Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Check for updates

Cite this: *Org. Biomol. Chem.*, 2018, **16**, 8791

Synthesis and mass spectrometric analysis of disaccharides from methanolysis of heparan sulfate†

Qi Qi He,[a] Paul J. Trim,[b] Marten F. Snel,[b] John J. Hopwood[b] and Vito Ferro *[a]

Received 9th September 2018,
Accepted 30th October 2018

DOI: 10.1039/c8ob02225a

rsc.li/obc

The quantification of heparan sulfate (HS) in biological matrices, *e.g.*, urine, cerebrospinal fluid, tissue samples *etc.*, is of great importance for the diagnosis and prognosis of several of the mucopolysaccharidosis (MPS) disorders, which are lysosomal storage diseases of impaired glycosaminoglycan metabolism. The development of suitable assays for this purpose is challenging due to the high molecular weight and complexity of HS. Recent efforts towards this goal include the acid catalysed methanolysis of HS, which desulfates the polymer and results in the formation of disaccharide cleavage products which can be detected and quantified by LC-MS/MS. We have synthesized a library of 12 HS-derived disaccharides as methanolysis standards *via* the stereoselective 1,2-*cis* glycosylation of suitably protected GlcA and IdoA acceptors with a 2-deoxy-2-azido thioglucoside donor. This facilitated identification of the major peaks in the LC-MS/MS chromatograms, and potentially will allow the monitoring of specific metabolites as surrogate markers for genotype. This work also paves the way towards a fully quantitative LC-MS/MS assay for HS *via* the preparation of a suitably labelled derivative.

Introduction

Heparan sulfate (HS) is a highly complex polysaccharide of the glycosaminoglycan (GAG) family that is a marker for a number of lysosomal storage diseases, most notably several of the mucopolysaccharidoses (MPS), including MPS types I, II, IIIA–D and VII.[1] The MPS disorders are rare diseases that affect approximately 1 in 25 000 people and are characterized by genetic defects in GAG-degrading enzymes. This results in impaired GAG degradation and lysosomal accumulation of GAGs with associated abnormalities in multiple organ systems and reduced life expectancy. As a direct result of disease, there are higher levels of HS in biological samples from MPS patients, such as urine, blood and cerebrospinal fluid.[2] The quantification of HS concentrations in such samples is thus very important for diagnosis and prognosis of MPS disorders and for monitoring the efficacy of new therapies.

HS is a complex, linear polysaccharide composed of repeating disaccharide subunits of uronic acid, β-D-glucuronic acid (GlcA) or α-L-iduronic acid (IdoA), (1→4)-linked to D-glucosamine (GlcN) (Fig. 1).[3,4] The high molecular weight and remarkable degree of structural heterogeneity of HS makes its analysis and quantification extremely challenging. In order to simplify the analytical problem, a number of depolymerisation methods using enzymatic[5,6] and chemical methods[7] have been employed. Of particular note is the development of a simple method for methanolysis of HS in urine and cerebrospinal fluid samples of MPS patients,[8–11] and its recent adaptation for use with solid tissue samples.[12] Recently, this method has been used to monitor the effectiveness of enzyme replacement therapies in animal models of MPS disorders.[13–15] Methanolysis degrades HS into simple desulfated and deacetylated disaccharides (Scheme 1), which can then be analysed by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

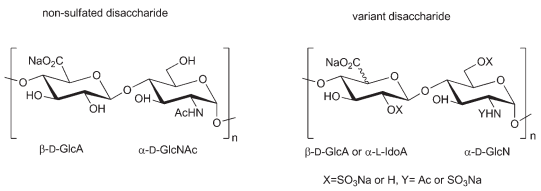
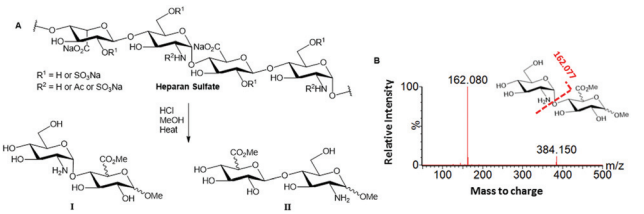


Fig. 1 The structure of heparan sulfate (HS) depicting the major non-sulfated and variant disaccharide sequences.

[a]*School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD 4072, Australia. E-mail: v.ferro@uq.edu.au*
[b]*Hopwood Centre for Neurobiology, South Australian Health and Medical Research Institute, Adelaide, SA 5000, Australia*

†Electronic supplementary information (ESI) available: Experimental and characterization details for compounds **13**, **17–24**, **29**, **37**, **42**, **44**, **46**. Copies of ^{1}H and ^{13}C NMR spectra for compounds **1–14**, **17–30**, **32**, **34–48**. LC-MS/MS chromatograms for compounds **1–12**. See DOI: 10.1039/c8ob02225a



Scheme 1 A. Methanolysis of HS to give desulfated, methylated disaccharides resulting from cleavage of uronic acid glycosidic bonds (type I) or GlcN glycosidic bonds (type II); B. MS/MS of the major peak observed from the methanolysis of heparan sulfate.

The methanolysis of HS greatly reduces the complexity of the sample to be analysed, however, it can yield several isobaric and isomeric disaccharide products. Previous MS/MS studies[12] indicate that the GlcN-(1→4)-uronic acid disaccharides (Scheme 1, type I) are the major reaction products of HS methanolysis of HS, however, the identities of the minor disaccharide products are unknown. The identities of these minor products could provide additional disease specific information and are thus of great interest. The lack of authentic disaccharide standards has also hampered attempts to develop a quantitative assay and to control assay variability. Therefore, the aims of the current study were to synthesize and fully characterize a series of methylated HS disaccharide standards to (i) verify the identity of the major peaks in the LC-MS/MS chromatograms from the methanolysis assay, (ii) to determine the structures of the products corresponding to the minor peaks, and (iii) to facilitate HS quantitation by the methanolysis digestion followed by LC-MS/MS analysis with the addition of known internal standards.

Results and discussion

The disaccharides targeted for synthesis (**1–12**) are shown in Fig. 2. A flexible synthetic strategy was developed in order to maximise the number of possible disaccharides obtained from common intermediates. As discussed above, the major disaccharides are of "type I" where cleavage has occurred at the more labile β-D- or α-L-glycosidic bonds. Under the conditions of the methanolysis reaction (3 N methanolic HCl, reflux) the newly formed methyl glycosidic bonds are expected to be predominantly α. However, as the formation of minor amounts of β-linked products cannot be ruled out, these anomers were also targeted for synthesis. Similarly, while previous MS/MS analyses indicated that *N*-acetyl groups are cleaved under the reaction conditions, the presence of minor *N*-acetylated products could not be ruled out. Such compounds should in any case be readily accessible *via* simple *N*-acetylation of the major disaccharides. Disaccharides of "type II" were only expected as minor products, if present at all. Therefore, only the more readily available, and likely more abundant, α-methyl glycosides were prepared.

Synthesis of methyl GlcN-GlcA disaccharides

Synthetic access to the target disaccharides requires the use of suitable glycosyl donors and acceptors with the correct combination of protecting groups to ensure stereocontrol of the key glycosylation reaction. The readily available[16,17] thioglycoside **13** was converted into the glycosyl donor **14** in 80% yield by treatment with benzyl bromide and sodium hydride. Donor **14** has a non-participating 2-azido group to facilitate formation of the desired 1,2-*cis* glycosidic linkage and ready installation of the desired 2-amino or acetamido groups. The α-methyl GlcA acceptor[18] **23** and the β-methyl GlcA acceptor[19] **24** were both prepared, respectively, *via* a similar sequence of reactions from commercially available methyl α-D-glucopyranoside **15** and methyl β-D-glucopyranoside **16**, respectively, as shown in Scheme 2. 4,6-*O*-*p*-Methoxybenzylidenation,[20] followed by benzylation[21] and acid hydrolysis[22] gave the 4,6-diols **21** and **22** in good yield (91–94%). Next, TEMPO oxidation of the primary alcohol in the presence of excess BAIB as co-oxidant,[23,24] followed by base-promoted esterification[25] gave the target acceptors **23** and **24** in acceptable overall yield (52–80%).

The preparation of the target methyl GlcN-GlcA disaccharides was carried out as illustrated in Scheme 3. The NIS/TfOH-

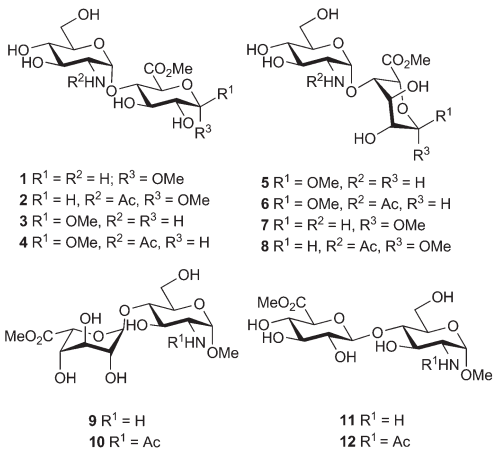
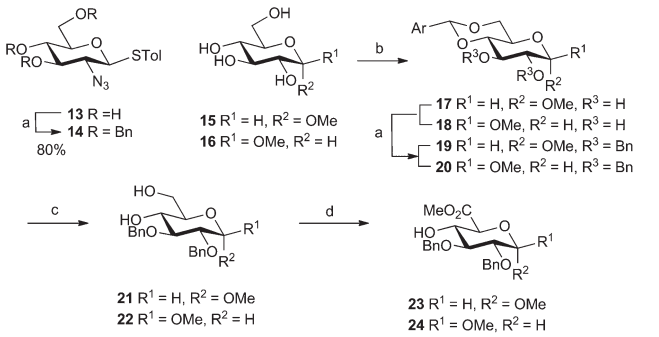
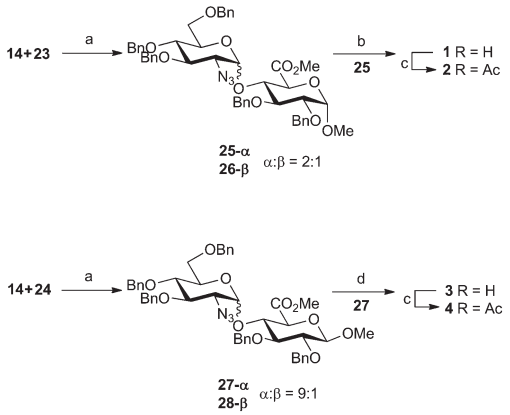


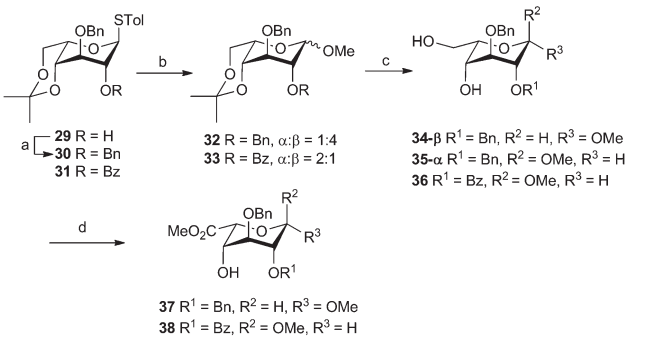
Fig. 2 Structures of target disaccharides from methanolysis of HS.



Scheme 2 Preparation of glycosyl donor **14** and α- and β-methyl GlcN acceptors **23** and **24**. Reagents and conditions: (a) NaH, BnBr, DMF, r.t., 3 h, 98–100%; (b) anisaldehyde dimethyl acetal, CSA, DMF, r.t., 18 h, 39–52%; (c) TFA/H_2O 1 : 1, r.t., 18 h, 91–94%; (d) i. TEMPO, BAIB, DCM/H_2O 3 : 1, r.t., 2 h; ii. MeI, $KHCO_3$, DMF, r.t., 18 h, 52–80%.



Scheme 3 Synthesis of methyl GlcN-GlcA disaccharides **1–4**. Reagents and conditions: (a) NIS/TfOH, DCM, −78 → −20 °C, 2 h, 94–95%; (b) H_2, 10% Pd/C, MeOH, r.t., 24 h, 51%; (c) Ac_2O, Et_3N, MeOH, r.t., 90 min, 100% (**2**), 62% (**4**, 2 steps); (d) H_2, 20% $Pd(OH)_2$/C, MeOH, conc. HCl, r.t., 21 h, 60%.



Scheme 4 Preparation of methyl IdoA acceptors **37** and **38**. Reagents and conditions: (a) NaH, BnBr, DMF, r.t., 4 h, 82%; (b) MeOH, NIS/TfOH, DCM, −78 → −20/−10 °C, 2–3 h, 98% (**32**), 68% (**33**); (c) DCM/TFA/H_2O 100 : 10 : 1, r.t., 30 min, 91–100% (**35-α**, **36**); or 80% HOAc/H_2O, 100 °C, 24 h, 51% (**34-β**) (d) i. TEMPO, BAIB, DCM/H_2O 3 : 1, r.t., 3 h; ii. MeI, $KHCO_3$, DMF, r.t., 24 h, 46–67% (2 steps).

activated glycosylation[26,27] of thioglycoside donor **14** and α-methyl GlcA acceptor **23** proceeded in excellent yield (94%) but with moderate selectivity, giving a ∼2 : 1 mixture of α:β-linked GlcN-GlcA disaccharides (**25-α**, **26-β**). Following careful flash chromatography, the desired[28] α-isomer **25** ($J_{1',2'}$ = 3.7 Hz) was obtained in 49% yield along with **26** (24%, $J_{1',2'}$ = 7.9 Hz) and a mixed fraction (21%). The structure of **25** was confirmed by comparison of the NMR and MS data with the literature.[28] Next, **25** was subjected to hydrogenolysis with 10% Pd/C catalyst to cleave all the benzyl protecting groups and simultaneously reduce the azide to the free amine, to furnish the target disaccharide **1** in moderate yield (51%). A sample of amine **1** was converted into the acetamide **2** in quantitative yield by treatment with acetic anhydride and Et_3N in methanol.

The β-methyl disaccharides **3** and **4** were similarly prepared (Scheme 3). Glycosylation of thioglycoside **14** with acceptor **24** promoted by NIS and TfOH also proceeded in excellent yield (95%) but with significantly improved selectivity (α : β = 9 : 1). The desired disaccharide **27** was separated by flash chromatography and subjected to global deprotection by hydrogenolysis to give disaccharide **3** in 60%, while a sample was acetylated to give disaccharide **4** in 62% overall yield. The target methyl GlcN-GlcA disaccharides **1–4** were fully characterized *via* 1D and 2D NMR spectroscopy and HRMS experiments.

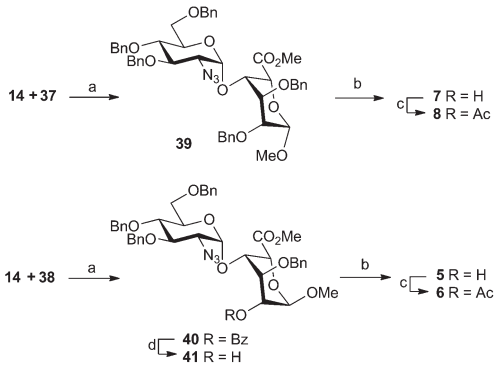
Synthesis of methyl GlcN-IdoA disaccharides

Initially it was decided to attempt to prepare both the required α- and β-L-IdoA acceptors from a common intermediate, *i.e.*, a glycosyl donor with a non-participating group at C-2 to favour formation of an anomeric mixture of methyl glycosides (Scheme 4). Thus, donor **30**, prepared by benzylation of the alcohol **29**,[17] was glycosylated with MeOH using NIS/TfOH as the promoter to furnish **32** as a 1 : 4 mixture of α/β-anomers which were separated by flash chromatography. Throughout this study, the anomeric configuration of L-idose or L-IdoA derivatives was confirmed by 1D NOESY spectroscopy: all β-L-configured glycosides, which favour the $^1C_4$ chair conformation in solution, show an NOE signal from H-1 to H-5, while the corresponding α-L-glycosides do not.[29] Interestingly, the anomeric configuration had a marked effect on the conformation of the product (Table 1). The β-anomer (**32-β**) was shown to favour the $^1C_4$ conformation in solution, while the α-anomer (**32-α**) favoured the ^2S_o skew-boat conformation,[30,31] based on its unusual coupling constants ($J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 4.7 Hz)[32] and diagnostic NOE signal between H-2 and H-5.[33] After acid catalysed hydrolysis of the isopropylidene protecting group, the product **35-α** favoured the $^1C_4$ chair conformation ($J_{2,3}$ = 3.8 Hz) in solution. This route provided insufficient material to continue through to the desired α-linked acceptor, however, similar acid catalysed hydrolysis of **32-β** followed by TEMPO/BAIB oxidation and base-promoted esterification gave the desired β-methyl IdoA acceptor **37**,[34] in 67% yield.

To access an α-methyl IdoA acceptor, donor **31** was glycosylated with MeOH using NIS/TfOH as the promoter to give a 2 : 1 mixture of α- and β-methyl glycosides **33** in 68% yield.[29] Although **31** has a participating benzoyl group at C-2 which favours formation of the α-L-glycoside, recent studies[29] have shown that neighbouring group participation is of lesser importance in glycosylations between Ido-configured donors and primary alcohol acceptors. The products **33-α** and **-β** were separated by flash chromatography, and **33-α** was taken

Table 1 Coupling constants (J, Hz) measured from ^{1}H NMR spectra ($CDCl_3$ or CD_3OD) for the L-idose or L-iduronic acid rings of selected compounds, with indicative conformations in parentheses

	32-α (^2S_o)	**32-β** ($^1C_4$)	**35-α** ($^1C_4$)	**39** (^2S_o/$^1C_4$)	**7** ($^1C_4$)	**8** ($^1C_4$)
$J_{1,2}$	4.9	1.5	1.4	3.1	1.6	1.6
$J_{2,3}$	9.8	3.9	3.8	7.3	3.4	3.3
$J_{3,4}$	4.7	2.1	—	7.3	3.4	3.3
$J_{4,5}$	—	2.1	—	4.5	2.0	2.3



Scheme 5 Synthesis of target GlcN-IdoA disaccharides **5–8**. Reagents and conditions: (a) NIS/TfOH, DCM, −78 → −20 °C, 2 h, 52% (**39**), 72% (**40**); (b) H_2, 20% $Pd(OH)_2/C$, +/−conc. HCl, MeOH, r.t., 24 h, 98–100%; (c) Ac_2O, Et_3N, MeOH, r.t., 90 min, 39–72; (d) NaOMe/MeOH, r.t., 5 h, 82%.

through the following synthetic steps. Acid catalysed hydrolysis of the isopropylidene group to give the diol **36** (91%) was followed by regioselective TEMPO/BAIB oxidation of the primary alcohol and base-promoted esterification with iodomethane to furnish α-methyl IdoA acceptor **38** [35] in 46% yield.

The GlcN-IdoA disaccharides were then prepared as shown in Scheme 5. Unlike the glycosylations with gluco-configured acceptors, the NIS/TfOH-activated coupling of the thioglycoside **14** with acceptors **37** and **38** proceeded with excellent stereocontrol and gave exclusively the α-linked disaccharide products ($J_{1',2'}$ = 3.5–3.6 Hz) **39** and **40** in acceptable yield (52–72%). The disaccharides were deprotected and acetylated in a similar fashion to the GlcN-GlcA disaccharides in good overall yields to furnish the target disaccharides **5–8**, which were fully characterized by 1D and 2D NMR spectroscopy and HRMS. Once again, interesting conformational effects were evident in the NMR spectra. The conformations of the α-L-IdoA series are consistent with $^1C_4$ chairs for the IdoA ring, however, the protected β-L-IdoA ring of **39** is in between a $^1C_4$ and ^2S_o conformation,[30] as indicated by a large coupling constant for $J_{2,3} = J_{3,4}$ = 7.3 Hz, while there are small coupling constants for $J_{1,2}$ = 3.1 Hz and $J_{4,5}$ = 4.5 Hz (Table 1). Upon deprotection to **7** and **8** the IdoA rings are once again observed in the $^1C_4$ chair conformation.

Synthesis of methyl IdoA-GlcN disaccharides

The target methyl IdoA-GlcN disaccharides were readily prepared from disaccharide **42** [17] *via* removal of the benzoate protecting groups under Zemplén conditions followed by hydrogenolysis (Pd/C) in methanol, either in the presence of conc. HCl to give **9** (79%), or Ac_2O to give **10** (58%), respectively (Fig. 3). The structures of **9** and **10** were confirmed by 1D and 2D NMR spectroscopy and HRMS.

Synthesis of methyl GlcA-GlcN disaccharides

For the preparation of the methyl GlcA-GlcN disaccharides, the thioglycoside **44** [36] was benzoylated under standard conditions

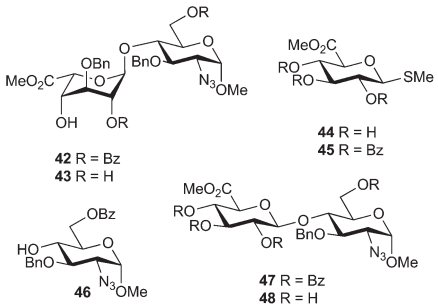


Fig. 3 Structures of intermediates for the synthesis of methyl IdoA-GlcN and GlcA-GlcN disaccharides.

to give **45** in excellent yield (93%). It was recognized that methyl thioglycoside **45** could be converted into a more efficient donor (*e.g.*, a trichloroacetimidate), however, given the small quantities of final product required, it was decided to utilise it directly in the next step. Thus, glycosylation of donor **45** with the readily available acceptor **46** [17,29] with NIS and TMSOTf as the promoter gave the β-linked disaccharide **47** in a modest but acceptable 31% yield ($J_{1',2'}$ = 7.9 Hz). Debenzoylation followed by hydrogenolysis (with Pearlman's catalyst) in the presence of HCl or Ac_2O as described above for **42** gave the target disaccharides **11** and **12** in 90% and 48% yield, respectively. The structures were confirmed by 1D and 2D NMR spectroscopy and HRMS.

LC-MS/MS analyses

The pure synthesized disaccharides **1–12** were each subjected to LC-MS/MS analysis (see ESI†) which demonstrated their purity. They were then spiked into a sample of HS that had been subjected to methanolysis according to the published protocol[12] in order to identify the individual peaks in the chromatogram of the latter. The results are summarized in Fig. 4. Interestingly, the major peak at 1.56 min corresponds to disaccharide **7** with a

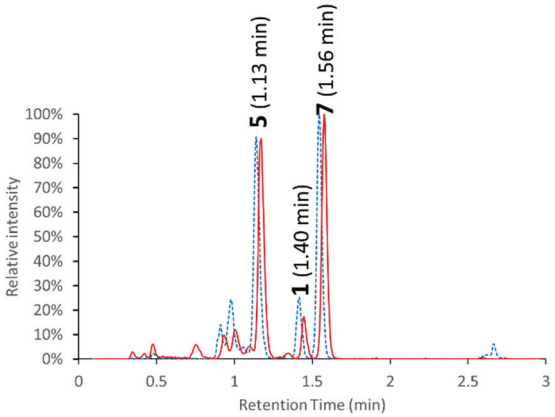


Fig. 4 Identification of disaccharides by LC-MS/MS produced from the methanolysis of heparan sulfate (solid red trace, MRM *m/z* 384–162) compared to an internal standard of d_6 deuterated heparan sulfate digest (dotted blue trace, MRM *m/z* 390–162). Peaks have been identified by retention time comparison of the synthesised disaccharides spiked with the d_6 internal standard.

β-linked methyl IdoA at the reducing end. The other major peak at 1.13 min corresponds to disaccharide **5**. Disaccharide **1**, containing an α-linked methyl GlcA, was expected to be a major component due to the anomeric effect, yet it is only present in minor amounts (small peak at 1.40 min). Disaccharide identifications were confirmed by performing an additional experiment. HS (10 μg) was digested using the methanolysis method and analysed. It was then spiked with disaccharides **1**, **5** or **7** to a final concentration of 50 ng mL^{-1} and the peaks observed from the digestion increased in area with each respective disaccharide spiking experiment, further confirming the identification (see ESI, p. S37†). Given that GlcA is known to be the major uronic acid component of HS,[3] these results suggest that the glycosidic bond of IdoA is more labile than that of GlcA. The results also indicate that the disaccharides have not equilibrated fully to the more stable α-methyl glycosides during the methanolysis reaction. In fact, they are largely in agreement with a recent study[37] during which the time course of HS methanolysis was determined. The amount of disaccharides produced during the reaction continually rose over a 24 hour period, not reaching a maximum, and was at lower levels compared with the disaccharides produced at higher temperature *via* butanolysis.[37] *N*-Acetylated disaccharides were not present in significant amounts. As expected, the four synthesized methyl uronic acid-(1→4)-GlcN disaccharides **9–12** were not detected in the chromatograms, confirming that glycosidic bond cleavage during methanolysis occurs preferentially at the uronic acid glycosidic bond.

Conclusions

In conclusion, acid catalysed methanolysis of HS results in exhaustive desulfation and cleavage of the polymer into a mixture of methylated disaccharides, which can be analysed by LC-MS/MS. This process forms the basis of an assay for the quantitation of HS in biological samples with utility for the diagnosis and prognosis of MPS disorders and for monitoring the efficacy of new therapies. We have synthesized a library of HS-based disaccharides predicted to be the likely major products of the methanolysis reaction. The key step in the synthesis of the library was the stereoselective 1,2-*cis* glycosylation of GlcA or IdoA acceptors with a 2-deoxy-2-azido thioglucoside donor, promoted by NIS/TMSOTf (or TfOH). The disaccharides were used to identify the major peaks in the LC-MS/MS chromatograms from methanolysis of HS and labelled versions may be suitable as internal standards for HS quantitation.

Experimental section

General methods

Melting points were determined on a DigiMelt MSRS apparatus. Optical rotations were determined on a JASCO P-2000 polarimeter at ambient temperature and are given in units of 10^{-1} deg cm^2 g^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer at 20 °C. The residual solvent peaks ($CDCl_3$: δ_H 7.26 and δ_C 77.0; CD_3OD: δ_H 3.3 and δ_C 49.0) served as internal standards. Coupling constants in Hz were measured from one-dimensional spectra. The analyses of ^1H and ^{13}C NMR spectra were assisted by COSY, HSQC ad NOE experiments. Analytical LRMS and HRMS were performed in a positive or negative ion ESI mode on a Bruker HCT spectrometer and a Bruker micrOTOF$_Q$ spectrometer, respectively. All reagents and solvents were obtained from Sigma-Aldrich, Australia, and were used without further purification, except EtOAc, *n*-hexane, MeOH and DCM which were distilled prior to use. Reactions were monitored by analytical thin layer chromatography (TLC) on silica gel 60 F_{254} plates and visualized by charring with 10% sulfuric acid in ethanol. Flash chromatography was performed on silica gel under positive pressure with specified solvent systems. Compounds **13**, **29**, **31**, **42**, **44**, **46** were gifts from Dr Mike West, Alchemia Ltd. Preparation of deuterated heparan sulfate digested internal standard was made using a previously published method.[12] LC-MS/MS analyses were performed on an AP14000 QTrap (Sciex, Concord, Ontario, Canada) coupled to a Waters Acquity UPLC system (Milford, MA, USA) equipped with a BEH C_{18} column (2.1 mm × 50 mm). Data were obtained in positive electrospray ionization multiple reaction monitoring (MRM) mode. Transitions were monitored based on product/ion pairs for the measurement of disaccharides. LC-MS/MS chromatograms were selected based on the MRM transitions for amino disaccharides and *N*-acetate disaccharides, and they are noted in figure captions. All disaccharides were made up to 1 μg mL^{-1}, injection volume of 10 μL was used. Chromatographic separation was by means of a 8.5 min gradient going from 1% B to 5% B or 2 min, followed by a 0.01 min step to 20% B followed by a gradient to 25% B over 3 min, a 2.5 min wash step of 99% B followed by a re-equilibration for 1 min at 1% B (mobile phase A = 0.1% formic acid (aq.) and mobile phase B = acetonitrile 0.1% formic acid).

4′-Methylphenyl 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-1-thio-β-D-glucoside (14)

Thioglycoside **13** (1.0 g, 3.2 mmol) was dissolved in anhydrous DMF (10 mL). A suspension of NaH (462 mg, 11.6 mmol, prewashed with *n*-hexane (3 × 20 mL)) in anhydrous DMF (10 mL) was added. The mixture was stirred at r.t. for 10 min, and then benzyl bromide (1.4 mL, 11.6 mmol) was added dropwise under ice cooling. The stirring was continued for 10 min under ice cooling, and for an additional 1 h at r.t. under N_2. MeOH (10 mL) was added to quench the reaction. The resulting mixture was then diluted with EtOAc, washed with H_2O, 1 M HCl, dried ($MgSO_4$), and concentrated. It was then purified by flash chromatography (EtOAc/*n*-hexane, 1 : 9) to give the tribenzyl ether **14** as colourless oil (1.50 g, 80%); R_f = 0.56 (EtOAc/*n*-hexane, 1 : 4); $[\alpha]_D$ −53.6 (*c* 9.4, $CHCl_3$; lit.[21] −65.94, *c* 1.0, $CHCl_3$). The NMR data of **14** were in accord with the literature.[21] ^1H NMR (500 MHz, $CDCl_3$): δ 7.50–7.02 (m, 19H, Ar), 4.84, 4.81 (ABq, 2H, J_{AB} = 10.5 Hz, CH_2Ph), 4.76, 4.57 (ABq, 2H, J_{AB} = 10.9 Hz, CH_2Ph), 4.60, 4.52 (ABq, 2H, J_{AB} = 11.9 Hz, CH_2Ph), 4.34 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 3.76 (dd, 1H, A part of

ABX, $J_{5,6a}$ = 2.2 Hz, $J_{6a,6b}$ = 11.0 Hz, H-6a), 3.73 (dd, 1H, B part of ABX, $J_{5,6b}$ = 3.9 Hz, H-6b), 3.57 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 3.49 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.3 Hz, H-3), 3.46–3.43 (m, 1H, H-5), 3.28 (dd, 1H, H-2), 2.31 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.7, 138.2, 137.8, 137.6, 134.3, 129.7, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.8, 127.5, 126.9 (Ar), 85.8 (C-1), 85.0 (C-3), 79.3 (C-5), 77.5 (C-4), 75.9, 75.0, 73.4 (3 × CH$_2$Ph), 68.7 (C-6), 64.8 (C-2), 21.1 (CH_3); LRMS: m/z = 604.3 $[M + Na]^+$, 620.3 $[M + K]^+$.

4′-Methylphenyl 2,3-di-*O*-benzyl-4,6-*O*-isopropylidene-α-L-idoside (30)

The alcohol **29** (2.0 g, 4.8 mmol) was benzylated as described for compound **14**. Following work-up, the residue was purified by flash chromatography (EtOAc/*n*-hexane, 1 : 9) to give the *benzyl ether* **30** as a pale yellow oil (2.0 g, 82%); R_f = 0.56 (EtOAc/*n*-hexane, 1 : 4); $[\alpha]_D$ −60.3 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38–7.27 (m, 12 H, Ar), 7.12–7.05 (m, 2H, Ar), 5.50 (d, 1H, $J_{1,2}$ = 4.5 Hz, H-1), 4.73, 4.66 (ABq, 4H, $J_{A,B}$ = 11.8 Hz, 2 × CH_2Ph), 4.24 (ddd, 1H, $J_{4,5}$ = 3.0 Hz, $J_{5,6a}$ = 4.4 Hz, $J_{5,6b}$ = 4.0 Hz, H-5), 4.03 (dd, A part of ABX, 1H, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.00 (dd, 1H, $J_{3,4}$ = 4.1 Hz, H-4), 3.80 (dd, 1H, B part of ABX, H-6b), 3.76 (dd, 1H, $J_{2,3}$ = 6.6 Hz, H-3), 3.65 (dd, 1H, H-2), 2.31 (s, 3H, ArCH_3), 1.41 (s, 3H, CH_3), 1.43 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.1, 138.0, 137.0, 132.0, 131.2, 129.6, 128.3, 128.2, 127.8, 127.7, 127.6 (C–Ar), 98.9 (C(CH$_3$)$_2$), 87.5 (C-1), 78.3 (C-3), 77.2 (C-2), 73.4 (CH$_2$Ph), 73.1 (CH$_2$Ph), 70.0 (C-4), 63.1 (C-5), 61.6 (C-6), 27.5 (CH_3), 21.0 (ArCH$_3$), 20.2 (CH_3); LRMS: m/z = 524.2 $[M + NH_4]^+$, 529.2 $[M + Na]^+$, 545.2 $[M + K]^+$; HRMS: m/z calcd for $C_{30}H_{34}O_5SNa$ $[M + Na]^+$: 529.2019; found: 529.2004.

Methyl(2-azido-3,4,6,-tri-*O*-benzyl-2-deoxy-D-glucopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-α-D-glucopyranosid)uronate (25, 26)

A solution of donor **14** (943 mg, 1.62 mmol) and acceptor **23** (544 mg, 1.35 mmol) and freshly dried AW300 mol sieves (1.5 g) in anhydrous DCM (15 mL) was stirred at r.t. for 30 min under N_2. NIS (426 mg, 1.89 mmol) was added to the solution. The solution was then stirred at −78 °C for 10 min. TfOH (24 μL, 0.27 mmol) was added at −78 °C, and the mixture was slowly warmed up to −20 °C over 2 h under N_2. The reaction was then quenched by addition of Et_3N (5 mL), sat. aq. $NaHCO_3$ (7 mL) and 10% $Na_2S_2O_3$ in H_2O (7 mL) to pH 8. The resulting solution was filtered through Celite and the filter cake was washed with DCM. The filtrate was then washed with 10% $Na_2S_2O_3$ in H_2O solution, dried ($MgSO_4$), filtered and concentrated. The residue was then purified by flash chromatography (EtOAc/*n*-hexane, 1 : 9) to give the α-linked disaccharide **25** as a colourless oil (576 mg), the β-linked isomer **26** as a colourless oil (275 mg) and an α/β-mixture (**25**, **26**) as yellow oil (248 mg). In total, it was a 2 : 1 α/β mixture in 94% yield. **α-Isomer 25**: R_f = 0.18 (EtOAc/*n*-hexane, 1 : 4); $[\alpha]_D$ +32.6 (*c* 1.1, $CHCl_3$; lit.[28] +32.0); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34–7.08 (m, 25H, Ph), 5.57 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1′), 5.01, 4.85 (ABq, 2H, $J_{A,B}$ = 10.6 Hz, CH_2Ph), 4.83 (s, 2H, CH_2Ph), 4.75, 4.58 (ABq, 2H, $J_{A,B}$ = 12.1 Hz, CH_2Ph), 4.73, 4.47 (ABq, 2H, $J_{A,B}$ = 11.0 Hz, CH_2Ph), 4.57, 4.40 (ABq, 2H, $J_{A,B}$ = 12.1 Hz, CH_2Ph), 4.55 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.16 (d, 1H, $J_{4,5}$ = 9.3 Hz, H-5), 4.03–4.00 (m, 2H, H-3, H-4), 3.84 (dd, 1H, $J_{2',3'}$ = 10.3 Hz, $J_{3',4'}$ = 9.0 Hz, H-3′), 3.73–3.69 (m, 2H, H-4′, H-6′a), 3.65 (s, 3H, CO_2CH_3), 3.57–3.55 (m, 2H, H-6′b, H-2), 3.43 (ddd, 1H, $J_{5',6'a}$ = 2.1 Hz, $J_{5',6'b} = J_{5',4'}$ = 10.0 Hz, H-5′), 3.37 (s, 3H, OCH_3), 3.29 (dd, 1H, H-2′). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9 (C═O), 138.3, 138.1, 137.8, 137.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4 (Ph), 98.5 (C-1), 97.8 (C-1′), 81.1 (C-3), 79.9 (C-3′), 79.5 (C-2), 77.8 (C-4′), 75.3 (C-4), 75.4, 74.9, 74.7, 73.6, 73.5 (5 × CH$_2$Ph), 71.1 (C-5′), 70.0 (C-5), 67.6 (C-6′), 63.2 (C-2′), 55.8 (OCH_3), 52.6 (CO_2CH_3); LRMS: m/z = 877.3 $[M + NH_4]^+$, 882.3 $[M + Na]^+$, 898.3 $[M + K]^+$; HRMS: m/z calcd for $C_{49}H_{53}N_3O_{11}Na$ $[M + Na]^+$: 882.3572, found: 882.3568; **β-isomer 26**: R_f = 0.17 (EtOAc/*n*-hexane, 1 : 4); $[\alpha]_D$ +7.4 (*c* 0.1, $CHCl_3$; lit.[28] +0.6, *c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33–7.10 (m, 25H, Ph), 5.02, 4.77 (ABq, 2H, $J_{A,B}$ = 11.4 Hz, CH_2Ph), 4.81, 4.76 (ABq, 2H, $J_{A,B}$ = 10.8 Hz, CH_2Ph), 4.73, 4.51 (ABq, 2H, $J_{A,B}$ = 11.0 Hz, CH_2Ph), 4.72, 4.55 (ABq, 2H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.54 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.34, 4.30 (ABq, 2H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.31 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1′), 4.21 (d, 1H, $J_{4,5}$ = 9.8 Hz, H-5), 4.03 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4), 3.90 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.4 Hz, H-3), 3.79 (s, 3H, CO_2CH_3), 3.62 (dd, 1H, $J_{2',3'}$ = 9.7 Hz, $J_{3',4'}$ = 8.7 Hz, H-3′), 3.56 (dd, 1H, $J_{5',6'a}$ = 2.1 Hz, $J_{6a',\ 6b'}$ = 10.9 Hz, H-6′a), 3.50 (dd, 1H, $J_{6b',5}$ = 4.1 Hz, $J_{6b',6a'}$ = 10.9 Hz, H-6′b), 3.48 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.39 (s, 3H, OCH_3), 3.35 (dd, 1H, $J_{3',4'} = J_{4',5'}$ = 8.7 Hz, H-4′), 3.29 (dd, 1H, H-2′), 3.30–3.27 (m, 1H, H-5′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1 (C═O), 139.2, 138.1, 137.9, 137.8, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4, 127.1 (Ph), 101.7 (C-1′), 98.8 (C-1), 83.2 (C-4′), 79.7 (C-3), 79.1 (C-4), 78.6 (C-2), 77.6 (C-3′), 75.3 (C-5′), 75.4, 75.2, 74.8, 73.7, 73.2 (5 × CH$_2$Ph), 69.8 (C-5), 68.4 (C-6′), 66.8 (C-2′), 55.7 (OCH_3), 52.7 (CO_2CH_3).

Methyl(2-amino-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl α-D-glucopyranosid)uronate (1)

The protected disaccharide **25** (108 mg, 0.13 mmol) was dissolved in anhydrous MeOH (5 mL) and 10% Pd/C (65 mg) was added. The resulting mixture was stirred at r.t. under an atmosphere of H_2 for 24 h. The catalyst was removed by filtration (Celite), and the filtrate was concentrated. The residue was then purified by flash chromatography (NH_4OH/i-PrOH, 1 : 4) to give the *disaccharide* **1** as white foam (25 mg, 51%); R_f = 0.14 (NH_4OH/i-PrOH, 1 : 4); $[\alpha]_D$ +132.9 (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.28 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1′), 4.70 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.09 (d, 1H, $J_{4,5}$ = 9.6 Hz, H-5), 3.83 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.3 Hz, H-3), 3.78 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 3.77 (s, 3H, CO_2CH_3), 3.72–3.70 (m, 2H, H-6′), 3.46 (dd, 1H, H-2), 3.42–3.40 (m, 2H, H-3′, H-5′), 3.41 (s, 3H, OCH_3), 3.33 (dd, 1H, $J_{3'4'} = J_{4',5'}$ = 8.9 Hz, H-4′), 2.65 (dd, $J_{2',3'}$ = 9.9 Hz, H-2′); ^{13}C NMR (125 MHz, CD_3OD): δ 171.3 (C═O), 101.7 (C-1), 101.3 (C-1′), 80.1 (C-4), 74.8 (C-3′), 74.5 (C-3), 74.4 (C-5′), 72.8 (C-2), 71.8 (C-5), 71.2 (C-4′), 61.9 (C-6′), 57.0 (C-2′), 56.1 (OCH_3),

53.2 (CO_2CH_3); LRMS: m/z = 384.1 $[M + H]^+$; HRMS: m/z calcd for $C_{14}H_{26}NO_{11}$ $[M + H]^+$: 384.1500, found: 384.1493.

Methyl(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl α-D-glucopyranosid)uronate (2)

To a solution of amine **1** (5.8 mg, 0.02 mmol) in anhydrous MeOH (0.5 mL) at 0 °C under N_2 was added Et_3N (4.2 μL, 0.03 mmol) and Ac_2O (21 μL, 0.23 mmol). The mixture was stirred at r.t. for 90 min, and then concentrated *in vacuo* to give the *amide* **2** as a colourless oil (6.4 mg, 100%); R_f = 0.5 (NH_4OH/i-PrOH, 1 : 4); $[\alpha]_D$ +116.2 (*c* 0.32, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.20 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1′), 4.68 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.09 (d, 1H, $J_{4,5}$ = 9.1 Hz, H-5), 3.86 (dd, 1H, $J_{2',3'}$ = 10.6 Hz, H-2′), 3.81–3.78 (m, 2H, H-3, H-4), 3.77 (s, 3H, CO_2CH_3), 3.72–3.71 (m, 2H, H-6′), 3.56 (dd, 1H, $J_{3',4'}$ = 8.5 Hz, H-3′), 3.47–3.42 (m, 3H, H-2, H-4′, H-5′), 3.41 (s, 3H, OCH_3), 1.98 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, CD_3OD): δ 173.9, 171.2 (2 × C═O), 101.7 (C-1), 100.2 (C-1′), 79.8 (C-4), 74.31 (C-5′), 74.29 (C-3), 73.1 (C-2), 72.9 (C-3′), 72.1 (C-5), 71.4 (C-4′), 61.8 (C-6′), 56.1 (OCH_3), 55.5 (C-2′), 53.2 (CO_2CH_3), 22.7 ($COCH_3$); LRMS: m/z = 448.1 $[M + Na]^+$, 464.1 $[M + K]^+$; HRMS: m/z calcd for $C_{16}H_{27}NO_{12}$ $[M + H]^+$: 448.1425, found: 448.1413.

Methyl(2-azido-3,4,6,-tri-*O*-benzyl-2-deoxy-D-glucopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-β-D-glucopyranosid)uronate (27, 28)

The donor **14** (702 mg, 1.21 mmol) and acceptor **24** (405 mg, 1.01 mmol) were glycosylated as described for compounds **25** and **26**. Following work-up, the residue was purified by flash chromatography (EtOAc/*n*-hexane, 1 : 9) to give the α-linked disaccharide **27** as colourless oil (558 mg), β-isomer **28** as colourless oil (66 mg) and α,β-mixture (**27**, **28**) as yellow oil (197 mg). In total, it was a 9 : 1 α/β mixture in 95% yield. **α-Isomer 27**: R_f = 0.18 (EtOAc/*n*-hexane, 1 : 4); $[\alpha]_D$ +23.7 (*c* 8.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33–7.22 (m, 23 H, Ph), 7.13–7.11 (m, 2H, Ph), 5.57 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1′), 4.99, 4.80 (ABq, 2H, $J_{A,B}$ = 10.7 Hz, CH_2Ph), 4.88, 4.75 (ABq, 2H, $J_{A,B}$ = 11.1 Hz, CH_2Ph), 4.84, 4.82 (ABq, 2H, $J_{A,B}$ = 10.7 Hz, CH_2Ph), 4.66, 4.49 (ABq, 2H, $J_{A,B}$ = 11.1 Hz, CH_2Ph), 4.60, 4.43 (ABq, 2H, $J_{A,B}$ = 12.1 Hz, CH_2Ph), 4.37 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.12 (dd, 1H, $J_{3,4}$ = 9.1 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 3.93 (d, 1H, H-5), 3.84 (dd, 1H, $J_{3',4'}$ = 9.0 Hz, $J_{2',3'}$ = 10.3 Hz, H-3′), 3.76–3.70 (m, 3H, H-3, H-4′, H-6′a), 3.65 (s, 3H, CO_2CH_3), 3.59 (dd, 1H, $J_{5',6'b}$ = 2.1 Hz, $J_{6'a,\ 6'b}$ = 10.8 Hz, H-6′b), 3.54 (s, 3H, OCH_3), 3.49 (dd, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 3.40 (ddd, 1H, $J_{5',6'a}$ = $J_{4',5'}$ = 10.0 Hz, H-5′), 3.27 (dd, 1H, H-2′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.9 (C═O), 138.1, 138.0, 137.8, 137.7, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4 (Ph), 104.8 (C-1), 97.7 (C-1′), 83.8 (C-3), 81.8 (C-2), 79.8 (C-3′), 77.8 (C-4′), 75.3, 75.2, 74.7, 73.6 (5 × CH_2Ph), 74.5 (C-4), 74.3 (C-5), 71.1 (C-5′), 67.6 (C-6′), 63.1 (C-2′), 57.3 (OCH_3), 52.5 (CO_2CH_3); LRMS: m/z = 877.2 $[M + NH_4]^+$, 882.2 $[M + Na]^+$, 898.2 $[M + K]^+$; HRMS: m/z calcd for $C_{49}H_{53}N_3O_{11}Na$ $[M + Na]^+$: 882.3572, found: 882.3571. **β-Isomer 28**: R_f = 0.17 (EtOAc/*n*-hexane, 1 : 4); $[\alpha]_D$ −3.2 (*c* 5.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.32–7.11 (m, 25H, Ph), 5.00, 4.72 (ABq, 2H, $J_{A,B}$ = 11.6 Hz, CH_2Ph), 4.81, 4.75 (ABq, 2H, $J_{A,B}$ = 11.0 Hz, CH_2Ph), 4.78, 4.62 (ABq, 2H, $J_{A,B}$ = 11.0 Hz, CH_2Ph), 4.73, 4.51 (ABq, 2H, $J_{A,B}$ = 11.0 Hz, CH_2Ph), 4.37 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.36, 4.28 (ABq, 2H, $J_{A,B}$ = 12.1 Hz, CH_2Ph), 4.35 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1′), 4.15 (dd, 1H, $J_{4,5}$ = 9.5 Hz, $J_{3,4}$ = 8.8 Hz, H-4), 3.95 (d, 1H, H-5), 3.81 (s, 3H, CO_2CH_3), 3.64 (dd, 1H, $J_{3',4'}$ = $J_{2',3'}$ = 9.1 Hz, H-3′), 3.62 (dd, 1H, $J_{3,4}$ = $J_{2,3}$ = 8.8 Hz, H-3), 3.57–3.52 (m, 2H, H-6′), 3.52 (s, 3H, OCH_3), 3.41 (dd, 1H, H-2), 3.34 (dd, 1H, $J_{4',5'}$ = 9.9 Hz, H-4′), 3.31 (dd, 1H, H-2′), 3.31–3.28 (m, 1H, H-5′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.1 (C═O), 139.0, 138.3, 138.1, 137.9, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 127.2 (Ph), 104.9 (C-1), 101.7 (C-1′), 83.1 (C-4′), 82.0 (C-3), 81.2 (C-2), 78.9 (C-4), 77.6 (C-3′), 75.5, 75.0, 74.9, 74.8, 73.3 (5 × CH_2Ph), 75.1 (C-2′), 74.3 (C-5), 68.4 (C-6′), 66.8 (C-5′), 57.4 (OCH_3), 52.7 (CO_2CH_3).

Methyl(2-amino-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl β-D-glucopyranosid)uronate (3)

The disaccharide **27** (159 mg, 0.19 mmol) was dissolved in anhydrous MeOH (10 mL), 20% $Pd(OH)_2$/C (160 mg) and one drop of conc. HCl were added. The solution was stirred at r.t. under an atmosphere of H_2 for 21 h. The catalyst was then removed by filtration over Celite, and the filtrate was concentrated. The residue was then purified by flash chromatography (NH_4OH/i-PrOH, 1 : 4) to give *disaccharide* **3** as white foam (42 mg, 60%); R_f = 0.11 (NH_4OH/i-PrOH, 1 : 4); $[\alpha]_D$ −11.4 (*c* 1.1, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.27 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1′), 4.24 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 3.96 (d, 1H, $J_{4,5}$ = 9.6 Hz, H-5), 3.78 (dd, 1H, $J_{3,4}$ = 9.1 Hz, H-4), 3.78 (s, 3H, CO_2CH_3), 3.71–3.70 (m, 2H, H-6′), 3.62 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.1 Hz, H-3), 3.49 (s, 3H, OCH_3), 3.43–3.35 (m, 3H, H-3′, H-5′, H-4′), 3.23 (dd, 1H, H-2), 2.64 (dd, 1H, $J_{2',3'}$ = 10.1 Hz, H-2′); ^{13}C NMR (125 MHz, CD_3OD): δ 170.7 (C═O), 105.7 (C-1), 101.7 (C-1′), 80.0 (C-4), 77.4 (C-3), 75.9 (C-5), 75.1 (C-3′), 74.4 (C-5′), 74.3 (C-2), 71.2 (C-4′), 61.9 (C-6′), 57.5 (C-2′), 57.0 (OCH_3), 53.2 (CO_2CH_3); LRMS: m/z = 384.1 $[M + H]^+$, 406.1 $[M + Na]^+$; HRMS: m/z calcd for $C_{14}H_{25}NO_{11}Na$ $[M + Na]^+$: 406.1320, found: 406.1319.

Methyl(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl β-D-glucopyranosid)uronate (4)

The disaccharide **27** (79 mg, 0.09 mmol) was hydrogenated as described for compound **1**. The crude amine was then dissolved in anhydrous MeOH (5 mL) and Ac_2O (195 μL, 2.07 mmol) and Et_3N (586 μL, 4.1 mmol) were added. The solution was stirred at r.t. for 24 h and was then concentrated. The residue was purified by flash chromatography (NH_4OH/i-PrOH, 1 : 4) to give the *amide* **4** as a colourless oil (24 mg, 62%); R_f = 0.55 (NH_4OH/i-PrOH, 2 : 3); $[\alpha]_D$ +8.0 (*c* 0.4, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.21 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1′), 4.23 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 3.96 (d, 1H, $J_{4,5}$ = 9.6 Hz, H-5), 3.86 (dd, 1H, $J_{2',3'}$ = 10.7 Hz, H-2′), 3.79 (dd, 1H, $J_{3,4}$ = 9.1 Hz, H-4), 3.78 (s, 3H, CO_2CH_3), 3.73–3.72 (m, 2H, H-6′), 3.60 (dd, 1H, $J_{3,4}$ = $J_{2,3}$ = 9.1 Hz, H-3), 3.58 (dd, 1H, $J_{3',4'}$ = 8.4 Hz, H-3′), 3.48 (s, 3H, OCH_3), 3.45–3.40 (m, 2H, H-4′, H-5′), 3.21 (dd, 1H, H-2), 1.99 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, CD_3OD):

δ 173.9, 170.6 (2 × C═O), 105.7 (C-1), 100.2 (C-1′), 79.6 (C-4), 77.1 (C-3), 62.2 (C-5), 74.9 (C-2), 74.2 (C-5′), 72.8 (C-3′), 71.3 (C-4′), 61.8 (C-6′), 57.6 (OCH_3), 55.4 (C-2′), 53.2 (CO_2CH_3), 23.0 ($COCH_3$); LRMS: m/z = 426.1 $[M + H]^+$, 448.1 $[M + Na]^+$, 464.1 $[M + K]^+$; HRMS: m/z calcd for $C_{16}H_{27}NO_{12}Na$ $[M + Na]^+$: 448.1425, found: 448.1422.

Methyl 2,3-di-*O*-benzyl-4,6-*O*-isopropylidene-L-idopyranoside (32)

The donor **30** (217 mg, 0.4 mmol) and anhydrous MeOH (87 μL, 2.1 mmol) were glycosylated as described for compounds **25** and **26**. Following work-up the residue was purified by flash chromatography (EtOAc/*n*-hexane, 1 : 9) to give **32-α** as colourless oil (33 mg) and **32-β** as colourless oil (141 mg). In total, it was a 1 : 4 α/β mixture in 98% yield. **α-Isomer (32-α)**: R_f = 0.55 (EtOAc/*n*-hexane, 2 : 3); $[\alpha]_D$ −30.5 (*c* 2.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33–7.24 (m, 10H, Ph), 4.74–4.67 (m, 5H, 2 × CH_2Ph, H-1), 3.98–3.94 (m, 2H, H-4, H-6a), 3.88–3.86 (m, 1H, H-5), 3.73 (dd, 1H, B part of ABX, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6b), 3.69 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 4.7 Hz, H-3), 3.49 (dd, 1H, $J_{1,2}$ = 4.9 Hz, H-2), 3.37 (s, 3H, OCH_3), 1.39 (s, 6H, 2 × CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.6, 138.5, 128.3, 128.2, 127.8, 127.7, 127.5 (Ph), 103.5 (C-1), 99.1 (C$H(CH_3)_2$), 80.2 (C-3), 79.4 (C-2), 73.6, 73.5 (2 × CH_2Ph), 72.7 (C-4), 64.0 (C-5), 61.0 (C-6), 55.3 (OCH_3), 26.9 (CH_3), 20.9 (CH_3); **β-isomer (32-β)**: R_f = 0.37 (EtOAc/*n*-hexane, 2 : 3); $[\alpha]_D$ +55.1 (*c* 1.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38–7.20 (m, 10H, Ph), 4.78, 4.64 (ABq, 2H, $J_{A,B}$ = 12.2 Hz, CH_2Ph), 4.63 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.55, 4.53 (ABq, 2H, $J_{A,B}$ = 11.8 Hz, CH_2Ph), 4.03 (dd, 1H, A part of ABX, $J_{5,6a}$ = 2.7 Hz, $J_{6a,6b}$ = 12.7 Hz, H-6a), 3.96 (dd, 1H, B part of ABX, $J_{5,6b}$ = 2.1 Hz, H-6b), 3.87 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 2.1 Hz, H-4), 3.76 (dd, 1H, $J_{2,3}$ = 3.9 Hz, H-3), 3.59–3.58 (m, 1H, H-5), 3.55 (dd, 1H, H-2), 3.51 (s, 3H, OCH_3), 1.59 (s, 3H, CH_3), 1.43 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.8, 137.7, 128.4, 128.0, 127.9, 127.6, 127.5, 127.1 (Ph), 100.3 (C-1), 98.3 (C$H(CH_3)_2$), 77.3 (C-3), 73.8 (C-2), 72.6, 72.4 (2 × CH_2Ph), 67.0 (C-4), 66.6 (C-5), 62.7 (C-6), 56.5 (OCH_3), 28.9 (CH_3), 18.8 (CH_3). LRMS: m/z = 432.1 $[M + NH_4]^+$, 437.1 $[M + Na]^+$, 453.1 $[M + K]^+$; HRMS: m/z calcd for $C_{24}H_{30}O_6Na$ $[M + Na]^+$: 437.1935, found: 437.1948.

Methyl 2,3-di-*O*-benzyl-β-L-idopyranoside (34)

The isopropylidene **32-β** (2.24 g, 5.4 mmol) was dissolved in 80% AcOH in H_2O solution (30 mL) and heated at 100 °C for 24 h. The mixture was then cooled to r.t., neutralised with solid $NaHCO_3$ and diluted with a mixture of EtOAc/H_2O (1 : 1, 30 mL). The organic layer was separated, dried ($MgSO_4$), filtered and concentrated. The residue was then purified by flash chromatography (EtOAc/*n*-hexane, 1 : 1) to give the diol **34** as colourless oil (1.0 g, 50%); R_f = 0.22 (EtOAc/*n*-hexane, 7 : 3); $[\alpha]_D$ +51.9 (*c* 4.5, $CHCl_3$), lit.[34] +86 (*c* 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29–7.20 (m, 10H, Ph), 4.83, 4.60 (ABq, 2H, $J_{A,B}$ = 12.1 Hz, CH_2Ph), 4.68 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1), 4.49, 4.45 (ABq, 2H, $J_{A,B}$ = 11.8 Hz, CH_2Ph), 3.98 (dd, 1H, A part of ABX, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6a}$ = 7.2 Hz, H-6a), 3.93–3.91 (m, 1H, H-5), 3.79 (dd, 1H, B part of ABX, $J_{5,6b}$ = 4.1 Hz, H-6b), 3.75 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 3.3 Hz, H-3), 3.63 (dd, 1H, $J_{4,5}$ = 1.5 Hz, H-4), 3.61 (dd, 1H, H-2), 3.56 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 137.4, 137.3, 128.5, 128.4, 128.1, 128.0, 127.6 (Ph), 100.9 (C-1), 75.6 (C-3), 75.2 (C-5), 74.5 (C-2), 74.2, 72.2 (2 × CH_2Ph), 67.2 (C-4), 62.7 (C-6), 57.1 (OCH_3); LRMS: m/z = 397.2 $[M + Na]^+$; HRMS: m/z calcd for $C_{21}H_{26}O_6Na$ $[M + Na]^+$: 397.1622, found: 397.1632.

Methyl 2,3-di-*O*-benzyl-α-L-idopyranoside (35)

The isopropylidene **32-α** (14 mg, 0.03 mmol) was dissolved in a mixture of DCM/TFA/H_2O (100 : 10 : 1, 3 mL) and the solution stirred at r.t. for 30 min. The solution was then concentrated and the residue purified by flash chromatography (EtOAc/*n*-hexane, 1 : 1) to give the *diol* **35** as a colourless oil (12.6 mg, 100%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34–7.23 (m, 10 H, Ph), 4.80 (app s, 1H, H-1), 4.59, 4.52 (ABq, 2H, $J_{A,B}$ = 12.2 Hz, CH_2Ph), 4.53, 4.51 (ABq, 2H, $J_{A,B}$ = 11.8 Hz, CH_2Ph), 4.12–4.10 (m, 1H, H-5), 3.93 (dd, 1H, A part of ABX, $J_{5,6a}$ = 6.3 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a), 3.78 (dd, 1H, B part of ABX, $J_{5,6b}$ = 3.9 Hz, H-6b), 3.69–3.68 (m, 2H, H-3, H-4), 3.54 (dd, 1H, $J_{1,2}$ = 1.4 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 3.41 (s, 3H, OCH_3), 3.28 (br. s, 1H, OH-4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 137.7, 136.7, 128.6, 128.5, 128.2, 127.9, 127.7 (Ph), 99.8 (C-1), 73.6 (C-2), 73.5 (C-3), 72.5, 71.8 (2 × CH_2Ph), 68.2 (C-4), 66.9 (C-5), 63.7 (C-6), 55.5 (OCH_3); LRMS: m/z = 397.2 $[M + Na]^+$; HRMS: m/z calcd for $C_{21}H_{26}O_6Na$ $[M + Na]^+$: 397.1622, found: 397.1621.

Methyl(2-azido-3,4,6,-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-β-L-idopyranosid)uronate (39)

The donor **14** (819 mg, 1.41 mmol) and acceptor **37** (472 mg, 1.17 mmol) were glycosylated as described for compounds **25** and **26**. Following work-up the residue was purified by flash chromatography (EtOAc/*n*-hexane, 1 : 9) to give the *disaccharide* **39** as colourless oil (520 mg, 52%); R_f = 0.2 (EtOAc/*n*-hexane, 1 : 4); $[\alpha]_D$ +40.9 (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36–7.11 (m, 25H, Ph), 5.12 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1′), 4.85, 4.77 (ABq, 2H, $J_{A,B}$ = 10.8 Hz, CH_2Ph), 4.73, 4.47 (ABq, 2H, $J_{A,B}$ = 11.1 Hz, CH_2Ph), 4.74, 4.73 (ABq, 2H, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.72, 4.63 (ABq, 2H, $J_{A,B}$ = 12.1 Hz, CH_2Ph), 4.55, 4.43 (ABq, 2H, $J_{A,B}$ = 12.1 Hz, CH_2Ph), 4.51 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1), 4.36 (d, 1H, $J_{4,5}$ = 4.5 Hz, H-5), 4.34 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 7.3 Hz, H-3), 3.88 (dd, 1H, H-4), 3.84 (dd, 1H, $J_{2',3'}$ = 10.3 Hz, $J_{3',4'}$ = 8.8 Hz, H-3′), 3.74–3.72 (m, 1H, H-5′), 3.70 (s, 3H, CO_2CH_3), 3.67 (dd, 1H, A part of ABX, $J_{5',6a'}$ = 3.4 Hz, $J_{6a',6b'}$ = 10.6 Hz, H-6a′), 3.63 (dd, 1H, $J_{4',5'}$ = 9.9 Hz, H-4′), 3.54 (dd, 1H, B part of ABX, $J_{5',6b'}$ = 1.9 Hz, H-6b′), 3.47 (dd, 1H, H-2), 3.40 (s, 3H, OCH_3), 3.32 (dd, 1H, H-2′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.4 (C═O), 138.3, 138.2, 138.0, 137.9, 137.8, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6 (Ph), 100.9 (C-1), 99.5 (C-1′), 79.8 (C-3′), 78.1 (C-4′), 77.2 (C-2), 76.2 (C-4), 76.1 (C-3), 75.2, 74.9, 74.5, 73.6, 73.5 (5 × CH_2Ph), 72.0 (C-5), 71.5 (C-5′), 67.9 (C-6′), 63.5 (C-2′), 57.4 (OCH_3), 52.0 (CO_2CH_3); LRMS: m/z = 877.3 $[M + NH_4]^+$, 882.2 $[M + Na]^+$, 898.2 $[M + K]^+$; HRMS: m/z calcd for $C_{49}H_{53}O_{11}N_3Na$ $[M + Na]^+$: 882.3572, found: 882.3576.

Methyl(2-amino-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl β-L-idopyranosid)uronate (7)

The disaccharide **39** (66 mg, 0.08 mmol) was hydrogenated as described for compound **3** to give the *disaccharide* **7** as a colourless oil (29 mg, 98%); R_f = 0.08 (NH_4OH/i-PrOH, 1 : 4); $[\alpha]_D$ +63.7 (*c* 0.6, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.31 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1′), 4.71 (d, 1H, $J_{4,5}$ = 2.0 Hz, H-5), 4.69 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 4.24 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 3.4 Hz, H-3), 4.02 (dd, 1H, H-4), 3.79 (s, 3H, CO_2CH_3), 3.75–3.68 (m, 3H, H-6′, H-3′), 3.65 (dd, 1H, H-2), 3.58 (s, 3H, OCH_3), 3.37–3.36 (m, 2H, H-4′, H-5′), 3.09 (dd, 1H, $J_{2',3'}$ = 10.6 Hz, H-2′); ^{13}C NMR (125 MHz, CD_3OD): δ 171.0 (C=O), 101.8 (C-1), 93.8 (C-1′), 74.7 (C-5′), 74.0 (C-5), 73.4 (C-4), 71.1 (C-4′), 70.8 (C-3′), 70.4 (C-2), 66.6 (C-3), 61.8 (C-6′), 57.4 (OCH_3), 55.9 (C-2′), 52.9 (CO_2CH_3); LRMS: *m*/*z* = 384.1 $[M + H]^+$, 406.1 $[M + Na]^+$, 422.0 $[M + K]^+$; HRMS: *m*/*z* calcd for $C_{14}H_{26}O_{11}N$ $[M + Na]^+$: 384.1500, found: 384.1492.

Methyl(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl β-L-idopyranosid)uronate (8)

The amine **7** (5.2 mg, 0.014 mmol) was acetylated as described for compound **2**. The crude product was purified by flash chromatography (NH_4OH/i-PrOH, 1 : 4) to give the *amide* **8** as a white foam (4.1 mg, 72%); R_f = 0.44 (NH_4OH/i-PrOH, 1 : 4); $[\alpha]_D$ +97.6 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.97 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1′), 4.68 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 4.66 (d, 1H, $J_{4,5}$ = 2.3 Hz, H-5), 4.11 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 3.3 Hz, H-3), 3.91–3.89 (m, 2H, H-4, H-2′), 3.78 (s, 3H, CO_2CH_3), 3.73–3.72 (m, 2H, H-6′), 3.60 (dd, H-2), 3.57 (s, 3H, OCH_3), 3.54 (dd, 1H, $J_{2',3'}$ = 10.6 Hz, $J_{3',4'}$ = 8.4 Hz, H-3′), 3.41–3.36 (m, 2H, H-4′, H-5′), 1.96 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, CD_3OD): δ 174.1, 171.2 (2 × C=O), 101.9 (C-1), 97.1 (C-1′), 74.5 (C-5), 74.4 (C-4), 74.2 (C-5′), 73.1 (C-3′), 71.6 (C-4′), 70.7 (C-2), 67.6 (C-3), 62.3 (C-6′), 57.3 (OCH_3), 55.1 (C-2′), 52.8 (CO_2CH_3), 22.8 ($COCH_3$); LRMS: *m*/*z* = 448.1 $[M + Na]^+$, 464.1 $[M + K]^+$; HRMS: *m*/*z* calcd for $C_{16}H_{27}O_{12}NNa$ $[M + Na]^+$: 448.1425; found: 448.1443.

Methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside (36)

The isopropylidene **33-α** [5] (500 mg, 1.17 mmol) was hydrolysed as described for compound **35**. The crude product was purified by flash chromatography (EtOAc/*n*-hexane, 1 : 1) to give the *diol* **36** as colourless oil (411 mg, 91%); R_f = 0.19 (EtOAc/*n*-hexane, 1 : 1); $[\alpha]_D$ −24.9 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.01–7.97 (m, 2H, Ph), 7.61–7.24 (m, 10H, Ph), 5.20 (dd, 1H, $J_{1,2}$ = 1.2 Hz, $J_{2,3}$ = 2.4 Hz, H-2), 4.86 (app. s, 1H, H-1), 4.82, 4.62 (ABq, 2H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.27 (ddd, 1H, $J_{5,6a}$ = 6.2 Hz, $J_{5,6b}$ = 4.2 Hz, $J_{4,5}$ = 1.0 Hz, H-5), 3.95 (ddd, 1H, $J_{6a,6b}$ = 11.9 Hz, $J_{6a,OH}$ = 4.6 Hz, H-6a), 3.85 (ddd, 1H, $J_{6a,OH}$ = 7.8 Hz, H-6b), 3.78–3.74 (m, 2H, H-3, H-4), 3.46 (s, 3H, OCH_3), 2.71 (d, 1H, $J_{4,OH}$ = 9.3 Hz, OH-4), 2.09 (dd, 1H, OH-6); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.0 (C=O), 137.6, 133.5, 129.7, 129.2, 128.6, 128.4, 127.9, 127.7 (Ph), 99.8 (C-1), 74.9 (C-3), 71.9 (CH_2Ph), 68.2 (C-4), 68.1 (C-2), 66.8 (C-5), 63.4 (C-6), 55.7 (OCH_3); LRMS *m*/*z* = 411.1 $[M + Na]^+$, 427.1 $[M + K]^+$; HRMS: *m*/*z* calcd for $C_{21}H_{24}O_7Na$ $[M + Na]^+$: 411.1414; found: 411.1420.

Methyl(methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosid) uronate (38)

The diol **36** (456 mg, 1.2 mmol) was dissolved in a mixture of DCM/H_2O (3 : 1, 16 mL), and to this mixture, TEMPO (37 mg, 0.23 mmol) and BAIB (757 mg, 2.3 mmol) were added. The resulting mixture was stirred vigorously at r.t. for 3 h. The solution was washed with sat. aq. $Na_2S_2O_3$ solution, dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in anhydrous DMF (12 mL), and to this mixture, iodomethane (0.11 mL, 1.76 mmol) and $KHCO_3$ (235 mg, 2.35 mmol) were added. The resulting mixture was stirred at r.t. for 24 h in the dark under argon. The mixture was then concentrated and the residue purified by flash chromatography (EtOAc/*n*-hexane, 1 : 1) to give the ester **38** as yellow oil (223 mg, 46%); R_f = 0.23 (EtOAc/*n*-hexane, 1 : 1); $[\alpha]_D$ −10.5 (*c* 0.4, $CHCl_3$), lit.[35] −19 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.01–7.94 (m, 2H, Ph), 7.58–7.24 (m, 10H, Ph), 5.18 (dd, 1H, $J_{1,2}$ = 1.1 Hz, $J_{2,3}$ = 2.4 Hz, H-2), 4.99 (d, 1H, H-1), 4.88 (d, 1H, $J_{4,5}$ = 1.5 Hz, H-5), 4.82, 4.66 (ABq, 2H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.06 (dd, 1H, $J_{4,5}$ = $J_{3,4}$ = 1.5 Hz, H-4), 3.82 (dd, 1H, H-3), 3.81 (s, 3H, CO_2CH_3), 3.49 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9, 164.9 (2 × C=O), 137.4, 133.7, 129.7, 128.9, 128.6, 128.4, 127.9, 127.7 (Ph), 99.9 (C-1), 74.1 (C-3), 71.9 (CH_2Ph), 67.9 (C-4), 67.4 (C-2, C-5), 56.4 (OCH_3), 52.4 (CO_2CH_3); LRMS: *m*/*z* = 439.1 $[M + Na]^+$; HRMS: *m*/*z* calcd for $C_{22}H_{24}O_8Na$ $[M + Na]^+$: 439.1363; found: 439.1371.

Methyl(2-azido-3,4,6,-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosid) uronate (40)

The donor **14** (188 mg, 0.32 mmol) and acceptor **38** (112 mg, 0.27 mmol) were glycosylated as described for compounds **25** and **26**. Following work-up the residue was purified by flash chromatography (EtOAc/*n*-hexane, 1 : 9) to give the *disaccharide* **40** as yellow oil (170 mg, 72%); R_f = 0.08 (EtOAc/*n*-hexane, 1 : 4); $[\alpha]_D$ −47.5 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.09–8.05 (m, 2H, Ph), 7.44–7.06 (m, 23H, Ph), 5.09 (app t, 1H, $J_{2,3}$ = 2.5 Hz, H-2), 5.04 (app. s, 1H, H-1), 4.89, 4.73 (ABq, 2H, $J_{A,B}$ = 11.7 Hz, CH_2Ph), 4.80 (d, 1H, $J_{4,5}$ = 2.5 Hz, H-5), 4.67 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1′), 4.63, 4.46 (ABq, 2H, $J_{A,B}$ = 10.9 Hz, CH_2Ph), 4.56, 4.43 (ABq, 2H, $J_{A,B}$ = 12.2 Hz, CH_2Ph), 4.12, 3.90 (ABq, 2H, $J_{A,B}$ = 10.5 Hz, CH_2Ph), 4.08 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 2.5 Hz, H-3), 3.99 (dd, 1H, H-4), 3.88 (ddd, 1H, $J_{4',5'}$ = $J_{5',6b'}$ = 9.6 Hz, $J_{5',6a'}$ = 2.4 Hz, H-5′), 3.78 (dd, 1H, A part of ABX, $J_{6a',6b'}$ = 10.9 Hz, H-6a′), 3.71 (s, 3H, CO_2CH_3), 3.64 (dd, 1H, B part of ABX, H-6b′), 3.63 (dd, 1H, $J_{4',5'}$ = $J_{3',4'}$ = 9.6 Hz, H-4′), 3.49 (dd, 1H, $J_{3',4'}$ = 9.6 Hz, $J_{2',3'}$ = 10.2 Hz, H-3′), 3.46 (s, 3H, OCH_3), 3.18 (dd, 1H, H-2′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.6, 165.5 (2 × C=O), 138.3, 137.8, 137.4, 133.1, 129.9, 129.6, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.7, 127.6 (Ph), 100.3 (C-1), 99.9 (C-1′), 80.0 (C-3′), 77.6 (C-4′), 76.0 (C-4), 74.7 (CH_2Ph), 74.6 (CH_2Ph), 73.5 (CH_2Ph), 72.9 (C-3), 72.4 (CH_2Ph), 71.6 (C-5′), 67.8 (C-6′, C-2), 67.0 (C-5), 63.7 (C-2′), 56.2 (OCH_3), 52.2

(CO_2CH_3); LRMS: m/z = 896.3 $[M + Na]^+$, 912.3 $[M + K]^+$; HRMS: m/z calcd for $C_{49}H_{51}O_{12}N_3Na$ $[M + Na]^+$: 896.3365; found: 896.3365.

Methyl(2-azido-3,4,6,-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 3-*O*-benzyl-α-L-idopyranosid)uronate (41)

The benzoate **40** (15 mg, 0.02 mmol) was dissolved in anhydrous MeOH (3 mL), and a solution of 1 M NaOMe in MeOH (2 mL) was added. The resulting solution was stirred at r.t. for 5 h. It was then neutralized (Amberlite IR-120, H^+ form, prewashed with MeOH (3 × 20 mL) before use) in small portions to pH 7. The mixture was then filtered and the filtrate concentrated under reduced pressure. The residue was then purified by flash chromatography (EtOAc/*n*-hexane, 1 : 9) to give the *alcohol* **41** as colourless oil (10.8 mg, 82%); R_f = 0.14 (EtOAc/*n*-hexane, 3 : 7); $[\alpha]_D$ −16.0 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34–7.10 (m, 20 H, Ph), 4.95 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1′), 4.93 (app. s, 1H, H-1), 4.85 (d, 1H, $J_{4,5}$ = 1.7 Hz, H-5), 4.78 (s, 2H, CH_2Ph), 4.75, 4.48 (ABq, 2H, $J_{A,B}$ = 11.4 Hz, CH_2Ph), 4.71, 4.56 (ABq, 2H, $J_{A,B}$ = 11.9 Hz, CH_2Ph), 4.58, 4.42 (ABq, 2H, $J_{A,B}$ = 12.0 Hz, CH_2Ph), 4.15 (dd, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 3.84 (dd, 1H, $J_{2,3}$ = 2.4 Hz, H-3), 3.79 (dd, 1H, $J_{2',3'}$ = 9.1 Hz, $J_{3',4'}$ = 8.0 Hz, H-3′), 3.74 (ddd, 1H, $J_{2,OH}$ = 11.8 Hz, $J_{2,3}$ = 2.4 Hz, $J_{1,2}$ = 1.1 Hz, H-2), 4.72 (s, 3H, CO_2CH_3), 3.71–3.68 (m, 2H, H-4′, H-6a′), 3.61–3.54 (m, 3H, H-6b′, H-2′, H-5′), 3.53 (d, 1H, $J_{2,OH}$ = 11.8 Hz, OH-2), 3.45 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1 (C═O), 138.1, 137.7, 137.5, 137.3, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.2 (Ph), 102.9 (C-1), 94.9 (C-1′), 81.0 (C-3′), 77.5 (C-4′), 75.8, 74.6, 73.5, 71.9 (4 × *C*H_2Ph), 71.5 (C-5′), 71.4 (C-3, C-4), 67.7 (C-6′), 66.7 (C-5), 66.3 (C-2), 63.6 (C-2′), 56.2 (OCH_3), 52.4 (CO_2CH_3); LRMS: m/z = 792.3 $[M + Na]^+$, 808.3 $[M + K]^+$; HRMS: m/z calcd for $C_{42}H_{47}O_{11}N_3Na$ $[M + Na]^+$: 792.3103; found: 792.3105.

Methyl(2-amino-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl α-L-idopyranosid)uronate (5)

The disaccharide **41** (19 mg, 0.025 mmol) was dissolved in anhydrous MeOH (5 mL) and 20% $Pd(OH)_2$/C (120 mg) was added. The mixture was then stirred under an atmosphere of H_2 at r.t. for 24 h. The catalyst was removed by filtration over Celite, the filtrate was concentrated to give the *disaccharide* **5** as a colourless oil (9.5 mg, 100%); R_f = 0.06 (NH_4OH/i-PrOH = 1 : 4); $[\alpha]_D$ +11.6 (*c* 0.3, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.30 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1′), 4.82 (d, 1H, $J_{4,5}$ = 2.7 Hz, H-5), 4.74 (d, 1H, $J_{1,2}$ = 2.4 Hz, H-1), 4.06 (dd, 1H, $J_{4,5}$ = 2.7 Hz, $J_{3,4}$ = 4.0 Hz, H-4), 4.03 (dd, 1H, $J_{3,4}$ = $J_{2,3}$ = 4.0 Hz, H-3), 3.79 (s, 3H, CO_2CH_3), 3.77 (dd, 1H, A part of ABX, $J_{5',6a'}$ = 2.4 Hz, $J_{6a',6b'}$ = 11.9 Hz, H-6a′), 3.71 (dd, 1H, B part of ABX, $J_{5',6b'}$ = 4.5 Hz, H-6b′), 3.61 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 8.9 Hz, H-3′), 3.56 (dd, 1H, H-2), 3.44 (ddd, 1H, $J_{4',5'}$ = 8.9 Hz, H-5′), 3.40 (s, 3H, OCH_3), 3.36 (dd, 1H, H-4′), 3.02 (dd, 1H, H-2′); ^{13}C NMR (125 MHz, CD_3OD): δ 171.6 (C═O), 104.1 (C-1), 95.3 (C-1′), 74.7 (C-5′), 74.4 (C-4), 71.7 (C-3′), 71.2 (C-4′), 70.5 (C-2), 68.6 (C-5), 67.8 (C-3), 61.9 (C-6′), 56.3 (OCH_3), 56.0 (C-2′), 52.9 (CO_2CH_3); LRMS: m/z = 406.1 $[M + Na]^+$; HRMS: m/z calcd for $C_{14}H_{25}O_{11}NNa$ $[M + Na]^+$: 406.1320; found: 406.1326.

Methyl(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl α-L-idopyranosid)uronate (6)

The amine **5** (5.5 mg, 0.014 mmol) was acetylated as described for compound **2**. The crude product was purified by flash chromatography (NH_4OH/i-PrOH, 1 : 4) to give the *amide* **6** as a colourless oil (2.4 mg, 39%); R_f = 0.28 (NH_4OH/i-PrOH, 1 : 4); $[\alpha]_D$ +8.5 (*c* 0.05, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.04 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1′), 4.79 (d, 1H, $J_{4,5}$ = 2.9 Hz, H-5), 4.74 (d, 1H, $J_{1,2}$ = 2.7 Hz, H-1), 3.99 (dd, 1H, $J_{3,4}$ = 3.3 Hz, H-4), 3.92–3.89 (m, 2H, H-3, H-2′), 3.79 (s, 3H, CO_2CH_3), 3.76 (dd, 1H, A part of ABX, $J_{5',6a'}$ = 2.3 Hz, $J_{6a',6b'}$ = 11.6 Hz, H-6a′), 3.71 (dd, 1H, B part of ABX, $J_{5',6b'}$ = 4.1 Hz, H-6b′), 3.55–3.51 (m, 2H, H-3′, H-2), 3.42–3.39 (m, 2H, H-5′, H-4′), 3.40 (s, 3H, OCH_3), 1.95 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, CD_3OD): δ 174.0 (C═O), 171.7 (C═O), 104.1 (C-1), 97.2 (C-1′), 74.9 (C-4), 74.3 (C-5′), 73.1 (C-3′), 71.6 (C-4′), 70.7 (C-2), 68.7 (C-5), 68.5 (C-3), 62.4 (C-6′), 56.3 (OCH_3), 55.1 (C-2′), 52.9 (CO_2CH_3), 22.7 (NAc); LRMS: m/z = 448.1 $[M + Na]^+$, 464.1 $[M + K]^+$; HRMS: m/z calcd for $C_{16}H_{27}O_{12}NNa$ $[M + Na]^+$: 448.1425; found: 448.1420.

Methyl(methyl 3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (43)

The disaccharide **42** (500 mg, 0.62 mmol) was deprotected as described for compound **41**. The crude product was purified by flash chromatography (EtOAc/*n*-hexane, 3 : 1) to give the *triol* **43** as white solid (325 mg, 88%); R_f = 0.64 (EtOAc/MeOH, 9 : 1); m.p. 77–80 °C; $[\alpha]_D$ +24.1 (*c* 3.0, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD): δ 7.45–7.19 (m, 10H, Ph), 5.12 (d, 1H, $J_{1',2'}$ = 2.5 Hz, H-1′), 4.81 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.74, 4.66 (ABq, 2H, $J_{A,B}$ = 11.1 Hz, CH_2Ph), 4.71 (d, 1H, $J_{4',5'}$ = 2.7 Hz, H-5′), 4.68, 4.51 (ABq, 2H, $J_{A,B}$ = 11.2 Hz, CH_2Ph), 3.92–3.88 (m, 2H, H-4, H-4′), 3.84–3.81 (m, 2H, H-2′, H-6a), 3.78 (dd, 1H, B part of ABX, $J_{5,6b}$ = 3.8 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6b), 3.73 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.3 Hz, H-3), 3.72 (dd, 1H, $J_{2',3'}$ = $J_{3',4'}$ = 3.5 Hz, H-3′), 3.68–3.65 (m, 1H, H-5), 3.42 (s, 3H, CO_2CH_3), 3.40 (dd, 1H, H-2), 3.38 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, CD_3OD): δ 171.9 (C═O), 139.6, 139.5, 129.4, 129.3, 129.0, 128.9, 128.3, 128.2 (Ph), 102.1 (C-1′), 100.0 (C-1), 79.7 (C-3), 78.0 (C-3′), 75.8 (C-4′), 75.1 (CH_2Ph), 73.3 (CH_2Ph), 73.3 (C-5), 70.8 (C-5′), 70.1 (C-4), 68.5 (C-2′), 64.9 (C-2), 61.6 (C-6), 55.5 (CO_2CH_3), 52.2 (OCH_3); LRMS: m/z = 607.2 $[M + NH_4]^+$, 612.2 $[M + Na]^+$, 628.2 $[M + K]^+$; HRMS: m/z calcd for $C_{29}H_{35}N_3O_{11}$ $[M + Na]^+$: 612.2164; found: 612.2166.

Methyl(methyl α-L-idopyranosyluronate)-(1→4)-2-amino-2-deoxy-α-D-glucopyranoside (9)

The disaccharide **43** (50 mg, 0.085 mmol) was hydrogenated as described for compound **3** to give the *amine* **9** as yellow oil (25.7 mg, 79%); R_f = 0.36 (i-PrOH/NH_4OH, 3 : 1); $[\alpha]_D$ +14.6 (*c* 2.2, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.04 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1′), 4.98 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.91 (d, 1H, $J_{4',5'}$ = 3.5 Hz, H-5′), 3.90 (dd, 1H, $J_{3',4'}$ = 4.9 Hz, H-4′), 3.85–3.78 (m, 4H, H-3, H-6a, H-6b, H-3′), 3.75 (s, 3H, CO_2CH_3), 3.66–3.65

(m, 2H, H-4, H-5), 3.52 (dd, 1H, $J_{2',3'}$ 5.7 Hz, H-2′), 3.45 (s, 3H, OCH_3), 3.22 (dd, 1H, $J_{2,3}$ = 10.4 Hz, H-2); ^{13}C NMR (100 MHz, CD_3OD): δ 172.1 (C═O), 103.4 (C-1′), 97.6 (C-1), 79.3 (C-4), 72.7 (C-5), 72.4 (C-3′), 71.9 (C-5′), 71.8 (C-4′), 71.7 (C-2′), 70.3 (C-3), 61.3 (C-6), 55.8 (C-2), 55.7 (OCH_3), 52.6 (CO_2CH_3); LRMS: m/z = 384.1 $[M + H]^+$; HRMS: m/z calcd for $C_{14}H_{25}NO_{11}$ $[M + H]^+$: 384.1500; found: 384.1511.

Methyl(methyl α-L-idopyranosyluronate)-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranoside (10)

The disaccharide **43** (50 mg, 0.085 mmol) was dissolved in anhydrous MeOH (5 mL) and 10% Pd/C (50 mg) and Ac_2O (0.1 mL, 1.7 mmol) were added. The mixture was then stirred at r.t. under an atmosphere of H_2 for 24 h. The catalyst was removed by filtration over Celite, and the filtrate was concentrated. The residue was then purified by flash chromatography (EtOAc/MeOH/H_2O, 7 : 2 : 1) to give the *amide* **10** as a colourless oil (20.8 mg, 58%); R_f = 0.4 (EtOAc/MeOH/H_2O, 6 : 3 : 1); $[\alpha]_D$ +26.48 (c 1.67, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.03 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1′), 4.94 (d, 1H, $J_{4',5'}$ = 3.4 Hz, H-5′), 4.64 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 3.93 (dd, 1H, $J_{2,3}$ = 10.6 Hz, H-2), 3.87 (dd, 1H, $J_{4',5'}$ = 3.4 Hz, $J_{3',4'}$ = 4.9 Hz, H-4′), 3.81–3.76 (m, 3H, H-6a, H-6b, H-3′), 3.74 (s, 3H, CO_2CH_3), 3.71 (dd, 1H, $J_{3,4}$ = 8.6 Hz, $J_{2,3}$ = 10.6 Hz, H-3), 3.64–3.59 (m, 2H, H-4, H-5), 3.50 (dd, 1H, $J_{2',3'}$ = 5.3 Hz, H-2′), 3.34 (s, 3H, OCH_3), 1.96 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, CD_3OD): δ 173.6, 172.1 (2 × C═O), 103.3 (C-1′), 99.6 (C-1), 80.3 (C-4), 72.3 (C-5), 72.2 (C-3′), 71.9 (C-5′), 71.8 (C-4′), 71.7 (C-2′), 71.4 (C-3), 61.8 (C-6), 55.5 (C-2), 55.4 (OCH_3), 52.4 (CO_2CH_3), 22.5 ($COCH_3$); LRMS: m/z = 426.1 $[M + H]^+$, 448.1 $[M + Na]^+$, 464.1 $[M + K]^+$; HRMS: m/z calcd for $C_{16}H_{27}NO_{12}$ $[M + Na]^+$: 448.1425; found: 448.1417.

Methyl(methyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranosid) uronate (45)

The triol **44** (1 g, 4.2 mmol) was dissolved in anhydrous pyridine (10 mL) and BzCl (1.76 mL, 15.1 mmol) was added dropwise to the solution at 0 °C under N_2 over 10 min. The ice bath was removed and the reaction mixture was stirred at r.t. for 2 h under N_2. The mixture was then diluted with $CHCl_3$ (10 mL), washed with H_2O (10 mL), 0.1 M HCl (10 mL), sat. aq. $NaHCO_3$ (10 mL) and dried ($MgSO_4$), filtered and concentrated. The residue was then recrystallized from EtOAc/n-hexane to give the *tribenzoate* **45** as colourless crystals (2.13 g, 93%); R_f = 0.68 (EtOAc/n-hexane, 1 : 1); m.p. 164 °C; $[\alpha]_D$ +4.0 (c 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.96–7.91 (m, 4H, Ph), 7.84–7.81 (m, 2H, Ph), 7.54–7.25 (m, 9H, Ph), 5.94 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, H-3), 5.67 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 5.61 (dd, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.7 Hz, H-2), 4.74 (d, 1H, H-1), 4.36 (d, 1H, H-5), 3.70 (s, 3H, CO_2CH_3), 2.29 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.0, 165.6, 165.1, 165.0 (4 × C═O), 133.4, 133.3, 133.2, 129.9, 129.8, 129.7, 128.9, 128.7, 128.6, 128.4, 128.3, 128.2 (Ph), 83.5 (C-1), 76.6 (C-5), 73.3 (C-3), 70.1 (C-4), 69.3 (C-2), 52.8 (OCH_3), 11.6 (SCH_3); LRMS: m/z = 568.1 $[M + NH_4]^+$, 573.1 $[M + Na]^+$, 589.1 $[M + K]^+$; HRMS: m/z calcd for $C_{29}H_{32}O_9S$ $[M + Na]^+$: 573.1190; found: 573.1208.

Methyl(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→4)-2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (47)

The glycosyl acceptor **46** (250 mg, 0.6 mmol) and thioglycoside donor **45** (333 mg, 0.6 mmol) were dissolved in anhydrous DCM (10 mL), and then activated 3 Å mol sieves (200 mg) were added. The mixture was stirred at r.t. for 30 min under argon. The mixture was then cooled to −78 °C, and NIS (135 mg, 0.6 mmol) and TMSOTf (108 μL, 0.6 mmol) were added. The resulting mixture was stirred at −78 °C under argon for 17 h. Sat. aq. $Na_2S_2O_3$ was added to quench the reaction, and the purple colour faded. The mixture was then filtered through Celite and the filter cake was washed with DCM. The combined filtrate and washings were washed with H_2O, $NaHCO_3$, brine, dried ($MgSO_4$), filtered and concentrated. The residue was then purified by flash chromatography (EtOAc/n-hexane, 3 : 7) to give the *disaccharide* **47** as a white solid (172 mg, 31%); R_f = 0.4 (EtOAc/n-hexane, 1 : 3); m.p. 211 °C; $[\alpha]_D$ +46.83 (c 1.16, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95–7.80 (m, 8H, Ph), 7.59–7.26 (m, 17H, Ph), 5.83 (dd, 1H, $J_{3',4'}$ = $J_{2',3'}$ = 9.6 Hz, H-3′), 5.64 (dd, 1H, $J_{4',5'}$ = $J_{3',4'}$ = 9.6 Hz, H-4′), 5.61 (dd, 1H, $J_{1',2'}$ = 7.9 Hz, H-2′), 5.24, 4.82 (ABq, 2H, $J_{A,B}$ = 11.2 Hz, CH_2Ph), 5.08 (d, 1H, H-1′), 4.72 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.53 (dd, 1H, A part of ABX, $J_{5,6a}$ = 1.9 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.37 (dd, 1H, B part of ABX, $J_{5,6b}$ = 4.1 Hz, H-6b), 4.13 (d, 1H, H-5′), 4.00–3.97 (m, 2H, H-3, H-4), 3.82–3.80 (m, 1H, H-5), 3.49 (s, 3H, CO_2CH_3), 3.37 (dd, 1H, $J_{2,3}$ = 10.2 Hz, H-2), 3.35 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.6, 165.7, 165.4, 165.0, 164.8 (4 × C═O), 138.2, 133.4, 133.3, 133.2, 129.7, 129.6, 129.5, 128.6, 128.5, 128.4, 128.3, 127.8, 127.5 (Ph), 101.1 (C-1′), 98.5 (C-1), 78.6 (C-4), 78.2 (C-3), 75.4 (CH_2Ph), 73.1 (C-5′), 72.1 (C-3′), 71.9 (C-2′), 70.1 (C-4′), 68.5 (C-5), 63.1 (C-2), 61.9 (C-6), 55.4 (OCH_3), 52.7 (CO_2CH_3); LRMS: m/z = 933.2 $[M + NH_4]^+$, 938.2 $[M + Na]^+$, 954.2 $[M + K]^+$; HRMS: m/z calcd for $C_{49}H_{45}N_3O_{15}$ $[M + Na]^+$: 938.2743; found: 938.2709.

Methyl(methyl β-D-glucopyranosyluronate)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (48)

The disaccharide **47** (126 mg, 0.14 mmol) was deprotected as described for compound **41**. The crude product was purified by flash chromatography (EtOAc/MeOH, 4 : 1) to give the *tetrol* **48** as a colourless oil (60.6 mg, 88%); R_f = 0.44 (EtOAc/MeOH, 4 : 1); $[\alpha]_D$ +1.36 (c 0.44, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD): δ 7.45–7.21 (m, 5H, Ph), 5.13, 4.68 (ABq, 2H, $J_{A,B}$ = 10.9 Hz, CH_2Ph), 4.77 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.59 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1′), 3.98–3.93 (m, 2H, H-4, H-6a), 3.90 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 8.9 Hz, H-3), 3.82 (dd, 1H, B part of ABX, $J_{5,6b}$ = 2.0 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6b), 3.68 (ddd, 1H, $J_{5,6a}$ = 3.5 Hz, $J_{4,5}$ = 9.8 Hz, H-5), 3.58 (d, 1H, $J_{4',5'}$ = 9.6 Hz, H-5′), 3.50 (dd, 1H, $J_{3',4'}$ = 9.0 Hz, H-4′), 3.39 (s, 3H, CO_2CH_3), 3.37 (dd, 1H, $J_{2',3'}$ = $J_{3',4'}$ = 9.0 Hz, H-3′), 3.33 (s, 3H, OCH_3), 3.31 (dd, 1H, H-2), 3.26 (dd, 1H, H-2′); ^{13}C NMR (125 MHz, CD_3OD): δ 172.1 (C═O), 139.8, 129.5, 129.2, 128.5 (Ph), 104.1 (C-1′), 100.1 (C-1), 79.6 (C-3), 77.7 (C-4), 77.6 (C-3′), 75.7 (C-5′), 75.6 (C-2′, OCH_2), 73.5 (C-4′), 72.9 (C-5), 64.5 (C-2), 61.7 (C-6), 55.4 (CO_2CH_3), 49.6

(OCH_3); LRMS: m/z = 522.2 $[M + Na]^+$; HRMS: m/z calcd for $C_{21}H_{29}N_3O_{11}$ $[M + Na]^+$: 522.1694; found: 522.1695.

Methyl(methyl β-D-glucopyranosyluronate)-(1→4)-2-amino-2-deoxy-α-D-glucopyranoside (11)

The disaccharide **48** (20.3 mg, 0.04 mmol) was hydrogenated as described for compound **3**. The crude product was purified by flash chromatography (EtOAc/MeOH, 1 : 1) to give the *amine* **11** as a colourless oil (14.5 mg, 90%); R_f = 0.28 (EtOAc/MeOH, 1 : 1); $[\alpha]_D$ +17.29 (*c* 1.67, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.88 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.48 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1′), 3.94 (d, 1H, $J_{4',5'}$ = 9.6 Hz, H-5′), 3.90 (dd, 1H, A part of ABX, $J_{5,6a}$ = 3.7 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a), 3.85 (dd, 1H, $J_{3,4}$ = 8.5, $J_{2,3}$ = 10.4 Hz, H-3), 3.83 (dd, 1H, B part of ABX, $J_{5,6b}$ = 1.7 Hz, H-6b), 3.76 (s, 3H, CO_2CH_3), 3.68–3.65 (m, 1H, H-5), 3.61 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 3.49 (dd, 1H, $J_{3',4'}$ = 9.1 Hz, H-4′), 3.44 (s, 3H, OCH_3), 3.41 (dd, 1H, $J_{2',3'}$ = $J_{3',4'}$ = 9.1 Hz, H-3′), 3.25 (dd, 1H, H-2′), 3.15 (dd, 1H, 1H, H-2); ^{13}C NMR (125 MHz, CD_3OD): δ 171.2 (C═O), 104.6 (C-1′), 97.8 (C-1), 80.4 (C-4), 77.1 (C-3′), 76.3 (C-5′), 74.6 (C-2′), 73.0 (C-4′), 72.3 (C-5), 70.3 (C-3), 61.0 (C-6), 55.8 (OCH_3), 55.6 (C-2), 52.9 (CO_2CH_3); LRMS: m/z = 384.0 $[M + H]^+$; HRMS: m/z calcd for $C_{14}H_{25}NO_{11}$ $[M + H]^+$: 384.1500; found: 384.1500.

Methyl(methyl β-D-glucopyranosyluronate)-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranoside (12)

The disaccharide **48** (20 mg, 0.04 mmol) was dissolved in anhydrous MeOH (3 mL) and 20% $Pd(OH)_2$/C (20 mg) and Ac_2O (75 μL, 0.4 mmol) were added. The resulting solution was stirred at r.t. under an atmosphere of H_2 for 24 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated. The residue was then purified by flash chromatography (EtOAc/MeOH, 4 : 1) to give the *amide* **12** as a syrup (8.1 mg, 48%); R_f = 0.52 (EtOAc/MeOH, 1 : 1); $[\alpha]_D$ +13.09 (*c* 0.53, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.64 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.48 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1′), 3.94 (d, 1H, $J_{4',5'}$ = 9.8 Hz, H-5′), 3.91 (dd, 1H, $J_{2,3}$ = 10.7 Hz, H-2), 3.86 (dd, 1H, A part of ABX, $J_{5,6a}$ = 3.9 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a), 3.81 (dd, 1H, B part of ABX, $J_{5,6b}$ = 2.4 Hz, H-6b), 3.78 (dd, 1H, $J_{3,4}$ = 8.5 Hz, H-3), 3.76 (s, 3H, CO_2CH_3), 3.66–3.63 (m, 1H, H-5), 3.57 (dd, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 3.49 (dd, 1H, $J_{3',4'}$ = 9.2 Hz, H-4′), 3.40 (dd, 1H, $J_{2',3'}$ = $J_{3',4'}$ = 9.2 Hz, H-3′), 3.35 (s, 3H, OCH_3), 3.25 (dd, 1H, H-2′), 1.96 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, CD_3OD): δ 173.5, 171.0 (2 × C═O), 104.7 (C-1′), 99.5 (C-1), 81.6 (C-4), 77.2 (C-3′), 76.4 (C-5′), 74.6 (C-2′), 73.0 (C-4′), 71.9 (C-5), 71.0 (C-3), 61.7 (C-6), 55.6 (OCH_3), 55.0 (C-2), 52.9 (CO_2CH_3), 22.5 ($COCH_3$); LRMS: m/z = 426.1 $[M + H]^+$, 448.1 $[M + Na]^+$, 464.0 $[M + K]^+$; HRMS: m/z calcd for $C_{16}H_{27}NO_{12}$ $[M + Na]^+$: 448.1425; found: 448.1420.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We thank the National MPS Society (USA) and the Australian Research Council (DP170104431 to VF) for funding. QQH gratefully acknowledges the University of Queensland for a UQ International PhD Scholarship and Arlo D. Harris Travel Scholarship.

References

1 S. Tomatsu, T. Fujii, M. Fukushi, T. Oguma, T. Shimada, M. Maeda, K. Kida, Y. Shibata, H. Futatsumori, A. M. Montano, R. W. Mason, S. Yamaguchi, Y. Suzuki and T. Orii, *Mol. Genet. Metab.*, 2013, **110**, 42–53.
2 A. C. Crawley, B. L. Gliddon, D. Auclair, S. L. Brodie, C. Hirte, B. M. King, M. Fuller, K. M. Hemsley and J. J. Hopwood, *Brain Res.*, 2006, **1104**, 1–17.
3 C. C. Griffin, R. J. Linhardt, C. L. Van Gorp, T. Toida, R. E. Hileman, R. L. Schubert 2nd and S. E. Brown, *Carbohydr. Res.*, 1995, **276**, 183–197.
4 J. E. Turnbull and J. T. Gallagher, *Biochem. J.*, 1991, **273**(Pt 3), 553–559.
5 R. Lawrence, S. K. Olson, R. E. Steele, L. Wang, R. Warrior, R. D. Cummings and J. D. Esko, *J. Biol. Chem.*, 2008, **283**, 33674–33684.
6 H. Tsuda, S. Yamada, Y. Yamane, K. Yoshida, J. J. Hopwood and K. Sugahara, *J. Biol. Chem.*, 1996, **271**, 10495–10502.
7 U. Lindahl and O. Axelsson, *J. Biol. Chem.*, 1971, **246**, 74–82.
8 C. Auray-Blais, P. Bherer, R. Gagnon, S. P. Young, H. H. Zhang, Y. An, J. T. Clarke and D. S. Millington, *Mol. Genet. Metab.*, 2011, **102**, 49–56.
9 C. Auray-Blais, P. Lavoie, H. Zhang, R. Gagnon, J. T. Clarke, B. Maranda, S. P. Young, Y. An and D. S. Millington, *Clin. Chim. Acta*, 2012, **413**, 771–778.
10 H. Zhang, S. P. Young, C. Auray-Blais, P. J. Orchard, J. Tolar and D. S. Millington, *Clin. Chem.*, 2011, **57**, 1005–1012.
11 C. K. Chuang, H. Y. Lin, T. J. Wang, C. C. Tsai, H. L. Liu and S. P. Lin, *Orphanet J. Rare Dis.*, 2014, **9**, 135.
12 P. J. Trim, A. A. Lau, J. J. Hopwood and M. F. Snel, *Rapid Commun. Mass Spectrom.*, 2014, **28**, 933–938.
13 S. Hassiotis, H. Beard, A. Luck, P. J. Trim, B. King, M. F. Snel, J. J. Hopwood and K. M. Hemsley, *Eur. J. Neurosci.*, 2014, **39**, 2139–2150.
14 B. King, N. Marshall, H. Beard, S. Hassiotis, P. J. Trim, M. F. Snel, T. Rozaklis, R. D. Jolly, J. J. Hopwood and K. M. Hemsley, *J. Inherited Metab. Dis.*, 2015, **38**, 341–350.
15 N. R. Marshall, S. Hassiotis, B. King, T. Rozaklis, P. J. Trim, S. K. Duplock, L. K. Winner, H. Beard, M. F. Snel, R. D. Jolly, J. J. Hopwood and K. M. Hemsley, *Exp. Neurol.*, 2015, **263**, 79–90.
16 L. Singh, A. Lam, R. Premraj and J. Seifert, *Tetrahedron Lett.*, 2008, **49**, 4854–4856.
17 M. L. West, N. B. Drinnan, T. E. Ramsdale, L. Singh and J. Seifert, Synthetic heparin pentasaccharides, *Australian Patent*, AU2002/331426B2Pat., 2008.

18 C. Puchner, T. Eixelsberger, B. Nidetzky and L. Brecker, *Carbohydr. Res.*, 2017, **437**, 50–58.
19 N. J. Davis and S. L. Flitsch, *Tetrahedron Lett.*, 1993, **34**, 1181–1184.
20 X.-S. Ye and C.-H. Wong, *J. Org. Chem.*, 2000, **65**, 2410–2431.
21 K.-K. T. Mong, Y.-F. Yen, W.-C. Hung, Y.-H. Lai and J.-H. Chen, *Eur. J. Org. Chem.*, 2012, 3009–3017.
22 R. Hevey, X. Chen and C.-C. Ling, *Carbohydr. Res.*, 2013, **376**, 37–48.
23 L. J. van den Bos, J. D. C. Codée, J. C. van der Toorn, T. J. Boltje, J. H. van Boom, H. S. Overkleeft and G. A. van der Marel, *Org. Lett.*, 2004, **6**, 2165–2168.
24 J. B. Epp and T. S. Widlanski, *J. Org. Chem.*, 1999, **64**, 293–295.
25 W. Pilgrim and P. V. Murphy, *Org. Lett.*, 2009, **11**, 939–942.
26 M. M. L. Zulueta, S.-Y. Lin, Y.-T. Lin, C.-J. Huang, C.-C. Wang, C.-C. Ku, Z. Shi, C.-L. Chyan, D. Irene, L.-H. Lim, T.-I. Tsai, Y.-P. Hu, S. D. Arco, C.-H. Wong and S.-C. Hung, *J. Am. Chem. Soc.*, 2012, **134**, 8988–8995.
27 T.-Y. Huang, M. M. L. Zulueta and S.-C. Hung, *Org. Lett.*, 2011, **13**, 1506–1509.
28 J. Li, Y. Dai, W. Li, S. Laval, P. Xu and B. Yu, *Asian J. Org. Chem.*, 2015, **4**, 756–762.
29 S. Mohamed, Q. Q. He, R. J. Lepage, E. H. Krenske and V. Ferro, *Eur. J. Org. Chem.*, 2018, 2214–2227.
30 D. R. Ferro, A. Provasoli, M. Ragazzi, G. Torri, B. Casu, G. Gatti, J.-C. Jacquinet, P. Sinay, M. Petitou and J. Choay, *J. Am. Chem. Soc.*, 1986, **108**, 6773–6778.
31 B. Casu, J. Choay, D. R. Ferro, G. Gatti, J.-C. Jacquinet, M. Ragazzi, P. Sinay and G. Torri, *Nature*, 1986, **322**, 215–216.
32 M. Hricovini and F. Bizik, *Carbohydr. Res.*, 2007, **342**, 779–783.
33 J. C. Munoz-Garcia, C. Solera, P. Carrero, J. L. de Paz, J. Angulo and P. M. Nieto, *Org. Biomol. Chem.*, 2013, **11**, 8269–8275.
34 T. Chiba, J.-C. Jacquinet and P. Sinay, *Carbohydr. Res.*, 1988, **174**, 253–264.
35 N. Barroca and J.-C. Jacquinet, *Carbohydr. Res.*, 2000, **329**, 667–679.
36 T. Nakano, Y. Ito and T. Ogawa, *Tetrahedron Lett.*, 1990, **31**, 1597–1600.
37 P. J. Trim, J. J. Hopwood and M. F. Snel, *Anal. Chem.*, 2015, **87**, 9243–9250.