Supporting Information

Protein-Engineering of an Amine Transaminase for the Stereoselective Synthesis of a Pharmaceutically Relevant Bicyclic Amine

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Materials and Methods

Materials

The ketone and amine substrates/reference compounds were made available by F. Hoffmann - La Roche as indicated in Scheme S1. Acylation of the commercially available ketoamine hydrochloride starting material (Ark Pharm Inc.) with PhCOCl/Et$_3$N/CH$_2$Cl$_2$, as described by S. Bollinger$^1$, had a propensity to generate a chloride mediated ring opened side product which could be precluded when the reaction was conducted in a two-phase system$^2$. Oxime formation followed by hydrogenation was conducted in analogy to H. Hamilton$^3$ and delivered a mixture of 3:2 endo/exo isomers. Non-selective reduction conditions were applied deliberately to render adequate amounts of both amine isomers required for this study. Efficient separation of the endo & exo amines was achieved on a Chiralpak IA (100x250mm) column employing a MeCN eluent containing 5% EtOH & 0.2% Et$_2$NH with UV-detection at 254 nm. The isolated isomers were ~99% pure. All reagents were of analytical grade.

Scheme S1: Synthesis overview of the amine preparation from the corresponding ketone precursor.
Figure S1: Calibration curves of different glycine standards at pH 9.5 (black, triangle) and pH 9.0 (grey, circles).

Figure S2. SDS-PAGE of purified variants of the amine transaminase 3FCR. Lane 1: 3FCR_QM, M: Marker, lane 2 = 3FCR_QM/Y59L, lane 3: 3FCR_QM/I234M, lane 4: 3FCR_QM/I234M/L382M, lane 5 3FCR_QM/S86A/I234M/L382M, lane 6: 3FCR_QM/Y59L/S86A/I234M/L382M.
HPLC Chromatograms of preparative reductive amination

4 h IPC

9 d IPC

Isolated exo amine

SFC chromatogram of preparative reductive amination

Isolated exo amine

Figure S3. HPLC and SFC chromatograms.
1H-NMR Spectra

1H-NMR spectrum - overview

1H-NMR (600 MHz, DMSO-d6): 8 ppm 7.33 - 7.55 (m, 5 H), 4.56 (br s, 1 H), 3.92 (br s, 1 H), 2.97 - 3.12 (m, 1 H), 1.58 - 2.00 (m, 6 H), 1.45 (s, 3 H), 1.35 (s, 1 H), 1.25 (s, 3 H)

1H-NMR spectrum - expansion region 1

NMR spectrum with detailed peaks and chemical shifts.
References:

