Advanced Synthesis & Catalysis

Very Important Publication

DOI: 10.1002/adsc.201500311

On-Column Reaction Set-Up for High-Throughput Screenings and Mechanistic Investigations

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Received: March 28, 2015; Revised: June 5, 2015; Published online: September 11, 2015

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/adsc.201500311.

Abstract: A screening platform, which offers a high-throughput approach as well as an easy investigation of kinetic isotope effects, applicable to a wide range of reactions is presented. To illustrate the high potential of this approach, the asymmetric transfer hydrogenation of methyl benzoylformate with copper(II) bis(oxazoline) and Hantzsch ester was examined. Accordingly, the enantioselectivities of the reaction performed on-column in a microcapillary were comparable to standard reaction conditions, however, we were able achieve catalysis and analysis in a single step in less than 30 min. The throughput can be increased by simultaneous investigation of different substrates without increasing the overall analysis time. Use of di-deuterated Hantzsch ester allowed us to investigate the kinetic isotope effect of the transfer hydrogenation reaction only requiring a minute amount of the deuterated transfer hydrogenation reagent. Hence we were able to get further insights into the mechanism of the asymmetric transfer hydrogenation using Hantzsch ester as hydrogen source. The here presented technique is broadly applicable to study isotope effects on a very small scale, which is a rapid and an inexpensive alternative compared to conventional experiments.

Keywords: asymmetric transfer hydrogenation; copper(II) bis(oxazoline); Hantzsch ester; high-throughput; kinetic isotope effect; on-column reaction gas chromatography

Introduction

To optimize the yield and selectivity of a reaction requires the detailed knowledge of the mechanism and all factors influencing the reaction. Conventionally, this information is obtained by tedious synthetic and consequent analytical steps in combination with systematic variation of the reaction conditions, reactants, catalysts and their concentrations. However, this procedure is often highly time consuming and associated with high experimental costs. To minimize the number of steps, methods for a statistical experimental design were developed and represent a highly promising approach.[1] Despite this progress there is a demand for techniques to speed up the monitoring process, for example, by combination of synthesis and analysis in a single step. In this area microfluidic devices (lab on a chip) and flow-through microreactors are broadly used[2] for various applications in organic synthesis[3] and total analysis.[4] Microcapillaries used routinely in gas chromatography (GC), open-tubular liquid chromatography (OTLC) and capillary electrophoresis (CE) for highly efficient and fast separations, can also be used as microreactor devices.[5] Coating of the inner surface of a capillary with a catalyst species enables one to screen catalyzed reactions. This integration of catalytic activity and separation selectivity in a single or consecutive microreactor column offers many advantages, like the investigation of interconversions of stereoisomers by dynamic chromatography[6] or as screening platform under varying reaction conditions, substrates or catalysts.[5] Furthermore, the overall screening process can be accelerated by simultaneous investigation of several reactants at the same time by injection of reactant libraries, because of the simultaneous separation no competitive reactions can take place allowing the determination of reaction kinetics and selectivities under exactly the same reaction conditions.[7] Additionally, such a set-up is predestinated for mechanistic investigations using isotope labelled compounds because only small amounts are required and analysis by mass spectrometry can be directly performed.

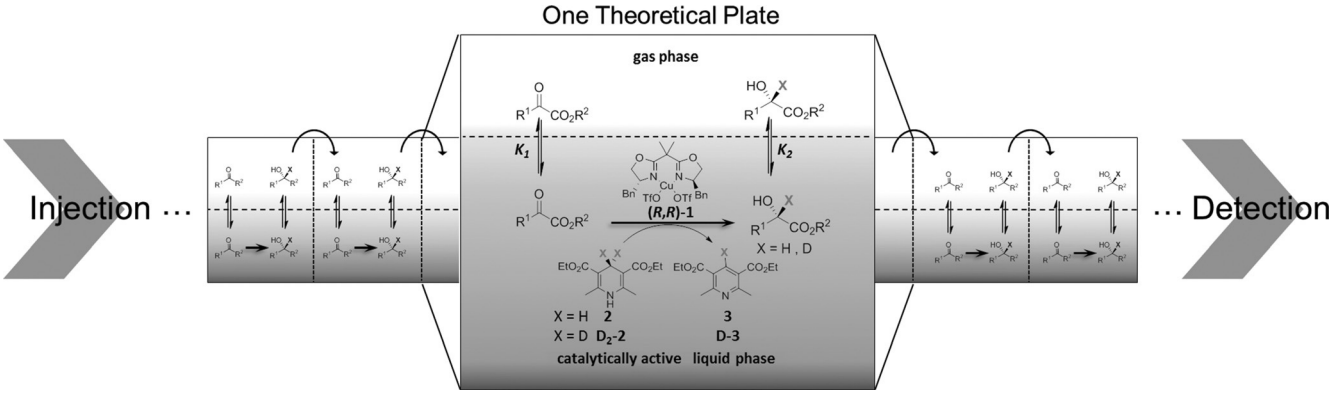


Figure 1. Schematic overview of the reaction process taking place in a chromatographic reactor based on the theoretical plate model.

Here we present an on-column reaction gas chromatographic (ocRGC) set-up applicable for high-throughput measurements as well as for mechanistic investigations based on kinetic isotope experiments. We chose the asymmetric transfer hydrogenation[8] of ketones by a copper(II) bis(oxazoline) catalyst and Hantzsch ester to demonstrate the features of such an on-column reaction chromatographic set-up.

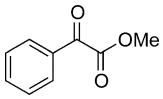
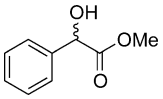
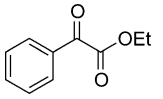
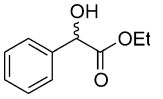
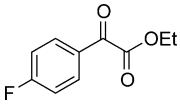
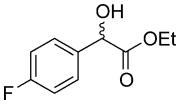
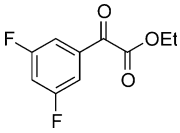
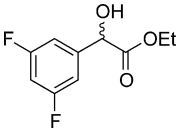
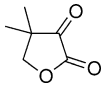
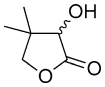
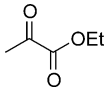
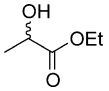
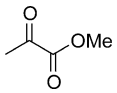
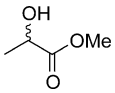
Results and Discussion

Establishing the Transfer Hydrogenation Screening Set-Up

To transfer the conventional batch reaction set-up to a nanoliter capillary reactor we prepared in a first step catalytically active capillaries with Cu(II) bis(oxazoline) {2,2-bis[(*4R*)-benzyl-4,5-dihydro-1,3-oxazol-2-yl)propane]copper(II) bistriflate} (***R*,*R*)-1** embedded as catalyst and Hantzsch ester **2** as hydrogen donor. Catalyst and transfer hydrogenation reagent were dissolved in an inert polysiloxane of medium polarity (PS086 containing 12–15% diphenylsiloxy and 85–88% dimethylsiloxy groups) and coated on the inner surface of fused-silica capillaries (I.D. 250 μm) by the static method described by Grob[9] resulting in a catalytically active film with a defined thickness of 250 nm. The final (***R*,*R*)-1** catalyst loading was only 9.82×10^{-5} mg (1.36×10^{-10} mol) and 2.62×10^{-4} mg (1.03×10^{-9} mol) for the Hantzsch ester **2** per cm of capillary. These nanoliter capillary reactors were employed for asymmetric transfer hydrogenations in an on-column reactions gas chromatographic set-up (ocRGC), combining stereoselective catalysis and stereoselective analysis in a single step. Figure 1 shows schematically the theoretical background of this set-up, considering the theoretical plates of a chromatographic separation column as chromatographic flow-through reactor, where phase distribution, catalysis and phase shift from one plate to the next plate takes place.

The experimental set-up we used in this study consists of the catalytically active capillary coupled between a pre-separation capillary (GE-SE-30; length 0.5 m, I.D. 250 μm; film thickness 500 nm) and an enantioselective separation capillary (chiral stationary phase heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin;[10] length 1.5–8 m, I.D. 250 μm; film thickness 250 nm). The pre-separation column achieves an optimal equilibration after the injection (split injection at 250 °C) and spatial separation of the injected reactants, enabling high-throughput investigations due to the absence of competing reactions, whereas the (post)-separation column enables a complete separation of the formed enantiomers. Reagents and products were detected by flame ionization detection (FID) for quantification and identified by MS (quadrupole–ion trap MS) in a single step. The absolute configuration[11] of the catalytically formed major enantiomers was assigned by co-injection of the pure (*S*)-enantiomers. Optimal reaction and separation conditions were found to be at 60 °C and 250 kPa helium to achieve reaction and separation at the same time. A temperature increase to 80 °C leads to an 8% decrease of conversion, which can be attributed to thermal decomposition of the catalyst (see the Supporting Information). The enantioselectivity of the reaction shows on the other hand no temperature dependence (30–80 °C). The slight decrease in enantioselectivity compared to the results published by List et al.[8] might be contributed to the here applied higher reaction temperatures. Remarkably, the reactor capillary, stored under argon, was stable for several days without any loss of catalytic activity or selectivity (see the Supporting Information).

Table 1. Summarized results of the asymmetric transfer hydrogenation by enantioselective ocRGC.[a]

Entry	Substrate	Product	Major Product	Conversion [%]	*er* [%]
1			(*S*)	>99.5	79:21
2			(*S*)	98.8	76:24
3			(*S*)	>99.5	77:23
4			(*S*)	>99.5	65:35
5[b]			(*S*)	49.9	70:30
6[b]			(*S*)	43.3	70:30
7[c]			(*S*)	26.4	67:33

[a] *Reaction conditions:* 60 °C, inlet pressure 250 kPa He as inert carrier gas; experimental set-up: see text or the Supporting Information.
[b] Chiral stationary phase of the separation capillary was heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-*β*-cyclodextrin; length 11.0 m; I.D. 250 μm; film thickness 250 nm at 60 °C, 40 kPa He.
[c] Inlet pressure 40 kPa.

Substrate Screening

Various aromatic and aliphatic α-keto esters (Table 1) were investigated as substrates. Aryl alkyl α-keto esters were hydrogenated with high conversions and moderate enantioselectivites. EWG groups, for example, F,F groups, led to a slight decrease in the catalytic selectivity. Hydrogenation of the investigated aliphatic α-keto ester resulted in lower conversions and enantioselectivities.

Even with capillaries as long as only 12 cm we observed very high conversions (>96%) at contact times as short as 55 s because of the high specific interfacial area per volume. In comparison, ocRGC reactions performed under conventional conditions require much longer reaction times to achieve these conversions.[8]

Performing hydrogenation experiments using different capillary lengths (1.1, 2.3, 4.0, 5.2, 7.0, 10.0 cm ± 0.05 cm) at a constant loading of (***R***,***R***)**-1** resulted in contact times between 8 and 54 s ± 0.3 s. Here, the contact time and conversion correlate linearly with the length of the capillary (see the Supporting Information). To compare the results, which we obtained by ocRGC we performed the (***R***,***R***)**-1**-catalyzed asymmetric transfer hydrogenation reactions in a flask under conventional reaction conditions with four of the on-column analyzed α-keto esters. Because of its similar polarity compared to polysiloxane, toluene was chosen as solvent and the reaction temperature was set to room temperature. The obtained selectivities were comparable to the results published by List et al.[8] and were slightly higher than the selectivities determined by ocRGC (Figure 2). In agreement with the on-column measurements, the F,F group at the aromatic system lowered the catalyst's selectivity. Additionally, no temperature influence on the selectivity was observed in the investigated temperature range between 25 °C and 60 °C. Interestingly, ketopantolactone gave not only lower enantioselectivities compared to the here investigated aromatic α-keto esters but was also in very good agreement with the results obtained by ocRGC catalysis.

To demonstrate the possibility of achieving a higher throughput, a small substrate library containing 3 different α-keto esters (entries 1, 3 and 4 in Table 1) was simultaneously injected onto the experimental set-up. All three compounds were stereoselectively trans-

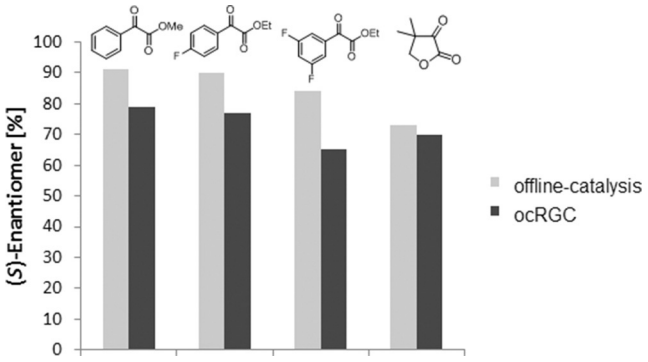


Figure 2. Comparison of enantioselectivites achieved by batch catalysis in a flask and ocRGC catalysis.

formed to the corresponding (*S*)-α-hydroxy ester with complete conversion by performing the reaction in a 50-cm reactor capillary. This capillary length was chosen to achieve a complete conversion of all substrates.

It has to be pointed out that catalysis and analysis could be achieved in less than 34 min and each product could be identified by three-dimensional MS analysis (Figure 3). Mass spectrometric detection allows us to deconvolute overlapping peaks of different compounds eluting at the same retention time and makes quantitative analysis and determination of enantiomeric ratios even in complex cases possible.

Study of Isotope Effects by On-Column Reaction Gas Chromatography

To demonstrate the advantages for mechanistic studies we investigated the kinetic isotope effect using at position 4 di-deuterated Hantzsch ester **D_2-2** (Figure 4). Deuterated Hantzsch ester was synthesized according to Westheimer et al.[12] and crystallized in the monoclinic space group C2m in contrast to the triclinic P$\bar{1}$ space group described in the literature.[13] The di-deuterated Hantzsch ester **D_2-2** was used under the same conditions like the undeuterated

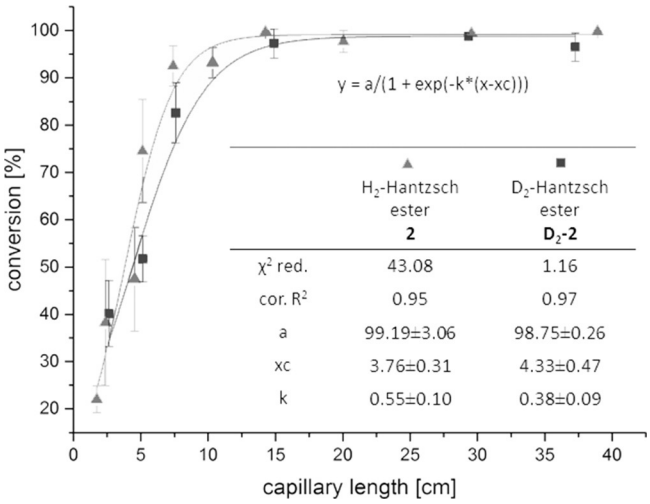


Figure 4. Conversions in dependence on the capillary length of methyl benzoylformate **4** in presence of the catalyst (***R***,***R***)**-1** and Hantzsch ester **2** or **D_2-2** dissolved in an inert polysiloxane matrix.

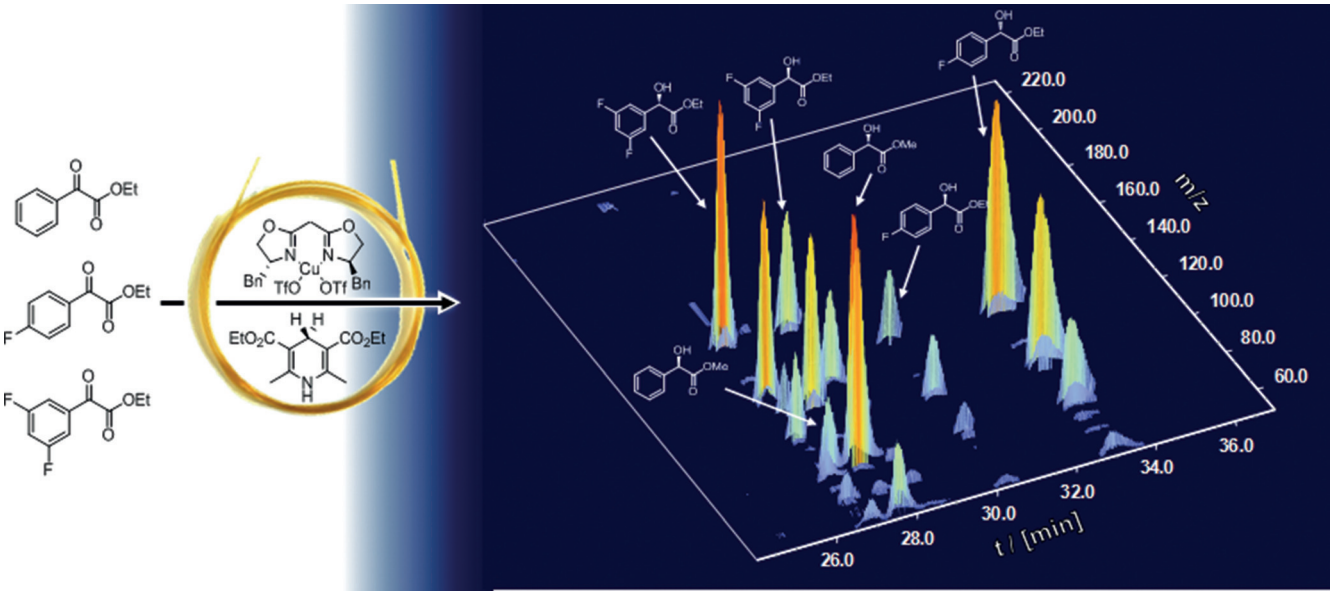
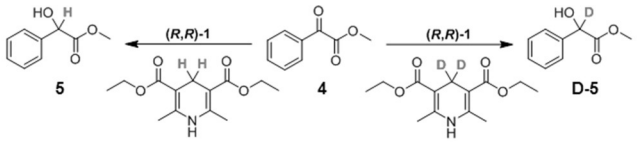


Figure 3. An example of a high-throughput measurement of a substrate library containing 3 different α-keto esters (entries 1, 3 and 4 in Table 1), which were simultaneously injected.



2			D_2-2		
Conv. [%]	*er* [%]		Conv. [%]	*er* [%]	Kinetic isotope effect
79.5	92:8	batch	52.8	92:8	1.51±0.29
38-93	79:21	ocRGC <10cm	40-83	81:19	None
>96.0	79:21	ocRGC >10cm	>93.3	79:21	None

Figure 5. Comparison of batch (offline) and ocRGC catalysis using either Hantzsch ester **2** or **D_2-2**. For the tabulated values obtained by the online measurements reactor capillaries with 2 to 10 cm for the category <10 cm and 15 to 40 cm for the category >10 cm are summarized. The conversion trend related to all measured capillary lengths is plotted as Figure 4.

compound **2** for the ocRGC catalysis as well as for the batch synthesis of methyl benzoylformate **4**. The mono-deuterated methyl mandelate **D-5** was identified as product (Figure 5 and Supporting Information). In ocRGC the conversions for both Hantzsch esters depend on the capillary lengths as expected and complete conversions are achieved for 10 and 15 cm capillary lengths (Figure 4), respectively. Statistical analysis of the experimental data shows an excellent agreement of the dependences in the range of experimental error (Table in Figure 5). Surprisingly, this suggests that the conversions are isotope independent. As shown in Figure 5 also the enantioselectivity seems to be independent. Only when shorter capillaries (<10 cm) and di-deuterated Hantzsch ester **D_2-2** were employed did the enantiomeric ratio increase slightly from 79:21 to 81:19 ($\Delta = 4\%$ *ee*). Although the conversion is not affected by the isotope labelling, it suggests that the enantioselectivity is influenced. Generally we observed that the enantioselectivities of ocRGC are slightly lower compared to conventional batch catalysis, which is due to higher reaction temperatures in ocRGC. Thus the increase of 4% *ee* by using Hantzsch ester **D_2-2** can be explained by an isotope effect, which only affects the post-hydrogenation racemization and therefore is independent of the original hydrogenation rate. With increasing capillary length there is a higher probability to interact with the catalysts, which in turn decreases the influence of the isotope effect, resulting in a constant enantiomeric ratio for deuterated and non-deuterated Hantzsch esters.

For comparison, the conventional batch catalysis was investigated for the non-deuterated **2** and the deuterated Hantzsch ester **D_2-2** under comparable conditions (Figure 5). In contrast to the on-column measurements, the batch catalysis showed a kinetic isotope effect of 1.51 ± 0.29.

Hitherto only investigations of kinetic isotope effects of the hydrogenation of ethyl α-cyanocinnamates and benzylidenemalononitriles with Hantzsch ester under non-catalytic conditions have been described.[14] Kinetic isotope effects in the range of 1.2 to 1.3 for mono-deuterated Hantzsch ester at position 1 (ND) and 5.3 to 6.0 for an at position 4 di-deuterated Hantzsch ester **D_2-2** were observed. Consequently it was assumed that the C–H bond dissociation at position 4 of the Hantzsch ester is the rate-limiting step, in terms of a direct hydride transfer.[15]

Taking into account that we only detect a kinetic isotope effect for the batch reaction, it seems that in the ocRGC this rate-limiting step is already completed before the substrate is converted into the product. Therefore no kinetic isotope effect is detectable. The only difference is that in ocRGC the catalyst (***R***,***R***)-**1** together with the Hantzsch esters **2** or **D_2-2** are embedded in an inert polysiloxane. We made the observation that if methyl benzoylformate **4** is added to a pre-mixture of catalyst (***R***,***R***)-**1** and Hantzsch ester **2** or **D_2-2** in the conventional batch experiment without the addition of a polysiloxane, the reaction is significantly slowed down compared to the classical protocol where methyl benzoylformate **4** is added to catalyst (***R***,***R***)-**1** followed by the Hantzsch ester. Interestingly, if polysiloxane is present no influence on the reaction rate is detectable when the reactant order is changed. Goodman et al. proposed for the hydrogenation of imines using Hantzsch ester under BINOL-phosphoric acid catalysis the formation of a three molecules-containing transition state, where the imine

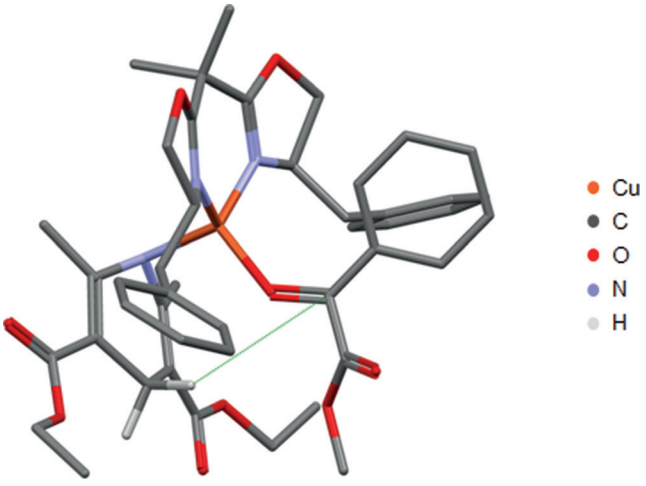


Figure 6. Calculated transition state (DFT calculations at the B3LYP/6-31G** level of theory) for the transfer hydrogenation of methyl benzoylformate **4** with (***R***,***R***)-**1** and Hantzsch ester **2**. The path of the hydride transfer is illustrated by a dotted green line. For clarity the innocent hydrogen atoms were not plotted.

and the Hantzsch ester are coordinated across the nitrogen proton to the phosphoric acid moiety of the catalyst.[16] Transferring this concept to the system presented here we get a transition state as depicted in Figure 6. Here, both the Hantzsch ester and the methyl benzoylformate **4** are coordinated to Cu of catalyst (***R***,***R***)-**1**. According to the mentioned kinetic influence in the batch synthesis and the ocRGC experiment, the rate-limiting step has to be the coordination of the Hantzsch ester to catalyst (***R***,***R***)-**1**. In the ocRGC approach such a complex can be preformed. This explanation fits to the batch synthesis experiments applying a different reactant order whereas when catalyst and Hantzsch ester were premixed in presence of the polysiloxane matrix before addition of methyl benzoylformate. In this case no isotope effect was detectable. In addition without polysiloxane the reaction was significantly slower.

Conclusions

In summary, we have presented a reactor set-up, which integrates catalysis with direct analysis in a nanoliter capillary and which can be used as a powerful tool to rapidly explore kinetic data (e.g., reactivity and enantioselectivity) as well as mechanistic data (e.g., isotope labelling experiments). Therefore both catalyst and hydrogen donor were dissolved in an inert polysiloxane matrix. In contrast to previously published results, a time-consuming modification of the catalyst's ligand backbone was not necessary to obtain an immobilized catalytic system. The here described catalytically active system is easy to handle, stable for several days and shows great activity exemplified in quantitative conversions in less than 60 s. Both aromatic and aliphatic α-keto esters were enantioselectively hydrogenated to the corresponding products. High-throughput applications can be realized with this set-up as demonstrated by injection of a substrate library containing 3 different substrates, which reduced the overall experimental time to just 34 min.

By straightforward use of deuterated Hantzsch ester as transfer hydrogenation reagent kinetic isotope effects could be analyzed. In combination with the chosen model reaction it enabled us not only to synthesize mono-deuterated α-hydroxy esters with high stereoselectivity but also to propose a model for the asymmetric transfer hydrogenation using Cu(II) bis(oxazoline) catalyst (***R***,***R***)-**1** with Hantzsch ester **2**. The here presented reactor set-up is not limited to the here investigated reaction, but can be used for other catalytic transformations using a reactive carrier gas, i.e., hydrogen in hydrogenations, or using reagents in the stationary phase. Limitations are the volatility of the substrates and reaction products, which is mandatory to obtain quantitative data.

Experimental Section

Materials and Methods

α-Keto esters which were used for ocRGC experiments were purchased from Sigma–Aldrich (St. Louis, USA) and were used without any further purification. Ethyl pyruvate and methyl pyruvate were freshly distilled before use. The inert polysiloxane PS086 which was used as stabilizing polymer matrix to prepare catalyst capillaries was purchased from ABCR chemicals (Karlsruhe, Germany). Anhydrous dichloromethane was used after boiling over calcium hydride and distillation under an inert atmosphere of argon.

GC and GC-MS-measurements were performed on a Thermo Trace GC-PolarisQ MS (Thermo, San Jose, California, USA) equipped with a split/splitless (SSL) injector (at 250 °C), an autosampler (Thermo AS 3000) a flame ionization detector (FID, operated at 250 °C) and a quadrupole–ion trap mass spectrometer. Electron impact mass data were recorded at an ion source temperature of 200 °C and electron energy of 70 eV. Gas chromatograms and mass traces were recorded using the Xcalibur software package (Thermo, San Jose, California, USA). Enantioselective GC was performed using fused-silica columns coated with the chiral stationary phase (CSP) heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin[10] (length 1.5–11 m, I.D. 250 μm, O.D. 365 μm, 250 nm film thickness). Helium was used as inert carrier gas. Fused-silica capillaries to prepare catalytically active reactor columns (I.D. 250 μm, O.D. 365 μm) were purchased from Microquartz (Munich, Germany).

Preparation of 4,4′-D_2-Hantzsch Ester (D_2-2)

4,4′-Dideuterio-2,6-dimethyl-3,5-dicarboethoxy-1,4-dihydropyridine **D_2-2** was prepared according to the procedure reported by Norcross, Klinedinst and Westheimer.[12] Paraformaldehyde-d_2 (100 mg, 3.10 mmol), *p*-toluenesulfonic acid (2.30 mg, 10.0 μmol), ethyl acetoacetate (754 mg, 5.80 μmol) and ammonia (2.00 mL of 2 N solution in ethanol, 3.2 μmol) was transformed to 4,4′-D_2 Hantzsch ester; yield: 298 mg (1.17 mmol, 38%). ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 1.28$ (t, $J = 7.15$ Hz, 6H), 2.19 (s, 6H), 4.17 (q, $J = 6.97$ Hz, 4H), 5.21 (bs, 1H); HR-MS (EI+): $m/z = 255.1435$, calcd. for $C_{13}H_{17}D_2NO_4$ [M]: 255.1440. Crystal data: $C_{13}H_{19}NO_4$, $M_r = 253.29$, $0.25 \times 0.24 \times 0.15$ mm^3, monoclinic, space group *C*2*m*, $a = 24.3328(5)$ Å, $b = 6.9207(2)$ Å, $c = 7.4758(2)$ Å, $\beta = 91.6095(10)°$, $V = 1258.43(6)$ Å^3, $Z = 4$, $\delta_{calcd.} = 1.337$ g cm^{-3}, $\lambda = 0.71073$ Å, $T = 200(2)$ K, $\theta_{range} = 1.674$–$27.507°$. Reflections measured 18735, independent 1557, $R_{int} = 0.0228$. Final *R* indices [$I > 2\sigma(I)$]: $R1 = 0.043$, $wR2 = 0.132$. Details of data collection, structure solution and refinement of crystal structures of **D_2-2** are contained in CCDC 1016121. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

Preparation of Cu(II) Bis(oxazoline) Complex (*R*,*R*)-1

{2,2-Bis[(4*R*)-benzyl-4,5-dihydro-1,3-oxazol-2-yl]-propane}-copper(II) bistriflate (*R*,*R*)-**1** was prepared according to the procedure reported by List et al.[8] $Cu(OTf)_2$ (5.80 mg, 16.0 μmol) and 2,2-bis[(4*R*)-4-benzyloxazoline]propane (5.80 mg, 16.0 μmol) were added to a flame-dried Schlenk tube. After drying the mixture under vacuum for 0.5 h freshly distilled dichloromethane (2.0 mL) was added and the mixture was stirred for 1 h.

Coating of Catalytically Active Capillaries

(*R*,*R*)-**1**, freshly prepared by the procedure described above (0.6 mg, 0.82 μmol), Hantzsch ester **2** (1.6 mg, 6.2 μmol) or **D_2-2** (1.6 mg, 6.2 μmol) and PS086 (12 mg) were dissolved in anhydrous dichloromethane (3 mL) and fused-silica capillaries were coated with this solution by the static method described by Grob.[9] This capillary (I.D. 250 μm, O.D. 365 μm) was filled with this solution and the solvent was removed under high vacuum after closing one end of the capillary with silicon to obtain a 250 nm polymer film on the inner surface of the capillary. Afterwards the capillary was flushed with argon for several minutes at room temperature to remove volatile components.

On-Column Reaction Gas Chromatographic Experiments of the Asymmetric Transfer Hydrogenation

On-column reaction gas chromatographic experiments of the asymmetric transfer hydrogenation were performed by enantioselective gas chromatography on a Thermo Trace PolarisQ GC-MS. For the stereoselective asymmetric transfer hydrogenation of the α-keto esters a fused-silica capillary (0.01–1 m in length) coated with the catalyst (***R***,***R***)-**1** and the Hantzsch ester **2** or **D_2-2** embedded in the polysiloxane matrix PS086 (I.D. 250 μm, film thickness 250 nm) was employed. This reactor column was coupled between a pre-separation capillary coated with GE-SE-30 (0.50 m, I.D. 250 μm, film thickness 500 nm) and an enantioselective separation capillary coated with heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin (1.5–11.0 m, I.D. 250 μm, film thickness 250 nm) to quantify reactants and products of the hydrogenation. Helium was used as inert carrier gas. All measurements were repeated 3 times.

Batch Reactions of the Asymmetric Transfer Hydrogenation

The batch synthesis measurements were realized based on the work of List et al.[8] Therefore freshly prepared (***R***,***R***)-**1** (1.8 mg, 5.0 μmol), following the procedure described above with chloroform as solvent unless otherwise indicated, were added to methyl benzoylformate **4** (4.1 mg, 25 μmol). After addition of Hantzsch ester **2** (8.86 mg, 35 μmol) or **D_2-2** (8.94 mg, 35 μmol) the sealed vials were stirred for 19 h under argon atmosphere. After filtration over celite the sample was analyzed by GC/MS using a pre-separation capillary coated with GE-SE-30 (0.50 m, I.D. 250 μm, film thickness 500 nm) and an enantioselective separation capillary coated with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin (1.5–11.0 m, I.D. 250 μm, film thickness 250 nm). Helium was used as inert carrier gas. All measurements were repeated 3 times.

Acknowledgements

We thank the European Research Council for financial support (ERC Grant Agreement No. StG 258740). We thank Ute Gärtner for coating fused-silica capillaries.

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