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Supporting Information

Artificial Metalloenzymes based on TetR Proteins and Cu(II) for Enantioselective Friedel-Crafts Alkylation Reactions

Cora Gutiérrez de Souza, Manuela Bersellini, and Gerard Roelfes*© 2020 The Authors.
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1. Materials and methods

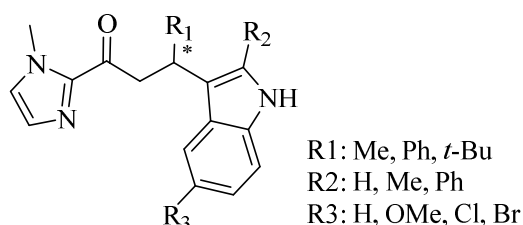
All the chemicals and reagents were purchased from commercial suppliers (Sigma Aldrich or Acros) and used without further purification. Flash chromatography was performed using Reveleris® X2 Flash Chromatography System. ¹H-NMR and ¹³C-NMR spectra were recorded on a Varian 400 (400 MHz) spectrometer in CDCl₃. Chemical shifts values (δ) are denoted in ppm using residual solvent peaks as the internal standard (CHCl₃: δ 7.26 for 1H). ¹H-NMR results are reported in the conventional form: chemical shifts, multiplicity (br = broad, d = doublet, dd = doublet of doublet, ddd = doublet of doublet of doublets, dq=doublet of quartets, m=multiplet, q=quartet, s=singlet, t=triplet), coupling constants (Hz), and integration. Data for ¹³C-NMR are reported in terms of chemical shift (δ ppm) and multiplicity. Enantiomeric excess determinations were performed by HPLC analysis using UV-detection (Shimadzu SCL-10Avp) on Chiralpak AD, n-heptane:iPrOH 90:10, 1.0 ml/min. UPLC-MS on protein samples was performed on a Acquity TQ Detector (ESITQD-MS) coupled to Waters Acquity Ultra Performance LC using a Acquity BEH C8 (1.7 μm 2.1 x 150 mm). Water (solvent A) and acetonitrile (solvent B) containing 0.1% formic acid by volume, were used as the mobile phase at a flow rate of 0.3 mL/min. Gradient: 90% A for 2 min, linear gradient to 50% A in 2 min, linear gradient to 20% A in 5 min, followed by 2 min at 5% A. Re-equilibration of the column with 2 min at 90% A. E. coli strain NEB5α-T1 Phage Resistant (Stratagene) was used for cloning and E. coli BL21(DE3)-C43-T1 Phage Resistant (Stratagene) strain was used for protein expression. Primers were synthesized by Eurofins MWG Operon (Ebesberg, Germany). Pfu Turbo polymerase was purchased from Agilent and DpnI was purchased from New England Biolabs. Plasmid isolation Kit was purchased from Roche. DNA sequencing was carried out by Eurofins Genomics. FPLC columns were purchased from GE Healthcare. Size exclusion chromatography was performed using AKTApurifier 10 apparatus. The concentration of the proteins was measured with Nanodrop 2000 (Thermo Scientific).

2. Synthetic procedures and characterization

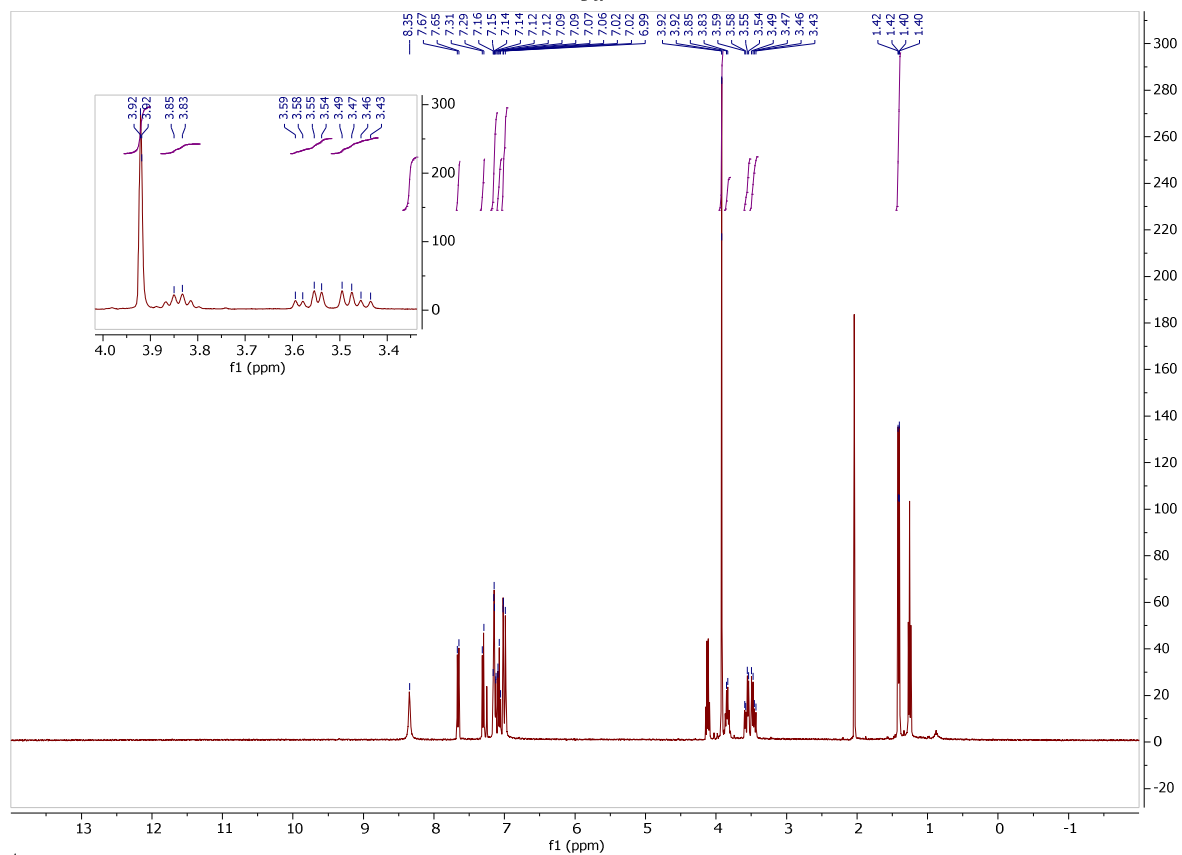
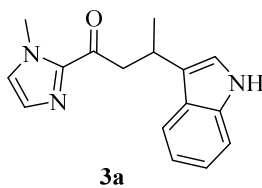
General procedure for the preparation of the Friedel-Crafts products as reference material

Procedure was adapted from literature.^{1,2} 50 mg of the corresponding α,β-unsaturated-2-acyl imidazole and 2 equivalents of the corresponding indole, predissolved in 10 mL acetonitrile, were added to 1 L of water containing 2.3 g sodium dodecyl sulfate (8 mM final concentration) and 15 mol% Cu(NO₃)₂·3H₂O. The reaction was stirred at room temperature for 16 hours. 10 g of NaCl was added and the aqueous phase was extracted 3 times with 100 mL diethyl ether. Subsequently, the organic phase was washed with 100 mL brine and dried on Na₂SO₄. The product was purified by column chromatography with a gradient heptane:ethyl acetate.

Some of the reference products present a peak at 7.26 ppm corresponding to benzene, which was used as internal standard to calculate the amount of impurity present in the sample and correct for it.

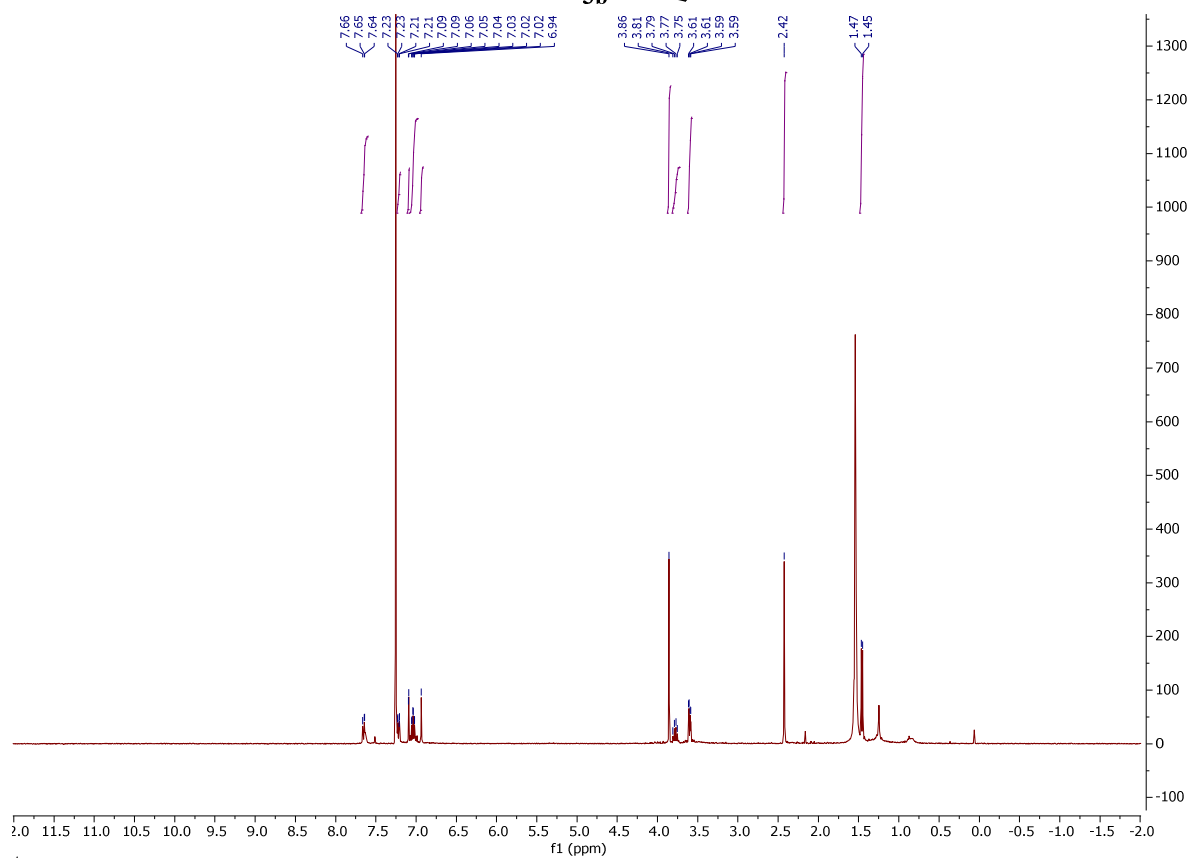
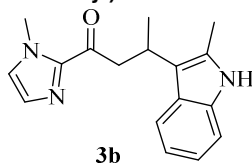


3-(1H-indol-3-yl)-1-(1-methyl-1H-imidazol-2-yl)butan-1-one (3a)^{3,4}



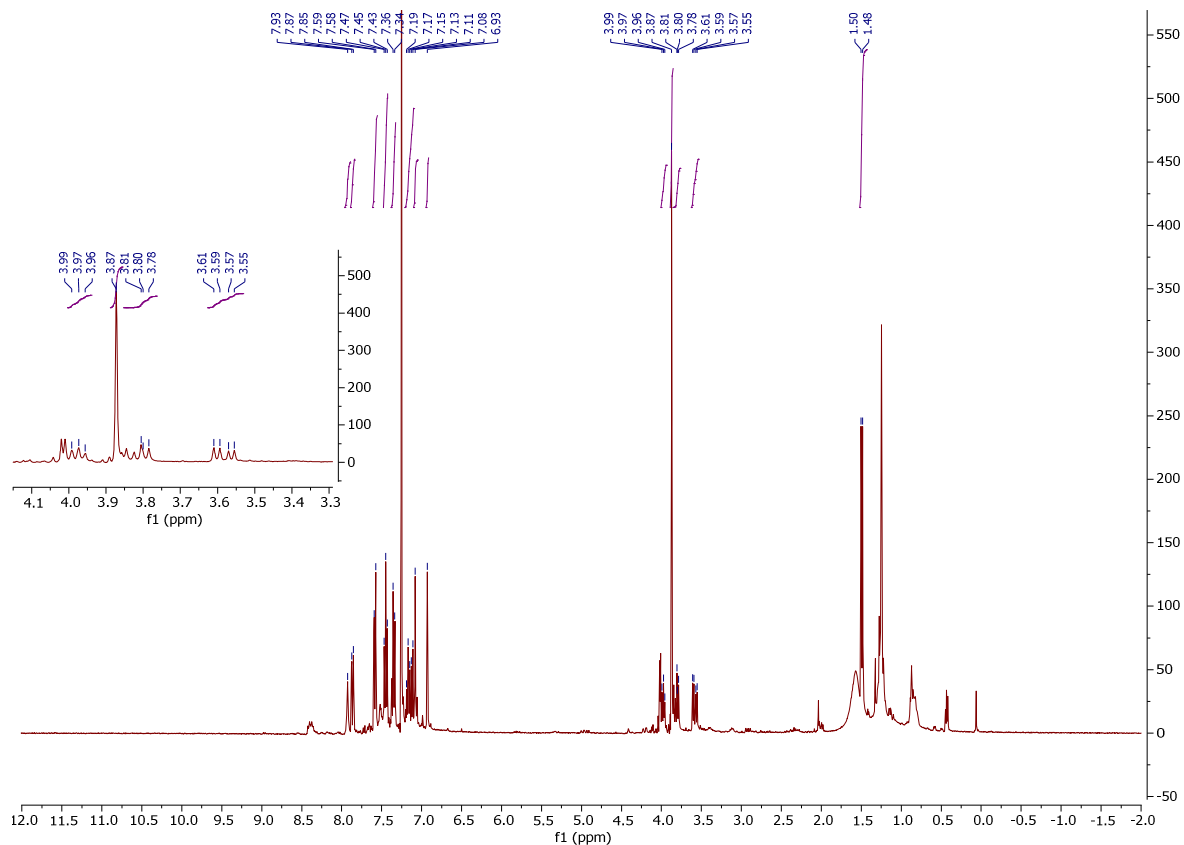
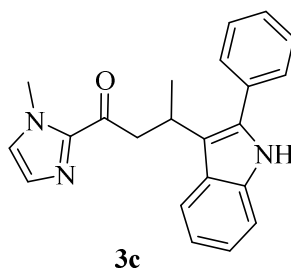
¹H NMR (400 MHz, Chloroform-*d*) δ 8.35 (s, 1H), 7.66 (d, J = 7.9 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.19 – 7.10 (m, 2H), 7.10 – 7.04 (m, 1H), 7.04 – 6.96 (m, 2H), 3.92 (d, J = 1.2 Hz, 3H), 3.84 (d, J = 7.1 Hz, 1H), 3.57 (dd, J = 15.9, 6.3 Hz, 1H), 3.46 (dd, J = 15.9, 8.2 Hz, 1H), 1.41 (dd, J = 6.9, 1.3 Hz, 3H).

1-(1-methyl-1H-imidazol-2-yl)-3-(2-methyl-1H-indol-3-yl)butan-1-one (3b)⁴⁻⁶



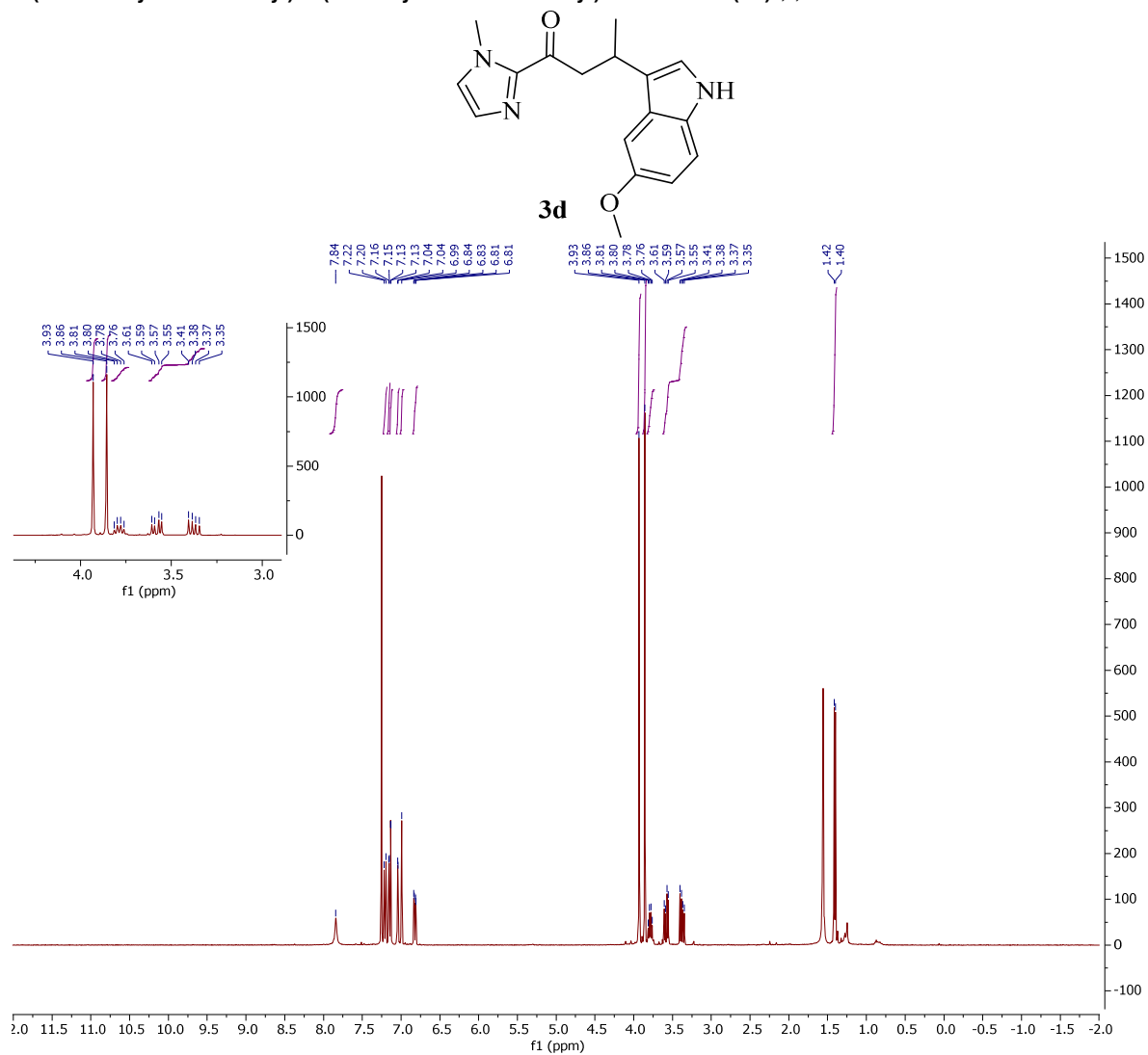
¹H-NMR (400 MHz, Chloroform-*d*) δ 7.68 – 7.60 (m, 2H), 7.24 – 7.20 (m, 1H), 7.09 (d, $J = 1.0$ Hz, 1H), 7.04 (td, $J = 6.9, 1.5$ Hz, 2H), 6.94 (s, 1H), 3.86 (s, 3H), 3.78 (m, $J = 7.2$ Hz, 1H), 3.60 (dd, $J = 7.4, 2.8$ Hz, 2H), 2.42 (s, 3H), 1.46 (d, $J = 7.1$ Hz, 3H).

1-(1-methyl-1H-imidazol-2-yl)-3-(2-phenyl-1H-indol-3-yl)butan-1-one (3c)

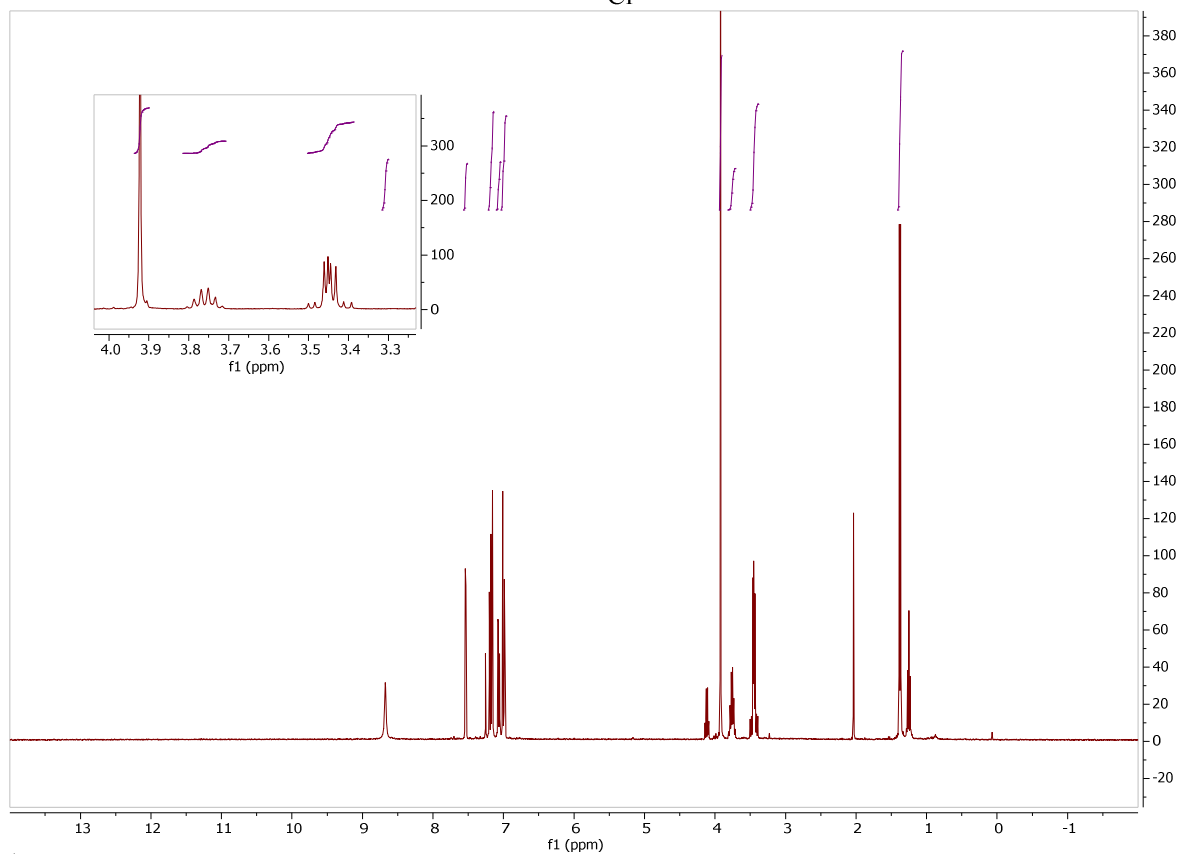
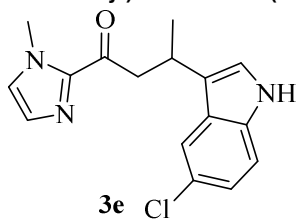


¹H NMR (400 MHz, Chloroform-*d*) δ 7.98 (s, 1H), 7.87 (d, *J* = 7.9 Hz, 1H), 7.62 – 7.57 (m, 3H), 7.44 (d, *J* = 7.9 Hz, 2H), 7.40 – 7.31 (m, 2H), 7.21 – 7.06 (m, 2H), 6.93 (s, 1H), 4.02 – 3.94 (m, 1H), 3.88 (s, 3H), 3.81 (dd, *J* = 15.8, 8.5 Hz, 1H), 3.58 (dd, *J* = 15.8, 6.3 Hz, 1H), 1.49 (d, *J* = 7.0 Hz, 3H). HRMS (ES⁺): Exact mass calcd for C₂₂H₂₁N₃O [M+H]⁺ 343.1684, found 342.1607.

3-(5-methoxy-1H-indol-3-yl)-1-(1-methyl-1H-imidazol-2-yl)butan-1-one (3d)^{2,5,6}

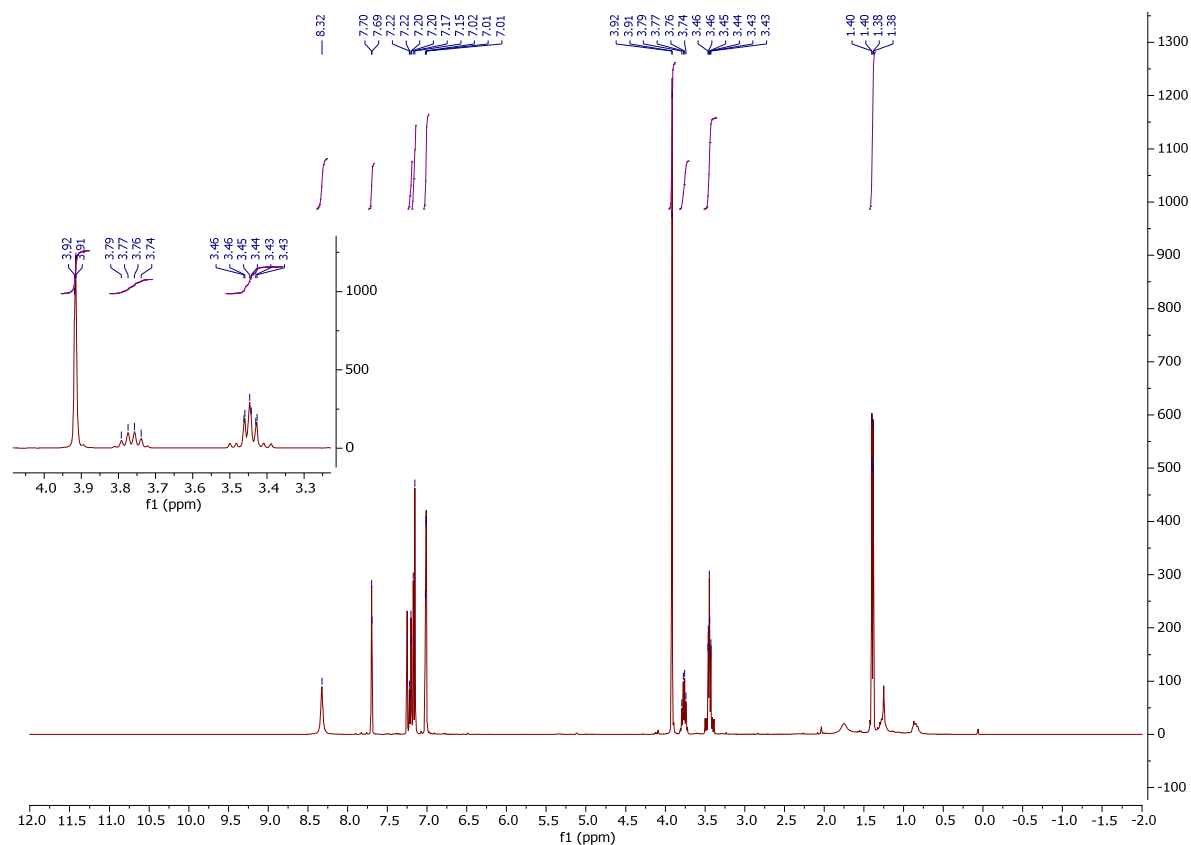
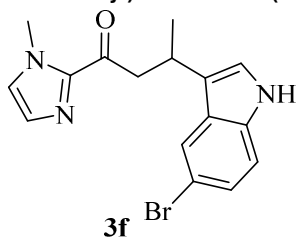


3-(5-chloro-1H-indol-3-yl)-1-(1-methyl-1H-imidazol-2-yl)butan-1-one (3e)⁴⁻⁶



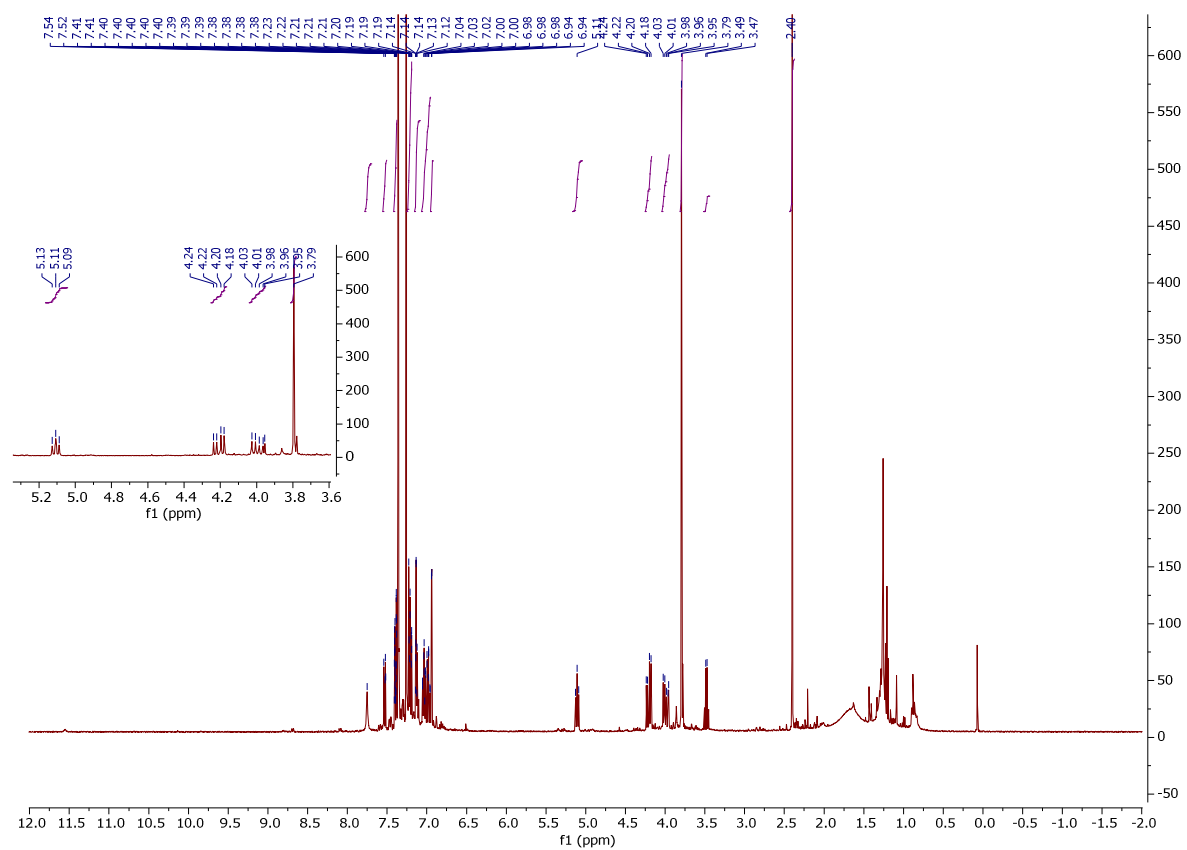
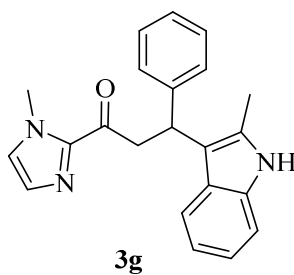
¹H NMR (400 MHz, Chloroform-*d*) δ 8.68 (s, 1H), 7.54 (d, J = 2.0 Hz, 1H), 7.22 – 7.14 (m, 2H), 7.07 (dd, J = 8.6, 2.0 Hz, 1H), 7.03 – 6.96 (m, 2H), 3.92 (s, 3H), 3.81 – 3.71 (m, 1H), 3.50 – 3.39 (m, 2H), 1.38 (d, J = 6.9 Hz, 3H).

3-(5-bromo-1H-indol-3-yl)-1-(1-methyl-1H-imidazol-2-yl)butan-1-one (3f)



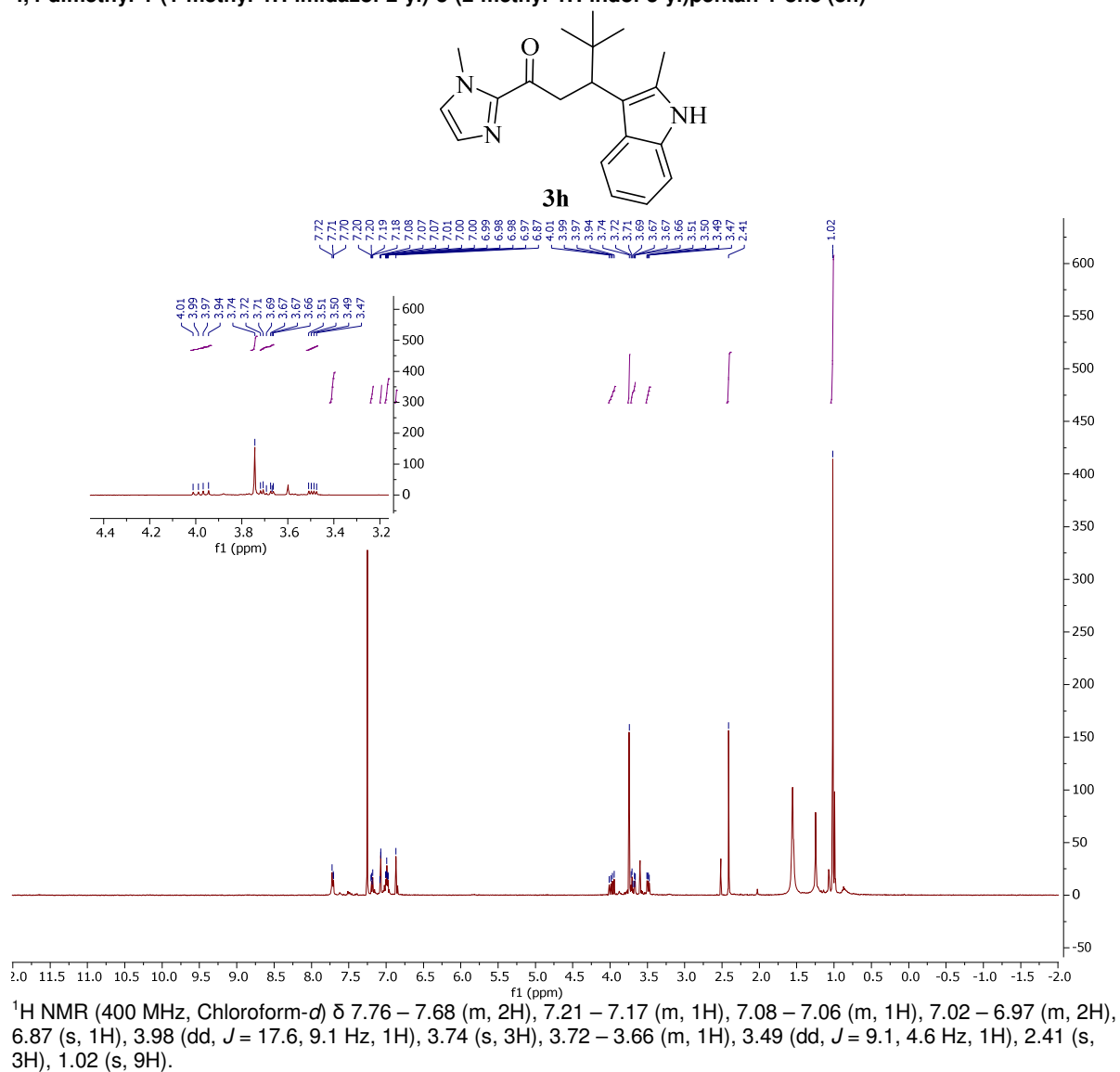
¹H-NMR (400 MHz, Chloroform-*d*) δ 8.32 (s, 1H), 7.69 (d, *J* = 2.0 Hz, 1H), 7.21 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 2H), 7.04 – 6.98 (m, 2H), 3.92 (d, *J* = 1.3 Hz, 3H), 3.77 (m, *J* = 7.1 Hz, 1H), 3.44 (m, *J* = 6.2, 1.2 Hz, 2H), 1.39 (dd, *J* = 6.9, 1.2 Hz, 3H). ¹³C-NMR (101 MHz, Chloroform-*d*) δ 194.6, 145.8, 137.5, 131.5, 131.0, 129.6, 127.3, 124.4, 124.1, 123.8, 115.1, 115.0, 49.6, 38.8, 29.7, 24.2.

1-(1-methyl-1H-imidazol-2-yl)-3-(2-methyl-1H-indol-3-yl)-3-phenylpropan-1-one (3g)⁶



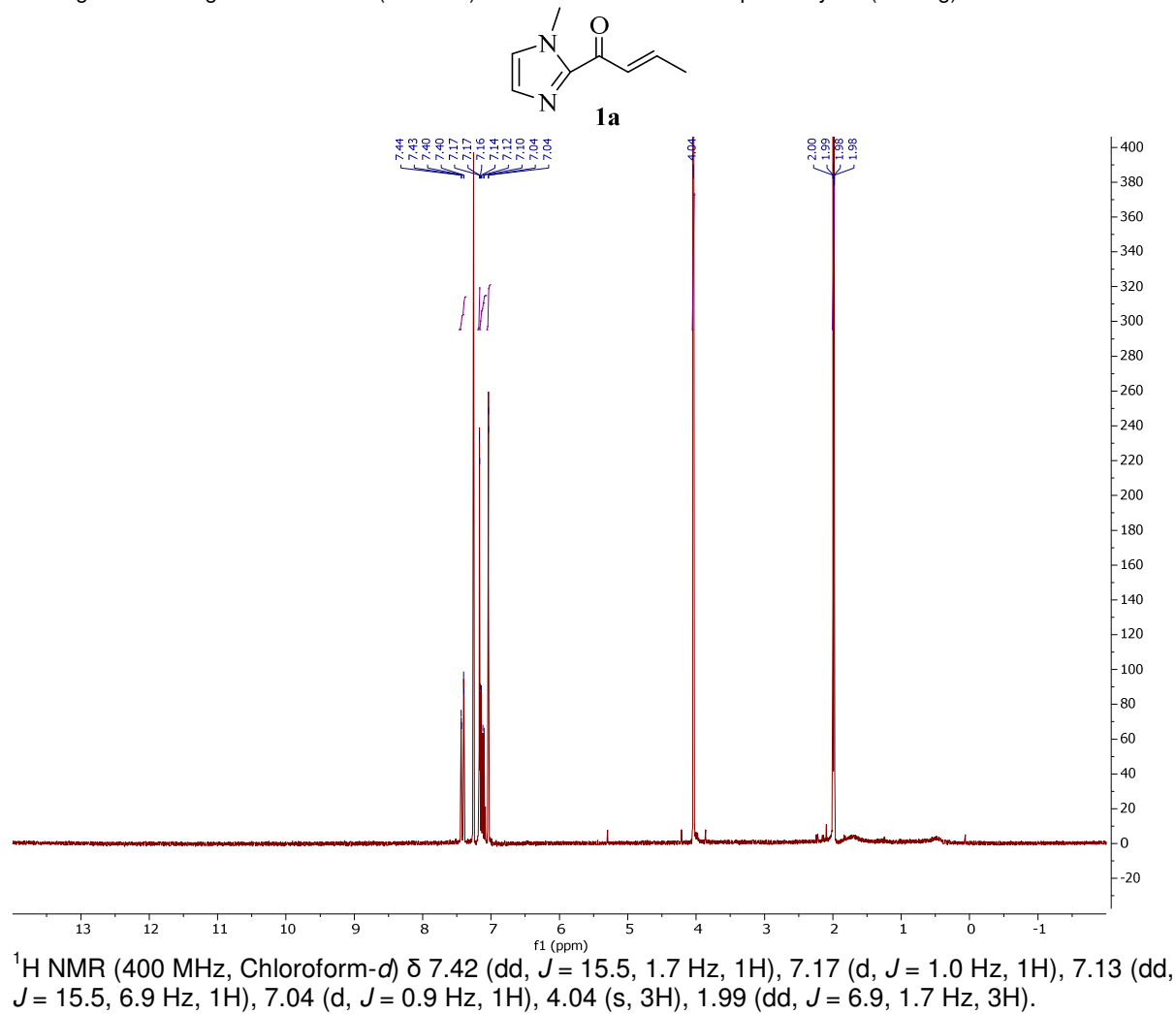
¹H-NMR (400 MHz, Chloroform-*d*) δ 7.75 (s, 1H), 7.55 – 7.50 (m, 1H), 7.42 – 7.38 (m, 2H), 7.25 – 7.19 (m, 3H), 7.16 – 7.09 (m, 2H), 7.07 – 6.95 (m, 2H), 6.94 (d, $J = 1.0$ Hz, 1H), 5.11 (t, $J = 7.6$ Hz, 1H), 4.21 (dd, $J = 16.6, 7.0$ Hz, 1H), 4.04 – 3.95 (m, 1H), 3.79 (s, 3H), 2.40 (s, 3H).

4,4-dimethyl-1-(1-methyl-1H-imidazol-2-yl)-3-(2-methyl-1H-indol-3-yl)pentan-1-one (3h)

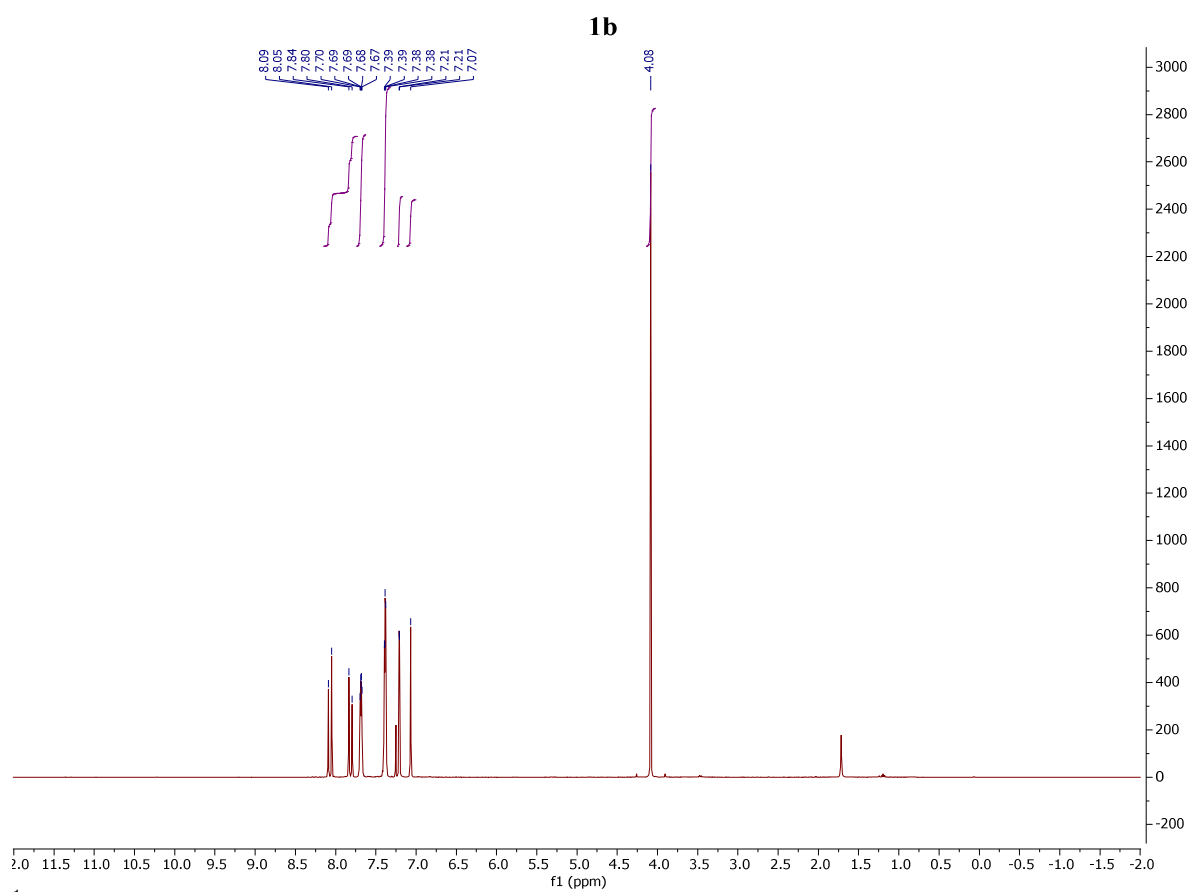
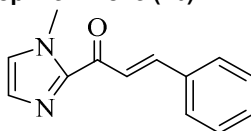


General procedure for the preparation of the Friedel-Crafts substrate

(E)-1-(1-Methyl-1H-imidazole-2-yl)-but-2-en-1-one (**1a**) was synthesized according to a literature procedure.^{3,5} Starting from 861 mg of crotonic acid (10 mmol) **1a** was obtained in a 20% purified yield (300 mg).

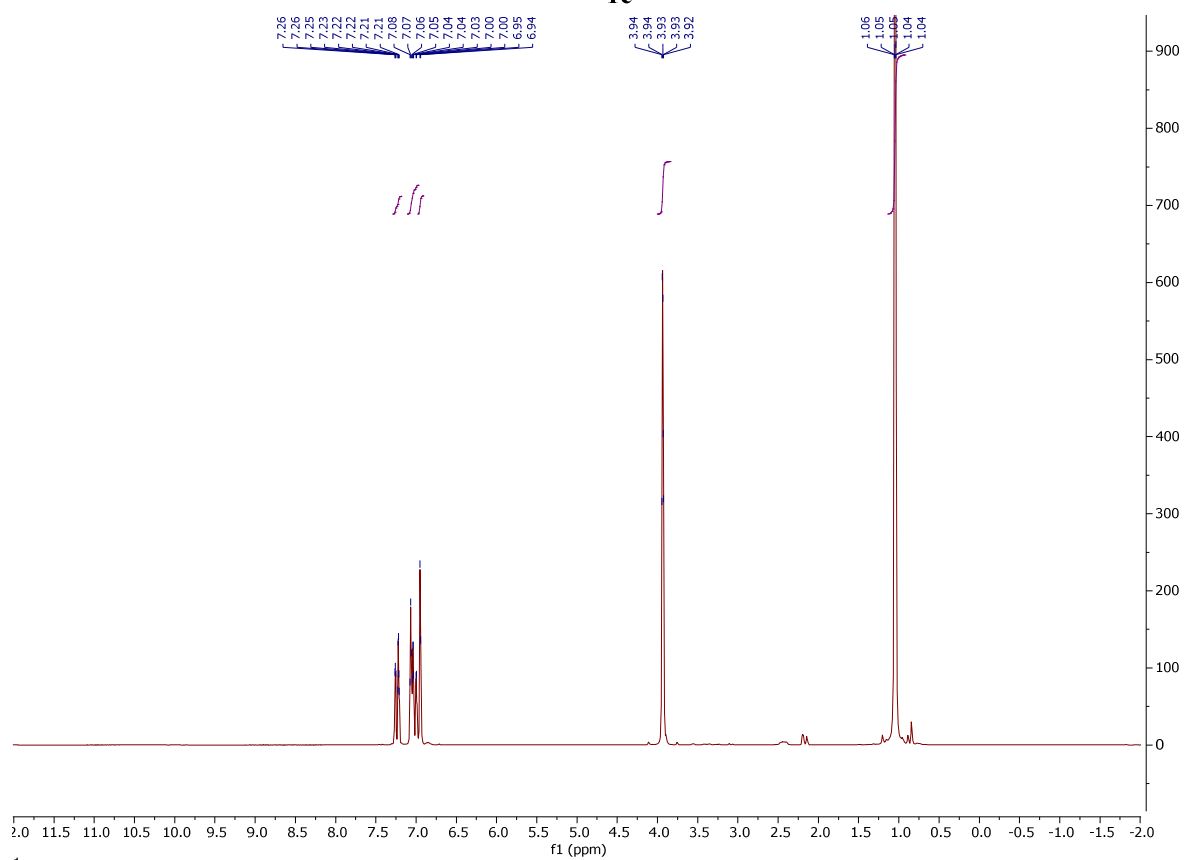
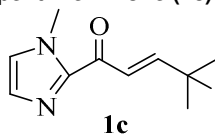


(E)-1-(1-methyl-1H-imidazol-2-yl)-3-phenylprop-2-en-1-one (**1b**)³



¹H-NMR (400 MHz, Chloroform-*d*) δ 7.94 (dd, J = 101.5, 16.0 Hz, 2H), 7.74 – 7.63 (m, 2H), 7.39 (dd, J = 4.9, 1.9 Hz, 3H), 7.21 (d, J = 1.0 Hz, 1H), 7.07 (s, 1H), 4.08 (s, 3H).

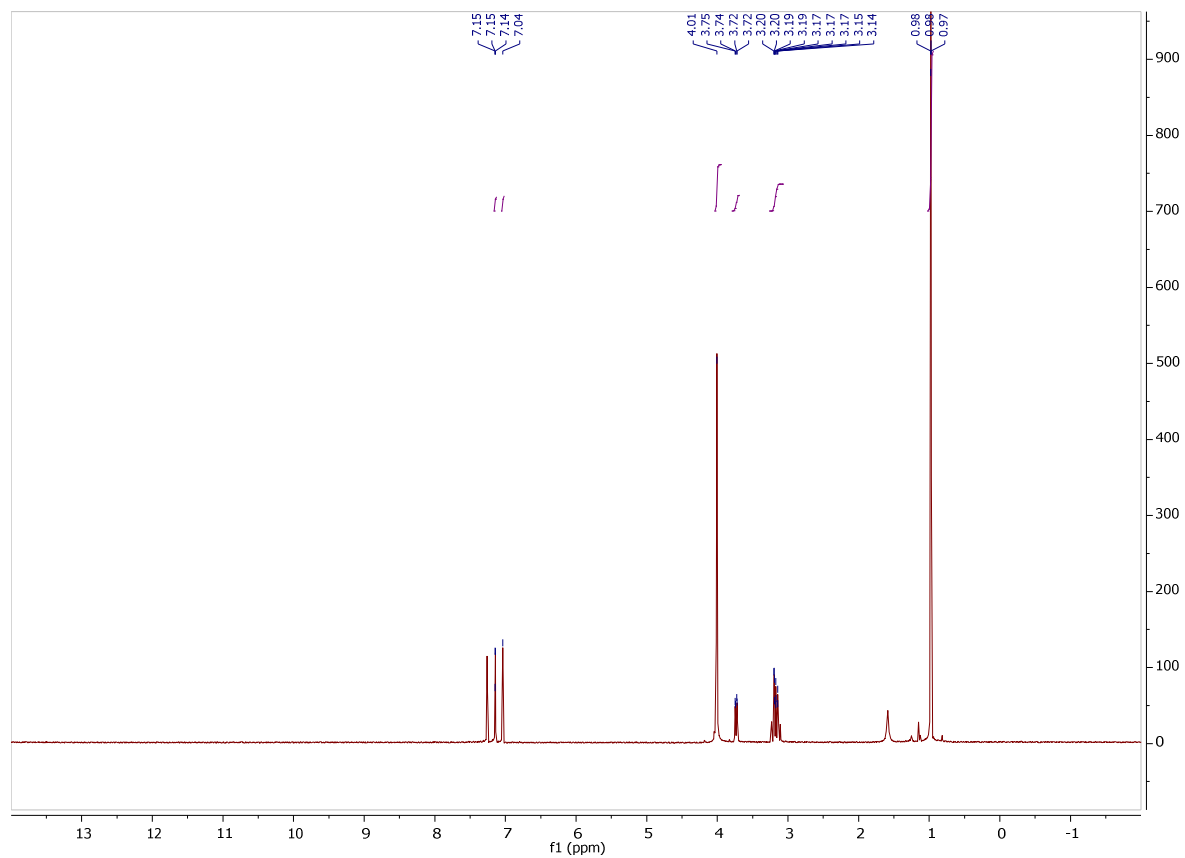
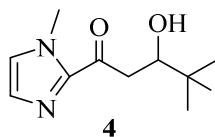
(E)-4,4-dimethyl-1-(1-methyl-1H-imidazol-2-yl)pent-2-en-1-one (**1c**)⁷



¹H-NMR (400 MHz, Chloroform-*d*) δ 7.29 – 7.18 (m, 1H), 7.11 – 6.97 (m, 2H), 6.95 (d, J = 3.7 Hz, 1H), 3.93 (s, 3H), 1.05 (s, 9H).

Water-addition product

3-hydroxy-4,4-dimethyl-1-(1-methyl-1H-imidazol-2-yl)pentan-1-one (4)



$^1\text{H-NMR}$ (400 MHz, Chloroform- d) δ 7.16 – 7.13 (s, 1H), 7.04 (s, 1H), 4.01 (s, 3H), 3.73 (dd, $J = 9.4, 2.2$ Hz, 1H), 3.26 – 3.07 (m, 2H), 0.98 (s, 9H).

3. Molecular biology

pET 17b plasmids encoding for wt-QacR ,wt-CgmR and wt-RamR were available from a previous work.⁴ Plasmids were purchased from Genescript (USA) as codon optimized sequences for E.Coli expression and included a C-terminal Strep-tag for purification purposes. Gene sequences:

RamR, Codon optimized: Host expression organism: Escherichia coli, Length: 624 bp,
CATATGGTGGCGCTCCGAAGAGCGAGGACAAGAAACAAGCGCTGCTGGAAGCGGCGACCCAGGCGATTGCGCAAAGCGGTATT
GCGGCGAGCACCGCGGTGATTGCGCGTAACGCGGGTGTTCGGGAGGGTACCCTGTTCCGTTACTTTGCGACCAAGGACGAACTGA
TTAACACCCTGTATCTGCACCTGAAACAGGATCTGAGCCAAAGCATGATCATGGAGCTGGACCGTAGCATTACCGATGCGAAAATGA
TGACCCGTTTCATCTGGAAACAGCTACATTAGCTGGGGCCTGAACCATCCGGCGCGTCACCGTGCATCCGTCAGCTGGCGGTTAG
CGAGAAGCTGACCAAAGAAACCGAACCAACGTGCGGACGATATGTTCCCGAACTGCGTGATCTGAGCCACCGTAGCGTGCTGATG
GTTTTTATGAGCGACGAGTACCGTGCCTTCGGTGTATGGCCTGTTTCTGGCGCTGGCGGAAACCACCATGGATTTTGGCGCGCTGA
TCCGGCGCGTGCGGGCGAGTATATTGCGCTGGGCTTTGAAGCGATGTGGCGTGCGCTGACCCGTGAGGAACAGGCGCGCTGGAG
CCACCCGCAATTTGAAAAGTAACTCGAG

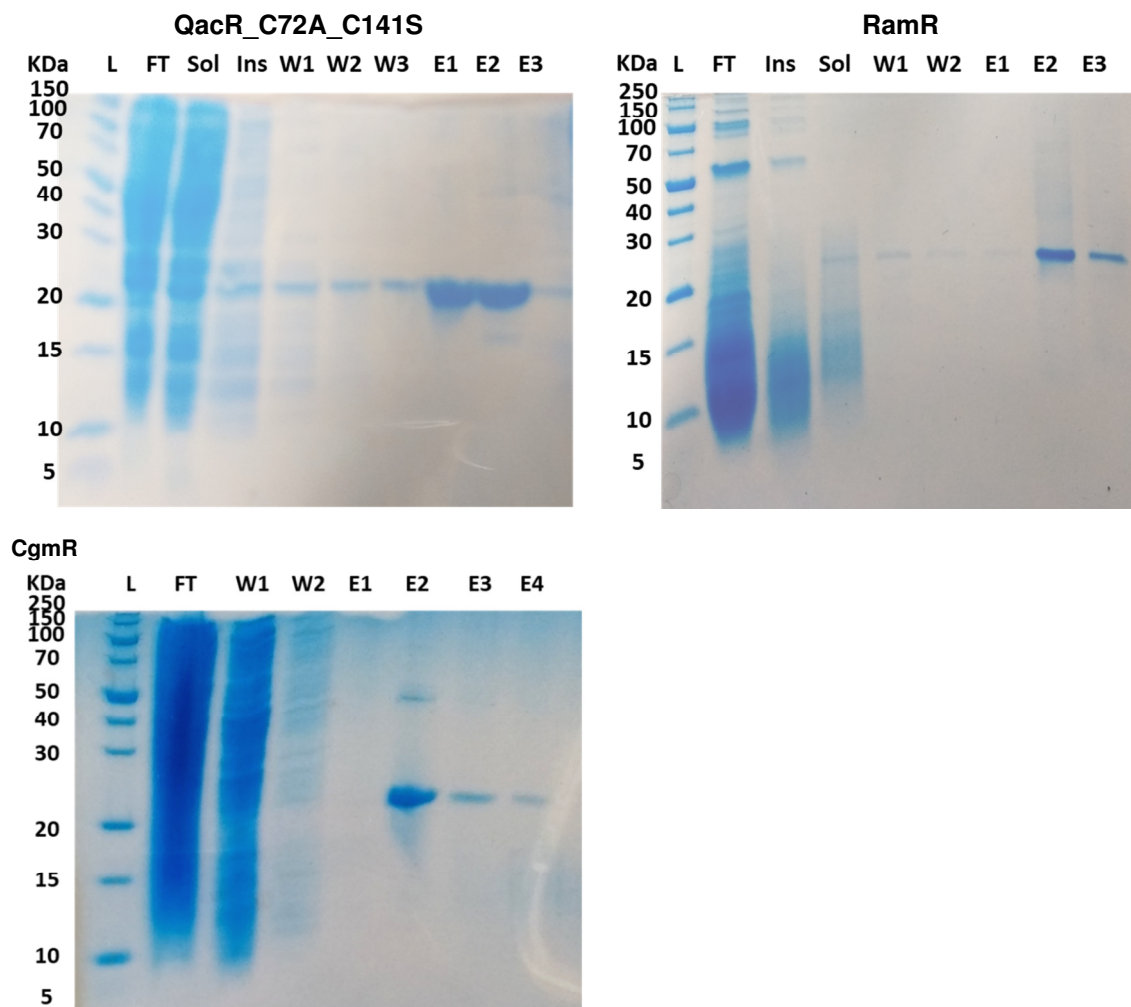
QacR, Codon optimized: Host expression organism: Escherichia coli, Length: 606 bp,
CATATGAACCTGAAGGACAAAATCCTGGGTGTGGCGAAGGAGCTGTTCAATAAAAACGGTTATAACGCGACCACCACCGGCGAGAT
CGTTAAGCTGAGCGAAAGCAGCAAAGGCAACCTGTACTATCACTTCAAGACCAAAGAGAACCTGTTTCTGGAATCCTGAACATTGA
GGAAAGCAAGTGGCAGGAGCAATGGAAAAGCGAACAGATTAAGTGCAAACCAACCGTGAGAAGTTCTATCTGTACAACGAACTGA
GCCTGACCACCCAGTACTATTACCCGCTGCAAAACGCGATCATCGAGTTCTACACCGAATACTACAAGACCAACAGCATCAACGAGA
AGATGAACAAACTGGAAAACAAGTATATCGACGCGTACCACGTGATTTTCAAAGAGGGTAACCTGAACGGCGAATGGTGCATTAACG
ATGTGAACGCGGTTAGCAAGATCGCGGCGAACGCGGTGAACGGTATTGTTACCTTTACCCACGAGCAGAACATCAACGAACGTATT
AAGCTGATGAACAAATTCAGCCAAATCTTCTGAACGGCCTGAGCAAGGCGGCGTGGAGCCACCCGCAATTTGAAAAATAACTCGA
G

CgmR, Codon optimized: Host expression organism: Escherichia coli, Length: 573 bp,
CATATGCGTACCAGCAAGAAAGAGATGATCCTGCGTACCGCGATCGACTACATTGGCGAGTATAGCCTGGAACCCTGAGCTACGA
TAGCCTGGCGGAAGCGACCCGGTCTGAGCAAGAGCGGCCGTGATCTATCACTTTCCGAGCCGTCATGCGCTGCTGCTGGGTATGCAC
GAGCTGCTGGCGGACGATTGGGACAAGAAGTGCCTGATATTACCCGTGACCCGGAGGATCCGCTGGAACGTTCTGCGTGCGGTGG
TTGTGACCCTGGCGGAGAACGTTAGCCGTCGGAAGTGCCTGCTGCTGATTGATGCGCCGAGCCACCCGGATTTCTGAACGCGTG
GCGTACCGTGAACCACAGTGGATTCCGGACACCGACGATCTGGAGAACGATGCGCACAAAGCGTGCGGTTTACCTGGTGCAACTG
GCGGCGGACGGTCTGTTTGTTCAGATTATATTCACGACGATGTGCTGAGCAAGAGCAAACGTCAGGCGATGCTGGAACCATCCT
GAACTGATTCCGAGCGAGCTGGCGCGTGGAGCCACCCGCAATTCGAAAAATAACTCGAG

4. Protein production, purification and characterization

pET17b plasmids encoding for wt-CgmR, wt-RamR and QacR_C72A_C141S, were transformed into *E. coli* BL21(DE3)C43 cells. The cells were spread onto an agar plate containing 100 µg/mL of ampicillin. 5 mL of LB media with µg/mL of ampicillin were inoculated with a single colony from the transformation and grown at 37 °C overnight. The entire starter culture was used to inoculate 500 mL of fresh LB medium containing 100 µg/mL of ampicillin. When the culture reached an optical density at 600 nm between 0.8-0.9, isopropyl β-D-1thiogalactopyranoside (IPTG) at a final concentration of 1 mM was added to induce the expression of the target proteins. Protein expression was performed at 30 °C overnight. Cells were harvested by centrifugation (6000 rpm, JA-10, 20 min, 4 °C, Beckman) and the pellet was resuspended in 50 mM NaH₂PO₄, pH 8.0, 150 mM NaCl and protease inhibitor cocktail (Complete, Roche, 1 tablet, for 50 mL of resuspension buffer). Cells were sonicated (70% (200 W) for 15 min (10 sec on, 10 sec off) and afterwards incubated for 30 min on ice with PMSF solution (final concentration 0.1 mM), DNaseI (0.1 mg/mL) and 10 mM MgCl₂. After centrifugation (16000 rpm, JA-17, 45 min, 4°C, Beckman), the supernatant was equilibrated with 5 mL of pre-equilibrated Strep-tag Tactin column material for 1 h (mixed at 200 rpm on a rotatory shaker) at 4°C. The column was washed with three times with 3XCV (column volume) of the re-suspension buffer, and the protein was eluted with five 1X CV of resuspension buffer containing 5 mM desthiobiotin. Elution fractions were analyzed on a 12% polyacrylamide SDS-Tris Tricine gel followed by Coomassie staining (InstantBlue, Expedeon). Fractions containing protein were pulled concentrated using Vivaspin Turbo (5000 MWCO, Sartorius) centrifugation filters and they were dialyzed twice against 20 mM MOPS, 500 mM NaCl, pH 7 buffer. Concentration of the proteins was measured on a Nanodrop 2000 (Thermo Scientific), using the calculated extinction coefficient for the monomer (Protparam, ExPASy server). The proteins with A²⁶⁰/A²⁸⁰ higher than 0.7, were purified *via* cation exchange chromatography on a Hitrap Heparin HP column by a gradient of NaCl concentration from 0 to 1 M in 5 min with a flow of 1 mL min⁻¹. Typical expression yields were between 15 and 30 mg/L. Analytical size exclusion chromatography was performed on a Superdex 75 10/300 GL (GE Healthcare). 100 µL of the sample was injected using 20 mM MOPS, pH 7.0, 500 mM NaCl as buffer (flow 0.5 mL min⁻¹).

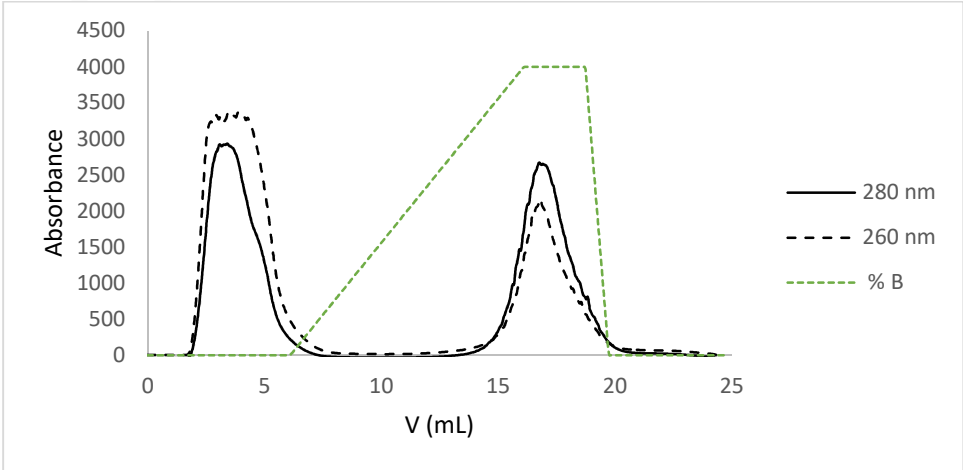
Figure S1. SDS-PAGE after Strep-Tag purification



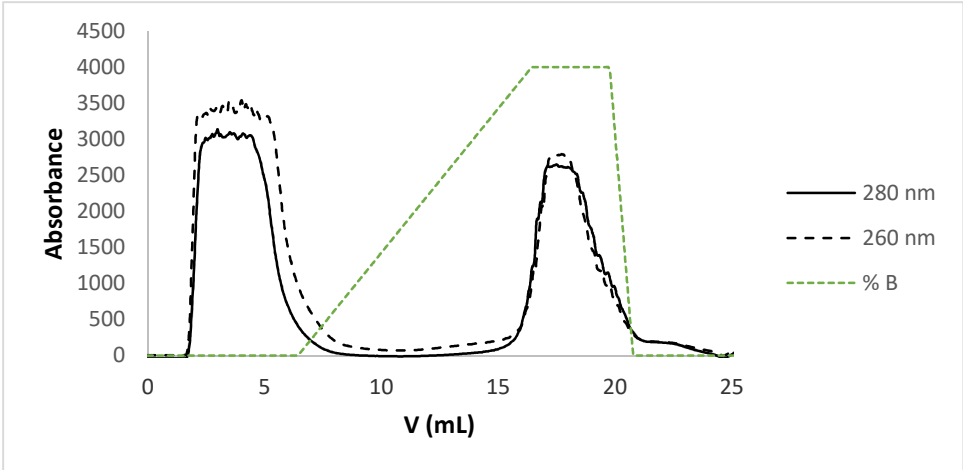
L: protein ladder (Unstained PageRuler Broad Range Protein Ladder), FT: flow through, sol: soluble fraction; ins: insoluble fraction W: wash fraction; E: elution fraction

Figure S2. Heparin purification chromatography traces

QacR_C72A_C141S



RamR



CgmR

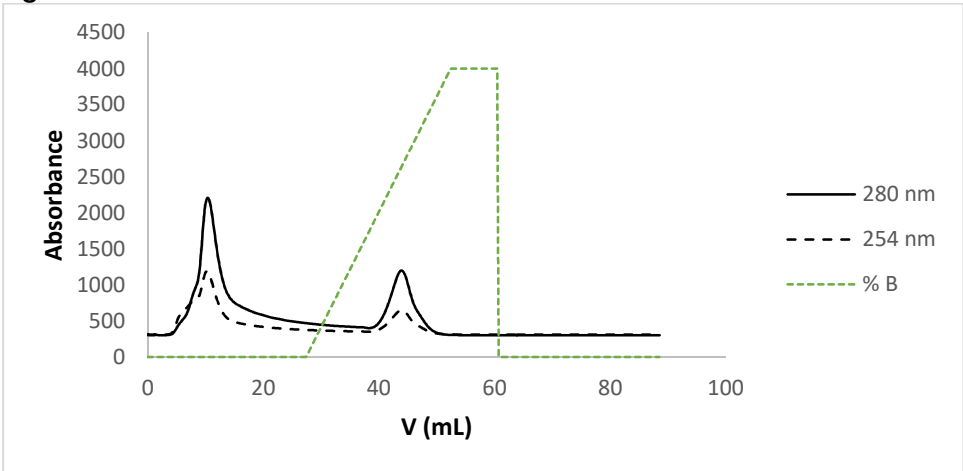
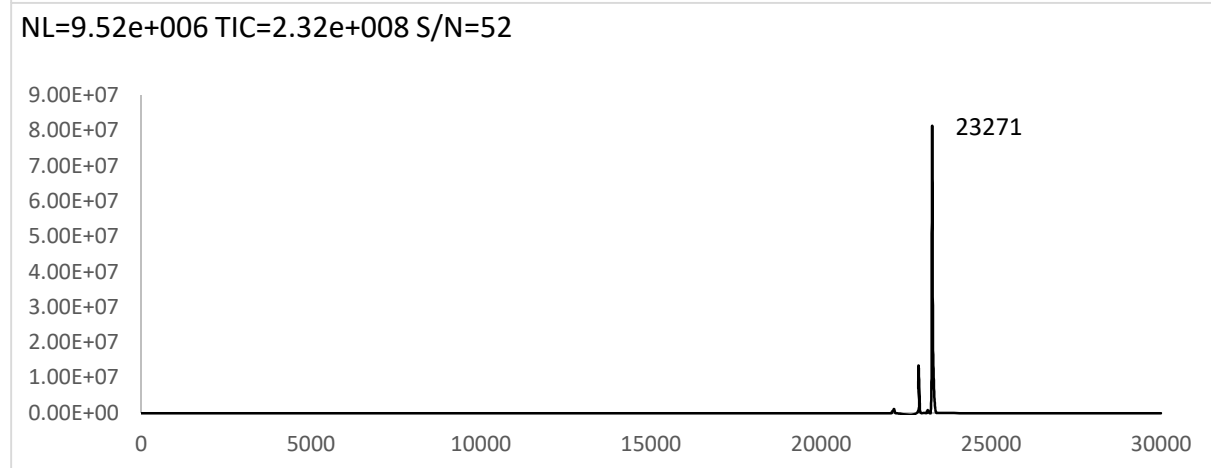
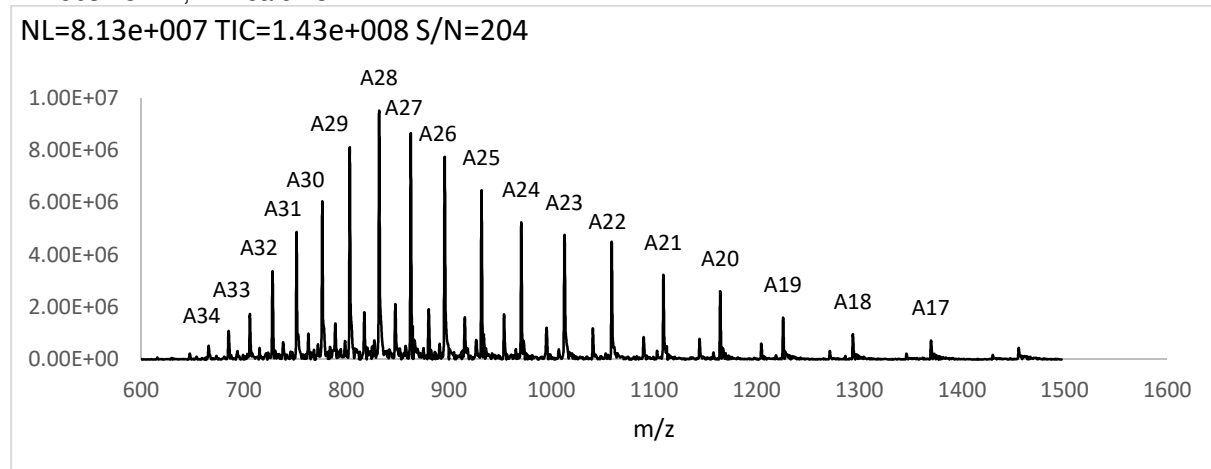


Figure S3. Mass spectra from UPLC-MS ESI(+) analysis

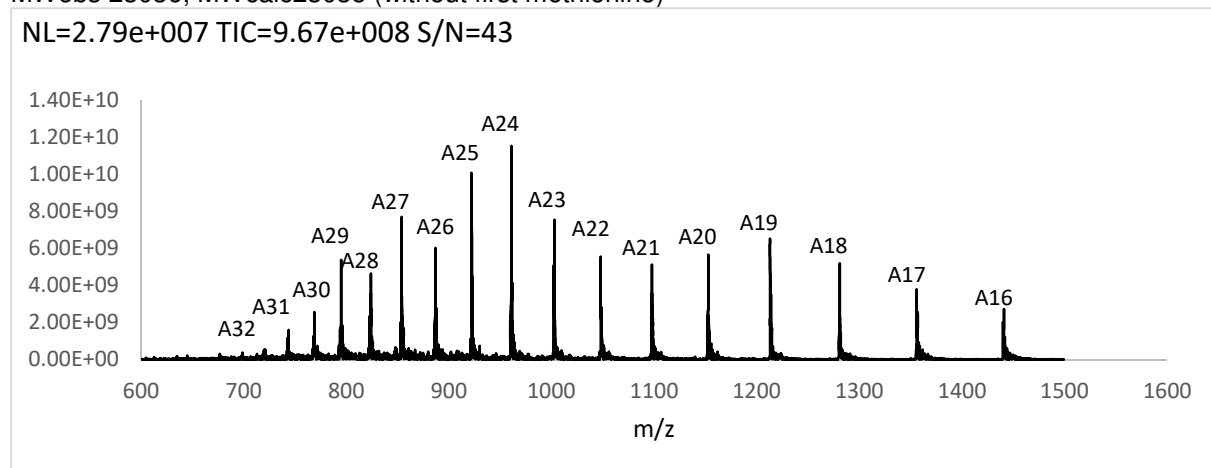
QacR_C72A_C141S

MWobs 23272, MWcalc 23271

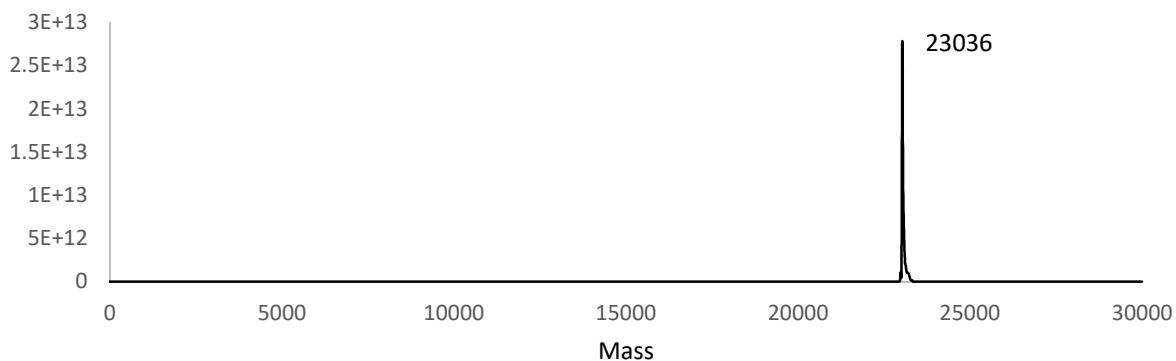


RamR

MWobs 23036, MWcalc 23035 (without first methionine)



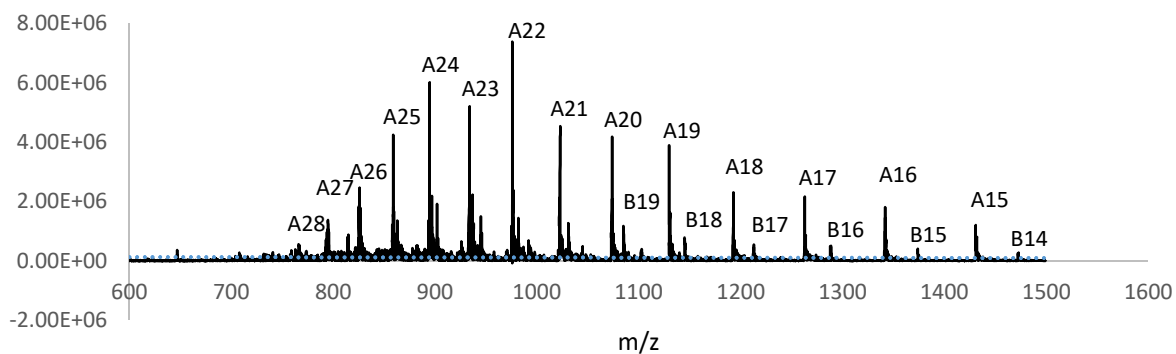
NL=2.77e+008 TIC=5.86e+008 S/N=224



CgmR

MWobs 21454, MWcalc 21456

NL=1.09e+008 TIC=7.14e+009 S/N=107



NL=1.79e+007 TIC=6.92e+008 S/N=42

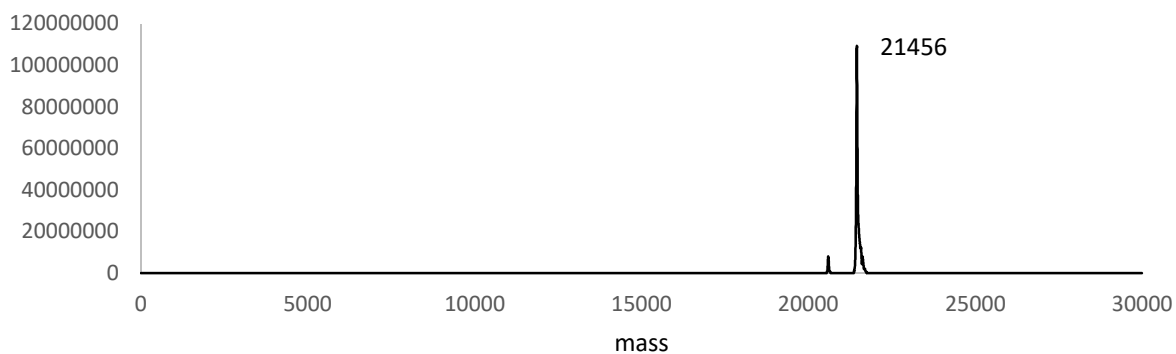
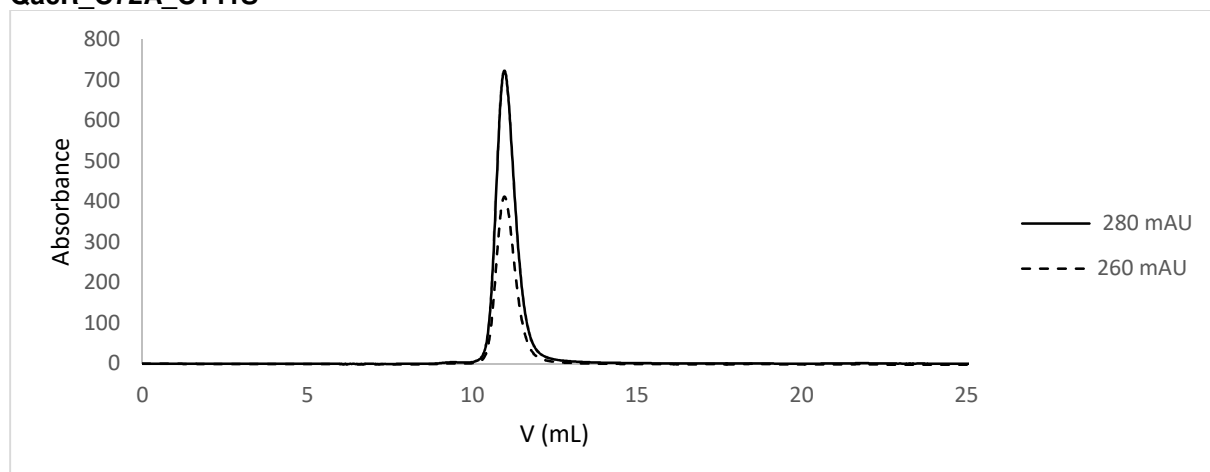
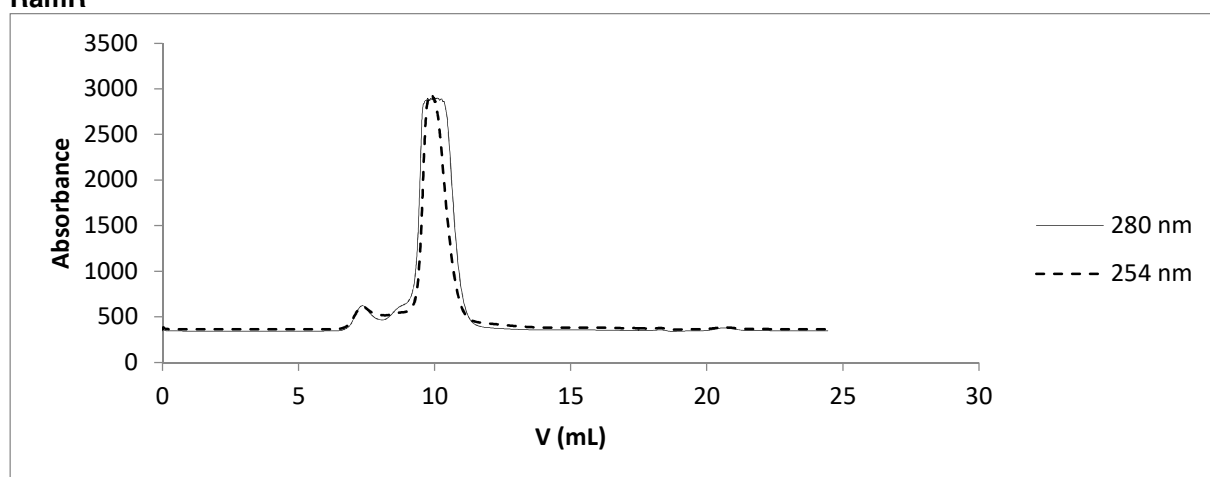


Figure S4. Size exclusion chromatography

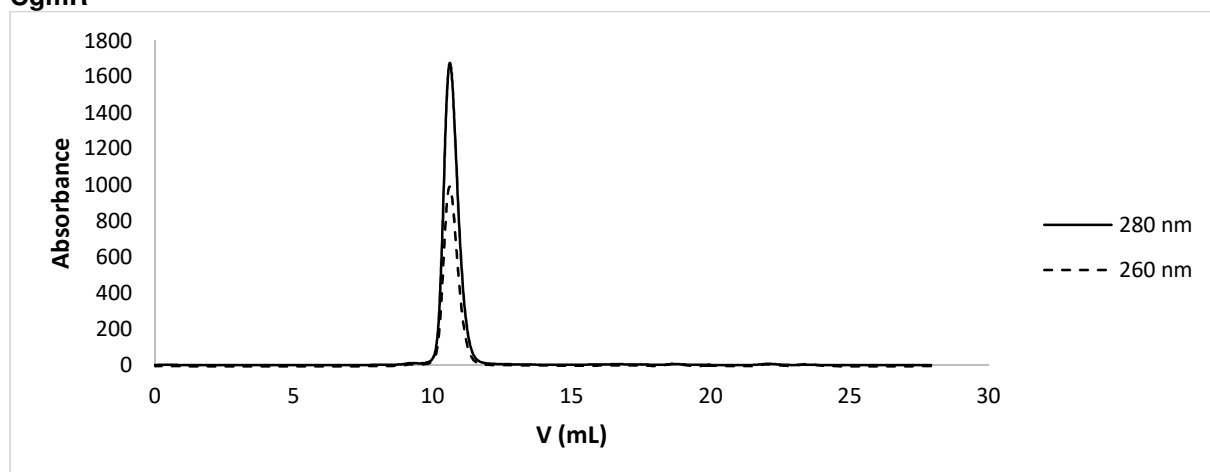
QacR_C72A_C141S



RamR



CgmR

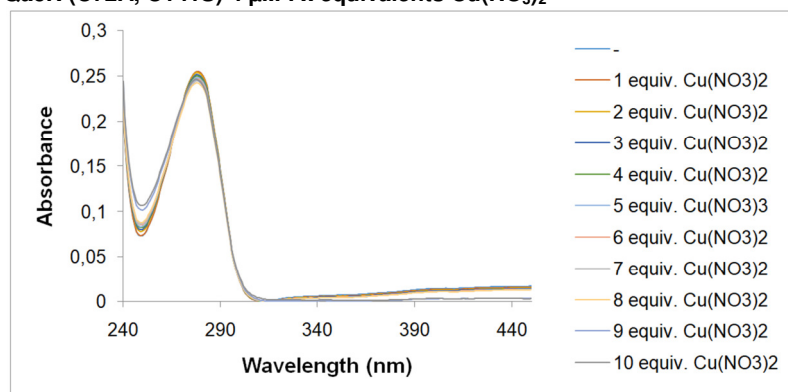


5. UV-visible titrations

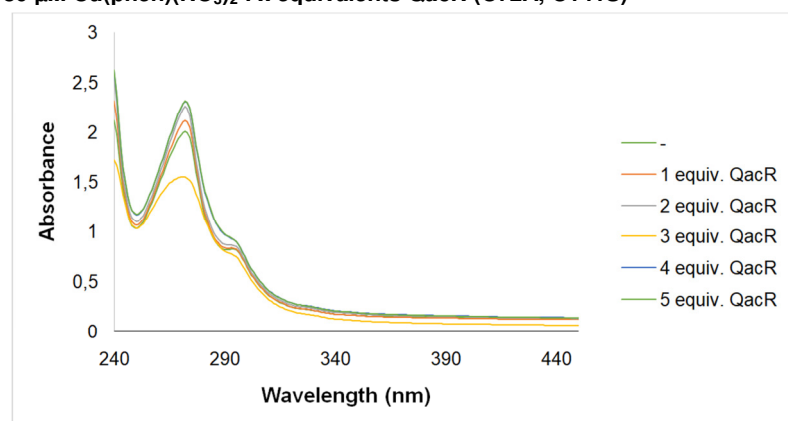
UV-visible titrations were performed with 4 μM solution of the dimeric proteins titrated with 400 μM solution of $\text{Cu}(\text{NO}_3)_2$ in 20 mM MOPS, pH 7.0 containing 500 μM NaCl. The experiments with $\text{Cu}(\text{phen})(\text{NO}_3)_2$ were performed with 80 μM solution of $\text{Cu}(\text{phen})(\text{NO}_3)_2$ titrated with 400 μM solution of the dimeric proteins.

Figure S5. UV-visible titrations

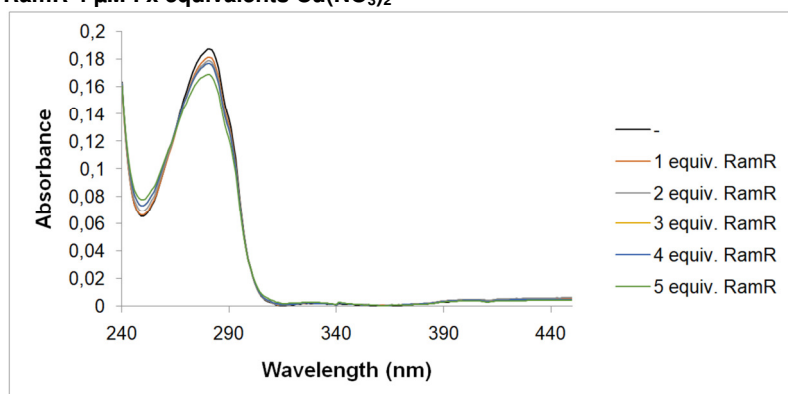
QacR (C72A, C141S) 4 μM : x equivalents $\text{Cu}(\text{NO}_3)_2$



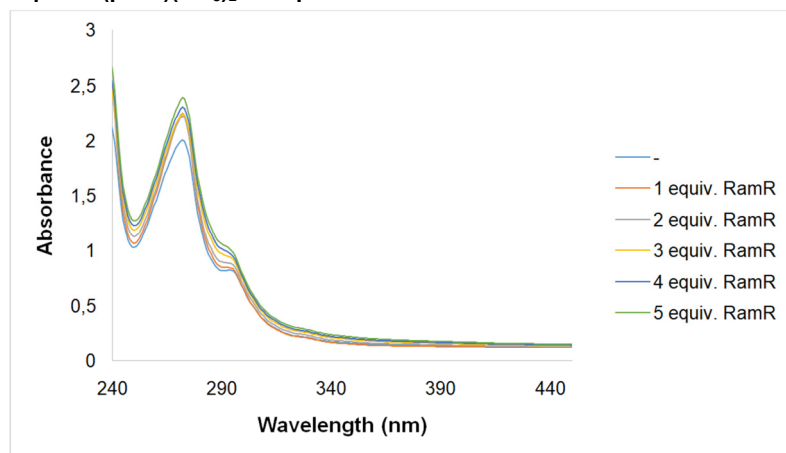
80 μM $\text{Cu}(\text{phen})(\text{NO}_3)_2$: x equivalents QacR (C72A, C141S)



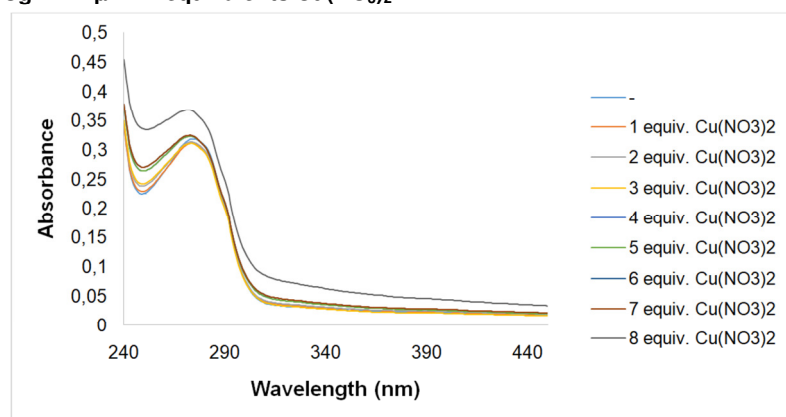
RamR 4 μM : x equivalents $\text{Cu}(\text{NO}_3)_2$



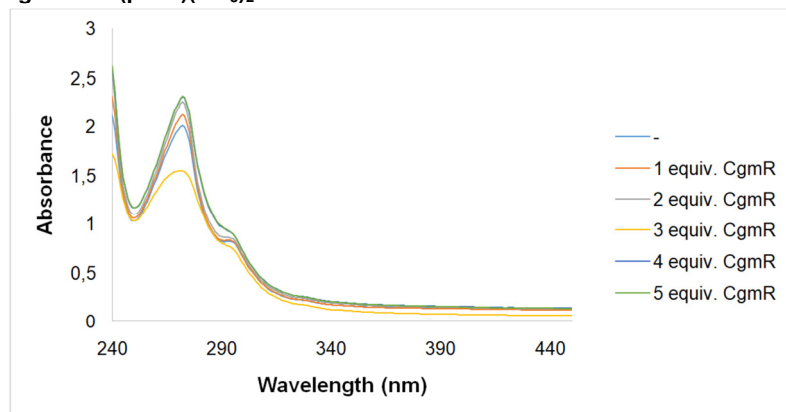
80 μM $\text{Cu}(\text{phen})(\text{NO}_3)_2$: x equivalents RamR



CgmR 4 μM : x equivalents $\text{Cu}(\text{NO}_3)_2$



CgmR : $\text{Cu}(\text{phen})(\text{NO}_3)_2$



6. Catalysis: Friedel-Crafts alkylation

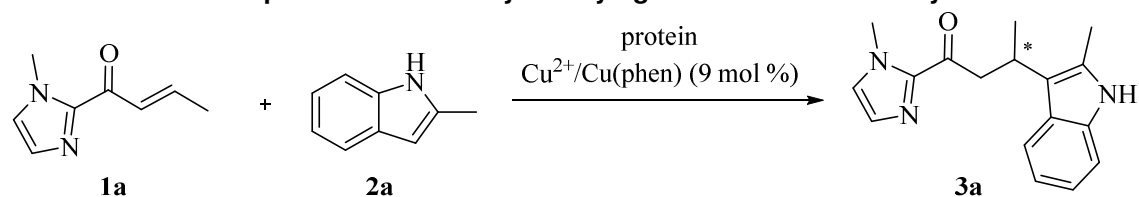
Catalytic reactions were performed in 150 μL total volume containing 90 μM $\text{Cu}(\text{NO}_3)_2$ (9 mol%) and 120 μM proteins (monomer, 1.3 equivalents), 1 mM of substrate **1** and **2** in 20 mM MOPS buffer pH 7.0 (or 20 mM MES buffer pH 5.0), 500 mM NaCl. In a typical procedure, the protein and the metal solutions were incubated for an hour under continuous inversion at 4 $^\circ\text{C}$ prior to addition of the substrates. Reactions were then incubated under continuous inversion at 4 $^\circ\text{C}$ for 72 h after which 50 μL of a 1 mM solution of 2-phenylquinoline in 20 mM MOPS buffer pH 7.0, 500 mM NaCl, 20% CH_3CN were added. Reactions were extracted 3 times with 600 μL diethylether and the organic layers were dried over Na_2SO_4 and evaporated *in vacuo*. The resulting products were re-dissolved in 150 μL heptane:isopropanol 9:1 and analyzed by chiral HPLC (Chiralpak AD). Yields of all the catalytic reactions are based on peak areas at 275 nm using 2-phenylquinoline as internal standard.

Table S1. Enone and indole scope of Friedel-Crafts reactions catalyzed by QacR, RamR and CgmR-based artificial metalloenzymes

1a R1: methyl
1b R1: phenyl
1c R1: *tert*-butyl
2a R2: methyl; R3 H
2b R2: H; R3: H
2c R2: phenyl; R3: H
2d R2: H; R3: methoxyl
2e R2: H; R3: chlorine
2f R2: H; R3: bromine

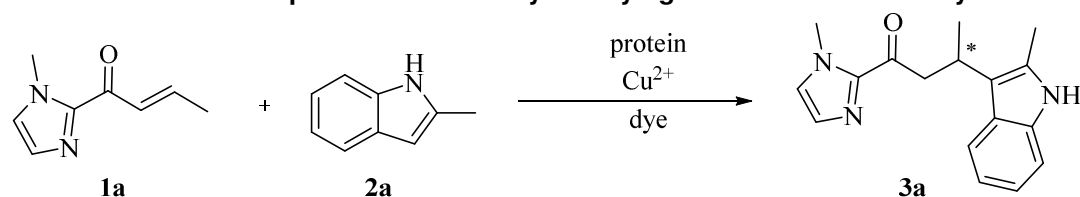
Entry	catalyst	enone	indole	product	Yield (%)	ee (%)
1	Cu^{2+}	1a	2a	3a	22 \pm 7	-
2	Cu^{2+} -RamR	1a	2a	3a	57 \pm 9	29 \pm 3 (-)
3	Cu^{2+} -CgmR	1a	2a	3a	52 \pm 12	13 \pm 3 (-)
4	Cu^{2+} -QacR	1a	2a	3a	78 \pm 11	34 \pm 3 (-)
5	Cu^{2+}	1a	2b	3b	7 \pm 8	-
6	Cu^{2+} -RamR	1a	2b	3b	5 \pm 5	n.d.
7	Cu^{2+} -CgmR	1a	2b	3b	7 \pm 3	27 \pm 3 (+)-R
8	QacR- Cu^{2+}	1a	2b	3b	27 \pm 12	26 \pm 2 (+)-R
9	Cu^{2+}	1a	2c	3c	< 5	-
10	Cu^{2+} -RamR	1a	2c	3c	< 5	n.d.
11	Cu^{2+} -CgmR	1a	2c	3c	< 5	n.d.
12	Cu^{2+} -QacR	1a	2c	3c	< 5	n.d.
13	Cu^{2+}	1a	2d	3d	10 \pm 6	-
14	Cu^{2+} -RamR	1a	2d	3d	19 \pm 3	35 \pm 3 (+)
15	Cu^{2+} -CgmR	1a	2d	3d	31 \pm 14	37 \pm 1 (+)
16	Cu^{2+} -QacR	1a	2d	3d	39 \pm 10	9 \pm 4 (+)
17	Cu^{2+}	1a	2e	3e	Y < 5	-
18	Cu^{2+} -RamR	1a	2e	3e	Y < 5	n.d.
19	Cu^{2+} -CgmR	1a	2e	3e	Y < 5	n.d.
20	Cu^{2+} -QacR	1a	2e	3e	17 \pm 8	38 \pm 5 (+)
21	Cu^{2+}	1a	2f	3f	< 5	-
22	Cu^{2+} -RamR	1a	2f	3f	< 5	n.d.
23	Cu^{2+} -CgmR	1a	2f	3f	< 5	n.d.
24	Cu^{2+} -QacR	1a	2f	3f	< 5	n.d.
25	Cu^{2+}	1b	2b	3g	54 \pm 9	-
26	Cu^{2+} -RamR	1b	2b	3g	< 5	n.d.
27	Cu^{2+} -CgmR	1b	2b	3g	18 \pm 4	6 \pm 2
28	Cu^{2+} -QacR	1b	2b	3g	59 \pm 7	75 \pm 4
29	Cu^{2+}	1c	2b	3h	< 5	-
30	Cu^{2+} -RamR	1c	2b	3h	< 5	n.d.
31	Cu^{2+} -CgmR	1c	2b	3h	< 5	n.d.
32	Cu^{2+} -QacR	1c	2b	3h	< 5	n.d.

Typical conditions: 90 μM $\text{Cu}(\text{NO}_3)_2$ (9 mol%) loading with 1.3 eq of protein (120 μM), 1 mM of substrate **1** and **2** in 20 mM MOPS buffer pH 7.0, 500 mM NaCl, for 72 h at 4 $^\circ\text{C}$. All the results listed correspond to the average of two independent experiments, each carried out in duplicate. Errors listed are standard deviations.^a Yields were determined by HPLC and using 2-phenylquinoline as internal standard.^b Sign of rotation was assigned by comparison with elution order in chiral HPLC.^{3,6} For yields <5% ee's were not determined.

Table S2. Control experiments for catalytic vinylogous Friedel–Crafts alkylation reactions

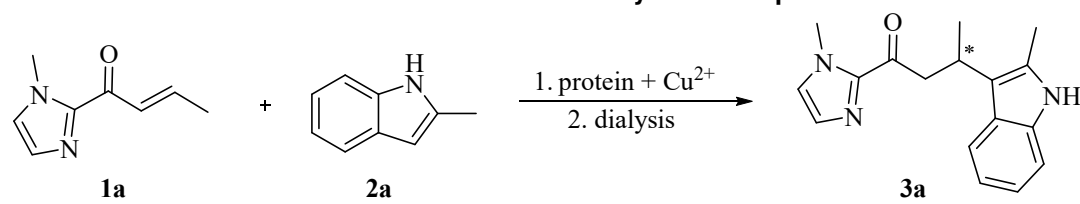
Entry	catalyst	Yield (%)	ee (%)
1	-	< 5	n.d.
2	Cu^{2+}	22 ± 7	-
3	$\text{Cu}(\text{phen})$	43 ± 2	7 ± 3 (-)
4	RamR	5 ± 6	8 ± 6 (-)
5	Cu^{2+} cRamR	57 ± 9	29 ± 3 (-)
6	$\text{Cu}(\text{phen})$ cRamR	21 ± 13	34 ± 7 (-)
7	CgmR	6 ± 3	10 ± 21 (-)
8	Cu^{2+} cCgmR	52 ± 12	13 ± 3 (-)
9	$\text{Cu}(\text{phen})$ cCgmR	30 ± 3	15 ± 1 (-)
10	QacR	11 ± 7	13 ± 19 (-)
11	Cu^{2+} cQacR	78 ± 11	34 ± 3 (-)
12	$\text{Cu}(\text{phen})$ cQacR	36 ± 6	30 ± 2 (-)

Typical conditions: 90 μM $\text{Cu}(\text{NO}_3)_2$ (9 mol %) loading with 1.3 eq of protein (120 μM), 1 mM of substrate **1** and **2** in 20 mM MOPS buffer pH 7.0, 500 mM NaCl, for 72 h at 4 °C. All the results listed correspond to the average of two independent experiments, each carried out in duplicate. Errors listed are standard deviations.^a Yields were determined by HPLC and using 2-phenylquinoline as internal standard. ^b Sign of rotation was assigned by elution order in chiral HPLC. For yields <5% ee's were not determined.

Table S3. Inhibition experiments for catalytic vinylogous Friedel–Crafts alkylation reactions

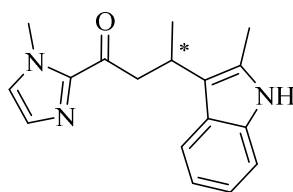
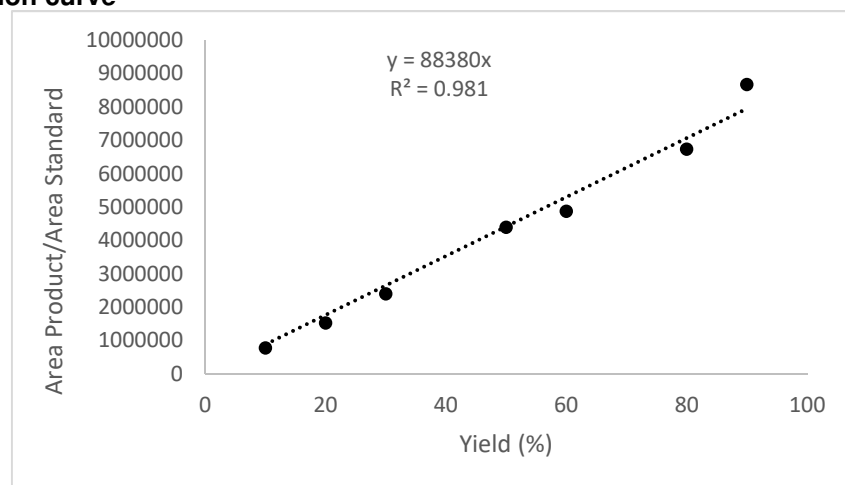
Entry	Catalyst	Dye/equivalents	Yield (%)	ee (%)
1	$\text{Cu}(\text{NO}_3)_2$	-	22 ± 7	-
2 ^a	$\text{Cu}(\text{NO}_3)_2$	Ethidium/1	13 ± 0	-
3 ^a	$\text{Cu}(\text{NO}_3)_2$	Ethidium/4	13 ± 1	-
4 ^a	$\text{Cu}(\text{NO}_3)_2$	Ethidium/8	12 ± 0	-
5 ^a	$\text{Cu}(\text{NO}_3)_2$	R6G/1	12 ± 1	-
6 ^a	$\text{Cu}(\text{NO}_3)_2$	R6G/4	10 ± 1	-
7 ^a	$\text{Cu}(\text{NO}_3)_2$	R6G/8	12 ± 1	-
8	Cu^{2+} cQacR	-	78 ± 11	34 ± 3 (-)
9	Cu^{2+} cQacR	Ethidium/1	60 ± 7	25 ± 1 (-)
10	Cu^{2+} cQacR	Ethidium/4	36 ± 9	5 ± 3 (+)
11	Cu^{2+} cQacR	Ethidium/8	27 ± 10	9 ± 2 (+)
12	Cu^{2+} cQacR	R6G/1	76 ± 9	32 ± 1 (-)
13	Cu^{2+} cQacR	R6G/4	44 ± 4	15 ± 4 (-)
14	Cu^{2+} cQacR	R6G/8	33 ± 17	7 ± 9 (-)
15	Cu^{2+} cRamR	-	57 ± 9	29 ± 3 (-)
16	Cu^{2+} cRamR	Ethidium/1	32 ± 8	32 ± 4 (-)
17	Cu^{2+} cRamR	Ethidium/4	26 ± 6	31 ± 0 (-)
18	Cu^{2+} cRamR	Ethidium/8	17 ± 3	29 ± 2 (-)
19	Cu^{2+} cCgmR	-	52 ± 12	13 ± 3 (-)
20	Cu^{2+} cCgmR	Ethidium/1	39 ± 11	3 ± 2 (-)
21	Cu^{2+} cCgmR	Ethidium/4	25 ± 9	6 ± 1 (+)
22	Cu^{2+} cCgmR	Ethidium/8	18 ± 4	11 ± 1 (+)

^aThe reactions were performed in duplicate.

Table S4. Control Friedel-Crafts reaction with dialyzed Cu²⁺-protein

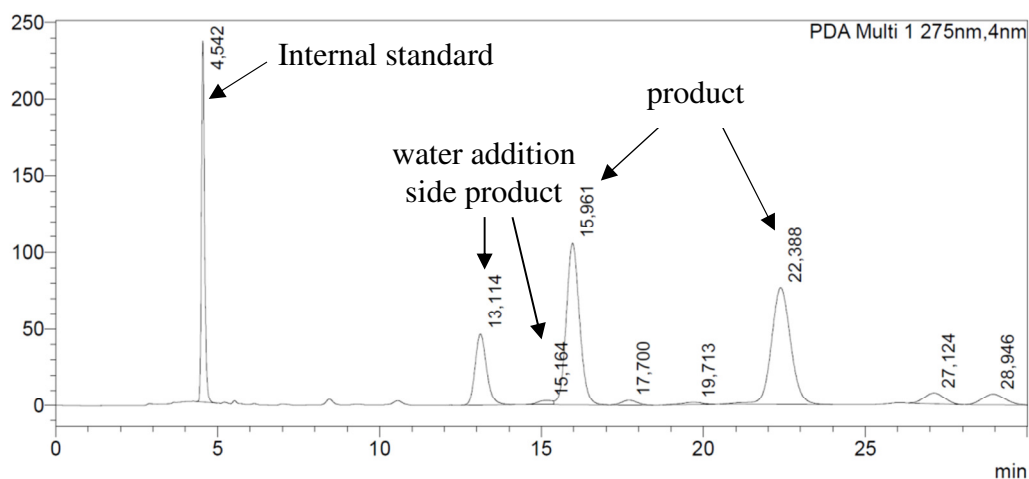
Entry	Protein	Dialysis	Yield (%)	ee (%)
1	Cu ²⁺ -QacR	NO	78 ± 11	34 ± 3
2	Cu ²⁺ -QacR	YES	29 ± 1	48 ± 0
3	Cu ²⁺ -RamR	NO	57 ± 9	29 ± 3
4	Cu ²⁺ -RamR	YES	13 ± 2	43 ± 4
5	Cu ²⁺ -CgmR	NO	52 ± 12	13 ± 3
6	Cu ²⁺ -CgmR	YES	24 ± 8	15 ± 2

Typical conditions: The proteins were incubated with 1.1 excess of Cu(NO₃)₂ for 1 h. Subsequently, the 1 mL samples were dialyzed against 1 L of 20 mM MOPS, 500 mM NaCl, pH7 (reaction buffer) overnight and the recovered samples were then used for catalysis as previously described. The reactions were performed in duplicates.

Figure S6: calibration curve and chiral HPLC traces obtained for biocatalytic reactions with product 3a**3a****S6.1. Calibration curve**

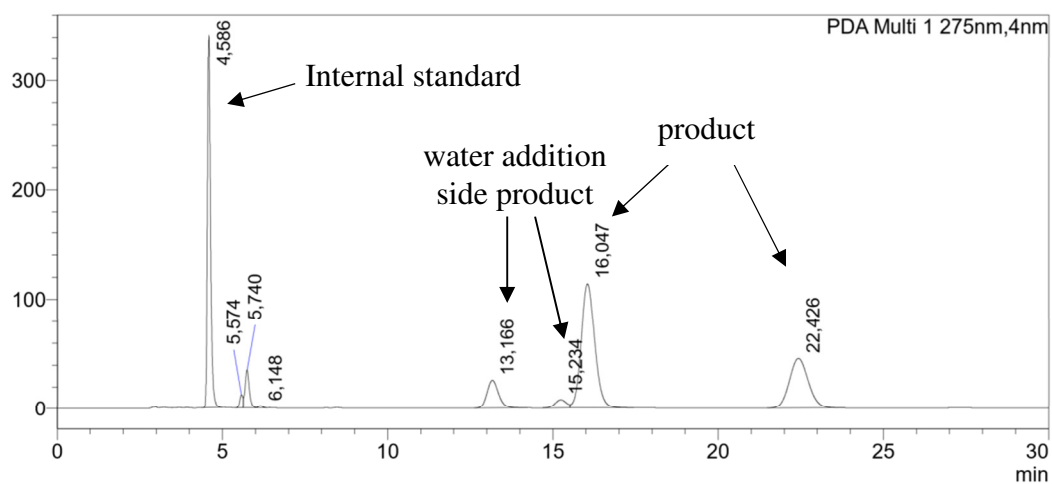
S6.2. Catalyst: $\text{Cu}(\text{NO}_3)_2$

mAU



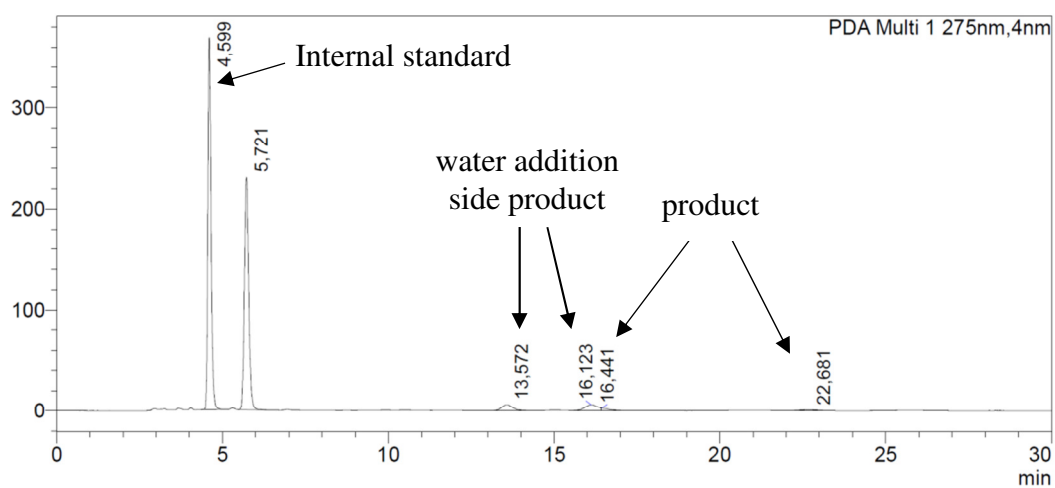
S6.3. Catalyst: Cu^{2+} -RamR

mAU

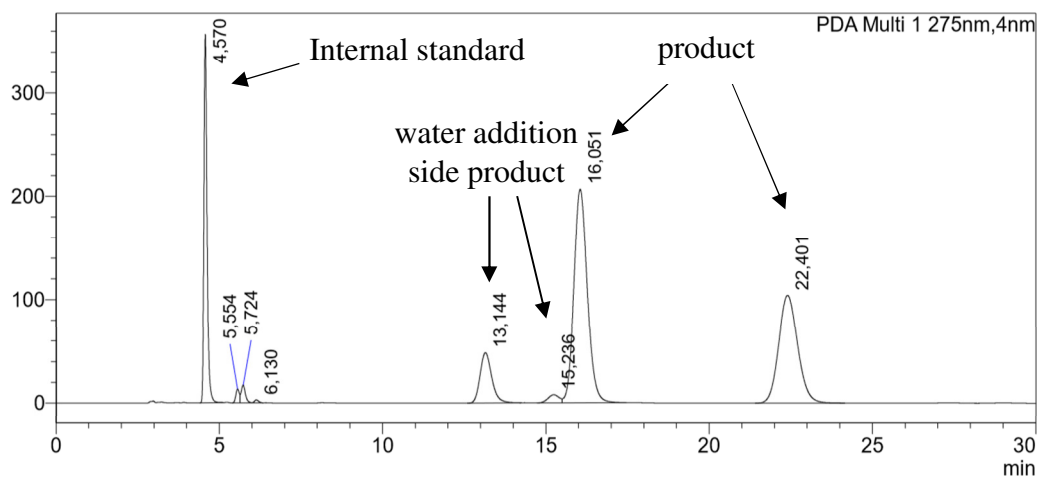


S6.4. Catalyst: RamR

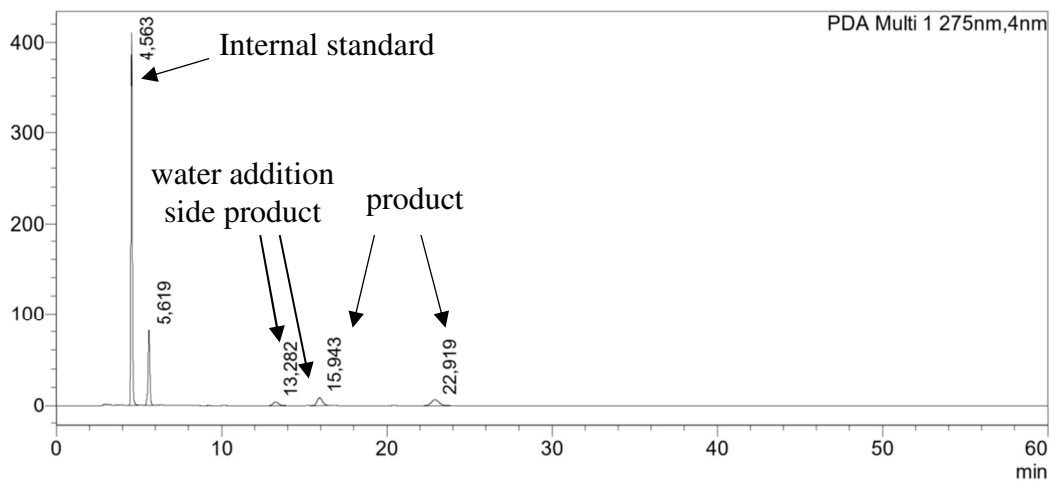
mAU



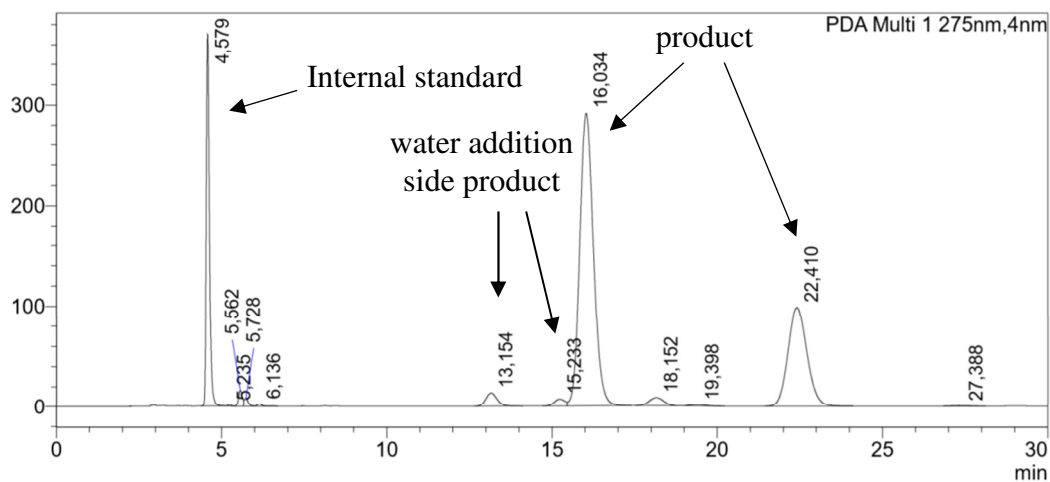
S6.5. Catalyst: Cu²⁺-CgmR
mAU



S6.6. Catalyst: CgmR
mAU



S6.7. Catalyst: Cu²⁺-CQacR
mAU



S6.8. Catalyst: QacR

mAU

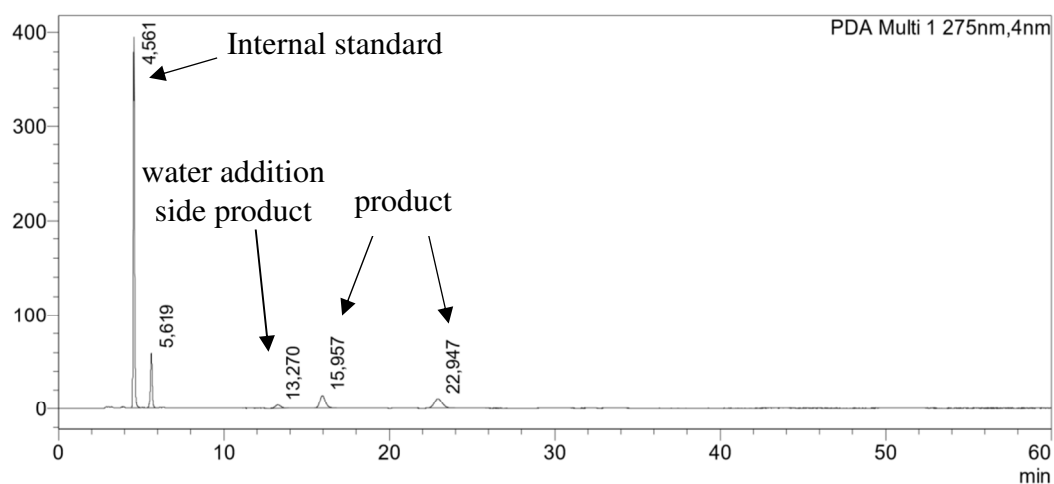
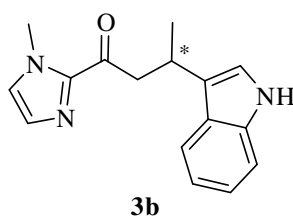
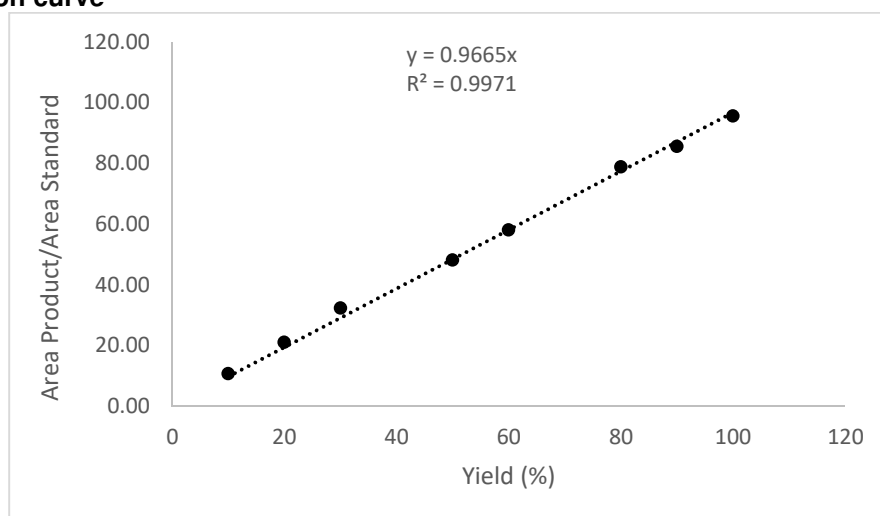


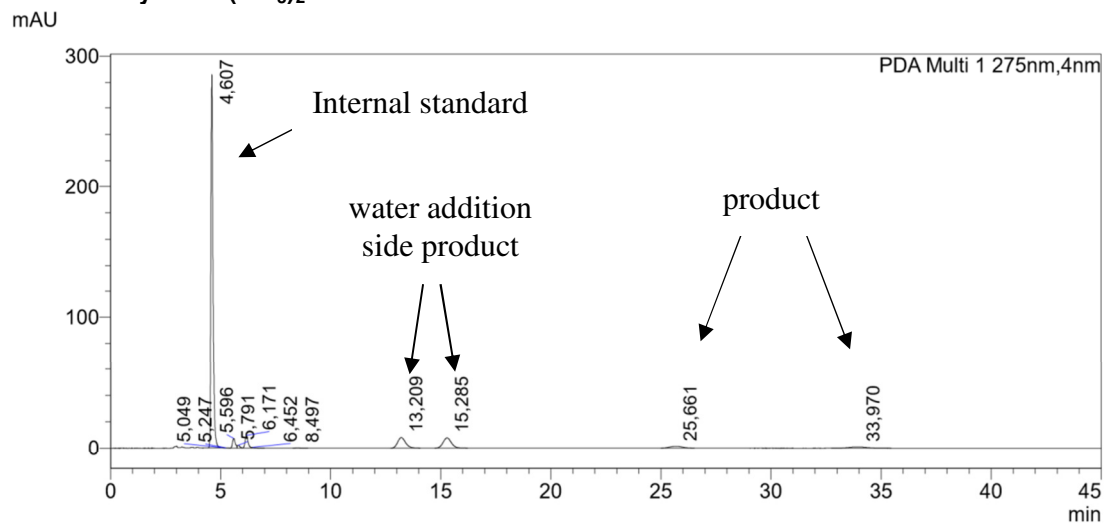
Figure S7: calibration curve and chiral HPLC traces obtained for biocatalytic reactions with product 3b



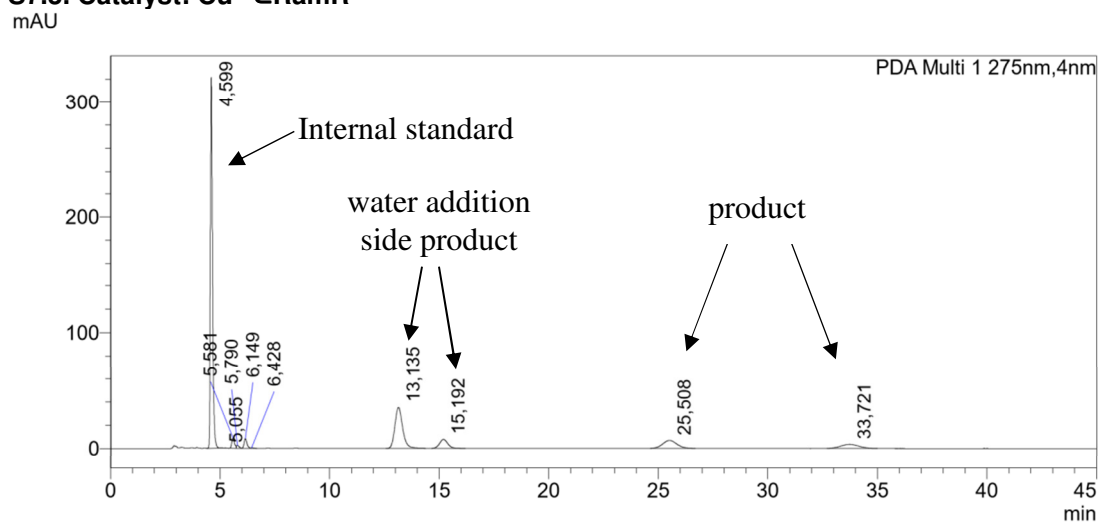
S7.1. Calibration curve



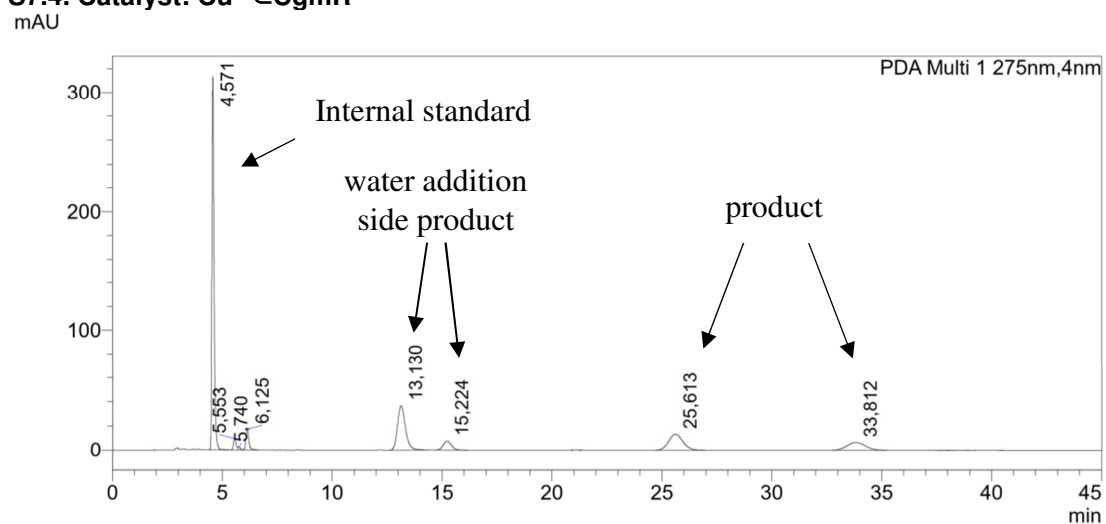
S7.2. Catalyst: $\text{Cu}(\text{NO}_3)_2$



S7.3. Catalyst: Cu^{2+} -cRamR



S7.4. Catalyst: Cu^{2+} -cCgmR



S7.5. Catalyst: Cu²⁺-CQacR

mAU

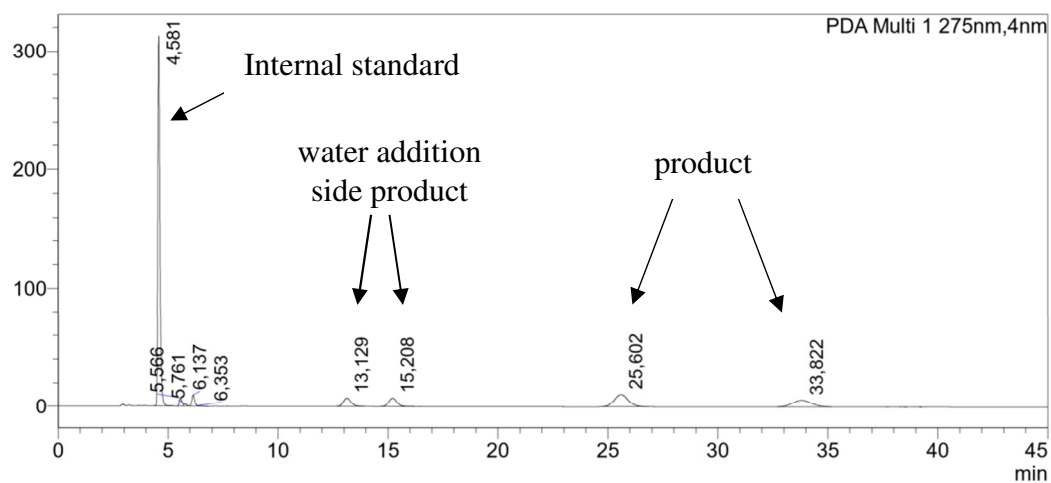
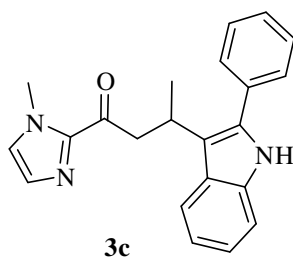
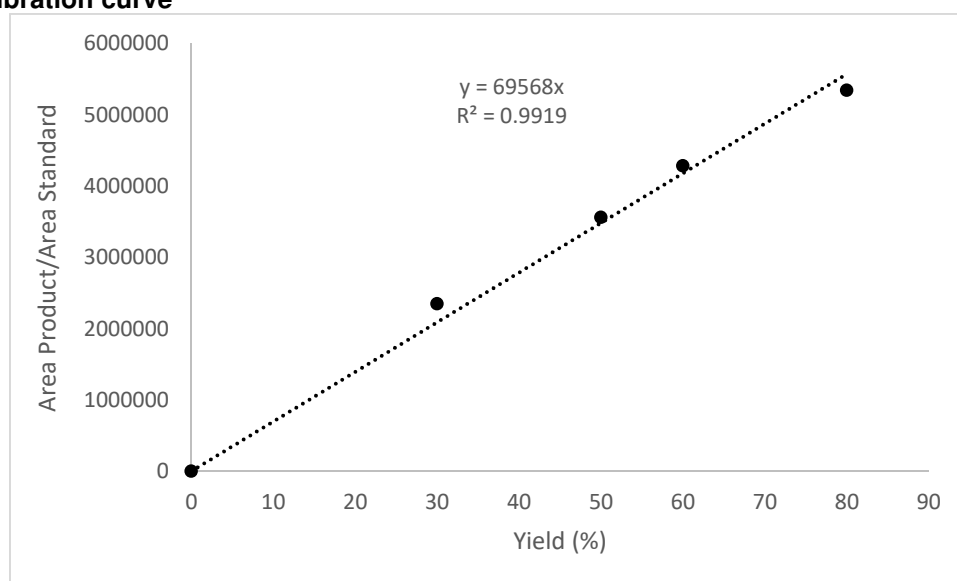


Figure S8: calibration curve and chiral HPLC traces obtained for biocatalytic reactions with product 3c

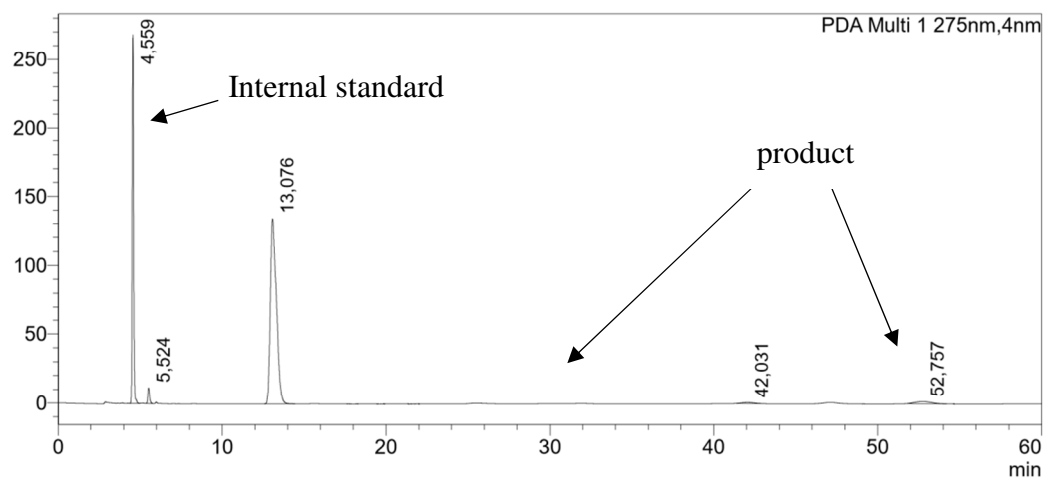


S8.1. Calibration curve



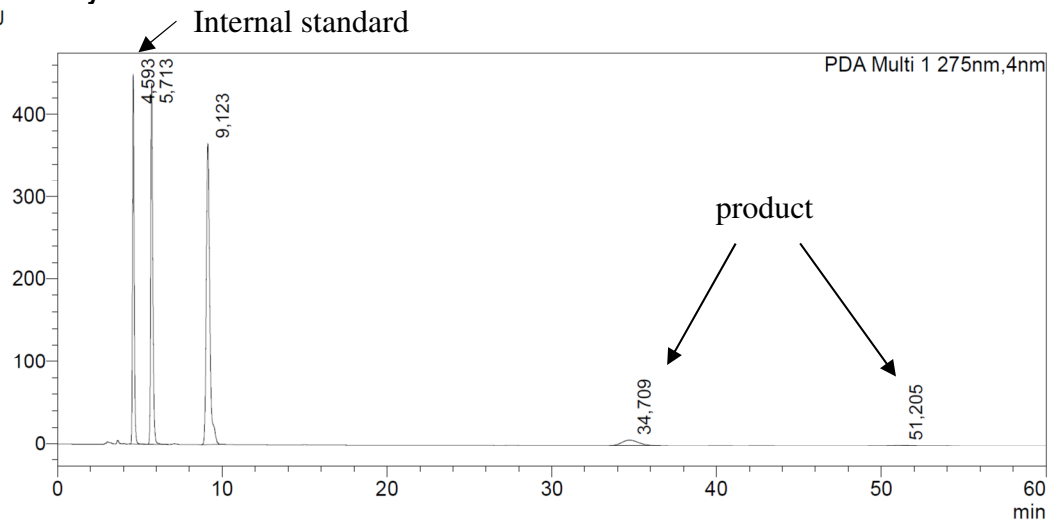
S8.2. Catalyst: $\text{Cu}(\text{NO}_3)_2$

mAU



S8.3. Catalyst: Cu^{2+} -cRamR

mAU



S8.4. Catalyst: Cu^{2+} -cGmR

mAU

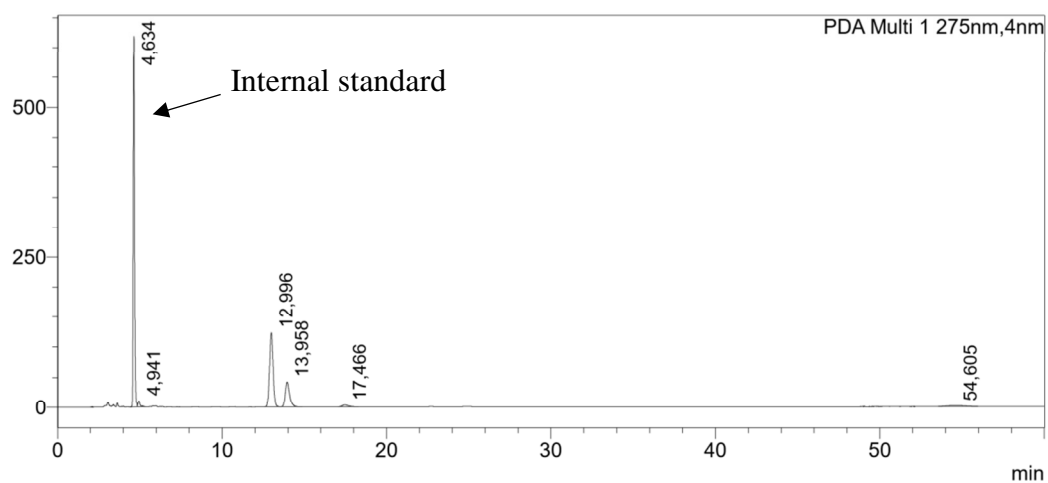
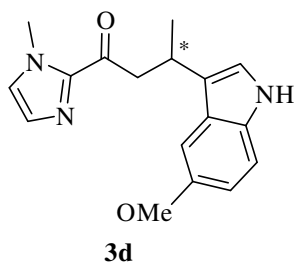
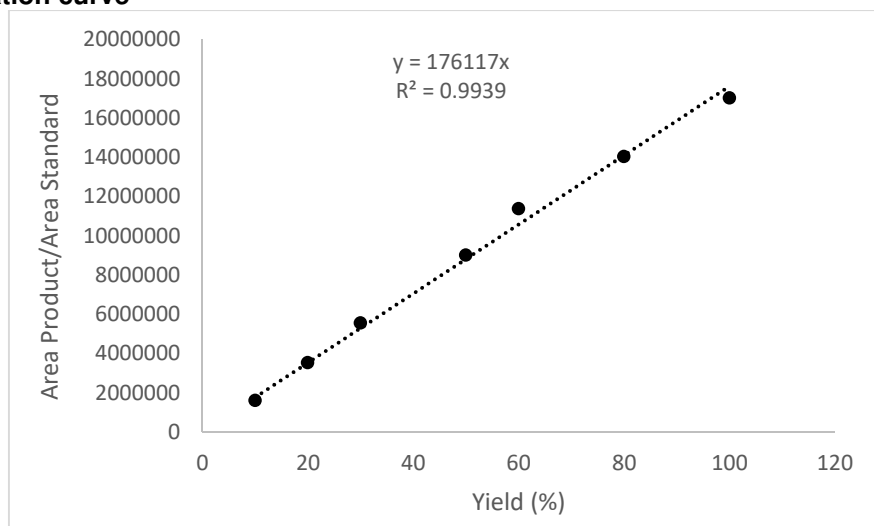


Figure S9: calibration curve and chiral HPLC traces obtained for biocatalytic reactions with product 3d

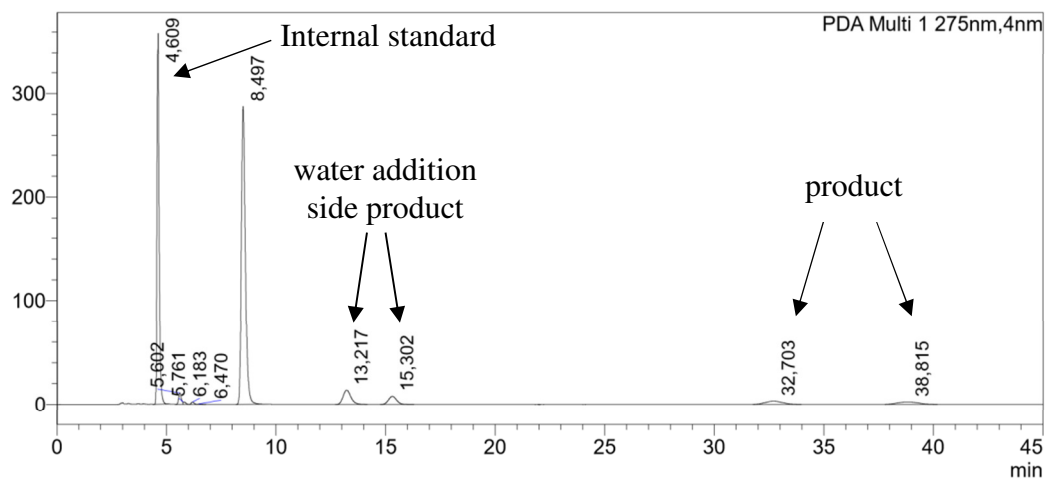


S9.1. Calibration curve

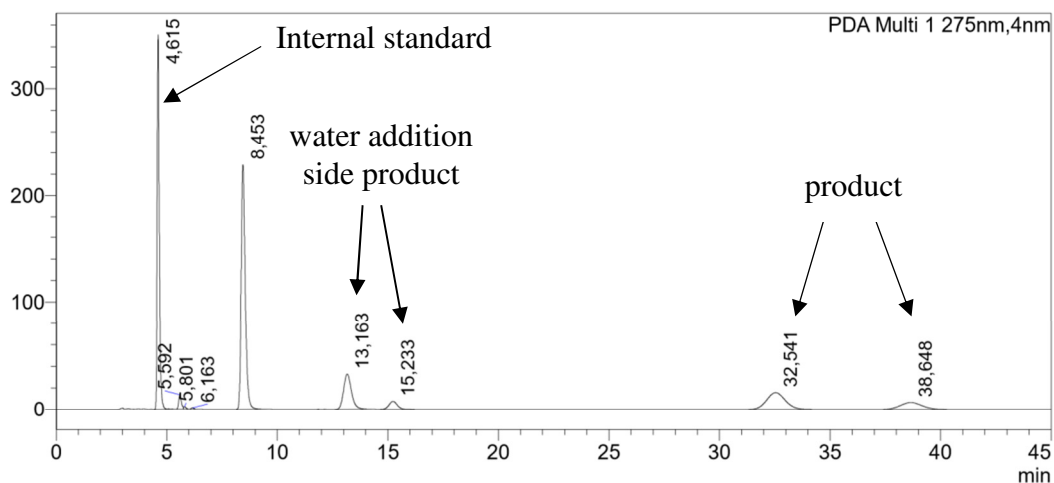


S9.2. Catalyst: $\text{Cu}(\text{NO}_3)_2$

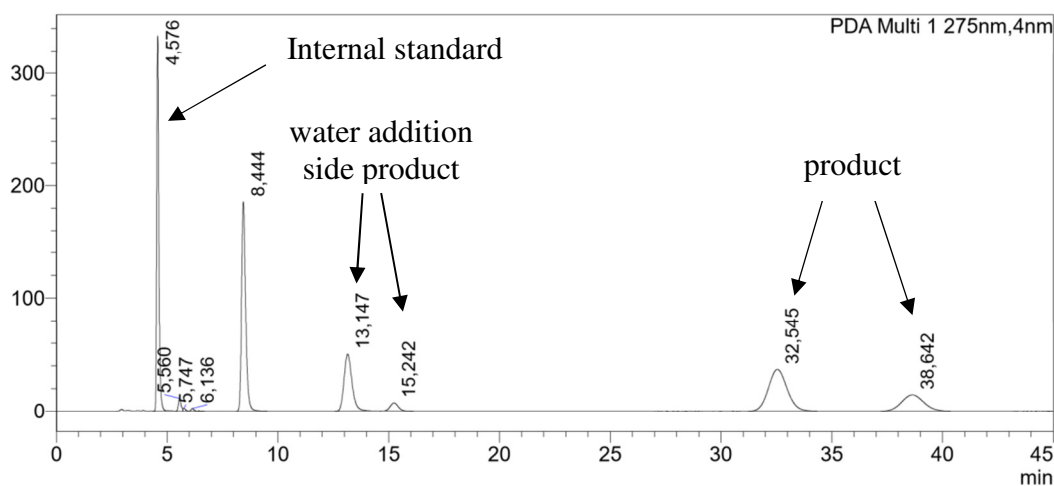
mAU



S9.3. Catalyst: Cu²⁺-cRamR
mAU



S9.4. Catalyst: Cu²⁺-cCgmR
mAU



S9.5. Catalyst: Cu²⁺-cQacR
mAU

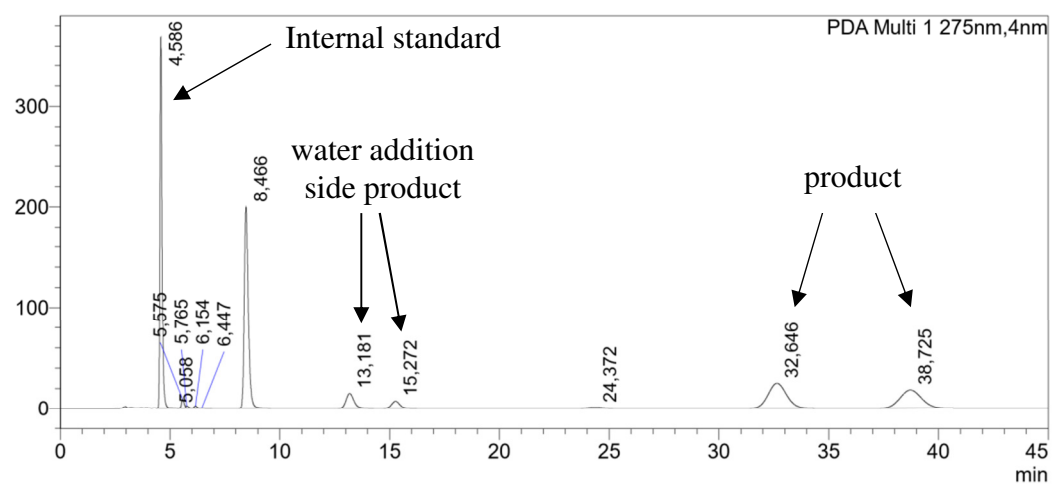
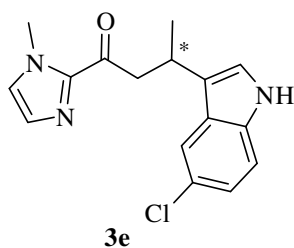
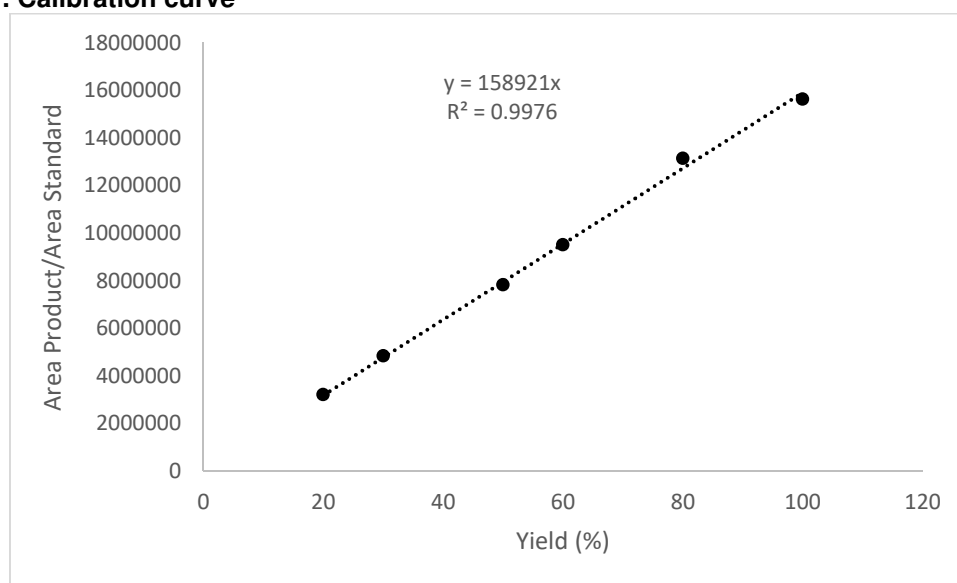


Figure S10: calibration curve and chiral HPLC traces obtained for biocatalytic reactions with product 3e

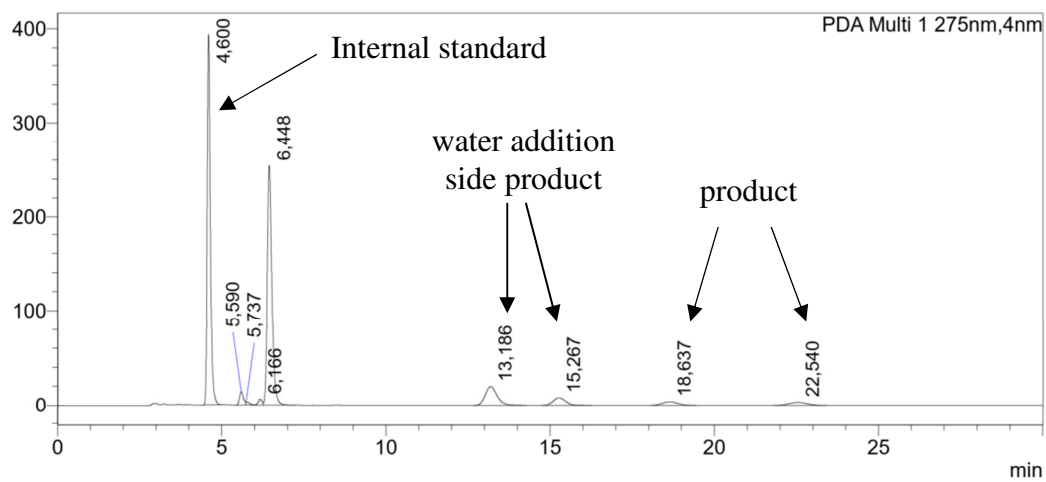


S10.1. Calibration curve



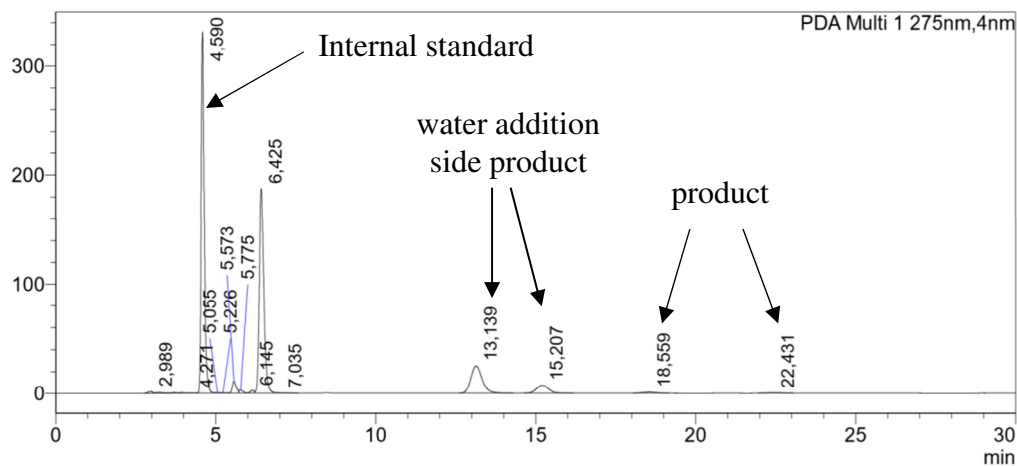
S10.2. Catalyst: Cu(NO₃)₂

mAU



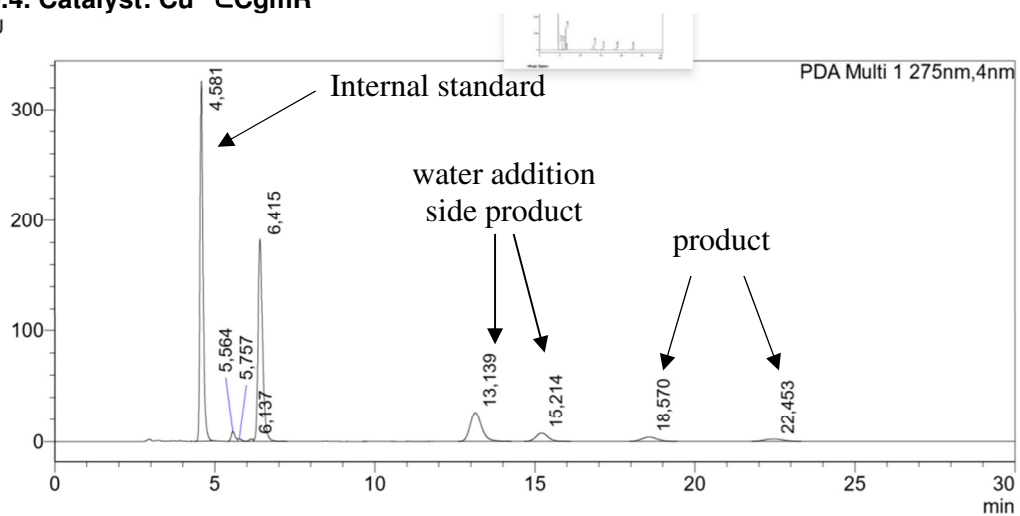
S10.3. Catalyst: Cu²⁺cRamR

mAU



S10.4. Catalyst: Cu²⁺cCgmR

mAU



S10.5. Catalyst: Cu²⁺cQacR

mAU

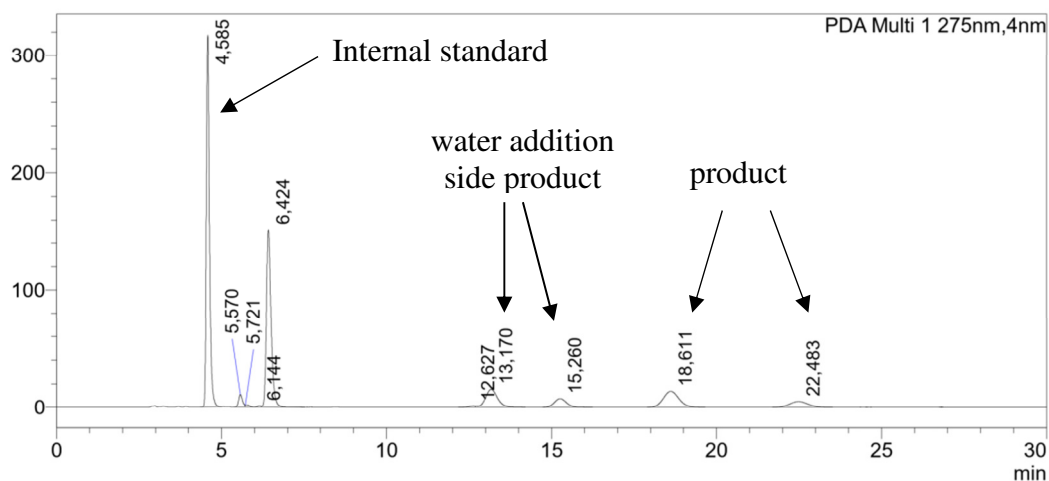
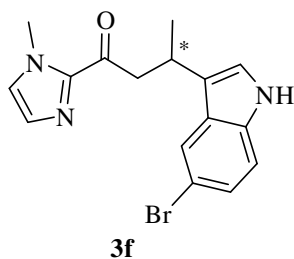
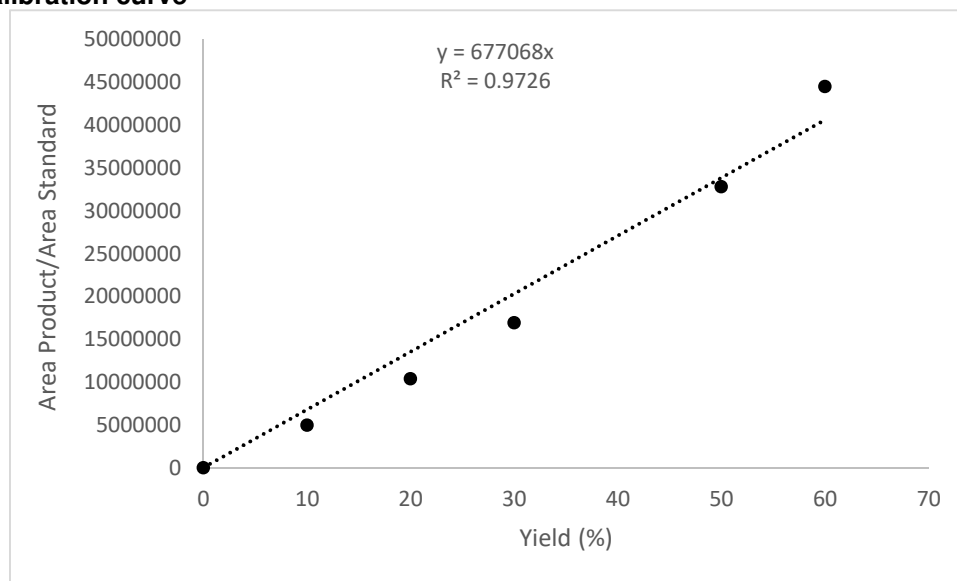


Figure S11: calibration curve and chiral HPLC traces obtained for biocatalytic reactions with product 3f

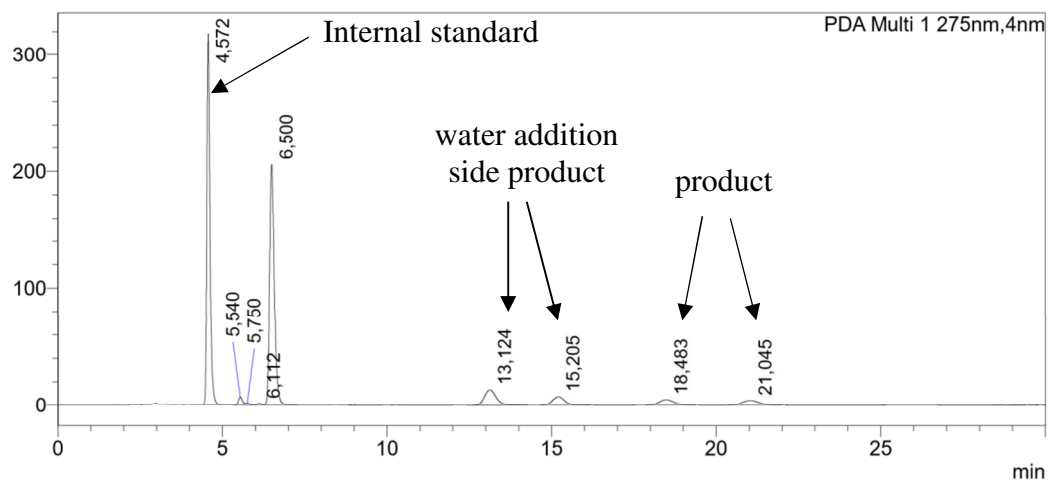


S11.1. Calibration curve

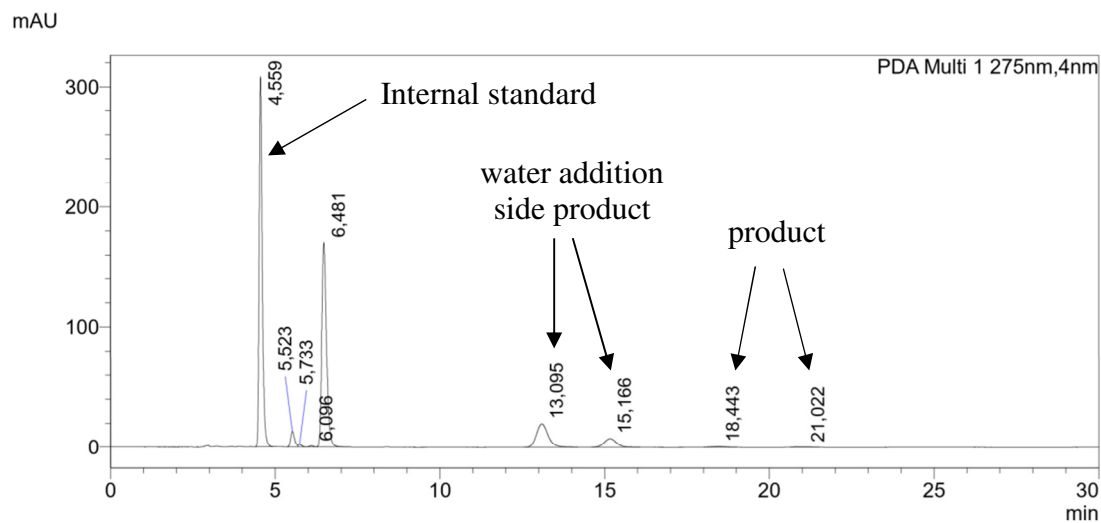


S11.2. Catalyst: $\text{Cu}(\text{NO}_3)_2$

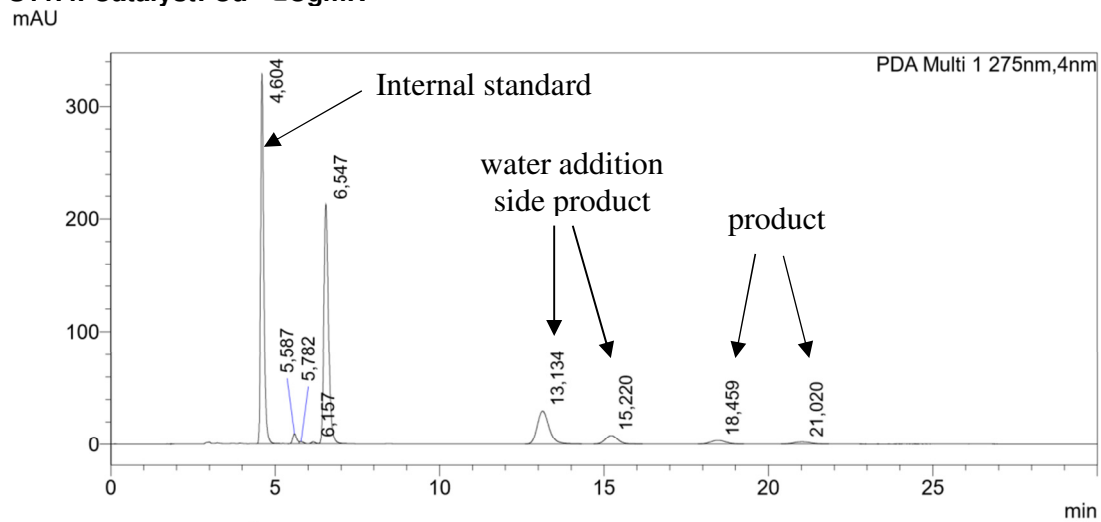
mAU



S11.3. Catalyst: Cu²⁺-cRamR



S11.4. Catalyst: Cu²⁺-cGmR



S11.5. Catalyst: Cu²⁺-cQacR

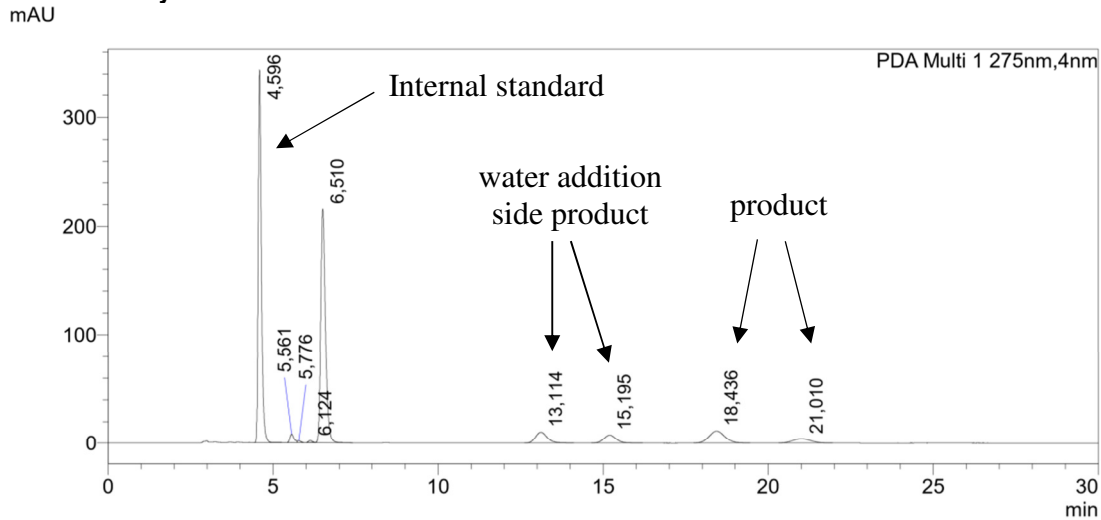
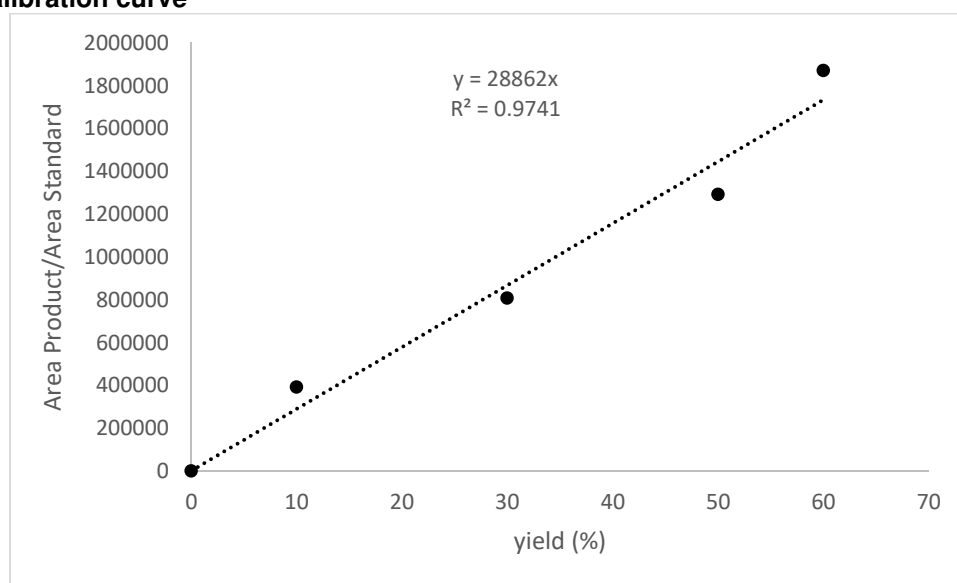


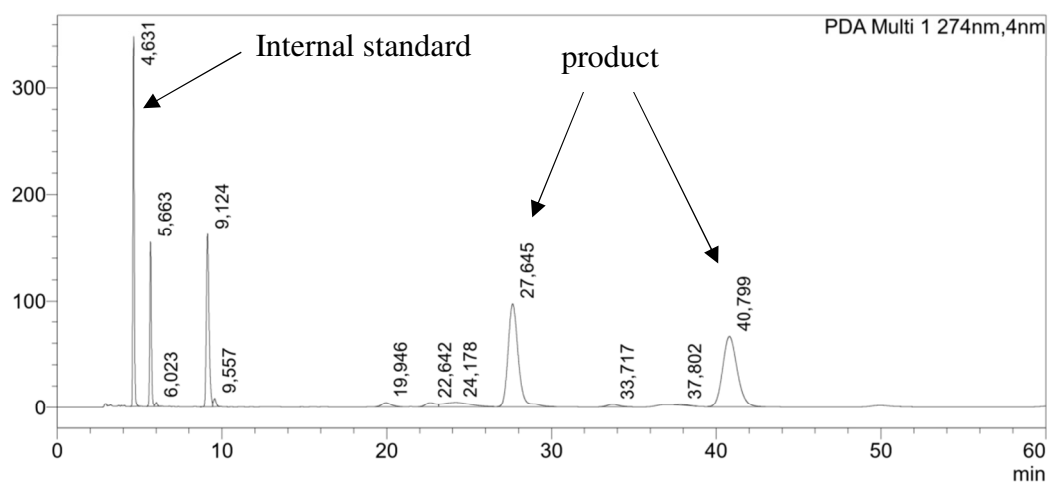
Figure S12: calibration curve and chiral HPLC traces obtained for biocatalytic reactions with product 3g

S12.1. Calibration curve

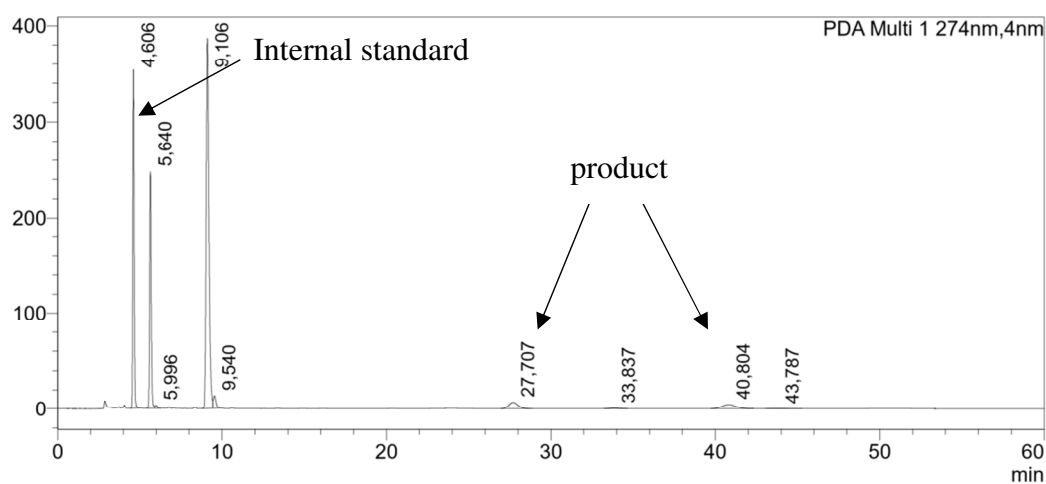


S12.2. Catalyst: $\text{Cu}(\text{NO}_3)_2$

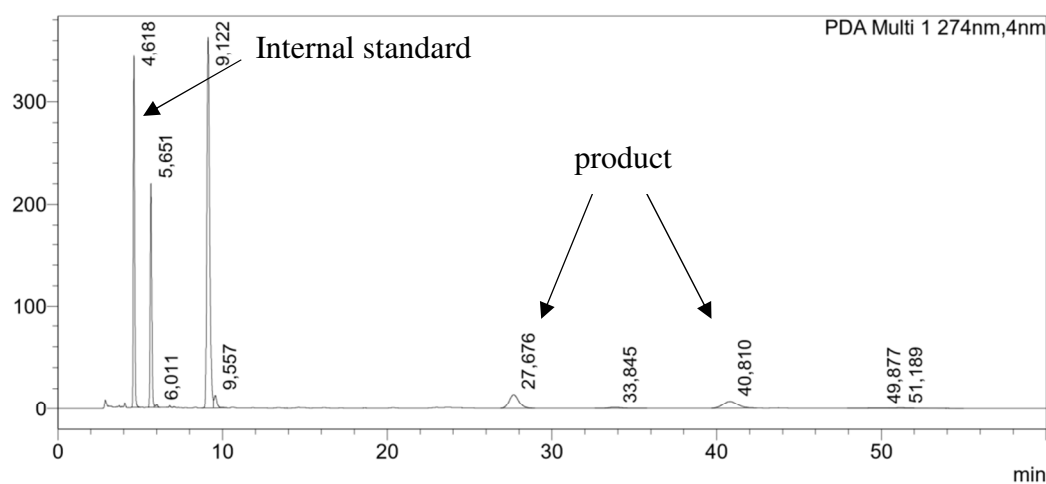
mAU



S12.3. Catalyst: Cu²⁺cRamR
mAU



S12.4. Catalyst: Cu²⁺cGmR
mAU



S12.5. Catalyst: Cu²⁺cQacR
mAU

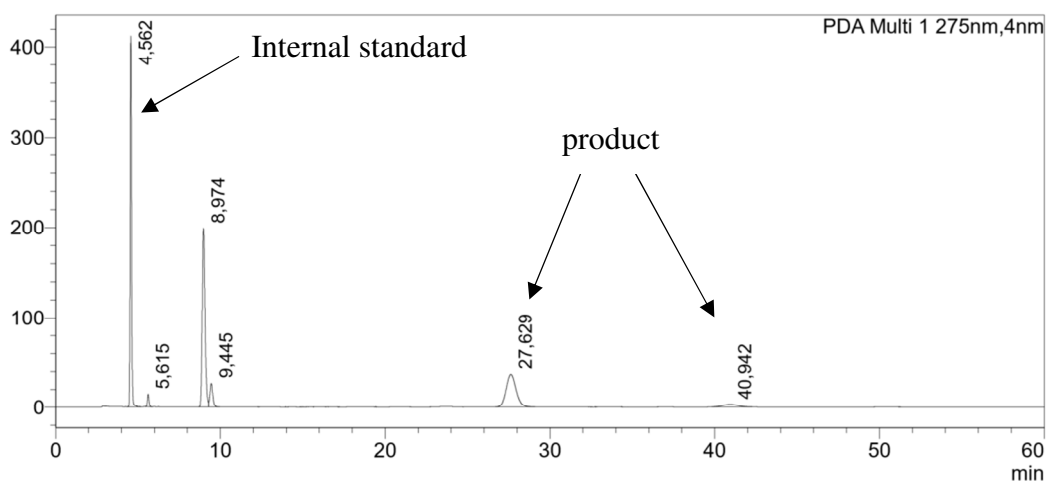
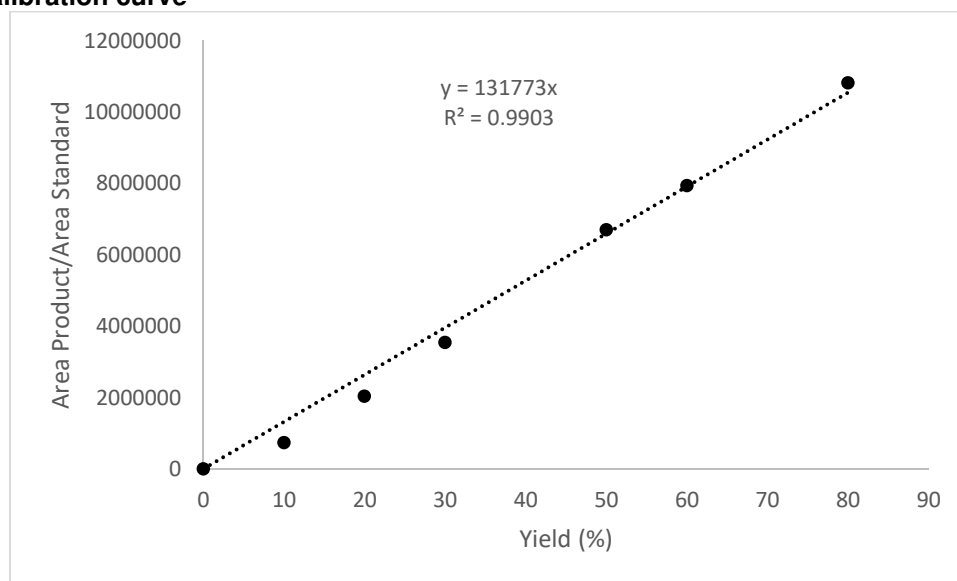


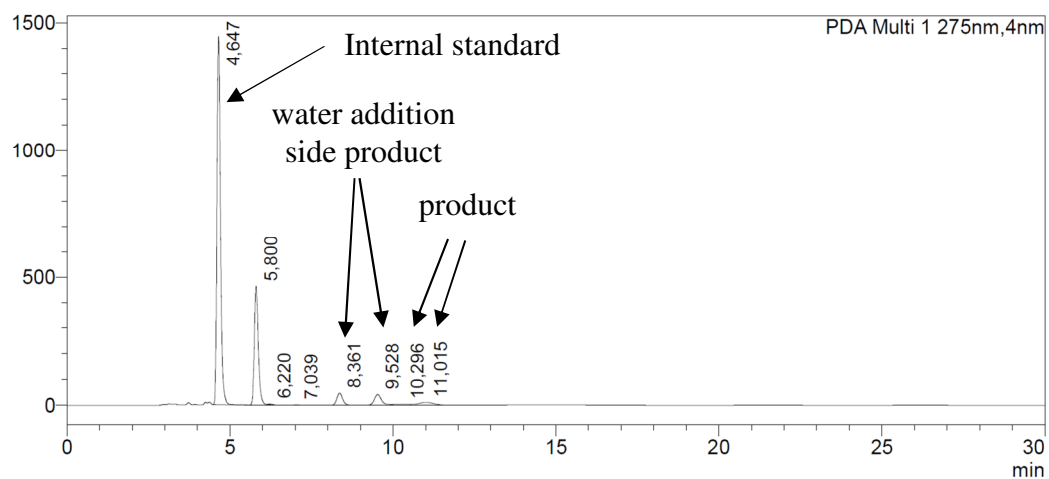
Figure S13: calibration curve and chiral HPLC traces obtained for biocatalytic reactions with product 3h

S13.1. Calibration curve

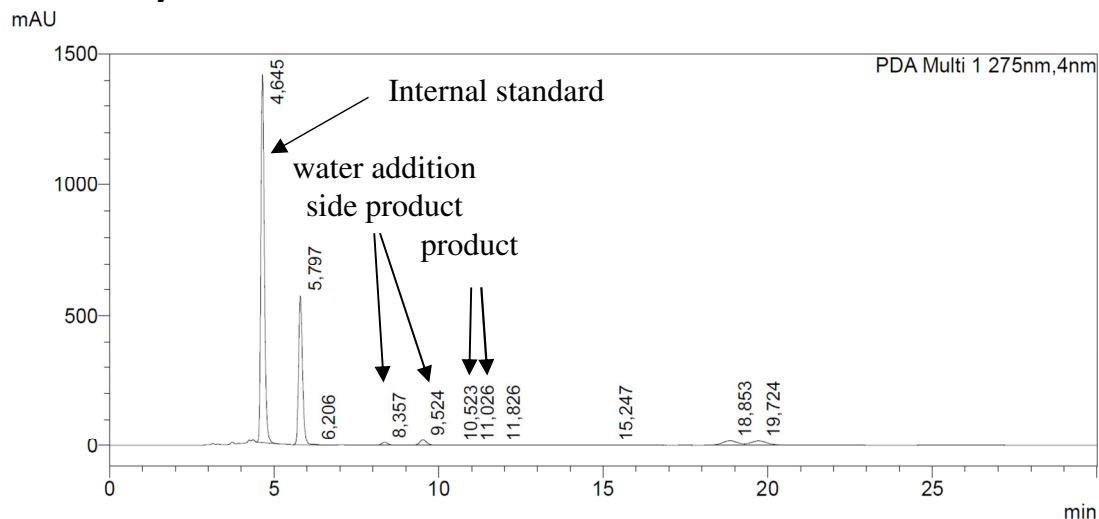


S13.2. Catalyst: $\text{Cu}(\text{NO}_3)_2$

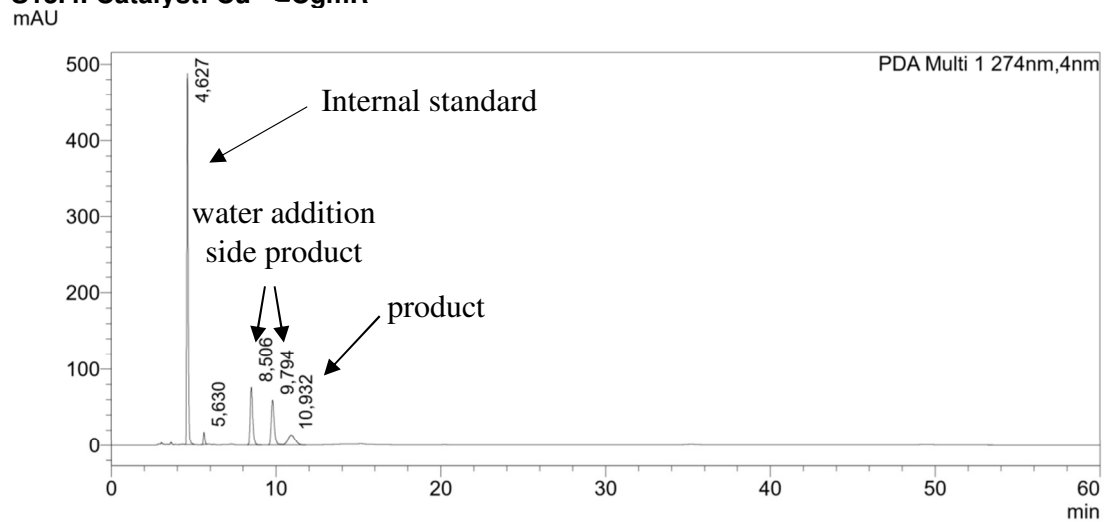
mAU



S13.3. Catalyst: Cu²⁺-cRamR



S13.4. Catalyst: Cu²⁺-cGmR



S13.5. Catalyst: Cu²⁺-cQacR

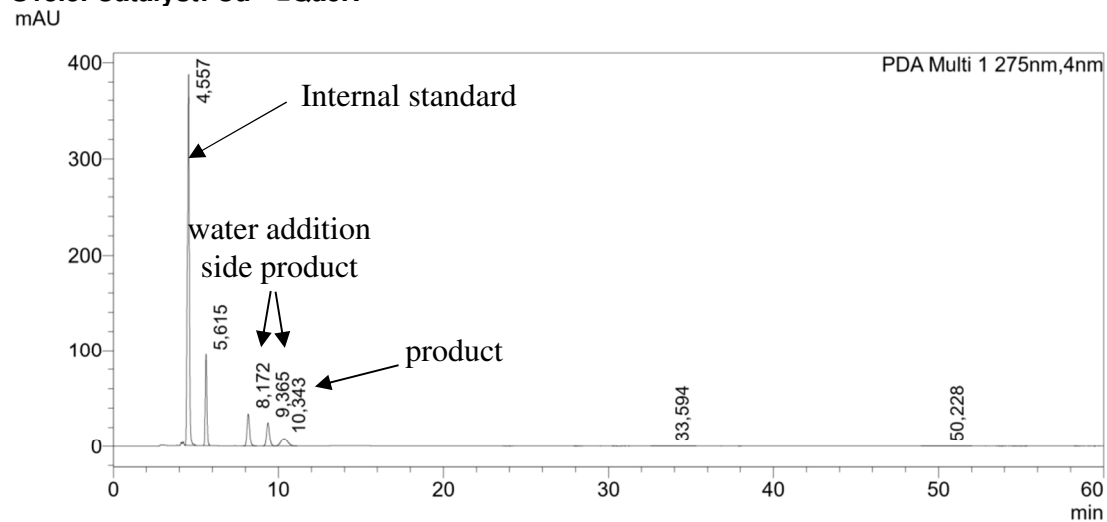
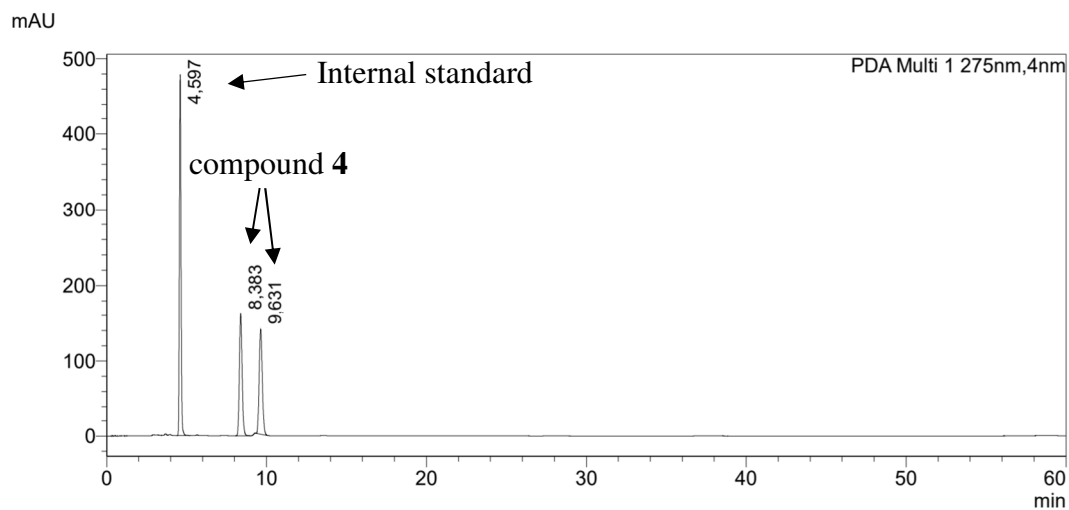
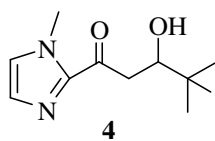


Figure S14. Chiral HPLC traces for water addition product (4)



7. References

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