Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Rational creation of mutant enzyme showing remarkable enhancement of catalytic activity and enantioselectivity toward poor substrates

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[A] General.

All the DNA manipulations and bacterial transformation were carried out according to the standard protocols[1] or manufacturers' instructions, unless otherwise stated. *E. coli* BL21(DE3) (Stratagene) was used as a host for pET-11a (Stratagene) and their derivatives. *TaKaRa Ex Taq* DNA Polymerase was purchased from Takara Bio. Restriction enzymes and T4 DNA ligase were purchased from Toyobo or New England Biolabs. Agarose for electrophoresis was purchased from Nacalai Tesque. QIAprep Spin Miniprep Kit, QIAquick PCR Purification Kit, and QIAquick Gel Extraction Kit (Qiagen) were routinely used for DNA isolation and purification. Synthetic oligonucleotides were obtained from Sigma Genosys Japan. DNA sequencing was performed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) at Advanced Science Research Center of Okayama University, and analyzed with MacVector 9.5 (MacVector, Inc.). All the genes cloned into the plasmids were fully sequenced in both directions. The molecular-weight marker for SDS-PAGE (lysozyme (14 kDa), β-lactoglobulin (18 kDa), trypsinogen (24 kDa), ovalbumin (45 kDa), albumin (66 kDa)) was purchased from Sigma. The amount of proteins was determined by the method of Bradford using BSA as the standard.[2] TLC was performed on Merck silica gel 60 F_{254}, and silica gel column chromatography was performed on Fuji Silysia BW-127 ZH (100–270 mesh).

[B] Site-Directed Mutagenesis.

The mutation was introduced by the overlap-extension PCR method as described previously.[3] The expression plasmid called pELIP(I287F) having the I287F mutation was used as a template to prepare pELIP(I287F/I290A) and pELIP(I287F/I290F), while pELIP encoding the wild-type enzyme was used as a template to prepare pELIP(I290A). The mutagenic oligonucleotides used as primers and the plasmids used as templates are shown in Table S1.

Table S1 Primers and templates used for site-directed mutagenesis

Mutant	Primer	Sequence	Template
I287F/I290A	BC-I290A-1F	5'-GACGAGGCCAACCAGTTGC-3'	pELIP(I287F)
	BC-I290A-3R	5'-CAACTGGTTGGCCTCGTCG-3'	
I287F/I290F	BC-I290F-1F	5'-CGACGAGTTCAACCAGTTGC-3'	pELIP(I287F)
	BC-I290F-2R	5'-GCAACTGGTTGAACTCGTCG-3'	
I290A	BC-I290A-1F	5'-GACGAGGCCAACCAGTTGC-3'	pELIP
	BC-I290A-3R	5'-CAACTGGTTGGCCTCGTCG-3'	

Together with the above mutagenic primers, in the first PCRs, BC-LIP-9F (5'-CCGCCACGTACAACCAGAACTATC-3') and PET-2R (5'-GTTATTGCTCAGCGGTGG-3') were also used, and in the second PCR, BC-LIP-9F and PET-2R were used. The conditions for the 100 μL PCR mixture were as follows: 0.5 μM each primer, 0.2 mM each dNTP, pELIP(I287F) or

pELIP (1 ng) (first PCR) or the DNA fragments (1 ng) amplified in the first PCR (second PCR), 2.5 units of *TaKaRa Ex Taq* DNA Polymerase, 1.5 mM $MgCl_2$, and 10 μL of PCR buffer. PCR was done for 30 cycles of 98 °C for 10 s and 57 °C for 30 s followed by a final extension at 72 °C for 1 min. The DNA fragments were separated by 1.2% agarose gel electrophoresis and purified with QIAquick Gel Extraction Kit. After the second PCR, the amplified DNA fragment was digested with *Asc* I and *Bam* HI. The DNA fragment was purified as described above, and then ligated into pELIP that had been treated with the same restriction enzymes.[3] *E. coli* BL21(DE3) strain harboring a mutated plasmid DNA was obtained by transformation. Introduction of the mutation was confirmed by DNA sequencing.

[C] Preparation of Recombinant Lipases.

Recombinant lipases were overexpressed, refolded, purified, and immobilized as reported previously.[3]

[D] Synthesis of Racemic Alcohols.

1-Phenyl-1-hexanol (1a).[4] To a solution of *n*-pentyl phenyl ketone (5.28 g, 30.0 mmol) in dry EtOH (45 mL) was added $NaBH_4$ (574 mg, 15.2 mmol) in an ice bath. The mixture was stirred at room temperature for 3 h. The solution was adjusted to pH 4. After EtOH had been removed under reduced pressure, brine (9 mL) was added. The solution was neutralized, and the product was extracted with EtOAc (15 mL × 3). The mixture was dried over $MgSO_4$, and concentrated. The product was purified by distillation (4 mmHg, 110 °C) to afford **1a** as a colorless oil (4.35 g, 81%): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.87 (t, J = 6.8 Hz, 3H), 1.29–1.30 (m, 5H), 1.39–1.43 (m, 1H), 1.68–1.83 (m, 3H), 4.67 (dd, J = 5.9, 7.5 Hz, 1H), 7.27–7.35 (m, 5H).

1-Phenyl-1-heptanol (1b).[4] To a solution of *n*-hexyl phenyl ketone (5.71 g, 30.0 mmol) in dry EtOH (45 mL) was added $NaBH_4$ (580 mg, 15.3 mmol) in an ice bath. The mixture was stirred at room temperature for 3 h. The solution was adjusted to pH 4. After EtOH had been removed under reduced pressure, brine (10 mL) was added. The product was extracted with EtOAc (30 mL × 4). The mixture was dried over $MgSO_4$, and concentrated. The product was purified by distillation (4 mmHg, 120 °C) to afford **1b** as a colorless oil (3.00 g, 52%): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.87 (t, J = 7.0 Hz, 3H), 1.24–1.33 (m, 7H), 1.38–1.43 (m, 1H), 1.58 (br s, 1H), 1.67–1.73 (m, 1H), 1.77–1.83 (m, 1H), 4.67 (dd, J = 5.9, 7.5 Hz, 1H), 7.27–7.35 (m, 5H).

1-Phenyl-6,6,6-trifluoro-1-hexanol (1c). To a mixture of Mg (579 mg, 23.8 mmol) in dry Et_2O (12 mL) under N_2 was added I_2 (a few pieces), and subsequently a solution of 5-iodo-1,1,1-trifluoropentane (4.58 g, 18.2 mmol) in dry Et_2O (12 mL) was added dropwise over 50 min at room temperature. The mixture was stirred for 2 h. To the slurry was added benzaldehyde

(1.00 g, 9.46 mmol) dropwise over 45 min in an ice bath, and the mixture was stirred at room temperature for 2 h. The reaction was quenched with saturated aqueous NH_4Cl. After the mixture had been filtered, the filtrate was adjusted to pH 4. The mixture was extracted with Et_2O (30 mL × 3), dried over $MgSO_4$, and concentrated. The product was purified by silica gel column chromatography (hexane/EtOAc (15:1) to (5:1)) to afford **1c** as a colorless oil (1.68 g, 77%): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.30–1.38 (m, 1H), 1.45–1.52 (m, 1H), 1.55–1.60 (m, 2H), 1.67–1.73 (m, 1H), 1.77–1.83 (m, 1H), 2.01–2.09 (m, 2H), 2.31 (br s, 1H), 4.63 (dd, J = 6.0, 7.3 Hz, 1H), 7.28–7.37 (m, 5H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 21.7 (q, J_{CF} = 3.0 Hz), 24.9, 33.6 (q, J_{CF} = 28.3 Hz), 38.4, 74.2, 125.8, 127.1 (q, J_{CF} = 274.9 Hz), 127.6, 128.5, 144.5; ^{19}F NMR ($CDCl_3$, 565 MHz) δ –67.5 (t, J_{FH} = 11.0 Hz, 3F); IR (neat) 3366, 3032, 2945, 2874, 1495, 1456, 1391, 1256, 1136, 1042, 839, 762, 700 cm^{-1}; HRMS (EI) calcd for $C_{12}H_{15}F_3O$ 232.1075, found 232.1075 (M^+).

1-Phenyl-4,4,5,5,6,6,6-heptafluoro-1-hexanol (1d). To a mixture of Mg (249 mg, 10.2 mmol) in dry Et_2O (4 mL) under Ar was added I_2 (a few pieces), and subsequently a solution of 1,1,1,2,2,3,3-heptafluoro-5-iodopentane (3.72 g, 11.5 mmol) in dry Et_2O (4 mL) was added dropwise over 30 min at room temperature. The mixture was stirred for 2 h. To the slurry was added a solution of benzaldehyde (859 mg, 8.09 mmol) in dry Et_2O (4 mL) dropwise over 15 min in an ice bath, and the mixture was stirred at room temperature for 3.5 h. The reaction was quenched with saturated aqueous NH_4Cl in an ice bath. After the mixture had been filtered, the filtrate was adjusted to pH 4. The mixture was extracted with Et_2O (30 mL × 6), dried over $MgSO_4$, and concentrated. The product was purified by silica gel column chromatography (hexane/Et_2O (10:1)) to afford **1d** as a white solid (1.75 g, 70%): mp 39–40 ºC; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.80 (br s, 1H), 2.00–2.09 (m, 2H), 2.14–2.17 (m, 1H), 2.29–2.30 (m, 1H), 4.77 (dd, J = 4.8, 7.8 Hz, 1H), 7.30–7.40 (m, 5H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 27.2 (t, J_{CF} = 22.2 Hz), 29.4 (t, J_{CF} = 3.5 Hz), 73.2, 108.8 (t of sextet, J_{CF} = 36.4, 262.4 Hz), 117.8 (tt, J_{CF} = 30.9, 251.4 Hz), 117.9 (qt, J_{CF} = 33.7, 285.9 Hz), 125.6, 128.1, 128.8, 143.5; ^{19}F NMR ($CDCl_3$, 565 MHz) δ –128.9 (s, 2F), –116.4 (m, 2F), –81.8 (t, J = 9.6 Hz, 3F); IR (KBr) 3258, 3032, 2953, 2876, 1495, 1456, 1391, 1356, 1223, 1182, 1117, 1047, 980, 916, 770, 700 cm^{-1}; HRMS (EI) calcd for $C_{12}H_{11}F_7O$ 304.0698, found 304.0703 (M^+).

[E] Lipase-Catalyzed Kinetic Resolution.

General Procedure. A mixture of alcohol **1** (0.50 mmol), immobilized lipase (700 mg, 0.5% (w/w) enzyme/Toyonite-200M), and molecular sieves 3A (three pieces) in dry *i*-Pr_2O (5.0 mL) in a test tube with a rubber septum was stirred in a thermostat at 30 °C. The reaction was started by adding vinyl acetate (93 μL, 1.0 mmol) via a syringe. The progress of the reaction was monitored by TLC. The reaction was stopped by filtration at an appropriate conversion, and the filtrate was concentrated under reduced pressure. Alcohol **1** and ester **2** were separated by silica gel column chromatography. To a solution of **2** (0.1–0.2 mmol) in EtOH (1 mL) was added aqueous NaOH (1 N, 1 mL). The mixture was stirred at room temperature overnight and then acidified with 3% HCl.

After the solvent was removed under reduced pressure, the product was extracted with EtOAc (4 mL × 3). The mixture was dried over $MgSO_4$, and concentrated. The alcohol **1** obtained was purified by silica gel column chromatography.

Determination of Enantiomeric Purities and Absolute Configurations. The enantiomeric purities of **1a–d** were determined by HPLC using chiral columns (Daicel Chemical Industries), and those of **2a–d** were determined after conversion to the corresponding alcohols. HPLC for **1a**: Chiralcel OB-H, hexane/*i*-PrOH = 9:1, 0.5 mL/min, 254 nm, (*S*) 10.6 min, (*R*) 12.2 min. HPLC for **1b**: Chiralcel OD-H, hexane/*i*-PrOH = 98:2, 0.5 mL/min, 254 nm, (*R*) 29.3 min, (*S*) 35.3 min. HPLC for **1c**: Chiralcel OB-H, hexane/*i*-PrOH = 9:1, 0.5 mL/min, 254 nm, (*S*) 12.2 min, (*R*) 14.3 min. HPLC for **1d**: Chiralcel OB-H, hexane/*i*-PrOH = 98:2, 0.5 mL/min, 254 nm, (*S*) 15.1 min, (*R*) 29.9 min. The absolute configurations of **1a** and **1b** were determined by comparison with the signs of the reported optical rotation, and those of **1c** and **1d** were determined by the Mosher method with MTPA.[5] The alcohols **1c** and **1d** were derivatized to the corresponding MTPA esters, and the absolute configurations were assigned by using the ^{19}F NMR signals for the terminal CF_3 group: ^{19}F NMR ($CDCl_3$, 282 MHz) δ –67.64 (t, J = 11.6 Hz, 3F) for (*R*)-MTPA ester of (*R*)-**1c**, –67.57 (t, J = 11.6 Hz, 3F) for (*R*)-MTPA ester of (*S*)-**1c**. ^{19}F NMR ($CDCl_3$, 282 MHz) δ –81.79 (t, J = 9.3 Hz, 3F) for (*R*)-MTPA ester of (*R*)-**1d**, –81.76 (t, J = 9.3 Hz, 3F) for (*R*)-MTPA ester of (*S*)-**1d**.

Kinetic Resolution of 1-Phenyl-1-hexanol (1a). (*S*)-**1a**: Colorless oil; $[\alpha]^{35}_D$ –6.4 (*c* 1.18, $CHCl_3$), 18.2% ee, lit.[4] $[\alpha]^{28}_D$ +35.3 (*c* 1.04, $CHCl_3$) for (*R*)-**1a** with 94% ee. (*R*)-**2a**:[6] Colorless oil; $[\alpha]^{34}_D$ +44.1 (*c* 0.673, $CHCl_3$), 61.3% ee; ^1H NMR ($CDCl_3$, 600 MHz) δ 0.86 (t, J = 7.1 Hz, 3H), 1.22–1.32 (m, 6H), 1.73–1.78 (m, 1H), 1.86–1.91 (m, 1H), 2.07 (s, 3H), 5.72 (dd, J = 6.5, 7.6 Hz, 1H), 7.27–7.35 (m, 5H).

Kinetic Resolution of 1-Phenyl-1-heptanol (1b). (*S*)-**1b**: Colorless oil; $[\alpha]^{23}_D$ –16.1 (*c* 1.05, $CHCl_3$), 44.9% ee, lit.[4] $[\alpha]^{30}_D$ +32.0 (*c* 1.02, $CHCl_3$) for (*R*)-**1b** with 93% ee. (*R*)-**2b**:[6] Colorless oil; $[\alpha]^{23}_D$ +48.1 (*c* 1.19, $CHCl_3$), 70.8% ee; ^1H NMR ($CDCl_3$, 600 MHz) δ 0.86 (t, J = 7.0 Hz, 3H), 1.21–1.31 (m, 8H), 1.74–1.78 (m, 1H), 1.87–1.91 (m, 1H), 2.07 (s, 3H), 5.72 (dd, J = 6.5, 7.5 Hz, 1H), 7.27–7.35 (m, 5H).

Kinetic Resolution of 1-Phenyl-6,6,6-trifluoro-1-hexanol (1c). (*S*)-**1c**: Colorless oil; $[\alpha]^{35}_D$ –17.0 (*c* 1.07, $CHCl_3$), 41.5% ee. (*R*)-**2c**: Colorless oil; $[\alpha]^{34}_D$ +48.2 (*c* 1.22, $CHCl_3$), 80.8% ee; ^1H NMR ($CDCl_3$, 600 MHz) δ 1.29–1.33 (m, 1H), 1.39–1.43 (m, 1H), 1.54–1.59 (m, 2H), 1.76–1.82 (m, 1H), 1.90–1.96 (m, 1H), 2.00–2.06 (m, 2H), 2.07 (s, 3H), 5.73 (dd, J = 6.4, 7.4 Hz, 1H), 7.28–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 21.2, 21.6 (q, J_{CF} = 3.1 Hz), 24.6, 33.5 (q, J_{CF} = 28.3 Hz), 35.9, 75.7, 126.4, 127.0 (q, J_{CF} = 275.0 Hz), 128.0, 128.5, 140.3, 170.3; ^{19}F NMR ($CDCl_3$, 565 MHz) δ –67.5 (t, J_{FH} = 11.0 Hz, 3F); IR (neat) 3034, 2949, 2874, 1736, 1497, 1437, 1375, 1240, 1140, 1040,

837, 761, 700 cm^{-1}; HRMS (EI) calcd for $C_{14}H_{17}F_3O_2$ 274.1181, found 274.1179 (M^+).

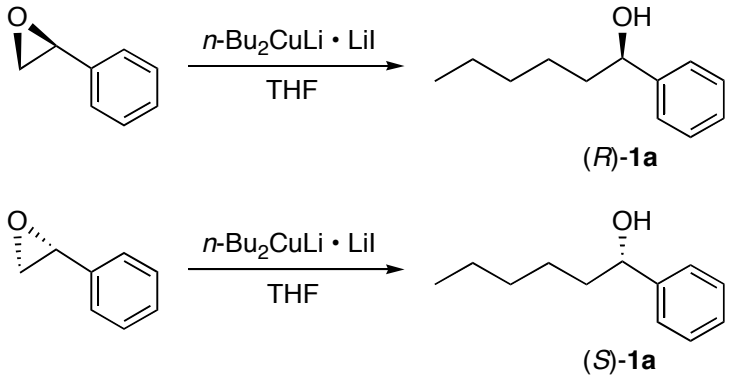
Kinetic Resolution of 1-Phenyl-4,4,5,5,6,6,6-heptafluoro-1-hexanol (1d). (*S*)-**1d**: White solid; $[\alpha]^{27}_D$ –4.5 (*c* 0.97, $CHCl_3$), 18.6% ee. (*R*)-**2d**: Colorless oil; ^1H NMR ($CDCl_3$, 600 MHz) δ 2.04–2.21 (m, 4H), 2.12 (s, 3H), 5.79 (dd, J = 4.8, 7.8 Hz, 1H), 7.31–7.39 (m, 5H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 21.0, 27.02 (t, J_{CF} = 21.8 Hz), 27.04 (t, J_{CF} = 3.5 Hz), 74.4 (t, J_{CF} = 10.9 Hz), 108.7 (t of sextet, J_{CF} = 36.5, 262.1 Hz), 117.4 (tt, J_{CF} = 31.2, 251.8 Hz), 117.8 (qt, J_{CF} = 33.7, 285.7 Hz), 126.2, 128.4, 128.7, 139.2, 170.1; ^{19}F NMR ($CDCl_3$, 565 MHz) δ –128.8 (s, 2F), –116.4 (m, 2F), –81.8 (t, J = 9.3 Hz, 3F); IR (neat) 3068, 3037, 2951, 1747, 1454, 1354, 1227, 1173, 1115, 1026, 702 cm^{-1}; HRMS (EI) calcd for $C_{14}H_{13}F_7O_2$ 346.0804, found 346.0810 (M^+).

Table S2 Kinetic resolution of **1** with wild-type and mutant enzymes[a]

					% Yield[c] (% ee)		
Entry	**1**	Lipase	Time/h	c[b]	(*R*)-**2**	(*S*)-**1**	*E* value[d]
1	**1a**	wild-type	41	0.23	21 (61.3)	73 (18.2)	5
2	**1a**	I287F	41	0.46	49 (87.1)	44 (72.8)	32
3	**1a**	I287A	41	0.20	13 (13.5)	78 (3.4)	1.4
4	**1a**	I287F/I290A	2.5	0.50	43 (98.9)	50 (98.4)	>200
5	**1a**	I287F/I290F	41	0.10	18 (56.4)	80 (6.4)	4
6	**1a**	I290A	41	0.41	35 (95.1)	51 (65.3)	79
7	**1b**	wild-type	41	0.39	35 (70.8)	58 (44.9)	9
8	**1b**	I287F	22	0.47	39 (92.8)	43 (83.9)	71
9	**1b**	I287F/I290A	4	0.47	40 (98.7)	46 (86.6)	>200
10	**1c**	wild-type	41	0.34	35 (80.8)	61 (41.5)	14
11	**1c**	I287F	22	0.47	40 (91.4)	38 (80.2)	55
12	**1c**	I287F/I290A	4	0.50	41 (98.4)	46 (96.5)	>200
13	**1d**	I287F/I290A	75	0.19	9 (78.9)	44 (18.6)	10

[a] Conditions: immobilized lipase (700 mg, 0.5% (w/w) enzyme/Toyonite-200M), **1** (0.50 mmol), vinyl acetate (1.0 mmol), molecular sieves 3A (three pieces), dry *i*-Pr_2O (5 mL), 30 ºC. [b] Conversion calculated from c = ee(**1**)/(ee(**1**) + ee(**2**)). [c] Isolated yield. [d] Calculated from $E = \ln[1 - c(1 + \text{ee}(\mathbf{2}))]/\ln[1 - c(1 - \text{ee}(\mathbf{2}))]$.

[F] Synthesis of Enantiomerically Pure Alcohols.



Enantiomerically pure alcohol (*R*)-**1a** was synthesized according to the literature.[7] To a mixture of CuI (4.97 g, 26.1 mmol) in dry THF (50 mL) at –50 ºC under Ar was added *n*-BuLi (1.59 M in hexane, 32 mL, 51 mmol). The mixture was stirred at –50 ºC for 30 min. A solution of (*S*)-styrene oxide (2.3 mL, 20 mmol) in dry THF (40 mL) was added dropwise over 10 min at –78 ºC. The mixture was stirred at –78 ºC for 2.5 h. The reaction was quenched with saturated aqueous NH_4Cl, and the solution was adjusted to pH 3. The mixture was filtered through Celite. The product was extracted with EtOAc (50 mL × 4), dried over $MgSO_4$, and concentrated. Purification by silica gel column chromatography (hexane/$CHCl_3$/EtOAc (70:25:5)) followed by bulb-to-bulb distillation (130 ºC, 10 mmHg) gave (*R*)-**1a** as a white solid (1.54 g, 43%, >99% ee, $[\alpha]^{22}_D$ +35.8 (*c* 1.6, $CHCl_3$)). Enantiomerically pure alcohol (*S*)-**1a** was also prepared in the same way (46%, >99% ee, $[\alpha]^{21}_D$ –38.6 (*c* 1.1, $CHCl_3$)).

[G] Determination of Kinetic Constants.

A mixture of enantiomerically pure alcohol **1a** (typically, ca. 0.05–0.5 M), immobilized lipase (typically, 300 mg, 0.5% (w/w) enzyme/Toyonite-200M), and molecular sieves 3A (one piece) in dry *i*-Pr_2O (1.0 mL) in a 5-mL screw cap vial was stirred in a thermostat at 30 ºC for 30 min. The reaction was started by adding vinyl acetate (93 μL, 1.0 mmol, 1.0 M) via a syringe. At an appropriate time interval, aliquots (10 μL) were withdrawn and added to EtOAc (0.5 mL). After the diluted solution had been centrifuged at 6,400 rpm for 2 min, the supernatant was filtered through a syringe filter (Nacalai Tesque, Cosmonice Filter S (pore size 0.45 μm, ϕ 4 mm)). The filtrate was then analyzed by GC to obtain the conversion (<15%, calibrated), and five data points were routinely collected to determine the initial rate (v_0) at each substrate concentration $[S]_0$. Plot of v_0 against $[S]_0$ afforded a saturation curve, and the apparent V_{max} and K_m values were obtained by the nonlinear least-squares method applied to the Michaelis–Menten type of equation as follows: $v_0 = V_{max}\,(E)_{mg}\,[S]_0\,/(K_m + [S]_0)$. The V_{max} value, which is normalized by the weight of the enzyme powder $(E)_{mg}$, corresponds to the k_{cat} value in homogeneous enzymatic reactions. The kinetic constants determined are given in the text.

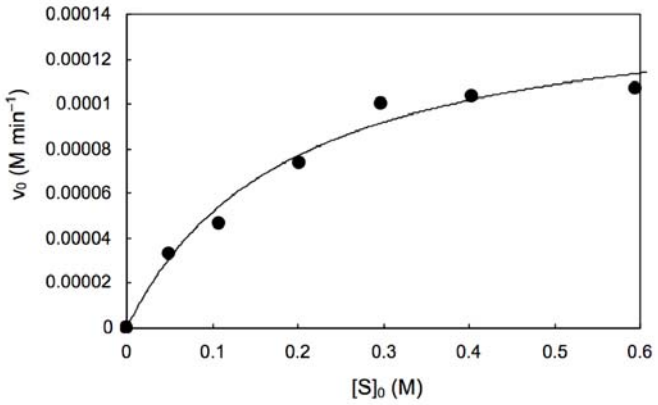


Fig. S1 Initial rates v_0 plotted against substrate concentrations $[S]_0$ for the determination of the kinetic constants for the acylation of (*R*)-**1a** catalyzed by the wild-type enzyme (300 mg).

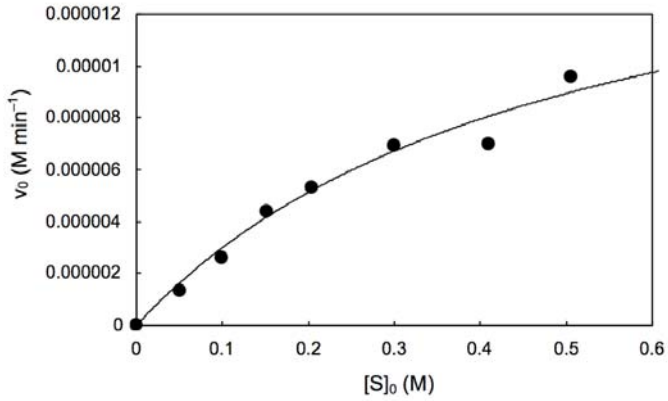


Fig. S2 Initial rates v_0 plotted against substrate concentrations $[S]_0$ for the determination of the kinetic constants for the acylation of (*S*)-**1a** catalyzed by the wild-type enzyme (300 mg).

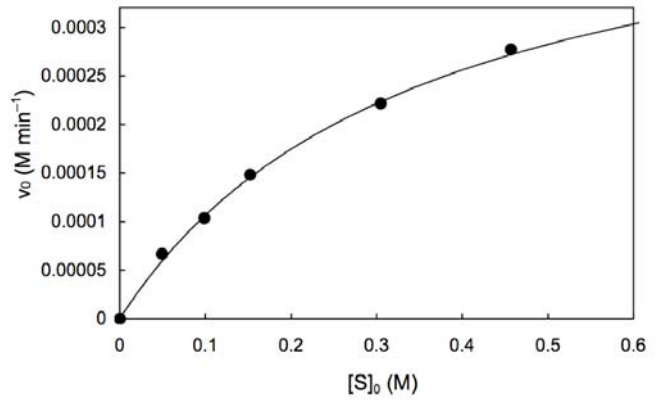


Fig. S3 Initial rates v_0 plotted against substrate concentrations $[S]_0$ for the determination of the kinetic constants for the acylation of (*R*)-**1a** catalyzed by the I287F mutant (300 mg).

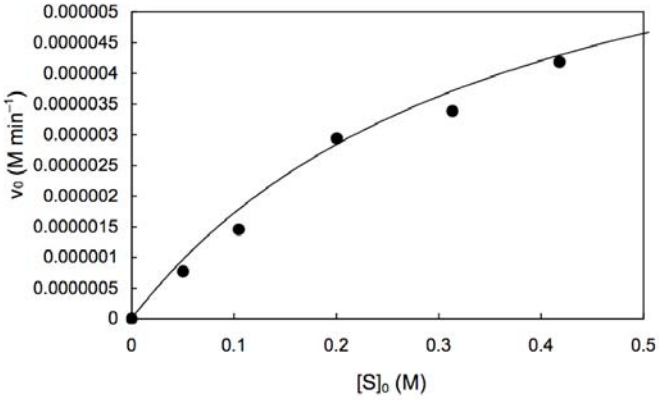


Fig. S4 Initial rates v_0 plotted against substrate concentrations $[S]_0$ for the determination of the kinetic constants for the acylation of (*S*)-**1a** catalyzed by the I287F mutant (300 mg).

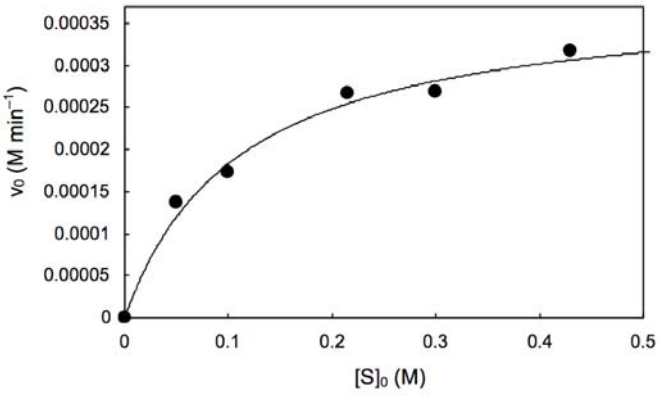


Fig. S5 Initial rates v_0 plotted against substrate concentrations $[S]_0$ for the determination of the kinetic constants for the acylation of (*R*)-**1a** catalyzed by the I287F/I290A double mutant (50 mg).

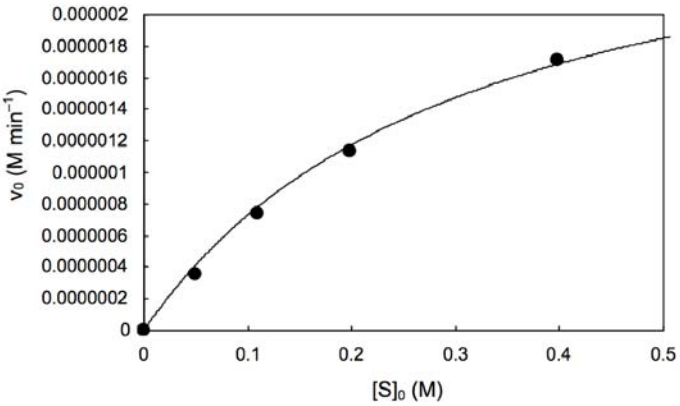


Fig. S6 Initial rates v_0 plotted against substrate concentrations $[S]_0$ for the determination of the kinetic constants for the acylation of (*S*)-**1a** catalyzed by the I287F/I290A double mutant (300 mg).

[H] Design of Mutant Enzymes.

SYBYL 6.4 (Tripos Inc.) was used to design the mutant enzymes. The crystal structure of a *Burkholderia cepacia* lipase (PDB code 1OIL) having 96% sequence identity with that used in this study was used after the thirteen different residues had been replaced. The tetrahedral intermediate for (*R*)- or (*S*)-**1** covalently linked to the catalytic residue Ser87 was generated, and the oxyanion moiety of this tetrahedral intermediate was located in the oxyanion hole by rotating the dihedral angles. Typically, the distance between the oxyanion O atom and the peptide backbone N atom of the oxyanion hole was ca. 2.8 Å. The conformation of the alkoxy moiety of the docked substrate was manually adjusted to *gauche* as represented by the transition-state model (Fig. 1a in the text).[8,9] The other dihedral angles were manually adjusted to see which amino acid residue was close to the substituent of the docked substrate and which amino acid residue was suitable as a mutation site for improving the catalytic function. To replace an amino acid residue with another one, a Mutate Monomers command of a Biopolymer module of SYBYL was employed. We did not perform any computational calculation because the active site involving the bulky substrate moiety was too congested to perform reliable calculations and because the stereoelectronic effect, which needs MO calculations, is known to play a crucial role in the transition-state conformation.[8,9] Despite such a primitive method, this manual docking experiment enabled us to find useful mutants very efficiently. Actually, almost all the mutants that we have prepared so far are shown in Table S2.

References

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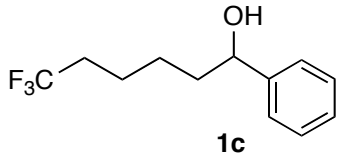
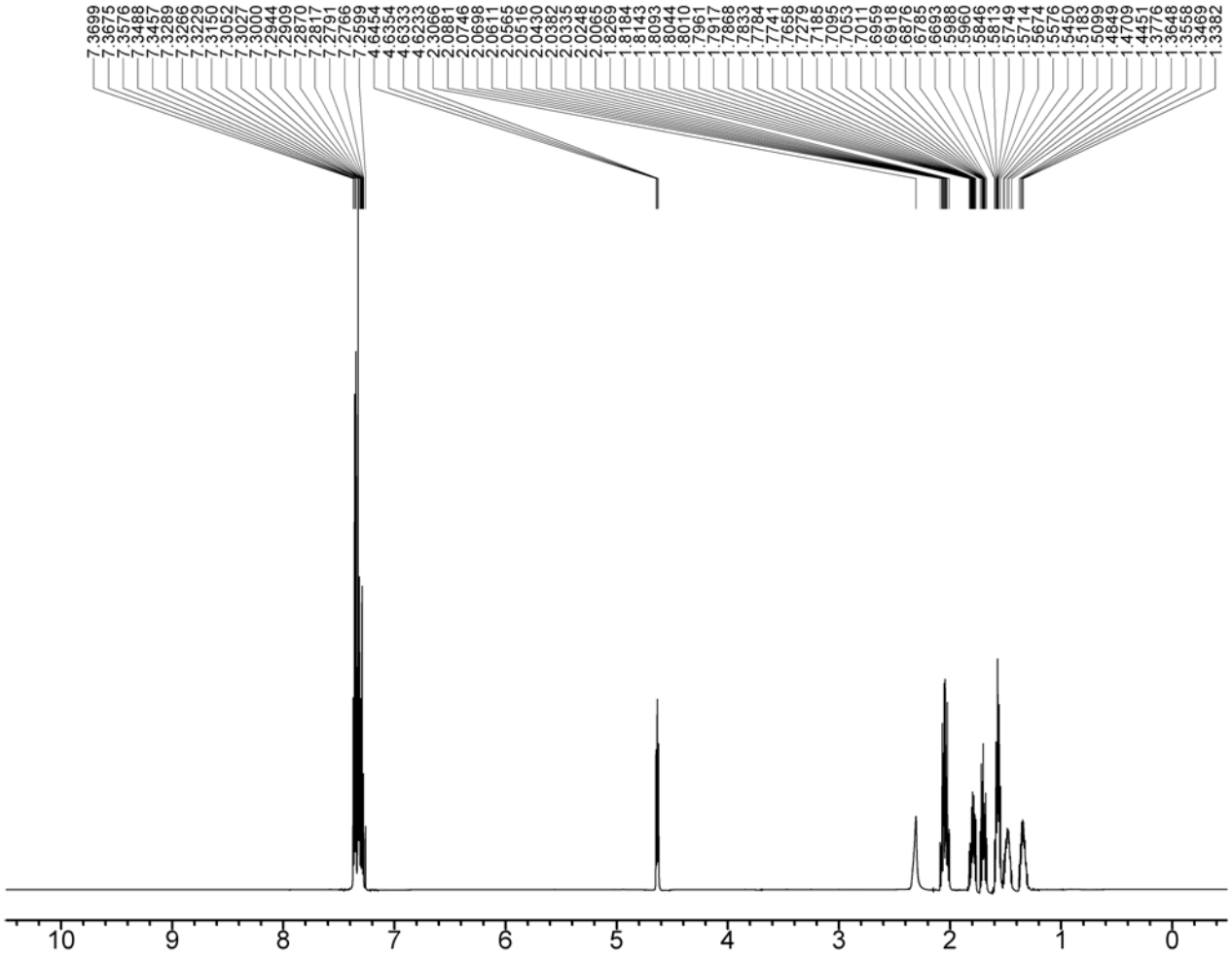
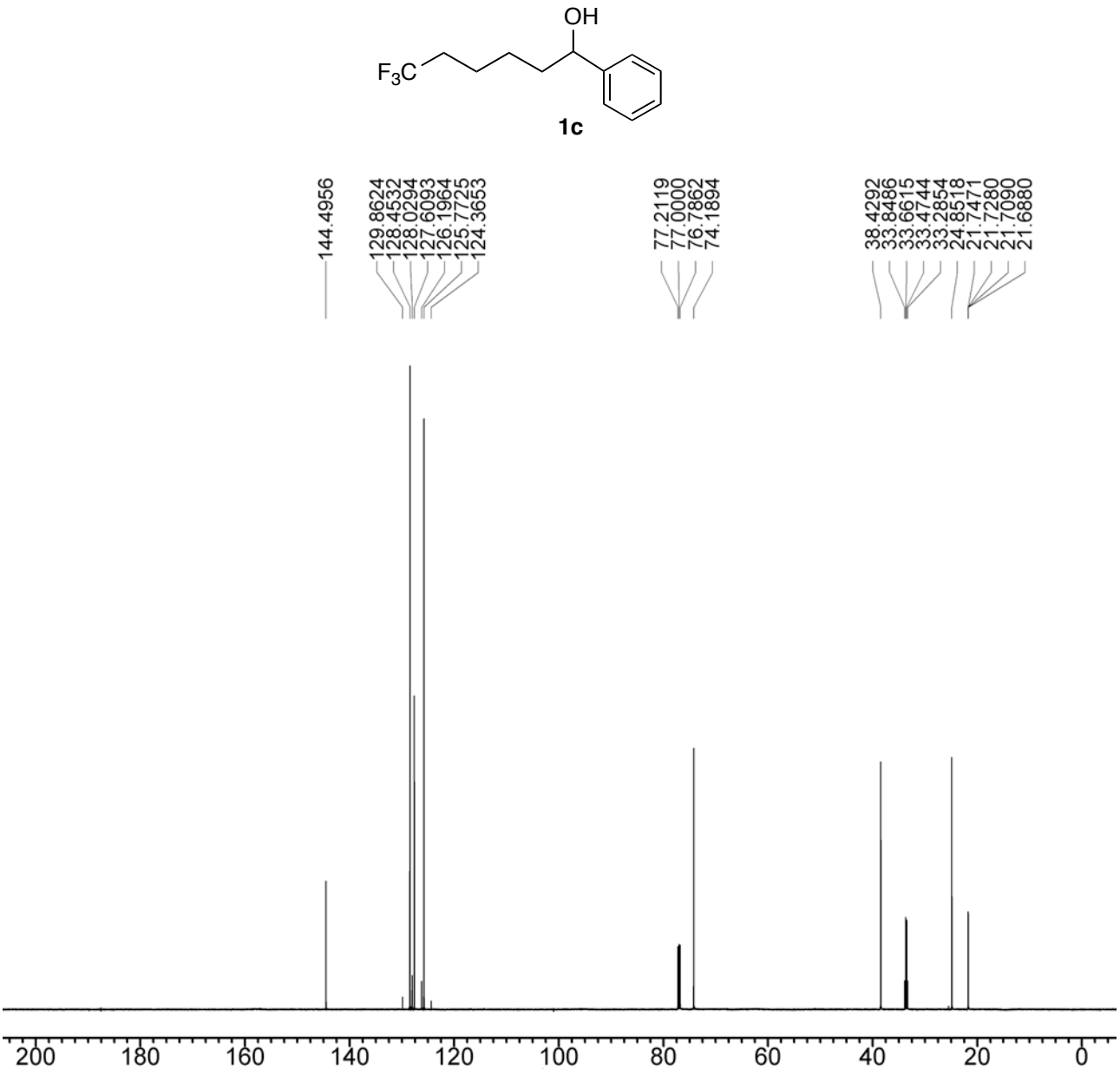
[I] NMR Spectra.



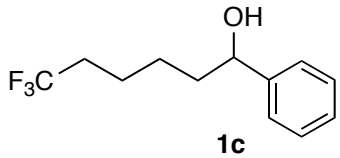
1c



600 MHz ^{1}H NMR of **1c** in $CHCl_3$.



150 MHz ^{13}C NMR of **1c** in $CDCl_3$.



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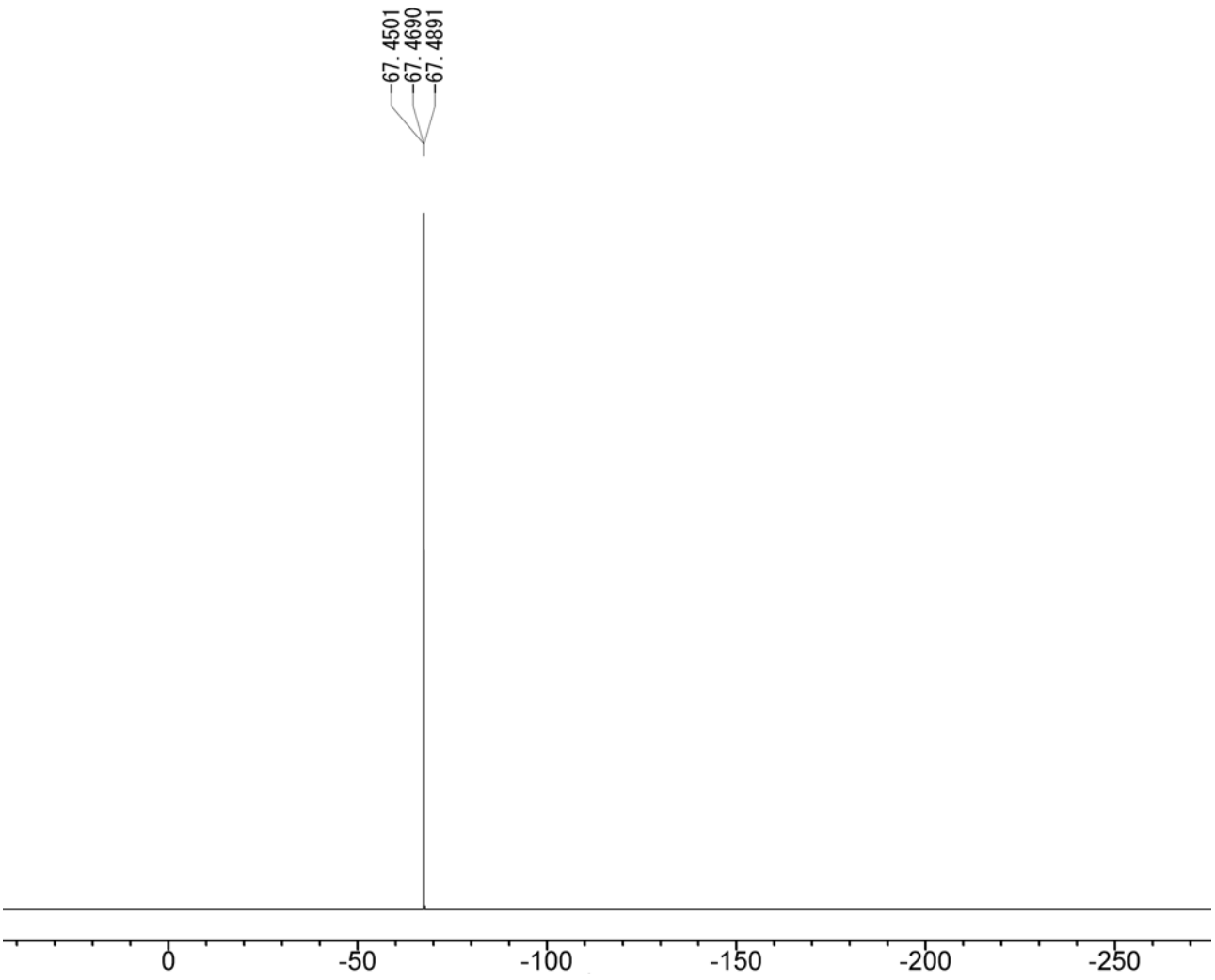
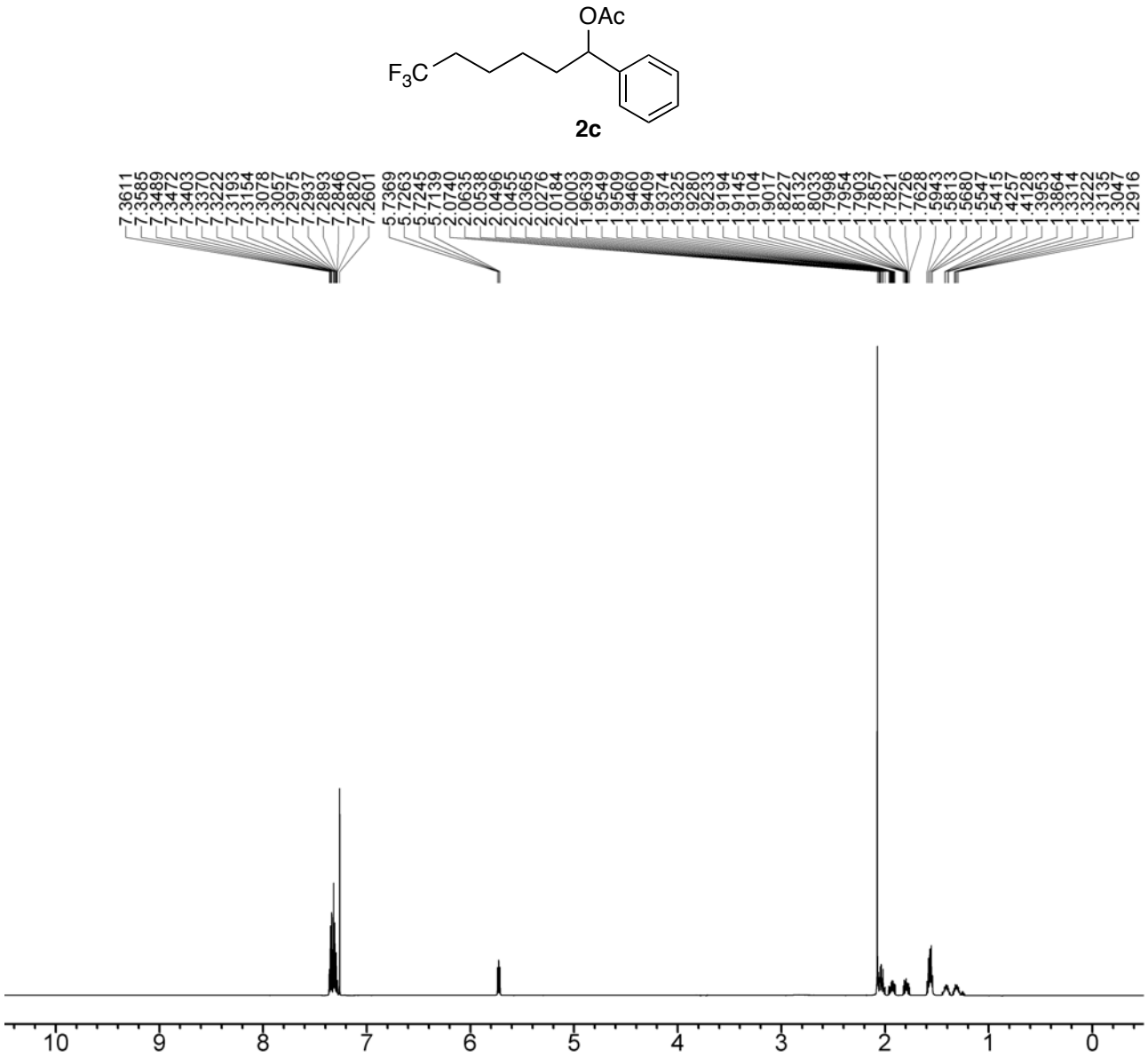
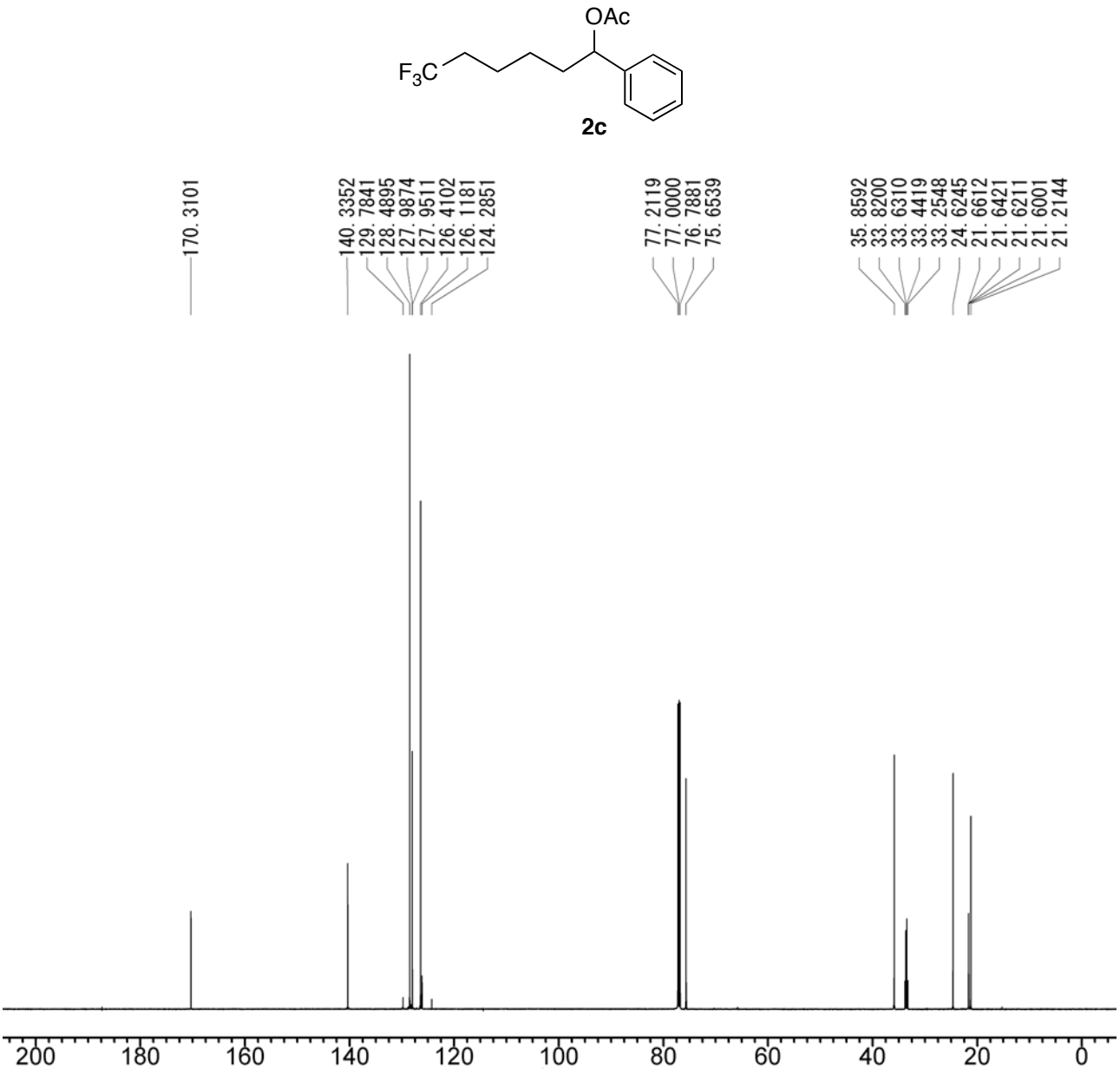


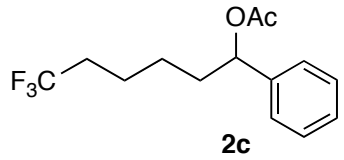
565 MHz ^{19}F NMR of **1c** in $CDCl_3$.



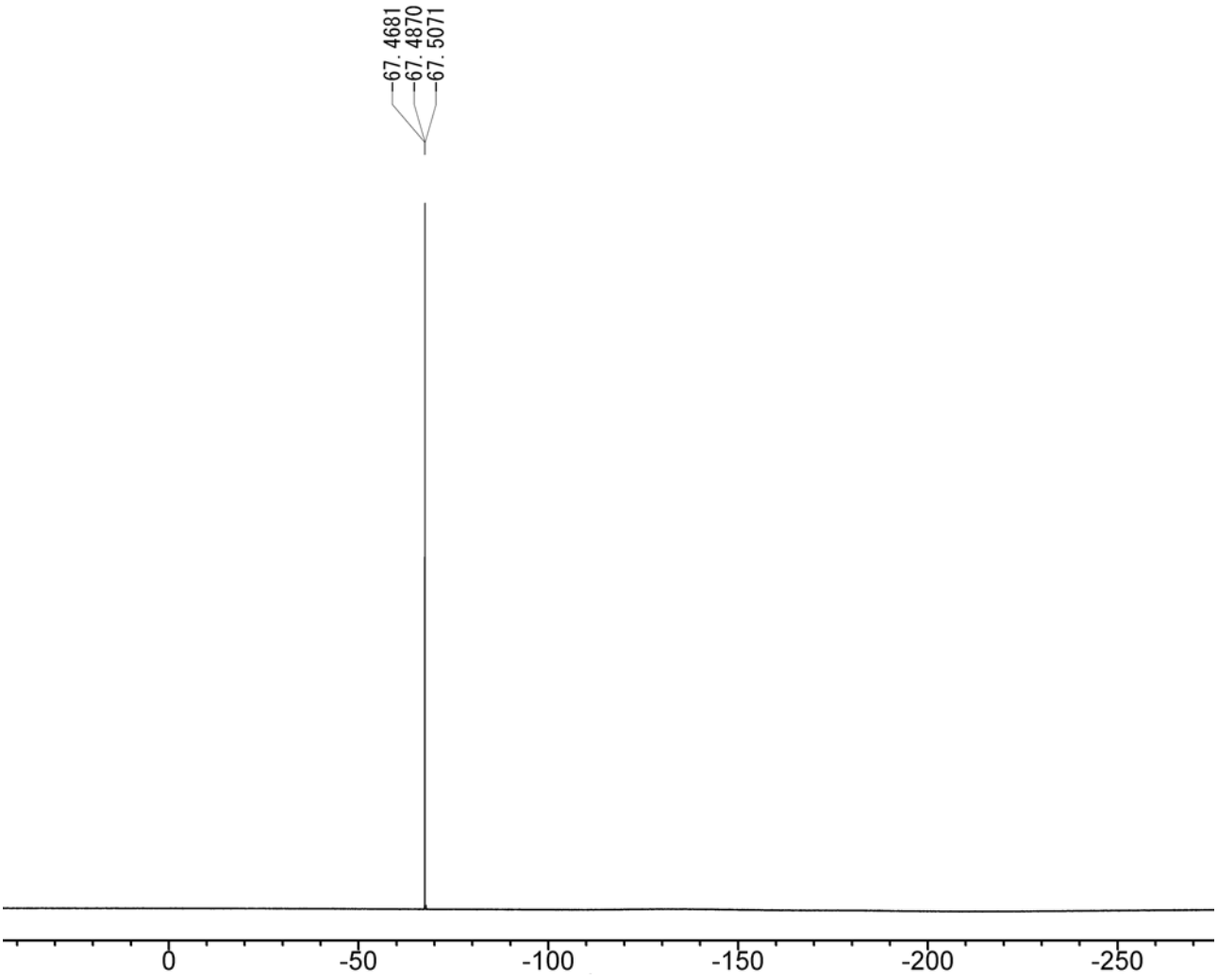
600 MHz ^{1}H NMR of **2c** in $CHCl_3$.



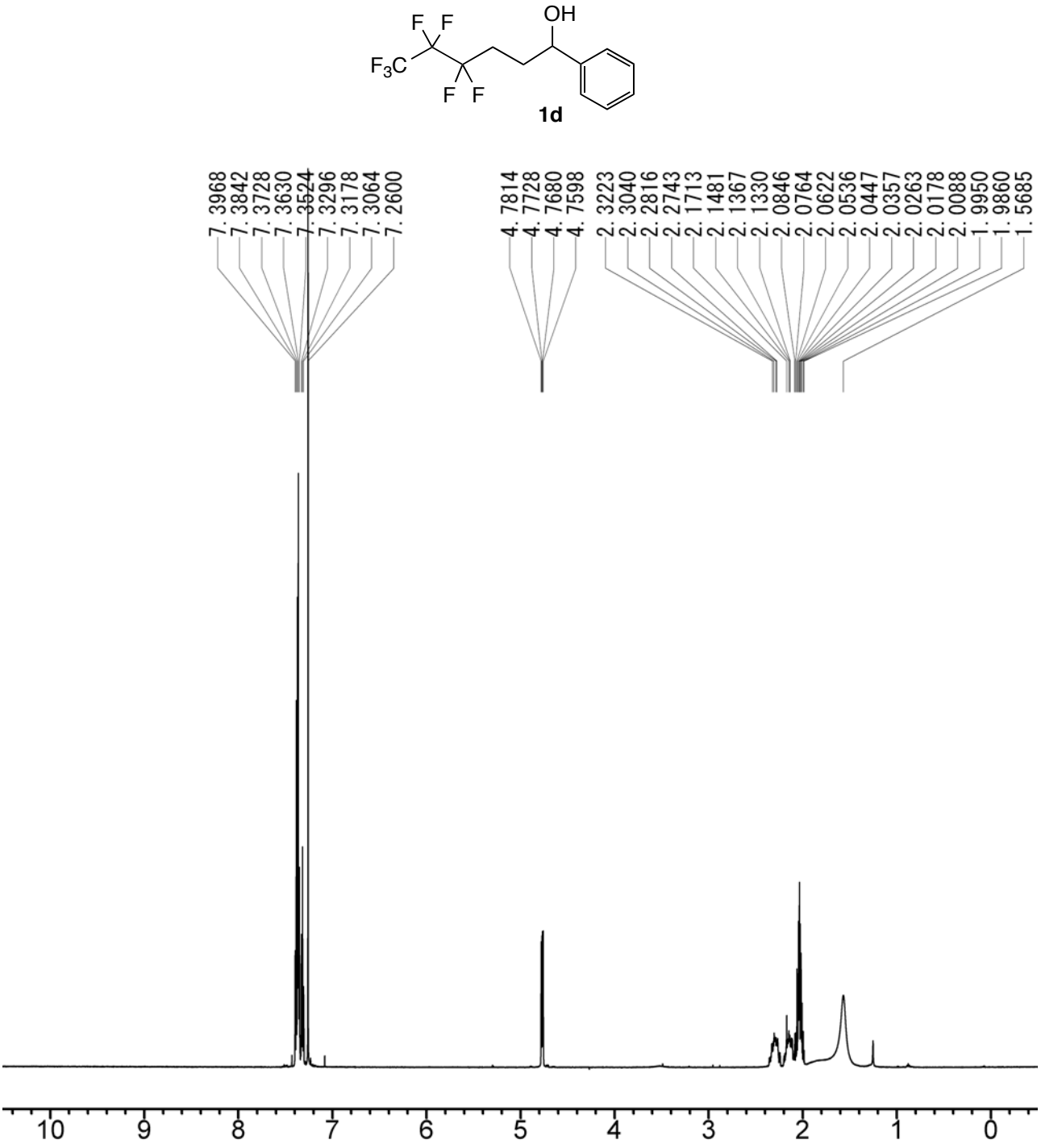
150 MHz ^{13}C NMR of **2c** in $CDCl_3$.



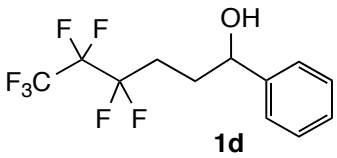
2c



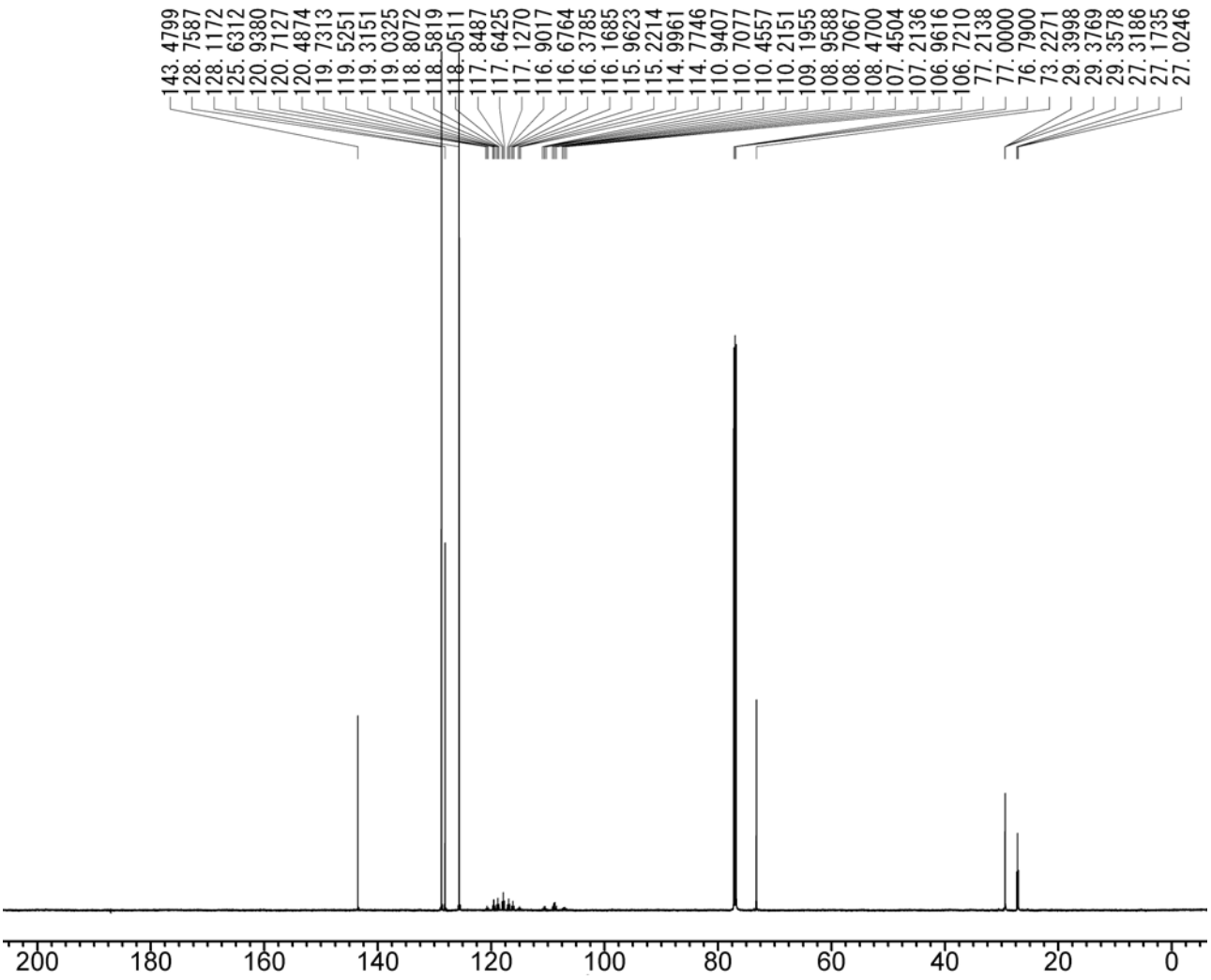
565 MHz ^{19}F NMR of **2c** in $CDCl_3$.



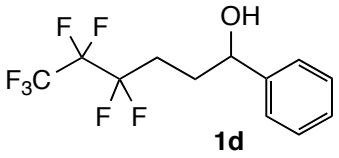
600 MHz ^{1}H NMR of **1d** in $CHCl_3$.



1d



150 MHz ^{13}C NMR of **1d** in $CDCl_3$.



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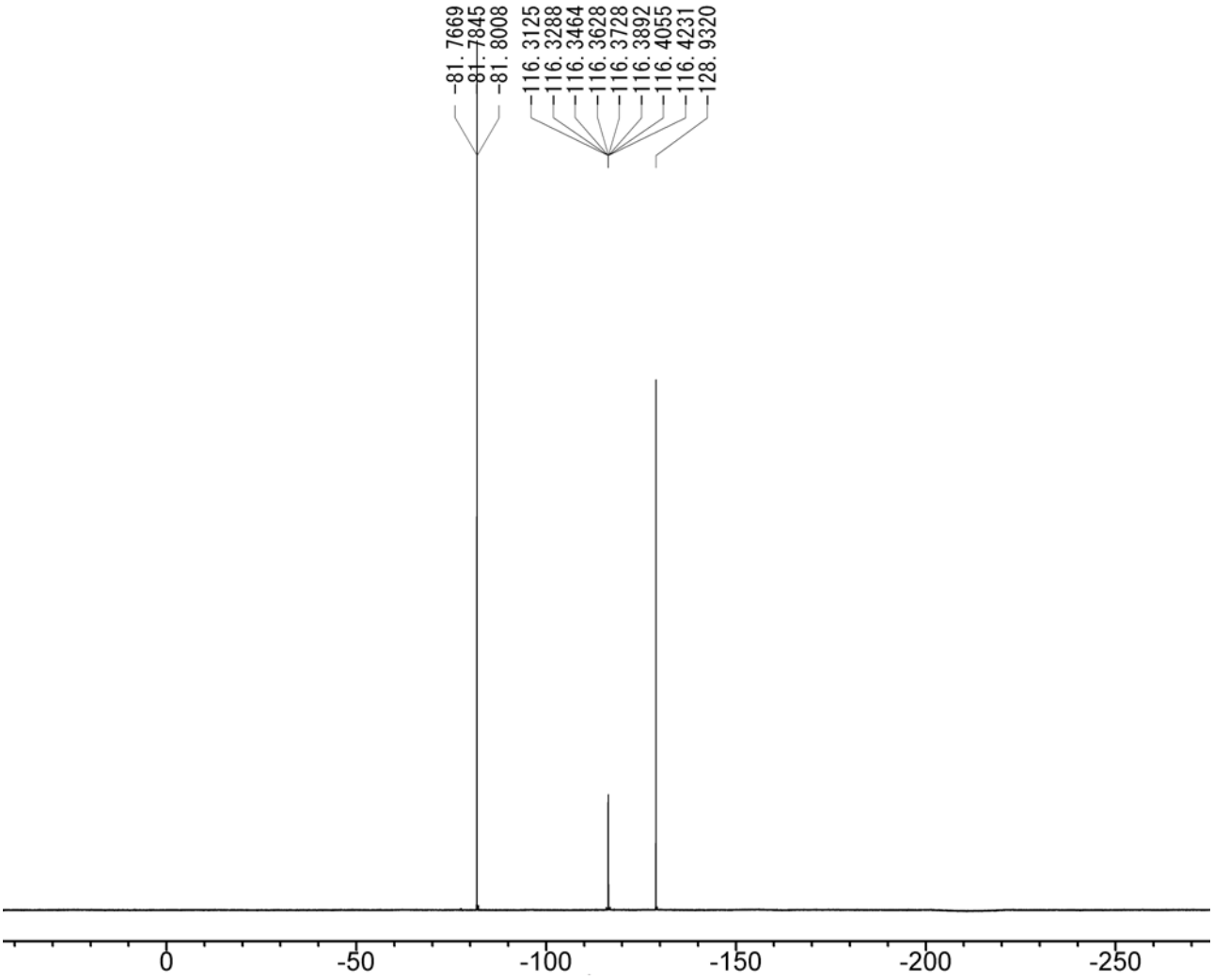
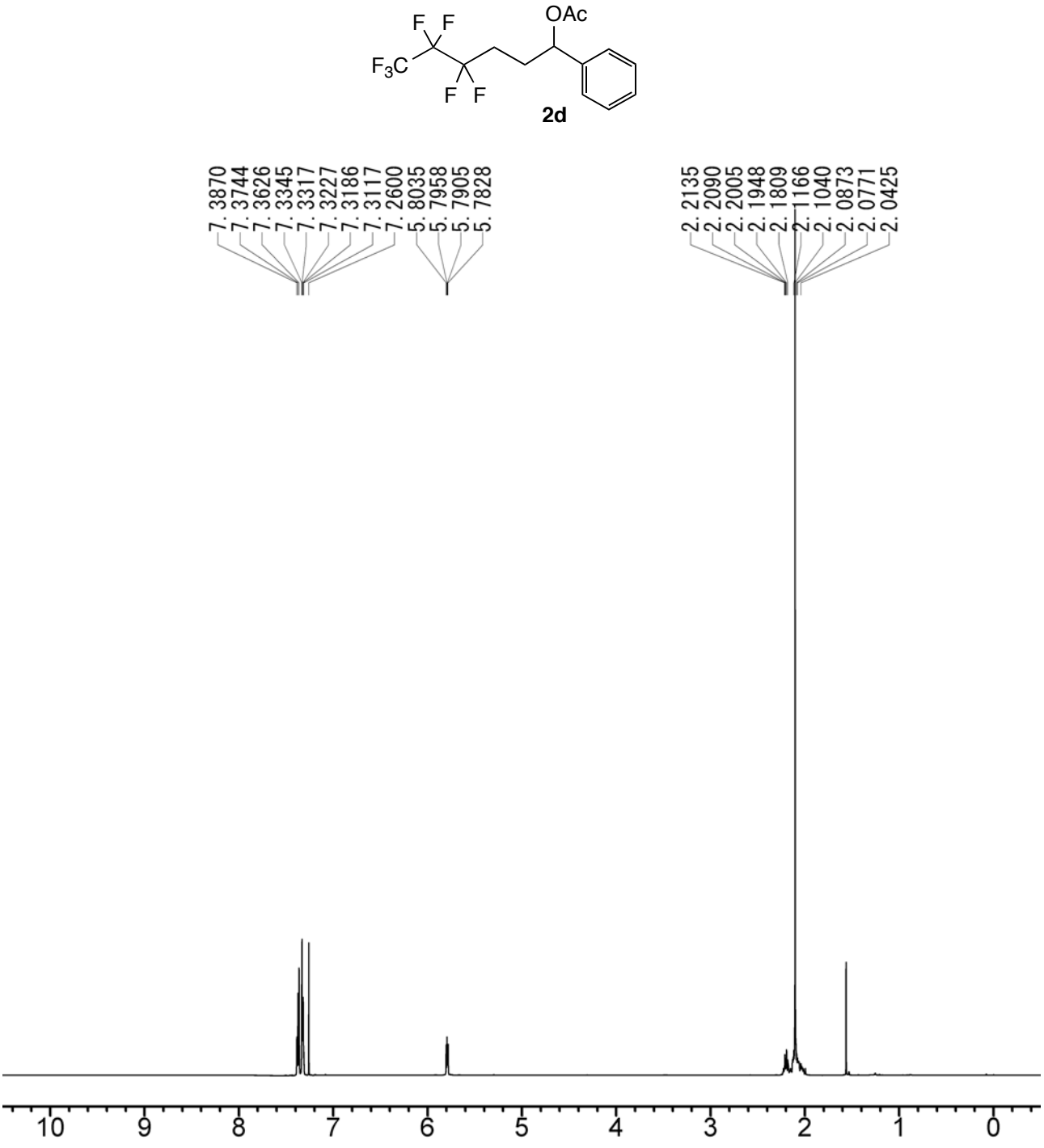
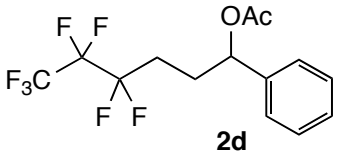


565 MHz ^{19}F NMR of **1d** in $CDCl_3$.



600 MHz ^{1}H NMR of **2d** in $CHCl_3$.



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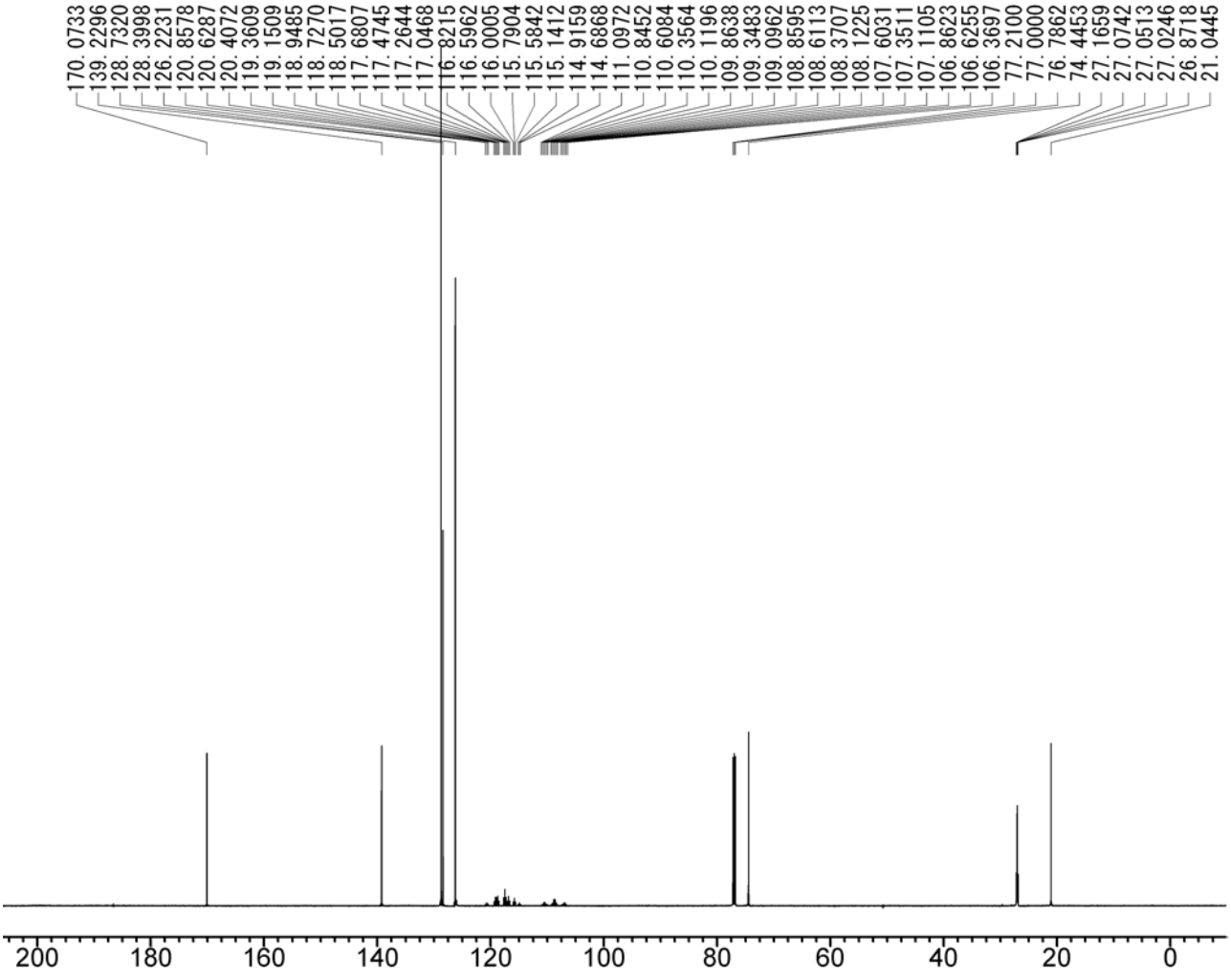

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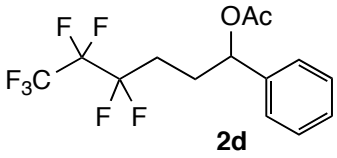

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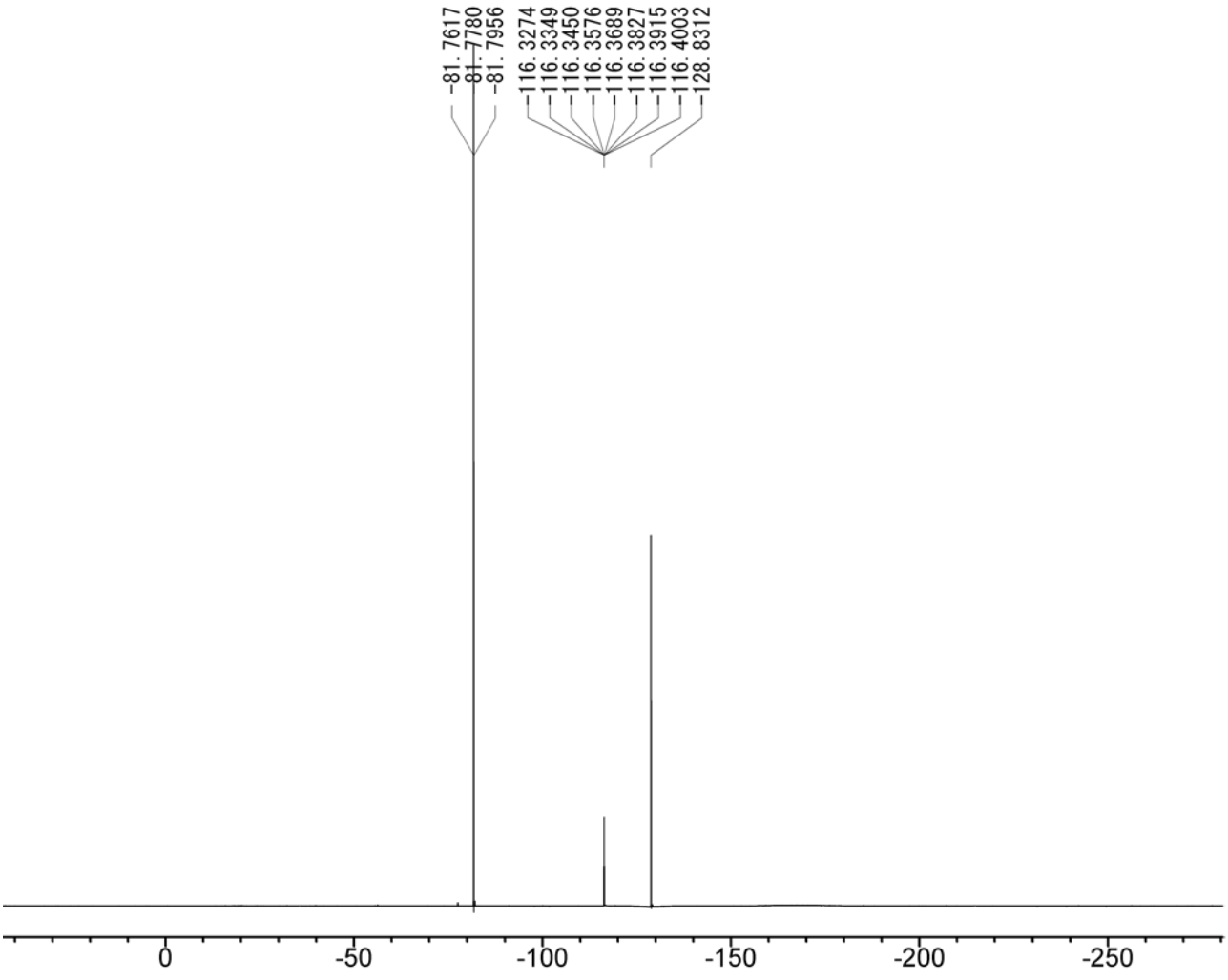

565 MHz ^{19}F NMR of **2d** in $CDCl_3$.