A Practical High-Throughput Screening System for Enantioselectivity by Using FTIR Spectroscopy

Patrick Tielmann,[a] Matthias Boese,[b] Martin Luft,[b] and Manfred T. Reetz*[a]

Dedicated to Professor Reinhard W. Hoffmann on the occasion of his 70th birthday

Abstract: For the first time FTIR spectroscopy has been applied to the measurement of enantiomeric purity. The underlying concept is based on the use of *pseudo*-enantiomers that are ^{13}C-labeled at appropriate positions. Upon applying Lambert–Beer's law in the determination of the concentrations of both enantiomers, the *ee* values are accessible, accuracy to within $\pm 5\,\%$ of the true values being possible. The application of a commercially available high-throughput FTIR system results in a slightly decreased accuracy ($\pm 7\,\%$ for the *ee* values), but this allows a throughput of up to 10000 samples per day. The method is of interest in the area of combinatorial asymmetric catalysis and directed evolution of enantioselective enzymes.

Keywords: asymmetric catalysis · combinatorial chemistry · directed evolution · high-throughput screening · IR spectroscopy

Introduction

The catalytic synthesis of enantiomerically pure or enriched organic compounds is of great interest to industry and academia. Two major options for reaching this goal are available, namely synthetic catalysts such as transition-metal complexes[1] or biocatalysts such as enzymes[2] or catalytic antibodies.[3] In the former case a lot of trial-and-error is still needed despite rational approaches such as ligand design by molecular modeling. Recently, an alternative approach has been developed which makes use of combinatorial methods in the parallel synthesis of large numbers of chiral homogeneous catalysts.[4, 5] Nevertheless, the actual size of the libraries has been limited so far to less then 50–100 catalysts. Intriguing opportunities appear possible if larger libraries could be generated and screened. The challenges in this area of research include the development of new strategies for the synthesis of modular ligands and the development of high-throughput screening systems for the rapid determination of enantioselectivity.[5] In addition to initial progress in this field, which has been loosely called "combinatorial asymmetric catalysis", recent research in the area of directed evolution already has provided large libraries of potentially enantioselective biocatalysts[6] which likewise need to be evaluated by using high-throughput *ee*-screening systems. This fundamentally new approach to the creation of asymmetric catalysts is based on the combination of appropriate molecular biological methods for random gene mutagenesis and protein expression coupled with screening systems for the determination of thousands of *ee* values in a short period of time. Last but not least the opportunities that metagenome DNA panning[7] offers to organic chemists interested in asymmetric catalysis also depend on fast and precise *ee*-screening systems. In this regard it has been estimated that more than 99% of all enzymes occurring in nature are still unexplored.[7] It can be estimated that an enormous biodiversity can be investigated by collecting genes in the environment and expressing the corresponding enzymes in recombinant microorganisms. Figure 1 summarizes these three main approaches to the creation of large libraries of enantioselective catalysts.

We and others have previously developed various high-throughput screening systems for enantioselectivity which are in most cases complementary. For example, *ee* assays based on UV/Vis spectroscopy,[8] IR thermography,[9] capillary array electrophoresis,[10] and even special forms of GC[11] were devised in our laboratory, allowing between 700 and 20000 *ee* determinations per day. The accuracy in the *ee* value ranges between $\pm 2\,\%$ and $\pm 5\,\%$. Further *ee*-screening systems were developed by other groups by applying pH indicators or fluorescence,[12] circular dichroism,[13] enzymatic methods,[14] DNA arrays,[15] immunoassays,[16] or mass spectrometry of mass-tagged diastereomeric substrates.[17] In most cases a precision of $\pm 10\,\%$ was reported, which suffices in some, but not all, cases.

In addition to these methods we have also developed a new and convenient approach for measuring *ee* values based on

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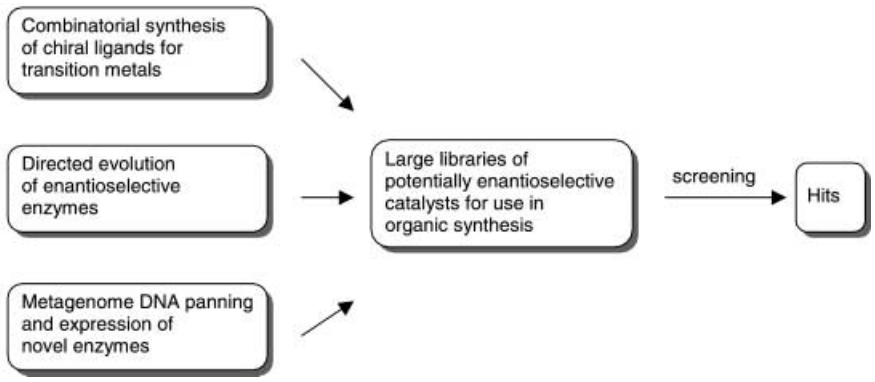
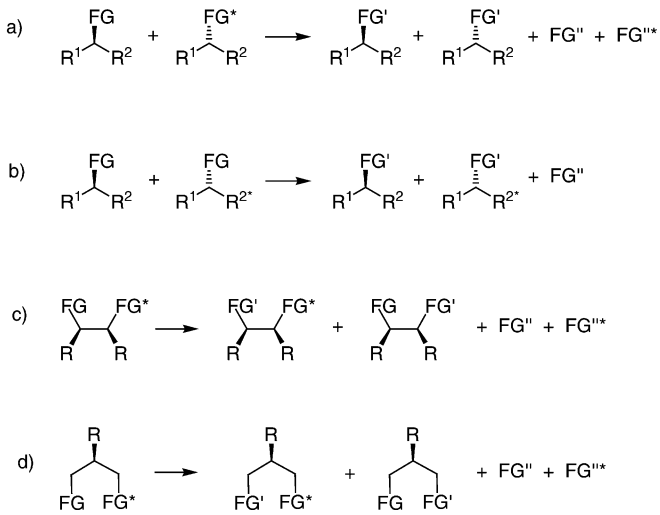


Figure 1. Sources of large libraries of potentially enantioselective catalysts.

isotopic labeling which leads to so called *pseudo*-enantiomers and *pseudo-meso*-compounds (Scheme 1). The underlying principle is restricted to the desymmetrization of prochiral compounds bearing reactive enantiotopic groups and to the kinetic resolution of chiral coumpounds.



Scheme 1. a) 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 group FG and labeled FG*. d) Asymmetric transformation of a *pseudo*-prochiral substrate involving cleavage of the functional group FG and labeled FG*.

To determine the *ee* values of such isotopically labeled compounds in a fast and precise manner, two different approaches have been put into practice. The first one makes use of deuterated substrates that can be easily analyzed by standard MS techniques like electrospray ionization (ESI) or matrix-assisted laser-desorption ionization (MALDI).[18] A throughput of up to 10000 samples per day can be reached with an accuracy of better than ±5%.

The second method makes use of ^{1}H NMR spectroscopy of ^{13}C-labeled compounds, which allows the analysis of up to 1400 samples per day with an exceptionally high degree of precision (±2–3%).[19] For this purpose flow-through NMR systems need to be used, these being commercially available. We also developed a second NMR-based *ee*-screening system that also makes use of flow-through NMR systems, derivatization by a chiral reagent being necessary in this case. Precision amounts to ±5% of the true *ee* value.[19]

We now wish to present yet another high-throughput *ee*-screening system based on the same general principle, but in this case using FTIR spectroscopy. Appropriate isotopic labeling of one enantiomer of a given (*R*)/(*S*) pair leads to *pseudo*-enantiomers which in a mixture can be distinguished by FTIR spectroscopy. As will be seen, this allows the determination of as many as 10000 *ee* values per day with a good degree of accuracy (±7%). Since FTIR spectroscopy is a cheap and readily available analytical technique that is available in almost all laboratories, we expect this method to become frequently applied. It is applicable to the evaluation of large libraries of synthetic chiral catalysts. Moreover, it is of particular interest in the analysis of biocatalytic reactions because the *ee* values can be measured directly in culture supernatants, making a time-consuming and expensive work-up unnecessary.

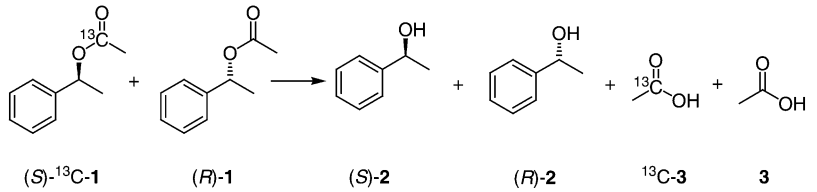
Results and Discussion

To evaluate the applicability of FTIR spectroscopy for the determination of *ee* values for a given substrate, especially with regard to accuracy, the "best" position at which isotopes are introduced needs to be determined. To illustrate the method, we considered the kinetic resolution of esters, which is often accomplished by the catalytic action of lipases.[21] In doing so ^{13}C labeling of carbonyl groups was chosen for several reasons:

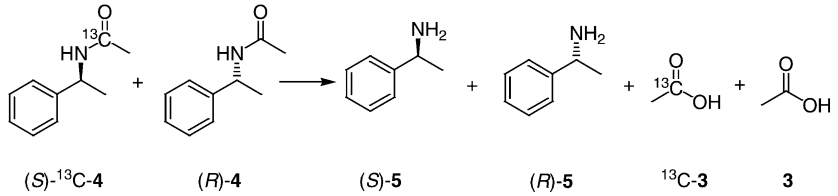
a) Carbonyl groups provide intensive vibrational bands in an IR spectrum, allowing for easy and precise determination of the concentration of the compounds by applying Lambert–Beer's law.
b) In the spectral region between 1600 and 1800 cm^{-1}, which is typical for carbonyl stretching vibrations, almost no absorption of other functional groups appear, eliminating interferences with other vibrational bands.
c) The ^{13}C-labeled compounds can be easily prepared because reactive reagents with ^{13}C-labeled carbonyl groups such as 1-^{13}C-acetyl chloride are commercially available.
d) The absorption maxima of the carbonyl stretching vibration is shifted by 40 to 50 cm^{-1} to lower wavenumbers by introducing a ^{13}C-label, which prevents the overlap of the two carbonyl bands.[22]

Keeping these advantages in mind we synthesized (*R*)-1-phenylethyl acetate ((*R*)-**1**), (*S*)-(1-phenylethyl)-1-^{13}C-acetate

((*S*)-^{13}C-**1**), (*R*)-*N*-1-phenylethylacetamide ((*R*)-**4**) and (*S*)-*N*-(1-phenylethyl)-1-^{13}C-acetamide ((*S*)-^{13}C-**4**), which are possible *pseudo*-enantiomeric substrates for enzyme-catalyzed hydrolytic kinetic resolutions (Schemes 2 and 3). Figure 2 shows part of the FTIR spectrum of a 1:1 mixture of (*R*)-**1** and (*S*)-^{13}C-**1**, illustrating the anticipated shift of the respective carbonyl stretching vibration which allows quantification of the *pseudo*-enantiomers.



Scheme 2.



Scheme 3.

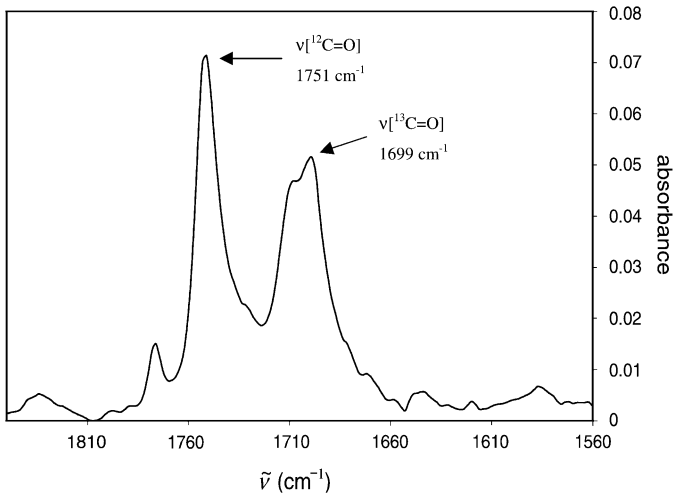


Figure 2. Part of an FTIR spectrum of a 1:1 mixture of (*R*)-**1** and (*S*)-^{13}C-**1**.

These compounds were used to evaluate the accuracy of a possible high-throughput screening system by measuring different synthetic mixtures of the *pseudo*-enantiomers with a standard FTIR spectrometer and checking the *ee* values by chiral GC analysis. To apply Lambert–Beer's law in the calculation of the concentrations of the *pseudo*-enantiomeric substances, the molar coefficients of absorbance had to be determined. For this reason we prepared solutions of (*R*)-**1** and (*S*)-^{13}C-**1** in cyclohexane and (*R*)-**4** and (*S*)-^{13}C-**4** in 1,2-dichloroethane with different concentrations. After measuring the corresponding absorbances at the absorption maxima of the carbonyl stretching vibration, we were able to calculate the molar coefficients of absorbance by applying Lambert–Beer's law $E = \varepsilon \cdot c \cdot d$ (Figure 3, Table 1).

With these coefficients in hand, the exploitation of the FTIR spectra of different synthetic mixtures of the labeled and nonlabeled enantiomeric compounds was possible. After applying an automated baseline correction to the spectra and correcting the absorbance of one enantiomer in the synthetic mixtures by the absorbance of the other enantiomer at this position, the accuracy of the *pseudo*-enantiomeric system based on 1-phenylethyl acetate turned out to be excellent, specifically within ±3% in comparison to the *ee* values determined by chiral GC (Table 2).

In the case of the phenylethyl acetamides (*R*)-**4** and (*S*)-^{13}C-**4**, the precision decreased slightly (±5% of the true *ee* values), but this is still quite acceptable (Table 3). It is currently not clear why the small deviations are all positive, that is, whether a systematic source of error is involved.

Encouraged by these results, we decided to carry out high-throughput measurements with commercially available HTS-

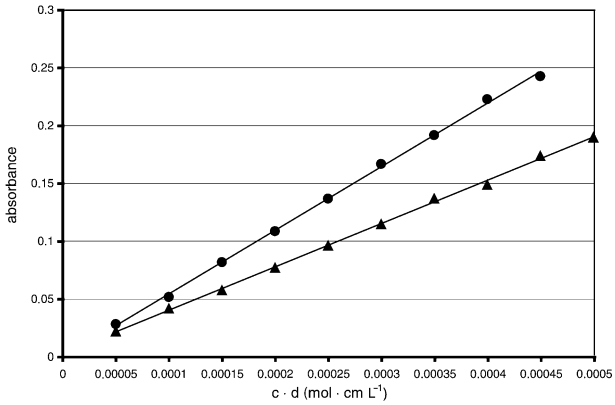


Figure 3. Diagram for the determination of the molar coefficients of absorbance of (*R*)-**1** (●, absorption maximum: 1751 cm^{-1}) and (*S*)-^{13}C-**1** (▲, absorption maximum: 1699 cm^{-1}) by linear regression.

FTIR systems. The analysis was performed on a Tensor 27 FTIR spectrometer coupled to a HTS-XT system (Bruker Optik GmbH) which is able to analyze the samples on 96 or 384 well microtiter plates. The plates are equipped with a silicon plate for IR transmittance. Moreover, we attempted to measure the *ee* values in culture supernatants, which would avoid a time-consuming and expensive workup of biocatalytic reactions in water. In the case of the MS- or NMR-based assays this additional step is indispensable. Such a simplifica-

Table 1. Molar coefficients of absorbance (ε) of the *pseudo*-enantiomers calculated by linear regression.

	Compound	ε [L cm^{-1} mol^{-1}]	Correlation coefficient
1	(*R*)-**1**	550.6	0.999
2	(*S*)-^{13}C-**1**	374.9	0.999
3	(*R*)-**4**	477.1	0.995
4	(*S*)-^{13}C-**4**	403.2	0.996

Table 2. Comparison of the *ee* values of synthetic mixtures of (*R*)-**1** and (*S*)-^{13}C-**1** determined by chiral GC and FTIR using a standard FTIR spectrometer.

	ee by GC [%]	*ee* by FTIR [%]	Deviation [%]
1	100 (*S*)	100 (*S*)	0
2	94.4 (*S*)	93.9 (*S*)	0.5
3	89.7 (*S*)	87.5 (*S*)	2.2
4	71.0 (*S*)	70.1 (*S*)	0.9
5	40.2 (*S*)	37.8 (*S*)	2.4
6	2.6 (*S*)	0.2 (*R*)	2.8
7	39.8 (*R*)	42.7 (*R*)	2.9
8	71.0 (*R*)	73.9 (*R*)	2.9
9	89.7 (*R*)	93.1 (*R*)	3.4
10	94.0 (*R*)	95.7 (*R*)	1.7
11	99.7 (*R*)	100 (*R*)	0.3

Table 3. Comparison of the *ee* values of synthetic mixtures of (*R*)-**4** and (*S*)-^{13}C-**4** determined by chiral GC and FTIR using a standard FTIR spectrometer.

	ee by GC [%]	*ee* by FTIR [%]	Deviation [%]
1	100 (*S*)	100 (*S*)	0
2	93.4 (*S*)	95.1 (*S*)	1.7
3	89.3 (*S*)	90.2 (*S*)	0.9
4	69.8 (*S*)	70.3 (*S*)	1.5
5	40.0 (*S*)	37.1 (*S*)	2.9
6	0.4 (*S*)	4.7 (*R*)	5.1
7	39.6 (*R*)	45.1 (*R*)	5.5
8	71.4 (*R*)	75.4 (*R*)	4.0
9	88.6 (*R*)	93.3 (*R*)	4.7
10	93.4 (*R*)	96.5 (*R*)	3.1
11	100 (*R*)	100 (*R*)	0

tion would be of great value in projects concerning the directed evolution of enantioselective enzymes.

For this reason 19 synthetic mixtures of (*R*)-**4** and (*S*)-^{13}C-**4** in DMSO (0.20 M) were prepared, of which 14 were used to calibrate the system, while the remaining samples were used as unknown mixtures. We diluted 20 μL of each mixture with 180 μL of culture supernatant (*E. coli* in LB-medium) containing extracellular proteins, nutrients like glucose and other components. After placing 3 μL of each mixture three times on a 384 microtiterplate and drying in an desiccator for 30 min at 220 mbar and ambient temperature, the microtiter plate was transferred into the HTS-XT system. The measurements were performed with a resolution of 8 cm^{-1} and 10 scans per sample resulting in a total time for each sample of 8.9 s. We were pleased to discover that the system works surprisingly well. The accuracy of the *ee* values of the unknown samples, which were automatically calculated by the software Opus,[23] decreased slightly (±7% of the true *ee* value determined by chiral GC) (Table 4).

Table 4. Comparison of the *ee* values of synthetic mixtures of (*R*)-**4** and (*S*)-^{13}C-**4** determined by chiral GC and FTIR using a high-throughput FTIR spectrometer.

	ee by FTIR [%]	*ee* by GC [%]	Deviation [%]
1	87.1 (*S*)	90.8 (*S*)	3.7
2	85.3 (*S*)		5.5
3	87.0 (*S*)		3.8
4	57.4 (*S*)	52.0 (*S*)	5.4
5	56.9 (*S*)		4.9
6	52.3 (*S*)		0.3
7	97.4 (*R*)	90.2 (*R*)	7.2
8	97.5 (*R*)		7.3
9	94.5 (*R*)		4.3
10	11.5 (*R*)	12.4 (*R*)	0.9
11	10.4 (*R*)		2.0
12	11.1 (*R*)		1.3
13	33.5 (*S*)	29.8 (*S*)	3.7
14	30.5 (*S*)		0.7
15	33.4 (*S*)		3.6

Although the present method has not yet been applied in a specific project concerning combinatorial asymmetric transition metal catalysis or directed evolution of enantioselective enzymes, high-throughput in the range of 10000 samples per day is technically possible under these conditions. For this purpose Bruker has already coupled the microplate stacking device TWISTER 1 (Zymark) to its microplate reader. In this combination which is controlled by OPUS software (Bruker), 40 IR microplates can be measured automatically. To perform the sample loading at high throughput, the autosampler microlab 4000 SP (Hamilton) was tested successfully. Both formats (96 and 384) of the Bruker silicon microplates were suitable to be loaded automatically with different types of samples (proteins, cells, culture media).

The surprisingly good accuracy connected with the high sample throughput and the opportunity to measure the *ee* values directly in culture supernatants makes this approach very valuable for the analysis of biocatalytic reactions in water. Furthermore, the selectivity factors *E* in kinetic resolutions can also be obtained because this system provides the concentration of each enantiomer which allows the conversion of the reaction to be calculated.[24]

Conclusion

We have devised a high-throughput FTIR system for the fast and precise determination of the enantiomeric purity of chiral acetates or acetamides. In the case of enzyme catalysis, supernatants can be used without any workup. A throughput of up to 10000 samples per day is possible by directly measuring the *ee* values in culture supernatants, the accuracy amounting to ±7% of the true value as checked by GC. This avoids the time-consuming and expensive workup of the

samples in biocatalytic reactions in water. Extension to other substrates should also be feasible, provided appropriate ^{13}C labeling is possible. Thus, we view this new and cheap *ee* assay to be a practical screening technique in enantioselective catalytic and biocatalytic reactions.

Experimental Section

General remarks: The reagents and solvents were obtained from commercial sources and the reagents were generally used without further purification. All solvents were distilled and stored under argon. The conditions for gas chromatographic analyses are given in the analytical data of the relevant compounds. ^1H NMR spectra were recorded on a Bruker AMX 300 (300 MHz) spectrometer. Chemical shifts are reported in ppm using tetramethylsilane (TMS, $\delta = 0.00$ ppm) as internal standard. ^{13}C NMR spectra were recorded at 75 MHz with $CDCl_3$ as internal reference. Mass spectra were recorded on a Finnigan MAT 8200 and IR data were obtained using a Perkin–Elmer FT 1600. High-throughput FTIR measurements were performed using a Bruker Tensor 27 spectrometer in connection with a High-Throughput-Screening Extension (HTS-XT) system. For data post processing Opus© software (Bruker) was used. Elemental analysis was carried out in an external laboratory (Mikroanalytisches Labor 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 the solution was cooled with an ice bath, the corresponding 1-^{13}C-labeled or unlabeled acetyl chloride (0.97 g, 12.3 mmol) was slowly added with a syringe with appearance of a white solid (pyridine hydrochloride). The reaction mixture was stirred overnight 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 1M HCl and brine. After the organic phase was dried with $MgSO_4$, the solvent was evaporated and the crude products were purified by 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 ppm (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 ppm; MS: *m/z* (rel. int.): 164 (25) [M^+], 122 (77), 104 (100), 77 (43); IR (neat): $\tilde{\nu} = 3064$, 3034 (C–H, ArH), 2982, 2934 (C–H, CH + CH_3), 1744 (C=O), 1242 (C–O) cm^{-1}; elemental analysis (%) calcd: C 73.3, H 7.3; found: C 72.9, H 7.4.

(S)-(1-Phenylethyl)-1-^{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, $J_{C,H} = 6.8$ Hz, 3H; $^{13}C(O)CH_3$); 5.88 (q, $J_{C,H} = 3.2$ Hz, $J = 6.6$ Hz, 1H; CH), 7.24–7.37 ppm (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 ppm; MS: *m/z* (rel. int.): 165 (38) [M^+], 122 (91), 104 (100), 77 (35), 44 (53); IR (neat): $\tilde{\nu} =$ 3065, 3034 (C–H, ArH), 2982, 2934 (C–H, CH + CH_3), 1691 (^{13}C=O), 1206, 1066 (C–O) cm^{-1}; elemental analysis (%) calcd: C + ^{13}C 73.3, H 7.3; found: C + ^{13}C 73.2, H 7.4.

GC-analysis: Hewlett–Packard 5890, column: 25 m TBCD/OV-1701, detector: FID, temperature programme: 230 °C, 60 °C – 2 °C per min – 180 °C, 5 min isotherm, 350 °C, gas: 1 bar hydrogen, retention time: 21.7 min ((*S*)-(1-phenylethyl)-1-^{13}C-acetate), 23.3 min ((*R*)-1-phenylethyl acetate), >99% ee in both cases.

Synthesis of *N*-1-phenylethyl acetamides 4: Enantiomerically pure (*S*)- or (*R*)-1-phenylethylamine (**5**) (0.90 g, 7.5 mmol) and pyridine (4 mL) were dissolved in dichloromethane (30 mL) in a 50 mL N_2 flask under argon. After the solution was cooled with an ice bath the corresponding 1-^{13}C-labeled or unlabeled acetyl chloride (0.59 g, 7.6 mmol) was slowly added with a syringe. The reaction mixture was stirred overnight at ambient temperature and the resulting yellow solution was quenched with water while being cooled with an ice bath. The organic phase was separated and subsequently washed three times with 1M HCl and once with brine. After the organic phase was dried with $MgSO_4$, the solvent was evaporated and the crude products were recrystallized from hexane. After filtration and drying in vacuum the desired products were obtained as white solids. Yield: 0.73 g (64%).

(*R*)-*N*-1-Phenylethyl acetamide ((*R*)-4): ^1H NMR ($CDCl_3$): $\delta = 1.46$ (d, $J =$ 6.9 Hz, 3H; CH_3), 1.95 (s, 3H; $C(O)CH_3$), 5.10 (q, $J = 7.0$ Hz, 1H; CH), 6.03 (s, 1H; NH) 7.24–7.37 ppm (m, 5H; ArH); ^{13}C NMR ($CDCl_3$): $\delta = 21.7$, 23.4, 48.8, 126.2, 127.3, 128.6, 143.3, 169.2 ppm; MS: *m/z* (rel. int.): 163 (54) [M^+], 148 (13), 120 (30), 106 (100), 77 (19), 43 (22); IR (KBr): $\tilde{\nu} = 3265$ (N-H) 3071, 3022 (C–H, ArH), 2980, 2972, 2929 (C–H, CH + CH_3), 1642 (C=O), 1557 (N-H) cm^{-1}; elemental analysis (%) calcd: C 73.6, H 8.0, N 8.6; found: C 73.5, H 8.1, N 8.5.

(*S*)-*N*-(1-Phenylethyl)-1-^{13}C-acetamide ((*S*)-4): ^1H NMR ($CDCl_3$): $\delta = 1.46$ (d, $J = 6.9$ Hz, 3H; CH_3), 1.95 (s, $J = 6.0$ Hz, 3H; $^{13}C(O)CH_3$), 5.10 (q, $J_{C,H} = 2.2$ Hz, $J = 7.0$ Hz, 1H; CH), 6.04 (s, 1H; NH) 7.24–7.37 ppm (m, 5H; ArH); ^{13}C NMR ($CDCl_3$): $\delta = 21.7$, 23.4, 48.8, 126.2, 127.3, 128.6, 143.3, 169.1 ppm; MS: *m/z* (rel. int.): 164 (46) [M^+], 149 (13), 120 (30), 106 (100), 77 (22), 44 (40); IR (KBr): $\tilde{\nu} = 3265$ (N–H) 3049, 3023 (C–H, ArH), 2972, 2929 (C–H, CH + CH_3), 1606 (^{13}C=O), 1545 (N-H) cm^{-1}; elemental analysis (%) calcd: C + ^{13}C 73.7, H 8.0, N 8.5; found: C + ^{13}C 73.7, H 8.0, N 8.5.

GC-analysis: Hewlett Packard 5890, column: 25 m Ivadex-1/PS086 G/375, detector: FID, temperature programme: 220 °C, 80 °C – 2 °C per min – 210 °C, 5 min isotherm, 320 °C, gas: 0.8 bar hydrogen, retention time: 26.7 min ((*S*)-*N*-(1-phenylethyl)-1-^{13}C-acetamide), 27.9 min ((*R*)-*N*-1-phenylethyl acetamide), >99% *ee* in both cases.

Procedure for the determination of molar coefficients of absorbance of the labeled and unlabeled 1-phenylethyl acetates: After preparation of a stock solution (0.200 M) of (*R*)-1-phenylethyl acetate ((*R*)-**1**) and (*S*)-(1-phenylethyl)-1-^{13}C-acetate ((*S*)-^{13}C-**1**) in cyclohexane, the solutions were diluted with cyclohexane to concentrations of 0.180, 0.160, 0.140, 0.120, 0.100, 0.080, 0.060, 0.040 and 0.020 M, respectively (total volume: 1 mL). The absorbance of the resulting samples was measured with a FTIR spectrometer at the corresponding absorption maxima of the carbonyl stretching vibration ((*R*)-**1**: 1751 cm^{-1}, (*S*)-^{13}C-**1**: 1699 cm^{-1}) with a thickness of the layers of 25.0 μm. The measurements were carried out with 32 scans and a resolution of 4 cm^{-1}. The molar coefficients of absorbance were determined by linear regression. The correlation coefficient were in both cases better than 0.995.

The analysis of synthetic mixtures of the *pseudo*-enantiomers of 1-phenylethyl acetate was performed under the same conditions at a concentration of 0.10 M.

Procedure for the determination of molar coefficients of absorbance of the labeled and unlabeled *N*-1-phenylethyl acetamides: After preparation of a stock solution (0.200 M) of (*R*)-*N*-1-phenylethyl acetamide ((*R*)-**4**) and (*S*)-*N*-(1-phenylethyl)-1-^{13}C-acetamide ((*S*)-^{13}C-**4**) in 1,2-dichloroethane, the solutions were diluted with 1,2-dichloroethane to concentrations of 0.180, 0.160, 0.140, 0.120, 0.100, 0.080, 0.060, 0.040 and 0.020 M, respectively (total volume: 1 mL). The absorbance of the resulting samples was measured with a FTIR-spectrometer at the corresponding absorption maxima of the carbonyl stretching vibration ((*R*)-**4**: 1676 cm^{-1}, (*S*)-^{13}C-**4**: 1635 cm^{-1}) with a thickness of the layers of 106.7 μm. The measurements were carried out with 32 scans and a resolution of 4 cm^{-1}. The molar coefficients of absorbance were determined by linear regression. The correlation coefficient were in both cases better than 0.995.

The analysis of synthetic mixtures of the *pseudo*-enantiomers of *N*-1-phenylethyl acetamide was performed under the same conditions at a concentration of 0.10 M.

High-throughput FTIR measurements: The high-throughput measurements were performed on a Tensor 27 spectrometer in connection with a HTS-XT system provided by Bruker Optik GmbH. For this reason 19 different synthetic mixtures of the *pseudo*-enantiomers of *N*-1-phenylethyl acetamide were dissolved in DMSO (total concentration: 0.20 M) and aliquots of 20 μL were diluted in 180 μL of a culture supernatant (*E. coli* in LB-medium). A 3 μL portion of each mixture were transferred onto a 384 microtiterplate equipped with a silicon plate for IR transmittance. This procedure was repeated three times for every sample. After the microtiterplate was dried in a desiccator for 30 min at 220 mbar and ambient temperature, the plate was placed in the HTS-XT system. Every sample was measured with a resolution of 8 cm^{-1} and a scan number of 10, so that the total time for the analysis of each sample amounted to 8.9 s, allowing a throughput of 9700 samples per day. The resulting spectra were analyzed

with the software Opus and Opus Lab.[23] The first 14 samples were used for calibration purposes, while the remaining probes were taken as unknown samples. To evaluate the accuracy of the system, the *ee* value of each mixture was independently determined by chiral GC analysis.

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