Supporting Information:

Enantioselective imidation of sulfides via enzyme-catalyzed intermolecular nitrogen-atom transfer

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Experimental Procedures

General. Unless otherwise noted, all chemicals and reagents for chemical reactions were obtained from commercial suppliers (Sigma-Aldrich, VWR, Alfa Aesar) and used without further purification. Silica gel chromatography purifications were carried out using AMD Silica Gel 60, 230-400 mesh. ^{1}H spectra were recorded on a Varian Inova 500 MHz instrument in CDCl3, and are referenced to the residual solvent peak. Synthetic reactions were monitored using thin layer chromatography (Merck 60 gel plates) using an UV-lamp for visualization.

Chromatography. Analytical high-performance liquid chromatography (HPLC) was carried out using an Agilent 1200 series, and a Kromasil 100 C18 column (Peeke Scientific, 4.6 x 50 mm, 5 μm). Semi-preparative HPLC was performed using an Agilent XDB-C18 (9.4 x 250 mm, 5 μm). Analytical chiral HPLC was conducted using a supercritical fluid chromatography (SFC) system with isopropanol and liquid CO_2 as the mobile phase. Chiralcel OB-H and OJ columns were used to separate sulfimide enantiomers (4.6 x 150 mm, 5 μM). Sulfides were all commercially available and sulfimide standards were prepared as reported.[1] e.r. values determined by dividing the major peak area by the sum of the peak areas determined by SFC chromatography.

Cloning and site-directed mutagenesis. pET22b(+) was used as a cloning and expression vector for all enzymes described in this study. Site-directed mutagenesis on P411$_{BM3}$-CIS T438S to generate P411$_{BM3}$-CIS I263A T438S was performed using a modified QuickChange^{TM} mutagenesis protocol. The PCR products were gel purified, digested with DpnI, and directly transformed into *E. coli* strain BL21 (DE3).

Determination of P450 concentration. Concentration of P450/P411 enzymes was accomplished by quantifying the amount of free hemin present in purified protein using the pyridine/hemochrome assay.[2]

Protein expression and purification. Enzymes used in purified protein experiments were expressed in BL21(DE3) *E. coli* cultures transformed with plasmid encoding P450 or P411 variants. Expression and purification was performed as described elsewhere,[3] except that the shake rate was lowered to 130 RPM during expression. Following expression, cells were pelleted and frozen at -20 °C. For purification, frozen cells were resuspended in buffer A (20 mM tris, 20 mM imidazole, 100 mM NaCl, pH 7.5, 4 mL/g of cell wet weight) and disrupted by sonication (2 x 1 min, output control 5, 50% duty cycle; Sonicator 3000, Misonix, Inc.). To pellet insoluble material, lysates were centrifuged at 24,000 x *g* for 0.5 h at 4 °C. Proteins were expressed in a construct containing a 6x-His tag and were consequently purified using a nickel NTA column (5 mL HisTrap HP, GE Healthcare, Piscataway, NJ) using an AKTAxpress purifier FPLC system (GE healthcare). P450 or P411 enzymes were then eluted on a linear gradient from 100% buffer A 0% buffer B (20 mM tris, 300 mM imidazole, 100 mM NaCl, pH 7.5) to 100 % buffer B over 10 column volumes (P450/P411 enzymes elute at around 80 mM imidazole). Fractions containing P450 or 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 °C until later use.

Typical procedure for small-scale sulfimidation bioconversions under anaerobic conditions using purified enzymes. Small-scale reactions (400 μL) were conducted anaerobically in 2 mL crimp vials as described previously.[3] A solution of aryl sulfide in DMSO or methanol (100 mM, 10 μL) was added to the reaction vial via syringe, followed by arylsulfonyl azide (100 mM, 10 μL, DMSO). Final concentrations of the reagents were typically: 2.5 mM aryl sulfide, 2.5 mM arylsulfonyl azide, 10 mM NADPH, 25 mM glucose, 5-20 μM P450. To the vials were then added acetonitrile (460 μL) and internal standard (1,3,5 trimethoxybenzene, 10 mM in 10% DMSO/90% acetonitrile, 1 mM final concentration). This mixture was then transferred to a microcentrifuge tube, and centrifuged at 17,000 x *g* for 5 minutes. A portion (20 μL) of the supernatant was then analyzed by HPLC. Sulfimide formation was quantified by comparison of integrated peak areas of internal standard (1,3,5-trimethoxy benzene, 1 mM or 1,3,5-trichlorobenzene, 1 mM) and sulfimide at 220 nm to a calibration curve made using synthetically produced sulfimide and internal standard. Coefficients determined from standard curves were multiplied by a dilution factor in order to obtain sulfimide concentrations in the reaction mixture. Standard curves and response factors for products 8a-8d are presented in Figures S12-S15 below. For chiral HPLC, the quenched reaction mixture was extracted twice with ethyl acetate (2 x 350 μL), dried under a light argon stream and resuspended in acetonitrile (100 μL).

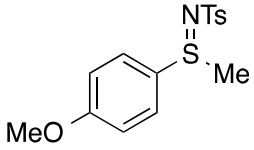
Controls to confirm the enzymatic sulfimidation activity of variant P411$_{BM3}$CIS T438S. Small-scale reactions (400 μL total volume) were set up and worked up as described above. For the reaction containing hemin as catalyst, 10 μL of a hemin solution (1 mM in 50% DMSO-H_2O) was added to a final concentration of 25 μM. TTNs were determined as described above

and are presented in Table S2. CS denotes 'complete system' in which all components of the reactions as described above are present. Variations from the complete system are denoted with a "- X" where X is the component removed.

Synthesis of substrates and standards

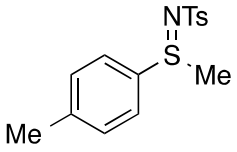
All sulfides presented in Table 2 were obtained from commercial sources (Sigma Aldrich, Alfa Aesar). Sulfimide standards were synthesized as previously reported.[1]

8a



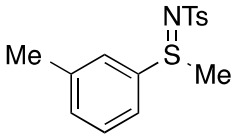
^{1}H NMR (500 MHz; $CDCl_3$): δ = 7.71 (d, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 8.9 Hz, 2H), 7.15 (d, *J* = 8.35 Hz, 2H), 6.96 (d, *J* = 8.9 Hz, 2H), 3.83 (s, 3H), 2.81 (s, 3H), 2.34 (s, 3H)

8b



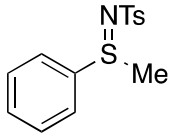
^{1}H NMR (500 MHz; $CDCl_3$): δ = 7.72 (d, *J* = 8.2 Hz, 2H), 7.57 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 7.3 Hz, 2H), 7.16 (d, *J* = 8.1 Hz, 2H), 2.82 (s, 3H), 2.39 (s, 3H), 2.35 (s, 3H)

8c



^{1}H NMR (500 MHz; $CDCl_3$): δ = 7.73 (d, *J* = 8.0 Hz, 2H), 7.48-7.33 (m, 4H), 7.17 (d, *J* = 8.1 Hz, 2H), 2.82 (s, 3H), 2.36 (s, 3H), 2.35 (s, 3H)

8d



^{1}H NMR (500 MHz; $CDCl_3$): δ = 7.72 (d, *J* = 7.8 Hz, 2H), 7.55-7.46 (m, 5H), 7.16 (d, *J* = 8.1 Hz, 2H), 2.83 (s, 3H), 2.34 (s, 3H)

The ^{1}H NMR listings above for products **8a-8d** matched those of characterized compounds.[4]

Determination of initial rates. Four 2-ml vials were charged with a stir bar, 10x oxygen depletion system[3] (40 μL), and a solution of enzyme prior to crimp sealing with a silicon septum. Once sealed, the headspace was flushed with argon for at least 10 minutes. Concurrently, a sealed 6-mL vial charged with glucose (250 mM, 400 μL), NADPH (20 mM, 400 μL), and KPi (pH = 8.0, 0.1 M, 2.6 mL) was sparged for 10 minutes with argon. After degassing was complete, 340 μL of the reaction solution was transferred to the 2-mL vial via syringe. Sulfide (100 mM, 10 μL) was added to all four 2-mL vials followed quickly (less than 20 seconds) by tosyl azide (100 mM, 10 μL). The reactions were quenched at 1-2 minute intervals over 5-10

minutes by decapping and adding acetonitrile (460 μL). After 5 minutes of stirring, the vials were charged with internal standard and the reaction mixtures were transferred to 1.8 mL tubes, which were vortexed and centrifuged (14,000 x *g*, 5 min). The supernatant was transferred to a vial for analysis by HPLC. Initial rates are plotted for individual enzymes referenced in the text in Figure S2, and for various substituted aryl sulfides in Figure S7.

Visible absorbance spectroscopy and observation of resting states. To a semi-micro anaerobic cuvette, 8 μL of P411$_{BM3}$CIS I263A T438S (400 μM) was added. To obtain a spectrum of the ferric protein, 0.5 mL of degassed phosphate buffer was added to the cuvette and the visible spectrum was recorded from 650 to 400 nm. To obtain a spectrum of the ferrous protein, the cuvette was sealed with a cap equipped with rubber septa and the headspace of the cuvette was purged with a gentle stream of Ar for 3 min. A solution of NADPH (5 mM) was added to a 6 mL crimp vial and made anaerobic by sparging with Ar for 5 min. The NADPH solution (0.5 mL) was then added to the anaerobic cuvette containing protein. Visible spectra of the protein sample are recorded until a stable ferrous state is reached. Representative spectra of the Fe(III)- and Fe(II)-protein are shown below (Figure S8 green and red lines, respectively).

To determine the resting state of the protein in the unproductive catalytic cycle, a degassed solution of tosyl azide (2 μL, 400 mM in DMSO) was added to a fully reduced sample of ferrous protein. The visible spectrum of the protein shifted to the ferric heme immediately and remained unchanged for 20 min. Addition of an aliquot of organic solvent of similar volume did not cause the observed change in iron oxidation state. At the end of 20 minutes (Figure S8, blue line), the cuvette was uncapped and the reaction mixture was worked up following the general procedure

for small scale reactions. HPLC of the resulting solution confirmed that a substantial amount of azide is reduced to the corresponding sulfonamide.

To determine the resting state of the protein during the sulfimidation reaction, a degassed solution of sulfide **7a** (2 μL, 400 mM in DMSO) was added to the cuvette containing $P411_{BM3}$CIS I263A T438S in the presence of NADPH. A visible absorbance spectrum of the mixture was recorded to ensure that the oxidation state of iron heme is unchanged. Next, a degassed solution of tosyl azide (2 μL, 400 mM in DMSO) was added to the cuvette. Visible absorbance spectra of the solution were recorded at 5, 7, 12, 18, 22, 25 and 30 min (Figure S9). The appearance of ferrous heme is observed over time. HPLC confirmed formation of both sulfimide and sulfonamide.

Excess sulfide and azide slow addition experiments. To assess the impact of sulfide concentration on overall productivity of reaction, sulfide was added to reaction ranging from 0.5 eq to 4 eq relative to azide. 1 eq of azide denotes 1 mM in the small scale reactions described above. Results are plotted in Figure S10 below as a ratio of the TTN for sulfimide vs. TTN for sulfonamide. Slow addition was accomplished by adding 1 μL of a 100 mM (100 nmol) tosyl azide solution (DMSO) at 15 minute intervals to a reaction set up as described previously with 0.4% catalyst loading, containing 2.5 mM **7a**. Azide was added over 150 minutes until equimolar final concentrations of sulfide and azide were achieved. Results of the slow addition are presented in Figure S11.

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

Enzyme	Mutations relative to wild-type $P450_{BM3}$
$P450_{BM3}$	none
$P450_{BM3}$-T268A	T268A
$P411_{BM3}$	C400S
$P411_{BM3}$-T268A	T268A, C400S
$P450_{BM3}$-CIS T438S	V78A, F87V, P142S, T175I, A184V, S226R, H236Q, E252G, A290V, L353V, I366V, T438S, E442K
$P411_{BM3}$-CIS T438S	P450BM3-CIS C400S, T438S
$P411_{BM3}$-CIS A268T T438S	P450BM3-CIS, A268T, C400S, T438S
$P411_{BM3}$ H2-A-10	P450BM3-CIS, L75A, L181A, C400S,
$P411_{BM3}$ H2-5-F10	P450BM3-CIS, L75A, I263A, C400S, L437A
$P411_{BM3}$ H2-4-D4	P450BM3-CIS, L75A, M177A, L181A, C400S, L437A, T438S

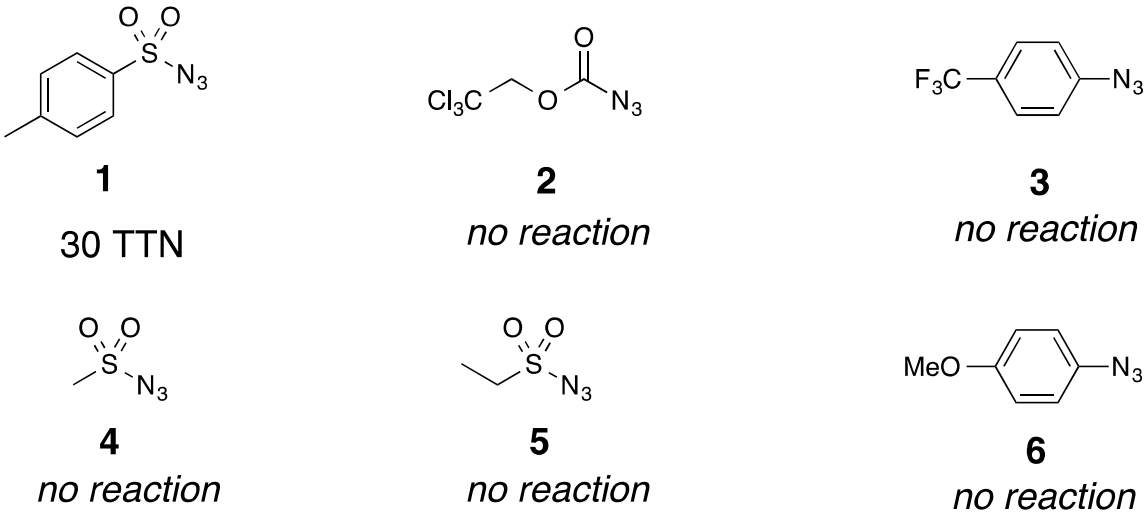
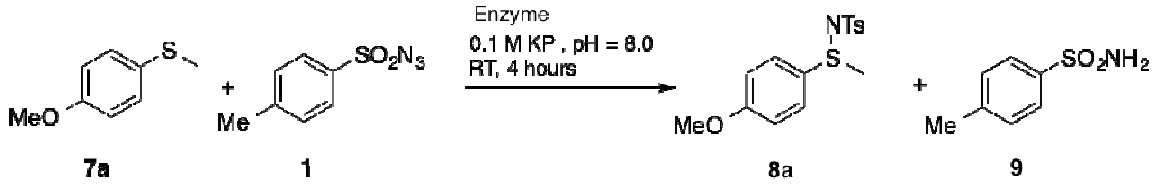


Figure S1. Azides tested as nitrene sources for sulfimidation of thioanisole using the $P411_{BM3}$-CIS T438S enzyme. TTN = total turnover number.

Table S2. Control experiments using substrate **7c** yielding products **8c** (sulfimide) and **9** (sulfonamide). CS = complete system.

Condition	TTN 8c	TTN 9
$P411_{BM3}$-CIS T438S (CS)	90	300
CS - NADPH	1	2
CS + $Na_2S_2O_4$ + CO	2	40
CS boiled enzyme	1	53
CS aerobic	2.3	16
Hemin + $Na_2S_2O_4$	0	42
CS - $P411_{BM3}$-CIS T438S	0	21



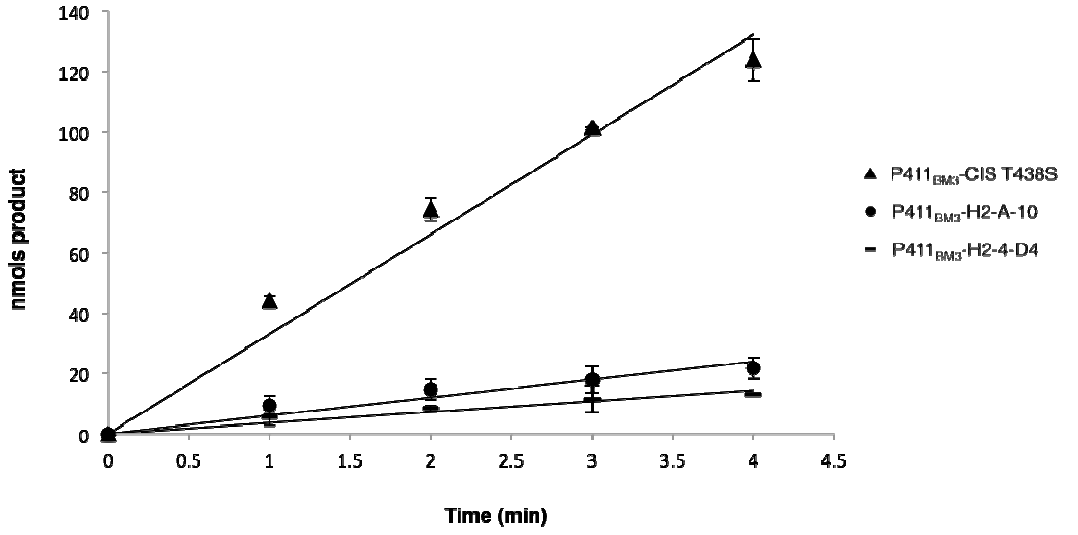


Figure S2. Data used to determine initial rates of the reaction depicted in scheme above using the enzymes listed in figure legend.

Table S3. Initial rates determined from data presented in Figure S2 and TTNs presented in Table 1.

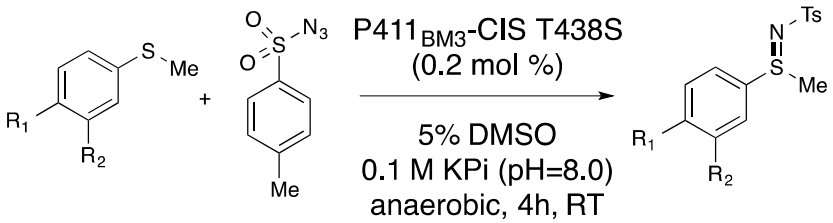
Enzyme	Rate (nmol min^{-1})	TTN
$P411_{BM3}$-CIS T438S	33	300
$P411_{BM3}$-H2-A-10	3.5	32
$P411_{BM3}$-H2-4-D4	5.9	84

Table S4. Qualitative description of reaction productivity using para-methoxy aryl sulfide **7a** and azides shown in Figure S1.

Azide	Productivity [a]
1	+++
2	+
3	nd [b]
5	+
6	nd

[a] + denotes relative amount of product, where +++ is the most productive reaction. See Figures S26 and S27 for LC-MS verification of product formation for azides **2** and **4**. [b] nd = none detected.

Table S5. Enantioselectivity of products **8a**–**8d** with $P411_{BM3}$-CIS T438S enzyme.



7a-d **1** **8a-d**

Product	e.r.	R1	R2
8a	74:26	-OMe	-H
8b	80:20	-Me	-H
8c	59:41	-H	-Me
8d	87:13	-H	-H

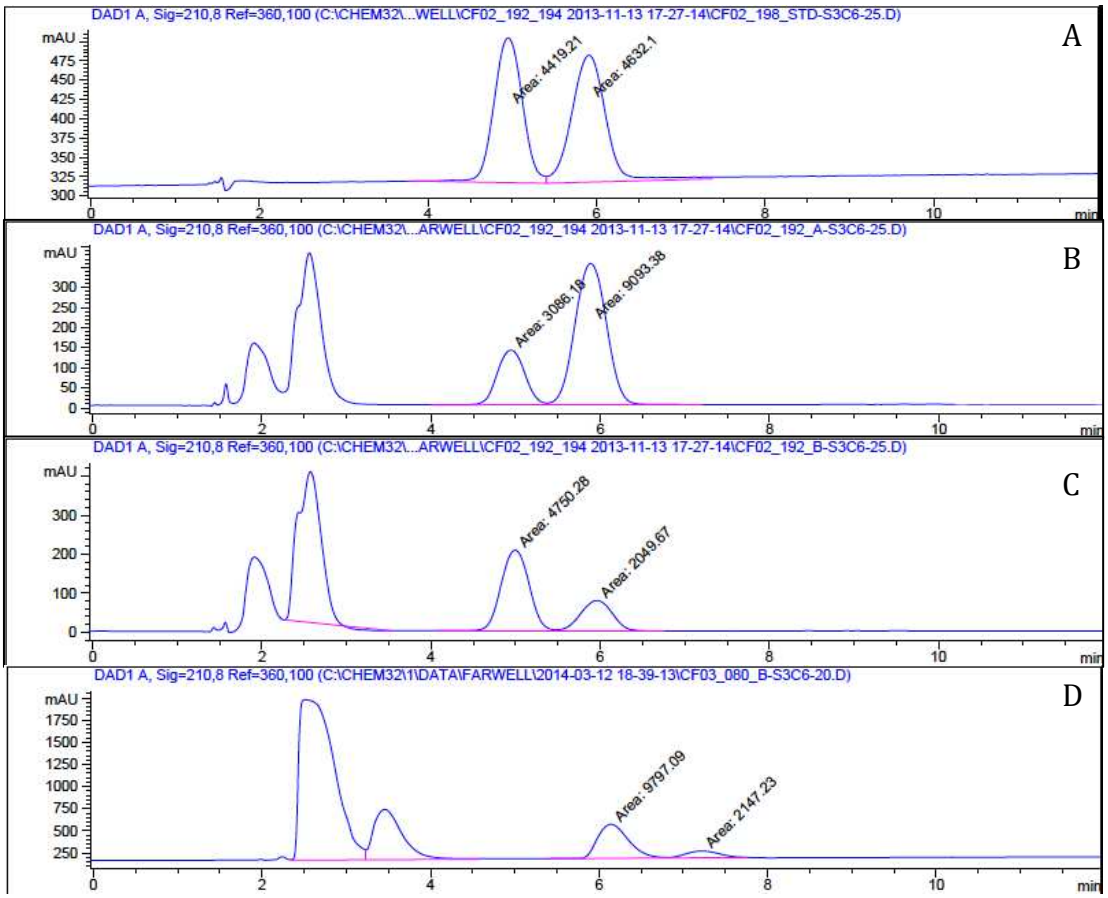


Figure S3. A) SFC trace of synthetic standard of **8a** using Chiralpak OD-H column with 25% isopropanol/75% supercritical CO_2 mobile phase. B) Trace of $P411_{BM3}$-CIS T438S produced **8a** under same conditions as synthetic standard. C) Trace of $P411_{BM3}$-H2-5-F10 produced **8a**. D) Trace of $P411_{BM3}$-CIS I263A T438S produced **8a**, using 20 % isopropanol/80% supercritical CO_2 mobile phase.

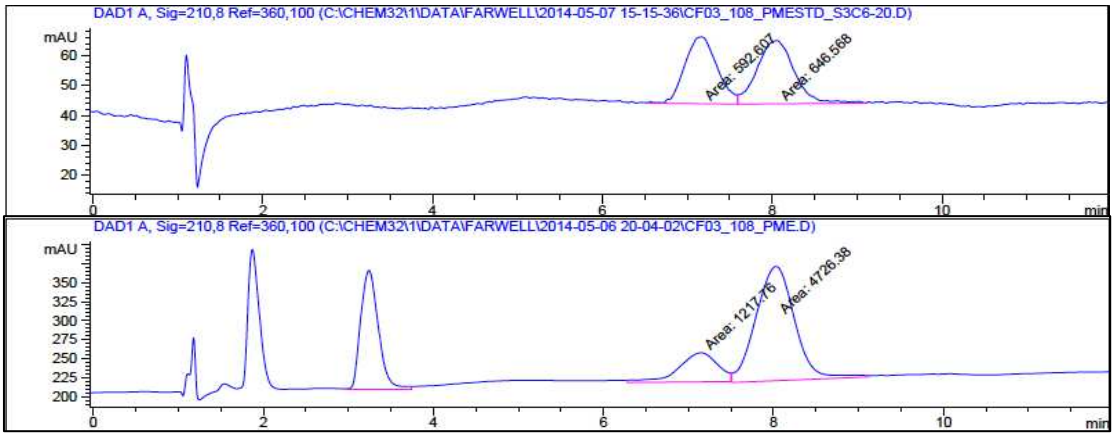


Figure S4. Top: SFC trace of **8b** synthetic standard using Chiralpak OD-H column with 20% isopropanol/80% supercritical CO_2 mobile phase. Bottom: Trace of enzyme produced **8b.**

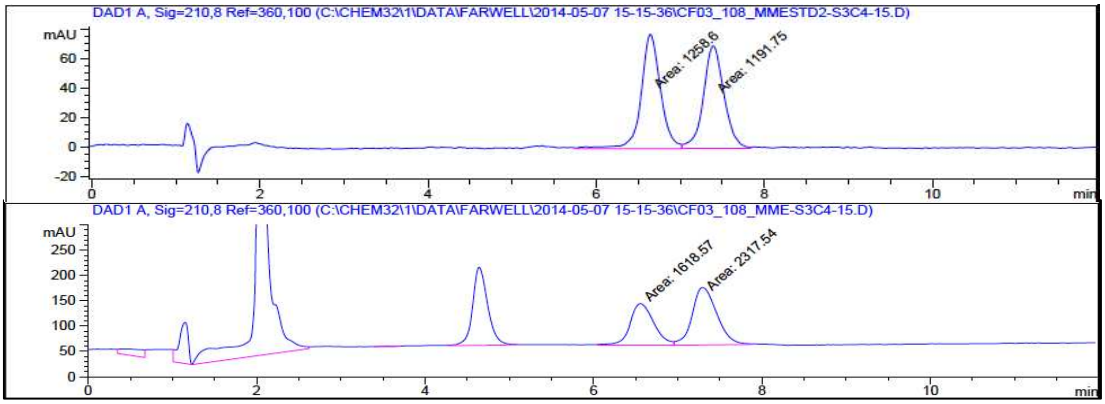


Figure S5. Top: SFC trace of **8c** synthetic standard using Chiralpak OJ column with 15% isopropanol/85% supercritical CO_2 mobile phase. Bottom: Trace of enzyme produced **8c**.

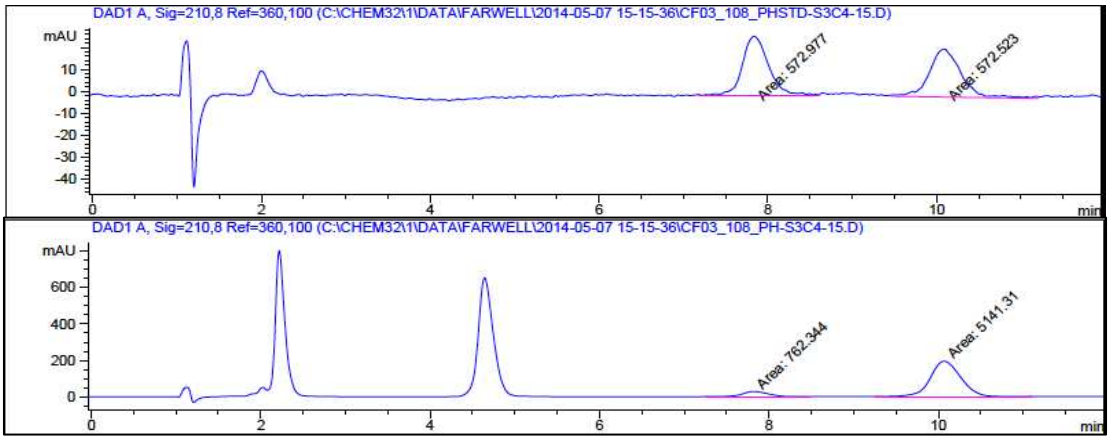


Figure S6. Top: SFC trace of **8d** synthetic standard using Chiralpak OJ column with 15% isopropanol/85% supercritical CO_2 mobile phase. Bottom: Trace of enzyme produced **8d**.

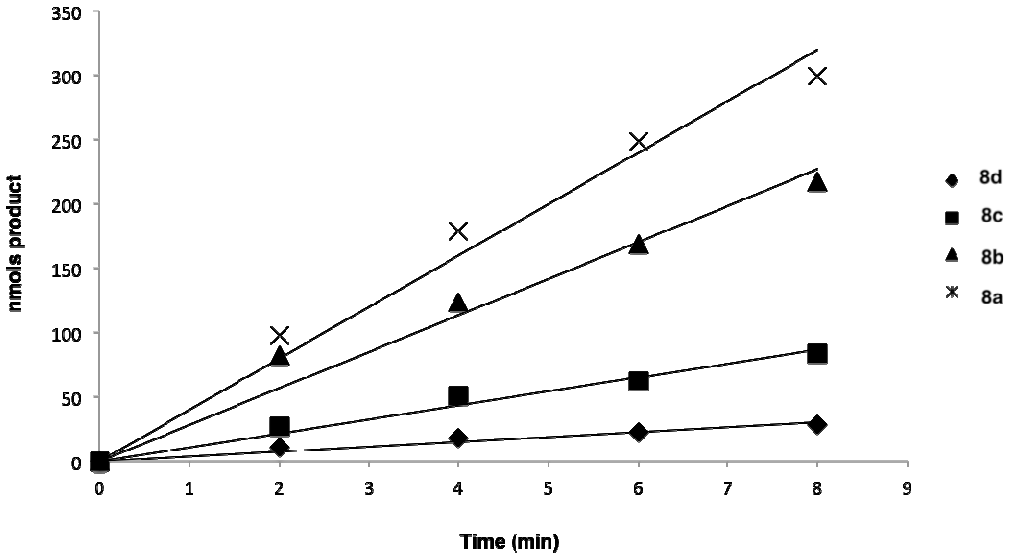


Figure S7. Data used to determine initial rates for reaction of substituted aryl sulfides with tosyl azide, using P411$_{BM3}$-CIS T438S as catalyst. Sulfimide products measured are as listed in Table 2 of the text.

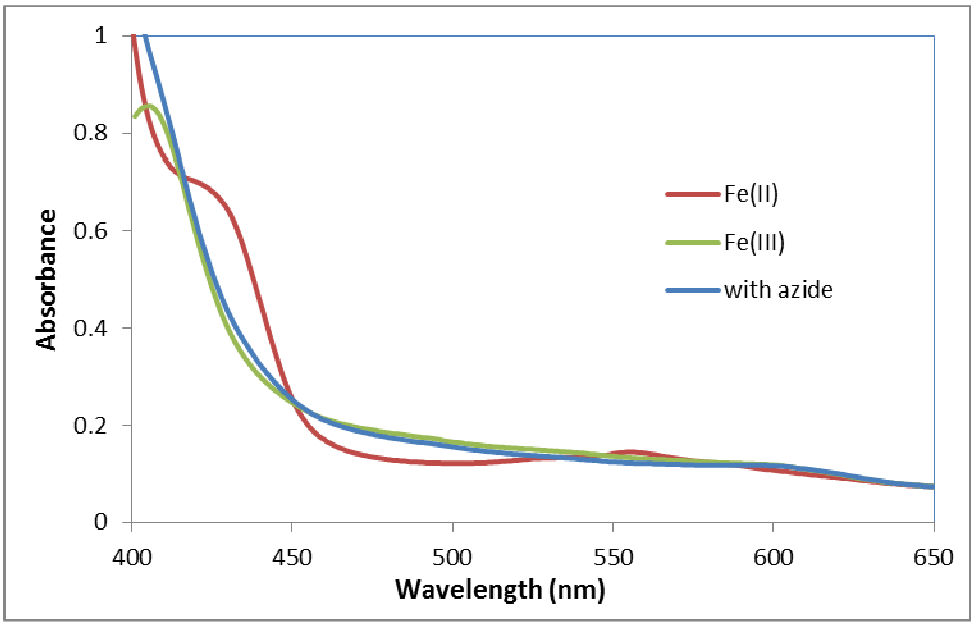


Figure S8. Visible spectroscopy of Fe(III) (green) and Fe(II)-P411$_{BM3}$CIS I263A T438S in the presence of NADPH (red), followed by azide addition (blue).

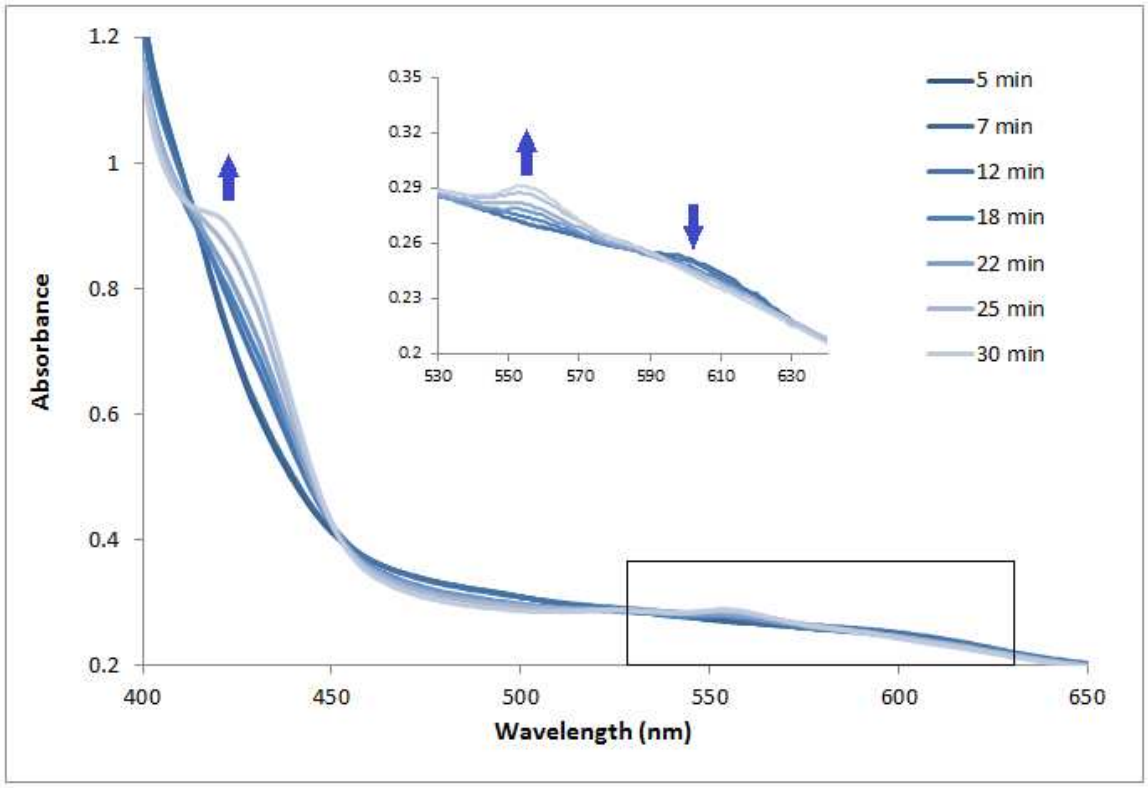
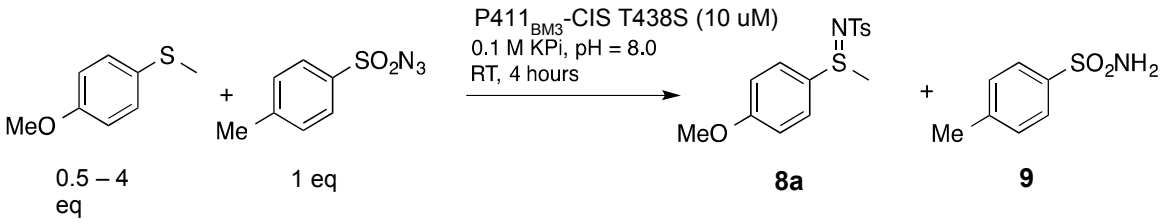


Figure S9. Monitoring the iron heme during the sulfimidation reaction with azide and sulfide. As shown in Figure S8, the Soret peak for the Fe(III) state is obscured by NADPH. Therefore the Q-band region (enlarged box from 530-640 nm) was used to assess the transition from Fe(III) to Fe(II) as reaction proceeds as described in the supplemental text.



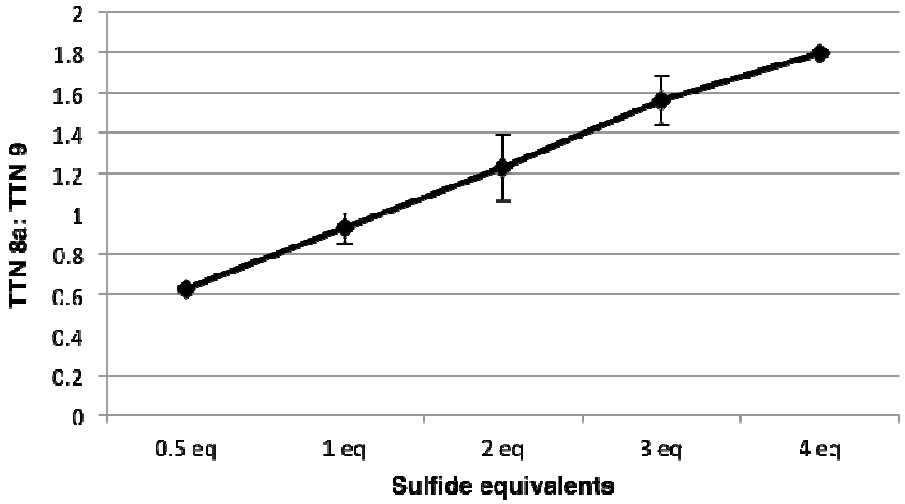


Figure S10. Ratio of sulfimide TTN to sulfonamide TTN as sulfide concentration is varied, using P411$_{BM3}$-CIS T438S enzyme, as shown in scheme. Reactions were assembled as described above, with azide concentration held constant at 1 mM and sulfide concentration varied from 0.5 mM to 4 mM.

Table S6: Production of sulfimide **8a** and sulfonamide **9** in the presence of varying levels of sulfide acceptor substrate, as described above.

Sulfide	Azide	TTN 8a	TTN 9
0.5eq	1 eq	71	110
1 eq	1 eq	97	100
2 eq	1 eq	110	93
3 eq	1 eq	130	85
4 eq	1 eq	140	80

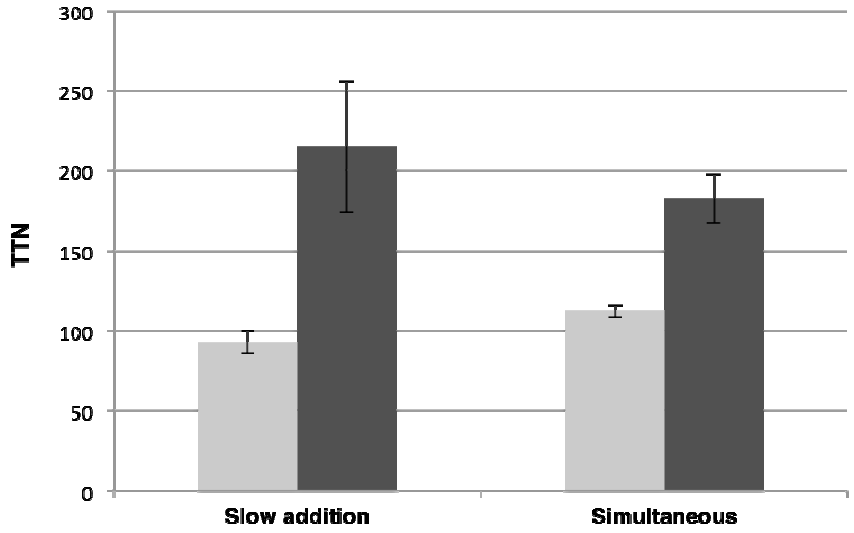


Figure S11. TTN values measured for slow addition vs. adding substrates simultaneously, using P411BM3-CIS T438S enzyme and the substrates shown in Figure S10. Light gray bar shows TTN for sulfonamide, **9**, dark gray shows TTN for the sulfimide, **8a**.

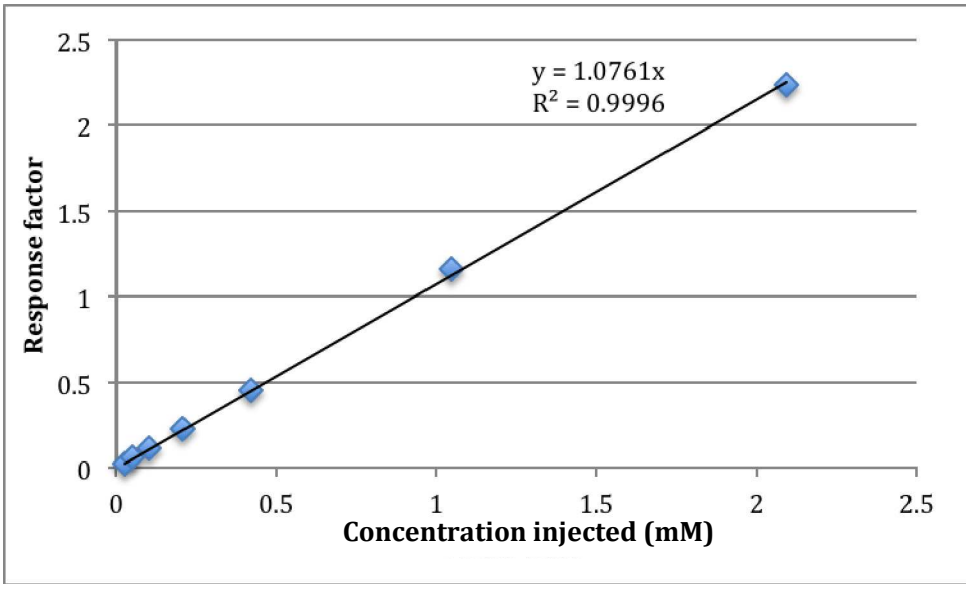


Figure S12: Standard curve for product **8a**, showing response factor as ratio of product area to internal standard area as described above (y-axis) and concentration of product injected (x-axis).

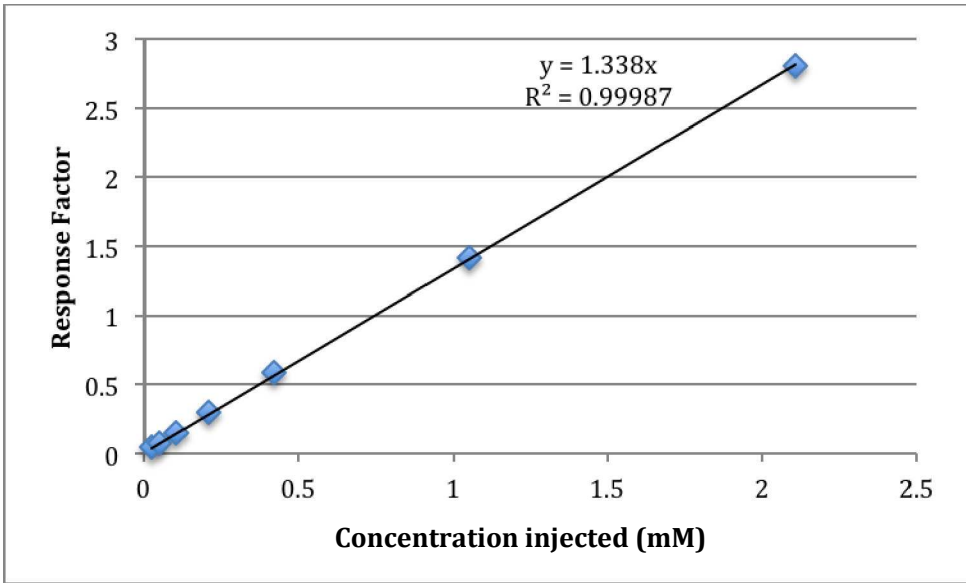


Figure S13. Standard curve for product **8b**.

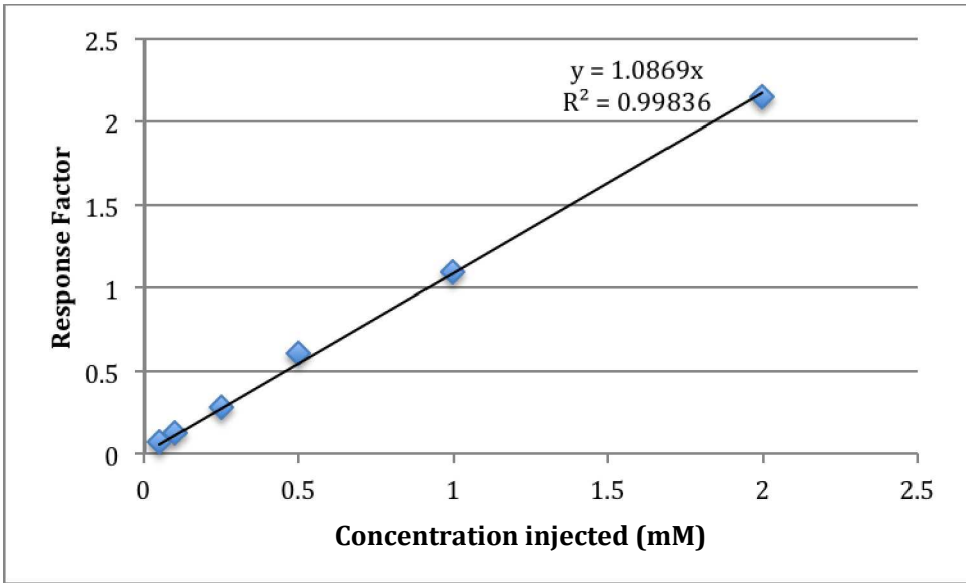


Figure S14. Standard curve for product **8c**.

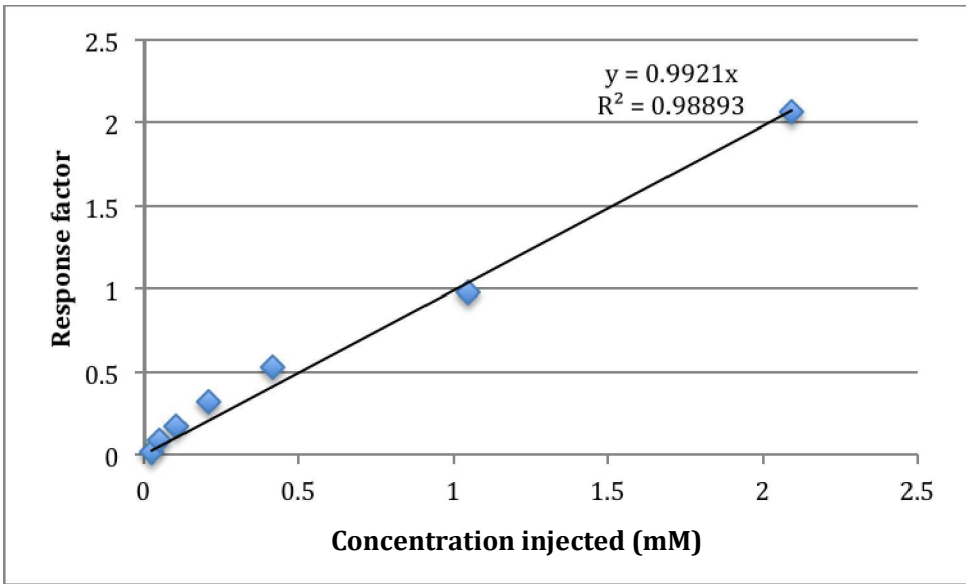


Figure S15. Standard curve for product **8d**.

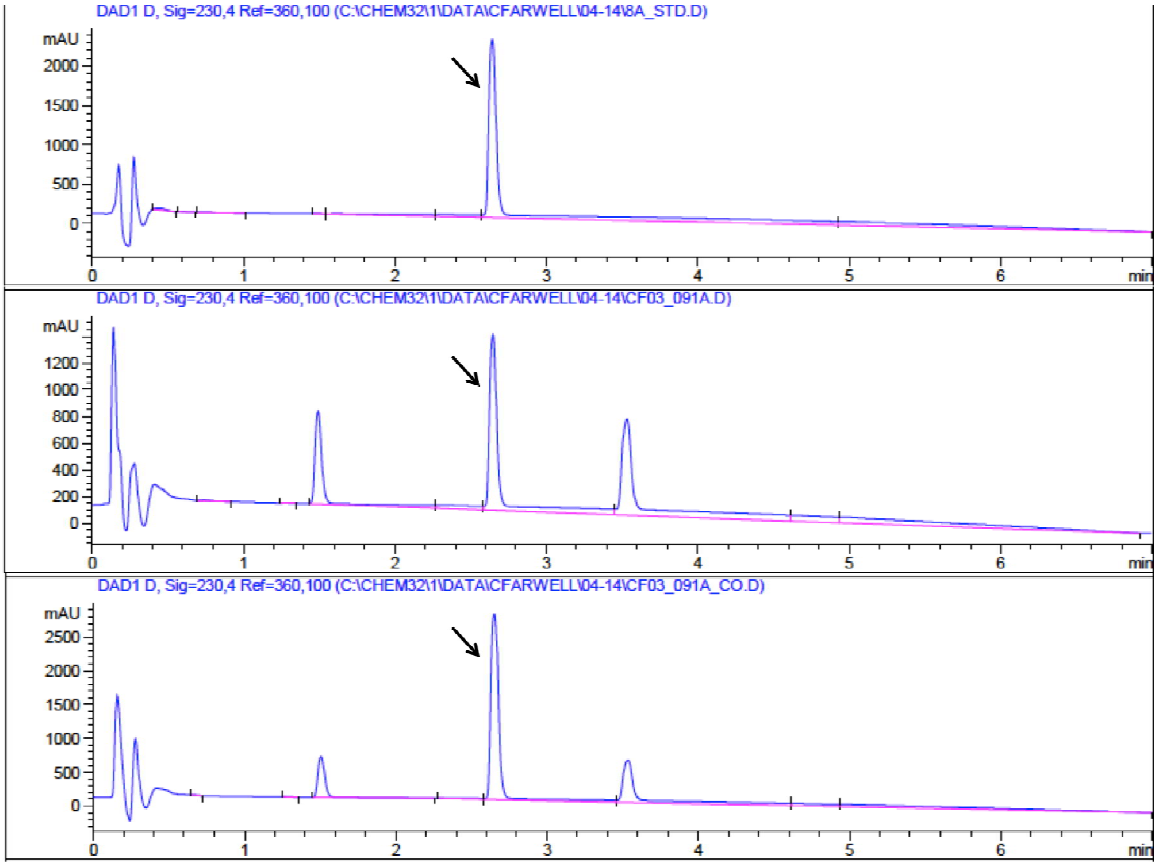


Figure S16. Demonstration of enzymatic production of 8a
Top: LC-MS 230 nm chromatogram of synthetic standard of **8a**, confirmed by NMR. Middle: Enzyme reaction containing putative **8a**. Bottom: Mixture of enzyme reaction and synthetic **8a**, showing coelution.

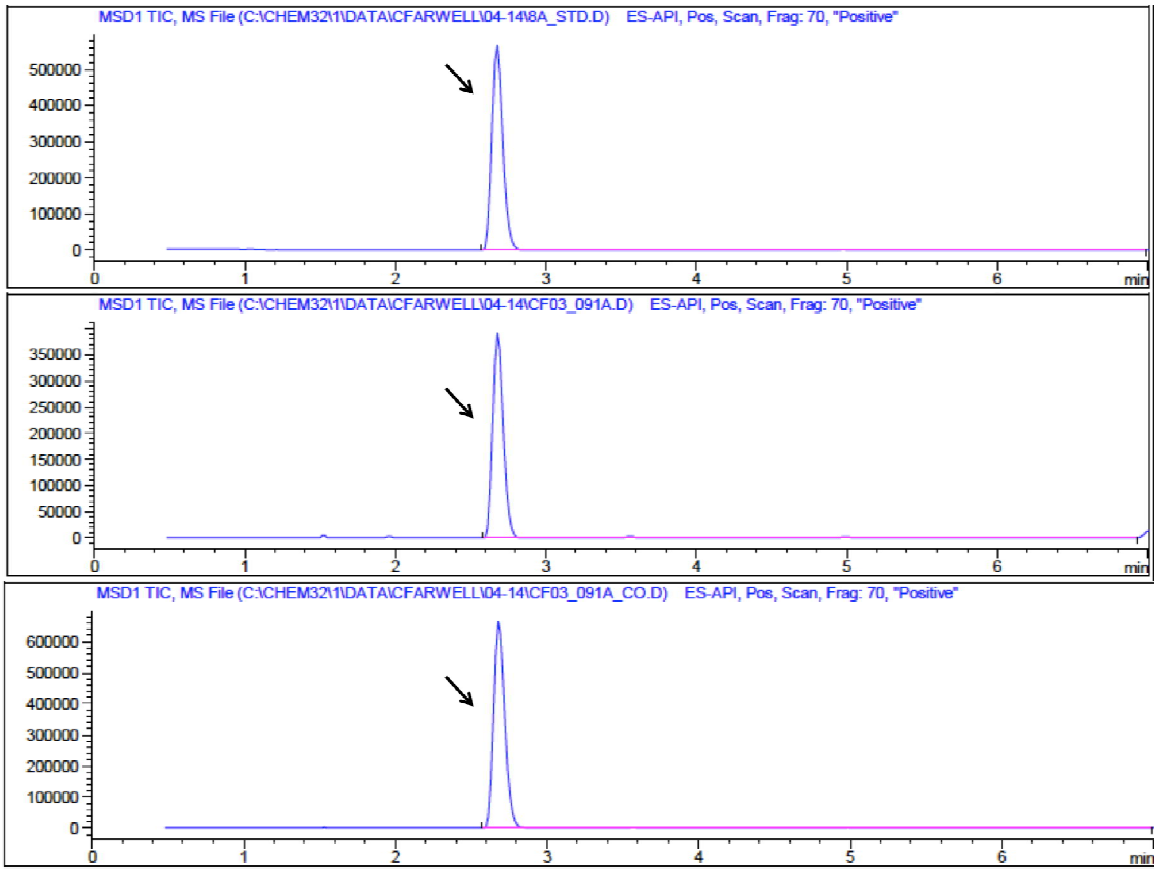


Figure S17. LC runs from figure S16 showing ESI-MS-(+) detection of total ion chromatogram (TIC) (major peak = 324, corresponding to **8a** M+H+).

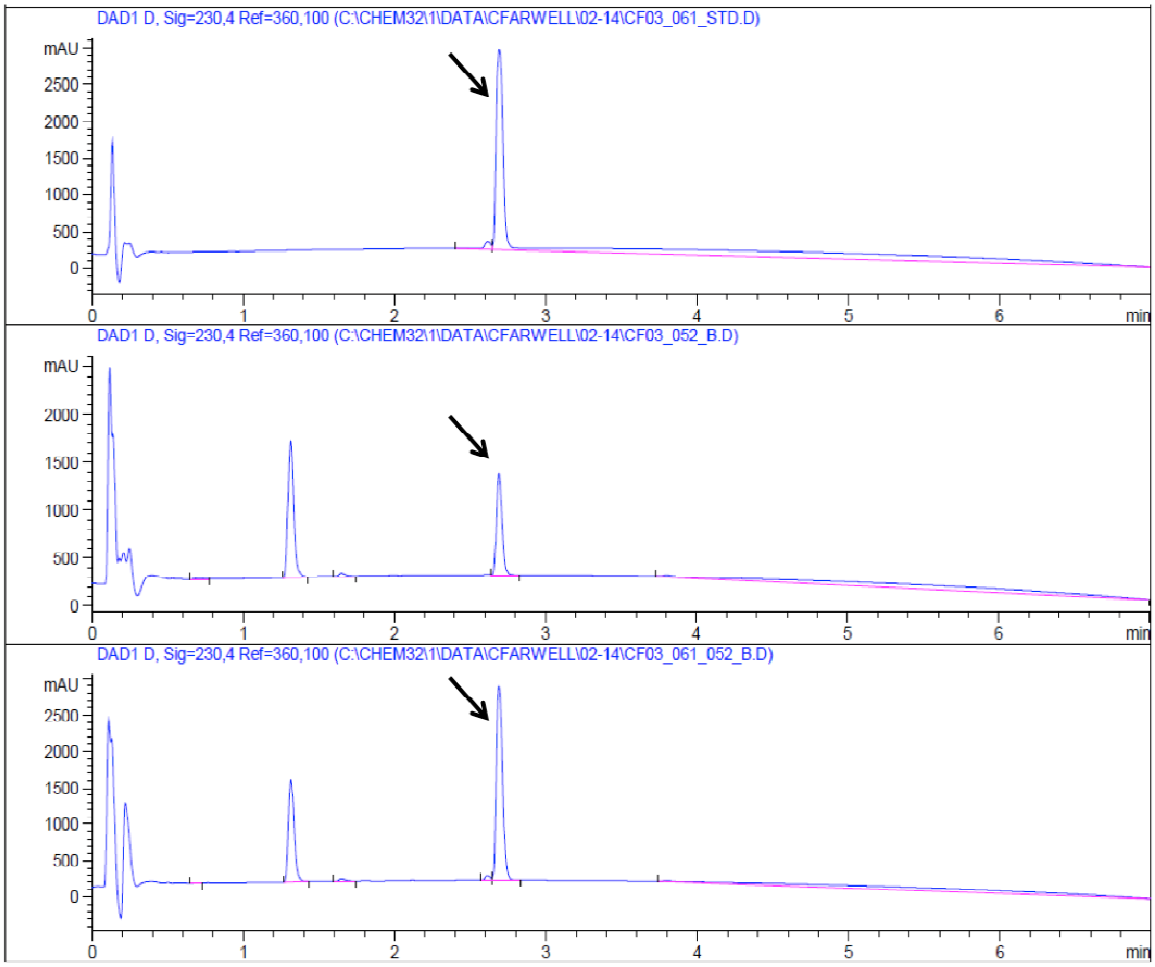


Figure S18. Demonstration of enzymatic production of 8b
Top: LC-MS 230 nm chromatogram of synthetic standard of **8b**, confirmed by NMR. Middle: Enzyme reaction containing putative **8b**. Bottom: Mixture of enzyme reaction and synthetic **8b**, showing coelution.

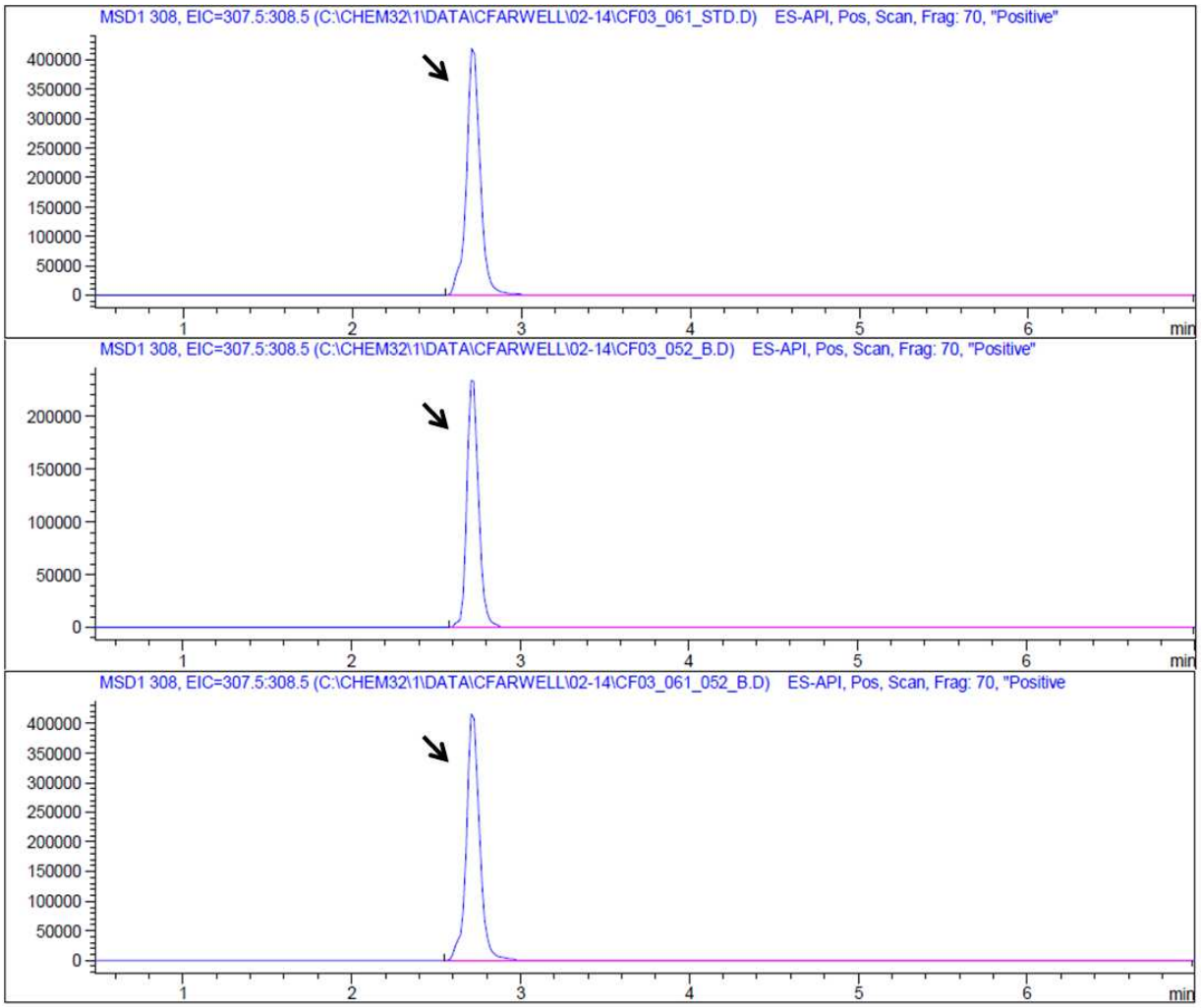


Figure S19. LC runs from figure S18 showing ESI-MS-(+) detection of selected ions (mass window 307.5-308.5).

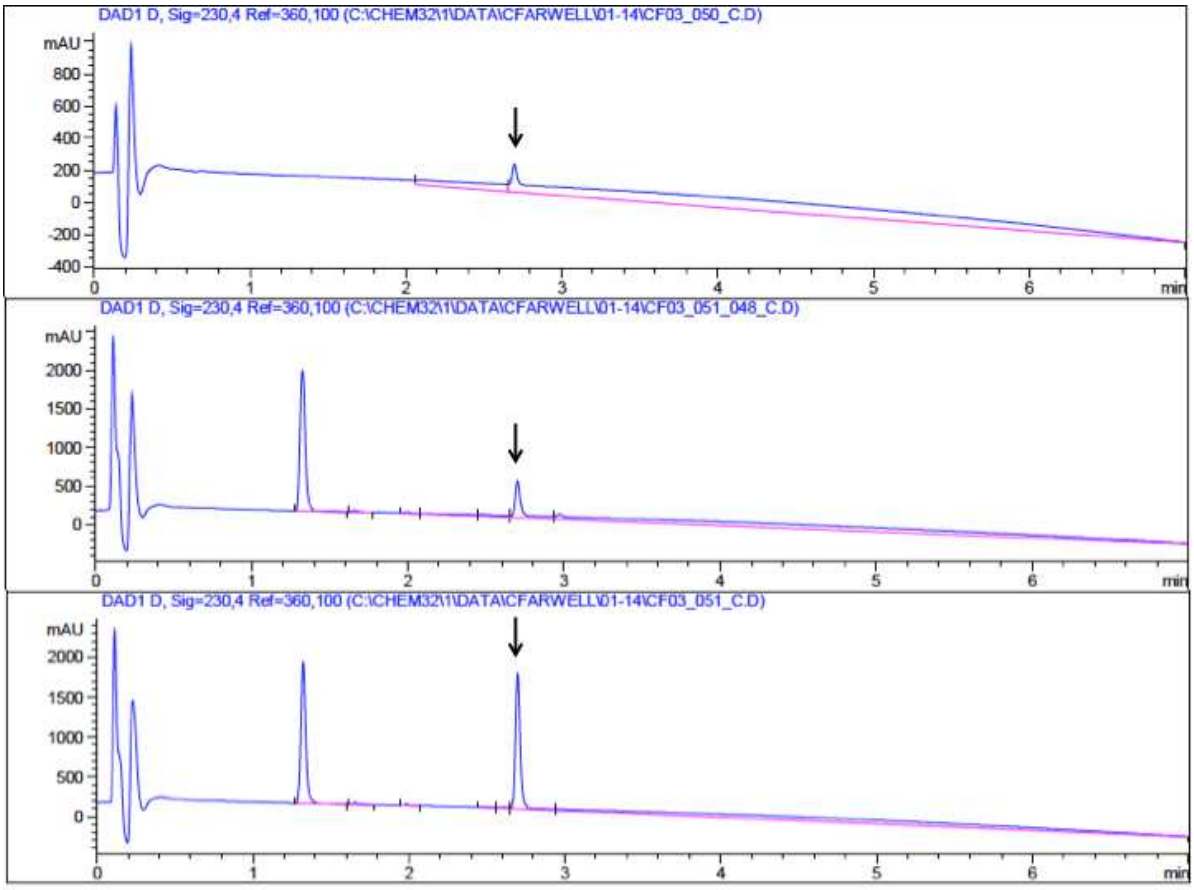


Figure S20. Demonstration of enzymatic production of 8c.
Top: LC-MS 230 nm chromatogram of synthetic standard of **8c**, confirmed by NMR. Middle: Enzyme reaction containing putative **8c**. Bottom: Mixture of enzyme reaction and synthetic **8c**, showing coelution.

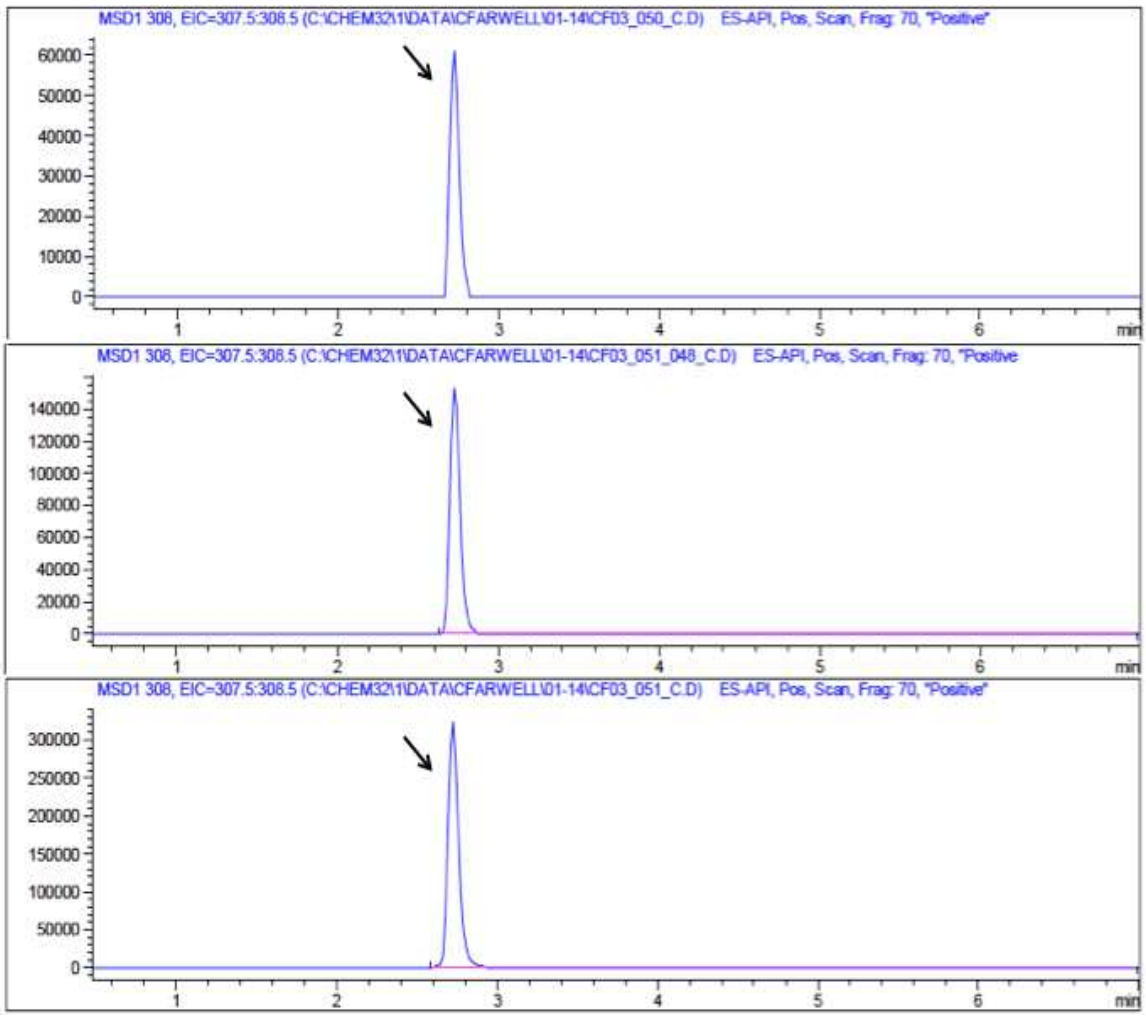


Figure S21. LC runs from figure S20 showing ESI-MS-(+) detection of selected ions (mass window 307.5-308.5).

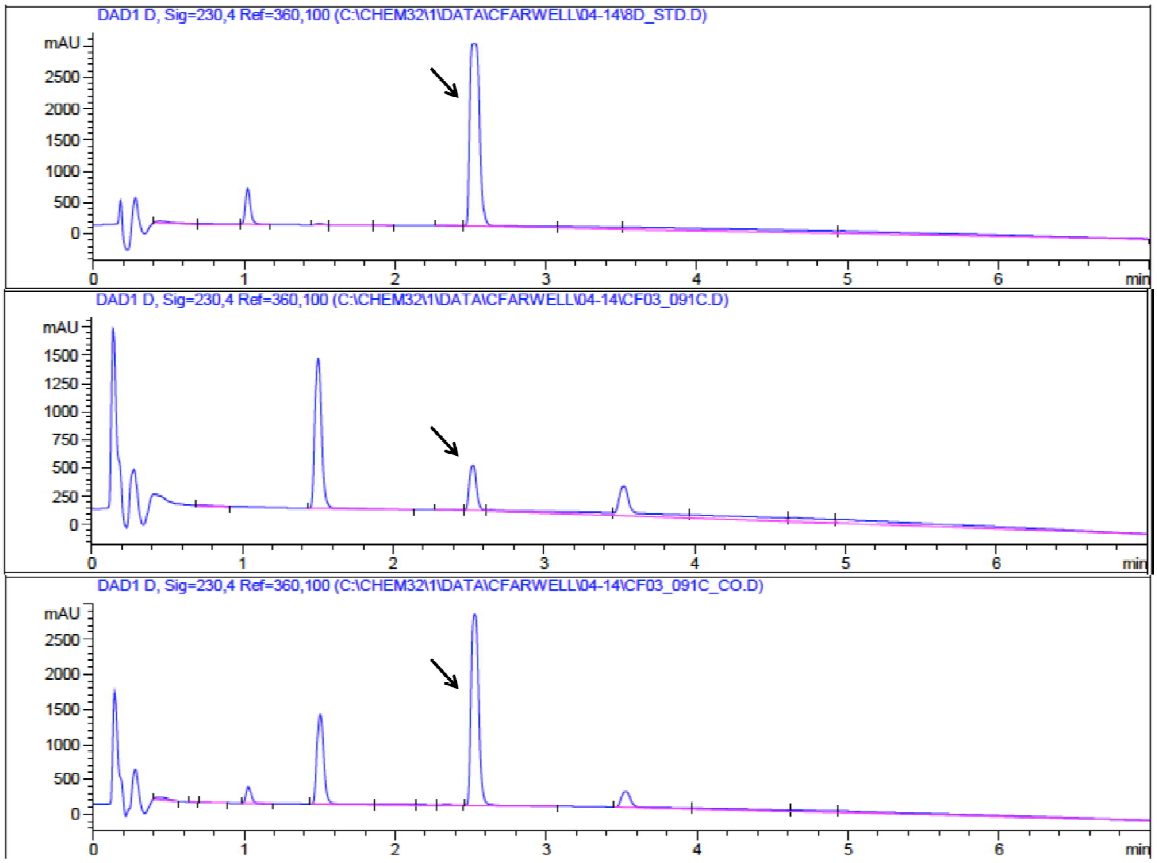


Figure S22. Demonstration of enzymatic production of 8d
Top: LC-MS 230 nm chromatogram of synthetic standard of **8d**, confirmed by NMR. Middle: Enzyme reaction containing putative **8d**. Bottom: mixture of enzyme reaction and synthetic **8d**, showing coelution.

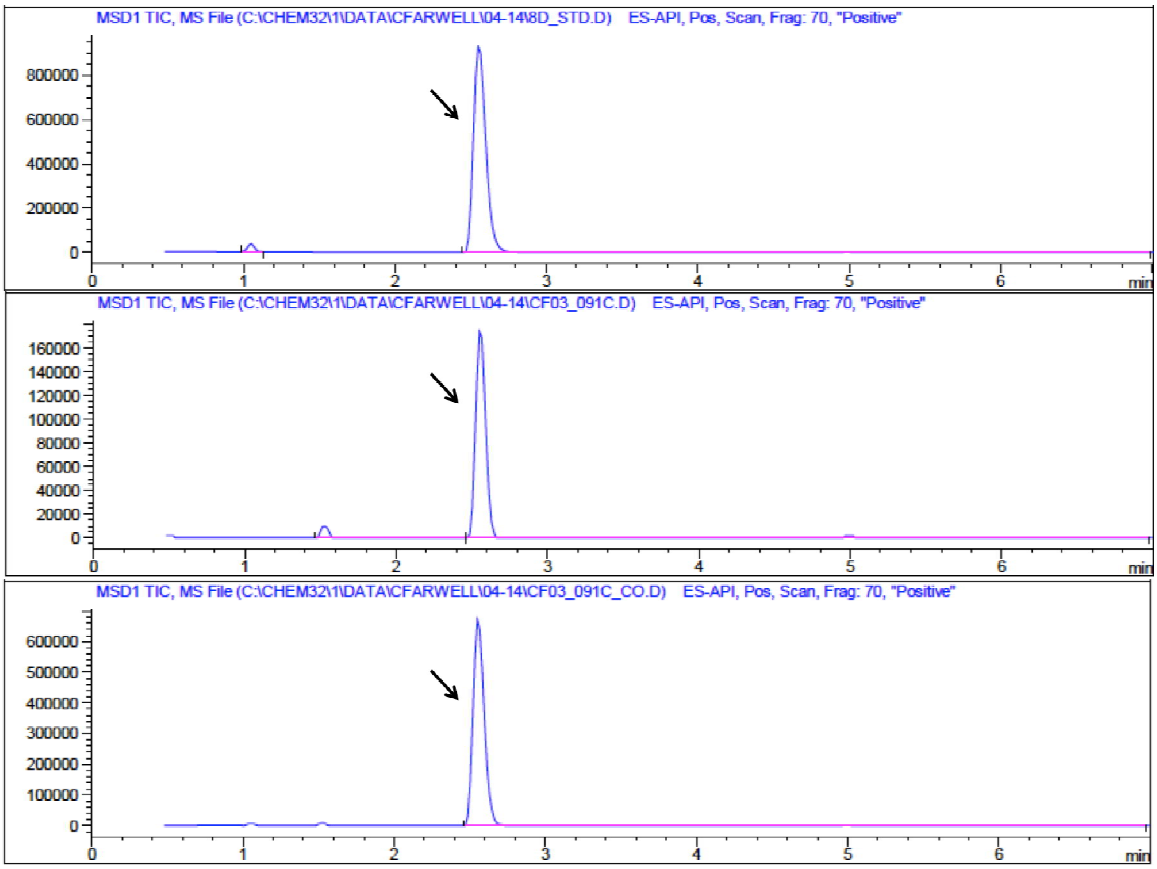


Figure S23. LC runs from figure S22 showing ESI-MS-(+) detection of total ion chromatogram (major mass peak = 294 m/z corresponding to **8d** M+H+).

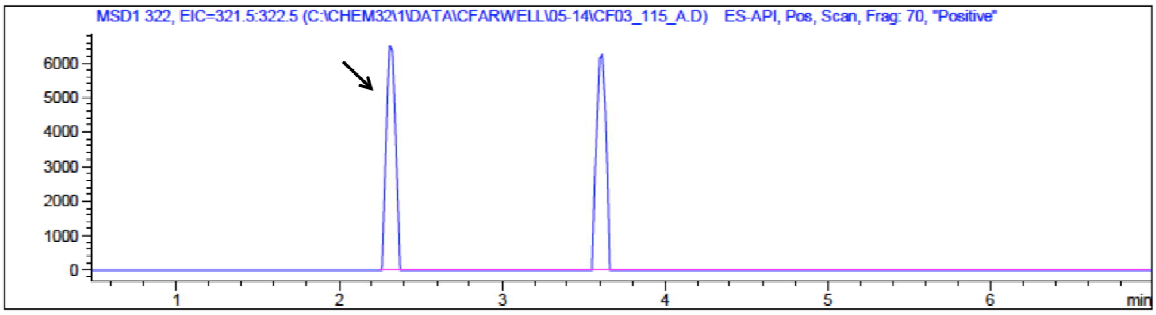


Figure S24. LC runs from reaction with P411$_{BM3}$-CIS T438S with substrate **7e** showing ESI-MS-(+) detection of selected ions (mass window 321.5 – 322.5 m/z), demonstrating production of **8e**.

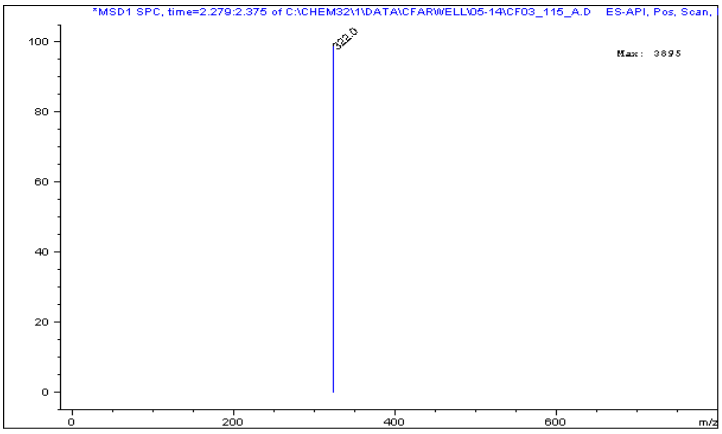


Figure S25. Mass spectrum of peak identified in figure S24, showing M+H+ of product **8e**.

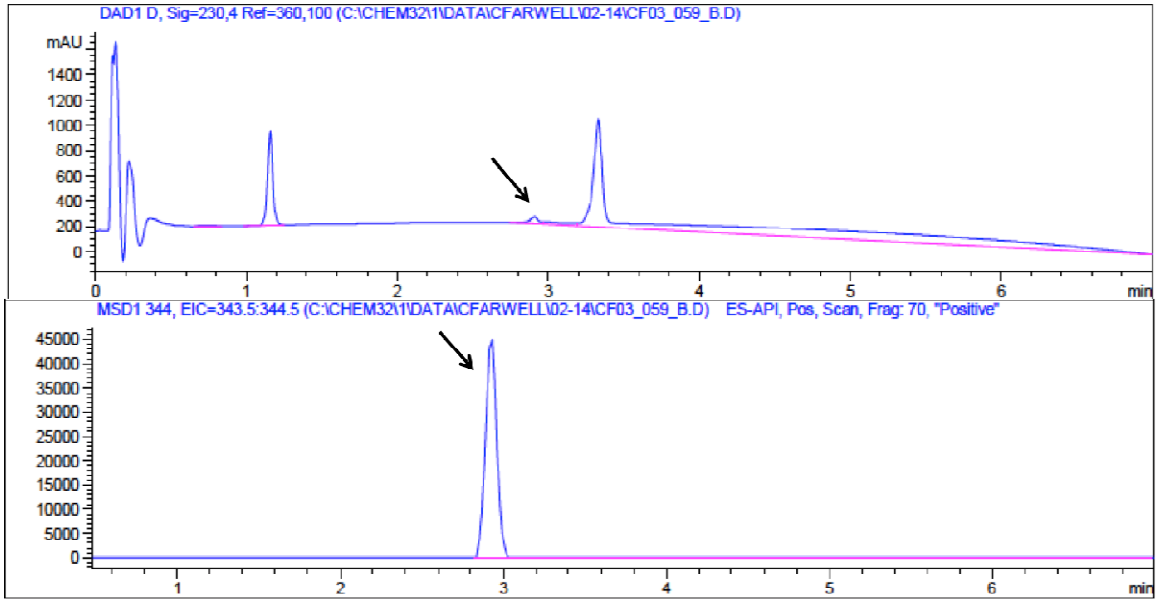


Figure S26. LC-MS chromatogram showing UV trace at 230 nm (top) and selected ions for 344 m/z , corresponding to the M+H+ mass of the product of azide **2** with sulfide **7a**. Note TIC timescale differs from due to instrument solvent delay.

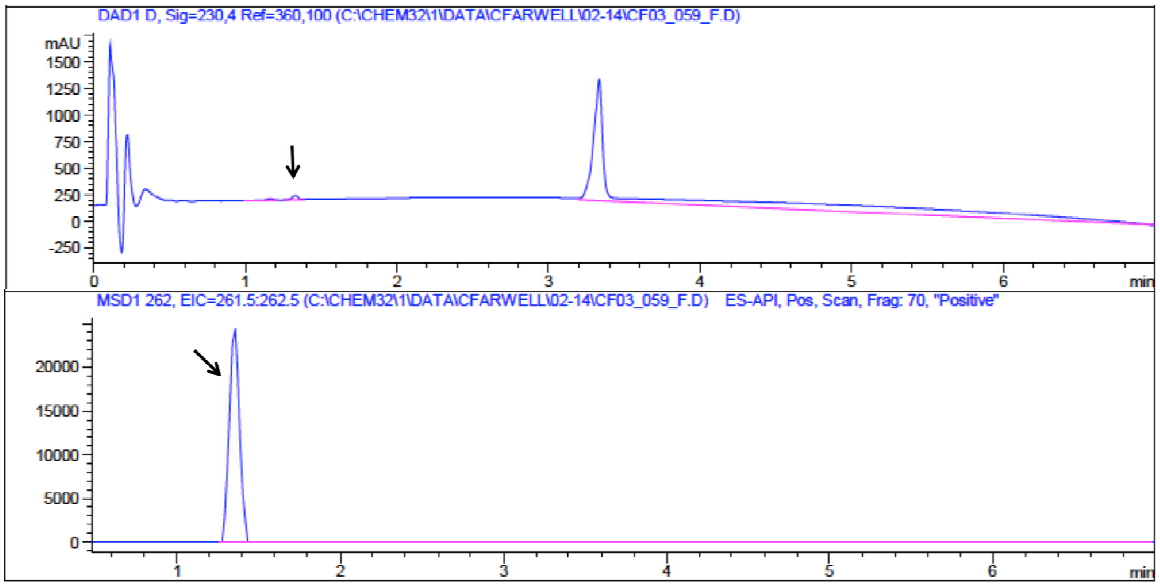


Figure S27. LC-MS chromatogram showing UV trace at 230 nm (top) and selected ions for 262 m/z, corresponding to the M+H+ mass of the product of azide **4** with sulfide **7a**. Note TIC timescale differs from chromatogram due to instrument solvent delay.

References

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