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An Improved Chemo-Enzymatic Synthesis of 1-β-*O*-Acyl Glucuronides: Highly Chemoselective Enzymatic Removal of Protecting Groups from Corresponding Methyl Acetyl Derivatives

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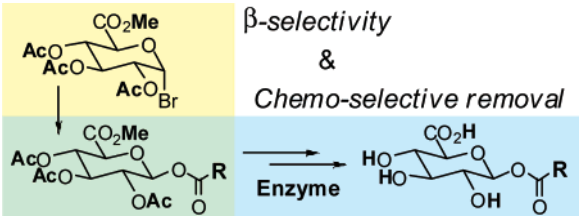

An improved and widely applicable chemo-enzymatic method for the synthesis of a series of 1-β-*O*-acyl glucuronides **5a**−**f** has been developed from the corresponding methyl acetyl derivatives **3a**−**f**, which were stereospecifically synthesized from cesium salts of carboxylic acids **1a**−**f** and methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (**2**). Chemoselectivity of lipase AS Amano (LAS) in the hydrolytic removal of *O*-acetyl groups of **3a**−**f** to provide methyl esters **4a**−**f** was influenced by the nature of their 1-β-*O*-acyl groups; high selectivity was evident only for **3b** and **3f**. Carboxylesterase from *Streptomyces rochei* (CSR), newly screened as an alternative to LAS, showed much greater chemoselectivity toward the *O*-acetyl groups than LAS; **3a**, **3d**, and **3e** were chemoselectively hydrolyzed only by CSR. The combination of CSR with LAS yielded better results in the hydrolysis of **3c** and **3f** than did single usage of CSR. Final deprotection of the methyl ester groups of **4a**−**f** to provide **5a**−**f** was chemoselectively achieved by using lipase from *Candida antarctica* type B (CAL-B) as well as esterase from porcine liver (PLE), although CAL-B possessed higher chemoselectivity and catalytic efficiency than did PLE. CSR also exhibited high chemoselectivity in the synthesis of (*S*)-naproxen 1-β-*O*-acyl glucopyranoside (**7**) from its 2,3,4,6-tetra-*O*-acetyl derivative **6**.

Introduction

In drug discovery and development and for drug safety, it is important to understand drug metabolism and the underlying mechanism of drug-induced toxicity, including pharmacological actions, necrotic activity, immune-mediated response, idiosyncratic reactions, and potential cancer-inducing properties.[1−7] Recent articles have reviewed bioactivation pathways for drug metabolism to electrophilic metabolites, after covalent binding to target macromolecules (proteins and/or DNA), which has toxicological consequences.[8−13] These chemically reactive

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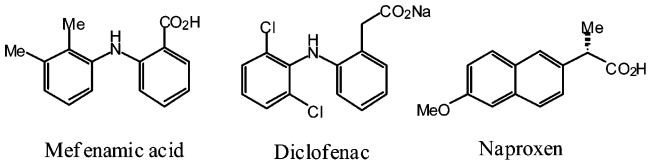
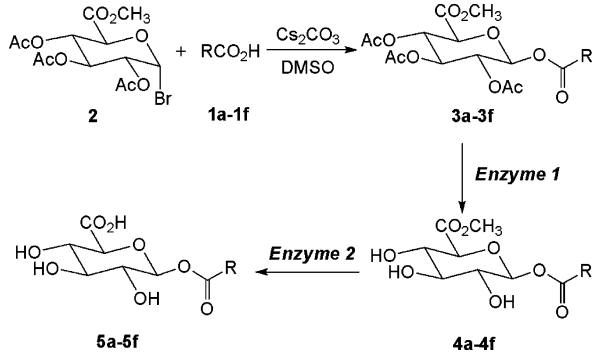
CHART 1. Three NSAIDs Used in the Preceding Study[20]



SCHEME 1. Chemo-Enzymatic Synthetic Method for 1-β-*O*-Acyl Glucuronides

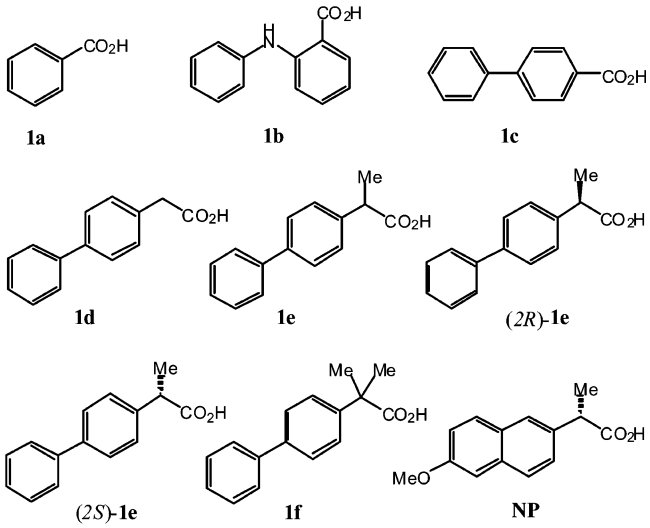


metabolites include 1-β-*O*-acyl glucuronides, associated with many carboxylate drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs). Because these acyl glucuronides are, in general, electrophilic species,[9,14−19] covalent binding of acyl glucuronides to tissue proteins is believed to cause hypersensitivity and idiosyncratic reactions. Some acyl glucuronides have been implicated in the adverse effects of some NSAIDs which have been withdrawn from the market,[1,8,10,12] although their toxicological relevance is not well understood.

A previous study[20] has reported a facile chemo-enzymatic synthesis of 1-β-*O*-acyl glucuronides from commercially available methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate **2** and three NSAIDs [diclofenac (DF), mefenamic acid (MF), and (*S*)-naproxen (NP)] as model carboxylate drugs (Chart 1), which provides a useful synthetic method for toxicological research on 1-β-*O*-acyl glucuronides. Two characteristics of this method, among the synthetic methods reported for 1-β-*O*-acyl glucuronides,[19,21] include stereospecific glucuronidation with nearly exclusive β-configuration and highly chemoselective enzymatic removal of sugar ester-type protection groups without affecting 1-β-*O*-acyl functions by using lipase AS Amano (LAS) and porcine liver esterase (PLE) (Scheme 1).

In the present study, we extended this synthetic chemo-enzymatic method using carboxylic acids **1a**−**f** with a wide range of structures (Chart 2), while focusing on the validation and improvement of the synthetic method, especially for chemoselectivity in the enzymatic hydrolytic deprotection of methyl acetyl derivatives of 1-β-*O*-acyl glucuronides **3a**−**f** and **4a**−**f**.

CHART 2. Wide-Ranging Carboxylic Acids Used in This Study



The enzymatic deprotection of these compounds has been achieved by using newly screened enzymes, carboxylesterase from *Streptomyces rochei* (CSR) and lipase from *Candida antarctica* type B (CAL-B), as well as LAS and PLE. In addition, because of recent reports on a glucosidation pathway for carboxylic acid drugs,[22−24] we applied this chemo-enzymatic method for the synthesis of 1-β-*O*-acyl glucopyranoside derivative **7** of NP, starting from its per-*O*-acetylated compound **6**, as a model compound.

Results and Discussion

Synthesis of Methyl Acetyl Derivatives of 1-β-*O*-Acyl Glucuronides 3a−f. Diverse carboxylic acids **1a**−**f**, having a wide range of size and numbers of methyl substituents at the α-carbon of 4-biphenylyl carboxylic acid derivatives **1d**−**f**, were chosen for validating the chemo-enzymatic synthetic strategy. The condensation of **1a**−**f** with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (**2**) was performed by using a previously reported procedure[20] to provide the corresponding 1-β-*O*-acyl glucuronides **3a**−**f** in moderate yields, without production of α-anomers (based on ^{1}H NMR data). The yields of **3a**−**f** and their ^{1}H-chemical shifts and *J* values for anomeric protons are summarized in Table 1. The β-configuration of **3a**−**f** was confirmed by *J* values of 7.8−8.3 Hz.[25,26] Compound **3e**, obtained from the racemic carboxylic acid **1e**, was a 1:1 mixture of diastereoisomers (2*R*)-**3e** and (2*S*)-**3e**, based

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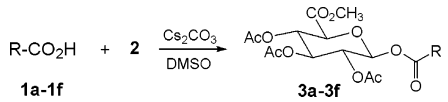
TABLE 1. Stereospecific Synthesis of Methyl Acetyl Derivatives of 1-β-O-Acyl Glucuronides 3a−f



R−CO_2H	product	yield (%)[a]	C_1−H (δ)[b]	J (Hz)[c]
1a	**3a**	50	6.25	8.1
1b	**3b**	67	6.24	7.8
1c	**3c**	55	6.28	7.8
1d	**3d**	58	6.04	8.2
1e	**3e**	52	5.99	8.3
			6.05	8.3
(2*R*)-**1e**	(2*R*)-**3e**	44	5.99	8.3
(2*S*)-**1e**	(2*S*)-**3e**	45	6.05	8.3
1f	**3f**	52	6.00	8.3

[a] Isolated yields, as recrystallized form, based on **2** as the limiting reagent. [b] Chemical shifts of the anomeric protons measured in DMSO-d_6. [c] Coupling constants of the anomeric protons observed as doublets.

TABLE 2. Enzymatic Hydrolysis of 3a−f with LAS

substrate	concn (mM)[a]	time (h)[b]	products and yields (%)[c]
3a	1.00[d]	2.0	**4a** (17), **1a** (80), others[e] (3)
3b	0.50[d]	6.0	**4b** (95), **1b** (3)
3c	0.40	4.5	**4c** (57), **1c** (21), others (21)
3d	0.20	0.25	**4d** (3), **1d** (92), others (6)
3e	0.50[d]	1.5	**4e** (41), **1e** (35), **3e** (11), others (13)
3f	0.50[d]	1.5	**4f** (14), **1f** (nd[f]), others (85)

[a] Initial concentration of substrate in 20 mM sodium citrate buffer (pH 5.0) containing 20% (v/v) DMSO. [b] Incubation time at 40 °C. [c] Yields based on HPLC analysis. [d] The reaction mixture was a suspension. [e] Others = partially *O*-deacetylated compounds. [f] nd = not detectable.

on comparison of ^{1}H NMR spectra. The methyl protons of the 2-*O*-acetyl group of (2*R*)-**3e**, assigned to a singlet peak at 1.52 ppm by the HMBC method (data not shown), are markedly shifted ca. 0.4 ppm to higher field compared to that of the (2*S*)-**3e** isomer whose 2-*O*-acetyl methyl protons resonate at 1.91 ppm. This upfield shift may be due in part to the anisotropic effect of the aromatic ring of (2*R*)-**3e**.

Chemoselective Enzymatic Removal of *O*-Acetyl Groups of 3a−f. In a preceding paper,[20] enzyme screening revealed that lipase AS Amano (LAS) showed high chemoselectivity toward the *O*-acetyl groups of methyl acetyl derivatives of 1-β-*O*-acyl glucuronides of MF, DF, and NP. Therefore, the chemoselectivity of LAS toward a new series of the substrates **3a**−**f** was first examined under the same conditions to verify the scope of the chemoselectivity of LAS. Incubation was conducted in 20 mM sodium citrate buffer (pH 5.0) containing 20% (v/v) DMSO as a cosolvent at 40 °C by the addition of 10 mg/mL LAS in the incubation mixture. Results shown in Table 2 indicate that the chemoselectivity of LAS was influenced by the nature of the substrate 1-*O*-acyl groups.

Compounds **3a** and **3d** released the corresponding carboxylic acids **1a** and **1d** in 80% and 92% yields, respectively, indicating much lower chemoselectivity of LAS toward both **3a** and **3d**. Compound **4d** also was confirmed to be hydrolyzed by LAS, providing **1d** (data not shown). The effect of cosolvents other than DMSO on chemoselectivity toward **3d** was investigated with use of acetone, *t*-BuOH, CH_3CN, dioxane, DMF, diglyme, MeOH, methyl cellosolve, methyl carbitol, and THF at a concentration of 20% (v/v). Except for THF and methyl carbitol, in which LAS was significantly inactivated, carboxylic acid **1d** was released in 30% yield or greater, indicating chemoselectivity of LAS toward **3d** was not improved by the nature of the cosolvent. Compounds **3c** and **3e** provided products **4c** and **4e** in moderate yields (57% and 41%, respectively) but also released carboxylic acids **1c** (21%) and **1e** (35%), although in lower yields than those from **3a** and **3d.** High chemoselectivity with LAS was found only for **3b** and **3f**. While **3b** provided **4b** in 95% yield after 6.0 h, **3f** provided **4f** in only 14% yield after 1.5 h because of an accumulation of the corresponding partially *O*-deacetylated intermediates (85%); however, the corresponding carboxylic acid **1f** was not detected. These results indicate that high chemoselectivity of LAS can be achieved, especially for substrates that have rather bulky groups near 1-β-*O*-acyl functions, such as the *o*-phenylamino substituent (of **3b**) and α,α-dimethyl substituents (of **3f**).

Enzyme Screening for Chemoselective Hydrolysis of 3d as a Model Substrate. It is important to remove the protecting groups of sugar moieties without affecting their 1-β-*O*-acyl linkage. Therefore, enzymes were newly screened to achieve the chemoselective deprotection of *O*-acetyl groups of the substrates other than **3b**. Screening was performed with 0.1 mM **3d**, as a model substrate, in 20 mM sodium citrate buffer (pH 5.0) containing 20% (v/v) DMSO at 40 °C for 2 h in the presence of each enzyme at an amount of 10 mg/mL of incubation mixture. Of 17 enzymes tested, a carboxylesterase from *Streptomyces rochei* (CSR) showed the highest chemoselectivity. Compound **3d** provided **4d** in 65% yield along with partially *O*-deacetylated intermediates (20%), unreacted **3d** (10%), and the released carboxylic acid **1d** in only 4% yield. Equine liver acetone powder provided **4d** (35%) as well as **1d** (30%). Acylase Amano, lipase AP6, lipase AYS Amano, acylase I, and lipase from *Candida cylindracea* showed higher catalytic activity but lower chemoselectivity, resulting in the release of **1d** in 60−95% yields. Ten additional enzymes tested showed much lower catalytic actitivity toward **3d**, leaving 60−90% of **3d** unreacted.

CSR-Catalyzed Removal of *O*-Acetyl Groups of 3a−f. Newly screened CSR was examined for chemoselectivity and catalytic efficiency toward **3a**−**f**. The catalytic efficiency of CSR toward **3d** was optimal at 50−55 °C; the efficiency at 50 °C was about 3-fold greater than that at 40 °C; activity was deactivated at 60 °C (Figure 1).

Therefore, incubation with CSR was performed at the optimal temperature of 50 °C. Table 3 summarizes the results, obtained in 20 mM sodium citrate buffer (pH 5.0) containing 20% (v/v) DMSO as a cosolvent after addition of CSR at 10 mg/mL of incubation mixture. Compounds **3a**, **3d**, and **3e**, which did not yield good results with LAS, were chemoselectively hydrolyzed to **4a**, **4d**, and **4e**, respectively, using CSR. Compound **3a**, at an initial amount of 1.00 μmol/mL of the incubation mixture, was chemoselectively hydrolyzed to provide **4a** in 92% yield after 4 h (run 1). When the amount of **3a** was increased to 2.00 μmol/mL of incubation mixture for preparative scale (run 2), the incubation mixture became a suspension. CSR-catalyzed hydrolysis of the resultant suspension was prolonged for 9.0 h, probably due in part to the dissolution of **3a** being a rate-limiting

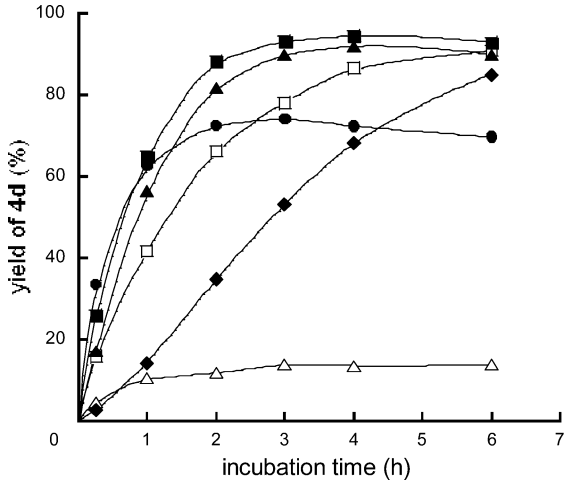
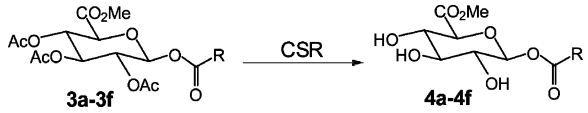


FIGURE 1. Effect of incubation temperature on CSR-catalyzed hydrolysis of **3d** to **4d** in 20 mM sodium citrate buffer (pH 5.0) containing 20% (v/v) DMSO at pH 4.0 and 55 °C (Δ), at pH 4.5 and 55 °C (□), at pH 5.0 and 40 °C (◆), at pH 5.0 and 50 °C (▲), at pH 5.0 and 55 °C (■), or at pH 5.0 and 60 °C (●).

TABLE 3. Enzymatic Hydrolysis of 3a−f with CSR



substrate	run	concn (mM)[a]	time (h)[b]	products and yields (%)[c]
3a	1	1.00	4.0	**4a** (92), **1a** (6)
	2	2.00[d]	9.0	**4a** (82), **1a** (16)
3b	1	0.50[d]	4.0	**4b** (13), **1b** (1), **3b** (84)
3c	1	0.40	2.0	**4c** (10), **1c** (nd[e]), **3c** (89), others[f] (2)
3d	1	0.80	4.5	**4d** (92), **1d** (7)
	2	1.50[d]	7.5	**4d** (86), **1d** (10)
3e	1	0.20	3.0	**4e** (95), **1e** (2)
	2	0.50[d]	6.0	**4e** (96), **1e** (4)
(2*R*)-**3e**	1	0.20	4.0	(2*R*)-**4e** (95), (2*R*)-**1e** (3)
	2	1.00[d]	30	(2*R*)-**4e** (89), (2*R*)-**1e** (11)
(2*S*)-**3e**	1	0.20	2.0	(2*S*)-**4e** (96), (2*S*)-**1e** (2)
	2	1.00[d]	3.0	(2*S*)-**4e** (98), (2*S*)-**1e** (2)
3f	1	0.50[d]	1.5	**4f** (14), **1f** (nd), **3f** (83), others (4)

[a] Initial concentration of substrate in 20 mM sodium citrate buffer (pH 5.0) containing 20% (v/v) DMSO. [b] Incubation time at 50 °C. [c] Yields based on HPLC analysis. [d] The reaction mixture was a suspension. [e] nd = not detectable. [f] Others = partially *O*-deacetylated compounds.

step. The initial concentration of substrate **3a** should be approximately 1.0 mM, near saturated concentration under the incubation conditions. Compounds **3d** and **3e**, at initial amounts of 0.80 and 0.50 μmol/mL, respectively, also successfully underwent chemoselective hydrolysis to provide **4d** and **4e** in yields of 92% and 96%, respectively. Both (2*R*)-**3e** and (2*S*)-**3e** also were chemoselectively hydrolyzed in high yields, although (2*S*)-**3e** was a better substrate than (2*R*)-**3e**. A remarkable difference in the rates of CSR-catalyzed hydrolysis of (2*R*)-**3e** and (2*S*)-**3e** was observed when the reaction was started from a suspension at a concentration of 1.00 μmol/mL (run 2 in each case). Similarly, starting from a suspension of **3d** at an initial amount of 1.50 μmol/mL (run 2), the reaction time again was prolonged for 7.5 h and the yield of **4d** was decreased to 86% with an increased production of carboxylic acid **1d** (10%). These results indicate that the initial concentration of the substrates should be near their saturated concentrations.

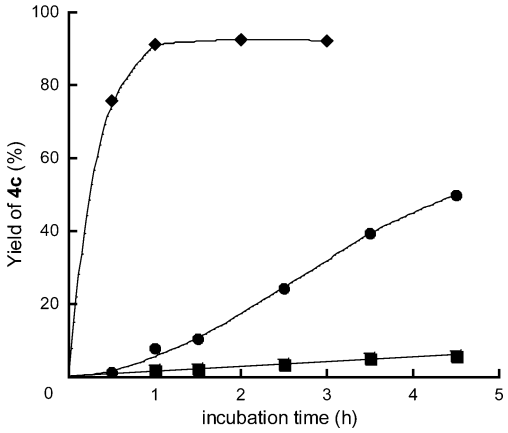


FIGURE 2. Time courses of enzymatic hydrolysis of **3c** to **4c** with CSR (■), LAS (●), or both CSR and LAS (◆) in 20 mM sodium citrate buffer (pH 5.0) containing 20% (v/v) DMSO at 40 °C.

TABLE 4. Enzymatic Hydrolysis of 3a−f with Both CSR and LAS

substrate	run	concn (mM)[a]	time (h)[b]	products and yields (%)[c]
3c	1	0.40	2.0	**4c** (94), **1c** (4), others[e] (2)
	2	2.00[d]	9.0	**4c** (93), **1c** (6)
(2*R*)-**3e**	1	1.00[d]	3.0	(2*R*)-**4e** (84), (2*R*)-**1e** (11), others (5)
3f	1	1.00[d]	3.0	**4f** (98)

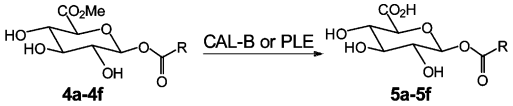
[a] Initial concentration of substrate in 20 mM sodium citrate buffer (pH 5.0) containing 20% (v/v) DMSO. [b] Incubation time at 40 °C. [c] Yields based on HPLC analysis. [d] The reaction mixture was a suspension. [e] Others = partially *O*-deacetylated compounds.

In contrast, the ortho-substituted compound **3b**, which was chemoselectively hydrolyzed by LAS (Table 2), was minimally hydrolyzed by CSR. Similarly, CSR-catalyzed hydrolysis of both **3c** and **3f** was very slow. For these cases, a considerable amount of substrates **3b**, **3c**, and **3f** remained unreacted; however, significant amounts of the corresponding carboxylic acids **1b**, **1c**, and **1f** were not released. For **3c** and **3f**, therefore, concurrent use of CSR and LAS was examined next at 40 °C, because LAS was deactivated at 50 °C. Figure 2 shows the time course of formation of **4c** from **3c** through enzymatic hydrolysis with CSR, LAS, or both CSR and LAS (each 10 mg/mL); catalytic activity of CSR for hydrolysis of **3c** to **4c** was lower than that of LAS. In the CSR-catalyzed reaction no significant accumulation of the corresponding partially *O*-deacetylated intermediates occurred, whereas these intermediates accumulated in the LAS-catalyzed reaction (data not shown). This indicates that catalytic activity of CSR toward the intermediates is much greater than that of LAS. Therefore, concurrent use of the enzymes synergistically accelerated the rate of formation of **4c** and resulted in 94% yield in 2 h (Table 4, run 1).

Similarly, **3f** effectively provided **4f** (98%) by concurrent use of both enzymes (Table 4). Furthermore, concurrent usage of CSR and LAS shortened the reaction time required for the hydrolysis of (2*R*)-**3e**; (2*R*)-**4e** was readily obtained in 84% yield in only 3 h (Table 4).

Products **4a**−**f** were isolated easily through EtOAc extraction from the incubation mixture or by passage through an XAD-4 column, as reported previously.[20] The structures of **4d** (isolated

TABLE 5. Enzymatic hydrolysis of 4a−f with PLE or CAL-B



substrate	enzyme (mg/mL of incubation mixture)	concn (mM)[a]	time (h)[b]	products and yields (%)[c]
4a	CAL-B (2)	1.00	2.0	**5a** (87), **1a** (8)
4b	CAL-B (1)	1.00	2.5	**5b** (98), **1b** (nd[d])
4c	CAL-B (1)	1.00	9.0	**5c** (93), **1c** (nd)
4d	PLE (0.4)	0.100	1.0	**5d** (68), **1d** (17), **4d** (15)
	CAL-B (0.4)	0.100	0.25	**5d** (97)
	CAL-B (1)	1.00	2.0	**5d** (99)
4e	PLE (0.4)	0.100	1.5	**5e** (82), **1e** (nd), **4e** (15)
	CAL-B (0.4)	0.100	0.5	**5e** (99)
	CAL-B (0.4)	1.00	0.75	**5e** (99)
(2*R*)-**4e**	CAL-B (0.4)	1.00	1.0	(2*R*)-**5e** (99)
(2*S*)-**4e**	CAL-B (0.4)	2.00	2.0	(2*S*)-**5e** (99)
4f	CAL-B (0.8)	2.00	3.0	**5f** (97)

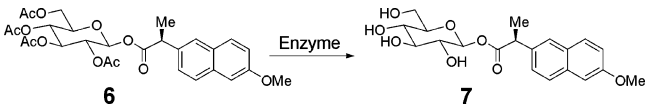
[a] Initial concentration of substrate in 20 mM sodium citrate buffer (pH 5.0) containing 20% (v/v) DMSO. [b] Incubation time at 40 °C. [c] Yields based on HPLC analysis. [d] nd = not detectable.

by an XAD-4 column method) and **4e** (isolated by the EtOAc extraction method) were confirmed by ^{1}H and ^{13}C NMR studies (see the Experimental Section). ^{1}H NMR of **4e** showed it to be a 1:1 mixture of diastereoisomers of (2*R*)-**4e** and (2*S*)-**4e**.

Enzyme Screening for Chemoselective Hydrolysis of the Methyl Ester of 4d as a Model Substrate. Chemoselective hydrolysis of the methyl esters of **4a**−**f** was investigated. Porcine liver esterase (PLE), an effective enzyme for the hydrolysis of methyl esters of 1-β-*O*-acyl glucuronides[20] of DF, MF, and NP, was applied first to the hydrolysis of the methyl ester of **4d**. As shown in Table 5, the product **5d** was obtained in 68% yield with concomitant formation of the corresponding carboxylic acid **1d** (17%), indicating that chemoselectivity of PLE was influenced by the structure of the 1-β-*O*-acyl group. Lipases have been used for the enzymatic removal of carboxylic methyl esters,[27] and the lipase from *Candida antarctica* type B (CAL-B) has been utilized in the chemo-enzymatic synthesis of 1-β-*O*-acyl glucuronide.[28] Therefore, screening was performed by using 0.1 mM **3d** as a model substrate, in 20 mM sodium citrate buffer (pH 5.0) containing 20% (v/v) DMSO at 40 °C for 1.5 h in the presence of 10 mg/mL of each enzyme. Of the 17 enzymes tested, CAL-B showed the highest chemoselectivity, leading to the formation of **4d** in 97% yield within 15 min. Acylase Amano and acylase I showed the worst chemoselectivity for our purpose, resulting in the release of **1d** in almost quantitative yield. The other 14 enzymes were either low in chemoselectivity or showed almost no catalytic activity toward **4d**. Table 5 summarizes the results of hydrolytic cleavage of **4a**−**f** with CAL-B or PLE.

PLE showed high chemoselectivity toward **4b** and **4f**, although the catalytic activity was lower than that of CAL-B (data not shown). CAL-B-catalyzed hydrolysis of **4e** provided a 1:1 mixture of (2*R*)-**5e** and (2*S*)-**5e**, which were readily isolated by preparative-HPLC with a C_{18} column (see the Experimental Section); the retention times of these compounds isolated were identical with those of compounds (2*R*)-**5e** and (2*S*)-**5e** obtained from CAL-B-catalyzed hydrolysis of (2*R*)-**4e** and (2*S*)-**4e**, respectively. No significant epimerization was observed for the final products (2*R*)-**5e** and (2*S*)-**5e**, based on HPLC analysis and NMR spectra (see the Supporting Information).

SCHEME 2. Chemo-Enzymatic Synthesis of (*S*)-Naproxen (NP) 1-β-*O*-Acyl Glucopyranoside 6



Because these four enzymes are commercially available and inexpensive, the overall reaction could be readily scaled up to millimole amounts of starting material.

Chemo-Enzymatic Synthesis of 7 from Its 2,3,4,6-Tetra-*O*-acetyl Derivative 6. As shown in Scheme 2, the chemo-enzymatic method was applied to the synthesis of (*S*)-naproxen 1-β-*O*-acyl glucopyranoside **6** as the model compound.

In carbohydrate chemistry, many ester-hydrolytic enzymes have been reported as catalysts for mainly regioselective synthetic reactions.[29] Recently, CAL-B was reported as a biocatalyst for the chemoselective deacetylation of acylglucopyranosides.[30] Therefore, CAL-B and PLE as well as LAS and CSR were examined for activity in the chemoselective deacetylation of **6**. LAS and PLE showed low chemoselectivity under the optimum conditions used for abovementioned 1-β-*O*-acyl glucuronides, releasing a considerable amount of NP. CAL-B was chemoselective for **6**, but showed low catalytic activity. CSR-catalyzed deacetylation of **6** in 20 mM MES-NaOH buffer (pH 5.5) containing 20% (v/v) DMF at 50 °C for 40 h provided **7** in 64% yield, along with mono-*O*-acetates (34%). Modification of the incubation conditions is needed for a better result and is now in progress.

In conclusion, our chemo-enzymatic method was improved for the synthesis of 1-β-*O*-acyl glucuronides by using CSR and CAL-B as well as LAS and PLE as catalysts. This efficient method is applicable to a wide range of carboxylic acids, providing 1-β-*O*-acyl glucuronides with complete β-selectivity and in good yields. Differences in chemoselectivity and substrate specificity between CSR and LAS are currently under investigation.

Experimental Section

Materials. Optically active 2-(4-biphenylyl)propionic acids, (2*R*)-**2e** and (2*S*)-**2e**, with optical purities being 98.2% and 99.2% (reported by HPLC method), respectively, were kindly gifted from Nagase ChemteX Corporation (Osaka, Japan) and were used without further purification. Racemic 2-(4-biphenylyl)propionic acid (**2e**),[31] methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (**1**),[32] and 2, 3, 4, 6-tetra-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopy-

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ranose[33] were synthesized according to the reported procedures. Amberlite XAD-4 was used after grinding (80−200 mesh). The 17 commercially available enzymes, used in the screening for their chemoselective hydrolytic activities, were as follows. Liver acetone powder from equine, lipase from porcine pancreas, acylase I (from *Aspergillus* sp.), pectin esterase from orange peel (PE), acylase Amano (from *Aspergillus* sp.), lipase CAL-B (from *Candida Antarctica* type B), lipase M Amano 10 (from *Mucor javanicus*), lipase MML (from *Mucor miehei*), lipase from *Phycomyces nitens*, lipase AK Amano (from *Pseudomonas fluorescence*), lipase AYS Amano (from *Candida rugosa*), CSR (carboxylesterase from *Streptomyces rochei*, crude), lipase AP6 (from *Aspergillus niger*), lipase PS Amano (from *Burkholderia cepacia*), lipase R Amano (from *Penicillium roqueforti*), newlase (from *Rhizopus niveus*), and lipase (from *Candida cylindracea*). All other chemicals used were analytical grade commercial products.

HPLC Analysis. Enzymatic reactions were analyzed with a Shimadzu HPLC system, equipped with a column of Symmetry C_{18} (5 μm, 4.6 × 150 mm^2, Waters). Mobile phases used were aqueous CH_3CN (except for **3a** and **4a** with MeOH) containing 50 mM ammonium acetate (pH 4.5) and 10 mM tetra-*n*-butylammonium bromide, as reported previously.[20]

Synthesis of 2-(4-Biphenylyl)-2-methylpropionic Acid (1f). A solution of **1d** (1.25 g, 5.9 mmol) in MeOH (10 mL) was refluxed in the presence of a catalytic amount of TsOH (50 mg) for 1 h to provide the corresponding methyl ester in 96% isolated yield, which was treated with NaH (60%, 0.64 g, 16 mmol) in THF (18 mL) followed by the dropwise addition of CH_3I (0.83 mL, 13.3 mmol) and then the mixture was stirred for overnight. After concentration in vacuo, the residue was worked up with water and then the miture was extracted with EtOAc (2 × 25 mL) to give the corresponding dimethylated ester in 83% isolated yield. The ester was refluxed in MeOH (15 mL) and 2 M NaOH (20 mL) for 3 h. After concentration in vacuo, 1 M HCl (15 mL) was added to the residue and the mixture was extracted with EtOAc (2 × 25 mL). The combined organic layer was dried over Na_2SO_4 and then concentrated to provide **1f** (1.13 g) in 80% overall yield from **1d**: mp 175−176 °C (a white solid from benzene/hexane) (lit.[34] mp 175−177 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58−7.55 (m, 4H), 7.48 (d, 2H, J = 8.3 Hz), 7.42 (t, 2H, J = 7.8 Hz), 7.36−7.31 (m, 1H), 1.64 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.0, 142.8, 140.7, 139.9, 128.8, 127.3, 127.2, 127.1, 126.3, 46.1, 26.3.

General Procedure for the Synthesis of Methyl 2,3,4-Tri-*O*-acetyl-1-β-*O*-acyl-D-glucopyranuronates (3a−f). These compounds were readily synthesized within 2 h at room temperature by the reaction of cesium salts of the corresponding carboxylic acids (**1a**−**f**) with commercially available methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (**2**, 0.9 equiv) in DMSO as reported previously.[20] Analytical and spectral data of the products are as follows.

Methyl 2,3,4-tri-*O*-acetyl-1-β-*O*-benzoyl-D-glucopyranuronate (3a): mp 141−143 °C (white needles from EtOH). Found: C, 54.80; H, 5.08; $C_{20}H_{22}O_{11}$ requires C, 54.80; H, 5.06. *m*/*z* (EI) 438 (M^+), 378, 317, 228, 186, 157, 106, 77 (base peak); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 (dd, 2H, J = 1.2 and 8.3 Hz), 7.72 (tt, 1H, J = 1.2 and 7.3 Hz), 7.57 (t, 2H, J = 7.3 Hz), 6.25 (d, 1H, J = 8.1 Hz), 5.60 (t, 1H, J = 9.3 Hz), 5.19 (dd, 1H, J = 8.1 and 9.3 Hz), 5.10 (1H, t, J = 9.3 Hz), 4.77 (d, 1H, J = 9.5 Hz), 3.61 (s, 3H), 2.01 (s, 6H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 169.2, 169.1, 166.9, 163.7, 134.4, 129.5, 129.0, 127.9, 91.3, 71.4, 70.4, 69.7, 68.7, 52.5, 20.3, 20.2.

Methyl 2,3,4-tri-*O*-acetyl-1-β-*O*-(2-phenylamino)benzoyl-D-glucopyranuronate (3b): mp 154−155.5 °C (pale yellow needles from EtOH). Found: C, 59.04; H, 5.08; N, 2.57; $C_{26}H_{27}O_{11}N$ requires C, 58.98; H, 5.14; N, 2.65. *m*/*z* (EI) 529 (M^+), 469, 213, 196 (base peak); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H), 7.80 (dd, 1H, J = 1.5 and 7.8 Hz), 7.45 (dt, 1H, J = 2.0 and 8.8 Hz), 7.37 (t, 2H, J = 8.3 Hz), 7.27 (d, 2H, J = 7.3 Hz), 7.19 (d, 1H, J = 8.3 Hz), 7.12 (t, 1H, J = 7.3 Hz), 6.82 (t, 1H, J = 7.3 Hz), 6.24 (d, 1H, J = 7.8 Hz), 5.63 (t, 1H, J = 9.8 Hz), 5.22 (dd, 1H, J = 7.8 and 9.3 Hz), 5.11 (1H, t, J = 9.3 Hz), 4.77 (d, 1H, J = 9.5 Hz), 3.61 (s, 3H), 2.012 (s, 3H), 2.008 (s, 3H), 1.995 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 169.3, 169.2, 167.0, 165.2, 147.9, 139.8, 135.7, 131.3, 129.5, 123.8, 122.3, 117.7, 114.1, 109.6, 91.0, 71.4, 70.4, 69.5, 68.8, 52.6, 20.30, 20.26, 20.20.

Methyl 2,3,4-tri-*O*-acetyl-1-β-*O*-(4-phenyl)benzoyl-D-glucopyranuronate (3c): mp 211−212 °C (white needles from EtOH). Found: C, 60.79; H, 5.06; $C_{26}H_{26}O_{11}$ requires C, 60.70; H, 5.09. *m*/*z* (EI) 514 (M^+), 317, 257, 181 (base peak); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 (d, 2H, J = 8.8 Hz), 7.88 (d, 2H, J = 8.8 Hz), 7.76 (d, 2H, J = 7.3 Hz), 7.51 (t, 2H, J = 7.3 Hz), 7.44 (t, 1H, J = 7.3 Hz), 6.28 (d, 1H, J = 7.8 Hz), 5.62 (t, 1H, J = 9.3 Hz), 5.21 (dd, 1H, J = 7.8 and 9.3 Hz), 5.11 (1H, t, J = 9.3 Hz), 4.79 (d, 1H, J = 9.8 Hz), 3.62 (s, 3H), 2.01 (s, 6H), 1.98 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 169.3, 169.2, 167.0, 163.6, 145.7, 138.5, 130.2, 129.1, 128.6, 127.2, 127.0, 126.7, 91.4, 71.4, 70.4, 69.7, 68.8, 52.6, 20.3, 20.24, 20.21.

Methyl 2,3,4-tri-*O*-acetyl-1-β-*O*-(4-phenyl)phenylacetyl-D-glucopyranuronate (3d): mp 149−151 °C (white needles from EtOAc/Hexane). Found: C, 61.04; H, 5.35; $C_{27}H_{28}O_{11}$ requires C, 61.36; H, 5.34. *m*/*z* (EI) 528 (M^+), 468, 317, 257, 194, 167 (base peak); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.66−7.60 (m, 4H), 7.48−7.43 (m, 1H), 7.38−7.31 (m, 4H), 6.04 (d, 1H, J = 8.2 Hz), 5.49 (t, 1H, J = 9.6 Hz), 5.05−4.94 (m, 2H), 4.68 (d, 1H, J = 9.9 Hz), 3.79 (s, 2H), 3.63 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.41, 169.38, 169.25, 168.8, 166.9, 139.7, 139.0, 132.6, 129.9, 128.9, 127.4, 126.7, 126.6, 90.8, 71.4, 70.8, 69.7, 68.8, 52.6, 20.24, 20.17, 20.1.The methlene carbon of **3d** was overlapping with the solvent peaks of DMSO-d_6.

Methyl 2,3,4-tri-*O*-acetyl-1-β-*O*-(2-(4-biphenylyl))propionyl-D-glucopyranuronate (3e) (mixture of diastereoisomers): mp 118−122 °C (white needles from EtOH). Found: C, 61.92; H, 5.57; $C_{28}H_{30}O_{11}$ requires C, 62.03; H, 5.58. *m*/*z* (EI) 542 (M^+), 482, 317, 257, 181 (base peak); ^{1}H NMR (400 MHz, DMSO-d_6) (mixture of diastereoisomers) δ 7.65−7.60 (m, 4H), 7.45 (t, 2H, J = 7.8 Hz), 7.37−7.30 (m, 3H), 6.05 (d, 0.5H, J = 8.3 Hz), 5.99 (d, 0.5H, J = 8.3 Hz), 5.51 (t, 0.5H, J = 9.8 Hz), 5.44 (t, 0.5H, J = 9.8 Hz), 5.00 (t, 0.5H, J = 9.8 Hz), 4.99 (dd, 0.5H, J = 7.8 and 9.3 Hz), 4.97 (t, 0.5H, J = 9.8 Hz), 4.87 (dd, 0.5H, J = 8.3 and 9.8 Hz), 4.67 (d, 1H, J = 9.8 Hz), 3.95−3.88 (m, 1H), 3.63 (s, 1.5H), 3.61 (s, 1.5H), 1.97 (s, 1.5H), 1.97 (s, 1.5H), 1.96 (s, 1.5H), 1.93 (s, 1H), 1.90 (s, 1.5H), 1.52 (s, 1.5H), 1.42 (d, 1.5H, J = 6.8 Hz), 1.40 (d, 1.5H, J = 6.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.0, 171.9, 169.4, 169.3, 169.2, 168.9, 168.3, 166.8, 139.67, 139.65, 139.18, 139.14, 138.9, 138.4, 128.93, 128.90, 128.1, 127.8, 127.4, 127.0, 126.9, 126.6, 126.5, 91.0, 90.8, 71.4, 70.7, 69.7, 69.4, 68.8, 52.60, 52.57, 43.93, 43.7, 20.3, 20.19, 20.15, 19.6, 18.0, 17.7.

Methyl 2,3,4-tri-*O*-acetyl-1-β-*O*-{(2*R*)-2-(4-biphenylyl)}-propionyl-D-glucopyranuronate ((2*R*)-3e): mp 90−92 °C (white needles from EtOH). Found: C, 61.89; H, 5.56; $C_{28}H_{30}O_{11}$ requires C, 62.03; H, 5.58. *m*/*z* (EI) 542 (M^+), 482, 317, 257, 181 (base peak); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63−7.60 (m, 4H), 7.45 (t, 2H, J = 7.7 Hz), 7.37−7.30 (m, 3H), 5.99 (d, 1H, J = 8.3 Hz), 5.44 (t, 1H, J = 9.5 Hz), 4.98 (t, 1H, J = 9.8 Hz), 4.87 (dd, 1H, J = 8.3 and 9.5 Hz), 4.67 (d, 1H, J = 10.0 Hz), 3.92 (q, 1H, J = 7.1 Hz), 3.63 (s, 3H), 1.97 (s, 3H), 1.90 (s, 3H), 1.52 (s, 3H), 1.40 (d, 3H, J = 7.1 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.0, 169.3, 169.2, 168.3, 166.8, 139.6, 139.1, 138.8, 128.9, 127.8, 127.4, 127.0, 126.5, 90.8, 71.4, 70.7, 69.4, 68.8, 52.6, 43.7, 20.2, 20.1, 19.6, 17.6.

Methyl 2,3,4-tri-*O*-acetyl-1-β-*O*-{(2*S*)-2-(4-biphenylyl)}-propionyl-D-glucopyranuronate ((2*S*)-3e): mp 152−154 °C (white needles from EtOH). Found: C, 62.03; H, 5.59; $C_{28}H_{30}O_{11}$ requires C, 62.03; H, 5.58. *m*/*z* (EI) 542 (M^+), 482, 317, 257, 181 (base

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peak); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65−7.60 (m, 4H), 7.47−7.43 (m, 2H), 7.37−7.34 (m, 3H), 6.05 (d, 1H, J = 8.3 Hz), 5.50 (t, 1H, J = 9.5 Hz), 5.01 (t, 1H, J = 9.8 Hz), 4.99 (dd, 1H, J = 8.3 and 9.5 Hz), 4.67 (d, 1H, J = 9.8 Hz), 3.91 (q, 1H, J = 7.1 Hz), 3.61 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.43 (d, 3H, J = 7.1 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.8, 169.3, 169.2, 168.9, 166.7, 139.7, 139.2, 138.4, 128.9, 128.0, 127.4, 126.8, 126.6, 91.0, 71.4, 70.8, 69.7, 68.8, 52.5, 43.9, 20.2, 20.1, 18.0.

Methyl 2,3,4-tri-*O*-acetyl-1-β-*O*-{2-(4-biphenylyl)-2-methyl}-propionyl-D-glucopyranuronate (3f): mp 165−166.5 °C (white needles from EtOH). Found: C, 62.37; H, 5.83; $C_{29}H_{32}O_{11}$ requires C, 62.62; H, 5.80. m/z (EI) 556 (M^+), 496, 257, 195 (base peak); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65−7.61 (m, 4H), 7.46 (t, 2H, J = 7.3 Hz), 7.37−7.34 (m, 3H), 6.00 (d, 1H, J = 8.3 Hz), 5.48 (dd, 1H, J = 9.3 and 9.6 Hz), 4.98 (t, 1H, J = 9.3 Hz), 4.91 (dd, 1H, J = 8.3 and 9.3 Hz), 4.67 (d, 1H, J = 9.8 Hz), 3.63 (s, 3H), 1.97 (s, 3H), 1.93 (s, 3H), 1.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.9, 169.3, 169.2, 168.6, 166.8, 142.4, 139.5, 138.8, 128.9, 127.4, 126.7, 126.5, 126.0, 91.1, 71.4, 70.7, 69.4, 68.8 52.6, 45.8, 25.9, 25.4, 20.2, 20.1, 19.9.

Screening of Enzymes for Chemoselective Hydrolysis of the Model Substrates 3d and 4d. Each incubation was carried out at the initial concentration of 0.1 mM for **3d** and **4d** in 25 mM sodium citrate buffer (pH 5.0) containing 20% (v/v) DMSO as a cosolvent at 40 °C. Each enzyme of the commercially available 17 enzymes was added to the incubation mixture at the final amount of 10 mg/mL of incubation mixture, regardless of their solubilities in the incubation mixture. For enzyme assays, an aliquot of the incubation mixture was appropriately diluted with an HPLC carrier and then analyzed by the abovementioned reversed-phase HPLC. The concentration of CH_3CN in the mobile phase was 55% (v/v) and 35% (v/v) for **3d** and **4d**, respectively.

General Procedure for the Enzymatic Hydrolysis of Substrates 3d and 3e to 4d and 4e: Methyl 1-β-*O*-(4-phenyl)-phenylacetyl-D-glucopyranuronate (4d). To a solution of **3d** (20.7 mg, 39.2 μmol) in DMSO (10 mL) was added a solution of CSR (500 mg; 10 mg/ mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (40 mL) then the solution was stirred for 4.5 h at 50 °C. The conversion yield to **4d** was 92% by HPLC analysis and the amount of the liberated acid **1d** was 7%. The product **4d** was isolated by passing through a short XAD-4 column (1.5 g, 1.6 × 3 cm^2), which had been washed thoroughly with acetone and then equilibrated with water. After the incubation mixture was loaded onto the column, the column was washed with water (30 mL) and then 20% (v/v) aqueous CH_3CN (30 mL). The product **4d** was eluted with 75% (v/v) aqueous CH_3CN (30 mL) with a recovery yield of 98% (by HPLC analysis). **4d**: ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.61−7.55 (m, 4H), 7.43−7.29 (m, 5H), 5.52 (d, 1H, J = 7.8 Hz), 3.95 (t, 1H, J = 9.5 Hz), 3.79 (s, 2H), 3.75 (s, 3H), 3.54 (t, 1H, J = 9.3 Hz), 3.47−3.38 (m, 2H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 172.0, 170.8, 142.1, 141.4, 134.0, 131.1, 129.8, 128.3, 128.1, 127.9, 95.9, 77.33, 77.28, 73.6, 72.9, 52.9, 41.1; m/z (SIMS, positive) $[M + Na]^+$ 425.1225 (error 1.4 mmu). $C_{21}H_{22}NO_8Na$ requires m/z 425.1211.

Methyl 1-β-*O*-(2-(4-Biphenylyl))propionyl-D-glucopyranuronate (4e) (Mixtures of Diastereoisomers). To a solution of **3e** (27.1 mg, 50.0 μmol) in DMSO (20 mL) was added a solution of CSR (1.00 g; 10 mg/ mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (80 mL) then the solution was stirred for 6 h at 50 °C. The conversion yield to **4e** was 96% by HPLC analysis. The incubation mixture was successfully extracted with EtOAc (2 × 100 mL) and the combined organic layer was dried over Na_2SO_4. After removal of the solvent in vacuo, the residue was purified by recrystallization from CH_3CN. **4e**: ^{1}H NMR (400 MHz, DMSO-d_6) (mixture of diastereoisomers) δ 7.65−7.60 (m, 4H), 7.47−7.33 (m, 5H), 5.47 (d, 0.5H, J = 5.4 Hz, OH), 5.45 (d, 0.5H, J = 8.3 Hz), 5.44 (d, 0.5H, J = 8.1 Hz), 5.40 (d, 0.5H, J = 5.6 Hz, OH), 5.39 (d, 0.5H, J = 5.6 Hz, OH), 5.33 (d, 0.5H, J = 5.6 Hz, OH), 5.27 (d, 0.5H, J = 5.1 Hz, OH), 5.25 (d, 0.5H, J = 5.4 Hz, OH), 3.91−3.87 (m, 2H), 3.66 (s, 1.5 H), 3.62 (s, 1.5H), 3.38−3.27 (m, 2H), 3.18−3.15 (m, 1H), 1.45 (d, 1.5H, J = 6.6 Hz), 1.43 (d, 1.5H, J = 6.9 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 172.6, 168.9, 168.8, 139.8, 139.2, 139.0, 138.9, 128.9, 128.1, 127.4, 126.9, 126.8, 126.60, 126.57, 94.5 75.9, 75.5, 75.3, 72.1, 72.0, 71.2, 52.0, 51.9, 44.0, 18.7, 18.5; m/z (EI, positive) $[M]^+$ 416.1490 (error 2.0 mmu). $C_{22}H_{24}O_8$ requires m/z 416.1470.

Consecutive Enzymatic Hydrolysis for the Synthesis of 1-β-*O*-Acyl-D-glucopyranuronates (5a−f): 1-β-*O*-Benzoyl-D-glucopyranuronate (5a). To a solution of **3a** (39.8 mg, 93.3 μmol) in DMSO (9.1 mL) was added a solution of CSR (456 mg; 10 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (37 mL) then the solution was stirred for 9 h at 50 °C. The initial concentration of **3a** was 2.00 mM and the conversion yield to **4a** was 82% by HPLC analysis. The yield was increased to 92% for 4 h of incubation, when starting at the initial concentration of **3a** of 1.00 mM. The product **4a** was extracted with EtOAc (3 × 60 mL) in a recovery yield of 98%. After removal of the organic solvent in vacuo, the residue was taken up into DMSO (7.4 mL) and then a solution of CALB (148 mg; 2 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (67 mL) was added and the resulting solution was stirred for 2 h at 40 °C. The conversion yield to **5a** was 87% by HPLC analysis. The isolation of **5a** was performed by passing through a XAD-4 column (5 g, 1.6 × 10 cm^2) as described above. The incubation mixture was acidified to pH around 2.5 by the addition of 1 M HCl and then the solution was loaded onto the column, which was then washed with water (80 mL) followed by elution with 20% (v/v) aqueous CH_3CN. The fractions containing **5a** were pooled and concentrated in vacuo to give **5a** in recovery yield of 93%: ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.11−8.08 (m, 2H), 7.63 (tt, 1H, J = 1.2 and 7.6 Hz), 7.51−7.47 (m, 2H), 5.75 (d, 1H, J = 7.8 Hz), 3.96 (d, 1H, J = 9.3 Hz), 3.67−3.50 (m, 3H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 172.6, 166.6, 134.8, 131.0, 130.6, 129.6, 96.1, 77.6, 77.2, 73.7, 73.0; m/z (SIMS, positive) $[M + H]^+$ 299.0782 (error 1.6 mmu). $C_{13}H_{15}O_8$ requires m/z 299.0766.

1-β-*O*-(2-Phenylamino)benzoyl-D-glucopyranuronate (5b). To a solution of **3b** (53.0 mg, 100 μmol) in DMSO (20 mL) was added a solution of LAS (1.00 g; 10 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (80 mL) then the solution was stirred for 6 h at 40 °C. The conversion yield to **4b** was 92% by HPLC analysis. The product **4b** was almost quantitatively extracted with EtOAc (3 × 60 mL). After removal of the organic solvent in vacuo, the residue was taken up into DMSO (5 mL) and then a solution of CALB (35 mg; 1 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (30 mL) was added and the resulting solution was stirred for 2.5 h at 40 °C. The conversion yield to **5b** was 98% by HPLC analysis. After acidification of the incubation mixture with 1 M HCl to pH around 2.5, **5b** was extracted quantitatively with EtOAc (2 × 35 mL). The isolation of **5b** was performed by passing through a XAD-4 column (2.5 g, 1.0 × 12 cm^2) as described above. After removal of the organic solvent in vacuo, the residue was taken up into water (8 mL) and then the solution was loaded onto the column, which was then washed with water (20 mL)and 30% CH_3CN (20 mL) followed by elution with 50% (v/v) aqueous CH_3CN. The fractions containing **5b** were pooled and concentrated in vacuo to give **5b** in recovery yield of 98%: ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.07 (dd, 1H, J = 1.7 and 8.1 Hz), 7.36−7.32 (m, 3H), 7.24−7.19 (m, 3H), 7.09 (dt, 1H, J = 1.7 and 7.3 Hz), 6.75 (t, 1H, J = 8.1 Hz), 5.77 (d, 1H, J = 7.6 Hz), 3.99 (d, 1H, J = 9.5 Hz), 3.64−3.51 (m, 3H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 172.2, 168.1, 149.8, 141.8, 135.9, 133.1, 130.5, 125.0, 123.7, 118.2, 114.9, 112.0, 95.7, 77.6, 77.2, 73.7, 73.0; m/z (SIMS, positive) $[M + H]^+$ 390.1139 (error −0.6 mmu). $C_{19}H_{20}NO_8$ requires m/z 390.1187.

1-β-*O*-(4-Phenyl)benzoyl-D-glucopyranuronate (5c). To a solution of **3c** (41.2 mg, 80.0 μmol) in DMSO (8 mL) was added a solution of LAS (400 mg; 10 mg/mL of incubation mixture) and CSR (400 mg, 10 mg/mL of incubation mixture) in 25 mM sodium

citrate buffer (pH 5.0) (32 mL) then the solution was stirred for 9 h at 40 °C. The conversion yield to **4c** was 93% by HPLC analysis. The product **4c** was quantitatively extracted with EtOAc (2 × 50 mL). After removal of the organic solvent in vacuo, the residue was taken up into DMSO (15 mL) and then a solution of CALB (76 mg; 1 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (61 mL) was added and the resulting solution was stirred for 9 h at 40 °C. The conversion yield to **5c** was 93% by HPLC analysis. After acidification of the incubation mixture with 1 M HCl to pH around 2.5, the solution was loaded onto a XAD-4 column (5 g, 1.6 × 10 cm^2) as described above. The column was washed with water (100 mL) and 30% CH_3CN (50 mL) followed by elution with 50% (v/v) aqueous CH_3CN. The fractions containing **5c** were pooled and concentrated in vacuo to give **5c** in recovery yield of 98%: ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.17 (d, 2H, J = 8.8 Hz), 7.76 (d, 2H, J = 8.8 Hz), 7.68 (d, 2H, J = 8.8 Hz), 7.47 (d, 2H, J = 7.4 Hz), 7.41−7.37 (m, 1H), 5.77 (d, 1H, J = 7.8 Hz), 3.98 (d, 1H, J = 9.5 Hz), 3.63−3.51 (m, 3H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 172.4, 166.4, 147.7, 141.1, 131.6, 130.1, 129.4, 129.3, 128.3, 128.1, 96.1, 77.6, 77.3, 73.8, 73.0; *m*/*z* (SIMS, positive) $[M + H]^+$ 375.1093 (error 1.4 mmu). $C_{19}H_{19}O_8$ requires *m*/*z* 375.1079.

1-β-*O*-(4-Phenyl)phenylacetyl-D-glucopyranuronate (5d). To a solution of **3d** (45.4 mg, 85.9 μmol) in DMSO (12 mL) was added a solution of CSR (570 mg, 10 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (45 mL) then the solution was stirred for 7.5 h at 50 °C. The conversion yield to **4d** was 86% and the ratio of liberated **1d** was 10% by HPLC analysis. The product **4d** was quantitatively extracted with EtOAc (2 × 50 mL). After removal of the organic solvent in vacuo, the residue was taken up into DMSO (15 mL) and then a solution of CALB (59 mg; 0.8 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (59 mL) was added and the resulting solution was stirred for 2 h at 40 °C to provide quantitatively **5d** by HPLC analysis. After acidification of the incubation mixture with 1 M HCl to pH around 2.5, the solution was loaded onto a XAD-4 column (5 g, 1.6 × 10 cm^2) as described above. The column was washed with water (100 mL) and 20% CH_3CN (100 mL) followed by elution with 45% (v/v) aqueous CH_3CN. The fractions containing **5d** were pooled and concentrated in vacuo to provide quantitatively **5d**: ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.59−7.57 (m, 2H), 7.56−7.54 (m, 2H), 7.40 (t, 2H, J = 7.3 Hz), 7.37 (d, 2H, J = 8.3 Hz), 7.30 (tt, 1H, J = 1.2 and 7.3 Hz), 5.55 (d, 1H, J = 7.8 Hz), 3.91 (d, 1H, J = 9.8 Hz), 3.79 (s, 2H), 3.55 (t, 1H, J = 9.0 Hz), 3.47 (t, 1H, J = 9.0 Hz), 3.43 (t, 1H, J = 9.0 Hz); ^{13}C NMR (100 MHz, MeOH-d_4) δ 172.13, 172.06, 142.1, 141.3, 134.0, 131.1, 129.8, 128.3, 128.0, 127.9, 95.8, 77.5, 77.2, 73.6, 72.9, 41.0; *m*/*z* (SIMS, positive) $[M + H]^+$ 389.1262 (error 2.7 mmu). $C_{20}H_{21}O_8$ requires *m*/*z* 389.1235.

1-β-*O*-(2-(4-Biphenylyl))propionyl-D-glucopyranuronate (5e) (Mixture of Diastereoisomers). To a solution of **3e** (27.1 mg, 50.0 μmol) in DMSO (20 mL) was added a solution of CSR (1.00 g, 10 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (80 mL) then the solution was stirred for 6.0 h at 50 °C to provide **4e** (96%) by HPLC analysis. The product **4e** was quantitatively extracted with EtOAc (2 × 100 mL). After removal of the organic solvent in vacuo, the residue was taken up into DMSO (10 mL) and then a solution of CAL-B (19 mg; 0.4 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (38 mL) was added and the resulting solution was stirred for 45 min at 40 °C to provide **5e** (99%) by HPLC analysis. After acidification of the incubation mixture with 1 M HCl to pH around 2.5, **5e** was quantitatively extracted with EtOAc (2 × 50 mL). After removal of the organic solvent in vacuo, the residue was loaded onto a semipreparative column of Symmetry C_{18} (7 μm, 19 × 150 mm^2, Waters) to isolate the diastereoisomers (2*R*)-**5e** and (2*S*)-**5e**, with the HPLC mobile phase being 30% (v/v) CH_3CN containing 10 mM ammonium acetate (pH 4.5) and 1 mM tetra-*n*-butylammonium bromide at a flow rate of 3 mL/min with detection at 260 nm. The retention time of (2*S*)-**5e** was shorter than that of (2*R*)-**5e** and both the compounds were fully separated to provide sufficiently pure compounds (2*R*)-**5e** and (2*S*)-**5e**.

1-β-*O*-{(2*R*)-2-(4-Biphenylyl)}propionyl-D-glucopyranuronate ((2*R*)-5e). To a solution of (2*R*)-**3e** (40.0 mg, 73.7 μmol) in DMSO (15 mL) was added a solution of CSR (740 mg; 10 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (59 mL) then the solution was stirred for 30 h at 50 °C. The conversion yield to (2*R*)-**4e** was 89% by HPLC analysis. The product (2*R*)-**4e** was quantitatively extracted with EtOAc (2 × 75 mL). After removal of the organic solvent in vacuo, the residue was taken up into DMSO (12.5 mL) and then a solution of CALB (26 mg; 0.4 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (53 mL) was added and the resulting solution was stirred for 1 h at 40 °C to provide quantitatively the product (2*R*)-**5e** by HPLC analysis. After acidification of the incubation mixture with 1 M HCl to pH around 2.5, (2*R*)-**5e** was quantitatively extracted with EtOAc (2 × 50 mL). ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.54−7.49 (m, 4H), 7.37−7.33 (m, 4H), 7.27−7.23 (m, 1H), 5.46 (d, 1H, J = 7.8 Hz), 3.85 (d, 1H, J = 9.5 Hz), 3.83 (d, 1H, J = 7.3 Hz), 3.48 (t, 1H, J = 8.8 Hz), 3.36 (t, 1H, J = 9.0 Hz), 3.30 (dd, 1H, J = 8.3 and 9.0 Hz), 1.48 (d, 3H, J = 7.3 Hz); ^{13}C NMR (100 MHz, MeOH-d_4) δ 174.8, 172.1, 142.0, 141.4, 140.5, 129.8, 129.2, 128.3, 128.2, 127.9, 95.8, 77.6, 77.2, 73.6, 72.9, 46.2, 19.1; *m*/*z* (SIMS, positive) $[M + H]^+$ 403.1394 (error 0.2 mmu). $C_{21}H_{23}O_8$ requires *m*/*z* 403.1392.

1-β-*O*-{(2*S*)-2-(4-Biphenylyl)}propionyl-D-glucopyranuronate ((2*S*)-5e). To a solution of (2*S*)-**3e** (40.0 mg, 73.7 μmol) in DMSO (15 mL) was added a solution of CSR (740 mg; 10 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (59 mL) then the solution was stirred for 3 h at 50 °C. The conversion yield to (2*S*)-**4e** was 98% by HPLC analysis. The product (2*R*)-**4e** was quantitatively extracted with EtOAc (2 × 75 mL). After removal of the organic solvent in vacuo, the residue was taken up into DMSO (7.2 mL) and then a solution of CALB (14.4 mg; 0.4 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (29 mL) was added and the resulting solution was stirred for 3.5 h at 40 °C to provide quantitatively the product (2*S*)-**5e** by HPLC analysis. After acidification of the incubation mixture with 1 M HCl to pH around 2.5, (2*S*)-**5e** was quantitatively extracted with EtOAc (2 × 50 mL). ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.53−7.47 (m, 4H), 7.35−7.29 (m, 4H), 7.25−7.21 (m, 1H), 5.45 (d, 1H, J = 7.8 Hz), 3.82 (d, 1H, J = 7.1 Hz), 3.80 (d, 1H, J = 8.8 Hz), 3.43 (t, 1H, J = 8.8 Hz), 3.37 (t, 1H, J = 8.8 Hz), 3.30 (t, 1H, J = 8.3 Hz), 1.45 (d, 3H, J = 7.1 Hz); ^{13}C NMR (100 MHz, MeOH-d_4) δ 174.7, 172.2, 142.1, 141.4, 140.6, 129.8, 129.2, 128.3, 128.2, 127.9, 95.8, 77.5, 77.3, 73.6, 72.9, 46.2, 19.2; *m*/*z* (SIMS, positive) $[M + H]^+$ 403.1367 (error −2.5 mmu). $C_{21}H_{23}O_8$ requires *m*/*z* 403.1392.

1-β-*O*-{2-(4-Biphenylyl)-2-methyl}propionyl-D-glucopyranuronate (5f). To a solution of **3f** (40.0 mg, 71.9 μmol) in DMSO (14 mL) was added a solution of CSR (720 mg; 10 mg/mL of incubation mixture) and LAS (720 mg; 10 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (58 mL) then the solution was stirred for 3 h at 40 °C. The conversion yield to **4f** was 95% by HPLC analysis. The product **4f** was quantitatively extracted with EtOAc (2 × 70 mL). After removal of the organic solvent in vacuo, the residue was taken up into DMSO (7 mL) and then a solution of CALB (29 mg; 0.8 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (29 mL) was added and the resulting solution was stirred for 3 h at 40 °C to provide quantitatively **5f** by HPLC analysis. After acidification of the incubation mixture with 1 M HCl to pH around 2.5, (2*S*)-**5e** was quantitatively extracted with EtOAc (2 × 35 mL). ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.60−7.54 (m, 4H), 7.46−7.38 (m, 4H), 7.32−7.28 (m, 1H), 5.54 (d, 1H, J = 8.1 Hz), 3.90 (d, 1H, J = 9.8 Hz), 3.52 (t, 1H, J = 9.0 Hz), 3.43 (t, 1H, J = 9.0 Hz), 3.34 (t, 1H, J = 8.1 Hz), 1.64 (s, 3H), 1.61 (s, 3H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 176.9, 172.1, 144.7, 142.0, 140.9, 129.8, 128.3, 127.91, 127.86, 127.5, 96.0, 77.7, 77.3, 73.5, 72.9, 47.8, 27.2, 26.9; *m*/*z*

(SIMS, positive) $[M + H]^+$ 417.1522 (error −2.5 mmu). $C_{22}H_{25}O_8$ requires *m*/*z* 417.1547.

2,3,4,6-Tetra-*O*-acetyl-D-1-β-*O*-(6-methoxy-α-methyl-2-naphthaleneacetyl)- glucopyranoside (6). To a solution of (*S*)-naproxen (247 mg, 1.07 mmol) in MeOH (2 mL) was added an aqueous 3 M Cs_2CO_3 solution (178 μL, 1.07 mmol) then the solution was stirred for 5 min. After removal of the solvent in vacuo, the residue was dissolved in DMSO (2.5 mL) and then 2,3,4,6-tetra-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranose (352 mg, 0.8 equiv) was added then the resulting solution was stirred for 1 h at room temperature and HPLC assay showed the reaction was complete. The reaction mixture was diluted with EtOAc (50 mL) and the organic layer was washed with water (30 mL) and then aqueous 2% Na_2CO_3 solution (2 × 30 mL). After being dried over Na_2SO_4, the organic solvent was removed in vacuo to give a crude product, which was purified by recrystallization. Yield 51%; mp 181−182 °C (white needles from EtOH). Found: C, 60.05; H, 5.80; $C_{28}H_{32}O_{12}$ requires C, 60.03; H, 5.76. *m*/*z* (EI) 560 (M^+), 331, 272, 185 (base peak); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (d, 2H, *J* = 8.8 Hz), 7.70 (d, 1H, *J* = 1.7 Hz), 7.36 (dd, 1H, *J* = 1.7 and 8.8 Hz), 7.30 (d, 1H, *J* = 2.4 Hz), 7.16 (dd, 1H, *J* = 2.4 and 8.8 Hz), 6.00 (d, 1H, *J* = 8.3 Hz), 5.43 (t, 1H, *J* = 9.5 Hz), 4.94 (t, 1H, *J* = 9.5 Hz), 4.93 (dd, 1H, *J* = 8.3 and 9.8 Hz), 4.21 (ddd, 1H, *J* = 2.2, 4.6, and 12.2 Hz), 4.15 (dd, 1H, *J* = 4.6 and 12.2 Hz), 4.00−3.94 (m, 1H), 3.97 (q, 1H, *J* = 7.1 Hz), 3.86 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.87 (s, 3H), 1.47 (d, 3H, *J* = 7.1 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 169.9, 169.4, 169.1, 168.9, 157.3, 134.4, 133.4, 129.1, 128.3, 127.0, 126.2, 125.9, 118.8, 105.7, 91.2, 71.6, 71.4, 70.0, 67.6, 61.3, 55.2, 44.2, 20.4, 20.3, 20.2, 20.1, 18.1.

1-β-*O*-(6-Methoxy-α-methyl-2-naphthaleneacetyl)-D-glucopyranoside (7). To a solution of **6** (20.2 mg, 36.0 μmol) in DMF (9 mL) was added a solution of CSR (360 mg; 10 mg/mL of incubation mixture) in 25 mM sodium citrate buffer (pH 5.0) (27 mL) then the solution was stirred for 40 h at 50 °C. The conversion yield to **7** was 64% by HPLC analysis. Other products were the corresponding mono-*O*-acetates (total 34%). After adding NaCl to saturate the reaction mixture, compound **6** was extracted with EtOAc (3 × 40 mL) in recovery yield of 96%. After removal of the organic solvent in vacuo, the residue was loaded onto a XAD-4 column (1 g, 1.0 × 8 cm^2) as described above. The column was washed with water (20 mL) and then eluted with 30% (v/v) aqueous CH_3CN. The fractions containing **7** were pooled and concentrated in vacuo to provide quantitatively **7**: ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.64−7.59 (m, 3H), 7.31 (dd, 1H, *J* = 2.0 and 8.5 Hz), 7.10 (d, 1H, *J* = 2.4 Hz), 7.01 (dd, 1H, *J* = 2.4 and 9.0 Hz), 5.41 (d, 1H, *J* = 8.1 Hz), 3.85 (1H, q, *J* = 7.1 Hz), 3.80 (s, 3H), 3.64 (dd, 1H, *J* = 2.0 and 12.2 Hz), 3.51 (dd, 1H, *J* = 4.4 and 12.2 Hz), 3.33−3.20 (m, 4H), 1.47 (d, 3H, *J* = 7.1 Hz); ^{13}C NMR (100 MHz, MeOH-d_4) δ 175.1, 159.2, 136.6, 135.3, 130.4, 130.2, 128.3, 127.3, 127.2, 119.9, 106.6, 96.1, 78.9, 78.1, 74.0, 70.9, 62.2, 55.7, 46.5, 19.2; *m*/*z* (SIMS, positive) $[M + H]^+$ 393.1543 (error −0.4 mmu). $C_{20}H_{25}O_8$ requires *m*/*z* 393.1547.

Assay of Purity of 1-β-*O*-Acyl Glucuronides 5a−f and 1-β-*O*-Acyl Glucopyranoside 7. Compounds **5a**−**f** and **7** were treated with 0.1 M NaOH at 37 °C for 15 min to complete the hydrolysis of the ester linkage and the amounts of the corresponding carboxylic acids **1a**−**f** and NP formed were determined by HPLC. In addition, the same amounts of the glucuronides **5a**−**f** and **7** were incubated with β-glucuronidase (from bovine liver) and β-glucosidase (from almond), respectively, in 100 mM AcOH/NaOH buffer (pH 5.0) at 37 °C for 30 min and the amounts of liberated carboxylic acids were also determined as described previously.[20] Purities of the glucuronides **5a**−**f** and **7** as 1-β-*O*-acyl glucuronides and 1-β-*O*-acyl glucopyranoside, respectively, were calculated from the amounts of the corresponding carboxylic acids formed under both sets of conditions. The calculated purities (%) of these compounds were 99.8 ± 1.1 for **5a**, 98.3 ± 0.2 for **5b**, 99.9 ± 0.9 for **5c**, 96.6 ± 0.5 for **5d**, 94.2 ± 1.3 for (2*R*)-**5e**, 96.1 ± 1.8 for (2*S*)-**5e**, 98.2 ± 0.4 for **5f**, and 97.4 ± 1.2 for **7**.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **3a**−**f**, **4d**, **4e**, **5a**−**f**, **6**, and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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