Supporting Information for Evans, M.J. et al.

General Synthetic Methods

All compounds were purchased from commercial sources (Aldrich, Acros, Argonaut, Maybridge, Molecular Probes) and used without further purification, unless otherwise noted. Dry tetrahydrofuran (THF), acetonitrile (MeCN), methylene chloride (CH_2Cl_2), triethylamine (NEt_3), and *N,N'*-dimethyformamide (DMF) were obtained by passing commercially available pre-dried, oxygen-free formulations though activated alumina columns. Unless otherwise noted, reactions were run under an atmosphere of dry argon. Analytical thin layer chomatography (TLC) was carried out on Whatman silica gel plates (catalog no. 4861-820), and developed plates were examined under a UV light source and stained with cerium molybdate stain or ninhydrin. Flash chomatography was performed with EMD silica gel (catalog no. 11567-1). Preparative scale HPLC was performed by using a Hitachi L-7150 pump equipped with a Higgins Analytical C18, 5 μm, 150 x 10 mm reverse phase column. Purifications were achieved by using a binary gradient with A (95/5 H_2O/MeCN 0.05% TFA) and B (95/5 MeCN/H_2O 0.05% TFA) with a flow rate of 5 ml/min. NMR spectra were recorded on either a Bruker AMX 400 or a DRX 500 spectrometer. Chemical shifts are reported in δ ppm values relative to the residual undeuterated solvent, and coupling constants (*J*) are reported in Hz. Mass spectra were obtained with an Agilent ESI-TOF and IonSpec Ultima FTMS at the Center for Mass Spectrometry at The Scripps Research Institute. Optical rotations were obtained with a Perkin-Elmer 431 Polarimeter. The rhodamine-azide and the trifunctional rhodamine-biotin azide reporter tags were prepared as previously described.[15] All library members (**MJE1-64, -67**) were stored as 10 mM DMSO stocks at -20° C. Abbreviations: DIC, diisopropylcarbodiimide; DMAP, *N,N'*-dimethylaminopyridine; DMSO, dimethylsulfoxide; EDC, *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride; *m*-CPBA, 3-chloro-peroxybenzoic acid; TBAF, tetrabutylammonium fluoride.

Synthetic protocols and characterization data for representative members of the spiroepoxide probe library (other library members were characterized by HRMS, data shown in Supporting Information Table 1)

MJE4: To a stirring solution of **10** (0.004 g, 0.0095 mmol, 1.0 equiv) in MeCN (0.45 mL) was added L-tryptamine (0.002 g, 0.0095 mmol, 1.0 equiv) and MP-Carbonate™ polystyrene resin (0.0036 g, 0.01 mmol, 1.1 equiv). The reaction was stirred at rt for 30 min, and **MJE4** (0.0026 g, 60%) was purified by silica chromatography. R_f = 0.7 (50% EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (br, 1H), 7.59 (d, 1H, *J* = 7.6 Hz), 7.38 (d, 1H, *J* = 8.2 Hz), 7.20 (td, 1H, *J* = 7.0, 1.2 Hz), 7.12 (m, 1H), 7.01 (d, 1H, *J* = 2.3 Hz), 5.03 (dd, 1H, *J* = 12.0, 7.3 Hz), 4.84 (t, 1H, *J* = 5.8 Hz), 3.51 (m, 2H), 2.95 (t, 2H, *J* = 6.7), 2.82 (d, 1H, *J* = 4.4 Hz), 2.60 (d, 1H, *J*=4.7 Hz), 2.37 (t, 2H, *J* = 7.6 Hz), 2.24 (td, 2H, *J*=7.0, 2.6 Hz), 2.15 (td, 1H, *J*=13.5, 4.0 Hz), 1.96 (t, 1H, *J* = 2.6 Hz), 1.99 (m, 1H), 1.94 (m, 1H), 1.80 (m, 2H), 1.71 (m, 1H), 1.63 (s, 1H), 1.54-1.40 (m, 2H), 1.46 (s, 3H), 1.44 (s, 3H). HR-MS (ESI-TOF) Calcd for $C_{27}H_{34}N_2O_5$ $(M+Na)^+$ 489.2360, found 489.2368.

MJE10: To a stirring solution of **10** (0.0040 g, 0.0095 mmol, 1.0 equiv) in MeCN (0.45 mL) was added diphenylethylamine (0.0020 g, 0.0095 mmol, 1.0 equiv) and MP-Carbonate™ polystyrene resin (0.0036 g, 0.01 mmol, 1.1 equiv). The reaction was stirred at rt for 30 min, and **MJE10** (0.0040 g, 83%) was purified by silica chromatography. R_f = 0.8 (50% EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58-7.52 (m, 5H), 7.43 (t, 2H, *J* = 18 Hz), 7.34 (m, 1H), 7.27 (m, 1H), 7.24 (s, 1H), 5.03 (dd, 1H, *J* = 12.0, 7.3 Hz), 3.48 (m, 2H), 2.84 (t, 2H, *J* = 7.0 Hz), 2.81 (d, 1H, *J* = 4.4 Hz), 2.61 (d, 1H, *J* = 4.7 Hz), 2.37 (t, 2H, *J* = 7.6 Hz), 2.24 (td, 2H, *J* = 7.0, 2.6 Hz), 2.15 (td, 1H, *J* = 13.5, 4.0 Hz), 1.96 (t, 1H, *J* = 2.6 Hz), 1.99 (m, 1H), 1.94 (m, 1H), 1.80 (m, 2H), 1.71 (m, 1H), 1.54-1.40 (m, 2H), 1.46 (s, 3H), 1.45 (s, 3H). HR-MS (ESI-TOF) Calcd for $C_{31}H_{37}NO_5$ $(M+Na)^+$ 526.2564, found 526.2561.

MJE16: To a stirring solution of **10** (0.0050 g, 0.012 mmol, 1.0 equiv) in MeCN (0.9 mL) was added (*S*)-(_)-2-amino-4-methyl-1,1-diphenylpentane (0.0030 g, 0.012 mmol, 1.0 equiv) and MP-Carbonate™ polystyrene resin (0.0047 g, 0.013 mmol, 1.1 equiv). The reaction was stirred at rt for 30 min, and **MJE16** (0.0061 g, 91%) was purified by silica chromatography**.** R_f = 0.9 (50% EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29-7.28 (m, 6H), 7.25-7.18 (m, 3H), 7.12 (m, 1H), 4.88 (dd, 1H, *J* = 12.0, 7.3 Hz), 4.55 (m, 1H), 4.43 (m, 1H), 3.82 (d, 1H, *J* = 9.4 Hz), 2.77 (d, 1H, *J* = 4.4 Hz), 2.57 (d, 1H, *J* = 4.4 Hz), 2.38 (t, 2H, *J* = 7.6 Hz), 2.24 (td, 2H, *J*=7.0, 2.6 Hz), 2.08 (m, 1H), 1.96 (t, 1H, *J* = 2.6 Hz), 1.90 (m, 1H), 1.80 (m, 2H), 1.67 (m, 2H), 1.46 (s, 3H), 1.45 (m, 3H), 1.44 (s, 3H), 1.24 (m, 3H), 0.94 (m, 3H), 0.84 (m, 3H). HR-MS (ESI-TOF) Calcd for $C_{35}H_{45}NO_5$ $(M+Na)^+$ 582.3190, found 582.3193.

MJE24: To a stirring solution of **10** (0.0070 g, 0.016 mmol, 1.0 equiv) in MeCN (0.9 mL) was added (S)-(-)-1,1-diphenyl-2-aminopropane (0.0033 g, 0.016 mmol, 1.0 equiv) and MP-Carbonate™ polystyrene resin (0.0065 g, 0.018 mmol, 1.1 equiv). The reaction was stirred at rt for 30 min, and **MJE24** (0.0067 g, 82%) was purified by silica chromatography**.** R_f = 0.8 (50% EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21

(m, 8H), 7.19 (m, 2H), 4.93 (dd, 1H, *J* = 12.0, 7.3 Hz), 4.58 (m, 2H), 3.80 (d, 1H, *J* = 9.6 Hz), 2.77 (d, 1H, *J* = 4.4 Hz), 2.58 (d, 1H, *J* = 4.4 Hz), 2.38 (t, 2H, *J* = 7.6 Hz), 2.26 (td, 2H, *J* = 7.0, 2.6 Hz), 2.09 (m, 1H), 1.97 (t, 1H, *J* = 2.6 Hz), 1.92 (m, 1H), 1.81 (m, 2H), 1.75-1.71 (m, 1H), 1.46 (s, 3H), 1.42 (s, 3H), 1.45-1.38 (m, 2H), 1.35-1.25 (m, 2H), 1.13 (d, 3H, *J* = 6.1 Hz). HR-MS (ESI-TOF) Calcd for $C_{32}H_{39}NO_5$ $(M+Na)^+$ 540.2720, found 540.2716.

MJE40: To a stirring solution of **10** (0.0060 g, 0.014 mmol, 1 equiv) in MeCN (0.9 mL) was added 1-adamantanamine hydrochloride (0.0022 g, 0.014 mmol, 1.0 equiv) and NEt_3 (0.0020 mL, 0.015 mmol, 1.1 equiv) via syringe. The reaction was stirred at rt for 30 min, and **MJE40** (0.0030 g, 46%) was purified by silica chromatography**.** R_f = 0.7 (50% EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.04 (dd, 1H, *J* = 12.0, 7.3 Hz), 4.73 (t, 1H, *J* = 5.8 Hz), 2.95 (d, 1H, *J* = 3.5 Hz), 2.71 (d, 1H, *J* = 3.5 Hz), 2.47 (t, 2H, *J* = 5.8 Hz), 2.33 (m, 2H), 2.24 (s, 2H), 2.20 (m, 1H), 2.15 (m, 2H), 2.04 (t, 1H, *J* = 2.6 Hz), 1.98 (m, 5H), 1.88 (m, 2H), 1.80 (m, 2H), 1.74 (s, 5H), 1.63 (s, 1H), 1.54-1.40 (m, 2H), 1.54 (s, 3H), 1.53 (s, 3H), 1.33 (m, 2H). HR-MS (ESI-TOF) Calcd for $C_{27}H_{39}N_2O_5$ $(M+Na)^+$ 489.2360, found 489.2368.

MJE50: To a solution of **10** (0.020 g, 0.047 mmol, 1.0 equiv), (S)-2-amino-N-benzyl-3-(1H-indol-3-yl)propanamide[9] (0.015 g, 0.052 mmol, 1.1 equiv) in 4.5 mL MeCN was added NEt_3 (0.0070 mL, 0.052 mmol, 1.1 equiv) dropwise via syringe. After 1 h, the reaction was concentrated under reduced pressure, and the crude mixture was separated by silica chromatography to afford 0.0040 g (25%) of **MJE50**. R_f = 0.6 (50% EtOAc in hexanes) ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (br, 1H), 7.67 (d, 1H, *J* = 7.92 Hz), 7.37 (d, 1H, *J* = 8.2 Hz), 7.23 (m, 4H), 7.14 (m, 1H), 6.97 (m, 2H), 6.93 (d, 1H, *J* = 2.10 Hz), 5.86 (m, 1H), 5.55 (m, 1H), 4.98 (dd, 1H, *J* = 12.0, 7.3 Hz), 4.8 (m, 1H), 4.49 (m, 1H), 4.30 (m, 2H), 3.32 (m, 1H), 3.18 (m, 1H), 2.67 (d, 1H, *J* = 4.12 Hz), 2.55 (d, 1H, *J* = 4.12 Hz), 2.35 (m, 2H), 2.23 (m, 2H), 2.14 (m, 1H), 1.95 (t, 1H, *J* = 5.3 Hz), 1.95-1.90 (m, 1H), 1.78 (m, 2H), 1.58 (m, 1H), 1.46-1.42 (m, 3H) 1.45 (s, 3H) 1.43 (s, 3H). HR-MS (ESI-TOF) Calcd for $C_{35}H_{41}N_3O_6$ $(M+H)^+$ 600.3068, found 600.3066.

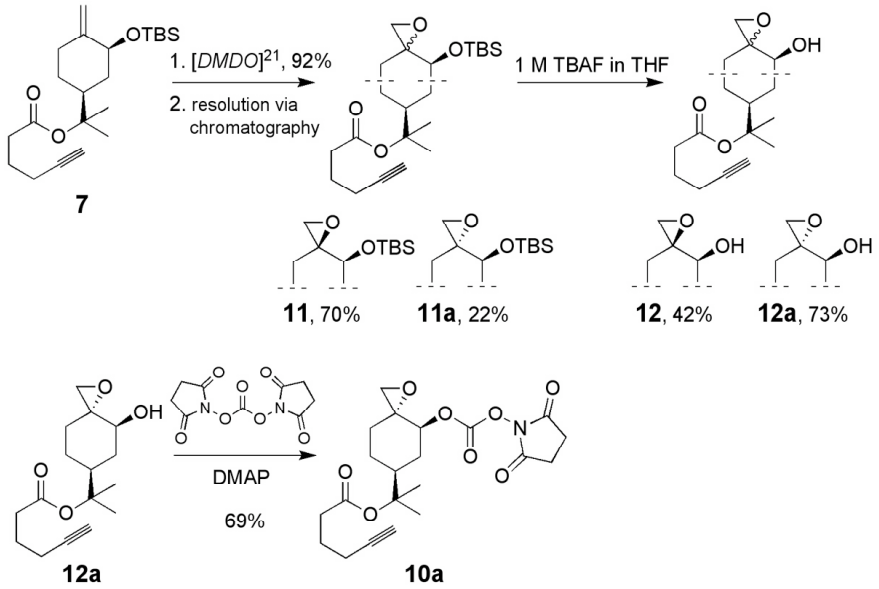
MJE51: To a solution of **10** (0.004 g, 0.0095 mmol, 1.0 equiv), L-tryptophan (0.010 g, 0.0095 mmol, 1.0 equiv) in 0.45 mL DMF was added NEt_3 (0.0027 mL, 0.019 mmol, 2.1 equiv) dropwise via syringe. After 1 h, the reaction volume was reduced to 0.3 mL, and the crude mixture was separated with HPLC (gradient 0–100% B over 40 min, with the product eluting at 20 min, and LC/MS was used to identify the product containing fractions) to afford 0.0039 g (80%) of **MJE51**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (br, 1H), 7.56 (d, 1H, *J* = 7.92 Hz), 7.33 (d, 1H, *J* = 5.88 Hz), 7.17 (m, 1H), 7.11 (d, 1H, *J* = 7.92 Hz), 6.95 (m, 1H), 4.98 (dd, 1H, *J* = 12.0, 7.3 Hz), 3.26 (m, 3H), 2.65 (d, 1H, *J* = 4.68 Hz), 2.53 (d, 1H, *J* = 4.72 Hz), 2.33 (m, 2H), 2.21 (m, 2H), 2.14 (m, 1H), 1.95 (t, 1H, J = 5.3 Hz), 1.95-1.9 (m, 2H), 1.78 (m, 2H), 1.68 (m, 1H), 1.45-1.42 (m, 4H), 1.44 (s, 3H), 1.42 (s, 3H). HR-MS (ESI-TOF) Calcd for $C_{28}H_{34}N_2O_7$ $(M+Na)^+$ 533.2258, found 533.2258.

MJE52: To a solution of **10** (0.010 g, 0.023 mmol, 1.0 equiv), L-tryptophan methyl ester (0.0050 g, 0.023 mmol, 1.0 equiv) in MeCN (0.9 mL) was added MP-Carbonate™

(0.0095 g, 0.025 mmol, 1.1 equiv). After stirring for 1 h at rt, **MJE52 (**0.0095 g, 76%) was isolated using the protocol outlined for **MJE3.** R_f = 0.4 (50% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (br, 1H), 7.53 (d, 1H, *J* = 7.64 Hz), 7.36 (d, 1H, *J* = 8.2 Hz), 7.19 (t, 1H, *J* = 14.96 Hz), 7.11 (m, 1H), 6.97 (d, 1H, *J* = 2.04 Hz), 5.33 (d, 1H, J = 8.24 Hz), 4.99 (m, 1H), 4.69 (m, 1H), 3.69 (s, 3H), 3.28 (m, 2H), 2.63 (d, 1H, *J* = 4.4 Hz), 2.53 (d, 1H, *J* = 4.4 Hz), 2.36 (m, 2H), 2.23 (m, 3H), 2.12 (m, 1H), 2.01 (m, 1H), 1.95 (t, 1H, J = 5.28 Hz), 1.91 (m, 1H), 1.78 (m, 1H), 1.45-1.43 (m, 4H) 1.44 (s, 3H), 1.43 (s, 3H). HR-MS (ESI-TOF) Calcd for $C_{29}H_{36}N_2O_7$ $(M+H)^+$ 524.2522, found 524.2523.

MJE55: To a stirring solution of **10a** (0.010 g, 0.023 mmol, 1 eq) in MeCN (0.9 mL) was added L-tryptophan benzyl ester (0.006 g, 0.023 mmol, 1.0 eq) and MP-Carbonate™ polystyrene resin (0.009 g, 0.025 mmol, 1.1 equiv). The reaction was stirred at rt, and reaction progress was monitored by TLC. After 30 min, **MJE55** (0.0030 g, 20%) was isolated using the protocol outlined for **MJE3.** R_f = 0.5 (50% EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (br, 1H), 7.55 (d, 1H, *J* = 7.7 Hz), 7.34 (m, 4H), 7.24 (m, 2H), 7.18 (m, 1H), 7.10 (m, 1H), 6.78 (d, 1H, *J* = 2.0 Hz), 5.19 (d, 1H, *J* = 8.4 Hz), 5.09 (s, 2H), 5.03 (m, 1H), 4.77 (m, 1H), 3.32 (m, 2H), 2.89 (m, 1H), 2.51 (d, 1H, *J* = 5.1 Hz), 2.36 (t, 2H, *J* = 7.6 Hz), 2.24 (td, 2H, *J* = 7.0, 2.6 Hz), 2.15 (m, 1H), 2.07 (m, 1H), 1.98 (m, 1H), 1.97 (t, 1H, *J* = 2.6 Hz), 1.89 (m, 1H), 1.78 (m, 1H), 1.56 (br, 1H), 1.46-1.43 (m, 2H), 1.44 (s, 3H), 1.43 (s, 3H) 1.27 (m, 1H). HR-MS (ESI-TOF) Calcd for $C_{35}H_{40}N_2O_7$ $(M+Na)^+$ 623.2728, found 623.2729.

Supporting Information Scheme 1.



11, 11a: A solution containing, **7** (1.1 g, 3.0 mmol, 1.0 equiv), $NBu_4{\cdot}HSO_4$ (0.26 g, 0.80 mmol, 0.25 equiv) and acetone (6.8 mL, 90 mmol, 30 equiv) in CH_2Cl_2 (15 mL) was prepared and cooled in an ice bath. OXONE™ (5.7 g, 9.1 mmol, 3.0 equiv) and $NaHCO_3$ (3.4 g, 40 mmol, 13 equiv) were weighed in separate vials and each dissolved in H_2O (12.5 mL). The solution containing $NaHCO_3$ was added dropwise via syringe over 10 min to the solution containing **7**. The solution containing OXONE™ was then added dropwise via syringe to initiate the formation of dimethydioxirane in situ,[21] and the reaction cocktail was allowed to warm to rt. After 18 h, the reaction was complete, and CH_2Cl_2 (15 mL) was added. The organic layer was separated, washed (1 x 10 mL H_2O), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified with flash chromatography (6% EtOAc in hexanes) to afford 0.82 g (70%) and 0.26 g (22%) of **11** and **11a**, respectively. **11**: R_f = 0.5 (25% EtOAc in hexanes); $[\alpha]^D$ = -5.5 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (dd, 1H, *J* = 11.4, 4.4 Hz), 3.04 (d, 1H, *J* = 5.5 Hz), 2.55 (d, 1H, *J* = 5.5 Hz), 2.38 (t, 2H, *J* = 7.3 Hz), 2.24 (td, 2H, *J* = 7.0, 2.6 Hz), 2.26-2.20 (m, 1H), 2.15 (m, 1H), 1.96 (t, 1H, *J* = 2.9 Hz), 1.93 (td, 1H, *J* = 13.5, 4.0 Hz), 1.79 (m, 2H), 1.69 (m, 2H), 1.52-1.40 (m, 2H), 1.46 (s, 3H), 1.44 (s, 3H) 0.88 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H). HR-MS (ESI-TOF) Calcd for $C_{22}H_{38}O_4Si$ $(M+H)^+$ 395.2612, found 395.2615. **11a**: R_f = 0.55 (25% EtOAc in hexanes); $[\alpha]^D$ = -6.0 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.77 (m, 1H), 2.95 (m, 1H), 2.41 (d, 1H, *J* = 5.5 Hz), 2.38 (t, 2H, *J* = 7.3 Hz), 2.24 (td, 2H, *J* = 7.0, 2.6 Hz), 2.26-2.20 (m, 1H), 2.15 (m, 1H), 1.96 (t, 1H, *J* = 2.9 Hz), 1.93 (td, 1H, *J* = 13.5, 4.0 Hz), 1.79 (m, 2H), 1.69 (m, 2H), 1.52-1.40 (m, 2H), 1.46 (s, 3H), 1.44 (s, 3H) 0.88 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H). HR-MS (ESI-TOF) Calcd for $C_{22}H_{38}O_4Si$ $(M+H)^+$ 395.2612, found 395.2614.

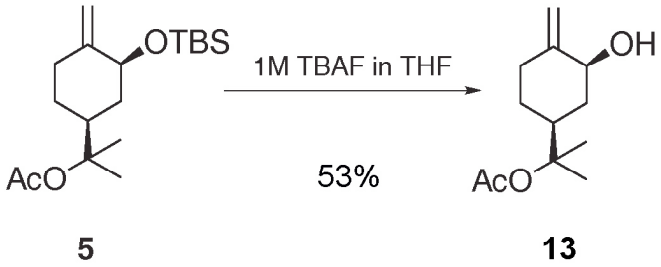
12: To a solution of **11** (0.13 g, 0.33 mmol, 1.0 eq) in THF (3 mL) was added TBAF (0.70 mL, 1 M in THF, 2.1 eq) via syringe. The reaction was stirred at rt for 1 h, at which time EtOAc (5 mL) was added. The reaction was washed with H_2O (1 x 10 mL) and aqueous saturated NaCl (1 x 10 mL). The organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Flash chromatography (40% EtOAc in hexanes) afforded 0.038 g (42%) of **12** as a colorless oil. R_f = 0.2 (25% EtOAc in hexanes); $[\alpha]^D$ = -15.0 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.78 (m, 1H), 3.12 (d, 1H, J = 4.6 Hz), 2.60 (d, 1H, J = 4.6 Hz), 2.37 (t, 2H, J = 7.3 Hz), 2.24 (td, 2H, *J* = 7.0, 2.6 Hz), 2.24 (m, 1H), 2.10 (m, 1H), 1.96 (t, 1H, *J* = 2.9 Hz), 1.93 (td, 1H, *J* = 13.5, 4.0 Hz), 1.79 (m, 2H), 1.78-1.69 (m, 2H), 1.64 (m, 1H),1.52-1.47 (m, 1H), 1.46 (s, 3H), 1.44 (s, 3H) 1.38 (m, 1H). HR-MS (ESI-TOF) Calcd for $C_{16}H_{24}O_4$ $(M+Na)^+$ 303.1567, found 303.1569.

12a: Compound **11a** (0.046 g, 0.12 mmol, 1.0 equiv) in THF (1.5 mL) was treated with TBAF (0.2 mL, 1M in THF, 2.1 equiv) and compound **12a** (0.024 g, 73%) was isolated as a colorless oil using the protocol outlined for compound **12**. R_f = 0.25 (25% EtOAc in hexanes); $[\alpha]^D$ = -11.0 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 3.81 (m, 1H), 3.07 (m, 1H), 2.55 (d, 1H, J = 4.6 Hz), 2.37 (t, 2H, J = 7.3 Hz), 2.26 (td, 2H, *J* = 7.0, 2.6 Hz), 2.20 (m, 1H), 2.17 (m, 1H), 2.04 (br, 1H), 1.97 (t, 1H, *J* = 2.9 Hz), 1.88 (m, 2H), 1.80 (m, 2H), 1.61 (br, 1H), 1.45-1.41 (m, 2H), 1.46 (s, 3H), 1.44 (s, 3H) 1.28 (m, 1H),

1.24 (m, 1H). HR-MS (ESI-TOF) Calcd for $C_{16}H_{24}O_4$ $(M+Na)^+$ 303.1567, found 303.1568.

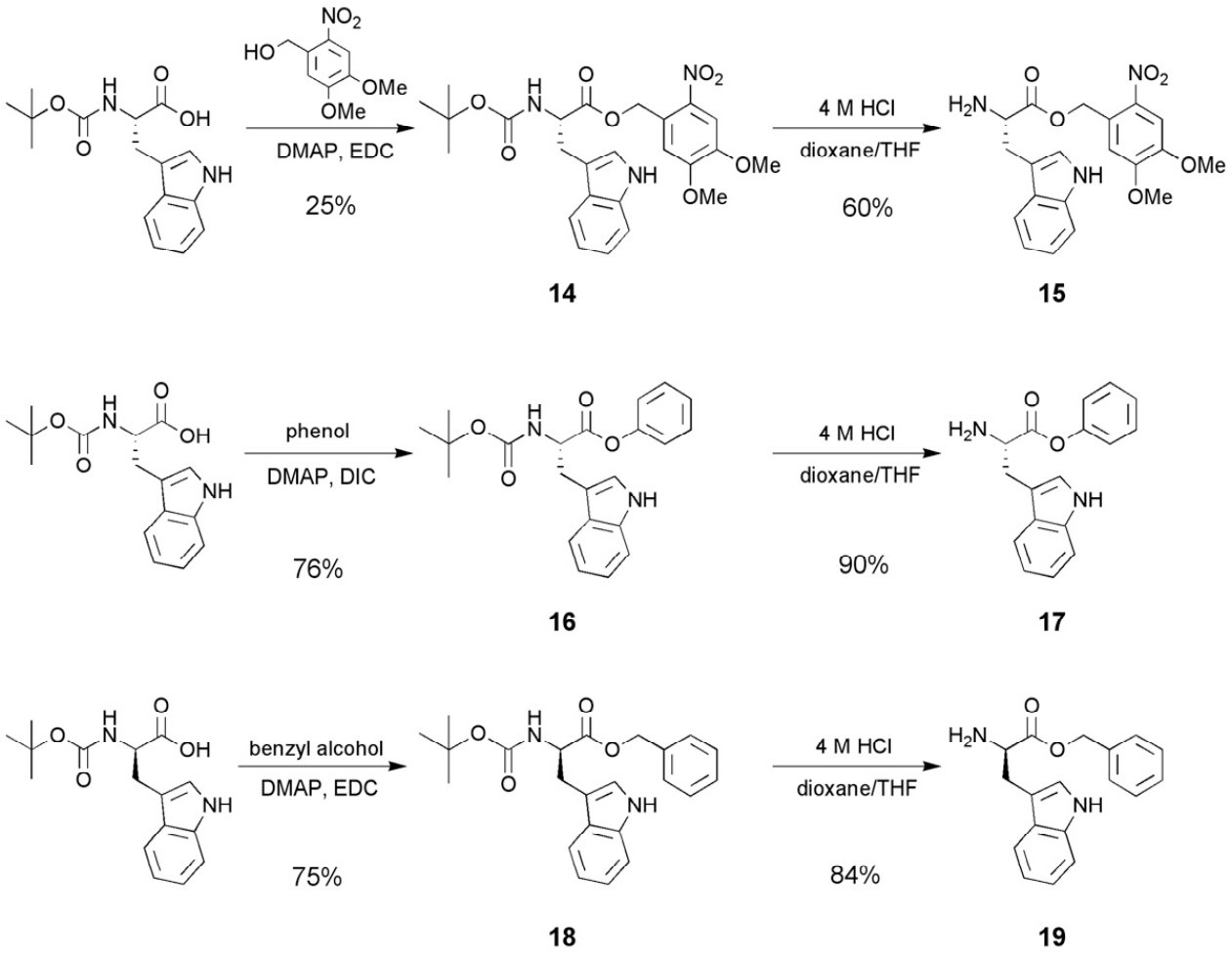
10a: To a solution of **12a** (0.076 g, 0.27 mmol, 1.0 equiv) and DMAP (0.066 g, 0.54 mmol, 2.0 equiv) in MeCN (1.0 mL) was added *N,N'*-disuccinimidyl carbonate (0.34 g, 1.4 mmol, 5.0 equiv). The reaction stirred at rt for 4 h, and diluted with EtOAc (10 mL). The solution was washed with aqueous saturated citric acid (2 x 10 mL) and aqueous saturated NaCl (1 x 10 mL). The organic extracts were dried (Na_2SO_4), filtered, and adsorbed onto silica. Flash chromatography (30% EtOAc in hexanes) afforded 0.079 g (69%) of **10a** as a colorless oil. R_f = 0.2 (25% EtOAc in hexanes); $[\alpha]^D$ = -1.8 (c = 1.0, $CHCl_3$); ^{1}H (500 MHz, $CDCl_3$) δ 4.97 (m, 1H), 3.04 (m, 1H), 2.81 (s, 4H), 2.57 (d, 1H, *J* = 4.6 Hz), 2.37 (t, 2H, *J* = 7.3 Hz), 2.32 (m, 1H), 2.25 (td, 2H, *J* = 7.0, 2.6 Hz), 2.16 (m, 1H), 2.17 (m, 1H), 2.04 (m, 1H), 1.98 (t, 1H, *J* = 2.9 Hz), 1.90 (m, 1H), 1.80 (m, 2H), 1.64 (br, 1H), 1.45-1.41 (m, 2H), 1.46 (s, 3H), 1.44 (s, 3H) 1.33 (m, 1H), 1.26 (m, 1H). HR-MS (ESI-TOF) Calcd for $C_{21}H_{27}NO_8$ $(M+Na)^+$ 444.1629, found 444.1634

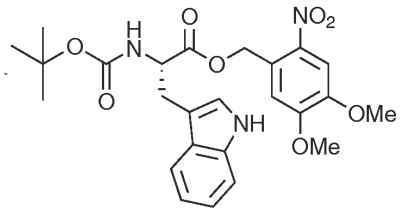
Supporting Information Scheme 2.



13: To a solution of **5** (0.050 g, 0.015 mmol, 1.0 equiv) in THF (0.050 mL) was added TBAF (0.50 mL, 1 M in THF, 33 equiv) via syringe. The reaction was stirred at room temperature for 1 hr, at which time EtOAc (1 mL) was added. The reaction was washed with H_2O (1 x 50 mL) and aqueous saturated NaCl (1 x 50 mL). The organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Flash chromatography (30% EtOAc in hexanes) afforded 0.017 g (53%) of **13** as a colorless oil. R_f = 0.2 (50% EtOAc in hexanes); $[\alpha]^D$ = -15.0 (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.93 (d, 1H, *J* = 1.4 Hz), 4.78 (d, 1H, *J* = 1.8 Hz), 4.05 (dd, 1H, *J* = 11.4, 4.8 Hz), 2.45 (dt, 1H, *J* = 12.8, 3.6 Hz, 2.24, (td, 1H, *J* = 7.0, 2.6 Hz), 2.15 (m, 1H), 2.01 (td, 1H, *J* = 13.2, 4.0 Hz), 1.97 (s, 3H), 1.82 (m, 1H), 1.78 (m, 1H), 1.41 (s, 3H), 1.40 (s, 3H), 1.15 (m, 2H). HR-MS (ESI-TOF) Calcd for $C_{12}H_{20}O_3$ $(M+H)^+$ 212.1412, found 212.1408.

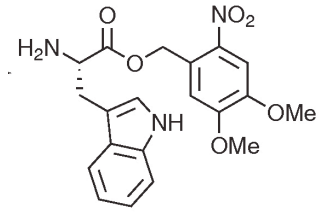
Supporting Information Scheme 3.





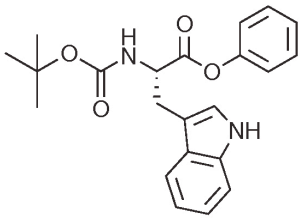
14

(S)-4,5-dimethoxy-2-nitrobenzyl 2-(tert-butoxycarbonyl)-3-(1H-indol-3-yl) propanoate (14): *N*-α-BOC-L-tryptophan (0.100 g, 0.32 mmol, 1.0 equiv), (4,5-dimethoxy-2-nitrophenyl)methanol (0.140 g, 0.65 mmol, 2.0 equiv), DMAP (0.0400 g, 0.32 mmol, 1.0 equiv), and EDC (0.125 g, 0.65 mmol, 2.0 equiv) were combined in a dry round bottom flask. After the flask was purged of oxygen, THF (0.65 mL) was added via syringe. The reaction was stirred for 2 h at rt and concentrated onto a plug of silica. The reaction mixture was purified via silica gel chromatography (40% EtOAc in hexanes), affording 0.041 g of **14** (25%). R_f = 0.6 (50% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (br, 1H), 7.66 (s, 1H), 7.58 (d, 1H, *J* = 7.9 Hz), 7.33 (d, 1H, *J* = 7.9 Hz), 7.17 (m, 1H), 7.10 (t, 1H, *J* = 7.3 Hz), 7.02 (d, 1H, *J* = 2.0 Hz), 6.90 (s, 1H), 5.52 (m, 2H), 5.11 (d, 1H, *J* = 7.6 Hz), 3.94 (s, 3H), 3.85 (s, 3H), 3.31 (d, 2H, *J* = 5.8 Hz), 1.67 (br, 1H), 1.40 (s, 9H). HRMS (ESI-TOF) Calcd for $C_{25}H_{29}N_3O_8$ $(M+H)^+$ 500.2027, found 500.2023.



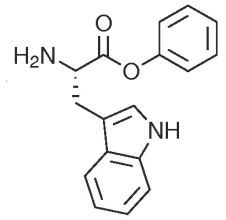
15

(S)-4,5-dimethoxy-2-nitrobenzyl 2-amino-3-(1H-indol-3-yl)propanoate (15): To a stirring mixture of **14** (0.04 g, 0.08 mmol, 1.0 equiv) in THF (0.5 mL) was added 4 M HCl in dioxanes (0.1 mL, 0.4 mmol, 5.0 equiv). The reaction was stirred at rt for 4 h, and concentrated under a stream of nitrogen. The resulting solid (**15**, 0.019 g, 60%) was carried forward without further purification. ^{1}H NMR (400 MHz, MeOD) δ 7.79 (s, 1H), 7.58 (d, 1H, *J* = 7.9 Hz), 7.40 (d, 1H, *J* = 7.9 Hz), 7.24 (s, 1H), 7.17 (t, 1H, *J* = 7.0 Hz), 7.08 (d, 1H, *J* = 7.0 Hz), 6.96 (s, 1H), 5.58 (m, 2H), 4.48 (m, 1H), 3.98 (s, 3H), 3.88 (s, 3H), 3.55 (m, 2H). HRMS (ESI-TOF) Calcd for $C_{20}H_{21}N_3O_6$ $(M+H)^+$ 400.1503, found 400.1508.



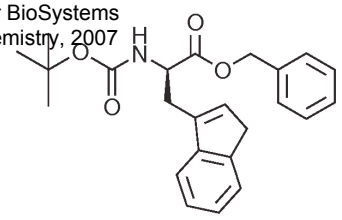
16

(S)-phenyl-2-(tert-butoxycarbonyl)-3-(1H-indol-3-yl)propanoate (16): To a stirring solution of *N*-α-BOC-L-tryptophan (0.10 g, 0.32 mmol, 1.0 equiv), phenol (0.058 g, 0.62 mmol, 1.9 equiv) and DMAP (0.0040 g, 0.032 mmol, 0.10 equiv) in THF (0.65 mL) was added DIC (0.10 mL, 0.65 mmol, 2.0 equiv) via syringe. The reaction was stirred for 30 min at ambient temperature, then diluted with EtOAc (5 mL), and then washed with 1M NaOH (2 x 5 mL). The organic extract was dried (Na_2SO_4), filtered, and concentrated. The resulting residue was purified via silica gel chromatography (10% EtOAc in hexanes), affording 0.095 g of **16** (76%). R_f = 0.5 (50% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (br, 1H), 7.66 (d, 1H, *J* = 7.9 Hz), 7.40 (d, 1H, *J* = 8.5 Hz), 7.33 (t, 2H, *J* = 7.9 Hz), 7.22 (m, 2H), 7.14-7.11 (m, 2H), 6.93 (t, 1H, *J* = 1.4 Hz), 6.90 (m, 1H), 5.17 (m, 1H), 4.90 (m, 1H), 3.45 (d, 2H, *J* = 5.8 Hz), 1.45 (s, 9H). HRMS (ESI-TOF) Calcd for $C_{22}H_{24}N_2O_4$ $(M+H)^+$ 381.1809, found 381.1800.



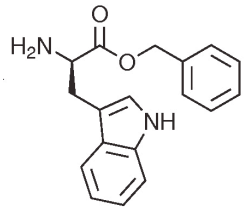
17

(S)-phenyl-2-amino-3-(1H-indol-3-yl)propanoate (17): To a stirring mixture of **16** (0.050 g, 0.13 mmol, 1.0 equiv) in THF (0.26 mL) was added 4 M HCl in dioxanes (0.13 mL, 0.52 mmol, 4.0 equiv). The reaction was stirred at rt for 3 h, and concentrated under a stream of nitrogen. The resulting solid (**17**, 0.032g, 90%) was carried forward without further purification. ^{1}H NMR (400 MHz, MeOD) δ 7.71 (d, 1H, *J* = 7.9 Hz), 7.51 (d, 1H, *J* = 7.9 Hz), 7.42 (t, 2H, *J* = 7.6 Hz), 7.38 (s, 1H), 7.32 (t, 1H, *J* = 7.6 Hz), 7.24 (t, 1H, *J* = 7.3 Hz), 7.14 (t, 1H, *J* = 7.6 Hz), 7.01 (d, 2H, *J* = 7.9 Hz), 4.66 (t, 1H, *J* = 6.7 Hz), 3.65 (m, 2H). HRMS (ESI-TOF) Calcd for $C_{17}H_{16}N_2O_2$ $(M+H)^+$ 281.1282, found 281.1292.



18

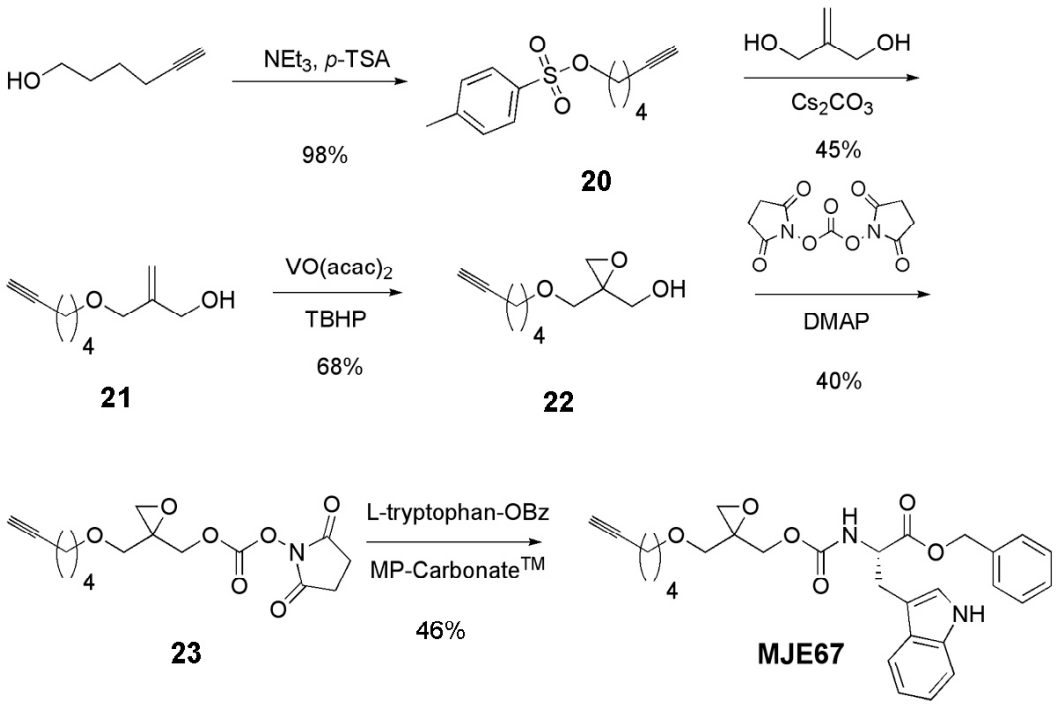
(R)-benzyl 2-(tert-butoxycarbonyl)-3-(1H-indol-3-yl)propanoate(18): To a stirring solution of *N*-α-BOC-D-tryptophan (0.10 g, 0.32 mmol, 1.0 equiv), benzyl alcohol (0.070 mL, 0.65 mmol, 2.0 equiv), DMAP (0.040 g, 0.32 mmol, 1 equiv), and EDC (0.12 g, 0.65 mmol, 2.0 equiv) in MeCN (1.0 mL). The reaction was stirred for 2 h at ambient temperature and concentrated onto a plug of silica. The reaction mixture was purified via silica gel chromatography (40% EtOAc in hexanes), affording 0.097 g of **18** (75%). R_f = 0.55 (50% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (br, 1H), 7.56 (d, 1H, *J* = 7.9 Hz), 7.38-7.31 (m, 5H), 7.24 (m, 2H), 7.19 (m, 1H), 7.10 (t, 1H, *J* = 7.3 Hz), 6.81 (br, 1H), 5.1 (m, 3H), 4.71 (m, 1H), 3.3 (d, 1H, *J* = 5.2 Hz), 1.42 (s, 9H). HRMS (ESI-TOF) Calcd for $C_{23}H_{26}N_2O_4$ $(M+H)^+$ 395.1965, found 395.1959.

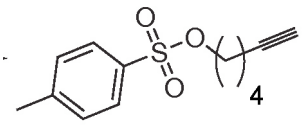


19

(R)-benzyl 2-amino-3-(1H-indol-3-yl)propanoate (19): To a stirring mixture of **18** (0.040 g, 0.080 mmol, 1.0 equiv) in THF (0.50 mL) was added 4 M HCl in dioxanes (0.10 mL, 0.40 mmol, 5.0 equiv). The reaction was stirred at room temperature for 4 h, and concentrated under a stream of nitrogen. The resulting solid (**19**, 0.031 g, 84%) was carried forward without further purification. ^{1}H NMR (400 MHz, MeOD) δ 7.61 (d, 1H, *J* = 7.9 Hz), 7.48 (d, 1H, *J* = 7.9 Hz), 7.40 (m, 3H), 7.31 (m, 2H), 7.21 (t, 1H, *J* = 7.3 Hz), 7.17 (s, 1H), 7.12 (t, 1H, *J* = 7.3 Hz), 5.25 (m, 2H), 4.42 (t, 1H, *J* = 6.7 Hz), 3.47 (m, 2H). HRMS (ESI-TOF) Calcd for $C_{18}H_{18}N_2O_2$ $(M+H)^+$ 295.1441, found 295.1445.

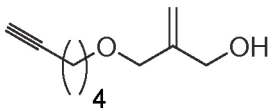
Supporting Information Scheme 3.





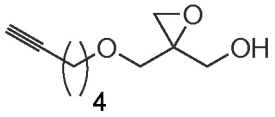
20

20: NEt_3 (6.4 mL, 46 mmol, 1.5 equiv) was added dropwise via syringe to a stirring solution of 5-hexyn-1-ol (3.0 g, 31 mmol, 1.0 equiv) and *p*-toluene sulfonic acid (12 g, 62 mmol, 2.0 equiv) in CH_2Cl_2 (60 mL). The reaction was stirred at rt for 18 h. The reaction was washed 1 x 30 mL with aqueous 10% HCl and 1 x 30 mL aqueous saturated $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered and concentrated. The crude residue was purified with flash chromatography (10% EtOAc in hexanes) to afford 7.6 g (98%) of **20**. $R_f = 0.8$ (50% EtOAc in hexanes). ^1H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, 2H, $J = 8.1$ Hz), 7.35 (d, 2H, $J = 11$ Hz), 4.05 (t, 2H, $J = 7.7$ Hz), 2.45 (s, 3H), 2.16 (td, 2H, $J = 8.4, 3.3$ Hz), 1.92 (t, 1H, $J = 2.9$ Hz), 1.76 (m, 2H), 1.61 (m, 2H). HRMS (ESI-TOF) Calcd for $C_{13}H_{16}O_3S$ $(M+Na)^+$ 275.0712, found 275.0712.



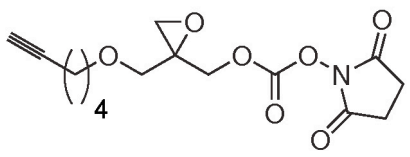
21

21: To a mixture of **20** (0.70 g, 2.7 mmol, 1.0 equiv) and Cs_2CO_3 (1.4 g, 4.1 mmol, 1.5 equiv) in MeCN (140 mL), was added 2-methylene-1,3-propanediol (1.2 g, 14 mmol, 5.0 equiv). The reaction was refluxed for 24 h. After cooling to rt, MeCN was removed under reduced pressure with a rotary evaporator. The crude residue was dissolved in EtOAc, and washed with H_2O (1 x 50 mL). The organic layer was dried and concentrated. Flash chomatography (10% EtOAc in hexanes) afforded 0.21 g (45%) of **21**. R_f = 0.65 (50% EtOAc in hexanes). ^{1}H (500 MHz, $CDCl_3$) δ 5.13 (s, 1H), 5.09 (s, 1H), 4.17 (s, 2H), 4.04 (s, 2H), 3.44 (t, 2H, *J* = 7.7 Hz), 2.23 (m, 1H), 2.20 (td, 2H, *J* = 8.4, 3.3 Hz), 1.95 (t, 1H, *J* = 2.9 Hz), 1.69 (m, 2H), 1.62 (m, 2H). HRMS (ESI-TOF) Calcd for $C_{10}H_{16}O_2$ (M+H) 169.1223, found 169.1222.



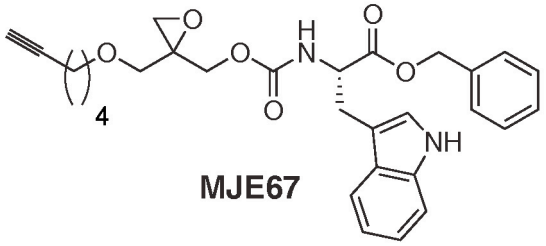
22

22: Vanadyl acetoacetonate (0.0090 g, 0.035 mmol, 0.030 equiv) was dissolved in CH_2Cl_2 (30 mL), and the reaction vessel was cooled to 0 ºC. *tert*-Butyl hydrogen peroxide (0.43 mL, 5.5 M in decanes, 2.0 equiv) was added to the solution dropwise via syringe. The solution stirred for 5 min at 0 ºC, at which time a solution of **21** (0.20 g, 1.2 mmol, 1.0 equiv) in CH_2Cl_2 (1.0 mL) was added slowly via syringe. The reaction was stirred at rt for 18 h, and diluted with CH_2Cl_2 (30 mL). The solution was washed with H_2O (2 x 10 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Flash chromatography (40% EtOAc in hexanes) purified 0.15 g (68%) of compound **22**. R_f = 0.35 (50% EtOAc in hexanes). ^{1}H (500 MHz, $CDCl_3$) δ 3.88 (d, 1H, *J* = 12.1 Hz), 3.73 (d, 1H, *J* = 12.1 Hz), 3.64 (d, 1H, *J* = 11.0 Hz), 3.57 (d, 1H, *J* = 11.0 Hz), 3.52 (m, 2H), 2.88 (d, 1H, *J* = 4.7 Hz), 2.75 (d, 1H, *J* = 4.7 Hz), 2.19 (td, 2H, *J* = 8.4, 3.3 Hz), 2.09 (m, 1H), 1.94 (t, 1H, *J* = 2.9 Hz), 1.69 (m, 2H), 1.58 (m, 2H). HRMS (ESI-TOF) Calcd for $C_{10}H_{16}O_3$ $(M+Na)^+$ 207.0992, found 207.0992.



23

23: To a solution of **22** (0.15 g, 0.80 mmol, 1.0 equiv) and DMAP (0.19 g, 1.6 mmol, 2.0 equiv) in MeCN (5.3 mL) was added *N,N'*-disuccinimidyl carbonate (1.0 g, 3.9 mmol, 5.0 equiv). The reaction was stirred at rt for 2 h, and adsorbed onto a pad of silica. Flash chromatography (40% EtOAc in hexanes) afforded 0.10 g (40%) of **23**. R_f = 0.4 (50% EtOAc in hexanes). ^{1}H (500 MHz, $CDCl_3$) δ 4.45 (s, 2H), 3.62 (d, 1H, *J* = 11.1 Hz), 3.55 (d, 1H, *J* = 11.1 Hz), 3.48 (m, 2H), 2.87-2.80 (m, 2H), 2.87 (s, 4H), 2.20 (td, 2H, *J* = 8.4, 3.3 Hz), 1.94 (t, 1H, *J* = 2.9 Hz), 1.69-1.66 (m, 2H), 1.59-1.55 (m, 2H). HRMS (ESI-TOF) Calcd for $C_{15}H_{19}NO_7$ $(M+Na)^+$ 348.1054, found 348.1057.



MJE67

MJE67: To a stirring solution of **23** (0.010 g, 0.030 mmol, 1.0 equiv) in MeCN (0.90 mL) was added L-tryptophan benzyl ester (0.0088 g, 0.030 mmol, 1.0 equiv) and MP-Carbonate™ polystyrene resin (0.012 g, 0.033 mmol, 1.1 equiv). The reaction was stirred at ambient temperature, and reaction progress was monitored by TLC. After 30 min, the reaction was complete, and the solution was removed from the reaction vessel with a syringe, and transferred to a fresh vial. The solution was concentrated under a stream of nitrogen. The resulting residue was purified with flash chomatography (40% EtOAc in hexanes) to afford 0.0070 g of **MJE67** (46%). R_f = 0.7 (50% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (br, 1H), 7.52 (d, 1H, *J* = 8.2 Hz), 7.34 (m, 1H), 7.33 (m, 3H), 7.22 (m, 2H), 7.18 (m, 1H), 7.10 (m, 1H), 6.79 (s, 1H), 5.33 (t, 1H, *J* = 6.7 Hz), 5.09 (s, 2H), 4.75 (m, 1H), 4.30-4.19 (m, 2H), 3.59-3.44 (m, 4H), 3.32 (d, 2H, *J* = 5.3 Hz), 2.74 (m, 2H), 2.22 (td, 2H, *J* = 7.0, 2.6 Hz), 1.96 (t, 1H, *J* = 2.6 Hz), 1.67 (m, 2H), 1.59 (m, 2H). HRMS (ESI-TOF) Calcd for $C_{29}H_{32}N_2O_6$ $(M+H)^+$ 505.2333, found 505.2322.

Supporting Information Table 1. Characterization data for the spiroepoxide library.

Entry	Molecular Formula	Yield (%)	R_f [a]	Predicted Mass[b,c,d]	Detected Mass
MJE1	$C_{28}H_{36}N_2O_6$	42	0.1	519.2465[c]	519.2472
MJE2	$C_{28}H_{36}N_2O_6$	63	0.4	519.2465[c]	519.2471
MJE5	$C_{26}H_{34}N_2O_9$	61	0.1	519.2337[b]	519.2341
MJE6	$C_{28}H_{38}N_2O_5$	44	0.2	483.2835[b]	483.2841
MJE7	$C_{29}H_{40}N_2O_7$	59	0.2	529.2908[b]	529.2909
MJE8	$C_{25}H_{33}FNO_5$	73	0.8	432.2181[b]	432.2188
MJE9	$C_{24}H_{29}Cl_2NO_5$	59	0.8	504.1315[c]	504.1305
MJE11	$C_{26}H_{35}NO_6$	69	0.7	480.2356[c]	480.2350
MJE12	$C_{26}H_{33}NO_8$	43	0.4	510.2098[c]	510.2091
MJE13	$C_{37}H_{41}NO_6$	35	0.9	596.3006[b]	596.3020
MJE14	$C_{37}H_{41}NO_5$	38	0.9	580.3057[b]	580.3048
MJE15	$C_{38}H_{43}NO_6$	52	0.9	632.2982[c]	632.2979
MJE17	$C_{22}H_{29}NO_6$	78	0.8	426.1887[c]	426.1890
MJE18	$C_{23}H_{34}NO_7$	72	0.1	460.2306[c]	460.2310
MJE19	$C_{26}H_{33}NO_7$	78	0.8	494.2149[c]	494.2151
MJE20	$C_{38}H_{44}N_2O_7S$	44	0.8	695.2761[c]	695.2756
MJE21	$C_{26}H_{33}N_3O_5$	45	0.2	468.2493[b]	468.2499
MJE22	$C_{26}H_{33}NO_7$	55	0.5	494.2149[c]	494.2153
MJE23	$C_{30}H_{42}N_2O_7$	56	0.5	565.2884[c]	565.2883
MJE25	$C_{21}H_{33}NO_5$	67	0.8	402.2251[c]	402.2248
MJE26	$C_{24}H_{36}N_2O_6$	46	0.2	471.2465[c]	471.2468
MJE27	$C_{21}H_{31}N_2O_7$	51	0.1	432.1993[c]	432.1993
MJE28	$C_{34}H_{40}N_2O_6$	22	0.7	595.2778[c]	595.2784
MJE29	$C_{27}H_{33}FN_2O_5$	37	0.4	507.2266[c]	507.2271
MJE30	$C_{24}H_{32}N_2O_5$	68	0.2	429.2384[b]	429.2381
MJE31	$C_{28}H_{41}NO_6$	66	0.4	496.2570[c]	496.2568
MJE32	$C_{23}H_{35}NO_6$	60	0.4	444.2536[c]	444.2358
MJE33	$C_{27}H_{45}NO_5$	86	0.8	486.3190[c]	486.3195
MJE34	$C_{28}H_{37}NO_9$	35	0.1	530.2395[d]	530.2385
MJE35	$C_{28}H_{33}NO_7S$	22	0.1	550.1870[c]	550.1865
MJE36	$C_{28}H_{33}NO_5$	38	0.8	486.2251[c]	486.2248
MJE37	$C_{26}H_{33}NO_6$	45	0.2	487.2200[c]	487.2202
MJE38	$C_{23}H_{31}NO_5S$	72	0.8	456.1815[c]	456.1821
MJE39	$C_{24}H_{32}N_4O_7$	49	0.6	489.2344[b]	489.2341
MJE41	$C_{27}H_{34}N_2O_5$	35	0.6	477.2344[b]	477.2342
MJE42	$C_{24}H_{30}INO_5$	62	0.9	540.1241[b]	540.1239
MJE43	$C_{25}H_{39}F_4NO_5$	63	0.9	522.1874[c]	522.1875
MJE44	$C_{27}H_{34}N_2O_5$	73	0.8	436.2094[c]	436.2092
MJE45	$C_{27}H_{41}NO_5$	34	0.8	460.3057[b]	460.3058
MJE46	$C_{27}H_{31}ClN_2O_5S$	43	0.7	531.1715[b]	531.1715

Supporting Information Table 1, continued.

Entry	Molecular Formula	Yield (%)	R_f [a]	Predicted Mass[b,c,d]	Detected Mass
MJE47	$C_{24}H_{29}N_3O_5S$	48	0.7	494.1720[c]	494.1718
MJE48	$C_{27}H_{34}N_2O_5$	34	0.7	434.2901[b]	434.2890
MJE49	$C_{30}H_{37}NO_6S$	28	0.7	552.2414[b]	552.2403
MJE53	$C_{37}H_{43}N_3O_{11}$	45	0.8	706.2970[b]	706.2961
MJE54	$C_{34}H_{38}N_2O_7$	37	0.5	587.2752[b]	587.2756
MJE56	$C_{35}H_{40}N_2O_7$	50	0.5	601.2908[b]	601.2904
MJE57	$C_{26}H_{33}NO_7$	45	0.7	472.2330[b]	472.2318
MJE58	$C_{24}H_{37}NO_7$	69	0.8	452.2643[b]	452.2638
MJE59	$C_{28}H_{37}NO_8$	20	0.5	538.2411[c]	538.2404
MJE60	$C_{27}H_{35}NO_7$	52	0.8	486.2486[b]	486.2468
MJE61	$C_{33}H_{39}NO_8$	43	0.5	578.2748[b]	578.2731
MJE62	$C_{27}H_{35}NO_7$	43	0.7	486.2486[b]	486.2477
MJE63	$C_{35}H_{43}NO_8$	35	0.9	606.3061[b]	606.3057
MJE64	$C_{32}H_{44}N_2O_9$	49	0.3	601.3116[b]	601.3118

[a] R_f values were obtained in a solution of 50% EtOAc in hexanes. [b] HRMS values refer to the $(M+H)^+$ ion. [c] HRMS values refer to the $(M+Na)^+$ ion. D. HRMS values refer to the $(M-H)^-$ ion.

Supporting Information Figure Legends.

Supporting Information Figure 1. A summary of the ^{1}H-NMR data (400 MHz, $CDCl_3$) used to determine the relative stereochemistry of **13**. Chemical shifts and coupling constants for the natural metabolites 2-((1S, 3S)-3-hydroxy-4-methylene-cyclohexyl)propan-2-yl (syn), and 2-((1S, 3R)-3-hydroxy-4-methylene-cyclohexyl)propan-2-yl (anti) were obtained from Ngo, K. S.; Wong, W. T.; Brown, G. D. *J Nat Prod* **1999**, *62*, 549-53.

Supporting Information Figure 2. Representative partial images of ^{1}H-NMR spectra showing first-order coupling to H1 on **12** (500 MHz, MeOD), and evidence for long-range coupling between H1 and H2 on compound **12a** (500 MHz, $CDCl_3$). Careful analysis of the spectra will reveal minor quantities of the axial product (~3.1 ppm) in the equatorial spectrum and minor quantities of the equatorial product (~3.15 ppm) in the axial spectrum. Importantly, these smaller peaks show the expected splitting pattern (i.e. long range splitting for the axial peak in the equatorial spectrum) to further support the assignment of the two isomers. Residual undeuterated solvent is indicated with an arrow. Similar results were obtained for **10/10a** and **11/11a** (data not shown).

Supporting Information Figure 3. Structures of the amine substituents of various MJE probes #1-52.

Supporting Information 4. Mass spectrometry fragmentation pattern for MJE3 probe. (A) Predicted chemical structures of observed fragments. (B) Major neutral loss probe fragments observed in tandem mass spectrometry profiles of the MJE3-aa 91-106 peptide adduct.

Supporting Information 5. Kinetic analysis of the in situ proteome reactivity profile of MJE3. (A) Fluorescent gel analysis (shown in greyscale) of time course for MJE3 labeling of PGAM1 in MUM2B cells. (B) Plot of labeling intensity for MJE3-PGAM1 reaction versus time. Data represent the average values $\pm$ standard deviation for three independent experiments. Based on these results, a time point of 1 h was selected to obtain *in situ* labeling profiles of maximal intensity.

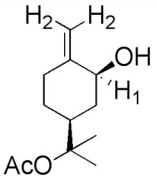
Supporting Information 6. Clustering histogram for the docked conformations from 200 independent dockings of the MJE3-PGAM1 interaction. Binned clusters contain every conformation that has an RMSD of 3 Å or less relative to every other member of the cluster. Each bin is plotted at the docking energy of the tightest binding member. It can be seen that the lowest energy cluster is well separated from the next highest energy clusters, and it is also highly populated. Inspection of the conformations in the five most populated clusters reveals that three of the five share similar binding modes for the

carboxylate and indole groups of the ligand, while the cyclohexane ring and acetylene-containing sidechain have more variability in binding mode (see Supporting Information Figure 7).

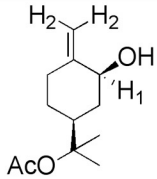
Supporting Information Figure 7. Each of the five panels A-E shows all the conformations in each of the five lowest-energy conformational clusters shown in the histogram in Supporting Information Figure 6. Panels A (cluster 1), B (cluster 2) and C (cluster 5) show strong similarities in the binding preferences of the carboxylate and indole ring of MJE3, while panels D (cluster 3) and E (cluster 4) are more similar in that the acetylene-containing sidechain prefers to bind outside the citrate-binding pocket and over the Lys-100 to which MJE is covalently bound.

Supporting Information Figure 1

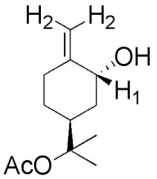
Supplementary Material (ESI) for Molecular BioSystems



13



2-((1S,3S)-3-hydroxy-4-methylene-cyclohexyl)propan-2-yl acetate (syn)

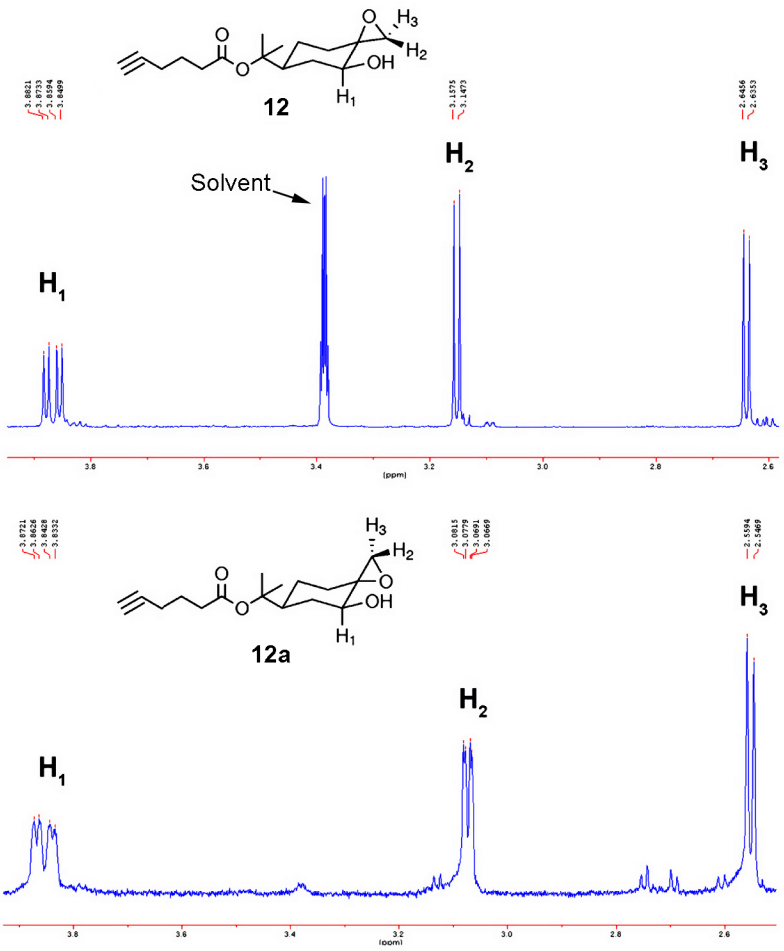


2-((1S,3R)-3-hydroxy-4-methylene-cyclohexyl)propan-2-yl acetate (anti)

	H_1	H_2
13	4.08 (m, 1H)	4.78 (d, 1H, *J* =1.76 Hz), 4.93 (d, 1H, *J* = 1.55 Hz)
syn	4.08 (ddd, 1H, *J* = 11.4, 2.5, 2.5 Hz)	4.78 (d, 1H, *J* = 1.7 Hz), 4.93 (d, 1H, *J* = 1.6 Hz)
anti	4.38 (br, 1H)	4.76 (t, 1H, *J* = 2.0 Hz), 4.85 (d, 1H, *J* = 1.6 Hz)

Supporting Figure 2

Supplementary Material (ESI) for Molecular BioSystems



Supporting Information Figure 3

Supplementary Material (ESI) for Molecular BioSystems

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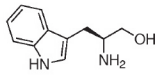

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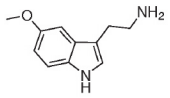

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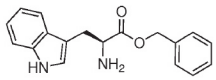

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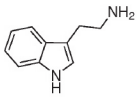

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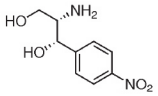

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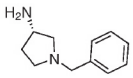

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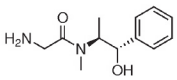

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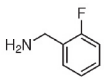

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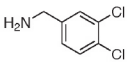

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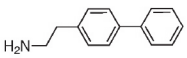

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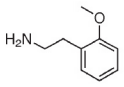

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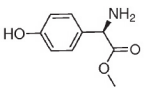

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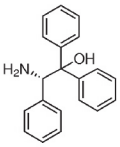

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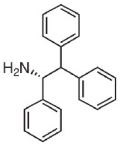

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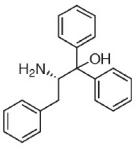

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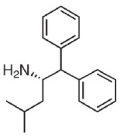

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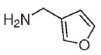

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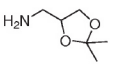

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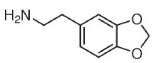

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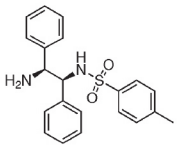

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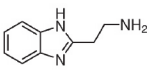

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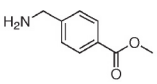

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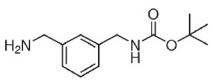

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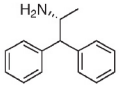

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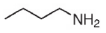

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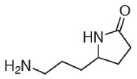

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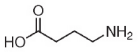

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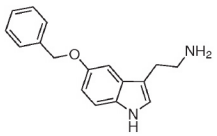

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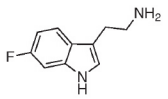

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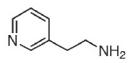

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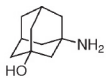

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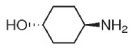

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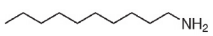

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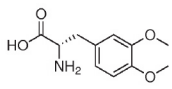

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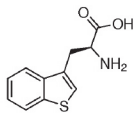
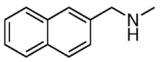


Supporting Information Figure 3 continued

MJE36



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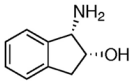

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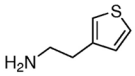

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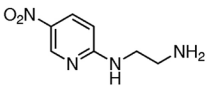

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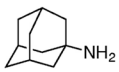

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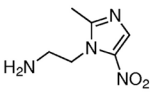

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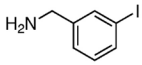

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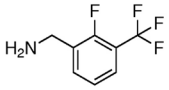

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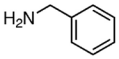

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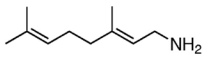

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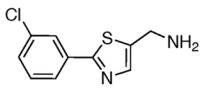

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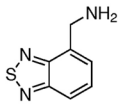

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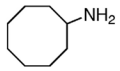

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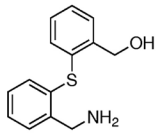

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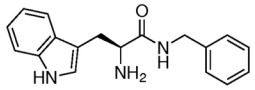

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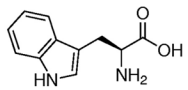

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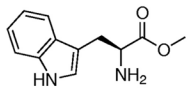
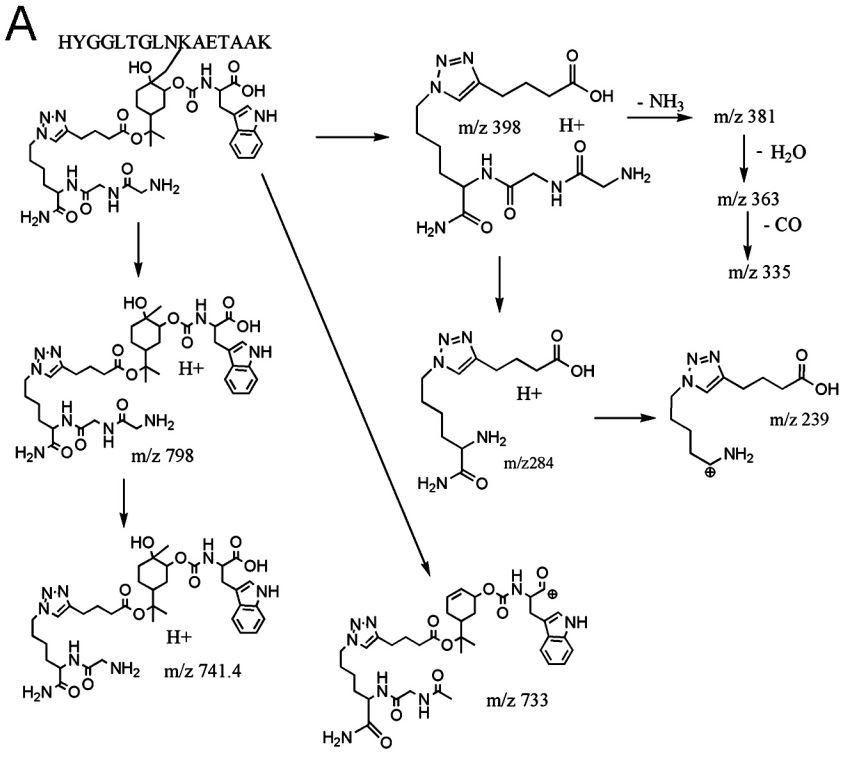
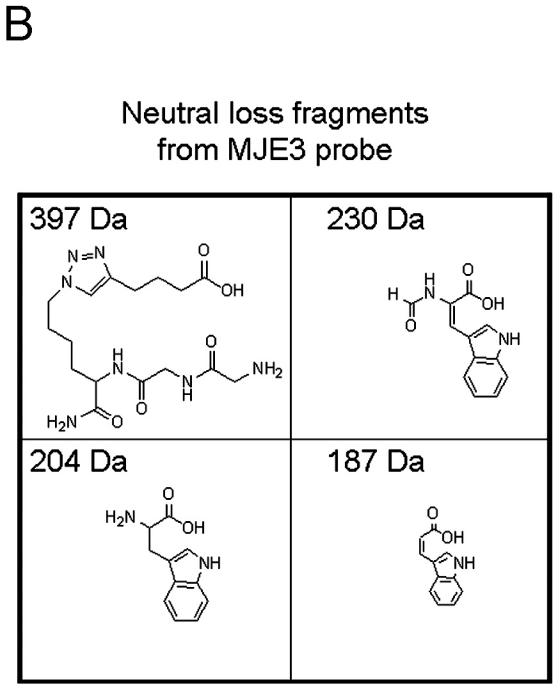


Supporting Information Figure 4

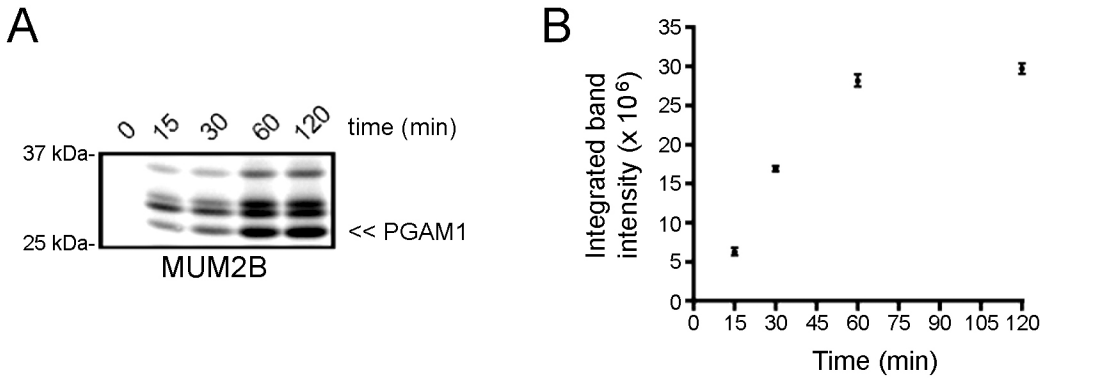
Supplementary Material (ESI) for Molecular BioSystems





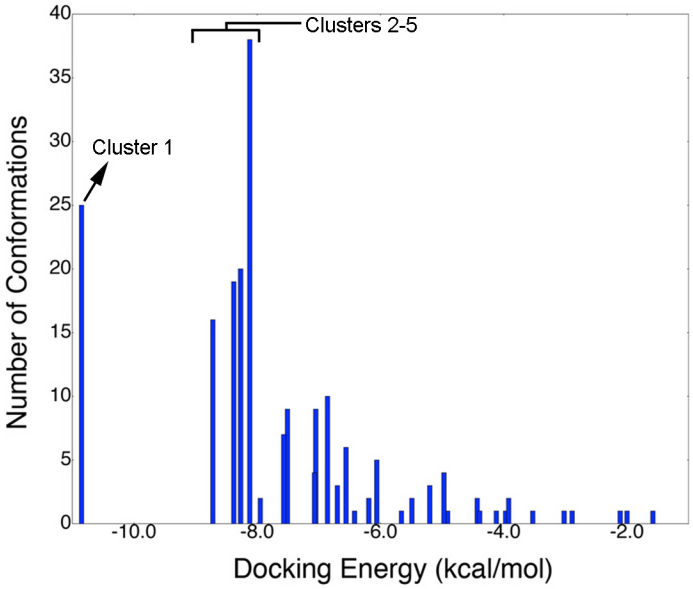
Supporting Information Figure 5

Supplementary Material (ESI) for Molecular BioSystems



Supporting Information Figure 6

Supplementary Material (ESI) for Molecular BioSystems



Supporting Information Figure 7

Supplementary Material (ESI) for Molecular BioSystems