Experimental Lineage and Functional Analysis of a Remotely Directed Peptide Epoxidation Catalyst

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General information

NMR spectra were acquired at ambient temperature. The concentration of the peptide NMR samples was 20 mM with respect to peptide, unless otherwise specified. Spectra were processed using MestReNova 8.1 using default phasing and baseline correction features. $^1$H NMR spectra are calibrated to an internal TMS standard (0.00 ppm) or residual solvent peak (7.26 ppm in CDCl$_3$). $^{13}$C NMR spectra were completely proton decoupled and are calibrated to CHCl$_3$ (77.16 ppm). NMR spectral data are reported as chemical shift (multiplicity, coupling constants, integration). Multiplets were reported from the “multiplet analysis” feature of MestreNova with some manual adjustment. Multiplicity is reported as follows: singlet (s), doublet (d), doublet of doubles (dd), triplet (t), quartet (q), and multiplet (m), etc.

Gas chromatography (GC) was performed on an instrument with two HP-5 columns (50 m, 0.320 mm diameter, 0.25 µm film thickness) and flame ionization detectors using He as a carrier gas. Site-selectivity ratios for the monoepoxides of farnesol are reported as the area of the indicated peak to the sum of the areas of all the monoepoxide products. For method detail and characterization of farnesol oxidation products, see the Supplementary Information of Lichtor, P. A.; Miller, S. J. *Nature Chem.* 2012, 4, 990-995. Details pertaining to other GC methods are included where relevant.

Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 Å F$_{254}$ pre-coated plates (0.25 mm thickness) and visualized using irradiation by a UV lamp and/or staining with I$_2$/silica, and/or cerium ammonium molybdate (CAM). Flash column chromatography was performed using Silica Gel 60 Å (32-63 micron).

Peptide sequencing and high-resolution liquid chromatography-mass spectrometry (HRMS) was performed on a Waters XEVO instrument equipped with ESI, a QToF mass spectrometer, and a photodiode array detector.

Reverse phase chromatography was performed with a Biotage instrument.
On-bead library synthesis and screening

Study of split-and-pool libraries

Split-and-pool libraries were synthesized and screened as described previously. Briefly described, the peptide libraries were synthesized on Polystyrene A NH₂ macrobeads (500-560 µm) using double deprotections and double couplings to Fmoc-protected amino acids (except when coupling to the N-terminal Boc-Asp(OFm)-OH). Before synthesizing peptide libraries, the resin was first coupled to Fmoc-Met-OH and then to three Fmoc-Ahx-OH (Ahx = 6-aminohexanoic acid) residues.

Study of parallel synthesis library

Peptides sequences that were evaluated in Table 1 were synthesized on RAM macrobeads (500-560 µm), which were first coupled to a single Fmoc-Ahx-OH residue (3 equiv; 3 equiv HOBt, 3 equiv HBTU; 6 equiv iPr₂EtN; DMF).

Peptide sequences were then attached to the resin by first deprotecting with two ~20 min treatments with 20% (v/v) piperidine in DMF and then using single couplings in DMF with Fmoc-protected amino acids (4 equiv), HBTU (4 equiv), HOBt•H₂O (4 equiv), and iPr₂EtN (8 equiv). Upon addition of the coupling reagents, the resin mixture was sealed and generally mixed for 3-6 h on a rotary mixer, and then drained, washing with a combination of DMF, DCM, and/or CH₃OH. Following the final coupling to Boc-Asp(OFm)-OH, the resin was then treated with two ~20 min treatments with 20% (v/v) piperidine in DMF, and then washed with copious DMF, DCM, and/or CH₃OH.

1 Lichtor, P. A.; Miller, S. J. Nature Chem. 2012, 4, 990-995.
Some of the on-bead synthesized peptides were checked by treating a single bead with 100% TFA for 30 min and then concentrating the resulting mixture under N₂. The resulting cleaved material was analyzed by LC/MS/MS and confirmed to contain expected peptide/fragment masses.

To 400 µL glass reaction vials containing a single bead was added 0.5 µL of an aqueous solution of H₂O₂ (2 M, 1 equiv). Immediately following the H₂O₂ addition, 5.05 µL of a DCM solution containing farnesol (1 µmol, 1 equiv), DIC (0.3 µmol, 0.3 equiv), HOBt•H₂O (0.1 µmol, 0.1 equiv), and DMAP (0.1 µmol, 0.1 equiv) was then added to start the reaction. The vials were sealed and allowed to stand for 12 h before quenching with 20 µL of a saturated aqueous solution of Na₂SO₃, vortexing with additional 220 µL hexanes, and analyzing by GC.

Testing, synthesis, and characterization of truncated peptides

General procedure for screening truncated catalyst analogs

Peptide (0.1 equiv) was added into a tared screw cap test tube with DCM. The solvent was concentrated, the tube was weighed, and the peptide mass was determined. Farnesol (1.0 equiv) was weighed into the tube and then a stir bar was added. A solution of HOBt•H₂O (0.1 equiv) and DMAP (0.1 equiv) in CHCl₃ (to bring reaction to 0.2 M with respect to farnesol; solvent passed through basic alumina) was then added, washing down the sides of the tube, followed by H₂O₂ (1.5 equiv, 30% w/w aqueous solution). The tube was placed in an ice bath and chilled. DIC (1.1 equiv) was added and the tube was immediately placed in an iPrOH bath maintained at –20 ºC by cryostat. The reaction was stirred for 48 h before quenching with saturated aqueous Na₂SO₃, warming to room temperature, and then mixing vigorously with additional saturated aqueous NaHCO₃ and hexanes. After the layers
separated, an aliquot of the organic layer was removed, diluted with hexanes and EtOAc and analyzed by GC.

**General protocols for truncated peptide synthesis**

**Solution peptide synthesis.** To peptide acid (1 equiv) was added amine coupling partner (1.0-1.1 equiv), EDC•HCl (1.1 equiv), HOBt•H2O (1.1 equiv), and DCM (to 0.1 M in peptide acid). iPr2EtN or Et3N (1.2 equiv) was also added if the amine coupling partner was the hydrochloride salt. The resulting solution was allowed to stir until the reaction was deemed complete as determined by LCMS, usually 3-48 h. The reaction solution was diluted with additional DCM and then washed with aqueous 0.5 M citric acid, half-saturated brine, and saturated aqueous NaHCO3. The combined organics were dried over Na2SO4, filtered, and concentrated.

**Solid phase peptide coupling protocol.** To pre-swollen resin was added Fmoc-protected amino acid (3 equiv), HBTU (3 equiv), HOBt•H2O (3 equiv) with DMF, followed by iPr2EtN (6 equiv). The resin mixture was sealed and mixed for 3-6 h on a rotary mixer and then drained, washing with a combination of DMF, DCM, and/or CH3OH. If required for the next coupling, the Fmoc-protecting group was removed with two treatments of 20% piperidine in DMF (v/v) for 20 min each.

**Standard Fm-ester deprotection.** To protected peptide was added 1:1 (v/v) solution of Et2NH and DCM. The resulting solution was swirled occasionally over the course of 30-60 min before concentrating via rotary evaporator. The resulting material was dissolved in DCM and then concentrated further. Typically, the crude material was loaded on a silica gel column as a solution in DCM, where it was eluted with a gradient of 1% CH3OH to 4% CH3OH in 1% AcOH/DCM.

**Hydrogenolysis Protocol.** To a flask containing peptide and stir bar was added a slurry of 10% (wt.) Pd/C in CH3OH (~0.1 M). The flask was sealed with a septum and the reaction atmosphere was flushed
first with $N_2$, and then $H_2$. The reaction was allowed to stir under pressure of a balloon filled with $H_2$. Once the reaction was deemed complete as determined by LCMS, the reaction atmosphere was flushed with $N_2$. The crude reaction mixture was then filtered through celite and concentrated.

**Synthesis and characterization of truncated peptides**

![Synthesis diagram]

**SI Figure 1.** Synthesis of peptides 7 and 8.

Synthesis of peptide 7 and 8 began from common intermediate SI-1 (SI Figure 1), which was synthesized starting with 2-chlorotrityl resin, preloaded with Asn(Trt), and then coupling the appropriate amino acid monomers (Fmoc-Thr(Bn)-OH, Fmoc-dPro-OH, and then Boc-Asp(Ofm)-OH) using the Solid Phase Peptide Coupling protocol. Resin was cleaved with a 4:1:1 mixture of DCM: 2,2,2-trifluoroethanol: acetic acid, treating for 30-60 min before draining. The resulting solution, along with washes, was concentrated a couple of times with additional toluene.

Intermediate SI-1 was coupled to HCl•H$_2$N-Tyr(tBu)-OCH$_3$ (1.1 equiv) using the Solution Peptide Synthesis protocol with additional Et$_3$N. After work-up, the crude material was deprotected using the...
Standard Fm-ester Deprotection protocol, and upon concentration, was purified with a silica gel column eluted with a gradient of 1% to 4% CH₃OH in 1% AcOH/DCM (as described in the standard protocol). A portion of this material was further purified by reverse phase chromatography using a Biotage C18 column eluting with a gradient of 40% CH₃OH to 100% CH₃OH in water with 0.1% formic acid.

Peptide 7: white solid. TLC: 4% CH₃OH, 1% AcOH in DCM (Rₜ =0.38). \(^{1}H\) NMR (500 MHz, CDCl₃): δ 7.62 (d, J = 7.9 Hz, 1H), 7.52 (s, 1H), 7.46 (d, J = 7.4 Hz, 1H), 7.32 – 7.10 (m, 20H), 6.97 (d, J = 8.5 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H), 6.64 (d, J = 9.2 Hz, 1H), 6.22 (d, J = 10.3 Hz, 1H), 4.89 – 4.82 (m, 1H), 4.81 – 4.73 (m, 1H), 4.63 – 4.55 (m, 1H), 4.49, 4.41 (ABq, J_AB = 11.4 Hz, 2H), 4.47 – 4.42 (m, 1H), 4.36 (d, J = 8.7 Hz, 1H), 3.98 (t, J = 7.4 Hz, 1H), 3.71 – 3.50 (m, 5H), 3.12 (dd, J = 15.1, 9.6 Hz, 1H), 3.02 (dd, J = 13.9, 6.0 Hz, 1H), 2.96 (dd, J = 13.8, 6.4 Hz, 1H), 2.74 – 2.65 (m, 1H), 2.63 – 2.54 (m, 2H), 2.17 – 1.97 (m, 3H), 1.90 – 1.79 (m, 1H), 1.43 (s, 9H), 1.31 (s, 10H), 1.17 (d, J = 6.4 Hz, 3H). \(^{13}C\) NMR (126 MHz, CDCl₃): δ 173.5, 172.4, 171.6, 171.1, 170.7, 170.1, 169.1, 155.5, 154.4, 144.8, 137.9, 130.9, 130.0, 129.0, 128.6, 128.2, 128.0, 127.9, 127.0, 124.3, 80.4, 78.4, 73.8, 72.4, 70.7, 61.5, 57.9, 54.6, 52.3, 50.3, 49.6, 47.9, 37.6, 37.1, 36.7, 29.0, 28.9, 28.4, 25.9, 17.3. IR (film in/from DCM, cm⁻¹): 3311, 2977, 1672, 1632, 1505, 1447, 1366, 1236, 1208, 1161, 1091, 1048, 1026. HRMS: calculated mass for [C₆₂H₇₄N₆O₁₃+H]⁺: 1111.5387, ESI+ found 1111.5427.

Peptide 8: white solid. Intermediate SI-1 was coupled to methanol using the Solution Peptide Synthesis protocol, except instead of performing the reaction in DCM, it was performed in 0.2 M methanol. After work-up, the crude reaction mixture was purified by flash silica gel chromatography with a gradient of 0% to 30% acetone in toluene (TLC: 25% acetone in toluene, Rₜ = 0.45, visualized by UV, I₂, and CAM). The resulting material was deprotected using the Standard Fm-ester Deprotection protocol, and upon concentration, was purified with a silica gel column eluted with a gradient of 1% to 4% CH₃OH in 1% AcOH/DCM (as described in the standard protocol).
4% CH₂OH in 1% AcOH/DCM (as described in the protocol). A portion of this material was further purified by reverse phase chromatography using a Biotage C18 column eluting with a gradient of 40% CH₂OH to 100% CH₂OH in water with 0.1% formic acid.

Peptide 8: white solid. **TLC:** 4% CH₂OH, 1% AcOH, DCM (R<sub>f</sub> = 0.32). ¹H NMR (500 MHz, CDCl₃): δ 8.35 (d, J = 6.9 Hz, 1H), 7.33 (d, J = 4.3 Hz, 4H), 7.36 – 7.18 (m, 20H), 7.16 (s, 1H), 6.65 (d, J = 9.2 Hz, 1H), 6.26 (d, J = 10.4 Hz, 1H), 4.67 (dt, J = 10.5, 4.0 Hz, 1H), 4.53 (apparent d, J = 11.3 Hz, 1H), 4.49 – 4.42 (m, 2H), 4.37 – 4.27 (m, 2H), 4.12 (t, J = 7.8 Hz, 1H), 3.73 – 3.65 (m, 1H), 3.65 – 3.53 (m, 4H), 3.38 (dd, J = 14.8, 10.1 Hz, 1H), 2.75 (dd, J = 14.8, 3.7 Hz, 1H), 2.61 (dd, J = 16.8, 3.5 Hz, 1H), 2.39 (dd, J = 16.7, 4.4 Hz, 1H), 2.22 – 2.00 (m, 3H), 1.91 – 1.79 (m, 1H), 1.47 (s, 9H), 1.15 (d, J = 6.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃): δ 174.0, 172.5, 171.9, 171.3, 170.9, 170.2, 155.4, 144.2, 138.1, 128.9, 128.6, 128.1, 128.0, 127.2, 80.3, 73.2, 72.1, 71.2, 62.3, 57.6, 52.5, 50.7, 49.9, 48.0, 38.8, 35.4, 29.0, 28.5, 26.2, 18.1. **IR** (film in/from DCM, cm⁻¹): 3322, 2978, 1669, 1632, 1493, 1447, 1367, 1285, 1265, 1231, 1208, 1162, 1080, 1047, 1026. **HRMS:** calculated mass for [C₄₉H₅₇N₅O₁₁+H]⁺: 892.4127, ESI+ found 892.4189.

**SI Figure 2.** Synthesis of peptide 9.

The standard Solution Peptide Coupling protocol was followed using Boc-Thr(Bn)-OH and CH₃NH₂•HCl, with iPr₂EtN (SI Figure 2). After 16 h, the reaction was worked up and then concentrated.
to ultimately yield a white powder. The N-terminus of Boc-Thr(Bn)-NHCH₃ was deprotected by treating with 4.0 M HCl/dioxanes for ~1.5 h before concentrating the resulting solution under a stream of N₂. The resulting yellow oil was dissolved in DCM and concentrated further. The material was then coupled to Boc-dPro-OH using the standard Solution Peptide Synthesis protocol with iPr₂EtN. After running for ~21 h, the reaction was worked up and concentrated to yield a whitish foam.

The resulting material was deprotected using 4.0 M HCl/dioxanes as before for ~2 h. Following HCl removal and concentration, DCM was added and the peptide was coupled to Boc-Asp(OFm)-OH using the standard Solution Peptide Coupling protocol with iPr₂EtN, running the reaction for ~32 h. Following work-up, the resulting yellowish thick oil was purified by flash column chromatography with a silica gel column packed in 10% acetone in toluene. The material was eluted with a gradient of 10% to 30% acetone in toluene (TLC: 20% acetone in toluene, Rᵣ = ~0.15, visualized by UV).

The tripeptide solid was subjected to the Standard Fm-ester Deprotection protocol. After a silica gel column, as the protocol describes, the resulting material was further purified on a C18 reverse phase column with a gradient of 40% MeOH in 0.1% (v/v) aqueous formic acid to 100% MeOH. Desired fractions were concentrated.

Peptide 9: white solid. TLC: 4% CH₃OH, 1% AcOH, DCM (Rᵣ =0.23). NMR chemical shift assignments made by ¹H-¹H gCOSY spectrum. ¹H NMR (500 MHz, CDCl₃): δ 7.39 – 7.27 (m, 6H, Ar-H’s and NHCH₃), 7.15 (d, J = 8.6 Hz, 1H, NH-Thr), 5.55 (d, J = 9.3 Hz, 1H, NHBoc), 4.85 – 4.77 (m, 1H, α-Asp), 4.54, 4.51 (ABq, J_AB = 11.6 Hz, 2H, -OCH₂Ph), 4.46 – 4.35 (m, 2H, α/β-Thr), 4.31 (t, J = 6.8 Hz, 1H, α-DPro), 3.78 – 3.63 (m, 2H, δ-DPro), 2.85 – 2.76 (m, 4H, -NHCH₃ and β-Asp), 2.69 (dd, J = 16.0, 4.4 Hz, 1H, β-Asp), 2.21 – 2.03 (m, 3H, β/γ-DPro), 1.99 – 1.88 (m, 1H, γ-DPro), 1.44 (s, 9H, Boc), 1.15 (d, J = 6.3 Hz, 3H, γ-Thr). ¹³C NMR (126 MHz, CDCl₃): δ 174.0, 172.5, 170.5, 170.1, 155.3, 137.9, 128.6, 128.1, 128.0, 80.6, 74.2, 72.3, 61.4, 57.6, 49.3, 47.8, 36.8, 29.0, 28.4, 26.7, 25.6,
17.1. **IR** (film in/from DCM, cm⁻¹): 3284, 3094, 2978, 2878, 1708, 1688, 1630, 1572, 1517, 1452, 1420, 1391, 1366, 1293, 1246, 1206, 1159, 1101, 1048, 1002. **HRMS**: calculated mass for \([C_{26}H_{38}N_{4}O_{8}+H]^+\): 535.2762, ESI+ found 535.2778.

**SI Figure 3.** Synthesis of peptide **10**.

The standard Solution Peptide Coupling protocol was followed using Boc-Thr(Bn)-OH and CH₃OH, which was used as a solvent instead of DCM (SI Figure 3). After 21 h, the crude reaction was concentrated using a rotary evaporator. The resulting material was dissolved in DCM and then worked up as described in the standard protocol. The N-terminus of Boc-Thr(Bn)-OCH₃ was deprotected by treating with 4.0 M HCl/dioxanes for ~50 min before concentrating the resulting solution under a stream of N₂. The resulting yellow oil was dissolved in DCM and concentrated further by rotary evaporation. The material was then coupled to Boc-DPro-OH using the standard Solution Peptide Coupling protocol with iPr₂EtN. After running for ~14 h, the reaction was worked up and concentrated.

The resulting off-white solid was deprotected using 4.0 M HCl/dioxanes as before, though for ~1.2 h. Following HCl removal and concentration, the resulting yellowish solid was dissolved in DCM and then coupled to Boc-Asp(OFm)-OH using the standard Solution Peptide Synthesis protocol with iPr₂EtN, running the reaction for ~20 h. Following work-up, the resulting white solid was purified by flash column chromatography with a silica gel column packed in toluene. The material was eluted with a gradient of 0% to 17% acetone in toluene to yield, upon concentration, a clear, colorless oil, which
turned to a solid upon standing under vacuum (TLC: 20% acetone in toluene (Rf =0.45)). The tripeptide solid was subjected to the Standard Fm-ester Deprotection protocol. After a silica gel column, as the protocol describes, the resulting yellow oil was further purified on a C18 reverse phase column with a gradient of 40% MeOH in 0.1% (v/v) aqueous formic acid to 100% MeOH. Desired fractions were concentrated.

Peptide 10: Clear, colorless, oily looking solid. TLC: 4% CH3OH, 1% AcOH in DCM (Rf =0.23), visualized with CAM. Material was determined to contain a mixture of conformational isomers using a TOCSY experiment. 1H NMR (500 MHz, CDCl3): δ 7.42 (d, J = 9.5 Hz, 1H), 7.38 – 7.32 (m, 2H), 7.32 – 7.23 (m, 3H), 5.72 (d, J = 9.2 Hz, 1H), 4.84 (td, J = 8.9, 4.2 Hz, 1H), 4.79 – 4.67 (m, 1H), 4.66 – 4.53 (m, 2H), 4.39 (d, J = 12.1 Hz, 1H), 4.09 (qd, J = 6.3, 2.3 Hz, 1H), 3.73 – 3.65 (m, 2H), 3.62 (s, 3H), 2.93 (dd, J = 16.4, 8.4 Hz, 1H), 2.75 (dd, J = 16.3, 4.1 Hz, 1H), 2.38 – 2.28 (m, 1H), 2.18 – 2.06 (m, 1H), 2.06 – 1.93 (m, 2H), 1.43 (s, 9H), 1.21 (d, J = 6.3 Hz, 3H). Minor conformer peaks were also observed: 7.00 (d, J = 9.3 Hz), 5.42 (d, J = 9.4 Hz), 5.01 (bs), 4.22 – 4.14 (m), 3.64 (s), 2.28 – 2.20 (m).

13C NMR (126 MHz, CDCl3): δ 173.5, 171.6, 171.2, 170.9, 155.4, 137.9, 128.5, 127.9, 127.9, 80.5, 73.9, 70.7, 60.1, 56.8, 52.4, 49.3, 47.2, 37.0, 28.4, 27.3, 25.0, 16.3. IR (film in/from DCM, cm⁻¹): 3307, 2978, 1710, 1685, 1640, 1518, 1437, 1367, 1268, 1249, 1207, 1159, 1084, 1048, 1026, 1000. HRMS: calculated mass for [C26H37N3O9+H]+: 536.2603, ESI+ found 536.2643.

SI Figure 4. Synthesis of peptide 11.
Boc-dPro-OH was coupled to \( n \)-butyl amine using the Solution Peptide Synthesis Protocol (SI Figure 4). Following work-up, the Boc group was removed by treatment with a 4.0 M solution of HCl in dioxanes. After 30 min, the solution was concentrated, dissolved in DCM, and then concentrated again. To the resulting crude oil was added coupling reagents, iPr\(_2\)EtN, and Boc-Asp(Bn)-OH, following the Solution Peptide Synthesis Protocol. After work-up, the material was dissolved in CH\(_3\)OH and purified using a Biotage C18 column with a gradient of 20% to 100% CH\(_3\)CN in water. Desired fractions were concentrated and the resulting material was deprotected following the Standard Hydrogenolysis Protocol. Finally, the resulting material was purified on a Biotage C18 column running a gradient of 20% to 100% CH\(_3\)CN in water with 0.1% formic acid. Desired fractions were collected and concentrated.

Peptide 11: white solid. Compound exists as a putative mixture of conformational isomers. **TLC**: 4% CH\(_3\)OH, 1% AcOH in DCM (\( R_f = 0.18 \)), visualized with I\(_2\)/silica. **\(^1\)H NMR** (500 MHz, CDCl\(_3\)): \( \delta \) 6.96 – 6.85 (m, 1H), 5.65 – 5.53 (m, 1H), 4.65 (apparent q, \( J = 6.9 \) Hz, 1H), 4.53 (d, \( J = 5.4 \) Hz, 1H), 3.90 – 3.81 (m, 1H), 3.70 (q, \( J = 7.9 \) Hz, 1H), 3.23 (dt, \( J = 13.2, 8.0 \) Hz, 1H), 3.18 – 3.07 (m, 1H), 2.85 (dd, \( J = 16.4, 7.3 \) Hz, 1H), 2.69 (dd, \( J = 16.4, 5.8 \) Hz, 1H), 2.31 – 2.20 (m, 1H), 2.08 – 1.92 (m, 3H), 1.53 – 1.36 (m, 11H), 1.30 (q, \( J = 7.4 \) Hz, 2H), 0.90 (t, \( J = 7.3 \) Hz, 3H). Minor conformer peaks also observed at 5.49 (d, \( J = 9.8 \) Hz), 4.73 – 4.69 (m), 3.59 (d, \( J = 8.3 \) Hz), 2.18 – 2.08 (m). **\(^{13}\)C NMR** (126 MHz, CDCl\(_3\)): \( \delta \) 173.0, 171.2, 171.0, 155.8, 80.8, 61.0, 49.4, 47.7, 39.8, 39.5, 36.4, 31.6, 28.9, 28.4, 24.7, 20.2, 13.9. **IR** (film in/from DCM, cm\(^{-1}\)): 3341, 2965, 2935, 2875, 1713, 1691, 1547, 1506, 1418, 1408, 1393, 1365, 1348, 1327, 1269, 1255, 1181, 1158, 1077, 1046, 1015. **HRMS**: calculated mass for [C\(_{18}\)H\(_{31}\)N\(_3\)O\(_6\)\(+\)H\(^+\)]\(^+\): 386.2286, ESI+ found 386.2314.
The Solution Peptide Synthesis protocol was used to couple Boc-Asp(Bn)-OH to pyrrolidine (SI Figure 5). Following work-up, the resulting oil was purified by flash silica gel chromatography, eluting with a gradient of 15% to 60% EtOAc in hexanes. Desired fractions were concentrated to yield a clear, colorless oil (TLC: 60% EtOAc in hexanes ($R_f=0.40$)). Much of the resulting material was deprotected using the Standard Hydrogenolysis Protocol, reacting for ~6.5 h. Material was further purified by elution through a Biotage C18 column with a gradient of 20% to 100% CH$_3$OH in aqueous 0.1% formic acid.

Peptide 12: white solid. **TLC**: 4% CH$_3$OH, 1% AcOH in DCM ($R_f=0.23$), visualized with I$_2$/silica. 

$^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 5.64 (d, $J=9.3$ Hz, 1H), 4.87 – 4.77 (m, 1H), 3.72 – 3.57 (m, 2H), 3.47 (td, $J=6.9$, 1.6 Hz, 2H), 2.82 (dd, $J=15.9$, 6.6 Hz, 1H), 2.71 (dd, $J=15.9$, 5.1 Hz, 1H), 2.02 – 1.93 (m, 2H), 1.93 – 1.81 (m, 2H), 1.44 (s, 9H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 173.1, 169.9, 155.4, 80.5, 49.0, 47.0, 46.6, 37.8, 28.5, 26.2, 24.3. **IR** (film in/from DCM, cm$^{-1}$): 3308, 2977, 1706, 1609, 1515, 1454, 1392, 1367, 1342, 1250, 1160, 1046, 1023, 1001. **HRMS**: calculated mass for [C$_{13}$H$_{22}$N$_2$O$_5$+H]$^+$: 287.1601, ESI+ found 287.1596.
NMR experiments and modeling of peptide 1

NMR experiments

NMR spectra were acquired on a Varian Inova 500 MHz spectrometer running VnmrJ 2.2D with pulse sequences provided by Varian. A combination of 1D $^1$H NMR with 2D gCOSY, TOCSY, and ROESY experiments (provided by Varian) were used to assign proton chemical shifts. T1 relaxation times were measured by performing an inversion-recovery experiment (INVREC, provided by Varian), acquiring $^1$H NMR spectra with different relaxation delay times ($d_1$) ranging from (0.0625 s to 30 s). The intensity ($M_z$) of each peak was measured as a function of time ($t$) and fit to Equation 1 using Prism Graphing Software. Measurements are shown in SI Table 1. All reported NMR data were collected on 20 mM samples of peptide in CDCl$_3$.

$$M_z = M_{01} + 2M_{02}e^{-t/T1} \quad (1)$$

SI Table 1. T1 relaxation values determined for protons within peptide 1.

| Entry | Signal       | T1 (s) | Entry | Signal       | T1 (s) |
|-------|--------------|--------|-------|--------------|--------|
| 1     | Boc(tBu)     | 0.395  | 18    | Aryl peaks   | 0.854  |
| 2     | NH-Asp       | 0.576  | 19    | NH-Asn(Trt) | 0.392  |
| 3     | $\alpha$-Asp | 0.496  | 20    | $\alpha$-Asn(Trt) | 0.590 |
| 4     | $\beta$-Asp  | 0.337  | 21    | $\beta$-Asn(Trt) | 0.279 |
| 5     | $\beta$-Asp  | 0.310  | 22    | $\beta$-Asn(Trt) | 0.285 |
| 6     | $\alpha$-DPro| 0.406  | 23    | $\delta$-Asn(Trt) | 0.319 |
| 7     | $\gamma$-DPro| 0.441  | 24    | NH-Tyr       | 0.434  |
| 8     | $\beta$-DPro | 0.419  | 25    | $\alpha$-Tyr(tBu) | 0.713 |
| 9     | $\gamma$-DPro| 0.408  | 26    | $\beta$-Tyr(tBu) | 0.337 |
| 10    | $\gamma$-DPro| 0.341  | 27    | $\beta$-Tyr(tBu) | 0.341 |
| 11    | $\delta$-DPro| 0.440  | 28    | $\delta$-Tyr(tBu) | 0.885 |
| 12    | NH-Thr(Bn)   | 0.350  | 29    | e-Tyr(tBu)   | 1.068  |
| 13    | $\alpha$-Thr(Bn) | 0.798  | 30    | Tyr(tBu)     | 0.796  |
| 14    | $\beta$-Thr(Bn) | 0.669  | 31    | NH-Gly       | 0.593  |
| 15    | $\gamma$-Thr(Bn) | 0.396  | 32    | $\alpha$-Gly | 0.510  |
| 16    | $\delta$-Thr(Bn) | 0.556  | 33    | $\alpha$-Gly | 0.494  |
| 17    | $\delta$-Thr(Bn) | 0.550  | 34    | OCH$_3$      | 0.884  |
ROESY data was acquired with a 200 ms mixing time; spectral width of 5102.4 Hz in both F1 and F2; 765 points in t2 and 512 points in t1; 128 scans; relaxation delay (d1) of 3.06 s (three times the highest T1 relaxation of 1.07 s less acquisition time of 0.15 s); and at 25 ºC. Data were processed using MestReNova version 8.1.1-11591 using zero-filling to bring the spectral size to 1024, 1024; automatic phasing with manual adjustments; apodization with a Gaussian function; and automatic baseline correction with a polynomial fit.

\(^1\)H NMR (500 MHz, CDCl\(_3\), 25 ºC) δ 7.61 – 7.53 (m, 2H, NH-Asn and Asn(NH)), 7.31 – 7.14 (m, 20H, Trt and Bn aryl peaks), 7.14 – 7.09 (m, 1H, NH-Gly), 7.07 (d, J = 8.4 Hz, 2H, δ-Tyr), 6.95 (d, J = 8.2 Hz, 1H, NH-Tyr), 6.89 (d, J = 8.4 Hz, 1H, ε-Tyr), 6.69 (d, J = 9.1 Hz, 1H, NH-Thr), 6.35 (d, J = 10.4 Hz, 1H, NH-Asp), 4.80 (dt, J = 10.2, 5.6 Hz, 1H, α-Asp), 4.67 (ddd, J = 11.2, 8.2, 5.3 Hz, 1H, α-Asn), 4.59 – 4.52 (m, 1H, α-Tyr), 4.49, 4.38 (ABq, J\(_{AB}\) = 11.4 Hz, 2H, Thr(CH\(_2\)Ph), 4.49 – 4.43 (m, 1H, β-Thr), 4.33 – 4.27 (m, 1H, α-Thr), 4.12 (t, J = 7.3 Hz, 1H, α-dPro), 3.77 (dd, J = 17.6, 6.8 Hz, 1H, α-Gly), 3.73 – 3.61 (m, 2H, δ-dPro, individual protons assigned in F2 of TOCSY spectrum to be 3.69 and 3.64), 3.58 (s, 3H, OCH\(_3\)), 3.42 (dd, J = 15.9, 11.2 Hz, 1H, β-Asn), 3.29 (dd, J = 14.2, 6.1 Hz, 1H, β-Tyr), 2.99 (dd, J = 14.2, 5.6 Hz, 1H, β-Tyr), 2.84 (dd, J = 17.6, 4.9 Hz, 1H, α-Gly), 2.77 (dd, J = 16.5, 6.1 Hz, 1H, β-Asp), 2.57 (dd, J = 16.4, 4.0 Hz, 1H, β-Asp), 2.47 (dd, J = 15.8, 5.3 Hz, 1H, β-Asn), 2.23 – 2.11 (m, 2H, β/γ-dPro), 2.11 – 1.98 (m, 1H, β-dPro), 1.95 – 1.82 (m, 1H, γ-dPro), 1.47 (s, 9H, Boc-tBu), 1.32 (s, 9H, Tyr(tBu)), 1.19 (d, J = 6.3 Hz, 3H, γ-Thr).
**SI Figure 6.** Unabridged ROESY correlation map of peptide 1 in CDCl₃ at 25 ºC. The crosspeaks assigned to both NH-Asn and Asn(NH) were difficult to resolve; thus, ROESY assignment of crosspeaks were made by analogy to the those observed with peptide 7.

**Modeling of peptide 1.** Distances between protons diagramed in SI Figure 6 were estimated by manually integrating the peak volumes (generally the average of both peaks across the diagonal) and fitting to Equation 2, where \( r_{ij} \) (the distance between \( H_i \) and \( H_j \)) is a function of a reference distance \( r_{ref} \), the reference crosspeak volume \( v_{ref} \), and the crosspeak volume between \( H_i \) and \( H_j \) \( v_{ij} \).

\[
\begin{align*}
  r_{ij} &= r_{ref} \sqrt{\frac{v_{ref}}{v_{ij}}} \\

\end{align*}
\]

Three different sets of crosspeaks and distance restraints were considered for internal distance calibration. While two sets of methylene peaks assigned to \( \alpha \)-Gly and \( \beta \)-Asn are resolved well enough for clear integration, the relative volumes of these sets differ substantially \( \left( v_{Asn}/v_{Gly} = 1.8 \right) \) and we were not comfortable referencing to these distances alone if possible. We established that only a limited number of conformations were accessible given the ROESY data of the \( \alpha \)-Asp and \( \delta \)-DPro and the stereochemical configuration of the N-terminal Boc-Asp. These data led us to estimate the approximate distances between the \( \alpha \)-Asp and \( \delta \)-DPro protons that satisfied the possible conformations. We

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2 The basic approach and equations employed are described in: (a) Wüthrich, K. *NMR of Proteins and Nucleic Acids* John Wiley & Sons, Inc.: New York, 1986. (b) Cavanagh, J.; Fairbrother, W. J.; Palmer, A. G., III; Rance, M.; Skelton, N. J. *Protein NMR Spectroscopy: Principles and Practice*, 2nd ed., Inc.: Burlington, 2007.
reasoned that the distance between Hα-Asp and Hδ2-DPro were in the vicinity of 2.2 or 2.3 Å (defined as Ref1 and Ref2, respectively), which were also in line with those determined from another similar peptide structure that we had observed, and we used these values in our calculations. Importantly, these two reference values provided some agreement with the distances derived from the α-Gly and β-Asn crosspeaks. Specifically, the reference distance between methylene protons would be 1.8 Å. With Ref1, the calculated distances of α-Gly and β-Asn are 1.9 and 1.7 Å; and with Ref2 these calculated distances round to 2.0 and 1.8 Å, respectively.

The distance estimates were then assigned to bins, which were used as restraints (SI Table 2). The bins were defined according to the ROESY crosspeak intensity such that the strongest peaks were assigned to the narrowest range of distances as follows: very strong (1.8 to 2.5 Å), strong (1.8 to 3.0 Å), medium (1.8 to 3.5 Å), and weak (1.8 to 4.5 Å). In the case of some H-H correlations, while crosspeaks were observed, the corresponding distances were not calculated due to inconclusive integrations (i.e., there was overlap with neighboring peaks or excessive noise). In such cases, the peaks were assigned to the largest bin (1.8 to 4.5 Å).

The distance restraints were then input into the Crystallography & NMR System (CNS) modeling program, version 1.21. The amino acid monomer topology/parameter files were built manually (except Gly) using the distances and angles from existing crystal structures in combination with the provided

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3 Blank, J. T.; Miller, S. J. Biopolymers 2006, 84, 38-47. See Cambridge Structural Databank, entry WEDBUD.
4 The calculated distance was derived from integration of the crosspeak where Hδ2-DPro could be resolved from Hδ1-DPro in F2.
5 (a) Brunger, A. T.; Adams, P. D.; Clore, G. M.; Delano, W. L.; Gros, P. Grosse-Kunstleve, R. W.; Jiang, J.-S.; Kuszewski, J.; Nilges, N.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. Acta Cryst. 1998, D54, 905-921. (b) Brunger, A. T. Nature Protocols 2007, 2, 2728-2733.
amino acid topology/parameter files, where appropriate. For each reference, 1000 structures were generated during the annealing, ten of which were accepted for further analysis.

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6 Some of the amino acid monomer topology/parameter files were built with Dr. Michael Giuliano and Nadia Abascal.
SI Table 2. Calculated distances between protons within peptide 1, and the associated bin, derived from ROESY crosspeaks with different reference distances.

| Entry | Peak 1  | Peak 2    | Ref1 distance (Å) | Ref1 bin  | Ref2 distance (Å) | Ref2 bin  |
|-------|---------|-----------|-------------------|-----------|-------------------|-----------|
| 1     | NH-Asp  | Hδ1-DPro  | 3.5               | weak      | 3.7               | weak      |
| 2     | NH-Asp  | Hδ2-DPro  | 3.5               | weak      | 3.7               | weak      |
| 3     | Hα-Asp  | Hδ1-DPro  | 2.5               | very strong | 2.6               | strong    |
| 4     | Hα-Asp  | Hδ2-DPro  | 2.2               | very strong | 2.3               | very strong |
| 5     | Hβ-Asp  | Hβ-Asn(Trt) | 3.0         | strong     | 3.1               | medium    |
| 6     | Hα-DPro | NH-Thr(Bn) | 2.1             | very strong | 2.2               | very strong |
| 7     | Hα-DPro | NH-Asn(Trt) | 3.6           | weak       | 3.7               | weak      |
| 8     | NH-Thr(Bn) | NH-Asn(Trt) | 2.6           | strong     | 2.7               | strong    |
| 9     | Hα-Thr(Bn) | NH-Asn(Trt) | 3.0           | strong     | 3.1               | medium    |
| 10    | Hα-Thr(Bn) | Hδ-Tyr(tBu) | 3.7         | weak       | 3.9               | weak      |
| 11    | Hα-Thr(Bn) | He-Tyr(tBu) | 3.6         | weak       | 3.7               | weak      |
| 12    | Hβ-Thr(Bn) | NH-Asn(Trt) | ND            | weak       | ND                | weak      |
| 13    | Hδ-Thr(Bn) | NH-Asn(Trt) | ND            | weak       | ND                | weak      |
| 14    | Hα-Asn  | NH-Tyr(tBu) | 2.2           | very strong | 2.5               | very strong |
| 15    | Hα-Asn(Trt) | NH-Gly      | ND            | weak       | ND                | weak      |
| 16    | Hε-Tyr(tBu) | COOCH₃      | ND            | weak       | ND                | weak      |
NMR experiments with peptide 7

NMR spectra were acquired on a Varian Inova 500 MHz spectrometer running VnmrJ 2.2D with pulse sequences provided by Varian. Peak assignments for peptide 7 acquired at −20 ºC were based upon 2D gCOSY, TOCSY, and ROESY data.

ROESY data was acquired with a 200 ms mixing time; spectral width of 5102.4 Hz in both F1 and F2; 765 points in t2 and 512 points in t1; 32 scans; relaxation delay (d1) of 3.328 s and at −20 ºC. Data were processed using MestReNova version 8.1.1-11591 using zerofilling to bring the spectral size to 1024, 1024; automatic phasing with manual adjustments; apodization with a Gaussian function; and automatic baseline correction with a polynomial fit.

$^1$H NMR (500 MHz, CDCl$_3$, −20 ºC): δ 7.74 (d, J = 8.0 Hz, 1H, NH-Asn), 7.70 (s, 1H, Asn(NHTrt)), 7.56 (d, J = 7.3 Hz, 1H, NH-Tyr), 7.41 – 7.19 (m, 20H, Trt and Bn aryl peaks), 7.18 – 7.03 (m, 1H, NH-Thr), 6.98 (d, J = 8.4 Hz, 2H, δ-Tyr), 6.90 (d, J = 8.4 Hz, 1H, ε-Tyr), 6.67 (d, J = 8.2 Hz, 1H, NH-Asp), 4.93 – 4.84 (m, 1H, α-Asn), 4.81 (ddd, J = 10.9, 7.1, 4.0 Hz, 1H, α-Asp), 4.57 (apparent q, J = 6.7 Hz, 1H, α-Tyr), 4.51 – 4.38 (m, 3H, β-Thr and benzylic CH$_2$), 4.35 (d, J = 9.6 Hz, 1H, α-Thr), 4.03 (t, J = 7.5 Hz, 1H, α-DPro), 3.72 – 3.51 (m, 5H, δ-DPro and OCH$_3$, one δ-DPro proton is resolved/assigned in F2 of TOCSY spectrum to be 3.65), 3.19 (dd, J = 15.6, 10.1 Hz, 1H, β-Asn), 3.04 – 2.88 (m, 2H, β-Tyr), 2.77 (dd, J = 16.6, 7.4 Hz, 1H, β-Asp), 2.66 – 2.48 (m, 2H, β-Asp and β-Asn, assigned in F2 of TOCSY spectrum to be 2.59 and 2.56, respectively), 2.17 – 2.07 (m, 2H, β/γ-DPro), 2.05 – 1.94 (m, 1H, β-DPro), 1.89 – 1.74 (m, 1H, γ-DPro), 1.44 (s, 9H, Boc-tBu), 1.31 (s, 9H, Tyr(tBu)), 1.18 (d, J = 6.3 Hz, 3H, γ-Thr).
**SI Figure 7.** Unabridged ROESY correlation map of peptide 7 in CDCl₃ at −20 ºC.

**SI Figure 8.** Composite map of ROESY correlations found in peptide 7 at −20 ºC (blue), 1 at 25 ºC (red), and those shared by both 1 and 7 (purple).
Experiments with farnesyl methyl ether\(^7\) (13)

**Preliminary experiments**

\[
\begin{align*}
\text{Entry} & \quad \text{Catalyst} & \quad \text{GC Trace} & \quad \text{Selectivity} \\
1^a & \quad \text{H}_3\text{C} & \quad & \text{(10,11-:6,7-)} \\
2^b & \quad & \text{peptide} & \quad \text{1.0 : 1.0}
\end{align*}
\]

**SI Figure 9.** Comparison of selectivity derived from acid catalysts with farnesyl methyl ether and one theoretical equivalent of oxidant (controlled by DIC stoichiometry) as determined by GC (acquisition method is the same as for farnesol). \(^a\)Performed with 0.3 equiv propionic acid, run for approximately 2 h at room temperature. \(^b\)Performed at 4 °C with 0.1 equiv peptide, run for 5 h.

Peptide 17 was prepared analogously to peptide 1. Fmoc-Ile-OH was used in the solid-phase peptide synthesis to be present in the \(i+2\) position. Following the Fm-ester deprotection and purification by silica gel chromatography (see the Standard Fm-ester deprotection protocol, SI-5), peptide 17 was used without further purification and used in most reaction studies. This batch of material was found to

\(^7\) Schwartz, M. A.; Dunn, T. J. *J. Am. Chem. Soc.* **1972**, *94*, 4205-4211.
contain an impurity and was further purified by reversed phase chromatography for characterization (H NMR spectrum included). When tested with 13 and 18, this further purified batch of 17 did not indicate any appreciable change in performance, as determined by GC of the crude reactions.

Peptide 17: white solid. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.10 – 7.73 (m, 2H), 7.31 – 7.11 (m, 16H), 6.97 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 8.3 Hz, 2H), 6.55 (bs, 1H), 5.91 (bs, 1H), 4.92 – 4.84 (m, 1H), 4.51 (apparent t, J = 9.8 Hz, 1H), 4.36 (bs, 1H), 4.22 (dd, J = 9.0, 3.7 Hz, 1H), 4.06 (apparent t, J = 7.2 Hz, 1H), 3.70 – 3.57 (m, 6H), 3.52 (apparent t, J = 8.8 Hz, 1H), 3.43 – 3.26 (m, 1H), 3.10 (dd, J = 14.0, 6.4 Hz, 1H), 3.05 – 2.94 (m, 2H), 2.66 (dd, J = 16.8, 3.9 Hz, 1H), 2.33 (d, J = 14.1 Hz, 1H), 2.07 – 1.74 (m, 4H), 1.52 – 1.44 (m, 10H), 1.31 (s, 9H), 1.07 (bs, 1H), 0.83 – 0.78 (m, 4H), 0.75 (d, J = 6.8 Hz, 3H). A TOCSY spectrum was used to assist the assignment of proton integer values given the peak broadening.
**SI Figure 10.** Comparison of selectivity derived from acid catalysts with farnesyl methyl ether as determined by GC (acquisition method is the same as for farnesol). a. Performed in DCM b. Prepared from selective bromohydration,\(^8\) followed by epoxide-forming ring closer with K\(_2\)CO\(_3\) in CH\(_3\)OH.\(^9\) GC retention times differ from those shown in SI Figure 9 on account of a newer column being installed and used for these experiments.

Reactions of farnesyl methyl ether (13) and peptide 17 were performed as described for the screening of truncated catalyst analogs (see SI-4), except DCM was used as a reaction solvent. Following work-up and GC analysis, the remaining organics, along with two additional hexanes extracts of the aqueous material, were combined, concentrated. The resulting material was loaded onto a silica gel column, eluting with a gradient of 0% to 30% Et\(_2\)O in hexanes.

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\(^8\) van Tamelen, E. E.; Sharpless, K. B. *Tetrahedron Lett.* **1967**, *8*, 2655-2659.

\(^9\) Ceruti, M.; Balliano, G.; Viola, F.; Cattel, L.; Gerst, N.; Schuber, F. *Eur. J. Med. Chem.* **1987**, *22*, 199-208.
Epoxide 15: (major product, isolated as an enriched mixture with epoxide 14): **TLC**: 30% Et₂O in hexanes (R_f =0.43), visualized with I₂/silica. **¹H NMR** (600 MHz, CDCl₃): δ 5.39 (tdt, J = 6.8, 2.8, 1.3 Hz, 1H), 5.07 (ddp, J = 8.6, 5.8, 1.5 Hz, 1H), 3.95 – 3.91 (m, 2H), 3.33 (s, 2H), 2.71 (t, J = 6.2 Hz, 1H), 2.24 – 2.01 (m, 4H), 1.73 – 1.57 (m, 12H), 1.41 (ddd, J = 13.6, 9.5, 7.1 Hz, 1H), 1.25 (s, 3H). **¹³C NMR** (151 MHz, CDCl₃): δ 139.6, 132.0, 123.8, 121.3, 69.0, 63.4, 61.0, 58.1, 38.9, 36.3, 27.2, 25.9, 24.0, 17.8, 16.6, 16.6.

Epoxide 14 (minor product of isolated mixture with epoxide 15, partially reported): **¹H NMR** (600 MHz, CDCl₃): δ 5.34 (tdt, J = 6.7, 2.6, 1.3 Hz, 1H), 5.18 – 5.13 (m, 1H), 3.32 (s, 3H), 1.30 (s, 3H), 1.26 (s, 3H).
Experiments with difarnesyl ether (18)

SI Figure 11. Comparison of selectivity derived from oxidants/catalysts with difarnesyl ether as determined by GC (275 ºC injector and detector; 1.5 mL/min He flow through column; 60 ºC initial oven temp, ramp at 10 ºC/min to 200 ºC, hold for 1 min, ramp at 1 ºC/min to 260 ºC with 3 min hold at 237 ºC and 2 min hold at 238 ºC; final hold at 260 ºC for 3 min; 84 min total). Selectivities are reported as the ratio of area of 19 to that of the next most intense peak, presumed to be 20.

| Entry | Oxidant/Catalyst/Source | GC Trace | Selectivity |
|-------|------------------------|----------|-------------|
| 1<sup>a</sup> | mCPBA | | 1 : 1 |
| 2<sup>b</sup> | | | 1 : 4 |
| 3<sup>c</sup> | Crude reaction from preparation of epoxide 14 standard | | |
| 4 | An isolated sample | | |

1<sup>a</sup> Prashad, M.; Kathawala, F. G.; Scallen, T. J. Med. Chem. 1993, 36, 1501-1504.

1<sup>b</sup> Lichtor, P. A.; Miller, S. J. Nature Chem. 2012, 4, 990-995.
Reactions of difarnesyl ether (18) and peptide 17 were performed as described for the screening of truncated catalyst analogs (see SI-4), except DCM was used as a reaction solvent and either 1.1 or 1.5 equiv DIC (higher equiv results in slightly higher conversion of starting material) with 2.0 equiv H₂O₂ were used. Following work-up and GC analysis, the remaining organics, along with two additional hexanes extracts of the aqueous material, were combined, concentrated. The resulting material was loaded onto a silica gel column, eluting with a gradient of 0% to 30% Et₂O in hexanes. The monoepoxide products were found to be somewhat unstable to silica gel, as was evident from a two-dimensional TLC of a crude reaction run with mCPBA, where products were allowed to stand for 25 min on the TLC plate following the first elution, and then separated in the perpendicular direction. Additionally, trace amounts of unidentified products were sometimes isolated along with mixtures of 19 and 20. The ratio of epoxide products following isolation was >3:1 (19:20), as determined by NMR. Furthermore, we note that the estimation of the crude ratios by both NMR and GC is similar to that observed in the isolated material by NMR (~3:1; See page S-51).

We note further that epoxide ratios from the crude reactions with 18 are challenging to ascertain. As in previous reactions, the site selectivity was estimated by comparing the GC spectra of the crude reactions and measuring the ratio of the most intense peak areas eluting at the retention time characteristic of the monoepoxides. We confirmed the GC assignment of the major product (19) by synthesizing it independently (SI Figure 11, entry 3). We presume that the other major peak in the GC to be 20 based upon its analogy to GC spectra/studies with 13 in addition to the observation that epoxide 19 is isolated as a minor product along with 20. We note, however, that reinjection of the isolated mixture of 19 and 20 results in the slightly different epoxide ratio (SI Figure 11, entry 4) and the appearance of other neighboring peaks that seem to have increased intensity relative to the main products (compared to the crude reaction), the cause of which may be the instability of products under
the high temperature conditions of this GC assay. Nonetheless, the GC trace of the crude reactions is illustrative of the comparative selectivities observed with peptide-based catalysts 1 and 17, and mCPBA (entry 1) and 17 (entry 2); moreover, the selectivity estimated from these traces is corroborated by the $^1$H NMR of the crude reactions where we estimate a ratio of what attribute to all “6,7-” and “10,11-” epoxides (that is, integrating over the di- and potentially higher epoxidized products as well; see page S-53). Furthermore, we note that the estimation of the crude ratios by both NMR and GC is similar to that observed in the isolated material by NMR (~3:1).

Epoxide 19: (major product, isolated as an enriched mixture with epoxide 20): TLC: 20% Et$_2$O in hexanes (R$_f$ =0.45), visualized with I$_2$/silica. $^1$H NMR (500 MHz, CDCl$_3$): δ 5.41 (tdd, J = 6.8, 2.8, 1.4 Hz, 1H), 5.37 (tdd, J = 6.8, 2.6, 1.3 Hz, 1H), 5.15 – 5.04 (m, 3H), 3.98 (d, J = 6.7 Hz, 4H), 2.70 (t, J = 6.2 Hz, 1H), 2.24 – 1.94 (m, 12H), 1.76 – 1.58 (m, 24H), 1.41 (ddd, J = 13.6, 9.3, 7.2 Hz, 1H), 1.25 (s, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$): δ 140.3, 139.2, 135.4, 132.0, 131.4, 124.5, 124.1, 123.9, 121.8, 121.1, 66.7, 66.5, 63.4, 60.9, 39.9, 39.8, 38.9, 36.3, 27.3, 26.9, 26.5, 25.8, 24.0, 17.8, 17.8, 16.7, 16.7, 16.6, 16.1. MS: calculated mass for [C$_{30}$H$_{50}$O$_2$+H]+: 443.388, ESI+ found 443.389; [C$_{30}$H$_{50}$O$_2$+NH$_4$]+: 460.415, ESI+ found 460.414; [C$_{30}$H$_{50}$O$_2$+Na]+: 465.370, ESI+ found 465.374.

Epoxide 20 (minor product of isolated mixture with epoxide 19, partially reported): $^1$H NMR (500 MHz, CDCl$_3$): δ 5.19 – 5.14 (m, 1H), 1.30 (s, 3H), 1.25 (s, 3H).

NMR spectra follow beginning on page S-29.
25 °C
$25 \, ^\circ \mathrm{C}$

ROESY
Crude reaction of mCPBA and difarnesyl ether (18)
Crude reaction of catalyst 17 and difarnesyl ether (18)
