2-Deoxy-2-trichloroacetamido-D-glucopyranose derivatives in oligosaccharide synthesis: from hyaluronic acid to chondroitin 4-sulfate trisaccharides

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Suitably protected derivatives of phenyl 2-deoxy-1-thio-2-trichloroacetamido-β-D-glucopyranoside, **6**, **15** and **16**, a new class of glycosyl donors, were tested in the reaction with sugar acceptors of low reactivity (*i.e.* the methyl uronate **2**). This methodology was applied to the stereocontrolled and high-yielding construction of the hyaluronic acid trisaccharide derivative **26**. Selective inversion of configuration at C-4 of the central D-glucosamine unit, transformation of the *N*-trichloroacetyl group into *N*-acetyl, *O*-sulfation, and final deprotection afforded the corresponding chondroitin 4-sulfate trisaccharide derivative **30** in high yield.

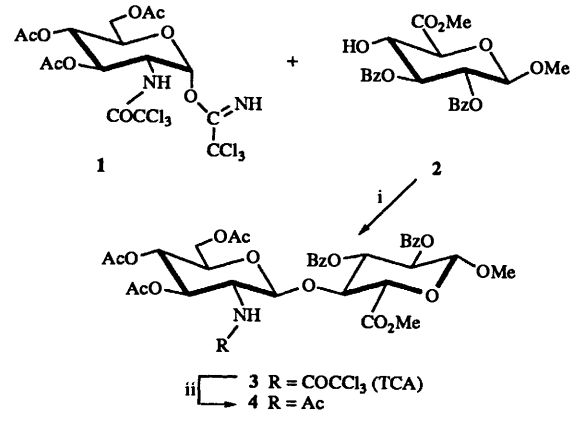
Introduction

Chondroitin sulfate proteoglycans are found in various body fluids, intracellularly in secretory granules, at the cell surface, or in the extracellular matrix.[1] Structural studies showed chondroitins to be essentially linear copolymers built from dimeric units (Fig. 1) composed of D-glucuronic acid (GlcA) and 4- or 6-*O*-sulfated 2-acetamido-2-deoxy-D-galactose (GalNAc). Although chondroitin 4-sulfate is the major variant, articular cartilage, particularly of older individuals,[2] has high contents of the 6-sulfated variant. However, copolymeric chondroitin 4-/6-sulfate may be a common form, even if over- and under-sulfated structures have also been described.

Their biological roles are highly diversified, ranging from simple mechanical support functions to more intricate, still poorly understood effects, such as cell recognition,[3] development of osteoarthritis,[4] AT III-mediated anticoagulant activity.[5] and inhibition of factor Clq.[6] Most of these effects depend on binding of proteins to the glycosaminoglycan chains. These associations vary from charge interactions of low affinity to highly specific, high-affinity bondings involving a particular oligosaccharide region of definite structure. In the case of heparin, another glycosaminoglycan, it has been demonstrated[7] that a specific pentasaccharide sequence is responsible for binding to antithrombin III. Determination of the precise structure of such sequences is highly complicated by the microheterogeneity of the polymers. Chemically or enzymically controlled degradations afford complex mixtures of products for which analysis is hampered by the lack of appropriate techniques. In addition, few methods have been developed that enable sequences of sulfation within the chains to be determined. However, it has been recently reported[8] that monoclonal antibodies recognize specific epitopes within the chondroitin chains, and this should be an attractive tool for the determination of the sulfation patterns.

However, as we demonstrated for the heparin–antithrombin binding sequence,[7] chemical synthesis of fragments of definite size and structure remains one of the most efficient ways to answer these questions.

One of us recently reported[9] the synthesis of the methyl glycosides of the repeating units of chondroitin 4- and 6-sulfate using substituted 2-azido-2-deoxy-α-D-galactopyranosyl trichloroacetimidates, prepared from D-galactal, and D-glucopyranose derivatives as sugar acceptors. Selective oxidation at C-6 of the D-glucose unit was achieved after coupling, a route still reported by others[10] for the synthesis of hyaluronic acid fragments. We also demonstrated[11] (Scheme 1) that 3,4,6-tri-



Scheme 1 *Reagents and conditions:* i, TMSOTf, 4 Å mol. sieves, 1,2-dichloroethane, 0 °C, 1 h; ii, TBTH, AIBN, benzene, reflux, 1 h

O-acetyl-2-deoxy-2-trichloroacetamido-α-D-glucopyranosyl trichloroacetimidate **1** was a very efficient glycosyl donor for the synthesis of 1,2-*trans*-2-amino-2-deoxy-D-glucosides, and that the N-trichloroacetyl group in the disaccharide product **3** could be easily transformed into N-acetyl **4** under neutral conditions by reduction with tributylstannane (TBTH). In addition, this method allowed the direct glycosylation of the low-reactive 4-OH group of D-glucuronic acid derivative **2**, thus avoiding the tedious oxidation of the synthetic oligosaccharides.

Besides the presence of sulfate esters, chondroitin differs from hyaluronic acid (HA), a related proteoglycan, by the nature of the amino sugar, *i.e.* D-galactosamine instead of D-glucosamine (Fig. 1). Since D-galactosamine is a rare, thus expensive sugar, it is generally prepared by azidonitration of D-galactal[12] followed by subsequent protection and/or activation. We used this strategy in our syntheses of chondroitin fragments.[9] Since our methodology employing 2-deoxy-2-trichloroacetamido-D-glucose derivatives allows the preparation, in an expeditious way, of hyaluronic acid oligosaccharides,[13] the question is whether it should be possible to invert selectively the stereochemistry at C-4 of the amino sugar moiety in such structures,

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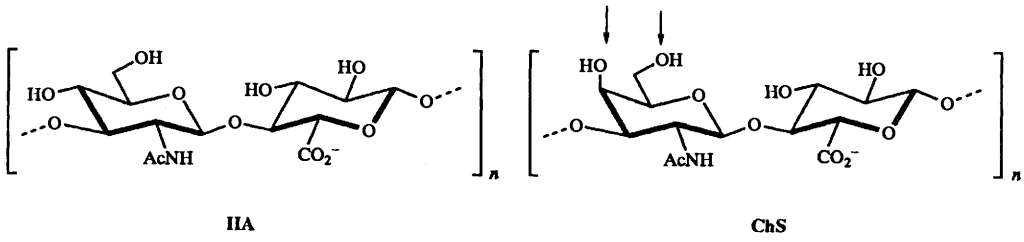


HA ChS

Fig. 1 The structure of hyaluronic acid (HA) and chondroitin sulfates (ChS). The arrows indicate possible substitutions with sulfate groups.

thus opening up the route to the chondroitin series. Inversion of configuration at C-4 of D-glucosamine monomers[14] as well as of neutral disaccharides containing D-glucosamine[15] have already been reported. However, these techniques were never applied to structures containing uronic acid moieties.

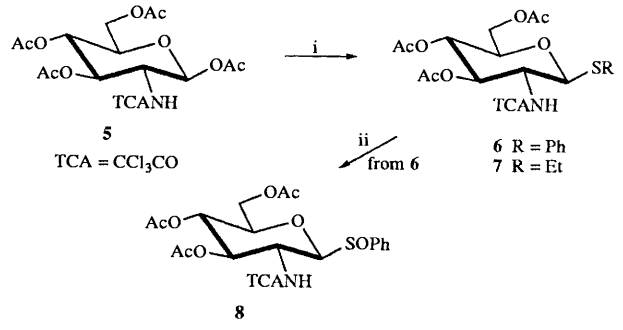
We now report on the use of this new strategy for the stereoselective and high-yielding construction of chondroitin 4-sulfate oligosaccharides from hyaluronic acid derivatives. New expeditious syntheses of D-glucuronic acid derivatives as donors and acceptors are also described.

Results and discussion

To test the validity of this strategy, we needed at least a trisaccharide structure in which the central D-glucosamine moiety was surrounded by two D-glucuronic acid residues. In lengthy synthetic routes such as those required for the construction of glycosaminoglycan fragments, it is important that the coupling reactions be as stereoselective and high-yielding as possible, and that the number of steps be kept to a minimum. For these reasons, we first looked at the preparation of D-glucosamine donors which could be potentially activated at C-1, and selectively protected on the other hydroxy groups, thus avoiding numerous transformations after coupling. Suitably protected thioglycosides fulfil these requirements, but have never been prepared as 2-deoxy-2-trichloroacetamido-D-glucopyranose derivatives.

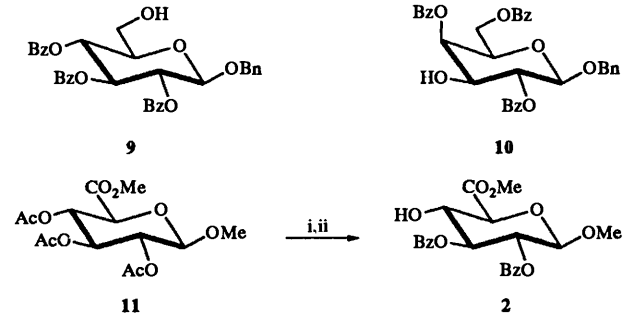
Synthesis of suitably protected monosaccharide derivatives

Treatment of known tetracetate **5**[11,16] with thiophenol and boron trifluoride–diethyl ether afforded the crystalline thioglycoside **6** in 87% yield. A similar reaction with ethanethiol gave crystalline sulfide **7** in 87% yield (Scheme 2). No corresponding α-isomers were isolated in these reactions. We were first attracted by a new and promising method of glycosylation using anomeric phenylsulfoxides[17] as glycosyl donors. Thus, treatment of sulfide **6** with *m*-chloroperbenzoic acid (MCPBA) in buffered medium afforded the sulfoxide **8** as a mixture of isomers which could be partially separated on silica gel. Attempted coupling of sulfoxide **8** with the reactive alcohol **9**[18] under the catalysis of triflic anhydride[17] or trimethylsilyl triflate failed, mainly because substrate **8** is sparingly soluble in the solvents usually employed, even at room temperature. Thus, a trivial problem of solubility prevented the exploration of the potentialities of such compounds.



Scheme 2 *Reagents and conditions:* i, RSH, BF_3·Et_2O, dichloromethane, 1 h; ii, MCPBA, $NaHCO_3$, dichloromethane, 0 °C, 1 h

These frustrating results prompted us to test the glycosylating ability of the much more soluble thioglycosides **6** and **7**. For this purpose, we first looked for an expeditious preparation of a suitably protected D-glucuronic acid derivative having only the 4-OH free, and that could be used as a terminal reducing acceptor in our oligosaccharide synthesis. Thus, known[19] compound **11**, easily prepared from commercial D-glucofuranurono-6,3-lactone, was *O*-deacetylated with methanolic sodium methoxide and the resulting triol was directly submitted to the tin procedure[20] (dibutyltin oxide in refluxing benzene). Treatment of the intermediary stannylene acetal with benzoyl chloride (2.1 mol equiv.) and triethylamine (1.5 mol equiv.) afforded the 2,3-di-*O*-benzoyl derivative **2** in 66% yield (Scheme 3), along with the corresponding 2,4- (3%) and 3,4- (6%) isomers



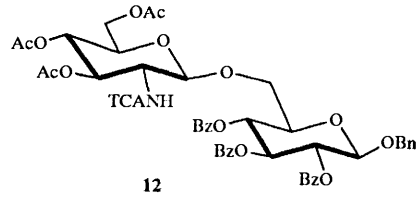
Scheme 3 *Reagents and conditions:* i, MeONa, MeOH, 1 h; ii, Bu_2-SnO, benzene, reflux, 15 h; then PhCOCl (2.1 mol equiv.), Et_3N (1.5 mol equiv.), THF, 1 h

which were *O*-debenzoylated and recycled. The rather high regioselectivity observed for this reaction could be explained by the following reasons. First, the equatorial 4-hydroxy group is obviously deactivated by the presence of the neighbouring 5-methoxycarbonyl group, and a 2,3-*O*-dibutylstannylene acetal is very likely the major intermediate in the reaction. In such a derivative, which exists probably as a dimer, there is one dicoordinated (O-2) and one tricoordinated (O-3) oxygen atom.[21] Acylation is thought to occur at the dicoordinated oxygen. Addition of a base (triethylamine) then causes ring closure of the resulting dibutylchlorostannyl ether to afford a 3,4-*O*-stannylene acetal which undergoes mainly acylation at O-3. The salient feature of this one-pot reaction is the possibility of obtaining a 2,3-di-*O*-acylated derivative of D-glucuronic acid such as compound **2** in an expeditious way. These results contrast with those obtained with an anomeric mixture of the corresponding methylthio glycosides.[22]

Disaccharide synthesis

The coupling of donors **6** and **7** with various partially protected nucleophiles **9**,[18] **10**[23] and **2**, unsubstituted respectively at O-6, O-3 and O-4, was then studied. Activation of the sulfur was achieved by using a modification of the method employing *N*-iodosuccinimide (NIS)–catalytic triflic acid.[24] We found that

Table 1 Coupling reactions between the donors and the corresponding nucleophiles. For a general procedure, see the Experimental section of this paper.



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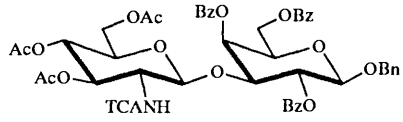

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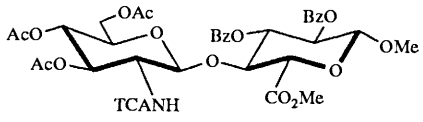

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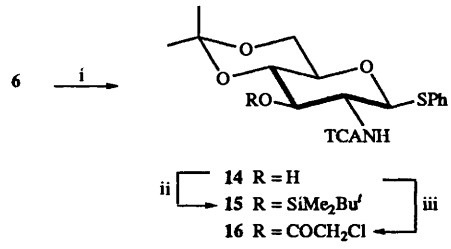
Donor	Nucleophile	Product (linkage)	Yield (%)
6	**9**	**12** (β 1 ⟶ 6)	93
6	**10**	**13** (β 1 ⟶ 3)	85
6	**2**	**3** (β 1 ⟶ 4)	82
7	**2**	**3** (β 1 ⟶ 4)	83

triflic acid could be advantageously replaced by trimethylsilyl triflate, a reagent much more convenient to handle.

The results are reported in Table 1. Good yields were obtained with a moderate excess (1.2 mol equiv.) of the donor, and no marked difference of behaviour was experienced between the donors **6** and **7**, though the reactivity of phenyl compound **6** was expected to be slightly lower than that of ethyl compound **7**. Pure 1,2-*trans*-glycosides were obtained, and no formation of 1,2-*cis*-isomers was observed. The anomeric configuration of the disaccharide products **3**, **12**, **13** was evident from the ^{1}H NMR spectra ($J_{1',2'}$ ~ 8 Hz).

These results prompted us to undertake the preparation of suitably protected D-glucosamine donors which could serve as a central unit in our trisaccharide synthesis. Transesterification of compound **6** with methanolic sodium methoxide afforded quantitatively the corresponding triol, which was directly treated with 2-methoxypropene in *N*,*N*-dimethylformamide (DMF) under acid catalysis to give the crystalline 4,6-*O*-isopropylidene derivative **14** in 90% yield. Temporary protection at O-3 required the use of a group which could be removed under neutral conditions after coupling with the methyl uronate **2**, thus avoiding undesired side-reactions such as β-elimination on the uronic acid ester residue, or hydrolysis of the labile acetal on the amino sugar moiety (Scheme 4). Treatment of **14** with *tert*-



Scheme 4 *Reagents and conditions:* i, MeONa, MeOH, 1 h; then 2-methoxypropene, CSA, DMF, 1 h; ii, TBDMSCl, imidazole, DMF, 4 h; iii, $(ClCH_2CO)_2O$, pyridine, 0 °C, 30 min

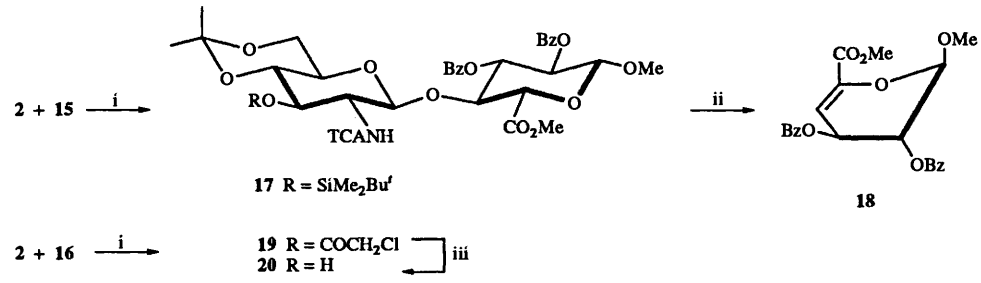
butyldimethylsilyl chloride (TBDMSCl) and imidazole in DMF afforded crystalline product **15** in 94% yield. Similarly, treatment of compound **14** with chloroacetic anhydride in pyridine gave crystalline compound **16** in 90% yield.

The glycosyl donors **15** and **16** were then coupled with the methyl uronate **2** as previously reported to give the crystalline disaccharide derivatives **17** and **19**, in 91 and 90% yield, respectively (Scheme 5). The anomeric configurations of the interglycosidic linkages were evident from the ^{1}H NMR spectra ($J_{1',2'}$ 8 and 8.5 Hz, respectively). Attempted *O*-desilylation of compound **17** with tetrabutylammonium fluoride (TBAF) (2 mol equiv.) in tetrahydrofuran (THF) at 0 °C led spontaneously to the formation of the unsaturated derivative **18** which resulted from a β-elimination reaction (details not presented in the Experimental section), possibly because fluoride ions in THF are sufficiently basic[25] to affect the sensitive β-ketol system in compound **17**. In contrast, selective *O*-dechloroacetylation was readily achieved by treatment of compound **19** with thiourea to give crystalline alcohol **20** in 92% yield.

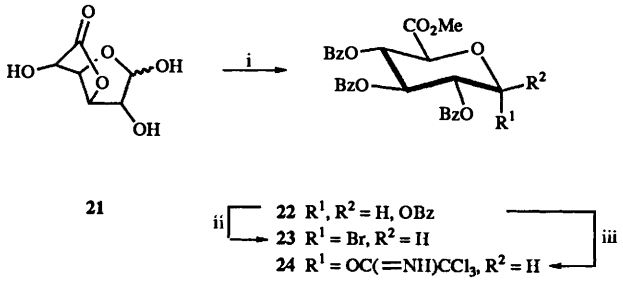
Construction of a hyaluronic acid trisaccharide derivative

We have previously reported[23] that *O*-benzoylated derivatives of D-glucuronic acid activated through their corresponding trichloroacetimidate[26] were powerful glycosyl donors for the preparation of β-D-glucuronides. Treatment of commercial D-glucofuranurono-6,3-lactone in methanol with a catalytic amount of sodium hydroxide[19] afforded the corresponding methyl glucopyranuronate that was directly *O*-benzoylated (benzoyl chloride in pyridine) to give **22** as a ~ 1 : 1 mixture of anomers (Scheme 6), as judged by integrated ^{1}H NMR spectroscopy. Treatment of compound **22** with hydrobromic acid in acetic acid (33%, w/v) afforded the crystalline bromide **23**, another potentially useful donor, in 88% yield. The anomeric configuration of compound **23** was evident from its ^{1}H NMR spectrum ($J_{1,2}$ 4 Hz). Introduction of the trichloroacetimidoyl group at C-1 was achieved by selective 1-*O*-debenzoylation of tetrabenzoate **22** using hydrazine acetate in DMF, followed by treatment with trichloroacetonitrile and 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) to give the α-imidate **24** in 60% overall yield, the structure of which was deduced from its ^{1}H NMR spectrum ($J_{1,2}$ 3.5 Hz).

Condensation of the imidate **24** (1.4 mol equiv.) with the alcohol **20** (1 mol equiv.) in dichloromethane at room temp., in the presence of trimethylsilyl triflate (10% based on **24**), afforded the crystalline trisaccharide derivative **25** in 92% yield (Scheme 7). The ^{1}H NMR spectrum of product **25**, recorded in deuteriochloroform, showed a doublet at δ 5.09 (*J* 8 Hz), attributed by



Scheme 5 *Reagents and conditions:* i, NIS (1 mol equiv.), TMSOTf (0.1 mol equiv.), 4 Å mol. sieves, dichloromethane, 0 °C, 15 min; ii, Bu_4NF, THF, 0 °C, 10 min; iii, thiourea, pyridine–EtOH, 80 °C, 1.5 h



Scheme 6 *Reagents and conditions:* i, MeOH, cat. NaOH, 1 h; then PhCOCl, pyridine, 15 h; ii, 33% HBr in AcOH, 8 h; iii, H_2NNH_2·HOAc, DMF, 1 h; then CCl_3CN, DBU, dichloromethane, 30 min

spin-decoupling experiments to 1″-H, and characteristic of a 1,2-*trans* linkage. It is relevant to note that attempted coupling of alcohol **20** with the bromide **23** in the presence of silver triflate (AgOTf) and 2,4,6-trimethyl pyridine (*sym*-collidine) as an acid scavenger (details not presented) gave a complex mixture of products in which trisaccharide **25** could be isolated in ~30% yield. Mild hydrolysis of the *O*-isopropylidene acetal in compound **25** with aq. acetic acid afforded the corresponding diol, which was directly treated with benzoyl cyanide[27] in pyridine to give the crystalline 6-*O*-benzoylated derivative **26** in 90% overall yield. The ^{1}H NMR spectrum of compound **26**, showed, *inter alia*, a doublet at δ 4.14 (J 1.5 Hz), attributed after exchange with deuterium oxide (D_2O) to 4′-OH, confirming that *O*-benzoylation occurred exclusively at O-6.

From hyaluronic acid to chondroitin 4-sulfate trisaccharides

Crucial inversion of configuration at C-4 of the central D-glucosamine unit was then studied. Treatment of compound **26** with triflic anhydride (Tf_2O) in pyridine at −15 °C afforded the corresponding 4′-*O*-triflyl derivative in 96% yield (Scheme 8). Comparison of the ^{1}H NMR spectra of the triflate product and substrate **26** in deuteriochloroform showed the expected downfield shift (1.28 ppm) of the signal for 4′-H in the triflate. Attempted displacement of the 4′-*O*-triflyl group by various nucleophiles in DMF (details not presented) led to complex mixtures of products, in which unsaturated compounds (resulting from a β-elimination on the uronic acid moieties) were identified by ^{1}H NMR spectroscopy. However, treatment of the crude triflate with tetrabutylammonium nitrite (TBAN) (10 mol equiv.), a reagent known[28] to give directly the *epi*-hydroxy analogue, afforded smoothly the crystalline *galacto* product **27** in 87% yield (from original substrate **26**). The ^{1}H NMR spectrum of product **27** in deuteriochloroform showed signals at δ 2.64 (d, J 3.5 Hz) and 4.11 (m, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1 Hz) attributed, respectively, by spin-decoupling experiments and exchange with D_2O, to 4′-OH and 4′-H. The J-values observed for 4′-H fit quite well with those expected for a D-*galacto* structure for the central amino sugar unit.

The *N*-trichloroacetyl group in compound **27** was easily transformed into *N*-acetyl under neutral conditions[11] using TBTH and azoisobutyronitrile (AIBN) to give the crystalline acetamide **28** in 92% yield. The free hydroxy group in compound **28** was then *O*-sulfated by treatment with the sulfur trioxide–trimethylamine complex in DMF, followed by ion-exchange chromatography (Na^+ resin) to give the sodium salt **29** in 93% yield. Comparison of the ^{1}H NMR spectra of compounds **29** and **28**, recorded, respectively, in deuteriated methanol and deuteriochloroform, showed the expected[9] downfield shift (0.94 ppm) of the signal for 4′-H in compound **29**. Final deprotection was achieved by treatment of compound **29** with aq. sodium hydroxide in methanol–water, followed by purification on Sephadex G-10 in water to afford the target molecule **30** in 87% yield. The ^{1}H and ^{13}C NMR spectra of product **30** are in full agreement with the expected structure, and in accord with those reported both for synthetic disaccharide fragments[9] and for oligosaccharide fragments isolated[29] from commercial chondroitin sulfates after digestion with chondroitinase ABC.

In conclusion, a stereocontrolled and high-yielding synthesis of the chondroitin 4-sulfate trisaccharide derivative **30** starting from 2-deoxy-2-trichloroacetamido-D-glucopyranose precursors is reported. Application of this new methodology for the synthesis of chondroitin 4- and 6-sulfate fragments of higher relative molecular mass is currently under investigation in our group.

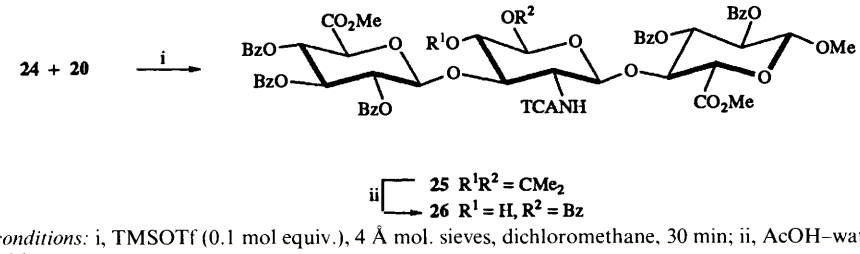
Experimental

General

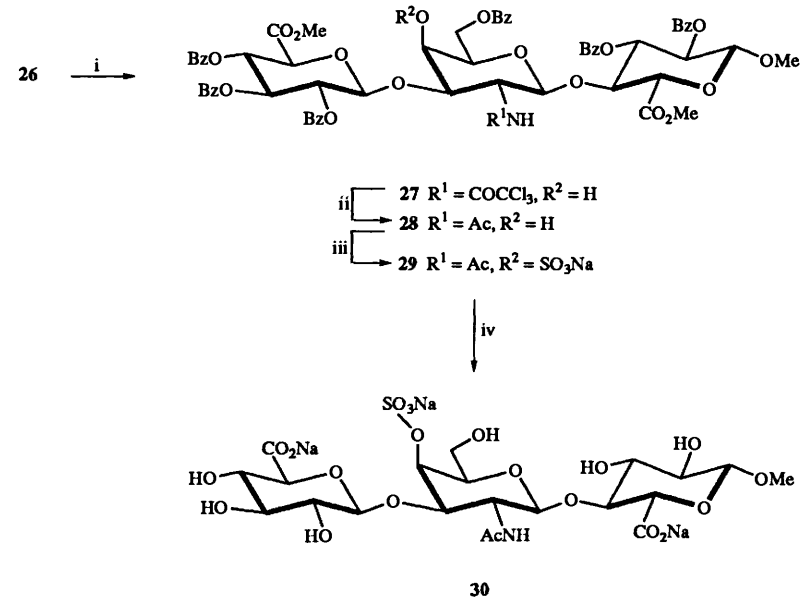
Mps were recorded with a Buchi apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 polarimeter and $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectra were recorded at 300 K on a Bruker AM-300 WB (300 MHz) spectrometer. Chemical shifts are reported in δ values relative to tetramethylsilane using the solvents stated, and J-values are given in Hz. ^{13}C NMR spectra were recorded on a Bruker AM-300 WB spectrometer operating at 75.4 MHz. ^{13}C spectra run in D_2O were referenced to internal acetone (δ_C 30.5). Mass spectra were recorded with a Ribermag R-10-10 instrument in the desorption, chemical ionization (NH_3) mode. TLC was conducted on Merck 60 F_{254} precoated plates. Flash silica chromatography was performed using Merck silica gel C60 (40–60 μ). Elemental analyses were carried out at the Service Central de Microanalyse du Centre National de la Recherche Scientifique (Vernaison, France).

Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-2-trichloroacetamido-β-D-glucopyranoside 6

Boron trifluoride–diethyl ether (1.85 cm^3, 15 mmol) was added dropwise to a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranose[11,16] **5** (4.92 g, 10 mmol) and thiophenol (1.75 cm^3, 17 mmol) in dry dichloromethane (30 cm^3), and the mixture was stirred for 1 h at room temp. The mixture was treated with an excess of saturated aq. sodium



Scheme 7 *Reagents and conditions:* i, TMSOTf (0.1 mol equiv.), 4 Å mol. sieves, dichloromethane, 30 min; ii, AcOH–water (3:1), 100 °C, 30 min; then PhCOCN, pyridine, 20 h



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Scheme 8 *Reagents and conditions:* i, $(CF_3SO_2)_2O$, pyridine, −15 °C, 1 h; then $Bu_4N^+NO_2^-$ (8 mol equiv.), DMF, 15 h; ii, TBTH, AIBN, benzene–*N*,*N*-dimethylacetamide (1:1), 80 °C, 1 h; iii, SO_3·NMe_3, DMF, 65 °C, 24 h; then ion-exchange resin (Na^+) in EtOAc–MeOH–water (5:2:1); iv, 3 mol dm^{-3} NaOH, MeOH–water (5:1), 6 h

hydrogen carbonate, and diluted with dichloromethane (100 cm^3): the organic phase was washed with water, dried (Na_2SO_4), and concentrated. The residue was crystallized from ethyl acetate–heptane to give *compound* **6** (4.72 g, 87%), mp 168–169 °C; $[\alpha]_D^{22}$ −14 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 1.98, 2.02 and 2.10 (9 H, 3 s, Ac), 3.76 (1 H, m, 5-H), 4.02 (1 H, m, *J* 9.5, 10.5 and 11, 2-H), 4.19 (1 H, dd, *J* 2.5 and 12, 6-H^b), 4.25 (1 H, dd, *J* 5 and 12, 6-H^a), 4.85 (1 H, d, *J* 10.5, 1-H), 5.07 (1 H, t, *J* 9.5, 4-H), 5.33 (1 H, dd, *J* 9.5 and 11, 3-H), 6.90 (1 H, d, *J* 9.5, NH) and 7.40 (5 H, m, Ph) (Found: C, 44.2; H, 3.9; N, 2.3. $C_{20}H_{22}Cl_3NO_8S$ requires C, 44.2; H, 4.1; N, 2.6%).

Ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-2-trichloroacetamido-β-D-glucopyranoside 7

Compound **5** (492 mg, 1 mmol) was treated with ethanethiol as described for the preparation of the phenylsulfanyl derivative **6** to give *compound* **7** (433 mg, 87%), mp 135–136 °C (from ethyl acetate–heptane); $[\alpha]_D^{22}$ −34 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 1.28 (3 H, t, CH_2Me), 2.04, 2.05 and 2.09 (9 H, 3 s, Ac), 2.75 (2 H, m, CH_2Me), 3.75 (1 H, m, 5-H), 4.08 (1 H, m, *J* 9.5, 10 and 10.5, 2-H), 4.16 (1 H, dd, *J* 2.5 and 12.5, 6-H^b), 4.27 (1 H, dd, *J* 5 and 12.5, 6-H^a), 4.67 (1 H, d, *J* 10.5, 1-H), 5.13 (1 H, t, *J* 10, 4-H), 5.31 (1 H, dd, *J* 10 and 10.5, 3-H) and 6.77 (1 H, d, *J* 9.5, NH) (Found: C, 38.7; H, 4.6; N, 2.9. $C_{16}H_{22}Cl_3NO_8S$ requires C, 38.8; H, 4.5; N, 2.8%).

Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl sulfoxide 8

A mixture of compound **6** (0.2 g, 0.37 mmol), 85% MCPBA (0.1 g, 0.59 mmol) and solid sodium hydrogen carbonate (0.16 g, 1.9 mmol) in dichloromethane (4 cm^3) was stirred at 0 °C for 1 h. The reaction mixture was diluted with dichloromethane (20 cm^3), washed successively with water, brine and water, dried ($MgSO_4$), and concentrated under reduced pressure to give the *sulfoxide* **8** (mixture of isomers) as a solid (187 mg, 91%) (Found: C, 42.8; H, 4.0; N, 2.3. $C_{20}H_{22}Cl_3NO_9S$ requires C, 43.0; H, 4.0; N, 2.5%).

Flash silica chromatography with dichloromethane–ethyl acetate (3:2) as eluent allowed partial separation of the isomers:

(i) Faster moving isomer (R_f 0.35); $\delta_H(CDCl_3)$ 1.92, 2.03 and 2.05 (9 H, 3 s, Ac), 3.69 (1 H, m, 5-H), 4.07 (3 H, m, 6-H_2 and 2-H), 4.94 (1 H, d, *J* 10.5, 1-H), 5.04 (1 H, t, *J* 9.5, 4-H), 5.73 (1 H, dd, *J* 9.5 and 10.5, 3-H), 7.60 (5 H, m, Ph) and 8.00 (1 H, d, *J* 7.5, NH); *m*/*z* 576 (M^+ + 18).

(ii) Slower moving isomer (R_f 0.30); $\delta_H[(CD_3)_2SO]$ 1.86, 1.94 and 1.95 (9 H, 3 s, Ac), 4.05 (4 H, m, 6-H_2, 5- and 2-H), 4.68 (1 H, t, *J* 9.5, 4-H), 5.07 (1 H, d, *J* 10.5, 1-H), 5.35 (1 H, dd, *J* 9.5 and 10.5, 3-H) and 7.60 (6 H, m, Ph and NH); *m*/*z* 576 (M^+ + 18).

Methyl (methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosid)uronate 2

Methanolic sodium methoxide (1 mol dm^3, 0.1 cm^3) was added to a solution of methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate[19] **11** (1 g, 2.87 mmol) in dry methanol (20 cm^3), and the mixture was stirred for 1 h at room temp., then was neutralized with Amberlite IR-120 (H^+) resin, filtered, concentrated, and dried over phosphorus pentaoxide under reduced pressure.

A mixture of the residue and dibutyltin oxide (747 mg, 3 mmol) was heated for 15 h in refluxing benzene (30 cm^3) with azeotropic removal of water. Solvent (15 cm^3) was then slowly distilled off at atmospheric pressure, and the mixture was

cooled, then diluted with dry THF (10 cm^3). To this solution were added successively benzoyl chloride (0.69 cm^3, 6 mmol) and triethylamine (0.6 cm^3, 4.3 mmol), and the mixture was stirred for 1 h at room temp., then was concentrated under reduced pressure. Flash silica chromatography [toluene–ethyl acetate (4:1)] afforded first the 2,4-*di*-O-*benzoylated isomer* (37 mg, 3%), mp 164–165 °C (from ethyl acetate–heptane); $[\alpha]_D^{22}$ −13 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 3.00 (1 H, d, *J* 3, 3-OH), 3.57 (3 H, s, OMe), 3.72 (3 H, s, CO_2Me), 4.13 (1 H, m, 3-H), 4.26 (1 H, d, *J* 9.5, 5-H), 4.70 (1 H, d, *J* 7, 1-H), 5.21 (1 H, dd, *J* 7 and 9.5, 2-H), 5.47 (1 H, t, *J* 9.5, 4-H) and 7.40–8.10 (10 H, m, Ph) (Found: C, 61.2; H, 5.1. $C_{22}H_{22}O_9$ requires C, 61.4; H, 5.1%).

Next eluted was the *title* 2,3-*di*-O-*benzoylated derivative* **2** as a foam (815 mg, 66%), $[\alpha]_D^{22}$ +70 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 3.31 (1 H, d, *J* 3, 4-OH), 3.55 (3 H, s, OMe), 3.87 (3 H, s, CO_2Me), 4.09 (1 H, d, *J* 10, 5-H), 4.21 (1 H, m, 4-H), 4.66 (1 H, d, *J* 7.5, 1-H), 5.43 (1 H, dd, *J* 7.5 and 9.5, 2-H), 5.54 (1 H, t, *J* 9.5, 3-H) and 7.40–8.00 (10 H, m, Ph) (Found: C, 61.3; H, 5.2%).

Last eluted was the 3,4-*di*-O-*benzoylated isomer* (75 mg, 6%), mp 129–130 °C (from ethyl acetate–heptane); $[\alpha]_D^{22}$ −95 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 2.75 (1 H, d, *J* 3, 2-OH), 3.64 and 3.67 (2 × 3 H, 2 s, OMe and CO_2Me), 3.84 (1 H, m, 2-H), 4.23 (1 H, d, *J* 9.5, 5-H), 4.49 (1 H, d, *J* 7.5, 1-H), 5.60 (2 H, m, 3- and 4-H) and 7.40–8.00 (10 H, m, Ph) (Found: C, 61.5; H, 5.1%).

General procedure for coupling

A mixture of the donor (0.24 mmol), the nucleophile (0.2 mmol), NIS (0.24 mmol) and 4 Å powdered molecular sieves (0.2 g) in dry dichloromethane (2 cm^3) was stirred for 30 min at room temp. under dry argon. A solution of trimethylsilyl triflate in dry toluene (1 mol dm^3, 0.024 cm^3) was added, and the mixture was stirred for 30 min. Triethylamine (0.014 cm^3, 0.1 mmol) was added, and the mixture was filtered, and concentrated under reduced pressure. The coupled product was isolated through flash silica chromatography using the solvents stated.

Benzyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 ⟶ 6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside 12. Compound **6** (130 mg, 0.24 mmol) and the alcohol **9**[18] (116 mg, 0.2 mmol) were coupled as described above. The reaction mixture was purified by flash silica chromatography [heptane–ethyl acetate (4:3)] to give *compound* **12** (188 mg, 93%), mp 188–189 °C (from ethyl acetate–heptane); $[\alpha]_D^{22}$ −12 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 2.04, 2.05 and 2.06 (9 H, 3 s, Ac), 3.58 (1 H, dd, *J* 5 and 12, 6-H^b), 3.62 (1 H, m, 5-H), 3.85 (1 H, m, 5′-H), 4.09 (1 H, dd, *J* 2.5 and 12.5, 6′-H^b), 4.19 (1 H, m, 2′-H), 4.21 (2 H, m, 6′- and 6-H^a), 4.57 (1 H, d, *J* 8.5, 1′-H), 4.78 (1 H, d, *J* 8, 1-H), 4.80 (2 H, ABq, $PhCH_2$), 5.12 (1 H, t, *J* 9.5, 4′-H), 5.22 (1 H, dd, *J* 9.5 and 10.5, 3′-H), 5.50 (1 H, dd, *J* 8 and 9.5, 2-H), 5.58 (1 H, t, *J* 9.5, 4-H), 5.81 (1 H, t, *J* 9.5, 3-H) and 7.20–8.0 (21 H, m, Ph and NH) (Found: C, 56.9; H, 4.6; N, 1.4. $C_{48}H_{46}Cl_3NO_{17}$ requires C, 56.8; H, 4.6; N, 1.4%).

Benzyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 ⟶ 3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside 13. Compound **6** (130 mg, 0.24 mmol) and the alcohol **10**[23] (116 mg, 0.2 mmol) were coupled as described above. The reaction mixture was purified by flash silica chromatography [ethyl acetate–heptane (1:1)] to give compound **13** (173 mg, 85%), mp 193–194 °C (from ethyl acetate–heptane) (lit.,[11] 193–194 °C). NMR and $[\alpha]_D$ data are in agreement with those previously reported.[11]

Methyl [methyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 ⟶ 4)-2,3-di-*O*-benzoyl-β-D-glucopyranosid]uronate 3. *Method A.*—Compound **6** (130 mg, 0.24 mmol and the alcohol **2** (86 mg, 0.2 mmol) were coupled as described above. The reaction mixture was purified by flash silica chromatography [toluene–ethyl acetate (2:1)] to give *compound* **3** (142 mg, 82%), mp 147–148 °C (from ethyl acetate–hexane); $[\alpha]_D^{22}$ −13 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 1.88, 1.95 and 1.98 (9 H, 3 s, Ac), 3.47 (1 H, dd, *J* 2.5 and 12, 6-H^b), 3.53 (3 H, s, OMe), 3.55 (1 H, m, 5-H), 3.62 (1 H, dd, *J* 4.5 and 12, 6-H^a), 3.87 (3 H, s, CO_2Me), 3.94 (1 H, m, 2-H), 4.13 (1 H, d, *J* 9.5, 5-H), 4.32 (1 H, t, *J* 9.5, 4-H), 4.67 (1 H, d, *J* 7.5, 1-H), 4.86 (1 H, t, *J* 9.5, 4′-H), 4.96 (1 H, d, *J* 8.5, 1′-H), 5.17 (1 H, dd, *J* 9.5 and 10.5, 3′-H), 5.35 (1 H, dd, *J* 7.5 and 9.5, 2-H), 5.68 (1 H, t, *J* 9.5, 3-H), 6.81 (1 H, d, *J* 9, NH) and 7.30–8.0 (10 H, m, Ph) (Found: C, 50.2; H, 4.5; N, 1.4. $C_{36}H_{38}Cl_3NO_{17}$ requires C, 50.1; H, 4.4; N, 1.6%).

Method B.—Compound **7** (119 mg, 0.24 mmol) was treated as described above to yield compound **3** (144 mg, 83%).

Phenyl 2-deoxy-4,6-*O*-isopropylidene-1-thio-2-trichloroacetamido-β-D-glucopyranoside 14

A solution of compound **6** (542 mg, 1 mmol) in dry methanol (10 cm^3) was treated with methanolic sodium methoxide (1 mol dm^3, 0.1 cm^3) for 1 h at room temp. The mixture was then neutralized (pH paper) with Amberlite IR-120 (H^+) resin, filtered, concentrated, and dried over phosphorus pentaoxide *in vacuo*.

To a solution of the residue in DMF (3 cm^3) were added 2-methoxypropene (0.2 cm^3, 2 mmol) and (±)-camphor-10-sulfonic acid (CSA) (30 mg). The mixture was stirred for 1 h at room temp., and triethylamine (0.2 cm^3) was added. After concentration, the residue was purified by flash silica chromatography [ethyl acetate–heptane 1:1), containing 0.2% of triethylamine] to afford *compound* **14** (411 mg, 90%) mp 193–194 °C (from ethyl acetate–heptane); $[\alpha]_D^{22}$ −24 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 1.42 and 1.52 (6 H, 2 s, CMe_2), 2.74 (1 H, d, *J* 3, 3-OH), 3.39 (1 H, m, 5-H), 3.55 (1 H, t, *J* 10, 4-H), 3.57 (1 H, m, 2-H), 3.81 (1 H, t, *J* 10, 6-H^b), 3.97 (1 H, dd, *J* 5.5 and 10, 6-H^a), 4.09 (1 H, dt, *J* 3 and 10, 3-H), 5.09 (1 H, d, *J* 10.5, 1-H), 6.89 (1 H, d, *J* 8.0, NH) and 7.40 (5 H, m, Ph) (Found: C, 44.5; H, 4.4; N, 3.0. $C_{17}H_{20}Cl_3NO_5S$ requires C, 44.7; H, 4.4; N, 3.1%).

Phenyl 3-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-4,6-*O*-isopropylidene-1-thio-2-trichloroacetamido-β-D-glucopyranoside 15

A mixture of compound **14** (457 mg, 1 mmol), imidazole (171 mg, 2.5 mmol) and TBDMSCl (190 mg, 1.25 mmol) in dry DMF (3 cm^3) was stirred for 4 h at room temp. The mixture was diluted with dichloromethane (20 cm^3), washed successively with brine and water, dried ($MgSO_4$), and concentrated. The residue was purified by flash silica chromatography [heptane–ethyl acetate (2:1), containing 0.2% of triethylamine] to give *compound* **15** (537 mg, 94%), mp 183–184 °C (from diethyl ether); $[\alpha]_D^{22}$ −23 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 0.04 and 0.05 (6 H, 2 s, $SiMe_2$), 0.86 (9 H, s, SiBu^t), 1.40 and 1.49 (6 H, 2 s, CMe_2), 3.35 (1 H, m, 5-H), 3.51 (1 H, t, *J* 10, 4-H), 3.59 (1 H, m, 2-H), 3.78 (1 H, dd, *J* 10 and 12, 6-H^b), 3.95 (1 H, dd, *J* 5.5 and 12, 6-H^a), 4.04 (1 H, dd, *J* 10 and 10.5, 3-H), 5.09 (1 H, d, *J* 10.5, 1-H), 6.85 (1 H, d, *J* 8.5, NH) and 7.40 (5 H, m, Ph) (Found: C, 48.4; H, 6.0; N, 2.3. $C_{23}H_{34}Cl_3NO_5SSi$ requires C, 48.4: H, 6.0; N, 2.4%).

Phenyl 3-*O*-chloroacetyl-2-deoxy-4,6-*O*-isopropylidene-1-thio-2-trichloroacetamido-β-D-glucopyranoside 16

A mixture of compound **14** (457 mg, 1 mmol) and chloroacetic anhydride (256 mg, 1.5 mmol) in dry pyridine (8 cm^3) was stirred for 30 min at 0 °C. Ice-cold water (1 cm^3) was added, and the mixture was diluted with dichloromethane (30 cm^3), washed successively with water, saturated aq. sodium hydrogen carbonate, and water, dried ($MgSO_4$), and concentrated. The residue was crystallized from ethyl acetate–heptane to give *compound* **16** (480 mg, 90%), mp 216–217 °C; $[\alpha]_D^{22}$ −34 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 1.24 and 1.45 (6 H, 2 s, CMe_2), 3.43 (1 H, m, 5-H), 3.79 (1 H, t, *J* 10, 4-H), 3.81 (1 H, dd, *J* 10 and 11,

6-H^b), 3.97 (1 H, dd, *J* 5.5 and 11, 6-H^a), 4.04 (2 H, ABq, $COCH_2Cl$), 4.11 (1 H, m, 2-H), 4.83 (1 H, d, *J* 10.5, 1-H), 5.23 (1 H, dd, *J* 10 and 10.5, 3-H), 7.14 (1 H, d, *J* 9.5, NH) and 7.40 (5 H, m, Ph) (Found: C, 42.7; H, 4.0; N, 2.8. $C_{19}H_{21}Cl_4NO_6S$ requires C, 42.7; H, 4.1; N, 2.6%).

Methyl {methyl *O*-[3-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-4,6-*O*-isopropylidene-2-trichloroacetamido-β-D-glucopyranosyl]-(1 ⟶ 4)-2,3-di-*O*-benzoyl-β-D-glucopyranosid}uronate 17

A mixture of uronate **2** (430 mg, 1 mmol), donor **15** (656 mg, 1.15 mmol), NIS (259 mg, 1.15 mmol) and 4 Å powdered molecular sieves (0.5 g) in dry dichloromethane (8 cm^3) was stirred for 30 min at room temp. under dry argon, then was cooled to 0 °C. A solution of trimethylsilyl triflate in dry toluene (1 mol dm^3, 0.115 cm^3) was added, and the mixture was stirred for 15 min at 0 °C. Triethylamine (0.14 cm^3) was added, and the mixture was filtered and concentrated. The residue was directly purified by flash silica chromatography [heptane–ethyl acetate (3:1), containing 0.2% of triethylamine] to give *compound* **17** (811 mg, 91%), mp 126–127 °C (from diethyl ether–heptane); $[\alpha]_D^{22}$ −3 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 0.05 (6 H, s, $SiMe_2$), 0.81 (9 H, s, SiBu^t), 1.15 and 1.28 (6 H, 2 s, CMe_2), 2.41 (1 H, t, *J* 10.5, 6′-H^b), 3.02 (1 H, m, 5′-H), 3.15 (1 H, t, *J* 10, 4′-H), 3.18 (1 H, dd, *J* 5 and 10.5, 6′-H^a), 3.52 (3 H, s, OMe), 3.66 (2 H, m, 2′- and 3′-H), 3.85 (3 H, s, CO_2Me), 4.09 (1 H, d, *J* 9.5, 5-H), 4.29 (1 H, t, *J* 9.5, 4-H), 4.65 (1 H, d, *J* 7.5, 1-H), 4.81 (1 H, d, *J* 8, 1′-H), 5.34 (1 H, dd, *J* 7.5 and 9.5, 2-H), 5.63 (1 H, t, *J* 9.5, 3-H), 6.69 (1 H, d, *J* 8.5, NH) and 7.30–8.0 (10 H, m, Ph) (Found: C, 52.6; H, 5.8; N, 1.7. $C_{39}H_{50}Cl_3NO_{14}Si$ requires C, 52.5; H, 5.6; N, 1.6%).

Methyl [methyl *O*-(3-*O*-chloroacetyl-2-deoxy-4,6-*O*-isopropylidene-2-trichloroacetamido-β-D-glucopyranosyl)-(1 ⟶ 4)-2,3-di-*O*-benzoyl-β-D-glucopyranosid]uronate 19

Uronate **2** (430 mg, 1 mmol) and donor **16** (614 mg, 1.15 mmol) were coupled as described for the preparation of compound **17**. The residue was purified by flash silica chromatography [ethyl acetate–heptane (1:1), containing 0.1% of triethylamine] to give *compound* **19** (768 mg, 90%), mp 165–166 °C (from ethyl acetate–heptane); $[\alpha]_D^{22}$ −5 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 1.18 and 1.24 (6 H, 2 s, CMe_2), 2.45 (1 H, t, *J* 10.5, 6′-H^b), 3.12 (1 H, m, 5′-H), 3.45 (2 H, m, 6′-H^a and 4′-H), 3.53 (3 H, s, OMe), 3.85 (3 H, s, CO_2Me), 3.92 (1 H, m, 2′-H), 4.04 (2 H, ABq, $COCH_2Cl$), 4.11 (1 H, d, *J* 9.5, 5-H), 4.24 (1 H, t, *J* 9.5, 4-H), 4.66 (1 H, d, *J* 7.5, 1-H), 4.83 (1 H, d, *J* 8.5, 1′-H), 5.09 (1 H, dd, *J* 9.5 and 10.5, 3′-H), 5.36 (1 H, dd, *J* 7.5 and 9.5, 2-H), 5.66 (1 H, t, *J* 9.5, 3-H), 6.86 (1 H, d, *J* 9, NH) and 7.30–8.0 (10 H, m, Ph) (Found: C, 49.3; H, 4.4; N, 1.6. $C_{35}H_{37}Cl_4NO_{15}$ requires C, 49.2; H, 4.5; N, 1.6%).

Methyl [methyl *O*-(2-deoxy-4,6-*O*-isopropylidene-2-trichloroacetamido-β-D-glucopyranosyl)-(1 ⟶ 4)-2,3-di-*O*-benzoyl-β-D-glucopyranosid]uronate 20

A mixture of compound **19** (854 mg, 1 mmol) and thiourea (228 mg, 3 mmol) in pyridine (6 cm^3) and ethanol (6 cm^3) was stirred for 16 h at 80 °C, then was cooled and concentrated. The residue was purified by flash silica chromatography [ethyl acetate–heptane (1:1), containing 0.1% of triethylamine] to give compound **20** (715 mg, 92%), mp 197–198 °C (from ethyl acetate–heptane); $[\alpha]_D^{22}$ −7 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 1.19 and 1.30 (6 H, 2 s, CMe_2), 2.42 (1 H, t, *J* 10.5, 6′-H^b), 2.88 (1 H, d, *J* 3, 3′-OH), 3.05 (1 H, m, 5′-H), 3.18 (1 H, dd, *J* 5.5 and 10.5, 6′-H^a), 3.22 (1 H, t, *J* 9.5, 4′-H), 3.53 (3 H, s, OMe), 3.58 (1 H, m, 2′-H), 3.79 (1 H, m, *J* 3, 8.5 and 10.5, 3′-H), 3.87 (3 H, s, CO_2Me), 4.14 (1 H, d, *J* 9.5, 5-H), 4.28 (1 H, t, *J* 9.5, 4-H), 4.67 (1 H, d, *J* 7.5, 1-H), 4.91 (1 H, d, *J* 8.5, 1′-H), 5.38 (1 H, dd, *J* 7.5 and 9.5, 2-H), 5.64 (1 H, t, *J* 9.5, 3-H), 7.05 (1 H, d, *J* 7.5, NH) and 7.30–8.0 (10 H, m, Ph) (Found: C, 51.1; H, 4.8; N, 2.0. $C_{33}H_{36}Cl_3NO_{14}$ requires C, 51.0; H, 4.7; N, 1.8%).

Methyl 1,2,3,4-tetra-*O*-benzoyl-D-glucopyranuronate 22

Commercial D-glucofuranurono-6,3-lactone **21** (2 g, 11.3 mmol) was added portionwise to a solution of powdered sodium hydroxide (10 mg) in dry methanol (20 cm^3), and the mixture was stirred for 2 h at room temperature, then was concentrated, and dried *in vacuo*. Benzoyl chloride (8 cm^3, 68 mmol) was added at 0 °C to a solution of the residue in pyridine (25 cm^3), and the mixture was stirred overnight at room temp. Ice-cold water (20 cm^3) was added, and the mixture was diluted with dichloromethane (200 cm^3), washed successively with water, saturated aq. sodium hydrogen carbonate and water, dried (Na_2SO_4), and concentrated. The residue was purified by flash silica chromatography [heptane–ethyl acetate 3:2)] and crystallized from diethyl ether–heptane to give *compound* **22** (mixture of anomers) as a pale yellow solid (5.32 g, 75%); $\delta_H(CDCl_3)$ 3.61 and 3.69 (3 H, 2 s, CO_2Me α and β), 4.62 (d, *J* 9, 5-H α), 4.78 (d, *J* 10, 5-H β), 5.61 (dd, *J* 3.5 and 10, 2-H α), 5.78 (dd, *J* 9 and 10, 4-H β), 5.82 (dd, *J* 7.5 and 9, 2-H β), 5.84 (5, *J* 9, 4-H α), 6.01 (t, *J* 9, 3-H β), 6.33 (dd, *J* 9 and 10, 3-H α), 6.35 (d, *J* 7.5, 1-H β), 6.83 (d, *J* 3.5, 1-H α) and 7.30–8.20 (20 H, m, Ph) (Found: C, 67.1; H, 4.6. $C_{35}H_{28}O_{11}$ requires C, 67.3; H, 4.5%); *m/z* 642 (M^+ + 18).

Methyl 2,3,4-tri-*O*-benzoyl-1-bromo-1-deoxy-α-D-glucopyranuronate 23

A mixture of compound **22** (mixture of anomers, 5 g) and hydrobromic acid in acetic acid (20 cm^3 of a 33% w/v solution) was stirred at room temperature for 8 h, then was cooled to 0 °C. The mixture was diluted with cold dichloromethane (100 cm^3), washed with ice-cold water (4 × 100 cm^3), dried ($MgSO_4$), and concentrated. The residue was crystallized from diethyl ether–heptane to give *bromide* **23** (4.11 g, 88%), mp 76–77 °C; $[\alpha]_D^{22}$ +125 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 3.68 (3 H, s, CO_2Me), 4.85 (1 H, d, *J* 10, 5-H), 5.33 (1 H, dd, *J* 4 and 10, 2-H), 5.73 (1 H, t, *J* 10, 4-H), 6.26 (1 H, t, *J* 10, 3-H), 6.88 (1 H, d, *J* 4, 1-H) and 7.30–8.0 (15 H, m, Ph) (Found: C, 57.5; H, 4.0. $C_{28}H_{23}BrO_9$ requires C, 57.6; H, 4.0%).

Methyl 2,3,4-tri-*O*-benzoyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate 24

A mixture of compound **22** (625 mg, 1 mmol) and hydrazine acetate (230 mg, 2.5 mmol) in dry DMF (7 cm^3) was stirred for 1 h at room temp., then was diluted with ethyl acetate (30 cm^3), washed twice with water, dried ($MgSO_4$), and concentrated. The residue was purified by flash silica chromatography [toluene–ethyl acetate (4:1)] to give the corresponding hemiacetal (343 mg, 66%); $\delta_H(CDCl_3)$ 3.52 (1 H, d, *J* 4, 1-OH), 3.66 (3 H, s, CO_2Me), 4.87 (1 H, d, *J* 10, 5-H), 5.33 (1 H, dd, *J* 4 and 10, 2-H), 5.66 (1 H, t, *J* 10, 4-H), 5.84 (1 H, t, *J* 4, 1-H), 6.24 (1 H, t, *J* 10, 3-H) and 7.30–8.0 (15 H, m, Ph).

A mixture of the hemiacetal (343 mg, 0.66 mol), trichloroacetonitrile (0.66 cm^3, 6.6 mmol) and DBU (0.03 cm^3, 0.2 mmol) in dichloromethane (5 cm^3) was stirred for 20 min at room temp., then was directly purified by flash silica chromatography [heptane–ethyl acetate (5:2), containing 0.1% of triethylamine] to give *compound* **24** (390 mg, 89%); $[\alpha]_D^{22}$ +59 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 3.78 (3 H, s, CO_2Me), 4.22 (1 H, d, *J* 10, 5-H), 5.53 (1 H, dd, *J* 3.5 and 10, 2-H), 5.62 (1 H, t, *J* 10, 4-H), 6.13 (1 H, t, *J* 10, 3-H), 6.86 (1 H, d, *J* 3.5, 1-H), 7.30–8.0 (15 H, m, Ph) and 8.70 (1 H, s, C=NH) (Found: C, 54.1; H, 3.5; N, 2.0. $C_{30}H_{24}Cl_3NO_{10}$ requires C, 54.2; H, 3.6; N, 2.1%).

Methyl [methyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 ⟶ 3)-*O*-(2-deoxy-4,6-*O*-isopropylidene-2-trichloroacetamido-β-D-glucopyranosyl)-(1 ⟶ 4)-2,3-di-*O*-benzoyl-β-D-glucopyranosid]uronate 25

A mixture of the alcohol **20** (443 mg, 0.57 mmol), imidate **24** (532 mg, 0.8 mmol) and powdered 4 Å molecular sieves (0.5 g) in

dry dichloromethane (8 cm^3) was stirred for 1 h at room temp. under dry argon. A solution of trimethylsilyl triflate in dry toluene (1 mol dm^3; 0.08 cm^3, 0.08 mmol) was added, and the mixture was stirred for 30 min, then treated with triethylamine (0.028 cm^3, 0.2 mmol), filtered, and concentrated. The residue was purified by flash silica chromatography [ethyl acetate–heptane (1 : 1)] to give *compound* **25** (673 mg, 92%), mp 225–226 °C (from ethyl acetate–heptane); $[\alpha]_D^{22}$ +4 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 1.10 and 1.25 (6 H, 2 s, CMe_2), 2.49 (1 H, t, *J* 10.5, 6′-H^b), 3.11 (1 H, m, 5′-H), 3.31 (1 H, dd, *J* 5 and 10.5, 6′-H^a), 3.37 (1 H, m, 2′-H), 3.42 (1 H, t, *J* 9, 4′-H), 3.51 (3 H, s, OMe), 3.70 and 3.79 (6 H, 2 s, CO_2Me), 4.07 (1 H, d, *J* 9.5, 5-H), 4.21 (1 H, d, *J* 9.5, 5″-H), 4.23 (1 H, dd, *J* 9 and 10.5, 3′-H), 4.36 (1 H, t, *J* 9, 4-H), 4.64 (1 H, d, *J* 8, 1-H), 5.08 (1 H, d, *J* 8, 1′-H), 5.09 (1 H, d, *J* 8, 1″-H), 5.37 (1 H, dd, *J* 8 and 9.5, 2-H), 5.41 (1 H, dd, *J* 8 and 9.5, 2″-H), 5.59 (1 H, t, *J* 9.5, 3-H), 5.63 (1 H, t, *J* 9.5, 4″-H), 5.79 (1 H, t, *J* 9.5, 3″-H), 6.76 (1 H, d, *J* 8, NH) and 7.30–8.0 (25 H, m, Ph) (Found: C, 57.2; H, 4.5; N, 1.0. $C_{61}H_{58}Cl_3NO_{23}$ requires C, 57.3; H, 4.6; N, 1.1%).

Methyl [methyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 ⟶ 3)-*O*-(6-*O*-benzoyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 ⟶ 4)-2,3-di-*O*-benzoyl-β-D-glucopyranosid]uronate 26

A solution of compound **25** (640 mg, 0.5 mmol) in acetic acid (18 cm^3) was heated at 100 °C. Water (6 cm^3) was added dropwise, and the mixture was stirred at 100 °C for 30 min, then was cooled, concentrated, and dried *in vacuo*. Benzoyl cyanide (132 mg, 1 mmol) was added to a solution of the residue in dry pyridine (6 cm^3), and the mixture was stirred for 20 h at room temp. Methanol (0.5 cm^3) was then added, and the mixture was concentrated. The residue was purified by flash silica chromatography [ethyl acetate–heptane (3 : 2)] to give *compound* **26** (605 mg, 90%), mp 189–190 °C (from ethyl acetate–heptane); $[\alpha]_D^{22}$ −10 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 3.19 (1 H, m, *J* 7.5, 8.5 and 10, 2′-H), 3.35 (1 H, m, 4′-H), 3.48 (3 H, s, OMe), 3.52 (1 H, dd, *J* 7.5 and 12, 6′-H^b), 3.62 and 3.81 (6 H, 2 s, CO_2Me), 3.63 (1 H, m, 5′-H), 4.10 (1 H, d, *J* 9.5, 5-H), 4.14 (1 H, d, *J* 1.5, 4′-OH), 4.37 (1 H, d, *J* 9.5, 5″-H), 4.38 (1 H, dd, *J* 8.5 and 10, 3′-H), 4.41 (1 H, t, *J* 9.5, 4-H), 4.50 (1 H, dd, *J* 2 and 12, 6′-H^a), 4.61 (1 H, d, *J* 7, 1-H), 4.86 (1 H, d, *J* 7.5, 1″-H), 5.11 (1 H, d, *J* 8.5, 1′-H), 5.31 (1 H, dd, *J* 7 and 9.5, 2-H), 5.51 (1 H, dd, *J* 7.5 and 9.5, 2″-H), 5.58 (1 H, t, *J* 9.5, 3-H), 5.64 (1 H, t, *J* 9.5, 4″-H), 5.82 (1 H, t, *J* 9.5, 3″-H), 6.72 (1 H, d, *J* 7.5, NH) and 7.15–8.10 (30 H, m, Ph) (Found: C, 58.2; H, 4.3; N, 1.0. $C_{65}H_{58}Cl_3NO_{24}$ requires C, 58.1; H, 4.3; N, 1.0%).

Methyl [methyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 ⟶ 3)-*O*-(6-*O*-benzoyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1 ⟶ 4)-2,3-di-*O*-benzoyl-β-D-glucopyranosid]uronate 27

Trifluoromethanesulfonic anhydride (0.086 cm^3, 0.5 mmol) was added at −15 °C to a solution of the alcohol **26** (0.5 g, 0.37 mmol) and dry pyridine (0.16 cm^3, 2 mmol) in dichloromethane (5 cm^3), and the mixture was stirred for 2 h at this temperature. Dichloromethane (20 cm^3) was then added, and the mixture was washed successively with ice-cold hydrochloric acid (1 mol dm^3), brine and water, dried ($MgSO_4$), and concentrated. A solution of the residue in toluene–ethyl acetate (4 : 1) was filtered through a pad (1 × 2 cm) of silica gel and concentrated to give the 4′-*O*-triflyl derivative (538 mg, 96%); $\delta_H(CDCl_3)$ 4.63 (1 H, t, *J* 9.5, 4′-H).

A mixture of the above isolated triflate and dried TBAN (1.15 g, 4 mmol) in dry DMF (6 cm^3) was stirred for 15 h at room temp. Ethyl acetate (40 cm^3) was then added, and the mixture was washed successively with brine and water, dried ($MgSO_4$), and concentrated. The residue was purified by flash silica chromatography [toluene–ethyl acetate (4 : 1)] to afford *compound* **27** (435 mg, 87% from **26**), mp 234–235 °C (from MeOH); $[\alpha]_D^{22}$ +10 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 2.64 (1 H, d, *J* 3.5, 4′-OH), 3.49 (3 H, s, OMe), 3.53 (1 H, m, 2′-H), 3.57 and 3.78 (6 H, 2 s, CO_2Me), 3.72 (2 H, m, 6′-H^b and 5′-H), 4.07 (1 H, dd, *J* 6 and 12, 6′-H^a), 4.10 (1 H, d, *J* 9.5, 5-H), 4.11 (1 H, m, *J* 1 and 3.5, 4′-H), 4.32 (1 H, d, *J* 9.5, 5″-H), 4.43 (1 H, t, *J* 9.5, 4-H), 4.50 (1 H, dd, *J* 3.5 and 10.5, 3′-H), 4.65 (1 H, d, *J* 7, 1-H), 5.02 (1 H, d, *J* 7, 1″-H), 5.09 (1 H, d, *J* 8.5, 1′-H), 5.37 (1 H, dd, *J* 7 and 9.5, 2-H), 5.49 (1 H, dd, *J* 7 and 9.5, 2″-H), 5.60 (1 H, t, *J* 9.5, 3-H), 5.66 (1 H, t, *J* 9.5, 4″-H), 5.80 (1 H, t, *J* 9.5, 3″-H), 6.72 (1 H, d, *J* 7.5, NH) and 7.30–8.10 (30 H, m, Ph) (Found: C, 58.0; H, 4.4; N, 1.0. $C_{65}H_{58}Cl_3NO_{24}$ requires C, 58.1; H, 4.4; N, 1.0%).

Methyl [methyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 ⟶ 3)-*O*-(2-acetamido-6-*O*-benzoyl-2-deoxy-β-D-galactopyranosyl)-(1 ⟶ 4)-2,3-di-*O*-benzoyl-β-D-glucopyranosid]uronate 28

A mixture of compound **27** (403 mg, 0.3 mmol), TBTH (0.52 cm^3, 1.8 mmol) and AIBN (10 mg) in dry benzene (4 cm^3) and dry *N*,*N*-dimethylacetamide (2 cm^3) was stirred for 30 min under a flow of argon, then heated for 1 h at 80 °C, cooled and concentrated. The solid residue was washed with hexane (3 × 5 cm^3), and crystallized from methanol to give *compound* **28** (342 mg, 92%), mp 231–232 °C; $[\alpha]_D^{22}$ +16 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 1.64 (3 H, s, NAc), 2.36 (1 H, d, *J* 3, 4′-OH), 3.05 (1 H, m, *J* 7, 8 and 11, 2′-H), 3.38 (3 H, s, OMe), 3.56 and 3.78 (6 H, 2 s, CO_2Me), 3.60 (2 H, m, 6′-H^b and 5′-H), 4.01 (1 H, dd, *J* 4 and 11, 6′-H^a), 4.02 (1 H, m, *J* 1, 3 and 3.5, 4′-H), 4.09 (1 H, d, *J* 9.5, 5-H), 4.28 (1 H, d, *J* 9.5, 5″-H), 4.31 (1 H, t, *J* 9.5, 4-H), 4.62 (1 H, d, *J* 7.5, 1-H), 4.72 (1 H, dd, *J* 3.5 and 11, 3′-H), 4.92 (1 H, d, *J* 8, 1′-H), 4.93 (1 H, d, *J* 7.5, 1″-H), 5.32 (1 H, d, *J* 7, NH), 5.39 (1 H, dd, *J* 7.5 and 9.5, 2-H), 5.52 (1 H, dd, *J* 7.5 and 9.5, 2″-H), 5.55 (1 H, t, *J* 9.5, 3-H), 5.62 (1 H, t, *J* 9.5, 4″-H), 5.86 (1 H, t, *J* 9.5, 3″-H) and 7.30–8.10 (30 H, m, Ph) (Found: C, 62.8; H, 4.9; N, 1.0. $C_{65}H_{61}NO_{24}$ requires C, 62.9; H, 4.9; N, 1.1%).

Methyl [methyl *O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 ⟶ 3)-*O*-(2-acetamido-6-*O*-benzoyl-2-deoxy-4-*O*-sulfo-β-D-galactopyranosyl)-(1 ⟶ 4)-2,3-di-*O*-benzoyl-β-D-glucopyranosid]uronate sodium salt 29

A solution of alcohol **28** (0.25 g, 0.2 mmol) and the sulfur trioxide–trimethylamine complex (0.14 g, 1 mmol) in dry DMF (3 cm^3) was stirred at 65 °C for 24 h, then was cooled. Methanol (0.5 cm^3) was added, and the mixture was layered onto a column (3 × 80 cm) of Sephadex LH-20 and eluted with dichloromethane–methanol (1 : 1). The residue was then eluted from a column (1 × 20 cm) of Sephadex SP-C25 (Na^+) with ethyl acetate–methanol–water (5 : 2 : 1) to give the *sodium salt* **29** as a foam (252 mg, 93%); $[\alpha]_D^{22}$ −0.5 (*c* 1, MeOH); $\delta_H(CD_3OD)$ 1.57 (3 H, s, NAc), 3.46 (3 H, s, OMe), 3.58 and 3.80 (6 H, 2 s, CO_2Me), 3.70 (3 H, m, 2′-, 5′- and 6′-H^b), 4.20 (1 H, dd, *J* 5 and 11, 6′-H^a), 4.23 (1 H, d, *J* 9.5, 5-H), 4.31 (1 H, t, *J* 9.5, 4-H), 4.33 (1 H, dd, *J* 3.5 and 11, 3′-H), 4.53 (1 H, d, *J* 9.5, 5″-H), 4.68 (1 H, d, *J* 8, 1′-H), 4.80 (1 H, d, *J* 7.5, 1-H), 4.96 (1 H, dd, *J* 1 and 3.5, 4′-H), 5.21 (1 H, dd, *J* 7.5 and 9.5, 2-H), 5.25 (1 H, d, *J* 7.5, 4″-H), 5.57 (1 H, dd, *J* 7.5 and 9.5, 2″-H), 5.62 (1 H, t, *J* 9.5, 3-H), 5.74 (1 H, t, *J* 9.5, 4″-H), 5.95 (1 H, t, *J* 9.5, 3″-H) and 7.20–8.10 (30 H, m, Ph) (Found: C, 58.0; H, 4.7; N, 1.0. $C_{65}H_{60}NNaO_{27}S$ requires C, 58.2; H, 4.7; N, 1.0%).

Methyl *O*-(β-D-glucopyranosyluronic acid)-(1 ⟶ 3)-*O*-(2-acetamido-2-deoxy-4-*O*-sulfo-β-D-galactopyranosyl)-(1 ⟶ 4)-β-D-glucopyranosyluronic acid trisodium salt 30

Aq. sodium hydroxide (3 mol dm^3, 2 cm^3) was added at 0 °C to a solution of compound **29** (0.2 g, 0.15 mmol) in methanol–water (5 : 1; 6 cm^3), and the mixture was stirred for 6 h at room temp. The pH of the solution was brought to ~8 (pH paper) with dil. hydrochloric acid, and the mixture was concentrated.

The residue was eluted from a column (2 × 150 cm) of Sephadex G-10 with water and freeze-dried to give the *target compound* **30** as an hygroscopic foam (96 mg, 87%); $[\alpha]_D^{22}$ −35 (*c* 1, water); $\delta_H(D_2O)$ 2.09 (3 H, s, NAc), 3.36 (1 H, dd, *J* 8 and 9.5, 2-H), 3.38 (1 H, dd, *J* 7.5 and 9.5, 2″-H), 3.52 (1 H, t, *J* 9.5, 3″-H), 3.58 (1 H, t, *J* 9.5, 4″-H), 3.59 (3 H, s, OMe), 3.66 (1 H, t, *J* 9.5, 3-H), 3.70 (1 H, d, *J* 9.5, 5″-H), 3.75 (1 H, d, *J* 9.5, 5-H), 3.82 (1 H, t, *J* 9.5, 4-H), 3.86 (3 H, m, 5′-H and 6′-H_2), 4.08 (2 H, m, 2′- and 3′-H), 4.42 (1 H, d, *J* 8, 1-H), 4.51 (1 H, d, *J* 7.5, 1″-H), 4.63 (1 H, d, *J* 8, 1′-H) and 4.84 (1 H, dd, *J* 1 and 3, 4′-H); $\delta_C(D_2O)$ 22.79 (CO*Me*), 51.9 (C-2′), 57.4 (OMe), 61.3 (C-6′), 72.1 (C-2), 72.8 (C-2″ and -4″), 74.2 (C-3), 74.9 (C-5′), 75.4 (C-4′ and -3″), 76.5 (C-5 and -5″), 76.7 (C-3′), 80.4 (C-4), 101.1 (C-1), 103.6 (C-1′ and -1″) and 174.4, 175.1 and 176.1 (C=O) (Found: C, 33.4; H, 4.5; N, 1.7. $C_{21}H_{30}NNa_3O_{21}S{\cdot}H_2O$ requires C, 33.6; H, 4.3; N, 1.8%).

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