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RESEARCH ARTICLE

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Synthesis of ^{13}C-labelled sulfated *N*-acetyl-D-lactosamines to aid in the diagnosis of mucopolysaccharidosis diseases

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Morquio A syndrome is an autosomal mucopolysaccharide storage disorder that leads to accumulation of keratan sulfate. Diagnosis of this disease can be aided by measuring the levels of keratan sulfate in the urine. This requires the liquid chromatography tandem mass spectrometry (LCMS/MS) measurement of sulfated *N*-acetyl-D-lactosamines in the urine after cleavage of the keratan sulfate with keratanase II. Quantification requires isotopically-labelled internal standards. The synthesis of these $^{13}C_6$-labelled standards from $^{13}C_6$-galactose and *N*-acetylglucosamine is described. The required protected disaccharide is prepared utilising a regioselective, high yielding β-galactosylation of a partially protected glucosamine acceptor and an inverse addition protocol. Subsequent synthesis of the $^{13}C_6$-labelled mono and disulfated *N*-acetyllactosamines was achieved in five and eight steps, respectively, from this intermediate to provide internal standards for the LCMS/MS quantification of keratan sulfate in urine.

KEYWORDS

^{13}C-labelled, keratan sulfate, lactosamine, Morquio A, MPS IVA, sulfated

1 | INTRODUCTION

Morquio A syndrome (mucopolysaccharidosis IVA, MPS IVA) is a rare autosomal recessive lysosomal storage disease disorder caused by deficient *N*-acetylgalactosamine-6-sulfatase (GALNS; EC 3.1.6.4) activity.[1,2] GALNS catabolises keratan sulfate (KS), a glycosaminoglycan that consists of sulfated β(1 → 3) repeats of a galactosyl β(1 → 4)-*N*-acetylglucosamine disaccharide (Figure 1).[3,4] Deficiencies in GALNS lead to accumulation of KS resulting in progressive tissue and organ dysfunction. Early and accurate diagnosis of this condition is critical for improved patient outcomes, particularly as enzyme replacement therapy has recently become available.[5] KS level in urine is a biomarker for MPS IVA and so a liquid chromatography tandem mass spectrometry (LCMS/MS) method has been developed that provides a sensitive and specific means for quantitation of urinary KS.[6,7] Additionally, enzyme activity testing of GALNS is essential in diagnosing MPS IVA with fibroblasts and leukocytes being the recommended sample types.[8] However, there is still a need in the screening and enzyme replacement therapy dosage monitoring settings for an accurate and rapid measurement, meaning that LCMS/MS analysis of urine is still a key diagnostic tool.

In this method, keratanase II[9] is used to selectively hydrolyse urinary KS into its key disaccharides, mono and disulfated *N*-acetyllactosamine, and this mixture is analysed by LCMS/MS. Addition of isotopically-labelled versions of these disaccharides is required for quantification of the results. Synthetic non-labelled material is also valuable for use in calibrating the method. Because of the appreciable natural abundance of ^{34}S (4.2%), and the fact that the disulfated *N*-acetyllactosamines contains two sulfur atoms, there needs to be sufficient isotopic labelling to increase the mass by five or greater Daltons to fully

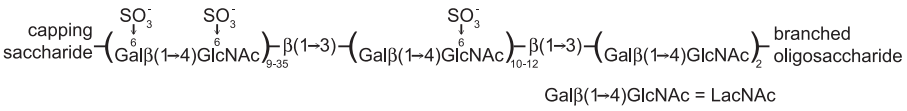


FIGURE 1 Structure of keratan sulfate

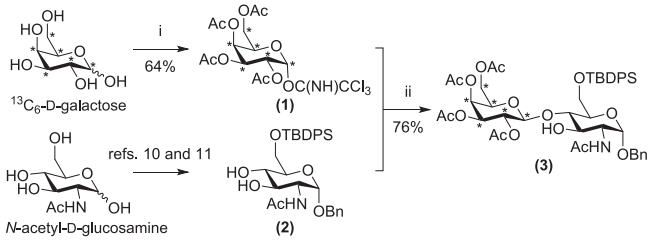
separate the internal standard mass peaks from the analyte natural abundance isotope peaks to facilitate quantification. Lactosamine derivatives labelled to this extent are not readily available, and therefore, herein, we describe syntheses of mono and disulfated *N*-acetyllactosamines (**8** and **16**, Schemes 3 and 4) from their constituent monosaccharides, allowing the introduction of six heavy atom carbons via the use of the readily available $^{13}C_6$-galactose as a key raw material.

2 | RESULTS AND DISCUSSION

There have been several syntheses of unlabelled keratan sulfate oligosaccharide fragments[10-14] as well as more specifically, unlabelled sulfated lactosamines.[15-18] Apart from Ogawa's work,[12] these reports generate the final products as glycosides, or in one case, the oxazoline rather than the *N*-acetyllactosamine required here where the glucosamine *O*-1 is not substituted. As part of our synthetic plan, for efficiency in monosaccharide building block preparation, we decided to take advantage of a selective glycosylation of a partially protected *N*-acetylglucosamine 3,4-diol previously employed by Roy.[19] Route development was undertaken using D-galactose, allowing the generation of the unlabelled variants for use as calibration controls in urine analysis. The labelled variants were then produced in an analogous manner using $^{13}C_6$-D-galactose.

2.1 | Preparation of key intermediate (3)

The galactosyl trichloroacetimidate donor (**1**) (Scheme 1) was synthesised in three steps from D-galactose in 64% yield. Following established methods for unlabelled galactose,[20] peracetylation of $^{13}C_6$-labelled galactose in anhydrous pyridine followed by anomeric deprotection with ethanolamine in ethyl acetate, followed by treatment with trichloroacetonitrile and DBU afforded the donor (**1**) as an anomeric mixture. The α-anomer was isolated by flash chromatography on silica gel, generating a highly-crystalline product as the major product, and this was subsequently used in the glycosylation reaction. Following literature procedures, the glycosylation acceptor (**2**) was prepared by anomeric *O*-benzyl protection of *N*-acetylglucosamine,[21] followed by selective 6-*O*-*tert*-butyldiphenylsilylation.[22] Glycosylation of the TBDPS-protected diol acceptor (**2**) with donor (**1**) in anhydrous DCM in the presence of TMSOTf at -50°C using a conventional procedure in which the catalyst was added to a mixture of donor and acceptor yielded very little desired product. However, when the reaction was carried out using an inverse addition procedure, in which the acceptor (**2**) and TMSOTf were premixed prior to donor (**1**) addition,[23] it proved to be highly regio- and stereo-selective in accordance with literature findings.[19] The glycosylation reaction using the inverse addition procedure, under conditions of excess donor (two equivalents), furnished the product as a single β-linked anomer (**3**) in quantitative yield after chromatography. As the $^{13}C_6$-labelled donor was deemed valuable, this reaction was modified to use just one equivalent of donor (**1**) and then the labelled disaccharide product was isolated in a reduced but acceptable yield of 76%. Regio- and stereo-chemistry were confirmed by nuclear magnetic resonance (NMR).[19] Assignment of anomeric protons by a heteronuclear single quantum correlation (HSQC) experiment revealed a $J_{1',2'} = 8.1$ Hz in the ^{1}H NMR, which is indicative of the desired β-anomer stereochemistry for the newly formed glycosidic bond. A large down field shift from 3.80 to 5.17 of *H*-3 (as assigned by HSQC and ^{1}H—^{1}H homonuclear correlation spectroscopy (COSY)) upon acetylation of *O*-3 in the preparation of compound (**4**) confirms the regiochemistry of the glycosylation to be at *O*-4.



SCHEME 1 Synthesis of the key disaccharide (**3**) from $^{13}C_6$-D-galactose and *N*-acetyl-D-glucosamine. Conditions: (i) a. Ac_2O/Py, 50°C, 5 h, 20°C overnight; b. ethanolamine, 20°C, overnight; c. trichloroacetonitrile, DBU, 20°C, 2 h; (ii) TMSOTf, DCM, 4 Å molecular sieves, -50°C, 1 h → 20°C, 1 h. * denotes ^{13}C-labelling

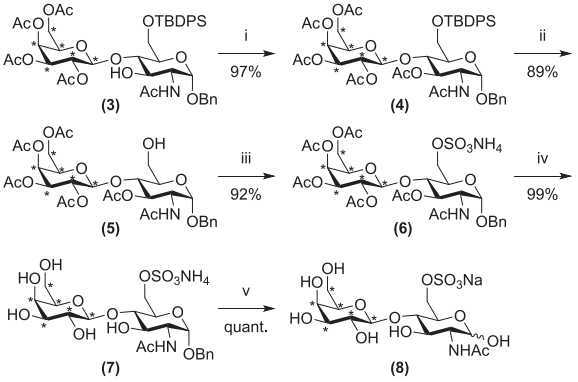
2.2 | Preparation of 6-O-sulfated LacNAc

The target $^{13}C_6$-labelled monosulfated LacNAc (**8**) was prepared from the key intermediate (**3**) in five steps

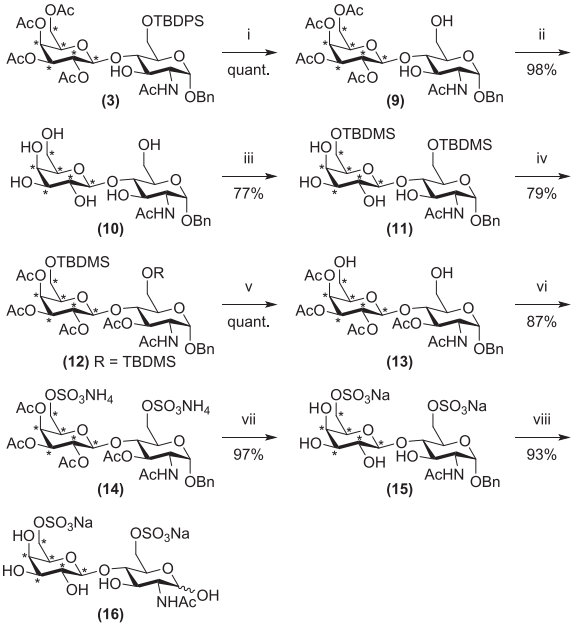
(Scheme 2). Acetylation of key intermediate (**3**) gave the fully-protected disaccharide (**4**) in 86% yield. Attempts to remove the TBDPS protecting group with tetrabutylammonium fluoride led to deacetylation. Treatment of disaccharide (**4**) with HF-pyridine in THF overnight at room temperature yielded the desired monohydroxy disaccharide (**5**) in good yield. Sulfation of the monohydroxy disaccharide (**5**) with sulfur trioxide trimethylamine complex in anhydrous *N*,*N*-dimethylformamide at 50°C gave the monosulfated disaccharide (**6**) as the ammonium salt following chromatographic purification. Zemplén deacetylation with sodium methoxide in methanol gave the desired deacetylated product (**7**) in excellent yield following chromatography on silica gel. Hydrogenolysis in the presence of $Pd(OH)_2/C$ in ethanol and aqueous ammonia afforded the target monosulfated LacNAc as the ammonium salt. Ion exchange chromatography (Dowex 50WX8-200, sodium form) of the ammonium salt provided the final product (**8**) as the desired sodium salt.

2.3 | Preparation of 6,6-O-disulfated LacNAc

The target $^{13}C_6$-disulfated LacNAc (**16**) was prepared from the key disaccharide (**3**) in eight steps (Scheme 3). Initially, we attempted the preparation of the unlabelled target material (equivalent of **16**) using similar chemistry to that shown in Scheme 3, but without first removing the TBDPS group from disaccharide (**3**). This led to the unlabelled version of protected disaccharide (**12**) where R = TBDPS. During the silyl deprotection of this material (**12**, R = TBDPS, unlabelled) using HF-pyridine, we



SCHEME 2 Synthesis of monosulfated LacNAc. Conditions: (i) Ac_2O, Py, 0°C, 3 h → 20°C, overnight; (ii) HF·Py, THF, Py, 0°C → 20°C, overnight; (iii) SO_3·NMe_3, DMF, 50°C, 16 h; (iv) NaOMe, MeOH, 20°C, 1 h; (v) $Pd(OH)_2/C$, H_2, EtOH, aq. NH_4OH, 20°C, 5 h, Dowex 50WX8-200 (Na^+ form). * denotes ^{13}C-labelling



SCHEME 3 Synthesis of disulfated LacNAc (**16**). Conditions: (i) HF·Py, THF, Py, 0°C → 20°C, 2 days; (ii) NaOMe, MeOH, 20°C, 1 day; (iii) TBDMSCl, DMF, imidazole, 0°C, 3 h; (iv) Ac_2O, Py, 0°C → 20°C, overnight; (v) HF·Py, THF, Py, 0°C → 20°C, 2 h; (vi) SO_3·NMe_3, DMF, 50°C, 20 h; 7 M NH_3/MeOH, 20°C 15 min; (vii) NaOMe, MeOH, 20°C, 1 h; (viii) Pd $(OH)_2/C$, H_2, EtOH, aq. NH_4OH, 20°C, overnight, Dowex 50WX8-200 (Na^+ form). * denotes ^{13}C-labelling

observed rapid removal of the TBDMS group, but extended reaction times were required to remove the remaining TBDPS group. The prolonged reaction time resulted in significant amounts (approximately 15%) of a side-product resulting from an acetyl migration from *O*-4 to *O*-6 on the galactosyl unit. Hence, the synthesis was modified to install two labile TBDMS groups at the 6-positions of both sugars (unlabelled version of **11**). In the future, we would trial the preparation and use of the TBDMS-protected version of the *N*-acetylglucosamine building block (**2**), to improve yields and shorten the synthesis of disulfate (**16**) by one step.

Therefore, deprotection of disaccharide (**3**) was carried out using HF-pyridine and yielded disaccharide (**9**) in quantitative yield. Complete de-*O*-acetylation of disaccharide (**9**) was achieved under Zemplén conditions (sodium methoxide in methanol). Selective 6-*O* disilyl protection followed by peracetylation afforded the fully protected LacNAc (**12**). Desilylation using HF-pyridine provided diol (**13**), which was subsequently sulfated using sulfur trioxide trimethylamine complex in anhydrous *N*,*N*-dimethylformamide at 50°C to give the disulfated product (**14**), which was isolated as the ammonium salt. Saponification with sodium methoxide

in anhydrous methanol at ambient temperature led to the desired deacetylated product (**15**) in good yield. Hydrogenolysis of benzyl glycoside (**15**) in the presence of $Pd(OH)_2/C$ in ethanol and aqueous ammonia afforded the target disulfated $^{13}C_6$-labelled LacNAc (**16**), which was passed as an aqueous solution through a strong anion-exchange resin in the sodium form (Dowex 50WX8-200) to ensure the product was the disodium salt.

3 | CONCLUSIONS

A highly regio- and stereo-selective glycosylation of a partially protected *N*-acetylglucosamine acceptor was used to prepare a key $^{13}C_6$-labelled disaccharide (**3**). This key disaccharide (**3**) was then used as a common intermediate for the reasonably high-yielding synthesis of $^{13}C_6$-labelled mono and disulfated *N*-acetyllactosamine derivatives for use as internal standards to aid in the diagnosis of MPS IVA. The synthesis of monosulfated *N*-acetyllactosamine (**8**) was achieved in eight steps with 38% overall yield from $^{13}C_6$-labelled galactose, whereas the synthesis disulfated *N*-acetyllactosamine (**16**) was achieved in 11 steps with 23% overall yield from the labelled galactose.

4 | EXPERIMENTAL

4.1 | General methods

Thin-layer chromatography (TLC) was performed on aluminium sheets coated with 60 F_{254} silica gel and visualised under UV light and/or with a ceric ammonium molybdate dip. Chromatography (flash column or an automated system with continuous gradient facility) was performed on silica gel (40-63 μm). NMR spectra were recorded on a Bruker Advance^{III} 500 instrument at 500 MHz (^{1}H) referenced to TMS δ 0.0 ppm and 125 MHz (^{13}C) referenced to residual solvent peak, $CDCl_3$, δ 77.0 ppm; CD_3OD δ 49.0 ppm. High resolution mass spectra (HRMS) were recorded on a Waters Q-TOF Premier Mass Spectrometer with electro-spray ionisation (ESI). Unlabelled 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose was purchased from Aldrich and used as received. $^{13}C_6$-Galactose was purchased from Omicron Biochemicals, Inc. The ^{1}H NMR are ^{13}C decoupled. Coupling constants observed in ^{13}C-NMR spectra are indicated for the ^{13}C containing products.

4.2 | Synthesis of benzyl (2,3,4,6-tetra-*O*-acetyl-β-$^{13}C_6$-D-galactopyranosyl)-(1 → 4)-2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (3)

4.2.1 | 2,3,4,6-Tetra-O-acetyl-β-$^{13}C_6$-D-galactopyranosyl 2,2,2-trichloroacetimidate (1)

Acetic anhydride (12 mL, 7.7 eq) was added to a slurry of $^{13}C_6$-D-galactose (3.00 g, 1 eq) in anhydrous pyridine (24 mL) at 20°C. The mixture was stirred at 50°C for 5 h then overnight at 20°C. The solvent was removed under reduced pressure and the resulting residue was redissolved in ethyl acetate (150 mL) and washed with 0.5 M HCl (100 mL). The aqueous phase was re-extracted with ethyl acetate (150 mL), and the combined organic phases were washed with saturated aqueous $NaHCO_3$ (200 mL) followed by water (200 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield $^{13}C_6$-1,2,3,4,6-penta-*O*-acetyl-D-galactose as a colourless oil (6.40 g, quantitative), which crystallised over time. HRMS (ESI) calcd for $C_{10}{}^{13}C_6H_{22}O_{11}Na$ $[M + Na]^+$ m/z 419.1261, found: 419.1252.

A solution of 1,2,3,4,6-penta-*O*-acetyl-$^{13}C_6$-D-galactose (6.05 g, 1 eq) and ethanolamine (1.9 mL, 2.0 eq) in ethyl acetate (100 mL) was stirred at 20°C overnight. The solution was washed with water and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure, giving the tetra-acetate as a colourless syrup. This was dissolved in anhydrous dichloromethane (60 mL), then trichloroacetonitrile (5.30 mL, 4.88 eq), and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.080 mL, 0.050 eq) were added. After 2 h, the solution was diluted with dichloromethane, washed with saturated aqueous $NaHCO_3$ and water, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure, yielding a sticky orange solid. This mixture was purified by column chromatography (0:1 to 1:1 ethyl acetate: petroleum ether), giving a white solid **1** (α isomer, 3.36 g, 64%) and then a colourless oil, which slowly crystallised (β isomer, 0.78 g, 15%). HRMS (ESI) calcd for $C_{10}{}^{13}C_6H_{20}NO_{10}Cl_3Na$ $[M + Na]^+$ m/z 520.0259, found: 520.0246. Alpha anomer: ^{1}H NMR ($CDCl_3$) δ (ppm): 2.01 (s, 3H), 2.02 (s, 3H), 2.02 (s, 3H), 2.16 (s, 3H), 4.08 (dd, J = 11.4, 6.6 Hz, 1H), 4.16 (dd, J = 11.3, 6.6 Hz, 1H), 4.44 (td, J = 6.7, 1.3 Hz, 1H), 5.36 (dd, J = 10.8, 3.5 Hz, 1H), 5.43 (dd, J = 10.8, 3.2 Hz, 1H), 5.56 (dd, J = 3.1, 1.3 Hz, 1H), 6.60 (d, J = 3.5 Hz, 1H), 8.66 (s, 1H). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.8, 20.8, 20.9, 20.9, 61.5 (d, J = 47.0 Hz), 67.2 (m), 67.7 (m), 67.8 (m), 69.3 (m), 91.1, 93.8 (d, J = 42.6 Hz), 161.2, 170.2, 170.3,

170.3, 170.5. Beta anomer: ^{1}H NMR ($CDCl_3$) δ (ppm): 1.95 (s, 3H), 1.97 (s, 3H), 1.99 (s, 3H), 2.13 (s, 3H), 4.05–4.10 (m, 2H), 4.14–4.17 (m, 1H), 5.09 (dd, J = 10.4, 3.4 Hz, 1H), 5.42 (dd, J = 3.5, 1.2 Hz, 1H), 5.44 (dd, J = 10.4, 8.3 Hz, 1H), 5.80 (d, J = 8.2 Hz, 1H), 8.71 (s, 1H). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.5, 20.6, 20.6, 20.6, 60.9 (dt, J = 47.3, 4.6 Hz), 66.8 (t, J = 38.9 Hz), 67.8 (dd, J = 48.5, 42.8 Hz), 70.7 (br. t, J = 40.8 Hz), 71.8 (dd, J = 47.3, 38.9 Hz), 90.4, 96.1 (dt, J = 48.2, 5.9 Hz), 161.0, 169.0, 170.0, 170.2, 170.3.

4.2.2 | Benzyl 2-acetamido-2-deoxy-6-O-tert-butyldiphenylsilyl-4-O-(2,3,4,6-tetra-O-acetyl-β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranoside (3)

A solution of **1** (5.47 g, 1 eq) in anhydrous dichloromethane (15 mL) was stirred under argon at 20°C with 4 Å molecular sieves (2 g) for 30 min, then cooled to −50°C. TMSOTf (1.89 mL, 1.03 eq) was added and the solution stirred at −50°C for 15 min. A solution of **2** (6.58 g, 1.34 eq)[21,22] in anhydrous dichloromethane (15 mL) was added dropwise over 30 min, with stirring at −45°C continued for a further 45 min. The resulting solution was then allowed to warm to 20°C over 1 h, before excess solid $NaHCO_3$ and water (3 mL) were added, and the reaction mixture was stirred for 20 min, diluted with dichloromethane, filtered, and the solvent removed under reduced pressure, yielding a white foam. This crude product was purified by column chromatography (2:1 ethyl acetate:petroleum ether), giving the desired product (**3**) as a crystalline white solid (6.63 g, 76%). HRMS (ESI) calcd for $C_{39}{}^{13}C_6H_{57}NO_{15}SiNa$ $[M + Na]^+$ m/z 908.3591, found: 908.3608. (Unlabelled: HRMS [ESI] calcd for $C_{45}H_{57}NO_{15}SiNa$ $[M + Na]^+$ m/z 902.3395, found: 902.3385.) ^{1}H NMR ($CDCl_3$) δ (ppm): 1.08 (s, 9H, *t*Bu), 1.68 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 2.06 (s, 3H), 2.14 (s, 3H), (5Ac), 3.65 (dt, J = 9.2, 2.6 Hz, 1H, H-5), 3.75–3.87 (m, 4H, C*Ha*Ph, H-6′a, H-3, H-4), 3.90 (td, J = 6.6, 1.2 Hz, 1H, H-5′), 4.08–4.20 (m, 3H, H-2, H-6′a, C*Hb*Ph), 4.44 (d, J = 12.0 Hz, 1H, H-6a), 4.63 (d, J = 12.0 Hz, 1H, H-6b), 4.66 (d, J = 8.1 Hz, 1H, H-1′), 4.94 (dd, J = 10.5, 3.5 Hz, 1H, H-3′), 4.97 (d, J = 3.7 Hz, 1H, H-1), 5.19 (dd, J = 10.5, 8.1 Hz, 1H, H-2′), 5.35 (dd, J = 3.5, 1.2 Hz, 1H, H-4′), 5.67 (d, J = 8.8 Hz, 1H, NH), 7.22–7.36 (5H), 7.36–7.49 (m, 6H), 7.68–7.76 (4H) (SiPh, Bn). ^{13}C NMR ($CDCl_3$) δ (ppm): 19.5, 20.4, 20.6, 20.6, 20.7, 23.5, 27.0, 53.4, 61.3 (dt, J = 46.5, 4.4 Hz), 61.8, 67.0 (t, J = 38.9 Hz), 69.0 (dd, J = 49.0, 42.6 Hz), 69.8, 70.4, 70.7, 71.0 (br. t, J = 40.1 Hz), 71.4 (dd, J = 46.5, 38.9 Hz), 80.6, 96.6 (C-1), 101.2 (dt, J = 49.0, 5.0 Hz, C-1′), 127.8, 128.0, 128.1, 128.6, 130.0, 130.0, 132.9, 133.7, 135.7, 136.1, 137.2, 169.3, 169.9, 170.2, 170.2, 170.5.

4.3 | Synthesis of sodium 2-acetamido-2-deoxy-4-*O*-(β-$^{13}C_6$-D-galactopyranosyl)-D-glucopyranose-6-sulfate (8)

4.3.1 | Benzyl 2-acetamido-3-O-acetyl-2-deoxy-6-O-tert-butyldiphenylsilyl-4-O-(2,3,4,6-tetra-O-acetyl-β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranoside (4)

A solution of **3** (1.98 g, 1 eq) in anhydrous pyridine (25 mL) was cooled to 0°C. Acetic anhydride (5 mL, 22.8 eq) was added dropwise. The reaction was stirred at 0°C for 3 h, then at 20°C overnight. The solution was concentrated and then purified by column chromatography (1:1 ethyl acetate:petroleum ether), giving the product (**4**) as a white foam (2.02 g, 97%). (Unlabelled: HRMS [ESI] calcd for $C_{47}H_{59}NO_{16}SiNa$ $[M + Na]^+$ m/z 944.3501, found: 944.3495.) ^{1}H NMR ($CDCl_3$) δ (ppm): 1.09 (s, 9H, *t*Bu), 1.76 (s, 3H), 1.91 (s, 3H), 1.98 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.13 (s, 3H) (6Ac), 3.58 (dt, J = 9.9, 2.0 Hz, 1H, H-5), 3.70–3.79 (m, 2H, H-5′, H-6′a or C*Ha*Bn), 3.87 (dd, J = 11.6, 2.7 Hz, 1H, H-6′a or C*Ha*Bn), 4.05–4.15 (m, 3H, H-4, H-6′b, C*Hb*Bn), 4.25 (ddd, J = 10.9, 9.4, 3.8 Hz, 1H, H-2), 4.47 (d, J = 12.0 Hz 1H, H-6a), 4.61 (d, J = 12.0 Hz, 1H, H-6b), 4.74 (d, J = 8.0 Hz, 1H, H-1′), 4.88 (dd, J = 10.3, 3.5 Hz, 1H, H-3′), 4.93 (d, J = 3.8 Hz, 1H, H-1), 5.04 (dd, J = 10.3, 8.0 Hz, 1H, H-2′), 5.17 (dd, J = 10.9, 9.3 Hz, 1H, H-3), 5.30 (d, J = 3.5 Hz, 1H, H-4′), 5.73 (d, J = 9.4 Hz, 1H, NH), 7.23–7.35 (m, 5H), 7.38–7.50 (m, 6H), 7.69–7.77 (m, 4H) (SiPh, Bn). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.6, 20.7, 20.8, 20.8, 21.1, 23.3, 27.0, 29.8, 52.4, 61.2, 61.2 (d, J = 47.6 Hz), 67.1 (t, J = 38.8 Hz), 69.5 (dd, J = 48.4, 42.6 Hz), 70.1, 70.7 (dd, J = 47.5, 38.9 Hz), 71.3 (br. t, J = 40.7 Hz), 71.4, 71.5, 74.4, 96.7, 100.4 (dt, J = 48.4, 5.4 Hz), 127.8, 128.1, 128.3, 128.4, 128.7, 130.0, 130.1, 132.5, 133.6, 135.6, 136.1, 137.0, 168.9, 170.1, 170.2, 170.3, 170.4, 171.5.

4.3.2 | Benzyl 2-acetamido-3-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranoside (5)

Hydrogen fluoride-pyridine (70% solution in pyridine) (4 mL) was added to a solution of **4** (2.00 g) in THF (35 mL) and anhydrous pyridine (3 mL) contained in a

Nalgene vial at 0°C. The mixture was warmed to 20°C, then stirred overnight. The solution was diluted with ethyl acetate, washed with saturated aqueous $NaHCO_3$ and water, then dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure, giving a tan solid. This material was purified by column chromatography (2:1 to 1:0 ethyl acetate:petroleum ether), yielding the product (**5**) as a white solid (1.32 g, 89%). (Unlabelled: HRMS [ESI] calcd for $C_{31}H_{41}NO_{16}Na$ $[M + Na]^+$ m/z 706.2323, found: 706.2318.) ^1H NMR ($CDCl_3$) δ (ppm): 1.88 (s, 3H), 1.97 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.06 (s, 3H), 2.14 (s, 3H), 3.68 (dt, J = 9.9, 2.5 Hz, 1H), 3.73 (dd, J = 6.8, 2.6 Hz, 1H), 3.89 (td, J = 10.8, 6.9 Hz, 1H), 3.93 (dd, J = 9.9 Hz, 1H), 4.08 (dd, J = 10.8, 6.9 Hz, 1H), 4.11 (dd, J = 10.8, 6.9 Hz, 1H), 4.18 (ddd, J = 10.8, 9.5, 3.7 Hz, 1H), 4.49 (d, J = 12.0 Hz, 1H), 4.64 (d, J = 7.9 Hz, 1H), 4.67 (d, J = 12.0 Hz, 1H), 4.89 (d, J = 3.7 Hz, 1H), 4.99 (dd, J = 10.5, 3.5 Hz, 1H), 5.12 (dd, J = 10.5, 7.9 Hz, 1H), 5.25 (dd, J = 10.8, 9.2 Hz, 1H), 5.34 (dd, J = 3.5, 1.1 Hz, 1H), 5.69 (d, J = 9.5 Hz, 1H), 7.24–7.39 (m, 5H). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.6, 20.7, 20.8, 21.0, 23.2, 52.4, 60.4, 61.1 (dt, J = 47.2, 4.4 Hz), 66.9 (t, J = 38.9 Hz), 69.5 (dd, J = 48.6, 42.5 Hz), 70.3, 70.7 (dd, J = 47.5, 38.7 Hz), 71.1, 71.2 (br. t, J = 40.8 Hz), 71.9, 75.2, 96.8, 101.2 (dt, J = 48.3, 5.5 Hz), 128.2, 126.4, 128.7, 136.8, 169.4, 170.2, 170.2, 170.3, 170.5, 171.2.

4.3.3 | Benzyl 2-acetamido-3-O-acetyl-2-deoxy-6-O-sulfo-4-O-(2,3,4,6-tetra-O-acetyl-β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranoside, ammonium salt (6)

The starting material (**5**) and trimethylamine sulfur trioxide complex were co-evaporated with anhydrous acetonitrile three times, then put under high vac for 8 h. Anhydrous *N*,*N*-dimethylformamide (20 mL) was added to the dried trimethylamine sulfur trioxide complex (1.20 g, 4.53 eq) and **5** (1.30 g, 1 eq). This mixture was heated at 50°C for 16 h. MeOH (5 mL) was added, and the mixture was stirred for 15 min. The solvents were removed under high vac at 40°C. The crude material was purified by column chromatography. The silica column was first equilibrated using 1% NH_3 in MeOH, then flushed with ethyl acetate with 1% of the 1% NH_3 in MeOH mixture. At this stage, the material was loaded onto the column and eluted with 99:1 to 4:1 ethyl acetate: (1% NH_3 in MeOH), yielding a white solid (1.36 g, 92%). (Unlabelled: HRMS [ESI] calcd for $C_{31}H_{40}NO_{19}SNa_2$ $[M + 2Na]^{2+}$ m/z 404.0850, found: 404.0853.) ^1H NMR ($CDCl_3$) δ (ppm): 1.82 (s, 3H), 1.94 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 2.12 (s, 3H), 3.84 (dt, J = 10.4, 2.2 Hz, 1H), 4.00–4.09 (m, 2H), 4.20 (ddd, J = 11.0, 9.4, 3.7 Hz, 1H), 4.26 (dd, J = 10.9, 1.4 Hz, 1H), 4.34 (dd, J = 11.2, 2.8 Hz, 1H), 4.50 (d, J = 12.0 Hz, 1H), 4.70 (d, J = 12.0 Hz, 1H), 4.87 (d, J = 8.0 Hz, 1H), 4.98–5.05 (m, 2H), 5.13–5.21 (m, 2H), 5.33 (d, J = 3.5 Hz, 1H), 5.80 (d, J = 9.2 Hz, 1H), 7.25–7.38 (m, 5H). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.7, 20.8, 20.8, 20.9, 21.0, 23.2, 52.2, 61.4 (d, J = 47.5 Hz), 65.5, 67.5 (t, J = 38.6 Hz), 69.5 (dd, J = 48.2, 42.5 Hz), 70.0, 70.1, 70.4, (dd, J = 47.4, 38.6 Hz), 71.1 (br t, J = 40.4 Hz), 71.6, 75.0, 96.9, 100.6 (d, J = 48.4 Hz), 128.2, 128.3, 128.7, 137.1, 170.1, 170.3, 170.6, 170.7, 171.2, 171.4.

4.3.4 | Benzyl 2-acetamido-2-deoxy-4-O-β-$^{13}C_6$-D-galactopyranosyl-6-O-sulfo-α-D-glucopyranoside, ammonium salt (7)

The ammonium salt of **6** (470 mg) was dissolved in anhydrous MeOH (10 mL) and sodium methoxide in methanol (30 mass%) was added until pH was 11. After 1 h, the reaction was complete by TLC and so was neutralised with Amberlite 15 resin (H^+ form). The mixture was filtered, concentrated to remove methanol, and then loaded onto a column of silica gel and eluted with 7:2:0.5 dichloromethane: MeOH: aq NH_4OH (26%), yielding the ammonium salt of the target material (**7**) as a clear syrup (0.341 g, 99%). (Unlabelled: HRMS [ESI] calcd for $C_{21}H_{30}NO_{14}S$ $[M - H]^-$ m/z 552.1387, found: 552.1386.) ^1H NMR (CD_3OD) δ (ppm): 1.94 (s, 3H), 3.49–3.56 (m, 2H), 3.63 (ddd, J = 7.5, 4.6, 1.0 Hz, 1H), 3.66 (dd, J = 10.0, 8.5 Hz, 1H), 3.70 (dd, J = 11.5, 4.6 Hz, 1H), 3.76 (dd, J = 11.5, 7.5 Hz, 1H), 3.82–3.84 (m, 1H), 3.87 (dd, J = 10.8, 8.4 Hz, 1H), 3.94 (dd, J = 10.8, 3.6 Hz, 1H), 3.99 (ddd, J = 10.0, 4.3, 2.0 Hz, 1H), 4.27 (dd, J = 10.9, 2.0 Hz, 1H), 4.35 (dd, J = 11.0, 4.3 Hz, 1H), 4.48-4.54 (m, 2H), 4.73 (d, J = 12.1 Hz, 1H), 4.85 (d, J = 3.6 Hz, 1H), 7.26–7.30 (m, 1H), 7.31–7.36 (m, 2H), 7.37–7.40 (m, 2H). ^{13}C NMR (CD_3OD) δ (ppm): 22.5, 54.9, 62.5 (br d, J = 44.5 Hz), 67.4, 70.4 (t, J = 38.6 Hz), 70.5, 71.0, 72.8 (dd, J = 46.1, 39.6 Hz), 74.8 (br t, J = 38.9 Hz), 77.0 (dd, J = 44.7, 38.6 Hz), 80.8, 97.3, 104.7 (d, J = 46.2 Hz), 128.9, 129.3, 129.4, 138.8, 173.4.

4.3.5 | Sodium 2-acetamido-2-deoxy-4-O-(β-$^{13}C_6$-D-galactopyranosyl)-D-glucopyranose-6-sulfate (8)

Palladium hydroxide (20%) on carbon (65 mg) was added to a solution of the ammonium salt of **7** (197 mg) in EtOH (16 mL) and aqueous NH_4OH (28%, 4 mL), and the solution was stirred under a hydrogen gas atmosphere at 20°C

for 5 h. Celite was washed using the same solvent ratio as above, and then the mixture was filtered through it. The filtrate was concentrated to dryness, then passed through a Dowex 50WX8-200, Na^+ form resin exchange column, then lyophilized giving the sodium salt of the product (**8**, mixture of anomers) as a white foam, (174 mg, quant). HRMS (ESI) calcd for $C_8{}^{13}C_6H_{24}NO_{14}S$ $[M - Na]^-$ m/z 468.1119, found: 468.1125. (Unlabelled: HRMS [ESI] calcd for $C_{14}H_{24}NO_{14}S$ $[M - Na]^-$ m/z 462.0923, found: 462.0918.) ^{1}H NMR (D_2O) δ (ppm): 2.04 (s, 3H), 3.51–3.57 (m, 1H), 3.64–3.82 (m, 6H), 3.83–3.96 (m, 2H), 4.17 (d, J = 9.8 Hz, 0.5H), 4.28–4.42 (m, 2H), 4.51–4.55 (m, 1H), 4.65 (under H_2O peak, HSQC), 4.73–4.77 (m, obscured by H_2O peak), 5.20 (d, J = 2.9 Hz, 0.5H). ^{13}C NMR (D_2O) δ (ppm): 22.1, 22.4, 53.7, 56.2, 61.1 (dt, J = 44.3, 4.3 Hz), 61.3, 66.5, 66.6, 68.6, 68.7 (t, J = 38.3 Hz), 68.9, 69.3, 71.1 (dd, J = 46.2, 39.7 Hz), 71.1, 72.4, 72.6, 72.6 (t, J = 39.1 Hz), 72.7, 75.4 (dd, J = 44.4, 38.3 Hz), 75.4, 77.6, 78.0, 90.7, 95.0, 102.7 (d, J = 46.2 Hz), 102.8, 174.5, 174.8. Isotope enrichment by mass spectrometry ion intensities: 99.0%.

4.4 | Synthesis of 2-acetamido-2-deoxy-6-*O*-sulfo-4-*O*-(6-*O*-sulfo-β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranose, disodium salt (16)

4.4.1 | Benzyl 2-acetamido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranoside (9)

Hydrogen fluoride-pyridine (70% solution in pyridine) (3 mL) was added to a solution of **3** (1.14 g) in THF (20 mL) and anhydrous pyridine (2 mL) contained in a Nalgene vial at 0°C. The mixture was warmed to 20°C, then stirred for 2 days. The solution was diluted with ethyl acetate, washed with saturated aqueous $NaHCO_3$ and water, then dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure, yielding a tan solid. This material was purified by column chromatography (2:1 to 1:0 ethyl acetate:petroleum ether), giving the product (**9**) as colourless oil. On further drying, this gave a white solid (0.80 g, quantitative). HRMS (ESI) calcd for $C_{23}{}^{13}C_6H^{39}NO_{15}Na$ $[M + Na]^+$ m/z 670.2413, found: 670.2413. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.96 (s, 3H), 1.98 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.14 (s, 3H), 3.63 (d, J = 11.7 Hz, 1H), 3.64–3.70 (m, 2H), 3.71 (d, J = 11.7 Hz, 1H), 3.81–3.86 (m, 1H), 3.97–4.04 (m, 2H), 4.08–4.16 (m, 1H), 4.11 (ddd, J = 10.5, 8.8, 3.7 Hz, 1H), 4.46 (d, J = 11.8 Hz, 1H), 4.65 (d, J = 8.0 Hz, 1H), 4.67 (d, J = 11.8 Hz, 1H), 4.95 (d, J = 3.6 Hz, 1H), 5.00–5.05 (m, 1H), 5.20–5.26 (m, 1H), 5.36–5.40 (m, 1H), 5.63 (d, J = 8.8 Hz, 1H), 7.27–7.38 (m, 5H). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.6, 20.6, 20.7, 20.8, 23.4, 53.1, 60.8, 61.7 (dt, J = 45.6, 4.7 Hz), 67.1 (t, J = 38.9 Hz), 69.1 (dd, J = 49.0, 42.7 Hz), 70.2, 70.4, 70.5, 71.0 (br t, J = 40.8 Hz), 71.5 (dd, J = 45.9, 39.2 Hz), 81.8, 97.0, 102.0 (dt, J = 48.9, 4.7 Hz), 128.1, 128.3, 128.7, 137.1, 169.6, 170.0, 170.2, 170.3, 170.6.

4.4.2 | Benzyl 2-acetamido-2-deoxy-4-O-(β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranoside (10)

To a solution of **9** (0.83 g) in anhydrous MeOH (30 mL), 8 drops of NaOMe in MeOH (5.4 M) was added, resulting in pH = 10 solution. This was stirred at 20°C for 20 h, then neutralised by stirring with Amberlyst-15 resin (H^+ form) for 15 min. The solution was then filtered through Celite and the solvent removed under reduced pressure, yielding the product (**10**) as a white solid (0.60 g, 98%). HRMS (ESI) calcd for $C_{15}{}^{13}C_6H_{31}NO_{11}Na$ $[M + Na]^+$ m/z 502.1996, found: 502.1994. ^{1}H NMR (CD_3OD) δ (ppm): 1.94 (s, 3H), 3.48 (dd, J = 11.4, 7.4 Hz, 1H), 3.54 (dd, J = 9.7, 7.6 Hz, 1H), 3.58 (ddd, J = 7.4, 4.7, 1.1 Hz, 1H), 3.63 (dd, J = 9.9, 8.3 Hz, 1H), 3.69 (dd, J = 11.4, 4.7 Hz, 1H), 3.76 (dd, J = 11.4, 7.4 Hz, 1H), 3.77 (ddd, J = 9.7, 4.1, 2.7 Hz, 1H), 3.82 (dd, J = 3.3, 1.0 Hz, 1H), 3.82-3.90 (m, 3H), 3.93 (dd, J = 10.8, 3.5 Hz, 1H), 4.37 (d, J = 7.6 Hz, 1H), 4.51 (d, J = 12.0 Hz, 1H), 4.72 (d, J = 12.0 Hz, 1H), 4.87 (d, J = 3.5 Hz, 1H), 7.23–7.30 (m, 1H), 7.31–7.35 (m, 2H), 7.36–7.39 (m, 2H). ^{13}C NMR (CD_3OD) δ (ppm): 22.5, 55.0, 61.9, 70.3 (t, J = 38.7 Hz), 70.9, 72.6 (dd, J = 46.8, 40.0 Hz), 74.9 (br t, J = 39.2 Hz), 77.1 (dd, J = 44.6, 38.8 Hz), 81.4, 97.4, 105.1 (dt, J = 46.7, 4.8 Hz), 128.8, 129.2, 129.4, 139.0, 173.4. Two natural abundance carbon peaks are not listed because of being obscured by high-abundance ^{13}C signals.

4.4.3 | Benzyl 2-acetamido-2-deoxy-6-O-tert-butyldiphenylsilyl-4-O-(6-O-tert-butyldimethylsilyl-β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranoside (11)

A solution of **10** (0.52 g, 1 eq) in anhydrous *N,N*-dimethylformamide (15 mL) under argon was cooled to 0°C. Imidazole (0.30 g, 4.1 eq) was added followed by *tert*-butyldimethylsilyl chloride (0.38 g, 2.3 eq). The resulting solution was stirred at 0°C for 3 h. The solvent was removed under high vac. The crude material was purified by column chromatography (1:0 petroleum

ether:ethyl acetate to 1:0 ethyl acetate:MeOH to 10:1 ethyl acetate:MeOH), giving several fractions containing the desired product. These fractions were combined and the solvent removed under reduced pressure, yielding the product (**11**) as a colourless oil (0.59 g, 77%). HRMS (ESI) calcd for $C_{27}{}^{13}C_6H_{59}NO_{11}Si_2Na$ $[M + Na]^+$ m/z 730.3726, found: 730.3726. ^1H NMR (CD_3OD) δ (ppm): 0.09 (s, 6H), 0.11 (s, 3H), 0.11 (s, 3H), 0.91 (s, 9H), 0.93 (s, 9H), 1.94 (s, 3H), 3.45 (dd, *J* = 9.7, 3.3 Hz, 1H), 3.53 (dd, *J* = 6.5, 1.1 Hz, 1H), 3.55 (dd, *J* = 9.7, 7.7 Hz, 1H), 3.64 (dd, *J* = 9.9, 8.4 Hz, 1H), 3.75 (ddd, *J* = 9.8, 4.3, 1.8 Hz, 1H), 3.79 (dd, *J* = 10.2, 6.4 Hz, 1H), 3.81–3.87 (m, 3H), 3.90 (dd, *J* = 11.5, 1.8 Hz, 1H), 3.92 (dd, *J* = 10.8, 3.5 Hz, 1H), 3.99 (dd, *J* = 11.5, 4.2 Hz, 1H), 4.38 (d, *J* = 7.7 Hz, 1H), 4.49 (d, *J* = 12.0 Hz, 1H), 4.70 (d, *J* = 12.0 Hz, 1H), 4.85 (d, *J* = 3.5 Hz, 1H), 7.26–7.30 (m, 1H), 7.31–7.37 (m, 4H). ^{13}C NMR (CD_3OD) δ (ppm): −5.3, −5.2, −5.0, −4.9, 22.5, 26.4, 26.5, 31.7, 36.9, 55.1, 62.9, 62.9 (dt, *J* = 46.7, 4.2 Hz), 69.6 (t, *J* = 38.9 Hz), 70.4, 70.8, 72.5 (dd, *J* = 46.9, 40.0 Hz), 75.0 (t, *J* = 39.4 Hz), 76.9 (dd, *J* = 46.7, 39.1 Hz), 80.8, 97.5, 105.0 (dt, *J* = 47.0, 4.8 Hz), 128.8, 129.1, 129.4, 139.0, 173.3. Two natural abundance carbon peaks are not listed because of being obscured by high-abundance ^{13}C signals.

4.4.4 | Benzyl 2-acetamido-3-O-acetyl-2-deoxy-6-O-tert-butyldiphenylsilyl-4-O-(2,3,4-tri-O-acetyl-6-O-tert-butyldimethylsilyl-β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranoside (12)

To a solution of **11** (0.59 g) in anhydrous pyridine (10 mL) at 0°C was added acetic anhydride (5 mL) dropwise. The solution was stirred at 20°C overnight. TLC (1:1 petroleum ether:ethyl acetate) indicated a small amount of intermediate was still present and so the reaction was heated at 40°C for 2 h to drive it to completion. The solvent was removed under reduced pressure, and the crude material purified by column chromatography (2:1 to 1:1 petroleum ether:ethyl acetate), giving the product (**12**) as a clear syrup, which on drying gave a white foam (0.58 g, 79%). HRMS (ESI) calcd for $C_{35}{}^{13}C_6H_{68}NO_{15}Si_2$ $[M + H]^+$ m/z 876.4329, found: 876.4339. ^1H NMR ($CDCl_3$) δ (ppm): 0.01 (s, 3H), 0.03 (s, 3H), 0.86 (s, 9H), 0.93 (s, 9H), 1.88 (s, 3H), 1.96 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.11 (s, 3H), 3.54 (t, *J* = 8.9 Hz, 1H), 3.57–3.64 (m, 2H), 3.68–3.74 (m, 2H), 3.79 (dd, *J* = 11.5, 3.3 Hz, 1H), 3.89 (t, *J* = 9.6 Hz, 1H), 4.18 (ddd, *J* = 10.8, 9.5, 3.8 Hz, 1H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.63 (d, *J* = 7.9 Hz, 1H), 4.67 (d, *J* = 12.0 Hz, 1H), 4.85 (d, *J* = 3.7 Hz, 1H), 4.95 (dd, *J* = 10.3, 3.5 Hz, 1H), 5.06 (dd, *J* = 10.4, 7.9 Hz, 1H), 5.18 (dd, *J* = 10.8, 9.3 Hz, 1H), 5.45 (dd, *J* = 3.4, 1.1 Hz, 1H), 5.78 (d, *J* = 9.5 Hz, 1H), 7.28–7.38 (m, 5H). ^{13}C NMR ($CDCl_3$) δ (ppm): −5.7, −5.6, −5.3, −5.0, 18.0, 18.3, 20.6, 20.7, 20.8, 20.9, 23.2, 25.7, 25.9, 52.1, 60.0 (d, *J* = 47.8 Hz), 60.9, 66.7 (t, *J* = 39.2 Hz), 69.8 (dd, *J* = 48.5, 42.6 Hz), 71.5 (br t, *J* = 41.0 Hz), 73.2 (dd, *J* = 47.7, 39.5 Hz), 96.6, 100.5 (dt, *J* = 48.4, 5.4 Hz), 128.1, 128.1, 128.5, 136.9, 169.0, 169.9, 170.0, 170.1, 171.1. Four natural abundance carbon peaks are not listed because of being obscured by high-abundance ^{13}C signals.

4.4.5 | Benzyl 2-acetamido-3-O-acetyl-2-deoxy-4-O-(2,3,4-tri-O-acetyl-β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranoside (13)

Hydrogen fluoride-pyridine (70% solution in pyridine) (1.5 mL) was added to a solution of **12** (0.58 g) in THF (6.7 mL) and anhydrous pyridine (0.70 mL) contained in a Nalgene vial at 0°C. The mixture was taken out of the ice bath and stirred for 2 h. TLC (in ethyl acetate) indicated complete reaction after 2 h. The solution was diluted with ethyl acetate, cooled to 0°C, washed with saturated aqueous $NaHCO_3$ and water, then dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure, giving a yellow syrup. This crude material was purified by column chromatography (solid loaded, 1:0 to 4:1 ethyl acetate: MeOH), giving the product (**13**) as a colourless oil, which on drying gave a white solid (0.38 g, quantitative). HRMS (ESI) calcd for $C_{23}{}^{13}C_6H_{39}NO_{15}Na$ $[M + Na]^+$ m/z 670.2419, found: 670.2421. (Unlabelled: HRMS (ESI) calcd for $C_{29}H_{39}NO_{15}Na$ $[M + Na]^+$ m/z 664.2217, found: 664.2220.) ^1H NMR ($CDCl_3$) δ (ppm): 1.89 (s, 3H), 1.97 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 2.13 (s, 3H), 3.49 (dd, *J* = 11.6, 5.2 Hz, 1H), 3.63–3.81 (m, 5H), 4.01 (t, *J* = 9.5 Hz, 1H), 4.22 (ddd, *J* = 10.8, 9.4, 3.7 Hz, 1H), 4.46 (d, *J* = 12.0 Hz, 1H), 4.66 (d, *J* = 11.9 Hz, 1H), 4.70 (d, *J* = 7.8 Hz, 1H), 4.85 (d, *J* = 3.6 Hz, 1H), 5.02 (dd, *J* = 10.4, 3.4 Hz, 1H), 5.11 (dd, *J* = 10.4, 7.8 Hz, 1H), 5.24 (dd, *J* = 10.8, 9.2 Hz, 1H), 5.33 (dd, *J* = 3.4, 1.0 Hz, 1H), 5.76 (d, *J* = 9.4 Hz, 1H), 7.27–7.38 (m, 5H). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.7, 20.8, 20.9, 21.1, 23.2, 52.3, 60.3, 61.1 (d, *J* = 43.4 Hz), 68.0 (t, *J* = 38.6 Hz), 69.9 (dd, *J* = 48.1, 42.4 Hz), 70.2, 71.1, 71.3 (br. t, *J* = 40.4 Hz), 72.4, 74.2 (dd, *J* = 43.7, 38.5 Hz), 74.6, 96.7, 100.9 (dt, *J* = 48.1, 5.0 Hz), 128.2, 128.4, 128.8, 136.8, 169.6, 170.1, 170.4, 170.9, 171.6.

4.4.6 | Benzyl 2-acetamido-3-O-acetyl-2-deoxy-6-O-sulfo-4-O-(2,3,4-tri-O-acetyl-6-O-sulfo-β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranoside, diammonium salt (14)

The diol starting material and sulfating reagent were dried under high vac for 5 h. A mixture of **13** (0.38 g, 1 eq) and sulfur trioxide trimethylamine complex (0.20 g, 2.4 eq) in anhydrous *N,N*-dimethylformamide (6.5 mL) were heated at 50°C for 20 h. MeOH (5 mL) and 7 M NH_3 in MeOH (1 mL) was added and the mixture was stirred for 15 min. The solvent was removed under high vac. The resulting crude material was purified by column chromatography (dry loaded, 65:35 $CHCl_3$:MeOH), giving the ammonium salt of the product (**14**) as a white solid (0.43 g, 87%). HRMS (ESI) calcd for $C_{23}{}^{13}C_6H_{37}NO_{21}S_2Na$ $[M - H]^-$ m/z 828.1403, found: 828.1389. (Unlabelled: HRMS (ESI) calcd for $C_{29}H_{38}NO_{21}S_2$ $[M - H]^-$ m/z 800.1378, found: 800.1379.) ^{1}H NMR (CD_3OD) δ (ppm): 1.89 (s, 3H), 1.91 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 2.12 (s, 3H), 3.83 (ddd, J = 10.1, 4.1, 2.0 Hz, 1H), 3.88 (dd, J = 10.1, 8.6 Hz, 1H), 4.01 (dd, J = 9.8, 7.7 Hz, 1H), 4.06 (dd, J = 9.8, 5.7 Hz, 1H), 4.12 (ddd, J = 7.2, 5.7, 1.2 Hz, 1H), 4.16 (dd, J = 11.0, 5.8 Hz, 1H), 4.17 (d, J = 11.0 Hz, 1H), 4.22 (dd, J = 10.9, 4.1 Hz, 1H), 4.59 (d, J = 12.1 Hz, 1H), 4.74 (d, J = 12.1 Hz, 1H), 4.81 (d, J = 3.7 Hz, 1H), 4.85 (d, J = 7.9 Hz, 1H), 5.00 (dd, J = 10.4, 7.9 Hz, 1H), 5.10 (dd, J = 10.4, 3.5 Hz, 1H), 5.20 (dd, J = 11.0, 8.6 Hz, 1H), 5.44 (dd, J = 3.5, 1.2 Hz, 1H), 7.27–7.32 (m, 1H), 7.33–7.38 (m, 2H), 7.39–7.44 (m, 2H). ^{13}C NMR (CD_3OD) δ (ppm): 20.5, 20.6, 20.9, 21.4, 22.4, 53.0, 65.7 (dt, J = 47.7, 4.1 Hz), 66.5, 68.8 (t, J = 38.4 Hz), 70.7 (dd, J = 48.5, 42.8 Hz), 70.8, 70.9, 72.2 (dd, J = 47.5, 38.7 Hz), 72.3, 72.8 (br t, J = 40.3 Hz), 77.0, 97.3, 101.7 (dt, J = 48.5, 5.5 Hz), 129.0, 129.5, 129.6, 138.6, 171.4, 171.6, 172.0, 172.6, 173.3.

4.4.7 | Benzyl 2-acetamido-2-deoxy-6-O-sulfo-4-O-(6-O-sulfo-β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranoside, disodium salt (15)

To a solution of **14** (0.120 g) in anhydrous MeOH (5 mL), 8 drops of NaOMe in MeOH (5.4 M) was added, resulting in a pH = 10–11 solution. The solution was left at 20°C for 1 h, at which point TLC indicated complete reaction. The solution was neutralised by stirring with Amberlyst-15 (H^+ form) resin for 15 min (added in small portions until pH neutral). The solution was filtered through Celite and the solvent removed under reduced pressure, yielding the sodium salt of the product (**15**) as a white solid (0.095 g, 97%). HRMS (ESI) calcd for $C_{15}{}^{13}C_6H_{29}NO_{17}S_2Na$ $[M - H]^-$ m/z 660.0981, found: 660.0971. (Unlabelled: HRMS [ESI] calcd for $C_{21}H_{30}NO_{17}S_2$ $[M - H]^-$ m/z 632.0955, found: 632.0950.) ^{1}H NMR (CD_3OD) δ (ppm): 1.95 (s, 3H), 3.50–3.57 (m, 2H), 3.64 (dd, J = 10.0, 8.0 Hz, 1H), 3.85-3.91 (m, 3H), 3.93 (dd, J = 10.8, 3.4 Hz, 1H), 3.99 (ddd, J = 10.0, 4.4, 2.0 Hz, 1H), 4.18 (d, J = 6.3 Hz, 2H), 4.28 (dd, J = 11.0, 2.1 Hz, 1H), 4.35 (dd, J = 11.0, 4.4 Hz, 1H), 4.51 (m, 2H), 4.74 (d, J = 12.0 Hz, 1H), 4.86 (d, J = 3.4 Hz, 1H), 7.26–7.30 (m, 1H), 7.31–7.36 (m, 2H), 7.37–7.40 (m, 2H). ^{13}C NMR (CD_3OD) δ (ppm): 22.6, 54.8, 67.5, 67.9 (dt, J = 46.3, 4.3 Hz), 70.1 (t, J = 38.4 Hz), 70.4, 70.5, 71.1, 72.7 (dd, J = 46.3, 39.6 Hz), 74.5 (br t, J = 38.7 Hz), 74.6 (dd, J = 46.2, 38.5 Hz), 81.5, 97.3, 104.9 (dt, J = 46.4, 4.7 Hz), 128.9, 129.3, 129.4, 138.8, 173.6.

4.4.8 | 2-Acetamido-2-deoxy-6-O-sulfo-4-O-(6-O-sulfo-β-$^{13}C_6$-D-galactopyranosyl)-α-D-glucopyranose, disodium salt (16)

Palladium hydroxide (20%) on carbon (100 mg) was added to a solution of disodium salt of **15** (0.310 g) in EtOH (20 mL) and aqueous NH_4OH (28%) (5 mL), and the solution was stirred under a hydrogen gas atmosphere at ambient temperature overnight. Celite was washed using the same solvent ratio as above. The mixture was filtered through this, and the filtrate was concentrated to dryness. The resulting residue was dissolved in water, passed through a Dowex exchange column (Na^+ form, 50WX8-200), then lyophilized (approximately 60 mL of water), giving the disodium salt of the product (**16**) as a white solid (0.255 g, 93%). HRMS (ESI) calcd for $C_8{}^{13}C_6H_{23}NO_{17}S_2Na$ $[M - Na]^-$ m/z 570.0506, found: 570.0514. (Unlabelled: HRMS [ESI] calcd for $C_{14}H_{23}NO_{17}S_2Na$ $[M - Na]^-$ m/z 564.0311, found: 564.0300.) ^{1}H NMR (D_2O) δ (ppm): 2.03 (s, 3H), 3.53 (ddd, J = 9.9, 7.8, 4.4 Hz), 3.69 (dt, J = 9.9, 3.4 Hz), 3.71–3.77 (m), 3.82 (ddd, J = 9.6, 5.1, 2.3 Hz), 3.88–3.92 (m), 3.95–4.00 (m), 4.14-4.22 (m), 4.28 (dd, J = 11.1, 5.1 Hz), 4.30–4.37 (m), 4.40 (dd, J = 11.1, 2.1 Hz), 4.54 (d, J = 7.9 Hz, 4.73 (s), 5.21 (d, J = 2.5 Hz). ^{13}C NMR (D_2O) δ (ppm): 22.0, 22.3, 53.7, 56.2, 66.7, 67.1 (dt, J = 46.3, 4.6 Hz), 68.3 (t, J = 38.2 Hz), 68.4, 69.3, 70.9 (dd, J = 46.2, 39.8 Hz), 72.3, 72.4 (br t, J = 39.5 Hz), 72.7, 72.8 (dd, J = 46.6, 38.0 Hz), 78.8, 79.2, 90.6, 95.0, 102.8, 103.0 (ddt, J = 46.3, 21.0, 5.2 Hz), 174.5, 174.8. Isotope enrichment by mass spectrometry ion intensities: 98.7%.

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