Glycosides Having Chromophores as Substrates for Sensitive Enzyme Analysis. II. Synthesis of Phenolindophenyl-β-D-glucopyranosides Having an Electron-Withdrawing Substituent as Substrates for β-Glucosidase[1)]

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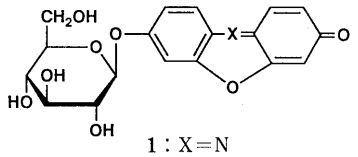
Five glucopyranosides, resorufinyl- (1), resazurinyl- (2), *N*-oxyphenolindophenyl- (3), phenolindo-3′-fluorophenyl- (4), and phenolindo-3′-azaphenyl-β-D-glucopyranoside (5), were synthesized through two routes. Compounds 1, 2, and 3 were synthesized by direct glycosidation of phenolindophenols. Compounds 4 and 5 were synthesized *via* the condensation of 4-aminophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosides (11 and 15) with *p*-quinone. These glucopyranosides (1—4) were hydrolyzed by β-glucosidase to give blue products showing high absorbance (ε: 22000—39000). They are considered to be potential substrates for the assay of β-glucosidase.

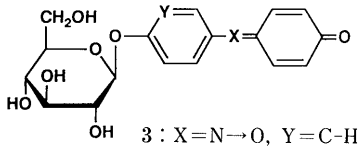
Keywords glucosidase activity; colorimetric assay; phenolindophenyl-β-D-glucopyranoside; polar effect

Glycosides bearing chromophores are very important as substrates for sensitive enzyme analysis of glycosyl hydrolase activities. For example, we have patented some convenient and useful substrates for the assay of α-amylase (1,4-α-D-glucan 4-glucanohydrase, EC 3.2.1.1).[2)] In the previous paper[1)] we presented the hypothesis that a good substrate with high affinity and large V_{max} in the β-glucosidase reaction must have a polar group in the benzene ring and must not have bulky substituents at the 3′- and 5′-positions of its phenolindophenol moiety. From this point of view, we have synthesized five glucosides (**1**—**5**, Chart 1) having an electron-withdrawing substituent in the phenolindophenyl group and tested them for hydrolysis by β-glucosidase.

Synthesis Our first route to the synthesis of the desired glucosides was the direct introduction of phenolindophenyl groups into a glycosyl donor as shown in Chart 2. By means of the Koenigs–Knorr reaction,[3)] resorufinyl (**7**), resazurinyl (**8**), and *N*-oxyphenolindophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosides (**9**) were obtained from the bromosugar (**6**)[4)] in 45, 78, and 31% yields, respectively. Deacetylation of these compounds with K_2CO_3 in absolute



1 : X=N
2 : X=N→O



3 : X=N→O, Y=C-H
4 : X=N, Y=C-F
5 : X=Y=N

Chart 1

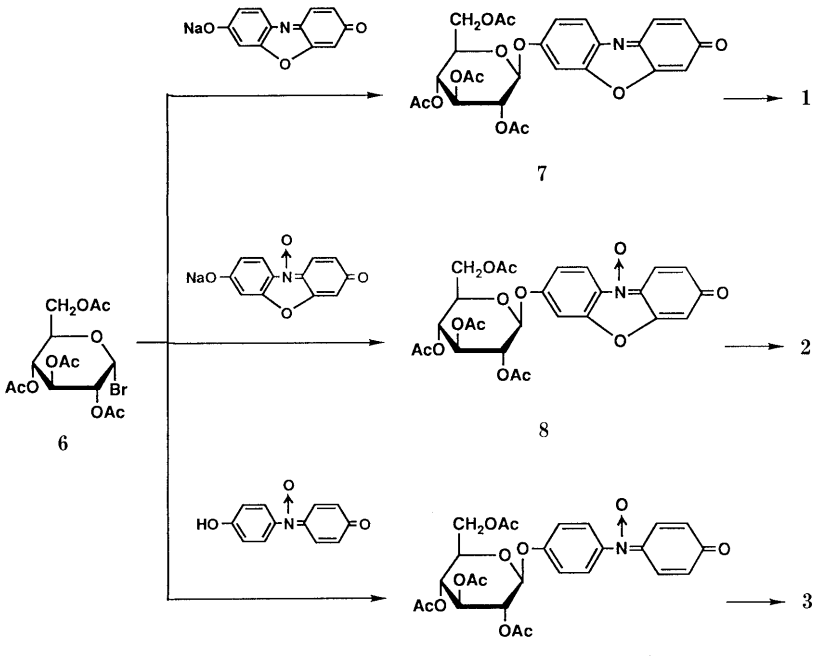


Chart 2

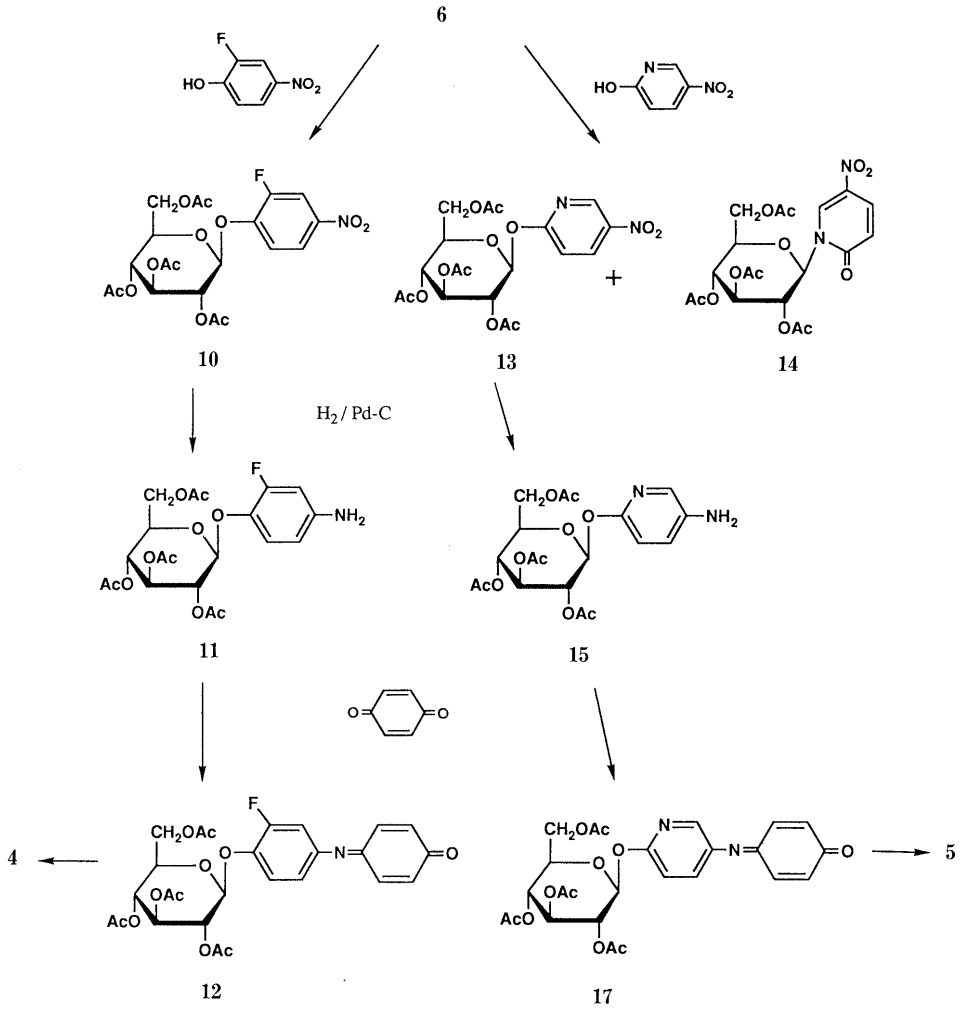


Chart 3

TABLE I. Physical Properties and Spectral Data for **1**—**5**

Compd. No.	Yield (%)	mp (°C) (Recrystn. solv.)	UV λ_{max}^{MeOH} nm (log ε)	IR (KBr) cm^{-1}	Formula	Elemental analysis (%) Calcd (Found)		
						C	H	N
1	90	218.0—220.0 (dec.) (MeOH–H_2O)	249 (4.15), 382 (4.01), 445 (4.26)	3370, 1610, 1570, 1506, 1404, 1352, 1322, 1258, 1214, 1046	$C_{18}H_{17}NO_8$	57.60 (57.56	4.57 4.59	3.73 3.73)
2	78	185.5—187.0 (dec.) (MeOH–H_2O)	270 (3.95), 349 (3.97), 359 (3.98), 490 (4.15), 522 (4.16)	3370, 1616, 1596, 1540, 1466, 1406, 1374, 1252, 1212, 1082	$C_{18}H_{17}NO_9$	55.25 (55.22	4.38 4.41	3.58 3.55)
3	74	186.0—186.5 (MeOH–H_2O)	203 (4.20), 259 (3.64), 376 (4.32)	3370, 1618, 1548, 1504, 1442, 1388, 1248, 1084, 1038	$C_{18}H_{19}NO_8$	57.29 (57.31	5.07 5.13	3.71 3.65)
4	76	185.5—186.5 (dec.) (MeOH–H_2O)	261 (4.30), 468 (3.74)	3352, 1646, 1580, 1506, 1276, 1218, 1086, 1038, 1014	$C_{18}H_{18}FNO_7 \cdot 1/10H_2O$	56.72 (56.40	4.81 4.83	3.67 3.67)
5	34	117.5—123.0 (MeOH–H_2O)	201 (4.01), 261 (4.18), 454 (3.69)	3390, 1638, 1582, 1470, 1372, 1270, 1066	$C_{17}H_{18}N_2O_7 \cdot 1/2H_2O$	54.98 (54.88	5.16 5.30	7.54 7.54)

MeOH gave resorufinyl- (**1**), resazurinyl- (**2**), and *N*-oxyphenolindophenyl-β-D-glucopyranosides (**3**) in 90, 78, and 74% yields, respectively.

Some attempts to obtain **9** or **3** by oxidation of a non-substituted derivative,[1] phenolindophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside or phenolindophenyl-β-D-glucopyranoside[1] gave a complex mixture of products which were difficult to separate. Similarly, **8** or **2** could not be produced by oxidation of **7** or **1**, respectively.

In order to synthesize other glucosides, our second route was stepwise method shown in Chart 3. The first step was the preparation of 2-fluoro-4-nitrophenyl and 2-(5-nitro)-pyridyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosides (**10** and **13**) by the Koenigs–Knorr reaction of **6** with 2-fluoro-

TABLE II. ^{1}H-NMR Spectral Data (199.5 MHz) for **1**—**5**[a]

Compd. No.	Phenolindophenyl moiety								Glucosyl moiety	
	H-2	H-6	H-3	H-5	H-2′	H-6′	H-3′	H-5′	Anomeric proton	Others
1	6.78 1H, dd (9.8, 2.0)	6.26 1H, d (2.0)	7.52 1H, d (9.8)	—	7.74 1H, d (8.6)	—	7.10 1H, dd (8.6, 2.4)	7.14 1H, d (2.4)	5.09 1H, d (7.1)	3.10—5.33 10H, m
2	6.67 1H, dd (10.0, 2.0)	6.16 1H, d (2.0)	7.98 1H, d (10.0)	—	8.08 1H, d (9.3)	—	7.12 1H, br d (9.3)	7.22 1H, br s	5.13 1H, d (7.3)	3.10—5.38 10H, m
3	6.22 1H, dd (10.3, 2.0)	6.60 1H, dd (10.3, 2.0)	7.29 1H, dd (10.3, 2.9)	7.94 1H, dd (10.3, 2.9)	7.20 2H, d (8.9)		7.58 2H, d (8.9)		4.96 1H, d (6.7)	3.10—5.29 10H, m
4	6.60 1H, dd (10.3, 2.2)	6.71 1H, dd (9.9, 2.2)	7.17 1H, dd (10.3, 2.7)	7.34 1H, dd (9.9, 2.7)	6.97 1H, dd (12.0, 2.4)	6.75 1H, br d (8.1)	—	7.36 1H, dd (8.1, 8.1)	4.96 1H, d (7.0)	3.17—5.29 10H, m
5	6.63 1H, dd (10.4, 2.1)	6.73 1H, dd (10.0, 2.1)	7.22 1H, dd (10.4, 2.4)	7.38 1H, dd (10.0, 2.4)	7.86 1H, d (2.2)	7.52 1H, dd (8.8, 2.2)	—	6.98 1H, d (8.8)	5.73 1H, d (6.8)	3.03—5.32 10H, m

a) All spectra were taken in DMSO-d_6. Chemical shifts are in δ units. Coupling constants (in Hz) are given in parentheses.

TABLE III. Properties of Phenolindophenyl-β-D-glucopyranosides (**1**—**4**) as β-Glucosidase Substrates

Compd. No.	K_m (M)	V_{max} (M/min)
1	7.7×10^{-3}	3.6×10^{-6}
2	4.4×10^{-3}	6.5×10^{-6}
3	7.9×10^{-3}	3.9×10^{-6}
4	7.0×10^{-3}	5.6×10^{-6}

4-nitrophenol and 2-hydroxy-5-nitropyridine. In the latter case *N*-(5-nitro)pyridonyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**14**) was obtained as a minor product. The second and third steps were the catalytic reduction of the nitro group to the amino group to yield **11** and **15**, and their condensation with *p*-quinone in the presence of trifluoroacetic acid (TFA) to yield the desired phenolindo-3′-fluorophenyl and phenolindo-3′-azaphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosides (**12** and **17**) from **6** in 53 and 42% yields, respectively. The last step of the deacetylation of **12** and **17** gave phenolindo-3′-fluorophenyl- (**4**, yield 76%) and phenolindo-3′-azaphenyl-β-D-glucopyranosides (**5**, yield 34%).

The structures of the five glucosides (**1**—**5**) were established from the results of elemental analyses and the spectral data as shown in Tables I and II. The syntheses of **1** and **2** have already been reported,[5] but their detailed physical properties and spectral data have not been presented. The proton nuclear magnetic resonance (^{1}H-NMR) spectra of **1**—**5** in dimethyl sulfoxide (DMSO)-d_6 showed signals at δ 4.96—5.73 assigned to an anomeric proton having a large coupling constant ($J_{1,2}=6.7$—7.3 Hz). The presence of the β-glucosidic bond[6] in **1**—**5** was suggested by the above ^{1}H-NMR data and also by the results of β-glucosidase hydrolysis.

Enzyme Assay Since the hydrolysis product of the compound (**5**) decomposed rapidly in water, the other four glucosides (**1**—**4**) were examined for their suitability for the colorimetric assay of β-glucosidase activity. The assay was carried out in 5 mM phosphate buffer (pH 6.8, which was the optimum pH for α-amylase action[7]) at 37 °C. All four substrates released phenolindophenols and the absorption of the blue color was measured at regular intervals under an alkaline condition (pH 11) to ensure sufficient dissociation. The K_m values and maximum velocities (V_{max}) for **1**—**4** obtained from Lineweaver–Burk plots are summarized in Table III. It was found that all four glucosides had the desired levels of affinity and V_{max} values in the β-glucosidase reaction. However, **1** and **2** were only slightly soluble in water. Consequently, we concluded that **3** and **4** were suitable substrates for the assay of β-glucosidase.

Although the inductive effect of a fluorine atom or *N*-oxide group did not influence the properties of the glucosides as strongly as we had expected, the validity of our assumption that a good substrate must have a polar group in the benzene ring and must not have bulky substituents at the 3′- and 5′-positions of its phenolindophenol moiety was confirmed. Since the hydrolysis of β-glucosides is the last step in the coupled enzymic assay system of α-amylase,[7] phenolindophenyl maltooligosides, which have a similar electron-withdrawing substituent in the chromophores to that of **3** or **4**, should be very useful substrates for α-amylase assay.

Experimental

General Procedures Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. ^{1}H-NMR spectra were taken at 199.5 MHz with a JEOL JNM-FX200 NMR spectrometer with tetramethylsilane as an internal standard. The infrared (IR) spectra were taken with a JASCO A-202 spectrometer. Ultraviolet (UV) spectra were recorded with a Hitachi 557 spectrometer. Column chromatography was performed on Merck Kieselgel 60 (SiO_2, 230—400 mesh).

***N*-Oxyphenolindophenol** *m*-Chloroperbenzoic acid (4.36 g, 25.3 mmol) was added to a stirred solution of phenolindophenol (2.52 g, 12.7 mmol) in MeOH (30 ml). The mixture was kept at 35 °C for 4 h. The solvent was evaporated off *in vacuo* to give a syrupy residue, which was chromatographed on silica gel with AcOEt–CH_2Cl_2 (1 : 1, v/v). *N*-Oxyphenolindophenol was produced as a red solid (2.00 g, 73.5%).

Preparation of Phenolindophenyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosides (7, Resorufinyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside; 8, Resazurinyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside; 9, *N*-Oxyphenolindophenyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside) A phenolindophenol (12.2 mmol) and Ag_2O (2.83 g, 12.2 mmol) were added to a solution

TABLE IV. Physical Properties and Spectral Data for **7**—**9**, **12**, and **17**

Compd. No.	Yield (%)	mp (°C) (Recrystn. solv.)	UV λ_{max}^{MeOH} nm (log ε)	IR (KBr) cm^{-1}	Formula	Elemental analysis (%) Calcd (Found)		
						C	H	N
7	45	167.0—168.0 (EtOH)	248 (4.18), 374 (4.05), 450 (4.26)	1745, 1610, 1570, 1510, 1364, 1304, 1246, 1206	$C_{26}H_{25}NO_{12}$	57.46 (57.36)	4.64 4.64	2.58 2.62)
8	78	218.0—220.0 (dec.) (EtOH)	271 (3.99), 348 (4.03), 357 (4.02), 488 (4.17), 519 (4.17)	1750, 1630, 1600, 1550, 1492, 1416, 1372, 1230, 1248, 1074, 1038	$C_{26}H_{25}NO_{13}$	55.82 (55.80)	4.50 4.45	2.50 2.51)
9	31	179.0—180.0 (EtOH)	203 (4.19), 220 (sh), 257 (3.60), 376 (4.35)	1744, 1616, 1500, 1456, 1432, 1372, 1222, 1064, 1018	$C_{26}H_{27}NO_{12}$	57.25 (57.15)	4.99 4.99	2.57 2.61)
12	87	117.0—119.0 (EtOH)	261 (4.31), 460 (3.66)	1748, 1644, 1504, 1372, 1220, 1078, 1068, 1044	$C_{26}H_{26}FNO_{11}$	57.04 (56.81)	4.79 4.77	2.56 2.58)
17	73	An oil	261 (4.20), 289 (4.18), 448 (3.68)	1748, 1640, 1582, 1470, 1366, 1230, 1062, 1032	$C_{25}H_{26}N_2O_{11}$	56.60 (56.40)	4.94 4.99	5.28 5.33)

TABLE V. ^{1}H-NMR Spectral Data (199.5 MHz) for **7**—**9**, **12**, and **17**[a]

Compd. No.	Phenolindophenyl moiety								Glucosyl moiety					
	H-2	H-6	H-3	H-5	H-2′	H-6′	H-3′	H-5′	Me in acetyl	H-5	H-6a	H-6b	H-1	H-2—4
7	6.84 1H, dd (9.8, 2.2)	6.95 1H, d (2.2)	7.42 1H, d (9.8)	—	7.73 1H, d (8.3)	—	6.99 1H, dd (8.3, 2.0)	6.29 1H, d (2.0)	2.05, 2.07, 2.08, 2.10 each 3H, s	3.90—4.00 1H, m	4.22—4.28 2H, m		5.10—5.35 4H, m	
8	6.77 1H, dd (10.0, 2.0)	6.24 1H, d (2.0)	8.04 1H, d (10.0)	—	8.17 1H, d (10.0)	—	7.00 1H, dd (10.0, 2.4)	7.01 1H, d (2.4)	2.05, 2.07, 2.08, 2.11 each 3H, s	3.90—4.00 1H, m	4.22—4.28 2H, m		5.10—5.35 4H, m	
9	6.26 1H, dd (10.4, 1.8)	6.66 1H, dd (10.0, 1.8)	7.23 1H, dd (10.4, 2.8)	8.01 1H, dd (10.0, 2.8)	7.13 2H, d (8.9)		7.46 2H, d (8.9)		2.05, 2.07, 2.08, 2.08 each 3H, s	3.90—3.97 1H, m	4.19 1H, dd (12.3, 2.5)	4.30 1H, dd (12.3, 5.0)	5.10—5.35 4H, m	
12	6.56 1H, dd (10.2, 2.2)	6.70 1H, dd (10.0, 2.2)	7.09 1H, dd (10.2, 2.5)	7.28 1H, dd (10.0, 2.5)	6.73 1H, dd (11.2, 2.7)	6.60 1H, ddd (8.8, 2.7, 1.5)	—	7.24 1H, dd (8.8, 8.8)	2.05, 2.05, 2.09, 2.11 each 3H, s	3.79—3.88 1H, m	4.20 1H, dd (12.5, 2.4)	4.33 1H, dd (12.5, 4.9)	5.00—5.36 4H, m	
17	6.60 1H, dd (10.3, 2.2)	6.71 1H, dd (10.0, 2.2)	7.14 1H, dd (10.3, 2.7)	7.30 1H, dd (10.0, 2.7)	7.74 1H, d (2.7)	7.34 1H, dd (8.4, 2.7)	—	6.91 1H, d (8.4)	2.02, 2.05, 2.06, 2.07 each 3H, s	3.91—3.99 1H, m	4.15 1H, dd (12.5, 2.2)	4.33 1H, dd (12.5, 4.4)	6.18 1H, d (8.1)	5.00—5.36 3H, m

a) All spectra were taken in $CDCl_3$. Chemical shifts are in δ units. Coupling constants (in Hz) are given in parentheses.

TABLE VI. Physical Properties and Spectral Data for **10**—**11** and **13**—**15**

Compd. No.	Yield (%)	mp (°C) (Recrystn. solv.)	UV λ_{max}^{MeOH} nm (log ε)	IR (KBr) cm^{-1}	Formula	Elemental analysis (%) Calcd (Found)		
						C	H	N
10	67	168.0—170.0 (MeOH)	201 (4.02), 217 (3.92), 283 (3.98)	1754, 1734, 1602, 1532, 1502, 1376, 1348, 1280, 1216, 1060, 1034	$C_{20}H_{22}FNO_{12}$	49.29 (49.31)	4.55 4.52	2.87 2.84)
11	91	157.0—158.5	203 (4.21), 235 (4.07), 291 (3.30)	3430, 3390, 1754, 1626, 1518, 1368, 1212, 1068, 1042	$C_{20}H_{24}FNO_{10}$	52.52 (52.51)	5.29 5.27	3.06 3.10)
13	58	145.5—146.5	209 (4.03), 283 (4.03)	1746, 1606, 1582, 1524, 1472, 1382, 1350, 1306, 1218, 1076, 1034	$C_{19}H_{22}N_2O_{12}$	48.51 (48.24	4.71 4.73	5.96 5.77)
14	30	An oil	297 (4.04)	1752, 1688, 1618, 1564, 1516, 1440, 1368, 1348, 1228, 1100, 1038	$C_{19}H_{22}N_2O_{12}$	48.51 (48.55	4.71 4.66	5.96 5.89)
15	98	An oil	203 (3.92), 235 (4.04), 307 (3.43)	3390, 3360, 1752, 1624, 1586, 1490, 1422, 1370, 1230, 1036	$C_{19}H_{24}N_2O_{10}$	51.82 (52.01	5.49 5.40	6.36 6.24)

of **6** (1.00 g, 2.44 mmol) in CH_3CN (20 ml). The mixture was stirred overnight at 40 °C and was filtered through a pad of Celite, then insoluble material was washed with CH_2Cl_2. The filtrate and washings were evaporated *in vacuo* to leave a syrupy residue, which was chromatographed on silica gel with AcOEt–toluene (1 : 1, v/v) to give the corresponding product (**7**—**9**). Yields, physical properties and spectral data are summarized in Tables IV and V.

Preparation of 2-Fluoro-4-nitrophenyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (10), 2-(5-Nitro)pyridyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (13), and *N*-(5-Nitro)pyridonyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (14) Reaction of **6** (2.00 g, 4.88 mmol) with 2-fluoro-4-nitrophenol or 2-hydroxy-5-nitropyridine (each 24.4 mmol) and Ag_2O (5.66 g,

TABLE VII. ^{1}H-NMR Spectral Data (199.5 MHz) for **10**—**11** and **13**—**15**[a]

Compd. No.	Aglycone moiety					Glucosyl moiety					
	H-4	H-3	H-5	H-6	NH_2	Me in acetyl	H-5	H-6a	H-6b	H-1	H-2—4
10	—	7.92—8.13 2H, m		7.28 1H, dd (8.8, 7.2)	—	2.04, 2.05, 2.08, 2.08 each 3H, s	3.84—3.94 1H, m	4.19 1H, dd (12.2, 2.7)	4.29 1H, dd (12.2, 5.3)	5.03—5.41 4H, m	
11	—	6.41 1H, dd (12.0, 2.7)	6.33 1H, ddd (8.8, 2.7, 1.5)	6.99 1H, dd (8.8, 8.8)	3.57 2H, br s	2.03, 2.03, 2.08, 2.10 each 3H, s	3.68—3.76 1H, m	4.15 1H, dd (12.2, 2.4)	4.29 1H, dd (12.2, 4.8)	4.79 1H, d (7.6)	5.10—5.32 3H, m
13	8.45 1H, dd (9.0, 2.7)	6.95 1H, d (9.0)	9.08 1H, d (2.7)	—	—	2.01, 2.05, 2.06, 2.06 each 3H, s	3.92—4.00 1H, m	4.12 1H, dd (12.5, 2.2)	4.32 1H, dd (12.5, 4.3)	5.16—5.42 4H, m	
14	8.06 1H, dd (10.3, 2.9)	6.52 1H, d (10.3)	8.70 1H, d (2.9)	—	—	1.93, 2.03, 2.07, 2.11 each 3H, s	3.98—4.06 1H, m	4.22—4.77 2H, m		6.24 1H, d (9.6)	5.19—5.48 3H, m
15	7.03 1H, dd (8.8, 2.9)	6.65 1H, d (8.8)	7.62 1H, d (2.9)	—	3.49 1H, br s	1.99, 2.03, 2.04, 2.05 each 3H, s	3.87—3.95 1H, m	4.10 1H, dd (12.5, 2.4)	4.31 1H, dd (12.5, 4.4)	6.04 1H, d (6.5)	5.14—5.38 3H, m

a) All spectra were taken in $CDCl_3$. Chemical shifts are in δ units. Coupling constants (in Hz) are given in parentheses.

24.4 mmol) in CH_3CN (40 ml) was carried out using a similar procedure to that described above, giving **10** or **13** and **14**. Yields, physical properties and spectral data are summarized in Tables VI and VII.

Preparation of 4-Amino-2-fluorophenyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (11) and 2-(5-Amino)pyridyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (15) A stirred solution of **10** or **13** (each 5.00 mmol) in 1,4-dioxane (80 ml) was hydrogenated immediately after the addition of 200 mg of Pd/C (10%) at ordinary pressure at 35 °C for 20 h. The mixture was filtered through a pad of Celite, then insoluble material was washed with CH_2Cl_2. The filtrate and washings were evaporated *in vacuo* to leave a syrupy residue, which was chromatographed on silica gel with AcOEt–toluene (1 : 1, v/v) to give **11** or **15**. Yields, physical properties and spectral data are summarized in Tables VI and VII.

Preparation of Phenolindophenyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosides (12, Phenolindo-3′-fluorophenyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside; 17, Phenolindo-3′-azaphenyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside) **11** or **15** (each 4.50 mmol) was added to a solution of *p*-benzoquinone (45.0 mmol) in 1,4-dioxane (60 ml) containing TFA (1.2 ml) and molecular sieves (10.0 g), and the mixture was stirred for 2 h at room temperature. The mixture was cooled, neutralized with K_2CO_3 and filtered through a glass filter, then insoluble material was washed with CH_2Cl_2. The filtrate and washings were evaporated *in vacuo* to leave a syrupy residue, which was chromatographed on silica gel with AcOEt–toluene (1 : 1, v/v) to give **12** or **17**. Yields, physical properties and spectral data are summarized in Tables IV and V.

Preparation of Phenolindophenyl-β-D-glucopyranosides (1, Resorufinyl-β-D-glucopyranoside; 2, Resazurinyl-β-D-glucopyranoside; 3, *N*-Oxyphenolindophenyl-β-D-glucopyranoside; 4, Phenolindo-3′-fluorophenyl-β-D-glucopyranoside; 5, Phenolindo-3′-azaphenyl-β-D-glucopyranoside) Deacetylation of **7**—**9**, **12** or **17** (each 2.00 mmol) with anhydrous K_2CO_3 (0.50 mmol) in absolute MeOH (120 ml) was carried out using a similar procedure to that described in the previous paper[1] and **1**—**5** were obtained. Yields, physical properties and spectral data are summarized in Tables I and II.

Evaluation of 1—4 as β-Glucosidase Substrates Solutions of **1**—**4** (2.0 ml) in H_2O were each mixed with 30 mM phosphate buffer (pH 6.8, 0.5 ml) and a solution of β-glucosidase (0.06 U/ml, 0.5 ml). The mixture was incubated at 37 °C for 1, 2.5, 4, and 5.5 min. At a designated time, 1.2 ml of 350 mM Na_2CO_3 solution was added and the optical density at 580—620 nm against H_2O was immediately measured. For the blank, H_2O was added instead of the indicated volume of β-glucosidase solution. The K_m values and maximum velocities (V_{max}) of hydrolysis of **1**—**4** by β-glucosidase were obtained from Lineweaver–Burk plots.

Molar absorptivities (ε) of hydrolysis products of the glucosides (**1**—**4**) were obtained by complete hydrolyses of the glucosides with excess enzyme. ε (λ_{max} nm): **1**, 34000 (580); **2**, 39000 (600); **3**, 22000 (620); **4**, 29000 (620).

References

1) S. Tokutake, N. Yamaji, and M. Kato, *Chem. Pharm. Bull.*, **38**, 13 (1990).
2) Kikkoman Corp., Japan Kokai Patent 202893 (1985) [*Chem. Abstr.*, **105**, 6764*k* (1986)]; Kikkoman Corp., Japan Kokai Patent 283989 (1987) [*Chem. Abstr.*, **108**, 22048*i* (1988)].
3) W. Koenigs and E. Knorr, *Chem. Ber.*, **34**, 957 (1901).
4) C. E. Redemann and C. Nielmann, "Organic Syntheses," Coll. Vol. III, ed. by E. C. Horning, John Wiley and Sons, Inc., New York, 1955, pp. 11—14; H. J. Koeners, J. Verhoeven, and J. H. von Boom, *Tetrahedron Lett.*, **21**, 381 (1980).
5) Boehringer Mannheim GmbH., Japan Kokai Patent 218397 (1985) [*Chem. Abstr.*, **104**, 168764*s* (1986)].
6) H. S. Gutowsky, M. Karplus, and D. M. Grant, *J. Chem. Phys.*, **31**, 1278 (1959).
7) J. Bergmeyer (ed.), "Methods of Enzymatic Analysis," 3rd ed., Vol. 4, Verlag Chemie, Weinheim, 1984, pp. 146—177.