Experimental Details for New Compounds

General

$^1$H and $^{13}$C NMR spectra were obtained using Bruker 300 MHz spectrometer at 300 and 75.5 MHz respectively. Signals are reported in terms of their chemical shift ($\delta$ in ppm) relative to CDCl$_3$ ($^1$H, 7.26 and $^{13}$C, 77.0) and D$_2$O ($^1$H, 4.78 and $^{13}$C external reference used). For $^1$H spectra, multiplicity, integration intensity, coupling constants and assignment values are reported, two dimensional COSY and HSQC spectra were used to aid assignment. Mass spectral analysis was performed using a Bruker Esquire 3000 electrospray ionisation mass spectrometer. Infrared spectra were recorded on a Bruker Alpha-P spectrophotometer.

Monitoring of reactions was performed by thin layer chromatography using Merck pre-coated aluminium-backed silica plates. Plates were observed under UV light at 254 nm and then visualised after charring using H$_2$SO$_4$. All solvents and reagents used were of analytical grade or distilled prior to use.

Synthesis of C-5 modified arabinose derivatives

1,2,3-tri-$O$-acetyl-5-$O$-mesyl-$D$-arabinofuranose (5)

The alcohol 2 (4.0 g, 14.4 mmol) was dissolved in dry CH$_2$Cl$_2$ (150 mL) at 0 °C under N$_2$. N,N-Diisopropylethylamine (7.7 mL, 30.3 mmol) was added, and then methanesulfonyl chloride (MsCl) (1.7 mL, 15.15 mmol) was added dropwise. The reaction mixture was then allowed to stir for 30 minutes in an ice bath, followed by 1 hour at RT. The reaction mixture was quenched with MeOH, dissolved in chloroform (200 mL) and was hed with 1M HCl (200 mL) and water (100 mL), dried (Na$_2$SO$_4$) and concentrated. Purification by column chromatography (EtOAc:Hex 2:3 $\rightarrow$ 1:1, $R_f$ 0.3), gave the desired compound in a yield of 5.1 g (97%).

$^1$H NMR (CDCl$_3$) $\delta$ α anomer 6.18 (s, 1H, H-1), 5.20 (s, 1H, H-2), 5.02 (d, 1H, $J_{3,4}$ 4.2 Hz, H-3), 4.50- 4.40 (m, 2H, $J_{5,5'}$ 11.4 Hz, H-5, H-5'), 4.35 (dd, 1H, $J_{4,5}$ 8.1 Hz, $J_{4,3}$ 4.2 Hz, H-4), 3.05 (s, 3H, Ms-CH$_3$), 2.11 (s, 9H, 3 x OAc); β anomer 6.36 (d, 1H, $J_{1,2}$ 4.2 Hz, H-1), 5.34- 5.26 (m, 2H, $J_{2,1}$ 4.2 Hz, $J_{2,3}$ 7.2 Hz, H-2, H-3), 4.51- 4.39 (m, 2H, $J_{5,5'}$ 11.4 Hz, H-5, H-5'), 4.20 (dd, 1H, H-4), 3.05 (s, 3H, Ms-CH$_3$), 2.10 (s, 9H, 3 x OAc).

$^{13}$C NMR (CDCl$_3$) $\delta$ α anomer 170.1, 169.4, 169.1 (O=C(O)Me), 99.1 (C-1), 82.6 (C-4), 80.1 (C-3), 76.4 (C-2), 74.9, 74.0 (C-2, C-3), 69.7 (C-5), 37.5 (Ms-CH$_3$), 20.8, 20.5, 20.3 (OC(O)Me); β anomer 170.5, 169.6, 169.4 (O=C(O)Me), 95.7 (C-1), 79.4 (C-2, C-3), 69.7 (C-5), 37.5 (Ms-CH$_3$), 20.8, 20.5, 20.3 (OC(O)Me). m/z 377 [M + Na]$^+$

1,2,3-tri-$O$-acetyl-5-azido-5-deoxy-$D$-arabinofuranose (6)

To a stirred solution of the mesylate 5 (1.30 g, 3.66 mmol) in dry DMF (20 mL) under N$_2$ was added sodium azide (NaN$_3$) (480 mg, 7.38 mmol). The reaction mixture was heated to 60 °C and was allowed to stir at this temperature for 24 hours before being concentrated in vacuo. The residue was diluted with EtOAc (150 mL), washed with water (150 mL), dried with Na$_2$SO$_4$ and concentrated. Purification by column chromatography (EtOAc:Hex 1:2, $R_f$ 0.3), gave the desired compound in a yield of 805 mg (73%).

$^1$H NMR (CDCl$_3$) $\delta$ α anomer 6.22 (s, 1H, H-1), 5.21 (d, 1H, $J_{2,3}$ 1.2 Hz, H-2), 5.04 (dd, 1H, $J_{3,4}$ 4.8 Hz, H-3), 4.29 (ddd, 1H, $J_{4,5}$ 4.8 Hz, $J_{3,4}$ 3.3 Hz, H-4), 3.68 (dd, 1H, $J_{5,5'}$ 13.5 Hz, $J_{5,4}$ 3.3 Hz, H-5), 3.45 (dd, 1H, $J_{5,5'}$ 13.5 Hz, $J_{5,4}$ 4.8 Hz, H-5'), 2.14, 2.12, 2.11 (3s, 9H, 3 x OAc); β anomer 6.40 (d, 1H, $J_{1,2}$ 4.2 Hz, H-1), 5.40 – 5.34 (m, 2H, H-2, H-3), 4.15 – 4.10 (m, 1H, H-4), 3.60 (dd, 1H, $J_{5,5'}$ 13.2 Hz, $J_{5,4}$ 3.6 Hz, H-5), 3.47 (dd, 1H, $J_{5,5'}$ 13.2 Hz, $J_{5,4}$ 4.5 Hz, H-5'), 2.11, 2.10, 2.08 (3s, 9H, 3 x OAc). $^{13}$C NMR (CDCl$_3$) $\delta$ α anomer 170.3, 169.4, 169.1 (OC(O)Me), 93.4 (C-1), 80.7 (C-4), 75.0, 74.7 (C-2, C-3), 52.9 (C-5), 20.9, 20.6, 20.3 (OC(O)Me); β anomer 170.1, 169.5,
169.1 (OC(O)Me), 99.3 (C-1) 84.1 (C-4), 80.6 (C-3), 77.3 (C-2), 51.2 (C-5), 20.9, 20.6, 20.3 (OC(O)Me). m/z 346.3 [M + 2Na].

1,2,3-tri-O-acetyl-5-thioacetyl-D-arabinofuranose (7)
The mesylate 5 (3.0 g, 8.46 mmol) was dissolved in dry acetone (150 mL) at RT under N₂, and KSAc (7.2 g, 63.45 mmol) was added. The mixture was heated to 40 °C and stirred for 72 hours. After cooling to RT, the reaction mixture was filtered through celite, concentrated in vacuo, and the residue dissolved in EtOAc (250 mL) and washed with 1M HCl (250 mL) and water (150 mL), dried (Na₂SO₄) and concentrated. The title compound 7 was obtained by column chromatography (EtOAc:Hex 1:3; R₁ 0.45) in a yield of 2.3g (82%).

1H NMR (CDCl₃) δ α anomer 6.17 (s, 1H, H-1), 5.12 (s, 1H, H-2), 4.94 (dd, 1H, J₃,₄ 5.1 Hz, H-3), 4.26 (ddd, 1H, J₄,₅ 5.1 Hz, J₅,₅’ 6.6 Hz, J₅,₅” 5.1 Hz, H-4), 3.32 (dd, 1H, J₅,₅’ 5.1 Hz, J₅,₅” 14.1 Hz, H-5), 3.13 (dd, 1H, J₅’,₅” 6.6 Hz, J₅”,₅’ 14.1 Hz, H-5”), 2.32 (s, 3H, SAc), 2.09- 2.06 (m, 9H, 3x OAc); β anomer 6.30 (d, 1H, J₁,₂ 6.0 Hz, H-1), 5.31- 5.24 (m, 2H, J₂,₁ 6.0 Hz, J₂₃ 6.3 Hz, J₃,₂ 6.3 Hz, J₃,₄ 6.3 Hz, H-2, H-3), 4.05 (ddd, 1H, J₃,₄ 6.3 Hz, J₄,₅ 4.5 Hz, J₄,₅ 6.3 Hz, H-4), 3.32 (dd, 1H, J₅,₄ 5.4 Hz, J₅,₅’ 14.1 Hz, H-5), 3.13 (dd, 1H, J₅’,₅” 6.3 Hz, J₅”,₅’ 14.1 Hz, H-5”), 2.32 (s, 3H, SAc), 2.09-2.06 (m, 9H, 3x OAc). 13C NMR (CDCl₃) δ α anomer 194.1 (SC(O)Me), 169.8, 169.4, 168.8 (OC(O)Me), 100.2 (C-1), 82.6 (C-4), 80.5 (C-2), 78.0 (C-3), 31.0 (C-5), 30.6 (SC(O)Me), 20.6, 20.3, 20.2 (OC(O)Me); β anomer 193.9 (SC(O)Me), 169.8, 169.5, 168.8 (OC(O)Me), 93.4 (C-1), 80.2 (C-4), 76.6, 75.3 (C-2, C-3), 32.5 (C-5), 30.9 (SC(O)Me), 20.7, 20.4, 20.0 (OC(O)Me). m/z 356.3 [M+Na]⁺. IR (EtOAc) 2980, 1750, 1700, 1200, 900, 730.

1,2,3-tri-O-acetyl-5-thiomethyl-D-arabinofuranose (8)
A solution of the 5-thioacetyl derivative 7 (250 mg, 0.75 mmol) in dry DMF (8 mL) was degassed by bubbling N₂ for 15 minutes. Hydrazine acetate (H₂NNH₂•HOAc) (336 mg, 3.74 mmol) and dimethyl sulfate (706 µL, 7.47 mmol) were then added under N₂ atmosphere, and the mixture stirred for 4 hours at RT. The reaction mixture was diluted with EtOAc (50 mL) and washed using 1M HCl (50 mL) and water (40 mL), dried (Na₂SO₄) and concentrated. Column chromatography (EtOAc : Hex 1:1; R₁ 0.6), gave the desired compound in a yield of 140 mg (61%).

1H NMR (CDCl₃) δ α anomer 6.17 (s, 1H, H-1), 5.18 (d, 1H, J₂,₃ 1.5 Hz, H-2), 5.09 (dd, 1H, J₃,₄ 1.5 Hz, J₃,₄ 5.4 Hz, H-3), 4.35 (dd, 1H, J₄,₅ 5.4 Hz, J₄,₅ 5.7 Hz, H-4), 2.86 (dd, 1H, J₅,₅’ 5.7 Hz, J₅,₅” 14.1 Hz, H-5), 2.80 (dd, 1H, J₅’,₅” 14.1 Hz, H-5”), 2.17 (s, 3H, SAc), 2.19-2.03 (m, 9H, 3x OAc); β anomer 6.37 (d, 1H, J₁,₂ 3.9 Hz, H-1), 5.38 (dd, 1H, J₂,₃ 6.6 Hz, J₃,₄ 12.0 Hz, H-3), 5.32 (dd, 1H, J₂,₁ 3.9 Hz, J₂,₃ 6.3 Hz, H-2), 4.18 (dd, 1H, J₃,₄ 12.0 Hz, J₄,₅ 6.6 Hz, H-4), 2.86 (dd, 1H, J₅,₅’ 14.1 Hz, H-5), 2.80 (dd, 1H, J₅’,₅” 14.1 Hz, H-5”), 2.14 (s, 3H, SAc), 2.19-2.03 (m, 9H, 3x OAc). m/z 329.0 [M+Na]⁺. IR (EtOAc) 2980, 1730, 1370, 1230, 1050.

1,2,3-tri-O-benzyl-5-deoxy-5-fluoro-D-arabinofuranose (13)
To a stirred solution of 1,2,3-O-tri-O-benzyl-D-arabinofuranose (12) (290 mg, 0.69 mmol) in dry CH₂Cl₂ (7 mL), was added diethylamino sulfur trifluoride (DAST) (360 µL, 2.76 mmol) slowly under argon atmosphere at –10 °C. The mixture was allowed to stir for 15 minutes at –10 °C, then allowed to warm to room temperature and stirred for an additional 3 hours. The reaction was carefully quenched by the addition of saturated aqueous sodium bicarbonate (NaHCO₃). The solution was then diluted in chloroform and the organic layer was washed with water and
concentrated under vacuum. Column chromatography (EtOAc:Hexane 1:3; Rf 0.5) gave the 5-fluoro derivative 13 in a yield of 169 mg (58%).

1H NMR (CDCl3) α-anomer: δ 7.40-7.29 (15H, m, 3 x Ph); 5.15 (1H, s, H-1); 4.71 (2H, ABq, PhCH2O); 4.68-4.40 (6H, m, H-5; 2 x PhCH2O); 4.35-4.23 (1H, m, H-4, J4,F 23Hz); 4.13 (1H, d, J2,3 = 3.0Hz, H-2); 3.94 (1H, dd, J3,4 6.0, J3,2 3.0Hz, H-3). Assignments confirmed by COSY.

13C NMR (CDCl3) α-anomer: δ 137.5, 137.4, 137.3 (all ipso Ph); 128.5, 128.4, 128.3, 128.0, 127.9, 127.9, 127.8, 127.7 (all Ph); 105.1 (C-1); 87.9 (C-2); 82.4 (C-3, 1JCF 6.5Hz); 82.0 (C-5, 1JCF 174Hz); 80.0 (C-4, 2JCF 19.6Hz), 72.3, 71.9, 69.0 (3 x PhCH2O).

1,2,3-tri-O-Benzyl-5-O-methyl-d-arabinofuranose (14)

1,2,3-O-tri-O-Benzyl-d-arabinofuranose (12) (370 mg, 0.88 mmol) was dissolved in DMF (20 mL) under N2 in an ice water bath. Sodium hydride (60% dispersion in oil, 104 mg, 2.6 mmol) was added and the mixture was stirred for 50 min before the addition of methyl iodide (62.2 µl, 1.0 mmol). After stirring for 16h at RT, the reaction was quenched by the addition of MeOH, and concentrated. The residue was diluted with EtOAc (50 mL) and washed with 1M HCl (50 mL) and water (40 mL), dried (Na2SO4) and concentrated. Column chromatography (EtOAc:Hexane 1:5; Rf 0.4) gave the desired product in a yield of 310 mg (81%).

1H NMR(CDCl3) δ β anomer- 7.43-7.30 (m, 15H, Ph-H), 5.01 (d, 1H, J1,2 4.2 Hz, H-1), 4.84-4.51 (m, 6H, OCH3Ph), 4.18-4.15 (m, 1H, J4,5 3.3 Hz, J4,5 4.8 Hz, H-4), 4.09-4.05 (m, J2,1 4.2 Hz J2,3 7.2 Hz, H-2), 3.89 (dd, 1H, J3,2 7.2 Hz, J3,4 3.3 Hz, H-3) 3.60 (dd, 1H J5,5 3.3 Hz, J5,5 10.2 Hz, H-5), 3.51 (dd, 1H, J5,5 10.2 Hz, J5,4 4.8 Hz, H-5') 3.40 (s, 3H, OCH3). 13C NMR (CDCl3) δ 138.3, 137.7, 137.5 (ipso Ph-C), 128.5, 128.45, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7 (all Ph), 99.2 (C-1), 84.3 (C-3), 83.3, 80.3 (C-4, C-2), 75.4 (C-5), 72.4, 68.8 (3 x CH2Ph), 59.2 (CH3). m/z 437.1 [M]+

Synthesis of C-8 modified KDO derivatives

2-keto-3-deoxy-d-manno-octulosonic acid (KDO, 1)

D-Arabinose (500mg, 3.3 mmol) was added to a solution of sodium carbonate (860mg, 8.1 mmol) in water (8 mL). Oxalacetic acid (525mg, 4.0 mmol) was added portionwise over 5 min, and the solution adjusted to pH 11 using NaOH (10M). After stirring for 2h at RT the solution was acidified to pH 5 using AcOH, NiCl2 (7.5mg, 0.03 mmol) added, and the mixture heated at 50 °C for 1h. After cooling to RT the reaction was neutralized (to pH 8) with ammonia and the KDO isolated by column chromatography using CG-400 (HCOO−) resin, washing first with water and then eluting with ammonium hydrogen carbonate solution (0.5M). The eluant was concentrated under reduced pressure, and then freeze-dried. The lyophilized residue was purified using reversed-phase (C18) silica gel with water as the mobile phase. Fractions containing KDO can be visualized as bluish-grey spots on silica gel (t.l.c. plates) using CHCl3:MeOH:H2O (5:5:1) as the mobile phase, and staining with anisaldehyde–sulfuric acid dip. For spectroscopic purposes, the material from above was purified using Dowex 1x8 (200-400mesh) anion exchange resin, eluting with a linear gradient of ammonium bicarbonate (0-0.15M). Fractions containing carbohydrate (charred with H2SO4 dip on TLC) were concentrated separately, analysed by nmr and then combined according to their identity. In this way, the following compounds were obtained (data given for major anomer only):

KDO pyranose 1H NMR (D2O) δ 3.91- 3.81 (m, 2H, J4,5 3.0 Hz, J4,3 5.4 Hz, J5,4 3.0 Hz, J5,6 6.3 Hz, H-4, H-5), 3.75- 3.60 (m, 3.0 Hz, J6,7 8.4 Hz, J6,5 6.3 Hz, J7,6 8.4 Hz, J7,8' 5.7 Hz, H-7, H-8, H-6), 3.43 (dd, 1H, J8,7 5.7 Hz, J8,'8 12.0 Hz, H-8'), 1.79 (t, 1H, J3a,3eq 12.6 Hz, H-3a), 1.69 (dd, 1H, J3eq,3ax 12.6

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Hz, $J_{3eq,4} 5.4$ Hz, H-3eq). $^{13}$C NMR (D$_2$O) $\delta$ 176.8 (C-1), 96.4 (C-2), 71.5 (C-6), 69.2 (C-7), 66.6 (C-4), 66.2 (C-5), 63.0 (C-8), 33.6 (C-3). $m/z = 261.9$ [M+Na]$^+$

KDO furanose $^1$H NMR (D$_2$O) $\delta$ 4.30 (dd, 1H, $J_{5,4} 3.6$ Hz, H-5) 3.96 (dd, 1H, $J_{4,3ax} 5.4$ Hz, H-3ax), 2.00 (dd, 1H, H-3eq). $^{13}$C NMR (D$_2$O) $\delta$ 176.8 (C-1), 96.4 (C-2), 71.5 (C-6), 69.2 (C-7), 66.6 (C-4), 66.2 (C-5), 63.0 (C-8), 33.6 (C-3). $m/z = 261.9$ [M+Na]$^+$

In a similar manner were prepared:

8-azido-8-deoxy-KDO (15, X=N$_3$)

Obtained in 87% yield by condensation between 5-azido-5-deoxy-arabinose (450mg) and oxalacetic acid.

$^1$H NMR (D$_2$O) $\delta$ 4.09 – 3.92 (m, 3H, H-4, H-5, H-7), 3.80 – 3.74 (m, 1H, H-6), 3.51 (dd, 1H, $J_{8,8'} 13.2$ Hz, $J_{8,7} 2.7$ Hz, H-8), 3.35 (dd, 1H, $J_{8',8} 13.2$ Hz, $J_{8',7} 6.0$ Hz, H-8'), 1.91 (dd, 1H, $J_{3ax,3eq} 12.3$ Hz, $J_{3ax,4} 12.3$ Hz, H-3ax), 1.80 (dd, 1H, $J_{3eq,3ax} 12.3$ Hz, $J_{3eq,4} 5.1$ Hz, H-3eq). $^{13}$C NMR (D$_2$O) $\delta$ 176.6 (C-1), 96.4 (C-2), 71.6 (C-4), 70.0 (C-5), 68.0 (C-6), 66.1 (C-7), 53.6 (C-8), 33.6 (C-3). $m/z = 263.1$ [M-H]$^-$. IR (H$_2$O) 3200 – 2900 (br), 2100, 1600, 1450, 1100.

8-amino-8-deoxy-KDO (15, X = NH$_2$)

8-Azido-8-deoxy-KDO was dissolved in water with acetic acid which was degassed with bubbling N$_2$. The compound was passed through a "ThalesNano H-cube" instrument (Pd/C cartridge) at 30 bar with 40 bar H$_2$ gas pressure at 30 °C. The amine was obtained in a quantitative yield.

$^1$H NMR (D$_2$O) $\delta$ 4.10 – 3.98 (m, 3H, H-7, H-5, H-7), 3.72 (dd, 1H, H-6), 3.12 (dd, 1H, $J_{8,8'} 12.9$ Hz, $J_{8,7} 3.6$ Hz, H-8), 2.92 (dd, 1H, $J_{8',8} 4.5$ Hz, H-8'), 1.82-1.75 (m, 2H, H-3ax, H-3eq). $^{13}$C NMR (D$_2$O) $\delta$ 176.4 (C-1), 96.1 (C-2), 73.0 (C-6), 66.2, 65.9, 65.6 (C-4, C-5, C-7), 41.1 (C-8), 33.5 (C-3). IR (H$_2$O) 3400 – 3300 (br), 1560, 1410. $m/z$ 238.1 [M+H]$^+$

8-O-methyl-KDO (15, X = OMe)

Obtained in 87% yield by condensation between 5-O-methyl-arabinose (280mg) and oxalacetic acid.
$^1$H NMR (D$_2$O) $\delta$ 3.99 (ddd, 1H, $J_{4,3ax}$ 12.3 Hz, $J_{4,3eq}$ 5.1 Hz, $J_{4,5}$ 3.6 Hz, H-4), 3.95 – 3.88 (m, 2H, H-5, H-7), 3.74 – 3.60 (m, 2H, H-6, H-8), 3.42 (dd, 1H, $J_{8,8'}$ 12.0 Hz, $J_{8',7}$ 8.4 Hz, H-8'), 3.33 (s, 3H, OCH$_3$), 1.93 (dd, 1H, $J_{3ax,4}$ 12.3 Hz, $J_{3ax,3eq}$ 12.3 Hz, H-3ax), 1.81 (dd, 1H, $J_{3eq,3ax}$ 12.3 Hz, $J_{3eq,4}$ 5.1 Hz, H-3eq). $^{13}$C NMR (D$_2$O) $\delta$ 175.0 (C-1), 126.1 (C-2), 74.5 (C-8), 71.4 (C-4), 69.7 (C-5), 67.7 (C-6), 67.0 (C-7), 58.3 (OCH$_3$), 33.0 (C-3). m/z 251.1 [M-H]$^-$

8-deoxy-8-thio-KDO (15, X = SH)
Obtained in 67% yield by condensation between 5-thio-5-deoxy-arabinose (830mg) and oxalacetic acid.

$^1$H NMR (D$_2$O) $\delta$ 3.36 (dd, 1H, H-5), 2.79 – 2.58 (m, 3H, H-4, H-6, H-7), 2.50 – 2.18 (m, 2H, H-8, H-8'), 1.60 – 1.28 (m, 2H, H-3ax, H-3eq). $^{13}$C NMR (D$_2$O) $\delta$ 172.3 (C-1), 121.2 (C-2), 66.6, 65.6, 65.2, 62.2 (C-4, C-5, C-6, C-7), 23.2 (C-8), 22.6 (C-3). m/z 253.1 [M-H]$^-$ IR (H$_2$O) 3050-2900(br), 1590, 1455, 1450.