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Supporting Information

Table of Contents

Section 1. Materials and general methods	2
Section 2. Production of artUPO and mutants	3
Section 3. Synthetic methods	4
Section 4. NMR Spectra	27
Section 5. References	33

Section 1. Materials and General Methods

General Techniques and Equipment

Residual solvents were removed under high vacuum. Column chromatography was performed on silica gel (VWR International, 40-63 μM mesh particle size). Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F₂₅₄ coated aluminium plates and the compounds were visualized under UV (wavelength 254 nm). Preparative thin layer chromatography was performed on silica gel coated glass plates and the compounds were visualized under UV (wavelength 254 nm). A Razel A 99 syringe pump was used for the slow addition of solutions. Melting points were measured on a Gallenkamp Melting Point Measuring Apparatus in capillary tubes and data are reported uncorrected. NMR measurements were taken on Jeol ECS and Bruker AV and AM spectrometers. All spectra were acquired at room temperature, unless otherwise noted. The ¹H and ¹³C chemical shifts are stated on a δ internal scale in parts per million (ppm) units. Residual solvent was used as the standard. Coupling constants are reported in Hz. MS analysis was performed on a Bruker compact[®] time of flight mass spectrometer (ESI and APCI), GC-MS spectra were acquired on an Agilent 5975C MSD system equipped with a 60 m DB-5[™] column. For GC-FID analysis, an Agilent 7890B equipped with a 30 m DB-5MS[™] column was used, ee values were measured on the same instrument using chiral columns, as described. UV-VIS spectra were collected on an Agilent Cary[®] 100 UV-VIS spectrophotometer. Infrared spectra were acquired on a Perkin Elmer Spectrum Two FT-IR Spectrometer. Optical rotation was measured on a Bellingham & Stanley ADP450 Polarimeter.

<u>Materials</u>

All chemicals were purchased from Sigma Aldrich (Irvine, UK), Alfa Aesar (Heysham, UK), Tokyo Chemical Industry (Oxford, UK) or Fluorochem (Hadfield, UK) and used without further purification, GPR-grade and HPLC-grade solvents were bought from VWR International (Lutterworth, UK). Anhydrous solvents were collected from a solvent tower, purchased, or dried using standard methods. Ultrapure water was produced by a Milli-Q[®] Integral Water Purification System.

Section 2. Production of artUPO and mutants

The cloning and expression of artUPO and its preparation from fermentations of *Pichia pastoris* has been described previously.¹

The artUPO mutant constructs were generated through mutagenesis of the original WT artUPO construct¹ by InFusion cloning, as previously described.² The inverse PCR reactions were performed with the WT artUPO template and primers containing the required mutated sequences.

The resulting constructs were transformed into *E. coli* Stellar Cells (TakaRa) and the cells grown at 37 °C overnight on low salt LB agar plates containing 25 μ g mL⁻¹ zeocin. Single colonies were used to inoculate 10 mL starter cultures of low-salt LB with 25 μ g mL⁻¹ zeocin and grown overnight at 37 °C with shaking at 180 rpm. Plasmid DNA was extracted using a miniprep kit (Qiagen) and the artUPO mutant sequences were confirmed by Sanger sequencing.

The mutant plasmids were linearised with *Pmel* and used to transform *Pichia pastoris* strain X-33 (Invitrogen) by electroporation. Transformed cells were plated onto Yeast extract Peptone Dextrose plates with 100 μ g mL⁻¹ zeocin (YPDz) and incubated at 30 °C for 3 d. Single colonies were picked and streaked onto fresh YPDz plates and incubated at 30 °C for a further 3 d. Clean single colonies were used to inoculate 5 mL Buffered Glycerol-complex Medium (BMGY) containing 1 % yeast extract; 2 % peptone; 100 mM potassium phosphate pH 6.0; 1.34 % (w/v) yeast nitrogen base; 4×10⁻⁵ % biotin and 1 % glycerol, and grown for 24 h at 30 °C with shaking at 220 rpm. 50 % glycerol stocks were prepared and stored at -80 °C.

The artUPO mutants were expressed in 0.5 L MiniBio fermenters (Applikon) as previously described.¹ The duration of the methanol feed was extended up to 160 h, at which point the cultures were harvested and centrifuged at 6,000 × g for 20 min. The supernatant was concentrated approximately 5-fold by centrifugation at 4,000 × g using 10 kDa MWCO centrifugal spin filters and stored at -80 °C.

Section 3. Synthetic methods

3.1 The experimental procedure for results in Table 1



Entry 1: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO expression secretate (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO expression secretate by syringe under an argon atmosphere. To the resulting mixture, styrene **4a** (33 µL, 0.3 mmol) and ethyldiazoacetate **2b** (11 µL, 0.1 mmol, 5 mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard, and isomer distributions was measured by GC-FID (BGB175 column, flow rate: 2 mL/min, column temperature: 80–120/40 min, 120–180/17.5 min).



P	eaks	Summary									
1	Ŧ	 Name 	RT (min)	Area%	Area	Height	Height%	Amount	Concentration	Start time (min)	End time (min)
		1	38.504	36.123	128.710	14.060	30.89			38.230	38.807
		2	39.377	35.963	128.142	14.047	30.86			39.113	39.638
		3	41.347	13.939	49.666	8.458	18.58			41.192	41.445
		4	41.538	13.975	49.795	8.951	19.67			41.445	41.702



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Injection Results

	reaks	3	ummary									
G	#	*	Name	RT (min)	Area%	Area	Height	Height%	Amount	Concentration	Start time (min)	End time (min)
		1		39.484	23.862	10.402	1.156	20.53			39.270	39.655
		2		40.329	68.415	29.825	3.694	65.59			40.133	40.510
		3		41.960	3.274	1.427	0.320	5.68			41.867	42.040
		4		42.125	4.450	1.940	0.462	8.19			42.040	42.256

Entry 2: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO expression secretate (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (9 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO expression secretate by syringe under an argon atmosphere. To the resulting mixture, styrene **4a** (33 µL, 0.3 mmol) and ethyldiazoacetate **2b** (11 µL, 0.1 mmol, 5 mM) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard, and isomer distributions was measured by GC-FID (BGB175 column, flow rate: 2 mL/min, column temperature: 80–120/40 min, 120–180/17.5 min).



Injection Results

ſ	Peaks	Summary									
Ŀ	#	 Name 	RT (min)	Area%	Area	Height	Height%	Amount	Concentration	Start time (min)	End time (min)
		1	38.925	19.371	6.805	0.720	17 .9 9			38.681	39.096
		2	39.799	77.092	27.083	2.995	74.88			39.580	40.020
		3	41.627	1.522	0.535	0.117	2.92			41.540	41.706
		4	41.798	2.015	0.708	0.168	4.20			41.732	41.871

Entry 3: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO expression secretate (10 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 100 mL) and deionised water (50 mL) containing Na₂S₂O₄ (180 mg, 1 mmol) was degassed under an argon atmosphere for 1 h, and then transferred to liquid artUPO expression secretate by syringe under an argon atmosphere. To the resulting mixture, styrene **4a** (330 µL, 3 mmol) and ethyldiazoacetate **2b** (110 µL, 1 mmol, 5mM) in degassed MeOH (40 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 16 h. The reaction mixture was extracted with ethyl acetate (3 × 20 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture, which was purified by flash column chromatography on silica gel (eluent: hexane: EtOAc = 50:1) to yield 8% product. The isomer distribution was measured by GC-FID (BGB175 column, flow rate: 2 mL/min, column temperature: 80–120/40 min, 120–180/17.5 min).



Injection Results

	Peaks	Summary									
B	#	 Name 	RT (min)	Area%	Area	Height	Height%	Amount	Concentration	Start time (min)	End time (min)
		1	39.341	15.189	36.835	4.004	11.59			39.156	39.553
		2	40.211	52.301	126.837	15.003	43.44			39.920	40.407
		3	41.890	15.164	36.774	7.111	20.59			41.686	41.969
		4	42.050	17.346	42.065	8.421	24.38			41.969	42.206

Entry 4: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO expression secretate (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (9 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO expression secretate by syringe under an argon atmosphere. To the resulting mixture, styrene 4a (11 μ L, 0.1 mmol) and ethyldiazoacetate 2b (33 μ L, 0.3 mmol, 15 mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO4 and concentrated in vacuo to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard, and isomer distributions was measured by GC-FID (BGB175 column, flow rate: 2 mL/min, column temperature: 80–120/40 min, 120-180/17.5 min).



Injection Results

	Peaks	SL	ummary									
Œ	. #	*	Name	RT (min)	Area%	Area	Height	Height%	Amount	Concentration	Start time (min)	End time (min)
		1		38.782	3.310	0.766	0.090	3.37			38.662	38.946
		2		39.680	90.574	20.948	2.277	85.18			39.465	39.851
		3		41.552	2.596	0.600	0.121	4.51			41.446	41.619
		4		41 721	3 519	0.814	0 185	6.94			41,649	41.801

3.2 The experimental procedure for results in Table 2



Entry 1: see procedure for Table 1 entry 3.

Entry 2: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO E164A mutants (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 mins. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 mins, and then slowly transferred to liquid artUPO E164A mutants by syringe under an argon atmosphere. To the resulting mixture, styrene **4a** (33 µL, 0.3 mmol) and ethyldiazoacetate **2b** (11 µL, 0.1 mmol, 5 mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard, and isomer distributions was measured by GC-FID (BGB175 column, flow rate: 2 mL/min, column temperature: 80–120/40 min, 120–180/17.5 min).

Ini	ection	Resu	ts

ſ	Peaks	Su	ummary									
Đ	#	*	Name	RT (min)	Area%	Area	Height	Height%	Amount	Concentration	Start time (min)	End time (min)
		1		37.493	10.915	48.123	5.309	9.64			37.233	37.738
		2		38.368	43.602	192.240	21.224	38.56			38.127	38.614
		3		40.581	18.946	83.534	11.217	20.38			40.388	40.696
		4		40.824	26.537	117.002	17.298	31.42			40.696	41.026

Entry 3: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO I62F mutants (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO I62F mutants by syringe under an argon atmosphere. To the resulting mixture, styrene **4a** (33 µL, 0.3 mmol) and ethyldiazoacetate **2b** (11 µL, 0.1 mmol, 5 mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 hours. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard, and isomer distributions was measured by GC-FID (BGB175 column, flow rate: 2 mL/min, column temperature: 80–120/40 min, 120–180/17.5 min).

Injection Results

	Peaks	Su	ummary									
Œ	#	*	Name	RT (min)	Area%	Area	Height	Height%	Amount	Concentration	Start time (min)	End time (min)
		1		37.501	1.346	0.468	0.066	1.63			37.364	37.590
		2		38.377	84.068	29.192	3.287	81.28			38.190	38.572
		3		40.576	6.362	2.209	0.300	7.43			40.391	40.695
		4		40.813	8.223	2.855	0.391	9.67			40.695	40.975

Entry 4: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO I160F mutants (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO I160F mutants by syringe under an argon atmosphere. To the resulting mixture, styrene **4a** (33 µL, 0.3 mmol) and ethyldiazoacetate **2b** (11 µL, 0.1 mmol, 5mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard, and isomer distributions was measured by GC-FID (BGB175 column, flow rate: 2 mL/min, column temperature: 80–120/40 min, 120–180/17.5 min).

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Peaks Summary

G	#	 Name 	RT (min)	Area%	Area	Height	Height%	Amount	Concentration	Start time (min)	End time (min)
		1	37,497	1.330	1.312	0.149	1.33			37.336	37.650
		2	38.363	86.171	84.987	9.254	82.83			38.131	38.607
		3	40.567	5.399	5.325	0.730	6.54			40.406	40.694
		4	40.807	7.100	7.002	1.040	9.31			40.694	40.953

Entry 5: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO I62F/I160F mutants (0.5 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5.5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO I160F mutants by syringe under an argon atmosphere. To the resulting mixture, styrene **4a** (33 µL, 0.3 mmol) and ethyldiazoacetate **2b** (11 µL, 0.1 mmol, 5 mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard, and isomer distributions was measured by GC-FID (BGB175 column, flow rate: 2 mL/min, column temperature: 80–120/40 min, 120–180/17.5 min).

	Peaks	SU	ummary									
G	#		Name	RT (min)	Area%	Area	Height	Height%	Amount	Concentration	Start time (min)	End time (min)
		1		36.677	2.435	2.339	0.266	2.59			36.532	36.848
		2		37.572	70.035	67.275	6.826	66.49			37.373	37.785
		3		39.781	13.127	12.609	1.473	14.34			39.614	39.930
		4		40.088	14.403	13.836	1.702	16.57			39.930	40.341

Entry 6: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO E164A/I62F mutants (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO E164A/I62F mutants by syringe under an argon atmosphere. To the resulting mixture, styrene 4a (33 μ L, 0.3 mmol) and ethyldiazoacetate 2b (15 μ L, 0.14 mmol, 5 mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture, which was purified by flash column chromatography on silica gel (eluent: hexane: EtOAc = 100:1–50:1) to afford trans: (8.3 mg, 32% yield); $[\alpha]^{20}_{D} = 0.35$ (c = 0.8, CHCl₃), Lit. $[\alpha]^{20}_{D} = 223$ (c = 1.0, CHCl₃); ¹H NMR (400 MHz, CHLOROFORM-D) δ 7.31 – 7.26 (m, 2H), 7.23 – 7.17 (m, 1H), 7.14 – 7.06 (m, 2H), 4.17 (q, J = 7.2 Hz, 2H), 2.52 (ddd, J = 8.7, 6.0, 4.2 Hz, 1H), 1.90 (ddd, J = 8.7, 6.0, 4.2 Hz, 1H), 1.63 - 1.58 (m, 1H), 1.34 -1.25 (m, 4H); ¹³C NMR (101 MHz, CHLOROFORM-D) δ 173.5, 140.2, 128.5, 126.5, 126.2, 60.8, 26.3, 24.3, 17.2, 14.4, 14.3; GC data: 8% ee, determined by GC-FID (BGB175 column, flow rate: 2 mL/min, column temperature: 80–120/40 min, 120–180/17.5 min): tr(major) = 40.505 min; tr(minor) = 40.239 min. and *cis*: (11.7 mg, 45% yield); $[\alpha]^{20}_{D} = 4.85$ (c = 1.0, CHCl₃), Lit. $[\alpha]^{20}_{D} = 26$ (c = 1.0, CHCl₃); ¹H NMR (400 MHz, CHLOROFORM-D) δ 7.28 – 7.23 (m, 4H), 7.21 – 7.15 (m, 1H), 3.86 (q, J = 7.2 Hz, 2H), 2.57 (td, J = 9.0, 7.5 Hz, 1H), 2.06 (ddd, J = 9.0, 7.5, 5.4 Hz, 1H), 1.70 (dt, J = 7.5, 5.4 Hz, 1H), 1.31 (ddd, J = 9.0, 7.5, 5.4 Hz, 1H), 0.96 (t, J = 7.2 Hz, 3H); ¹³C NMR (101 MHz, CHLOROFORM-D) δ 171.1, 136.6, 129.4, 128.0, 126.7, 60.3, 25.5, 21.9, 14.1, 11.2; GC data: 80% ee, determined by GC-FID (BGB175 column, flow rate: 2 mL/min, column temperature: 80–120/40 min, 120–180/17.5 min): tr(major) = 37.962 min; tr(minor) = 37.095 min.^3

Injection Results

Peaks Summary

- E		1 7										
G	#	*	Name	RT (min)	Area%	Area	Height	Height%	Amount	Concentration	Start time (min)	End time (min)
		1		37.095	6.415	50.414	5.388	6.09			36.897	37.292
		2		37,962	53.498	420.427	44.518	50.31			37.750	38.236
		3		40.239	18.523	145.567	16.955	19.16			40.043	40.365
		4		40.505	21.565	169.474	21.620	24.43			40.365	40.688

Entry 7: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO E164A/I160F mutants (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO E164A/I160F mutants by syringe under an argon atmosphere. To the resulting mixture, styrene **4a** (33 µL, 0.3 mmol) and ethyldiazoacetate **2b** (11 µL, 0.1 mmol, 5 mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard, and isomer distributions was measured by GC-FID (BGB175 column, flow rate: 2 mL/min, column temperature: 80–120/40 min, 120–180/17.5 min).

Injection Results

	PEaks	SU	ummary									
B	#		Name	RT (min)	Area%	Area	Height	Height%	Amount	Concentration	Start time (min)	End time (min)
		1		37.094	17.077	55.211	5.755	15.63			36.903	37.307
		2		37,972	23.744	76.766	8.115	22.03			37.756	38.133
		3		40.225	29.893	96.647	11.085	30,10			40.008	40.360
		4		40.493	29.286	94.685	11.875	32.24			40.360	40.703

Entry 8: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO E164A/I62F/I160F mutants (0.5 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5.5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO E164A/I62F/I160F mutants by syringe under an argon atmosphere. To the resulting mixture, styrene **4a** (33 µL, 0.3 mmol) and ethyldiazoacetate **2b** (11 µL, 0.1 mmol, 5 mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard, and isomer distributions was measured by GC-FID (BGB175 column, flow rate: 2 mL/min, column temperature: 80–120/40 min, 120–180/17.5 min).

} #	*	Name	RT (min)	Area%	Area	Height	Height%	Amount	Concentration	Start time (min)	End time (min)
	1		36.669	6.365	66.926	7.239	6.16			36.475	36.894
	2		37.550	16.608	174.625	19.141	16.28			37.344	37.756
	3		39.812	33.811	355.501	38.249	32.54			39.545	39.955
	4		40.124	43.215	454.375	52.912	45.02			39.955	40.372

3.3 The experimental procedure for results in Table 3

Entry 1: no conversion was observed. The procedure used was the same as for the other biotransformations in this section, using artUPO (wt).

Entry 2: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO E164A mutants (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO E164A mutants by syringe under an argon atmosphere. To the resulting mixture, 4-OMe-styrene **4c** (41 µL, 0.3 mmol) and ethyldiazoacetate **2b** (11 µL, 0.1 mmol, 5mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard. Isomer distributions were measured by HPLC, with two different HPLC columns needed to resolved the enantiomers of the *cis* and *trans* diastereoisomers in this case (IA column, flow rate 1.0 mL/min, hexane/IPA 98/2 for *trans*; IC column, flow rate 1.0 mL/min, hexane/IPA 98/2 for *cis*).

No.	Name	tR	Peak Area (Y units*ms)	Area (%)	Width
1	S,S	6.22	28171.844	24.424	0.252
2	R,R	6.50	87172.828	75.576	0.259

Entry 3: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO E164A/I62F mutants (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO E164A/I62F mutants by syringe under an argon atmosphere. To the resulting mixture, 4-OMe-styrene **4c** (41 μ L, 0.3 mmol) and ethyldiazoacetate **2b** (11 μ L, 0.1 mmol, 5 mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard, and isomer distributions was measured by HPLC HPLC (IA column, flow rate 0.5mL/min, hexane/IPA 99.5/0.5).

No.	Name	tR	Peak Area (Y units*ms)	Area (%)	Width
1	S,S	13.43	7980043.030	33.592	0.543
2	R,R	14.08	8284335.938	34.873	0.568
3	S,R	15.87	3925828.857	16.526	0.635
4	R,S	16.65	3565461.273	15.009	0.666

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12 13 14 15 16 17 18 Retention Time (min)

No.	Name	tR	Peak Area (Y units*ms)	Area (%)	Width
1	S,S	13.77	45085.625	2.748	0.535
2	R,R	14.43	799251.250	48.721	0.558
3	S,R	16.29	700704.188	42.714	0.625
4	R,S	17.05	95427.617	5.817	0.717

Entry 4: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO E164A/I160F mutants (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO E164A/I160F mutants by syringe under an argon atmosphere. To the resulting mixture, 4-OMe-styrene 4c (41 μ L, 0.3 mmol) and ethyldiazoacetate **2b** (15 μL, 0.14 mmol, 5 mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture, which was purified by flash column chromatography on silica gel (eluent: hexane: EtOAc = 100:1–50:1) to afford *trans*: (10.8 mg, 35% yield); $[\alpha]^{20}_{D} = -160.04$ (c = 1.0, CHCl₃), Lit. $[\alpha]^{20}_{D} = -29.6$ (c = 1.0, CHCl₃); ¹H NMR (400 MHz, CHLOROFORM-*D*) δ 7.03 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 4.16 (q, J = 7.2 Hz, 2H), 3.78 (s, 3H), 2.48 (ddd, J = 9.0, 5.4, 4.2 Hz, 1H), 1.82 (ddd, J = 9.0, 5.4, 4.2 Hz, 1H), 1.55 (ddd, J = 9.0, 5.4, 4.2 Hz, 1H), 1.31 – 1.23 (m, 4H); ¹³C NMR (101 MHz, CHLOROFORM-D) δ 173.7, 158.4, 132.2, 127.4, 114.0, 60.7, 55.4, 25.7, 24.0, 16.8, 14.4; HPLC data: 91% ee, determined by HPLC (IA column, flow rate 0.5mL/min, hexane/IPA 99.5/0.5): tr(major) = 13.98 min; tr(minor) = 13.36 min. and *cis*: (7.2 mg, 23% yield); $[\alpha]^{20}_{D} = -2.01$ (c = 0.7, CHCl₃), Lit. $[\alpha]^{20}_{D} = -6$ (c = 1.0, CHCl₃); ¹H NMR (400 MHz, CHLOROFORM-D) δ 7.18 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 3.89 (q, J = 7.2 Hz, 2H), 3.77 (s, 3H), 2.52 (q, J = 9.0 Hz, 1H), 2.03 (ddd, J = 9.0, 7.8, 5.6 Hz, 1H), 1.69 – 1.61 (m, 1H), 1.30 (ddd, J = 9.0, 7.8, 5.6 Hz, 1H), 1.69 – 1.61 (m, 1H), 1.30 (ddd, J = 9.0, 7.8, 5.6 Hz, 1H), 1.69 – 1.61 (m, 1H), 1.60 (ddd, J = 9.0, 7.8, 5.6 Hz, 1H), 1.69 (m, 1H), 1.60 (ddd, J = 9.0, 7.8, 5.6 Hz) J = 9.0, 7.8, 5.6 Hz, 1H), 1.02 (t, J = 7.2 Hz, 3H); ¹³C NMR (101 MHz, CHLOROFORM-D) δ 171.2, 158.4, 130.3, 128.6, 113.4, 60.3, 55.3, 24.9, 21.8, 14.2, 11.4; HPLC data: 63% ee, determined by HPLC (IA column, flow rate 0.5mL/min, hexane/IPA 99.5/0.5): tr(major) = 15.69 min; tr(minor) = 16.39 min. ^{4,5}

Entry 5: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO E164A/I62F mutants (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO E164A/I62F mutants by syringe under an argon atmosphere. To the resulting mixture, 4-OMe-styrene **4c** (41 μ L, 0.3 mmol) and ethyldiazoacetate **2b** (11 μ L, 0.1 mmol, 5 mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard, and isomer distributions was measured by HPLC HPLC (IA column, flow rate 0.5mL/min, hexane/IPA 99.5/0.5).

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4

S.R

R.S

15.86

16.52

61500.285

9488.564

19.362

2.987

0.625

0.842

3.4 The experimental procedure for results in Table 4

Entry 1: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO E164A/I62F mutants (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO E164A/I62F mutants by syringe under an argon atmosphere. To the resulting mixture, 2-vinylnaphthalene **4e** (32 mg, 0.21 mmol) and ethyldiazoacetate **2b** (7.5 µL, 0.07 mmol, 5mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture. The yield was measured by ¹H NMR, using 2Br-methyl naphthalene as an internal standard, and isomer distributions was measured by HPLC (IC column, flow rate 1.0 mL/min, hexane/IPA 99.3/0.7).

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7 8	9 10 11	12	13 14	Retention	Time (min)	
7 8 No.	9 10 11 Name	12 12	13 14 Peak Area (Y units*ms)	Retention Area (%)	Time (min) Width	
7 8 No.	9 10 11 Name S,S	12 12 18 8.77	13 14 Peak Area (Y units*ms) 94088.539	Retention Area (%) 4.188	Width 0.305	
7 8 No. 1 2	9 10 11 Name S,S R,R	12 12 18 8.77 9.42	13 14 Peak Area (Y units*ms) 94088.539 1408530.000	Retention Area (%) 4.188 62.690	Width 0.305 0.328	
7 8 No. 1 2 3	9 10 11 Name S,S R,R S,R	12 12 18 8.77 9.42 12.12	13 14 Peak Area (Y units*ms) 94088.539 1408530.000 650457 188	Retention Area (%) 4.188 62.690 28.950	Vidth 0.305 0.424	

Entry 2: To a round bottom flask containing a magnetic stirring bar was added liquid artUPO E164A/I160F mutants (1.0 mL, prepared as described in Section 2), which was degassed under an argon atmosphere for 5 min. Meanwhile, a mixture of KPi buffer (100 mM, pH = 7, 10 mL) and deionised water (5 mL) containing Na₂S₂O₄ (18 mg, 0.1 mmol) was degassed under an argon atmosphere for 30 min, and then slowly transferred to liquid artUPO E164A/I62F mutants by syringe under an argon atmosphere. To the resulting mixture, 2-vinylnaphthalene 4e (32 mg, 0.21 mmol) and ethyldiazoacetate **2b** (7.5 μL, 0.07 mmol, 5 mM) in degassed MeOH (4 mL) was added in one portion. The mixture was then stirred at room temperature under Ar atmosphere for 2.5 h. The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture, which was purified by flash column chromatography on silica gel (eluent: hexane: EtOAc = 100:1–50:1) to give *trans*: (6.7 mg, 40% yield); $[\alpha]^{20}_{D} = -101.59$ (c = 0.6, CHCl₃), Lit. $[\alpha]^{20}_{D} = -30.7$ (c = 1.0, CHCl₃); ¹H NMR (400 MHz, CHLOROFORM-D) δ 7.82 – 7.73 (m, 3H), 7.59 – 7.56 (m, 1H), 7.49 – 7.39 (m, 2H), 7.21 (dd, J = 8.5, 1.8 Hz, 1H), 4.19 (q, J = 7.2 Hz, 2H), 2.69 (ddd, J = 9.0, 5.4, 4.2 Hz, 1H), 2.01 (ddd, J = 9.0, 5.4, 4.2 Hz, 1H), 1.67 (ddd, J = 9.0, 5.4, 4.2 Hz, 1H), 1.43 (ddd, J = 9.0, 5.4, 4.2 Hz, 1H), 1.30 (t, J = 7.2 Hz, 3H); ¹³C NMR (101 MHz, CHLOROFORM-D) δ 173.5, 137.6, 133.4, 132.4, 128.3, 127.7, 127.5, 126.3, 125.6, 124.9, 124.6, 60.9, 26.5, 24.2, 17.1, 14.4; HPLC data: 90% ee, determined by HPLC (IC column, flow rate 1.0 mL/min, hexane/IPA 99.3/0.7): tr(major) = 9.42 min; tr(minor) = 8.77 min. and *cis*: (3.4 mg, 20% yield); $[\alpha]^{20}_{D} = -11.84$ (c = 0.3, CHCl₃); ¹H NMR (400 MHz, CHLOROFORM-*D*) δ 7.83 – 7.67 (m, 4H), 7.48 – 7.37 (m, 3H), 3.82 (q, J = 7.2 Hz, 2H), 2.78 – 2.67 (m, 1H), 2.16 (ddd, J = 9.0, 7.8, 5.6 Hz, 1H), 1.89 – 1.80 (m, 1H), 1.42 (ddd, J = 9.0, 7.8, 5.6 Hz, 1H), 0.91 (t, J = 7.2 Hz, 3H); ¹³C NMR (101 MHz, CHLOROFORM-D) δ 171.0, 134.2, 133.3, 132.5, 128.0, 127.7, 127.7, 127.5, 125.9, 125.5, 60.3, 25.8, 22.1, 14.1, 11.4; HPLC data: 75% ee, determined by HPLC (IC column, flow rate 1.0 mL/min, hexane/IPA 99.3/0.7): tr(major) = 12.12 min; tr(minor) = 14.50 min.⁴

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8	9	10	11	12	13	14 Reter	ntion Time	(min)

No.	Name	tR	Peak Area (Y units*ms)	Area (%)	Width
1	S,S	8.77	75922.602	4.005	0.299
2	R,R	9.43	867682.562	45.774	0.321
3	S,R	11.99	823727.875	43.455	0.412
4	R,S	14.23	128256.555	6.766	0.487

3.2 General procedure for the racemic cyclopropane synthesis⁵

To a round bottom flask containing a magnetic stirring bar was added $Rh_2(OAc)_4$ (1 mg, 2 µmol, 1 mol%) in DCM (5 mL), styrene **4a** (1.1 mL, 1 mmol, 5 equiv.) was added one portion and to the resulting reaction mixture was added ethyldiazoacetate **2b** (22µL, 0.2 mmol, 1 equiv.) very slowly. The solution was stirred at room temperature for 1 h. Upon completion, the reaction mixture was quenched by adding 1M HCl (2 mL) and washed by DCM (3 × 10 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous MgSO₄ and concentrated *in vacuo* to give the crude product mixture, which is pure enough for GC and HPLC trace analysis.

Section 4. NMR Spectra

Ph_{4.} ____CO₂Et Ph CO₂Et cis-(R,S)-5a cis-(S,R)-5a

1.05-I 2.00-1 1.01 H <u>F66:0</u> 1.20-I 1.01 2.98-I 4.5 4.0 f1 (ppm) 6.5 6.0 5.5 5.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 9.0 8.5 8.0 7.5 7.0 n5496jdj Jason Li - LJC-506-2 -171.08 -136.64 -25.55 -21.88 --14.10 --11.20 Ph_{4.} ____CO₂Et Ph CO₂Et cis-(S,R)-5a cis-(R,S)-5a

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Section 5. References

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