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Chemical Synthesis of Uridine Diphospho-D-xylose and UDP-L-arabinose

Christiane Ernst[†] and Werner Klaffke*[,‡]

Organisch-Chemisches Institut der Westfälischen Wilhelms-Universität, Corrensstrasse 40, D-48149 Münster, Germany, and Unilever Research and Development, Unilever Health Institute, Olivier van Noortlaan 120, NL-3130 AT Vlaardingen, The Netherlands

werner.klaffke@unilever.com

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Abstract: Leloir transferases, like UDP-D-xylosyl transferase and arabinosyl transferase, utilize nucleoside diphosphate sugars to build up plant oligo- and polysaccharides. By the described, scalable three-step synthesis a simple route is described to arrive at the respective enzyme substrates, which are otherwise difficult to obtain.

Nucleotide-depending glycosyltransferases that glycosylate aglycons are widespread in Nature and can be found throughout the eucaryotic and procaryotic world. Overall, glycosylation is of paramount biological importance, not only in mammalians but also in plants and microorganisms. In higher plants, polysaccharides such as pectins and xylans are constituents of the cell wall, and oligomers serve as plant hormones and fulfill signaling functions. Glycosylation in general changes physicochemical properties of the aglycon, thereby changing transport properties and bioactivity.

Sequencing of the *Arabidopsis thaliana* gene has revealed a superfamily of more than 100 uridine diphosphate glycosyltransferases (UGT's),[1,2] which can also be identified in other higher plants. The exact substrate specificities, however, will only become evident if both aglycon and substrate are easily obtained, and the respective reaction can be followed at the molecular level.

In a recent paper, Pauly et al.[3] describe the synthesis of previously described UDP-β-L-arabinopyranose[4] by enzymatic epimerization from UDP-xylose.[5] Here we wish to disclose a novel method for obtaining both building blocks for the biosynthesis of plant polysaccharides, UDP-arabinose and UDP-xylose, in a simple and scalable procedure.

By and large, the syntheses for activated nucleotide sugars can be summarized by two concepts, either starting off from a glycosyl phosphate and a suitably activated nucleotide to form the pyrophosphate linkage or by glycosylation of an activated glycosyl donor with the nucleoside diphosphate serving as aglycon.[6−9]

We have recently described a ring-opening route on 1,2-anhydro sugars to introduce the nucleoside diphosphate moiety.[10] This paper explicitly describes the procedure for the pentopyranoses, L-arabinose and D-xylose.

The acetylated glycals **1** and **2**, synthesized in a one-pot procedure from the reducing sugars D-xylose and L-arabinose,[9] were deprotected under basic conditions (MeONa/MeOH) and subsequently reacted with chlorotriethylsilane to yield the silyl ethers **3** and **4**. Both were reacted with freshly prepared dimethyldioxirane (DMDO)[12] in a solution of acetone and dichloromethane to quantitatively give β-L-*arabino*-configured pentopyranose **5** and α-D-*xylo*-configured pentopyranose **6**. The oxiranes were formed stereoselectively due to the sensitivity of DMDO to the bulky protecting group at C-3. The stereoselectivity of both reactions was proven by methanolysis. In both cases a nucleophilic attack by methanol opens the epoxide ring trans-selectively to render a single product. The configuration of the resulting methyl glycosides **7** and **8** was assigned from the ^{1}H NMR spectra. The $J_{1,2}$ and $J_{2,3}$ coupling constants correspond to an α-arabinopyranoside for **7** ($J_{1,2} = 7.1$ Hz, $J_{2,3} = 9.0$ Hz) and to a β-xylopyranoside for **8** ($J_{1,2} = 5.1$ Hz, $J_{2,3} = 7.1$ Hz).

Prior to the coupling reaction, commercially available UDP (sodium salt) was converted into its tetrabutylammonium salt via the respective free acid by passing through a column of DOWEX-50 (H^+). The eluent was subsequently titrated against a tetrabutylammonium hydroxide solution to pH 6. This had to be carried out with great care, since any deviation from the recommended pH significantly decreased the yields.

The 1,2-anhydrosugars **5** and **6** were coupled to the lyophilized UDP-TBA-salt (UDP×1.9 TBA, titrated to pH 6) to give the silyl-protected nucleoside diphosphate sugars **9** and **11**, after column chromatography on Iatrobeads silica gel. These compounds could be easily deprotected by treatment with tetrabutylammonium fluoride (TBAF) without any observable decomposition. Uridin diphospho-L-arabinose (**10**) and uridine diphospho-D-xylose (**12**) were obtained as α/β mixtures, with the enzymatically active anomer prevailing (**10**, α/β = 1:3; **12**, α/β = 5:3).

L-Arabinosyl transferases utilize β-L-configured activated substrates, D-xylosyl transferases the respective α-D-configured counterparts. It has previously been shown that glycosyltransferases selectively pick the

[†] Organisch-Chemisches Institut der Westfälischen Wilhelms-Universität.

[‡] Unilever Health Institute.

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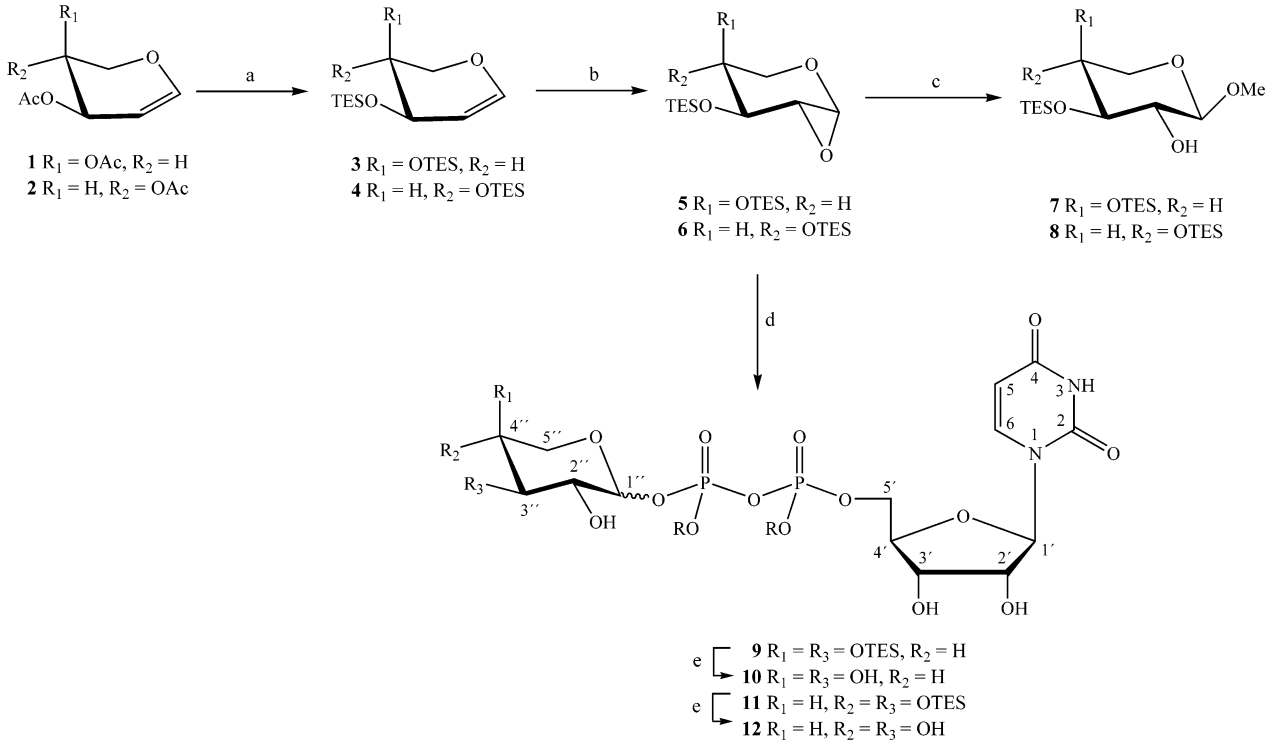
SCHEME 1. Synthesis of UDP-xylose and UDP-arabinose Starting from D-Xylal or L-Arabinal[a]



[a] Reagents and conditions: (a) (i) MeONa, MeOH, rt, 1 h, quant.; (ii) TESCl, DMF, rt, 5 h (78%); (b) DMDO, CH_2Cl_2, 1 h, 98%; (c) MeOH, rt, 1 h (86%); (d) UDP TBA salt, CH_2Cl_2, rt, 16 h (30%); (e) TBAF/THF, 0 °C → rt.

correct anomers from the respective mixtures, rendering a separation step unnecessary.[7] To our knowledge the described procedure comprises an easy and straightforward path to both activated xylose and arabinose, which can be widely applied also to glycals of the hexose family.[10]

Experimental Section

Instrumentation and General Methods. ^{1}H and ^{13}C NMR spectra and GCOSY and GHSQC experiments were performed at 400 or 100.6 MHz, respectively, or at 600 (^{1}H) and 125 MHz (^{13}C). ^{31}P NMR spectra were recorded with an external standard (85% H_3PO_4, δ 0). Chemical shifts (δ) are given in ppm relative to the signal for internal TMS. Optical rotations were measured at 20 °C at 589 nm (Na), using a 10 cm × 1 mL cell. Mass spectra were recorded by ESI. Reactions were monitored by TLC on silica plates (Merck Kieselgel 60 F_{254}) and detected by spraying with a solution of naphthoresorcin (200 mg) in sulfuric acid (2 N, 100 mL) and ethanol (100 mL) and subsequent heating. Medium-pressure liquid chromatography (MPLC, 3−5 bars) was performed on Kieselgel (Merck, 230−400 mesh, 0.040−0.063 μm). Anhydrous solvents (CH_2Cl_2, MeOH) were prepared by passing solvents (analytical grade, p.a.) through molecular sieves (4 Å) under argon.

General Procedure for Epoxidation. The glycal (1 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL) under argon, and the resulting solution was cooled to 0 °C. A freshly prepared solution of dimethyldioxirane in acetone (20 mL) was added and the reaction mixture was stirred at 0 °C for 1 h or until TLC indicated complete consumption of the glycal. The solution was evaporated with a stream of dry argon and the residue was dried in vacuo to afford the 1,2-anhydro sugar quantitatively.

General Procedure for Coupling of 1,2-Anhydro Sugars with UDP. UDP trisodium salt (100 mg, 0.2 mmol) was dissolved in water (1 mL) and the solution was passed through DOWEX 50W×8 (H^+). Fractions containing protonated UDP were detected by UV absorption, pooled, and titrated, using aqueous $Bu_4N(OH)$ (40% w/v), to pH 6. The resulting solution was lyophilized to yield UDP TBA salt as a white powder. The lyophilized UDP TBA salt was dispersed in anhydrous CH_2Cl_2 (approximately 5 mL) under argon and a solution of the glycosyl donor was added. In a stream of argon, the reaction mixture was concentrated to yield a syrup, which was stirred overnight. After this, the reaction was complete, and the reaction mixture was diluted with a small amount of CH_2Cl_2 and applied directly to a column of Iatrobeads silica gel. Elution by $CHCl_3$:MeOH:H_2O:concd aq NH_4OH 160:70:5:0.25 yielded the desired nucleotide sugars which were obtained after solvent removal in vacuo.

1,5-Anhydro-2-deoxy-3,4-di-*O*-triethylsilyl-L-erythro-pent-1-enitol (3). Acetylated glycal **1** (1.6 g, 8 mmol) was dissolved in methanol (20 mL) and sodium methoxide (160 μL, 30 wt % solution in MeOH) was added. The solution was stirred at room temperature for 2 h and the solvent evaporated subsequently. Then the residue was redissolved in anhydr DMF (25 mL) and imidazole (1.36 g, 20 mmol) and chlorotriethylsilane (3.22 mL, 19 mmol, 1.2 equiv per OH group) were added. After 2 h TLC indicated completion. The mixture was poured into H_2O (50 mL) and extracted with ether (3 × 70 mL). The combined organic layers were dried over anhydr $MgSO_4$, filtered, and concentrated under reduced pressure. The crude silylated derivative was purified by MPLC (cyclohexane/ethyl acetate 35:1) to yield 2.51 g (91%) of compound **3** as a colorless oil. [α_D^{20}] −167.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (d, 1H, H-1), 4.77 (dd−t, 1H, H-2), 4.05 (m, 1H, H-3), 3.92 (dd, 1H, H-5b), 3.81 (ddd−dt, 1H, H-4), 3.72 (ddd, 1H, H-5a), 0.97, 0.96 (2 t, 18H, CH_3−CH_2−Si), 0.62 (m, 12H, CH_3−CH_2−Si) ppm; $J_{1,2}$ = 5.9 Hz, $J_{2,3}$ = 5.9 Hz, $J_{3,4}$ = 3.1 Hz, $J_{3,5}$ =1.0 Hz, $J_{4,5a}$ = 3.1 Hz, $J_{4,5b}$ = 10.7 Hz, $J_{5a,5b}$ = 8.9 Hz; ^{13}C NMR ($CDCl_3$) δ 144.9 (C-1), 102.2 (C-2), 68.4 (C-4), 64.7 (C-5), 63.9 (C-3), 6.7, 6.6 (CH_3−CH_2−Si), 5.1, 4.8 (CH_3−CH_2−Si) ppm; MS (ESI$^+$) *m/z* 367 (M + Na^+).

1,5-Anhydro-2-deoxy-3,4-di-*O*-triethylsilyl-D-threo-pent-1-enitol (4). Acetylated glycal **2** (521 mg, 2.6 mmol) was dissolved in methanol (10 mL) and sodium methoxide (50 μL, 30 wt % solution. in MeOH) was added. The solution was stirred

at room temperature for 1.5 h after which the solvent was evaporated. After the residue was carefully dried it was redissolved in anhydr DMF (12 mL) and imidazole (477 mg, 7 mmol) and chlorotriethylsilane (1.05 mL, 6.2 mmol, 1.2 equiv per OH group) were added. The mixture was stirred overnight at room temperature, poured into H_2O (50 mL), and extracted with ether (3 × 70 mL). The combined organic layers were dried over anhydr $MgSO_4$, filtered, and concentrated under reduced pressure. The crude silylated derivative was purified by MPLC (cyclohexane/ethyl acetate 40:1) to yield 715 mg (80%) of compound **4** as a colorless oil. $[\alpha_D^{20}]$ −138.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.39 (d, 1H, H-1), 4.72 (m, 1H, H-2), 3.93 (dd, 1H, H-5a), 3.87 (dd−t, 1H, H-3), 3.83 (ddd, 1H, H-5b), 3.71 (m, 1H, H-4), 0.97 (m, 18H, CH_3−CH_2−Si), 0.62 (m, 12H, CH_3−CH_2−Si) ppm; $J_{1,2}$ = 6.1 Hz, $J_{2,3}$ = 4.4 Hz, $J_{2,5b}$ = 1.2 Hz, $J_{4,5a}$ = 2.2 Hz, $J_{4,5b}$ = 4.4 Hz, $J_{5a,5b}$ = 11.0 Hz; ^{13}C NMR ($CDCl_3$) δ 145.0 (C-1), 101.9 (C-2), 69.6 (C-4), 66.4 (C-5), 63.6 (C-3), 6.7, 6.6 (CH_3−CH_2−Si), 5.0, 4.8 (CH_3−CH_2−Si) ppm; MS (ESI$^+$) *m*/*z* 367 (M + Na^+). Anal. Calcd for $C_{17}H_{36}O_3Si_2$: C, 59.25; H, 10.53. Found: C, 58.87; H, 10.55.

1,2-Anhydro-2-deoxy-3,4-di-*O*-(triethylsilyl)-*β*-L-arabinopentopyranose (5). Compound **5** was prepared according to the general epoxidation protocol. The regio- and stereoselective product formation was proven by methanolysis to compound **7**.

1,2-Anhydro-2-deoxy-3,4-di-*O*-(triethylsilyl)-α-D-xylo-pentopyranose (6). Compound **6** was prepared according to the general epoxidation protocol. The regio- and stereoselective product formation was proven by methanolysis to compound **8**.

Methyl-3,4-di-*O*-(triethylsilyl)-α-L-arabino-pentopyranoside (7). 1,2-Anhydro sugar **5** (360 mg, 1.0 mmol) was dissolved in anhydrous methanol (3 mL) and stirred for 1 h at room temperature. The solvent was evaporated and the residue was purified by MPLC (cyclohexane/ethyl acetate 9:1) to yield 285 mg (73%) of compound **7** as a colorless oil. $[\alpha_D^{20}]$ +20.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.06 (d, 1H, H-1), 3.85−3.80 (m, 2H, H-4, H-5a), 3.67 (dd, 1H, H-2), 3.53 (dd, 1H, H-3), 3.52 (s, 3H, OCH_3), 3.43 (dd, 1H, H-5b), 2.30 (br s, 1H, OH), 1.00−0.93 (m, 18H, CH_3−CH_2−Si), 0.70−0.58 (m, 12H, CH_3−CH_2−Si) ppm; $J_{1,2}$ = 7.1 Hz, $J_{2,3}$ = 9.0 Hz, $J_{3,4}$=2.9 Hz, $J_{4,5b}$ = 2.4 Hz, $J_{5a,5b}$ = 13.1 Hz; ^{13}C NMR ($CDCl_3$) δ 103.6 (C-1), 74.2 (C-3), 70.2 (C-2), 69.5 (C-4), 65.9 (C-5), 55.6 (OCH_3), 5.6, 5.6 (CH_3−CH_2−Si), 3.8, 3.8 (CH_3−CH_2−Si) ppm; MS (ESI$^+$) *m*/*z* 415 (M + Na^+). Anal. Calcd for $C_{18}H_{40}O_5Si_2$: C, 55.06; H, 10.27. Found: C, 54.82; H, 10.43.

Methyl-3,4-di-*O*-(triethylsilyl)-*β*-D-xylo-pentopyranoside (8). 1,2-Anhydro sugar **6** (177 mg, 0.5 mmol) was dissolved in anhydrous methanol (2 mL) and stirred for 2.5 h at room temperature. The solvent was evaporated and the residue was purified by MPLC (cyclohexane/ethyl acetate 20:1) to yield 169 mg (86%) of compound **8** as a colorless oil. $[\alpha_D^{20}]$ −50.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30 (d, 1H, H-1), 3.97 (dd, 1H, H-5a), 3.61−3.57 (m, 2H, H-3, H-4), 3.45 (s, 3H, OCH_3), 3.31 (ddd, 1H, H-2), 3.23 (ddd, 1H, H-5b), 1.00−0.93 (m, 18H, CH_3−CH_2−Si), 0.68−0.59 (m, 12H, CH_3−CH_2−Si) ppm; $J_{1,2}$ = 5.1 Hz, $J_{2,3}$ = 7.1 Hz, $J_{2,5b}$ = 2.0 Hz, $J_{4,5a}$ = 3.2 Hz, $J_{4,5b}$ = 4.8 Hz, $J_{5a,5b}$ = 12.1 Hz; ^{13}C NMR ($CDCl_3$) δ 103.5 (C-1), 74.2, 71.1 (C-3, C-4), 72.5 (C-2), 63.5 (C-5), 56.2 (OCH_3), 6.7, 6.6 (CH_3−CH_2−Si), 4.9, 4.8 (CH_3−CH_2−Si) ppm; MS (ESI$^+$) *m*/*z* 415 (M + Na^+). Anal. Calcd for $C_{18}H_{40}O_5Si_2$: C, 55.06; H, 10.27. Found: C, 54.82; H, 10.25.

Uridine 5′-(3″,4″-Di-*O*-(triethylsilyl)-α/*β*-L-arabinopyranosyl)-diphosphate (9). 1,2-Anhydro sugar **5** was coupled to UDP TBA salt according to the general procedure. The desired nucleotide sugar **9** was obtained after lyophilization in 30% yield as a white powder. α/*β* ratio 1:3; ^{1}H NMR (600 MHz, MeOD) δ 8.04 (2 d, 2H, H-6), 5.97 (2 d, 2H, H-1′), 5.86, 5.85 (2 d, 2H, H-5), 5.62 (br s, 1H, H-1″*β*), 5.05 (br s, 1H, H-1″α), 4.35 (m, 2H, H-3′), 4.18−4.31 (m, 6H, H-2′ and H-5′), 4.12 (m, 2H, H-4′), 3.98−3.52 (m, H-2″, H-3″, H-4″, H-5″), 3.24 (m, 16H, TBA), 1.66 (m, 16H, TBA), 1.42 (m, 16H, TBA), 1.03 (t, 24H, TBA), 0.96−1.01 (18H, m, CH_3CH_2Si), 0.61−0.72 (12H, m, CH_3CH_2Si) ppm; $J_{5,6}$ = 8.0 Hz, $J_{1',2'}$ = 5.1 Hz; ^{13}C NMR (125 MHz, MeOD) δ 166.2, (C-4), 152.6 (C-2), 142.9 (C-6), 103.5 (C-5), 98.4 (C-1″*β*), 89.9 (C-1′), 85.2, (C-4′), 75.8 (C-2′), 73.3 (C-3″), 72.2 (C-4″), 71.3 (C-3′), 70.7 (C-2″), 66.4 (C-5″), 66.2 (C-5′), 64.1 (C-5″*β*), 59.5, 24.8, 20.7, 13.9 (TBA), 6.9 (CH_3CH_2Si), 5.0 (CH_3CH_2Si) ppm; ^{31}P NMR (121.5 MHz, MeOD) δ −10.35 and −11.95 ppm; MS (ESI-MS, Nanospray) (*m*/*z*) 763 $[M]^-$, 381 $[M]^{2-}$; exact mass calcd for $[C_{26}H_{48}N_2O_{16}P_2Si_2]^{2-}$ 381.1014, found 381.1027.

Uridine 5′-(α/*β*-L-Arabinopyranosyl)-diphosphate (UDP-α/*β*-L-arabinose, 10). The protected nucleotide sugar **9** (50 mg) was dissolved in THF (2 mL) under Argon and the solution was cooled to 0 °C. After adding tetrabutylammonium fluoride (375 *μ*L, 1 M solution in THF) the mixture was kept at this temperature for 0.5 h, then stirred at room temperature for an additional 3.5 h. Upon removal of the solvent, the residue was taken up in H_2O and the aqueous solution was washed with pentane (3 × 4 mL). The resulting aqueous layer was passed over Amberlite IR 120 (Na^+) and lyophilized. Nucleotide sugar **10** was obtained quantitatively as a white powder. α/*β* ratio 1:3; ^{1}H NMR (600 MHz, D_2O) δ 7.94 (d, 1H, H-6), 5.97 (d, 1H, H-1′), 5.96 (d, 1H, H-5), 5.59 (dd, 1H, H-1″*β*), 4.88 (dd−t, 1H, H-1″α), 4.34−4.37 (m, 2H, H-2′ and H-3′), 4.27 (m, 1H, H-4′), 4.23, 4.18 (m, 2H, H-5′a and H-5′b), 4.10 (dd, 1H, H-5″a, *β*), 4.01 (m, 1H, H-4″*β*), 3.91 (dd, 1H, H-3″*β*), 3.80 (dt, 1H, H-2″*β*), 3.71 (dd, 1H, H-5″b, *β*) ppm; $J_{5,6}$ = 8.2 Hz, $J_{1',2'}$ = 4.8 Hz, $J_{1''\beta,2''\beta}$ = 3.3 Hz, $J_{1''\beta,P}$ = 6.9 Hz, $J_{2''\beta,3''\beta}$ = 10.2 Hz, $J_{3''\beta,4''\beta}$ = 3.3 Hz, $J_{4''\beta,5''a}$ = 1.1 Hz, $J_{4''\beta,5''b}$ = 2.1 Hz, $J_{5''a,5''b,\beta}$ = 13.0 Hz, $J_{1''\alpha,2''\alpha}$ = 7.6 Hz, $J_{1''\alpha,P}$ = 7.6 Hz; ^{13}C NMR (125 MHz, D_2O) δ 166.2 (C-4), 151.7 (C-2), 141.5 (C-6), 102.6 (C-5), 98.6 (C-1″α), 96.2 (C-1″*β*), 88.3, 88.3 (C-1′), 83.1 (C-4′, d, $^3J_{C-C-O-P}$ = 9.2 Hz), 73.7 (C-2′), 69.6 (C-3′), 68.8, 68.6, 68.3 (C-2″*β*, C-3″*β*, C-4″*β*), 64.9, 64.8 (C-5′, 2 d, $^2J_{C-O-P}$ = 5.2 Hz), 63.9 (C-5″*β*) ppm; ^{31}P NMR (242.5 MHz, D_2O) δ −10.56 (d, attached to ribose, J_{P-O-P} = 20.3 Hz) and −12.04, −12.35 (2 d, attached to arabinose, J_{P-O-P} = 20.3 Hz) ppm; MS (ESI-MS, Nanospray) (*m*/*z*) 535 $[M]^-$, 267 $[M]^{2-}$; exact mass calcd for $[C_{14}H_{20}N_2O_{16}P_2]^{2-}$ 267.0150, found 267.0140.

Uridine 5′-(3″,4″-Di-*O*-(triethylsilyl)-α/*β*-D-xylopyranosyl)-diphosphate (11). 1,2-Anhydro sugar **6** was coupled to UDP TBA salt according to the general procedure. The desired nucleotide sugar **11** was obtained after lyophilization in 34% yield as a white powder. α/*β* ratio 5:3; ^{1}H NMR (600 MHz, MeOD) δ 8.09 (2 d, 2H, H-6), 6.02, 6.01 (2 d, 2H, H-1′), 5.91, 5.89 (2 d, 2H, H-5), 5.65 (br s, 1H, H-1″α), 5.00 (dd−t, 1H, H-1″*β*), 4.42, 4.39 (2 dd−2 t, 2H, H-3′), 4.27−4.34 (m, 4H, H-2′ and H-5′), 4.24 (m, 2H, H-5′), 4.16 (m, 2H, H-4′), 3.85 (dd, 1H, H-5″*β*), 3.82 (dd, 1H, H-5″α), 3.76 (dd∼t, 1H, H-3″α), 3.69 (m, 1H, H-4″*β*), 3.61 (m, 1H, H4″α), 3.50 (dd∼t, 1H, H-3″*β*), 3.32−3.39 (m, 2H, H-2″α, H-5″α), 3.22−3.31 (m, 18H, H-2″*β*, H-5″*β*, TBA), 1.70 (m, 16H, TBA), 1.46 (m, 16H, TBA), 1.06 (t, 24H, TBA), 0.99−1.05 (18H, m, CH_3CH_2Si), 0.65−0.77 (12H, m, CH_3CH_2Si) ppm; $J_{5,6}$ = 8.0 Hz, $J_{1',2'}$ = 5.2 Hz, $J_{1''\alpha,2''\alpha}$ = 3.3 Hz, $J_{2''\alpha,3''\alpha}$ = 8.4 Hz, $J_{3''\alpha,4''\alpha}$ = 8.4 Hz, $J_{4''\alpha,5''\alpha}$ = 5.1 Hz, $J_{5''a,5''b,\alpha}$ = 12.5 Hz, $J_{2''\beta,3''\beta}$ = 8.7 Hz, $J_{3''\beta,4''\beta}$ = 8.7 Hz, $J_{4''\beta,5''\beta}$ = 5.5, $J_{5''a,6''b,\beta}$ = 12.1 Hz; ^{13}C NMR (125 MHz, MeOD) δ 166.2, 166.2 (C-4), 152.7, 152.6 (C-2), 142.8, 142.7 (C-6), 103.4, 103.3 (C-5), 100.7 (C-1″*β*, d, $^2J_{C-O-P}$ = 5.2 Hz), 97.7 (C-1″α, d, $^2J_{C-O-P}$ = 5.4 Hz), 89.70, 89.50 (C-1′), 85.3, 85.1 (C-4′, 2 d, $^3J_{C-C-O-P}$ = 8.9 Hz), 77.6 (C-3″α), 75.6, 75.4 (C-2′), 75.5 (C-2″*β*), 75.4 (C-4″*β*), 74.3 (C-2″α, d, $^3J_{C-C-O-P}$ = 8.0 Hz), 71.5 (C-4″α), 71.2, 71.1 (C-3′), 71.0 (C-3″*β*), 67.1 (C-5″*β*), 66.1, 66.0 (C-5′, 2 d), 64.1 (C-5″α), 59.5, 24.8, 20.7, 13.9 (TBA), 6.9 (CH_3CH_2Si), 4.8 (CH_3CH_2Si) ppm; ^{31}P NMR (121.5 MHz, MeOD) δ −10.60 and −12.10 ppm; MS (ESI-MS, Nanospray) (*m*/*z*) 763 $[M]^-$, 381 $[M]^{2-}$; exact mass calcd for $[C_{26}H_{48}N_2O_{16}P_2Si_2]^{2-}$ 381.1014, found 381.0979.

Uridine 5′-(α/*β*-D-Xylopyranosyl)-diphosphate (UDP-α/*β*-D-xylose, 12). The protected nucleotide sugar **9** (103 mg) was dissolved in THF (3 mL) under Argon and the solution was cooled to 0 °C. After adding tetrabutylammonium fluoride (750 *μ*L, 1 M solution in THF) the mixture was kept at this temperature for 0.5 h, then stirred at room temperature for another 2.5 h. Upon removal of the solvent, the residue was taken up in H_2O and the aqueous solution was washed with pentane (3 × 4 mL). The resulting aqueous layer was passed over Amberlite IR 120 (Na^+) and lyophilized. Nucleotide sugar **12** was obtained quantitatively as a white powder. α/*β* ratio 5:3; ^{1}H NMR (600 MHz, D_2O) δ 7.80 (2 d, 2H, H-6), 5.84 (2 d, 2H, H-1′), 5.82 (2 d, 2H, H-5), 5.40 (dd, 1H, H-1″α), 4.79 (dd−t, 1H,

H-1″β), 4.24−4.20 (m, 4H, H-2′ and H-3′), 4.14 (m, 2H, H-4′), 4.24−4.20 (m, 4H, H-5′a and H-5′b), 3.82 (dd, 1H, H-5″a, β), 3.60 (m, 1H, H-4″α), 3.56 (dd−t, 1H, H-3″α), 3.45−3.51 (m, 3H, H4″β, H5″a and b, α), 3.37 (dt, 1H, H-2″α), 3.33 (dd−t, 1H, H-3″β), 3.22 (dd, 1H, H-2″β), 3.20 (dd, 1H, H-5″b, β) ppm; $J_{5,6} = 8.0$ Hz, $J_{1',2'} = 4.8$ Hz, $J_{1''\alpha,2''\alpha} = 3.3$ Hz, $J_{1''\alpha,P} = 7.0$ Hz, $J_{2''\alpha,3''\alpha} = 9.3$ Hz, $J_{3''\alpha,4''\alpha} = 9.3$ Hz, $J_{1''\beta,2''\beta} = 8.0$ Hz, $J_{1''\beta,P} = 8.0$ Hz, $J_{2''\beta,3''\beta} = 9.1$ Hz, $J_{3''\beta,4''\beta} = 9.1$ Hz, $J_{4''\beta,5''a} = 5.5$ Hz, $J_{4''\beta,5''b} = 7.1$ Hz, $J_{5''a,5''b,\beta} = 11.6$ Hz; ^{13}C NMR (90 MHz, D_2O) δ 167.1 (C-4), 152.7 (C-2), 142.5 (C-6), 103.5, 103.5 (C-5), 99.5 (C-1″β, d, $^2J_{C-O-P} = 5.5$ Hz), 96.5 (C-1″α, d, $^2J_{C-O-P} = 6.8$ Hz), 89.2, 89.2 (C-1′), 84.2, 84.1 (C-4′, 2 d, $^3J_{C-C-O-P} = 8.3$ Hz), 76.0 (C-3″β), 74.6 (C-2′), 74.3 (C-2″β, d, $^3J_{C-C-O-P} = 8.4$ Hz), 73.9 (C-3″α), 72.5 (C-2″α, d, $^3J_{C-C-O-P} = 8.4$ Hz), 70.6, 70.5 (C-3′), 70.0 (C-4″α), 69.9 (C-4″β), 66.3 (C-5″β), 65.8, 64.7 (C-5′, 2 d, $^2J_{C-O-P} = 5.6$ Hz), 63.1 (C-5″α) ppm; ^{31}P NMR (242.5 MHz, D_2O) δ −10.60 (d, attached to ribose, $J_{P-O-P} = 20.8$ Hz) and −12.27, −12.42 (2 d, attached to xylose, $J_{P-O-P} = 20.8$ Hz) ppm; MS (ESI-MS, Nanospray) (*m*/*z*) 535 $[M]^-$, 267 $[M]^{2-}$; exact mass calcd for $[C_{14}H_{20}N_2O_{16}P_2]^{2-}$ 267.0150, found 267.0137.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra of compounds **3**, **4**, **7**, **8**, **9**, **10**, **11**, and **12.** This material is available free of charge via the Internet at http://pubs.acs.org.

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