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New Strategy in the Synthesis of 3–Deoxy–D-manno–2–octulosonic acid (KDO), 2–Deoxy–KDO and Thioglycoside of KDO

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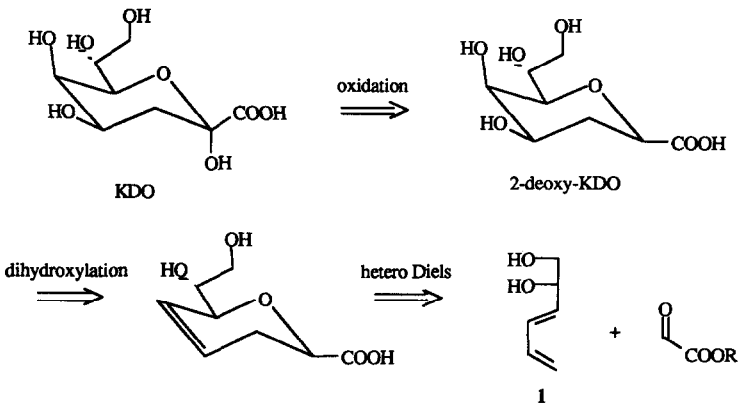
Abstract: To take advantage of synthetic intermediates on the way to KDO, we developed a new strategy based on an aqueous hetero Diels-Alder reaction with a water-soluble diene derived from D-glyceraldehyde, followed by a dihydroxylation of the newly created double bond. The stereoselectivities of these reactions were investigated to give rise to 2-deoxy-KDO, which was sulfenylated via the enolate to yield β-thioglycoside, the hydrolysis of which led to KDO.

Introduction

The eight-carbon monosaccharide 3-deoxy-D-manno-2-octulosonic acid (KDO) is an essential constituant of the outer membrane lipopolysaccharide (LPS) of all Gram-negative bacteria.[1] KDO has also been found in the primary cell wall of higher plants,[2] but has never been detected in Gram-positive bacteria.[3] While Gram-positive strains are susceptible to several antibacterial agents, Gram-negative bacteria are more difficult to combat. Since the incorporation of KDO appears to be a vital step in growth of Gram-negative bacteria, KDO seems to be an important target for the design of successful inhibitors for enzymatic cell wall assembly. There are several biosynthetic steps involved, but most interest has been focused on CMP-KDO synthetase. This synthetase is considered to be the enzyme of the rate-limiting step in the biosynthetic incorporation of KDO into LPS.[4] The preparation of KDO analogues as potent inhibitors of this enzyme includes the preparation of 2-deoxy-β-KDO and derivatives, known to be such inhibitors.[5-7] The importance of 2-deoxy-KDO comes from the possibility to use it as a precursor[8-10] of glycosyl donors of KDO, leading to a large diversity of compounds.

Several total syntheses of KDO have been previously reported[11-13] but none goes through 2-deoxy-KDO as a synthetic intermediate. Our strategy is based upon three key-steps leading to an activated form of KDO. This goal could be achieved via an hetero Diels-Alder reaction, in which C_2-C_3 and C_6-O bonds would be created (Scheme 1). To our knowledge, this strategy, which differs from the one developed by David[14] and Danishefsky,[13c] was never used for the construction of carbohydrates.[15] The second step is the cis dihydroxylation of the newly created double bond; the third one deals with the oxidation of an 2-deoxy-ulosonic acid, which still remains a problem in carbohydrate chemistry.

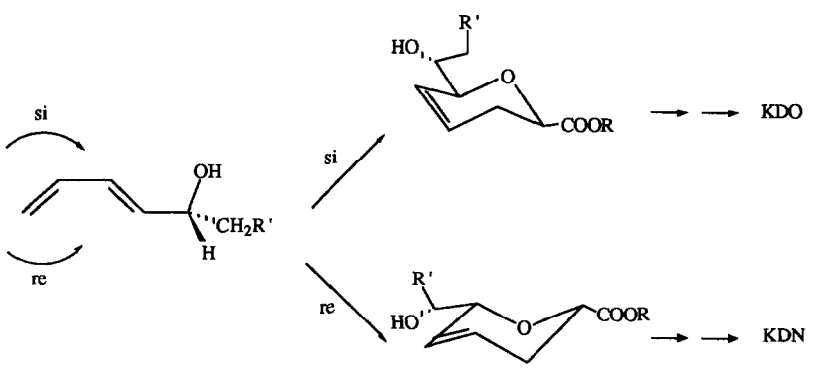


Scheme 1: Retrosynthetic analysis

Results and discussion

Hetero Diels-Alder Reaction

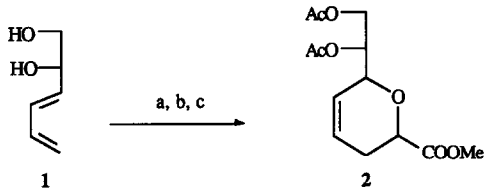
The facial selectivity of the heterocycloaddition was controlled by the asymmetric carbon of the diene **1**, prepared in 3 steps from 2,3-isopropylidene-D-glyceraldehyde; we have previously shown[16] that such a diene which could be considered as the simplest diene obtainable from the simplest sugar, induced *si* selectivity when reacting with acrolein. With glyoxylate as the dienophile, this selectivity should afford the configuration of KDO at the 6 position. The reversal of the face selectivity, i.e. the attack on the *re* face of the diene **1**, could lead to the skeleton of sialic acid and 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN), a naturally-occurring deaminated sialic acid (Scheme 2). The large coupling constant ($J_{6,7}$ ~ 8 Hz) observed in most KDO derivatives contrasts with the small one ($J_{6,7}$ ~ 4 Hz) observed in KDN and sialic acid derivatives.[17] This observation allows us to assign, at the outset, the absolute configuration of the cycloadducts.



Scheme 2: Facial selectivity in the hetero Diels-Alder reaction

Relying on the convenience of the water-promoted hetero Diels-Alder reaction with glyoxylic acid[18], we have studied the reactivity of the diene **1** towards this electrophile. The aqueous solution of glyoxylic acid was used at different pH by progressive addition of sodium hydroxide to the commercial solution. No cycloadducts

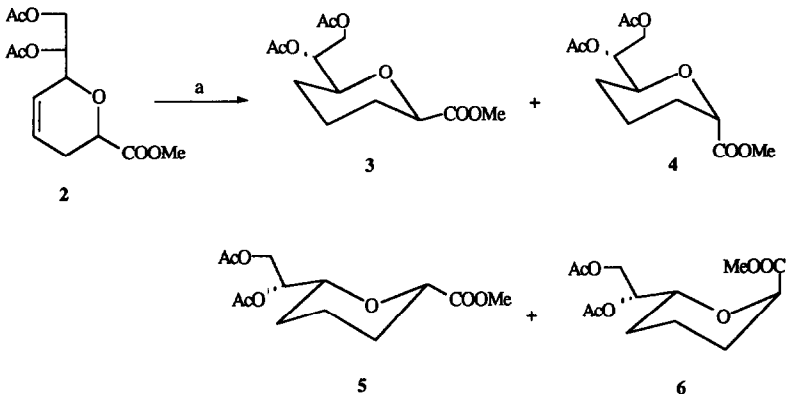
were detected under acidic conditions, because of the sensitivity of the diene **1**. As a matter of fact, the aqueous hetero Diels-Alder reaction (Scheme 3) of the water-soluble diene **1** with sodium glyoxylate (pH 6) went to completion at 110°C within 18 hours, giving after esterification, in 54 % yield, an inseparable mixture **2** of four cycloadducts in the proportions 42/32/19/7 as indicated by ^{1}H and ^{13}C NMR spectroscopies.



1 2

Scheme 3: a. $HCOCOO^{-}Na^{+}$, H_2O; b. MeOH, H^{+}; c. Ac_2O, pyridine

The facial selectivity of the reaction (*si/re* = 74/26) was determinated as following. The mixture **2** was hydrogenated (H_2, Pd/C) to afford a new mixture of four products in the same proportions (Scheme 4). Three fractions could be isolated by flash chromatography, the minor one being a mixture of two diastereomers. The coupling constants in the ^{1}H NMR spectrum of the first isomer **3** ($J_{2,3}$ 11 and 2.5 Hz, $J_{6,7}$ 6.5 Hz) indicated this compound was issued from an *endo, si* transition state. The stereochemistry of the second isomer was assigned as depicted in structure **4**, resulting from an *exo, si* transition state, based upon the coupling constants ($J_{2,3}$ 4 and 2 Hz, $J_{6,7}$ 6.5 Hz). The minor fraction was a mixture of diastereomers **5** and **6** arising from *endo, re* and *exo, re* transition states, as indicated by ^{1}H NMR spectrum ($J_{6,7}$ 4 Hz).



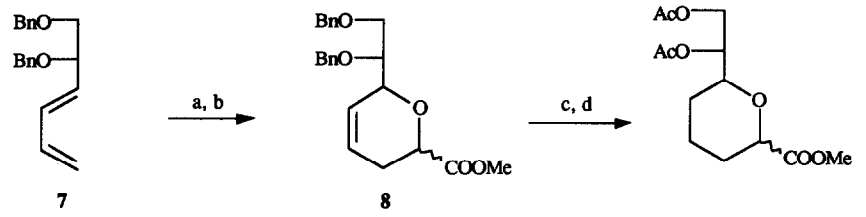
2 3 4 5 6

Scheme 4: a. H_2, Pd/C

The heterocycloaddition was also investigated under several other conditions for the purposes of comparison, by modification of the solvent, the glyoxylate and the protecting groups attached to the diene **1**. The best result was obtained when the diene **7** resulting from benzylation (NaH, $PhCH_2Br$, 97 % yield) of the diene **1**, was allowed to react at 130°C with butyl glyoxylate in neat conditions (Scheme 5).

The temperature required for that reaction is 20°C higher than the one required for the aqueous phase reaction, but the yield (60%) is improved to some slight extent; however, compared to the aqueous cycloaddition, these conditions required the tedious preparation of alkyl glyoxylate. In order to ascertain the stereochemistries of the cycloadducts, the mixture was hydrogenated (H_2, Pd/C), then acetylated (Ac_2O, pyridine) to afford four

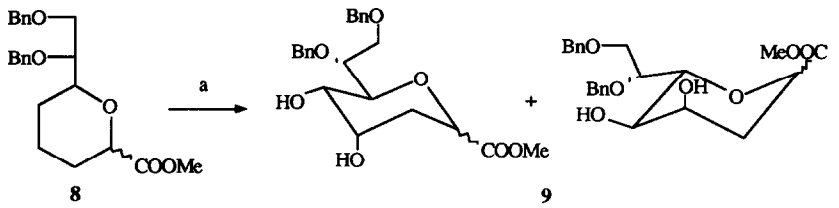
diastereomers in the proportions 50/24/16/10, as indicated by ^{1}H and ^{13}C NMR spectroscopies. The facial selectivity was found to be identical to the preceding one.



Scheme 5: a. HCOCOOBu, 18 h, 130°C; b. MeOH, TsOH; c. H_2, Pd/C; d. Ac_2O, pyr.

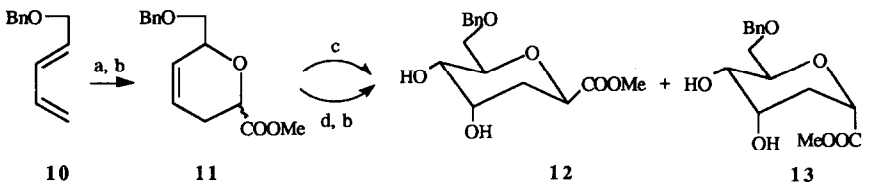
Dihydroxylation and functionalisation

The mixture of benzylated cycloadducts **8** was hydroxylated with osmium tetraoxide as catalyst in the presence of N-methylmorpholine oxide to afford an inseparable mixture of four diols in 96 % yield (Scheme 6).



Scheme 6: a. OsO_4 cat., NMO, acetone-water

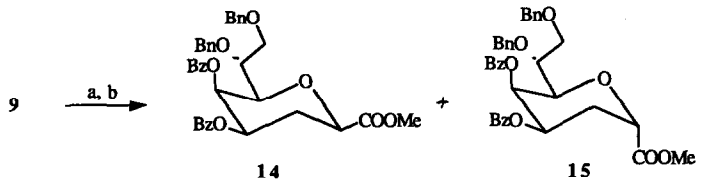
The total stereospecificity of the reaction (hydroxylation onto the *si* face of the *si* cycloadducts, onto the *re* face of the *re* cycloadducts), which should require a bis-inversion to attain the configuration of KDO derivatives, was revealed by the small coupling constants $J_{4,5}$ detected in the ^{1}H NMR spectrum of the mixture. However, in order to confirm this salient feature, we have studied the dihydroxylation on a simplified model. For this purpose, the cycloadducts **11**, isolated as a mixture of *endo* and *exo* isomers in the ratio 66:34, were transesterified (CH_3ONa/CH_3OH) without epimerisation, then subjected to osmylation producing in 95 % yield a mixture of diols. Its ^{1}H NMR spectrum allowed us to ascertain the stereochemistries as depicted in structures **12** and **13** (Scheme 7), which confirmed that the dihydroxylation occurred on the least hindered face of the olefin.



Scheme 7: a. HCOCOOBu, 18 h, 130°C; b. MeONa, MeOH; c. OsO_4, NMO, 3.5 h; d. AgOAc, I_2, 4.5 h

At this stage, it was interesting to attempt the Prévost-Winstein-Woodward reaction which gave generally the reversal of face selectivity. The reaction was conducted according to the method previously described[19] (iodine-silver acetate in wet acetic acid, 4 h 30 at 95°C), then deacetylated (CH_3ONa/CH_3OH) to afford **12** and **13** rather than the expected isomers. Such an unusual result was already mentioned.[20]

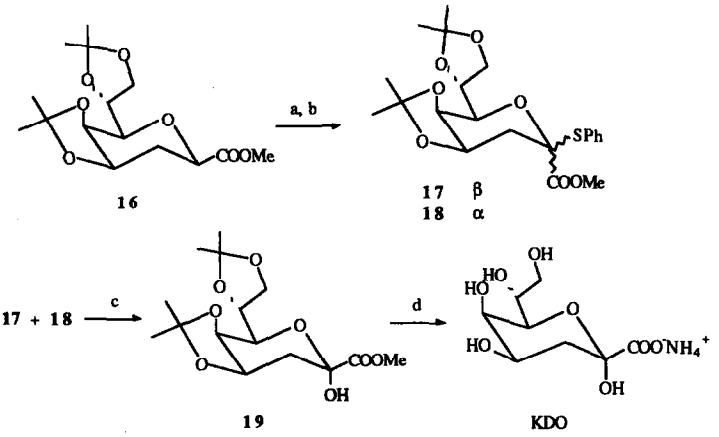
Coming back to the osmylation giving rise to the diols **9**, we chose to develop the bis-inversion at 4 and 5 position, to reach the configuration of KDO derivatives.[21] This was performed by esterification of the diols **9** with triflic anhydride-pyridine in dichloromethane at −10°C, followed by nucleophilic substitution with tetrabutylammonium benzoate. Two pure fractions in the ratio 2:1 were separated by flash chromatography in 51% overall yield (Scheme 8).



Scheme 8: a. Tf_2O, -10°C, CH_2Cl_2, pyr.; b. $PhCOO^-NBu_4^+$, toluene

The major one **14** appeared to be a protected derivative of 2-deoxy-α-KDO as evidenced by 2D-NMR spectroscopy. Cross peaks between H-2, H-4, H-5 and H-6 are visible in the phase sensitive NOESY spectrum. The structure for the minor one **15** was elucidated by NOESY spectrum, showing cross peaks between H-4, H-5 and H-6, but none between H-2 and these protons. The compound **15** turned out to be a protected derivative of 2-deoxy-β-KDO. In order to confirm definitively this assignment and all the preceding ones, the major compound **14** was converted to diisopropylidene-2-deoxy-α-KDO methyl ester **16**. Our sample displayed identical spectroscopic data with those reported in the literature.[8]

Oxidation



Scheme 9: a. LDA, THF; b. PhSSPh; c. NBS, THF-H_2O; d. Dowex 50 (H^+); e. NaOH; f. Dowex 50 (NH_4^+)

Until recently [8], nothing had been published on enolates from tetrahydropyran-2-carboxylic ester systems. The α or β configuration of such derivatives gave the same stereochemical result on alkylation, indicating that the same enolate intermediate was involved in both reaction.[8] In order to create an activated KDO derivative, we investigated the sulfenylation of 2-deoxy-KDO via the enolate. The reaction of the lithium enolate from **16** with diphenyldisulfide gave in 51 % yield a 85:15 mixture of anomeric sulfides **17** and **18**, identified as β and α anomers, respectively, according to their spectroscopic data[9] (Scheme 9). The β/α selectivity was superior to the one observed when an homologue enolate was quenched with methylthiomethane sulfonate.[10] The major β sulfide was already used as a glycosyl donor in O-and C-glycosylation.[9,10] The anomeric mixture of sufides could be easily hydrolyzed in the presence of N-bromosuccinimide to give rise to the diisopropylidene-α-KDO methyl ester **19** in 91 % yield. Deprotection of **19** (Dowex 50 (H^+) resin, then NaOH) gave KDO, which was isolated as the ammonium salt, according to a procedure already described[12m].

Conclusion

This new access to KDO and KDO analogues allowed us to develop an hetero Diels-Alder reaction in water; the heterocycloaddition, both in water or in neat conditions, displayed a modest facial stereoselectivity (74/26), but the two next steps, i.e. the osmylation and the bis-inversion, were totally stereospecific. Diastereomers were separated only after these steps, yielding pure 2-deoxy-α-and -β-KDO. Both could be converted to an activated KDO, such as β-thioglycoside, the hydrolysis of which led to pure protected α-KDO (10% total yield based on starting diene **1**). It must be emphasized that the aqueous hetero Diels-Alder reaction allows the use of the very cheap commercial aqueous solution of glyoxylic acid; compared to the tedious preparation of butylglyoxylate, the aqueous hetero Diels-Alder reaction should find other applications in synthetic organic chemistry.

Experimental part

General methods

Solvents were distilled prior to use: toluene and dichlomethane from CaH_2; THF from Na-benzophenone; methanol from Mg-iodine. Preparative flash column chromatographies were carried out using Merck kieselgel 60 (230-400 mesh). Solvent compositions are quoted as v/v. Melting points are uncorrected. Optical rotations were measured with a Roussel-Jouan or a Jasco DIP 370 micropolarimeter. ^1H and ^{13}C NMR spectra were recorded on Brucker spectrometers (AC 200 or AC 250); ^1H and ^{13}C NMR spectra were reported in ppm relative to tetramethylsilane. ^{13}C NMR spectra were obtained fully decoupled and multiplicities were determined using DEPT. Elemental analyses were performed by the Service Central de Microanalyse du CNRS.

Hetero Diels-Alder reaction in water

To a solution of the diene **1** (1.95 g, 17 mmol) in water (17 mL) was added sodium glyoxylate (5.85 g, 51 mmol); the solution was heated for 18 h at 110°C and was then lyophilised. The mixture was dissolved in anhydrous methanol (100 mL) and stirred with Dowex 50 (H^+) resin during 24 h at room temperature. After filtration and evaporation, the residue was acetylated with an equal volume of acetic anhydride and pyridine (20 mL) to afford an inseparable mixture **2** of diastereomers (2.62 g, 54 %). Spectroscopic analysis (^1H and ^{13}C NMR) gave the proportions of the cycloadducts (42/32/19/7).

Methyl 2,6-anhydro-7,8-di-O-acetyl-3,4,5-trideoxy-D-*ribo*- and -D-*arabino*-octonate (3) and (4)

To a solution of the mixture **2** (1.3 g, 4.54 mmol) in EtOH (14 mL) was added 10 % Pd/C (200 mg). The suspension was stirred under hydrogen at room temperature for 3 h, filtered through celite, and concentrated under reduced pressure to afford a mixture of 4 diastereomers in the proportions 42/32/19/7 (^{1}H NMR). Flash chromatography (hexane-EtOAc, 80:20, v/v) gave compounds **3** (462 mg, 35 %), **4** (352 mg, 27 %) and a mixture of compounds **5** and **6** (281 mg, 21 %) identified as methyl 2,6-anhydro-7,8- di-O-acetyl-3,4,5-trideoxy-D-*lyxo*- and D-*xylo*-octonate, respectively.

Compound **3** : $[\alpha]_D^{20}$ + 46 (*c* 1.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MH_2) : δ 1.65 (m, 6H, H-3a, H-3e, H-4a, H-4e, H-5a and H-5e), 2.05 and 2.09 (s, 6H, CH_3, acetyl), 3.55 (ddd, 1H, H-6, $J_{5a,6}$ 2Hz, $J_{5a,6}$ 11 Hz, $J_{6,7}$ 6.5 Hz), 3.77 (s, 3H, CH_3, methyl ester), 4.00 (dd, 1H, H-2, $J_{2,3a}$ 11H_Z, $J_{2,3e}$ 2.5 Hz), 4.23 (dd, 1H, H-8', $J_{7,8'}$ 6.5 Hz, $J_{8,8'}$12 Hz), 4,48 (dd, 1H, H-8, $J_{7,8}$ 2.5 Hz), 5.06 (dt, 1H, H-7) ; ^{13}C NMR ($CDCl_3$, 62.5 MHz) : δ 20.60 and 20.75 (CH_3 acetyl), 22.46, 26.71 and 28.23 (C-3, C-4 and C-5), 51.82 (CH_3, ester), 62.32 (C-8), 72.91, 75.91 and 76.60 (C-2, C-6 and C-7), 169.95, 170.56 and 171.12 (CO, ester).

Anal. Calcd. for C_{13} H_{20} O_7 : C 54.16, H 6.99 ; found : C 54.12, H 6.94 %.

Compound **4**: $[\alpha]_D^{20}$ + 44 (*c* 0.8, CH_2 Cl_2) . ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.65 (m, 6H, H-3a, H-3e, H-4a, H-4e, H-5a and H-5e), 2.08 and 2.11 (s, 6H, CH_3, acetyl), 4.02 (ddd, 1H, H-6, $J_{5a,6}$ 9Hz, $J_{5e,6}$ 3Hz, $J_{6,7}$ 6.5 Hz), 3.77 (s, 3H, CH_3, methyl ester), 4.23 (dd, 1H, H-8', $J_{7,8'}$ 7Hz, $J_{8,8'}$ 12 Hz), 4.45 (dd, 1H, H-8, $J_{7,8}$ 2.5 Hz), 4.49 (dd, 1H, H-2, $J_{2,3a}$ 4 Hz, $J_{2,3e}$ 2Hz), 5.03 (dt, 1H, H-7) ; ^{13}NMR ($CDCl_3$, 62.5 MHz) δ 20.70 and 20.85 (CH_3, acetyl), 19.26, 26.46 and 26.55 (C-3, C-4 and C-5), 51.76 (CH_3, ester), 62.57 (C-8), 71.45, 72.68 and 72.98 (C-2, C-6 and C-7), 170.19, 170.71 and 172.18 (CO, ester).

Anal. Calcd. for C_{13} H_{20} O_7 : C 54.16, H 6.99 ; found : C 53.67, H 6.88 %.

Compound **5**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.65 (m, 6H, H-3a, H-3e, H-4a, H-4e, H-5a and H-5a), 2.06 and 2.11 (s, 6H, CH_3, acetyl), 3.62 (ddd, 1H, H-6, $J_{5a,6}$ 11Hz, $J_{5e,6}$ 2Hz, $J_{6,7}$ 4 Hz), 3.77 (s, 3H, CH_3, ester), 3.98 (dd, 1H, H-2, $J_{2,3e}$ 2.5 Hz, $J_{2,3a}$ 11 Hz), 4.2 (dd, 1H, H-8', $J_{7,8'}$ 7.5 Hz, $J_{8,8'}$ 12 Hz), 4.4 (dd, 1H, H-8, $J_{7,8}$ 2.7 Hz), 5.17 (td, 1H, H-7) ; ^{13}C NMR ($CDCl_3$, 62.5 MHz) 20.76 and 20.92 (CH_3, acetyl), 22.78, 25.89 and 28.15 (C-3, C-4 and C-5), 51.99 (CH_3, ester), 62.94 (C-8), 72.31, 76.50 and 77.00 (C-2, C-6 and C-7), 170.03, 170.52 and 172.25 (CO, ester).

Compound **6**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.65 (m, 6H, H-3a, H-3e, H-4a, H-4e, H-5a and H-5e), 2.07 and 2.09 (s, 6H, CH_3, acetyl), 3.77 (s, CH_3, ester), 4.1 (ddd, 1H, H-6, $H_{5a,6}$ 10 Hz, $J_{5e,6}$ 2.5 Hz, $J_{6,7}$ 3.5 Hz), 4.25 (dd, 1H, H-8', $J_{7,8'}$ 7.5 Hz, $J_{8,8'}$ 12 Hz), 4.45 (dd, 1H, H-8, $J_{7,8}$ 3.5 Hz), 4.55 (dd, 1H, H-2, $J_{2,3a}$ 4 Hz, $H_{2,3e}$ 2 Hz), 5.15 (dt, 1H, H-7) ; ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 20.71 and 20.84 (CH_3, acetyl), 19.57, 26.30 (C-3, C-4 and C-5), 51.79 (CH_3, ester), 63.27 (C-8), 71.79, 72.44 and 72.93 (C-2, C-6 and C-7), 170.49, 170.66 and 172.27 (CO, ester).

(3E, 2S)-1,2-di-O-benzyl-hexa-3,5-diene-1,2-diol (7)

To a solution of diene **1** (544 mg, 4.77 mmol) in THF (8mL) was added at 0°C sodium hydride (252 mg, 10.49 mmol). The mixture was stirred and allowed to warm to room temperature during 1 h before the addition of benzyl bromide (1.79 g, 10.47 mmol). The mixture was refluxing overnight, diluted with diethyl ether, then

quenched with ammonium chloride. After extraction with diethyl ether, washing, drying over $MgSO_4$, the reaction mixture was evaporated, then chromatographed (hexane/ EtOAc, 90:10, v/v) to afford the compound **7** as an oil (1,36 g, 97 %). $[\alpha]_D^{20}$ + 21 (*c* 1.2, CH_2Cl_2) ; ^1H NMR ($CDCl_3$, 250 MHz) δ 3.52 (dd, 1H, H-1', $J_{1',2}$ 4.5 Hz, $J_{1,1'}$ 10.5 Hz), 3.61 (dd, 1H, H-1, $J_{1,2}$ 6.5 Hz), 4.08 (ddd, 1H, H-2, $J_{2,3}$ 7.5 Hz), 4.55 (m, 4H, CH_2 benzyl), 5.12 (dd, 1H, H-6', J_{cis} 9.5 Hz, J_{gem} 1.5 Hz), 5.23 (dd, 1H, H-6, J_{trans} 16.5 Hz), 5.65 (dd, 1H, H-3, $J_{3,4}$ 14.5 Hz), 6.3 (m, 2H, H-4 and H-5), 7.3 (m, 10H, CH aromat.); ^{13}C NMR ($CDCl_3$, 62.5 MHz) 70.39 (C-1), 72.94 and 73.19 (CH_2, benzyl), 78.39 (C-2), 117,84 (C-6), 131.07, 133.95 and 136.09 (C-3, C-4 and C-5).

Anal. Calcd for $C_{20}H_{22}O_2$: C 81.60, H 7.53 ; found : C 80.80, H 7.52 %.

Hetero Diels-Alder reaction with the benzylated diene 7

A mixture of diene **7** (2.94 g 10 mmol), butylglyoxylate (3.12 mL, 30 mmol) and hydroquinone (20 mg) were heated at 130°C for 18 h. The reaction mixture was taken up with methanol (60 mL) and p-toluenesulfonic acid (20 mg). After 3 h at reflux, the solution was diluted with diethyl ether, neutralised with a saturated solution of potassium hydrogenocarbonate. The organic layer was washed with water, dried over $MgSO_4$, filtered off, then evaporated. Flash chromatrography (hexane/EtOAc, 50:50, v/v) gave an inseparable mixture of cycloadducts **8** (2.29 g, 60 %).

Anal. Calcd for $C_{23}H_{26}O_5$: C 72.23, H 6.85 ; found : C 72.28, H 6.95 %.

Identification of the cycloadducts 8

To the preceding mixture (325 mg, 0,87 mmol) dissolved in EtOH (8mL) was added palladium on charcoal (10 %, 125 mg). The suspension was stirred under hydrogen (3 atm.) at room temperature for 5 h, filtered through celite, and concentrated under vaccuum. The residue was treated with acetic anhydride (2 mL) and pyridine (2 mL) to afford after 24 hours, a mixture of diastereomers in the ratio 50/24/16/10. These diastereomers were identified (^{13}C and ^1H NMR) as compounds **3**, **4**, **5**, and **6**, respectively.

Dihydroxylation of the cycloadducts 8

To the mixture of cycloadducts **8** (2.5 g, 6.5 mmol) dissolved in acetone/water (8:1, v/v, 10 mL) was added 4-methylmorpholine N-oxide (1.052 g, 8.98 mmol) and osmium tetraoxide dissolved in ^{t}BuOH (0.05 M, 3.9 mL). The solution was kept at room temperature for 3,5 h. The osmate was reduced by adding a saturated solution of sodium bisulfite. The mixture was stirred for 15 min, diluted with water (30 mL). After extraction with ethyl acetate (7 x 50 mL), the organic layer was dried over $MgSO_4$, then filtered off. Concentration of the filtrate afforded a mixture of the diols **9** (2.595 g, 96 %).

Anal. Calcd for $C_{23}H_{28}O_7$: C 66.33, H 6.78 ; found : C 66.37, H 6.85 %.

(E) Benzyl 2,4-pentadienyl ether (10)

A solution of (E) 2,4-pentadienol (3 g, 35 mmol) in THF (58 mL) was cooled to 0°C, sodium hydride (925 mg, 38.5 mmol) and then benzyl bromide (4.58 ml, 38.5 mmol) were added, the mixture was allowed to warm up and kept for 15 min at room temperature. The excess hydride was destroyed with water, the reaction

mixture was extracted with ether, the organic layer was washed with water and evaporated to dryness. The residue was adsorbed on a silica gel column, then eluted with hexane/EtOAc (95:5, v/v) to afford the compound **10** as an oil (4.58 g, 75 %). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 4.05 (m, 2H, H-1 and H-1'), 4.5 (d, 2H, CH_2, benzyl), 5.1 (dd, 1H, H-5, J_{cis} 9 Hz, J_{gem} 1.5 Hz), 5.22 (dd, 1H, H-5', J_{trans} 16 Hz), 5.81 (dt, 1H, H-2, J_{trans} 14.5 Hz, $J_{1,2}$ 6 Hz), 6.31 (m, 2H, H-3 and H-4), 7.3 (m, 5H, H-aromat.).

Anal. Calcd for $C_{12}H_{14}O$: C 82.72, H 8.10 ; found : C 82.27, H 8.33 %.

Hetero Diels-Alder with the diene 10

A mixture of the diene **10** (9 g, 51 mmol) and butyl glyoxylate (16 mL, 150 mmol) were heated at 130°C for 18 hours to afford a mixture of endo and exo cycloadducts in the ratio 66:34 (^{13}C NMR). The reaction mixture was then diluted with a solution of CH_3ONa in methanol (1 M, 50 mL) and kept at room temperature for 8 hours. After neutralisation with HCl, extraction with ether, and evaporation, flash chromatography of the residue gave an inseparable mixture **11** (9.07 g, 67 %) of *endo* and *exo* cycloadducts in the same ratio.

Anal. Calcd for $C_{15}H_{18}O_4$: C 68.68, H 6.98 ; found : C 68.43, H 6.91 %.

Spectroscopic data of the *endo* cycloadduct : ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.4 (m, 2H, H-3 and H-3') 3.6 (m, 2H, H-7 and H-7'), 3.75 (s, 3H, CH_3, ester), 4.25 (dd, 1H, H-2, $J_{2,3}$ 10 Hz, $J_{2,3'}$ 4.5 Hz), 4.45 (m, 1H, H-6), 4.6 (s, 2H, CH_2, benzyl), 5,75 (m, 2H, H-4 and H-5), 7.3 (m, 5H, H-aromat.) ; ^{13}C NMR ($CDCl_3$, 50 MHz) δ 28.02 (C-3), 52.13 (CH_3, ester), 72.09, 72.71, 73.41 and 74.75 (C-2, C-6, C-7 and CH_2, benzyl), 124.68 and 127.18 (C-4 and C-5), 170.98 (CO, ester).

Spectroscopic data of the *exo* cycloadduct : ^{13}C NMR ($CDCl_3$, 50 MHz) δ 26.92 (C-3), 52.13 (CH_3, ester), 69.44, 71.76, 73.26 (C-2, C-6, C-7 and CH_2, benzyl), 124.22 and 126.37 (C-4 and C-5), 171.76 (CO, ester).

Methyl 2,6-anhydro-7-0-benzyl-3-deoxy-D,L-*allo*- and -*altro*-heptonate (12) and (13)

The osmylation of the cycloadducts **11** (1.558 g, 5.95 mmol) was conducted as above to afford an inseparate mixture of the diols **12** and **13** (1.671 g, 95 %) in the ratio 65:35.

Anal. Calcd for $C_{15}H_{20}O_6$: C 60.80, H 6.80 ; found : C 60.54, H 6.95 %.

Spectroscopic data of compound **12** : ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.81 (ddd, 1H, H-3a, $J_{2,3a}$ 12 Hz, $J_{3a,3e}$ 14 Hz, $J_{3a,4}$ 2.5 Hz), 2.22 (ddd, 1H, H-3e, $J_{2,3e}$ 2.5 Hz, $J_{3e,4}$ 3.5 Hz), 3.63 (ddd, 1H, H-6, J 3, 8.5 and 12 Hz), 3.74 (s, 3H, CH_3, ester), 3.8 (m, 2H, H-7 and H-7'), 4.45 (dd, 1H, H-2), 4.58 (s, 2H, CH_2, benzyl), 7.3 (m, 5H, H aromat.); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 34.01 (C-3), 52.15 (CH_3, ester), 66.03, 70.13, 70.51 and 71.82 (C-2, C-4, C-5, C-6), 71.83 (C-7), 73.86 (CH_2, benzyl), 127-137 (C aromat.), 171.61 (CO, ester).

Spectroscopic data of compound **13** : ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.49 (ddd, 1H, H-3e, $J_{2,3e}$ 1.5 Hz, $J_{3a,3e}$ 14.5 Hz, $J_{3e,4}$ 4Hz), 3.75 (s, 3H, CH_3, ester), 4.58 (s, 2H, CH_2, benzyl), 7.3 (m, 5H, H aromat.); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 32.2 (C-3), 66.50, 68.89, 68.97 and 70.94 (C-2, C-4, C-5, C-6), 174.75 (CO, ester).

Dihydroxylation of cycloadducts 11 via iodonium

The mixture of the adducts **11** (1 g, 3.8 mmol) was dissolved in glacial acetic acid (18 mL); after the

addition of silver acetate (1.42 g, 8.51 mmol) and iodine (1.013 g, 3.99 mmol), the reaction mixture was heated at 95°C during 4 h 30, cooled, then treated with an excess of sodium chloride and finally filtered. The filtrate was evaporated and dissolved in methanol in the presence of sodium methoxide (540 mg, 10 mmol); after neutralisation with Dowex 50 (H^+) resin, filtration, evaporation, the residue was purified by flash chromatography to give **12** and **13** as a mixture (698 mg, 62%) in the ratio 65:35.

Methyl 2,6-anhydro-4,5 di-O-benzoyl-7,8-di-O-benzyl-3-deoxy-D-*glycero*-D-*galacto*-and-*talo*-octonate (14) and (15)

To the mixture of diols **9** (3.567 g, 8.57 mmol) dissolved in dichloromethane (30 mL) and pyridine (5.5 mL) was added at –10°C a solution of triflic anhydride in dichloromethane (1 M, 35 mL). The formation of monotriflates and then ditriflates was monitored by thin-layer chromatography. After 3 h at –10°C, the reaction mixture was quenched with a solution of sodium bicarbonate. After extraction with dichloromethane, the organic layer was dried over $MgSO_4$, then filtered off. Concentration of the filtrate gave ditriflates which were dissolved in toluene (18 ml). Tetrabutyl ammonium benzoate (12,37 g, 34.28 mmol) was added to the solution. After 2 h at room temperature, the mixture was diluted with dichloromethane, then washed with water and evaporated. Flash chromatography (hexane/EtOAc, 80:20, v/v) afforded **14** as a crystalline compound (1,818 g, 34 %) and **15** as a syrup (910 mg, 17 %).

Compound **14**: m.p. 165°C (ether-hexane); $[\alpha]_D^{20}$ + 18 (*c* 0.85, EtOH) ; ^1H NMR ($CDCl_3$, 250 MHz) δ 2.2 (q, 1H, H-3a, $J_{2,3a}$ 12 Hz, $J_{3a,3e}$ 12 Hz, $J_{3a,4}$ 12 Hz), 2.3 (ddd, 1H, H-3e, $J_{2,3e}$ 2.5 Hz, $J_{3e,4}$ 5 Hz), 3.7 (m, 2H, H-8 and H-8'), 3.77 (s, 3H, CH_3, ester), 3.9 (m, 1H, H-6), 4.25 (dd, 1H, H-2), 4.33 (m, 1H, H-7), 4.6 (m, 2H, CH_2, benzyl), 5.39 (ddd, 1H, H-4, $J_{3a,4}$ 12 Hz, $J_{3e,4}$ 5 Hz, $J_{4,5}$ 3 Hz), 6.02 (d, 1H, H-5), 7.6 (m, 20 H, H aromat.) ; ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 29.35 (C-3), 52.26 (CH_3, ester), 66.27, 70.65, 74.46, 74.80 and 75.84 (C-2, C-4, C-5, C-6, C-7), 67.72, 72.22 and 73.35 (C-8 and CH_2, benzyl), 127-138 (C aromat.), 165.34, 165.41 and 169.87 (CO, ester).

Anal. Calcd for $C_{37}H_{36}O_9$: C 71.14, H 5.81 ; found : C 71.18, H 5.89 %.

Compound **15**: $[\alpha]_D^{20}$ +78 (*c* 0.83, CH_2Cl_2); ^1H NMR ($CDCl_3$, 250 MHz) δ 2.45 (m, 2H, H-3a and H-3e), 3.75 (m, 2H, H-8 and H-8'), 3.78 (s, 3H, CH_3, ester), 4.24 (d, 1H, H-6, $J_{6,7}$ 7.5 Hz), 4.33 (d, 1H, H-7), 4.6 (m, 2H, CH_2, benzyl), 4.77 (t, 1H, H-2, $J_{2,3a}$ and $J_{2,3e}$ 4 Hz), 5.33 (dt, 1H, H-4, $J_{3a,4}$ 8 Hz, $J_{3e,4}$ 8 Hz, $J_{4,5}$ 2.5 Hz), 6.01 (dd, 1H, H-5, $J_{5,6}$ 1Hz), 7.6 (m, 20 H, H aromat.); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 26.95 (C-3), 52.41 (CH_3, ester), 66.78, 68.35, 72.55, 72.86 and 75.77 (C-2, C-4, C-5, C-6, C-7), 70.61, 72.86 and 73.53 (CH_2, benzyl), 127-138 (C aromat.), 165.34 and 171.29 (CO, ester).

Anal. Calcd for $C_{37}H_{36}O_9$: C 71.14, H 5.81 ; found : C 70.85, H 5.91 %.

Methyl 2,6-anhydro-3-deoxy-4:5,7:8-di-O-isopropylidene-D-*glycero*-D-*galacto*-octonate (16)

Compound **14** (1.7 g, 2.72 mmol) was dissolved in a solution of CH_3ONa in methanol (0.25 M, 20 mL). After 20 min, the reaction mixture was neutralised with Dowex 50 (H^+) resin, then filtered off. The filtrate was evaporated; to the residue dissolved in ethanol (10 mL) was added 10 % Pd/C (150 mg). The suspension was stirred under hydrogen at room temperature for 3 h, filtered through celite, concentrated under vacuum and then

treated with dimethoxypropane (1.2 mL, 10 mmol) in acetone (10 mL) in the presence of p-toluenesulfonic acid (3 mg). The mixture was stirred for 1 h at room temperature. After evaporation, the residue was chromatographed (hexane/EtOAc, 50:50, v/v) to afford compound **16** (688 mg, 80%), the spectroscopic data of which were identical to those published in the literature.[8]

Methyl 4,5:7,8-di-O-isopropylidene-2,3-dideoxy-2-phenylthio-β- and -α-D-*manno*-2-octulosonate (17) and (18)

A solution of n BuLi in hexane (1.5 M, 1 mL) was added via a syringe into a solution of diisopropylamine (2.4 mL, 1.25 mmol) in THF (50 mL) at –20°C. The solution was then cooled to –50°C. After stirring for 15 min, compound **16** (316 mg, 1 mmol) dissolved in THF was added dropwise. After the end of the addition, the reaction mixture was kept at –50°C during 30 min. Diphenyldisulfide (327 mg, 1.5 mmol) was then added dropwise. The mixture was allowed to warm up to room temperature, then quenched with a saturated ammonium chloride solution. After extraction with ether, the organic fraction was dried over $MgSO_4$. After concentration the residue was chromatographed on silica gel with hexane/EtOAc (90:10, v/v) as eluent, yielding **17** and **18** (216 mg, 51%) as a mixture of anomers β and α in the ratio 85:15.

Anal. Calcd for $C_{21}H_{28}O_7S$: C 59.42, H 6.65 ; found : C 59.62, H 6.74 %.

Compound **17** : Spectroscopic data were identical to those reported in the literature.[9]

Compound **18** : ^1H NMR ($CDCl_3$, 200 MHz) δ 1.96 (dd, 1H, H-3a, $J_{3a,3e}$ 15.5 Hz, $J_{4,3a}$ 2.5 Hz), 3.15 (dd, 1H, H-3e, $J_{4,3e}$ 3.5 Hz), 3.49 (s, 3H, CH_3, ester), 3.79 (dd, 1H, H-6, $J_{6,7}$ 8 Hz, $J_{6,5}$ 1.5 Hz), 4.56 (ddd, 1H, H-7, $J_{7,8}$ 2.5 Hz, $J_{7,8'}$ 4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.87 and 26.94 (CH_3, isopropylidene), 32.58 (C-3), 52.07 (CH_3, ester), 67.23 (C-8), 72.04, 72.22 and 73.40 (C-2, C-4, C-5, C-6 and C-7), 109.29 and 109.67 (C isopropylidene and aromat.), 125,52, 130,15 and 135.46 (C aromat.), 169.28 (CO, ester).

Methyl 3-deoxy-4:5,7-8-di-O-isopropylidene-α-D-*manno*-2-octulosonate (19)

The mixture of compounds **17** and **18** (60 mg, 0.14 mmol) was dissolved in THF (1.4 mL). Water (0.1 mL) and then N-bromosuccinimide (36 mg, 0.2 mmol) were added. The mixture was stirred at room temperature for 30 min, neutralised with saturated sodium bicarbonate solution and then extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and then evaporated to afford compound **19** as a pure anomer (26 mg, 91 %). Spectroscopic data were identical to those reported in the literature.[12m]

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