A One-Bead-One-Catalyst Approach to Aspartic Acid-Based Oxidation Catalyst Discovery

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Supporting Information

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General Information.

Proton NMR spectra were recorded on a Bruker 500 MHz spectrometer and carbon NMR spectra were recorded on a Bruker 126 MHz or 101 MHz spectrometer, all at ambient temperature. All NMR chemical shifts are referenced in ppm relative to residual solvent or internal tetramethylsilane according to Gottlieb *et al*.[1] Solvent reference ppm in ^{1}H-NMR and ^{13}C-NMR for $CDCl_3$ are 7.26 ppm and 77.16 ppm, respectively. Carbon NMR spectra were completely proton decoupled. NMR spectral data are reported as chemical shift (multiplicity, coupling constants, integration). Multiplicity is reported as follows: singlet (s), doublet (d), doublet of doubles (dd), triplet (t), quartet (q), and multiplet (m).

Attenuated total reflectance-infrared spectra (ATR-IR) were obtained on a Nicolet 6700 FT-IR spectrometer; υ_{max} (cm^{-1}) are partially reported.

Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 Å F254 pre-coated plates (0.25 mm thickness) and visualized using irradiation by a UV lamp and/or staining with I_2/silica, cerium ammonium molybdate (CAM), ninhydrin, or $KMnO_4$ solutions. Flash chromatography was perfomed using Silica Gel 60 Å (32-63 micron).

Optical rotations were recorded on a Perkin-Elmer Polarimeter 341 at the sodium D line (1.0 dm path length) at 20 ºC.

Mass spectrometry was acquired using ultra high performace liquid chromatography-mass spectrometry (UPLC/MS) using a Waters UPLC/MS instrument equipped with a reverse-phase C_{18} column (1.7 μm particle size, 2.1 x 50 mm), dual atmospheric pressure chemical ionization (API)/electrospray ionization (ESI) mass spectrometry detector and a photodiode array detector.

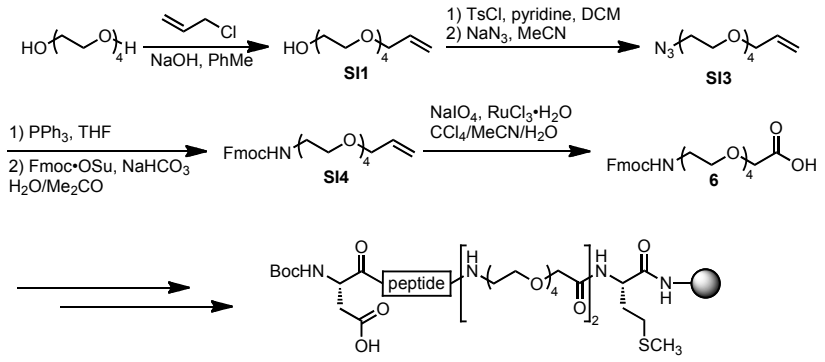
MALDI-TOF spectra were acquired using an Applied Biosystems Voyager-DE Pro BioSpectrometry Workstation. Spectra were acquired in the positive-ion linear mode with 800-1000 shots per spectrum.

Chiral analytical HPLC was performed using a column at ambient temperature on a Hewlett-Packard or Agilent 1100 Series HPLC instrument with a diode array detector (210 nm, 230 nm, and/or 254 nm).

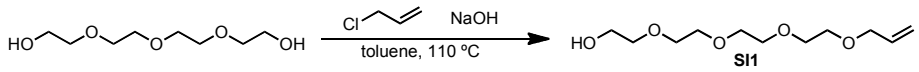
Many reaction solvents were purified using a Seca Solvent Purification System by GlassContour. All other chemicals were purchased commercially and used as received, unless otherwise indicated.

Synthesis of PEG-linker, 6.

The synthesis of **6** was carried out in analogy to work reported elsewhere for such PEG-amino acids.[2] Characterization and alternate preparations of intermediates to **6** may also be found elsewhere.[3] The overall scheme is as follows:



Synthesis of SI1:

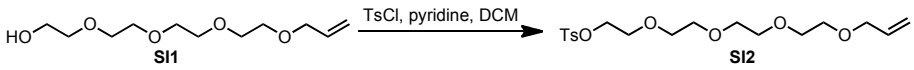


Tetraethylene glycol (87 mL, 500 mol, 2.5 equiv.) was added with toluene (180 mL) to a 500 mL round bottom flask with stir bar. Sodium hydroxide (8.4 g, 210 mmol, 1.05 equiv.) was added while stirring and the flask was fitted with a condenser. As the solution was heating to reflux, allyl chloride (15.3 g, 163 mL, 200 mol, 1.0 equiv.) was added slowly via syringe. The mixture turned an orange color, which persisted while refluxing overnight. The crude reaction mixture was washed three times with water (150 mL each) and the combined aqueous layer was acidified in portions with 1 M HCl and each extracted three or four times with DCM generally until the resulting aqueous layer lost its orange color. The combined organic layers were then concentrated *in vacuo* and loaded on a column containing 500 mL silica gel in Et_2O and pentane (1:1) for flash chromatography, after which the solvent gradient was gradually brought to 100% Et_2O. Fractions containing the monoallylated

tetraethylene glycol and a little of the bisallyl product were collected and concentrated *in vacuo* to yield **SI1** as a faint yellow oil (23.4 g, 100 mmol, *50%*).

TLC: 100% diethyl ether (R_f = 0.12). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 5.84 (ddt, *J* = 5.7, 11.2, 16.9 Hz, 1H), 5.20 (d, *J* = 17.2 Hz, 1H), 5.10 (d, *J* = 10.6 Hz, 1H), 3.95 (d, *J* = 5.7 Hz, 2H), 3.68 – 3.62 (m, *J* = 5.1, 9.5 Hz, 2H), 3.62 – 3.57 (m, *J* = 7.3 Hz, 10H), 3.56 – 3.51 (m, 4H), 2.86 (t, *J* = 6.0 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$): δ 134.7, 117.1, 72.5, 72.2, 70.6, 70.6, 70.5, 70.3, 69.4, 61.6. **IR** (film, cm^{-1}): 3452, 2865, 1453, 1348, 1291, 1248, 1093, 995, 924, 884, 842. **MS**: calculated mass $[C_{11}H_{23}O_5+H]^+$: 235.15; ESI+ found 235.08.

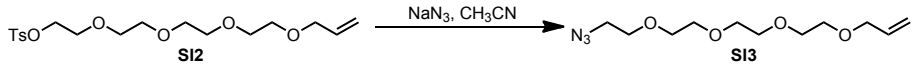
Synthesis of SI2:



In a 1 L two-neck round bottom flask with magnetic stir bar, 4-toluenesulfonyl chloride (28.8 g, 151.0 mmol, 2 equiv.), **SI1** (17.7 g, 75.5 mmol, 1 equiv.), and DCM (58 mL, 1.3 M without pyridine) were allowed to stir over ice. An addition funnel with pyridine (23.9 g, 24.3 mL, 302 mmol, 4 equiv.) was affixed to the top of the reaction flask and pyridine slowly added to the reaction (2-5 seconds/drop, 50 minutes for complete addition), after which the reaction was allowed to warm to room temperature. After five total hours of stirring, the reaction was again put in an ice bath and 140 mL 1 M HCl was added drop-wise via the addition funnel to the vigorously stirring reaction. The reaction was poured into a separatory funnel and extracted thrice with DCM (about 300 mL each). The combined organic extracts were concentrated *in vacuo*, dissolved in DCM, and then washed once with water and once with buffered acetic acid/K_2CO_3 solution (1 L 0.1 M K_2CO_3 with 4.5 mL glacial acetic acid: pH ~9). After concentrating via rotary evaporator, the pale yellow oil was purified by silica gel chromatography (400 mL), eluted using a gradient of 50% Et_2O in pentane to 100% Et_2O. The fractions were concentrated *in vacuo* to yield **SI2** as pale yellow oil (29.2 g, 75.2 mmol, *>99%*).

TLC: 100% Et_2O (R_f = 0.44). **^{1}H NMR** (500 MHz, $CDCl_3$): 7.79 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 5.90 (ddt, *J* = 5.7, 10.9, 22.3 Hz, 1H), 5.26 (dd, *J* = 1.6, 17.2 Hz, 1H), 5.17 (dd, *J* = 0.9, 10.4 Hz, 1H), 4.21 – 4.09 (m, 2H), 4.01 (d, *J* = 5.7 Hz, 2H), 3.71 – 3.56 (m, 14H), 2.44 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$): δ 144.6, 134.6, 132.7, 129.6, 127.7, 116.7, 71.9, 70.4, 70.3, 70.3, 69.2, 69.1, 68.4, 21.4. **IR** (film, cm^{-1}): 2867, 1598, 1451, 1352, 1292, 1248, 1189, 1175, 1095, 1012. **MS**: calculated mass [$C_{18}H_{28}O_7S$+H]: 389.16; ESI+ found 389.1.

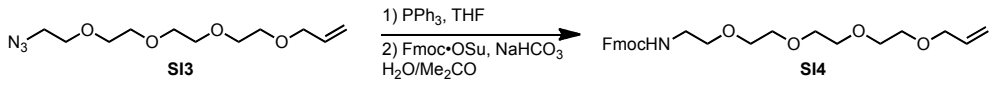
Synthesis of SI3:



In a round bottom flask with magnetic stir bar, **SI2** (65.4 g, 168 mmol, 1.0 equiv.), CH_3CN (170 mL, ~1 M), and NaN_3 (21.9 g, 337 mmol, 2.0 equiv.) were allowed to stir at room temperature. The flask was fitted with a reflux condenser and heated to reflux. After 1.5 hours, an additional 100 mL of CH_3CN was added. After 39 hours, the reaction mixture was cooled to room temperature, filtered, and concentrated. The resulting oil/precipitate was dissolved in Et_2O and washed with H_2O. The organic layer was concentrated *in vacuo*, yielding **SI3**, as gold oil (30.3 g, 117 mmol, *69%*).

TLC: 50% Et_2O in pentane (R_f = 0.18). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 5.88 (ddd, *J* = 5.7, 10.8, 16.1 Hz, 1H), 5.24 (dd, *J* = 1.5, 17.2 Hz, 1H), 5.14 (dd, *J* = 1.2, 10.4 Hz, 1H), 4.05 – 3.94 (m, 2H), 3.70 – 3.61 (m, 14H), 3.60 – 3.52 (m, 2H), 3.35 (t, *J* = 5.0 Hz, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$): δ 134.8, 117.1, 72.3, 70.7, 70.7, 70.7, 70.7, 69.5, 50.7. **IR** (film, cm^{-1}): 2866, 2099, 1450, 1346, 1285, 1249, 1094, 1038. **MS**: calculated mass for $[C_{11}H_{21}N_3O_4+H]^+$: 260.16, ESI+ found 260.20. Other mass peaks were found as well, including the following: calculated mass for $[C_{11}H_{21}N_3O_4+Na]^+$: 282.14, ESI+ found 282.15; and calculated mass for $[C_{11}H_{21}NO_4+H]^+$: 232.15, ESI+ found 232.16.

Synthesis of SI4:

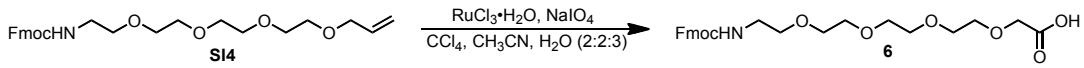


To a 1 L round bottom flask with stir bar was added **SI3** (30.3 g, 117 mmol, 1.0 equiv.) and THF (470 mL). The flask was placed in an ice bath and the solution was allowed to stir. Triphenylphosphine (33.9 g, 129 mmol, 1.1 equiv.) and H_2O (2.3 g, 129 mmol, 1.1 equiv.) were added and the reaction was allowed to warm to ambient temperature. After two days, the reaction was concentrated. The crude reaction mixture was dissolved in H_2O and poured into a separatory funnel with toluene. The aqueous layer and two additional H_2O washes were combined and concentrated. The concentrated aqueous layer was then washed with toluene. The resulting aqueous solution was concentrated *in vacuo* to yield the free amine as a yellow oil (26.8 g, 115 mmol, *98%*).

A 500 mL round bottom flask was charged with a stir bar, Fmoc-OSu (6.32 g, 18.7 mmol, 1.0 equiv.), H_2O (30 mL), and acetone (95 mL). A portion of the resulting amine (4.37 g, 18.7 mmol, 1.0 equiv.) was added with four H_2O rinses (40 mL total), followed by $NaHCO_3$ (3.15 g, 37.6 mmol, 2.0 equiv) and an additional 5 mL H_2O. After stirring for ~20 hours, the reaction mixture was concentrated via rotary evaporator. The resulting white goo was loaded on a silica column (250 mL) and eluted using a gradient of 50% Et_2O in pentane to 100% Et_2O. Fractions containing pure **SI4** were concentrated *in vacuo* to yield 1.74 g of a yellow oil. Fractions containing impure **SI4** were concentrated and subjected to another silica column to provide an additional 1.79 g, bringing the total yield to 3.53 g (7.75 mmol, *41%*) **SI4**.

TLC: 5% MeOH in DCM (R_f = 0.24). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.74 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.37 (t, *J* = 7.5 Hz, 2H), 7.29 (td, *J* = 7.4, 1.0 Hz, 2H), 5.88 (m, 1H), 5.47 (bs, 1H), 5.24 (d, *J* = 15.9 Hz, 1H), 5.15 (d, *J* = 10.4 Hz, 1H), 4.39 (d, *J* = 6.9 Hz, 2H), 4.20 (t, *J* = 6.8 Hz, 1H), 3.98 (d, *J* = 5.5 Hz, 2H), 3.77 – 3.51 (m, 14H), 3.41 – 3.31 (m, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$): δ 156.6, 144.0, 141.3, 134.8, 127.7, 127.0, 125.1, 119.9, 117.0, 72.2, 70.6, 70.6, 70.6, 70.4, 70.0, 69.4, 66.5, 47.3, 41.0. **IR** (film, cm^{-1}): 3329, 2868, 1716, 1529, 1450, 1348, 1244, 1093, 1031. **MS**: calculated mass $[C_{26}H_{33}NO_6+H]^+$: 456.24; ESI+ found 456.22.

Synthesis of 6:

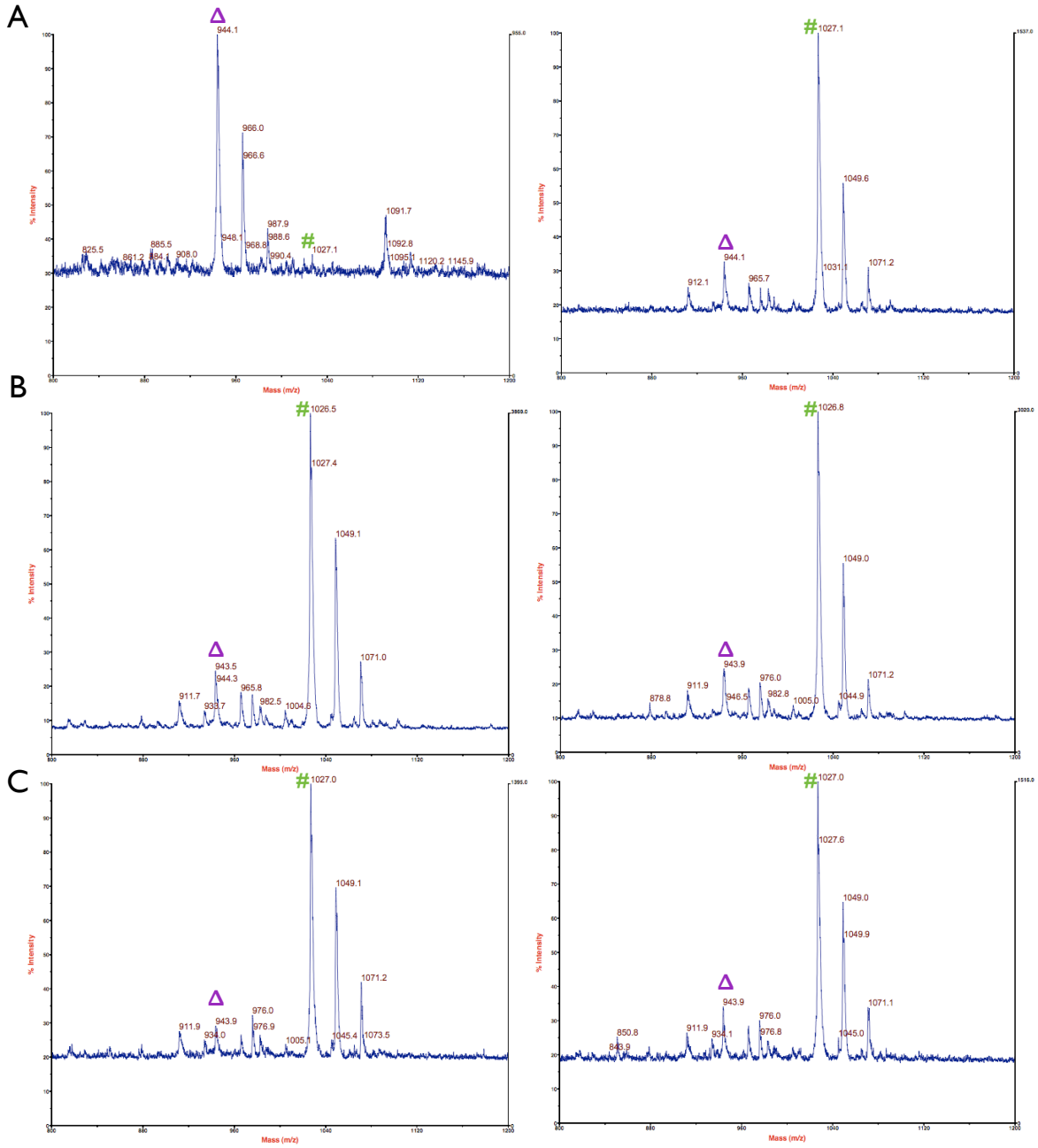


In a 200 mL round bottom flask with magnetic stir bar, **SI4** (3.54 g, 7.76 mmol, 1 equiv.) and solvents (15.4 mL CCl_4, 15.4 mL CH_3CN, and 23 mL H_2O) were allowed to stir. A mixture of $RuCl_3•H_2O$ (35 mg, 0.17 mmol, 0.02 equiv.) and $NaIO_4$ (6.82 g, 32 mmol, 4.1 equiv.) were added slowly while stirring. After about eight minutes, the reaction became hot, so the reaction flask was put into an ice bath and allowed to stir overnight. After 12.8 hours, additional $NaIO_4$ (4.98 g, 23.28 mmol, 3.0 equiv.) was added to the reaction mixture. At 15.3 hours total time, an additional portion of $NaIO_4$ (830 mg, 3.88 mmol, 0.5 equiv.) was added. Then at 18.6 hours, yet another portion $NaIO_4$ (830 mg, 3.88 mmol) and a dash of $RuCl_3•H_2O$ were added. After a total reaction time of 19.9 hours, the reaction mixture was diluted with DCM and washed with water. The organic layer was dried over Na_2SO_4, concentrated, and then loaded on a silica gel column three times (200-500 mL silica) and eluted using a gradient of 0% to 20% MeOH in DCM. Still impure, the resulting oil was dissolved in DCM and treated with sat. aq. $NaHCO_3$, resulting in an emulsion that was left overnight. Upon standing, the emulsion had not cleared completely, yet the clear aqueous solution was removed, treated with 1 M HCl, extracted with DCM, and concentrated *in vacuo* to yield **6** as a highly viscous yellow oil (1.27 g, 2.67 mmol, *34%*).

Two putative rotamers were identified by NMR. **TLC**: 10% MeOH in DCM (R_f = 0.18). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 10.21 (bs, 1H), 7.75 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.2 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 2H), 6.32 (bs, 0.2H), 5.53 (s, 0.8H), 4.50 – 4.33 (m, 2H), 4.21 (s, 1H), 4.13 (s, 2H), 3.82 – 3.23 (m, 16H). **^{13}C NMR** (126 MHz, $CDCl_3$): δ 172.7, 156.8, 144.1, 143.9, 141.4, 127.7, 127.1, 125.2, 120.0, 71.4, 70.7, 70.5, 70.4, 70.2, 69.7, 68.9, 68.5, 67.4, 66.7, 47.3, 41.5, 40.9. **IR** (film, cm^{-1}): 3337, 2873, 1711, 1532, 1449, 1349, 1246, 1097. **MS**: calculated mass $[C_{25}H_{31}NO_8+H]^+$: 474.21; ESI+ found 474.14.

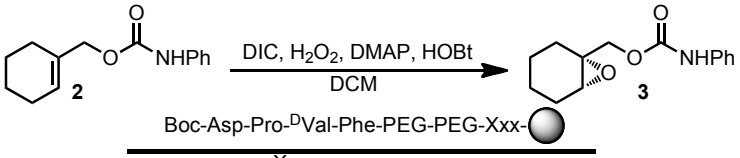
Linker Oxidation Studies.

Six polystyrene macrobeads functionalized with Boc-Asp-Pro-^{D}Val-Phe-PEG-PEG-Met were transferred to individual PCR tubes. Two beads were treated with 20 μL H_2O, two other beads were treated with 20 μL 30% aq. H_2O_2, and the remaining two were left alone. After ten hours, the liquid in each of the tubes was removed, first by pipetting off the excess and then by placing the tubes under vacuum. A bead from each category was then treated with the reduction solution (1 mL anhydrous TFA, >25 mg NH_4I, and ~50 μL DMS) and the tubes containing these beads were allowed to sit in ice for 20 minutes. The excess reduction solution was then removed from these tubes and the beads were washed at least three times each with ~4 mL H_2O and once with methanol. After washing, these beads were placed in fresh tubes and dried. All six beads were then treated with a few drops of 20 mg/mL CNBr in 70% TFA (aq.) overnight in the dark for at least 12 hours. After drying the beads under vacuum, the resulting white solid was dissolved in 30% H_2O/MeCN. 0.5 μL of the peptide solution was mixed with 0.5 μL of 30% H_2O/MeCN containing 4.5 mg/mL α-cyano-4-hydroxycinnamic acid.



SI Figure 1. MALDI-TOF spectra of beads functionalized to Boc-Asp-Pro-^{D}Val-Phe-PEG-PEG-Met following cleavage with CNBr solution. In each panel, the left spectrum is from the unreduced bead and the spectrum on the right corresponds to beads that had been cleaved following treatment with the reduction solution. Green # indicates the expected mass (~1027 amu) for Boc-Asp-Pro-^{D}Val-Phe-PEG-PEG-Met* (Met* = homoserine lactone) and the purple Δ indicates the expected reduced mass (~944 amu) of Boc-Asp-Pro-^{D}Val-Phe-PEG-PEG-OH (less Met*). A. Beads treated with H_2O_2. B. Beads treated with H_2O. C. Beads treated with nothing.

SI Table 1.



Entry[a]	Xxx Met : Ala	Avg. yield[b]	Avg. ee
1	1 : 0	2%	14%
2	1 : 1	4%	26%
3	1 : 4	10%	37%
4	0 : 1	6%	19%
5[c]	0 : 0	2%	26%
7[c]	No Linker	74%	57%

[a]Average of three runs. [b]Uncorrected, crude HPLC yield. [c]No Xxx in peptide.

Partial Edman Degradation (PED) Protocol.[4]

The bead(s) was transferred to a fritted glass reaction vessel in methanol and washed with DCM, then dried. The bead(s) was treated twice with 1-2 mL anhydrous TFA for 20 minutes each to remove the N-terminal protecting group. The resin was washed with DCM. Finally, the following steps were repeated three times:

1. washed several times with H_2O
2. washed several times with pyridine
3. washed several times with 2:1 pyridine/H_2O with 0.1% Et_3N
4. treated with 160 μL of 2:1 pyridine/H_2O with 0.1% Et_3N and then with 160 μL of a solution resulting from mixing 10 μL of phenyl isothiocyanate (PITC) with 160 μL of 31 mM FmocOSu in pyridine and allowed to stand for 6 minutes.
5. after six minutes, the resin was washed with pyridine
6. washed several times with DCM
7. dried under a stream of nitrogen
8. washed once with TFA
9. treated with TFA for six minutes
10. after draining, treated a second time with TFA for six minutes

11. washed several times with DCM after draining TFA

12. rinsed with pyridine

After completing these cycles three times, the beads were washed with H_2O, MeOH, DCM, then DMF. They were then treated with 20% piperidine in DMF during two 20 minute cycles, then rinsed with DMF, DCM, and MeOH. The bead(s) were moved to a PCR tube and dried.

Reduction protocol. The bead(s) were treated with about 50 uL of a 1 mL solution of TFA containing 25 mg NH_4I (does not dissolve well) and >20 μL DMS. After 20 minutes, the bead was removed, washed exhaustively with H_2O, DCM, and MeOH, then dried in a PCR tube.

Analysis. Beads were treated with the CNBr cleavage solution in the dark overnight. After drying the beads under vacuum, the resulting white solid was dissolved in a 30% H_2O in MeCN solution. 0.5 μL of the peptide solution was mixed with 0.5 μL 33% H_2O in MeCN solution saturated with α-cyano-4-hydroxycinnamic acid, and dried on a MALDI plate for analysis.

Synthesis of Split-and-Pool Library.

Polystyrene macrobeads (Polystyrene A NH_2, 500-560 μm, 0.85 mmol/g loading, Rapp Polymere, Batch No. 122.816) were swelled in DMF for 20 minutes and then coupled twice for 3 hours each to amino acid coupling partner using Fmoc-protected amino acid monomer (4 equiv.), HBTU (equiv.), HOBt•H_2O (4 equiv.), $^{i}Pr_2EtN$ (8 equiv.). After coupling, the resin was washed several times with DMF and DCM. Deprotections commenced with two 20 minutes treatments of 20% piperidine in DMF, and were followed by exhaustive washing with DMF and DCM.

All beads were first coupled to a mixture of Fmoc-Met-OH (PerSeptive Biosystems) and Fmoc-Ala-OH•H_2O (Advanced ChemTech) in a molar ratio of 1:4. Next, the library was coupled to **6** twice. The beads were then split into eight tubes for coupling to either Fmoc-Asn(Trt)-OH (CreoSalus and Advanced ChemTech), Fmoc-His(Bn)-OH (Peptides International), Fmoc-Ile-OH (Advaned ChemTech), Fmoc-Phe-OH (Novabiochem), Fmoc-Pro-OH (Novabiochem), Fmoc-D-Val-OH

(Advanced ChemTech), Fmoc-Thr(OBn)-OH (Advanced ChemTech), or Fmoc-Tyr(OtBU)-OH (Bachem). After a round of double couplings, all of the beads were then combined and deprotected together in one reaction vessel. After washing exhaustively, the beads were then split again into eight separate reaction vessels and the split-and-pool process was performed two more times. The final coupling was performed with Boc-Asp(OFm)-OH (AAPPTEC). The Asp-side chain was deprotected by treatment with 20% piperidine/DMF four times for 10 minutes each. Finally, beads were washed exhaustively with DMF, DCM, and MeOH; then dried under N_2.

Confirmation of Library by Sequencing.

Seven beads from the split-and-pool library were subjected to the PED protocol with reduction and found to have had the following sequences (see reference 4 for methodological approach):

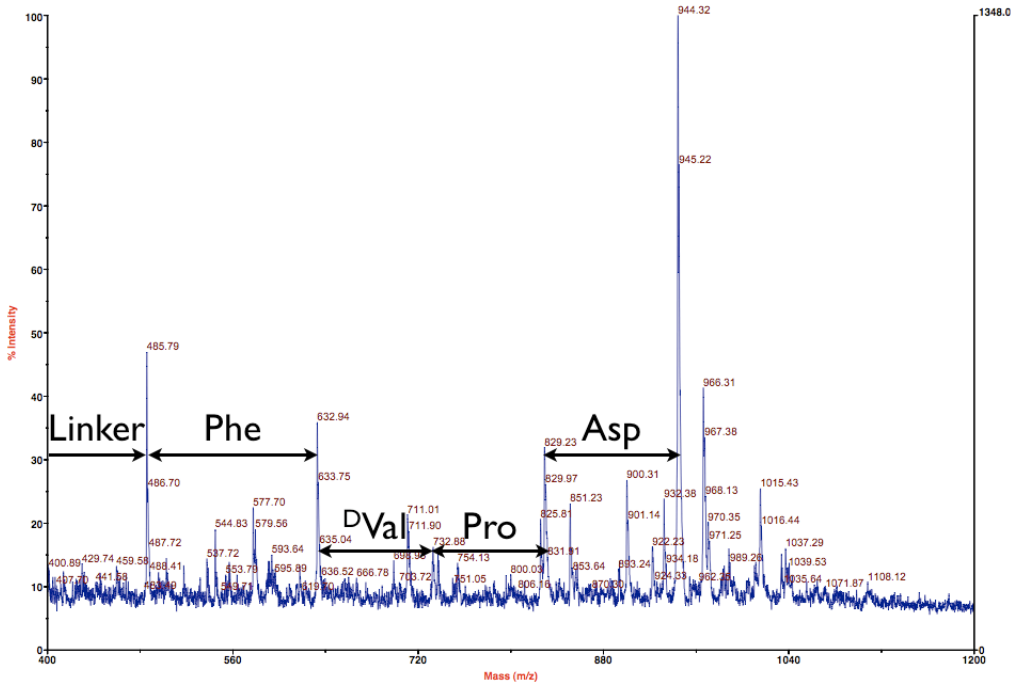
Sequence	*i*	*i*+1	*i*+2	*i*+3
A	Boc-Asp	Tyr(OtBu)	Pro	Tyr(OtBu)
B	Boc-Asp	Ile	Phe	^{D}Val
C	Boc-Asp	Phe	Pro	Thr(OBn)
D	Boc-Asp	Asn(Trt)	^{D}Val	Ile
E	Boc-Asp	Thr(OBn)	Phe	His(Bn)
F	Boc-Asp	Phe	Phe	Ile
G	Boc-Asp	Pro	Ile	Ile

Library Interpretation.

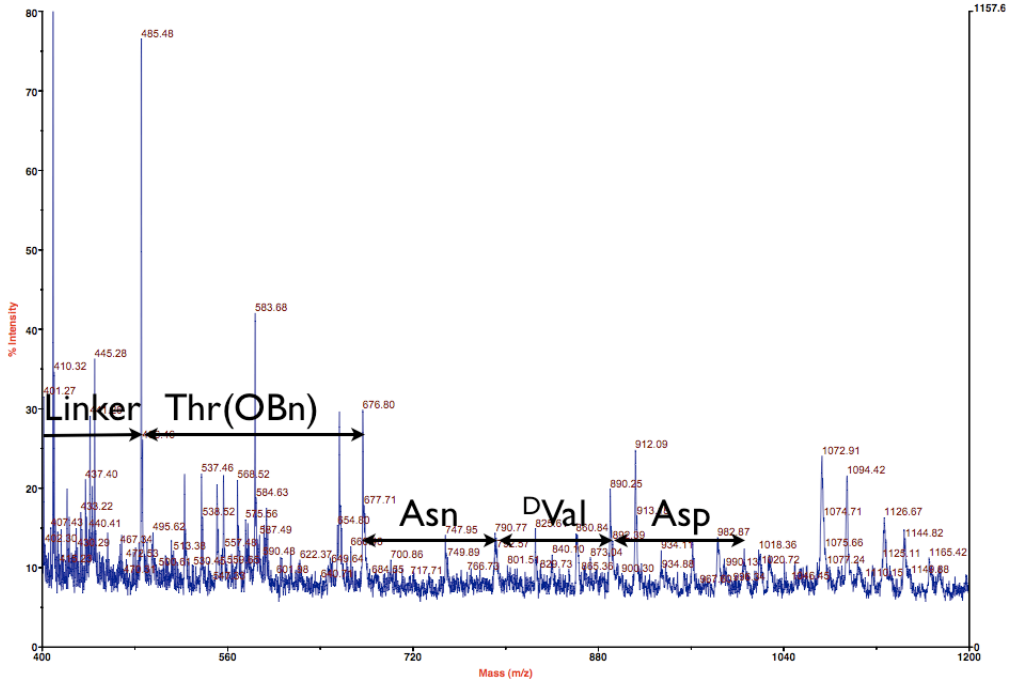
SI Table 2. Expected monoisotopic masses of library residues and library residues functionalized to linker/degraded linker.

	Monoisotopic mass	Monomer with linker	$[M+H]^+$	$[M+Na]^+$	$[M-M^*+H]^+$	$[M-M^*+Na]^+$
Pro	97.05	664.74	665.74	687.73	582.65	604.64
Val	99.07	666.76	667.76	689.75	584.67	606.66
Thr	191.09	758.85	759.85	781.84	676.76	698.75
Asn	114.04	681.73	682.73	704.72	599.64	621.63
Ile	113.08	680.78	681.78	703.77	598.69	620.68
Phe	147.07	714.80	715.8	737.79	632.71	654.7
Tyr	163.03	730.80	731.8	753.79	648.71	670.7
His(Bn)	227.11	794.89	795.89	817.88	712.8	734.79
His	137.06	704.77	705.77	727.76	622.68	644.67
Linker		567.63	568.63	590.62	485.54	507.53

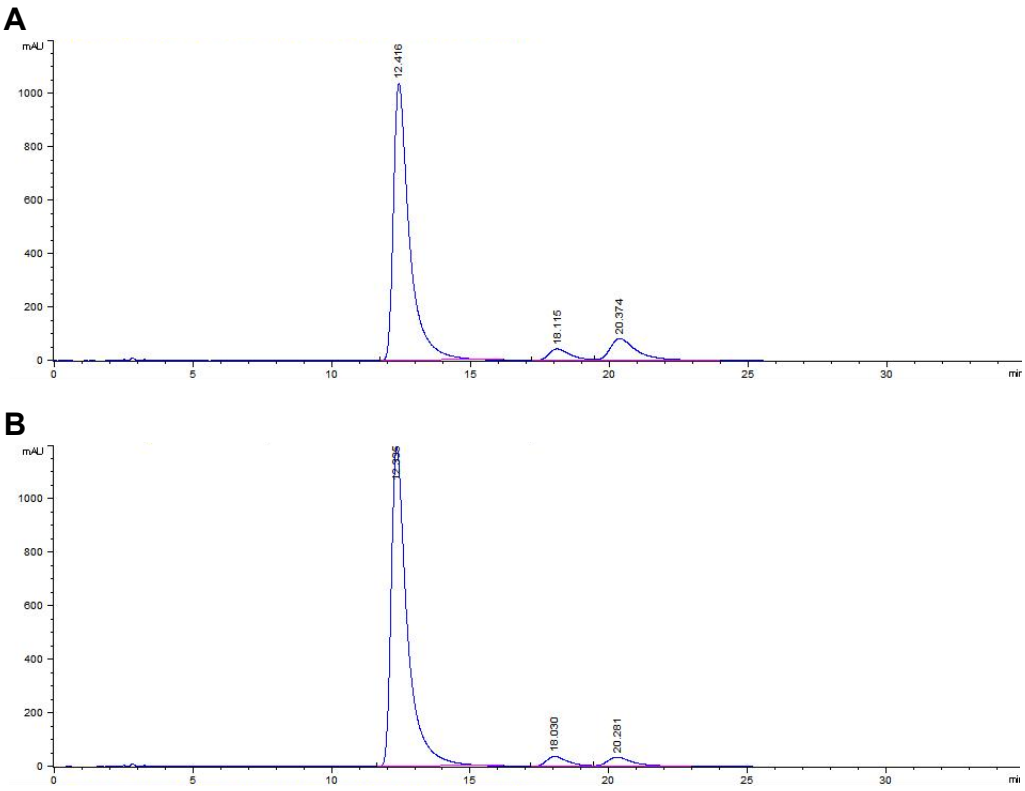
Sample MALDI spectra of peptides from 50 bead screen after PED and no reduction.



SI Figure 2. Bead 30: Boc-Asp-Pro-^{D}Val-Phe (control).



SI Figure 3. Another peptide identified from the 50-bead screen.



SI Figure 4. Sample HPLC traces from 50-bead screen analyzed on a Chiralpak AD column and monitored at 230 nm. A. Hit peptide exhibiting high selectivity. B. Peptide providing racemic product.

Synthesis of 9 and 10.

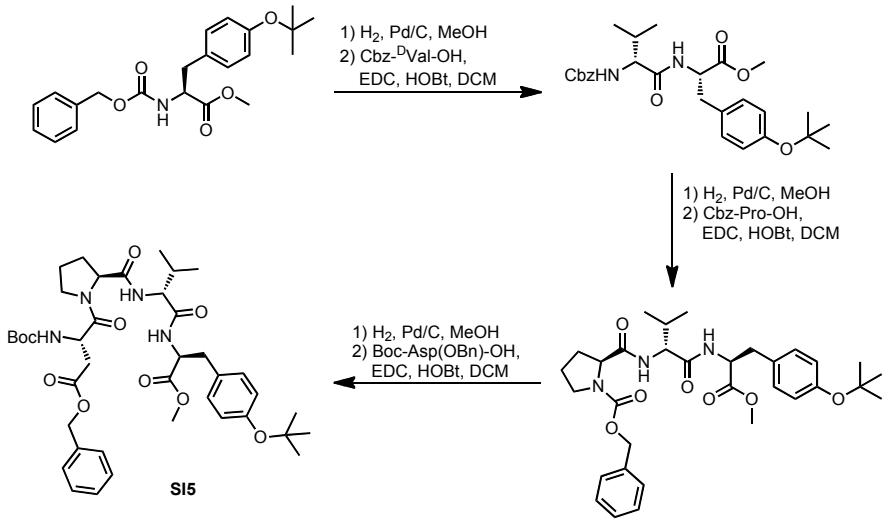
General procedure A: Cbz-deprotection and benzyl ester hydrogenolysis.

A flask charged with palladium on charcoal was fitted with a septum and purged of air using a stream of N_2 and a vent needle. After flushing with N_2 for a few minutes, a portion of methanol was injected through the septum into this flask. Meanwhile, the benzyl ester was added to a separate flask (the reaction flask) with a portion of methanol and a stir bar. The flask containing the methanol-palladium slurry was then opened to atmosphere and the slurry was transferred by pipet to the reaction flask with additional portions of methanol. After all of the slurry and solvent (~0.1 M in methanol) were transferred, the reaction flask was sealed with a septum and purged with N_2 for a few minutes, as before. A needle affixed to a balloon filled with H_2 was used to pierce the reaction flask septum. The N_2 line was removed and the reaction flask was purged with H_2 for a few seconds before the vent needle was removed. After about three hours of stirring, the balloon was removed and the reaction flask was flushed with N_2 for a few minutes. The reaction mixture was filtered through a pad of celite and concentrated into a flask.

General procedure B: solution phase peptide synthesis.

A flask charged with a peptide or amino acid (1.0 equiv.) with a free amine was added to a flask with stir bar, EDC•HCl (1.1 equiv.), HOBt•H_2O (1.1 equiv.), and the free carboxylic acid of the amino acid coupling partner (1.0 equiv.). DCM (0.1 M with respect to amine) was added and the solution was allowed to stir overnight. Coupling reactions were diluted with DCM, and then transferred to a seperatory funnel to be washed with sat. aq. $NaHCO_3$, 50% brine, and aq. 10% citric acid. The resulting organic layer is dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The purity is assessed after work-up (LC/MS), and the reactions are generally clean enough to be carried forward without flash column chromatography

Synthesis of SI5:

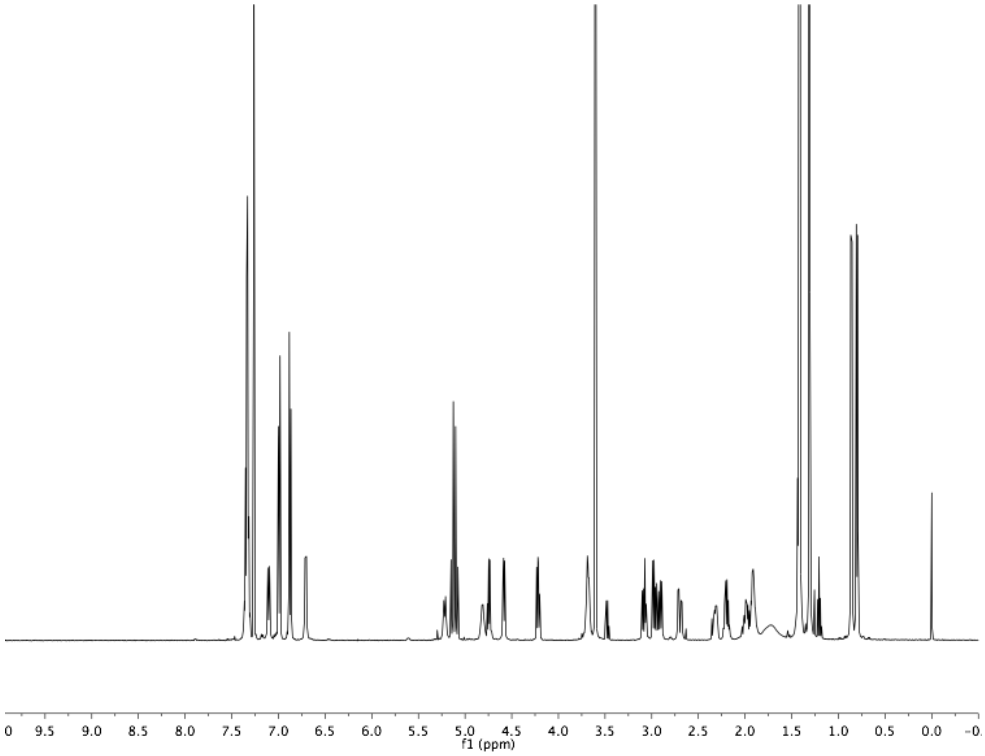


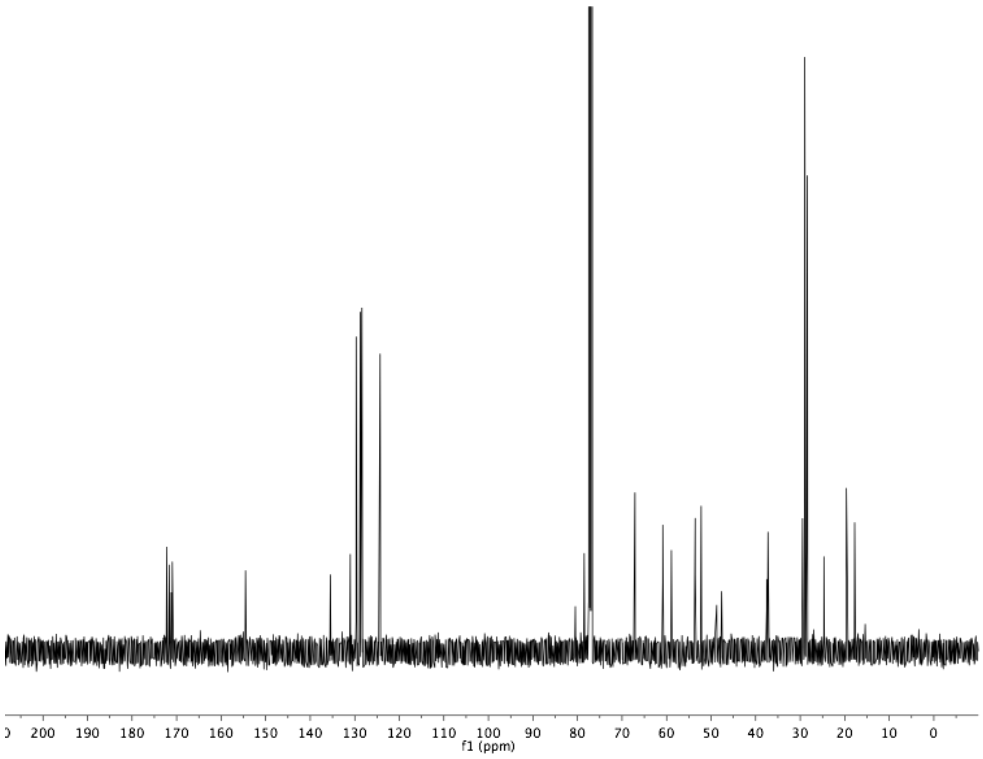
Cbz-Tyr(OtBu)-OMe (Bachem, 385 mg, 1.0 mmol) was deprotected according to General Procedure A. The resulting amino ester was coupled to Cbz-D-Val-OH (Bachem, 251 mg, 1.0 mmol) using General Procedure B. The dipeptide was subjected to General Procedure A to Cbz-deprotect, then coupled to Cbz-Pro-OH (Advanced ChemTech, 249 mg, 1.0 mmol) according to General Procedure B. Cbz-Pro-D-Val-Tyr(OtBu)-OMe was obtained and then deprotected according to General Procedure A and coupled to Boc-Asp(OBn)-OH (NovaBiochem, 323 mg, 1.0 mmol) according to General Procedure B. **SI5** was obtained after loading on to a silica gel column in 15% acetone in toluene, and then eluting with 25% acetone in toluene. In total, 362 mg (0.48 mmol) of **SI5** was isolated in an overall yield of *48%*.

TLC: 25% acetone in toluene (R_f =0.38). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.38 – 7.30 (m, 5H), 7.10 (d, *J* = 8.7 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 6.71 (d, *J* = 7.6 Hz, 1H), 5.22 (d, *J* = 9.2 Hz, 1H), 5.11 (q, *J* = 12.3 Hz, 2H), 4.81 (dd, *J* = 14.0, 8.1 Hz, 1H), 4.74 (dd, *J* = 13.5, 7.3 Hz, 1H), 4.58 (dd, *J* = 8.1, 2.7 Hz, 1H), 4.22 (dd, *J* = 8.6, 6.5 Hz, 1H), 3.69 (dd, *J* = 9.4, 6.4 Hz, 2H), 3.60 (s, 3H), 3.08 (dd, *J* = 14.1, 5.9 Hz, 1H), 2.97 (dd, *J* = 14.1, 7.4 Hz, 1H), 2.92 (dd, *J* = 16.3, 8.2 Hz, 1H), 2.70 (dd, *J* = 16.3 Hz, 5.4, 1H), 2.70 (dd, *J* = 16.3, 5.4 Hz, 1H), 2.33 (dd, *J* = 15.1, 10.6 Hz, 1H), 2.26 – 2.15 (m, 1H), 1.42 (s, 9H), 1.31 (s, 9H), 0.86 (d, *J* = 6.8 Hz, 3H), 0.80 (d, *J* = 6.8 Hz, 3H) . **^{13}C NMR** (126 MHz, $CDCl_3$): δ 172.2, 171.7, 171.2, 171.0, 154.5,

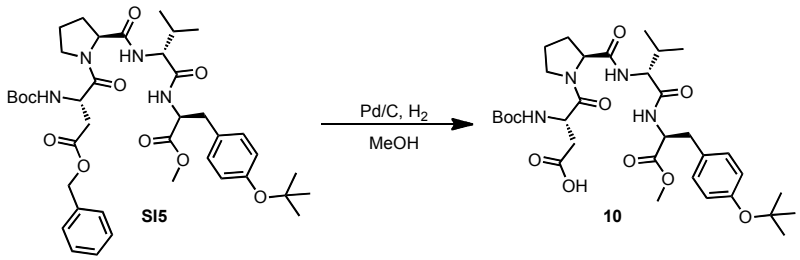
135.5, 131.0, 129.7, 128.7, 128.5, 128.4, 124.3, 80.5, 78.5, 67.1, 60.9, 58.9, 53.6, 52.2, 48.7, 47.6, 37.5, 37.2, 29.5, 29.0, 28.6, 28.4, 24.7, 19.6, 17.8. **MS**: calculated mass for $[C_{40}H_{56}N_4O_{10}+H]^+$: 753.41, ESI+ found 753.33.

*^{1}H NMR, ^{13}C NMR spectra for **SI5**:*





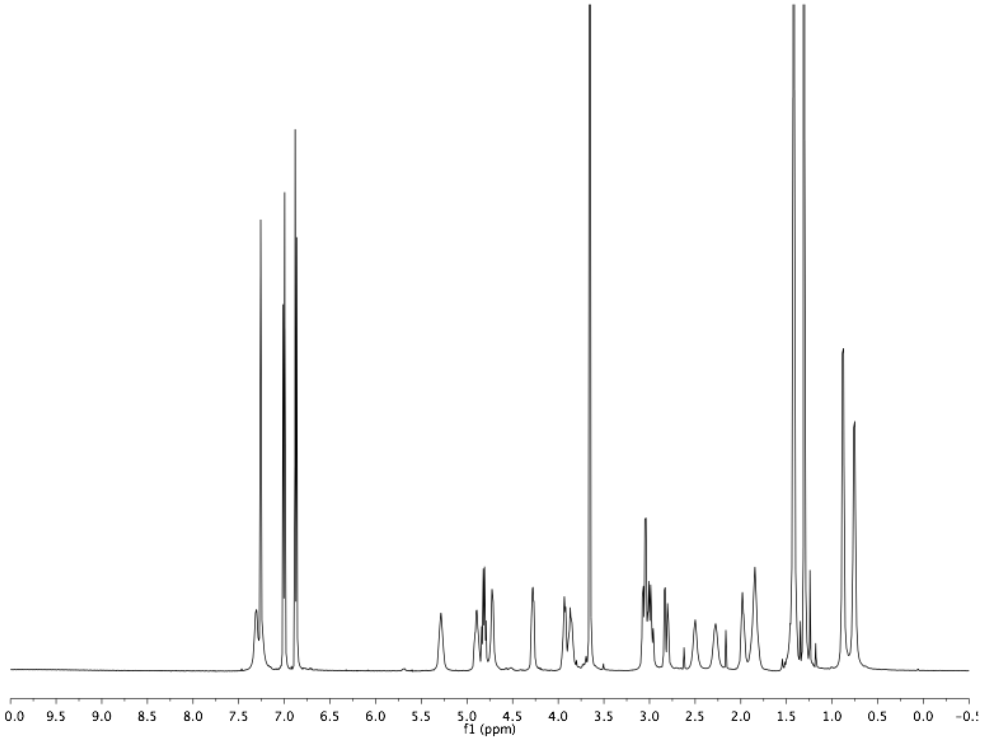
Deprotection of SI5 to afford 10:

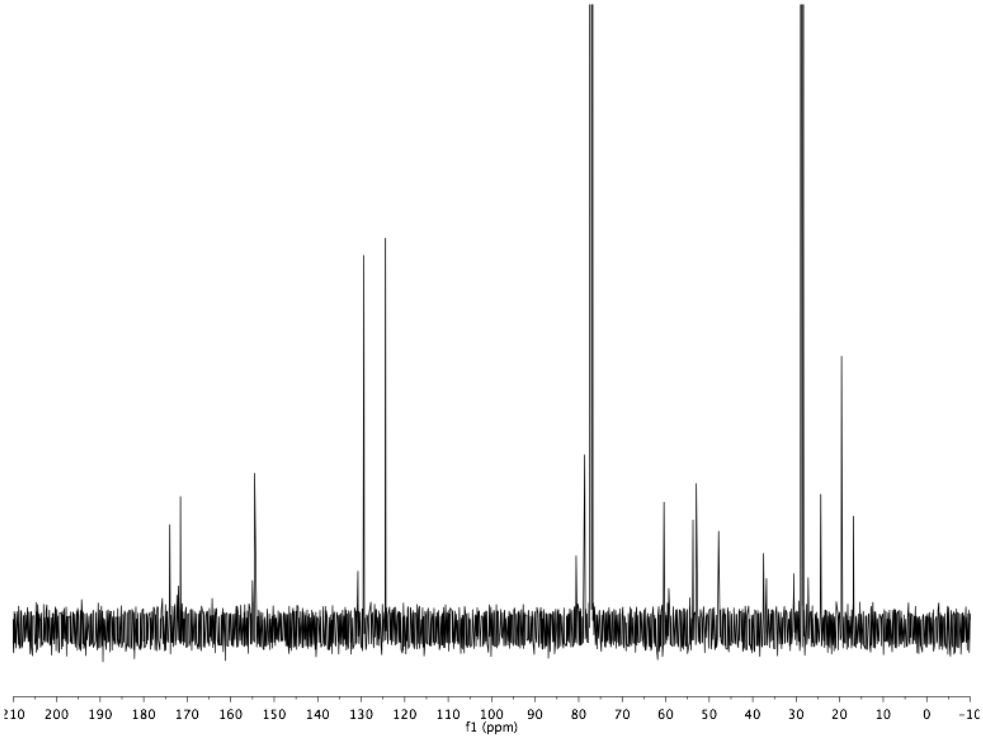


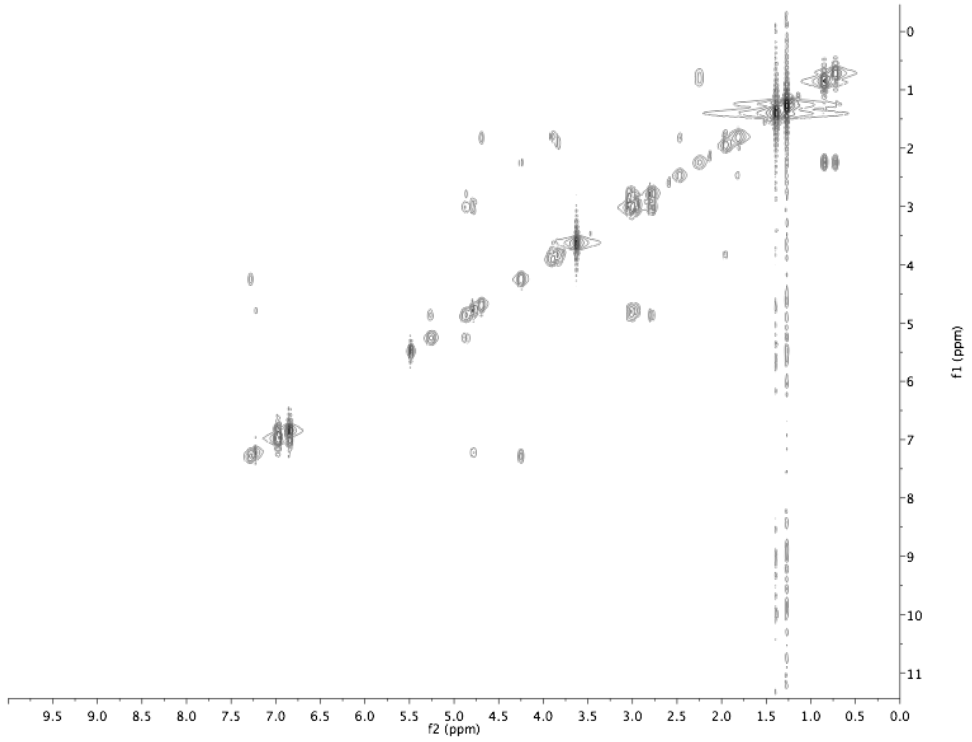
SI5 (362.5 mg, 0.48 mmol) was subjected to General Procedure A and concentrated *in vacuo* to yield **10** as a white solid (314.7 mg, 0.48 mmol, *99%*).

TLC: 50% acetone in toluene (R_f = 0.18). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.31 (s, 1H), 7.00 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 5.29 (s, 1H), 4.89 (s, 1H), 4.81 (d, *J* = 6.9 Hz, 1H), 4.72 (s, 1H), 4.28 (s, 1H), 3.90 (d, *J* = 32.4 Hz, 2H), 3.65 (s, 3H), 3.16 – 2.93 (m, 3H), 2.82 (d, *J* = 13.0 Hz, 1H), 2.50 (s, 1H), 2.27 (s, 1H), 2.16 (s, 1H), 1.98 (s, 1H), 1.85 (s, 2H), 1.42 (s, 9H), 1.37 – 1.24 (s, 9H), 0.88 (d, *J* = 6.5 Hz, 3H), 0.75 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$): δ 174.0, 171.6, 154.5, 130.8, 129.4, 124.4, 80.6, 78.6, 60.4, 53.7, 53.0, 47.8, 37.5, 30.6, 28.9, 28.4, 24.4, 19.6, 16.8. **IR** (solid, cm^{-1}): 3318, 2975, 1713, 1636, 1506, 1437, 1391, 1365, 1282, 1236, 1159, 1046, 1017. **MS**: calculated mass for $[C_{33}H_{50}N_4O_{10}+H]^+$: 663.36, ESI+ found 663.36. **$[\alpha]_D$** = –92 (c = 0.62 g/100 mL distilled $CHCl_3$).

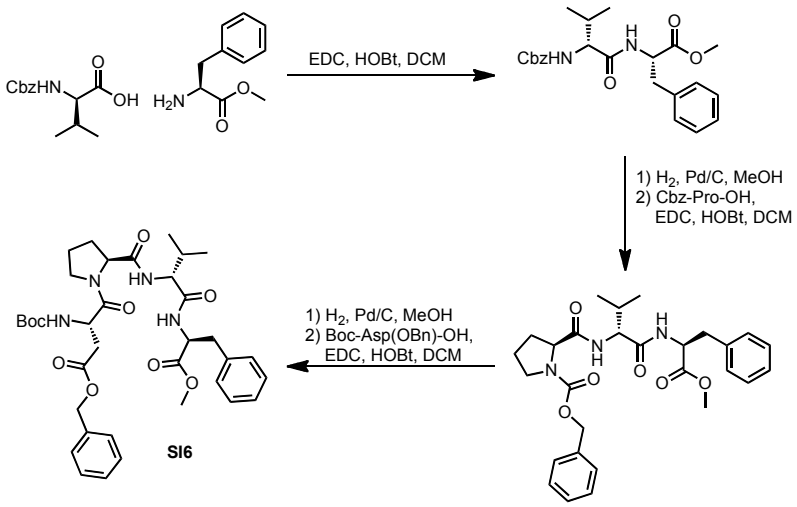
*^{1}H NMR, ^{13}C NMR and COSY spectra for **10**:*


0.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



Synthesis of SI6:

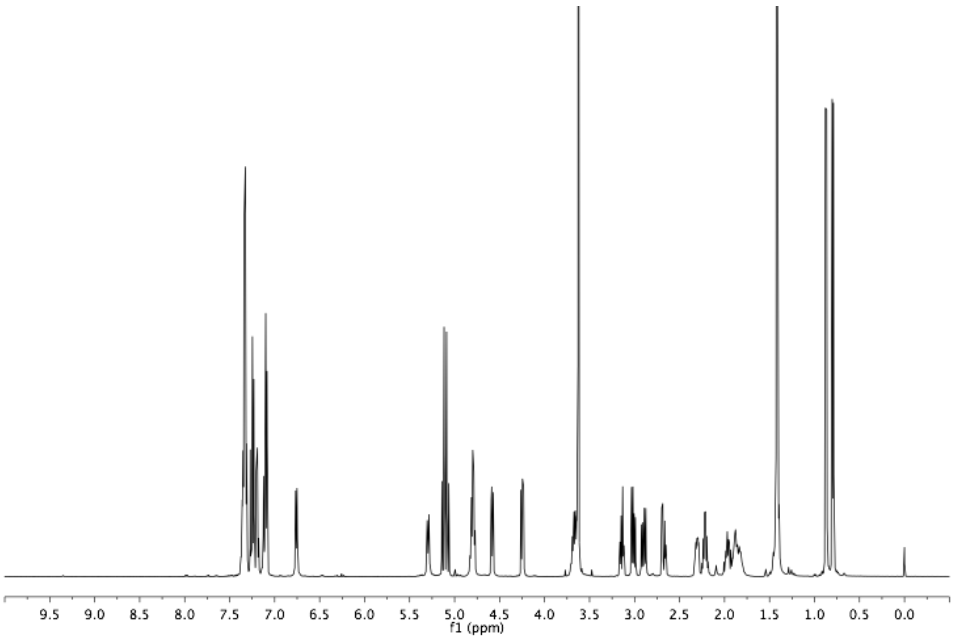


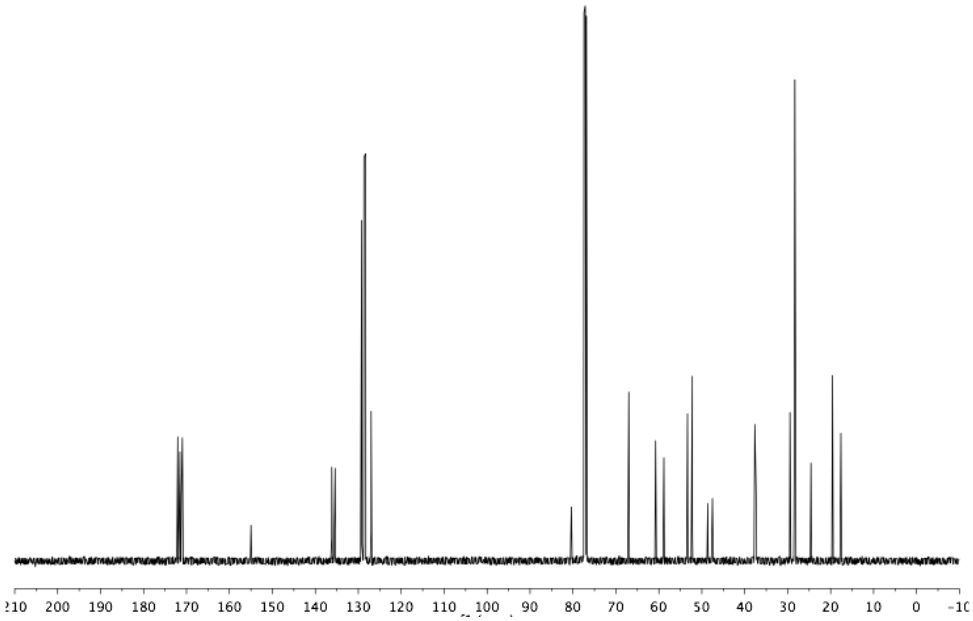
H-Phe-OMe•HCl (NovaBiochem, 216 mg, 1.0 mmol) was coupled to Cbz-D-Val-OH (Bachem, 251 mg, 1.0 mmol) with the addition of Et_3N (121 mg, 167 μL, 1.2 equiv.) using General Procedure B. The resulting dipeptide was subjected to General Procedure A, then coupled to Cbz-Pro-OH (Advanced ChemTech, 249 mg, 1.0 mmol) according to General Procedure B. Cbz-Pro-D-Val-Phe-OMe was obtained and then deprotected according to General Procedure A and coupled to Boc-Asp(OBn)-OH (NovaBiochem, 323 mg, 1.0 mmol) according to General Procedure B. **SI6** was obtained after loading

on to a silica gel column in 15% acetone in toluene, and then eluting with 25% acetone in toluene. In total, 416 mg (0.61 mmol) of **SI6** was isolated in an overall yield of *61%*.

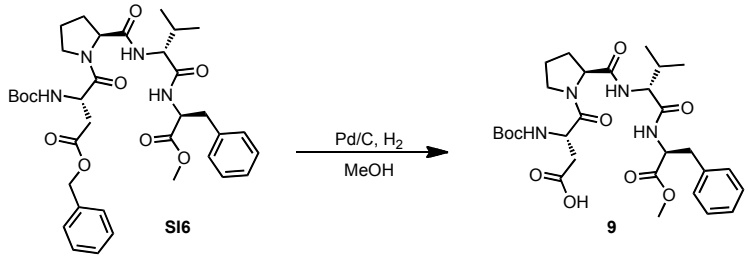
TLC: 25% acetone in toluene (R_f =0.30). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.38 – 7.29 (m, 5H), 7.25 (t, *J* = 7.3 Hz, 2H), 7.21 – 7.16 (m, 1H), 7.10 (dd, *J* = 10.1, 8.7 Hz, 3H), 6.76 (d, *J* = 7.6 Hz, 1H), 5.30 (d, *J* = 9.2 Hz, 1H), 5.10 (q, *J* = 12.3 Hz, 2H), 4.86 – 4.69 (m, 2H), 4.58 (dd, *J* = 8.2, 2.6 Hz, 1H), 4.25 (dd, *J* = 8.7, 6.3 Hz, 1H), 3.74 – 3.63 (m, 2H), 3.63 (s, 3H), 3.14 (dd, *J* = 14.0, 5.7 Hz, 1H), 3.01 (dd, *J* = 14.0, 7.3 Hz, 1H), 2.90 (dd, *J* = 16.3, 8.2 Hz, 1H), 2.68 (dd, *J* = 16.3, 5.4 Hz, 1H), 2.38 – 2.26 (m, 1H), 2.22 (dd, *J* = 13.4, 6.7 Hz, 1H), 2.01 – 1.91 (m, 1H), 1.87 (m, 2H), 1.42 (s, 9H), 0.87 (d, *J* = 6.8 Hz, 3H), 0.80 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$): δ 172.0, 171.5, 171.2, 171.1, 171.0, 155.0, 136.2, 135.4, 129.2, 128.7, 128.6, 128.5, 128.3, 127.0, 80.4, 67.0, 60.8, 58.8, 53.3, 52.2, 48.6, 47.5, 37.7, 37.4, 29.5, 28.4, 28.4, 24.6, 19.6, 17.6. **MS**: calculated mass for $[C_{36}H_{48}N_4O_9+H]^+$: 681.35, ESI+ found 681.25.

*^{1}H NMR, ^{13}C NMR spectra for **SI6**:*





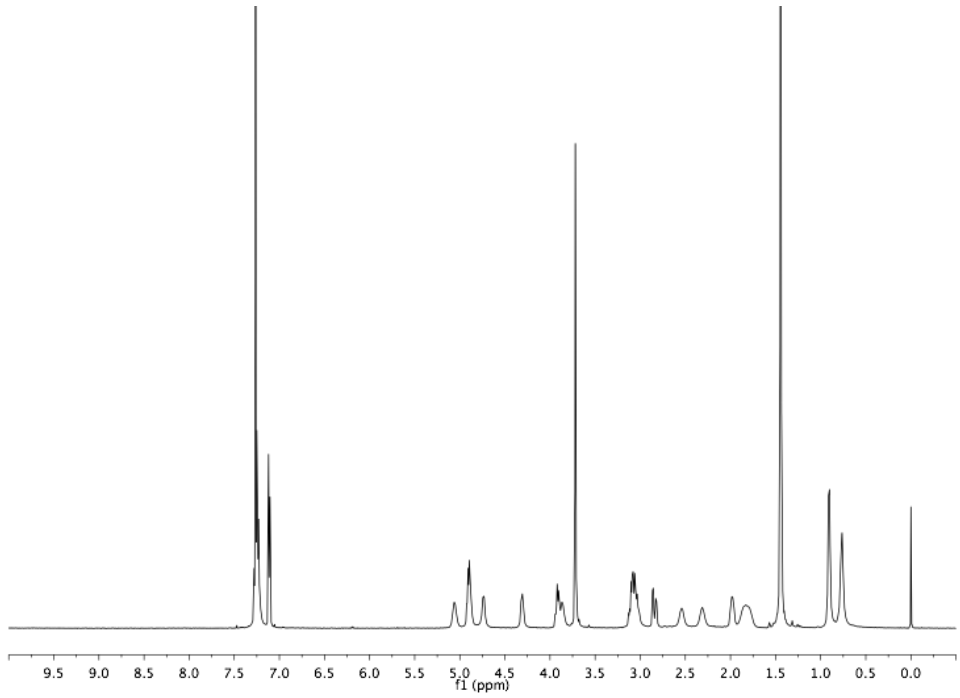
Deprotection of SI6 to afford 9:

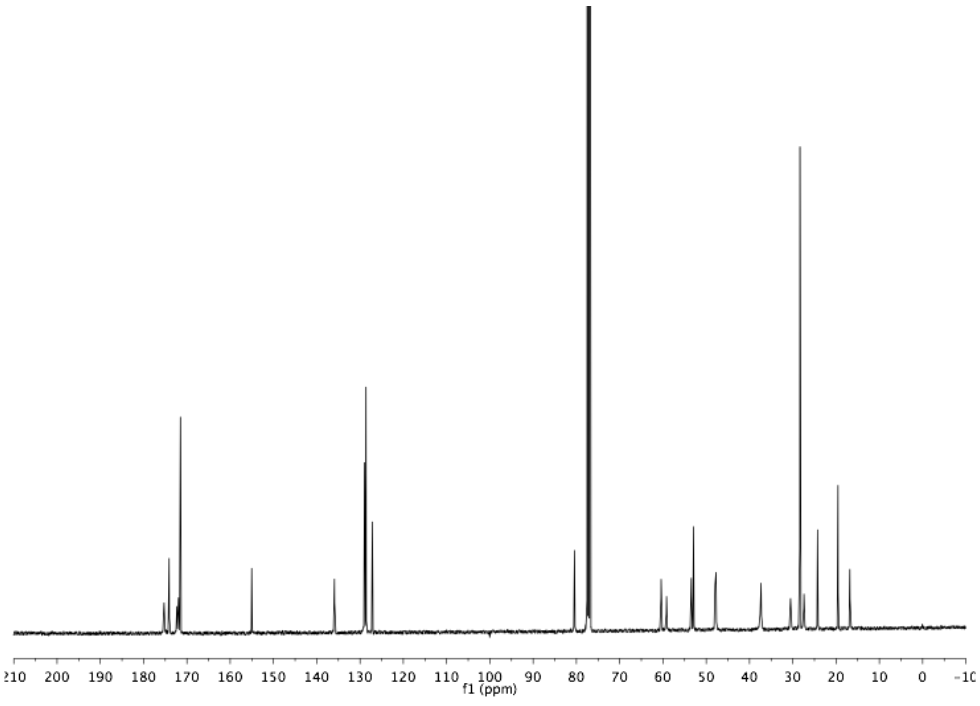


SI6 (416 mg, 0.61 mmol) was subjected to General Procedure A and concentrated *in vacuo* to yield **9** as a white solid (354 mg, 0.60 mmol, *98%*).

TLC: 50% acetone in toluene (R_f =0.16). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.35 – 7.16 (m, 5H), 7.11 (d, *J* = 6.6 Hz, 2H), 5.06 (s, 1H), 4.96 – 4.82 (m, 2H), 4.73 (s, 1H), 4.31 (s, 1H), 3.91 (dd, *J* = 21.3, 13.0 Hz, 2H), 3.72 (s, 3H), 3.21 – 2.95 (m, 3H), 2.84 (dd, *J* = 17.0, 4.0 Hz, 1H), 2.54 (s, 1H), 2.31 (s, 1H), 1.98 (s, 1H), 1.83 (s, 2H), 1.44 (s, 9H), 0.91 (d, *J* = 6.4 Hz, 3H), 0.76 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 175.3, 174.1, 172.3, 172.0, 171.5, 155.0, 135.9, 120.0, 128.6, 127.2, 80.4, 77.4, 60.4, 59.2, 53.5, 52.9, 48.0, 47.7, 37.5, 37.3, 30.5, 28.4, 27.4, 24.3, 19.6, 16.8. **IR** (film, cm^{-1}): 3305, 2971, 1712, 1635, 1522, 1436, 1392, 1367, 1281, 1248, 1213, 1161, 1046, 1024, 1006. **MS**: calculated mass for $[C_{29}H_{42}N_4O_9+H]^+$: 591.30, ESI+ found 591.31. **$[\alpha]_D$** = –106 (c = 0.51 g/100 mL $CHCl_3$).

*^{1}H NMR, ^{13}C NMR spectra for **9**:*



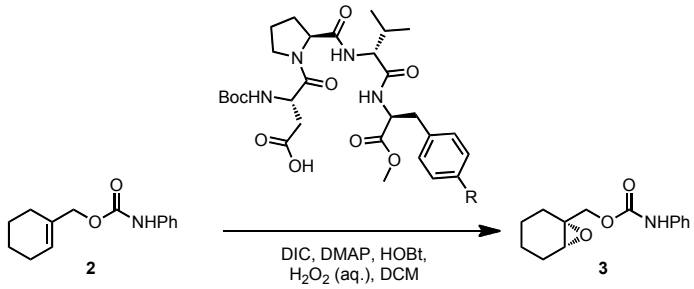


Validation of Peptide Hits.

Polystyrene macrobeads functionalized either Boc-Asp-Pro-^{D}Val-Phe (**7**) or Boc-Asp-Pro-^{D}Val-Tyr(O^{t}Bu) (**8**) were transferred individually to 200 μL PCR tubes and inspected for uniformity of size. An aqueous solution of H_2O_2 (2.54 μL, 2.97 M, 5 equiv.) was added to each tube followed by 2.65 μL

of a THF solution containing **2** (1 μmol, 1 equiv.), DIC (2 μmol, 2 equiv.), and HOBt•H_2O (0.1 μmol, 0.1 equiv.), DMAP (0.1 μmol, 0.1 equiv.), bringing the total reaction concentration of **2** to ~0.2 M. After the last addition, the tubes were closed, centrifuged, and allowed to stand for 12 hours. Reactions were quenched with ~3 drops of sat. aq. Na_2SO_3, centrifuged, then 175 μL HPLC grade hexanes was added. The biphasic mixture was vortexed, centrifuged, and the organic layer was carefully removed to an HPLC vial with insert. The samples were concentrated to dryness under a stream of N_2, dissolved in 125 μL HPLC hexanes, and resolved by HPLC using a Chiralpak® AD-H column, eluting with 1.0% isopropanol in hexanes.

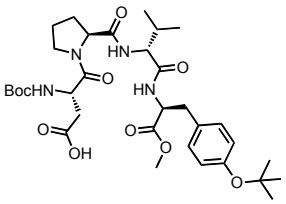
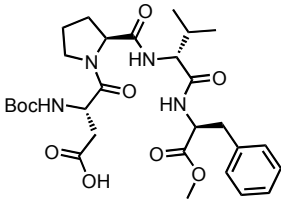
Peptide		Uncorr. Conversion	ee	Avg. Uncorr. Conversion	Avg. ee
7	Replicate 1	58%	46%	55%	46%
	Replicate 2	56%	46%		
	Replicate 3	52%	46%		
8	Replicate 1	55%	49%	55%	50%
	Replicate 2	53%	50%		
	Replicate 3	57%	51%		
No bead		0%	–		



2 **3**

Peptide (0.025 mmol, 0.1 equiv.), **2**[5] (57.8 mg, 0.25 mmol, 1.0 equiv.), DMAP (3.1 g, 0.025 mmol, 0.1 equiv.), and HOBt•H_2O (3.8 mg, 0.025 mmol, 0.1 equiv.) were added to a screw-cap vial with stir bar. DCM (0.25 mL), DIC (63 mg, 78 μL, 0.50 mmol, 2.0 equiv.), and H_2O_2 (63.8 μL of a 9.79 M solution) were then added. The solutions were allowed to stir for 16 hours at room temperature and a

precipitate formed while the reaction mixture turned a yellowish color. The mixtures were each diluted with ~2 mL Et_2O and loaded directly on a silica gel column in 15% Et_2O in pentane. The column was eluted with 25% Et_2O in pentane. Fractions containing **3** were collected, concentrated *via* rotary evaporation, filtered through celite, then concentrated *in vacuo* into a tared vial to yield the following results:

Catalyst	Mass of catalyst used	isolated yield	ee
	16.6 mg	45.6 mg (*74%*)	65%
	14.8 mg	50.0 mg (*81%*)	64%

[1] Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512-7515.
[2] Lim, Y.-B.; Park, S.; Lee, E.; Jeong, H.; Ryu, J.-H.; Sup Lee, M.; Lee, M. *Biomacromolecules* **2007**, *8*, 1404-1408.
[3] Busch, B. B *et al. Macromolecules* **2002**, *35*, 8330-8337. Alonso, J. M. *et al. Langmuir* **2008**, *24*, 448-457.
[4] Following the protocol outilined in: Thakkar, A.; Wavreille, A.-S.; Pei, D. *Anal. Chem.* **2006**, *78*, 5935-5939.
[5] For synthetic protocol and characterization, see: Peris, G.; Jakobsche, C. E.; Miller, S. J. *J. Am. Chem. Soc.* **2007**, *129*, 8710–8711.