Facile Conversion of Cysteine and AlkylCysteines to Dehydroalanine on Protein Surfaces: Versatile and Switchable Access to Functionalized Proteins

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General procedures

Melting points were recorded on a Kofler hot block and are uncorrected. Proton nuclear magnetic resonance (δ_H) spectra were recorded on a Bruker AV400 (400 MHz), or by Dr. B. Odell or Dr. T. Claridge on a Bruker AVII500 (500 MHz) spectrometer. Carbon nuclear magnetic resonance (δ_C) spectra were recorded on a Bruker AV400 (100.7 MHz) spectrometer or by Dr. B. Odell or Dr. T. Claridge on a Bruker AVII500 (125.8 MHz) spectrometer. Spectra were fully assigned using COSY and HMQC; multiplicities were assigned using DEPT 135. All chemical shifts are quoted on the δ scale in ppm using residual solvent as the internal standard (1H NMR: $CDCl_3$ = 7.26, CD_3OD = 4.87; ^{13}C NMR: $CDCl_3$ = 77.0; CD_3OD = 49.0). The following splitting abbreviations were used: s = singlet, d = doublet, t = triplet, q = quartet, a = apparent.

Infrared spectra were recorded on a Bruker Tensor 27 Fourier Transform spectrophotometer using thin films on NaCl plates for oils and KBr discs for solids and crystals. Absorption maxima (υ_{max}) are reported in wavenumbers (cm^{-1}) and classified as strong (s) or broad (br).

Low resolution mass spectra were recorded on a Micromass Platform 1 spectrometer using electrospray ionization (ESI) or by Mr. Robin Proctor using a Walters 2790-Micromass LCT electrospray ionization mass spectrometer. High resolution mass spectra were recorded by Mr. Robin Proctor on a Walters 2790-Micromass LCT electrospray ionization mass spectrometer. m/z values are reported in Daltons.

Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm and are reported with implied units of 10^{-1} deg cm^2 g^{-1}. Concentrations (c) are given in g/100 ml.

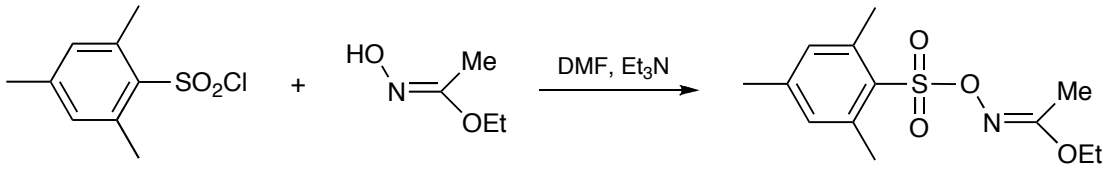
Thin layer chromatography (TLC) was carried out using Merck aluminium backed sheets coated with $60F_{254}$ silica gel. Visualization of the silica plates was achieved using a UV lamp (λ_{max} = 254 nm), and/or ammonium molybdate (5% in 2M H_2SO_4), or potassium permanganate (5% in 1M NaOH). Flash column chromatography was carried out using BDH PROLAB® 40-63 mm silica gel (VWR).

Anhydrous solvents were purchased from Fluka or Acros except dichloromethane which was distilled over calcium hydride. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Distilled water was used for chemical reactions and Milli-Q water for protein modifications. Reagents were purchased from Aldrich and used as supplied. 'Petrol' refers to the fraction of light petroleum ether boiling in the range 40-60 ºC. All reactions using anhydrous conditions were performed using flame-dried apparatus under an atmosphere of argon or nitrogen.

Protein Mass Spectrometry: Liquid chromatography-mass spectrometry (LC-MS) was performed on a Micromass LCT (ESI-TOF-MS) coupled to a Waters Alliance 2790 HPLC using a Phenomenex Jupiter C4 column (250 x 4.6 mm x 5µm). Water:acetonitrile, 95:5 (solvent A) and acetonitrile (solvent B), each containing 0.1% formic acid, were used as the mobile phase at a flow rate of 1.0 mL min^{-1}. The gradient was programmed as follows: 95% A (5 min isocratic) to 100% B after 15 min then isocratic for 5 min. The electrospray source of LCT was operated with a capillary voltage of 3.2 kV and a cone voltage of 25 V. Nitrogen was used as the nebulizer and desolvation gas at a total flow of 600 l hr^{-1}. Spectra were calibrated using a calibration curve constructed from a minimum of 17 matched peaks from the multiply charged ion series of equine myoglobin, which was also obtained at a cone voltage of 25V. Total mass spectra were reconstructed from the ion series using the MaxEnt algorithm preinstalled on MassLynx software (v. 4.0 from Waters) according to manufacturer's instructions.

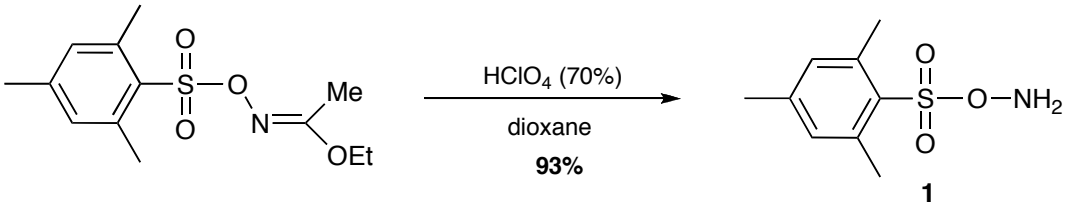
Preparation of MSH

Ethyl-*O*-(mesitylenesulfonyl)acetohydroxamate (MSH precursor)



Ethyl *N*-hydroxyacetimidate (2.36 g, 22.9 mmol) was dissolved in DMF (6 mL). Triethylamine (3 mL) was added and the stirred solution was cooled to 0 ºC. 2-Mesitylenesulfonyl chloride (5.00 g, 22.9 mmol) was added in two portions and the resulting white slurry was stirred vigorously for 15 min. The mixture was then diluted with DCM (100 mL) and washed repeatedly with water. The organic layer was dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to give the titled compound as a white solid that was used without further purification; spectroscopic data was identical to that previously reported;[1] m.p. 51-53 ºC [Lit. 57-58 ºC][1]; δ_H (400 MHz, $CDCl_3$) 1.19 (3H, t, *J* 7.1 Hz, $CH_2C\underline{H}_3$), 2.04 (3H, s, CH_3), 2.31 (3H, s, CH_3Ar), 2.64 (6H, s, 2 x CH_3Ar), 3.90 (2H, q, *J* 7.1 Hz, $C\underline{H}_2CH_3$), 6.97 (2H, s, Ar-H); δ_C (100.7 MHz, $CDCl_3$) 14.0 (q, $OCH_2\underline{C}H_3$), 14.9 (q, CH_3), 21.1 (q, CH_3Ar), 22.8 (q, 2 x CH_3Ar), 63.6 (t, $O\underline{C}H_2CH_3$), 130.3, 131.5, 140.7, 143.3 (Ar), 169.2 (C=N).

***O*-Mesitylsulfonylhydroxylamine[2] 1 (MSH)**

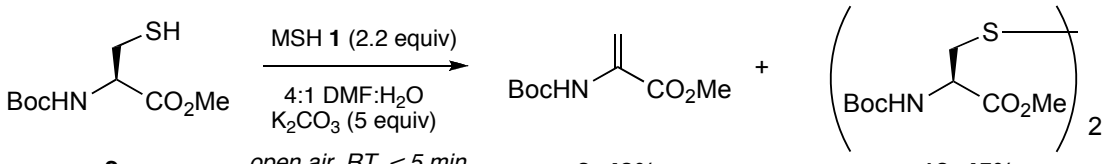


Caution: MSH can detonate. To prevent explosion, the crystallization procedure must be followed precisely to produce small crystals. Slow crystallization provides large crystals that are prone to explosive decomposition.[2] The product should be stored in a plastic container that is sealed with no more than wax film. To date MSH prepared in the manner described below has not led to any difficulty.

A solution of ethyl-*O*-(mesitylsulfonyl)acetohydroxamate (4.42 g, 15.49 mmol) in dioxane (4 mL) was cooled to 0 ºC. Perchloric acid (70%, 1.80 mL) was added dropwise *via* pipette over 2 minutes. After stirring for 5 minutes the mixture solidified. The contents of the reaction were transferred to 200 mL of ice water and the flask

rinsed with water (50 mL) and diethyl ether (50 mL). The contents were transferred to a separatory funnel and extracted with diethyl ether (40 mL). The organic layer was neutralized and partially dried with anhydrous potassium carbonate and then filtered. The filtrate was concentrated to less than 100 mL total volume and then poured into 150 mL of ice cold petrol and left to crystallize for 30 min. The white crystals (small needles) were isolated by filtration, transferred to a plastic Falcon tube, and dried under vacuum. The dried product (3.11 g, 93% yield) was stored at -20 ºC and sealed with no more than wax film; m.p. 90-91 ºC [Lit. 95-96 ºC][1]; δ_H (400 MHz, $CDCl_3$) 2.32 (3H, s, CH_3Ar), 2.63 (6H, s, 2 x CH_3Ar), 6.58 (2H, br s, NH_2), 6.98 (2H, s, Ar-H); δ_H (100.7 MHz, $CDCl_3$) 21.1 (q, CH_3Ar), 22.7 (q, 2 x CH_3Ar), 128.9, 131.7, 141.0, 143.9 (Ar); Found: C, 50.18%; H, 5.90%, N, 6.27%. $C_9H_{15}NO_4$ requires: C, 50.21%; H, 6.09%; N, 6.51%.

Chemical conversion of BocCysOMe 2 to BocDhaOMe 3

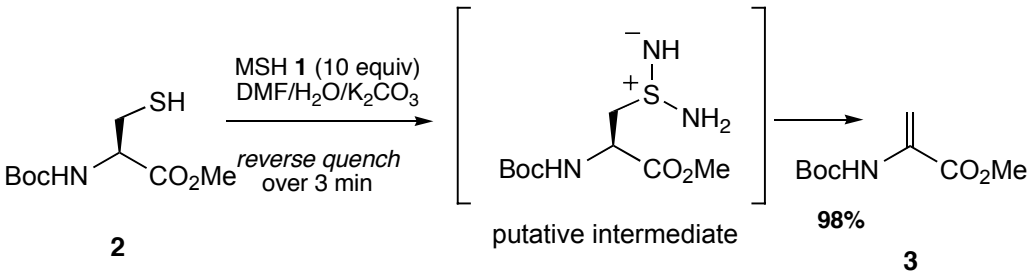


2 **3, 48%** **18, 45%**

N-(*tert*-Butoxycarbonyl)-L-cysteine methyl ester **2** (100 mg, 0.42 mmol) was added to a 50 mL round bottom flask and dissolved in DMF (8 mL, open air, room temperature). A solution of potassium carbonate (294 mg, 2.12 mmol) in water (2 mL) was added by pipette and stirred 30 seconds. MSH **1** (198 mg, 0.92 mmol) was then added in one portion and stirred for 1 minute at which time the reaction mixture changed from a cloudy suspension to a clear solution. The reaction was checked immediately by TLC (ethyl acetate:petrol; 3:7), showing complete consumption of the starting material and the formation of two products: a strongly UV active material (R_f 0.6) and another product (R_f 0.2). After 2 minutes of total reaction time, the mixture was diluted with diethyl ether (80 mL) and water (80 mL). The organic layer was separated and the aqueous layer was extracted with diethyl ether (3 × 40 mL). The combined organics were washed with brine (100 mL), dried ($MgSO_4$), and filtered. After removal of solvent, the residue was purified by column chromatography by eluting first with 3% ethyl acetate in petrol to give methyl 2-[(*tert*-butoxycarbonyl)amino]acrylate **3** (41 mg, 48%) as a clear oil and then 30% ethyl acetate in petrol to give *N,N*-bis(*tert*-butoxycarnonyl)-L-cysteine dimethyl ester **18** (45 mg, 45%) as small white needles;

Data for **Methyl 2-[(*tert*-butoxycarbonyl)amino]acrylate (BocCysOMe) 3**: υ_{max} (thin film) 3423, 2980, 1719, 1634, 1513, 1328, 1159, 1068 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.46 (9H, s, $C(CH_3)_3$), 3.80 (3H, s, OCH_3), 5.70 (1H, d, *J* 1.5 Hz, C=C<u>H</u>H), 6.13 (1H, app s, C=CH<u>H</u>), 7.00 (1H, br s, NH); δ_C (100.7 MHz, $CDCl_3$) 28.2 (q, $C(\underline{C}H_3)_3$), 52.8 (q, OCH_3), 80.6 (s, $\underline{C}(CH_3)_3$), 105.1 (t, C=$\underline{C}H_2$), 131.3 (s, $\underline{C}$=CH_2), 152.5, 164.4 (2 x s, 2 x CO); Found: C, 53.95%; H, 7.63%, N, 6.83%. $C_9H_{15}NO_4$ requires: C, 53.72%; H, 7.51%; N, 6.96%.

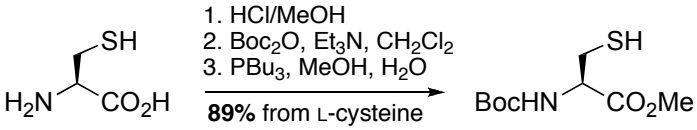
Data for ***N,N*-Bis(*tert*-butoxycarnonyl)-disulfide-L-cysteine dimethyl ester 18** (BocCysOMe disulfide): m.p. 89-90 ºC [Lit. 96-97 ºC][3]; $[\alpha]_D^{20}$ +91.4 (c, 1 in $CHCl_3$); υ_{max} (KBr disc) 3377, 2983, 1748, 1686, 1515, 1367, 1167 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.45 (18H, s, 2 x $C(CH_3)_3$), 3.16 (4H, d, *J* 5.1 Hz, 2 x $C\underline{H}_2S$), 3.77 (6H, s, 2 x $OC\underline{H}_3$), 4.60 (2H, m, 2 x αH), 5.40 (2H, d, *J* 7.6 Hz, NH); δ_C (100.7 MHz, $CDCl_3$) 28.3 (q, $C(\underline{C}H_3)_3$), 41.3 (t, CH_2S), 52.6 (q, OCH_3), 52.8 (d, αC), 80.3 (s, $\underline{C}(CH_3)_3$),155.0, 171.2 (2 x s, 2 x CO); *m/z* (ES^+) 469 $[M+H]^+$ 486 $[M+NH_4]^+$ 491 $[M+MeCN+NH_4]^+$.



2 **3**

MSH **1** (439 mg, 2.0 mmol) was added to a 10 mL round bottom flask and dissolved in DMF (3 mL). In a separate vial, *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester **2** (48 mg, 0.20 mmol) was added and dissolved in DMF (3 mL). The vial was cooled to 0 ºC and a solution of potassium carbonate (138 mg, 1.0 mmol) in water (3.0 mL) was added. The resulting solution was added dropwise by pipette over a period of 3 min to the stirred MSH solution at room temperature. The vial was rinsed with DMF (2 × 1 mL) to ensure complete transfer. TLC (petrol:ethyl acetate, 4:1) analysis after completion of the addition revealed a single, UV active product (R_f 0.6). The reaction mixture was transferred to a separatory funnel and diluted with diethyl ether (150 mL) and water (100 mL). After separation, the organic layer was washed successively with water (80 mL) and brine (80 mL) before drying ($MgSO_4$) and filtering. The solvent was removed under reduced pressure and the resulting residue purified by column chromatography to provide methyl 2-[(*tert*-butoxycarbonyl)amino]acrylate **3** as a clear oil (40 mg, 98%); spectroscopic data were identical to that reported above.

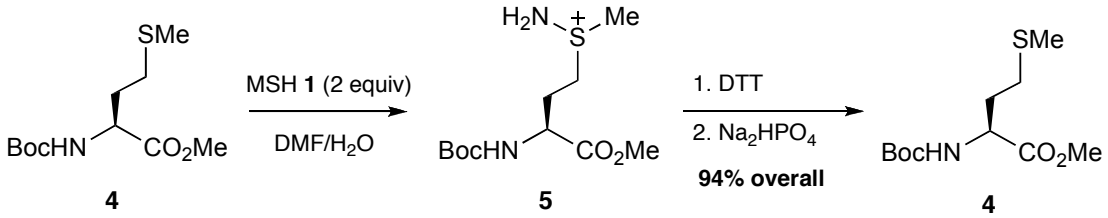
Synthesis of ***N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (BocCysOMe) 2**



2

Methanol (100 mL) was added to a flame dried 250 mL round bottom flask equipped with a teflon stir bar. The solvent was stirred and cooled to 0 ºC and acetyl chloride (17.6 mL, 248 mmol) was added dropwise over 5 minutes. The solution was stirred an additional 10 minutes at 0 ºC to give a concentrated solution of HCl.L-cysteine (2.00 g, 16.51 mmol) was then added in one portion and the flask flushed with argon. The ice bath was removed and the reaction was stirred at room temperature for 24 h. The solvent was then removed under reduced pressure to give the crude cysteine methyl ester hydrochloride as a pale yellow solid. This material was used immediately in the next step without purification. The crude ester was suspended in DCM (100 mL) and cooled to 0 ºC. Triethylamine (5.06 mL, 36.3 mmol) was added carefully followed by di-*tert*-butyl dicarbonate (4.32 g, 19.81 mmol). The reaction was stirred at room temperature for 3.25 h after which time TLC (ethyl acetate:petrol, 3:7) revealed the desired product (R_f 0.6) and its corresponding disulfide (R_f 0.3). The solvent was removed under reduced pressure and the resulting residue was redissolved in methanol (40 mL) and water (8 mL). Tributylphosphine (2.0 mL, 8.1 mmol) was added dropwise to the stirred solution. TLC revealed reduction of the disulfide. The reaction was diluted with diethyl ether (100 mL) and water (50 mL). The organic layer was separated and the aqueous layer was extracted with diethyl ether (2 × 50 mL). The combined organics were washed with brine (100 mL), dried over $MgSO_4$, and filtered. The solvent was removed by rotary evaporation and the residue purified by column chromatography eluting first with 5% ethyl acetate in petrol and then 20% ethyl acetate in petrol. The titled compound **2** was isolated as a clear oil (3.48 g, 89% from L-cysteine); $[\alpha]^{20}_D$ +28.3 (c = 7.5, $CHCl_3$) [Lit. $[\alpha]_D^{20}$ +28.5 (c, 7.5 in $CHCl_3$)][4]; δ_H (400 MHz, $CDCl_3$) 1.42 (10H, s, includes SH, $C(CH_3)_3$), 2.94 (2H, atd, *J* 4.3 Hz, *J* 8.7 Hz, $C\underline{H}_2SH$), 3.76 (3H, s, CO_2CH_3), 4.58 (1H, m, αH), 5.44 (1H, d, *J* 5.8 Hz, NH); δ_C (100.7 MHz, $CDCl_3$) 27.3 (t, CH_2SH), 28.2 (q, $C(\underline{C}H_3)_3$), 52.6 (q, OCH_3), 54.8 (d, αC), 80.2 (s, $\underline{C}(CH_3)_3$), 155.1, 170.8 (2 x s, 2 × CO); *m/z* (ES^-) 234 $[M-H]^+$.

Methionine recovery from corresponding sulfilimine



4 **5** **4**

N-(*tert*-Butoxycarbonyl)-L-methionine methyl ester **4** (245 mg, 0.93 mmol) was added to a 50 mL round bottom flask and then dissolved in DMF (5 mL). The solution was stirred vigorously while water (5 mL) was added by pipette. MSH **1** (400 mg, 1.86 mmol) was added to the solution in one portion and the cloudy suspension homogenized after 30 seconds of stirring. After 5 minutes, TLC analysis revealed complete consumption of **4**. All material was located on the baseline, and no sulfoxide **19** or sulfone **20** was detected. After 20 minutes of stirring, DTT (1.43 g, 1.86 mmol) was added as a solid. TLC analysis revealed no change after 1 hour of stirring. After 1 hour of total reaction time, $Na_2HPO_4 \cdot 12H_2O$ (3.33 g, 9.30 mmol) was added to give a saturated solution of phosphate salts. After 2 hours of total reaction time (1 hour with base), TLC (30% ethyl acetate in petrol) revealed the regeneration of **4**. A final hour of reaction time revealed no further change. The reaction was then diluted with diethyl ether (150 mL) and water (150 mL) and separated. The organic layer was washed sequentially with water (150 mL) and brine (150 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The product was purified by column chromatography (30% ethyl acetate in petrol) to give recovered *N*-(*tert*-butoxycarbonyl)-L-methionine methyl ester **4** (230 mg, 94%). A similar experiment without DTT returned **4** more slowly suggesting that both thiol and base are necessary for rapid recovery in DMF. Interestingly, we have observed that while Met recovery is most efficient in the presence of thiol (either using DTT or the reagents in conjugate addition) it may also be achieved simply through the use of basic buffer at sufficient concentrations. The full mechanistic details of the Met recovery are currently under investigation;

Data for ***N*-(*tert*-butoxycarbonyl)-L-methionine methyl ester 4**: $[\alpha]_D^{20}$ -29.5 (c, 1 in MeOH) [Lit. $[\alpha]_D^{25}$ -34.0 (c, 1.0 in MeOH)][5]; δ_H (400 MHz, $CDCl_3$) 1.39 (9H, s, $C(CH_3)_3$), 1.88 (1H, m, $CH\underline{H}CH_2SCH_3$), 2.04-2.12 (4H, m, SCH_3, $C\underline{H}HCH_2SCH_3$), 2.49 (2H, t, *J* 8.0, $C\underline{H}_2SCH_3$), 3.70 (3H, s, CO_2CH_3), 4.37 (1H, q, *J* 7.1, αH), 5.20 (1H, d, *J*

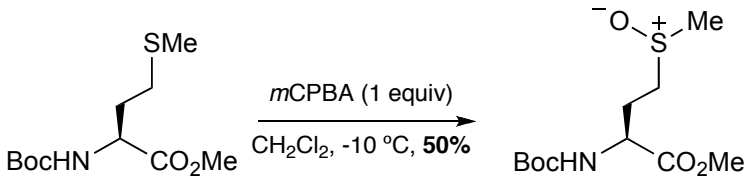
7.1, NH); δ_C (100.7 MHz, $CDCl_3$) 15.3 (q, SCH_3), 28.1 (q, $C(\underline{C}H_3)_3$), 29.8 (t, $\underline{C}H_2CH_2SCH_3$), 31.9 (t, $\underline{C}H_2SCH_3$), 52.2 (q, $CO_2\underline{C}H_3$), 52.6 (d, αC), 79.8 (s, $\underline{C}(CH_3)_3$), 155.2 (s, CO), 172.7 (s, $\underline{C}O_2CH_3$).

Synthesis of ***N*-(*tert*-butoxycarbonyl)-L-methionine methyl ester 4**

4

To a flame dried flask under nitrogen was added methanol (30 mL). The stirred solution was cooled to 0 ºC before thionyl chloride (3.70 mL, 50.67 mmol) was added dropwise. The solution was stirred 10 min at 0 ºC before methionine (5.04 g, 33.8 mmol) was added in one portion. The reaction was stirred at room temperature overnight after which time the volatiles were removed under reduced pressure to give the crude methionine methyl ester hydrochloride as a yellow-white solid. This solid was dissolved in DCM (150 mL) and cooled to 0 ºC. Triethylamine (17.50 mL) was added carefully followed by di-*tert*-butyl dicarbonate (13.6 g, 62.5 mmol). After stirring for 7 hours at room temperature, the reaction was diluted with DCM (100mL) and washed with water (2 × 100 mL). The organic layer was dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure. The product (R_f 0.6 in ethyl acetate: petrol, 1:4) was purified by flash column chromatography to afford the titled compound **4** as a clear oil (7.75 g, 87% yield); spectroscopic data was identical to that reported above.

Synthesis of **BocMetOMe sulfoxide 19**

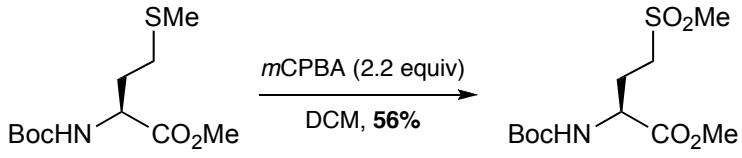


4 **19**

N-(*tert*-Butoxycarbonyl)-L-methionine methyl ester **4** (1.52 g, 5.76 mmol) was added to a 100 mL round bottom flask and dissolved in DCM (30 mL). The stirred solution was cooled to -10 ºC (sodium chloride/ice bath) and 3-chloroperoxybenzoic acid (1.36 g, 5.76 mmol) was added by pipette as a suspension in DCM (10 mL) over a

period of 10 min. The temperature was maintained between -5 ºC and -10 ºC over 1 h. TLC (ethyl acetate) revealed consumption of the starting material and formation of the sulfoxide (R_f = 0.2). The reaction was then diluted with ethyl acetate (250 mL) and sodium hydrogen carbonate (200 mL of a saturated aqueous solution). After separation, the organics were washed successively with sodium hydrogen carbonate (200 mL of a saturated aqueous solution) and brine (200 mL) and then dried ($MgSO_4$). The solution was filtered and the solvent removed under reduced pressure. The residue was purified by column chromatography (ethyl acetate to ethyl acetate:methanol, 9:1) to give the corresponding sulfoxide **19** as a thick clear oil, and as a mixture of diastereomers (806 mg, 50%); $[\alpha]_D^{20}$ +17.1 (c, 0.6 in $CHCl_3$); υ_{max} (thin film) 1699, 1165, 1017 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.25 (9H, s, $C(CH_3)_3$), 1.94 (1H, m, $CH\underline{H}CH_2S(O)CH_3$), 2.13 (1H, m, $C\underline{H}HCH_2S(O)CH_3$), 2.42 (3H, s, $S(O)CH_3$), 2.55-2.70 (2H, m, $C\underline{H}_2S(O)CH_3$), 3.57 (3H, s, CO_2CH_3), 4.16-4.24 (1H, m, αH), 5.78 (1H, at, *J* 9.4, NH); δ_C (100.7 MHz, $CDCl_3$) 25.3 (t, $\underline{C}H_2CH_2S(O)CH_3$), 28.2 (q, $C(\underline{C}H_3)_3$), 38.3 (q, $S(O)CH_3$), 50.3 (t, $\underline{C}H_2S(O)CH_3$), 52.4 (q, $CO_2\underline{C}H_3$), 52.6 (d, αC), 79.8 (s, $\underline{C}(CH_3)_3$), 155.5 (s, CO), 172.0 (s, $\underline{C}O_2CH_3$); HRMS (ES^+) Calcd. for $C_{11}H_{21}NO_6SNa$ $[MNa]^+$ 302.1038. Found: 302.1033.

Synthesis of **BocMetOMe sulfone 20**

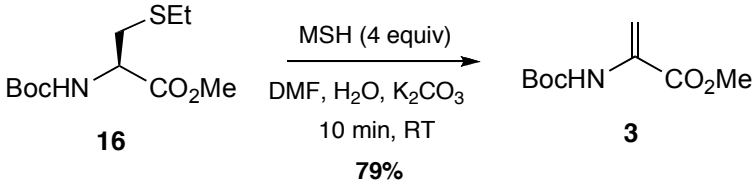


4 **20**

N-(*tert*-Butoxycarbonyl)-L-methionine methyl ester **4** (876 mg, 3.33 mmol) was added to a 100 mL round bottom flask and dissolved in DCM (15 mL). The stirred solution was cooled to 0 ºC and 3-chloroperoxybenzoic acid (70%, 1.80 g, 7.32 mmol) was added as a suspension in 10 mL of DCM by pipette over a period of 5 min. An additional 3 mL of DCM was used to complete the transfer of oxidant. The reaction was stirred for 1.5 hours at room temperature at which time it was diluted with diethyl ether (200 mL) and sodium hydrogen carbonate (100 mL of a saturated aqueous solution). The layers were separated and the organic fraction was washed successively with sodium hydrogen carbonate (2 × 100 mL of a saturated aqueous solution) and brine (150 mL). The organics were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The product was purified by flash column chromatography (ethyl acetate:petrol, 6:4) to afford the corresponding sulfone **20**

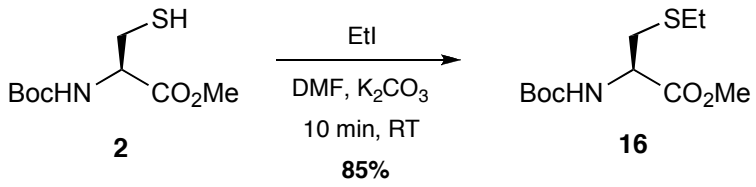
(R_f 0.3) as a thick clear oil (547 mg, 56%); $[\alpha]_D^{20}$ +17.8 (c, 1.8 in $CHCl_3$); υ_{max} (thin film) 3357, 3000, 2380, 1712, 1520, 1368, 1299, 1165 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.41 (9H, s, $C(CH_3)_3$), 2.13 (1H, m, $CH\underline{H}CH_2SO_2CH_3$), 2.38 (1H, m, $C\underline{H}HCH_2SO_2CH_3$), 2.91 (3H, s, SO_2CH_3), 3.03-3.18 (2H, m, $C\underline{H}_2SO_2CH_3$), 3.74 (3H, s, CO_2CH_3), 4.38 (1H, br s, αH), 5.38 (1H, d, *J* 7.6, NH); δ_C (100.7 MHz, $CDCl_3$) 28.2 (q, $C(\underline{C}H_3)_3$), 25.6 (t, $\underline{C}H_2CH_2SO_2CH_3$), 40.7 (q, SO_2CH_3), 51.0 (t, $\underline{C}H_2SO_2CH_3$), 52.0 (d, αC), 52.8 (q, $CO_2\underline{C}H_3$), 80.5 (s, $\underline{C}(CH_3)_3$), 155.4 (s, CO), 171.7 (s, $\underline{C}O_2CH_3$); HRMS (ES^+) Calcd. for $C_{11}H_{21}NO_6SNa$ $[MNa]^+$ 318.0987. Found: 318.0982.

Regeneration of BocDhaOMe 3 from BocCys(SEt)OMe 16



N-(*tert*-Butoxycarbonyl)-ethylthio-L-cysteine methyl ester **16** (106 mg, 0.40 mmol) was added to a 50 mL round bottom flask and dissolved in DMF (5 mL). Potassium carbonate (278 mg, 2.01 mmol) was added by pipette as a solution in water (1.0 mL). MSH **1** (172 mg, 0.80 mmol) was added as a solid in one portion (open air, room temperature). TLC analysis (ethyl acetate:petrol; 1:4) after 1 min of reaction revealed a strongly UV active product (R_f 0.6) and a trace of starting material (R_f 0.5). A second dose of MSH **1** (172 mg, 0.80 mmol) was added after 5 min of reaction time and TLC analysis revealed only the UV active product. After 10 min of total reaction time, the reaction mixture was diluted with diethyl ether (100 mL) and water (50 mL). The organic layer was separated and the aqueous layer was extracted with diethyl ether (2 x 50 mL). The combined organics were dried ($MgSO_4$), filtered, and the solvent removed by rotary evaporation. Column chromatography (3% ethyl acetate in petrol) provided methyl 2-[(*tert*-butoxycarbonyl)amino]acrylate **3** (63 mg, 79%); this material was spectroscopically identical to that obtained from *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester **2**.

Synthesis of ***N*-(*tert*-butoxycarbonyl)-ethylthio-L-cysteine methyl ester 16**



N-(*tert*-Butoxycarbonyl)-L-cysteine methyl ester **2** (573 mg, 2.44 mmol) was added to a 100 mL round bottom flask and dissolved in DMF (15 mL). Potassium carbonate (674 mg, 4.88 mmol) was added to the solution followed by ethyl iodide (0.30 mL, 3.66 mmol). The reaction was stirred at room temperature for 10 min. TLC (15% ethyl acetate in petrol) revealed complete consumption of starting material (R_f 0.3) and the formation of new product (R_f 0.4). The reaction mixture was diluted with diethyl ether (150 mL) and water (100 mL). The organic layer was separated and the aqueous layer extracted with diethyl ether (2 x 100 mL). The combined organics were dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure. Purification by column chromatography (15% ethyl acetate in petrol) gave compound **16** as a clear oil (546 mg, 85%); $[\alpha]_D^{20}$ +22.0 (c, 1.1 in $CHCl_3$); υ_{max} (thin film) 2972, 2932, 2361, 2342, 1748, 1717, 1505, 1167 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.23 (3H, t, *J* 7.4 Hz, $SCH_2C\underline{H}_3$), 1.44 (9H, s, $C(CH_3)_3$), 2.53 (2H, q, *J* 7.4 Hz, $SC\underline{H}_2CH_3$), 2.96 (2H, br s, $C\underline{H}_2SCH_2CH_3$), 3.75 (3H, s, OCH_3), 4.52 (1H, m, αH), 5.36 (1H, d, *J* 7.1 Hz, NH); δ_C (100.7 MHz, $CDCl_3$) 14.6 (q, $SCH_2\underline{C}H_3$), 26.5 (q, $S\underline{C}H_2CH_3$), 28.3 (q, $C(\underline{C}H_3)_3$), 33.9 (t, $C\underline{H}_2SCH_2CH_3$), 52.5 (q, OCH_3), 53.2 (d, αC), 80.1 (s, $(\underline{C}(CH_3)_3$), 155.1, 171.6 (2 x s, 2 x CO); HRMS (ES^+) Calcd. for $C_{11}H_{21}NaNO_4S$ $[MNa]^+$ 286.1089. Found: 286.1083; Found: C, 49.88%; H, 8.09%, N, 5.01%. $C_9H_{15}NO_4$ requires: C, 50.17%; H, 8.04%; N, 5.32%.

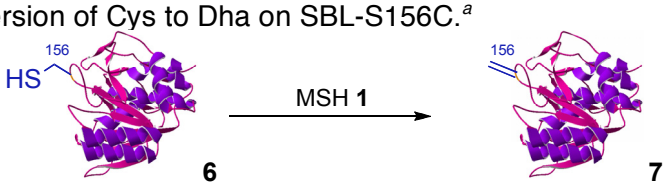
Protein modification

Sequence of SBL-S156C (BPN' numbering, PDB code for wild type SBL = 1GCI)

AQSVPWGISRVQAPAAHNRGLTGSGVKVAVLDTGISTHPDLNIRGGASFVPGEPST
QDGNGHGTHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWA
GNNGMHVANLSLGSPSPSATLEQAVNSATSRGVLVVAASGNCGAGSISYPARYAN
AMAVGATDQNNNRASFSQYGAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVA
GAAALVKQKNPSWSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR

Calculated average isotopic mass = 26714.5

Table S1. Conversion of Cys to Dha on SBL-S156C.[a]



6 SBL-S156C — SBL-C156Dha **7**

Entry	MSH (equiv)	Buffer	pH	time (min)	Conv. %
1	100	CHES (70mM), MES (5mM), $CaCl_2$ (2mM)	9.5	120	0
2	100	TRIS (50mM)	8.0	20	50
3	100	TAPS (50mM)	8.0	20	60
4	100	Carbonate (50mM)	8.0	20	50
5	60	Carbonate (100mM)	9.6	10	40
6	100	Carbonate (100mM)	9.6	120	50
7	20	Phosphate (50mM)	8.0	20	10
8	50	Phosphate (50mM)	8.0	20	20
9	100	Phosphate (50mM)	8.0	1	25
10	100	Phosphate (50mM)	8.0	10	60
11	**100**	**Phosphate (50mM)**	**8.0**	**20**	**>95**
12	100	Phosphate (50mM)	7.5	20	90
13	100	Phosphate (50mM)	6.5	20	40

[a] All reaction were carried out at 4 ºC.

General procedure for conversion of Cys156 to Dha156 on SBL

All manipulations were carried out in a cold room at 4 ºC. Lyophilized SBL-S156C **6** (2.5 mg, 0.094 μmol) was dissolved in 2.50 mL of pH 8.0 sodium phosphate buffer (50 mM) in a 1.5 mL plastic tube. A solution of MSH was prepared in a separate tube by dissolving 4.0 mg (18.6 μmol) in 250 μL DMF. 125 μL of the MSH solution (9.3 μmol) was added by micropipette to the protein solution and the reaction was vortexed periodically over 1 minute. The tube was left to shake for an additional 19 minutes after which time a 30 μL aliquot was analyzed by LC-MS. A single protein species was detected with a mass of 26681, corresponding to the mass of SBL-

C156Dha **7** (26681 = calculated mass). Small molecules were removed from the reaction mixture by loading the sample onto a PD10 desalting column (GE Healthcare) previously equilibrated with 10 column volumes of pH 8.0 sodium phosphate buffer (50 mM) and eluting with 3.50 mL of the same buffer. The collected sample (now diluted to 0.7 mg/mL) was split into 200 μL aliquots, flash frozen with liquid nitrogen, and stored at -80 ºC.

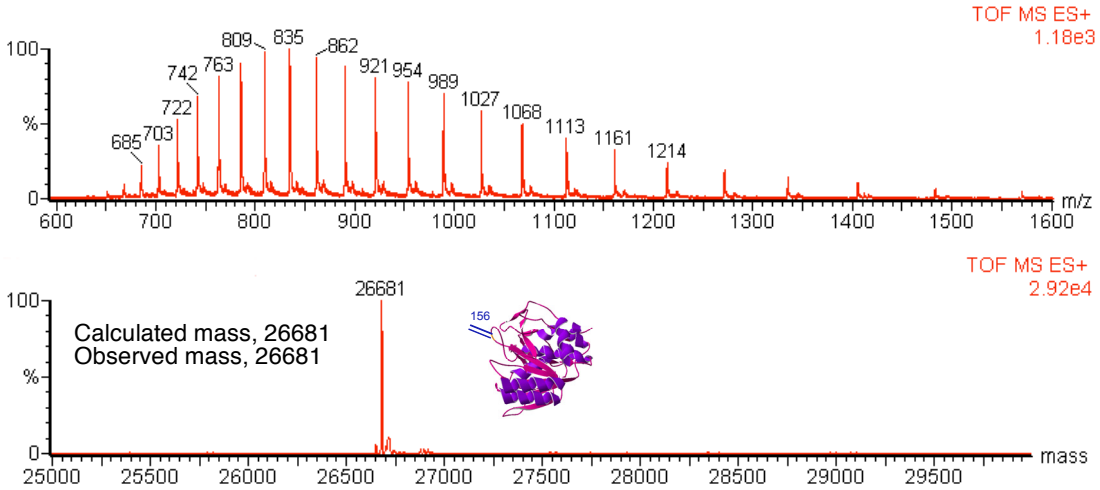
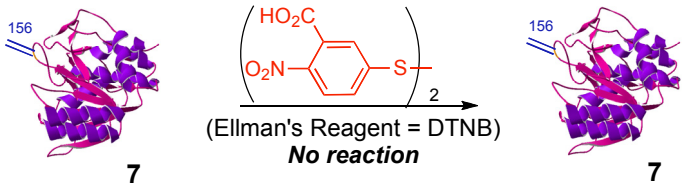


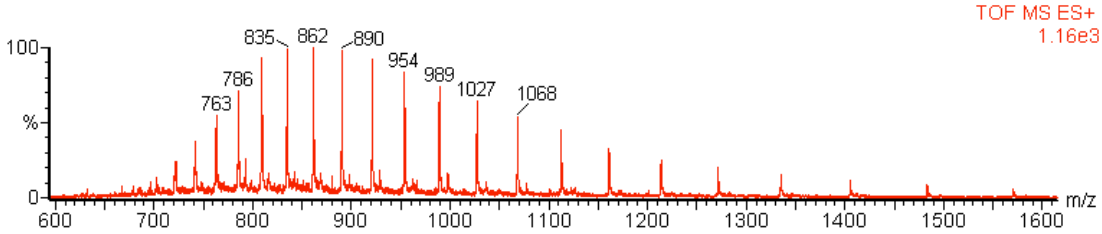
Figure S1 ESI-MS spectrum of SBL-C156Dha **7**.

Modified Ellman's assay

*Treatment of SBL-C156Dha **7** with Ellman's reagent*



A 200 μL aliquot of 0.7 mg/mL SBL-C156Dha **7** previously prepared was thawed and kept on ice until needed. Ellman's reagent (DTNB, 0.52 mg, 1.3 μmol, 250 eq) was added as a solid to the protein solution, vortexed and placed on a lab rotisserie for 20 min at room temperature. LC-MS analysis of a 50 μL aliquot revealed unchanged SBL-C156Dha **7** indicating absence of free thiol (calculated mass, 26681; observed mass, 26683).



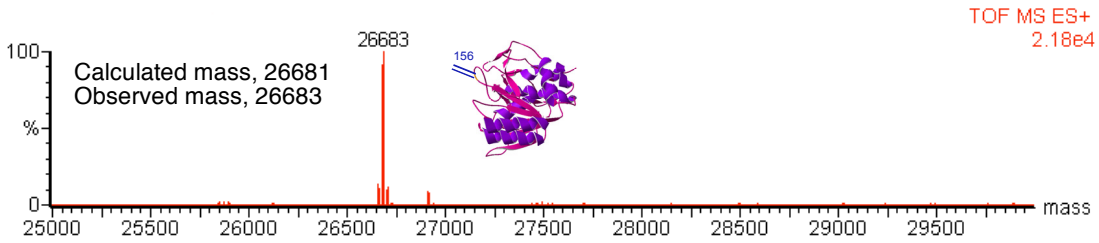
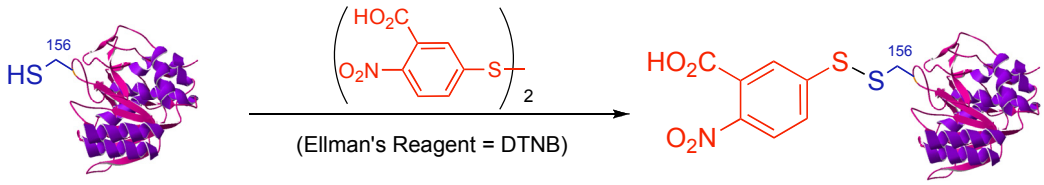


Figure S2 ESI-MS spectrum of **7** after treatment with Ellman's reagent.

*Treatment of SBL-S156C **6** with Ellman's reagent*



6 **21**

SBL-S156C **6** was prepared as a 1 mg/mL solution in 50 mM potassium phosphate buffer (pH 8.0) and 200 μL were added to a 1.5 mL plastic tube. In a separate tube, Ellman's reagent (DTNB, 1.2 mg, 3 μmol) was dissolved in 200 μL of the same buffer. A 50 μL aliquot of the Ellman's solution was added to the protein and the reaction vortexed to homogenize. The resulting yellow solution was then shaken at 4 ºC for 20 minutes. LC-MS analysis revealed full conversion to the Ellman adduct **21** (calculated mass, 26911; observed mass, 26911).

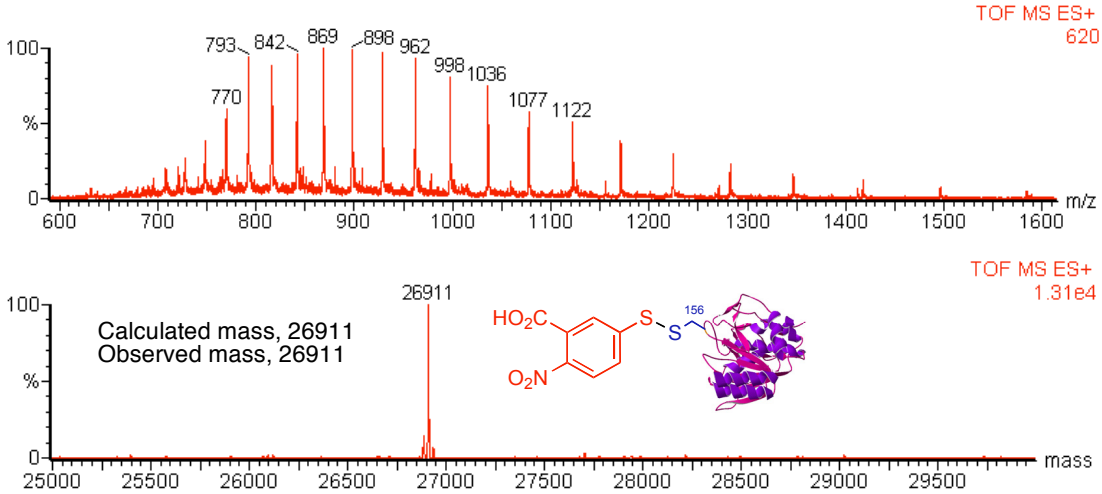
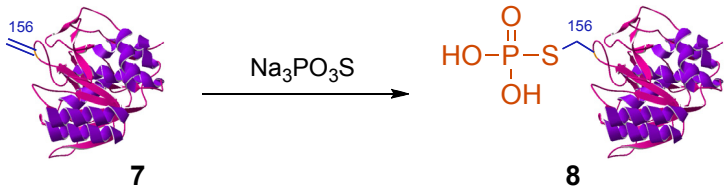


Figure S3 ESI-MS spectrum of SBL-S156C-SS-"Ellman" **21**.

Conjugate addition of sulfur nucleophiles to SBL-C156Dha 7

*Conjugation of sodium thiophosphate to SBL-C156Dha **7***



7 **8**

All manipulations were carried out in a cold room maintained at 4 ºC. A 250 μL aliquot of 1.0 mg/mL SBL-C156Dha **7** (pH 8.0 sodium phosphate, 50 mM) previously prepared was thawed and kept on ice until needed. Sodium thiophosphate (8.4 mg, 0.05 mmol) was added as a solid along with 100 μL of pH 8.0 sodium phosphate buffer (50 mM). The reaction was vortexed to homogenize and then shaken at 4 ºC for 1 h. LC-MS analysis of the reaction mixture revealed complete conversion to SBL-156phosphocysteine **8** (calculated mass, 26795, observed mass, 26794).

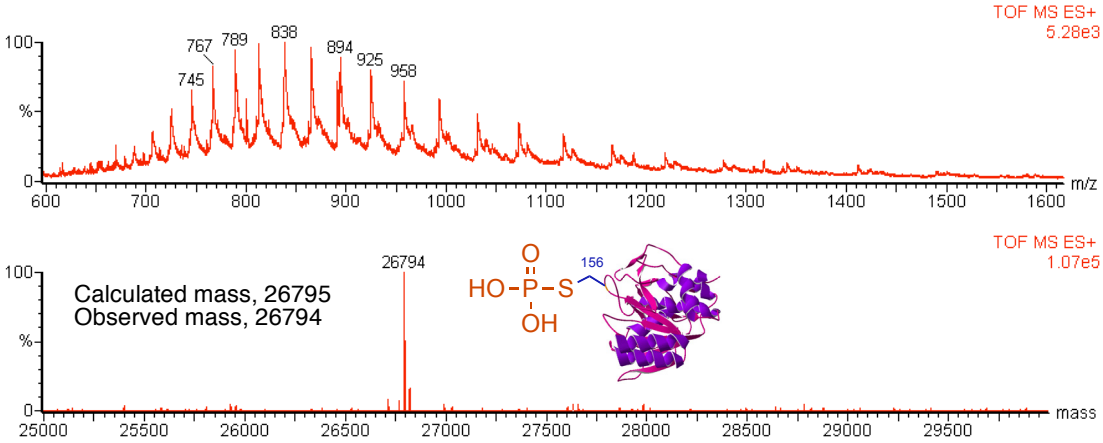
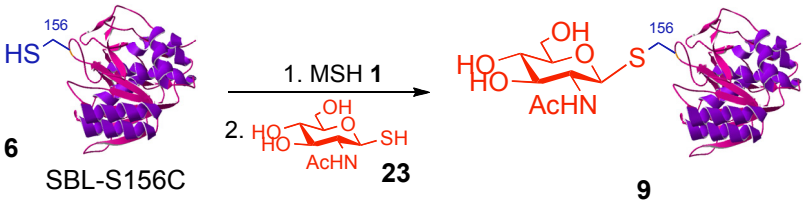


Figure S4 ESI-MS spectrum of SBL-C156SPO_3H_2 **8**.

*One-Pot Conversion of SBL-S156C **6** to SBL-C156SGlcNAc **9***



6 SBL-S156C **23** **9**

All manipulations were carried out in a cold room maintained at 4 ºC. A 1 mg sample of lyophilized SBL-S156C **6** (0.037 μmol) was dissolved in 1.0 mL in pH 8.0 sodium phosphate buffer (50 mM). A solution of MSH **1** was prepared by dissolving 1.8 mg (8.36 μmol) in 100 μL DMF. A 50 μL portion of the MSH solution was added to the protein by micropipette. The reaction was vortexed periodically over 1 minute and

then rotated on a lab rotisserie for an additional 19 minutes at 4 ºC. A 50 μL aliquot was analyzed by LC MS to confirm the conversion of Cys156 to Dha156 (26681 calculated, 26681 found). To the reaction mixture was added 2-acetamido-2-deoxy-1-thio-β-D-glucopyranose **23** as a solid (8.8 mg, 1000 eq) to give a 39 mM solution in thiol. After 90 minutes of shaking at 4 ºC, the reaction was analyzed directly by LC-MS. Complete conversion to SBL-C156SGlcNAc **9** was observed (calculated mass, 26918; observed mass, 26918).

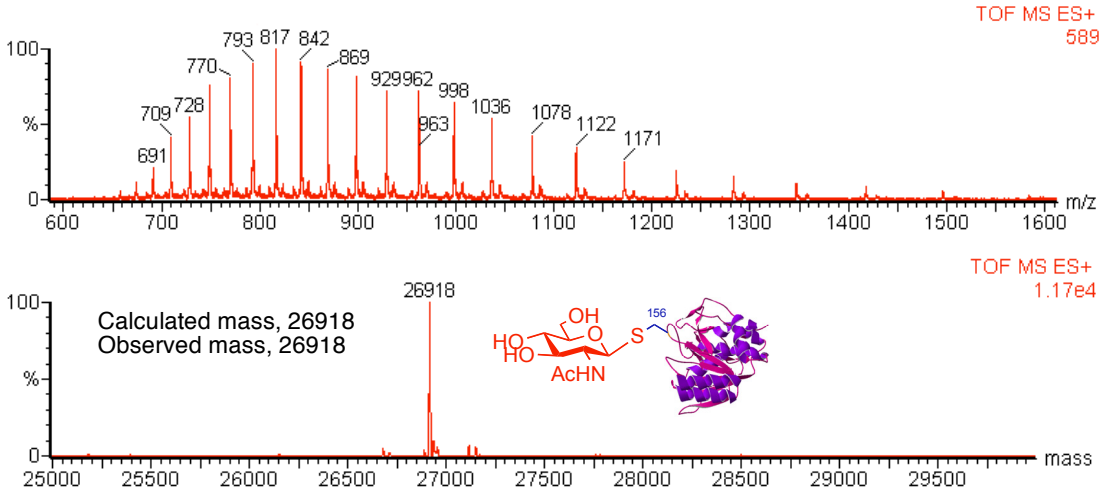
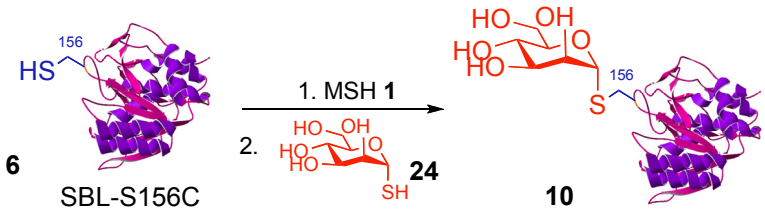
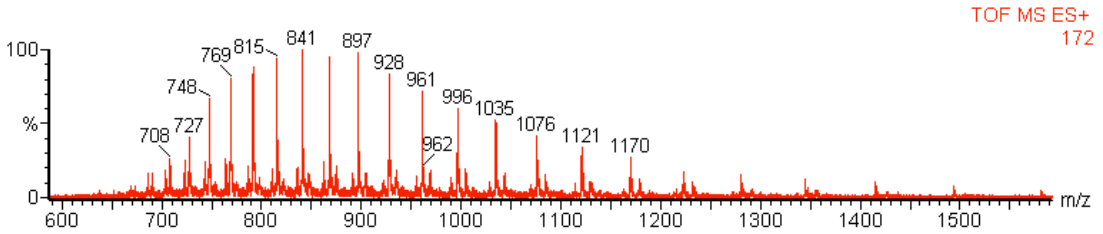


Figure S5 ESI-MS spectrum of SBL-C156SGlcNAc **9**.

*One-Pot Conversion of SBL-S156C **6** to SBL-C156SMan **10***



An analogous procedure to that above was followed for the conversion of SBL-S156C **6** to SBL-C156SMan **10**. LC-MS analysis revealed full conversion to the desired glycoprotein **10** (calculated mass, 26877; observed mass, 26877).



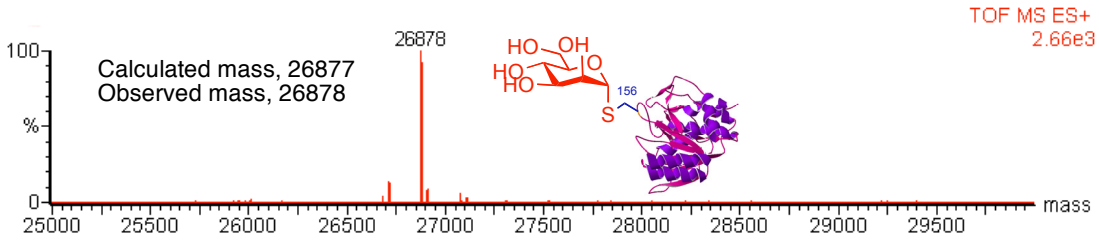
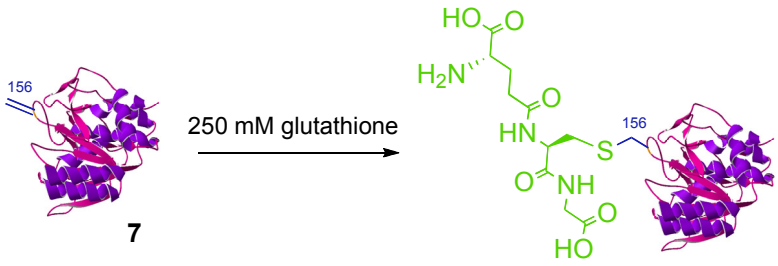


Figure S6 ESI-MS spectrum of SBL-C156SMan **10**.

*Conjugation of glutathione to SBL-C156Dha **7***



7 **11**

All manipulations were carried out in a cold room maintained at 4 ºC. A 200 μL aliquot of 0.7 mg/mL SBL-C156Dha **7** previously prepared was thawed and kept on ice until needed. Glutathione (GSH) (16.1 mg, 0.05 mmol) and potassium phosphate dibasic (46 mg) were both added as solids to a 1.5 mL plastic tube and dissolved in 150 μL water (MilliQ). The solution of GSH was then added to the protein solution (pH of reaction 9.0) and vortexed over 1 min. The reaction was shaken for an additional 90 minutes. LC-MS analysis of the reaction mixture revealed near complete conversion to SBL-C156SGSH **11** (calculated mass, 26988, observed mass, 26987).

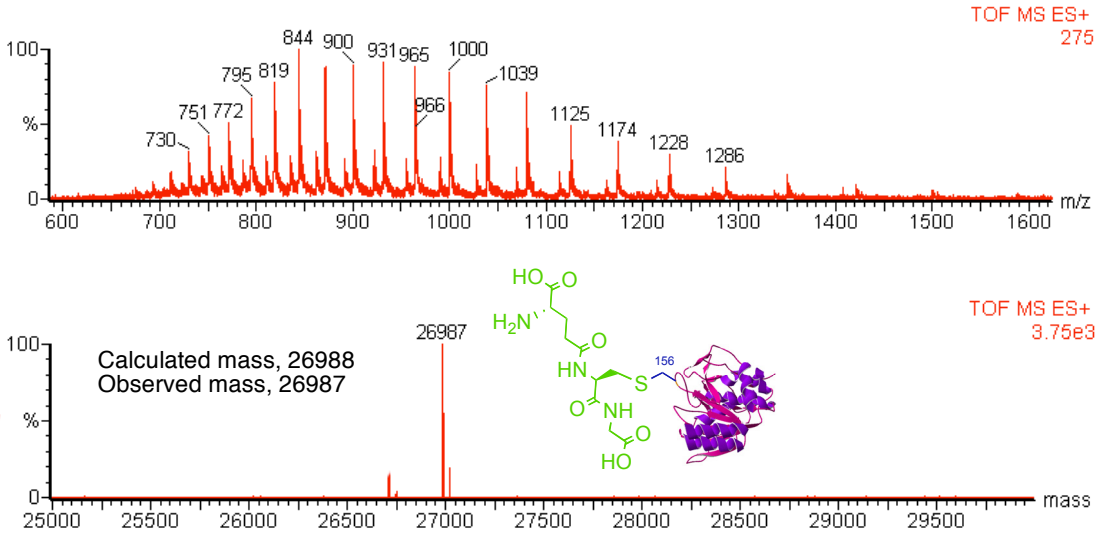
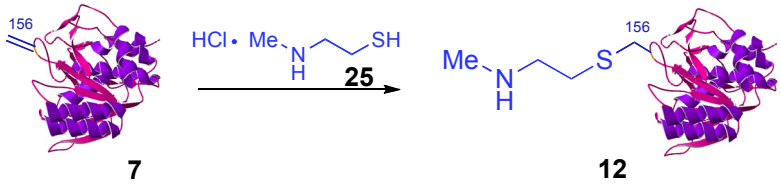


Figure S7 ESI-MS spectrum of SBL-C156SGSH **11**.

*Monomethyl lysine analog **12***



7 **12**

All manipulations were carried out in a cold room maintained at 4 ºC. A 200 μL aliquot of 0.7 mg/mL SBL-C156Dha **7** previously prepared was thawed and kept on ice until needed. A solution of 2-(methylamino)ethanethiol hydrochloride **25** was prepared by dissolving 6.6 mg (0.052 mmol) in 200 μL water (MilliQ). Potassium phosphate dibasic (45 mg, 0.26 mmol) was added to the thiol solution as a solid and the solution vortexed. All of the thiol solution was transferred to the protein by micropipette to give a reaction mixture of pH 9.0. The reaction was vortexed and then shaken at 4 ºC for 90 minutes. LC-MS analysis revealed full conversion to the monomethyl lysine analog **12** (calculated mass, 26772; observed mass 26773).

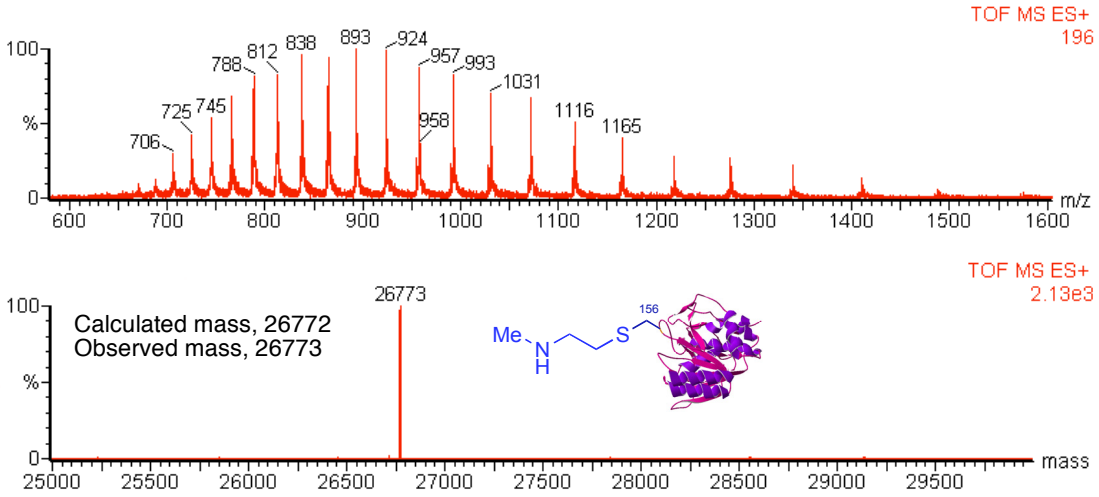
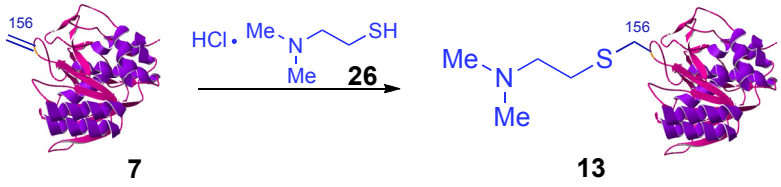


Figure S8 ESI-MS spectrum of **12**.

*Dimethyl lysine analog **13***



7 **13**

All manipulations were carried out in a cold room maintained at 4 ºC. A 200 μL aliquot of 0.7 mg/mL SBL-C156Dha **7** previously prepared was thawed and kept on ice until needed. A solution of 2-(dimtheylamino)ethanethiol hydrochloride **26** was prepared by dissolving 3.2 mg (0.022 mmol) in 150 μL of pH 8.0 phosphate buffer

(50 mM). A 50 μL aliquot of the thiol solution was added to the protein and the reaction was shaken at 4 ºC for 90 minutes at which time a 40 μL aliquot was taken for LC-MS analysis. Full conversion to the desired dimethyl lysine analog **13** was observed (calculated mass, 26786; observed mass, 26787).

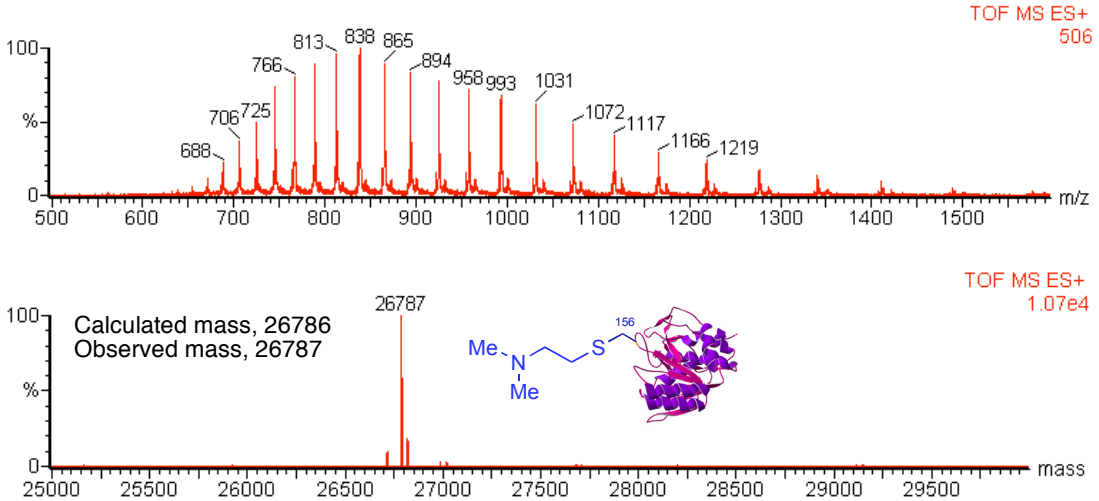
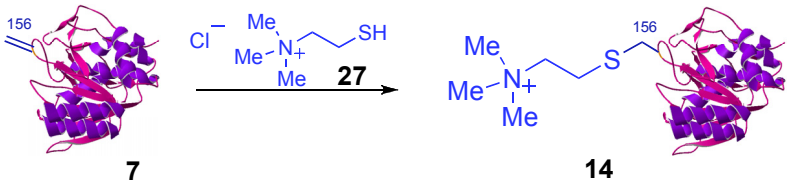


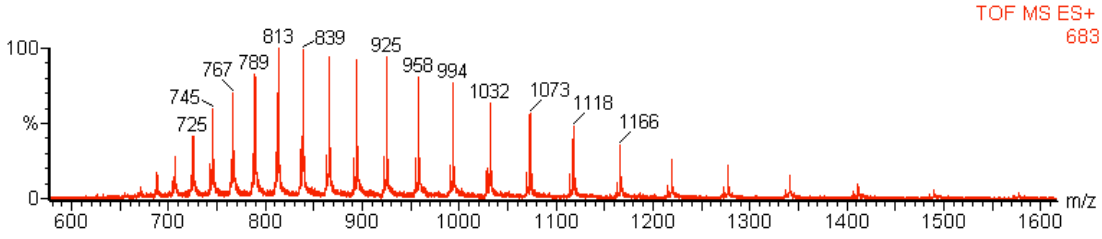
Figure S9 ESI-MS spectrum of **13**.

*Trimethyl lysine analog **14***



7 **14**

All manipulations were carried out in a cold room maintained at 4 ºC. A 200 μL aliquot of 0.7 mg/mL SBL-C156Dha **7** previously prepared was thawed and kept on ice until needed. A solution of 2-(mercaptoethyl)trimethylammonium chloride **27** was prepared by dissolving 8.2 mg of **27** (0.052 mmol) and 27 mg potassium phosphate dibasic (0.20 mmol) in 200 μL of water (MilliQ). All of the thiol solution was added to protein to give a reaction mixture at pH 9.0. The reaction was shaken at 4 ºC for 90 min before a 50 μL aliquot was analyzed by LC-MS. Full conversion to the trimethyl lysine analog **14** was observed (calculated mass, 26801; observed mass, 26801).



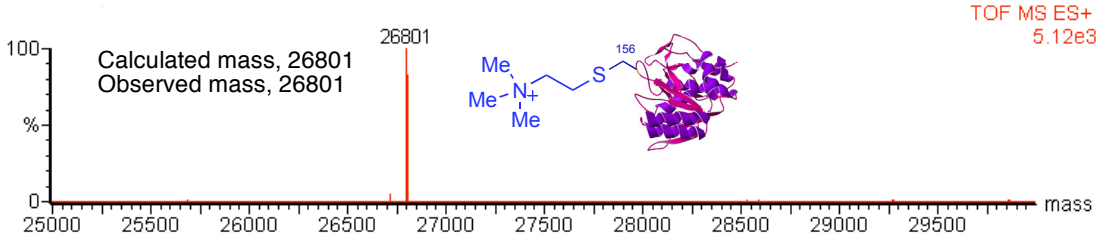
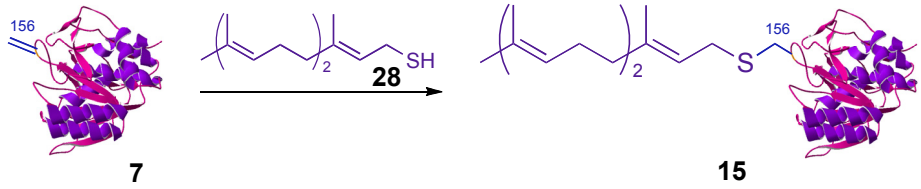


Figure S10 ESI-MS spectrum of **14**.

*SBL-C156Farnesyl **15***



A 200 μL aliquot of 0.7 mg/mL SBL-C156Dha **7** previously prepared was thawed. A (0.35 M) farnesyl thiol **28** solution in DMSO was prepared alongside an aqueous solution of TCEP•HCl (tris(2-carboxyethyl) phosphine chloride). The TCEP was neutralized to pH 7.0 with sodium hydroxide to give a final concentration of 0.20 M TCEP. The farnesyl thiol **28** (15 μL) and TCEP solution (52 μL) were added in succession to the protein to give a cloudy emulsion. The reaction was rotated on a lab rotisserie for 90 minutes at room temperature and then analyzed directly by LC-MS. A protein species with a mass of 26940 was found which corresponds to the farnesyl thioether sodium adduct **15** (calculated mass, 26941).

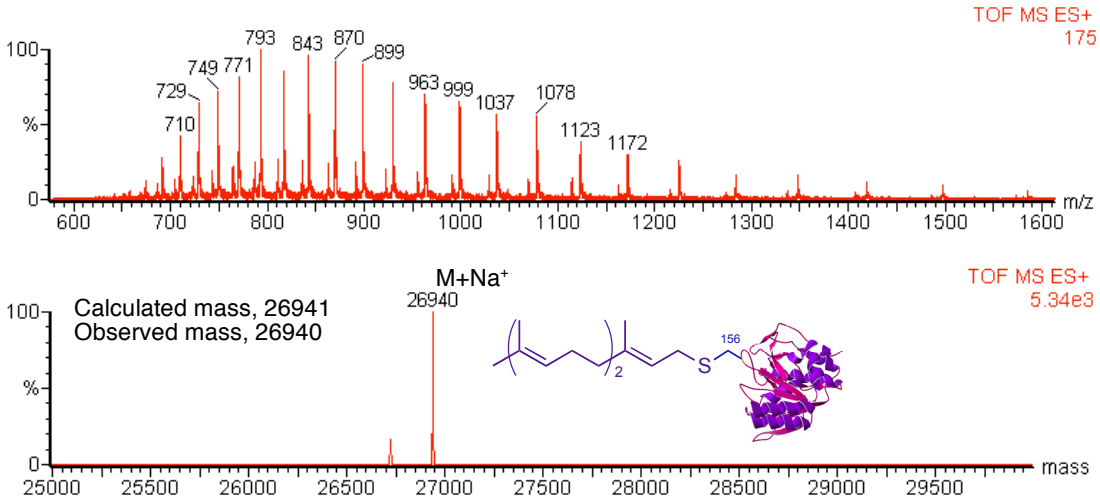


Figure S11 ESI-MS spectrum of SBL-C156SFarnesyl **15**.

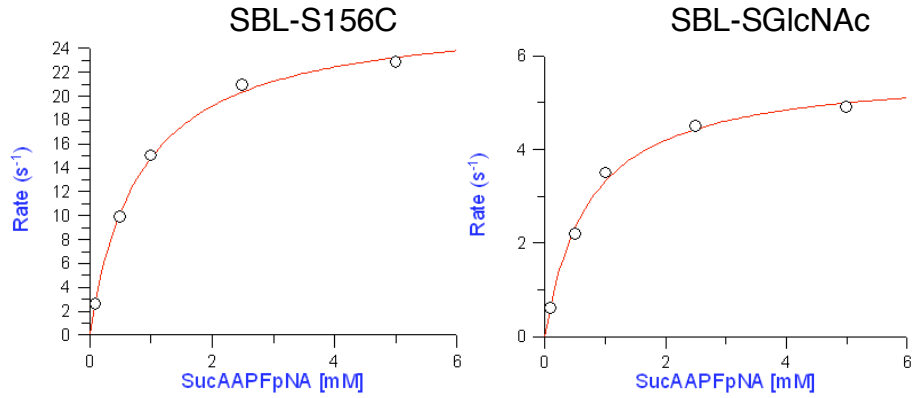
Activity assay for SBL-SGlcNAc 9

Determination of protein concentration

The enzyme concentration was determined using the bicinchoninic acid protein assay (Pierce) with bovine serum albumin as a standard. Turnover numbers are based on an enzyme monomer.

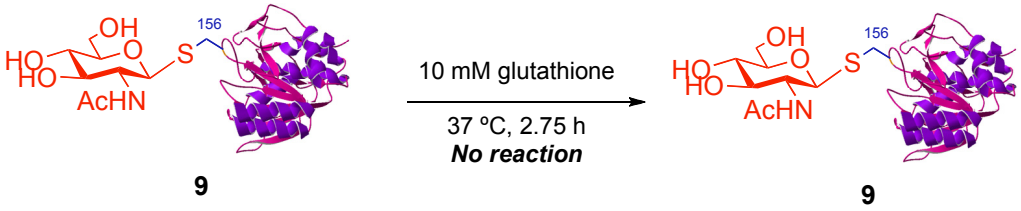
Measurement of Enzyme Activity

Initial velocities for SBL-S156C **6** and SBL-SGlcNAc **9** were determined using suc-AAPF-pNA (Bachem Biosciences Inc) with continuous detection of the formation of the product pNA at 410 nm (pNA: ε = 8,800 M^{-1} cm^{-1}) at 25°C. A typical reaction mixture contained 100 mM sodium phosphate, pH 7.5, 500 mM NaCl, 1 mM suc-AAPF-pNA in a final volume of 1 ml. Reactions were initiated by the addition of enzyme, typically 15 nM final concentration. Initial velocity kinetic data were fitted using GraFit 5.

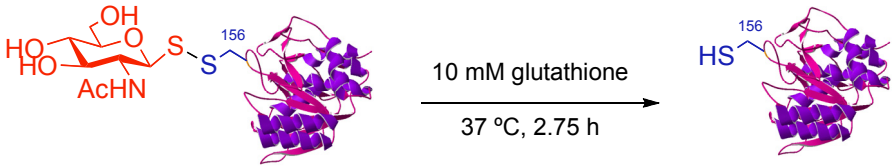


SBL	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})
SBL-S156C **6**	0.83 ± 0.07	27.1 ± 0.7	(3.3 ± 0.1) × 10^4
SBL-SGlcNAc **9**	0.72 ± 0.09	5.7 ± 0.2	(7.9 ± 0.3) × 10^3

Demonstration of Chemical Stability of S-Glycoprotein to Glutathione

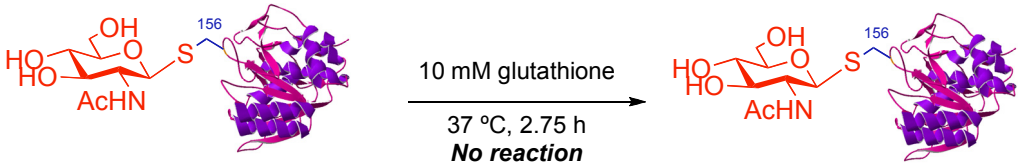


9 **9**



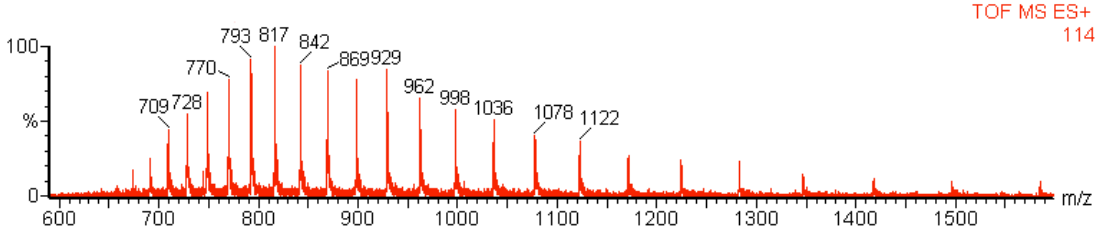
22 **6**

Thioether SBL-GlcNAc **9** Incubation with Glutathione



9 **9**

Thioether-linked GlcNAc-SBL **9** was prepared as described above. The product protein was purified by loading the sample in 2.50 mL of pH 8.0 sodium phosphate buffer (50 mM) onto a PD10 desalting column (GE Healthcare) previously equilibrated with 10 column volumes of pH 8.0 sodium phosphate buffer (50 mM) and eluting with 3.50 mL of the same buffer. The collected sample was concentrated to 1 mg/L using 10 kDa molecular weight cutoff Vivaspin column. A 250 μL aliquot of the SBL-C156SGlcNAc **9** was added to a 1.5 mL plastic tube and diluted to 500 μL with pH 8.0 sodium phosphate buffer (50 mM). Glutathione (1.5 mg, 5.0 μmol) was added as a solid to the tube (GSH concentration = 10 mM) and the mixture incubated in a 37 °C shaker for 2.75 hours. A 40 μL aliquot was analyzed by LC-MS to show that the thioether-linked SBL-GlcNAc **9** was still intact and the only protein species detected. (calculated mass, 26918, observed mass, 26918).



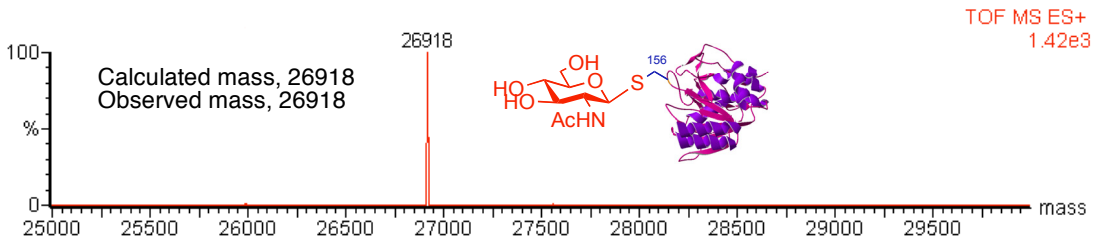
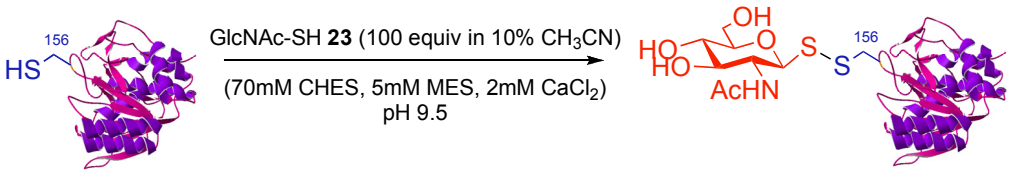


Figure S12 ESI-MS spectrum of SBL-C156SGlcNAc **9** after incubation with GSH.

Preparation of SBL-C156SSGlcNAc **22**



6 **22**

SBL-S156C **6** (3.0 mg, 0.11μmol) was added to a 1.5 mL plastic tube as a lyophilized solid and dissolved in 1.0 mL buffer comprised of 70 mM CHES, 5.0 mM MES, and 2.0 mM $CaCl_2$ (pH 9.5). 1-Thio-2-acetamido-2-deoxy-β-D-glucopyranose **23** (2.7 mg, 11μmol) was added as a solid to the protein solution along with 200 μL acetonitrile. The reaction was vortexed to homogenize and rotated on a lab rotisserie for 1 h. LC-MS analysis of a 50 μL aliquot of the reaction mixture revealed complete conversion to the corresponding disufide (SBL-SS-GlcNAc) **22** (calculated mass, 26949; observed mass 26951).

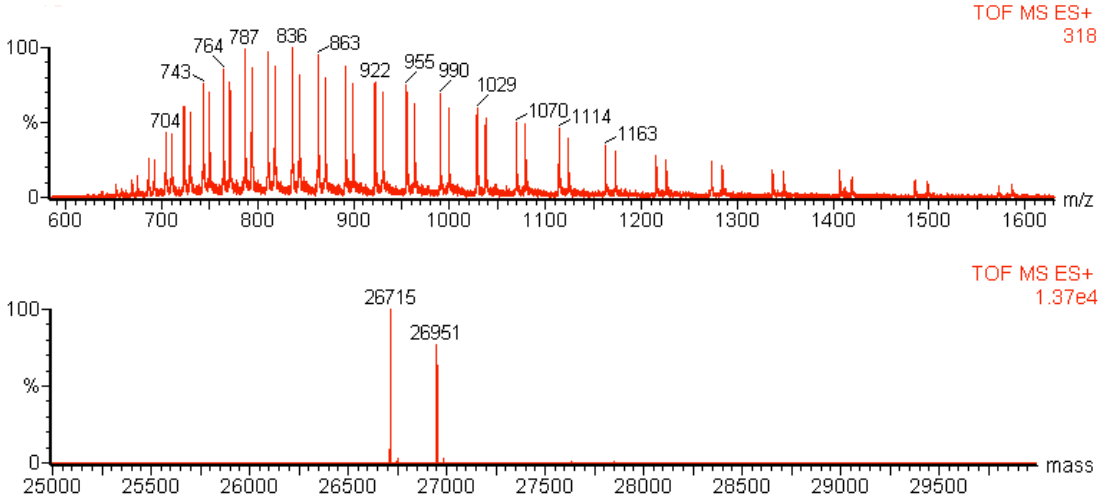


Figure S13 ESI-MS spectrum from reaction of SBL-S156C **6** with GlcNAc-SH **23** after 30 min.

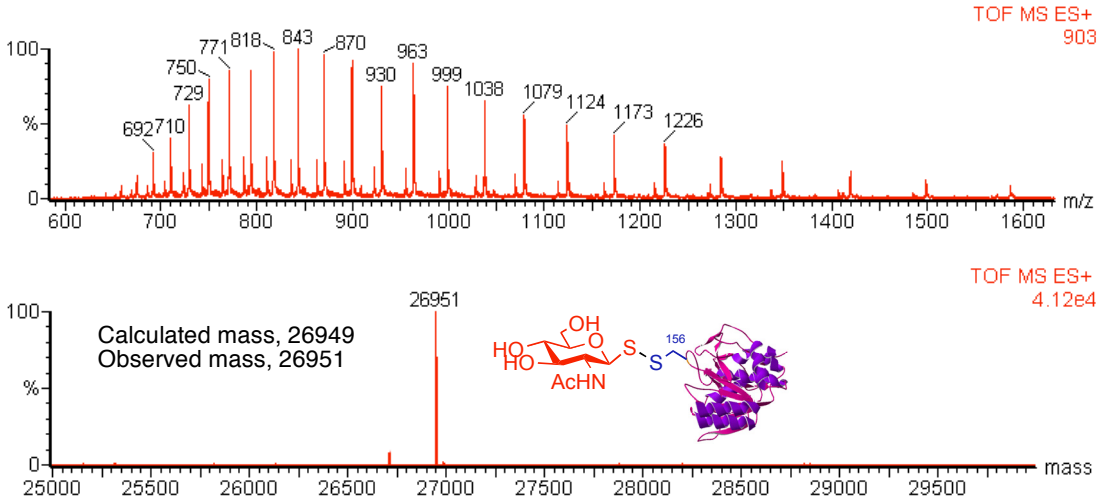
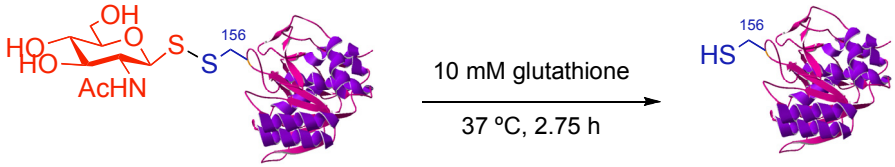


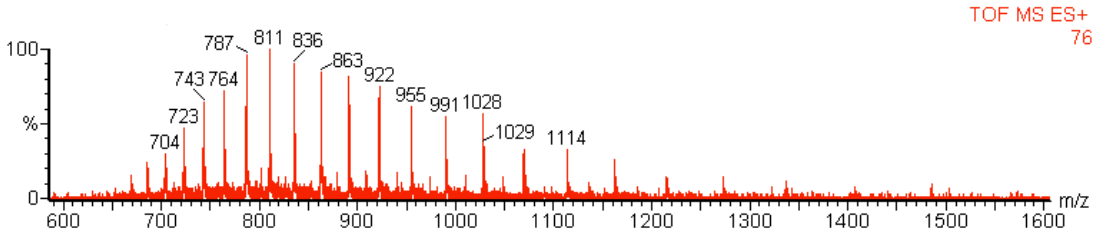
Figure S14 ESI-MS spectrum from reaction of SBL-S156C **6** with GlcNAc-SH **23** after 1 h.

Disulfide SBL-GlcNAc **22** Incubation with Glutathione



22 **6**

SBL-C156SSGlcNAc **22** was prepared as described above. A 500 μL solution of the disulfide-linked SBL-GlcNAc **22** was prepared at a concentration of 0.5 mg/mL in pH 8.0 sodium phosphate buffer (50 mM) and added to a 1.5 mL plastic tube along with glutathione (1.5 mg, 5 μmol). The GSH concentration was 10 mM. Incubating this mixture for 2.75 h in a 37 ºC shaker led to reduction of the disulfide as the only protein species detected by LC-MS analysis was SBL-S156C **6** (Calculated mass, 26714, observed mass, 26716).



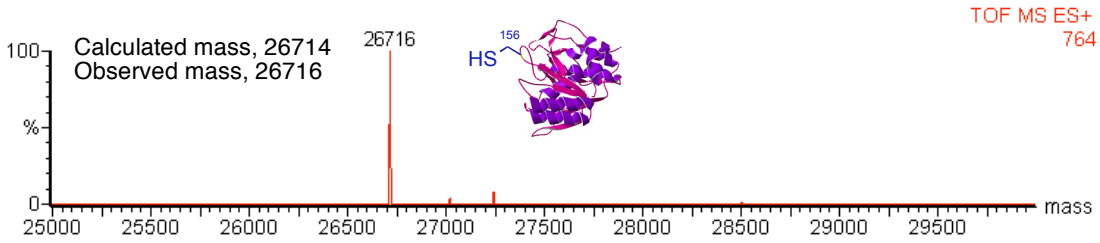
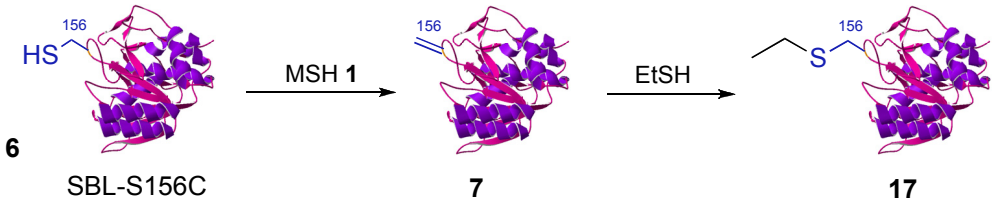


Figure S15 ESI-MS spectrum of SBL-S156C **6**, formed from incubation of SBL-S156SSGlcNAc **22** with GSH.

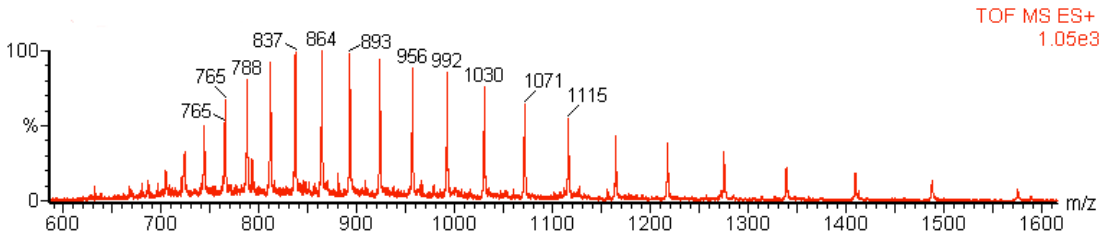
Functional Switch: regeneration of Dha from SEt-Cys on SBL

Chemical incorporation SEt-Cys



6 SBL-S156C **7** **17**

A fresh sample of SBL-156Dha **7** was prepared as described above by the action of MSH on cysteine and used directly. Accordingly, 35 μL of ethanthiol was added directly to a 625 μL of a 1 mg/mL solution of SBL-156Dha **7** (0.05 μmol) in 50 mM sodium phosphate (pH 8.0). The sample was vortexed to homogenize and then rotated for 30 minutes at room temperature. LC-MS analysis of the reaction mixture showed full conversion to the ethyl thioether protein **17** (calculated mass, 26743, observed mass, 26746). The reaction mixture was passed through a PD10 column to remove the bulk of small molecules, eluting with pH 8.0 sodium phosphate buffer (50 mM) and then purified twice by dialysis against 4 L of the same buffer to remove remaining small molecules. After dialysis the sample concentration was ~0.36 mg/mL.



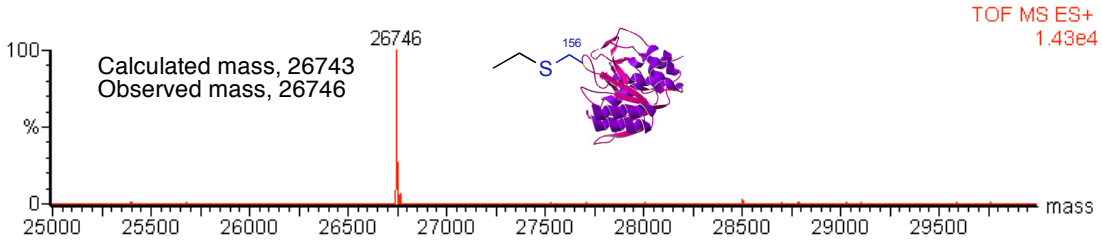
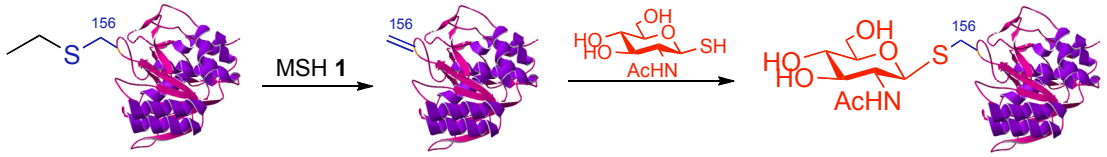


Figure S16 ESI-MS spectrum of SBL-C156SEt **17**.



17 **7** **9**

A 500 μL sample of SBL-156SEt **17** was thawed and kept on ice until needed. An MSH solution (1.8 mg, 8 μmol) was prepared in DMF (720 μL) and 14 μL (0.16 μmol) was added to a 250 μL sample of SBL-156SEt **17**. The reaction was vortexed to homogenize and then shaken for 20 minutes at 4 ºC. A 40 μL aliquot was taken for LC-MS analysis that showed full conversion to SBL-C156Dha **7** (calculate mass, 26681; observed mass, 26685). To verify that this material corresponds to the dehydroalanine containing protein, GlcNAc-SH **23** (4 mg, 16.9 μmol) was added as a solid to the reaction mixture and rotated at room temperature for 30 min. Full conversion to SBL-C156SGlcNAc **9** confirmed the regeneration of dehydroalanine (calculated mass, 26918; observed mass, 26920).

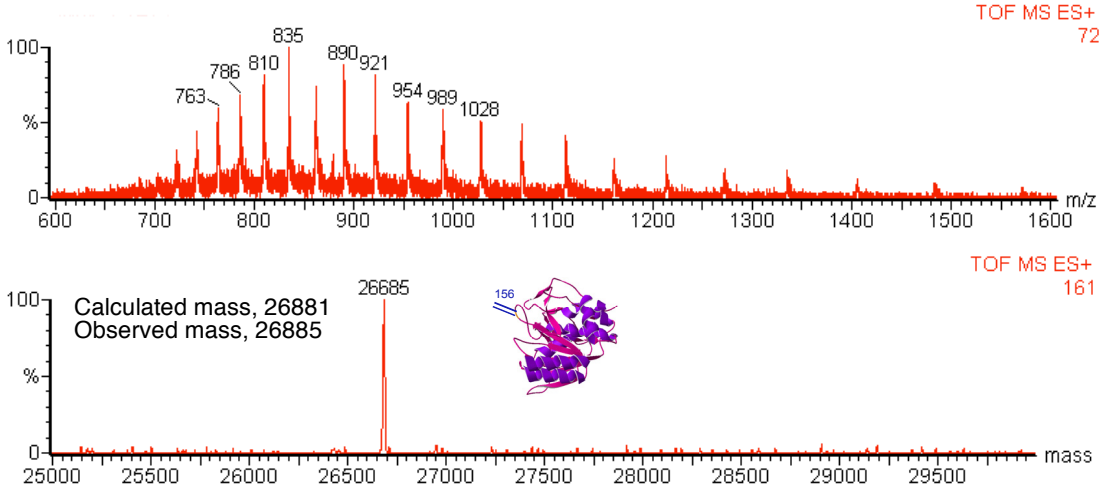


Figure S17 ESI-MS spectrum of SBL-C156Dha **7**, formed from reaction of SBL-C156SEt **17** with MSH **1**.

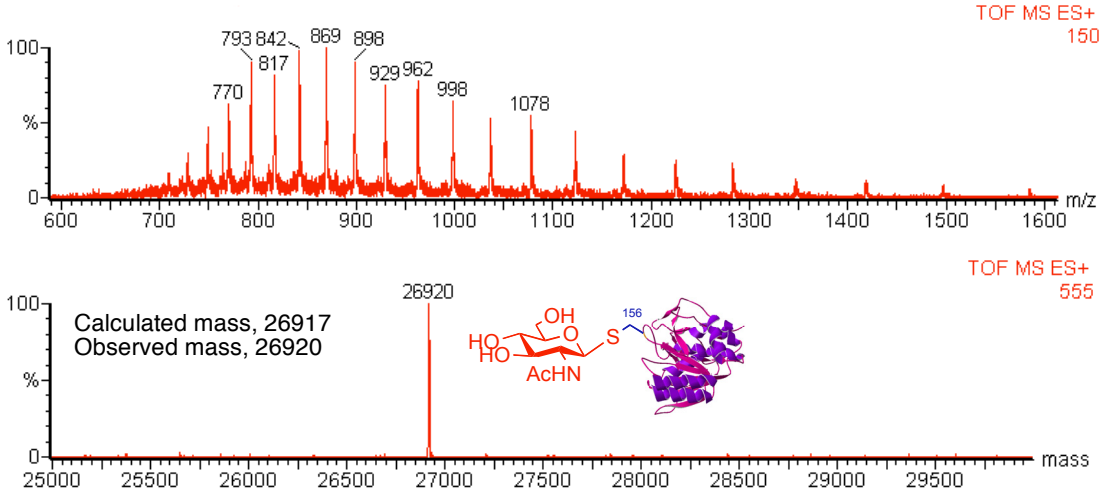
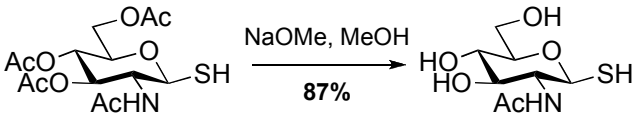


Figure S18 ESI-MS spectrum of SBL-C156GlcNAc **9**.

Substrate Synthesis for Protein Modification

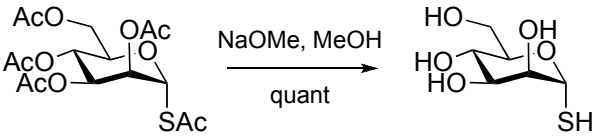
1-Thio-2-acetamido-2-deoxy-β-D-glucopyranose 23



29 **23**

1-Thio-3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranose[6] **29** (0.66 g, 1.83 mmol) was dissolved in anhydrous methanol (10 mL) and sodium methoxide (0.32 g, 5.96 mmol) was added. After 30 min, TLC (ethyl acetate) indicated complete consumption of starting material (R_f 0.2). Ion exchange resin (DOWEX H^+ form 50WX8-200) was added portionwise until the solution was neutralized, at which point the reaction mixture was concentrated *in vacuo*. Recrystallization from methanol/ethyl acetate yielded 1-thio-2-acetamido-2-deoxy-β-D-glucopyranose **23** (0.38 g, 87%) as a white crystalline solid; m.p. 174-176 ºC (methanol/ethyl acetate) [Lit. 177-179 ºC (methanol/ethyl acetate)][6]; $[\alpha]_D^{21}$ -12.1 (c, 1 in MeOH) [Lit. $[\alpha]_D^{22}$ -10.4 (c, 1 in MeOH)][6]; δ_H (400 MHz, CD_3OD) 2.00 (3H, s, HNC(O)C$\underline{H}_3$), 3.27-3.37 (2H, m, H-4, H-5), 3.41 (1H, at, *J* 9.0 Hz, H-3), 3.65 (1H, dd, $J_{5,6}$ 5.6 Hz, $J_{6,6'}$ 11.9 Hz, H-6), 3.87 (1H, dd, $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 12.0 Hz, H-6'), 4.56 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 8.15 (1H, d, $J_{\underline{N}H,H\text{-}2}$ 8.8 Hz, $\underline{H}$NC(O)CH_3); δ_C (100.7 MHz, CD_3OD) 21.9 (q, HNC(O)$\underline{C}H_3$), 58.9 (d, C-2), 61.8 (t, C-6), 70.8 (d, C-4), 76.0 (d, C-3), 79.9 (d, C-1), 81.5 (d, C-5), 172.8 (s, HN$\underline{C}$(O)CH_3); *m/z* (ES^-) 236 $[M\text{-}H]^+$.

1-Thio-α-D-mannopyranose 24

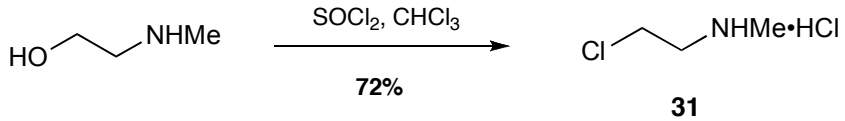


30 **24**

To a stirred solution of 1-*S*-acetyl-2,3,4,6-tetra-*O*-acetyl-1-α-thio-D-mannopyranose[7] **30** (200 mg, 0.49 mmol) in anhydrous methanol (4 mL), sodium methoxide (54 mg, 0.98 mmol) was added. After 1 h, TLC (petrol:ethyl acetate, 1:1) indicated the formation of a product (R_f 0). Ion exchange resin (DOWEX H^+ form 50WX8-200) was added portionwise until the solution was neutralized, at which point the reaction mixture was concentrated *in vacuo* to yield 1-thio-α-D-mannopyranose **24** (96 mg, 100%) as an oil; $[\alpha]_D^{22}$ +73.4 (c, 1.0 in MeOH) [Lit. $[\alpha]_D^{20}$ +76.1 (c, 1 in MeOH)][7];

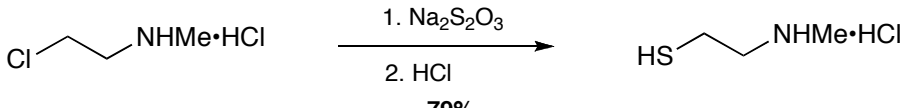
υ_{max} (thin film) 3375 (br, OH) 2532 (br, SH) cm^{-1}; δ_H (400 MHz, CD_3OD) 2.72 (1H, d, $J_{1,SH}$ 7.4 Hz, SH), 3.65 (1H, at, J 9.7 Hz, H-4), 3.69-3.85 (3H, m, H-5, H-6, H-6'), 3.92 (1H, dd, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.3 Hz, H-2), 4.05 (1H, dd, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 9.7 Hz, H-3), 5.51 (1H, d, $J_{1,2}$ 1.6 Hz, H-1); δ_C (100.7 MHz, CD_3OD) 61.7 (t, C-6), 67.7 (d, C-4), 71.0 (d, C-5), 74.0 (d, C-2), 74.4 (d, C-3), 79.9 (d, C-1); *m/z* (ES^-) 195 $[M-H]^+$; HRMS (ES^+) Calcd. for $C_6H_{12}NaO_5S$ $[M+Na]^+$ 219.0298. Found: 219.0304.

2-(Methylamino)ethanethiol hydrochloride 25



31

2-(Methylamino)-ethanol (7.51 g, 100 mmol) was added to a 250 mL round bottom flask equipped with a Teflon coated stir bar along with chloroform (50 mL). The flask was then equipped with a 100 mL addition funnel and a solution of thionyl chloride (9.43 mL, 130 mmol) in chloroform was added to the funnel. The stirred solution was cooled to 0 ºC and the thionyl chloride was added dropwise over 25 minutes. After the addition was complete, the ice bath was removed and the addition funnel replaced by a condenser. The reaction was heated to reflux (oil bath temp = 70 ºC) and the white slurry gradually changed to a yellow solution. After 3 h, the reaction was cooled to room temperature and the solvent removed to give orange crystals which were recrystallized from acetone/ethanol to give large, clear flakes (9.38 g, 72%); m.p. 109-110 ºC [Lit. 115-117 ºC (acetone)][8]; υ_{max} (KBr disc) 1872, 1586, 1469, 1145 cm^{-1}; δ_H (400 MHz, CD_3OD) 2.77 (3H, s, NCH_3), 3.44 (2H, t, J 5.6 Hz, $C\underline{H}_2NCH_3$), 3.93 (2H, t, J 5.6 Hz, CH_2Cl); δ_C (100.7 MHz, CD_3OD) 32.7 (q, NCH_3), 39.3 (t, $\underline{C}H_2NCH_3$), 50.5 (t, CH_2Cl).

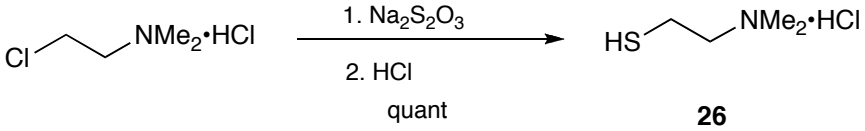


31 **25**

(2-Chloroethyl)methylamine hydrochloride **31** (2.00 g, 15.38 mmol) and sodium thiosulfate pentahydrate (3.82 g, 15.38 mmol) were added to a 100 mL round bottom flask and dissolved in 20 mL of water (MilliQ). The flask was equipped with a condenser and heated to reflux and stirred for 26 h (oil bath temp = 120 ºC). The reaction was then cooled to room temperature and the solvent removed under reduced pressure to give a white residue. The crude Bunte salt was dissolved in

hydrochloric acid (50 mL of a 6M aqueous solution) and heated at 85 ºC for 3 h. The solvent was removed by rotary evaporation to give a white residue. DCM (100 mL) and ethanol (15 mL) were added to the residue to extract the product. Sodium chloride and sulfate salts were removed by filtration and the filtrate was concentrated to give **25** as an extremely hygroscopic residue (1.55 g, 79%); υ_{max} (thin film) 1609, 1467, 1186, 1047, 860, 582 cm^{-1}; δ_H (400 MHz, CD_3OD) 2.75 (3H, s, NCH_3), 2.85 (2H, t, *J* 7.0 Hz, $C\underline{H}_2SH$), 3.22 (2H, t, *J* 7.0 Hz, $C\underline{H}_2NCH_3$); δ_C (100.7 MHz, CD_3OD) 21.1 (t, CH_2SH), 33.8 (q, NCH_3), 52.9 (t, $\underline{C}H_2NCH_3$).

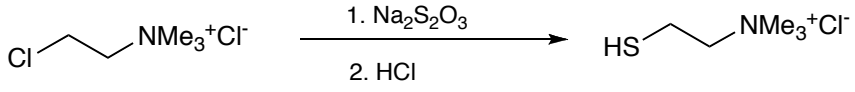
2-(Dimethylamino)ethanethiol hydrochloride 26



26

(2-Chloroethyl)dimethylamine hydrochloride (3.00 g, 20.83 mmol) and sodium thiosulfate pentahydrate (5.17 g, 20.82 mmol) were added to a 100 mL round bottom flask equipped with a Teflon coated stir bar and dissolved in water (30 mL, MilliQ). The flask was equipped with a condenser, heated to reflux (oil bath temp = 120 ºC), and stirred for 10 h. The reaction was cooled to room temperature and the solvent removed by rotary evaporation to give the crude Bunte salt. The residue was dissolved in hydrochloric acid (30 mL of a 6M aqueous solution) and heated to 85 ºC. After stirring for 2 h, the reaction was cooled and the solvent removed. The product was extracted with a mixture of DCM (60 mL) and ethanol (10 mL). Sodium chloride and sulfate salts were removed by filtration and the solvent removed to give 2.95 g (quant) of **26** as a highly hygroscopic residue, which could be used for protein modifications without further purification. 2-(Dimethylamino)ethanethiol hydrochloride **26** could be obtained as a hygroscopic white solid by extraction with boiling DCM and subsequent decantation and evaporation, though the limited solubility in DCM alone leads to diminished yields; m.p. 188-190 ºC then decomposition (started observations on hot block above 110 ºC to prevent absorption of water); υ_{max} (KBr disc) 2963, 2713, 1633, 1476 cm^{-1}; δ_H (400 MHz, CD_3OD) 2.89-2.94 (8H, m, includes $N(CH_3)_2$, s, and $C\underline{H}_2SH$), 3.36 (2H, t, *J* 7.1 Hz, $C\underline{H}_2N(CH_3)_2$); δ_C (100.7 MHz, CD_3OD) 19.7 (t, CH_2SH), 43.6 (q, $N(CH_3)_2$), 61.2 (t, $\underline{C}H_2N(CH_3)_2$).

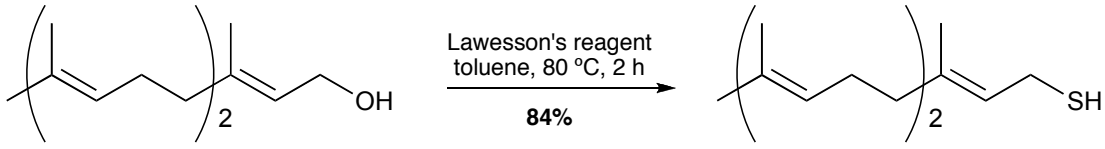
2-(Mercaptoethyl)trimethylammonium chloride 27



27

(2-Chloroethyl)-trimethylammonium chloride (3.00 g, 18.98 mmol) and sodium thiosulfate pentahydrate (4.71 g, 18.98 mmol) were added to a 100 mL round bottom flask equipped with a Teflon coated stir bar and dissolved in water (30 mL, MilliQ). The reaction was heated to reflux (oil bath temp = 120 ºC) and stirred for 13 h. The reaction was cooled to room temperature and the solvent removed under reduced pressure to give the crude Bunte salt as a white residue. This residue was dissolved in hydrochloric acid (30 mL of a 6M aqueous solution) and stirred at 85 ºC for 2 h. The reaction was cooled and the solvent removed under reduced pressure. DCM (150 mL) was added and heated with stirring. Ethanol was added slowly until the solution was no longer cloudy (~50 mL ethanol). Sodium chloride and sulfate salts were removed by filtration and the filtrate concentrated to white solid. The solid was recrystalized from aqueous ethanol to give **27** as clear needles (1.17 g, 40 %); m.p. 133-135 ºC; υ_{max} (KBr disc) 1651, 1486, 1224, 887 cm^{-1}; δ_H (400 MHz, CD_3OD) 2.93 (2H, m, C<u>H</u>$_2$SH), 3.18 (9H, s, N$(CH_3)_3$), 3.56 (2H, m, C<u>H</u>$_2$N$(CH_3)_3$); δ_C (100.7 MHz, CD_3OD) 17.9 (t, CH_2SH), 53.8 (q, N$(CH_3)_3$), 69.6 (t, <u>C</u>H_2N$(CH_3)_3$).

***trans,trans*-Farnesylmercaptan 28**



28

trans,trans-Farnesol (0.40 mL, 1.58 mmol) was dissolved in anhydrous toluene (5 mL). Lawesson's reagent (0.42 g, 0.95 mmol) was added, and the reaction mixture heated to 80 ºC under an atmosphere of argon. After 2 h, TLC (petrol) indicated the formation of a product (R_f 0.7). The reaction mixture was cooled to room temperature, filtered through Celite® and concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol) to yield *trans,trans*-farnesylmercaptan **28** (0.32 g, 84%) as a clear oil; spectroscopic data was identical to that previously reported;[9] υ_{max} (thin film) 2564 (br, SH) 1446, 1380 (2 x s, 2 x C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.40 (1H, t, *J* 7.1 Hz, SH), 1.61, 1.66, 1.69 (12H, 3 x s, 4 x CH_3) 1.96-2.13 (8H, m, 4 x CH_2), 3.17 (2H, at, *J* 7.4 Hz, C<u>H</u>$_2$SH), 5.08-5.12 (2H, m, 2 x CH=C), 5.35 (1H, dt,

J_{vic} 7.8 Hz, J 1.2 Hz, C<u>H</u>CH_2SH); δ_C (100.7 MHz, $CDCl_3$) 15.8, 16.1, 17.7 (3 x q, 3 x CH_3), 22.2 (t, CH_2SH), 25.7 (q, CH_3), 26.3, 26.7 (2 x t, 2 x CH<u>C</u>H_2), 39.4, 39.7 (2 x t, 2 x C<u>C</u>H_2), 123.3, 123.7, 124.3 (3 x d, 3 x C<u>C</u>H), 131.3, 135.3, 137.5 (3 x s, 3 x <u>C</u>CH).

References

[1] Tamura, Y.; Minamikawa, J.; Sumoto, K.; Fujii, S.; Ikeda, M. *J. Org. Chem.* **1973**, *38*, 1239-1241.

[2] Johnson, C. R.; Kirchhoff, R. A.; Corkins, H. G. *J. Org. Chem.* **1974**, *39*, 2458-2459.

[3] Kuligowski, C.; Bezzenine-Lafollee, S.; Chaume, G.; Mahuteau, J.; Barriere, J. C.; Bacque, E.; Pancrazi, A.; Ardisson, J. *J. Org. Chem.* **2002**, *67*, 4565-4568.

[4] Threadgill, M. D.; Gledhill, A. P. *J. Org. Chem.* **1989**, *54*, 2940-2949.

[5] Dhaon, M. K.; Olsen, R. K.; Ramasamy, K. *J. Org. Chem.* **1982**, *47*, 1962-1965.

[6] Gamblin, D. P.; Garnier, P.; Kasteren, S. v.; Oldham, N. J.; Fairbanks, A. J.; Davis, B. G. *Angew. Chem. Int. Ed.* **2004**, *43*, 828-833.

[7] Bernardes, G. J. L.; Gamblin, D. P.; Davis, B. G. *Angew. Chem. Int. Ed.* **2006**, *45*, 4007-4011.

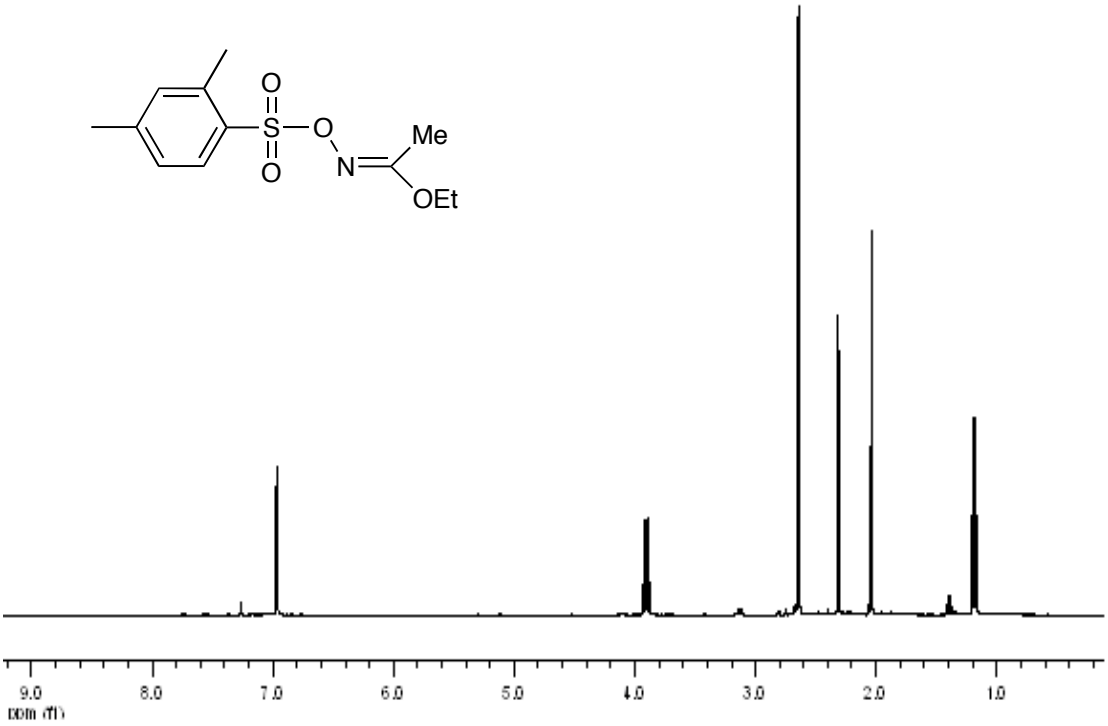
[8] Li, R.; Farmer, P. S.; Xie, M.; Quilliam, M. A.; Pleasance, S.; Howlett, S. E.; Yeung, P. K. F. *J. Med. Chem.* **1992**, *35*, 3246-3253.

[9] Epstein, W. W.; Wang, Z. *Chem. Commun.* **1997**, 863.

Spectral data

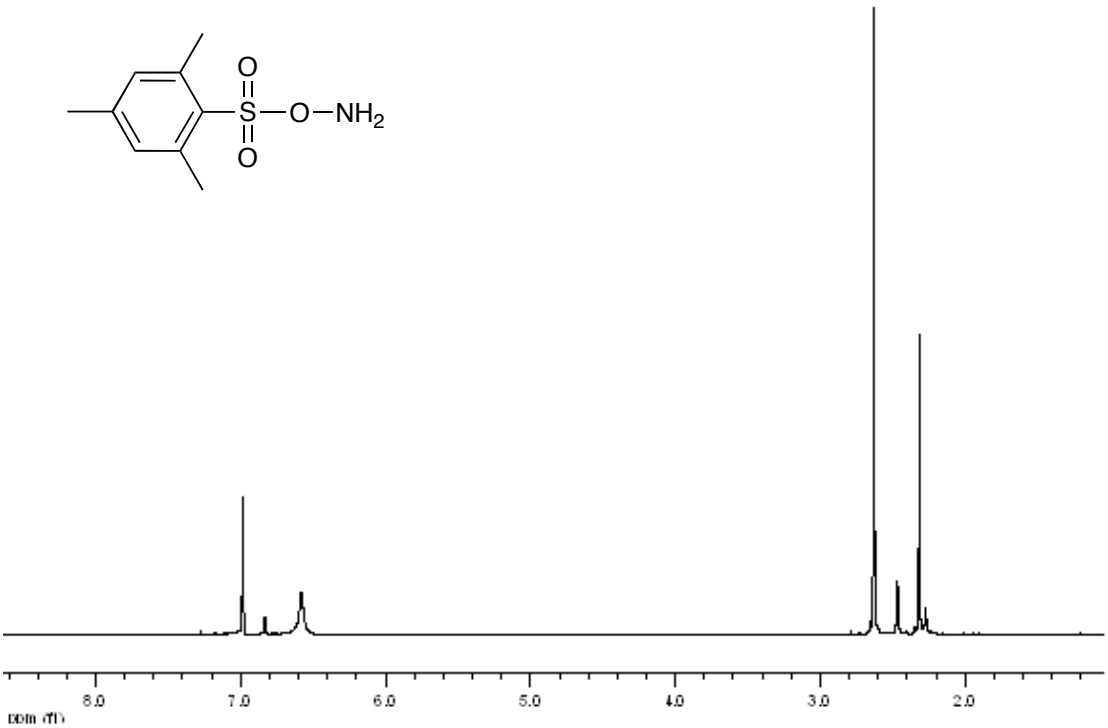
Ethyl-*O*-(mesitylenesulfonyl)hydroxyacetimidate (MSH precursor)

^{1}H NMR

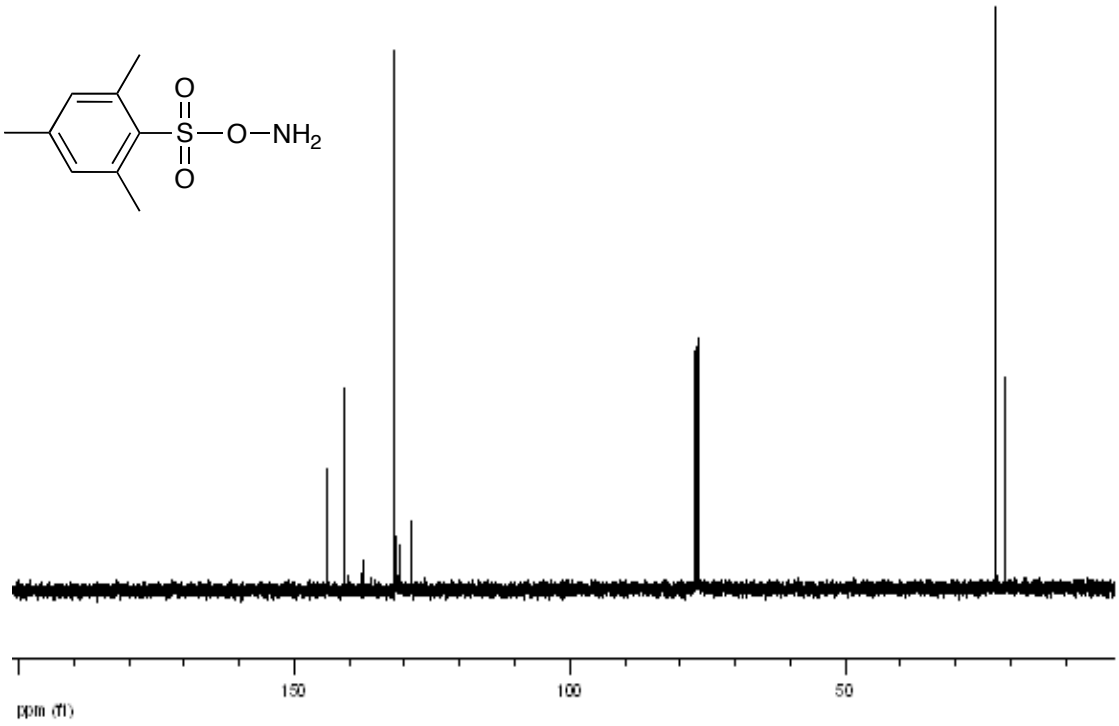


***O*-Mesitylsulfonylhydroxylamine 1 (MSH)**

^{1}H NMR

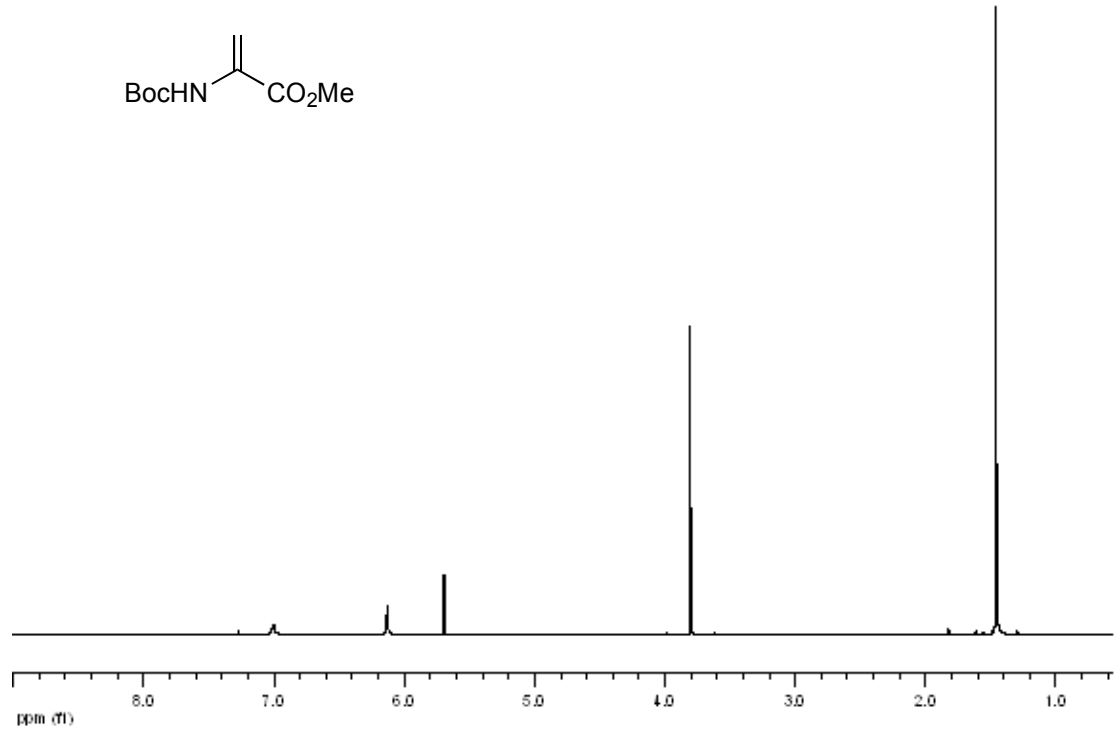


^{13}C NMR

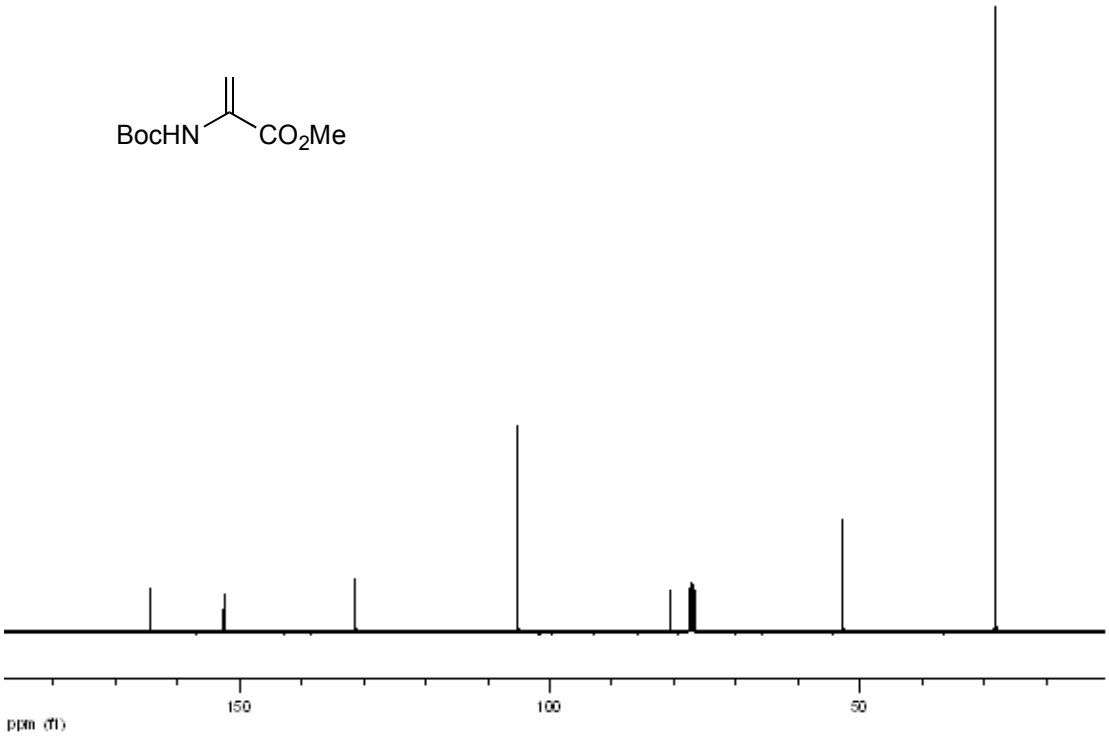


Methyl 2-[(*tert*-butoxycarbonyl)amino]acrylate 3

^{1}H NMR

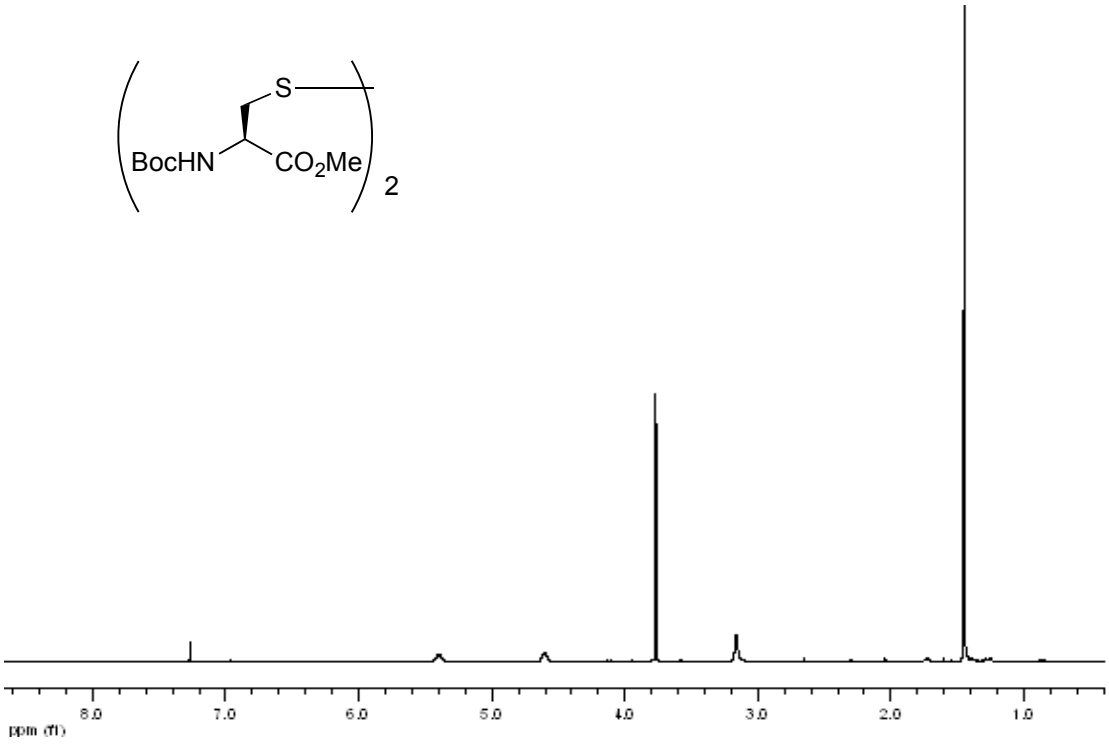


^{13}C NMR



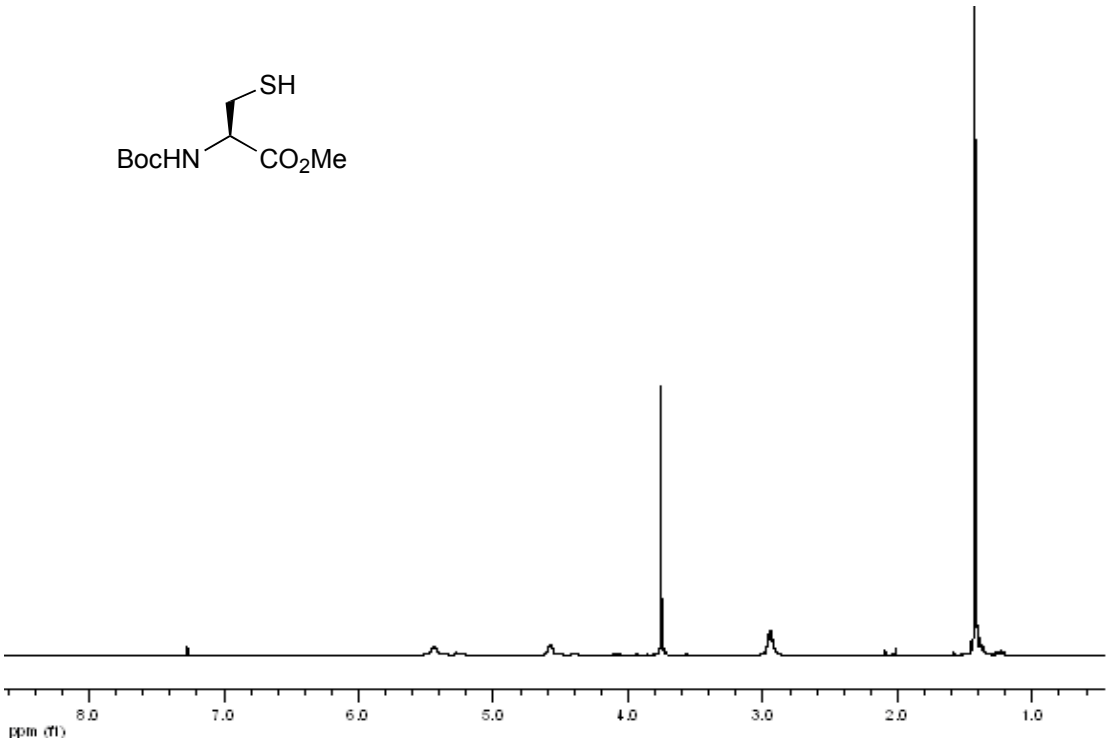
***N,N*-Bis(*tert*-butoxycarnonyl)-L-cysteine dimethyl ester 18**

^{1}H NMR



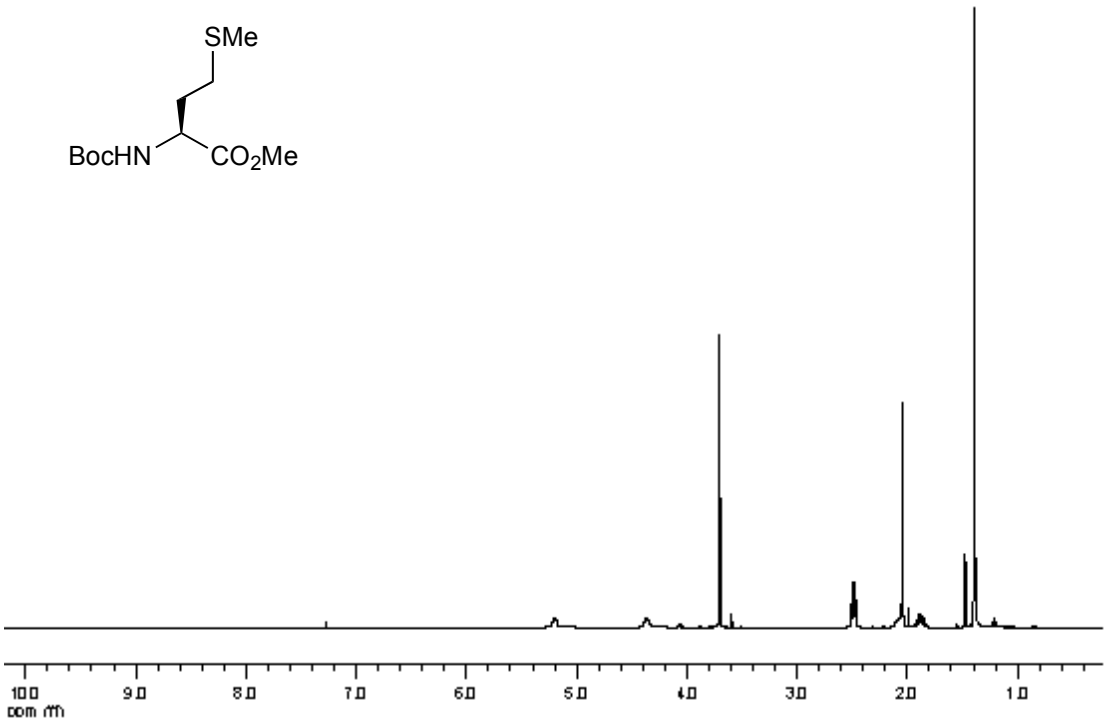
***N*-(*tert*-Butoxycarbonyl)-L-cysteine methyl ester 2**

^{1}H NMR



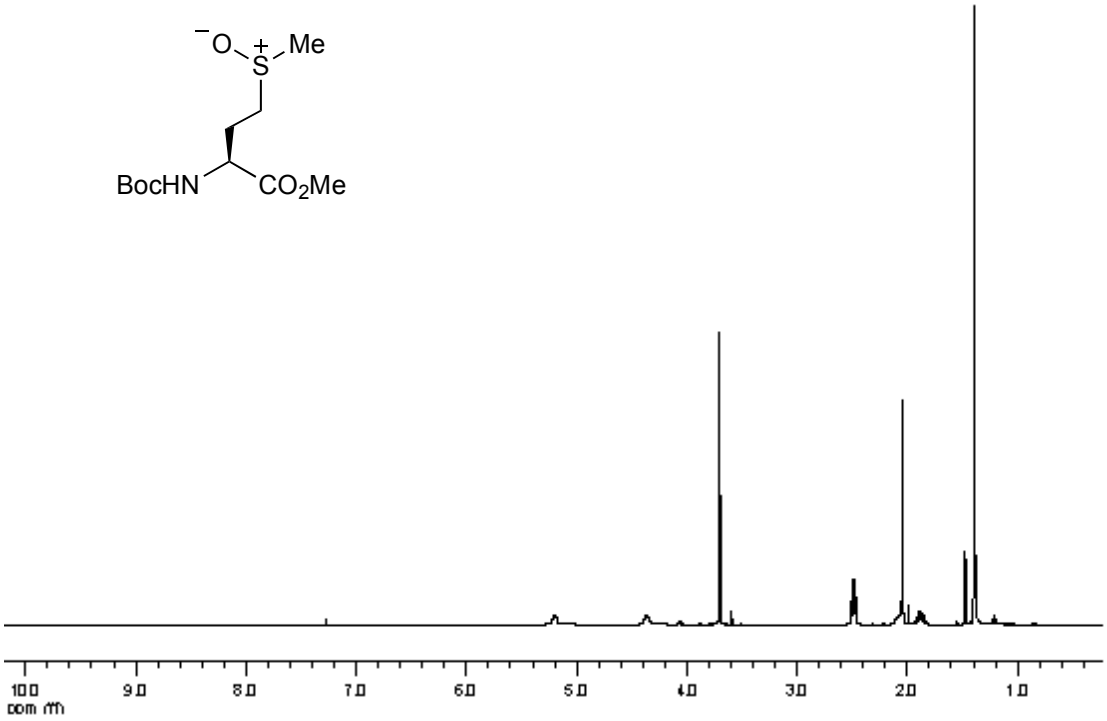
***N*-(*tert*-butoxycarbonyl)-L-methionine methyl ester 4**

^{1}H NMR

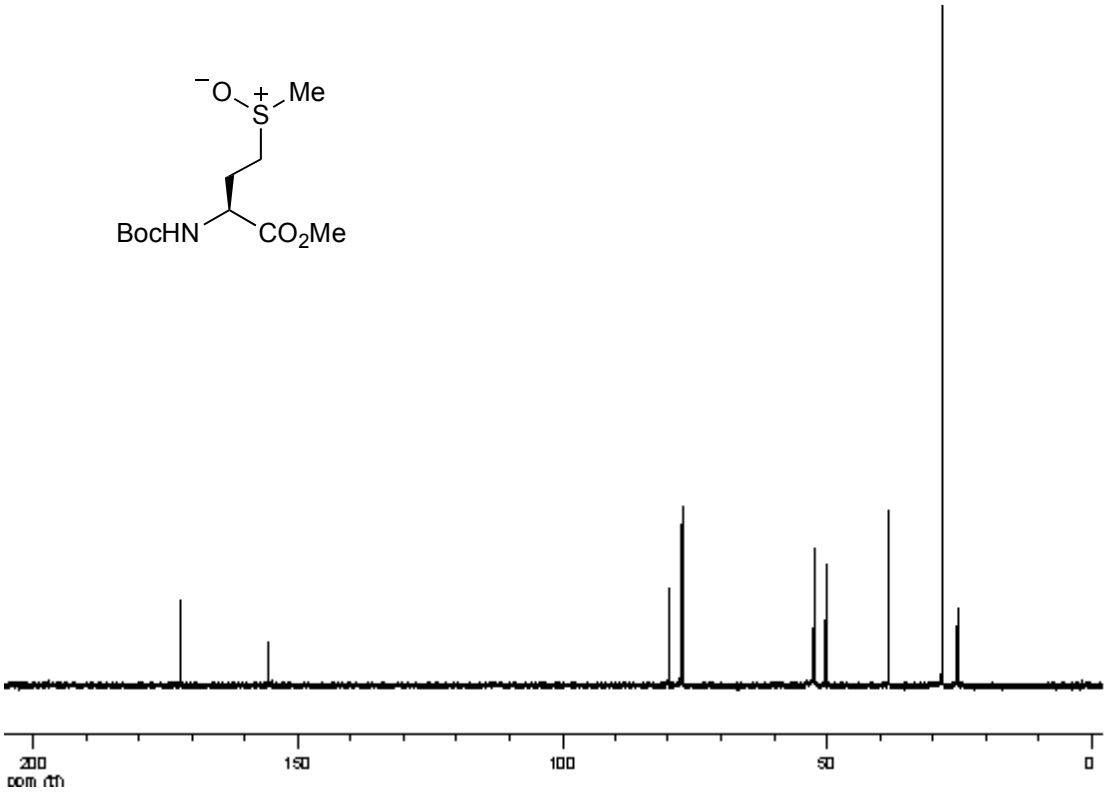


BocMetOMe sulfoxide 19

^{1}H NMR

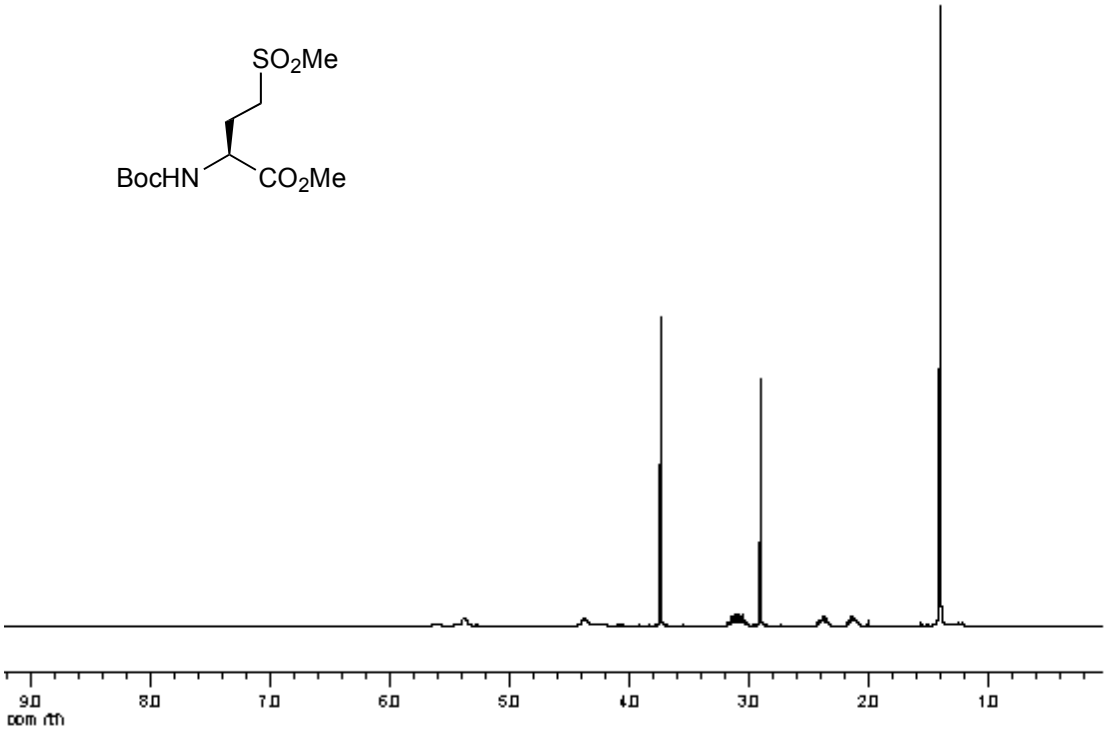


^{13}C NMR

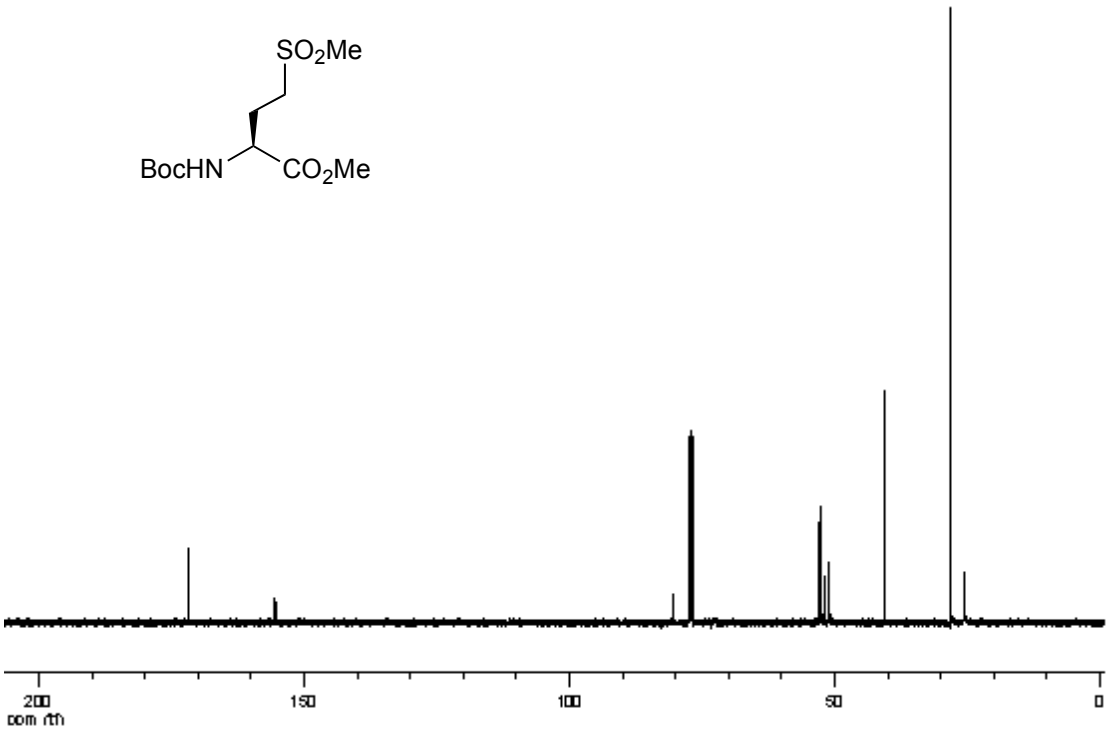


BocMetOMe sulfone 20

^{1}H NMR

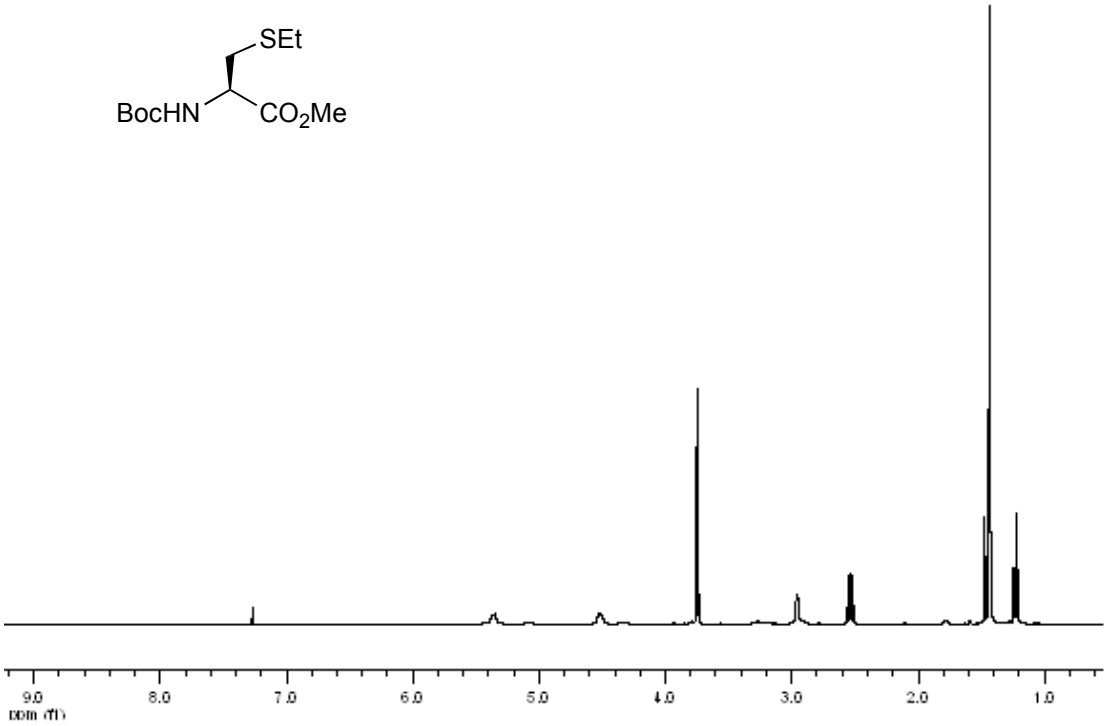


^{13}C NMR

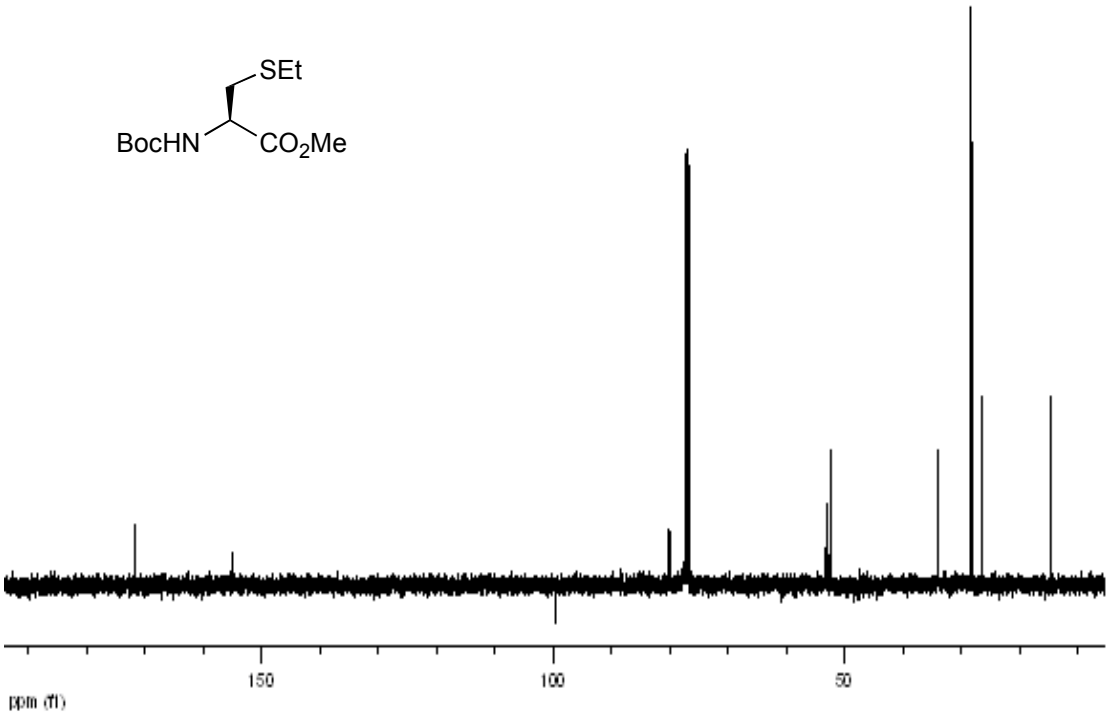


_N_-(_tert_-Butoxycarbonyl)-ethylthio-L-cysteine methyl ester 16

^{1}H NMR

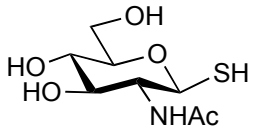


^{13}C NMR



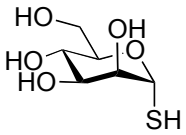
1-Thio-2-acetamido-2-deoxy-β-D-glucopyranose 23

^{1}H NMR



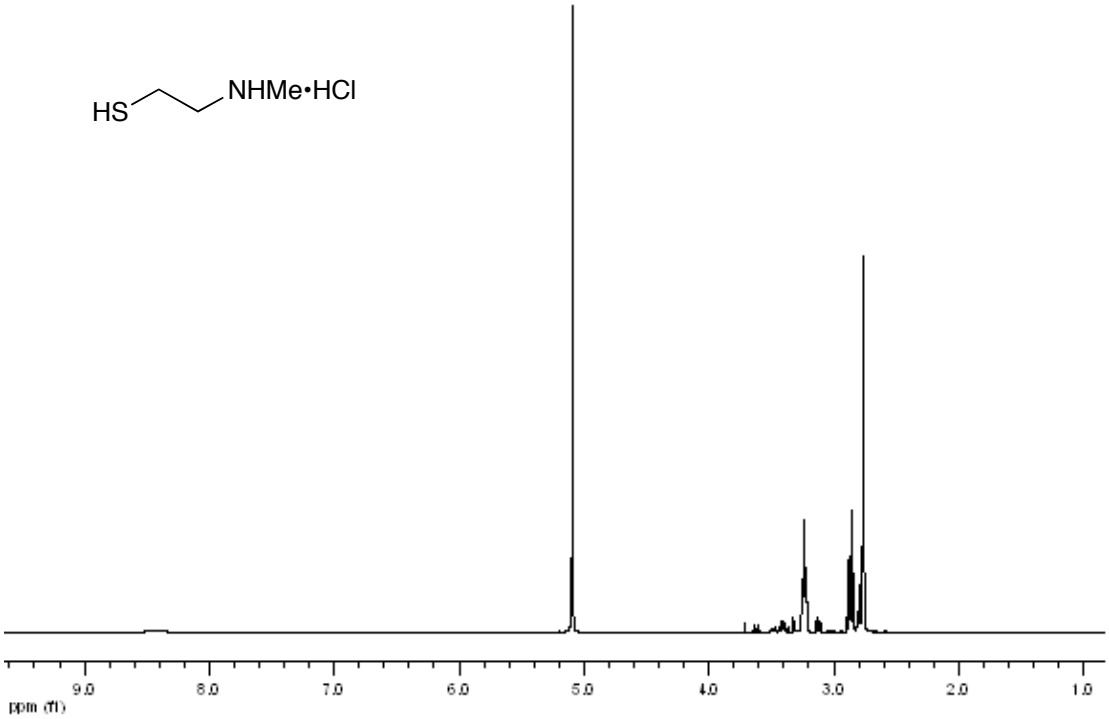
1-Thio-α-D-mannopyranose 24

^{1}H NMR

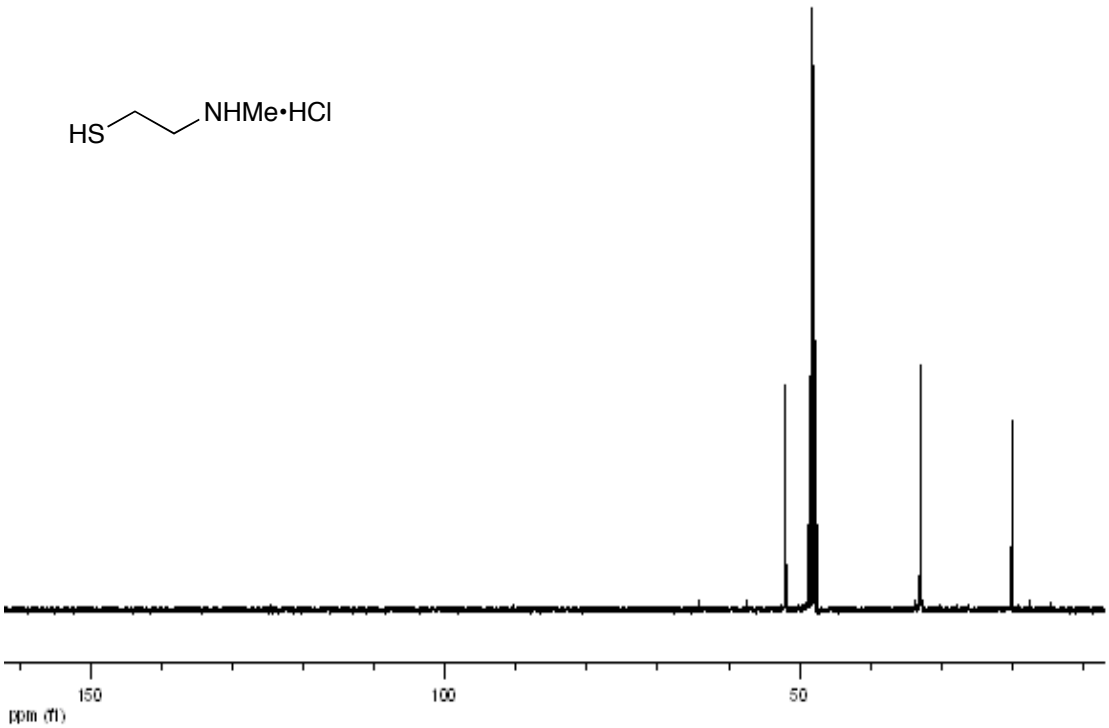


2-(Methylamino)ethanethiol hydrochloride 25

^{1}H NMR

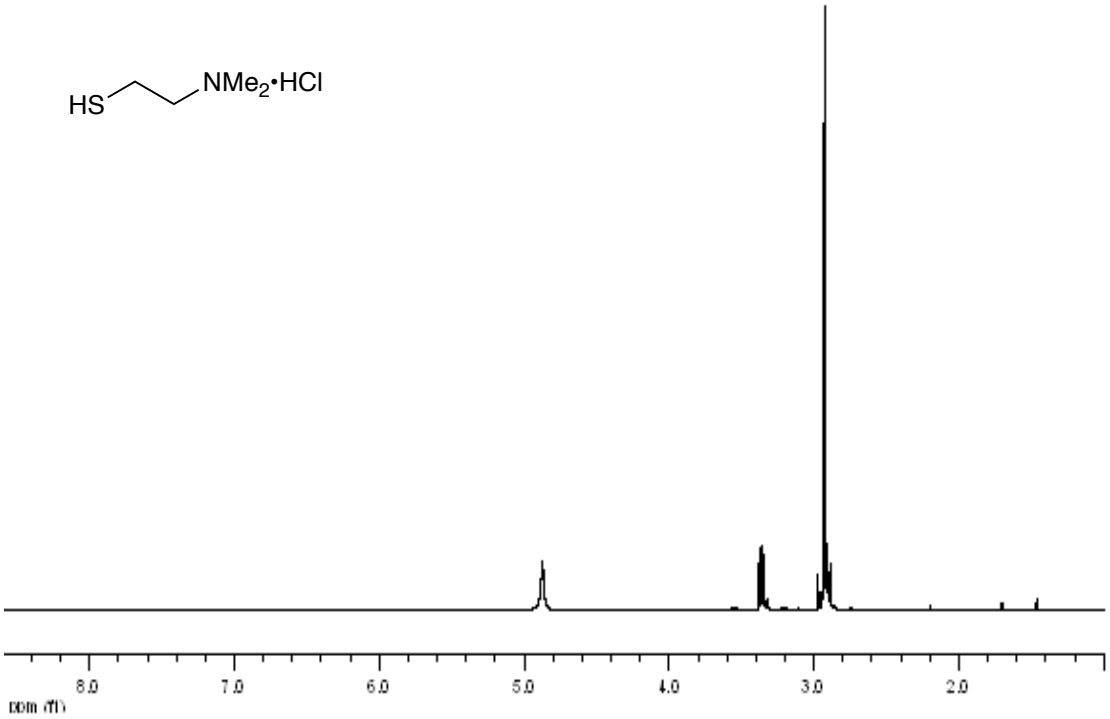


^{13}C NMR

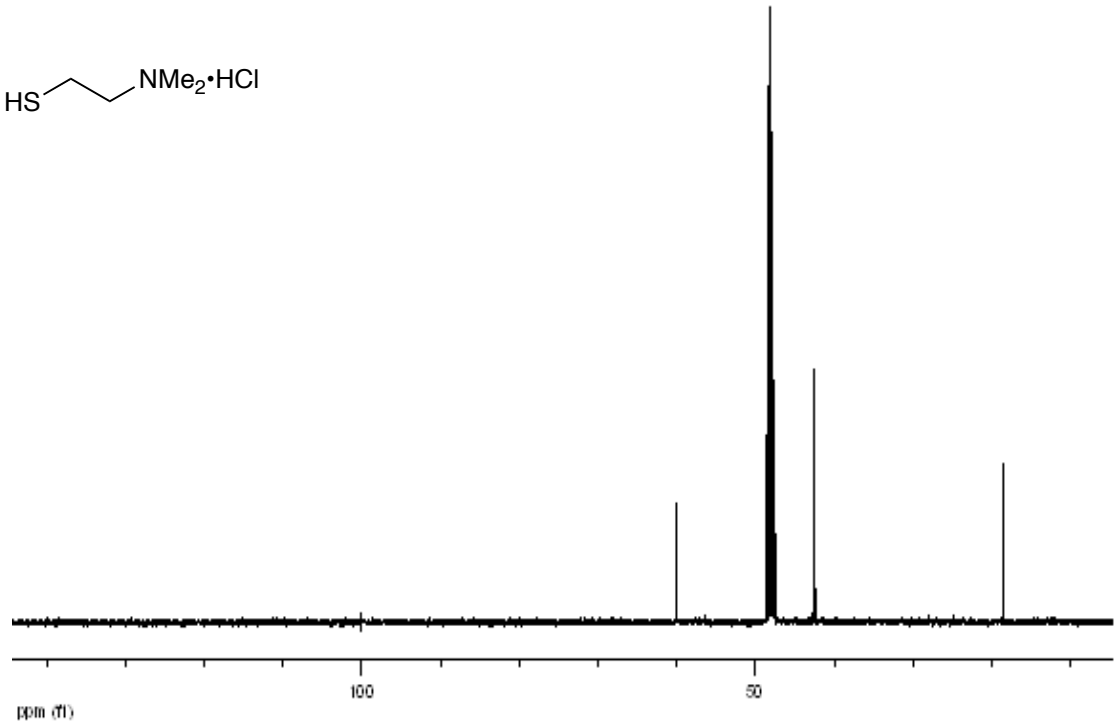


2-(Dimethylamino)ethanethiol hydrochloride 26

^{1}H NMR

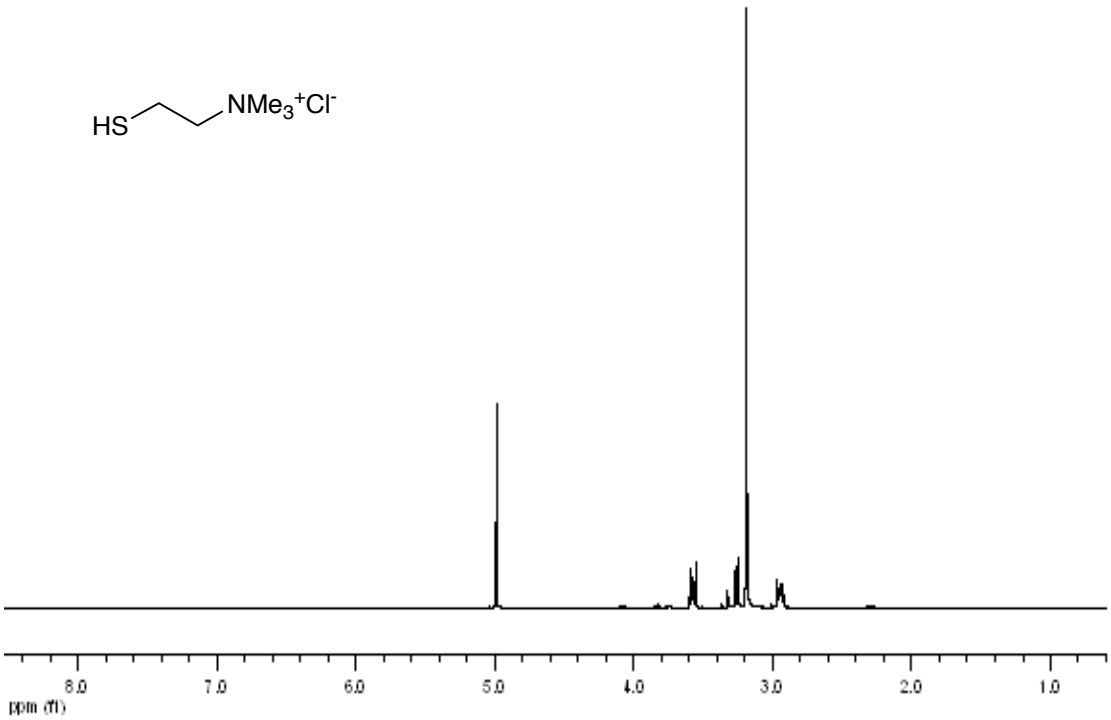


^{13}C NMR

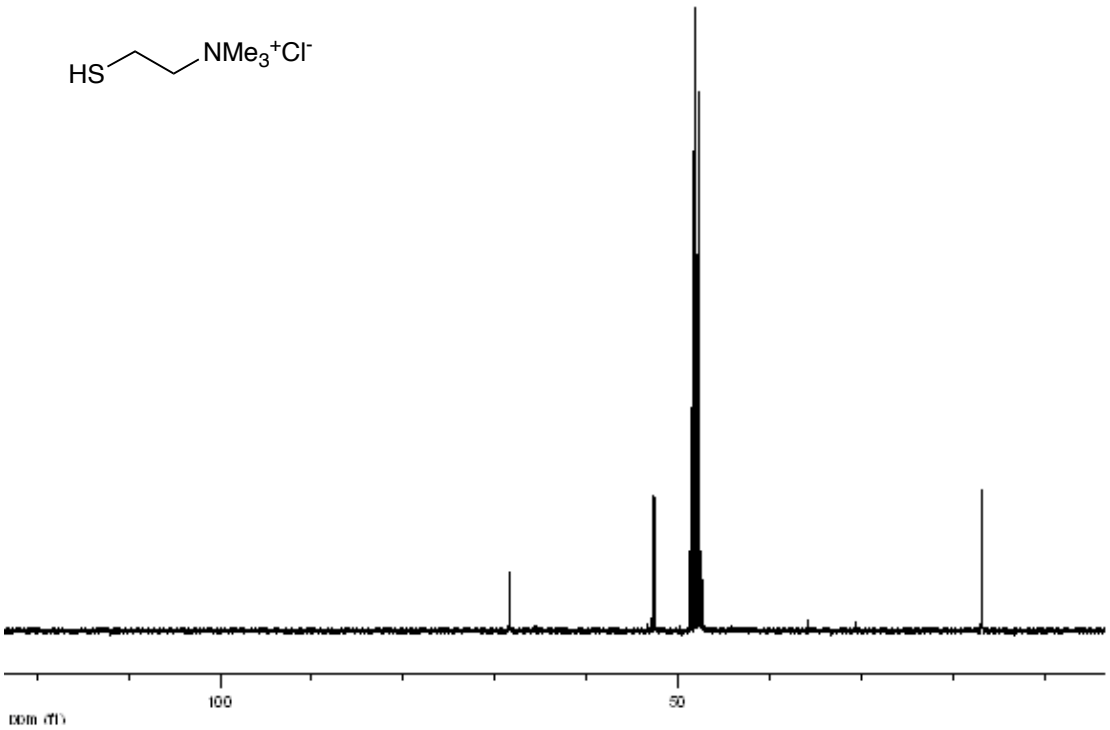


2-(Mercaptoethyl)trimethylammonium chloride 27

^{1}H NMR



^{13}C NMR



***trans,trans*-Farnesylmercaptan 28**

^{1}H NMR