Supplemental Material

Stable-Isotope Labeled Hydrophobic Hydrazide Reagents for the Relative Quantification of N-linked Glycans by Electrospray Ionization Mass Spectrometry

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Submitted to:

Analytical Chemistry

Revised: July 15, 2011

Running Title: Stable-isotope labeled reagents for *N*-linked glycans

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Glycan Composition	Retention Time Average (Light) (min)	Retention Time Average (Heavy) (min)	Average Retention Time Difference (s)
$Hex_5HexNAc_2$	29.26	29.29	3.4
$Hex_3HexNAc_4$	28.51	28.47	-1.4
$Hex_6HexNAc_2$	32.48	32.48	-1.0
$Hex_3HexNAc_4Fuc_1$	29.33	29.33	2.6
$Hex_3HexNAc_5$	29.14	29.16	0.0
$Hex_7HexNAc_2$	35.86	35.87	-1.8
$Hex_4HexNAc_4Fuc_1$	32.37	32.37	0.0
$Hex_5HexNAc_4$	34.59	34.53	0.0
$Hex_3HexNAc_5Fuc_1$	30.38	30.36	2.0
$Hex_4HexNAc_3NeuAc_1Fuc_1$	35.15	35.11	-1.0
$Hex_8HexNAc_2$	38.23	38.19	1.0
$Hex_5HexNAc_3NeuAc_1$	35.93	35.91	0.0
$Hex_5HexNAc_3NeuAc_1$	35.93	35.91	2.8
$Hex_4HexNAc_5Fuc_1$	32.96	32.93	0.8
$Hex_5HexNAc_5$	34.87	34.86	-0.6
$Hex_9HexNAc_2$	39.85	39.88	0.8
$Hex_5HexNAc_4NeuAc_1$	37.25	37.25	0.0
$Hex_5HexNAc_4NeuAc_1Fuc_1$	38.35	38.34	1.0
$Hex_4HexNAc_5NeuAc_1Fuc_1$	36.56	36.54	3.0
$Hex_5HexNAc_5NeuAc_1$	37.91	37.92	0.8
$Hex_5HexNAc_4NeuAc_2$	39.41	39.41	2.0
$Hex_5HexNAc_5NeuAc_1Fuc_1$	38.69	38.68	1.0
$Hex_6HexNAc_5NeuAc_1$	40.23	40.26	0.8
$Hex_5HexNAc_4NeuAc_2Fuc_1$	40.50	40.51	-0.8
$Hex_5HexNAc_5NeuAc_2Fuc_1$	40.84	40.79	-1.6
$Hex_6HexNAc_5NeuAc_3$	42.63	42.65	-1.4

Table S1 presents the retention times for the ‘light’ and ‘heavy’ glycans detected in human plasma. The data presented are average (n=3) retention times for technical replicates. The average retention time difference was calculated by first calculating the retention time difference for each of the replicates. Then, the retention time differences for each of the replicates were averaged.

Glycan Composition	Uncorrected Ratio (H:L) Average (n = 3)	Corrected Ratio (H:L) Average (n=3)
Hex_7 (Internal Standard)	1.30	1.00
$Hex_5HexNAc_2$	1.38	1.05
$Hex_3HexNAc_4$	1.29	0.98
$Hex_6HexNAc_2$	1.48	1.13
$Hex_3HexNAc_4Fuc_1$	1.31	1.00
$Hex_3HexNAc_5$	1.27	0.98
$Hex_7HexNAc_2$	1.39	1.05
$Hex_4HexNAc_4Fuc_1$	1.40	1.08
$Hex_5HexNAc_4$	1.33	1.03
$Hex_3HexNAc_5Fuc_1$	1.31	1.01
$Hex_4HexNAc_3NeuAc_1Fuc_1$	1.31	1.03
$Hex_8HexNAc_2$	1.40	1.04
$Hex_5HexNAc_3NeuAc_1$	1.40	1.08
$Hex_5HexNAc_3NeuAc_1$	1.34	1.02
$Hex_4HexNAc_5Fuc_1$	1.34	1.05
$Hex_5HexNAc_5$	1.34	1.02
$Hex_9HexNAc_2$	1.53	1.14
$Hex_5HexNAc_4NeuAc_1$	1.52	1.13
$Hex_5HexNAc_4NeuAc_1Fuc_1$	1.38	1.07
$Hex_4HexNAc_5NeuAc_1Fuc_1$	1.41	1.12
$Hex_5HexNAc_5NeuAc_1$	1.22	0.97
$Hex_5HexNAc_4NeuAc_2$	1.33	1.06
$Hex_5HexNAc_5NeuAc_1Fuc_1$	1.26	1.02
$Hex_6HexNAc_5NeuAc_1$	1.33	1.03
$Hex_5HexNAc_4NeuAc_2Fuc_1$	1.39	1.05
$Hex_5HexNAc_5NeuAc_2Fuc_1$	1.55	1.10
$Hex_6HexNAc_5NeuAc_3$	1.66	1.19

Table S2 presents the glycan corrected and uncorrected abundance ratios. The data were corrected by using the ratio of the spiked-in internal standard.

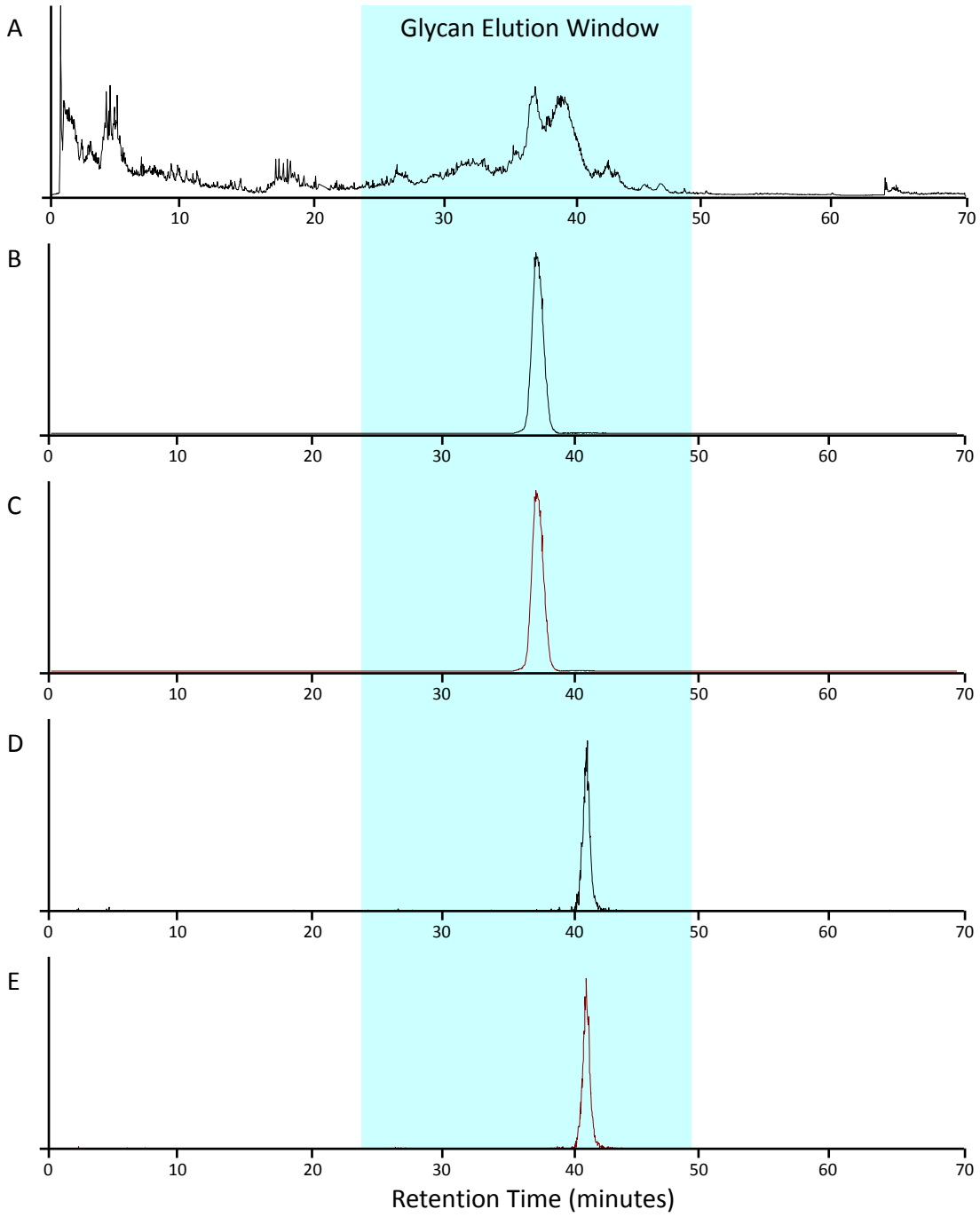
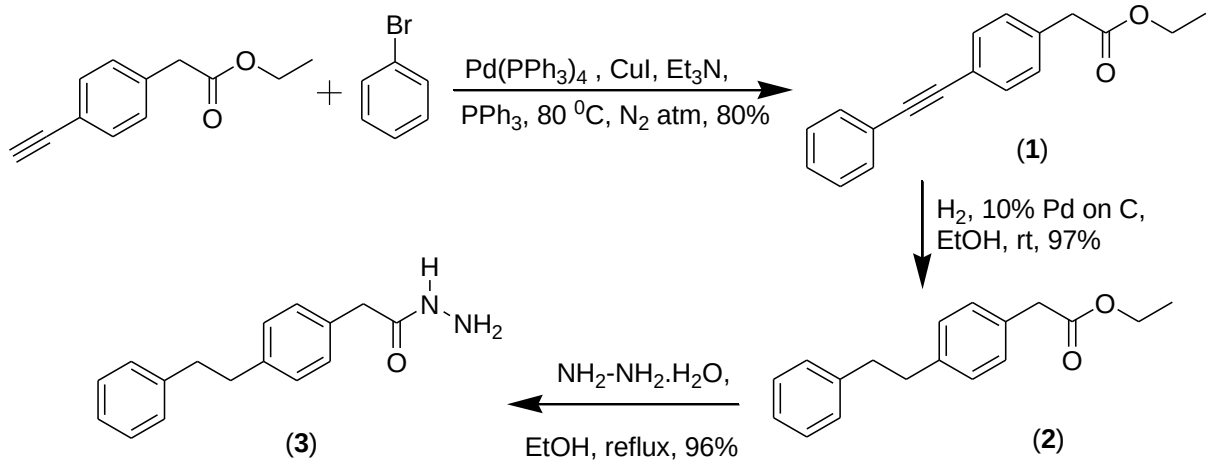


Figure S1 shows a total ion chromatogram in part A. Parts B and C are the 'light' and 'heavy' EIC's of the $Hex_5HexNAc_4NeuAc$ glycan, respectively. Parts D and E are the 'light' and 'heavy' EIC's of the $Hex_5HexNAc_5NeuAc_2Fuc$ glycan, respectively.

Reaction Scheme:

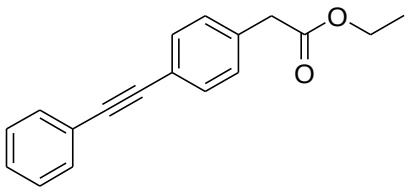


Experimental Section

General. The chemicals; bromobenzene, hydrazine monohydrate, tetrakis(triphenyl phosphine) palladium(0), triphenyl phosphine, copper(I)iodide, triethyl amine, palladium on charcoal were purchased from Sigma-Aldrich. Ethyl-4-ethynylphenylacetate was purchased from Spectra Group. Ltd. Inc. Isotopically labeled bromobenzene (^{13}C6, 99%) was purchased from Cambridge Isotope Laboratories. Triethyl amine was dried with anhydrous sodium sulphate prior to use and all other chemicals were used as received. The Sonogashira coupling reaction was performed either inside a dry box filled with nitrogen gas or under nitrogen atmosphere. ^{1}H and ^{13}C NMR data were recorded on Mercury 300 or 400 MHz spectrometers (300 or 400 MHz for ^{1}H, 75 or 100 MHz for ^{13}C NMR) at room temperature. The chemical shift values were reported relative to TMS (δ = 0.00 ppm) as an internal standard. IR spectra were obtained from JASCO FT/IR-410. Wave numbers in cm^{-1} are reported for characteristic peaks. Mass spectra were obtained at the NCSU Department of Chemistry Mass Spectrometry Facility using electro spray ionization (ESI) on an Agilent Technologies 6210 LC-TOF mass spectrometer.

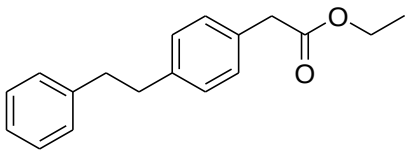
(A) Synthesis of unlabeled compounds:

Synthesis of ethyl-2-(4-(2-phenylethynyl) phenyl) acetate, (**1**)



Bromobenzene (0.464g, 2.848 mmol) was added to ethyl-2-(4-ethynylphenyl)acetate (0.50g, 2.66mmol) taken in a clean and dry vial with a magnetic stir bar. $Pd(PPh_3)_4$ (0.150g, 0.129 mmol), PPh_3 (0.120g, 0.457 mmol) and triethyl amine (5.0mL) were added in sequence to the reaction mixture while stirring under nitrogen atmosphere. The reaction mixture was then heated in an oil bath at 80 °C for six hours. The completion of the reaction was determined from FTIR analysis of the reaction mixture by complete disappearance of the sharp absorption band at 3290 cm^{-1} of terminal alkyne C−H. The crude product was extracted from the heterogeneous mixture by hexane and further purified by column chromatography on silica gel using hexane: ethyl acetate mixture (9:1 v/v) to give **1** (0.580g, 80% yield) as clear oil. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) 7.53-7.18(m, 9H), 4.14(q, *J* = 7.2 Hz, 2H), 3.60(s, 2H), 1.24(t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 171.8, 134.9, 132.3, 132.2, 129.9, 128.9, 128.8, 123.8, 122.6, 90.08, 89.7, 61.6, 41.9, and 14.8; IR (KBr film, cm^{-1}) 3062, 3033, 2981, 2931, 1735(carbonyl ester).

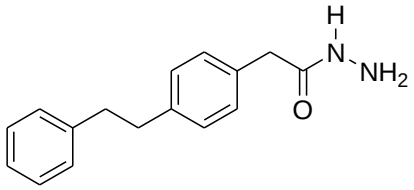
Synthesis of ethyl-2-(4-phenethylphenyl) acetate, (**2**)



To the stirring solution of (**1**),ethyl-2-(4-(2-phenylethynyl) phenyl) acetate, (0.56g, 2.072 mmol) in 5.0 mL absolute ethanol was added 10% Pd on charcoal (0.05g, ~9% w/w). The mixture was stirred

under hydrogen atmosphere for four hours. The completion of the reaction was determined by TLC on silica gel plate using mixture of hexane and ethyl acetate (9:1 v/v). The mixture was filtered through a short column of silica gel. The solvent (ethanol) was removed by rotavap and the product was dried under reduced pressure to give **2** (0.551g, 97% yield) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) 7.30-7.12 (m, 9H), 4.14(q, *J* = 7.2 Hz, 2H), 3.57(s, 2H), 2.89(s, 4H), 1.24(t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 172.4, 142.3, 141.1, 132.2, 129.8, 129.2, 129.0, 128.9, 126.5, 61.4, 41.6, 38.4, 38.1, 14.8; IR (KBr film, cm^{-1}) 3085, 3058, 3025, 2981, 2931, 2857, 1735(carbonyl ester).

Synthesis of ethyl-2-(4-phenethylphenyl)acetohydrazide, (**3**)

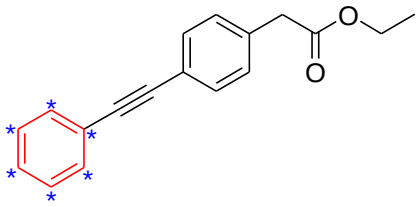


To the stirring solution of ethyl-2-(4-phenethylphenyl) acetate (**2**) (0.535g, 1.95 mmol) in 0.7mL absolute ethanol was added hydrazine hydrate (0.488g, 9.75 mmol) at room temperature under nitrogen atmosphere. The mixture was then refluxed for two hours. The completion of the reaction was determined by TLC on silica gel using mixture of hexane and ethyl acetate (9:1 v/v). Solvent was removed by rotavap and the product was purified by recrystalization from isopropanol. The white crystalline solid was dried under reduced pressure to give **3** (0.487g, 96% yield). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) 9.22(s, 1H), 7.29-7.12(m, 9H), 4.20 (s, 2H), 3.29 (s, 2H), 2.83 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm) 170.3, 142.2, 140.2, 134.3, 129.4, 129.0, 128.9, 128.8, 126.4, 40.7, 37.7, 37.3; IR (KBr film, cm^{-1}) 3351, 3208, 3080, 3050, 3023, 2981, 2969, 2933, 2911, 2850, 1648, 1619.

(B) Synthesis and characterization of Stable Isotope Labeled (SIL) compounds

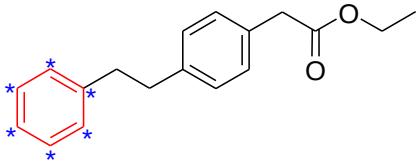
The similar procedure as above was followed except that the SIL bromobenzene (13C6, 99%) was used as a starting material.

Characterization of **SIL** (**1**); **ethyl-2-(4-(2-phenylethynyl) phenyl) acetate**



^{1}H NMR (400MHz, $CDCl_3$, δ ppm) 7.73-7.71(m, br, 1H), 7.53-7.48(m, overlap, 3H), 7.33-7.26 (m, overlap, 3H), 7.15-7.12(m, br, 2H), 4.15(q, *J* = 7.2 Hz, 2H), 3.62(s, 2H), 1.25(t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 171.8, 134.9, 132.8-130.7(m overlap), 129.5-127.5(m, overlap), 124.0-122.7(m, overlap), 61.2, 41.5, and 14.4; IR (KBr film, cm^{-1}) 3052, 2981, 1733(carbonyl ester). HRMS: Calculated for [M+H]: 271.1424; found: 271.1425.

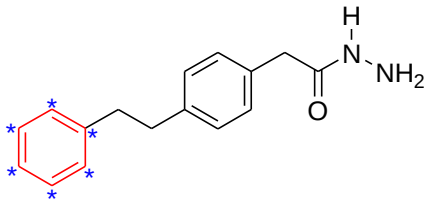
Characterization of **SIL**(**2**); **ethyl-2-(4-phenethylphenyl) acetate**



^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 6.98-7.47 (m, 9H), 4.14 (q, *J* = 7.2 Hz, 2H), 3.58 (s, 2H), 2.90 (s, 4H), 1.25(t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 171.8, 142.5-141.3(m, overlap), 132.2, 129.4, 129.3-128.3(m, overlap), 128.0-125.2(m, overlap), 61.0, 41.2, 38.0, 37.7, 14.4; IR (KBr

film, cm^{-1}) 3046, 2981, 2929, 2857, 1735(carbonyl ester); HRMS: Calculated for [M+H]: 275.1737; found: 275.1740

Characterization of SIL(**3**); **ethyl-2-(4-phenethylphenyl)acetohydrazide**



^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) 9.18(s, 1H), 7.46-6.98 (m, 9H), 4.20 (s, 2H), 3.29 (s, 2H), 2.83 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm) 169.6, 142.3-140.5(m, overlap), 139.8, 133.6, 129.0-124.8(m, overlap), 37.2, 36.8, 36.6; IR (KBr film, cm^{-1}) 3349, 3208, 3048, 3031, 2933, 2911, 2850, 1647, 1618.; HRMS: Calculated for [M+H]: 261.1693; found: 261.1693.