SUPPORTING INFORMATION

Direct aqueous synthesis of cyanomethyl thioglycosides from reducing sugars; ready access to reagents for protein glycosylation.

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Table S1 Extent of protein modification as a percentage of total lysozyme

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<th>Sugar</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Average (mean)</th>
<th>Conversion</th>
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<td>1</td>
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<td>43</td>
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Fig. S1 HRMS of lysozyme modified with glucose reagent 3a

Fig. S2 Deconvoluted HRMS of lysozyme modified with glucose reagent 3a
Fig. S3 HRMS of lysozyme modified with galactose reagent 3b

Fig. S4 Deconvoluted HRMS of lysozyme modified with galactose reagent 3b
**Fig. S5** HRMS of lysozyme modified with mannose reagent 3c

**Fig. S6** Deconvoluted HRMS of lysozyme modified with mannose reagent 3c
**Fig. S7** HRMS of lysozyme modified with lactose reagent 3d

**Fig. S8** Deconvoluted HRMS of lysozyme modified with lactose reagent 3d
Fig. S9 HRMS of lysozyme modified with maltotriose reagent 3e

Fig. S10 Deconvoluted HRMS of lysozyme modified with maltotriose reagent 3e
General Experimental

Reagents were used as supplied without further purification unless otherwise stated. Lysozyme from hens’ egg white was purchased from Sigma-Aldrich and used as received. HPLC–grade solvents were used for reactions. Petroleum ether (Petrol) refers to the fraction of light petroleum ether boiling in the range 40–60 °C. Solvent was removed under reduced pressure using a Buchi™ rotary evaporator. Thin Layer Chromatography (t.l.c.) was carried out on Merck Silica Gel 60F254 aluminium-backed plates. Visualisation of the plates was achieved using a UV lamp (λ<sub>max</sub> = 254 or 365 nm), and/or ammonium molybdate (5% in 2 M H<sub>2</sub>SO<sub>4</sub>). Flash column chromatography was carried out using Sorbsil C60 40/60 silica. Melting points were recorded on an Electrothermal melting point apparatus and are uncorrected. Proton and carbon nuclear magnetic resonance (1H, 13C) spectra were recorded on either an Agilent 400–MR instrument operating for 1H NMR at 400 MHz, and at 100 MHz for 13C NMR, or an Agilent 600–MR instrument operating for 1H NMR at 600 MHz, and at 150 MHz for 13C NMR. All chemical shifts are quoted on the δ-scale in ppm using residual solvent as an internal standard. 1H and 13C spectra were assigned using COSY, DEPT, HSQC, and HMBC. High resolution mass spectra were recorded by Dr. Marie Squire and Dr. Amelia Albrecht on either a DIONEX Ultimate 3000 or Bruker MaXis 4G spectrometer, operated in high resolution positive ion electrospray mode. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a 1 cm<sup>3</sup> cell with a path length of 1 dm, and are quoted in units of °.cm<sup>2</sup>.g<sup>-1</sup>. Concentrations (c) are given in g/100 cm<sup>3</sup>, solvent and temperature are recorded. Infrared spectra were recorded on a Bruker FTIR spectrometer with Alpha’s Platinum ATR single reflection diamond as neat samples.

Protein Mass Spectrometry Analysis

High resolution accurate mass samples were analysed on a maXis 3G UHR-Qq-TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) coupled to a Dionex Ultimate 3000 LC system (ThermoFisher). 5 μL sample was injected. ESI-L Low Concentration Tuning Mix (Agilent Technologies) injected after each sample as a calibrant. Processed using Compass software (Bruker Daltonik GmbH, Bremen, Germany)

LC MS analysis used the following column: Agilent ZORBAX, SB-C18, 5μ, 2.1 x 150 mm; A flow rate of 200 μL/min and the following solvent profile of acetonitrile (Scharlau, Acetonitrile, Multisolvent<sup>®</sup>, HPLC grade) with water (purified using a MilliQ deionising
system) with 0.5% formic acid was utilised: a hold on 100% water for five minutes, followed by a gradient from 0% - 80% CH₃CN/H₂O (0.5% formic acid) over 10 minutes, an isocratic hold on 80% CH₃CN for two minutes and then a restoration to 0% CH₃CN over two minutes. The method was finished with an isocratic hold on 0% CH₃CN over five minutes. The ESI source was set at 4 kV, 200°C, 1 bar nebulisation gas and 8 L/min dry gas.

Mercaptoacetonitrile¹

\[
\text{HS} \quad \equiv \quad \text{N}
\]

Chloroacetonitrile (6.3 mL, 0.1 mol) and thioacetic acid (7.05 mL, 0.1 mol) were dissolved in CH₂Cl₂ (70 mL) and cooled to -30 °C under an atmosphere of N₂. Triethylamine (16.7 mL, 0.12 mol) was added dropwise over 0.5 h. The reaction was stirred at -30 °C for another 1 h, and then gradually warmed to RT. Water (5 mL) was added, and the organic layer was washed with acetic acid (10%, 2 x 15 mL) followed by water (2 x 15 mL). The organic layer was dried (MgSO₄), filtered, and concentrated in vacuo. The liquid produced was dissolved in MeOH (100 mL) and Amberlyst® 15 (H⁺ form, 3 g) was added. The reaction was heated to reflux under an atmosphere of N₂ and stirred for 20 h. The reaction was filtered, Amberlyst® 15 (H⁺ form, 0.3 g) was added, and the mixture was concentrated in vacuo at RT to afford mercaptoacetonitrile 45 (5.96 g, 82%) as a pale yellow liquid; \( \nu_{\text{max}} \) (neat) 2247 cm⁻¹ (C≡N); \( \delta_H \) (400 MHz, CDCl₃) 2.37 (1H, t, J 8.2 Hz, SH), 3.29 (2H, d, J 8.2 Hz, CH₂); \( \delta_C \) (100.5 MHz, CDCl₃) 9.6 (t, CH₂), 118.2 (s, CN).

Cyanomethyl 1-thio-β-D-glucopyranoside 2a

D-Glucose (200 mg, 1.11 mmol) was dissolved in water (4 mL). Triethylamine (2.5 mL, 34 mmol) was added, the reaction was cooled to 0 °C, and stirred. 2-Chloro-1,3-dimethylimidazolinium chloride (0.92 g, 5.5 mmol) was dissolved in mercaptoacetonitrile
(1.3 g, 17.8 mmol) and the resulting solution was added dropwise to the aqueous D-glucose solution over 2 min. The reaction was stirred at 0 °C for 0.5 h, and was then diluted with water (5 mL) and washed with CH$_2$Cl$_2$ (5 x 10 mL). The aqueous layer was filtered through a column of Amberlite® IR-120 (Na$^+$ form, produced by treating the H$^+$ form with 1 M aqueous NaOH solution and then washing with water until pH 7), lyophilised, pre-adsorbed onto silica, and purified by flash column chromatography (CHCl$_3$:MeOH, 5:1) to afford cyanomethyl 1-thio-β-D-glucopyranoside 2a (232 mg, 89%) as a pale yellow oil; [α]$_{D}^{20}$ -67 (c, 1.0 in MeOH) [lit. [α]$_{D}^{22}$ -60.2 (c, 5.05 in water)]$^2$; $\nu_{\text{max}}$ (neat) 2248 cm$^{-1}$ (C≡N); $\delta_{\text{H}}$(400 MHz, D$_2$O) 3.37 - 3.47 (2H, m, H-2 & H-3), 3.49 - 3.55 (2H, m, H-4 & H-5), 3.68, 3.79 (2H, ABq, $J_{17.6}$ 17.6 Hz, CH$_2$CN) 3.72 (1H, dd, $J_{5,6'}$ 5.5 Hz, $J_{6,6'}$ 12.1 Hz, H-6), 3.91 (1H, dd, $J_{5,6'}$ 1.8 Hz, $J_{6,6'}$ 12.3 Hz, H-6'), 4.72 (1H, d, $J_{1,2}$ 9.8 Hz, H-1); $\delta_{\text{C}}$(100.5 MHz, D$_2$O) 14.4 (t, CH$_2$CN), 60.7 (t, C-6), 69.2 (d, C-2), 71.8 (d, C-3), 77.0, 80.0 (2 x d, C-4 & C-5), 84.4 (d, C-1), 118.6 (s, CH$_2$CN); HRMS (ESI-TOF): calcd. for C$_8$H$_{13}$NO$_5$SNa$^+$: 258.0407. Found: 258.0414 (MNa$^+$).

2-Imino-2-methoxyethyl 1-thio-β-D-glucopyranoside 3a$^2$

Sodium metal (8 mg, 0.36 mmol) was dissolved in MeOH (3.6 mL). Cyanomethyl 1-thio-β-D-glucopyranoside 2a (83.6 mg, 0.36 mmol) was added and the reaction was stirred for 64 h. The reaction mixture was then concentrated in vacuo and analysed by $^1$H NMR to reveal the formation of 2-imino-2-methoxyethyl 1-thio-β-D-glucopyranoside 47 (35% conversion). The crude material was subsequently used without purification for conjugation experiments; $\nu_{\text{max}}$ (neat) 1654 cm$^{-1}$ (C=N); $\delta_{\text{H}}$(400 MHz, CD$_3$OD) 4.32 Hz (1H, d, $J$ 10.2 Hz, H-1); HRMS (ESI-TOF): calcd. for C$_9$H$_{18}$NO$_6$S$^+$: 268.0849. Found: 268.0860 (MH$^+$).
Cyanomethyl 1-thio-β-D-galactopyranoside 2b

D-Galactose (200 mg, 1.11 mmol) was dissolved in water (4 mL). Triethylamine (2.4 mL, 33 mmol) was added, the reaction was cooled to 0 °C, and stirred. 2-Chloro-1,3-dimethylimidazolinium chloride (1.12 g, 6.7 mmol) was dissolved in mercaptoacetonitrile (1.6 g, 22 mmol) and the resulting solution was added dropwise to the aqueous D-galactose solution over 2 min. The reaction was stirred at 0 °C for 0.5 h, and was then diluted with water (5 mL) and washed with CH₂Cl₂ (5 x 10 mL). The aqueous layer was filtered through a column of Amberlite® IR-120 (Na⁺ form, produced by treating the H⁺ form with 1 M aqueous NaOH solution and then washing with water until pH 7), lyophilised, pre-adsorbed onto silica, and purified by flash column chromatography (CHCl₃:MeOH, 5:1) to afford cyanomethyl 1-thio-β-D-galactopyranoside 2b (106 mg, 41%) as a pale yellow oil; [α]D²⁰ +13 (c, 0.1 in MeOH) [lit. [α]D²⁰ 51.5 (c, 5.05 in water)]; νmax ( neat) 2252 cm⁻¹ (C≡N); δH (400 MHz, D₂O) 3.62 - 3.80 (5H, m, H-2, H-4, H-5, H-6 & H-6’), 3.69, 3.81 (2H, ABq, J 17.6 Hz, CH₂) 3.99 (1H, d, J₁₂ 3.1 Hz, H-4), 4.66 (1H, d, J₁₂ 9.4 Hz, H-1); δC (100.5 MHz, D₂O) 14.5 (t, CH₂CN), 61.0 (t, H-6), 68.6 (d, C-4), 69.2 (d, C-2), 73.8, 79.1 (2 x d, C-3 & C-5), 84.9 (d, C-1), 118.6 (s, CH₂CN); HRMS (ESI-TOF): calcd. for C₈H₁₃NO₂SNa⁺: 258.0407. Found: 258.0413 (MNa⁺).

2-Imino-2-methoxyethyl 1-thio-β-D-galactopyranoside 3b²

Sodium metal (5 mg, 0.22 mmol) was dissolved in MeOH (2.5 mL). Cyanomethyl 1-thio-β-D-galactopyranoside 2b (52 mg, 0.22 mmol) was added and the reaction was stirred for 64 h. The reaction mixture was then concentrated in vacuo and analysed by ¹H NMR to reveal the formation of 2-imino-2-methoxyethyl 1-thio-β-D-galactopyranoside 3b (50% conversion).
The crude material was subsequently used without purification for the conjugation experiments; $\nu_{\text{max}}$ (neat) 1651 cm$^{-1}$ (C=N); $\delta_H$ (400 MHz, CD$_3$OD)$^1$ 4.27 (1H, d, $J_{1,2}$ 9.4 Hz, H-1); HRMS (ESI-TOF): calcd. for C$_9$H$_{18}$NO$_6$S$^+$: 268.0849. Found: 268.0853 (M$^+$).

Cyanomethyl 1-thio-\(\alpha\)-d-mannopyranoside 2c

![](image)

\(\alpha\)-D-Mannose (100 mg, 0.56 mmol) was dissolved in water (2 mL). Triethylamine (1.2 mL, 11.1 mmol) was added, the reaction was cooled to 0 °C, and stirred. 2-Chloro-1,3-dimethylimidazolinium chloride (0.56 g, 3.3 mmol) was dissolved in mercaptoacetonitrile (0.8 g, 11.1 mmol) and the resulting solution was added dropwise to the aqueous \(\alpha\)-mannose solution over 2 min. The reaction was stirred at 0 °C for 0.5 h, and was then diluted with water (2 mL) and washed with CH$_2$Cl$_2$ (5 x 10 mL). The aqueous layer was filtered through a column of Amberlite® IR-120 (Na$^+$ form, produced by treating the H$^+$ form with 1 M aqueous NaOH solution and then washing with water until pH 7), lyophilised, pre-adsorbed onto silica, and purified by flash column chromatography (CHCl$_3$:MeOH, 5:1) to afford cyanomethyl 1-thio-\(\alpha\)-d-mannopyranoside 2c (103 mg, 78%) as a yellow oil; $[\alpha]_D^{20}$ +80 (c, 1.0 in MeOH); $\nu_{\text{max}}$ (neat) 3327 cm$^{-1}$ (OH), 2255 cm$^{-1}$ (CN); $\delta_H$ (400 MHz, D$_2$O) 3.60, 3.65 (2H, ABq, $J$ 17.6 Hz, SCH$_2$CN), 3.71 - 3.76 (2H, m, H-3 & H-4), 3.80 (1H, dd, $J_{5,6}$ 5.5 Hz, $J_{6,6'}$ 12.5 Hz, H-6), 3.88 (1H, dd, $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 12.1 Hz, H-6′), 3.91 - 3.97 (1H, m, H-5), 4.08 (1H, d, $J_{2,3}$ 1.2 Hz, H-2), 5.47 (1H, s, H-1); $\delta_C$ (100.5 MHz, D$_2$O) 15.3 (t, SCH$_2$CN), 60.4 (t, C-6), 66.7 (d, C-3), 70.8 (d, C-2), 71.0 (d, C-4), 73.5 (d, C-5), 84.8 (d, C-1), 118.2 (s, SCH$_2$CN); HRMS (ESI-TOF): calcd. for C$_9$H$_{13}$NO$_5$SNa$^+$: 258.0407. Found: 258.0401 (MNa$^+$).
2-Imino-2-methoxyethyl 1-thio-α-D-mannopyranoside 3c

Sodium metal (13.7 mg, 0.60 mmol) was dissolved in MeOH (6 mL). Cyanomethyl 1-thio-α-D-mannopyranoside 2c (140 mg, 0.60 mmol) was added and the reaction was stirred for 64 h. The reaction mixture was then concentrated in vacuo and analysed by $^1$H NMR to reveal the formation of 2-imino-2-methoxyethyl 1-thio-α-D-mannopyranoside 3c (22% conversion). The crude material was subsequently used without purification for conjugation experiments; $\nu_{\text{max}}$ (neat) 3311 cm$^{-1}$ (OH), 1649 cm$^{-1}$ (C=NH); $\delta_{\text{H}}$ (400 MHz, CD$_2$OD) 5.34 (1H, s, H-1); HRMS (ESI-TOF): calcd. for C$_9$H$_{18}$NO$_5$S$: 268.0849$. Found: 268.0848 (MH$^+$).

Cyanomethyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 2d

β-D-Galactopyranosyl-(1→4)-D-glucopyranose (103 mg, 0.3 mmol) was dissolved in water (2 mL). Triethylamine (0.84 mL, 6 mmol) was added, the reaction was cooled to 0°C, and stirred. 2-Chloro-1,3-dimethylimidazolinium chloride (0.303 g, 1.8 mmol) was dissolved in mercaptoacetonitrile (0.439 g, 6 mmol) and the resulting solution was added dropwise to the aqueous β-D-galactopyranosyl-(1→4)-D-glucopyranose solution over 2 min. The reaction was stirred at 0°C for 0.5 h, and was then diluted with water (2 mL) and washed with CH$_2$Cl$_2$ (5 x 10 mL). The aqueous layer was filtered through a column of Amberlite® IR-120 (Na$^+$ form, produced by treating the H$^+$ form with 1 M aqueous NaOH solution and then washing with water until pH 7), lyophilised, pre-adsorbed onto silica, and purified by flash column chromatography (CHCl$_3$:MeOH, 5:1) to afford cyanomethyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 2d (77 mg, 65%) as a yellow oil; $[\alpha]_D^{20}$ -25 (c, 0.1 in MeOH); $\nu_{\text{max}}$ (neat) 3298 cm$^{-1}$ (OH), 2251 cm$^{-1}$ (CN); $\delta_{\text{H}}$ (400 MHz, CD$_3$OD) 3.37 (1H, t, $J$ 9.8 Hz, H-2$_\alpha$),
3.42 - 3.65 (6H, m, H-3<sub>A</sub>, H-4<sub>A</sub>, H-5<sub>A</sub>, H-2<sub>B</sub>, H-3<sub>B</sub> & H-5<sub>B</sub>), 3.66 - 3.88 (6H, m, CH<sub>2</sub>, H-6<sub>A</sub>, H-4<sub>B</sub>, H-6<sub>B</sub> & H-6<sub>'B</sub>), 3.94 (1H, dd, J<sub>5,6</sub> 2.0 Hz, J<sub>6,6'</sub> 12.1 Hz, H-6<sub>'A</sub>), 4.37 (1H, d, J<sub>1,2</sub> 7.4 Hz, H-1<sub>B</sub>), 4.60 (1H, d, J<sub>1,2</sub> 9.4 Hz, H-1<sub>A</sub>); δ<sub>C</sub> (100.5 MHz, CD<sub>3</sub>OD) 13.1 (t, CH<sub>2</sub>CN), 60.5 (t, C-6<sub>A</sub>), 61.1 (t, C-6<sub>B</sub>), 68.9 (d, C-4<sub>B</sub>), 71.1, 72.6, 73.3, 75.6, 76.4, 78.8, 79.4 (7 × d, C-2<sub>A</sub>, C-3<sub>A</sub>, C-4<sub>A</sub>, C-5<sub>A</sub>, C-2<sub>B</sub>, C-3<sub>B</sub> & C-5<sub>B</sub>), 83.9 (d, C-1<sub>A</sub>), 103.6 (d, C-1<sub>B</sub>), 117.3 (s, CH<sub>2</sub>CN); HRMS (ESI-TOF): calcd. for C<sub>14</sub>H<sub>24</sub>NO<sub>10</sub>S<sup>+</sup>: 398.1115. Found: 398.1109 (MH<sup>+</sup>).

2-Imino-2-methoxyethyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 3d<sup>2</sup>

Sodium metal (7.6 mg, 0.33 mmol) was dissolved in MeOH (3.3 mL). Cyanomethyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 2d (130 mg, 0.33 mmol) was added and the reaction was stirred for 64 h. The reaction mixture was then concentrated in vacuo and analysed by <sup>1</sup>H NMR to reveal the formation of 2-imino-2-methoxyethyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 3d (85% conversion). The crude material was subsequently used without purification for conjugation experiments; ν<sub>max</sub> (neat) 3279 cm<sup>-1</sup> (OH), 1652 cm<sup>-1</sup> (C=NH); δ<sub>H</sub> (400 MHz, CD<sub>3</sub>OD) 4.36 (1H, d, J 7.4 Hz, H-1<sub>B</sub>), 4.45 (1H, d, J 7.4 Hz, H-1<sub>A</sub>); HRMS (ESI-TOF): calcd. for C<sub>15</sub>H<sub>28</sub>NO<sub>11</sub>S<sup>+</sup>: 430.1378. Found: 430.1392 (MH<sup>+</sup>).
Cyanomethyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 2e

α-D-Glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-β-D-glucopyranose (101 mg, 0.2 mmol) was dissolved in D$_2$O (1.3 mL). Triethylamine (0.56 mL, 4 mmol) was added, the reaction was cooled to 0 °C, and stirred. 2-Chloro-1,3-dimethylimidazolinium chloride (0.202 g, 1.2 mmol) was dissolved in mercaptoacetonitrile (0.292 g, 4 mmol) and the resulting solution was added dropwise to the aqueous α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-β-D-glucopyranose solution over 2 min. The reaction was stirred at 0 °C for 0.5 h, and was then diluted with water (2 mL) and washed with CH$_2$Cl$_2$ (5 x 10 mL). The aqueous layer was filtered through a column of Amberlite® IR-120 (Na$^+$ form, produced by treating the H$^+$ form with 1 M aqueous NaOH solution and then washing with water until pH 7), lyophilised, pre-adsorbed onto silica, and purified by flash column chromatography (CHCl$_3$:MeOH, 5:1) to afford cyanomethyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 2e (101 mg, 90%) as a yellow oil; [α]$_D^{20} +54$ (c, 1.0 in MeOH); $\nu_{\text{max}}$ (neat) 3279 cm$^{-1}$ (OH), 2248 cm$^{-1}$ (CN); δ$_H$ (600 MHz, CD$_3$OD) 3.26 (1H, t, J 9.2 Hz), 3.33 (1H, t, J 9.6 Hz), 3.42 - 3.47 (2H, m), 3.48 - 3.53 (2H, m), 3.57 (1H, t, J 9.2 Hz), 3.61 (1H, t, J 9.4 Hz), 3.63 - 3.70 (3H, m), 3.73 - 3.84 (7H, m), 3.84 - 3.88 (2H, m), 3.90 (1H, dd, J 12.4 Hz, J 2.0 Hz), 4.59 (1H, d, J$_{1,2}$ 9.6 Hz, H-1$_A$), 5.15 (1H, d, J$_{1,2}$ 3.7 Hz, H-1$_B$), 5.19 (1H, d, J$_{1,2}$ 3.9 Hz, H-1$_C$); δ$_C$ (151 MHz, CD$_3$OD) 13.0 (t, SCH$_2$CN), 60.7, 60.9, 61.3 (3 x t, 3 x C-6), 70.1, 71.9, 72.4, 72.5, 72.8, 73.4, 73.5, 73.6, 77.8, 79.3, 79.4, 79.9, 83.9 (d, C-1$_A$), 101.2 (d, C-1$_C$), 101.5 (d, C-1$_B$), 117.2 (SCH$_2$CN); HRMS (ESI-TOF): calcd. for C$_{20}$H$_{33}$NO$_{15}$SNa$^+$: 582.14633. Found: 582.1461 (MNa$^+$).
2-Imino-2-methoxyethyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 3e

Sodium metal (4 mg, 0.17 mmol) was dissolved in MeOH (1.7 mL). cyanomethyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 2e (97 mg, 0.17 mmol) was added and the reaction was stirred for 64 h. The reaction mixture was then concentrated in vacuo and analysed by ¹H NMR to reveal the formation of 2-imino-2-methoxyethyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 3e (60% conversion). The crude material was subsequently used without purification for conjugation experiments; νmax (neat) 3289 cm⁻¹ (OH), 1652 cm⁻¹ (C=NH); δH (400 MHz, D₂O) 4.69 (1H, d, J₁₂ 10.6 Hz, H-1A), 5.29 - 5.35 (2H, m, H-1B & H-1C); HRMS (ESI-TOF): calcd. for C₂₁H₃₈NO₁₆S⁺: 592.1906. Found: 592.1907 (MH⁺).

Conjugation of 2-imino-2-methoxyethyl 1-thio-β-D-glucopyranoside 3a to lysozyme
Lysozyme (1 mg, 0.070 µmol) was dissolved in sodium borate buffer (0.2 mL, 0.2 M, pH 8.5). A portion of the 2-imino-2-methoxyethyl 1-thio-β-D-glucopyranoside 3a crude reaction mixture (50 µL, approximately 1.8 µmol of 3a) was added to the lysozyme solution and the reaction was stirred. After 5 h the reaction was dialysed against water (2 x 500 mL), and filtered; HRMS (ESI-TOF): 15246, 54% (calcd. for lysozyme-(C(NH)CH₂SC₆H₁₁O₅)₄: 15247), 15481, 100% (calcd. for lysozyme-(C(NH)CH₂SC₆H₁₁O₅)₅: 15482), 15715, 54% (calcd. for lysozyme-(C(NH)CH₂SC₆H₁₁O₅)₆: 15717).

Conjugation of 2-imino-2-methoxyethyl 1-thio-β-D-galactopyranoside 3b to lysozyme
Lysozyme (1 mg, 0.070 µmol) was dissolved in sodium borate buffer (0.2 mL, 0.2 M, pH 8.5). A portion of the 2-imino-2-methoxyethyl 1-thio-β-D-galactopyranoside 3b crude reaction mixture (50 µL, approximately 2.2 µmol of 3b) was added to the lysozyme solution
and the reaction was stirred. After 5 h the reaction was dialysed against water (2 x 500 mL), and filtered; HRMS (ESI-TOF): 15010, 2% (calcd. for lysozyme-(C(NH)CH$_2$SC$_6$H$_{11}$O$_5$)$_3$: 15012), 15246, 61% (calcd. for lysozyme-(C(NH)CH$_2$SC$_6$H$_{11}$O$_5$)$_4$: 15247), 15481, 100% (calcd. for lysozyme-(C(NH)CH$_2$SC$_6$H$_{11}$O$_5$)$_5$: 15482), 15717, 54% (calcd. for lysozyme-(C(NH)CH$_2$SC$_6$H$_{11}$O$_5$)$_6$: 15717).

Conjugation of 2-imino-2-methoxyethyl 1-thio-α-D-mannopyranoside 3c to lysozyme
Lysozyme (1 mg, 0.070 µmol) was dissolved in sodium borate buffer (0.2 mL, 0.2 M, pH 8.5). A portion of the 2-imino-2-methoxyethyl 1-thio-α-D-mannopyranoside 3c crude reaction mixture (50 µL, approximately 1.1 µmol of 3c) was added to the lysozyme solution and the reaction was stirred. After 5 h the reaction was dialysed against water (2 x 500 mL), and filtered; HRMS (ESI-TOF): 14305, 25% (calcd. for lysozyme: 14307), 14540, 87% (calcd. for lysozyme-(C(NH)CH$_2$SC$_6$H$_{11}$O$_5$)$_2$: 14542), 14775, 100% (calcd. for lysozyme-(C(NH)CH$_2$SC$_6$H$_{11}$O$_5$)$_3$: 14777), 15011, 47% (calcd. for lysozyme-(C(NH)CH$_2$SC$_6$H$_{11}$O$_5$)$_4$: 15012), 15247, 6% (calcd. for lysozyme-(C(NH)CH$_2$SC$_6$H$_{11}$O$_5$)$_6$: 15247).

Conjugation of 2-imino-2-methoxyethyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 3d to lysozyme
Lysozyme (1 mg, 0.070 µmol) was dissolved in sodium borate buffer (0.2 mL, 0.2 M, pH 8.5). A portion of the 2-imino-2-methoxyethyl 1-thio-α-D-mannopyranoside 3d crude reaction mixture (50 µL, approximately 1.7 µmol of 3d) was added to the lysozyme solution and the reaction was stirred. After 5 h the reaction was dialysed against water (2 x 500 mL), and filtered; HRMS (ESI-TOF): 14307, 9% (calcd. for lysozyme: 14307), 14703, 25% (calcd. for lysozyme-(C(NH)CH$_2$SC$_{12}$H$_{21}$O$_{10}$)$_2$: 14704), 15099, 78% (calcd. for lysozyme-(C(NH)CH$_2$SC$_{12}$H$_{21}$O$_{10}$)$_3$: 15091), 15497, 100% (calcd. for lysozyme-(C(NH)CH$_2$SC$_{12}$H$_{21}$O$_{10}$)$_4$: 15498), 15894, 47% (calcd. for lysozyme-(C(NH)CH$_2$SC$_{12}$H$_{21}$O$_{10}$)$_5$: 15895), 16293, 27% (calcd. for lysozyme-(C(NH)CH$_2$SC$_{12}$H$_{21}$O$_{10}$)$_6$: 16292).

Conjugation of 2-imino-2-methoxyethyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-1-thio-β-D-glucopyranoside 3e to lysozyme
Lysozyme (1 mg, 0.070 µmol) was dissolved in sodium borate buffer (0.2 mL, 0.2 M, pH 8.5). A portion of crude 2-imino-2-methoxyethyl 1-thio-α-D-mannopyranoside 3e (3 mg, approximately 3 µmol of 3e) was added to the lysozyme solution and the reaction was stirred.
After 5 h the reaction was dialysed against water (2 x 500 mL), and filtered; HRMS (ESI-TOF): 14305, 9% (calcd. for lysozyme: 14307), 14864, 61% (calcd. for lysozyme-(C(NH)CH$_2$SC$_{18}$H$_{31}$O$_{15}$): 14866), 15042, 100% (calcd. for lysozyme-(C(NH)CH$_2$SC$_{18}$H$_{31}$O$_{15}$)$_2$: 15425), 15983, 50% (calcd. for lysozyme-(C(NH)CH$_2$SC$_{18}$H$_{31}$O$_{15}$)$_3$: 15984), 16543, 15% (calcd. for lysozyme-(C(NH)CH$_2$SC$_{18}$H$_{31}$O$_{15}$)$_4$: 16543), 17103, 1% (calcd. for lysozyme-(C(NH)CH$_2$SC$_{18}$H$_{31}$O$_{15}$)$_5$: 17102).

References

Mercaptoacetonitrile