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Synthesis of highly deuterium-labeled (*R*)-K-13675, PPAR α agonist, for use as an internal standard for low-level quantification of drugs in plasma

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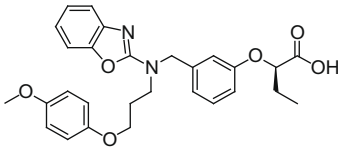
ABSTRACT

Two highly deuterium-labeled compounds, (*R*)-K-13675-d_{11} and (*R*)-K-13675-d_7, were prepared for use as internal standards for low-level quantification of plasma drugs by LC/MS/MS. We successfully demonstrated their utility in pharmacokinetic studies for sensitive and precise drug quantification.

1. Introduction

Peroxisome proliferator-activated receptor α (PPARα) is a member of one of the nuclear receptor superfamilies, activation of which leads to a decrease in triglyceride levels and an increase in HDL-cholesterol levels in humans.[1] During the course of our drug discovery program, we identified (*R*)-2-[3-[[benzoxazol-2-yl[3-(4-methoxyphenoxy)propyl]amino]methyl]phenoxy]butanoic acid ((*R*)-K-13675 (**1**), Fig. 1) as a highly potent and selective PPARα agonist, and its in vivo efficacy was shown to be more than 100 times higher than that of fenofibrate.[2]

As we might anticipate a clinical dose as low as one hundredth that of fenofibrate, it was necessary to develop and validate a method for quantifying trace amounts (less than ng/mL) of **1** in human plasma for pharmacokinetic studies. Deuterium-labeled compounds are well known to be useful as internal standards for such applications.[3] However, for precise low-level quantifications, the purities of deuterated compounds must be very high. Otherwise residual non-deuterated compounds that can still be detected with high sensitivity, might interfere. Therefore the efficient synthesis of such compounds from appropriate reagents is a significant challenge in medicinal chemistry. Although a number of deuteration methods have been reported,[4] most reactions require harsh reaction conditions or special equipment, are limited by substrate availability, and result in unsatisfactory deuterium incorporation. Despite these obstacles we succeeded in the synthesis of highly pure deuterium-labeled (*R*)-K-13675 as an excellent internal standard for sensitive and precise quantification in pharmacokinetic studies. Herein we report details of synthetic procedures and preliminary pharmacokinetic studies.



(*R*)-K-13675 (**1**)

Figure 1. Structure of (*R*)-K-13675.

2. Results and discussion

Our synthetic strategy towards deuterated (*R*)-K-13675 was based on the construction of two major precursors, 2-aminophenol-d_4 (**2**) and 4-methoxyphenol-d_7 (**3**) (Scheme 1). Envisaging that post-synthetic deuteration would be feasible in this synthetic scheme. We planned to synthesize **2** by hydrogen–deuterium (H–D) exchange displacement of 2-aminophenol (**5**) in deuterium oxide (D_2O),[5] while **3** was to be derived from a synthetic combination of the commercially available 1,4-hydroquinone-d_6 (**9**) and methanol-d_4.

First, we focused on the H–D exchange reaction of Sajiki et al.,[6] because it is applicable to a variety of aromatic compounds and its implementation is straightforward. Thus we conducted the H–D exchange reaction of 2-aminophenol (**5**) using 5% Pt/C (Aldrich, Bach#03505ME) in D_2O (99.9 atom % D; Aldrich) under a hydrogen

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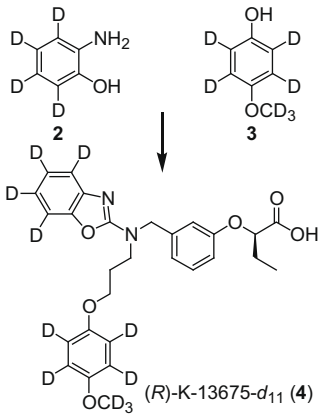
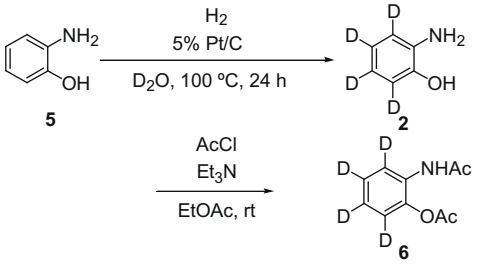


Scheme 1. Synthetic strategy of (*R*)-K-13675-d_{11}.

atmosphere at 100 °C for 24 h. The deuterium content was determined on the basis of acetyl groups by ^{1}H NMR after diacetylation of 2-aminophenol-d_4 (**2**) (Scheme 2, Table 1). Surprisingly, we could achieve a deuterium content of only 86% D, significantly lower than the values reported in the literature (89% D at 80 °C, 97% D at 180 °C).[6d] After these unsatisfying results, we decided on a different approach.

We then devised a method for exploiting the kinetic isotope effect,[7] in which almost full deuterium incorporation can be achieved by multiple cycles of Pt/C-catalyzed deuteration. The ratio of deuterium content was increased markedly in each cycle and reached 97% D by the third cycle. A slight increase was observed from the fourth cycle (fourth cycle, 97% D; fifth cycle, 98% D). As detailed in Table 1, this efficient method let to easily reproducible results even on a 10-g-scale (third cycle, 94% D; fifth cycle, 98% D). In summary, we have evidenced that it is possible to obtain highly deuterated **2** by repeating the Pt/C-catalyzed reaction under a hydrogen atmosphere, without requiring either extremely high temperatures or specialized equipment.



Scheme 2. Synthesis of 2-aminophenol-d_4 (**2**) and the diacetylated 2-aminophenol-d_4 **6**.

Table 1
Deuterium incorporation of 2-aminophenol-d4 (**2**) after H–D exchange reaction[a]

Reaction cycle	Deuterium content[b] (% D)
1	86
2	91
3	97
4	97
5	98

[a] The first reaction involved treatment of **5** (1.0 g) with 5% Pt/C (200 mg) in D_2O (50 mL, 99.9 atom% D) at 100 °C under a hydrogen atmosphere for 24 h. From the second cycle, the reactions were performed with the same ratio of reagents under the same conditions.

[b] Deuterium content (% D) of **6** was determined on the basis of the acetyl groups by ^{1}H NMR after diacetylation of **2**.

Next, we turned our attention to construction of the two key parts of (*R*)-K-13675-d_{11} (**4**) (Scheme 3).[8] Treatment of **2** with potassium xanthogenate in ethanol afforded the cyclized 2-mercaptobenzoxazole-d_4 (**7**), subsequent chlorination of which with thionyl chloride furnished 2-chlorobenzoxazole-d_4 (**8**) in moderate yield. For the other key part, after several attempts[9] we achieved the synthesis of 4-methoxyphenol-d_7 (**3**) by application of Bellas' method.[10] Exposure of **9** to benzoquinone-d_4 and sulfuric acid in methanol-d_4 at room temperature proceeded smoothly to provide the desired product **3** in 95% yield. Thus obtained, **3** was alkylated by Michael reaction with acrylonitrile in the presence of Triton B at 80 °C to give the nitrile **10** (64%), followed by reduction with borane-THF at 80 °C to provide the amine **11** (73%). Reductive alkylation of **11** with 3-hydroxybenzaldehyde (**12**) and sodium borohydride yielded the secondary amine **13** (90%). Interestingly, the ratio of deuterium content was unaffected in a series of reductive reaction conditions. The seven-deuterium-labeled amine **13** was reacted with **8** to afford **14** in 83% yield. Etherification of the labeled phenol **14** with *n*-butyl (*S*)-2-trifluoromethanesulfonyloxybutanoate in the presence of K_2CO_3 in acetonitrile at room temperature gave the phenyl ether **15** in 97% yield. Finally, hydrolysis of **15** in aqueous NaOH provided (*R*)-K-13675-d_{11} (**4**) in 72% yield.

Analogously (*R*)-K-13675-d_7 (**18**) was prepared from the non-deuterated 2-chlorobenzoxazole following the same synthetic operation (Scheme 4).

The deuterium-labeled compounds **4** and **18** were then analyzed by monitoring with LC/MS/MS.[11] The multiple reaction monitoring (MRM) ion chromatograms of (*R*)-K-13675-d_7 (**18**) and (*R*)-K-13675-d_{11} (**4**) are shown in Figure 2a and b. When both compounds were measured after injection of 5 ng, **18** was observed to contain a small amount (0.19%) of non-deuterated (*R*)-K-13675, and **4** showed a trace amount (0.02%) of (*R*)-K-13675, as summarized in Table 2.

For quantification of (*R*)-K-13675 (**1**) in rat plasma using isotope dilution mass spectrometry, a calibration curve for **1** was constructed by spiking 100 μL of rat plasma with 0.25 ng of (*R*)-K-13675-d_{11} (**4**) and 0.02, 0.04, 0.1, 0.2, 0.4, 1, 2, or 4 ng of **1**, respectively (Fig. 3 and Table 3).[12] The calibration curve showed excellent linearity (accuracy: 97.4–102%) and the correlation coefficient was 0.9999. Interestingly, compounds labeled with more deuterium were shown to be superior to those with less deuterium for validating low-level quantifications of plasma drugs by LC/MS/MS. Indeed, we carried out the quantifications of drugs in plasma using (*R*)-K-13675-d_{11} (**4**) as an internal standard administered to rats and dogs at a dose of 0.1 mg/kg. The results indicated that we have successfully established a method to determine low-level concentrations (0.1 ng/mL) of drugs in plasma in each species. Details of these pharmacokinetic studies will be reported elsewhere in due time.

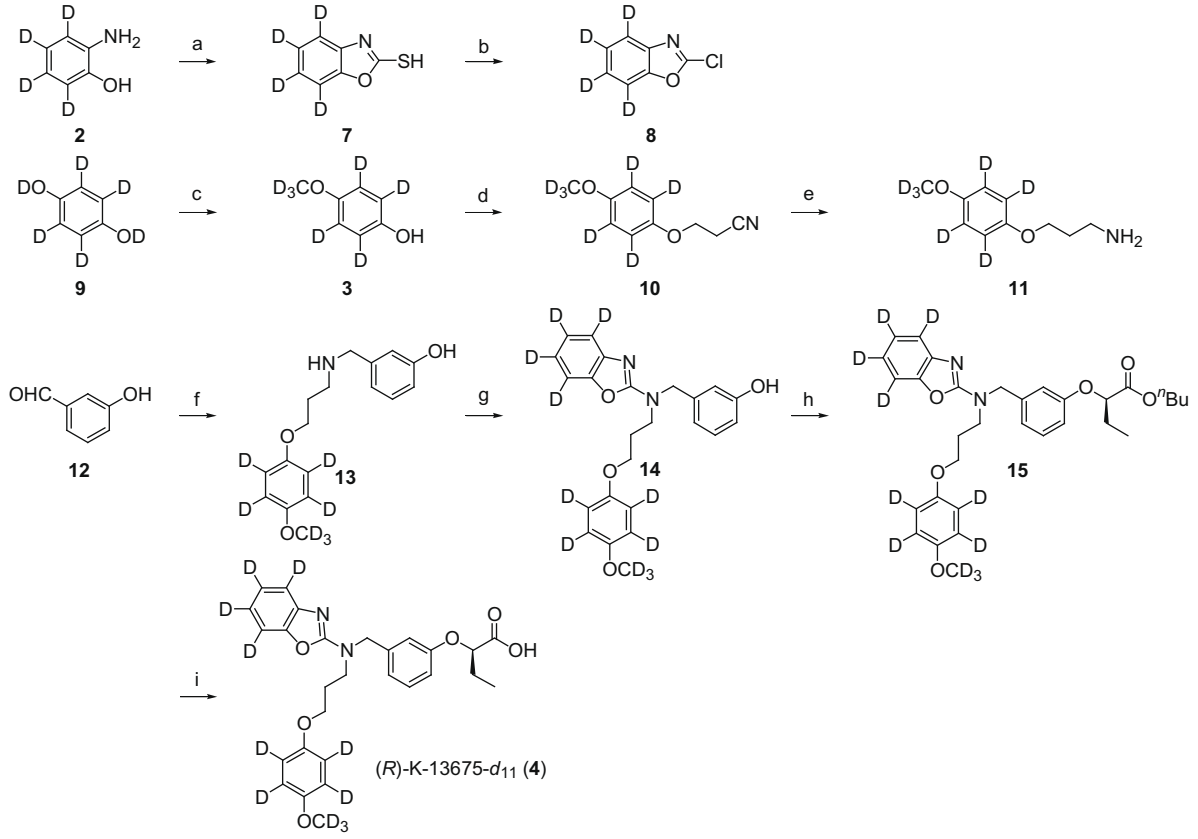
3. Conclusions

We have developed a useful method to obtain 2-aminophenol-d_4 with excellent deuterium incorporation by repetitive cycles of H–D exchange, and have succeeded in the synthesis of highly deuterated (*R*)-K-13675-d_{11} and (*R*)-K-13675-d_7. Furthermore, we demonstrated that (*R*)-K-13675-d_{11} served as an excellent internal standard for sensitive and precise quantification in pharmacokinetic studies.

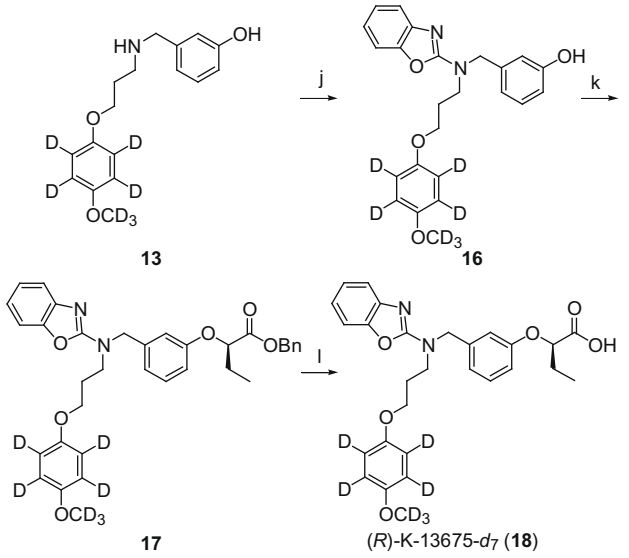
4. Experimental

4.1. General

Commercially available reagents and solvents were used without further purification. TLC analyses were carried out on silica gel 60 F_{254} plates (Merck). ^{1}H NMR and ^{13}C NMR spectra were re-



Scheme 3. Reagents and conditions: (a) potassium xanthogenate, EtOH, 55%; (b) $SOCl_2$, DMF, 49%; (c) benzoquinone-d_4, H_2SO_4, methanol-d_4 , 95%; (d) acrylonitrile, Triton B, 64%; (e) BH_3–THF, THF, 73%; (f) **11**, $NaBH_4$, MeOH, 90%; (g) **8**, $^iPr_2NEt$, DMF, 83%; (h) *n*-butyl 2-(*S*)-trifluoromethanesulfonyloxybutanoate, K_2CO_3, MeCN, 97%; (i) 4 M aq NaOH, EtOH, 72%.



Scheme 4. Reagents and conditions: (j) 2-chlorobenzoxazole, $^iPr_2NEt$, DMF, 87%; (k) benzyl (*S*)-2-trifluoromethanesulfonyloxybutanoate, K_2CO_3, MeCN, 100%; (l) 4 M aq NaOH, EtOH, 85%.

corded on a JEOL JNM-LA 400 MHz and JEOL GSX-270. Tetramethylsilane was used as an internal standard. Chemical shifts (δ) are given in parts per million (ppm), coupling constants *J* values are given in Hertz (Hz) and are reported to the nearest 0.1 Hz. Starred (*) values in the ^{13}C NMR are for small peaks. Infrared (IR) spectra were recorded on a Thermo Nicolet 370 FT-IR (ATR) spectrometer. Mass spectra were obtained on a JEOL MS-BU20 mass spectrometer. Elemental analyses (C, H, N) were performed by Yanaco MT-5. Melting points were determined in open glass capillaries on a Buchi B-545 melting point apparatus.

4.2. Synthesis of 2-aminophenol-d_4 (2) (large scale)

T o a suspension of 2-aminophenol (**5**) (10.0 g, 91.6 mmol) in D_2O (70 mL, 99.9 atom % D; Aldrich) was added 5% Pt/C (2.0 g, Aldrich, Bach; #03505ME) under an argon atmosphere. The reaction mixture was stirred at 100 °C under a hydrogen atmosphere for 24 h and diluted with EtOAc. The mixture was filtered through a pad of Celite. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo to give **2** as a brown solid (8.77 g). This obtained product was used in the next reaction without purification. The second reaction was performed with the same ratio of reagents under the same conditions. Finally, the desired product **2** was obtained after the fifth reaction cycle (5.57 g, 54%): ^{13}C NMR (68 MHz, DMSO-d_6) δ 113.71*, 114.20*, 118.63*, 119.25*, 136.41, 143.90; IR (solid sample): 3376, 3351, 3328, 3305, 1578, 1438, 1300 cm^{-1}; HRMS (EI): *m/z* [M^+] calcd for $C_6H_3D_4NO$: 113.0775; found: 113.0773.

4.3. Synthesis of the diacetylated 2-aminophenol-d_4 6. Determination of deuterium content (% D)

To a solution of 2-aminophenol-d_4 (**2**) (295 mg, 2.61 mmol) and triethylamine (527 mg, 5.21 mmol) in EtOAc (10 mL) was added acetyl chloride (409 mg, 5.21 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h and quenched with water. The organic layer was washed with water and brine, dried over

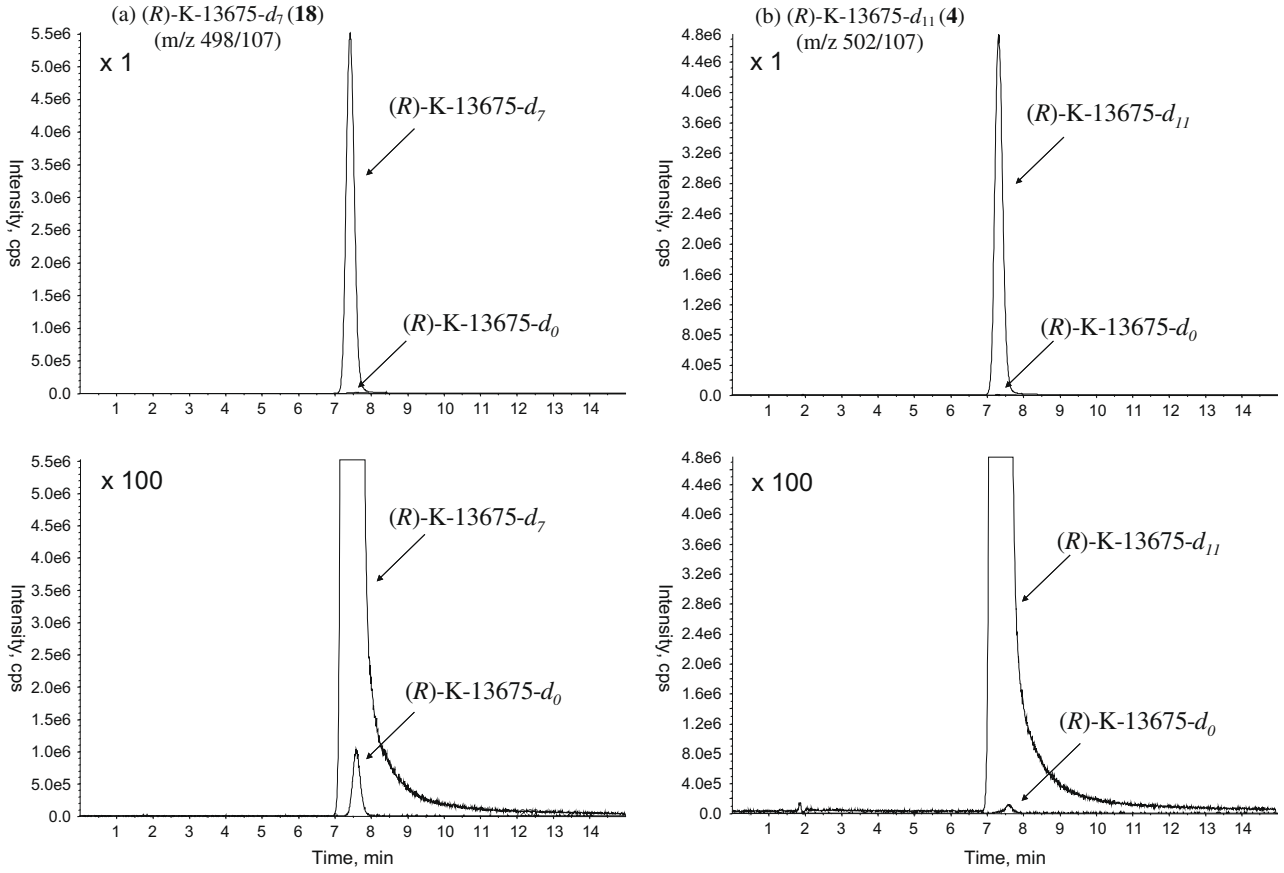


Figure 2. MRM ion chromatograms of the deuterated and non-deuterated peaks.

Table 2
Peak area and ratio in MRM ion chromatograms.[11]

Compounds	Peak area[a]		Ratio[b] (%)
	Deuterated	Non-deuterated	
4 (d_{11})	71,200,000 ± 1,100,000	16,900 ± 800	0.02 ± 0.00
18 (d_7)	88,800,000 ± 7,000,000	167,000 ± 7000	0.19 ± 0.01

[a] Values are means ± S.D. (n = 10).
[b] Ratio of non-deuterated compound (%) = (peak area of non-deuterated compound/ deuterated compound) × 100.

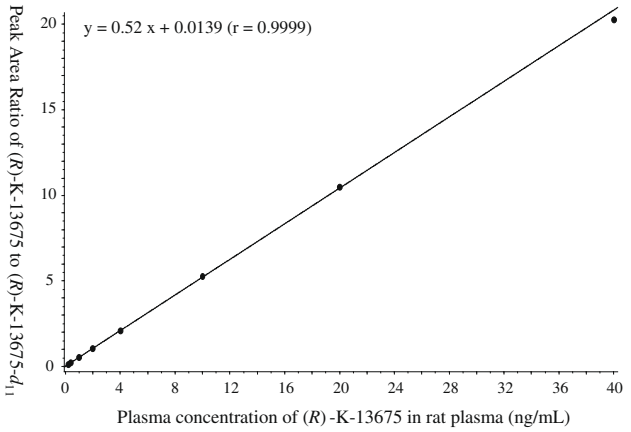


Figure 3. Calibration curve for (*R*)-K-13675 (**1**) in rat plasma.

Na_2SO_4 and concentrated in vacuo. The residue was purified by preparative TLC (*n*-hexane/EtOAc = 1:2) to give **6** as a yellow solid

Table 3
Accuracy of (*R*)-K-13675 (**1**) for calibration curve samples in rat plasma

	1	2	3	4	5	6	7	8
Concentration (ng/mL)	0.2	0.4	1	2	4	10	20	40
Accuracy[a] (%)	99.8	99.6	102	100	99.7	101	101	97.4

[a] Accuracy (%) = (calculated concentration/nominal concentration) × 100.12.

(131 mg, 26%): ^{1}H NMR (400 MHz, CD_3OD) δ 2.13 (s, 3H), 2.30 (s, 3H), 7.12 (s, 0.02H), 7.18 (s, 0.02H), 7.21 (s, 0.02H), 7.74 (s, 0.02H); Deuterium content on aromatic ring = 98% D; Anal. Calcd for $C_{10}H_7D_4NO_3$: C, 60.90; H, 3.58; D, 4.08; N, 7.10. Found: C, 60.92; H, 3.64; D, 4.04; N, 7.12; IR (solid sample) 3334, 1736, 1686, 1588, 1509, 1439, 1413 cm^{-1}; HRMS (EI): *m/z* [M^+] calcd for $C_{10}H_7D_4NO_3$: 197.09858; found: 197.09783; mp 123–124 °C.

4.4. Synthesis of 2-mercaptobenzoxazole-d_4 (7)

To a solution of 2-aminophenol-d_4 (**2**) (5.15 g, 45.5 mmol) in ethanol (100 mL) was added potassium xanthogenate (8.03 g, 50.1 mmol) at room temperature. The reaction mixture was stirred at 100 °C for 5 h and cooled to room temperature. Solvent was evaporated under reduced pressure. The residue was dissolved in water. The aqueous solution was acidified with 4 M HCl and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was recrystallized from EtOAc/*n*-hexane to give **7** as a red-brown crystal (2.94 g, 42%). The filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 4:1) to give **7** as a red-brown crystal (933 mg, 13%. Total yield; 3.87 g, 55%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.03 (s,

0.07H), 7.25 (s, 0.07H), 7.30 (s, 0.07H), 7.51 (s, 0.07H), 13.86 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 109.88*, 110.33*, 123.56*, 124.92*, 131.08, 148.07, 180.12; Anal. Calcd for C_7HD_4NOS: C, 54.17; H, 0.65; D, 5.19; N, 9.02. Found: C, 54.33; H, 0.68; D, 5.29; N, 8.91; IR (solid sample): 3341, 1599, 1476, 1368, 1327, 1112, 932 cm^{-1}; MS (EI) *m/z* 155 [M$^+$]; mp 186–189 °C.

4.5. Synthesis of 2-chlorobenzoxazole-d_4 (8)

To a solution of 2-mercaptobenzoxazole-d_4 (**7**) (2.00 g, 12.9 mmol) in thionyl chloride (4 mL) was added *N*,*N*-dimethylformamide (940 mg, 12.9 mmol) dropwise over 5 min at 0 °C under an argon atmosphere. The reaction mixture was stirred at same temperature for 2 h and concentrated in vacuo. The residue was dissolved in EtOAc. Water was added. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 10:1) to give **8** as a yellow oil (999 mg, 49%): ^{13}C NMR (68 MHz, $CDCl_3$) δ 110.11*, 119.39*, 124.07*, 125.11*, 143.73, 150.78, 151.39; Anal. Calcd for C_7D_4ClNO: C, 53.35; D, 5.11; N, 8.89. Found: C, 53.21; D, 5.39; N, 8.77; IR (neat): 1783, 1584, 1523, 1436, 1370, 1208, 1113 cm^{-1}; MS (EI) *m/z* 157 [M$^+$], 159 [M$^+$+2].

4.6. Synthesis of 4-methoxyphenol-d_7 (3)

To a solution of 1,4-hydroquinone-d_6 (**9**) (98 atom% D, 4.48 g, 38.6 mmol) and benzoquinone-d_4 (98 atom% D, 325 mg, 2.90 mmol) in methanol-d_4 (99.8 atom % D, 25 mL) was added sulfuric acid (4.80 g) over 5 min at room temperature. The reaction mixture was stirred for 14 h, poured into ice-water and neutralized with 4 M aqueous NaOH. The aqueous solution was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5:1) to give **3** as a pale yellow solid (4.83 g, 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.72–3.76 (m, 0.06H), 4.87 (s, 1H), 6.76 (s, 0.10H), 6.79 (s, 0.10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.06*, 114.59*, 115.80*, 149.41, 153.48; IR (solid sample): 3381, 2521, 2262, 2069, 1419, 1141, 1112 cm^{-1}; HRMS (EI): *m/z* [M$^+$] calcd for $C_7HD_7O_2$: 131.09564; found: 131.09642; mp 56–57 °C.

4.7. Synthesis of 3-(4-methoxyphenoxy-d_7)propionitrile (10)

To acrylonitrile (3.50 g, 65.3 mmol) were added 4-methoxyphenol-d_7 (**3**) (4.28 g, 32.7 mmol) and Triton B (0.3 mL) at room temperature. The reaction mixture was stirred at 80 °C under an argon atmosphere for 22 h and diluted with EtOAc and water. The organic layer was washed with 1 M aqueous NaOH, water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was recrystallized from EtOAc/*n*-heptane to give **10** as colorless needles (3.87 g, 64%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.79 (t, *J* = 6.4 Hz, 2H), 3.72–3.75 (m, 0.01H), 4.13 (t, *J* = 6.4 Hz, 2H), 6.84 (s, 0.03H), 6.86 (s, 0.03H); deuterium content = 99% D; ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.63, 54.87*, 63.55, 114.38*, 115.66*, 117.35, 151.66, 154.55; Anal. Calcd for $C_{10}H_4D_7NO_2$: C, 65.19; H, 2.19; D, 7.65; N, 7.60. Found: C, 65.09; H, 2.22; D, 7.54; N, 7.54; IR (solid sample): 2930, 2886, 2255, 2219, 2069, 1446, 1420 cm^{-1}; MS (EI) *m/z* 184 [M$^+$]; mp 65–66 °C.

4.8. Synthesis of 3-(4-methoxyphenoxy-d_7)propylamine (11)

To a solution of **10** (3.82 g, 20.7 mol) in THF (20 mL) was added BH_3-THF (1.17 mol/L, 21.3 mL, 24.9 mmol) dropwise at room temperature under an argon atmosphere. The reaction mixture was stirred at 80 °C for 3 h and cooled to room temperature. Methanol (50 mL) was added slowly to the mixture at 0 °C. The mixture was stirred at 80 °C for 0.5 h, acidified with 4 M HCl until pH 1 at 0 °C and stirred at 80 °C for 14 h. Solvent was evaporated under reduced pressure. The residue was dissolved in water. The aqueous solution was washed with Et_2O. The aqueous layer was alkalinized with 4 M aqueous NaOH and extracted with toluene. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo to give **11** as a colorless solid (2.87 g, 73%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (br s, 2H), 1.90 (quintet, *J* = 6.1 Hz, 2H), 2.90 (t, *J* = 6.1 Hz, 2H), 3.60-3.64 (m, 0.01H), 4.00 (t, *J* = 6.1 Hz, 2H), 6.82 (s, 0.03H), 6.83 (s, 0.03H); ^{13}C NMR (100 MHz, CD_3OD) δ 33.48, 39.82, 55.24*, 67.64, 115.25*, 116.08*, 154.37, 155.20; IR (solid sample): 2937, 2872, 2216, 2070, 1600, 1426, 1378 cm^{-1}; HRMS (EI): *m/z* [M$^+$] calcd for $C_{10}H_8D_7NO_2$: 188.15349; found: 188.15294.

4.9. Synthesis of 3-[[[3-(4-methoxyphenoxy-d_7)propyl] amino]methyl]phenol (13)

To a solution of **11** (1.88 g, 10.0 mmol) in MeOH (25 mL) was added 3-hydroxybenzaldehyde (1.22 g, 10 mmol) at room temperature. The reaction mixture was stirred at 60 °C for 3 h. A solution of $NaBH_4$ (567 mg, 15.0 mmol) in water (10 mL) was added slowly to this mixture at 0 °C. The mixture was stirred at room temperature for 14 h and filtered off. The isolated solid was rinsed with water and dried in vacuo to give **13** as a colorless crystal (2.66 g, 90%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.82 (quintet, *J* = 6.8 Hz, 2H), 2.06 (br s, 1H), 2.60 (t, *J* = 6.8 Hz, 2H), 3.60 (s, 2H), 3.94 (t, *J* = 6.8 Hz, 2H), 6.59 (dd, *J* = 7.8, 2.2 Hz, 1H), 6.72 (d, *J* = 7.8 Hz, 1H), 6.74 (s, 1H), 7.07 (t, *J* = 7.8 Hz, 1H), 9.24 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.33, 45.50, 53.08, 54.43*, 66.37, 113.38, 114.72, 114.15*, 114.89*, 118.44, 128.92, 142.51, 152.57, 153.10, 157.26; Anal. Calcd for $C_{17}H_{14}D_7NO_3$: C, 69.36; H, 4.79; D, 4.79; N, 4.76. Found: C, 69.34; H, 4.90; D, 4.76; N, 4.75; IR (solid sample): 3275, 1580, 1480, 1431, 1390, 1156, 1109 cm^{-1}; MS (EI) *m/z* 294 [M$^+$]; mp 142–143 °C.

4.10. Synthesis of 3-[[benzoxazol-2-yl-d_4[3-(4-methoxy phenoxy-d_7)propyl]amino]methyl]phenol (14)

To a solution of **13** (1.87 g, 6.34 mmol) in DMF (15 mL) and *N*,*N*-diisopropylethylamine (983 mg, 7.61 mmol) was added **8** (999 mg, 6.34 mmol) at room temperature. The reaction mixture was stirred at 80 °C for 15 h and diluted with EtOAc and water. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2:1) to give **14** as an amorphous brown solid (2.19 g, 83%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 (quintet, *J* = 6.8 Hz, 2H), 3.45 (t, *J* = 6.8 Hz, 2H), 3.89 (t, *J* = 6.8 Hz, 2H), 4.65 (s, 2H), 6.70–6.73 (m, 2H), 6.75 (s, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 8.75 (s, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 27.62, 45.01, 52.07, 54.89*, 65.32, 108.84*, 113.12, 114.25*, 114.81*, 115.08*, 115.33, 118.77, 120.22*, 123.53*, 129.79, 137.60, 141.40, 148.07, 152.61, 153.77, 157.82, 162.28; Anal. Calcd for $C_{24}H_{13}D_{11}N_2O_4$: C, 69.37; H, 3.15; D, 5.33; N, 6.74. Found: C, 69.47; H, 3.24; D, 5.32; N, 6.75; IR (solid sample): 2941, 1633, 1567, 1424, 1393, 1153, 1111 cm^{-1}; HRMS (EI): *m/z* [M$^+$] calcd for $C_{24}H_{13}D_{11}N_2O_4$: 415.24150; found: 415.24342.

4.11. Synthesis of *n*-butyl (*R*)-2-[3-[[benzoxazol-2-yl-d_4 [3-(4-methoxyphenoxy-d_7)propyl]amino]methyl]phenoxy] butanoate (15)

To a solution of **14** (2.16 g, 5.20 mmol) and K_2CO_3 (1.08 g, 7.79 mmol) in MeCN (30 mL) was added a solution of *n*-butyl

(*S*)-2-trifluoromethanesulfonyloxybutanoate (1.97 mg, 6.76 mmol) in MeCN (10 mL) under an argon atmosphere. The reaction mixture was stirred at room temperature for 19 h and filtered off. The filtrate was concentrated in vacuo. The residue was dissolved in EtOAc. Water was added. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5:1) to give **15** as a brown oil (2.82 g, 97%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (t, J = 7.3 Hz, 3H), 1.05 (t, J = 7.1 Hz, 3H), 1.22–1.28 (m, 2H), 1.49–1.55 (m, 2H), 1.96 (quintet, J = 6.7 Hz, 2H), 2.14 (quintet, J = 6.8 Hz, 2H), 3.70 (t, J = 6.7 Hz, 2H), 3.96 (t, J = 6.7 Hz, 2H), 4.00-4.14 (m, 2H), 4.53 (t, J = 6.5 Hz, 1H), 4.72 (d, J = 15.6 Hz, 1H), 4.77 (d, J = 15.6 Hz, 1H), 6.76 (dd, J = 7.8, 2.2 Hz,1H), 6.86 (s, 1H), 6.89 (d, J = 7.8 Hz, 1H), 7.21 (t, J = 7.8 Hz, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 9.67, 13.59, 18.92, 26.17, 27.51, 30.48, 45.11, 52.06, 55.19*, 64.96, 65.50, 77.60, 108.64*, 113.73, 113.86*, 114.66, 115.32*, 115.78*, 120.15*, 120.68, 123.44*, 129.82, 138.67, 143.41, 148.56, 152.69, 153.76, 158.31, 162.15, 171.67; Anal. Calcd for $C_{32}H_{27}D_{11}N_2O_6$: C, 68.91; H, 4.88; D, 3.97; N, 5.02. Found: C, 68.80; H, 4.97; D, 3.94; N, 4.94; IR (neat): 2960, 2067, 1751, 1633, 1563, 1425, 1154 cm^{-1}; HRMS (EI): *m/z* [M$^+$] calcd for $C_{32}H_{27}D_{11}N_2O_6$: 557.34087; found: 557.34196; $[\alpha]_D^{20}$ +19.1 (*c* 0.89, $CHCl_3$).

4.12. Synthesis of (*R*)-2-[3-[[benzoxazol-2-yl-d_4 [3-(4-methoxyphenoxy-d_7)propyl]amino]methyl]phenoxy] butanoic acid; (*R*)-K-13675-d_{11} (4)

To a solution of **15** (2.70 g, 4.84 mmol) in EtOH (20 mL) was added 4 M aqueous NaOH (2.4 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h, diluted with water, acidified with 4 M HCl and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 10:1) and recrystallized from EtOAc/*n*-heptane to give (*R*)-K-13675 d_{11} as a colorless crystal (1.74 g, 72%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.96 (t, J = 7.1 Hz, 3H), 1.79-1.89 (m, 2H), 2.06 (quintet, J = 6.8 Hz, 2H), 3.65 (t, J = 6.8 Hz, 2H), 3.94 (t, J = 6.8 Hz, 2H), 4.60 (t, J = 6.4 Hz, 1H), 4.72 (s, 2H), 6.78 (dd, J = 7.8, 2.4 Hz, 1H), 6.86 (s, 1H), 6.89 (d, J = 7.8 Hz, 1H), 7.25 (t, J = 7.8 Hz, 1H), 13.1 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 9.53, 25.42, 27.12, 45.29, 51.27, 65.47, 76.43, 113.30, 114.13, 119.77, 129.76, 139.02, 143.19, 148.43, 152.31, 153.25, 158.04, 162.24, 172.46; Anal. Calcd for $C_{28}H_{19}D_{11}N_2O_6$: C, 67.04; H, 3.82; D, 4.42; N, 5.58. Found: C, 66.91; H, 3.86; D, 4.34; N, 5.50; IR (solid sample): 2959, 2939, 2887, 1716, 1628, 1426, 1377 cm^{-1}; MS (FAB) *m/z* 502 [M$^+$+1]; mp 95–96 °C; $[\alpha]_D^{24}$ +17.1 (*c* 0.67, MeOH).

4.13. Synthesis of 3-[[benzoxazol-2-yl[3-(4-methoxyphenoxy-d_7)propyl]amino]methyl]phenol (16)

To a solution of **13** (6.22 g, 21.1 mmol) in DMF (60 mL) and *N*,*N*-diisopropylethylamine (2.73 g, 21.1 mmol) was added 2-chlorobenzoxazole (3.24 g, 21.1 mmol) at room temperature. The reaction mixture was stirred at 80 °C for 14 h and diluted with EtOAc and water. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2:1) to give a yellow oil. The oil was recrystallized from *tert*-butyl methyl ether to give **16** as a colorless crystal (7.56 g, 87%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.06 (quintet, J = 6.8 Hz, 2H), 3.65 (t, J = 6.8 Hz, 2H), 3.94 (t, J = 6.8 Hz, 2H), 4.70 (s, 2H), 6.70 (dd, J = 7.8, 1.7 Hz, 1H), 6.69-6.76 (m, 2H), 7.00 (td, J = 7.8, 1.2 Hz, 1H), 7.15 (t, J = 7.8 Hz, 2H), 7.30 (d, J = 7.8 Hz, 1H), 7.36 (d, J = 7.8 Hz, 1H), 9.43 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.13, 45.22, 51.31, 54.45*, 65.50, 108.81, 113.92, 114.16*, 114.42, 115.04*, 115.59, 117.90, 120.13, 123.88, 129.64, 138.73, 143.31, 148.46, 152.30, 153.27, 157.63, 162.26; Anal. Calcd for $C_{24}H_{17}D_7N_2O_4$: C, 70.05; H, 4.16; D, 3.43; N, 6.81. Found: C, 70.05; H, 4.27; D, 3.42; N, 6.77; IR (solid sample): 2937, 1638, 1600, 1582, 1459, 1424, 1394 cm^{-1}; MS (EI) *m/z* 411 [M$^+$]; mp 100–101 °C.

4.14. Synthesis of benzyl (*R*)-2-[3-[[benzoxazol-2-yl-d_4 [3-(4-methoxyphenoxy-d_7)propyl]amino]methyl]phenoxy] butanoate (17)

To a solution of **16** (4.59 g, 11.2 mmol) and K_2CO_3 (2.31 g, 16.7 mmol) in MeCN (60 mL) was added a solution of benzyl (*S*)-2-trifluoromethanesulfonyloxybutanoate (4.00 g, 12.3 mmol) under an argon atmosphere. The reaction mixture was stirred at room temperature for 11 h and filtered off. The filtrate was concentrated in vacuo. The residue was dissolved in EtOAc. Water was added. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5:1) to give **17** as a colorless oil (6.97 g, 100%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (t, J = 7.2 Hz, 3H), 1.96 (quintet, J = 7.1 Hz, 2H), 2.13 (quintet, J = 6.5 Hz, 2H), 3.68 (t, J = 6.5 Hz, 2H), 3.95 (t, J = 6.5 Hz, 2H), 4.57 (t, J = 6.7 Hz, 1H), 4.69 (d, J = 15.9 Hz, 1H), 4.74 (d, J = 15.9 Hz, 1H), 5.06 (d, J = 12.2 Hz, 1H), 5.15 (d, J = 12.2 Hz, 1H), 6.74 (dd, J = 7.8, 2.4 Hz, 1H), 6.84 (s, 1H), 6.89 (d, J = 7.8 Hz, 1H), 7.00 (d, J = 7.8 Hz, 1H), 7.14-7.30 (m, 8H), 7.37 (d, J = 7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.52, 26.03, 27.27, 45.12, 52.00, 54.48*, 65.47, 66.64, 77.48, 108.69, 113.83, 114.19*, 114.61, 115.23*, 116.10, 120.32, 120.66, 123.88, 128.15, 128.27, 128.45, 129.78, 135.30, 138.65, 143.43, 148.85, 152.63, 153.72, 158.17, 162.56, 171.30; Anal. Calcd for $C_{35}H_{29}D_7N_2O_6$: C, 71.53; H, 4.97; D, 2.40; N, 4.77. Found: C, 71.42; H, 5.11; D, 2.40; N, 4.75; IR (neat): 2938, 1752, 1638, 1459, 1578, 1425, 1153 cm^{-1}; HRMS (EI): *m/z* [M$^+$] calcd for $C_{35}H_{29}D_7N_2O_6$: 587.30052; found: 587.30184; $[\alpha]_D^{20}$ +16.4 (*c* 1.00, $CHCl_3$).

4.15. Synthesis of (*R*)-2-[3-[[benzoxazol-2-yl [3-(4-methoxy phenoxy-d_7)propyl]amino]methyl]phenoxy]butanoic acid; (*R*)-K-13675-d_7 (18)

To a solution of **17** (6.30 g, 10.7 mmol) in EtOH (60 mL) was added 4 M aqueous NaOH (5.4 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h and diluted with water. The aqueous solution was washed with Et_2O, acidified with *c*HCl at 0 °C and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 10:1) and recrystallized from EtOAc–*n*-heptane to give (*R*)-K-13675-d_7 as colorless needles (4.55 g, 85%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.98 (t, J = 7.0 Hz, 3H), 1.83-1.92 (m, 2H), 2.07 (quintet, J = 6.5 Hz, 2H), 3.67 (t, J = 6.5 Hz, 2H), 3.95 (t, J = 6.5 Hz, 2H), 4.64 (t, J = 6.3 Hz, 1H), 4.74 (s, 2H), 6.80 (dd, J = 7.8, 2.0 Hz, 1H), 6.90 (s, 1H), 6.91 (d, J = 7.8 Hz, 1H), 7.00 (t, J = 7.8 Hz, 1H), 7.15 (t, J = 7.8 Hz, 1H), 7.26 (t, J = 7.8 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.36 (d, J = 7.8 Hz, 1H), 13.1 (br s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 9.46, 25.41, 27.14, 45.29, 51.30, 54.45*, 65.49, 76.41, 108.85, 113.36, 114.17, 114.18*, 115.05*, 115.67, 119.85, 120.18, 123.89, 129.74, 139.01, 143.29, 148.51, 152.34, 153.29, 158.04, 162.25, 172.39; Anal. Calcd for $C_{28}H_{23}D_7N_2O_6$: C, 67.59; H, 4.66; D, 2.83; N, 5.63. Found: C, 67.66; H, 4.77; D, 2.82; N, 5.54; IR (solid sample): 2967, 2935, 1725, 1635, 1584, 1426, 1382 cm^{-1}; MS (FAB) *m/z* 498 [M$^+$+1]; mp 91–93 °C; $[\alpha]_D^{240}$ +16.5 (*c* 0.62, MeOH).

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5. Vaidyanathan et al. reported a simple and efficient method for post-synthesis of 4-aminophenol-d_4 by microwave irradiation in a CEM microwave oven at 140 W and 175 °C for 20 min in the presence of 35% deuterium chloride in D_2O.[4o] We attempted to apply this method for preparation of 2-aminophenol-d_4 (**2**) under the same reaction conditions except using a Biotage microwave oven. Unfortunately, satisfactory results could not be obtained due to the mechanistic differences in the microwave oven. The maximum pressure of the Biotage microwave oven was set as 22 bar. When the deuteration reaction was carried out at 140 W and 175 °C, the reaction stopped within 2 min because the pressure exceeded 22 bar.
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8. Refer to synthesis of (*R*)-K-13675: (a) Yamazaki, Y.; Araki, T.; Koura, M.; Shibuya, K. *Synthesis* **2008**, *7*, 1017–1022; (b) Yamazaki, Y.; Araki, T.; Koura, M.; Shibuya, K. *Tetrahedron* **2008**, *64*, 8155–8158.
9. We initially attempted the post-synthetic deuterium reaction of 4-methoxyphenol to give 4-methoxyphenol-d_7 (**3**) using 5% Pt/C in D_2O under a hydrogen atmosphere at 100 °C for 24 h. However, the H–D exchange displacement of the phenol occurred only at the aromatic ring and afforded 4-methoxyphenol-d_4 (64%, 66% D). When we conducted the methylation of 1,4-hydroquinone-d_6 (**9**) with iodomethane-d_3 and K_2CO_3 in acetonitrile, **9** was easily transformed into benzoquinone-d_4 via oxidation.
10. Bellas, M.; Cahill, R.; Hayes, L. G.B. Patent 1557237.
11. Liquid chromatography: Autosampler; 3133 HTS autosampler Z (Shiseido), degasser/binary pump/column compartment; Agilent 1100 Series (Agilent Technologies), Column; Symmetry C18 2.1 × 50 mm id, 5 μm (Waters). Mass spectrometer: API4000 triple-quadrupole mass spectrometer (Applied Biosystems) was equipped with an electrospray source, operating in the positive ion mode. Quantification was performed in multiple reaction monitoring (MRM) mode. The MS/MS was set to monitor at a transition of *m/z* 491/107 for (*R*)-K-13675, *m/z* 498/107 for (*R*)-K-13675-d_7 and *m/z* 502/107 for (*R*)-K-13675-d_{11}, respectively. MRM data were acquired and integrated using Analyst software (Ver.1.4.1, Applied Biosystems).
12. Methanol solution containing different amounts of **1** (0.02, 0.04, 0.1, 0.2, 0.4, 1, 2, and 4 ng/tube) was added to 250 μL of 2% propylene glycol in ethanol. After evaporation under nitrogen at 40 °C, rat plasma (100 μL) was added to the dried residue, and then 100 μL of aqueous solution of **4** (2.5 ng/mL) and 900 μL of 0.1% aqueous acetic acid were added to the sample. Each sample was vortexed, extracted with *tert*-butylmethyl ether (5 mL) with shaking for 10 min and centrifuged at 3000 rpm for 10 min. The organic layer (3 mL) was transferred into a glass tube and 250 μL of 2% propylene glycol in ethanol was added to the solution. After evaporation under nitrogen at 40 °C, the dried residue was dissolved in 150 μL of 50% aqueous methanol. The samples thus obtained (20 μL) were analyzed using LC/MS/MS and monitored at *m/z* 491 and 502.[11]