Enantioselective epoxide hydrolysis: catalysis involving microbes, mammals and metals

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Although alkyl epoxides are difficult to synthesize in enantiomerically pure form they can often be prepared by the enantioselective hydrolysis of racemic epoxide. The best methods for this transformation are all catalytic and they illustrate the complementary role that biological and synthetic catalysts can play in organic chemistry.

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Why should researchers in the biological sciences care about epoxides?

Organic chemists have devoted tremendous effort to the preparation of enantiomerically pure epoxides and the use of epoxides in organic synthesis. Although it is assumed that the organic chemists among the readership of *Chemistry & Biology* are probably convinced of the value of this functional group, the epoxides may not seem as important to those scientists who aren't in the business of making molecules. There are, for purposes of this discussion, two practical reasons to care about epoxides. First, epoxides are a common structural element in a great many biologically active molecules, both simple and complex (Figure 1). Second, they undergo facile, stereoselective ring-opening reactions with a wide range of nucleophiles, making them versatile starting materials and intermediates for the synthesis of compounds that have interesting biological activity (Figure 2) [1,2].

The nature of the ring-opening reactions raises an important issue — that of chirality. The vast majority of compounds that have an interesting biological function are chiral, and the different stereoisomers often have different biological activities. As epoxides are involved in the preparation of biologically useful compounds, it is advantageous that epoxides are stereochemically pure when used as a substrate.

Why should anyone care about epoxide hydrolysis?

Effective methods have now been discovered for the enantioselective conversion of several classes of alkenes to the corresponding epoxides. The two most extensively developed reactions — the Sharpless [3] and Jacobsen [4] epoxidations — allow the catalytic, enantioselective preparation of epoxides from allylic alcohols and conjugated olefins, respectively (Figure 2), and these processes now provide routine access to many of the substituted epoxides within these structural categories. Although the enzymatic oxidation of alkenes has met with some success [5], methods for the enantioselective preparation of epoxides from monosubstituted or alkyl olefins have largely remained elusive. As illustrated in Figure 1, this is not for want of importance.

In the absence of direct chemical methods for the enantioselective synthesis of terminal or alkyl epoxides, numerous alternative procedures have been explored, including indirect synthesis and stoichiometric resolution using an external chiral reagent [6–8]. An intriguing alternative to these strategies is the preparation of enantiomerically pure epoxides by kinetic resolution [9], a process in which one of the enantiomers of a racemic epoxide is selectively degraded, leaving behind the other enantiomer. When the selective process is the catalytic hydrolysis of the epoxide with water (Figure 3), the transformation has the additional appeal of using an environmentally benign compound (water) as the only stoichiometric reagent, and providing a potentially valuable byproduct, the chiral diol. (Effective methods for the direct enantioselective synthesis of diols from olefins are available, however [10].)

Figure 1

Biologically important epoxides.

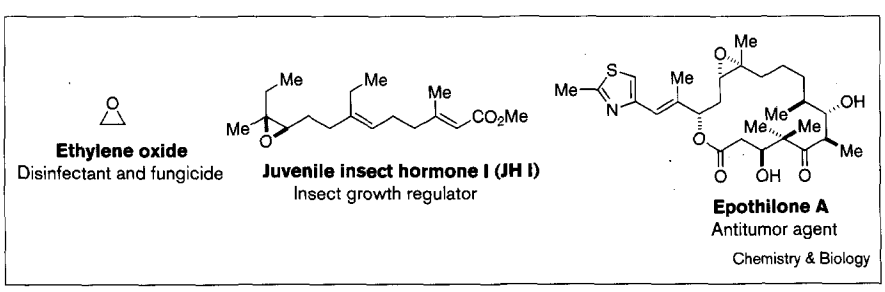
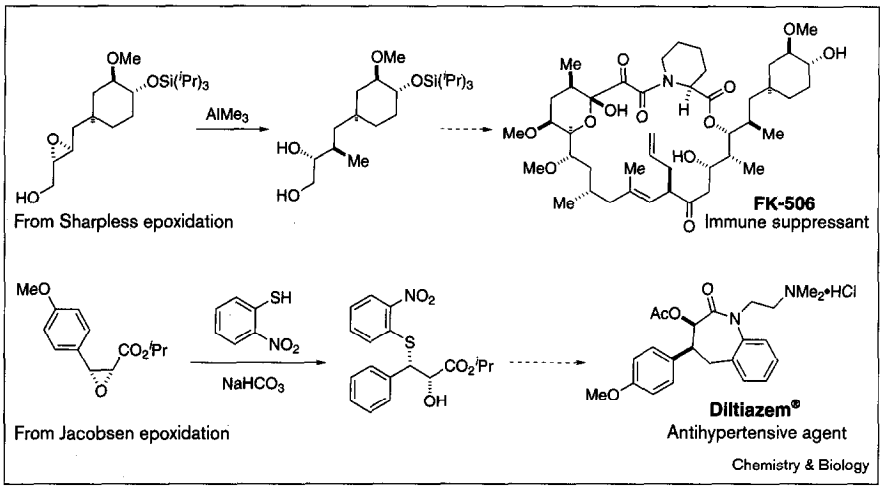


Figure 2



The use of epoxides in the synthesis of biologically active molecules. The enantioselective epoxidation of an allylic alcohol was a key transformation early in the synthesis of the immune suppressant FK-506 by Schreiber *et al.* [1]. Similarly, the enantioselective epoxidation of a cinnamate ester served as the basis for Jacobsen's synthesis of the antihypertensive agent Diltiazem® [2]. (Ac, acetyl; Me, methyl; Pr, isopropyl.)

As elegant as the hydrolytic kinetic resolution (HKR) shown in Figure 3 may be in principle, it has a built-in limitation: the yield of enantiomerically pure epoxide is limited to a maximum of 50%. However, many useful alkyl epoxides are either commercially available at low cost or can be readily prepared from the corresponding olefins, making HKR processes currently one of the most effective methods for gaining enantioselective access to this important class of compounds. Indeed, as will be discussed, for some classes of alkyl epoxides HKR is far and away the method of choice.

Available methods for HKR

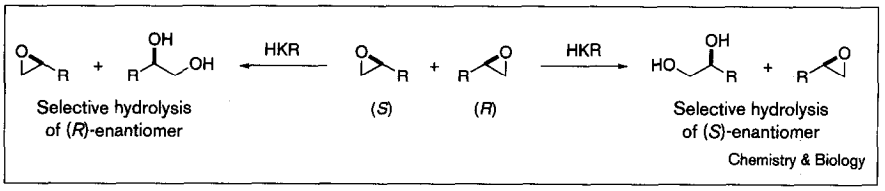
To date, all of the effective methods for the HKR of epoxides are catalytic, so the benefits of catalytic reagents for asymmetric transformation will not be discussed at length here. (Among these benefits are lowered net reagent consumption, minimized waste generation, and often, as in the present case, access to enantioselective reactions for which there is no noncatalytic alternative.) The catalysts known to effect epoxide HKR may be divided into three categories: enzymes, of both microbial and mammalian origin, catalytic antibodies derived from the mammalian immune system, and synthetic catalysts.

The first class of catalysts — enzymes — has been the most extensively studied, whereas the use of catalytic antibodies and synthetic catalysts in epoxide HKR have only recently been demonstrated, each by a single published example. As might be expected, each type of catalyst has characteristic strengths and weaknesses. These will be discussed with an eye towards practical utility. Where possible, literature examples will be evaluated using several criteria previously enumerated for the practical comparison of asymmetric catalytic systems: enantioselectivity (expressed as % enantiomeric excess (ee), % ee = % major enantiomer – % minor enantiomer); the amount of catalyst required to transform a given amount of starting material (and the volume of the solution in which the transformation is carried out); the accessibility and cost of the catalyst; and how the method compares to alternative strategies [11]. The last criterion will be addressed, in part, by focusing on reactions for which equivalent direct alternatives do not exist.

Enzymatic catalysts for HKR – an overview

Unlike the other methods for HKR, enzymatic resolution has been known long enough to have a history to relate. The class of enzymes known to be active in the hydrolysis of epoxides are, unsurprisingly, called epoxide hydrolases. Epoxide hydrolases are present in most living organisms, including fungi, bacteria, plants, insects and mammals [12,13]. Although certain specialized epoxide hydrolases have very specific regulatory functions (such as the enzyme responsible for hydrolyzing juvenile insect hormone I,

Figure 3



Hydrolytic kinetic resolution (HKR) of an alkyl epoxide.

Figure 4

General mechanism for the enzymatic hydrolysis of epoxides.

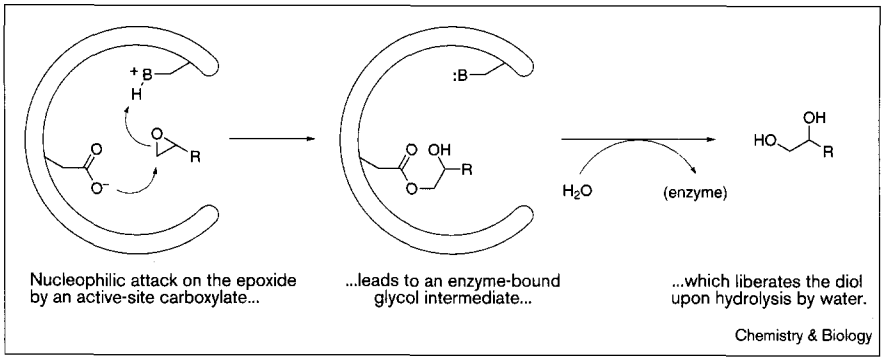


Figure 1) [14], the predominant collective role of epoxide hydrolases is xenobiotic detoxification by degrading exogenous epoxides or the oxidation products of exogenous olefinic compounds. Although the enzymes from different organisms, and even enzymes from within the same organism, do not behave in exactly the same way, the general consensus is that they are all derived from a common evolutionary ancestor, a predecessor that also gave rise to the haloalkane hydrolases. Several of the epoxide hydrolases have been characterized at the level of the gene (corroborating the preceding assertion of a common ancestor), and in some cases have been successfully overexpressed [12,13].

A general mechanism for enzymatic epoxide hydrolysis is shown in Figure 4. The most extensive mechanistic studies have been conducted on enzymes of mammalian origin [15], but in the light of the qualitative similarities among the epoxide hydrolases of different species, it is widely accepted that most epoxide hydrolases function in a similar manner. Two features of the reaction in Figure 4 should be noted, as the illustration somewhat understates their importance. First, epoxide opening by the active-site carboxylate proceeds with inversion of configuration at the center undergoing nucleophilic attack. Second, nucleophilic opening of the epoxide can occur at either carbon center (even with terminal epoxides), and enzymatic hydrolysis is not uniformly regioselective. Epoxide hydrolases from different species have widely differing regioselectivities, and span the range from completely selective to completely nonselective.

Enzymatic catalysts for HKR – selected case studies

Enzymatic catalysis is often characterized by exquisite selectivity coupled with limited substrate scope. HKR by epoxide hydrolases is no exception, and it is usually the case that a given enzyme, if highly selective, exhibits this selectivity for only a few substrates. The number of known epoxide hydrolases partially offsets this limitation, however; there are now established, if not always practical, enzymatic resolutions for several terminal epoxides and at least representative members of most structural classes of alkyl epoxides (Figure 5). To illustrate the salient features of HKR by epoxide hydrolases, three of these substrates will be examined in greater detail.

The HKR of *trans*-2-butene oxide (Figure 6) demonstrates the potential of epoxide hydrolases in organic chemistry — an inexpensive racemic epoxide is resolved to a precious chiral compound with yield and ee close to the theoretical maximum, a result for which there is simply no nonenzymatic equivalent [16]. Several limitations are also apparent, however. First, the yeast strain in question (*Rhodotorula glutinis* CIMW 147) is not commercially available. Second, only one enantiomer of the epoxide can be prepared in this manner, another signature limitation of enzymatic synthesis. Third, the reaction is carried out by incubating the epoxide in the fungal fermentation. Although this is not a concern for those already in the habit of routinely culturing fungi, it represents a significant impediment for synthetic chemists who have no background in such techniques. Finally, even those for

Figure 5

Representative epoxides that can be resolved to > 95% ee by HKR with epoxide hydrolases.

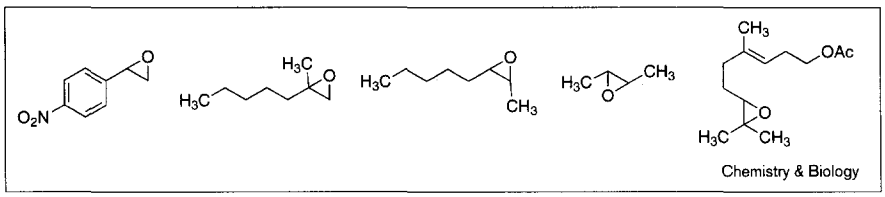
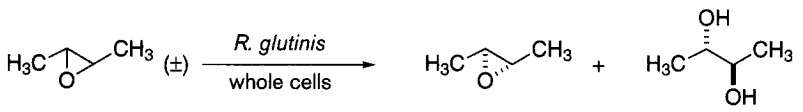


Figure 6



Weight of substrate	Weight of catalyst	Volumetric productivity	Yield Epoxide	Yield Diol	ee Epoxide	ee Diol
0.014g	1 g	≈ 0.4 g l^{-1}	47%	–	> 98%	(meso)

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Nearly perfect HKR of a *trans*-dialkyl epoxide by a fungal epoxide hydrolase. *R. glutinis*, *Rhodotorula glutinis* CIMW 147.

whom culturing yeast is not an intimidating prospect will have to come to terms with the fact that the volumetric productivity is rather low, and a large quantity of catalyst is required to transform a proportionally small quantity of substrate. (Extrapolating from the literature report, preparing 1 g of enantiomerically pure epoxide would require over 150 g, dry weight, of yeast cells.)

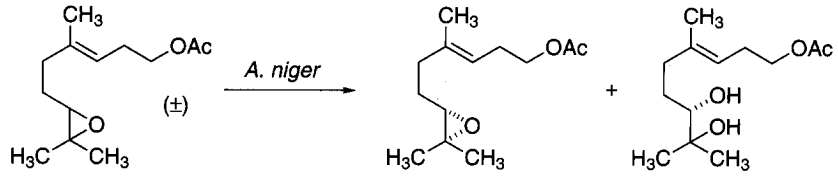
It should be noted that the large quantity of catalyst required (the catalyst weight-loading) for the above procedure is a reflection of the use of whole fungal cells — the active enzyme is only a small proportion of the weight of the entire organism. In practice, however, the amount of material that a researcher must handle is really the relevant parameter. The use of purified, or even partially purified, preparations of microbial and mammalian epoxide hydrolases leads to significantly lower catalyst weight-loadings, but the requirement of an isolated enzyme that is not commercially available moves the application even further out of the reach of most experimental chemists. (This is especially the case for mammalian epoxide hydrolases, which are typically isolated in very small quantities from fresh liver.)

Cautionary notes aside, epoxide hydrolases do have practical application; two illustrative examples are shown in Figures 7 and 8, both mediated by the fungus *Aspergillus niger*. In the first case (Figure 7) [17], which employs a whole-cell preparation of the fungus, an analog of the juvenile insect hormones known as Bower's compound was resolved on preparative scale. Although the diol products of this type can be prepared with greater enantioselectivity by other means [10], the ability to obtain gram quantities of nearly enantiomerically pure epoxide from a 1 l fermentation is quite attractive. Even more impressive is the recently reported resolution of *p*-nitrostyrene oxide (Figure 8) [18], a precursor to the β-blocker Nifénalol. This reaction has the highest volumetric productivity reported for an epoxide-hydrolase-mediated HKR.

Catalytic antibodies for HKR

For those not familiar with this area of research, antibody catalysts are usually identified by the following sequence: inoculating an animal with a hapten (such as bovine serum albumin) to which a transition-state analog for the reaction of interest has been attached; isolating the antibodies raised by the immune system in response to this hapten; identifying the antibodies capable of binding the transition-state analog and catalyzing the reaction; and cloning the antibodies to obtain sufficient amounts of monoclonal antibody for further study [19,20]. The potential of antibodies to serve as catalysts for important

Figure 7



Weight of substrate	Weight of catalyst	Volumetric productivity	Yield Epoxide	Yield Diol	ee Epoxide	ee Diol
5g	–	≈ 2g l^{-1}	42%	43%	96%	40%

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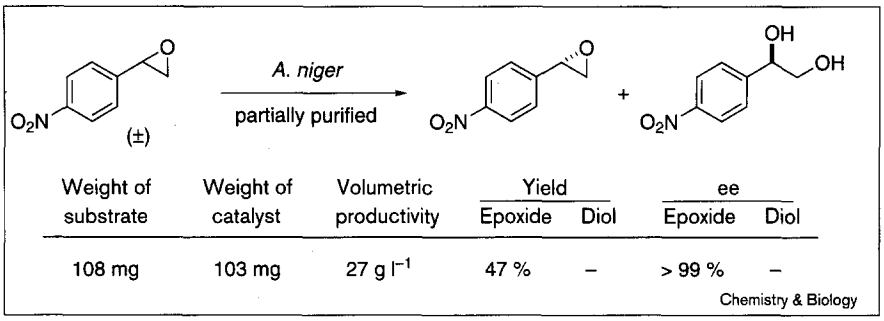
Application of epoxide hydrolases to the preparation of Bower's compound, an analog of the juvenile insect hormones.

Figure 8

Preparative epoxide-hydrolase-mediated resolution of a styrene oxide.



Weight of substrate	Weight of catalyst	Volumetric productivity	Yield Epoxide	Yield Diol	ee Epoxide	ee Diol
108 mg	103 mg	27 g l^{-1}	47 %	–	> 99 %	–

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synthetic transformations has been a topic of extensive discussion in recent years. This potential has seen preparative (i.e., gram-scale) realization in only a few cases, such as the HKR of a fluorinated ester [21], but catalytic antibodies still hold the tantalizing prospect of bridging the gap between the tremendous selectivities of enzymes and the broad substrate scope more often associated with synthetic catalysts.

To date, there has been only one report of the application of catalytic antibodies to enantioselective epoxide hydrolysis (Figure 9) [22]. Reactions employing catalytic antibodies usually have many of the same restrictions associated with enzymatic catalysis: their execution requires significant expertise generally not possessed by the researchers most interested in preparing enantiomerically pure epoxides, and the procedures are often plagued by high catalyst weight-loading and low volumetric productivity. Although the present system does not appear to be exempt from these limitations, the data presented in Figure 9 were extracted from a preliminary communication, and the reaction has almost certainly been optimized further.

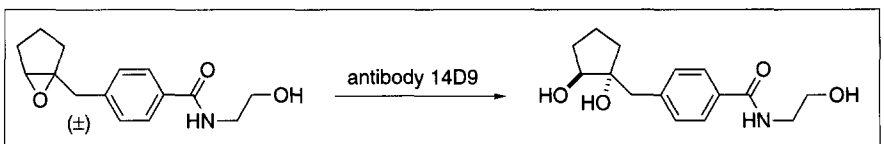
Synthetic catalysts for HKR

The recent report of the HKR of terminal epoxides by chiral (salen)Co catalysts represents the first successful application of synthetic catalysts to this reaction (Figure 10; [23], E.N. Jacobsen, unpublished observations). The catalyst system is remarkable in almost every respect: it employs relatively low weight-loadings of an inexpensive, reusable, commercially available catalyst; it exhibits enzyme-like selectivity for a range of terminal epoxides, and can be applied to the preparation of either epoxide enantiomer; and it requires no solvent (just epoxide, ≈ 0.5 equivalents of water, and the catalyst), and, as such, its volumetric productivity exceeds that of almost any catalytic reaction known.

The method is currently the most convenient and effective for the large-scale preparation of optically active monosubstituted epoxides. The practical use of this reaction is demonstrated by the hydrolysis of propylene oxide to provide completely resolved epoxide and diol, a process that has been used to prepare a cumulative total of > 700 kg of resolved propylene oxide (Chirex, Dudley, UK; E.N. Jacobsen, personal communication). It is also worth noting that for this particular substrate, the (salen)Co system represents the most enantioselective route for the preparation of the diol as well as the epoxide. The only apparent limitation of this chemistry is the narrow substrate scope, which is, ironically, enzyme-like. The system is still under investigation, however; efforts to expand the method to include other epoxide substitution patterns are underway, as are synthetic applications (E.N. Jacobsen, unpublished observations).

Figure 9

HKR of a trisubstituted alkyl epoxide using a catalytic antibody. Catalyst weight was calculated by assuming a molecular weight of 150,000 (150 kDa) for the monoclonal antibody.



Weight of substrate	Weight of catalyst	Volumetric productivity	Yield Epoxide	Yield Diol	ee Epoxide	ee Diol
0.24 mg	11 mg	≤ 0.13 g l^{-1}	–	–	–	87%

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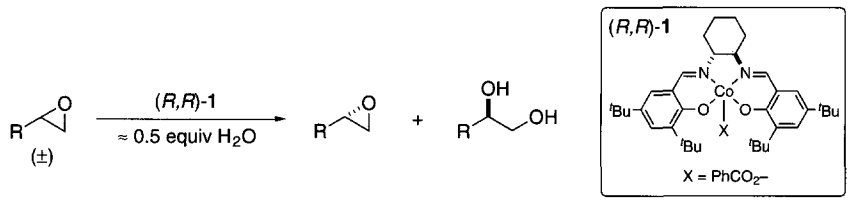
Figure 10



R	Weight of substrate	Weight of catalyst	Volumetric productivity	Yield		ee	
				Epoxide	Diol	Epoxide	Diol
CH_3	58 g	1.4 g	≈ 325 g l^{-1}	44%	50%	>98%	98%
CH_2Cl	100 g	2.3 g	≈ 225 g l^{-1}	44%	38%	98%	86%
Ph	40 g	0.5 g	≈ 375 g l^{-1}	38%	39%*	98%	98%*

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HKR of terminal epoxides with a synthetic catalyst. Volumetric productivities were calculated based on epoxide yield only; * denotes data after one recrystallization. 'Salen' is the common name for the type of organic chelating ligand wrapped around the cobalt centre in *(R,R)*-1, and the catalyst is thus referred to as a '(salen)Co' complex. The ligand shown is commercially available, but can also be readily prepared by the condensation of a chiral diamine (in this case, *(R,R)*-cyclohexanediamine) with two equivalents of the appropriate *ortho*-hydroxy aromatic aldehyde. This ligand has been successfully employed in the Jacobsen asymmetric epoxidation and asymmetric ring-opening reactions as well [4,26].

Future directions and opportunities

Enzymatic and antibody catalysts will have to become more accessible to synthetic chemists to become more widely used. There are a number of ways in which this change might be effected. For example, the current experimental procedures could be brought into synthetic chemists' regimen through increased training or collaboration. It would also help if enzyme or antibody preparations were commercially available. (Note that to provide access to a wide substrate pool, a number of these catalysts would have to be commercialized). Although not a trivial undertaking, the success of commercially available hydrolytic enzymes for the kinetic resolution of racemic chiral esters suggests that this is not out of the question. Alternatively, current methodology could be improved to the point that it can be carried out on an industrial scale. At least for simple epoxides, which are to be used at the early stages of a synthesis, many chemists would actually be happier purchasing the enantiomerically pure material rather than making it themselves. Again, although this would be no small task, the laudable resolution of *p*-nitrostyrene oxide (Figure 8) and the interest of a major pharmaceutical company in the epoxide hydrolase-catalyzed resolution of indene oxide indicate that such possibilities are being actively investigated [24].

Further application of the (salen)Co-catalyzed HKR will probably depend on whether the method can be extended to substrates other than terminal epoxides. Although it is not possible to predict the future of a new asymmetric catalyst system on the basis of a single report, the fact that (salen)Co complexes catalyze the enantioselective ring-opening of substituted epoxides (such as cyclohexene oxide) with other nucleophiles bodes well for future efforts [25].

The HKR of terminal and alkyl epoxides will clearly have a secure place among valued asymmetric catalytic methods, at least until direct enantioselective epoxidation of olefins becomes a viable option. Even if such direct epoxidations are developed, they will have to match or exceed the efficiencies of current HKR procedures to be truly competitive and with the standard now set by the (salen)Co HKR system, this will be no mean feat.

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