Chemoenzymatic syntheses of carbasugar analogues of nucleoside diphosphate sugars: UDP-carba-Gal, UDP-carba-GlcNAc, UDP-carba-Glc, and GDP-carba-Man

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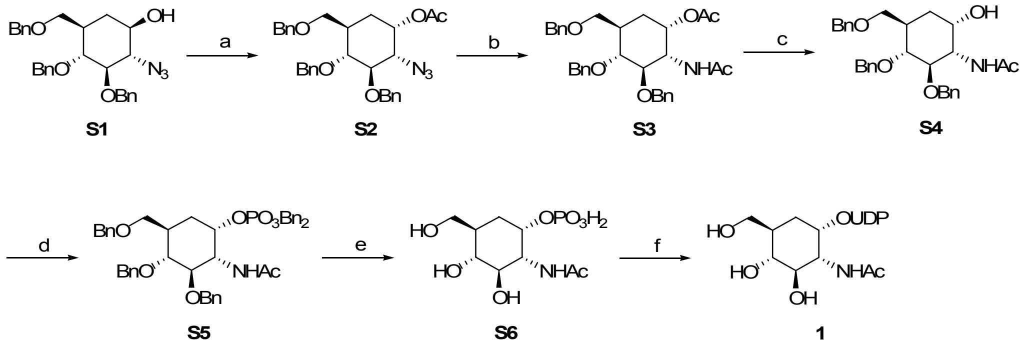
Supporting Information

Synthetic Part.

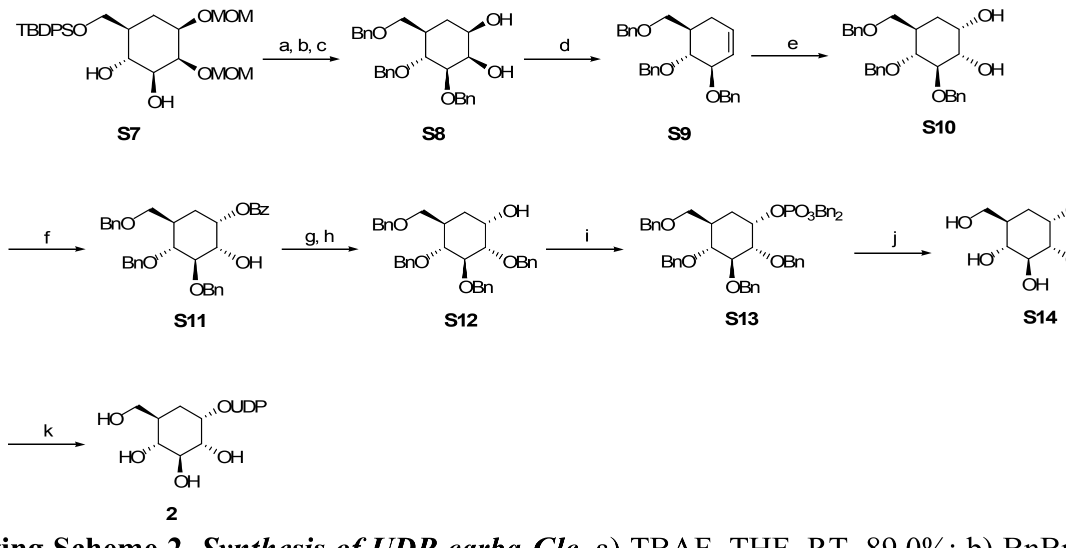
l. Materials and reagents

General methods. All nonhydrolytic reactions were carried out in oven-dried glassware under an inert atmosphere of dry argon or nitrogen. All commercial chemicals were used as received except solvents, which were purified and dried by means of standard methods prior to use. Analytical thin-layer chromatography (TLC) was performed on Merck 60 F254 silica gel plates (0.25mm thickness); visualization was carried out be using UV light (λ=254 and 365nm) or by spraying the plates with 5% solution of phosphomolybdic acid or ninhydrin solution followed by charring with a heat gun. Column chromatography was performed on Merck 60 silica gel (70-230 or 230-400mesh). Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded on Bruker DPX 300 (^{1}H NMR at 300MHz; ^{13}C NMR at 75MHz; ^{31}P NMR at 121.5MHz) spectrometer. Tetramethylsilane and phosphoric acid (85%) were used as internal and external standards for ^{1}H and ^{31}P NMR spectra. The abbreviation "app" signifies an apparent peak or set of peaks. High-resolution mass spectra (FAB) were determined on a JMS-700 instrument at the Korea Basic Science Support Center. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. The standard extractive work-up procedure consisted of pouring into a large amount of water, extracting with organic solvent indicated, washing the combined extracts successively with water and brine, drying the extract on anhydrous Na_2SO_4 or $MgSO_4$, and evaporating the solvent.

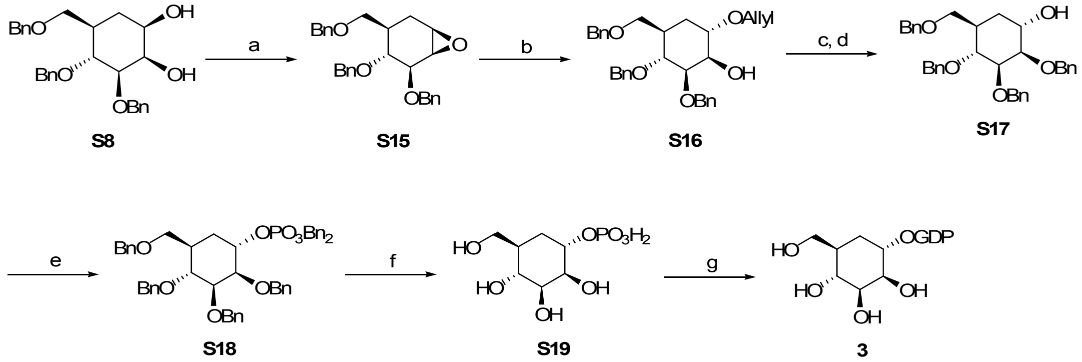
ll. Experimental and spectral data



Supporting Scheme 1. ***Synthesis of UDP-carba-GlcNAc.*** a) (i) MsCl, pyridine, RT, (ii) CsOAc, 18-crown-6, toluene, reflux, 94.8%; b) (i) H_2 (1atm), Pd/C, AcOH, THF, RT, (ii) AcCl, Et_3N, CH_2Cl_2, RT, 75.3%; c) $HBF_4 \cdot Et_2O$, MeOH, RT, 85.7%; d) (i) dibenzyl diisopropylphosphoramidite, 1H-tetrazole, CH_2Cl_2, RT, (ii) mCPBA, RT, 85.7%; e) H_2(40psi), Pearlman's catalyst, MeOH, RT, Quant; f) enzyme extract (UMP kinase, acetate kinase, GlcNAc-1-phosphate uridyltransferase), UMP, ATP, $MgCl_2 \cdot 6H_2O$, acetyl phosphate, Tris-HCl, 37℃, 23%



Supporting Scheme 2. ***Synthesis of UDP-carba-Glc.*** a) TBAF, THF, RT, 89.0%; b) BnBr, NaH, TBAI, THF, RT, 74.5%; c) conc'd HCl, MeOH, water, reflux, 98.1%; d) iodine, PPh_3, imidazole, toluene, reflux, 91.0%; e) OsO_4, NMO, acetone, water, RT, 92.7%.; f) triethyl orthobenzoate, TSA, CH_2Cl_2, RT, quant.; g) benzyl trichloroacetimidate, TfOH, hexane, CH_2Cl_2, RT, quant.; h) NaOMe, MeOH, RT, 72.8%; i) (i) dibenzyl diisopropylphosphoramidite, 1H-tetrazole, CH_2Cl_2, RT, (ii) mCPBA, RT, 70.0%; j) H_2(50psi), Pd/C, MeOH, CH_2Cl_2, RT, 97.2%; k) enzyme extract (UMP kinase, acetate kinase, Glucose-1-phosphate uridyltransferase), UMP, ATP, $MgCl_2 \cdot 6H_2O$, acetyl phosphate, Tris-HCl, 37℃, 84.4%.



Supporting Scheme 3. ***Synthesis of GDP-carba-Man.*** a) (i) trimethyl orthoacetate, PPTS, CH_2Cl_2, RT, (ii) AcBr, TEA, RT, (iii) NaOMe, MeOH, RT, 95.4%; b) allyl alcohol, $BF_3 \cdot OEt_2$, CH_2Cl_2, RT, quant.; c) BnBr, NaH, TBAI, THF, RT, 65.5%; d) $PdCl_2$, NaOAc, aq. AcOH, RT, 76.1%; e) (i) dibenzyl diisopropylphosphoramidite, 1H-tetrazole, CH_2Cl_2, RT, (ii) mCPBA, RT, 76.5%; f) H_2(50psi), Pd/C, MeOH, CH_2Cl_2, RT, 98.0%; g) enzyme extract (GMP kinase, acetate kinase, Mannose-1-phosphate guanylyltransferase), GMP, ATP, $MgCl_2 \cdot 6H_2O$, acetyl phosphate, Tris-HCl, 37℃, 83.0%.

Uridine 5'-(5a-carba-α-D-*N*-acetylglucosaminopyranosyl diphosphate) (1)

In order to determine one-pot enzymatic synthesis, in vitro enzyme reactions were conducted in 20ml of reaction mixture including UMP (10mM), ATP (0.25mM), $MgCl_2 \cdot 6H_2O$ (20mM), acetyl phosphate (50mM), Tris-HCl (100mM, pH7.5), compound **S6** (25mM) and enzyme extracts from *E. coli*. The activities of UMK (UMP kinase), ACK (Acetate kinase) and GlmU (GlcNAc-1-phosphate uridyltransferase) were measured as previously reported. [(a) A. Matsuyama, H. Yamamoto, E. Nakano, *J. Bacteriol.* **1989**, *171*, 577. (b) J. Smallshaw, R. A. Kellin, *Genetics* **1992**, *11*, 59. (c) D. Mengin-Lecreulx, H. van Heijenoort, *J. Bacteriol.* **1993**, *175*, 6150. (d) S. W. Chung, H. S. Joo, K. S. Jang, H. J. Lee, S. G. Lee, B. G. Kim, *Enzyme and Microbial Technology* **2006**, *39*, 60.] The reaction was carried out for 5 hours at 37℃ and then was stopped by heating the reaction mixture at 100℃. The reactions were monitored by HPLC using a strong anion exchange column (Hypersil ODS 4.6x250mm, 5μm particle size) with potassium phosphate buffer (100mM, pH7.0) : MeOH = 95 : 5 (v/v) at 270nm absorbance and a flow rate of 1.0ml/min. The reaction mixture was purified using SHIMADZU SPD-10Avp (source Q15 resin 200ml, FineLine Pilot 35 column, Kyoto chromato co,. ltd). After lyophilization, final product **1**

(30mg, 23%) was obtained as white solid, and the purity of product was proved to be more than 98% by HPLC peak integration.

$[\alpha]_D^{28}$ +24.5° (*c* 0.40, H_2O); mp (too high to be measured due to decomposition); ^{1}H NMR (300MHz, D_2O): 7.84 (dd, *J* = 11.4, 1.2Hz, 1H), 5.86-5.83 (m, 2H), 4.39 (br s, 1H), 4.25-4.24 (m, 2H), 4.15-4.09 (m, 3H), 3.72-3.51 (m, 4H), 3.27 (app t, *J* = 9.8Hz, 1H), 2.11-1.90 (m, 6H), 1.41 (t, *J* = 13.6Hz, 1H, H-5aβ); ^{13}C NMR (75MHz, D_2O): 173.9, 165.7, 151.3, 141.2, 102.2, 88.0, 82.7, 73.8, 73.3, 73.0, 72.5, 69.2, 64.4, 61.6, 54.2, 37.6, 29.8, 21.7; ^{31}P NMR (121.5MHz, D_2O): - 9.883 (d, *J* = 21.3Hz), - 10.407 (d, *J* = 21.1Hz); APIESMS m/z calcd. for $C_{18}H_{27}N_3O_{16}P_2Na_2$ 626.1, found 626.2 $[M-Na]^-$.

Uridine 5'-(5a-carba-α-D- glucopyranosyl diphosphate) (2)

Enzymatic UDP introduction to carba-α-D-Glucose-1-phosphate **S14** according to the procedures described for UDP-carba-GlcNAc **1** gave UDP-carba-Glc **2** (219mg, 84.4%) as a white solid, and the purity of product was proved to be more than 98% by HPLC peak integration.

$[\alpha]_D^{23}$ +22.5° (*c* 1.50, H_2O); mp (too high to be measured due to decomposition); ^{1}H NMR (300MHz, D_2O): 7.84 (d, *J* = 8.1Hz, 1H), 5.89-5.84 (m, 2H), 4.50 (br s, 1H, H-1), 4.28-4.25 (m, 2H), 4.16-4.15 (m, 1H), 4.11-4.07 (m, 2H), 3.62-3.57 (m, 2H, H-6A, H-6B), 3.53 (t, J = 9.5Hz, 1H, H-3), 3.29 (dt, *J* = 9.8Hz, 2.8Hz, 1H, H-2), 3.18(td, J = 10.7Hz, 2.2Hz, 1H, H-4), 2.03 (dt, J = 14.6Hz, 3.8Hz, 1H, H-5aα) 1.92-1.83 (m, 1H, H-5), 1.34 (br t, *J* = 13.8Hz, 1H, H-5aβ); ^{13}C NMR (75MHz, D_2O): 165.5, 151.4, 141.1, 102.2, 87.8, 82.7, 74.7, 74.4, 73.2, 73.0, 72.4, 69.1, 64.3, 61.5, 37.5, 28.8; ^{31}P NMR (121.5MHz, D_2O): - 10.353 (d, *J* = 21.0Hz), - 10.849 (d, *J* = 21.0Hz); APIESMS m/z calcd. for $C_{16}H_{24}N_2O_{16}P_2Na$ 585.1, found 585.1 $[M-Na]^-$.

Guanosine 5'-(5a-carba-α-D- mannopyranosyl diphosphate) (3)

Enzymatic GDP introduction to carba-α-D-Mannose-1-phosphate **S19** according to the procedures described for UDP-carba-GlcNAc **1** gave GDP-carba-Man **3** (192mg, 83.0%) as a white solid, and the purity of product was proved to be more than 96% by HPLC peak integration.

$[\alpha]_D^{23}$ +14.5° (*c* 1.50, H_2O); mp (too high to be measured due to decomposition); ^{1}H NMR (300MHz, D_2O): 7.97 (s, 1H), 5.79 (d, J = 6.2Hz, 1H), 4.38 (dd, J = 5.1Hz, 3.3Hz, 1H), 4.32-4.29 (m, 1H, H-1), 4.22-4.20 (m, 1H), 4.08-4.05 (m, 2H), 4.01-3.98 (m, 1H, H-2), 3.64 (dd, J = 9.7Hz, 3.2Hz, 1H), 3.55-3.51 (m, 2H), 3.41 (t, *J* = 10.1Hz, 1H, H-4), 1.82-1.72 (m, 2H), 1.54 (br t, *J* = 13.8Hz, 1H, H-5aα); ^{13}C NMR (75MHz, D_2O): 138.0, 87.1, 84.3, 84.2, 74.4, 74.3, 73.9, 73.5, 71.7, 71.6, 70.8, 70.5, 65.5, 62.7, 47.0, 39.0, 27.8, 8.6; ^{31}P NMR (121.5MHz, D_2O): - 10.759 (d, *J* = 21.1Hz), - 11.346 (d, *J* = 21.1Hz); APIESMS calcd. for $C_{17}H_{25}N_5O_{15}P_2Na$ 624.1, found 624.1 $[M-Na]^-$.

(*1S, 2S, 3R, 4R, 5R*)-2-Azido-5-(benzyloxymethyl)-3,4-(dibenzyloxy)-cyclohexane-1-acetate (S2)

To a solution of **S1** [S. H. Yu, S. K. Chung, *Tetrahedron:Asymmetry*, **2004**, *15*, 581.](790mg, 1.67mmol) in pyridine (10ml) was added MsCl (0.40ml, 5.01mmol). The reaction mixture was stirred for 5 hours at RT and then worked up by a standard extractive procedure with EtOAc to provide chloromethanesulfonate compound. To a solution of CsOAc (1.20g, 5.01mmol) and 18-crown-6 (1.60g, 3.34mmol) in toluene was added this chloromethanesulfonate. The mixture was stirred and refluxed for 5 hours, then extracted with ether, dried with $MgSO_4$, filtered, concentrated in *vacuo*, and purified by column chromatography to give **S2** (806mg, 94.8%) as white solid.

$[\alpha]_D^{28}$ +40.2° (*c* 1.95, CH_2Cl_2); mp 94.5-95.0℃ (hexane/EtOAc); ^{1}H NMR (300MHz, $CDCl_3$): 7.40-7.22 (m, 15H, Ar-H), 5.30 (app d, *J* = 2.4Hz, 1H, H-1), 4.93-4.39 (m, 6H, C**H$_2$**Ph), 3.86 (t, *J* = 9.6Hz, 1H, H-3 or H-4), 3.71 (dd, *J* = 9.0, 3.9Hz, 1H, H-2), 3.60 (t, *J* = 9.8Hz, 1H, H-3 or H-4), 3.42 (dd, *J* = 10.4, 2.8Hz, 1H, H-6A), 3.39 (dd, *J* = 11.0, 1.7Hz, 1H, H-6B), 2.09 (s, 3H, O**Ac**), 2.05-1.92 (m, 2H, H-5, H-5aα), 1.71 (app t, *J* = 13.9Hz, 1H, H-5aβ); ^{13}C NMR (75MHz, $CDCl_3$): 169.5, 137.9, 137.7, 137.6, 128.0, 127.9, 127.6, 127.39, 127.35, 127.30, 127.2, 127.1, 82.2, 80.7, 75.2, 74.9, 72.7, 70.0, 68.68, 64.6, 37.4, 29.3, 20.7; HRFABMS calcd. for $C_{30}H_{33}N_3O_5$ 516.2493, found 516.2498 $[M+H]^+$.

(*1S, 2S, 3R, 4R, 5R*)-2-Acetamido-5-(benzyloxymethyl)-3,4-(dibenzyloxy)-cyclohexane-1-acetate (S3)

A mixture of **S2** (640mg, 1.24mmol), Pd/C (120mg) and AcOH (0.078ml, 1.36mmol) in THF (10ml) was hydrogenated by using a balloon (1atm). After stirring for 15 hours at RT, the catalyst was filtered and the filtrate was evaporated. To the residue dissolved in pyridine (10ml), Ac_2O (0.529ml, 7.44mmol) was added, and the resulting solution was stirred at RT for 4 hours. The reaction mixture was subjected to the standard extraction workup using EtOAc to give the crude product, which was purified by using flash column chromatography on silica gel to afford **S3** (497mg, 75.3%) as white solid.

$[\alpha]_D^{28}$ +76.3° (*c* 1.65, CH_2Cl_2); mp 160.0-161.0℃(hexane/EtOAc); ^{1}H NMR (300MHz, $CDCl_3$): 7.38-7.24 (m, 15H, Ar-H), 5.19 (d, *J* = 8.3Hz, 1H, NH), 5.30 (app d, *J* = 3.0Hz, 1H, H-1), 4.87-4.40 (m, 6H, C**H₂**Ph), 4.16-4.10 (m, 1H, H-2), 3.69-3,61 (m, 3H, H-3, H-4, H-6A), 3.41 (dd, *J* = 9.0, 2.7Hz, 1H, H-6B), 2.07-1.93 (m, 5H, H-5, H-5aα, OAc), 1.77-1.75 (m, 4H, N**H**Ac, H-5aβ); ^{13}C NMR (75MHz, $CDCl_3$): 169.4, 169.2, 137.9, 137.8, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 127.3, 127.12, 127.06, 80.6, 80.2, 74.6, 74.1, 72.6, 71.3, 69.0, 51.9, 37.6, 28.6, 22.8, 20.7; HRFABMS calcd. for $C_{32}H_{37}NO_6$ 532.2694, found 532.2699 $[M+H]^+$.

(*1S, 2S, 3R, 4R, 5R*)-2-Acetamido-5-(benzyloxymethyl)-3,4-(dibenzyloxy)-cyclohexane-1-ol (S4)

A solution of **S3** (460mg, 0.87mmol) in MeOH (6ml) was treated with HBF_4 (~54% in Et_2O, 0.5ml) at 0℃ and stirred at RT. After 3 days, the solution was treated with Et_3N at 0℃, and then most of the volatiles were removed under vacuum. Column chromatography afforded **S4** (363mg, 85.7%) as white solid.

$[\alpha]_D^{28}$ +59.8° (*c* 1.05, CH_2Cl_2); mp 144.0-144.5℃(hexane/EtOAc); ^{1}H NMR (300MHz, $CDCl_3$): 7.36-7.24 (m, 15H, Ar-H), 5.73 (d, *J* = 7.7Hz, 1H, NH), 4.85-4.43 (m, 6H, C**H₂**Ph), 4.10 (app br s, 1H, H-1), 3.87 (ddd, *J* = 10.1, 8.0, 2.6Hz, 1H, H-2), 3.72 (t, *J* = 9.1Hz, 1H, H-3 or H-4), 3.64 (dd, *J* = 8.9, 5.1Hz, 1H, H-6A), 3.56 (t, *J* = 9.2Hz, 1H, H-3 or H-4), 3.46 (dd, *J* = 8.9, 3.0Hz, 1H, H-6B), 2.24-2.16 (m, 1H, H-5), 1.83 (dt, *J* = 14.5, 4.1Hz, 1H, H-5aα), 1.78 (s, 3H, N**H**Ac), 1.67 (app td, *J* = 13.2, 2.0Hz, 1H, H-5aβ); ^{13}C NMR (75MHz, $CDCl_3$): 170.1, 138.1, 138.0, 137.97, 128.1, 128.0, 127.9, 127.7, 127.5, 127.4, 127.2, 127.0, 81.1, 80.2, 74.3, 74.2, 72.6, 69.6, 67.7, 54.9, 36.8, 31.8, 23.0; HRFABMS calcd. for $C_{30}H_{35}NO_5$ 490.2588, found 490.2593 $[M+H]^+$.

(*1S, 2R, 3R, 4R, 5R*)-2-Acetamido-5-(benzyloxymethyl)-3,4-(dibenzyloxy)-cyclohexane-1-dibenzylphosphate (S5)

To a solution of **S4** (320mg, 0.66mmol) and 1H tetrazole (0.45M in acetonitrile, 10ml) in CH_2Cl_2 (15ml) was added dibenzyl diisopropylphosphoramidite (0.659ml, 1.98mmol) at RT. After 6 hours, mCPBA (902mg, 2.64mmol) was added to the mixture at 0℃. After being stirred 1 hour at rt, the mixture was diluted with CH_2Cl_2 and washed with aq. Na_2SO_3, aq. $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$), concentrated, and chromotographed to give **S5** (420mg, 85.7%) as white solid.

$[\alpha]_D^{28}$ +45.2° (*c* 1.00, CH_2Cl_2); mp 93.0-94.0℃(hexane/EtOAc); ^{1}H NMR (300MHz, $CDCl_3$): 7.37-7.21 (m, 25H, Ar-H), 5.77 (d, *J* = 9.0Hz, 1H, NH), 5.04-4.40 (m, 10H, C**H₂**Ph), 4.63 (app br s, 1H, H-1), 4.08 (app t, *J* = 9.5Hz, 1H, H-2), 3.63 (dd, *J* = 8.7, 4.2Hz, 1H, H-6A), 3.59 (dd, *J* = 9.4Hz, 1H, H-3 or H-4), 3.52 (t, *J* = 9.6Hz, 1H, H-3 or H-4), 3.28 (dd, *J* = 9.0, 2.1Hz, 1H, H-6B), 2.03-1.88 (m, 2H, H-5aα), 1.74-1.72 (m, 1H, H-5aβ), 1.63 (s, 3H, NH**Ac**); ^{13}C NMR (75MHz, $CDCl_3$): 169.5, 138.04, 137.98, 137.87, 128.31, 128.25, 128.0, 127.93, 127.89, 127.54, 127.51, 127.48, 127.25, 127.20, 127.1, 127.0, 80.8, 80.7, 77.6, 74.71, 74.65, 72.5, 69.20, 69.16, 68.7, 53.1, 36.9, 30.7, 22.6; ^{31}P NMR (121.5MHz, $CDCl_3$): 0.2520; HRFABMS calcd. for $C_{44}H_{48}NO_8P$ 750.3190, found 750.3196 $[M+H]^+$.

(*1S, 2R, 3R, 4R, 5R*)-2-Acetamido-3,4-dihydroxy-5-hydroxymethyl-cyclohexane-1-phosphate (S6)

A mixture of **S5** (350mg, 0.47mmol), Pearlman's catalyst (100mg) in MeOH (15ml) was hydrogenated (40psi) at RT, overnight. The reaction mixture was filtered through Celite and washed with MeOH and the filtrate was diluted with water. After lyophilization, compound **S6** (140mg, Quantitative) was obtained as foamy solid.

$[\alpha]_D^{28}$ +79.1° (*c* 0.70, MeOH); ^{1}H NMR (300MHz, D_2O): 4.37 (app d, *J* = 7.0Hz, 1H, H-1), 3.67-3.48 (m, 4H), 3.25 (t, *J* = 9.9Hz, 1H), 1.99 (app d, *J* = 14.7Hz, 1H, H-5aα), 1.90 (s, 3H, NH**Ac**), 1.90-1.78 (m, 1H, H-5), 1.40 (t, *J* = 13.7Hz, 1H, H-5aβ); ^{13}C NMR (75MHz, D_2O): 173.9, 73.3, 73.0, 71.9, 61.5, 54.3, 37.4, 29.5, 21.5; ^{31}P NMR (121.5MHz, D_2O): 0.5862; HRFABMS calcd. for $C_9H_{18}NO_8P$ 300.0843, found 300.0848 $[M+H]^+$.

(*1R, 2R, 3R, 4R, 5R*)-3,4-bis(benzyloxy)-5-(benzyloxymethyl)-cyclohexane-1,2-diol (S8)

To a solution of **S7** [S. H. Yu, S. K. Chung, *Tetrahedron:Asymmetry* **2005**, *16*, 2729.] (8.65g, 17.1mmol) in THF (400ml) at 0°C, was added TBAF (1.0M in THF, 34.4ml, 34.2mmol). The reaction mixture was stirred for 1 hours at RT and then the reaction mixture was quenched with H_2O (4ml). The mixture was concentrated, and chromatographed on silica gel to give triol (4.06g, 89.0%) as colorless oil.

$[\alpha]_D^{23}$ -32.2° (*c* 1.84, CH_2Cl_2); ^{1}H NMR (300MHz, $CDCl_3$): 4.83-4.67 (m, 4H, OC**H₂**OCH₃), 4.01 (br s, 1H, H-2), 3.77-3.66 (m, 3H, H-1, H-6A, H-6B), 3.61 (t, *J* = 9.5Hz, 1H, H-4), 3.46 & 3.37 (2s, 6H, $OCH_2OC\mathbf{H_3}$), 3.32 (app d, J = 9.5Hz, 1H, H-3), 1.73-1.57 (m, 3H, H-5, H-5aα, H-5aβ); ^{13}C NMR (75MHz, $CDCl_3$): 110.1, 99.2, 95.7, 82.1, 75.7, 75.1, 74.9, 67.2, 56.7, 56.2, 40.9, 28.2; HRFABMS calcd. for $C_{11}H_{23}O_7$ 267.1438, found 267.1441 $[M+H]^+$.

To a solution of triol (4.06g, 15.2mmol) in dry THF (180ml) at 0°C, was added NaH (3.99g, 55% in paraffin liquid, 60.8mmol). After stirring for 30 minutes at RT, BnBr (11.0ml, 60.8mmol) and TBAI (1.71g, 3.04mmol) were added. After stirring for 30 hours at RT, the reaction mixture was quenched with drops of sat'd aq. $NaHCO_3$ and the reaction mixture was subjected to the standard extraction workup using EtOAc to give the crude product, which was purified by using column chromatography on silica gel to afford fully protected carba-β-D-mannose (6.12g, 74.5%) as colorless oil.

$[\alpha]_D^{23}$ +8.7° (*c* 1.20, CH_2Cl_2); ^{1}H NMR (300MHz, $CDCl_3$): 7.39-7.23 (m, 15H, Ar-H), 4.94-4.49 (m, 10H, $OC\mathbf{H_2}OCH_3$, $C\mathbf{H_2}Ph$), 4.31 (br s, 1H, H-2), 3.74 (t, *J* = 10.0Hz, 1H, H-4), 3.63-3.50 (m, 3H), 3.43 & 3.40 (2s, 6H, $OCH_2OC\mathbf{H_3}$), 3.43-3.40 (m, 1H), 1.98-1.91 (m, 2H, H-5aα, H-5aβ), 1.73-1.69 (m, 1H, H-5); ^{13}C

NMR (75MHz, $CDCl_3$): 138.4, 138.1, 138.0, 127.9, 127.7, 127.2, 127.1, 96.5, 93.8, 83.4 77.5, 74.8, 73.5, 72.7, 72.6, 71.6, 70.2, 55.1, 53.0, 39.1, 28.2; HRFABMS calcd. for $C_{32}H_{41}O_7$ 537.2847, found 537.2854 $[M+H]^+$.

A solution of this carba-mannose derivative (6.02g, 11.2mmol) in MeOH-water-conc'd. HCl (300ml, 10 : 1 : 0.1) was refluxed at 70°C. After 2 days, the solution was concentrated, and subjected to the standard extraction workup using EtOAc to give the crude product, which was purified by using column chromatography on silica gel to afford **S8** (5.02g, 98.1%) as white solid.

$[\alpha]_D^{23}$ +20.5° (*c* 1.05, CH_2Cl_2); mp 108.0-109.0°C; ^1H NMR (300MHz, $CDCl_3$): 7.48-7.33 (m, 15H, Ar-H), 5.00-4.57 (m, 6H, C**H₂**Ph), 4.27 (br s, 1H, H-2), 3.83 (t, *J* = 9.4Hz, 1H, H-4), 3.70-3.61 (m, 3H), 3.54 (dd, J = 9.2Hz, 2.7Hz, 1H), 2.01-1.95 (m, 2H, H-5aα, H-5aβ), 1.80-1.70 (m, 1H, H-5); ^{13}C NMR (75MHz, $CDCl_3$): 139.1, 138.9, 138.5, 129.0, 128.8, 128.5, 128.3, 128.0, 127.9, 83.5, 77.6, 75.3, 73.1, 72.3, 70.9, 70.4, 69.7, 39.2, 30.9; HRFABMS calcd. for $C_{28}H_{33}O_5$ 449.2323, found 449.2326 $[M+H]^+$.

((*1R, 2R, 6R*)-6-(benzyloxymethyl)cyclohex-3-ene-1,2-diyl)bis(oxy)-bis(methylene)dibenzene (S9)

To a refluxed mixture of **S8** (3.71g, 8.27mmol), PPh_3 (8.68g, 33.1mmol), and imidazole (2.42g, 33.1mmol) in toluene (200ml), was added a solution of iodine (6.93g, 27.3mmol) in toluene (50ml). After 25 minutes, imidazole (2.23g) in toluene (50ml) was added to the mixture. After refluxing for 4 hours, the reaction mixture was cooled to RT, diluted with EtOAc (500ml), washed with half-saturated aqueous $Na_2S_2O_3$ (300ml x 2). The organic layer was dried ($MgSO_4$), concentrated, and chromatographed on silica gel column to give compound **S9** (3.12g, 91.0%) as colorless oil.

$[\alpha]_D^{23}$ -2.2° (*c* 1.00, CH_2Cl_2); ^1H NMR (300MHz, $CDCl_3$): 7.45-7.36 (m, 15H, Ar-H), 5.84 (br d, J = 11.7Hz, 1H, H-1, olefin), 5.77 (br d, J = 11.7Hz, 1H, H-2, olefin), 4.99-4.56 (m, 6H, C**H₂**Ph), 4.27 (dd, J = 7.1Hz, 2.1Hz, 1H, H-3), 3.81-3.72 (m, 2H, H-4, H-6A), 3.66 (dd, J = 8.8Hz, 3.0Hz, 1H, H-6B), 2.35-2.33 (m, 2H, H-5aα, H-5aβ), 2.17-2.16 (m, 1H, H-5); ^{13}C NMR (75MHz, $CDCl_3$): 139.5, 139.2, 139.1, 129.0, 128.9, 128.8, 128.4, 128.3, 128.0, 126.6, 111.0, 81.6, 80.0, 74.9, 73.6, 71.9, 70.9, 39.8, 29.3; HRFABMS calcd. for $C_{28}H_{31}O_3$ 415.2268, found 415.2278 $[M+H]^+$.

(*1S, 2S, 3R, 4R, 5R*)-3,4-bis(benzyloxy)-5-(benzyloxymethyl)-cyclohexane-1,2-diol (S10)

To a solution of **S9** (3.12g, 7.53mmol) and NMO (882mg, 15.1mmol) in acetone-water (70ml, 6:1), was added a catalytic amount of OsO_4 at RT. After stirring for 18 hours, Na_2SO_3 (9.5g) was added and the resulting mixture was further stirred for 30 minutes at RT. The reaction mixture was diluted with ethyl acetate (500ml x 2) and washed with 1N HCl (500ml) and sat'd aq. $NaHCO_3$ (500ml). The organic layers were dried ($MgSO_4$), concentrated, and chromatographed on silica gel column to give compound **S10** (3.13g, 92.7%) as white solid.

$[\alpha]_D^{23}$ +50.3° (*c* 0.70, CH_2Cl_2); mp 85.0-89.0°C; ^{1}H NMR (300MHz, $CDCl_3$): 7.39-7.32 (m, 15H, Ar-H), 5.05-4.50 (m, 6H, C**H₂**Ph), 4.09 (pseudo q, J = 2.9Hz, 1H, H-1), 3.81 (dd, J = 9.2Hz, 4.1Hz, 1H, H-6A), 3.77 (t, J = 9.2Hz, 1H, H-3), 3.57 (t, J = 10.4Hz, 1H, H-4), 3.54 (dd, J = 9.3Hz, 3.0Hz, 1H, H-2), 3.47 (dd, J = 9.0Hz, 2.5Hz, 1H, H-6B), 2.28-2.19 (m, 1H, H-5), 1.95 (dt, J = 14.6Hz, 3.7Hz, 1H, H-5aα), 1.70 (td, J = 13.7Hz, 2.4Hz, 1H, H-5aβ); ^{13}C NMR (75MHz, $CDCl_3$): 139.1, 139.0, 138.9, 129.1, 128.9, 128.8, 128.3, 128.1, 128.0, 84.0, 81.6, 75.7, 75.3, 74.9, 73.5, 70.2, 68.7, 37.8, 31.0; HRFABMS calcd. for $C_{28}H_{33}O_3$ 449.2323, found 449.2325 $[M+H]^+$.

(*1S, 2S, 3R, 4R, 5R*)-3,4-bis(benzyloxy)-5-(benzyloxymethyl)-2-hydroxycyclohexyl benzoate (S11)

To a mixture of **S10** (3.13g, 6.98mmol) and triethyl orthobenzoate (3.44ml, 14.0mmol) in CH_2Cl_2 (120ml) at RT, was added portionwise TSA (133mg, 0.70mmol) over 1 hour. The resulting mixture was concentrated, and 80% aq. AcOH (50ml) added and then stirred for 10 minutes. After removal of AcOH by evaporation, the residue was diluted with EtOAc (300ml) and washed with sat'd aq. $NaHCO_3$ (300ml). The organic layer was dried ($MgSO_4$), concentrated, and chromatographed on silica gel to give **S11** (4.17g, quantitative yield) as oil.

$[\alpha]_D^{23}$ +74.6° (*c* 1.07, CH_2Cl_2); ^{1}H NMR (300MHz, $CDCl_3$): 7.57-7.13 (m, 20H, Ar-H), 5.52 (d, J = 2.2Hz, 1H, H-1), 5.00-4.46 (m, 6H, C**H₂**Ph), 3.88 (t, J = 9.3Hz, 1H, H-3), 3.78-3.72 (m, 2H, H-6A, H-6B), 3.64 (t, J = 9.5Hz, 1H, H-4), 3.41 (dd, J = 8.9Hz, 2.1Hz, 1H, H-2), 2.14-2.00 (m, 2H, H-5, H-5aα), 1.81 (td, J =

14.6Hz, 1.5Hz, 1H, H-5aβ); ^{13}C NMR (75MHz, $CDCl_3$): 166.3, 138.7, 138.5, 138.4, 133.3, 130.4, 129.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 84.2, 80.9, 75.6, 75.5, 73.7, 73.3, 72.1, 69.5, 38.5, 29.3; HRFABMS calcd. for $C_{35}H_{37}O_6$ 553.2585, found 553.2589 $[M+H]^+$.

(*1S, 2S, 3S, 4R, 5R*)-2,3,4-tris(benzyloxy)-5-(benzyloxymethyl)-cyclohexanol (S12)

To a solution of **S11** (2.97g, 5.37mmol) in dry CH_2Cl_2 (30ml) at 0°C, was added hexane (60ml) and benzyl trichloroacetimidate (2.00ml, 10.7mmol). After stirring for 10 minutes, TfOH (2~3 drop with 1ml syringe) were added. After stirring for 20 hours at RT, the reaction mixture was diluted with CH_2Cl_2 (300ml) and washed with sat'd aq. $NaHCO_3$ (300ml), water (300ml), and brine (300ml). The organic layer was dried (Na_2SO_4), concentrated, and chromatographed on silica gel to give fully protected carba-α-D-glucose (3.71g, quantitative yield) as colorless oil.

$[\alpha]_D^{23}$ +45.4° (*c* 0.75, CH_2Cl_2); ^{1}H NMR (300MHz, $CDCl_3$): 7.46-7.16 (m, 25H, Ar-H), 5.79 (d, J = 2.1Hz, 1H, H-1), 5.01-4.46 (m, 8H, C**H₂**Ph), 4.00 (t, J = 9.5Hz, 1H, H-3), 3.77 (dd, J = 8.9Hz, 3.1Hz, 1H, H-6A), 3.63 (t, J = 10.6Hz, 1H, H-4), 3.61 (dd, J = 9.5Hz, 2.9Hz, 1H, H-6B), 3.45 (dd, J = 8.9Hz, 2.4Hz, 1H, H-2), 2.23-2.15 (m, 1H, H-5), 2.05 (dt, J = 14.7Hz, 3.7Hz, 1H, H-5aα), 1.77 (td, J = 14.0Hz, 1.9Hz, 1H, H-5aβ); ^{13}C NMR (75MHz, $CDCl_3$): 166.2, 139.0, 133.4, 130.2, 129.4, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 84.3, 81.9, 16.2, 76.1, 73.5, 72.4, 69.9, 69.0, 60.8, 53.9, 38.3, 29.7; HRFABMS calcd. for $C_{42}H_{43}O_6$ 643.3054, found 643.3057 $[M+H]^+$.

To a solution of this carba-glucose derivative (3.71g, 5.77mmol) in MeOH (65ml), was added NaOMe (4.37M in MeOH, 0.13ml). After strring for 4 hours at RT, the reaction mixture was treated with AcOH (2~3 drops) and concentrated. The residue was directly chromatographed on silica gel to give **S12** (2.26g, 72.8%) as colorless oil.

$[\alpha]_D^{23}$ +37.5° (*c* 1.12, CH_2Cl_2); ^{1}H NMR (500MHz, $CDCl_3$): 7.41-7.22 (m, 20H, Ar-H), 4.96-4.48 (m, 8H, C**H₂**Ph), 4.18 (d, J = 2.5Hz, 1H, H-1), 3.90 (t, J = 9.0Hz, 1H, H-3), 3.81 (dd, J = 9.0Hz, 4.0Hz, 1H, H-6A), 3.55 (td, J = 10.7Hz, 1.0Hz 1H, H-4), 3.50 (dd, J = 10.0Hz, 3.0Hz, 1H, H-2), 3.48 (dd, J = 9.0Hz, 2.5Hz, 1H, H-6B), 2.27-2.19 (m, 1H, H-5), 2.00 (dt, J = 14.5Hz, 3.6Hz, 1H, H-5aα), 1.64 (pseudo td, J =

13.1Hz, 1H, H-5aβ); ^{13}C NMR (75MHz, $CDCl_3$): 139.5, 139.3, 139.0, 138.6, 129.4, 129.0, 128.8, 128.7, 128.3, 127.9, 84.01, 83.8, 81.3, 76.2, 75.7, 73.4, 73.0, 70.2, 67.0, 37.1, 30.6; HRFABMS calcd. for $C_{35}H_{39}O_5$ 539.2792, found 539.2793 $[M+H]^+$.

Dibenzyl-(*1S, 2R, 3S, 4R, 5R*)-2,3,4-tris(benzyloxy)-5-(benzyloxymethyl)cyclohexyl phosphate (S13)

To a solution of **S12** (2.26g, 4.20mmol) and 1H tetrazole (0.45M in acetonitrile, 43.7ml) in CH_2Cl_2 (100ml) was added dibenzyl diisopropylphosphoramidite (4.3ml, 12.6mmol) at RT. After 6 hours, mCPBA (4.3g, 12.6mmol) was added to the mixture at 0℃. After being stirred 1 hour at RT, the mixture was diluted with CH_2Cl_2 (200ml) and washed with sat'd aq. Na_2SO_3 (200ml), sat'd aq. $NaHCO_3$ (200ml), and brine (200ml). The organic layer was dried ($MgSO_4$), concentrated, and chromotographed to give **S13** (2.34g, 70.0%) as colorless oil.

$[\alpha]_D^{23}$ +44.7° (*c* 0.60, CH_2Cl_2); ^{1}H NMR (500MHz, $CDCl_3$): 7.41-7.27 (m, 30H, Ar-H), 5.13-4.45 (m, 12H, C**H₂**Ph), 4.95 (dd, J = 11.0Hz, 4.5Hz, 1H, H-1), 3.91 (t, J = 9.5Hz, 1H, H-3), 3.73 (dd, J = 9.0Hz, 4.0Hz, 1H, H-6A), 3.58 (td, J = 10.5Hz, 1.0Hz 1H, H-4), 3.49 (dt, J = 9.5Hz, 2.5Hz, 1H, H-2), 3.36 (dd, J = 9.0Hz, 2.5Hz, 1H, H-6B), 2.15-2.05 (m, 1H, H-5), 2.04 (dt, J = 15.0Hz, 4.0Hz, 1H, H-5aα), 1.64 (t, J = 13.5Hz, 1H, H-5aβ); ^{13}C NMR (75MHz, $CDCl_3$): 139.3, 139.1, 138.8, 138.4, 129.1, 128.9, 128.8, 128.7, 128.6, 128.3, 128.1, 128.0, 127.9, 83.6, 82.0, 80.7, 76.1, 75.8, 74.8, 73.4, 72.6, 69.6, 69.5, 69.4, 37.6, 30.7; ^{31}P NMR (121.5MHz, $CDCl_3$): 0.3336; HRFABMS calcd. for $C_{49}H_{52}O_8P$ 799.3394, found 799.3391 $[M+H]^+$.

(*1S, 2R, 3S, 4R, 5R*)-2,3,4-trihydroxy-5-(hydroxymethyl)cyclohexyl dihydrogen phosphate (S14)

A mixture of **S13** (1.04g, 1.30mmol) and Pd/C (10wt%, 511mg) in MeOH-CH_2Cl_2 (88ml, 10:1) was hydrogenated (50psi) at RT. After 8 hours, the reaction mixture was filtered through cotton. After concentration, compound **S14** (327mg, 97.2%) was obtained as colorless oil.

$[\alpha]_D^{23}$ +31.6° (*c* 1.12, CH_2Cl_2); ^{1}H NMR (300MHz, D_2O): 4.40 (br s, 1H, H-1), 3.59-3.50 (m, 2H, H-6A, H-6B), 3.45 (t, J = 9.4Hz, 1H, H-3), 3.31 (dt, J = 9.9Hz, 2.8Hz, 1H, H-2), 3.14 (td, J = 9.8Hz, 1.5Hz, 1H, H-4), 1.96 (dt, J = 14.7Hz, 3.7Hz, 1H, H-5aα), 1.80-1.72 (m, 1H, H-5), 1.30 (td, J = 13.3Hz, 1.1Hz, 1H, H-5aβ); ^{13}C NMR (75MHz, D_2O): 75.7, 75.6, 74.8, 73.5, 73.4, 73.1, 62.3, 48.2, 38.3, 29.4; ^{31}P NMR (121.5MHz, D_2O): 1.3126; HRFABMS calcd. for $C_7H_{16}O_8P$ 259.0577, found 259.0580 $[M+H]^+$.

(*1R, 2S, 3R, 4R, 6R*)-2,3-bis(benzyloxy)-4-(benzyloxymethyl)-7-oxa-bicyclo[4.1.0]heptane (S15)

To a solution of **S8** (2.95g, 6.58mmol) in CH_2Cl_2 (50ml) at RT, were added trimethyl orthoacetate (1.25ml, 9.87mmol) and catalytic amount of PPTS (ca. 0.01eq). After stirring for 40 minutes at RT, the reaction mixture was treated with few drops of TEA and concentrated and dried in vacuo. To the residue dissolved in CH_2Cl_2 (50ml), were added TEA (90ul, 0.66mmol) and acetyl bromide (611ul, 16.5mmol) at 0°C. After stirring for 4 hours at RT, the reaction mixture was poured into sat'd aq. $NaHCO_3$ and extracted with CH_2Cl_2 (500ml x 2). The organic phase was dried ($MgSO_4$) and concentrated to give crude mixture of bromoacetoxy compound. To the crude mixture in methanol (100ml), was added NaOMe (4.41ml, 25% in methanol) at RT. After stirring for 30 minutes, the reaction mixture was diluted with EtOAc (500ml x 2) and washed with sat'd aq. $NaHCO_3$. The organic phase was dried ($MgSO_4$), concentrated, and chromatographed on silica gel column to give **S15** (2.70g, 95.4%) as white solid.

$[\alpha]_D^{23}$ +19.4° (*c* 1.07, CH_2Cl_2); mp 63.0-64.0°C; ^{1}H NMR (300MHz, $CDCl_3$): 7.46-7.26 (m, 15H, Ar-H), 4.89-4.48 (m, 6H, C**H₂**Ph), 3.86 (dd, J = 8.1Hz, 1.8Hz, 1H, H-3), 3.69 (dd, *J* = 10.8Hz, 8.2Hz, 1H, H-4), 3.60-3.48 (m, 2H, H-6A, H-6B), 3.36 (dd, J = 4.0Hz, 1.8Hz, 1H, H-2), 3.28 (t, J = 4.1Hz, 1H, H-1), 2.21-2.03 (m, 2H, H-5aα, H-5aβ), 1.90-1.82 (m, 1H, H-5); ^{13}C NMR (75MHz, $CDCl_3$): 139.1, 139.0, 138.9, 128.8, 128.7, 128.5, 128.3, 128.1, 128.0, 127.9, 81.9, 78.2, 75.6, 73.5, 72.8, 70.5, 55.7, 53.9, 40.3, 27.4; HRFABMS calcd. for $C_{28}H_{31}O_4$ 431.2217, found 431.2227 $[M+H]^+$.

(*1R, 2R, 3R, 4R, 6S*)-6-(allyloxy)-2,3-bis(benzyloxy)-4-(benzyloxymethyl)-cyclohexanol (S16)

To a solution of **S15** (1.86g, 4.32mmol) in CH_2Cl_2 (50ml) at RT, were added allyl alcohol (2.0ml, 28mmol) and BF_3OEt_2 (1.14ml, 9.07mmol). After 4 hours, the reaction mixture was quenched by Et_3N, diluted with CH_2Cl_2 (200ml), and washed with water (200ml). The organic phase was dried (Na_2SO_4), concentrated, and chromatographed on silica gel column to give **S16** (2.17g, quantitative yield) as colorless oil.

$[\alpha]_D^{23}$ +24.2° (*c* 0.65, CH_2Cl_2); ^{1}H NMR (300MHz, $CDCl_3$): 7.38-7.26 (m, 15H, Ar-H), 5.88 (m, 1H, $OCH_2C\mathbf{H}=CH_2$), 5.25 (ddd, J = 17.3Hz, 1.8Hz, 1.6Hz, 1H, $-OCH_2CH=C\mathbf{H_\alpha}H_\beta$), 5.16 (ddd, J = 10.3Hz, 1.4Hz, 1.2Hz, 1H, $OCH_2CH=CH_\alpha\mathbf{H_\beta}$), 4.88-4.50 (m, 6H, $C\mathbf{H_2}Ph$), 4.13 (t, J = 3.0Hz, 1H, H-2), 4.06-3.88 (m, 2H, $OC\mathbf{H_2}CH=CH_2$), 3.82 (dd, J = 8.9Hz, 3.0Hz, 1H, H-3), 3.77-3.71 (m, 2H, H-1, H-4), 3.68 (dd, J = 9.0Hz, 5.2Hz, 1H, H-6A), 3.51 (dd, J = 8.9Hz, 2.9Hz, 1H, H-6B), 2.13-2.03 (m, 1H, H-5), 1.93 (td, J = 14.3Hz, 2.6Hz, 1H, H-5aβ), 1.91-1.85 (m, 1H, H-5aα); ^{13}C NMR (75MHz, $CDCl_3$): 139.4, 139.1, 138.8, 135.4, 128.9, 128.8, 128.3, 128.2, 128.0, 116.9, 82.8, 78.2, 78.9, 75.9, 75.4, 73.4, 73.0, 70.8, 70.1, 69.9, 37.7, 27.3; HRFABMS calcd. for $C_{31}H_{37}O_5$ 489.2636, found 489.2644 $[M+H]^+$.

(*1S, 2R, 3S, 4R, 5R*)-2,3,4-tris(benzyloxy)-5-(benzyloxymethyl)-cyclohexanol (S17)

To a solution of **S16** (2.17g, 4.43mmol) in dry THF (100ml) at 0°C, was added NaH (0.290mg, 55% in paraffin liquid, 6.65mmol). After stirring for 30 minutes at RT, BnBr (0.79ml, 6.65mmol) and TBAI (162mg, 0.44mmol) were added. After stirring for 24 hours at RT, the reaction mixture was quenched with drops of sat'd aq. $NaHCO_3$ and the reaction mixture was subjected to the standard extraction workup using EtOAc to give the crude product, which was purified by using column chromatography on silica gel to afford fully protected carba-α-D-mannose (1.68g, 65.5%) as colorless oil.

$[\alpha]_D^{23}$ +16.8° (*c* 1.07, CH_2Cl_2); ^{1}H NMR (300MHz, $CDCl_3$): 7.41-7.26 (m, 20H, Ar-H), 5.81 (m, 1H, $OCH_2C\mathbf{H}=CH_2$), 5.18 (ddd, J = 17.3Hz, 1.7Hz, 1.6Hz, 1H, $OCH_2CH=C\mathbf{H_\alpha}H_\beta$), 5.12 (ddd, J = 10.4Hz, 1.5Hz, 1.4Hz, 1H, $OCH_2CH=CH_\alpha\mathbf{H_\beta}$), 4.96-4.51 (m, 8H, $C\mathbf{H_2}Ph$), 3.96-3.77 (m, 2H, $OC\mathbf{H_2}CH=CH_2$), 3.88-3.84 (m, 3H), 3.69-3.64 (m, 2H), 3.55 (dd, J = 8.9Hz, 2.9Hz, 1H, H-6B), 2.13-2.04 (m, 1H, H-5), 1.97-1.88 (m, 2H, H-5aα, H-5aβ); ^{13}C NMR (75MHz, $CDCl_3$): 139.6, 139.34, 139.3, 139.2, 128.7, 128.5, 128.3, 128.1, 127.9, 127.8, 116.8, 82.7, 78.7, 76.8, 75.5, 74.9, 73.3, 73.2, 73.1, 71.1, 69.9, 38.2, 27.8; HRFABMS calcd. for $C_{38}H_{43}O_5$ 579.3105, found 579.3115 $[M+H]^+$.

To a solution of this carba-mannose derivative (1.68g, 2.90mmol) in aq. AcOH (42ml, AcOH : water = 20 : 1) at RT, were added $PdCl_2$ (771mg, 4.35mmol) and NaOAc (1.19g, 14.5mmol). After 20 hours, the reaction mixture was diluted with EtOAc (300ml) and washed with sat'd aq. $NaHCO_3$ (300ml). The organic layers were dried ($MgSO_4$), concentrated, and chromatographed on silica gel column to give compound **S17** (1.19g, 76.1%) as colorless oil.

$[\alpha]_D^{23}$ +14.1° (*c* 1.12, CH_2Cl_2); ^1H NMR (300MHz, $CDCl_3$): 7.38-7.25 (m, 20H, Ar-H), 4.77-4.47 (m, 8H, C**H₂**Ph), 4.03 (m, 1H), 3.88-3.86 (m, 2H), 3.74(pseudo dd, J = 5.4Hz, 1H), 3.61-3.58 (m, 2H), 2.25-2.15 (m, 1H, H-5), 2.05-1.94 (m, 1H, H-5aβ), 1.78 (dt, J = 14.1Hz, 5.2Hz, 1H, H-5aα); ^{13}C NMR (75MHz, $CDCl_3$): 139.4, 139.2, 139.1, 128.7, 128.5, 128.3, 128.1, 128.0, 127.9, 127.8, 126.3, 82.0, 78.4, 76.8, 76.6, 76.3, 75.4, 75.1, 74.8, 73.4, 73.3, 73.1, 73.0, 71.0, 38.3, 27.6, 26.9; HRFABMS calcd. for $C_{35}H_{39}O_5$ 539.2792, found 539.2794 $[M+H]^+$.

Dibenzyl-(*1S, 2S, 3S, 4R, 5R*)-2,3,4-tris(benzyloxy)-5-(benzyloxymethyl)cyclohexyl phosphate (S18)

To a solution of **S17** (1.19g, 2.21mmol) and 1H tetrazole (0.45M in acetonitrile, 23ml) in CH_2Cl_2 (50ml) was added dibenzyl diisopropylphosphoramidite (2.3ml, 6.63mmol) at RT. After 6 hours, mCPBA (2.3g, 6.63mmol) was added to the mixture at 0℃. After being stirred 1 hour at RT, the mixture was diluted with CH_2Cl_2 (200ml) and washed with sat'd aq. Na_2SO_3 (200ml), sat'd aq. $NaHCO_3$ (200ml), and brine (200ml). The organic layer was dried ($MgSO_4$), concentrated, and chromotographed to give **S18** (1.35g, 76.5%) as colorless oil.

$[\alpha]_D^{23}$ +4.6° (*c* 1.30, CH_2Cl_2); ^1H NMR (300MHz, $CDCl_3$): 7.32-7.22 (m, 30H, Ar-H), 5.05-4.45 (m, 12H, C**H₂**Ph), 4.69-4.66 (m, 1H, H-1), 3.92 (t, J = 2.9Hz, 1H, H-2), 3.86 (t, J = 9.5Hz, H-4), 3.72 (dd, J = 9.3Hz, 2.8Hz 1H, H-3), 3.61 (dd, J = 9.0Hz, 4.5Hz, 1H, H-6A), 3.43 (dd, J = 8.9Hz, 1.9Hz, 1H, H-6B), 2.05-2.01 (m, 2H, H-5, H-5aβ), 1.88-1.84 (dd, J = 10.3Hz, 1.9Hz, 1H, H-5aα); ^{13}C NMR (75MHz, $CDCl_3$): 139.4, 139.1, 138.8, 129.0, 128.7, 128.4, 128.3, 128.1, 128.1, 128.0, 127.9, 12.78, 81.7, 78.0, 76.5, 76.4, 75.5, 74.9, 74.8, 73.4, 72.9, 70.4, 69.8, 69.8, 69.7, 69.6, 38.0, 29.3; ^{31}P NMR (121.5MHz, $CDCl_3$): -0.4528; HRFABMS calcd. for $C_{49}H_{52}O_8P$ 799.3394, found 799.3398 $[M+H]^+$.

(*1S, 2S, 3S, 4R, 5R*)-2,3,4-trihydroxy-5-(hydroxymethyl)cyclohexyl dihydrogen phosphate (S19)

A mixture of **S18** (0.83g, 1.04mmol) and Pd/C (10wt%, 407mg) in MeOH-CH_2Cl_2 (70ml, 10:1) was hydrogenated (50psi) at RT. After 8 hours, the reaction mixture was filtered through cotton. After concentration, compound **S19** (263mg, 98.0%) was obtained as colorless oil.

$[\alpha]_D^{23}$ +7.9° (*c* 0.80, CH_2Cl_2); ^{1}H NMR (300MHz, D_2O): 4.26-4.22 (m, 1H, H-1), 3.91 (t, J = 3.0Hz, 1H, H-2), 3.60-3.45 (m, 3H, H-3, H-6A, H-6B), 3.39 (t, J = 10.0Hz, 1H, H-4), 1.73 (td, J = 13.1Hz, 3.0Hz, 1H, H-5aβ), 1.69-1.66 (m, 1H, H-5), 1.57 (br d, J = 13.6Hz, 1H, H-5aα); ^{13}C NMR (75MHz, D_2O): 74.3, 74.2, 72.5, 71.7, 71.6, 70.3, 62.6, 49.2, 38.9, 27.8, 27.7, 18.6; ^{31}P NMR (121.5MHz, D_2O): -0.0475; HRFABMS calcd. for $C_7H_{16}O_8P$ 259.0577, found 259.0586 $[M+H]^+$.

***in vitro* OGT Inhibition Assay Part** [(a) W. Lubas, J. A. Hanover, *J. Biol. Chem.* **2000**, *275*, 10983. (b) S. Marshall, T. Duong, R. J. Orbus, J. M. Rumberger, R. Okuyama, *Anal. Biochem.* **2003**, *314*, 169.]

The FLAG tagged human ncOGT was expressed in 293T cell line and immunoprecipitated using FLAG/agarose bead (Sigma, F2426). The following reagents were added: ncOGT binding bead (20μl), purified CKII protein (BioLabs P6010, 2.2μg), UDP-GlcNAc (20μM), assay buffer (25mM Hepes pH7.0, 10mM $MgCl_2$, 1mM EDTA) and H_2O up to 40μl. The reaction mixture was incubated for 1hour at 37°C and mixed gently at each 10 minutes. The reaction was stopped by adding SDS sample buffer (0.0642M Tris pH6.8, 10% glycerol, 2% SDS, 0.002% BPB, 5% β-mercaptoethanol). After quick centrifugation, the supernatant was subjected to SDS-PAGE and transfered to nitrocellular membrane (Hybond ECL, Amersham Biosciences, RPN303D). And then Western blot was preformed with casein kinase IIα antibody (Santa Cruz Biotechnology, Inc. Sc-12738), *O*-GlcNAc monoclonal antibody (CTD110.6, Covance, MMS-248R) and anti-FLAG M2 monoclonal antibody (Sigma, F3165). West-one™ solution (iNtRON biotechnology) was sprayed to the membrane and the image was analyzed by LAS-4000 (FUJIFILM corporation). The density of band was calculated by Multi-guage V3.1 program.
The amount of *O*-GlcNAcylation on CKIIα after OGT assay was calculated as density of band visualized by CTD110.6 was devided by densities of CKIIα and FLAG band.