A Practical NMR-Based High-Throughput Assay for Screening Enantioselective Catalysts and Biocatalysts

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Dedicated to Roger Sheldon on the occasion of his 60th birthday.

Abstract: Two NMR-based approaches for high-throughput screening of enantioselective catalysts and biocatalysts are described. One version makes use of *pseudo*-enantiomers or *pseudo-meso*-compounds based on ^{13}C-labeling. A throughput of at least 1400 ee determinations per day is possible by using an appropriate flow-through cell and an autosampler. The other approach is based on traditional diastereomer formation using a chiral reagent or complexing agent. The ee values are accurate to within $\pm 2\%$ and $\pm 5\%$ of the true values.

Keywords: asymmetric catalysis; combinatorial catalysis; directed evolution; enzyme screening; NMR spectroscopy

Introduction

The enantioselective catalytic synthesis of chiral organic compounds is of substantial academic and industrial interest, as evidenced *inter alia* by the award of the Nobel Prize for Chemistry 2001 to K. B. Sharpless, R. Noyori and W. S. Knowles. Currently most industrial syntheses of optically pure intermediates involve classical antipode separation,[1] but asymmetric catalysis is likely to gain in importance. Two options are available, namely synthetic catalysts such as transition metal complexes[2] or biocatalysts such as enzymes.[3] In the former case ligand tuning is necessary, which requires intuition, design (molecular modeling), kinetics and/or some degree of trial and error. Recently, a new approach has emerged which has been loosely called "combinatorial asymmetric catalysis".[4,5] This involves the parallel preparation and testing of libraries of chiral catalysts. Although the actual size of the libraries has thus far been limited to a few dozen catalysts, this method offers genuine opportunities, especially if larger libraries of chiral catalysts were to be generated. One of the prerequisites for practical implementation is the availability of high-throughput screening systems for determining enantioselectivity, which is one of the reasons for current research in developing rapid ee assays.[5] In the case of biocatalysis, recent research in the area of directed evolution of enantioselective enzymes also requires high-throughput ee assays.[6] In this approach it is the combination of appropriate molecular biological methods for random mutagenesis and expression coupled with screening systems for the determination of thousands of ee values that forms the basis of a fundamentally new method in asymmetric catalysis. A major part of this research effort has been devoted to devising new and efficient ee screening systems.[5,6] A further reason for developing such assays lies in the opportunities that metagenome DNA panning[7] offers to organic chemists interested in asymmetric catalysis. It has been estimated that $>99\%$ of nature's enzymes have not yet been discovered.[7] In this area of research, methods have been developed which allow access to the biodiversity hidden in uncultured microorganisms. This is in fact possible by collecting genes in the environment and expressing the encoded enzymes in recombinant organisms.[7] Figure 1 summarizes the three main sources of large libraries of potentially enantioselective catalysts.

We have previously developed several high-throughput ee assays based on UV/Vis spectroscopy,[8] mass spectrometry (MS) using isotopically labeled substrates,[9] IR-thermography,[10] capillary array electrophoresis[11] and even special forms of GC.[12] In most cases the methods, which allow between 700 and 20,000 ee determinations per day, are complementary. Other recent high-throughput ee screening systems are based on pH indicators or fluorescence,[13] circular dichroism,[14] enzymatic methods,[15] DNA arrays,[16] immunoassays[17] or MS of mass-tagged diastereomeric substrates.[18] Again, no system is universally applicable. In addition to the degree of throughput, precision also varies from system to system, $\pm 10\%$ of the true ee value being common. Whereas in many applications such precision may suffice in order to identify hits, in other

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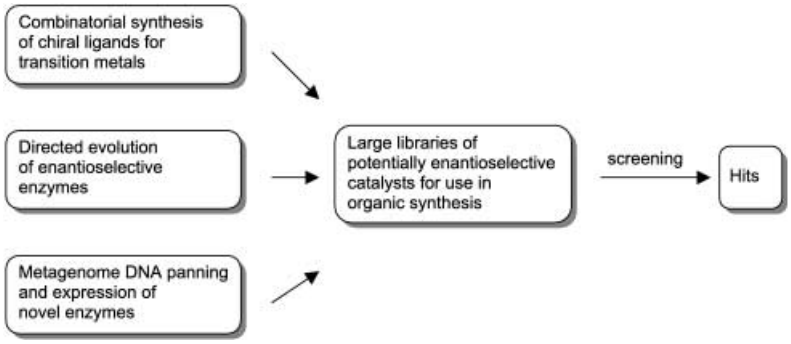


Figure 1. Sources of large libraries of potentially enantioselective catalysts.

cases it is insufficient, as in the late stages of directed evolution of enantioselective enzymes when attempting to improve the ee from typically 90% to $>98\%$.[5b]

In this paper we present a new high-throughput screening system which is based on NMR spectroscopy, allowing for at least 1400 ee determinations per day with an exceptionally high degree of accuracy ($\pm 2\%$ to $\pm 5\%$).[19] Since NMR instrumentation is available in essentially all laboratories, we expect this assay to become a standard technique. The only additional investment necessary is an appropriate flow-through cell coupled with an autosampler.

Results and Discussion

Although NMR measurements are usually considered to be slow processes, recent advances in the design of flow-through cells have allowed the method to be applied in the combinatorial search for therapeutic drugs.[20] In our ee assay we make use of such systems. Two versions are possible, the first being based on isotopic labeling of *pseudo*-enantiomers or *pseudo*-prochiral compounds. This means that in this case the method is restricted to two fundamentally different processes, namely kinetic resolution of racemates and the desymmetrization of prochiral substrates bearing enantiotopic groups, which is similar to the scope and limitation of our MS-based ee assay[9] (Figure 2).

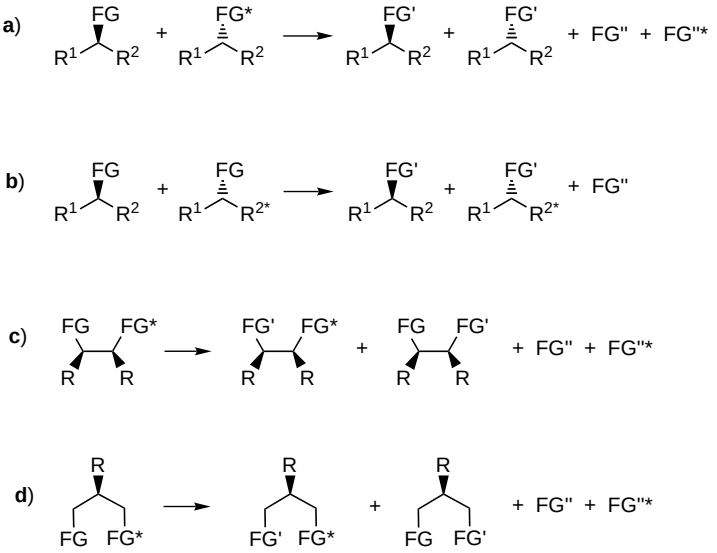
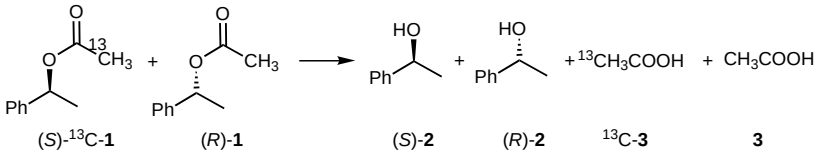


Figure 2. Asymmetric transformation of a mixture of *pseudo*-enantiomers involving cleavage of the functional groups FG and isotopically labeled FG*. **b**) Asymmetric transformation of a mixture of *pseudo*-enantiomers involving either cleavage or bond formation at the functional group FG; isotopic 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 FG*. **d**) Asymmetric transformation of a *pseudo*-prochiral substrate involving cleavage of the functional groups FG and labeled FG*.

The most practical form of our new assay utilizes ^{1}H NMR spectroscopy, ^{13}C-labeling being used to distinguish between the (*R*)- and (*S*)-forms of a chiral compound under study. Practically any carbon atom in the compound of interest can be labeled, but methyl groups of which the ^{1}H signals are not split by ^{1}H,^{1}H coupling are preferred because the relevant peaks to be integrated are the singlet arising from the CH_3 group of one enantiomer and the doublet due to the splitting of the $^{13}CH_3$ group of the other. A case in point concerns the hydrolytic kinetic resolution of *rac*-1-phenylethyl acetate, derived from *rac*-1-phenylethanol, catalyzed by appropriate enzymes or synthetic catalysts. However, the acetate of any chiral alcohol or the acetamide of any chiral amine can be used. Labeling can be carried out in any position of a compound (Figure 2). When applying our screening system in order to assay thousands of samples, it is necessary to label one of the enantiomeric forms, as in (*S*)-^{13}C-**1**. The synthesis is trivial, since it simply involves acylation of the (*S*)-alcohol by ^{13}C-labeled acetyl chloride, which is commercially available. Then a 1:1 mixture of labeled and non-labeled compounds (*S*)-^{13}C-**1** and (*R*)-**1** is prepared which simulates a racemate. It is used in the actual catalytic hydrolytic kinetic resolution, leading to a mixture of true enantiomers (*S*)- and (*R*)-**2** as well as labeled and non-labeled acetic acid, ^{13}C-**3** and **3**, together with unreacted starting esters. At 50% conversion (or at any other point of the



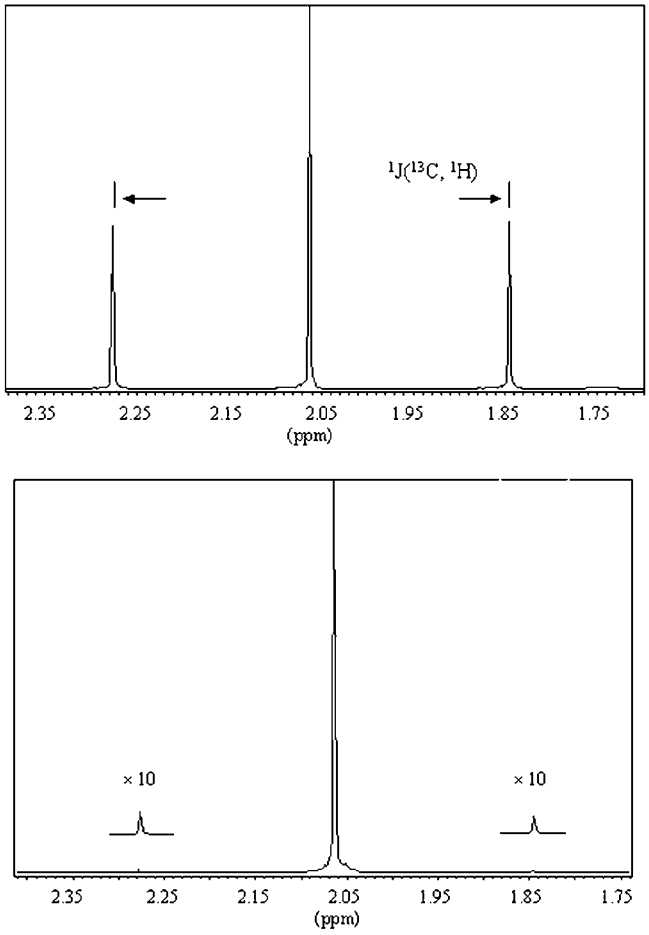


Figure 3. Expanded region of the ^{1}H NMR spectra of **a**) racemic mixture of (*S*)-^{13}C-**1**/(*R*)-**1** and **b**) (*R*)-**1**.

reaction) the ratio of (*S*)-^{13}C-**1** to (*R*)-**1** reveals the enantiomeric purity of the starting ester, while the ratio of ^{13}C-**3** to **3** correlates with the relative amounts of (*S*)-**2** and (*R*)-**2**, respectively.

In order to establish the screening system, a number of experiments were performed using various mixtures of (*S*)-^{13}C-**1** and (*R*)-**1** which were checked by gas chromatography (GC). Figure 3a shows a part of the ^{1}H NMR spectrum of a racemic mixture of (*S*)-^{13}C-**1** and (*R*)-**1** featuring the expected doublet of the ^{13}C-labeled methyl group and the singlet of the non-labeled methyl group. Figure 3b displays the singlet of the non-labeled methyl group of (*R*)-**1**, including the ^{13}C-satellites due to the presence of natural ^{13}C in the sample.

Thus, the two *pseudo*-enantiomers are nicely distinguishable by ^{1}H NMR spectroscopy, the exact ratio of the two being accessible by simple integration of the respective peaks. This ultimately provides the ee value. The quantitative analysis can be accomplished automatically by suitable software such as AMIX™.[21] The presence of naturally occurring ^{13}C in the non-labeled (*R*)-substrate needs to be accounted for when assaying a mixture. Table 1 and Figure 4 summarize the results of 11 control experiments using various mixtures of (*S*)-^{13}C-**1** and (*R*)-**1.** The agreement with the corresponding ee values obtained by independent GC analysis is excellent, the correlation coefficient amounting to $R^2 = 0.9998$.

Since the ee value in an actual kinetic resolution depends on the conversion, it has become customary to report the selectivity factor *E* (sometimes designated as *s*), which reflects the relative rate of reaction of the two enantiomers, the value being independent of conversion.[22] If the ee value is known (as in the present

Table 1. Analysis of 11 mixtures of (*S*)-^{13}C-**1**/(*R*)-**1**.

Entry	ee [%] per GC	ee [%] per ^{1}H NMR
1	100.0 (*S*)	100.0 (*S*)
2	88.5 (*S*)	89.2 (*S*)
3	71.2 (*S*)	71.0 (*S*)
4	39.2 (*S*)	38.5 (*S*)
5	13.4 (*S*)	13.8 (*S*)
6	0.4 (*S*)	1.6 (*S*)
7	13.6 (*R*)	14.5 (*R*)
8	42.8 (*R*)	45.0 (*R*)
9	69.6 (*R*)	72.1 (*R*)
10	87.8 (*R*)	89.1 (*R*)
11	100.0 (*R*)	100.0 (*R*)

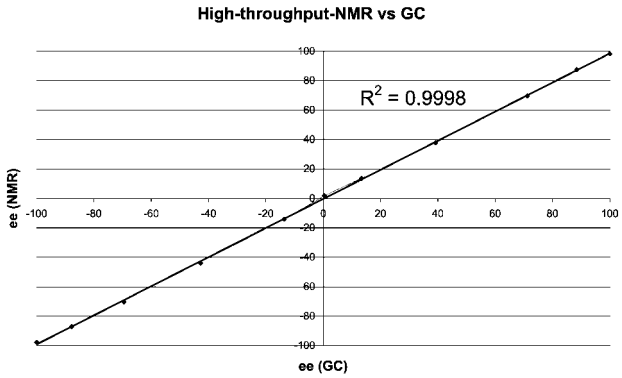


Figure 4. Comparison of ee values of 11 mixtures of (*S*)-^{13}C-**1**/(*R*)-**1** obtained by NMR and GC analyses.

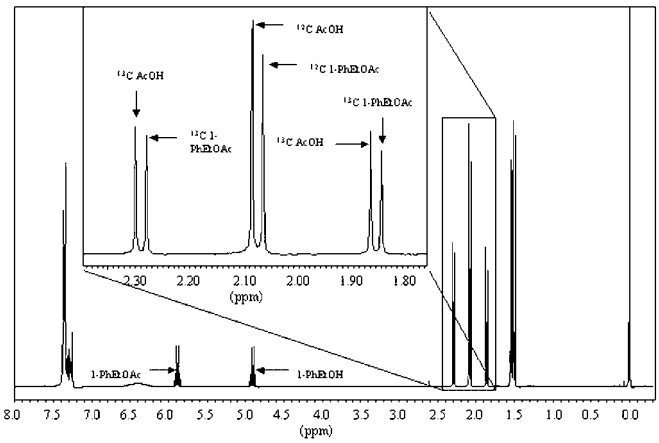


Figure 5. ^{1}H NMR spectrum of a 1:1:1 mixture of labeled and unlabeled 1-phenylethyl acetate, acetic acid, and 1-phenylethanol.

experiments), the *E* value can be ascertained, provided the conversion or the ratio of the two product enantiomers can be measured. In the present system this is possible by integration of the corresponding methine signals of the unreacted substrate ester at 5.9 ppm and the product alcohol at 4.9 ppm (Figure 5) which is a measure of conversion. Then the *E* value can be estimated according to the method of Sih et al. [22]

In order to test how general our approach is, the potential kinetic resolution of *rac*-2-phenylpropionic acid methyl ester was considered. In this case various mixtures of *pseudo*-enantiomers (*S*)-^{13}C-**4** and (*R*)-**4** were prepared and analyzed by ^{1}H NMR spectroscopy. Here again agreement with the corresponding ee values determined by GC turned out to be excellent (Table 2), the correlation coefficient amounting to $R^2 = 0.9998$. Again, the respective ee values differ by only $\pm 2\%$.

Finally, the desymmetrization of *meso*-1,4-diacetoxycyclopent-2-ene (**7**) was considered. In this case the products (1*S*,4*R*)-**8** and (1*R*,4*S*)-**8** are true enantiomers, indistinguishable by ^{1}H NMR spectroscopy. Therefore, the ^{13}C-labeled substrate (1*S*,4*R*)-^{13}C-**7**, which is a *pseudo-meso* compound, was prepared.

The two products of a potentially enantioselective transformation, (1*S*,4*R*)-^{13}C-**8** and (1*R*,4*S*)-**8**, are now *pseudo*-enantiomers which are easily distinguished in the ^{1}H NMR spectrum. The former shows a doublet centered at $\delta = 2.07$, while the latter displays a singlet at this position. Table 3 reveals the ee values determined by ^{1}H NMR spectroscopy and by classical GC, the agreement once again being excellent ($\pm 3\%$).

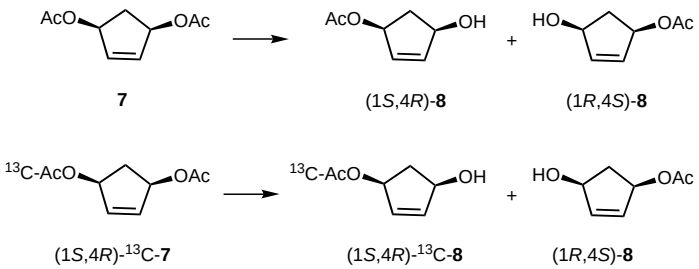
Table 2. Analysis of 11 mixtures of (*S*)-^{13}C-**4**/(*R*)-**4**.

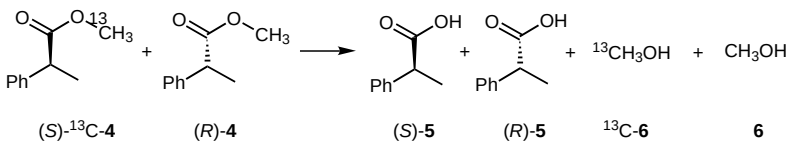
Entry	ee [%] per GC	ee [%] per ^{1}H NMR
1	100.0 (*S*)	100.0 (*S*)
2	82.6 (*S*)	84.5 (*S*)
3	76.4 (*S*)	78.6 (*S*)
4	58.0 (*S*)	60.0 (*S*)
5	29.8 (*S*)	31.0 (*S*)
6	0	0.7 (*R*)
7	31.0 (*R*)	29.7 (*R*)
8	58.4 (*R*)	58.5 (*R*)
9	74.6 (*R*)	75.6 (*R*)
10	81.2 (*R*)	83.2 (*R*)
11	100.0 (*R*)	100.0 (*R*)

Table 3. Analysis of 11 mixtures of (1*S*,4*R*)-^{13}C-**8**/(1*R*,4*S*)-**8**.

Entry	ee [%] per GC	ee [%] per ^{1}H NMR
1	100.0 (*S*)	99.5 (*S*)
2	82.4 (*S*)	82.6 (*S*)
3	63.0 (*S*)	63.8 (*S*)
4	43.0 (*S*)	44.3 (*S*)
5	6.4 (*S*)	9.2 (*S*)
6	2.6 (*S*)	3.6 (*S*)
7	19.6 (*R*)	17.3 (*R*)
8	41.6 (*R*)	38.3 (*R*)
9	64.4 (*R*)	63.9 (*R*)
10	82.2 (*R*)	81.8 (*R*)
11	99.9 (*R*)	97.5 (*R*)

The above results illustrate a new way of measuring enantiopurity, but the actual goal of this research is to develop an assay suitable for high-throughput. Miniaturization and automation are necessary while maintaining sensitivity. In order to accomplish this, the above substrates were used in an adapted form of a flow-through NMR cell. Accordingly, a sample manager is needed (e.g., a Gilson 215 liquid handler)[23] in order to transfer samples from 96-well plates to a 300 MHz ^{1}H NMR instrument which is equipped with a flow-





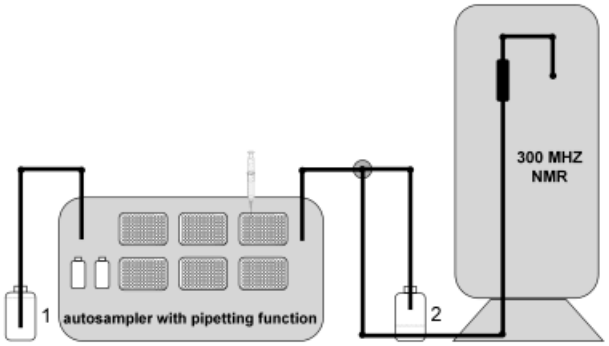
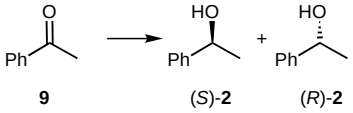


Figure 6. High-throughput NMR combines an autosampler with a pipetting function (for example, Gilson 215) connected with a flow-through NMR probe head. The sample is transported into the NMR cell and stopped during the measuring time. When the dataset is complete, the system is flushed in both directions with the solvent **1** into the waste **2** and the cycle is complete.



9 (*S*)-**2** (*R*)-**2**

Table 4. Analysis of 11 mixtures of (*S*,*R*)-**11**/(*R*,*R*)-**11**.

Entry	ee [%] per GC	ee [%] per ^{1}H NMR
1	100.0 (*S*)	100.0 (*S*)
2	82.7 (*S*)	86.0 (*S*)
3	65.0 (*S*)	66.7 (*S*)
4	47.7 (*S*)	55.0 (*S*)
5	35.4 (*S*)	38.7 (*S*)
6	11.4 (*S*)	16.3 (*S*)
7	6.6 (*R*)	3.5 (*R*)
8	25.2 (*R*)	21.9 (*R*)
9	49.6 (*R*)	45.9 (*R*)
10	74.8 (*R*)	75.4 (*R*)
11	100.0 (*R*)	100.0 (*R*)

through cell system. We chose the commercially available BEST™ cell system[24,25] (Figure 6). It was possible in all cases to reduce the measuring time per cycle down to one minute without loss in precision. This means that at least 1400 ee determinations are possible per day.

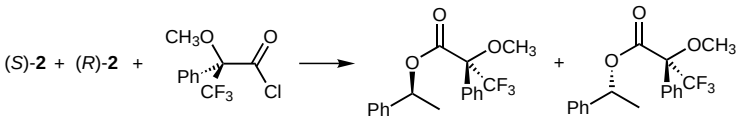
The underlying principle of the above screening system cannot be used to evaluate the enantiopurity of products obtained by the transformation of prochiral compounds not involving enantiotopic groups, as in the catalytic hydrogenation of prochiral ketones with formation of chiral alcohols. An example is the catalytic enantioselective transformation of acetophenone (**9**) leading to the chiral alcohols (*S*)- and (*R*)-**2**.

In cases of this kind we implemented classical ee determination by NMR using an appropriate flow-through cell (e.g., the BEST™ cell system[24,25]). This involves covalent derivatization using known chiral reagents or formation of complexes employing chiral H-bond forming agents or NMR shift reagents.[26] Integration of appropriate peaks in the ^{1}H (or ^{19}F) NMR spectra of the relevant diastereomers is known to provide reliable ee values.[26] We tested this traditional principle under the conditions of miniaturization and automation, again keeping the crucial time factor as well as accuracy in mind. In this case the model compound was chosen to be (*S*)- and (*R*)-**2**, present as enantiomeric mixtures. Using Mosher's reagent [(*R*)-**10**],[26,27] various enantiomeric mixtures were transformed into the corresponding diastereomers (*S*,*R*)-**11** and (*R*,*R*)-**11**. This was easily accomplished in the wells of a 96 microtiter plate using standard robotic equipment. As far as the NMR spectroscopy utilizing short measurement times is concerned, automation using the flow-through cells as described above led to excellent results (Table 4). In this case a precision of $\pm 5\%$ (except entry 4) in the ee values was observed under these stringent conditions. As before, about one sample per minute can be analyzed. The slightly larger deviation of the ee value is due the concentration of substance in solution (1 mg per mL) being very low. A higher precision can be achieved by raising the concentration. Similar results were obtained using mixtures of (*S*)- and (*R*)-2-butanol.[19]

In all of the experiments described here test mixtures of enantiomers were prepared and assayed by the high-throughput ee systems. In real applications robotic extractions following enzymatic transformation or work-up in the case of transition metal-catalyzed reactions are necessary. Since these are standard robotic procedures known to work well with microtiter plates, they pose no problems in the NMR-based ee screening systems.

Conclusions

We have developed two rapid NMR-based ee assays. Suitable instrumentation in both cases is commercially available and consists of a standard NMR spectrometer



(*R*)-**10** (*S*,*R*)-**11** (*R*,*R*)-**11**

equipped with a sample manager and an appropriate flow-through cell, which allows for at least 1400 samples to be evaluated per day. In the case of ^{13}C-labeling the principle involved is basically the same as in the previously published ESI-MS-based ee screening system.[9] However, in most cases the present NMR-based assay is even more precise. The decision as to which of these two assays is to be used depends on several factors, including the availability of the instruments in a given laboratory. The second NMR-based ee assay which we describe here is simply an appropriate adaptation of known ee determination methods based on diastereomer formation, i.e., we demonstrate that traditional NMR methods can be adapted for high-throughput in a practical and precise manner.

Catalytic reaction types and substrate families other than the ones described here should pose no problems, which means that the NMR approach to high-throughput ee analysis may well constitute the most general and practical method currently available. Thus, it is likely to be of considerable interest to those researchers involved in the combinatorial search for asymmetric transition metal catalysts, in the directed evolution of enantioselective enzymes and in exploring nature's diversity with respect to the stereoselectivity of the huge number of untapped wild-type enzymes.[28]

Experimental Section

General Methods

The reagents and solvents were obtained from commercial sources and the reagents were generally used without further purification. The solvents were distilled and stored under argon. The conditions for gas chromatographic analyses are given in the analytical data of the desired compounds. ^1H NMR spectra were recorded on a Bruker Avance 300 (300 MHz) spectrometer. All high-throughput measurements were performed using a Bruker Efficient Sample Transfer BEST™ (300 MHz) system[24] and a Gilson 215 autosampler.[23] For data post-processing AMIX™ software (Bruker) was used.[21] Chemical shifts are reported in ppm using tetramethylsilane (TMS, 0.00 ppm) as an internal standard. ^{13}C NMR spectra were recorded at 75 MHz with $CDCl_3$ as an internal reference ($\delta_C \equiv 77.0$). Mass spectra were recorded on a Finnigan MAT 8200 and IR data were obtained using a Perkin-Elmer FT 1600. Elemental analysis was carried out in an external laboratory (microanalytical laboratory Kolbe in Mülheim/Ruhr). In all cases the amount of ^{13}C-labeling was $> 99\%$.

Synthesis of 1-Phenylethyl Acetates 1

Enantiomerically pure (*S*)- or (*R*)-1-phenylethanol (**2**) (1.0 g, 8.2 mmol) and pyridine (4 mL) were dissolved in dichloromethane (30 mL) in a 50 mL N_2-flask under argon. After cooling the solution with an ice bath the corresponding 2-^{13}C-labeled or unlabeled acetyl chloride (0.97 g, 12.3 mmol) was slowly added via syringe with appearance of a white solid (pyridine hydrochloride). The reaction mixture was stirred over night at ambient temperature and the resulting red solution was quenched with water while being cooled with an ice bath. The organic phase was separated and subsequently washed with 1 M HCl and saturated brine. After drying of the organic phase with $MgSO_4$ the solvent was evaporated and the crude products were purified *via* column chromatography (SiO_2) using dichloromethane as eluent. After removal of the solvent in vacuum the desired products were obtained as colorless oils. Yield: 1.24 g (92%).

***(R)*-1-Phenylethyl Acetate [(*R*)-1]:** ^1H NMR ($CDCl_3$): $\delta =$ 1.53 (d, $J = 6.6$ Hz, 3H, CH_3), 2.06 [s, 3H, $C(O)CH_3$], 5.88 (q, $J = 6.6$ Hz, 1H, CH), 7.24–7.37 (m, 5H, ArH); ^{13}C NMR ($CDCl_3$): $\delta = 21.3, 22.2, 72.3, 126.1, 127.9, 128.5, 141.7, 170.3$; MS: *m/z* (rel. int.) = 164 (M^+, 25), 122 (77), 104 (100), 77 (43); IR (neat): $\nu = 3064, 3034$ (C-H, ArH), 2982, 2934 (C-H, CH + CH_3), 1744 (C=O), 1242 (C-O) cm^{-1}; Anal: C 72.9% (calcd. 73.3%), H 7.4% (calcd. 7.3%).

***(S)*-1-Phenylethyl 2-^{13}C-Acetate [(*S*)-^{13}C-1]:** ^1H NMR ($CDCl_3$): $\delta = 1.53$ (d, $J = 6.6$ Hz, 3H, CH_3), 2.06 [d, $J_{C,H} =$ 129.4 Hz, 3H, $C(O)^{13}CH_3$], 5.88 (q, $J = 6.6$ Hz, 1H, CH), 7.24–7.37 (m, 5H, ArH); ^{13}C NMR ($CDCl_3$): $\delta = 21.3, 22.2, 72.3, 126.1, 127.9, 128.5, 141.7, 170.7$; MS: *m/z* (rel. int.) = 165 (M^+, 31), 122 (93), 104 (100), 77 (25), 44 (45); IR (neat): $\nu =$ 3064, 3034 (C-H, ArH), 2982, 2934 (C-H, CH + CH_3), 1743 (C=O), 1239 (C-O) cm^{-1}; Anal.: C + ^{13}C 72.6% (calcd. 73.3%), H 7.5% (calcd. 7.3%). *GC-analysis:* Hewlett Packard 5890, column: 25 m TBCD/OV-1701, detector: FID, temperature program: 230 °C, 60 °C at 2 °C/min to 180 °C, 5 min isotherm, 350 °C, gas: 1 bar hydrogen, retention time: 21.7 min [(*S*)-1-phenylethyl 2-^{13}C-acetate], 23.3 min [(*R*)-1-phenylethyl acetate], $> 99\%$ ee in both cases.

Synthesis of Methyl 2-Phenylpropanoates 4

Enantiomerically pure (*S*)- or (*R*)-2-phenylpropanoic acid (600 mg, 4.0 mmol) and CsF (912 mg, 6.0 mmol) were suspended in DMF (12 mL) in a 25-mL N_2-flask under argon. The reaction mixture was cooled with a cryostat to 13 ± 1 °C and the corresponding ^{13}C-labeled or unlabeled methyl iodide (1.93 g, 13.6 mmol) was slowly added via syringe. After stirring at this temperature for 46 h ethyl acetate (20 mL) was added to the solution and the excess methyl iodide and the solvents were removed in vacuum. The residue was dissolved in ethyl acetate and extracted with saturated $NaHCO_3$ solution. The organic phase was dried with $MgSO_4$ and the solvent was evaporated. The crude products were purified *via* column chromatography (SiO_2) using hexane/ethyl acetate (8:2) as eluent. After removal of the solvents in vacuum the desired products were obtained as colorless oils. Yield: 454 mg (69%).

Methyl *(R)*-2-phenylpropanoate [(*R*)-4]: ^1H NMR ($CDCl_3$): $\delta = 1.50$ (d, $J = 7.2$ Hz, 3H, CH_3), 3.65 (s, 3H, OCH_3), 3.72 (q, $J = 7.2$ Hz, 1H, CH), 7.23–7.35 (m, 5H, ArH); ^{13}C NMR ($CDCl_3$): $\delta = 18.6, 45.4, 52.0, 127.1, 127.5, 128.6, 140.6, 175.0$; MS: *m/z* (rel. int.) = 164 (M^+, 24), 105 (100), 77 (12), 51 (5); IR (neat): $\nu = 3087, 3063, 3030$ (C-H, ArH), 2981, 2952, 2877 (C-H, CH + CH_3), 1738 (C=O), 1208, 1166 (C-O) cm^{-1}; Anal.: C 73.2% (calcd. 73.3%), H 7.5% (calcd. 7.3%).

^{13}C-Methyl *(S)*-2-phenylpropanoate [(*S*)-^{13}C-4]: ^1H NMR ($CDCl_3$); $\delta = 1.50$ (d, $J = 7.2$ Hz, 3H CH_3), 3.65 (d, $J_{C,H} =$

146.9 Hz, 3H, OCH_3), 3.71 (q, $J = 7.1$ Hz, 1H, CH), 7.22 – 7.35 (m, 5H, ArH); ^{13}C NMR ($CDCl_3$): $\delta = 18.6$, 45.4, 52.0, 127.1, 127.5, 128.6, 140.6, 175.0; MS: *m/z* (rel. int.) = 165 (M^+, 28), 105 (100), 77 (10), 51 (3); IR (neat): $\nu = 3063$, 3030 (C-H, ArH), 2982, 2946, 2878 (C-H, $CH + CH_3$), 1737 (C=O), 1206 (C-O) cm^{-1}; Anal.: C + ^{13}C 72.8% (calcd. 73.3%), H 7.4% (calcd. 7.3%). *GC analysis:* Hewlett Packard 5890, column: 30 m GTA, detector: FID, temperature program: 200 °C, 60 °C at 0.2 °C/min to 100 °C at 5 °C/min to 180 °C, 300 °C, gas: 0.9 bar hydrogen, retention time: 37.7 min [methyl (*R*)-2-phenylpropanopate), 38.7 [^{13}C-methyl (*S*)-2-phenylpropanoate), > 98% ee in both cases.

Synthesis of (1*S*,4*R*)-*cis*-1-(2-^{13}C-Acetoxy)-4-acetoxycyclopent-2-ene [(1*S*,4*R*)-^{13}C-7]

In a 250-mL N_2-flask (1*S*,4*R*)-*cis*-4-acetoxycyclopent-2-en-1-ol (5.00 g, 35.2 mmol), pyridine (4.27 mL, 6.95 mmol) and dichloromethane (100 mL) were cooled to 0 °C. Under stirring 2-^{13}C-acetyl chloride (3.00 mL, 42.2 mmol) was added dropwise. The reaction mixture was stirred overnight at ambient temperature and was then extracted with 2 × 50 mL 1 M HCl, 2 × 50 mL saturated $NaHCO_3$ and 2 × 50 mL brine. The organic phase was dried over $MgSO_4$, filtered and the solvent was evaporated. Purification of the crude product by flash chromatography (hexane/ethyl acetate, 5:1) gave a colorless oil; yield: 6.38 g (97%). ^1H NMR ($CDCl_3$): $\delta = 1.71 – 1.78$ (m, 1H, CH_2), 2.07 [s, 3H, $C(O)CH_3$], 2.07 [d, $J_{C,H} = 130$ Hz, 3H, $C(O)^{13}CH_3$], 2.87 (dd, $^3J = 3.8$ Hz, $^2J = 7.5$ Hz, 1H, CH_2), 5.55 [m, 2H, CHOC(O)], 6.10 (s, 2H, =CH); ^{13}C NMR ($CDCl_3$): $\delta =$ 21.5, 37.5, 76.9, 135.0, 171.1; MS: *m/z* (rel. int.) = 184 (M^+, < 1), 126 (5), 125 (5), 124 (2), 83 (20), 82 (100), 81 (21), 65 (5), 54 (12), 53 (9), 44 (32), 43 (31); IR (neat): $\nu = 3073$ (C-H, =CH), 2991, 2951 (CH, $CH_2 + CH_3$), 1737 (C=O), 1231 (C-O) cm^{-1}; Anal.: C + ^{13}C 59.3% (calcd. 59.2%), H 6.1% (calcd. 6.0%).

Synthesis of (1*S*,4*R*)-*cis*-4-(2-^{13}C-Acetoxy)-cyclopent-2-en-1-ol [(1*S*,4*R*)-^{13}C-8]

In a 500-mL N_2-flask (1*S*,4*R*)-*cis*-1-(2-^{13}C-acetoxy)-4-acetoxycyclopent-2-ene (15.0 g, 81.5 mmol) and *Novo SP 435* Lipase (2.0 g) were mixed in NaH_2PO_4/K_2HPO_4 buffer (400 mL, 50 mM, pH 7.5) and shaken for 18 hours until the educt was completely converted to the monoacylated product (GC control). The reaction was stopped by filtration and the immobilized enzyme was washed with distilled H_2O (100 mL) and MTBE (200 mL). The reaction mixture was extracted with 2 × 200 mL MTBE, the united organic phases were dried over $MgSO_4$, filtered and the solvent was evaporated. The resulting colorless liquid was purified by crystallization in MTBE/pentane. After several steps of crystallization the product was obtained as white crystals with an enantiomeric purity of > 99% ee (chiral GC); yield: 11.1 g (96%). ^1H NMR ($CDCl_3$): $\delta = 1.67$ (dt, $^3J = 3.8$ Hz, $^2J =$ 14.6 Hz, 1H, CH_2), 1.84 (s, 1H, OH), 2.06 [d, $J_{C,H} = 130$ Hz, 3H, $C(O)^{13}CH_3$], 2.76 (dt, $^3J = 7.4$ Hz, $^2J = 14.6$ Hz, 1H, CH_2), 4.71 (m, 1H, C<u>H</u>OH), 5.49 – 5.51 [m, 1H, CHOC(O)], 5.97 – 6.00 (m, 1H, =C<u>H</u>CHOH), 6.10 – 6.13 [m, 1H, =C<u>H</u>CHOC(O)]; ^{13}C NMR ($CDCl_3$): $\delta = 21.2$, 40.5, 74.9, 77.1, 132.6, 138.5, 170.8; MS *m/z* (rel. int.): 126 (2), 101 (4), 100 (6), 83 (18), 82 (100), 81 (36), 55 (22), 54 (18), 53 (20), 44 (40), 43 (13); IR (KBr): $\nu = 3391$ (O-H), 3076, 3061 (C-H, =CH), 2996, 2978, 2950, 2921 (C-H, $CH_2 + CH_3$), 1726 (C=O), 1257 (C-O) cm^{-1}; Anal.: C + ^{13}C 59.3% (calcd. 59.4%), H 7.1% (calcd. 7.0%). *GC analysis:* Hewlett Packard 6890-2, column: 25 m IVADEX 5, detector: FID, temperature program: 230 °C, 60 °C at 5 °C/min to 140 °C at 15 °C/min to 200 °C, 5 min isotherm, 340 °C, gas: 0.98 bar hydrogen, retention time: 12.6 min, > 99% ee.

Derivatization of Mixtures of (*S*)/(*R*)-2 using Mosher's Acid Chloride [(*R*)-10]

Mixtures of (*R*)/(*S*)-1-phenyl ethanol were derivatized *in situ* with enantiomerically pure Mosher's acid chloride [(*R*)-**10**] (1.2 equiv., > 99% ee) in the presence of pyridine (1 equiv.), resulting in the corresponding diastereomeric esters (*S*,*R*)-**11** and (*R*,*R*)-**11**, respectively, using the known procedure[26,27] at a scale of 0.1 mmol. The diastereomeric protons in the ^1H NMR spectrum appear at 1.42 and 1.55 ppm corresponding to (*S*,*R*)-**11** and (*R*,*R*)-**11**, respectively.

High-Throughput NMR Measurements using BEST™

In order to achieve a high sample throughput without additional sample preparation, it is necessary to work with an autosampler which can "load and prep", which means that it is equipped with a pipetting function. In the present study a Gilson 215 autosampler[23] was employed, which is a standard feature in the commercially available BEST™ set-up provided by Bruker Biospin GmbH.[24] For experiments using ^{13}C-labeling, the relative amounts of the *pseudo*-enantiomers can be calculated if the naturally occurring ^{13}C is taken into account and an accurate value of the extent of enrichment in the labeled substrate is known. In the case of non-isotopically labeled compounds the samples can be readily prepared in 96-well microtiter plates (MTP), standard GC-flasks or 384 MTP. Every sample can be derivatized *in situ* with a base (for example, pyridine) and enantiomerically pure (*R*)- or (*S*)-Mosher's acid chloride.[27] The derivatization step is complete without additional reaction time by mixing the solution several times with the syringe. A crucial point is the use of an excess of Mosher's reagent in order not to undergo the risk of a kinetic resolution. While one sample is measured, the next can be prepared automatically without losing time, keeping the cycle time in the range of one minute. The autosampler is equipped with a small LC pump for washing the system. It can also deliver a continuous flow of solvent, making an additional HPLC pump unnecessary. As solvents $CDCl_3$, DMSO-d_6 or D_2O are used. Although the flow-through system does not need much solvent (~1 L in 24 hours), the solvents can be mixed with undeuterated solvent in 1:9 ratio to reduce costs.

In order to achieve reproducible and precise results, a total concentration of about 6 μmol/mL substance in the deuterated solvent is necessary. The samples are transferred into 2 mL GC bottles or 96 deep well plates, which are placed in the Gilson 215 autosampler of the BEST™ NMR system. For performing the high-throughput measurements, a total volume of 300 μL is injected into the flow through system of the BEST™ NMR by a XYZ needle. Then the probe is moved into the flow cell inside the NMR magnet. After measuring the sample the probe is

moved back to the Gilson 215 autosampler and eliminated into the waste.

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