Synthetic strategies for non-hydrolyzable pyrophosphate analogues

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Chapter 3

Design and Synthesis of UDP-GlcNAc Mimics as Inhibitors of GlcNAc Transferases

Introduction

N-acetylglucosamine (GlcNAc) transferases constitute a diverse family of enzymes that utilize uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc) to transfer a GlcNAc residue onto a wide range of acceptors, including proteins, lipids, and polysaccharides.[1-3] They participate in essential biosynthetic and regulatory processes in all kingdoms of life. Despite sharing the same donor substrate, these enzymes act in remarkably different biological contexts, such as MurG,[4] a bacterial glycosyltransferase, which participates in peptidoglycan synthesis, and eukaryotic glycosyltransferases responsible for a wide variety of complex carbohydrates, glycans and glycoconjugates.[5] Among this broad family, *O*-GlcNAc transferase (OGT) is an essential mammalian enzyme responsible for a dynamic post-translational modification,[6] in which GlcNAc is reversibly attached to the serine and threonine side chains of various proteins (Figure 3.1).[7-8] This *O*-GlcNAcylation plays crucial roles in cellular processes such as transcription, signal transduction, protein stability and degradation, and cell death.[9-12] Disruption of *O*-GlcNAc homeostasis has been implicated in the pathogenesis of various human diseases, including cancer, neurodegenerative, and proliferative disorders.[9, 13-16] Nevertheless, understanding of the molecular basis of *O*-GlcNAcylation remains incomplete, partly due to the lack of robust chemical tools for its precise identification and mechanistic study.

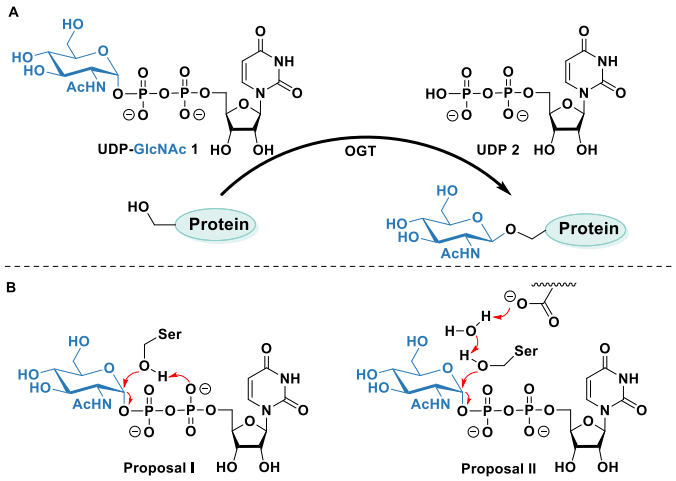


Figure 3.1. (A) *O*-GlcNAcylation. (B) Two proposed catalytic mechanisms of hOGT for *O*-GlcNAcylation. Proposal I: Substrate-assisted catalysis in which the α-phosphate of UDP-GlcNAc acts as the general base to activate the acceptor hydroxyl group. Proposal II: A conserved aspartate residue functions as the general base via a network of ordered water molecules, relaying the proton from the acceptor hydroxyl to the enzyme.

The diversity of GlcNAc transferases across biological systems gives rise to differences in their donor and acceptor binding modes and overall catalytic mechanisms. Human *O*-GlcNAc transferase (hOGT) catalyzes glycosyl transfer through a sequentially ordered mechanism, where UDP-GlcNAc binds first, followed by the acceptor peptide.[17-18] The reaction proceeds via an

overall S_N2-type inverting mechanism, generating a β-*O*-glycosidic linkage. Two mechanistic models have been proposed for this enzyme. In a substrate-assisted mechanism, the α-phosphate oxygen abstracts the proton from the acceptor, facilitating nucleophilic attack on the anomeric carbon.[19] Alternatively, a conserved aspartate residue functions as a general base through a chain of ordered water molecules that relay the proton from the acceptor hydroxyl to the enzyme. Structural studies showing water molecules positioned near this residue, together with mutagenesis experiments resulting in reduced catalytic activity, support this model.[20-22]

Building on these mechanistic and structural insights, significant efforts have been directed toward the discovery of small-molecule inhibitors. Some inhibitors have been identified by high-throughput screening (HTS) approaches, including inhibitor **3** (Figure 3.2),[23] which displays micromolar potency, and inhibitor **4**,[24] which is characterized as a reversible hOGT inhibitor. Inhibitor **5**,[19] despite its distinct chemical structure, has been proposed to mimic the uridine moiety of UDP-GlcNAc. In addition, a natural product (inhibitor **6**)[25] was identified as an hOGT inhibitor through structure-based virtual screening.

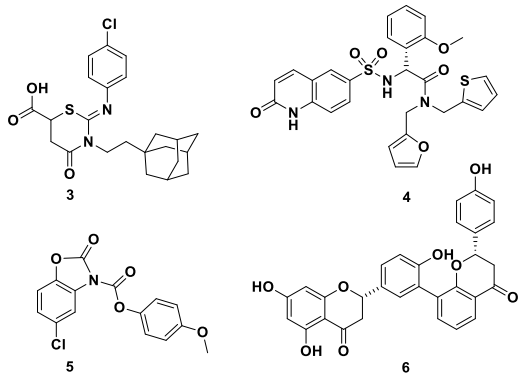


Figure 3.2. Structures of OGT inhibitors discovered through high-throughput screening.

Beyond screening-based discovery, rational design of donor substrate analogues represents a promising approach for developing new OGT inhibitors. As a natural nucleoside-based UDP-GlcNAc mimic, tunicamycin **7** (Figure 3.3) illustrates the feasibility of substrate mimetic inhibition and has stimulated interest in this strategy.[26] For instance, UDP and its analogue P–CH_2–P linked UDP **8** have been identified as competitive inhibitors of hOGT, exhibiting IC_{50} values of 1.8 μM and 9 μM, respectively.[27] In addition, replacement of the glycosidic oxygen in UDP or UDP-GlcNAc with non-hydrolyzable groups such as methylene (inhibitor **9**)[28] or sulfur (inhibitor **10**)[27] has yielded further UDP-GlcNAc analogues. Another donor-based inhibitor is UDP-5SGlcNAc **11**,[18, 29] in which the endocyclic oxygen of the sugar is replaced with sulfur. UDP-5SGlcNAc acts as a competitive hOGT inhibitor *in vitro* (IC_{50} = 11 μM). Structural studies show that its binding mode closely resembles that of the natural substrate, with the UDP moiety forming multiple hydrogen-bonding interactions.[18] UDP-GlcNAc analogue **12**,[30] contains an electrophilic substituent on the *N*-acyl group of the thiosugar and acts as an irreversible hOGT inhibitor by

covalently targeting the active site. Such rationally designed analogues not only deepen understanding of the OGT catalytic mechanism but also provide valuable structural insights into enzyme–substrate recognition, serving as a framework for developing selective and mechanistically informed inhibitors.

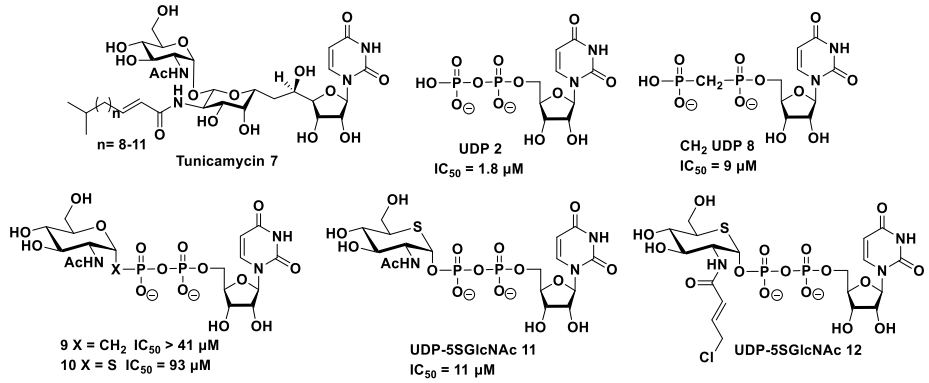


Figure 3.3. Substrate analogue inhibitors of OGT.

Carbasugars are carbocyclic analogues of carbohydrates in which the endocyclic oxygen atom of the sugar ring is replaced by a methylene group, and they have been extensively used in synthetic, conformational, and biological studies.[31-33] Their inhibitory activities towards carbohydrate-processing enzymes, such as α-amylase,[34-35] α-arabinofuranosidases,[36] α-glucosidases,[37-39] and galactosyltransferase,[40] have been well explored. Furthermore, the development of pyrophosphate mimetics represents an effective approach for probing enzymatic mechanisms and catalysis. Inspired by these carbasugar and pyrophosphate mimetics, this chapter describes the synthesis of a new class of UDP-GlcNAc mimetics. UDP-carbaGlcNAc **13** (Figure 3.4) was designed, in which the GlcNAc moiety was replaced with non-hydrolyzable carbaGlcNAc, while retaining the essential pyrophosphate and nucleoside motifs required for substrate recognition by OGT, or other related GlcNAc transferases. In particular, P–CH_2–P linked UDP-carbaGlcNAc analogue **14** was designed in which the glycosidic and pyrophosphate linkages are both non-hydrolyzable, allowing it to serve as a valuable probe for elucidating the catalytic mechanism of OGT, as well as for exploring structure–activity relationships and supporting the further development of more potent inhibitors.

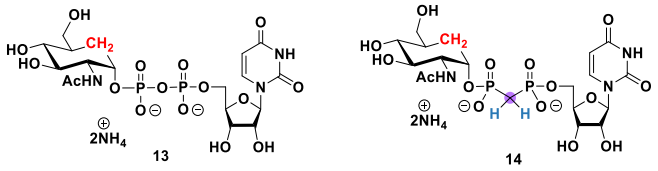
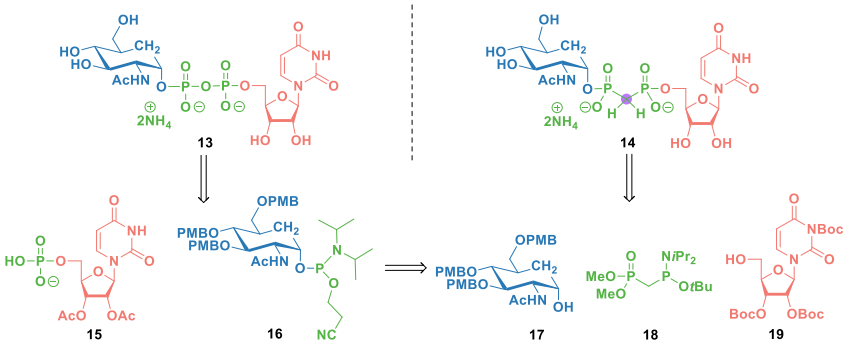


Figure 3.4. Chemical structures of proposed UDP-GlcNAc mimics.

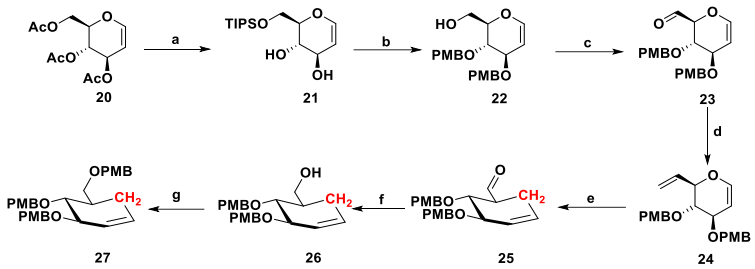
Results and discussion

As depicted in the retrosynthetic analysis (Scheme 3.1), UDP-carbaGlcNAc **13** could be synthesized from uridine-5′-*O*-phosphate **15** and phosphoramidite **16** using P^{III}-P^{V} coupling chemistry. CarbaGlcNAc **17** with PMB protecting groups could be used to prepare phosphoramidite **16** and also serve as the key reagent to synthesize the P–CH_2–P UDP-carbaGlcNAc analogue **14**. By employing the orthogonally protected phosphoramidite-phosphodiester reagent **18** as the key linkage unit,[41] α-carbaGlcNAc **17** and uridine derivative **19** could be coupled in a stepwise manner to furnish the protected P–CH_2–P linked UDP-carbaGlcNAc. Acid-labile *para*-methoxybenzyl (PMB) and Boc protecting groups were employed on the building blocks to enable the global deprotection in one acidic operation at the end of the synthesis.



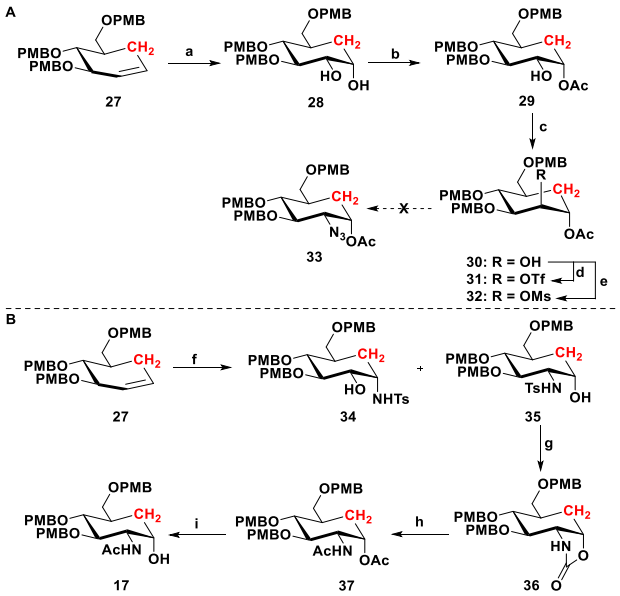
Scheme 3.1. Retrosynthesis of UDP-GlcNAc mimics.

The synthesis of the UDP-GlcNAc analogues was initiated from commercially available tri-*O*-acetyl-D-glucal **20** (Scheme 3.2).[31] Following a previously described procedure, deprotection of the acetyl groups using K_2CO_3 in methanol, followed by treatment with TIPSCl and imidazole afforded compound **21**, in which the 6-OH was protected as TIPS ether. Alkylation with PMBCl and NaH in DMF, and subsequent removal of the TIPS group using TBAF, gave the corresponding 3,4-di-*O*-(*p*-methoxybenzyl)-glucal **22** in 68% yield over two steps. The primary hydroxyl of compound **22** was oxidized to aldehyde **23** by treatment with Dess-Martin periodinane. Aldehyde **23** was transformed into alkene **24** under Wittig conditions in good yield. Thermal Claisen rearrangement of alkene **24** at 220 °C cleanly afforded aldehyde **25**,[42] which was immediately reduced with $NaBH_4$ to give compound **26** in 93% yield. Subsequent protection of 6-OH as PMB ether under standard Williamson etherification conditions afforded cyclohexene **27** in 88% yield.



Scheme 3.2. Synthesis of cyclohexene **27**. Reagents and conditions: a) (i) MeOH, K_2CO_3, 40 °C, 3 h; (ii) TIPSCl, imidazole, DMF, rt, 78% over two steps. b) (i) PMBCl, NaH, DMF, 0 °C to rt; (ii) TBAF, THF, rt, 68% over two steps. c) DMP, $NaHCO_3$, CH_2Cl_2, 0 °C. d) Ph_3PCH_3Br, BuLi, THF, -78 °C to rt, 65% over two steps. e) diphenylether, 210 °C. f) $NaBH_4$, THF/EtOH (2:1, v/v), 93% over two steps. g) PMBCl, NaH, DMF, 0 °C to rt, 88%.

The first strategy that was explored to install the C2 acetamide and C1 alcohol entailed Upjohn dihydroxylation of **27** with a catalytic amount of OsO_4 and an excess of NMO, which gave carba-α-D-glucose derivative **28** as the sole product in excellent yield (Scheme 3.3A). Regioselective acetylation of diol **28** provided alcohol **29** in 91% yield. The remaining 2-hydroxy group could be inverted by treatment with Tf_2O in pyridine at -10 °C, followed by addition of sodium nitrite and subsequent hydrolysis, to furnish the carba-α-D-mannose derivative **30** in 71% yield over 2 steps. With compound **30** in hand, a synthetic route toward a 2-azido carbaglucose derivative **33** was envisaged via an S_N2 displacement. The resulting azide would then be reduced to the corresponding amine through a Staudinger reaction, followed by acetyl protection and subsequent deacetylation at the anomeric position with sodium methoxide to give α-carbaGlcNAc **17**. Similar transformations have been reported in the literature for mannose derivatives, demonstrating the feasibility of this approach,[43] however, attempts to apply this strategy to the system at hand were unsuccessful. A Mitsunobu reaction was attempted to convert the 2-hydroxy group into an azide, but no desired product was obtained. The transformation of the hydroxyl group into triflate **31** was next investigated, anticipating that the highly reactive triflate could undergo substitution with sodium azide. Unfortunately, the triflate decomposed rapidly by hydrolysis under the reaction conditions, and only trace amounts of product were detected by TLC-MS. The more stable mesylate derivative **32** was also tested, but even when heated to reflux, it did not undergo the substitution reaction. This lack of reactivity was most likely due to steric hindrance preventing backside attack by the azide nucleophile. Based on these findings and literature precedents,[44] this route was abandoned and an alternative route was pursued (Scheme 3.3B).

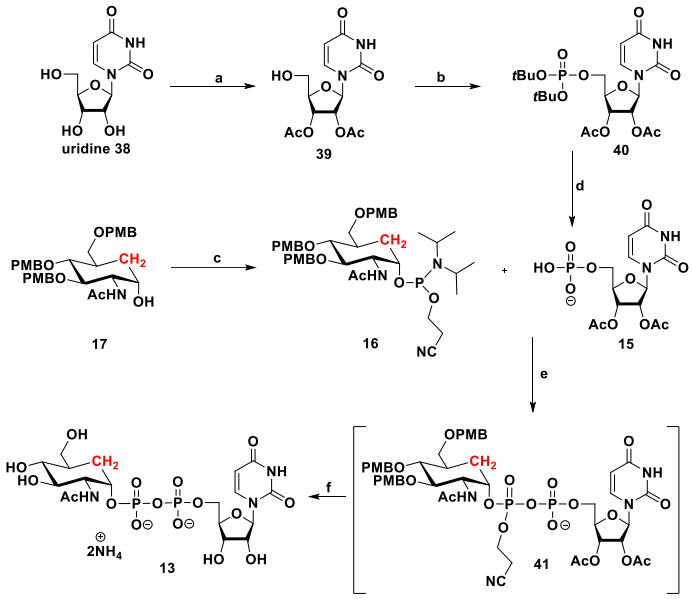


Scheme 3.3. Synthesis of α-carbaGlcNAc **17**. Reagents and conditions: a) OsO_4, Me_3NO, acetone/H_2O (4:1, v/v), rt, 93%. b) (i) $(MeO)_3CMe$, CH_3CN, PTSA, rt, 15 min; (ii) 80% HOAc, rt, 15 min, 91% over two steps. c) (i) Tf_2O, pyridine, CH_2Cl_2, -10 °C; (ii) $NaNO_2$, DMF, H_2O, 71% over two steps. d) Tf_2O, pyridine, CH_2Cl_2, -10 °C. e) MsCl, Me-imidazole, Et_3N, $CHCl_3$, rt, 85%. f) Chloramine-T, benzyl triethylammonium chloride, OsO_4 (5 mol %), $CHCl_3/H_2O$ (1:1, v/v), **34**, 46%, **35**, 30%. g) (i) triphosgene, pyridine, CH_2Cl_2, rt; (ii) Na, naphthalene, THF, -78 °C, 71% over two steps. h) (i) LiOH, MeOH/H_2O (4:6 v/v); (ii) pyridine, Ac_2O. i) MeONa, MeOH, rt, 89% over 3 steps.

Implementation of the modified Sharpless aminohydroxylation reaction[45] on alkene **27** stereospecifically afforded a mixture of **34** and **35**, with the desired alcohol **35** as a key intermediate for the subsequent synthetic sequence. *N*-tosylated *cis*-amino alcohol **35** could be transformed into the corresponding cyclic carbamate by treatment with triphosgene. Subsequent reductive detosylation using a sodium naphthalenide solution, provided cyclic carbamate **36** in 71% yield over two steps. Removal of the cyclic carbamate using LiOH/MeOH, followed by acetylation with acetic anhydride in pyridine, and final deacetylation of compound **37** with sodium methoxide in methanol, provided the carba-GlcNAc **17** in 89% yield over 3 steps.

With carbaGlcNAc **17** in hand, attempts were made to synthesize the UDP-carbaGlcNAc **13** (Scheme 3.4). For this purpose, protected diacetyl uridine **39** was prepared from commercially available uridine **38** via its trityl derivative. The 5′-position of compound **39** was phosphorylated to afford compound **40** in 85% yield, which was subsequently converted into the 5′-phosphate **15**. The target UDP-carbaGlcNAc analogue **13** was then synthesized via a P^{III}-P^{V} pyrophosphate coupling, employing the phosphoramidite of α-carbaGlcNAc **16** as the P^{III} component. Installation of the phosphoramidite on α-carbaGlcNAc **17** furnished the desired P^{III} building block **16** in 48% yield. Coupling of this phosphoramidite with phosphate **15**, and subsequent oxidation

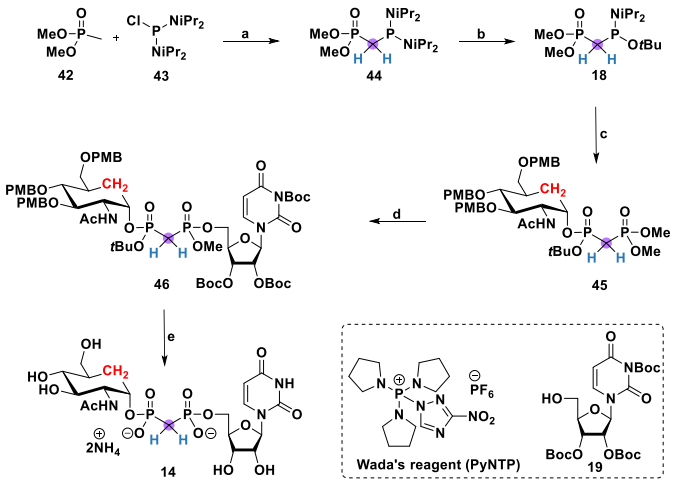
of the intermediate phosphite moiety gave protected UDP-carbaGlcNAc **41**. Removal of the cyanoethyl group using an excess of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), followed by cleavage of the PMB groups using TFA/TES, and subsequent treatment with aqueous NH_4OH to remove the acetyl groups afforded the crude UDP-CarbaGlcNAc. Purification by size-exclusion chromatography and HPLC yielded the desired UDP-CarbaGlcNAc **13** in 27% yield over five steps.



Scheme 3.4. Synthesis of UDP-carbaGlcNAc **13**. Reagents and conditions: a) Triphenylmethyl chloride, DCE, pyridine, 50°C, overnight; (ii) acetic anhydride, overnight; (iii) aqueous acetic acid (80%), reflux, 93% over 3 steps b) 1-methylimidazole hydrochloride, 1-methylimidazole, di-tert-butyl diisopropylphosphoramidite, DMF, 85%. c) 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DMF, DIPEA, rt, 48%. d) TFA, CH_2Cl_2, 0 °C. e) (i) DCI, MeCN, rt, 30 min; (ii) *t*BuOOH, 30 min. f) (i) DBU; (ii) TFA, CH_2Cl_2, 0 °C to rt, 5 h; (iii) NH_4OH, overnight, 27% over 5 steps.

After successfully obtaining UDP-CarbaGlcNAc **13**, attention was turned to the assembly of P-CH_2-P UDP-CarbaGlcNAc **14** (Scheme 3.5). To incorporate the methylene bisphosphonate into the UDP-GlcNAc analogue, mixed phosphoramidite–phosphodiester reagent **18** was used, which was synthesized in two steps from methylphosphonate **42** and chlorophosphoramidite **43** following a procedure previously developed by Engelsma *et al*.[41] Compound **42** was first treated with 1.0 equiv of LDA, and then reacted with compound **43** to afford intermediate **44** in 72% yield. Subsequent activation of compound **44** with 4,5-dicyanoimidazole (DCI, 0.6 equiv), followed by reaction with *tert*-butanol, afforded reagent **18** in 76% yield. Condensation of α-carba-GlcNAc **17** with phosphoramidite **18** was accomplished using DCI as an activator. Subsequent in situ oxidation of the phosphonite-phosphonate intermediate with *t*BuOOH yielded the methylene bisphosphonate derivative **45** in 59% yield over two steps. Next, removal of one

of the methyl groups was achieved using thiophenol in a mixture of MeCN/Et_3N, and this was followed by a condensation reaction with partially protected uridine **19** using Wada's reagent to give compound **46** in 28% yield over two steps. Global deprotection of **46** was achieved by removal of the methyl esters using thiophenol in MeCN/Et_3N, followed by cleavage of all the *tert*-butyl, PMB and Boc groups using TFA/TES to deliver P-CH_2-P linked UDP-carbaGlcNAc **14** in 46% yield over two steps.



Scheme 3.5. Synthesis of P-CH_2-P linked UDP-carbaGlcNAc **14**. Reagents and conditions: a) *n*-BuLi, diisopropylamine, THF, 72%. b) DCI, *t*BuOH, CH_2Cl_2, rt, 76%. c) (i) **17**, DCI, MeCN; (ii) *t*BuOOH, 59% over 2 steps. d) (i) MeCN/Et_3N/PhSH (3:3:2), 35 °C; (ii) **19**, PyNTP, DIPEA, MeCN, rt, 28% over 2 steps. e) (i) MeCN/Et_3N/PhSH (3:3:2), 35 °C; (ii) TFA, TES, CH_2Cl_2, 0 °C, overnight, 46% over two steps.

Conclusion

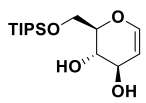
In conclusion, this Chapter has described the synthesis of two UDP-GlcNAc mimetics. The key α-carba-GlcNAc **17** was prepared for the construction of UDP-GlcNAc analogues. The UDP-carbaGlcNAc **13** was assembled using a phosphoramidite-phosphate coupling strategy. The P–CH_2–P linked UDP-carbaGlcNAc **14** was obtained by using a new orthogonally protected phosphonylmethylphosphonate reagent **18**. These UDP-carbaGlcNAc derivatives can serve as stable structural mimics and as chemical tools for mechanistic studies and future inhibitor development targeting OGT. Furthermore, the P–CH_2–P linked analogue **14** may also serve as probes or potential inhibitors for enzymes that transfer GlcNAc-phosphate involved in the biosynthesis of capsular polysaccharides in bacterial pathogens,[46-48] thereby extending their applicability.

Experimental section

General information

All chemicals were commercial grade and used as received and all moisture sensitive reactions were performed under an argon or nitrogen atmosphere, at ambient temperature (21°C), unless stated otherwise. For TLC analysis were used aluminium sheets (Merck, TLC silica gel 60 F_{254}), sprayed with a solution of H_2SO_4 (20%) in EtOH or with a solution of $(NH_4)_6Mo_7O_{24}$•$4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4$•$2H_2O$ (10g/L) in 10% aqueous H_2SO_4 or with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in H_2O and then heated at ~150°C. For column chromatography was used 60Å silica gel (40-63 μm, SD Screening Devices). NMR spectra (^1H, ^{13}C, ^{19}F and ^{31}P) were recorded with a Bruker AV-400liq or Bruker AV-500. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard (^1H NMR in $CDCl_3$) or the residual signal of the deuterated solvent. Chemical shifts for ^{31}P spectra are indirectly referenced to H_3PO_4 (0.00 ppm) according to the IUPAC method. ^{31}P NMR spectra measured to monitor reactions were made by charging an NMR tube with an aliquot of the reaction mixture and fitting the tube with an acetone-d_6 capillary. Coupling constants (*J*) are given in Hz. All ^{13}C NMR and ^{31}P NMR spectra are proton decoupled. NMR peak assignments were made using COSY and HSQC experiments. LC/MS analysis was performed on a Surveyor HPLC system (Thermo Finnigan) equipped with a C18 column (Gemini, 4.6 mm x 50 mm, 5 μm particle size, Phenomenex), coupled to a LCQ Adventage Max (Thermo Finnigan) ion-trap spectrometer (ESI^+). Solvent system: A: Milli-Q H_2O, B: MeCN, C: 1% TFA (aq). High resolution mass spectra were recorded by direct injection on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive ion mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275°C) with resolution R = 60000 at m/z 400 (mass range m/z = 150 - 4000) and dioctyl phthalate (m/z = 391.28428) as a lock mass. Size-exclusion chromatography was performed on Sephadex™ LH-20 gel using CH_2Cl_2/MeOH (1:1, v/v) as the eluent, or on a TOYOPEARL HW-40-S resin column (16 × 600 mm) using an aqueous NH_4OAc buffer (0.15 M) containing 10% MeCN at a flow rate of 1 mL/min. Semi-preparative high-performance liquid chromatography (semiprep-HPLC) was performed on a Gilson system (322-H2 Pump, GX-281 Liquid Handler, UV/VIS-156 detector) equipped with a C30 column (Fusion C30, 5 μm, 250 × 10 mm, NOMURA (via Phenomenex) using Solvent A: 50 mM $HNEt_3OAc$ in Milli-Q® H_2O; solvent B: acetonitrile; linear gradient of B in A (0 → 20% A/B), 5 mL/min.

Glucal 21



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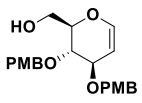
A solution of 3,4,6-Tri-*O*-acetyl-D-glucal **20** (55 g, 200 mmol) in MeOH (200 mL, 1.0 M) was added K_2CO_3 (16.6 g, 120 mmol, 0.3 eq) and the reaction mixture was stirred for 3 h at 40 °C. The reaction mixture was concentrated, co-evaporated twice using DMF, and then dissolved in DMF (400 mL, 0.5 M). Imidazole (40.8 g, 600 mmol, 3.0 eq) and Triisopropylsilyl chloride (28 mL, 260 mmol, 1.3 eq) were added at 0 °C and the solution was stirred overnight at room temperature. The reaction was quenched with MeOH (5 mL) and subsequently concentrated. The residue was extracted with EtOAc (3x) and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography (R_f = 0.25, EtOAc/pentane 1:1, v/v) yielded compound **21** (48 g, 159 mmol, 78% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.30 (dd, *J* = 6.1, 1.7 Hz, 1H, H-1), 4.73 (dd, *J* = 6.1, 2.3 Hz, 1H, H-2), 4.31 – 4.25 (m, 1H, H-3), 4.12 – 4.05 (m, 1H, H-6), 4.01 – 3.95 (m, 1H, H-6), 3.87 – 3.79 (m, 2H, H-4, H-5), 3.42 (d, *J* = 1.9 Hz, 1H, 4-OH), 2.46 (d, *J* = 5.3 Hz, 1H, 3-OH), 1.19 – 1.04 (m, 24H, CH(C*H*$_3$)$_2$ TIPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.0 (C-1), 102.5 (C-2), 76.1 (C-5), 73.1 (C-4), 69.3 (C-3), 64.8 (C-6), 17.9, 17.9 (CH(*C*H$_3$)$_2$ TIPS), 11.8 (*C*H(CH$_3$)$_2$ TIPS). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{15}H_{30}O_4SiNa$ 325.1811, found 325.1806.

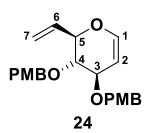
Glucal 22



22

Compound **21** (45 g, 149 mmol) was dissolved in dry DMF (250 mL, 0.6 M) and cooled on ice, NaH (60% dispersion in mineral oil, 21 g, 522 mmol, 3.5 eq) was added and stirred for 30 min. Next, *p*-methoxybenzylchloride (61 mL, 462 mmol, 3.0 eq) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction was quenched with water (25 mL) on ice. The crude product was extracted with diethyl ether, and the organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was redissolved in THF (250 mL) and treated with 1.0 M tetrabutylammonium fluoride (TBAF) in THF (156 mL, 156 mmol, 1.05 eq). The reaction mixture was stirred at room temperature for 2 h. The product was concentrated and extracted with diethyl ether, the organic layer was washed with brine and dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography (R_f = 0.2, EtOAc/pentane 3:7, v/v) afforded compound **22** as a colorless oil (39 g, 101 mmol, 68% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.16 (m, 4H, CH_{arom}), 6.93 – 6.82 (m, 4H, CH_{arom}), 6.39 (dd, *J* = 6.1, 1.4 Hz, 1H, H-1), 4.87 (dd, *J* = 6.1, 2.7 Hz, 1H, H-2), 4.78 (d, *J* = 11.1 Hz, 1H, C*H*H PMB), 4.68 – 4.58 (m, 2H, CH*H* PMB, C*H*H PMB), 4.50 (d, *J* = 11.2 Hz, 1H, C*H*H PMB), 4.23 – 4.15 (m, 1H, H-3), 3.96 – 3.87 (m, 1H, H-5), 3.83 (dd, *J* = 6.7, 4.2 Hz, 2H, H-6), 3.81 (d, *J* = 2.2 Hz, 6H, OMe), 3.78 – 3.73 (m, 1H, H-4), 2.00 (t, *J* = 6.7 Hz, 1H, 6-OH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.5, 159.4 ($C_{q\text{-}arom}$), 144.6 (C-1), 130.3, 130.2 ($C_{q\text{-}arom}$), 129.8, 129.5 (CH_{arom}), 114.0, 114.0 (CH_{arom}), 100.3 (C-2), 77.3 (C-5), 75.3 (C-3), 74.3 (C-4), 73.5, 70.4 (CH_2 PMB), 62.0 (C-6), 55.4 (OMe PMB). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{22}H_{26}O_6Na$ 409.1627, found 409.1621.

Glucal 24

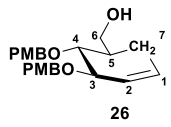


24

Compound **22** (21 g, 54.3 mmol) was co-evaporated with toluene and dissolved in dry DCM (270 mL, 0.2 M). $NaHCO_3$ (68 g, 815 mmol, 15 eq) was added and the mixture was cooled on ice. Dess-Martin periodinane (46 g, 109 mmol, 2.0 eq) was added, the reaction mixture was allowed to warm to room temperature and stirred for 2 hours. The reaction was quenched with sat. aq. $NaHCO_3$ and sat. aq. $Na_2S_2O_3$. The mixture was filtered over Celite. The mixture was extracted with DCM, the organic layer was washed with water and brine, dried over $MgSO_4$, filtered over Celite, and concentrated *in vacuo* to obtain the crude aldehyde as a yellow oil.

A solution of 2.5 M *n*-BuLi in hexane (30.4 mL, 76 mmol, 1.4 eq) was added dropwise to a solution of Ph_3PMeBr (29.1 g, 81.4 mmol, 1.5 eq) in anhydrous THF (140 mL) at −78 °C, and the mixture was stirred at the same temperature for 30 min and at 0 °C for 1 h. After cooling at –78 °C, a solution of the crude aldehyde (co-evaporated with toluene thrice before using) in anhydrous THF (100 mL) was added dropwise, and the reaction mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was quenched with sat. aq. $NaHCO_3$ and filtered over Celite. The mixture was extracted with EtOAc and the organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography (R_f = 0.2, Et_2O/pentane 1:1, v/v) afforded compound **24** (13.5 g, 35.3 mmol, 65% over 2 steps) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.20 (m, 4H, CH_{arom}), 6.91 – 6.82 (m, 4H, CH_{arom}), 6.40 (dd, *J* = 6.2, 1.4 Hz, 1H, H-1), 6.10 – 5.97 (m, 1H, H-7), 5.47 – 5.37 (m, 1H, H-6), 5.34 – 5.26 (m, 1H, H-6), 4.86 (dd, *J* = 6.2, 2.7 Hz, 1H, H-2), 4.70 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.61 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.57 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.52 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.34 – 4.26 (m, 1H, H-5), 4.20 – 4.13 (m, 1H, H-3), 3.56 (dd, *J* = 8.5, 6.2 Hz, 1H, H-4). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.4, 159.3 ($C_{q\text{-}arom}$), 144.5 (C-1), 134.5 (C-7), 130.6, 130.3 ($C_{q\text{-}arom}$), 129.8, 129.5 (CH_{arom}), 118.4 (C-6), 113.9, 113.9 (CH_{arom}), 100.6 (C-2), 78.2 (C-5), 78.0 (C-4), 75.2 (C-3), 73.6, 70.5 (CH_2 PMB), 55.4 (OMe PMB). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{23}H_{26}O_5Na$ 405.1678, found 405.1672.

Carba-glucal 26

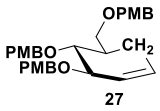


26

Compound **24** (2.9 g, 7.4 mmol) was co-evaporated with toluene three times and dissolved in anhydrous diphenyl ether (11 mL, 0.25 M). The mixture was heated to 210 °C for 3 hours. The reaction was cooled to room temperature and poured into a stirring suspension of $NaBH_4$ (429 mg, 11.1 mmol, 1.5 eq) in a mixture of THF/EtOH (2:1 v/v, 72 mL, 0.1 M). The reaction was stirred at room temperature for 30 minutes. The reaction was quenched with sat. aq. $NaHCO_3$. The mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude compound was purified by flash chromatography (R_f = 0.2, pentane/EtOAc 3:7, v/v) to yield compound **26** as a yellow oil (2.6 g, 6.9 mmol, 93% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.29 (m, 2H, CH_{arom}), 7.28 – 7.27 (m, 1H, CH_{arom}), 7.26 – 7.24 (m, 1H, CH_{arom}), 6.92 – 6.85 (m, 4H, CH_{arom}), 5.77 – 5.71 (m, 1H, H-1), 5.71 – 5.65

(m, 1H, H-2), 4.91 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.70 – 4.63 (m, 2H, CH*H* PMB, C*H*H PMB), 4.58 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.22 – 4.17 (m, 1H, H-3), 3.81 (s, 3H, OMe PMB), 3.80 (s, 3H, OMe PMB), 3.66 – 3.50 (m, 3H, H-4, H-6), 2.71 (bs, 1H, 6-OH), 2.15 – 2.05 (m, 1H, H-7), 2.05 – 1.94 (m, 1H, H-5), 1.85 (m, 1H, H-7). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.4, 159.3, 130.5, 130.4 ($C_{q\text{-}arom}$), 129.9, 129.5 (CH_{arom}), 128.1 (C-1), 126.1 (C-2), 114.0, 113.9 (CH_{arom}), 82.0 (C-4), 81.0 (C-3), 73.9, 71.0 (CH_2 PMB), 65.9 (C-6), 55.3 (OMe PMB), 40.6 (C-5), 28.1 (C-7). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{23}H_{28}O_5Na$ 407.1834, found 407.1829.

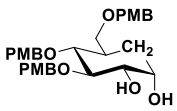
Carba-glucal 27



27

Compound **26** (11 g, 28.6 mmol) was dissolved in DMF (143 mL, 0.2 M) and cooled on ice. PMBCl (5.7 mL, 42.9 mmol, 1.5 eq) and NaH (60% dispersion in mineral oil, 2.3 g, 57.2 mmol, 2.0 eq) were added. The reaction mixture was slowly warmed to room temperature and stirred for 3 hours. After full conversion, the reaction was cooled back to 0 °C and quenched with sat. aq. $NaHCO_3$ and diluted with water. The mixture was extracted with EtOAc thrice. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography (R_f =0.2, pentane/EtOAc 1:9, v/v) to yield compound **27** as a yellow oil (12.7 g, 25.2 mmol, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 – 7.27 (m, 2H, CH_{arom}), 7.26 – 7.21 (m, 2H, CH_{arom}), 7.21 – 7.17 (m, 2H, CH_{arom}), 6.88 – 6.81 (m, 6H, CH_{arom}), 5.77 – 5.70 (m, 1H, H-1), 5.67 – 5.61 (m, 1H, H-2), 4.79 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.65 – 4.56 (m, 2H, CH*H* PMB, C*H*H PMB), 4.53 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.42 (s, 2H, CH_2 PMB), 4.16 – 4.10 (m, 1H, H-3), 3.79 (s, *J* = 0.7 Hz, 9H, OMe), 3.65 – 3.57 (m, 2H, H-4, H-6), 3.54 (dd, *J* = 9.0, 3.3 Hz, 1H, H-6), 2.27 – 2.15 (m, 2H, H-5, H-7), 2.08 – 2.00 (m, 1H, H-7). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.1, 159.1, 131.2, 130.9, 130.8 ($C_{q\text{-}arom}$), 129.6, 129.4, 129.1 (CH_{arom}), 128.4 (C-1), 126.2 (C-2), 113.8, 113.7 (CH_{arom}), 80.9 (C-3), 79.3 (C-4), 74.0, 72.8, 71.2 (CH_2 PMB), 70.2 (C-6), 55.3, 55.3 (OMe), 39.4 (C-5), 28.9 (C-7). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{31}H_{36}O_6Na$ 527.2410, found 527.2404.

Carba-glucose 1,2-diol 28

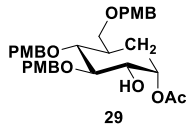


28

Compound **27** (3.0 g, 6 mmol) was dissolved in a mixture of acetone (240 mL) and H_2O (30 mL), then a solution of OsO4 (76.2 mg, 0.3 mmol) was added at room temperature, followed by Me_3NO (900 mg, 12 mmol), and the reaction mixture was stirred for 4 h at room temperature. A sat. aq. $Na_2S_2O_3$ (50 mL) was added and the mixture was stirred for 30 min to reduce OsO_4. The mixture was extracted with $CHCl_3$. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography (R_f =0.2, pentane/EtOAc 1:1, v/v) to yield **28** (3.0 g, 5.6 mmol, 93%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.22 (m, 4H, CH_{arom}), 7.20 – 7.14 (m, 2H, CH_{arom}), 6.91 – 6.81 (m, 6H, CH_{arom}), 4.93 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.76 (d, *J* = 10.4 Hz, 1H, CH*H* PMB), 4.60 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.49 (d, *J* = 10.5 Hz, 1H, CH*H* PMB), 4.45 – 4.35 (m, 2H, C*H*H PMB, CH*H* PMB), 4.07 – 4.00 (m, 1H, H-1), 3.82 (s, 9H, OMe), 3.72

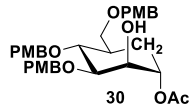
(dd, *J* = 9.1, 4.1 Hz, 1H, H-6), 3.67 (t, *J* = 9.2 Hz, 1H, H-3), 3.50 – 3.41 (m, 2H, H-2, H-4), 3.38 (dd, *J* = 9.0, 2.6 Hz, 1H, H-6), 2.41 (bs, 2H, 2-OH, 1-OH), 2.20 – 2.09 (m, 1H, H-5), 1.88 (m, 1H, H-7), 1.62 (m, 1H, H-7). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.5, 159.3, 159.2, 130.9, 130.9, 130.6 ($C_{q\text{-arom}}$), 129.6, 129.6, 129.4, 114.2, 113.9, 113.8 (CH_{arom}), 83.1 (C-3), 80.9 (C-4), 74.9, 74.6 (CH_2 PMB), 74.4 (C-2), 72.8 (CH_2 PMB), 69.4 (C-6), 68.3 (C-1), 55.4, 55.3 (OMe), 37.5 (C-5), 30.5 (C-7). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{31}H_{38}O_8Na$ 561.2464, found 561.2459.

Carba-glucose 29



Compound **28** (3.2 g, 6 mmol) was dissolved in dry MeCN (60 mL) under nitrogen. Trimethyl orthoacetate (1.44 g, 12 mmol) was added at room temperature, followed by a catalytic amount of *p*-toluenesulfonic acid (15 mg, 0.06 mmol). After 15 min, an 80% aqueous solution of acetic acid (60 mL) was added and stirring was continued for 15 min. The reaction mixture was diluted with DCM and the organic layer was washed with H_2O and a sat. aq. $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (R_f = 0.25, pentane/EtOAc 1:1, v/v) to yield **29** (3.2 g, 5.5 mmol, 91%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.29 (m, 2H, CH_{arom}), 7.27 – 7.22 (m, 2H, CH_{arom}), 7.20 – 7.15 (m, 2H, CH_{arom}), 6.93 – 6.85 (m, 6H, CH_{arom}), 5.29 – 5.25 (m, 1H, H-1), 4.92 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.81 (d, *J* = 10.3 Hz, 1H, CH*H* PMB), 4.72 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.49 (d, *J* = 10.3 Hz, 1H, CH*H* PMB), 4.46 – 4.38 (m, 2H, C*H*H PMB, CH*H* PMB), 3.85 – 3.80 (m, 9H, OMe), 3.73 (t, *J* = 9.2 Hz, 1H, H-3), 3.68 (dd, *J* = 9.0, 4.3 Hz, 1H, H-6), 3.64 – 3.59 (m, 1H, H-2), 3.56 – 3.49 (m, 1H, H-4), 3.39 (dd, *J* = 9.0, 2.7 Hz, 1H, H-6), 2.41 (d, *J* = 3.5 Hz, 1H, 2-OH), 2.08 (s, 3H, OAc), 2.05 – 1.98 (m, 1H, H-5), 1.92 – 1.84 (m, 1H, H-7), 1.67 – 1.56 (m, 1H, H-7). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7 (C=O OAc), 159.3, 159.3, 159.2, 130.8, 130.6, 130.4 ($C_{q\text{-arom}}$), 129.6, 129.5, 129.3, 114.0, 113.9, 113.8, 113.7 (CH_{arom}), 83.7 (C-3), 80.3 (C-4), 75.1, 74.8 (CH_2 PMB), 73.2 (C-2), 72.8 (CH_2 PMB), 71.3 (C-1), 69.0 (C-6), 55.3, 55.3 (OMe), 38.0 (C-5), 28.9 (C-7). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{31}H_{38}O_8Na$ 561.2464, found 561.2459

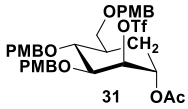
Carba-mannose 30



Compound **29** (260 mg, 0.45 mmol) was co-evaporated 3 times with toluene and dissolved in DCM (1 mL). Pyridine (715 µL, 9.0 mmol) was added and the reaction mixture was cooled to -10 °C. trifluoromethanesulfonic anhydride (376 µL, 2.2 mmol, 5 eq) was added dropwise and the reaction was stirred for 3 h. After the starting material was fully converted, the reaction was quenched with sat. aq. $NaHCO_3$ and extracted with DCM. The organic layer was washed with sat. aq. $CuSO_4$ three times and brine, dried over anhydrous Na_2SO_4, filtered and concentrated below 20 °C. The residue was used directly in the next step without further purification. The crude triflate was dissolved in dry DMF (1 mL), $NaNO_2$ (152 mg, 2.2 mmol) was added, and the reaction mixture was stirred at 50 °C for 5 h. The mixture was diluted with DCM and washed with brine. The organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. The crude residue was purified by flash

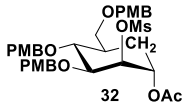
chromatography (R_f= 0.25, pentane/EtOAc 1:1, v/v) to afford compound **30** (185 mg, 0.32 mmol, 71% over 2 steps) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.20 (m, 4H, CH_{arom}), 7.18 – 7.12 (m, 2H, CH_{arom}), 6.89 – 6.82 (m, 6H, CH_{arom}), 5.12 – 5.04 (m, 1H, H-1), 4.73 (d, *J* = 10.5 Hz, 1H, C*H*H PMB), 4.66 – 4.55 (m, 2H, C*H*H PMB, CH*H* PMB), 4.46 – 4.37 (m, 3H, C*H*H PMB, CH*H* PMB, C*H*H PMB), 4.00 – 3.94 (m, 1H, H-2), 3.82 – 3.76 (m, 9H, OMe PMB, OMe PMB, OMe PMB), 3.72 – 3.65 (m, 2H, H-3, H-4), 3.56 (dd, *J* = 9.0, 4.4 Hz, 1H, H-6), 3.44 (dd, *J* = 9.1, 2.5 Hz, 1H, H-6), 2.58 (bs, 1H, 2-OH), 2.04 – 1.93 (m, 5H, H-5, H-7, OAc), 1.85 – 1.73 (m, 1H, H-7). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.1, 159.5, 159.3, 159.2, 130.9, 130.7, 130.2 ($C_{q\text{-}arom}$), 129.7, 129.6, 129.3, 114.0, 113.9, 113.8 (CH_{arom}), 81.7 (C-4), 76.8 (C-3), 74.7, 72.8, 72.4 (CH_2 PMB), 71.1 (C-1), 69.8 (C-6), 68.9 (C-2), 55.4, 55.3 (OMe PMB), 37.9 (C-5), 27.3 (C-7), 21.3 (OAc). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{31}H_{38}O_8Na$ 561.2464, found 561.2462.

Triflate 31



Compound **30** (120 mg, 0.21 mmol) was co-evaporated three times with toluene and dissolved in DCM (1 mL). Pyridine (334 µL, 4.2 mmol) was added and the reaction mixture was cooled to -10 °C. Trifluoromethanesulfonic anhydride (202 µL, 1.2 mmol, 5.5 eq) was added dropwise and the reaction was stirred for 4 h. After the starting material was fully converted, the reaction was quenched with sat. aq. $NaHCO_3$ and extracted with DCM. The organic layer was washed with sat. aq. $CuSO_4$ three times and brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* at temperatures below 20 °C. The resulting crude **31** was evaporated three times with toluene at temperatures below 20 °C before use.

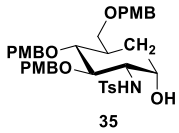
Mesylate 32



Compound **30** (527 mg, 0.9 mmol) was dissolved in $CHCl_3$ (3 mL, 0.3 M), and Et_3N (628 µL, 4.5 mmol, 5 eq), Me-imidazole (717 µL, 9 mmol, 10 eq) and methanesulfonyl chloride (348 µL, 4.5 mmol, 5 eq) were added to the solution at 0 °C. The reaction mixture was stirred overnight at room temperature. Upon full conversion of the starting material, the reaction mixture was diluted with water. The mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography (R_f = 0.2, pentane/EtOAc 6:1, v/v) yielded compound **32** (504 mg, 0.76 mmol, 85%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.27 (m, 2H, CH_{arom}), 7.25 – 7.21 (m, 2H, CH_{arom}), 7.16 – 7.11 (m, 2H, CH_{arom}), 6.89 – 6.82 (m, 6H, CH_{arom}), 5.19 – 5.12 (m, 1H, H-1), 4.96 (ddd, *J* = 4.4, 3.0, 1.1 Hz, 1H, H-2), 4.73 (d, *J* = 10.5 Hz, 1H, C*H*H PMB), 4.69 – 4.57 (m, 2H, CH*H* PMB, C*H*H PMB), 4.45 – 4.38 (m, 3H, CH*H* PMB, C*H*H PMB, CH*H* PMB), 3.82 – 3.79 (m, 9H, OMe), 3.78 – 3.75 (m, 1H, H-3), 3.65 (t, *J* = 9.0 Hz, 1H, H-4), 3.57 (dd, *J* = 9.0, 4.5 Hz, 1H, H-6), 3.42 (dd, *J* = 9.0, 2.5 Hz, 1H, H-6), 3.03 (s, 3H, Me), 2.01 (s, 3H, OAc), 2.00 – 1.95 (m, 2H, H-5, H-7), 1.89 – 1.84 (m, 1H, H-7). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.4 (C=O OAc), 159.6, 159.4, 159.3, 130.6, 130.5 ($C_{q\text{-}arom}$), 130.1, 129.8, 129.5, 114.1, 114.0, 113.9 (CH_{arom}), 79.3 (C-3), 78.3 (C-2), 76.7 (C-4), 74.7, 73.2, 72.9 (CH_2 PMB), 69.7 (C-1), 69.4 (C-6), 55.4, 55.4 (OMe), 38.9

(Me), 38.2 (C-5), 27.6 (C-7), 21.1 (OAc). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{34}H_{42}O_{11}SNa$ 681.2340, found 681.2345.

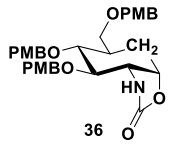
Carba-glucosamine 35



35

Compound **27** (6.5 g, 12.8 mmol) was dissolved in a mixture of $CHCl_3/H_2O$ (1:1 v/v; 256 mL, 0.05 M). Chloramine-T trihydrate (7.2 g, 25.6 mmol, 2.0 eq), benzyltriethylammonium chloride (174 mg, 0.75 mmol, 6 mol%) and potassium osmate (235 mg, 0.64 mmol, 5 mol%) were added. The biphasic reaction mixture was heated to 60 °C and stirred vigorously overnight. Upon full conversion of the starting material, the reaction was quenched with sat. aq. $Na_2S_2O_3$. The mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography (R_f = 0.2, EtOAc/pentane 2:8, v/v) yielded **34** (4.1 g, 5.9 mmol, 46%) as a colorless oil, **35** (2.7 g, 3.8 mmol, 30%) as an off-white solid. **34**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 – 7.71 (m, 2H, CH_{arom}), 7.31 – 7.27 (m, 2H, CH_{arom}), 7.25 – 7.14 (m, 6H, CH_{arom}), 6.93 – 6.82 (m, 6H, CH_{arom}), 4.94 (d, *J* = 3.1 Hz, 1H, NH), 4.79 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.65 (d, *J* = 10.7 Hz, 1H, C*H*H PMB), 4.50 (dd, *J* = 12.2, 11.0 Hz, 2H, CH*H* PMB, C*H*H PMB), 4.44 – 4.32 (m, 2H, C*H*H PMB, CH*H* PMB), 3.83 (s, 9H, OMe, OMe, OMe), 3.68 (dd, *J* = 9.2, 4.3 Hz, 1H, H-6), 3.59 – 3.48 (m, 2H, H-3, H-4), 3.48 – 3.38 (m, 2H, H-2, H-1), 3.34 (dd, *J* = 9.2, 3.5 Hz, 1H, H-6), 2.66 (s, 1H, 2-OH), 2.42 (s, 3H, Me Ts), 2.19 – 2.05 (m, 2H, H-5, H-7), 1.53 (m, 1H, H-7). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.5, 159.4, 159.2, 143.6, 136.6, 130.5, 130.4, 130.4 ($C_{q\text{-}arom}$), 129.8, 129.6, 129.5, 129.5, 129.4, 127.5, 114.2, 113.9, 113.8 (CH_{arom}), 81.7 (C-3), 79.3 (C-4), 74.3, 73.9, 72.8 (CH_2 PMB), 71.9 (C-2), 69.2 (C-6), 55.4, 55.4, 55.4 (OMe PMB), 51.8 (C-1), 37.4 (C-5), 27.5 (C-7), 21.6 (Me Ts). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{38}H_{45}NO_9SNa$ 714.2713, found 714.2712. **35**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.62 (m, 2H, CH_{arom}), 7.26 – 7.18 (m, 4H, CH_{arom}), 7.17 – 7.04 (m, 4H, CH_{arom}), 6.91 – 6.79 (m, 6H, CH_{arom}), 4.76 – 4.67 (m, 2H, NH, CH*H* PMB), 4.64 (d, *J* = 10.4 Hz, 1H, CH*H* PMB), 4.50 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.43 – 4.32 (m, 3H, CH*H* PMB, C*H*H PMB, CH*H* PMB), 4.16 – 4.10 (m, 1H, H-1), 3.83 (s, 3H, OMe PMB), 3.79 (s, 6H, OMe, PMB), 3.70 – 3.65 (m, 1H, H-6), 3.57 – 3.51 (m, 1H, H-3), 3.40 (dd, *J* = 10.1, 8.7 Hz, 1H, H-4), 3.34 (dd, *J* = 9.1, 2.9 Hz, 1H, H-6), 2.95 – 2.88 (m, 1H, H-2), 2.42 (s, 3H, Me Ts), 2.21 – 2.16 (m, 1H, 1-OH), 2.14 – 2.03 (m, 1H, H-5), 1.86 – 1.76 (m, 1H, H-7), 1.63 – 1.52 (m, 1H, H-7). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.6, 159.6, 159.3, 143.8, 136.4, 130.6, 130.5, 130.2 ($C_{q\text{-}arom}$), 129.9, 129.7, 129.6, 129.5, 127.4, 114.3, 113.9, 113.9 (CH_{arom}), 81.1 (C-4), 79.7 (C-3), 74.5, 72.9 (CH_2 PMB), 69.4 (C-6), 66.8 (C-1), 59.7 (C-2), 55.5, 55.4, 55.4 (OMe), 37.2 (C-5), 31.2 (C-7), 21.7 (Me Ts). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{38}H_{45}NO_9SNa$ 714.2713, found 714.2712.

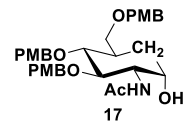
Carbamate 36



36

Compound **35** (100 mg, 0.14 mmol) was co-evaporated with toluene and dissolved in anhydrous DCM (10 mL, 0.1 M) and cooled to 0 °C. Pyridine (52 µL, 0.65 mmol, 4.5 eq) and triphosgene (25.6 mg, 96 µmol, 0.6 eq) were added. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. When full conversion was observed, the reaction was quenched with sat. aq. $NaHCO_3$. The mixture was extracted with EtOAc, the organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to obtain the crude *N*-tosyl protected cyclic carbamate. In an oven-dried round-bottomed flask equipped with a magnetic stirring bar, naphthalene (215 mg, 1.7 mmol, 12 eq) was dissolved in anhydrous THF (3 mL, 0.05 M), and freshly cut sodium (32 mg, 1.4 mmol, 10 eq) was added under a N_2 atmosphere. The reaction mixture was then sonicated for 15 minutes to obtain a dark-green Na/naphthalenide solution. The reaction mixture was cooled to –78 °C, and the crude *N*-Ts-protected cyclic carbamate intermediate was co-evaporated with toluene three times. The residue was then dissolved in anhydrous THF (1 mL) and added dropwise to the pre-cooled Na/naphthalenide solution at –78 °C. The reaction mixture was stirred for 30 minutes. After complete conversion, the reaction was quenched at –78 °C by the dropwise addition of sat. aq. NH_4Cl until the reaction mixture turned completely colorless.The reaction mixture was allowed to warm to room temperature and extracted with EtOAc, the organic layer was washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated in vacuo. Flash column chromatography (R_f = 0.2, DCM:acetone 1:5, v/v) yielded the title compound **36** (58 mg, 0.10 mmol, 71% over two steps) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 – 7.20 (m, 4H, CH_{arom}), 7.20 – 7.16 (m, 2H, CH_{arom}), 6.93 – 6.89 (m, 2H, CH_{arom}), 6.88 – 6.83 (m, 4H, CH_{arom}), 4.86 (d, *J* = 11.5 Hz, 1H, C*H*H PMB), 4.83 (s, 1H, 2-NH), 4.74 – 4.68 (m, 2H, C*H*H PMB, CH*H* PMB), 4.65 (m, 1H, H-1), 4.50 (t, *J* = 10.8 Hz, 2H, C*H*H PMB, CH*H* PMB), 4.39 (d, *J* = 2.7 Hz, 2H, C*H*H PMB, CH*H* PMB), 3.81 (s, 3H, OMe PMB), 3.79 (s, 6H, OMe PMB, OMe PMB), 3.67 (dd, *J* = 9.0, 4.0 Hz, 1H), 3.51 – 3.46 (m, 1H), 3.46 – 3.42 (m, 2H), 3.37 (dd, *J* = 9.1, 2.9 Hz, 1H, H-6), 2.14 (m, 1H, H-7), 1.98 (m, 1H, H-5), 1.90 (m, 1H, H-7). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.7 (C=O), 159.3, 159.2, 158.9, 130.4, 130.3, 130.2 ($C_{q\text{-}arom}$), 129.5, 129.5, 129.3, 114.3, 113.8, 113.8 (CH_{arom}), 84.4 (C-3), 78.7 (C-4), 76.0 (C-1), 74.4, 74.1, 72.8 (CH_2 PMB), 69.4 (C-6), 58.0 (C-2), 55.3, 55.3, 55.2 (OMe PMB), 37.3 (C-5), 28.2 (C-7). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{32}H_{37}NO_8Na$ 586.2411, found 586.2417.

Carba-glucosamine 17

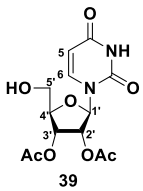


17

Carbamate **36** (2.7 g, 4.8 mmol) was dissolved in a mixture of MeOH/H_2O (4:6, v/v, 90 mL), LiOH (3.3 g, 144 mmol) was added, and the mixture was heated to reflux overnight. Upon full conversion of the starting material, the reaction was cooled to room temperature. The reaction was extracted with EtOAc, the organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was co-evaporated with toluene three times and dissolved in

dry pyridine (10 mL, 0.5 M). Ac_2O (1.7 mL, 19.2 mmol, 4 eq) was added and the reaction mixture was stirred overnight. The mixture was evaporated and the residue was poured into the sat. aq. $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, concentrated *in vacuo* to afford crude acylation product as a yellow oil. The resulting oil was then dissolved in MeOH (10 mL, 0.5M), NaOMe (4.1 mL, 19.2 mmol, 25 wt. % in methanol, 4.0 eq) was added and the reaction mixture was stirred for 4 h. Upon full conversion of the starting material, Dowex 50W-X8 H^+ resin was added gradually until the pH of the solution reached neutral (pH 7). The mixture was filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography (R_f = 0.2, DCM/acetone 1:1, v/v) to yield compound **17** (2.5 g, 4.3 mmol, 89% over 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 – 7.20 (m, 4H, CH_{arom}), 7.19 – 7.14 (m, 2H, CH_{arom}), 6.90 – 6.82 (m, 6H, CH_{arom}), 5.64 (d, *J* = 7.6 Hz, 1H, NH), 4.79 (d, *J* = 11.5 Hz, 1H, C*H*H PMB), 4.73 (d, *J* = 10.4 Hz, 1H, CH*H* PMB), 4.56 (d, *J* = 11.5 Hz, 1H, C*H*H PMB), 4.46 (d, *J* = 10.5 Hz, 1H, CH*H* PMB), 4.43 – 4.35 (m, 2H, CH_2 PMB), 4.13 – 4.08 (m, 1H, H-1), 3.85 – 3.77 (m, 10H, OMe, H-2), 3.71 – 3.64 (m, 1H, H-3), 3.60 (dd, *J* = 9.0, 5.2 Hz, 1H, H-6), 3.51 (dd, *J* = 10.1, 8.4 Hz, 1H, H-4), 3.44 (dd, *J* = 8.9, 3.2 Hz, 1H, H-6), 2.41 – 2.36 (m, 1H, 1-OH), 2.21 – 2.11 (m, 1H, H-5), 1.86 – 1.78 (m, 4H, H-7, OAc), 1.69 – 1.60 (m, 1H, H-7). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6 (C=O NHAc), 159.4, 159.3, 159.2, 130.8, 130.8, 130.6 ($C_{q\text{-}arom}$), 130.0, 129.8, 129.4, 129.3, 114.1, 113.9, 113.9 (CH_{arom}), 81.4 (C-4), 80.2 (C-3), 74.6, 74.3, 72.8 (CH_2 PMB), 69.8 (C-6), 68.2 (C-1), 55.5 (C-2), 55.4, 55.4, 55.4 (OMe), 37.3 (C-5), 32.4 (C-7), 23.7 (NHAc). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{32}H_{37}NO_8Na$ 602.2724, found 602.2724.

2',3'-Di-*O*-acetyl-uridine 39

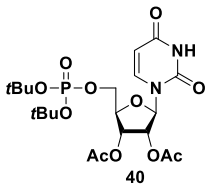


39

To a solution of uridine (12.2 g, 36.7 mmol) in 300 mL dry DCE/pyridine 1:1 was added triphenylmethyl chloride (13.6 g, 48.9 mmol) under argon. The mixture was heated to 50 °C and stirred overnight. Upon full conversion of the starting material, the reaction mixture was cooled to room temperature. Acetic anhydride (10.6 mL, 113 mmol) was added. The reaction mixture was stirred at room temperature overnight. The mixture was concentrated *in vacuo* and redissolved in 300 mL DCM. The organics were washed with 1M HCl, sat. aq. $NaHCO_3$ and brine. The water layers were back-extracted using DCM. The combined organic layers were dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in 80% aqueous acetic acid (300 mL) and the mixture was heated to refluxe until TLC showed full conversion of staring material. The reaction mixture was cooled to room temperature and the solids were removed by suction filtration on a Büchner funnel. The solids were washed with acetic acid/water (1:1 v/v, 100 mL). The filtrate was concentrated *in vacuo* and purified by flash column chromatography (R_f = 0.2, DCM/MeCN 7:3, v/v), to yield **39** (11.7g, 35.6 mmol, 93% over three steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.42 (s, 1H, NH), 7.79 (d, *J* = 8.1 Hz, 1H, H-6), 6.12 – 6.05 (m, 1H, H-1'), 5.80 (d, *J* = 8.1 Hz, 1H, H-5), 5.52 – 5.44 (m, 2H, H-2', H-3'), 4.22 (q, *J* = 2.4 Hz, 1H, H-4'), 3.95 (d, *J* = 12.1 Hz, 1H, H-5'), 3.86 (d, *J* = 12.2 Hz, 1H, H-5'), 3.27 (s, 1H, 5'-OH), 2.14 (s, 3H, OAc), 2.09 (s, 3H, OAc). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 170.0 (C=O, OAc), 163.4

(C=O, cytosine), 150.6 (C=O, cytosine), 140.8 (C-6), 103.3 (C-5), 87.5 (C-1'), 83.5 (C-4'), 73.0 (C-2'), 71.3 (C-3'), 61.8 (C-5'), 20.7, 20.50 (OAc). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{13}H_{16}N_2O_8Na$ 351.0799, found 351.0802.

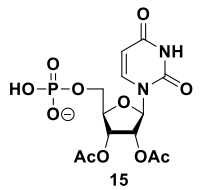
Uridine phosphate 40



40

Compound **39** (328 mg, 1 mmol) was co-evaporated with acetonitrile thrice and dissolved in dry DMF (5 mL, 0.2M). 1-Methyl-imidazole·HCl (533 mg, 4.5 mmol) and 1-methyl-imidazole (246 mg, 3 mmol) were added. Di-*tert*-butyl-*N,N*-diisopropylphosphoramidite (1.1 g, 4.5 mmol) was added and the reaction was stirred at room temperature for 20 minutes. Then *t*BuOOH in decane (0.54 mL, 3 mmol, 5.5 M in decane) was added and the reaction mixture was stirred for 1 h. The reaction was quenched by the addition of sat. aq. $NaHCO_3$ and extracted with DCM. The organic layer was washed with H_2O, dried over $MgSO_4$, concentrated *in vacuo* and purified by silica gel chromatography (R_f = 0.3, pentane/EtOAc, 3:1, v/v) to obtain **40** (442 mg, 0.85 mmol, 85%) as a white foam. ^{1}H NMR (500 MHz, CD_3CN) δ 9.14 (bs, 1H, NH), 7.57 (d, *J* = 8.1 Hz, 1H, H-6), 6.01 (d, *J* = 6.2 Hz, 1H, H-1'), 5.65 (d, *J* = 8.2 Hz, 1H, H-5), 5.36 (dd, *J* = 5.9, 3.8 Hz, 1H, H-3'), 5.29 (t, *J* = 6.0 Hz, 1H, H-2'), 4.32 – 4.25 (m, 1H, H-4'), 4.17 – 4.08 (m, 2H, H-5'), 2.08 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.46 (s, 18H, $C(CH_3)_3$). ^{13}C NMR (126 MHz, CD_3CN) δ 170.7, 170.6 (C=O, OAc), 163.6, 151.4 (C=O, cytosine), 141.1 (C-6), 103.6 (C-5), 87.7 (C-1'), 82.0 (d, *J* = 8.7 Hz, C-4'), 73.4 (C-2'), 71.3 (C-3'), 66.7 (d, *J* = 6.0 Hz, C-5'), 30.1, 30.0 ($C(CH_3)_3$), 20.8, 20.6 (OAc). ^{31}P NMR (202 MHz, CD_3CN) δ -10.21. HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{21}H_{33}N_2O_{11}Na$ 543.1714, found 543.1714.

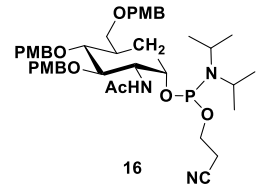
Uridine phosphate 15



15

Compound **40** (51 mg, 0.1 mmol) was dissolved in DCM (2 mL) and TFA (0.12 mL, 1.6 mmol) was added. The reaction was stirred for 1 hour followed by co-evaporation with pyridine (2x) and toluene (3x) after which it was used crude in the next reaction without further purification. ^{31}P NMR (121 MHz, acetone-d_6 capillary) δ 1.08 (s) (Acetone-d_6 in a sealed capillary, centered in a 5 mm NMR tube, was used as an external reference for the ^{31}P chemical shifts).

Phosphoramidite 16

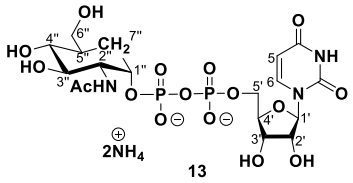


16

Compound **17** (100 mg, 0.17 mmol) was co-evaporated with toluene, dissolved in dry DMF (1.7 mL, 0.1M), and put under argon atmosphere. DIPEA (87 µL, 0.51 mmol) was added and the reaction was cooled on ice. 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (43 µL, 0.19 mmol) was added and the mixture was stirred for 2 h. After ^{31}P-NMR monitoring indicated full conversion of the chlorophosphoramidite into the corresponding phosphoramidite (180 ppm →150 ppm). The reaction was quenched by the addition of sat. aq. $NaHCO_3$ solution and extracted with EtOAc. The organic layer was dried over

$MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography using neutralized silica gel (R_f = 0.3, pentane/EtOAc 3:1 + 1% Et_3N, v/v) gave compound **16** (64 mg, 82 µmol, 48%) as a colourless oil. *A mixture of diastereoisomers.* ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 – 7.20 (m, 8H, CH_{arom}, CH_{arom}*), 7.19 – 7.13 (m, 4H, CH_{arom}, CH_{arom}*), 6.90 – 6.79 (m, 12H, CH_{arom}, CH_{arom}*), 5.66 (d, *J* = 8.8 Hz, 1H, NH), 5.32 (d, *J* = 8.8 Hz, 1H, NH*), 4.85 – 4.73 (m, 4H, CH*H* PMB, C*H*H PMB, CH*H* PMB*, C*H*H PMB*), 4.60 (dd, *J* = 11.4, 7.1 Hz, 2H, CH*H* PMB, CH*H* PMB*), 4.49 – 4.32 (m, 6H, CH*H* PMB, C*H*H PMB, , CH*H* PMB, CH*H* PMB*, C*H*H PMB* CH*H* PMB*), 4.23 – 4.09 (m, 2H, H-1, H-1*), 4.02 – 3.91 (m, 2H, H-2, H-2*), 3.82 – 3.73 (m, 26H, OMe PMB, OMe PMB, OMe PMB, OMe PMB* OMe PMB*, OMe PMB*, CH_2CH_2CN, CH_2CH_2CN*), 3.71 – 3.62 (m, 4H, CH_2CH_2CN, CH_2CH_2CN*, H-6, H-6*), 3.60 – 3.50 (m, 4H, H-3, H-3*, H-4, H-4*), 3.46 – 3.35 (m, 2H, H-6, H-6*), 2.59 – 2.55 (m, 2H, CH_2CH_2CN), 2.52 (t, *J* = 6.2 Hz, 2H, CH_2CH_2CN*), 2.10 – 1.97 (m, 3H, H-5. H-5*, H-7), 1.93 – 1.83 (m, 4H, H-7*, NHAc), 1.77 (s, 3H, NHAc*), 1.71 – 1.60 (m, 2H, H-7, H-7*), 1.18 – 1.07 (m, 24H, $CH(CH_3)_2$, $CH(CH_3)_2$*). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.8 (C=O NHAc), 169.6 (C=O NHAc*), 159.4, 159.3, 159.2, 159.2, 131.0, 131.0, 130.9, 130.7 ($C_{q\text{-}arom}$, $C_{q\text{-}arom}$*), 130.2, 130.1, 130.0, 129.9, 129.3, 129.1, 118.0, 117.7, 114.0, 113.9, 113.9, 113.9, 113.8, 113.8 (CH_{arom}, CH_{arom}*), 81.9, 81.5, 81.0, 80.5 (C-4, C-4*), 75.0 (C-1), 74.9, 74.4, 74.3, 72.8 (CH_2 PMB, CH_2 PMB*), 72.7 (C-1), 72.2 (d, *J* = 13.9 Hz, C-1*), 69.6, 69.6 (C-6. C-6*), 58.2, 58.1 (CH_2CH_2CN, CH_2CH_2CN*), 55.4, 55.4, 55.4 (OMe, PMB), 54.5, 54.4 (C-2, C-2*), 43.4, 43.3, 43.2, 43.1 (C-3, C-3*), 37.7, 37.5 (C-5, C-5*), 32.3, 31.7 (C-7, C-7*), 24.9, 24.9, 24.7, 24.7, 24.7, 24.6, 24.6 ($CH(CH_3)_2$, $CH(CH_3)_2$*), 23.7, 23.5 (NHAc, NHAc*), 20.6 (d, *J* = 2.6 Hz, CH_2CH_2CN), 20.50 (d, *J* = 2.3 Hz, CH_2CH_2CN*). ^{31}P NMR (202 MHz, $CDCl_3$) δ 148.81, 147.52, 14.22 (H-phosphate). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{32}H_{37}NO_8Na$ 602.2724, found 602.2724.

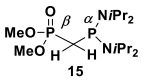
UDP-CarbaGlcNAc 13



Crude phosphate **15** (~0.10 mmol) was co-evaporated thrice with pyridine after which it was co-evaporated one more time with dry MeCN. The residue was dissolved in dry MeCN (1 mL). DCI (19 mg, 0.16 mmol) and molecular sieves were added and the mixture was left to stand for 12 h under an argon atmosphere. Phosphoramidite **16** (64 mg, 82 µmol) was co-evaporated with toluene and MeCN and added to the mixture of **15** and DCI. The reaction was stirred for 30 minutes and analyzed with ^{31}P-NMR spectroscopy. *t*BuOOH (5.5 M in decane) (60 µL, 0.33 mmol, 4.0 eq) was added. After stirring at room temperature for an additional 30 minutes, reaction progress was checked by ^{31}P-NMR which indicated full oxidation of all P^{III} species to P^V and DBU (61 µL, 0.4 mmol, 5.0 eq) was added to the solution. The reaction mixture was stirred for 10 minutes, and concentrated *in vacuo.* The residue was dissolved in dry DCM (1.2 mL) and tri-*iso*-propylsilane (43 µL, 0.41 mmol, 5.0 eq) and TFA (0.3 mL) were added at 0 °C. The mixture was stirred at this temperature, and the reaction was monitored by LC-MS until all the PMB groups were removed. The reaction was concentrated *in vacuo*. The residue was dissolved in ammonia (30%, 5 mL) and stirred overnight. The excess ammonia was removed

under reduced pressure. The resulting solution was diluted with water, washed with ethyl acetate until the ethyl acetate returned clear and colourless. The aqueous phase was then concentrated *in vacuo*. Purification by size-exclusion chromatography (HW-40, NH_4OAc buffer) followed by semiprep-HPLC yielded compound **13** (17.2 mg, 26.9 μmol, 27% over 5 steps) as a white powder. ^{1}H NMR (500 MHz, D_2O) δ 7.95 (d, J = 8.1 Hz, 1H, H-6), 5.99 – 5.93 (m, 2H, H-5, H-1'), 4.52 – 4.46 (m, 1H, H-1''), 4.38 – 4.32 (m, 2H, H-2', H-3'), 4.29 – 4.25 (m, 1H, H-4'), 4.25 – 4.20 (m, 1H, H-5'), 4.20 – 4.14 (m, 1H, H-5'), 3.85 – 3.78 (m, 1H, H-2''), 3.74 – 3.69 (m, 2H, H-6''), 3.65 (dd, J = 10.6, 9.0 Hz, 1H, H-3''), 3.38 (dd, J = 10.8, 9.0 Hz, 1H, H-4''), 2.18 – 2.12 (m, 1H, H-7''), 2.06 (s, 3H, NHAc), 2.05 – 1.97 (m, 1H, H-5''), 1.57 – 1.48 (m, 1H, H-7''). ^{13}C NMR (126 MHz, D_2O) δ 174.4 (C=O NHAc), 166.3 (C=O uracil), 151.8 (C=O uracil), 141.6 (C-6), 102.6 (C-5), 88.4 (C-1'), 83.2 (d, J = 9.0 Hz, C-4'), 74.3 (d, J = 5.8 Hz, C-1''), 73.7 (C-2'), 73.4 (C-4''), 72.9 (C-3''), 69.6 (C-3'), 64.8 (d, J = 5.4 Hz, C-5'), 62.1 (C-6''), 54.7 (d, J = 6.9 Hz, C-2''), 38.0 (C-5''), 30.3 (C-7''), 22.1 (NHAc). ^{31}P NMR (202 MHz, D_2O) δ -11.31 (d, $J_{P\text{-}P}$ = 20.7 Hz), -11.82 (d, $J_{P\text{-}P}$ = 20.7 Hz). HRMS $[M+Na]^+$ calcd for $C_{18}H_{29}N_3O_{16}P_2Na$ 628.0915, found 628.0921.

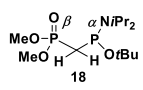
Reagent 44



15

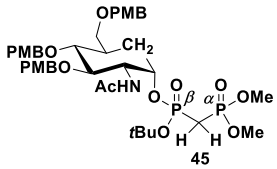
n-BuLi (2.5M in n-hexane, 3.4 mL, 8.4 mmol) was carefully added to a cooled solution (-78 ˚C) of diisopropylamine (1.2 mL, 8.4 mmol) in dry THF (3 mL), under argon atmosphere. The reaction mixture was stirred for 30 minutes. A solution of dimethyl methylphosphonate (**42**, 437 μL, 4.0 mmol) in THF (2 mL) was added dropwise over 5 minutes, and the reaction mixture was stirred for an additional 30 minutes. Next, a solution of bis(diisopropylamino)chlorophosphoamidite (**43**, 1.3 g, 4.8 mmol) in n-hexane:THF (2:1 v/v, 3 mL) was prepared and added dropwise through a syringe fitted with a silica filter to remove insoluble diisopropylamine hydrochloride originating from the commercial P^{III} reagent. After addition, the reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was poured into sat. aq. $NaHCO_3$ and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. Purification by silica gel column chromatography (R_f = 0.3, pentane/EtOAc 8:2 + 2% Et_3N) yielded the compound **44** as a colorless oil (1.3 g, 2.9 mmol, 72%). ^{1}H NMR (400 MHz, CD_3CN) δ 3.66 (s, 3H, P(O)OMe), 3.63 (s, 3H, P(O)OMe), 3.44 (m, 4H, C*H*$(CH_3)_2$), 2.25 (d, J = 3.3 Hz, 1H, P-C*H*H-P), 2.21 – 2.19 (m, 1H, P-C*H*H-P), 1.19 (s, 6H, C*H*$(CH_3)_2$), 1.18 (s, 6H, CH$(CH_3)_2$), 1.13 (s, 6H, CH$(CH_3)_2$), 1.11 (s, 6H, CH$(CH_3)_2$) (The isopropyl methyls appear as four singlets (6 H each) due to high concentration of the sample). ^{13}C NMR (101 MHz, CD_3CN) δ 52.6 (d, $J_{C\text{-}P}$ = 2.3 Hz, P(O)OMe), 52.5 (d, $J_{C\text{-}P}$ = 2.2 Hz, P(O)OMe), 47.8, 47.7 (*C*H$(CH_3)_2$), 26.6 (dd, $J_{C\text{-}P}$ = 131.2, 131.4 Hz, P-*C*H_2-P), 24.5, 24.5, 24.1, 24.1 (CH(*C*$H_3)_2$). ^{31}P NMR (162 MHz, CD_3CN) δ 38.25 (d, $J_{P\text{-}P}$ = 79.6 Hz, α-P), 32.85 (d, $J_{P\text{-}P}$ = 78.8 Hz, β-P). HRMS (ESI) M/Z: $[M+Na]^+$ Calcd for $C_{15}H_{36}N_2O_3P_2Na$ 377.2093 $[M+Na]^+$; Found 377.2091.

Reagent 18



Compound **44** (1.6 g, 4.5 mmol) was co-evaporated with toluene, dissolved in MeCN and put under argon atmosphere. Dry *t*BuOH (337 mg, 4.5 mmol) and DCI (319 mg, 2.7 mmol, 0.6 eq) were added. The reaction progression was followed by ^{31}P NMR, which indicated completion after 5 h. The reaction mixture was concentrated in vacuo and purified by silica gel column chromatography (R_f = 0.2, DCM/acetone 4:1 + 2% Et_3N) to yield compound **18** as a colorless oil (1.0 g, 2.7 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.75 (d, *J* = 2.1 Hz, 3H, P(O)OMe), 3.72 (d, *J* = 2.1 Hz, 3H, P(O)OMe), 3.59 – 3.44 (m, 2H, C*H*(CH_3)$_2$), 2.39 – 2.24 (m, 1H, P-C*H*H-P), 1.94 – 1.79 (m, 1H, P-C*H*H-P), 1.31 (s, 9H, C(CH_3)$_3$), 1.18 (d, *J* = 6.8 Hz, 6H, CH(CH_3)$_2$), 1.10 (d, *J* = 6.8 Hz, 6H, CH(CH_3)$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 52.2 (d, $J_{P\text{-}P}$ = 6.3 Hz, P(O)OMe), 44.8, 44.7 (*C*H(CH_3)$_2$), 31.6 (dd, $J_{C\text{-}P}$ = 130.5, 130.6 Hz, P-*C*H_2-P), 30.5 (d, $J_{C\text{-}P}$ = 8.2 Hz, C(*C*H_3)$_3$), 23.7, 23.4, 22.7 (CH(*C*H_3)$_2$). ^{31}P NMR (162 MHz, $CDCl_3$) δ 99.55 (d, $J_{P\text{-}P}$ = 45.6 Hz, α-P), 31.41 (d, $J_{P\text{-}P}$ = 45.4 Hz, β-P). HRMS (ESI) M/Z: $[M+Na]^+$ Calcd for $C_{13}H_{31}NO_4P_2Na$ 350.1621; Found 350.1623.

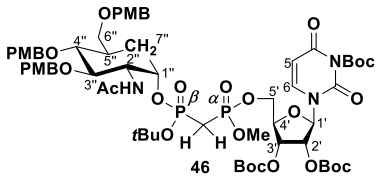
Carba-glucosamine 45



Compound **17** (450 mg, 0.78 mmol) and phosphanyl methylphosphonate **18** (330 mg, 1.0 mmol, 1.3 eq) was co-evaporated with toluene, dissolved in MeCN (4 mL, 0.2M), and put under argon atmosphere. DCI (184 mg, 1.56 mmol, 2.0 eq) was added and the reaction mixture was stirred for 30 min at room temperature. The mixture was cooled to 0 °C, tert-butyl hydroperoxide (5.0-6.0 M in decane, 0.28 mL, 1.6 mmol, 2 eq) was added. The reaction mixture was stirred for an additional 30 minutes. The solution was poured into DCM and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by silica gel column chromatography (R_f = 0.2, DCM/acetone 1:1, v/v) yielded **45** as a colorless oil (188 mg, 0.23 mmol, 59% over 2 steps). *A mixture of diastereoisomers.* ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (d, *J* = 9.5 Hz, 1H, NH), 7.30 – 7.25 (m, 2H, CH_{arom}), 7.25 – 7.20 (m, 2H, CH_{arom}), 7.15 – 7.10 (m, 2H, CH_{arom}), 6.89 – 6.81 (m, 6H, CH_{arom}), 4.81 – 4.73 (m, 3H, H-1, CH*H* PMB, C*H*H PMB), 4.71 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.44 – 4.34 (m, 3H, C*H*H PMB, CH*H* PMB, C*H*H PMB), 4.30 – 4.24 (m, 1H, H-2), 3.83 (d, *J* = 11.3 Hz, 3H, P(O)OMe), 3.84 – 3.76 (m, 9H, OMe PMB, OMe PMB, OMe PMB), 3.79 (d, *J* = 11.3 Hz, 3H, P(O)OMe), 3.71 (dd, *J* = 10.5, 9.1 Hz, 1H, H-3), 3.59 (dd, *J* = 8.8, 5.1 Hz, 1H, H-6), 3.54 (dd, *J* = 10.6, 9.1 Hz, 1H, H-4), 3.45 (dd, *J* = 8.9, 2.6 Hz, 1H, H-6), 2.50 – 2.25 (m, 2H, P-CH_2-P), 2.19 – 2.10 (m, 2H, H-5, H-7), 2.03 (s, 3H, OAc), 1.78 – 1.69 (m, 1H, H-7), 1.49 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7 (C=O NHAc), 159.2, 159.1, 159.0, 131.1, 130.9, 130.5 ($C_{q\text{-}arom}$), 129.8, 129.2, 129.1, 113.8, 113.7 (CH_{arom}), 84.6 (d, $J_{C\text{-}P}$ = 7.9 Hz, C(CH_3)$_3$), 82.5 (C-3), 80.8 (C-4), 76.6 (d, $J_{C\text{-}P}$ = 6.6 Hz, C-1), 75.2, 74.9, 72.7 (CH_2 PMB), 69.5 (C-6), 55.3, 55.3 (OMe PMB), 53.7 (d, $J_{C\text{-}P}$ = 6.7 Hz, P(O)OMe), 53.4 (d, $J_{C\text{-}P}$ = 6.5 Hz, C-2), 53.0 (d, $J_{C\text{-}P}$ = 6.6 Hz, P(O)OMe), 37.5 (C-5), 31.9 (C-7), 30.3, 30.2 (C(CH_3)$_3$), 26.86 (dd, $J_{C\text{-}P}$ = 140.8, 134.8

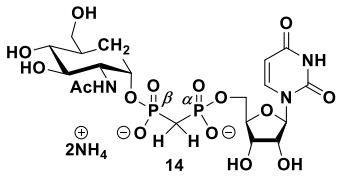
Hz, P-CH_2-P), 23.0 (NHAc). ^{31}P NMR (202 MHz, $CDCl_3$) δ 23.22 (d, $J_{P\text{-}P}$ = 6.9 Hz, α-P), 14.30 (d, $J_{P\text{-}P}$ = 6.9 Hz, β-P). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{40}H_{57}NO_{13}P_2Na$ 844.3197, found 844.3200.

Protected UDP-carbaGlcNAc analogue 46



Compound **45** (162 mg, 0.20 mmol) was dissolved in MeCN/Et_3N/PhSH (3:3:2, 1.6 mL) and stirred for 6 hours at 35 °C. After complete consumption of the starting material, the solution was concentrated in vacuo. The residue was quickly purified by flash silica gel column chromatography (100% DCM to 100% methanol). The product and the protected uridine **19** (163 mg, 0.3 mmol)) were co-evaporated with toluene, dissolved in dry MeCN (1 mL, 0.2 M), and DIPEA (0.35 mL, 2 mmol) and PyNTP (300 mg, 0.6 mmol) were added, and the mixture was stirred at room temperature. After 2 h, the reaction was complete as indicated by TLC-analysis. The reaction mixture was poured into DCM and washed with phosphate buffer (pH = 7). The water layer was extracted with DCM. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography over silica gel (R_f = 0.2, DCM/ acetone 1:2, v/v) and then by size exclusion (LH-20) (DCM/MeOH 1:1) to obtain **46** (73 mg, 43 μmol, 28% over two steps) as a white solid. *A mixture of diastereoisomers.* ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 9.6 Hz, 1H, NH), 7.36 (d, *J* = 8.2 Hz, 1H, H-6), 7.25 – 7.23 (m, 2H, CH_{arom}), 7.23 – 7.19 (m, 2H, CH_{arom}), 7.14 – 7.08 (m, 2H, CH_{arom}), 6.87 – 6.79 (m, 6H, CH_{arom}), 5.89 (dd, *J* = 3.1, 1.0 Hz, 1H, H-1'), 5.76 (d, *J* = 8.2 Hz, 1H, H-5), 5.78 – 5.73 (m, 2H, H-2', H-3'), 4.80 – 4.65 (m, 4H, H-1'', CH*H* PMB, C*H*H PMB, CH*H* PMB), 4.42 – 4.34 (m, 4H, H-4', C*H*H PMB, CH*H* PMB, C*H*H PMB), 4.34 – 4.29 (m, 2H, H-5'), 4.28 – 4.21 (m, 1H, H-2''), 3.87 (d, *J* = 11.3 Hz, 3H, P(O)OMe), 3.80 – 3.76 (m, 9H, OMe PMB, OMe PMB, OMe PMB), 3.73 – 3.65 (m, 1H, H-3''), 3.58 – 3.49 (m, 2H, H-4'', H-6''), 3.43 (dd, *J* = 8.8, 2.6 Hz, 1H, H-6''), 2.51 – 2.31 (m, 2H, P-CH_2-P), 2.14 – 2.08 (m, 2H, H-5'', H-7''), 2.00 (s, 3H, NHAc), 1.76 – 1.68 (m, 1H, H-7''), 1.59 (s, 9H, $C(CH_3)_3$), 1.49 – 1.45 (m, 27H, $C(CH_3)_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6 (C=O NHAc), 160.0 (C=O uracil), 159.2, 159.1, 159.0 ($C_{q\text{-}arom}$), 152.0, 152.0 (C=O uracil), 148.1, 147.3 (C=O carbonate), 139.7 (C-6), 131.1, 130.8, 130.5 ($C_{q\text{-}arom}$), 129.8, 129.2, 129.1, 113.8, 113.7 (CH_{arom}), 102.8 (C-5), 89.2 (C-1'), 87.1, 84.9, 84.9, 83.9, 83.7 ($C(CH_3)_3$), 82.4 (C-3''), 80.8 (C-4''), 80.0 (d, $J_{C\text{-}P}$ = 6.7 Hz, C-4'), 76.4 (d, $J_{C\text{-}P}$ = 6.5 Hz, C-1''), 75.2, 74.9 (CH_2 PMB), 74.8 (C-2'), 72.7 (C-5'), 71.6 (C-3'), 69.5 (C-6''), 64.6 (C-5'), 55.3 (OMe), 54.3 (d, $J_{C\text{-}P}$ = 6.4 Hz, P(O)OMe), 53.7 (d, $J_{C\text{-}P}$ = 6.7 Hz, C-2''), 37.4 (C-5''), 31.7 (C-7''), 30.3, 30.2, 27.6, 27.6, 27.4 ($C(CH_3)_3$), 23.1 (NHAc). ^{31}P NMR (202 MHz, $CDCl_3$) δ 22.92 (d, $J_{P\text{-}P}$ = 5.3 Hz, α-P), 13.76 (d, $J_{P\text{-}P}$ = 5.3 Hz, β-P). HRMS (ESI) M/Z: $[M+Na]^+$ calcd for $C_{63}H_{89}N_3O_{24}P_2Na$ 1356.5203, found 1356.5203.

UDP-carbaGlcNAc analogue 14



Compound **46** (40 mg, 30 µmol) was dissolved in MeCN: Et_3N: PhSH (3:3:2, 0.8 mL) and stirred for 6 hours at 35 °C. After complete consumption of starting material, the solution was concentrated in vacuo. The residue was quickly purified by flash silica gel column chromatography (100% DCM to 100% Methanol), the product was then dissolved in 30% TFA (210 µL) in DCM (0.5 mL) at 0 °C, followed by the addition of TES (48 µL, 0.3 mmol, 10.0 eq). The reaction mixture was stirred overnight at 0 °C. When the reaction was complete, as indicated by LC-MS analysis, the reaction was neutralized by the addition of 30% ammonia. The mixture was concentrated and the residue was purified by size-exclusion chromatography (HW40-column, 0.15 M aqueous NH_4OAc in water) to yield UDP-carbaGlcNAc analogue **14** (8.7 mg, 14 µmol, 46% over two steps) as a white powder. ^{1}H NMR (500 MHz, D_2O) δ 7.97 (d, J = 8.1 Hz, 1H, H-6), 5.95 – 5.90 (m, 2H, H-1', H-5), 4.48 – 4.41 (m, 1H, H-1''), 4.39 – 4.31 (m, 2H, H-2', H-3'), 4.27 – 4.21 (m, 1H, H-4'), 4.20 – 4.08 (m, 2H, H-5'), 3.82 – 3.77 (m, 1H, H-2''), 3.74 – 3.65 (m, 3H, H-3'', H-6''), 3.35 (dd, J = 10.5, 9.0 Hz, 1H, H-4''), 2.25 – 2.09 (m, 2H, P-CH_2-P), 2.07 – 1.99 (m, 5H, H-5'', H-7'', NHAc), 1.56 – 1.47 (m, 1H, H-7''). ^{13}C NMR (126 MHz, D_2O) δ 174.3 (C=O NHAc), 166.2 (C=O uracil), 151.7 (C=O uracil), 141.8 (C-6), 102.5 (C-5), 88.6 (C-1'), 83.3 (d, $J_{C\text{-}P}$ = 9.0 Hz, C-4') , 73.7 (C-2'), 73.5 (C-4''), 73.0 (C-3''), 72.5 (d, $J_{C\text{-}P}$ = 6.3 Hz, C-1''), 69.5 (C-3'), 63.3 (d, $J_{C\text{-}P}$ = 5.4 Hz, C-5'), 62.2 (C-6''), 54.7 (d, $J_{C\text{-}P}$ = 6.2 Hz, C-2''), 38.0 (C-5''), 31.3 (C-7''), 26.7 (dd, $J_{C\text{-}P}$ = 129.1, 130.2 Hz, P-CH_2-P), 22.1 (NHAc). ^{31}P NMR (162 MHz, D_2O): δ 18.09 (d, $J_{P\text{-}P}$= 11.2 Hz, α-P), 16.38 (d, $J_{P\text{-}P}$ = 11.2 Hz, β-P). HRMS (ESI) M/Z: $[M+H]^+$ Calcd for $C_{19}H_{32}N_3O_{15}P_2$ 604.1303 $[M+H]^+$; Found 604.1300.

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