Synthesis of macrocyclic inhibitors of trypanosomal cysteine proteases

Yen Ting Chen,[a,b] Ricardo Lira,[a.b] Elizabeth Hansell,[c] James H. McKerrow[c] and William R. Roush[a.b]*

[a] *Department of Chemistry, Scripps Florida, Jupiter, FL 33458, USA*
[b] *Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA*
[c] *Sandler Center for Basic Research in Parasitic Diseases, University of California, San Francisco, CA 94143, USA*

e-mail: roush@scripps.edu

SUPPORTING INFORMATION

Experimental details for synthesis of macrocyclic inhibitors **4** and **5**, and procedures for enzyme inhibition

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General experimental details

All reaction solvents were of reagent grade and used as received. Tetrahydrofuran, dichloromethane, diethyl ether, and toluene were purified by passing through a solvent column composed of activated A-1 alumina. Unless indicated otherwise, all reactions were conducted under an atmosphere of nitrogen using flame-dried or oven-dried (170 °C) glassware. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon-13 (^{13}C) NMR spectra were recorded on either Varian VXR-400 or Bruker Avance-400 spectrometer at 400 MHz and 100 MHz, respectively. The proton signal for residual, non-deuterated solvent (δ 7.26 ppm for $CHCl_3$, δ 3.31 ppm for MeOD) was used as an internal reference for ^{1}H NMR spectra. For ^{13}C NMR spectra, chemical shifts are reported relative to the δ 77.0 ppm resonance of $CDCl_3$ or the δ 49.0 ppm resonance of MeOD. Coupling constants are reported in Hertz (Hz). Infrared (IR) spectra were recorded as thin films on a Perkin-Elmer Spectrum 1000 FTIR. Mass spectra were recorded on a ZVG 70-250-s spectrometer manufactured by Micromass Corp. (Manchester UK), at the University of Michigan Mass Spectrometry Laboratory.

Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F_{254} glass plates pre-coated with a 0.25 mm thickness of silica gel. The TLC plates were visualized with UV light and/or by staining with either Hannesian solution (ceric sulfate and ammonium molybdate in aqueous sulfuric acid) or permanganate solution (potassium permanganate in aqueous sodium hydroxide). Column chromatography was generally performed using Kieselgel 60 (230-400 mesh) silica gel, typically using a 50-100:1 weight ratio of silica gel to crude product.

(*R*,*R*)-Pseudoephedrine (*S*)-2-Amino-5-hexenoic amide (16). To a -78 °C solution of anhydrous LiCl (3.7 g, 87.0 mmol) and freshly distilled diisopropylamine (4.1 mL, 29.0 mmol) in THF (50 mL) was added dropwise a solution of 2.5 N *n*-BuLi (11.0 mL, 27.6 mmol) in hexanes. This mixture was stirred at -78 °C for 40 min, then pseudoephedrine glycinamide (**14**) in THF (25 mL) was added dropwise to the inner edge of the flask over 20 minutes. The bright orange reaction mixture was stirred for 40 minutes at -78 °C, then allowed to warm to -5 °C. After being stirred at -5 °C for 1 h, 4-bromo-1-butene (**15**, 1.6 mL, 16.0 mmol) was added dropwise and the mixture was stirred at -5 °C for 6 h, then at 23 °C for 18 h. The mixture was cooled to 0 °C, quenched with an aqueous 1 N HCl solution (100 mL), and treated with ethyl acetate (130 mL). The organic layer was separated and extracted with a second portion of an aqueous 1 N HCl solution (100 mL). The combined aqueous extracts were cooled in an ice bath and carefully basified to pH 14 by the addition of an aqueous 40% NaOH solution. The basic solution was then extracted with dichloromethane (4 × 100 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. The residue was purified by flash column chromatography (4% MeOH and 4% Et_3N in chloroform) to yield **16** (2.5 g, 62%) as a yellow

syrup. This material was obtained as a mixture of amide rotamers (4:1 ratio) as determined by ^{1}H NMR analysis: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24-7.38 (m, 5 H), 5.88 (m, 0.2 H), 5.75 (m, 0.8 H), 4.97-5.13 (m, 2 H), 4.62 (d, *J* = 8.0, 0.8 H), 4.59 (d, *J* = 9.2 Hz, 0.2 H), 4.45 (br s, 0.8 H), 4.03 (m, 0.2 H), 3.69 (dd, *J* = 8.8, 4.0 Hz, 0.2 H), 3.61 (dd, *J* = 8.0, 4.8 Hz, 0.8 H), 2.95 (s, 0.7 H), 2.84 (s, 2.3 H), 2.6 (br s, 2 H), 2.30-1.96 (m, 3 H), 1.66-1.40 (m, 2 H), 1.10 (d, *J* = 7.2 Hz, 2.3 H), 0.97 (d, *J* = 7.2 Hz, 0.7 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 176.1, 142.2, 141.8, 138.0, 137.6, 128.6, 128.3, 128.2, 127.6, 126.9, 126.4, 115.3, 115.2, 75.6, 75.0, 57.9, 57.5, 51.0, 50.9, 45.9, 34.3, 34.0, 31.7, 30.1, 29.8, 26.9, 15.5, 14.3; HRMS (ES+) *m/z* $(M + Na)^+$ calcd for $C_{16}H_{24}N_2O_2Na$ 299.1735, found 299.1732.

(*S*)-2-[N^2-[(*tert*-Butyloxy)carbonyl]amino]-*N*-methoxyl-*N*-methyl-5-hexenoic amide (18). Compound **16** (450 mg, 1.6 mmol) was dissolved in aqueous 1 N NaOH solution (3.3 mL, 3.3 mmol) and heated to reflux for 3 h, during which a white precipitate began to form. The mixture was cooled to room temperature, diluted with water (3.6 mL), and washed twice with dichloromethane (25 mL). The combined organic layers were extracted with water (15 mL) and the combined aqueous extracts were washed once more with dichloromethane (20 mL). The combined aqueous extracts were placed in an ice bath and treated with dioxane (25 mL) and di-*tert*-butyl dicarbonate (430 mg, 2.0 mmol). The reaction mixture was stirred for 16 h and allowed to warm to 23 °C. The mixture was washed once with dichloromethane (40 mL) and the aqueous layer was carefully acidified to pH 2 by addition of aqueous 1 N HCl solution at 0 °C. The aqueous mixture was extracted with dichloromethane (3 × 30 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to give acid **17** (307 mg, 82%) as a pale yellow syrup. Without further purification, acid **17** (275 mg, 1.2 mmol) was dissolved in chloroform (5 mL) and cooled in an ice bath. To this solution was added *N,O*-dimethylhydroxylamine hydrochloride (180 mg, 1.8 mmol), *N*-methylmorpholine (0.33 mL, 3.0 mmol), HOBT (190 mg, 1.3 mmol), and EDC (250 mg, 1.3 mmol). The resulting reaction mixture was stirred for 14 h and allowed to warm to 23 °C. After removal of the solvent, the residue was purified by flash column chromatography (50% ethyl acetate in hexanes) to afford Weinreb amide **18** (288 mg, 88%) as a colorless foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (dddd, *J* = 17.2, 10.4, 6.8, 6.8 Hz, 1 H), 5.16 (d, *J* = 9.2 Hz, 1 H), 5.02 (d, *J* = 17.2 Hz, 1 H), 4.96 (d, *J* = 10.4 Hz, 1 H), 4.66 (br s, 1 H), 3.73 (s, 3 H), 3.17 (s, 3 H), 2.10 (m, 2 H), 1.77 (m, 1 H), 1.58 (m, 1 H), 1.4 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 155.4, 137.3, 115.2, 79.4, 61.5, 49.8, 32.1, 32.0, 29.4, 28.3; HRMS (ES+) *m/z* $(M + Na)^+$ calcd for $C_{18}H_{24}N_2O_4Na$ 295.1634, found 295.1733.

(*S*)-2-Amino-*N*-methoxyl-*N*-methyl-5-hexenoic amide (9). To a solution of Weinreb amide **18** (280 mg, 1.0 mmol) in dichloromethane (2.0 mL) was added TFA (1.0 mL) dropwise.

The mixture was stirred at 23 °C for 1 h, then the solvent was removed, and the excess acid was removed by repeated evaporation from benzene *in vacuo*. The residue was dissolved in dichloromethane (100 mL) and washed with aqueous 0.5 NaOH (2 × 20 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated to give amine **9** as a pale-yellow liquid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 (dddd, J = 17.2, 10.4, 6.8, 6.8 Hz, 1 H), 4.95 (dq, J = 17.2, 3.6, 1.6 Hz, 1 H), 4.88 (m, 1 H), 3.67 (m, 1 H), 3.61 (s, 3 H), 3.11 (s, 3 H), 2.09 (m, 2 H), 1.68 (ddd, J = 9.2, 7.2, 5.2 Hz, 1 H), 1.65 (ddd, J = 8.8, 7.2, 4.8 Hz, 1 H), 1.44 (m, 2 H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 137.7, 114.8, 61.2, 50.3, 33.8,. 32.2, 29.8; HRMS (ES+) *m/z* $(M + Na)^+$ calcd for $C_8H_{16}N_2O_2Na$ 195.1109, found 195.1109.

(*S*)-2-Hydroxy-4-pentenoic acid *tert*-butyl ester (11). To a suspension of activated 4 Å molecular sieves (6.0 g) in toluene (100 mL) was added (*S*,*S*)-**20** (50 mL of a 0.6 N solution in toluene, 30.0 mmol). The mixture was stirred at 23 °C for 20 min, and then cooled to -78 °C and stirred for 15 min. A solution of *tert*-butyl glyoxylate (**19**, 60 mmol) in toluene (50 mL) was added dropwise. The reaction was stirred at -78 °C for 2 h during which a colorless gel formed. The mixture was quenched by addition of saturated aqueous sodium bicarbonate solution (100 mL), diluted with diethyl ether (100 mL), warmed to 23 °C and stirred for 0.5 h. The layers were separated and the aqueous layer was extracted with diethyl ether (3 × 200 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography (20% diethyl ether in pentane) to yield ester **11** (3.6 g, 70%) in 74% ee (determined by Mosher ester analysis) as a pale yellow liquid.

(*R*)-2-((*S*)-1-Methoxycarbonyl-3-methyl-butylamino)-4-pentenoic acid *tert*-butyl ester (21). A solution of compound **11** (800 mg, 4.6 mmol) and pyridine (0.38 mL, 4.6 mmol) in dichloromethane (5 mL), was cooled to –78 °C and trifluoromethanesulfonic anhydride (0.78 mL, 4.6 mmol) dissolved in dichloromethane (5 mL) was added dropwise down the inner edge of the flask. The reaction mixture was stirred at –78 °C for 10 minutes then allowed to warm to room temperature. After being stirred for 0.5 h at room temperature, the solution was concentrated in vacuo to *ca.* 2 mL and diluted with pentane (15 mL) to promote the precipitation of the pyridinium-triflate salt. The white solid was filtered through a pipette cotton plug, washed with pentane (3 × 5 mL), and the filtrate was concentrated in vacuo. The residue was dissolved in chloroform (4 mL) and added dropwise to a solution of L-leucine methyl ester (880 mg, 6.0 mmol) and proton sponge (500 mg, 2.3 mmol) in chloroform (4 mL) at –78 °C. The resulting solution was warmed to room temperature and stirred for 14 h during which a white precipitate formed. Purification of the crude product by flash column chromatography (20% diethyl ether in hexanes) gave diastereomerically (and enantiomerically) pure diester **21** (1.2 g, 70%) as a colorless liquid: ^{1}H NMR (400 MHz,

$CDCl_3$) δ 5.78 (dddd, *J* = 17.2, 10.4, 7.6, 7.6 Hz, 1 H), 5.15-5.08 (m, 2 H), 3.70 (s, 3 H), 3.34 (dd, *J* = 7.2, 6.4 Hz, 1 H), 3.17 (dd, *J* = 7.2, 7.2 Hz, 1 H), 2.38 (m, 1 H), 1.79 (br s, 1 H), 1.73 (d, *J* = 6.4 Hz, 1 H), 1.48 (m, 1 H), 1.45 (s, 9H), 0.91 (d, *J* = 6.4 Hz, 3 H), 0.90 (d, *J* = 6.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 173.1, 133.6, 128.4, 118.1, 81.2, 59.7, 58.2, 51.7, 51.7, 42.7, 37.9, 28.0, 24.8, 22.8, 22.1; HRMS (ES+) *m/z* $(M + Na)^+$ calcd for $C_{16}H_{29}NO_4Na$ 322.1994, found 322.1987.

(*R*)-2-[Benzyloxycarbonyl-((*S*)-1-methoxycarbonyl-3-methyl-butyl)-amino]-pent-4-enoic acid *tert*-butyl ester (22). A solution of amine **21** (570 mg, 1.9 mmol) in diethyl ether (20 mL), was cooled to -78 °C and *n*-BuLi (2.4 N in hexanes, 0.5 mL, 1.2 mmol) was added dropwise. The resulting mixture was stirred for 10 minutes, and then benzyl chloroformate (0.8 mL, 5.7 mmol) was added dropwise. The reaction was stirred for 10 min, then was allowed to warm to room temperature and stirred for 72 h. The reaction was quenched with brine (20 mL), and the aqueous layer was extracted with ether (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (16% diethyl ether in hexanes) to give compound **22** (520 mg, 55%) as a mixture of amide rotamers: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (m, 5 H), 5.81 (m, 1 H), 5.23 (d, *J* = 12.4 Hz, 1 H), 5.18-5.00 (m, 3 H), 4.73 (dd, *J* = 7.2, 7.2 Hz, 0.6 H), 4.48 (t, *J* = 6.4 Hz, 0.4 H), 4.08 (dd, *J* = 7.2, 7.2 Hz, 1 H), 3.67 (s, 1.8 H), 3.57 (s, 1.2 H), 2.84 (m, 1 H), 2.62-2.45 (m, 1 H), 1.79 (m, 1 H), 1.66 (m, 1 H), 1.52 (m, 1 H), 1.39 (s, 3 H), 1.35 (s, 6 H), 0.95-0.88 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 171.3, 169.4, 155.9, 154.9, 136.3, 136.0, 135.2, 128.4, 128.1, 128.01, 127.99, 117.3, 81.5, 67.7, 67.4, 59.8, 58.6, 57.2, 57.1, 52.0, 40.3, 39.7, 36.3, 35.1, 27.8, 24.9, 22.6, 22.4; HRMS (ES+) *m/z* $(M + Na)^+$ calcd for $C_{24}H_{35}NO_6Na$ 456.2362, found 456.2357.

(*R*)-2-(Benzyloxycarbonyl-{(*S*)-1-[(*S*)-1-(methoxy-methyl-carbamoyl)-pent-4-enylcarbamoyl]-3-methyl-butyl}-amino)-pent-4-enoic acid *tert*-butyl ester (24). To a stirred solution of methyl ester **22** (480 mg, 1.1 mmol) in THF (15 mL) and methanol (4.5 mL) at 0 °C was added aqueous 0.5 N NaOH (2.4 mL, 1.2 mmol). After allowing the reaction to warm up to room temperature and stirring for 13 h, the solution was diluted with saturated aqueous ammonium chloride solution (15 mL). The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The aqueous phase was then acidified to pH 2 with an aqueous 1 N HCl solution and extracted once more with dichloromethane (10 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. Without further purification, the residue was dissolved in chloroform (10 mL) and cooled to 0 °C. To this solution was added amine **19** (230 mg, 1.3 mmol), *N*-methylmorpholine (0.24 mL, 2.2 mmol), HOBT (160 mg, 1.2 mmol), and EDC (250 mg, 1.3 mmol). The resulting reaction mixture was slowly warmed to 23 °C over 14 h. The solvent was removed by rotary evaporation and the residue

was purified by flash column chromatography (33% ethyl acetate in hexanes) to give diene **24** (430 mg, 68 % for two steps) as a mixture of rotamers: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (br d, *J* = 6.4, 0.4 H), 7.47 (br d, *J* = 5.6, 0.6 H), 7.33-7.26 (m, 5 H), 5.80 (m, 2 H), 5.19-4.81 (m, 7 H), 4.60 (br s, 0.6 H), 4.36 (br s, 0.4 H), 3.92 (dd, *J* = 6.8, 6.8, 1 H), 3.76 (s, 3 H), 3.18 (s, 3 H), 3.00 (br s, 0.5 H), 2.83 (m, 0.5 H), 2.43 (m, 1 H), 2.12-1.92 (m, 3 H), 1.85-1.57 (m, 3 H), 1.47-1.39 (m, 5 H), 1.32 (s, 5 H), 0.96-0.81 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 171.1, 170.7, 170.2, 156.5, 155.4, 137.5, 137.3, 135.9, 135.3, 128.3, 128.1, 128.0, 117.3, 115.1, 82.8, 82.0, 67.9, 67.7, 61.4, 59.9, 58.8, 58.6, 58.4, 49.3, 48.9, 39.1, 38.4, 36.9, 35.6, 32.0, 31.1, 30.7, 29.7, 29.5, 28.0, 27.9, 27.8, 24.9, 22.7, 22.4, 22.2; HRMS (ES+) *m/z* $(M + Na)^+$ calcd for $C_{31}H_{47}N_3O_7Na$ 596.3312, found 596.3326.

(*R*)-2-(Benzyloxycarbonyl-{(*S*)-1-[(*S*)-1-(methoxy-methyl-carbamoyl)-but-3-enylcarbamoyl]-3-methyl-butyl}-amino)-pent-4-enoic acid *tert*-butyl ester (23). Compound **23** was synthesized using a procedure similar to that used to prepare compound **24**: ^{1}H NMR (400 MHz, MeOD) δ 7.38 (m, 5 H), 5.94-5.61 (m, 2 H), 5.21-4.95 (m, 7 H), 4.81 (br s, 0.6 H), 4.66 (br s, 0.4 H), 3.84 (s, 3 H), 3.19 (s, 3 H), 2.95 (m, 0.4 H), 2.83 (m, 0.6 H), 2.68 (m, 0.4 H), 2.60 (m, 0.6 H), 2.51-2.26 (m, 4 H), 1.94-1.81 (m, 1 H), 1.67-1.53 (m, 2 H), 1.49-1.41 (m, 6 H), 1.36-1.27 (m, 2 H), 0.96-0.84 (m, 6 H); LRMS (ES+) *m/z* $(M + H)^+$ calcd for $C_{31}H_{47}N_3O_7Na$ 560, found 560.

(3*S*,5*R*,11*S*)-3-Isobutyl-11-(methoxy-methyl-carbamoyl)-2-oxo-1,4diaza-cycloundecane-5-carboxylic acid *tert*-butyl ester (27). To a solution of diene **24** (300 mg, 1.9 mmol) in 1,2-dichloroethane (600 mL, purged with nitrogen) was added Grubb's 2nd generation catalyst (**25**, 80 mg, 0.1 mmol). The resulting clear burgundy reaction mixture was heated to 95 °C for 45 min, then cooled to 23 °C and passed through a plug of silica gel (washed with ethyl acetate). The solvent was removed in vacuo to give pale brown foam. Without further purification, the residue was dissolved in ethanol (4.5 mL) and purged with nitrogen. To this solution was added 10% palladium on carbon and the reaction was held under a hydrogen atmosphere using a balloon for 36 h. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography (66% ethyl acetate in hexanes) to give macrocyclic amine **27** (145 mg, 67% for three steps) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 3.6 Hz, 1 H), 4.68 (m, 1 H), 3.74 (s, 3 H), 3.35 (dd, *J* = 11.2, 2.8 Hz, 1 H), 3.22 (s, 3 H), 2.97 (dd, *J* = 9.2, 4.8 Hz, 1 H), 2.12 (m, 1 H), 1.98-1.89 (m, 3 H), 1.82-1.34 (m, 10 H), 1.42 (s, 9 H), 0.90 (d, *J* = 6.4 Hz, 3 H), 0.85 (d, *J* = 6.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 173.6, 170.4, 81.2, 61.5, 60.9, 58.9, 50.5, 42.0, 32.4, 32.1, 28.0, 25.0, 24.6, 23.9, 23.5, 23.3; HRMS (ES+) *m/z* $(M + H)^+$ calcd for $C_{21}H_{40}N_3O_5$ 414.2968, found 414.2958.

(3*S*,5*R*,10*S*)-3-Isobutyl-10-(methoxy-methyl-carbamoyl)-2-oxo-[1,4]diazecane-5-carboxylic acid *tert*-butyl ester (26). Compound **26** was synthesized from **23** using a procedure similar to that used to prepare compound **27**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, J = 6.8 Hz, 1 H), 4.74 (d, J = 4.8 Hz, 1 H), 3.73 (s, 3 H), 3.36 (t, J = 5.2 Hz, 1 H), 3.22 (s, 3 H), 3.01 (t, J = 7 Hz, 1 H), 2.06-1.99 (m, 1 H), 1.96-1.88 (m, 1 H), 1.77-1.35 (m, 10 H), 1.44 (s, 9 H), 0.90 (d, J = 6.4 Hz, 3 H), 0.87 (d, J = 6.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 173.6, 171.3, 81.3, 62.3, 61.7, 59.6, 50.1, 41.6, 31.5, 28.2, 26.2, 25.0, 23.2, 23.0, 22.7, 20.7; HRMS (ES+) *m/z* $(M + H)^+$ calcd for $C_{20}H_{38}N_3O_5$ 400.2806, found 400.2791.

(2*S*,5*S*,11*R*)-2-Isobutyl-3-oxo-1,4diaza-cycloundecane-5,11-dicarboxylic acid 11-benzylamide 5-(methoxy-methyl-amide) 29. To a solution of macrocyclic *tert*-butyl ester **27** (125 mg, 0.3 mmol) in dichloromethane (1.0 mL) at 0 °C was added TFA (1.0 mL) dropwise. The reaction was stirred at 0 °C for 10 min then allowed to warm to room temperature and stirred for 6 h. The solution was concentrated in vacuo, and the excess acid was removed by repeated evaporation from benzene. The residue was dissolved in chloroform (2 mL) and cooled to 0 °C. To this solution was added benzylamine (45 μL, 0.39 mmol), *N*-methylmorpholine (0.10 mL, 0.90 mmol), HOBT (45 mg, 0.33 mmol), and EDC (70 mg, 0.36 mmol). The resulting mixture was stirred at 0 °C then warmed to room temperature over 14 h. Removal of solvent and purification of the crude product by flash column chromatography (75% ethyl acetate in hexanes) gave macrocyclic amide **29** (98 mg, 73% for two steps) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (br s, 1 H), 7.30-7.20 (m, 5 H), 7.06 (br s, 1 H), 4.90 (br s, 1 H), 4.46 (dd, J = 14.4, 6.0 Hz, 1 H), 4.30 (dd, J = 14.8, 5.2 Hz, 1 H), 3.69 (s, 3 H), 3.35 (br s, 1H), 3.16 (s, 3 H), 2.86 (br s, 1 H), 2.36 (t, J = 12.4 Hz, 1 H), 1.93 (m, 1 H), 1.66-1.54 (m, 3 H), 1.51-1.30 (m, 9 H), 0.77 (d, J = 6.0 Hz, 3 H), 0.69 (d, J = 6.0 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.0, 174.2, 171.8, 138.6, 128.6, 127.6, 127.3, 62.6, 61.6, 60.6, 51.4, 43.2, 42.3, 32.6, 32.3, 26.9, 26.1, 24.8, 24.4, 22.8, 22.7, 22.2; HRMS (ES+) *m/z* $(M + H)^+$ calcd for $C_{24}H_{39}N_4O_4$ 447.2971, found 447.2970.

(2*S*,5*S*,10*R*)-2-Isobutyl-3-oxo-[1,4]diazecane-5,10-dicarboxylic acid 10-benzylamide 5-(methoxy-methyl-amide) (28). Compound **28** was synthesized from **26** using a procedure similar to that used to prepare compound **29**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (br s, 1 H), 7.35-7.25 (m, 5 H), 7.11 (br s, 1 H), 5.11 (br s, 1 H), 4.49 (dd, J = 15.0, 6.0 Hz, 1 H), 4.30 (dd, J = 14.8, 5.6 Hz, 1 H), 3.75 (s, 3 H), 3.46 (br s, 1H), 3.19 (s, 3 H), 2.97 (br s, 1 H), 2.09-2.02 (m, 2 H), 1.78-1.14 (m, 10 H), 0.84 (d, J = 6.4 Hz, 3 H), 0.78 (d, J = 6.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 173.5, 172.7, 138.6, 128.7, 127.6, 127.4, 62.9, 61.6, 59.0, 48.1, 43.2, 41.0, 32.3, 26.9, 24.8, 23.6, 22.8, 22.5, 19.4; HRMS (ES+) *m/z* $(M + H)^+$ calcd for $C_{23}H_{37}N_4O_4$ 433.2815, found 433.2820.

(3*S*,5*R*,11*S*)-11-((E)-2-Benzenesulfonyl-vinyl)-3-isobutyl-2-oxo-1,4-diaza-cycloundecane-5-carboxylic acid benzylamide (5). To a solution of **29** (74 mg, 0.17 mmol) in THF (7.5 mL) at –10 °C was added a 1 N solution of $LiAlH_4$ in THF (0.18 mL, 0.18 mmol) dropwise. The mixture was stirred vigorously at -10 °C for 0.5 h, then the reaction was quenched with aqueous 1 N $KHSO_4$ solution (1 mL) and diluted with ethyl acetate (20 mL). The mixture was stirred for 5 min prior to the slow addition of a 0.2 N aqueous NaOH solution to *ca.* pH 9. The organic phase was separated and the aqueous phase was extracted with ethyl acetate (2 × 10 mL). The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to give the crude aldehyde as a colorless solid which was used immediately in the next reaction.

To a 0 °C suspension of 60% NaH (12.8 mg, 0.32 mmol) in THF (5 mL) was added phosphonate **30** (100 mg, 0.35 mmol) in two portions. The mixture was stirred at 0 °C for 0.5 h, then a solution of the above crude aldehyde in THF (5 mL) was added dropwise and the resulting solution allowed to warm to 23 °C over 4 h. The solvent was removed by rotary evaporation and the residue was purified by flash chromatography (75% ethyl acetate in hexanes) to give vinyl sulfone **5** (43 mg, 51% for two steps) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23-8.18 (m, 2 H), 7.75 (s, 1 H), 7.73 (s, 1 H), 7.52 (dd, *J* = 7.6, 7.6 Hz, 1 H), 7.43-7.39 (m, 2 H), 7.31-7.22 (m, 3 H), 7.16-7.14 (m, 2 H), 6.94 (dd, *J* = 15.2, 4.8, 1 H), 6.57 (d, *J* = 14.8 Hz, 1 H), 4.76 (br s, 1 H), 4.37 (dd, *J* = 14.8, 6.0 Hz, 1 H), 4.27 (dd, *J* = 14.8, 5.2 Hz, 1 H), 3.49 (d, *J* = 10.0 Hz, 1 H), 2.92 (br s, 1 H), 2.22 (m, 1 H), 1.65 (m, 3 H), 1.46-1.22 (m, 10 H), 0.64 (d, *J* = 9.2 Hz, 3 H), 0.62 (d, *J* = 9.2 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9, 174.5, 146.0, 140.5, 137.8, 133.3, 130.3, 129.2, 128.8, 127.53, 127.50, 127.2, 61.7, 60.1, 51.1, 43.2, 42.1, 33.0, 30.6, 26.6, 24.9, 24.6, 23.3, 22.4; HRMS (ES+) *m/z* $(M + H)^+$ calcd for $C_{29}H_{40}N_3O_4S$ 526.2740, found 526.2756.

(3*S*,5*R*,10*S*)-10-((*E*)-2-Benzenesulfonyl-vinyl)-3-isobutyl-2-oxo-[1,4]diazecane-5-carboxylic acid benzylamide (**4**). Compound **4** was prepared from **28** using a procedure similar to that used to prepare compound **5**: ^{1}H NMR (400 MHz, MeOD) δ 7.88 (d, *J* = 7.2 Hz, 2 H), 7.68 (t, *J* = 7.4 Hz, 1 H), 7.60 (t, *J* = 7.6 Hz, 2 H), 7.33-7.20 (m, 5 H), 7.03 (dd, *J* = 15.2, 4.6, 1 H), 6.56 (dd, *J* = 15.2, 2.0 Hz, 1 H), 4.73 (br s, 1 H), 4.45 (d, *J* = 14.8, Hz, 1 H), 4.30 (d, *J* = 14.8 Hz, 1 H), 3.40 (dd, *J* = 7.8, 3.8 Hz, 1 H), 2.99 (t, J = 7.2 Hz, 1 H), 1.91-1.84 (m, 2 H), 1.74-1.40 (m, 8 H), 1.22 (m, 1 H), 0.86 (d, *J* = 6.4 Hz, 3 H), 0.80 (d, *J* = 6.0 Hz, 3 H); ^{13}C NMR (100 MHz, MeOD) δ 177.8, 177.2, 148.3, 141.7, 139.8, 134.8, 130.9, 130.6, 129.6, 128.7, 128.5, 128.3, 63.4, 61.2, 50.2, 44.0, 42.4, 29.4, 26.1, 23.1, 23.0, 22.9, 21.6; HRMS (ES+) *m/z* $(M + Na)^+$ calcd for $C_{28}H_{37}N_3NaO_4S$ 534.2402, found 534.2404.

Cruzain and rhodesain IC_{50} determinations. Cruzain[1] and rhodesain[2] were recombinantly expressed as described previously. Cruzain (2 nM) or rhodesain (3 nM) was incubated with 0.5 to 10 μM inhibitor concentration in 100 mM sodium acetate, pH 5.5, containing 5 mM DTT (buffer A), for 5 min at room temperature. Then Z-Phe-Arg-AMC (Bachem, K_m = 1 μM) was added to the enzyme-inhibitor mixture to give 20 μM substrate concentration in 200 μL, and the increase in fluorescence (excitation at 355 nm and emission at 460 nm) was followed with an automated microtiter plate spectrofluorimeter (Molecular Devices, Flex station). Inhibitor stock solutions were prepared at 20 mM in DMSO, and serial dilutions were made in DMSO (0.7% DMSO in assay). Controls were performed using enzyme alone and enzyme with DMSO. IC_{50} values were determined graphically using inhibitor concentrations in the linear portion of a plot of inhibition versus log[I] (seven concentrations tested with at least two in the linear range).

References and notes

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