (manuscript preparation).

Supporting Information Available: Synthesis and spectroscopic characterization of probes **1**−**8**, experimental protocols describing the assays (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA003786+