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Double-Cuvette ISES: In Situ Estimation of Enantioselectivity and Relative Rate for Catalyst Screening

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Combinatorial methods have made an important impact on catalyst discovery in recent years.[1] Notable examples include the discovery of catalysts for asymmetric acylation[2a] and Stetter-type chemistry,[2b,c] Pd(0)-[2d] and Cu(I)-mediated[2e] allylic alkylations, Ag-based carbene insertion,[2f] $FeCl_2$-mediated epoxidation,[2g] and early transition-metal-based additions to imines.[2h,i] These successes have spurred interest in catalyst screening.[3] Screens for active lead catalysts, based upon IR thermography,[4a,b] fluorescence,[4c−f] and dye formation[4g]/bleaching[4h] have been reported. Particularly valuable screens also provide information on *enantioselectivity*.[5] To predict kinetic resolution efficiency in situ, several elegant parallel enantiomer competition assays are available.[4a,6] In situ screens for organic catalysts that detect product handedness and thereby apply to enantioselective catalysis (achiral educts) or actual resolutions (racemic educts) are much rarer. One such method has recently been reported by Morken and employs an isotopically chiral ^{13}C NMR probe substrate.[7]

We have described the use of enzymes to monitor relative rates in real time for allylic substitution catalysts.[8,9] In its first iteration, ISES (in situ enzymatic screening) was run in a bilayer, with an aqueous enzymatic layer reporting on the turnover of an allylic ethyl carbonate substrate in an organic layer. An asymmetric, Ni-(0)-mediated allylic amination was uncovered in the process.[8a,b]

Herein, we disclose a second iteration of the ISES technique, in which the reporting enzymes provide information on both relative rate and enantioselectivity. This method differs significantly from the first version of ISES: (i) One uses the reporting enzymes to observe the reaction product directly (a chiral 1,2-diol here) rather than a byproduct of catalyst turnover (e.g., EtOH previously); this allows one to take full advantage of the chirality in the enzymatic "sensor". (ii) Because one wishes to glean information on enantioselectivity, as well as relative rates, two reporting enzymes are employed, in parallel cuvettes. Two ISES reporting rates are needed to distinguish the situation in which catalyst A has the same rate as catalyst B but greater *R*:*S* selectivity from the one in which the two catalysts display similar enantioselectivities, with catalyst A possessing the greater rate. We term this approach "double-cuvette" ISES (Scheme 1).

To demonstrate proof of principle, we chose the hydrolytic kinetic resolution (HKR) of (±)-propylene oxide, a reaction known to be catalyzed efficiently by chiral Co(III)−salen complexes from the pioneering work of Jacobsen.[11] To provide an information-rich data set requires that the two reporting enzymes display different−ideally opposite−enantiomeric preferences. Screening revealed that alcohol dehydrogenase from horse liver (HLADH) and from *Thermoanaerobium brockii* (TBADH) fulfill this criterion. The former enzyme prefers *(S)*-1,2-propanediol,[12] whereas the latter favors the (*R*)-antipode.[13]

A focused 7 × 7 "salen" array was designed (Table 1) so as to explore the interplay of novel chiral diamine scaffolds (from terpenoid, amino acid, and carbohydrate skeletons) with sterically and electronically diverse "salicylaldehydes".

Scheme 1. Double-Cuvette ISES

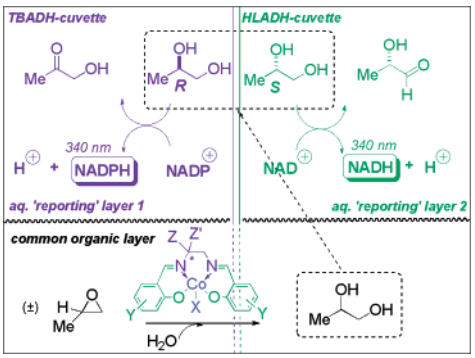

Table 1. Focused 7 × 7 Chiral Salen Array for Co(III)−Salen-Mediated HKR of *rac*-Propylene Oxide[a]

	a	b	c	d	e	f	g
1	+56 +72 6.9	+68 +75 10	§	+75 +81 12	+47† +28 2.1	§	§
2	-97 -93 -37	-73 -75 -9.4	§	-15 -54 -3.7	+4 -41 -2.6	§	-41# -71 -5.9
3	+48 +55 4.8	+70 +57 4.1	§	-54 -30 1.9	§	§	¶
4	+77 +76 9.5	+91 +59 5.1	§	¶	+43# +14 1.3	+57 +51 3.6	§
5	+87 +66 6.4	+65 +68 7.6	§	+9 +11 1.3	+35 -5 -1.1	§	§
6	+77* +69* 6.2	+70* +42* 2.6	§	¶	¶	¶	¶
7	-33* -64* -4.7	-28* -40* -2.5	§	§	+87 (+85)* +81 (+83)* 11 13	§	¶

[a] Each box provides HKR data for the Co(III)−salen acetate derived from the indicated salen. Presented are the % ee of the 1,2-propanediol product ["+" = (*S*) and "−" = (*R*)] as predicted by double-cuvette ISES (indigo) and as observed by chiral HPLC (black). Where available, observed catalyst *S* values[10] are also provided (enclosed boxes). The cuvette experiments are run in a bilayer of pH 8.6 buffer over 7.2 M epoxide in $CHCl_3$, containing 0.25 mol % catalyst, for 15−35 min. "Inherent" catalyst ee's are judged by running the HKR in neat propylene oxide, containing 0.55 equiv of H_2O, also at 0.25 mol % catalyst. §These catalysts gave ISES signals < 20 mAbs min^{-1} over 35 min. †This catalyst was tested at 0.05 mol %, as it was especially fast. #The catalysts derived from **2g** and **4e** displayed ISES rates of 14.9 and 18.1 mAbs min^{-1}, respectively, in the HLADH cuvette, over 35 min. ¶Difficulty was encountered in synthesizing appreciable quantities of these salens. *The 3,5-dinitrobenzoate counterion was employed for these Co(III) catalysts.

In the experiment, each Co(III)-salen catalyst (at 0.25 mol %) is placed in a lower organic layer ($CHCl_3$ and expoxide, 300 μL total volume) in each of two parallel cuvettes. Aqueous reporting layers containing TBADH/$NADP^+$ (cuvette 1) and HLADH/NAD^+

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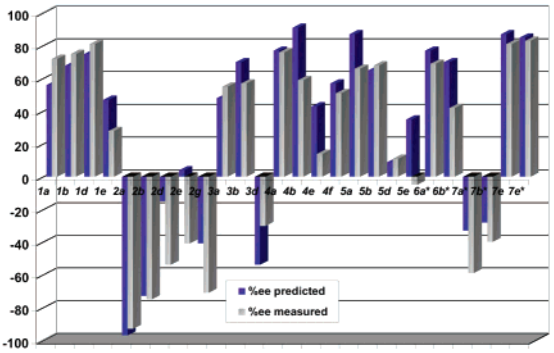

Figure 1. Predicted (double-cuvette ISES) vs observed enantioselectivities for the HKR of (±)-propylene oxide.

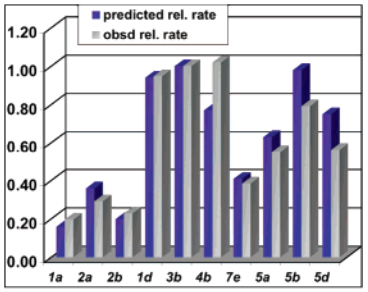

Figure 2. Predicted (ISES) vs observed (NMR) relative rates (normalized to **4b**) for the Co(III)−salen-mediated HKR of (±)-propylene oxide.

(cuvette 2), respectively, are then added. The increase in absorbance at 340 nm can be followed for several such sets in parallel by UV−vis spectrophotometry. The kinetic data are then used to make the double-cuvette ISES predictions. A comparison of the predicted and measured ee's is presented in Figure 1. Of 49 new salens targeted, 42 were obtained, and 25 gave active Co(III)-catalysts. Above a 65% ee threshold in either direction, ISES found 9 of 11 true hits (18% false negatives) and succeeded in 9 of its 12 predictions (25% false positives). Note that the measured ee's are for the HKR under the neat conditions typically used in Jacobsen's studies,[11] whereas the predicted ee's are for the bilayer used in double-cuvette ISES. Figure 2 compares the relative rates predicted by ISES with those observed in a bilayer by ^{1}H NMR. Observed relative rates agree with ISES predictions to within 25% in 9 of 10 cases measured, with the Co(III)-**5d** catalyst displaying slightly greater variance.

Interesting "combinatorial hits" include the finding that 2-hydroxy-1-naphthaldehyde (**c**) yields catalysts with very low activity, whereas 1-hydroxy-2-naphthaldehyde (**d**) is the best partner for β-pinene-derived diamine **1**. Furthermore, a remarkable inversion of stereoselectivity is observed for salens emanating from the new β-D-fructopyranose-based diamine **7**, upon going from the 3,5-di-*tert*-butylsalicylaldehyde partner to the sterically less encumbered 3,5-diiodo congener.

More work is needed to define the scope and limitations of double-cuvette ISES. Candidate reactions must tolerate some water, if run under biphasic conditions, but may be run under inert atmosphere.[8] The ISES method has the advantage that the reactant under study need not be altered by installing a chromophore. However, appropriate reporting enzymes (e.g., dehydrogenases) must be available that recognize the reaction product. In addition to other kinetic resolutions related to the HKR, it should be possible to apply double-cuvette ISES to reactions in which chirality is installed de novo in achiral substrates. Transformations such as carbonyl additions, ketone reductions, and alkene oxidations, for example, would appear to be good target reaction types with which to explore this approach.

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Supporting Information Available: Experimental methods and synthetic characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) HLADH displays an *S*:*R* relative velocity that increases from 2.6 to 3.9, in going from 100 to 4 mM fixed diol concentrations, whereas TBADH displays an *R*:*S* relative velocity that ranges from 8.4 to 10.7 between 80 and 3 mM. Consistent with this, for HLADH, $[(V_{max}/K_m)_S \div (V_{max}/K_m)_R] = 3.6$ and, for TBADH, $[(V_{max}/K_m)_R \div (V_{max}/K_m)_S] = 11$. Relative velocities do not vary greatly with concentration as the antipodal diol K_m's for each enzyme are in the range of 20 ± 5 mM. We thus set an approximate (enantio)selectivity factor for each enzyme across the concentration range of the ISES experiment. Expressions were then derived for the enantioselectivity (i.e., [(*R*)-diol]/[(*S*)-diol]) and for relative rate (i.e., total diol), assuming that observed ISES rates (ΔAbs/time) could be approximated as the {*R*-Units} × {[(*R*)-diol] + ([(*S*)-diol]/Sel)} (see SI for details).

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