From Disulfide- to Thioether-linked Glycoproteins

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**Scheme S1.** $\delta$-attack followed by thio-mitsunobu mechanism.

**Scheme S2.** $\gamma$-attack followed by thio-mitsunobu mechanism.

**Scheme S3.** $\gamma$-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 ($\delta_H$) 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 ($\delta_C$) 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 $\delta$ scale in ppm using residual solvent as the internal standard (1H NMR: CDCl$_3$ = 7.26, CD$_3$OD = 4.87; $^{13}$C NMR: CDC$_3$ = 77.0; CD$_3$OD = 49.0; D$_2$O = 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 ($\nu_{\text{max}}$) 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 ($\lambda_{\text{max}}$ = 254 nm), and/or ammonium molybdate (5% in 2M H$_2$SO$_4$), 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 disulfide¹ 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 (Rₚ 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; [α]D²⁵ +184 (c, 1 in CHCl₃); υmax (KBr disc) no significant peaks; δH (400 MHz, CDCl₃) 3.58 (1H, dd, J₅,₆ 1.8 Hz, J₆,₆’ 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, J₁₂ 5.3 Hz, J₂₂ 9.6 Hz, H-2), 4.17 (1H, ddd, J₄,₅ 10.0 Hz, J₅,₆ 1.8 Hz, J₆,₆’ 3.6 Hz, H-5), 4.42, 4.58 (2H, ABq, J₆,₆’ 11.9 Hz, OCH₂Ph), 4.50, 5.00 (2H, ABq, J₅,₆ 10.7 Hz, OCH₂Ph), 4.74 (2H, s, OCH₂Ph), 4.83, 4.87 (2H, ABq, J₆,₆’ 9.6 Hz, OCH₂Ph), 5.80 (1H, d, J₁₂ 5.3 Hz, H-1), 7.14-7.38 (20H, m, Ar-H), 7.56 (2H, d, J 8.8 Hz, o-PhNO₂), 8.08 (2H, d, J 8.8 Hz, p-PhNO₂); δC (100.7 MHz, CDCl₃) 68.3 (t, C-6), 71.7 (d, C-5), 73.0, 73.5, 75.2, 75.9 (4 x t, 4 x OCH₂Ph), 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 C₄₀H₃₉NNaO₇S (MNa⁺) 700.2340. Found: 700.2339; Found: C, 72.44%; H, 5.68%; N, 2.09%. C₄₀H₃₉NO₅S 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-glucopyranoside) methyl ester 4**

Using the general procedure, **N-acetyl-DL-cysteine-S-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranoside) 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, 0.4 (petrol:ethyl acetate, 1:1); $[\alpha]_D^{18}$ -13.6 (c, 0.5 in CHCl₃); $\nu_{\text{max}}$ (thin film) 3361 (br, NH) 1744 (s, C=O) 1661 (s, C=O) cm$^{-1}$; $\delta_H$ (500 MHz, CDCl₃) 1.84, 1.87 (6H, 2 x s, HNC(O)CH₃, HNC(O)CH₂L), 3.10, 3.14 (2H, 2 x dd, $J_{CH,N}$ 14.6 Hz, $J_{CH,H'}$ 7.2 Hz, CH₂H'D, CH₂H'L), 3.22, 3.23 (2H, dd, $J_{CH,H'}$ 14.6 Hz, $J_{CH,H''}$ 4.2 Hz, CH₂H'D, CH₂H'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, OCH₃D, OCH₃L), 4.38, 4.40 (2H, 2 x d, $J_{\alpha,C}$ 9.8 Hz, H-1D, H-1L), 4.49-4.99 (18H, m, 4 x OCH₃PhD, 4 x OCH₃PhL, $\alpha$HD, $\alpha$HL) 6.85, 6.95 (2H, 2 x d, $J_{NH,H''}$ 7.9 Hz, HNC(O)CH₃D, HNC(O)CH₃L), 7.15-7.36 (40H, m, 20 x Ar-HD, 20 x Ar-HL); $\delta_C$ (125.8 MHz, CDCl₃) 22.5, 22.6 (2 x q, HNC(O)CH₃D, HNC(O)CH₃L), 32.8, 34.6 (2 x t, CH₂H'D, CH₂H'L), 52.4, 52.5 (2 x d, $\alpha$CD, $\alpha$CL), 52.6, 52.9 (2 x q, OCH₃D, OCH₃L), 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 OCH₃PhD, 4 x OCH₃PhL), 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-CO, 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, HNC(O)CH₃D, HNC(O)CH₃L, CO₂CH₃D, CO₂CH₃L); $m/z$ (ES$^+$) 758 (MMeCNNH₄⁺, 100%); HRMS (ES$^+$) Calcd. for C₄₀H₄₆NNaO₈S (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%; Rf 0.4 (ethyl acetate); [α]D22 -4.3 (c, 0.5 in CHCl₃); νmax (thin film) 3299 (br, NH) 1746 (s, C=O) 1654 (s, C=O) cm⁻¹; δH (500 MHz, CDCl₃) δH (500 MHz, CDCl₃) 1.14 (1H, d, J 6.5 Hz, CH₃D), 1.22 (1H, d, J 6.4 Hz, CH₃L), 1.97 (3H, s, HNC(O)CH₃D), 1.98 (3H, s, HNC(O)CH₃L), 2.77 (1H, dd, JCH,HH 14.5 Hz, JCH,HL 3.3 Hz, CH₃H′D), 2.85 (1H, dd, JCH,HH 14.0 Hz, JCH,HL 5.7 Hz, CH₃H′L), 3.07 (1H, dd, JCH,HH 13.8 Hz, JCH,HL 4.3 Hz, CH₃H′L), 3.29 (1H, dd, JCH,HH 14.7 Hz, JCH,HL 4.7 Hz, CH₃H′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, OCH₃D, OCH₃L), 4.07 (1H, q, J 6.5 Hz, H-5D), 4.15 (1H, q, J 6.5 Hz, H-5L), 4.28 (2H, dd, J₁,₂ 5.7 Hz, J₂,₃ 9.9 Hz, H-2D, H-2L), 4.64-4.99 (14H, m, 3 x OCH₂PhD, 3 x OCH₂PhL, αDd, αDL), 5.21 (1H, d, J₁,₂ 5.6 Hz, H-1D), 5.48 (1H, d, J₁,₂ 5.4 Hz, H-1L), 6.21 (1H, br d, JHH,HH 7.8 Hz, HNC(O)CH₃D), 7.07 (1H, br d, JHH,HL 9.0 Hz, HNC(O)CH₃L), 7.29-7.40 (30H, m, 15 x Ar-HD, 15 x Ar-HL); δC (125.8 MHz, CDCl₃) 14.1, 16.5 (2 x q, CH₃D, CH₃L), 22.7, 23.1 (2 x q, HNC(O)CH₃D, HNC(O)CH₃L), 35.0, 37.3 (2 x t, CH₃H′D, CH₃H′L), 52.1, 52.2 (2 x d, αCD, αCL), 52.5, 52.7 (2 x q, OCH₂PhD, OCH₂PhL), 67.8, 68.0 (2 x d, C-5D, C-5L), 72.6, 72.8, 73.5 (3 x t, 3 x OCH₂PhD, 3 x OCH₂PhL), 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, COOCH₃D, COOCH₃L, HNCOCH₃D, HNCOCH₃L); m/z (ES⁺) 615 (MNa⁺, 100%); HRMS (ES⁺) Calcd. for C₃₃H₃₉NNaO₇S (MNa⁺) 616.2339. Found: 616.2340.
**N-Acetyl-DL-cysteine-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) methyl ester 8**

Using the general procedure, *N*-acetyl-DL-cysteine-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) 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%; Rf 0.3 (petrol:ethyl acetate, 1:4); [α]D<sup>18</sup> +4.0 (c, 0.75 in CHCl₃); δ<sub>H</sub> (500 MHz, CDCl<sub>3</sub>) 2.01, 2.02, 2.03, 2.04, 2.06, 2.07, 2.11, 2.13 (30H, 9 x s, 4 x C(O)CH₃, 4 x C(O)CH₂L, HNC(O)CH₃D, HNC(O)CH₃L), 3.05, 3.08 (2H, 2 x dd, J<sub>CH,H</sub> 14.2 Hz, J<sub>CH,iH</sub> 5.7 Hz, CHH'D, CH'H'L), 3.20, 3.23 (2H, dd, J<sub>CH,H</sub> 14.1 Hz, J<sub>CH,iH</sub> 3.3 Hz, CHH'D, CH'H'L), 3.70-3.75 (2H, m, H-5D, H-5L), 3.77, 3.78 (6H, 2 x s, OCH₃D, OCH₃L), 4.17, 4.20 (2H, 2 x dd, J<sub>5,6</sub> 2.1 Hz, J<sub>5,6</sub> 12.4 Hz, H-6D, H-6L), 4.24, 4.26 (2H, 2 x dd, J<sub>5,6</sub> 5.4 Hz, J<sub>5,6</sub> 12.4 Hz, H-6'D, H-6'L), 4.49, 4.53 (2H, 2 x d, J<sub>1,2</sub> 10.1 Hz, H-1D, H-1L), 4.78-4.84 (2H, m, αHD, αHL), 4.98, 4.99 (2H, 2 x at, J<sub>9,7</sub> Hz, H-2D, H-2L), 5.04, 5.08 (2H, 2 x at, J<sub>9,5</sub> Hz, H-4D, H-4L), 5.20, 5.23 (2H, 2 x at, J<sub>9,4</sub> Hz, H-3D, H-3L), 6.46, 6.53 (2H, 2 x d, J<sub>NH,iH</sub> 7.4 Hz, HNC(O)CH₃D, HNC(O)CH₃L); δ<sub>C</sub> (125.8 MHz, CDCl<sub>3</sub>) 20.6, 20.7 (2 x q, 4 x C(O)CH₃D, 4 x C(O)CH₃L), 22.9 (q, HNC(O)CH₃D, HNC(O)CH₃L), 31.7, 32.4 (2 x t, CHH'D, CHH'L), 51.8, 52.2 (2 x d, αCD, αCL), 52.7, 52.8 (2 x q, OCH₃D, OCH₃L), 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 C(O)CH₃D, 4 x C(O)CH₃L, HNC(O)CH₃D, HNC(O)CH₃L, CO₂CH₃D, CO₂CH₃L); m/z (ES<sup>+</sup>) 566 (MMeCNNH<sub>4</sub>+, 100%); HRMS (ES<sup>+</sup>) Calcd. for C<sub>20</sub>H<sub>29</sub>NaO₁₂S (MNa<sup>+</sup>) 530.1303. Found: 530.1296.
**N-Acetyl-DL-cysteinamide-S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) 10**

Using the general procedure, N-acetyl-DL-cysteinamide-S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) 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%; R₉ 0.3 (ethyl acetate:methanol, 95:5); [α]D° = -1.6 (c, 0.5 in CHCl₃); ν_{max} (KBr disc) 3057 (br, NH NH δ) 2543 (s, C=O) cm⁻¹; δ_H (500 MHz, CDCl₃) 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)CH₃D, 4 x C(O)CH₃L, HNC(O)CH₃D, HNC(O)CH₃L), 2.69 (1H, dd, J_{CH,H'} 14.2 Hz, J_{CH,H} 7.4 Hz, CH_H', δ), 2.79 (1H, dd, J_{CH,H'} 14.2 Hz, J_{CH,H} 9.0 Hz, CH_H'L), 3.16 (1H, dd, J_{CH,H'} 14.2 Hz, J_{CH,H} 4.4 Hz, CH_H'L), 3.34 (1H, dd, J_{CH,H'} 14.3 Hz, J_{CH,H} 5.3 Hz, CH_H'L), 3.76-3.83 (1H, m, H-5D), 3.88 (1H, ddd, J_{H/H'}, J_{H/D}), 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-3L), 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_{N/H,L} 7.4 Hz, HNC(O)CH₃D, HNC(O)CH₃L), δ_C (125.8 MHz, CDCl₃) 20.5, 20.6, 20.7, 20.8 (4 x q, 4 x C(O)CH₃D, 4 x C(O)CH₃L, 23.0, 23.1 (2 x q, HNC(O)CH₃D, HNC(O)CH₃L), 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 C(O)CH₃D, 4 x C(O)CH₃L, HNC(O)CH₃D, HNC(O)CH₃L), 172.3, 173.9 (2 x s, CONH₂D, CONH₂L); m/z (ES⁺) 515 (MNa⁺, 100%); HRMS (ES⁺) Calcd. for C₁₉H₂₈N₂NaO₁₁S (MNa⁺) 515.1306. Found: 515.1304.
**N-Acetyl-DL-cysteine-S-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside) methyl ester 12**

Using the general procedure, N-Acetyl-DL-cysteine-S-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside) 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%; Rf 0.5 (ethyl acetate:methanol, 9:1); \( \nu_{\text{max}} \) (thin film) 3366 (br, NH) 1749 (s, C=O) 1664 (s, C=O) cm\(^{-1}\);

δH (500 MHz, CDCl\(_3\)) 1.98, 1.99, 2.05, 2.06, 2.07, 2.08, 2.09, 2.16, 2.17 (30H, 10 x s, 4 x C(O)CH\(_3\)D, 4 x C(O)CH\(_3\)L, HNC(O)CH\(_3\)D, HNC(O)CH\(_3\)L), 3.04 (1H, dd, J\( \text{CH,CH} \) 14.1 Hz, J\( \text{CH,CH} \) 6.4 Hz, CH\(_2\)H\('D'), 3.09 (1H, dd, J\( \text{CH,CH} \) 14.1 Hz, J\( \text{CH,CH} \) 4.4 Hz, CH\(_2\)H\('D'), 3.21 (1H, dd, J\( \text{CH,CH} \) 13.9 Hz, J\( \text{CH,CH} \) 5.4 Hz, CH\(_2\)H\('D'), 3.24 (1H, dd, J\( \text{CH,CH} \) 13.9 Hz, J\( \text{CH,CH} \) 4.5 Hz, CH\(_2\)H\('D'), 3.77, 3.82 (6H, 2 x s, OCH\(_3\)), 5.18 (1H, at, J 6.5 Hz, H-5D, H-5L), 4.13 (2H, dd, J\( \text{5,6} \) 2.6 Hz, J\( \text{6,6} \) 11.4 Hz, H-6D, H-6L), 4.18 (2H, dd, J\( \text{5,6} \) 4.4 Hz, J\( \text{6,6} \) 11.4 Hz, H-6D, H-6L), 4.49 (2H, 2 x d, J\( \text{1,2} \) 10.3 Hz, H-1D, H-1L), 4.79-4.85 (2H, m, \( \alpha\)H\(_{\text{D}}\), \( \alpha\)H\(_{\text{L}}\)), 5.03 (1H, dd, J\( \text{2,3} \) 10.4 Hz, J\( \text{3,4} \) 3.4 Hz, H-3D), 5.07 (1H, dd, J\( \text{2,3} \) 10.4 Hz, J\( \text{3,4} \) 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, HNC(O)CH\(_3\)D), 6.55 (1H, d, J 7.3 Hz, HNC(O)CH\(_3\)L); δC (125.8 MHz, CDCl\(_3\)) 20.3, 20.5, 20.7, 20.8 (4 x q, 4 x C(O)CH\(_3\)D, 4 x C(O)CH\(_3\)L), 22.9, 23.0 (2 x q, HNC(O)CH\(_3\)D, HNC(O)CH\(_3\)L), 31.5, 32.6 (2 x t, CH\(_2\)H\('D\), CH\(_2\)H\('L\)), 51.7, 52.2 (2 x d, \( \alpha\)CD, \( \alpha\)CL), 52.7, 52.8 (2 x q, OCH\(_3\)D, OCH\(_3\)L), 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 C(O)CH\(_3\)D, 4 x C(O)CH\(_3\)L, HNC(O)CH\(_3\)D, HNC(O)CH\(_3\)L, CO\(_2\)CH\(_3\)D, CO\(_2\)CH\(_3\)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-glucopyranoside) methyl ester 14

Using the general procedure, N-acetyl-DL-cysteine-S-(3,4,6-tetra-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside) 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%; Rf 0.3 (ethyl acetate); \([\alpha]_D^{24} +15.6\) (c 1 in CHCl₃); \(\nu_{\text{max}}\) (thin film) 3386 (br, NH) 1743 (s, C=O) 1656 (s, C=O) cm⁻¹; \(\delta_H\) (400 MHz, CDCl₃) 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)CH₃, 3 x C(O)CH₂L, 2 x HNC(O)CH₃D, 2 x HNC(O)CH₂L), 3.02 (1H, dd, \(J_{\text{CH,CH}}\) 14.2 Hz, \(J_{\text{CH,CH'}}\) 6.5 Hz, CH₃L), 3.21 (1H, dd, \(J_{\text{CH,CH}}\) 14.2 Hz, \(J_{\text{CH,CH'}}\) 6.5 Hz, CH₃L), 3.29 (1H, dd, \(J_{\text{CH,CH}}\) 14.2 Hz, \(J_{\text{CH,CH'}}\) 3.9 Hz, CH₃L), 3.76-3.77 (6H, 2 x s, OCH₃D, OCH₃L), 4.04 (1H, at, J 9.8 Hz, H-2D), 4.06 (1H, at, J 9.8 Hz, H-2L), 4.14 (2H, dd, \(J_{\text{CH,CH}}\) 2.2 Hz, \(J_{\text{CH,CH}}\) 12.5 Hz, H-6D, H-6L), 4.24 (2H, dd, \(J_{\text{CH,CH}}\) 4.7 Hz, \(J_{\text{CH,CH}}\) 12.5 Hz, H-6'D, H-6'L), 4.57 (1H, d, \(J_{\text{CH,CH}}\) 10.4 Hz, H-1D), 4.59 (1H, d, \(J_{\text{CH,CH}}\) 10.0 Hz, H-1L), 4.88-4.94 (2H, m, \(\alpha\)HD, \(\alpha\)HL), 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, \(J_{\text{NH,CH}}\) 9.4 Hz, HNC(O)CH₃D, H-2D), 5.81 (1H, d, \(J_{\text{NH,CH}}\) 9.3 Hz, HNC(O)CH₃L, H-2L), 6.58 (1H, d, \(J_{\text{NH,CH}}\) 8.1 Hz, HNC(O)CH₃D, \(\alpha\)HD), 6.68 (1H, d, \(J_{\text{NH,CH}}\) 7.3 Hz, HNC(O)CH₃L, \(\alpha\)HL); \(\delta_C\) (125.8 MHz, CDCl₃) 20.5, 20.6, 20.7, 20.8 (4 x q, 3 x C(O)CH₃D, 3 x C(O)CH₂L), 22.9, 23.0, 23.2, 23.3 (4 x q, 2 x HNC(O)CH₃D, 2 x HNC(O)CH₂L), 31.9, 32.2 (2 x t, CH₃L, CH₃L), 50.8, 52.2 (2 x d, \(\alpha\)CD, \(\alpha\)CL), 52.7, 52.8 (2 x q, OCH₃D, OCH₃L), 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 C(O)CH₃D, 3 x C(O)CH₂L, 2 x HNC(O)CH₃D, 2 x HNC(O)CH₂L, CO₂CH₃D, CO₂CH₂L); \(m/z\) (ES⁺) 565 (MMeCNHH₄⁺, 100%). HRMS (ES⁺) Calcd. for C₂₀H₃₀N₃O₁₁S (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%; Rf 0.3 (ethyl acetate:iso-propanol:water, 5:3:1); [α]D$^{22}$ +0.0 (c, 0.5 in MeOH); $\nu_{\text{max}}$ (thin film) 3442 (br, NH OH) 1643 (s, C=O) cm$^{-1}$; $\delta$H (500 MHz, CD$_3$OD) 2.03, 2.04 (6H, 2 x s, HNC(O)C$_2$H$_5$), 2.95 (1H, dd, J$_{\text{CH,CH}}$ 14.2 Hz, J$_{\text{CH,CH}}$ 8.1 Hz, CH$_2$H$^\alpha$D), 3.09 (1H, dd, J$_{\text{CH,CH}}$ 14.3 Hz, J$_{\text{CH,CH}}$ 8.3 Hz, CH$_2$H$^\alpha$L), 3.20 (1H, dd, J$_{\text{CH,CH}}$ 14.5 Hz, J$_{\text{CH,CH}}$ 7.8 Hz, CH$_2$H$^\alpha$L), 3.26-3.40 (7H, m, H-2D, H-2L, H-3D, H-3L, H-4D, H-4L, CH$_2$H$^\alpha$L), 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, OCH$_3$D, OCH$_3$L), 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, $\alpha$H), 4.76-4.78 (1H, m, $\alpha$H), $\delta$C (125.8 MHz, CD$_3$OD) 22.4, 22.5 (2 x q, HNC(O)C$_2$H$_5$D, HNC(O)C$_2$H$_5$L), 31.9, 33.0 (2 x t, CH$_2$H$^\alpha$D, CH$_2$H$^\alpha$L), 52.9, 54.3 (2 x d, $\alpha$C$_\text{D}$, $\alpha$C$_\text{L}$), 54.6, 55.5 (2 x q, OCH$_3$D, OCH$_3$L), 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, HNC(O)C$_2$H$_5$D, HNC(O)C$_2$H$_5$L, CO$_2$CH$_3$D, CO$_2$CH$_3$L); m/z (ES$^+$) 362 (M$^+$, 100%); HRMS (ES$^+$) Calcd. for C$_{12}$H$_{21}$NNaO$_5$S (M$^+$) 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%; Rf 0.3 (ethyl acetate:iso-propanol:water, 5:3:1); [α]D$^{18}$ +1.6 (c, 1 in MeOH); $\nu_{\text{max}}$ (KBr disc) 3413 (br, NH OH) 1735 (s, C=O) 1646 (s, C=O) cm$^{-1}$; $\delta$H (500 MHz, CD$_3$OD) 1.99,
N-Acetyl-DL-cysteine-S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside)-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-glucopyranoside)-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%; Rf 0.6 (petrol:ethyl acetate, 1:4); [α]D^22^ +0.1 (c, 0.5 in CHCl₃); νmax (thin film) 3334 (br, NH) 1751 (s, C=O) 1661 (s, C=O) cm⁻¹; δH (500 MHz, CDCl₃) 1.19, 1.22 (18H, 2 x s, C(CH₃)₂D, C(CH₃)₂L), 1.26 (6H, t, J 7.2 Hz, OCH₂CH₃D, OCH₂CH₃L), 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)CH₃D, 4 x C(O)CH₃L, HNC(O)CH₃D, HNC(O)CH₃L), 2.90 (2H, 2 x d, JCH,CH 14.0 Hz, JCH₃D, H₂NCH₃D, H₂NCH₃L, 9.5 Hz, CH₃DCH₂, CH₃LCH₂, 3.11 (2H, dd, JCH₂CH₃, 14.0 Hz, JCH₂CH₃, 4.9 Hz, CH₃DCH₂, CH₃LCH₂), 3.35-3.48 (4H, m, CH₃DCH₂, CH₃LCH₂), 3.75-3.80 (1H, m, H-5D), 3.85-3.93 (1H, m, H-5L), 4.01-4.06 (4H, m, 2 x CH₂NCH₃D, 2 x CH₂NCH₃L), 4.16-4.26 (6H, m, OCH₂CH₃D, OCH₂CH₃L, H-6D, H-6L), 4.38 (1H, dd, Jδ,δ 2.0 Hz, Jδ,δ 12.6 Hz, H-6'), 4.41 (1H, dd, Jδ,δ 2.0 Hz, Jδ,δ 12.6 Hz, H-6'), 4.46-4.56 (2H, m, αHser)}
\(\alpha\text{HserL}, 4.59 \text{ (1H, d, } J_{1,2} 9.8 \text{ Hz, } H-1\text{D}), 4.77 \text{ (1H, d, } J_{1,2} 10.2 \text{ Hz, } H-1\text{L}), 4.85-4.88 \text{ (2H, } m, \alpha\text{HcysD, } \alpha\text{HcysL}), 5.03 \text{ (2H, at, } J 9.8 \text{ Hz, } H-2\text{D, } H-2\text{L}), 5.11 \text{ (1H, at, } J 9.7 \text{ Hz, } H-4\text{D), 5.17 \text{ (1H, at, } J 9.7 \text{ Hz, } H-4\text{L}), 5.26 \text{ (2H, at, } J 9.9 \text{ Hz, } H-3\text{D, } H-3\text{L}), 6.51 \text{ (2H, d, } J_{\text{NH,OH}} 7.4 \text{ Hz, } \text{HNC(O)CH}_3\text{cysD, } \text{HNC(O)CH}_3\text{cysL}), 6.65 \text{ (2H, d, } J_{\text{NH,OH}} 6.9 \text{ Hz, } \text{HNC(O)CH}_3\text{SerD, } \text{HNC(O)CH}_3\text{SerL}), 7.44 \text{ (2H, d, } J_{\text{NH,OH}} 7.8 \text{ Hz, } \text{HNC(O)CH}_3\text{glyD, } \text{HNC(O)CH}_3\text{glyL}); \delta_C \text{ (125.8 MHz, } \text{CDCl}_3) 14.1, 14.2 \text{ (2 x q, } \text{OCH}_3\text{CH}_3\text{D, } \text{OCH}_3\text{CH}_3\text{L}, 20.5, 20.6, 20.7, 20.8, 21.0 \text{ (5 x q, } 4 \times \text{C(O)}\text{CH}_3\text{D, } 4 \times \text{C(O)}\text{CH}_3\text{L}), 22.9, 23.0 \text{ (2 x q, } \text{HNC(O)CH}_3\text{D, } \text{HNC(O)CH}_3\text{L}), 27.3, 27.4 \text{ (2 x q, } \text{C(CH}_3)_3\text{D, } \text{C(CH}_3)_3\text{L}), 34.6 \text{ (2 x t, } \text{CH}_3\text{H'cysD, } \text{CH}_3\text{H'cysL}), 41.2, 41.4 \text{ (2 x t, } \alpha\text{CglyD, } \alpha\text{CglyL}), 52.4, 53.1, 53.4, 54.1 \text{ (4 x d, } \alpha\text{CcysD, } \alpha\text{CcysL, } \alpha\text{CserD, } \alpha\text{CserL}), 60.4, 60.9, 61.1, 61.3, 61.4, 62.3 \text{ (6 x t, } \text{OCH}_2\text{CH}_2\text{D, } \text{OCH}_2\text{CH}_2\text{L, } \text{C-6D, } \text{C-6L, } \text{CH}_3\text{H'serD, } \text{CH}_3\text{H'serL}), 67.8, 68.3 \text{ (2 x d, } \text{C-4D, } \text{C-4L}), 69.0, 69.7 \text{ (2 x d, } \text{C-2D, } \text{C-2L}), 73.6, 74.1 \text{ (d, } \text{C-3D, } \text{C-3L}), 76.4, 76.5 \text{ (2 x d, } \text{C-5D, } \text{C-5L}), 83.1, 85.6 \text{ (2 x d, } \text{C-1D, } \text{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 \times \text{C(O)}\text{CH}_3\text{D, } 4 \times \text{C(O)}\text{CH}_3\text{L, } \text{HNC(O)CH}_3\text{D, } \text{HNC(O)CH}_3\text{L, } \text{C(O)HN\text{\alpha}CserD, } \text{C(O)HN\text{\alpha}CserL, } \text{C(O)HN\alphaCglyD, } \text{C(O)HN\alphaCglyL, } \text{CO}_2\text{CH}_3\text{D, } \text{CO}_2\text{CH}_3\text{L}); m/z \text{ (ES') 780 (MMe\text{CNNH}_3^+, } 100\%); \text{HRMS (ES') Calcd. for } C_{30}H_{47}N_3NaO_{15}S (\text{MNa}^+) 744.2620. \text{Found: 744.2608.}

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

Using the general procedure, \textit{N}-acetyl-DL-cysteine-S-(2,3,4,6-tetra-O-acetyl-\beta-D-galactopyranoside)-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\(_3\));

\(v_{\text{max}}\) (thin film) 3382 (br, NH) 1725 (s, C=O) 1652 (s, C=O) cm\(^{-1}\); \(\delta_H\) (500 MHz, CDCl\(_3\)) 1.05, 1.07 (6H, 2 x d, \(J\ 6.5\) Hz, \text{CHCH}_4\text{thrd}, \text{CHCH}_3\text{thrl}), 1.28-1.54 (24H, m, \text{C(CH}_3)_3\text{D, C(CH}_3)_3\text{L, OCH}_2\text{CH}_3\text{D, OCH}_2\text{CH}_3\text{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\(_3\)D, 4 x C(O)CH\(_3\)L), 2.18, 2.22 (6H, 2 x s, \text{HNC(O)CH}_3\text{D, HNC(O)CH}_3\text{L}), 2.87-3.00 (3H, m, \text{CH}_3\text{H'cysd}, \text{CH}_3\text{H'cysl, CH}_3\text{H'cysl}), 3.13 (1H, dd, \(J_{\text{CH,rt}}\) 14.4 Hz, \(J_{\text{CH,rt}}\) 5.4 Hz, \text{CH}_3\text{H'cysd}), 3.53-4.23 (16H, m, H-5D, H-5L, H-6D, H-6L, H-6'D, H-6'L, \text{OCH}_2\text{CH}_3\text{D, OCH}_2\text{CH}_3\text{L}, 2 x \alpha\text{HglyD, 2 x } \alpha\text{HglyL, CHCH}_3\text{thrd, CHCH}_3\text{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)CH₃D, 4 x HNC(O)CH₃L); δC (125.8 MHz, CDCl₃) 14.1, 14.2 (2 x q, OCH₂CH₃D, OCH₂CH₃L), 17.3, 17.4 (2 x q, CHCH₃thrD, CHCH₃thrL), 20.5, 20.6, 20.7, 20.8, 20.9, 21.0 (6 x q, 4 x C(O)CH₃D, 4 x C(O)CH₃L), 22.9, 23.0 (2 x q, HNC(O)CH₃D, HNC(O)CH₃L), 28.1, 28.2 (6 x q, C(CH₃)₃D, C(CH₃)₃L), 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, OCH₂CH₃D, OCH₂CH₃L), 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(CH₃)₃D, C(CH₃)₃L), 74.9, 75.4 (2 x d, C-5D, C-5L), 75.7, 75.9 (2 x d, CHCH₃thrD, CHCH₃thrL), 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)CH₃D, 4 x C(O)CH₃L, HNC(O)CH₃D, HNC(O)CH₃L); m/z (ES⁺) 851 (MMeCNNH₄⁺, 100%); HRMS (ES⁺) Calcd. for C₃₃H₅₂N₄NaO₁₆S (MNa⁺) 815.2991. Found: 815.2990.
Protein Modification

**Preparation of SBL-S156C-SS-Glc(OAc)₄ 23**

SBL-S156C mutant 25 (2.5 mg) was dissolved in buffer (500 µL, 70 mM CHES, 5 mM MES, 2 mM CaCl₂, 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).

**Scheme S4.**

![Scheme S4](image)

**Figure S1.** ESI-MS spectrum of SBL-S156C-SS-Glc(OAc)₄ 23.

**Desulfurization of SBL-S156C-SS-Glc(OAc)₄ 23**

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 CaCl₂, 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**

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 CaCl₂, 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 toioether-linked glycoprotein 24 was shown to be stable under reducing conditions (calculated mass, 27044; observed mass 27046).

**Scheme S6.**

**Figure S3.** ESI-MS spectrum of SBL-S156C-S-Glc(OAc)₄ 24 when treated with TCEP.
**Treatment of 23 with TCEP**

![Scheme S7.](image)

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 CaCl₂, 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.](image) 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 NH₄HCO₃, pH 8.0) was incubated with 1 µg 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 H₂O 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).
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Disulfide-linked glycoaminoacids and glycopeptides - prepared using glycoPTS$^2$ or glycoSeS$^3$ strategy

Preparation of Sodium phenylthiosulfonate (Na-PTS)$^4$ 26

Sodium benzenesulfinic acid (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. Recrystallisation from anhydrous ethanol afforded sodium phenylthiosulfonate 26 (9.8 g, 83%) as a white crystalline solid; m.p. 282-284 °C (ethanol) [Lit. 287 °C]$^4$; δH (400 MHz, D$_2$O) 3.26 (3H, s, CH$_3$); m/z (ES$^-$) 173 (M-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 °C (ethyl acetate) [Lit. 79-80 °C (ethyl acetate)]$^5$; [α]$_D$ $^22$ -23.2 (c, 1 in CD$_3$OD) [Lit. [α]$_D$ $^{25}$ -24 (c, 1 in MeOH)]$^6$; δH (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$_2$CH$_3$), 4.88-4.92 (1H, m, αH), 6.40 (1H, br s, NH); m/z (ES$^-$) 173 (M-H$^+$, 100%).
Synthesis of N-Acetyl-L-cysteine (2,3,4,6-tetra-O-benzyl-1-dithio-β-D-glucopyranosyl disulfide) methyl ester 3

Methyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside 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 °C. 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 (Rf 0.4) with complete consumption of the starting material (Rf 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 (MgSO4), 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; [α]D²¹ +19.3 (c, 1 in CHCl₃) [Lit. [α]D²¹ +21.2 (c, 1 in H₂O)]; δH (400 MHz, CDCl₃) 3.39 (3H, s, OCH₃), 3.57 (1H, dd, J₁,₂ 3.6 Hz, J₂,₃ 9.6 Hz, H-2), 3.63 (1H, d, J 9.6 Hz, H-4), 3.64 (1H, dd, J₅,₆ 2.3 Hz, J₆,₆ 13.1 Hz, H-6), 3.71-3.78 (2H, m, H-5, H-6′), 4.00 (1H, at, J₂,₃ 9.6 Hz, J₃,₄ 9.2 Hz, H-3), 4.48, 4.84 (2H, ABq, Jₐ,b 11.0 Hz, OCH₂Ph), 4.49, 4.68 (2H, ABq, Jₐ,b 12.1 Hz, OCH₂Ph), 4.59, 4.81 (2H, ABq, Jₐ,b 8.9 Hz, OCH₂Ph), 4.64 (1H, d, 4.65 (2H, ABq, Jₐ,b 10.2 Hz, OCH₂Ph), 4.74 (1H, br s) ppm.
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\[ J_{1,2} 3.6 \text{ Hz, H-1}, 4.91, 4.92 (2H, ABq, J_{A,B} 10.9 \text{ Hz, OCH}_2\text{Ph}), 7.13-7.16 (2H, m, Ar-H), 7.27-7.39 (18H, m, Ar-H); m/z (ES\textsuperscript{+}) 577 (MNa\textsuperscript{+}, 50\%) 613 (MMMeCNNH\textsubscript{4}\textsuperscript{+}, 100\%). \]

2,3,4,6-Tetra-O-benzyl-\(\alpha\)-d-glucopyranose\textsuperscript{8} 30

![Chemical structure of 2,3,4,6-Tetra-O-benzyl-\(\alpha\)-d-glucopyranose](image)

Methyl 2,3,4,6-tetra-O-benzyl-\(\alpha\)-d-glucopyranoside 29 (15.33 g, 27.64 mmol) was dissolved in glacial acetic acid (300 mL). The mixture was heated to 90 °C 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\textsubscript{f} 0.4) and complete consumption of starting material (R\textsubscript{f} 0.5). Water (200 mL) was added and the reaction mixture cooled to 0 °C, 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-\(\alpha\)-d-glucopyranose 30 (10.25 g, 68\%) as a white crystalline solid, being a mixture of anomers (\(\alpha\):\(\beta\), 1:1); m.p. 148-150 °C (ethyl acetate/petrol) [Lit. 151-152 °C\textsuperscript{8}; \([\alpha]\textsubscript{D}\textsuperscript{23} +20.7 (c, 1 in CHCl\textsubscript{3}) [Lit. \([\alpha]\textsubscript{D}\textsuperscript{25} +22 (c, 1.0 in CHCl\textsubscript{3})]\textsuperscript{8}; \(\delta\)\textsubscript{H} (400 MHz, CDCl\textsubscript{3}) 3.42 (1H, dd, \(J\textsubscript{1,2} 7.8 \text{ Hz, } J\textsubscript{2,3} 9.1 \text{ Hz, H-2} \beta\)), 3.47-3.74 (8H, m, H-4\(\alpha\), H-6\(\alpha\), H-6'\(\alpha\), H-3\(\beta\), H-4\(\beta\), H-5\(\beta\), H-6\(\beta\), H-6'\(\beta\)), 3.60 (1H, dd, \(J\textsubscript{1,2} 3.7 \text{ Hz, } J\textsubscript{2,3} 9.5 \text{ Hz, H-2} \alpha\)), 3.99 (1H, at, \(J\textsubscript{2,3} 9.5 \text{ Hz, } J\textsubscript{3,4} 9.2 \text{ Hz, H-3} \alpha\)), 4.05 (1H, ddd, \(J\textsubscript{4,5} 10.1 \text{ Hz, } J\textsubscript{5,6} 3.8 \text{ Hz, } J\textsubscript{5,6'} 2.1 \text{ Hz, H-5} \alpha\)), 4.48-4.97 (16H, m, 4 x OCH\textsubscript{2}Ph\(\alpha\), 4 x OCH\textsubscript{2}Ph\(\beta\)), 4.73 (1H, d, \(J\textsubscript{1,2} 7.8 \text{ Hz, H-1} \alpha\)), 5.24 (1H, d, \(J\textsubscript{1,2} 3.7 \text{ Hz, H-1} \beta\)), 7.15-7.38 (40H, m, 20 x Ar-H\(\alpha\), 20 x Ar-H\(\beta\)).

2,3,4,6-Tetra-O-benzyl-\(\alpha\)-d-glucopyranose bromide 31

![Chemical structure of 2,3,4,6-Tetra-O-benzyl-\(\alpha\)-d-glucopyranose bromide](image)

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 °C, 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\textsubscript{f} 0.6). The reaction was cooled to 0 °C 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 (MgSO₄), 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; δH (400 MHz, CDCl₃) 3.55 (1H, dd, J₁,₂ 3.7 Hz, J₂,₃ 9.2 Hz, H-2), 3.66 (1H, dd, J₅,₆ 1.8 Hz, J₅,₆' 11.0 Hz, H-6), 3.76-3.80 (1H, m, H-4, H-6'), 4.04 (1H, at, J₂,₃ 9.2 Hz, J₃,₄ 9.2 Hz, H-3), 4.55-5.00 (8H, m, 4 x OCH₂Ph), 6.44 (1H, d, J₁,₂ 3.7 Hz, H-1), 7.14-7.42 (20H, m, Ar-H).

2,3,4,6-Tetra-O-benzyl-β-D-glucopyranosyl phenylthiosulfonate 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 (Rf 0.5) with complete consumption of the starting material (Rf 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-β-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; [α]D²² +47.8 (c, 1 in CHCl₃) [Lit. [α]D²² +21.4 (c, 0.35 in CHCl₃)]²; δH (400 MHz, CDCl₃) 3.45 (1H, ddd, J₄,₅ 9.6 Hz, J₅,₆ 1.6 Hz, J₅,₆' 3.6 Hz, H-5), 3.49 (1H, dd, J₅,₆ 1.4 Hz, J₆,₆' 11.7 Hz, H-6), 3.54 (1H, dd, J₁,₂ 9.9 Hz, J₂,₃ 8.7 Hz, H-2), 3.57 (1H, dd, J₅,₆ 3.6 Hz, J₆,₆' 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 OCH₂Ph), 5.13 (1H, d, J₁,₂ 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 (MMeCNNH₄⁺, 100%).
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 \( \text{N-acetyl-L-cysteine methyl ester} \) 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 \( \text{N-acetyl-L-cysteine (2,3,4,6-tetra-O-benzyl-1-dithio-β-D-glucopyranosyl disulfide) methyl ester} \) 3 (0.52 g, 89%) as a white crystalline solid; m.p. 108-110 °C (ethyl acetate/petrol); [\( \alpha \)]\(_D\)\(^{23} \) +35.2 (c, 1 in CHCl\(_3\)); \( \nu \)\(_{\text{max}} \) (KBr disc) 3330 (br, NH) 1740 (s, C=O) 1651 (s, C=O) cm\(^{-1} \); \( \delta \)\(_H\) (400 MHz, CDCl\(_3\)) 1.99 (3H, s, HNC(O)C\(_3\)H\(_3\)), 3.24 (1H, dd, \( \text{J}_{\text{CH,H}} \) 13.9 Hz, \( \text{J}_{\text{CH,H'}} \) 7.7 Hz, CH\(_3\)H'), 3.45 (1H, dd, \( \text{J}_{\text{CH,H}} \) 13.9 Hz, \( \text{J}_{\text{CH,H'}} \) 3.9 Hz, CH\(_3\)H'), 3.54 (1H, m, H-5), 3.68 (1H, at, \( \text{J}_{1,2} \) 9.1 Hz, \( \text{J}_{2,3} \) 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, \( \text{J}_{5,6} \) 1.9 Hz, \( \text{J}_{5,6'} \) 11.4 Hz, H-6), 3.80 (1H, dd, \( \text{J}_{6,6'} \) 3.8 Hz, \( \text{J}_{6,6'} \) 11.4 Hz, H-6'), 4.48 (1H, d, \( \text{J}_{1,2} \) 9.1 Hz, H-1), 4.52-4.93 (8H, m, 4 x OCH\(_2\)Ph), 4.77 (1H, d, \( \text{J}_{7,7} \) 7.7 Hz, \( \alpha \)H), 6.86 (1H, d, \( \text{J}_{\text{NH,H}} \) 8.0 Hz, HNC(O)CH\(_3\)), 7.13-7.16 (2H, m, Ar-H), 7.27-7.35 (18H, m, Ar-H); \( \delta \)\(_C\) (100.7 MHz, CDCl\(_3\)) 23.0 (q, HNC(O)CH\(_3\)), 41.4 (t, CH\(_3\)H'), 52.3 (d, \( \alpha \)C), 52.6 (q, OCH\(_3\)), 68.7 (t, C-6), 73.5, 75.1, 75.5, 75.7 (4 x t, 4 x OCH\(_2\)Ph), 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, COOCH\(_3\), HNCOCH\(_3\)); \( m/z \) (ES\(^+\)) 790 (MMeCNNH\(_4^+\), 100%); HRMS (ES\(^+\)) Calcd. for C\(_{40}\)H\(_{45}\)NNaO\(_8\)S\(_2\) (MNa\(^+\)) 754.2479. Found: 754.2479.; Found: C, 64.78%; H, 6.30%, N, 1.75%. C\(_{40}\)H\(_{45}\)NNaO\(_8\)S\(_2\) requires: C, 64.64%; H, 6.20%; N, 1.91%.
Synthesis of \( N \)-Acetyl-L-cysteine (2,3,4-tri-O-benzyl-1-dithio-\( \alpha \)-L-fucopyranosyl disulfide) methyl ester 5

\[
\begin{align*}
\text{Phenyl} & \quad \text{AcO} \quad \text{AcO} \\
& \quad \text{S} \quad \text{OMe} \\
& \quad \text{S-S} \\
& \text{NHAc} \quad \text{AcCN} \\
& \quad \text{DCM/MeOH} \quad \text{Et}_2\text{N} \quad 31\% \\
& \quad \text{NaPTS} \quad \text{CH}_3\text{CN}, \text{TBAB}, 50^\circ\text{C}, 4\text{h} \\
& \quad \text{Br} \\
& \quad \text{OBn} \quad \text{OBn} \quad \text{OBn} \quad \text{OBn} \\
& \quad \text{AcO} \quad \text{AcO} \\
\end{align*}
\]

Scheme S9.

1,2,3,4-Tetra-\( O \)-acetyl-\( \alpha \)-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 (\( R_r 0.4 \)) with complete consumption of starting material (\( R_r 0 \)). 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-\( \alpha \)-L-fucopyranoside 34 (3.3 g, 81%) as a white crystalline solid; m.p. 94-96 °C (diethyl ether/petrol) [Lit. 93 °C (diethyl ether/petrol)]\(^9\); [\( \alpha \)]\( D \) \(-105.7\) (c, 1 in CHCl\(_3\)) [Lit. [\( \alpha \)]\( D \) \(-129.9\) (c, 2 in acetone)]\(^9\); \( \delta \) (400 MHz, CDCl\(_3\)) 1.16 (3H, d, \( J = 6.5 \text{ Hz, CH}_3 \)), 2.00, 2.01, 2.14, 2.17 (12H, 4 x s, 4 x C(O)CH\(_3\)), 4.27 (1H, q, \( J = 6.5 \text{ Hz, H}-5 \)), 5.29-5.37 (3H, m, H-2, H-3, H-4), 6.34 (1H, d, \( J_{1,2} = 2.7 \text{ Hz, H}-1 \)); \( m/z \) (ES\(^+\)) 391 (MMeCNH4+, 100%).

Phenyl 2,3,4-tri-\( O \)-acetyl-1-thio-\( \alpha \)-L-fucopyranoside\(^10\) 35

\[
\begin{align*}
\text{Ph} & \quad \text{AcO} \quad \text{AcO} \\
& \quad \text{SPh} \\
\end{align*}
\]

1,2,3,4-Tetra-\( O \)-acetyl-\( \alpha \)-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 (Rf 0.6) and the complete consumption of starting material (Rf 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 (MgSO4), 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; δH (400 MHz, CDCl3) 1.14 (3H, d, J 6.5 Hz, CH3α), 1.25 (3H, d, J 6.4 Hz, CH3β), 1.98, 2.09, 2.15 (9H, 3 x s, 3 x C(O)CH3β), 2.02, 2.05, 2.17 (9H, 3 x s, 3 x C(O)CH3α), 3.84 (1H, q, J 6.4 Hz, H-5β), 4.62 (1H, q, J 6.5 Hz, H-5α), 4.71 (1H, d, J1,2 9.9 Hz, H-1β), 5.06 (1H, dd, J2,3 9.9 Hz, J3,4 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, J1,2 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**

![Structure](image)

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 (Rf 0) and complete consumption of starting material (Rf 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 (Rf 0.5) with complete consumption of the starting material (Rf 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 (MgSO4), 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_{4}S (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; [α]_D^18 -35.1 (c, 1 in CHCl_3); _υ_ \text{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$_2$Ph), 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$_6$S$_2$ (MNa$^+$) 613.1689. Found: 613.1692.

$N$-Acetyl-L-cysteine (2,3,4-tri-O-benzyl-1-dithio-$\alpha$-L-fucopyranosyl disulfide) methyl ester 5

A solution of 2,3,4-tri-O-benzyl-1-thio-$\alpha$-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-$\alpha$-L-fucopyranosyl disulfide) methyl ester 5 (48 mg, 31%) as white foam; [\(\alpha\)]$_D^{25}$ -37.3 (c, 0.5 in CHCl$_3$); \(\nu_{\text{max}}\) (KBr disc) 3299 (br, NH) 1746 (s, C=O) 1654 (s, C=O) cm$^{-1}$; \(\delta_H\) (500 MHz, CDCl$_3$) 1.17 (1H, d, J = 6.4 Hz, CH$_3$), 2.03 (3H, s, HNC(O)C$_3$H$_3$), 3.34 (1H, dd, J$_{CH,H'}$ 14.5 Hz, J$_{CH,CH'}$ 5.0 Hz, CH$_3$H'), 3.41 (1H, dd, J$_{CH,H'}$ 14.5 Hz, J$_{CH,\alpha}$ 4.5 Hz, CH$_3$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$_{t,2}$ 5.4 Hz, J$_{t,3}$ 9.6 Hz, H-2), 4.66-5.00 (6H, m, 3 x OCH$_2$Ph), 4.93-4.96 (1H, m, \(\alpha\)H), 5.63 (1H, d, J$_{t,2}$ 5.5 Hz, H-1), 6.36 (1H, br d, J$_{NH,CH}$ 7.7 Hz, HNC(O)CH$_3$), 7.29-7.39 (15H, m, Ar-H); \(\delta_C\) (125.8 MHz, CDCl$_3$) 16.3 (q, CH$_3$), 23.2 (q, HNC(O)CH$_3$), 41.3 (t, CH$_3$H'), 51.8 (d, \(\alpha\)C), 52.8 (q, OCH$_3$), 68.5 (d, C-5), 72.7, 73.3, 74.9 (3 x t, 3 x OCH$_2$Ph), 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, COOCH$_3$, HNCOCH$_3$); m/z (ES$^+$) 684 (MMeCNNH$_4^+$, 100%); HRMS (ES$^+$) Calcd. for C$_{33}$H$_{34}$NaO$_6$S$_2$ (MNa$^+$) 648.2060. Found: 648.2067.
Synthesis of N-Acetyl-L-cysteine (2,3,4,6-tetra-O-acetyl-1-dithio-β-D-glucopyranosyl disulfide) methyl ester 7

1,2,3,4,6-Penta-O-acetyl-D-glucopyranoside\textsuperscript{12,13} 40

\textit{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\textsubscript{f} 0.6) with complete consumption of starting material (R\textsubscript{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 °C (ethanol) [Lit. 100-102 °C]\textsuperscript{12}; [α]\textsubscript{D}\textsuperscript{21} +51.3 (c, 1.01 in CHCl\textsubscript{3}) [Lit. [α]\textsubscript{D} +54.5 (c, 3.8 in CHCl\textsubscript{3})]\textsuperscript{13}; δ\textsubscript{H} (400 MHz, CDCl\textsubscript{3}) 2.02, 2.03, 2.09, 2.11, 2.18 (15H, 5 x s, 5 x C(O)CH\textsubscript{3}α), 2.02, 2.03, 2.09, 2.12, 2.18 (15H, 5 x s, 5 x C(O)CH\textsubscript{3}β), 3.82-3.86 (1H, 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\textsubscript{1,2} 8.3 Hz, H-1β), 6.33 (1H, d, J\textsubscript{1,2} 3.6 Hz, H-1α).
2,3,4,6-Tetra-O-acetyl-\(\alpha\)-D-glucopyranosyl bromide\(^{14}\) 41

![Image of 2,3,4,6-Tetra-O-acetyl-\(\alpha\)-D-glucopyranosyl bromide](image)

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-\(\alpha\)-D-glucopyranosyl bromide 41 (17.5 g, 83%) as a white crystalline solid; m.p. 84-86 °C (ethyl acetate/petrol) [Lit. 89.5-90.5 °C\(^{14}\); [\(\alpha\)]\(_D\)\(^{22}\) +182.1 (c, 1.01 in CHCl\(_3\)) [Lit. [\(\alpha\)]\(_D\) +186 (c, 6 in CH\(_2\)Cl\(_2\))]\(^{14}\); \(\delta_H\) (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, \(J_{2,2}\) 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-\(\beta\)-D-glucopyranosyl phenylthiosulfonate\(^2\) 42

![Image of 2,3,4,6-Tetra-O-acetyl-\(\beta\)-D-glucopyranosyl phenylthiosulfonate](image)

2,3,4,6-Tetra-O-acetyl-\(\alpha\)-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 °C. 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 °C (ethyl acetate/petrol) [Lit. 129-130 °C (ethyl acetate/petrol)]²; [α]₂³ +48.3 (c, 1 in CHCl₃) [Lit. [α]₂⁵ +51.2 (c, 1 in CHCl₃)]²; δ₂ (400 MHz, CDCl₃) 1.99, 2.00, 2.02, 2.05 (12H, 4 x s, 4 x C(O)CH₃), 3.74 (1H, ddd, J₄,5 10.1 Hz, J₅,6 2.3 Hz, J₆,6 4.3 Hz, H-5), 3.91 (1H, dd, J₅,6 2.3 Hz, J₆,6 12.5 Hz, H-6), 4.11 (1H, dd, J₅,6 4.3 Hz, J₆,6 12.5 Hz, H-6'), 4.99-5.05 (2H, m, H-2, H-4), 5.26 (1H, d, J₁,₂ 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-glucopyranosyl 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 (Rₜ 0.4) along with complete consumption of the starting material (Rₜ 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-glucopyranosyl disulfide) methyl ester 7 (164 mg, 76%) as a white amorphous solid; [α]₂⁰ +19.8 (c, 1 in CHCl₃); νₗₘₜₐₓ (KBr disc) 3290 (br, NH) 1749 (s, C=O) 1663 (s, C=O) cm⁻¹; δ₂ (400 MHz, CDCl₃) 2.00, 2.02, 2.03, 2.04, 2.08 (15H, 5 x s, 4 x C(O)CH₃), HNC(O)(CH₃), 3.09 (1H, ddd, J₂,₃₄ 14.2 Hz, J₃₄,₃₅ 6.8 Hz, CH₂H'), 3.33 (1H, dd, J₃₄,₃₅ 14.2 Hz, J₃₅,₃₆ 4.4 Hz, CH₃H'), 3.77 (3H, s, OCH₃), 3.81-3.85 (1H, m, H-5), 4.18 (1H, dd, J₅,₆ 2.2 Hz, J₆,₆ 12.5 Hz, H-6), 4.27 (1H, dd, J₅,₆ 4.7 Hz, J₆,₆ 12.5 Hz, H-6'), 4.59 (1H, d, J₁,₂ 9.5 Hz, H-1), 4.95-4.99 (1H, m, aH), 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, J₉,₁₀ 8.0 Hz, HNC(O)(CH₃)); δ₁ (100.7 MHz, CDCl₃) 20.6, 20.7, 20.8 (3 x q, 4 x C(O)CH₃), 23.1 (q, HNC(O)(CH₃), 41.9 (t, CH₂H'), 51.9 (d, αC), 52.8 (q, OCH₃), 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,
Synthesis of N-Acetyl-L-cysteinamide\textsuperscript{15} 43

\begin{center}
\begin{tikzpicture}
\node (a) [circle, draw] at (0,0) {AchN};
\node (b) [circle, draw] at (1,0) {\(\text{NH}_2\)};
\node (c) [circle, draw] at (-0.5,0.5) {SH};
\node (d) [circle, draw] at (1,0.5) {O};
\draw (a) -- (b);
\draw (a) -- (c);
\draw (a) -- (d);
\end{tikzpicture}
\end{center}

\(N\)-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 consumption of starting material (R\textsubscript{f} 0.3) and formation of a product (R\textsubscript{f} 0). The reaction mixture was concentrated \textit{in vacuo} at 60 \degree 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 \degree C (ethanol) [Lit. 148-150 \degree C (ethanol)]\textsuperscript{15}; \([\alpha]_D^{25}\) -44.0 (c, 1 in H\textsubscript{2}O) [Lit. \([\alpha]_D^{25}\) -12.28 (c, 5 in H\textsubscript{2}O)]\textsuperscript{15}; \(\delta\) (400 MHz, CDCl\textsubscript{3}) 2.03 (3H, s, HNC(O)CH\textsubscript{3}), 2.80 (1H, dd, \textit{J}_{\text{CH,H}} 13.9 Hz, \textit{J}_{\text{CH,\alpha H}} 7.3 Hz, CH\textsubscript{\alpha H}), 2.92 (1H, dd, \textit{J}_{\text{CH,H}} 13.8 Hz, \textit{J}_{\text{CH,\alpha H}} 9.2 Hz, CH\textsubscript{\alpha H}), 4.72 (1H, dd, J 4.9 Hz, J 9.2 Hz, \alpha H); \(\delta\) (100.7 MHz, CDCl\textsubscript{3}) 21.5 (q, HNC(O)CH\textsubscript{3}), 25.9 (t, CH\textsubscript{\alpha H}), 55.8 (d, \alpha C), 172.4 (s, HNC(O)CH\textsubscript{3}), 174.2 (s, C(O)NH\textsubscript{2}); \textit{m/z} (ES\textsuperscript{+}) 161 (M-H\textsuperscript{+}, 100\%).

\textbf{Synthesis of \(N\)-Acetyl-L-cysteinamide} (2,3,4,6-tetra-\(O\)-acetyl-1-dithio-\(\beta\)-d-glucopyranosyl disulfide) 9

\begin{center}
\begin{tikzpicture}
\node (a) [circle, draw] at (0,0) {AcO};
\node (b) [circle, draw] at (0.5,0) {O};
\node (c) [circle, draw] at (1,0) {AcO};
\node (d) [circle, draw] at (1.5,0) {O};
\node (e) [circle, draw] at (2,0) {AcO};
\node (f) [circle, draw] at (2.5,0) {S\text{SO}_2\text{Ph}};
\node (g) [circle, draw] at (3,0) {AcO};
\node (h) [circle, draw] at (3.5,0) {AcO};
\draw (a) -- (b) -- (c) -- (d) -- (e) -- (f) -- (g) -- (h);
\end{tikzpicture}
\end{center}

\textbf{Scheme S11.}
Supporting Information

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

![Chemical Structure](image)

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-glucopyranosyl disulfide) 9 (260 mg, 61%) as a white amorphous solid; [α]_D^25 -173 (c, 1 in MeOH); ν_max (KBr disc) 3046 (br, NH NH) 1748 (s, C=O) 1638 (s, C=O) cm⁻¹; δ_H (400 MHz, CD₃OD) 1.99, 2.03, 2.07 (15H, 3 x s, 4 x C(O)CH₃, HNC(O)CH₃), 2.97 (1H, dd, J₃,4H' 13.8 Hz, J₅,6H' 9.7 Hz, CH,H'), 3.36 (1H, dd, J₈,9H 13.9 Hz, J₇,8H 4.6 Hz, CH,H'), 3.97 (1H, ddd, J₆,₇H 10.2 Hz, J₅,₆H 2.3 Hz, J₅,₆' 4.3 Hz, H-5), 4.33 (1H, dd, J₅,₆ 2.2 Hz, J₆,₆' 12.5 Hz, H-6), 4.38 (1H, dd, J₅,₆ 4.4 Hz, J₆,₆' 12.5 Hz, H-6'), 4.75-4.78 (1H, m, αH), 4.79 (1H, d, J₁,₂ 9.5 Hz, H-1), 5.07 (1H, at, J₉,₉ Hz, H-4), 5.27 (1H, at, J₉,₉ Hz, H-2), 5.34 (1H, at, J₉,₉ Hz, H-3); δ_C (100.7 MHz, CD₃OD) 19.5, 19.6, 19.7, 19.8 (4 x q, 4 x C(O)CH₃), 21.6 (q, HNC(O)CH₃), 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 C(O)CH₃, HNC(O)CH₃), 174.1 (s, CONH₂); m/z (ES⁺) 547 (MNa⁺, 100%) 583 (MMeCNNH₄⁺, 70%); HRMS (ES⁺) Calcd. for C₁₉H₂₈N₂O₁₁S₂ (MNa⁺) 547.1027. Found: 547.1027; Found: C, 44.85%; H, 5.73%, N, 5.34%. C₁₉H₂₈N₂O₁₁S₂ 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-galactopyranosyl disulfide) methyl ester 11

**Scheme S12.**

1,2,3,4,6-Penta-O-acetyl-α-D-galactopyranoside\(^\text{16}\) 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 °C (ethanol) \([\text{Lit.} \ 92-94 \ °C]\)\(^\text{16}\); \([\alpha]_D^{21}\) +87.7 (c, 1.06 in CHCl\(_3\)) \([\text{Lit.} \ [\alpha]_D^{25}\] +107 (c, 1.0 in CHCl\(_3\)))\(^\text{16}\); \(\delta_H\) (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\(^\text{17}\) 46

1,2,3,4,6-Penta-O-acetyl-α-D-galactopyranoside 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 (Rf 0.3) with complete consumption of starting material (Rf 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 (MgSO4), 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 °C (diethyl ether/petrol) [Lit. 84-85 °C]17; [α]21D +212 (c, 1 in CHCl3); δH (400 MHz, CDCl3) 2.02, 2.06, 2.12, 2.16 (12H, 4 x s, 4 x C(O)CH3), 4.09-4.21 (2H, m, H-6, H-6'), 4.49 (1H, at, J6,6' 6.6 Hz, H-5), 5.05 (1H, dd, J1,2 3.9 Hz, J2,3 10.6 Hz, H-2), 5.41 (1H, dd, J2,3 10.6 Hz, J3,4 3.3 Hz, H-3), 5.53 (1H, br d, J3,3 Hz, H-4), 6.76 (1H, d, J1,2 3.9 Hz, H-1).

2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl phenylthiosulfonate2 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 °C. After a 5 h period, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a major product (Rf 0.4) with complete consumption of the starting material (Rf 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 (MgSO4), 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 °C [Lit. 53-54 °C]2; [α]25D +21.2 (c, 1 in CHCl3) [Lit. [α]27D +24.2 (c, 1 in CHCl3)]; δH (400 MHz, CDCl3) 1.98, 2.03, 2.06, 2.11 (12H, 4 x s, 4 x C(O)CH3), 3.85 (1H, dd, J5,6 8.8 Hz, J6,6' 13.6 Hz, H-6), 3.93-3.99 (2H, m, H-5, H-6'), 5.11 (1H, dd, J2,3 9.6 Hz, J2,3 9.6 Hz,
\[ J_{3,4} 3.3 \text{ Hz, H-3}, \ 5.22 \ (1\text{H, at, } J 9.9 \text{ Hz, H-2}), \ 5.28 \ (1\text{H, d, } J_{1,2} 9.9 \text{ Hz, H-1}), \ 5.43 \ (1\text{H, br d, } J 3.3 \text{ Hz, H-4}), \ 7.54-7.68 \ (3\text{H, m, Ar-H}), \ 7.94-7.97 \ (2\text{H, m, Ar-H}). \]

**N-Acetyl-L-cysteine (2,3,4,6-tetra-O-acetyl-1-dithio-β-D-galactopyranosyl 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 (Rf 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-galactopyranosyl disulfide) methyl ester 11 (228 mg, 63%) as a white amorphous solid; [\( \alpha \)]\(_D\)\(^{20} \) +27.3 (c, 1 in CHCl\(_3\)); \( \nu_{\text{max}} \) (KBr disc) 3373 (br, NH) 1665 (s, C=O) cm\(^{-1}\); \( \delta_H \) (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\(_3\)H\(_3\)), 3.13 (1H, dd, \( J_{\text{C,H}} \) 14.3 Hz, \( J_{\text{CH,CH}} \) 6.8 Hz, \( \text{CH,H}' \)), 3.36 (1H, dd, \( J_{\text{C,H}} \) 14.2 Hz, \( J_{\text{CH,CH}} \) 14.2 Hz, \( J_{\text{CH,CH}} \) 4.3 Hz, \( \text{CH,H}' \)), 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, dd, \( J_{1,2} \) 9.9 Hz, H-1), 4.97-5.02 (1H, m, \( \alpha \text{H} \)), 5.09 (1H, dd, \( J_{2,3} \) 10.0 Hz, \( J_{3,4} \) 3.4 Hz, H-3), 5.36 (1H, at, \( J 9.9 \text{ Hz, H-2} \)), 5.45 (1H, br d, \( J 3.3 \text{ Hz, H-4} \)), 6.39 (1H, \( J_{\text{NH,u,H}} \) 8.0 Hz, HNC(O)CH\(_3\)); \( \delta_C \) (100.7 MHz, CDCl\(_3\)) 20.5, 20.6, 20.7, 20.8 (4 x q, 4 x C(O)CH\(_3\)), 23.2 (q, HNC(O)CH\(_3\)), 42.1 (t, CH,H\(_{3}\)), 52.0 (d, cC), 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 C(O)CH\(_3\), HNC(O)CH\(_3\), CO\(_2\)CH\(_3\)); \( m/z \) (ES\(^+\)) 598 (MMeCNNH\(_4\)^+, 100%); HRMS (ES\(^+\)) Calcd. for C\(_{29}\)H\(_{39}\)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-glucopyranosyl disulfide) methyl ester 13

Acetyl chloride (40 mL, 563 mmol) was added through an air condenser into a round bottom flask containing 2-acetamido-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 (Rf 0.3) with complete consumption of the starting material (Rf 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 (MgSO₄), 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 °C (diethyl ether/DCM) [Lit. 122-123 °C]

\[ \begin{align*}
\text{ HO } & \quad \text{ AcCl} \\
\text{ AcO } & \quad \text{ AcO} \\
\text{ OH } & \quad \text{ AcO} \\
\text{ OH } & \quad \text{ AcO} \\
\text{ AcO } & \quad \text{ AcO} \\
\text{ SH } & \quad \text{ Cl}
\end{align*} \]

Scheme S13.

3,4,6-Tri-O-acetyl-2-N-acetylamido-2-deoxy-α-D-glucopyranosyl chloride 49
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-α-D-glucopyranosyl chloride 50 (4.5 g, 75%) as a white crystalline solid; m.p. 134–136 ºC (acetone/petrol) [Lit. 134–137 ºC (acetone/petrol)]: [α]D25 -24.6 (c, 1 in H2O) [Lit. [α]D25 -25.2 (c, 1 in H2O)]; δH (400 MHz, DMSO-d6) 1.80 (3H, s, HNC(O)C(CH3)3), 1.94, 1.97, 2.01 (9H, 3 x s, 3 x C(O)CH3), 4.00 (1H, at, J 9.9 Hz, H-2), 4.06 (1H, dd, J5,6 2.0 Hz, J6,6 11.4 Hz, H-6), 4.17 (1H, dd, J5,6 4.8 Hz, J6,6 11.4 Hz, H-6'), 4.22 (1H, ddd, J6,5 9.9 Hz, J5,6 2.0 Hz, J6,6 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, J1,2 10.5 Hz, H-1), 8.43 (1H, d, J 9.3 Hz, NH), 9.20 (2H, br s, NH2), 9.39 (2H, br s, NH2); δC (100.7 MHz, DMSO-d6) 21.1, 21.3, 21.4 (3 x q, 3 x C(O)CH3), 23.4 (q, HNC(O)CH3), 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=O), 170.1, 170.5, 170.8, 170.9 (4 x s, 3 x C(O)CH3, HNC(O)CH3).

1-Thio-3,4,6-Tri-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranose 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 (MgSO4), 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 °C (ethyl acetate/petrol) [Lit. 165-187 °C (ethyl acetate/petrol)\(^3\); \([\alpha]_D^{20} +25.3\) (c, 1 in CHCl\(_3\)) [Lit. \([\alpha]_D^{25} +24.8\) (c, 1 in CHCl\(_3\))\(^3\); \(\delta_H\) (400 MHz, CDCl\(_3\)) 1.99, 2.03, 2.05, 2.10 (12H, s, 3 x C(O)CH\(_3\), HNC(O)CH\(_3\)), 2.57 (1H, d, J\(_{1,3} 9.4\) 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, HNC(O)CH\(_3\)); \(\delta_C\) (100.7 MHz, CDCl\(_3\)) 20.6, 20.7, 20.8 (3 x q, 3 x C(O)CH\(_3\)), 23.3 (q, HNC(O)CH\(_3\)), 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 C(O)CH\(_3\), HNC(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 °C [Lit. 177-179 °C\(^3\); \([\alpha]_D^{20} +141.3\) (c, 1 in CHCl\(_3\)) [Lit. \([\alpha]_D^{25} +134.0\) (c, 1 in CHCl\(_3\))\(^3\); \(\delta_H\) (400 MHz, CDCl\(_3\)) 1.89 (3H, s, HNC(O)CH\(_3\)), 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, HNC(O)CH\(_3\)), 7.26-7.29 (3H, m, Ar-H), 7.69-7.71 (2H, m, Ar-H); \(\delta_C\) (100.7 MHz, CDCl\(_3\)) 20.6, 20.7 (2 x q, 3 x C(O)CH\(_3\)), 23.3 (q,
N-Acetyl-L-cysteine (3,4,6-tetra-O-acetyl-2-acetamido-2-deoxy-1-dithio-β-D-glucopyranosyl 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<sub>t</sub> 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-glucopyranosyl disulfide) methyl ester 13 (126 mg, 74%) as a white amorphous solid; [α]<sub>D</sub> <sup>21</sup> -28.9 (c 1 in CHCl<sub>3</sub>); <i>υ</i><sub>max</sub> (KBr disc) 3309 (br, NH) 1746 (s, C=O) 1659 (s, C=O) cm<sup>-1</sup>; δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>) 1.95, 2.03, 2.04, 2.06, 2.09 (15H, 5 x s, 3 x C(O)CH<sub>3</sub>, 2 x HNC(O)CH<sub>3</sub>), 3.12 (1H, dd, J<sub>CH,H</sub> 14.2 Hz, J<sub>CH,αH</sub> 6.7 Hz, CH<sub>3</sub>), 3.34 (1H, dd, J<sub>CH,αH</sub> 14.2 Hz, J<sub>CH,H</sub> 4.8 Hz, CH<sub>3</sub>), 3.79 (3H, s, OCH<sub>3</sub>), 3.82 (1H, ddd, J<sub>5,6</sub> 9.9 Hz, J<sub>5,6α</sub> 2.4 Hz, J<sub>5,6β</sub> 4.6 Hz, H-5), 4.18 (1H, at, J 9.8 Hz, H-2), 4.20 (1H, dd, J<sub>5,6</sub> 2.4 Hz, J<sub>6,6α</sub> 12.5 Hz, H-6), 4.26 (1H, dd, J<sub>5,6</sub> 4.6 Hz, J<sub>6,6</sub> 12.5 Hz, H-6), 4.77 (1H, d, J<sub>1,2</sub> 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<sub>α2α1</sub> 9.2 Hz, HNC(O)CH<sub>3</sub>, H-2), 6.46 (1H, d, J<sub>α2α1</sub> 7.9 Hz, HNC(O)CH<sub>3</sub>, αH); δ<sub>C</sub> (100.7 MHz, CDCl<sub>3</sub>) 26.0, 20.7, 20.8 (3 x q, 3 x C(O)CH<sub>3</sub>), 23.1, 23.2 (2 x q, 2 x HNC(O)CH<sub>3</sub>), 42.0 (t, CH<sub>3</sub>), 52.7 (d, αC), 52.7 (d, C-2), 52.8 (q, OCH<sub>3</sub>), 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 C(O)CH<sub>3</sub>, 2 x HNC(O)CH<sub>3</sub>, CO<sub>2</sub>CH<sub>3</sub>); <i>m/z</i> (ES<sup>+</sup>) 597 (MMeCNNH<sub>4</sub><sup>+</sup>, 100%); HRMS (ES<sup>+</sup>) Calcd. for C<sub>20</sub>H<sub>39</sub>N<sub>2</sub>O<sub>11</sub>S<sub>2</sub> (MH<sup>+</sup>) 539.1364. Found: 539.1372.
Synthesis of \( N\)-Acetyl-L-cysteine (1-dithio-\( \beta\)-d-glucopyranosyl disulfide) methyl ester 15

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 °C 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 (MgSO₄), 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-\( \beta\)-d-glucopyranoside 53 (0.2 g, 44%) as a white solid; m.p. 100-102 °C [Lit. 119-120 °C]²⁰, \([\alpha]_D^{20}\) +11.4 (c, 1.10 in CHCl₃) [Lit. \([\alpha]_D^{23}\) +10.5 (c, 0.6 in CHCl₃)]²⁰, δ\( _H \) (400 MHz, CDCl₃) 2.00, 2.02, 2.03, 2.08 (12H, 4 x s, 4 x C(O)CH₃), 2.39 (3H, s, S(O)CH₃), 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 (MMeCNH₄⁺, 100%).

Scheme S14.
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 \textit{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); \(\delta\)\(_H\) (400 MHz, CD\(_3\)OD) 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); \(\delta\)\(_C\) (100.7 MHz, CD\(_3\)OD) 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-H\(^+\), 100%).

Phenyl 1-selenenylsulfide-β-D-glucopyranoside\(^{3}\) 55

1-Thio-β-D-glucopyranoside 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 \textit{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)]; \(\delta\)\(_H\) (400 MHz, CD\(_3\)OD) 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); \(\delta\)\(_C\) (100.7 MHz, CD\(_3\)OD) 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<sub>f</sub> 0.2) along with complete consumption of the starting material (R<sub>f</sub> 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-glucopyranosyl disulfide) methyl ester 15 (75 mg, 79%) as a white amorphous solid; [α]<sub>D</sub><sup>21</sup> -12.5 (c, 1 in MeOH); <i>υ</i><sub>max</sub> (KBr disc) 3363 (br, NH OH) 1764 (s, C=O) cm<sup>-1</sup>; δ<sub>H</sub> (400 MHz, CD<sub>3</sub>OD) 2.00 (3H, s, HNC(O)CH<sub>3</sub>), 3.02 (1H, dd, <i>J</i><sub>CH,H'13.8 Hz, H</sub>-5), 3.31-3.34 (1H, m, H<sub>-5</sub>), 3.35-3.37 (2H, m, CH<sub>-4</sub>, H<sub>-4</sub>), 3.42 (1H, dd, <i>J</i> 8.0 Hz, J 10.3 Hz, H<sub>-3</sub>), 3.52 (1H, at, J 9.0 Hz, H-2), 3.71 (1H, dd, <i>J</i> 5.6 5.3 Hz, <i>J</i> 5.6· 11.9 Hz, H-6), 3.75 (3H, s, OCH<sub>3</sub>), 3.89 (1H, dd, <i>J</i> 5.6· 1.9 Hz, <i>J</i> 5.6· 11.9 Hz, H-6'), 4.36 (1H, d, <i>J</i><sub>1,2</sub> 9.3 Hz, H-1), 4.90-4.93 (1H, m, αH); δ<sub>C</sub> (100.7 MHz, CD<sub>3</sub>OD) 21.4 (q, HNC(O)CH<sub>3</sub>), 40.6 (t, CH<sub>-4</sub>, H'), 51.9 (d, αC), 52.4 (q, OCH<sub>3</sub>), 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, HNC(O)CH<sub>3</sub>, CO<sub>2</sub>CH<sub>3</sub>); m/z (ES<sup>+</sup>) 394 (MNa<sup>+</sup>, 90%), 430 (MMeCNNH<sub>4</sub>+, 100%); HRMS (ES<sup>+</sup>) Calcd. for C<sub>12</sub>H<sub>21</sub>NNaO<sub>8</sub>S<sub>2</sub> (MNa<sup>+</sup>) 394.0601. Found: 394.0601.
Synthesis of \( N\)-Acetyl-L-cysteine (2-acetamido-2-deoxy-1-dithio-\( \beta \)-d-glucopyranosyl disulfide) methyl ester 17

\[
\begin{align*}
\text{AcO} & \quad \text{AcO} \\
\text{SH} & \quad \text{OH} \\
\text{SH} & \quad \text{OH} \\
\text{AcHN} & \quad \text{AcHN} \\
\end{align*}
\]

87% 66% 94%

Scheme S15.

\( 1 \)-Thio-2-acetamido-2-deoxy-\( \beta \)-d-glucopyranose\(^3 \) 56

1-thio-3,4,6-Tri-O-acetyl-2-acetamido-2-deoxy-\( \beta \)-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. Recrystalisation from methanol/ethyl acetate yielded 1-thio-2-acetamido-2-deoxy-\( \beta \)-d-glucopyranose 56 (0.38 g, 87%) as a white crystalline solid; m.p. 174-176 °C (methanol/ethyl acetate) [Lit. 177-179 °C (methanol/ethyl acetate)]; \([\alpha]_D^{21} \) -12.1 (c, 1 in MeOH) [Lit. \([\alpha]_D^{22} \) -10.4 (c, 1 in MeOH)]; \( \delta_H \) (400 MHz, CD\(_3\)OD) 2.00 (3H, s, HNC(O)CH\(_3\)), 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, \( J_{\text{NH},H-2} \) 8.8 Hz, HNC(O)CH\(_3\)); \( \delta_C \) (100.7 MHz, CD\(_3\)OD) 21.9 (q, HNC(O)CH\(_3\)), 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, HNC(O)CH\(_3\)); \( m/z \) (ES\(^-\)) 236 (M-H\(^+\), 100%).
Phenyl 2-acetamido-2 deoxy-1-selenenylsulfide-β-D-glucopyranoside\(^3\) \(^5\)

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 a white amorphous solid; [\(\alpha\)]\(_D\)\(^{21}\) -171.3 (c, 1 in MeOH) \[^3\] (Lit. [\(\alpha\)]\(_D\)\(^{22}\) -174.0 (c, 1 in MeOH) \[^3\]); \(\delta\)\(_H\) (400 MHz, CD\(_3\)OD) 1.96 (3H, s, H\(_N\)C(O)\(\text{CH}_3\)), 3.31-3.34 (1H, m, H-5), 3.36 (1H, dd, \(J_{5,6} 5.3 \text{ Hz}, J_{6,6}' 11.9 \text{ Hz}, \text{ H-6}\)), 3.84 (1H, dd, \(J_{5,6} 2.0 \text{ Hz}, J_{6,6}' 11.9 \text{ Hz}, \text{ H-6}'\)), 3.87 (1H, at, \(J 10.0 \text{ Hz}, \text{ H-2}\)), 4.64 (1H, d, \(J_{1,2} 10.3 \text{ Hz}, \text{ H-1}\)), 7.27-7.34 (3H, m, Ar-H), 7.71-7.74 (2H, m, Ar-H); \(\delta\)\(_C\) (100.7 MHz, CD\(_3\)OD) 21.9 (q, H\(_N\)C(O)\(\text{CH}_3\)), 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, H\(_N\)C(O)\(\text{CH}_3\)).

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; [α]D25 -74.1 (c, 1 in MeOH); vmax (KBr disc) 3363 (br, NH 3OH) 1737 (s, C=O) 1654 (s, C=O) cm⁻¹; δH (500 MHz, CD3OD) 1.99, 2.00 (6H, 2 x s, 2 x HNC(O)CH3), 3.07 (1H, dd, JCH,H 13.9 Hz, JCH,H' 8.7 Hz, CH,H'), 3.30-3.39 (3H, m, H-3, H-4, CH,H'), 3.50-3.54 (1H, m, H-5), 3.72-3.78 (2H, m, H-6, H-6'), 3.79 (3H, s, OCH3), 3.92 (1H, at, J 10.3 Hz, H-2), 4.57 (1H, d, J1,2 10.3 Hz, H-1), 4.73-4.77 (1H, m, αH), 7.32 (1H, d, JH,H-2 7.5 Hz, HNC(O)CH3,H-2), 7.74 (1H, d, JH,H-4 7.0 Hz, HNC(O)CH3,αH); δC (125.8 MHz, CD3OD) 22.4, 22.9 (2 x q, 2 x HNC(O)CH3), 41.9 (t, CH,H'), 52.9 (q, OCH3), 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 HNC(O)CH3, CO₂CH3); m/z (ES⁺) 471 (MMeCNNH₄⁺, 100%); HRMS (ES⁺) Calcd. for C14H24N2O₈S₂ (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 (MgSO4), filtered and concentrated in vacuo.

General procedure 2 (GP 2): a ~0.1M solution of the Fmoc building block in DCM (~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 (~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 (MgSO₄), filtered and concentrated in vacuo.

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

![Chemical diagram](image)

**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) Ac₂O, pyridine (yield 62: 84%). (v) TFA/DMC (5/95 v/v), iPr₅SiH (yield 58: 73%).

**N-fluorenyl methoxycarbonyl-<i>O</i>-<i>tert</i>-butyl-L-serine-glycine ethyl ester 59**

Fmoc-Ser(tBu)-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(tBu)-Gly-OEt 59 as a white solid; m.p. 119 °C; Yield: 99%; Rᵢ 0.6 (diethyl ether); [α]<sub>d</sub><sup>18</sup> +23.2 (c, 1 in CHCl₃); ν<sub>max</sub> (KBr disc) 3300 (br, NH) 1726 (s, C=O) cm⁻¹; δ<sub>υ</sub> (400 MHz, CDCl₃) 11.5 (9H, s, C(CH₃)₃), 1.21 (3H, dd, J 7.1 Hz, partially obscured by following resonance, OCH₂CH₃), 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<sub>Fmoc</sub>, OCH₂CH₃), 4.32 (2H, d, J 6.8 Hz, CH₂<sub>Fmoc</sub>), 5.72 (1H, d, J 5.2 Hz, NH), 7.18-7.36 (5H, m, 4xH<sub>Fmoc</sub>, NH<sub>amide</sub>), 7.55 (2H, d, J 7.4 Hz, H<sub>Fmoc</sub>), 7.70 (1H, d, J 7.5 Hz, NH<sub>Fmoc</sub>); δ<sub>c</sub> (100.7 MHz, CDCl₃) 13.5 (q, OCH₂CH₃), 26.7 (q, C(CH₃)₃), 40.9 (t, αCgly), 46.5 (d, CH<sub>Fmoc</sub>), 54.1 (d, αCser), 60.7 (t, CH,H'ser), 61.4 (t, OCH₂CH₃), 66.5 (s, C(CH₃)₃), 73.4 (t, CH₂<sub>Fmoc</sub>), 119.4, 124.6, 126.5, 127.1 (4 x d, 4 x C<sub>Fmoc</sub>), 140.6, 143.2, 143.3 (3 x s, 3 x C<sub>Fmoc</sub>), 155.2 (s, CO<sub>Fmoc</sub>), 169.2,
171.2 (2 x s, 2 x CO); \textit{m/z} (ES\textsuperscript{+}) 527 (MMeCNNH\textsubscript{4}\textsuperscript{+}, 100%); HRMS (ES\textsuperscript{+}) Calcd. for C\textsubscript{26}H\textsubscript{32}N\textsubscript{2}NaO\textsubscript{6} (MNa\textsuperscript{+}) 491.2153. Found: 491.2136.

\textit{N-}fluorenyl methoxycarbonyl-\textit{S-trityl-L-cysteine-O-tert-buty-l-L-serine-glycine ethyl ester} 60

Fmoc-Ser(tBu)-Gly-OEt \textit{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 \textit{60} as a colourless foam; Yield: 89%; \textit{R}_f 0.6 (diethyl ether); \textit{[\alpha]}\textsubscript{D}\textsuperscript{18} +2.0 (c, 0.5 in CHCl\textsubscript{3}; \textit{\nu}\textsubscript{max} (thin film) 3370 (br, NH) 1750 (s, C=O) 1650 (s, C=O) cm\textsuperscript{-1}; \textit{\delta}_H (400 MHz, CDCl\textsubscript{3}) 1.09 (9H, s, C(CH\textsubscript{3})\textsubscript{3}), 1.16 (3H, at, J 7.1 Hz, OCH\textsubscript{2}CH\textsubscript{3}), 2.64 (2H, d, J 6.8 Hz, CH,H\textsubscript{cys}), 3.25 (1H, dd, J 6.2 Hz, J 8.8 Hz CH,H\textsubscript{ser}), 3.51 (1H, dd, J 3.3 Hz, J 8.8 Hz, CH,H\textsubscript{ser}), 3.80 (1H, dd, J 3.3 Hz, J 8.8 Hz, CH,H\textsubscript{ser}), 3.84, 3.88 (1H, ABX system, J 5.8 Hz, \textit{\alpha}Hgly), 4.04 (2H, dd, J 6.9 Hz, J 7.2 Hz, OCH\textsubscript{2}CH\textsubscript{3}), 4.10 (1H, at, J 7.0 Hz, CH\textit{Fmoc}), 4.28 (2H, dd, J 5.4 Hz, J 7.2 Hz, CH\textsubscript{2}\textit{Fmoc}), 4.37 (1H, ddd, J 3.3 Hz, J 6.5 Hz, J 6.9 Hz, \textit{\alpha}Hser) 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 x H\textit{Fmoc}, 13 x H\textsubscript{Tr}), 7.49 (2H, d, J 7.0 Hz, H\textit{Fmoc}), 7.65 (1H, d, J 3.5 Hz, H\textsubscript{Tr}), 7.69 (1H, d, J 3.9 Hz, H\textsubscript{Tr}); \textit{\delta}_C (100.7 MHz, CDCl\textsubscript{3}) 13.8 (q, OCH\textsubscript{2}CH\textsubscript{3}), 27.0 (q, C(CH\textsubscript{3})\textsubscript{3}), 33.5 (t, CH,H\textsubscript{cys}), 40.8 (t, \textit{\alpha}Cgly), 46.7 (d, CH\textit{Fmoc}), 52.9, 53.7 (2 x d, \textit{\alpha}Cser, \textit{\alpha}Ccys), 60.8 (2 x t, OCH\textsubscript{2}CH\textsubscript{3}, CH,H\textsubscript{ser}), 66.9 (t, CH\textsubscript{2}\textit{Fmoc}), 73.6 (s, C(CH\textsubscript{3})\textsubscript{3}), 119.6, 124.7 (2 x d, 3 x C\textsubscript{Tr}), 126.6-127.8 (C\textit{Fmoc}, C\textsubscript{Tr}), 140.9, 143.3, 143.5, 143.9 (4 x s, 3 x C\textit{Fmoc}, C\textsubscript{Tr}), 155.5 (s, CO\textit{Fmoc}), 168.9, 169.6, 169.8 (3 x s, 2 x CO\textit{amide}, CO\textit{ester}); \textit{m/z} (ES\textsuperscript{+}) 872 (MMeCNNH\textsubscript{4}\textsuperscript{+}, 100%); HRMS (ES\textsuperscript{+}) Calcd. for C\textsubscript{48}H\textsubscript{51}N\textsubscript{2}NaO\textsubscript{7}S (MNa\textsuperscript{+}) 836.3340. Found: 836.3316.

\textit{S-trityl-L-cysteine-O-tert-butyl-L-serine-glycine ethyl ester} \textit{61}

Fmoc-Cys(Tr)-Ser(tBu)-Gly-OEt \textit{60} (1.00 g, 1.23 mmol) was deprotected according to GP 2; purification by flash column chromatography (DCM→DCM:methanol, 9:1) gave H-
Cys(Tr)-Ser(fBu)-Gly-OEt 61 as a yellow oil; Yield: 86%; R, 0.5 (DCM:methanol, 9:1); δH (400 MHz, CDCl₃) 1.17 (9H, s, C(CH₃)₃), 1.25 (3H, at, J 7.2 Hz, OCH₂CH₃), 2.55 (1H, at, J 8.1 Hz, CH'Hcys), 2.75 (1H, dd, J 4.6 Hz, J 12.8 Hz, CH'Hcys), 2.83 (1H, dd, J 4.2 Hz, J 8.1 Hz, C,Hcys), 3.33 (1H, at, J 7.7 Hz, CH'Hser), 3.80 (1H, dd, J 4.0 Hz, J 8.8 Hz, CH'Hser), 3.95 (2H, d, J 5.2 Hz, αHgly), 4.18 (2H, at, J 7.2 Hz, OCH₂CH₃), 4.40 (1H, dd, J 4.0 Hz, J 7.3 Hz, αHser), 7.20-7.45 (16 H, m, 15 x HTr, NHamide), 7.62 (1H, d, J 7.2 Hz, NHamide); δC (100.7 MHz, CDCl₃) 13.7 (q, OCH₂CH₃), 27.0 (q, C(CH₃)₃), 36.9 (t, CH'Hcys), 40.9 (t, αCgly), 52.4, 53.7 (2 x d, αCser, αCcys), 60.8, 66.4 (2 x t, OCH₂CH₃, CH'Hser), 73.5 (s, C(C(CH₃)₃), 126.7, 127.6, 129.1 (3 x d, 3 x C̃Tr), 144.1 (s, C̃Tr), 160.0, 170.0, 173.0 (3 x s, 2 x COamide, COester); m/z (ES⁺) 650 (MMeCNNH₄⁺, 100%); HRMS (ES⁺) Calcd. for C₃₅H₄₅N₅NaO₆S (MNa⁺) 656.2659. Found: 614.2647.

N-acetyl-S-trityl-L-cysteine-O-tert-butyl-L-serine-glycine ethyl ester 62

H-Cys(Tr)-Ser(fBu)-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(fBu)-Gly-OEt 62 as a white solid; m.p. 191 °C; Yield: 84%; R, 0.5 (6% methanol in DCM); [α]D²⁰ +14.4 (c, 0.25 in CHCl₃); νmax (KBr disc) 3260 (br, NH) 1757 (s, C=O) 1640 (s, C=O) cm⁻¹; alternatively, Fmoc-Cys(Tr)-Ser(fBu)-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; δH (400 MHz, CDCl₃) 1.18 (9H, s, C(CH₃)₃), 1.25 (3H, at, J 7.2 Hz, OCH₂CH₃), 1.90 (3H, s, C(O)CH₃), 2.65 (1H, at, J 6.6 Hz, CH'Hcys), 2.75 (1H, dd, J 5.9 Hz, J 6.6 Hz, CH'Hcys), 3.35 (1H, dd, J 6.0 Hz, J 8.8 Hz, CH'Hser), 3.75 (1H, dd, J 5.4 Hz, J 8.0 Hz, αHgly), 3.88 (3H, m, αHgly, CH'Hser, αHcys), 4.18 (2H, dd, J 7.0 Hz, J 14.0 Hz, OCH₂CH₃), 4.40 (1H, dd, J 3.3 Hz, J 7.3 Hz, αHser), 6.05 (1H, d, J 7.1 Hz, NHamide), 6.70 (1H, d, J 7.4 Hz, NHamide), 7.20-7.45 (16H, m, 15 x HTr, NHamide); δH (100.7 MHz, CDCl₃) 13.9 (q, OCH₂CH₃), 22.7 (q, C(O)CH₃), 27.1 (q, C(C(CH₃)₃), 33.3 (t, CH'Hcys), 40.9 (t, αCgly), 52.1, 53.1 (2 x d, αCser, αCcys), 60.7, 60.9 (2 x t, OCH₂CH₃, CH'Hser), 73.7 (s, C(C(CH₃)₃), 126.7, 127.9, 129.2 (3 x d, 3 x C̃Tr), 144.0 (s, C̃Tr), 160.0, 169.8, 169.9 (4 x CO); m/z (ES⁺) 692 (MMeCNNH₄⁺, 100%); HRMS (ES⁺) Calcd. for C₃₅H₄₅N₅NaO₆S (MNa⁺) 656.2765. Found: 656.2766.
Synthesis of Ac-Cys-Gly-Thr(tBu)-Gly-OEt 63

\[
\text{Fmoc-Thr(tBu)-OH} + \text{HCl H-Gly-OEt} \rightarrow \text{Fmoc-Thr(tBu)-Gly-OEt}
\]

\[
\text{Fmoc-Thr(tBu)-OH} + \text{HCl H-Gly-OEt} \rightarrow \text{Fmoc-Thr(tBu)-Gly-OEt}
\]

Scheme S17. Reagents and Conditions: (i) HBTU, HOBr, DiPEA, DCM, DMF (yield 64: 89%). (ii) (a) DBU, DCM; (b) HOBr; (c) Fmoc-Gly-OH, HBTU, HOBr, DiPEA, DCM, DMF (yield 65: 85%); (iii) DBU, DCM (yield 66: 82%); (iv) Fmoc-Cys(Tr)-OH, HBTU, HOBr, DiPEA, DCM, DMF (yield: 85%); (v) DBU, DCM (yield 67: 70% from 65); (vi) Ac₂O, pyridine (yield 69: 82%); (vii) TFA/DCM (5/95 v/v), iPr₂SiH (yield 63: 38%).

\[\text{N-fluorenyl methoxycarbonyl-L-threonine-glycine ethyl ester 64}\]

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 °C; Yield: 89%; R₉ 0.8 (diethyl ether); [α]D²⁺ +19.2 (c, 1 in CHCl₃); νmax (KBr disc) 3310 (br, NH) 1742 (s, C=O) 1670 (s, C=O) cm⁻¹; δH (400 MHz, CDCl₃) 1.10 (3H, d, J 6.4 Hz, CH₃Thr), 1.30 (12H, m, OCH₂CH₃, C(CH₃)₃), 4.07 (2H, ABX system, δHgly), 4.18-4.26 (5H, m, δHthr, CHthhr, H₁⁵Fmoc, OCH₂CH₃), 4.40 (2H, d, J 7.5 Hz, CH₂₁⁵Fmoc), 6.03 (1H, d, J 5.0 Hz, NH), 7.31 (2H, at, J 7.4 Hz, H₁⁵Fmoc), 7.40 (2H, at, J 7.5 Hz, H₁⁵Fmoc), 7.61 (2H, d, J 7.6 Hz, H₁⁵Fmoc), 7.64 (1H, m, NH), 7.75 (2H, d, J 7.5 Hz, H₁⁵Fmoc); δC (100.7 MHz, CDCl₃) 13.9 (q, OCH₂CH₃), 16.6 (q, CH₃thr), 27.9 (q, C(CH₃)₃), 41.3 (t, Cgly), 46.9 (d, CH₁⁵Fmoc), 58.3 (d, Cthhr), 61.1 (t, OCH₂CH₃), 66.4 (d, Cthhr), 66.6 (t, CH₂₁⁵Fmoc), 75.3 (q, C(CH₃)₃), 119.7, 124.9, 126.8, 127.4 (4 x d, 4 x C₁⁵Fmoc), 141.0 (s, 2 x C₁⁵Fmoc), 143.4, 143.7, (2 x s, 2 x C₁⁵Fmoc), 155.7 (s, CO₁⁵Fmoc), 169.1,
169.3 (2 x d, 2 x CO); m/z (ES\textsuperscript{+}) 505 (MNa\textsuperscript{+}, 100%); HRMS (ES\textsuperscript{+}) Calcd. for C\textsubscript{27}H\textsubscript{34}N\textsubscript{2}O\textsubscript{6} (MNa\textsuperscript{+}) 505.2309. Found: 505.2304.

\textit{N}-fluorenyl methoxycarbonyl-glycine-\textit{O-}tert-butyl-L-threonine-glycine ethyl ester 65

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\text{Fmoc-Thr(tBu)-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(tBu)-Gly-OEt 65 as a foam; Yield: 85%; R\textsubscript{f} 0.4 (8% methanol in DCM); [\alpha]_D^{18} +23.6 (c, 0.5 in CHCl\textsubscript{3}) 1726 (s, C=O) 1656 (s, C=O) cm\textsuperscript{-1}; \delta_H (400 MHz, CDCl\textsubscript{3}) 1.05 (3H, d, J = 6.3 Hz, CH\textsubscript{3} thr), 1.25 (3H, t, J = 7.2 Hz, OCH\textsubscript{2}CH\textsubscript{3}), 1.28 (9H, s, C(CH\textsubscript{3})\textsubscript{3}), 4.05-4.11 (4H, ABX system, \alpha Hgly), 4.16-4.22 (4H, m, CH\textsuperscript{Fmoc} Fmoc, CHthr, OC\textsubscript{H}\textsubscript{2}CH\textsubscript{3}), 4.38 (2H, d, J = 7.0 Hz, CH\textsubscript{2} Fmoc), 4.44 (1H, at, J = 4.6 Hz, \alpha Hser), 5.80 (1H, m, NH), 7.23 (1H, d, J = 5.6 Hz, NH\textsuperscript{amide}), 7.30 (2H, m, H\textsuperscript{Fmoc}), 7.37 (2H, at, J = 7.4 Hz, H\textsuperscript{Fmoc}), 7.58 (2H, d, J = 7.4 Hz, H\textsuperscript{Fmoc}), 7.68 (1H, at, J = 5.0 Hz, NH\textsuperscript{amide}), 7.74 (2H, d, J = 7.5 Hz, NH\textsuperscript{Fmoc}); \delta_C (100.7 MHz, CDCl\textsubscript{3}) 14.0 (q, OCH\textsubscript{2}CH\textsubscript{3}), 17.1 (q, CH\textsubscript{3}thr), 28.0 (q, C(CH\textsubscript{3})\textsubscript{3}), 41.4, 44.2 (2 x t, 2 x \alpha Cgly), 46.9 (d, CH\textsuperscript{Fmoc}), 57.4 (d, \alpha Cthr), 61.4 (t, OCH\textsubscript{2}CH\textsubscript{3}), 66.0, 67.1 (2 x t, CH\textsubscript{2} Fmoc, CHthr), 75.5 (s, C(CH\textsubscript{3})\textsubscript{3}), 119.8, 125.0, 126.9, 127.6 (4 X d, C\textsuperscript{Fmoc}), 141.1 (s, 2 x C\textsuperscript{Fmoc}), 143.7 (s, 2 x C\textsuperscript{Fmoc}), 156.5 (s, CO\textsuperscript{Fmoc}), 168.8, 169.3, 169.5 (3 x s, 3 x CO); m/z (ES\textsuperscript{+}) 598 (MMeCNNH\textsubscript{4}\textsuperscript{+}, 100%); HRMS (ES\textsuperscript{+}) Calcd. for C\textsubscript{29}H\textsubscript{37}N\textsubscript{2}NaO\textsubscript{7} (MNa\textsuperscript{+}) 562.2524. Found: 562.2525.

\textit{N}-fluorenyl methoxycarbonyl-S-trityl-L-cysteine-glycine-\textit{O-}tert-butyl-L-threonine-glycine ethyl ester 67

\[
\text{Fmoc-Gly-Thr(tBu)-Gly-OEt 65 (108 mg, 200 \mu mol) was deprotected following GP 2; (H-Gly-Thr(tBu)-Gly-OEt: R\textsubscript{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 stirring 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%; Rf 0.4 (5% methanol in DCM); [α]D18 +14.6 (c, 0.5 in CHCl₃); νmax (KBr disc) 3300 (br, NH) 1742 (s, C=O) 1632 (s, C=O) cm⁻¹; δH (400 MHz, CDCl₃) 0.91 (3H, d, J 6.2 Hz, CH₃th), 1.13 (3H, t, J 7.1 Hz, OCH₂CH₃), 1.17 (9H, s, C(CH₃)₃), 2.61 (2H, m, CH₂,H'cys), 3.83 (2H, m, αHgly), 3.88-4.00 (3H, m, 2 x αHgly, αHcys), 4.00-4.13 (4H, m, CH₃Fmoc, OCH₂CH₃, CHth), 4.30 (3H, m, αHth, CH₂Fmoc), 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, H₂Fmoc, NH), 7.65 (2H, dd, J 5.2 Hz, J 7.4 Hz, NHFmoc); δC (100.7 MHz, CDCl₃) 14.0 (q, OCH₂CH₃), 17.1 (q, CH₂th), 28.0 (q, C(CH₂)₃), 41.4, 43.0 (2 x t, 2 x αCgly), 33.6 (t, CH₂,H'cys), 47.0 (d, CH₃Fmoc), 53.8 (d, αCcys), 57.3 (d, αCth), 61.3 (t, OCH₂CH₃), 66.0, 67.1 (2 x t, CH₂Fmoc, CHth), 75.4 (s, C(CH₂)₃), 119.8, 125.0, 126.7, 126.9, 127.6, 129.5 (6 x, d, C₁Fmoc,Tr), 141.1, 143.6, 143.7, 144.3 (4 x s, C₂Fmoc,Tr), 155.9 (s, COFmoc), 168.0, 169.3, 170.5 (3 x s, 4 x CO); m/z (ES⁺) 907 (MNa⁺, 100%); HRMS (ES⁺) Calcd. for C₅₁H₅₆N₂O₇S (MNa⁺) 907.3711. Found: 907.3712.

**N-acetyl-S-trityl-L-cysteine-glycine-O-tert-butyl-L-threonine-glycine ethyl ester 69**

![Diagram](attachment:image.png)

Fmoc-Cys(Tr)-Gly-Thr(βBu)-Gly-OEt 67 was synthesised from Fmoc-Gly-Thr(βBu)-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(βBu)-Gly-OEt (68, 2.03 g, 3.06 mmol) as a foam; Yield: 70% from Fmoc-Gly-Thr(βBu)-Gly-OEt; Rf 0.5 (8% methanol in DCM); m/z (ES⁺) 721 (MMeCNNH₄⁺, 100%);

Next, H-Cys(Tr)-Gly-Thr(βBu)-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 (MgSO₄), 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%; Rf 0.5 (8% methanol in DCM); [α]D +15.0 (c, 1 in CHCl3); νmax (KBr disc) 3312 (br, NH) 1734 (s, C=O) 1656 (s, C=O) cm⁻¹; δH (400 MHz, CD3OD) 1.01 (3H, d, J 6.4 Hz, CH3thrr), 1.13-1.17 (12H, m, OCH2CH3, C(CH3)3), 1.91 (3H, s, C(O)CH3), 2.58 (1H, dd, JCH=H 13.0 Hz, JCH,αH 5.9 Hz, CH3Hcys), 2.71 (1H, dd, JCH,αH 10/1 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%; Rf 0.4 (ethyl acetate:methanol, 9:1); δH (400 MHz, CD3OD) 1.12 (3H, d, J 6.3 Hz, CH3thrr), 1.24 (9H, s, C(CH3)3), 1.30 (3H, at, J 7.1 Hz, OCH2CH3), 2.05 (3H, s, C(O)CH3), 2.83 (2H, d, J 6.1 Hz, CH3Hcys), 3.88-4.06 (4H, m, C(O)CH3), 4.17 (1H, m, Cthrr), 4.23 (2H, dd, J 7.1 Hz, J 14.1 Hz, OCH2CH3), 4.45 (1H, at, J 3.3 Hz, Cthrr), 4.58 (1H, at, J 6.1 Hz, Ccys), 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); δC (100.7 MHz, CD3OD) 13.8 (q, OCH2CH3), 17.8 (q, CH3thrr), 22.4 (q, C(O)CH3), 27.9 (q, C(CH3)3), 34.3 (t, CH3Hcys), 41.2, 42.9 (2 x t, 2 x Cgly), 54.6, 57.6 (2 x d, Cthrr, Ccys), 61.4 (t, OCH2CH3), 66.3 (t, Cthrr), 75.3 (s, C(CH3)3), 169.1-
171.3 (CO); \textit{m/z} (ES\textsuperscript{+}) 521 (MMeCNNH_{4}\textsuperscript{+}, 100\%); HRMS (ES\textsuperscript{+}) Calcd. for C_{19}H_{34}N_{4}NaO_{7}S (MNa\textsuperscript{+}) 485.2040. Found: 485.2037.

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

\[
\begin{align*}
\text{AcO} & \quad \text{AcO} & \quad \text{AcO} & \quad \text{SSO}_{2}\text{Ph} \\
\text{SH} & \quad \text{H} & \quad \text{N} & \quad \text{O} & \quad \text{N} & \quad \text{O} & \quad \text{Et} \\
\text{AcHN} & \quad \text{O} & \quad \text{Ac} & \quad \text{O} & \quad \text{O} & \quad \text{S} & \quad \text{S} & \quad \text{O} \\
\text{O} & \quad \text{Ac} & \quad \text{O} & \quad \text{O} & \quad \text{Ac} & \quad \text{O} & \quad \text{Ac} & \quad \text{O} \\
\end{align*}
\]

\textbf{Scheme S18.}

\textit{N}-Acetyl-L-cysteine-(2,3,4,6-tetra-O-acetyl-1-dithio-\textit{β}-D-glucopyranosyl disulfide)-O-\textit{tert}-butyl-L-serine-glycine ethyl ester 19

Ac-Cys(Tr)-Ser(\textit{tBu})-Gly-OEt \textit{58} (250 mg, 394 \textmu mol) was dissolved in DCM (10 mL) and treated with \textit{iPr}_{3}\text{SiH} (513 \textmu mol, 105 \textmu L) and trifluoroacetic acid (0.5 mL). After 1 h the reaction mixture was co-evaporated with dry toluene (2\times25 mL) and a solution of this tripeptide and triethylamine (394 \textmu mol, 55 \textmu L) 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-\textit{β}-D-glucopyranosyl phenyl thiosulfonate \textit{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 (\textit{R}_{f} 0.4). The reaction mixture was concentrated \textit{in vacuo} and the resulting residue purified by flash column chromatography (DCM:methanol, 9:1) yielding \textit{19} (137 mg, 46\% over 2 steps) as an oil; [\textit{α}]_{D}^{18} -159.2 (c, 0.5 in CHCl_{3}); \textit{υ}_{\text{max}} (thin film) 3300 (br, NH) 1750 (s, C=O) 1643 (s, C=O) cm\textsuperscript{-1}; \textit{δ}_{H} (400 \text{MHz, CDCl}_{3}) 1.16 (9H, s, C(CH_{3})_{3}), 1.23 (3H, at,
J 7.2 Hz, OCH₃CH₃), 1.96-2.02 (15H, m, 4 x C(O)CH₃, HNC(O)CH₃), 3.01 (1H, dd, J₆₃H₆ = 14.1 Hz, J₆₃H₅ = 8.5 Hz, CH₃H'cys), 3.22 (1H, dd, J₆₃H₆ = 14.2 Hz, J₆₃H₅ = 5.2 Hz, CH₃H'cys), 3.40, 3.73 (2H, CH₃H'ser), 3.79-3.83 (1H, ddd, J₆₃H₆ = 2.2 Hz, J₆₃H₅ = 4.1 Hz, J₆₃H₄ = 6.2 Hz, H-5), 3.93-4.05 (2H, ABX system, 2 x Hgly), 4.13-4.19 (3H, m, OCH₂CH₃, H-6), 4.27 (1H, dd, J₆₃H₆ = 2.0 Hz, J₆₃H₅ = 12.5 Hz, H-6'), 4.44 (1H, m, Hser), 4.58 (1H, d, J₆₃H₆ = 9.4 Hz, H-1), 4.85 (1H, m, Hcys), 5.09 (1H, at, J₆₃H₆ = 9.5 Hz, H-4), 5.21 (2H, m, H₂-2, H-3), 7.05 (1H, d, J₆₃H₆ = 7.5 Hz, NH), 7.35 (1H, m, NH), 7.46 (1H, d, J₆₃H₆ = 7.1 Hz, NH); δC (100.7 MHz, CDCl₃) 14.0 (q, OCH₂CH₃), 20.3, 20.4, 20.5, 20.6 (4 x q, 4 x C(O)CH₃), 22.8 (q, HNC(O)CH₃), 27.2 (q, C(CH₃)₃), 41.3 (2 x t, Hgly, CH₃H'Cys), 52.4, 53.3 (2 x d, Cser, Ccys), 61.0, 61.2, 61.3 (3 x t, OCH₂CH₃, C-6, CH₃H'ser), 67.8 (d, C-4), 69.0, 73.4 (C-2, C-3) 76.0 (d, C-5), 77.2 (s, C(CH₃)₃), 88.3 (d, C-1), 169.1-170.8 (8 x s, 8 x CO); m/z (ES⁺) 812 (MMeCNNH₄⁺, 100%); HRMS (ES⁺) Calcd. for C₃₀H₄₇N₃NaO₁₅S₂ (MNa⁺) 776.2341. Found: 776.2345.

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

A solution of Ac-Cys-Gly-Thr(tBu)-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, 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; \([\alpha]_D^{18} -58.0 \text{ (c, 1 in CHCl}_3\); \(\nu_{\text{max}}\) (thin film) 3321 (br, NH) 1749 (s, C=O) 1654 (s, C=O) cm\(^{-1}\); \(\delta_H\) (500 MHz, CDCl\(_3\)) 1.05 (3H, d, J 6.3 Hz, CH\(_3\)thr), 1.27 (12H, m, OCH\(_2\)CH\(_3\), 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)CH\(_3\)), 3.04 (1H, dd, J\(_{\text{CH,H}}\) 14.2 Hz, J\(_{\text{CH,H,cys}}\) 8.2 Hz, CH\(_{\text{H',cys}}\)), 3.26 (1H, dd, J\(_{\text{CH,H',cys}}\) 14.2 Hz, J\(_{\text{CH,H,cys}}\) 5.4 Hz, CH\(_{\text{H',cys}}\)), 3.85-4.23 (10H, m, H-5, H-6, OCH\(_2\)CH\(_3\), 2 x \(\alpha\)Hgly, CH,H\(_{\text{H',thr}}\)), 4.40 (1H, dd, J 3.6 Hz, J 6.2 Hz, \(\alpha\)Hthr), 4.62 (1H, d, J\(_{1,2}\) 10.1 Hz, H-1), 4.90 (1H, dd, J 2.5 Hz, J 7.6 Hz, \(\alpha\)Hcys), 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); \(\delta_C\) (125.7 MHz, CDCl\(_3\)) 14.2 (q, OCH\(_2\)CH\(_3\)), 17.3 (q, CH\(_3\)thr), 20.5, 20.6, 20.7, 20.8 (4 x q, 4 x C(O)CH\(_3\)), 23.1 (q, HNC(O)CH\(_3\)), 28.1 (q, C(CH\(_3\))\(_3\)), 41.5, 41.7, 43.3 (3 x t, 2 x \(\alpha\)Cgly, CH,H\(_{\text{H',cys}}\)), 52.5 (d, \(\alpha\)Ccys), 57.5 (d, \(\alpha\)Cthr), 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(CH\(_3\))\(_3\)), 75.6 (t, OCH\(_2\)CH\(_3\)), 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\(_4\)NaO\(_{16}\)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

\[
\text{Scheme S20.}
\]

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\(_3\)); \(\nu_{max}\) (thin film) no significant peaks; \(\delta\) \(_H\) (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\(_2\)Ph), 7.13-7.46 (25H, m, Ar-H); \(\delta\) \(_C\) (128.7 MHz, CDCl\(_3\)) 69.0 (t, C-6), 73.6, 75.0, 75.4, 75.7 (4 x t, 4 x OCH\(_2\)Ph), 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

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-β-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\textsuperscript{17} 72: \([\alpha]_0^{20} +124\) (c, 1 in CHCl\textsubscript{3}) [Lit. \([\alpha]_0^{25} +128.2\) (c, 1 in CHCl\textsubscript{3})\textsuperscript{17}; \(\delta_H\) (500 MHz, CDCl\textsubscript{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\textsubscript{2}Ph), 5.66 (1H, d, \(J_{1,2} 4.8\) Hz, H-1), 7.17-7.45 (25H, m, Ar-H); \(m/z\) (ES\textsuperscript{+}) 650 (MNH\textsubscript{4}+, 80%) 655 (MNa+, 100%).

2,3,4,6-Tetra-O-benzyl-1-thio-β-D-glucopyranose\textsuperscript{21} 73: m.p. 48-50 °C (ether/petrol) [Lit. 47-50 °C\textsuperscript{22}; \([\alpha]_0^{22} +42.5\) (c, 0.75 in CHCl\textsubscript{3})]; \(\delta_H\) (500 MHz, CDCl\textsubscript{3}) 1.95 (1H, d, \(J_{1,SH} 4.8\) Hz, SH(α), 2.37 (1H, d, \(J_{1,SH} 8.1\) Hz, SH(β)), 3.43 (1H, at, J 9.0 Hz, H-2(β)), 3.50-3.58 (1H, m, H-5(β)), 3.67-3.73 (2H, m, H-3(α), H-3(β)), 3.78-3.88 (6H, m, H-4(α), H-6(α), H-6'(α), H-4(β), H-6(β), H-6'(β)), 3.92 (1H, dd, \(J_{1,2} 4.9\) Hz, \(J_{2,3} 9.0\) Hz, H-2(α)), 4.25-4.28 (1H, m, H-5(α)), 4.56 (1H, dd, \(J_{1,SH} 8.1\) Hz, \(J_{1,2} 9.0\) Hz, H-1(β)), 4.57-5.01 (16H, m, 4 x OCH\textsubscript{2}Ph(α), 4 x OCH\textsubscript{2}Ph(β)), 5.80 (1H, at, J 4.8 Hz, H-1(α)), 7.19-7.43 (40H, m, 20 x Ar-H(α), 20 x Ar-H(β)); \(m/z\) (ES\textsuperscript{+}) 574 (MNH\textsubscript{4}+, 100%) 579 (MNa+, 80%).
Crossover experiment

Preparation of \(N(\text{tert-Butoxycarbonyl})-\text{L-cysteine methyl ester}\)^{23} 74

\[
\begin{align*}
\text{H}_2\text{N}-\text{CO}_2\text{H} & \quad \text{1. HCl/MeOH} \\
\text{SH} & \quad \text{2. Boc}_2\text{O, Et}_3\text{N, CH}_2\text{Cl}_2 \\
& \quad \text{3. PBu}_3, \text{MeOH, H}_2\text{O} \\
\text{BocHN} & \quad \text{89\% from L-Cysteine} \\
\text{SH} & \quad \text{CO}_2\text{Me}
\end{align*}
\]

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 °C and acetyl chloride (17.6 mL, 248 mmol) was added dropwise over 5 minutes. The solution was stirred an additional 10 minutes at 0 °C 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 °C. 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 (Rf 0.6) and its corresponding disulfide (Rf 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); [\(\alpha\)]\(_D^{20}\) +28.3 (c = 7.5, CHCl\(_3\)) [Lit. [\(\alpha\)]\(_D^{20}\) +28.5 (c, 7.5 in CHCl\(_3\))]; \(\delta\)\(_H\) (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, CH\(_2\)SH), 3.76 (3H, s, CO\(_2\)CH\(_3\)), 4.58 (1H, m, \(\alpha\)H), 5.44 (1H, d, J 5.8 Hz, NH); \(\delta\)\(_C\) (100.7 MHz, CDCl\(_3\)) 27.3 (t, CH\(_2\)SH), 28.2 (q, C(CH\(_3\))\(_3\)), 52.6 (q,
OCH₃), 54.8 (d, JαC), 80.2 (s, C(CH₃)₃), 155.1, 170.8 (2 x s, 2 x CO); m/z (ES⁻) 234 (M-H⁺, 100%).

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

![Scheme S22.](image)

N-(tert-butoxycarbonyl)-L-cysteine (2,3,4,6-tetra-O-acetyl-1-dithio-β-D-glucopyranosyl 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 µL, 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 (Rf 0.5) along with complete consumption of the starting material (Rf 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-glucopyranosyl disulfide) methyl ester 75 (155 mg, 66%) as a white amorphous solid; [α]D²² +105 (c, 1 in CHCl₃); νmax (KBr disc) 3416 (br, NH) 1750 (s, C=O) cm⁻¹; δH (400 MHz, CDCl₃) 1.46 (9H, s, C(CH₃)₃), 2.01, 2.03, 2.04, 2.09 (12H, 4 x s, 4 x C(O)CH₃), 3.06 (1H, dd, JCH₃,αH 13.8 Hz, JCH,CαH 7.7 Hz, CH₃H'), 3.31 (1H, dd, JCH₃H' 13.9 Hz, JCH',αH 4.7 Hz, CH₃H'), 3.77 (3H, s, OCH₃), 3.80 (1H, m, H-5), 4.16 (1H, dd, J5,6 2.1 Hz, J6,6' 12.4 Hz, H-6), 4.27 (1H, dd, J5,6' 4.6 Hz, J5,6' 12.4 Hz, H-6'), 4.57 (1H, d, J1,2 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); δC (100.7 MHz, CDCl₃) 20.6, 20.7, 20.8 (3 x q, 4 x C(O)CH₃), 28.3 (q, C(CH₃)₃), 42.6 (t, CH,H'), 52.6 (d, αC), 52.8 (q, OCH₃), 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, O(CH₃)₃), 87.8 (d, C-1), 169.2, 169.4, 170.2, 170.6 (4 x s, 4 x C(O)CH₃, CO₂CH₃); m/z (ES⁺) 615 (MNH₄⁺, 100%), 620 (MNa⁺, 95%); HRMS (ES⁺) Calcd. for C₂₃H₃₅NNaO₁₃S₂ (MNa⁺) 620.1451. Found: 6201451.

Scheme S23. Crossover experiment.

N-Acetyl-L-cysteine (2,3,4,6-tetra-O-benzyl-1-dithio-β-D-glucopyranosyl 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-glucopyranosyl disulfide) methyl ester 75 (10 mg, 17 µmol) was dissolved in degassed anhydrous methanol (0.5 mL). Hexamethylphosphorusr (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:

NMR kinetics on desulfurization reaction

N-Acetyl-L-cysteine (2,3,4,6-tetra-O-benzyl-1-dithio-β-D-glucopyranosyl 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.
References

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-\(\beta\)-D-glucopyranoside)

methylester 4
$N$-Acetyl-DL-cysteine-$S$(2,3,4-tri-$O$-benzyl-1-thio-$\alpha$-$L$-fucopyranoside) methylester 6
$N$-Acetyl-DL-cysteine-$S$-(2,3,4,6-tetra-$O$-acetyl-$\beta$-$D$-glucopyranoside)

methylester 8
**N-Acetyl-dL-cysteinamide-S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside) 10**

![Chemical structure diagram]
$N$-Acetyl-DL-cysteine-$S(2,3,4,6$-tetra-$O$-acetyl-$\beta$-$D$-galactopyranoside)

methylester 12
$N$-Acetyl-DL-cysteine-$S$(3,4,6-tetra-$O$-acetyl-2-acetamido-2-deoxy-$\beta$-$D$-glucopyranoside) methylester 14
**Supporting Information**

*N-Acetyl-DL-cysteine-S-(β-D-glucopyranoside) methylester 16*

![Chemical Structure](image)

![NMR Spectra](image)
$N$-Acetyl-DL-cysteine-S-(2-acetamido-2-deoxy-$\beta$-D-glucopyranoside)
methylester 18
$N$-Acetyl-DL-cysteine-S-(2,3,4,6-tetra-O-acetyl-$\beta$-D-glucopyranoside)-O-tert-butyl-L-serine-glycine ethylester 20
$N$-Acetyl-DL-cysteine-$S$-(2,3,4,6-tetra-$O$-acetyl-$\beta$-$D$-galactopyranoside)-glycine-

$O$-tert-butyl-$L$-serine-glycine ethylester 22
**N-Acetyl-L-cysteine (2,3,4,6-tetra-O-benzyl-1-dithio-β-D-glucopyranosyl disulfide) methylester 3**
2,3,4-Tri-O-benzyl-α-L-fucopyranosyl phenylthiosulfonate 38

![Chemical Structure](image-url)
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-glucopyranosyl disulfide) methylester 7
*N*-Acetyl-L-cysteinamide (2,3,4,6-tetra-O-acetyl-1-dithio-β-D-glucopyranosyl disulfide) 9
$N$-Acetyl-$L$-cysteine \textit{(2,3,4,6-tetra-O-acetyl-1-dithio-$\beta$-d-galactopyranosyl disulfide) methylester 11}
$N$-Acetyl-$\text{l}$-cysteine (3,4,6-tetra-$O$-acetyl-2-acetamido-2-deoxy-1-dithio-\(\beta\)-d-glucopyranosyl disulfide) methylester 13
$N$-Acetyl-$L$-cysteine (1-dithio-$\beta$-$d$-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-$L$-glycine ethyl ester 64

![N-fluorenyl methoxycarbonyl-L-threonine-glycine ethyl ester 64]
*N*-fluorenly methoxycarbonyl-glycine-\textit{O}-\textit{tert}-butyl-L-threonine-glycine ethyl ester 65
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$N$-fluorenyl methoxycarbonyl-S-trityl-L-cysteine-glycine-O-tert-butyl-L-threonine-glycine ethyl ester 67
$N$-acetyl-$S$-trityl-$L$-cysteine-$O$-tert-butyl-$L$-threonine-glycine ethyl ester
$N$-acetyl-$L$-cysteine-$L$-glycine-$O$-tert-butyl-$L$-threonine-$L$-glycine ethyl ester 63
*N*-Acetyl-L-cysteine-(2,3,4,6-tetra-O-acetyl-1-dithio-β-D-glucopyranosyl
disulfide)-O-tert-butyl-L-serine-glycine ethyl ester 19
$N$-Acetyl-$l$-cysteine-(2,3,4,6-tetra-$O$-acetyl-1-dithio-$\beta$-$D$-galactopyranosyl)-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-glucopyranosyl disulfide) methyl ester 75