Supplementary Material (ESI) for Chemical Communications

Supplementary Information

General techniques and reagents

NMR analysis was performed on an Avance 300 machine (Bruker, Coventry, U.K.). HPLC analysis was performed using a Bio-Rad Aminex HPX-87H organic analysis column (300 mm x 7.8 mm) linked to an RID-10A refractive index detector (Shimadzu, Milton Keynes, U.K.). Samples were eluted in 8 mM H_2SO_4 at a flow rate of 0.55 ml.min^{-1}. Polarimetry was carried out using an AA-10 automatic polarimeter (Optical Activity Ltd., Huntingdon, U.K.). Matrix 60 silica gel for flash chromatography was from Fluorogen Ltd., Glossop, U.K., L-gulonolactone was from Acros, Loughborough, U.K., and all other chemicals and reagents were obtained from Sigma-Aldrich Ltd., Poole, U.K.

D-Glyceraldehyde-acetonide (*R*)-5 and D-glyceraldehyde (*R*)-2

D-Glyceraldehyde-acetonide (*R*)-**5** was synthesised by periodate cleavage of 1,2-5,6-di-*O*-isopropylidene-D-mannitol as described previously,[1] purified by silica gel flash chromatography using dichloromethane:methanol (20:1) as eluant and analysed by polarimetry: $[\alpha]^{25}_D$ = +77 (*c* 1.5, EtOAc), Lit.[2] $[\alpha]^{25}_D$ = +80 (*c* 1.5, EtOAc). D-Glyceraldehyde (*R*)-**2** was synthesised by treatment of D-glyceraldehyde-acetonide (*R*)-**5** with 0.5 M H_2SO_4 and purified by silica gel flash chromatography using dichloromethane:methanol (9:1) as eluant. Its enantiomeric purity was determined from its specific rotation $[\alpha]^{25}_D$ = +9.75 (*c* 2, H_2O), Lit.[3] $[\alpha]^{22}_D$ = +7 to +14 (*c* 12, H_2O); and confirmed via diastereoisomeric derivatisation with L-(-)-α-methylbenzylamine as described previously.[4]

L-Glyceraldehyde-acetonide (*S*)-5 and L-glyceraldehyde (*S*)-2

L-Glyceraldehyde-acetonide (*S*)-**5** was synthesised from L-gulonolactone as described previously,[5] purified by silica gel flash chromatography using dichloromethane:methanol (20:1) as eluant and analysed by polarimetry: $[\alpha]^{25}_D$ = -64.7 (*c* 1, benzene), Lit.[3] $[\alpha]^{22}_D$ = -67.9 (*c* 8, benzene). L-Glyceraldehyde (*S*)-**2** was synthesised by treatment of L-glyceraldehyde-acetonide (*S*)-**5** with 0.5 M H_2SO_4 and purified by silica gel flash chromatography using dichloromethane:methanol (9:1) as eluant. Its enantiomeric purity was determined from its specific rotation $[\alpha]^{25}_D$ = -9.17 (*c* 2, H_2O), Lit.[3] $[\alpha]^{22}_D$ = -7 to -14 (*c* 12, H_2O), and confirmed via diastereoisomeric derivatisation with L-(-)-α-methylbenzylamine as described previously.[4]

Determination of kinetic parameters

100 µl reactions were prepared in 50 mM sodium phosphate (pH6.0) containing 20 mM sodium pyruvate, 0-40 mM D-glyceraldehyde-acetonide (*R*)-**5** or L-glyceraldehyde-acetonide (*S*)-**5** and an appropriate quantity of recombinant KDGA.[4] Reactions were heated at 70^{o}C for 10 min and quenched by addition of 10 µl 12% (w/v) trichloracetic acid. The amount of aldol product produced was quantified by the oxidative thiobarbituric acid assay as described previously,[6] and kinetic constants determined by the direct linear method.[7] Kinetic parameters for (*R*)-**5** were K_m = 22.7mM, k_{cat} = 324min^{-1}, whilst for (*S*)-**5** K_m = 5.5mM and k_{cat} = 42min^{-1}. These parameters are listed alongside previous values determined for D-glyceraldehyde (*R*)-**2** and L-glyceraldehyde (*S*)-**2**.[6]

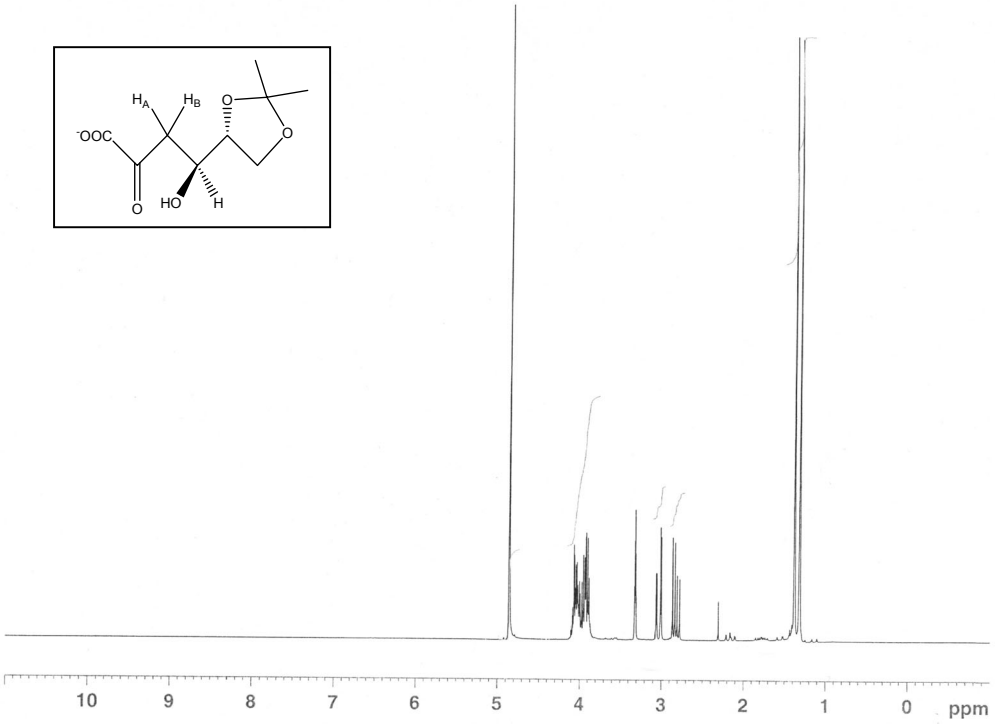
Biotransformations using D-glyceraldehyde (*R*)-2 and L-glyceraldehyde (*S*)-2 as substrates for KDGA

2 ml reactions were prepared in 50 mM Tris/HCl (pH 7.7) containing 35 mM of the glyceraldehyde substrates and 70 mM sodium pyruvate. 1 mg recombinant KDGA was added and the reactions were incubated at 50°C. The progress of each biotransformation was monitored by quenching 500 µl samples of each reaction with 100 µl 12% (w/v) trichloracetic acid at various time points up to 2 h. Precipitated enzyme was removed by centrifugation and the resultant supernatant was analysed by HPLC to determine diastereomeric excess values. As found previously,[4] using D-glyceraldehyde (*R*)-**2** as a substrate for KDGA gave an approximately 50:50 mixture of (4*S*,5*R*)-3-deoxy-2-hexulosonic acid **3** (Retention time = 10.75 min) and (4*R*,5*R*)-3-deoxy-2-hexulosonic acid **4** (Retention time = 10.01 min). Similarly, employing L-glyceraldehyde (*S*)-**2** as a substrate for KDGA gave a 53:47 mixture of (4*R*,5*S*)-3-deoxy-2-hexulosonic acid **3** and (4*S*,5*S*)-3-deoxy-2-hexulosonic acid **4**. To ensure there was no racemisation under the reaction conditions, solutions of D- or L-glyceraldehyde in 50 mM Tris/HCl (pH 7.7) were heated at 50°C for up to 2 h. The specific rotation measurements of the solutions were monitored throughout the incubation and found not to change. D-Glyceraldehyde: $[\alpha]^{25}_{D}$ = +9.18 (*c* 2, Tris buffer), Lit.[3] $[\alpha]^{22}_{D}$ = +7 to +14 (*c* 12, H_2O). L-Glyceraldehyde: $[\alpha]^{25}_{D}$ = -9.11 (*c* 2, Tris buffer), Lit.[3] $[\alpha]^{22}_{D}$ = -7 to -14 (*c* 12, H_2O).

Sodium 5,6-*O*-isopropylidene-(4*S*,5*R*)-3-deoxy-2-hexulosonate 6

50 ml 25 mM sodium phosphate (pH 7.0) containing 1 g D-glyceraldehyde-acetonide (*R*)-**5**, 1.2 g sodium pyruvate and 10 mg KDGA was heated at 50°C for 3 h with shaking. The reaction was then filtered and lyophilised before being dissolved in methanol, filtered again and dried by rotary evaporation to afford (4*S*,5*R*)-**6** in >92% d.e. as determined by ^{1}H NMR spectroscopic analysis. The crude product was then dissolved in ~100 ml boiling absolute ethanol and allowed to crystallise for 20 h at 4°C to afford a white crystalline powder that was washed with ice-cold absolute ethanol and dried to give the title compound (4*S*,5*R*)-**6** (1.126 g) in 61% yield and >99% d.e.; m.p. 178 dec. $[\alpha]^{25}_{D}$ = +6.0 (*c* 3, H_2O); ^{1}H NMR (MeOD) δ 1.31 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 2.82 (dd, 1H, J_{AB} = 16.3 Hz, $J_{3A,4}$ = 8.8 Hz, H_{3A}), 3.03 (dd, 1H, J_{AB} = 16.3 Hz, $J_{3B,4}$ = 3.1 Hz, H_{3B}), 3.88-4.10 (m, 4H, H_4, H_5 and 2xH_6); ^{13}C NMR (D_2O) δ 24.4 (C_8), 25.7 (C_9), 43.2 (C_3), 65.7 (C_4), 67.7 (C_6), 78.2 (C_5), 110.6 (C_7), 169.5 (C_1), 203.8 (C_2); I.R. (KBr) *v* 3427 (OH), 1721 (C=O), 1635 (CO_2^-) cm^{-1}; HRMS: Calcd for $C_9H_{13}O_6$ 217.0718, Found 217.0715.

^{1}H NMR spectrum for sodium 5,6-*O*-isopropylidene-(4*S*,5*R*)-3-deoxy-2-hexulosonate **6** in MeOD

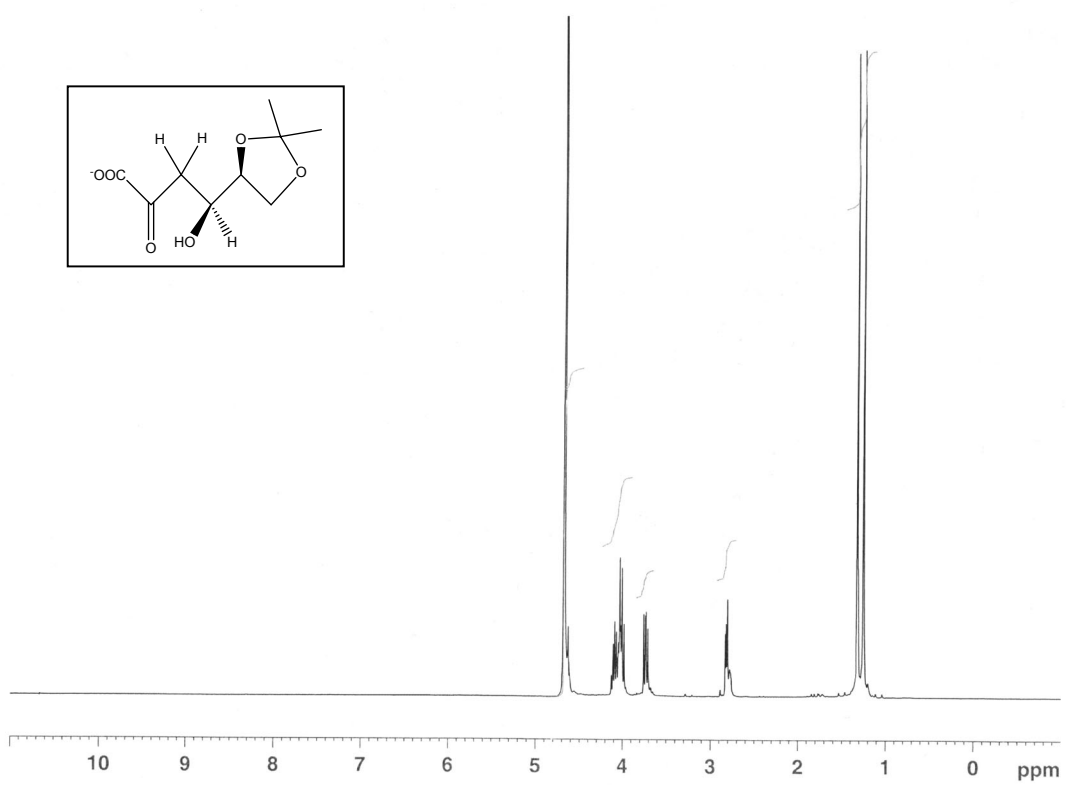


Treatment of sodium 5,6-*O*-isopropylidene-(4*S*,5*R*)-3-deoxy-2-hexulosonate **6** with 0.5 M H_2SO_4 afforded (4*S*,5*R*)-3-deoxy-2-hexulosonic acid **3** in >99% d.e., as determined via comparison of its ^{1}H NMR spectrum, HPLC retention time, and specific rotation with an authentic sample of (4*S*,5*R*)-**3** prepared previously.[4,8]

Sodium 5,6-*O*-isopropylidene-(4*S*,5*S*)-3-deoxy-2-hexulosonate 7

12.5 ml 25 mM sodium phosphate (pH 7.0) containing 250 mg L-glyceraldehyde-acetonide (*S*)-**5**, 300 mg sodium pyruvate and 7 mg KDGA was heated at 50^{o}C for 3 h with shaking. The reaction was then filtered and lyophilised before being dissolved in methanol, filtered again and dried by rotary evaporation, to afford (4*S*,5*S*)-**7** in >92% d.e. as determined by ^{1}H NMR spectroscopic analysis. It was then dissolved in ~20 ml boiling absolute ethanol and allowed to crystallise for 20 h at 4^{o}C to afford a white crystalline powder that was washed in ice-cold absolute ethanol and dried to give the title compound (4*S*,5*S*)-**7** (0.194 g) in 42% yield and in >99% d.e.; m.p. 172 dec., $[\alpha]^{25}_D$ = +6.7 (*c* 1.5, H_2O); ^{1}H NMR (D_2O): δ 1.25 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 2.75-2.83 (m, 2H, 2 x H_3), 3.73 (dd, 1H, *J* = 8.5, 6.2Hz), 3.98-4.13 (m, 3H); ^{13}C NMR (D_2O): δ 24.3 (C_8), 25.5 (C_9), 43.2 (C_3), 65.5 (C_4), 67.1 (C_6), 78.3 (C_5), 110.5 (C_7), 169.6 (C_1), 203.3 (C_2); I.R. (KBr) *v* 3447 (OH), 1718 (C=O), 1636 (CO_2^-) cm^{-1}; HRMS: Calcd for $C_9H_{13}O_6$ 217.0718, Found 217.0721.

^{1}H NMR spectrum for sodium 5,6-*O*-isopropylidene-(4*S*,5*S*)-3-deoxy-2-hexulosonate **7** in D_2O



Treatment of sodium 5,6-*O*-isopropylidene-(4*S*,5*S*)-3-deoxy-2-hexulosonate **7** with 0.5 M H_2SO_4 afforded (4*S*,5*S*)-3-deoxy-2-hexulosonic acid **4** in >99% d.e., as determined via comparison of its ^{1}H NMR spectrum, HPLC retention time, and specific rotation with an authentic sample of its enantiomer (4*R*,5*R*)-**4** that had been prepared previously.[4,9]

Glyceraldehyde-acetonide (*rac*)-5

2.5 g glyceraldehyde (*rac*)-**2** was dissolved in 20 ml dimethylformamide, cooled to 10^{o}C and 25 mg *p*-toluene sulfonic acid was added. 2 ml 2-methoxypropene was then added dropwise over 20 min and the reaction was stirred at room temperature for 20 h. 2.5 g sodium carbonate was added and the reaction was stirred vigorously for 2 h. It was then filtered and dried under vacuum before product purification by silica gel flash chromatography using dichloromethane:methanol (50:1) as an eluant to afford the title compound (*rac*)-**5** which gave identical ^{1}H and ^{13}C NMR spectra to those observed previously for (*R*)-**5**.[2]

Parallel kinetic resolution of glyceraldehyde-acetonide (*rac*)-5

12.5 ml 25 mM sodium phosphate (pH 7.0) containing 250 mg glyceraldehyde-acetonide (*rac*)-**5**, 500 mg pyruvate and 10 mg KDGA was heated at 50^{o}C for 3 h with shaking. After incubation, the reaction pH was adjusted to ~1 with H_2SO_4, neutralized with NaOH(aq) and then filtered and lyophilised. 57% of (*rac*)-**5** had been converted to product as assessed by HPLC and afforded a 59:41 mixture of (4*S*,5*R*)-3-deoxy-2-hexulosonic acid **3** and (4*S*,5*S*)-3-deoxy-2-hexulosonic acid **4** that were separated by DOWEX 1X8-formate anion exchange

chromatography using a 0-0.6 M formic acid elution gradient. Diastereoisomerically pure samples of (4*S*,5*R*)-**3** and (4*S*,5*S*)-**4** were dried over P_2O_5 before analysis by ^{1}H NMR spectroscopy, giving spectra that were identical to those observed previously.[4,8,9] Polarimetry was used to determine enantiomeric excess values that were assigned as ≥90% e.e. in both cases: (4*S*,5*R*)-3-deoxy-2-hexulosonic acid **3** $[\alpha]^{25}_D$ = -31.5 (*c* 1.0, H_2O), Lit.[10] $[\alpha]^{25}_D$ = -33.1 (*c* 1.3, H_2O); (4*S*,5*S*)-3-deoxy-2-hexulosonic acid **4** $[\alpha]^{25}_D$ = -7.5 (*c* 1, H_2O), Lit.[11] (4*R*,5*R*)-**4** $[\alpha]^{25}_D$ = +7.9 (*c* 1.65, H_2O). This biotransfromation was replicated two more times using (*rac*)-**5** which afforded samples of (4*S*,5*R*)-**3** and (4*S*,5*S*)-**4** that exhibited specific rotations that were essentially **i**dentical (+/-2%) to those achieved in the first run. To ensure that no hydrolysis of the acetonide protecting group of (*rac*)-**5** was occurring under the biotransformation conditions, solutions of (*rac*)-**5** in 50 mM Tris/HCl (pH 7.7) were heated at 50°C for 3 hrs, and then analysed by ^{1}H NMR spectroscopy which revealed a clean spectra of (*rac*)-**5** with no evidence of any glyceraldehyde (*rac*)-**2** being present.

Notes

Preparative scale reactions were performed in pH 7.0 phosphate buffer at 50°C, to minimise the likelihood of acetonide hydrolysis and facilitate workup. The small-scale biotransformations were performed at 50°C in pH 7.7 Tris/HCl buffer, although it should be noted that Tris buffer pH decreases with increasing temperature to give a reaction pH of 7.0. The reason Tris buffer was used in this case is because phosphate buffer was found to give a peak on the HPLC profile that overlapped with the product peaks. Enzyme kinetic assays were performed under standard assay conditions of pH6.0 phosphate buffer at 70°C to allow comparison of the kinetic parameters with other substrates. Given the 10 min assay, hydrolysis of the acetonide was considered unlikely to have a significant effect.

References

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