Supplementary Materials

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Contents:

Experimental procedures

Extraction of 5-thiomannosides from treated cells

Confluent monolayers of CHO K1 cells (*ca*. 1x10^{7} cells) were grown for 14 h in the presence or absence of 5-thioMan-(1,3)-*N*-Man. Cells were harvested into 2 mL methanol, sonicated in a water bath sonicator, and dried under a gentle stream of N_2. Lipids were removed by sonicating samples in chloroform : methanol (2:1, 2x5mL) and the remaining pellets were extracted by sonicating the samples in 4 mL 18MΩ H_2O. All samples were then passed through graphite-containing Hypersep SPE cartridges (Thermo Scientific). The flow through and aqueous washes (2x2mL), predicted to contain only monosaccharides,

were pooled. Disaccharides including the 5-thiomannosides were eluted with 5% CH_3CN (2x2mL) while oligosaccharides were eluted using 50% CH_3CN (2x2mL). All fractions were lyophilized. Non-retained material (*i.e.* the aqueous wash) were spiked with lactose and desalted using AG W50-X8(H^+) and AG 1X4 (formate) ion exchange resins (1mL each). Samples were analyzed on an Agilent 6210 TOF LC/MS high resolution magnetic sector mass spectrometer using the electrospray ionization method. Of interest were ionic masses corresponding 5-thiomannoside hydrolysis or those in which the mass of the 5-thioMan-(1,3)-*N*-Man had increased by one or more hexose residue.

*NMR data for 5-thioMan-(1-6)-N-Man (**3**).*

Methyl 6-amino-6-deoxy-6-*N*-(5-thio-β-D-mannopyranosyl)-α-D-mannopyranoside (major)

^{1}H NMR (D_2O, 500 MHz): δ 4.66 (1H, d, $J_{1,2}$ = 1.5 Hz, H-1), 4.27 (1H, d, $J_{1',2'}$ = 1.5 Hz, H-1'), 4.12 (1H, dd, $J_{2',3'}$ = 2.5 Hz, H-2'), 3.89 (1H, dd, $J_{6'a,6'b}$ = 12.0, $J_{6'a,5'}$ = 3.0 Hz, H-6'a), 3.86-3.83 (1H, m, H-2), 3.76-3.57 (4H, m, H-6'b, H-4', H-3, H-5), 3.50-3.45 (1H, m, H-4), 3.38 (1H, dd, $J_{3',4'}$ = 10.0 Hz, H-3'), 3.33 (3H, s, -OCH_3), 3.14 (1H, dd, $J_{6a,6b}$ = 13.0, $J_{6a,5}$ = 3.0 Hz, H-6a), 2.84 (1H, dt, $J_{5',6'b}$ = $J_{5',4'}$ = 7.0 Hz, H-5'), 2.77 (1H, dd, $J_{6b,5}$ = 8.0 Hz, H-6b). ^{13}C NMR (D_2O, 125 MHz): δ 100.8 (C-1), 75.0 (C-3'), 73.1 (C-2), 71.4 (C-5), 70.3 (C-3), 69.9 (C-4'), 69.8 (C-2'), 68.7 (C-4), 63.9 (C-1'), 60.8 (C-6'), 54.9 (-OCH_3), 47.7 (C-6), 46.4 (C-5').

Methyl 6-amino-6-deoxy-6-*N*-(5-thio-α-D-mannopyranosyl)-α-D-mannopyranoside (minor)

^{1}H NMR (D_2O, 500 MHz): δ 4.67 (1H, d, $J_{1,2}$ = 1.5 Hz, H-1), 4.10 (1H, dd, $J_{2',1'}$ = 3.5, $J_{2',3'}$ = 3.0 Hz, H-2'), 3.92 (1H, d, H-1'), 3.86-3.83 (2H, m, H-6'a, H-2), 3.76-3.57 (4H, m, H-6'b, H-4', H-3, H-5), 3.55 (1H, dd, $J_{3',4'}$ = 3.0 Hz, H-3'), 3.50-3.45 (1H, m, H-4), 3.32 (3H, s, -OCH_3), 3.01-2.95 (3H, m, H-6a, H-6b, H-5'). ^{13}C NMR (D_2O, 125 MHz): δ 100.9 (C-1), 73.0 (C-2), 71.8 (C-3'), 70.3 (C-3), 69.83, 69.80, 69.7 (C-2', C-4', C-5), 68.8 (C-4), 65.4 (C-1'), 60.7 (C-6'), 54.9 (-OCH_3), 47.3 (C-6), 43.6 (C-5').

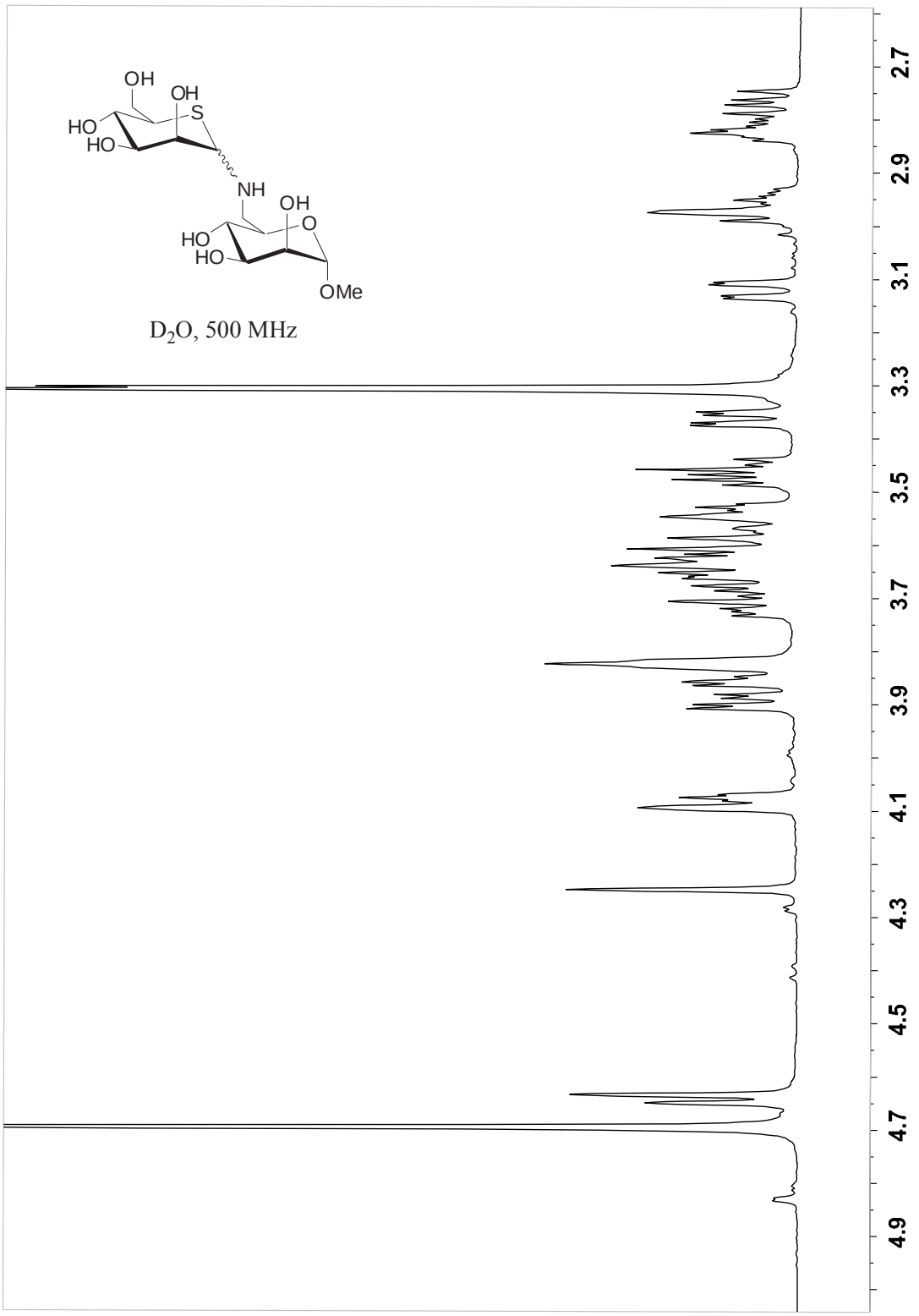

Figure S-1. ^{1}H-NMR spectrum for 5-thioMan-(1-6)-*N*-Man

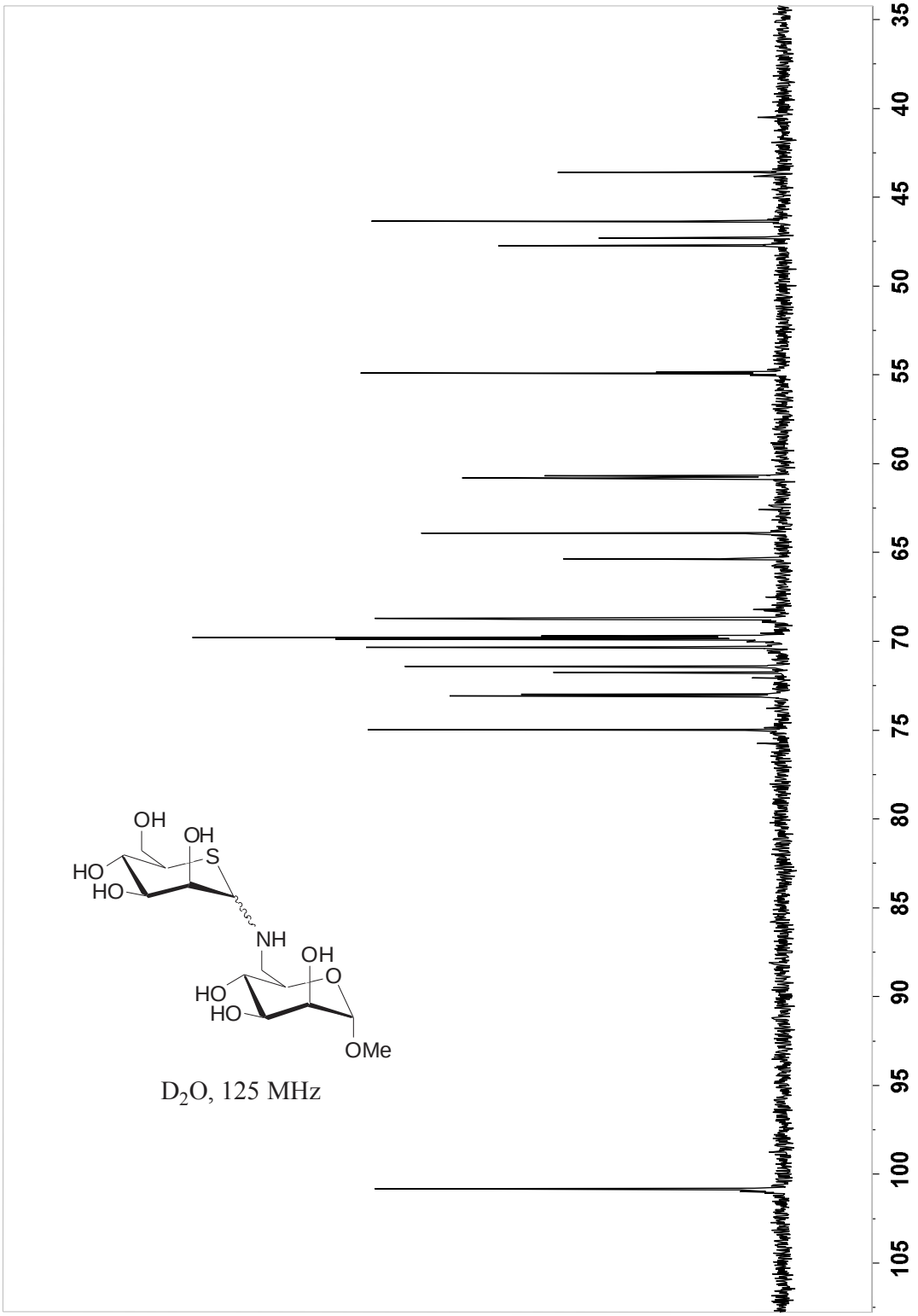

Figure S-2. ^{13}C-NMR spectrum for 5-thioMan-(1-6)-*N*-Man.

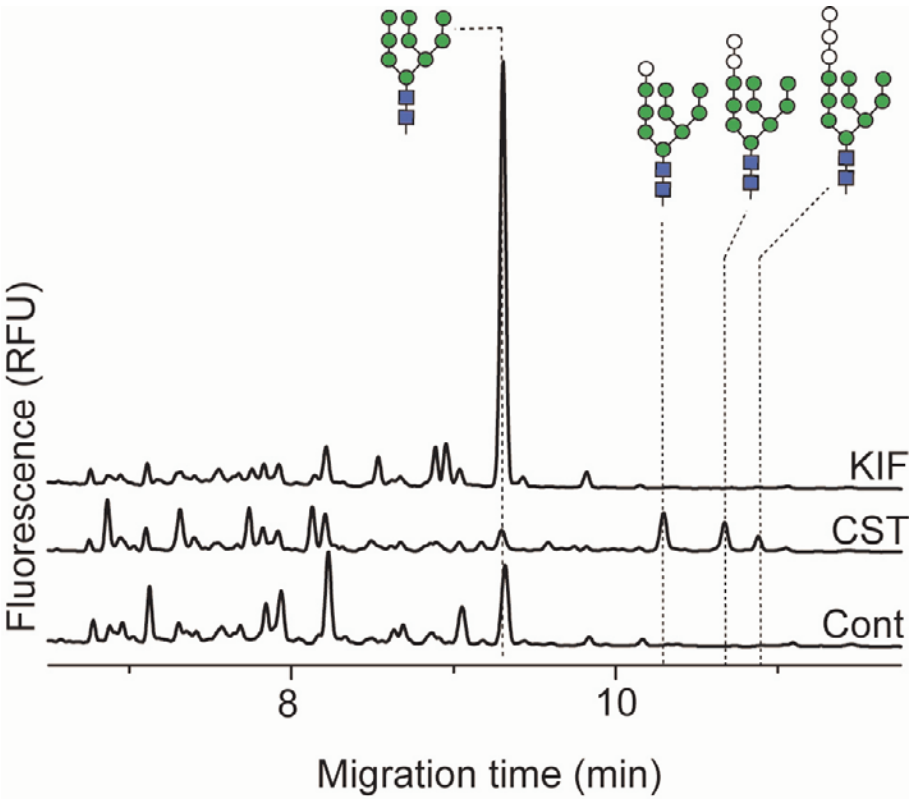

Figure S-3. CE analysis of *N*-glycans obtained from canstanospermine (CST)- or kifunensine (KIF)-treated cells.

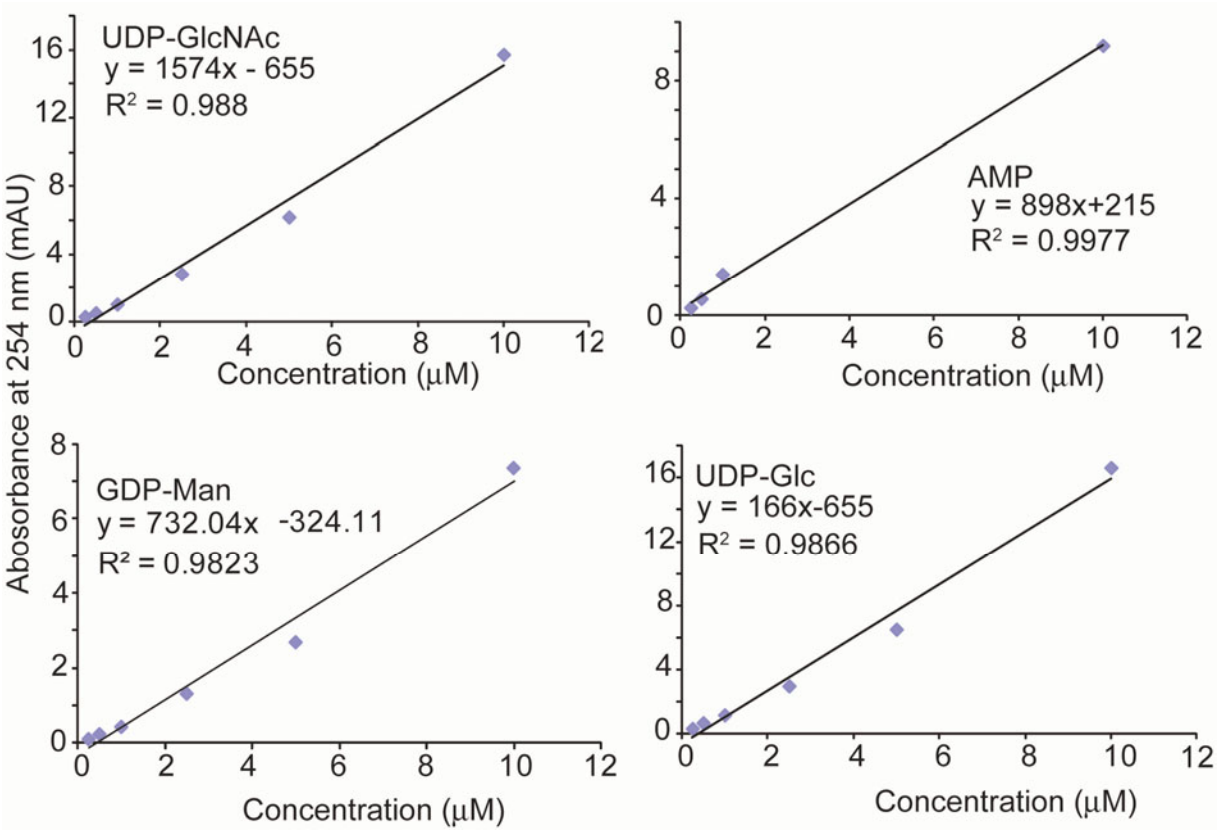

Figure S-4. Calibration curves for UDP-GlcNAc, UDP-Glc, GDP-Man and AMP quantification.

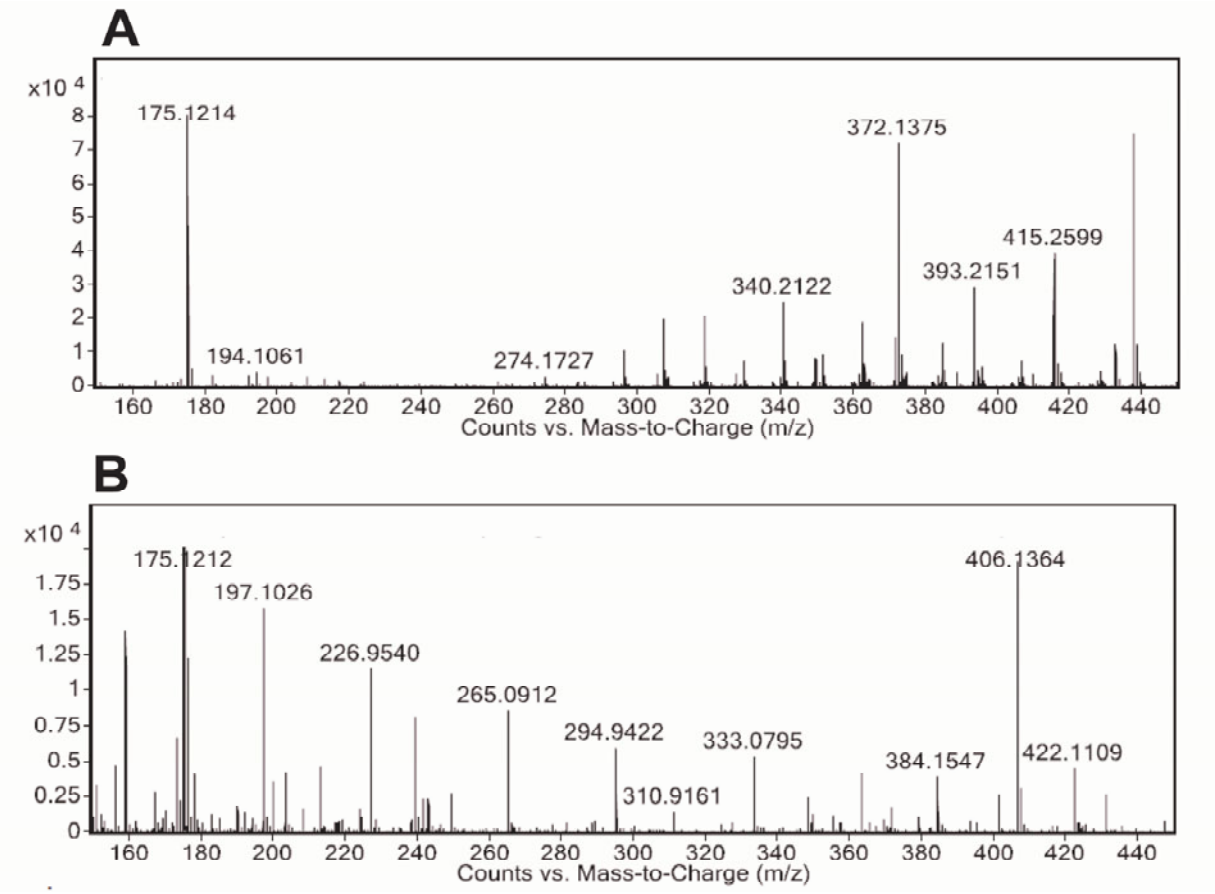

Figure S-5. Mass spectrometric analysis of 5-thiomannosides extracted from cells treated with (**A**) or without (**B**) 5-thioMan-(1-3)-*N*-Man (**3**). Compared are Hypersep SPE fractions (5% CH_3CN) which had been confirmed to contain **3** but not monosaccharides such as 5-thiomannose or oligosaccharides. m/z ($M+H^+$) for **3** is 372.13.