Pergamon

Tetrahedron: Asymmetry, Vol. 8, No. 24, pp. 4111–4120, 1997
© 1997 Published by Elsevier Science Ltd. All rights reserved.
Printed in Great Britain
PII: S0957-4166(97)00604-6
0957-4166/97 $17.00 + 0.00

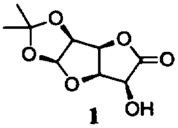
Mannuronolactone acetonide: easy access to C-3 OH and C-5 OH of mannose

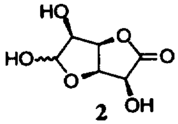
Mark P. Watterson,[a] Angeles Martin,[a] Thomas M. Krülle,[a] Juan C. Estevez[b] and George W. J. Fleet[a,*]

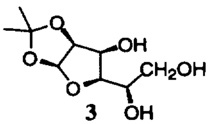
[a] Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY, UK
[b] Departamento de Química Orgánica, Universidade de Santiago de Compostela, Facultade de Ciencias, 15706 Santiago de Compostela, Spain

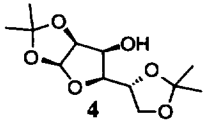
Abstract: The synthesis of D-mannuronolactone acetonide **1** is described from alginic acid and provides an efficient route for the manipulation at C-5 of mannose. Reduction gives a new acetonide of mannose, 1,2-*O*-isopropylidene-β-D-mannofuranose which, on further acetonation, gives 1,2:5,6-di-*O*-isopropylidene-β-D-mannofuranose [giving easy access to C-3 OH of mannose] together with a small amount of 1,2:3,5-di-*O*-isopropylidene-β-D-mannofuranose. Some silylated derivatives of mannuronolactone allow immediate access to the C-6 of mannose. Such intermediates are likely to be of value in the synthesis of derivatives of mannose. © 1997 Published by Elsevier Science Ltd. All rights reserved.

This paper reports the first synthesis of the acetonide **1** of D-mannuronolactone **2** to allow access in one step to an intermediate with only C-5 OH of mannose unprotected. Reduction of **1** gave a new acetonide of mannose **3**. Further acetonation of **3** gave the mannose diketal **4** as the major product, together with a small amount of **5**, containing a six ring acetonide. This two step synthesis of **4** from mannuronolactone acetonide **1** in 63% yield gives an intermediate in which only C-3 OH is unprotected. A fully protected persilylated lactone **6** is readily available in one step in over 80% yield. Further elaborations and illustrations of the uses of these acetonides are described; the ease of accessibility of C-3 and C-5 OH in these protected derivatives of mannose, coupled with the very facile acid hydrolysis of the isopropylidene unit in the crowded frameworks, makes them attractive new synthons for the manipulation of mannose derivatives.

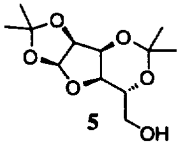

1

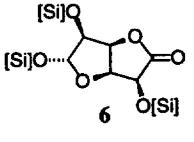

2

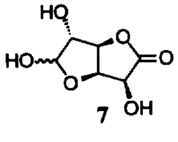

3

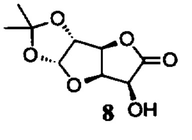

4

[Si] = *tert*-BuMe$_2$Si


5


6


7


8

D-Glucuronolactone **7** is a cheap[1] carbohydrate lactone which efficiently forms the acetonide **8**, first used in an elegant synthesis of vitamin C.[2] Because of its low cost, glucuronolactone has been widely used in the synthesis of many compounds[3] other than carbohydrates themselves, including oxacephems,[4] pseudosugars,[5] *C*-linked disaccharides,[6] castanospermine[7] and other glycosidase inhibitors,[8] bulgecinine[9] and other amino acids,[10] highly substituted cyclopentanes,[11] and 6*C*-alkylated glucose derivatives as inhibitors of phosphomutases and other enzymes[12] of primary metabolism.[13]

---

* Corresponding author.

In contrast, mannuronolactone **2** is expensive [1 g costs £345.50 in the 1997 Sigma catalogue] and, unsurprisingly, has not been used at all as a synthetic starting material. However D-mannuronic acid is the main component of alginic acid, a vital structural polysaccharide of many marine plants.[14] The industrially useful gelling properties of alginic acid have led to its large scale production from macroalgae crops; it is commercially and cheaply[15] available as the free acid isolated from the seaweed *Macrocystis pyrifera* (kelp). Alginic acid is a binary block co-polymer of (1→4)-linked β-D-mannopyranuronic acid and α-L-gulopyranuronic acid residues, the typical composition of which in *Macrocystis pyrifera* is 61% D-mannopyronate and 39% L-gulopyronate residues.[16] Nelson and Cretcher were the first to demonstrate that acid hydrolysis of alginic acid from kelp gave D-mannuronolactone **2**.[17] Subsequent studies[18] established that the highest yields of **2** were obtained using formic acid. Very minor modification of the literature procedure[19] of the treatment of alginic acid with 90% formic acid at reflux afforded the crystalline lactone **2** with NMR,[14] melting point and rotation data consistent with literature values [33% yield, based on the D-mannopyronate composition of alginic acid].

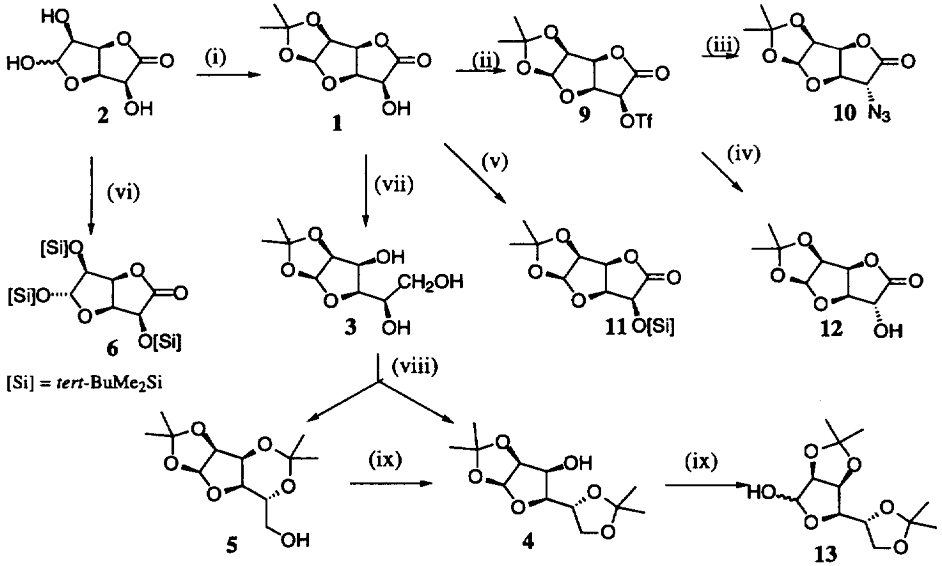
In order to attempt to exploit the potential of mannuronolactone as a synthetic starting material, procedures for the synthesis of the acetonide **1** were investigated. D-Glucuronolactone **7** forms[2] the 1,2-isopropylidene ketal **8** in high yield using acetone and sulfuric acid. Various conditions were investigated for the protection of **2** as the 1,2-isopropylidene ketal **1**, and in all cases the yields were low (<45%). Treatment of a solution of D-mannuronolactone **2** in acetone at reflux with camphorsulfonic acid (CSA) and anhydrous copper sulfate proved to be the optimum conditions for the conversion (Scheme 1); in particular, dimethoxypropane procedures gave alternative methyl mannuronate derivatives. D-Mannuronolactone **2** has low solubility in acetone and highest yields (40–45%) of the ketal **1** were achieved only when the starting material was first completely dissolved in a large volume of the reaction solvent at reflux. Under these conditions, significant amounts of unreacted D-mannuronolactone **1** were recovered by flash column chromatography; this allowed **1** to be isolated in 43% yield (or 68% based on unrecovered starting material). The ketal quaternary carbon of **1** gave a singlet in the ^{13}C NMR spectrum at δ 115.2, characteristic[20] of a five-membered acetonide fused to a furanose ring.

The instability of the *manno*-acetonide **1** [relative to the *gluco*- epimer **8**] is reflected in the high sensitivity of the reaction to moisture; without copper sulfate, yields of **1** were reduced considerably. Attempts to recover unreacted starting material via an aqueous wash resulted in complete reversion of the product **1** to D-mannuronolactone **2**. The *manno*- isomer **1** is much less stable to acid and to base than the *gluco*- epimer **8**. Although this instability may be a disadvantage in obtaining an efficient synthesis of **1**, it may also have significant advantages in the ease of removal of the acetonide protecting group at a later stage in a synthesis. Energy minimisation calculations[21] indicate that the ketal **1** is a highly crowded molecule (Figure 1), having all *cis* substituents on the furanose ring. The high energy of this structure results in an equilibrium that lies in favour of the starting material **1**. By comparison, the same crowding does not exist in the ketal **8** since the D-*gluco*- stereochemistry creates a *trans* relationship between the isopropylidene and lactone rings fused to the furanose ring.

The acetonide **1**, in which only the C-5 OH is unprotected, should prove useful for chemical modification at C-5 of mannose. Esterification of the free hydroxyl function in **1** with trifluoromethanesulfonic (triflic) anhydride and pyridine in dichloromethane afforded the triflate **9**, which was immediately treated with sodium azide in dimethylformamide to give a single azide **10** in 83% yield over the two steps. α-Azidolactones can undergo unanticipated epimerisations,[22] but the indicated L-*gulurono* configuration for the azide **10** is strongly supported by the observation of an NOE enhancement of 3% between H-5 and one of the methyl groups of the isopropylidene group (Figure 2).

Similarly, treatment of the triflate **9** with caesium trifluoroacetate[23] in butanone, followed by work-up of the resulting trifluoroacetate with methanol, gave the inverted alcohol **12** in 56% overall yield.

Treatment of the acetonide **1** with *tert*-butyldimethylsilyl triflate in pyridine at 0°C gave the fully



Scheme 1. (i) Me$_2$CO, CSA, CuSO$_4$, 43% (68% on unrecovered **2**); (ii) (CF$_3$SO$_2$)$_2$O, pyridine, CH$_2$Cl$_2$; (iii) NaN$_3$, DMF, 83% from **1**; (iv) CsOCOCF$_3$, butanone; then MeOH, 56% from **1**; (v) *tert*-BuMe$_2$SiOSO$_2$CF$_3$, pyridine, 87%; (vi) *tert*-BuMe$_2$SiCl, imidazole, 83%; (vii) LiBH$_4$, THF, 72%; (viii) Me$_2$CO, Me$_2$(OMe)$_2$, PPTS, 87% of **4** and 4% of **5**; (ix) Me$_2$CO, CSA, 95/92%.

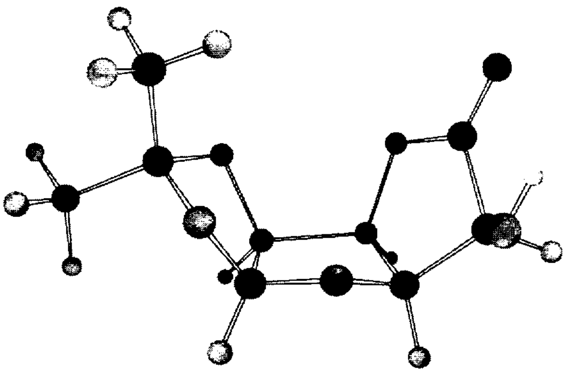
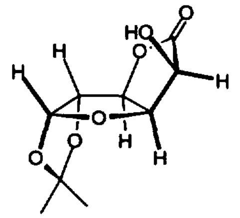
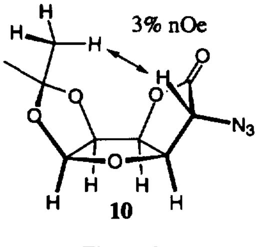


Figure 1. Energy minimised structure of the *manno*-acetonide **1**.

protected lactone **11** in 87% yield, a suitable intermediate for nucleophilic attack on C-6 of mannose. The low yields of formation of the 1,2-isopropylidene ketal **1** obtained indicated that direct protection by silyl ether formation would be likely to provide an alternative route to a fully protected derivative of mannuronolactone **2**. Treatment of **2** with excess *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide at 0°C formed the fully protected trisilyl ether **6** in one step as the sole product in 83% yield. The stereochemistry at the anomeric position C-1 in **6** is tentatively assigned as shown, due to the absence of an NOE enhancement between H-1 and either of the protons H-3 and H-4; such enhancements would clearly be anticipated in the β-anomer **14**. Additionally, the steric crowding between the silyl ethers in **14** make this an inherently less likely product in the reaction on either kinetic or thermodynamic considerations. In contrast, trisilyl protection of glucuronolactone **7** gave the β-trisilyl ether **15** as the major product in 65% isolated yield.[13]

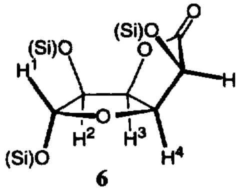


gluco-acetonide **8**

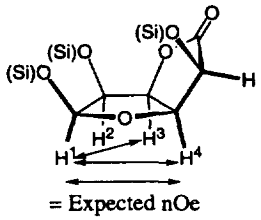


10

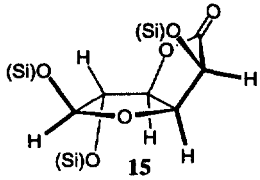
Figure 2.



6



14



15

Thus, protection of all positions of D-mannuronolactone **2** allows access to C-6 of mannose either in an overall yield of up to 39% over two steps via the acetonide **1**, or in a single step in 83% yield. Among other uses such intermediates would, on the basis of studies on 6*C*-alkylglucoses, provide easy access to 6*C*-alkylmannose analogues which are likely to be of interest in the study of the primary metabolism of mannose.[24]

Reduction of **1** with lithium borohydride in tetrahydrofuran gave a new monoacetonide of mannofuranose **3** in 72% yield, in which C-3, C-5 and C-6 of mannose are unprotected. Reaction of **3** with dimethoxypropane in the presence of pyridinium *p*-toluenesulfonate (PPTS) gave two diacetonides **4**[25] and **5** in a combined isolated yield of 91%. The reaction of mannose itself with acetone gives the 2,3:5,6-diacetonide **13** as the thermodynamic acetonide efficiently[26] and on a large scale.[27] The major diacetonide **4** [87% yield], displayed two singlets in the ^{13}C NMR spectrum at δ 109.1 and 114.2 indicating the presence of two five ring ketals, whereas the minor product **5** [4% yield] showed two singlets in the ^{13}C NMR spectrum at δ 101.3 [for a six ring ketal] and δ 114.8 [for a five ring ketal]. Under the above conditions, none of the most stable diacetonide **13** was formed.

The relative stability of the three diacetonides **4**, **5** and **13** was investigated by treatment of the acetonides with CSA in acetone. Under these conditions, it was found that the six ring acetonide **5** completely rearranged to the less stable of the two five ring diacetonides **4** before any of **13** was formed. Longer treatment of **4** with acid induced essentially quantitative isomerisation, as has been previously reported,[25] to the well-known and thermodynamically stable diacetonide **13**. Thus the diacetonide **4** can be formed reasonably efficiently from **1** in an overall yield of 63%; only the C-3 hydroxyl group in **4** is unprotected and may be an ideal intermediate for the construction of oligosaccharides linked to C-3 of mannose. The only previous synthesis[25,28] of **4** involved reduction of a ketone derived from 1,2:5,6-di-*O*-isopropylidenealtrose, the preparation of which is too lengthy to be attractive for the generation of **4** as a synthetic intermediate. The ease of removal of the 1,2-isopropylidene protecting group in such derivatives is likely to be a major attraction in the synthesis of such oligosaccharides and further work in this direction is in progress. In fact, all new materials in this paper are likely to prove useful in the preparation of mannose containing oligosaccharides and of mimics of mannose and other sugars.

In summary, this paper describes the first preparation of mannuronolactone acetonide **1**, a very different species in terms of its chemical stability to the epimeric glucuronolactone acetonide **8**, but nonetheless one which is readily available and likely to provide easy access to individual hydroxyl groups of mannose which have hitherto been much more difficult to access.

Experimental

Melting points were recorded on a Kofler hot block and are corrected. Proton nuclear magnetic resonance (δ_H) spectra were recorded on a Varian Gemini 200 (200 MHz), Bruker AC 200 (200 MHz) or a Bruker AM 500 (500 MHz) spectrometer. ^{13}C nuclear magnetic resonance (δ_C) spectra were recorded on a Varian Gemini 200 (50 MHz), a Bruker AC 200 (50 MHz) or a Bruker AM 500 (125 MHz) spectrometer and multiplicities were assigned using DEPT sequence. All chemical shifts are quoted on the δ-scale. The following abbreviations were used to explain multiplicities: s, singlet; d, doublet; dd, double–doublet; ddd, double–double–doublet; t, triplet; q, quartet; m, multiplet; br, broad; app, apparent. Infra-red spectra were recorded on a Perkin–Elmer 1750 IR FT spectrophotometer. Mass spectra were recorded on a VG PLATFORM (APCI, positive or negative as stated), a VG Autospec spectrometer or a VG 20-250 spectrometer (chemical ionisation [NH_3, CI] as stated), or a BIO-Q spectrometer (electrospray as stated). Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml. Microanalyses were performed by the microanalysis service of the Dyson Perrins Laboratory. Thin layer chromatography (t.l.c.) was carried out on plastic or aluminium sheets coated with $60F_{254}$ silica, and plates were developed using a spray of 0.2% w/v cerium (IV) sulfate and 5% ammonium molybdate in 2 M sulfuric acid. Flash chromatography was carried out using Sorbsil C60 40/60 silica. Solvents and commercially available reagents were dried and purified before use according to standard procedures; hexane was distilled at 68°C before use to remove less volatile fractions. Alginic acid was purchased from Avocado Chemicals and from Sigma, and used without any purification.

D-*Mannurono-6,3-lactone 2*

This procedure contains only minor modifications of the literature procedure.[19] A suspension of alginic acid (50.0 g) in a mixture of 90% formic acid (AR grade, 800 ml) and ethanol (40 ml) was stirred at room temperature for 2 h, then heated under reflux for 16 h. T.l.c. (15% ethanol/ethyl acetate) indicated the formation of a major product (R_f 0.1). After cooling to room temperature the mixture was filtered through Celite and the filtrate concentrated *in vacuo* by co-evaporation with ethanol. The residual gum was dissolved in 95% ethanol (1 l), decolourising charcoal (30 g) was added and the suspension brought briefly to reflux. The mixture was filtered without cooling and the filtrate concentrated to approximately 20 ml *in vacuo* to afford a pale precipitate which was collected by filtration and washed with acetone:ethanol (70:30 v/v). The precipitate was dissolved in water (100 ml), decolourising charcoal (30 g) was added and the suspension brought briefly to reflux. The mixture was filtered without cooling and the solvent removed *in vacuo* to afford mannuronolactone **2** (10.0 g, 20% based on mass of alginic acid used, 33% on the content of mannuronopyronate) as a white crystalline solid; m.p. 185–186°C (decomp., 90% ethanol) [lit.[19] 186–187°C, 197–199°C]; $[\alpha]_D^{21}$ +97.1 (*c* 1.00, H_2O, after 40 minutes) [lit.[19] $[\alpha]_D^{21}$ +92 (*c* 1.00, H_2O, after 20 minutes)]; δ_H (200 MHz; D_2O) 4.04 (1H, app t, H-2, α-anomer, J=4.5 Hz), 4.12 (1H, app t, H-2, β-anomer, J=4.7 Hz), 4.39–4.66 (3H, m, H-5, α-anomer, β-anomer, H-4, α-anomer), 4.74 (1H, dd, H-4, α-anomer, $J_{4,3}$=3.2, $J_{4,5}$=5.0 Hz), 4.84–5.01 (2H, m, H-3, α-anomer, β-anomer), 5.04 (1H, d, H-1, α-anomer, $J_{1,2}$=5.6 Hz), 5.19 (1H, d, H-1, β-anomer, $J_{1,2}$=4.5 Hz).

1,2-O-Isopropylidene-β-D-mannurono-6,3-lactone 1

A suspension of mannuronolactone **2** (3.0 g, 17.0 mmol) in acetone (AR grade, 2 l) was heated to reflux and stirred for 3 h, by which time the mixture had become homogeneous. Anhydrous copper sulphate (9.0 g, 56.3 mmol) and camphorsulfonic acid (1.0 g, 4.31 mmol) were added and the mixture refluxed for 21 h. T.l.c. (ethyl acetate) indicated the presence of starting material (baseline) and the formation of a major product (R_f 0.3). The mixture was allowed to cool to room temperature, stirred with sodium hydrogen carbonate (1.81 g, 21.5 mmol) for 16 h then filtered through silica gel. The solvent was removed *in vacuo*, the residue pre-adsorbed onto silica gel and purified by flash column chromatography (ethyl acetate) to afford 1,2-*O*-isopropylidene-β-D-mannurono-6,3-lactone **1** (1.59 g, 43%; 68% based on unrecovered starting material — see below) as a white crystalline solid; m.p. 175–177°C (ethyl acetate); $[\alpha]_D^{21}$ +32.5 (*c* 1.02, MeCN); ν_{max} (KBr) 3477 (OH), 1780 (C=O) cm^{-1}; δ_H (500 MHz; CD_3CN) 1.36, 1.46 (6H, 2×s, $C(CH_3)_2$), 3.75 (1H, d, OH-5, $J_{OH,5}$=8.7 Hz), 4.48 (1H, dd, H-5, $J_{5,4}$=5.7, $J_{5,OH}$=8.7 Hz), 4.68 (1H, dd, H-4, $J_{4,3}$=4.2, $J_{4,5}$=5.7 Hz), 4.81 (1H, dd, H-3, $J_{3,2}$=5.6, $J_{3,4}$=4.2 Hz), 4.86 (1H, dd, H-2, $J_{2,1}$=4.2, $J_{2,3}$=5.6 Hz), 5.95 (1H, d, H-1, $J_{1,2}$=4.2 Hz); δ_C (50.3 MHz, CD_3CN) 25.8, 26.9 (2×q, $C(CH_3)_2$), 69.4, 76.8, 79.3, 80.8 (4×d, C-2, C-3, C-4, C-5), 106.8 (d, C-1), 115.3 (s, *C*Me$_2$), 175.2 (s, C-6); *m/z* (NH_3, CI) 217 ($M+H^+$, 27%), 234 ($M+NH_4^+$, 100%). Found: C, 50.01; H, 5.51; $C_9H_{12}O_6$ requires C, 50.00; H, 5.59%.

Further elution (15% ethanol/ethyl acetate) gave recovered starting material **2** (1.09 g, 36%).

5-Azido-5-deoxy-1,2-O-isopropylidene-α-L-gulurono-6,3-lactone 10

Triflic anhydride (0.10 ml, 0.59 mmol) was added to a stirred solution of the acetonide **1** (102 mg, 0.47 mmol) and pyridine (0.11 ml, 1.36 mmol) in dichloromethane (20 ml) at −25°C. T.l.c. (ethyl acetate) after 5 min indicated complete conversion of starting material (R_f 0.3) to a single product (R_f 0.5). A drop of water was added and the mixture filtered through silica gel, eluting with ethyl acetate. The solvent was removed *in vacuo* by co-evaporation with toluene and the crude product dissolved in dry DMF (3 ml). Sodium azide (31 mg, 0.48 mmol) was added with stirring at −40°C. T.l.c. (hexane:ethyl acetate=1:1) after 3 h indicated complete conversion of starting material (R_f 0.2) to a single product (R_f 0.4). The reaction mixture was diluted with water (100 ml) and extracted with ethyl acetate (3×30 ml). The combined organic phases were dried over $MgSO_4$, filtered, concentrated *in vacuo* and the residue purified by flash column chromatography (hexane:ethyl acetate=3:2) to afford the azidolactone **10** (95 mg, 83% over two steps) as a white crystalline solid; m.p. 65–67°C (hexane:diethyl ether); $[\alpha]_D^{22}$ +211.6 (*c* 1.00, $CHCl_3$); ν_{max} (KBr) 2126 (N_3), 1798 (C=O) cm^{-1}; δ_H (500 MHz; $CDCl_3$) 1.38, 1.61 (6H, 2×s, $C(CH_3)_2$), 4.54 (1H, d, H-5, $J_{5,4}$=5.2 Hz), 4.65 (1H, dd, H-4, $J_{4,3}$=7.7, $J_{4,5}$=5.2 Hz), 4.71 (1H, dd, H-2, $J_{2,1}$=3.4, $J_{2,3}$=5.4 Hz), 5.05 (1H, dd, H-3, $J_{3,2}$=5.4, $J_{3,4}$=7.7 Hz), 5.92 (1H, d, H-1, $J_{1,2}$=3.4 Hz); δ_C (50.3 MHz, $CDCl_3$) 25.8, 26.5 (2×q, $C(CH_3)_2$), 76.5, 77.3, 77.9, 83.4 (4×d, C-2, C-3, C-4, C-5), 107.6 (d, C-1), 114.8 (s, *C*Me$_2$), 172.1 (s, C-6); *m/z* (APCI+) 214 ($M-N_2+H^+$, 44%), 112 (100%). Found: C, 45.02; H, 4.41; N, 17.21; $C_9H_{11}N_3O_6$ requires C, 44.82; H, 4.60; N, 17.35%.

1,2-O-Isopropylidene-α-L-gulurono-6,3-lactone 12

Triflic anhydride (0.13 ml, 0.77 mmol) was added in two portions to a solution of lactone **1** (97 mg, 0.45 mmol) in dichloromethane (10 ml) and pyridine (0.10 ml, 1.24 mmol) at 0°C. After stirring for 30 min in the cold, the reaction mixture was filtered through silica gel (eluent: dichloromethane) and concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane:ethyl acetate=1:1) to afford the triflate **9** (121 mg, 78%) as a relatively unstable colourless solid; ν_{max} (film) 1802 (C=O), 1422 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.42, 1.58 (6H, 2×s, $C(CH_3)_2$), 4.86 (1H, dd, $J_{2,1}$=3.8, $J_{2,3}$=5.5 Hz, H-2), 4.91 (1H, dd, $J_{4,3}$=5.2, $J_{4,5}$=6.4 Hz, H-4), 5.03 (1H, app t, H-3), 5.28 (1H, d, $J_{5,4}$=6.4 Hz, H-5), 5.94 (1H, d, $J_{1,2}$=3.8 Hz, H-1); which was used immediately. Caesium trifluoroacetate (150 mg, 0.61 mmol) was added to a solution of the triflate **9** (95 mg, 0.27 mmol) in butanone (5 ml). The reaction was stirred for 12 hours at room temperature before methanol (0.10 ml) was added. After stirring for a further 60 minutes, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (hexane:ethyl acetate=1:1) to afford the acetonide of guluronolactone **12** (45 mg, 76%) as a crystalline solid; m.p. 128–130°C (hexane:ethyl acetate); $[\alpha]_D^{24}$ +56.7 (*c* 0.36, MeCN); ν_{max} (film) 3401 (OH), 1794 (C=O) cm^{-1}; δ_H (500 MHz, CD_3CN) 1.33, 1.51 (6H, 2×s, $C(CH_3)_2$), 4.16 (1H, d, $J_{OH,5}$=6.1 Hz, OH), 4.55 (1H, app t, J=5.8 Hz, H-5), 4.68 (1H, dd, $J_{4,5}$=5.5, $J_{4,3}$=7.9 Hz, H-4), 4.71 (1H, dd, $J_{2,1}$=3.4, $J_{2,3}$=5.4 Hz, H-2), 5.08 (1H, dd, $J_{3,2}$=5.4, $J_{3,4}$=7.9 Hz, H-3), 5.87 (1H, d, $J_{1,2}$=3.4 Hz, H-1); δ_H (500 MHz, $CDCl_3$) 1.37, 1.58 (6H, 2×s, $C(CH_3)_2$), 3.15 (1H, br s, OH), 4.70 (1H, dd, $J_{2,1}$=3.4, $J_{2,3}$=5.4 Hz, H-2), 4.74 (1H, d, $J_{5,4}$=5.3 Hz, H-5), 4.83 (1H, dd, $J_{4,3}$=7.9, $J_{4,5}$=5.3 Hz, H-4), 5.07 (1H, dd, $J_{3,2}$=5.4, $J_{3,4}$=7.9 Hz, H-3), 5.93 (1H, d, $J_{1,2}$=3.4 Hz, H-1); δ_C (50.3 MHz, CD_3CN) 26.2, 26.8 (2×q, $C(CH_3)_2$), 73.2, 78.1, 78.4, 86.7 (4×d, C-2, C-3, C-4, C-5), 108.7 (d, C-1), 114.8 (s, CMe_2), 176.2 (s, C-6); *m/z* (electrospray) 261 ($M+HCO_2^-$, 100%). Found: C, 49.88; H, 5.50%; $C_9H_{12}O_6$ requires C, 50.00; H, 5.59%.

5-O-tert-Butyldimethylsilyl-1,2-O-isopropylidene-β-D-mannurono-6,3-lactone **11**

tert-Butyldimethylsilyl triflate (1.65 ml, 7.19 mmol) was added to a stirred solution of the acetonide **1** (1.38 g, 6.39 mmol) in dry pyridine (20 ml) at 0°C. T.l.c. (ethyl acetate) after 10 min indicated complete conversion of starting material (R_f 0.3) to a single product (R_f 0.7). The mixture was quenched with ethanol (2 ml) and the solvent removed *in vacuo* by co-evaporation with toluene. The residue was dissolved in ethyl acetate (250 ml) and washed with pH 7 buffer (100 ml). The organic phase was dried over $MgSO_4$, filtered, concentrated *in vacuo*, and the residue purified by flash column chromatography (hexane:ethyl acetate=2:1) to afford the fully protected lactone **11** (1.84 g, 87%) as a white crystalline solid; m.p. 91–92°C; $[\alpha]_D^{21}$ +46.3 (*c* 1.06, $CHCl_3$); ν_{max} (KBr) 1786 (C=O) cm^{-1}; δ_H (500 MHz; $CDCl_3$) 0.22, 0.24 (6H, 2×s, $Si(CH_3)_2$), 0.99 (9H, s, $SiC(CH_3)_3$), 1.45, 1.61 (6H, 2×s, $C(CH_3)_2$), 4.52 (1H, d, H-5, $J_{5,4}$=5.7 Hz), 4.65 (1H, dd, H-4, $J_{4,3}$=3.9, $J_{4,5}$=5.7 Hz), 4.82–4.86 (2H, m, H-2, H-3), 5.92 (1H, d, H-1, $J_{1,2}$=3.3 Hz); δ_C (50.3 MHz, $CDCl_3$) −5.1, −5.4 (2×q, $Si(CH_3)_2$, 18.2 (s, $SiCMe_3$), 25.5 (q, $SiC(CH_3)_3$), 27.6, 26.5 (2×q, $C(CH_3)_2$), 70.6, 76.6, 78.6, 80.5 (4×d, C-2, C-3, C-4, C-5), 106.9 (d, C-1), 116.0 (s, CMe_2), 173.9 (s, C-6); *m/z* (NH_3, CI) 273 (100%), 348 ($M+NH_4^+$, 61%). Found: C, 54.29; H 7.98; $C_{15}H_{26}O_6Si$ requires C, 54.52; H, 7.93%.

1,2,5-Tri-O-tert-butyldimethylsilyl-α-D-mannurono-6,3-lactone **6**

tert-Butyldimethylsilyl chloride (1.41 g, 9.34 mmol) was added to a stirred solution of D-mannurono-6,3-lactone **1** (300 mg, 1.39 mmol) and imidazole (0.90 g, 13.2 mmol) in dry DMF (9 ml) at 0°C. T.l.c. (hexane:ethyl acetate=4:1) after 24 h indicated the absence of starting material (baseline) and the formation of a major product (R_f 0.7). The solvent was removed *in vacuo* by co-evaporation with toluene, the residue dissolved in ethyl acetate (150 ml) and washed with pH 7 buffer solution (150 ml). The organic phase was dried over $MgSO_4$, filtered, concentrated *in vacuo* and the residue purified by flash column chromatography (hexane:ether=20:1) to afford the trisilyl lactone **6** (731 mg, 83%) as a white crystalline solid; m.p. 64–65°C; $[\alpha]_D^{21}$ +107.5 (*c* 1.11, $CHCl_3$); ν_{max} (KBr) 1775 (C=O) cm^{-1}; δ_H (500 MHz; $CDCl_3$) 0.08–0.19 (18H, 6×s, 3×$Si(CH_3)_2$), 0.91, 0.92, 0.94 (27H, 3×s, 3×$SiC(CH_3)_3$), 4.13 (1H, dd, H-2, $J_{2,1}$=3.4, $J_{2,3}$=4.4 Hz), 4.37 (1H, d, H-5, $J_{5,4}$=5.2 Hz), 4.68–4.72 (2H, m, H-3, H-4), 5.23 (1H, d, H-1, $J_{1,2}$=3.4 Hz); δ_C (50.3 MHz, $CDCl_3$) −5.4 to −4.7 (6×q, 3×$Si(CH_3)_2$), 17.8, 18.0, 18.2 (3×s, 3×$SiCMe_2$), 25.5 (3×q, 3×$SiC(CH_3)_3$), 70.7, 75.8, 78.8, 79.6 (4×d, C-2, C-3, C-4, C-5) 103.5 (d, C-1), 174.3 (s, C-6); *m/z* (APCI+) 519 ($M+H^+$, 100%). Found: C, 55.47; H, 9.87; $C_{24}H_{50}O_6Si_3$ requires C, 55.55; H, 9.71%.

1,2-O-Isopropylidene-β-D-mannofuranose **3**

Lithium borohydride (257 mg, 11.8 mmol) was added to a stirred solution of the acetonide **1** (850 mg, 3.93 mmol) in dry THF (28 ml) at 0°C. T.l.c. (ethyl acetate) after 1 hour at 0°C indicated complete conversion of starting material (R_f 0.3) to a single product (baseline). A saturated solution of ammonium chloride was added, and the mixture stirred for 30 min then concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate:methanol=9:1) to yield the monoacetonide **3** (626 mg, 72%) as a colourless oil which crystallised *in vacuo*; m.p. 60–62°C; $[\alpha]_D^{21}$ +4.8 (*c* 1.7, MeOH); ν_{max} (film) 3401 (OH) cm^{-1}; δ_H (500 MHz, CD_3CN) 1.33, 1.53 (6H, 2×s, $C(CH_3)_2$), 2.75 (1H, t, J=5.3 Hz, OH-6), 3.41 (1H, d, $J_{OH,5}$=3.9 Hz, OH-5), 3.50 (1H, m, H-6), 3.65–3.69 (2H, m, H-6′, OH-3), 3.81 (1H, dd, $J_{4,3}$=6.3, $J_{4,5}$=9.2 Hz, H-4), 4.10 (1H, m, H-5), 4.35 (1H, app q, J=5.8 Hz, H-3), 4.61 (1H, dd, $J_{2,1}$=4.1, $J_{2,3}$=5.7 Hz, H-2), 5.67 (1H, d, $J_{1,2}$=4.1 Hz, H-1); δ_C (125 MHz, CD_3CN) 26.5, 26.9 (2×q, $C(CH_3)_2$), 64.3 (t, C-6), 71.3, 71.6, 80.5, 80.6 (4×d, C-2, C-3, C-4, C-5), 105.8 (d, C-1), 114.4 (s, CMe_2); *m/z* (APCI−) 219 ($M-H^-$, 100%). Found: C, 48.70; H, 7.46; $C_9H_{16}O_6$ requires C, 49.09; H, 7.32%.

1,2:5,6-Di-O-isopropylidene-β-D-mannofuranose 4 and 1,2:3,5-di-O-isopropylidene-β-D-mannofuranose 5

2,2-Dimethoxypropane (1 ml, 8.18 mmol) and pyridinium *p*-toluenesulfonate (68 mg, 0.27 mmol) were added to a stirred solution of the monoacetonide **3** (600 mg, 2.73 mmol) in acetone (AR grade, 27 ml) at room temperature. T.l.c. (ethyl acetate) after 90 min indicated the absence of starting material (R_f 0.1) and the formation of two products (R_f 0.6 [major] and R_f 0.4 [minor]). Solid sodium hydrogen carbonate was added and the mixture stirred overnight, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane:ethyl acetate=11:9) to afford 1,2:5,6-di-*O*-isopropylidene-β-D-mannofuranose **4** (619 mg, 87%) as a colourless oil which crystallised *in vacuo*; m.p. 48–50°C; $[\alpha]_D^{21}$ +8.3 (*c* 1.00, MeOH) [lit.[25] m.p. 51–53°C; $[\alpha]_D^{21}$ +12.5 (*c* 0.4, H_2O); lit.[28] m.p. 51–53.°C; $[\alpha]_D^{21}$ +3 (*c* 1.27, Me_2CO)]; ν_{max} (film) 3498 (OH) cm^{-1}; δ_H (500 MHz, CD_3CN) 1.31, 1.35, 1.36, 1.53 (12H, 4×s, $2\times C(CH_3)_2$), 3.06 (1H, d, $J_{OH,3}$=5.7 Hz, OH-3), 3.83 (1H, dd, $J_{4,3}$=5.4, $J_{4,5}$=7.7 Hz, H-4), 3.88 (1H, dd, $J_{6,5}$=6.1, $J_{6,6'}$=8.5 Hz, H-6), 4.04 (1H, dd, $J_{6,5}$=6.4, $J_{6,6'}$=8.5 Hz, H-6′), 4.20 (1H, app q, J=5.7 Hz, H-3), 4.48 (1H, ddd, $J_{5,6}$=6.2, $J_{5,6'}$=6.2, $J_{5,4}$=7.7 Hz, H-5), 4.64 (1H, dd, $J_{2,1}$=4.2, $J_{2,3}$=5.8 Hz, H-2), 5.65 (1H, d, $J_{1,2}$=4.2 Hz, H-1); δ_C (50.3 MHz, CD_3CN) 24.7, 26.0, 26.0, 29.2 (4×q, $2\times C(CH_3)_2$), 66.8 (t, C-6), 69.8, 73.5, 80.3, 82.0 (4×d, C-2, C-3, C-4, C-5), 105.9 (d, C-1), 109.1, 114.2 (2×s, $2\times CMe_2$); *m/z* (NH_3, CI) 261 ($M-H^+$, 3%), 245 (M^+-CH_3, 36%), 187 (100%). Found: C, 55.23; H, 7.85; $C_{12}H_{20}O_6$ requires C, 55.37; H, 7.74%.

1,2:3,5-Di-*O*-isopropylidene-β-D-mannofuranose **5**: (30 mg, 4%) as a colourless oil; $[\alpha]_D^{21}$ −4.3 (*c* 1.05, MeOH); ν_{max} (film) 3498 (OH) cm^{-1}; δ_H (500 MHz, CD_3CN) 1.33, 1.35, 1.37, 1.52 (12H, 4×s, $2\times C(CH_3)_2$), 2.77 (1H, t, J=6.1 Hz, OH-6), 3.50 (1H, ddd, $J_{6,OH}$=6.1, $J_{6,5}$=6.1, $J_{6,6'}$=12.2 Hz, H-6), 3.67 (1H, ddd, $J_{6',5}$=2.6, $J_{6',OH}$=5.9, $J_{6',6}$=11.9 Hz, H-6′), 3.71 (1H, ddd, $J_{5,6'}$=2.6, $J_{5,6}$=6.2, $J_{5,4}$=8.8 Hz, H-5), 3.93 (1H, dd, $J_{4,3}$=5.6, $J_{4,5}$=8.7 Hz, H-4), 4.22 (1H, dd, $J_{3,4}$=5.7, $J_{3,2}$=6.6 Hz, H-3), 4.74 (1H, dd, $J_{2,1}$=4.1, $J_{2,3}$=6.7 Hz, H-2), 5.70 (1H, d, $J_{1,2}$=4.1 Hz, H-1); δ_C (125 MHz, CD_3CN) 24.1, 25.4, 27.2, 27.9 (4×q, $2\times C(CH_3)_2$), 63.1 (t, C-6), 69.7, 73.2, 79.1, 81.4 (4×d, C-2, C-3, C-4, C-5), 101.3 (s, CMe_2), 106.8 (d, C-1), 114.8 (s, CMe_2); *m/z* (APCI+) 283 ($M-Na^+$, 30%), 244 (68%), 145 (100%). Found: C, 55.41; H, 7.72; $C_{12}H_{20}O_6$ requires C, 55.37; H, 7.74%.

Isomerization of 1,2:5,6-di-O-isopropylidene-β-D-mannofuranose 4

Camphorsulfonic acid (9 mg, 0.038 mmol) was added to a stirred solution of 1,2:5,6-di-*O*-isopropylidene-β-D-mannofuranose **4** (100 mg, 0.39 mmol) in acetone (AR grade, 5 ml) at room temperature. T.l.c. (hexane:ethyl acetate=1:1) after 46 h indicated complete conversion of starting material (R_f 0.3) to a single product (R_f 0.5). Solid sodium hydrogen carbonate was added and the mixture stirred for 1 h, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane:ethyl acetate=4:1) to afford 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose **13** (95 mg, 95%) as a white crystalline solid; δ_H (200 MHz, CD_3CN) data identical to those from an authentic sample.[26]

Isomerization of 1,2:3,5-di-O-isopropylidene-β-D-mannofuranose 5

Camphorsulfonic acid (3.6 mg, 0.015 mmol) was added to a stirred solution of 1,2:3,5-di-*O*-isopropylidene-β-D-mannofuranose **5** (40 mg, 0.15 mmol) in acetone (AR grade, 2 ml) at room temperature. T.l.c. (hexane:ethyl acetate=1:1) after 2 h indicated that a trace of starting material (R_f

0.1) remained and a single product (R_f 0.3) had been formed. Solid sodium hydrogen carbonate was added and the mixture stirred for 1 h, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane:ethyl acetate=3:1) to afford 1,2:5,6-di-*O*-isopropylidene-β-D-mannofuranose **4** (37 mg, 92%) as a colourless oil which crystallised *in vacuo*; δ_H (200 MHz, CD_3CN) data identical to those given for the major product **4** above.

Acknowledgements

This work was supported by EPSRC and European Community post-doctoral fellowships (Programme Training Mobility Research) contract no. ERBFMBICT961664.

References

1. Price of 100 g of glucuronolactone from Avocado £16.50; Sigma £23.50 (1997 catalogues).
2. T. Kitihara, T. Ogawa, T. Naganuma, M. Matsui, *Agr. Biol. Chem.*, **1974**, *38*, 2189.
3. E. Kaji, Y. Osa, K. Takahashi, M. Hirooka, S. Zen, F. W. Lichtenthaler, *Bull. Chem. Soc. Jpn*, **1994**, *67*, 1130.
4. B. Furman, S. Molotov, R. Thurmer, Z. Kaluza, W. Voelter, M. Chmielewski, *Tetrahedron*, **1997**, *53*, 5883.
5. M. Yoshikawa, N. Murakami, Y. Yokokawa, Y. Inoue, Y. Kuroda, I. Kitagawa, *Tetrahedron*, **1994**, *50*, 9619; M. Yoshikawa, N. Murakami, Y. Inoue, Y. Kuroda, I. Kitagawa, *Chem. Pharm. Bull*, **1993**, *41*, 1197.
6. A. Deraadt, A. E. Stutz, *Carbohydr. Res.*, **1991**, *220*, 101.
7. P. B. Anzeveno, L. J. Creemer, *Tetrahedron Lett.*, **1990**, *15*, 2085; P. B. Anzeveno, P. T. Angell, L. J. Creemer, M. R. Whalon, *Tetrahedron Lett.*, **1990**, *30*, 4321.
8. S. Takahashi, H. Kuzuhara, *Biosci. Biotech. Biochem.*, **1995**, *59*, 762; I. Bruce, G. W. J. Fleet, I. C. Di Bello, B. G. Winchester, *Tetrahedron*, **1992**, *48*, 10191.
9. B. P. Bashyal, H. F. Chow, G. W. J. Fleet, *Tetrahedron Lett.*, **1987**, *27*, 3205; B. P. Bashyal, H. F. Chow, G. W. J. Fleet, *Tetrahedron*, **1987**, *43*, 423.
10. B. P. Bashyal, H. F. Chow, L. E. Fellows, G. W. J. Fleet, *Tetrahedron*, **1987**, *43*, 415; S. Yuasa, *Clinica Chim. Acta*, **1986**, *160*, 331.
11. K. Y. Hsia, P. Ward, R. B. Lamont, P. M. de Q. Lilley, D. J. Watkin, G. W. J. Fleet, *Tetrahedron Lett.*, **1994**, *35*, 4823.
12. Y. Blériot, K. H. Smelt, J. Cadefau, M. Bollen, W. Stalmans, K. Biggadike, L. N. Johnson, N. G. Oikonomakos, A. L. Lane, S. Crook, D. J. Watkin, G. W. J. Fleet, *Tetrahedron Lett.*, **1996**, *37*, 7155; Y. Blériot, C. Veighey, K. H. Smelt, J. Cadefau, W. Stalmans, K. Biggadike, A. L. Lane, M. Muller, D. J. Watkin, G. W. J. Fleet, *Tetrahedron: Asymmetry*, **1996**, *7*, 2761.
13. Y. Blériot, C. F. Masaguer, J. Charlwood, B. G, Winchester, A. L. Lane, S. Crook, D. J. Watkin, G. W. J. Fleet, *Tetrahedron*, **1997**, *53*, 15135; C. F. Masaguer, Y. Blériot, J. Charlwood, B. G. Winchester, G. W. J. Fleet, *Tetrahedron*, **1997**, *53*, 15147.
14. M. A. Shalaby, F. R. Fronczek, D. Vargas, E. S. Younathon, *Carbohydr. Res.*, **1994**, *265*, 197.
15. 1997 Sigma catalogue lists alginic acid sodium salt at £58.50 for 1 kg; it is considerably less expensive from Avocado, at £88.00 for 2.5 kg.
16. O. Smidsrød, K. I. Draget, *Carbohydrates in Europe*, **1996**, *14*, 6.
17. W. L. Nelson, L. H. Cretcher, *J. Am. Chem. Soc.*, **1930**, *52*, 2130.
18. H. A. Spoehr, *Arch. Biochem.*, **1947**, *14*, 153.
19. R. L. Whistler, J. N. BeMiller, *Methods in Carbohydrate Chemistry*, Vol. II, Eds R. L. Whistler, M. L. Wolfram. Academic Press, 1963, p. 35.
20. J. P. Clayton, R. S. Oliver, N. H. Rogers, T. J. King, *J. Chem. Soc., Perkin Trans. 1*, **1979**, 838; J. G. Buchanan, M. E. Chacun-Fuertes, A. R. Edgar, S. J. Moorhouse, D. I. Rawson, R. H. Wightman, *Tetrahedron Lett.*, **1980**, *21*, 1793.

21. Molecular modelling was performed using a CAChe Worksystem Version 2.5 manufactured by Tektronix. Energy calculations used an MM2 force field, with a conjugate gradient to locate the energy minimum. Optimisation was continued until the change between successive iterations was less than 0.00001 kcal/mol. The energy of the final structure of **1** was 19.3980 kcal/mol.
22. T. M. Krülle, B. Davis, H. Ardron, D. D. Long, N. A. Hindle, C. Smith, D. Brown, A. L. Lane, D. J. Watkin, D. G. Marquess, G. W. J. Fleet, *J. Chem. Soc., Chem. Commun.*, **1996**, 1271.
23. A. A. Bell, L. Pickering, M. Finn, C. de la Fuente, T. M. Krülle, B. G. Davis, G. W. J. Fleet, *Synlett*, **1997**, 1077.
24. B. G. Winchester, G. W. J. Fleet, in preparation.
25. K. N. Slessor, A. S. Tracey, *Can. J. Chem.*, **1969**, *47*, 3889; D. Hall, S. A. Black, K. N. Slessor, A. S. Tracey, *Can. J. Chem.*, **1972**, *50*, 1912.
26. O. T. Schmidt, *Methods in Carbohydrate Chemistry, Vol. II*, Eds. R. L. Whistler, M. L. Wolfram. Academic Press, 1963, p. 319.
27. A. R. Beacham, I. Bruce, S. Choi, O. Doherty, A. J. Fairbanks, G. W. J. Fleet, B. M. Skead, J. M. Peach, J. Saunders, D. J. Watkin, *Tetrahedron: Asymmetry*, **1991**, *2*, 883.
28. J. S. Brimacombe, P. A. Gent, *Carbohydr. Res.*, **1970**, *12*, 1.

(Received in UK 27 October 1997)