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Supporting Information

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From Disulfide- to Thioether-linked Glycoproteins

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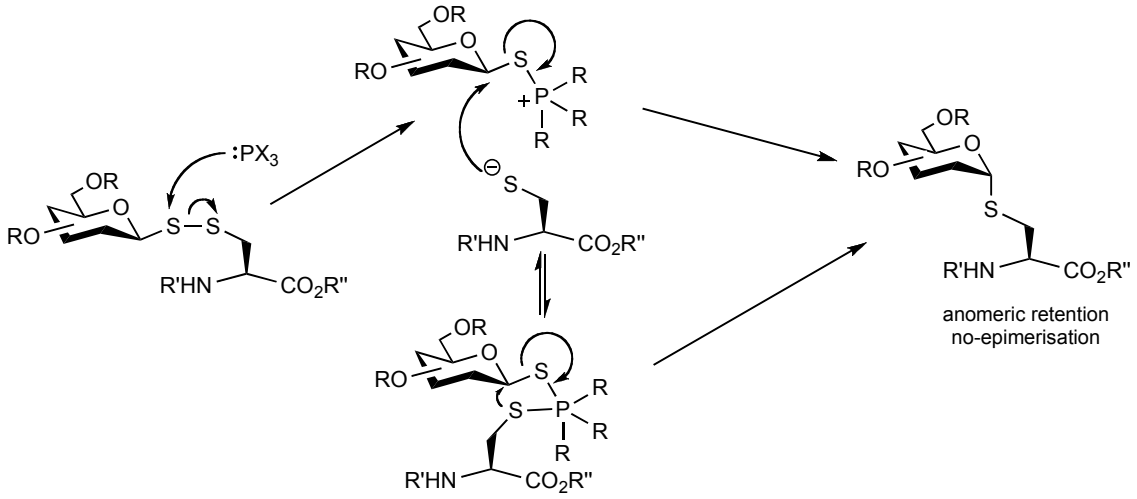
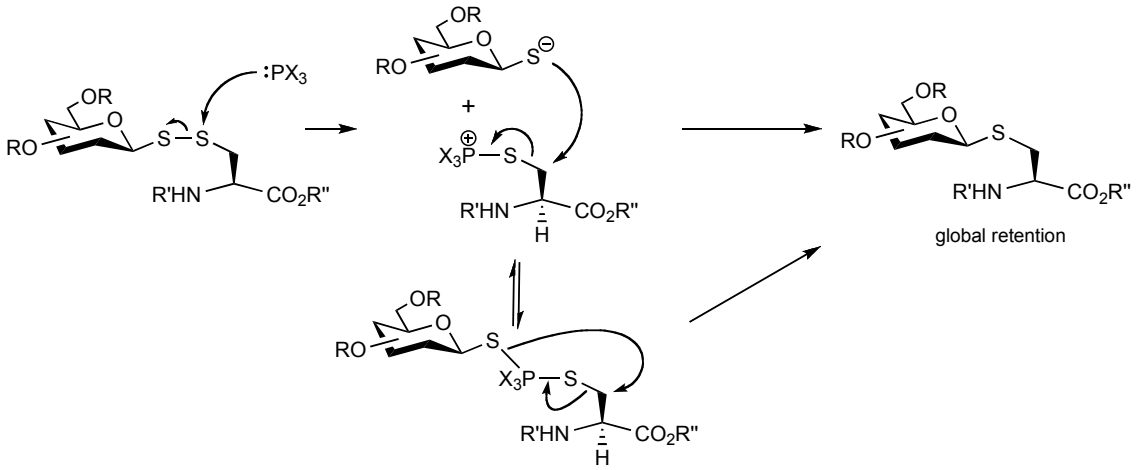
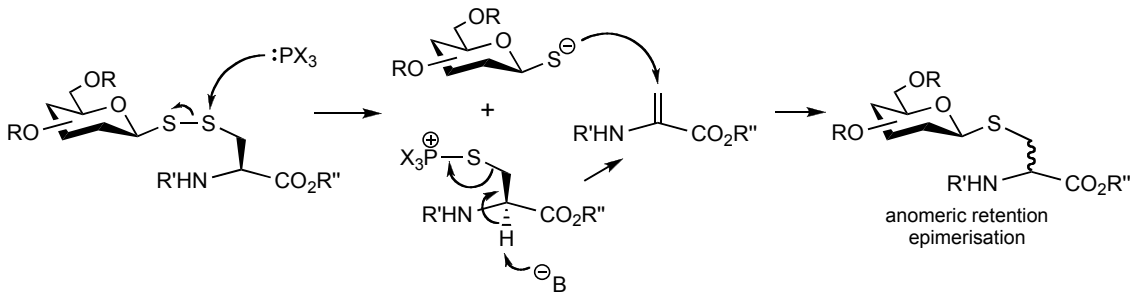
Scheme S1. Sδ-attack followed by thio-mitsunobu mechanism.



Scheme S2. Sγ-attack followed by thio-mitsunobu mechanism.



Scheme S3. Sγ-attack followed by elimination-conjugate addition mechanism.



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; D_2O = 4.80). 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). Only signals representing functional groups are reported; C-H absorptions as well as the fingerprint region are not listed.

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.

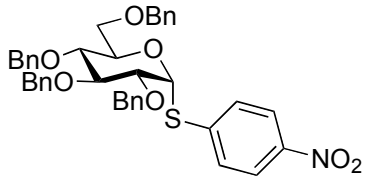
Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm and are reported with implied units of 10^{-1} deg cm^2 g^{-1}. Concentrations (c) are given in g/100 ml.

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.

***p*-Nitrophenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside 2**

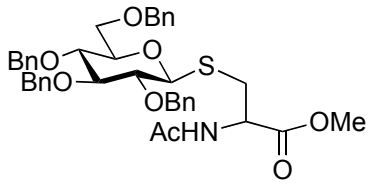


Tributylphosphine (51 μl, 0.206 mmol) was added to a stirred solution of *p*-nitrophenyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl disulfide[1] **1** (73 mg, 0.103 mmol) in anhydrous dichloromethane (2 ml) under an atmosphere of argon. The reaction mixture instantly became a dark orange red, but the colour faded rapidly to very pale yellow within 15 min. After 1 h, t.l.c. (petrol:ethyl acetate, 8:2) showed formation of a major product (R_f 0.4). The reaction mixture was concentrated *in vacuo* and the resulting residue purified by flash column chromatography (petrol:ethyl acetate, 9:1) to afford *p*-nitrophenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside **2** (52 mg, 74%) as a pale yellow solid; $[\alpha]_D^{25}$ +184 (c, 1 in $CHCl_3$); υ_{max} (KBr disc) no significant peaks; δ_H (400 MHz, $CDCl_3$) 3.58 (1H, dd, $J_{5,6}$ 1.8 Hz, $J_{6,6'}$ 10.7 Hz, H-6), 3.69-3.76 (2H, m, H-4, H-6'), 3.90 (1H, at, *J* 9.1 Hz, H-3), 3.98 (1H, dd, $J_{1,2}$ 5.3 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.17 (1H, ddd, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 1.8 Hz, $J_{5,6'}$ 3.6 Hz, H-5), 4.42, 4.58 (2H, ABq, $J_{A,B}$ 11.9 Hz, OCH_2Ph), 4.50, 5.00 (2H, ABq, $J_{A,B}$ 10.7 Hz, OCH_2Ph), 4.74 (2H, s, OCH_2Ph), 4.83, 4.87 (2H, ABq, $J_{A,B}$ 9.6 Hz, OCH_2Ph), 5.80 (1H, d, $J_{1,2}$ 5.3 Hz, H-1), 7.14-7.38 (20H, m, Ar-H), 7.56 (2H, d, *J* 8.8 Hz, *o*-$PhNO_2$), 8.08 (2H, d, *J* 8.8 Hz, p-$PhNO_2$); δ_C (100.7 MHz, $CDCl_3$) 68.3 (t, C-6), 71.7 (d, C-5), 73.0, 73.5, 75.2, 75.9 (4 x t, 4 x OCH_2Ph), 77.1 (d, C-4), 79.4 (d, C-2), 82.4 (d, C-3), 85.3 (d, C-1), 123.8, 127.8, 127.9, 128.0, 128.1, 128.4, 128.5, 128.6, 129.1 (9 x d, 24 x Ar-C), 137.3, 137.6, 137.9, 138.4, 144.9, 145.9 (6 x s, 6 x Ar-C); *m/z* (ES^+) 700 (MNa^+, 100%); HRMS (ES^+) Calcd. for $C_{40}H_{39}NNaO_7S$ (MNa^+) 700.2340. Found: 700.2339; Found: C, 72.44%; H, 5.68%, N, 2.09%. $C_{40}H_{39}NO_7S$ requires: C, 71.88%; H, 5.80%; N, 2.07%.

A) General procedure for desulfurization reaction

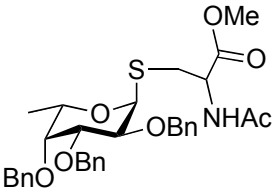
Typically, the disulfide-linked glycoaminoacid/glycopeptide was dissolved in degassed anhydrous methanol (1 mL for a 50 mg scale reaction). The phosphine reagent (2.0-2.2 equivalents) was added *via* microsyringe, and the resulting solution stirred under an atmosphere of argon. After t.l.c. (petrol:ethyl acetate) showed complete consumption of starting material and formation of a major product, the reaction mixture was concentrated *in vacuo* and the resulting residue purified by flash column chromatography.

_N_-Acetyl-DL-cysteine-_S_-(2,3,4,6-tetra-_O_-benzyl-β-D-glucopyaranoside) methyl ester 4



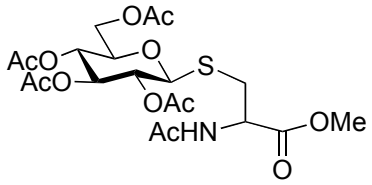
Using the general procedure, *N*-acetyl-DL-cysteine-*S*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyaranoside) methyl ester **4** was prepared as a thin film being a mixture of epimers (D:L, 1:1) on a 0.068 mmol (substrate) scale; Yield: 73%; R_f 0.4 (petrol:ethyl acetate, 1:1); $[\alpha]_D^{18}$ -13.6 (c, 0.5 in $CHCl_3$); υ_{max} (thin film) 3361 (br, NH) 1744 (s, C=O) 1661 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.84, 1.87 (6H, 2 x s, HNC(O)C$\underline{H}_3$D, HNC(O)C$\underline{H}_3$L), 3.10, 3.14 (2H, 2 x dd, $J_{C\underline{H},H'}$ 14.6 Hz, $J_{CH,\alpha H}$ 7.2 Hz, C$\underline{H}$,H'D, C$\underline{H}$,H'L), 3.22, 3.23 (2H, dd, $J_{CH,\underline{H}'}$ 14.6 Hz, $J_{CH',\alpha H}$ 4.2 Hz, CH,$\underline{H}$'D, CH,$\underline{H}$'L), 3.25-3.29 (2H, m, H-5D, H-5L), 3.32, 3.36 (2H, 2 x at, *J* 9.1 Hz, H-2D, H-2L), 3.40-3.49 (2H, m, H-4D, H-4L), 3.66-3.68 (2H, 2 x at, *J* 8.6 Hz, H-3D, H-3L), 3.70-3.77 (4H, m, H-6D, H-6L, H-6'D, H-6'L), 3.75, 3.78 (6H, 2 x s, OCH_3D, OCH_3L), 4.38, 4.40 (2H, 2 x d, $J_{1,2}$ 9.8 Hz, H-1D, H-1L), 4.49-4.99 (18H, m, 4 x OCH_2PhD, 4 x OCH_2PhL, αHD, αHL) 6.85, 6.95 (2H, 2 x d, $J_{NH,\alpha H}$ 7.9 Hz, $\underline{H}$NC(O)CH_3D, $\underline{H}$NC(O)CH_3L), 7.15-7.36 (40H, m, 20 x Ar-HD, 20 x Ar-HL); δ_C (125.8 MHz, $CDCl_3$) 22.5, 22.6 (2 x q, HNC(O)$\underline{C}H_3$D, HNC(O)$\underline{C}H_3$L), 32.8, 34.6 (2 x t, CH,H'D, CH,H'L), 52.4, 52.5 (2 x d, αCD, αCL), 52.6, 52.9 (2 x q, OCH_3D, OCH_3L), 68.7, 69.7 (2 x t, C-6D, C-6L), 73.6, 73.7, 75.1, 75.2, 75.5, 75.6, 75.7, 75.8 (8 x t, 4 x OCH_2PhD, 4 x OCH_2PhL), 77.5, 77.7 (2 x d, C-4D, C-4L), 78.5, 78.8 (2 x d, C-2D, C-2L), 81.0, 81.7 (2 x d, C-5D, C-5L), 85.6, 86.1 (2 x d, C-1D, C-1L), 86.4, 86.5 (2 x d, C-3D, C-3L), 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5 (10 x d, 20 x Ar-CD, 20 x Ar-CL), 137.3, 137.5, 137.6, 137.7, 137.8, 138.2, 138.3 (7 x s, 4 x Ar-CD, 4 x Ar-CL), 170.2, 170.3, 170.5, 170.9 (4 x s, HN$\underline{C}$(O)CH_3D, HN$\underline{C}$(O)CH_3L, $\underline{C}O_2CH_3$D, $\underline{C}O_2CH_3$L); *m/z* (ES^+) 758 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{40}H_{45}NNaO_8S$ (MNa^+) 722.2758. Found: 722.2759.

_N_-Acetyl-DL-cysteine-_S_-(2,3,4-tri-_O_-benzyl-1-thio-α-L-fucopyranoside) methyl ester 6



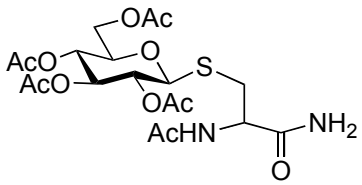
Using the general procedure, *N*-acetyl-DL-cysteine-*S*-(2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside) methyl ester **6** was prepared as a colourless oil being a mixture of epimers (D:L, 1:1) on a 0.048 mmol (substrate) scale; Yield: 61%; R_f 0.4 (ethyl acetate); $[\alpha]_D^{22}$ -4.3 (c, 0.5 in $CHCl_3$); υ_{max} (thin film) 3299 (br, NH) 1746 (s, C=O) 1654 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) δ_H (500 MHz, $CDCl_3$) 1.14 (1H, d, *J* 6.5 Hz, CH_3D), 1.22 (1H, d, *J* 6.4 Hz, CH_3L), 1.97 (3H, s, HNC(O)C$\underline{H}_3$D), 1.98 (3H, s, HNC(O)C$\underline{H}_3$L), 2.77 (1H, dd, $J_{C\underline{H},H'}$ 14.5 Hz, $J_{CH,\alpha H}$ 3.3 Hz, C$\underline{H}$,H'D), 2.85 (1H, dd, $J_{C\underline{H},H'}$ 14.0 Hz, $J_{CH,\alpha H}$ 5.7 Hz, C$\underline{H}$,H'L), 3.07 (1H, dd, $J_{CH,\underline{H}'}$ 13.8 Hz, $J_{CH',\alpha H}$ 4.3 Hz, CH,$\underline{H}$'L), 3.29 (1H, dd, $J_{CH,\underline{H}'}$ 14.7 Hz, $J_{CH',\alpha H}$ 4.7 Hz, CH,$\underline{H}$'D), 3.63-3.67 (2H, m, H-3D, H-4D), 3.74-3.79 (2H, m, H-3L, H-4L), 3.76, 3.77 (6H, 2 x s, OCH_3D, OCH_3L), 4.07 (1H, q, *J* 6.5 Hz, H-5D), 4.15 (1H, q, *J* 6.5 Hz, H-5L), 4.28 (2H, dd, $J_{1,2}$ 5.7 Hz, $J_{2,3}$ 9.9 Hz, H-2D, H-2L), 4.64-4.99 (14H, m, 3 x OCH_2PhD, 3 x OCH_2PhL, αHD, αHL), 5.21 (1H, d, $J_{1,2}$ 5.6 Hz, H-1D), 5.48 (1H, d, $J_{1,2}$ 5.4 Hz, H-1L), 6.21 (1H, br d, $J_{NH,\alpha H}$ 7.8 Hz, $\underline{H}$NC(O)CH_3D), 7.07 (1H, br d, $J_{NH,\alpha H}$ 9.0 Hz, $\underline{H}$NC(O)CH_3L), 7.29-7.40 (30H, m, 15 x Ar-HD, 15 x Ar-HL); δ_C (125.8 MHz, $CDCl_3$) 14.1, 16.5 (2 x q, CH_3D, CH_3L), 22.7, 23.1 (2 x q, HNC(O)$\underline{C}H_3$D, HNC(O)$\underline{C}H_3$L), 35.0, 37.3 (2 x t, CH,H'D, CH,H'L), 52.1, 52.2 (2 x d, αCD, αCL), 52.5, 52.7 (2 x q, OCH_3D, OCH_3L), 67.8, 68.0 (2 x d, C-5D, C-5L), 72.6, 72.8, 73.5 (3 x t, 3 x OCH_2PhD, 3 x OCH_2PhL), 75.0, 75.9 (2 x d, C-2D, C-2L), 76.0, 76.7, 76.9, 77.2 (4 x d, C-3D, C-3L, C-4D, C-4L), 86.5 (d, C-1D, C-1L), 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 128.6 (12 x d, 15 x Ar-CD, 15 x Ar-CL), 138.2, 138.6 (2 x s, 3 x Ar-CD, 3 x Ar-CL), 169.9, 170.0, 170.9 (3 x s, $\underline{C}OOCH_3$D, $\underline{C}OOCH_3$L, HN$\underline{C}OCH_3$D, HN$\underline{C}OCH_3$L); *m/z* (ES^+) 615 (MNa^+, 100%); HRMS (ES^+) Calcd. for $C_{33}H_{39}NNaO_7S$ (MNa^+) 616.2339. Found: 616.2340.

_N_-Acetyl-DL-cysteine-_S_-(2,3,4,6-tetra-_O_-acetyl-β-D-glucopyaranoside) methyl ester 8



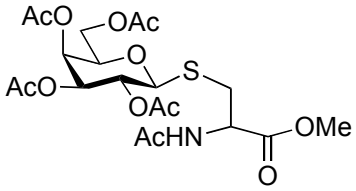
Using the general procedure, *N*-acetyl-DL-cysteine-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyaranoside) methyl ester **8** was prepared as a colourless oil being a mixture of epimers (D:L, 1:1) on a 0.093 mmol (substrate) scale; Yield: 72%; R_f 0.3 (petrol:ethyl acetate, 1:4); $[\alpha]_D^{18}$ +4.0 (c, 0.75 in $CHCl_3$); υ_{max} (thin film) 3383 (br, NH) 1750 (s, C=O) 1668 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 2.01, 2.02, 2.03, 2.04, 2.06, 2.07, 2.08, 2.11, 2.13 (30H, 9 x s, 4 x C(O)CH_3D, 4 x C(O)CH_3L, HNC(O)C$\underline{H}_3$D, HNC(O)C$\underline{H}_3$L), 3.05, 3.08 (2H, 2 x dd, $J_{C\underline{H},H'}$ 14.2 Hz, $J_{CH,\alpha H}$ 5.7 Hz, C$\underline{H}$,H'D, C$\underline{H}$,H'L), 3.20, 3.23 (2H, dd, $J_{CH,\underline{H}'}$ 14.1 Hz, $J_{CH',\alpha H}$ 3.3 Hz, CH,$\underline{H}$'D, CH,$\underline{H}$'L), 3.70-3.75 (2H, m, H-5D, H-5L), 3.77, 3.78 (6H, 2 x s, OCH_3D, OCH_3L), 4.17, 4.20 (2H, 2 x dd, $J_{5,6}$ 2.1 Hz, $J_{6,6'}$ 12.4 Hz, H-6D, H-6L), 4.24, 4.26 (2H, 2 x dd, $J_{5,6'}$ 5.4 Hz, $J_{6,6'}$ 12.4 Hz, H-6'D, H-6'L), 4.49, 4.53 (2H, 2 x d, $J_{1,2}$ 10.1 Hz, H-1D, H-1L), 4.78-4.84 (2H, m, αHD, αHL), 4.98, 4.99 (2H, 2 x at, *J* 9.7 Hz, H-2D, H-2L), 5.04, 5.08 (2H, 2 x at, *J* 9.5 Hz, H-4D, H-4L), 5.20, 5.23 (2H, 2 x at, *J* 9.4 Hz, H-3D, H-3L), 6.46, 6.53 (2H, 2 x d, $J_{NH,\alpha H}$ 7.4 Hz, $\underline{H}$NC(O)CH_3D, $\underline{H}$NC(O)CH_3L); δ_C (125.8 MHz, $CDCl_3$) 20.6, 20.7 (2 x q, 4 x C(O)$\underline{C}H_3$D, 4 x C(O)$\underline{C}H_3$L), 22.9 (q, HNC(O)$\underline{C}H_3$D, HNC(O)$\underline{C}H_3$L), 31.7, 32.4 (2 x t, CH,H'D, CH,H'L), 51.8, 52.2 (2 x d, αCD, αCL), 52.7, 52.8 (2 x q, OCH_3D, OCH_3L), 61.8, 62.1 (2 x t, C-6D, C-6L), 68.0, 68.1 (2 x d, C-4D, C-4L), 69.7, 69.9 (2 x d, C-2D, C-2L), 73.5 (d, C-3D, C-3L), 76.0, 76.1 (2 x d, C-5D, C-5L), 83.3, 83.7 (2 x d, C-1D, C-1L), 169.3, 169.4, 169.5, 169.6, 169.8, 169.9, 170.0, 170.1, 170.6, 170.7, 170.8, 170.9 (12 x s, 4 x $\underline{C}$(O)CH_3D, 4 x $\underline{C}$(O)CH_3L, HN$\underline{C}$(O)CH_3D, HN$\underline{C}$(O)CH_3L, $\underline{C}O_2CH_3$D, $\underline{C}O_2CH_3$L); *m/z* (ES^+) 566 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{20}H_{29}NNaO_{12}S$ (MNa^+) 530.1303. Found: 530.1296.

***N*-Acetyl-DL-cysteinamide-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyaranoside) 10**



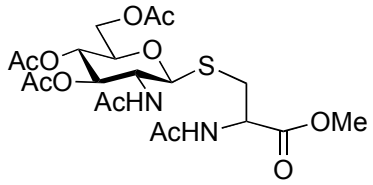
Using the general procedure, *N*-acetyl-DL-cysteinamide-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyaranoside) **10** was prepared as a colourless oil being a mixture of epimers (D:L, 1:1) on a 0.067 mmol (substrate) scale; Yield: 75%; R_f 0.3 (ethyl acetate:methanol, 95:5); $[\alpha]_D^{25}$ -1.6 (c, 0.5 in $CHCl_3$); υ_{max} (KBr disc) 3057 (br, NH NH_2) 1752 (s, C=O) 1643 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 2.01, 2.03, 2.04, 2.05, 2.06. 2.07, 2.08, 2.09, 2.10, 2.11 (30H, 10 x s, 4 x C(O)CH_3D, 4 x C(O)CH_3L, HNC(O)C$\underline{H}_3$D, HNC(O)C$\underline{H}_3$L), 2.69 (1H, dd, $J_{C\underline{H},H'}$ 14.2 Hz, $J_{CH,\alpha H}$ 7.4 Hz, C$\underline{H}$,H'D), 2.79 (1H, dd, $J_{C\underline{H},H'}$ 14.2 Hz, $J_{CH,\alpha H}$ 9.0 Hz, C$\underline{H}$,H'L), 3.16 (1H, dd, $J_{CH,\underline{H}'}$ 14.2 Hz, $J_{CH',\alpha H}$ 4.4 Hz, CH,$\underline{H}$'L), 3.34 (1H, dd, $J_{CH,\underline{H}'}$ 14.3 Hz, $J_{CH',\alpha H}$ 5.3 Hz, CH,$\underline{H}$'D), 3.76-3.83 (1H, m, H-5D), 3.88 (1H, ddd, $J_{4,5}$ 10.1 Hz, $J_{5,6}$ 5.2 Hz, $J_{5,6'}$ 2.0 Hz, H-5L), 4.12 (1H, dd, $J_{5,6}$ 5.3 Hz, $J_{6,6'}$ 12.4 Hz, H-6L), 4.22 (1H, dd, $J_{5,6}$ 4.4 Hz, $J_{6,6'}$ 12.5 Hz, H-6D), 4.29 (1H, dd, $J_{5,6}$ 2.1 Hz, $J_{6,6'}$ 12.5 Hz, H-6'D), 4.37 (1H, dd, $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 12.4 Hz, H-6'L), 4.59 (1H, d, $J_{1,2}$ 9.9 Hz, H-1D), 4.62-4.67 (1H, m, αHD), 4.76-4.80 (1H, m, αHL), 4.77 (1H, d, $J_{1,2}$ 10.3 Hz, H-1L), 4.99 (1H, at, *J* 9.8 Hz, H-2L), 5.07 (1H, at, *J* 9.8 Hz, H-2D), 5.13 (1H, at, *J* 9.7 Hz, H-4L), 5.18 (1H, at, *J* 9.7 Hz, H-4D), 5.26, 5.27 (2H, 2 x at, *J* 9.3 Hz, H-3D, H-3L), 6.54, 6.63 (2H, 2 x d, $J_{NH,\alpha H}$ 7.4 Hz, $\underline{H}$NC(O)CH_3D, $\underline{H}$NC(O)CH_3L); δ_C (125.8 MHz, $CDCl_3$) 20.5, 20.6, 20.7, 20.8 (4 x q, 4 x C(O)$\underline{C}H_3$D, 4 x C(O)$\underline{C}H_3$L), 23.0, 23.1 (2 x q, HNC(O)$\underline{C}H_3$D, HNC(O)$\underline{C}H_3$L), 31.0, 33.9 (2 x t, CH,H'D, CH,H'L), 52.2, 52.7 (2 x d, αCD, αCL), 61.6, 61.8 (2 x t, C-6D, C-6L), 67.9, 68.1 (2 x d, C-4D, C-4L), 69.2, 69.7 (2 x d, C-2D, C-2L), 73.5, 73.6 (2 x d, C-3D, C-3L), 76.0, 76.5 (2 x d, C-5D, C-5L), 83.1, 85.7 (2 x d, C-1D, C-1L), 169.4, 169.5, 169.6, 170.0, 170.1, 170.2, 170.3, 170.7 (8 x s, 4 x $\underline{C}$(O)CH_3D, 4 x $\underline{C}$(O)CH_3L, HN$\underline{C}$(O)CH_3D, HN$\underline{C}$(O)CH_3L), 172.3, 173.9 (2 x s, $\underline{C}ONH_2$D, $\underline{C}ONH_2$L); *m/z* (ES^+) 515 (MNa^+, 100%); HRMS (ES^+) Calcd. for $C_{19}H_{28}N_2NaO_{11}S$ (MNa^+) 515.1306. Found: 515.1304.

_N_-Acetyl-DL-cysteine-_S_-(2,3,4,6-tetra-_O_-acetyl-β-D-galactopyaranoside) methyl ester 12



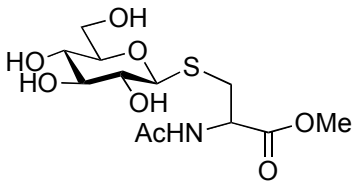
Using the general procedure, *N*-Acetyl-DL-cysteine-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyaranoside) methyl ester **12** was prepared as a colourless oil being a mixture of epimers (D:L, 1:1) on a 0.124 mmol (substrate) scale; Yield: 70%; R_f 0.5 (ethyl acetate:methanol, 9:1); υ_{max} (thin film) 3366 (br, NH) 1749 (s, C=O) 1664 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.98, 1.99, 2.05, 2.06, 2.07, 2.08, 2.08, 2.09, 2.16, 2.17 (30H, 10 x s, 4 x C(O)CH_3D, 4 x C(O)CH_3L, HNC(O)C$\underline{H}_3$D, HNC(O)C$\underline{H}_3$L), 3.04 (1H, dd, $J_{C\underline{H},H'}$ 14.1 Hz, $J_{CH,\alpha H}$ 6.4 Hz, C$\underline{H}$,H'D), 3.09 (1H, dd, $J_{CH,\underline{H}'}$ 14.1 Hz, $J_{CH',\alpha H}$ 4.4 Hz, CH,$\underline{H}$'D), 3.21 (1H, dd, $J_{C\underline{H},H'}$ 13.9 Hz, $J_{CH,\alpha H}$ 5.4 Hz, C$\underline{H}$,H'L), 3.24 (1H, dd, $J_{CH,\underline{H}'}$ 13.9 Hz, $J_{CH',\alpha H}$ 4.5 Hz, CH,$\underline{H}$'L), 3.77, 3.82 (6H, 2 x s, OCH_3D, OCH_3L), 3.96 (2H, at, *J* 6.5 Hz, H-5D, H-5L), 4.13 (2H, dd, $J_{5,6}$ 2.6 Hz, $J_{6,6'}$ 11.4 Hz, H-6D, H-6L), 4.18 (2H, dd, $J_{5,6'}$ 4.4 Hz, $J_{6,6'}$ 11.4 Hz, H-6'D, H-6'L), 4.49 (2H, 2 x d, $J_{1,2}$ 10.3 Hz, H-1D, H-1L), 4.79-4.85 (2H, m, αHD, αHL), 5.03 (1H, dd, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.4 Hz, H-3D), 5.07 (1H, dd, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.4 Hz, H-3L), 5.18 (1H, at, *J* 10.0 Hz, H-2D), 5.21 (1H, at, *J* 10.0 Hz, H-2L), 5.44 (2H, br s, H-4D, H-4L), 6.44 (1H, d, *J* 7.4 Hz, $\underline{H}$NC(O)CH_3D), 6.55 (1H, d, *J* 7.3 Hz, $\underline{H}$NC(O)CH_3L); δ_C (125.8 MHz, $CDCl_3$) 20.3, 20.5, 20.7, 20.8 (4 x q, 4 x C(O)$\underline{C}H_3$D, 4 x C(O)$\underline{C}H_3$L), 22.9, 23.0 (2 x q, HNC(O)$\underline{C}H_3$D, HNC(O)$\underline{C}H_3$L), 31.5, 32.6 (2 x t, CH,H'D, CH,H'L), 51.7, 52.2 (2 x d, αCD, αCL), 52.7, 52.8 (2 x q, OCH_3D, OCH_3L), 61.4, 61.8 (2 x t, C-6D, C-6L), 66.8, 67.1 (2 x d, C-2D, C-2L), 67.2, 67.4 (2 x d, C-4D, C-4L), 71.6, 71.7 (2 x d, C-3D, C-3L), 74.6, 74.9 (2 x d, C-5D, C-5L), 83.6, 84.2 (2 x d, C-1D, C-1L), 169.6, 169.7, 169.8, 169.9, 169.9, 170.0, 170.1, 170.2, 170.3, 170.4, 170.6, 170.9 (12 x s, 4 x $\underline{C}$(O)CH_3D, 4 x $\underline{C}$(O)CH_3L, HN$\underline{C}$(O)CH_3D, HN$\underline{C}$(O)CH_3L, $\underline{C}O_2CH_3$D, $\underline{C}O_2CH_3$L); *m/z* (ES^+) 566 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{20}H_{29}NNaO_{12}S$ (MNa^+) 530.1303. Found: 530.1321.

_N_-Acetyl-DL-cysteine-_S_-(3,4,6-tetra-_O_-acetyl-2-acetamido-2-deoxy-β-D-glucopyaranoside) methyl ester 14



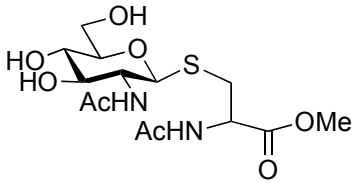
Using the general procedure, *N*-acetyl-DL-cysteine-*S*-(3,4,6-tetra-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyaranoside) methyl ester **14** was prepared as a colourless oil being a mixture of epimers (D:L, 6:5) on a 0.082 mmol (substrate) scale; Yield: 73%; R_f 0.3 (ethyl acetate); $[\alpha]_D^{24}$ -15.6 (c, 1 in $CHCl_3$); υ_{max} (thin film) 3386 (br, NH) 1743 (s, C=O) 1656 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.96, 1.97, 1.98, 2.03, 2.04, 2.06, 2.07, 2.10, 2.11, 2.20 (30H, 10 x s, 3 x C(O)CH_3D, 3 x C(O)CH_3L, 2 x HNC(O)C$\underline{H}_3$D, 2 x HNC(O)C$\underline{H}_3$L), 3.02 (1H, dd, $J_{C\underline{H},H'}$ 14.2 Hz, $J_{CH,\alpha H}$ 6.5 Hz, C$\underline{H}$,H'D), 3.05 (1H, dd, $J_{CH,\underline{H}'}$ 14.2 Hz, $J_{CH',\alpha H}$ 4.8 Hz, CH,$\underline{H}$'D), 3.21 (1H, dd, $J_{C\underline{H},H'}$ 14.2 Hz, $J_{CH,\alpha H}$ 5.7 Hz, C$\underline{H}$,H'L), 3.29 (1H, dd, $J_{CH,\underline{H}'}$ 14.2 Hz, $J_{CH',\alpha H}$ 3.9 Hz, CH,$\underline{H}$'L), 3.67-3.73 (2H, m, H-5D, H-5L), 3.76, 3.77 (6H, 2 x s, OCH_3D, OCH_3L), 4.04 (1H, at, *J* 9.8 Hz, H-2D), 4.06 (1H, at, *J* 9.8 Hz, H-2L), 4.14 (2H, dd, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.5 Hz, H-6D, H-6L), 4.24 (2H, dd, $J_{5,6'}$ 4.7 Hz, $J_{6,6'}$ 12.5 Hz, H-6'D, H-6'L), 4.57 (1H, d, $J_{1,2}$ 10.4 Hz, H-1D), 4.59 (1H, d, $J_{1,2}$ 10.0 Hz, H-1L), 4.88-4.94 (2H, m, αHD, αHL), 5.06 (1H, at, *J* 9.8 Hz, H-4D), 5.10 (1H, at, *J* 9.8 Hz, H-4D), 5.13 (1H, at, *J* 9.6 Hz, H-3D), 5.16 (1H, at, *J* 9.5 Hz, H-3L), 5.71 (1H, d, $J_{N\underline{H},H\text{-}2}$ 9.4 Hz, $\underline{H}$NC(O)CH_3D, H-2D), 5.81 (1H, d, $J_{N\underline{H},H\text{-}2}$ 9.3 Hz, $\underline{H}$NC(O)CH_3L, H-2L), 6.58 (1H, d, $J_{N\underline{H},\alpha H}$ 8.1 Hz, $\underline{H}$NC(O)CH_3D, αHD), 6.68 (1H, d, $J_{N\underline{H},\alpha H}$ 7.3 Hz, $\underline{H}$NC(O)CH_3L, αHL); δ_C (125.8 MHz, $CDCl_3$) 20.5, 20.6, 20.7, 20.8 (4 x q, 3 x C(O)$\underline{C}H_3$D, 3 x C(O)$\underline{C}H_3$L), 22.9, 23.0, 23.2, 23.3 (4 x q, 2 x HNC(O)$\underline{C}H_3$D, 2 x HNC(O)$\underline{C}H_3$L), 31.9, 32.2 (2 x t, CH,H'D, CH,H'L), 50.8, 52.2 (2 x d, αCD, αCL), 52.7, 52.8 (2 x q, OCH_3D, OCH_3L), 52.9, 53.5 (2 x d, C-2D, C-2L), 62.0, 62.1 (2 x t, C-6D, C-6L), 67.9, 68.2 (2 x d, C-4D, C-4L), 73.5, 73.8 (2 x d, C-3D, C-3L), 76.1, 76.3 (2 x d, C-5D, C-5L), 84.2, 84.3 (2 x d, C-1D, C-1L), 169.2, 169.3, 169.4, 170.1, 170.4, 170.5, 170.6, 170.7, 170.8, 170.9, 171.0, 171.3 (12 x s, 3 x $\underline{C}$(O)CH_3D, 3 x $\underline{C}$(O)CH_3L, 2 x HN$\underline{C}$(O)CH_3D, 2 x HN$\underline{C}$(O)CH_3L, $\underline{C}O_2CH_3$D, $\underline{C}O_2CH_3$L); *m/z* (ES^+) 565 ($MMeCNNH_4^+$, 100%). HRMS (ES^+) Calcd. for $C_{20}H_{30}N_2NaO_{11}S$ (MNa^+) 529.1463. Found: 529.1462.

_N_-Acetyl-DL-cysteine-_S_-(β-D-glucopyranoside) methyl ester 16



Using the general procedure, *N*-acetyl-DL-cysteine-*S*-(β-D-glucopyranoside) methyl ester **16** was prepared as a white foam being a mixture of epimers (D:L, 1:1) on a 0.064 mmol (substrate) scale; Yield: 68%; R_f 0.3 (ethyl acetate:*iso*-propanol:water, 5:3:1); $[\alpha]_D^{22}$ +0.0 (c, 0.5 in MeOH); υ_{max} (thin film) 3442 (br, NH OH) 1643 (s, C=O) cm^{-1}; δ_H (500 MHz, CD_3OD) 2.03, 2.04 (6H, 2 x s, HNC(O)C$\underline{H}_3$D, HNC(O)C$\underline{H}_3$L), 2.95 (1H, dd, $J_{C\underline{H},H'}$ 14.2 Hz, $J_{CH,\alpha H}$ 8.1 Hz, C$\underline{H}$,H'D), 3.09 (1H, dd, $J_{C\underline{H},H'}$ 14.3 Hz, $J_{CH,\alpha H}$ 8.3 Hz, C$\underline{H}$,H'L), 3.20 (1H, dd, $J_{CH,\underline{H}'}$ 14.5 Hz, $J_{CH',\alpha H}$ 7.8 Hz, CH,$\underline{H}$'L), 3.26-3.40 (7H, m, H-2D, H-2L, H-3D, H-3L, H-4D, H-4L, CH,$\underline{H}$'D), 3.59-3.66 (2H, m, H-5D, H-5L), 3.69 (1H, dd, $J_{5,6}$ 6.0 Hz, $J_{6,6'}$ 13.0 Hz, H-6D), 3.76 (6H, s, OCH3D, OCH3L), 3.77-3.79 (1H, m, H-6L), 3.83 (1H, dd, $J_{5,6'}$ 2.4 Hz, $J_{6,6'}$ 12.0 Hz, H-6'D), 3.90 (1H, dd, $J_{5,6'}$ 1.7 Hz, $J_{6,6'}$ 12.0 Hz, H-6'L), 4.41 (1H, d, $J_{1,2}$ 9.7 Hz, H-1D), 4.43 (1H, d, $J_{1,2}$ 9.7 Hz, H-1L), 4.69-4.72 (1H, m, αHD), 4.76-4.78 (1H, m, αHL); δ_C (125.8 MHz, CD_3OD) 22.4, 22.5 (2 x q, HNC(O)$\underline{C}H_3$D, HNC(O)$\underline{C}H_3$L), 31.9, 33.0 (2 x t, CH,H'D, CH,H'L), 52.9, 54.3 (2 x d, αCD, αCL), 54.6, 55.5 (2 x q, OCH_3D, OCH_3L), 62.7, 62.9 (2 x t, C-6D, C-6L), 71.6, 71.8 (2 x d, C-2D, C-2L), 74.2, 74.4 (2 x d, C-5D, C-5L), 78.1, 79.5 (2 x d, C-4D, C-4L), 82.1, 82.3 (2 x d, C-3D, C-3L), 86.8, 87.8 (2 x d, C-1D, C-1L), 172.7, 173.5 (2 x s, HN$\underline{C}$(O)CH_3D, HN$\underline{C}$(O)CH_3L, $\underline{C}O_2CH_3$D, $\underline{C}O_2CH_3$L); *m/z* (ES^+) 362 (MNa^+, 100%); HRMS (ES^+) Calcd. for $C_{12}H_{21}NNaO_8S$ (MNa^+) 362.0880. Found: 362.0870.

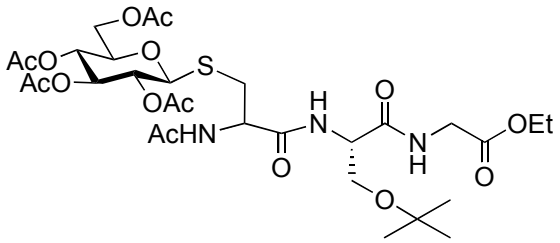
_N_-Acetyl-DL-cysteine-_S_- (2-acetamido-2-deoxy-β-D-glucopyranoside) methyl ester 18



Using the general procedure, *N*-acetyl-DL-cysteine-*S*-(2-acetamido-2-deoxy-β-D-glucopyranoside) methyl ester **18** was prepared as a white amorphous solid being a mixture of epimers (D:L, 1:1) on a 0.072 mmol (substrate) scale; Yield: 74%; R_f 0.3 (ethyl acetate:*iso*-propanol:water, 5:3:1); $[\alpha]_D^{18}$ +1.6 (c, 1 in MeOH); υ_{max} (KBr disc) 3413 (br, NH OH) 1735 (s, C=O) 1646 (s, C=O) cm^{-1}; δ_H (500 MHz, CD_3OD) 1.99,

2.00, 2.04, 2.18 (12H, 4 x s, 2 x HNC(O)C$\underline{H}_3$D, 2 x HNC(O)C$\underline{H}_3$L), 2.83 (1H, dd, $J_{C\underline{H},H'}$ 14.2 Hz, $J_{CH,\alpha H}$ 9.1 Hz, C$\underline{H}$,H'D), 3.07 (1H, dd, $J_{C\underline{H},H'}$ 14.1 Hz, $J_{CH,\alpha H}$ 7.8 Hz, C$\underline{H}$,H'L), 3.11 (1H, dd, $J_{CH,\underline{H}'}$ 14.2 Hz, $J_{CH',\alpha H}$ 5.5 Hz, CH,$\underline{H}$'L), 3.33-3.37 (3H, m, CH,$\underline{H}$'D, H-4D), 3.48 (2H, at, *J* 9.0 Hz, H-3D, H-3L), 3.54-3.59 (1H, m, H-5D), 3.74, 3.75 (6H, 2 x s, OCH_3D, OCH_3L), 3.66-3.76 (4H, m, H-2L, H-5L, H-6D, H-6L), 3.80-3.97 (3H, m, H-2D, H-6'D, H-6'L), 4.52 (1H, d, $J_{1,2}$ 10.3 Hz, H-1D), 4.58 (1H, d, $J_{1,2}$ 10.3 Hz, H-1L), 4.67-4.76 (2H, m, αHD, αHL); δ_C (125.8 MHz, CD_3OD) 22.5, 22.6, 23.0, 25.3 (4 x q, 2 x HNC(O)$\underline{C}H_3$D, 2 x HNC(O)$\underline{C}H_3$L), 32.0, 33.3 (2 x t, CH,H'D, CH,H'L), 52.9, 53.0 (2 x q, OCH_3D, OCH_3L), 54.1, 54.5 (2 x d, αCD, αCL), 55.8, 56.3 (2 x d, C-2D, C-2L), 62.7, 62.8 (2 x t, C-6D, C-6L), 73.0, 73.7 (2 x d, C-4D, C-4L), 77.1, 77.2 (2 x d, C-5D, C-5L), 82.2, 82.4 (2 x d, C-3D, C-3L), 85.3, 86.8 (2 x d, C-1D, C-1L), 172.7, 172.8, 173.5, 173.6, 173.7, 173.8 (6 x s, 2 x HN$\underline{C}$(O)CH_3D, 2 x HN$\underline{C}$(O)CH_3L, $\underline{C}O_2CH_3$D, $\underline{C}O_2CH_3$L); *m/z* (ES^+) 403 (MNa^+, 100%); HRMS (ES^+) Calcd. for $C_{14}H_{24}N_2NaO_8S$ (MNa^+) 403.1146. Found: 403.1141.

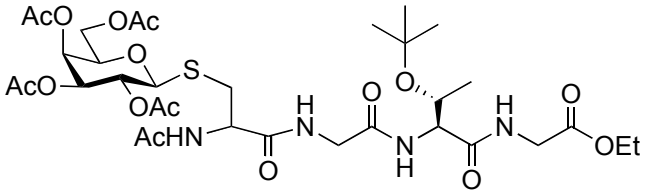
_N_-Acetyl-DL-cysteine-_S_-(2,3,4,6-tetra-_O_-acetyl-β-D-glucopyaranoside)-_O-tert_-butyl-L-serine-glycine ethyl ester 20



Using the general procedure, *N*-acetyl-DL-cysteine-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyaranoside)-*O*-*tert*-butyl-L-serine-glycine ethyl ester **20** was prepared as a colourless oil being a mixture of epimers (D:L, 1:1) on a 0.066 mmol (substrate) scale; Yield: 73%; R_f 0.6 (petrol:ethyl acetate, 1:4); $[\alpha]_D^{22}$ +0.1 (c, 0.5 in $CHCl_3$); υ_{max} (thin film) 3334 (br, NH) 1751 (s, C=O) 1661 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.19, 1.22 (18H, 2 x s, $C(CH_3)_3$D, $C(CH_3)_3$L), 1.26 (6H, t, *J* 7.2 Hz, $OCH_2C\underline{H}_3$D, $OCH_2C\underline{H}_3$L), 1.99, 2.01, 2.03, 2.04, 2.05, 2.06, 2.07, 2.08, 2.09, 2.12 (30H, 10 x s, 4 x C(O)CH_3D, 4 x C(O)CH_3L, HNC(O)C$\underline{H}_3$D, HNC(O)C$\underline{H}_3$L), 2.90 (2H, 2 x dd, $J_{C\underline{H},H'}$ 14.0 Hz, $J_{CH,\alpha H}$ 9.5 Hz, C$\underline{H}$,H'cysD, C$\underline{H}$,H'cysL), 3.11 (2H, dd, $J_{CH,\underline{H}'}$ 14.0 Hz, $J_{CH',\alpha H}$ 4.9 Hz, CH,$\underline{H}$'cysD, CH,$\underline{H}$'cysL), 3.35-3.48 (4H, m, CH,H'serD, CH,H'serL), 3.75-3.80 (1H, m, H-5D), 3.85-3.93 (1H, m, H-5L), 4.01-4.06 (4H, m, 2 x αHglyD, 2 x αHglyL), 4.16-4.26 (6H, m, $OC\underline{H}_2CH_3$D, $OC\underline{H}_2CH_3$L, H-6D, H-6L), 4.38 (1H, dd, $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 12.6 Hz, H-6'D), 4.41 (1H, dd, $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 12.6 Hz, H-6'L), 4.46-4.56 (2H, m, αHserD,

αHserL), 4.59 (1H, d, $J_{1,2}$ 9.8 Hz, H-1D), 4.77 (1H, d, $J_{1,2}$ 10.2 Hz, H-1L), 4.85-4.88 (2H, m, αHcysD, αHcysL), 5.03 (2H, at, *J* 9.8 Hz, H-2D, H-2L), 5.11 (1H, at, *J* 9.7 Hz, H-4D), 5.17 (1H, at, *J* 9.7 Hz, H-4L), 5.26 (2H, at, *J* 9.9 Hz, H-3D, H-3L), 6.51 (2H, d, $J_{NH,\alpha H}$ 7.4 Hz, HNC(O)CH_3cysD, HNC(O)CH_3cysL), 6.65 (2H, d, $J_{NH,\alpha H}$ 6.9 Hz, HNC(O)CH_3serD, HNC(O)CH_3serL), 7.44 (2H, d, $J_{NH,\alpha H}$ 7.8 Hz, HNC(O)CH_3glyD, HNC(O)CH_3glyL); δ_C (125.8 MHz, $CDCl_3$) 14.1, 14.2 (2 x q, $OCH_2\underline{C}H_3$D, $OCH_2\underline{C}H_3$L), 20.5, 20.6, 20.7, 2.08, 21.0 (5 x q, 4 x C(O)$\underline{C}H_3$D, 4 x C(O)$\underline{C}H_3$L), 22.9, 23.0 (2 x q, HNC(O)$\underline{C}H_3$D, HNC(O)$\underline{C}H_3$L), 27.3, 27.4 (2 x q, C($\underline{C}H_3)_3$D, C($\underline{C}H_3)_3$L), 34.6 (2 x t, CH,H'cysD, CH,H'cysL), 41.2, 41.4 (2 x t, αCglyD, αCglyL), 52.4, 53.1, 53.4, 54.1 (4 x d, αCcysD, αCcysL, αCserD, αCserL), 60.4, 60.9, 61.1, 61.3, 61.4, 62.3 (6 x t, O$\underline{C}H_2CH_3$D, O$\underline{C}H_2CH_3$L, C-6D, C-6L, CH,H'serD, CH,H'serL), 67.8, 68.3 (2 x d, C-4D, C-4L), 69.0, 69.7 (2 x d, C-2D, C-2L), 73.6, 74.1 (d, C-3D, C-3L), 76.4, 76.5 (2 x d, C-5D, C-5L), 83.1, 85.6 (2 x d, C-1D, C-1L), 169.0, 169.2, 169.4, 169.5, 169.6, 169.7, 169.8, 169.9, 170.0, 170.1, 170.2, 170.4, 170.9, 171.1 (14 x s, 4 x $\underline{C}$(O)CH_3D, 4 x $\underline{C}$(O)CH_3L, HN$\underline{C}$(O)CH_3D, HN$\underline{C}$(O)CH_3L, $\underline{C}$(O)HNαCserD, $\underline{C}$(O)HNαCserL, $\underline{C}$(O)HNαCglyD, $\underline{C}$(O)HNαCglyL, $\underline{C}O_2CH_3$D, $\underline{C}O_2CH_3$L); *m/z* (ES^+) 780 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{30}H_{47}N_3NaO_{15}S$ (MNa^+) 744.2620. Found: 744.2608.

***N*-Acetyl-DL-cysteine-*S*- (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyaranoside)-glycine-*O*-*tert*-butyl-L-threonine-glycine ethyl ester 22**

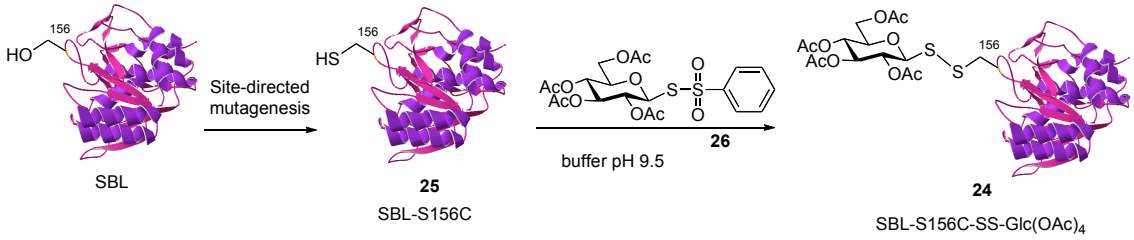


Using the general procedure, *N*-acetyl-DL-cysteine-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyaranoside)-glycine-*O*-*tert*-butyl-L-threonine-glycine ethyl ester **22** was prepared as a colourless oil being a mixture of epimers (D:L, 1:1) on a 0.057 mmol (substrate) scale; Yield: 67%; R_f 0.5 (DCM:methanol, 9:1); $[\alpha]_D^{18}$ -1.7 (c, 0.5 in $CHCl_3$); υ_{max} (thin film) 3382 (br, NH) 1725 (s, C=O) 1652 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.05, 1.07 (6H, 2 x d, *J* 6.5 Hz, CHC$\underline{H}_3$thrD, CHC$\underline{H}_3$thrL), 1.28-1.54 (24H, m, C($CH_3)_3$D, C($CH_3)_3$L, $OCH_2C\underline{H}_3$D, $OCH_2C\underline{H}_3$L), 1.98, 1.99, 2.00, 2.01, 2.05, 2.06, 2.07, 2.09 (24H, 8 x s, 4 x C(O)CH_3D, 4 x C(O)CH_3L), 2.18, 2.22 (6H, 2 x s, HNC(O)C$\underline{H}_3$D, HNC(O)C$\underline{H}_3$L), 2.87-3.00 (3H, m, C$\underline{H}$,H'cysD, C$\underline{H}$,H'cysL, CH,$\underline{H}$'cysL), 3.13 (1H, dd, $J_{CH,\underline{H}'}$ 14.4 Hz, $J_{CH',\alpha H}$ 5.4 Hz, CH,$\underline{H}$'cysD), 3.53-4.23 (16H, m, H-5D, H-5L, H-6D, H-6L, H-6'D, H-6'L, O$\underline{C}H_2CH_3$D, O$\underline{C}H_2CH_3$L, 2 x αHglyD, 2 x αHglyL, C$\underline{H}CH_3$thrD, C$\underline{H}CH_3$thrL),

4.49 (2H, dd, *J* 3.6 Hz, *J* 6.2 Hz, αHthrD, αHthrL), 4.66 (2H, 2 x d, $J_{1,2}$ 10.1 Hz, H-1D, H-1L), 4.77 (1H, dd, *J* 3.8 Hz, *J* 6.3 Hz, αHcysD), 4.83 (1H, dd, *J* 3.6 Hz, *J* 5.8 Hz, αHcysL), 4.99-5.47 (6H, m, H-2D, H-2L, H-3D, H-3L, H-4D, H-4L), 6.60, 6.97, 7.10, 7.17, 7.37, 7.66 (6H, 6 x d, *J* 4.2 Hz, *J* 5.4 Hz, *J* 5.8 Hz, *J* 6.7 Hz, *J* 8.3 Hz, 4 x HNC(O)CH_3D, 4 x HNC(O)CH_3L); δ_C (125.8 MHz, $CDCl_3$) 14.1, 14.2 (2 x q, OCH_2CH_3D, OCH_2CH_3L), 17.3, 17.4 (2 x q, $CHCH_3$thrD, $CHCH_3$thrL), 20.5, 20.6, 20.7, 2.08, 20.9, 21.0 (6 x q, 4 x C(O)CH_3D, 4 x C(O)CH_3L), 22.9, 23.0 (2 x q, HNC(O)CH_3D, HNC(O)CH_3L), 28.1, 28.2 (6 x q, $C(CH_3)_3$D, $C(CH_3)_3$L), 41.3, 41.4, 41.5, 41.6, 41.7, 41.8 (6 x t, 2 x CH,H'glyD, 2 x CH,H'glyL, CH,H'cysD, CH,H'cysL), 52.3, 52.7 (2 x d, αCcysD, αCcysL), 57.5, 57.6 (2 x d, αCthrD, αCthrL), 61.4, 61.5 (2 x t, OCH_2CH_3D, OCH_2CH_3L), 62.0, 62.4 (2 x t, C-6D, C-6L), 65.9, 66.1, 66.4, 66.7, 67.2, 67.6 (6 x d, C-2D, C-2L, C-3D, C-3L, C-4D, C-4L), 71.3, 71.6 (2 x s, $C(CH_3)_3$D, $C(CH_3)_3$L), 74.9, 75.4 (2 x d, C-5D, C-5L), 75.7, 75.9 (2 x d, $CHCH_3$thrD, $CHCH_3$thrL), 83.1, 84.9 (2 x d, C-1D, C-1L), 169.9, 170.1, 170.3, 170.8, 170.9, 172.4, 172.5 (7 x s, 4 x C(O)CH_3D, 4 x C(O)CH_3L, HNC(O)CH_3D, HNC(O)CH_3L); *m/z* (ES^+) 851 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{33}H_{52}N_4NaO_{16}S$ (MNa^+) 815.2991. Found: 815.2990.

Protein Modification

Preparation of SBL-S156C-SS-Glc(OAc)$_4$ ***23***



Scheme S4.

SBL-S156C mutant **25** (2.5 mg) was dissolved in buffer (500 μL, 70 mM CHES, 5 mM MES, 2 mM $CaCl_2$, pH 9.5). A solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside phenylthiosulfonate **26** (100 μL of a 10 mM solution in acetonitrile) was added and placed on an end-over-end rotator. After 30 min, the reaction mixture was purified by size exclusion chromatography Sephadex® G25 column against the above buffer. The protein fraction was analysed by LC-mass spectrometry to afford the modified protein **23** (calculated mass, 27078; observed mass, 27075).

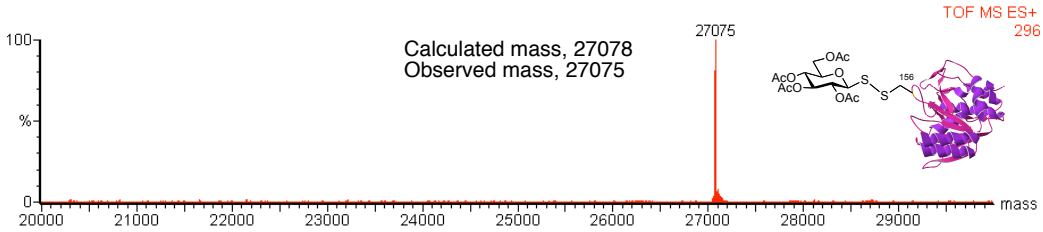
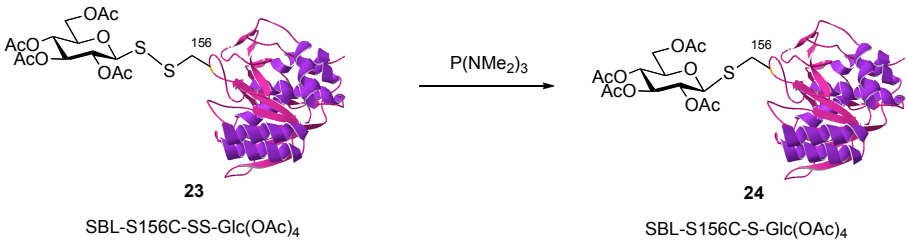


Figure S1. ESI-MS spectrum of SBL-S156C-*SS*-Glc(OAc)$_4$ **23**.

Desulfurization of SBL-S156C-SS-Glc(OAc)$_4$ ***23***



Scheme S5.

To a degassed solution of SBL-S156C-SS-Glc(OAc)$_4$ **23** (250 μL of a 2 mg/mL solution in buffer (70 mM CHES, 5 mM MES, 2 mM $CaCl_2$, pH 9.5)) hexamethylphosphorus triamide was added (6.0 μL, 2 equivalent). The reaction was placed on an end-over-

end rotator. After 12 h, the reaction mixture was purified by size exclusion chromatography Sephadex® G25 column against the above buffer and analysed by LC-mass spectrometry to afford SBL-S156C-S-Glc(OAc)$_4$ **24** (calculated mass, 27046; observed mass 27044).

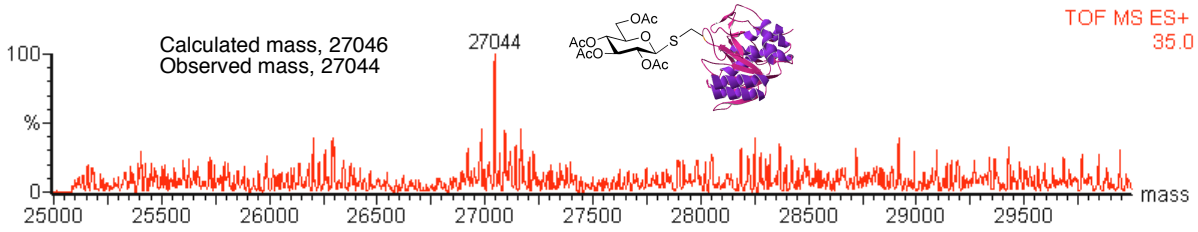
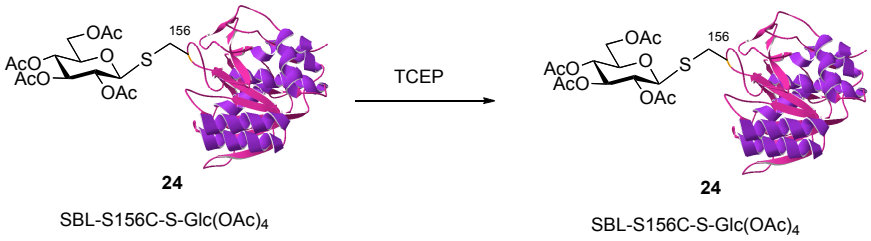


Figure S2. ESI-MS spectrum of SBL-S156C-*S*-Glc(OAc)$_4$ **24**.

*Treatment of **24** with TCEP*



Scheme S6.

To a degassed solution of SBL-S156C-S-Glc(OAc)$_4$ **24** (80 μL of a 2 mg/mL solution in buffer (70 mM CHES, 5 mM MES, 2 mM $CaCl_2$, pH 9.5)) tris(2-carboxyethyl) phosphine (TCEP) (0.4 μL of a 200 mmol aqueous solution, pH 7.0). The reaction was placed on an end-over-end rotator for 10 min, purified by size exclusion chromatography Sephadex® G25 column against the above buffer and analysed by LC-mass spectrometry and the tioether-linked glycoprotein 24 was shown to be stable under reducing conditions (calculated mass, 27044; observed mass 27046).

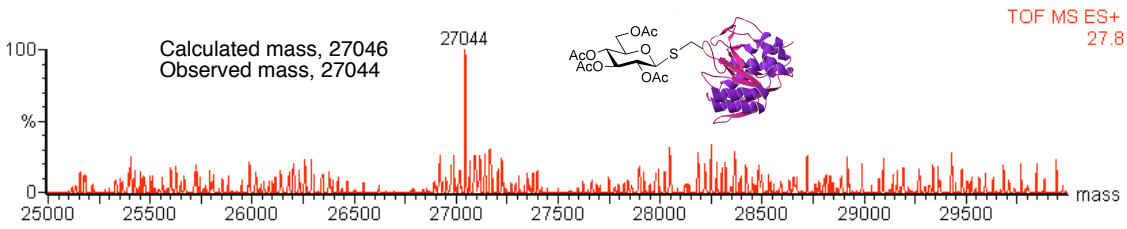
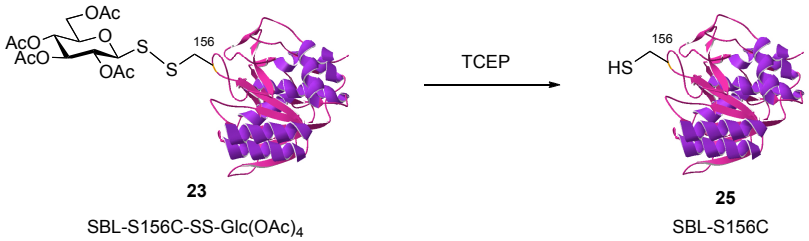


Figure S3. ESI-MS spectrum of SBL-S156C-*S*-Glc(OAc)$_4$ **24** when treated with TCEP.

*Treatment of **23** with TCEP*



Scheme S7.

To a degassed solution of SBL-S156C-SS-Glc(OAc)$_4$ **23** (150 μL of a 2 mg/mL solution in buffer (70 mM CHES, 5 mM MES, 2 mM $CaCl_2$, pH 9.5)) tris(2-carboxyethyl) phosphine (TCEP) (1 μL of a 200 mmol aqueous solution, pH 7.0). The reaction was placed on an end-over-end rotator for 10 min, purified by size exclusion chromatography Sephadex® G25 column against the above buffer and analysed by LC-mass spectrometry to afford SBL-S156C **25** (calculated mass, 26714; observed mass 26718).

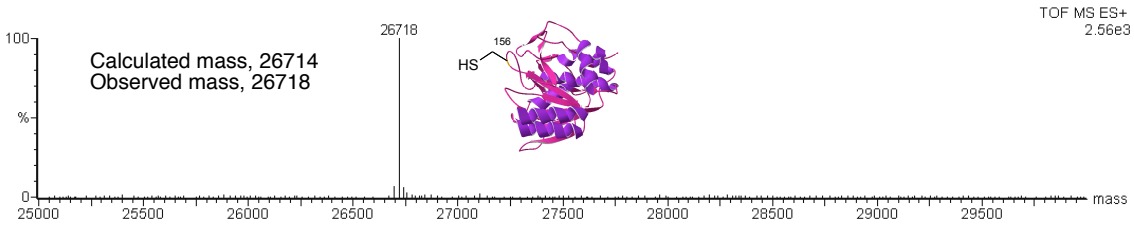
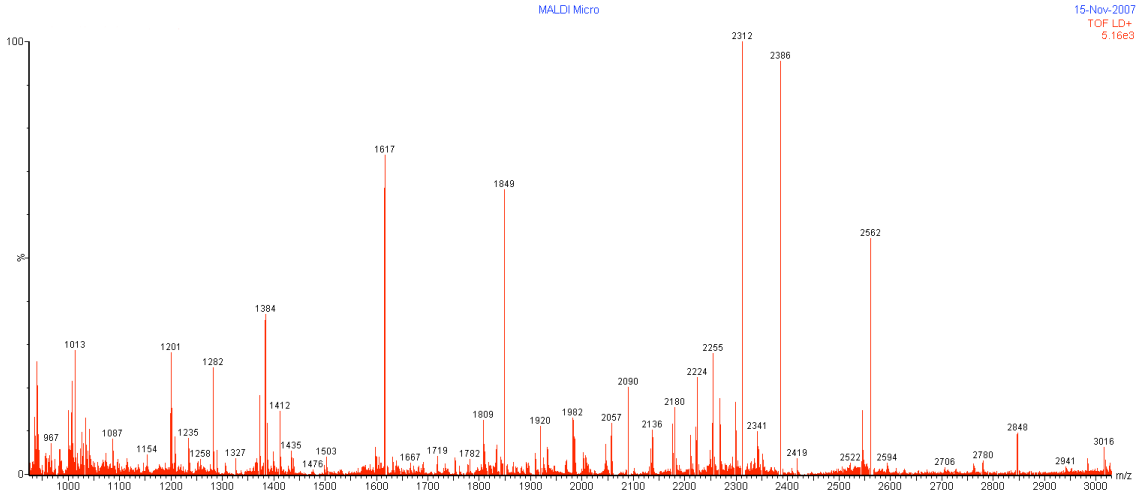


Figure S4. ESI-MS spectrum of SBL-S156C **25**, formed from reaction of **23** with TCEP.

Trypsin digestion and MALDI analysis of 24

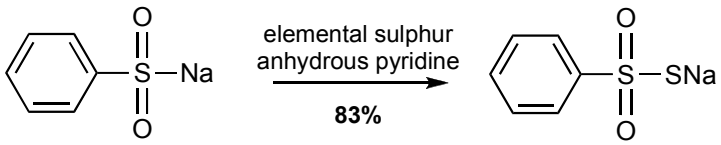
Thioether protein **24** (20 μl of 1mg/mL in 100 mM NH_4HCO_3, pH 8.0) was incubated with 1μg of trypsin (Promega) overnight at 37 °C. Peptides were extracted and desalted with a C18 ZipTip (Millipore Corp.) according to the manufacturer's specifications. Eluted peptides were mixed 1:1 (v/v) with a solution of α-cyano-4-hydroxycinnamic acid (saturated in 50% MeCN in H_2O with 0.1% TFA). From this mixture, 2 μl were spotted onto a steel target and analyzed in positive mode on a Waters Micro-Mass MALDI. A three point calibration curve of Angiotensin (1296.5), Renin (1759.0), and ACTH (18-34 clip, 2465.7) was applied to data with ACTH as the lock mass. 8 of 13 predicted peptides of **24** were observed including the peptide containing Cys156Glc(OAc)$_4$. (2312 calculated, 2312 found).



Peptide Fragment (cut predicted after all R and K residues)	Calcd. Mass	Observed Mass
AQSVPWGISR	1101	1087
VQAPAAHNR	964	967
GLTGSGVK	718	-
VAVLDTGISTHPDLNIR	1822	1849
GGASFVPGEPSTQDGNGHGTHVAGTIAALNNSIGVLGVAPSAELYAVK	4591	-
VLGASGSGSVSSIAQGLEWAGNNGMHVANLSLGSPSPSATLEQAVNSATSR	4927	-
GVLVVAASGN[CGlc(OAc)$_4$]GAGSISYPAR	2313	2312
YANAMAVGATDQNNNR	1711	1719
ASFSQYGAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVK	4800	-
QK	275	-
NPSWSNVQIR	1201	1201
NHLK	511	516
NTATSLGSTNLYGSGLVNAEAATR	2370	2386

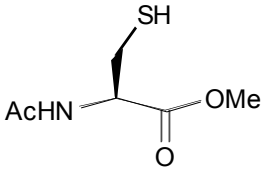
Disulfide-linked glycoaminoacids and glycopeptides - prepared using glycoPTS[2] or glycoSeS[3] strategy

Preparation of Sodium phenylthiosulfonate (Na-PTS)[4] **26**



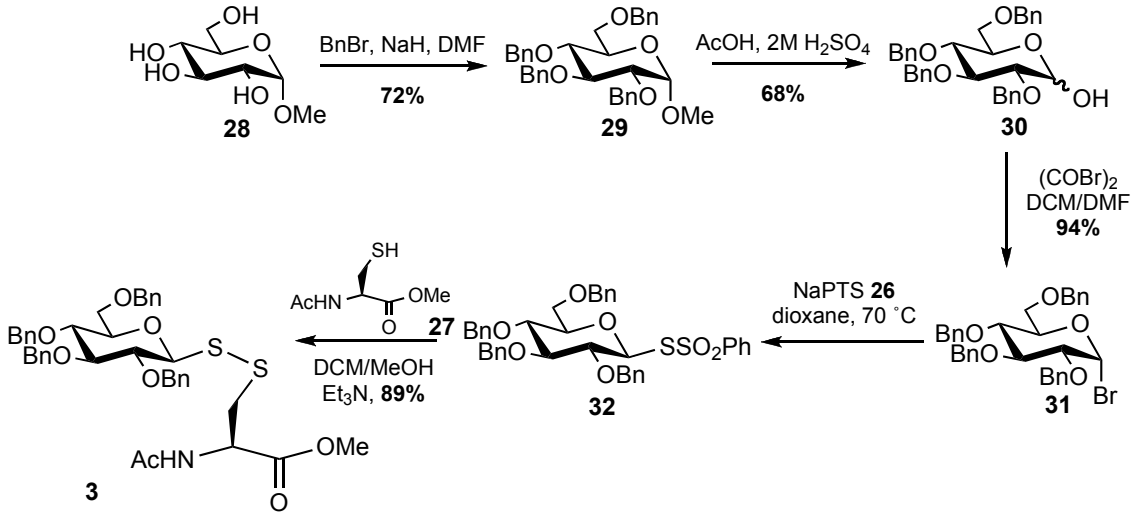
Sodium benzenesulfinate (10.0 g, 61 mmol) and elemental sulphur (1.9 g, 61 mmol) was dissolved in anhydrous pyridine (60 mL) to give a yellow solution. The reaction was stirred under argon and after 1 h gave a white suspension. The reaction was filtered and washed with anhydrous diethyl ether. Recrystalisation from anhydrous ethanol afforded sodium phenylthiosulfonate **26** (9.8 g, 83%) as a white crystalline solid; m.p. 282-284 ºC (ethanol) [Lit. 287 ºC][4]; δ_H (400 MHz, D_2O) 3.26 (3H, s, CH_3); m/z (ES^-) 173 ($M\text{-}Na^+$, 100%).

Synthesis of *N*-acetyl-L-cysteine-methyl ester[5] **27**



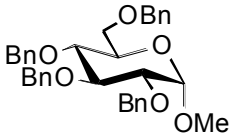
Thionyl chloride (2 mL, 26.99 mmol) was carefully added to a solution of *N*-acetyl-L-cysteine (4 g, 24.54 mmol) in anhydrous methanol, and the resulting mixture was stirred for 3 h at RT. The reaction mixture was concentrated *in vacuo*, diluted with ethyl acetate (75 mL), and washed with sodium hydrogen carbonate (50 mL of a saturated aqueous solution). The aqueous layer was re-extracted with ethyl acetate (2 x 75 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford *N*-acetyl-L-cysteine methyl ester **27** (2.32 g, 54%) as a white crystalline solid; m.p. 78-80 ºC (ethyl acetate) [Lit. 79-80 ºC (ethyl acetate)][5]; $[\alpha]_D^{22}$ -23.2 (c, 1 in CD_3OD) [Lit. $[\alpha]_D^{25}$ -24 (c, 1 in MeOH)][6]; δ_H (400 MHz, $CDCl_3$) 1.34 (1H, t, *J* 9.0 Hz, SH), 2.08 (3H, s, $COCH_3$), 3.00-3.04 (2H, m, CH_2), 3.80 (3H, s, CO_2CH_3), 4.88-4.92 (1H, m, αH), 6.40 (1H, br s, NH); *m/z* (ES^-) 176 ($M\text{-}H^+$, 100%).

Synthesis of *N*-Acetyl-L-cysteine (2,3,4,6-tetra-*O*-benzyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester 3



Scheme S8.

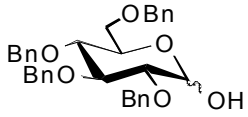
Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside[7] 29



Methyl α-D-glucopyranoside **28** (25 g, 129 mmol) was dissolved in anhydrous DMF (250 mL), and sodium hydride (60% dispersed in mineral oil) (31 g, 774 mmol) was added portionwise for a period of 10 min at 0 ºC. Benzyl bromide (92 mL, 770 mmol) was then added dropwise and the mixture left to stir under an atmosphere of argon at room temperature. After a 24 h period, t.l.c. (petrol:ethyl acetate, 3:1) indicated the formation of a product (R_f 0.4) with complete consumption of the starting material (R_f 0). The reaction mixture was quenched by the slow addition of methanol (150 mL) and stirred for 30 min, at which point the resulting solution was concentrated *in vacuo*. The residue was dissolved in DCM (800 mL), washed with water (2 x 500 mL), and brine (500 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash column chromatography (petrol:ethyl acetate, 6:1) afforded methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside **29** (51.5 g, 72%) as a viscous yellow oil; $[\alpha]_D^{21}$ +19.3 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{22}$ +21.2 (c, 1 in H_2O)][7]; δ_H (400 MHz, $CDCl_3$) 3.39 (3H, s, OCH_3), 3.57 (1H, dd, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.6 Hz, H-2), 3.63 (1H, d, *J* 9.6 Hz, H-4), 3.64 (1H, dd, $J_{5,6}$ 2.3 Hz, $J_{6,6'}$ 13.1 Hz, H-6), 3.71-3.78 (2H, m, H-5, H-6'), 4.00 (1H, at, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 9.2 Hz, H-3), 4.48, 4.84 (2H, ABq, $J_{A,B}$ 11.0 Hz, OCH_2Ph), 4.49, 4.68 (2H, ABq, $J_{A,B}$ 12.1 Hz, OCH_2Ph), 4.59, 4.81 (2H, ABq, $J_{A,B}$ 8.9 Hz, OCH_2Ph), 4.64 (1H, d,

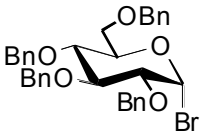
$J_{1,2}$ 3.6 Hz, H-1), 4.91, 4.92 (2H, ABq, $J_{A,B}$ 10.9 Hz, OCH_2Ph), 7.13-7.16 (2H, m, Ar-H), 7.27-7.39 (18H, m, Ar-H); *m/z* (ES^+) 577 (MNa^+, 50%) 613 ($MMeCNNH_4^+$, 100%).

2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose[8] 30



Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside **29** (15.33 g, 27.64 mmol) was dissolved in glacial acetic acid (300 mL). The mixture was heated to 90 ºC with stirring, at which point sulfuric acid (2M, 75 mL) was added. After a further 2 h period, sulfuric acid (2M, 75 mL) was added. After 22 h, t.l.c. (petrol:ethyl acetate, 4:1) indicated formation of a major product (R_f 0.4) and complete consumption of starting material (R_f 0.5). Water (200 mL) was added and the reaction mixture cooled to 0 ºC, at which point a crystalline solid precipitated. The crystals were filtered off and washed with methanol (80% V/V) affording 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose **30** (10.25 g, 68%) as a white crystalline solid, being a mixture of anomers (α:β, 1:1); m.p. 148-150 ºC (ethyl acetate/petrol) [Lit. 151-152 ºC][8]; $[\alpha]_D^{23}$ +20.7 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{25}$ +22 (c, 1.0 in $CHCl_3$)][8]; δ_H (400 MHz, $CDCl_3$) 3.42 (1H, dd, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.1 Hz, H-2β), 3.47-3.74 (8H, m, H-4α, H-6α, H-6'α, H-3β, H-4β, H-5β, H-6β, H-6'β), 3.60 (1H, dd, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.5 Hz, H-2α), 3.99 (1H, at, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 9.2 Hz, H-3α), 4.05 (1H, ddd, $J_{4,5}$ 10.1 Hz, $J_{5,6}$ 3.8 Hz, $J_{5,6'}$ 2.1 Hz, H-5α), 4.48-4.97 (16H, m, 4 x $OCH_2Ph\alpha$, 4 x $OCH_2Ph\beta$), 4.73 (1H, d, $J_{1,2}$ 7.8 Hz, H-1β), 5.24 (1H, d, $J_{1,2}$ 3.7 Hz, H-1α), 7.15-7.38 (40H, m, 20 x Ar-Hα, 20 x Ar-Hβ).

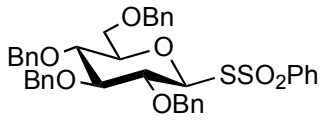
2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose bromide 31



2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose **30** (4.0 g, 7.39 mmol) was dissolved in anhydrous DCM (24 mL) and anhydrous DMF (2 mL). The resulting solution was cooled to 0 ºC, at which point oxalyl bromide (16 mL, 2M in DCM, 29.56 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and left to stir under an atmosphere of argon. After 2 h, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a major product (R_f 0.6). The reaction was cooled to 0 ºC and quenched with ice cold water (60 mL) added over a 5 min period. The mixture was

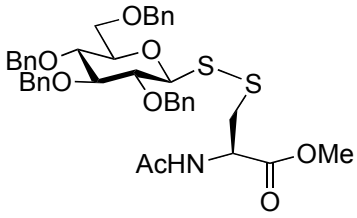
partitioned between DCM (80 mL) and water. The aqueous layer was re-extracted with DCM (3 x 80 mL), and the combined organic layers were washed with brine (150 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose bromide **31** (4.20 g, 94%) as a crude yellow oil which was used without any further purification; δ_H (400 MHz, $CDCl_3$) 3.55 (1H, dd, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.2 Hz, H-2), 3.66 (1H, dd, $J_{5,6}$ 1.8 Hz, $J_{5,6'}$ 11.0 Hz, H-6), 3.76-3.80 (1H, m, H-4, H-6'), 4.04 (1H, at, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 9.2 Hz, H-3), 4.55-5.00 (8H, m, 4 x OCH_2Ph), 6.44 (1H, d, $J_{1,2}$ 3.7 Hz, H-1), 7.14-7.42 (20H, m, Ar-H).

2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl phenylthiosulfonate[2] 32



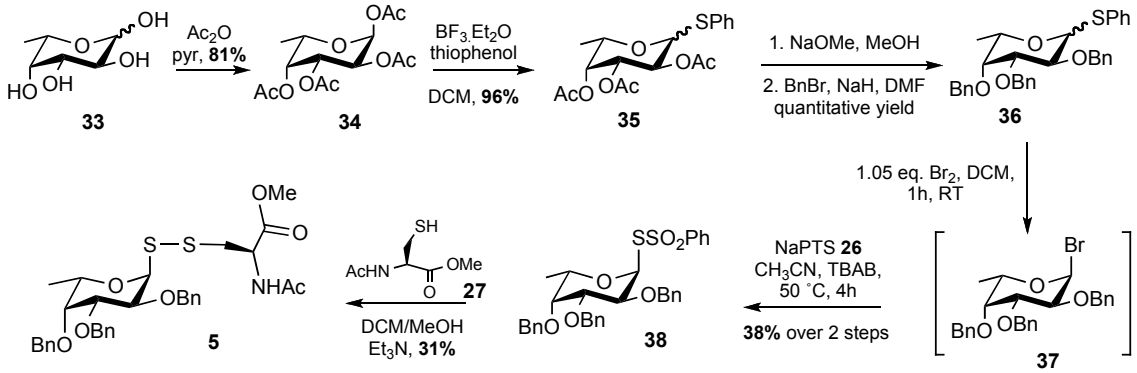
2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose bromide **31** (3.5 g, 5.804 mmol) and sodium phenylthiosulfonate **26** (4.41 g, 22.47 mmol) were dissolved in anhydrous dioxane (90 mL). The resulting reaction mixture was heated to 70 ºC under an atmosphere of argon. After a 24 h period, t.l.c. (petrol:ethyl acetate, 2:1) indicated the formation of a major product (R_f 0.5) with complete consumption of the starting material (R_f 0.6). The reaction mixture was cooled to room temperature and filtered. The precipitate was washed with petrol/ethyl acetate and the filtrate concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 4:1) to afford 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl phenylthiosulfonate **32** (3.26 g, 81%) as a white viscous gum being a mixture of anomers (α:β, 1:2). Selective recrystallisation from ethyl acetate/petrol afforded pure 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl phenylthiosulfonate **X** as a white crystalline solid; m.p. 106-108 ºC (ethyl acetate/petrol) [Lit. 106-108 ºC (ethyl acetate/petrol)][2]; $[\alpha]_D^{23}$ +47.8 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{22}$ +21.4 (c, 0.35 in $CHCl_3$)][2]; δ_H (400 MHz, $CDCl_3$) 3.45 (1H, ddd, $J_{4,5}$ 9.6 Hz, $J_{5,6}$ 1.6 Hz, $J_{5,6'}$ 3.6 Hz, H-5), 3.49 (1H, dd, $J_{5,6}$ 1.4 Hz, $J_{6,6'}$ 11.7 Hz, H-6), 3.54 (1H, dd, $J_{1,2}$ 9.9 Hz, $J_{2,3}$ 8.7 Hz, H-2), 3.57 (1H, dd, $J_{5,6'}$ 3.6 Hz, $J_{6,6'}$ 11.6 Hz, H-6'), 3.62 (1H, at, J 9.4 Hz, H-3), 3.72 (1H, at, J 8.8 Hz, H-4), 4.34-4.90 (8H, m, 4 x OCH_2Ph), 5.13 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 7.09-7.15 (3H, m, Ar-H), 7.27-7.54 (20H, m, Ar-H), 7.90-7.98 (2H, m, Ar-H); *m/z* (ES^+) 718 (MNa^+, 20%) 755 ($MMeCNNH_4^+$, 100%).

***N*-Acetyl-L-cysteine (2,3,4,6-tetra-*O*-benzyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester 3**



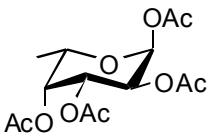
2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl phenylthiosulfonate **32** (1.10 g, 1.581 mmol) and triethylamine (74 μL, 0.527 mmol) were dissolved in anhydrous DCM (15 mL), and the resulting solution stirred at room temperature under an atmosphere of argon. A solution of *N*-acetyl-L-cysteine methyl ester **27** (0.15 g, 0.847 mmol) in a mixture of anhydrous DCM (15 mL) and anhydrous methanol (12 mL) was added slowly to the above solution. After a 2 h period, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a product (R_f 0.2) along with complete consumption of the starting material (R_f 0.1). The reaction mixture was concentrated *in vacuo* and the resulting residue purified by flash column chromatography (petrol:ethyl acetate, 1:1) to afford *N*-acetyl-L-cysteine (2,3,4,6-tetra-*O*-benzyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester **3** (0.52 g, 89%) as a white crystalline solid; m.p. 108-110 ºC (ethyl acetate/petrol); $[\alpha]_D^{23}$ +35.2 (c, 1 in $CHCl_3$); υ_{max} (KBr disc) 3330 (br, NH) 1740 (s, C=O) 1651 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.99 (3H, s, HNC(O)C$\underline{H}_3$), 3.24 (1H, dd, $J_{CH,H'}$ 13.9 Hz, $J_{CH,\alpha H}$ 7.7 Hz, C$\underline{H}$,H'), 3.45 (1H, dd, $J_{CH,H'}$ 13.9 Hz, $J_{CH',\alpha H}$ 3.9 Hz, CH,$\underline{H}$'), 3.54 (1H, m, H-5), 3.68 (1H, at, $J_{1,2}$ 9.1 Hz, $J_{2,3}$ 9.0 Hz, H-2), 3.69-3.72 (2H, m, H-3, H-4), 3.74 (3H, s, OCH_3), 3.77 (1H, dd, $J_{5,6}$ 1.9 Hz, $J_{6,6'}$ 11.4 Hz, H-6), 3.80 (1H, dd, $J_{5,6'}$ 3.8 Hz, $J_{6,6'}$ 11.4 Hz, H-6'), 4.48 (1H, d, $J_{1,2}$ 9.1 Hz, H-1), 4.52-4.93 (8H, m, 4 x OCH_2Ph), 4.77 (1H, d, *J* 7.7 Hz, αH), 6.86 (1H, d, $J_{NH,\alpha H}$ 8.0 Hz, $\underline{H}$NC(O)CH_3), 7.13-7.16 (2H, m, Ar-H), 7.27-7.35 (18H, m, Ar-H); δ_C (100.7 MHz, $CDCl_3$) 23.0 (q, HNC(O)$\underline{C}H_3$), 41.4 (t, CH,H'), 52.3 (d, αC), 52.6 (q, OCH_3), 68.7 (t, C-6), 73.5, 75.1, 75.5, 75.7 (4 x t, 4 x OCH_2Ph), 78.9, 79.5 (3 x d, C-2, C-4, C-5), 86.4 (d, C-3), 89.9 (d, C-1), 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5, 128.6 (9 x d, 20 x Ar-C), 137.5, 137.6, 137.8, 138.2 (4 x s, 4 x Ar-C), 170.2, 171.0 (2 x s, $\underline{C}OOCH_3$, HN$\underline{C}OCH_3$); *m/z* (ES^+) 790 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{40}H_{45}NNaO_8S_2$ (MNa^+) 754.2479. Found: 754.2479.; Found: C, 64.78%; H, 6.30%, N, 1.75%. $C_{40}H_{45}NO_8S_2$ requires: C, 64.64%; H, 6.20%; N, 1.91%.

Synthesis of *N*-Acetyl-L-cysteine (2,3,4-tri-*O*-benzyl-1-dithio-α-L-fucopyranosyl disulfide) methyl ester 5



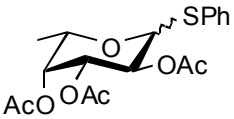
Scheme S9.

1,2,3,4-Tetra-*O*-acetyl-α-L-fucopyranoside[9] 34



L-Fucose **33** (2.0 g, 12.2 mmol) was dissolved in pyridine (25 mL) under an atmosphere of argon. Acetic anhydride (25 mL) was added portionwise over 30 min and the mixture was left to stir at room temperature. After 16 h, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a product (R_f 0.4) with complete consumption of starting material (R_f 0). The reaction mixture was co-evaporated with toluene until no pyridine or acetic anhydride remained. The residue was recrystallised (diethyl ether/petrol) to afford 1,2,3,4-tetra-*O*-acetyl-α-L-fucopyranoside **34** (3.3 g, 81%) as a white crystalline solid; m.p. 94-96 ºC (diethyl ether/petrol) [Lit. 93 ºC (diethyl ether/petrol)][9]; $[\alpha]_D^{21}$ -105.7 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{14}$ -129.9 (c, 2 in acetone)][9]; δ_H (400 MHz, $CDCl_3$) 1.16 (3H, d, *J* 6.5 Hz, CH_3), 2.00, 2.01, 2.14, 2.17 (12H, 4 x s, 4 x C(O)CH_3), 4.27 (1H, q, *J* 6.5 Hz, H-5), 5.29-5.37 (3H, m, H-2, H-3, H-4), 6.34 (1H, d, $J_{1,2}$ 2.7 Hz, H-1); *m/z* (ES^+) 391 (MMeCNNH4+, 100%).

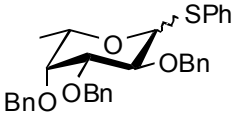
Phenyl 2,3,4-tri-*O*-acetyl-1-thio-L-fucopyranoside[10] 35



1,2,3,4-Tetra-*O*-acetyl-α-L-fucopyranoside **34** (1.91 g, 5.74 mmol) was dissolved in anhydrous DCM (12 mL) under argon. Thiophenol (1.2 mL, 11.49 mmol) and boron trifluoride diethyl etherate (1.7 mL, 14.37 mmol) were added and the mixture was left to

stir at room temperature. After 16 h, t.l.c. (petrol:ethyl acetate, 3:1) indicated the formation of a major product (R_f 0.6) and the complete consumption of starting material (R_f 0.1). Triethylamine was added dropwise until the effervescence ceased. The reaction mixture was diluted with DCM (40 mL), washed with sodium hydrogen carbonate (2 x 30 mL of a saturated aqueous solution), water (30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 2:1) to give phenyl 2,3,4-tri-*O*-acetyl-1-thio-L-fucopyranoside **35** (2.10 g, 96%) as a colourless oil; δ_H (400 MHz, $CDCl_3$) 1.14 (3H, d, *J* 6.5 Hz, $CH_3\alpha$), 1.25 (3H, d, *J* 6.4 Hz, $CH_3\beta$), 1.98, 2.09, 2.15 (9H, 3 x s, 3 x $C(O)CH_3\beta$), 2.02, 2.05, 2.17 (9H, 3 x s, 3 x $C(O)CH_3\alpha$), 3.84 (1H, q, *J* 6.4 Hz, H-5β), 4.62 (1H, q, *J* 6.5 Hz, H-5α), 4.71 (1H, d, $J_{1,2}$ 9.9 Hz, H-1β), 5.06 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.4 Hz, H-3β), 5.23 (1H, at, *J* 9.9 Hz, H-2β), 5.27 (1H, br d, *J* 3.4 Hz, H-4β), 5.30-5.37 (3H, m, H-2α, H-3α, H-4α), 5.94 (1H, d, $J_{1,2}$ 5.2 Hz, H-1α), 7.27-7.53 (10H, m, 5 x Ar-Hα, 5 x Ar-Hβ).

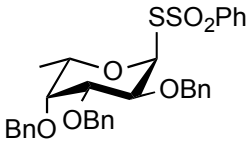
Phenyl 2,3,4-Tri-*O*-benzyl-1-thio-L-fucopyranoside[11] 36



A solution of phenyl 2,3,4-tri-*O*-acetyl-1-thio-L-fucopyranoside **35** (2.01 g, 5.27 mmol) in anhydrous methanol (12 mL) was treated with sodium methoxide (57 mg, 1.05 mmol). The mixture was stirred under an atmosphere of argon for 10 min, when t.l.c. (petrol:ethyl acetate, 1:1) indicated formation of a single product (R_f 0) and complete consumption of starting material (R_f 0.5). The reaction mixture was then concentrated *in vacuo*. The resulting residue was dissolved in anhydrous DMF (20 mL), and sodium hydride (60% dispersed in mineral oil) (1.82 g, 45.50 mmol) was added portionwise for a period of 10 min at 0 ºC. Benzyl bromide (3.8 mL, 31.62 mmol) was then added dropwise and the mixture left to stir under an atmosphere of argon at RT. After a 16 h period, t.l.c. (petrol:ethyl acetate, 7:3) indicated the formation of a major product (R_f 0.5) with complete consumption of the starting material (R_f 0). The reaction mixture was quenched by the slow addition of methanol (8 mL) and stirred for 10 min. The resulting solution was diluted with water (15 mL) and extracted with ether (3 x 20 mL). The organic layers were combined and washed with sodium hydrogen carbonate (40 mL of a saturated aqueous solution), and water (40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography

(petrol:ethyl acetate, 6:1) to afford phenyl 2,3,4-tri-*O*-benzyl-1-thio-L-fucopyranoside **36** (2.79 g) being a mixture of anomers (α:β, 3:2) in quantitative yield over two steps; α anomer: δ_H (400 MHz, $CDCl_3$) 1.13 (3H, d, *J* 6.5 Hz, CH_3), 3.71 (1H, br d, *J* 2.3 Hz, H-4), 3.84 (1H, dd, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 2.9 Hz, H-3), 4.34 (1H, q, *J* 6.5 Hz, H-5), 4.38 (1H, dd, $J_{1,2}$ 5.5 Hz, $J_{2,3}$ 10.1 Hz, H-2), 4.66-5.05 (6H, m, 3 x OCH_2Ph), 5.74 (1H, d, $J_{1,2}$ 5.5 Hz, H-1), 7.21-7.62 (20H, m, Ar-H); β anomer: δ_H (400 MHz, $CDCl_3$) 1.29 (3H, d, *J* 6.4 Hz, CH_3), 3.55 (1H, q, *J* 6.4 Hz, H.5), 3.62 (1H, dd, $J_{2,3}$ 9.1 Hz, $J_{3,4}$ 2.8 Hz, H-3), 3.66 (1H, br d, *J* 2.7 Hz, H-4), 3.95 (1H, at, *J* 9.4 Hz, H-2), 4.62 (1H, d, $J_{1,2}$ 9.7 Hz, H-1), 4.66-5.05 (6H, m, 3 x OCH_2Ph), 7.21-7.62 (20H, m, Ar-H); *m/z* (ES^+) 585 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{33}H_{34}NaO_4S$ (MNa^+) 549.2070. Found: 549.2060.

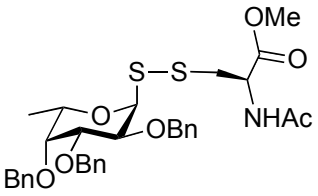
2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl phenylthiosulfonate 38



Bromine (38 μL, 0.799 mmol) was added to a solution of phenyl 2,3,4-tri-*O*-benzyl-1-thio-L-fucopyranoside **36** (363 mg, 0.699 mmol) in anhydrous DCM (3 mL), and after stirring for 1 h at room temperature cyclohexane (100 μL) was added. The resulting glycosyl bromide solution **37** was added dropwise to a solution of sodium phenylthiosulfonate **26** (315 mg, 1.605 mmol) and tetrabutylammonium bromide (23 mg, 0.069 mmol) in anhydrous acetonitrile (6 mL). The resulting mixture heated to 50 ºC and stirred under an atmosphere of argon. After 4 h, t.l.c. (petrol:ethyl acetate, 3:1) indicated the formation of a major product (R_f 0.3). The reaction mixture was diluted with DCM (20 mL), washed with brine (15 mL) and the aqueous layer re-extracted with DCM (2 x 20 mL). The organic layers were combined and washed with water (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 8:1) to afford 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl phenylthiosulfonate **38** (155 mg, 38% yield over two steps) as a colourless oil; $[\alpha]_D^{18}$ -35.1 (c, 1 in $CHCl_3$); υ_{max} (thin film) 1325 (s, SO_2) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.66 (3H, d, *J* 6.4 Hz, CH_3), 3.45 (1H, dd, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 2.8 Hz, H-3), 3.53 (1H, br d, *J* 2.0 Hz, H-4), 3.67 (1H, q, *J* 6.4 Hz, H-5), 4.32 (1H, dd, $J_{1,2}$ 5.5 Hz, $J_{2,3}$ 10.0 Hz, H-2), 4.57-4.94 (6H, m, 3 x OCH_2Ph), 6.20 (1H, d, $J_{1,2}$ 5.5 Hz, H-1), 7.27-7.37 (15H, m, Ar-H), 7.47-7.60 (3H, m, Ar-H), 7.93-7.96 (2H, m, Ar-H); δ_C (100.7 MHz, $CDCl_3$) 15.8 (q, CH_3), 69.3 (d, C-5), 72.5, 73.5, 75.0 (3 x t,

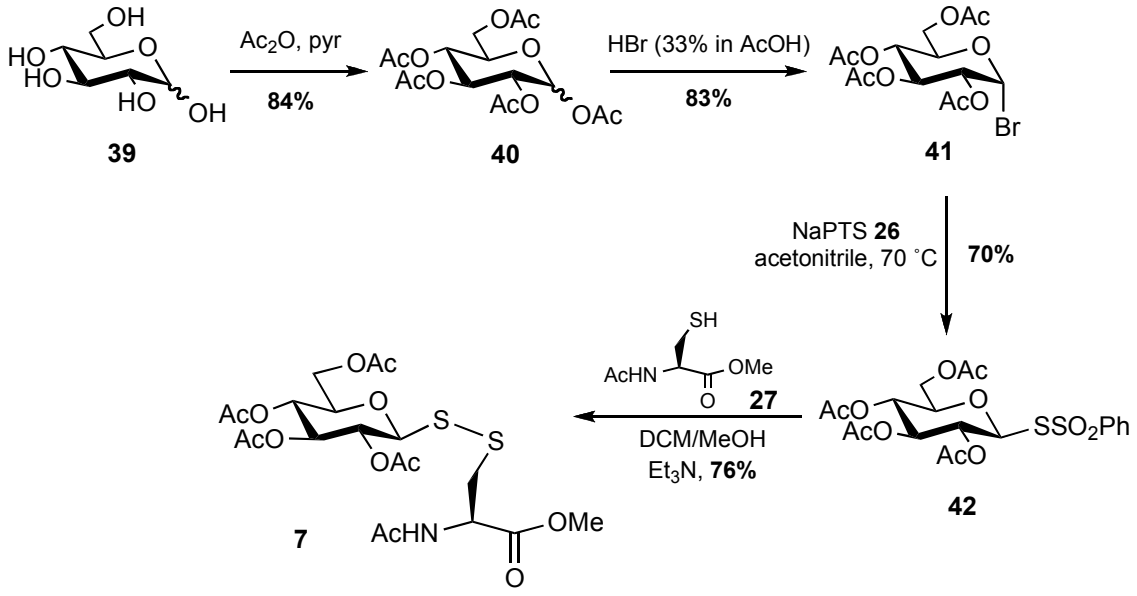
3 x OCH_2Ph), 75.5 (d, C-2), 76.9 (d, C-4), 79.9 (d, C-3), 90.9 (d, C-1), 127.3, 127.5, 127.7, 127.8, 127.9, 128.0, 128.2, 128.5, 128.9 (9 x d, 16 x Ar-C), 133.4, 137.3, 138.1, 138.3 (4 x s, 4 x Ar-C); *m/z* (ES^+) 649 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{33}H_{34}NaO_6S_2$ (MNa^+) 613.1689. Found: 613.1692.

_N_-Acetyl-L-cysteine (2,3,4-tri-_O_-benzyl-1-dithio-α-L-fucopyranosyl disulfide) methyl ester 5



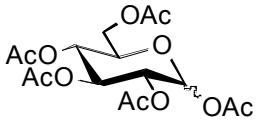
A solution of 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranosyl phenylthiosulfonate **38** (148 mg, 0.251 mmol) and triethylamine (12 μL, 0.084 mmol) were dissolved in anhydrous DCM (3 mL), and the resulting solution stirred at room temperature under an atmosphere of argon. A solution of *N*-acetyl-L-cysteine methyl ester **27** (16 mg, 0.084 mmol) in a mixture of anhydrous DCM (3 mL) and anhydrous methanol (2 mL) was slowly added *via* a syringe pump over a 2 h period. After 2 h, t.l.c. (petrol:ethyl acetate, 3:7) indicated the formation of a product (R_f 0.4) along with complete consumption of the starting material (R_f 0.1). The reaction mixture was concentrated *in vacuo* and the resulting residue purified by flash column chromatography (petrol:ethyl acetate, 4:6) to afford *N*-acetyl-L-cysteine (2,3,4-tri-*O*-benzyl-1-dithio-α-L-fucopyranosyl disulfide) methyl ester **5** (48 mg, 31%) as white foam; $[\alpha]_D^{25}$ -37.3 (c, 0.5 in $CHCl_3$); υ_{max} (KBr disc) 3299 (br, NH) 1746 (s, C=O) 1654 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.17 (1H, d, *J* 6.4 Hz, CH_3), 2.03 (3H, s, HNC(O)C$\underline{H}_3$), 3.34 (1H, dd, $J_{C\underline{H},H'}$ 14.5 Hz, $J_{CH,\alpha H}$ 5.0 Hz, C$\underline{H}$,H'), 3.41 (1H, dd, $J_{CH,\underline{H}'}$ 14.5 Hz, $J_{CH',\alpha H}$ 4.5 Hz, CH,$\underline{H}$'), 3.71 (1H, br s, H-4), 3.73-3.75 (1H, m, H-3), 3.76 (3H, s, OCH_3), 4.06 (1H, q, *J* 6.5 Hz, H-5), 4.35 (1H, dd, $J_{1,2}$ 5.4 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.66-5.00 (6H, m, 3 x OCH_2Ph), 4.93-4.96 (1H, m, αH), 5.63 (1H, d, $J_{1,2}$ 5.5 Hz, H-1), 6.36 (1H, br d, $J_{NH,\alpha H}$ 7.7 Hz, $\underline{H}NC(O)CH_3$), 7.29-7.39 (15H, m, Ar-H); δ_C (125.8 MHz, $CDCl_3$) 16.3 (q, CH_3), 23.2 (q, HNC(O)$\underline{C}H_3$), 41.3 (t, CH,H'), 51.8 (d, αC), 52.8 (q, OCH_3), 68.5 (d, C-5), 72.7, 73.3, 74.9 (3 x t, 3 x OCH_2Ph), 76.5 (d, C-2), 77.6 (d, C-4), 79.6 (d, C-3), 90.2 (d, C-1), 127.4, 127.6, 127.7, 127.8, 127.9, 128.2, 128.3, 128.4, 128.5 (9 x d, 15 x Ar-C), 137.7, 138.3, 138.5 (3 x s, 3 x Ar-C), 169.7, 170.8 (2 x s, $\underline{C}OOCH_3$, HN$\underline{C}OCH_3$); *m/z* (ES^+) 684 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{33}H_{39}NNaO_7S_2$ (MNa^+) 648.2060. Found: 648.2067.

Synthesis of *N*-Acetyl-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester 7



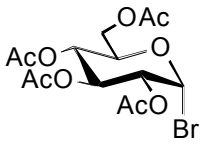
Scheme S10.

1,2,3,4,6-Penta-*O*-acetyl-D-glucopyranoside[12,13] 40



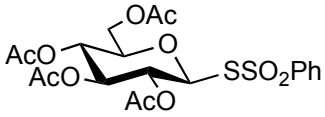
D-Glucose **39** (50.0 g, 278 mmol) was dissolved in pyridine (200 mL) under an atmosphere of argon. Acetic anhydride (250 mL) was added portionwise over 30 min and the mixture was left to stir at room temperature. After 22 h, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a product (R_f 0.6) with complete consumption of starting material (R_f 0). The reaction mixture was co-evaporated with ethanol until no pyridine or acetic anhydride remained. The residue was recrystallised (ethanol) to afford 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranoside **40** (90.5 g, 84%) as a white crystalline solid being a mixture of anomers (α:β, 1:1.2); m.p. 98-100 ºC (ethanol) [Lit. 100-102 ºC][12]; $[\alpha]_D^{21}$ +51.3 (c, 1.01 in $CHCl_3$) [Lit. $[\alpha]_D$ +54.5 (c, 3.8 in $CHCl_3$)][13]; δ_H (400 MHz, $CDCl_3$) 2.02, 2.03, 2.09, 2.11, 2.18 (15H, 5 x s, 5 x C(O)$CH_3\alpha$), 2.02, 2.03, 2.09, 2.12, 2.18 (15H, 5 x s, 5 x C(O)$CH_3\beta$), 3.82-3.86 (1H, m, H-5β), 4.08-4.14 (3H, m, H-5α, H-6'α, H-6'β), 4.25-4.31 (2H, m, H-6α, H-6β), 5.08-5.17 (4H, m, H-2α, H-4α, H-2β, H-4β), 5.25 (1H, at, *J* 9.4 Hz, H-3β), 5.47 (1H, at, *J* 9.8 Hz, H-3α), 5.72 (1H, d, $J_{1,2}$ 8.3 Hz, H-1β), 6.33 (1H, d, $J_{1,2}$ 3.6 Hz, H-1α).

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide[14] 41



1,2,3,4,6-Penta-*O*-acetyl-D-glucopyranoside **40** (20.0 g, 51.2 mmol) was dissolved in anhydrous DCM (200 mL) and to this hydrogen bromide (33% w/w in acetic acid, 150 mL) was added. The mixture was stirred under argon at room temperature. After a 2 h period, t.l.c. (petrol:ethyl acetate, 3:1) indicated the formation of a product (R_f 0.4) with complete consumption of starting material (R_f 0.1). Ice water (250 mL) was added and the mixture stirred for 10 min. The two phases were separated and the aqueous layer re-extracted with DCM (3 x 50 mL). The combined organic layers were washed with sodium hydrogen carbonate (saturated aqueous solution) until pH 8 was obtained. The combined organics were washed with brine (200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Recrystallisation (ethyl acetate/petrol) afforded 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **41** (17.5 g, 83%) as a white crystalline solid; m.p. 84-86 ºC (ethyl acetate/petrol) [Lit. 89.5-90.5 ºC][14]; $[\alpha]_D^{22}$ +182.1 (c, 1.01 in $CHCl_3$) [Lit. $[\alpha]_D$ +186 (c, 6 in CH_2Cl_2)][14]; δ_H (400 MHz, $CDCl_3$) 2.04, 2.06, 2.10, 2.11 (12H, 4 x s, 4 x C(O)CH_3), 4.14 (1H, dd, $J_{5,6}$ 1.9 Hz, $J_{6,6'}$ 12.6 Hz, H-6), 4.28-4.36 (2H, m, H-5, H-6'), 4.85 (1H, dd, $J_{1,2}$ 4.0 Hz, $J_{2,3}$ 10.0 Hz, H-2), 5.17 (1H, at, J 9.8 Hz, H-4), 5.56 (1H, at, J 9.7 Hz, H-3), 6.25 (1H, d, $J_{1,2}$ 4.0 Hz, H-1).

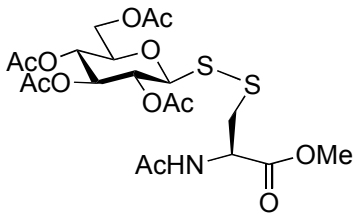
2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl phenylthiosulfonate[2] 42



2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranose bromide **41** (7.90 g, 19.21 mmol) was dissolved in anhydrous acetonitrile (80 mL). To this solution sodium phenylthiosulfonate (7.51 g, 38.42 mmol) and tetrabutylammonium bromide (0.62 g, 1.92 mmol) were added. The resulting mixture was stirred under argon at 70 ºC. After a 4.5 h period, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a major product (R_f 0.2) with complete consumption of the starting material (R_f 0.3). The reaction mixture was concentrated *in vacuo*. The crude solid was partitioned between DCM (100 mL) and water (80 mL), and the aqueous layer re-extracted with DCM (2 x 100 mL). The combined organic layers were washed with brine (80 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Recrystallisation (ethyl acetate/petrol) afforded 2,3,4,6-tetra-*O*-

acetyl-β-D-glucopyranosyl phenylthiosulfonate **42** (6.81 g, 70 %) as a white crystalline solid; m.p. 128-130 ºC (ethyl acetate/petrol) [Lit. 129-130 ºC (ethyl acetate/petrol)][2]; $[\alpha]_D^{23}$ +48.3 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{25}$ +51.2 (c, 1 in $CHCl_3$)][2]; δ_H (400 MHz, $CDCl_3$) 1.99, 2.00, 2.02, 2.05 (12H, 4 x s, 4 x C(O)CH_3), 3.74 (1H, ddd, $J_{4,5}$ 10.1 Hz, $J_{5,6}$ 2.3 Hz, $J_{5,6'}$ 4.3 Hz, H-5), 3.91 (1H, dd, $J_{5,6}$ 2.3 Hz, $J_{6,6'}$ 12.5 Hz, H-6), 4.11 (1H, dd, $J_{5,6'}$ 4.3 Hz, $J_{6,6'}$ 12.5 Hz, H-6'), 4.99-5.05 (2H, m, H-2, H-4), 5.26 (1H, d, $J_{1,2}$ 10.4 Hz, H-1), 5.27 (1H, at, *J* 9.3 Hz, H-3), 7.53-7.67 (3H, m, Ar-H), 7.93-7.95 (2H, m, Ar-H).

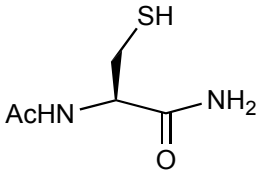
_N_-Acetyl-L-cysteine (2,3,4,6-tetra-_O_-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester 7



2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl phenylthiosulfonate **42** (200 mg, 0.397 mmol) was dissolved in anhydrous DCM (8 mL) and stirred at room temperature under an atmosphere of argon. A solution of *N*-acetyl-L-cysteine methyl ester **27** (70 mg, 0.397 mmol) and triethylamine (55 μL, 0.397 mmol) in a mixture of anhydrous DCM (15 mL) and anhydrous methanol (2 mL) was slowly added *via* a syringe pump over a 2 h period. After 2 h, t.l.c. (ethyl acetate) indicated the formation of a product (R_f 0.4) along with complete consumption of the starting material (R_f 0.3). The reaction mixture was concentrated *in vacuo* and the resulting residue purified by flash column chromatography (ethyl acetate) to afford *N*-acetyl-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester **7** (164 mg, 76%) as a white amorphous solid; $[\alpha]_D^{18}$ +19.8 (c, 1 in $CHCl_3$); υ_{max} (KBr disc) 3290 (br, NH) 1749 (s, C=O) 1663 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.00, 2.02, 2.03, 2.04, 2.08 (15H, 5 x s, 4 x C(O)CH_3, HNC(O)C$\underline{H}_3$), 3.09 (1H, dd, $J_{C\underline{H},H'}$ 14.2 Hz, $J_{CH,\alpha H}$ 6.8 Hz, C$\underline{H}$,H'), 3.33 (1H, dd, $J_{CH,\underline{H}'}$ 14.2 Hz, $J_{CH',\alpha H}$ 4.4 Hz, CH,$\underline{H}$'), 3.77 (3H, s, OCH_3), 3.81-3.85 (1H, m, H-5), 4.18 (1H, dd, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.5 Hz, H-6), 4.27 (1H, dd, $J_{5,6'}$ 4.7 Hz, $J_{6,6'}$ 12.5 Hz, H-6'), 4.59 (1H, d, $J_{1,2}$ 9.5 Hz, H-1), 4.95-4.99 (1H, m, αH), 5.10 (1H, at, *J* 9.7 Hz, H-4), 5.21 (1H, at, *J* 9.4 Hz, H-2), 5.25 (1H, at, *J* 9.2 Hz, H-3), 6.41 (1H, d, $J_{NH,\alpha H}$ 8.0 Hz, $\underline{H}$NC(O)CH_3); δ_C (100.7 MHz, $CDCl_3$) 20.6, 20.7, 20.8 (3 x q, 4 x C(O)$\underline{C}H_3$), 23.1 (q, HNC(O)$\underline{C}H_3$), 41.9 (t, CH,H'), 51.9 (d, αC), 52.8 (q, OCH_3), 61.9 (t, C-6), 67.9 (d, C-4), 68.9 (d, C-2), 73.7 (d, C-3), 76.1 (d, C-5), 88.9 (d, C-1), 169.4,

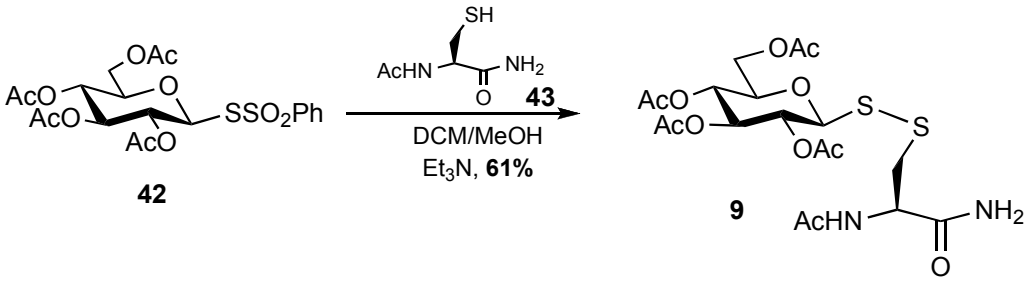
169.8, 170.1, 170.6, 171.0, 172.4 (6 x s, 4 x $\underline{C}(O)CH_3$, $HN\underline{C}(O)CH_3$, $\underline{C}O_2CH_3$); *m/z* (ES^+) 598 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{20}H_{29}NNaO_{12}S_2$ (MNa^+) 562.1017. Found: 562.1023; Found: C, 44.43%; H, 5.49%, N, 2.58%. $C_{40}H_{45}NO_8S_2$ requires: C, 44.52%; H, 5.42%; N, 2.60%.

Synthesis of *N*-Acetyl-L-cysteinamide[15] **43**



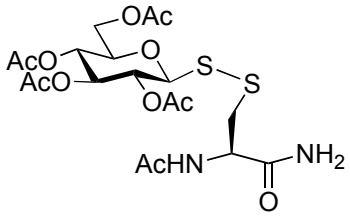
N-acetyl-L-cysteine methyl ester (387 mg, 2.19 mmol) was stirred in a 1:1 mixture of toluene and ammonium hydroxide. After 19 h, t.l.c. (ethyl acetate) showed comsumption of starting material (R_f 0.3) and formation of a product (R_f 0). The reaction mixture was concentrated *in vacuo* at 60 °C and the resulting residue purified by recrystallisation (ethanol) to afford *N*-acetyl-L-cysteinamide **43** (322 mg, 91%) as a white crystalline solid; m.p. 147-149 ºC (ethanol) [Lit. 148-150 ºC (ethanol)][15]; $[\alpha]_D^{25}$ -44.0 (c, 1 in H_2O) [Lit. $[\alpha]_D^{25}$ -12.28 (c, 5 in H_2O)][15]; δ_H (400 MHz, $CDCl_3$) 2.03 (3H, s, $HNC(O)C\underline{H}_3$), 2.80 (1H, dd, $J_{C\underline{H},H'}$ 13.9 Hz, $J_{CH,\alpha H}$ 7.3 Hz, $C\underline{H}$,H'), 2.92 (1H, dd, $J_{CH,\underline{H}'}$ 13.8 Hz, $J_{CH',\alpha H}$ 9.2 Hz, CH,$\underline{H}$'), 4.72 (1H, dd, *J* 4.9 Hz, *J* 9.2 Hz, αH); δ_C (100.7 MHz, $CDCl_3$) 21.5 (q, $HNC(O)\underline{C}H_3$), 25.9 (t, CH,H'), 55.8 (d, αC), 172.4 (s, $HN\underline{C}(O)CH_3$), 174.2 (s, $C(O)NH_2$); *m/z* (ES^-) 161 ($M\text{-}H^+$, 100%).

Synthesis of *N*-Acetyl-L-cysteinamide (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) 9



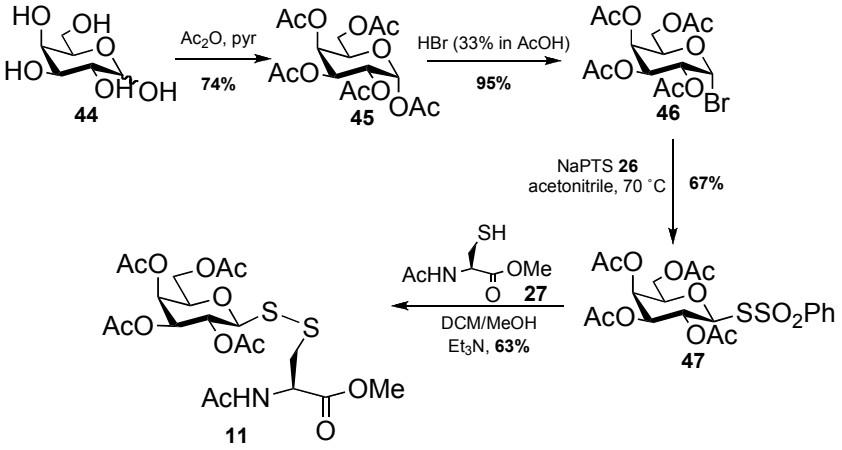
Scheme S11.

***N*-Acetyl-L-cysteinamide (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) 9**



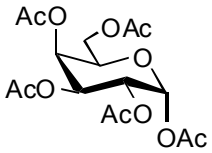
2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl phenylthiosulfonate **42** (407 mg, 0.807 mmol) was dissolved in anhydrous DCM (8 mL) and stirred at room temperature under an atmosphere of argon. A solution of *N*-acetyl-L-cysteinamide **43** (131 mg, 0.807 mmol) and triethylamine (0.11 mL, 0.807 mmol) in a mixture of anhydrous DCM (10 mL) and anhydrous methanol (8 mL) was slowly added *via* a syringe pump over a 2 h period. After 3 h, t.l.c. (ethyl acetate:MeOH, 9:1) indicated the formation of a product (R_f 0.4) along with complete consumption of the starting material (R_f 0.3). The reaction mixture was concentrated *in vacuo* and the resulting residue purified by recrystallisation (ethyl acetate) to afford *N*-acetyl-L-cysteinamide (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) **9** (260 mg, 61%) as a white amorphous solid; $[\alpha]_D^{25}$ -173 (c, 1 in MeOH); υ_{max} (KBr disc) 3046 (br, NH NH_2) 1748 (s, C=O) 1638 (s, C=O) cm^{-1}; δ_H (400 MHz, CD_3OD) 1.99, 2.03, 2.07 (15H, 3 x s, 4 x C(O)CH_3, HNC(O)$C\underline{H}_3$), 2.97 (1H, dd, $J_{C\underline{H},H'}$ 13.8 Hz, $J_{CH,\alpha H}$ 9.7 Hz, C$\underline{H}$,H'), 3.36 (1H, dd, $J_{CH,\underline{H}'}$ 13.9 Hz, $J_{CH',\alpha H}$ 4.6 Hz, CH,$\underline{H}$'), 3.97 (1H, ddd, $J_{4,5}$ 10.2 Hz, $J_{5,6}$ 2.3 Hz, $J_{5,6'}$ 4.3 Hz, H-5), 4.33 (1H, dd, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.5 Hz, H-6), 4.38 (1H, dd, $J_{5,6'}$ 4.4 Hz, $J_{6,6'}$ 12.5 Hz, H-6'), 4.75-4.78 (1H, m, αH), 4.79 (1H, d, $J_{1,2}$ 9.5 Hz, H-1), 5.07 (1H, at, *J* 9.7 Hz, H-4), 5.27 (1H, at, *J* 9.4 Hz, H-2), 5.34 (1H, at, *J* 9.3 Hz, H-3); δ_C (100.7 MHz, CD_3OD) 19.5, 19.6, 19.7, 19.8 (4 x q, 4 x C(O)$\underline{C}H_3$), 21.6 (q, HNC(O)$\underline{C}H_3$), 41.8 (t, CH,H'), 52.5 (d, αC), 62.1 (t, C-6), 68.3 (d, C-4), 69.4 (d, C-2), 74.2 (d, C-3), 76.0 (d, C-5), 87.4 (d, C-1), 169.9, 170.1, 170.5, 171.4, 172.5 (5 x s, 4 x $\underline{C}$(O)CH_3, HN$\underline{C}$(O)CH_3), 174.1 (s, $\underline{C}ONH_2$); *m/z* (ES^+) 547 (MNa^+, 100%) 583 ($MMeCNNH_4^+$, 70%); HRMS (ES^+) Calcd. for $C_{19}H_{28}N_2NaO_{11}S_2$ (MNa^+) 547.1027. Found: 547.1027; Found: C, 44.85%; H, 5.73%, N, 5.34%. $C_{19}H_{28}N_2O_{11}S_2$ requires: C, 44.50%; H, 5.38%; N, 5.34%.

Synthesis of *N*-Acetyl-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-galactopyaranosyl disulfide) methyl ester 11



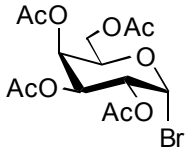
Scheme S12.

1,2,3,4,6-Penta-*O*-acetyl-α-D-galactopyranoside[16] 45



A solution of D-galactose **44** (30.0 g, 167 mmol) in pyridine (200 mL) was treated with acetic anhydride (250 mL), and stirred at room temperature under an atmosphere of argon. After 24 h, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a product (R_f 0.5) with complete consumption of starting material (R_f 0). The reaction mixture was co-evaporated with ethanol until no pyridine or acetic anhydride remained. The residue was recrystallised (ethanol) to afford 1,2,3,4,6-penta-*O*-acetyl-α-D-galactopyranoside **45** (82.3 g, 74%) as white crystalline solid; m.p. 88-90 ºC (ethanol) [Lit. 92-94 ºC][16]; $[\alpha]_D^{21}$ +87.7 (c, 1.06 in $CHCl_3$) [Lit. $[\alpha]_D^{25}$ +107 (c, 1.0 in $CHCl_3$)][16]; δ_H (400 MHz, $CDCl_3$) 2.00, 2.02, 2.04, 2.16 (15H, 4 x s, 5 x C(O)CH_3), 4.08 (1H, dd, $J_{5,6}$ 4.2 Hz, $J_{6,6'}$ 11.3 Hz, H-6), 4.12 (1H, dd, $J_{5,6'}$ 6.7 Hz, H-6'), 4.34 (1H, dt, $J_{4,5}$ 0.6 Hz, H-5), 5.33 (2H, at, *J* 1.3 Hz, *J* 1.7 Hz, H-2, H-4), 5.50 (1H, br s, H-3), 6.38 (1H, d, $J_{1,2}$ 1.6 Hz, H-1).

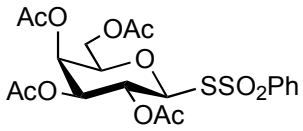
2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide[17] 46



1,2,3,4,6-Penta-*O*-acetyl-α-D-galactpyranoside **45** (1.0 g, 2.56 mmol) was dissolved in anhydrous DCM (10 mL) and to this hydrogen bromide (33% w/w in acetic acid, 10 mL)

was added. The mixture was stirred under argon at room temperature. After a 18 h period, t.l.c. (petrol:ethyl acetate, 3:1) indicated the formation of a product (R_f 0.3) with complete consumption of starting material (R_f 0.2). Ice water (15 mL) was added and the mixture stirred for 10 min. The two phases were separated and the aqueous layer re-extracted with DCM (3 x 10 mL). The combined organic layers were washed with sodium hydrogen carbonate (saturated aqueous solution) until pH 8 was obtained. The combined organics were washed with brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Recrystallisation (diethyl ether/petrol) afforded 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **46** (1.0 g, 95%) as a white crystalline solid; m.p. 84-86 ºC (diethyl ether/petrol) [Lit. 84-85 ºC][17]; $[\alpha]_D^{21}$ +212 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{25}$ +210 (c, 1 in $CHCl_3$)][17]; δ_H (400 MHz, $CDCl_3$) 2.02, 2.06, 2.12, 2.16 (12H, 4 x s, 4 x C(O)CH_3), 4.09-4.21 (2H, m, H-6, H-6'), 4.49 (1H, at, *J* 6.6 Hz, H-5), 5.05 (1H, dd, $J_{1,2}$ 3.9 Hz, $J_{2,3}$ 10.6 Hz, H-2), 5.41 (1H, dd, $J_{2,3}$ 10.6 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.53 (1H, br d, *J* 3.3 Hz, H-4), 6.76 (1H, d, $J_{1,2}$ 3.9 Hz, H-1).

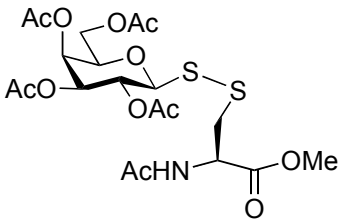
2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl phenylthiosulfonate[2] 47



2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranose bromide **46** (1.0 g, 2.43 mmol) was dissolved in anhydrous acetonitrile (40 mL). To this solution sodium phenylthiosulfonate **26** (0.95 g, 4.86 mmol) and tetrabutylammonium bromide (78 mg, 0.243 mmol) were added. The resulting mixture was stirred under argon at 70 ºC. After a 5 h period, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a major product (R_f 0.4) with complete consumption of the starting material (R_f 0.6). The reaction mixture was concentrated *in vacuo*. The crude solid was partitioned between DCM (50 mL) and water (30 mL), and the aqueous layer re-extracted with DCM (2 x 50 mL). The combined organic layers were washed with brine (80 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate:petrol, 2:1) afforded 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl phenylthiosulfonate **47** (0.82 g, 67%) as a white crystalline solid; m.p. 56-58 ºC [Lit. 53-54 ºC][2]; $[\alpha]_D^{21}$ +21.2 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{27}$ +24.2 (c, 1 in $CHCl_3$)][2]; δ_H (400 MHz, $CDCl_3$) 1.98, 2.03, 2.06, 2.11 (12H, 4 x s, 4 x C(O)CH_3), 3.85 (1H, dd, $J_{5,6}$ 8.8 Hz, $J_{6,6'}$ 13.6 Hz, H-6), 3.93-3.99 (2H, m, H-5, H-6'), 5.11 (1H, dd, $J_{2,3}$ 9.6 Hz,

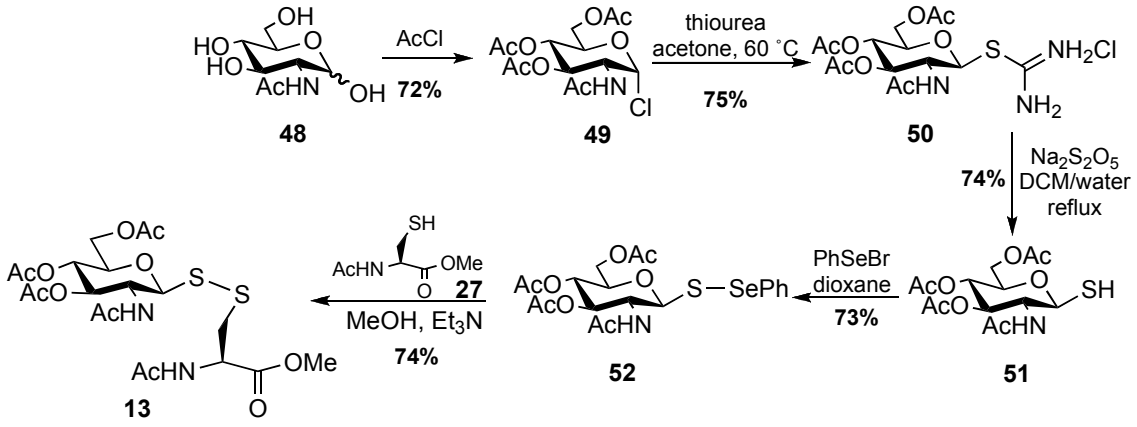
$J_{3,4}$ 3.3 Hz, H-3), 5.22 (1H, at, *J* 9.9 Hz, H-2), 5.28 (1H, d, $J_{1,2}$ 9.9 Hz, H-1), 5.43 (1H, br d, *J* 3.3 Hz, H-4), 7.54-7.68 (3H, m, Ar-H), 7.94-7.97 (2H, m, Ar-H).

_N_-Acetyl-L-cysteine (2,3,4,6-tetra-_O_-acetyl-1-dithio-β-D-galactopyaranosyl disulfide) methyl ester 11



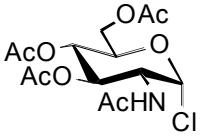
2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl phenylthiosulfonate **47** (339 mg, 0.672 mmol) was dissolved in anhydrous DCM (10 mL) and stirred at room temperature under an atmosphere of argon. A solution of *N*-acetyl-L-cysteine methyl ester **27** (119 mg, 0.672 mmol) and triethylamine (0.1 mL, 0.672 mmol) in a mixture of anhydrous DCM (10 mL) and anhydrous methanol (2 mL) was slowly added *via* a syringe pump over a 2 h period. After a 2 h period, t.l.c. (ethyl acetate) indicated the formation of a product (R_f 0.4). The reaction mixture was concentrated *in vacuo* and the resulting residue purified by flash column chromatography (ethyl acetate) to afford *N*-acetyl-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-galactopyaranosyl disulfide) methyl ester **11** (228 mg, 63%) as a white amorphous solid; $[\alpha]_D^{20}$ +27.3 (c, 1 in $CHCl_3$); υ_{max} (KBr disc) 3373 (br, NH) 1749 (s, C=O) 1665 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.99, 2.05, 2.06, 2.07, 2.17 (15H, 5 x s, 4 x C(O)CH_3, HNC(O)C$\underline{H}_3$), 3.13 (1H, dd, $J_{C\underline{H},H'}$ 14.3 Hz, $J_{CH,\alpha H}$ 6.8 Hz, C$\underline{H}$,H'), 3.36 (1H, dd, $J_{CH,\underline{H}'}$ 14.2 Hz, $J_{CH',\alpha H}$ 4.3 Hz, CH,$\underline{H}$'), 3.79 (3H, s, OCH_3), 4.06-4.09 (1H, m, H-5), 4.11-4.17 (2H, m, H-6, H-6'), 4.62 (1H, d, $J_{1,2}$ 9.9 Hz, H-1), 4.97-5.02 (1H, m, αH), 5.09 (1H, dd, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.4 Hz, H-3), 5.36 (1H, at, *J* 9.9 Hz, H-2), 5.45 (1H, br d, *J* 3.3 Hz, H-4), 6.39 (1H, $J_{NH,\alpha H}$ 8.0 Hz, $\underline{H}$NC(O)CH_3); δ_C (100.7 MHz, $CDCl_3$) 20.5, 20.6, 20.7, 20.8 (4 x q, 4 x C(O)$\underline{C}H_3$), 23.2 (q, HNC(O)$\underline{C}H_3$), 42.1 (t, CH,H'), 52.0 (d, αC), 52.8 (q, OCH_3), 61.5 (t, C-6), 66.6 (d, C-2), 67.2 (d, C-4), 71.7 (d, C-3), 74.9 (d, C-5), 90.3 (d, C-1), 169.5, 169.8, 170.0, 170.1, 170.4, 171.0 (6 x s, 4 x $\underline{C}$(O)CH_3, HN$\underline{C}$(O)CH_3, $\underline{C}O_2CH_3$); *m/z* (ES^+) 598 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{20}H_{29}NNaO_{12}S_2$ (MNa^+) 562.1023. Found: 562.1025.

Synthesis of *N*-Acetyl-L-cysteine (3,4,6-tetra-*O*-acetyl-2-acetamido-2-deoxy-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester 13



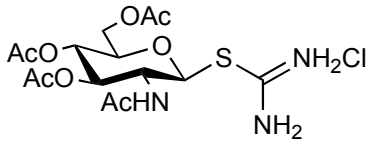
Scheme S13.

3,4,6-Tri-*O*-acetyl-2-*N*-acetylamido-2-deoxy-α-D-glucopyranosyl chloride[18,19] 49



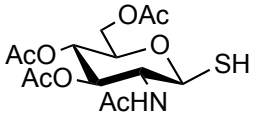
Acetyl chloride (40 mL, 563 mmol) was added through an air condenser into a round bottom flask containing 2-acetylamido-2-deoxy-D-glucose **48** (20.0 g, 90.41 mmol). The reaction mixture was heated for 1 h until a colour change was observed (pink) and the reaction mixture was stirred vigorously overnight. After 16 hours, t.l.c. (petrol:ethyl acetate, 1:2) indicated formation of a product (R_f 0.3) with complete consumption of the starting material (R_f 0).The reaction mixture was diluted with DCM (150 mL) and then poured on to ice water (100 mL). The organic layer was washed with ice cold sodium bicarbonate (3 x 100 mL) until no more gas was evolved. The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Recrystallization (diethyl ether/DCM) yielded 3,4,6-tri-*O*-acetyl-2-*N*-acetylamido-2-deoxy-α-D-glucopyranosyl chloride **49** as a crystalline solid (23.95 g, 72%); m.p. 120-122 ºC (diethyl ether/DCM) [Lit. 122-123 ºC][18]; $[\alpha]_D^{18}$ +127.0 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{26}$ +120.6 (c, 1.03 in $CHCl_3$)][19]; δ_H (400 MHz, $CDCl_3$) 1.99, 2.06, 2.11 (12H, 3 x s, 3 x C(O)CH_3, HNC(O)C$\underline{H}_3$), 4.05-4.14 (1H, m, H-6), 4.23-4.31 (2H, m, H-5, H-6'), 4.53 (1H, ddd, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 10.7 Hz, $J_{2,NH}$ 8.9 Hz, H-2), 5.22 (1H, at, *J* 9.8 Hz, H-4), 5.32 (1H, at, *J* 10.0 Hz, H-3), 5.83 (1H, d, *J* 8.6 Hz, $\underline{H}$NC(O)CH_3), 6.19 (1H, d, $J_{1,2}$ 3.7 Hz, H-1).

(3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-isothiouronium chloride[3] 50



3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl chloride **49** (5.0 g, 13.67 mmol) and thiourea (1.8 g, 23.24 mmol) were dissolved in anhydrous acetone (40 mL). The reaction mixture was stirred under an atmosphere of argon and heated to 60 ºC. After a 2 h period a white solid precipitated. The precipitate was removed by filtration, the filtrate was returned to reflux, and this process was repeated until the solid ceased to precipitate. The off white crystals were combined and recrystallised from acetone/petrol to afford (3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-isothiouronium chloride **50** (4.5 g, 75%) as a white crystalline solid; m.p. 134-136 ºC (acetone/petrol) [Lit. 134-137 ºC (acetone/petrol)][3]; $[\alpha]_D^{20}$ -24.6 (c, 1 in H_2O) [Lit. $[\alpha]_D^{25}$ -25.2 (c, 1 in H_2O)][3]; δ_H (400 MHz, DMSO-d_6) 1.80 (3H, s, HNC(O)C$\underline{H}_3$), 1.94, 1.97, 2.01 (9H, 3 x s, 3 x C(O)CH_3), 4.00 (1H, at, *J* 9.9 Hz, H-2), 4.06 (1H, dd, $J_{5,6}$ 2.0 Hz, $J_{6,6'}$ 11.4 Hz, H-6), 4.17 (1H, dd, $J_{5,6'}$ 4.8 Hz, $J_{6,6'}$ 11.4 Hz, H-6'), 4.22 (1H, ddd, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 2.0 Hz, $J_{5,6'}$ 4.8 Hz, H-5), 4.93 (1H, at, *J* 9.6 Hz, H-4), 5.13 (1H, at, *J* 9.8 Hz, H-3), 5.67 (1H, d, $J_{1,2}$ 10.5 Hz, H-1), 8.43 (1H, d, *J* 9.3 Hz, NH), 9.20 (2H, br s, NH_2), 9.39 (2H, br s, NH_2); δ_C (100.7 MHz, DMSO-d_6) 21.1, 21.3, 21.4 (3 x q, 3 x C(O)$\underline{C}H_3$), 23.4 (q, HNC(O)$\underline{C}H_3$), 52.0 (d, C-2), 62.3 (t, C-6), 68.7 (d, C-4), 73.5 (d, C-3), 75.6 (d, C-5), 81.5 (d, C-1), 168.0 (s, C=N), 170.1, 170.5, 170.8, 170.9 (4 x s, 3 x $\underline{C}(O)CH_3$, HN$\underline{C}(O)CH_3$).

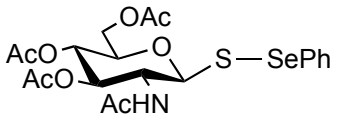
1-Thio-3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranose[3] 51



(3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-isothiouronium chloride **50** (4.4 g, 9.96 mmol) and sodium metabisulfite (2.3 g, 11.95 mmol) were dissolved in a mixture of DCM (60 mL) and water (30 mL). The mixture was heated to reflux under an atmosphere of argon. After a 2 h period the reaction was cooled to room temperature and the phases were separated. The aqueous layer was re-extracted with DCM (2 x 60 mL). The organics were combined and washed with water (60 mL) and brine (60 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Recrystallisation from

ethyl acetate/petrol afforded 1-thio-3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranose **51** (2.7 g, 74%) as a white solid; m.p. 166-168 ºC (ethyl acetate/petrol) [Lit. 165-187 ºC (ethyl acetate/petrol)][3]; $[\alpha]_D^{20}$ -25.3 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{25}$ -24.8 (c, 1 in $CHCl_3$)][3]; δ_H (400 MHz, $CDCl_3$) 1.99, 2.03, 2.05, 2.10 (12H, 4 x s, 3 x C(O)CH_3, HNC(O)C$\underline{H}_3$), 2.57 (1H, d, $J_{1,SH}$ 9.4 Hz, SH), 3.69 (1H, ddd, $J_{4,5}$ 9.5 Hz, $J_{5,6}$ 2.2 Hz, $J_{5,6'}$ 4.8 Hz, H-5), 4.00 (1H, at, *J* 9.9 Hz, H-2), 4.13 (1H, dd, $J_{5,6}$ 2.3 Hz, $J_{6,6'}$ 12.4 Hz, H-6), 4.24 (1H, dd, $J_{5,6'}$ 4.8 Hz, $J_{6,6'}$ 12.4 Hz, H-6'), 4.58 (1H, at, *J* 9.7 Hz, H-1), 5.08 (1H, at, *J* 9.4 Hz, H-3), 5.13 (1H, at, *J* 9.3 Hz, H-4), 5.70 (1H, d, *J* 9.5 Hz, $\underline{H}$NC(O)CH_3); δ_C (100.7 MHz, $CDCl_3$) 20.6, 20.7, 20.8 (3 x q, 3 x C(O)$\underline{C}H_3$), 23.3 (q, HNC(O)$\underline{C}H_3$), 56.8 (d, C-2), 62.1 (t, C-6), 68.0 (d, C-4), 73.5 (d, C-3), 76.3 (d, C-5), 80.4 (d, C-1), 169.2, 170.4, 170.8, 171.3 (4 x s, 3 x $\underline{C}$(O)CH_3, HN$\underline{C}$(O)CH_3).

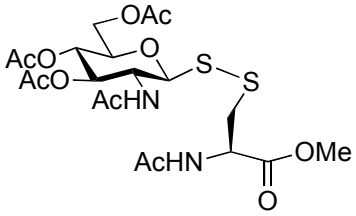
Phenyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-1-selenenylsulfide-β-D-glucopyranoside[3] 52



1-Thio-3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranose **51** (406 mg, 1.11 mmol) and phenylselenyl bromide (395 mg, 1.67 mmol) were dissolved in anhydrous DCM (10 mL). The resulting mixture was stirred under an atmosphere of argon at room temperature. After 10 min, t.l.c. (ethyl acetate) indicated the formation of a major product (R_f 0.4). The reaction was quenched with the addition of triethylamine (2 mL) and stirred for 5 min. The mixture was portioned between DCM (10 mL) and water (15 mL), the aqueous phase was separated and re-extracted with DCM (2 x 20 mL). The combined organic layers were washed with brine (25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate) to afford phenyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-1-selenenylsulfide-β-D-glucopyranoside **52** (424 mg, 73 %) as a white crystalline solid; m.p. 176-178 ºC [Lit. 177-179 ºC][3]; $[\alpha]_D^{20}$ -141.3 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{25}$ -134.0 (c, 1 in $CHCl_3$)][3]; δ_H (400 MHz, $CDCl_3$) 1.89 (3H, s, HNC(O)C$\underline{H}_3$), 2.01, 2.02, 2.04 (9H, 3 x s, 3 x C(O)CH_3), 3.75 (1H, ddd, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 2.3 Hz, $J_{5,6'}$ 4.7 Hz, H-5), 4.08 (1H, dd, $J_{5,6}$ 2.3 Hz, $J_{6,6'}$ 12.3 Hz, H-6), 4.16 (1H, dd, $J_{5,6'}$ 4.7 Hz, $J_{6,6'}$ 12.3 Hz, H-6'), 4.20 (1H, at, *J* 10.3 Hz, H-2), 4.78 (1H, at, *J* 10.1 Hz, H-1), 5.11 (1H, at, *J* 9.7 Hz, H-4), 5.28 (1H, at, *J* 9.8 Hz, H-3), 5.46 (1H, d, *J* 9.0 Hz, $\underline{H}$NC(O)CH_3), 7.26-7.29 (3H, m, Ar-H), 7.69-7.71 (2H, m, Ar-H); δ_C (100.7 MHz, $CDCl_3$) 20.6, 20.7 (2 x q, 3 x C(O)$\underline{C}H_3$), 23.3 (q,

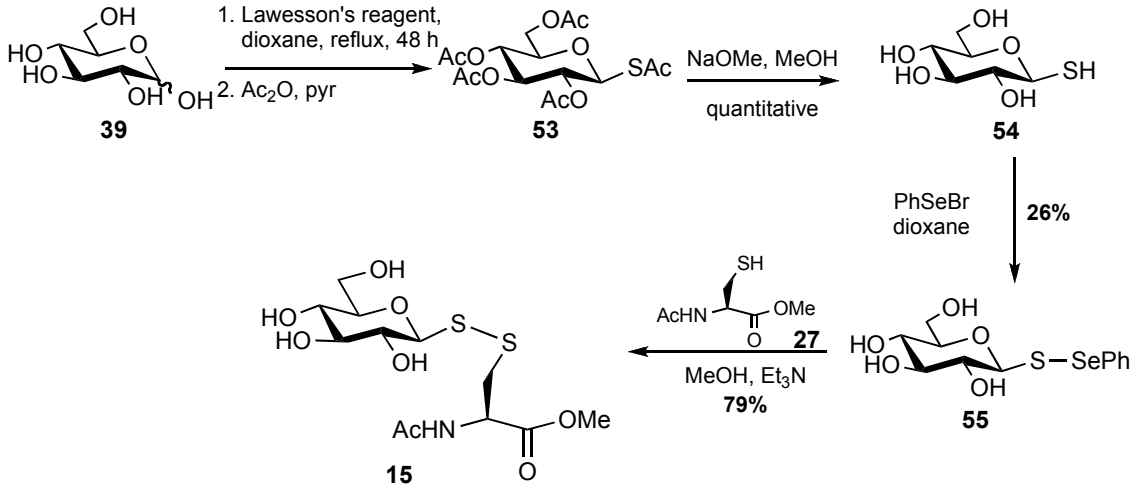
HNC(O)$\underline{C}H_3$), 54.3 (d, C-2), 62.1 (t, C-6), 68.1 (d, C-4), 73.3 (d, C-3), 76.0 (d, C-5), 86.9 (d, C-1), 127.9, 129.0, 131.3 (3 x d, 5 x Ar-C), 132.4 (s, Ar-C), 169.3, 170.0, 170.7, 171.0 (4 x s, 3 x $\underline{C}(O)CH_3$, HN$\underline{C}(O)CH_3$); *m/z* (ES^+) 578 ($MMeCNNH_4^+$, 100%).

_N_-Acetyl-L-cysteine (3,4,6-tetra-_O_-acetyl-2-acetamido-2-deoxy-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester 13



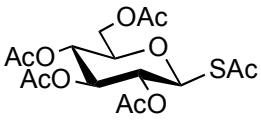
Phenyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-1-selenenylsulfide-β-D-glucopyranoside **52** (165 mg, 0.318 mmol) was dissolved in anhydrous DCM (5 mL) and stirred at room temperature under an atmosphere of argon. Triethylamine (50 μL, 0.318 mmol) was added to the above solution. A solution of *N*-acetyl-L-cysteine methyl ester **27** (56 mg, 0.318 mmol) in a mixture of anhydrous DCM (5 mL) and anhydrous methanol (4 mL) was slowly added *via* a syringe pump over a 2 h period. After a 2 h period, t.l.c. (ethyl acetate) indicated the formation of a major product (R_f 0.2). The reaction mixture was concentrated *in vacuo* and the resulting residue purified by flash column chromatography (ethyl acetate) to afford *N*-acetyl-L-cysteine (3,4,6-tetra-*O*-acetyl-2-acetamido-2-deoxy-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester **13** (126 mg, 74%) as a white amorphous solid; $[\alpha]_D^{21}$ -28.9 (c, 1 in $CHCl_3$); υ_{max} (KBr disc) 3309 (br, NH) 1746 (s, C=O) 1659 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.95, 2.03, 2.04, 2.06, 2.09 (15H, 5 x s, 3 x $C(O)CH_3$, 2 x HNC(O)C$\underline{H}_3$), 3.12 (1H, dd, $J_{C\underline{H},H'}$ 14.2 Hz, $J_{CH,\alpha H}$ 6.7 Hz, C$\underline{H}$,H'), 3.34 (1H, dd, $J_{CH,\underline{H}'}$ 14.2 Hz, $J_{CH',\alpha H}$ 4.8 Hz, CH,$\underline{H}$'), 3.79 (3H, s, OCH_3), 3.82 (1H, ddd, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 2.4 Hz, $J_{5,6'}$ 4.6 Hz, H-5), 4.18 (1H, at, *J* 9.8 Hz, H-2), 4.20 (1H, dd, $J_{5,6}$ 2.4 Hz, $J_{6,6'}$ 12.5 Hz, H-6), 4.26 (1H, dd, $J_{5,6'}$ 4.6 Hz, $J_{6,6'}$ 12.5 Hz, H-6'), 4.77 (1H, d, $J_{1,2}$ 10.4 Hz, H-1), 4.94-4.99 (1H, m, αH), 5.11 (1H, at, *J* 9.7 Hz, H-4), 5.24 (1H, at, *J* 9.8 Hz, H-3), 5.89 (1H, d, $J_{N\underline{H},H\text{-}2}$ 9.2 Hz, $\underline{H}NC(O)CH_3$, H-2), 6.46 (1H, d, $J_{N\underline{H},\alpha H}$ 7.9 Hz, $\underline{H}NC(O)CH_3$,αH); δ_C (100.7 MHz, $CDCl_3$) 20.6, 20.7, 20.8 (3 x q, 3 x $C(O)\underline{C}H_3$), 23.1, 23.2 (2 x q, 2 x $HNC(O)\underline{C}H_3$), 42.0 (t, CH,H'), 52.1 (d, αC), 52.7 (d, C-2), 52.8 (q, OCH_3), 62.0 (t, C-6), 68.0 (d, C-4), 73.4 (d, C-3), 76.1 (d, C-5), 90.3 (d, C-1), 169.3, 169.9, 170.2, 170.6, 170.6 (5 x s, 3 x $\underline{C}(O)CH_3$, 2 x HN$\underline{C}(O)CH_3$, $\underline{C}O_2CH_3$); *m/z* (ES^+) 597 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{20}H_{31}N_2O_{11}S_2$ (MH^+) 539.1364. Found: 539.1372.

Synthesis of *N*-Acetyl-L-cysteine (1-dithio-β-D-glucopyranosyl disulfide) methyl ester 15



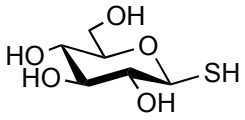
Scheme S14.

1-*S*-acetyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside[20] 53



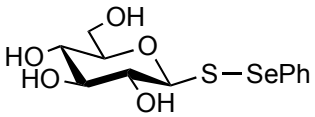
Lawesson's reagent (672 mg, 1.665 mmol) was added to a partial solution of D-glucose **39** (200 mg, 1.110 mmol) in anhydrous dioxane (6 mL) and the reaction mixture heated to 110 ºC under an atmosphere of argon for 48 h. After this time, the reaction mixture was cooled to room temperature, filtered through Celite® and concentrated *in vacuo*. The resulting crude product was then dissolved in pyridine (3 mL) and acetic anhydride (3 mL) added to the solution. The reaction was stirred for 16 h, after which time a few drops of water were added and the reaction left to stir for 10 min. The reaction mixture was extracted with ether (3 x 90 mL), washed with hydrochloric acid (50 mL of a 1M solution), sodium hydrogen carbonate (50 mL of a saturated aqueous solution), and water (2 x 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 3:1) to afford 1-*S*-acetyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **53** (0.2 g, 44%) as a white solid; m.p. 100-102 ºC [Lit. 119-120 ºC][20]; $[\alpha]_D^{20}$ +11.4 (c, 1.10 in $CHCl_3$) [Lit. $[\alpha]_D^{23}$ +10.5 (c, 0.6 in $CHCl_3$)][20]; δ_H (400 MHz, $CDCl_3$) 2.00, 2.02, 2.03, 2.08 (12H, 4 x s, 4 x $C(O)CH_3$), 2.39 (3H, s, $S(O)CH_3$), 3.82-3.86 (1H, m, H-5), 4.10 (1H, dd, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.5 Hz, H-6), 4.26 (1H, dd, $J_{5,6'}$ 4.5 Hz, H-6'), 5.09-5.15 (2H, m, H-3, H-4), 5.26 (1H, d, $J_{1,2}$ 10.6 Hz, H-1), 5.28 (1H, at, $J_{2,3}$ 9.3 Hz, H-2); *m/z* (ES^+) 465 ($MMeCNNH_4^+$, 100%).

1-Thio-β-D-glucopyranose[21] 54



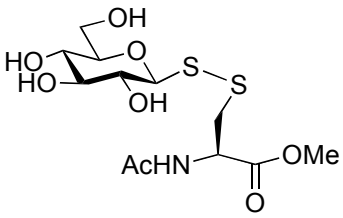
1-*S*-acetyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **53** (0.2 g, 0.50 mmol) was dissolved in anhydrous methanol (5 mL) and sodium methoxide (27 mg, 0.50 mmol) was added. The mixture was stirred under an atmosphere of argon for 1 h, when t.l.c. (petrol:ethyl acetate, 1:1) indicated formation of a single product (R_f 0.1) and complete consumption of starting material (R_f 0.4). Ion exchange resin (DOWEX 50WX8-200) was added portionwise until the solution was neutralised, at which point the reaction mixture was concentrated *in vacuo* to yield 1-thio-β-D-glucopyranose **54** (quantitative yield) which was used directly without further purification; $[\alpha]_D^{22}$ +6.0 (c, 1.0 in MeOH); δ_H (400 MHz, CD_3OD) 2.61 (1H, d, $J_{1,SH}$ 8.0 Hz, SH), 3.12 (1H, at, $J_{1,2}$ 9.3 Hz, $J_{2,3}$ 9.3 Hz, H-2), 3.30-3.36 (2H, m, H-3, H-5), 3.51 (1H, at, $J_{3,4}$ 9.4 Hz, $J_{4,5}$ 9.4 Hz, H-4), 3.64 (1H, dd, $J_{5,6}$ 1.9 Hz, $J_{6,6'}$ 12.0 Hz, H-6), 3.85 (1H, dd, $J_{5,6'}$ 5.3 Hz, $J_{6,6'}$ 12.0 Hz, H-6'), 4.42 (1H, d, $J_{1,2}$ 9.3 Hz, H-1); δ_C (100.7 MHz, CD_3OD) 61.8 (t, C-6), 72.1 (d, C-4), 76.4 (d, C-5), 77.2 (d, C-2), 78.2 (d, C-3), 80.9 (d, C-1); *m/z* (ES^-) 195 ($M\text{-}H^+$, 100%).

Phenyl 1-selenenylsulfide-β-D-glucopyranoside[3] 55



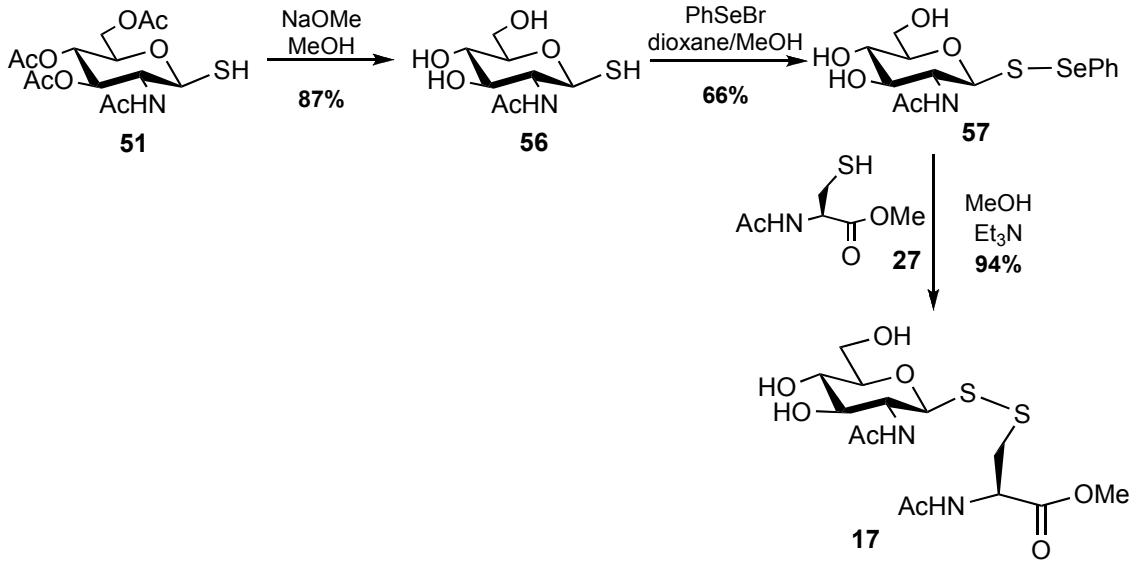
1-Thio-β-D-glucopyranose **54** (200 mg, 1.02 mmol) and phenylselenyl bromide (265 mg, 1.12 mmol) were dissolved in anhydrous dioxane (5 mL). The resulting mixture was stirred under an atmosphere of argon at room temperature. After 1 min, t.l.c. (ethyl acetate:methanol, 9:1) indicated the formation of a major product (R_f 0.4). The reaction was quenched with the addition of triethylamine (2 mL). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate) to afford phenyl 1-selenenylsulfide-β-D-glucopyranoside **55** (94 mg, 26 %) as an off white amorphous solid; $[\alpha]_D^{18}$ -93.8 (c, 1 in MeOH) [Lit. $[\alpha]_D^{22}$ -153.0 (c, 1 in MeOH)][3]; δ_H (400 MHz, CD_3OD) 3.53-3.55 (2H, m, H-3, H-5), 3.61-3.66 (2H, m, H-2, H-4), 3.84 (1H, dd, $J_{5,6}$ 5.1 Hz, $J_{6,6'}$ 11.7 Hz, H-6), 4.04 (1H, dd, $J_{5.6'}$ 1.7 Hz, $J_{6,6'}$ 11.3 Hz, H-6'), 4.69 (1H, d, $J_{1,2}$ 9.3 Hz, H-1), 7.47-7.56 (3H, m, Ar-H), 7.98-8.00 (2H, m, Ar-H); δ_C (100.7 MHz, CD_3OD) 62.2 (t, C-6), 70.6, 81.5 (2 x d, C-3, C-5), 73.8, 78.7 (2 x d, C-2, C-4), 89.8 (d, C-1), 127.8, 129.3, 130.9 (3 x d, 5 x Ar-C), 133.1 (s, Ar-C).

***N*-Acetyl-L-cysteine (1-dithio-β-D-glucopyranosyl disulfide) methyl ester 15**



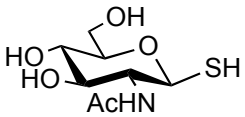
Phenyl 1-selenenylsulfide-β-D-glucopyranoside **55** (90 mg, 0.256 mmol) was dissolved in anhydrous methanol (8 mL) and stirred at room temperature under an atmosphere of argon. A solution of *N*-acetyl-L-cysteine methyl ester **27** (45 mg, 0.256 mmol) and triethylamine (40 μL, 0.256 mmol) in anhydrous methanol (5 mL) was slowly added *via* a syringe pump over a 2 h period. After a 2 h period, t.l.c. (ethyl acetate:methanol, 9:1) indicated the formation of a product (R_f 0.2) along with complete consumption of the starting material (R_f 0.4). The reaction mixture was concentrated *in vacuo* and the resulting residue purified by flash column chromatography (ethyl acetate:methanol, 9:1) to afford *N*-acetyl-L-cysteine (1-dithio-β-D-glucopyaranosyl disulfide) methyl ester **15** (75 mg, 79%) as a white amorphous solid; $[\alpha]_D^{21}$ -12.5 (c, 1 in MeOH); υ_{max} (KBr disc) 3363 (br, NH OH) 1764 (s, C=O) 1653 (s, C=O) cm^{-1}; δ_H (400 MHz, CD_3OD) 2.00 (3H, s, HNC(O)C<u>H</u>$_3$), 3.02 (1H, dd, $J_{C\underline{H},H'}$ 13.8 Hz, $J_{CH,\alpha H}$ 8.7 Hz, C<u>H</u>,H'), 3.31-3.34 (1H, m, H-5), 3.35-3.37 (2H, m, CH,<u>H</u>', H-4), 3.42 (1H, dd, *J* 8.0 Hz, *J* 10.3 Hz, H-3), 3.52 (1H, at, *J* 9.0 Hz, H-2), 3.71 (1H, dd, $J_{5,6}$ 5.3 Hz, $J_{6,6'}$ 11.9 Hz, H-6), 3.75 (3H, s, OCH_3), 3.89 (1H, dd, $J_{5,6'}$ 1.9 Hz, $J_{6,6'}$ 11.9 Hz, H-6'), 4.36 (1H, d, $J_{1,2}$ 9.3 Hz, H-1), 4.90-4.93 (1H, m, αH); δ_C (100.7 MHz, CD_3OD) 21.4 (q, HNC(O)<u>C</u>H$_3$), 40.6 (t, CH,H'), 51.9 (d, αC), 52.4 (q, OCH_3), 61.9 (t, C-6), 70.2 (d, C-4), 71.4 (d, C-2), 78.4 (d, C-3), 81.5 (d, C-5), 90.5 (d, C-1), 171.8, 172.4 (2 x s, HN<u>C</u>(O)CH_3, <u>C</u>O_2CH_3); *m/z* (ES^+) 394 (MNa^+, 90%), 430 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{12}H_{21}NNaO_8S_2$ (MNa^+) 394.0601. Found: 394.0601.

Synthesis of *N*-Acetyl-L-cysteine (2-acetamido-2-deoxy-1-dithio-β-D-glucopyranosyl disulfide) methyl ester 17



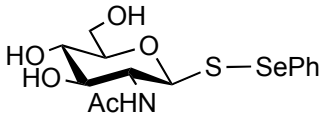
Scheme S15.

1-Thio-2-acetamido-2-deoxy-β-D-glucopyranose[3] 56



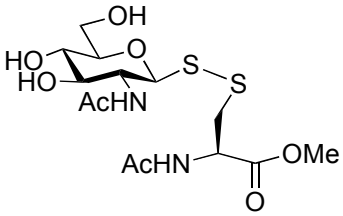
1-thio-3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranose **51** (0.66 g, 1.829 mmol) was dissolved in anhydrous methanol (10 mL) and sodium methoxide (0.32 g, 5.961 mmol) was added. After 30 min, t.l.c. (ethyl acetate) indicated the formation of a single product (R_f 0) and the absence of starting material (R_f 0.2). Ion exchange resin (DOWEX 50WX8-200) was added portionwise until the solution was neutralised, at which point the reaction mixture was concentrated *in vacuo*. Recrsytalisation from methanol/ethyl acetate yielded 1-thio-2-acetamido-2-deoxy-β-D-glucopyranose **56** (0.38 g, 87%) as a white crystalline solid; m.p. 174-176 ºC (methanol/ethyl acetate) [Lit. 177-179 ºC (methanol/ethyl acetate)][3]; $[\alpha]_D^{21}$ -12.1 (c, 1 in MeOH) [Lit. $[\alpha]_D^{22}$ -10.4 (c, 1 in MeOH)][3]; 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 ($M\text{-}H^+$, 100%).

Phenyl 2-acetamido-2 deoxy-1-selenenylsulfide-β-D-glucopyranoside[3] 57



1-Thio-2-acetamido-2-deoxy-β-D-glucopyranose **56** (248 mg, 1.05 mmol) and phenylselenyl bromide (271 mg, 1.15 mmol) were dissolved in a mixture of anhydrous dioxane (5 mL) and anhydrous methanol (3 mL). The resulting mixture was stirred under an atmosphere of argon at room temperature. After 1 min, t.l.c. (ethyl acetate:methanol, 9:1) indicated the formation of a major product (R_f 0.4). The reaction was quenched with the addition of triethylamine (3 mL). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate:methanol, 9:1) to afford phenyl 2-acetamido-2 deoxy-1-selenenylsulfide-β-D-glucopyranoside **57** (271 mg, 66 %) as an white amorphous solid; $[\alpha]_D^{21}$ -171.3 (c, 1 in MeOH) [Lit. $[\alpha]_D^{22}$ -174.0 (c, 1 in MeOH)][3]; δ_H (400 MHz, CD_3OD) 1.96 (3H, s, HNC(O)C$\underline{H}_3$), 3.31-3.34 (1H, m, H-5), 3.36 (1H, dd, $J_{5,6}$ 5.3 Hz, $J_{6,6'}$ 11.9 Hz, H-6), 3.84 (1H, dd, $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 11.9 Hz, H-6'), 3.87 (1H, at, *J* 10.0 Hz, H-2), 4.64 (1H, d, $J_{1,2}$ 10.3 Hz, H-1), 7.27-7.34 (3H, m, Ar-H), 7.71-7.74 (2H, m, Ar-H); δ_C (100.7 MHz, CD_3OD) 21.9 (q, HNC(O)C$\underline{H}_3$), 56.1 (d, C-2), 61.9 (t, C-6), 70.8 (d, C-5), 76.0 (d, C-3), 81.4 (d, C-4), 89.5 (d, C-1), 127.6, 129.1 (2 x d, 5 x Ar-C), 130.8 (s, Ar-C), 172.6 (s, HN$\underline{C}$(O)CH_3).

***N*-Acetyl-L-cysteine (2-acetamido-2-deoxy-1-dithio-β-D-glucopyranosyl disulfide) methyl ester 17**



Phenyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-1-selenenylsulfide-β-D-glucopyranoside **57** (112 mg, 0.286 mmol) and triethylamine (20 μL, 0.143 mmol) were dissolved in anhydrous methanol (8 mL) and stirred at room temperature under an atmosphere of argon. A solution of *N*-acetyl-L-cysteine methyl ester **27** (17 mg, 0.095 mmol) in anhydrous methanol (5 mL) was added dropwise over a 10 min period. After 6 h, t.l.c. (ethyl acetate:methanol, 9:1) indicated the formation of a product (R_f 0.1) along with complete consumption of the starting material (R_f 0.2). The reaction mixture was concentrated *in vacuo* and the resulting residue purified by flash column

chromatography (ethyl acetate:methanol, 9:1) to afford *N*-acetyl-L-cysteine (2-acetamido-2-deoxy-1-dithio-β-D-glucopyranosyl disulfide) methyl ester **17** (37 mg, 94%) as a white amorphous solid; $[\alpha]_D^{25}$ -74.1 (c, 1 in MeOH); υ_{max} (KBr disc) 3363 (br, NH OH) 1737 (s, C=O) 1654 (s, C=O) cm^{-1}; δ_H (500 MHz, CD_3OD) 1.99, 2.00 (6H, 2 x s, 2 x HNC(O)C$\underline{H}_3$), 3.07 (1H, dd, $J_{CH,H'}$ 13.9 Hz, $J_{CH,\alpha H}$ 8.7 Hz, C$\underline{H}$,H'), 3.30-3.39 (3H, m, H-3, H-4, CH,$\underline{H}$'), 3.50-3.54 (1H, m, H-5), 3.72-3.78 (2H, m, H-6, H-6'), 3.79 (3H, s, OCH_3), 3.92 (1H, at, *J* 10.3 Hz, H-2), 4.57 (1H, d, $J_{1,2}$ 10.3 Hz, H-1), 4.73-4.77 (1H, m, αH), 7.32 (1H, d, $J_{N\underline{H},H\text{-}2}$ 7.5 Hz, $\underline{H}$NC(O)CH_3,H-2), 7.74 (1H, d, $J_{N\underline{H},\alpha H}$ 7.0 Hz, $\underline{H}$NC(O)CH_3,αH); δ_C (125.8 MHz, CD_3OD) 22.4, 22.9 (2 x q, 2 x HNC(O)$\underline{C}H_3$), 41.9 (t, CH,H'), 52.9 (q, OCH_3), 53.6 (d, αC), 55.6 (d, C-2), 62.9 (t, C-6), 71.7 (d, C-4), 77.1 (d, C-5), 82.6 (d, C-3), 91.3 (d, C-1), 172.8, 173.4, 173.5 (3 x s, 2 x HN$\underline{C}$(O)CH_3, $\underline{C}O_2CH_3$); *m/z* (ES^+) 471 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{14}H_{24}N_2NaO_8S_2$ (MNa^+) 435.0869. Found: 435.0866.

General procedures for peptide synthesis

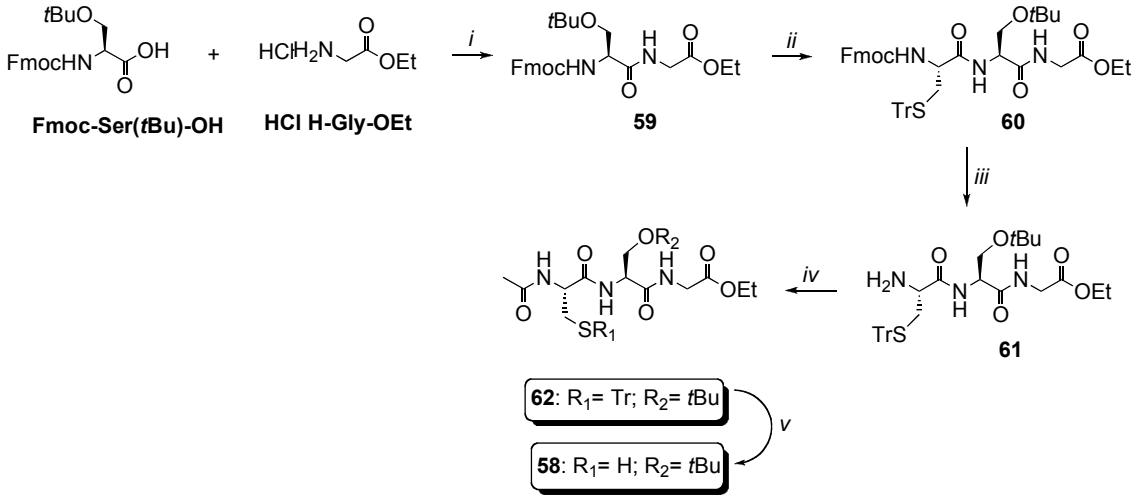
General procedure 1 **(GP 1)**: to a ~0.1M solution of the amine and appropriate amino acid (1.1 equivalents) in anhydrous DMF were added HBTU (1.1 equivalents), HOBt (0.37 equivalents) and DIPEA (2 equivalents). After t.l.c. analysis showed complete consumption of starting material and formation of a major product, the reaction mixture was concentrated *in vacuo*. The residue was diluted with DCM, washed with sodium hydrogen carbonate (saturated aqueous solution), potassium hydrogen sulfate (1N aqueous solution), and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*.

General procedure 2 **(GP 2)**: a ~0.1M solution of the Fmoc building block in DCM (*c*≈ 0.1M) was treated with 1.05 equiv. DBU. After t.l.c. analysis showed complete consumption of starting material and formation of a major product, the reaction mixture was concentrated *in vacuo* ~20% of the original volume. The amine was subsequently purified by flash column chromatography. In case of the "one-pot" procedure (*i.e.* deprotection-coupling), the reaction mixture was not concentrated after completion but treated with 1.0 equivalents of HOBt and this solution was used for the amino acid coupling as described in **GP 1**.

General procedure 3 **(GP 3)**: a solution of the amine in pyridine (*c*≈ 0.1M) was cooled to 0°C and treated with acetic anhydride (5 mL $mmol^{-1}$). To speed up the reaction 0.33 equivalents of DMAP were added. After t.l.c. analysis showed complete consumption of starting material and formation of a major product, the reaction mixture was concentrated *in vacuo*. The residue was diluted with DCM, washed with sodium

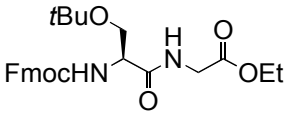
hydrogen carbonate (saturated aqueous solution), potassium hydrogen sulfate (1N aqueous solution), and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*.

Synthesis of Ac-Cys-Ser(*t*Bu)-Gly-OEt 58



Scheme S16. Reagents and Conditions: (*i*) HBTU, HOBt, DiPEA, DCM, DMF (yield **59**: 99%); (*ii*) (a) DBU, DCM; (b) HOBt; (c) Fmoc-Cys(Tr)-OH, HBTU, HOBt, DiPEA, DCM, DMF (yield **60**: 89%); (*iii*) DBU, DCM (yield **61**: 86%). (*iv*) Ac_2O, pyridine (yield **62**: 84%). (*v*) TFA/DCM (5/95 v/v), *i*Pr_3SiH (yield **58**: 73%).

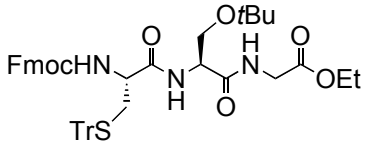
***N*-fluorenyl methoxycarbonyl-*O*-*tert*-butyl-L-serine-glycine ethyl ester 59**



Fmoc-Ser(*t*Bu)-OH (5.00 g, 13.0 mmol) and HCl•Gly-OEt (2.00 g, 14.3 mmol) were treated according to GP 1; purification by flash column chromatography (diethyl ether) gave Fmoc-Ser(*t*Bu)-Gly-OEt **59** as a white solid; m.p. 119 ºC; Yield: 99%; R_f 0.6 (diethyl ether); $[\alpha]_D^{18}$ +23.2 (c, 1 in $CHCl_3$); υ_{max} (KBr disc) 3300 (br, NH) 1726 (s, C=O) 1656 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.15 (9H, s, $C(CH_3)_3$), 1.21 (3H, dd, *J* 7.1 Hz, partially obscured by following resonance, $OCH_2C\underline{H}_3$), 3.34 (1H, at, *J* 8.4 Hz, CH,H'ser), 3.77 (1H, dd, *J* 3.8 Hz, *J* 8.4 Hz, CH,H'ser), 3.99 (2H, ABX system, *J* 13.3 Hz, *J* 18.3 Hz, αHgly), 4.10 (4H, m, αHser, H^{Fmoc}, $OC\underline{H}_2CH_3$), 4.32 (2H, d, *J* 6.8 Hz, CH_2^{Fmoc}), 5.72 (1H, d, *J* 5.2 Hz, NH), 7.18-7.36 (5H, m, 4×H^{Fmoc}, NH^{amide}), 7.55 (2H, d, *J* 7.4 Hz, H^{Fmoc}), 7.70 (1H, d, *J* 7.5 Hz, NH^{Fmoc}); δ_C (100.7 MHz, $CDCl_3$) 13.5 (q, $OCH_2\underline{C}H_3$), 26.7 (q, $C(\underline{C}H_3)_3$), 40.9 (t, αCgly), 46.5 (d, CH^{Fmoc}), 54.1 (d, αCser), 60.7 (t, CH,H'ser), 61.4 (t, $O\underline{C}H_2CH_3$), 66.5 (s, $\underline{C}(CH_3)_3$), 73.4 (t, CH_2^{Fmoc}), 119.4, 124.6, 126.5, 127.1 (4 x d, 4 x C^{Fmoc}), 140.6, 143.2, 143.3 (3 x s, 3 x C^{Fmoc}), 155.2 (s, CO^{Fmoc}), 169.2,

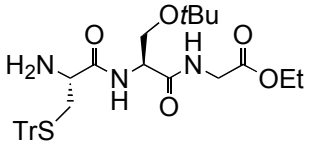
171.2 (2 x s, 2 x CO); *m/z* (ES^+) 527 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{26}H_{32}N_2NaO_6$ (MNa^+) 491.2153. Found: 491.2136.

_N_-fluorenyl methoxycarbonyl-_S_-trityl-L-cysteine-_O-tert_-butyl-L-serine-glycine ethyl ester 60



Fmoc-Ser(*t*Bu)-Gly-OEt **59** (2.50 g, 5.34 mmol) was deprotected according to GP 2 and coupled with Fmoc-Cys(Tr)-OH according to GP 1; purification by flash column chromatography (diethyl ether) gave **60** as a colourless foam; Yield: 89%; R_f 0.6 (diethyl ether); $[\alpha]_D^{18}$ +2.0 (c, 0.5 in $CHCl_3$); υ_{max} (thin film) 3370 (br, NH) 1750 (s, C=O) 1650 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.09 (9H, s, $C(CH_3)_3$), 1.16 (3H, at, *J* 7.1 Hz, $OCH_2C\underline{H}_3$), 2.64 (2H, d, *J* 6.8 Hz, CH,H'cys), 3.25 (1H, dd, *J* 6.2 Hz, *J* 8.8 Hz CH,H'ser), 3.51 (1H, dd, *J* 6.6 Hz, *J* 7.0 Hz, αHcys), 3.66, 3.71 (1H, ABX system, *J* 5.2 Hz, αHgly), 3.80 (1H, dd, *J* 3.3 Hz, *J* 8.8 Hz, CH,H'ser), 3.84, 3.88 (1H, ABX system, J 5.8 Hz, αHgly), 4.04 (2H, dd, *J* 6.9 Hz, *J* 7.2 Hz, $OC\underline{H}_2CH_3$), 4.10 (1H, at, *J* 7.0 Hz, CH^{Fmoc}), 4.28 (2H, dd, *J* 5.4 Hz, *J* 7.2 Hz, CH_2^{Fmoc}), 4.37 (1H, ddd, *J* 3.3 Hz, *J* 6.5 Hz, *J* 6.9 Hz, αHser)' 5.00 (1H, d, *J* 7.0 Hz, NH), 6.64 (1H, d, *J* 7.3 Hz, NH), 7.09-7.38 (19 H, m, NH, 6 × H^{Fmoc}, 13 × H^{Tr}), 7.49 (2H, d, *J* 7.0 Hz, H^{Fmoc}), 7.65 (1H, d, *J* 3.5 Hz, H^{Tr}), 7.69 (1H, d, *J* 3.9 Hz, H^{Tr}); δ_C (100.7 MHz, $CDCl_3$) 13.8 (q, $OCH_2\underline{C}H_3$), 27.0 (q, $C(\underline{C}H_3)_3$), 33.5 (t, CH,H'cys), 40.8 (t, αCgly), 46.7 (d, CH^{Fmoc}), 52.9, 53.7 (2 x d, αCser, αCcys), 60.8 (2 x t, $O\underline{C}H_2CH_3$, CH,H'ser), 66.9 (t, CH_2^{Fmoc}), 73.6 (s, $\underline{C}(CH_3)_3$), 119.6, 124.7 (2 x d, 3 x C^{Tr}), 126.6-127.8 (C^{Fmoc}, C^{Tr}), 140.9, 143.3, 143.5, 143.9 (4 x s, 3 x C^{Fmoc}, C^{Tr}), 155.5 (s, CO^{Fmoc}), 168.9, 169.6, 169.8 (3 x s, 2 × CO^{amide}, CO^{ester}); *m/z* (ES^+) 872 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{48}H_{51}N_3NaO_7S$ (MNa^+) 836.3340. Found: 836.3316.

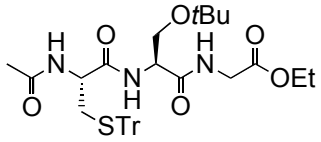
_S_-trityl-L-cysteine-_O-tert_-butyl-L-serine-glycine ethyl ester 61



Fmoc-Cys(Tr)-Ser(*t*Bu)-Gly-OEt **60** (1.00 g, 1.23 mmol) was deprotected according to GP 2; purification by flash column cromatography (DCM→DCM:methanol, 9:1) gave H-

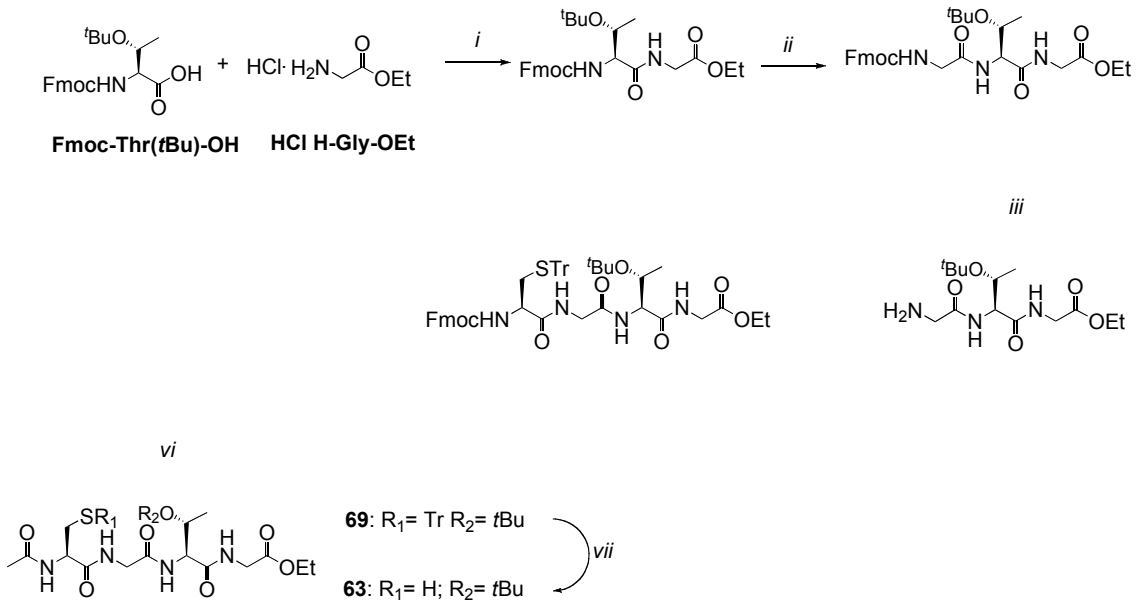
Cys(Tr)-Ser(*t*Bu)-Gly-OEt **61** as a yellow oil; Yield: 86%; R_f 0.5 (DCM:methanol, 9:1); δ_H (400 MHz, $CDCl_3$) 1.17 (9H, s, $C(CH_3)_3$), 1.25 (3H, at, *J* 7.2 Hz, $OCH_2C\underline{H}_3$), 2.55 (1H, at, *J* 8.1 Hz, CH,H'cys), 2.75 (1H, dd, *J* 4.6 Hz, *J* 12.8 Hz, CH,H'cys), 2.83 (1H, dd, *J* 4.2 Hz, J 8.1 Hz, αHcys), 3.33 (1H, at, *J* 7.7 Hz, CH,H'ser), 3.80 (1H, dd, *J* 4.0 Hz, *J* 8.8 Hz, CH,H'ser), 3.95 (2H, d, *J* 5.2 Hz, αHgly), 4.18 (2H, at, *J* 7.2 Hz, $OC\underline{H}_2CH_3$), 4.40 (1H, dd, *J* 4.0 Hz, *J* 7.3 Hz, αHser), 7.20-7.45 (16 H, m, 15 x H^{Tr}, NH^{amide}), 7.62 (1H, d, *J* 7.2 Hz, NH^{amide}); δ_C (100.7 MHz, $CDCl_3$) 13.7 (q, $OCH_2\underline{C}H_3$), 27.0 (q, $C(\underline{C}H_3)_3$), 36.9 (t, CH,H'cys), 40. 9 (t, αCgly), 52.4, 53.7 (2 x d, αCser, αCcys), 60.8, 66.4 (2 x t, $O\underline{C}H_2CH_3$, CH,H'ser), 73.5 (s, $\underline{C}(CH_3)_3$), 126.7, 127.6, 129.1 (3 x d, 3 x C^{Tr}), 144.1 (s, C^{Tr}), 169.0, 170.0, 173.0 (3 x s, 2 x CO^{amide}, CO^{ester}); *m/z* (ES^+) 650 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{33}H_{41}N_3NaO_5S$ (MNa^+) 614.2659. Found: 614.2647.

_N_-acetyl-_S_-trityl-L-cysteine-_O_-_tert_-butyl-L-serine-glycine ethyl ester 62



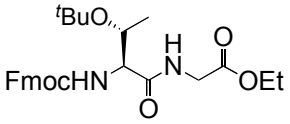
H-Cys(Tr)-Ser(*t*Bu)-Gly-OEt **61** (351 mg, 593 μmol) was treated according to GP 3; purification by flash column chromatography (6% methanol in DCM) furnished Ac-Cys(Tr)-Ser(*t*Bu)-Gly-OEt **62** as a white solid; m.p. 191 ºC; Yield: 84%; R_f 0.5 (6% methanol in DCM); $[\alpha]_D^{18}$ +14.4 (c, 0.25 in $CHCl_3$); υ_{max} (KBr disc) 3260 (br, NH) 1757 (s, C=O) 1640 (s, C=O) cm^{-1}; alternatively, Fmoc-Cys(Tr)-Ser(*t*Bu)-Gly-OEt **60** (1.00 g, 1.15 mmol) was deprotected according to GP 2 and acetylated following GP 3b; Yield: 81% over 2 steps; δ_H (400 MHz, $CDCl_3$) 1.18 (9H, s, $C(CH_3)_3$), 1.25 (3H, at, *J* 7.2 Hz, $OCH_2C\underline{H}_3$), 1.90 (3H, s, $C(O)CH_3$), 2.65 (1H, at, *J* 6.6 Hz, CH,H'cys), 2.75 (1H, dd, *J* 5.9 Hz, *J* 6.6 Hz, CH,H'cys), 3.35 (1H, dd, *J* 6.0 Hz, *J* 8.8 Hz, CH,H'ser), 3.75 (1H, dd, *J* 5.4 Hz, *J* 8.0 Hz, αHgly), 3.88 (3H, m, αHgly, CH,H'ser, αHcys), 4.18 (2H, dd, *J* 7.0 Hz, *J* 14.0 Hz, $OC\underline{H}_2CH_3$), 4.40 (1H, dd, *J* 3.3 Hz, *J* 7.3 Hz, αHser), 6.05 (1H, d, *J* 7.1 Hz, NH^{amide}), 6.70 (1H, d, *J* 7.4 Hz, NH^{amide}), 7.20-7.45 (16H, m, 15 x H^{Tr}, NH^{amide}); δ_H (100.7 MHz, $CDCl_3$) 13.9 (q, $OCH_2\underline{C}H_3$), 22.7 (q, $C(O)CH_3$), 27.1 (q, $C(\underline{C}H_3)_3$), 33.3 (t, CH,H'cys), 40.9 (t, αCgly), 52.1, 53.1 (2 x d, αCser, αCcys), 60.7, 60.9 (2 x t, $O\underline{C}H_2CH_3$, CH,H'ser), 73.7 (s, $\underline{C}(CH_3)_3$), 126.7, 127.9, 129.2 (3 x d, 3 x C^{Tr}), 144.0 (s, C^{Tr}), 169.0, 169.8, 169.9 (4 x CO); *m/z* (ES^+) 692 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{35}H_{43}N_3NaO_6S$ (MNa^+) 656.2765. Found: 656.2766.

Synthesis of Ac-Cys-Gly-Thr(*t*Bu)-Gly-OEt 63



Scheme S17. Reagents and Conditions: (*i*) HBTU, HOBt, DiPEA, DCM, DMF (yield **64**: 89%). (*ii*) (a) DBU, DCM; (b) HOBt; (c) Fmoc-Gly-OH, HBTU, HOBt, DiPEA, DCM, DMF (yield **65**: 85%); (*iii*) DBU, DCM (yield **66**: 82%); (*iv*) Fmoc-Cys(Tr)-OH, HBTU, HOBt, DiPEA, DCM, DMF (yield: 85%); (*v*) DBU, DCM (yield **67**: 70% from **65**); (*vi*) Ac_2O, pyridine (yield **69**: 82%); (*vii*) TFA/DCM (5/95 v/v), *i*Pr_3SiH (yield **63**: 38%).

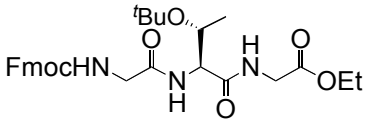
***N*-fluorenyl methoxycarbonyl-L-threonine-glycine ethyl ester 64**



Fmoc-Thr(tBu)-OH (5.00 g, 12.6 mmol) and HCl•H-Gly-OEt (2.11 g, 15.1 mmol) were treated according to GP 1; purification by flash column chromatography (petrol:diethyl ether, 1:3) gave **64** as a white solid; m.p. 93 ºC; Yield: 89%; R_f 0.8 (diethyl ether); $[\alpha]_D^{18}$ +19.2 (c, 1 in $CHCl_3$); υ_{max} (KBr disc) 3310 (br, NH) 1742 (s, C=O) 1670 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.10 (3H, d, *J* 6.4 Hz, CH_3thr), 1.30 (12H, m, $OCH_2\underline{C}H_3$, $C(CH_3)_3$), 4.07 (2H, ABX system, αHgly), 4.18-4.26 (5H, m, αHthr, CHthr, H^{Fmoc}, $OC\underline{H}_2CH_3$), 4.40 (2H, d, *J* 7.5 Hz, CH_2^{Fmoc}), 6.03 (1H, d, *J* 5.0 Hz, NH), 7.31 (2H, at, *J* 7.4 Hz, H^{Fmoc}), 7.40 (2H, at, *J* 7.5 Hz, H^{Fmoc}), 7.61 (2H, d, *J* 7.6 Hz, H^{Fmoc}), 7.64 (1H, m, NH), 7.75 (2H, d, *J* 7.5 Hz, H^{Fmoc}); δ_C (100.7 MHz, $CDCl_3$) 13.9 (q, $OCH_2\underline{C}H_3$), 16.6 (q, CH_3thr), 27.9 (q, $C(\underline{C}H_3)_3$), 41.3 (t, αCgly), 46.9 (d, CH^{Fmoc}), 58.3 (d, αCthr), 61.1 (t, $O\underline{C}H_2CH_3$), 66.4 (d, CHthr), 66.6 (t, CH_2^{Fmoc}), 75.3 (q, $\underline{C}(CH_3)_3$), 119.7, 124.9, 126.8, 127.4 (4 x d, 4 x C^{Fmoc}), 141.0 (s, 2 x C^{Fmoc}), 143.4, 143.7, (2 x s, 2 x C^{Fmoc}), 155.7 (s, CO^{Fmoc}), 169.1,

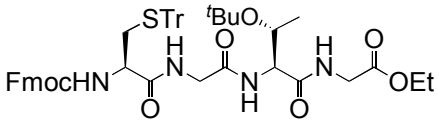
169.3 (2 x d, 2 x CO); *m/z* (ES^+) 505 (MNa^+, 100%); HRMS (ES^+) Calcd. for $C_{27}H_{34}N_2NaO_6$ (MNa^+) 505.2309. Found: 505.2304.

_N_-fluorenyl methoxycarbonyl-glycine-_O_-_tert_-butyl-L-threonine-glycine ethyl ester 65



Fmoc-Thr(*t*Bu)-Gly-OEt **64** (2.60 g, 5.39 mmol) was deprotected according to GP 1 and the free amine was coupled with Fmoc-Gly-OH following GP 2; purification by flash column chromatography (petrol:diethyl ether, 1:1→DCM→5% methanol in DCM) furnished Fmoc-Gly-Thr(*t*Bu)-Gly-OEt **65** as a foam; Yield: 85%; R_f 0.4 (8% methanol in DCM);); $[\alpha]_D^{18}$ +23.6 (c, 0.5 in $CHCl_3$); υ_{max} (thin film) 3320 (br, NH) 1726 (s, C=O) 1656 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.05 (3H, d, *J* 6.3 Hz, CH_3thr), 1.25 (3H, t, *J* 7.2 Hz, $OCH_2C\underline{H}_3$), 1.28 (9H, s, $C(CH_3)_3$), 4.05-4.11 (4H, ABX system, αHgly), 4.16-4.22 (4H, m, CH^{Fmoc}, CHthr, $OC\underline{H}_2CH_3$), 4.38 (2H, d, *J* 7.0 Hz, CH_2^{Fmoc}), 4.44 (1H, at, *J* 4.6 Hz, αHser), 5.80 (1H, m, NH), 7.23 (1H, d, *J* 5.6 Hz, NH^{amide}), 7.30 (2H, m, H^{Fmoc}), 7.37 (2H, at, *J* 7.4 Hz, H^{Fmoc}), 7.58 (2H, d, *J* 7.4 Hz, H^{Fmoc}), 7.68 (1H, at, *J* 5.0 Hz, NH^{amide}), 7.74 (2H, d, *J* 7.5 Hz, NH^{Fmoc}); δ_C (100.7 MHz, $CDCl_3$) 14.0 (q, $OCH_2\underline{C}H_3$), 17.1 (q, CH_3thr), 28.0 (q, $C(\underline{C}H_3)_3$), 41.4, 44.2 (2 x t, 2 x αCgly), 46.9 (d, CH^{Fmoc}), 57.4 (d, αCthr), 61.4 (t, $O\underline{C}H_2CH_3$), 66.0, 67.1 (2 x t, CH_2^{Fmoc}, CHthr), 75.5 (s, $\underline{C}(CH_3)_3$), 119.8, 125.0, 126.9, 127.6 (4 X d, C^{Fmoc}), 141.1 (s, 2 x C^{Fmoc}), 143.7 (s, 2 x C^{Fmoc}), 156.5 (s, CO^{Fmoc}), 168.8, 169.3, 169.5 (3 x s, 3 x CO); *m/z* (ES^+) 598 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{29}H_{37}N_3NaO_7$ (MNa^+) 562.2524. Found: 562.2525.

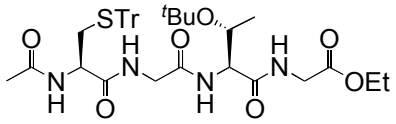
_N_-fluorenyl methoxycarbonyl-_S_-trityl-L-cysteine-glycine-_O_-_tert_-butyl-L-threonine-glycine ethyl ester 67



Fmoc-Gly-Thr(*t*Bu)-Gly-OEt **65** (108 mg, 200 μmol) was deprotected following GP 2; (H-Gly-Thr(*t*Bu)-Gly-OEt: R_f 0.3 (DCM:methanol, 9:1). After t.l.c. showed completion (30 min), the solution was concentrated *in vacuo* and the residue purified by flash column chromatography (DCM→DCM:methanol, 9:1). The amine (**66**, 52 mg,

164 μmol, 82%) was used directly for the coupling with Fmoc-Cys(Tr)-OH following GP 2; after strirring the reaction mixture overnight, t.l.c. analysis showed total consumption of starting material; purification by flash column chromatography (DCM→DCM:methanol, 9:1) gave **67** as a white solid; m.p. 117 ºC; Yield: 85%; R_f 0.4 (5% methanol in DCM);); $[\alpha]_D^{18}$ +14.6 (c, 0.5 in $CHCl_3$); υ_{max} (KBr disc) 3300 (br, NH) 1742 (s, C=O) 1632 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.91 (3H, d, *J* 6.2 Hz, CH_3thr), 1.13 (3H, t, *J* 7.1 Hz, $OCH_2C\underline{H}_3$), 1.17 (9H, s, $C(CH_3)_3$), 2.61 (2H, m, CH,H'cys), 3.83 (2H, m, αHgly), 3.88-4.00 (3H, m, 2 x αHgly, αHcys), 4.00-4.13 (4H, m, CH^{Fmoc}, $OC\underline{H}_2CH_3$, CHthr), 4.30 (3H, m, αHthr, CH_2^{Fmoc}), 5.40 (1H, d, *J* 6.9 Hz, NH), 7.10 (1H, m, NH), 7.10-7.22 (11H, m, 11 x Ar-H), 7.26-7.33 (8H, m, 8 x Ar-H), 7.51 (2H, m, H^{Fmoc}, NH), 7.65 (2H, dd, *J* 5.2 Hz, *J* 7.4 Hz, NH^{Fmoc}); δ_C (100.7 MHz, $CDCl_3$) 14.0 (q, $OCH_2\underline{C}H_3$), 17.1 (q, CH_3thr), 28.0 (q, $C(\underline{C}H_3)_3$), 41.4, 43.0 (2 x t, 2 x αCgly), 33.6 (t, CH,H'cys), 47.0 (d, CH^{Fmoc}), 53.8 (d, αCcys), 57.3 (d, αCthr), 61.3 (t, $O\underline{C}H_2CH_3$), 66.0, 67.1 (2 x t, CH_2^{Fmoc}, CHthr), 75.4 (s, $\underline{C}(CH_3)_3$), 119.8, 125.0, 126.7, 126.9, 127.6, 129.5 (6 x, d, $C^{Fmoc,Tr}$), 141.1, 143.6, 143.7, 144.3 (4 x s, $C^{Fmoc,Tr}$), 155.9 (s, CO^{Fmoc}), 168.0, 169.3, 170.5 (3 x s, 4 x CO); *m/z* (ES^+) 907 (MNa^+, 100%); HRMS (ES^+) Calcd. for $C_{51}H_{56}N_4NaO_8S$ (MNa^+) 907.3711. Found: 907.3712.

_N_-acetyl-_S_-trityl-L-cysteine-glycine-_O-tert_-butyl-L-threonine-glycine ethyl ester 69

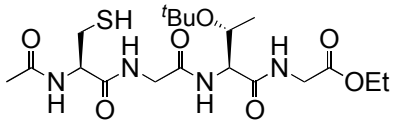


Fmoc-Cys(Tr)-Gly-Thr(*t*Bu)-Gly-OEt **67** was synthesised from Fmoc-Gly-Thr(*t*Bu)-Gly-OEt on a 4.36 mmol scale, however, in this case the crude tetrapeptide was used crude during the next removal of the Fmoc group on the cysteine residue according to GP 2; purification by flash column chromatography (DCM→DCM:methanol, 9:1) gave H-Cys(Tr)-Gly-Thr(*t*Bu)-Gly-OEt (**68**, 2.03 g, 3.06 mmol) as a foam; Yield: 70% from Fmoc-Gly-Thr(*t*Bu)-Gly-OEt; R_f 0.5 (8% methanol in DCM); *m/z* (ES^+) 721 ($MMeCNNH_4^+$, 100%);

Next, H-Cys(Tr)-Gly-Thr(*t*Bu)-Gly-OEt (1.00 g, 1.51 mmol) was treated with acetic anhydride (10 mL) and pyridine (10 mL) for 20 h. The reaction mixture was diluted with ethyl acetate (100 mL), washed with sodium hydrogen carbonate (saturated aqueous solution), hydrochloric acid (1N aqueous solution), and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 1/0→0/1→5% methanol in ethyl acetate) yielding **69** as a glassy

oil; Yield: 82%; R_f 0.5 (8% methanol in DCM);); $[\alpha]_D^{18}$ +15.0 (c, 1 in $CHCl_3$); υ_{max} (KBr disc) 3312 (br, NH) 1734 (s, C=O) 1656 (s, C=O) cm^{-1}; δ_H (400 MHz, CD_3OD) 1.01 (3H, d, *J* 6.4 Hz, CH_3thr), 1.13-1.17 (12H, m, $OCH_2C\underline{H}_3$, $C(CH_3)_3$), 1.91 (3H, s, $C(O)CH_3$), 2.58 (1H, dd, $J_{C\underline{H},H'}$ 13.0 Hz, $J_{CH,\alpha H}$ 5.9 Hz, C<u>H</u>,H'cys), 2.71 (1H, dd, $J_{CH,\underline{H}'}$ 13.0 Hz, $J_{CH',\alpha H}$ 6.7 Hz, CH,<u>H</u>'cys), 3.81-3.92 (1H, d, *J* 4.9 Hz, αHgly), 3.92-3.98 (1H, d, *J* 5.2 Hz, αHgly), 4.01-4.09 (2H, m, 2 x αHgly), 4.12 (1H, m, CHthr), 4.19 (3H, m, $OC\underline{H}_2CH_3$, αHcys), 4.40 (1H, dd, *J* 3.8 Hz, *J* 5.9 Hz, αHthr), 6.25 (1H, m, NH^{amide}), 6.88 (1H, at, *J* 5.0 Hz, NH^{amide}), 7.19-7.30 (11H, m, 10 x H^{Tr}, NH^{amide}), 7.63 (1H, t, *J* 5.1 Hz, NH^{amide}), 7.41 (5H, m, 5 x H^{Tr}); δ_C (100.7 MHz, CD_3OD) 14.1 (q, $OCH_2\underline{C}H_3$), 17.8 (q, CH_3thr), 23.0 (q, $C(O)\underline{C}H_3$), 28.1 (q, $C(\underline{C}H_3)_3$), 33.2 (t, CH,H'cys), 41.5, 43.2 (2 x t, 2 x αCgly), 52.0 (d, αCcys), 57.5 (d, αCthr), 61.4 (t, $O\underline{C}H_2CH_3$), 66.1 (t, CHthr), 75.5 (s, $\underline{C}(CH_3)_3$), 126.8, 128.0, 129.5 (3 x d, 3 x C^{Tr}), 144.4 (s, C^{Tr}), 168.1, 169.4, 169.5, 170.4, 170.6 (5 x s, 5 x CO); *m/z* (ES^+) 763 (MNa^+, 100%); HRMS (ES^+) Calcd. for $C_{38}H_{48}N_4NaO_7S$ (MNa^+) 727.3136. Found: 727.3135.

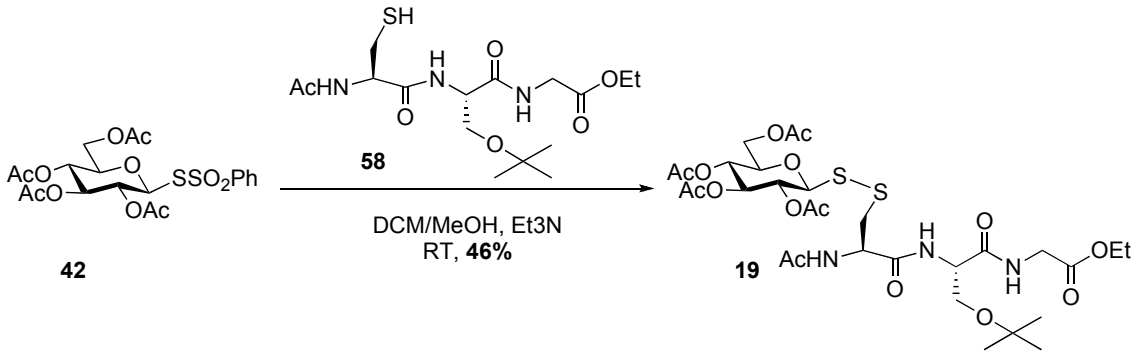
_N_-acetyl-L-cysteine-glycine-_O-tert_-butyl-L-threonine-glycine ethyl ester 63



Ac-Cys(Tr)-Gly-Thr(*t*Bu)-Gly-OEt **68** (868 mg, 1.23 mmol) was dissolved in DCM (14 mL) and treated with *i*Pr_3SiH (1.6 mmol, 327 μL) and trifluoroacetic acid (0.75 mL). After 6 h t.l.c. (DCM:methanol, 9:1) showed complete consumption of starting material and the formation of two lower running spots. The reaction mixture was co-evaporated with dry toluene and purified by flash column chromatography (petrol:ethyl acetate, 10/1→1/1→ethyl acetate→10% methanol in ethyl acetate→20% methanol in ethyl acetate) to give **63** as a foam; Yield: 38%; R_f 0.4 (ethyl acetate:methanol, 9:1); δ_H (400 MHz, CD_3OD) 1.12 (3H, d, *J* 6.3 Hz, CH_3thr), 1.24 (9H, s, $C(CH_3)_3$), 1.30 (3H, at, *J* 7.1 Hz, $OCH_2C\underline{H}_3$), 2.05 (3H, s, $C(O)CH_3$), 2.83 (2H, d, *J* 6.1 Hz, CH,H'cys), 3.88-4.06 (4H, m, αHgly), 4.17 (1H, m, $CHthr^r$), 4.23 (2H, dd, *J* 7.1 Hz, *J* 14.1 Hz, $OC\underline{H}_2CH_3$), 4.45 (1H, at, *J* 3.3 Hz, αHthr), 4.58 (1H, at, *J* 6.1 Hz, αHcys), 7.56 (1H, d, *J* 7.6 Hz, NH), 7.90 (1H, at, *J* 5.6 Hz, NH), 8.03 (1H, d, *J* 7.6 Hz, NH), 8.36 (1H, at, *J* 5.6 Hz, NH); δ_C (100.7 MHz, CD_3OD) 13.8 (q, $OCH_2\underline{C}H_3$), 17.8 (q, CH_3thr), 22.4 (q, $C(O)\underline{C}H_3$), 27.9 (q, $C(\underline{C}H_3)_3$), 34.3 (t, CH,H'cys), 41.2, 42.9 (2 x t, 2 x αCgly), 54.6, 57.6 (2 x d, αCthr, αCcys), 61.4 (t, $O\underline{C}H_2CH_3$), 66.3 (t, CHthr), 75.3 (s, $\underline{C}(CH_3)_3$), 169.1-

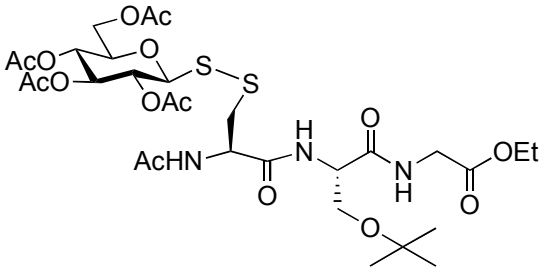
171.3 (CO); *m/z* (ES^+) 521 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{19}H_{34}N_4NaO_7S$ (MNa^+) 485.2040. Found: 485.2037.

Synthesis of *N*-Acetyl-L-cysteine-(2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyaranosyl disulfide)-*O*-*tert*-butyl-L-serine-glycine ethyl ester 19



Scheme S18.

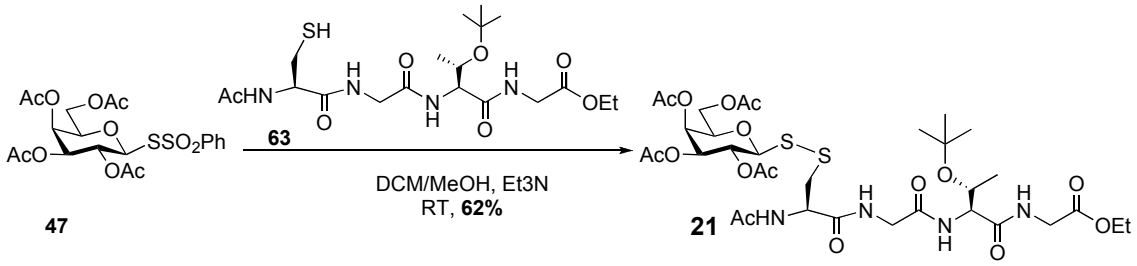
***N*-Acetyl-L-cysteine-(2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyaranosyl disulfide)-*O*-*tert*-butyl-L-serine-glycine ethyl ester 19**



Ac-Cys(Tr)-Ser(*t*Bu)-Gly-OEt **58** (250 mg, 394 μmol) was dissolved in DCM (10 mL) and treated with $i Pr_3SiH$ (513 μmol, 105 μL) and trifluoroacetic acid (0.5 mL). After 1 h the reaction mixture was co-evaporated with dry toluene (2×25 mL) and a solution of this tripeptide and triethylamine (394 μmol, 55 μL) in a mixture of DCM/methanol (9/1 v/v, 10 mL) was subsequently added dropwise (30 min period) to a solution of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl phenyl thiosulfonate **42** (398 mg, 0.79 mmol) in DCM/methanol (9/1 v/v, 10 mL). After stirring under an atmosphere of argon for 1 h 30 min, t.l.c. (6% methanol in DCM) showed complete consumption of starting material and formation of a major product (R_f 0.4). The reaction mixture was concentrated *in vacuo* and the resulting residue purified by flash column chromatography (DCM:methanol, 9:1) yielding **19** (137 mg, 46% over 2 steps) as an oil; $[\alpha]_D^{18}$ -159.2 (c, 0.5 in $CHCl_3$); υ_{max} (thin film) 3300 (br, NH) 1750 (s, C=O) 1643 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.16 (9H, s, $C(CH_3)_3$), 1.23 (3H, at,

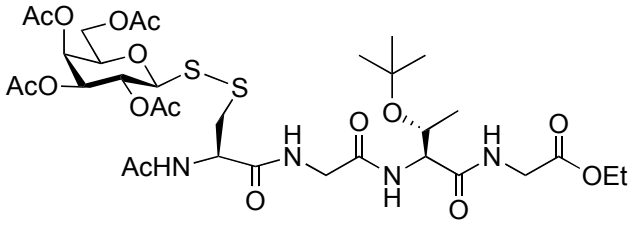
J 7.2 Hz,$OCH_2C\underline{H}_3$), 1.96-2.02 (15H, m, 4 x $C(O)CH_3$, $HNC(O)C\underline{H}_3$), 3.01 (1H, dd, $J_{C\underline{H},H'}$ 14.1 Hz, $J_{CH,\alpha H}$ 8.5 Hz, C<u>H</u>,H'cys), 3.22 (1H, dd, $J_{CH,\underline{H}'}$ 14.2 Hz, $J_{CH',\alpha H}$ 5.2 Hz, CH,<u>H</u>'cys), 3.40, 3.73 (2H, 2 x m, CH,H'ser), 3.79-3.83 (1H, ddd, *J* 2.2 Hz, *J* 4.1 Hz, *J* 6.2 Hz, H-5), 3.93-4.05 (2H, ABX system, 2 x αHgly), 4.13-4.19 (3H, m, $OC\underline{H}_2CH_3$, H-6), 4.27 (1H, dd, $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 12.5 Hz, H-6'), 4.44 (1H, m, αHser), 4.58 (1H, d, $J_{1,2}$ 9.4 Hz, H-1), 4.85 (1H, m, αHcys), 5.09 (1H, at, *J* 9.5 Hz, H-4), 5.21 (2H, m, H-2, H-3), 7.05 (1H, d, *J* 7.5 Hz, NH), 7.35 (1H, m, NH), 7.46 (1H, d, *J* 7.1 Hz, NH); δ_C (100.7 MHz, $CDCl_3$) 14.0 (q, $OCH_2\underline{C}H_3$), 20.3, 20.4, 20.5, 20.6 (4 x q, 4 x $C(O)\underline{C}H_3$), 22.8 (q, $HNC(O)\underline{C}H_3$), 27.2 (q, $C(\underline{C}H_3)_3$), 41.3 (2 x t, αCgly, CH,H'cys), 52.4, 53.3 (2 x d, αCser, αCcys), 61.0, 61.2, 61.3 (3 x t, $O\underline{C}H_2CH_3$, C-6, CH,H'ser), 67.8 (d, C-4), 69.0, 73.4 (C-2, C-3) 76.0 (d, C-5), 77.2 (s, $\underline{C}(CH_3)_3$), 88.3 (d, C-1)), 169.1-170.8 (8 x s, 8 x CO); *m/z* (ES^+) 812 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{30}H_{47}N_3NaO_{15}S_2$ (MNa^+) 776.2341. Found: 776.2345.

Synthesis of *N*-Acetyl-L-cysteine-(2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-galactopyaranosyl)-glycine-*O*-*tert*-butyl-L-threonine-glycine ethyl ester 21



Scheme S19.

***N*-Acetyl-L-cysteine-(2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-galactopyaranosyl)-glycine-*O*-*tert*-butyl-L-threonine-glycine ethyl ester 21**

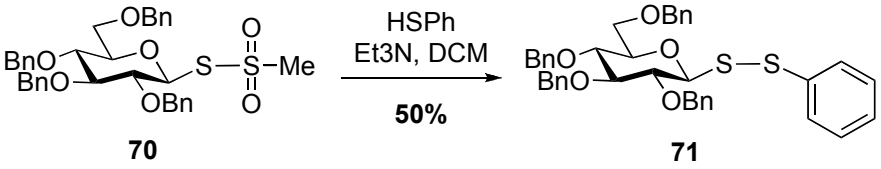


A solution of Ac-Cys-Gly-Thr(*t*Bu)-Gly-OEt **63** (214 mg, 463 μmol) in DCM/methanol (6/2 v/v, 8 mL) and triethylamine (463 μmol, 65 μL) was added dropwise (3 mL h^{-1}) to a solution of 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside phenylthiosulfonate **47** (0.70 g, 1.39 mmol) in DCM (10 mL). After stirring overnight, t.l.c. (DCM,methanol, 9:1) showed total consumption of starting material and formation of a major product (R_f 0.5). The

reaction mixture was concentrated *in vacuo* and purified by flash column chromatography (DCM:methanol, 9:1) to afford **21** (240 mg, 62%) as a glassy solid; $[\alpha]_D^{18}$ -58.0 (c, 1 in $CHCl_3$); υ_{max} (thin film) 3321 (br, NH) 1749 (s, C=O) 1654 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.05 (3H, d, J 6.3 Hz, CH_3thr), 1.27 (12H, m, $OCH_2C\underline{H}_3$, $C(CH_3)_3$), 1.98, 2.03, 2.04, 2.05 (12H, 4 x s, 4 x $C(O)CH_3$), 2.17 (3H, s, $HNC(O)C\underline{H}_3$), 3.04 (1H, dd, $J_{C\underline{H},H'}$ 14.2 Hz, $J_{CH,\alpha H}$ 8.2 Hz, C<u>H</u>,H'cys), 3.26 (1H, dd, $J_{CH,\underline{H}'}$ 14.2 Hz, $J_{CH',\alpha H}$ 5.4 Hz, CH,<u>H</u>'cys), 3.85-4.23 (10H, m, H-5, H-6, $OC\underline{H}_2CH_3$, 2 x αHgly, CH,H'thr), 4.40 (1H, dd, *J* 3.6 Hz, *J* 6.2 Hz, αHthr), 4.62 (1H, d, $J_{1,2}$ 10.1 Hz, H-1), 4.90 (1H, dd, *J* 2.5 Hz, *J* 7.6 Hz, αHcys), 5.10 (1H, dd, $J_{2,3}$ 10.1 Hz, $J_{3,4}$ 3.5 Hz, H-3), 5.38 (1H, at, *J* 9.9 Hz, H-2), 5.43 (1H, dd, *J* 0.6 Hz, *J* 3.5 Hz, H-4), 6.84 (1H, d, *J* 8.3 Hz, NH), 7.22 (1H, d, *J* 6.3 Hz, NH), 7.39 (1H, m, NH), 7.64 (1H, at, *J* 4.8 Hz, NH); δ_C (125.7 MHz, $CDCl_3$) 14.2 (q, $OCH_2\underline{C}H_3$), 17.3 (q, CH_3thr), 20.5, 20.6, 20.7, 20.8 (4 x q, 4 x $C(O)\underline{C}H_3$), 23.1 (q, $HNC(O)\underline{C}H_3$), 28.1 (q, $C(\underline{C}H_3)_3$), 41.5, 41.7, 43.3 (3 x t, 2 x αCgly, CH,H'cys), 52.5 (d, αCcys), 57.5 (d, αCthr), 61.5 (t, C-6), 66.1, 66.6, 67.4 (3 x d, C-2, C-4, C-5), 71.7 (d, C-3), 75.0 (s, $C(\underline{C}H_3)_3$), 75.6 (t, $O\underline{C}H_2CH_3$), 89.8 (d, C-1), 168.2-170.8 (9 x s, 9 x CO); *m/z* (ES^+) 883 ($MMeCNNH_4^+$, 100%); HRMS (ES^+) Calcd. for $C_{33}H_{52}N_4NaO_{16}S_2$ (MNa^+) 847.2712. Found: 847.2717.

Mechanistic Studies on Desulfurization Reaction

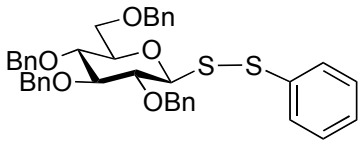
Synthesis of Phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl disulfide 71



70 **71**

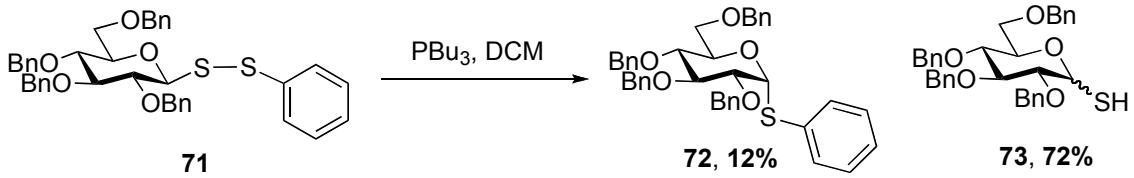
Scheme S20.

Phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl disulfide 71



A solution of thiophenol (34 μl, 0.34 mmol) in dichloromethane (20 ml) was added dropwise over 45 min to a stirred solution of 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl methanethiosulfonate[1] (0.22 g, 0.34 mmol) and triethylamine (47 μl, 0.34 mmol) in dichloromethane (10 ml) at 0 °C. The ice bath was removed. After a further 1 h, t.l.c. (petrol:ethyl acetate, 4:1) showed disappearance of most of the starting material. The reaction mixture was passed through a short silica plug and the plug washed with dichloromethane. The filtrate was evaporated and the residue purified by flash column chromatography (petrol:ethyl acetate, 9:1) to give phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl disulfide **71** (0.12 g, 50%) as a white crystalline solid; m.p. 80-82 °C; $[\alpha]_D^{25}$ -122 (c, 0.7 in $CHCl_3$); υ_{max} (thin film) no significant peaks; δ_H (500 MHz, $CDCl_3$) 3.48 (1H, m, H-5), 3.66-3.72 (4H, m, H-3, H-4, H-6, H-6'), 3.71 (1H, at, *J* 8.7 Hz, H-2), 4.41-4.90 (9H, m, H-1, 4 x OCH_2Ph), 7.13-7.46 (25H, m, Ar-H); δ_C (128.7 MHz, $CDCl_3$) 69.0 (t, C-6), 73.6, 75.0, 75.4, 75.7 (4 x t, 4 x OCH_2Ph), 77.6, 79.5, 80.1 (3 x d, C-2, C-4, C-5), 86.7 (d, C-3), 89.4 (d, C-1), 127.0, 127.5, 127.6, 127.7, 127.8, 127.9, 128.2, 128.3, 128.4, 128.7 (10 x d, Ar-C), 137.4, 137.8, 138.0, 138.2, 138.4 (5 x s, Ar-C); *m/z* (ES^+) 687 (MNa^+, 100%); HRMS (ES^+) Calcd. for $C_{40}H_{40}NaO_5S_2$ (MNa^+) 687.2209. Found: 687.2212.

Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside 72



71 **72**, **12%** **73**, **72%**

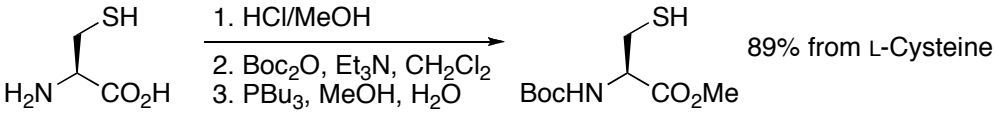
Tributylphosphine (44 μl, 0.18 mmol) was added to a stirred solution of phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl disulfide **71** (59 mg, 0.09 mmol) in anhydrous dichloromethane (2 ml) under an atmosphere of argon. After 5 h, t.l.c. (petrol:ethyl acetate, 8:2) showed incomplete consumption of the starting material along with the formation of two products. A further portion of tributylphosphine (44 μl, 0.18 mmol) was added and the reaction mixture stirred for 12 h. The solvent was removed *in vacuo* and the resulting residue purified by flash column chromatography (petrol:ethyl acetate, 95:5) to afford phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside **72** (7 mg, 12%) as a white foam and 2,3,4,6-tetra-*O*-benzyl-1-thio-D-glucopyranose **73** (35 mg, 72%) as an oil being a mixture of anomers (α:β, 1:2);

Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside[17] **72**: $[\alpha]_D^{20}$ +124 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{25}$ +128.2 (c, 1 in $CHCl_3$)][17]; δ_H (500 MHz, $CDCl_3$) 3.59 (1H, dd, $J_{5,6}$ 2.0 Hz, $J_{6,6'}$ 10.8 Hz, H-6), 3.70-3.76 (2H, m, H-4, H-6'), 3.93 (1H, at, *J* 9.0 Hz, H-3), 3.99 (1H, dd, $J_{1,2}$ 4.7 Hz, $J_{2,3}$ 9.5 Hz, H-2), 4.28 (1H, ddd, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 1.8 Hz, $J_{5,6'}$ 3.6 Hz, H-5), 4.43-4.87 (8H, m, 4 x OCH_2Ph), 5.66 (1H, d, $J_{1,2}$ 4.8 Hz, H-1), 7.17-7.45 (25H, m, Ar-H); *m/z* (ES^+) 650 (MNH_4^+, 80%) 655 (MNa^+, 100%).

2,3,4,6-Tetra-*O*-benzyl-1-thio-D-glucopyranose[21] **73**: m.p. 48-50 °C (ether/petrol) [Lit. 47-50 °C][22]; $[\alpha]_D^{22}$ +42.5 (c, 0.75 in $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.95 (1H, d, $J_{1,SH}$ 4.8 Hz, SHα), 2.37 (1H, d, $J_{1,SH}$ 8.1 Hz, SHβ), 3.43 (1H, at, *J* 9.0 Hz, H-2β), 3.50-3.58 (1H, m, H-5β), 3.67-3.73 (2H, m, H-3α, H-3β), 3.78-3.88 (6H, m, H-4α, H-6α, H-6'α, H-4β, H-6β, H-6'β), 3.92 (1H, dd, $J_{1,2}$ 4.9 Hz, $J_{2,3}$ 9.0 Hz, H-2α), 4.25-4.28 (1H, m, H-5α), 4.56 (1H, dd, $J_{1,SH}$ 8.1 Hz, $J_{1,2}$ 9.0 Hz, H-1β), 4.57-5.01 (16H, m, 4 x $OCH_2Ph\alpha$, 4 x $OCH_2Ph\beta$), 5.80 (1H, at, *J* 4.8 Hz, H-1α), 7.19-7.43 (40H, m, 20 x Ar-Hα, 20 x Ar-Hβ); *m/z* (ES^+) 574 (MNH_4^+, 100%) 579 (MNa^+, 80%).

Crossover experiment

Preparation of ***N*-(*tert*-Butoxycarbonyl)-L-cysteine methyl ester[23] 74**



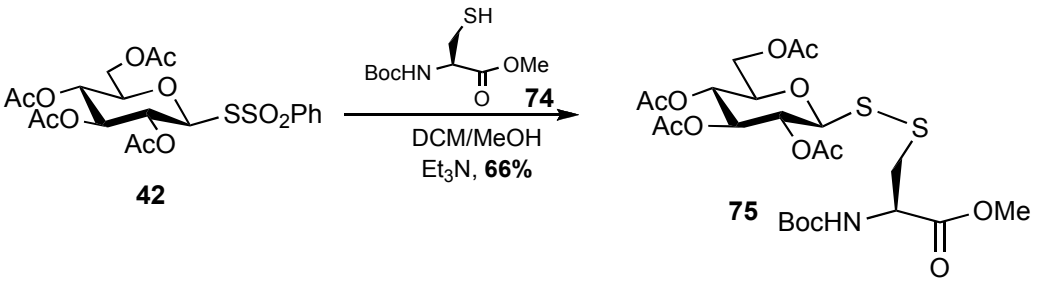
74

Scheme S21.

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 (2α.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 dichloromethane (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 t.l.c. (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. t.l.c. 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 **74** 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 $CHCl_3$)][23]; δ_H (400 MHz, $CDCl_3$) 1.42 (10H, s, 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,

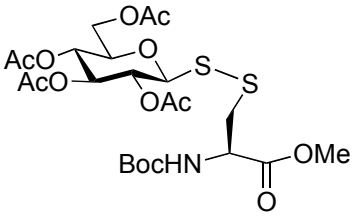
OCH_3), 54.8 (d, αC), 80.2 (s, $\underline{C}(CH_3)_3$), 155.1, 170.8 (2 x s, 2 × CO); *m/z* (ES^-) 234 ($M\text{-}H^+$, 100%).

Synthesis of *N*-(*tert*-butoxycarbonyl)-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester 75



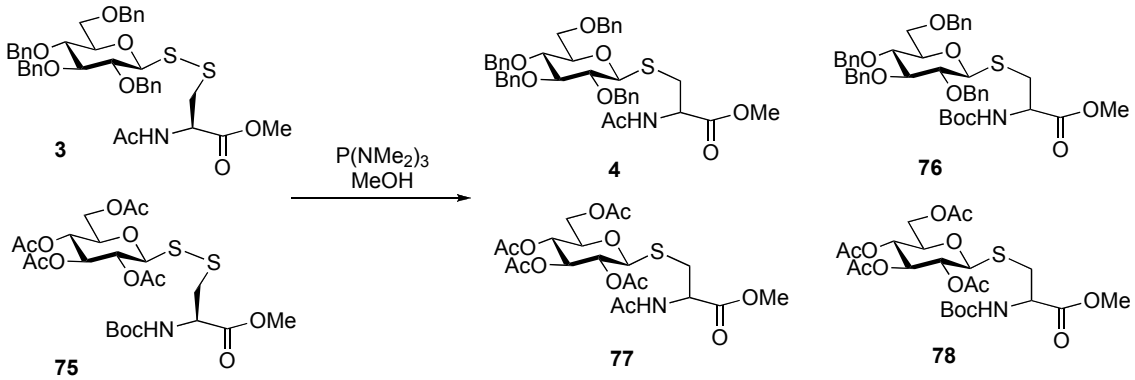
Scheme S22.

***N*-(*tert*-butoxycarbonyl)-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester 75**



2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl phenylthiosulfonate **42** (200 mg, 0.397 mmol) was dissolved in anhydrous DCM (8 mL) and stirred at room temperature under an atmosphere of argon. A solution of *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester **74** (93 mg, 0.397 mmol) and triethylamine (55 *μ*L, 0.397 mmol) in a mixture of anhydrous DCM (10 mL) and anhydrous methanol (2 mL) was slowly added *via* a syringe pump over a 2 h period. After 1 h, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a product (R_f 0.5) along with complete consumption of the starting material (R_f 0.4). The reaction mixture was concentrated *in vacuo* and the resulting residue purified by flash column chromatography (ethyl acetate:petrol, 1:2) to afford *N*-(*tert*-butoxycarbonyl)-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester **75** (155 mg, 66%) as a white amorphous solid; $[\alpha]_D^{22}$ +105 (c, 1 in $CHCl_3$); υ_{max} (KBr disc) 3416 (br, NH) 1750 (s, C=O) 1645 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.46 (9H, s, $C(CH_3)_3$), 2.01, 2.03, 2.04, 2.09 (12H, 4 x s, 4 x $C(O)CH_3$), 3.06 (1H, dd, $J_{C\underline{H},H'}$ 13.8 Hz, $J_{CH,\alpha H}$ 7.7 Hz, C<u>H</u>,H'), 3.31 (1H, dd, $J_{CH,\underline{H}'}$ 13.9 Hz, $J_{CH',\alpha H}$ 4.7 Hz, CH,<u>H</u>'), 3.77 (3H, s, OCH_3), 3.80 (1H, m, H-5), 4.16 (1H, dd, $J_{5,6}$ 2.1 Hz, $J_{6,6'}$ 12.4 Hz, H-6), 4.27 (1H, dd, $J_{5,6'}$ 4.6 Hz, $J_{6,6'}$ 12.4 Hz, H-6'), 4.57 (1H, d, $J_{1,2}$ 9.3 Hz, H-1), 4.68 (1H, m, αH), 5.14 (1H, at, *J* 9.7 Hz,

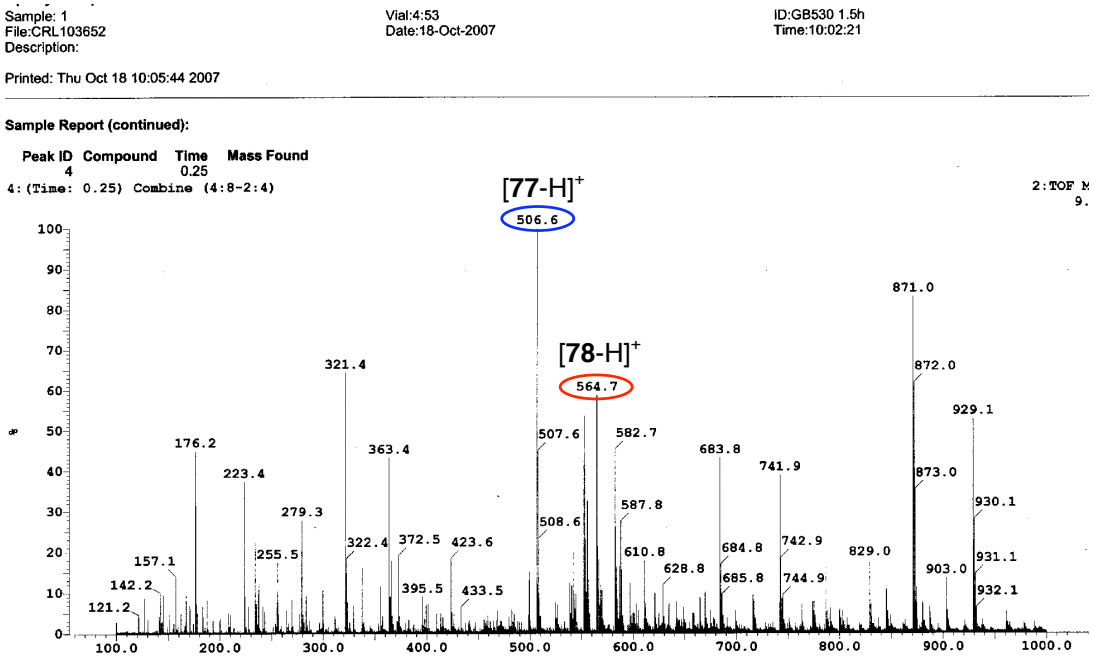
H-4), 5.24-5.34 (2H, m, H-2, H-3); δ_C (100.7 MHz, $CDCl_3$) 20.6, 20.7, 20.8 (3 x q, 4 x C(O)<u>C</u>H_3), 28.3 (q, C(<u>C</u>H_3)$_3$), 42.6 (t, CH,H'), 52.6 (d, αC), 52.8 (q, OCH_3), 61.9 (t, C-6), 67.8 (d, C-4), 68.9 (d, C-2), 73.8 (d, C-3), 76.1 (d, C-5), 80.3 (s, <u>C</u>$(CH_3)_3$), 87.8 (d, C-1), 169.2, 169.4, 170.2, 170.6 (4 x s, 4 x <u>C</u>(O)CH_3, <u>C</u>O_2CH_3); *m/z* (ES^+) 615 (MNH_4^+, 100%), 620 (MNa^+, 95%); HRMS (ES^+) Calcd. for $C_{23}H_{35}NNaO_{13}S_2$ (MNa^+) 620.1451. Found: 6201451.



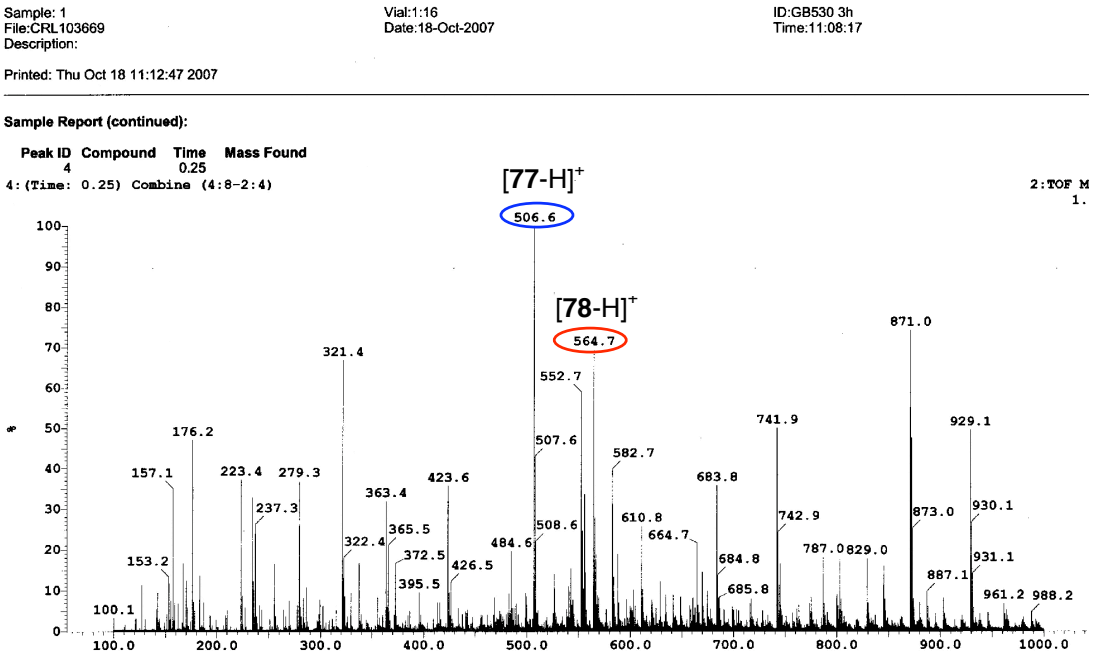
Scheme S23. Crossover experiment.

N-Acetyl-L-cysteine (2,3,4,6-tetra-*O*-benzyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester **3** (12 mg, 17 μmol) and *N*-(*tert*-butoxycarbonyl)-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester **75** (10 mg, 17 μmol) was dissolved in degassed anhydrous methanol (0.5 mL). Hexamethylphosphorus (7 μL, 37 μmol) was added *via* microsyringe, and the resulting solution stirred under an atmosphere of argon. The reaction was analysed directly by ESI mass spectrometry and distinct peaks were observed for **77** and **78**.

ESI (negative mode) after 1.5 h:



ESI (negative mode) after 3 h:



NMR kinetics on desulfurization reaction

N-Acetyl-L-cysteine (2,3,4,6-tetra-*O*-benzyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester **3** (20 mg, 27 μmol) was dissolved in degassed deuterated methanol (0.5 mL) in a precision NMR tube. Hexamethylphosphorus triamide (11 μL, 62 μmol) was added *via* microsyringe, and the resulting solution placed in a Bruker AV II 500 spectrometer at 30

ºC equipped with a triple resonance (TBI) inverse probe. The ^{1}H NMR spectra were collected at 6 minute intervals which included 1 minute data acquisition. Integrated peak intensities were analysed as a function of the reaction time course.

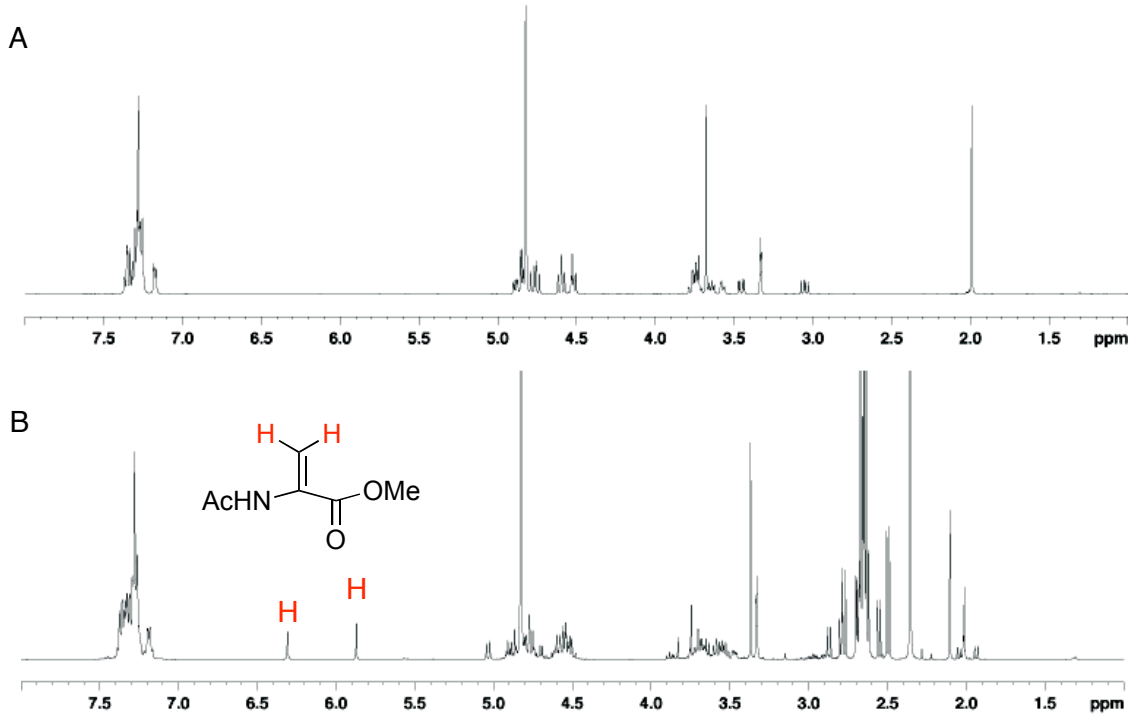


Figure S5. ^{1}H-NMR before (A) and immediately (B) after addition of HMPT.

The spectroscopic data for the dehydroalanine intermediate (δ 5.86 and 6.31 ppm) was identical to that previously reported in the literature.[24]

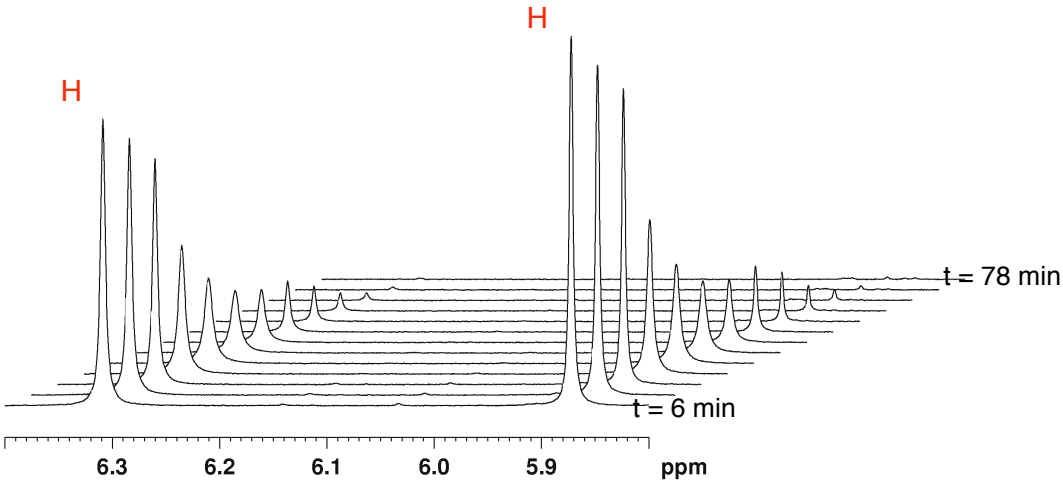


Figure S6. Formation and consumption of dehydroalanine during the desulfurization reaction.

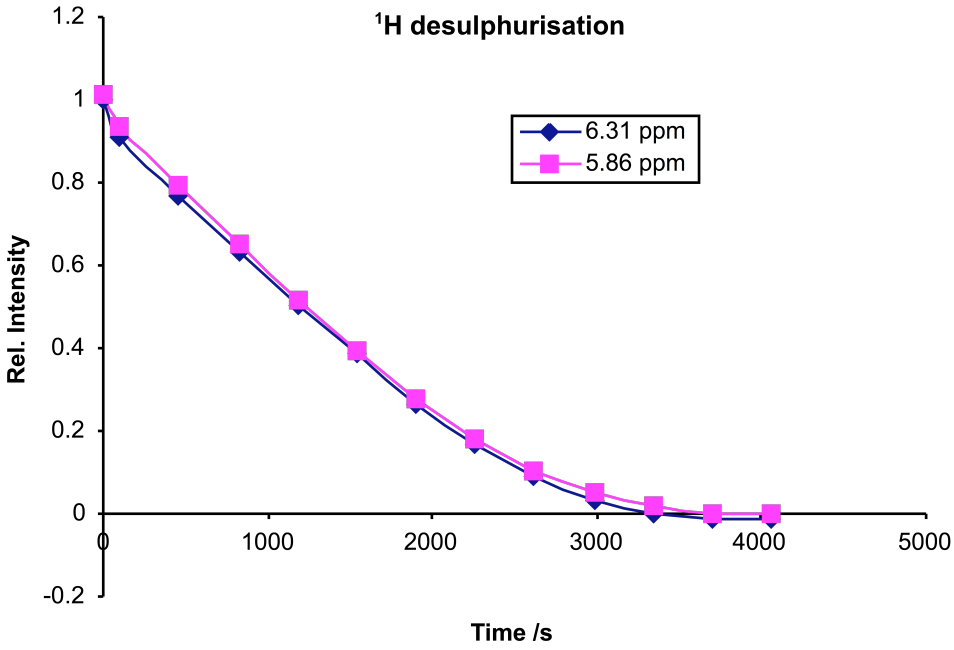


Figure S7. Plot of ^{1}H NMR signal intensities (relative to those in the first spectrum) showing the kinetics of the consumption of dehydroalanine.

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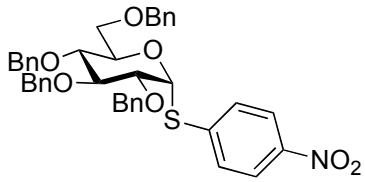
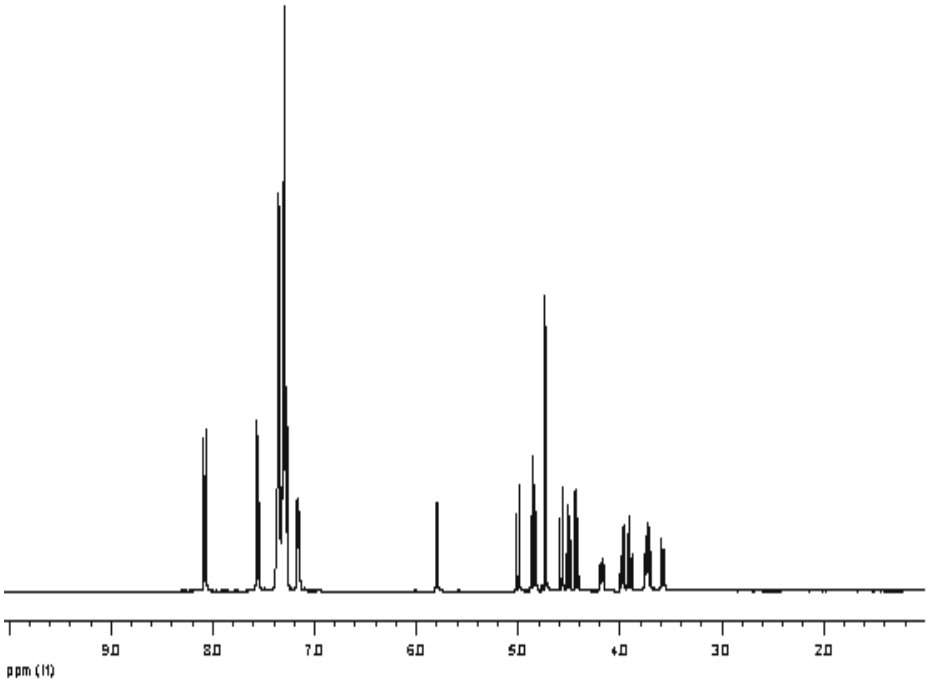
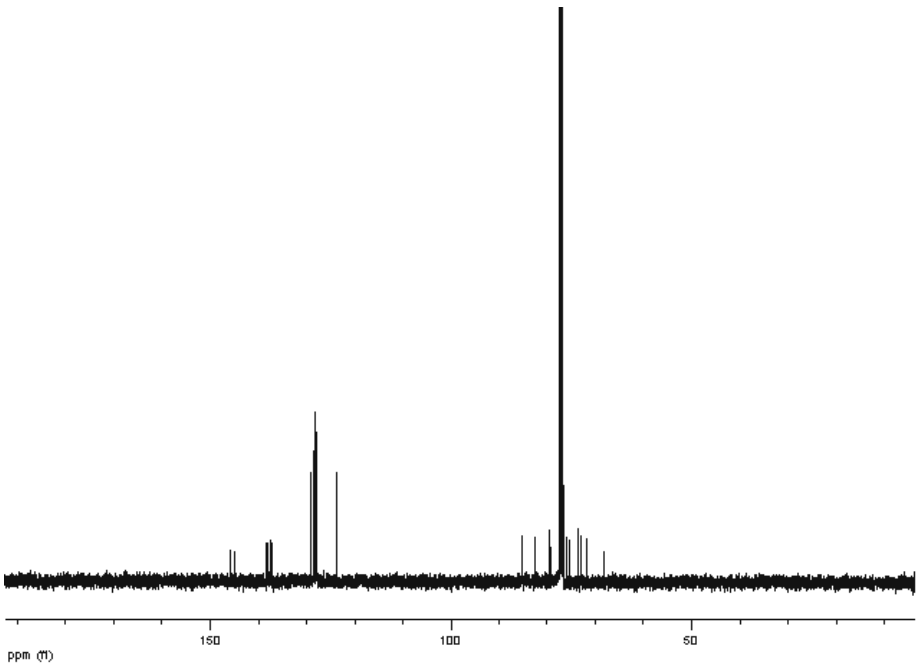
Spectral Data for all new compounds

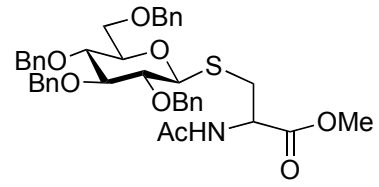
***p*-Nitrophenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside 2**

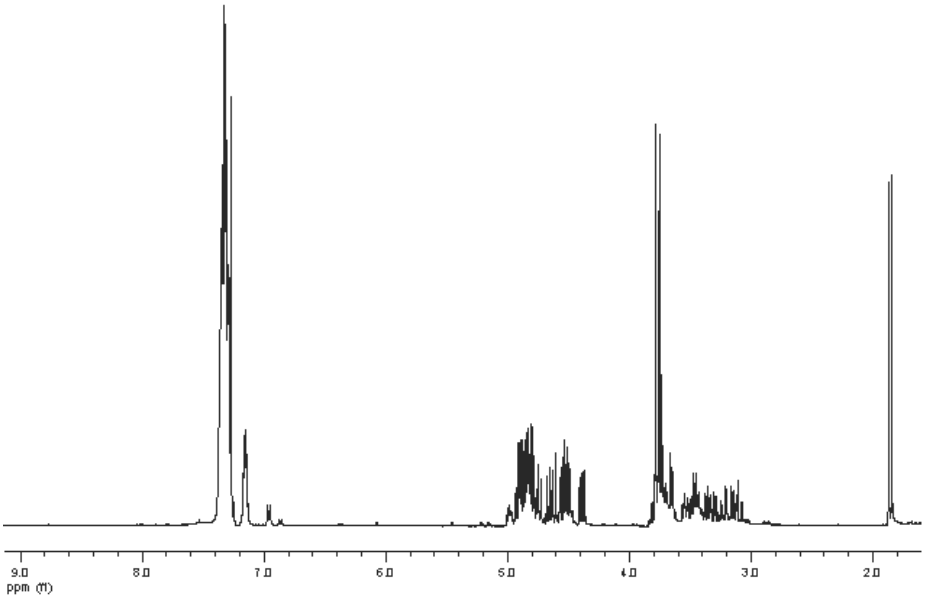


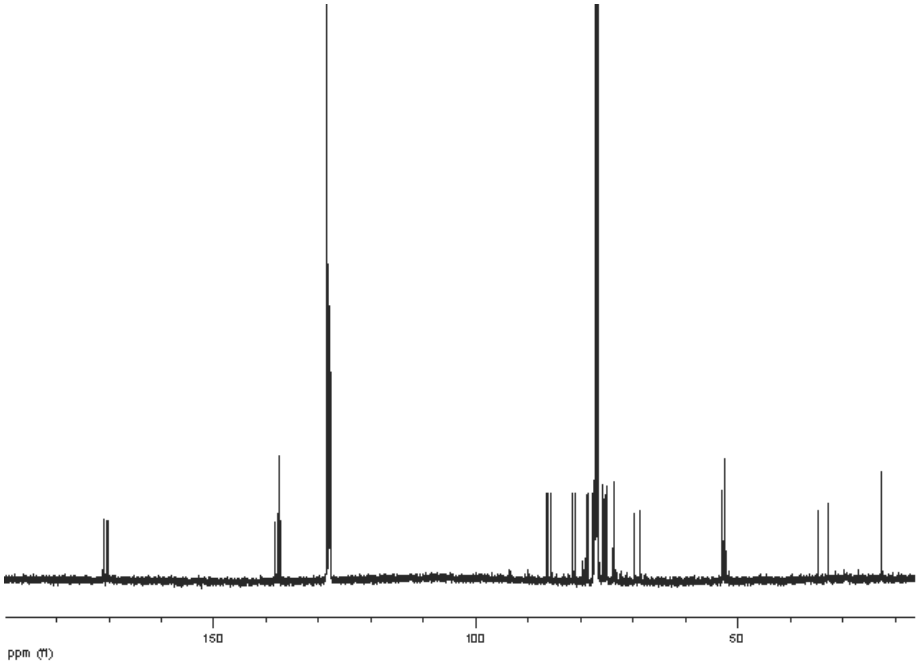




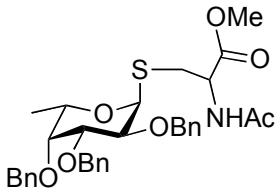
_N_-Acetyl-DL-cysteine-_S_-(2,3,4,6-tetra-_O_-benzyl-β-D-glucopyaranoside) methylester 4

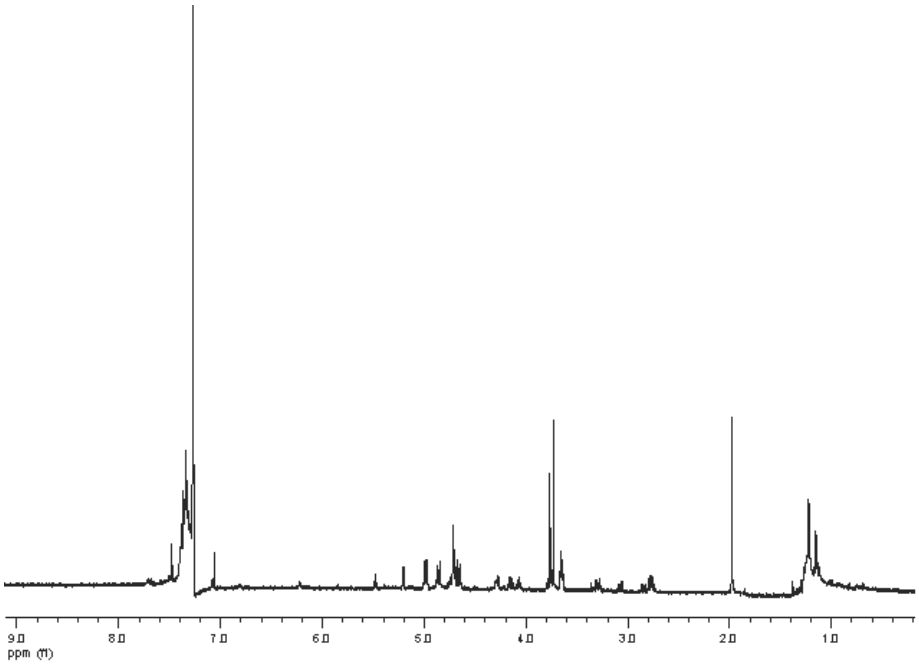


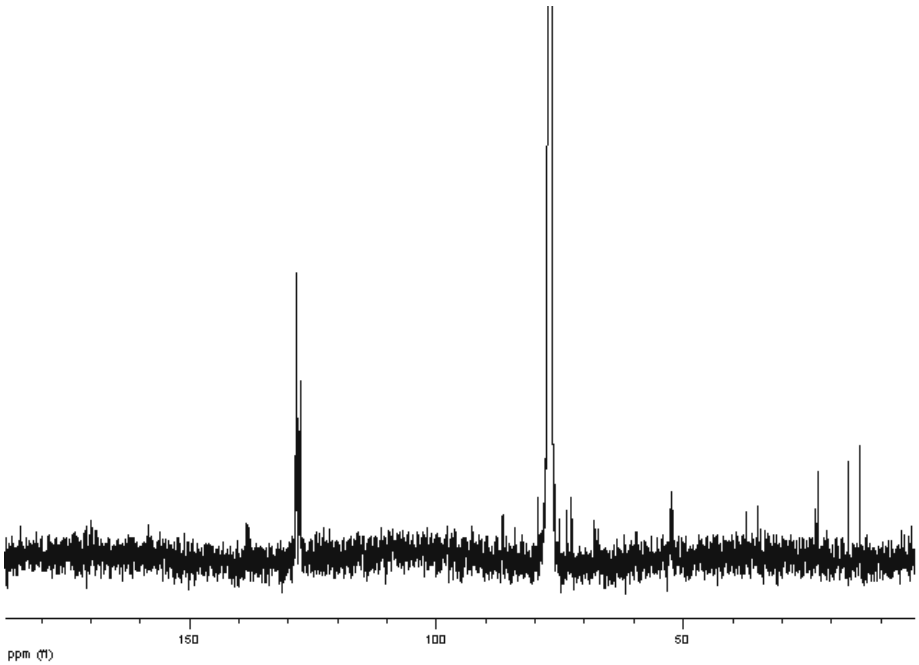




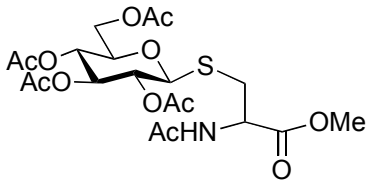
_N_-Acetyl-DL-cysteine-_S_-(2,3,4-tri-_O_-benzyl-1-thio-α-L-fucopyranoside) methylester 6

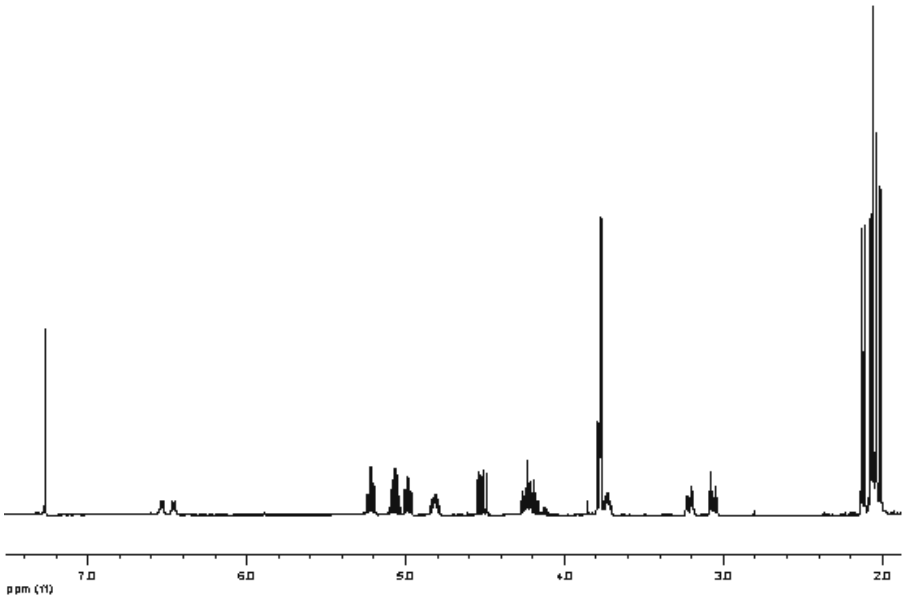


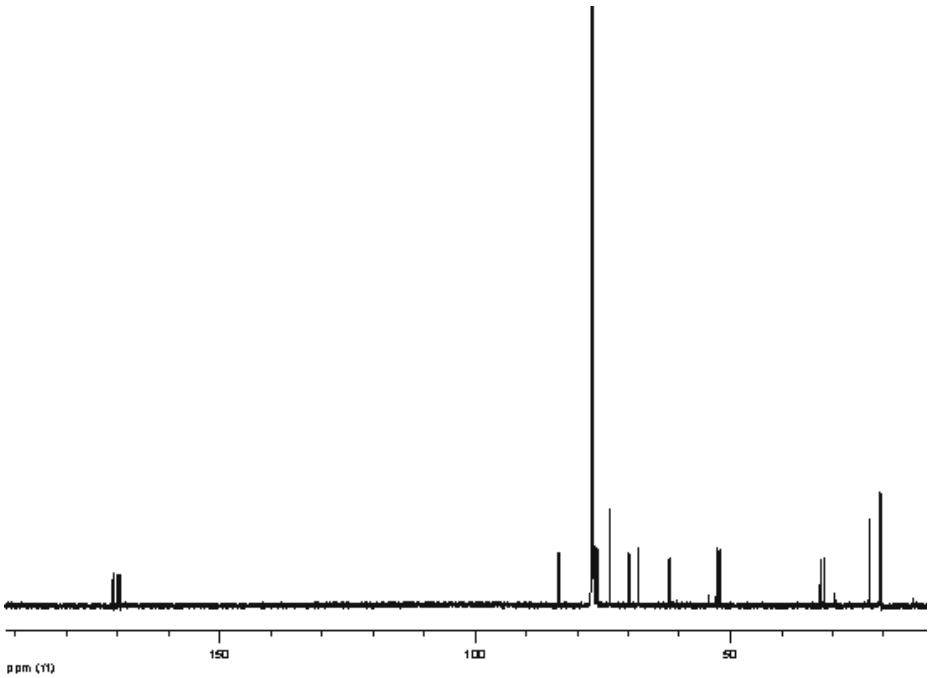




_N_-Acetyl-DL-cysteine-_S_-(2,3,4,6-tetra-_O_-acetyl-β-D-glucopyaranoside) methylester 8







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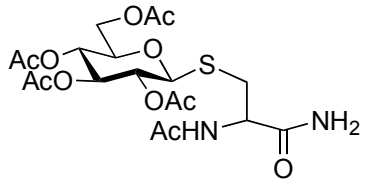
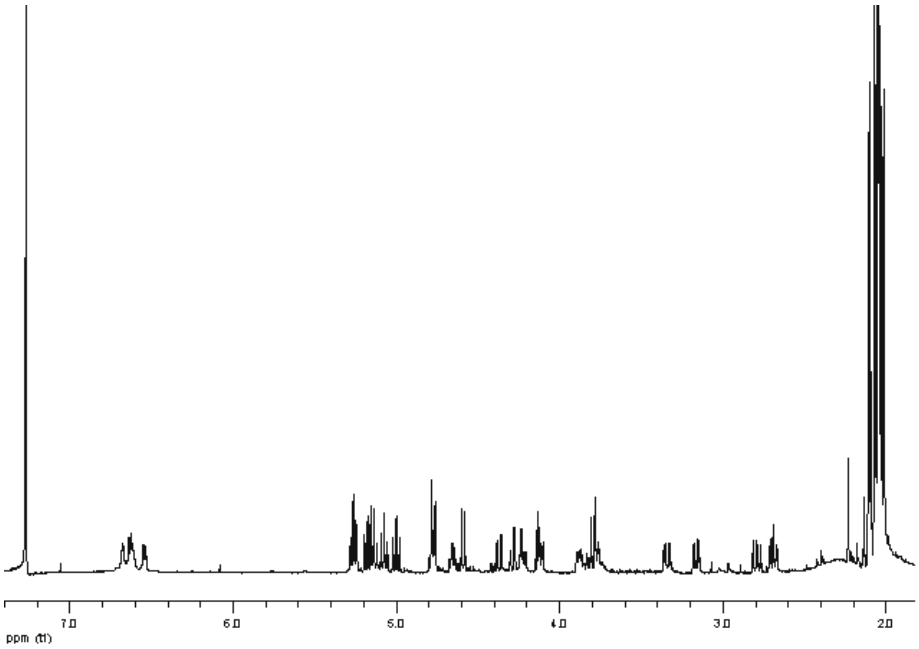
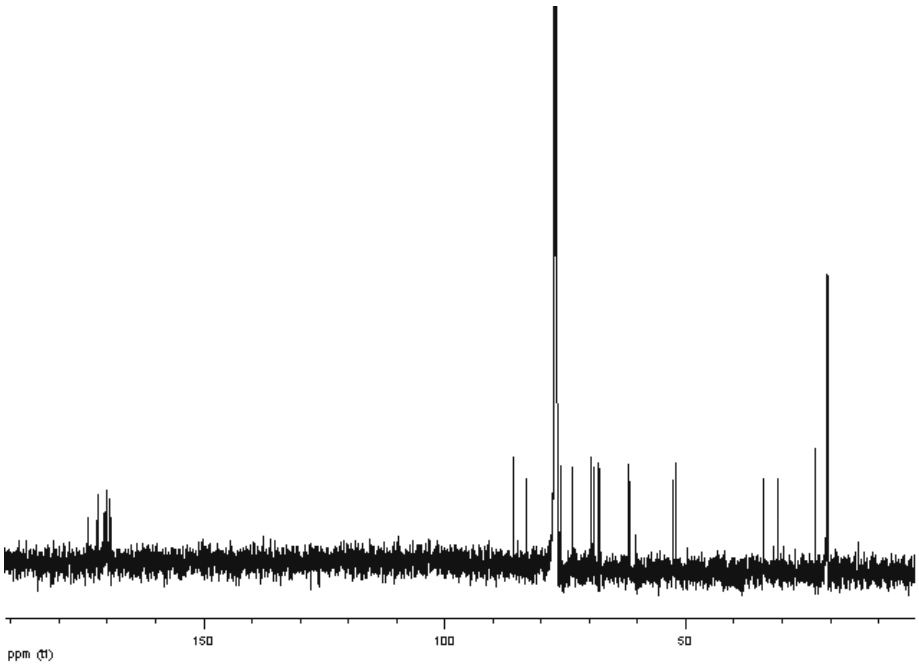
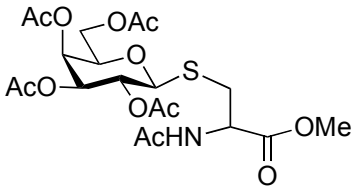
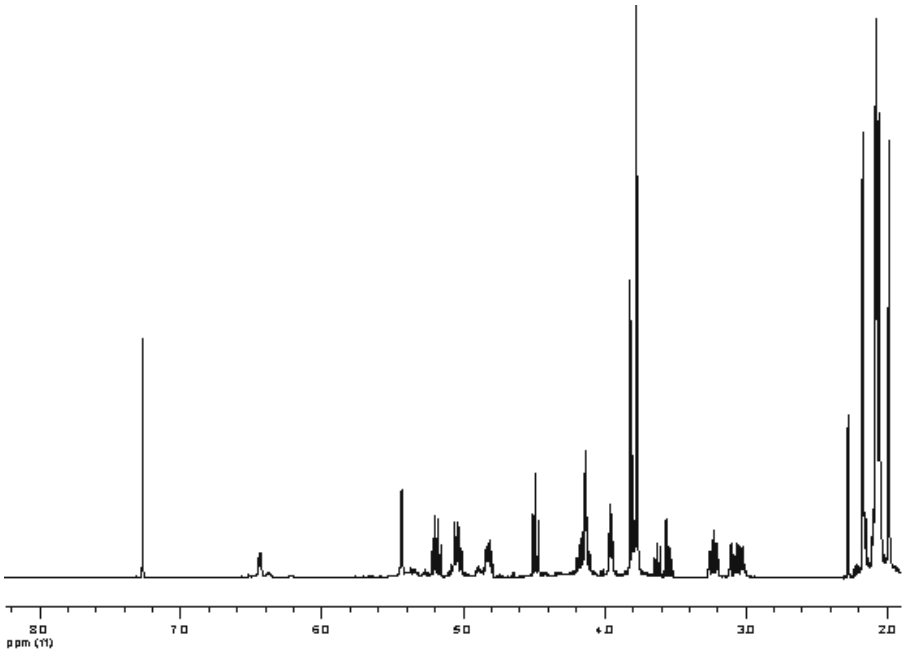


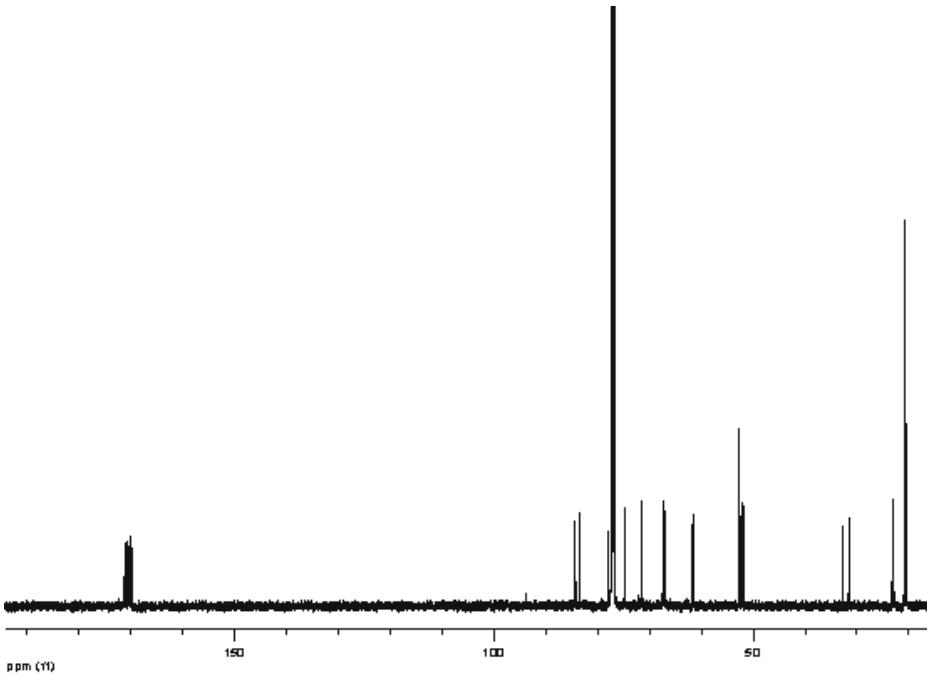




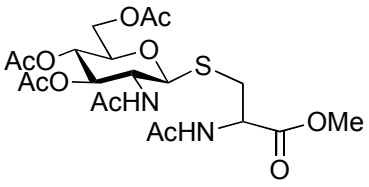
_N_-Acetyl-DL-cysteine-_S_-(2,3,4,6-tetra-_O_-acetyl-β-D-galactopyaranoside) methylester 12

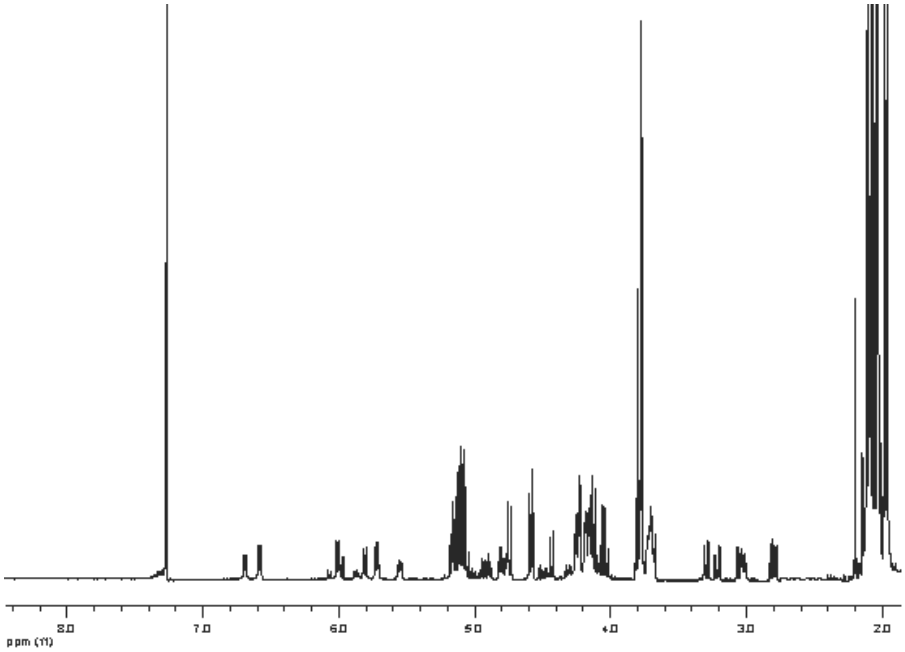


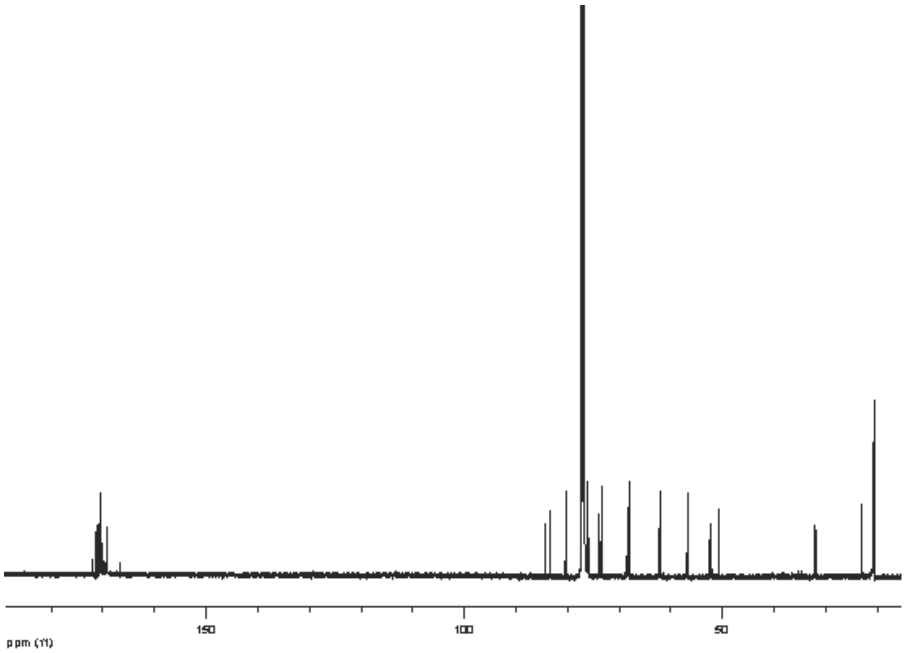




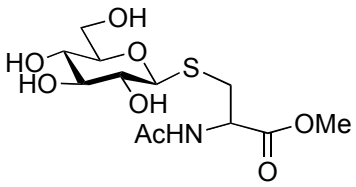
_N_-Acetyl-DL-cysteine-_S_-(3,4,6-tetra-_O_-acetyl-2-acetamido-2-deoxy-β-D-glucopyaranoside) methylester 14

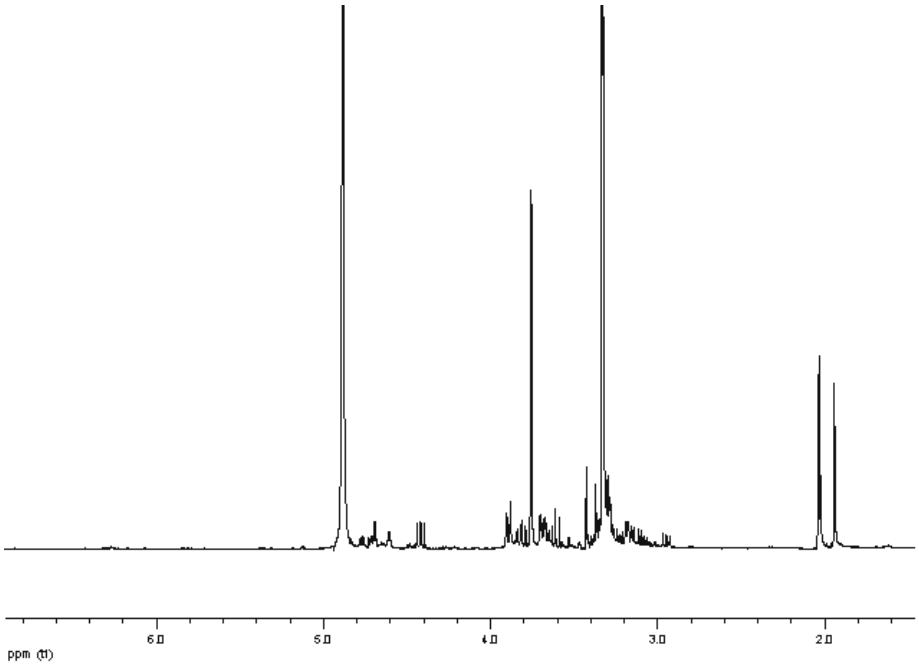


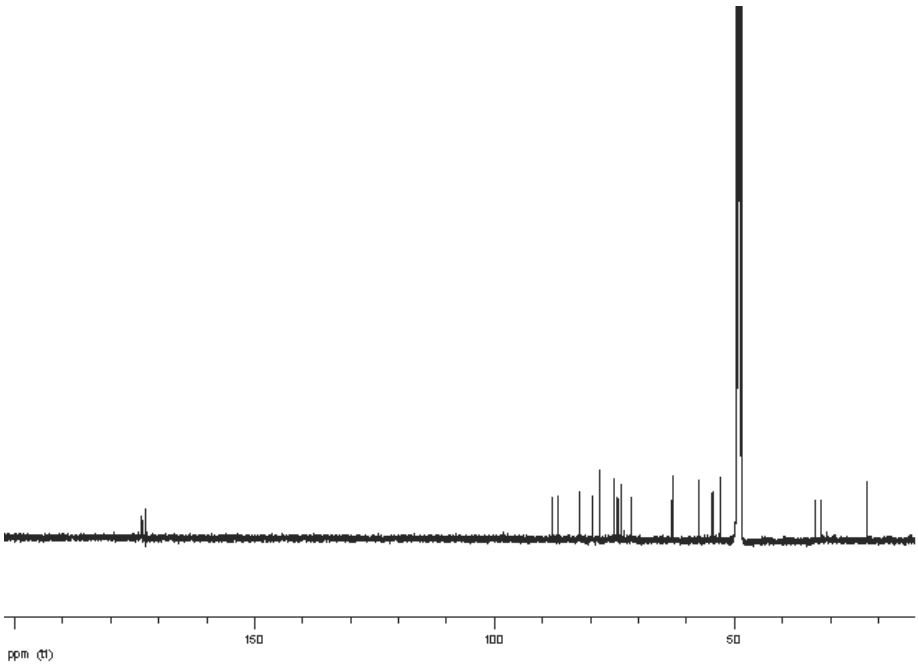




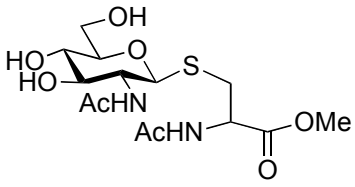
_N_-Acetyl-DL-cysteine-_S_-(β-D-glucopyranoside) methylester 16

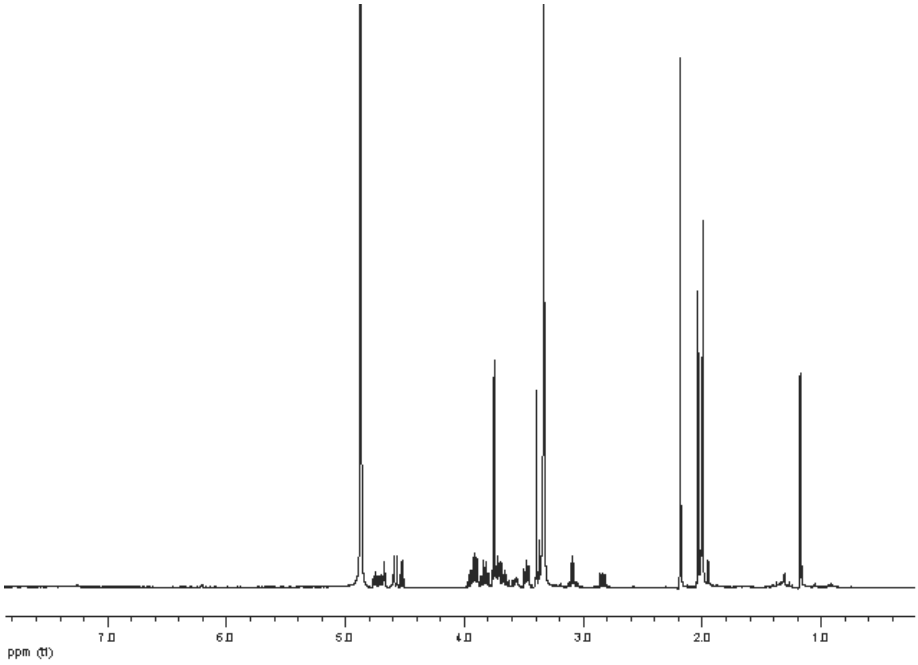


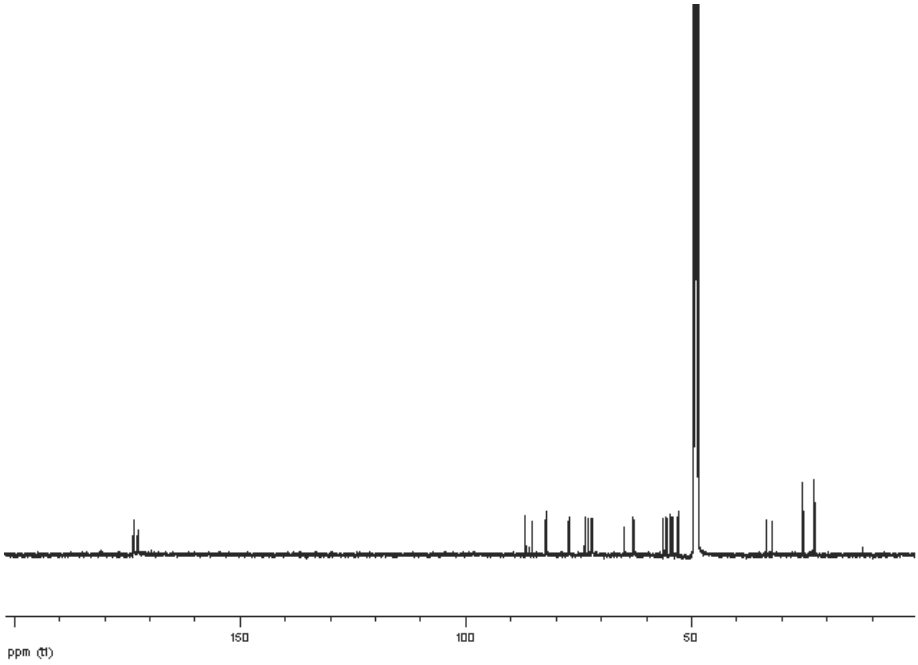




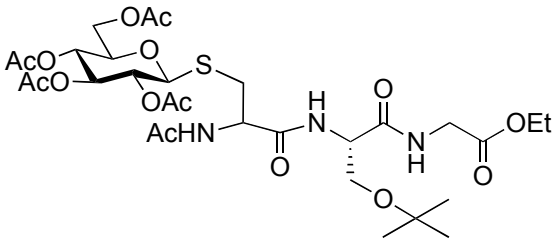
_N_-Acetyl-DL-cysteine-_S_-(2-acetamido-2-deoxy-β-D-glucopyranoside) methylester 18

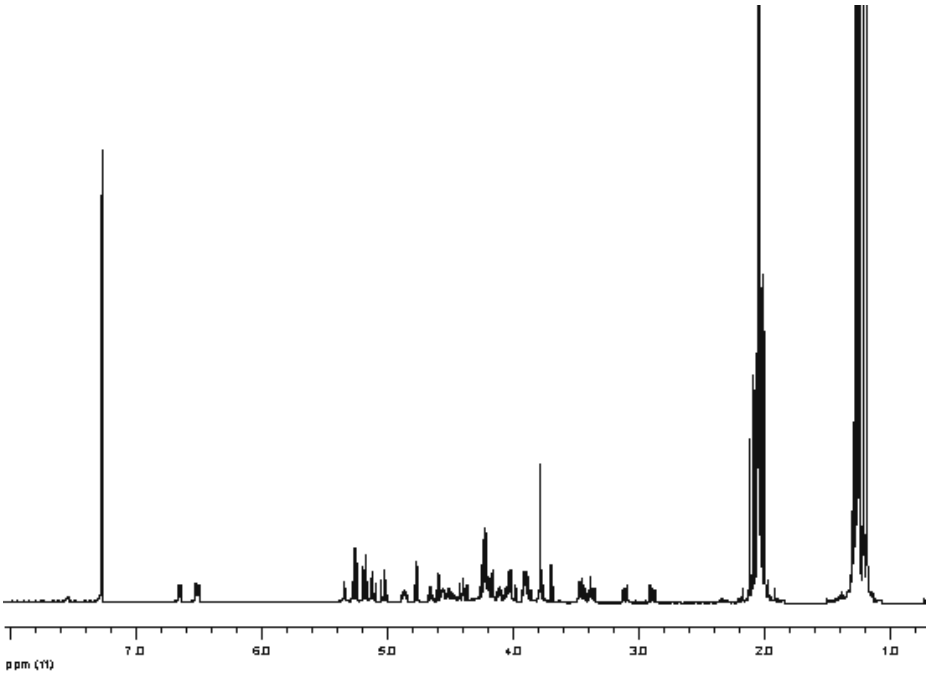


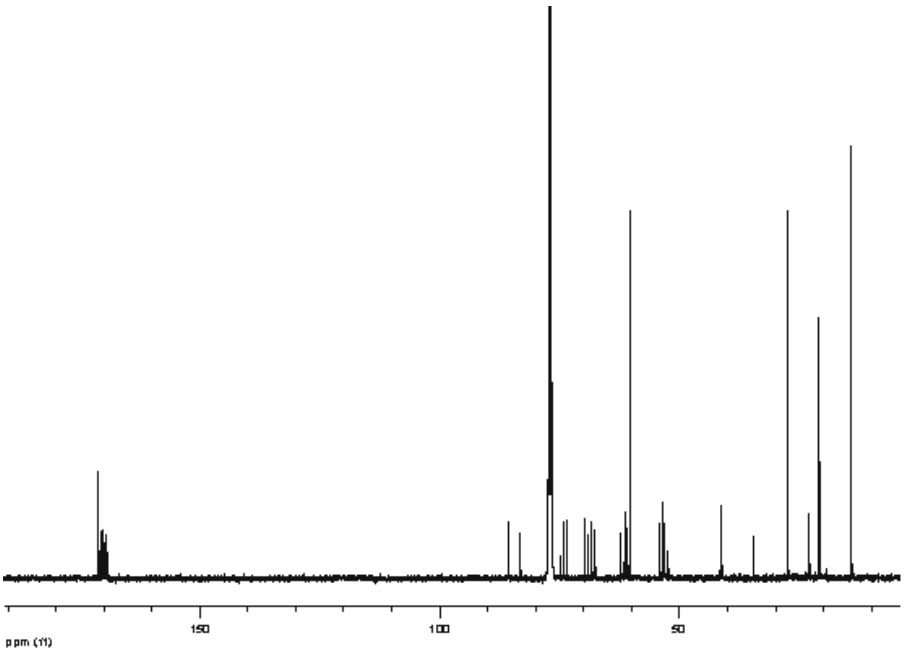




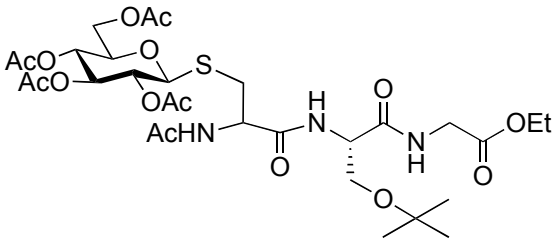
_N_-Acetyl-DL-cysteine-_S_-(2,3,4,6-tetra-_O_-acetyl-β-D-glucopyaranoside)-_O_-_tert_-butyl-L-serine-glycine ethylester 20

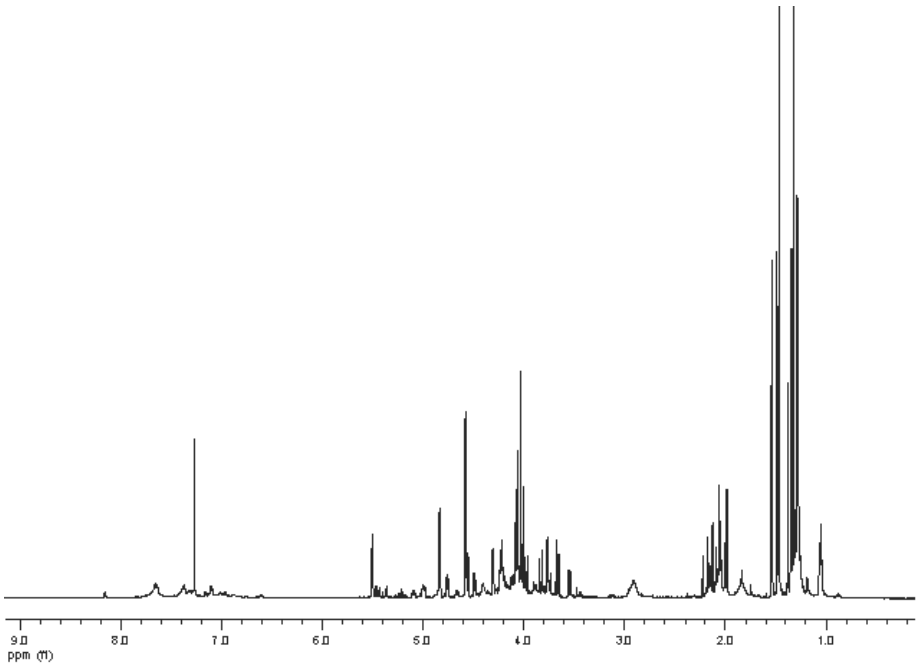


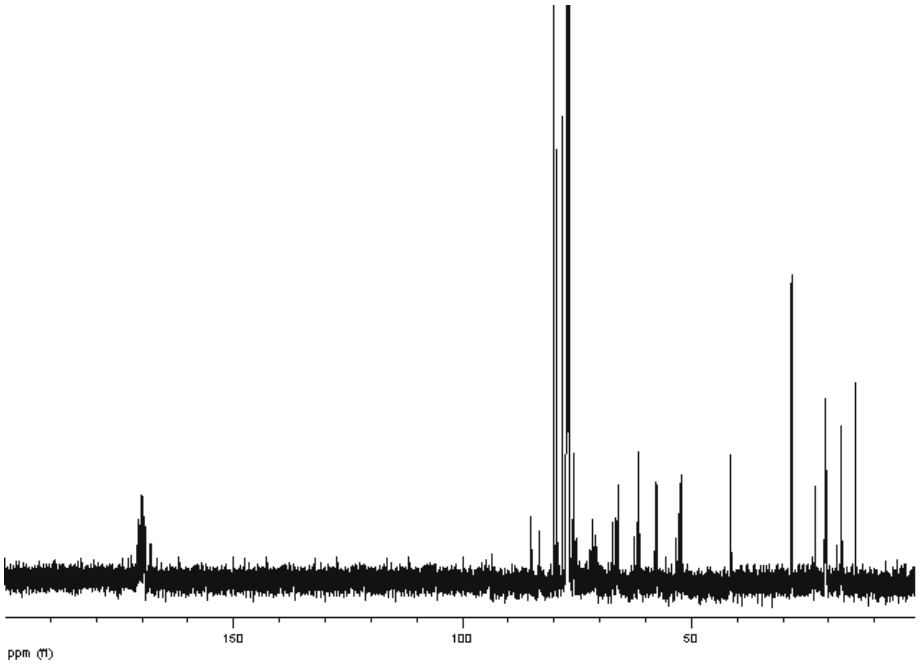




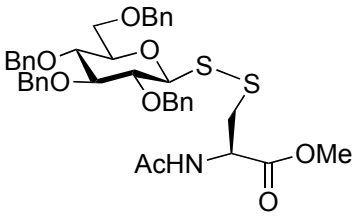
_N_-Acetyl-DL-cysteine-_S_-(2,3,4,6-tetra-_O_-acetyl-β-D-galactopyaranoside)-glycine-_O-tert_-butyl-L-serine-glycine ethylester 22

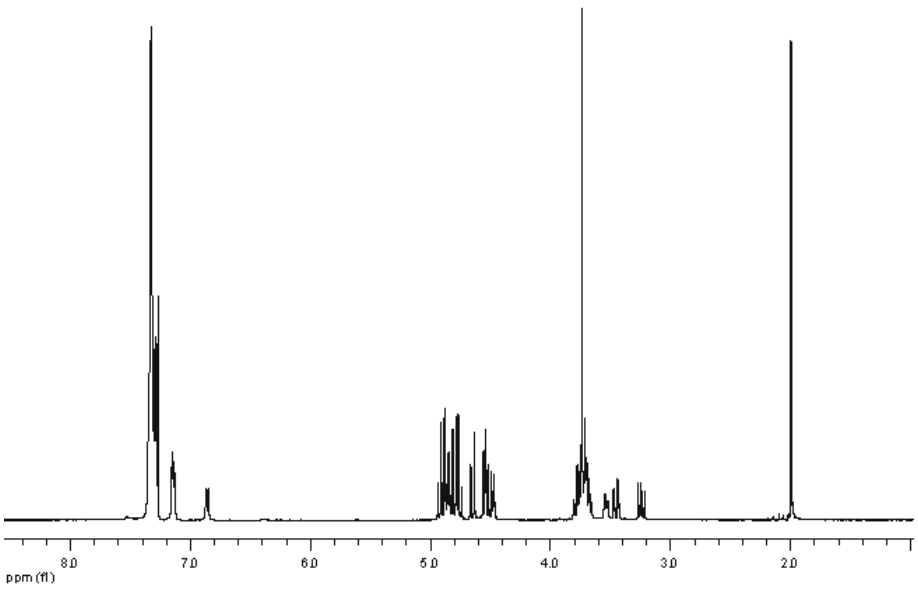


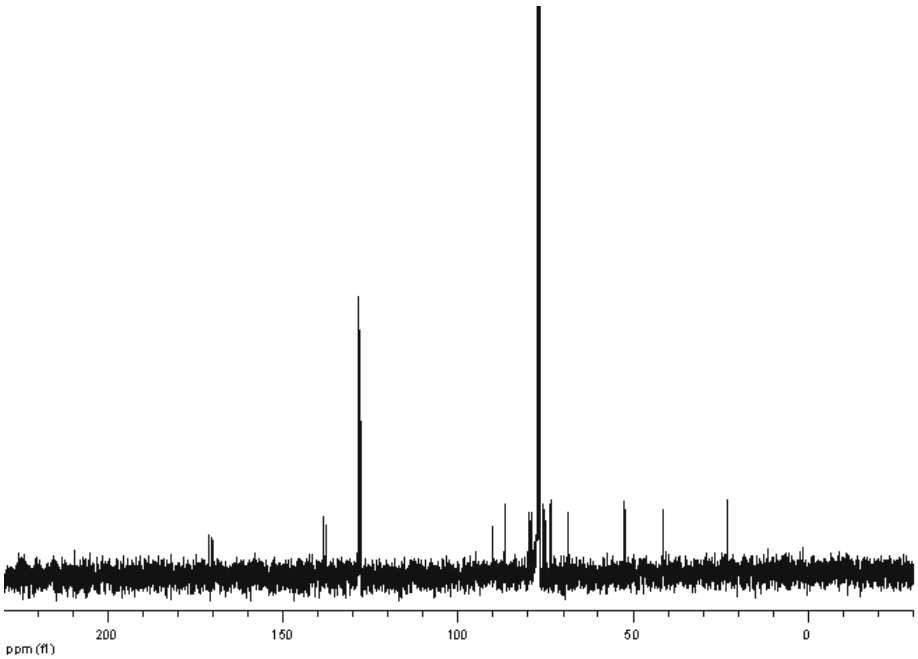




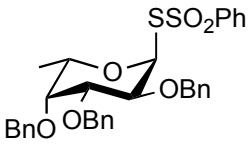
_N_-Acetyl-L-cysteine (2,3,4,6-tetra-_O_-benzyl-1-dithio-β-D-glucopyaranosyl disulfide) methylester 3

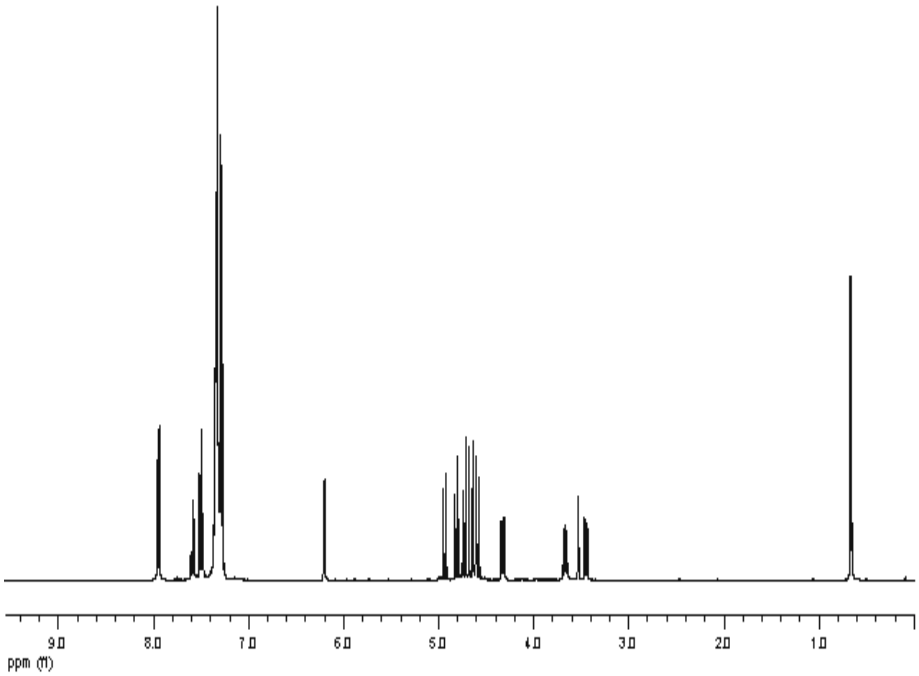


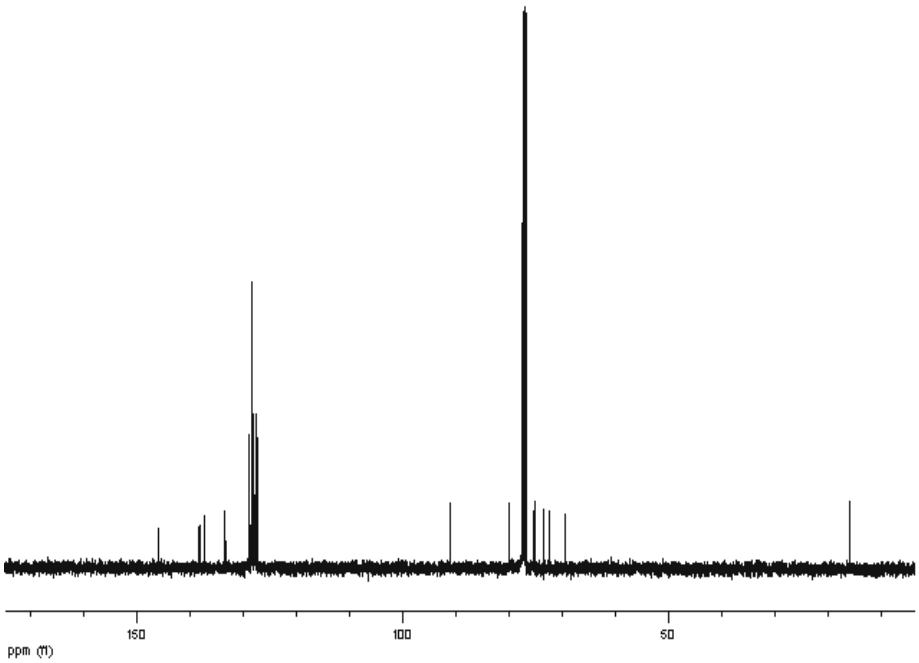




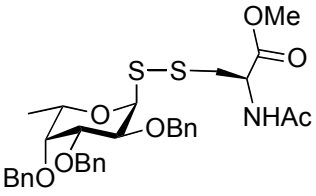
2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl phenylthiosulfonate 38

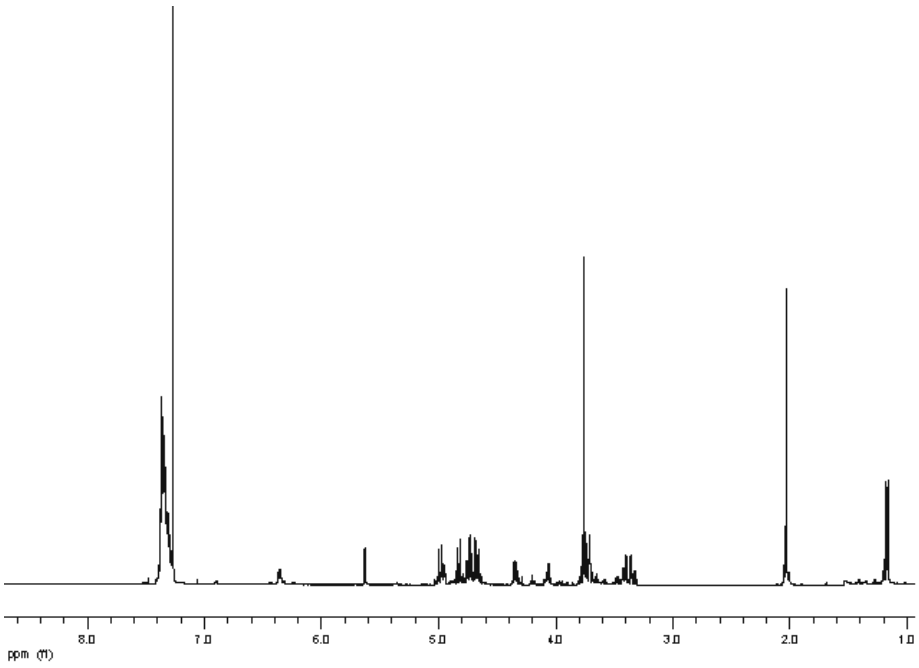


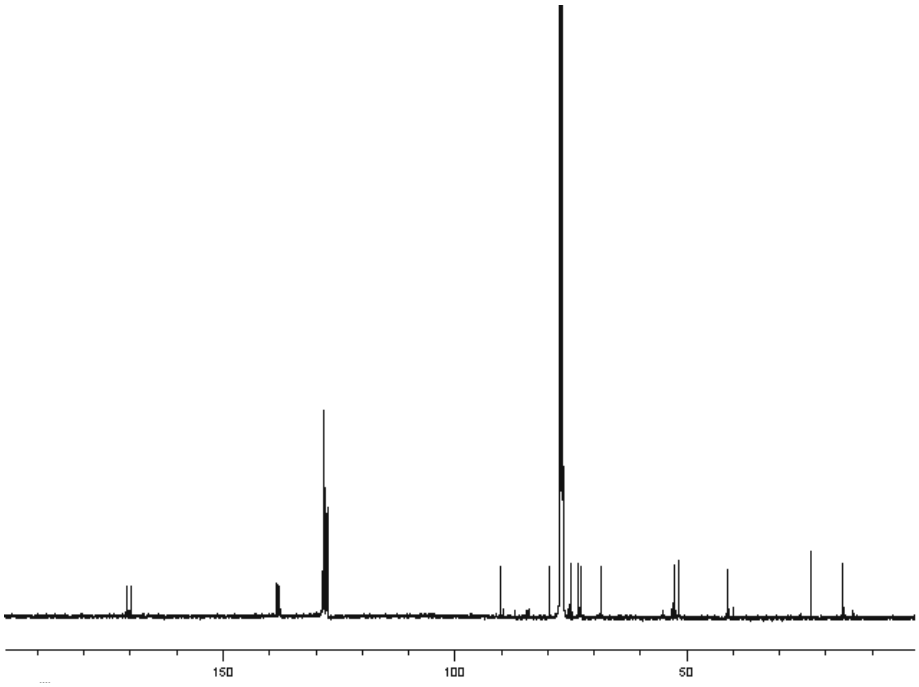




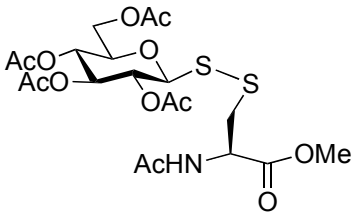
_N_-Acetyl-L-cysteine (2,3,4-tri-_O_-benzyl-1-dithio-α-L-fucopyranosyl disulfide) methylester 5

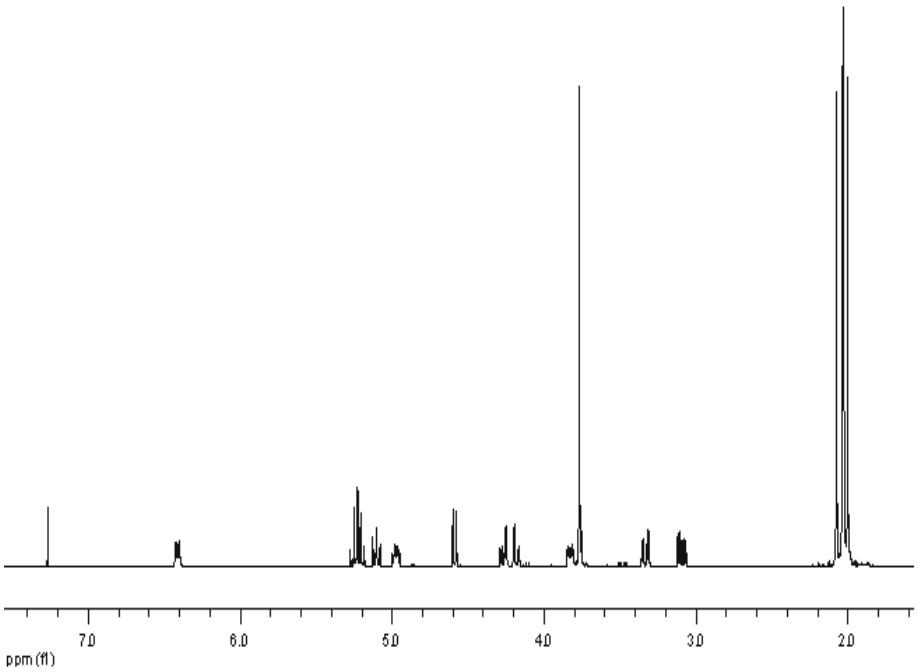


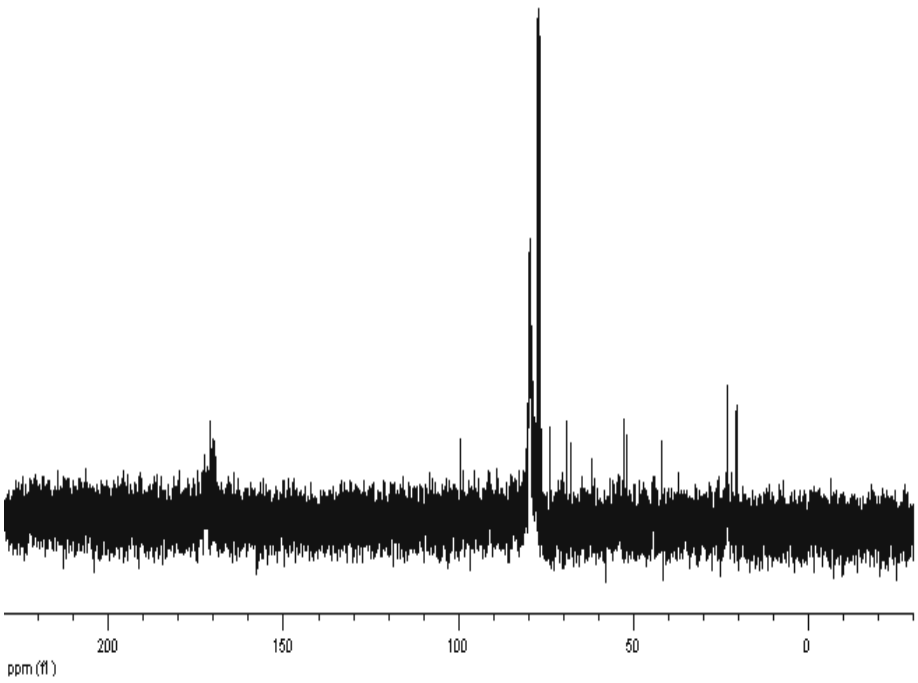




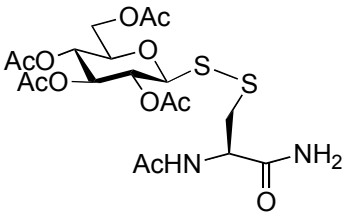
_N_-Acetyl-L-cysteine (2,3,4,6-tetra-_O_-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) methylester 7

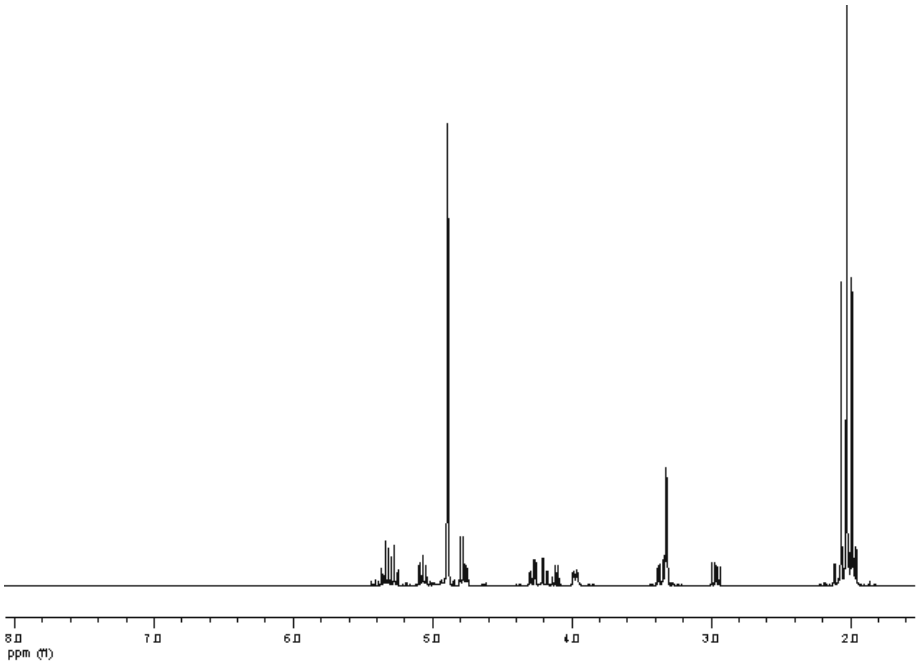


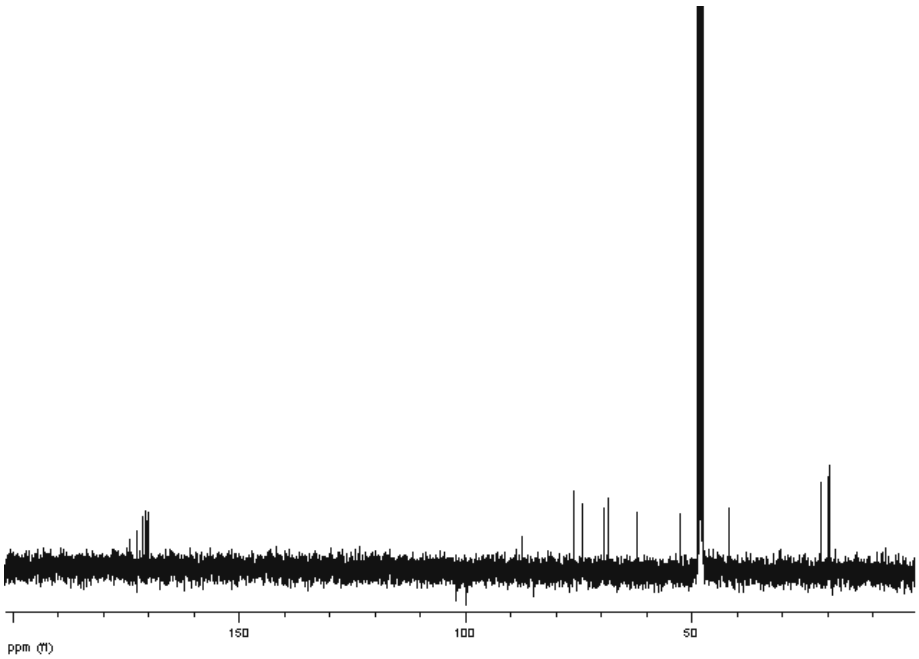




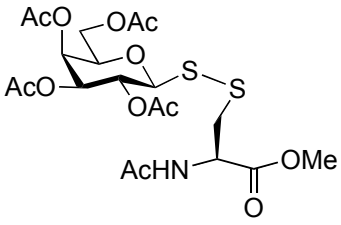
_N_-Acetyl-L-cysteinamide (2,3,4,6-tetra-_O_-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) 9

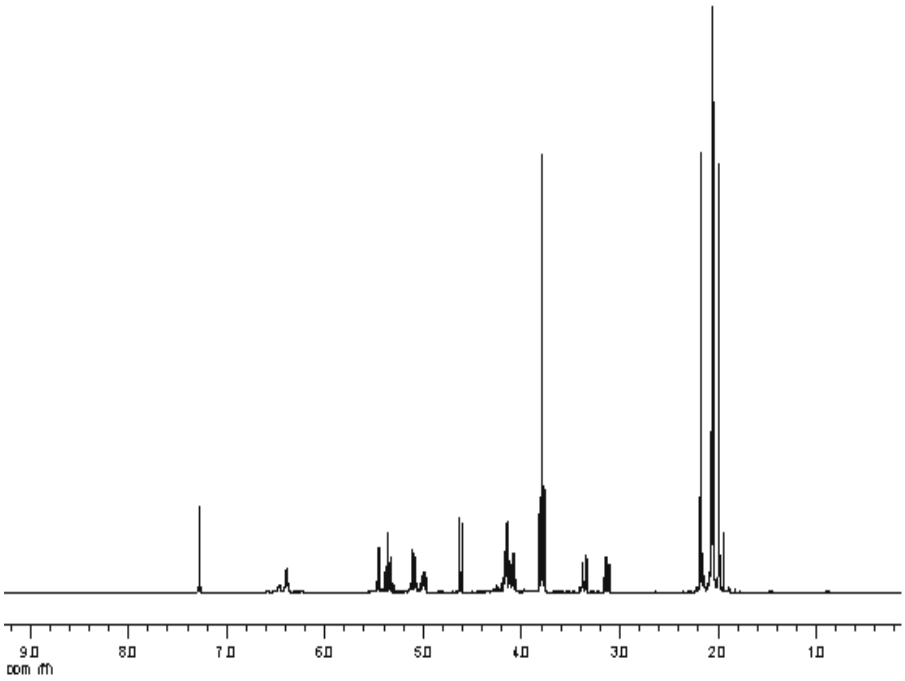


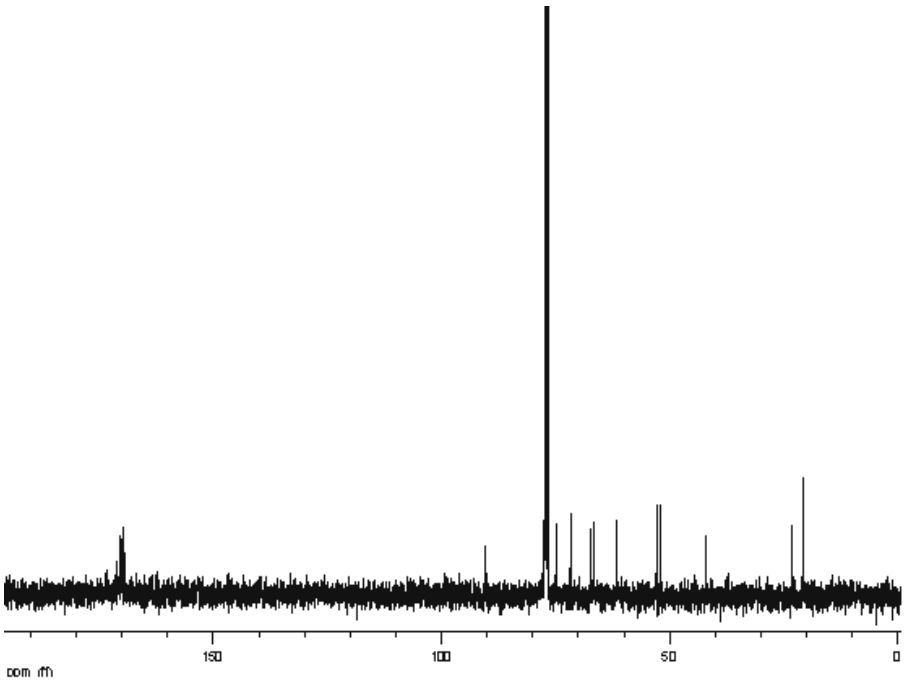




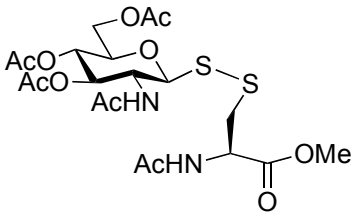
_N_-Acetyl-L-cysteine (2,3,4,6-tetra-_O_-acetyl-1-dithio-β-D-galactopyaranosyl disulfide) methylester 11

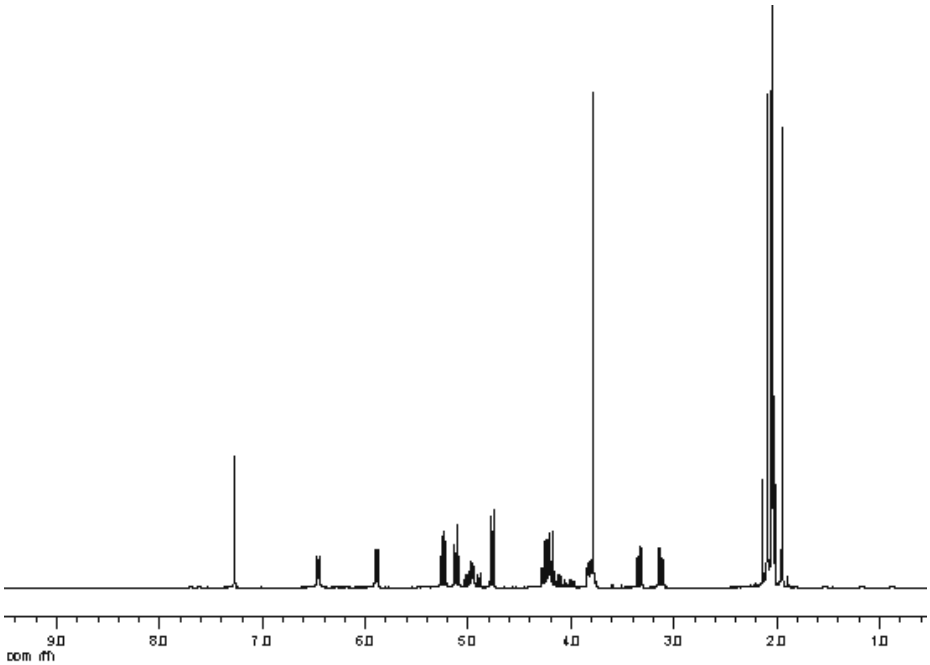


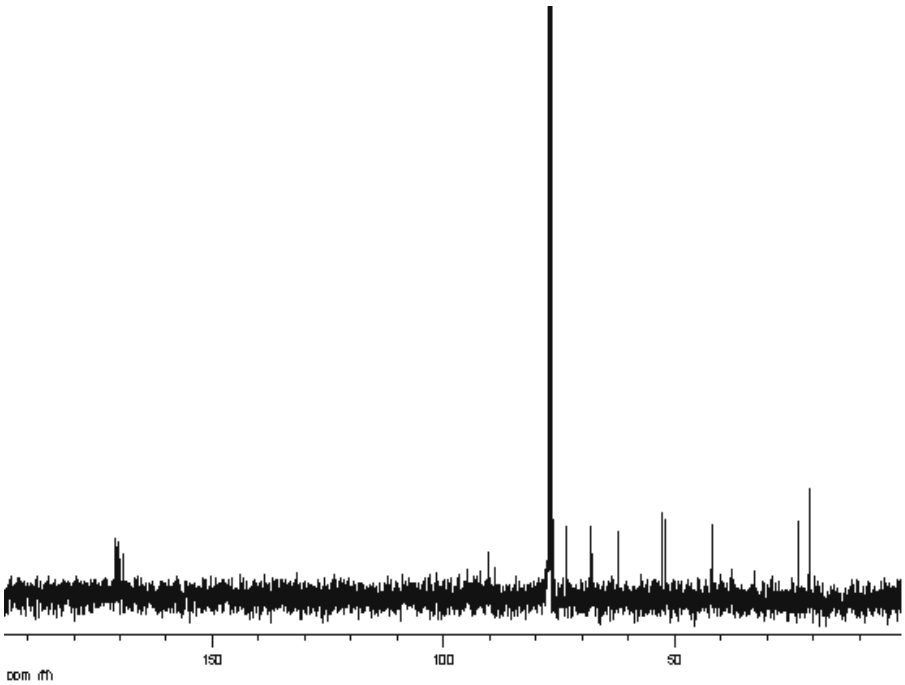




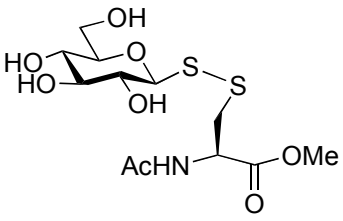
_N_-Acetyl-L-cysteine (3,4,6-tetra-_O_-acetyl-2-acetamido-2-deoxy-1-dithio-β-D-glucopyaranosyl disulfide) methylester 13

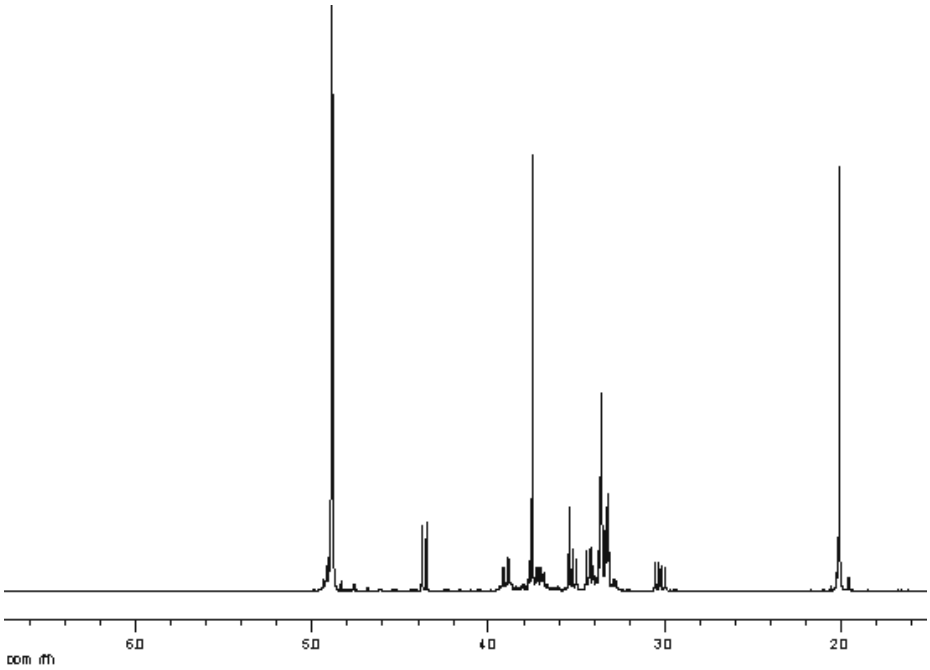


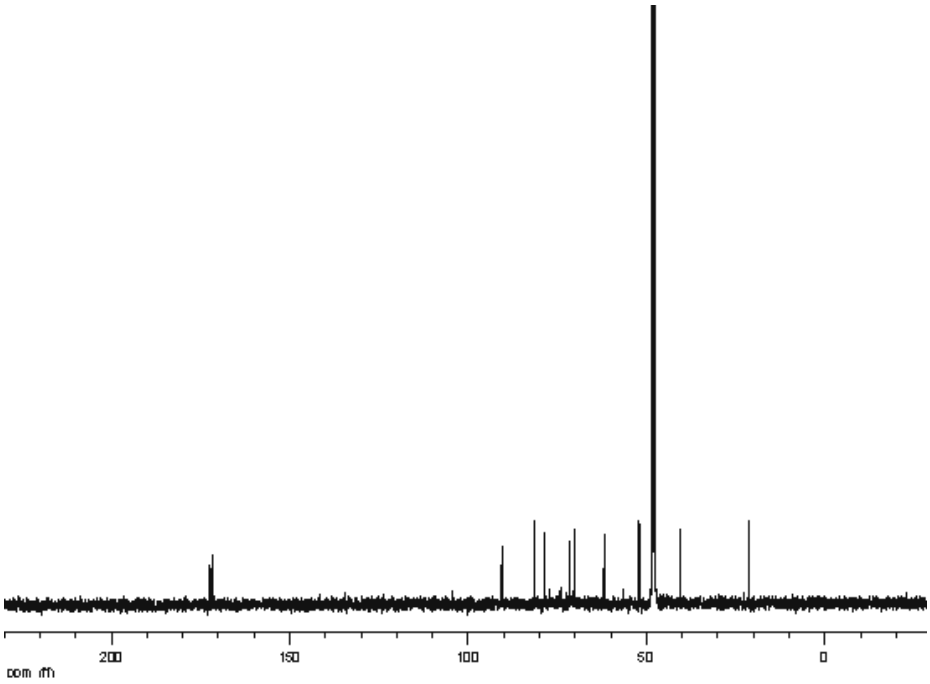




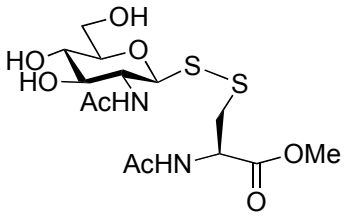
_N_-Acetyl-L-cysteine (1-dithio-β-D-glucopyranosyl disulfide) methylester 15

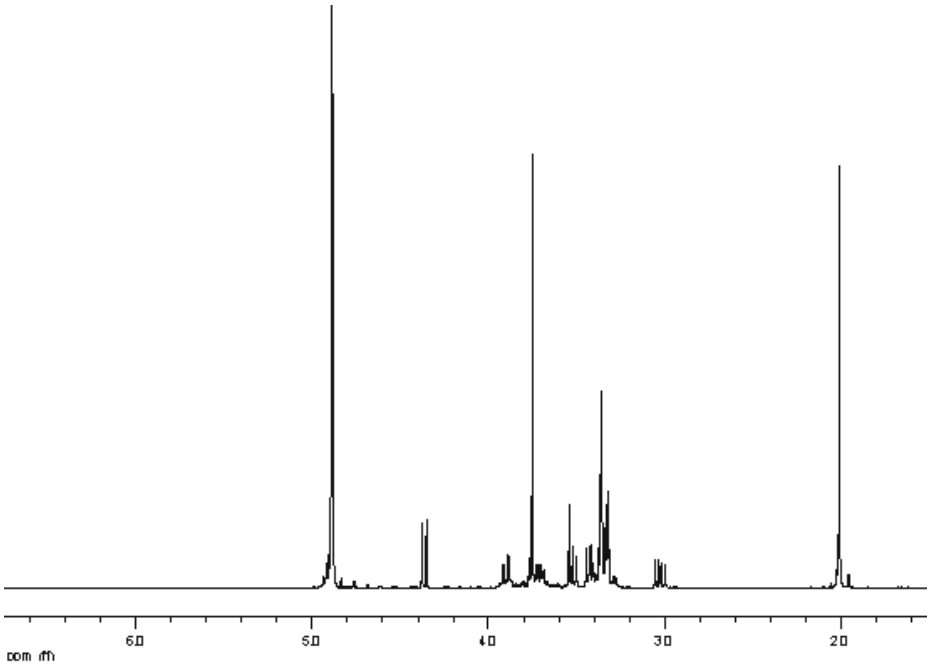


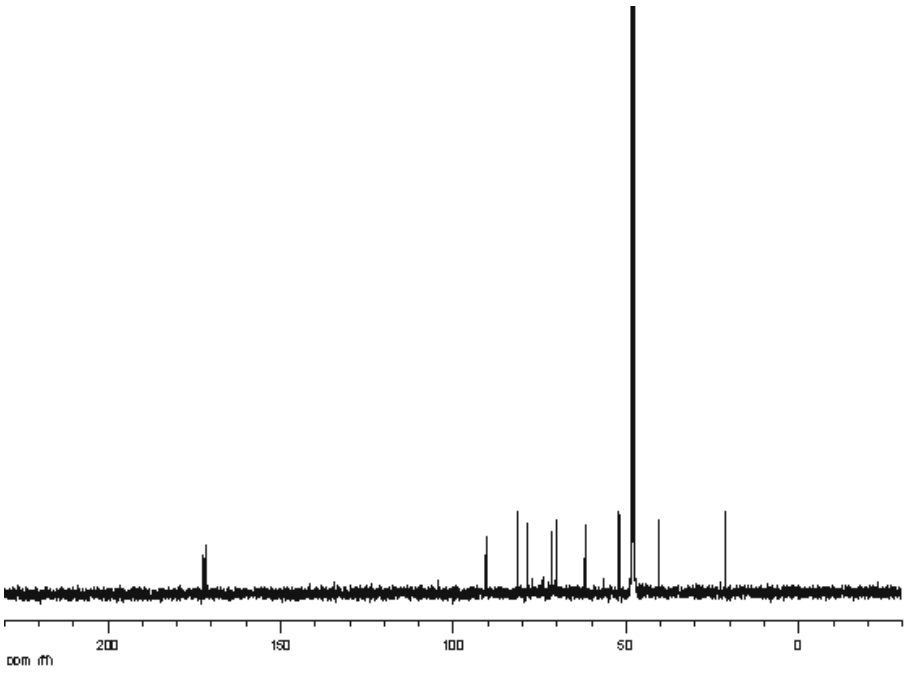




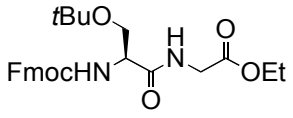
_N_-Acetyl-L-cysteine (2-acetamido-2-deoxy-1-dithio-β-D-glucopyranosyl disulfide) methylester 17

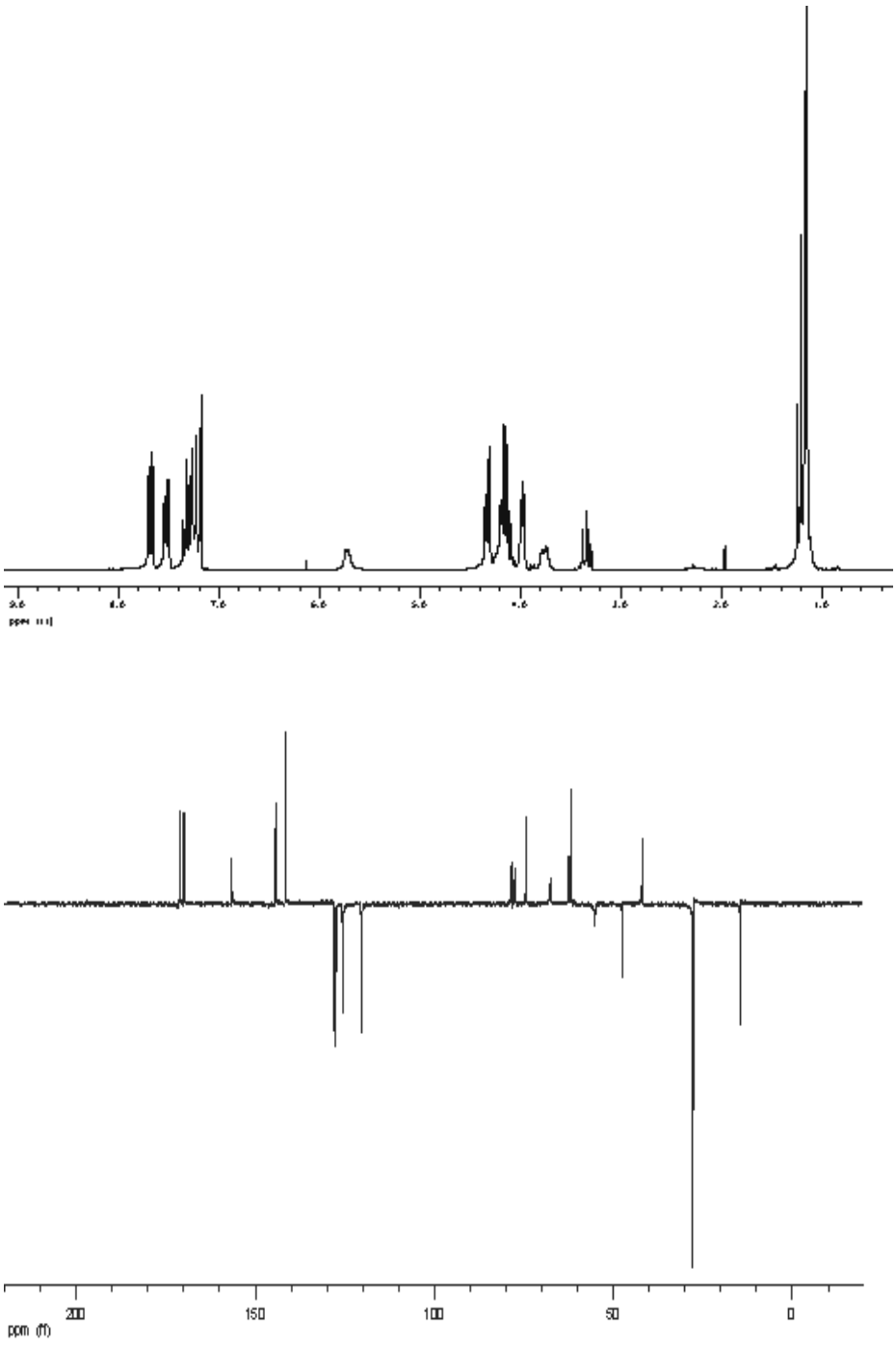




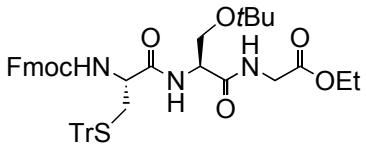


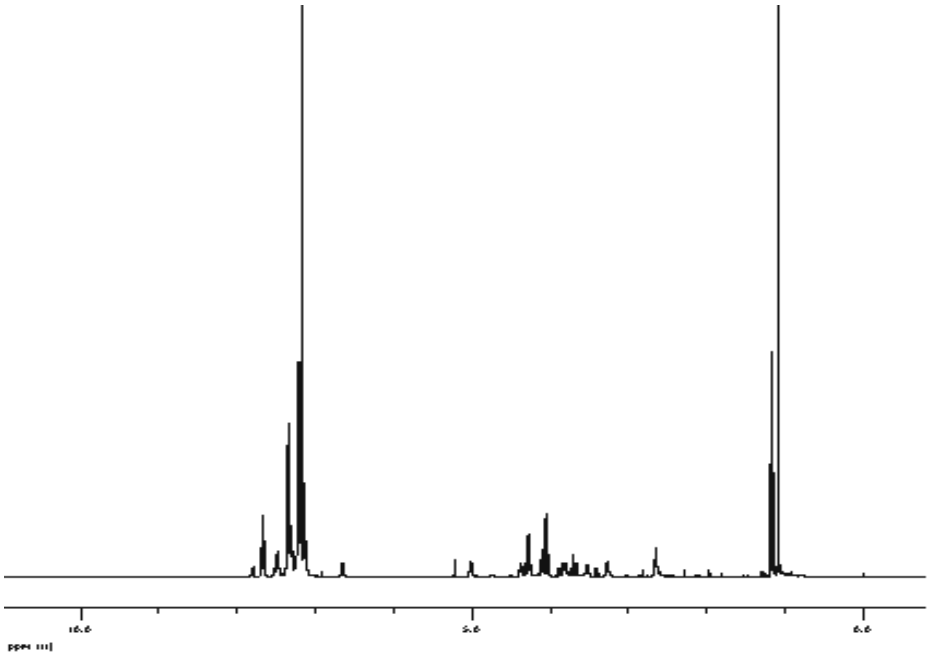
_N_-fluorenyl methoxycarbonyl-_O-tert_-butyl-L-serine-glycine ethyl ester 59

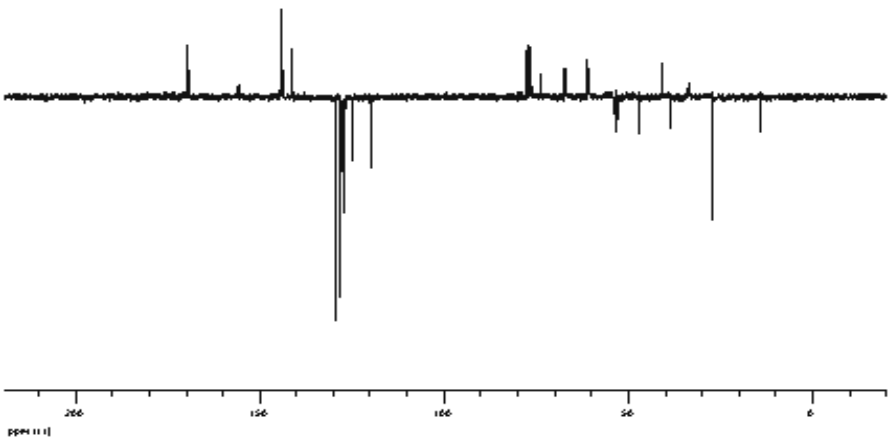




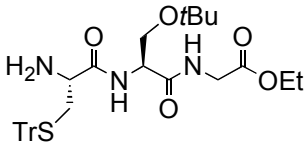
_N_-fluorenyl methoxycarbonyl-_S_-trityl-L-cysteine-_O-tert_-butyl-L-serine-glycine ethyl ester 60

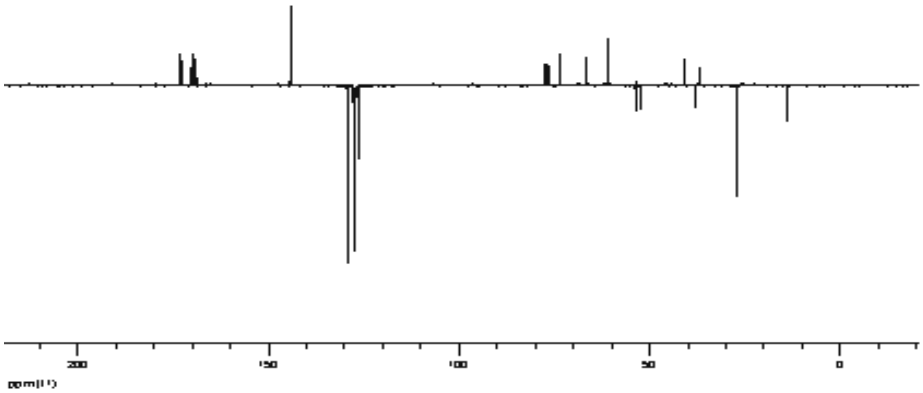




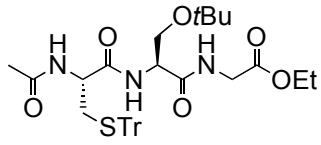


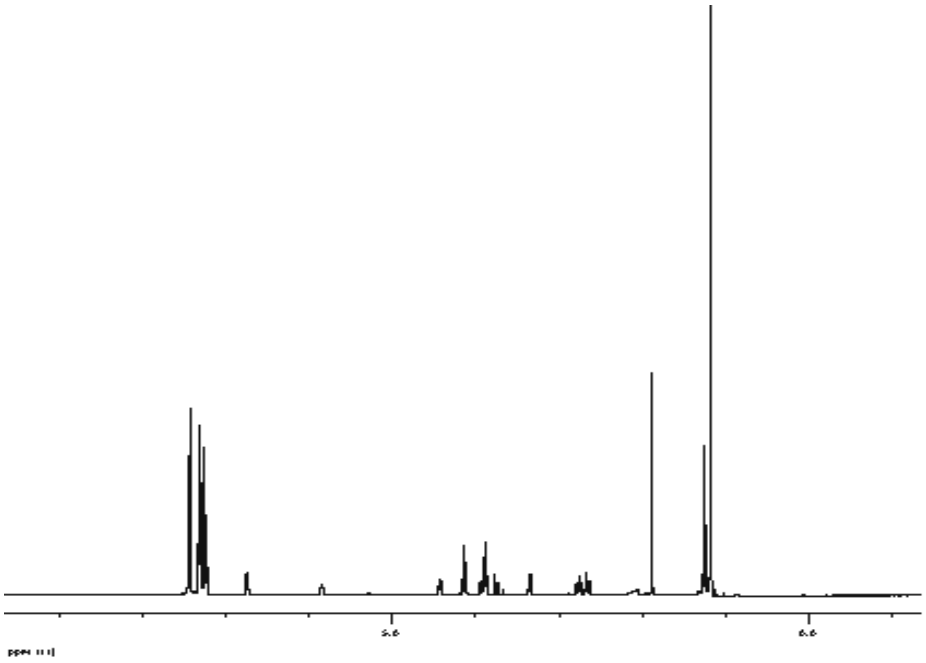
_S_-trityl-L-cysteine-_O_-_tert_-butyl-L-serine-glycine ethyl ester 61

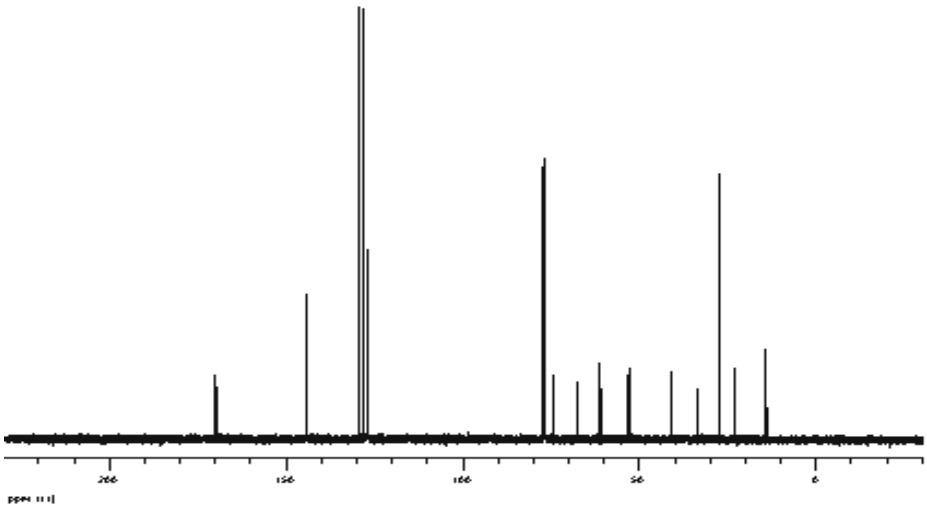




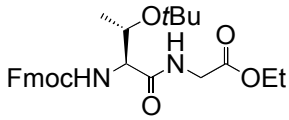
_N_-acetyl-_S_-trityl-L-cysteine-_O-tert_-butyl-L-serine-glycine ethyl ester 62

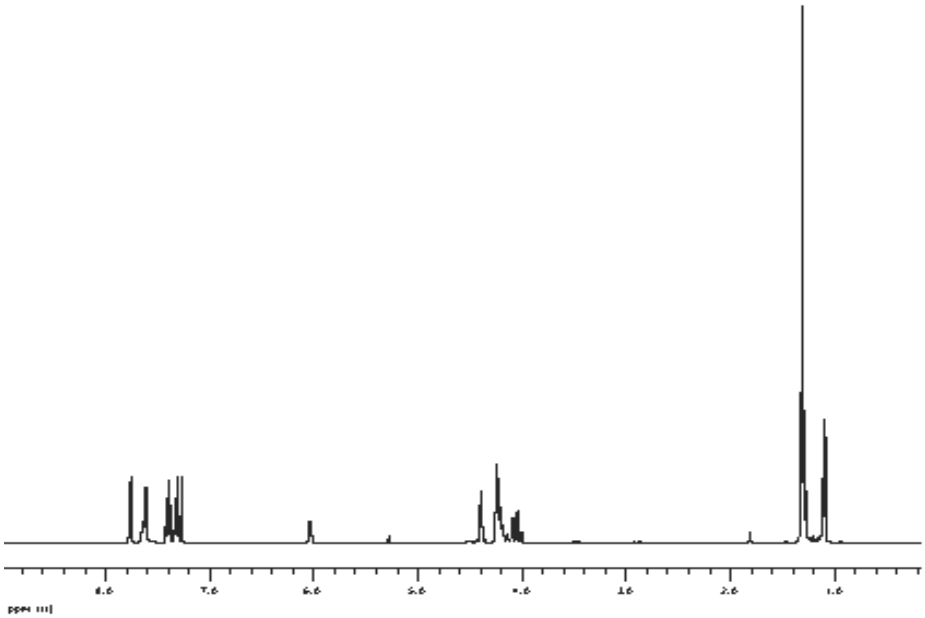


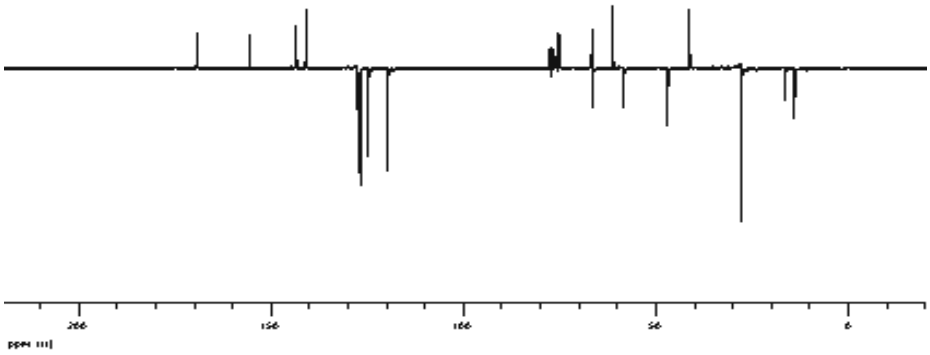




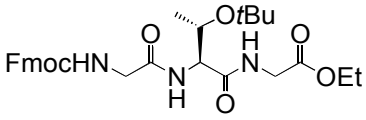
_N_-fluorenyl methoxycarbonyl-L-threonine-glycine ethyl ester 64

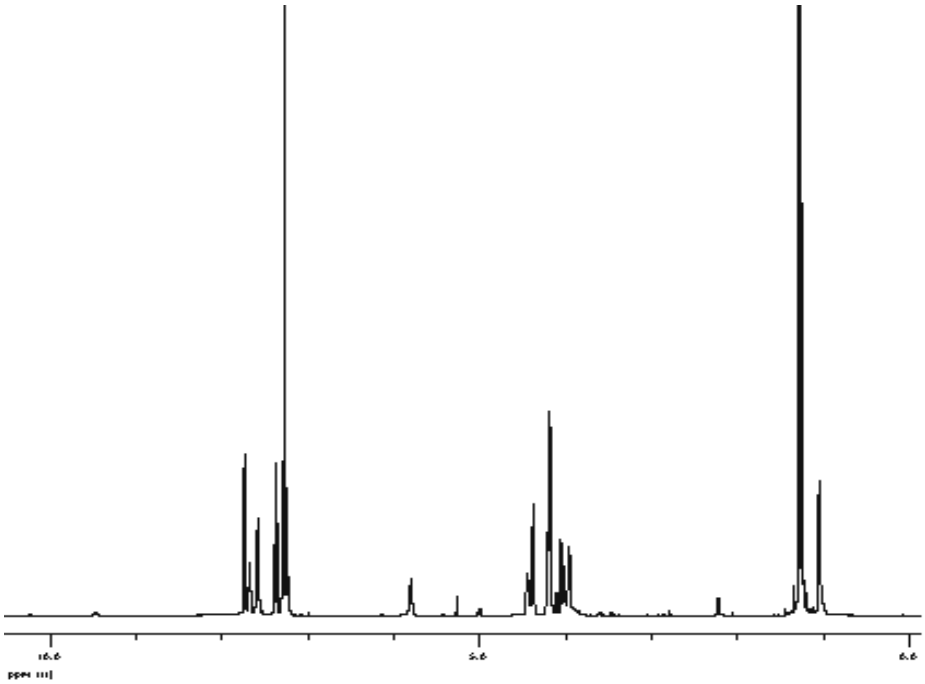


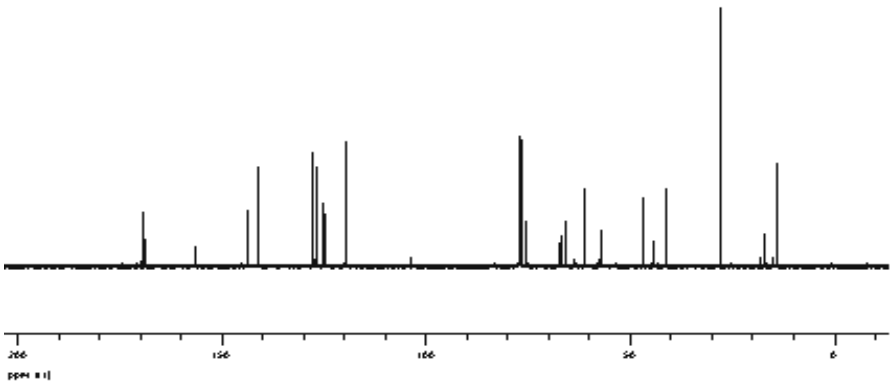




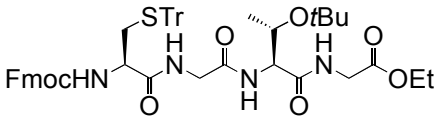
_N_-fluorenyl methoxycarbonyl-glycine-_O-tert_-butyl-L-threonine-glycine ethyl ester 65

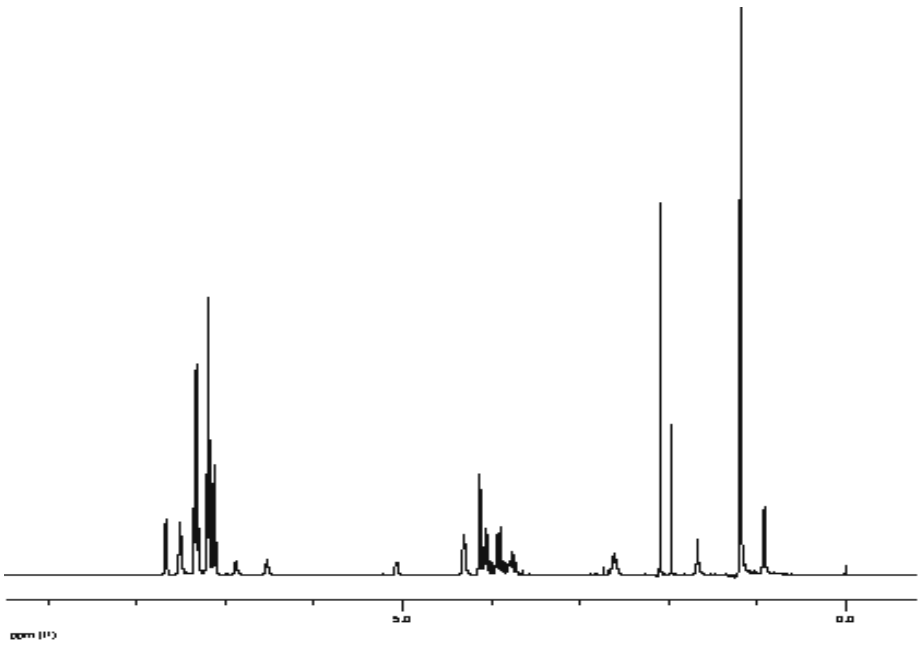


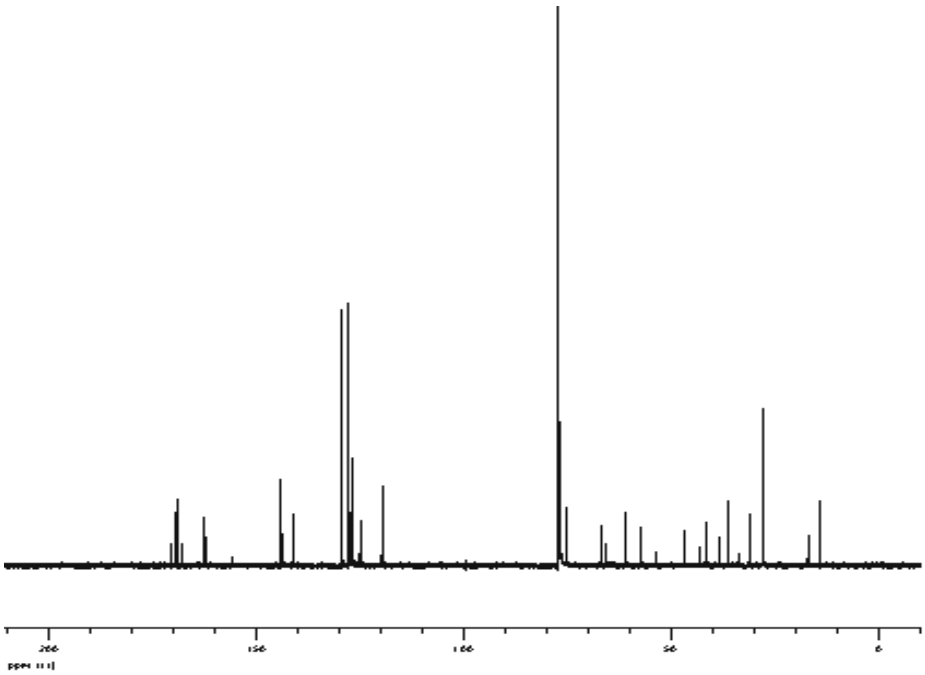




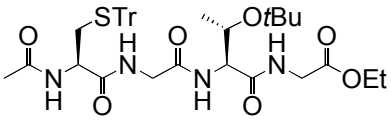
_N_-fluorenyl methoxycarbonyl-_S_-trityl-L-cysteine-glycine-_O-tert_-butyl-L-threonine-glycine ethyl ester 67

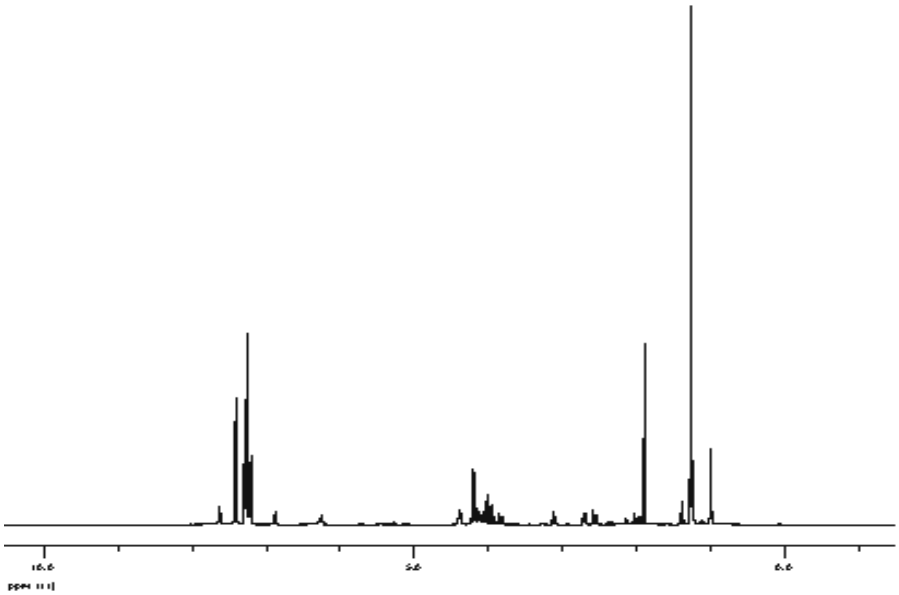


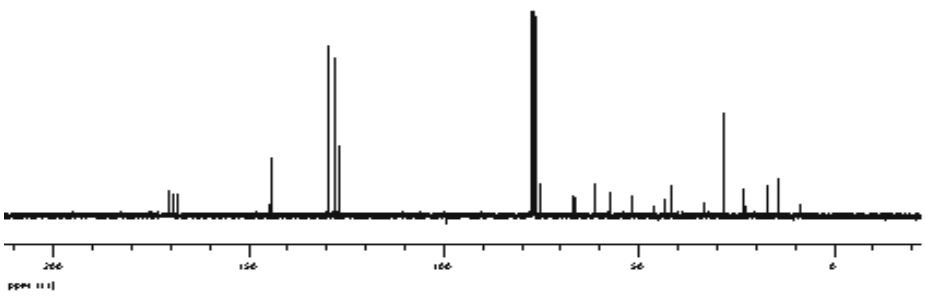




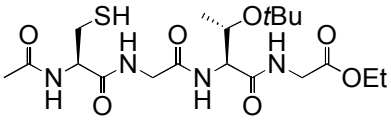
_N_-acetyl-_S_-trityl-L-cysteine-glycine-_O-tert_-butyl-L-threonine-glycine ethyl ester 69

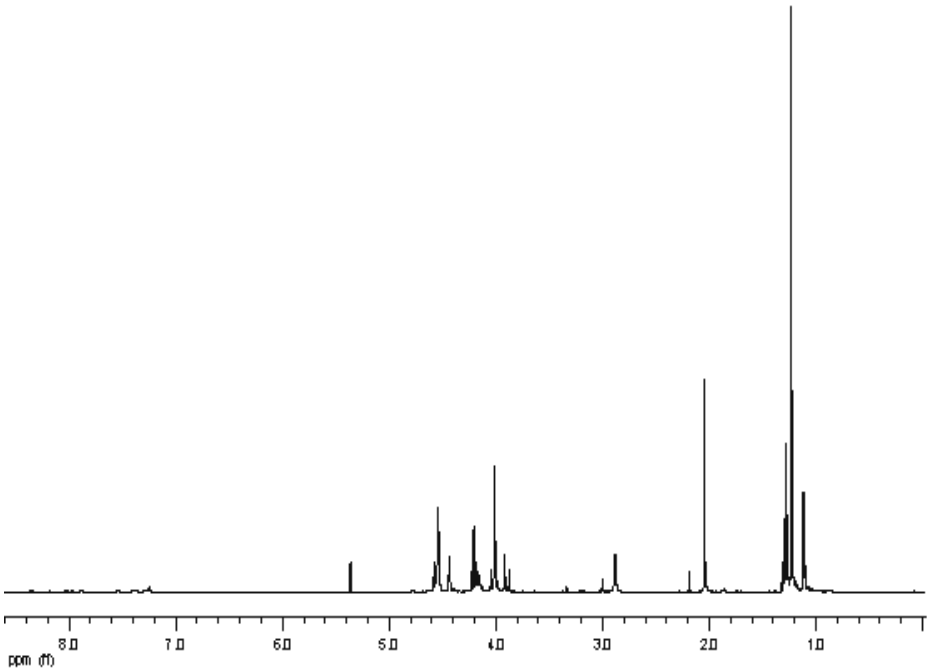


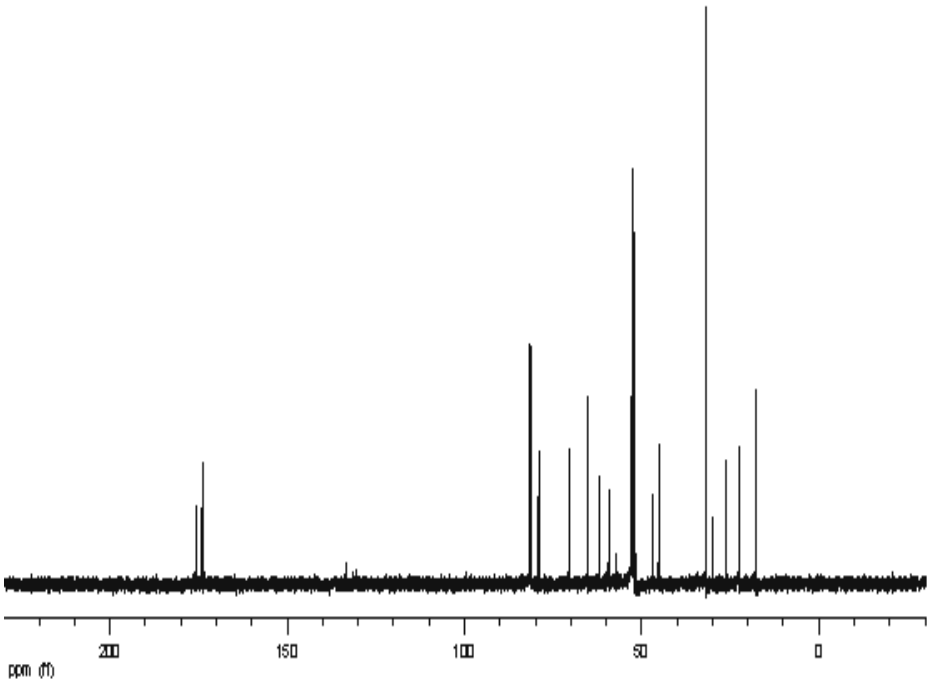




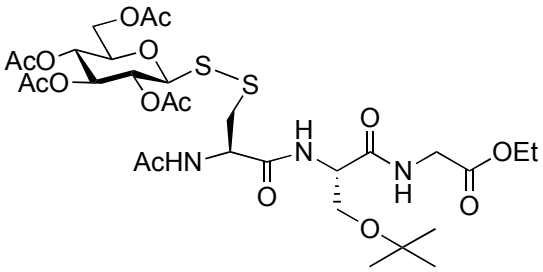
_N_-acetyl-L-cysteine-glycine-_O-tert_-butyl-L-threonine-glycine ethyl ester 63

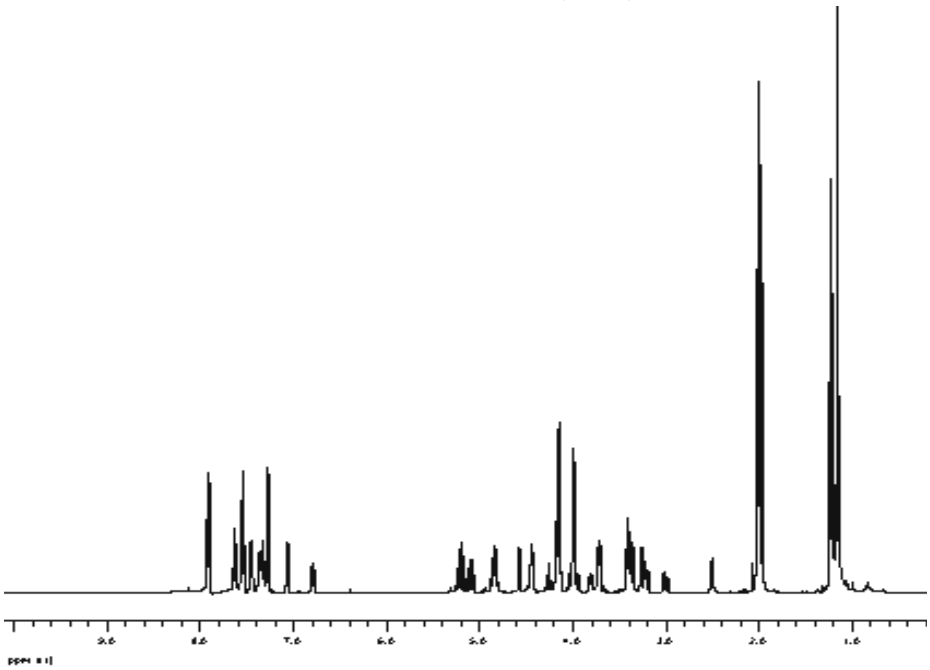


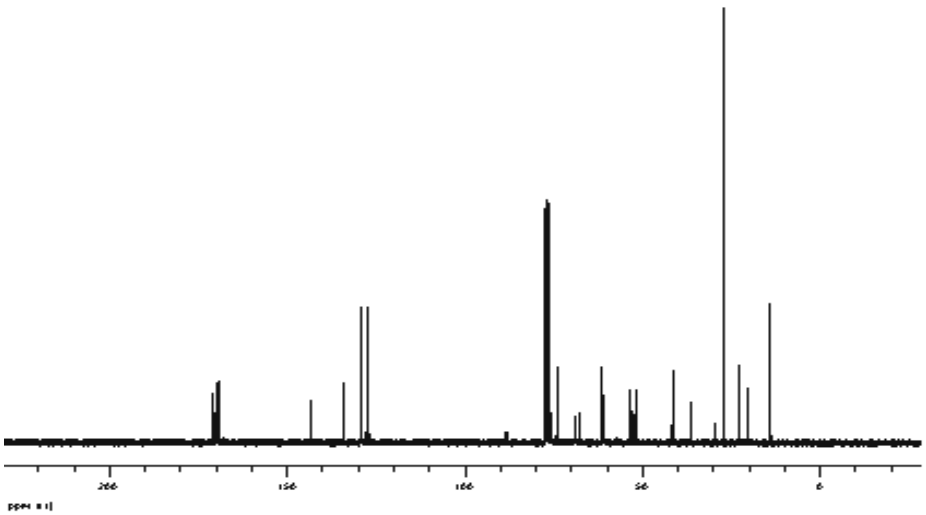




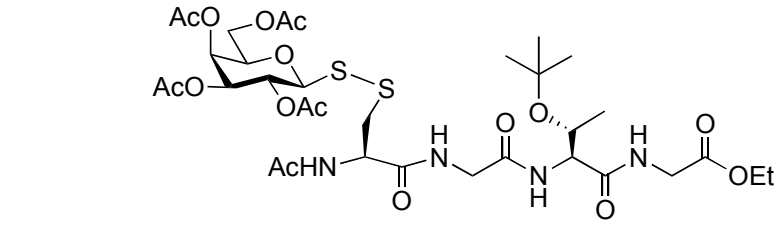
_N_-Acetyl-L-cysteine-(2,3,4,6-tetra-_O_-acetyl-1-dithio-β-D-glucopyaranosyl disulfide)-_O_-_tert_-butyl-L-serine-glycine ethyl ester 19

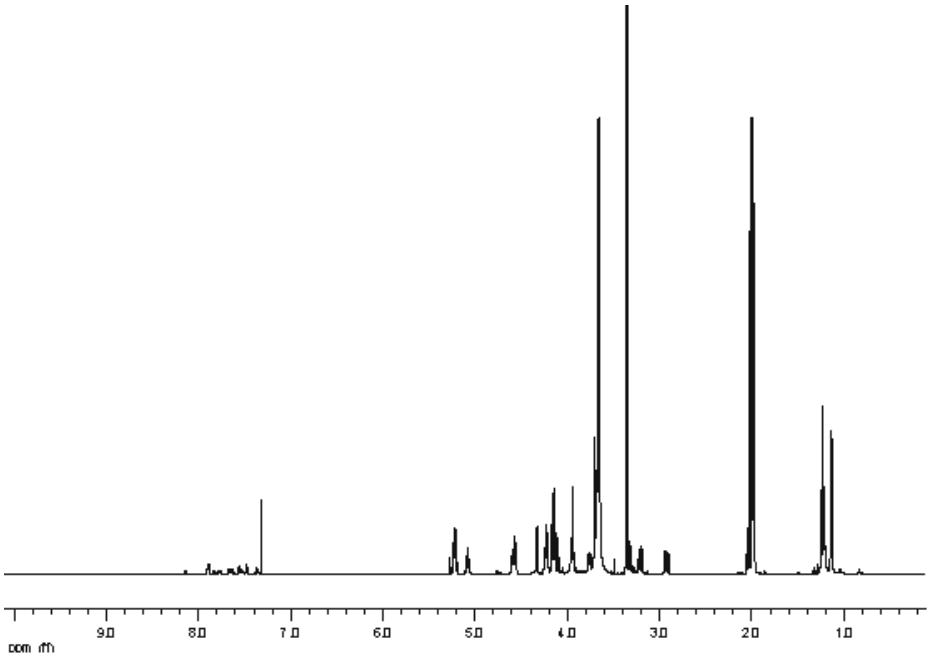


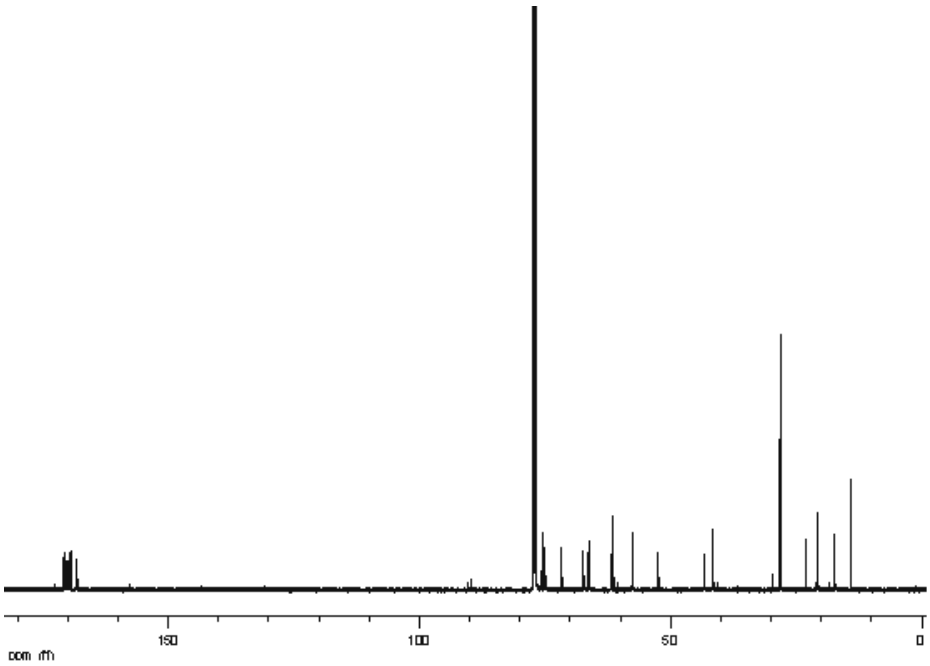




_N_-Acetyl-L-cysteine-(2,3,4,6-tetra-_O_-acetyl-1-dithio-β-D-galactopyaranosyl)-glycine-_O_-_tert_-butyl-L-threonine-glycine ethyl ester 21







_N_-(_tert_-butoxycarbonyl)-L-cysteine (2,3,4,6-tetra-_O_-acetyl-1-dithio-β-D-glucopyaranosyl disulfide) methyl ester 75