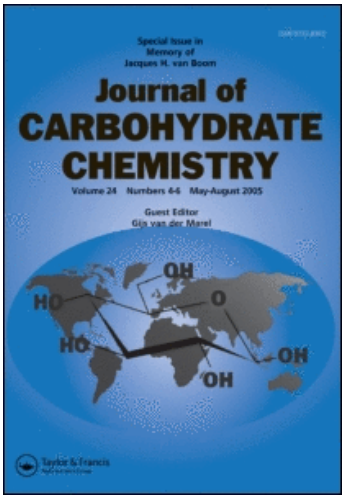

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THE SYNTHESIS OF SOME C-4 AND C-9 SUBSTITUTED DERIVATIVES OF KDN2EN METHYL ESTER

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Dedicated to Dr. Joachim Thiem on the occasion of his 60th birthday.

ABSTRACT

The synthesis of a number of C-4 and C-9 substituted derivatives of KDN2en methyl ester **2** is reported. 9-Deoxy-9-iodo, 9-azido-9-deoxy and 9-*O*-methyl derivatives of **2** (compounds **5**, **7** and **9**) were prepared from the corresponding 9-*O*-tosylate, methyl 2,6-anhydro-3-deoxy-9-*O*-*p*-toluenesulfonyl-D-*glycero*-D-*galacto*-non-2-enonate (**3**). These compounds have been fully characterised as the peracetates **6**, **8** and **10**. Treatment of **3** with KSAc gave the 9-thioacetyl derivative which was isolated as the peracetate **11**. 4-*C*-Ethenyl-4-deoxy (**14**), 4-*C*-phenyl-4-deoxy (**15**) and 4-*C*-[1-(methoxycarbonyl)ethenyl]-4-deoxy (**16**) derivatives of **2** were prepared via the palladium-catalysed coupling of the 4-*epi*-chloride, methyl 5,7,8,9-tetra-*O*-acetyl-2,6-anhydro-4-chloro-3,4-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (**12**) with the appropriate organostannanes.

INTRODUCTION

Prior to its discovery in nature some 15 years ago from the membrane polysialoglycoproteins (PSGP) of fish eggs,[1] KDN (3-deoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid) was considered to be the result of C-5 deamination

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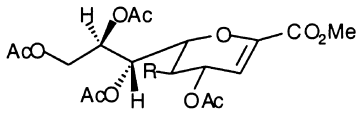
of Neu5Ac, the more ubiquitously distributed member of the ulosonic acid family. As terminal constituents of glycoproteins and glycolipids, these carbohydrates have been implicated in a diverse array of biological processes such as cell adhesion and differentiation.[2] In particular, as a terminal unit of PSGP chains, KDN plays a unique role by protecting the membrane from bacterial degradation since only sialic acid residues with an acylamino group at C-5 are cleaved by bacterial sialidases.[3] In recent years, Li and coworkers have found new sialidases that have unique KDN metabolism. For example, the sialidase, KDNase, first isolated from the liver of the loach, specifically hydrolyses KDN-bearing glycoconjugates and not *N*-acylneuraminyl linkages.[4] More recent studies have found the coexistence of two different sialidases in the starfish *Asterina pectinifera*; namely, a regular sialidase (RS) which cleaves sialic acid from sialoglycoconjugates, and a KDN-sialidase (KS) which hydrolyses KDN from KDN-containing glycoconjugates.[5] A KDN-specific sialidase has also been isolated from *Sphingobacterium multivorum*.[6,7]

The 2,3-unsaturated analogues of the ulosonic acids have received much interest in recent years as inhibitors of sialidases. For example, the C-4 nitrogen-containing Neu5Ac2en derivative, 5-acetamido-2,6-anhydro-3,4,5-trideoxy-4-guanidino-D-*glycero*-D-*galacto*-non-2-enonic acid, is a potent inhibitor of a viral sialidase.[8] Neu5Ac2en itself, but not KDN2en, is still one of the most potent inhibitors of bacterial sialidase.[5,9] However, both Neu5Ac2en and KDN2en are moderate inhibitors of KS.[5] Therefore, the synthesis of more complex derivatives and analogues of KDN is required, both for further study of the functional role of KDN-containing glycoconjugates, and for probing the role played by the unique enzymes involved in their metabolism. The synthesis of KDN has been achieved by various chemistries.[2,10,11]

We report here the synthesis of several C-4 and C-9 derivatives of the peracetylated methyl ester of KDN2en, methyl 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-3-deoxy-D-*glycero*-D-*galacto*-non-2-enonate (**1**).



1 R = OAc

RESULTS AND DISCUSSION

Our initial approach towards the synthesis of C-9 functionalised KDN2en derivatives was based on some of our earlier work on the preparation of Neu5Ac2en derivatives. [12,13] In that study we reported a facile approach into functionalised Neu5Ac2en derivatives via acetolysis of the corresponding substituted glycosides of Neu5Ac methyl ester.[12,13] Very recently we have reported the synthesis of a range of C-9 substituted derivatives of the methyl glycoside of KDN methyl ester.[14] Based on our earlier study,[12,13] we thought it reasonable

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that this series of compounds may be directly converted into the corresponding C-9 substituted KDN2en via the same acetolysis conditions. Indeed, an initial investigation was carried out using the α-methyl glycoside of KDN methyl ester, methyl (methyl 3-deoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid)onate, as a template. A cursory examination of the ^{1}H NMR spectrum of the crude product showed an insignificant amount of the desired KDN2en derivative; namely, methyl 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-3-deoxy-D-*glycero*-D-*galacto*-non-2-enonate. Interestingly, a compound consistent with the known furanosonate,[15] methyl 5-[{D-*erythro*-(1,2,3,4-tetra-*O*-acetyl-1,2,3,4-tetrahydroxy)butyl}-2-furanosid]onate was obtained as the major product (data not shown).

The failure of the acetolysis chemistry to provide us direct entry into KDN2en derivatives prompted us to search for an alternative approach to these valuable probes. As part of an investigation of the chemistry of KDN derivatives, Sun and coworkers reported that, not unlike the Neu5Ac system, the 2-β-chloride of peracetylated KDN methyl ester, methyl 4,5,7,8,9-penta-*O*-acetyl-2-chloro-3-deoxy-D-*glycero*-β-D-*glycero*-non-2-ulopyranosonate, readily undergoes base-promoted dehydrochlorination. This chemistry readily provides the 2,3-unsaturated analogue of KDN methyl ester **1** in high yield.[16] Subsequent de-*O*-acetylation of **1** under standard Zemplén conditions gave **2**. The 2,3-unsaturated analogue of KDN methyl ester **2** makes an ideal starting template for elaboration into a range of functionalised KDN2en derivatives.

Our approach to the introduction of new functionalities at C-9 of the KDN2en template required activation of the C-9 hydroxyl group. This was readily achieved by the use of well-established sulfonate ester chemistry. Accordingly, compound **2** was treated with *p*-toluenesulfonyl chloride in pyridine which resulted in selective tosylation at C-9 to provide methyl 2,6-anhydro-3,9-dideoxy-9-*p*-toluenesulfonyl-D-*glycero*-D-*galacto*-non-2-enonate (**3**), in 92% yield. The 9-*O*-tosylate **3** was further characterised as the peracetate **4**.

The readily available 9-*O*-tosylate **3** provides a common starting material for the synthesis of all of the C-9 derivatives of KDN2en in this study. Thus, nucleophilic displacement of the sulfonate ester **3** with azide at 50°C proceeded smoothly giving the corresponding 9-azide **5** which was characterised as the peracetate **6** (76%, 2 steps). Similarly, treatment of the 9-*O*-tosylate **3** with NaI in acetone under reflux gave the corresponding 9-iodide **7** which was also fully characterised as the peracetate **8** (65%, 2 steps). An attempt to displace the sulfonate ester **3** with methoxide (unoptimised) resulted in partial conversion to the desired 9-*O*-methyl derivative **9**. Compound **9** was isolated from the unreacted starting material **3** (62%) in 38% yield after column chromatography and was further characterised as the peracetate **10**.

To achieve introduction of sulfur at C-9, the 9-*O*-tosylate **3** was treated with KSAc in acetone for 2 days at rt. This resulted in the isolation of the 9-thioacetyl peracetate **11** after chromatography in high yield (95%). It was found that a prolonged reaction time gave an appreciable amount of the corresponding disulfide as a by-product (data not shown).

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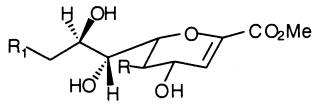
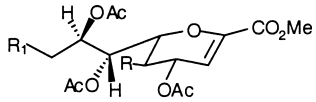
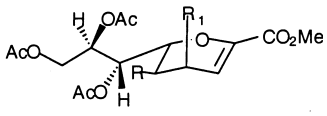


2 R_1= OH
3 R_1 = O Ts
5 R_1 = N_3
7 R_1 = I
9 R_1 = O Me



4 R_1 = OTs
6 R_1 = N_3
8 R_1 = I
10 R_1 = OMe
11 R_1 = SAc

With a selection of C-9 derivatives of KDN2en in hand, our focus turned to the preparation of C-4 modified KDN2en derivatives. Recently, we have reported the facile synthesis of C-4 halogen-substituted derivatives of Neu5Ac and KDN2en.[17] We have also shown that the 4-chloride, methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-chloro-5,7,8-trideoxy-D-*glycero*-D-*talo*-non-2-enonate can be elaborated into a range of C-4 *C*-branched 2,3-unsaturated Neu5Ac derivatives by using a palladium(0)-mediated coupling with different organostannanes.[18] In analogous reactions, using the readily accessible 4-*epi*-chloride of KDN2en, methyl 5,7,8,9-tetra-*O*-acetyl-2,6-anhydro-4-chloro-3,4-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (**12**) as a coupling partner, we have successfully prepared several C-4 *C*-branched derivatives of KDN2en. Not unexpectedly, this reaction results in inversion of stereochemistry at C-4, as is evident from the smaller $^3J_{3,4}$ coupling constant for the D-*glycero*-D-*galacto* configuration. As representative examples of this reaction we selected the readily available organnostannanes, tributylvinyltin, tributylphenyltin and methyl α-(tributylstannyl)propenoate.[18,19] Accordingly, 4-*C*-ethenyl-4-deoxy, 4-*C*-phenyl-4-deoxy and 4-*C*-[1-(methoxycarbonyl)ethenyl]-4-deoxy derivatives, **14**–**16**, have been prepared in modest yields. Interestingly, while the reaction with either tributylvinyltin and methyl α-(tributylstannyl)propenoate gave only the coupled products, the same reaction with tributylphenyltin also gave 20% of the reduced product, methyl 5,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,4-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate (**17**), which was shown to be identical to known material.[16]



12 R_1 = Cl
13 R_1 = OAc

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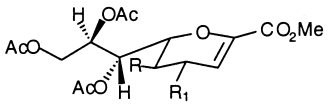

14 $R_1 = CH{=}CH_2$
15 $R_1 = Ph$
16 $R_1 = C(CO_2Me){=}CH_2$
17 $R_1 = H$

In conclusion, a range of C-9 and C-4 modified KDN2en derivatives have been prepared from the 2,3-unsaturated analogue of KDN methyl ester. These compounds, upon deprotection, may provide useful probes for KDN-recognising proteins.

EXPERIMENTAL

General Methods. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra (in δ ppm) were recorded on a Bruker AMX300 spectrometer at 303 K and were referenced using solvent residues. *J*-Values are in hertz (Hz). Both low resolution (LR) and high resolution (HR) fast atom bombardment (FAB) mass spectra were obtained on a JEOL JMS-DX 300 spectrometer. Specific optical rotations $[\alpha]_D$, quoted in 10^{-1} deg cm^2 g^{-1}, were measured at room temperature using a JASCO DIP-370 polarimeter with a path length 50 mm. Concentrations are quoted in 10^{-2} g cm^{-3}. Elemental analyses were performed by the Chemical and Microanalytical Service, Essendon, Victoria. IR spectra were recorded using a Hitachi 270-30 spectrophotometer. Column chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). Reactions were monitored by TLC on Kieselgel 60 F_{254} plates (Merck 5554) and the spots were detected under ultraviolet (UV) irradiation and by spraying with 95% aq ethanol containing 5% H_2SO_4 and charring for several minutes at 180°C. Triphenylphosphine (Aldrich) and bis(dibenzylideneacetone)palladium(0) (Lancaster Synthesis) were used as received. Chloroform was put through a short plug of basic alumina just prior to use. All reactions were performed under anhydrous conditions in flame-dried glassware under an atmosphere of nitrogen or argon.

Preparation of the Organostannanes. Methyl α-(tributylstannyl) propenoate was prepared from methyl propynoate according to the literature.[18,19] Tributylphenyltin (Fluka) and tributylvinyltin (Aldrich) were used as supplied, without prior purification.

Preparation of the Starting Materials. KDN was prepared by aldolase (EC 4.1.3.3) condensation of D-mannose and pyruvate according to the literature,[20] with slight modification. Rather than using an immobilised system, the enzyme was contained within a dialysis bag. Methanolysis of KDN and subsequent treatment of the resulting methyl ester with neat AcCl gave the 2-β-chloride, methyl 4,5,7,8,9-penta-*O*-acetyl-2-chloro-3-deoxy-D-*glycero*-β-D-*glycero*-non-2-ulopy-

ranosonate. DBU-promoted dehydrochlorination of the latter compound according to the literature,[16] followed by chromatography (EtOAc/hexane, 1:1) gave the p eracetylated KDN2en methyl ester, methyl 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-3-deoxy-D-*glycero*-D-*galacto*-non-2-enonate (**1**). Standard Zemplén de-*O*-acetylation (NaOMe/MeOH) of **1** afforded **2** in 97% yield. The 4-*epi*-chloride, methyl 5,7,8,9-tetra-*O*-acetyl-2,6-anhydro-4-chloro-3,4-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (**12**) was prepared from a 1:1 epimeric mixture of the peracetylated methyl esters of KDN2en, methyl 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-3-deoxy-D-*glycero*-D-*galacto*-non-2-enonate (**1**) and methyl 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-3-deoxy-D-*glycero*-D-*talo*-non-2-enonate (**13**) in 56% yield, according to the method as previously described.[16]

General Procedure for Acetylation. Ac_2O (0.5 mL) and DMAP (ca. 5 mg) were added to a sample of the compound (amount as specified) to be acetylated in pyridine (1 mL). After stirring at rt for 16 h, the mixture was concentrated and the residue purified by column chromatography on silica using the solvent(s) specified.

General Procedure for Palladium-Catalysed Cross-Coupling Reactions of the Chloride 12 and Organostannanes. To a solution of **12** (100 mg, 0.022 mmol) in chloroform (5 mL) was added triphenylphosphine (3.5 mg, approx. 6 mol%) and bis(dibenzylideneacetone)palladium(0) (4 mg, approx. 3 mol%). The tin reagent (0.34 mmol, 1.5 mol equiv) was then added and the resulting solution heated under reflux for 18 h. The reaction mixture was cooled; saturated aq KF (5 mL) was added and the mixture stirred for 24 h. The solids were filtered off and concentrated. The residue was extracted with ether (20 mL × 3), the ethereal extracts combined and washed successively with H_2O and brine, and dried ($MgSO_4$). After removal of the volatiles, the residue obtained was purified by chromatography on silica. The product was eluted using a combination of EtOAc and hexane (1:1).

Methyl 2,6-anhydro-3-deoxy-9-*O*-*p*-toluenesulfonyl-D-*glycero*-D-*galacto*-non-2-enonate (3). A mixture of **1** (1.20 g; 4.53 mmol) and *p*-TsCl (1.34 g; 7.03 mmol) in pyridine (20 mL) was stirred at 0 °C for 2 h. More *p*-TsCl (0.25 g; 1.36 mmol) was added and the mixture left to stir at 5 °C for a further 10 h. MeOH (20 mL) was added and the mixture concentrated. The residue was purified by column chromatography (EtOAc/*i*-PrOH/H_2O, 6:2:1) which gave the 9-*O*-tosylate **3** as a colourless amorphous mass (1.74 g; 92%): $[\alpha]_D$ −11.6° (*c* 5.63, MeOH); ^{1}H NMR (D_2O) δ 2.47 (3 H, s, Ph-CH_3), 3.76 (1 H, dd, $J_{9,8}$ 7.9, $J_{9,9'}$ 10.4, H-9), 3.82 (3 H, s, $COOCH_3$), 3.85 (1 H, dd, $J_{6,5}$ 9.5, $J_{6,7}$ 2.1, H-6), 3.99 (1 H, dd, $J_{9',8}$ 1.0, H-9′), 4.10 (1 H, dd, $J_{4,5}$ 10.4, H-5), 4.30 (1 H, dd, $J_{8,7}$ 5.3, H-8), 4.41 (1 H, dd, H-7), 4.44 (1 H, dd, $J_{4,3}$ 2.6, H-4), 5.97 (1 H, d, H-3), 7.50–7.88 (4 H, m, Ph); ^{13}C NMR (D_2O) δ 21.8 (Ph-*C*H_3), 52.9 ($COOCH_3$), 68.3, 68.5, 69.3, 69.8, 77.6 (C-4, C-5, C-6, C-7, C-8), 72.6 (C-9), 112.6 (C-3), 128.3, 130.3, 132.9, 143.5 (aromatic carbons), 145.3

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(C-2), 163.5 (carbonyl); IR (υ_{max}, KBr): 3448, 1731, 1653, 1446, 1356, 1257, 1173, 1092, 972, 903, 972, 903, 810 cm^{-1}; LRMS *m/z*: 419 (MH^+, 6%), 383 (31), 365 (37), 351 (51), 257 (53), 227 (39), 211 (100).

Methyl 4,5,7,8-tetra-*O*-acetyl-2,6-anhydro-3-deoxy-9-*O*-*p*-toluenesulfonyl-D-*glycero*-D-*galacto*-non-2-enonate (4). Acetylation of **3** (151 mg, 0.36 mmol) according to the general acetylation conditions gave, after chromatography (EtOAc/hexane, 1:1), **4** as a colourless amorphous mass (202 mg; 99%): $[\alpha]_D$ +21.2° (*c* 6.00, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.00, 2.02, 2.04, 2.05 (each 3 H, s, $OCOCH_3 \times 4$), 2.45 (3 h, s, Ph-CH_3), 3.82 (3 H, s, $COOCH_3$), 4.15 (1 H, dd, $J_{9,8}$ 6.6, $J_{9,9'}$ 11.4, H-9), 4.29 (1 H, dd, $J_{6,5}$ 9.9, $J_{6,7}$ 2.7, H-6), 4.69 (1 H, dd, $J_{9',8}$ 2.7, H-9′), 5.17 (1 H, dd, $J_{5,4}$ 7.2, H-5), 5.29 (1 H, dd, $J_{8,7}$ 5.1, H-8), 5.49 (1 H, dd, H-7), 5.58 (1 H, dd, $J_{4,3}$ 2.8, H-4), 5.95 (1 H, d, H-3); ^{13}C NMR ($CDCl_3$) δ 20.7, 20.9, 21.0 ($OCOCH_3 \times 4$), 21.9 (Ph-CH_3), 52.9 ($COOCH_3$), 65.8, 67.1, 69.3, 70.5 (C-5, C-6, C-7, C-8), 67.4 (C-9), 108.3 (C-3), 128.3, 130.1, 132.5, 145.2 (aromatic carbons), 145.6 (C-2), 169.9, 170.7 (carbonyls); IR (υ_{max}, KBr): 2998, 1740, 1660, 1440, 1378, 1268, 1178, 1110, 1048, 1024, 984, 821, 786, 660 cm^{-1}; LRMS: *m/z*, 587 (MH^+, 5%), 527 (100), 485 (21), 453 (26), 425 (31), 371 (21), 365 (100);

Anal. Calcd for $C_{25}H_{30}O_{14}S$: C, 51.19; H, 5.16. Found: C, 51.11; H, 5.21.

Methyl 4,5,7,8-tetra-*O*-acetyl-2,6-anhydro-9-azido-3,9-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate (5). To a solution of the 9-*O*-tosylate **3** (133 mg, 0.32 mmol) in DMF (10 mL) was added LiN_3 (93 mg, 1.91 mmol) and the resulting solution heated at 50°C for 3.5 days. The reaction mixture was concentrated to dryness and the residue acetylated according to the general conditions. After column chromatography (EtOAc/hexane, 1:1), the title compound **5** was obtained as a colourless amorphous mass (110 mg; 76%): $[\alpha]_D$ +30.4° (*c* 3.17, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.01, 2.04, 2.07, 2.09 (each 3 H, s, $OCOCH_3 \times 4$), 3.46 (1 H, dd, $J_{9,8}$ 7.4, $J_{9,9'}$ 13.5, H-9), 3.82 (3 H, s, $COOCH_3$), 3.87 (1 H, dd, $J_{9',8}$ 2.9, H-9′), 4.34 (1 H, dd, $J_{6,5}$ 9.8, $J_{6,7}$ 2.8, H-6), 5.23 (2 H, m, H-5, H-8), 5.49 (1 H, dd, $J_{7,8}$ 4.5, H-7), 5.59 (1 H, dd, $J_{4,3}$ 2.8, $J_{4,5}$ 7.2, H-4), 5.97 (1 H, d, H-3); ^{13}C NMR ($CDCl_3$) δ 20.8, 20.9, 21.0 ($OCOCH_3 \times 4$), 50.6 (C-9), 52.9 ($COOCH_3$), 65.9, 67.8, 69.4, 72.2 (C-5, C-6, C-7, C-8), 108.3 (C-3), 145.6 (C-2), 170.0, 170.1 (carbonyls); IR (υ_{max}, KBr): 2104, 1752, 1660, 1438, 1370, 1234, 1146, 1110, 1062, 1022, 972, 936 cm^{-1}; LRMS: *m/z*, 475 (MNH_4^+, 100%), 415 (8), 398 (6);

Anal. Calcd for $C_{18}H_{23}N_3O_{11}$: C, 47.27; H, 5.07; N, 9.19. Found: C, 47.19; H, 4.95; N, 8.97.

Methyl 4,5,7,8-tetra-*O*-acetyl-2,6-anhydro-3,9-dideoxy-9-*O*-iodo-D-*glycero*-D-*galacto*-non-2-enonate (8). A mixture of the 9-*O*-tosylate **3** (108 mg, 0.26 mmol), NaI (154 mg, 1.03 mmol) and acetone (20 mL) was heated under reflux for 3.5 days. The mixture was cooled and the solids filtered off. The resulting solution was concentrated to dryness which gave crude methyl 2,6-anhydro-3,9-dideoxy-9-*O*-iodo-D-*glycero*-D-*galacto*-non-2-enonate (**7**) (96 mg; 99%).

The 9-iodide **7** (96 mg, 0.26 mmol) was acetylated under the general acetylation conditions which, after chromatography (EtOAc/hexane, 1:1), afforded the title compound **8** (93 mg; 66%).

7: ^{1}H NMR (D_2O) δ 3.63 (1 H, dd, $J_{9,9'}$ 10.9, $J_{9,8}$ 5.4, H-9), 3.76 (1 H, dd, $J_{9',8}$ 2.3, H-9′), 3.83 (1 H, dd, $J_{6,5}$ 8.9, $J_{6,7}$ 2.2, H-6), 3.92 (1 H, m, H-8), 3.92 (3 H, s, $COOCH_3$), 4.00 (1 H, pseudo t, $J_{7,9}$, H-5), 4.34 (1 H, m, H-7), 4.61 (1 H, dd, $J_{4,3}$ 2.2, H-4), 6.09 (1 H, d, H-3); IR (υ_{max}, KBr): 3440, 1722, 1650, 1438, 1272, 1136, 1076 cm^{-1}.

8: $[\alpha]_D$ +42.1° (*c* 1.76, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.04, 2.06, 2.10, 2.11 (each 3 H, s, $OCOCH_3 \times 4$), 3.25 (1 H, dd, $J_{9,8}$ 8.5, $J_{9,9'}$ 11.2, H-9), 3.82 (3 H, s, $COOCH_3$), 3.89 (1 H, dd, $J_{9',8}$ 3.0, H-9′), 4.33 (1 H, dd, $J_{6,5}$ 9.6, $J_{6,7}$ 2.9, H-6), 5.14 (1 H, m, H-8), 5.20 (1 H, dd, $J_{5,4}$ 7.1, H-5), 5.43 (1 H, dd, $J_{7,8}$ 4.3, H-7), 5.58 (1 H, dd, $J_{4,3}$ 3.0, H-4), 5.96 (1 H, d, H-3); ^{13}C NMR ($CDCl_3$) δ 2.7 (C-9), 20.8, 20.9, 21.0 ($OCOCH_3 \times 4$), 52.9 ($COOCH_3$), 66.0, 68.9, 69.3, 72.9, 76.3 (C-4, C-5, C-6, C-7, C-8), 108.2 (C-3), 145.6 (C-2), 170.0, 170.6 (carbonyls); IR (υ_{max}, KBr): 1746, 1662, 1438, 1370, 1236, 1148, 1110, 1062, 1022, 970, 934, 910, 590 cm^{-1}; LRMS: *m/z*, 560 (MNH_4^+, 100%), 531 (18), 423 (10), 374 (20), 321 (11), 297 (16);

Anal. Calcd for $C_{18}H_{23}O_{11}I$: C, 39.87; H, 4.28. Found: C, 40.02; H, 4.38.

Methyl 4,5,7,8-tetra-*O*-acetyl-2,6-anhydro-3,9-dideoxy-9-*O*-methyl-D-*glycero*-D-*galacto*-non-2-enonate (10). NaOMe (2 mL of a 0.04 M solution in MeOH, 0.8 mmol) was added to a stirring solution of the 9-*O*-tosylate **3** (108 mg, 0.26 mmol) in MeOH (6 mL). After 30 min, a further 2 mL of the NaOMe solution was added and the solution stirred for a further 30 min. Dowex 50W (H^+) resin was added to pH 2 and the mixture stirred for 1 h. After filtration, all volatiles were removed *in vacuo* and the residue purified by chromatography (EtOAc/hexane, 1:15) to give methyl 2,6-anhydro-3,9-dideoxy-9-*O*-methyl-D-*glycero*-D-*galacto*-non-2-enonate (**9**) as a colourless amorphous mass (27 mg; 38%) and unreacted starting material (68 mg; 62%).

Acetylation of **9** (27 mg, 0.1 mmol) according to the general procedure gave, after chromatography (EtOAc/hexane, 1:1), the title compound **10** as a colourless amorphous mass (22 mg; 50%): $[\alpha]_D$ +23.8° (*c* 1.73, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.03, 2.05, 2.06, 2.08 (each 3 H, s, $OCOCH_3 \times 4$), 3.35 (3 H, s, OCH_3), 3.48 (1 H, dd, $J_{9,8}$ 6.0, $J_{9,9'}$ 10.9, H-9), 3.80 (3 H, s, $COOCH_3$), 3.82 (1 H, dd, $J_{9',8}$ 5.9, H-9′), 4.36 (1 H, dd, $J_{6,5}$ 9.0, $J_{6,7}$ 3.5, H-6), 5.22 (1 H, dd, $J_{5,4}$ 6.5, H-5), 5.30 (2 H, m, $J_{7,8}$ 5.7, H-7, H-8), 5.52 (1 H, dd, $J_{4,3}$ 3.1, H-4), 5.96 (1 H, d, H-3); ^{13}C NMR ($CDCl_3$) δ 21.0 ($OCOCH_3 \times 4$), 52.7, 59.4 (OCH_3, $COOCH_3$), 66.2, 67.7, 68.9, 70.6, 70.9 (C-4, C-5, C-6, C-7, C-8), 107.8 (C-3); IR (υ_{max}, KBr): 3550, 1756, 1742, 1656, 1458, 1438, 1372, 1310, 1252, 1144, 1110, 1062, 1046, 1034, 972 cm^{-1}; LRMS: *m/z*, 469 (MNa^+, 30%), 391 (8), 257 (11), 240 (62)

Anal. Calcd for $C_{19}H_{26}O_{12}$: C, 51.12; H, 5.87. Found: C, 51.07; H, 5.78.

Methyl 4,5,7,8-tetra-*O*-acetyl-9-*S*-acetyl-2,6-anhydro-3,9-dideoxy-9-thio-D-*glycero*-D-*galacto*-non-2-enonate (11). KSAc (20 mg, 0.45 mmol) was added to a stirred mixture of the 9-*O*-tosylate **3** (64 mg, 0.15 mmol) and acetone

(5 mL). After 2 days at rt, the solids were filtered off and the resulting solution concentrated. The residue was acetylated according to the general conditions which gave, after chromatography (hexane/acetone, 1:1), the title compound **11** as a colourless amorphous mass (71 mg; 95%): $[\alpha]_D$ +33.3° (*c* 4.45, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.00, 2.03, 2.04, 2.10 (each 3 H, s, $OCOCH_3$ × 4), 2.31 (1 H, dd, $J_{9,8}$ 8.2, $J_{9,9'}$ 14.5, H-9), 3.67 (1 H, dd, $J_{9',8}$ 3.2, H-9′), 3.81 (3 H, s, $COOCH_3$), 4.35 (1 H, dd, $J_{6,5}$ 8.7, $J_{6,7}$ 3.6, H-6), 5.28 (2 H, m, H-5, H-8), 5.43 (1 H, dd, $J_{7,8}$ 5.1, H-7), 5.52 (1 H, dd, $J_{4,3}$ 3.2, $J_{4,5}$ 6.4, H-4), 5.96 (1 H, d, H-3); ^{13}C NMR ($CDCl_3$) δ 20.9 ($OCOCH_3$ × 4), 29.3 (C-9), 52.8 ($COOCH_3$), 66.3, 66.5, 68.3, 70.8, 76.0 (C-4, C-5, C-6, C-7, C-8), 107.8 (C-3), 169.8 (carbonyls); IR (υ_{max}, KBr): 1750, 1698, 1436, 1370, 1226, 1108, 1038, 1024, 974, 944, 622 cm^{-1};

Anal. Calcd for $C_{20}H_{26}O_{12}S.0.25CHCl_3$: C, 46.75; H, 5.09. Found: C, 46.93; H, 5.10.

Methyl 5,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,4-dideoxy-4-*C*-ethenyl-D-*glycero*-D-*galacto*-non-2-enonate (14). According to the general coupling conditions, **12** and tributylvinyltin gave **14** in 29% yield; $[\alpha]_D$ +16.9° (*c* 0.90, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.03, 2.07, 2.08, 2.09 (each 3 H, s, $OCOCH_3$ × 4), 3.09 (1 H, ddd, $J_{3,4}$ 2.5, $J_{4,5}$ 9.4, $J_{4,10}$ 9.4, H-4), 3.80 (3 H, s, $COOCH_3$), 4.15 (1 H, dd, $J_{6,5}$ 9.6, $J_{6,7}$ 2.0, H-6), 4.20 (1 H, dd, $J_{9,8}$ 6.8, $J_{9,9'}$ 12.5, H-9), 4.68 (1 H, dd, $J_{9',8}$ 2.6, H-9′), 4.98 (1 H, dd, H-5), 5.14 (1 H, d, $J_{11,11'}$ 9.5, H-11), 5.19 (1 H, d, $J_{10,11}$ 16.6, H-10), 5.37 (1 H, dd, $J_{8,7}$ 5.5, H-8), 5.50 (1 H, dd, H-7), 5.62 (1 H, m, H-11′), 5.93 (1 H, d, H-3); ^{13}C NMR ($CDCl_3$) δ 20.6, 20.8 ($OCOCH_3$ × 4), 44.8 (C-4), 52.3 ($COOCH_3$), 62.1 (C-9), 66.5, 67.1, 70.8, 76.1 (C-5, C-6, C-7, C-8), 111.8 (C-3), 118.4 (C-10), 135.7 (C-11), 143.4 (C-2), 169.7, 169.9, 170.1, 170.6 (carbonyls); IR (υ_{max}, KBr): 1746, 1700, 1258 cm^{-1}; LRMS: *m/z*, 443 (MH^+, 8%), 383 (28), 291 (100);

HRMS: Calcd for $C_{20}H_{27}O_{11}$:[M^+ + 1], 443.1553. Found: *m/z*, 443.1527.

Anal. Calcd for $C_{20}H_{26}O_{11}.1.5H_2O$: C, 51.17; H, 6.23. Found: C, 51.27; H, 6.01.

Methyl 5,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,4-dideoxy-4-*C*-phenyl-D-*glycero*-D-*galacto*-non-2-enonate (15). According to the general coupling conditions, **12** and phenyltributyltin gave **15** in 32% yield; $[\alpha]_D$ +19.3° (*c* 4.46, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.88, 2.02, 2.05, 2.07 (each 3 H, s, $OCOCH_3$ × 4), 3.65 (1 H, dd, $J_{4,3}$ 2.5, $J_{4,5}$ 9.3, H-4), 3.80 (3 H, s, $COOCH_3$), 4.19 (1 H, dd, $J_{9,8}$ 6.9, $J_{9,9'}$ 12.5, H-9), 4.30 (1 H, dd, $J_{6,5}$ 9.8, $J_{6,7}$ 2.1, H-6), 4.68 (1 H, dd, $J_{9',8}$ 2.6, H-9′), 5.13 (1 H, dd, H-5), 5.38 (1 H, dd, $J_{8,7}$ 5.4, H-8), 5.48 (1 H, dd, H-7), 6.10 (1 H, d, H-3); ^{13}C NMR ($CDCl_3$) δ 20.4, 20.6, 20.7, 20.8 ($OCOCH_3$ × 4), 46.2 (C-4), 52.3 ($COOCH_3$), 62.2 (C-9), 67.2, 68.3, 70.9, 76.9 (C-5, C-6, C-7, C-8), 112.9 (C-3), 127.8, 127.9, 128.8, 138.8 (aromatic carbons), 144.0 (C-2), 162.0, 168.8, 169.9, 170.1, 170.5 (carbonyls); IR (υ_{max}, KBr): 2968, 1854, 1438, 1136, 1098, 964, 946, 760, 698 cm^{-1}; LRMS: *m/z*, 493 (MH^+, 11%), 433 (28), 357 (46), 271 (45), 253 (21), 215 (96), 185 (100);

HRMS: Calcd for $C_{24}H_{29}O_{11}$:[M^+ + 1], 493.1710. Found: *m/z*, 493.1719.

Methyl 5,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,4-dideoxy-4-*C*-[1-(methoxycarbonyl)ethenyl]-D-*glycero*-D-*galacto*-non-2-enonate (16). The reaction of **12** with (*Z*)-methyl 1-(tributylstannyl)propenoate, according to the general coupling conditions, gave 49% **16** and 20% of the 4-deoxy compound, methyl 5,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,4-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate (**17**).

16: $[\alpha]_D$ +53.9° (*c* 2.17, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.00, 2.06, 2.07, 2.08 (each 3 H, s, $OCOCH_3 \times 4$), 3.69 (1 H, dd, $J_{4,3}$ 2.6, $J_{4,5}$ 9.0, H-4), 3.76, 3.80 (each 3 H, s, $COOCH_3 \times 2$), 4.21 (1 H, dd, $J_{9,8}$ 6.4, $J_{9,9'}$ 12.5, H-9), 4.21 (1 H, dd, $J_{6,5}$ 9.2, $J_{6,7}$ 2.2, H-6), 4.66 (1 H, dd, $J_{9',8}$ 2.5, H-9′), 5.14 (1 H, dd, H-5), 5.39 (1 H, dd, $J_{8,7}$ 5.8, H-8), 5.51 (1 H, dd, H-7), 5.78 (1 H, s, H-11), 5.93 (1 H, d, H-3), 6.32 (1 H, s, H-11′); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.7, 20.8 ($OCOCH_3 \times 4$), 42.4 (C-4), 52.1, 52.3 ($COOCH_3 \times 2$), 62.1 (C-9), 66.8, 67.1, 70.6, 76.4 (C-5, C-6, C-7, C-8), 112.3 (C-3), 128.0 (C-11), 138.7 (C-10), 143.4 (C-2), 161.9, 166.1, 169.8, 170.0, 170.5 (carbonyls); IR (υ_{max}, KBr): 2964, 1658, 1628, 1438, 1310, 1092, 988, 952 cm^{-1}; LRMS: *m/z*, 501 (MH^+, 16%), 441 (75), 279 (51), 265 (26), 223 (100);

HRMS: Calcd for $C_{22}H_{29}O_{13}$:[M^+ + 1], 501.1608. Found: *m/z*, 501.1631.

17: physical and spectral data were consistent with the literature.[16]

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