[13] Under DCC-mediated esterification conditions, we observe a small degree of epimerization of the *N*-acylprolines, but this does not interfere with the present application.
[14] The use of more concentrated samples, containing approximately 25 nmol of desired esters or amides along with the other components of the acylation reaction mixtures, showed poorer sensitivity and gave less accurate results (standard deviations for repeat injections of approximately 5%).
[15] $s = [\%\ ee(y+1) + 100(y-1)]/[\%\ ee(y+1) - 100(y-1)]$ for 100% *ee*, $s = y$.
[16] a) T. F. Spande, H. M. Garraffo, M. W. Edwards, H. J. C. Yeh, L. Pannell, J. W. Daly, *J. Am. Chem. Soc.* **1992**, *114*, 3475–3478; b) J. W. Daly, *J. Nat. Prod.* **1998**, *61*, 162–172; c) Z. M. Chen, M. L. Trudell, *Chem. Rev.* **1996**, *96*, 1179–1193.
[17] For example, diol **7** was analyzed using the masses corresponding to its derived diesters containing two units of each mass tag (and ignoring diesters having one of each of the mass tags). It appears that the first derivatization of the primary alcohol occurs with little or no diastereoselectivity, whereas the secondary alcohol is esterified with $s \approx 2$. Thus, the analysis of chiral compounds such as diols containing multiple functionalities should also be feasible.
[18] Note, for example, that not all of the compounds analyzed here could have been accomodated by a single chiral HPLC or GC column. Of course, the techniques are related in that the chemical interactions responsible for molecular discrimination in chromatography are likely to be similar to those giving rise to diastereoselectivity in the derivatization process that preceeds mass spectrometry readout.
[19] Each mass spectrometry injection presently requires approximately two minutes. The analysis can be made more efficient by combining compounds of differing molecular weights in each injection.
[20] Our present sensitivity limit for quantitative ESI-MS of small molecules is approximately 500 fmol per μL.[6]

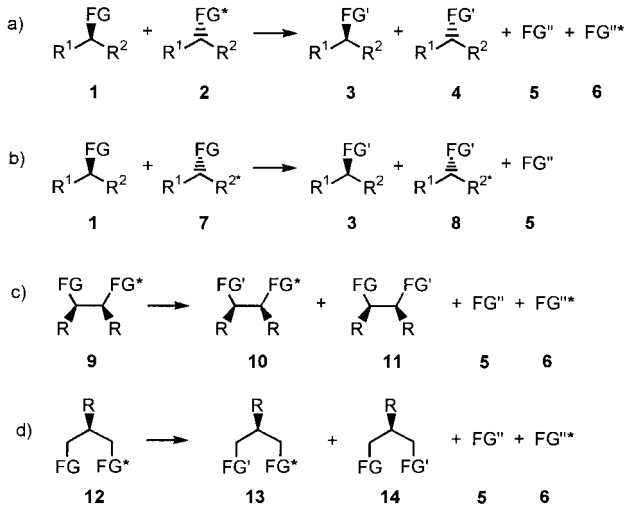
A Method for High-Throughput Screening of Enantioselective Catalysts**

Manfred T. Reetz,* Michael H. Becker, Heinz-Werner Klein, and Detlef Stöckigt

Whereas the principles of combinatorial chemistry are well established in pharmaceutical research,[1] extension to the area of catalysis is not as advanced.[2] One reason is that few general methods for high-throughput screening of heterogeneous and homogeneous catalysts have been devised. This applies all the more to enantioselective catalysts.[3] We have previously developed a screening system for the catalytic enantioselective hydrolysis of chiral *p*-nitrophenol esters in which the course of the reactions of the *R*- and *S*-configured substrates is monitored in a parallel manner by UV/Vis spectroscopy.[4] With the use of microtiter plates crude screening of about 800 different enantioselective catalysts is possible per day, in this case mutant lipases created by directed evolution.[4, 5] Accordingly, following expression of a library of mutant genes in *E. coli*/*P. aeruginosa*, the bacterial colonies on agar plates were collected and cultivated individually in the wells of microtiter plates, each supernatant containing a mutant lipase suitable for screening. By nature this particular screening system is restricted to chiral acids and cannot be used in the evaluation of asymmetric catalytic reactions involving chiral alcohols, diols, amines, amino alcohols, alkyl halides, or epoxides. Recently, IR thermography was introduced as a means to detect metal- or enzyme-catalyzed enantioselective reactions, but quantification still needs to be accomplished.[6]

We now describe a method based on electrospray ionization mass spectrometry (ESI-MS)[7] which enables the determination of enantioselectivity in about 1000 catalytic or stoichiometric asymmetric reactions per day. Two basically different stereochemical processes can be monitored by this approach, namely, kinetic resolution of racemates and asymmetric transformation of substrates which are prochiral due to the presence of enantiotopic groups.

The underlying principle is based on the use of isotopically labeled substrates in the form of *pseudo*-enantiomers or *pseudo*-prochiral compounds (Scheme 1).[8] The course of the asymmetric transformation—that is, the relative amounts of reactants and/or products—is detected by ESI-MS.[9, 10]



Scheme 1. a) Asymmetric transformation of a mixture of *pseudo*-enantiomers involving cleavage of the functional groups FG and labeled functional groups FG*. b) Asymmetric transformation of a mixture of *pseudo*-enantiomers involving either cleavage or bond formation at the functional group FG; isotopic labeling at R^2 is indicated by the asterisk. c) Asymmetric transformation of a *pseudo-meso* substrate involving cleavage of the functional groups FG and labeled functional groups FG*. d) Asymmetric transformation of a *pseudo*-prochiral substrate involving cleavage of the functional group FG and labeled functional group FG*.

In the case of kinetic resolution, compounds **1** and **2**, differing in absolute configuration and in labeling at the functional group FG*, are prepared in enantiomerically pure form and then mixed in a 1:1 manner, simulating a racemate (Scheme 1a). Following asymmetric functional group transformation (in an ideal kinetic resolution up to 50% conversion), true enantiomers **3** and **4** are formed, in addition to

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[**] We thank H. Husmann for performing GC analyses and H. Hinrichs for performing LC analyses as well as Novo Nordisk (Denmark) for enzyme samples.

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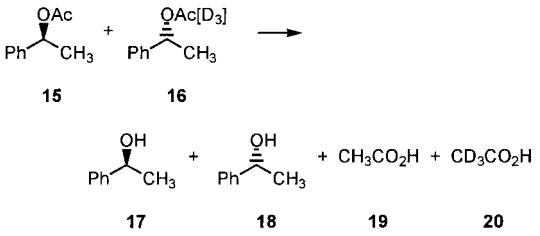
nonlabeled and labeled achiral products **5** and **6**. The ratios of the total intensities of **1**/**2** and **5**/**6** in the mass spectra (*m*/*z* intensities of the quasi-molecular ions) allow for the determination of enantioselectivity. In some cases it may be advantageous to use an internal standard in order to determine the conversion.[11]

As a variation of this theme, kinetic resolution of the *pseudo*-enantiomers **1** and **7**, in which labeling occurs at residue R^2, affords a new pair of *pseudo*-enantiomers **3** and **8** (Scheme 1b). Based on the *m*/*z* intensities of the quasi-molecular ions of **1**/**7** and **3**/**8**, the conversion, the enantioselectivity, and the selectivity factor (*s* or *E* value) can be obtained. An internal standard is not necessary.

In the case of prochiral substrates having enantiotopic groups, for example *meso* compounds (Scheme 1c), the synthesis of a single *pseudo-meso* compound suffices, for example **9**, since the stereodifferentiating reaction of interest delivers a mixture of two *pseudo*-enantiomers **10** and **11** that are detectable by MS. The same applies to other *pseudo*-prochiral substrates of the type **12** (Scheme 1d).

Although our method is independent of the type of catalyst or reagent used, we restrict ourselves in this paper to lipase-catalyzed reactions. The lipase-catalyzed hydrolytic kinetic resolution of racemic 1-phenylethyl acetate, corresponding to Scheme 1a, served as the first example. In exploratory experiments *pseudo*-enantiomers **15** and **16** were prepared in enantiomerically pure form and mixed in various ratios [Eq. (1)]. The resulting mixtures were analyzed by gas



(1)

chromatography in order to ascertain the exact *pseudo-ee* values. Thereafter the samples were analyzed employing ESI-MS.[12] A typical ESI-mass spectrum is shown in Figure 1. Comparison of the two sets of data shows an agreement of ±5% (Table 1). Similar observations were made in control experiments involving other substrates, thereby demonstrating the accuracy and precision of such *ee* determinations.

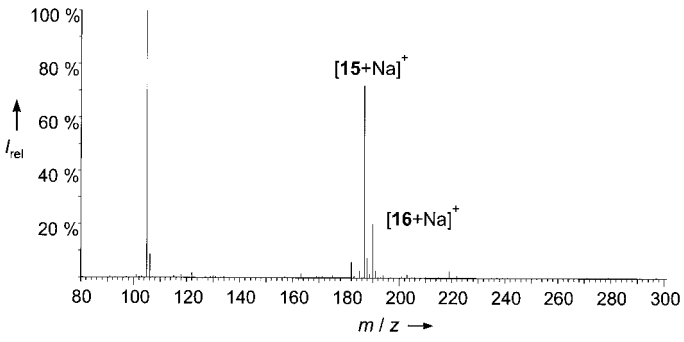
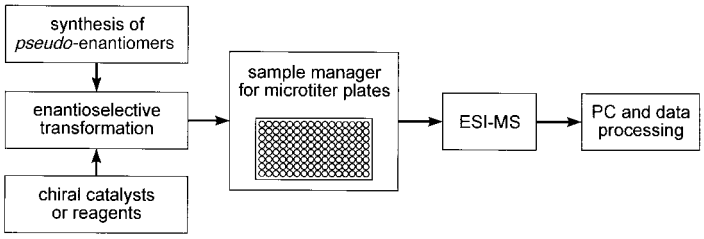


Figure 1. ESI mass spectrum of **15** and **16** (sample 6, Table 1).

Table 1. Enantiomeric excesses of samples prepared by mixing **15** and **16** as determined by GC and ESI-MS.

Sample	*ee* [%] (GC)	*ee* [%] (ESI-MS)
1	100	100
2	91	90
3	81	79
4	74	73
5	60	57
6	56	54
7	48	48
8	28	27
9	10	10
10	23	20
11	40	38
12	45	43
13	55	53
14	65	60
15	75	74
16	95	93
17	100	100

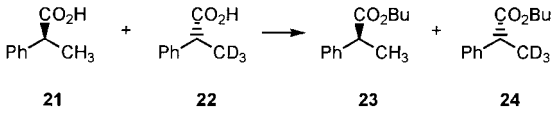
Following these encouraging results, the task remained to devise an experimental setup capable of high-throughput screening of enantioselective reactions. This was achieved by combining an automated liquid sampler for microtiter plates with an ESI-MS system, both commercially available (Scheme 2). With such a configuration about 1000 precise



Scheme 2. Schematic description of the experimental setup for the determination of *ee* values.

determinations of the *ee* value and the conversion of the reaction in Equation (1) can be performed in a single day.[13] In other cases the number varies between 700 and 1400, depending upon the particular substrate. It should be emphasized that neither chromatographic separation of enantiomers or *pseudo*-enantiomers nor of the products is involved. Thus, the method constitutes a fast and accurate way to determine the enantioselectivity and conversion of lipase-catalyzed (or otherwise induced) hydrolysis reactions leading to chiral alcohols.

Our approach can be used in the kinetic resolution of all types of chiral compounds, for example in the lipase-catalyzed stereoselective esterification of 2-phenylpropionic acid using a 1:1 mixture of **21** and **22** [Eq. (2)],[13] which correspond



(2)

to Scheme 1b. As before, about 1000 *ee* determinations can be performed per day. To exclude possible secondary kinetic isotope effects, the reaction of the *pseudo*-enantiomers **21** and

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22 was compared to that of the true racemate of **21**. The data in Figure 2 demonstrate agreement within the limits of experimental error.

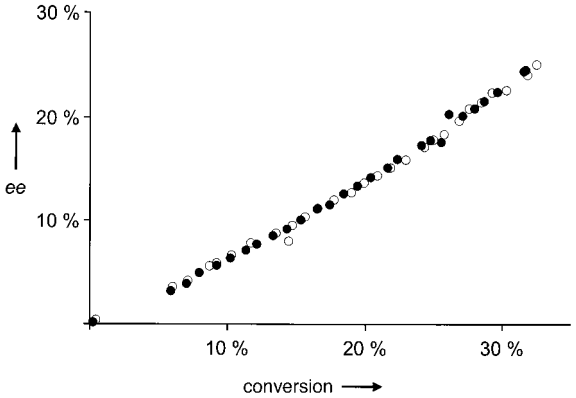
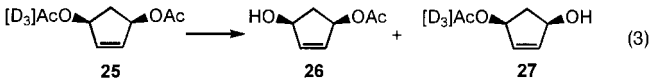


Figure 2. Comparison of the *ee* values obtained for the lipase-catalyzed (Novo SP 435 lipase, Novo Nordisk) esterification of the racemate of **21** (○) with that of a 1:1 mixture of **21** and **22** (●).

Finally, the lipase-catalyzed enantioselective hydrolysis of *meso*-1,4-diacetoxy-2-cyclopentene was investigated,[13] which is an example of Scheme 1c. Accordingly, the *pseudo-meso* compound **25** was first prepared by acylation of (1*S*,4*R*)-4-acetoxycyclopent-2-ene-1-ol (**26**) using $CD_3C(O)Cl$ (>99 atom % D). Hydrolysis of **25** afforded the expected mixture of *pseudo*-enantiomeric products **26** and **27** [Eq. (3)], which



(3)

were analyzed by MS as described above for the other substrates. In Figure 3 ESI mass spectra of two representative samples are depicted, one involving a mutant lipase (*P. aeruginosa*)[4] having no enzyme activity (no conversion; Figure 3a) and the other involving an active enzyme (porcine liver esterase, Sigma) leading to an *ee* value of 73 % (Figure 3b). In control experiments several samples were also analyzed by gas chromatography using a chiral stationary phase. Again, the correspondence between the traditional chromatographic (slow) and the present method (fast) turned out to be excellent.

In summary, we have developed a highly efficient method to assay the enantioselectivity of asymmetric reactions of chiral compounds or of prochiral substrates bearing enantiotopic groups.[14] Although isotopically labeled *pseudo*-enantiomers or *pseudo*-prochiral compounds need to be prepared, once this task has been performed a large number of enantioselective biocatalysts or chemical catalysts can be screened for the particular reaction in question within a short time. Since the position of isotopic labeling is chosen so as to preclude primary isotope effects which might influence the results, reliable data are obtained. The present method is not restricted to ESI-MS; preliminary studies show that other ionization techniques such as matrix-assisted laser desorption/ionization (MALDI) are also efficient. Other screening methods are required for systems involving prochiral substrates having enantiotopic faces, as in the enantioselective reduction of ketones or olefins.

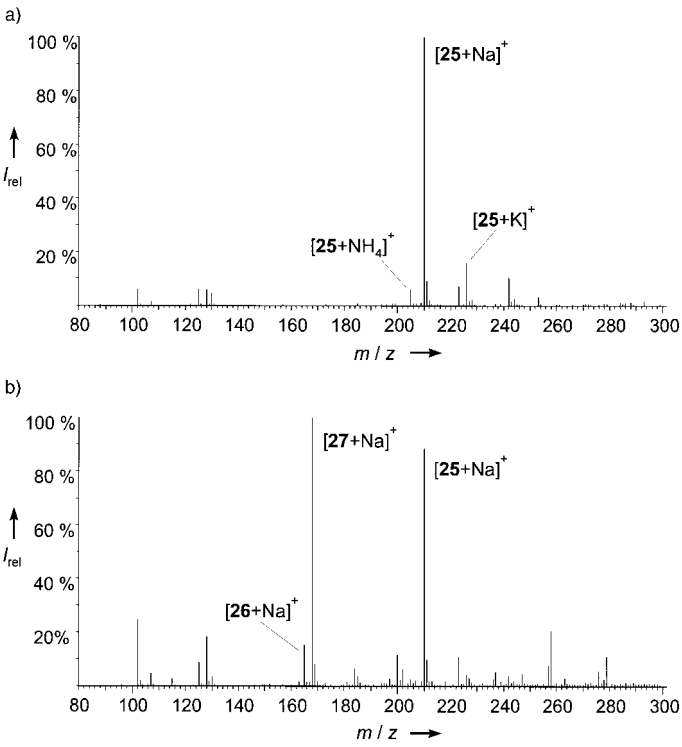


Figure 3. a) ESI mass spectrum of a sample obtained from the use of a nonactive mutant lipase in the attempted hydrolysis of **25**. b) ESI mass spectrum of a sample obtained from the use of an active esterase in the hydrolysis of **25** (ESI-MS: pseudo-*ee* = 73 %; GC: *ee* = 73 %).

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[8] *pseudo*-Enantiomers are chiral compounds which differ in two respects only: opposite absolute configuration and isotopic labeling. A 1:1 mixture of such compounds has been called a *pseudo*-racemate (B. Testa, P. Jenner in *Drug Fate and Metabolism: Methods and Techniques*, *Vol. 2* (Eds.: E. R. Garrett, J. L. Hirtz), Dekker, New York, **1978**, p. 143), but this has been declared a misnomer (E. L. Eliel, S. H. Wilen, L. N. Mander, *Stereochemistry of Organic Compounds*, Wiley, New York, **1994**, p. 159).

[9] Horeau et al. have described the application of MS to the detection of isotopically labeled diastereomers, prepared by the reaction of a compound of unknown configuration with an excess of a 1:1 mixture of isotopically labeled *pseudo*-enantiomers: a) A. Horeau, A. Nouaille, *Tetrahedron Lett.* **1990**, *31*, 2707 – 2710; other use of *pseudo*-enantiomers: b) L. R. Sousa, G. D. Y. Sogah, D. H. Hoffman, D. J. Cram, *J. Am. Chem. Soc*. **1978**, *100*, 4569 – 4576; c) T. Walle, M. J. Wilson, U. K. Walle, S. A. Bai, *Drug Metab. Dispos.* **1983**, *11*, 544 – 549; d) D. W. Armstrong, *Anal. Chem*. **1987**, *59*, 84A – 91A; e) M. A. Baldwin, S. A. Howell, K. J. Welham, F. J. Winkler, *Biomed. Environ. Mass Spectrom*. **1988**, *16*, 357 – 360; f) M. Sawada, H. Yamaoka, Y. Takai, Y. Kawai, H. Yamada, T. Azuma, T. Fujioka, T. Tanaka, *Chem. Commun.* **1998**, 1569 – 1570.

[10] In principle it should be possible to extend Horeau's method[9a] to the determination of *ee* values in high-throughput screening. However, this requires an additional step (derivatization) and an excess of a 1:1 mixture of the pseudo-enantiomeric reagent as well as the assumption that conversion is complete without any enrichment of one of the diastereomers.

[11] Determination of conversion by ESI-MS using an internal standard: a) S. Takayama, S. T. Lee, S.-C. Hung, C.-H. Wong, *Chem. Commun.* **1999**, 127 – 128; b) S. A. Gerber, C. R. Scott, F. Turecek, M. H. Gelb, *J. Am. Chem. Soc*. **1999**, *121*, 1102 – 1103.

[12] Aliquots (10 μL) of 1 mM 1:1 mixtures of **15** and **16** in CH_3OH were injected into a Rheodyne port of an ESI-MS system (ESI-MS conditions: MS: Hewlett Packard 5989B MS engine quadrupole mass spectrometer equipped with a Hewlett Packard 59987A API electrospray source II with hexapole ion guide (Analytica of Branford) and ChemStation data system; data acquisition: positive-ion mode scan spectra; *m*/*z* 90 – 300; step size *m*/*z* 0.1, unit resolution, gaussian mass filter *m*/*z* 0.3; gaussian time filter 0.05 min; API source conditions: potential difference between spray needle and first electrode – 5250 V, pressure of N_2 nebulization gas: 4140 Torr, flow of N_2 drying gas ca. 9 L min^{-1} (150 °C), solvent flow 0.06 mL min^{-1}, CH_3OH/H_2O 8/2). ESI mass spectra were collected, and the ratios of **15** and **16** were determined on the basis of absolute intensities of the peaks of the corresponding sodium adducts ([**15**+Na]$^+$ and [**16**+Na]$^+$, Figure 1). The ratios of the peak intensities (and thus the *ee* values of the synthetic mixtures of **15** and **16**) were obtained automatically using a macro to submit the data from the *m*/*z* intensity table of each measurement to an Excel spread sheet.

[13] All enzyme-catalyzed hydrolytic reactions and esterifications were performed in deep-well microtiter plates (total volume 1.2 mL). For ESI-MS analysis a defined volume of the resulting product mixtures was extracted with diethyl ether. The extracts were automatically transferred in microtiter plates and diluted with methanol to a final concentration of 0.5 – 2.0 mM. The microtiter plates were placed in an automated sample manager (Scheme 2) equipped with a Rheodyne port for the injections.

[14] M. T. Reetz, M. H. Becker, H.-W. Klein, D. Stöckigt, **1999**, patent application submitted.

[{[(η^7-$C_2B_{10}H_{12}$)(η^6-$C_2B_{10}H_{12}$)U][K_2(thf)$_5$]}$_2$]: A Metallacarborane Containing the Novel η^7-$C_2B_{10}H_{12}^{4-}$ Ligand**

Zuowei Xie,* Chaoguo Yan, Qingchuan Yang, and Thomas C. W. Mak

It has been well-documented that $C_2B_{10}H_{10}R_2$ (R = H, alkyl, aryl) can be reduced by alkali metals to form the *nido*-$C_2B_{10}H_{10}R_2^{2-}$ dianion, which can be bound in a η^6 manner to transition metals to afford a series of 13-vertex *closo*-metallacarboranes.[1] Treatment of [(η^6-$C_2B_{10}H_{12}$)Co(η^5-C_5H_5)] with Na/naphthalene followed by reaction with C_5H_5Na and $CoCl_2$ gave the 14-vertex *closo*-metallacarborane [(η^6:η^6-$C_2B_{10}H_{12}$)-{Co(η^5-C_5H_5)}$_2$].[2] The proposed geometry of the cage is the bicapped hexagonal antiprism; X-ray confirmation of this species has not been reported. We are interested in this tetraanion ligand and its bonding mode to transition metals, and describe herein the isolation and structural characterization of the first metallacarborane bearing a η^7-$C_2B_{10}H_{12}^{4-}$ ligand.

Interaction between *o*-$C_2B_{10}H_{12}$ and excess K metal in THF at room temperature followed by treatment with a suspension of UCl_4 in THF gave, after workup, **1** as deep red crystals in 58% yield [Eq. (a)]. Compound **1** is extremely air- and

$$4\,o\text{-}C_2B_{10}H_{12} + 12\,K + 2\,UCl_4 \xrightarrow{THF} \underset{\mathbf{1}}{[\{[(\eta^7\text{-}C_2B_{10}H_{12})(\eta^6\text{-}C_2B_{10}H_{12})U][K_2(thf)_5]\}_2]} + 8\,KCl \quad \text{(a)}$$

moisture-sensitive, but remains stable for months at room temperature under an inert atmosphere. Contact with traces of air immediately results in conversion of the intensely colored **1** into a yellow powder. Compound **1** is soluble in polar organic solvents such as THF and pyridine, sparely soluble in toluene, and insoluble in hexane.

An X-ray diffraction study[3] reveals that **1** is a centrosymmetric dimer with a bent sandwich structural motif. As shown in Figure 1, each U atom is η^6-bound to *nido*-$C_2B_{10}H_{12}^{2-}$, η^7-bound to *arachno*-$C_2B_{10}H_{12}^{4-}$, and coordinated to two B–H bonds from the C_2B_5 bonding face of the neighboring *arachno*-$C_2B_{10}H_{12}^{4-}$ ligand. This results in a highly distorted tetrahedral geometry at U with a cent(S)–U–cent(L) angle of 136.3° (cent(S) and cent(L) are the centroids of the C_2B_4 and C_2B_5 bonding faces, respectively). Compound **1** represents not only the first metallacarborane containing a novel η^7-$C_2B_{10}H_{12}^{4-}$ ligand, but also the first organoactinide compound bearing a η^6-$C_2B_{10}H_{12}^{2-}$ ligand.

The average distance between U and a cage atom of the C_2B_4 bonding face in **1** (2.867(7) Å) is longer than that between U and a cage atom of the C_2B_3 bonding face in

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