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SYNTHESIS OF SALACINOL-d_4 AS AN INTERNAL STANDARD FOR MASS-SPECTROMETRIC QUANTITATION OF SALACINOL, A POTENT α-GLUCOSIDASE INHIBITOR FOUND IN A TRADITIONAL AYURVEDIC MEDICINE "*SALACIA*"

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Abstract – Accurate quantitative analysis of trace principles in extracts of biologically active natural medicines relies on the use of reliable internal standards (ISs), which, in the case of liquid chromatography–mass spectrometry (LC-MS) analysis, commonly correspond to isotope-labeled stable analogues of the analytes. Herein, we describe the synthesis of salacinol-1,1,5,5-*C*-d_4 (**7**), an isotope-labeled stable analogue of salacinol (**1**), which, in turn, is a potent α-glycosidase inhibitor isolated from *Salacia* (a traditional Ayurvedic medicine), and show that the isotopic purity of the labeled standard is satisfactory for LC-MS analysis.

INTRODUCTION

Diabetes mellitus is one of the largest global health emergencies of the 21st century, currently affecting ~425 million people worldwide, including more than 200 million undiagnosed people according to the International Diabetes Federation. Over the past three decades, the global number of people living with diabetes has dramatically increased and is expected to reach ~693 million by 2045 if no remedial measures are taken.[1] Diabetes is classified as either Type 1 or Type 2, with the latter accounting for ~90% of all diagnosed cases. Patients suffering from Type 2 diabetes rely on the management of blood glucose

levels, which typically involves delaying the digestion of ingested carbohydrates and thus lowers postprandial blood glucose levels. α-Glucosidase inhibitors are particularly useful for managing mild hyperglycemia in people newly diagnosed with Type 2 diabetes as well as for managing postprandial hyperglycemia; three anti-diabetics (voglibose, acarbose, and miglitol) are currently clinically used worldwide. In the late 1990s, salacinol (**1**), which shows potent α-glucosidase inhibitory activity equal to that of voglibose and acarbose, was isolated from *Salacia reticulata*, traditionally used for diabetes treatment in Ayurveda.[2] Thereafter, kotalanol[3] (**2**) and ponkoranol[4] (**3**) as well as their de-*O*-sulfonated variations neosalacinol[5] (**4**), neokotalanol[6] (**5**), and neoponkoranol[7] (**6**), were subsequently isolated from plants of the same genus as other principles exhibiting potent antidiabetic activities. Clinical trials revealed that the extract of *Salacia reticulata* was effective for treatment of patients with Type 2 diabetes and exhibited minimal side effects.[8] Based on this evidence, the plants of "*Salacia*" genus have recently gained considerable attention as a possible functional food for diabetic patients and people with impaired glucose tolerance (i.e., those at high risk of developing the disease in the future). In view of the above, an efficient quality control method is urgently required to ensure the authenticity of these functional foods and their active constituent content, as well as to verify manufacturer claims. To evaluate the quality of *Salacia* extracts, we developed two protocols for the quantitative analysis of sulfonium sulfate inner salts **1**–**3**[9a,9c] and their de-*O*-sulfonates **4**–**6**[9b,9c] based on liquid chromatography–mass spectrometry (LC-MS). Extracts prepared from a variety of raw *Salacia* samples collected in different geographical regions (e.g., Thailand, India, and Sri Lanka) were evaluated by determining the distribution of sulfonium salts in stems, roots, leaves, and fruits. Isotope dilution mass spectrometry (IDMS) is widely accepted as a highly accurate analytical technique, being an ideal method for the quantitative analysis of small amounts of bioactive chemicals in biological fluids,[10] natural medicine,[11] agriculture[12] or environment.[13] Notably, IDMS features the use of isotopically labeled stable internal standards (ISs) behaving nearly identically to target analytes at all steps of the isolation and chromatographic procedures, which significantly reduces the systematic error due to losses occurring at every stage of analysis, including post-extraction sample work-up and calibration. So, the availability of such ISs would not only allow one to evaluate the quality of *Salacia* raw materials themselves, but also permit the identification and quantitation of all sulfonium salt conjugates found in biological fluids. However, despite these advantages, no isotopically labeled ISs of salacinol-type α-glucosidase inhibitors **1**–**6** have been synthesized to date. To prevent mass peak overlap, the molecular masses of prospective ISs are required to be at least three mass units larger than those of the target analytes. To address the above issue, we have designed and synthesized a deuterated salacinol derivative, salacinol-1,1,5,5-*C*-d_4 (**7**), as a part of our continuing study on the quantitative analysis of *Salacia* constituents (Figure 1).

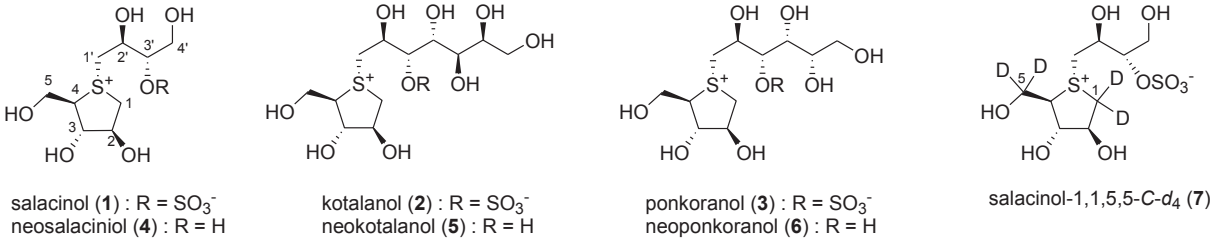
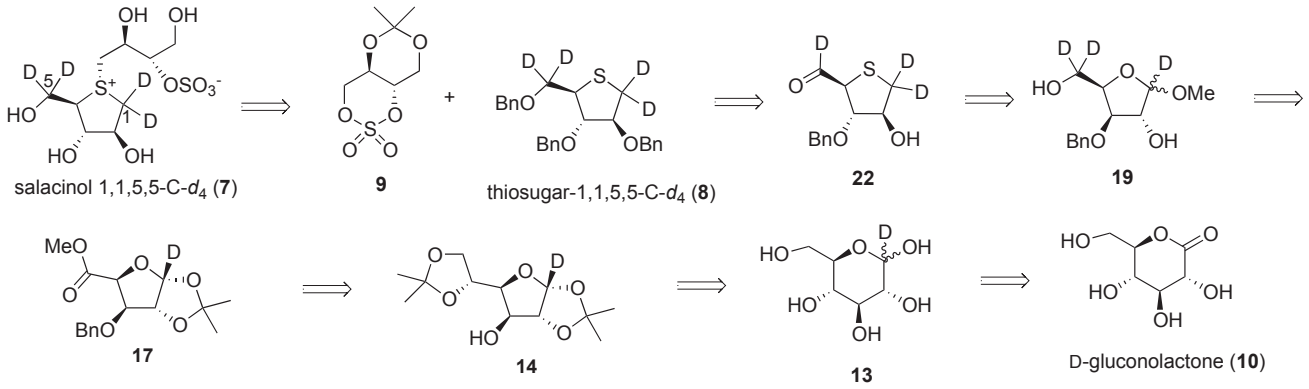


Figure 1. A new class of natural α-glucosidase inhibitors (**1**–**6**) from *Salacia* genus plants and a stable isotope-labeled salacinol-1,1,5,5-*C*-d_4 (**7**)

RESULTS AND DISCUSSION

The retrosynthetic route to **7** is provided in Scheme 1. A thiosugar, 2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-epithio-D-arabinitol-1,1,5,5-*C*-d_4 (**8**), which is a common motif of natural α-glucosidase inhibitors **1**–**6**, was selected as a coupling a partner for the known cyclic sulfate[14] (**9**) in consideration of the syntheses of multiply deuterated analogues of other natural α-glucosidase inhibitors **2**–**6**. The four deuterium atoms of **8** were introduced stepwise by deuteride reductions. Initially, one deuterium was introduced by reducing D-glucono-1,5-lactone (**10**) with sodium borodeuteride ($NaBD_4$), with the second reduction featuring the double deuteration of ester **17** by lithium aluminum deuteride ($LiAlD_4$), and the final reduction corresponding to the monodeuteration of aldehyde **22** by $NaBD_4$ (Scheme 1).

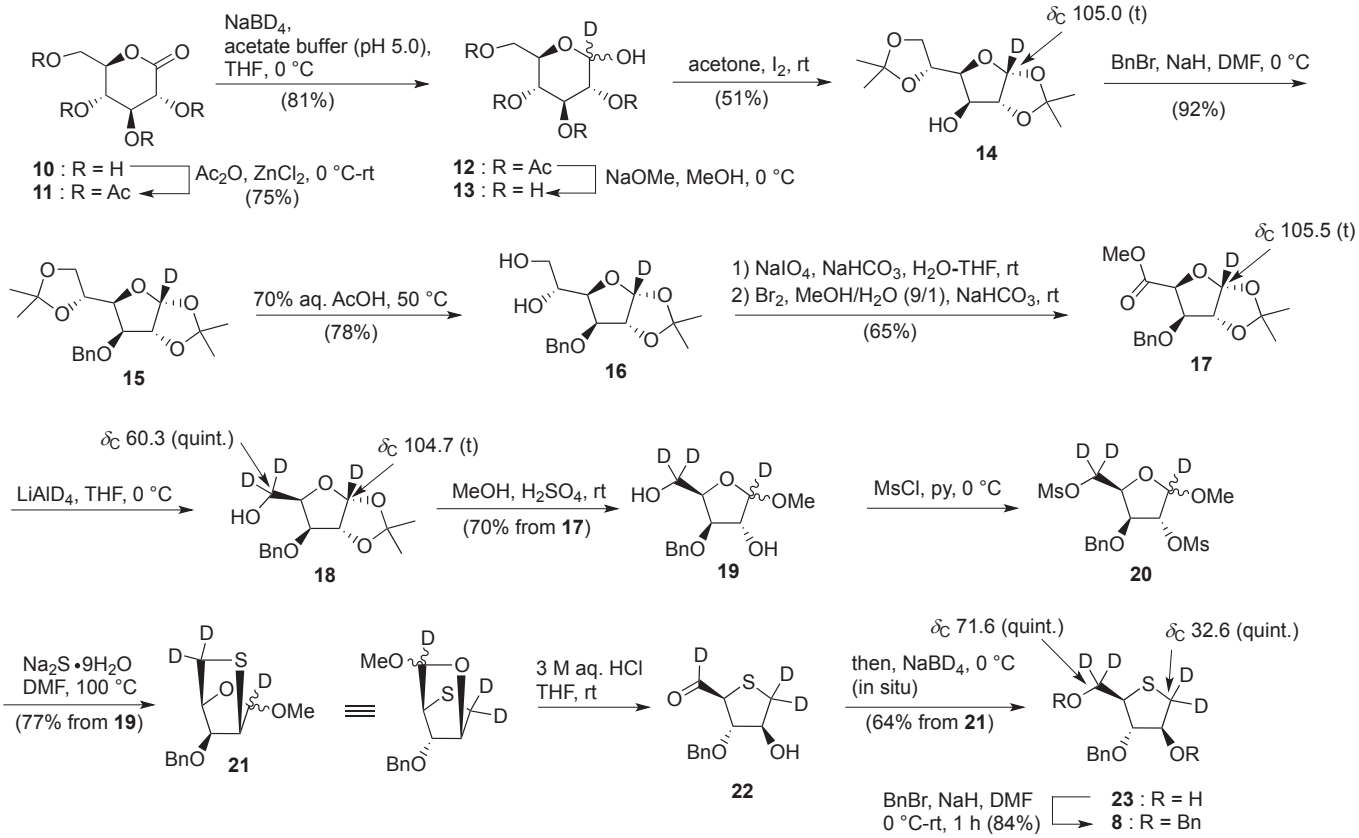


Scheme 1

According to literature,[15] **10** was acetylated with acetic anhydride in the presence of zinc chloride to give practically pure D-gluconolactone 2,3,4,6-tetraacetate (**11**) in 75% yield, which was subsequently converted to D-glucose-1-*C*-*d* 2,3,4,6-tetraacetate[16] (**12**) by treatment with $NaBD_4$ in a mixture of THF and H_2O. Even after several attempts, the yield of 82% reported for the reduction of **11** could not be reproduced and ended up being low or moderate (around 47–65%), despite the reaction mixture being

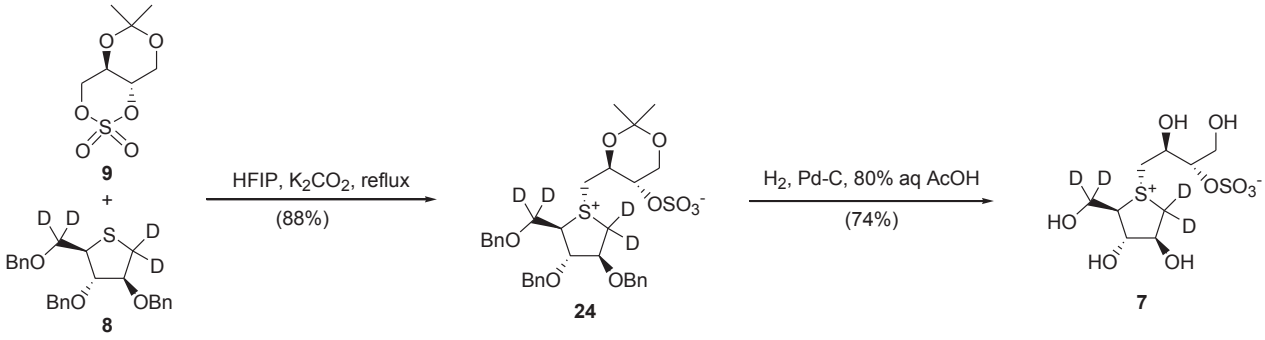
quenched with an ion-exchange resin (H^+-form) according to the reported work-up protocol.[16] This result suggested that the acetyl moieties of **11** or **12** were partially hydrolyzed by the basic aqueous $NaBD_4$, which decreased the yield of **12**. Therefore, $NaBD_4$ reduction was performed under pH-controlled conditions in an acetate buffer (pH = 5), reproducibly affording a ~2:1 anomeric mixture of **α-** and **β-12** in approximately 80% yield after column chromatography. Based on these results, the reduction of **11** with $NaBD_4$ was scaled up, and subsequent deacetylation of the resultant hemiacetal **12** with sodium methoxide afforded D-glucose-1-*C*-*d* (**13**), which was acetalized with acidic acetone to give 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose-1-*C*-*d*[15] (**14**, 51% yield over three steps from **11**). The remaining hydroxyl in **14** was protected with benzyl bromide, and one of the two acetonide moieties of the resultant benzyl ether, 3-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose-1-*C*-*d* (**15**), was selectively removed by hydrolysis with 70% aqueous acetic acid to give 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose-1-*C*-*d* (**16**, 72% yield over two steps from **14**). The *vic*-diol moiety of **16** was oxidized with sodium metaperiodate in the presence of sodium hydrogen carbonate, with oxidation of the resultant aldehyde by hypobromous acid affording methyl 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranuronate-1-*C*-*d* (**17**) in 65% yield. Compound **17** was further reduced with $LiAlD_4$ to give 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose-1,5,5-*C*-d_3 (**18**). In addition to the signals of three methine carbons [C-2 (δ_C 82.3), C-3 (δ_C 82.7), and C-4 (δ_C 79.9)], the ^{13}C NMR spectrum of **18** featured characteristic C-1 and C-5 signals [(δ_C 104.7, t, J = 27.4) and (δ_C 60.3, quint, J = 21.5), respectively], which supported the 1,5,5-trideuterated structure of **18**. Upon treating compound **18** with methanol in the presence of a catalytic amount of sulfuric acid, deacetalization and methyl glycosidation proceeded simultaneously to give a ~1:1 anomeric mixture of methyl 3-*O*-benzyl-α- and β-D-xylofuranoside-1,5,5-*C*-d_3 (**α-** and **β-19**) in 70% yield over two steps from **17**. Compounds **α-** and **β-19** were converted to **8** by applying the protocol previously reported[17] for the preparation of the non-deuterated version of **8**. Thus, a mixture of **α-** and **β-19** was treated with methanesulfonyl chloride to afford the corresponding bismesylates, methyl 3-*O*-benzyl-2,5-di-*O*-mesyl-D-xylofuranoside-*1,5,5*-*C*-d_3 (**α-** and **β-20**), which were subjected to an S_N2 substitution reaction with sodium sulfide in DMF to afford a ~1:1 anomeric mixture of methyl 2,5-dideoxy-2,5-epithio-3-*O*-benzyl-D-lyxofuranoside-1,1,5-*C*-d_3 (**α-** and **β-21**; 77% yield from **19**). Hydrolysis of **α-** and **β-21** in a mixture of 3 M hydrochloric acid and THF followed by $NaBD_4$ reduction of the resultant aldehyde **22** gave 3-*O*-benzyl-1,4-dideoxy-1,4-epithio-D-arabinitol-1,1,5,5-*C*-d_4 (**23**) in 64% yield from **21**. Finally, the two hydroxyls of **23** were protected by treatment with benzyl bromide in the presence of sodium hydride to afford the key thiosugar **8** in 84% yield. The IR spectrum of **8** showed absorptions due to C–D stretching at 2719 and 2075 cm^{-1}, whereas the corresponding ^1H NMR spectrum displayed three signals at δ_{H-2} 4.18 (d, $J_{2,3}$ = 4.0 Hz), δ_{H-3}

4.11 (dd, $J_{3,2}$ = 4.0, $J_{3,4}$ = 3.4 Hz), and δ_{H-4} 3.55 (d, $J_{4,3}$ = 3.4 Hz), with the doublets of H-2 and H-4 implying that C-1 and C-5 carbons were both CD_2 moieties. The above carbons were also observed as two quintets at δ_{C-1} 32.6 (J = 21.1 Hz) and δ_{C-5} 71.6 (J = 23.0 Hz) in the ^{13}C NMR spectrum. Moreover, the ESI-MS spectrum of **8** displayed a peak of the quasimolecular ion of the [d_4]-form at m/z 447 $[M+Na]^+$ (100%) along with those due to [d_3]- and [d_2]-forms at m/z 446 (14%) and m/z 445 (0.6%), respectively (Scheme 2).



Scheme 2

The thus obtained thiosugar **8** with a moderate degree of deuteration was subsequently converted to salacinol-1,1,5,5-*C*-d_4 (**7**) according to the conventional protocol for salacinol synthesis.[14,18] Specifically, *S*-alkylation of **8** with cyclic sulfate[14] **9** in gently refluxed 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) in the presence of potassium carbonate gave sulfonium salt **24** in 88% yield. Finally, hydrogenolysis of **24** over Pd-C in 80% acetic acid smoothly afforded **7** in 74% yield (Scheme 3).



Scheme 3

The ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectra of **7** in D_2O are displayed in Figures 2 and 3, respectively, along with those of salacinol (**1**). As summarized in Table 1, the chemical shifts of all carbons of **7** were perfectly identical to those of **1** (deviations of 0.01 ppm) except for those of C-1 and C-5 (CD_2), which were observed as quintets at $\delta_{C\text{-}1}$ 50.2 and $\delta_{C\text{-}5}$ 61.2 and were slightly shifted upfield compared to those of **1** ($\delta_{C\text{-}1}$ 50.5 and $\delta_{C\text{-}5}$ 61.7) due to the effect of deuterium substitution. Similarly, the ^{1}H NMR spectrum of **7** was similar to that of **1**, except for the coupling patterns of H-2, H-3, and H-4 signals being simplified by the introduction of deuterium at C-1 and C-5. The negative-mode ESI-MS spectrum of **7** showed a peak at *m/z* 337.0530 {$[M–H]^-$, base peak (100%)} along with those due to [d_3]- and [d_2]-forms [*m/z* 336.0509 (19%) and *m/z* 335.0456 (1.5%), respectively]. Notably, signals at *m/z* 333 and 334, corresponding to [d_0] and [d_1] forms, respectively, were not detected. Thus, based on the observed ion intensities, the isotopic substitution grade of **7** was estimated as ~99% ([d_4]- and [d_3]-forms) (Figure 4).

Table 1. ^{1}H and ^{13}C NMR data of salacinol-1,1,5,5-*C*-d_4 (**7**) and salacinol (**1**) in D_2O

Salacinol-1,1,5,5-*C*-d_4 (**7**)			Salacinol (**1**)		Deviations of the chemical shifts	
Position	δ_H[1]	δ_C[2]	δ_H[1]	δ_C[2]	$\Delta\delta_H$	$\Delta\delta_C$
H-1a,1b	–	50.2 (quint., *J* = 19.4)	3.89 (dd, *J* = 13.4, 4.0)	50.5		0.30
	–		3.91 (dd, *J* = 13.4, 3.6)			
H-2	4.82 (d-like, *J* = ca. 3.4)	79.4	4.75 (ddd, *J* = 4.0, 3.6, 3.4)	79.5	0.07	0.01
H-3	4.51 (dd, *J* = 3.4, 2.6)	80.3	4.46 (dd., *J* = 3.4, 2.7)	80.3	0.05	0
H-4	4.14 (d, *J* = 2.6)	72.6	4.10 (ddd, *J* = 8.5, 4.9, 2.7)	72.7	0.04	0.01
H-5a	–	61.2 (quint., *J* = 18.7)	3.96 (dd, *J* = 12.0, 8.5)	61.7		0.50
H-5b	–		4.13 (dd, *J* =12.0, 4.9)			
H-1'a	3.90 (dd, *J* = 13.5, 8.4)	52.4	3.84 (dd, *J* = 13.5, 8.4)	52.4	0.06	0
H-1'b	4.06 (dd, *J* = 13.5, 3.2)		4.00 (dd, *J* = 13.5, 3.2)		0.06	
H-2'	4.47 (ddd, *J* = 8.4, 7.3, 3.2)	68.3	4.41 (ddd, *J* = 8.4, 7.4, 3.2)	68.3	0.06	0
H-3'	4.42 (ddd, *J* = 7.6, 3.4, 3.2)	82.7	4.36 (dt, *J* = 7.4, 3.2)	82.6	0.06	0.01
H-4'a	3.93 (dd, *J* = 12.8, 3.2)	62.3	3.87 (dd, *J* = 12.8, 3.2)	62.2	0.06	0.01
H-4'b	4.03 (dd, *J* = 12.8, 3.4)		3.97 (dd, *J* = 12.8, 3.2)		0.06	

[1]800 MHz. [2]200 MHz.

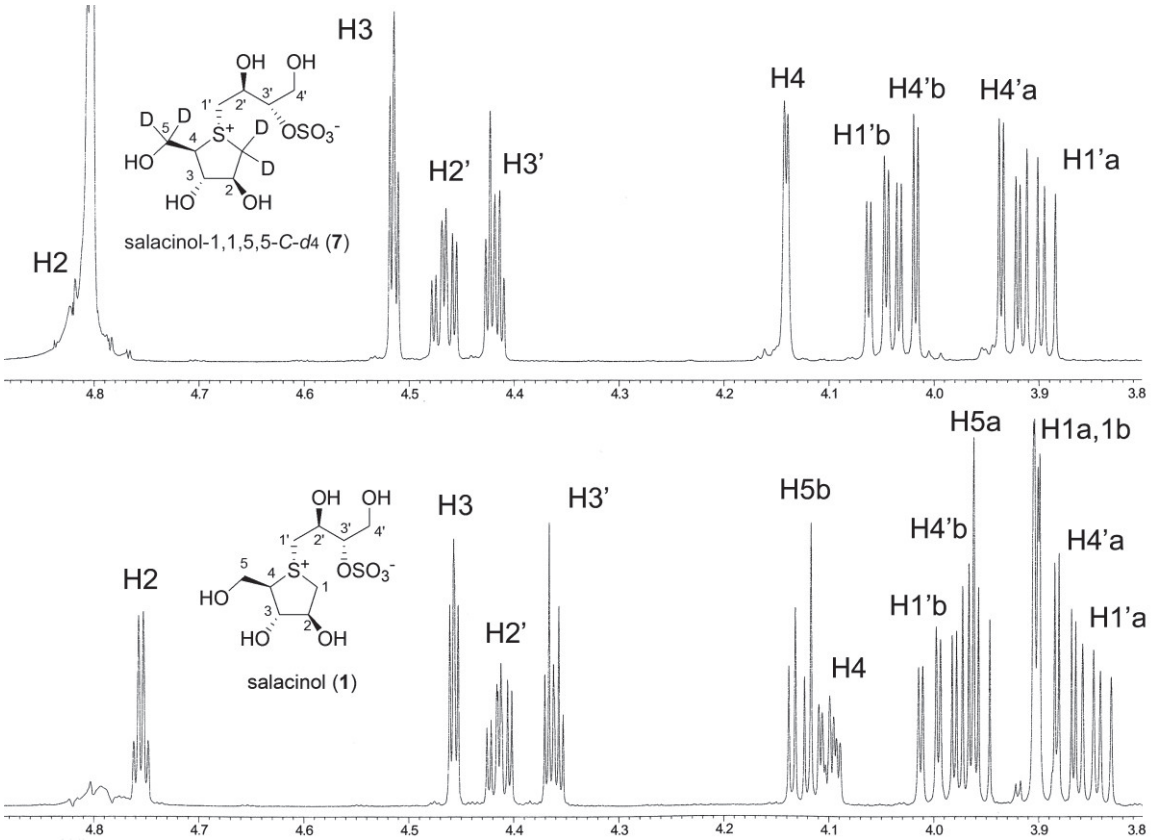


Figure 2. ^{1}H NMR spectra (800 MHz) of **1** and **7** in D_2O

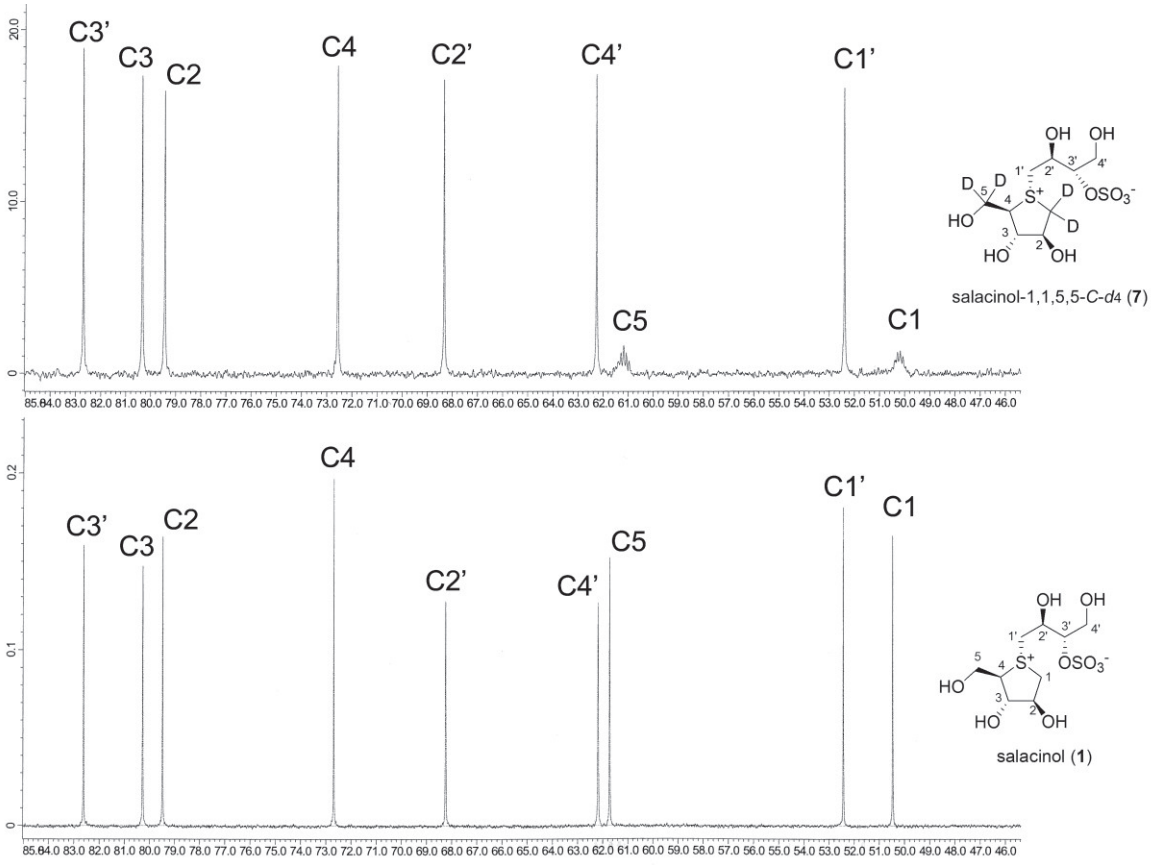


Figure 3. ^{13}C NMR spectra (200 MHz) of **1** and **7** in D_2O

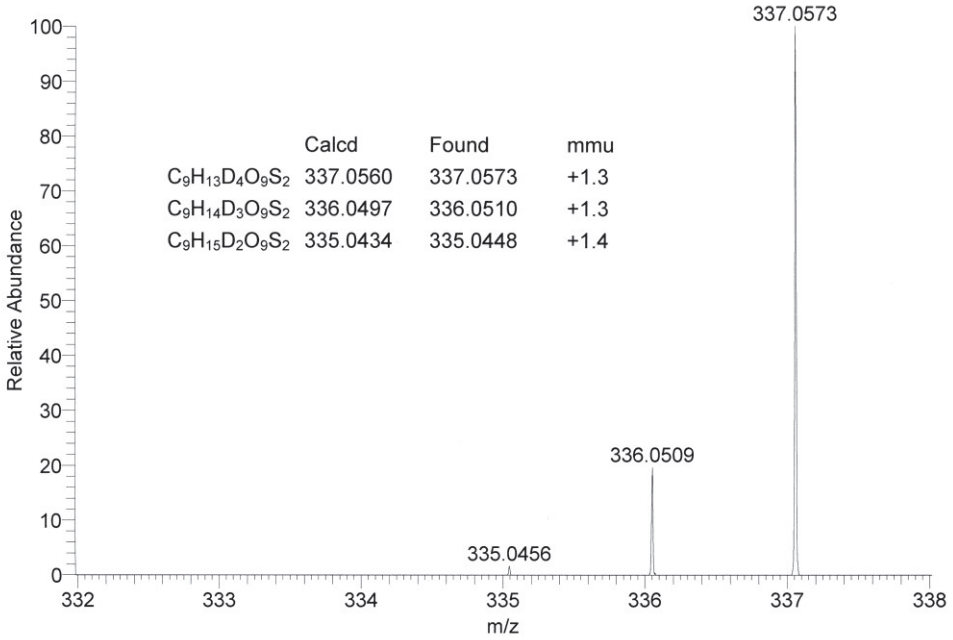


Figure 4. MS spectrum (ESI, negative) of **7**

Finally, the behavior of **7** under the conditions of LC-MS analysis was compared to that of **1**. According to a previously reported protocol,[9a] the quasi-molecular $[M–H]^-$ ions of **7** (*m/z* 337) and **1** (*m/z* 333) were monitored in selected ion monitoring (SIM) mode using negative-ion ESI ionization. The retention time (t_R 6.7 min) of **7** in the SIM chromatogram was perfectly identical to that (t_R 6.7 min) of **1**, indicating that **7** can be used as an IS for stable IDMS of *Salacia* (Figure 5).

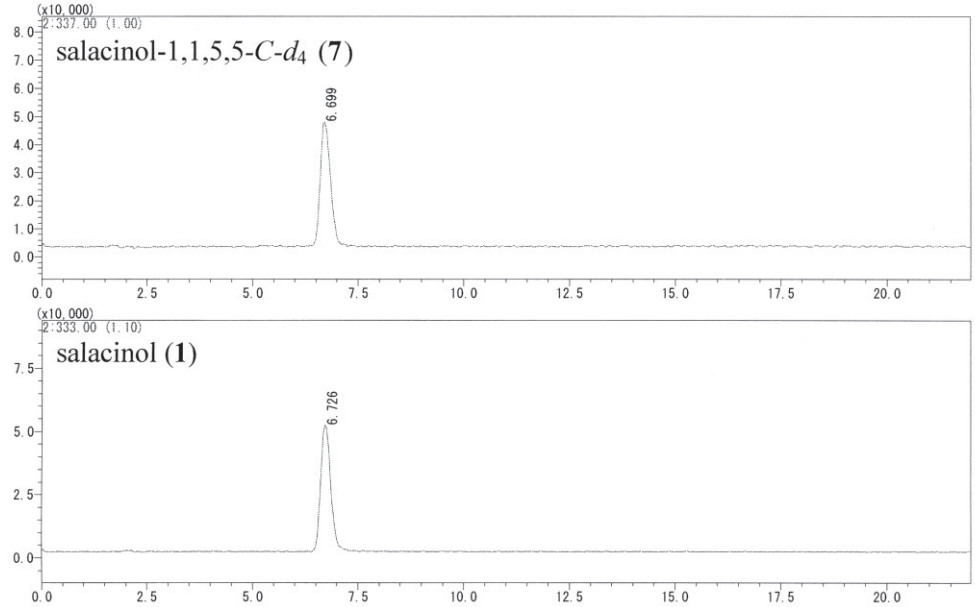


Figure 5. SIM chromatograms (negative-ESI-MS) of salacinol-1,1,5,5-*C*-d_4 (**7**, *m/z* 337, t_R 6.7 min) and salacinol (**1**, *m/z* 333, t_R 6.7 min)

In conclusion, thiosugar-1,1,5,5-*C*-d_4 (**8**), a common motif of natural α-glucosidase inhibitors **1**–**6**, was successfully synthesized starting from commercially available D-gluconolactone (**10**) by three-fold deuteride reduction and was subsequently converted to salacinol-1,1,5,5-*C*-d_4 (**7**) via *S*-alkylation with cyclic sulfate **9**. ^{1}H and ^{13}C NMR spectroscopy demonstrated that four deuterium atoms were introduced into the structure of **7**. The good isotopic purity of **7**, estimated based on the intensities of the quasimolecular ion peaks in MS spectra, proved that **7** could function as a stable isotopically labeled IS for the IDMS of *Salacia*. Further studies on the synthetic application of **8** to other natural sulfonium salts **2**–**6** as well as those on its analytical application to the quality evaluation of *Salacia* raw materials and assessment of the disposition dynamics of natural sulfonium salts conjugates in biological fluids are in progress.

EXPERIMENTAL

Mps were determined on a hot-stage melting point apparatus and are uncorrected. IR spectra were measured on a FT-IR spectrophotometer. NMR spectra were recorded on a FT-NMR spectrometers (^{1}H, 500 or 800 MHz; ^{13}C, 125 or 200 MHz). Chemical shifts (δ) and coupling constants (J) are given in ppm and Hz, respectively. Tetramethylsilane (TMS) was used as an internal standard for ^{1}H NMR measurements in $CDCl_3$, whereas ^{13}C NMR measurements utilized the solvent signal (77.0 ppm) of $CDCl_3$ for this purpose. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as an external standard in the measurement of ^{1}H and ^{13}C NMR spectra in D_2O. Low-resolution and high-resolution mass spectra were recorded on a double-focusing mass spectrometer (FAB) or a orbitrap mass spectrometer (ESI). Optical rotations were determined with a digital polarimeter. Sodium borodeuteride ($NaBD_4$, isotopic purity: 99%) and lithium aluminum deuteride ($LiAlD_4$, isotopic purity: 98%) were purchased from Cambridge Isotope Laboratories, Inc. and Strem Chemicals, Inc., respectively. Column chromatography was performed over silica gel (45–106 μM). All the organic extracts were dried over anhydrous Na_2SO_4 prior to evaporation.

D-Glucono-1,5-lactone 2,3,4,6-tetraacetate (11). According to the literature,[15] a mixture of D-glucono-1,5-lactone (**10**, 30 g, 168 mmol), acetic anhydride (106 mL, 1.0 mol), and zinc chloride (5.0 g, 37 mmol) was stirred at room temperature for 2.5 h. The reaction mixture was poured in to ice-cooled water (500 mL), and the resulting mixture was neutralized with sodium hydrogen carbonate. The deposited solid was filtered off, and the filtrate was extracted with AcOEt. The extract was washed with brine and condensed to give the title compound **11** (43.8 g, 75%) as a colorless oil. ^{1}H NMR (800 MHz, $CDCl_3$) δ: 2.08/2.09/2.12/2.17 (each 3H, s, COCH_3), 4.27 (1H, dd, J = 12.8, 2.4, H-6a), 4.41 (1H, dd, J = 12.8, 4.0, H-6b), 4.62 (1H, ddd, J = 8.8, 4.0, 2.4, H-5), 5.12 (1H, J = 8.8, H-2), 5.36 (1H, dd, J = 8.8, 8.8,

H-4), 5.56 (1H, dd, J = 8.8, 8.8, H-3). ^{13}C NMR (200 MHz, $CDCl_3$) δ: 20.3/20.4/20.5/20.6 (CO*C*H$_3$), 61.3 (C-6), 66.6 (C-4), 70.3 (C-2), 70.4 (C-3), 75.8 (C-5), 164.5 (C-1), 169.1/169.5/169.9/170.2 (*C*OCH$_3$).

D-Glucose-1-*C*-*d* 2,3,4,6-tetraacetate (12).

Method A: Sodium borodeuteride ($NaBD_4$, 25 mg, 0.60 mmol) was added portionwise to a mixture of compound **11** (412 mg, 1.19 mmol), THF (10 mL), and H_2O (2 mL) at 0 °C. After being stirred at 0 °C for 1 h, the reaction mixture was neutralized with ion exchange resin IR-120 (H^+-form). The ion exchange resin was filtered off, and washed with AcOEt. The combined filtrate and washings were diluted with water (5 mL) and extracted with AcOEt. The extract was washed with brine and condensed to give a colorless viscous oil (327 mg), which on column chromatography (*n*-hexane/AcOEt = 10/1→5/1→3/1) gave the title compound **12**[16] (269 mg, 65%) as a colorless viscous oil.

Method B: Sodium borodeuteride ($NaBD_4$, 27.4 mg, 0.65 mmol) was added portionwise to a mixture of compound **11** (453 mg, 1.31 mmol), THF (8 mL), and acetate buffer (pH = 5.0, 2 mL) at 0 °C. After being stirred at 0 °C for 2 h, the reaction mixture was diluted by addition of water (10 mL) and the resulting mixture was extracted with AcOEt. The extract was washed with brine and condensed to give a colorless viscous oil (407 mg), which on column chromatography (*n*-hexane/AcOEt = 10/1→5/1→3/1) gave the title compound **12** (369 mg, 81%) as a colorless viscous oil.

A mixture of α- and β-12 (α/β = *ca.* 2/1): ^{1}H NMR (800 MHz, $CDCl_3$) δ : 2.024/2.041/2.090/2.101 (each 2H, s, COC*H*$_3$-α), 2.025/2.035/2.094/2.098 (each 1H, s, COC*H*$_3$-β), 3.49 (0.66H, d, $J_{OH\text{-}H2}$ = 1.6, OH-α), 3.76 (0.33H, ddd, J = 10.4, 4.8, 2.4, H-5-β), 3.85 (0.33H, s, OH-β), 4.14 (0.66H, dd, J = 12.0, 2.4, H-6a-α), 4.16 (0.33H, dd, J = 12.0, 2.4, H-6a-β), 4.24 (0.66H, dd, J = 12.0, 4.0, H-6b-α), 4.26 (0.33H, dd, J = 12.0, 4.8, H-6b-β), 4.27 (0.66H, ddd, J = 10.4, 4.0, 2.4, H-5-α), 4.89 (0.33H, d, J = 9.6, H-2-β), 4.90 (0.66H, dd, J = 10.4, $J_{OH\text{-}2}$ = 1.6, H-2-α), 5.086 (0.33H, dd, J = 10.4 9.6, H-4-β), 5.088 (0.66H, dd, J = 10.4, 9.6, H-5-α), 5.26 (0.33H, dd, J = 9.6, 9.6, H-3-β), 4.26 (0.66H, dd, J = 10.4, 9.6, H-3-α). ^{13}C NMR (200 MHz, $CDCl_3$) δ: 20.6/20.65/20.68/20.72 (CO*C*H$_3$-α), 20.6/20.7 (CO*C*H$_3$-β, two signals overlapped with those of α-isomer), 61.9 (C-6α and C-6β), 67.4 (C-5α), 68.4 (C-4β), 68.5 (C-4α), 69.8 (C-3α), 71.0 (C-2α), 72.1 (C-3β), 72.2 (C-5β), 73.2 (C-2β), 89.8 (t, J = 25.8, C-1α), 95.1 (t, J = 25.0, C-1β), 169.6/170.1/170.2/170.8 (*C*OCH$_3$-α), 169.5/170.8/170.9 (*C*OCH$_3$-β, one signal overlapped with that of α-isomer).

1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose-1-*C*-*d* (14). According to the method B used for the preparation of **12**, to a mixture of compound **11** (9.87 g, 28.5 mmol), THF (160 mL), and acetate buffer (pH = 5.0, 40 mL) was added portionwise $NaBD_4$ (597 mg, 14.3 mmol) at 0 °C, and the mixture was stirred at 0 °C for 2 h. Work-up gave **12** (8.67 g) as a colorless viscous oil, which was used without

further purification.

A mixture of the crude **12** (8.67 g), sodium methoxide (537 mg, 9.9 mmol), and dry MeOH (90 mL) was stirred at room temperature for 5 h. The reaction mixture was neutralized with ion exchange resin IR-120 (H^+-form). The ion exchange resin was filtered off, and washed with wet MeOH. The combined filtrate and washings were condensed to give D-glucose-1-*C*-*d* (**13**, 4.79 g) as a colorless amorphous, which was used without further purification.

A mixture of the crude **13** (4.79 g), iodine (1.62 g, 6.4 mmol), and acetone (500 mL) was stirred at room temperature for 3.5 days. After the reaction mixture was poured into aqueous sodium thiosulfate-sodium hydrogen carbonate (300 mL), acetone was evaporated *in vacuo* from the resulting mixture, and the residue was extracted with AcOEt. The extract was washed with brine and condensed to give a colorless solid (4.19 g), which on column chromatography (*n*-hexane/AcOEt = 10/1→5/1→3/1) gave the title compound **14** (3.79 g, 51% from **11**) as a pale yellow solid. Colorless needles (from *n*-hexane). Mp 103–105 °C, lit.[15] mp 107–109 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.32/1.37/1.45/1.50 [each 3H, s, $(CH_3)_2C$], 2.61 (1H, d, J = 5.4, OH), 3.99 (1H, dd, J = 8.6, 5.2, H-6a), 4.07 (1H, dd, J = 7.5, 2.9, H-4), 4.17 (1H, dd, J = 8.6, 6.3, H-6b), 4.33 (1H, dd, J = 5.4, 2.9, H-3), 4.34 (1H, ddd. J = 7.5, 6.3, 5.2, H-5), 4.53 (1H, s, H-2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.1/26.2/26.7/26.8 [$(CH_3)_2C$], 67.6 (C-6), 73.5 (C-5), 75.2 (C-3), 81.1 (C-4), 85.0 (C-2), 105.0 (t, J = 27.4, C-1), 109.6/111.8 [$(CH_3)_2C$].

3-*O*-Benzyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose-1-*C*-*d* (15). A solution of **14** (3.47 g, 13.3 mmol) in DMF (30 mL) was added dropwise to a mixture of sodium hydride (636 mg, 15.9 mmol, 60% in liquid paraffin), benzyl bromide (1.9 mL, 16.0 mmol), and DMF (20 mL) at 0 °C. After being stirred at 0 °C for 1.5 h, the reaction mixture was poured into ice-cooled water (250 mL) and extracted with AcOEt. The extract was washed with brine and condensed to give a pale yellow oil (5.71 g), which on column chromatography (*n*-hexane/AcOEt = 10/1) gave the title compound **15** (4.28 g, 92%) as a colorless oil. $[\alpha]_D^{24}$ –40.4 (c 1.10, $CHCl_3$). IR (neat): 2986, 2936, 2203 (C–D), 1496, 1454, 1373, 1254, 1215, 1114, 1076, 1034, 1003 cm^{-1}. ^{1}H NMR (800 MHz, $CDCl_3$) δ: 1.31/1.37/1.43/1.49 [each 3H, s, $(CH_3)_2C$], 4.00 (1H, dd, J = 8.8, 5.6 H-6a), 4.02 (1H, d, J = 3.2, H-3), 4.11 (1H, dd, J = 8.8, 6.4, H-6a), 4.15 (1H, dd, J = 7.2, 3.2, H-4), 4.37 (1H, ddd, J = 7.2, 6.4, 5.6, H-5), 4.58 (1H, s, H-2), 4.64/4.68 (each 1H, d, J = 11.2, CH_2Ph), 7.27–7.37 (5H, m, arom.). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 25.4/26.2/26.77/26.83 [s, $(CH_3)_2C$], 67.4 (C-6), 72.3 (CH_2Ph), 72.5 (C-5), 81.3 (C-4), 81.7 (C-3), 82.6 (C-2), 105.0 (t, J = 27.4, C-1), 109.0/111.8 [$(CH_3)_2C$)], 127.6/127.8/128.4 (d, arom.), 137.6 (s, arom.). LRMS (FAB) *m/z*: 352 $[M+H]^+$, 374 $[M+Na]^+$. HRMS (FAB) *m/z*: $[M+H]^+$ Calcd for $C_{19}H_{26}DO_6$ 352.1869; Found 352.1872.

3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose-1-*C*-*d* (16). A mixture of **15** (4.28 g, 12.2 mol) and 70% aqueous acetic acid (60 mL) was heated at 50 °C for 4.5 h. After the reaction mixture was

poured into ice-cooled water (400 mL), the resulting mixture was neutralized with sodium hydrogen carbonate and extracted with AcOEt. The extract was washed with brine and condensed to give a pale yellow oil (3.54 g), which on column chromatography (*n*-hexane/AcOEt = 10/1) gave the title compound **15** (2.96 g, 78%) as a colorless oil. $[\alpha]_D^{24}$ –47.7 (c 1.10, $CHCl_3$). IR (neat): 3418, 2985, 2935, 2877, 2199 (C–D), 1497, 1454, 1377, 1354, 1303, 1253, 1215, 1192, 1153, 1076, 1029 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.32/1.49 [each 3H, s, $(CH_3)_2C$], 2.27/2.56 (each 1H, br s, OH), 3.69 (1H, dd, *J* = 11.5, 5.5, H-6a), 3.81 (1H, dd, *J* = 11.5, 3.5, H-6b), 4.03 (1H, ddd, *J* = 7.8, 5.5, 3.5, H-5), 4.10 (1H, d, *J* = 3.2, H-3), 4.13 (1H, dd, *J* = 7.8, 3.2, H-4), 4.55/4.74 (each d, *J* = 11.8, CH_2Ph), 4.63 (1H, s, H-2), 7.30–7.38 (5H, m). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 26.2/26.7 [$(CH_3)_2C$], 64.3 (C-6), 69.3 (C-5), 72.1 (CH_2Ph), 79.9 (C-4), 82.0 (C-2/C-3), 104.9 (t, *J* = 27.4, C-1), 111.8 [$(CH_3)_2C$], 127.9/128.3/128.7 (d, arom.), 137.1 (s, arom.). LRMS (FAB) *m/z*: 312 $[M+H]^+$, 334 $[M+Na]^+$. HRMS (FAB) *m/z*: $[M+H]^+$ Calcd for $C_{16}H_{22}DO_6$ 312.1556; Found 312.1546.

Methyl 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranuronate-1-*C*-*d* (17). A mixture of **16** (2.96 g, 9.5 mmol), sodium metaperiodate (7.14 g, 33.3 mmol), sodium hydrogen carbonate (1.2 g, 14.3 mmol), water (45 mL), and THF (45 mL) was stirred at room temperature for 4 h. The precipitates were filtered off, and the filtrate was extracted with AcOEt. The extract was washed with brine and condensed to give the corresponding aldehyde as a pale yellow oil (2.66 g), as a pale yellow oil, which was then dissolved in a mixture of MeOH and water (9/1, 40 mL) and basified with sodium hydrogen carbonate (8.0 g, 95 mmol). To the mixture was added dropwise a solution of bromine (9.1 g, 56.9 mmol) in a mixture of MeOH and water (9/1, 20 mL) at room temperature, and the resulting mixture was stirred at room temperature for 7.5 h. After the reaction mixture was poured into aqueous sodium thiosulfate-sodium hydrogen carbonate (250 mL), the deposited precipitate was filtered off, and the filtrate was extracted with AcOEt. The extract was washed with brine and condensed to give a pale yellow oil (2.73 g), which on column chromatography (*n*-hexane/AcOEt = 10/1) gave the title compound **17** (1.92 g, 65%) as a colorless oil. $[\alpha]_D^{24}$ –43.9 (c 1.16, $CHCl_3$). IR (neat): 2990, 2935, 2206 (C–D), 1767, 1736, 1497, 1454, 1439, 1373, 1300, 1211, 1115, 1076, 1038 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.32/1.48 [each 3H, s, $(CH_3)_2C$], 3.75 (3H, s, CO_2CH_3), 4.26 (1H, dd, *J* = 3.7, 0.6, H-3), 4.52/4.66 (each 1H, d, *J* = 12.0, CH_2Ph), 4.61 (1H, s-like, H-2), 4.83 (1H, d, *J* = 3.7, H-4), 7.24–7.37 (5H, m, arom.). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 26.3/26.9 [$(CH_3)_2C$], 52.2 (CO_2CH_3), 72.3 (CH_2Ph), 79.6 (C-4), 81.7 (C-2), 82.8 (C-3), 105.5 (t, *J* = 27.4, C-1), 112.4 [$(CH_3)_2C$], 127.7, 128.0, 128.4 (d, arom.), 136.9 (s, arom.), 168.3 (CO_2CH_3). LRMS (FAB) *m/z*: 310 $[M+H]^+$, 332 $[M+Na]^+$. HRMS (FAB) *m/z*: $[M+H]^+$ Calcd for $C_{16}H_{20}DO_6$ 310.1400; Found 310.1421.

3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-xylofurannose-1,5,5-*C*-*d*$_3$ (18). A solution of **17** (2.51 g, 8.13

mmol) in THF (10 mL) was added dropwise to a stirred suspension of lithium aluminum deuteride ($LiAlD_4$, 341 mg, 8.12 mmol) in THF (10 mL) at 0 °C, and the mixture was stirred at 0 °C for 30 min. The excess of deuteride was decomposed successively with AcOEt and 10% aqueous sodium hydroxide. The deposited gel was filtered off and was washed with THF. The combined filtrate and washings were condensed to give the title compound **18** (2.23 g) as a pale blown oil as an oil, which was used in the next step without purification. Analytical samples of the compound was obtained by means of column chromatography (*n*-hexane/AcOEt = 10/1→5/1→3/1) as a colorless oil. $[\alpha]_D^{24}$ –60.5 (c 1.27, $CHCl_3$). IR (neat): 3480, 2990, 2936, 2203/2110 (C–D), 1497, 1454, 1377, 1308, 1254, 1215, 1192, 1107, 1072, 1034, 1003 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.33/1.49 [each 3H, s, $(CH_3)_2C$], 2.18 (1H, s, OH), 4.02 (1H, d, J = 3.5, H-3), 4.27 (1H, dd, J = 3.5, H-4), 4.49/4.72 (each d, J = 12.0, CH_2Ph), 4.64 (1H, s, H-2), 7.29–7.38 (5H, m). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 26.3/26.8 [$(CH_3)_2C$], 60.3 (quint., J = 21.5, C-5), 71.8 (CH_2Ph), 79.9 (C-4), 82.3 (C-2), 82.7 (C-3), 104.7 (t, J = 27.4, C-1), 111.7 [$(CH_3)_2C$], 127.7/128.2/128.6 (d, arom.), 137.0 (s, arom.). LRMS (FAB) *m/z*: 284 $[M+H]^+$, 306 $[M+Na]^+$. HRMS (FAB) *m/z*: $[M+H]^+$ Calcd for $C_{15}H_{18}D_3O_5$ 284.1574 Found 284.1558.

Methyl 3-*O*-benzyl-α- and β-D-xylofurannoside-1,5,5-*C*-d_3 (α- and β-19). A mixture of the crude **18** (2.23 g), MeOH (90 mL), and concentrated sulfuric acid (1.5 mL) was stirred at room temperature for 15 h. The reaction was quenched by addition of excess sodium hydrogen carbonate, and the MeOH insoluble materials were filtered of and washed with MeOH. The combined filtrate and washings were condensed to give a pale yellow paste, which was extracted with $CHCl_3$. The $CHCl_3$ layer was condensed to give pale yellow oil (1.91 g), which on column chromatography (*n*-hexane/AcOEt = 10/1→5/1→3/1) gave a *ca*. 1:1 anomeric mixture of the title compounds **α-** and **β-19** (1.46 g, 70% from **17**) as a colorless oil. Pure two anomers of **19** partially separated by the column chromatography were used for analytical purpose.

Less polar isomer α-19: colorless oil. $[\alpha]_D^{24}$ +83.8 (c 0.26, $CHCl_3$). IR (neat): 3441, 2931, 2909, 2191/2106 (C–D), 1497, 1454, 1400, 1361, 1315, 1211, 1188, 1126, 1041 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.35 (1H, br s, OH), 2.65 (1H, d, J = 8.9, OH), 3.47 (3H, s, OCH_3), 4.13 (1H, dd, J = 6.9, 4.9, H-3), 4.24 (1H, d, J = 6.9, H-4), 4.29 (1H, dd, J = 8.9, 4.9, H-2), 4.61/4.84 (each 1H, d, J = 12.0, CH_2Ph), 7.29–7.39 (5H, m, arom.). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 55.5 (OCH_3), 61.4 (quint., J = 21.5, C-5), 72.0 (CH_2Ph), 77.3 (C-4), 77.9 (C-2), 84.3 (C-3), 101.1 (t, J = 26.2, C-1), 127.7/128.0/128.5 (d, arom.), 137.4 (s, arom.). LRMS (FAB) *m/z*: 258 $[M+H]^+$, 280 $[M+Na]^+$. HRMS (FAB) *m/z*: $[M+H]^+$ Calcd for $C_{13}H_{16}D_3O_5$ 258.1418 Found 258.1439.

More polar isomer β-19: colorless oil. $[\alpha]_D^{24}$ –85.2 (c 0.40, $CHCl_3$). IR (neat): 3406, 2927, 2160/2102 (C–D), 1496, 1454, 1396, 1334, 1211, 1176, 1099, 1049 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.44 (1H,

d, J = 4.3, OH), 2.55 (1H, br s, OH), 3.42 (3H, s, OCH_3), 4.08 (1H, dd, J = 6.9, 3.8, H-3), 4.30 (1H, dd, J = 4.3, 3.8, H-2), 4.33 (1H, d, J = 6.9, H-4), 4.57/4.71 (each 1H, d, J = 12.0, CH_2Ph), 7.28–7.38 (5H, m, arom.). ^{13}C-NMR (125 MHz, $CDCl_3$) δ : 55.8 (OCH$_3$), 61.5 (quint., J = 21.5, C-5), 72.6 (CH$_2$Ph), 79.8 (C-2), 80.3 (C-4), 84.3 (C-3), 108.9 (t, J = 26.2, C-1), 127.8/128.1/128.6 (d, arom.), 137.4 (s, arom.). LRMS (FAB) *m/z*: 258 $[M+H]^+$, 280 $[M+Na]^+$. HRMS (FAB) *m/z*: $[M+H]^+$ Calcd for $C_{13}H_{16}D_3O_5$ 258.1418 Found 258.1441.

Methyl 3-*O*-benzyl-2,5-di-*O*-mesyl-D-xylofuranoside-*1,5,5-C-d*$_3$ (α- and β-20). Mesyl chloride (0.97 mL, 12.5 mmol) was added dropwise to a solution of the mixture of **α-** and **β-19** (1.46 g) in pyridine (10 mL) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was poured into ice-cooled water (50 mL) and extracted with AcOEt. The extract was washed with brine and condensed to give a ca. 1:1 anomeric mixture of the title compounds **α-** and **β-20**, as a pale yellow oil (2.33 g), which was used in the next step without purification. As two isomers **α-** and **β-20** were found hardly separable, analytical sample was obtained as a *ca*. 1:1 anomeric mixture of **α-** and **β-20** by column chromatography (*n*-hexane/acetone = 10/1→5/1→3/1) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 2.99/3.00/3.04/3.06 (each 3H, OSO$_2$CH_3), 3.450/3.453 (each 3H, s, OCH_3), 4.31 (0.5H, dd, J = 6.3, 2.0, Hβ-3), 4.41–4.46 (1H, m, Hα-3 and Hα-4), 4.56 (0.5H, d, J = 6.3, Hβ-4), 4.57 (1H, d, J = 12.0, CH_2Ph x 2), 4.73/4.75 (each 0.5 H, d, J = 12.0, CH_2Ph), 4.96 (0.5H, d, J = 4.9, Hα-2), 5.03 (0.5H, d, J = 2.0, Hβ-2), 7.30–7.40 (5H, m, arom.). ^{13}C-NMR (125 MHz, $CDCl_3$) δ : 37.48/37.54/38.4/38.7 (OSO$_2$$CH_3$), 55.78/55.83 (O$CH_3$), 67.6/68.4 (each quint., J = 21.5, Cα-5/Cβ-5), 72.7/73.0 (CH$_2$Ph), 74.1/78.6 (Cα-4/Cβ-4), 79.5/80.7 (Cα-3/Cβ-3), 81.6/84.2 (Cα-2/Cβ-2), 99.9/106.7 (each t, J = 26.2, Cα-1/Cβ-1), 128.0/128.2/128.3/128.6/128.7 (d, arom.), 136.5/136.6 (s, arom.). LRMS (FAB) *m/z*: 436 $[M+Na]^+$. HRMS (FAB) *m/z*: $[M+Na]^+$ Calcd for $C_{15}H_{19}D_3NaO_9S_2$ 436.0788 Found 436.0762.

2,5-Dideoxy-2,5-epithio-3-*O*-benzyl-α- and β-D-lyxofuranoside-1,1,5-*C-d*$_3$ (α- and β-21). A mixture of the crude dimesylate **α-** and **β-20** (2.33 g), sodium sulfide nonahydrate (2.71 g, 11.3 mmol), and DMF (35 mL) was heated at 100 °C for 4 h. After being cooled, the mixture was poured into ice-cooled water (180 mL) and extracted with AcOEt. The extract was washed with brine and condensed to a pale brown oil (1.49 g), which on column chromatography (*n*-hexane/AcOEt = 10/1→5/1) gave a *ca*. 1:1 anomeric mixture of the title compounds **α-** and **β-21** (1.12 g, 77% from **19**) as a colorless oil. Pure two anomers of **21** partially separated by the column chromatography were used for analytical purpose.

Less polar isomer α-21: colorless oil. $[\alpha]_D^{25}$ +30.5 (c 1.27, $CHCl_3$). IR (neat): 2927, 2909, 2168 (C–D), 1497, 1454, 1350, 1288, 1242, 1203, 1146, 1033 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 3.33 (1H, d, J = 2.3 Hz, H-2), 3.34 (3H, s, OCH$_3$), 4.36 (1H, d, J = 2.9 Hz, H-4), 4.48 (1H, dd, J = 2.9, 2.3 Hz, H-3), 4.50/4.63 (1H, d, J = 11.5 Hz, CH_2Ph), 7.27–7.37 (5H, m). ^{13}C-NMR (125 MHz, $CDCl_3$) δ : 34.4 (quint.,

J = 21.4, C-5), 48.4 (C-2), 55.0 (O*C*H$_3$), 72.1 (*C*H$_2$Ph), 76.0 (C-4), 79.8 (C-3), 109.3 (t, *J* = 27.4, C-1), 127.9/128.0/128.5 (d, arom.), 137.5 (s, arom.). LRMS (FAB) *m/z*: 256 $[M+H]^+$, 278 $[M+Na]^+$. HRMS (FAB) *m/z*: $[M+H]^+$ Calcd for $C_{13}H_{14}D_3O_3S$ 256.1084 Found 256.1089.

More polar isomer β-21: colorless oil. $[\alpha]_D^{24}$ –47.5 (c 1.32, $CHCl_3$). IR (neat): 2956, 2927, 2126 (C–D), 1496, 1454, 1358, 1300, 1242, 1207, 1153, 1130, 1107, 1080, 1033 cm^{-1}. ^{1}H-NMR (800 MHz, $CDCl_3$) δ: 3.48 (3H, s, O*C*H$_3$), 3.50 (1H, d, *J* = 2.0, H-2), 4.29 (1H, dd, *J* = 3.2, 2.0, H-3), 4.35 (1H, d, *J* = 3.2, H-4), 4.54/4.66 (each 1H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 7.30–7.39 (5H, m). ^{13}C-NMR (200 MHz, $CDCl_3$) δ: 34.5 (quint., *J* = 22.0, C-5), 50.4 (C-2), 56.4 (OCH_3), 71.7 (*C*H$_2$Ph), 78.1 (C-4), 80.0 (C-3), 105.9 (t, *J* = 25.8, C-1), 127.9/128.1/128.5 (d, arom.), 137.2 (s, arom.). LRMS (FAB) *m/z*: 256 $[M+H]^+$, 278 $[M+Na]^+$. HRMS (FAB) *m/z*: $[M+H]^+$ Calcd for $C_{13}H_{14}D_3O_3S$ 256.1084 Found 256.1092.

3-*O*-Benzyl-1,4-dideoxy-1,4-epithio-D-arabinitol-*1,1,5,5-C-d*$_4$ (23). The crude mixture of **α-** and **β-21** (1.12 g) was dissolved in THF (4 mL), and was treated with 3*M* hydrochloric acid (8 mL) at room temperature for 2 h. The reaction was quenched with sodium hydrogen carbonate, and $NaBD_4$ (223 mg, 5.3 mmol) was added in small portionwise at 0 °C, and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with water (30 mL) and extracted with AcOEt. The extract was washed with brine and condensed to give a pale brown oil (968 mg), which on column chromatography (*n*-hexane/AcOEt = 10/1→5/1→3/1) gave the title compound **23** (683 mg, 64%) as a pale yellow oil. $[\alpha]_D^{24}$ +18.9 (c 1.19, $CHCl_3$). IR (neat): 3360, 2920, 2207/2099 (C–D), 1497, 1454, 1354, 1072 cm^{-1}. ^{1}H-NMR (800 MHz, $CDCl_3$) δ: 2.75/3.74 (each br s, OH), 3.58 (1H, d, *J* = 2.4, H-4), 3.95 (1H, dd, *J* = 2.6, 2.4, H-3), 4.38 (1H, br d-like, *J* = 2.6, H-2), 4.64 (2H, s, C*H*$_2$Ph), 7.29–7.37 (5H, m). ^{13}C-NMR (200 MHz, $CDCl_3$) δ: 37.6 (quint., *J* = 21.0, C-1), 53.3 (C-4), 62.6 (quint., *J* = 22.0, C-5), 72.0 (*C*H$_2$Ph), 76.6 (C-2), 89.0 (C-3), 127.7/127.9/128.5 (d, arom.), 137.7 (s, arom.) HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{12}H_{12}D_4O_3NaS$ 267.0963; Found 267.0965.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-epithio-D-arabinitol-*1,1,5,5-C-d*$_4$ (8). A solution of thiosugar (**23**, 552 mg, 2.27 mmol) in DMF (4 mL) was added dropwise to a mixture of sodium hydride (273 g, 6.8 mmol, 60% in liquid paraffin), benzyl bromide (0.8 mL, 6.73 mmol), and DMF (2 mL) at 0 °C. After being stirred at 0 °C for 30 min, the mixture was poured into ice-cooled water (30 mL) and extracted with AcOEt. The extract was washed with brine and condensed to give a pale yellow oil (1.2 g), which on column chromatography (*n*-hexane/AcOEt = 50/1→2/1) gave the title compound **8** (808 mg, 84%) as a colorless oil. $[\alpha]_D^{24}$ +5.3 (c 0.75, $CHCl_3$). IR (neat): 2862, 2172/2075 (C–D), 1605, 1497, 1454, 1389, 1358, 1323, 1207, 1096, 1026, 910 cm^{-1}. ^{1}H-NMR (800 MHz, $CDCl_3$) δ: 3.55 (1H, d, *J* = 3.4, H-4), 4.11 (1H, dd, *J* = 4.0, 3.4, H-3), 4.18 (1H, d, *J* = 4.0, H-2), 4.47/4.50 (each 1H, d, *J* = 11.9, CH_2Ph), 4.48/4.53 (each 1H, d, *J* = 12.0, CH_2Ph), 4.60/4.62 (each 1H, d, *J* = 12.0, CH_2Ph), 7.25–7.33 (15H, m, arom.).

^{13}C-NMR (200 MHz, $CDCl_3$) δ : 32.6 (quint., J = 21.1, C-1), 48.8 (C-4), 71.5/71.9/73.0 (CH_2Ph), 71.6 (quint., J = 23.0, C-5), 85.0/85.1 (C-2/C-3) 127.6/127.67/127.68/127.72/127.82/128.35/128.36/128.41 (d, arom.), 137.9/138.1/138.2 (s, arom.). HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{26}H_{24}D_4NaO_3S$ 447.1902; Found 447.1902.

2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-{(*S*)-[4-deoxy-1,3-*O*-isopropylidene-3-sulfooxy-D-erythritol-1-yl]-episulfoniumylidene}-D-arabinitol-1,1,5,5-*C*-*d*$_4$ (24). A mixture of thiosugar-1,1,5,5-*C*-*d*$_4$ (**8**, 200 mg, 0.47 mmol), cyclic sulfate (**9**, 180 mg, 0.80 mmol), potassium carbonate (26 mg, 0.19 mmol), and HFIP (1.5 mL) was heated under reflux for 24 h. After removal of the solvent at reduced pressure, the residue was purified on column chromatography ($CHCl_3$→$CHCl_3$/MeOH = 80/1→50/1) to give title compound (**24**, 269 mg, 88%) as a colorless amorphous. $[\alpha]_D^{24}$ –6.98 (c 1.96, $CHCl_3$). IR (neat): 2997, 2940, 2357/2330 (C–D), 1497, 1454, 1381, 1273, 1227, 1092, 1018 cm^{-1}. ^{1}H-NMR (800 MHz, $CDCl_3$) δ : 1.26/1.45 [each 3H, s, $(CH_3)_2C$], 3.79 (dd, J = 11.2, 10.0, H-4'a), 3.83 (1H, dd, J = 13.4, 3.3, H-1'a), 3.96 (1H, br s-like, H-4), 4.15 (1H, dd, J = 11.2, 5.8, H-4'b), 4.27 (1H, ddd-like, J = 9.9, 3.3, 2.3, H-2'), 4.36 (1H, m, H-3), 4.37 (1H, dd, J = 13.4, 2.3, H-1'b), 4.38/4.50 (each 2H, J = 11.7, CH_2Ph), 4.45/4.50 (each 2H, J = 12.0, CH_2Ph), 4.50 (1H, ddd, J = 10.0, 9.9, 5.8, H-3'), 4.56/4.61 (each 2H, J = 12.0, CH_2Ph), 4.54 (1H, m, H-2), 7.15–7.36 (15H, m, arom.). ^{13}C-NMR (200 MHz, $CDCl_3$) δ : 19.0/28.4 [$(CH_3)_2C$], 47.3 (quint.-like, J = ca. 22.0, C-1), 50.0 (C-1'), 62.3 (C-4'), 65.3 (C-4), 66.0 (quint.-like, J = *ca*. 23.9, C-5), 67.0 (C-3'), 69.8 (C-2'), 72.2/72.5/73.7 (CH_2Ph), 82.0 (C-2), 83.3 (C-3), 99.8 [$(CH_3)_2C$], 127.9/128.0/128.4/128.5/128.6/128.7/128.8/128.9 (d, arom.), 135.8/136.0/136.5 (s, arom.).

1,4-Dideoxy-1,4-{(*S*)-[(2*S*,3*S*)-2,4-dihydroxy-3-(sulfooxy)butyl]episulfoniumylidene}-D-arabinitol-1,1,5,5-*C*-*d*$_4$ Inner Salt [Salacinol-1,1,5,5-*C*-*d*$_4$ (7)]. A suspension of 10% palladium-on-carbon (200 mg) in 90% aqueous acetic acid (4 mL) was pre-equilibrated with hydrogen. To the suspension was added a solution of **23** (260 mg, 0.40 mmol) in 90% aqueous acetic acid (4 mL), and hydrogenation was continued at room temperature and atmospheric pressure until the hydrogen uptake ceased. The catalyst was filtered off, and the catalyst was washed with water. The combined filtrate and washings were condensed at recused pressure to give a pale yellow oil (165 mg), which on column chromatography (AcOEt/MeOH = 20/1→10/1→5/1→AcOEt/MeOH/H_2O = 5/1/0.5) gave the title compound **7** (100 mg, 74%) as a colorless prisms, mp 139–140 °C (from MeOH). $[\alpha]_D^{23}$ –1.60 (c 0.69, CH_3OH). IR (KBr): 3356, 1924, 2893, 2264/2118 (C–D), 1420 1303, 1246, 1184, 1111, 1092, 1056, 1011 cm^{-1}. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data of **7** were summarized in Table 1. MS spectrum (ESI, negative mode) and HRMS data of **7** were given in Figure 4.

LC-MS Instruments and Conditions. A series LC-20A Prominence HPLC system (Shimadzu Co., Kyoto, Japan) was equipped with a binary pump, a degasser, an autosampler, a thermostated column

compartment and a control module connected with a LCMS-2010EV mass spectrometer equipped with an electrospray ionization (ESI) interface. The chromatographic separation was performed on an Asahipak NH2P-50 column (5 μm particle size, 2.0mm i.d.×150mm, Showa Denko K.K., Tokyo, Japan) operated at 40 °C. The mobile phase was consisted of acetonitrile and water (78:22, v/v), and was delivered at a flow rate of 0.2 mL/min. The injection volume was 1 μL. The mass spectrometer was operated at negative mode with selected ion monitoring (SIM). The optimal operating parameters of ESI for the maximum signal intensity of the molecular ions were obtained by direct infusion of the standard solutions of salacinol (**1**) and salacinol 1,1,5,5-*C*-d_4 (**7**), and were as follows; neburizing gas flow: 1.5 L/min, drying gas pressure: 0.15 MPa, CDL temperature: 250 °C, block heater temperature: 200 °C, CDL voltage: Constant-mode (−25 V), Q-array DS & RF voltage: Scan-mode. Under SIM mode, deprotonated molecular ions ($[M-H]^-$) for each compound were observed at following retention times (**1** *m/z* 333, t_R 6.7 min and **7** *m/z* 337, t_R 6.7 min).

Standard Solution Preparation. Accurately weighed 2.00 mg of **1** or **7** was introduced into a 20 mL volumetric flask and made up to the volume with water, the solution being used as a stock standard solution (100 μg/mL). Aliquots of 1.0 mL of the stock standard solution were transferred into a 20 mL volumetric flask and made up to the volume with 50% MeOH, the solutions being used as working solution (5.0 μg/mL) for constructing calibration curves. For the calibration an aliquot of 1 μL of the solution was injected into the LC-MS system.

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