

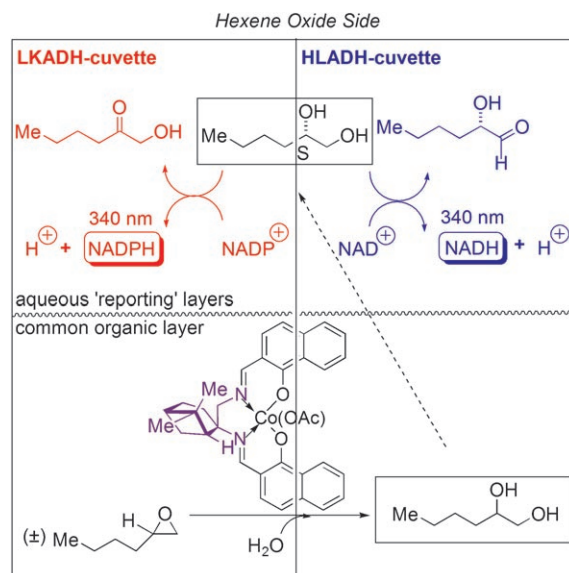
“Cassette” In Situ Enzymatic Screening Identifies Complementary Chiral Scaffolds for Hydrolytic Kinetic Resolution Across a Range of Epoxides**

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For combinatorial catalysis,^[1,2] rapid and information-rich screening methods are very useful. Toward this end, we describe herein a new “cassette” in situ enzymatic screening (ISES) approach that allows the experimentalist to obtain a parallel readout on substrate specificity, as well as a sense and magnitude of enantioselectivity.^[3] In its first iteration, cassette-ISES is used to “cherry pick” only those catalysts in the array that show high ISES *ee*-value readouts across both test substrates. Two such catalysts are then investigated further, yielding promising results.

In our ISES approach, typically the reaction product^[4] or by-product^[5] diffuses from an organic layer into an aqueous layer containing the “reporting enzyme”.^[6–9] There, an enzyme-catalyzed reaction leads to a spectroscopic signal that is monitored in real time. The approach complements other emerging screens by using chiroptical techniques,^[10] liquid crystalline arrays,^[11] IR thermography,^[12] mass,^[13] NMR,^[14] IR^[15] and fluorescence spectroscopy.^[16] The technique is sensitive (i.e. 10 nmol of product gives rise to $\Delta A_{340} \approx 0.12$ for a dehydrogenase reporting enzyme in a 500 μL aqueous volume), allowing one to get information on catalyst performance at relatively early conversions/short reaction times. Catalysts may be screened in parallel in a standard spectrophotometer with a multicell changer without the need to draw aliquots or work up the reaction. Moreover, the need to install a chromophore (adding steps and potentially altering substrate reactivity) is obviated.

Herein, we describe a new pair of reporting enzymes (Scheme 1) that are capable of differentiating the 1,2-



Scheme 1. A suitable pair of “reporting enzymes” for 1,2-hexanediol permits a cassette-ISES evaluation of HKR catalyst candidates. Each “side” of the cassette screens for a particular substrate, and comprises a pair of cuvettes with identical (lower-CH₂Cl₂) organic layers but distinct reporting dehydrogenases in the (upper) aqueous layer. NADPH = nicotinamide adenine dinucleotide phosphate, NADH = nicotinamide adenine dinucleotide.

hexanediol antipodes (*Lactobacillus kefir* alcohol dehydrogenase (LKADH): highly *S* selective $k_S/k_R \approx 20$, and horse-liver alcohol dehydrogenase (HLADH): modestly *S* selective $k_S/k_R \approx 2.2$). This allows one to obtain simultaneous enantioselectivity readouts on two distinct substrates for the Co^{III}-salen-mediated (salen = (salicylidene)ethylenediamine) hydrolytic kinetic resolution (HKR) of epoxides,^[17] presenting both “short” (propylene oxide; R = Me)^[4] and long (hexene oxide; R = Bu) R groups. In this way, one can begin to address the question of substrate generality, which is so important in asymmetric catalysis today.^[18,19]

To demonstrate proof of principle for cassette-ISES, we employed a focused chiral salen array (Figure 1) that crosses chiral space variation in the constituent 1,2-diamines with considerable steric^[20] and electronic variation in the “salicylaldehyde partners” (including the benzoylacetalddehyde (baen) precursor^[21]). Figure 2 illustrates the “four coordinate” structure–enantioselectivity relationship (SER) data that one obtains from such a cassette-ISES protocol. The *x* and *y* axes represent the two structural variables in the salen array. The directionality and length of the *z* vector provide the

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 Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

1	56(S) 72(S) 6.9(S)	93(S) 80(S) 12(S)	47(S) 28(S) 2.1(S)	36(S) 53(S) 4.6(S)	** 56(S) 71(S) 6.8(S)	75(S) 81(S) 12(S)	94(S) 76(S) 13(S)	** □
	87(S) 66(S) 6.4(S)	64(S) 67(S) 5.8(S)	35(S) 5(R) 1.1(R)	□	** □	9(S) 11(S) 1.3(S)	17(S) 0 1(S)	** □
2								
	81(R) 74(R) 9.2(R)	96(R) 72(R) 7.0(R)	8(R) 12(R) 1.3(R)	29(R) 5(R) 1.1(R)	** □	8(R) 20(R) 1.5(R)	27(R) 21(R) 1.7(R)	** □
3								
	77(R) 82(R) 15(R)	84(R) 86(R) 15(R)	22(R) 5(R) 1.4(R)	□	** □	31(R) 14(R) 1.3(R)	18(R) 22(R) 1.6(R)	** □
4								

Figure 1. Structure versus enantioselectivity profile for the Co^{III}-acetate catalysts derived from this 5 × 4 salen/baen ligand library. Within a box, the entries (top to bottom) represent the ISES-estimated *ee* values, the observed *ee* values (flask conditions: neat, RT), and the calculated *E* value. Black (left) and blue (right) columns designate results with propylene oxide and hexene oxide, respectively. ** and □ denote slow catalysts showing enzymatic reporting rates of 15 mAbs min⁻¹ for propylene oxide and hexene oxide, respectively.

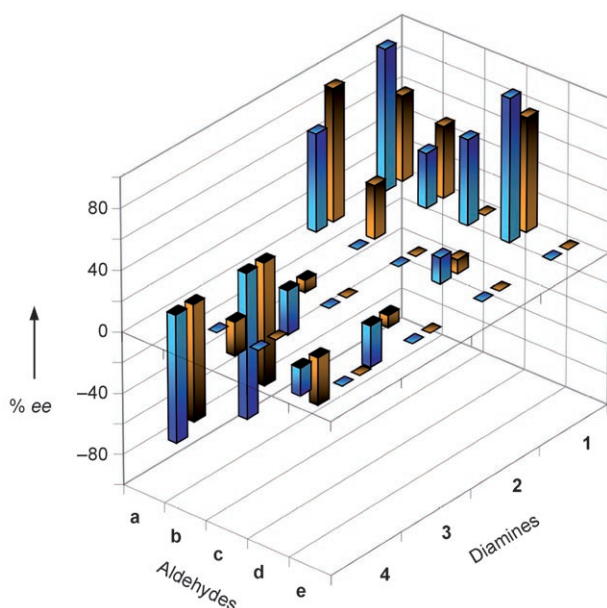


Figure 2. Three-dimensional view of the enantioselectivity output from cassette-ISES for the 5 × 4 ligand array. For a given catalyst, brown and blue bars represent predicted *ee* values for propylene oxide and hexene oxide, respectively. A positive deflection indicates *S* selectivity, whereas a negative deflection indicates *R* selectivity (convention follows the 1,2-propanediol optical rotation sign).

sense and magnitude of enantioselection, respectively. The fourth dimension is substrate variation, which is represented by color-coded bars.

Several trends are apparent from this cassette-ISES readout. Unfortunately, the baen ligands (**e**) do not appear to produce effective Co^{III}-based HKR catalysts. Conversely, the most versatile diamine partner in the array is **1**, which successfully confers *S* selectivity, particularly for the hexene oxide test substrate, upon all of its derivative salens. This chiral element was first constructed through a clever Mn^{III}-mediated diazidation of β -pinene by Snider,^[22] and appears from these findings to have significant (as yet untapped) potential in asymmetric catalysis.

In seeking to “cherry pick” the catalysts with the most promise for both high enantioselectivity and generality,^[18] one quickly gravitates to ligands **1d**, **3a**, and **4a**. We chose **1d** and **4a**, as representative *S*- and *R*-selective cassette-ISES hits, for further development. These were next exposed to a more-extensive battery of epoxides that differ in functionality, sterics, polarity, and position and electronic nature of π surfaces presented.

As can be seen in Table 1, catalyst Co^{III}-**1d**-OAc does indeed appear to retain “*S*” antipodal selectivity across the epoxide library. Several substrates presenting π surfaces at intermediate chain lengths from the epoxide are well matched with this catalyst. Particularly striking are the HKR’s with *O*-phenylglycidol (*E* value $\approx$ 20) and 4-benzyloxybut-1-ene oxide (*E* value $\approx$ 65!). Moreover, the selectivity associated with the HKR of the 2'-acetyl-4'-nitro-*O*-phenylglycidol [Table 1, entry 7. The remaining epoxide (96% *ee*) has the correct handedness^[23] for the drug), though not quite as spectacular, provides a formal new route^[24] into the β -blocker celiprolol.

Table 1: Kinetic resolutions with the Co^{III}-**1d**-OAc Catalyst.

Entry	Epoxide	Loading [mol %]	Conditions ^[a]	Isolated epoxide (yield [%], <i>ee</i> [%], <i>E</i> value)	Isolated diol (yield [%], <i>ee</i> [%], <i>E</i> value)
1		5	A	51,45 (<i>R</i>), 4	37, 52 (<i>S</i>), ^[b] 4
2		5	B	46,70 (<i>R</i>), 8	52, 67 (<i>S</i>), ^[c] 11
3		2	B	71,27 (<i>R</i>),6	29, 71 (<i>S</i>), ^[c] 8
4		2	B	70,33 (<i>R</i>), 11	29, 79 (<i>S</i>), ^[c] 12
5		2	B	50,80 (<i>S</i>), ^[d] 22	45, 80 (<i>R</i>), ^[d,e] 18
6		3	B	48,96 (<i>R</i>), 65	50, 91 (<i>S</i>), ^[e] 67
7		10	C	37, 96 (<i>S</i>), ^[d,f] 13	63, 55 (<i>R</i>), ^[d,g] 11

[a] Conditions: A: 12 h, 0°C, neat; B: 12 h, 0°C, THF; C: 24 h, RT, THF. [b] Stereochemistry assigned by comparison of HPLC retention time with that of authentic standards^[17] (see the Supporting Information for details). [c] In these cases, absolute stereochemistry was assigned by analogy with catalyst selectivity with structurally related substrates. [d] The sense of enantioselection is the same for these examples, but substituent priorities lead to the opposite configurational assignment. [e] Absolute stereochemistry assigned by comparison of the observed optical rotation with literature values (see reference [17]). [f] The *R* epoxide was independently synthesized from (*S*)-glycidol and matches the minor peak found here (Chiralcel OD). [g] Stereochemistry assigned by relative HPLC retention time (Chiralcel OD, reference [24]).

In a complementary fashion, the Co^{III}-**4a**-OAc catalyst apparently displays rather general “*R*” bias for epoxide ring opening (Table 2). Here, epoxides bearing long-chain alkyl groups such as 1,7-octadien-1-oxide,^[25] and 6-*tert*-butyldiphenylsilyloxy-hex-1-ene oxide appear to be especially well resolved. Although *O*-phenylglycidol shows almost perfectly mirrored enantioselectivity here, the celiprolol substrate is processed much less selectively.

Three-dimensional X-ray crystal structures of both Co^{II}-**1d** and Co^{II}-**4a** have been obtained. If one assumes that upon oxidation to Co^{III}, the acetate ligand coordinates axially to the least-hindered face,^[26] then one can begin to examine the available chiral epoxide-binding surface at the opposite face. A schematic model^[27] wherein the preferred approach of the *S*-antipode from the “front left quadrant”, as shown in Figure 3, is proposed for the Co^{III}-**1d**-OAc system. This raises the intriguing possibility of favorable π - π interactions between the α -hydroxy- β -naphthaldehyde platform and aryl substituents on the best-resolved substrates. On the other hand, for Co^{III}-**4a**-OAc, a “front-right quadrant” approach of the opposite enantiomer is suggested. In this case, no such π - π interactions would be accessible to intermediately spaced aryl substituents. Rather, these might have to “thread the

needle” in avoiding the bulky *tert*-butyl groups upon coordinating to the Co^{III} center.

In conclusion, the cassette procedure described herein, with read-out on both enantio- and substrate selectivity, makes for an especially information-rich in situ enzyme-based parallel screen. An interesting subtlety is the fact that the two 1,2-hexanediol reporting enzymes introduced herein (LKADH and HLADH) are both *S* selective but differ greatly in the magnitude of that selectivity. This difference in selectivity allows us to pick up both *S* and *R* selective catalysts for hexene oxide ring opening. On the discovery side, this combinatorial approach has uncovered some rather unconventional scaffolds for asymmetric catalysis. For example, one of our best HKR ligands, **1d**, is assembled from a non-*C*₂-symmetric, terpene-derived chiral diamine and a sterically unencumbered α -hydroxy- β -naphthaldehyde partner and yet shows remarkable enantio-discrimination (*E* value $\approx$ 65 for 4-benzyloxybutene oxide!). Given the rapidly expanding menu of metal-salen-mediated C-X bond-forming reactions, this ligand scaffold

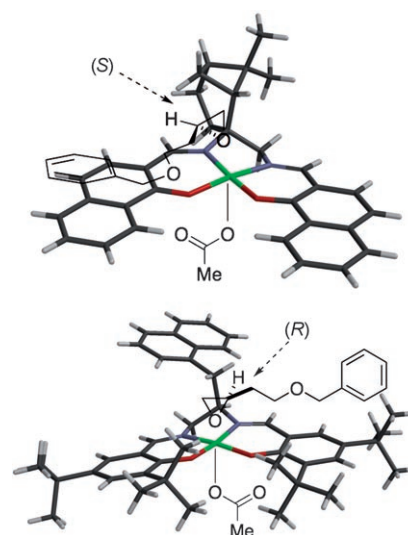


Figure 3. Three-dimensional tube representation (Spartan 1.0.3) of X-ray crystallographic structures determined for the Co^{II}-**1d** (top) and Co^{II}-**4a** (bottom) complexes. Superimposed upon the structures is presented a model that is consistent with the observed Co^{III}-salen enantiopreferences.

Table 2: Kinetic resolutions with the Co^{III}-4a-OAc Catalyst.

Entry	Epoxide	Loading [mol %]	Conditions ^[a]	Isolated epoxide (yield [%], ee [%], E value)	Isolated diol (yield [%], ee [%], E value)
1		1	A	70, 33 (S), 11	26, 72 (R), ^[b] 8
2		1	A	44, 92 (S), 20	56, 72 (R), ^[c] 19
3		4	B	59, 55 (S), 16	39, 80 (R), ^[d] 15
4		2	B	59, 53 (S), 13	37, 75 (R), ^[d] 11
5		2	B	45, 93 (R), ^[e] 25	55, 72 (S), ^[e,f] 17
6		1	B	82, 17 (S), 9	18, 80 (R), ^[f] 11
7		10	B	62, 28 (R), ^[g] 3.5	37, 46 (S), ^[e,h] 3.5

[a],[b] as described in Table 1. [c] Assigned by comparison of optical rotation with that of the known R diol.^[25] [d]–[f] See Table 1, footnotes [c]–[e], Table 1. [g] The sign of the optical rotation was found to differ with that reported (the S epoxide is said to be levorotatory^[24]), however, the R epoxide was found to be levorotatory here). To confirm the absolute stereochemistry, the R epoxide was synthesized independently from (S)-glycidol. [h] See Table 1, footnote [g].

fold will likely find application well beyond the HKR chemistry reported herein.

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