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SYNTHESES OF PARTIAL STRUCTURES OF GLYCOSYL PHOSPHATIDYLINOSITOL ANCHOR#

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Abstract-The triethylamine salts of $AcNHCH_2CH_2PO_4H\rightarrow6Man\alpha(1\rightarrow2)Man\alpha1\rightarrow OCH_2CH_2CH_3$ and $AcNHCH_2CH_2PO_4H\rightarrow6Man\alpha(1\rightarrow2)Man\alpha(1\rightarrow6)Man\alpha1\rightarrow OCH_2CH_2CH_3$ were synthesized. These mannosyl residues were constructed by the dimethylphosphinothioate method. The introduction of the phosphodiester function was achieved by the hydrogen-phosphate method.

INTRODUCTION

Many proteins are associated with the cell membrane through a glycosyl phosphatidylinositol (GPI) anchor. Although the functional significance of GPI anchor is unclear, there is much interest about the properties of the GPI anchor.[1] The first total synthesis of the GPI anchor of *Trypanosoma brucei* was achieved by Murakata *et al.* in 1992.[2] Recently, it was found that some cytokines have lectin-like characteristics by the recognition of the mannosyl residues of the glycans of the GPI anchor, and that the ^{35}S-labeled recombinant human interleukin-1β (rhIL-1β) binds to phosphatidylinositol-specific phospholipase C-treated human placental alkaline phosphatase (ALP). To determine the recognition part of the GPI anchor, we investigated the inhibitory activity of rhIL-1β binding to ALP using several saccharides. Among them, a wide variety of compounds including the biantennary sugar chains derived from glycoproteins as well as ethanolamine phosphate, inositol phosphate, mannose 6-sulfate, mannose 1-phosphate, glucose 6-phosphate, and mannitol 6-phosphate did not show any inhibitory effect. On the other hand, specific saccharides, such as mannose 6-phosphate and synthetic $AcNHCH_2CH_2PO_4H\rightarrow6Man\alpha(1\rightarrow\pm2)Man\alpha(1\rightarrow\pm6)Man\alpha1\rightarrow OCH_2CH_2CH_3$, have a relatively strong inhibitory activity of rhIL-1β binding to ALP. This results showed that rhIL-1β can directly interact with the mannose 6-phosphate diester component of the intact glycan of the GPI anchors.[3]

#*Dedicated with best wishes to Professor Teruaki Mukaiyama on the occasion of his 73rd birthday.*

In this paper, we describe the syntheses of $AcNHCH_2CH_2PO_4H \rightarrow 6Man\alpha(1\rightarrow 2)Man\alpha 1\rightarrow OCH_2CH_2CH_3$ (**1**) and $AcNHCH_2CH_2PO_4H \rightarrow 6Man\alpha(1\rightarrow 2)Man\alpha(1\rightarrow 6)Man\alpha 1\rightarrow OCH_2CH_2CH_3$ (**2**) in detail. We have reported a series of glycosylations using the dimethylphosphinothioate method,[4a-c] and with regard to mannosylations, have achieved the syntheses of stereocontrolled 1,2-*trans*-α- and 1,2-*cis*-β-mannopyranosides using two different activation systems. This method using $AgClO_4$ as the activation system afforded only the α-mannopyranosides.[4b] We applied this α-mannopyranosylation to the construction of the mannnosy residues of the compounds (**1**) and (**2**).

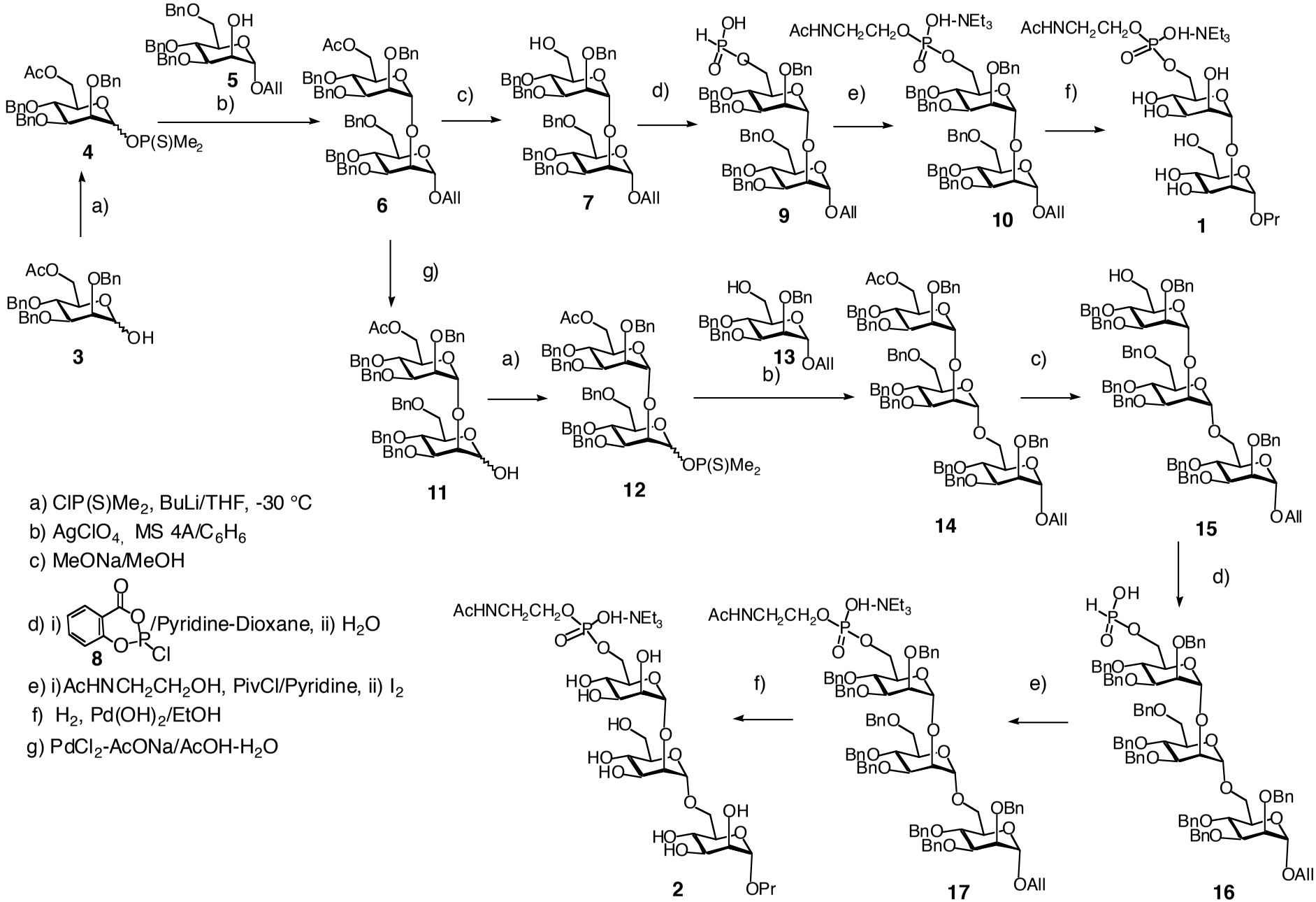
RESULTS AND DISCUSSION

The synthetic routes of compounds (**1**)and (**2**) are shown in Scheme 1.

6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-**D**-mannopyranosyl dimethylphosphinothioate (**4**) was prepared in 78% yield with the anomer ratio of α/β=89/11 from the reaction of 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-**D**-mannopyranose (**3**)[2] with dimethylphosphinothioyl chloride using *n*-butyllithium (BuLi) in THF at -30 °C. The coupling reaction of **4** with allyl 3,4,6-tri-*O*-benzyl-α-**D**-mannopyranoside (**5**)[5] in benzene using $AgClO_4$ as an activator afforded the disaccharide (**6**) in 90% yield with α-stereoselectivity. This glycosylation was successfully achieved in spite of using **5** having the hindered axial hydroxyl function. The stereochemistry of the formed glycosidic bond of **6** was assigned on the basis of the ^{13}C-NMR data and was confirmed to be α by the signal of the anomeric carbon ($J_{C1\text{-}H1}$=174.6 Hz) in the ^{13}C-NMR spectrum that agreed with the observations of Bock and Pedersen.[6] The deacetylation of **6** using sodium methoxide in methanol quantitatively gave the corresponding **7**. Following the literature of Marugg *et al.*[7] and Pannecoucke *et al.*,[8] we investigated the synthesis of the phosphoric acid diester of *N*-acetylethanolamine and of **7**. The reaction of **7** with 2-chloro-1,3,2-benzodioxaphosphorin-4-one (**8**) using anhydrous pyridine in dioxane at room temperature gave the hydrogen phosphonate (**9**) in 74% yield. The mixed anhydride formed by the reaction of **9** with pivaloyl chloride was coupled with *N*-acetylethanolamine, and the following oxidation using iodine afforded the phosphonate diester (**10**) in 73% yield. After the deprotection of the benzyl groups and the reduction of the allyl group of **10** using $H_2/Pd(OH)_2$ in ethanol, the purification by gel chromatography on Sephadex LH-20 in methanol gave the desired compound (**1**) in 82% yield.

The deallylation of **6** using $PdCl_2$-CH_3COONa in acetic acid-water afforded the disaccharide (**11**) in 89% yield. The dimethylphosphinothioate (**12**) was obtained in 69% yield with the anomer ratio of α/β=90/10 by the reaction of **11** with dimethylphosphinothioyl chloride using BuLi in THF. The reaction of **12** with allyl 2,3,4-tri-*O*-benzyl-α-**D**-mannopyranoside (**13**) using $AgClO_4$ in benzene gave the trisaccharide (**14**) in 56% yield with α-stereoselectivity. From the measurement of the ^{13}C-NMR spectrum of **14**, it was found that three signals of the anomeric carbons have $J_{C1\text{-}H1}$ values of ~169 Hz. Based on this fact, the new mannosidic linkage formed by this glycosidation was confirmed to be α.

Using a similar synthetic procedure from **6** to **1**, we synthesized the desired compound (**2**) in several steps; the deacetylation of **14** proceeded quantitatively, and the yields of the hydrogen-phosphate (**16**) and the


a) ClP(S)Me2, BuLi/THF, -30 °C
b) AgClO4, MS 4A/C6H6
c) MeONa/MeOH
d) i) /Pyridine-Dioxane, ii) H2O
e) i) AcHNCH2CH2OH, PivCl/Pyridine, ii) I2
f) H2, Pd(OH)2/EtOH
g) PdCl2-AcONa/AcOH-H2O

Scheme 1

hydrogen-phosphonate (**17**) were 73% and 58%, respectively, and deprotection of the benzyl groups and the reduction of the allyl group of **17** using $Pd(OH)_2/H_2$ gave **2** in 87% yield.

As mentioned above, we synthesized compounds (**1**) and (**2**) in satisfactory yields. These mannosyl residues were constructed with α-stereoselectivities using the dimethylphosphinothioate method. The introduction of the phosphodiester function was efficiently achieved by the hydrogen-phosphate method.

EXPERIMENTAL

^{1}H-NMR, ^{13}C -NMR and ^{31}P-NMR spectra were recorded on JEOL EX-400 spectrometers. ^{1}H-NMR and ^{13}C -NMR spectra were measured with tetramethylsilane as the internal standard in $CDCl_3$ or CD_3OD, and ^{31}P-NMR spectra were measured with trimethylphosphite as the internal standard in $CDCl_3$. by Optical rotations were recorded on a JASCO DIP-360 digital polarimeter. MS numbers were determined MALDI-TOF MS spectrometry using a Voyager® RP (PerSeptive Biosystems Inc.) with 2,5-dihydroxybenzoic acid as the matrix.

6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-D-mannopyranosyl dimethylphosphinothioate (**4**). To a solution of 6-*O*-acetyl-2,3,4-tri-*O*-bennzyl-**D**-mannopyranose (**3**) (1.06 g, 2.2 mmol) in dry THF (5 mL) at -30 °C was added a 1.68 M (1 M=1 mol· dm^{-3}) hexane solution of butyllithium (1.3 mL, 2.2 mmol) under an argon atmosphere. After stirring for 15 min, dimethylphosphinothioyl chloride (0.277 g, 2.2mmol) was added to the solution. The mixture was stirred for 2.5 h at -30 °C, then water and dichloromethane were added, and the organic layer was separated and washed with water. After drying over sodium sulfate, the organic solvent was evaporated *in vacuo* to afford the crude oil. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate=4/1) to give **4** (0.974 g, 78%) as a colorless oil with an anomer ratio of α/β=89/11.

^{1}H-NMR ($CDCl_3$): δ 6.02 (0.89H, dd, $J_{1,2}$=2.0 Hz, J_{HCOP}=12.2 Hz, H-1α), 5.34 (0.11H, d, J_{HCOP}=11.7 Hz, H-1β) , 2.04 (0.33H, s, CH_3β), 2.03 (2.67H, s, CH_3α), ^{13}C-NMR ($CDCl_3$): δ 170.7 (C=O), 94.5 (C-1β, J_{COP}=5.5 Hz), 92.8 (C-1α, J_{COP}=5.6 Hz), 20.8 (CH_3α), 20.9 (CH_3β), ^{31}P-NMR ($CDCl_3$): δ 95.2 (β), 92.5 (α), MALDI-TOF MS: Found: m/z $[M+Na]^+$ 605.2. Calcd for $C_{31}H_{37}O_7PS[M+Na]^+$ 607.2, Anal. Calcd for $C_{31}H_{37}O_7PS$: C, 63.68; H, 6.38. Found: C, 63.41; H, 6.42.

Allyl 2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**6**). To a solution of **4** (300 mg, 0.51 mmol) and ally 3,4,6-tri-*O*-bennzyl-α-**D**-mannopyranose (**5**) (208 mg, 0.42 mmol) in benzene (3 mL) in the presence of MS 4A (*ca.* 100 mg) was added silver perchlorate (110 mg, 0.53 mmol) and the mixture was stirred overnight in a dark place. A 5% sodium sulfide solution and ethyl acetate were added to the reaction mixture. The insoluble materials were filtered off, and the mixture was extracted with ethyl acetate. The organic layer was washed with a 5% sodium sulfide solution, a saturated sodium chloride solution and dried over anhydrous sodium sulfate. After filtration, the organic layer was concentrated *in vacuo* to afford the crude glycoside. The crude

glycoside was purified by preparative thin-layer chromatography (hexane/ethylacetate=2/1) on silica gel to give **6** (367 mg, 90%) as a colorless oil.
^{1}H-NMR ($CDCl_3$): δ 5.86 (1H, m, CH_2=CH-), 2.05 (3H, s, CH_3), ^{13}C-NMR ($CDCl_3$): δ 170.9 (C=O), 99.3 (C-1', J_{CH}=174.6 Hz), 98.1 (C-1, J_{CH}=169.2 Hz), 20.9 (CH_3), $[\alpha]_D^{25}$+24.7。(*c* 1.5, $CHCl_3$), MALDI-TOF MS: Found: m/z $[M+Na]^+$ 985.5. Calcd for $C_{59}H_{64}O_{12}$ $[M+Na]^+$ 987.4.

Allyl 2-*O*-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**7**). To a solution of **6** (60.8 mg, 0.063 mmol) in methanol (5 mL) was added sodium methoxide (*ca*. 5 mg) for 5 h. After the addition of Amberlite IR-122, the organic layer was filtered, and concentrated *in vacuo* to afford the crude product. The crude product was purified by preparative thin-layer chromatography (hexane/ethylacetate=2/1) on silica gel to quantitatively give **7** (57.9 mg) as a colorless oil.
^{1}H-NMR ($CDCl_3$): δ 5.86 (1H, m, CH_2=CH-), 2.04 (1H, OH), ^{13}C-NMR ($CDCl_3$): δ 99.4 (C-1', J_{CH}=171.0 Hz), 97.9 (C-1, JCH=169.1 Hz), $[\alpha]_D^{23}$+23.2。(*c* 1.5, $CHCl_3$), MALDI-TOF MS: Found: m/z $[M+Na]^+$ 945.5. Calcd for $C_{57}H_{62}O_{11}$ $[M+Na]^+$ 945.5.

Allyl 2-*O*-([6-*O*-{2-(*N*-acetyl)amino}ethyl phosphono]-2,3,4-tri-*O*-benzyl-α-D-mannopyranosy)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside NEt3 salt (**10**). To a solution of **7** (126.7 mg, 0.137 mmol) in pyridine (1 mL)-dioxane (3 mL) was added 2-chloro-1,3,2-benzodioxaphosphorin-4-one (**8**) (83 mg, 0.41 mmol), and stirred overnight at r.t. After water (3 mL)-pyridine (3 mL) was added to the reaction mixture, the resulting mixture was stirred for 30 min. The solvent was evaporated *in vacuo* to afford the crude **9**. The crude residue was purified by preparative thin-layer chromatography (trichloromethane/methanol=9/1 containing 1% Et_3N) on silica gel to give the hydrogen-phosphonate (**9**) (102.5 mg, 69%). Pivaloyl chloride (0.0465 mL, 0.377 mmol) was added dropwise during 20 min to a solution of **9** (102.5 mg, 0.0943 mmol) and *N*-acetylethanolamine (19.5 mg, 0.189 mmol) in pyridine (3 mL) under an Ar atmosphere at -20 °C. The reaction was kept at -20 °C for 2 h, then quenched by water (1 mL) and warmed to r.t. Iodine (13.2 mg, 0.052 mmol) was added and the reaction mxture was stirred overnight. After the addition of water and ethyl acetate, the organic layer was separated and washed with a saturated sodium hydrogen carbonate solution, a saturated sodium thiosulfate solution, a saturated sodium chloride solution, and dried over anhydrous sodium sulfate. The organic solvents were evaporated *in vacuo* to afford the crude product. The crude product was purified by preparative thin-layer chromatography (trichloromethane/methanol=9/1 containing 1% Et_3N) on silica gel to give **10** (68.2 mg, 61%) as a colorless oil.
^{1}H-NMR ($CDCl_3$): δ 5.87 (1H, m, CH_2=CH-), 3.05 (6H, m, NCH_2CH_3), 1.89 (3H, s, CH_3CO), 1.35 (9H, t, *J*=7.3 Hz , NCH_2CH_3), ^{13}C-NMR ($CDCl_3$): δ 99.7 (C-1'), 98.1 (C-1), ^{31}P-NMR ($CDCl_3$): δ -0.28, MALDI-TOF MS: Found: m/z $[M-HNEt_3]^+$1086.8: Calcd for $C_{67}H_{85}N_2O_{15}P$ $[M-HNEt_3]^+$ 1086.4.

Propyl 2-*O*-([6-*O*-{2-(*N*-acetyl)amino}ethyl phosphono]-α-D-mannopyranosyl)-α-D-mannopyranoside NEt3 salt (**1**). A mixture of **10** (59.9 mg, 0.0504 mmol) and palladium hydroxide (30 mg, 0.21 mmol) in ethanol (8 mL) was stirred under hydrogen overnight at r.t. After filtration, the solvent was concentrated *in vacuo*. The residue was purified by gel chromatography on Sephadex LH-20 in methanol to give **1** (22.8 mg, 82%) as a colorless oil.
^{1}H-NMR (CD_3OD) δ 1.96 (3H, s, CH_3CO), 1.59 (2H, m, CH_2CH_3), 0.96 (3H, t, *J*=7.3 Hz, CH_2CH_3), MALDI-TOF MAS: Found: m/z $[M\text{-}HNEt_3]^+$ 548.2 : Calcd for $C_{25}H_{50}N_2O_{15}P[MHNEt_3]^+$ 547.2.

2-*O*-(6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranose (**11**). To a solution of **6** (322 mg, 0.33 mmol) and sodium acetate (70 mg, 0.40 mmol) in acetic acid (10 mL)-water (1 mL) was added palladium dichloride (71 mg, 0.40 mmol), and the mixture was stirred overnight. After the insoluble materials were filtered off, water and ethyl acetate were added to the mixture, and the organic layer was separated and washed with a saturated sodium hydrogen carbonate solution and a saturated sodium chloride solution, and then dried over anhydrous sodium sulfate. After filtration, the organic solvents were evaporated *in vacuo* to afford the crude product. The crude product was purified by silica gel column chromatography (hexane/ethylacetate=4/1) to give **11** (272 mg, 89%) as a colorless oil.
^{1}H-NMR ($CDCl_3$): δ 2.06 (0.2H, s, $CH_3\beta$), 2.03 (0.8H, s, $CH_3\alpha$), ^{13}C-NMR ($CDCl_3$): δ 170.9 (C=O),100.0 (C-1'β, J_{CH}=165.5 Hz), 99.1 (C-1'α, J_{CH}=170.1 Hz), 93.6 (C-1β), 93.5 (C-1α, J_{CH}=169.2 Hz), 20.8 (CH_3), $[\alpha]_D^{23}$+4.4° (*c* 0.90, $CHCl_3$), MALDI-TOF MS: Found: m/z $[M+Na]^+$ 946.0: Calcd for $C_{56}H_{60}O_{12}$ $[M+Na]^+$ 947.4.

6-*O*-(6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-D-mannopyranosyl dimethylphosphinothioate (**12**). To a solution of **11** (249 mg, 0.27 mmol) in dry THF (5 mL) at -30 °C was added a 1.5 M (1 M=1 mol·dm^{-3}) hexane solution of butyllithium (0.17 mL, 0.26 mmol) under an argon atmosphere. After stirring for 15 min, dimethylphosphinothioyl chloride (36 mg, 0.28 mmol) was added to the solution and the mixture was stirred for 1.5 h at -30 °C. Water (10 mL) and ethyl acetate (10 mL) were then added to the reaction mixture. The organic layer was separated and washed with water. After drying over sodium sulfate, the organic solvent was evaporated *in vacuo* to afford the crude oil. The crude product was purified by preparative thin-layer chromatography (hexane/ethyl acetate=4/1) on silica gel to give **12** (183 mg, 69%) as a colorless oil with an anomer ratio of α/β=90/10.
^{1}H-NMR ($CDCl_3$): δ 5.89 (0.9H, dd, H-1α, $J_{1,2}$=2.0 Hz, J_{HCOP}=12.2 Hz), 5.18 (0.9H, d, H-1'α, $J_{1,2}$=1.5 Hz), 2.09 (2.7H, s, $CH_3\alpha$), 2.05 (0.3H, s, $CH_3\beta$), ^{13}C-NMR ($CDCl_3$): δ 171.0 (C=O), 99.3 (C-1'α), 99.1 (C-1'β), 94.1 (C-1α, J_{COP}=3.6 Hz), 92.8 (C-1β, J_{COP}=4.8 Hz), 21.1 ($CH_3\alpha$), 21.0 ($CH_3\beta$), ^{31}P-NMR=93.5 (α), 92.1 (β), MALDI-TOF MS: Found: m/z $[M+Na]^+$ 1040.0: Calcd for $C_{58}H_{65}O_{12}PS$ $[M+Na]^+$ 1039.4, Anal. Calcd for $C_{58}H_{65}O_{12}PS\cdot H_2O$: C, 67.29; H, 6.52. Found: C, 67.52; H, 6.39.

Allyl 6-*O*-{2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-atri-*O*-benzyl-α-D-mannopyranosyl}-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**14**). To a solution of **12** (123 mg, 0.12 mmol) and allyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**13**) (65 mg, 0.13 mmol) in benzene (3 mL) in the presence of MS 4A (*ca.* 100 mg) was added $AgClO_4$ (125 mg, 0.60 mmol) , and the mixture was stirred overnight in the dark. A 5% sodium sulfide solution and ethyl acetate were added to the reaction mixture. The insoluble materials were filtered off, and the organic layer was separated and washed with a 5% sodium sulfide solution, a saturated sodium chloride solution and then dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated *in vacuo* to afford the crude glycoside. The crude product was purified by preparative thin-layer chromatography (hexane/ethylacetate=5/2) on slica gel to give **14** (94 mg, 56%) as a coloress oil.
^{1}H-NMR ($CDCl_3$): δ 5.82 (1H, m, CH_2=CH-), 1.99 (3H, s, CH_3), ^{13}C-NMR ($CDCl_3$): δ 170.9 (C=O), 99.4, 99.3 (C-1', J_{CH}=169.2 Hz, C-1", J_{CH}=170.7 Hz), 96.9(C-1, J_{CH}=169.2 Hz), 20.9 (CH_3), $[\alpha]_D^{25}$+16.9。(*c* 1.6, $CHCl_3$), MALDI-TOF MS: Found: m/z $[M+Na]^+$ 1419.2: Calcd for $C_{86}H_{92}O_{17}$ $[M+Na]^+$ 1419.6.

Allyl 6-*O*-{2-*O*-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl}-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**15**). To a solution of **14** (21.8 mg, 0.0155 mmol) in methanol (5 mL) was added sodium methoxide (*ca.* 3 mg) for 5 h. After the addition of Amberlite IR-122, the solvent was filtered, and evaporated *in vacuo* to give the crude product. The crude product was purified by preparative thin-layer chromatography (hexane/ethylacetate=2/1) on silica gel to quantitatively give **15** (20.9 mg) as a colorless oil.
^{1}H-NMR: δ 5.81 (1H, m, CH_2=CH-), 2.04 (1H, OH), ^{13}C-NMR: δ 99.7, 99.4 (C-1', J_{CH}=169.1 Hz, C-1", J_{CH}=172.8 Hz), 97.0 (C-1, J_{CH}=169.1 Hz), $[\alpha]_D^{25}$+40.0。(*c* 1.0, $CHCl_3$), MALDI-TOF MS: Found: m/z $[M+Na]^+$ 1374.5: Calcd for $C_{84}H_{90}O_{16}$ $[M+Na]^+$ 1377.6.

Allyl 6-*O*-[2-*O*-{(6-*O*-[{2-(*N*-acetyl)amino}ethyl phosphono]-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl}-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside NEt3 salt (**17**). To a solution of **15** (68.8 mg, 0.0493 mmol) in pyridine (0.5 mL)-dioxane (2 mL) was added 2-chloro-1,3,2-benzodioxaphosphorin-4-one (**8**) (12 mg, 0.0592 mmol), then this reaction mixture was stirred overnight at r.t. After the addition of water (1 mL)-pyridine (1 mL), the mixture was stirred for 30 min and the solvents were evaporated *in vacuo*. The residue was purified by preparative thin-layer chromatography (trichloromethane/methanol=9/1 containing 1% Et_3N) on silica gel to give the hydrogen-phosphonate (**16**) (56.7 mg, 74%). Pivaoly chloride (0.024 mL, 0.197 mmol) was added dropwise during 20 min to a solution of **6** (51.2 mg, 0.0328 mmol) and *N*-acetylethanolamine (11.2 mg, 0.109 mmol) in pyridine (3 mL) under an Ar atmosphere at -20 °C. The reaction was kept at -20 °C for 2 h, and then quenched by introducing water (1 mL) and warmed to r.t. Iodine (0.036 mmol) was added to this reaction, and this reaction mixture was stirred overnight. After the addition of water and ethyl acetate, the organic layer was separated and washed with a saturated sodium hydrogen carbonate solution, a

saturated sodium thiosulfate solution, a saturated sodium chloride solution, and dried over anhydrous sodium sulfate. The organic solvents were evaporated *in vacuo* to afford the crude product. The crude product was purified by preparative thin-layer chromatography (trichloromethane/methanol=9/1 containing 1% Et_3N) on silica gel to give **2** (31.5 mg, 58%) as a colorless oil.
^{1}H-NMR: δ 5.80 (1H, m, CH_2=CH-), 2.93 (6H, m, NCH_2CH_3), 1.83 (3H, s, CH_3CO), 1.26 (9H, t, *J*=7.3Hz , NCH_2CH_3), ^{13}C-NMR: δ 99.3, 98.9 (C-1', C-1''), 96.9 (C-1), 45.5 (NCH_2CH_3), 23.0 (CH_3CO), 8.52 (NCH_2CH_3), ^{31}P-NMR: δ -1.66, MALDI-TOF MS: Found: m/z $[M\text{-}HNEt_3+Na]^+$ 1556.9: Calcd for $C_{94}H_{113}N_2O_{21}P$ $(M\text{-}HNEt_3+Na^+)$ 1557.6.

Propyl 6-*O*-[2-*O*-{6-*O*-[{2-(*N*-acetyl)amino}ethyl phosphono]-α-D-mannopyranosyl}-3,4,6-α-D-mannopyranosyl]-α-D-mannopyranoside NEt_3 salt (**2**). A mixture of **17** (31.5 mg, 0.0191 mmol) and palladium hydroxide (60 mg) in ethanol (5 mL) was stirred under hydrogen overnight at r.t., and then filtered, and concentrated *in vacuo*. The residue was purified by gel chromatography on Sephadex LH-20 in methanol to give **2** (12.1 mg, 87%) as a colorless oil.
^{1}H-NMR: δ 1.97 (3H, s, CH_3CO), 1.61 (2H, m, CH_2CH_3), 0.95 (3H, t, *J*=7.3 Hz , CH_2CH_3), ^{13}C-NMR: δ 105.0, 101.7, 100.1 (C-1, C-1', C-1''), MALDI-TOF MAS: Found: m/z $[M\text{-}HNEt_3+Na]^+$ 710.4: Calcd for $C_{25}H_{45}NO_{20}P$ $[M\text{-}HNEt_3+Na]^+$ 710.2.

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