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A high-throughput NMR-based ee-assay using chemical shift imaging†

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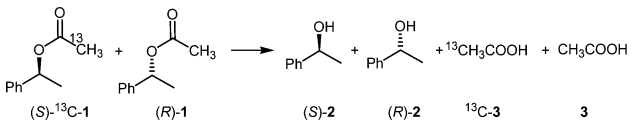
The throughput of a previously described NMR-based ee-assay has been increased by a factor of at least 4 as a consequence of adapting the system to chemical shift imaging; by using a 19-capillary system the enantiomeric purity of 5600 samples can be measured per day.

The development of efficient high-throughput screening systems for assaying the enantiomeric purity of chiral compounds is crucial in three different areas, namely combinatorial asymmetric transition metal catalysis,[1] directed evolution of enantioselective enzymes[1,2] and metagenome DNA panning.[3] Although a number of such ee-assays have been described, none is general, which is why research needs to continue in this area. We have previously reported an NMR-based assay for screening enantioselective catalysts in which either *pseudo*-enantiomers (or *pseudo-meso*-compounds) are ^{13}C-labeled,[4] or diastereomers are formed classically using chiral reagents or complexing agents.[5] A throughput of about 1400 ee determinations per day is possible in both cases by using an appropriate flow-through NMR-cell connected to an auto-sampler, the ee-values being accurate to ±5% of the true values. We now report that throughput of the NMR approach can be increased by a factor of at least 4 as a consequence of employing chemical shift imaging (CSI).[6]

We adapted CSI originally for NMR based structural characterization of large compound libraries.[7] In order to increase parallelization and sample throughput while circumventing the need for additional costly hardware, CSI was combined with a compartmented detection volume realized by using a bundle of nine 1 mm NMR-suited glass capillaries placed inside a 5 mm standard NMR-tube. Recently a 19-capillary system has been devised.[8] The CSI-experiment can be performed on any standard 5 mm NMR probe equipped with a triple-axis gradient system widely used in analytical chemistry nowadays. We describe here the application of this setup as a new high-throughput ee-screening system.

The model reaction that we considered in our original NMR-based ee-assay and in the present study concerns the lipase-catalyzed hydrolytic kinetic resolution of *rac*-1-phenylethyl acetate derived from *rac*-1-phenylethanol. It is necessary to label one of the enantiomeric forms as in (*S*)-^{13}C-**1**, and to prepare a 1 : 1 mixture of (*S*)-^{13}C-**1** and (*R*)-**1** which simulates a racemate. As kinetic resolution proceeds, the ratio of (*S*)-^{13}C-**1** to (*R*)-**1** changes and selectivity can be measured quantitatively by ^{1}H NMR spectroscopy. This is possible because the ^{13}C-labeled methyl group appears as a doublet while in non-labeled (*R*)-**1** it is a singlet at δ = 2.06 ppm. In this expanded region of the ^{1}H NMR spectrum the two *pseudo*-enantiomers are nicely distinguishable. The ratio of the two is accessible by simple integration of the respective peaks and thus provides the ee-value. The presence of naturally occurring ^{13}C in the non-labeled (*R*)-substrate can be taken into account if high accuracy is needed. An internal standard can be used to monitor the degree of conversion.

In order to test the present CSI-based ee-assay, we prepared 11 different mixtures of (*S*)-^{13}C-**1** and (*R*)-**1** so as to produce samples characterized by different enantiopurity. The exact ee-values were first measured by conventional gas chromatography (GC) using a



chiral stationary phase. Subsequently the samples were dissolved at 1 mM concentration in d_6-DMSO. Finally 25 µl of each sample was injected into the bundle of 1 mm capillaries. 256 free induction decays (FIDs) were acquired as described[7] by independent incrementation of *x*- and *y*-direction gradient-pulse strengths. The 16 × 16 sized data matrix allows for a spatial resolution high enough to resolve all individual capillaries in the image at high precision. According to the theory of NMR imaging[6] the "cross-talk" of amplitudes between extracted traces is well below 6% at the spatial resolution given. Cross-talk can be further reduced by application of optimized spatial sampling schemes. Fig. 1 shows the spatial skyline projection of the data after Fourier-Transformation (FT) with respect to *x*- and *y*-direction gradient-strength only. The expected 11 capillaries filled with test mixtures are clearly visible, whereas no NMR signal arises in the case of the remaining 8 empty capillaries of the 19-membered bundle. If all 19 capillaries are filled, the corresponding results are of the same quality.

Expanded regions of extracted ^{1}H spectra showing the methyl peaks indicative of the enantiomeric forms are displayed in Fig. 2. Here FT with respect to the acquisition time was done on traces taken orthogonally out of the 3D data set at *x*, *y* coordinates of the maxima in the centers of all capillaries. This process can be automated using known peak-picking algorithms.

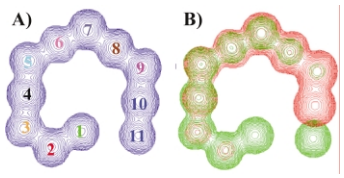


Fig. 1 Spatial images of a 19-capillary bundle. Only 11 capillaries are filled with samples of different (*S*)-^{13}C-**1**–(*R*)-**1** composition (see Table 1); 19 can also be used. Contours code for signal amplitude. Panel A shows an image obtained by FT along the spatial dimensions only. For comparison in panel B an image of one (*S*)-^{13}C-**1** satellite signal (in green) is superimposed with an image of the (*R*)-**1** signal (red).

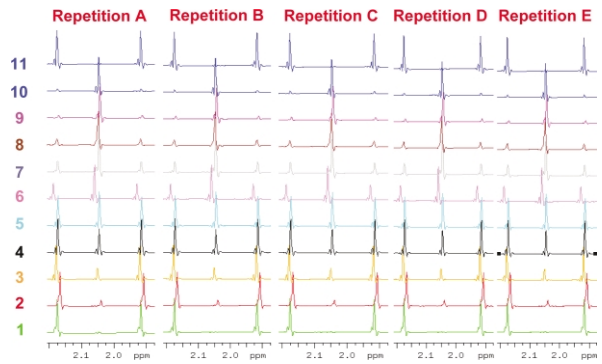


Fig. 2 Expanded region of the extracted ^{1}H 1D NMR spectra of the 11 mixtures of (*S*)-^{13}C-**1**–(*R*)-**1** (see Table 1).

† Dedicated to Jean-Marie Lehn on the occasion of his 65th birthday.

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At the given signal-to-noise level only spurious cross-talk between the traces was seen. In order to check reproducibility, the measurement was repeated five times (Repetitions A–E). The results are excellent (Fig. 2).

The ee-values were calculated on the basis of the integrated peak intensities and compared with the corresponding GC-values (Table 1). The results show that the assay is highly accurate, with the largest deviation from the true ee-values derived from GC measurements of ±6.4%. Improving this to ±5% is probably possible by further optimization. In order to reliably identify hits in thousands of samples obtained from directed evolution studies or combinatorial asymmetric catalysis,[1,2] such a degree of accuracy suffices. If needed, hits can be characterized more precisely by conventional analytical tools.

The NMR experimental time needed, if a single scan of 1s is performed per gradient setting, is 4 minutes per 19 samples. It was shown that 19 samples can be measured in an optimized flow-through setup within 18 minutes. Thus a decrease of NMR experimental time needed by a factor of 4 has been realised here, if a fast sample changing system is used for the exchange of the 19-capillary bundles. The CSI-based ee-assay can be performed on any commercially available NMR spectrometer. In order to inject samples from 384-format deep-well microtiter plates into the capillaries of the bundle automatically, an in-house developed pipetting robot with a modified tray can be used. Price worthy disposable glass capillaries fulfil quality requirements imposed by the NMR experiment. Alternatively a bundle of flow-through capillaries can be constructed. In either case at least 5600 ee-determinations are possible per day.

In summary, since the NMR approach to high-throughput determination of enantiomeric purity is the most general ee-assay currently available,[5] the present CSI-adaptation constitutes a significant advancement.

Table 1 ee-Values of 11 samples of (*S*)-^{13}C-**1**–(*R*)-**1** determined by GC and CSI

Sample	Ee (%) by GC	Ee (%) by CSI	Deviation (%)
1	100 (*S*)	94.0 (*S*)	6.0
2	84.7 (*S*)	82.6 (*S*)	2.1
3	72.4 (*S*)	66.0 (*S*)	6.4
4	48.2 (*S*)	48.0 (*S*)	0.2
5	24.6 (*S*)	27.3 (*S*)	2.7
6	8.4 (*R*)	2.0 (*R*)	6.4
7	26.2 (*R*)	23.4 (*R*)	2.8
8	50.6 (*R*)	45.4 (*R*)	5.2
9	74.6 (*R*)	68.4 (*R*)	6.2
10	81.8 (*R*)	75.7 (*R*)	6.1
11	99.6 (*S*)	96.7(*S*)	2.9

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