C-Glycosidic UDP-GlcNAc Analogues as Inhibitors of UDP-GlcNAc 2-Epimerase

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The first step in the biosynthesis of neuraminic acid, the "epimerisation" of UDP-GlcNAc to ManNAc, is catalyzed by UDP-GlcNAc 2-epimerase. In this paper we report the synthesis of the *C*-glycosidic UDP-GlcNAc analogues **1**–**5** as substrate-based inhibitors of this enzyme. The focus is on the optimal distance and geometry of the connection between the sugar and the UDP-moiety, which are both important for recognition by UDP-GlcNAc 2-epimerase.

Introduction

Owing to their position at the nonreducing end of oligosaccharide chains in vertebrate glycoconjugates, sialic acids play a key role in a wide range of biological processes, such as cell-cell recognition, the protection of cells from pathogen attachment and degradation, cellular adhesion processes, or virus-host recognition. Furthermore, cancer cells often show an increased level of sialylation on their glycocalix and the metastatic potential of tumor cells has been correlated with their surface sialylation.[1−3]

The most frequently occurring sialic acid is *N*-acetylneuraminic acid (Neu5Ac), which is also the biosynthetic precursor for this family of 3-deoxy-2-keto-acids. The mammalian biosynthesis of Neu5Ac starts with the epimerisation of UDP-*N*-acetylglucosamine (UDP-GlcNAc) into *N*-acetylmannosamine (ManNAc), followed by phosphorylation of the 6-hydroxy group. These two steps are catalyzed by one bifunctional enzyme, UDP-GlcNAc 2-epimerase/ManNAc kinase.[4,5] This enzyme has been found to serve as the key regulator of cell surface sialylation as it catalyzes the rate-limiting step of the biosynthetic pathway.[6] The UDP-GlcNAc 2-epimerase activity is controlled by a feedback inhibition by CMP-Neu5Ac, the final product of its biosynthetic pathway. Point mutations of this enzyme result in the human diseases "hereditary inclusion body myopathy" (HIBM)[7] or sialuria, an inborn error in the feedback inhibition.[8]

Recently, a mechanistic study of UDP-GlcNAc 2-epimerase was reported,[9] which clearly indicates that the reaction starts with an *anti*-elimination of UDP to form 2-acetamidoglucal, followed by the *syn*-addition of water. On this basis a mechanism for the epimerisation was postulated.[9,10]

We recently reported the design and synthesis of some transition-state-based inhibitors for UDP-GlcNAc 2-epimerase, which showed binding affinities in the range of the natural substrate UDP-GlcNAc.[10] In this study we followed a different approach and turned our interest to mimics of the enzyme substrate, UDP-GlcNAc. The results of previous studies[10,11] clearly indicated that the sugar moiety and the UDP residue are both important for binding to the active site of UDP-GlcNAc 2-epimerase. Therefore, a potent inhibitor should mimic both moieties, but the optimal distance and geometry of their connection is still unclear. Therefore, we have designed and synthesized the potential inhibitors **1**−**5**, which have different diphosphate-type connections between the *N*-acetylglucosamine and the uridine moiety (see Scheme 1).

In compound **1** the formal distance between the two parts is one atom shorter than in the natural substrate UDP-GlcNAc. In compounds **2** and **4** the distance between the two parts is the same as in UDP-GlcNAc and in compounds **3** and **5** the distance is increased by one atom. In all the compounds a *C*-glycosidic linkage replaces the labile natural glycosidic phosphate bond to increase the resistance to chemical and enzymatic hydrolysis.

Results and Discussion

The target molecules **1**−**3** should all be accessible from the same precursor, the heptitol **12**, which has recently been

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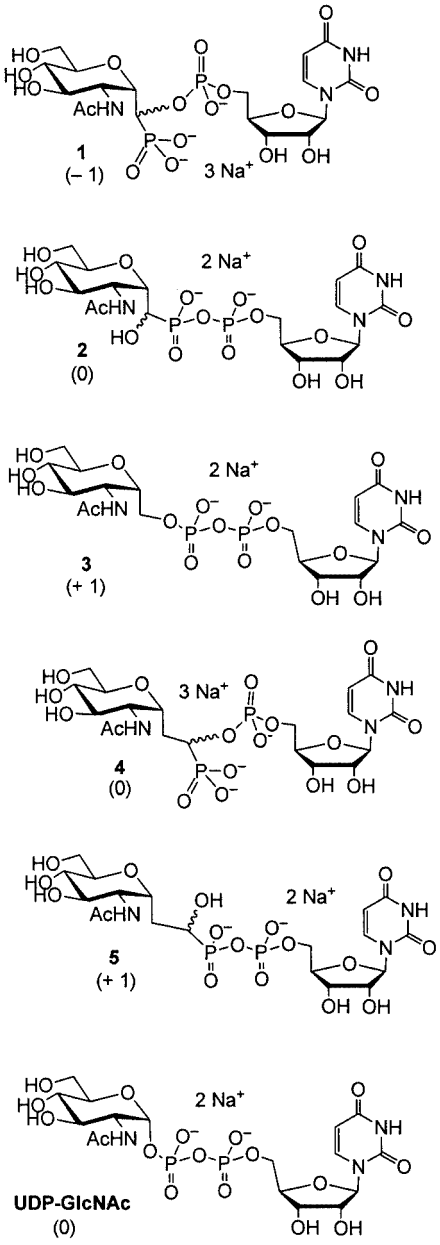


Scheme 1. Formal distance between the uridine and the sugar moiety in the synthesized inhibitors **1**–**5** compared with the substrate UDP-GlcNAc

prepared by Schäfer and Thiem.[12] Their procedure includes a demanding addition reaction of carbon dioxide to a dilithio intermediate. We followed a different approach. Starting from benzyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**6**), which is accessible by a known one-step procedure from *N*-acetylglucosamine,[13] the *N*-acetyl group and the anomeric benzyl ether could be cleaved under reflux in HCl/dioxane (Scheme 2).[14] The free amine that was generated was then converted by diazo transfer[15] into the known azido derivative **7**[16] in 52% yield over the two steps. This procedure is a convenient alternative route to the versatile azido building block **7**,[17,18] which is usually prepared by azidonitration of glycals, leading to a mixture of *gluco*- and *manno*-derivatives.[16,19]

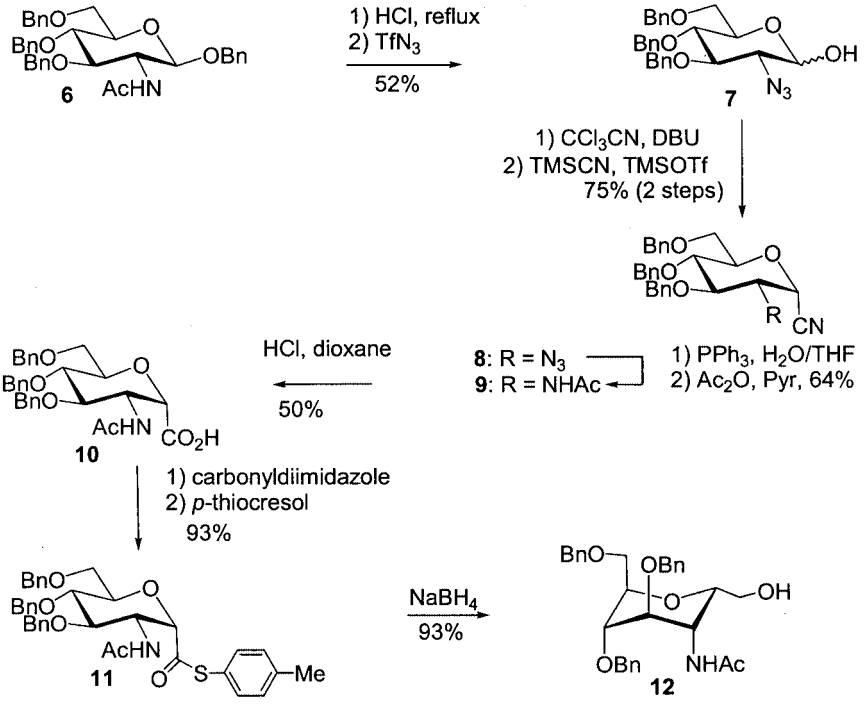
Compound **7** was then transformed into the cyanide **8** via the *O*-glycosyl trichloroacetimidate following a published procedure.[20] The azido group was reduced to the free amine under Staudinger conditions, which was then acetylated to afford the acetamido derivative **9**. Treatment of cyanide **9** with HCl in dioxane led to the known heptonic acid **10**.[12] Its reduction to the desired heptitol **12** was performed in two steps. In contrast to the behaviour of the reported methyl ester, which could be reduced only in moderate yields,[12] we prepared the activated thiocresol ester **11**, which could be smoothly reduced with sodium borohydride to afford heptitol **12** in 93% yield. The very small coupling constants ($J_{2,3} < 1$, $J_{3,4} = 1$, $J_{5,6} < 1$, $J_{4,5} = 2.9$ Hz.) in the ^{1}H NMR spectrum indicate that the heptitol **12** adopts a $^1C_4$ conformation.

To complete the synthesis of **1** and **2**, heptitol **12** had to be transformed into the phosphonate **13** (Scheme 3). Oxidation of the alcohol **12** using Dess–Martin periodinane led to an aldehyde intermediate,[21] which was treated in an Arbuzov-type reaction with dibenzyl phosphonate and triethylamine to yield the phosphonate **13** in 94% yield as a single stereoisomer. A similar reaction with the β-anomer was very recently reported by Wen and Hultin.[22]

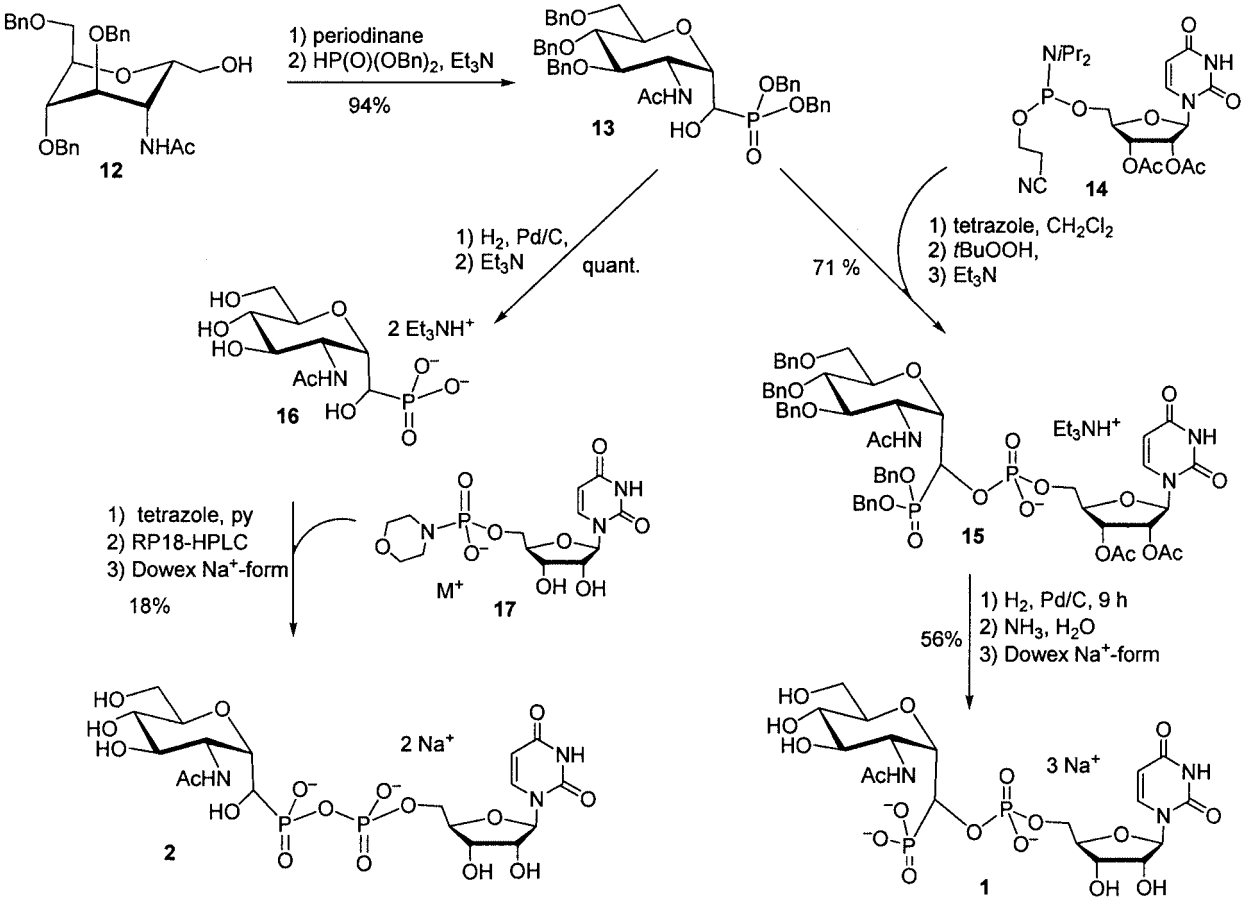
The phosphonate **13** was coupled with the known phosphoramidite **14**[23–25] and oxidized with *tert*-butyl hydroperoxide. After cleavage of the cyanoethoxy group with triethylamine the protected target molecule **15** was obtained in 71% yield. All the benzyl groups were cleaved by using standard hydrogenolysis conditions with Pd/C/H_2 for 9 h without affecting the double bond of the uracil moiety. However, prolonged reaction times (2 days) also led to the reduction of the uracil double bond. After cleavage of the acetyl protecting groups with ammonia in water and transformation into the sodium salt the target molecule **1** was obtained in 56% yield.

To reach target molecule **2** the phosphonate **13** was deprotected by hydrogenolysis to yield phosphonate **16**, which was coupled without purification with the UMP-morpholidate **17** by using standard conditions.[26,27] After isolation by RP-18-HPLC and ion-exchange to yield the sodium form, the target molecule **2** was obtained in moderate yield in accordance with previous results with this procedure.[12,28,29]

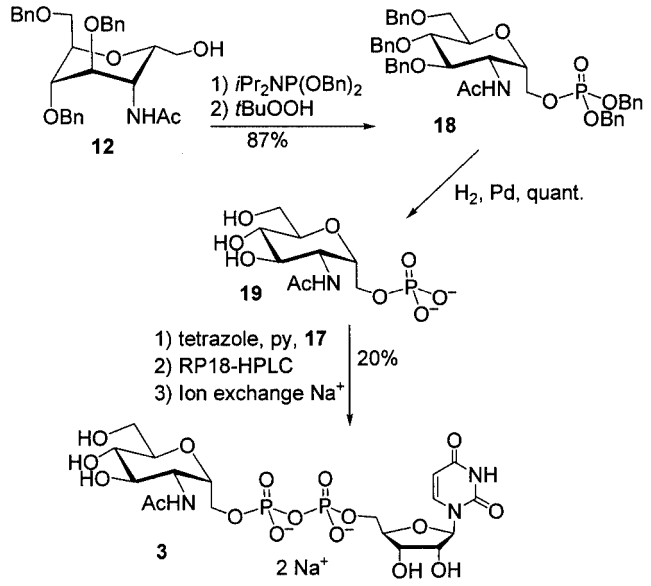
The synthesis of target molecule **3** was achieved by closely following the reported procedure (Scheme 4).[12] Heptitol **12** was transformed into the phosphate **18** by using the phosphoramidite method. We used benzyl protecting groups (instead of phenyl esters),[12] which can be easily removed with Pd/C/H_2 to afford phosphate **19** in quantitative yield. After coupling with the UMP-morpholidate **17**, RP-18-HPLC, ion exchange and precipitation the target compound **3** was obtained as the sodium salt in 20% yield, which had physical data identical to those reported.[12]



Scheme 2. Synthesis of heptitol **12**



Scheme 3. Synthesis of the target molecules **1** and **2**



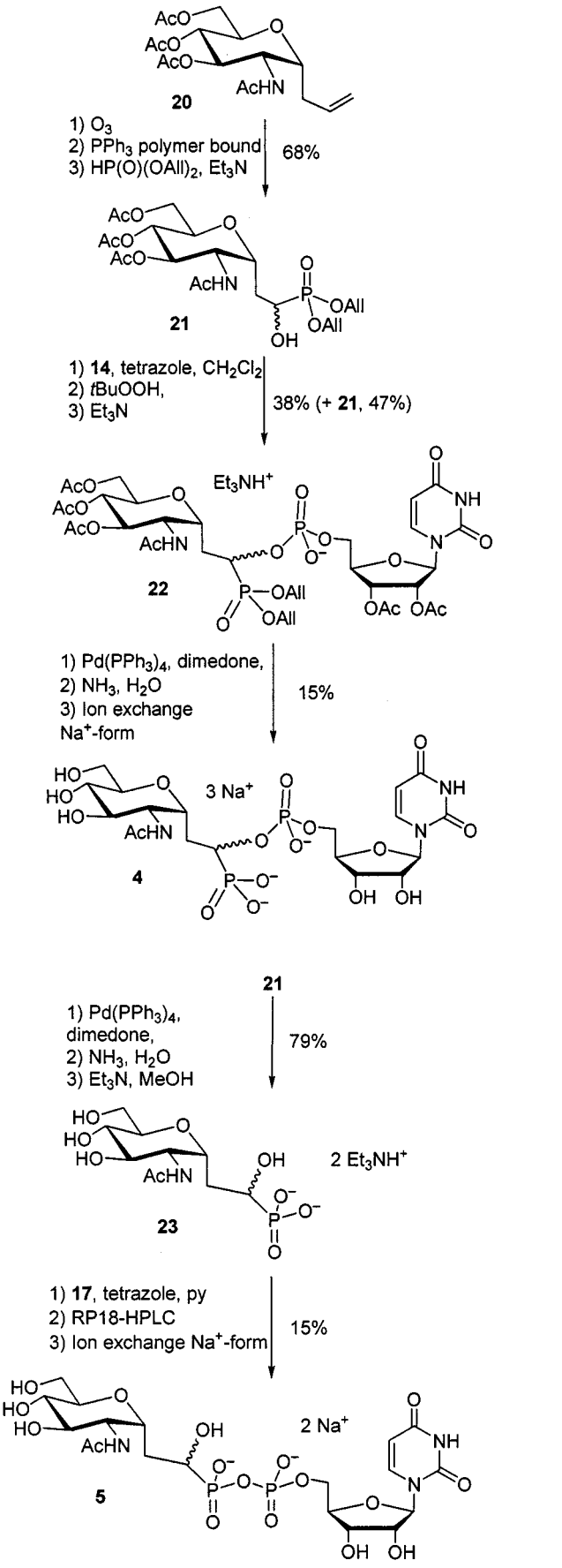
Scheme 4. Synthesis of the known *C*-glycoside **3**

For the synthesis of the homologous compounds **4** and **5** a similar approach to that used for the synthesis of **1** and **2** was followed (Scheme 5).

Starting from the known allyl *C*-glycoside **20**,[30] oxidative cleavage of the double bond and subsequent treatment with triphenylphosphane (on a polymer support) led to the aldehyde intermediate, which was treated with diallyl phosphonate and triethylamine to afford the phosphonate **21** as a mixture of diastereoisomers (2:1). Coupling of phosphonate **21** with the phosphoramidite **14** and oxidation led to the protected target molecule **22**. Deprotection of the allyl protecting groups with $[Pd(PPh_3)_4]$ and dimedone, followed by deprotection of the acetyl protecting groups with aqueous ammonia afforded the target compound **4** in moderate yield.

For the synthesis of target molecule **5**, the phosphonate **21** was deprotected with $[Pd(PPh_3)_4]$ and dimedone, and then treated with aqueous ammonia; the intermediate obtained was transformed into the triethylammonium salt **23**, which was coupled with UMP-morpholidate **17**. After isolation by RP-18-HPLC, ion exchange and precipitation the target compound **5** was obtained as the disodium salt in 15% yield.

All the synthesized target compounds **1**−**5** were tested as inhibitors of UDP-GlcNAc 2-epimerase. Under the conditions employed,[11] using the natural substrate UDP-GlcNAc and the inhibitor in the same concentration (1.25 mM), compounds **1** and **2** showed up to 85% inhibition. By comparing the homologous compounds **1** and **4**, elongation of the distance between the uridine and the sugar moiety was found to decrease the inhibition activity from 85% to 16%. Similar results were found by comparing the inhibition activity of the elongated compounds **3** (60% inhibition) and **5** (40%) with **2** (85%). The details of the biological evaluations will be published elsewhere.



Scheme 5. Synthesis of inhibitors **4** and **5**

Experimental Section

Solvents were purified according to standard procedures. NMR spectra were recorded at 22 °C on a Bruker AC 250 Cryospec, JEOL JNM-GX 400 or Bruker DRX 600 spectrometer. Tetramethylsilane (TMS) or the resonance of the deuterated solvent was used as the internal standard; solvent: $CDCl_3$, δ = 7.24; D_2O, δ = 4.63; [D_6]DMSO, δ = 2.49 ppm. For ^{31}P NMR, phosphoric acid was used as the external standard. ^{31}P NMR spectra were broadband ^1H-decoupled. MALDI-mass spectra were recorded on a Kratos Kompact Maldi 2 spectrometer and 2,5-dihydroxybenzoic acid (DHB) or 6-aza-2-thiothymine (ATT) were used as matrices. Thin-layer chromatography was performed on Merck 60 F_{254} silica gel plastic plates or Merck RP-18 glass plates; compounds were visualized by treatment with a solution of [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% sulfuric acid (400 mL). Flash chromatography was performed on J.T. Baker silica gel 60 (40−63 μm) at a pressure of 0.3 bar. Preparative HPLC separations were performed on an Autochrom System with a Shimadzu LC8A preparative pump and a Rainin Dynamax UV 1 Detector at 260 nm. The column used was a LiChrosorb RP-18, 7 μm, 250 × 16 mm (Knauer, Germany). Mixtures of acetonitrile and 0.05 M triethylammonium hydrogen carbonate (TEAB) (pH 7.0−7.2) were used as the mobile phase. Optical rotations were measured at 25 °C with a Perkin-Elmer 241/MS polarimeter at the sodium D line.

Trisodium (3-Acetamido-2,6-anhydro-3-deoxy-1-phosphoryl-D-*erythro*-L-*idol*L-*gulo*-heptitol-1-yl) (Uridin-5′-yl) Phosphate (1): A mixture of **15** (40 mg, 32 μmol) and Pd/C (10%, 20 mg) in methanol (8 mL) and water (0.5 mL) was stirred at room temperature for 9 h under a positive pressure of hydrogen (the reaction was monitored by MALDI-MS; a longer reaction time leads to reduction of the double bond of the uracil ring). The catalyst was removed by filtration and the resulting solution was concentrated under reduced pressure. The remaining residue was dissolved in water (5 mL) and was treated with aqueous NH_3 (25%, 4 mL). After stirring at room temperature for 16 h the reaction mixture was concentrated under reduced pressure. The resulting residue was purified by RP-18-HPLC (0.05 M TEAB buffer, 1% CH_3CN, flow rate: 14 mL·min^{-1}, t_R = 6 min) to afford the triethylammonium salt of **1**, which was transformed to the sodium salt by ion exchange chromatography (Amberlite IR-120 Na^+ form). The crude salt was dissolved in water (2 drops) and ethanol (10 mL) was added to precipitate compound **1** (16 mg, 17 μmol, 56%) as a white solid. ^1H NMR (600 MHz, D_2O): δ = 1.92 (s, 3 H, Ac), 3.37 (dd, $J_{5'',6''} = J_{5'',4''}$ = 7 Hz, 1 H, 5''-H), 3.62 (d, $J_{7a,b''}$ = 12 Hz, 1 H, 7a′′-H), 3.74 (dd, $J_{7b,a''}$ = 12, $J_{7b'',6''}$ = 6.8 Hz, 1 H, 7b′′-H), 3.85 (m, 1 H, 6′′-H), 3.90 (dd, $J_{4'',5''} = J_{4'',3''}$ = 7 Hz, 1 H, 4′′-H), 4.02 (m, 1 H, 3′′-H), 4.14 (m, 3 H, 4′-H, 5a,b′-H), 4.24 (m, 3 H, 2′-H, 3′-H, 2′′-H), 4.49 (m, 1 H, 1′′-H), 5.83 (m, 2 H, 5-H, 1′-H), 7.81 (d, $J_{6,5}$ = 7.9 Hz, 1 H, 6-H) ppm. ^{13}C NMR (151 MHz, D_2O, HMQC): δ = 51.8 (1 C, 3′′-C), 60.4 (1 C, 7′′-C), 65.2 (1 C, 5′-C), 69.7 (1 C, 3′-C), 70.0 (1 C, 5′′-C), 71.0 (1 C, 4′′-C), 71.4 (1 C, 2′′-C), 73.8 (d, $J_{C,P}$ = 130 Hz, 1 C, 1′′-C), 74.0 (1 C, 2′-C), 76.4 (1 C, 6′′-C), 83.3 (1 C, 4′-C), 88.9 (1 C, 1′-C), 102.9 (1 C, 5-C), 142.8 (1 C, 6-C) ppm. ^{31}P NMR (243 MHz, D_2O): δ = 14.9 (br. s, 1 P, CPO_3), 0.33 (br. s, 1 P, PO_4) ppm. MALDI-MS (negative mode, ATT): *m*/*z* = 620.5 [M − 3 Na^+ + 2 H^+]$^-$. $C_{18}H_{26}N_3Na_3O_{17}P_2$ (687.4).

Disodium (3-Acetamido-2,6-anhydro-3-deoxy-D-*erythro*-L-*idol*L-*gulo*-heptitol-1-*C*-ylphosphono) (Uridin-5′-yl) Phosphate (2): Compound **16** (35 mg, 68 μmol) was coevaporated 3 times with dry pyridine. Then, 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphomorpholidate (66 mg, 96 μmol) was added and the mixture was again coevaporated with dry pyridine. The resulting residue was dissolved in dry pyridine (2 mL) and dry 1*H*-tetrazole (14 mg, 0.20 mmol) was added. After stirring for 3 days at room temperature the reaction was quenched by adding water (2 mL) and Hünig′s base (0.1 mL). The mixture was concentrated under reduced pressure and coevaporated with water/Hünig′s base. The resulting residue was purified by RP-18-HPLC [0.05 M TEAB buffer + 0.5% CH_3CN, flow rate: 10 mL·min^{-1}, t_R = 11 min] to afford the triethylammonium salt of **2**, which was transformed to the sodium salt by ion exchange chromatography (Amberlite IR-120 Na^+ form). The crude salt was dissolved in water (2 drops) and ethanol (10 mL) was added to precipitate compound **2** (8.0 mg, 12 μmol, 18%) as a white solid. ^1H NMR (600 MHz, D_2O): δ = 1.92 (s, 3 H, Ac), 3.40 (dd, $J_{5'',6''} = J_{5'',4''}$ = 6.9 Hz, 1 H, 5′′-H), 3.62 (m, 1 H, 7a′′-H), 3.76 (m, 2 H, 7b′′-H, 6′′-H), 3.90 (m, 1 H, 4′′-H), 4.02 (d, $J_{3'',4''}$ = 3.0 Hz, 1 H, 3′′-H), 4.10−4.18 (m, 5 H, 4′-H, 5a,b′-H, 1′′-H, 2′′-H), 4.24 (m, 2 H, 2′-H, 3′-H), 5.84 (m, 2 H, 5-H, 1′-H), 7.84 (d, $J_{6,5}$ = 8.0 Hz, 1 H, 6-H) ppm. ^{13}C NMR (151 MHz, D_2O, HMQC): δ = 52.1 (1 C, 3′′-C), 60.6 (1 C, 7′′-C), 65.2 (1 C, 5′-C), 69.6 (d, *J* = 166 Hz, 1 C, 1′′-C), 69.8 (1 C, 5′′-C), 69.9, 74.2 (2 C, 2′-C, 3′-C), 71.2 (1 C, 2′′-C), 71.8 (1 C, 4′′-C), 76.8 (1 C, 6′′-C), 83.5 (1 C, 4′-C), 88.7 (1 C, 1′-C), 102.9 (1 C, 5-C) ppm. ^{31}P NMR (243 MHz, D_2O): δ = −8.8 (br. d, 1 P, PO_4), 12.4 (br. d, 1 P, CPO_3) ppm. MALDI-MS (negative mode, ATT): *m*/*z* = 620.5 [M − 2 Na^+ + H^+]$^-$. $C_{18}H_{27}N_3Na_2O_{17}P_2$ (665.4).

Disodium (3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-D-*glycero*-D-*ido*-heptitol-1-yl) (Uridin-5′-yl) Diphosphate (3): The procedure described for the synthesis of **2** was followed using **19** (34 mg, 66 μmol), 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphomorpholidate (77 mg, 0.11 mmol) and 1*H*-tetrazole (23 mg, 0.33 mmol) in abs. pyridine (2 mL) to afford the sodium salt **3** (8.7 mg, 13 μmol, 20%) as a white powder. Purification was achieved with RP-18-HPLC: [0.05 M TEAB buffer + 0.1% CH_3CN, flow rate: 10 mL·min^{-1}, t_R = 6.0 min]. The ammonium salt of **3** has already been reported in the literature.[12] Data for the disodium salt: ^1H NMR (250 MHz, D_2O): δ = 1.87 (s, 3 H, Ac), 3.24 (dd, $J_{5'',6''}$ = 9.0, $J_{5'',4''}$ = 8.3 Hz, 1 H, 5′′-H), 3.55 (dd, $J_{7a,b''}$ = 12.2, $J_{7a'',6''}$ = 5.1 Hz, 1 H, 7a′′-H), 3.69 (dd, $J_{7b,a''}$ = 12.2, $J_{7b'',6''}$ = 2.2 Hz, 1 H, 7b′′-H), 3.75 (m, 1 H, 6′′-H), 3.83−4.12 (m, 8 H, 3′′-H, 4′′-H, 1a,b′′-H, 2′′-H, 5a,b′-H, 4′-H), 4.20 (m, 2 H, 2′-H, 3′-H), 5.80 (m, 2 H, 5-H, 1′-H), 7.79 (d, $J_{6,5}$ = 8.1 Hz, 1 H, 6-H) ppm. MALDI-MS (negative mode, ATT): *m*/*z* = 621 [M − 2 Na^+ + H^+]$^-$. $C_{18}H_{27}N_3Na_2O_{17}P_2$ (665.4).

Trisodium (4-Acetamido-3,7-anhydro-2,4-dideoxy-1-phosphoryl-D-*erythro*-L-*idol*L-*gulo*-octitol-1-yl) (Uridin-5′-yl) Phosphate (4): Dimedone (100 mg, 0.7 mmol) and tetrakis(triphenylphosphane)palladium (70 mg, 60 μmol) were added to a solution of **22** (100 mg, 97 μmol) in abs. THF/MeOH (1:1, 12 mL) and the mixture was stirred under argon and protected from light for 20 h at room temperature. After concentration under reduced pressure, the remaining residue was purified by RP-18 flash chromatography (EtOH/H_2O, 1:3). The resulting intermediate was dissolved in water (1.5 mL) and treated with an aqueous solution of NH_3 (25%, 1.5 mL). After stirring at room temperature for 16 h, the mixture was concentrated under reduced pressure. The resulting triethylammonium salt was converted into the sodium salt by ion exchange chromatography (Amberlite IR-120 Na^+ form). The crude salt was dissolved in water (2 drops) and ethanol (10 mL) was added to precipitate a diastereomeric mixture [major (*ma*)/minor (*mi*), 2:1] of **4** (10 mg, 14 μmol, 15%) as a white powder. ^1H NMR (600 MHz, D_2O): δ = 1.70/2.04 (2 m, 1 H, 2a′′-H), 1.88/1.92 (2 s, 3 H, Ac), 2.16 (m, 1 H, 2b′′-H), 3.23 (m, 1 H, 6′′-H), 3.47 (m, 0.3 H, 7′′$_{mi}$-

H), 3.56−3.75 (m, 3.7 H, 7′′$_{ma}$-H, 5′′-H, 8a,b′′-H), 3.87/3.90 (2 m, 1 H, 4′′-H), 4.04−4.29 (m, 7 H, 2′-H, 3′-H, 4′-H, 5a,b′-H, 1′′-H, 3′′-H), 5.80−5.86 (m, 2 H, 1′-H, 5-H), 7.89/7.92 (2 d, $J_{6,5}$ = 8.1 Hz, 1 H, 6-H). ^{13}C NMR (151 MHz, D_2O): δ = 21.4/21.5 (1 C, Ac), 27.3/29.5 (1 C, 2′′-C), 52.4/52.7 (1 C, 4′′-C), 60.7/61.0 (1 C, 8′′-C), 62.9/63.6 (1 C, 5′-C), 67.8/68.6 (1 C, 3′-C), 70.3−70.7 (m, 4 C, 1′′-C, 3′′-C, 5′′-C, 6′′-C), 72.2/72.5 (1 C, 7′′-C), 73.5/73.7 (1 C, 2′-C), 82.3/82.9 (1 C, 4′-C), 88.1/88.6 (1 C, 1′-C), 101.7/101.8 (1 C, 5-C), 141.2 (1 C, 6-C), 173.9 (1 C, Ac) ppm. ^{31}P NMR (243 MHz, D_2O): δ = 1.56 (m, 1 P, PO_4), 15.5 (d, J = 26.7 Hz, 0.7 P, *ma*: PO_3), 16.5 (br. d, 0.3 P, *mi*: PO_3) ppm. MALDI-MS (negative mode, ATT): m/z = 634.3 [M − 3 Na^+ + 2 H^+]$^-$, 656.3 [M − 2 Na^+ + H^+]$^-$. $C_{19}H_{28}N_3Na_3O_{17}P_2$ (701.4).

Disodium (4-Acetamido-3,7-anhydro-2,4-dideoxy-D-*erythro*-L-*ido*/L-*gulo*-octitol-1-*C*-ylphosphono) (Uridin-5′-yl) Phosphate (5): The procedure described for the synthesis of **3** was followed using **23** (65 mg, 0.12 mmol), 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphomorpholidate (103 mg, 0.15 mmol) and 1*H*-tetrazole (15 mg, 0.22 mmol) in abs. pyridine (2 mL) to afford a diastereomeric mixture (*ma*/*mi*, 2:1) of the sodium salt **5** (12 mg, 18 μmol, 15%) as a white solid. Purification was achieved with RP-18-HPLC (0.05 M TEAB buffer + 0.5−1% CH_3CN, flow rate: 10 mL·min^{-1}, t_R = 15 min). ^{1}H NMR (600 MHz, D_2O): δ = 1.62/1.89 (2 m, 1 H, 2a′′-H), 1.92 (2 s, 3 H, Ac), 2.00/2.11 (2 m, 1 H, 2b′′-H), 3.30 (m, 1 H, 6′′-H), 3.44/3.54 (m, 1 H, 7′′-H), 3.61−3.88 (m, 5 H, 1′′-H, 4′′-H, 5′′-H, 8a,b′′-H), 4.09−4.18 (m, 4 H, 4′-H, 5a,b′-H, 3′′-H), 4.24−4.26 (m, 2 H, 2′-H, 3′-H), 5.83−5.88 (m, 2 H, 1′-H, 5-H), 7.83/8.00 (2 d, $J_{6,5}$ = 8.1 Hz, 1 H, 6-H) ppm. ^{13}C NMR (151 MHz, D_2O, HMQC): δ = 22.2 (1 C, Ac), 26.8/29.1 (1 C, 2′′-C), 53.2/53.7 (1 C, 4′′-C), 61.1/61.3 (1 C, 8′′-C), 63.4/67.9 (2 d, J = 136 Hz, 1 C, 1′′-C), 65.0 (1 C, 5′-C), 69.7/72.7 (1 C, 3′′-C), 69.8 (1 C, 3′-C), 70.9 (1 C, 5′′-C), 71.0 (1 C, 6′′-C), 72.8/73.5 (1 C, 7′′-C), 74.1 (1 C, 2′-C), 83.3/83.9 (1 C, 4′-C), 88.6 (1 C, 1′-C), 102.9 (1 C, 5-C) ppm. ^{31}P NMR (243 MHz, D_2O): δ = −9.6 (m, 1 P, PO_4), 12.8/13.9 (2 d, J = 30 Hz, 1 P, PO_3) ppm. MALDI-MS (negative mode, ATT): m/z = 634.4 [M − 2 Na^+ + H^+]$^-$; (positive mode, DHB): m/z = 658.1 [M − Na^+ + 2 H^+]$^+$, 674.1 [M − 2 Na^+ + K^+ + 2 H^+]$^+$, 680.1 [M + H^+]$^+$. $C_{19}H_{29}N_3Na_2O_{17}P_2$ (679.4).

Benzyl 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucoside (6): Compound **6** was prepared following a procedure reported by Harrison and Fletcher[13] starting with *N*-acetylglucosamine (yield 76%).

2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucose (7): A solution of **6** (15 g, 26 mmol) in THF (800 mL) and 3 N HCl (400 mL) was refluxed for 2 days. The THF was removed by distillation (30 °C bath temperature) and the resulting crystalline magma was stored at +5 °C overnight. The solid mass was then removed by filtration, washed with cold 1 N aqueous hydrochloric acid and dried in air. The resulting hydrochloride **6A**[14] was used without purification in the next step. Trifluoromethanesulfonic anhydride (8.5 mL, 51 mmol) was slowly (15 min) added to a cooled (0 °C) mixture of sodium azide (16.4 g, 250 mmol), CH_2Cl_2 (70 mL) and water (42 mL) . After stirring for 2 h, the aqueous layer was extracted with CH_2Cl_2 (40 mL). The pooled organic layer was washed with water and added to a mixture of **6A**, potassium carbonate (8.4 g, 85 mmol) and $CuSO_4$·$5H_2O$ (42 mg) in CH_2Cl_2/H_2O/MeOH (90/90/310 mL). After stirring for 2 days the solution was concentrated in vacuo. The remaining residue was extracted with CH_2Cl_2/water. The pooled organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification by flash chromatography (toluene/acetone, 12:1) afforded compound **7** (6.4 g, 13.5 mmol, 52%) as a colourless solid. The physical data are in good agreement with the reported data.[16]

(2-Azido-3,4,6-tri-*O*-benzyl-1,2-dideoxy-α-D-glucopyranosyl) Cyanide (8): Compound **8** was prepared from **7** in 75% yield (over 2 steps) following a procedure reported by Hoffmann and Schmidt.[20]

(2-Acetamido-3,4,6-tri-*O*-benzyl-1,2-dideoxy-α-D-glucopyranosyl) Cyanide (9): Triphenylphosphane (4.1 g, 15.7 mmol) was added to a solution of **8** (3.8 g, 7.8 mmol) in THF (70 mL) and the solution was stirred for 1 h at room temperature. Then, water (7 mL) was added and the mixture was stirred for 2 days. The solution was concentrated in vacuo and the remaining residue was dissolved in pyridine (12 mL) and Ac_2O (10 mL). After stirring for 2 days at room temperature the solution was concentrated in vacuo. Purification by flash chromatography (toluene/acetone, 9:1) afforded compound **9** (2.5 g, 5.0 mmol, 64%) as a colourless solid. TLC (toluene/acetone, 3:1): R_f = 0.41. $[\alpha]_D$ = +59.6 (c = 0.5, dioxane). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.72 (s, 3 H, Ac), 3.66−3.78 (m, 5 H, 2-H, 3-H, 4-H, 6a,b-H), 3.93 (m, 1 H, 5-H), 4.51−4.69 (m, 4 H, CH_2Ph), 4.82−4.96 (m, 3 H, NH, CH_2Ph), 5.23 (d, $J_{1,2}$ = 5.7 Hz, 1 H, 1-H), 7.25−7.46 (m, 15 H, Ph) ppm. MALDI-MS (positive mode, DHB, dioxane): m/z = 524.0 [M + Na^+]. $C_{30}H_{32}N_2O_5$ (500.59): calcd. C 71.98, H 6.44, N 5.59; found C 71.77, H 6.64, N 5.24.

3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-D-*glycero*-D-*ido*-heptonic Acid (10): A solution of **9** (1.5 g, 3.0 mmol) in dioxane/water (10:1, 13 mL) was treated with HCl (4 M in dioxane, 12 mL) and stirred at 60 °C for 20 h. The solution was concentrated under reduced pressure and the resulting residue was dissolved in 1 N aqueous hydrochloric acid. The solution was extracted with CH_2Cl_2 and the pooled organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography (toluene/ethyl acetate, 1:1, + 1% acetic acid) afforded compound **10** (770 mg, 1.5 mmol, 50%) as a white solid. The physical data are in good agreement with the reported data.[12]

***S*-(4-Methylphenyl) 3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-D-*glycero*-D-*ido*-hepto-pyranosonothioate (11):** Carbonyldiimidazole (31 mg, 0.19 mmol) was added to a cooled (0 °C) solution of **10** (45 mg, 88 μmol) in DMF (5 mL) and the mixture was stirred at 0 °C → room temperature for 2.5 h. Then, *p*-thiocresol (24 mg, 0.19 mmol) was added and the solution was stirred at room temperature for 1.5 h. The reaction mixture was poured into water and the organic layer was extracted with ethyl acetate. The pooled organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography (toluene/acetone, 6:1) afforded compound **11** (51 mg, 82 μmol, 93%) as a white solid. TLC (toluene/acetone, 2:1): R_f = 0.62. $[\alpha]_D$ = +30.8 (c = 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.78 (s, 3 H, Ac), 2.38 (s, 3 H, Me), 3.71 (m, 2 H, 4-H, 5-H), 3.78 (dd, $J_{7a,b}$ = 10.7, $J_{7a,6}$ = 3.8 Hz, 1 H, 7a-H), 3.86 (dd, $J_{7b,a}$ = 10.7, $J_{7b,6}$ = 5.3 Hz, 1 H, 7b-H), 4.06 (m, 1 H, 6-H), 4.55−4.70 (m, 8 H, 2-H, 3-H, CH_2Ph), 6.19 (d, J = 9.7 Hz, 1 H, NH), 7.23−7.35 (m, 19 H, Ar) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 21.3 (1 C, Me), 23.3 (1 C, Ac), 49.5 (1 C, 3-C), 67.8 (1 C, 7-C), 73.5, 74.0 (3 C, *C*H_2Ph), 76.2, 78.0 (2 C, 4-C, 5-C), 76.3 (1 C, 6-C), 77.5 (1 C, 2-C), 122.8−140.1 (24 C, Ar), 169.7 (1 C, Ac), 200.1 (1 C, 1-C) ppm. MALDI-MS (positive mode, DHB, dioxane): m/z = 627.1 [M + H]$^+$, 648.9 [M + Na]$^+$, 665.2 [M + K]$^+$. $C_{37}H_{39}NO_6S$ (625.78): calcd. C 71.02, H 6.28, N 2.24; found C 70.96, H 6.07, N 1.92.

3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-D-*glycero*-D-*ido*-heptitol (12): Sodium borohydride (60 mg, 1.6 mmol) was added

to a cooled (0 °C) solution of **11** (500 mg, 0.8 mmol) in abs. dichloromethane (20 mL) and dry ethanol (10 mL) and the stirred solution was allowed to warm to room temperature over a period of 3 h. After complete conversion (TLC: toluene/acetone, 2:1, R_f = 0.40) the reaction mixture was poured into an aqueous ammonium chloride solution (50 mL). The aqueous layer was extracted with dichloromethane; the pooled organic layer was washed with water and dried over Na_2SO_4. After concentration under reduced pressure the remaining residue was purified by flash chromatography (toluene/acetone, 6:1) to afford compound **12** (375 mg, 0.74 mmol, 93%) as a white solid. The physical data are in good agreement with the reported data.[12] Additional data: ^{1}H NMR: $J_{2,3} < 1$, $J_{3,4} = 1$, $J_{5,6} < 1$, $J_{4,5} = 2.9$ Hz.

(3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-D-*erythro*-L-*idol*/L-*gulo*-heptitol-1-*C*-yl) Dibenzyl Phosphonate (13): A solution of **12** (130 mg, 0.26 mmol) in abs. dichloromethane (5 mL) was treated with Dess−Martin periodinane (150 mg, 0.35 mmol). After stirring at room temperature for 2 h, the reaction was quenched by adding saturated aqueous solutions of $NaHCO_3$ (10 mL) and $Na_2S_2O_3$ (10 mL). The mixture was then diluted with diethyl ether (50 mL) and stirred at room temperature for 30 min. The aqueous layer was extracted with diethyl ether; the pooled organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The remaining residue was dissolved in dichloromethane (3 mL) and treated with triethylamine (0.1 mL) and diallyl phosphonate (250 μL, 1.6 mmol). After stirring for 2 h at room temperature under argon, the reaction mixture was concentrated under reduced pressure. Purification by flash chromatography (toluene/acetone, 4:1) afforded compound **13** (375 mg, 1.15 mmol, 94%) as a colourless oil. TLC (toluene/acetone, 3:2): R_f = 0.52. $[\alpha]_D = -9.0$ (c = 1, $CDCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.89 (s, 3 H, Ac), 3.68 (m, 1 H, 1-H), 3.74 (m, 2 H, 4-H, 5-H), 3.76 (dd, $J_{7a,b}$ = 9.0, $J_{7a,6}$ = 6.0 Hz, 1 H, 7a-H), 3.94 (dd, $J_{7b,6} = J_{7b,a}$ = 9.0 Hz, 1 H, 7b-H), 4.28 (m, 2 H, 6-H, 3-H), 4.35−4.65 (m, 7 H, 2-H, CH_2Ph), 5.05−5.15 (m, 4 H, POCH_2Ph), 5.25 (br. d, 1 H, OH), 7.22−7.37 (m, 26 H, Ph, NH) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 23.1 (1 C, Ac), 45.9 (d, $J_{3,P}$ = 13 Hz, 1 C, 3-C), 66.7 (d, $J_{1,P}$ = 173 Hz, 1 C, 1-C), 67.6, 68.3 (2 d, J = 6.5 Hz, 2 C, POCH$_2$Ph), 68.0 (1 C, 7-C), 68.7 (1 C, 2-C), 71.8, 71.9, 73.3 (3 C, CH$_2$Ph), 72.7, 73.3 (2 C, 4-C, 5-C), 74.6 (1 C, 6-C), 127.5−138.2 (30 C, Ph), 172.1 (1 C, Ac) ppm. ^{31}P NMR (243 MHz, $CDCl_3$): δ = 26.8 (1 P) ppm. MALDI-MS (positive mode, DHB): m/z = 787.9 $[M + Na]^+$, 804.0 $[M + K]^+$. $C_{44}H_{48}NO_9P$ (765.86): calcd. C 69.01, H 6.32, N 1.83; found C 68.89, H 6.33, N 1.77.

2′,3′-Di-*O*-acetyluridine-5′-*O*-yl(2-cyanethoxy)(diisopropylamino)-phosphane (14): Compound **14** was prepared similarly to the reported procedure.[23−25] A solution of 2,3-di-*O*-acetyluridine (700 mg, 2.1 mmol) in abs. dichloromethane (18 mL) was treated with diisopropylammonium tetrazolide (180 mg, 1.1 mmol) and bis(diisopropylamido)phosphoric acid 2-cyanoethyl ester (1.2 mL, 4 mmol) and stirred at room temperature under argon for 16 h. The mixture was concentrated under reduced pressure and the remaining residue was purified by flash chromatography (toluene/ethyl acetate, 2:1) to afford the diastereomeric mixture of **14** (1.1 g, 2.0 mmol, 95%) as a colourless foam. Additional physical data: TLC (toluene/acetone, 3:2): R_f = 0.59. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.10−1.23 (m, 12 H, iPr), 2.02−2.14 (4 s, 6 H, Ac), 2.62 (m, 2 H, CH_2CH$_2$CN), 3.49−3.93 (m, 7 H, 4′-H, 5a,b′-H, CH_2CH$_2$CN, iPr), 5.22−5.42 (m, 2 H, 2′-H, 3′-H), 5.73 (2 d, J = 4.0 Hz, 1 H, 1′-H), 6.25 (2 d, J = 7.9 Hz, 1 H, 5-H), 7.76 (2 d, J = 7.9 Hz, 1 H, 6-H), 9.5 (br. s, 1 H, NH).

Triethylammonium [3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-1-(di-*O*-benzyl phosphoryl)-D-*erythro*-L-*idol*/L-*gulo*-heptitol-1-*O*-yl] (2′,3′-Di-*O*-acetyluridin-5′-yl) Phosphate (15): 1*H*-Tetrazole (28 mg, 0.40 mmol) and **14** (160 mg, 0.30 mmol) was added to a solution of **13** (210 mg, 0.27 mmol) in abs. CH_2Cl_2 (5 mL) under argon. After stirring at room temperature for 2 h, the reaction mixture was cooled to 0 °C and a 5.5 M solution of *t*BuOOH in decane (55 μL, 0.30 mmol) was added slowly. After stirring at room temperature for 40 min, triethylamine (0.5 mL) was added and the mixture was stirred for 16 h. After concentration under reduced pressure, the remaining residue was purified by flash chromatography (ethyl acetate/MeOH, 4:1, + 1% Et_3N) to afford compound **15** (240 mg, 0.19 mmol, 71%) as a colourless oil. TLC (ethyl acetate/MeOH, 3:1, + 1% Et_3N): R_f = 0.30. ^{1}H NMR (250 MHz, [D$_4$]MeOH): δ = 1.21 (t, 9 H, NCH$_2$CH_3), 1.91, 1.96, 2.05 (3 s, 9 H, Ac), 3.02 (q, 6 H, NCH_2CH$_3$), 3.58−3.62, 3.89, 4.05−4.30 [3 m, (3 H, 1 H, 7 H), 4′-H, 5′a,b-H, 1′′-H, 2′′-H, 3′′-H, 4′′-H, 5′′-H, 6′′-H, 7a,b′′-H], 4.43 (m, 4 H, CH_2Ph), 4.54, 4.66 (2 d, J = 11.7 Hz, 2 H, CH_2Ph), 5.05−5.12 (m, 4 H, POCH_2Ph), 5.37−5.45 (m, 2 H, 2′-H, 3′-H), 5.75 (d, $J_{5,6}$ = 8.1 Hz, 1 H, 5-H), 6.05 (d, $J_{1',2'}$ = 6.2 Hz, 1 H, 1′-H), 7.18−7.27 (m, 25 H, Ph), 7.90 (d, $J_{6,5}$ = 8.1 Hz, 1 H, 6-H), 8.12 (br. d, NH) ppm. MALDI-MS (ATT, negative mode): m/z = 1154.3 $[M - HNEt_3^+]^-$. $C_{57}H_{62}N_3O_{19}P_2 \cdot C_6H_{16}N$ (1257.3).

Bis(triethylammonium) (3-Acetamido-2,6-anhydro-3-deoxy-D-*erythro*-L-*idol*/L-*gulo*-heptitol-1-*C*-yl) Phosphonate (16): A mixture of **13** (100 mg, 130 μmol) and Pd/C (10%, 40 mg) in methanol (16 mL) and water (0.5 mL) was stirred at room temperature for 1 day under a positive pressure of hydrogen. The catalyst was removed by filtration and the resulting solution was treated with Et_3N (0.1 mL), stirred at room temperature for 5 min and concentrated in vacuo to afford compound **16** in quantitative yield, which was used in the next step without purification. ^{1}H NMR (250 MHz, D_2O): δ = 1.34 (t, 18 H, Et_3N), 2.02 (s, 3 H, Ac), 3.21 (q, 12 H, Et_3N), 3.43 (dd, $J_{5,4} = J_{5,6}$ = 4.5 Hz, 1 H, 5-H), 3.52 (dd, $J_{7a,b}$ = 9.1, $J_{7a,6}$ = 4.1 Hz, 1 H, 7a-H), 3.77 (dd, $J_{7b,a} = J_{7b,6}$ = 9.1 Hz, 1 H, 7b-H), 3.92 (m, 2 H, 4-H, 6-H), 4.12 (m, 2 H, 1-H, 3-H), 4.28 (m, 1 H, 2-H) ppm. MALDI-MS (negative mode, ATT): m/z = 315.0 $[M - 2\,HNEt_3^+ + H^+]^-$. $C_9H_{16}NO_9P \cdot 2C_6H_{16}N$ (517.6).

(3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-D-*glycero*-D-*ido*-heptitol-1-yl) Dibenzyl Phosphate (18): 1*H*-Tetrazole (63 mg, 0.90 mmol) was added to a solution of **12** (230 mg, 0.45 mmol) in abs. CH_2Cl_2 (10 mL) under argon. Then, bis(benzyloxy)(diisopropylamino)phosphane (222 μL, 0.68 mmol) was added slowly to the reaction mixture. After stirring at room temperature for 3 h, the solution was cooled to 0 °C and a solution of 5.5 M *t*BuOOH in nonane (120 μL, 0.68 mmol) was added slowly to the reaction mixture. After stirring at room temperature for 1 h the reaction mixture was poured into an aqueous solution of $Na_2S_2O_3$ (50 mL). The organic layer was washed with a NaCl solution, a $NaHCO_3$ solution and water (each 50 mL). The aqueous layer was extracted again with CH_2Cl_2 (2 × 25 mL). The pooled organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The remaining residue was purified by flash chromatography (toluene/acetone, 2:1) to afford compound **18** (300 mg, 0.39 mmol, 87%) as a colourless oil. TLC (toluene/acetone, 2:1): R_f = 0.38. $[\alpha]_D = -7.1$ (c = 1, dioxane). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.79 (s, 3 H, Ac), 3.63, 3.68 (2 m, 2 H, 4-H, 5-H), 3.77 (m, 2 H, 7a,b-H), 4.05 (m, 2 H, 1a,b-H), 4.18 (ddd, $J_{2,3} = J_{2,1a}$ = 5.6, $J_{2,1b}$ = 2 Hz, 1 H, 2-H), 4.26 (m, 2 H, 3-H, 6-H), 4.44−4.51 (m, 4 H, CH_2Ph), 4.59 (d, J = 11.7 Hz, 1 H, CH_2Ph), 4.61 (d, J = 11.7 Hz, 1 H CH_2Ph), 5.02−5.05 (m, 4 H, POCH_2Ph), 6.54 (d, J = 9.9 Hz, 1 H, NH),

7.26−7.35 (m, 25 H, Ar) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 23.3 (1 C, Ac), 45.9 (1 C, 3-C), 67.8 (1 C, 7-C), 67.9 (1 C, 1-C), 67.9 (1 C, 2-C), 69.3 (2 C, PO*C*H_2Ph), 72.0, 72.2, 73.3 (3 C, *C*H_2Ph), 73.4, 74.4 (2 C, 4-C, 5-C), 75.1 (1 C, 6-C), 127.6−138.1 (30 C, Ar), 169.7 (1 C, Ac) ppm. ^{31}P NMR (243 MHz, $CDCl_3$): δ = 0.24 (1 P) ppm. MALDI-MS (positive mode, DHB, dioxane): *m*/*z* = 788.1 $[M + Na]^+$, 804.2 $[M + K]^+$. $C_{44}H_{48}NO_9P{\cdot}0.5H_2O$ (774.85): calcd. C 68.21, H 6.37, N 1.81; found C 68.12, H 6.45, N 1.97.

Bis(triethylammonium) (3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-D-*glycero*-D-*ido*-heptitol-1-yl) Phosphate (19): A mixture of **18** (250 mg, 0.33 mmol) and Pd/C (10%, 30 mg) in methanol (10 mL) was stirred at room temperature for 1 day under a positive pressure of hydrogen. The catalyst was removed by filtration and the resulting solution was treated with Et_3N (0.1 mL), stirred at room temperature for 5 min and concentrated in vacuo to afford compound **19** in quantitative yield. The physical data are in good agreement with the reported data.[12]

3-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyrano-1-*C*-yl)-1-propene (20): Compound **20** was prepared following a procedure reported by Cui and Horton.[30]

Diallyl (4-Acetamido-5,6,8-tri-*O*-acetyl-3,7-anhydro-2,4-dideoxy-D-*erythro*-L-*ido*/L-*gulo*-octitol-1-*C*-yl)] Phosphonate (21): A cooled (−80 °C) solution of **20** (415 mg, 1.1 mmol) in dry methanol (30 mL) was treated with ozone for 10 min (blue colour). Then, argon was bubbled into the solution for 10 min. Triphenylphosphane on a polymer support (720 mg, 2.2 mmol) was added to the reaction mixture. After stirring at room temperature for 2 h, the mixture was filtered and the filtrate was concentrated under reduced pressure. Purification by flash chromatography (petroleum ether/ethyl acetate, 1:5) afforded the aldehyde **20A** (360 mg, 0.96 mmol), which was dissolved in abs. dichloromethane (10 mL) and treated with triethylamine (0.2 mL) and diallyl phosphonaate (195 μL, 1.25 mmol). After stirring at room temperature for 20 h the solution was concentrated under reduced pressure. Purification by flash chromatography (toluene/acetone, 4:3) yielded a diastereomeric mixture (*ma*/*mi*, 2:1) of **21** (350 mg, 0.65 mmol, 68%) as a colourless oil. TLC (toluene/acetone, 1:3): R_f = 0.39 (*ma* + *mi*). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.81−2.25 (m, 14 H, 2a,b-H, Ac), 3.87 (m, 0.3 H, 7_{mi}-H), 4.05−4.16 (m, 2.7 H, 7_{ma}-H, 1-H, 8a-H), 4.27 (m, 1 H, 4-H), 4.34−4.41 (m, 3 H, 2-H, 3-H, 8b-H), 4.56 (m, 4 H, OCH_2CH=CH_2), 4.89 (m, 1 H, 6-H), 5.00 (m, 1 H, 5-H), 5.22−5.35 (m, 4 H, $OCH_2CH=CH_2$), 5.91−5.95 (m, 2 H, $OCH_2CH=CH_2$), 6.25/6.39 (2 d, *J* = 9.2 Hz, 1 H, NH) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 20.5−23.1 (4 C, Ac), 29.7/30.0 (1 C, 2-C), 49.2/49.6 (1 C, 4-C), 61.2/61.4 (1 C, 8-C), 63.5/66.5 (2 d, *J* = 169 Hz, 1 C, 1-C), 67.0−67.2 (2.3 C, 3_{mi}-C, O*C*H_2CH=CH_2), 67.5/67.6 (1 C, 6-C), 69.1/69.4 (1 C, 5-C), 70.7 (0.7 C, 3_{ma}-C), 71.6 (1 C, 7-C), 117.9−118.3 (2 C, $OCH_2CH=CH_2$), 132.7−123.8 (2 C, $OCH_2CH=CH_2$), 168.9−171.4 (4 C, Ac) ppm. ^{31}P NMR (243 MHz, $CDCl_3$): δ = 25.2/26.2 (2 s, 1 P) ppm. MALDI-MS (positive mode, DHB): *m*/*z* = 558.6 $[M + Na]^+$, 574.6 $[M + K]^+$. $C_{22}H_{34}NO_{12}P$ (535.5): calcd. C 49.35, H 6.40, N 2.61; found C 48.82, H 6.59, N 2.74.

Triethylammonium [4-Acetamido-5,6,8-tri-*O*-acetyl-3,7-anhydro-2,4-dideoxy-1-(di-*O*-benzylphosphoryl)-D-*erythro*-L-*ido*/L-*gulo*-octitol-1-*O*-yl] (2′,3′-Di-*O*-acetyluridin-5′-yl) Phosphate (22): 1*H*-Tetrazole (34 mg, 0.48 mmol) and **14** (150 mg, 0.28 mmol) were added to a solution of **21** (170 mg, 0.32 mmol) in abs. CH_2Cl_2 (5 mL) under argon. After stirring at room temperature for 4 h, the reaction mixture was cooled to 0 °C and a 5.5 M solution of *t*BuOOH in decane (58 μL, 0.32 mmol) was added slowly. After stirring at room temperature for 30 min, triethylamine (0.3 mL) was added and the mixture was stirred for 16 h. After concentration under reduced pressure, the remaining residue was purified by flash chromatography (ethyl acetate/MeOH, 6:1, + 1% Et_3N → ethyl acetate/MeOH, 3:1, + 1% Et_3N) to afford a diastereomeric mixture (*ma*/*mi*, 2:1) of **22** (110 mg, 0.11 mmol, 38% calcd. on **14**) as a colourless oil. Additionally, some starting material **21** (80 mg, 0.15 mmol, 47%) could be reisolated (yield 71%, based on converted starting material **21**). ^{1}H NMR (250 MHz, [D$_4$]MeOH): δ = 1.33 (t, 9 H, Et_3NH), 1.88−2.15 (m, 20 H, 2a,b′′-H, Ac), 3.22 (q, 6 H, Et_3NH), 3.90−4.51 (m, 9 H, 1′′-H, 3′′-H, 4′′-H, 7′′-H, 8a,b′′-H, 4′-H, 5a,b′-H), 4.58−4.64 (m, 4 H, OCH_2CH=CH_2), 5.11−5.58 (m, 8 H, 5′′-H, 6′′-H, 2′-H, 3′-H, $OCH_2CH=CH_2$), 5.82 (m, 1 H, 1′-H), 5.91−6.09 (m, 2 H, $OCH_2CH=CH_2$), 6.17 (m, 1 H, 5-H), 7.99 (m, 1 H, 6-H) ppm. MALDI-MS (negative mode, ATT): *m*/*z* = 924.6 $[M - HNEt_3^+]^-$. $C_{35}H_{48}N_3O_{22}P_2{\cdot}C_6H_{16}N$ (1026.9).

Bis(triethylammonium) (4-Acetamido-3,7-anhydro-2,4-dideoxy-D-*erythro*-L-*ido*/L-*gulo*-octitol-1-*C*-yl) Phosphonate (23): Dimedone (84 mg, 0.6 mmol) and tetrakis(triphenylphosphane)palladium (23 mg, 20 μmol) were added to a solution of **21** (90 mg, 0.17 mmol) in abs. THF (5 mL) and the mixture was stirred at room temperature under argon and protected from light for 20 h. After concentration under reduced pressure, the remaining residue was purified by flash chromatography ($CHCl_3$/MeOH/H_2O, 4:2:0.25, + 1% Et_3N). The resulting intermediate was dissolved in water (2 mL) and treated with an aqueous solution of NH_3 (25%, 2 mL). After stirring at room temperature for 6 h the mixture was concentrated under reduced pressure. The remaining residue was dissolved in water and was treated with triethylamine (0.1 mL). After stirring at room temperature for 2 min, the mixture was concentrated under reduced pressure. The resulting diastereomeric mixture (*ma*/*mi*, 2:1) of triethylammonium salt **23** (71 mg, 0.13 mmol, 79%) was used without further purification for the next step. ^{1}H NMR (250 MHz, D_2O): δ = 1.12 (t, 18 H, Et_3NH), 1.81, 1.87 (2 s, 3 H, Ac), 1.80−2.06 (m, 2 H, 2a,b-H), 3.03 (q, 12 H, Et_3NH), 3.25 (m, 1 H, 6-H), 3.33−3.90 (m, 6 H, 1-H, 4-H, 5-H, 7-H, 8a,b-H), 4.15 (m, 1 H, 3-H). $C_{10}H_{18}NO_9P{\cdot}2C_6H_{16}N$ (531.6).

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