Inhibition of Trypanothione Reductase by Substrate Analogs

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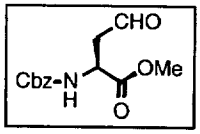
Supporting Information

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-yloxytripyrrolidinophophonium hexafluorophosphate (PyBOP)[1] and bromotripyrrolidinophosphonium hexafluorophosphate (PyBrop)[2] were purchased from Novabiochem. Schöllkopf's bislactim ether **13** was prepared according to the literature procedure[3] or purchased from Fluka. Dimethyldioxirane was prepared by the method of Murray and Singh.[4] Wilkinson's catalyst ($Rh(PPh_3)_3Cl$) 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: tetrahydrofuran (THF) was distilled from potassium-benzophenone or calcium hydride, and dichloromethane (CH_2Cl_2), diisopropylethylamine (DIEA), and triethylamine (TEA) were distilled from calcium hydride. Flash chromatography was performed by the method of Still, Kahn, and Mitra[6] 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-d_4, the chemical shifts are reported relative to residual $CD_2\underline{H}OD$ at 3.30 ppm. ^{13}C NMR spectra were obtained at the indicated field strength using broad-band decoupling. Chemical shifts are reported relative to $CDCl_3$ 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.

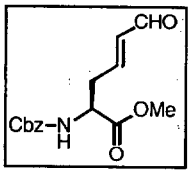


(2S)-Methyl 2-(Benzyloxycarbonylamino)-4-oxobutanoate (9). To a cold (–5 °C) solution of 5.11 g (18.2 mmol) of *N*-(benzyloxy)carbonyl-aspartic acid α-methyl ester (**8**, Sigma) in 10 mL of THF was added 37 mL (37 mmol) of 1M borane in THF (Aldrich), over a 15 min period. After 5.5 h, the excess borane 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 portions of ether and the combined ether layers were washed once with 50% brine and dried over Na_2SO_4. The solvents were removed under reduced pressure and the residue was purified by flash chromatography (40% ethyl acetate/hexane) to afford 3.67 g (75% yield) of Cbz-homoserine methyl ester as a clear oil: IR ($CHCl_3$) 3425, 2957, 2990, 1725, 1512, 1440, 1348, 1236, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{13}H_{18}NO_5$ $(M+H)^+$ 268.1185, found 268.1201. Anal. Calcd for $C_{13}H_{17}NO_5$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.40; H, 6.40; N, 5.21.

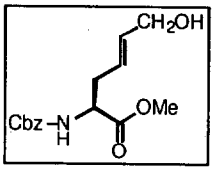
Cbz-homoserine methyl ester was converted to aldehyde **9** via a TEMPO mediated bleach oxidation.[7] Thus, 3.67 g (13.7 mmol) of Cbz-homoserine methyl ester was added to a mixture of 1.46 g of NaBr, 25 mg (0.16 mmol) of TEMPO free radical (Aldrich), 40 mL of toluene, and 40 mL of H_2O. The mixture was 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 h period. The bleach solution was prepared as follows: 50 mL of Clorox bleach, 50 mL of H_2O, and 7.8 g of $NaHCO_3$ 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% $KHSO_4$, 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 Na_2SO_4 and removing the solvents under reduced pressure, the crude product was purified by flash chromatography (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 was recrystallized from ether/hexane: mp 58–61 °C; IR

($CHCl_3$) 3430, 3960, 2845, 1740, 1515, 1438, 1340, 1235, 1050 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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 $C_{13}H_{15}NO_5$: 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 (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 $C_{15}H_{18}NO_5$ $(M+H)^+$ 292.1185, found 292.1167.

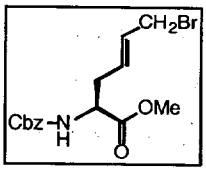


Methyl (2S,4E)-2-(Benzyloxycarbonylamino)-6-hydroxyhex-4-enoate (11). To a cold (0 °C) solution of 2.04 g of α,β-unsaturated aldehyde **10** (about 95% pure, as described in the preceding experimental procedure) in 5 mL of THF was added 30 mL of a 0.5 M 9-BBN solution in THF (15 mmol, Aldrich), dropwise, over a 10 minute period. After stirring for 2 h the excess borane reagent 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 com-



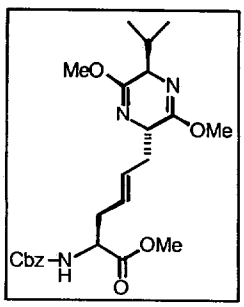
bined organic layers were washed with 20 mL of pH 7.0 phosphate buffer, 15 mL of brine, and dried over Na_2SO_4. After removal of the solvents under reduced pressure, the residue was purified by flash chromatography (45%, 50%, and finally 60% ethyl acetate/hexane) to afford 1.80 g (87% yield) of allylic 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 $C_{15}H_{20}NO_5$ $(M+H)^+$ 294.1341, found 294.1329.

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



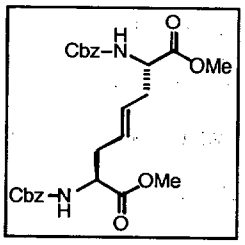
To a cold (0 °C) solution of 1.20 g (4.09 mmol) of allylic alcohol **11** in 8 mL of CH_2Cl_2 was added 1.93 g (5.83 mmol) of CBr_4 (Aldrich), followed by 1.60 g (6.11 mmol) of triphenylphosphine. The dark red/brown solution was stirred for 30 min and then diluted with 60 mL of ether. A precipitate formed 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 $C_{15}H_{18}BrNO_4$: 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).



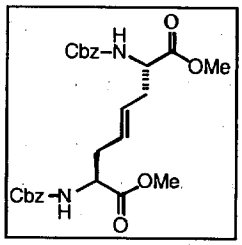
To a –78 °C solution of 1.09 g (5.90 mmol) of bislactim ether **13**[3] in 10 mL of THF was added 3.7 mL of 1.6 M *n*-butyl lithium in hexane, dropwise, over a 10 min

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 Na_2SO_4. 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, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{24}H_{33}N_3O_6$: C, 62.73; H, 7.24; N, 9.14. Found: C, 62.65; H, 7.28; N, 9.08.



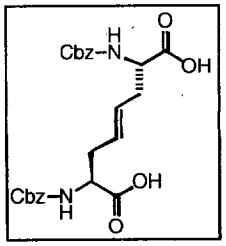
Dimethyl (2S,4E,7S)-2,7-Bis(Benzyloxycarbonylamino)oct-4-en-1,8-dioate (15) (prepared from bislactim ether **14**). To a solution of 1.07g (2.32 mmol) of bislactim ether **14** in 9.5 mL of THF was added 9.7 mL (4.9 mmol) of 0.5 M HCl. After stirring for 12.5 h, the reaction mixture was partitioned between 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. $NaHCO_3$. The basic solution was extracted with ether (3x25 mL) and the combined ether layers were dried over K_2CO_3. 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 without further purification: ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{18}H_{25}N_2O_6$ $(M+H)^+$ 365.1713, found 365.1699.

To a cold solution (0 °C) of 750 mg of the crude amine in 10 mL of THF was added 430 μL (3.1 mmol) of TEA 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% $KHSO_4$, 10 mL of 50% brine, 10 mL brine, and dried over Na_2SO_4. 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 was recrystallized from ether/hexane. The ^{1}H-NMR spectrum of **15** showed that about 10% of this material exists in solution as a minor amide rotamer: mp. 92-93 °C; IR ($CHCl_3$) 3430, 3040, 1720, 1510, 1440, 1350, 1225, 1057 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) 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-CH_3 of minor rotamer), 2.51-2.41 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 155.7, 136.2, 128.5, 128.2, 128.1, 67.0, 53.4, 52.4, 35.4. Anal. Calcd for $C_{26}H_{30}N_2O_8$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.78; H, 6.15; N, 5.54.

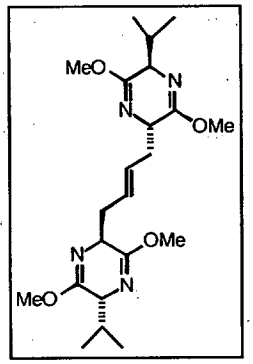


Dimethyl (2S,4E,7S)-2,7-Bis(Benzyloxycarbonylamino)oct-4-en-1,8-di-oate (15) (prepared from bis-dihydropyrazine **17**). To a solution of 415 mg (0.987 mmol) of bis-dihydropyrazine **17** in 8.5 mL of THF was added 8.5 mL (4.25 mmol) of 0.5M HCl. After stirring overnight, the solvents were removed under reduced pressure to afford 636 mg of an oil. The crude diamine was contaminated with 2 equiv of valine methyl ester, which was not removed, and the crude diamine was taken on without purification. ^{1}H-NMR of the desired diamine (400 MHz, CD_3OD) 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 in 5 mL of CH_2Cl_2 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% $KHSO_4$, 25 mL of 50% brine, and 25 mL of brine. The organic layer was dried over Na_2SO_4, 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).



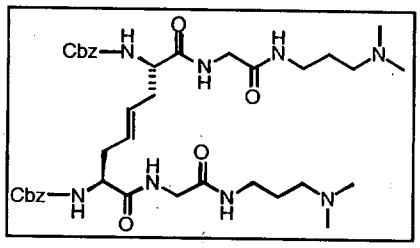
(2S,4E,7S)-2,7-Bis(benzyloxycarbonylamino)oct-4-en-1,8-dioic Acid (16). To a cold (0 °C) solution of 100 mg (0.20 mmol) of diester **15** in 3 mL of methanol was added 2.0 mL (1.0 mmol) of 0.5 M LiOH. Upon addition of the aqueous base, the reaction mixture became cloudy. The mixture was allowed to slowly warm to room temperature overnight. 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 Na_2SO_4. Filtration and removal of the solvent under reduced pressure afforded 98 mg of diacid **16** as a clear, glassy solid. The ^{1}H-NMR spectrum showed the presence of a minor rotamer of **16**. Acquiring the ^{1}H-NMR spectrum at 55 °C resulted in the near coalescence of rotamer resonances: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 10.35 (bs, 2H), 7.51-7.29 (m, 10H), 6.90-6.70 (m, 0.6H, N-H, minor rotomer), 5.92 (bs, 0.4H, N-H, minor rotomer), 5.70 (d, 1H, J=7.9 Hz, N-H, major rotomer), 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 rotomer), 2.50-2.40 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{24}H_{27}N_2O_8$ $(M+H)^+$ 471.1767, found 471.1782.



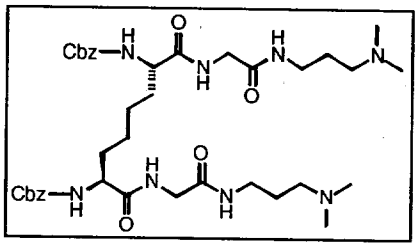
Bis-dihydropyrazine 17. To a cold (-78 °C) solution of 1.03 g (5.59 mmol) of Schöllkopf's bislactim ether **13**[3] in 15 mL of THF was added 4.75 mL (5.93 mmol) of 1.25M *n*-butyl lithium, dropwise over 15 min. After stirring at –78 °C for 40 minutes, a solution of 531 mg (2.48 mmol) of *trans*-1,4-dibromobutene 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 NH_4Cl and extracted with 90 mL of ether. The aqueous phase was extracted with two more 30 mL portions of ether and the combined organic layers were dried over Na_2SO_4. 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, $CDCl_3$) 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, $CDCl_3$) 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 $C_{22}H_{36}N_4O_4$ $(M+H)^+$ 421.2815, found 421.2803.

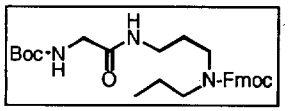
Inhibitor 5a. To a cold (0 °C) solution of 82 mg (0.174 mmol) of diacid **16**, 77 mg (0.490 mmol) of glycine-3-dimethylamino-propylamide[8], and 125 μL (0.718 mmol) of DIEA in 2.0 mL of CH_2Cl_2 was added 170 mg (0.365 mmol) of PyBrop. The mixture was stirred overnight at 0 °C, filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (6:3:1 $CHCl_3$/MeOH/TEA) to afford 121 mg of impure material. The impure product was purified a second time by flash chromatography (80:20:5 $CHCl_3$/MeOH/conc. NH_3) to afford 75 mg (57% yield) of **5a** as thick oil: TLC R_f = 0.41 (80:20:5 $CHCl_3$/MeOH/conc. NH_3); ^1H NMR (400 MHz, 5% CD_3OD in $CDCl_3$) 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); ^{13}C NMR (100 MHz, 5% CD_3OD in $CDCl_3$) 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 $C_{38}H_{57}N_8O_8$ $(M+H)^+$: 753.4299, found: 753.4325.



Inhibitor 6a. A solution of 28 mg (0.037 mmol) of alkene **5a** in 5 mL of 1:1 benzene/EtOH (which had been degassed by bubbling argon through the solvent mixture for 20 min) was transferred to a high pressure bomb reaction vessel, and 40 mg (0.043 mmol) of Wilkinson's catalyst was added. The bomb was flushed three times with 200 psi of H_2 gas and then filled with 480 psi of H_2. After five days, the crude product was concentrated under reduced pressure. The residue was purified by flash chromatography (80:23:5 $CHCl_3$/MeOH/conc. NH_3) to afford 22 mg (78 % yield) of **6a** as an oil: TLC R_f = 0.29 (80:23:5 $CHCl_3$/MeOH/conc. NH_3); ^1H NMR (400 MHz, 5% CD_3OD in $CDCl_3$) 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),



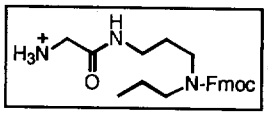
2.23 (s, 12H), 1.80-1.74 (m, 2H), 1.70-1.61 (m, 6H), 1.37 (m, 4H); ^{13}C NMR (100 MHz, 10% CD_3OD in $CDCl_3$) δ 173.1, 169.3, 156.7, 136.0, 128.3, 127.9, 127.6, 66.8, 56.7, 54.9, 44.6, 42.4, 37.4, 31.3, 26.3, 24.5; HRMS (FAB) *m/z* calcd for $C_{38}H_{59}N_8O_8$ $(M+H)^+$ 755.4456, found 755.4449.



***N*-(*tert*-Butoxycarbonyl)glycine 3-[*N*-(9-Fluorenylmethyoxycarbonyl)propylamino]propylamide (18).** To a cold (0 °C) solution of 500 mg of Boc-glycine *N*-hydroxysuccinimde ester (Novabiochem) in 5 mL of CH_2Cl_2 was added 260 μL 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 CH_2Cl_2 and 20 mL of water. The aqueous layer was extracted with 20 mL of CH_2Cl_2 and the water was removed from the aqueous phase under reduced pressure to afford 564 mg of a white solid. The ^{1}H-NMR spectrum of this solid showed it to be composed of nearly a 1:1 ratio of the desired secondary amide and *N*-hydroxy succinimde. The impure Boc-gly-PAPA amide was taken on without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) 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).

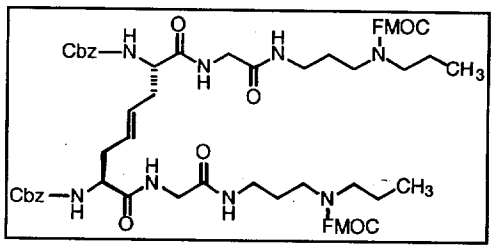
To a solution of 564 mg of crude Boc-gly-PAPA amide in 10 mL of CH_2Cl_2 was added 475 mg of 9-fluorenylmethyl chloroformate (Aldrich), followed by 320 μL of DIEA. After stirring for 3 h at room temperature, the mixture was diluted with 30 mL of ethyl acetate and washed with 15 mL of 10% citric acid, 10 mL of water, 10 mL of sat. aq. $NaHCO_3$, 10 mL of brine, and then dried over Na_2SO_4. After filtration, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (80%, 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 ^{1}H- and ^{13}C-NMR spectra show that **18** exists in solution as a 75:25 mixture of amide rotamers. A ^{1}H-NMR spectrum acquired at 55 °C showed the near coalscence 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}; ^{1}H NMR (400 MHz, $CDCl_3$, 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, glycyl C<u>H</u>$_2$, 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 C<u>H</u>$_2$, major rotamer), 3.68 (m, 0.5H, Fmoc-carbamate C<u>H</u>$_2$, 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, -C<u>H</u>$_3$, minor rotamer), 0.67 (t, 2.25H, J=7.2 Hz, -C<u>H</u>$_3$, 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 $C_{28}H_{38}N_3O_5$ $(M+H)^+$ 496.2811, found 496.2810, *m/z* calcd for $C_{28}H_{37}N_3O_5Na$ $(M+Na)^+$ 518.2631, found 518.2653.



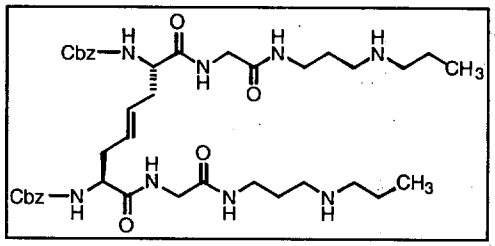
Glycine 3-[N-(9-Fluorenylmethyoxycarbonyl)propylamino]propylamide Trifluoracetate Salt (19). To a solution of 231 mg (0.467 mmol) of **18** in 1 mL of CH_2Cl_2 was added 2 mL of trifluoroacetic acid. After 1.5 h, the solvents were removed under reduced pressure. The residue was taken up in a small amount 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 ^{1}H-NMR spectrum of **19** shows that this material exists in $CDCl_3$ solution as a 85:15 mixture of amide rotamers: ^{1}H 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 C<u>H</u>$_2$, minor rotamer), 4.43 (d, 1.7H, J=5.2 Hz, Fmoc-carbamate C<u>H</u>$_2$, 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, -C<u>H</u>$_3$ minor rotamer), 0.62 (t, 2.55H, J=7.2 Hz, -C<u>H</u>$_3$ 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. The mixture was cooled to 0 °C and 309 mg (0.594 mmol) of PyBOP (Novabiochem) was added, 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

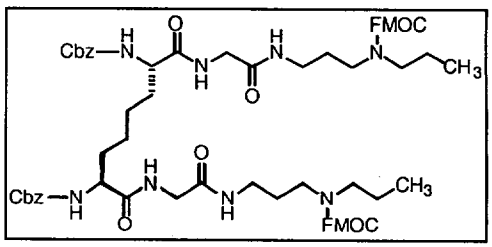


pressure to afford 681 mg of a thick yellow oil. The oil was purified by flash chromatography (6%, 10%, then 20% MeOH/ethyl acetate) to afford 274 mg of a white foamy solid. TLC (10% MeOH/CH_2Cl_2) showed this material to be contaminated by a lower R_f material. The impure solid was repurified by flash chromatography (60:40, then 75:25 acetone/toluene) to afford 230 mg (96% yield) of **20** as a white solid: TLC R_f = 0.55 (10% MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, 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); ^{13}C NMR (100 MHz, $CDCl_3$, 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 $C_{70}H_{80}N_8O_{12}Na$ $(M+Na)^+$ 1247.5793, found 1247.5800.

Inhibitor 5b. To a solution of 110 mg (0.0898 mmol) of Fmoc protected **20** in 1 mL of CH_2Cl_2 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 $CHCl_3$/MeOH/TEA) 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 ^{1}H-NMR spectrum of **5b** 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 $CHCl_3$/MeOH/TEA); ^{1}H-NMR (400 MHz, $CDCl_3$) 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 $C_{40}H_{61}N_8O_8$ $(M+H)^+$ 781.4612, found 781.4655.

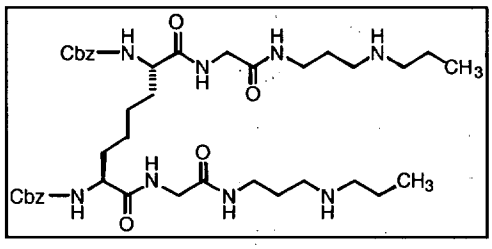


Fmoc Protected Saturated Diamine (21). A solution was prepared of 52.3 mg (0.0427 mmol) of **20** in 5 mL of 1:1 benzene/ethanol, which had been previously degassed by bubbling argon through the solvent mixture for 20 min.

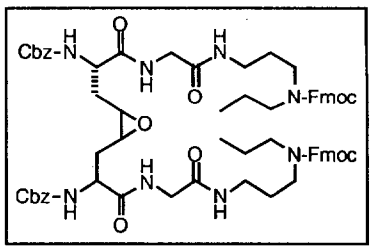


The degassed solution was transferred to a high pressure bomb reaction vessel and 40 mg of Wilkinson's catalyst was added. The bomb was sealed, flushed twice with 200 psi of H_2 gas, and filled with 400 psi of H_2 gas. After 4 days, the mixture was concentrated under reduced pressure and purified by flash chromatography (75% acetone/hexanes) to afford 45 mg (86% yield) of **21**: ^{1}H-NMR (400 MHz, $CDCl_3$, the spectrum showed 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 $C_{70}H_{82}N_8O_{12}Na$ $(M+Na)^+$ 1249.5949, found 1249.6051.

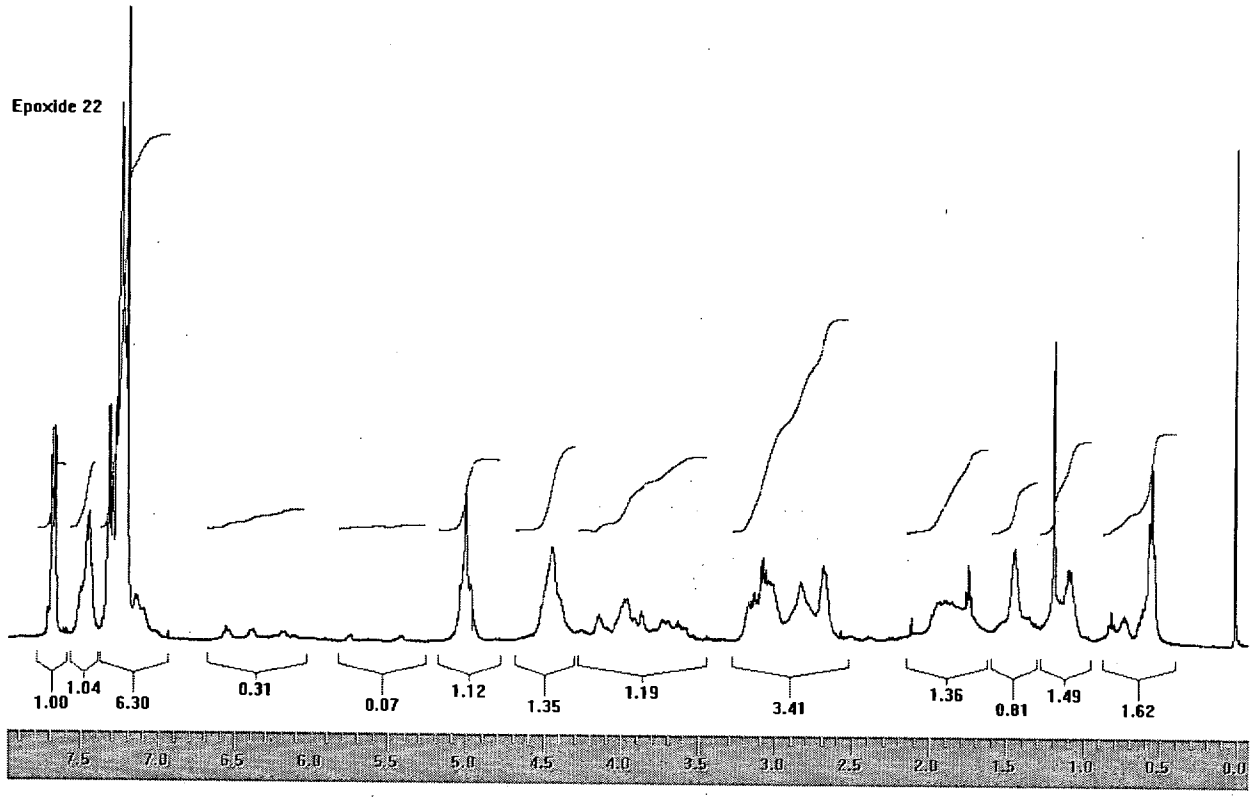
Inhibitor 6b. A solution of 42 mg (0.034 mmol) of **21** in 4 mL of 1:1 diethylamine/acetonitrile was stirred at room temperature for 1 h. The solvents were removed under reduced pressure and the residue was purified by flash chromatography (6:3:1 $CHCl_3$/MeOH/TEA) to afford 20 mg (74% yield) of **6b** as an oil. TLC R_f = 0.21 (6:3:1 $CHCl_3$/MeOH/TEA), R_f = 0.53 (6:3:1 $CHCl_3$/MeOH/conc. NH_3); ^{1}H NMR (400 MHz, 10% CD_3OD in $CDCl_3$) 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); ^{13}C NMR (100 MHz, 10% CD_3OD in $CDCl_3$) 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 $C_{40}H_{63}N_8O_8$ $(M+H)^+$: 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 dimethyldioxirane[4] (0.045 mmol) in acetone. The mixture was allowed to stir overnight. The solvent was removed under reduced pressure to afford 39 mg of crude epoxide **22**: ^{1}H NMR (400



MHz, $CDCl_3$), 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 μM NADPH. All assay solutions were also adjusted to 2% MeOH, since inhibitor stock solutions required 10% MeOH for complete dissolution of our compounds. Control experiments indicated that 2% MeOH 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 μM to 105 μM, in the presence of five substrate concentrations, varied from 2.5 μM to 36 μM. 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% MeOH, since inhibitor stock solutions required 10% MeOH for complete dissolution of our compounds. Control experiments indicated that 2% MeOH 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.

References:

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