A Shortcut to Mycothiol Anallogues

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Supporting Information

N-(tert-Butoxycarbonyl)-S-acetyl-L-cysteine. Zinc dust (2.0 g, 30.58 mmol) was added slowly in portions to a stirred solution of 500 mg (1.135 mmol) of N,N’-bis-Boc-L-cysteine in 10 mL of 5% ethereal acetic acid at 0 °C. After 4 h, 2.5 mL of acetic anhydride was added dropwise, followed by 10 mL of pyridine, and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was filtered through a Celite pad, which was washed with methanol. The combined filtrate and washings were concentrated to afford a yellow oil, which was dissolved in 12 ml of ethyl acetate. The organic solution was washed with cold aq 5% H₂SO₄ (5 x 4 mL), dried over Na₂SO₄, and then concentrated. Column chromatography with 2:98 methanol/dichloromethane as the eluant provided 598 mg (100%) of the protected amino acid as a yellow syrup: \( R_f = 0.71 \) (silica, 8:2 dichloromethane/methanol); \(^1\)H NMR (300 MHz, CDCl₃) \( \delta 6.30 \) (br s, 1 H), 5.31 (d, \( J = 5.7 \) Hz, 1 H), 4.40–4.50 (br m, 1 H), 3.44 (dd, \( J = 2.8, 13.8 \) Hz, 1 H), 3.31 (dd, \( J = 6.6, 14.1 \) Hz, 1 H), 2.37 (s, 3 H), 1.45 (s, 9 H); \(^13\)C NMR (75 MHz, CDCl₃) \( \delta 174.11, 155.42, 155.42, 80.61, 54.05, 53.25, 31.00, 30.55, 28.32; \) LC-MS \( m/z \) 286 (M+Na)⁺.
Cyclohexyl 2-Acetamido-2-deoxy-1-thio-3,4,6-tri-O-acetyl-α-D-glucopyranoside (12). A solution of 140 mg (0.385 mmol) of mercaptan 11 [Knapp, S.; Myers, D. S. J. Org. Chem. 2001, 66, 3636-3638] and 40 mg (0.243 mmol) of 2,2'-azobis(isobutyronitrile) in 2.5 mL of 1:1 cyclohexene/chloroform was heated at reflux. An additional 40 mg of AIBN was added after 1 h. After 2 h total reaction time, the mixture was cooled, concentrated, and then chromatographed on silica with 3:1 dichloromethane/ethyl acetate as the eluant to give 135 mg (79%) of 12 as a colorless foam: \( R_f 0.50 \) (7:3 dichloromethane/ethyl acetate); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 5.69 (br d, \( J = 9.2 \) Hz, 1 H), 5.45 (d, \( J = 5.2 \) Hz, 1 H), 5.09 (t, \( J = 9.6 \) Hz, 1 H), 5.02 (t, \( J = 10 \) Hz, 1 H), 4.49 (ddd, \( J = 5.6, 9.2, 10.8 \) Hz, 1 H), 4.38 (dd, \( J = 2.2, 4.8, 10.0 \) Hz, 1 H), 4.24 (dd, \( J = 4.8, 12.4 \) Hz, 1 H), 4.07 (dd, \( J = 2.4, 12.4 \) Hz, 1 H), 2.81 (tt, \( J = 3.4, 10.2 \) Hz, 1 H), 2.07, 2.02, 1.94, and 1.94 (four s, 3 H each), 1.94–2.10 (m, 2 H), 1.67–1.79 (m, 2 H), 1.54–1.63 (m, 1 H), 1.22–1.42 (m, 5 H); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 171.05, 170.31, 169.53, 169.00, 83.45, 71.36, 68.30, 68.17, 61.98, 52.13, 44.87, 34.16, 33.59, 25.87, 25.78, 25.48, 23.19, 20.65 (2 C), 20.56; LR-FAB-MS \( m/z \) 452.1 (M+Li)^+. 

Cyclohexyl 2-Amino-2-deoxy-1-thio-α-D-glucopyranoside (13). A mixture of 126 mg (0.282 mmol) of 12 and 3.5 mL of hydrazine monohydrate was heated in a sealed tube at 120 °C for 24 h. The solution was cooled, concentrated, and then dissolved in methanol. A small portion of Iatrobead silica (available from Iatron Laboratories, Inc., Japan) was added, the solvent was removed, and the pre-adsorbed reaction mixture was loaded onto an Iatrobead column and pre-eluted with 19:1 dichloromethane/methanol. Chromatography using 3:1 dichloromethane/methanol as the eluant provided 66 mg (84%) of the amino triol 13 as a clear film: \( R_f 0.31 \) (3:2 dichloromethane/methanol); \(^1\)H NMR (400 MHz, D\(_2\)O) \( \delta \) 5.44 (d, \( J = 5.6 \) Hz, 1 H), 4.05 (ddd, \( J = 2.8, 6.0, 8.8 \) Hz, 1 H), 3.84 (dd, \( J = 2.0, 12.4 \) Hz, 1 H), 3.77 (dd, \( J = 5.4, 12.2 \) Hz, 1 H).
Hz, 1 H), 3.36–3.39 (m, 2 H), 3.01 (dd, J = 5.4, 9.2 Hz, 1 H), 2.86–2.92 (m, 1 H), 2.36 (s, ~4 H, unidentified), 1.92–2.40 (m, 2 H), 1.66–1.78 (m, 2 H), 1.51–1.62 (m, 1 H), 1.20–1.48 (m, 5 H); 13C NMR (175 MHz, CD3OD) δ 85.75, 75.32, 73.53, 71.00, 61.38, 55.98, 44.25, 34.48, 33.74, 25.85, 25.78, 25.72, 8.52 (unidentified); LC-MS m/z 278.2 (M+H)+.

Cyclohexyl 2-Amino-2-N-(N-tert-butylcarbonyl-S-acetyl-L-cysteinyl)-2-deoxy-1-thio-α-D-glucopyranoside (14). N-(tert-Butoxycarbonyl)-S-acetyl-L-cysteine (22.7 mg, 0.0865 mmol) and amine 13 (12 mg, 0.0433 mmol) were azeotroped together with anhydrous toluene. A solution of 17.4 mg (0.0909 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) in 400 µL of DMF was added at 0 ºC, and the reaction mixture was allowed to warm to room temperature over a 1 h period. The reaction mixture was quenched with 3 drops of water and then concentrated. The residue was dissolved in dichloromethane and purified by column chromatography using 19:1 dichloromethane/methanol as the eluant to provide 18.5 mg (82%) of 14 as a white powder, mp 131–132 ºC; Rf 0.23 (19:1 dichloromethane/methanol); 1H NMR (500 MHz, CD3OD) δ 5.52 (d, J = 5.0 Hz, 1 H), 4.25 (dd, J = 5.2, 8.4 Hz, 1 H), 3.95–3.99 (m, 2 H), 3.79 (dd, J = 2.2 12.0 Hz, 1 H), 3.73 (dd, J = 5.3, 11.9 Hz, 1 H), 3.52 (t, J = 9.8 Hz, 1 H), 3.33–3.37 (m, 2 H), 3.08 (dd, J = 8.9, 13.7 Hz, 1 H), 2.80–2.84 (m, 1 H), 2.34 (s, 3 H), 1.93–2.10 (m, 2 H), 1.70–1.79 (m, 2 H), 1.55–1.62 (m, 1 H), 1.46 (s, 9 H), 1.24–1.40 (m, 5 H); 13C NMR (175 MHz, CD3OD) δ 195.93, 171.82, 83.08, 79.75, 73.41, 71.83, 71.26, 61.38, 54.80, 54.20, 44.06, 34.23, 33.80, 30.77, 29.23, 27.56, 25.90, 25.71; LC-MS m/z 523.0 (M+H)+.

Cyclohexyl 2-N-(N-Acetyl-L-cysteiny1)-2-amino-2-deoxy-1-thio-α-D-glucopyranoside (2). Cold anhydrous trifluoroacetic acid (1 mL) was added to 10.4 mg (0.0198 mmol) of 14 at 0 ºC. After 5 min the reaction mixture was allowed to warm to room temperature over a 15 min period. The mixture was concentrated, and 0.5 mL
of pyridine was added. The reaction mixture was stirred at room temperature for 30 min, concentrated, dissolved in methanol, and then passed through a short plug of Sephadex with methanol as the eluant to afford 8.41 mg (100%) of \( \text{2} \) as a colorless film:

\[ ^1H \text{ NMR (500 MHz, D}_2\text{O)} \delta 5.46 (d, J = 5.3 Hz, 1 H), 4.30 (t, J = 6.7 Hz, 1 H), 4.05 (dd, J = 5.4, 10.9 Hz, 1 H), 4.01 (ddd, J = 2.4, 5.0, 7.4 Hz, 1 H), 3.83 (dd, J = 2.3, 12.4 Hz, 1 H), 3.77 (dd, J = 5.2, 12.5 Hz, 1 H), 3.62 (dd, J = 8.9, 11 Hz, 1 H), 3.43 (t, J = 9.5 Hz, 1 H) 2.85 (dd, J = 6.1, 13.5 Hz, 1 H) 2.82–2.85 (m, 1 H), 2.76 (dd, J = 7.55, 13.5 Hz, 1 H), 2.03 (s, 3 H), 1.89–1.93 (m, 2 H), 1.64–1.71 (m, 2 H), 1.49–1.56 (m, 1 H), 1.20–1.37 (m, 5 H); LC-MS \text{ m/z 423 (M+H)}^+ \]

**Preparation of bimane derivative 18.** Five hundred µL of a 2 mM solution of bromobimane \( \text{16} \) (Molecular Probes) in 20 mM Tris HCl buffer (pH 8.0) was added to 500 µL of a 1 mM solution of \( \text{2} \) in the same buffer. After 20 min the reaction mixture was washed twice with 500 µL of dichloromethane. TLC analysis indicated that \( \text{18} \) and residual \( \text{16} \) partitioned cleanly to the aqueous and organic layers, respectively (respective \( R_f \)'s 0.6 and 0.8 with 80:30:1 chloroform / methanol / ammonium hydroxide as the eluant). The aqueous layer was concentrated to remove traces of dichloromethane, and the residue dissolved in 400 µL of 10 mM Tris buffer (pH 7.4). The solution of \( \text{18} \) was quantitated by fluorescence-detected reverse phase HPLC with authentic mycothiol-bimane (\( \text{17} \)) as internal standard.

**Enzyme assays.** The mycothiol conjugate amidase (MCA) assays followed procedures similar to those of a previous study [Nicholas, G. M.; Kovác, P.; Bewley, C. A. *J. Am. Chem. Soc.* **2002**, **124**, 3492-3493]. Recombinant MCA from *M. tuberculosis*, which cleaves mycothiol-bimane (\( \text{17} \), the bromobimane S-alkylation derivative of MSH, \( \text{1} \)) with rates comparable to those of natural MCA from *M. smegmatis* [Newton, G. L.; Av-Gay, Y.; Fahey, R. C. *Biochemistry* **2000**, **39**, 10739-10746], was used in the experiments in this study. Amidase reactions were carried out on 30 µM substrate (\( \text{17} \))
and 18) solutions in the presence of 22 ng of MCA in 50 mM Tris buffer, pH 7.4, in a final volume of 40 µL. Following incubation in a Perkin-Elmer thermocycler at 32 ºC for 20 min, the reactions were quenched with an equal volume of dilute aqueous methanesulfonic acid at 4 ºC, and then centrifuged at 5000 X g for 10 min. The extent of amide cleavage was measured directly by fluorescence-detected reverse phase HPLC (Agilent Technologies, Waters Spherisorb ODS column, λ<sub>exc</sub> = 250 nm, λ<sub>emis</sub> = 395 nm), eluting with a linear gradient comprised of 0.05% TFA (buffer A) and 0% to 35% CH<sub>3</sub>CN in A. With a flowrate of 1 mL/min, the bimane derivatives of N-acetylcysteine (8) and of 2 (=18) eluted at 14.9 and 22.8 min, respectively.