Chemo-Enzymatic Synthesis, Structural and Stereochemical Characterization, and Intrinsic Degradation Kinetics of Diastereomers of 1-β-O-Acyl Glucuronides Derived from Racemic 2-{4-[(2-Methylprop-2-en-1-yl)amino]phenyl}propanoic Acid

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ABSTRACT: Alminoprofen, (RS)-2-{4-[(2-methylprop-2-en-1-yl)amino]phenyl}propanoic acid (ALP) 1, is a racemic drug categorized as a 2-arylpropanoic acid-class nonsteroidal anti-inflammatory drug. Pharmacokinetic studies of 1 in patients have revealed that the corresponding acyl glucuronide 5 is a major urinary metabolite, but little is known about the structure and stereochemistry of 5. The present work describes the synthesis of a diastereomeric mixture of 1-β-O-acetyl glucuronid (2RS)-5 from 1 and methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate 2 using our chemo-enzymatic method that has complete specificity for the β-configuration. The structure of (2RS)-5 was characterized by 1H and 13C NMR spectroscopy and high-resolution mass spectrometry as well as by complete hydrolysis by β-glucuronidase. The absolute stereochemistry of (2RS)-5 was determined by comparison with (2R)-5 synthesized alternatively from (2R)-1 and 2. Compound (2R)-1 was prepared in two steps starting from chiral (R)-2-{4-nitrophenyl}propanoic acid (2R)-6. Chiral resolution of (2RS)-1 was achieved using a chiral high-performance liquid chromatography column, and its stereochemistry was determined by comparison with (2R)-1. The intrinsic degradation rate constant of (2R)-5 was 0.405 ± 0.002 h⁻¹, which is approximately twice that of (2S)-5 (the k value was 0.226 ± 0.002 h⁻¹) under physiological conditions (pH 7.40, 37 °C).

INTRODUCTION

Glucuronidation of carboxylic acid drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) is a major conjugation reaction catalyzed by multiple UDP-glucuronosyltransferases (UGTs).1−5 The resultant 1-β-O-acetyl glucuronides (AGs) are of great importance not only in drug metabolism and excretion but also because of their toxicological consequences. AGs are chemically reactive, electrophilic metabolites that undergo degradation reactions (hydrolysis and intramolecular migration of the 1-β-O-acyl linkage)6,7 as well as covalent binding to cellular macromolecules and thus may be implicated in adverse drug reactions (ADRs) such as drug-induced liver injury8−15 and intestinal injury.16−18 Some NSAIDs have been withdrawn from the market because of ADRs, including liver and renal toxicities and anaphylaxis.8,19−21 Since a pioneering study on the excellent correlation between the extents of covalent binding of AGs to albumin and their degradation rate constants,21 there have been several thorough studies on the structure−rate constant relationships of AGs as well as prediction and evaluation studies of the toxicological risks posed by AGs.23−25 The mechanism of the covalent binding of AGs to and the binding sites on albumin have been reported,30−33 and the modification of albumin has been demonstrated to proceed via both the direct acylation34 and the Schiff base-mediated glycation35 mechanisms. Recently, AG-mediated cytotoxic mechanisms involving oxidative stress have been reported.36,37 In addition, AGs play roles in drug−drug interactions via membrane transporters17,38,39 as well as through CYP2C8 inhibition.40−42 Various factors that affect the safety of AGs, including the rates of formation, elimination, and aglucuronidation; their intrinsic reactivity; and the potential proteins being targeted,43 as well as the molecular mechanisms underlying AG-induced toxicities,44 all remain to be elucidated fully.

ALP, (RS)-2-{4-[(2-methylallyl)amino]phenyl}propanoic acid 1 (Figure 1), is a racemic drug categorized as a 2-arylpropanoic acid-class NSAID and has the distinctive molecular characteristic of possessing a 4-monoalkenylnalamin group in a structure that closely resembles that of ibuprofen. The drug 1 has been used in several countries such as France,
Luxembourg, and Japan; in Japan, 1 was approved in 2014 as a switch OTC commonly used to treat conditions such as rheumatism, arthralgia, and lumbago. The drug 1 is reported to exhibit a more potent analgesic effect than ibuprofen 5° and to exert a dual anti-inflammatory action by inhibiting both cyclooxygenase-2 and phospholipase A₂ 5° thereby possibly causing interesting analgesic, antipyretic, and anti-inflammatory actions 1° different from those of commonly used NSAIDs. Pharmacokinetic studies in patients after p.o. administration of 1 have revealed rapid absorption, a short time to maximum plasma level, a short biological half-life, and excretion of the corresponding ALP acyl glucuronide 5 as a major urinary metabolite. 5° The acyl glucuronide 5 is most likely the diastereomers (2RS)-5 derived from 1, but little is known about the structure and stereochemistry of 5. Furthermore, the membrane transporter(s) for 1 and (2RS)-5, the UGT isozyme(s) involved in glucuronidation, and the possible stereoselectivities of these biological systems toward 1 (enantioselective) and (2RS)-5 (diastereoselective) all remain to be elucidated. However, 1 has been reported to show high permeability comparable to that of ibuprofen in Caco-2 cell studies. 52,53 The synthesis and stereochromatographic determination of (2RS)-5, as well as the chiral resolution of 1, are thus of great importance in pharmacokinetic and toxicological studies of 1.

Herein, we report the synthesis of (2RS)-5 from 1 using our chemo-enzymatic method, 54 the stereochemistry of both (2RS)-5 and 1, and the intrinsic degradation rate constants of (2R)-5 and (2S)-5 under physiological conditions (at pH 7.40 and 37 °C).

### RESULTS AND DISCUSSION

**Synthesis of ALP AGs (2RS)-5**. Two diastereomeric mixtures of (2RS)-5 were synthesized using our two-step chemo-enzymatic method, 54 as shown in Scheme 1. In the first step, condensation of the cesium salt of 1 with methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate 2 afforded the corresponding methyl acetyl derivatives (2RS)-3 as an approximately 1:1 mixture of the diastereomers in a modest 53% yield, without production of the corresponding α-anomers (based on 1H NMR data). The anomic β-configurations of the diastereomers (2RS)-3 were confirmed by 1H NMR. The J values of both anomic protons were 8.2 Hz, 5° and the chemical shifts were δ 5.85 and 5.78 for (2S)-3 and (2R)-3, respectively. We were unable to separate the diastereomers (2RS)-3 using conventional methods, and thus we examined enzymatic removal of the sugar-protecting groups as the next step toward (2RS)-3. It is important to remove the protecting groups without affecting the 1β-O-acyl linkage. As shown in Table 1, (2RS)-3 was not detected following treatment with lipase AS Amano (LAS) at the optimal temperature of 40 °C for 1.33 h of incubation, and the products and yields (%) were the partially O-deacetylated intermediates (48%), the fully O-deacetylated derivatives (2RS)-4 (43%), and the liberated parent carboxylic acid 1 (9%). The chemo-selectivity of LAS toward the O-acetyl groups was not improved by using cosolvents (1,4-dioxane, N,N-dimethylformamide, and MeOH) other than dimethyl sulfoxide (DMSO) (data not shown). In contrast, the use of carboxylesterase from Streptomyces rochei (CSR) at the optimal temperature of 50 °C for 1.33 h resulted in 16% unreacted (2RS)-3; the products were fully O-deacetylated derivatives (2RS)-4 (64%) and intermediates (20%). No liberation of the parent carboxylic acid 1 was detected, indicating that CSR exhibited much higher chemo-selectivity toward the O-acetyl groups than did LAS. Furthermore, CSR exhibited higher hydrolytic activity toward the partially O-deacetylated intermediates than toward (2RS)-3, whereas LAS exhibited the opposite preference for the substrates. Therefore, concurrent use of LAS and CSR (1:10, w/w) synergistically accelerated O-deacetylation to afford (2RS)-4 in 94% yield in 7 h and a small amount of 1 (%). To diminish the liberation of 1 catalyzed by LAS, the initial reaction temperature of 40 °C was maintained for 0.25 h and then was raised to 50 °C to deactivate the hydrolytic activity of LAS. As was reported previously, 54,55 DMSO was proved to be a good choice of cosolvent for the enzymatic deacetylation of the O-acetyl groups of (2RS)-3.

As the final step, enzymatic chemo-se lective hydrolysis of the methyl ester of (2RS)-4 was examined using pig liver esterase (PLE), lipase type-B from Candida antarctica (CALB), and Chirazymes L-5 CA and L-2 CB, using DMSO as a cosolvent. As shown in Table 2, Chirazyme L-5 CA showed the lowest hydrolytic activity toward (2RS)-4. PLE showed a high hydrolytic activity comparable with those of CALB and Chirazyme L-2 CB but lower chemo-selectivity; the parent carboxylic acid 1 (3%) was concomitantly liberated. DMSO was the best cosolvent for these enzymatic reactions: in the PLE-catalyzed reaction, the amount of 1 increased to 14% when MeOH was used as the cosolvent while keeping all other conditions unchanged and the hydrolytic activity of PLE was almost inhibited when tert-ButOH was used as the cosolvent (data not shown).

Both CALB and Chirazyme L-2 CB yielded (2RS)-5 efficiently and quantitatively, with both the enzymes showing extremely high chemo-selectivity toward the methyl ester group.
Table 1. Enzyme-Catalyzed Hydrolytic Deprotection of the O-Acetyl Groups of (2RS)-3

| enzyme       | concn (mM) | temp (°C) | time (h) | product yields (%) |
|--------------|------------|-----------|----------|--------------------|
| LAS          | 1.1        | 40        | 1.33     | (2RS)-4 (43), (2RS)-3 (nd<sup>d</sup>), 1 (9), others<sup>e</sup> (48) |
| CSR          | 1.1        | 50        | 1.33     | (2RS)-4 (64), (2RS)-3 (16), 1 (nd), others<sup>e</sup> (20) |
| LAS + CSR    | 1.1        | 40–50<sup>b</sup> | 7.0     | (2RS)-4 (94), (2RS)-3 (nd<sup>d</sup>), 1 (3), others<sup>e</sup> (3) |

<sup>a</sup>Initial concentration of (2RS)-3 in 20 mM sodium citrate buffer (pH 5.0) containing 15% (v/v) DMSO. <sup>b</sup>Incubation time. <sup>c</sup>Yields based on high-performance liquid chromatography (HPLC) analysis. <sup>d</sup>Enzyme: 10 mg/mL. <sup>e</sup>nd = not detected. Others: partially O-deacetylated intermediates.

Table 2. Enzyme-Catalyzed Hydrolytic Deprotection of the Methyl Ester of (2RS)-4

| enzyme<sup>a</sup> | time (h)<sup>b</sup> | product yields (%)<sup>c</sup> |
|---------------------|----------------------|-------------------------------|
| PLE                 | 1.0                  | (2RS)-5 (97), 1 (3), (2RS)-4 (nd<sup>d</sup>) |
| CALB                | 0.50                 | (2RS)-5 (99), 1 (nd<sup>d</sup>), (2RS)-4 (nd<sup>d</sup>) |
| Chirazyme L-5 CA    | 1.5                  | (2RS)-5 (17), 1 (nd<sup>d</sup>), (2RS)-4 (83) |
|                     | 3.0                  | (2RS)-5 (29), 1 (nd<sup>d</sup>), (2RS)-4 (71) |
| Chirazyme L-2 CB    | 1.0                  | (2RS)-5 (99), 1 (nd<sup>d</sup>), (2RS)-4 (nd<sup>d</sup>) |

<sup>a</sup>Enzyme concentration was 1.0 mg/mL. Initial concentration of (2RS)-4 was 1.0 mM in 20 mM sodium citrate buffer (pH 5.0) containing 10% (v/v) DMSO. <sup>b</sup>Incubation time at 40 °C. <sup>c</sup>Yields based on HPLC analysis. <sup>d</sup>nd = not detected.

Figure 2. Reversed-phase HPLC chromatograms of the synthesized (2RS)-5 (peaks a and b in A), ALP 1 (peak c in B) liberated from (2RS)-5 after treatment with β-glucuronidase (20 IU) for 20 min at pH 5.0 and 37 °C, and the synthesized (2RS)-5 (peak d in C) whose retention time was consistent with that of peak b in A.

of (2RS)-4 without affecting the 1-β-O-acyl linkage. Chirazyme L-2 CB, whose specific activity is approximately one-half that of CALB, might be more a convenient enzyme than CALB: a CALB-derived impurity coelutes with the product (2RS)-5 in the Amberlite XAD-4 column chromatography purification step, although this impurity can be removed prior to use of CALB as the enzymatic catalyst by adsorption onto XAD-4 resin (cf. Experimental Section).

The purity of (2RS)-5 as AG was assayed by <sup>1</sup>H NMR and HPLC analyses. The anomic β-configurations of the diastereomers (2RS)-5 were confirmed by <sup>1</sup>H NMR: the J values of both anomic protons of the (2S)- and (2R)-5 diastereomers were 8.2 Hz,<sup>56</sup> representative values for their β-configurations. HPLC analyses using an L-column2 ODS revealed that two peaks, corresponding to diastereomeric (2RS)-5 (peaks a and b in Figure 2A), were quantitatively hydrolyzed to the parent ALP 1 following treatment with β-glucuronidase at 37 °C for 20 min (peak c in Figure 2B). The purity of (2RS)-5 as AG was calculated to be over 99% (data not shown).

**Synthesis of (2R)-1 and Its AGs (2R)-5.** We synthesized (2R)-5 from (2R)-1 and 2 to determine the absolute configuration at the chiral center α to the 1-β-O-acyl linkage of (2RS)-5. Compound (2R)-1 was prepared in two steps starting from (RS)-2-(4-nitrophenyl)propanoic acid (2RS)-6, as shown in Scheme 2. Chiral resolution of (2RS)-6 was achieved using a diastereomeric salt method.<sup>57</sup> The racemate (2RS)-6 in ethyl acetate solution was treated with (S)-(−)-1-phenylethylamine to precipitate the (2R)-6-derived diastereomeric salt; after four recrystallizations, (2R)-6 with 95.3% ee was obtained. The R configuration was confirmed by optical rotation measurements. Catalytic hydrogenation of (2R)-6 to the corresponding amine (2R)-7 followed by N-alkenylation<sup>58</sup> using excess amounts of 3-chloro-2-methyl-1-propane and potassium fluoride on Celite afforded (2R)-1 with 95.4% ee. The chiral (2R)-5 was synthesized from (2R)-1 and 2 according to the methods shown in Scheme 1. Figure 2C shows a typical reversed-phase HPLC chromatogram of (2R)-5 (peak d) whose retention time was consistent with that of peak b in A.

**Chiral Resolution of ALP 1.** Methods for the chiral resolution of several NSAIDs have been reviewed.<sup>59</sup> To our knowledge, though, there is no published stereospecific assays for 1 other than one paper in a Japanese journal<sup>60</sup> that reported the pharmacological activities of the D- and L-isomers of 1. However, that paper did not describe the synthesis and structural characteristics of these isomers. We, therefore, attempted the chiral resolution of 1 and compared the...
retention times of the enantiomers with that of the synthesized (2R)-1 (Scheme 2). The enantiomers of 1 were well-separated from each other using a semipreparative CHIRALCEL OZ-H chiral HPLC column, and the comparison of the retention times was conducted using a CHIRALPAK IG analytical column. As shown in Figure 3, the retention time of (2S)-5 (peak c in Figure 3B) was consistent with that of the peak a in Figure 3A.

Figure 3. Chiral HPLC chromatograms of 1 (peaks a and b in A) and (2R)-1 (peak c in B). The retention times of the major peak c corresponding to (2R)-1 and the minor peak d were consistent with those of peaks a and b in A, respectively.

**Kinetic Studies on the Degradation Reaction of (2RS)-5.** As was reported previously, the degradation rate constant (k) values of AGs in sodium phosphate buffer (100 mM, pH 7.4) at 37 °C have been demonstrated to consist of rate constants of intramolecular acyl migration and hydrolysis of their 1-β-O-acyl linkages. Because both the reactions are electrophilic ones, the more the k value increases, the higher the electrophilic reactivity of the corresponding AG becomes. Therefore, to estimate the electrophilic reactivity of (2RS)-5, their k values were determined by incubating (2RS)-5 in sodium phosphate buffer (100 mM, pH 7.4) at 37 °C and analyzing changes with time by HPLC. As shown in Figure 4, the decreases in the concentrations of both (2R)-5 and (2S)-5 obeyed pseudo-first-order reaction kinetics in the time range spanning 3 or 4 half-lives. The major products were the corresponding intramolecular acyl-migrated isomers; the released ALP 1 was only 9% after 7 h (data not shown). The k value of (2R)-5 was 0.405 ± 0.002 h⁻¹ (the half-life was 1.71 h), which is approximately twice that of (2S)-5 (the k value and the half-life were 0.226 ± 0.002 h⁻¹ and 3.07 h, respectively). This result is in accordance with earlier reports that the (R)/(S) ratio of the k values for several 2-arylpropanoic acid-derived AGs are around 2. These k values for (2R)- and (2S)-5 are smaller than those of AGs derived from (2R)- and (2S)-phenylpropanoic acids, whose values were previously determined to be 0.604 ± 0.003 and 0.324 ± 0.008 h⁻¹, respectively, under the same conditions and methods. The lower reactivity of (2RS)-5 might be due partly to the electronic effect of the electron-donating (2-methylallyl)amino substituent on (2R)- and (2S)-5; the Hammett substituent constant σ_p of this substituent is not found in the literature but estimated to be a negative value, judging from the σ_p values of EtNH– and CH3(CH2)3NH– substituents being −0.61 and −0.51, respectively. Although there is no report for the Hammett reaction constant ρ value for AGs derived from m- and p-substituted (2RS)-phenylpropanoic acid derivatives and hence the theoretical k values of (2R)- and (2S)-5 cannot be calculated, the theoretical k values can be predicted to be lower than those of AGs derived from (2R)- and (2S)-phenylpropanoic acids based on the estimated ρ value being positive. Therefore, we next tried to calculate the k value of (2R)-5, based on the previously reported linear relationship between log k of AGs and δ_COOH values of the parent carboxylic acids, a good positive correlation with a slope of 0.79. From the δ_COOH values (12.04 and 12.33 ppm for ALP 1 and 2-phenylpropanoic acid, respectively) and a log k value of −0.219 for AG derived from (2R)-phenylpropanoic acid,62 the k value for (2R)-5 was calculated to be 0.356, the value being close to the measured value.

**CONCLUSIONS**

ALP-derived acyl glucuronides (2RS)-5 were successfully prepared using our chemo-enzymatic method, and their chemical structures were fully characterized; the complete β-configuration of (2RS)-5 was confirmed by 1H NMR as well as by exhaustive hydrolysis by β-glucuronidase. The intrinsic degradation rate constant of (2R)-5 was approximately twice that of (2S)-5 under physiological conditions (pH 7.40 and 37 °C).

Scheme 2. Synthesis of (2R)-ALP (2R)-1

4935

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The absolute stereochemistry of both ALP 1 and (2RS)-5 was determined by comparison with chemically synthesized (2R)-1 and (2S)-5, respectively. We have prepared the enantiomers of ALP 1 and developed the HPLC analytical conditions for these compounds. Further studies are in progress on the UGT isozyme(s) responsible for glucuronidation of I, the chiral inversion from (2R)-1 to (2S)-1, and the membrane transporter for both 1 and (2RS)-5 in Caco-2 cells.

**EXPERIMENTAL SECTION**

Materials and General Experimental Methods. Methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-a-D-glucopyranuronate 2 (Sigma), (S,)-1,1'-phenylenediamine (99%; Acros), 3-chloro-2-methyl-1-propene (TCI), potassium chloride (Wako), (Sigma), (2R,3S)-6-(2-(4-nitrophenyl)propanoic acid (2R)-6 (Aldrich) was purified by column chromatography (silica gel; CHCl 3/CH 3OH/10% Pd/C/AcOH = 90/10/0.1) followed by charcoal treatment to remove impurities and colored components. Optical resolution of (2R)-6 was performed by a diastereomeric salt method using (S,)-(-)-1-phenylethylamine (0.34 mL) to afford a white precipitate. The mixture was heated to reflux, and then the clear solution was allowed to stand at room temperature. The precipitated white needles were collected and recrystallized from EtOAc (50 mL) to afford (2R)-6-derived salt (552 mg). The optical purity (% ee) was determined using a CHIRALPAK IG analytical column equilibrated with 55% (v/v) MeOH containing 56 mM formic acid (pH 2.1) at a flow rate of 0.8 mL/min and monitored at 254 nm. The retention times of (2R)-1 and (2S)-1 were 15.9 and 18.8 min, respectively. The specific optical rotation [α]D 26 values of (2R)-1 and (2S)-1 were −57° (c = 0.42, EtOH) and +60° (c = 0.30, EtOH), respectively.

Synthesis of (2R)-1 from (2RS)-6 Obtained by Optical Resolution of (2RS)-6. Commercially available (2RS)-6 was purified by column chromatography (silica gel; CHCl 3/MeOH/AcOH = 100/1/1) followed by charcoal treatment to remove impurities and colored components. Optical resolution of purified (2RS)-6 was performed by a diastereomeric salt method using (S,)-(-)-1-phenylethylamine as follows: To a stirred solution of the purified (2RS)-6 (1.02 g, 5.23 mmol) in EtOAc (50 mL) was added (S,)-(-)-1-phenylethylamine (0.34 mL, 2.6 mmol) to afford a white precipitate. The mixture was heated to reflux, and then the clear solution was allowed to stand at room temperature. The precipitated white needles were collected and recrystallized from EtOAc (50 mL) to afford (2R)-6-derived salt (552 mg). The optical purity (% ee) was 87.4% as determined using a CHIRALPAK IG chiral column equilibrated with n-hexane/EtOH/TFA = 800:200:1 (v/v) at a flow rate of 0.6 mL/min. The retention times of (2R)-6 and (2S)-6 were 10.5 and 12.2 min, respectively. A fourth recrystallization using the same conditions as described above increased the percent enantiomeric excess (% ee) of the (2R)-6-derived salt (400 mg) to 95.2%. The salt was next treated with EtOAc (30 mL) and 0.33 M HCl (10 mL) saturated with NaCl, and then the organic layer was dried over Na 2SO 4. After evaporation of the organic solvent under reduced pressure, (2R)-6 (281 mg, 55%) was obtained as a white solid with a % ee of 95.3%. The R configuration was confirmed by its [α]D 25 value of −53.1° (c = 0.565, CHCl 3), which is comparable to the reported value (lit., 56 [α]D 25 = −55.3°). Catalytic hydrogenation of (2R)-6 (220 mg, 1.13 mmol) was performed in MeOH (3.0 mL) with 10% Pd–C (20 mg) at room temperature for 4 h to afford the corresponding (R)-2-(4-aminophenyl)propionic acid (2R)-7 as a pale pink solid (171 mg, 92%). HRMS (ESI,requires C, 71.21; H, 7.81; N, 6.39; HRMS (ESI, positive): [M + H] + calcd for C 13H 18NO 2, m/z 220.1332; found, m/z 220.1339 (error 0.7 mmu); 1H NMR (600 MHz, DMSO-d 6): δ 12.04 (br s, 1H, COOH, exchangeable with D 2O), 6.95 (d, 2H, J = 8.6 Hz, ArH 2), 6.48 (d, 2H, J = 8.6 Hz, ArH 2), 5.83 (br s, 1H, NH, exchangeable with D 2O), 4.88 (br s, 1H, C−CH 3), 4.79 (br s, 1H, C−CH 3), 3.55 (br s, 2H, NHCH 2), 3.44 (q, 1H, J = 7.2 Hz, CHCH 3), 1.70 (s, 3H, CH 3, C−CH 3), 1.27 (d, 3H, J = 7.2 Hz, CHCH 3); 13C NMR (151 MHz, DMSO-d 6): δ 175.9 (COOH), 147.6, 143.0, 127.9, 127.6, 111.9, 110.1, 48.7, 43.7, 20.1, 18.6. Optical Resolution of 1. Optical resolution was performed using a CHIRALCEL OZ-H chiral preparative column equilibrated with n-hexane/EtOH/AcOH = 90/10/0.1 (v/v) at a flow rate of 2.5 mL/min. A 200 μl aliquot of 9.0 mg/mL 1 in the mobile phase was injected onto the column for each separation. The retention times of (2R)-1 and (2S)-1 were 19.0 and 15.5 min, respectively. Both enantiomers were almost completely resolved, and their optical purities (% ee) were each >99%. The absolute configuration of the chiral center was unambiguously determined by confirming the retention time of the (2R)-1 enantiomer and that of authentic (2R)-1 synthesized from (2R)-2-(4-nitrophenyl)propionic acid (2R)-6 (see below). Chiral HPLC was conducted using a CHIRALPAK IG analytical column equilibrated with 55% (v/v) MeOH containing 56 mM formic acid (pH 2.1) at a flow rate of 0.8 mL/min and monitored at 254 nm. The retention times of (2R)-1 and (2S)-1 were 15.9 and 18.8 min, respectively. The specific optical rotation [α]D 26 values of (2R)-1 and (2S)-1 were −57° (c = 0.42, EtOH) and +60° (c = 0.30, EtOH), respectively.

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positive): [M + H]+ calc'd for C8H14NO2, m/z 168.0869; found, m/z 168.0863 (error 0.6 mmu). The final step of N-(2-
 methyl)allylation of (2R)-7 (96 mg, 0.58 mmol) was performed at 70 °C with 3-chloro-2-methyl-1-prope
 (83 μL, 0.85 mmol) and KF—Cellite11 (150 mg) in CH3CN (2.5 mL) for 12 h. After removal of the insoluble materials, the solvent from filtrate was evaporated under reduced pressure to afford crude solid, which was purified by preparative TLC to afford (2R)-1 (57 mg, 44%). The retention time and optical purity (% ee) of (2R)-1 obtained were 15.9 min and 95.4%, respectively, using a CHIRALPAK IG chiral column eluted with 55% (v/v) MeOH containing 56 mM formic acid (pH 2.1) at a flow rate of 0.8 mL/min and monitored at 254 nm.

**Synthesis of Methyl 1β-O-2-[4-(2-Methylprop-2-ene-1-yl)aminophenyl]propanoyl-2,3,4-tri-
acyl-1-yl)amino]phenyl}propanoyl-D-glucopyranuronate (2RS)-5 (Mixture of Two Diastereomers).**

To a stirred solution of (2RS)-1 (493 mg, 2.25 mmol) in 8.0
mL of EtOH, 2.25 mL of 0.50 mol/L aqueous Cs2CO3 was added. After brief sonication to dissolve the cesium salt, the mixture was concentrated under reduced pressure. To remove water, EtOH (3.0 mL) was added to the residue and the solvent was removed under reduced pressure. The residue was dissolved with 20 mL of DMSO, and then methyl 2,3,4-tri-ethyl-1-bromo-1-deoxy-α-D-glucopyranuronate 2 (1.14 g, 272 mmol) was added at room temperature with vigorous stirring. The reaction was monitored by HPLC using a symmetry C18 column eluted with 50% (v/v) CH3CN containing 25 mM ammonium acetate (pH 4.5) and 10 mM tetra-n-butylammonium bromide at a flow rate of 0.5 mL/min and monitored at 254 nm. The retention times of (2RS)-1 and (2RS)-3 were 8.15 and 36.5 min, respectively. After 3 h, the reaction mixture was diluted with 200 mL of ethyl acetate and then washed three times with water (each 70 mL), four times with 0.1 mol/L NaOH (each 40 mL), and then once with saturated saline (70 mL). After drying over Na2SO4, the organic solvent was evaporated under reduced pressure. Column chromatography (silica; EtOAc/benzene = 2/5) of the residue afforded (2RS)-3 (663 mg) as a colorless syrup in 53% yield. HRMS (ESI, positive): [M + Na]+ calc'd for C30H42NaNO5, m/z 558.1946; found, m/z 558.1954 (error 0.8 mmu). 1H NMR (600 MHz, MeOH-d4): δ 7.00 (d, 2H, J = 8.6 Hz), 6.97 (d, 2H, J = 8.6 Hz), 6.54 (d, 2H, J = 8.6 Hz), 6.53 (d, 2H, J = 8.6 Hz), 5.85 (d, 1H, J = 8.2 Hz), 5.78 (d, 1.06H, J = 8.2 Hz), 5.40 (t, 1H, J = 9.3 Hz), 5.35 (d, 1.06H, J = 9.3 Hz), 5.13 (t, 1H, J = 9.6 Hz), 5.10 (t, 1.06H, J = 9.6 Hz), 5.08 (dd, 1H, J = 8.2 and 9.6 Hz), 4.98 (dd, 1.06H, J = 8.2 and 9.6 Hz), 4.93−4.92 (m, 2.1H), 4.83−4.82 (m, 2.1H), 4.41 (d, 1.06H, J = 9.6 Hz), 4.39 (d, 1H, J = 9.6 Hz), 3.72 (s, 3.2H), 3.69 (s, 3H), 3.64−3.59 (m, 6.2H), 2.00 (s, 6.2H), 1.98 (s, 3H), 1.95 (s, 3.2H), 1.87 (s, 3H), 1.75 (s, 3.2H), 1.74 (s, 3H), 1.57 (s, 3.2H), 1.43 (d, 3H, J = 7.2 Hz), 1.38 (d, 3.2H, J = 7.2 Hz); 13C NMR (151 MHz, MeOH-d4): δ 174.6, 174.2, 171.4, 171.3, 171.2, 170.9, 168.9, 149.7, 144.5, 129.1, 129.0, 128.7, 128.1, 114.0, 113.9, 111.1, 121.9, 92.7, 92.6, 73.7, 73.65, 73.3, 73.1, 71.2, 70.7, 53.4, 50.6, 50.58, 46.0, 45.4, 20.6, 20.54, 20.5, 20.46, 20.4, 18.4, 18.3.

**Enzyme-Catalyzed Chemo-Selective Removal of the O-Acetyl Groups of (2RS)-3 to (2RS)-4.** Of the enzymes tested, CALB and Chirazyme L-2 CB showed high chemo-selective activities, yielding almost quantitatively the product (2RS)-5 (see Table 2). The hydrolytic reaction was monitored by HPLC using a symmetry C18 column eluted with 35% (v/v) CH3CN containing 25 mM ammonium acetate (pH 4.5) and 10 mM tetra-n-butylammonium bromide at a flow rate of 0.5 mL/min. (1) Method with CALB. CALB (2.0 mg/mL 25 mM sodium citrate buffer, pH 5.0) was treated with XAD-4 resin wetted (80−200 mesh, 40 mg) in an ice water bath for 5 min with gentle shaking to remove impurities. Hydrolysis of (2RS)-4 with the abovementioned CALB (a final concentration of 1.0 mg/mL) was performed at 40 °C with an initial concentration of 1.0 mM (2RS)-4 in 20 mM sodium citrate buffer (pH 5.0) containing 10% (v/v) DMSO. (2) Method with Chirazyme L-2 CB. Hydrolysis of (2RS)-4 with Chirazyme L-2 CB (a final concentration of 1.0 mg/mL) was performed at 40 °C with an initial concentration of 1.0 mM (2RS)-4 in 20 mM sodium citrate buffer (pH 5.0) containing 10% (v/v) DMSO. The concentration of the substrate (2RS)-4 was able to rise to 4 mM. (3) Isolation of (2RS)-5. After 1.5 h of the incubation starting from 0.22 mmol of (2RS)-4 and 55 mg of Chirazyme L-2 CB, the mixture (55 mL) was loaded onto an XAD-4 column (10 g, 1.6 cm i.d.), which had been washed thoroughly with acetone and then equilibrated with aqueous water. The column was washed with 50 mL of water, 200 mL of 4 mM HCl, and then 50 mL of water. The product (2RS)-5 was eluted with 200 mL of 20% (v/v) aqueous CH3CN. Fractions containing (2RS)-5 were pooled, the solvent was evaporated under reduced pressure, and the residue was dried over P2O5 in a vacuum desiccator to afford a pale yellow solid (83 mg, 95%). HRMS (ESI, negative): [M − H]+ calc'd for C19H24NO8, m/z
CH3CN containing 25 mM ammonium acetate (pH 4.5) and by HPLC using an L-column ODS eluted with 30% (v/v)

δ 7.06 (d, 2H, J = 8.6 Hz), 6.57 (d, 4H, J = 8.6 Hz), 5.48 (d, 1H, J = 7.9 Hz), 5.47 (d, 1H, J = 7.9 Hz), 4.93 (br s, 2H), 4.84 (br s, 2H), 3.89 (d, 1H, J = 9.6 Hz), 3.86 (d, 1H, J = 9.6 Hz), 3.69 (q, 1H, J = 7.2 Hz), 3.68 (q, 1H, J = 7.2 Hz), 3.64 (br s, 4H), 3.53 (t, 1H, J = 9.6 Hz), 3.51 (t, 1H, J = 9.6 Hz), 3.44 (t, 1H, J = 9.1 Hz), 3.39–3.34 (m, 2H), 1.75 (s, 6H), 1.44 (d, 3H, J = 7.2 Hz), 1.43 (d, 3H, J = 7.2 Hz).

13C NMR (151 MHz, MeOH-δ): δ 175.5, 175.4, 172.1, 149.2, 149.1, 144.3, 143.31, 129.5, 129.4, 129.1, 114.2, 114.1, 111.2, 111.1, 95.7, 77.5, 77.22, 77.16, 73.6, 72.92, 72.89, 50.85, 50.79, 45.7, 20.4, 19.3, 19.2.

**Synthesis of (2R)-5 through Methyl 1-β-O-2-[4(2-Methylprop-2-en-1-y)amino]phenyl]propanoyl-2,3,4-tri-O-acetyl-D-glucopyranuronate (2RS)-3.** In accordance to the abovementioned procedure for the synthesis of (2RS)-3, the cesium salt prepared from (2R)-1 (25 mg, 0.11 mmol) was reacted with methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-a-D-glucopyranuronate (2) (57 mg, 0.14 mmol) at room temperature for 2 h to afford (2R)-3 (25 mg) in 40% yield. HRMS (ESI positive): [M + Na]+ calcld for C31H35NO9Na, m/z 558.1946; found, m/z 558.1959 (error 1.3 mmu).

1H NMR (600 MHz, MeOH-d4): δ 9.69 (d, 2H, J = 8.6 Hz), 6.52 (d, 2H, J = 8.6 Hz), 5.76 (d, 1H, J = 8.2 Hz), 5.34 (t, 1H, J = 9.6 Hz), 5.09 (t, 1H, J = 9.6 Hz), 4.97 (dd, 1H, J = 8.2 and 9.6 Hz), 4.91 (br s, 1H), 4.82 (br s, 1H), 4.41 (d, 1H, J = 9.6 Hz), 3.71 (s, 3H), 3.62 (s, 2H), 3.59 (q, 1H, J = 7.2 Hz), 2.00 (s, 3H), 1.95 (s, 3H), 1.74 (s, 3H), 1.57 (s, 3H), 1.38 (d, 3H, J = 7.2 Hz).

13C NMR (151 MHz, MeOH-d4): δ 174.5, 171.2, 171.1, 170.6, 168.8, 149.6, 144.5, 129.0, 128.7, 113.9, 111.0, 92.5, 73.6, 73.1, 71.1, 70.7, 53.4, 50.5, 45.3, 20.44, 20.38, 20.36, 20.32, 18.3. According to the above procedures described for the preparation of (2RS)-5 from (2RS)-3, enzyme-catalyzed chemo-selective hydrolysis of the sugar-protecting groups of (2R)-3 was performed using LAS and CSR followed by using Chirazyme L-2 CB to afford (2R)-5. HRMS (ESI negative): [M − H]− calcld for C31H33NO9, m/z 394.1507; found, m/z 394.1513 (error 0.6 mmu).

**Measurement of the Intrinsic Degradation Rate Constants (k Values) of (2RS)-5.** The k values of (2RS)-5 (an initial concentration of 0.10 mM) were measured under physiological conditions (100 mM sodium phosphate buffer at pH 7.40 and 37 °C). The degradation reaction was monitored by HPLC using an L-column ODS eluted with 30% (v/v) CH3CN containing 25 mM ammonium acetate (pH 4.5) and by HPLC using an L-column ODS eluted with 30% (v/v) MeOH.

394.1507; found, m/z 394.1518 (error 1.1 mmu); 1H NMR (600 MHz, MeOH-d4): δ 7.06 (d, 2H, J = 8.6 Hz), 7.04 (d, 2H, J = 8.6 Hz), 6.57 (d, 4H, J = 8.6 Hz), 5.48 (d, 1H, J = 7.9 Hz), 5.47 (d, 1H, J = 7.9 Hz), 4.93 (br s, 2H), 4.84 (br s, 2H), 3.89 (d, 1H, J = 9.6 Hz), 3.86 (d, 1H, J = 9.6 Hz), 3.69 (q, 1H, J = 7.2 Hz), 3.68 (q, 1H, J = 7.2 Hz), 3.64 (br s, 4H), 3.53 (t, 1H, J = 9.6 Hz), 3.51 (t, 1H, J = 9.6 Hz), 3.44 (t, 1H, J = 9.1 Hz), 3.39–3.34 (m, 2H), 1.75 (s, 6H), 1.44 (d, 3H, J = 7.2 Hz), 1.43 (d, 3H, J = 7.2 Hz).

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**ABBREVIATIONS**

ALP, aliminoprofen; NSAID, nonsteroidal anti-inflammatory drug; UGT, UDP-glucuronosyltransferase; LAS, lipase AS Amano; CSR, carboxylesterase from Streptomyces rochei; PLE, pig liver esterase; CALB, lipase type-B from Candida antarctica; DMP, NN-dimethylformamide; DMSO, dimethyl sulfoxide

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**Notes**

The authors declare no competing financial interest.

**ASSOCIATED CONTENT**

Supporting Information

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**1H and 13C NMR spectra and mass spectra for ALP 1** and all new compounds (2RS)-3, (2R)-3, (2RS)-4, (2RS)-5, and (2RS)-7 (PDF)
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