Supporting Information

Amination of a Green Solvent via Immobilized Biocatalysis for the Synthesis of Nemtabrutinib

Christopher K. Prier[1], Karla Camacho Soto[1]*, Jacob H. Forstater [1]*, Nadine Kuhl[1], Jeffrey T. Kuethe[1], Wai Ling Cheung-Lee[1], Michael J. Di Maso[1], Claire M. Eberle[1], Shane T. Grosser[1], Hsing-I Ho[1], Erik Hoyt[2], Anne Maguire[1], Kevin M. Maloney[1], Amanda Makarewicz[1], Jonathan P. McMullen[1], Jeffrey C. Moore[1], Grant S. Murphy[1], Karthik Narsimhan[1], Weilan Pan[1], Nelo R. Rivera[2], Anumita Saha-Shah[2], David A. Thaisrivongs[1], Deeptak Verma[3], Adeya Wyatt[1], and Daniel Zewge[2]

Corresponding Authors:

*Karla Camacho Soto- Email: karla.camacho.soto@merck.com
*Jacob H. Forstater- Email: jacob.forstater@merck.com

1. Process Research and Development, Merck & Co., Inc., Rahway, New Jersey 07065, United States
2. Analytical Research and Development, Merck & Co., Inc., Rahway, New Jersey 07065, United States
3. Computational and Structural Chemistry, Merck & Co., Inc., Rahway, New Jersey 07065, United States

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General Experimental Details

Chemicals and reagents.

Unless otherwise noted, all chemicals and reagents were obtained from commercial suppliers and used without further purification. Diaion HP2MGL and HP20 (Mitsubishi) resin was obtained from Itochu Chemicals America; Amberlite XAD-7HP (Dupont) resin was obtained from Sigma Aldrich. Relizyme BU113 resin were obtained from Resindion SRL. IB-ADS-1 resin was obtained from ChiralVision BV. PurosorB PAD950 and ECR resins were obtained from Purolite Ltd. Cyrene™ was purchased from Sigma-Aldrich or Circa Group directly.

Enzymes

Transamines (ATA) were used as lyophilized powders of *E. coli* cell lysate produced by either Merck or Codexis.

Analytical Assays

NMR Spectra: NMR spectra were obtained on a 500 MHz Bruker instrument, using DMSO-d6 as the solvent, and are referenced to TMS. Data for ^{1}H NMR are reported in the conventional form: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (Hz), and integration.

Gas Chromatography (GC). GC-FID analysis was performed using an Agilent DB-624 (6% cyanopropylphenyl/94% dimethyl polysiloxane) GC column (20 m x 0.18 mm, 1 μm) and operating at an inlet port temperature of 220 °C and an FID detector temperature of 280 °C. The oven temperature was increased at a rate of 20 °C/minute from 80 °C at t = 0 to 250 °C, and then held for 5 min using a constant hydrogen flow of 1 mL/minute.

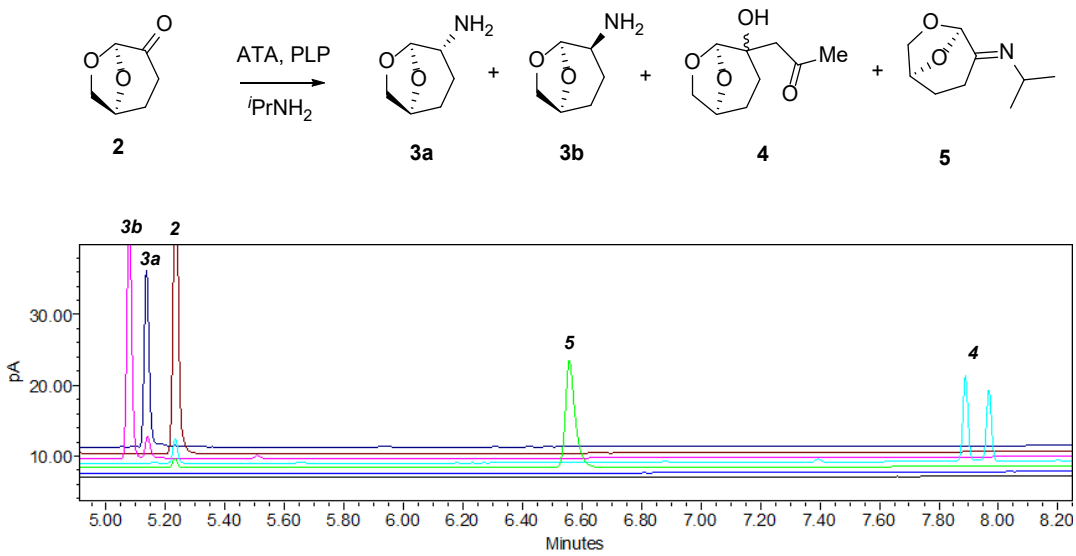


Figure S1. GC assay is used to measure the progress and selectivity of reactions with immobilized transaminase. Peak identities are labeled with the corresponding compound numbers in the scheme above.

UPLC Methods:

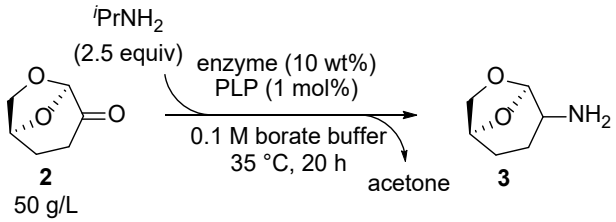
High Throughput UPLC Method: Reaction progress was monitored after derivatization with 1-fluoro-2,4-dinitrobenzene (DNFB, protocol below) using an Agilent 1290 Infinity II UPLC equipped with diode array detector (λ = 360 nm) and a CHIRALPAK IA-3 (3 µm, 50 x 4.6 mm) column, operating at 35 °C with a flow rate of 1.5 ml/min for 1.5 min. The method was run isocratically with a mobile phase of 90% Acetonitrile/10% 2mM Ammonium Formate in Water (pH 8.5). Derivatized (*R*)-Cyrene amine was observed to elute at 0.93 min, and derivatized (*S*)-Cyrene amine eluted at 1.2 min.

Long UPLC method*:* For reaction process development and determination of diastereoselectivity, was measured after derivatization with 1-fluoro-2,4-dinitrobenzene (DNFB, protocol below) using an Agilent 1290 Infinity II UPLC equipped with diode array detector (λ = 254 nm) and a CHIRALPAK IA-3 (3 µm, 50 x 4.6 mm) column, operating at 35 °C with a flow rate of 1.5 ml/min for 3.6 min. A binary eluent mixture of eluent A = 2 mM Ammonium Formate in water pH 8.5 and eluent B = 10 vol% Mobile Phase A in Acetonitrile was used with a gradient starting from 40%A:60%B to 20%A:80%B from 0 to 3.0 min, held isocratically at 20%A:80%B from 3.0 to 3.5 min, followed by a gradient from 20%A:80%B to 40%A:60%B from 3.5 to 3.6 min. Derivatized (*R*)-Cyrene amine was observed to elute at 2.1 min, and derivatized (*S*)-Cyrene amine eluted at 2.7 min.

DNFB Derivatization: To determine selectivity, a stock of 1-fluoro-2,4-dinitrobenzene (DNFB, 150 mg) and triethylamine (200 µL) was prepared in 9.6 mL acetonitrile. In vials, 990 µL of this reagent mixture was combined with 20 µL of each reaction. After at least 4 h of incubation, the derivatization reactions were filtered and analyzed by UPLC (method above).

Identification of a Cyrene™ transaminase

Screening protocol: Cyrene™ **2** (500 mg, (*1S,5R*)-6,8-dioxabicyclo[3.2.1]octan-4-one) and pyridoxal-5′-phosphate (PLP, 10 mg) were dissolved in 9.6 mL buffer (0.1 M borate, 1 M isopropylamine, pH 9.5). 500 µL of this solution was added to 2.5 mg of transaminase variant (lyophilized cell-free powder, 10 wt% relative to Cyrene™) in vials, and incubated at 35 °C with shaking. After 20 hr, 100 µL of each reaction was diluted with 600 µL of 0.5% maleic acid (internal standard) in D_2O for analysis by quantitative ^1H NMR.



Scheme S1. Aqeuous transamination of Cyrene™ **2** as detailed below and in Kuhl et al.[1]

Table S1. Conversion and selectivity of ATA-426 under aqueous conditions at (50 g/L and 100 g/L Cyrene™ **2**).

	50 g/L		**100 g/L**	
wt% ATA-426	**Conversion**	**Selectivity (*R*:*S*)**	**Conversion**	**Selectivity (*R*:*S*)**
5 wt%	42%	> 20:1	34%	19:1
10 wt%	57%	17:1	44%	17:1
15 wt%	62%	18:1	49%	15:1
20 wt%	71%	17:1	54%	16:1

Table S2. Temperature dependence and aldol content as a function of temperature and Cyrene™ **2** concentration (50 g/L or 100 g/L) using 15 wt% ATA-426.

	50 g/L			**100 g/L**		
Temperature	**Conversion**	**Selectivity (R:S)**	**Aldol**	**Conversion**	**Selectivity (R:S)**	**Aldol**
35 °C	62%	18:1	1%	49%	15:1	3%
45 °C	62%	>20:1	3%	45%	>20:1	5%
55 °C	45%	>20:1	6%	41%	>20:1	8%

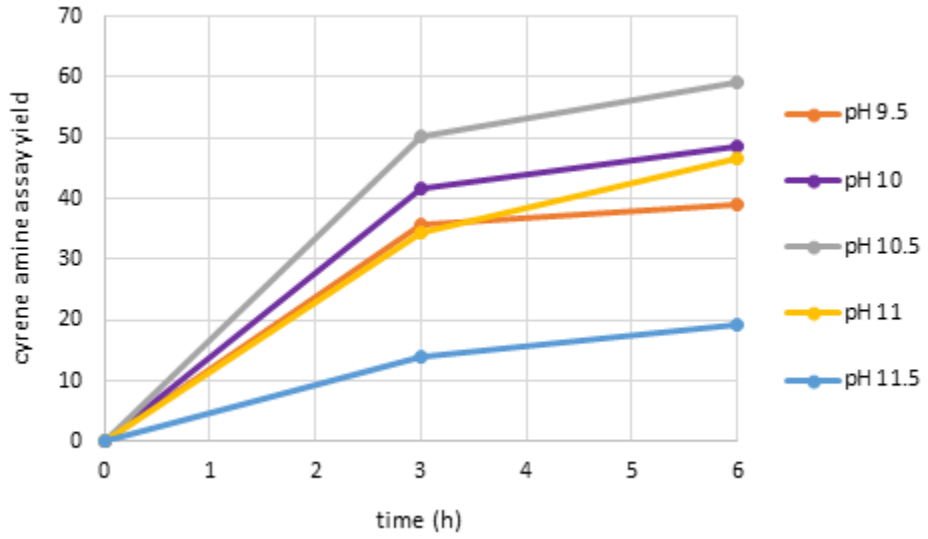


Figure S2: Impact of initial reaction pH on transamination reaction with ATA-426. Conditions: 100 g/L Cyrene™ **2**, 15 wt% ATA-426, 1 g/L PLP, 0.1 M sodium borate buffer with 1 M isopropylamine, 45 °C. Assay yield by quantitative ^{1}H NMR using maleic acid as internal standard.

Protein Engineering

Protein Engineering: Library Generation

Site saturation mutagenesis (SSM) libraries were constructed using the standard gene splicing by overlap extension (SOEing) method. Briefly, for each target site, two fragments flanking the site were generated using PCR. Next the fragments were joined together in a second PCR with appropriate 5' and 3' flanking primers. Oligonucleotides for the first PCR step encoding all 20 amino acids were designed using proprietary Codexis software with NNK degenerate codons. The proprietary software optimizes the length of overlap for SOEing to allow parallel construction of all targeted sites.

Assembled gene fragments were then cloned into pCK110900 vector[2] using NEBuilder HiFi DNA assembly (New England BioLabs). This vector has the gene of interest under the control of a lac promoter, a P15a origin of replication, and a chloramphenicol selection marker. The assembled plasmid was then transformed into W3110 *E. coli* cells and plated onto LB agar plates containing 34 mg/L chloramphenicol and 1% glucose (Teknova).

Combinatorial libraries were constructed using the QuikChange Lightning Multi Site-Directed Mutagenesis kit from Agilent Technologies. The transaminase variant in pCK110900 was used as template for the QuikChange multi site-directed mutagenesis reaction. The template was then removed using DpnI and the product was transformed into W3110 *E. coli* cells. Cells were plated onto LB agar plates containing 34 mg/L chloramphenicol and 1% glucose (Teknova).

Table S3. Transaminase evolution library design details

Round	Backbone	Library Type	Targeted sites	Details	Cyrene, g/L
1	ATA-426	SSM	Active site	96 sites	20
		SSM	Surface	96 sites	20
		SSM	Core	96 sites	20
2	Rd2BB	Combinatorial	Beneficial diversity from Rd1 and historical data	15 sites, 20 mutations	20
3	Rd3BB	SSM	Active Site	48 sites	50
4	Rd4BB	Combinatorial	Thermostable active, surface, and core residues	9 sites, 13 mutations; screening focus on thermostability	50

High Throughput Screening for Enzyme Evolution

Enzyme Preparation for Well-Plate Reactions. LB-agar supplemented with 34 micrograms per mL chloramphenicol and 1% (w/v) glucose in Q-tray plates was inoculated with a glycerol stock of E. coli W3110 strain cells harboring plasmid encoding for the transaminase enzyme in the pCK110900 vector. The plate was incubated at 37 °C overnight. The following day, a 96-well plate containing 0.2 mL per well of Luria-Bertani Broth (culture media for cells) supplemented with 34 micrograms per mL of chloramphenicol and 1% (w/v) glucose was inoculated with individual colonies. The 96-well plate was shaken at 250 RPM/30 °C overnight. The following day, the cells' optical density at 600 nm (OD600) was measured, and an aliquot of cells was diluted to an OD600 of 0.05 in ~390 µL of Terrific Broth media supplemented with 34 micrograms per mL of chloramphenicol and 0.1 mM pyridoxine in a new 96-deep well plate. This culture was grown at 30 °C/250 RPM until OD600 reached 0.6-0.8. Protein production was induced with 1 mM IPTG for 20 hr at 30 °C/250 RPM. Cells were pelleted by centrifugation and the supernatants were discarded. Cell pellets were frozen, thawed, and then resuspended in lysis buffer (0.4 mL per well of 100 mM triethanolamine-HCl pH 7.5, 0.25 mg/mL lysozyme, 0.2 mg/mL polymyxin B sulfate, 1.6 U/mL DNaseI, and 0.1 mM PLP). The plate was shaken for 2 h at room temperature at 1000 RPM. The lysate was clarified by centrifugation (4000 x g, 15 min). The supernatant was then used in subsequent well-plate reactions.

Transaminase Reactions in Well Plates. To each well of a 96-well plate were charged 1 g/L PLP, 1 M isopropylamine in 100 mM sodium borate buffer pH 10.5, appropriate concentration of (1*S*,5*R*)-6,8-dioxabicyclo[3.2.1]octan-4-one (Cyrene™ **2**), 0.5 g/L aniline, and appropriate concentration of lysate containing the transaminase enzyme. The plate was sealed and shaken at 45 °C, 1000 RPM overnight. 40 µL of the reaction mixture was then diluted with 160 µL 80/20 (v/v) acetonitrile: water containing 200 mM triethylamine and 27 mg/mL 1-fluoro-2,4-dinitrobenzene (DNFB) for derivatization. Derivatization reactions were aged for 1h at room temperature at 600 RPM. The reactions were then quenched by diluting 5-fold into 80% acetonitrile, filtered, and analyzed by UPLC.

Enzyme Preparation as Lyophilized Cell-Free Lysate Powder. 25 mL of LB broth supplemented with 34 micrograms per mL chloroamphenicol and 1% (w/v) glucose was inoculated with 20 microliters of a glycerol stock of *E. coli* W3110 strain cells harboring plasmid encoding for transaminase in the pCK110900 vector. Cells were grown until saturation for 18 hr at 30 °C/250RPM. The following day, a 2.8L flask containing 1 L of TB supplemented with 34 micrograms per mL of chloroamphenicol and 0.1 mM pyridoxine was subcultured with the overnight saturated culture to an initial OD600 of 0.05. This culture was grown at 30 °C/250RPM until the OD600 reached 0.6-0.8. Protein production was induced with 1 mM IPTG for 20 hr at 30 °C/250RPM. Cells were pelleted by centrifugation and the supernatants were discarded. Cell pellets were flash frozen in liquid nitrogen, thawed and resuspended with 5 mL (per gram of pellet) of ice-cold 50 mM triethanolamine-HCl buffer pH 7.5 supplemented with 0.1 mM PLP. This suspension was shaken at 20 °C for 30 min, after which time the cells were placed on ice to chill, and then disrupted by high-pressure homogenization (16,000 PSI). The resulting lysate was then clarified by centrifuging at 10,000 x g for 45 min at 4 °C. Following centrifugation, the supernatant was frozen and lyophilized to give a powder.

Enzyme Characterization

Initial Rate Measurements with Lyophilized Cell-Free Lysate Powder. Cyrene™ (1.5 g, (1*S*,5*R*)-6,8-dioxabicyclo[3.2.1]octan-4-one, **2**) was dissolved in buffer (0.1 M sodium borate, 1 M isopropylamine, pH 10.5, 10 mL) containing 1 mg/mL pyridoxal-5′-phosphate (PLP). Separately, transaminase enzyme (37.5 mg of lyophilized cell-free powder) was dissolved in buffer (2 mL, 0.1 M sodium borate, 1 M isopropylamine, pH 10.5) containing 1 mg/mL PLP. In vials, 300 µL of the Cyrene™/PLP stock were combined with 200 µL of the transaminase enzyme/PLP stock. Reactions were heated to 45 °C with shaking. After a given amount of time, 100 µL of each reaction was diluted with 700 µL of 0.5% maleic acid in D_2O for analysis by quantitative ^1H NMR.

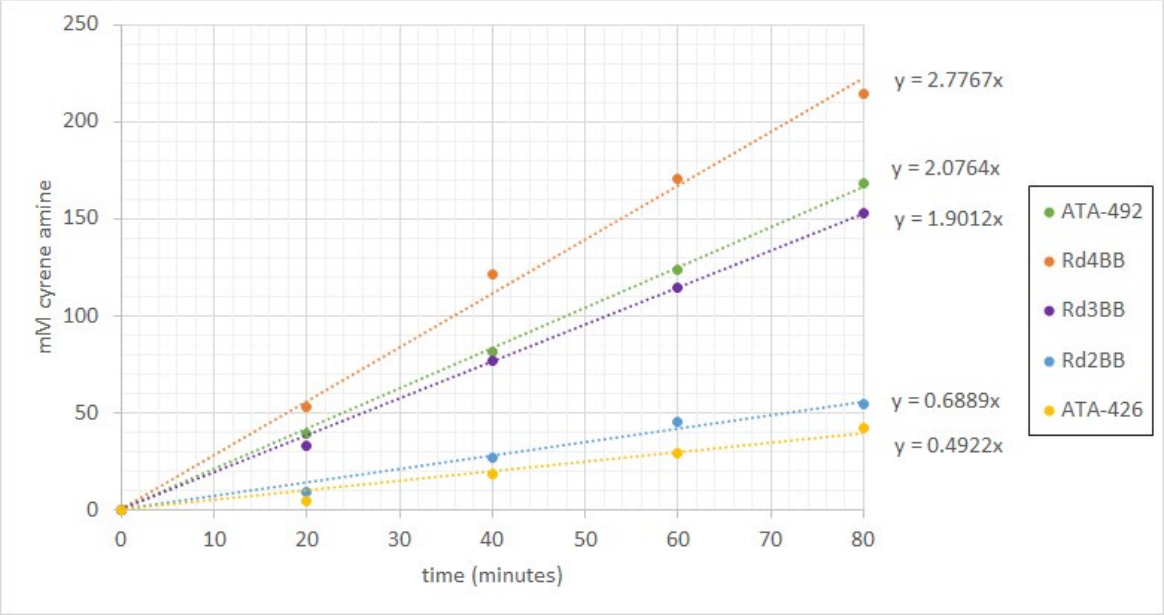
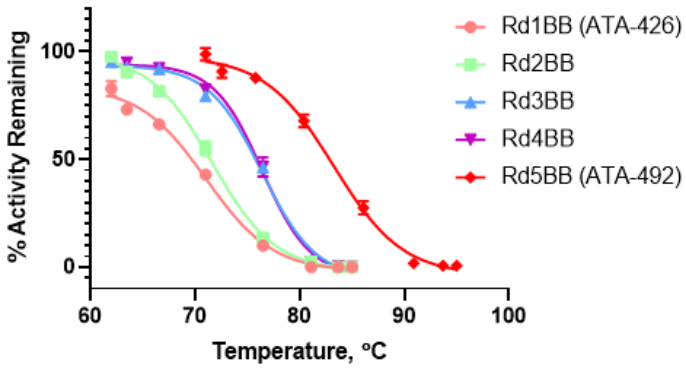


Figure S3: Initial rates of transaminase-catalyzed amination of Cyrene™ **2**, conditions as described above. Rates (in units of (mM cyrene amine **3**)/min) were calculated by a linear fit from 0 to 80 min, the y intercept was constrained to 0.

Measurement of T_{50}. A 10 mg/mL solution of transaminase enzyme lyophilized cell-free lysate was prepared in 100 mM triethanolamine pH 7.5 with 0.1 mM PLP. 50 µL of the enzyme solution was aliquoted into PCR plates in triplicate for each tested temperature. The enzyme was heat challenged for 10 min at temperatures ranging from 35 °C to 95 °C in a thermocycler. Plates were cooled to room temperature and enzymatic reactions were assembled at a final enzyme concentration of 0.5 g/L with heated and non-heat-challenged controls, following the reaction protocol described above ("Transaminase Reactions in Well Plates"). The percentage of remaining enzyme activity versus temperature in °C was plotted to determine T_{50} values.



Variant	T50, °C
Rd1BB (ATA-426)	71.1
Rd2BB	71.6
Rd3BB	76.4
Rd4BB	76.4
Rd5BB (ATA-492)	83.3

Figure S4: Thermostability of evolved transaminase variants, measured as temperature of half inactivation (T_{50}).

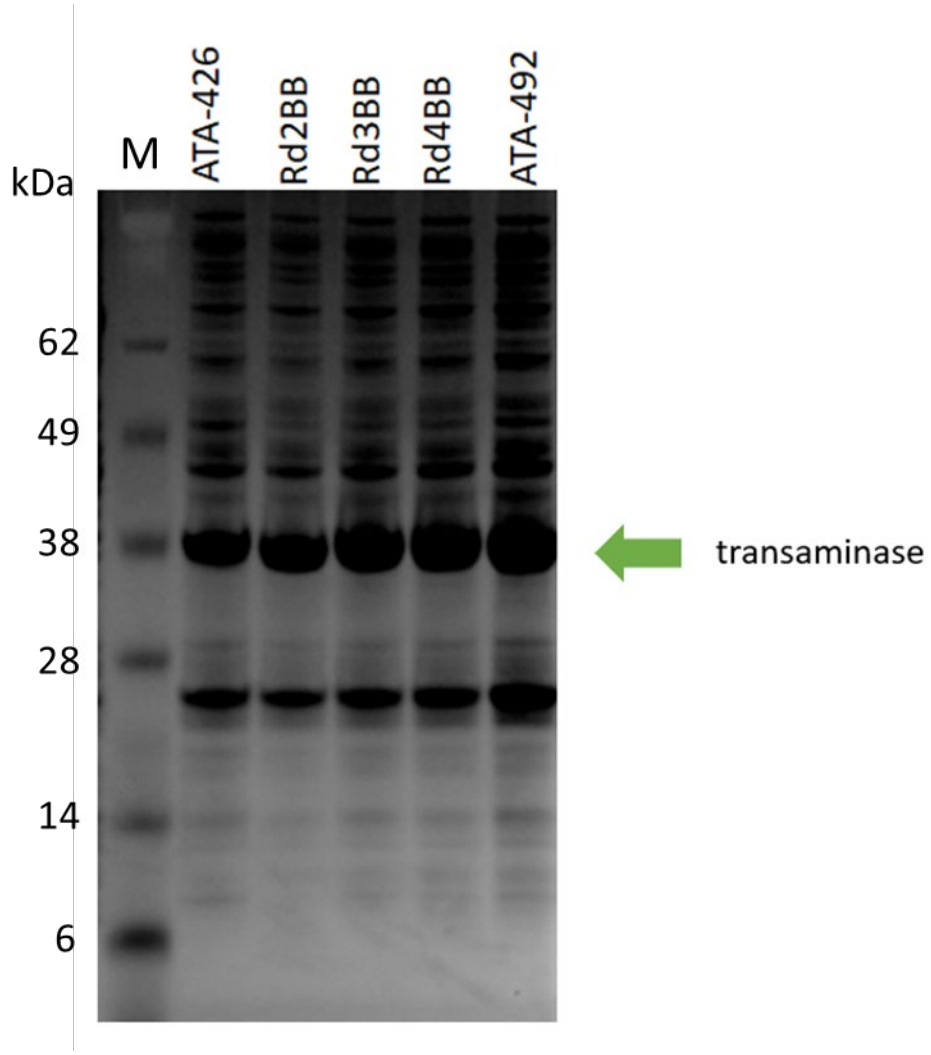


Figure S5. SDS-Page Gel of lyophilized shake flask powders of Transaminase variants. Molecular weight marker lane is indicated by M

DNA and Protein Sequences

ATA-426

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ATA-492

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CSTAGGVWPFVSVDGNSISDGVPGPVTQSIIRRYWELNVEPSCLLTPVQY

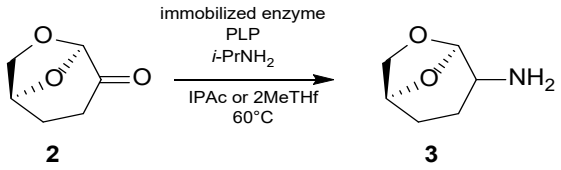
Immobilized Reactions

Gram-scale Enzyme Immobilization

Representative Immobilization on HP2MGL and Solvent Exchange. Pyridoxal 5′-phosphate monohydrate (0.96 g, 3.62 mmol) was added to a 100 mM potassium phosphate buffer pH 6.9 (130.4 mL) and the mixture was aged at 4°C for 0.25 hr Subsequently, the solution pH was re-adjusted to 6.9 by the addition of 1N KOH (6.58 mL). ATA-426 (24.19 g) was then charged into the same vessel over approximately 0.5 hr followed by a rinse with additional 100 mM potassium phosphate buffer pH 6.9 (5.98 mL) and the mixture was agitated for 2 hr The resin Diaion® HP2MGL (171.77 g, hydrated) was then added, followed by a rinse with 100 mM potassium phosphate buffer pH 6.9 (11.97 mL), and the mixture was aged for 48 hr at 4 °C. The resulting immobilized transaminase slurry mixture was diluted with chilled (4 - 8°C) water (150 mL), agitated for 15 min at 4 °C, and a portion transferred to a fritted filter funnel. Subsequently, the mother liquor was removed by filtering over vacuum to afford a wet cake of immobilized enzyme resin (approximately 30 g). The wet cake was then slurry washed as follows: chilled water was charged to the filter and agitated for approximately 3 min, subsequently, the mother liquor was removed by filtration. This process was repeated three times, the first wash utilized 120 mL water, and subsequent water washes used 90 mL water. Next, the immobilized transaminase was similarly slurry washed, three times, with 90 mL of a chilled isopropanol:PEG-400:water mixture (88:10:2 wt%). This was followed by three similar slurry washes with 90 mL water-saturated IPAc. Subsequently, the excess water-saturated IPAc was removed by gentle filtration yielding a wet cake of immobilized transaminase resin, a portion of which was utilized for the subsequent reaction. Based on quantification of the protein removed during each wash stage (as described in SI section “Resin Screen – Immobilization Capacity and Selectivity"), the final resin contains the equivelent of ~ 55 mg protein per gram hydrated resin.

Representative Immobilization on ECR8415F and Solvent Exchange. Pyridoxal 5′-phosphate monohydrate (0.244 g, 0.92 mmol) was added to a 100 mM potassium phosphate buffer pH 6.9 (34.1 mL) and the mixture was aged at 4°C for 0.25 hr Following dissolution, the solution was titrated with 1N KOH to a final pH of 6.8. Subsequently, ATA-492 (6.0 g) was then charged into the same vessel over approximately 0.5 hr and the mixture was agitated for 1 hr The resin Purolite® ECR8415F (50.4 g, hydrated) was then added and the mixture was aged for 48 hr at 4 °C and subsequently transferred to a bottle a portion subdivided for use. The mother liquor was removed from this portion by filtering over vacuum to afford a wet cake of immobilized enzyme resin (approximately 15.1 g). The wet cake was then slurry washed as follows: chilled water was charged to the filter and agitated for approximately 3 min, subsequently, the mother liquor was removed by filtration. This process was repeated three times, the first wash utilized 60 mL water, and subsequent water washes used 45 mL water. Next, the immobilized transaminase was similarly slurry washed, three times, with 45 mL of a chilled isopropanol:PEG-400:water mixture (85.4:10:4.6 wt%). This was followed by three similar slurry washes with 45 mL water-saturated 2-MeTHF. Subsequently, the excess water-saturated 2-MeTHF was removed by gentle filtration yielding a wet cake of immobilized transaminase resin. Based on quantification of the protein removed during each wash stage (as described in SI section “Resin Screen – Immobilization Capacity and Selectivity"), the final resin contains the equivalent of ~ 60 mg protein per gram hydrated resin.

Synthesis of (1S,4R,5R)-6,8-dioxabicyclo[3.2.1]octan-4-amine (3)

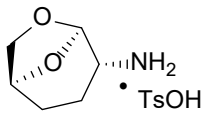


2 **3**

Representative Synthesis in IPAc (ATA-426 with HP2MGL Resin). ATA-426 was immobilized on HP2MGL resin as previously described. The resulting immobilized transaminase resin (15.02 g) was combined with a mixture of (1*S*,5*R*)-6,8-Dioxabicyclo[3.2.1]octan-4-one (**2**) (10.04 g, 78 mmol), isopropylamine (5.77 g, 98 mmol)), and water-saturated isopropyl acetate (IPAc, 60 mL). A SpinChem® S2 RBR (rotating bed reactor), with the internal retaining mesh removed, was placed into the jacketed vessel (Easymax 102). The RBR was progressively spun up to approximately 400 rpm to load the resin into the RBR, and rotation was maintained at 600 rpm throughout the reaction. The vessel was then heated to 60 °C and aged for approximately 75 hr. A condenser chilled to -10°C was attached to the vessel neck, the condenser outlet was sealed, and condensate was allowed to return to the vessel. The reaction was periodically sampled with an EasySampler (Mettler Toledo). Samples were quenched and diluted 80X in MeOH and subsequently analyzed by GC. At the end of the reaction, the reaction stream was cooled to room temperature, and the reaction stream was recovered by filtration to yield a solution of compound **3** (9.20 wt%, 10.46:1 *dr*).

Representative Synthesis in 2-MeTHF (ATA-492 with ECR8415 Resin). ATA-492 was immobilized on ECR8415F as previously described. The resulting immobilized transaminase resin (12.36 g) was combined with a mixture of (1*S*,5*R*)-6,8-Dioxabicyclo[3.2.1]octan-4-one (**2**) (8.42g, 65.7 mmol), isopropylamine (4.90 g, 83 mmol)), and water-saturated 2-MeTHF (70.42 mL). A SpinChem® S2 RBR with the internal retaining mesh removed, was placed into the jacketed vessel (Easymax 102). The RBR was progressively spun up to approximately 400 rpm to load the resin into the RBR, and rotation was maintained at 600 rpm throughout the reaction. The vessel was then heated to 60 °C and aged for approximately 7 hr. A condenser chilled to -10 °C was attached to the vessel neck, the condenser outlet was sealed and condensate was allowed to return to the vessel. The reaction was periodically sampled with an EasySampler (Mettler Toledo). Samples were quenched and diluted 80X in MeOH and subsequently analyzed by GC. At the end of the reaction, the reaction stream was cooled to room temperature, and the reaction stream was recovered by filtration to yield a solution of compound **3** (11.1 wt%, 22:1 *dr*).

Synthesis of (1*S*, 4*R*)-6,8-dioxabicyclo[3.2.1]octan-1-amine-4-methylbenzenesulfonate (3a·TsOH)



(3a•TsOH)

To a 250 mL 3-neck flask equipped with a mechanical stirrer, distillation apparatus, and an inlet for solvent addition was added a 9.45 wt% Cyrene amine reaction solution (containing approximately 6.2 g cyrene amine); the material was distilled to ~ 45 mL total solution and was flushed with 60 mL (7 vol) of dry 2-MeTHF to a final volume of ~ 45 mL. The distillation was stopped, and 0.75 mL of water was added, (0.12X based on starting material assay). The solution was then diluted with 16 mL of 2-MeTHF and warmed to approximately 40 °C. Next, a homogenous solution of *para*-toluenesulfonic acid (3.4M in 5.4:94.6 v/v H_2O in 2-MeTHF) was added dropwise over 1.5 hr. The resulting slurry was aged for 30 min at 40 °C, cooled to room temperature and aged overnight. The slurry was then filtered and the solid washed with 1 bed vol of 2-MeTHF, two times, and dried under vacuum/N_2 sweep for 18 h to provide 14.4 g of **3a·TsOH** as a solid (>99 % purity, 73.2% yield from **2**, *dr* > 51:1): mp 227 ℃ (DSC); **^{1}H NMR (DMSO-d$_6$, 500 MHz**) δ 7.96 (br s, 3H), 7.49 (d, 2H, *J* = 8.0 Hz), 7.13 (d, 2H, *J* = 8.0 Hz), 5.40 (s, 1H), 4.61 (s, 1H), 3.98 (d, 1H, *J* = 7.3 Hz), 3.68 (m 1H), 3.12 (br m, 1H), 2.30 (s, 3H), 2.04 (m, 2H), 1.57 (m, 1H), 1.43 (m, 1H); **^{13}C NMR (DMSO-d$_6$, 125 MHz)**, δ 145.6, 138.5, 128.7, 125.9, 99.1, 73.5, 67.5, 47.9, 23.7, 21.3, 19.1. Calculated for $C_{13}H_{19}NO_5S$: C, 51.81; H, 6.36; N, 4.65. Found: C, 51.69; H, 6.37; N, 4.57.

Reaction Optimization and Characterization

Variant Initial Rates. The initial rate of product formation for each variant immobilized on HP2MGL were obtained from SpinChem experiments in both IPAc and 2-MeTHF. A linear fit of cyrene amine **3** GCAP versus time, constrained to a y-intercept of 0, was used to quantify the conversion kinetics. Only the linear regime of the intial kinetics was used for the fit. The standard error of the fit parameter was calculated according to: $\sigma_m = m\sqrt{\frac{(\frac{1}{R^2}-1)}{n-2}}$,

where R^2 is calculated from the fit, m is the slope from the fit, and n is the number of data points.

Table S4. Initial Rate of cyrene amine 3 product formation (GCAP %/hr) obtained by a linear fit to the linear regime (0-2 hr for ATA-492, 0-4hr for ATA-426). Uncertainty in fit parameter is reported as standard error, calculated from R^2 value.

	IPAc	**2-MeTHF**
ATA-426	6.53 ± 0.49	8.04 ± 0.43
ATA-492	29.84 ± 4.75	23.95 ± 0.98

Solvent effect on diastereoselectivity of ATA-492 immobilized on HP2MGL

Representative Scaled-down Immobilization and Solvent Exchange: A protein stock solution containing 165 g/L ATA-492, 6.7 g/L pyridoxal 5′-phosphate was prepared in 100 mM KP*i* and pH adjusted to pH 6.7. This solution (218 µL) was charged to identical vials containing 300 mg of HP2MGL resin and mixed on a thermoblock (4 °C x 400 rpm) for 72 hr. Subsequently, the supernatant was removed, and the resins were washed three times with 4 mL of pre-chilled water (4°C), followed by three washes with 4 mL of an IPA/PEG/H_2O solution which was formulated to contain identical water content as the reaction solvent solution. Next, the resin was washed three times with 4 mL of the water reaction solvent (containing water contents as specified in the table below). Following the final wash, the resins were stored at room temperature overnight in an excess of reaction solvent.

Table S5. Conversion to Cyrene amine and *dr* for reactions run in different solvent/water systems (ATA-492, immobilized on HP2MGL. The water content of solvents miscible with water were set to 1.8 wt%, all others were saturated. The prepared solvent water content was determined by coulometric KF.

Solvent	KF (wt%)	18 hr Product GCAP (%)	*dr* (X:1)
2-MeTHF	4.5	94.6%	17.5
IPAc	1.8	84.3%	5.2
IPA	1.8	48.6%	18.0
MTBE	1.5	82.9%	9.3
Toluene	0.033	74.5%	14.5
NMP	1.84	2.3%	16.4
EtOAc	3.3	93.5%	14.6
DMSO	1.8	0.4%	ND[1]

[1] ND = not detected

Resin Screen – Immobilization Capacity and Selectivity:

Immobilization: 10.0 g of each resin was weighed out in 50 mL conical tubes. Prior to use, the resins were briefly washed with water, filtered, and returned to the conical tube. A protein stock solution containing 165 g/L enzyme, 7 g/L pyridoxal 5′-phosphate was prepared in 100 mM KP*i* and pH adjusted to pH 6.9. This solution (218 µL) was charged to identical vials containing 300 mg of resin and mixed on a thermoblock (4 °C x 400 rpm) for 72 hr. Following immobilization, the supernatant was recovered and assayed for protein content as described below.

Protein Quantification Assay: Total protein was quantified with size exclusion chromatography, and the response was calibrated with a transaminase standard using an Agilent 1290 Infinity II UPLC equipped with a diode array detector (λ = 210 nm) and a Sepax Zenix SEC-100 (3 µm, 150 mm x 4.6 mm) column heated to 35 °C using an isocratic elution in a mobile phase containing 10% MeOH, 90% Water (containing 200 mM sodium phosphate and 150 mM sodium chloride, pH 7.2).

Transaminase concentration determination: SDS-PAGE densitometry was used to determine the relative concentration of transaminase in protein solutions, per the manufacturers directions.Samples were denatured with Novex 4X LDS Loading buffer per the manfuacturers directions, and 15 µL of each sample run on a Bolt 4-12% Bis Tris gel (1.0 mm, Invitrogen) at 200V for 35 min. The gel was then stained for 90 min in Imperial Protein-Stain (Thermo Scientific), destained in water overnight, then imaged on a gel imager (ProteinSimple). A MW ladder and known transaminase standard were used to confirm the transaminase band. Based on these measurements, the transaminase concentration in a solution could be determined from the product of the total protein concentration (from SEC) and the relative percentage of transaminase.

Table S6. Initial screen of ATA immobilization on different resins. Relative difference in total protein depleted from solution and change in the transaminase content in supernatant (percentage (based on SDS-PAGE) of supernatant transaminase content of in transaminase content in supernatant

Resin	Polymer	Functionalization	Pore Size[1] (Å)	Relative Loss of Protein from Solution (%)[2]	Supernatant ATA (%)	Relative Difference in ATA Specificity (%)[3]
HP2MGL	*PMMA*	--	*900*	*control*	*77*	*control*
ECR8309F	PMMA	C2-NH_2	900	-106	41	47
ECR8409F	PMMA	C6-NH_2	900	-17	34	55
ECR8415F	PMMA	C6-NH_2	1500	-19	41	47
PurosorB PAD950	PMMA	--	120	24	74	4
ECR1030M	DVB/MA	DVB-MA	300	-95	56	27
ECR1508	PS/DVB	PS/DVB+NR_2	800	-173	34	55
Dianion HP-20	PS/DVB	PS/DVB		-269	33	56

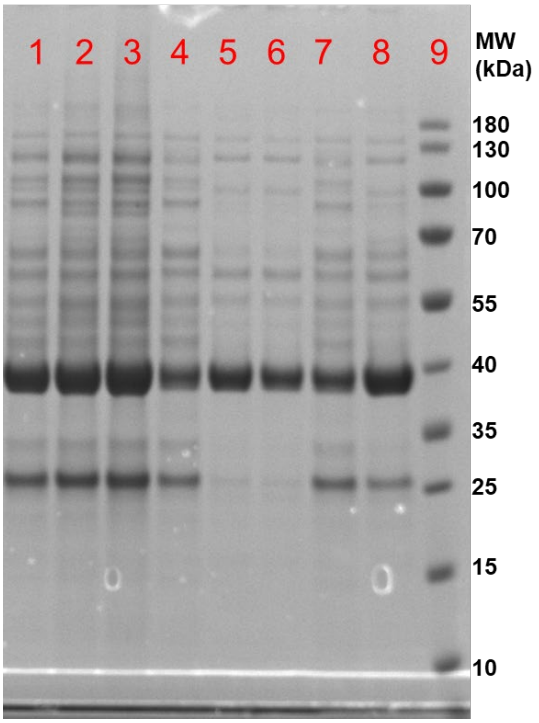
[1] Pore size is median pore size as reported by vendor

[2] Decrease in total protein in supernatant compared to HP2MGL

[3] Relative Difference in ATA Specificity is calculated as: ((ATA % in Supernatant HP2MGL)-(ATA % in Resin of interest))/(ATA % in Supernatant HP2MGL)*100

Table S7. Total conversion (**3a + 3b)** and dr for each immobilizatoin resin, as measured at 19 hr timepoint. Undesired diasteromer was not detectable in dr entries indicated by ND

Resin	Polymer	Functionalization	Conversion (3a+3b) GCAP%	dr
HP2MGL	PMMA	--	67%	15.69
ECR8309F	PMMA	C2-NH_2	82%	16.50
ECR8409F	PMMA	C6-NH_2	87%	15.30
ECR8415F	PMMA	C6-NH_2	90%	14.81
PurosorB PAD950	PMMA	--	79%	15.50
ECR1030M	DVB/MA	DVB-MA	46%	16.07
ECR1508	PS/DVB	PS/DVB+NR_2	33%	17.54
Dianion HP-20	PS/DVB	PS/DVB	0%	ND



Lane	Resin
1	ECR8309F
2	ECR1508
3	Dianion HP20
4	ECR8409F
5	HP2MGL
6	PurosorB PAD950
7	ECR8415F
8	ECR1030M
9	MW Ladder

Figure S6. SDS-PAGE page of residual protein in supernatant after immobilization of ATA-426. Samples were all identically diluted 100x prior to assay. Molecular weight of calibration ladder (Lane 9) is listed to right of gel image. Correspondence between lane number and resin used is shown in table on right.

Expanded Resin Screen – Reaction Performance:

Expanded resin screen: A protein stock solution containing 165 g/L ATA-492, 6.7 g/L Pyridoxal 5′-phosphate was prepared in 100 mM KPi, and pH adjusted to pH 6.7. This solution (218 µL) was charged to identical vials containing 300 mg of resin and mixed on a thermoblock (4 °C x 400 rpm) for 72 hr. Subsequently, the supernatant was removed, and the resins were washed three times with 4 mL of pre-chilled water (4°C), followed by three washes with 4 mL of an IPA/PEG/H_2O solution containing 4.5 wt% water. Next, the resin was washed three times with 4 mL with water-saturated 2-MeTHF. Following the final wash, the resins were stored at room temperature overnight in an excess of reaction solvent.

Immobilized Reaction Assay: A stock solution containing 100 g/L Cyrene™ **2** and 1.25 equivalent isopropylamine was prepared in water-saturated 2-MeTHF. For each vial, the supernatant was decanted, and 2.01 mL reaction stock solution was added to the vial. The vials were sealed with pierceable septa and incubated on a thermoblock (60 °C x 500 rpm). The reaction mixtures were sampled, through the septa, with a syringe (10 µL, quenched in 100 µL MeOH) and analyzed by GC. The conversion at 16.5 h is reported in **Figure 5** of the main text.

Adsorption Kinetics on ECR8415M:

Immobilizations: A protein stock solution containing 163.4 g/L lyophilized enzyme, 6.5 g/L pyridoxal 5′-phosphate was prepared in 100 mM KPi, and pH adjusted to pH 6.9. This solution (3.06 mL) was charged to identical vials containing 4.20 g of ECR8415M resin. and mixed on a thermoblock (4°C x 400 rpm). To monitor adsorption kinetics, individual (identical) vials were sacrificed at different times and the supernatant was assayed for total protein content by SEC.

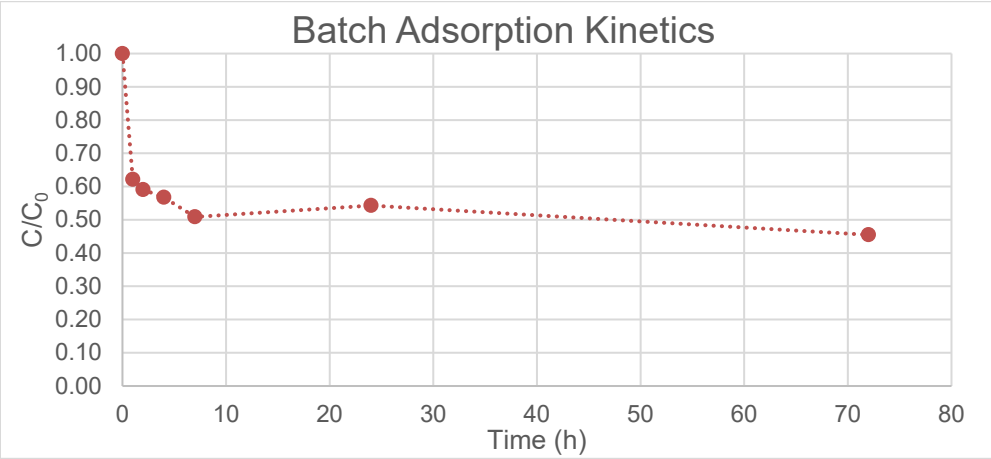


Figure S7. Batch adsorption kinetics of ATA-492 on ECR8415M. Concentration of protein in supernatant, C, relative to initial concentration, $C_{0,}$ vs time. Connecting lines are shown as a visual aid, not a fit.

Impact of Enzyme Loading on Reaction Performance:

Immobilization Protocol for ATA-426 w/ HP2MGL resin. A protein stock solution containing 5 g/L ATA-426 enzyme, 0.2 mg/mL pyridoxal 5′-phosphate was prepared in 100 mM KPi, and pH adjusted to pH 6.8. To evaluate the impact of increasing enzyme load, a varying volume of the protein stock solution was added (between 1 – 6 mL) to identical vials containing 330 mg of HP2MGL resin and incubated on a thermblock (4 °C x 400 rpm) for > 72 hr. Subsequently, the supernatant was removed, and the resins were washed three times with 4 mL of pre-chilled water (4°C), followed by three washes with 4 mL of an IPA/PEG/H_2O solution containing 10 wt% PEG and 2 wt% water. Next, the resin was washed three times with 4 mL with water-saturated IPAc. Following the final wash, the resins were stored at room temperature overnight in an excess of reaction solvent.

Immobilized Reaction Kinetics ATA-426/HP2MGL: The reaction performance of each immobilized enzyme was assesed at 150 wt% resin loading in 100 g/L Cyrene™ **2** and 1.25 equivalent isopropylamine in water-saturated IPAc. A reaction master mix solution containing 100 g/L Cyrene™ **2** and 1.25 equivalent isopropylamine was prepared in water-saturated IPAc. Next, the supernatant was decanted from each immobilized enzyme vial and master mix added to each vial (2.2 mL). The vials were sealed and shaken on a thermblock (60 °C x 500 rpm). The reaction mixtures were sampled, through the septa, with a syringe (10 µL, quenched in 100 µL MeOH) and analyzed by GC.

Immobilization Protocol for ATA-492 w/ ECR8415M resin. A protein stock solution containing 5 g/L ATA-492 enzyme, 0.2 mg/mL pyridoxal 5′-phosphate was prepared in 100 mM KPi, and pH adjusted to pH 6.83. To evaluate the impact of increasing enzyme load, a varying volume of the protein stock solution was added (between 1 – 6 mL) to identical vials containing 407 mg of ECR8415M resin and incubated on a thermoblock (4 °C x 400 rpm) for > 48 hr. Subsequently, the supernatant was removed, and the resins were washed four times with 4 mL of pre-chilled water (4 °C), followed by four washes with 4 mL of an IPA/PEG/H_2O solution containing 10 wt% PEG and 4.5 wt% water. Next, the resin was washed four times with 4 mL with water-saturated 2-MeTHF. Following the final wash, the resins were stored at room temperature overnight in an excess of reaction solvent.

Immobilized Reaction Kinetics ATA-492/ECR8415M: The reaction performance of each immobilized enzyme was assesed at 150 wt% resin loading in 100 g/L Cyrene™ **2** and 1.25 equivalent isopropylamine in water-saturated 2-MeTHF. A reaction master mix solution containing 100 g/L Cyrene™ **2** and 1.25 equivalent isopropylamine was prepared in water-satuared 2-Me-THF. Next, the supernatant was decanted from each immobilized enzyme vial and master mix added to each vial (2.7 mL). The vials were sealed with piercable septa and incubated on a thermoblock (60 °C x 500 rpm). The reaction mixtures were sampled, through the septa, with a syringe (10 µL, quenched in 100 µL MeOH) and analyzed by GC.

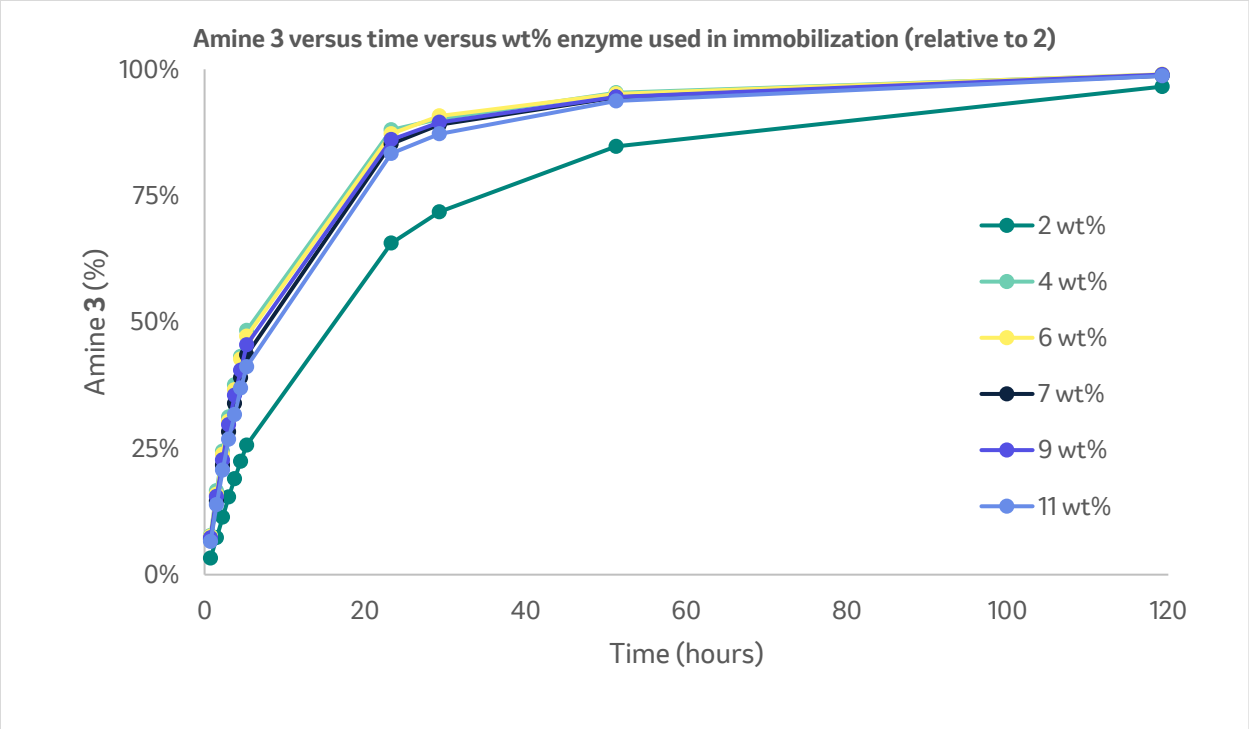


Figure S8. Amine (**3a** + **3b**) GC area percent versus time (hr) as a function of total enzyme used in immobilization (wt% relative to **2**). Lines shown as a visual aid only.

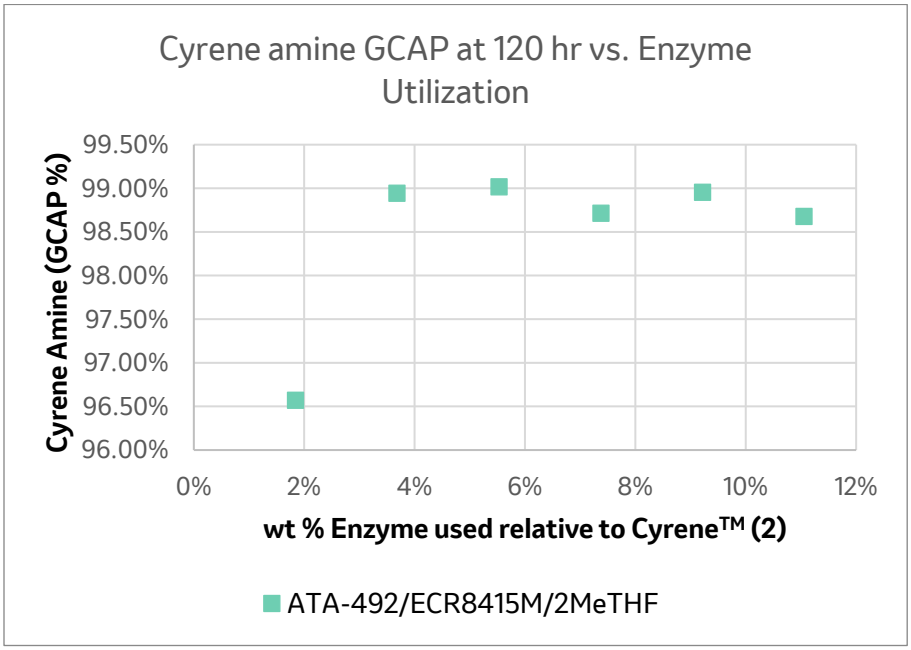


Figure S9. Impact of total enzyme used in immobilization (wt% relative to **2**) versus cyrene amine **3** GCAP (%) after 120 hr.

References

1. Kuhl, N.; Turnbull, B. W. H.; Ji, Y.; Larson, R. T.; Shevlin, M.; Prier, C. K.; Chung, C. K.; Desmond, R.; Guetschow, E.; He, C. Q.; Itoh, T.; Kuethe, J. T.; Newman, J. A.; Reibarkh, M.; Rivera, N. R.; Shang, G.; Wang, Z.; Zewge, D.; Thaisrivongs, D. A., Utilizing biocatalysis and a sulfolane-mediated reductive acetal opening to access nemtabrutinib from cyrene. *Green Chemistry* **2023,** *25* (2), 606-613.
2. Baidyaroy, D.; Clark, L.; Newman Lisa, M.; Ching, C. Beta-glucosidase variant enzymes and related polynucleotides. US 8715996 B2, 2010/02/26, 2014.