Synthesis of ^{13}C-Labeled Biosynthetic Precursor of Lipid A and Its Analogue with Shorter Acyl Chains

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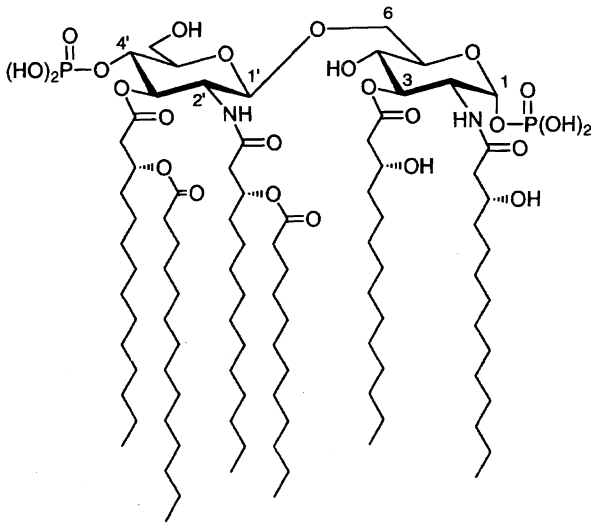
The synthesis of regiospecifically ^{13}C-labeled compounds of a biosynthetic precursor of lipid A and its analogue with shorter acyl chains is described. D-(6-^{13}C)Glucose was converted into a suitably protected glucosamine derivative via 1,6-anhydro-β-D-(6-^{13}C)glucose. After coupling with glycosyl donors, the desired compounds were synthesized through a 6-step reaction sequence. The total yields were 1.7% for the biosynthetic precursor, and 6.4% for the short acyl analogue, respectively, for a total of 18 steps from D-(6-^{13}C)glucose.

Lipid A (Chart 1) is a hydrophobic partial structure of lipopolysaccharide (LPS, endotoxin) located at the cell surface of Gram-negative bacteria, such as *Escherichia coli*, and is fully responsible for the endotoxic activity of LPS, including lethal toxicity, pyrogenicity, induction of hypotension and sepsis syndrome, enhancement of immunological responses, and antitumor activity.[1] By contrast, a biosynthetic precursor **1** that lacks two acyl groups of lipid A is known to have an antagonistic activity against the bioactivity of LPS and lipid A in human systems (Chart 2).[2,3] There are many other known structural variants of lipid A with different bioactivities, and it has been assumed that distinctive conformations of individual lipid A analogues are responsible for their bioactivities.[4,5] We have recently synthesized new analogues of **1** by a divergent pathway.[6] One analogue **2** among them with shorter acyl chains was found to exhibit neither toxic nor antagonistic activity. An analysis of the conformational difference between **1** and **2** has therefore been required to clarify the requisite conformation of lipid A analogues for their bioactivity.



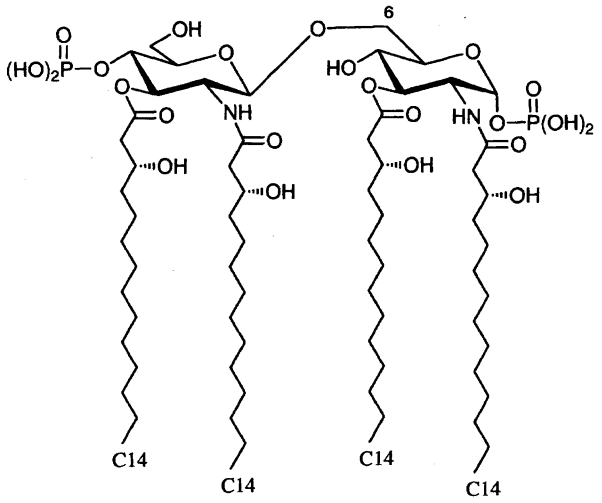
Lipid A from *Escherichia coli*

Chart 1.

In a conformational study of **1** and **2**, it was important to determine the relative arrangement of the two pyranoside rings which are united by three bonds, because the relative orientation of the glucosamine residues plays a central role in determining the shape of the molecule. For a precise analysis of the conformations around the C_5–C_6 (ω), C_6–O_6 (ψ), and O_6–$C_{1'}$ (ϕ) bonds, the respective long-range carbon–proton coupling constants in NMR, $^2J_{C6,H5}$, $^3J_{C1',H6}$, and $^3J_{C6,H1'}$ are required (Fig. 1).[7] In this paper we describe the synthesis of 6-^{13}C-**1** and 6-^{13}C-**2**, which were labeled at the 6-position with ^{13}C, for an unequivocal conformational study through a facile determination of the $^2J_{C6,H5}$ and $^3J_{C6,H1'}$ values.

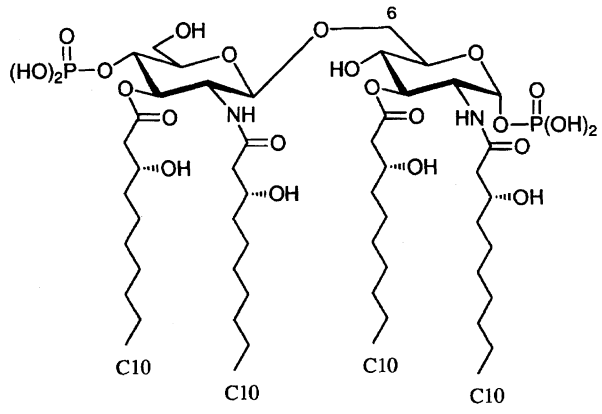
Results and Discussion

Synthesis of the ^{13}C-Labeled Glycosyl Acceptor (14). D-(6-^{13}C)Glucose was employed as the starting material for the ^{13}C-labeled reducing-side glucosamine. To transform the glucose into a suitably protected glucosamine derivative efficiently, 1,6-anhydro-β-D-glucose (levoglucosan, **6**) was expected to be the best synthetic intermediate readily available (vide infra).[8] Among several reports presented concerning the transformation of glucose into 1,6-anhydro-β-D-glucose,[9] we focused our attention on the method of Nagarayan et al.,[10] which involves Lewis acid-promoted intramolecular glycosidation between the trityl (Tr) ether and the glycosyl acetate functionalities of 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-D-glucopyranose (**A** in Scheme 1). It turned out, however, that the product yield depended upon the reaction scale when diethyl ether–boron trifluoride (1/1) (BF_3·OEt_2) was used as a Lewis-acid catalyst: 90% yield on 0.34 mmol of **A**, whereas 50% yield on 0.84 mmol of **A**. Concerning the use of tin



1 : Biosynthetic precursor of lipid A

6-^{13}C-1 (Carbon at 6-position is labeled with ^{13}C)



2 : Short acyl analogue

6-^{13}C-2 (Carbon at 6-position is labeled with ^{13}C)

Chart 2.

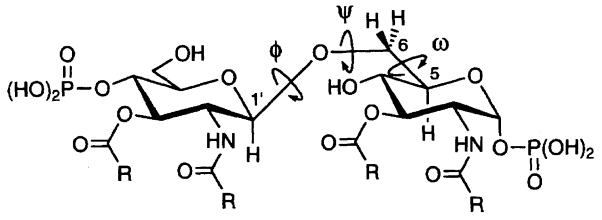
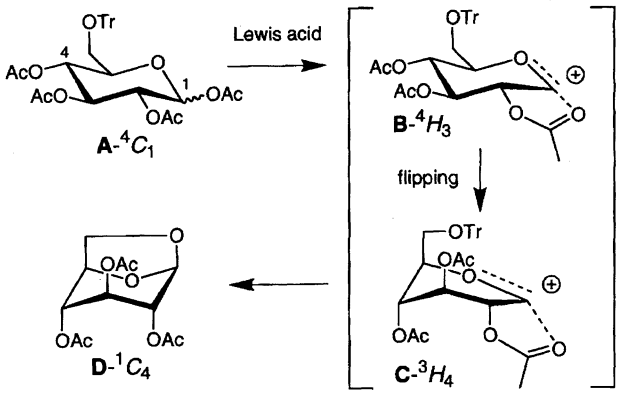


Fig. 1. Dihedral angles which determine the overall conformation of the molecule. The hydrophobic moieties are abbreviated as R (RCO = (*R*)-3-hydroxyalkanoyl).

(IV) chloride ($SnCl_4$), the yield was not reproducible either (55—82% on 0.34 mmol of **A**) by the reported procedure. The problem arises from intermolecular glycosidation, which competes with the desired reaction. The occurrence of the kinetically disfavored intermolecular reaction seemed to indicate that the rate of the intramolecular reaction is not much higher than that of the undesired intermolecular one, owing to the required conformational flipping of the hexose ring in the pseudo half-chair form before cyclization (**B**-$^4H_3$ →**C**-$^3H_4$ in Scheme 1).[11] The problem, however, was fortunately

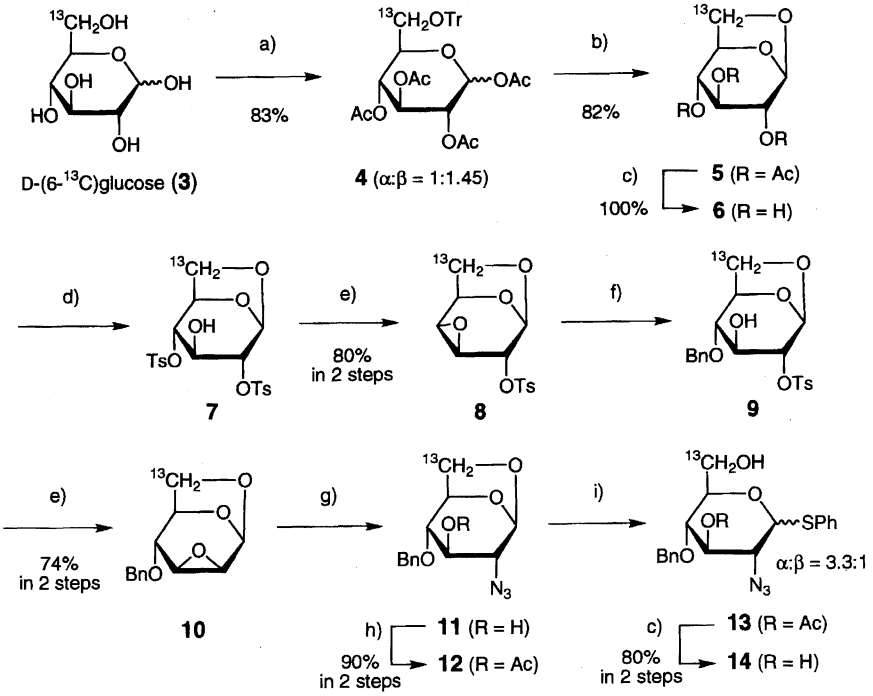


Scheme 1. Reaction mechanism for the formation of the 1,6-anhydro bridge.

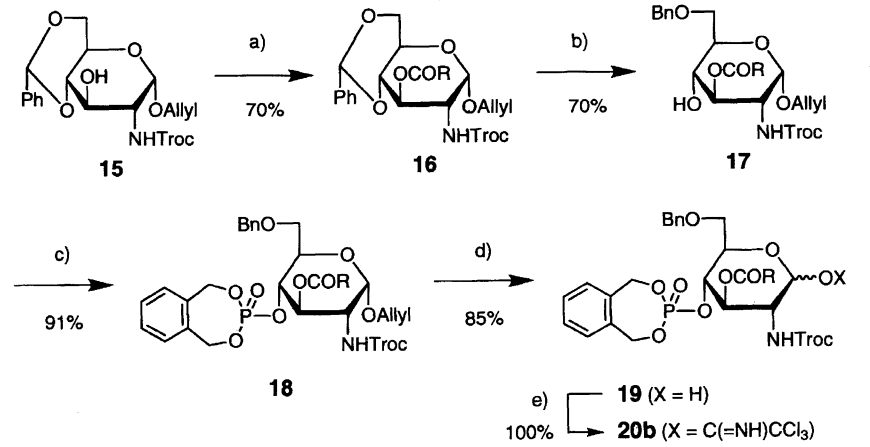
solved by inverse-mode mixing, wherein a 1,2-dichloroethane ($(CH_2Cl)_2$) solution of **4**, obtained from D-(6-^{13}C)glucose (**3**) by a one-pot procedure of tritylation followed by acetylation in 83% yield, was added dropwise into a solution of $SnCl_4$ at room temperature (Scheme 2). In this procedure the reaction proceeded cleanly with high reproducibility to yield **5** in over 80% yield.

The thus-obtained acetate **5** was quantitatively deacetylated by the action of sodium methoxide (MeONa) in methanol (MeOH) to afford 1,6-anhydro-β-D-(6-^{13}C)glucose (**6**). The regioselective tosylation of **6**[12] by *p*-toluenesulfonyl chloride (TsCl) and pyridine, followed by an alkaline treatment, gave the epoxide **8** in good yield (80%) and with complete selectivity. The epoxide **8**[13] was then cleaved by benzyl alcohol (BnOH) in the presence of a catalytic amount of 10-camphorsulfonic acid (CSA) to provide the 4-*O*-Bn ether **9**, which was converted into the epoxide **10** in 74% yield. To introduce a suitably protected nitrogen functionality at the 2-position, **10** was next treated with lithium azide (LiN_3)[14] in the presence of NH_4Cl to furnish, after acetylation, **12** in satisfactory yield. With the latent glucosamine derivative **12** in hand, an acidic opening of the anhydro bridge of **12** with (phenylthio)trimethylsilane[15] was then applied, and after subsequent deacetylation the ^{13}C-labeled glycosyl acceptor **14** for both 6-^{13}C-**1** and 6-^{13}C-**2** was obtained as a mixture of anomers (α/β = 3.3/1). The yield of **14** from D-(6-^{13}C)glucose (**3**) was 29% through 11 steps.[16]

Synthesis of the Glycosyl Donor (20b). The synthesis of the glycosyl donor **20a** for 6-^{13}C-**1** had been reported previously in an improved synthesis of the biosynthetic precursor **1** from our laboratory.[3c] The glycosyl donor **20b** for 6-^{13}C-**2** was prepared in the same manner from the protected glucosamine derivative **15**, which can be prepared from D-glucosamine hydrochloride in more than 50% yield in 3 steps. After acylation with (*R*)-3-(benzyloxy)decanoic acid[6] in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) (Scheme 3), the reductive opening of the benzylidene acetal functionality was effected by our method[17] (dimethylamine–borane (1/1), BF_3·OEt_2) in 70% yield. Thus, by employing these reagents in acetonitrile, the 6-*O*-Bn derivative **17** was formed exclusively, as expected



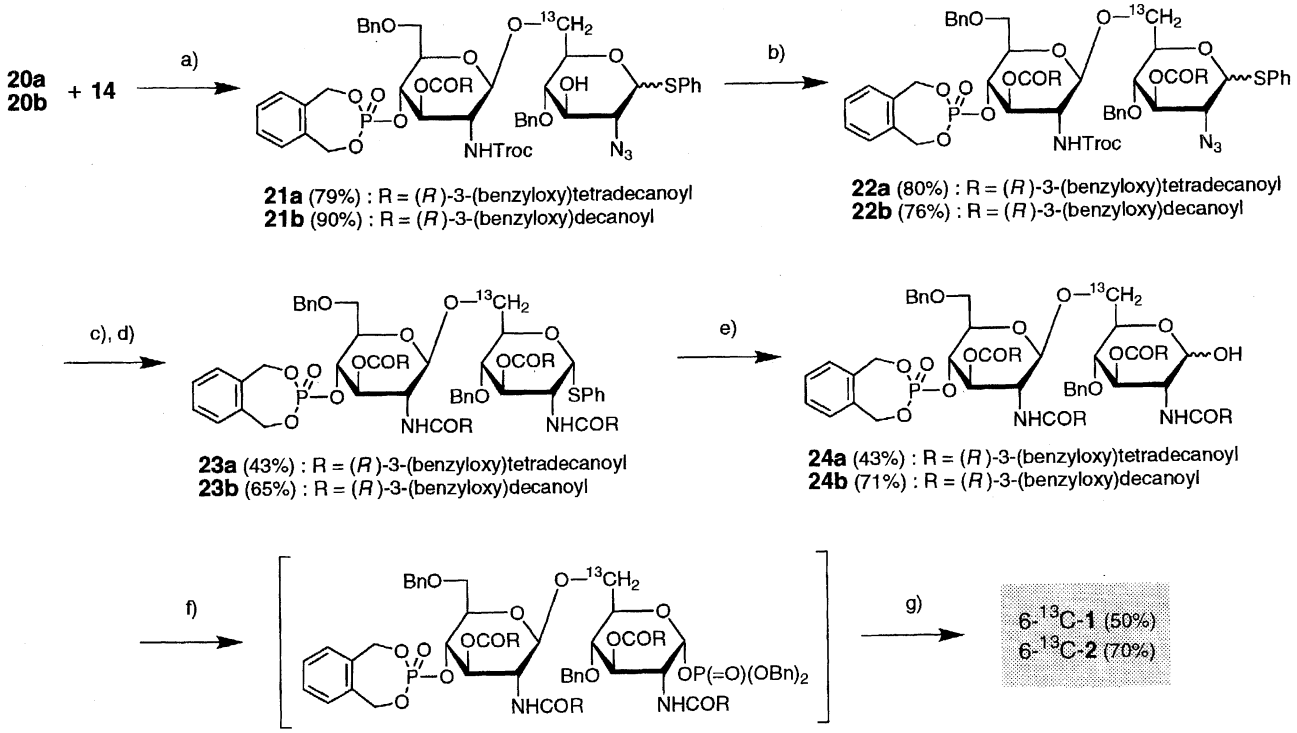
Scheme 2. Tr = triphenylmethyl. Ts = *p*-tolylsulfonyl. Bn = benzyl. a) TrCl, pyridine, 40 °C; Ac_2O, 0 → 23 °C. b) $SnCl_4$, $(CH_2Cl)_2$, 23 °C. c) MeONa, MeOH, 23 °C. d) TsCl, pyridine, 0 °C. e) MeONa, MeOH, $CHCl_3$, 23 °C. f) BnOH, 10-camphorsulfonic acid, toluene, 100 °C. g) LiN_3, NH_4Cl, DMF, 100 °C. h) Ac_2O, pyridine, 0 → 23 °C. i) (phenylthio)-trimethylsilane, ZnI_2, $(CH_2Cl)_2$, 0 → 23 °C; citric acid, MeOH, 23 °C.



Scheme 3. Troc = 2,2,2-trichloroethoxycarbonyl. RCO = (*R*)-3-(benzyloxy)decanoyl. a) (*R*)-3-(benzyloxy)decanoic acid, DCC, DMAP, $(CH_2Cl)_2$, 23 °C. b) BH_3·Me_2NH, BF_3·OEt_2, acetonitrile, −20 → 23 °C. c) *N,N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine, 1*H*-tetrazole, CH_2Cl_2, 23 °C; *m*CPBA, −20 °C. d) [Ir(cod)($MePh_2P$)$_2$]PF_6, THF, 23 °C; I_2, H_2O, 23 °C. e) CCl_3CN, Cs_2CO_3, CH_2Cl_2, 23 °C.

from our empirical rule. The alcohol **17** was successfully phosphitylated with Watanabe's reagent,[18] and the following oxidation with *m*-chloroperbenzoic acid (*m*CPBA) at −20 °C gave the phosphate **18** in 91% yield. Deallylation of **18** by a cationic iridium complex and a subsequent treatment with trichloroacetonitrile and Cs_2CO_3 led to the glycosyl donor **20b**[19] in 85% yield.

Disaccharide Formations and Synthesis of the Desired 6-^{13}C-Labeled Analogues. As shown in Scheme 4, trimethylsilyl trifluoromethanesulfonate (TMSOTf)-mediated coupling of the glycosyl donors, **20a** and **20b**,[19] with the ^{13}C-labeled glycosyl acceptor **14** proceeded regio- and stereoselectively to afford disaccharides **21a** and **21b** in good yields (78 and 90% yield, respectively). The stereochemistry of the disaccharides was controlled by the neighboring group participation of the *N*-carbamate protecting group. Indeed, the β-glycoside structure was confirmed by the large proton-proton coupling constants (7.8 Hz for **21a** and 8.3 Hz for **21b**) between $H_{1'}$ and $H_{2'}$,[7] and a (1 → 6) disaccharide linkage was confirmed by the next acylation at the 3-hydroxy group. Thus, **21a** and **21b** were acylated with (*R*)-3-(benzyloxy)alkanoic acids[6,20] by the action of 1-[3-(dimethylami-



Scheme 4. a) TMSOTf, MS4A, $(CH_2Cl)_2$, −35 °C. b) (*R*)-3-(benzyloxy)tetradecanoic acid or (*R*)-3-(benzyloxy)decanoic acid, WSCD·HCl, DMAP, CH_2Cl_2, 0 → 23 °C. c) Zn–Cu, acetic acid, 23 °C. d) (*R*)-3-(benzyloxy)tetradecanoic acid or (*R*)-3-(benzyloxy)decanoic acid, WSCD·HCl, HOAt, $CHCl_3$, 23 °C. e) NBS, acetone, water, −30 °C. f) $((BnO)_2PO)_2O$, $LiN(TMS)_2$, THF, −78 °C. g) H_2 (7 kg cm^{-2}), Pd black, THF, 23 °C.

no)propyl]-3-ethylcarbodiimide hydrochloride (water-soluble carbodiimide hydrochloride, WSCD·HCl) and DMAP to give the diacylated compounds **22a** and **22b**. Reductive diamine generation by a zinc–copper couple in acetic acid and the following *N*-acylation using WSCD·HCl and 3-hydroxy-3*H*-1,2,3-triazolo[4,5-*b*]pyridine (1-hydroxy-7-azabenzotriazole, HOAt) provided fully acylated **23a** and **23b** in moderate yields. At this stage, the α/β ratio, which originated from the acceptor **14**, changed to afford the α-anomers as the sole products, because the β-anomers were far less reactive toward *N*-acylation. To liberate the 1-hydroxy group for subsequent phosphorylation, the phenylthio group was removed with *N*-bromosuccinimide (NBS) in a mixture of acetone and water to give **24a** and **24b**.[21] α-Selective phosphorylation at the 1-position[22] was then effected at −78 °C by tetrabenzyl diphosphate in the presence of lithium bis (trimethylsilyl)-amide, which is not nucleophilic and can be more easily handled than butyllithium, which was often employed in our previous work. All of the benzyl-type protecting groups were removed[3,6,23] by hydrogenolysis to complete the synthesis of the 6-^{13}C-labeled lipid A analogues, which were identical to the respective authentic nonlabeled specimens[3,6] on TLC. Both synthetic materials were unambiguously characterized by the MS and NMR spectra, and were pure enough for a conformational analysis by NMR. The total yields were 1.7% for 6-^{13}C-**1** and 6.4% for 6-^{13}C-**2**, both in 18 steps from D-(6-^{13}C)glucose.

Experimental

The melting points were determined with a Yamato Melting Point Apparatus Model MP-21 and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were measured at 30 °C in the indicated solvent with a JEOL JNM-LA500 or Varian UNITY plus600 spectrometer, and analyzed using the Felix® program (version 97.0, Molecular Simulations). Proton chemical shifts were referenced to residual protons of solvents: $CDCl_3$ (7.24 ppm), D_2O (4.65 ppm), and DMSO-d_6 (2.565 ppm). Carbon chemical shifts were referenced to solvents ($CDCl_3$ = 77.0 ppm, DMSO-d_6 = 39.5 ppm), except that external tetramethylsilane (0 ppm) was employed for D_2O solutions. Mass spectra were obtained on a JEOL JMS-SX102 mass spectrometer for FAB. Used for ESI-MS were a API III Biomolecular Mass analyzer (*PE SCIEX*) or a ESI-TOF mass spectrometer (PerSeptive Biosystems, Mariner™). 3-Nitrobenzyl alcohol was employed for a matrix on every measurement. Elemental analyses were performed by the staff of our faculty. Silica-gel flash chromatography was performed on Kieselgel 60 Art.9385 (Merck) or Silica Gel 60 (nacalai tesque) using the indicated solvent system at medium-pressure (2—4 kg cm^{-2}).

Unless otherwise noted, nonaqueous reactions were carried out under a nitrogen atmosphere. D-(6-^{13}C)glucose was purchased from Cambridge Isotope Laboratories (Andover, MA 01810, USA). Anhydrous 1,2-dichloroethane and dichloromethane (CH_2Cl_2) were distilled from calcium hydride, whereas chloroform ($CHCl_3$) was distilled from phosphorus pentoxide. Other anhydrous solvents, such as *N*,*N*-dimethylformamide, acetonitrile, tetrahydrofuran (THF), and toluene, were purchased from Kanto Chemicals Co. Molecular sieves 4A were activated in vacuo at 250 °C for 3 h

before use. All other commercially obtained materials were used as received.

1,2,3,4-Tetra-*O*-acetyl-6-*O*-triphenylmethyl-D-(6-^{13}C)glucopyranose (4). To a solution of D-(6-^{13}C)glucose (**3**, 2.00 g, 11.0 mmol) in pyridine (15 mL) was added triphenylmethyl chloride (3.69 g, 13.2 mmol). The solution was stirred for 15 h at 40 °C. Triphenylmethyl chloride (0.24 g, 0.86 mmol) was added again and stirring was continued for 3 h. To the reaction mixture at 0 °C was added acetic anhydride (8.33 mL, 88.3 mmol), and stirring of the mixture was continued at room temperature for 3 h. Then, the reaction was quenched by the addition of ethanol (20 mL), and the mixture was concentrated by coevaporation with toluene (3×50 mL). The syrup was dissolved in $CHCl_3$ (50 mL), washed with saturated aqueous $NaHCO_3$ (2×80 mL) and brine (60 mL), and dried over Na_2SO_4. The solution was concentrated in vacuo and purified by silica-gel flash chromatography (240 g, hexane/EtOAc = 2/1) followed by precipitation in 95% ethanol to afford the fully protected compound **4** as a white powdered mixture of anomers (α/β = 1/1.45, 5.4 g, 83%). $[\alpha]_D^{25}$ +78.1 (*c* 1, $CHCl_3$); FAB-MS (positive) *m/z* 614 [(M+Na)$^+$]. Found: C, 67.14; H, 5.66%. Calcd for $C_{31}{}^{13}C_1H_{34}O_{10}$: C, 67.16; H, 5.79%.

Data for the α-Anomer of 4: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.43—7.41 (m, 6 H), 7.31—7.21 (m, 9 H), 6.44 (d, *J* = 3.7 Hz, 1 H), 5.41 (t, *J* = 9.8 Hz, 1 H), 5.31 (ddd, *J* = 10.2, 10.0, 3.0 ($^3J_{C6,H4}$) Hz, 1 H), 5.17 (dd, *J* = 10.3, 3.7 Hz, 1 H), 4.00—4.03 (m, 1 H), 3.31 (ddd, *J* = 145.0 ($^1J_{C6,H6a}$), 10.6, 2.3 Hz, 1 H), 3.03 (ddd, *J* = 143.5 ($^1J_{C6,H6b}$), 10.5, 3.7 Hz, 1 H), 2.15 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.73 (s, 3 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 61.8 (C_6).

Data for the β-Anomer of 4: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.43—7.41 (m, 6 H), 7.31—7.21 (m, 9 H), 5.72 (d, *J* = 8.0 Hz, 1 H), 5.23—5.28 (m, 1 H), 5.19—5.16 (m, 2 H), 3.67—3.71 (m, 1 H), 3.33 (ddd, *J* = 143.1 ($^1J_{C6,H6a}$), 10.5, 4.2 Hz, 1 H), 3.07 (ddd, *J* = 143.9 ($^1J_{C6,H6b}$), 10.7, 2.5 Hz, 1 H), 2.15 (s, 3 H), 2.04 (s, 3 H), 2.00 (s, 3 H), 1.74 (s, 3 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 62.0 (C_6).

2,3,4-Tri-*O*-acetyl-1,6-anhydro-β-D-(6-^{13}C)glucopyranose (5). A solution of **4** (1.00 g, 1.69 mmol) in anhydrous 1,2-dichloroethane (20 mL) was dropped into a vigorously stirred solution of $SnCl_4$ (0.39 mL, 3.3 mmol) in anhydrous 1,2-dichloroethane (30 mL) at room temperature over a period of 1.5 h. The mixture was stirred for another 4.5 h at room temperature, and then poured into saturated aqueous $NaHCO_3$ (300 mL) at 0 °C. To the mixture was added $CHCl_3$ (150 mL) and the organic layer was separated. The organic layer was washed with brine (80 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (100 g, hexane/EtOAc = 1/1) followed by lyophilization with benzene to give the anhydro compound **5** as a white powder (401 mg, 82%). $[\alpha]_D^{25}$ −62.4 (*c* 1, $CHCl_3$); FAB-MS (positive) *m/z* 290.0 [(M+H)$^+$]; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.47 (d, *J* = 5.2 Hz, 1 H), 4.88—4.86 (m, 1 H), 4.65 (br d, *J* = 1.2 Hz, 1 H), 4.63 (dt, *J* = 5.8, 1.1 Hz, 1 H), 4.63—4.61 (m, 1 H), 4.10 (ddd, *J* = 151.4 ($^1J_{C6,H6a}$), 7.8, 1.0 Hz, 1 H), 3.81 (ddd, *J* = 154.3 ($^1J_{C6,H6b}$), 7.9, 5.7 Hz, 1 H), 2.17 (s, 3 H), 2.15 (s, 3 H), 2.12 (s, 3 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 65.4 (C_6). Found: C, 50.45; H, 5.52%. Calcd for $C_{10}{}^{13}C_1H_{16}O_8$: C, 50.17; H, 5.58%.

1,6-Anhydro-β-D-(6-^{13}C)glucopyranose (6). To a solution of **5** (875 mg, 3.03 mmol) in MeOH (15 mL) was added sodium methoxide in MeOH (1 M = 1 mol dm^{-3}), 0.04 mL, 0.04 mmol). The mixture was stirred at room temperature for 3 h. The mixture was adjusted to pH 7.0 by the addition of Dowex® 50W-X8 (H$^+$ form), and all insoluble materials were removed by filtration. The filtrate was concentrated in vacuo and lyophilized from dioxane to give crude triol **6** as a glassy syrup (494 mg, 100%), which was used for the next reaction without purification. $[\alpha]_D^{25}$ −62.6 (*c* 0.5, H_2O); FAB-MS (positive) *m/z* 163.9 [(M+H)$^+$]; ^{1}H NMR (500 MHz, D_2O) δ = 5.35 (d, *J* = 5.0 Hz, 1 H), 4.53 (dt, *J* = 5.7, 1.4 Hz, 1 H), 4.00 (ddd, *J* = 151.5 ($^1J_{C6,H6a}$), 7.8, 1.1 Hz, 1 H), 3.66 (ddd, *J* = 154.2 ($^1J_{C6,H6b}$), 7.8, 5.8 Hz, 1 H), 3.60—3.57 (m, 2 H), 3.44—3.43 (m, 1 H); ^{13}C NMR (125.65 MHz, D_2O) δ = 66.4 (C_6). Found: C, 44.88; H, 6.07%. Calcd for $C_4{}^{13}C_1H_{10}O_5$: C, 44.78; H, 6.18%.

1,6-Anhydro-2,4-di-*O*-*p*-tolylsulfonyl-β-D-(6-^{13}C)glucopyranose (7) and 1,6:3,4-Dianhydro-2-*O*-*p*-tolylsulfonyl-β-D-(6-^{13}C)-galactopyranose (8). To a solution of **6** (239 mg, 1.46 mmol) in pyridine (7 mL) at 0 °C was added *p*-toluenesulfonyl chloride (860 mg, 4.51 mmol) in pyridine (2 mL). The mixture was stirred at 0 °C for 14 h. For completion of the reaction, another portion of *p*-toluenesulfonyl chloride (340 mg, 1.78 mmol) in pyridine (2 mL) was added and the mixture was stirred at room temperature for an additional 6 h. The reaction was quenched by the addition of water (7 mL) and saturated aqueous $NaHCO_3$ (5 mL). The solution was diluted with $CHCl_3$ (50 mL), and washed successively with saturated aqueous $NaHCO_3$ (20 mL), aqueous HCl (1 M, 20 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo, and the residual solvent coevaporated with toluene (3×50 mL) to give crude ditosylate **7** as a syrup (730 mg). Pure **7** (20 mg) for characterization was obtained as a glassy syrup by silica-gel flash chromatography (20 g, hexane/EtOAc = 1/1). $[\alpha]_D^{25}$ −39.8 (*c* 0.5, $CHCl_3$); ESI-MS (positive, Mariner™) *m/z* 494.1 [(M+Na)$^+$]; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.81—7.78 (m, 4 H), 7.40—7.37 (m, 4 H), 5.33 (d, *J* = 4.8 Hz, 1 H), 4.64 (d, *J* = 5.3 Hz, 1 H), 4.34 (m, 1 H), 4.18 (d, *J* = 3.7 Hz, 1 H), 3.98 (dd, *J* = 147.2 ($^1J_{C6,H6a}$), 8.1 Hz, 1 H), 3.94—3.91 (m, 1 H), 3.67 (ddd, *J* = 153.5 ($^1J_{C6,H6b}$), 8.1, 5.4 Hz, 1 H), 2.53 (d, *J* = 5.5 Hz, 1 H), 2.46 (s, 3 H), 2.45 (s, 3 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 66.4 (C_6).

The crude ditosylate **7** obtained as mentioned above was dissolved in $CHCl_3$ (3 mL), and sodium methoxide in MeOH (1 M, 1.50 mL, 1.50 mmol) was added at room temperature. The reaction was quenched by the addition of brine (5 mL) after 20 min. $CHCl_3$ (20 mL) was added to the mixture, and the organic layer was washed with brine (2×30 mL), dried over Na_2SO_4, and concentrated in vacuo. Purification by recrystallization from hot MeOH/CH_2Cl_2 afforded the epoxide **8** as white crystals (118 mg, 80% from **6**). Mp 145—147 °C; $[\alpha]_D^{25}$ −40.4 (*c* 1, $CHCl_3$); FAB-MS (positive) *m/z* 300.0 [(M+H)$^+$]; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.85 (dt, *J* = 2.0, 8.3 Hz, 2 H), 7.40—7.37 (m, 2 H), 5.17 (d, *J* = 5.5 Hz, 1 H), 4.83 (ddd, *J* = 4.9, 2.0 Hz, 1 H), 4.40 (s, 1 H), 3.95 (dd, *J* = 153.0 ($^1J_{C6,H6a}$), 6.6 Hz, 1 H), 3.62—3.59 (m, 1 H), 3.34 (ddd, *J* = 153.0 ($^1J_{C6,H6b}$), 6.7, 4.9 Hz, 1 H), 3.15 (dd, *J* = 4.0, 1.6 Hz, 1 H), 2.46 (s, 3 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 64.8 (C_6). Found: C, 52.12; H, 4.62%. Calcd for $C_{11}{}^{13}C_1H_{14}O_6S$: C, 52.50; H, 4.71%.

1,6-Anhydro-4-*O*-benzyl-2-*O*-*p*-tolylsulfonyl-β-D-(6-^{13}C)-glucopyranose (9) and 4-*O*-Benzyl-1,6:2,3-dianhydro-β-D-(6-^{13}C)mannopyranose (10). To a solution of **8** (396 mg, 1.32 mmol) and benzyl alcohol (0.680 mL, 6.57 mmol) in anhydrous toluene (2.5 mL) was added 10-camphorsulfonic acid (46 mg, 0.2 mmol); the mixture was stirred at 100 °C for 5.5 h. Then, the solution was poured into a cold mixture of saturated aqueous $NaHCO_3$ (10 mL) and $CHCl_3$ (10 mL) at 0 °C. The organic layer was separated, washed with brine (30 mL), dried over Na_2SO_4, and

concentrated in vacuo to give crude benzyl ether **9** as a syrup (1.1 g), which was used for the next reaction without purification. Pure **9** for a characterization was obtained by silica-gel flash chromatography (120 g, hexane/EtOAc = 1/1) as an opaque syrup. $[\alpha]_D^{25}$ −22.8 (*c* 0.5, $CHCl_3$); ESI-MS (positive, Mariner™) *m/z* 430.19 $[(M+Na)^+]$; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.83—7.78 (m, 2 H), 7.38—7.30 (m, 7 H), 5.33 (d, *J* = 4.8 Hz, 1 H), 4.69 and 4.61 (AB system, *J* = 12.2 Hz, 2 H), 4.56 (d, *J* = 5.2 Hz, 1 H), 4.22 (d, *J* = 3.9 Hz, 1 H), 3.98 (d, *J* = 3.7 Hz, 1 H), 3.83 (dd, *J* = 142.9 ($^1J_{C6,H6a}$), 7.8 Hz, 1 H), 3.63 (ddd, *J* = 153.3 ($^1J_{C6,H6b}$), 7.0, 5.4 Hz, 1 H), 3.33—3.32 (m, 1 H), 2.44 (s, 3 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 66.6 (C_6).

The monotosylate **9** obtained above was dissolved in $CHCl_3$ (3 mL), and to this was added sodium methoxide in MeOH (1 M, 2.6 mL, 2.6 mmol) at room temperature. After 20 min the reaction was quenched at 0 °C with water (5 mL). After $CHCl_3$ (30 mL) was added, the organic layer was separated, washed with brine (2×20 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography two times (50 g, $CHCl_3$/acetone = 20/1; then 30 g, toluene/EtOAc = 10/1) to yield the epoxide **10** as a colorless syrup (228 mg, 74% from **8**). $[\alpha]_D^{25}$ −25.2 (*c* 0.5, $CHCl_3$); ESI-MS (positive, Mariner™) *m/z* 258.1 $[(M+Na)^+]$; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.40—7.30 (m, 5 H), 5.71 (dd, *J* = 6.6, 3.2 ($^3J_{C6,H1}$) Hz, 1 H), 4.74 (s, 2 H), 4.51 (d, *J* = 6.5 Hz, 1 H), 3.70 (dd, *J* = 152.1 ($^1J_{C6,H6a}$), 6.9 Hz, 1 H), 3.66 (br s, 1 H), 3.67 (ddd, *J* = 151.1 ($^1J_{C6,H6b}$), 7.1, 2.1 Hz, 1 H), 3.45 (dd, *J* = 6.9, 3.5 Hz, 1 H), 3.19 (d, *J* = 3.9, 1.6 Hz, 1 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 65.8 (C_6).

1,6-Anhydro-2-azido-4-*O*-benzyl-2-deoxy-β-D-(6-^{13}C)glucopyranose (11) and 3-*O*-Acetyl-1,6-anhydro-2-azido-4-*O*-benzyl-2-deoxy-β-D-(6-^{13}C)glucopyranose (12). A solution of the epoxide **10** (208 mg, 0.882 mmol), LiN_3 (216 mg, 4.41 mmol) and NH_4Cl (147 mg, 2.75 mmol) in anhydrous *N,N*-dimethylformamide (5.5 mL) was stirred at 100 °C for 31 h. Other portions of LiN_3 (83 mg, 1.7 mmol) and NH_4Cl (194 mg, 3.63 mmol) were added and the solution was stirred at the same temperature for an additional 4 h. Water (10 mL) was added, and the mixture was extracted with $CHCl_3$ (3×10 mL). The combined extracts were washed with brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo to furnish crude azide **11** (230 mg). Pure **11** (30 mg) for a characterization was obtained as a glassy syrup by silica-gel flash chromatography (20 g, hexane/EtOAc = 1/1). $[\alpha]_D^{25}$ −7.40 (*c* 0.5, $CHCl_3$); ESI-MS (positive, Mariner™) *m/z* 301.13 $[(M+Na)^+]$; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.39—7.30 (m, 5 H), 5.47 (d, *J* = 4.7 Hz, 1 H), 4.70 (s, 1 H), 4.69 (s, 1 H), 4.61 (d, *J* = 5.1 Hz, 1 H), 3.94 (dd, *J* = 149.5 ($^1J_{C6,H6a}$), 7.4 Hz, 1 H), 3.90 (br s, 1 H), 3.75 (ddd, *J* = 153.7 ($^1J_{C6,H6b}$), 7.7, 5.4 Hz, 1 H), 3.39—3.37 (m, 1 H), 3.23 (br s, 1 H), 2.43 (br s, 1 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 66.3 (C_6).

The crude azide **11** obtained as mentioned above was dissolved in pyridine (4 mL), and the solution was cooled to 0 °C. Acetic anhydride (2.0 mL, 21 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of ethanol (10 mL) at 0 °C, and the solvents were removed in vacuo by coevaporation with toluene (2×10 mL). The residual syrup was purified by silica-gel flash chromatography (20 g, hexane/EtOAc = 2/1) to give the acetate **12** as an oily syrup (255 mg, 90% from **10**). $[\alpha]_D^{25}$ +72.6 (*c* 0.5, $CHCl_3$); ESI-MS (positive, Mariner™) *m/z* 343.11 $[(M+Na)^+]$; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.41—7.29 (m, 5 H), 5.51 (d, *J* = 5.1 Hz, 1 H), 5.06 (br s, 1 H), 4.83 and 4.70 (AB system, *J* = 12.1 Hz, 2 H), 4.61 (d, *J* = 5.7 Hz, 1 H), 3.94 (dd, 1 H, *J* = 148.6 ($^1J_{C6,H6a}$), 7.7 Hz), 3.75 (ddd, *J* = 153.9 ($^1J_{C6,H6b}$), 7.5, 5.9 Hz, 1 H), 3.28 (br s, 1 H), 3.21 (br s, 1 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 65.1 (C_6).

Phenyl 3-*O*-Acetyl-2-azido-4-*O*-benzyl-2-deoxy-1-thio-D-(6-^{13}C)glucopyranoside (13) and Phenyl 2-Azido-4-*O*-benzyl-2-deoxy-1-thio-D-(6-^{13}C)glucopyranoside (14). To a solution of **12** (219 mg, 0.68 mmol) and (phenylthio)trimethylsilane (0.515 mL, 2.72 mmol) in anhydrous 1,2-dichloroethane (4 mL) at 0 °C was added zinc iodide (760 mg, 2.38 mmol); the mixture was then stirred at room temperature for 3.5 h. EtOAc (10 mL) was added at 0 °C and all insoluble materials were removed by filtration. Saturated aqueous $NaHCO_3$ (15 mL) was added to the filtrate and the precipitate was filtered off. The filtrate was diluted with EtOAc (30 mL) and washed with saturated aqueous $NaHCO_3$ (30 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in MeOH (6 mL) and stirred with citric acid (70 mg) at room temperature for 1 h. The reaction mixture was concentrated, neutralized by the addition of saturated aqueous $NaHCO_3$ (30 mL), and extracted with $CHCl_3$ (30 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give the crude thioglycoside **13** as a mixture of anomers. A small portion of crude **13** was purified for characterization by silica-gel flash chromatography (30 g, $CHCl_3$/acetone = 30/1) to afford pure **13** (α/β = 3.3/1, 50 mg) as a colorless syrup. $[\alpha]_D^{25}$ +96.0 (*c* 0.3, $CHCl_3$); FAB-MS (positive) *m/z* 431.1 $[(M+H)^+]$.

Data for the α-Anomer of 13: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.49—7.47 (m, 2 H), 7.33—7.29 (m, 8 H), 5.59 (d, *J* = 5.5 Hz, 1 H), 5.43 (dd, *J* = 10.6, 9.0 Hz, 1 H), 4.65 (s, 2 H), 4.30 (dt, *J* = 9.8, 2.9 Hz, 1 H), 3.79 (m, only $^1J_{C6,H6}$ = 145.1 Hz was distinguishable, 2 H), 3.91 (dd, *J* = 10.5, 5.3 Hz, 1 H), 3.73—3.68 (ddd, *J* = 10.0, 9.5, 3.3 ($^3J_{C6,H4}$) Hz, 1 H), 2.05 (s, 3 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 61.3 (C_6).

Data for the β-Anomer of 13: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.56—7.54 (m, 2 H), 7.38—7.34 (m, 8 H), 5.16 (t, *J* = 9.6 Hz, 1 H), 4.64 (s, 2 H), 4.61 (d, *J* = 4.4 Hz, 1 H), 3.91 (m, only $^1J_{C6,H6}$ = 145.1 Hz was distinguishable, 2 H), 3.59 (ddd, *J* = 10.0, 9.5, 3.0 ($^3J_{C6,H4}$) Hz, 1 H), 3.44—3.41 (m, 1 H), 3.01 (t, *J* = 10.0 Hz, 1 H), 2.01 (s, 3 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 61.6 (C_6).

The crude thioglycoside **13** obtained as mentioned above was dissolved in MeOH (8 mL); to this at room temperature was added sodium methoxide in MeOH (1 M, 1 mL, 1 mmol). After 1.5 h, the solution was adjusted to pH 7.0 by the addition of Dowex® 50W-X8 (H^+ form). All insoluble materials were filtered off and the filtrate was concentrated in vacuo. Purification by silica-gel flash chromatography (55 g, $CHCl_3$/acetone = 30/1) gave the diol **14** as an opaque syrup (α/β = 3/1, 212 mg, 80% from **12**. FAB-MS (positive) *m/z* 389.0 $[(M+H)^+]$.

Data for the α-Anomer of 14: $[\alpha]_D^{25}$ +221 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.49—7.46 (m, 2 H), 7.40—7.29 (m, 8 H), 5.55 (d, *J* = 5.4 Hz, 1 H), 4.79 (s, 2 H), 4.22 (dt, *J* = 9.9, 3.2 Hz, 1 H), 3.96 (dd, *J* = 10.2, 9.0 Hz, 1 H), 3.81 (m, only $^1J_{C6,H6}$ = 152.3 Hz was distinguishable, 2 H), 3.79 (dd, *J* = 10.6, 5.4 Hz, 1 H), 3.55 (ddd, *J* = 10.2, 9.4, 3.3 ($^3J_{C6,H4}$) Hz, 1 H), 2.16 (br s, 1 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 61.6 (C_6). Found: C, 59.02; H, 5.46; N, 10.13%. Calcd for $C_{17}{}^{13}C_1H_{21}N_3O_4S$: C, 59.01; H, 5.45, N 10.82%.

Data for the β-Anomer of 14: $[\alpha]_D^{25}$ −38.3 (*c* 0.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.55—7.53 (m, 2 H), 7.36—7.32 (m, 8 H), 4.74 (s, 2 H), 4.49 (d, *J* = 10.0 Hz, 1 H), 3.92 (ddd, *J* = 144.2 ($^1J_{C6,H6a}$), 12.3, 2.5 Hz, 1 H), 3.74 (ddd, *J* = 143.8 ($^1J_{C6,H6b}$), 12.1, 4.4 Hz, 1 H), 3.63 (t, *J* = 9.1 Hz, 1 H), 3.44 (ddd, *J* = 10.0, 9.2, 3.3 ($^3J_{C6,H4}$) Hz, 1 H), 3.38—3.36 (m, 1 H), 3.26 (dd,

J = 10.3, 9.4 Hz, 1 H), 2.54 (br s, 1 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 62.0 (C_6). Found: C, 58.98; H, 5.42; N, 10.24%. Calcd for $C_{17}{}^{13}C_1H_{21}N_3O_4S$: C, 59.01; H, 5.45, N 10.82%.

Allyl 4,6-*O*-Benzylidene-3-*O*-[(*R*)-3-(benzyloxy)decanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (16). A solution of (*R*)-3-(benzyloxy)decanoic acid (1.16 g, 4.17 mmol),[6] allyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (**15**) (2.01 g, 4.17 mmol),[3] DCC (1.12 g, 5.43 mmol), and DMAP (66 mg, 0.54 mmol) in anhydrous CH_2Cl_2 (25 mL) was stirred at room temperature for 20 h. MeOH (1.5 mL) and acetic acid (0.5 mL) were added, and the mixture was stirred for an additional 30 min. All insoluble materials were filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in EtOAc (180 mL), and washed with saturated aqueous $NaHCO_3$ (150 mL) and brine (100 mL). After drying over Na_2SO_4, the organic layer was concentrated in vacuo. The residue was purified by silica-gel flash chromatography (200 g, toluene/EtOAc = 50/1), followed by precipitation from EtOAc/hexane to afford the ester **16** as a white powder (2.17 g, 70%). $[\alpha]_D^{24}$ +42.2 (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.42—7.23 (m, 10 H), 5.90 (m, 1 H), 5.48 (s, 1 H), 5.42 (t, J = 10.1 Hz, 1 H), 5.35 (d, J = 10.0 Hz, 1 H), 5.32 (dq, J = 17.1, 1.4 Hz, 1 H), 5.25 (dq, J = 9.4, 1.2 Hz, 1 H), 4.94 (d, J = 3.7 Hz, 1 H), 4.72 and 4.52 (AB system, J = 12.1 Hz, 2 H), 4.50 and 4.39 (AB system, J = 11.7 Hz, 2 H), 4.29 (dd, J = 10.3, 4.9 Hz, 1 H), 4.22 (ddt, J = 12.8, 5.3, 1.3 Hz, 1 H), 4.09—4.01 (m, 2 H), 3.95 (ddd, J = 10.3, 9.7, 4.9 Hz, 1 H), 3.82—3.77 (m, 1 H), 3.77 (t, J = 10.3 Hz, 1 H), 3.70 (t, J = 9.7 Hz, 1 H), 2.67 (dd, J = 15.3, 6.4 Hz, 1 H), 2.44 (dd, J = 15.3, 6.0 Hz, 1 H), 1.55—1.42 (m, 2 H), 1.36—1.13 (m, 10 H), 0.86 (t, J = 7.1 Hz, 3 H). Found: C, 57.99; H, 6.22; N, 1.95%. Calcd for $C_{36}H_{46}Cl_3NO_9$: C, 58.19; H, 6.24; N, 1.88%.

Allyl 6-*O*-Benzyl-3-*O*-[(*R*)-3-(benzyloxy)decanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (17). To a solution of **16** (1.50 g, 2.02 mmol) and BH_3·Me_2NH (595 mg, 10.1 mmol) in anhydrous acetonitrile (30 mL) at −20 °C was added BF_3·OEt_2 (1.28 mL, 10.1 mmol) over a period of 30 min. The mixture was stirred for an additional 2 h, and during this period was allowed to gradually warm to room temperature. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (50 mL) at 0 °C, and the reaction mixture was extracted with EtOAc (300 mL). The extract was washed successively with saturated aqueous $NaHCO_3$ (150 mL), brine (150 mL), and aqueous HCl (1 M, 150 mL). The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (150 g, hexane/EtOAc = 3/1) to give the 6-*O*-benzyl ether **17** as an oily, colorless syrup (1.05 g, 70%). $[\alpha]_D^{24}$ +45.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.36—7.25 (m, 10 H), 5.89 (m, 1 H), 5.32—5.26 (m, 2 H), 5.21 (dq, J = 10.3, 1.4 Hz, 1 H), 5.16 (dd, J = 10.8, 9.2 Hz, 1 H), 4.92 (d, J = 3.7 Hz, 1 H), 4.68 and 4.66 (AB system, J = 11.9 Hz, 2 H), 4.61 and 4.57 (AB system, J = 12.1 Hz, 2 H), 4.53 and 4.51 (AB system, J = 11.4 Hz, 2 H), 4.19 (ddt, J = 12.8, 5.5, 1.3 Hz, 1 H), 4.04—3.97 (m, 2 H), 3.88—3.80 (m, 2 H), 3.75—3.68 (m, 3 H), 2.82 (br s, 1 H), 2.64 (dd, J = 14.8, 7.6 Hz, 1 H), 2.49 (dd, J = 14.8, 5.0 Hz, 1 H), 1.66—1.49 (m, 2 H), 1.39—1.26 (m, 10 H), 0.88 (t, J = 6.9 Hz, 3 H). Found: C, 57.74; H, 6.42; N, 1.86%. Calcd for $C_{36}H_{48}Cl_3NO_9$: C, 58.02; H, 6.49; N, 1.88%.

Allyl 6-*O*-Benzyl-3-*O*-[(*R*)-3-(benzyloxy)decanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (18). To a solution of **17** (950 mg, 1.28 mmol) in anhydrous CH_2Cl_2 (30 mL) at room temperature were added *N,N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (622 mg, 2.60 mmol) and 1*H*-tetrazole (359 mg, 5.12 mmol). The mixture was stirred for 10 min, and then cooled to −20 °C. *m*-Chloroperbenzoic acid (80%, 552 mg, 2.56 mmol) was added and stirring was continued for 20 min. The reaction was quenched with saturated aqueous $NaHCO_3$ (30 mL), and the mixture was extracted with $CHCl_3$ (200 mL). The extract was washed with saturated aqueous $NaHCO_3$ (100 mL) and brine (2×50 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (200 g, hexane/EtOAc = 2/1) to yield the phosphate **18** as an oily, colorless syrup (1.08 g, 91%). $[\alpha]_D^{24}$ +38.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.39—7.12 (m, 13 H), 6.72 (d, J = 7.5 Hz, 1 H), 5.91 (m, 1 H), 5.41 (t, J = 9.6 Hz, 1 H), 5.31 (dq, J = 17.2, 1.4 Hz, 1 H), 5.30 (d, J = 11.0 Hz, 1 H), 5.23 (dq, J = 10.3, 1.4 Hz, 1 H), 5.10—4.96 (m, 5 H), 4.75 (q, J = 9.6 Hz, 1 H), 4.68 and 4.59 (AB system, J = 11.9 Hz, 2 H), 4.65 and 4.59 (AB system, J = 11.9 Hz, 2 H), 4.54 and 4.52 (AB system, J = 11.2 Hz, 2 H), 4.22 (ddt, J = 12.6, 5.3, 1.4 Hz, 1 H), 4.05—4.02 (m, 1 H), 4.02—3.99 (m, 2 H), 3.90—3.84 (m, 1 H), 3.80 (dd, J = 11.0, 2.1 Hz, 1 H), 3.74 (dd, J = 11.0, 5.0 Hz, 1 H), 2.74 (dd, J = 16.9, 7.8 Hz, 1 H), 2.54 (dd, J = 16.9, 4.4 Hz, 1 H), 1.58—1.54 (m, 2 H), 1.29—1.24 (m, 10 H), 0.88 (t, J = 6.9 Hz, 3 H). Found: C, 57.28; H, 5.98; N, 1.57%. Calcd for $C_{44}H_{55}Cl_3NO_{12}P$: C, 56.99; H, 5.98; N, 1.51%.

6-*O*-Benzyl-3-*O*-[(*R*)-3-(benzyloxy)decanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (19). To a degassed solution of **18** (1.00 g, 1.08 mmol) in anhydrous THF (15 mL) was added bis(methyldiphenylphosphine) (1,5-cyclooctadiene)iridium(I) hexafluorophosphate (93 mg, 0.11 mmol). After activation of the iridium catalyst with hydrogen two times (each about 5 s), the mixture was stirred under an argon atmosphere at room temperature for 15 min. Iodine (548 mg, 2.16 mmol) and water (8 mL) were added and the solution was stirred for an additional 20 min. The reaction was quenched with 5% aqueous $Na_2S_2O_3$ (20 mL), and the mixture was diluted with water (20 mL) and extracted with EtOAc (60 mL). The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (80 g, toluene/EtOAc = 2/1) followed by precipitation from hexane to give the deallylated product **19** of the α-anomeric configuration as a white powder (815 mg, 85%). $[\alpha]_D^{24}$ +12.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.37—7.11 (m, 13 H), 6.73 (d, J = 7.4 Hz, 1 H), 5.45 (t, J = 10.3, 9.4 Hz, 1 H), 5.37 (d, J = 9.6 Hz, 1 H), 5.30 (br s, 1 H), 5.07—4.92 (m, 4 H), 4.69—4.50 (m, 7 H), 4.26—4.23 (m, 1 H), 4.01—3.95 (ddd, J = 10.5, 9.8, 3.5 Hz, 1 H), 3.92—3.84 (m, 1 H), 3.80 (dd, J = 10.7, 1.0 Hz, 1 H), 3.74 (dd, J = 10.7, 5.9 Hz, 1 H), 3.44 (br s, 1 H), 2.73 (dd, J = 16.9, 9.1 Hz, 1 H), 2.54 (dd, J = 16.9, 4.3 Hz, 1 H), 1.58—1.51 (m, 2 H), 1.35—1.27 (m, 10 H), 0.88 (t, J = 6.9 Hz, 3 H). Found: C, 55.42; H, 5.77; Cl, 11.97; N, 1.61%. Calcd for $C_{41}H_{51}Cl_3NO_{12}P$: C, 55.51; H, 5.79; Cl, 11.99: N, 1.58%.

6-*O*-Benzyl-3-*O*-[(*R*)-3-(benzyloxy)decanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl Trichloroacetimidate (20b). To a solution of **19** (600 mg, 0.68 mmol) in anhydrous CH_2Cl_2 (8 mL) at room temperature were added Cs_2CO_3 (110 mg, 0.34 mmol) and trichloroacetonitrile (0.678 mL, 6.76 mmol). After being stirred for 1 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (10 mL) and the mixture was diluted with $CHCl_3$ (60 mL). The organic layer was separated, washed with saturated aqueous $NaHCO_3$ (30 mL) and brine (40 mL), dried over

Na_2SO_4, and concentrated in vacuo to give the crude imidate **20b** as a pale yellow syrup (701 mg, 100%) which was used for the subsequent glycosidation without further purification.

Phenyl 2-Azido-4-*O*-benzyl-6-*O*-[6-*O*-benzyl-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-2-deoxy-1-thio-D-(6-^{13}C)-glucopyranoside (21a). The imidate **20a** (380 mg, 0.402 mmol), the acceptor **14** (99.7 mg, 0.257 mmol), and molecular sieves 4A (200 mg) were stirred in anhydrous 1,2-dichloroethane (12 mL) at room temperature for 15 min. After the suspension was cooled to −35 °C, trimethylsilyl trifluoromethanesulfonate (0.007 mL, 0.04 mmol) was added. The mixture was stirred at this temperature for 13 min. After molecular sieves were removed by filtration, saturated aqueous $NaHCO_3$ (20 mL) and $CHCl_3$ (30 mL) were added to the filtrate. The organic layer was separated, washed with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (30 g, toluene/EtOAc = 3/1) to give the disaccharide **21a**, which is a mixture of isomers at the phenylthio group (α/β = 5/1), as a white powder (236 mg, 79%). $[\alpha]_D^{25}$ +81.6 (*c* 1.00, $CHCl_3$); ESI-MS (positive, API III) *m/z* 1415.7 [(M+Et_3N+H)$^+$].

Data for the α-Anomer of 21a: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.48—7.46 (m, 2 H), 7.40—7.20 (m, 22 H), 7.12 (d, *J* = 9.4 Hz, 1 H), 6.48 (d, *J* = 7.1 Hz, 1 H), 5.59 (d, *J* = 7.9 Hz, 1 H), 5.37 (t, *J* = 8.2 Hz, 1 H), 4.96 (d, *J* = 11.4 Hz, 1 H), 4.95—5.09 (m, 3 H), 4.82 (d, *J* = 8.2 Hz, 1 H), 4.77 (d, *J* = 11.4 Hz, 1 H), 4.68 (d, *J* = 11.4 Hz, 1 H), 4.57 (d, *J* = 7.8 Hz, 1 H), 4.64—4.52 (m, 7 H), 4.28 (d, *J* = 10.5 Hz, 1 H), 4.03 (dd, 2 H, *J* = 156.8 ($^1J_{C6,H6b}$), 10.5 Hz), 3.78 (dd, *J* = 150.0 ($^1J_{C6,H6b}$), 3.7 Hz, 2 H), 3.92 (dd, *J* = 10.2, 8.7 Hz, 1 H), 3.80 (dd, *J* = 10.3, 5.0 Hz, 1 H), 3.85—3.83 (m, 1 H), 3.72—3.65 (m, 4 H), 3.58—3.51 (m, 1 H), 3.36 (d, *J* = 8.3 Hz, 1 H), 2.78 (dd, *J* = 16.5, 7.1 Hz, 1 H), 2.62 (dd, *J* = 16.4, 4.8 Hz, 1 H), 1.66—1.51 (m, 2 H), 1.31—1.24 (m, 18 H), 0.88 (t, *J* = 6.9 Hz, 3 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 67.7 (C_6).

Data for the β-Anomer of 21a: ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 68.0 (C_6).

Phenyl 2-Azido-4-*O*-benzyl-6-*O*-[6-*O*-benzyl-3-*O*-[(*R*)-3-(benzyloxy)decanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-2-deoxy-1-thio-D-(6-^{13}C)glucopyranoside (21b). In a manner similar to that for the synthesis of **21a**, **20b** (697 mg, 0.68 mmol) was reacted with **14** (172 mg, 0.44 mmol) to yield the disaccharide **21b**, which is a mixture of isomers at the phenylthio group (α/β = 3/1), as a colorless syrup (500 mg, 90%). $[\alpha]_D^{24}$ +57.6 (*c* 0.33, $CHCl_3$); ESI-MS (positive, API III) *m/z* 1357.6 [(M+Et_3N+H)$^+$].

Data for the α-Anomer of 21b: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.48—7.46 (m, 2 H), 7.39—7.13 (m, 21 H), 6.84 (d, *J* = 7.1 Hz, 1 H), 5.59 (d, *J* = 5.3 Hz, 1 H), 5.38 (t, *J* = 9.6 Hz, 1 H), 5.09—4.87 (m, 5 H), 4.83 (d, *J* = 8.0 Hz, 1 H), 4.76—4.48 (AB system, 2 H), 4.73—4.48 (m, 7 H), 4.28 (d, *J* = 9.4 Hz, 1 H), 4.04 (dd, *J* = 155.0 ($^1J_{C6,H6a}$), 9.6 Hz, 1 H), 3.93—3.79 (m, 4 H), 3.86 (m, only $^1J_{C6,H6b}$ = 150.0 Hz was distinguishable, 1 H), 3.70—3.67 (m, 2 H), 3.57—3.51 (m, 1 H), 3.36 (br t, *J* = 9.6, 8.3 Hz, 1 H), 2.69 (dd, *J* = 16.3, 6.7 Hz, 1 H), 2.64 (dd, *J* = 16.3, 5.0 Hz, 1 H), 2.55 (br s, 1 H), 1.60—1.52 (m, 2 H), 1.36—1.27 (m, 10 H), 0.88 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 67.5 (C_6).

Data for the β-Anomer of 21b: ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 68.0 (C_6).

Phenyl 2-Azido-4-*O*-benzyl-6-*O*-[6-*O*-benzyl-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-1-thio-D-(6-^{13}C)glucopyranoside (22a). A solution of **21a** (200 mg, 0.152 mmol), (*R*)-3-(benzyloxy)-tetradecanoic acid (105 mg, 0.313 mmol),[20] WSCD·HCl (84 mg, 0.44 mmol), and DMAP (12 mg, 0.10 mmol) in anhydrous 1,2-dichloroethane (5 mL) was stirred at 0 °C to room temperature for 12 h. Saturated aqueous $NaHCO_3$ (10 mL) was added, and the mixture was extracted with $CHCl_3$ (3×5 mL). The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo to give a residue, which was purified by silica-gel flash chromatography (20 g, toluene/EtOAc = 5/1) to afford the diacyl compound **22a** as a colorless syrup (α/β = 5/1, 205 mg, 80%). $[\alpha]_D^{24}$ +53.2 (*c* 0.44, $CHCl_3$); ESI-MS (positive, API III) *m/z* 1732.0 [(M+Et_3N+H)$^+$].

Data for the α-Anomer of 22a: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.48 (dd, *J* = 8.3, 1.4 Hz, 2 H), 7.36—7.20 (m, 27 H), 7.13 (d, *J* = 7.3 Hz, 1 H), 5.62 (d, *J* = 5.3 Hz, 1 H), 5.46 (dd, *J* = 9.0, 8.6 Hz, 1 H), 5.46 (t, *J* = 9.9 Hz, 1 H), 5.10—4.93 (m, 3 H), 4.78 (d, *J* = 8.5 Hz, 1 H), 4.61—4.59 (m, 2 H), 4.56 (d, *J* = 10.9 Hz, 1 H), 4.54 (d, *J* = 9.7 Hz, 1 H), 4.51—4.39 (m, 6 H), 4.36 (br d, *J* = 9.7 Hz, 1 H), 3.98 (dd, *J* = 158.8 ($^1J_{C6,H6a}$), 9.8 Hz, 2 H), 3.92—3.85 (m, 1 H), 3.88 (dd, *J* = 10.5, 5.3 Hz, 1 H), 3.80 (d, *J* = 10.6 Hz, 1 H), 3.79 (dt, *J* = 159.1 ($^1J_{C6,H6b}$), 5.9 Hz, 2 H), 3.68 (d, *J* = 10.8 Hz, 1 H), 3.67 (d, *J* = 10.8 Hz, 1 H), 3.65—3.60 (m, 3 H), 3.39 (d, *J* = 10.4 Hz, 1 H), 3.27 (d, *J* = 6.9 Hz, 1 H), 2.65—2.52 (m, 2 H), 2.48 (dd, *J* = 15.8, 4.7 Hz, 1 H), 1.65—1.52 (m, 4 H), 1.30—1.24 (m, 36 H), 0.88 (t, *J* = 6.7 Hz, 6 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 67.4 (C_6).

Data for the β-Anomer of 22a: ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 67.5 (C_6).

Phenyl 2-Azido-4-*O*-benzyl-6-*O*-[6-*O*-benzyl-3-*O*-[(*R*)-3-(benzyloxy)decanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)decanoyl]-2-deoxy-1-thio-D-(6-^{13}C)glucopyranoside (22b). In a manner similar to that for the synthesis of **22a**, **21b** (260 mg, 0.21 mmol) was acylated with (*R*)-3-(benzyloxy)decanoic acid (85 mg, 0.31 mmol) to yield the diacyl compound **22b** as a colorless syrup (α/β = 3/1, 240 mg, 76%). $[\alpha]_D^{24}$ +68.6 (*c* 0.14, $CHCl_3$); ESI-MS (positive, API III) *m/z* 1617.8 [(M+Et_3N+H)$^+$].

Data for the α-Anomer of 22b: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.48—7.46 (m, 2 H), 7.37—7.20 (m, 26 H), 6.84 (d, *J* = 7.4 Hz, 1 H), 5.62 (d, *J* = 5.3 Hz, 1 H), 5.45 (dd, *J* = 10.6, 8.9 Hz, 1 H), 5.27 (t, *J* = 10.1 Hz, 1 H), 5.09—4.92 (m, 4 H), 4.79 (d, *J* = 8.7 Hz, 1 H), 4.61—4.40 (m, 12 H), 4.36 (d, *J* = 10.3 Hz, 1 H), 3.99 (dd, *J* = 150.0 ($^1J_{C6,H6a}$), 9.9 Hz, 1 H), 3.98—3.92 (m, 1 H), 3.89—3.83 (m, 2 H), 3.80 (dd, *J* = 11.0, 2.1 Hz, 1 H), 3.73 (m, only $^1J_{C6,H6b}$ = 150.0 Hz was distinguishable, 1 H), 3.68 (dd, *J* = 11.0, 6.0 Hz, 1 H), 3.68—3.59 (m, 2 H), 3.38 (m, 1 H), 2.67—2.62 (m, 2 H), 2.60—2.50 (m, 2 H), 1.61—1.52 (m, 4 H), 1.51—1.36 (m, 20 H), 0.90—0.86 (m, 6 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 67.5 (C_6).

Data for the β-Anomer of 22b: ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 67.6 (C_6).

Phenyl 4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-1-thio-α-D-(6-^{13}C)glucopyranoside (23a). To a solution of **22a** (193 mg, 0.118 mmol) in acetic acid (3 mL) was added zinc-

copper couple (600 mg); the solution was stirred at room temperature for 2.5 h. All insoluble materials were filtered off, and washed thoroughly with EtOAc (10 mL). The combined filtrate and washing were concentrated by coevaporation with toluene (3×30 mL). To the residual syrup was added saturated aqueous $NaHCO_3$ (5 mL), and the mixture was extracted with $CHCl_3$ (3×5 mL). The combined extracts were washed with brine (5 mL), dried over $MgSO_4$, and concentrated in vacuo to furnish the diamino compound ready for acylation without further purification.

The product obtained as mentioned above was dissolved in anhydrous $CHCl_3$ (3 mL), and to this solution at room temperature were added (*R*)-3-(benzyloxy)tetradecanoic acid (160 mg, 0.478 mmol), WSCD·HCl (99 mg, 0.52 mmol), and HOAt (49 mg, 0.36 mmol). After 12 h, the reaction was quenched and worked up in a manner similar to that for the compound **22a**. Silica-gel flash chromatography (30 g, $CHCl_3$/acetone = 15/1) afforded the tetraacyl compound **23a** as an opaque syrup (α-thioglycoside only, 105 mg, 43%). $[\alpha]_D^{24}$ +60.0 (*c* 0.45, $CHCl_3$); ESI-MS (positive, API III) *m/z* 2163.7 $[(M+Et_3N+H)^+]$; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.48—7.46 (m, 2 H), 7.35—7.20 (m, 39 H), 7.12 (d, *J* = 7.6 Hz, 1 H), 6.78 (d, *J* = 7.3 Hz, 1 H), 6.47 (d, *J* = 8.4 Hz, 1 H), 6.09 (d, *J* = 8.2 Hz, 1 H), 5.62 (d, *J* = 5.3 Hz, 1 H), 5.46 (dd, *J* = 9.0, 8.6 Hz, 1 H), 5.28 (t, *J* = 9.9 Hz, 1 H), 4.93—5.10 (m, 5 H), 4.61—4.59 (m, 10 H), 4.56 (d, *J* = 10.9 Hz, 1 H), 4.54 (d, *J* = 9.7 Hz, 1 H), 4.36 (d, *J* = 9.7 Hz, 1 H), 4.07 (br d, *J* = 10.5 Hz, 1 H), 3.98 (dd, *J* = 158.8 ($^1J_{C6,H6a}$), 9.8 Hz, 2 H), 3.93—3.74 (m, 5 H), 3.79 (dt, *J* = 150.0 ($^1J_{C6,H6b}$), 5.9 Hz, 2 H), 3.70—3.56 (m, 4 H), 2.70 (dd, *J* = 16.2, 6.9 Hz, 1 H), 2.54 (dd, *J* = 16.2, 6.2 Hz, 1 H), 2.39 (dd, *J* = 15.8, 5.4 Hz, 1 H),2.36—2.27 (m, 2 H), 2.31 (dd, *J* = 10.0, 6.9 Hz, 1 H), 2.19 (dd, *J* = 15.4, 7.3 Hz, 1 H),2.09 (dd, *J* = 15.0, 4.2 Hz, 1 H), 1.59—1.51 (m, 8 H), 1.31—1.19 (m, 72 H), 0.88 (t, *J* = 6.8 Hz, 12 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 67.5 (C_6).

Phenyl 4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-3-*O*-[(*R*)-3-(benzyloxy)-decanoyl]-2-[(*R*)-3-(benzyloxy)decanoylamino]-2-deoxy-4-*O*-(1, 5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)decanoyl]-2-[(*R*)-3-(benzyloxy)decanoylamino]-2-deoxy-1-thio-α-D-(6-^{13}C)glucopyranoside (23b). In a manner similar to that for the synthesis of **23a**, **22b** (240 mg, 0.16 mmol) was deprotected and acylated with (*R*)-3-(benzyloxy)decanoic acid (132 mg, 0.47 mmol) to yield the tetraacyl compound **23b** as an opaque syrup (α-thioglycoside only, 190 mg, 65%). $[\alpha]_D^{24}$ +32.2 (*c* 0.5, $CHCl_3$); ESI-MS (positive, API III) *m/z* 1938.5 $[(M+Et_3N+H)^+]$; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.34—7.19 (m, 37 H), 7.12 (d, *J* = 7.6 Hz, 1 H), 6.78 (d, *J* = 6.9 Hz, 1 H), 6.46 (d, *J* = 8.5 Hz, 1 H), 6.08 (d, *J* = 8.3 Hz, 1 H), 5.66 (d, *J* = 5.3 Hz, 1 H), 5.44 (dd, *J* = 10.3, 8.9 Hz, 1 H), 5.25 (dd, *J* = 10.4, 9.1 Hz, 1 H), 5.04—4.89 (m, 4 H), 4.71 (dd, *J* = 8.2, 4.1 Hz, 1 H), 4.59—4.42 (m, 14 H), 4.38 (dd, *J* = 10.3, 5.0 Hz, 1 H), 3.93 (dd, *J* = 145.0 ($^1J_{C6,H6a}$), 9.9 Hz, 1 H), 3.88—3.75 (m, 5 H), 3.75 (ddd, *J* = 150.0 ($^1J_{C6,H6b}$), 9.9, 4.4 Hz, 1 H), 3.69—3.60 (m, 4 H), 2.70 (dd, *J* = 16.7, 7.5 Hz, 1 H), 2.54 (dd, *J* = 16.0, 4.8 Hz, 1 H), 2.54 (dd, *J* = 15.8, 6.9 Hz, 1 H), 2.40 (dd, *J* = 15.8, 5.3 Hz, 1 H), 2.37—2.26 (m, 2 H), 2.19 (dd, *J* = 15.3, 7.1 Hz, 1 H), 2.09 (dd, 1 H, *J* = 15.5, 4.4 Hz), 1.60—1.40 (m, 8 H), 1.32—1.18 (m, 40 H), 0.88—0.85 (m, 12 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 67.4 (C_6).

4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-[(*R*)-3-(benzyloxy)tetradecanoylamino]-2-deoxy-D-(6-^{13}C)-glucopyranose(24a). To a solution of **23a** (193 mg, 0.118 mmol) in acetone (8 mL) at −30 °C were added *N*-bromosuccinimide(48.5 mg, 0.272 mmol) and water (0.3 mL). The mixture was stirred for 2 h, and then poured into 5% $Na_2S_2O_3$ (10 mL). The mixture was extracted with $CHCl_3$ (3×7 mL), and the combined extracts were washed with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (20 g, $CHCl_3$/acetone = 15/1) and the subsequent lyophilization from benzene gave the hemiacetal **24a** as a white powder (α/β = 5/1, 105 mg, 43%). $[\alpha]_D^{21}$ +15.0 (*c* 0.18, $CHCl_3$); ESI-MS (positive, API III) *m/z* 1991.3 $[(M+Na)^+]$.

Data for the α-Anomer of 24a: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.39—7.11 (m, 17 H), 6.76 (d, *J* = 7.0 Hz, 1 H), 6.35 (d, *J* = 7.4 Hz, 1 H), 6.23 (d, *J* = 9.5 Hz, 1 H), 5.48 (t, *J* = 9.7 Hz, 1 H), 5.32 (t, *J* = 9.7 Hz, 1 H), 5.13 (dd, *J* = 8.3, 3.7 Hz, 1 H), 4.97 (d, *J* = 3.3 Hz, 1 H), 5.06—4.78 (m, 4 H), 4.65—4.42 (m, 10 H), 4.54 (d, *J* = 11.2 Hz, 1 H), 4.38 (d, *J* = 11.2 Hz, 1 H), 4.15 (ddd, *J* = 10.9, 10.4, 3.4 Hz, 1 H), 3.92 (br d, *J* = 5.8 Hz, 1 H), 3.84—3.80 (m, 3 H), 3.73—3.69 (m, 4 H), 3.62 (m, only $^1J_{C6,H6a}$ = 141.4 Hz was distinguishable, 1 H), 3.56 (m, only $^1J_{C6,H6b}$ = 141.4 Hz was distinguishable, 1 H), 3.43—3.38 (m, 1 H), 3.27 (t, *J* = 9.6 Hz, 1 H), 2.78—2.22 (m, 4 H), 2.71 (dd, *J* = 17.0, 7.6 Hz, 1 H), 2.59—2.54 (m, 1 H), 2.39 (dd, *J* = 16.1, 5.4 Hz, 1 H), 2.31 (dd, *J* = 14.8, 8.0 Hz, 1 H), 2.16 (dd, *J* = 15.6, 7.9 Hz, 1 H), 1.60—1.35 (m, 8 H), 1.31—1.22 (m, 72 H), 0.88 (t, *J* = 6.9 Hz, 12 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 68.6 (C_6).

Data for the β-Anomer of 24a: ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 68.6 (C_6).

4-*O*-Benzyl-6-*O*-[6-*O*-benzyl-3-*O*-[(*R*)-3-(benzyloxy)decanoyl]-2-[(*R*)-3-(benzyloxy)decanoylamino]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-(benzyloxy)decanoyl]-2-[(*R*)-3-(benzyloxy)decanoylamino]-2-deoxy-D-(6-^{13}C)glucopyranose (24b). In a manner similar to that for the synthesis of **24a**, **23b** (77 mg, 0.042 mmol) was converted to the hemiacetal **24b** as a white powder (α/β = 4.5/1, 52 mg, 71%). $[\alpha]_D^{24}$ +12.0 (*c* 0.33, $CHCl_3$); ESI-MS (positive, API III) *m/z* 1767.1 $[(M+Na)^+]$.

Data for the α-Anomer of 24b: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.38—7.13 (m, 33 H), 6.78 (d, *J* = 7.1 Hz, 1 H), 6.33 (d, *J* = 7.6 Hz, 1 H), 6.21 (d, *J* = 9.4 Hz, 1 H), 5.47 (t, *J* = 9.2 Hz, 1 H), 5.32 (t, *J* = 9.2 Hz, 1 H), 5.12 (dd, *J* = 8.3, 3.2 Hz, 1 H), 5.04—4.87 (m, 5 H), 4.78 (br s, 1 H), 4.64—4.37 (m, 13 H), 4.19—4.12 (m, *J* = 10.8, 3.7 Hz, 1 H), 3.95 (br s, 1 H), 3.95 (dd, *J* = 154.2 ($^1J_{C6,H6a}$), 8.8 Hz, 1 H), 3.89—3.69 (m, 7 H), 3.59 (m, only $^1J_{C6,H6b}$ = 153.0 Hz was distinguishable, 1 H), 3.40 (m, 1 H), 3.27 (t, *J* = 9.6 Hz, 1 H), 2.69 (dd, *J* = 16.5, 7.1 Hz, 1 H), 2.58—2.37 (m, 2 H), 2.39 (dd, *J* = 15.8, 5.3 Hz, 1 H), 2.32—2.22 (m, 3 H), 2.15 (dd, *J* = 15.4, 7.8 Hz, 1 H), 1.60—1.36 (m, 8 H), 1.30—1.22 (m, 40 H), 0.88—0.85 (m, 12 H); ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 68.7 (C_6).

Data for the β-Anomer of 24b: ^{13}C NMR (125.65 MHz, $CDCl_3$) δ = 68.7 (C_6).

2-Deoxy-6-*O*-[2-*O*-deoxy-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-α-D-(6-^{13}C)glucopyranose 1,4′-Bisphosphate (6-^{13}C-1). To a solution of **24a** (20.0 mg, 0.101 mmol) and tetrabenzyl diphosphate (8.9 mg, 0.017 mmol) in anhydrous THF (2.5 mL) at −78 °C was added lithium bis(trimethylsilyl)amide in hexane (1.04 M, 0.034 mL, 0.034 mmol). After 5 h of the reaction period, saturated aqueous $NaHCO_3$ (10 mL) was added to the mixture, which was then extracted with $CHCl_3$ (2×10 mL). The combined extracts were washed with brine (10 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography

(15 g, $CHCl_3$/acetone = 15/1) to give the phosphorylated product as a colorless syrup, which was promptly subjected to the next deprotection reaction.

The thus-obtained syrup was dissolved in anhydrous THF (2 mL), and to this was added Pd-black (23 mg). The mixture was stirred under 7 kg cm^{-2} of hydrogen at room temperature for 12 h. To the mixture was then added THF (3 mL) and triethylamine in THF (10%, 0.1 mL). After removal of the Pd catalyst by filtration, the filtrate was concentrated in vacuo and lyophilized from water. The crude product was purified by liquid–liquid partition column chromatography on Sephadex LH-20® (1.4×48 cm, $CHCl_3$/MeOH/2-propanol/water/triethylamine = 20/20/2.5/22.5/0.001), wherein the organic layer was the stationary phase and the aqueous layer the mobile phase. After concentration of the eluate followed by lyophilization from water, triethylammonium salt (ca. 0.3 mol amt. to 6-^{13}C-**1**) of 6-^{13}C-**1** was obtained as a white powder (7.1 mg, 50% from **24a**). ESI-MS (negative, API III) *m/z* 1404.9 [(M−H)$^-$]; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.76 (br s, 1 H, 2′-NH), 7.32 (d, J = 9.0 Hz, 1 H, 2-NH), 5.24 (dd, J = 6.6, 1.8 Hz, 1 H, H_1), 5.03 (dd, J = 9.9, 9.9 Hz, 1 H, H_3), 4.89 (dd, J = 11.7, 6.4 Hz, 1 H, $H_{3'}$), 4.88 (d, J = 8.2 Hz, 1 H, $H_{1'}$), 4.03 (ddd, J = 6.4, 6.4, 6.4 Hz, 1 H, $H_{4'}$), 3.98 (m, 1 H, H_5), 3.95 (ddd, J = 9.9, 9.0, 1.8 Hz, 1 H, H_2), 3.87 (ddd, J = 136.0, 12.5, 2.7 Hz, 1 H, H_{6a}), 3.85, 3.83, 3.73, and 3.72 (m, 1 H each, one of oxymethine protons on acyl groups), 3.77 (dd, J = 11.7, 8.2 Hz, 1 H, $H_{2'}$), 3.75 (m, 1 H, $H_{6a'}$), 3.70 (ddd, J = 140.0, 12.5, 6.3 Hz, 1 H, H_{6a}), 3.53 (m, 1 H, $H_{6b'}$), 3.33 (m, 1 H, H_4), 3.13 (m, 1 H, $H_{5'}$), 2.47 (dd, J = 6.7, 13.1 Hz, 1 H, one of α-CH_2 protons on acyl groups), 2.41 (dd, J = 6.7, 15.8 Hz, 1 H, one of α-CH_2 protons on acyl groups), 2.34 (dd, J = 6.7, 15.8 Hz, 1 H, one of α-CH_2 protons on acyl groups), 2.31—2.26, 2.31—2.26, 2.18—2.13, and 2.18—2.13 (m, 1 H each, one of α-CH_2 protons on acyl groups), 2.08 (dd, J = 5.5, 13.1 Hz, 1 H, one of α-CH_2 protons on acyl groups), 1.44—1.21 (m, 80 H, CH_2 on acyl groups), 0.92 (t, J = 6.9 Hz, 12 H, CH_3 on acyl groups); ^{13}C NMR (125.65 MHz, DMSO-d_6) δ = 66.4 (C_6).

2-Deoxy-6-*O*-[2-*O*-deoxy-3-*O*-[(*R*)-3-hydroxydecanoyl]-2-[(*R*)-3-hydroxydecanoylamino]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-hydroxydecanoyl]-2-[(*R*)-3-hydroxydecanoylamino]-α-D-(6-^{13}C)glucopyranose 1,4′-Bisphosphate (6-^{13}C-2). In a manner similar to that for the synthesis of 6-^{13}C-**1**, **24b** (45 mg, 0.026 mmol) was phosphorylated and deprotected to yield 6-^{13}C-**2** as a white powder (22 mg, 70% from **24b**). To remove any excess triethylamine hydrochloride, which occasionally became contaminated into active fractions during the chromatography and remained even after concentration in vacuo, the thus-obtained product was passed quickly through a column (1×2 cm) of Dowex® resin (H^+ form); after lyophilization, 6-^{13}C-**2** was recovered quantitatively as a white, hygroscopic powder. $[\alpha]_D^{24}$ −21.0 (*c* 0.03, H_2O); ESI-MS (negative, API III) *m/z* 1180.7 [(M−H)$^-$]; ^{1}H NMR (500 MHz, D_2O) δ = 5.36 (br s, 1 H, H_1), 5.16 (m, 1 H, $H_{3'}$), 5.15 (m, 1 H, H_3), 4.63 (m, 1 H, $H_{1'}$), 4.11 (m, 1 H, H_2), 4.09 (m, 1 H, $H_{4'}$), 4.08 (m, only $^1J_{C6,H6a}$ = 144 Hz was distinguishable, 1 H, H_{6a}), 3.99 (m, 1 H, $H_{2'}$), 3.98, 3.97, 3.92, and 3.92 (m, 1 H each, one of oxymethine protons on acyl groups), 3.95 (m, 1 H, H_5), 3.84 (m, 1 H, $H_{6a'}$), 3.83 (m, only $^1J_{C6,H6b}$ = 144 Hz was distinguishable, 1 H, H_{6b}), 3.80 (m, 1 H, H_4), 3.75 (m, 1 H, $H_{6b'}$), 3.60 (m, 1 H, $H_{5'}$), 2.59 (dd, J = 15.9, 5.4 Hz, 1 H, one of α-CH_2 protons on acyl groups), 2.50, 2.45, 2.39, 2.38, 2.30, 2.25, and 2.25 (m, 1 H each, one of α-CH_2 protons on acyl groups), 1.50—1.16 (m, 48 H, CH_2 on acyl groups), 0.86—0.76 (m, 12 H, CH_3 on acyl groups); ^{13}C NMR (125.65 MHz, D_2O) δ = 68.5 (C_6).

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