Chemical synthesis of β-D-Glc*p*A(2SO_4)-(1→3)-D-Gal*p*NAc(6SO_4), the disaccharide repeating unit of shark cartilage chondroitin sulfate D, and of its methyl β-D-glycoside derivative

Nathalie Karst and Jean-Claude Jacquinet *

Institut de Chimie Organique et Analytique, UPRES-A CNRS 6005, UFR Faculté des Sciences, Université d'Orléans, BP 6759, F-45067 Orléans, France

Received (in Cambridge, UK) 10th April 2000, Accepted 19th June 2000
Published on the Web 19th July 2000

The syntheses of sodium *O*-(disodium 2-*O*-sulfonato-β-D-glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-6-*O*-sulfonato-D-galactopyranose **1**, which represents a structural element of shark cartilage chondroitin sulfate D, and of its methyl β-D-glycoside derivative **2** are reported for the first time. The glucuronyl donor **10** is prepared in a straightforward manner from D-glucose, whereas the glycosyl acceptors **20** and **21** are obtained from known 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-1-*O*-trichloroacetimidoyl-α-D-glucopyranose through glycosylation with benzyl alcohol and methanol, respectively, and subsequent transformation into D-*galacto* synthons by selective inversion of configuration at C-4. Unexpected pyranose ⟶ furanose ring contraction as well as 3,6-anhydro-derivative formation in the D-*galacto* series are also reported. Stereocontrolled coupling of the imidate **10** with the alcohols **20** and **21** afforded the corresponding β-linked disaccharide derivatives **24** and **25**, respectively, which are submitted to radical reduction of the *N*-trichloroacetyl groups, *O*-desilylation, saponification, *O*-sulfonation, and catalytic hydrogenation to provide the target molecules **1** and **2**, respectively, in high yields.

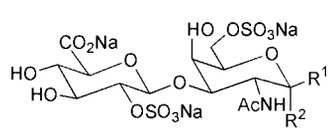
Introduction

Chondroitin sulfate (CS) proteoglycans are widely distributed among various tissues and exhibit a large variety of biological functions. They are found in the extracellular matrix of connective tissues, at the surface of many cells, and in intracellular secretory granules.[1,2] They are linear copolymers built essentially from dimeric units composed of D-glucuronic acid (GlcA) and 2-acetamido-2-deoxy-D-galactose (GalNAc), namely [4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→]$_n$. Several types of CS (A, B, C, D, E, and K) having sulfate(s) at various positions are known. These sulfation patterns give rise to biologically important functions deeply related to the position and the number of sulfate groups. However, homogeneous polymers (*i.e.* composed of regular repeating units) are not found in Nature, and species containing irregular sequences are often encountered. These microheterogeneities complicate to a larger extent accurate biological studies, but are potential sites for specific recognition processes. Whereas CS-A and CS-C (sulfated at C-4 and C-6 of the aminosugar moiety, respectively), the most abundant types, were extensively studied, the other variants, and especially CS-D (sulfated at C-6 of the GalNAc and C-2 of the GlcA units), draw less attention.

Shark cartilage is the major natural source of CS-D, and commercial preparations are now available. The structure of the basic disulfated disaccharide of CS-D was first established by Seno and Murakami,[3] and, more recently, several sulfated oligosaccharides were isolated[4–6] from shark cartilage CS-D, and their structures were determined by high-field ^{1}H NMR spectroscopy. The interest in shark cartilage, and *a fortiori* for CS-D, arose at the beginning of the eighties when it was claimed[7] that it contained a substance that strongly inhibits the growth of new blood vessels toward solid tumors. The discovery of this antiangiogenic activity led to numerous reports and to the development of new drugs[8,9] based on shark cartilage extracts. However, clinical trials that support their beneficial effects are anecdotal, and a matter of controversy.[10,11] Indeed, no structure–activity relationship studies were undertaken on these mixtures of molecules.

Besides this, CS-D was found to promote neurite outgrowth for hippocampal and rat embrionic mesencephalic neurons.[12] Since most of the observed effects depend on binding of glycosaminoglycan chains to proteins, determination of the precise structure and size of oligosaccharide sequences involved in such binding is of prime importance. Until now, the detailed structural and functional analyses of CS-D fragments have been hampered by a lack of analytical tools. Although various oligosaccharide fragments were prepared[4–6] using bacterial enzymes (chondroitinase, hyaluronidase), their structures were converted to unsaturated forms by the action of the eliminases, and consequently are not suitable for binding assays, or to be used as acceptor substrates for biosynthetic enzymes. Thus, chemical synthesis of molecules of definite size and structure remains one of the most efficient way to address these problems.

In the last decade, several syntheses of CS-A and CS-C fragments have been reported, such as those of di-,[13–15] tri-,[15–17] tetra-,[15] and pentasaccharides,[18] but, to the best of our knowledge, no synthetic work on the D-type appeared in the literature. As a primary target, we now report for the first time on the chemical synthesis of the basic disaccharide repeating unit of CS-D, compound **1**, and those of its methyl β-D-glycoside



1 R^1, R^2 = H, OH

2 R^1 = OMe, R^2 = H

derivative **2**, in which the methyl group is suitable as a marker for NMR studies.

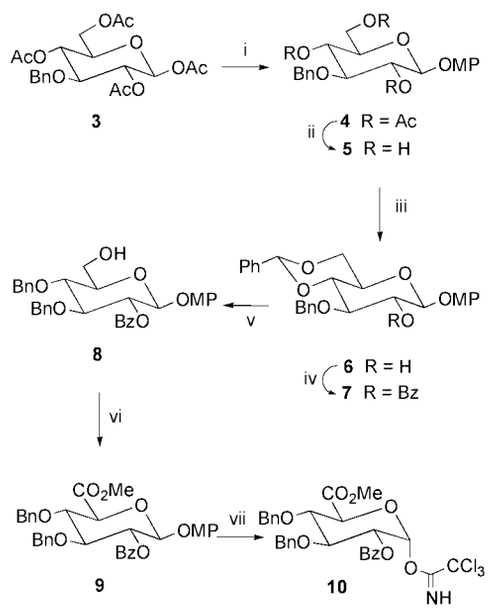
Results and discussion

For the syntheses of the target disaccharides **1** and **2**, a common glycosyl donor (**10**) was designed, which could be condensed

with suitably protected glycosyl acceptors, namely **20** and **21**. As far as structures **1** and **2** contain sulfate esters, we first planned to temporarily protect the hydroxy groups which would ultimately carry these substituents with ester groups, and those which should remain free with easily removable benzyl ethers. This provides a stereocontrolling auxiliary at C-2 of the glycosyl donor which would induce a 1,2-*trans* linkage formation. Since D-galactosamine is a rather expensive sugar, the glycosyl acceptors **20** and **21** were prepared by selective inversion of configuration at C-4 of easily attainable D-glucosamine-derived synthons. All glycosylation reactions were achieved by using trichloroacetimidates.[19]

Preparation of the glycosyl donor

Synthesis of the glycosyl donor **10** was achieved in a straightforward manner as follows (Scheme 1). The known tetraacetate

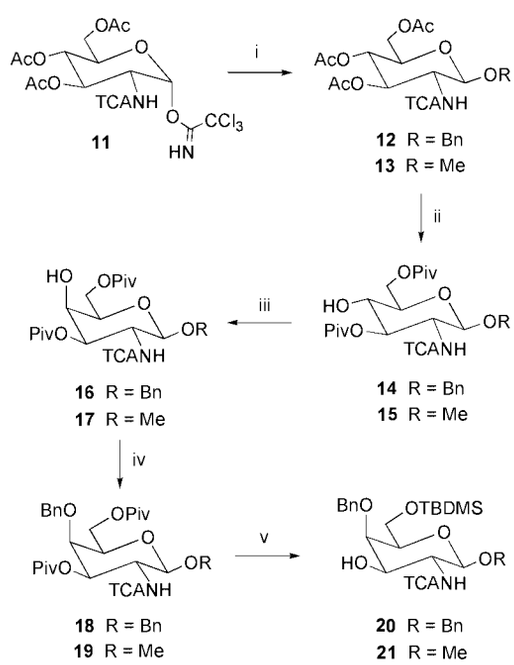


Scheme 1 *Reagents and conditions*: (i) 4-Methoxyphenol, 4 Å mol. sieves, TMSOTf (0.15 mol equiv.), CH_2Cl_2, 0 °C, 2 h; (ii) MeONa, MeOH, 16 h; (iii) PhCHO, TFA, 20 min; (iv) PhCOCl, pyridine, CH_2Cl_2, 0 °C, 1 h 30 min; (v) $Me_3N{\cdot}BH_3$, $AlCl_3$, diethyl ether–CH_2Cl_2, 0 °C, 2 h; (vi) CrO_3–H_2SO_4 (Jones reagent), acetone, 1 h; then MeOCOCl, Et_3N, DMAP, CH_2Cl_2, 0 °C ⟶ rt, 2 h; (vii) CAN, toluene–acetonitrile–water (1 : 1.5 : 1), 20 min; then CCl_3CN, DBU, CH_2Cl_2, 0 °C, 10 min. MP = 4-methoxyphenyl.

3,[20] easily prepared in three steps from commercially available 1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose, was treated with 4-methoxyphenol and trimethylsilyl triflate (TMSOTf, 0.15 mol equiv.), in dichloromethane, to afford the crystalline glycoside **4** in 69% yield. Transesterification of **4** with methanolic sodium methoxide gave the corresponding crystalline triol **5** in 89% yield, which was treated with benzaldehyde and trifluoroacetic acid (TFA, 5%, v/v) to afford the crystalline benzylidene acetal **6** in 84% yield. Treatment of **6** with benzoyl chloride in pyridine provided the crystalline benzoate **7** in 83% yield. Regioselective ring opening of the benzylidene acetal with borane–trimethylamine complex[21] and aluminium trichloride afforded the crystalline alcohol **8** in 64% yield. Jones oxidation (chromium trioxide in sulfuric acid) of **8** in acetone gave the intermediate acid, which was directly treated with methyl chloroformate, triethylamine, and 4-(dimethylamino)-pyridine (DMAP) in dichloromethane to afford the crystalline methyl uronate **9** in 68% overall yield. Introduction of the trichloroacetimidoyl group at C-1 was then achieved through oxidative removal of the 4-methoxyphenyl glycoside with cerium(IV) ammonium nitrate[22] (CAN), followed by imidoylation of the intermediate free hemiacetal with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give the crystalline α-imidate **10** in 71% overall yield, the anomeric configuration of which was deduced from its ^1H NMR spectrum ($J_{1,2}$ 3.7 Hz). In this sequence, most of the compounds were isolated by simple crystallization, thus avoiding tedious chromatographic separations, and allowing a multigram-scale preparation of the key intermediate **10**.

Preparation of the glycosyl acceptors

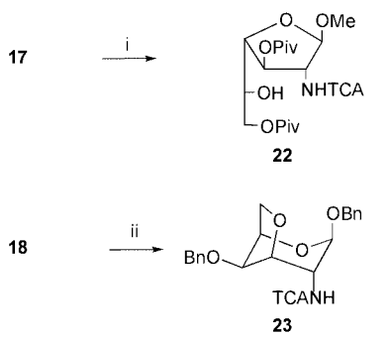
For the syntheses of the glycosyl acceptors **20** and **21**, the first requirement was the stereocontrolled and high yielding preparation of the benzyl (**12**) and methyl (**13**) β-D-glycosides. We previously demonstrated[16,23] that 2-deoxy-2-trichloroacetamido-D-glucopyranose derivatives activated at C-1 were very efficient glycosyl donors for the synthesis of 1,2-*trans*-2-amino-2-deoxy-D-glucosides, and that the *N*-trichloroacetyl group in the products could be easily transformed into *N*-acetyl under neutral conditions using tributylstannane (TBTH). In addition, the *N*-trichloroacetyl group provides a much better solubility in the usual organic solvents than does the *N*-acetyl group. Thus, treatment of easily available 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-1-*O*-trichloroacetimidoyl-α-D-glucopyranose[23] **11** with benzyl alcohol and methanol, in dichloromethane, in the presence of trimethylsilyl triflate (15% based on **11**), afforded readily the crystalline benzyl (**12**) and methyl (**13**) glycosides in 89 and 90% yield, respectively (Scheme 2). No



Scheme 2 *Reagents and conditions*: (i) $PhCH_2OH$, 4 Å mol. sieves, TMSOTf (0.15 mol equiv.), CH_2Cl_2, 1 h (for **11** ⟶ **12**); MeOH, 3 Å mol. sieves, TMSOTf (0.15 mol equiv.), CH_2Cl_2, 1 h (for **11** ⟶ **13**); (ii) MeONa, MeOH, 16 h; then $(CH_3)_3CCOCl$, pyridine, CH_2Cl_2, 0 °C, 1 h 30 min (for **12** ⟶ **14**, and **13** ⟶ **15**); (iii) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −15 °C ⟶ 0 °C, 2 h; then $NaNO_2$, DMF, 1 h 30 min (for **14** ⟶ **16**, and **15** ⟶ **17**); (iv) $PhCH_2Br$, $Bu_4N^+I^-$, NaH, THF, 0 °C ⟶ rt, 5 h (for **16** ⟶ **18**, and **17** ⟶ **19**); (v) MeONa, MeOH, 48 h; then TBDMSCl, imidazole, DMF, 0 °C, 40 min (for **18** ⟶ **20**, and **19** ⟶ **21**). TCA = trichloroacetyl; Piv = 2,2-dimethylpropionyl (pivaloyl).

corresponding α-isomers were obtained in these reactions, and the physical data for **13** are in agreement with those reported.[24] Transesterification of **12** and **13** with methanolic sodium methoxide gave the corresponding triols, which were treated with pivaloyl chloride (2 mol equiv.) and pyridine in dichloromethane at 0 °C to afford selectively the crystalline 3,6-di-*O*-pivaloyl derivatives **14** and **15** in 86 and 82% yield, respectively. Inversion of configuration at C-4 was then achieved by

treatment of **14** and **15** with trifluoromethanesulfonic anhydride and pyridine in dichloromethane at −15 °C, followed by nucleophilic displacement of the intermediate triflates with sodium nitrite[25] in *N*,*N*-dimethylformamide (DMF) to afford the crystalline, corresponding 3,6-di-*O*-pivaloyl-D-*galacto* derivatives **16** and **17** in 65 and 67% overall yield, respectively. The ^{1}H NMR spectra of **16** and **17** showed signals at δ 2.50 and 2.25, respectively, attributed, after exchange with D_2O, to 4-hydroxy groups. The *J*-values ($J_{3,4}$ 3.2, $J_{4,5}$ 0.8 Hz) observed for H-4 in both compounds fit quite well with those expected for a D-*galacto* structure. To follow the initial scheme, benzylation at O-4 was next studied. Since the alcohols **16** and **17** were substituted with ester groups, alkylation reactions under basic conditions had to be avoided to prevent acyl migration. Consequently, the alcohol **17** was treated with 4-methoxyphenyl trichloroacetimidate,[26] a reagent much more reactive than its corresponding benzyl analogue, in dichloromethane, in the presence of trifluoromethanesulfonic acid[27] (0.1 mol equiv.), but no 4-*O*-benzyl derivative could be isolated. The only detectable product was an isomer of the starting material, the structure of which was tentatively assigned, through ^{1}H NMR and mass analyses, to the furanoside **22** (Scheme 3). Such a



Scheme 3 *Reagents and conditions*: (i) 4-Methoxybenzyl trichloroacetimidate, CF_3SO_3H (0.1 mol equiv.), CH_2Cl_2, 0 °C ⟶ rt, 2 h; (ii) MeONa, MeOH, 48 h; then Ph_3P, DEAD, PhCOOH, THF, rt, 1 h, then 60 °C, 3 h.

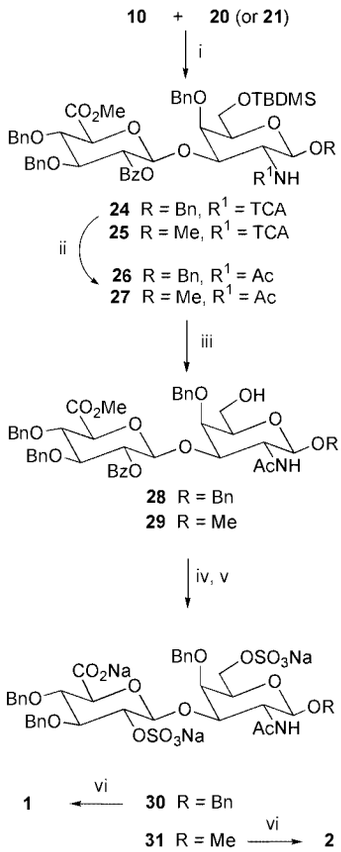
modification of structure should result from a ring-closure process involving *O*-4 participation in a transient open-chain methyloxonium ion, and was not unexpected in the D-*galacto* series. For instance, in the Fischer glycosylation reaction of D-galactose with methanol, up to 50% of methyl β-D-galactofuranoside can be isolated, and the presence of the axial 4-hydroxy group in the starting pyranose was postulated[28] to be the driving force of this glycosidic modification. In the present case, additional steric hindrance caused by the bulky pivaloyl groups at O-3 and O-6 should prevent alkylation from taking place at O-4, and favour the ring-contraction process. It is noteworthy that in the absence of alkylating reagent, or under the catalysis of trimethylsilyl triflate (details not presented in the Experimental section), similar ring contraction occurred. However, treatment of the alcohols **16** and **17** with benzyl bromide, tetrabutylammonium iodide, and sodium hydride in tetrahydrofuran (THF) afforded readily the 4-*O*-benzyl derivatives **18** and **19** in 81 and 80% yield, respectively. The ^{1}H NMR spectra for **18** and **19** are in complete agreement with the expected structures, and showed that no ester migration occurred under these basic conditions.

Transformation of the diesters **18** and **19** into the glycosyl acceptors **20** and **21** was then achieved as follows. Transesterification of compounds **18** and **19** with methanolic sodium methoxide afforded quantitatively the corresponding diol derivatives. Attempted selective 6-*O*-benzoylation of these 3,6-diols with benzoyl chloride in pyridine at low temperature, benzoyl cyanide[29] or benzoic anhydride in pyridine or acetonitrile, or *N*-benzoylimidazole in dichloromethane failed, and led to mixtures in which the 3,6-di-*O*-benzoyl derivatives were always preponderant. As far as esters can be obtained from non-hindered alcohols through the Mitsunobu reaction, the diol derived from **18** was treated with triphenylphosphine, diethyl azodicarboxylate (DEAD), and benzoic acid in THF, but no benzoylated derivative could be obtained. A major product was isolated from the mixture, and was identified through ^{1}H NMR and mass analyses as the 3,6-anhydro derivative **23** (Scheme 3). Such an intramolecular nucleophilic attack of O-3 upon C-6 to form a tetrahydrofuran ring has been reported[30] in the D-*galacto* series in the reaction with triphenylphosphine, and this occurred even when C-3 was substituted by a benzyloxy group.[31] Such a lack of selectivity in acylation reactions prompt us to modify our initial strategy, and to use another selective protection at O-6 which could be removed under non-basic conditions after coupling with the methyl uronate **10**. Thus, when the diols derived from **18** and **19** were treated with *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in DMF at 0 °C, the crystalline silyl ethers **20** and **21** were obtained in 88 and 85% yield, respectively.

Disaccharide syntheses

Condensation of the imidate **10** (1.25 mol equiv.) with the alcohols **20** and **21** (1 mol equiv.), in dichloromethane, at room temperature, and in the presence of TMSOTf (20% based on **10**), afforded smoothly the crystalline disaccharide derivatives **24** and **25** in 62 and 68% yield, respectively (Scheme 4). The anomeric configurations of the newly established interglycosidic linkages were deduced from their ^{1}H NMR spectra (δ 5.15 and 5.08, $J_{1',2'}$ 7.6 and 7.5 Hz, respectively). The *N*-trichloroacetyl groups in **24** and **25** were readily transformed into *N*-acetyl through treatment with tributylstannane[16,23] and azoisobutyronitrile (AIBN) in refluxing benzene to afford the crystalline acetamides **26** and **27** in 92 and 82% yield, respectively. We previously reported[16] that *O*-desilylation with tetrabutylammonium fluoride caused extensive β-elimination at the methyl uronate moiety in disaccharide structures, but treatment of compounds **26** and **27** with aq. acetic acid in THF afforded the crystalline alcohols **28** and **29** in 82 and 85% yield, respectively. Saponification of the ester groups in **28** and **29** was then achieved through treatment with lithium hydroperoxide[32] in THF at 0 °C, a reagent which readily saponifies the methyl ester groups without elimination side-reaction, followed by methanolic sodium hydroxide to give the corresponding hydroxy acids. These intermediates were directly *O*-sulfonated by treatment with the sulfur trioxide–trimethylamine complex in DMF at 50 °C, followed by ion-exchange chromatography (Na^+ resin) to provide the sodium salts **30** and **31** in 66 and 63% overall yield, respectively. While 6-*O*-sulfonation proceeded readily, 2′-*O*-sulfonation required a longer reaction time and a large excess of reagent to go to completion. Comparison of the ^{1}H NMR spectra, recorded in deuterated methanol, of **30** and **31** and of their non-sulfated hydroxy acid precursors, showed the expected[13] downfield shifts (≈0.5 ppm) of the signals for H^a-6 and H^b-6 in sulfates **30** and **31**, and downfield shifts (0.4 and 0.6 ppm, respectively) of the signals for H-2′ in **30** and **31**, in good agreement with those reported[33] for 2-*O*-sulfonated D-glucuronic acid residues in synthetic disaccharides. These chemical-shift differences indicated clearly that sulfation occurred at O-6 and O-2′. Final deprotection on **30** and **31** was then achieved by catalytic hydrogenation (Pd-C in methanol–water) to afford nearly quantitatively the target molecules **1** and **2**, respectively. The ^{1}H and ^{13}C NMR spectra of disaccharides **1** and **2** were in full agreement with the expected structures, and in accord with those reported[4,6] for oligosaccharide fragments isolated from shark cartilage after digestion with testicular hyaluronidase and containing non-modified CS-D disaccharide unit.

In conclusion, stereocontrolled and high yielding syntheses of the basic disaccharide unit of CS-D, **1**, and its methyl β-D-glycoside derivative **2** are reported for the first time, and the



Scheme 4 *Reagents and conditions*: (i) TMSOTf (0.2 mol equiv.), 4 Å mol. sieves, CH_2Cl_2, 30 min (for **10** + **20** ⟶ **20**, and **10** + **21** ⟶ **25**); (ii) TBTH, AIBN, benzene, 80 °C, 1 h (for **24** ⟶ **26**, and **25** ⟶ **27**); (iii) THF–acetic acid–water (3 : 3 : 1), rt, 72 h (for **26** ⟶ **28**); rt, 4 h; then 40 °C, 4 h (for **27** ⟶ **29**); (iv) LiOH-H_2O_2, THF, 0 °C ⟶ rt, 16 h; then 4 mol dm^{-3} NaOH, MeOH, rt, 6 h; (v) $Me_3N{\cdot}SO_3$, DMF, 50 °C, 72 h; then ion-exchange resin (Na^+) in CH_2Cl_2–MeOH–water (9 : 5 : 1) (for **28** ⟶ **30**, and **29** ⟶ **31**); (vi) H_2, 10% Pd on carbon, MeOH–water (for **30** ⟶ **1**, and **31** ⟶ **2**).

way is now open for the preparation of fragments of larger size. These sulfated molecules are currently being evaluated in biological assays, and the results of these studies will be reported elsewhere in due course.

Experimental

General

Mps were recorded with a Büchi apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter and $[a]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. NMR spectra were recorded at 300 K on a Bruker DPX 250 Avance (250 MHz) spectrometer with TMS as internal reference, unless otherwise stated, and *J*-values are quoted in Hz. Assignments were based on homo- and heteronuclear correlations using the supplier's software. Low-resolution mass spectra were recorded with a Perkin-Elmer SCIEX API 300 spectrometer in the ion-spray (IS) mode. TLC was performed on Merck 60 F_{254} precoated plates, and compounds were detected by spraying the plates with 5% H_2SO_4 in EtOH and heating. Flash silica chromatography was performed using Merck silica gel C60 (0.040–0.063 mm). Elemental analyses were carried out at the Service Central de Microanalyse du Centre National de la Recherche Scientifique (Vernaison, France). Light petroleum refers to the fraction with distillation range 40–60 °C.

4-Methoxyphenyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranoside 4

A mixture of 1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranose[20] **3** (10 g, 23 mmol), 4-methoxyphenol (5.67 g, 57 mmol), and 4 Å powdered molecular sieves (5 g) in dry dichloromethane (100 cm^3) was stirred for 30 min at rt under dry argon, then cooled to 0 °C. TMSOTf (0.63 cm^3, 3.45 mmol) was added, and the mixture was stirred for 2 h at 0 °C. Triethylamine (1.5 cm^3) was added, and the mixture was filtered, washed successively with water, saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was crystallized from ethyl acetate to give the *glycoside* **4** (7.94 g, 69%), mp 154 °C; $[a]_D^{22}$ −21 (*c* 1 in chloroform) (Found: C, 62.1; H, 5.9. $C_{26}H_{30}O_{10}$ requires C, 62.1; H, 6.0%); $\delta_H(CDCl_3)$ 2.08, 2.11, 2.15 (9 H, 3 s, 3 × $COCH_3$), 3.78 (1 H, m, H-5), 3.84 (1 H, t, $J_{2,3} = J_{3,4} = 9.3$, H-3), 3.85 (3 H, s, OCH_3), 4.23 (1 H, dd, $J_{5,6a}$ 2.7, $J_{6a,6b}$ 12.3, H^a-6), 4.32 (1 H, dd, $J_{5,6b}$ 5.5, $J_{6a,6b}$ 12.3, H^b-6), 4.65 (2 H, s, CH_2Ph), 4.94 (1 H, d, $J_{1,2}$ 7.9, H-1), 5.27 (1 H, dd, $J_{3,4}$ 9.3, $J_{4,5}$ 10.0, H-4), 5.37 (1 H, dd, $J_{1,2}$ 7.9, $J_{2,3}$ 9.3, H-2), and 6.80–7.50 (9 H, m, ArH); *m*/*z* 525 $[M + Na]^+$, 379 $[M - OC_6H_4OCH_3]^+$.

4-Methoxyphenyl 3-*O*-benzyl-β-D-glucopyranoside 5

Methanolic sodium methoxide (1 mol dm^{-3}; 1 cm^3) was added to a solution of the ester **4** (7 g, 14 mmol) in dry methanol (70 cm^3), and the mixture was stirred overnight at rt, then was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated under reduced pressure. The residue was crystallized from methanol to give the *triol* **5** (4.66 g, 89%), mp 136 °C; $[a]_D^{22}$ −23 (*c* 1 in methanol) (Found: C, 63.8; H, 6.4. $C_{20}H_{24}O_7$ requires C, 63.8; H, 6.4%); $\delta_H(CDCl_3)$ 2.06 (1 H, dd, *J* 6.5 and 6.7, HO-6), 2.44 (1 H, d, *J* 2.4, HO-4), 2.56 (1 H, d, *J* 2.6, HO-2), 3.50 (3 H, m, H-5, H_2-6), 3.74 (1 H, m, $J_{1,2}$ 7.9, $J_{2,3}$ 9.5, $J_{2,OH}$ 2.6, H-2), 3.86 (3 H, s, OCH_3), 3.95 (2 H, m, H-3, -4), 4.91 (1 H, d, $J_{1,2}$ 7.9, H-1), 5.0 (2 H, ABq, CH_2Ph), and 6.80–7.50 (9 H, m, ArH); *m*/*z* 399 $[M + Na]^+$, 253 $[M - OC_6H_4OCH_3]^+$.

4-Methoxyphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside 6

TFA (5%, v/v; 1.8 cm^3) was added at rt under dry argon to a suspension of the triol **5** (4.66 g, 12.4 mmol) in benzaldehyde (37 cm^3), and the mixture was stirred vigorously for 20 min. Triethylamine (5 cm^3), then cold diethyl ether (100 cm^3), were added, and the solids were filtered off and washed with cold diethyl ether to give the *benzylidene acetal* **6** (4.83 g, 84%), mp 201–202 °C (from ethanol); $[a]_D^{22}$ −26 (*c* 1 in chloroform) (Found: C, 69.7; H, 6.0. $C_{27}H_{28}O_7$ requires C, 69.8; H, 6.1%); $\delta_H(CDCl_3)$ 2.51 (1 H, d, *J* 2.4, HO-2), 3.53 (1 H, m, H-5), 3.70–3.88 (4 H, m, H-2, -3, -4, H^{ax}-6), 3.78 (3 H, s, OCH_3), 4.37 (1 H, dd, $J_{5,6eq}$ 4.9, $J_{6ax,6eq}$ 10.2, H^{eq}-6), 4.90 (1 H, d, $J_{1,2}$ 7.3, H-1), 4.91 (2 H, ABq, CH_2Ph), 5.60 (1 H, s, PhC*H*), and 6.80–7.50 (14 H, m, ArH); *m*/*z* 465 $[M + H]^+$, 341 $[M - OC_6H_4OCH_3]^+$.

4-Methoxyphenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside 7

Benzoyl chloride (3.6 cm^3, 31 mmol) was added dropwise at 0 °C to a solution of the alcohol **6** (4.83 g, 10.4 mmol) in dry pyridine (40 cm^3) and dry dichloromethane (20 cm^3), and the mixture was allowed to attain rt under stirring within 1 h 30 min. Methanol (10 cm^3) was then added, and the mixture was diluted with dichloromethane (100 cm^3), washed successively with water, saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was crystallized from ethyl acetate–light petroleum to give the *ester* **7** (4.9 g, 83%), mp 177 °C; $[a]_D^{22}$ +42 (*c* 1 in chloroform) (Found: C, 71.8; H, 5.4. $C_{34}H_{32}O_8$ requires C, 71.8; H, 5.7%); $\delta_H(CDCl_3)$ 3.61 (1 H, m, H-5), 3.73 (3 H, s, OCH_3), 3.86–3.97 (3 H, m, H-3, -4, H^{ax}-6), 4.43 (1 H, dd, $J_{5,6eq}$ 4.9, $J_{6ax,6eq}$ 10.4, H^{eq}-6), 4.79 (2 H, ABq, CH_2Ph), 5.07 (1 H, d, $J_{1,2}$ 7.8, H-1), 5.53 (1 H, dd, $J_{1,2}$ 7.8, $J_{2,3}$ 9.1, H-2), 5.64 (1 H, s, PhC*H*), and 6.70–8.10 (19 H, m, ArH); *m*/*z* 591 $[M + Na]^+$, 569 $[M + H]^+$.

4-Methoxyphenyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-glucopyranoside 8

A solution of aluminium trichloride (3.32 g, 24.8 mmol) in dry diethyl ether (30 cm^3) was added dropwise at 0 °C under dry argon to a mixture of the acetal **7** (3.54 g, 6.2 mmol), borane–trimethylamine complex (2.27 g, 31 mmol) and 4 Å powdered molecular sieves (5 g) in dry dichloromethane (60 cm^3). The mixture was stirred at 0 °C for 2 h, then was filtered through a pad of Celite, diluted with dichloromethane (100 cm^3), and stirred at rt for 1 h with aq. sulfuric acid (0.75 mol dm^{-3}; 10 cm^3). The organic layer was washed successively with saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated under reduced pressure. A solution of the residue in methanol (50 cm^3) and dichloromethane (5 cm^3) was stirred for 15 min at rt with Amberlite IR-120 resin (H^+, 20 cm^3), filtered, concentrated, and evaporated twice with methanol containing 1% of acetic acid, then with toluene. The solid residue was recrystallized from methanol to give the *alcohol* **8** (2.27 g, 64%), mp 114 °C; $[\alpha]_D^{22}$ +36 (*c* 1 in chloroform) (Found: C, 71.5; H, 6.2. $C_{34}H_{34}O_8$ requires C, 71.6; H, 6.0%); $\delta_H(CDCl_3)$ 1.92 (1 H, dd, *J* 6.2 and 7.9, HO-6), 3.58 (1 H, m, H-5), 3.73 (3 H, s, OCH_3), 3.78 (1 H, m, $J_{5,6a}$ 4.4, $J_{6a,6b}$ 12.2, $J_{6a,OH}$ 7.9, H^a-6), 3.90 (2 H, m, H-3, -4), 3.94 (1 H, m, $J_{5,6b}$ 2.7, $J_{6a,6b}$ 12.2, $J_{6b,OH}$ 6.2, H^b-6), 4.74, 4.82 (4 H, 2 ABq, 2 × CH_2Ph), 5.04 (1H, d, $J_{1,2}$ 7.9, H-1), 5.50 (1 H, dd, $J_{1,2}$ 7.9, $J_{2,3}$ 9.0, H-2), and 6.70–8.10 (19 H, m, ArH); *m*/*z* 607 $[M + Na]^+$, 461 $[M - OC_6H_4OCH_3]^+$.

Methyl (4-methoxyphenyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-glucopyranosid)uronate 9

Chromium trioxide in sulfuric acid (3.34 mol dm^{-3}; 8 cm^3) was added portionwise within 1 h at rt to a stirred solution of the alcohol **8** (2.33 g, 4 mmol) in acetone (58 cm^3). The mixture was poured into ice-cold water and extracted with ethyl acetate (3 × 50 cm^3). The combined extracts were washed with water, dried ($MgSO_4$), concentrated, and purified by flash silica chromatography [dichloromethane ⟶ dichloromethane–methanol (49 : 1)] to afford the corresponding acid (1.87 g, 79%) as a pale yellow syrup.

Methyl chloroformate (0.26 cm^3, 3.3 mmol) was added at 0 °C under dry argon to a solution of the above isolated acid and DMAP (38 mg, 0.3 mmol) in dry dichloromethane (50 cm^3) and triethylamine (0.5 cm^3), and the mixture was stirred for 1 h at 0 °C, then for 1 h at rt, after which it was washed successively with 5% aq. NH_4Cl and water, dried ($MgSO_4$), and concentrated under reduced pressure. Flash silica chromatography [ethyl acetate–light petroleum (4 : 1→2 : 1)] afforded the *methyl uronate* **9** (1.56 g, 68% from **8**), mp 97 °C (from ethyl acetate–light petroleum): $[\alpha]_D^{22}$ +21 (*c* 1 in chloroform) (Found: C, 70.0; H, 5.7. $C_{35}H_{34}O_9$ requires C, 70.2, H, 5.7%); $\delta_H(C_6D_6)$ 3.31, 3.39 (6 H, 2 s, CO_2CH_3, OCH_3), 3.95 (1 H, t, $J_{2,3} = J_{3,4} = 8.7$, H-3), 4.22 (1 H, d, $J_{4,5}$ 9.4, H-5), 4.31 (1 H, dd, $J_{3,4}$ 8.7, $J_{4,5}$ 9.4, H-4), 4.80, 4.82 (4 H, 2 ABq, 2 × CH_2Ph), 5.10 (1 H, d, $J_{1,2}$ 7.3, H-1), 6.09 (1 H, dd, $J_{1,2}$ 7.3, $J_{2,3}$ 8.7, H-2), and 6.70–8.30 (19 H, m, ArH); *m*/*z* 621 $[M + Na]^+$, 475 $[M - OC_6H_4OCH_3]^+$.

Methyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate 10

CAN (6.08 g, 11 mmol) was added to a solution of the glycoside **9** (1.33 g, 2.2 mmol) in toluene–acetonitrile–water (1 : 1.5 : 1; 52.5 cm^3), and the mixture was stirred for 20 min at rt, then poured into ice-cold water, and extracted with ethyl acetate (3 × 50 cm^3). The combined extracts were washed successively with saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated under reduced pressure. Flash silica chromatography [dichloromethane→dichloromethane–methanol (49 : 1)] afforded the corresponding hemiacetal (1 g, 90%) as a pale yellow solid.

A mixture of the above isolated hemiacetal, trichloroacetonitrile (2 cm^3, 20 mmol), and DBU (0.08 cm^3, 0.5 mmol) in dry dichloromethane (10 cm^3) was stirred for 10 min at 0 °C, then was directly purified by flash silica chromatography [light petroleum–ethyl acetate (4 : 1), containing 0.1% of triethylamine] to provide the *imidate* **10** (1 g, 71%), mp 102–104 °C (from diethyl ether–light petroleum); $[\alpha]_D^{22}$ +103 (*c* 1 in chloroform) (Found: C, 56.6; H, 4.4; N, 2.4. $C_{30}H_{28}Cl_3NO_8$ requires C, 56.6; H, 4.4; N, 2.2%); $\delta_H(CDCl_3)$ 3.72 (3 H, s, CO_2CH_3), 4.01 (1H, dd, $J_{3,4}$ 9.9, $J_{4,5}$ 10.1, H-4), 4.27 (1 H, t, $J_{2,3} = J_{3,4} = 9.9$, H-3), 4.48 (1 H, d, $J_{4,5}$ 10.1, H-5), 4.73, 4.81 (4 H, 2 ABq, 2 × CH_2Ph), 5.39 (1 H, dd, $J_{1,2}$ 3.7, $J_{2,3}$ 9.9, H-2), 6.64 (1 H, d, $J_{1,2}$ 3.7, H-1), 7.10–8.0 (15 H, m, Ph), and 8.57 (1 H, s, C=NH); δ_C(67.8 MHz; $CDCl_3$) 55.82 (CO_2CH_3), 72.12, 72.79 (C-3, -4), 75.69, 75.86 (CH_2Ph), 78.77, 79.15 (C-2, -5), 90.82 (CCl_3), 93.70 (C-1), 121.95–137.68 (aromatic C), and 160.45, 165.44, 168.80 (C=O, C=N); *m*/*z* 660 $[M + Na]^+$, 476 $[M - CCl_3CONH]^+$ for ^{35}Cl.

Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside 12

A mixture of 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-1-*O*-trichloroacetimidoyl-α-D-glucopyranose[23] **11** (2.85 g, 4.8 mmol), benzyl alcohol (1 cm^3, 9.6 mmol), and 4 Å powdered molecular sieves (2 g) in dry dichloromethane (30 cm^3) was stirred for 1 h at rt under dry argon. TMSOTf (0.14 cm^3, 0.7 mmol) was added, and the mixture was stirred for 1 h. Triethylamine (0.15 cm^3) was added, and the mixture was diluted with dichloromethane (50 cm^3), filtered through a pad of Celite, washed successively with saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was crystallized from ethyl acetate–light petroleum to give the *benzyl glycoside* **12** (2.37 g, 89%), mp 140–141 °C; $[\alpha]_D^{22}$ −41 (*c* 1 in chloroform) (Found: C, 46.7; H, 4.4; N, 2.6. $C_{21}H_{24}Cl_3NO_9$ requires C, 46.6; H, 4.5; N, 2.6%); $\delta_H(CDCl_3)$ 2.03, 2.04, 2.14 (9 H, 3 s, 3 × $COCH_3$), 3.69 (1 H, m, H-5), 4.06 (1 H, m, H-2), 4.20 (1 H, dd, $J_{5,6a}$ 2.5, $J_{6a,6b}$ 12.4, H^a-6), 4.32 (1 H, dd, $J_{5,6b}$ 4.7, $J_{6a,6b}$ 12.4, H^b-6), 4.65 (1 H, d, $J_{1,2}$ 8.4, H-1), 4.78 (2 H, ABq, CH_2Ph), 5.15 (1 H, t, $J_{3,4} = J_{4,5} = 9.5$, H-4), 5.27 (1 H, dd, $J_{2,3}$ 10.4, $J_{3,4}$ 9.5, H-3), 6.63 (1 H, d, $J_{2,NH}$ 8.2, NH), and 7.40 (5 H, m, Ph); *m*/*z* 559 $[M + NH_4]^+$, 434 $[M - OCH_2Ph]^+$ for ^{35}Cl.

Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside 13

A mixture of the imidate **11** (3.47 g, 5.83 mmol), dry methanol (1.15 cm^3, 29 mmol), and 3 Å powdered molecular sieves (4 g) in dry dichloromethane (35 cm^3) was treated as described for the preparation of **12**. The residue was crystallized from ethyl acetate–light petroleum to give the methyl glycoside **13** (2.44 g, 90%), mp 131–132 °C (lit.,[24] 131–133 °C); $[\alpha]_D^{22}$ −5 (*c* 1 in chloroform) [lit.,[24] −2.3 (*c* 2 in chloroform)]; $\delta_H(CDCl_3)$ 1.98, 2.08, 2.12 (9 H, 3 s, 3 × $COCH_3$), 3.50 (3 H, s, OCH_3), 3.74 (1 H, m, H-5), 3.98 (1 H, m, H-2), 4.16 (1 H, dd, $J_{5,6a}$ 3.0, $J_{6a,6b}$ 12.5, H^a-6), 4.29 (1 H, dd, $J_{5,6b}$ 5.0, $J_{6a,6b}$ 12.5, H^b-6), 4.61 (1 H, d, $J_{1,2}$ 8.0, H-1), 5.10 (1 H, t, $J_{3,4} = J_{4,5} = 9.0$, H-4), 5.36 (1 H, dd, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0, H-3), and 6.97 (1 H, d, $J_{2,NH}$ 9.0, NH); *m*/*z* 482 $[M + NH_4]^+$, 433 $[M - OCH_3]^+$ for ^{35}Cl.

Benzyl 2-deoxy-3,6-di-*O*-pivaloyl-2-trichloroacetamido-β-D-glucopyranoside 14

Methanolic sodium methoxide (1 mol dm^{-3}; 1 cm^3) was added to a solution of the ester **12** (3 g, 5.56 mmol) in dry methanol (30 cm^3), and the mixture was stirred overnight at rt, then was neutralized with Amberlite IR-120 (H^+) resin, filtered, concentrated, and dried over phosphorus pentaoxide under reduced pressure.

Pivaloyl chloride (2.7 cm^3, 22 mmol) was added at 0 °C to a solution of the above isolated triol in dry dichloromethane (20

cm^3) and dry pyridine (10 cm^3), and the mixture was stirred for 1 h 30 min at 0 °C. Methanol (3 cm^3) was then added, and the mixture was diluted with dichloromethane (50 cm^3), washed successively with water, saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was crystallized from ethyl acetate–light petroleum to give the *alcohol* **14** (2.79 g, 86%), mp 139–140 °C; $[\alpha]_D^{22}$ −52 (*c* 1 in chloroform) (Found: C, 51.6; H, 5.8; N, 2.7. $C_{25}H_{34}Cl_3NO_8$ requires C, 51.5; H, 5.9; N, 2.4%); $\delta_H(CDCl_3)$ 1.16, 1.28 [18 H, 2 s, 2 × $(CH_3)_3C$], 3.01 (1 H, br s, HO-4), 3.52 (2 H, m, H-4, -5), 4.04 (1 H, m, $J_{1,2}$ 8.2, $J_{2,3}$ 10.6, $J_{2,NH}$ 9.0, H-2), 4.35 (1 H, dd, $J_{5,6a}$ 5.0, $J_{6a,6b}$ 12.1, H^a-6), 4.46 (1 H, dd, $J_{5,6b}$ 2.0, $J_{6a,6b}$ 12.1, H^b-6), 4.62 (1 H, d, $J_{1,2}$ 8.2, H-1), 4.74 (2 H, ABq, CH_2Ph), 5.34 (1 H, dd, $J_{2,3}$ 10.6, $J_{3,4}$ 8.7, H-3), 6.80 (1 H, d, $J_{2,NH}$ 9.0, N*H*), and 7.50 (5 H, m, Ph); *m*/*z* 601 $[M + NH_4]^+$, 476 $[M - OCH_2Ph]^+$ for ^{35}Cl.

Methyl 2-deoxy-3,6-di-*O*-pivaloyl-2-trichloroacetamido-β-D-glucopyranoside 15

Compound **13** (4.64 g, 10 mmol) was treated as described for the preparation of **14** to give the *alcohol* **15** (4.16 g, 82%), mp 164–165 °C (from ethyl acetate–light petroleum); $[\alpha]_D^{22}$ −33 (*c* 1 in chloroform) (Found: C, 45.1; H, 6.0; N, 2.8. $C_{19}H_{30}Cl_3NO_8$ requires C, 45.0; H, 6.0; N, 2.8%); $\delta_H(CDCl_3)$ 1.19, 1.21 [18 H, 2 s, 2 × $(CH_3)_3C$], 3.21 (1 H, d, *J* 6.0, HO-4), 3.39 (3 H, s, OCH_3), 3.47 (1 H, m, H-5), 3.60 (1 H, m, $J_{3,4}$ 9.5, $J_{4,5}$ 10.0, $J_{4,OH}$ 6.0, H-4), 3.93 (1 H, m, $J_{1,2}$ 8.5, $J_{2,3}$ 10.0, $J_{2,NH}$ 9.0, H-2), 4.28 (1 H, dd, $J_{5,6a}$ 6.0, $J_{6a,6b}$ 12.5, H^a-6), 4.41 (1 H, dd, $J_{5,6b}$ 2.5, $J_{6a,6b}$ 12.5, H^b-6), 4.54 (1 H, d, $J_{1,2}$ 8.5, H-1), 5.52 (1H, dd, $J_{2,3}$ 10.0, $J_{3,4}$ 9.5, H-3), and 7.07 (1 H, d, $J_{2,NH}$ 9.0, NH); *m*/*z* 524 $[M + NH_4]^+$ for ^{35}Cl.

Benzyl 2-deoxy-3,6-di-*O*-pivaloyl-2-trichloroacetamido-β-D-galactopyranoside 16

Trifluoromethanesulfonic anhydride (3.3 cm^3, 15.4 mmol) was added dropwise at −15 °C under dry argon to a solution of the alcohol **14** (6.4 g, 11 mmol) in dry dichloromethane (60 cm^3) and dry pyridine (8 cm^3), and the mixture was allowed to warm up slowly to 0 °C within 2 h. Crushed ice was added, and the mixture was diluted with dichloromethane (30 cm^3), washed successively with ice-cold water, brine and water, dried ($MgSO_4$), and concentrated under reduced pressure to give the crude 4-*O*-triflyl intermediate (7.7 g, 98%) as a yellow foam, which was immediatly engaged in the next reaction.

A mixture of the above isolated crude triflate and dried sodium nitrite (3.7 g, 55 mmol) in dry DMF (70 cm^3) was stirred for 1 h 30 min at rt under dry argon, then was poured into ice-cold water, and extracted with ethyl acetate (4 × 80 cm^3). The combined extracts were washed successively with cold hydrochloric acid (1 mol dm^{-3}) and water, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was crystallized from ethyl acetate–light petroleum to afford the *alcohol* **16** (4.16 g, 65%), mp 169 °C; $[\alpha]_D^{22}$ +13 (*c* 1 in chloroform) (Found: C, 51.7; H, 5.8; N, 2.5. $C_{25}H_{34}Cl_3NO_8$ requires C, 51.5; H, 5.9; N, 2.4%); $\delta_H(CDCl_3)$ 1.22, 1.26 [18 H, 2 s, 2 × $(CH_3)_3C$], 2.50 (1 H, br s, HO-4), 3.75 (1 H, m, H-5), 3.95 (1 H, dd, $J_{3,4}$ 3.2, $J_{4,5}$ 0.8, H-4), 4.30 (3 H, m, H-2, H_2-6), 4.62 (1 H, d, $J_{1,2}$ 8.4, H-1), 4.74 (2 H, ABq, CH_2Ph), 5.10 (1 H, dd, $J_{2,3}$ 11.1, $J_{3,4}$ 3.2, H-3), 6.83 (1 H, d, $J_{2,NH}$ 8.9, NH), and 7.30 (5 H, m, Ph); *m*/*z* 601 $[M + NH_4]^+$, 474 $[M - OCH_2Ph]^+$ for ^{35}Cl.

Methyl 2-deoxy-3,6-di-*O*-pivaloyl-2-trichloroacetamido-β-D-galactopyranoside 17

Compound **15** (4.96 g, 9.8 mmol) was treated as described for the preparation of **16** to give the *alcohol* **17** (3.33 g, 67%), mp 177 °C (from ethyl acetate–light petroleum); $[\alpha]_D^{22}$ +19 (*c* 1 in chloroform) (Found: C, 45.2; H, 6.0; N, 3.0. $C_{19}H_{30}Cl_3NO_8$ requires C, 45.0; H, 6.0; N, 2.8%); $\delta_H(CDCl_3)$ 1.21, 1.27 [18 H, 2 s, 2 × $(CH_3)_3C$], 2.25 (1 H, br s, HO-4), 3.53 (3 H, s, OCH_3), 3.79 (1 H, m, H-5), 3.99 (1 H, m, $J_{3,4}$ 3.2, $J_{4,5}$ 0.8, H-4), 4.24 (1 H, m, $J_{1,2}$ 8.2, $J_{2,3}$ 11.2, $J_{2,NH}$ 7.9, H-2), 4.34 (2 H, m, H_2-6), 4.57 (1 H, d, $J_{1,2}$ 8.2, H-1), 5.20 (1 H, dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.2, H-3), and 6.83 (1 H, d, $J_{2,NH}$ 7.9, NH); *m*/*z* 525 $[M + NH_4]^+$, 474 $[M - OCH_3]^+$ for ^{35}Cl.

Benzyl 4-*O*-benzyl-2-deoxy-3,6-di-*O*-pivaloyl-2-trichloroacetamido-β-D-galactopyranoside 18

Sodium hydride (60% in mineral oil; 0.67 g, 17 mmol) was added portionwise at 0 °C under dry argon to a solution of the alcohol **16** (3.25 g, 5.6 mmol) in dry THF (33 cm^3), and the mixture was stirred at 0 °C for 30 min. Tetrabutylammonium iodide (0.41 g 1.1 mmol) and benzyl bromide (1.7 cm^3, 14 mmol) were then added, and the mixture was stirred for 1 h at 0 °C and 4 h at rt. Acetic acid (1 cm^3) was then added carefully, and the mixture was diluted with ethyl acetate (100 cm^3), washed successively with water, saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated under reduced pressure. Flash silica chromatography [toluene–ethyl acetate (9 : 1)] afforded the *benzyl ether* **18** (3.0 g, 81%) as a white solid, mp 150 °C (from ethyl acetate–light petroleum); $[\alpha]_D^{22}$ −54 (*c* 1 in chloroform) (Found: C, 57.2; H, 6.0, N, 2.0. $C_{32}H_{40}Cl_3NO_8$ requires C, 57.1; H, 6.0; N, 2.1%); $\delta_H(CDCl_3)$ 1.18, 1.20 [18 H, 2 s, 2 × $(CH_3)_3C$], 3.74 (1 H, m, H-5), 3.88 (1H, dd, $J_{3,4}$ 3.0, $J_{4,5}$ 0.9, H-4), 4.13 (1 H, dd, $J_{5,6a}$ 6.1, $J_{6a,6b}$ 11.2, H^a-6), 4.33 (1 H, dd, $J_{5,6b}$ 6.8, $J_{6a,6b}$ 11.2, H^b-6), 4.42 (1 H, m, $J_{1,2}$ 8.2, $J_{2,3}$ 11.2, $J_{2,NH}$ 8.7, H-2), 4.61 (1 H, d, $J_{1,2}$ 8.2, H-1), 4.73 (4 H, m, 2 × CH_2Ph), 5.20 (1 H, dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.0, H-3), 6.61 (1 H, d, $J_{2,NH}$ 8.7, NH), and 7.30 (10 H, m, Ph); *m*/*z* 691 $[M + NH_4]^+$, 566 $[M - OCH_2Ph]^+$ for ^{35}Cl.

Methyl 4-*O*-benzyl-2-deoxy-3,6-di-*O*-pivaloyl-2-trichloroacetamido-β-D-galactopyranoside 19

Compound **17** (4.1 g, 8.1 mmol) was treated as described for the preparation of **18**. Flash silica chromatography [light petroleum–ethyl acetate (5 : 1)] provided the *benzyl ether* **19** (3.88 g, 80%) as a white foam; $[\alpha]_D^{22}$ −4 (*c* 1 in chloroform) (Found: C, 52.3; H, 6.1; N, 2.5. $C_{26}H_{36}Cl_3NO_8$ requires C, 52.3; H, 6.1; N, 2.4%); $\delta_H(CDCl_3)$ 1.19, 1.22 [18 H, 2 s, 2 × $(CH_3)_3C$], 3.49 (3 H, s, OCH_3), 3.78 (1 H, m, H-5), 3.87 (1 H, dd, $J_{3,4}$ 3.0, $J_{4,5}$ 0.9, H-4), 4.11 (1 H, dd, $J_{5,6a}$ 6.2, $J_{6a,6b}$ 11.0, H^a-6), 4.30 (2 H, m, H-2, H^a-6), 4.57 (1 H, d, $J_{1,2}$ 8.4, H-1), 4.69 (2 H, ABq, CH_2Ph), 5.29 (1 H, dd, $J_{2,3}$ 11.1, $J_{3,4}$ 3.0, H-3), 6.70 (1 H, d, $J_{2,NH}$ 8.8, NH), and 7.40 (5 H, m, Ph); *m*/*z* 615 $[M + NH_4]^+$, 566 $[M - OCH_3]^+$ for ^{35}Cl.

Benzyl 4-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside 20

Methanolic sodium methoxide (1 mol dm^{-3}; 5 cm^3) was added to a solution of the diester **18** (3 g, 4.5 mmol) in dry methanol, and the mixture was stirred for 48 h, then was neutralized with Amberlite IR-120 (H^+) resin, filtered, concentrated and dried over phosphorus pentaoxide under reduced pressure.

A mixture of the residue, imidazole (1.1 g, 16.2 mmol) and TBDMSCl (1.85 g, 8.1 mmol) in dry DMF (25 cm^3) was stirred for 40 min at 0 °C, then was diluted with ethyl acetate (100 cm^3), washed successively with saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated under reduced pressure. Flash silica chromatography [toluene–ethyl acetate (7 : 1)] afforded the *alcohol* **20** (2.45 g, 88%) as a white solid, mp 157–158 °C (from ethyl acetate–light petroleum); $[\alpha]_D^{22}$ −16 (*c* 1 in chloroform) (Found: C, 54.3; H, 6.2; N, 2.5. $C_{28}H_{38}Cl_3NO_6Si$ requires C, 54.3; H, 6.2; N, 2.3%); $\delta_H(CDCl_3)$ 0.13 [6 H, s, $Si(CH_3)_2$], 0.95 [9 H, s, $(CH_3)_3CSi$], 2.56 (1 H, d, *J* 8.6, HO-3), 3.51 (1 H, m, H-5), 3.82 (2 H, m, H_2-6), 3.86 (1 H, m, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0, $J_{2,NH}$ 7.0, H-2), 3.92 (2 H, m, H-3, -4), 4.62 (1 H, d, $J_{1,2}$ 8.0, H-1), 4.70, 4.75 (4 H, 2 ABq, 2 × CH_2Ph), 6.73 (1 H, d,

$J_{2,NH}$ 7.0, NH), and 7.30 (10 H, m, Ph); *m*/*z* 636 $[M + NH_4]^+$, 512 $[M - OCH_2Ph]^+$ for ^{35}Cl.

Methyl 4-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside 21

The diester **19** (3.88 g, 6.5 mmol) was treated as described for the preparation of **20**. Flash silica chromatography [toluene–ethyl acetate (6:1)] provided the *alcohol* **21** (3 g, 85%) as a white solid, mp 158–159 °C (from diethyl ether–light petroleum); $[\alpha]_D^{22}$ +13 (*c* 1 in chloroform) (Found: C, 48.7; H, 6.3; N, 2.8. $C_{22}H_{34}Cl_3NO_6Si$ requires C, 48.7; H, 6.3; N, 2.6%); $\delta_H(CDCl_3)$ 0.11 [6 H, s, $Si(CH_3)_2$], 0.98 [9 H, s, $(CH_3)_3CSi$], 2.50 (1 H, br s, HO-3), 3.51 (3 H, s, OCH_3), 3.56 (1 H, m, H-5), 3.71 (1 H, m, $J_{1,2}$ 8.2, $J_{2,3}$ 10.6, $J_{2,NH}$ 7.0, H-2), 3.82 (2 H, m, H_2-6), 3.97 (1 H, dd, $J_{3,4}$ 3.4, $J_{4,5}$ 1.0, H-4), 4.05 (1 H, m, $J_{2,3}$ 10.6, $J_{3,4}$ 3.4, H-3), 4.58 (1 H, d, $J_{1,2}$ 8.2, H-1), 4.80 (2 H, ABq, CH_2Ph), 6.82 (1 H, d, $J_{2,NH}$ 7.0, NH), and 7.35 (5 H, m, Ph); *m*/*z* 561 $[M + NH_4]^+$, 512 $[M - OCH_3]^+$ for ^{35}Cl.

Methyl 2-deoxy-3,6-di-*O*-pivaloyl-2-trichloroacetamido-β-D-galactofuranoside 22

Triflic acid (0.1 mol dm^{-3}; 0.4 cm^3) was added at 0 °C to a solution of the alcohol **17** (0.2 g, 0.4 mmol) and 4-methoxybenzyl trichloroacetimidate (0.34 g, 1.2 mmol) in dry dichloromethane (10 cm^3), and the mixture was allowed to warm slowly to rt within 2 h. Triethylamine (0.15 cm^3) was added, and the mixture was concentrated under reduced pressure. Flash silica chromatography [toluene–ethyl acetate (6:1)] gave the furanoside **22** (64 mg, 32%) as a foam; $\delta_H(CDCl_3)$ 1.22, 1.24 [18 H, 2 s, 2 × $(CH_3)_3C$], 3.41 (3 H, s, OCH_3), 3.46 (1 H, br s, HO-5), 4.07 (1 H, m, $J_{4,5} = J_{5,6a} = 1.2$, $J_{5,6b}$ 2.5, H-5), 4.33 (1 H, dd, $J_{2,3}$ 2.4, $J_{2,NH}$ 8.0, H-2), 4.36 (2 H, m, H-4, H^a-6), 4.48 (1 H, dd, $J_{5,6b}$ 1.2, $J_{6a,6b}$ 8.4, H^b-6), 4.94 (1 H, s, H-1), 5.03 (1 H, dd, $J_{2,3}$ 2.4, $J_{3,4}$ 0.7, H-3), and 7.25 (1 H, d, $J_{2,NH}$ 8.7, NH); *m*/*z* 525 $[M + NH_4]^+$, 474 $[M - OCH_3]^+$ for ^{35}Cl.

Benzyl 3,6-anhydro-4-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside 23

DEAD (0.02 cm^3, 0.12 mmol) was added to a solution of the diol derived from **18** (51 mg, 0.1 mmol), together with triphenylphosphine (30 mg, 0.12 mmol) and benzoic acid (15 mg, 0.12 mmol) in dry THF (2 cm^3), and the mixture was stirred for 1 h at rt and for 3 h at 60 °C, then cooled, and concentrated under reduced pressure. Flash silica chromatography [toluene–ethyl acetate (6:1)] afforded the anhydro sugar **23** (29 mg, 60%) as a white powder; $\delta_H(CDCl_3)$ 3.93 (1 H, d, $J_{4,5}$ 1.8, H-4), 4.09 (1 H, dd, $J_{5,6a}$ 3.0, $J_{6a,6b}$ 9.7, H^a-6), 4.45 (1 H, d, $J_{6a,6b}$ 9.7, H^b-6), 4.50 (2 H, m, H-2, -5), 4.67 (1 H, br s, H-3), 4.68 (2 H, ABq, CH_2Ph), 4.73 (1 H, br s, H-1), 4.78 (2 H, ABq, CH_2Ph), 6.51 (1 H, d, $J_{2,NH}$ 8.2, NH), and 7.30 (10 H, m, Ph); *m*/*z* 504 $[M + NH_4]^+$, 487 $[M + H]^+$, 379 $[M - OCH_2Ph]^+$ for ^{35}Cl.

Benzyl *O*-(methyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-gluco-pyranosyluronate)-(1→3)-4-*O*-benzyl-6-*O*-(*tert*-butyldimethyl-silyl)-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside 24

A mixture of the donor **10** (0.64 g, 1 mmol), the acceptor **20** (0.5 g, 0.8 mmol) and 4 Å powdered molecular sieves (0.5 g) in dry dichloromethane (11 cm^3) was stirred for 1 h at rt under dry argon. A solution of TMSOTf in dry toluene (1 mol dm^{-3}; 0.2 cm^3) was added, and the mixture was stirred for 30 min. Triethylamine (0.15 cm^3) was added, and the mixture was filtered and concentrated under reduced pressure. Flash silica chromatography [toluene–ethyl acetate (15:1), containing 0.1% of triethylamine] afforded the *disaccharide* **24** (0.5 g, 62%), mp 161–162 °C (from ethanol); $[\alpha]_D^{22}$ −20 (*c* 1 in chloroform) (Found: C, 61.5; H, 5.9; N, 1.6. $C_{56}H_{64}Cl_3NO_{13}Si$ requires C, 61.5; H, 5.9; N, 1.3%); $\delta_H(C_6D_6)$ 0.22 [6 H, s, $(CH_3)_2Si$], 1.20 [9 H, s, $(CH_3)_3CSi$], 3.53 (3 H, s, CO_2CH_3), 3.56 (1 H, m, H-5), 3.82 (1 H, m, $J_{1,2}$ 8.4, $J_{2,3}$ 11.1, $J_{2,NH}$ 6.6, H-2), 3.86 (1 H, dd, $J_{3',4'}$ 8.5, $J_{4',5'}$ 9.5, H-4′), 4.02 (2 H, m, H_2-6), 4.15 (1 H, t, $J_{2',3'} = J_{3',4'} = 8.5$, H-3′), 4.22 (1 H, d, $J_{4',5'}$ 9.5, H-5′), 4.47 (1 H, dd, $J_{3,4}$ 3.0, $J_{4,5}$ 0.8, H-4), 4.75, 4.80, 4.89 (6 H, 3 ABq, 3 × CH_2Ph), 5.02 (1 H, dd, $J_{2,3}$ 11.1, $J_{3,4}$ 3.0, H-3), 5.15 (1 H, d, $J_{1',2'}$ 7.6, H-1′), 5.16 (1 H, d, $J_{1,2}$ 8.4, H-1), 5.30 (2 H, ABq, CH_2Ph), 5.96 (1 H, dd, $J_{1',2'}$ 7.6, $J_{2',3'}$ 8.5, H-2′), 6.48 (1 H, d, $J_{2,NH}$ 6.6, NH), and 7.10–8.40 (25 H, m, Ph); *m*/*z* 1112 $[M + NH_4]^+$ for ^{35}Cl.

Methyl *O*-(methyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-gluco-pyranosyluronate)-(1→3)-4-*O*-benzyl-6-*O*-(*tert*-butyldimethyl-silyl)-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside 25

Coupling of the donor **10** (0.82 g, 1.29 mmol) and the acceptor **21** (0.5 g, 0.92 mmol) as described for the preparation of **24** afforded the *disaccharide* **25** (0.64 g, 68%), mp 165–166 °C (from ethanol); $[\alpha]_D^{22}$ +0.5 (*c* 1 in chloroform) (Found: C, 58.9; H, 6.0; N, 1.4. $C_{50}H_{60}Cl_3NO_{13}Si$ requires C, 59.0; H, 5.9; N, 1.4%); $\delta_H(C_6D_6)$ 0.21 [6 H, s, $(CH_3)_2Si$], 1.26 [9 H, s, $(CH_3)_3CSi$], 3.31 (3 H, s, OCH_3), 3.46 (3 H, s, CO_2CH_3), 3.51 (1 H, m, H-5), 3.70 (1 H, m, H-2), 3.78 (1 H, dd, $J_{3',4'}$ 8.5, $J_{4',5'}$ 10.0, H-4′), 3.90 (2 H, m, H_2-6), 4.08 (1 H, t, $J_{2',3'} = J_{3',4'} = 8.5$, H-3′), 4.14 (1 H, d, $J_{4',5'}$ 10.0, H-5′), 4.41 (1 H, dd, $J_{3,4}$ 3.0, $J_{4,5}$ 0.8, H-4), 4.65, 4.71 (4 H, 2 ABq, 2 × CH_2Ph), 4.88 (1 H, d, $J_{1,2}$ 8.5, H-1), 4.98 (1 H, dd, $J_{2,3}$ 11.0, $J_{3,4}$ 3.0, H-3), 5.08 (1 H, d, $J_{1',2'}$ 7.5, H-1′), 5.30 (2 H, ABq, CH_2Ph), 5.88 (1 H, dd, $J_{1',2'}$ 7.5, $J_{2',3'}$ 8.5, H-2′), 6.42 (1 H, d, $J_{2,NH}$ 6.5, NH), and 7.10–8.40 (20 H, m, Ph); *m*/*z* 1035 $[M + NH_4]^+$ for ^{35}Cl.

Benzyl *O*-(methyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-gluco-pyranosyluronate)-(1→3)-2-acetamido-4-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-β-D-galactopyranoside 26

A mixture of the trichloroacetamide **24** (1.28 g, 1.17 mmol), tributylstannane (1.7 cm^3, 7 mmol) and AIBN (10 mg) in dry benzene (25 cm^3) was stirred for 30 min at rt under a flow of dry argon, then heated for 1 h at 80 °C, cooled, and concentrated under reduced pressure. The solid residue was stirred with light petroleum (30 cm^3) for 1 h at rt, filtered off, washed with light petroleum, and recrystallized from ethanol to provide the *acetamide* **26** (1.06, 92%), mp 175–176 °C; $[\alpha]_D^{22}$ −21 (*c* 1 in chloroform) (Found: C, 67.8; H, 6.9; N, 1.3. $C_{56}H_{67}NO_{13}Si$ requires C, 67.9; H, 6.8; N, 1.4%); $\delta_H(CDCl_3)$ 0.10 [6 H, s, $(CH_3)_2Si$], 0.85 [9 H, s, $(CH_3)_3CSi$], 1.35 (3 H, s, $COCH_3$), 3.22 (1 H, m, $J_{1,2}$ 8.2, $J_{2,3}$ 11.0, $J_{2,NH}$ 6.6, H-2), 3.53 (1 H, m, $J_{4,5}$ 0.8, $J_{5,6a}$ 6.0, $J_{5,6b}$ 6.2, H-5), 3.59 (1 H, dd, $J_{3',4'}$ 9.0, $J_{4',5'}$ 9.5, H-4′), 3.72 (3 H, s, CO_2CH_3), 3.84 (1 H, t, $J_{2',3'} = J_{3',4'} = 9.0$, H-3′), 4.0 (4 H, m, H-4, 5′, H_2-6), 4.60, 4.70 (4 H, 2 ABq, 2 × CH_2Ph), 4.74 (1 H, d, $J_{1,2}$ 8.2, H-1), 4.76 (5 H, m, H-3, 2 × CH_2Ph), 4.99 (1 H, d, $J_{1',2'}$ 7.5, H-1′), 5.25 (1 H, d, $J_{2,NH}$ 6.6, NH), 5.32 (1 H, dd, $J_{1',2'}$ 7.5, $J_{2',3'}$ 9.0, H-2′), and 7.0–8.0 (25 H, m, Ph); *m*/*z* 991 $[M + H]^+$, 882 $[M - OCH_2Ph]^+$.

Methyl *O*-(methyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-gluco-pyranosyluronate)-(1→3)-2-acetamido-4-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-β-D-galactopyranoside 27

The trichloroacetamide **25** (1.02 g, 1 mmol) was treated as described for the preparation of **26**, then the product was recrystallized from ethanol to afford the *acetamide* **27** (0.75 g, 82%), mp 85–86 °C; $[\alpha]_D^{22}$ −0.5 (*c* 1 in chloroform) (Found: C, 65.6; H, 6.9; N, 1.7. $C_{50}H_{63}NO_{13}Si$ requires C, 65.7; H, 7.0; N, 1.6%); $\delta_H(CDCl_3)$ 0.10 [6 H, s, $(CH_3)_2Si$], 0.80 [9 H, s, $(CH_3)_3CSi$], 1.40 (3 H, s, $COCH_3$), 3.12 (1 H, m, $J_{1,2}$ 8.5, $J_{2,3}$ 11.0, $J_{2,NH}$ 6.6, H-2), 3.36 (3 H, s, OCH_3), 3.46 (1 H, m, $J_{4,5}$ 1.0, $J_{5,6a}$ 6.5, $J_{5,6b}$ 6.6, H-5), 3.55 (1 H, t, $J_{3',4'} = J_{4',5'} = 9.5$, H-4′), 3.69 (3 H, s, CO_2CH_3), 3.82 (1H, t, $J_{2,3} = J_{3,4} = 9.5$, H-3′), 3.98 (4 H, m, H-4,- 5′, H_2-6), 4.67 (5 H, m, H-3, 2 × CH_2Ph), 4.71 (1 H, d, $J_{1',2'}$ 7.5, H-1′), 4.73 (2 H, ABq, CH_2Ph), 4.81 (1 H, d, $J_{1,2}$ 8.5, H-1), 5.24 (1 H, d, $J_{2,NH}$ 6.6, NH), 5.30 (1 H, dd, $J_{1',2'}$ 7.5, $J_{2',3'}$

9.5, H-2′), and 7.0–8.0 (20 H, m, Ph); *m/z* 937 $[M + Na]^+$, 915 $[M + H]^+$, 883 $[M - OCH_3]^+$.

Benzyl *O*-(methyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-gluco-pyranosyluronate)-(1→3)-2-acetamido-4-*O*-benzyl-2-deoxy-β-D-galactopyranoside 28

A solution of the silyl ether **26** (0.99 g, 1 mmol) in THF–acetic acid–water (3:3:1; 7 cm^3) was stirred at rt for 72 h, then was concentrated under reduced pressure, and evaporated with water (3 × 10 cm^3). The residue was crystallized from ethanol to afford the *alcohol* **28** (0.72 g, 82%), mp 208–209 °C; $[\alpha]_D^{22}$ −28 (*c* 1 in chloroform) (Found: C, 68.8; H, 6.0; N, 1.6. $C_{50}H_{53}NO_{13}$ requires C, 68.6; H, 6.1; N, 1.6%); $\delta_H(CDCl_3)$ 1.40 (3 H, s, $COCH_3$), 2.01 (1 H, br s, HO-6), 3.24 (1 H, m, $J_{1,2}$ 8.4, $J_{2,3}$ 11.0, $J_{2,NH}$ 6.6, H-2), 3.42 (2 H, m, H-5, H^a-6), 3.66 (1 H, m, H^b-6), 3.76 (3 H, s, CO_2CH_3), 3.90 (2 H, m, H-4, -4′), 4.03 (2 H, m, H-3′, -5′), 4.75 (9 H, m, H-3, 4 × CH_2Ph), 4.79 (1 H, d, $J_{1',2'}$ 7.7, H-1′), 5.02 (1 H, d, $J_{1,2}$ 8.4, H-1), 5.25 (1 H, d, $J_{2,NH}$ 6.6, NH), 5.35 (1 H, dd, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.5, H-2′), and 7.0–8.0 (25 H, m, Ph); *m/z* 894 $[M + NH_4]^+$, 877 $[M + H]^+$, 769 $[M - OCH_2Ph]^+$.

Methyl *O*-(methyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-gluco-pyranosyluronate)-(1→3)-2-acetamido-4-*O*-benzyl-2-deoxy-β-D-galactopyranoside 29

A solution of the silyl ether **27** (0.6 g, 0.66 mmol) in THF–acetic acid–water (3:3:1; 7 cm^3) was stirred for 4 h at rt, then was heated at 40 °C for 4 h, cooled, concentrated under reduced pressure, and evaporated with water (3 × 10 cm^3). The residue was crystallized from ethanol to give the *alcohol* **29** (0.43 g, 85%), mp 185–186 °C; $[\alpha]_D^{22}$ −8 (*c* 1 in chloroform) (Found: C, 66.0; H, 6.3; N, 1.7. $C_{44}H_{49}NO_{13}$ requires C, 66.1; H, 6.2; N, 1.7%); $\delta_H(CDCl_3)$ 1.50 (3 H, s, $COCH_3$), 2.05 (1 H, br s, HO-6), 3.17 (1 H, m, $J_{1,2}$ 8.2, $J_{2,3}$ 11.0, $J_{2,NH}$ 7.0, H-2), 3.41 (3 H, s, OCH_3), 3.58 (2 H, m, H-5, H^a-6), 3.76 (3 H, s, CO_2CH_3), 3.90 (2 H, m, H-4, H^b-6), 4.03 (1 H, dd, $J_{2',3'} = J_{3',4'} = 9.5$, H-3′), 4.05 (2 H, m, H-4′, -5′), 4.71 (1 H, dd, $J_{2,3}$ 11.0, $J_{3,4}$ 3.2, H-3), 4.76 (1 H, d, $J_{1',2'}$ 7.7, H-1′), 4.80 (6 H, m, 3 × CH_2Ph), 4.86 (1 H, d, $J_{1,2}$ 8.2, H-1), 5.29 (1 H, d, $J_{2,NH}$ 7.0, NH), 5.37 (1 H, dd, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.5, H-2′), and 7.0–8.0 (20 H, m, Ph); *m/z* 818 $[M + NH_4]^+$, 801 $[M + H]^+$, 769 $[M - OCH_3]^+$.

Sodium benzyl *O*-(disodium 3,4-di-*O*-benzyl-2-*O*-sulfonato-β-D-glucopyranosyluronate)-(1→3)-2-acetamido-4-*O*-benzyl-2-deoxy-6-*O*-sulfonato-β-D-galactopyranoside 30

A solution of the ester **28** (0.62 g, 0.71 mmol) in THF (16 cm^3) was treated at 0 °C with hydrogen peroxide (30 wt% solution in water; 1.8 cm^3) and lithium hydroxide (1 mol dm^{-3}; 3.6 cm^3), and the mixture was stirred at 0 °C for 1 h and at rt for 16 h, then was cooled to 0 °C. Methanol (10 cm^3) and sodium hydroxide (4 mol dm^{-3}; 4 cm^3) were then added, and the mixture was stirred at rt for 6 h, then was treated with Amberlite IR-120 (H^+) resin to pH 3 (pH meter control), filtered, and concentrated under reduced pressure. Flash silica chromatography [dichloromethane–methanol (5:1)] afforded the corresponding acid (484 mg, 90%) as a white powder; $\delta_H(CD_3OD)$ 1.95 (3 H, s, $COCH_3$), 3.60 (1 H, dd, $J_{5,6a}$ 5.0, $J_{6a,6b}$ 10.5, H^a-6), 3.68 (1 H, dd, $J_{1',2'}$ 7.5, $J_{2',3'}$ 9.5, H-2′), 3.70 (2 H, m, H-3′, -5), 3.86 (1 H, dd, $J_{5,6b}$ 6.4, $J_{6a,6b}$ 10.5, H^b-6), 3.98 (1 H, t, $J_{3',4'} = J_{4',5'} = 9.5$, H-4′), 4.03 (1 H, d, $J_{4',5'}$ 9.5, H-5′), 4.04 (1 H, dd, $J_{2,3}$ 10.3, $J_{3,4}$ 3.0, H-3), 4.18 (1 H, dd, $J_{3,4}$ 3.0, $J_{4,5}$ 0.8, H-4), 4.37 (1 H, dd, $J_{1,2}$ 8.4, $J_{2,3}$ 10.3, H-2), 4.62 (1 H, d, $J_{1',2'}$ 7.5, H-1′), 4.67 (1 H, d, $J_{1,2}$ 8.4, H-1), 4.75–5.20 (8 H, m, 4 × CH_2Ph), and 7.30 (20 H, m, Ph).

A solution of the above isolated acid and the sulfur trioxide–trimethylamine complex (1.06 g, 8 mmol) in dry DMF (10 cm^3) was stirred at 50 °C for 24 h. More reagent (0.7 g, 5.3 mmol) was then added, and the mixture was stirred at 50 °C for a further 48 h, then cooled. Methanol (1 cm^3) was added, and the mixture was concentrated under reduced pressure. Flash silica chromatography [ethyl acetate–methanol–water (12:2:1)] gave a fraction that was eluted from a column (1.5 × 20 cm) of Sephadex SP-C25 (Na^+) with dichloromethane–methanol–water (9:5:1) to afford the *sodium salt* **30** (0.49 g, 66% from **28**) as a white powder; $[\alpha]_D^{22}$ −20 (*c* 1 in methanol) (Found: C, 49.4; H, 4.9; N, 1.3. $C_{42}H_{44}NNa_3O_{18}S_2 \cdot 2H_2O$ requires C, 49.5; H, 4.7; N, 1.4%); $\delta_H(CD_3OD)$ 2.0 (3 H, s, $COCH_3$), 3.85 (2 H, m, H-3′, -5), 4.0 (1 H, dd, $J_{3',4'}$ 9.0, $J_{4',5'}$ 9.5, H-4′), 4.02 (1 H, dd, $J_{2,3}$ 11.0, $J_{3,4}$ 3.0, H-3), 4.03 (1 H, dd, $J_{1,2}$ 8.1, $J_{2,3}$ 11.0, H-2), 4.12 (3 H, m, H-2′, H_2-6), 4.15 (1 H, d, $J_{4',5'}$ 9.5, H-5′), 4.17 (1 H, dd, $J_{3,4}$ 3.0, $J_{4,5}$ 0.8, H-4), 4.61 (1 H, d, $J_{1',2'}$ 7.8, H-1′), 4.77 (1 H, d, $J_{1,2}$ 8.1, H-1), 4.85 (8 H, m, 4 × CH_2Ph), and 7.30 (20 H, m, Ph).

Sodium methyl *O*-(disodium 3,4-di-*O*-benzyl-2-*O*-sulfonato-β-D-glucopyranosyluronate)-(1→3)-2-acetamido-4-*O*-benzyl-2-deoxy-6-*O*-sulfonato-β-D-galactopyranoside 31

The ester **29** (0.55 g, 0.69 mmol) was treated as described for the preparation of **30**. Flash silica chromatography [dichloromethane–methanol (4:1→3:1)] afforded the corresponding acid (425 mg, 90%) as a white solid; $\delta_H(CD_3OD)$ 2.0 (3 H, s, $COCH_3$), 3.46 (3 H, s, OCH_3), 3.48 (1 H, dd, $J_{1',2'}$ 7.4, $J_{2',3'}$ 9.5, H-2′), 3.55 (2 H, m, H-3′, H^a-6), 3.66 (1 H, dd, $J_{5,6b}$ 5.0, $J_{6a,6b}$ 10.5, H^b-6), 3.70 (1 H, m, H-5), 3.78 (2 H, m, H-4′, -5′), 3.86 (1 H, dd, $J_{2,3}$ 11.0, $J_{3,4}$ 3.0, H-3), 4.01 (1 H, dd, $J_{3,4}$ 3.0, $J_{4,5}$ 0.8, H-4), 4.13 (1 H, dd, $J_{1,2}$ 8.2, $J_{2,3}$ 11.0, H-2), 4.36 (1 H, d, $J_{1,2}$ 8.2, H-1), 4.46 (1 H, d, $J_{1',2'}$ 7.4, H-1′), 4.80 (6 H, m, 3 × CH_2Ph), and 7.30 (15 H, m, Ph).

The above isolated acid was *O*-sulfonated as described for the preparation of **30**. Flash silica chromatography [ethyl acetate–methanol–water (6:2:1→5:2:1)] followed by ion-exchange chromatography as described above afforded the *sodium salt* **31** (0.42 g, 63% from **29**) as a white powder; $[\alpha]_D^{22}$ −7 (*c* 1 in methanol) (Found: C, 45.6; H, 4.8; N, 1.5. $C_{36}H_{40}NNa_3O_{18}S_2 \cdot 2H_2O$ requires C, 45.8; H, 4.7; N, 1.5%); $\delta_H(CD_3OD)$ 2.08 (3 H, s, $COCH_3$), 3.44 (3 H, s, OCH_3), 3.83 (1 H, m, $J_{4,5}$ 0.8, $J_{5,6a}$ 5.0, $J_{5,6b}$ 6.0, H-5), 3.93 (2 H, m, H-3′, -4′), 3.94 (1 H, dd, $J_{1,2}$, 8.1, $J_{2,3}$ 11.0, H-2), 4.05 (1 H, dd, $J_{2,3}$ 11.0, $J_{3,4}$ 3.0, H-3), 4.09 (3 H, m, H-2′, H_2-6), 4.15 (1 H, d, $J_{4',5'}$ 9.5, H-5′), 4.17 (1 H, dd, $J_{3,4}$ 3.0, $J_{4,5}$ 0.8, H-4), 4.57 (1 H, d, $J_{1,2}$ 8.1, H-1), 4.61 (1 H, d, $J_{1',2'}$ 7.5, H-1′), 4.80 (6 H, m, 3 × CH_2Ph), and 7.30 (15 H, m, Ph).

Sodium *O*-(disodium 2-*O*-sulfonato-β-D-glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-6-*O*-sulfonato-D-galactopyranose 1

A solution of the benzyl ether **30** (0.44 g, 0.42 mmol) in methanol–water (10:1, 11 cm^3) was hydrogenated in the presence of 10% Pd on carbon (0.1 g) at rt under one atmosphere pressure of hydrogen for 24 h. More water (5 cm^3) was then added, and the mixture was hydrogenated for a further 24 h. The catalyst was removed by filtration through Celite and the filtrate was freeze dried to give the *target compound* **1** (0.25 g, 96%) as a hygroscopic white foam; $[\alpha]_D^{22}$ +11 (*c* 1, equil., in water) (Found: C, 26.1; H, 3.6; N, 2.1. $C_{14}H_{20}NNa_3O_{18}S_2 \cdot H_2O$ requires C, 26.2; H, 3.4; N, 2.2%); $\delta_H(D_2O; H_2O)$ 1.95 (3 H, s, $COCH_3$), 3.53, 3.54 (1 H, 2 t, $J_{2',3'} = J_{3',4'} = 9.5$, H-3′α,β), 3.66, 3.67 (1 H, 2 t, $J_{3',4'} = J_{4',5'} = 9.5$, H-4′α,β), 3.80 (3 H, m, H-2, -5, -5′α,β), 3.84, 3.89 (1 H, 2 dd, $J_{2,3}$ 11.0, $J_{3,4}$ 3.0, H-3α,β), 4.01, 4.03 (1 H, 2 dd, $J_{1',2'}$ 7.5, $J_{2',3'}$ 9.5, H-2′α,β), 4.10 (2 H, m, H_2-6α,β), 4.16, 4.22 (1 H, 2 dd, $J_{3,4}$ 3.0, $J_{4,5}$ 0.8, H-4α,β), 4.65, 4.66 (1 H, 2 d, $J_{1',2'}$ 7.5, H-1′α,β), 4.76 (0.5 H, d, $J_{1,2}$ 8.0, H-1β), and 5.22 (0.5 H, d, $J_{1,2}$ 3.5, H-1α); δ_C(67.8 MHz; D_2O; acetone) 22.73, 23.11 ($COCH_3$), 52.85, 52.99 (C-2α,β), 68.10, 68.60, 68.90 (C-4, -6α,β), 71.92, 72.21 (C-3′α,β), 75.01, 75.12 (C-4′α,β), 76.01, 77.49 (C-5′α,β), 80.31, 80.61 (C-3, -2′α,β), 91.54 (C-1α), 96.05 (C-1β), 101.53, 101.85 (C-1′α,β), and 175.07, 175.31 (C=O).

Sodium methyl *O*-(disodium 2-*O*-sulfonato-β-D-glucopyranosyl-uronate)-(1→3)-2-acetamido-2-deoxy-6-*O*-sulfonato-β-D-galactopyranoside 2

The benzyl ether **31** (0.39 g, 0.4 mmol) was hydrogenated as

described for the preparation of **1**. The filtrate was freeze dried to afford the *target compound* **2** (0.24 g; 95%) as a hygroscopic white powder; $[a]_D^{22}$ −15 (*c* 1 in water) (Found: C, 27.3; H, 3.9; N, 2.0. $C_{15}H_{22}NNa_3O_{18}S_2 \cdot H_2O$ requires C, 27.5; H, 3.7; N, 2.1%); $\delta_H(D_2O; H_2O)$ 1.92 (3 H, s, $COCH_3$), 3.40 (3 H, s, OCH_3), 3.56 (1 H, t, $J_{2',3'} = J_{3',4'} = 9.5$, H-3′), 3.66 (1 H, t, $J_{3',4'} = J_{4',5'} = 9.5$, H-4′), 3.80 (1 H, dd, $J_{1,2}$ 8.0, $J_{2,3}$ 11.0, H-2), 3.84 (1 H, m, H-5), 3.85 (1 H, d, $J_{4',5'}$ 9.5, H-5′), 3.88 (1 H, dd, $J_{2,3}$ 11.0, $J_{3,4}$ 3.0, H-3), 4.01 (1 H, dd, $J_{1',2'}$ 7.5, $J_{2',3'}$ 9.5, H-2′), 4.10 (1 H, dd, $J_{3,4}$ 3.0, $J_{4,5}$ 0.8, H-4), 4.13 (2 H, m, H_2-6), 4.42 (1 H, d, $J_{1,2}$ 8.0, H-1), and 4.67 (1 H, d, $J_{1',2'}$ 7.5, H-1′); δ_C(67.8 MHz; D_2O; acetone) 23.11 ($COCH_3$), 51.62 (C-2), 57.78 (OCH_3), 67.88 (C-4), 67.99 (C-6), 71.72 (C-4′), 73.03 (C-3′), 75.02 (C-5′), 80.11, 80.40 (C-3, -2′), 101.92 (C-1′), 102.96 (C-1), and 173.90, 175.51 (C=O).

Acknowledgements

We are grateful for support by the CNRS and the Région Centre *via* a BDI studentship to N. K.

References

1 L. Å. Fransson, *Trends Biochem. Sci.*, 1987, **12**, 406.
2 L. Kjéllen and U. Lindahl, *Annu. Rev. Biochem.*, 1991, **60**, 443.
3 N. Seno and K. Murakami, *Carbohydr. Res.*, 1982, **103**, 190.
4 K. Sugahara, Y. Tanaka and S. Yamada, *Glycoconjugate J.*, 1996, **13**, 609.
5 K. Sugahara, S. Nadanaka, K. Takeda and T. Kojima, *Eur. J. Biochem.*, 1996, **239**, 871.
6 S. Nadanaka and K. Sugahara, *Glycobiology*, 1997, **7**, 253.
7 A. Lee and R. Langer, *Science*, 1983, **221**, 1185.
8 J. R. Shen, C. C. Fu, M. L. Tsai and W. J. Chung, *Anticancer Res.*, 1998, **18**, 4435.
9 E. Dupont, P. E. Savard, C. Jourdain, C. Juneau, A. Thibodeau, N. Ross, K. Marenus, D. H. Maess, G. Pelletier and D. N. Sauder, *J. Cutan. Med. Surg.*, 1998, **2**, 146.
10 D. R. Miller, G. T. Anderson, J. J. Starck, J. L. Granick and D. Richardson, *J. Clin. Oncol.*, 1998, **16**, 3649.
11 E. Ernst and B. R. Cassileth, *Eur. J. Cancer*, 1999, **35**, 1608.
12 S. Nadanaka, A. Clement, K. Masayama, A. Faissner and K. Sugahara, *J. Biol. Chem.*, 1998, **273**, 3296.
13 J.-C. Jacquinet, *Carbohydr. Res.*, 1990, **199**, 153.
14 J.-C. Jacquinet, L. Rochepeau-Jobron and J.-P. Combal, *Carbohydr. Res.*, 1998, **314**, 283.
15 J. Tamura, K. W. Neumann, S. Kuruno and T. Ogawa, *Carbohydr. Res.*, 1998, **305**, 43.
16 C. Coutant and J.-C. Jacquinet, *J. Chem. Soc.*, *Perkin Trans. 1*, 1995, 1573.
17 F. Bélot and J.-C. Jacquinet, *Carbohydr. Res.*, 2000, **325**, 93.
18 F. Bélot and J.-C. Jacquinet, *Carbohydr. Res.*, 2000, **326**, 88.
19 R. R. Schmidt, *Angew. Chem.*, *Int. Ed. Engl.*, 1986, **25**, 212.
20 S. David, A. Malleron and C. Dini, *Carbohydr. Res.*, 1989, **188**, 193.
21 M. Ek, P. J. Garegg, H. Hultberg and S. Oscarson, *J. Carbohydr. Chem.*, 1983, **2**, 305.
22 T. Fukuyama, A. A. Laird and L. M. Hotchkiss, *Tetrahedron Lett.*, 1985, **26**, 6291.
23 G. Blatter, J.-M. Beau and J.-C. Jacquinet, *Carbohydr. Res.*, 1994, **260**, 189.
24 A. M. Dempsey, L. Hough and A. C. Richardson, *Carbohydr. Res.*, 1975, **41**, 63.
25 R. Albert, K. Dax, R. W. Link and A. E. Stütz, *Carbohydr. Res.*, 1983, **118**, c5.
26 G. Jaurand, C. Tabeur and M. Petitou, *Carbohydr. Res.*, 1994, **255**, 295.
27 T. Iversen and D. R. Bundle, *J. Chem. Soc.*, *Chem. Commun.*, 1981, 1240.
28 P. Collins and R. J. Ferrier, *Monosaccharides*, John Wiley and Sons, Chichester, 1995, p. 63.
29 S. A. Abbas and A. H. Haines, *Carbohydr. Res.*, 1975, **39**, 358.
30 J. Christopher, I. M. McFarlane, C. G. Newton, R. Pitchen and D. H. R. Barton, *Tetrahedron*, 1991, **47**, 6381.
31 J. S. Brimacombe and O. A. Ching, *J. Chem. Soc. C*, 1968, 1642.
32 H. Lucas, J. E. M. Basten, T. G. Van Dinther, J. G. Meuleman, S. F. Van Aelst and C. A. A. Van Boeckel, *Tetrahedron*, 1990, **46**, 8207.
33 N. J. Davis and S. L. Flitsch, *J. Chem. Soc.*, *Perkin Trans. 1*, 1994, 359.