Experimental Procedures

General. Unless otherwise noted, all chemicals and reagents were obtained from commercial suppliers (Sigma-Aldrich, VWR, Alfa Aesar) and used without further purification. Silica gel chromatography was carried out using AMD Silica Gel 60, 230-400 mesh. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova 300 MHz or 500 MHz, or Bruker Prodigy 400 MHz instrument, in $CDCl_3$ and are referenced to residual protio solvent signals. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets, ddd = doublet of doublet of doublets, td = triplet of doublets), coupling constant (Hz), integration. Sonication was performed using a Qsonica Q500 sonicator. High-resolution mass spectra were obtained at the California Institute of Technology Mass Spectral Facility. Specific optical rotations were measured using a Jasco P-2000 Polarimeter, and are reported in units of $deg \cdot cm^3 \cdot g^{-1} \cdot dm^{-1}$. Synthetic reactions were monitored using thin layer chromatography (Merck 60 gel plates) using a UV-lamp for visualization.

Chromatography. Analytical high-performance liquid chromatography (HPLC) was carried out using an Agilent 1200 series instrument and a Kromasil 100 C18 column (4.6 x 50 mm, 5 μm) with water and acetonitrile as the mobile phase. Semi-preparative HPLC was performed using an Agilent XDB-C18 column (9.4 x 250 mm, 5 μm) with water and acetonitrile as the mobile phase. Analytical chiral HPLC was conducted using a supercritical fluid chromatography (SFC) system with isopropanol and liquid CO_2 as the mobile phase. Product enantiomers were separated using Chiralcel OD-H, Chiralcel OJ-H, and Chiralpak AS-H columns (4.6 x 150 mm, 5 μm) from Chiral Technologies Inc.

Cloning and site-directed mutagenesis. pET22b(+) was used as a cloning and expression vector for all enzymes described in this study. Site-directed mutagenesis was performed using a modified QuikChange™ mutagenesis protocol. The PCR products were gel purified, digested with DpnI, repaired using Gibson Mix™, and used to directly transform *E. coli* strain BL21(DE3).

Determination of P411 concentration. The concentration of P411 enzymes in whole cell experiments was determined from ferrous carbon monoxide binding difference spectra using the previously reported extinction coefficient for serine-ligated enzymes (ε = 103,000 M^{-1} cm^{-1}).[1] The concentration of purified P411 enzymes was determined by quantifying the amount of free hemin present using the pyridine/hemochrome assay.[2]

Expression and amination bioconversions using whole cells. *E. coli* BL21(DE3) cells transformed with plasmid encoding P411 variants were grown overnight in 5 mL Luria-Bertani medium with 0.1 mg/mL ampicillin (LB_{amp}, 37 ºC, 250 rpm). The preculture was used to inoculate 45 mL of Hyperbroth medium (prepared from AthenaES© powder, 0.1 mg/mL ampicillin) in a 125 mL Erlenmeyer flask; this culture was incubated at 37 ºC, 230 rpm for 2 h. Cultures were then cooled on ice (20 min) and induced with 0.5 mM IPTG and 0.5 mM 5-aminolevulinic acid (final concentrations). Expression was conducted at room temperature, 130 rpm, for 16 h. Cultures were then centrifuged (2,600 x g, 10 min, 4 ºC) and the pellets resuspended to OD_{600} = 30 in M9-N. Aliquots of the cell suspension (4 mL) were used to determine the P411 expression level after lysis by sonication.

For amination bioconversions, the cells at OD_{600} = 30 in M9-N were degassed by

sparging with argon in sealed 6 mL crimp vials for at least 40 minutes. Separately, glucose (250 mM in M9-N) was degassed by sparging with argon for at least five minutes. To 2 mL crimp vials were added an oxygen depletion system (20 μL of a stock solution containing 14,000 U/mL catalase and 1,000 U/mL glucose oxidase in 0.1 M KPi, pH 8.0). All solutions were uncapped and transferred into an anaerobic chamber. Resuspended cells (320 μL) were added to the vials, followed by glucose (40 μL, 250 mM in M9-N), sulfide (10 μL, 200 mM in DMSO), and tosyl azide (10 μL, 200 mM in DMSO). Final concentrations were 5 mM sulfide, 5 mM tosyl azide, and 25 mM glucose. The vials were sealed, removed from the anaerobic chamber, and shaken at room temperature, 40 rpm for 20 h. Reactions under aerobic conditions were performed by combining resuspended cells (340 μL), glucose (40 μL, 250 mM in M9-N), sulfide (10 μL, 200 mM in DMSO), and tosyl azide (10 μL, 200 mM in DMSO) on the benchtop, without any argon sparging. The reactions were quenched by adding acetonitrile (450 μL) and internal standard (10 μL of a DMSO stock). For reactions proceeding to >30% conversion (at which point the phenylthiosulfonamide is not completely degraded by cellular reductants), dithiothreitol was added (20 μL, 20 mM DTT in 0.1 M KPi, pH 8.0). This mixture was then transferred to a microcentrifuge tube and centrifuged at 14,000 rpm for 10 minutes. The supernatant was transferred to a vial and analyzed by HPLC.

Reaction screening in 96-well plate format. Site-saturation libraries were generated employing the "22c-trick" method.[3] *E. coli* libraries were cultured in LB_{amp} (300 μL/well) at 37 ºC, 220 rpm. Hyperbroth medium (1000 μL/well, 0.1 mg/mL ampicillin) was inoculated with the preculture (50 μL/well) and incubated at 37 ºC, 220 rpm, 80% humidity for 3 h. The plates were cooled on ice for 30 minutes and then induced with 0.5 mM IPTG and 1 mM 5-aminolevulinic acid (final

concentrations). Expression was conducted at 20 ºC, 120 rpm for 24 h. The cells were pelleted (3,000 x g, 5 min, 4 ºC) and resuspended in the oxygen depletion system (20 μL/well). The 96-well plate was then transferred to an anaerobic chamber. In the anaerobic chamber, argon-sparged reaction buffer (50 mM glucose in M9-N, 300 μL/well) was added, followed by sulfide (10 μL/well, 200 mM in DMSO) and tosyl azide (10 μL/well, 200 mM in DMSO). The plate was sealed with an aluminum foil, removed from the anaerobic chamber, and shaken at 40 rpm. After 16 hours, the seal was removed and acetonitrile (400 μL/well) and internal standard (10 μL/well of a DMSO stock) were added. The wells were mixed, the plate was centrifuged (5,000 x g, 10 min), and the supernatant was filtered through an AcroPrep 96-well filter plate (0.2 μm) into a shallow-well plate for HPLC analysis.

For the screening of site-saturation libraries for activity on sulfide **1**, we used batches of sulfide **1** favoring the *E*-alkene (3:1 or 4.5:1 *E:Z*) for the first three libraries (at residues A268, L437, and S438), while for the next set of libraries (at residues A78, A82, T260, and P329) we performing screening using *Z*-**1** (>15:1 *Z:E*).

Protein purification. *E. coli* BL21(DE3) cells transformed with plasmid encoding P411 variants were grown overnight in 25 mL LB_{amp} (37 ºC, 250 rpm). Hyperbroth medium (470 mL, 0.1 mg/mL ampicillin) in a 1 L flask was inoculated with 19 mL of the preculture and incubated at 37 ºC, 230 rpm for 2.5 h (OD_{600} ca. 1.8). Cultures were then cooled on ice (30 min) and induced with 0.5 mM IPTG and 1 mM 5-aminolevulinic acid (final concentrations). Expression was conducted at room temperature, 130 rpm, for 20–24 h. Cultures were then centrifuged (5,000 x g, 5 min, 4 ºC) and the cell pellets frozen at –20 ºC. Frozen cells were resuspended in buffer A (25 mM tris, 20 mM imidazole, 200 mM NaCl, pH 7.5, 4 mL/g of cell wet weight), loaded with 300

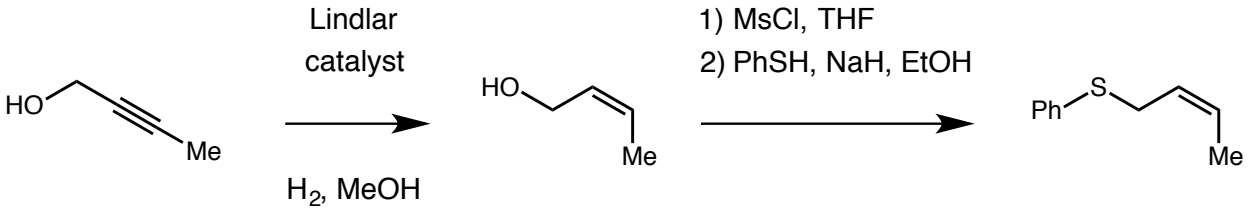
μg/mL hemin, and lysed by sonication. To pellet insoluble material, lysates were centrifuged (20,000 x g, 15 min, 4 ºC). Proteins were expressed in a construct containing a 6x-His tag and purified using a nickel NTA column (1 mL HisTrap HP, GE Healthcare, Piscataway, NJ) using an AKTAxpress purifier FPLC system (GE healthcare). P411 enzymes were eluted on a linear gradient from 100% buffer A/0% buffer B (25 mM tris, 300 mM imidazole, 200 mM NaCl, pH 7.5) to 100% buffer B over 10 column volumes. Fractions containing P411 enzymes were pooled, concentrated, and subjected to three exchanges of phosphate buffer (0.1 M KPi, pH 8.0) to remove excess salt and imidazole. Concentrated proteins were aliquoted, flash-frozen on powdered dry ice, and stored at –20 ºC.

Amination bioconversions using purified protein. Purified P411 enzyme (in 0.1 M KPi, pH 8.0, 30 μL) and the oxygen depletion system (20 μL) were added to 2 mL crimp vials. Portions of phosphate buffer (250 μL 0.1 M KPi, pH 8.0), glucose (40 μL, 250 mM), and NADPH (40 μL, 100 mM), or multiples thereof, were combined in a 6 mL crimp vial and degassed by sparging with argon for at least 10 min. All solutions were uncapped and transferred into an anaerobic chamber. The buffer/reductant/glucose solution (330 μL) was added to the reaction vials, followed by sulfide (10 μL, 200 mM in DMSO) and tosyl azide (10 μL, 200 mM in DMSO). Final concentrations were typically 5 mM sulfide, 5 mM tosyl azide, 10 mM NADPH, 25 mM glucose, and 10 μM P411. The vials were sealed, removed from the anaerobic chamber, and shaken at room temperature, 40 rpm for 20 h. The reactions were quenched by adding acetonitrile (450 μL) and internal standard (10 μL of a DMSO stock). For reactions proceeding to >10% conversion, dithiothreitol was added (20 μL, 20 mM DTT in 0.1 M KPi, pH 8.0). This mixture was then transferred to a microcentrifuge tube and centrifuged at 14,000 rpm for 10

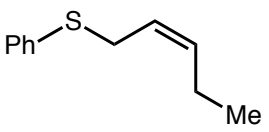
minutes. The supernatant was transferred to a vial and analyzed by HPLC. Sodium dithionite (5 mM) was used as the reductant for reactions with hemin, myoglobin, and cytochrome *c*. Myoglobin (equine heart) and cytochrome *c* (bovine heart) were purchased from Sigma-Aldrich.

Synthesis of substrates.

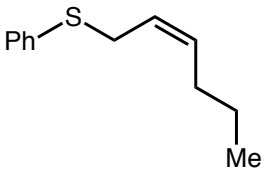
Thioanisole and ethyl phenyl sulfide were purchased from Sigma-Aldrich and used without further purification. Other sulfides were synthesized according to known procedures, and their spectral data are in agreement with reported values.[4] Tosyl azide was prepared according to de Nanteuil and Waser.[5]



(*Z*)-But-2-en-1-yl(phenyl)sulfide (*Z*-**1**) was prepared in two steps via Lindlar hydrogenation of 2-butyn-1-ol according to Balduzzi *et al*.[6] followed by mesylation and displacement with thiophenolate according to Hiroi and Makino.[7] The sulfide was obtained in 26% yield from (*Z*)-2-buten-1-ol as a >15:1 *Z:E* mixture of alkene isomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.24 (m, 4H), 7.22–7.14 (m, 1H), 5.87–5.47 (m, 2H), 3.58 (d, J = 6.6 Hz, 2H), 1.61–1.54 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.5, 130.0, 128.9, 127.8, 126.3, 125.5, 31.0, 12.8; HRMS (FAB) exact mass calculated for $C_{10}H_{12}S$ requires *m/z* 164.0660, found 164.0653.



(*Z*)-Pent-2-en-1-yl(phenyl)sulfide was prepared as above from *cis*-2-penten-1-ol in 15% yield, and obtained as a >15:1 *Z*:*E* mixture: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.14 (m, 5H), 5.56–5.42 (m, 2H), 3.57 (d, *J* = 6.4 Hz, 2H), 2.10–1.92 (m, 2H), 0.90 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.5, 135.4, 130.1, 128.9, 126.3, 123.9, 31.3, 20.7, 14.2; HRMS (FAB) exact mass calculated for $C_{11}H_{14}S$ requires *m/z* 178.0816, found 178.0822.

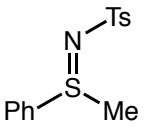


(*Z*)-Hex-2-en-1-yl(phenyl)sulfide was prepared as above from *cis*-2-hexen-1-ol in 53% yield, and obtained as a >15:1 *Z*:*E* mixture: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39–7.32 (m, 2H), 7.31–7.23 (m, 2H), 7.22–7.14 (m, 1H), 5.59–5.45 (m, 2H), 3.63–3.52 (m, 2H), 2.05–1.90 (m, 2H), 1.32 (ddt, *J* = 14.3, 7.9, 7.0, 2H), 0.87 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.6, 133.6, 130.0, 128.9, 126.3, 124.7, 31.4, 29.4, 22.7, 13.9; HRMS (FAB) exact mass calculated for $C_{12}H_{16}S$ requires *m/z* 192.0973, found 192.0964.

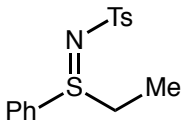
Characterization of reaction products.

Authentic standards corresponding to enzymatic reaction products were prepared by tosylation of the corresponding allylic amine or by nitrene transfer to the corresponding sulfide according to the method of Okamura and Bolm.[8] Phenylthiosulfonamides were cleaved to the corresponding sulfonamides by treatment with 0.5 M potassium hydroxide in methanol, according to Murakami

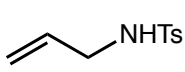
et al.[9] All products are known compounds, and their spectral data are in agreement with reported values.[9,10]



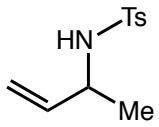
S-Methyl-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**11**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77–7.67 (m, 4H), 7.57–7.45 (m, 3H), 7.20–7.14 (m, 2 H), 2.84 (s, 3H), 2.35 (s, 3H).



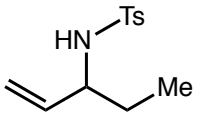
S-Ethyl-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**12**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76–7.70 (m, 2H), 7.69–7.62 (m, 2H), 7.55–7.43 (m, 3H), 7.19–7.10 (m, 2H), 3.12–2.90 (m, 2H), 2.33 (s, 3H), 1.17 (t, J = 7.3 Hz, 3H).



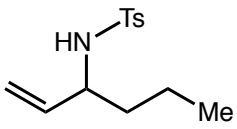
N-Allyl-4-methylbenzenesulfonamide (**13**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 8.3 Hz, 2H), 7.34–7.27 (m, 2H), 5.71 (ddt, J = 17.1, 10.2, 5.8 Hz, 1H), 5.16 (dq, J = 17.1, 1.5 Hz, 1H), 5.08 (dq, J = 10.2, 1.4 Hz, 1H), 4.73 (t, J = 6.2 Hz, 1H), 3.57 (tt, J = 6.0, 1.5 Hz, 2H), 2.42 (s, 3H).



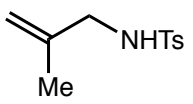
N-(But-3-en-2-yl)-4-methylbenzenesulfonamide (**10**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, J = 8.3 Hz, 2H), 7.30–7.26 (m, 2H), 5.63 (ddd, J = 17.2, 10.4, 5.8 Hz, 1H), 5.05 (ddd, J = 17.1, 1.5, 1.0 Hz, 1H), 4.96 (dt, J = 10.4, 1.2 Hz, 1H), 4.76 (d, J = 7.7 Hz, 1H), 3.93–3.84 (m, 1H), 2.42 (s, 3H), 1.17 (d, J = 6.8 Hz, 3H).



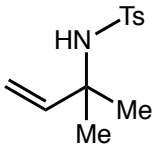
N-(Pent-1-en-3-yl)-4-methylbenzenesulfonamide (**14**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.3 Hz, 2H), 7.31–7.25 (m, 2H), 5.53 (ddd, *J* = 17.0, 10.3, 6.4 Hz, 1H), 5.03–4.94 (m, 2H), 4.49 (d, *J* = 8.0 Hz, 1H), 3.76–3.63 (m, 1H), 2.42 (s, 3H), 1.50 (p, *J* = 7.3 Hz, 2H), 0.82 (t, *J* = 7.4 Hz, 3H).



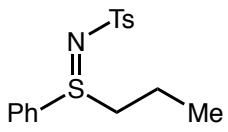
N-(Hex-1-en-3-yl)-4-methylbenzenesulfonamide (**15**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.3 Hz, 2H), 7.30–7.24 (m, 2H), 5.53 (ddd, *J* = 17.0, 10.3, 6.6 Hz, 1H), 5.01–4.91 (m, 2H), 4.63 (d, *J* = 7.9 Hz, 1H), 3.80–3.70 (m, 1H), 2.41 (s, 3H), 1.48–1.39 (m, 2H), 1.34–1.18 (m, 2H), 0.82 (t, *J* = 7.3 Hz, 3H).



N-(2-Methylallyl)-4-methylbenzenesulfonamide (**16**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.3 Hz, 2H), 7.33–7.28 (m, 2H), 4.67–4.79 (m, 2H), 4.64 (t, *J* = 6.4 Hz, 1H), 3.47 (d, *J* = 6.5 Hz, 2H), 2.43 (s, 3H), 1.67 (s, 3H).

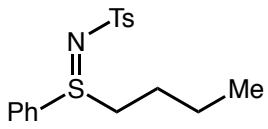


N-(2-Methylbut-3-en-2-yl)-4-methylbenzenesulfonamide (**17**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.3 Hz, 2H), 7.29–7.23 (m, 2H), 5.77 (dd, *J* = 17.3, 10.6 Hz, 1H), 5.08 (dd, *J* = 17.3, 0.7 Hz, 1H), 4.97–4.89 (m, 2H), 2.41 (s, 3H), 1.29 (s, 6H).



S-(*n*-Propyl)-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**18**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.2 Hz, 2H), 7.70–7.65 (m, 2H), 7.56–7.45 (m, 3H), 7.16–7.12 (m, 2H), 3.08 (ddd, *J* = 12.7, 8.3, 5.6 Hz, 1H),

2.85 (ddd, J = 12.7, 8.6, 7.1 Hz, 1H), 2.34 (s, 3H), 1.75–1.55 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H).



S-(*n*-Butyl)-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**19**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77–7.64 (m, 4H), 7.55–7.44 (m, 3H), 7.18–7.12 (m, 2H), 3.07 (ddd, J = 12.8, 8.5, 5.6 Hz, 1H), 2.87 (ddd, J = 12.7, 8.8, 7.0 Hz, 1H), 2.34 (s, 3H), 1.70–1.43 (m, 2H), 1.42–1.20 (m, 2H), 0.82 (t, J = 7.3 Hz, 3H).

Table S1. Mutations present in $P450_{BM3}$ variants used in this work.

Variant	Mutations relative to wild-type $P450_{BM3}$
$P411_{BM3}$-CIS T438S (P)	V78A, F87V, P142S, T175I, A184V, S226R, H236Q, E252G, T268A, A290V, L353V, I366V, C400S, T438S, E442K
P-I263F	V78A, F87V, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268A, A290V, L353V, I366V, C400S, T438S, E442K
P-I263F A328V	V78A, F87V, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268A, A290V, A328V, L353V, I366V, C400S, T438S, E442K
P-I263F V87A	V78A, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268A, A290V, L353V, I366V, C400S, T438S, E442K
P-I263F V87A A328V	V78A, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268A, A290V, A328V, L353V, I366V, C400S, T438S, E442K
P-I263F V87A A328V A268G	V78A, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K
P-I263F V87A A328V A268G A82L	V78A, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K
P-I263F V87A A328V A268G A82I	V78A, A82I, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K

Table S2. Yields and enantioselectivities of whole cell bioconversions represented in Figures 1 and 2 (and some not shown elsewhere).[a]

	Thioanisole	Phenyl ethyl sulfide	Phenyl allyl sulfide	Phenyl crotyl sulfide (3:1 *E:Z*)	Phenyl crotyl sulfide (>15:1 *Z:E*)
P	19% yield, 660 TTN	1.1% yield, 38 TTN	1.7% yield, 59 TTN	0.2% yield, 7 TTN	0.3% yield, 9 TTN
P-I263F	51% yield, 2800 TTN	12% yield, 650 TTN	3.1% yield, 170 TTN	0.8% yield, 45 TTN	1.4% yield, 77 TTN, 57% ee
P-I263F A328V	63% yield, 1600 TTN	27% yield, 680 TTN	9.0% yield, 230 TTN	4.6% yield, 120 TTN	10% yield, 260 TTN, 66% ee
P-I263F V87A	51% yield, 4300 TTN	43% yield, 3600 TTN, 48% ee	24% yield, 2000 TTN	4.5% yield, 380 TTN	5.5% yield, 460 TTN, 47% ee
P-I263F V87A A328V	46% yield, 1600 TTN	54% yield, 1800 TTN, 82% ee	27% yield, 930 TTN	6.6% yield, 220 TTN	14% yield, 490 TTN, 66% ee
P-I263F V87A A328V A268G	N.D.	75% yield, 2500 TTN, 39% ee	N.D.	17% yield, 580 TTN	44% yield, 1800 TTN, 66% ee
P-I263F V87A A328V A268G A82L	N.D.	N.D.	N.D.	N.D.	70% yield, 1500 TTN, 69% ee
P-I263F V87A A328V A268G A82I	N.D.	86% yield, 2200 TTN, 88% ee	N.D.	N.D.	77% yield, 2200 TTN, 68% ee

[a]Experiments were performed using whole cells overexpressing the P411 variant, resuspended to OD_{600} = 30, with 5 mM sulfide and 5 mM tosyl azide. Results are the average of experiments performed with duplicate cell cultures, each used to perform duplicate chemical reactions (total of four reactions). N.D. = Not determined; ee = enantiomeric excess.

Table S3. Reactions of other sulfide substrates with intermediate P411 variants. Conditions as in Table S2. The mutations introduced in the course of evolving a catalyst for amination of sulfide **1** are also strongly activating toward amination of related substrates.

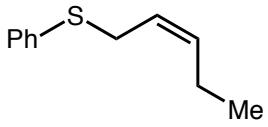
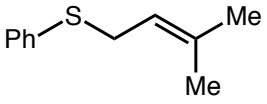
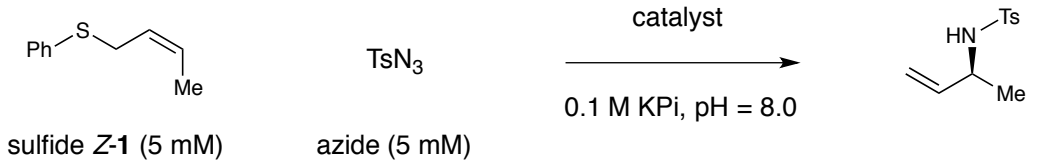
		
P-I263F V87A A328V	3.8% yield, 130 TTN	0.4% yield, 14 TTN
P-I263F V87A A328V A268G	18% yield, 600 TTN, 71% ee	7.9% yield, 260 TTN

Table S4. Reactions performed with purified proteins (and hemin).[a]



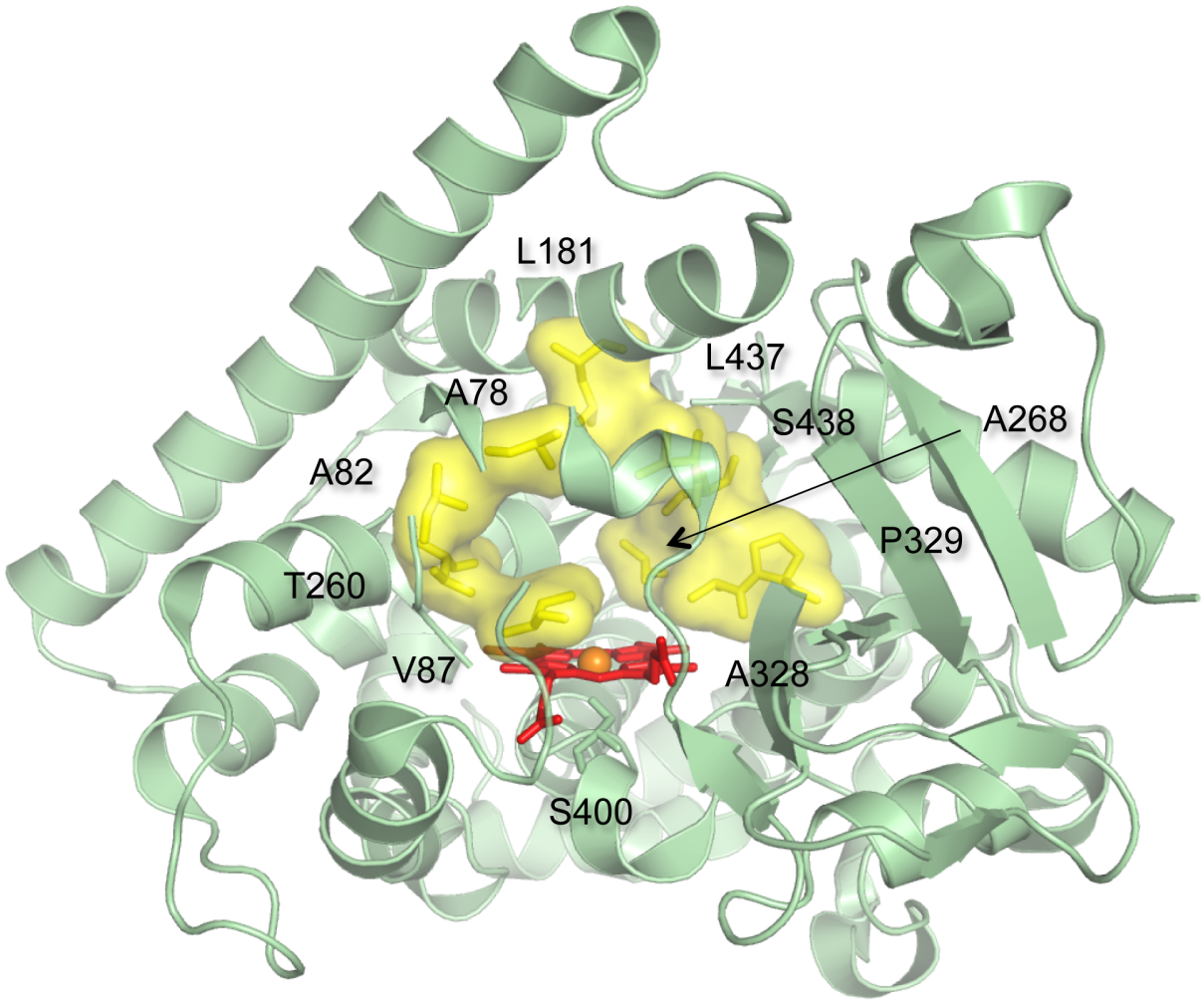
sulfide *Z*-**1** (5 mM) azide (5 mM)

catalyst	**percent yield, total turnovers**
hemin (25 μM)	0.2% yield, <1 TTN
hemin (25 μM) + imidazole (1 mM)	0.2% yield, <1 TTN
hemin (25 μM) + bovine serum albumin (10 μM)	0.1% yield, <1 TTN
Myoglobin (equine heart)	No reaction
Cytochrome *c* (bovine heart)	No reaction
$P411_{BM3}$ F87A T268A[b]	0.6% yield, 3 TTN
P-I263F	0.3% yield, 2 TTN
P-I263F A328V L437V[c]	1.7% yield, 9 TTN
P-I263F V87A A328V (P-3)	10% yield, 55 TTN, 63% ee
P-I263F V87A A328V A268G (P-4)	28% yield, 140 TTN, 63% ee
P-I263F V87A A328V A268G A82L	63% yield, 320 TTN, 70% ee
P-I263F V87A A328V A268G A82I (P-5, 10 μM)	52% yield, 260 TTN, 69% ee
P-I263F V87A A328V A268G A82I (P-5, 5.0 μM)	51% yield, 510 TTN, 69% ee
P-I263F V87A A328V A268G A82I (P-5, 2.5 μM)	43% yield, 860 TTN
P-I263F V87A A328V A268G A82I (P-5, 1.25 μM)	19% yield, 760 TTN

[a]Reactions performed in duplicate, with 10 μM enzyme unless indicated otherwise; ee = enantiomeric excess. As in whole cells, the intermediate **8** is converted to product **9** by the reductant. The *in vitro* conditions, however, provide fewer reducing equivalents compared to the whole cell conditions, necessitating a DTT work-up for all reactions that proceed to >10%

conversion. [b]Variant previously identified for intramolecular C–H amination.[11] [c]Variant previously identified for aziridination of styrenes.[12]

Figure S1. Location of residues subjected to site saturation mutagenesis, shown in yellow in the crystal structure of P-I263F; PBD: 4WG2.[11]



Semi-preparative scale reactions and assignment of absolute stereochemistry. *N*-(But-3-en-2-yl)-4-methylbenzenesulfonamide (**10**) was prepared by a semi-preparative enzymatic reaction. Two whole cell bioconversions were performed using 16 mL cells overexpressing variant P-5, resuspended to OD_{600} = 10 in M9-N. To the degassed cells in an anaerobic chamber were added

the oxygen depletion system (1.0 mL), glucose (2.0 mL, 250 mM in M9-N), sulfide *Z*-**1** (500 μL, 200 mM in DMSO), and tosyl azide (500 μL, 200 mM in DMSO). After 18 h, the reactions were diluted with 20 mL acetonitrile and 1.5 mL DTT solution (20 mM in 0.1 M KPi) and then centrifuged (5,000 rpm, 10 min). The supernatants were combined, extracted with EtOAc (2 x 30 mL), and the organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography (eluting with 20% EtOAc in hexanes). The product was isolated (32.0 mg, 71% yield, 6100 TTN, 71% ee) and found to have $[\alpha]_D^{25}$ –3.7 (*c* = 1.14 in $CHCl_3$). Based on the rotations and assignments of this compound made by Murakami *et al*.[9] and Moriwake *et al*.,[13] the enzymatically produced compound is assigned as the (*S*) enantiomer. Other allylic amine products (**14** and **15**) were assigned by analogy.

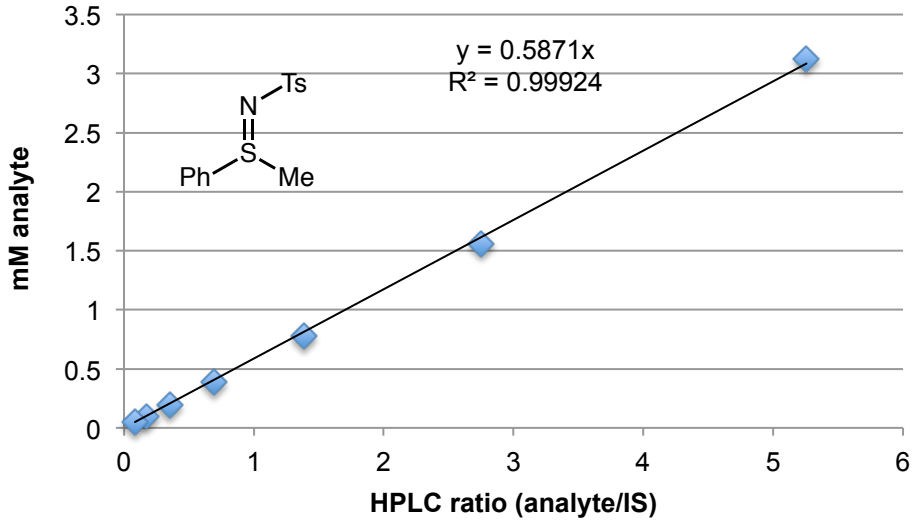
In addition, Boc-L-alanine methyl ester was derivatized according to the procedures of Moriwake *et al*.[13] and Velmourougane *et al*.,[14] providing **10** as the (*S*) enantiomer. The enantiopurity of the material was eroded to 68% ee during the reaction sequence, but the major peak matched that observed in the P411-catalyzed reactions, confirming assignment of the enzymatic product as the (*S*) enantiomer.

S-Ethyl-*S*-phenyl-*N*-(4-methylbenzenesulfonyl) sulfimide (**12**) was prepared racemically and separated by chiral HPLC to >99% ee using a Chiralcel OJ-H column. The enantiomer formed preferentially by variant P-5, which elutes first on the Chiralcel OJ-H column (using 12% isopropanol, SFC chromatography), was found to have $[\alpha]_D^{25}$ –248.2 (*c* = 0.38 in $CHCl_3$). Based on the rotation and assignment of this compound made by Wang *et al*.,[10a] the enzymatically produced compound is assigned as the (*S*) enantiomer.

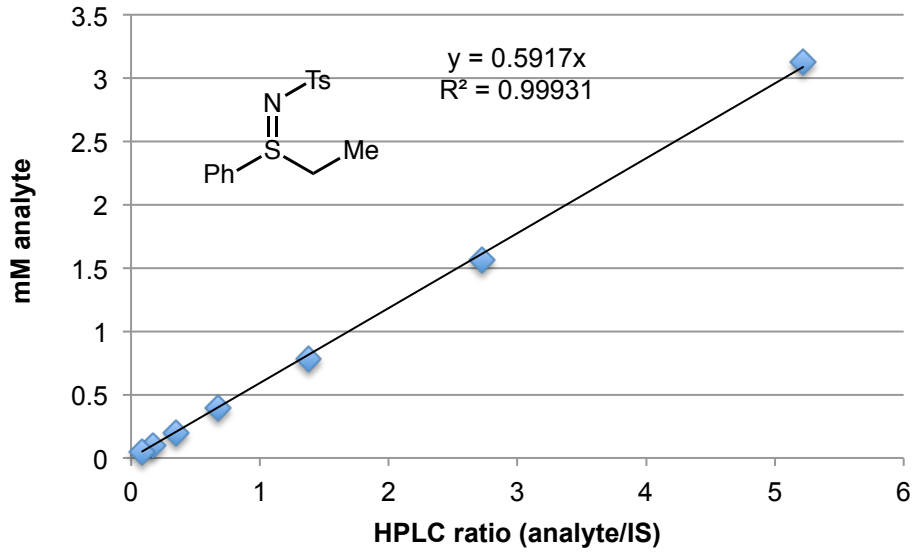
S-(*n*-Propyl)-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**18**) was prepared by a semi-preparative enzymatic reaction. Two whole cell bioconversions were performed using 12.8 mL cells overexpressing variant P-5, resuspended to OD_{600} = 10 in M9-N. To the degassed cells in an anaerobic chamber were added the oxygen depletion system (800 μL), glucose (1.6 mL, 250 mM in M9-N), phenyl *n*-propyl sulfide (400 μL, 300 mM in DMSO), and tosyl azide (400 μL, 300 mM in DMSO). After 18 h, the reactions were diluted with 16 mL acetonitrile and centrifuged (5,000 rpm, 10 min). The supernatants were combined, extracted with EtOAc (2 x 30 mL), and the organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography (eluting with 50% EtOAc in hexanes). The product was isolated (41.2 mg, 53% yield, 6100 TTN, >99% ee) and found to have $[\alpha]_D^{25}$ –277.2 (*c* = 0.44 in $CHCl_3$). Based on the rotation and assignment of this compound made by Wang *et al*.,[10a] the enzymatically produced compound is assigned as the (*S*) enantiomer. The absolute stereochemistry of *S*-(*n*-butyl)-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**19**) was assigned by analogy.

HPLC Calibration: Calibration curves with an internal standard were created for HPLC analysis of reaction products.

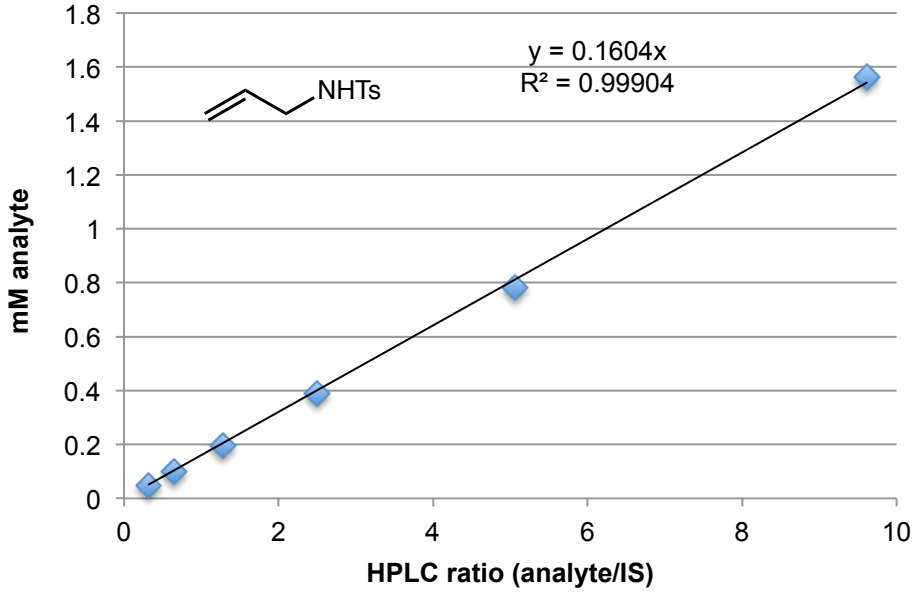
S-Methyl-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**11**), with 1.25 mM 1,3,5-trimethoxy-benzene as internal standard, at 230 nm.



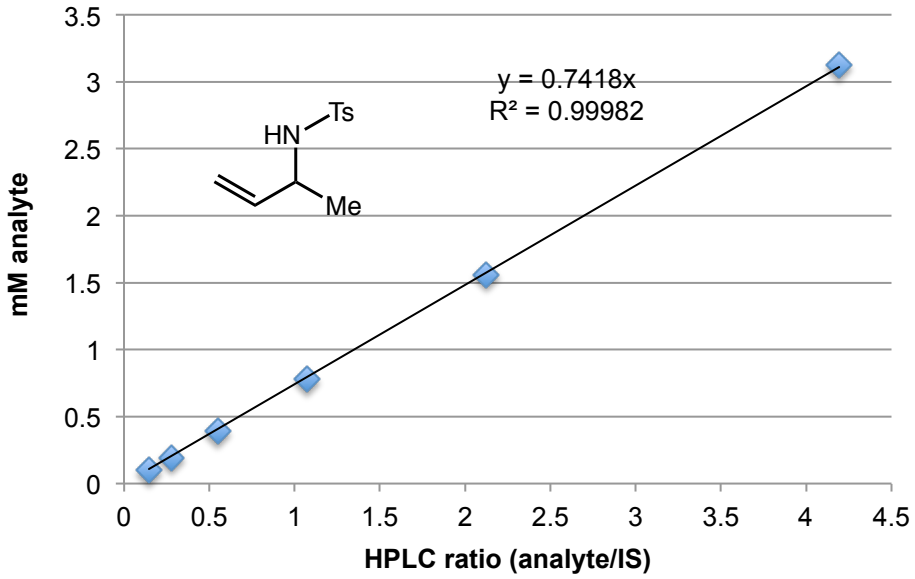
S-Ethyl-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**12**), with 1.25 mM 1,3,5-trimethoxy-benzene as internal standard, at 230 nm.



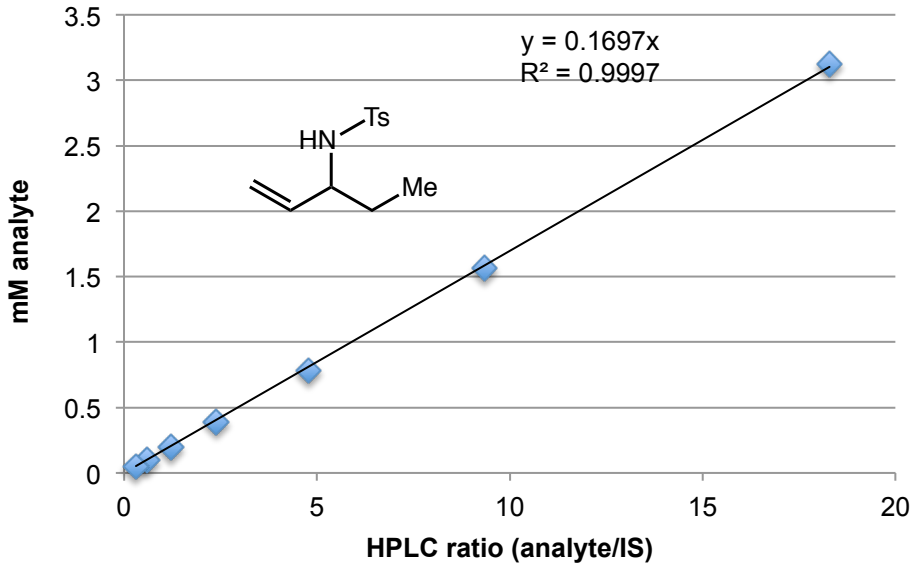
N-Allyl-4-methylbenzenesulfonamide (**13**), with 0.25 mM 1,3,5-trimethoxybenzene as internal standard, at 230 nm.



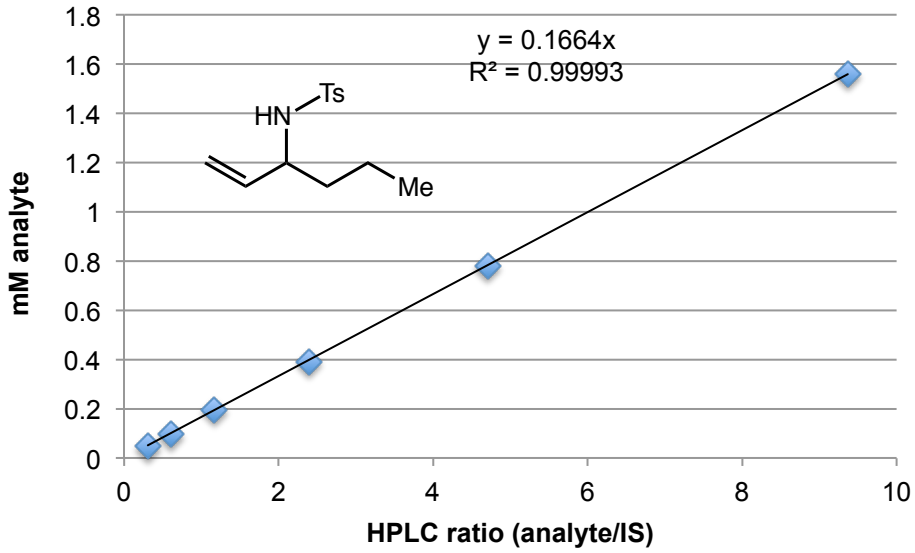
N-(But-3-en-2-yl)-4-methylbenzenesulfonamide (**10**), with 1.25 mM 4-ethylanisole as internal standard, at 230 nm.



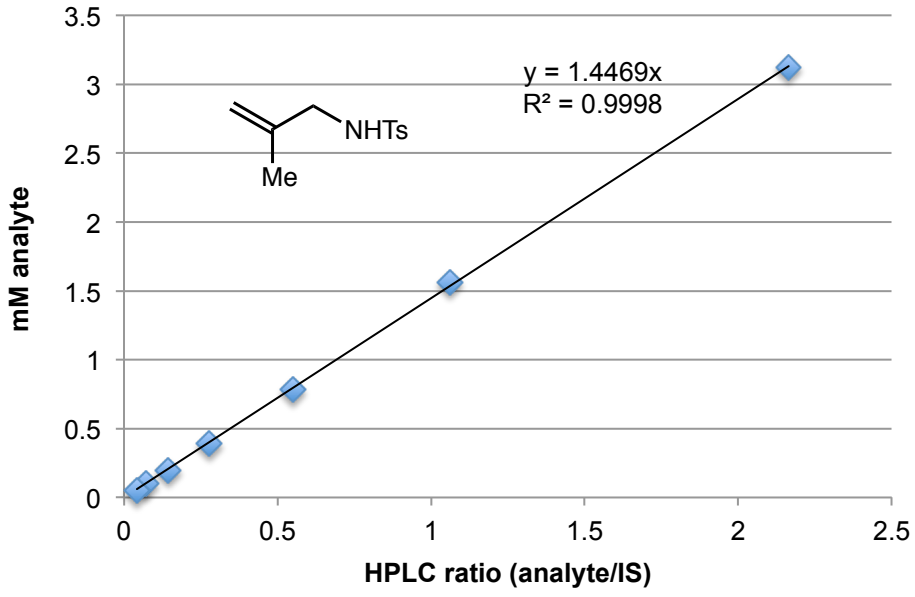
N-(Pent-1-en-3-yl)-4-methylbenzenesulfonamide (**14**), with 0.25 mM 1,3,5-trimethoxybenzene as internal standard, at 230 nm.



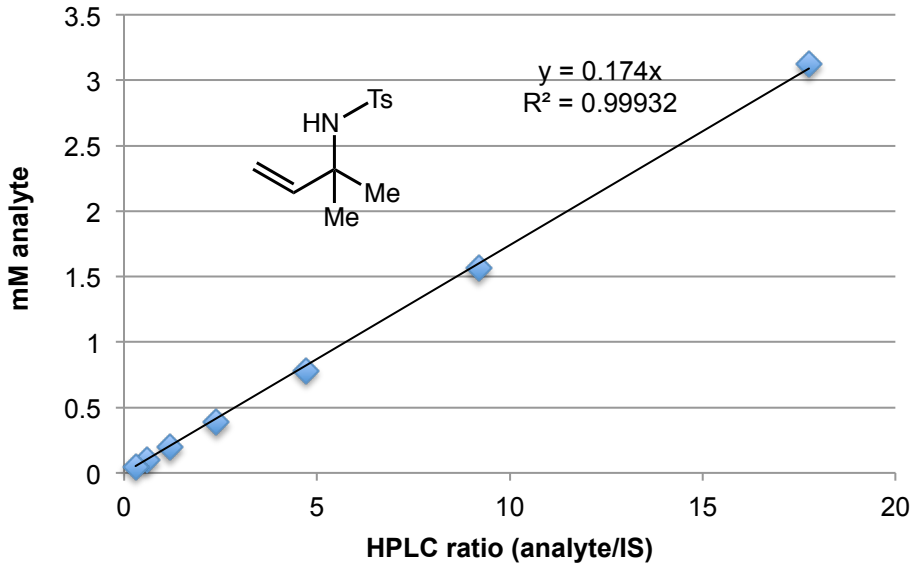
N-(Hex-1-en-3-yl)-4-methylbenzenesulfonamide (**15**), with 0.25 mM 1,3,5-trimethoxybenzene as internal standard, at 230 nm.



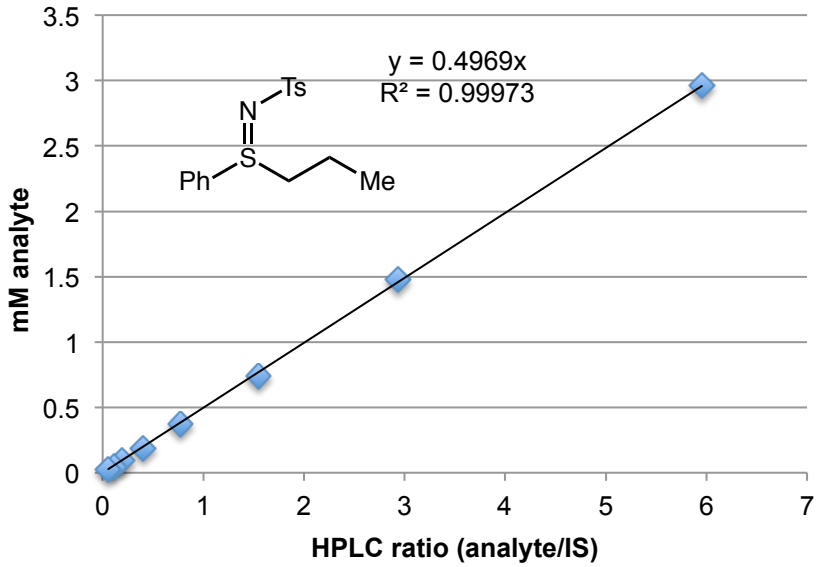
N-(2-Methylallyl)-4-methylbenzenesulfonamide (**16**), with 1.25 mM 3-phenyl-1-propanol as internal standard, at 210 nm.



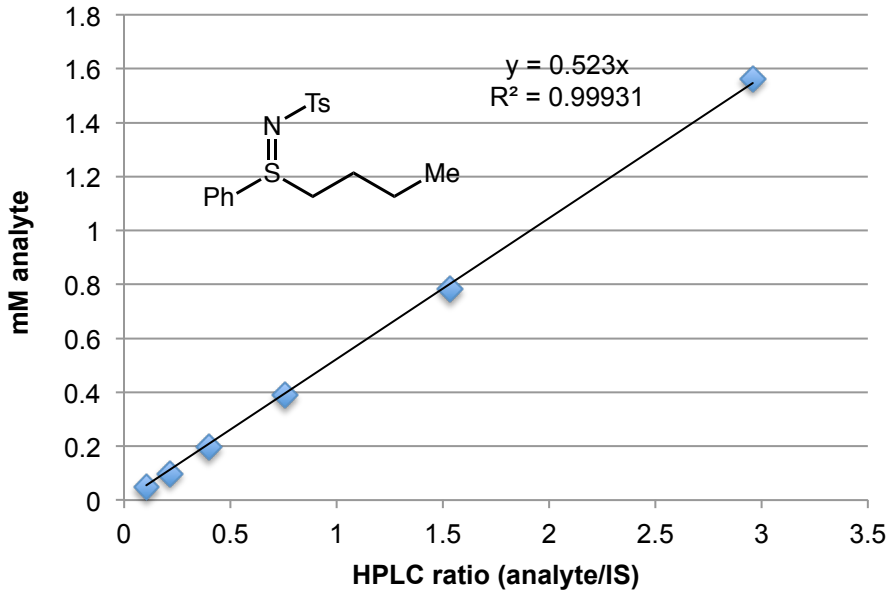
N-(2-Methylbut-3-en-2-yl)-4-methylbenzenesulfonamide (**17**), with 0.25 mM 1,3,5-trimethoxy-benzene as internal standard, at 230 nm.



S-(*n*-Propyl)-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**18**) with 1.25 mM 3-phenyl-1-propanol as internal standard, at 210 nm.

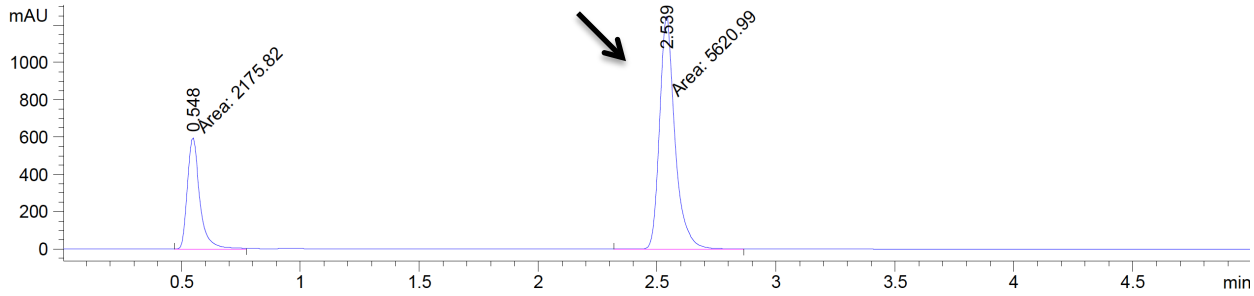


S-(*n*-Butyl)-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**19**) with 1.25 mM 3-phenyl-1-propanol as internal standard, at 210 nm.



HPLC co-injections of reaction mixtures with chemically synthesized authentic products.

S-Methyl-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**11**), synthetic:



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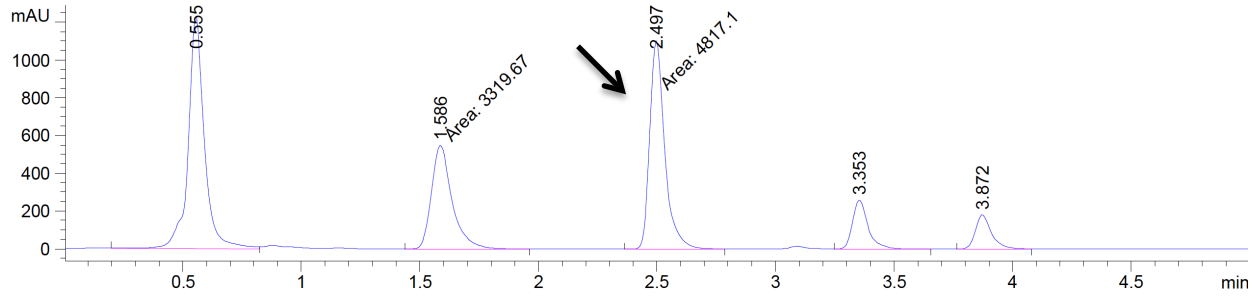
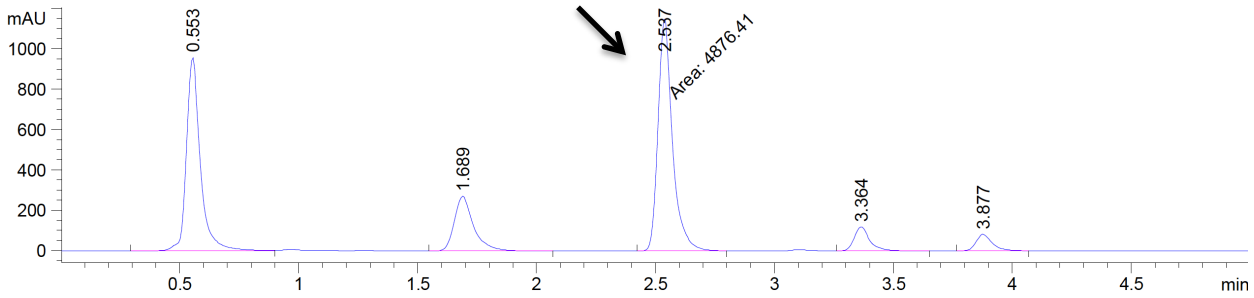
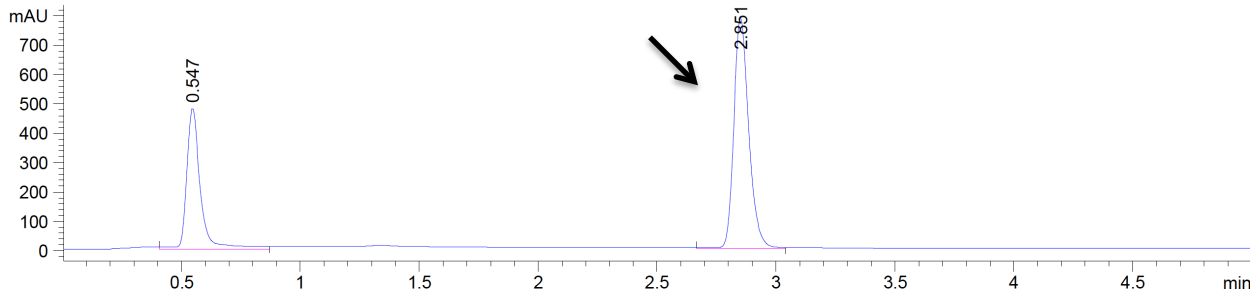


Co-injection:



S-Ethyl-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**12**), synthetic:



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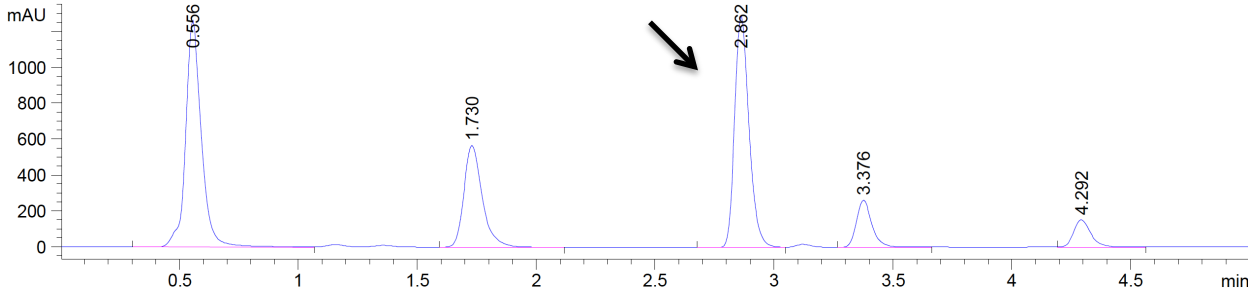

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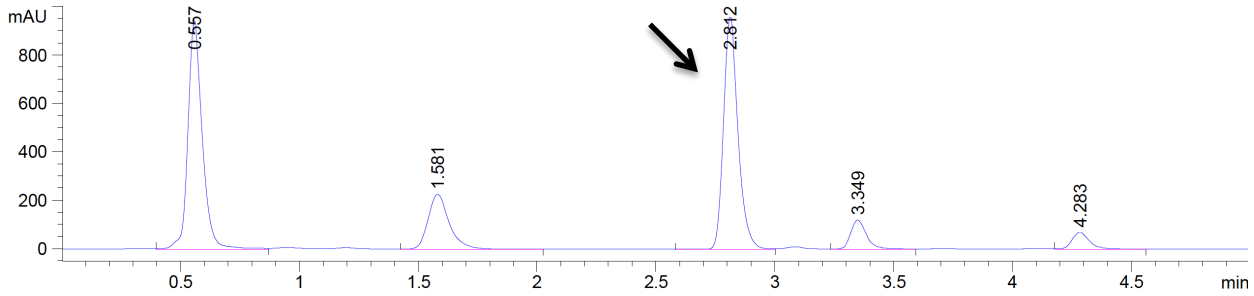
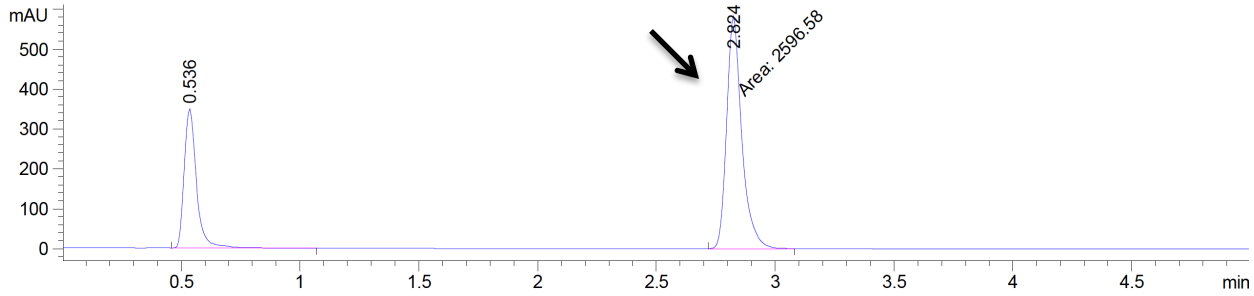


N-Allyl-4-methylbenzenesulfonamide (**13**), synthetic:



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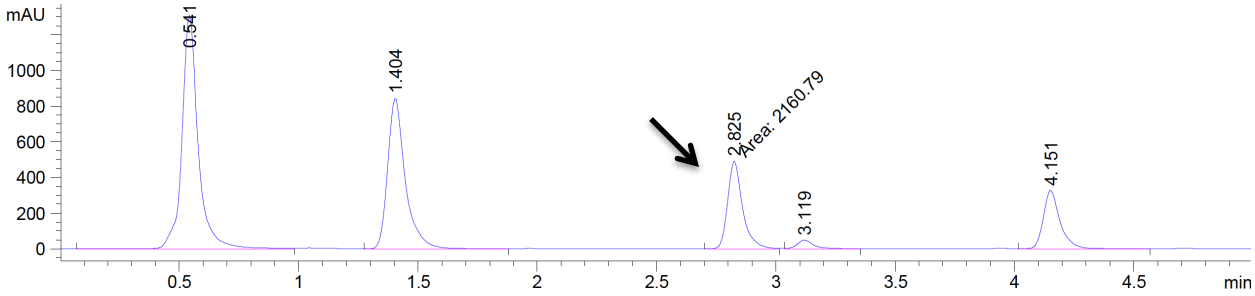

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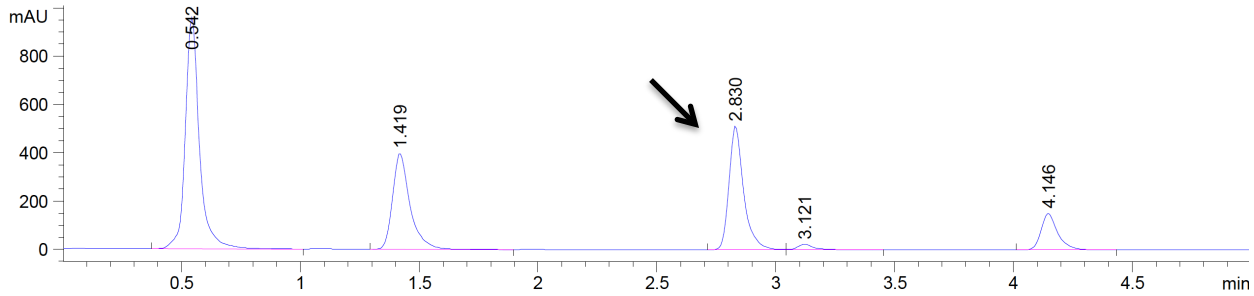
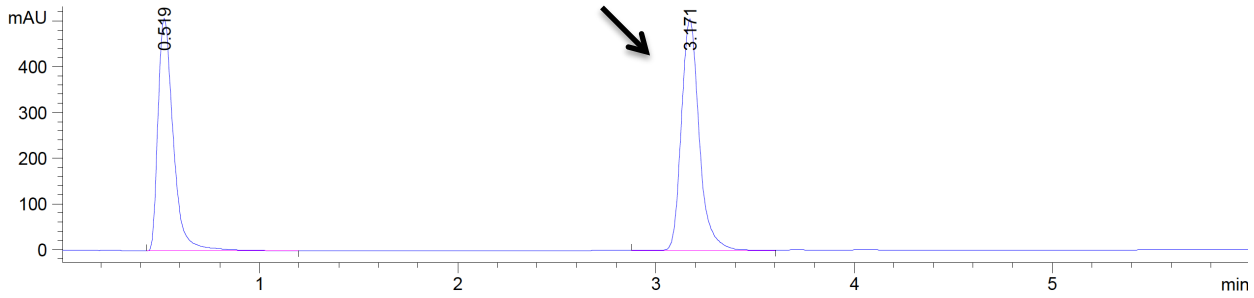


N-(But-3-en-2-yl)-4-methylbenzenesulfonamide (**10**), synthetic:



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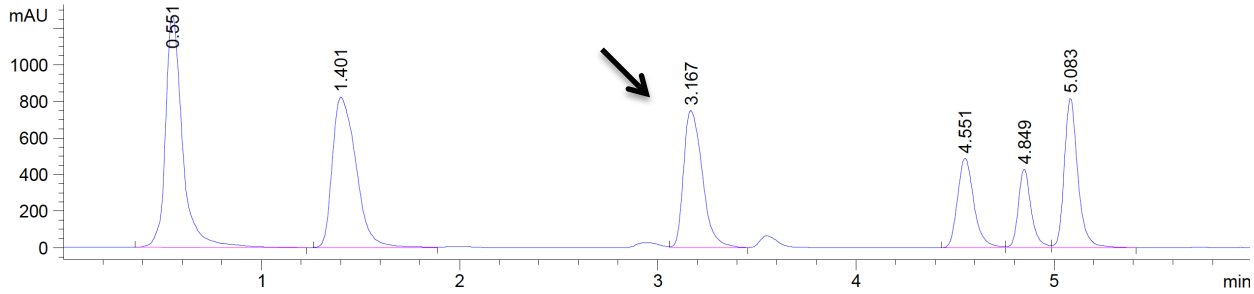

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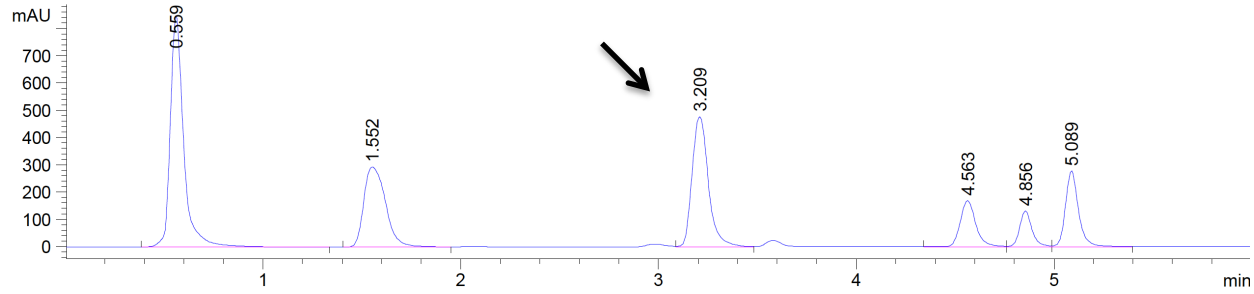
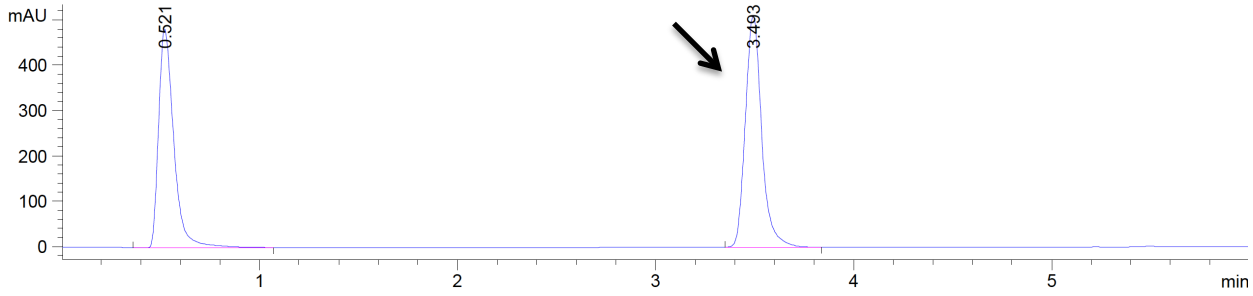


N-(Pent-1-en-3-yl)-4-methylbenzenesulfonamide (**14**), synthetic:



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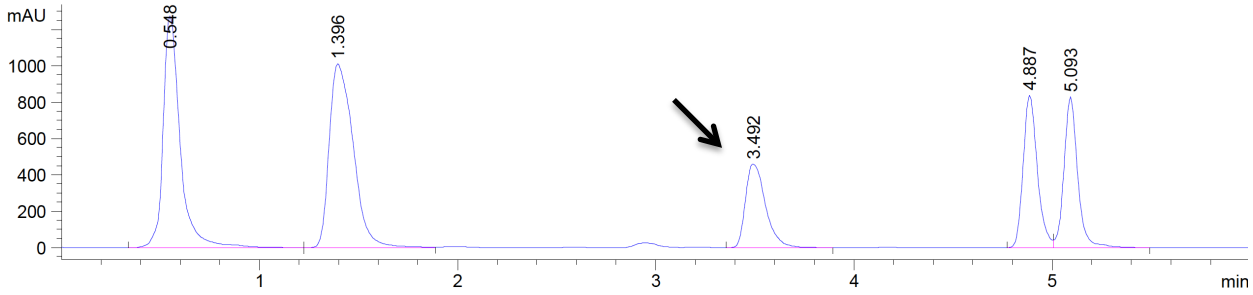

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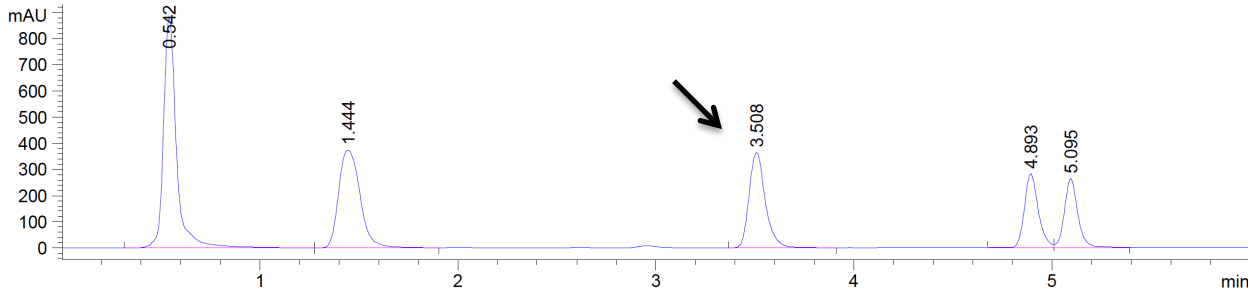
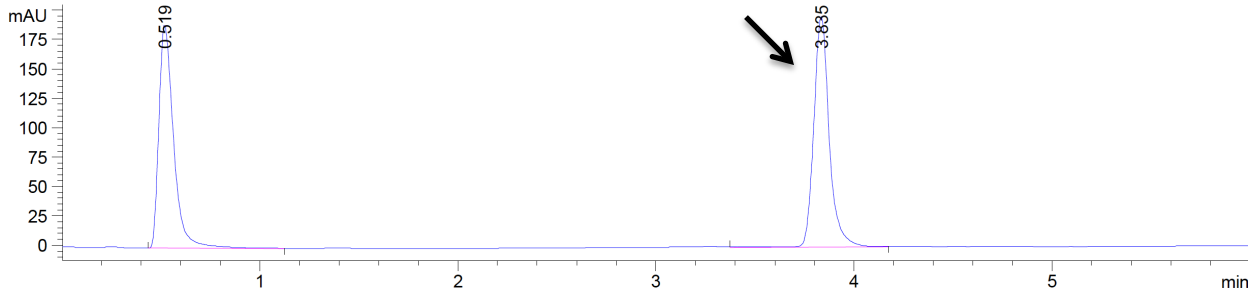


N-(Hex-1-en-3-yl)-4-methylbenzenesulfonamide (**15**), synthetic:



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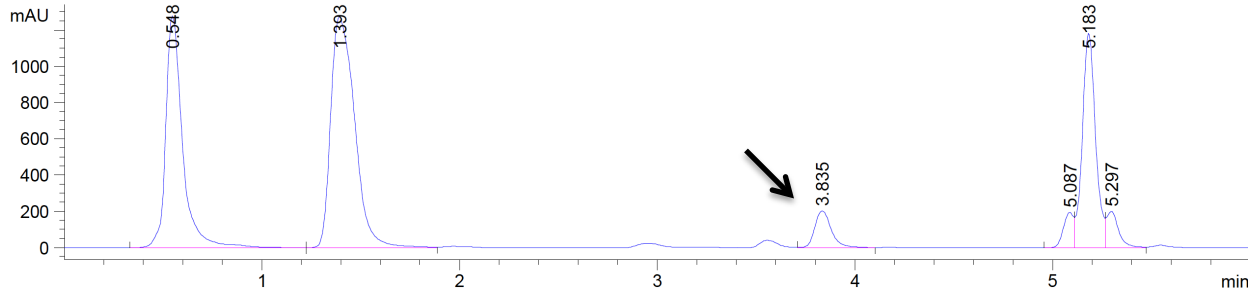

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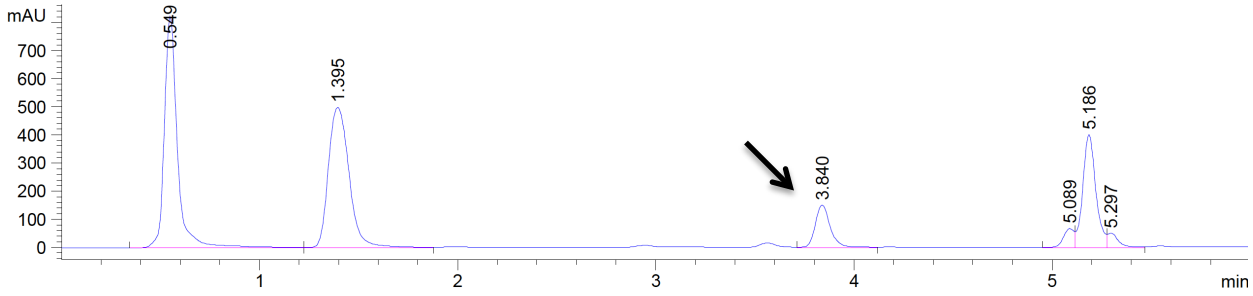
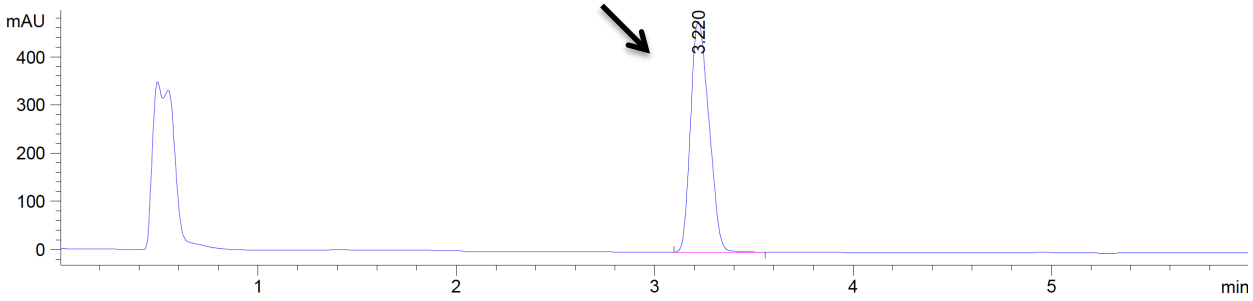


N-(2-Methylallyl)-4-methylbenzenesulfonamide (**16**), synthetic:



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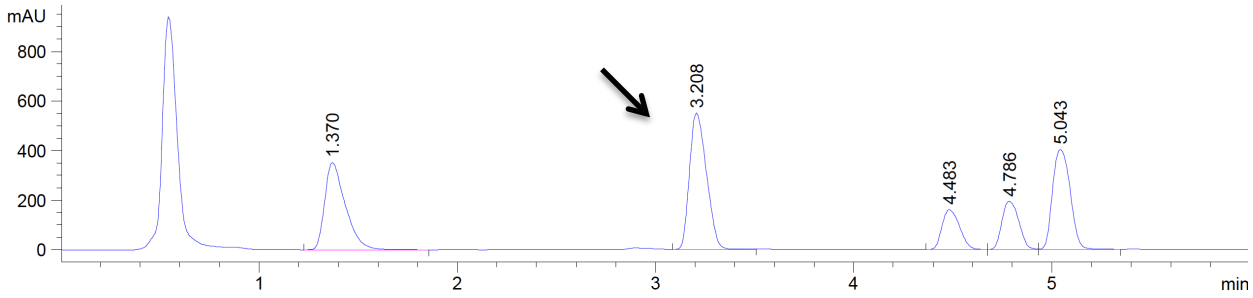

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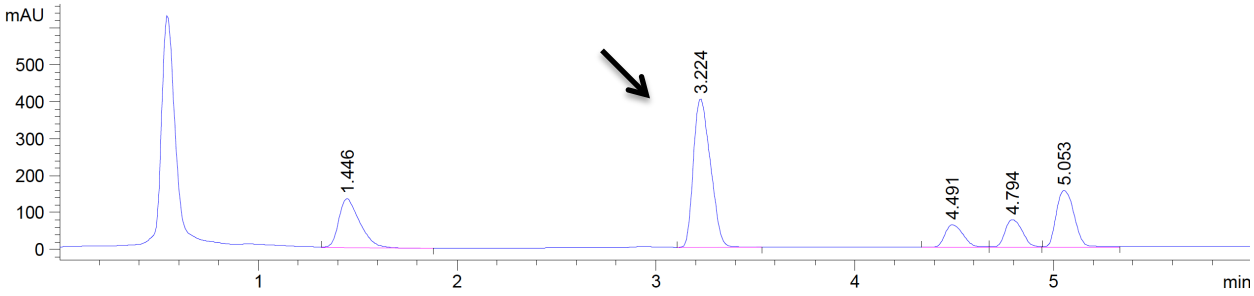
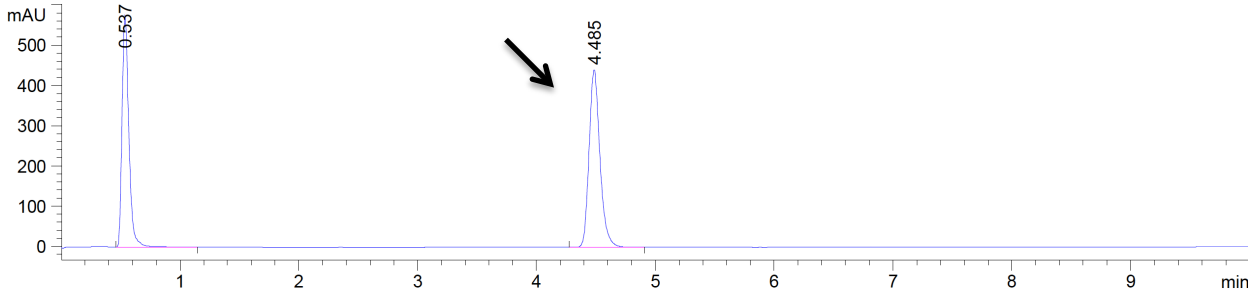


N-(2-Methylbut-3-en-2-yl)-4-methylbenzenesulfonamide (**17**), synthetic:



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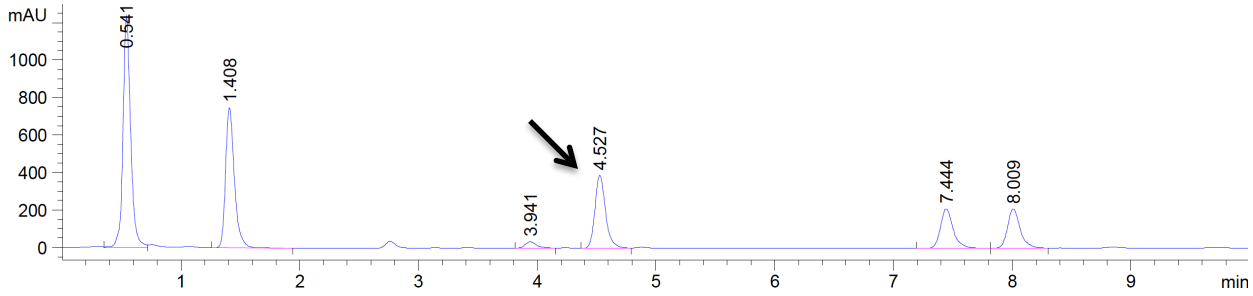

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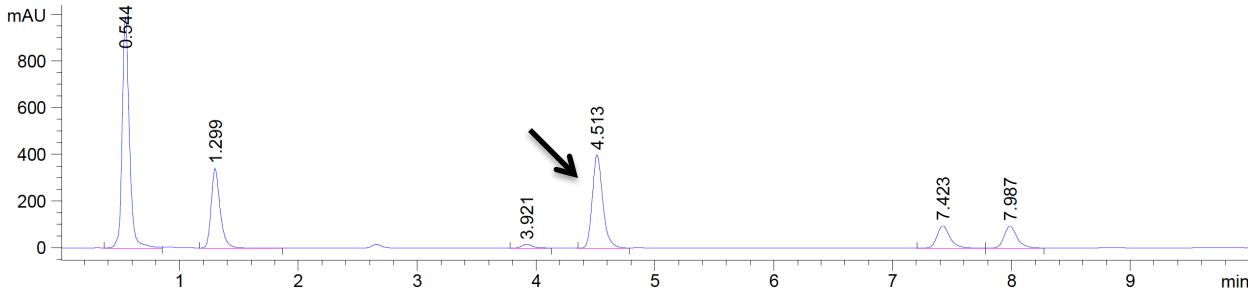
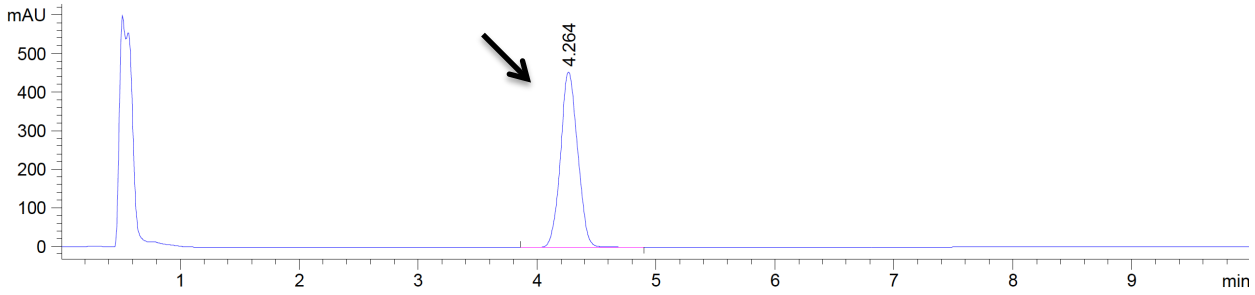
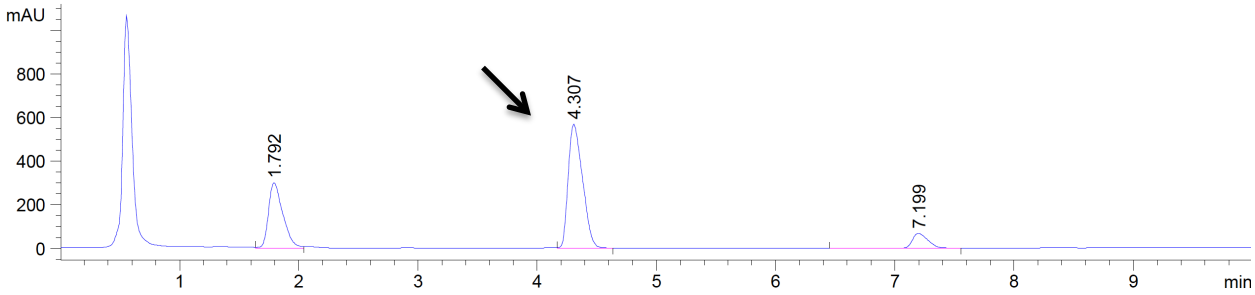


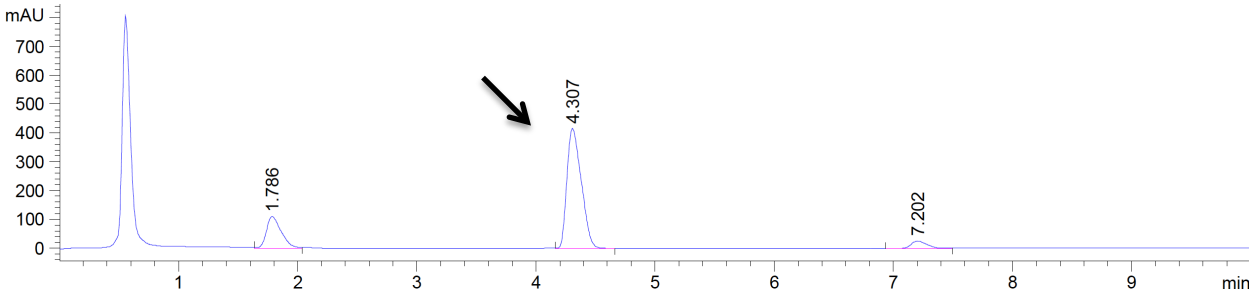
S-(*n*-Propyl)-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**18**), synthetic:



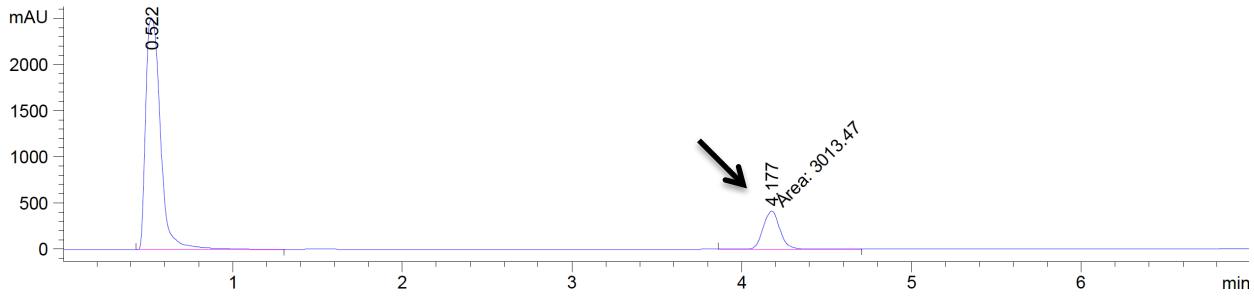
Enzymatic:



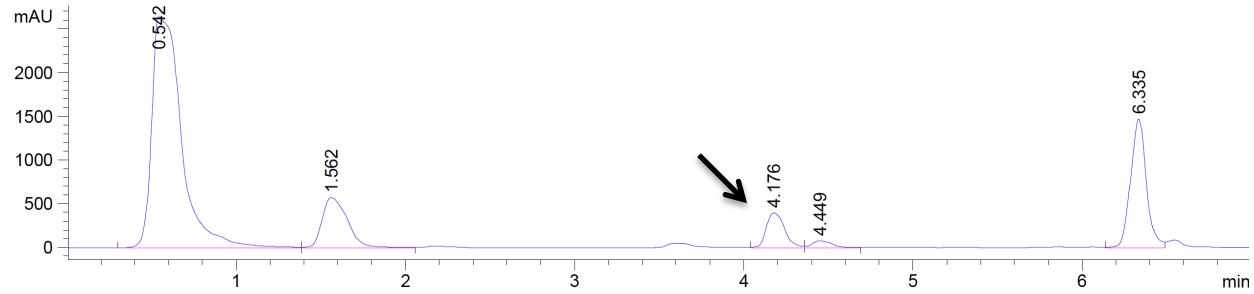
Co-injection:



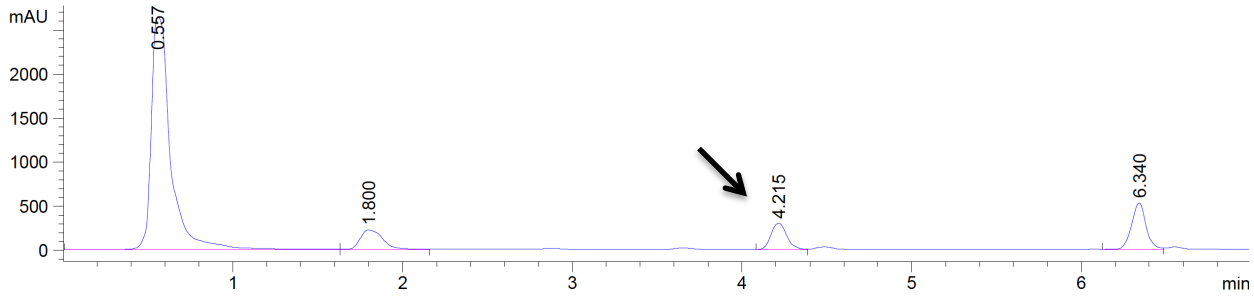
S-(*n*-Butyl)-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (**19**), synthetic:



Enzymatic:



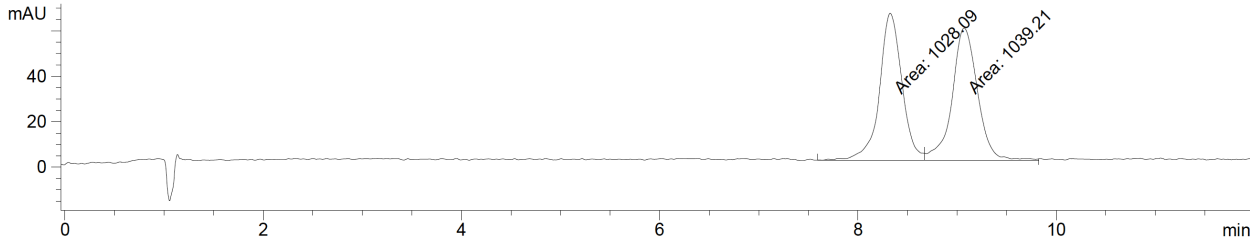
Co-injection:



Determination of enantioselectivity.

For chiral HPLC analysis, reaction mixtures were extracted with ethyl acetate, dried, and concentrated. For the sulfimidation of phenyl ethyl sulfide (**6**), the material was dissolved in 20% isopropanol in hexanes and analyzed directly by SFC for enantioselectivity. All other products were dissolved in acetonitrile and purified by C18 semi-preparative HPLC. The purified material was dried, dissolved in 20% isopropanol in hexanes, and analyzed by SFC for enantioselectivity.

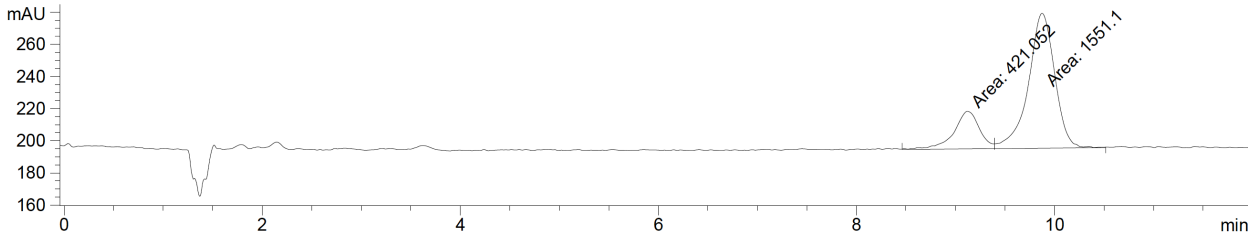
Enantioselectivity for enzyme-catalyzed formation of *N*-(but-3-en-2-yl)-4-methylbenzene sulfonamide (10), from the sulfide *Z*-1. Measured by SFC chromatography using Chiralpak AS-H (10% isopropanol). Racemic standard:



Signal 2: DAD1 B, Sig=235,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.326	MF	0.2644	1028.09180	64.79642	49.7312
2	9.071	FM	0.2976	1039.20654	58.20702	50.2688

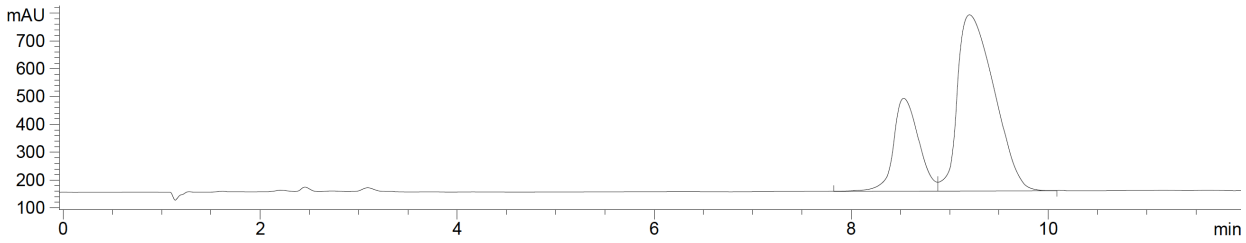
With P-I263F (57% ee):



Signal 2: DAD1 B, Sig=235,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.121	MF	0.3002	421.05237	23.37282	21.3499
2	9.875	FM	0.3082	1551.09827	83.87534	78.6501

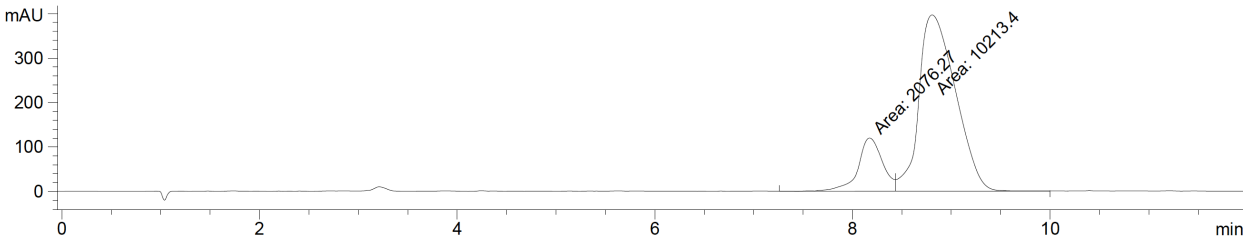
With P-I263F V87A (47% ee):



Signal 2: DAD1 B, Sig=235,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.533	VV	0.2775	5942.06885	334.16473	26.6094
2	9.201	VB	0.4119	1.63886e4	633.26636	73.3906

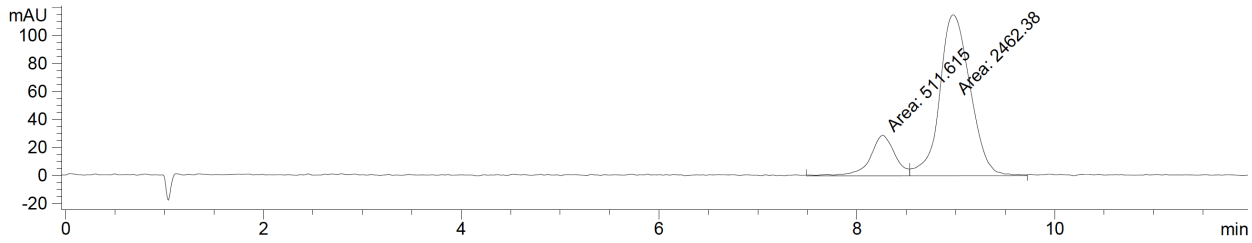
With P-I263F A328V (66% ee):



Signal 2: DAD1 B, Sig=235,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.173	MF	0.2890	2076.27002	119.74593	16.8944
2	8.806	FM	0.4294	1.02134e4	396.39926	83.1056

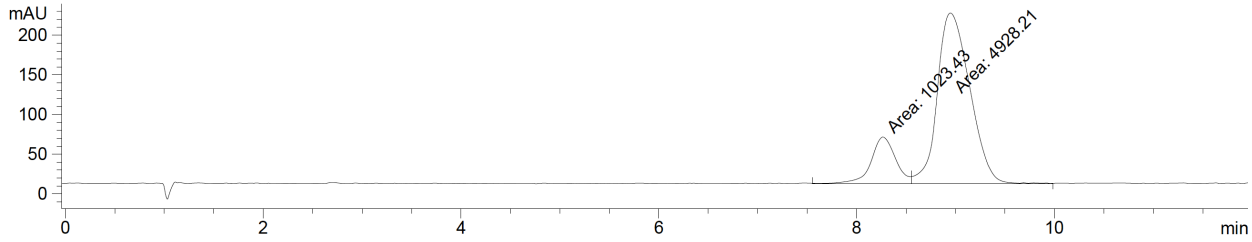
With P-I263F V87A A328V (66% ee):



Signal 2: DAD1 B, Sig=235,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.261	MF	0.2935	511.61548	29.04813	17.2030
2	8.973	FM	0.3573	2462.37500	114.85909	82.7970

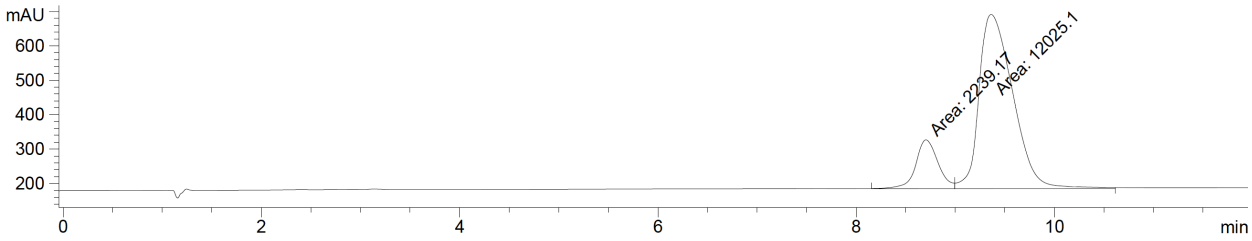
With P-I263F V87A A328V A268G (66% ee):



Signal 2: DAD1 B, Sig=235,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.267	MF	0.2892	1023.42816	58.98339	17.1957
2	8.950	FM	0.3816	4928.21045	215.23618	82.8043

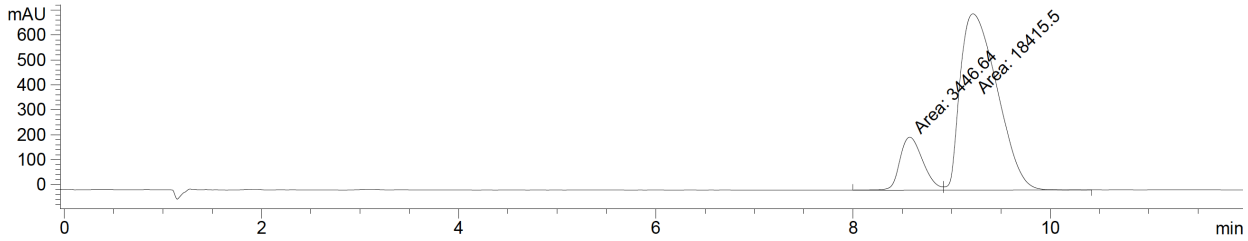
With P-I263F V87A A328V A268G A82L (69% ee):



Signal 2: DAD1 B, Sig=235,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.704	MF	0.2627	2239.16846	142.06618	15.6977
2	9.363	FM	0.3958	1.20251e4	506.30499	84.3023

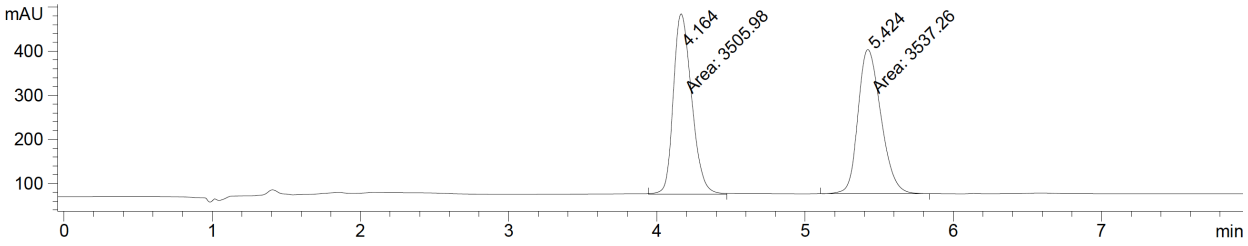
With P-I263F V87A A328V A268G A82I (68% ee):



Signal 2: DAD1 B, Sig=235,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.575	MF	0.2704	3446.63818	212.41125	15.7653
2	9.217	FM	0.4341	1.84155e4	706.99561	84.2347

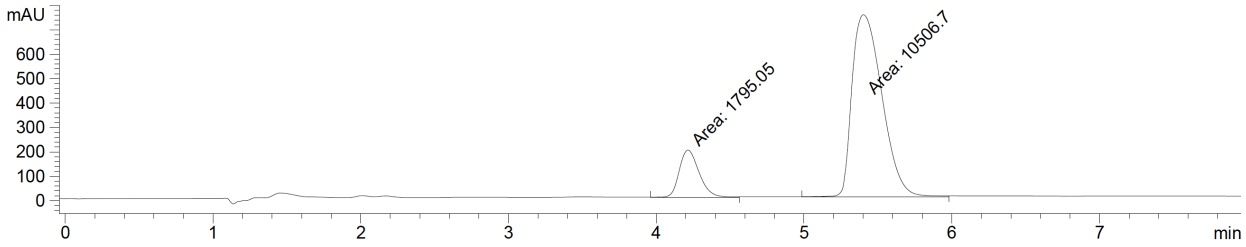
Enantioselectivity for enzyme-catalyzed formation of *N*-(pent-1-en-3-yl)-4-methylbenzene sulfonamide (14). Measured by SFC chromatography using Chiralpak AS-H (20% isopropanol). Racemic standard:



Signal 1: DAD1 A, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.164	MM	0.1429	3505.97510	408.89801	49.7779
2	5.424	MF	0.1800	3537.25537	327.43335	50.2221

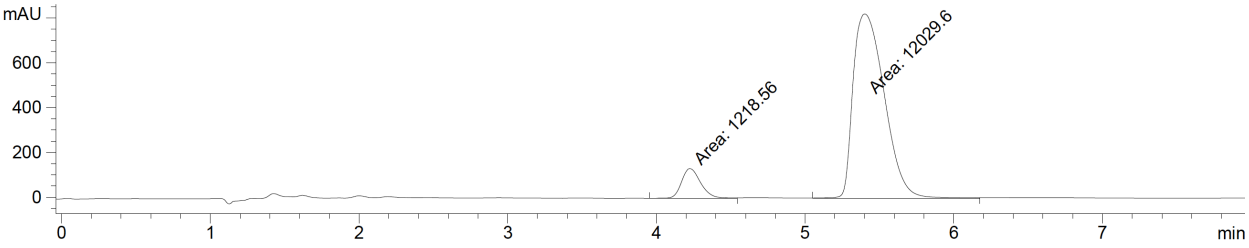
With P-I263F V87A A328V A268G (71% ee):



Signal 1: DAD1 A, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.214	MM	0.1545	1795.04980	193.69081	14.5919
2	5.402	MM	0.2348	1.05067e4	745.76099	85.4081

With P-I263F V87A A328V A268G A82I (82% ee):

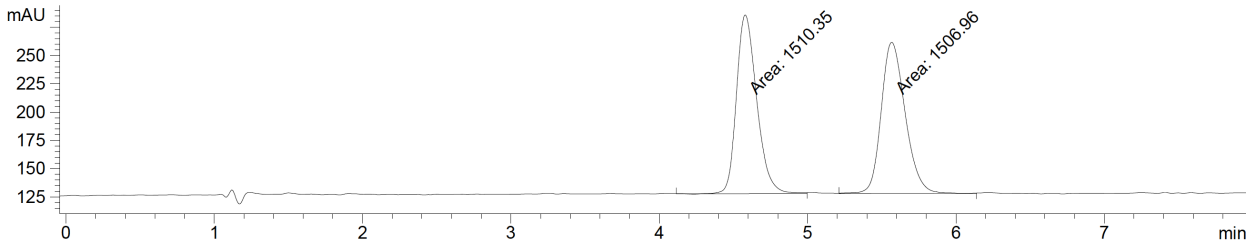


Signal 1: DAD1 A, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.225	MM	0.1518	1218.56250	133.75122	9.1980
2	5.401	MM	0.2437	1.20296e4	822.57532	90.8020

Enantioselectivity for enzyme-catalyzed formation of *N*-(hex-1-en-3-yl)-4-methylbenzene sulfonamide (15). Measured by SFC chromatography using Chiralpak AS-H (20% isopropanol).

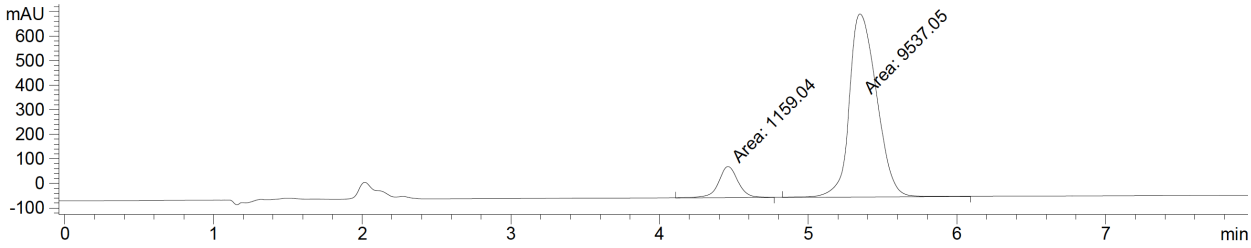
Racemic standard:



Signal 1: DAD1 A, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.579	MF	0.1590	1510.34705	158.28397	50.0561
2	5.567	FM	0.1876	1506.96082	133.89427	49.9439

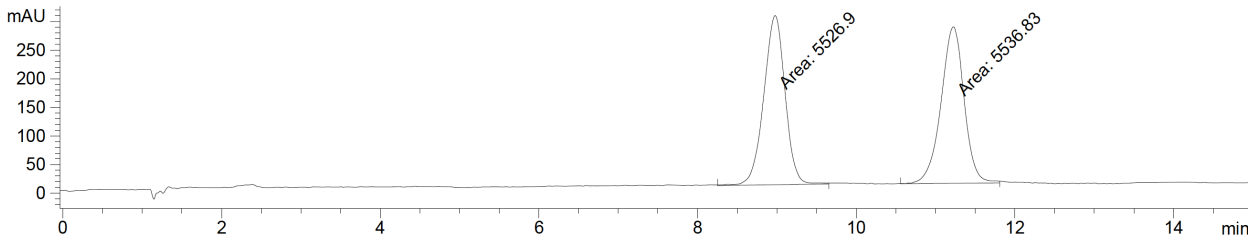
With P-I263F V87A A328V A268G A82I (78% ee):



Signal 1: DAD1 A, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.460	FM	0.1520	1159.04419	127.12802	10.8361
2	5.348	FM	0.2131	9537.04688	746.06012	89.1639

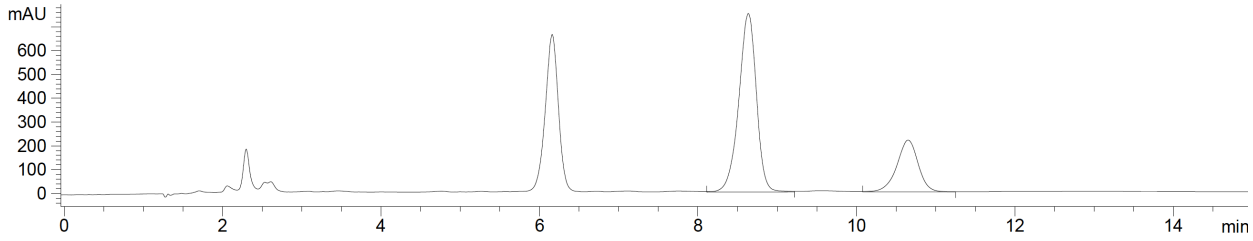
Enantioselectivity for enzyme-catalyzed formation of *S*-ethyl-*S*-phenyl-*N*-(4-methylbenzene sulfonyl)sulfimide (12). Measured by SFC chromatography using Chiralcel OJ-H (12% isopropanol). Racemic standard:



Signal 1: DAD1 A, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.977	MM	0.3114	5526.89941	295.84384	49.9551
2	11.224	MM	0.3374	5536.82764	273.46725	50.0449

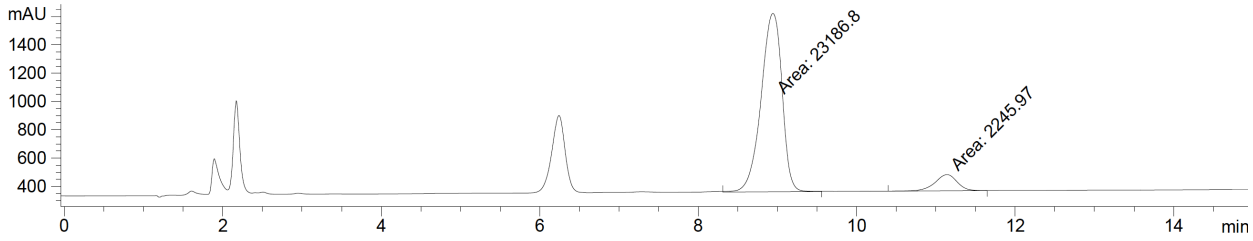
With P-I263F V87A (48% ee):



Signal 1: DAD1 A, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.637	VV	0.2405	1.16016e4	749.98163	74.0014
2	10.653	VV	0.2824	4075.94336	217.75298	25.9986

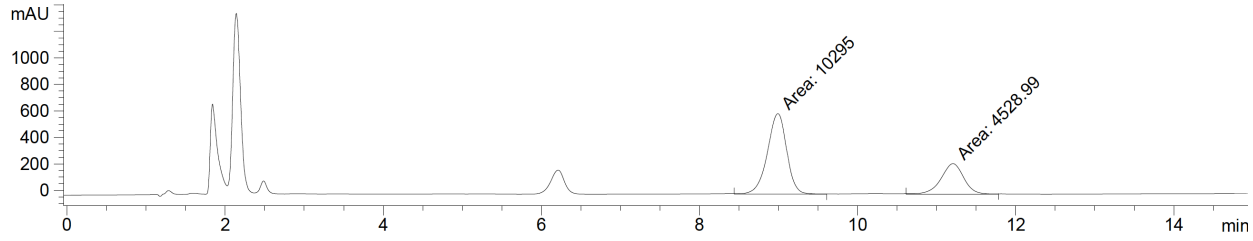
With P-I263F V87A A328V (82% ee):



Signal 1: DAD1 A, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.943	FM	0.3066	2.31868e4	1260.37512	91.1690
2	11.139	FM	0.3245	2245.97192	115.36353	8.8310

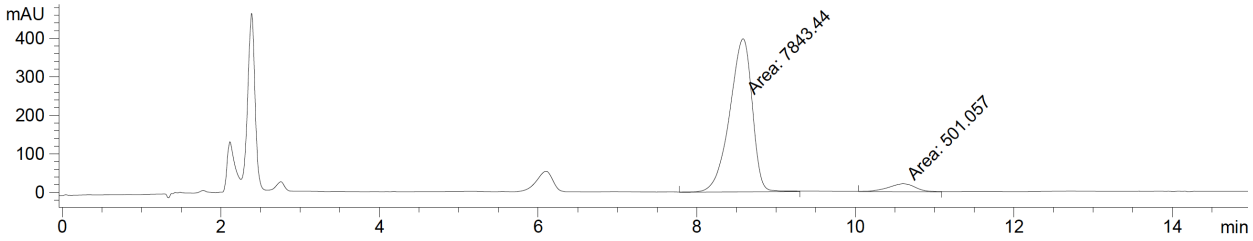
With P-I263F V87A A328V A268G (39% ee):



Signal 1: DAD1 A, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.991	MM	0.2824	1.02950e4	607.60736	69.4483
2	11.207	MM	0.3293	4528.98730	229.22603	30.5517

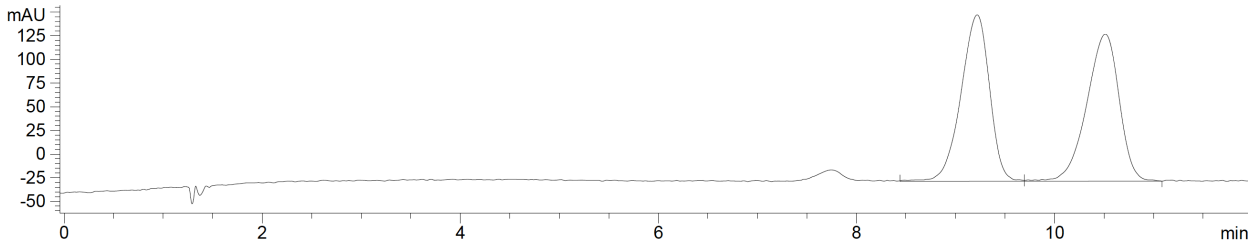
With P-I263F V87A A328V A268G A82I (88% ee):



Signal 1: DAD1 A, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.584	MM	0.3287	7843.44434	397.64984	93.9954
2	10.605	MM	0.4022	501.05716	20.76526	6.0046

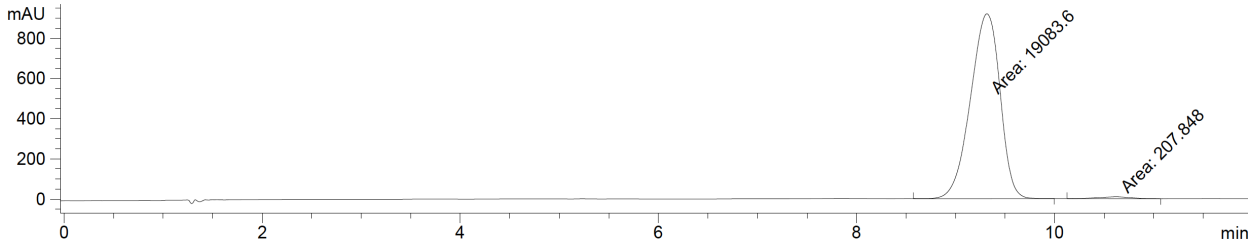
Enantioselectivity for enzyme-catalyzed formation of *S*-(*n*-propyl)-*S*-phenyl-*N*-(4-methylbenzenesulfonyl)sulfimide (18). Measured by SFC chromatography using Chiralcel OJ-H (10% isopropanol). Racemic standard:



Signal 1: DAD1 A, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.217	BV	0.3109	3538.38550	175.76720	49.9964
2	10.513	VV	0.3458	3538.90210	155.33209	50.0036

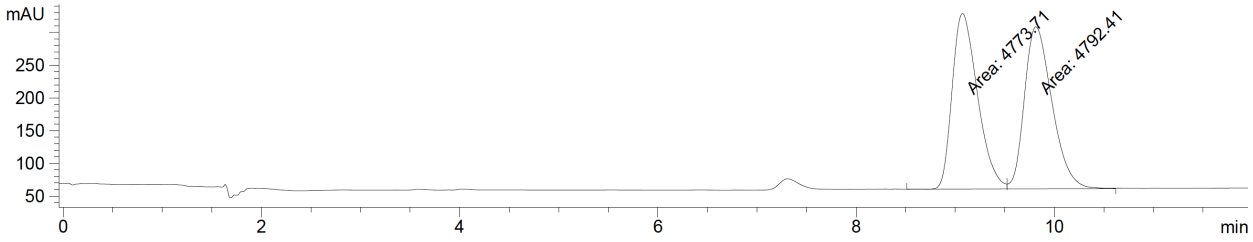
With P-I263F V87A A328V A268G A82I (98% ee):



Signal 1: DAD1 A, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.317	MM	0.3456	1.90836e4	920.32013	98.9226
2	10.627	MM	0.3657	207.84750	9.47129	1.0774

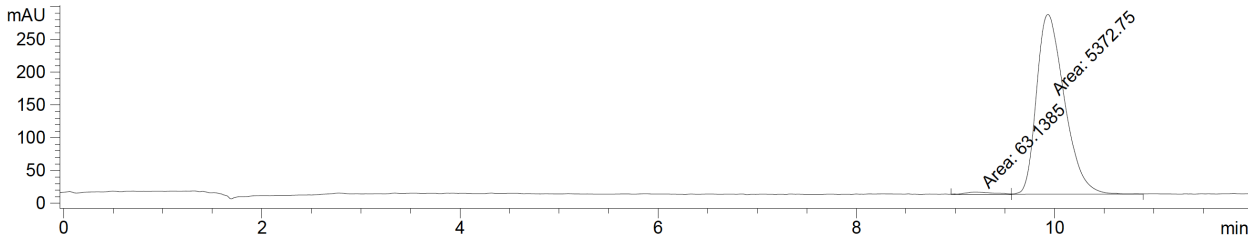
Enantioselectivity for enzyme-catalyzed formation of *S*-(*n*-butyl)-*S*-phenyl-*N*-(4-methyl benzenesulfonyl)sulfimide (19). Measured by SFC chromatography using Chiralcel OD-H (15% isopropanol). Racemic standard:



Signal 2: DAD1 B, Sig=235,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.073	FM	0.2966	4773.71387	268.26553	49.9023
2	9.814	MF	0.3226	4792.41309	247.61929	50.0977

With P-I263F V87A A328V A268G A82I (98% ee):



Signal 2: DAD1 B, Sig=235,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.222	MF	0.3311	63.13852	3.17796	1.1615
2	9.932	MF	0.3266	5372.74609	274.14957	98.8385

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