

Inhibition of Trypanothione Reductase by Substrate Analogs

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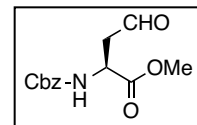
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Supporting Information

General. Glassware was oven dried and all reactions involving moisture-sensitive reagents were performed under a positive pressure of dry nitrogen. Peptide coupling reagents, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP)¹ and bromotripyrrolidinophosphonium hexafluorophosphate (PyBrop)² were purchased from Novabiochem. Schöllkopf's bislactim ether **11** was prepared according to the literature procedure³ or purchased from Fluka. Dimethyldioxirane was prepared by the method of Murray and Singh.⁴ Wilkinson's catalyst (Rh(PPh₃)₃Cl) was purchased from Aldrich. N-Butyl lithium (Aldrich) was titrated by the method of Winkle, et al., just prior to use.⁵ All other reagents and solvents obtained from commercial suppliers were used as received with the following exceptions: THF was distilled from potassium-benzophenone or calcium hydride, and dichloromethane (CH₂Cl₂), diisopropylethylamine (DIEA), and triethylamine (TEA) were distilled from calcium hydride. Flash chromatography was performed by the method of Still, Kahn, and Mitra⁶ on E. Merck silica gel 60 (230-400 mesh) using the indicated eluting solvent. Melting points are uncorrected.

Infrared spectra were recorded on a Mattson 4020 Galaxy Series FT-IR spectrometer. NMR spectra were obtained in the indicated solvent on a Varian Unity-plus 400 MHz spectrometer. ¹H NMR data are reported in the following manner: chemical shift (δ) in ppm, downfield from internal tetramethylsilane (multiplicity, integrated intensity, coupling constants in hertz). For spectra recorded in methanol-d₄, the chemical shifts are reported relative to residual CD₂HOD at 3.30 ppm. ¹³C NMR spectra were obtained at the indicated field strength using broad-band decoupling. Chemical shifts are reported relative to CDCl₃ at 77.00 ppm (downfield positive). Enzyme assays were carried out on a Hewitt-Packard 8453 Diode Array UV-visible spectrophotometer. High resolution FAB and MALDI mass spectral data were recorded at the University of Minnesota Mass Spectrometry Service Laboratory. Microanalyses were performed by Atlantic Microlabs, Inc, Norcross, GA.

(2S)-Methyl 2-(Benzyloxycarbonylamino)-4-oxobutanoate (9). To a cold (–5 °C) solution of 5.11 g (18.2 mmol) of N-(benzyloxy)carbonyl-aspartic acid α -methyl ester (**8**, Sigma) in 10 mL of THF was added 37 mL (37 mmol) of 1M



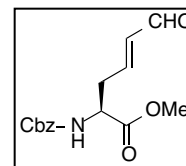
borane in THF (Aldrich), over a 15 min period. After 5.5 h the excess borane was quenched with 60 mL of 10% citric acid and the mixture was diluted with 70 mL of ether. The aqueous layer was extracted with three 50 mL portion of ether and the combined ether layers were washed once with 50% brine and dried over Na₂SO₄. The solvents were removed under reduced pressure and the residue was purified by flash chromatography (eluted with 40% ethyl acetate/hexane) to afford 3.67 g (75% yield) of Cbz-homoserine methyl ester as a clear oil: IR (CHCl₃) 3425, 2957, 2990, 1725, 1512, 1440, 1348, 1236, 1055 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.40-7.30 (m, 5H), 5.68 (d, 1H, J=7.5 Hz), 5.16-5.09 (m, 2H), 4.56 (ddd, J=3.9, 7.5, 8.5 Hz, 1H), 3.76 (s, 3H), 3.76-3.63 (m, 2H), 2.36 (bs, 1H), 2.20-2.12 (m, 1H), 1.76-1.68 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 173.0, 156.7, 136.0, 128.6, 128.3, 128.1, 67.2, 58.4, 52.6, 51.2, 35.7; HRMS (FAB) m/z calcd for C₁₃H₁₈NO₅ (M+H)⁺ 268.1185, found 268.1201. Anal. Calcd for C₁₃H₁₇NO₅: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.40; H, 6.40; N, 5.21.

Cbz-homoserine methyl ester was converted to aldehyde **9** via a TEMPO mediated bleach oxidation.⁷ Thus, 3.67 g (13.7 mmol) of Cbz-homoserine methyl ester was added to a mixture of 1.46 g of NaBr, 25 mg (0.16 mmol) of TEMPO free radical (Aldrich), 40 mL of toluene, and 40 mL of H₂O. The mixture was cooled to 0 °C, and with vigorous stirring, 45 mL (15.7 mmol NaOCl) of a buffered bleach solution was added via syringe pump, over a 1 hour period. The bleach solution was prepared as follows: 50 mL of Clorox bleach, 50 mL of H₂O, and 7.8 g of NaHCO₃ were shaken together until the bicarbonate had dissolved. After the biphasic mixture had stirred vigorously for 2 h after all the bleach solution had been added, the organic and aqueous phases were separated and the aqueous phase was extracted with 30 mL of ether. The combined organic layers were washed with 25 mL of 10% KHSO₄, to which had been added 0.25 g of KI. The iodine colored organic phase was washed with 20 mL of 10% sodium thiosulfate, followed by 20 mL of 0.2 M, pH 7, phosphate buffer and 15 mL of brine. After drying the organic layer over Na₂SO₄ and removing the solvents under reduced pressure, the crude product was purified by flash chromatography (eluted with 40%, 50%, 60% and finally 70% ethyl acetate/hexane) to afford 2.37 g (65% yield) of aldehyde **9** as a clear oil. On standing overnight in a -20 °C freezer, the oil crystallized. An analytical sample, recrystallized from ether/hexane, had a mp 58–61 °C: IR (CHCl₃) 3430, 3960, 2845, 1740, 1515, 1438, 1340, 1235, 1050 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 9.65

(s,1H), 7.34-7.26 (m, 5H), 5.76 (d, 1H, J=8.1 Hz), 5.07 (s, 2H), 4.63 (ddd, 1H, J=4.8, 5.2, 8.1 Hz), 3.70 (s, 3H), 3.08 (dd, 1H, J=5.2, 18.5 Hz), 2.98 (dd, 1H, J=4.8, 18.5 Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 199.1, 171.0, 155.8, 136.0, 128.4, 128.1, 128.0, 67.0, 52.7, 48.9, 45.6. Anal. Calcd for $\text{C}_{13}\text{H}_{15}\text{NO}_5$: C, 58.86; H, 5.70; N, 5.28. Found: C, 58.85; H, 5.70; N, 5.26.

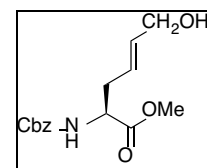
Methyl (2S,4E)-2-(Benzyloxycarbonylamino)-6-oxohex-4-enoate (10).

A solution of 2.37 g (8.93 mmol) of aldehyde **9** and 2.94 g (9.66 mmol) of (triphenylphosphoranylidene)acetaldehyde (Aldrich) in 50 mL of toluene was heated to 100 °C under a dry N_2 atmosphere. After 4.5 h, the mixture was cooled to room temperature, the solvent was removed under reduce pressure, and the residue was purified by flash chromatography (eluted with 30%, 40%, then 50% ethyl acetate/hexanes) to afford 2.17 g of α,β -unsaturated aldehyde **10**. This material was contaminated with ca. 5% of the side-product resulting from a second Wittig addition of (triphenylphosphoranylidene)acetaldehyde to aldehyde **10** (as judged by its ^1H -NMR spectrum), giving a yield of the desired α,β -unsaturated aldehyde **10** of 79%. The contaminated product was carried on to the next step without further purification: IR (CHCl_3) 3430, 3057, 2960, 1723, 1695, 1510, 1455, 1348, 1230, 1060 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 9.47 (d, 1H, J=7.8 Hz), 7.35-7.25 (m, 5H), 6.70 (ddd, 1H, J=7.2, 7.7, 15.6 Hz), 6.13 (dd, 1H, J=7.8, 15.6 Hz), 5.47 (d, 1H, J=7.6 Hz), 5.10 (s, 2H), 4.58 (ddd, 1H, J=6.0, 7.0, 7.6 Hz), 3.76 (s, 3H), 2.92 (ddd, 1H, J=6.0, 7.2, 14.8 Hz), 2.73 (ddd, 1H, J=7.0, 7.7, 14.8); ^{13}C NMR (100 MHz, CDCl_3) δ 193.2, 171.2, 155.6, 150.8, 135.9, 135.7, 128.6, 128.3, 128.1, 67.2, 52.8, 52.7, 35.7; HRMS (FAB) m/z calcd for $\text{C}_{15}\text{H}_{18}\text{NO}_5$ ($\text{M}+\text{H}$) $^+$ 292.1185, found 292.1167.



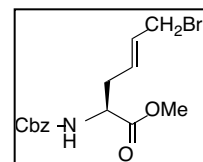
Methyl (2S,4E)-2-(Benzyloxycarbonylamino)-6-hydroxyhex-4-enoate (11).

To a cold (0 °C) solution of 2.04 g of α,β -unsaturated aldehyde **10** (about 95% pure, as described in the preceding experimental procedure) in 5 mL of THF was added 30 mL of a 0.5 M 9-BBN solution in THF (15 mmol, Aldrich), dropwise, over a 10 minute period. After stirring for 2 h the excess borohydride was carefully quenched with a few drops of 10% KHSO_4 . The mixture was partitioned between 20 mL of 10% KHSO_4 and 30 mL of ether. The aqueous layer was extracted with 50 mL of ether and the combined organic layers were washed with 20 mL of pH 7.0 phosphate buffer, 15 mL of brine, and dried over Na_2SO_4 . After removal of the solvents under reduced pressure, the residue was purified by flash chromatography (eluted with 45%, 50 %, and finally 60% ethyl acetate/hexane) to afford



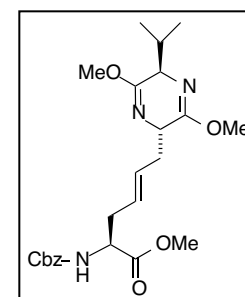
1.80 g (87% yield) of allylic alcohol **11** as a clear oil: IR (film) 3345, 3050, 2955, 2870, 1725, 1531, 1440, 1345, 1260, 1215, 1055 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.38-7.28 (m, 5H), 5.71 (ddd, 1H, $J=5.4, 5.5, 15.4$ Hz), 5.55 (ddd, 1H, $J=7.2, 7.2, 15.4$ Hz), 5.34 (d, 1H, $J=7.7$ Hz), 5.12-5.05 (m, 2H), 4.44 (ddd, 1H, $J=6.2, 7.2, 7.7$ Hz), 4.02 (m, 2H), 3.74 (s, 3H), 2.58 (ddd, 1H, $J=6.2, 7.2, 14.0$ Hz), 2.48 (ddd, 1H, $J=7.2, 7.2, 14.0$ Hz), 1.66 (bs, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 172.2, 155.8, 136.0, 133.9, 128.4, 128.0, 127.9, 125.0, 66.8, 62.6, 53.4, 52.3, 35.0; HRMS (FAB) m/z calcd for $\text{C}_{15}\text{H}_{20}\text{NO}_5$ ($\text{M}+\text{H}$) $^+$ 294.1341, found 294.1329.

Methyl (2S,4E)-2-(Benzyloxycarbonylamino)-6-bromohex-4-enoate (12).



To a cold (0 °C) solution of 1.20 g (4.09 mmol) of allylic alcohol **11** in 8 mL of CH_2Cl_2 was added 1.93 g (5.83 mmol) of CBr_4 (Aldrich), followed by 1.60 g (6.11 mmol) of triphenylphosphine. The dark red/brown solution was stirred for 30 min and then diluted with 60 mL of ether. A precipitate formed and the mixture was stirred for 5 min. The solid precipitate was removed by filtration through Celite and the filtrate was washed with two 25 mL portions of H_2O , followed by 25 mL of brine, and then dried over Na_2SO_4 . The solvents were removed under reduced pressure and the residue was purified by flash chromatography (30% ethyl acetate/hexane) to afford 1.05 g (72% yield) of bromide **12** as a clear oil: IR (CHCl_3) 3440, 3025, 2980, 1725, 1515, 1440, 1215, 1050 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.38-7.29 (m, 5H), 5.77 (ddd, 1H, $J=7.4, 7.5, 15.2$ Hz), 5.63 (ddd, 1H, $J=7.0, 7.5, 15.2$ Hz), 5.30 (d, 1H, $J=7.4$ Hz), 5.14-5.06 (m, 2H), 4.46 (ddd, 1H, $J=6.0, 6.4, 7.4$ Hz), 3.90-3.83 (m, 2H), 3.75 (s, 3H), 2.61 (ddd, 1H, $J=6.0, 7.0, 14.0$ Hz), 2.50 (ddd, 1H, $J=6.4, 7.5, 14.0$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 171.8, 155.6, 136.1, 130.9, 129.1, 128.5, 128.2, 128.1, 67.0, 53.2, 52.5, 35.1, 31.9. Anal. Calcd for $\text{C}_{15}\text{H}_{18}\text{BrNO}_4$: C, 50.58; H, 5.09; N, 3.93. Found: C, 50.67; H, 5.15; N, 3.95.

(2S,5R,2'E,5'S)-2-[5'-(Benzyloxycarbonylamino)-5'-carbomethoxy-pent-2-ene]-2,5-dihydro-5-isopropyl-3,6-dimethoxypyrazine (14). To a -78 °C solution of 1.09 g (5.90 mmol) of bislactim ether **13**³ in 10 mL of THF was added 3.7 mL of 1.6 M *n*-butyl lithium in hexane, dropwise, over a 10 min period. After stirring for 10 min, a solution of 1.05 g (2.94 mmol) of bromide **12** in 6 mL of THF was added dropwise, via cannula. The reaction mixture was

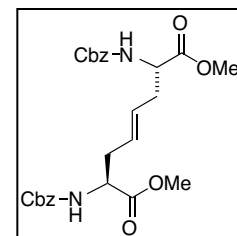


allowed to stir for 8 h at -78 °C. After warming to room temperature, the mixture was poured over 15 mL of sat. aq. NH_4Cl , and the resulting mixture was extracted with 130 mL of ether. The ether

layer was washed with 15 mL of 50% brine, followed by 15 mL of brine, and dried over Na₂SO₄. After removal of the solvents under reduced pressure, the crude residue was purified by flash chromatography (25%, followed by 30% ethyl acetate/hexane) to afford 1.21 g (89% yield) of **14**, as a colorless oil: IR (film) 3350, 2945, 1733, 1695, 1515, 1435, 1240, 1015 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.35-7.28 (m, 5H), 5.40 (ddd, 1H, J=7.1, 8.0, 15.3 Hz), 5.30 (ddd, 1H, J=7.0, 7.2, 15.3 Hz), 5.21 (d, 1H, J=8.0 Hz), 5.13-5.05 (m, 2H), 4.38 (ddd, 1H, J=5.4, 5.6, 8.0 Hz), 4.03 (dd, 1H, J=5.0, 8.6 Hz), 3.90 (dd, 1H, J=3.3, 3.5 Hz), 3.70 (s, 3H), 3.64 (s, 3H), 3.63 (s, 3H), 2.50-2.40 (m, 4H), 2.24-2.19 (m, 1H), 0.99 (d, 3H, J=6.9 Hz), 0.65 (d, 3H, J=6.96); ¹³C NMR (100 MHz, CDCl₃) δ 172.1, 163.9, 162.9, 155.7, 136.2, 130.6, 128.4, 128.1, 128.0, 126.5, 66.8, 60.6, 55.2, 53.2, 52.3, 52.2, 52.1, 37.1, 35.2, 31.6, 19.0, 16.4. Anal. Calcd for C₂₄H₃₃N₃O₆: C, 62.73; H, 7.24; N, 9.14. Found: C, 62.65; H, 7.28; N, 9.08.

Dimethyl (2S,4E,7S)-2,7-Bis(Benzyloxycarbonylamino)oct-4-en-1,8-

dioate (15) (prepared from bislactim ether **14**). To a solution of 1.07g (2.32 mmol) of bislactim ether **14** in 9.5 mL of THF was added 9.7 mL (4.9 mmol) of 0.5 M HCl. After stirring for 12.5 h, the reaction mixture was partitioned between 20 mL of ether and 20 mL of water. The ether layer was extracted

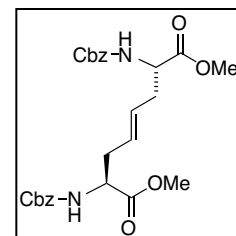


with 5 mL of water and the combined aqueous layers were made basic with 25 mL of sat. aq. NaHCO₃. The basic solution was extracted with ether (3x25 mL) and the combined ether layers were dried over K₂CO₃. Filtration and removal of the solvent under reduced pressure afforded 751 mg of the free amine as a pale yellow oil. The ¹H-NMR spectrum of the crude material showed that it was contaminated with a small amount of valine methyl ester. The crude amine was taken on further purification: ¹H NMR (400 MHz, CDCl₃) δ 7.36-7.29 (m, 5H), 5.56 (d, 1H, J=8.0 Hz), 5.52-5.36 (m, 2H), 5.10 (s, 2H), 4.42 (ddd, 1H, J=5.9, 5.9, 8.0 Hz), 3.74 (s, 3H), 3.69 (s, 3H), 3.49 (dd, 1H, J=5.9, 6.0 Hz), 2.59-2.43 (m, 2H), 2.42 (ddd, 1H, J=5.9, 7.4, 13.5 Hz), 2.31 (ddd, 1H, J=6.0, 7.0, 13.5 Hz), 1.65 (bs, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 175.3, 172.0, 155.6, 136.1, 129.8, 128.4, 128.0, 127.9, 127.6, 66.8, 53.9, 53.3, 52.2, 51.9, 37.7, 35.3; HRMS (FAB) *m/z* calcd for C₁₈H₂₅N₂O₆ (M+H)⁺ 365.1713, found 365.1699.

To a cold solution (0 °C) of 750 mg of the crude amine (contaminated with a small amount of valine methyl ester) in 10 mL of THF was added 430 μL (3.1 mmol) of triethylamine followed by 425 μL (2.83 mmol) of benzyl chloroformate (95%, Aldrich). The mixture was allowed to warm to room temperature and after 2.5 h, was diluted with 60 mL of ether. The resulting solution was

extracted with 10 mL of 10% KHSO₄, 10 mL of 50% brine, 10 mL brine, and dried over Na₂SO₄. After filtration and removal of the solvent under reduced pressure, the residue was purified by flash chromatography (40% ethyl acetate/hexanes) to afford 808 mg (70% overall yield from bislactim ether **14**) of **15** as a white solid. An analytical sample, recrystallized from ether/hexane, had a mp. 92-93 °C. The ¹H-NMR spectrum of **15** showed that ca. 10% of this material exists in solution as a minor amide rotamer: IR (CHCl₃) 3430, 3040, 1720, 1510, 1440, 1350, 1225, 1057 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.33-7.26 (m, 10H), 5.42-5.32 (m, 4H), 5.08 (s, 4H), 4.40 (ddd, 1.8H, J=5.6, 5.8, 7.0 Hz), 4.26 (m, 0.2H, C_α-H of minor rotamer), 3.69 (s, 5.4H), 3.64 (bs, 0.6H, ester-CH₃ of minor rotamer), 2.51-2.41 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 172.0, 155.7, 136.2, 128.5, 128.2, 128.1, 67.0, 53.4, 52.4, 35.4. Anal. Calcd for C₂₆H₃₀N₂O₈: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.78; H, 6.15; N, 5.54.

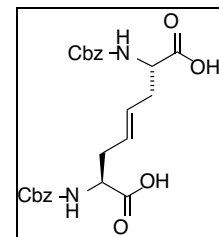
Dimethyl (2S,4E,7S)-2,7-Bis(Benzyloxycarbonylamino)oct-4-en-1,8-dioate (15) (prepared from bis-dihydropyrazine **17**). To a solution of 415 mg (0.987 mmol) of bis-dihydropyrazine **17** in 8.5 mL of THF was added 8.5 mL (4.25 mmol) of 0.5M HCl. After stirring overnight, the solvents were removed under reduced pressure to afford 636 mg of an oil. The crude diamine was contaminated with 2 eq. of valine methyl ester, which was not removed. Instead the crude diamine was taken on without purification. ¹H-NMR of the desired diamine (400 MHz, CD₃OD) δ 5.67 (m, 2H), 4.16 (t, 2H, J=6.2Hz), 3.84 (s, 6H), 2.70 (m, 4H).



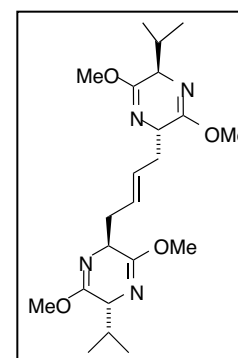
To a cold (0 °C) solution of 636 mg of crude diamine (contaminated with valine methyl ester) in 5 mL of CH₂Cl₂ was added 1.4 mL of DIEA followed by 0.58 mL of benzyl chloroformate (95%, Aldrich). The mixture was allowed to stir overnight in at 0 °C. The reaction mixture was diluted with 50 mL of ethyl acetate and extracted with 15 mL of 10% KHSO₄, 25 mL of 50% brine, and 25 mL of brine. The organic layer was dried over Na₂SO₄, filtered, and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography (30% ethyl acetate/ hexane), to afford 320 mg (65% yield) of **15** as a white solid. This material was identical in all respects to that obtained in the reaction from bislactim ether **14** (preceding experimental procedure).

(2S,4E,7S)-2,7-Bis(Benzyloxycarbonylamino)oct-4-en-1,8-dioic Acid

(16). To a cold (0 °C) solution of 100 mg (0.20 mmol) of diester **15** in 3 mL of methanol was added 2.0 mL (1.0 mmol) of 0.5 M LiOH. Upon addition of the aqueous base, the reaction mixture became cloudy. The cloudy suspension was allowed to slowly warm to room temperature overnight. The cloudy mixture slowly cleared and after a total of 19 h, the now clear solution was carefully acidified to pH 1 with 1M HCl. The acidic mixture was extracted with chloroform (4x20 mL), the combined organic layers were washed with 50% brine, and dried over Na₂SO₄. Filtration and removal of the solvent under reduced afforded 98 mg of diacid **16** as a clear glassy solid. The ¹H-NMR spectrum shows the presence of a minor rotamer of **16**. Acquiring the ¹H-NMR spectrum at 55 °C showed the near coalescence of rotamer resonances. ¹H NMR (400 MHz, CDCl₃, 25 °C) δ 10.35 (bs, 2H), 7.51-7.29 (m, 10H), 6.90-6.70 (m, 0.6H, N-H, minor rotamer), 5.92 (bs, 0.4H, N-H, minor rotamer), 5.70 (d, 1H, J=7.9 Hz, N-H, major rotamer), 5.42 (m, 2H), 5.10-5.03 (m, 4H, 4.50-4.40 (m, 1.4H), 4.40-4.30 (m, 0.6H, C_α-H, minor rotamer), 2.50-2.40 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 175.4, 174.9 (minor rotamer), 157.1 (minor rotamer), 156.0, 136.0, 135.4 (minor rotamer), 129.0 (minor rotamer), 128.4, 128.3 (minor rotamer), 128.1, 67.8 (minor rotamer), 67.1, 54.1 (minor rotamer), 53.5, 35.1, 34.8 (minor rotamer); HRMS (FAB) *m/z* calcd for C₂₄H₂₇N₂O₈ (M+H)⁺ 471.1767, found 471.1782.

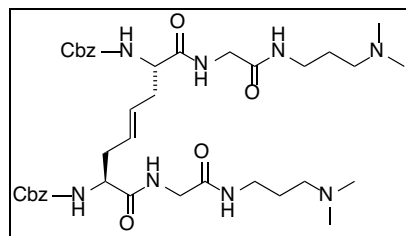


Bis-dihydropyrazine 17. To a cold (-78 °C) solution of 1.03 g (5.59 mmol) of Schöllkopf's bislactim ether **13**³ in 15 mL of THF was added 4.75 mL (5.93 mmol) of 1.25M *n*-butyl lithium, dropwise over 15 min. After stirring at -78 °C for 40 minutes, a solution of 531 mg (2.48 mmol) of *trans*-1,4-dibromobutene in 5 mL of THF was added, dropwise, via cannula. The mixture was allowed to stir overnight at -78 °C. The mixture was poured over 10 mL of saturated aqueous NH₄Cl, and extracted with 90 mL of ether. The aqueous phase was extracted with two more 30 mL portions of ether and the combined organic layer was dried over Na₂SO₄. After filtration of the drying agent, the solvents were removed under reduced pressure and the residue was purified by flash chromatography (20% ether/ hexane) to afford 540 mg (52% yield) of **17** as a clear oil: IR (film) 3007, 2959, 2945, 2930, 2871, 2846, 1694, 1462, 1436, 1382, 1365, 1355, 1337, 1310, 1238, 1196, 1142, 1118, 1014, 970 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 5.34 (m, 2H), 4.03 (dd, 2H, J=4.8, 8.6 Hz), 3.93 (dd, 2H, J=3.3, 3.6 Hz), 3.67 (s, 6H),



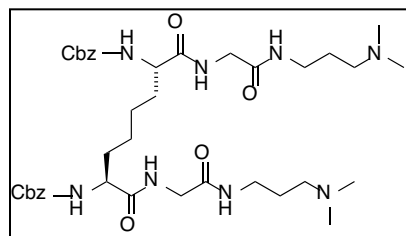
3.66 (s, 6H), 2.46 (m, 4H), 2.25 (dsep, 2H, J=3.2, 6.8), 1.04 (d, 6H, J=6.9), 0.66 (d, 6H, J=6.8); ¹³C-NMR (100 MHz, CDCl₃) δ 163.6, 162.9, 128.4, 60.5, 55.6, 52.2, 51.9, 37.0, 31.5, 19.0, 16.4; HRMS (FAB) *m/z* calcd for C₂₂H₃₆N₄O₄ (M+H)⁺ 421.2815, found 421.2803.

Inhibitor 5a. To a cold (0 °C) solution of 82 mg (0.174 mmol) of diacid **16**, 77 mg (0.490 mmol) of glycine-3-dimethylaminopropylamide⁸, and 125 μL (0.718 mmol) of DIEA in 2.0 mL of CH₂Cl₂ was added 170 mg (0.365 mmol) of PyBrop. The mixture was stirred overnight at 0 °C, then filtered through Celite



and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography eluting with 6:3:1 CHCl₃/methanol/triethylamine to afford 121 mg of impure material. The impure product was purified a second time by flash chromatography (80:20:5 CHCl₃/methanol/conc. NH₃) to afford 75 mg (57% yield) of **5a** as thick oil. TLC R_f = 0.41 (80:20:5 CHCl₃/methanol/conc. NH₃); ¹H NMR (400 MHz, 5% CD₃OD in CDCl₃) δ 7.33-7.28 (m, 10H), 5.51 (bs, 2H), 5.10-5.00 (m, 4H), 4.21 (t, 2H, J=5.9 Hz), 3.84 (d, 2H, J=16.6 Hz), 3.77 (d, 2H, 16.6 Hz), 3.22 (dd, 4H, J=6.7, 13.4 Hz), 2.55-2.38 (m, 4H), 2.32 (t, 4H, J=7.3 Hz), 2.22 (s, 12H), 1.65 (quin, 4H, J=6.9Hz); ¹³C NMR (100 MHz, 5% CD₃OD in CDCl₃) δ 172.2, 169.1, 156.4, 136.0, 128.8, 128.4, 128.1, 127.9, 67.0, 57.0, 55.0, 44.9, 42.6, 37.8, 35.2, 26.4; HRMS (FAB) *m/z* calcd for C₃₈H₅₇N₈O₈ (M+H)⁺: 753.4299, found: 753.4325.

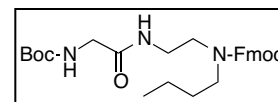
Inhibitor 6a. A solution was by dissolving 28 mg (0.037 mmol) of alkene **5a** in 5 mL of 1:1 benzene/ethanol which had been degassed by bubbling argon through the mixture for 20 minutes. This mixture was transferred to a high pressure bomb reaction vessel and 40 mg (0.043 mmol) of Wilkenson's catalyst was



added. The bomb was flushed three times with 200 psi of H₂ gas and then filled with 480 psi of H₂. After five days the crude product was concentrated under reduced pressure. The residue was purified by flash chromatography (80:23:5 CHCl₃/methanol/conc. NH₃) to afford 22 mg (78 % yield) of **6a** as an oil. TLC R_f = 0.29 (80:23:5 CHCl₃/methanol/conc. NH₃); ¹H NMR (400 MHz, 5% CD₃OD in CDCl₃) δ 7.34 (m, 10H), 5.11 (d, 2H, J=12.1 Hz), 5.07 (d, 2H, J=12.1 Hz); 4.12 (dd, 2H, J=6.1, 7.4 Hz), 3.91 (d, 2H, J=16.6 Hz), 3.89 (d, 2H, J=16.6 Hz), 3.28-3.17 (m, 4H), 2.33 (t, 4H, J=7.4 Hz), 2.23 (s, 12H), 1.80-1.74 (m, 2H), 1.70-1.61 (m, 6H), 1.37 (m, 4H); ¹³C NMR

(100 MHz, 10% CD₃OD in CDCl₃) δ 173.1, 169.3, 156.7, 136.0, 128.3, 127.9, 127.6, 66.8, 56.7, 54.9, 44.6, 42.4, 37.4, 31.3, 26.3, 24.5; HRMS (FAB) m/z calcd for C₃₈H₅₉N₈O₈ (M+H)⁺ 755.4456, found 755.4449.

N-(*tert*-Butoxycarbonyl)glycine 3-[N-(9-Fluorenylmethoxycarbonyl)propylamino]propylamide (18). To a cold (0 °C) solution of

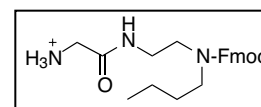


500 mg of BOC-glycine N-hydroxysuccinimide ester (Novabiochem) in 5 mL of CH₂Cl₂ was added 260 μ L of N-propyl-1,3-propanediamine (Aldrich). The mixture was allowed to warm to room temperature and was stirred overnight. After 19 h, the cloudy suspension was partitioned between 20 mL of CH₂Cl₂ and 20 mL of water. The aqueous layer was extracted with 20 mL of CH₂Cl₂ and the water was removed under reduced pressure to afford 564 mg of a white solid. The ¹H-NMR spectrum of this solid showed it to be composed of nearly a 1:1 ratio of the desired secondary amide and N-hydroxy succinimide. The impure Boc-gly-PAPA amide was taken on without further purification: ¹H NMR (400 MHz, CDCl₃) δ 7.48 (t, 1H, J=5.6 Hz), 6.2 (bs, 2H, amine N-H of Boc-gly-PAPA and O-H of N-hydroxy succinimide), 5.6 (bs, 1H), 3.72 (d, 2H, J=5.7 Hz), 3.30 (m, 2H), 2.94 (t, 2H, J=7.0), 2.77 (m, 2H), 1.87 (tt, 2H, J=6.5, 6.6 Hz), 1.57 (tq, 2H, J=7.5, 7.7), 1.39 (s, 9H), 0.87 (t, 3H, J=7.5).

To a solution of 564 mg of crude Boc-gly-PAPA amide in 10 mL of CH₂Cl₂ was added 475 mg of 9-fluorenylmethyl chloroformate (Aldrich), followed by 320 μ L of diisopropylethylamine. After stirring for 3 h at room temperature, the mixture was diluted with 30 mL of ethyl acetate and washed with 15 mL of 10% citric acid, 10 mL of water, 10 mL of sat. aq. NaHCO₃, 10 mL of brine, and then dried over Na₂SO₄. After filtration, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (80% ethyl acetate/hexane, followed by 90% ethyl acetate/hexane) to afford 664 mg (72% overall yield from BOC-glycine N-hydroxysuccinimide ester) of **18** as a foamy solid. Both the ¹H- and ¹³C-NMR spectra show that **18** exists in solution as a 75:25 mixture of amide rotamers. A ¹H-NMR spectrum acquired at 55 °C showed the near coalescence of rotamer resonances. TLC R_f 0.41 (ethyl acetate); IR (film): 3327, 3067, 2969, 2933, 2874, 1688, 1678, 1528, 1480, 1450, 1426, 1366, 1274, 1245, 1162, 1084, 1049, 1032, 946, 865, 759, 741 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, 25 °C) δ 7.73 (d, 2H, J=7.4 Hz), 7.54 (d, 2H, J=7.4 Hz), 7.37 (t, 2H, J=7.4 Hz), 7.29 (t, 2H, J=7.4 Hz), 7.04 (bs, 0.75H, Boc-carbamate N-H, major rotamer), 5.98 (bs, 0.25H, Boc-carbamate N-H, minor rotamer), 5.30-5.15 (m, 1H), 4.58 (m, 0.5H, glycy CH₂, minor rotamer), 4.51 (d, 1.5 H, J=5.5 Hz), 4.18 (t, 1H, J=5.4 Hz), 3.76 (m, 1.5H,

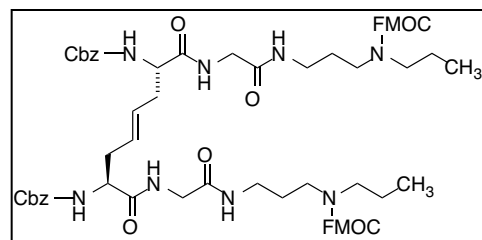
Fmoc-carbamate CH_2 , major rotamer), 3.68 (m, 0.5H, Fmoc-carbamate CH_2 , minor rotamer), 3.22-3.19 (m, 1.5H), 3.11-3.00 (m, 2H), 2.92-2.84 (m, 2.5H), 1.60-1.56 (m, 1.5H), 1.42 (s, 9H), 1.31-1.21 (m, 2.5H), 0.79 (m, 0.75H, $-\text{CH}_3$, minor rotamer), 0.67 (t, 2.25H, $J=7.2$ Hz, $-\text{CH}_3$, major rotamer); ^{13}C NMR (100 MHz, CDCl_3) δ 169.2, 156.8, 155.8, 143.9, 141.3, 127.6, 126.9, 124.6, 119.8, 79.7, 66.6, 66.0, 60.3, 49.2, 48.6, 47.4, 44.5, 44.2, 43.9, 36.8, 35.5, 28.2, 27.5, 21.5, 21.1, 21.0, 14.1, 11.0; HRMS (FAB) m/z calcd for $\text{C}_{28}\text{H}_{38}\text{N}_3\text{O}_5$ ($\text{M}+\text{H}$) $^+$ 496.2811, found 496.2810, m/z calcd for $\text{C}_{28}\text{H}_{37}\text{N}_3\text{O}_5\text{Na}$ ($\text{M}+\text{Na}$) $^+$ 518.2631, found 518.2653.

Glycine 3-[N-(9-Fluorenylmethoxycarbonyl)propylamino]-propylamide Trifluoroacetate Salt (19**).** To a solution of 231 mg (0.467 mmol) of **18** in 1 mL of CH_2Cl_2 was added 2 mL of trifluoroacetic acid.



After 1.5 h, the solvents were removed under reduced pressure. The residue was taken up in a small amount of toluene and re-concentrate under reduced pressure. The oil was placed under a high vacuum for 30 min to afford 235 mg (ca. 100%yield) of the trifluoroacetate salt of **19**, which was taken on without purification. The ^1H -NMR spectrum of **19** shows that this material exists in CDCl_3 solution as a 85:15 mixture of amide rotamers: ^1H NMR (400 MHz, CDCl_3) δ 8.04 (bs, 3H), 7.70 (d, 2H, $J=7.5$ Hz), 7.70-7.60 (m, 1H), 7.48 (d, 2H, $J=7.4$ Hz), 7.34 (t, 2H, $J=7.4$ Hz), 7.25 (t, 2H, $J=7.4$ Hz), 4.49 (m, 0.3H, Fmoc-carbamate CH_2 , minor rotamer), 4.43 (d, 1.7H, $J=5.2$ Hz, Fmoc-carbamate CH_2 , major rotamer), 4.13 (t, 1H, $J=5.2$ Hz), 3.74 (m, 2H), 3.18-2.98 (m, 4H), 2.98-2.82 (m, 0.6H), 2.82-2.72 (m, 1.4H), 1.60-1.43 (m, 2H), 1.43-1.25 (m, 0.6H), 1.20-1.10 (m, 0.4H), 0.78-0.68 (m, 0.45H, $-\text{CH}_3$ minor rotamer), 0.62 (t, 2.55H, $J=7.2$ Hz, $-\text{CH}_3$ minor rotamer).

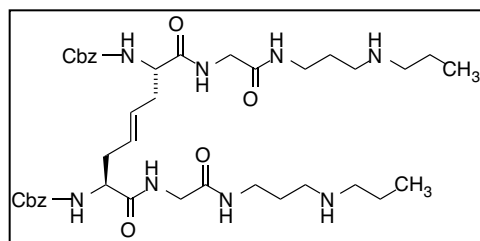
Fmoc Protected Unsaturated Diamine (20**).** To the 235 mg of crude **19**, taken on from the previous reaction, was added 92 mg (0.19 mmol) of diacid **16** and 3 mL of CH_2Cl_2 and the mixture was cooled to 0°C . To the cold mixture was added 309 mg (0.594 mmol) of PyBOP



(Novabiochem) followed by 215 μL (1.23 mmol) of DIEA. After stirring for 35 h, the mixture was diluted with 30 mL of ethyl acetate and washed with 15 mL portions of the following: 10% KHSO_4 , water, and brine. The organic layer was dried over Na_2SO_4 , filtered and concentrated under reduced pressure to afford 681 mg of a thick yellow oil. The oil was purified by flash chromatography (6% methanol/ethyl acetate, 10% methanol/ethyl acetate, followed by 20%

methanol/ethyl acetate) to afford 274 mg of a white foamy solid. TLC (10% methanol/ CH₂Cl₂) showed this material to be contaminated by a lower R_f material. The impure solid was repurified by flash chromatography (40:60 toluene/acetone, then 75:25 acetone/toluene) to afford 230 mg (96% yield) of **20** as a white solid: TLC R_f 0.55 (10% methanol/CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃, 55 °C) δ 7.78(d, 4H, J=7.5 Hz), 7.58 (d, 4H, J=7.4 Hz), 7.42 (t, 4H, J=7.4 Hz), 7.37-7.20 (m, 16H), 7.16-7.03 (m, 2H), 6.21 (bs, 2H), 5.54 (d, 2H, J=5.4 Hz), 5.15-5.07 (m, 4H), 4.57 (d, 4H, J=5.4 Hz), 4.39-4.25 (m, 2H), 4.22-4.13 (m, 2H), 4.00-3.78 (m, 4H), 3.37-2.80 (m, 12H), 2.58-2.42 (m, 4H), 1.68-1.43 (m, 4H), 1.40-1.20 (m, 4H), 0.88-0.62 (m, 6H); ¹³C NMR (100 MHz, CDCl₃, 55 °C) δ 171.7, 168.7, 156.3, 144.0, 141.4, 136.3, 129.1, 128.4, 128.1, 128.0, 127.6, 127.0, 124.6, 119.8, 67.0, 66.6, 55.1, 48.8, 47.5, 44.3, 43.1, 35.5, 27.6, 21.4, 10.9; HRMS (MALDI) *m/z* calcd for C₇₀H₈₀N₈O₁₂Na (M+Na)⁺ 1247.5793, found 1247.5800.

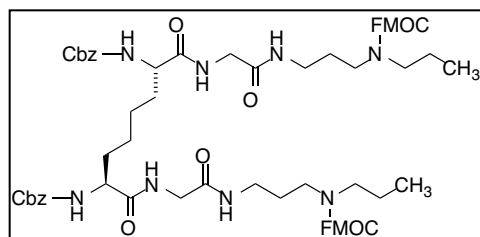
Inhibitor 5b. To a solution of 110 mg (0.0898 mmol) of Fmoc protected **20** in 1 mL of CH₂Cl₂ was added 30 μL (0.201 mmol) of DBU. After stirring for 1.5 h, the mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (6:3:1



CHCl₃/methanol/triethylamine) to afford 12.4 mg (18% yield) of **5b** as an oil. The low yield in this normally high-yielding transformation was due to accidental loss of material during purification.

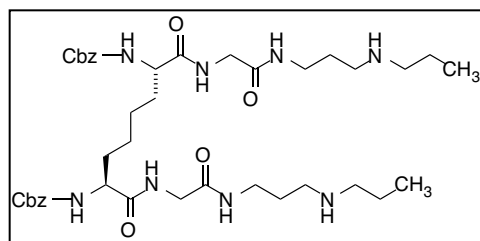
The ¹H-NMR spectrum of this material revealed that it exists as a mixture of rotamers. Only those signals for the major rotamer (which accounts for about 80% of the material) are reported below. TLC R_f 0.20 (6:3:1 CHCl₃/methanol/triethylamine). ¹H-NMR (400 MHz, CDCl₃) δ 7.77 (bs, 2H), 7.36-7.28 (m, 12H), 6.36 (d, 1H, J=6.9 Hz), 5.53 (m, 2H), 5.10-5.01 (m, 4H), 4.30-4.25 (m, 2H), 3.87-3.75 (m, 4H), 3.46, (bs, 2H), 3.33-3.26 (m, 4H), 2.73 (t, 4H, J=6.2 Hz), 2.61 (t, 3H, J=7.3 Hz), 2.47 (m, 2H), 2.41 (m, 2H), 1.72-1.65 (m, 4H), 1.54 (quin, 4H, J=7.4 Hz), 0.92 (t, 6H, J=7.4 Hz); HRMS (FAB) *m/z* calcd for C₄₀H₆₁N₈O₈ (M+H)⁺ 781.4612, found 781.4655.

Fmoc Protected Saturated Diamine (21). A solution was prepared of 52.3 mg (0.0427 mmol) of **20** in 5 mL of 1:1 benzene/ethanol, which had been previously degassed by bubbling argon through the solvent mixture for 20 minutes. The degassed solution was transferred to a high



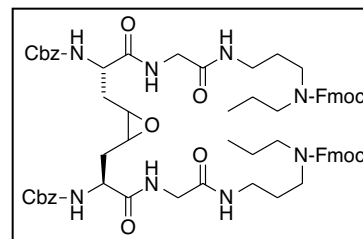
pressure bomb reaction vessel and 40 mg of Wilkinson's catalyst was added. The bomb was sealed, flushed twice with 200 psi of H₂ gas, and filled with 400 psi of H₂ gas. After 4 days, the mixture was concentrated under reduced pressure and purified by flash chromatography (75% acetone/hexanes) to afford 45 mg (86% yield) of **21**: ¹H-NMR (400 MHz, CDCl₃, the spectrum shows that **21** exists as one major rotamer along with a number of minor rotamers) δ 7.73 (d, 4H, J=7.4 Hz), 7.55-7.48 (m, 4H), 7.38 (t, 4H, J=7.4 Hz), 7.31-7.26 (m, 14H), 7.17 (t, 2H, J=6.0 Hz), 7.01 (t, 2H, J=6.0 Hz), 6.12 (d, 2H, J=7.6 Hz), 5.06 (m, 4H), 4.56-4.46 (m, 4H), 4.30-4.22 (m, 2H), 4.07 (dd, 2H, J=5.9, 16.8 Hz), 3.73 (dd, 2H, J=4.5, 16.8 Hz), 3.31-3.13 (m, 4H), 3.12-3.00 (m, 2H), 2.98-2.79 (m, 4H), 2.74 (t, 4H, J=7.7 Hz), 1.93-1.81 (m, 2H), 1.69-1.57 (m, 2H), 1.55-1.47 (m, 2H), 1.38-1.29 (m, 4H), 1.15 (quin, 4H, J=7.4 Hz), 0.62 (t, 6H, J=7.2 Hz); HRMS (MALDI) *m/z* calcd for C₇₀H₈₂N₈O₁₂Na (M+Na)⁺ 1249.5949, found 1249.6051.

Inhibitor 6b. A solution of 42 mg (0.034 mmol) of **21** in 4 mL of 1:1 diethylamine/acetonitrile was stirred at room temperature for 1 h. The solvents were removed under reduced pressure and the residue was purified by flash chromatography (6:3:1 CHCl₃/methanol/triethylamine) to

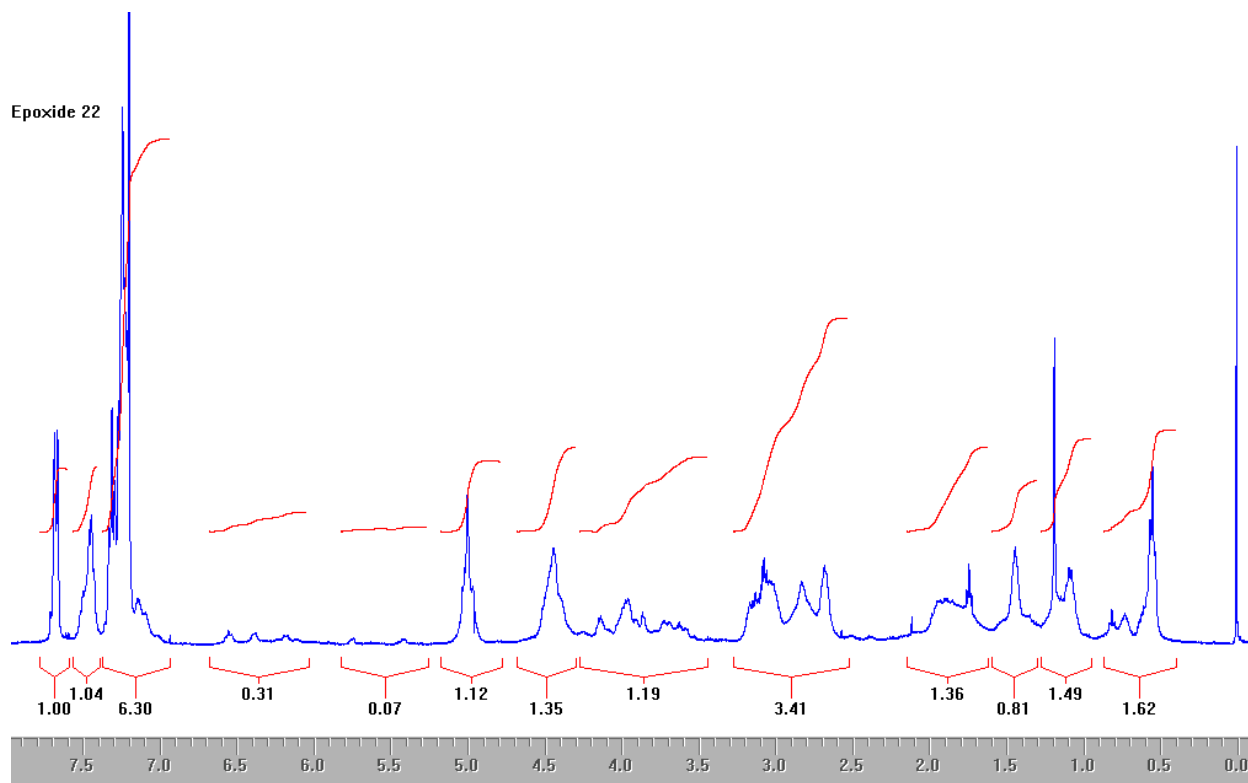


afford 20 mg (74% yield) of **6b** as an oil. TLC *R_f* 0.21 (6:3:1 CHCl₃/methanol/triethylamine), *R_f* 0.53 (6:3:1 CHCl₃/methanol/conc. NH₃); ¹H NMR (400 MHz, 10% CD₃OD in CDCl₃) δ 7.40-7.30 (m, 10H), 5.09 (m, 4H), 4.12 (dd, 2H, J=6.4, 6.8 Hz), 3.92 (d, 2H, J=16.7 Hz), 3.79 (d, 2H, J=16.7 Hz), 3.25 (m, 4H), 2.68 (t, 4H, J=7.0 Hz), 2.60 (dd, 4H, J=7.5, 7.7 Hz), 1.80-1.64 (m, 8H), 1.55 (h, 4H, J=7.5 Hz), 1.38 (m, 4H), 0.93 (t, 6H, J=7.4 Hz); ¹³C NMR (100 MHz, 10% CD₃OD in CDCl₃) δ 173.1, 169.8, 156.7, 136.1, 128.4, 128.1, 127.7, 66.9, 54.7, 50.8, 46.2, 42.7, 37.0, 31.4, 27.8, 24.3, 21.6, 11.3; HRMS (FAB) *m/z* calcd for C₄₀H₆₃N₈O₈ (M+H)⁺: 783.4769, found: 783.4811.

Epoxide 22. To a solution of 39 mg (0.032 mmol) of alkene **20** in 300 μL of acetone was added 750 μL of a 0.06 M solution of dimethyldioxirane⁴ (0.045 mmol) in acetone. The mixture was allowed to stir overnight. The solvent was removed under reduced pressure to afford 39 mg of epoxide **22**: ¹H NMR (400 MHz, CDCl₃), the spectrum of the crude product is reproduced below; (HRMS (FAB) *m/z* calcd for



$C_{70}H_{81}N_8O_{13}$ (M+H)⁺ 1241.5923, found 1241.5968, *m/z* calcd for $C_{70}H_{80}N_8O_{13}Na$ (M+Na)⁺ 1263.5743, found 1263.5767.



Enzyme Studies. *T. cruzi* trypanothione reductase (TR) was purified following the method of Walsh, et al.⁹ from an overproducing strain of *E. coli* SG5, a glutathione reductase deletion mutant, containing the expression vector pIBITczTR as described by Sullivan and Walsh.¹⁰ TR activity was assayed using **1**¹¹ as the disulfide substrate and by following the oxidation of NADPH spectrophotometrically at 340 nm.¹² Assays were run at 25 °C in 100mM HEPES (pH 7.8), 1mM EDTA, and 150 μ M NADPH. All assay solutions were also adjusted to 2% methanol, since inhibitor stock solutions required 10% methanol for complete dissolution of our compounds. Control experiments indicated that 2% methanol had no inhibitory effect on TR. K_i values for each inhibitor were determined by measuring the initial rates at three different inhibitor concentrations, ranging from 25 μ M to 105 μ M, in the presence of five substrate concentrations, varied from 2.5 μ M to 36 μ M. The data were fit to the competitive inhibition model ($Y = V_{max} * [S] / ((K_m(1+[I]/K_i) + S))$) using Cleland's COMP program.¹³ We also attempted to fit the data

to the uncompetitive and noncompetitive inhibition models (equations $Y = V_{\max} * [S] / (K_m + [S](1 + [I]/K_i))$ and $Y = V_{\max} * [S] / (K_m(1 + [I]/K_{is}) + S(1 + [I]/K_{ii}))$, respectively) using Cleland's UNCOMP and NCOMP programs.¹³ The data for each inhibitor either failed to fit these models or gave a fit significantly worse than that obtained with the competitive model. The K_m value for assay substrate **5** was 6.7 μM .

The inhibitory effects of compounds **5-6** on the rate of reduction of glutathione by yeast glutathione reductase (GR, Sigma) were determined by following the oxidation of NADPH spectrophotometrically at 340 nm. Assays were run at 25 °C in 100mM HEPES (pH 7.8), 1mM EDTA, and 150 μM NADPH. All assay solutions were also adjusted to 2% methanol, since inhibitor stock solutions required 10% methanol for complete dissolution of our compounds. Control experiments indicated that 2% methanol had no inhibitory effect on GR. The rate of reduction of glutathione, at a concentration of 30 μM , in the presence and absence of 250 μM inhibitor were determined in triplicate. Within experimental error, none of the compounds, **5-6**, showed a measurable inhibitory effect on GR.

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