Figure S1

(a)

(b)

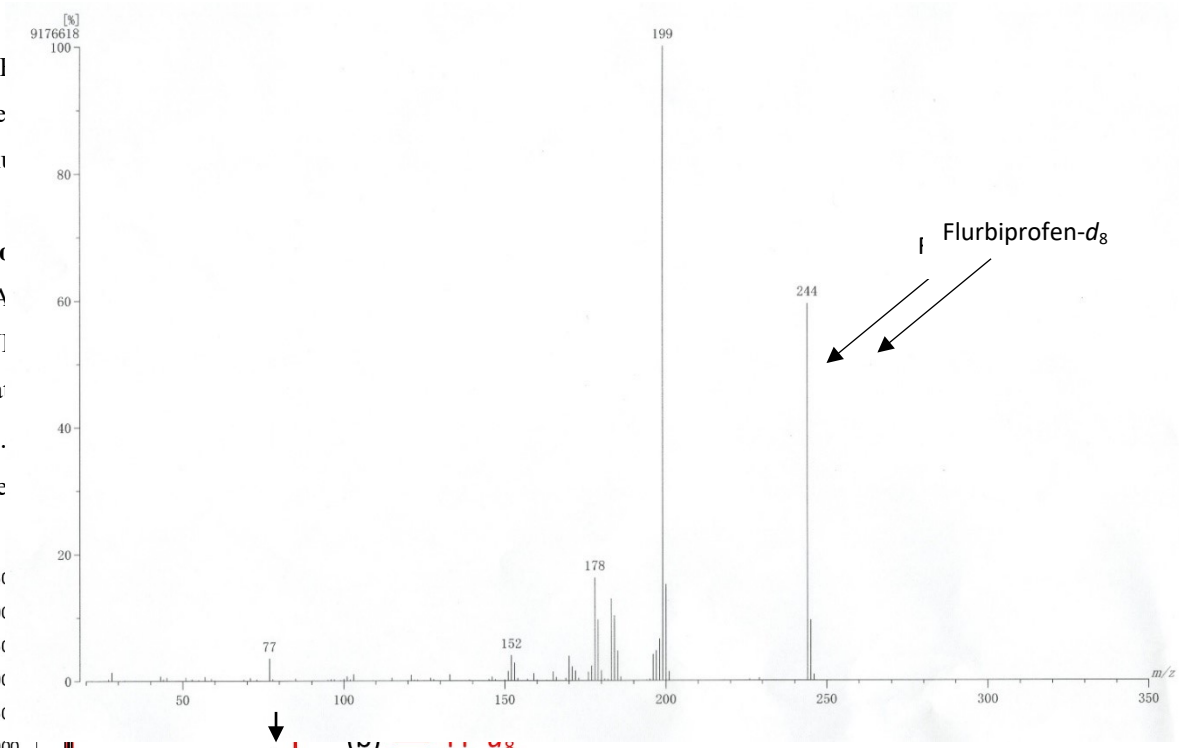

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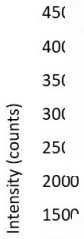
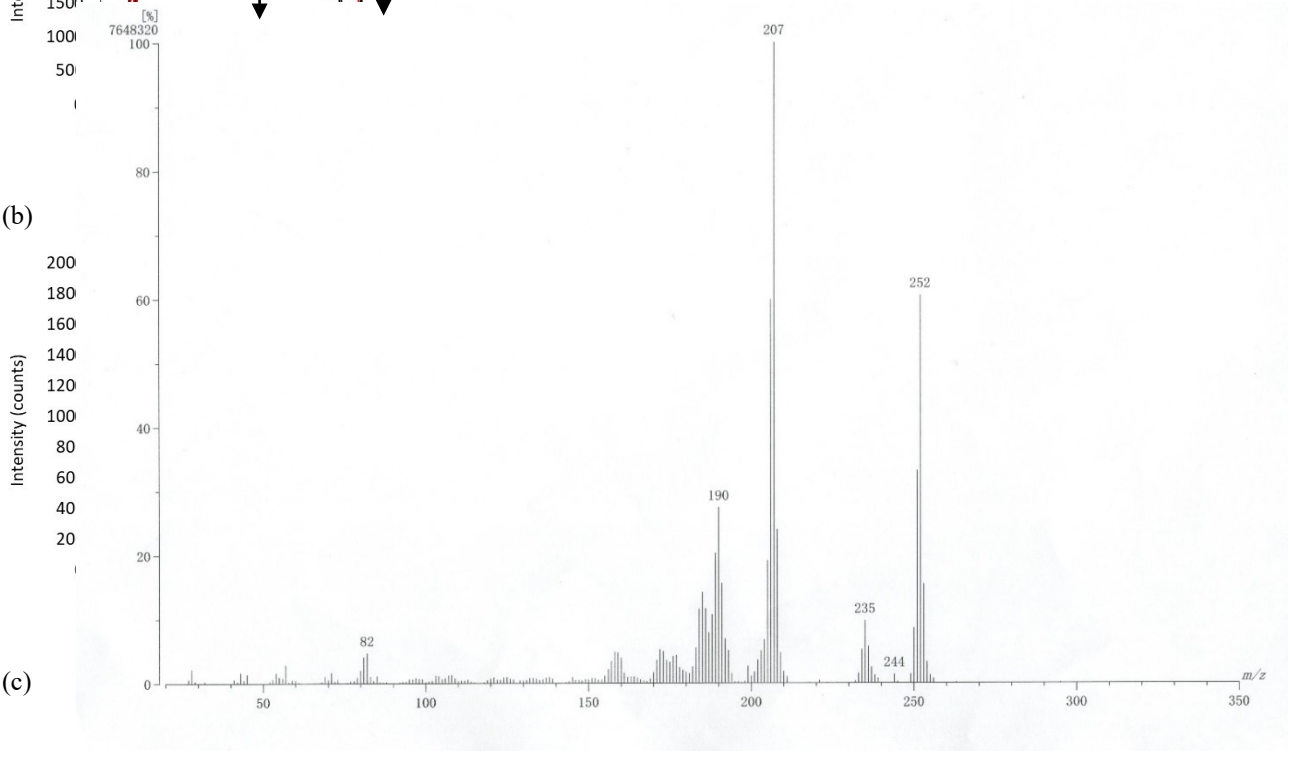
Figure

(a)



(b)



(c)

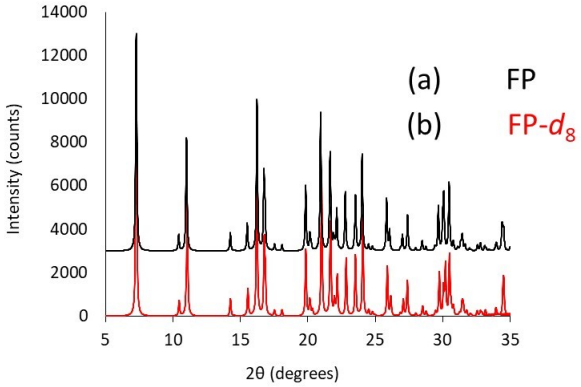


Figure S2 Powder X-ray diffraction (PXRD) patterns of flurbiprofen (FP) and flurbiprofen-d_8 (FP-d_8) from before recrystallization, (b) after recrystallization and (c) simulated single-crystal pattern.

Method

The Powder X-ray diffraction patterns of the powder samples were determined using an X-ray diffractometer (Miniflex; Rigaku Corporation, Tokyo, Japan) equipped with a split-detector and a Cu-Kα radiation source (30 kV, 15 mA, and λ = 0.15418 nm). The scanning rate was 2°/min over a 2θ range of 5–35°.

Figure S3

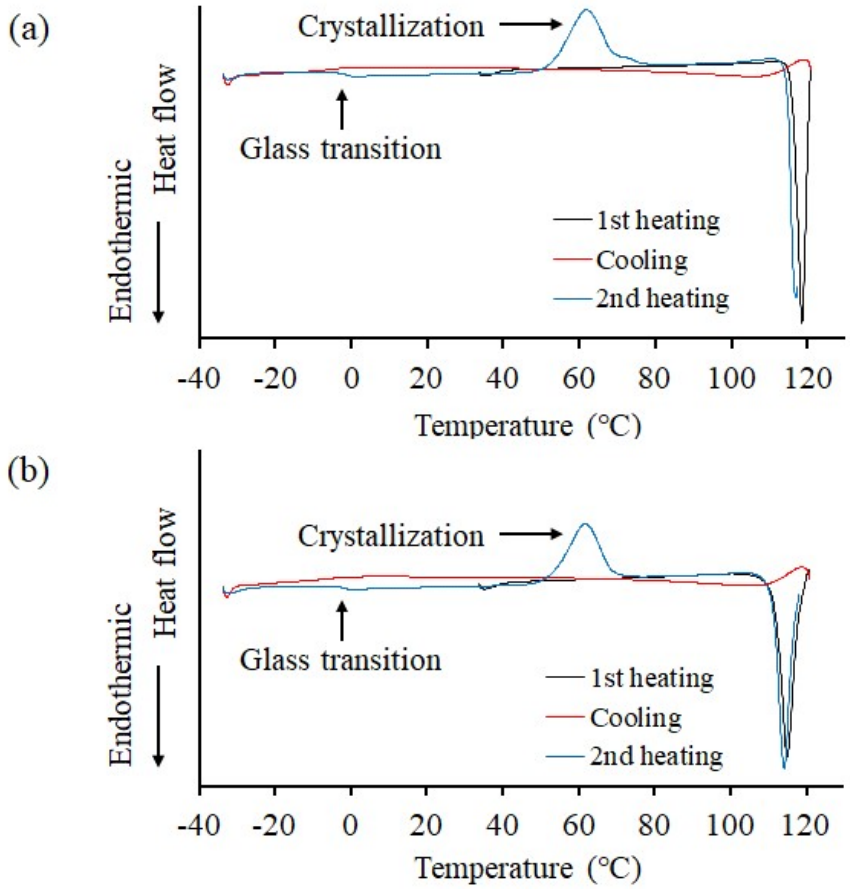


Figure S3 Differential scanning calorimetry (DSC) thermograms of (a) flurbiprofen (FP) and (b) flurbiprofen-d_8 (FP-d_8). The black line represents the first heating, blue line is the cooling profile, and the red line shows the second heating profile.

Method

DSC measurements were carried out with Hitachi DSC7000X (Hitachi High-Tech Science Corporation, Tokyo, Japan), and the cell was purged by nitrogen at a flow rate of 50 mL/min. For the evaluation of crystallization tendency, about 3 mg of the powders were put into aluminum pans. The samples were heated above melting temperature with the heating rate of 10°C/min and isothermally held for 3 min. The molten samples were cooled below −20°C with the cooling rate of 20°C/min and held for 5 min. The samples were reheated to above the melting temperature with a heating rate of 10°C/min.

Figure S4

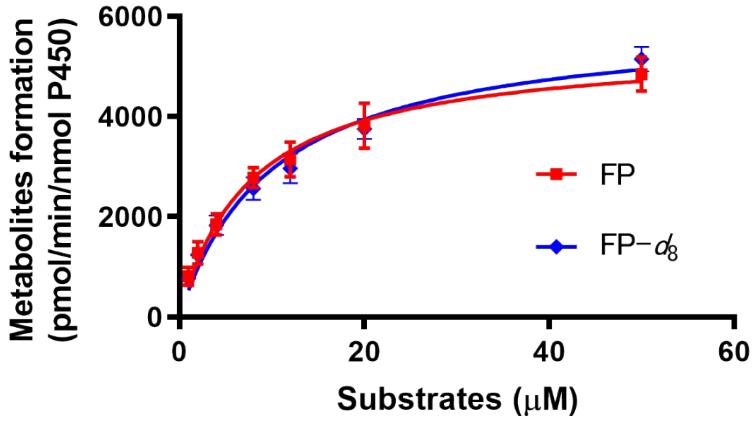


Figure S4 Michaelis-Menten plots of 4'-hydroxylation of flurbiprofen (FP) and flurbiprofen-d_8 (FP-d_8)

Supplemental Table S1

Table S1 Kinetic parameters of 4'-hydroxylation of flurbiprofen (FP) and flurbiprofen-d_8 (FP-d_8)

Substrate	K_m (μM)	V_{max} (pmol/min/nmol P450)	^{D}V	Clearance (V_{max}/K_m) (μL/min/nmol P450)	$^{D}(V/K)$
Flurbiprofen	7.67±0.72	5423±427	-	712.5±96.1	-

Flurbiprofen-d8	10.28±1.55	5957±313	0.911±0.069	587.0±69.1	1.243±0.341

Method

Flurbiprofen 4'-hydroxylation activities by recombinant CYP2C9 were compared between FP and FP-d_8 as substrates by noncompetitive intermolecular method. In brief, the mixture (0.5 ml) containing either FP or FP-d_8 (1.0 - 50 μM), 2.5 pmol of recombinant CYP2C9 (commercially available baculosomes coexpressing CYP2C9 and OR, Corning, Woburn, MA, USA) and an NADPH regenerating system were incubated at 37 °C for 10 min. Reactions were terminated by addition of 100 μl of 94 % acetonitrile and 6% acetic acid, followed by centrifugation at 3,000 g for 10 min at 4°C. The supernatants were filtered through polytetra-fluoroethylene membrane filters of 0.2 μm pore size (Millipore, Bedford, MA), and the aliquots (10 μl) were applied onto a InertSustain AQ-C18 HP column (3.0 μm; 2.1 x 100 mm, GL Sciences, Tokyo, Japan) kept at 40 °C. LC-MS analysis was performed using Fourier Transfom mass spectrometry (Q Exactive, Thermo Fisher Scientific). The kinetic parameters such as K_m, V_{max}, and intrinsic clearance (V_{max}/K_m) were estimated using a computer program designed for non-linear regression analysis of a hyperbolic Michaelis-Menten equation (Prism v.9, GraphPad Software, San Diego, CA, USA). Each value represents the mean ± S.D. of four separate experiments.