A Practical Method for the Stereoselective Generation of β -2-Deoxy Glycosyl Phosphates

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Supporting Information

General Methods. All chemicals were purchased from Aldrich or Sigma and used without further purification. Solvents were reagent grade and were further dried when necessary. Analytical thin-layer chromatography was performed on glass plates precoated with silica gel (250 μ m, Sorbent Technologies), with detection by UV and/or spraying with H₂SO₄ (50%). Flash chromatography was carried out on silica gel (60 Å, 32-63 μ m), purchased from Sorbent Technologies. Analytical HPLC of reaction mixtures was performed on a Hewlett-Packard 1100 series instrument using a Phenomenex Luna 5 μ m C18 column (250 mm x 4.6 mm). Compounds bearing a thymidine chromophore were monitored at an absorbance of 270 nm. Reactions were monitored by HPLC using method A (linear gradient from H₂O/0.1% NH₄HCO₃ to 100% MeOH/0.1% NH₄HCO₃ over the course of 20 min, flow-rate: 1 mL/min). Preparative HPLC was performed on a Varian ProStar instrument (flow-rate: 45 mL/min) using a Phenomenex Luna 10 μ m C18 column (250 x 50 mm) or on a Hitachi L6200 instrument (flow-rate: 7.5 mL/min) using a Phenomenex Luna 5 μ m C18 column (250 x 21.2 mm). NMR spectra were recorded on Varian Mercury 300 MHz and Inova 400 or 500 MHz spectrometers. Mass spectra (ESI) were obtained at the Mass Spectroscopy Facility at the Department of Chemistry, Princeton University.

*4-O-Acetyl-3-azido-2,3,6-trideoxy- β -L-arabino-hexopyranosyl phosphate (**8 β**):*

^1H NMR (500 MHz, CD_3OD): δ = 5.21 (ddd, $J_{1,\text{P}}$ = 8.0, $J_{1,2\text{a}}$ = 2.2, $J_{1,2\text{b}}$ = 9.6 Hz, 1 H, 1-H), 4.57 (t, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1 H, 4-H), 3.80 (ddd, $J_{2\text{a},3} = 4.8$, $J_{2\text{b},3} = 12.5$, $J_{3,4} = 9.7$ Hz, 1 H, 3-H), 3.69 (dq, $J_{4,5} = 9.7$, $J_{5,6} = 6.2$ Hz, 1 H, 5-H), 2.43 (ddd, $J_{1,2\text{a}} = 2.2$, $J_{2,2} = 12.7$, $J_{2,3} = 5.2$ Hz, 1 H, 2-H_a), 2.16 (s, 3H, COCH_3), 1.61 (dt, $J_{1,2\text{b}} = 9.6$, $J_{2,2} = J_{2\text{b},3} = 12.7$ Hz, 1 H, 2-H_b), 1.18 (d, $J_{5,6} = 6.2$ Hz, 3 H, 6-H₃); ^{31}P NMR (162 MHz, CD_3OD): δ = -0.50. LRMS (ESI) for $\text{C}_8\text{H}_{14}\text{N}_3\text{O}_7\text{P}_2$ (295.2): 293 $[\text{M}-2\text{H}]^-$.

*4-O-Acetyl-3-azido-2,3,6-trideoxy- α -L-arabino-hexopyranosyl phosphate (**8 α**):*

^1H NMR (500 MHz, CD_3OD): δ = 5.55 (m, 1 H, 1-H); ^{31}P NMR (162 MHz, CD_3OD): δ = -0.30.

Scheme S1. a) Ac_2O , pyridine; b) PhSH , $\text{BF}_3 \cdot \text{OEt}_2$; c) K_2CO_3 , MeOH ; d) 1. Tf_2O , pyridine, 2. KOAc , 18-crown-6; e) NIS , $\text{CH}_3\text{CN}/\text{H}_2\text{O}$; f) $(\text{COCl})_2$, DMF ; g) $\text{Bu}_4\text{NH}_2\text{PO}_4$, CH_3CN .

*Phenyl 4-O-acetyl-3-azido-1-thio-2,3,6-trideoxy- α/β -L-arabino-hexopyranoside (**25**):*

Lactol **5** (1.40 g, 6.5 mmol)^[S1] was dissolved in pyridine (10 mL), and Ac_2O (4 mL) and DMAP (20 mg) were added. The solution was stirred overnight, quenched with MeOH , concentrated, redissolved in CH_2Cl_2 and washed with 1 N HCl (2 x), H_2O , satd. aq. NaHCO_3 , and brine. The organic layer was dried (MgSO_4), filtered, evaporated and the oily residue was purified by flash chromatography (petroleum ether/ EtOAc 6:1) to give the anomeric acetate (1.67 g, quant.), R_f = 0.58 (α), 0.41 (β), petroleum ether/ EtOAc 6:1.

This compound (1.67 g, 6.5 mmol) was dissolved in CH₂Cl₂ (65 mL) and cooled to –78° C. Thiophenol (0.80 mL, 7.8 mmol) and BF₃·OEt₂ (1.15 mL, 9.1 mmol) were added to the solution. After the cooling bath was removed, the mixture was stirred for 1 h. The solution was poured into satd. aq. NaHCO₃ and extracted with CH₂Cl₂ (2 x 200 mL). The combined organic layers were washed with satd. aq. NaHCO₃ (2 x) and brine. The organic layer was dried (MgSO₄), filtered and concentrated to give an oily residue, which was purified by flash chromatography (petroleum ether/EtOAc 20:1, then 6:1) to give **25** as a mixture of anomers (1.82 g, 91%, α/β 2:1), R_f = 0.59 (**25 α**), 0.36 (**25 β**), petroleum ether/EtOAc 10:1. ¹H NMR (400 MHz, CDCl₃): **25 α** : δ = 7.48–7.23 (m, 5 H, aromatic H), 5.57 (bd, $J_{1,2b}$ = 5.5 Hz, 1 H, 1-H), 4.72 (t, $J_{3,4} = J_{4,5}$ = 9.7 Hz, 1 H, 4-H), 4.31 (dq, $J_{4,5}$ = 9.7, $J_{5,6}$ = 6.3 Hz, 1 H, 5-H), 3.87 (ddd, $J_{2a,3}$ = 4.9, $J_{2b,3}$ = 12.3, $J_{3,4}$ = 9.7 Hz, 1 H, 3-H), 2.37 (ddd, $J_{1,2}$ = 1.2, $J_{2,2}$ = 13.5, $J_{2,3}$ = 4.9 Hz, 1 H, 2-H_a), 2.15 (s, 3 H, COCH₃), 2.12 (ddd, $J_{1,2}$ = 5.5, $J_{2,2}$ = 13.5, $J_{2,3}$ = 2.5 Hz, 1 H, 2-H_b), 1.15 (d, $J_{5,6}$ = 6.2 Hz, 3 H, 6-H₃). **25 β** : δ = 7.48–7.23 (m, 5 H, aromatic H), 4.76 (dd, $J_{1,2a}$ = 2.0, $J_{1,2b}$ = 11.7 Hz, 1 H, 1-H), 4.65 (t, $J_{3,4} = J_{4,5}$ = 9.6 Hz, 1 H, 4-H), 3.57 (ddd, $J_{2a,3}$ = 5.1, $J_{2b,3}$ = 12.0, $J_{3,4}$ = 9.6 Hz, 1 H, 3-H), 3.47 (dq, $J_{4,5}$ = 9.6, $J_{2b,3}$ = 6.1 Hz, 1 H, 5-H), 2.35 (ddd, $J_{1,2}$ = 2.0, $J_{2,2}$ = 13.1, $J_{2,3}$ = 5.1 Hz, 1 H, 2-H_a), 2.09 (s, 3 H, COCH₃), 1.80 (app. q, 1 H, H-2_b), 1.22 (d, $J_{5,6}$ = 6.1 Hz, 3 H, 6-H₃); LRMS (ESI) for C₁₄H₁₇N₃O₃S (307.37): 307 [M]⁺.

Phenyl 4-O-acetyl-3-azido-1-thio-2,3,6-trideoxy- α/β -L-lyxo-hexopyranoside (26):

Thioglycoside **25** (1.72 g, 5.6 mmol) was dissolved in MeOH (50 mL) and K₂CO₃ (0.39 g, 2.8 mmol) was added to the solution. After stirring for 16 h, the solution was poured into sat. aq. NH₄Cl and extracted with CH₂Cl₂ (3 x). The organic layers were washed with sat. aq. NH₄Cl, sat. aq. NaHCO₃, brine and dried (MgSO₄). The solution was filtered, concentrated and purified by flash chromatography (petroleum ether/EtOAc 7:1) to give the 4-OH unprotected thioglycoside as a mixture of anomers (1.41 g, 95%, α/β 2:1), R_f = 0.45 (α), 0.35 (β), petroleum ether/EtOAc 7:1. This compound (1.41 g, 5.32 mmol) was dissolved in CH₂Cl₂ (20 mL) and cooled to 0°C. Pyridine (2 mL) and Tf₂O (1.1 mL, 6.5 mmol, dissolved in 2 mL of CH₂Cl₂) were added and the solution was stirred for 1 h. The solution was poured into 1 N HCl and extracted with CH₂Cl₂ (2 x 150 mL).

The combined organic layers were washed with brine and evaporated to dryness to give the crude 4-*O*-triflate as a brown oil, $R_f = 0.75$, petroleum ether/EtOAc 7:1. The 4-*O*-triflate and 18-crown-6 (2.0 g, 7.42 mmol) were azeotroped with toluene (3 x) and dissolved in DMF (40 mL). Potassium acetate (0.73 g, 7.42 mmol) was added, and the solution was stirred at room temp. for 2 h. The solution was diluted with EtOAc (300 mL) and washed with water (3 x). The combined water layers were extracted with EtOAc (2 x) and the organic layers were washed with brine and dried (MgSO₄). The solution was filtered, concentrated and purified by flash chromatography (petroleum ether/EtOAc 12:1) to give **26** as a mixture of anomers (1.50 g, 92%, α/β 2:1), $R_f = 0.60$ (α), 0.50 (β), petroleum ether/EtOAc 7:1. ¹H NMR (400 MHz, CDCl₃): **26** α : $\delta = 7.47$ – 7.22 (m, 5 H, aromatic H), 5.75 (d, $J_{1,2a} = 5.3$ Hz, 1 H, 1-H), 5.19 (bs, 1 H, 4-H) 4.60 (q, $J_{5,6} = 6.6$ Hz, 1 H, 5-H), 3.82 (ddd, $J_{2a,3} = 13.0$, $J_{2b,3} = 4.6$, $J_{3,4} = 3.0$ Hz, 1 H, 3-H), 2.44 (dt, $J_{1,2} = 5.3$, $J_{2,3} = J_{2,2} = 13.0$ Hz, 1 H, 2-H_a), 2.16 (s, 3 H, COCH₃), 2.14–2.08 (m, 1 H, 2-H_b), 1.12 (d, $J_{5,6} = 6.6$ Hz, 3 H, 6-H₃). **26** β : $\delta = 7.48$ – 7.23 (m, 5 H, aromatic H), 5.13 (bs, 1 H, 4-H), 4.84 (dd, $J_{1,2a} = 10.7$ Hz, $J_{1,2b} = 2.9$ Hz, 1 H, 1-H), 3.71 (q, $J_{5,6} = 6.6$ Hz, 1 H, 5-H), 3.57–3.50 (m, 1 H, 3-H), 2.37–2.34 (m, 1 H, 2-H_a), 2.19–2.10 (m, 4 H, 2-H_b, COCH₃), 1.25 (d, $J_{5,6} = 6.4$ Hz, 3 H, 6-H₃). LRMS (ESI) for C₁₄H₁₇N₃O₃S (307.37): 307 [M]⁺.

4-O-Acetyl-3-azido-2,3,6-trideoxy- α/β -L-lyxo-hexopyranosyl phosphate (11):

Thioglycoside **26** (710 mg, 2.3 mmol) was dissolved in CH₃CN/H₂O (20:1, 30 mL) and NIS (620 mg, 2.76 mmol) was added. After stirring for 30 min, the brown solution was diluted with EtOAc (300 mL) and washed with 10% aq. Na₂S₂O₃, sat. aq. NaHCO₃ and dried (MgSO₄). The solution was filtered, concentrated and the residue was purified by flash chromatography (petroleum ether/EtOAc, 10:1, then 3:1) to give the lactol as a mixture of anomers (300 mg, 61%, α/β 2:1), $R_f = 0.21$, petroleum ether/EtOAc 4:1. The lactol (300 mg, 1.4 mmol) was converted to chloride **10** and coupled with Bu₄NH₂PO₄ (0.4 M in CH₃CN, 7.0 mL, 2.8 mmol) as described for **5** $\rightarrow$ **8**. Purification by HPLC gave the ammonium salt of phosphate **11** (269 mg, 59%, β/α 5:1) as a white powder upon lyophilization. ¹H NMR (400 MHz, CD₃OD): **11** α : $\delta = 5.68$ (d, $J_{1,P} = 7.2$ Hz, 1 H, 1-H), 5.16 (d, $J_{4,5} = 3.6$ Hz, 1 H, 4-H), 4.33–4.29 (q, $J_{5,6} = 6.6$ Hz, 1 H, 5-H), 4.04–4.00 (m, 1

H, 3-H), 2.15–1.90 (m, 5 H, 2-H₂, COCH₃), 1.06 (d, $J_{5,6} = 6.6$ Hz, 3 H, 6-H₃). **11β**: $\delta = 5.18$ (dt, $J_{1,P} = J_{1,2} = 8.6$, $J_{1,2} = 2.0$ Hz, 1 H, 1-H), 5.05 (d, $J_{4,5} = 3.0$ Hz, 1 H, 4-H), 3.80–3.65 (m, 2 H, 3-H, 5-H), 2.15–1.80 (m, 5 H, 2-H₂, COCH₃), 1.14 (d, $J_{5,6} = 6.3$ Hz, 3 H, 6-H₃). ³¹P NMR (162 MHz, CD₃OD): **11α**: $\delta = -0.87$, **11β**: $\delta = -0.52$. LRMS (ESI) for C₉H₁₆N₃O₇P (309.07): 308 [M–H][–].

Scheme S2. a) HCl (g); b) Bu₄NH₂PO₄, CH₃CN.

3,4-Di-O-acetyl-2-deoxy-β-L-fucopyranosyl phosphate (13):

3,4-Di-O-acetyl-L-fucal (**27**, 0.100 g, 0.47 mmol)^[S2] was azeotroped with benzene (2 x), dissolved in benzene (2 mL), and HCl (g) was bubbled through the solution for 15 min. After evaporation of the solvent at reduced pressure without heating, the residue was azeotroped with benzene to remove excess HCl. Chloride **12** was obtained as an oily residue and was coupled with Bu₄NH₂PO₄ (0.4 M in CH₃CN, 2.35 mL, 0.94 mmol) as described for **5** → **8**. Purification by flash chromatography (CH₂Cl₂/MeOH/H₂O/Et₃N 16:8:0.2:0.3) gave the monotetrabutylammonium salt of phosphate **13** (135 mg, 52%, β/α 9:1) as a colorless syrup. ¹H NMR (400 MHz, CD₃OD) **13β**: $\delta = 5.21$ (dt, $J_{1,P} = J_{1,2} = 8.5$, $J_{1,2} = 2.0$ Hz, 1 H, 1-H), 5.06–5.02 (m, 2 H, 3-H, 4-H), 3.82 (q, $J_{5,6} = 6.4$ Hz, 1 H, 5-H), 2.12 (s, 3 H, COCH₃), 2.09–2.07 (m, 1 H, 2-H_a), 1.95 (s, 3 H, COCH₃), 1.90–1.82 (m, 1 H, 2-H_b), 1.16 (d, $J_{5,6} = 6.2$ Hz, 3 H, 6-H₃); ³¹P NMR (162 MHz, CD₃OD): $\delta = -0.25$; LRMS (ESI) for C₁₀H₁₇O₉P₂ (312.16): 311 [M–H][–].

Scheme S3. a) H₂, Pd-C; b) (COCl)₂, DMF; c) Bu₄NH₂PO₄, CH₃CN.

4-O-Acetyl-2,3,6-trideoxy- α/β -L-erythro-hexopyranosyl phosphate (15):

To a solution of benzyl hex-2-enopyranoside **28**^[S3] (260 mg, 1.0 mmol) in EtOH/EtOAc (1:1, 20 mL) was added Pd-C (10%, 60 mg). The mixture was hydrogenated (1 atm) for 10 h, filtered through Celite, concentrated and passed through a short silica gel column (petroleum ether/EtOAc, 2:1). Evaporation of product-containing fractions gave 4-*O*-acetyl-2,3,6-trideoxy- α/β -L-erythro-hexopyranose (148 mg, α/β 1:1, 85%) as a colorless oil, *R*_f = 0.41, petroleum ether/EtOAc 2:1. ¹H NMR (400 MHz, CDCl₃): δ = 5.20 (m, 0.5 H, 1-H _{α}), 5.20 (ddd, *J*_{1,OH} = 6.2, *J*_{1,2} = 9.6, *J*_{1,2} = 2.0 Hz, 0.5 H, 1-H _{β}), 4.48–4.38 (m, 1 H, 4-H _{α} , 4-H _{β}), 4.12 (d, *J*_{1,OH} = 6.2 Hz, 0.5 H, 1 _{β} -OH), 4.02 (dq, *J*_{4,5} = 9.5, *J*_{5,6} = 6.2 Hz, 0.5 H, 5-H _{α}), 3.62–3.51 (m, 1 H, 5-H _{β} , 1 _{α} -OH), 2.10–1.40 (m, 7 H, 2-H₂, 3-H₂, COCH₃), 1.12 and 1.10 (2 d, each 1.5 H, *J*_{5,6} = 6.5 Hz, 6-H₃).

The lactol (18 mg, 0.11 mmol) was converted to chloride **14** and coupled with Bu₄NH₂PO₄ (0.4 M in CH₃CN, 0.55 mL, 0.22 mmol) as described for **5** $\rightarrow$ **8**. Purification of the residue by flash chromatography (CH₂Cl₂/MeOH/H₂O/Et₃N 16:8:0.2:0.3) gave phosphate **15** (29 mg, 54%, β/α 9:1) as its monotetrabutylammonium salt, *R*_f = 0.3 (CH₂Cl₂/MeOH/H₂O/Et₃N 16:8:0.2:0.3). ¹H NMR (300 MHz, CDCl₃): **15 β** : δ = 5.25 (dt, *J*_{1,P} = *J*_{1,2b} = 8.1, *J*_{1,2a} = 1.6 Hz, 1 H, 1-H), 4.38–4.34 (m, 1 H, 4-H), 3.57–3.48 (m, 1 H, 5-H), 2.20–1.45 (m, 7 H, 2-H₂, 3-H₂, COCH₃), 1.12 (d, *J*_{5,6} = 6.2 Hz, 3 H, 6-H₃); ³¹P NMR (162 MHz, CD₃OD): δ = –0.48.

Scheme S4. a) HCl, H₂O; b) H₂, Pd-C; c) TfN₃, CuSO₄, K₂CO₃; d) Ac₂O, pyridine; e) HCl (g); f) Bu₄NH₂PO₄, CH₃CN.

1,4-Di-O-acetyl-3-azido-3-C-methyl-2,3,6-trideoxy- α/β -L-lyxo-hexopyranose (30):

Methyl glycoside **29** (500 mg, 1.63 mmol)^[S4] was dissolved in THF (30 mL) and 4 M HCl (aq., 20 mL) was added. After stirring for 3 h, the reaction mixture was slowly neutralized with solid NaHCO₃. The solution was extracted with CH₂Cl₂ (2 x) and the combined organic phases were washed with water (2 x), dried (MgSO₄), filtered and purified by flash chromatography (petroleum ether/EtOAc 2:1) to give the lactol as a mixture of anomers (412 mg, 86% yield, α/β = 1:2), R_f = 0.20, petroleum ether/EtOAc 1:1. The lactol (412 mg, 1.40 mmol) was dissolved in methanol (20 mL) and Pd/C (10%, 40 mg) was added. The mixture was hydrogenated (1 atm) for 3 h, filtered through a pad of Celite and concentrated to give L-vancosamine as a mixture of anomers (226 mg, quant., α/β = 1:2). ¹H NMR (400 MHz, CD₃OD): α -anomer: δ = 5.24 (d, $J_{1,2}$ = 4.0 Hz, 1 H, 1-H), 4.52 (q, $J_{5,6}$ = 6.4 Hz, 1 H, 5-H), 3.29 (s, 1 H, 4-H), 1.98–1.65 (m, 2-H, 2-H₂), 1.57 (s, 3 H, 3-CH₃), 1.21 (d, $J_{5,6}$ = 6.0 Hz, 3 H, 6-H₃) β -anomer: δ = 4.92–4.90 (m, 1 H, 1-H), 3.84 (q, $J_{5,6}$ = 6.0 Hz, 1 H, 5-H), 3.27 (s, 1 H, 4-H), 1.98–1.65 (m, 2 H, 2-H₂), 1.48 (s, 3 H, 3-CH₃), 1.24 (d, $J_{5,6}$ = 6.0 Hz, 3 H, 6-H₃); LRMS (ESI) for C₇H₁₅NO₃ (161.11): 162 [M+H]⁺.

L-vancosamine (266 mg, 1.65 mmol) was dissolved in H₂O (5 mL), and K₂CO₃ (340 mg, 2.5 mmol), CuSO₄ (20 mg, 0.082 mmol, dissolved in 0.28 mL of H₂O), and TfN₃^[S5] (0.37 M in CH₂Cl₂, 5.0 mmol, 13.4 mL) were added. MeOH was then added until a clear solution resulted. After 15 min the reaction mixture was concentrated and azeotroped with pyridine (2 x). The green residue was dissolved in pyridine (16 mL), and Ac₂O (12 mL) and DMAP (30 mg) were added. The solution was stirred for 2 h, quenched with MeOH, concentrated, redissolved in CH₂Cl₂ and washed with 1 N HCl (2 x), H₂O, satd. aq. NaHCO₃, and brine. The organic layer was dried (MgSO₄), filtered, evaporated and the oily residue was purified by flash chromatography (petroleum ether/EtOAc 4:1) to give **30** as a mixture of anomers (186 mg, 43%, α/β = 1:2), R_f = 0.80, CH₂Cl₂/MeOH 4:1. ¹H NMR (300 MHz, CDCl₃): **30 β** : δ = 5.82 (dd, $J_{1,2}$ = 2.5, $J_{1,2}$ = 10.1 Hz, 1 H, 1-H), 4.76 (bs, 1 H, 4-H), 3.95 (q, $J_{5,6}$ = 6.3 Hz, 1 H, 5-H), 2.20–2.03 (m, 7 H, 2-H_a, 2 COCH₃),

1.90–1.78 (m, 1 H, 2-H_b) 1.44 (s, 3 H, 3-CH₃), 1.25 (d, $J_{5,6}$ = 6.3 Hz, 3 H, 6-H₃). LRMS (ESI) for C₁₁H₁₇N₃O₅ (271.12): 294 [M + Na]⁺.

4-O-Acetyl-3-azido-3-C-methyl-2,3,6-trideoxy- α,β -L-lyxo-hexopyranoyl phosphate (17): Acetate **30** (117 mg, 0.43 mmol) was azeotroped with benzene (2 x), dissolved in benzene (5 mL), and HCl (g) was bubbled through the solution for 15 min. After evaporation of the solvent under reduced pressure without heating, the residue was azeotroped with benzene to remove excess HCl. Chloride **16** was obtained as an oily residue and was coupled directly with Bu₄NH₂PO₄ (0.4 M in CH₃CN, 2.15 mL, 0.86 mmol) as described for **5** $\rightarrow$ **8**. Purification by HPLC gave the ammonium salt of phosphate **17** (74 mg, 53%, β/α 5:1) as a white powder upon lyophilization. ¹H NMR (400 MHz, CD₃OD): **17 α** : δ = 5.53 (dd, $J_{1,2}$ = 3.9 Hz, $J_{1,P}$ = 6.3 Hz, 1 H, 1-H), 4.65 (bs, 1 H, 4-H), 4.26 (q, $J_{5,6}$ = 6.6 Hz, 1 H, 5-H), 2.20–1.90 (m, 5 H, 2-H₂, COCH₃), 1.39 (s, 3 H, 3-CH₃), 0.96 (d, $J_{5,6}$ = 6.6 Hz, 3 H, 6-H₃). **17 β** : δ = 5.28 (dt, $J_{1,2a}$ = $J_{1,P}$ = 8.7, $J_{1,2b}$ = 2.7 Hz, 1 H, 1-H), 4.57 (bs, 1 H, 4-H), 3.63 (q, $J_{5,6}$ = 6.3 Hz, 1 H, 5-H), 2.10–2.03 (m, 4 H, 2-H_a, COCH₃), 1.88–1.80 (m, 1 H, 2-H_b) 1.29 (s, 3 H, 3-CH₃), 1.00 (d, $J_{5,6}$ = 6.3 Hz, 3 H, 6-H₃). ³¹P NMR (162 MHz, CD₃OD): **17 α** : δ = –0.10 **17 β** : δ = –0.35. LRMS (ESI) for C₉H₁₆N₃O₇P (309.07): 308 [M–H][–].

Scheme S5. a) MeOH/H₂O/Et₃N; b) H₂, Pd-C; c) TfN₃, CuSO₄, K₂CO₃; d) Ac₂O, pyridine; e) HCl (g); f) Bu₄NH₂PO₄, CH₃CN.

1,4-Di-O-acetyl-3-azido-3-C-methyl-2,3,6-trideoxy- α,β -L-arabino-hexopyranose (32):

Lactol **31** (280 mg, 0.83 mmol)^[S6] was dissolved in MeOH/H₂O/Et₃N (2:2:1, 20 mL) and stirred for 16 h. The reaction mixture was evaporated to dryness and the residue was purified by flash chromatography (petroleum ether/EtOAc 1:1) to give *N*-Cbz-4-*epi*-vancosamine (243 mg, quant.) as a clear oil, *R*_f = 0.22 (petroleum ether/EtOAc 1:1). *N*-Cbz-4-*epi*-vancosamine (243 mg, 0.83 mmol) was dissolved in MeOH (15 mL) and Pd-C (10%, 30 mg) was added. The mixture was hydrogenated (1 atm) for 3 h, filtered through a pad of Celite and concentrated to give *L-epi*-vancosamine as a mixture of anomers (133 mg, quant, α/β = 1:2). ¹H NMR (400 MHz, CD₃OD): α -anomer: δ = 5.20 (d, *J*_{1,2b} = 3.8 Hz, 1 H, 1-H), 3.94–3.87 (m, 1 H, 5-H), 3.21 (d, *J*_{4,5} = 9.6 Hz, 1 H, 4-H), 2.98 (d, *J*_{2a,2b} = 13.8 Hz, 1 H, 2-H_a), 1.91–1.85 (m, 1 H, 2-H_b), 1.51 (s, 3 H, 3-CH₃), 1.21 (d, *J*_{5,6} = 6.0 Hz, 3 H, 6-H₃); β -anomer: δ = 4.92–4.88 (m, 1 H, 1-H), 3.57–3.51 (m, 1 H, 5-H), 3.19 (d, *J*_{4,5} = 9.4 Hz, 1 H, 4-H), 2.05 (dd, *J*_{1,2a} = 2.1, *J*_{2a,2b} = 12.6 Hz, 1 H, 2-H_a), 1.71 (dd, *J*_{1,2b} = 9.5, *J*_{2a,2b} = 12.4 Hz, 1 H, 2-H_b), 1.36 (s, 3 H, 3-CH₃), 1.25 (d, *J*_{5,6} = 6.0 Hz, 3 H, 6-H₃). LRMS (ESI) for C₇H₁₅NO₃ (161.11): 162 [M+H]⁺.

L-epi-vancosamine (133 mg, 0.83 mmol) was converted to the peracetylated 3-N₃ derivative **32** as described for **30**. Purification by flash chromatography (petroleum ether/EtOAc 4:1) gave **33** as a mixture of anomers (93 mg, 42%, α/β = 1:2), *R*_f = 0.89, CH₂Cl₂/MeOH 4:1. ¹H NMR (500 MHz, CDCl₃): **33** α : δ = 6.15 (bs, 1 H, 1-H), 4.86 (d, *J*_{4,5} = 10.0 Hz, 1 H, 4-H), 3.94 (dq, *J*_{4,5} = 10.0, *J*_{5,6} = 6.3 Hz, 1 H, 5-H), 2.20–1.90 (m, 8 H, 2-H₂, 2 COCH₃), 1.60 (s, 3 H, 3-CH₃), 1.19 (d, *J*_{5,6} = 6.3 Hz, 3 H, 6-H₃). **33** β : δ = 5.82 (dd, *J*_{1,2a} = 2.7, *J*_{1,2b} = 9.7 Hz, 1 H, 1-H), 4.82 (d, *J*_{4,5} = 9.9 Hz, 1 H, 4-H), 3.73–3.67 (dq, *J*_{4,5} = 9.9, *J*_{5,6} = 6.0 Hz, 1 H, 5-H), 2.20–1.90 (m, 8 H, 2-H₂, 2 COCH₃), 1.49 (s, 3 H, 3-CH₃), 1.22 (d, *J*_{5,6} = 6.0 Hz, 3 H, 6-H₃). LRMS (ESI) for C₁₁H₁₇N₃O₅ (271.12): 294 [M+Na]⁺.

4-O-Acetyl-3-azido-2,3,6-trideoxy-3-C-methyl- α,β -L-arabino-hexopyranosyl phosphate (**19**):

Acetate **33** (90 mg, 0.33 mmol) was converted to chloride **18** and coupled to give phosphate **19** as described for **31**. Purification by flash chromatography (CH₂Cl₂/MeOH/H₂O/Et₃N 16:8:0.2:0.3) gave **19** (110 mg, 60%, 5:1 β/α) as its

monotetrabutylammonium salt. ^1H NMR (400 MHz, CD_3OD): **19 β** : δ = 5.28 (dt, $J_{1,2}$ = $J_{1,\text{P}}$ = 8.8, $J_{1,2}$ = 2.1 Hz, 1 H, 1-H), 4.64 (d, $J_{4,5}$ = 10.0 Hz, 1 H, 4-H), 3.65–3.61 (dq, $J_{4,5}$ = 10.0, $J_{5,6}$ = 6.0 Hz, 1 H, 5-H), 2.15–1.90 (m, 5 H, 2- H_2 , COCH_3), 1.42 (s, 3 H, 3- CH_3), 1.09 (d, $J_{5,6}$ = 6.0 Hz, 3 H, 6- H_3); ^{31}P NMR (162 MHz, CD_3OD): δ = -0.40; LRMS (ESI) for $\text{C}_9\text{H}_{16}\text{N}_3\text{O}_7\text{P}$ (309.07): 308 $[\text{M}-\text{H}]^-$.

Scheme S6. a) TMP morpholidate, tetrazole; b) MeOH/ H_2O / Et_3N or NaOMe, MeOH; c) H_2 , Pd-C.

Thymidine 5'-(2,6-dideoxy- β -L-lyxo-hexopyranosyl diphosphate), *TDP β -2-deoxy-L-fucose (20)*: Phosphate **13** (monotetrabutylammonium salt, 49 mg, 90 μmol) and TMP morpholidate (96 mg, 0.14 mmol) were azeotroped with pyridine (3 x) and then dissolved in pyridine (0.5 mL). Tetrazole (22 mg, 0.28 mmol) was added to this solution and the reaction was stirred under argon at room temp. for 2 days. The progress of the reaction was monitored by analytical HPLC (method A, t_{R} (TMP morpholidate) = 8.8 min, t_{R} (TDP sugar, β -anomer) = 8.1 min, t_{R} (TDP sugar, α -anomer) = 8.2 min). After removal of the solvent, the residue was dissolved in 0.1% aq. NH_4HCO_3 (7 mL) and extracted with

diethyl ether (7 mL). The water layer was purified by reversed-phase HPLC and fractions containing the β -anomer were pooled to give the ammonium salt of 3,4-Di-*O*-acetyl fucose TDP (41 mg, 73%) as a white powder upon lyophilization; LRMS (ESI) for $C_{20}H_{30}N_2O_{16}P_2$ (616.33): 615 $[M-H]^-$. This TDP sugar (30 mg, 49 μ mol) was dissolved in MeOH/H₂O/Et₃N (2:2:1, 5 mL). The solution was stirred for 16 h, evaporated to dryness and purified by preparative HPLC, affording **20** (15 mg, 58%) as its ammonium salt; t_R = 6.15 min, method A. ¹H NMR (400 MHz, D₂O): δ = 7.70 (s, 1 H, 6-H), 6.36 (t, $J_{1,2a} = J_{1,2b} = 6.8$ Hz, 1 H, 1'-H) 5.19 (t, $J_{1,2} = J_{1,P} = 8.9$, 1 H, 1''-H), 4.63–4.59 (m, 1 H, 3'-H), 4.18–4.14 (m, 3 H, 4'-H, 5'-H₂), 3.87 (dt, $J_{3,4} = 4.2$, $J_{3,2} = 12.6$ Hz, 1 H, 3''-H), 3.69 (q, $J_{5,6} = 6.4$ Hz, 1 H, 5''-H), 3.57 (bs, 1 H, 4''-H), 2.40–2.33 (m, 1 H, 2'-H₂), 2.14–2.06 (m, 1 H, 2''-H_a), 1.91 (s, 3 H, thymidine CH₃), 1.73–1.65 (m, 1 H, 2''-H_b), 1.27 (d, $J_{5,6} = 6.41$ Hz, 3 H, 6''-H₃); ³¹P NMR (162 MHz, D₂O): δ = –10.78, –13.01; LRMS (ESI) for $C_{16}H_{26}N_2O_{14}P_2$ (531.03): 530 $[M-H]^-$.

Thymidine 5'-(3-amino-2,3,6-trideoxy- β -L-lyxo-hexopyranosyl diphosphate), TDP β -L-daunosamine (21):

Phosphate **11** (ammonium salt, 98 mg, 0.3 mmol) was coupled with TMP morpholidate as described for **13** (t_R (TMP morpholidate) = 8.8 min, t_R (TDP sugar, β -anomer) = 9.2 min, t_R (TDP sugar, α -anomer) = 9.3 min). HPLC purification of the residue gave the protected TDP daunosamine (91 mg, 49%) as a white powder upon lyophilization; LRMS (ESI) for $C_{18}H_{27}N_5O_{14}P_2$ (599.38): 598 $[M-H]^-$. This product (91 mg, 148 μ mol) was dissolved in MeOH/H₂O/Et₃N (2:2:1, 5 mL) and stirred for 16 h. The reaction mixture was concentrated and dissolved in MeOH (5 mL) and H₂O (5 mL). To this solution was added Pd/C (10%, 20 mg) and the mixture was hydrogenated (1 atm) for 1 h. The reaction mixture was filtered through a pad of Celite, concentrated and purified by reversed-phase HPLC. Product containing fractions were pooled, evaporated to a small volume and lyophilized to give the ammonium salt of **21** (54 mg, 67%) as a white solid, t_R = 6.2 min, method A. ¹H NMR (400 MHz, D₂O): δ = 7.69 (s, 1 H, 6-H), 6.36 (t, $J_{1,2a} = J_{1,2b} = 7.1$ Hz, 1 H, 1'-H) 5.20 (t, $J_{1,2} = J_{1,P} = 8.9$ Hz, 1 H, 1''-H), 4.67–4.61 (m, 1 H, 3'-H), 4.20–4.12 (m, 3 H, 4'-H, 5'-H₂), 3.72 (q, $J_{5,6} = 6.4$ Hz, 1 H, 5''-H), 3.46 (d, $J_{4,3} = 2.4$ Hz, 1

H, 4''-H), 3.02–3.00 (m, 1 H, 3''-H), 2.37–2.31 (m, 2 H, 2'-H₂), 2.03–1.97 (m, 1 H, 2''-H_a) 1.90 (s, 3 H, thymidine CH₃), 1.58–1.50 (m, 1 H, 2''-H_b), 1.21 (d, $J_{5,6} = 6.4$ Hz, 3 H, 6''-H₃); ³¹P NMR (162 MHz, D₂O): $\delta = -10.62, -12.95$; LRMS (ESI) for C₁₆H₂₇N₃O₁₃P₂ (531.10): 530 [M–H][–].

Thymidine 5'-(3-amino-2,3,6-trideoxy-β-L-arabino-hexopyranosyl diphosphate), TDP β-L-acosamine (22):

Phosphate **8** (97 mg, 0.31 mmol) was coupled with TMP morpholidate as described for **13**. Reversed-phase HPLC (t_R (TDP sugar) = 9.5 min, method A) of the residue gave the β-isomer of the protected TDP acosamine (80 mg, 44%), which was contaminated by a small amount of α-isomer (<10%) because of almost identical retention times of both anomers, as a white powder upon lyophilization; LRMS (ESI) for C₁₈H₂₇N₅O₁₄P₂ (599.38): 598 [M–H][–].

The protected TDP sugar (42 mg, 68 μmol) was dissolved in MeOH/H₂O (1:1, 5 mL) and Pd/C (10%, 20 mg) was added. The mixture was hydrogenated (1 atm) for 2 h, filtered, evaporated, and dried under high-vacuum for 2 h. The residue was dissolved in MeOH (5 mL), NaOMe (0.5 M in MeOH, 0.25 mL, 125 μmol) was added, and the mixture was stirred for 1 h at room temperature. The reaction was quenched by the addition of 0.1 % aq. NH₄OAc solution, evaporated, and purified by reversed-phase HPLC to give the ammonium salt of **22** (25 mg, 68%, $t_R = 6.0$ min, method A) as a white solid. ¹H NMR (400 MHz, D₂O): $\delta = 7.74$ (s, 1 H, 6-H), 6.37–6.33 (m, 1 H, 1'-H), 5.31 (dt, $J_{1'',2''a} = 1.9, J_{1'',2''b} = J_{1'',P} = 9.0$ Hz, 1 H, 1''-H), 4.52–4.47 (m, 1 H, 3'-H), 4.05–4.00 (m, 3 H, 4'-H, 5'-H₂), 3.38–3.42 (m, 1 H, 5''-H), 3.00–2.92 (m, 2 H, 3''-H, 4''-H), 2.30–2.20 (m, 3 H, 2'-H₂, 2''-H_a), 1.82 (s, 3 H, thymidine CH₃), 1.55–1.48 (m, 1 H, 2''-H_b), 1.18 (d, $J_{5,6} = 6.3$ Hz, 3 H, 6''-H₃); ³¹P NMR (162 MHz, D₂O): $\delta -10.7, -13.2$; LRMS (ESI) for C₁₆H₂₇N₃O₁₃P₂ (531.10): 530 [M–H][–].

Thymidine 5'-(3-amino-2,3,6-trideoxy-3-C-methyl-β-L-lyxo-hexopyranosyl diphosphate), TDP β-L-vancosamine (23):

Phosphate **17** (53 mg, 162 μ mol) was coupled with TMP morpholidate as described for **13**. Careful reversed-phase HPLC purification (t_R (β -anomer) = 10.7 min, t_R (α -anomer) = 10.9 min, method A) of the residue afforded the pure β -isomer (58 mg, 56% yield); LRMS (ESI) for $C_{19}H_{29}N_5O_{14}P_2$ (613.12): 612 $[M-H]^-$. The protected TDP vancosamine (15 mg, 24 μ mol) was dissolved in methanol (3 mL) and 0.5 M NaOMe (144 μ L, 72 μ mol) was added to the solution. After stirring for 48 h, the reaction was quenched with 0.2 M aq. NH_4HCO_3 (0.75 mL). Pd/C (10%, 3 mg) was added, and the mixture was hydrogenated (1 atm) for 1 h. The mixture was filtered, concentrated and purified by reversed-phase HPLC to afford TDP β -L-vancosamine **23** (7 mg, 62%) as a white powder upon lyophilization; t_R = 5.8 min, method A. 1H NMR (400 MHz, D_2O): δ = 7.68 (s, 1 H, 6-H), 6.31–6.28 (m, 1 H, 1'-H), 5.33–5.29 (dt, $J_{1,2b} = J_{1,P} = 9.6$ Hz, $J_{1,2a} = 2.4$ Hz, 1''-H), 4.54–4.49 (m, 1 H, 3'-H), 4.13–4.08 (m, 3 H, 4'-H, 5'-H₂), 3.98 (q, $J_{5,6} = 6.6$ Hz, 1 H, 5''-H), 3.37 (bs, 1 H, 4''-H), 2.36–2.27 (m, 2 H, 2'-H₂), 2.06 (dd, $J_{1,2a} = 2.4$, $J_{2,2} = 12.0$ Hz, 1 H, 2''-H_a), 1.87 (s, 3 H, thymidine CH₃), 1.92–1.81 (m, 1 H, 2''-H_b), 1.43 (s, 3 H, 3''-CH₃), 1.21 (d, $J_{5,6} = 6.6$ Hz, 3 H, 6''-H₃). ^{31}P NMR (162 MHz, D_2O): δ = –10.81, –13.02; LRMS (ESI) for $C_{17}H_{29}N_3O_{13}P_2$ (545.12): 543 $[M-2H]^-$

Thymidine 5'-(3-amino-2,3,6-trideoxy-3-C-methyl- α,β -L-arabino-hexopyranosyl diphosphate), TDP α,β -*epi*-L-vancosamine (**24**):

Phosphate **19** (monotetrabutylammonium salt, 98 mg, 178 μ mol) was coupled with TMP morpholidate as described for **13**. The residue was purified by reversed-phase HPLC (t_R (β -anomer) = 10.3 min, t_R (α -anomer) = 10.5 min, method A) to give the ammonium salt of **24** (45 mg, 40%) as a white powder; LRMS (ESI) for $C_{19}H_{29}N_5O_{14}P_2$ (613.12): 612 $[M-H]^-$. The protected TDP *epi*-vancosamine (38 mg, 60 μ mol) was dissolved in MeOH/ H_2O /Et₃N (2:2:1, 4 mL) and stirred at room temperature for 16 h. Following evaporation, the residue was redissolved in MeOH/ H_2O (2:1, 6 mL) and Pd-C (10%, 8 mg) was added to the solution. The mixture was hydrogenated (1 atm) for 1 h, filtered through a pad of Celite, evaporated to dryness and purified by reversed-phase HPLC. **24** (21 mg, 69%) was obtained as its ammonium salt, t_R = 5.8 min, method A. 1H NMR (400

MHz, D₂O): **24β**: δ = 7.73 (s, 1 H, 6-H), 6.33–6.29 (m, 1 H, 1'-H), 5.44–5.39 (dt, $J_{1,2b}$ = $J_{1,P}$ = 9.0, $J_{1,2a}$ = 2.1 Hz, 1 H, 1''-H), 4.59–4.54 (m, 1 H, 3'-H), 4.24–3.99 (m, 3 H, 4'-H, 5'-H₂), 3.73–3.69 (m, 1 H, 5''-H), 3.36 (d, $J_{4,5}$ = 10 Hz, 1 H, 4''-H), 2.32–2.17 (m, 3 H, 2'-H₂, 2''-H_a), 1.91 (s, 3 H, thymidine CH₃), 1.77–1.71 (dd, $J_{1,2b}$ = 9.0, $J_{2a,2b}$ = 12.6 Hz, 1 H, 2''-H_b), 1.39 (s, 3 H, 3''-CH₃), 1.29 (d, $J_{5,6}$ = 6.4 Hz, 3 H, 6''-H₃); ³¹P NMR (162 MHz, CD₃OD): δ = –10.71, –13.11. LRMS (ESI) for C₁₇H₂₉N₃O₁₃P₂ (545.12): 543 [M–2H][–].

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