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Complementary and Synergistic Roles in Enzyme-Catalyzed Regioselective and Complete Hydrolytic Deprotection of *O*-Acetylated β-D-Glucopyranosides of *N*-Arylacetohydroxamic Acids

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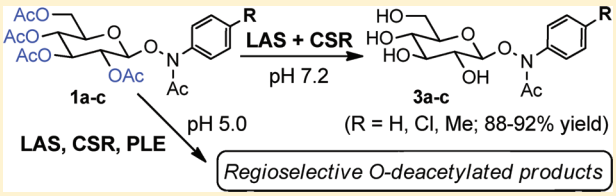
Supporting Information

ABSTRACT: An efficient chemoenzymatic synthesis of β-D-glucopyranosides of *N*-arylacetohydroxamic acids **3a−c** was achieved by the chemoselective *O*-deacetylation of **1a−c** under mild, neutral conditions, with no accompanying *N*-deacetylation. Lipase AS Amano from *Aspergillus niger* (LAS) and carboxylesterase from *Streptomyces rochei* (CSR) played complementary, synergistic roles in the *O*-deacetylation of **1a** and its partially *O*-deacetylated intermediates. An intramolecular *O*-acetyl migration, which proceeded simultaneously, also accelerated the overall reaction rate. Under weakly acidic conditions at pH 5.0, where the intramolecular *O*-acetyl migration is markedly slower, LAS, CSR, and porcine liver esterase (PLE) exhibited different regioselective *O*-deacetylation activities. LAS and PLE showed regioselective 3-*O*-deacetylation and 2-*O*-deacetylation activity, respectively, for **1a** and its tri-*O*-acetyl derivatives (**4**-**7**). CSR showed marked preferences for 3-*O*-deacetylation of 2,3,6-tri-*O*-acetyl intermediate **5** and 4-*O*-deacetylation of 2,4,6-tri-*O*-acetyl intermediate **6**. In contrast, CSR showed almost no *O*-deacetylation activity toward the other tri-*O*-acetyl intermediates **4** and **7**, which were efficiently *O*-deacetylated by LAS in a complementary manner. Using these enzyme-catalyzed regioselective *O*-deacetylation as well as chemical methods, we could synthesize all 14 partially *O*-acetylated intermediates (**4**−**17**) derived from **1a**.



INTRODUCTION

Glucuronidation is a well-known metabolic conjugation reaction that is also thought to contribute to the toxic bioactivation of some types of xenobiotics,[1] particularly carboxylic acid drugs and *N*-arylacetohydroxamic acids. The resulting glucuronides have been implicated as a cause of toxicological adverse reactions to carboxylic acid drugs[2] and the carcinogenicity of *N*-arylacetohydroxamic acids.[3] These glucuronides are alkali-labile, electrophilic metabolites that are capable of covalently binding to proteins and nucleic acids, which could elicit toxicological responses. However, the role of glucuronides in adverse reactions to carboxylic acid drugs[2] and the carcinogenic properties[4] of the *O*-glucuronide of *N*-arylacetohydroxamic acids remain largely unknown.

In addition to carcinogenic *N*-arylacetohydroxamic acids, *O*-glucuronides of biologically interesting hydroxamic acids have been also reported. These include urinary glucuronides of the anti-inflammatory drug bufexamac,[5] glucuronides of the matrix metalloprotease inhibitor trocade,[6] and the histone deacetylase inhibitor vorinostat.[7−9] *O*-Deacetylation of the acetylated *O*-glucuronide methyl ester of vorinostat was achieved in 67% yield under standard basic conditions using sodium methoxide in MeOH.[8] The moderate yield might be due in part to the alkali-labile nature of the *N*-acyl group on the hydroxamic acid *O*-glycosidic linkage.

We had previously reported the chemical synthesis of acetylated β-D-glucuronide methyl esters[10] and per-*O*-acetylated β-D-glucopyranosides[11] of *N*-arylacetohydroxamic acids using an orthoester glycosidation method.[12] The chemical *O*-deacetylation of the glucopyranosides, using a catalytic amount of barium methoxide in MeOH or in MeOH saturated with NH_3, afforded both *O*- and *N*-deacetylated derivatives, suggesting that the *N*-acetyl group on the aglycone moiety is alkali-labile. Consequently, the β-D-glucopyranosides of *N*-arylacetohydroxamic acids were synthesized via complete *O*- and *N*-deacetylation followed by selective *N*-acetylation[11] to study their chemical[13] and mutagenic activities.[11,14] Recently, we have developed a chemoenzymatic synthesis[15] for β-D-glucuronides of carboxylic acids from their acetylated methyl ester derivatives in order to examine the quantitative structure−activity relationships[16] for the electrophilicity of the 1-β-*O*-acyl linkages. The enzymatic hydrolytic deprotections of the sugar protecting groups were performed at pH 5.0 to minimize the nonenzymatic intramolecular acyl migration[17] of the 1-β-*O*-acyl groups. Two lipases from *Aspergillus niger* (LAS) and a carboxylesterase from *Streptomyces rochei* (CSR) were the biocatalysts of choice for the chemoenzymatic hydrolysis of the *O*-acetyl groups. Subsequent hydrolysis of the glucuronide methyl ester groups was achieved using either porcine liver esterase (PLE) or lipase type B from *Candida antarctica* (CAL-B).

We report the application of the enzyme-catalyzed chemoselective *O*-deacetylation method to model substrates, 2,3,4,6-

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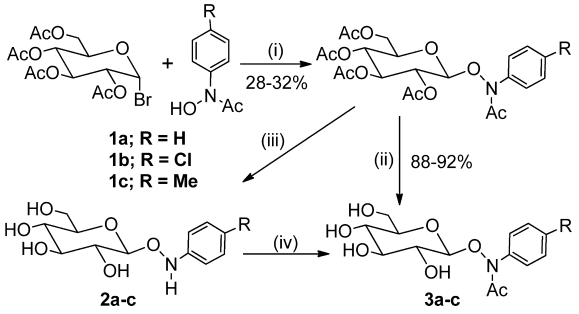
tetra-*O*-acetyl-*β*-D-glucopyranosides of *N*-arylacetohydroxamic acids **1a−c**, to obtain the targets **3a−c** directly, without *N*-deacetylation (Scheme 1). This enzymatic *O*-deacetylation

Scheme 1. Chemical and Chemoenzymatic Synthesis of *β*-D-Glucopyranosides of *N*-Arylacetohydroxamic Acids 3a−c[a]



[a]Reaction conditions: (i) aq KOH, EtOH, 25 °C, 32% (**1a**), 30% (**1b**), 28% (**1c**); (ii) LAS and CSR, phosphate buffer (pH 7.2), DMSO, 40 °C, 92% (**3a**), 90% (**3b**), 88% (**3c**); (iii)[11] MeOH saturated with NH_3, 0 °C; (iv)[11] AcCl, dioxane, $NaHCO_3$, 25 °C.

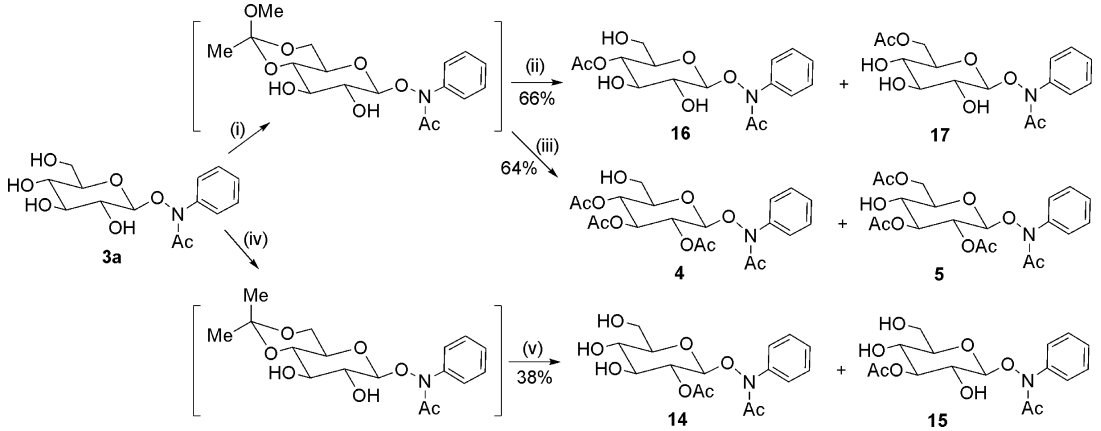
method may be useful as an alternative to the chemical method, particularly for removing *O*-acetyl groups on glycosides with an alkali-labile glycosidic bond or functional groups on the aglycone moiety. Furthermore, we have synthesized all the partially *O*-deacetylated derivatives of **1a** (**4−17**) and analyzed the kinetics of both the enzyme-catalyzed *O*-deacetylation of **1a** and the nonenzymatic intramolecular *O*-acetyl migration of the resultant *O*-deacetylated intermediates. LAS has recently been shown to exhibit regioselective 3-*O*-deacetylation activity;[18] therefore, the enzymatic regioselective *O*-deacetylation was also examined using **1a** and its partially *O*-deacetylated intermediates (**4−13**). LAS, CSR, and PLE exhibit different regioselective *O*-deacetylation activities, and thus several partially *O*-deacetylated compounds, including di-*O*-acetyl derivatives **9−13**, that were difficult to synthesize regioselectively using chemical methods, could be easily prepared chemoenzymatically.

RESULTS AND DISCUSSION

Chemical Synthesis of 1a−c and Partially *O*-Acetylated Glucopyranosides 4−17. 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranosides of *N*-arylacetohydroxamic acids **1a−c**, which have previously been synthesized by a two-step synthesis via orthoester intermediates,[12] were prepared directly from acetobromglucose and the corresponding potassium *N*-arylacetohydroxamates. The potassium salts of **1a−c**, prepared by the addition of a stoichiometric amount of KOH, showed far higher nucleophilic reactivity toward acetobromglucose than **1a−c**. However, the basic conditions could compete with the decomposition of acetobromglucose, which might lead to the poor yields between 28% and 32% (Scheme 1).

The basic conditions of chemical *O*-deacetylation of **1a−c**, using a catalytic amount of barium methoxide in MeOH or MeOH saturated with ammonia, proceeded with accompanying *N*-deacetylation to afford **2a−c**. When the *O*-deacetylation of **1** with barium methoxide in MeOH was performed at 0 °C until the starting material was consumed, HPLC monitoring showed a mixture of **2** and **3** as well as some mono-*O*-acetyl intermediates. The following products were obtained: **2a** (20%) and **3a** (70%) from **1a**; **2b** (54%) and **3b** (37%) from **1b**; and **2c** (25%) and **3c** (70%) from **1c**. These results led us to investigate the chemoenzymatic synthesis of **3a−c** without *N*-deacetylation. Compound **1a** was chosen as a model substrate for studying the enzyme-catalyzed *O*-deacetylation activity, and the 14 partially *O*-deacetylated derivatives (**4−17**) were synthesized as standard samples to investigate the *O*-deacetylation sequence. The mono-*O*-acetyl derivatives **16** and **17** were synthesized by 4,6-*O*-orthoesterification[19] of **3a** followed by the acidic opening of the orthoester group and deprotection (Scheme 2). Derivatives **14** and **15** were synthesized by 4,6-*O*-acetonidation of **3a** followed by mono-*O*-acetylation with acetic anhydride in pyridine[20] and then deprotection (Scheme 2). 2,3,4-Tri-*O*-acetyl and 2,3,6-tri-*O*-acetyl derivatives **4** and **5** were also synthesized from **3a** by 4,6-*O*-orthoesterification[19] and acetylation followed by deprotection (Scheme 2). In addition to **5**, the tri-*O*-acetyl derivatives **6** and **7** were prepared via intramolecular *O*-acetyl migration of **4** under weakly alkaline conditions (Scheme 3). Although 2,3-di-*O*-acetyl derivative **8** was synthesized through 4,6-*O*-acetonidation, the other di-*O*-acetyl derivatives (**9−13**) were prepared

Scheme 2. Chemical Synthesis of Tri-*O*-acetyl (4 and 5) and Mono-*O*-acetyl (14−17) Compounds[a]



[a]Reaction conditions: (i) $CH_3CH(OMe)_3$, TsOH (cat.), CH_3CN, 25 °C; (ii) H_2O, 25 °C; (iii) Ac_2O, pyridine, 25 °C then 80% (v/v) aq AcOH, 25 °C; (iv) dimethoxypropane, acetone, TsOH (cat.), 37 °C; (v) Ac_2O, pyridine, 4 °C then 80% (v/v) aq AcOH, 25 °C.

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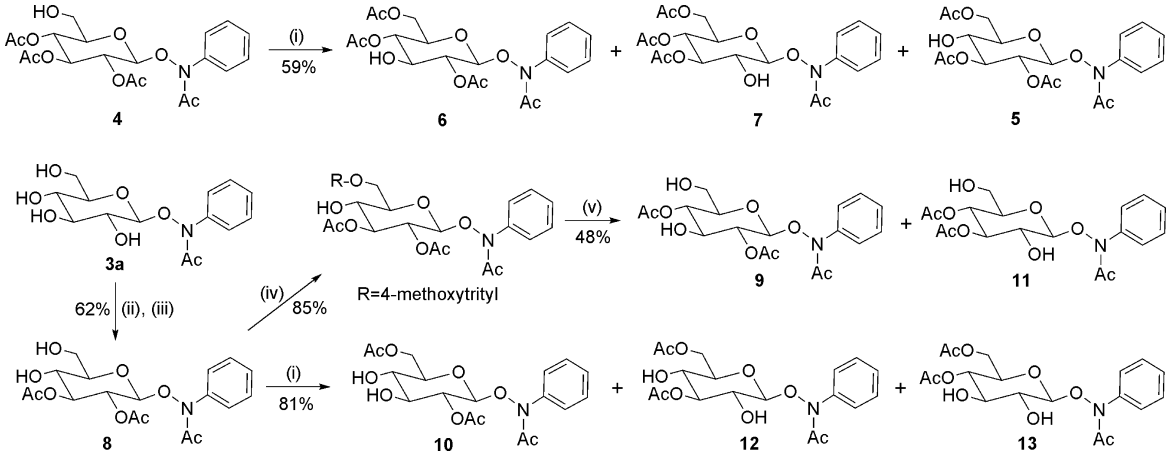
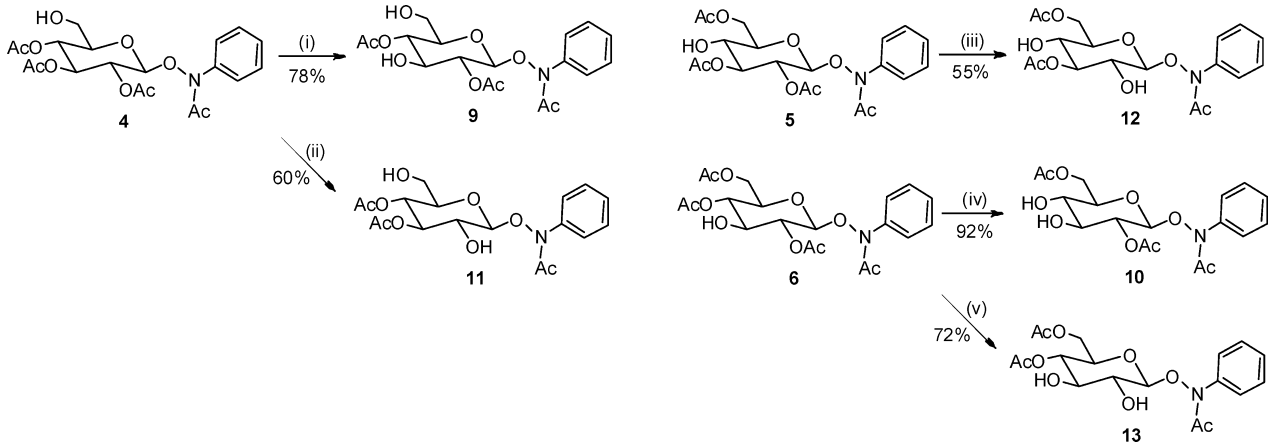
Scheme 3. Chemical Synthesis of Tri-*O*-acetyl Compounds 6 and 7 and Di-*O*-acetyl Compounds 8–13[a]



[a]Reaction conditions: (i) MOPS buffer (pH 7.5), CH_3CN, 37 °C; (ii) dimethoxypropane, acetone, TsOH (cat.); (iii) Ac_2O, pyridine, 25 °C then 80% (v/v) aq AcOH, 25 °C; (iv) 4-methoxytrityl chloride, pyridine, 25 °C; (v)50% (v/v) aq pyridine, 50 °C then 80% (v/v) aq AcOH.

Scheme 4. Chemoenzymatic Synthesis of Di-*O*-acetyl Compounds 9–13[a]



[a]Reaction conditions: (i) LAS, NaOAc buffer (pH 5.0), EtOH, 40 °C; (ii) PLE, NaOAc buffer (pH 5.0), EtOH, 40 °C; (iii) PLE, NaOAc buffer (pH 5.0), EtOH, 40 °C; (iv) CSR, NaOAc buffer (pH 5.0), EtOH, 40 °C; (v) PLE, NaOAc buffer (pH 5.0), EtOH, 40 °C.

by using intramolecular *O*-acetyl migrations of **8** and its 6-*O*-(4-methoxy)trityl derivative under weakly alkaline conditions (Scheme 3).

The acetonide and 4-methoxytrityl protecting groups were successfully removed by using 80% (v/v) aq AcOH with no accompanying hydrolysis or intramolecular *O*-acetyl migration. The structures of compounds **4**–**17** were characterized by ^1H and ^{13}C NMR and MS, together with analyses of the chemical shifts of the pyranose moiety based on the acylation and deacylation shifts.[21] The crystallographic data and ORTEP plots for **4** and **5** are provided in the Supporting Information. Although regioselective chemical *O*-acetylation of C_3-OH,[23] C_4-OH,[24] and C_6-OH[25] has been extensively exploited for α-D- or β-D-glucopyranosides,[22] the mono-*O*-acetyl derivatives **14**, **15** and **16**, **17** were synthesized and purified by chromatography with no problems. The synthesis of 3,6-di-*O*-acetyl derivative **12** from **3a** via an organotin-mediated direct *O*-acetylation[26] was unsuccessful because of *N*-deacetylation of **3a** during the reaction step of heating with dibutyltin oxide in MeOH at 70 °C. A mild and selective method for the cleaving both *O*-acetyl[27] and *N*-acetyl[28] groups has been reported under these conditions. To our knowledge, no regioselective chemical di-*O*-acetylation of glucopyranosides has been reported, with the exception of the 3,6-di-*O*-acetylation[26] and 2,6-di-*O*-acetylation.[29] As described later, tri-*O*-acetyl **6** and **7** and di-*O*-acetyl derivatives **9**-**13** (Scheme 4) were then individually synthesized by enzymatic regioselective *O*-deacetylation of their corresponding precursor substrates.

Enzyme Screening and Characterization for *O*-Deacetylation Activity toward 1a. To minimize the intramolecular *O*-acetyl migration, enzyme screening was performed under mild acidic conditions with 0.75 mM of **1a** in 85 mM sodium acetate buffer (pH 5.0), which contained 15% (v/v) DMSO, at 40 °C for 10 min. Of the 15 enzymes tested, LAS showed the highest *O*-deacetylation activity, followed by PLE, lipase AP6 from *Aspergillus niger*, CSR, and CAL-B. Acylase I showed weak activity and the other enzymes did not show any hydrolytic activity toward **1a** even when they were incubated at pH 7.2. Because LAS and lipase AP6 showed a similar HPLC pattern of hydrolytic products, the *O*-deacetylation activity of four enzymes, namely, LAS, PLE, CSR, and CAL-B, was examined more closely at pH 5.0 and at pH 7.2. DMSO was the best cosolvent, followed by 2-methoxyethanol, bis(2-methoxyethyl) ether, and EtOH. LAS

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and PLE exhibited high *O*-deacetylation activity and different regioselectivity at pH 5.0 (Table 1). LAS mainly gave the 3-*O*-deacetylated product **6** (60% and 47% yields after 0.5 and 2.0 h, respectively), as we have previously observed.[18] In contrast, PLE largely afforded the 2-*O*-deacetylated product **7** (63% and 23% yields after 0.5 and 2.0 h, respectively). The regioselectivity of LAS was calculated as 92% and the regioselectivity of PLE was 85%, after incubation for 0.5 h. At preparative scales, compounds **6** and **7** were successfully isolated in yields of 56% and 53%, respectively, under the optimized conditions. Conversely, CSR and CAL-B showed lower *O*-deacetylation activity at pH 5.0, leaving about 60% of the starting material **2** unreacted after 2.0 h incubation. The activity of CAL-B was low at both pH 7.2 and pH 5.0, whereas LAS, CSR, and PLE showed higher activity at pH 7.2. CSR was a particularly good catalyst at pH 7.2; the major products formed after 2.0 h were the mono-*O*-acetyl compound **17** (45%) and **3a** (33%). LAS and PLE showed higher activity toward **1a** and the tri-*O*-acetyl intermediates, and hence the simultaneous use of CSR and either LAS or PLE was investigated. The combination of CSR and LAS resulted in synergistic *O*-deacetylation to afford **3a**, and a higher conversion yield was observed at pH 7.2 than at pH 5.0 (Table 1). The combination of CSR and LAS was as good as or better than the combination of CSR and PLE (data not shown).

Table 1. Yields of Major Products Obtained from Enzyme-Catalyzed *O*-Deacetylation of 1a

enzyme	pH[a]	products and yields[b] (%)
LAS[c]	5.0	**1a** (nd[d]), **6** (47%), di-*O*-acetyl **9** + **10** + **12** + **13** (28%), mono-*O*-acetyl **14** + **17** (25%)
	7.2	**1a** (nd[d]), **6** (9%), di-*O*-acetyl **10** + **12** + **13** (31%), mono-*O*-acetyl **17** (52%)
CSR[c]	5.0	**1a** (58%), **5** (19%), **7** (7%), di-*O*-acetyl **10** + **12** + **13** (11%), mono-*O*-acetyls **17** (5%)
	7.2	**1a** (4%), **7** (5%), di-*O*-acetyl **10** + **12** + **13** (14%), mono-*O*-acetyl **17** (45%), **3a** (33%)
PLE[c]	5.0	**1a** (nd[d]), **7** (23%), di-*O*-acetyl **11** + **12** + **13** (57%), mono-*O*-acetyl **15** + **16** (19%)
	7.2	di-*O*-acetyl **12** + **13** (28%), mono-*O*-acetyl **14** + **15** + **16**+ **17** (65%), **3a** (7%)
CAL-B[c]	5.0	**1a** (64%), di-*O*-acetyl (29%)
	7.2	**1a** (56%), di-*O*-acetyl (36%)
LAS[c] + CSR[c]	5.0	di-*O*-acetyl **10** (27%), mono-*O*-acetyl **14** + **17** (48%), **3a** (25%)
	7.2	mono-*O*-acetyl **14** + **17** (39%), **3a** (60%)

[a]Incubation was performed at the indicated pH and incubation time at 40 °C for 2.0 h. [b]Yields based on HPLC analysis. [c]The concentration of LAS and CSR was 5.0 mg/mL of the incubation mixture and that of PLE and CAL-B was 2.5 mg/mL of the incubation mixture. [d]nd: not detected.

Kinetic Analysis of Regioselective *O*-Deacetylation and Complementary Roles of LAS and CSR. To confirm the enzyme-catalyzed regioselective *O*-deacetylation and to explain the synergistic effect of LAS and CSR, kinetic analyses were performed on the *O*-deacetylation of **1a** and the tri-*O*-acetyl compounds **4**–**7** using LAS, CSR, and PLE. The analyses were performed at pH 5.0 to minimize *O*-acetyl migration.[17] The first-order elimination rate constant k values for **1a** with LAS, PLE, and CSR were 2.27 ± 0.03, 2.30 ± 0.10, and 0.051 ± 0.004 h^{-1} $(mg/mL)^{-1}$, respectively, indicating that the *O*-deacetylation activity of LAS was as high as that of PLE and about 40-fold higher than that of CSR. On the basis of the k values of the tri-*O*-acetyl compounds **4**–**7**, LAS showed higher activity for **4** and **7** than for **5** and **6**, whereas CSR showed higher activity for **5** and **6** than for **4** and **7** (Table 2). These results demonstrate that LAS and CSR played complementary, synergistic roles in the *O*-deacetylation of **1a** to give **3a** via intermediates **4**–**7**, which explains the enhancement of *O*-deacetylation of **1a** by the combination of LAS and CSR (Table 1). The product ratios showed that LAS catalyzed the regioselective 3-*O*-deacetylation of **4**, **5**, and **7** and PLE efficiently catalyzed the regioselective 2-*O*-deacetylation of **4**–**6**. CSR preferentially catalyzed the 3-*O*-deacetylation of **5** and the 4-*O*-deacetylation of **6**, but did not catalyze the 3-*O*-deacetylation or the 4-*O*-deacetylation of **4** and **7**. The elimination rate constant k values of 3,4-di-*O*-acetyl compound **11** with LAS and CSR were 0.65 ± 0.04 and 0.039 ± 0.002 h^{-1} $(mg/mL)^{-1}$, respectively. This indicates that substrates possessing a 3,4-di-*O*-acetyl moiety such as **1a**, **4**, **7**, and **11** would be poor substrates for CSR, whereas the opposite substrate preference for LAS was observed. Di-*O*-acetyl compounds **9**–**13** were individually synthesized in yields between 55% and 92%, using the enzyme-catalyzed regioselective *O*-deacetylation activity of LAS, CSR, and PLE (Scheme 4). This method provides a convenient chemical and chemoenzymatic route for the synthesis of all 14 *O*-acetyl compounds (**4**–**17**).

Table 2. First-order Rate Constant k Values and Regioselectivity for *O*-Deacetylation of Compounds 4–7 at pH 5.0

enzyme	substrate	k[a]	product ratio[b]
LAS	**4**	1.54 ± 0.02	**9**:**8** (7.1:1)
	5	0.70 ± 0.01	**10**:**12** (6.3:1)
	6	0.033 ± 0.003	**13**:**10** (1.4:1)
	7	1.48 ± 0.02	**13**:**12** (2.5:1)
CSR	**4**	0.012 ± 0.001	nd[c]
	5	2.94 ± 0.12	**10**:**12** (20:1)
	6	9.36 ± 0.42	**10**:**13** (35:1)
	7	0.024 ± 0.002	**13**:**12** (3.8:1)
PLE	**4**	0.79 ± 0.03	**11**[d]
	5	0.164 ± 0.008	**12**[d]
	6	0.47 ± 0.01	**13**[d]
	7	0.225 ± 0.012	**13**:**12** (2.0:1)

[a]Incubation was performed at pH 5.0 and 40 °C and k values are shown as h^{-1} $(mg/mL)^{-1}$. [b]Product ratio was calculated from the HPLC peak areas of the *O*-deacetylation products in the early stages of the enzyme-catalyzed reaction. [c]Ratio was not determined because of low activity. [d]Obtained effectively as the sole product.

Kinetic Analysis of Nonenzymatic Intramolecular *O*-Acyl Migration at pH 7.2. Because the reaction rate at pH 7.2 was higher than at pH 5.0 (Table 1), the elimination rate constant k values of **1a** were determined at pH 7.2 as 2.24 ± 0.13 h^{-1} $(mg/mL)^{-1}$ for LAS, 9.23 ± 0.32 h^{-1} $(mg/mL)^{-1}$ for PLE, and 0.300 ± 0.003 h^{-1} $(mg/mL)^{-1}$ for CSR. Although the k value for LAS was as same as that at pH 5.0, the k values for CSR and PLE were 20-fold larger and 4-fold larger than those at pH 5.0, respectively. This may explain the enhancement of the k values observed at pH 7.2 compared with those at pH 5.0. Because intramolecular *O*-acyl migration has been observed at neutral or slightly alkaline pH,[17] kinetic studies on the intramolecular *O*-acetyl migration were then performed for

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the tri-*O*-acetyl and mono-*O*-acetyl intermediates. The *O*-acetyl migrations obeyed consecutive reversible first-order reaction kinetics; the *k* values are summarized in Table 3.

Table 3. First-order Rate Constant *k* Values for Intramolecular *O*-Acetyl Migration at pH 7.2[a]

migration	k (h^{-1})	migration	k (h^{-1})
4 to **5**	4.26 ± 0.03	**16** to **17**	1.51 ± 0.10
5 to **4**	0.11 ± 0.02	**17** to **16**	0.12 ± 0.03
5 to **6**	0.83 ± 0.01	**15** to **16**	0.23 ± 0.01
6 to **5**	0.86 ± 0.02	**16** to **15**	0.34 ± 0.07
6 to **7**	0.67 ± 0.03	**14** to **15**	0.17 ± 0.02
7 to **6**	0.63 ± 0.04	**15** to **14**	0.14 ± 0.01

[a]Incubation was performed under the same conditions as the enzymatic *O*-deacetylation at pH 7.2 and 40 °C.

The *k* values were highest for the *O*-acetyl migration from the 4-*O*-position to the 6-*O*-position, which gave 2,3,6-tri-*O*-acetyl compound **5** from 2,3,4-tri-*O*-acetyl compound **4** and 6-*O*-acetyl compound **17** from 4-*O*-acetyl compound **16**. However, the *k* values for the corresponding reverse migrations were the lowest. The *k* values for *O*-acetyl migrations between the secondary hydroxyl groups were similar; the tri-*O*-acetyl compounds had values of 0.63−0.86 h^{-1}, and the mono-*O*-acetyl compounds had values of 0.14−0.23 h^{-1}. Because the *O*-acetyl migration from the 4-*O*-position to the 6-*O*-position was the most favorable step, the major products in equilibrium were those with a 6-*O*-acetyl group: tri-*O*-acetyl compounds **5**−**7** and mono-*O*-acetyl compound **17**. For the di-*O*-acetyl compounds, **10**, **12**, and **13** were the major migration products in equilibrium (data not shown).

When **1a** was incubated with the combination of LAS and CSR at pH 5.0, 2,6-di-*O*-acetyl **10** remained after 2.0 h (Table 1), indicating the lower *O*-deacetylation activity of the enzymes toward 2-*O*- and 6-*O*-acetyl groups. However, at pH 7.2, no di-*O*-acetyl compounds were detected after 2 h (Table 1), probably because of the spontaneous *O*-acetyl migration in **10** which gave 3,6-di-*O*-acetyl **12** and 4,6-di-*O*-acetyl **13**, which subsequently underwent LAS- and CSR-catalyzed *O*-deacetylation to 6-*O*-acetyl compound **17**. CSR showed the highest *O*-deacetylation activity followed by LAS for mono-*O*-acetyl compounds **14**−**17**, and PLE exhibited the lowest activity (Table 4).

Table 4. First-Order Rate Constant *k* Values of *O*-Deacetylation of 14−17 at pH 7.2[a]

substrate	CSR[b]	LAS[b]	PLE[b]
14	0.17 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
15	4.8 ± 0.1	0.92 ± 0.04	0.19 ± 0.01
16	1.4 ± 0.1	0.15 ± 0.01	0.05 ± 0.01
17	0.14 ± 0.01	0.01 ± 0.00	0.01 ± 0.00

[a]Incubation was performed at pH 7.2 and 40 °C. [b]*k* values are shown as h^{-1} $(mg/mL)^{-1}$.

Enzyme-Catalyzed Complete *O*-Deacetylation of 1a−c to 3a−c. The combination of LAS and CSR at pH 7.2 resulted in the enhancement of *O*-deacetylation of **1a** to **3a** because of the complementary, synergistic roles of the enzymes and the nonenzymatic *O*-acetyl migrations. The sequence of LAS- and CSR-catalyzed *O*-deacetylation of **1a** to **3a** was as follows: LAS-catalyzed *O*-deacetylation to **6** and nonenzymatic *O*-acetyl migration to compounds **5** and **7** followed by LAS- and CSR-catalyzed complementary *O*-deacetylation to **10** which was *O*-deacetylated to **3a** via mono-*O*-acetyl intermediates **14** and **17**. Compounds **1a−c** were successfully converted to **3a−c** using the combination of LAS and CSR at pH 7.2 and 40 °C, with excellent chemoselectivity for *O*-deacetylation. Compound **3a** was obtained in 92% yield after incubation for 5.0 h, compound **3b** was obtained in 90% yield after incubation for 4.0 h, and compound **3c** was obtained in 88% yield after incubation for 4.0 h.

In summary, highly chemoselective *O*-deacetylation of alkali-labile per-acetylated *O*-glucopyranosides **1a−c** was achieved by the simultaneous use of enzymes LAS and CSR that exhibited complementary and synergistic roles in the enzyme-catalyzed *O*-deaceylation. This enzymatic *O*-deacetylation, under mild and neutral conditions, may be useful as an alternative to the chemical method, particularly for removing *O*-acetyl groups of alkali-labile glycosides. LAS, CSR, and PLE exhibit different regioselective *O*-deacetylation activities, Furthermore, several partially *O*-deacetylated derivatives of **1a** could be easily synthesized by using LAS, CSR, or PLE that exhibited different regioselective *O*-deacetylation activity.

EXPERIMENTAL SECTION

Materials and Methods. Compounds **3a−c** and **2a−c** were prepared according to the reported procedures.[11,30] Compounds **1a−c** were prepared by a slightly modified method of the reported procedure.[11] Fifteen commercially available enzymes, used in the screening for *O*-deacetylation activity toward **1a**, were as follows and used as received: lipase AS Amano (from *Aspergillus niger*) (LAS), carboxylesterase (from *Streptomyces rochei*, crude) (CSR), lipase type B (from *Candida antarctica*) (CAL-B), porcine liver esterase (PLE), lipase AP6 (from *Aspergillus niger*), lipase G Amano 50 (from *Penicillium camembertii*), lipase AYS Amano (from *Candida rugosa*), lipase PS Amano (from *Burkholderia cepacia*), lipase F-AP15 (from *Rhizopus oryzae*), lipase AK Amano (from *Pseudomonas fluorescens*), acylase I (from *Aspergillus* sp.), lipase from porcine pancreas, and three kinds of lipases from *Candida cylindracea* (lipases MY and OF from Meito Sangyo Co. Ltd. and CCL of 37 u/mg from Fluka). Amberlite XAD-4 was used after grinding (80−200 mesh). Column chromatography and preparative TLC were performed using silica gel 60 and silica gel plate, respectively. All other chemicals used were commercial products. ^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 with and without D_2O, and chemical shifts are presented by δ values with reference to the residual solvent signal of DMSO; that is, 2.49 and 39.50 ppm for ^1H and ^{13}C NMR, respectively.[31] Further analysis by 2D NMR (COSY, HMBC, HMQC) was performed to confirm the NMR peak assignments. MS spectra were recorded with a positive ionization mode. HPLC analyses were performed using a revered-phase column of Symmetry (C_{18}, 5 μm, 4.6 × 150 mm) with detection at 240 nm at 30 °C.

General Incubation Conditions for Enzyme-Catalyzed *O*-Deacetylation. Unless otherwise indicated, the incubation was performed with substrate at initial concentration of 0.75 mM in 85 mM sodium acetate buffer (pH 5.0) containing 15% (v/v) DMSO as a cosolvent and at 40.0 ± 0.1 °C, in the presence of an appropriate amount of enzyme(s). The progress of the reaction, monitored using HPLC, obeyed according to first-order reaction kinetics, from which the values of rate constant (*k*) were determined.

Kinetics of Intramolecular *O*-Acetyl Migration of 2,3,4-Tri-*O*-acetyl Compound 4 and 3-*O*-Acetyl Compound 15 and Enzyme-Catalyzed *O*-Deacetylation Reaction. Kinetic experiments of intramolecular *O*-acetyl migration of **4** and **15** at the initial concentration of 0.75 mM were performed at 40.0 ± 0.1 °C in 85 mM sodium phosphate buffer (pH 7.2) containing 15% (v/v) DMSO as a cosolvent. The progress of the reaction was monitored using HPLC. The values of individual *O*-acetyl migration rate constant (*k*) were

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computed on the assumption that the reactions obey according to first-order kinetics. The hydrolysis of *O*-acetyl groups was negligible during the reaction periods. The kinetics of the *O*-acetyl migration reactions of tri-*O*-acetyl compounds **4**–**7** are, therefore, represented by the following equations, where $k_{4\rightarrow6}$, for example, means the rate constant for the *O*-acetyl migration from the position 4-OH to 6-OH of the glucopyranoside ring.

$$\begin{aligned} d[\mathbf{4}]/dt &= -k_{4\rightarrow6}[\mathbf{4}] + k_{6\rightarrow4}[\mathbf{5}],\ d[\mathbf{5}]/dt \\ &= k_{4\rightarrow6}[\mathbf{4}] + k_{4\rightarrow3}[\mathbf{6}] - (k_{6\rightarrow4} + k_{3\rightarrow4})[\mathbf{5}],\ d[\mathbf{6}]/dt \\ &= k_{3\rightarrow4}[\mathbf{5}] + k_{3\rightarrow2}[\mathbf{7}] - (k_{4\rightarrow3} + k_{2\rightarrow3})[\mathbf{6}],\ d[\mathbf{7}]/dt \\ &= k_{2\rightarrow3}[\mathbf{6}] - k_{3\rightarrow2}[\mathbf{7}] \end{aligned}$$

The kinetics of the *O*-acetyl migration reactions of mono-*O*-acetyl compounds **14**–**17** are similarly represented by the following equations.

$$\begin{aligned} d[\mathbf{14}]/dt &= -k_{2\rightarrow3}[\mathbf{14}] + k_{3\rightarrow2}[\mathbf{15}],\ d[\mathbf{15}]/dt \\ &= k_{2\rightarrow3}[\mathbf{14}] + k_{4\rightarrow3}[\mathbf{16}] - (k_{3\rightarrow2} + k_{3\rightarrow4})[\mathbf{15}],\ d[\mathbf{16}]/dt \\ &= k_{3\rightarrow4}[\mathbf{15}] + k_{6\rightarrow4}[\mathbf{17}] - (k_{4\rightarrow3} + k_{4\rightarrow6})[\mathbf{16}],\ d[\mathbf{17}]/dt \\ &= k_{4\rightarrow6}[\mathbf{16}] - k_{6\rightarrow4}[\mathbf{17}] \end{aligned}$$

HPLC Analysis Conditions. All mobile phases, at a flow rate of 0.7 mL/min, were containing 0.1% (v/v) AcOH to minimize intramolecular acetyl migrations. The mobile phase used for analysis of tri-*O*-acetyl derivatives was EtOH/2-PrOH/H_2O (3:1:14, v/v) and the retention times of **4**, **5**, **6**, and **7** as well as **1a** were 13.3, 15.1, 9.8, 12.1, and 31.1 min, respectively. The mobile phase used for analysis of di-*O*-acetyl derivatives was EtOH/2-PrOH/H_2O (1:1:12, v/v) and the retention times of **8**, **9**, **10**, **11**, **12**, and **13** were 16.4, 9.3, 13.3, 8.8, 10.9, and 11.8 min, respectively. The mobile phase used for analysis of **3a** and mono-*O*-acetyl derivatives was 2-PrOH:H_2O (1:17, v/v) and the retention times of **14**, **15**, **16**, and **17** as well as **3a** were 19.0, 10.8, 11.7, 18.1, and 6.1 min, respectively. The mobile phases used for analysis of **3b** and **3c** were EtOH/2-PrOH/H_2O (3:1:10, v/v) and EtOH/2-PrOH/H_2O (3:1:12, v/v), respectively. The retention times of **3b** and **3c** were 6.1 and 4.6 min, respectively.

General Synthesis of 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosides of *N*-Arylacetohydroxamic Acids (1a–c). Besides our previous procedure,[11] the compounds **1a**–**c** were synthesized as follows. To a solution of *N*-arylacetohydroxamic acid (35 mmol) in EtOH (25 mL) and water (7 mL) containing KOH (35 mmol) was added acetobromglucose (28 mmol). After being stirred for 1 h, the reaction mixture was evaporated under reduced pressure, and the residue was dissolved in Et_2O and the organic layer was washed thoroughly with 1 M NaOH (about six times), dried over Na_2SO_4, and filtered. The filtrate was evaporated under reduced pressure to afford a crude product, which was purified by recrystallization from ethyl acetate–hexane to afford the compounds **1a**–**c**. **2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside of *N*-phenylacetohydroxamic acids (1a):** colorless fine needles (4.3 g, 32%); mp 109–110 °C (lit.[11] mp 109–110 °C); IR (Nujol) cm^{-1} 1760, 1740, 1690; $[\alpha]^{20}_D$ −56 (*c* 0.52, EtOH); ^1H NMR (400 MHz, DMSO-d_6) δ 1.92 (s, 3H), 1.93 (s, 3H), 1.97 (s, 3H), 2.00 (s, 3H), 2.19 (s, 3H), 4.04–4.15 (m, 3H, C_5-H, C_6-H and C_6-H′), 4.93 (dd, *J* = 8.3 and 9.3 Hz, 1H, C_2-H), 4.94 (t, *J* = 9.5 Hz, 1H, C_4-H), 5.32 (t, *J* = 9.5 Hz, 1H, C_3-H), 5.33 (d, *J* = 8.3 Hz, 1H, C_1-H), 7.29–7.36 (m, 3H, Ar-H), 7.41–7.49 (m, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.2, 20.3, 20.4, 20.4, 21.9, 61.5 (C_6), 67.8 (C_2 or C_4), 69.3 (C_2 or C_4), 70.8 (C_5), 71.7 (C_5), 102.5 (C_1), 124.9, 127.2, 128.5, 139.4, 168.7, 169.0, 169.2, 169.7, 171.2; MS (FAB, positive) *m*/*z* 482 $[M + H]^+$, 331, 169 (base), 109. Anal. Calcd for $C_{22}H_{27}NO_{11}$: C, 54.88; H, 5.65; N, 2.91. Found: C, 55.03; H, 5.59; N, 2.84. **2,3,4,6-Tetra-O-acetyl-β-D-glucopyranoside of *N*-(4-chloro)phenylacetohydroxamic acids (1b):** white solid (4.3 g, 30%); mp 117–119 °C; IR (Nujol) cm^{-1} 1750, 1700; $[\alpha]^{20}_D$ −44 (*c* 0.48, EtOH); ^1H NMR (400 MHz, DMSO-d_6) δ 1.94 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H × 2), 2.21 (s, 3H), 4.03–4.14 (m, 3H, C_5-H, C_6-H and C_6-H′), 4.94 (t, *J* = 9.6 Hz, 1H, C_4-H), 4.95 (dd, *J* = 8.5 and 9.6 Hz, 1H, C_2-H), 5.31 (t, *J* = 9.6 Hz, 1H, C_3-H), 5.34 (d, *J* = 8.5 Hz, 1H, C_1-H), 7.36 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.50 (d, *J* = 8.9 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.18, 20.23, 20.3, 21.8, 61.5 (C_6), 67.8 (C_2 or C_4), 69.3 (C_2 or C_4), 70.9 (C_5), 71.7 (C_5), 102.6 (C_1), 126.2, 128.6, 131.3, 138.4, 168.9, 169.2, 169.4, 169.8, 171.4. MS (FAB, positive) *m*/*z* 516 $[M + H]^+$, 331, 169 (base). Anal. Calcd for $C_{22}H_{26}NClO_{11}$: C, 51.22; H, 5.08; N, 2.72. Found: C, 51.42; H, 4.99; N, 2.80. **2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside of *N*-(4-methyl)phenylacetohydroxamic acid (1c):** white solid (3.9 g, 28%); mp 100–103 °C; IR (Nujol) cm^{-1} 1750, 1690; $[\alpha]^{20}_D$ −45 (*c* 0.56, EtOH); ^1H NMR (400 MHz, DMSO-d_6) δ 1.93 (s, 6H), 1.98 (s, 3H), 2.02 (s, 3H), 2.15 (s, 3H), 2.32 (s, 3H), 4.05–4.16 (m, 3H, C_5-H, C_6-H and C_6-H′), 4.90 (dd, *J* = 8.2 and 9.6 Hz, 1H, C_2-H), 4.93 (t, *J* = 9.4 Hz, 1H, C_4-H), 5.30 (d, *J* = 8.2 Hz, 1H, C_1-H), 5.32 (t, *J* = 9.6 Hz, 1H, C_3-H), 7.20 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.24 (d, *J* = 8.5 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.2, 20.3, 20.4, 20.6, 21.8, 61.5 (C_6), 67.9 (C_2 or C_4), 69.3 (C_2 or C_4), 70.9 (C_5), 71.7 (C_5), 102.5 (C_1), 125.5, 129.2, 137.0, 137.1, 168.9, 169.2, 169.2, 169.4, 169.9, 171.2; MS (FAB, positive) *m*/*z* 496 $[M + H]^+$, 331, 169 (base). Anal. Calcd for $C_{23}H_{29}NO_{11}$: C, 55.75; H, 5.90; N, 2.83. Found: C, 55.68; H, 6.00; N, 2.78.

2,3,4-Tri-*O*-acetyl-β-D-glucopyranoside of *N*-phenylacetohydroxamic Acid (4). To a solution of **3a** (510 mg, 1.63 mmol) in pyridine (6.0 mL) was added 4-methoxytrityl chloride (610 mg, 1.98 mmol). After 16 h at room temperature, acetic anhydride (0.56 mL, 5.9 mmol) was added to the reaction mixture. After 27 h, methanol was added to quench the excess reagents, and then the reaction mixture was evaporated under reduced pressure. The resultant residue was dissolved in 80% (v/v) aq AcOH (20 mL) and then kept at room temperature for 3 h. After evaporation of the reaction mixture, the residue was purified by silica gel column chromatography with ethyl acetate: benzene (1:1, v/v) to afford **4** (501 mg) in 70% overall yield from **3a**: mp 148–149 °C (colorless fine needles from ethyl acetate–hexane); IR (Nujol) cm^{-1} 3440, 1750, 1670; $[\alpha]^{20}_D$ −50 (*c* 0.52, EtOH); ^1H NMR (400 MHz, DMSO-d_6) δ 1.90 (s, 3H), 1.91 (s, 3H), 1.96 (s, 3H), 2.24 (s, 3H), 3.45 (dd, *J* = 5.4 and 12.0 Hz, 1H, C_6-H), 3.55 (ddd, *J* = 2.4, 5.4, and 12.0 Hz, 1H, C_6-H′), 3.81 (ddd, *J* = 2.4, 5.4, and 9.5 Hz, 1H, C_5-H), 4.72 (t, *J* = 5.4 Hz, 1H, C_6-OH), 4.88 (dd, *J* = 8.5 and 9.3 Hz, 1H, C_2-H), 4.92 (t, *J* = 9.5 Hz, 1H, C_4-H), 5.23 (d, *J* = 8.5 Hz, 1H, C_1-H), 5.25 (t, *J* = 9.5 Hz, 1H, C_3-H), 7.29–7.34 (m, 3H, Ar-H), 7.41–7.45 (m, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.1, 20.3, 22.0, 59.9 (C_6), 68.1 (C_4), 69.5 (C_2), 72.2 (C_3), 73.9 (C_5), 102.6 (C_1), 124.9, 127.1, 128.6, 139.4, 168.7, 169.0, 169.3, 171.6; MS (FAB, positive) *m*/*z* 440 $[M + H]^+$, 289 (base), 229, 169. Anal. Calcd for $C_{20}H_{25}NO_{10}$: C, 54.67; H, 5.73; N, 3.19. Found: C, 54.54; H, 5.67; N, 3.09.

2,3,6-Tri-*O*-acetyl-β-D-glucopyranoside of *N*-Phenylacetohydroxamic Acid (5). To a stirred suspension of **3a** (1.02 g, 3.26 mmol) in CH_3CN (16 mL) and trimethyl orthoacetate (2.0 mL, 16 mmol) was added a catalytic amount of TsOH (25 mg), and the reaction mixture was kept for 0.5 h at room temperature. After addition of pyridine (1 mL), the reaction mixture was evaporated under reduced pressure, and the resultant residue was acetylated with acetic anhydride (1.2 mL, 13 mmol) and pyridine (10 mL) for 2 h at room temperature. After addition of MeOH (2 mL), the reaction mixture was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and the solution was washed with 50 mM HCl and then water, dried with Na_2SO_4, and filtered. The filtrate was evaporated under reduced pressure to afford the corresponding crude 2,3-di-*O*-acetyl-4,6-orthoacetate derivative, which was recrystallized from ethyl acetate–hexane to afford the pure compound (1.09 g) in 74% overall yield: ^1H NMR (270 MHz, DMSO-d_6) δ 1.33 (s, 3H), 1.90 (s, 3H), 1.97 (s, 3H), 2.17 (s, 3H), 3.19 (s, 3H), 3.70–3.72 (m, 2H), 3.80–3.85 (m, 2H), 4.90 (t, *J* = 8.5 Hz, 1H), 5.19 (t, *J* = 9.5 Hz, 1H), 5.24 (d, *J* = 8.0 Hz, 1H), 7.27–7.34 (m, 3H, Ar-H), 7.41–7.46 (m, 2H, Ar-H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 20.2, 20.4, 21.3, 21.9, 50.2, 60.3, 64.9, 69.3, 70.3, 70.9, 102.9, 111.9, 125.0, 127.3, 128.7, 139.4, 168.9, 169.5, 171.4. Anal. Calcd for $C_{21}H_{27}NO_{10}$: C, 55.62; H, 6.00; N, 3.09. Found: C, 55.57; H, 6.04; N, 3.09. To the solution of this

compound (789 mg, 1.74 mmol) in AcOH (10 mL) was added H_2O (2.0 mL), and the resultant solution was kept at room temperature for 15 min. After evaporation of the solvent under reduced pressure, the residue was purified by silica gel column chromatography with ethyl acetate/benzene (1:1, v/v) to afford the desired product **5** (540 mg, 68%) along with **4** (150 mg, 19%). The analytical data of **5** are as follows: mp 124−125 °C (colorless block from ethyl acetate−hexane); IR (Nujol) cm^{-1} 3380, 1750, 1685; $[\alpha]^{20}_D$ −73 (*c* 0.61, EtOH); ^1H NMR (400 MHz, DMSO-d_6) δ 1.90 (s, 3H), 1.97 (s, 3H), 2.01 (s, 3H), 2.18 (s, 3H), 3.49 (dd, *J* = 5.6 and 9.5 Hz, 1H, C_4-H), 3.75 (ddd, *J* = 2.0, 6.3, and 9.5 Hz, 1H, C_5-H), 4.10 (dd, *J* = 6.3 and 12.0 Hz, 1H, C_6-H), 4.28 (dd, *J* = 2.0 and 12.0 Hz, 1H, C_6-H′), 4.77 (dd, *J* = 8.3 and 9.5 Hz, 1H, C_2-H), 5.02 (d, *J* = 9.5 Hz, 1H, C_3-H), 5.19 (d, *J* = 8.3 Hz, 1H, C_1-H), 5.68 (d, *J* = 5.6 Hz, 1H, C_4-OH), 7.28−7.33 (m, 3H, Ar-H), 7.39−7.43 (m, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.1, 20.4, 20.5, 21.7, 62.5 (C_6), 67.4 (C_4), 69.8 (C_2), 73.4 (C_3), 74.4 (C_5), 102.8 (C_1), 124.9, 127.1, 128.5, 139.6, 168.8, 169.4, 169.9, 171.3; MS (FAB, positive) *m*/*z* 440 $[M + H]^+$, 289 (base), 229. Anal. Calcd for $C_{20}H_{25}NO_{10}$: C, 54.67; H, 5.73; N, 3.19. Found: C, 54.86; H, 5.84; N, 3.20.

2,4,6-Tri-*O*-acetyl-β-D-glucopyranoside (6) and 3,4,6-Tri-*O*-acetyl-β-D-glucopyranoside (7) of *N*-Phenylacetohydroxamic Acid. (A) Intramolecular *O*-Acetyl Migration Method. To a solution of **4** (735 mg, 1.67 mmol) in CH_3CN (21 mL) was added 50 mM MOPS−NaOH (pH 7.5) buffer (400 mL), and the reaction mixture was kept at 37 °C for 4 h to afford the mixture composed mainly of **5**, **6**, and **7**. After the reaction mixture was extracted three times with ethyl acetate, the combined organic layers were dried over Na_2SO_4 and then filtered. The filtrate was evaporated under reduced pressure and the residue was purified by silica gel column chromatography with ethyl acetate−benzene (2:3, v/v), and the final purification was performed by preparative TLC developed with $CHCl_3$−MeOH (20:1, v/v) to afford **5** (133 mg, 18%), **6** (133 mg, 19%), and **7** (160 mg, 22%). The analytical data of **6** are as follows: mp 157−159 °C (colorless fine needles from ethyl acetate−hexane); IR (Nujol) cm^{-1} 3380, 1760, 1740, 1660; $[\alpha]^{20}_D$ −86 (*c* 0.50, EtOH); ^1H NMR (400 MHz, DMSO-d_6) δ 1.99 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 2.19 (s, 3H), 3.73 (dt, *J* = 5.9 and 9.5 Hz, 1H, C_3-H), 3.84−3.89 (m, 1H, C_5-H), 4.01 (dd, *J* = 2.4 and 12.3 Hz, 1H, C_6-H), 4.07 (dd, *J* = 5.6 and 12.3 Hz, 1H, C_6-H′), 4.69−4.75 (m, 2H, C_2-H and C_4-H), 5.07 (d, *J* = 8.5 Hz, 1H, C_1-H), 5.70 (d, *J* = 5.9 Hz, 1H, C_3-OH), 7.29−7.32 (m, 3H, Ar-H), 7.41−7.45 (m, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.5, 20.6, 20.7, 21.9, 61.8 (C_6), 70.2 (C_2 or C_4), 70.7 (C_3), 71.1 (C_5), 71.7 (C_2 or C_4), 103.0 (C_1), 124.8, 127.2, 128.7, 139.5, 169.0, 169.5, 169.9, 171.4; MS (FAB, positive) *m*/*z* 440 $[M + H]^+$, 289 (base). Anal. Calcd for $C_{20}H_{25}NO_{10}$: C, 54.67; H, 5.73; N, 3.19. Found: C, 54.58; H, 5.80; N, 3.18. The analytical data of **7** (a white solid) are as follows: mp 84−86 °C (colorless fine needles from ethyl acetate−hexane); IR (Nujol) cm^{-1} 3380, 1760, 1740, 1670; $[\alpha]^{20}_D$ −53 (*c* 0.47, EtOH); ^1H NMR (400 MHz, DMSO-d_6) δ 1.95 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 2.26 (s, 3H), 3.52−3.58 (m, 1H, C_2-H), 3.93−4.01 (m, 2H, C_5-H and C_6-H), 4.09 (dd, *J* = 5.4 and 12.0 Hz, 1H, C_6-H′), 4.80 (t, *J* = 9.5 Hz, 1H, C_4-H), 4.95 (d, *J* = 8.3 Hz, 1H, C_1-H), 5.07 (t, *J* = 9.5 Hz, 1H, C_3-H), 6.04 (d, *J* = 5.4 Hz, 1H, C_2-OH), 7.25 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.39 (t, *J* = 7.6 Hz, 2H, Ar-H), 7.50 (dd, *J* = 1.2 and 7.6 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.39, 20.41, 20.6, 22.1, 61.6 (C_6), 68.2 (C_4), 69.7 (C_2), 70.7 (C_5), 74.1 (C_3), 104.4 (C_1), 123.5, 126.3, 128.4, 139.3, 169.4, 169.5, 169.9, 171.6; MS (FAB, positive) *m*/*z* 440 $[M + H]^+$ (base), 398, 289, 229. Anal. Calcd for $C_{20}H_{25}NO_{10}$: C, 54.67; H, 5.73; N, 3.19. Found: C, 54.78; H, 5.88; N, 3.18.

Enzymatic Regioselective *O*-Deacetylation Method. Compound **6** was obtained in 56% yield as follows: the reaction mixture consisting of **1a** (16.6 mg, 34.5 μmol) in EtOH (3.5 mL) and LAS (117 mg) in 100 mM sodium acetate buffer (pH 5.0) (20 mL) was incubated at 40 °C for 20 min and then was extracted thoroughly with ethyl acetate. After evaporation of the combined organic layers, the residue was purified by preparative TLC with EtOAc/benzene (1:1, v/v). The compound **7** was obtained in 53% yield as follows: the reaction mixture consisting of **1a** (16.8 mg, 34.9 μmol) in EtOH (3.5 mL) and PLE (117 mg) in 100 mM sodium acetate buffer (pH 5.0) (20 mL) was incubated at 40 °C for 15 min and then was extracted thoroughly with ethyl acetate. After evaporation of the combined organic layer, the residue was purified by preparative TLC.

2,3-Di-*O*-acetyl-β-D-glucopyranoside of *N*-Phenylacetohydroxamic Acid (8). To a suspension of **3a** (2.59 g, 8.27 mmol) in acetone (20 mL) and dimethoxypropane (30 mL) was added a catalytic amount of TsOH (20 mg). After being stirred for 1 h at 37 °C, the resultant clear reaction mixture was evaporated under reduced pressure, the residue was dissolved in pyridine (6 mL), and then acetic anhydride (2.5 mL, 26 mmol) was added. After 2 h at room temperature, methanol was added to quench the excess anhydride, and then the reaction mixture was evaporated under reduced pressure to afford crude 2,3-di-*O*-acetyl-4,6-isopropylidene derivative, which was purified by recrystallization from ethyl acetate−hexane (2.49 g, 69%). The compound (2.17 g, 4.96 mmol) was dissolved in 80% (v/v) aq AcOH (12 mL) and then kept at 37 °C for 2 h. Evaporation of the reaction mixture afforded **8** (a white powder, 1.77 g, 90%): IR (Nujol) cm^{-1} 3420, 1760, 1730, 1690; $[\alpha]^{20}_D$ −78 (*c* 0.43, EtOH); ^1H NMR (270 MHz, DMSO-d_6) δ 1.89 (s, 3H), 1.96 (s, 3H), 2.25 (s, 3H), 3.47−3.50 (m, 3H), 3.68−3.72 (m, 1H), 4.63 (t, *J* = 5.6 Hz, 1H, C_6-OH), 4.73 (dd, *J* = 8.3 and 9.5 Hz, 1H, C_2-H), 4.97 (t, *J* = 9.5 Hz, 1H, C_3-H), 5.08 (d, *J* = 8.3 Hz, 1H, C_1-H), 5.49 (d, *J* = 4.9 Hz, C_4-OH), 7.27−7.33 (m, 3H, Ar-H), 7.40−7.45 (m, 2H, Ar-H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 20.3, 20.6, 22.2, 60.3 (C_6), 67.2 (C_4), 70.0 (C_2), 74.8 (C_3), 76.7 (C_5), 102.8 (C_1), 124.9, 127.1, 128.6, 139.4, 169.0, 169.6, 171.8; HRMS (FAB, positive) calcd for $C_{18}H_{24}NO_9$ $[M + H]^+$ *m*/*z* = 398.1451, found 398.1425 (error −2.6 mmu). Anal. Calcd for $C_{18}H_{23}NO_9$: C, 54.41; H, 5.83; N, 3.52. Found: C, 54.14; H, 5.96; N, 3.78.

2,6-Di-*O*-acetyl-β-D-glucopyranoside (10), 3,6-di-*O*-Acetyl-β-D-glucopyranoside (12), and 4,6-Di-*O*-acetyl-β-D-glucopyranoside (13) of *N*-Phenylacetohydroxamic Acid. To a solution of **8** (267 mg, 0.672 mmol) in CH_3CN (10 mL) was added 100 mM MOPS−NaOH (pH 7.5) buffer (190 mL), and the reaction mixture was kept at 37 °C for 8 h. The reaction mixture composed mainly of the title compounds was thoroughly extracted with ethyl acetate, and the combined organic layers were evaporated under reduced pressure. The residue was purified by silica gel column chromatography with $CHCl_3$−MeOH (20:1, v/v), and the final purification was performed by preparative TLC developed with $CHCl_3$−MeOH (10:1, v/v) to afford **10** (a white powder, 75 mg, 28%), **12** (a white powder, 85 mg, 32%), and **13** (a white powder, 55 mg, 21%). The analytical data of **10** are as follows: IR (Nujol) cm^{-1} 3420, 1745, 1680; $[\alpha]^{20}_D$ −65 (*c* 0.46, EtOH); ^1H NMR (270 MHz, DMSO-d_6) δ 1.97 (s, 3H), 2.00 (s, 3H), 2.17 (s, 3H), 3.17−3.25 (m, 1H), 3.36−3.45 (m, 1H), 3.52−3.56 (m, 1H), 4.05 (dd, *J* = 6.9 and 12.0 Hz, 1H, C_6-H), 4.28 (dd, *J* = 2.0 and 12.0 Hz, 1H, C_6-H′), 4.62 (t, *J* = 9.0 Hz, 1H, C_2-H), 4.93 (d, *J* = 8.5 Hz, 1H, C_1-H), 5.46 (d, *J* = 5.6 Hz, OH), 5.49 (d, *J* = 6.3 Hz, 1H, OH), 7.25−7.31 (m, 3H, Ar-H), 7.38−7.43 (m, 2H, Ar-H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 20.6, 20.8, 21.9, 62.9 (C_6), 69.7, 71.9, 73.4, 73.7, 103.2 (C_1), 124.6, 126.9, 128.4, 139.4, 168.9, 169.9, 171.2. HRMS (FAB, positive) calcd for $C_{18}H_{24}NO_9$ $[M + H]^+$ *m*/*z* = 398.1451, found 398.1464 (error 1.3 mmu). The analytical data of **12** are as follows; IR (Nujol) cm^{-1} 3490, 1720, 1660; $[\alpha]^{20}_D$ −72° (c 0.30, EtOH); ^1H NMR (270 MHz, DMSO-d_6) δ 1.98 (s, 3H), 2.02 (s, 3H), 2.24 (s, 3H), 3.28−3.41 (m, 2H), 3.54−3.60 (m, 1H, C_5-H), 4.07 (dd, *J* = 6.3 and 12.0 Hz, 1H, C_6-H), 4.21 (dd, *J* = 2.2 and 12.0 Hz, 1H, C_6-H′), 4.79 (d, *J* = 8.3 Hz, 1H, C_1-H), 4.80 (t, *J* = 10.0 Hz, 1H, C_3-H), 5.48 (d, *J* = 5.8 Hz, OH), 5.79 (d, *J* = 5.4 Hz, 1H, OH), 7.20−7.26 (m, 1H, Ar-H), 7.34−7.39 (m, 2H, Ar-H), 7.49 (dd, *J* = 1.3 and 8.8 Hz, 2H, Ar-H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 20.5, 21.0, 22.1, 62.7 (C_6), 67.6 (C_4), 69.9 (C_2), 73.4 (C_5), 76.9 (C_3), 104.8 (C_1), 123.6, 126.3, 128.4, 139.4, 169.6, 170.1, 171.6. HRMS (FAB, positive) calcd for $C_{18}H_{24}NO_9$ $[M + H]^+$ *m*/*z* = 398.1451, found 398.1433 (error −1.8 mmu). Anal. Calcd for $C_{18}H_{23}NO_9 \cdot H_2O$ requires C, 52.05; H, 6.07; N, 3.37. Found: C, 52.10; H, 6.10; N, 3.40. The analytical data of **13** are as follows; IR (Nujol) cm^{-1} 3410, 1750, 1680; $[\alpha]^{20}_D$ −108° (c 0.39, EtOH); ^1H NMR (270 MHz, DMSO-d_6) δ 1.96 (s, 3H), 2.00 (s, 3H), 2.25 (s, 3H), 3.26−3.31 (m, 1H, C_2-H, overlapped with H_2O

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peak), 3.45 (t, J = 9.5 Hz, 1H, C_3-H), 3.68−3.75 (m, 1H, C_5-H), 3.92 (dd, J = 2.4 and 12.2 Hz, 1H, C_6-H), 4.04 (dd, J = 5.9 and 12.2 Hz, 1H, C_6-H′), 4.62 (t, J = 9.5 Hz, 1H, C_4-H), 4.72 (d, J = 8.0 Hz, 1H, C_1-H), 5.47 (brs, 1H, OH), 5.77 (brs, 1H, OH), 7.22 (t, J = 7.4 Hz, 1H, Ar-H), 7.37 (t, J = 7.3 Hz, 2H, Ar-H), 7.50 (d, J = 7.8 Hz, 2H, Ar-H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 20.4, 20.8, 22.1, 61.9 (C_6), 70.5 (C_4), 71.0 (C_5), 72.0 (C_2), 73.2 (C_3), 104.7 (C_1), 123.3, 126.1, 128.4, 139.2, 169.6, 169.9, 171.5. HRMS (FAB, positive) calcd for $C_{18}H_{24}NO_9$ [M + H]$^+$ m/z = 398.1451, found 398.1432 (error −1.9 mmu).

2,4-Di-*O*-acetyl-β-D-glucopyranoside (9) and 3,4-di-*O*-acetyl-β-D-glucopyranoside (11) of *N*-phenylacetohydroxamic acid. To a solution of **8** (1.32 g, 3.32 mmol) in pyridine (20 mL) was added 4-methoxytrityl chloride (1.69 g, 5.47 mmol) and the reaction mixture was kept at room temperature for 15 h. After addition of MeOH to quench the excess reagent, the reaction mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography with ethyl acetate-benzene (1:2, v/v) to afford 6-(4-methoxytrityl) derivative (1.90 g, 85%). The compound (827 mg, 1.23 mmol) was dissolved in 50% (v/v) aq pyridine (20 mL) and the solution was kept at 50 °C for 24 h to afford a mixture composed mainly of the 6-(4-methoxytrityl) derivatives of **8**, **9** and **11**. After evaporation of the reaction mixture under reduced pressure, the residue was purified by silica gel column chromatography with ethyl acetate-benzene (1:2, v/v) to afford 6-(4-methoxytrityl) derivatives of **9** (a white powder, 178 mg, 22%) and **11** (a white powder, 218 mg, 26%), which were then deprotected with 80% (v/v) aq AcOH (7 mL) at 30 °C for 3 h. Evaporation of the each reaction mixture gave **9** and **11**, which were purified by silica gel column chromatography with $CHCl_3$-MeOH (40:1, v/v). The analytical data of **9** (a white powder) are as follows; IR (Nujol) cm^{-1} 3390, 1740, 1660; $[\alpha]^{20}_D$ −84° (c 0.38, EtOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.97 (s, 3H), 2.00 (s, 3H), 2.24 (s, 3H), 3.34−3.38 (m 1H, C_6-H), 3.43−3.47 (m, 1H, C_6-H′), 3.55−3.60 (m, 1H, C_5-H), 3.67 (dt, J = 5.8 and 9.3 Hz, 1H, C_3-H), 4.66 (t, J = 9.5 Hz, 1H, C_4-H), 4.69 (t, J = 8.5 Hz, 1H, C_2-H), 4.71 (d, J = 5.4 Hz, 1H, OH), 4.96 (d, J = 8.5 Hz, 1H, C_1-H), 5.58 (d, J = 5.6 Hz, 1H, OH), 7.27−7.32 (m, 3H, Ar-H), 7.40−7.45 (m, 2H, Ar-H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 20.7, 20.8, 22.2, 60.4 (C_6), 70.7 (C_4), 71.0 (C_3), 71.9 (C_2), 74.4 (C_5), 103.1 (C_1), 124.8, 127.1, 128.7, 139.4, 169.0, 169.4, 171.8. HRMS (FAB, positive) calcd for $C_{18}H_{24}NO_9$ [M + H]$^+$ m/z = 398.1451, found 398.1461 (error 1.0 mmu). Anal. Calcd for $C_{18}H_{23}NO_9$ requires C, 54.41; H, 5.83; N, 3.52. Found: C, 54.24; H, 5.85; N, 3.49. The analytical data of **11** (a white powder) are as follows; IR (Nujol) cm^{-1} 3470, 1750, 1650; $[\alpha]^{20}_D$ −48° (c 0.45, EtOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.93 (s, 3H), 1.96 (s, 3H), 2.29 (s, 3H), 3.36−3.52 (m, 3H, C_2-H, C_6-H, C_6-H′), 3.60−3.65 (m, 1H, C_5-H), 4.71 (brs, 1H, OH), 4.85 (d, J = 8.1 Hz, 1H, C_1-H), 4.80 (t, J = 9.5 Hz, 1H, C_3-H), 5.00 (t, J = 9.5 Hz, 1H, C_4-H), 5.99 (d, J = 5.3 Hz, 1H, OH), 7.23 (t, J = 7.3 Hz, 1H, Ar-H), 7.39 (t, J = 7.5 Hz, 2H, Ar-H), 7.52−7.55 (m, 2H, Ar-H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 20.5, 20.6, 22.5, 60.0 (C_6), 68.5 (C3), 69.8 (C_2), 73.8 (C_5), 74.6 (C_4), 104.3 (C_1), 123.4, 126.2, 128.4, 139.1, 169.2, 169.6, 171.7. HRMS (FAB, positive) calcd for $C_{18}H_{24}NO_9$ [M + H]$^+$ m/z = 398.1451, found 398.1479 (error 2.8 mmu). Anal. Calcd for $C_{18}H_{23}NO_9$: C, 54.41; H, 5.83; N, 3.52. Found: C, 54.20; H, 5.81; N, 3.48.

Enzymatic regioselective *O*-deacetylation for the synthesis of di-*O*-acetyl compounds 9−13. In general, the formation of each di-*O*-acetyl compound in the reaction mixture was analyzed and identified with the standard sample by using reversed-phase HPLC, as described below at the section of HPLC analysis conditions. Incubation mixture was composed of the appropriate precursor substrate in 85 mM sodium acetate buffer (pH 5.0) containing 15 (v/v) % EtOH and appropriate enzyme. Purification and isolation were performed by preparative TLC of the ethyl acetate extracts of the incubation mixtures. The precursor substrate and enzyme with their initial concentrations in the incubation mixture and the isolation yield of each di-*O*-acetyl compound were as follows: 2,4-di-*O*-acetyl compound **9** (78% after 40 min) from the substrate **4** (3.0 mM) with LAS (2.5 mg/mL), 2,6-di-*O*-acetyl compound **10** (92% after 6 min) from the substrate **6** (2.0 mM) with CSR (2.5 mg/mL), 3,4-di-*O*-acetyl compound **11** (60% after 20 min) from the substrate **4** (1.5 mM) with PLE (5.0 mg/mL), 3,6-di-*O*-acetyl ompound **12** (55% after 3.5 h) from the substrate **5** (1.5 mM) with PLE (5.0 mg/mL) and 4,6-di-*O*-acetyl ompound **13** (72% after 80 min) from the substrate **6** (1.5 mM) with PLE (5.0 mg/mL).

2-*O*-acety-β-D-glucopyranoside 14 and 3-*O*-acetyl-β-D-glucopyranoside 15 of *N*-phenylaceto-hydroxamic acid. As shown above for the synthesis of **8**, acetonidation of **3a** (1.53 g, 4.88 mmol) gave the corresponding 4,6-isopropylidene derivative, which was acetylated with acetic anhydride (0.42 mL, 4.38 mmol) in pyridine (2 mL) at 4 °C for 3 h. After evaporation of the reaction mixture, the residue was purified by silica gel column chromatography with ethyl acetate-benzene (1:3, v/v) to afford 4,6-isopropylidene derivatives of **14** (220 mg, 13%) and **15** (425 mg, 25%). The 4,6-isopropylidene derivatives of **14** (207 mg, 0.524 mmol) and **15** (200 mg, 0.506 mmol) were each deprotected with 80% (v/v) aq AcOH (3.0 mL) at 25 °C for 3 h. Evaporation of the reaction mixtures gave the title compounds **14** (175 mg, 94%) and **15** (167 mg, 93%). The analytical data of **14** (a white powder) are as follows; IR (Nujol) cm^{-1} 3400, 1740, 1660; $[\alpha]^{20}_D$ −68° (c 0.47, EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.97 (s, 3H), 2.25 (s, 3H), 3.19−3.22 (m, 1H, C_4-H), 3.27−3.39 (m, 2H, overlapped with H_2O peak, C_3-H and C_5-H), 3.45−3.51 (m, 1H, C_6-H′), 3.70 (dd, J = 5.4 and 12.0 Hz, 1H, C_6-H), 4.55 (t, J = 5.6 Hz, 1H, C_6-OH), 4.62 (t, J = 9.3 Hz, 1H, C_2-H), 4.81 (d, J = 8.5 Hz, 1H, C_1-H), 5.23 (brs, 1H, OH), 5.37 (brs, 1H, OH), 7.28−7.33 (m, 3H, Ar-H), 7.41−7.45 (m, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.8, 22.3, 60.7 (C_6), 69.5 (C_4), 72.1 (C_2), 73.8 (C_3), 77.3 (C_5), 103.3 (C_1), 124.7, 127.0, 128.6, 139.4, 169.2, 171.8. HRMS (FAB, positive) calcd for $C_{16}H_{22}NO_8$ [M + H]$^+$ m/z = 356.1345, found 356.1337 (error −0.9 mmu). Anal. Calcd for $C_{16}H_{21}NO_8 \cdot 1/2\ H_2O$: C, 52.74; H, 6.09; N, 3.84. Found: C, 52.88; H, 6.03; N, 3.83. The analytical data of **15** (a white powder) are as follows; IR (Nujol) cm^{-1} 3390, 1760, 1660; $[\alpha]^{20}_D$ −73° (c 0.30, EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.03 (s, 3H), 2.30 (s, 3H), 3.26−3.38 (m, 2H, overlapped with H_2O peak, C_2-H and C_4-H), 3.45−3.50 (m, 1H, C_6-H′), 3.65 (dd, J = 5.4 and 12.0 Hz, 1H, C_6-H), 4.52 (t, J = 5.6 Hz, 1H, C_6-OH), 4.67 (d, J = 8.0 Hz, 1H, C_1-H), 4.77 (t, J = 9.3 Hz, 1H, C_3-H), 5.24 (d, J = 5.8 Hz, 1H, OH), 5.75 (d, J = 5.4 Hz, 1H, OH), 7.24 (t, J = 7.6 Hz, 1H, Ar-H), 7.38 (t, J = 7.6 Hz, 2H, Ar-H), 7.54 (d, J = 7.6 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.1, 22.6, 60.4 (C_6), 67.3, 70.0, 76.8, 77.3 (C_3), 104.6 (C_1), 123.3, 126.1, 128.4, 139.0, 169.6, 171.7; HRMS (FAB, positive) calcd for $C_{16}H_{22}NO_8$ [M + H]$^+$ m/z = 356.1345, found 356.1347 (error 0.2 mmu). Anal. Calcd for $C_{16}H_{21}NO_8$: C, 54.08; H, 5.96; N, 3.94. Found: C, 54.11; H, 6.03; N, 3.92.

4-*O*-Acetyl-β-D-glucopyranoside 16 and 6-*O*-Acetyl-β-D-glucopyranoside 17 of *N*-Phenylacetohydroxamic Acid. To a suspension of **3a** (1.00 g, 3.19 mmol) in CH_3CN (20 mL) and trimethyl orthoacetate (1.2 mL, 9.6 mmol) was added a catalytic amount of *p*-TsOH (35 mg). After the mixture was stirred for 3 h at room temperature, water (20 mL) was added to hydrolyze the 4,6-orthoacetate group. After 1.5 h, the reaction mixture was evaporated under reduced pressure, and the residue was purified by preparative HPLC on a column of LiChrosorb RP-8 (7 μm, 10 × 250 mm) with MeOH/H_2O (1:4, v/v) to afford the title compounds **16** (410 mg, 33%) and **17** (417 mg, 33%). The analytical data of **16** (a white powder) are as follows: IR (Nujol) cm^{-1} 3420, 1740, 1670; $[\alpha]^{20}_D$ −102 (*c* 0.50, EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.01 (s, 3H), 2.30 (s, 3H), 3.26−3.34 (m, 2H, overlapped with H_2O), 3.37−3.44 (m, 3H), 4.58−4.63 (m 2H), 4.63 (d, J = 8.3 Hz, 1H, C_1-H), 5.36 (d, J = 5.6 Hz, 1H, OH), 5.70 (d, J = 5.1 Hz, 1H, OH), 7.23 (t, J = 7.6 Hz, 1H, Ar-H), 7.39 (t, J = 7.6 Hz, 2H, Ar-H), 7.56 (d, J = 7.6 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.9, 22.6, 60.6 (C_6), 71.0 (C_4), 72.2, 73.5, 74.3, 104.6 (C_1), 123.1, 126.0, 128.4, 139.0, 169.5, 171.7; HRMS (FAB, positive) calcd for $C_{16}H_{22}NO_8$ [M + H]$^+$ m/z = 356.1345, found 356.1349 (error 0.3 mmu). Anal. Calcd for $C_{16}H_{21}NO_8$: C, 54.08; H, 5.96; N, 3.94. Found: C, 54.11; H, 6.03; N, 3.92. The analytical data of **17** (a white powder) are as follows: IR (Nujol) cm^{-1} 3400, 1750, 1660; $[\alpha]^{20}_D$ −91 (*c* 0.60, EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.99 (s, 3H), 2.24 (s, 3H), 3.11−3.21 (m,

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3H, C_2-H, C_3-H and C_4-H), 3.37−3.41 (m, 1H, C_5-H), 4.07 (dd, 1H, J = 6.6 and 12.0 Hz, C_6-H), 4.22 (dd, 1H, J = 2.2 and 12.0 Hz, C_6-H′), 4.59 (d, J = 7.8 Hz, 1H, C_1-H), 5.22 (d, J = 5.4 Hz, 1H, OH), 5.26 (d, J = 5.4 Hz, 1H, OH), 5.55 (d, J = 5.4 Hz, 1H, OH), 7.23 (t, J = 7.6 Hz, 1H, Ar-H), 7.38 (t, J = 7.6 Hz, 2H, Ar-H), 7.51 (d, J = 7.6 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.5, 22.2, 63.0 (C_6), 69.8, 72.0, 73.7, 76.1, 104.9 (C_1), 123.3, 126.1, 128.4, 139.2, 170.1, 171.5; HRMS (FAB, positive) calcd for $C_{16}H_{22}NO_8$ $[M + H]^+$ m/z = 356.1345, found 356.1342 (error −0.3 mmu).

General Chemoenzymatic Synthesis of 3a−c from 1a−c Using LAS and CSR at pH 7.2. To a solution of **1a−c** (80 μmol) in DMSO (10 mL) was added a solution of LAS (335 mg; 5 mg/mL of incubation mixture) and CSR (335 mg, 5 mg/mL of incubation mixture) in 100 mM sodium phosphate (pH 7.2) buffer (57 mL) and the mixture stirred for 5.0 h with **1a** and for 4.0 h with **1b** and **1c** at 40 °C. After filtration, the filtrate was loaded onto a XAD-4 column (7 g, 1.6 × 10 cm), which had been washed thoroughly with acetone and then equilibrated with water. The column was washed with water (100 mL), and then the desired products **3a**, **3b**, and **3c** were eluted with 5, 10, and 7.5% (v/v) aqueous CH_3CN, respectively. The product-containing fractions were pooled and concentrated under reduced pressure. The residue was purified by preparative TLC developed with $CHCl_3$−acetone−H_2O (4:18:1, v/v) to afford **3a−c**. ***β*-D-Glucopyranoside of *N*-phenylacetohydroxamic acid (3a):** yield of **3a** (a white powder) (92%); IR (Nujol) cm^{-1} 3360, 1670; $[\alpha]^{20}_D$ −52 (c 0.36, H_2O); ^{1}H NMR (400 MHz, DMSO-d_6/D_2O) δ 2.29 (s, 3H), 3.10−3.22 (m, 4H), 3.39−3.47 (m, 1H, overlapped with DHO peak), 3.65 (d, J = 11.4 Hz, 1H), 4.50 (d, J = 7.8 Hz, 1H, C_1-H), 7.24 (t, J = 7.3 Hz, 1H, Ar-H), 7.39 (t, J = 7.6 Hz, 2H, Ar-H), 7.55 (dd, J = 1.2 and 7.6 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6/D_2O) δ 22.6, 60.8 (C_6), 69.6, 72.1, 76.4, 77.2, 104.8 (C_1), 123.0, 125.9, 128.4, 139.0, 171.7; HRMS (FAB, positive) calcd for $C_{14}H_{20}NO_7$ $[M + H]^+$ m/z = 314. 1240, found 314.1241 (error 0.1 mmu). ***β*-D-Glucopyranoside of *N*-(4-chloro)phenylacetohydroxamic acid (3b):** yield of **3b** (a white powder) (90%); IR (Nujol) cm^{-1} 3380, 1650; $[\alpha]^{20}_D$ −46 (c 0.42, H_2O); ^{1}H NMR (400 MHz, DMSO-d_6/D_2O) δ 2.31 (s, 3H), 3.11−3.24 (m, 4H), 3.44−3.47 (m, 1H), 3.64−3.67 (m, 1H), 4.52 (d, J = 7.8 Hz, 1H, C_1-H), 7.44 (d, J = 8.8 Hz, 2H, Ar-H), 7.57 (d, J = 8.8 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6/D_2O) δ 22.8, 61.0 (C_6), 69.8, 72.3, 76.4, 77.4, 105.4 (C_1), 125.4, 128.8, 130.7, 138.2, 172.5; HRMS (FAB, positive) calcd for $C_{14}H_{19}NO_7Cl$ $[M + H]^+$ m/z = 348.0850, found 348.0833 (error 0.1 mmu). ***β*-D-Glucopyranoside of *N*-(4-methyl)phenylacetohydroxamic acid (3c):** yield of **3c** (a white powder) (88%); IR (Nujol) cm^{-1} 3290, 1650; $[\alpha]^{20}_D$ −45 (c 0.38, H_2O); ^{1}H NMR (400 MHz, DMSO-d_6/D_2O) δ 2.26 (s, 3H), 2.31 (s, 3H), 3.13−3.18 (m, 4H), 3.43−3.46 (m, 1H), 3.65−3.68 (m, 1H), 4.48 (d, J = 7.3 Hz, 1H, C_1-H), 7.19 (d, J = 8.3 Hz, 2H, Ar-H), 7.39 (d, J = 8.3 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6/D_2O) δ 20.8, 22.6, 60.9 (C_6), 69.7, 72.2, 76.4, 77.3, 104.8 (C_1), 124.3, 129.2, 136.2, 136.6, 171.8; HRMS (FAB, positive) calcd for $C_{15}H_{22}NO_7$ $[M + H]^+$ m/z = 328. 1396, found 328.1389 (error 0.1 mmu).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of **1a−c**, **3a−c**, and **4−17** and crystallographic data and table of compounds **4** and **5**. This material is available free of charge via the Internet at http://pubs.acs/org.

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■ NOTE ADDED AFTER ASAP PUBLICATION

There were errors in the text on the fourth and fifth pages and in Table 1 in the version published ASAP on January 24, 2012; the correct version reposted on February 1, 2012.