Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

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

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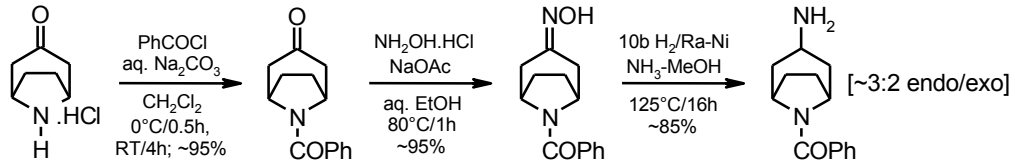
Materials and Methods

Materials



Scheme S1: Synthesis overview of the amine preparation from the corresponding ketone precursor.

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 $PhCOCl/Et_3N/CH_2Cl_2$, 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_2NH with UV-detection at 254 nm. The isolated isomers were ~99% pure. All reagents were of analytical grade.

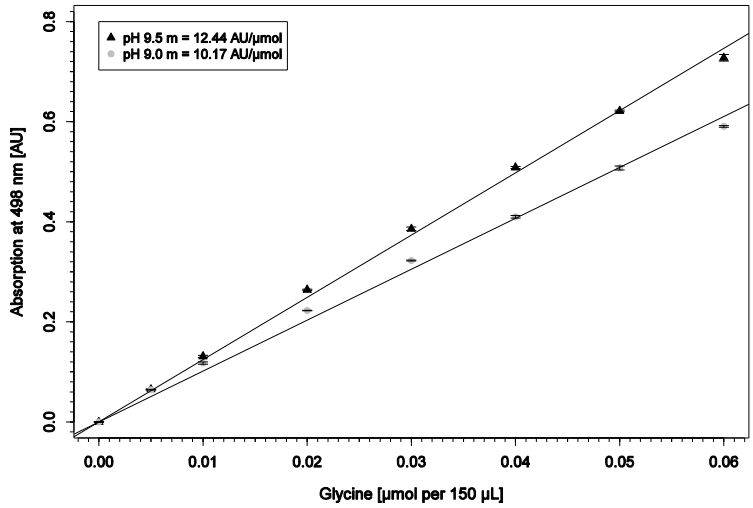


Figure S1: Calibration curves of different glycine standards at pH 9.5 (black, triangle) and pH 9.0 (grey, circles).

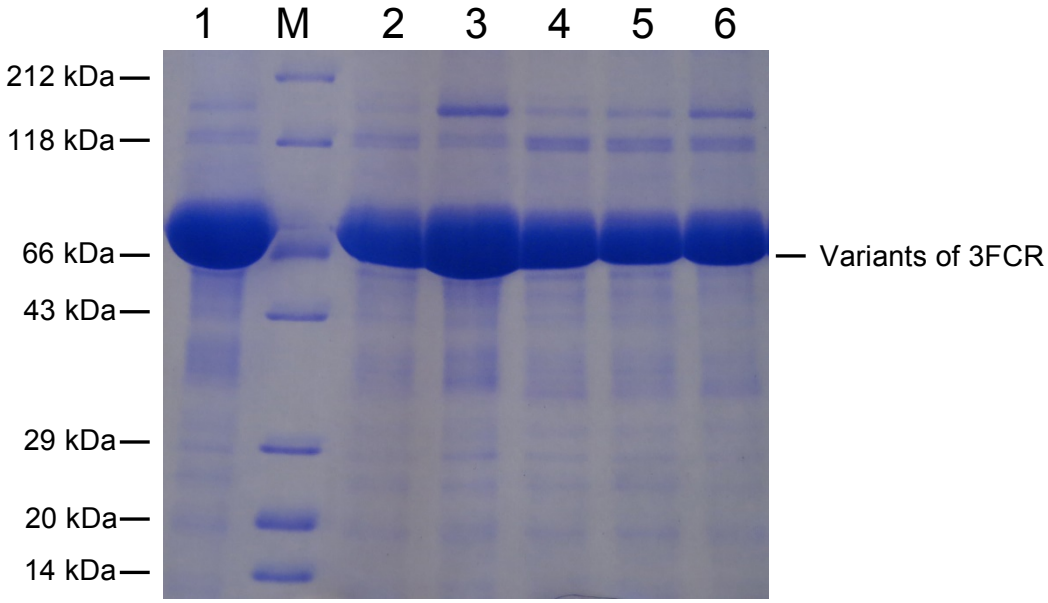
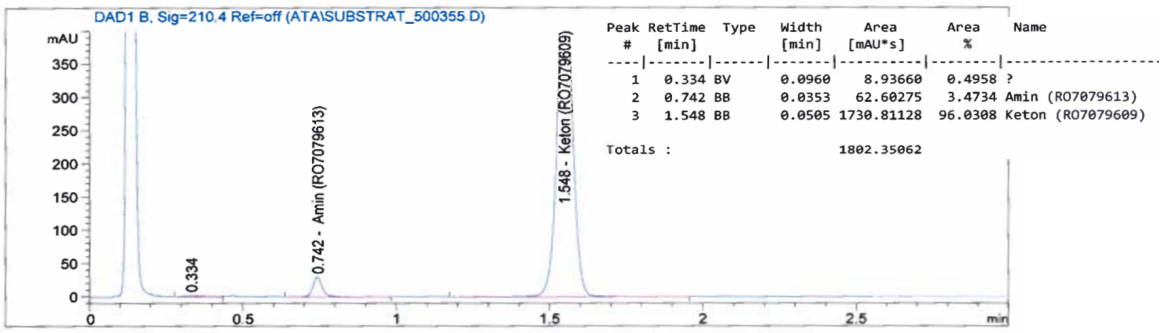


Figure S2. SDS-PAGE of purified variants of the amine transaminase 3FCR.Lane 1: 3FCR_QM, M: Marker, lane 2 = 3FCR_QM/Y59L, lane 3: 3FCR_QM/I234M, lane 4: 3FCR_QM/I234M/L382M, lane 5 3FCR_QM/S86A/I234M/L382M, lane 6: 3FCR_QM/Y59L/S86A/I234M/L382M.

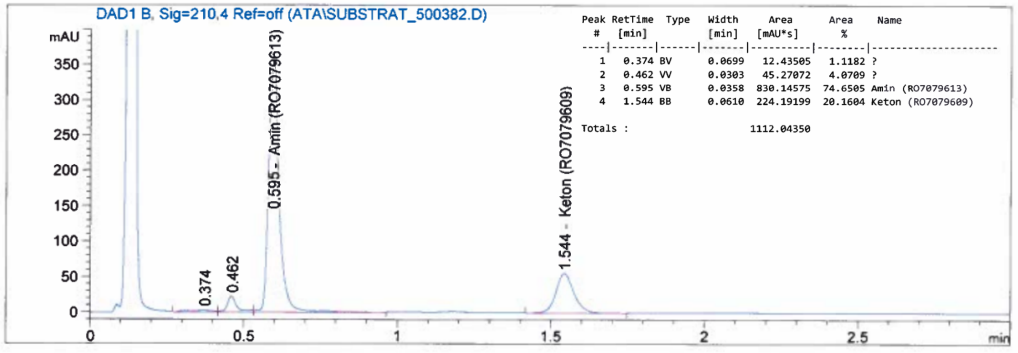
HPLC Chromatograms of preparative reductive amination

4 h IPC



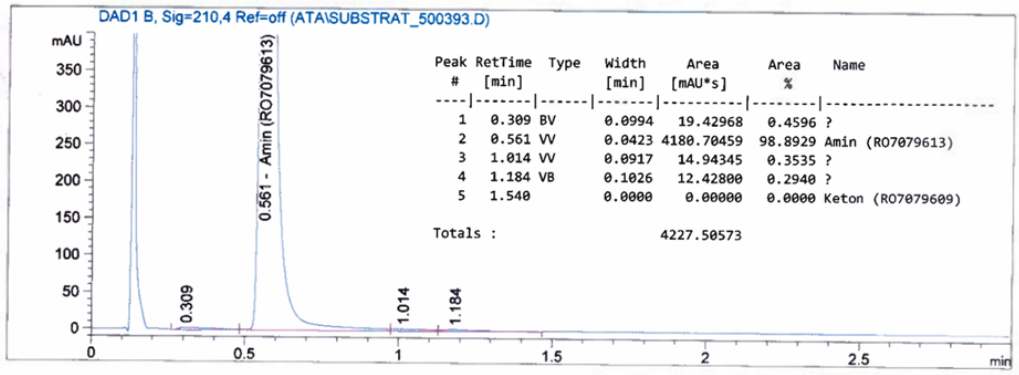
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	0.334	BV	0.0960	8.93660	0.4958	?
2	0.742	BB	0.0353	62.60275	3.4734	Amin (RO7079613)
3	1.548	BB	0.0505	1730.81128	96.0308	Keton (RO7079609)
Totals :				1802.35062		

9 d IPC



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	0.374	BV	0.0699	12.43505	1.1182	?
2	0.462	VV	0.0303	45.27072	4.0709	?
3	0.595	VB	0.0358	830.14575	74.6505	Amin (RO7079613)
4	1.544	BB	0.0610	224.19199	20.1604	Keton (RO7079609)
Totals :				1112.04350		

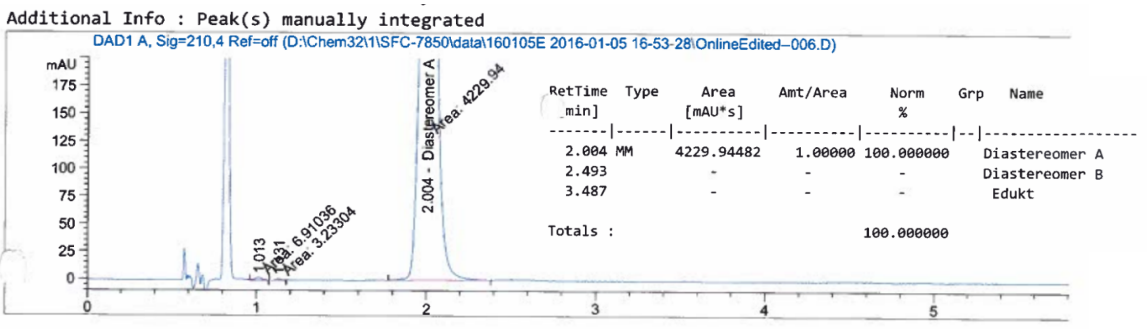
Isolated exo amine



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	0.309	BV	0.0994	19.42968	0.4596	?
2	0.561	VV	0.0423	4180.70459	98.8929	Amin (RO7079613)
3	1.014	VV	0.0917	14.94345	0.3535	?
4	1.184	VB	0.1026	12.42800	0.2940	?
5	1.540		0.0000	0.00000	0.0000	Keton (RO7079609)
Totals :				4227.50573		

SFC chromatogram of preparative reductive amination

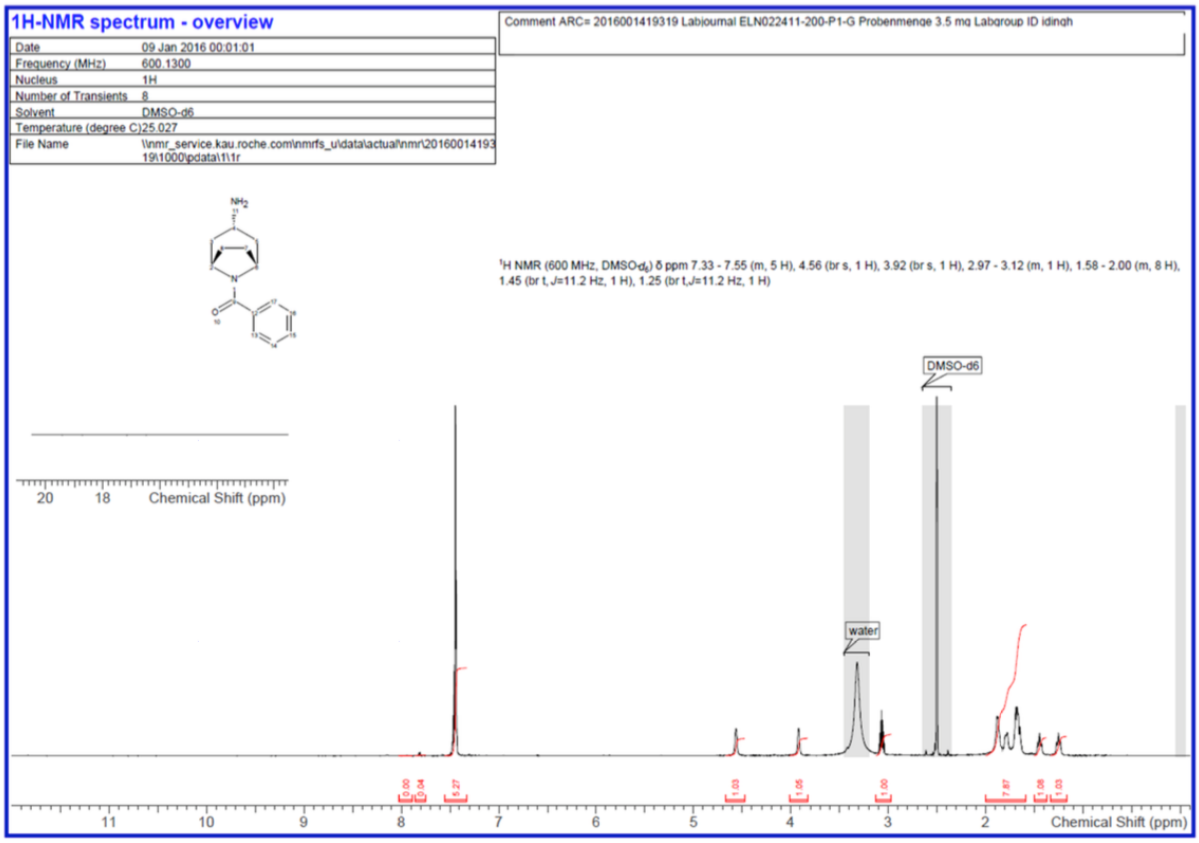
Isolated exo amine

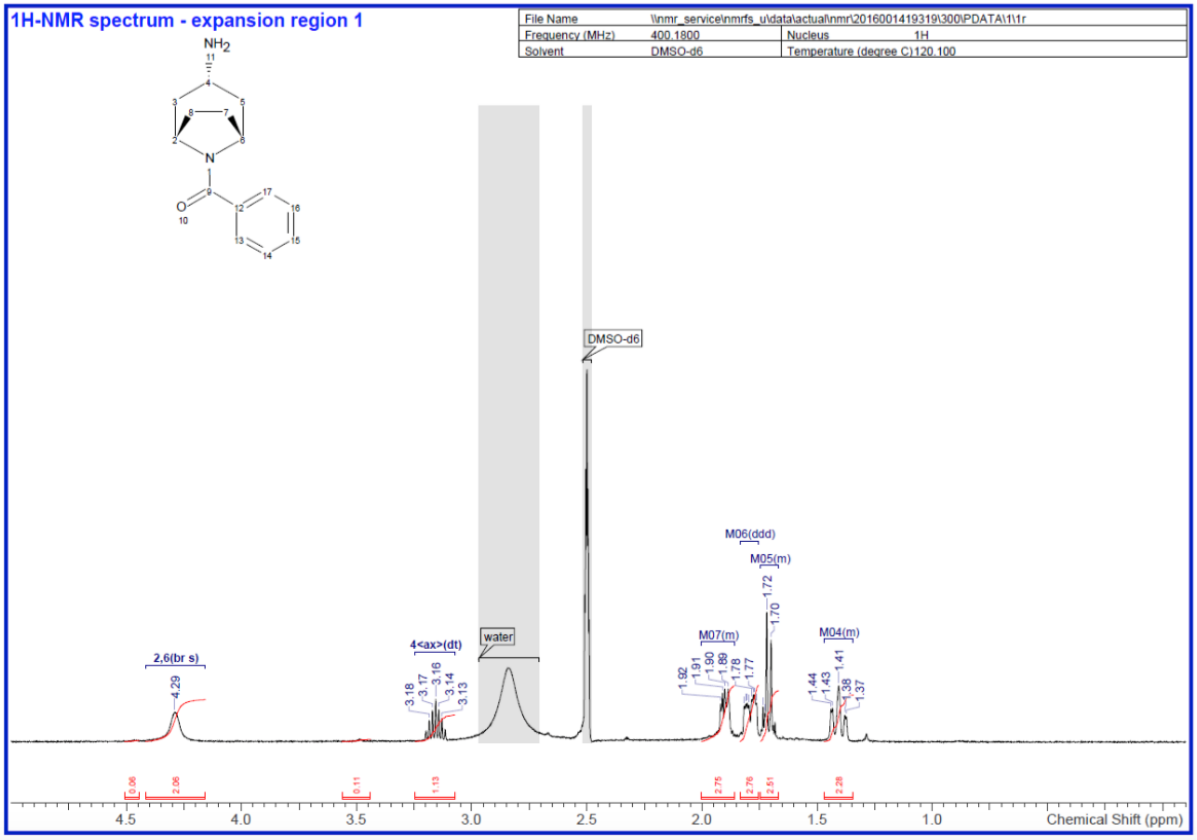


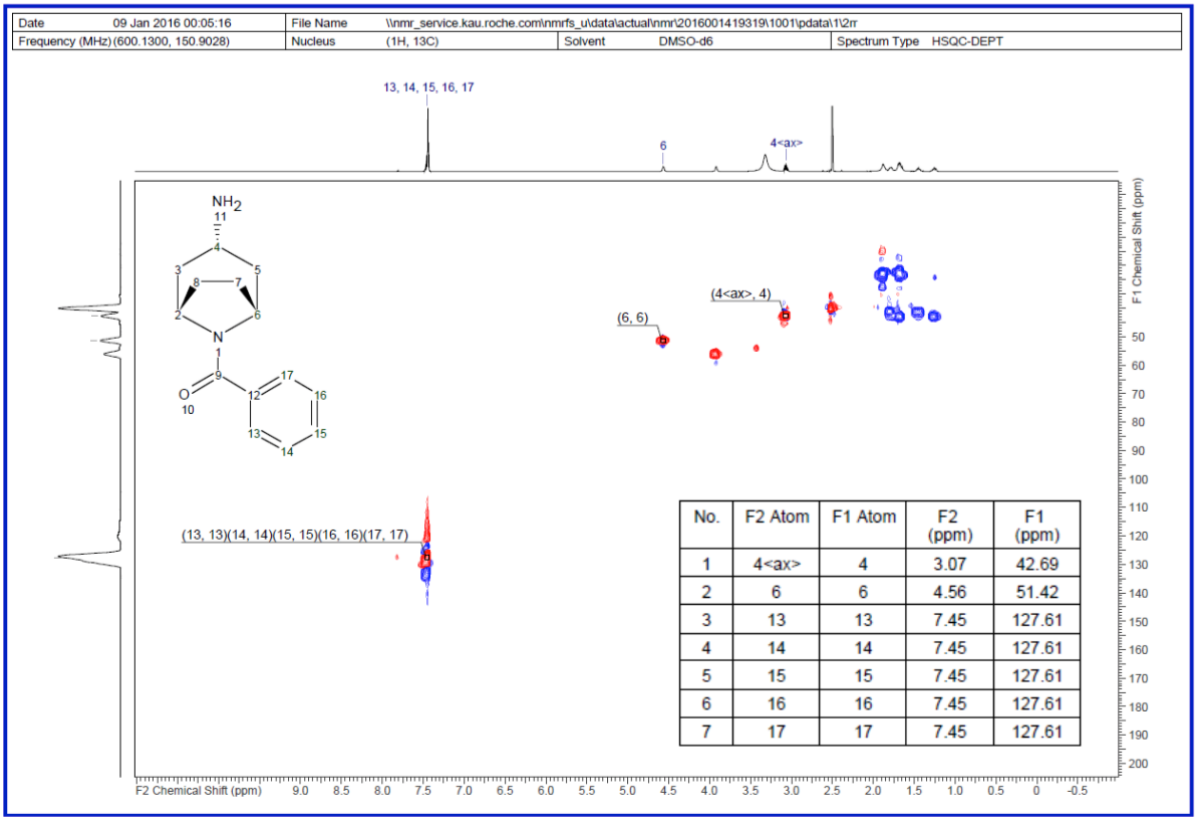
RetTime [min]	Type	Area [mAU*s]	Amt/Area	Norm %	Grp	Name
2.004	MM	4229.94482	1.00000	100.000000		Diastereomer A
2.493		-	-	-		Diastereomer B
3.487		-	-	-		Edukt
Totals :				100.000000		

Figure S3. HPLC and SFC chromatograms.

[1]H-NMR Spectra







No.	F2 Atom	F1 Atom	F2 (ppm)	F1 (ppm)
1	4<ax>	4	3.07	42.69
2	6	6	4.56	51.42
3	13	13	7.45	127.61
4	14	14	7.45	127.61
5	15	15	7.45	127.61
6	16	16	7.45	127.61
7	17	17	7.45	127.61

References:

1. S. Bollinger, H. Huebner, F. Heinemann, K. Meyer & P. Gmeiner, *J. Med. Chem.* **53**, 7167-7179 (2010).

2. L. Barton, J. Frazee, M. Hammond, P. Stoy, S. Thompson & D. Washburn, *WO 2008077089*.

3. H. Hamilton, W. Patt & B. Trivedi, EP0179631 (1985).