Supporting Information

On-line chiral analysis using the kinetic method

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Synthesis

Preparation of Compound **3**

This reaction was performed by combining magnesium tert-butoxide (542 mg, Sigma-Aldrich, St. Louis, MO) and **2 (**350 mg, Amgen Inc., Thousand Oaks, CA) with 60 mL of freshly distilled THF (Sigma-Aldrich) to a 250 mL round bottom flask which was then charged with argon. Then **1** (365 mg, Amgen Inc.) was added over 20 minutes, maintaining a batch temperature of 0 °C. Potassium tert-butoxide (2.2 mL of 20% in THF, Acros Organics, Geel, Belgium) was added in two aliquots over the course of an hour, while monitoring temperature. Between the two aliquot additions, the reaction mixture was brought to 20°C. After the second addition, the reaction mixture was heated slowly (ca. 45 minutes) to 35°C. Samples (50 µL each) were taken from the reaction vessel for analysis via syringe every 30 minutes. This procedure was modified from the work of Fang et. al.[1]

Racemization of Compound **3**

Forced racemization studies were conducted by addition of distilled water (20 mL) to the reaction vessel used to prepare compound **3** after the completion of the reaction in order to breakdown the catalytic tert-butomixides to butanol and hydroxide. The force degradation of the catalyst in turn provided conditions for base-catalyzed racemization of compound **3** via a keto-enol mechanism. This racemization was aided by refluxing the reaction mixture after the addition of water. Samples were taken intermittently for *ee* analysis of compound **3** and it was found that under the conditions used racemization of **3** is complete in ca. 1000 minutes. Note: the reaction did not show any signs of racemization after 24 hours when water was added to the reaction mixture and it was left at room temperature rather than under reflux in THF:water.

Racemization of (S)-ibuprofen

Racemization of ibuprofen has been previously characterized by Yuchun et al[2] and was therefore a desired target for this study for three reasons. 1) The concentration of strong base required to achieve racemization at room temperature on a manageable timescale was high, adding an additional challenge when performing on-line analysis of the reaction via MS, 2) the *ee* of ibuprofen has previously been studied on multiple occasions using the kinetic method[3,4] and 3) the mechanism of racemization involves the same keto-enol tautomerization as the racemization of compound **3**. The reaction was performed by adding (S)-Ibuprofen (99%, Sigma-Aldrich, St. Louis, MO, 0.1 M) to a DMSO:water (5:1, v:v) containing 0.5 M sodium hydroxide (Sigma-Aldrich, St. Louis, MO). Approximately 60 mL of this solution was loaded into the reaction mixture syringe of the on-line reaction monitoring apparatus for on-line chiral analysis.

On-line apparatus

In order to provide near-real-time chiral analysis using this methodology it was necessary to move from nESI to electrosonic spray ionization (ESSI) for the ionization process. ESSI (rather than nESI) allows for the continuous flow of reaction mixture into the mass spectrometer from the reaction syringe. In-line the alkaline reaction mixture is quenched and diluted by the first addition of dilute acid at the first mixing tee and further diluted by the addition of the ref* and M^{II}(Figure S1b). If the reagents had been more dilute or did not require the separate pH adjustment and addition of chiral reagents a simpler apparatus requiring only one mixing junction could have been used, further decreasing the dead volume of the apparatus (Figure S1a).

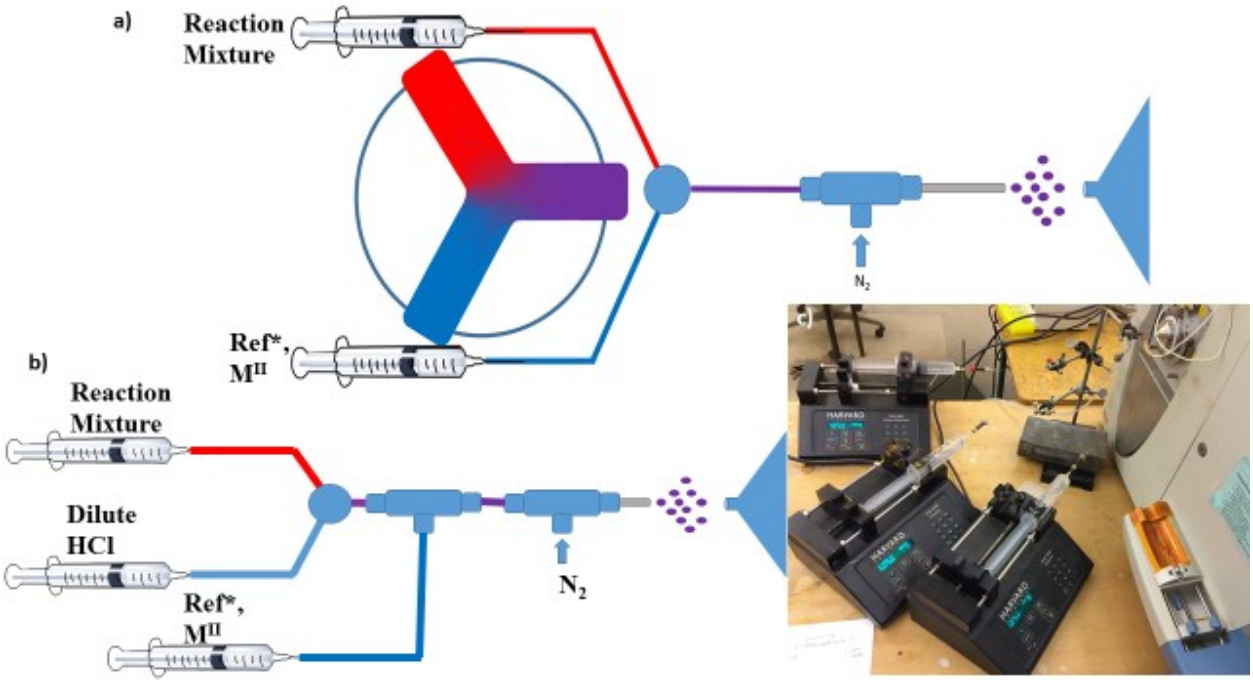

Figure S1: a) Simple in-line dilution and chiral analysis apparatus for ESSI analysis. b) Apparatus for successive in-line pH adjustment and addition of chiral reagents prior to ESSI and c) is a picture taken of the setup as used in this experiment.

The system used for chiral analysis used three 60 mL Luer-Lok tip syringes (BD, Franklin Lakes, NJ, REF 309653), two three-way mixing tees (Valco, Houston, TX and IDEX, Oak Harbor, WA), and three pH 2000 infusion syringe pumps (Harvard Apparatus, Holliston, MA) and an ESSI source constructed from 1/16' Swagelok. All lines were plumbed with 100 µm ID 259 µm OD fused-silica capillary (Polymicro Tech, Phoenix, AZ). The rather narrow ID of the fused silica was used to increase the speed of analysis and all tubing lines were kept as short as possible with this in mind.

Ligand selection for compound 4

From the spectra presented in Figure 6, it is apparent that, unlike chiral analysis method 1, only the product ion peaks for the loss of ref* and loss of A are present. This simplifies R for the relation between MS/MS ratio and ee (Eq. 4).

$$\ln(R) = \ln\left(\frac{I_{470}}{I_{472}}\right) \qquad \text{(Eq. 4)}$$

A calibration curve was developed using the same on-line apparatus as used for the on-line reaction monitoring experiment. This apparatus is described in the Supporting Information. This specific set of ref* and M^{II} shows more sensitivity for compound **4** than compound **3** and a precision of 7% *ee*.

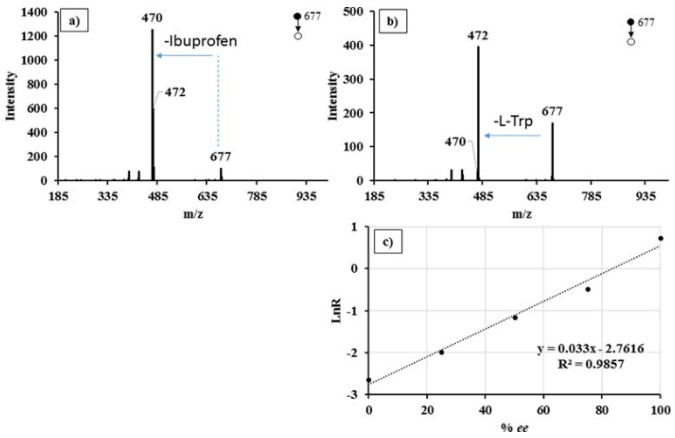

Fig. S2: Product ion MS of a) 100% *ee* (S)-**4** and b) 0% ee (S)-**4** where *m/z* 677 correlates to the ion cluster $[Cu^{II}(Ibu)(L\text{-}Trp)_2 - H]^+$ and *m/z* 472 and 470 correlate to the neutral loss of L-Trp and **4**, respectively. c) Calibration curve of % *ee* of **4** vs. the ln(R) where R is the ion current of *m/z* 470 divided by the current of *m/z* 472.

Sample preparation for off-line experiments

Preparation of compound 3

Sample preparation involved removal of 50 µL aliquots from the reaction mixture at ca. 30 minute intervals and mixing with diluent (1.0 mL water:methanol 1:1, v:v). Solvent addition was used to dilute the mixture and prevent carryover and to quench the catalyst to prevent any further reaction. This mixture was then adjusted with 3 M HCl to pH 3.5. For the chiral analyses, copper chloride (2.5 mM, 1.0 mL) and L-tryptophan (900 µM, 1.0 mL) were added with mixing and 10 µL of the mixture was loaded into a nanoESI tip.

Racemization of compound 3

To analyze the progress of the racemization reaction, 50 µL aliquots of reaction mixture were mixed with diluent (1.0 mL water:methanol 1:1, v:v). The mixture was then acidified by adding 10 µL of 3 M HCl. From this mixture 10 µL was withdrawn and combined with cobalt (II) chloride (7.5 mM, 10 µL), Gly-Gly-Ala-Gly (1.0 mM, 10 µL) and Gly-DAla (1.0 mM, 10 µL). After vortex mixing 10 µL of the mixture was loaded into a nanoESI tip.

[1] Fang, Y. Q.; Bio, M. M.; Hansen, K. B.; Potter, M. S.; Clausen, A. Magnesium Coordination-Directed N-Selective Stereospecific Alkylation of 2-Pyridones, Carbamates, and Amides Using α-Halocarboxylic Acids. *J. Am. Chem. Soc.* **2010**, *132* (44), 15525–15527.

[2] Yuchun, X.; Huizhou, L.; Jiayong, C. Kinetics of Base Catalyzed Racemization of Ibuprofen Enantiomers. *Int. J. Pharm.* **2000**, *196* (1), 21–26.

[3]Ranc, V.; Havlíček, V.; Bednar, P.; Lemr, K. Nano-Desorption Electrospray and Kinetic Method in Chiral Analysis of Drugs in Whole Human Blood Samples. *Eur. J. Mass Spectrom.* **2008**, *14* (2), 411.

[4]Augusti, D. V.; Augusti, R. Determination of the Enantiomeric Composition of Ibuprofen Solutions via a Rapid and Sensitive Mass Spectrometry Method. *Tetrahedron: Asymmetry* **2005**, *16* (10), 1881–1885.