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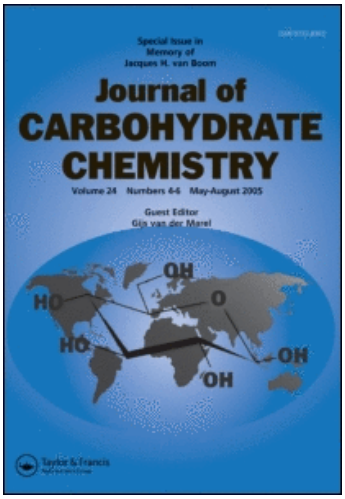

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SYNTHESIS OF MONOMETHYL DERIVATIVES OF *p*-NITROPHENYL α-D-GLUCO, GALACTO, AND MANNOPYRANOSIDES AND THEIR HYDROLYTIC PROPERTIES AGAINST α-GLYCOSIDASES

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ABSTRACT

All possible monomethyl derivatives of *p*-nitrophenyl α-D-gluco, galacto, and mannopyranosides were synthesized. Hydrolytic activities of α-glucosidase (rice), α-galactosidases (green coffee bean, *Mortierella vinacea*, and *Aspergillus niger*), and α-mannosidases (almond and jack bean) against them were elucidated. The 6-*O*-methyl galactopyranoside and mannopyranoside were hydrolyzed by the *M. vinacea* α-galactosidase and the almond and jack bean α-mannosidases, respectively, while these enzymes did not act on the 2-, 3-, and 4-*O*-methyl derivatives. On the other hand, rice α-glucosidase and green coffee bean and *A. niger* α-galactosidases had no hydrolyzing activities at all against the respective four monomethylated substrates.

INTRODUCTION

Various types of *exo*-glycosidases such as α-glucosidase (EC 3.2.1.20), galactosidase (EC 3.2.1.22), and mannosidase (EC 3.2.1.24), many of which were purified,[1-3] are distributed widely in microorganisms, plants, insects, and mammals. It is known that the

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hydrogen bonds between the active site of the enzyme and the hydroxyl groups of substrate glycoside are important in forming an enzyme-substrate complex. Using partially substituted monosaccharide containing glycosides as substrates, we undertook evaluation of the role of hydroxyl groups in the substrate recognizing mechanism by the α-glycosidases. Roeser and Legler discussed the role of sugar hydroxyl groups in hydrolysis by *Aspergillus* (*A.*) *wentii* β-glucosidase (EC 3.2.1.21) using some deoxy and amino sugars. Partially substituted maltose and isomaltose derivatives were used in order to investigate substrate specificity of *A. niger* glucoamylase (EC 3.2.1.3).[4-6] We have already elucidated the hydrolytic activities of α-glucosidase, galactosidases, and mannosidases against all possible monodeoxy derivatives of the corresponding *p*-nitrophenyl (PNP) α-D-glycopyranosides, and confirmed that some of them were good substrates for the enzymes.[7-9] Next, we planned to investigate the activities of the enzymes against partially methylated substrates. For these studies, efficient syntheses of various monomethylated glycopyranosides are required. In this report, we first describe the synthesis of all possible monomethyl derivatives of PNP α-D-gluco, galacto, and mannopyranosides. The hydrolytic activities of α-glucosidase (rice), α-galactosidases (*A. niger, Mortierella vinacea*, and green coffee bean), and α-mannosidases (almond and jack bean), toward these substrates were then determined.

There have been no reports on the syntheses of all the monomethyl derivatives of PNP α-D-gluco, galacto, and mannopyranosides, so far as we know.

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RESULTS AND DISCUSSION

Synthesis of monomethylated substrates

Methyl 4, 6-*O*-benzylidene-α-D-gluco (**16**), galacto (**30**), and mannopyranosides (**43**) were used as starting materials for the syntheses of possible monomethyl derivatives of PNP α-D-glucopyranoside (**1**), galactopyranoside (**6**), and mannopyranoside (**11**) (Scheme). The 2- and 3-*O*-methyl derivatives of **1**, **6**, and **11** were synthesized from **16**, **30**, and **43** through regioselective alkylation of 2-OH and 3-OH. In order to prepare 4- and 6-*O*-methyl derivatives of **1**, **6**, and **11**, the C2 and C3 hydroxyl groups of **16**, **30**, and **43**

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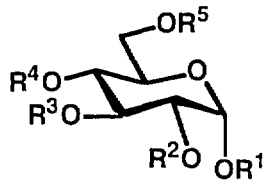
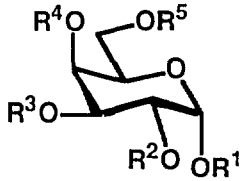
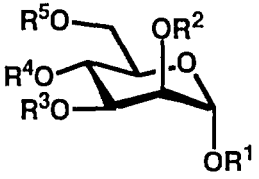


	R1	R2	R3	R4	R5
1	PNP	H	H	H	H
2	PNP	Me	H	H	H
3	PNP	H	Me	H	H
4	PNP	H	H	Me	H
5	PNP	H	H	H	Me
16	Me	H	H	-PhCH-	
17	Me	Me	H	-PhCH-	
18	Me	Me	Bz	Bz	Bz
19	PNP	Me	Bz	Bz	Bz
20	Me	Bn	H	-PhCH-	
21	Me	Bn	Me	-PhCH-	
22	PNP	Ac	Me	Ac	Ac
23	Me	Bn	Bn	-PhCH-	
24	Me	Bn	Bn	H	Bn
25	Me	Bn	Bn	Me	Bn
26	PNP	Ac	Ac	Me	Ac
27	Me	Bn	Bn	Bn	H
28	Me	Bn	Bn	Bn	Me
29	PNP	Ac	Ac	Ac	Me



	R1	R2	R3	R4	R5
6	PNP	H	H	H	H
7	PNP	Me	H	H	H
8	PNP	H	Me	H	H
9	PNP	H	H	Me	H
10	PNP	H	H	H	Me
30	Me	H	H	-PhCH-	
31	Me	Me	Bz	Bz	Bz
32	PNP	Me	Bz	Bz	Bz
33	Me	Bz	Me	Bz	Bz
34	PNP	Bz	Me	Bz	Bz
35	Me	Bn	Bn	-PhCH-	
36	Me	Bn	Bn	H	Bn
37	Me	Bn	Bn	Me	Bn
38	Me	Bz	Bz	Me	Bz
39	PNP	Bz	Bz	Me	Bz
40	Me	Bn	Bn	Bn	H
41	Me	Bn	Bn	Bn	Me
42	PNP	Ac	Ac	Ac	Me



	R1	R2	R3	R4	R5
11	PNP	H	H	H	H
12	PNP	Me	H	H	H
13	PNP	H	Me	H	H
14	PNP	H	H	Me	H
15	PNP	H	H	H	Me
43	Me	H	H	-PhCH-	
44	Me	H	Bn	-PhCH-	
45	Me	Me	Bn	-PhCH-	
46	PNP	Me	Ac	Ac	Ac
47	Me	H	Me	-PhCH-	
48	PNP	Ac	Me	Ac	Ac
49	Me	Bn	Bn	-PhCH-	
50	Me	Bn	Bn	H	Bn
51	Me	Bn	Bn	Me	Bn
52	PNP	Ac	Ac	Me	Ac
53	Me	Bn	Bn	Bn	H
54	Me	Bn	Bn	Bn	Me
55	PNP	Ac	Ac	Ac	Me

Scheme

were protected as their benzyl ethers, and then the 4, 6-*O*-benzylidene groups were regioselectively opened.

Each 2-*O*-methyl derivative was prepared as follows. Treatment of the 2, 3-*O*-stannylene derivative of glucopyranoside **16** with methyl iodide (MeI) in DMF, yielded the 2-methoxy compound **17**.[10] The benzylidene group of **17** was removed, followed by benzoylation of hydroxyl groups at C2, C3, and C6 to give methyl 3, 4, 6-tri-*O*-benzoyl-2-*O*-methyl-α-D-glucopyranoside (**18**). Reaction of galactopyranoside **30** with MeI under phase-transfer conditions (Bu_4NHSO_4, CH_2Cl_2-40% NaOH solution) gave 2- and 3-*O*-methyl derivatives, respectively, which yieled methyl 3, 4, 6-tri-*O*-benzoyl-2-*O*-methyl-α-D-galactopyranoside (**31**) and methyl 2, 4, 6-tri-*O*-benzoyl-3-*O*-methyl-α-D-galactopyranoside (**33**) via debenzylidenation and benzoylation. Treatment of the 2, 3-*O*-stannylene derivative of mannopyranoside **43** with benzyl bromide (BnBr) in DMF gave **44**.[11] Compound **44** was treated with NaH and MeI to afford **45**[11] in 96% yield. Benzyl and benzylidene groups of **45** were removed to give methyl 2-*O*-methyl-α-D-mannopyranoside.

Each 3-*O*-methyl derivative was prepared as follows. The reaction of the 2, 3-*O*-stannylene derivative of **16** with BnBr in DMF gave **20**,[10] followed by methylation of the 3-OH using NaH and MeI to afford methyl 2-*O*-benzyl-4, 6-*O*-benzylidene-3-*O*-methyl-α-D-glucopyranoside (**21**), and then benzyl and benzylidene groups of it were removed. Methylation of 3-OH of galactopyranoside **30** was performed under the phase-transfer condition as mentioned above. Treatment of 2, 3-*O*-stannylene derivatives of **43** with MeI in DMF gave methyl 4, 6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside (**47**) in good yield.[11]

Each 4-*O*-methyl derivative was prepared as follows. Glucopyranoside **24**, galactopyranoside **36**, and mannopyranoside **50**, which had a 4-OH, were prepared by the regioselective ring opening of 4, 6-*O*-benzylidene group of **23**,[12] **35**,[13] and **49**.[14] Methylation of 4-OH of **24**, **36**, and **50** were performed using NaH and MeI to afford methyl 2, 3, 6-tri-*O*-benzyl-4-*O*-methyl-α-D-glucopyranoside (**25**), methyl 2, 3, 6-tri-*O*-benzyl-4-*O*-methyl-α-D-galactopyranoside (**37**), and methyl 2, 3, 6-tri-*O*-benzyl-4-*O*-methyl-α-D-mannopyranoside (**51**) in good yields, respectively, followed by removal of the benzyl groups.

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Each 6-*O*-methyl derivative was synthesized as follows. In order to prepare compounds having 6-OH, **23**, **35**, and **49** were treated with lithium aluminum hydride (LAH) and $AlCl_3$ in the mixture of CH_2Cl_2 and Et_2O to give **27**, **40**, and **53** in good yields, respectively.[15] After methylation of 6-OHs of **27**, **40**, and **53** using NaH and MeI, the benzyl groups were removed.

For preparing of PNP α-D-glycopyranosides, corresponding methyl glycosides were subjected to acetolysis with sulfuric acid in the mixture of acetic anhydride and acetic acid. Glycosidation of 1-*O*-acetylated glucopyranoses, galactopyranoses, and mannopyranoses with *p*-nitrophenol were performed by the Helferich method[9,16] to afford α-glucopyranosides **19**, **22**, **26**, and **29**, galactopyranosides **32**, **34**, **39**, and **42**, and mannopyranosides **46**, **48**, **52**, and **55**. Treatment of the obtained PNP α-glycopyranosides with base gave 2-, 3-, 4-, and 6-*O*-monomethylated PNP α-D-glucopyranosides **2-5**, galactopyranosides **7-10**, and mannopyranoside **12-15**, respectively.

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The position of the methoxy group of each synthesized PNP glycopyranoside was confirmed by ^{13}C NMR spectroscopy, taking into account the downfield shifts about 10 ppm with respect to the δ values in corresponding PNP α-D-glycopyranoside (Table 5). The values of vicinal diaxial proton coupling constants ($J_{3,4}$) observed in ^1H NMR spectra of the glucopyranosides **1-5** and the mannopyranosides **11-15** were 9.2-9.6 Hz and 9.3-9.9 Hz, respectively. The $J_{2,3}$ values of PNP galactopyranosides **6-10** recorded 10.3-10.5 Hz. These values indicate that the conformations of all monomethylated PNP α-D-glycopyranosides were retained in $^4C_1$ chair form, in D_2O.[17]

Hydrolytic activities of α-glycosidases

We have a series of PNP α-D-glycopyranosides in hand. In order to elucidate the enzyme-substrate complexation mechanism, we examined the enzymatic hydrolysis of these α-glycopyranosides in the presence of different types of *exo*-α-glycosidases. The results of the hydrolytic activities of α-glycosidase against monodeoxy and monomethyl derivatives of PNP α-D-glycopyranosides are summarized in Table 1.

Rice α-glucosidase and green coffee bean and *Aspergillus niger* α-galactosidases did not hydrolyze all monomethyl derivatives of **1** and **6** at all, respectively. On the other hand, *Mortierella vinacea* α-galactosidase and almond and jack bean α-mannosidases

Table 1. Relative rates of hydrolysis of monodeoxygenated and monomethylated PNP α-D-glycopyranosides by α-glycosidases.

	Relative rate of hydrolysis (%)[a]					
	α-Glucosidase	α-Galactosidase			α-Mannosidase	
	Rice	Green coffee bean	*M. vinacea*	*A. niger*	Almond	Jack bean
Monomethyl						
2-*O*-Me	-	-	-	-	-	-
3-*O*-Me	-	-	-	-	-	-
4-*O*-Me	-	-	-	-	-	-
6-*O*-Me	-	-	1.10	-	<1	<1
Monodeoxy[b]						
2-Deoxy	175	15.0	19.0	230	-	-
3-Deoxy	-	-	-	-	-	-
4-Deoxy	-	-	-	-	-	-
6-Deoxy	-	3.27	8.77	-	118	92.2

a. Relative rate of hydrolysis was expressed by comparison with the amount of *p*-nitrophenol that was released from corresponding PNP α-D-glycopyranosides for 30 min by each α-glycosidase, which was taken as 100%. b. Data with α-glucosidase (rice), α-galactosidases (green coffee bean, *M. vinacea*, and *A. niger*), and α-mannosidases (almond and jack bean) were taken from references 7, 8, and 9, respectively. -: Not detectable.

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revealed hydrolyzing activities against only each 6-*O*-methylated compound, **10** and **15**, with low relative rates. Previously, we investigated the hydrolytic activities of α-glycosidases used in this study against possible monodeoxy derivatives of corresponding PNP α-D-glycopyranosides. The rice α-glucosidase and *A. niger* α-galactosidase hydrolyzed only each 2-deoxy substrate, and their activities with the 2-deoxy substrate appeared to be substantially higher than against corresponding PNP α-D-glycopyranosides, **1** and **6**.[7,8] α-Galactosidases from green coffee beans and *M. vinacea* showed activity not only for the 2-deoxy substrate, but also some still lower activity for the 6-deoxy derivative, PNP α-D-fucopyranoside.[8] Almond and jack bean α-mannosidases both hydrolyzed 6-deoxy derivative, PNP α-D-rhamnopyranoside, at a rate approaching that for **11**.[9]

Rice α-glucosidase and green coffee bean and *M. vinacea*, and *A. niger* α-galactosidases which have 2-deoxy substrate hydrolyzing activities did not act on each 2-*O*-

methylated compound at all. These facts indicate that the 2-OHs of the substrates do not have a crucial role in substrate discrimination by these enzymes and methylation of the 2-OH seem to result in serious steric hindrace for the enzymes. On the other hand, *M. vinacea* α-galactosidase and almond and jack bean α-mannosidases revealed hydrolyzing activities against both 6-deoxy substrate and 6-*O*-methyl one, respectively. These results suggest that these α-glycosidases have comparatively large space in the glycon 6-OH binding regions.

Further studies using the enzymes from another sources and various substituted substrates should be done to elucidate substrate specificity of α-glycosidase.

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EXPERIMENTAL..

General methods. Melting points were determined with a Yamato Model MP-21 capillary apparatus and are uncorrected. Optical rotations were measured with Perkin-Elmer 141 polarimeter at 20 °C. The ^{1}H and ^{13}C NMR spectra were recorded with a Varian VXR-400 spectrometer. Chemical shifts were expressed in ppm downfield shift from Me_4Si. Mass spectra were obtained with a Jeol JMX SX-102A instrument under positive ion FAB conditions. Column chromatography was performed on silica gel 60 (230-400 mesh, Merck). The progress of all reactions was monitored by thin-layer chromatography on silica gel 60 F_{254} (0.25 mm, Merck). For preparing of each monomethyl derivative of PNP α-D-glucopyranoside **1**, galactopyranoside **6**, and mannopyranoside **11**, the following methods were used.

Method A; to a stirred solution of NaH (7.5 mmol) in DMF (50 mL) was added the monool derivative of the carbohydrate (5.0 mmol). The mixture was stirred at 0 °C for 15 min and MeI (7.5 mmol) was slowly added. After the mixture was stirred at room temperature for 18 h, cold water was added. Product was extracted with Et_2O (3 x 150 mL) and the organic layer was washed with brine, and then concentrated after drying over Na_2SO_4.

Method B; a mixture of benzyloxy derivative (2.8 mmol) in EtOH (12 mL) and 1N HCl (100 μL) was hydrogenated at 5 atm under H_2 with 10% palladium charcoal (150

mg). After stirring the mixture for 18 h, the palladium charcoal was removed by filtration through Celite and the solvent was concentrated.

Method C; to a stirred mixture of AcOH:Ac_2O:H_2SO_4 (40:40:1, 16.4 mL) was added the sugar derivative (2.5 mmol) and the mixture was stirred overnight, and then poured into water (200 mL). The product was extracted with AcOEt (200 mL) and the organic layer was washed with water (3 x 200 mL), saturated $NaHCO_3$ solution, and brine, and then concentrated after drying over Na_2SO_4.

Method D; to a melt of 1-*O*-acetylated saccharide (2.0 mmol) and *p*-nitrophenol (8.0 mmol) was added 0.33 g of anhydrous $ZnCl_2$ (2.4 mmol) dissolved in 1 mL of AcOH-Ac_2O mixture (95:5). The flask was kept in an oil bath at 125 °C for 5 min over evacuation with a water pump. After dissolving the resulting brown syrup into 300 mL of AcOEt, $ZnCl_2$ and remaining *p*-nitrophenol were removed by successive washing with saturated $NaHCO_3$ solution and brine, and then concentrated after drying over Na_2SO_4.

Method E; to a stirred solution of 1-*O*-acetate (10 mmol) and *p*-nitrophenol (20 mmol) in dry toluene (200 mL) was added $BF_3 \cdot Et_2O$ (1.5 mL) under N_2, and the mixture was stirred at room temperature for 15 h. The mixture was washed with saturated $NaHCO_3$ solution and brine, and then concentrated after drying over Na_2SO_4.

Method F; a stirred solution of MeOH:NEt_3:H_2O (5:1:1, 10 mL) was added PNP glycopyranoside (16 mmol). After the mixture was stirred for 3 h at room temperature, solvent was evaporated.

Method G; sodium methoxide (28%) in MeOH (1.0 mL) was added to a stirred solution of PNP glycopyranoside (10 mmol) in dry MeOH (50 mL). The mixture was stirred for 5 h at room temperature, and Amberlite IR-120 (H^+ form) was added until neutrality.

Monomethyl derivatives of PNP α-D-glucopyranoside.

Methyl 3, 4, 6-Tri-*O*-benzoyl-2-*O*-methyl-α-D-glucopyranoside (18). According to method B, the benzylidene group of **17** (3.96 g, 13.4 mmol) was removed, and to the obtained product was added pyridine (50 mL) and benzoyl chloride (BzCl, 5.6 mL, 48.1 mmol). The solution was stirred for 30 h and poured into water (400 mL). The product was extracted with AcOEt (3 x 150 mL) and the organic layer was washed with 1N HCl, saturated $NaHCO_3$ solution, and brine, and then concentrated after drying over Na_2SO_4.

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The product was purified by column chromatography on silica gel (3:2 hexane-AcOEt) to afford 3.62 g (52.1%) of **18**: $[\alpha]_D$ +51.5° (*c* 0.52, $CHCl_3$); MS *m/z*: 521 (MH^+).

***p*-Nitrophenyl 3, 4, 6-Tri-*O*-benzoyl-2-*O*-methyl-α-D-glucopyranoside (19).** According to method C, compound **18** (3.42 g, 6.57 mmol) was 1-*O*-acetylated, and the product obtained was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 3.52 g (97.7%) of 1-*O*-acetyl-3, 4, 6-tri-*O*-benzoyl-2-*O*-methyl-α-D-glucopyranose. According to method D, the acetate (1.87 g, 3.41 mmol) was reacted with *p*-nitrophenol, and the product was purified by column chromatography on silica gel (4:1 hexane-AcOEt) to afford 1.20 g (56.1%) of **19**: $[\alpha]_D$ +121° (*c* 0.54, $CHCl_3$); mp 142-143 °C; MS *m/z*: 628 (MH^+).

***p*-Nitrophenyl 2-*O*-Methyl-α-D-glucopyranoside (2).** According to method E, compound **2** was prepared from **19** (1.20 g, 1.91 mmol). The product was recrystallized from hot EtOH (0.238 g, 39.5%): $[\alpha]_D$ +222° (*c* 0.52, H_2O); mp 183-186 °C; MS *m/z*: 307 (MH^+).

Methyl 2-*O*-Benzyl-4, 6-*O*-benzylidene-3-*O*-methyl-α-D-glucopyranoside (21). According to method A, compound **21** was prepared from **20** (3.76 g, 10.1 mmol), the product was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 2.53 g (64.8%) of **21** as crystals, that were recrystallized from CH_2Cl_2-hexane: $[\alpha]_D$ +14.7° (*c* 0.53, $CHCl_3$); mp 98.0-98.5 °C; MS *m/z*: 387 (MH^+).

***p*-Nitrophenyl 2, 4, 6-Tri-*O*-acetyl-3-*O*-methyl-α-D-glucopyranoside (22).** According to method B, the benzyl and benzylidene group of **21** (1.88 g, 4.86 mmol) were removed, followed by acetylation according to method C, and then the product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 1, 2, 4, 6-tetra-*O*-acetyl-3-*O*-methyl-D-glucopyranose in quantitative yield. According to method D, the acetate (1.83 g, 5.05 mmol) was reacted with *p*-nitrophenol, and the product was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 1.04 g (46.6%) of **22**: $[\alpha]_D$ +189° (*c* 0.50, $CHCl_3$); mp 126-127 °C; MS *m/z*: 442 (MH^+).

***p*-Nitrophenyl 3-*O*-Methyl-α-D-glucopyranoside (3).** According to method F, compound **3** was prepared from **22** (0.97 g, 2.2 mmol). The product was purified by column chromatography on silica gel (6:1 CH_2Cl_2-MeOH) to afford 0.49 g (71.5%) of **3** as crys-

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tals, that were recrystallized from hot EtOH: $[\alpha]_D$ +214° (*c* 0.55, H_2O); mp 158-159 °C; MS *m/z*: 316 (MH^+).

Methyl 2, 3, 6-tri-*O*-Benzyl-4-*O*-methyl-α-D-glucopyranoside (25). According to method A, compound **25** was prepared from **24** (5.20 g, 11.2 mmol). The product was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 5.07 g (94.7%) of **25**: $[\alpha]_D$ +36.8° (*c* 0.56, $CHCl_3$); MS *m/z*: 479 (MH^+).

***p*-Nitrophenyl 2, 3, 6-Tri-*O*-acetyl-4-*O*-methyl-α-D-glucopyranoside (26).** According to methods B and C, benzyl groups of **25** (4.83 g, 10.1 mmol) were removed and the product obtained was acetylated, and then the product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 3.41 g (93.3%) of 1, 2, 3, 6-tetra-*O*-acetyl-4-*O*-methyl-D-glucopyranose. According to method D, the acetate (1.02 g, 2.82 mmol) was reacted with *p*-nitrophenol, and the product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.987 g (79.3%) of **26**: $[\alpha]_D$ +162° (*c* 0.52, $CHCl_3$); mp 161-162 °C; MS *m/z*: 442 (MH^+).

***p*-Nitrophenyl 4-*O*-Methyl-α-D-glucopyranoside (4).** According to method F, compound **4** was prepared from **26** (0.55 g, 1.2 mmol). The product was recrystallized from hot EtOH (0.38 g, 96.2%): $[\alpha]_D$ +213° (*c* 0.27, H_2O); mp 152-153 °C; MS *m/z*: 316 (MH^+).

Methyl 2, 3, 4-tri-*O*-Benzyl-6-*O*-methyl-α-D-glucopyranoside (28). According to method A, compound **28** was prepared from **27** (4.30 g, 9.26 mmol). The product was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 3.91 g (88.3%) of **28**: $[\alpha]_D$ +10.2° (*c* 0.57, $CHCl_3$); MS *m/z*: 479 (MH^+).

***p*-Nitrophenyl 2, 3, 4-Tri-*O*-acetyl-6-*O*-methyl-α-D-glucopyranoside (29).** According to methods B and C, benzyl groups of **28** (3.91 g, 8.17 mmol) were removed and the product was acetylated, and then the product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 2.63 g (88.8%) of 1, 2, 3, 4-tetra-*O*-acetyl-6-*O*-methyl-D-glucopyranose. According to method D, the acetate (0.816 g, 2.25 mmol) was reacted with *p*-nitrophenol, and the product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.551 g (55.5%) of **29**: $[\alpha]_D$ +225° (*c* 0.53, $CHCl_3$); mp 117-119 °C; MS *m/z*: 442 (MH^+).

***p*-Nitrophenyl 6-*O*-Methyl-α-D-glucopyranoside (5).** According to method F, compound **5** was prepared from **29** (0.44 g, 1.0 mmol). The product was purified by

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Table 2. ^{1}H NMR Data of Glucopyranoside Derivatives.

Compound	δ (ppm), *J* (Hz)						
	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6b}$)	H-6a ($J_{5,6a}$)	H-6b ($J_{6a,6b}$)
2 [a]	6.09 (3.7)	3.55 (9.3)	3.98 (9.6)	3.56 (10.0)		3.64-3.75	
3 [a]	5.82 (3.6)	3.69-3.75 (9.2)	3.61 (9.2)		3.69-3.75 (3.4)		3.86 (9.8)
4 [a]	5.83 (3.7)	3.81 (9.4)	4.05 (9.4)	3.36 (9.4)		3.60-3.76	
5 [a]	5.83 (3.6)	3.79 (9.6)	3.96 (9.6)	3.53 (10.0)	3.81 (4.8)	3.62 (2.4)	3.67 (11.4)
18 [b]	5.03 (3.6)	3.65 (9.9)	5.92 (9.8)	5.52 (10.0)	4.33 (3.1)	4.45 (5.5)	4.56 (12.2)
19 [b]	5.89 (3.6)	3.85 (9.9)	6.11 (9.8)	5.56 (9.8)	4.37 (6.4)	4.44 (3.3)	4.47 (12.2)
21 [b]	4.57 (3.8)	3.46 (9.5)	3.69 (9.6)	3.50 (9.7)	3.81 (4.7)	3.76 (9.7)	4.25 (9.6)
22 [b]	5.82 (3.6)	4.97 (10.2)	3.92 (9.9)	5.10 (11.2)	3.92 (5.5)	4.03 (2.6)	4.18 (12.4)
25 [b]	4.60 (3.6)	3.51 (9.5)	3.86 (9.4)	3.33 (10.4)	3.68	3.61-3.65	
26 [b]	5.76 (3.6)	5.00 (10.4)	5.67 (9.3)	3.45 (10.1)	3.87 (3.0)	4.25 (4.4)	4.29 (12.0)
28 [b]	4.59 (3.5)	3.54 (9.5)	3.97 (9.2)	3.51-3.61	3.70 (10.0)	3.51-3.61	
29 [b]	5.86 (3.5)	5.07 (10.2)	5.69 (9.5)	5.25 (10.2)	3.94 (4.1)	3.40 (2.7)	3.46 (11.0)

a. Measured in D_2O. b. Measured in $CDCl_3$. The OCH_3 gave a singlet (3H) at δ 3.29-3.70 for **2-5**, **18**, **19**, **21**, **22**, **25**, **26**, **28** and **29**. The $OCOCH_3$ gave a singlet (3H) at δ 2.01-2.14 for **22**, **25**, and **29**. The PhCH gave a singlet (1H) at δ 5.52 for **21**. The CH_2 of benzyl groups gave a doublet (1H) at δ 4.51-4.94 for **21**, **25**, and **28**. The aromatic H gave a multiplet at δ 7.18-8.30 for **2-5**, **18**, **19**, **21**, **22**, **25**, **26**, **28** and **29**.

column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.27 g (85.3%) of **5**: $[\alpha]_D$ +201° (*c* 0.26, H_2O); mp 186-188 °C; MS *m/z*: 316 (MH^+).

The ^{1}H NMR data of glucopyranoside derivatives were shown in Table 2.

Monomethyl derivatives of PNP α-D-galactopyranoside.

Methyl 3, 4, 6-Tri-*O*-benzoyl-2-*O*-methyl-α-D-galactopyranoside (31) and Methyl 2, 4, 6-Tri-*O*-benzoyl-3-*O*-methyl-α-D-galactopyranoside (33). Forty percent of NaOH solution (80 mL) and Bu_4NHSO_4 (0.60 g, 1.8 mmol) were added to a solution of **30**

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(5.00 g, 17.7 mmol) in 80 mL of CH_2Cl_2 at room temperature, followed by a dropwise addition of MeI (1.22 mL, 19.5 mmol). The mixture was stirred for 3 days at room temperature and poured into CH_2Cl_2 (100 mL) and water (100 mL), and then the organic layer was washed with brine and concentrated after drying over Na_2SO_4. The products were purified by column chromatography on silica gel (AcOEt) to afford 5.41 g (80.4%) of the mixture of 2- and 3-*O*-methylated compounds. According to method B, benzylidene groups of the monomethylated compounds (3.05 g, 10.3 mmol) were removed. Then to the products were added pyridine (40 mL) and BzCl (4.20 mL, 36.4 mmol), and the solutions were stirred for 18 h and poured into water (300 mL). The products were extracted with AcOEt (3 x 150 mL) and the organic layer was washed with 1N HCl, saturated $NaHCO_3$ solution, and brine, and then concentrated after drying over Na_2SO_4. The products were purified by column chromatography on silica gel (4:1 hexane-AcOEt) to afford 2.16 g (40.3%) of **31** and 2.97 g (55.3%) of **33**. Compound **31**: $[\alpha]_D$ +123° (*c* 0.52, $CHCl_3$); MS *m/z*: 521 (MH^+). Compound **33**: $[\alpha]_D$ +108° (*c* 0.51, $CHCl_3$); MS *m/z*: 521 (MH^+).

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***p*-Nitrophenyl 3, 4, 6-Tri-*O*-benzoyl-2-*O*-methyl-α-D-galactopyranoside (32).** According to method C, compound **31** (2.00 g, 3.84 mmol) was 1-*O*-acetylated, and the obtained product was purified by column chromatography on silica gel (3:2 hexane-AcOEt) to afford 2.08 g (98.7%) of 1-*O*-acetyl-3, 4, 6-tri-*O*-benzoyl-2-*O*-methyl-D-galactopyranose. According to method D, the acetate (2.00 g, 3.65 mmol) was reacted with *p*-nitrophenol, and the product was purified by column chromatography on silica gel (10:1 toluene-AcOEt) to afford 1.48 g (64.8%) of **32**: $[\alpha]_D$ +178° (*c* 0.55, $CHCl_3$); mp 167-168 °C; MS *m/z*: 628 (MH^+).

***p*-Nitrophenyl 2-*O*-Methyl-α-D-galactopyranoside (7).** According to method G, compound **7** was prepared from **32** (1.21 g, 1.93 mmol). The product was purified by column chromatography on silica gel (6:1 CH_2Cl_2-MeOH) to afford 0.515 g (84.7%) of **7** as crystals, that were recrystallized from hot EtOH: $[\alpha]_D$ +257° (*c* 0.53, MeOH); mp 146-147 °C; MS *m/z*: 316 (MH^+).

***p*-Nitrophenyl 2, 4, 6-Tri-*O*-benzoyl-3-*O*-methyl-α-D-galactopyranoside (34).** According to method C, compound **33** (2.00 g, 3.75 mmol) was 1-*O*-acetylated, and the product was purified by column chromatography on silica gel (3:2 hexane-AcOEt) to afford 2.11 g (99.6%) of 1-*O*-acetyl-2, 4, 6-tri-*O*-benzoyl-3-*O*-methyl-D-galactopyranose.

According to method D, the acetate (2.00 g, 3.65 mmol) was reacted with *p*-nitrophenol, and the product was purified by column chromatography on silica gel (10:1 toluene-AcOEt) to afford 1.58 g (69.0%) of **34**: $[\alpha]_D$ +106° (*c* 0.53, $CHCl_3$); MS *m/z*: 628 (MH^+).

***p*-Nitrophenyl 3-*O*-Methyl-α-D-galactopyranoside (8).** According to method G, compound **8** was prepared from **34** (1.36 g, 2.19 mmol). The product was purified by column chromatography on silica gel (6:1 CH_2Cl_2-MeOH) to afford 0.360 g (52.7%) of **8** as crystals, that were recrystallized from hot EtOH: $[\alpha]_D$ +253° (*c* 0.50, MeOH); mp 150-151 °C; MS *m/z*: 316 (MH^+).

Methyl 2, 3, 6-Tri-*O*-benzyl-4-*O*-methyl-α-D-galactopyranoside (37). According to method A, compound **37** was prepared from **36** (2.00 g, 4.31 mmol). The product was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 2.06 g (99.9%) of **37**: $[\alpha]_D$ +18.1° (*c* 0.59, $CHCl_3$); MS *m/z*: 479 (MH^+).

Methyl 2, 3, 6-Tri-*O*-benzoyl-4-*O*-methyl-α-D-galactopyranoside (38). According to method B, benzyl groups of **37** (0.67 g, 1.4 mmol) were removed. Then to the product was added pyridine (15 mL) and BzCl (0.60 mL, 5.1 mmol), and the solution was stirred for 13 h and poured into water (100 mL). The product was extracted with AcOEt (3 x 50 mL) and the organic layer was washed with 1N HCl, saturated $NaHCO_3$ solution, and brine, and then concentrated after drying over Na_2SO_4. The product was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 0.72 g (99.1%) of **38**: $[\alpha]_D$ +65.5° (*c* 0.55, $CHCl_3$); MS *m/z*: 521 (MH^+).

***p*-Nitrophenyl 2, 3, 6-Tri-*O*-benzoyl-4-*O*-methyl-α-D-galactopyranoside (39).** According to method C, compound **38** (2.03 g, 3.90 mmol) was 1-*O*-acetylated, and the product was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 1.71 g (80.0%) of 1-*O*-acetyl-2, 3, 6-tri-*O*-benzoyl-4-*O*-methyl-D-galactopyranose. According to method D, the acetate (1.62 g, 2.96 mmol) was reacted with *p*-nitrophenol, and then the product was purified by column chromatography on silica gel (13:1 toluene-AcOEt) to afford 1.65 g (88.9%) of **39**: $[\alpha]_D$ +130° (*c* 0.52, $CHCl_3$); MS *m/z*: 628 (MH^+).

***p*-Nitrophenyl 4-*O*-Methyl-α-D-galactopyranoside (9).** According to method G, compound **9** was prepared from **39** (1.43 g, 2.27 mmol). The product was purified by column chromatography on silica gel (7:1 CH_2Cl_2-MeOH) to afford 0.423 g (59.1%) of **9**

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as crystals, that were recrystallized from hot EtOH: $[\alpha]_D$ +156° (*c* 0.52, MeOH); MS *m/z*: 316 (MH^+).

Methyl 2, 3, 4-Tri-*O*-benzyl-6-*O*-methyl-α-D-galactopyranoside (41). According to method A, compound **41** was prepared from **40** (2.33 g, 5.02 mmol). The product was purified by column chromatography on silica gel (3:2 hexane-AcOEt) to afford 2.35 g (97.8%) of **41**: $[\alpha]_D$ +22.8° (*c* 0.51, $CHCl_3$); MS *m/z*: 479 (MH^+).

***p*-Nitrophenyl 2, 3, 4-Tri-*O*-acetyl-6-*O*-methyl-α-D-galactopyranoside (42).** According to methods B and C, benzyl groups of **41** (2.44 g, 5.10 mmol) were removed and the product was acetylated, and then the product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 1.57 g (84.8%) of 1, 2, 3, 4-tetra-*O*-acetyl-6-*O*-methyl-D-galactopyranose. According to method D, the acetate (1.23 g, 3.39 mmol) was reacted with *p*-nitrophenol, the product was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 0.650 g (43.4%) of **42**: $[\alpha]_D$ +218° (*c* 0.51, $CHCl_3$); mp 146-147 °C; MS *m/z*: 442 (MH^+).

***p*-Nitrophenyl 6-*O*-Methyl-α-D-galactopyranoside (10).** According to method F, compound **10** was prepared from **42** (0.89 g, 2.0 mmol). The product was recrystallized from hot EtOH to afford 0.62 g (96.6%) of **10**: $[\alpha]_D$ +300° (*c* 0.57, MeOH); mp 158-161 °C; MS *m/z*: 316 (MH^+).

The ^1H NMR data of galactopyranoside derivatives were shown in Table 3.

Monomethyl derivatives of PNP α-D-mannopyranoside.

***p*-Nitrophenyl 3, 4, 6-Tri-*O*-acetyl-2-*O*-methyl-α-D-mannopyranoside (46).** According to methods B and C, benzyl and bezylidene groups of **45** (2.28 g, 5.90 mmol) were removed and the product was acetylated, and then the product was recrystallized from hexane-AcOEt to afford 1.55 g (72.5%) of 1, 3, 4, 6-tetra-*O*-acetyl-2-*O*-methyl-α-D-mannopyranose. According to method E, the acetate (1.38 g, 3.81 mmol) was reacted with *p*-nitrophenol, and the product was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 1.34 g (79.7%) of **46**: $[\alpha]_D$ +125° (*c* 0.51, $CHCl_3$); mp 123-126 °C; MS *m/z*: 442 (MH^+).

***p*-Nitrophenyl 2-*O*-Methyl-α-D-mannopyranoside (12).** According to method F, compound **12** was prepared from **46** (0.80 g, 1.8 mmol). The product was purified by

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Table 3. ^{1}H NMR Data of Galactopyranoside Derivatives.

Compound	δ (ppm), *J* (Hz)						
	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6b}$)	H-6a ($J_{5,6a}$)	H-6b ($J_{6a,6b}$)
7 [a]	6.13 (3.6)	3.77 (10.4)	4.15 (3.6)	4.06 (2.8)	3.95 (4.6)	3.68 (6.3)	3.71 (12.4)
8 [a]	5.86 (3.6)	4.08 (10.4)	3.82 (3.0)	4.34	3.96 (7.8)	3.69 (4.2)	3.74 (12.1)
9 [a]	5.85 (3.9)	4.00 (10.3)	4.18 (3.4)	3.78	3.99 (7.6)	3.72 (7.2)	3.83 (12.8)
10 [a]	5.89 (3.6)	4.05 (10.3)	4.13 (3.3)	4.03	4.17 (8.6)	3.58 (3.3)	3.67 (11.1)
31 [b]	5.12 (3.6)	3.97 (10.5)	5.70 (3.5)	5.95 (1.1)	4.51 (7.0)	4.34 (5.6)	4.59 (10.9)
32 [b]	6.00 (3.6)	4.17 (10.4)	5.90 (3.6)	5.98-6.01	4.55 (8.2)	4.32 (8.0)	4.56 (14.5)
33 [b]	5.23 (3.8)	5.42 (10.4)	4.02 (3.5)	5.95 (1.0)	4.42 (8.8)	4.42 (5.2)	4.54 (12.8)
34 [b]	6.13 (3.6)	5.61 (10.2)	4.22 (3.2)	6.00		4.43-4.51	
37 [b]	4.63 (3.2)	3.92 (10.0)	3.87 (2.7)	3.68 (1.0)	3.88 (7.2)	3.55 (6.7)	3.65 (8.9)
38 [b]	5.20 (3.6)	5.69 (10.8)	5.78 (3.2)	4.01 (1.2)	4.37 (7.2)	4.56 (6.0)	4.58 (11.2)
39 [b]	6.10 (3.6)	5.89 (10.8)	5.97 (3.1)	4.08 (1.0)	4.40 (7.0)	4.51 (2.9)	4.57 (11.2)
41 [b]	4.69 (3.6)	4.04 (9.8)	3.93 (2.8)	3.90 (1.0)	3.84 (6.4)	3.35 (6.4)	3.43 (9.6)
42 [b]	5.89 (3.8)	5.33 (11.6)	5.57 (2.4)	5.55 (1.0)	4.19 (6.2)	3.38 (5.9)	3.43 (10.1)

a. Measured in D_2O. b. Measured in $CDCl_3$. The OCH_3 gave a singlet (3H) at δ 3.19-3.60 for **7-10, 31-34, 37-39, 41**, and **42**. The $OCOCH_3$ gave a singlet (3H) at δ 2.00-2.18 for **42**. The CH_2 of benzyl groups gave a doublet (1H) at δ 4.51-4.96 for **37** and **41**. The aromatic H gave a multiplet at δ 7.18-8.30 for **7-10, 31-34, 37-39, 41**, and **42**.

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column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.50 g (87.2%) of **12**: $[\alpha]_D$ +125° (*c* 0.51, MeOH); mp 152-155 °C; MS *m/z*: 316 (MH^+).

***p*-Nitrophenyl 2, 4, 6-Tri-*O*-acetyl-3-*O*-methyl-α-D-mannopyranoside (48).** According to methods B and C, the benzylidene group of **47** (2.28 g, 5.88 mmol) was removed and the product was acetylated, and then the product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 1.11 g (88.0%) of 1, 2, 4, 6-tetra-

O-acetyl-3-*O*-methyl-α-D-mannopyranose. According to method E, the acetate (2.13 g, 5.88 mmol) was reacted with *p*-nitrophenol, and the product was purified by column chromatography on silica gel (3:2 hexane-AcOEt) to afford 1.91 g (73.6%) of **48**: $[\alpha]_D$ +102° (*c* 0.62, $CHCl_3$); MS *m/z*: 442 (MH^+).

***p*-Nitrophenyl 3-*O*-Methyl-α-D-mannopyranoside (13).** According to method F, compound **13** was prepared from **48** (1.61 g, 3.65 mmol). The product was recrystallized from hot EtOH to afford 0.907 g (74.3%) of **13**: $[\alpha]_D$ +158° (*c* 0.51, MeOH); mp 174-175 °C; MS *m/z*: 316 (MH^+).

Methyl 2, 3, 6-Tri-*O*-benzyl-4-*O*-methyl-α-D-mannopyranoside (51). According to method A, compound **51** was prepared from **50** (1.49 g, 3.21 mmol). The product was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 1.36 g (88.5%) of **51**: $[\alpha]_D$ +22.8° (*c* 0.51, $CHCl_3$); MS *m/z*: 479 (MH^+).

***p*-Nitrophenyl 2, 3, 6-Tri-*O*-acetyl-4-*O*-methyl-α-D-mannopyranoside (52).** According to methods B and C, benzyl groups of **51** (1.36 g, 2.84 mmol) were removed and the product was acetylated, and then the product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.920 g (89.4%) of 1, 2, 3, 6-tetra-*O*-acetyl-4-*O*-methyl-α-D-mannopyranose. According to method E, the acetate (1.00 g, 2.76 mmol) was reacted with *p*-nitrophenol, and the product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.790 g (64.9%) of **52**: $[\alpha]_D$ +71.2° (*c* 0.51, $CHCl_3$); MS *m/z*: 442 (MH^+).

***p*-Nitrophenyl 4-*O*-Methyl-α-D-mannopyranoside (14).** According to method F, compound **14** was prepared from **52** (0.56 g, 1.3 mmol). The product was purified by column chromatography on silica gel (7:1 CH_2Cl_2-MeOH) to afford 0.39 g (96.4%) of **14**: $[\alpha]_D$ +156° (*c* 0.51, MeOH); mp 140-142 °C; MS *m/z*: 316 (MH^+).

Methyl 2, 3, 4-Tri-*O*-benzyl-6-*O*-methyl-α-D-mannopyranoside (54). According to method A, compound **54** was prepared from **53** (7.74 g, 16.7 mmol). The product was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 7.79 g (97.7%) of **54**: $[\alpha]_D$ +32.7° (*c* 0.56, $CHCl_3$); MS *m/z*: 479 (MH^+).

***p*-Nitrophenyl 2, 3, 4-Tri-*O*-acetyl-6-*O*-methyl-α-D-mannopyranoside (55).** According to methods B and C, benzyl groups of **54** (5.27 g, 11.0 mmol) were removed and

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Table 4. ^{1}H NMR Data of Mannopyranoside Derivatives.

Compound	δ (ppm), *J* (Hz)						
	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6b}$)	H-6a ($J_{5,6a}$)	H-6b ($J_{6a,6b}$)
12 [a]	5.93 (1.8)	3.87 (3.6)	4.11 (9.5)	3.70 (10.1)	3.61 (2.5)	3.71 (5.7)	3.80 (12.3)
13 [a]	5.69 (2.0)	4.26 (3.3)	3.57 (9.5)	3.83 (9.6)	3.52 (2.4)	3.70 (5.4)	3.77 (12.1)
14 [a]	5.76 (1.8)	4.20 (3.5)	4.14 (9.6)	3.54 (10.1)	3.63 (2.8)	3.75-3.77	
15 [a]	5.78 (1.9)	4.21 (3.5)	4.08 (9.3)		3.65-3.78		
46 [b]	5.70 (2.0)	3.89 (2.4)	5.42-5.44		3.93 (5.0)	4.04 (2.2)	4.26 (12.2)
48 [b]	5.64 (1.6)	5.53 (3.4)	3.85 (9.8)	5.27 (10.0)	3.92 (6.0)	4.07 (2.4)	4.24 (12.4)
51 [b]	4.74 (1.2)		3.74-3.80			3.61-3.72	
52 [b]	5.57 (1.2)	5.43 (3.4)	5.45 (9.6)	3.61 (9.6)	3.86 (4.4)	4.30-4.31	
54 [b]	4.75 (1.6)	3.77 (2.8)	3.87 (9.4)	3.93 (9.4)	3.70	3.62-3.64	
55 [b]	5.65 (2.0)	5.45 (3.4)	5.54 (9.8)	5.46 (10.0)	3.92 (3.0)	3.43 (4.6)	3.49 (11.0)

a. Measured in D_2O. b. Measured in $CDCl_3$. The OCH_3 gave a singlet (3H) at δ 3.27-3.91 for **12-15**, **46**, **48**, **51**, **52**, **54**, and **55**. The $OCOCH_3$ gave a singlet (3H) at δ 2.00-2.21 for **46**, **48**, **52**, and **55**. The aromatic H gave a multiplet at δ 7.18-8.30 for **12-15**, **46**, **48**, **51**, **52**, **54**, and **55**.

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the product was acetylated, and then the product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 3.92 g (98.3%) of 1, 2, 3, 4-tetra-*O*-acetyl-6-*O*-methyl-α-D-mannopyranose. According to method E, the acetate (10.3 g, 2.84 mmol) was reacted with *p*-nitrophenol, and the product was purified by column chromatography on silica gel (2:1 hexane-AcOEt) to afford 0.800 g (63.8%) of **55**: $[\alpha]_D$ +117° (*c* 0.54, $CHCl_3$); MS *m/z*: 442 (MH^+).

***p*-Nitrophenyl 6-*O*-Methyl-α-D-mannopyranoside (15).** According to method F, compound **15** was prepared from **55** (0.57 g, 1.3 mmol). The product was recrystallized from hot EtOH to afford 0.33 g (82.3%) of **15**: $[\alpha]_D$ +152° (*c* 0.51, MeOH); mp 200-201 °C; MS *m/z*: 316 (MH^+).

Table 5. ^{13}C NMR Data of PNP mono-*O*-methyl-α-D-glycopyranosides.

	δ (ppm)[a]						
Compound	C-1	C-2	C-3	C-4	C-5	C-6	-OCH_3
2	96.2	82.1	74.0	71.1	74.8	62.2	59.2
3	99.2	72.8	84.7	70.7	75.0	62.1	61.4
4	99.0	73.0	74.9	80.5	74.1	61.8	61.0
5	99.1	72.9	74.7	71.3	73.7	72.1	59.4
7	96.7	79.0	73.6	70.6	70.5	62.3	59.2
8	99.3	68.6	80.7	66.4	73.6	62.3	57.3
9	99.4	69.9	71.7	80.6	73.9	61.6	62.2
10	99.4	71.8	69.5	70.7	71.0	72.8	59.3
12	96.9	81.3	72.1	68.4	75.9	62.5	59.6
13	99.9	67.5	81.8	66.9	75.8	62.4	57.6
14	99.9	71.8	72.2	77.8	74.9	62.1	61.1
15	99.9	71.3	72.1	68.1	74.4	72.8	59.4

a. Measured in CD_3OD. The aromatic C gave at δ 117.6-164.0 for **2-5**, **7-10**, and **12-15**.

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The ^1H NMR data of mannopyranoside derivatives were shown in Table 4. The ^{13}C NMR data of monomethylated PNP α-D-gluco, galacto, and mannopyranosides were shown in Table 5.

Assay of hydrolytic activity of α-glycosidase. Pure grade rice α-glucosidases (Sigma Chemical Co.), α-galactosidases of *M. vinacea* (Seikagaku Ind.), *A. niger* (Sigma Chemical Co.), and green coffee bean (Boehringer Mannheim Co.), and α-mannosidases of almond (Sigma Chemical Co.) and jack bean (Wako Chemicals Co.) were used in this study. PNP α-D-glucopyranoside, galactopyranoside, and mannopyranoside were purchased from Tokyo Kasei Ind., and they were recrystallized from EtOH.

The enzymatic hydrolysis of each glycosidic substrate was monitored by the amount of *p*-nitrophenol released under appropriate conditions as follows: rice; in 50 mM sodium acetate buffer (pH 4.0) at 37 °C, *M. vinacea*; in 50 mM sodium phosphate buffer (pH 5.9) at 40 °C, *A. niger*; in 50 mM sodium acetate buffer (pH 4.0) at 25 °C, green coffee bean; in

50 mM sodium phosphate buffer (pH 7.2) at 25 °C, almond; in 50 mM sodium acetate buffer (pH 4.5) at 25 °C, jack bean; in 50 mM sodium acetate buffer (pH 4.5) at 25 °C. The enzyme reaction was stopped by the addition of 0.3 M Na_2CO_3 solution, and then the *p*-nitrophenol released was measured spectrophotometrically at 405 nm.

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