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CS type A ((GlcAβ3GalNAc4Sulfateβ4)$_n$), CSB ((IdoAα3GalNAc4Sulfateβ4)$_n$) CSC ((GlcAβ3GalNAc6Sulfate)$_n$), and chondroitinase ABC, B and AC1 were obtained from Seikagaku America/Associates of Cape Cod (Falmouth, MA). Purified Δ-disaccharide standards were purchased from V-labs (Covington, LA). 2-anthranilic acid, Methylene Chloride (MC), and Piperdine (20 % in DMF) were purchased from Fluka Chemika (Buchs, Switzerland). Dimethyl formamide (DMF), ethanol (EtOH), *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), 1-Hydroxybenzotriazole (HOBt) , Fmoc- protected Rink resin, *N*-(9-Fluorenylmethoxycarbonyl)-L-alanine (Fmoc-d_0-Ala), diisopropylethyl amine (DIEA), piperdine (20 % in DMF), triisopropyl silane (TIPS), Trifluoroacetic acid (TFA), ninhydrin, 4-isopropylanaline, 1-1-adamantyl-ethyl amine, *p*-phenylenediamine, succinic anhydride, aminobenzamide, *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), and sodium cyanoborohydride were from Aldrich Chemicals Co (St. Louis, MO). *N*-(9-Fluorenylmethoxycarbonyl)-L-alanine (Fmoc-d_4-Ala) was from C/D/N Isotopes (Quebec, Canada). Solid phase extraction tubes (1 ml) were purchased from Supelco (Bellefonte, PA). Cellulose packing material Micro Spin Columns were purchased from Harvard Apparatus (Holliston, MA). Peptide-*N*-Glycosidase F (PNGase F) was purchased from PROzyme, (San Leandro, CA.) Porous graphitized carbon columns (SuperSil) were purchased from Thermo Fisher Scientific (Waltham, MA). C18 Macrospin columns were purchased from Nest Group (Southborough, MA).

In a typical reaction, Tags (**2-5**) were synthesized by standard manual solid phase peptide synthetic methods. Reactions were carried out in 1 ml polypropylene syringe barrels (Supelco) utilizing a polyethylene frit (0.2 μm). In a typical reaction sequence Rink resin (Aldrich, 0.6 mmol/g loading, 50 mg) was treated with piperdine:DMF (2:8) (1x min, 1x 20 min) to remove the Fmoc- protecting group. After removal of the piperidine solution the resin was washed with DMF (3x 1 min), methylene chloride (3x 1 min), ethanol (1x 3 min), methylene chloride (3x 1 min), DMF (3x 1 min) providing the resin bound free amine. Three equivalents of Fmoc protected amino acid (d_0 or d_4), HBTU, HOBt, and diisopropylethyl amine (DIEA) were preactivated for 5-8 min in DMF (0.6 ml, 300 mM final

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concentration), after which they were added to the resin and reacted for 2 hours. After 2 hours, the excess reagent was filtered off and the resin was washed with DMF (3x 1 min), MC (3x 1 min), and ethanol (1x 3 min) to remove any residual reactants. The resin was rinsed with MC (3x 1 min) and DMF (3x 1 min) in preparation for deprotection. The resin was treated with piperdine:DMF (2:8) (1x min, 1x 20 min). After washing, the resin was ready for the addition of the next amino acid. This cycle was repeated until the desired length had been achieved. The compounds were then cleaved from the resin using a trifluoroacetic acid (TFA): triisopropyl silane (TIS): water (95:2.5:2.5) solution for 2 hours under nitrogen, followed by precipitation in cold ether. The resulting compounds were purified to homogeneity by reverse-phase HPLC utilizing a C18 column (Vydac, 10 mm ID x 250 mm) on a 5-15% gradient of methanol:water. Purified compounds were analyzed by ESI-MS on a Quattro II mass spectrometer to confirm their identity.

Tags lacking a terminal carboxamide (**6-8**) were synthesized in solution phase. In a typical reaction, 0.25 mmol of succinic anhydride was dissolved in 1.5 ml ACN, followed by addition of the desired amine (0.25 mmol). The reaction was heated at 60°C for 4 hrs. The resulting monoacidic compound was purified by silica gel chromatography (ethyl acetate:hexane). The free acid was activated by *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) in MC in the presence of 2 eq. DIEA, followed by addition of 10 equivalents of 1,4-diaminobenzene for 2 hours at room temperature. The desired products were purified to homogeneity via silica gel chromatography and/or reverse-phase HPLC utilizing a C18 column (Vydac). Purified compounds were analyzed by ESI-MS on a Quattro II mass spectrometer to confirm their identity.

The partial digestion of chondroitin sulfate A, B, and C was achieved by the action of chondroitinase ABC on the desired substrate was done as previously described. A complete digestion was used to obtain the absorbance value equal to complete conversion to disaccharides. From this value a 10, 20, 30 and 40% digestion value was determined. Briefly, 10 μg of chondroitin substrate was digested in a solution containing 5 μl 1 M Tris-HCl (pH 7.4), 1 μl 1 M NH_4OAc, and 2 mU chondroitinase enzyme in

100 μl water. The digestion was terminated at the proper absorbance value by boiling in water for two minutes.

All oligosaccharides were derivatized with d_0-**1**-**8**, d_0-, d_4-, d_8- or d_{12}-**3** according to the method of Bigge et al.[28] with slight modification. Briefly, CSA samples were dried in the presence of the desired label (in 10% MeOH). The dried sample was then dissolved in 10 μL of a reaction reagent DMSO/glacial acetic acid (7:3) containing 1.0 M sodium cyanoborohydride. The glycan solutions were vortexed then centrifuged and incubated at 65 °C for 3 h. Excess reagents were removed with cellulose microspin columns as follows. The column was first hydrated with five 200 μL volumes of water, washed with five 200 μL volumes of 30% acetic acid solution, and then equilibrated with three 200 μL volumes of acetonitrile. The derivatized reaction mixture was applied to the column allowing 10 min for it to adsorb to the cellulose. Excess reagent was washed off with three 200 μL volumes of acetonitrile followed by two 200-μL volumes of 96% acetonitrile. The derivatized glycan was then eluted with two 100-μL volumes of water and dried. Derivatized glycan samples were fractionated using high performance SEC. Briefly, the column (Superdex Peptide 3.2/30, Amersham Biosciences, Piscataway, NJ) was equilibrated in 10% acetonitrile, 0.05 M ammonium formate solution at 40 μL/min and the oligosaccharide mixture (10 μL) was injected with UV detection at 232 nm. Samples were lyophilized three times in equal amounts of water to remove the volatile salts prior to MS analysis.

The *N*-Linked glycans of bovine fetuin (Sigma, St Louis, MO) were released from their proteins by the action of *N*-Glycanase® (Peptide-*N*-Glycosidase F, PROzyme, San Leandro, CA) according to the manufacturer's protocol. Briefly, 100 pmol (6 μg), 200 pmol (12 μg), 300 pmol (18 μg), and 400 pmol (24 μg) quantities of fetuin were dissolved in 45 μl reaction buffer (10 mM Tris-HCl, pH 8.0), 2.5 μl of denaturation solution was added (2% SDS, 1 M β-mercaptoethanol) for a final concentration of 0.1 % SDS, 50 mM β-mercaptoethanol. The tubes were heated at 100°C for 5 minutes. The denatured glycoproteins were allowed to cool to room temperature and 2.5 μL 15% NP-40 in water was added

(final concentration 0.75%). 1 µl of enzyme solution was added (2.5 mU enzyme) and the reaction was incubated at 37°C for 3 hours. Glycan release was confirmed by SDS-PAGE. Excess detergent, buffer salts, and the core proteins were removed by porous graphitized carbon chromatography (SuperSil, Thermo Fisher Scientific, Waltham, MA). Glycans were bound to a pre-equilibrated column in water containing 0.1% TFA. The column was washed three times with water/0.1 %TFA (1ml), the glycans were then eluted with 30% acetonitrile containing 0.1% TFA.

The glycan containing fractions were derivatized with d_0-, d_4-, d_8- or d_{12}-**3** previously described method. Briefly, *N*-linked samples were dried in the presence of the desired label (in 10% MeOH). The dried sample was dissolved in 10 µL of a reaction reagent DMSO/glacial acetic acid (7:3) containing 1.0 M sodium cyanoborohydride. The glycan solutions were vortexed then centrifuged and incubated at 65°C for 3 h. Samples were allowed to cool to room temperature and 200 µl of ddH2O was added. Excess tag was removed by a combination of solvent extraction and spin column chromatography. 200 µl portions of chloroform were added and the solution was allowed to partition, the chloroform layer was then removed, this step was repeated two times. The resulting aqueous solution was added to a pre-equilibrated (according to manufacturer's protocol) C18 MacroSpin column (Nest Group, Southborough, MA), the columns were washed with 250 µl water (3x), the tagged glycans were eluted in 5% methanol/water, and excess tag was eluted in 10% methanol/water fractions. The samples were dried in preparation for analysis.

Samples were analyzed off-line using nanospray introduction via a standard electrospray ion source into a QSTAR Pulsar i quadrupole orthoganol time-of-flight mass spectrometer (Applied Biosystems/MDS-Sciex, Toronto, Cananda). Samples were dissolved in water, then diluted in an isopropanol:water (1:9) solution containing 0.1% formic acid to a final concentration of 1 pmol/µl. Aliquots of 5 µl were sprayed using nanospray tips with a 1 µm orifice pulled in-house using a capillary puller (Sutter Instrument P80/PC micropipette puller, San Rafael, CA). An ionization potential of -1150 V produced a stable signal. All scans were acquired in the negative ion mode, and all spectra were

calibrated externally. The collision energy for tandem work involving CS tetramers were set to a level where the precursor ion remained the most abundant signal in the spectrum, the best results were obtained with a collision energy of -20 V, CAD gas 2. The MS/MS scans were summed for the -2 charge state for the CS oligosaccharides; this corresponded to one negative charge per sulfate group.