

## **A Red-Fluorescent Substrate Microarray for Lipase Fingerprinting**

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### **Supplementary Information**

- ***Synthetic procedures and spectral data for all compounds***
- ***NMR-spectra for all compounds***
- ***Images of lipase fingerprints on microarrays***

**Synthesis.** Reagents were purchased from Fluka, Aldrich, Sigma or Acros. Solvents were distilled from technical solvents for flash chromatography. CH<sub>2</sub>Cl<sub>2</sub>, THF were purified by passage through a column of activated alumina prior to use. Air- and moisture-sensitive liquids were transferred *via* syringe. All reactions were followed by TLC on pre-coated TLC plates SIL G-25 UV<sub>254</sub> silica gel sheets (Macherey-Nagel) with detection by UV or with revelators like 10 % phosphomolybdic acid solution in 90 % EtOH, 10 % ninhydrin in 90 % EtOH. All chromatographies (flash) were performed with SDS Silica gel 60 (0.04-0.063 mm, 230-400 mesh ASTM). NMR spectra were recorded on a BRUKER AC 300 or a BRUKER DMX 400 for <sup>1</sup>H (resp. 300 and 400 MHz) or <sup>13</sup>C (resp. 75 and 100 MHz) measurements. Chemical shifts  $\delta$  are given in ppm and coupling constants  $J$  in Hertz. Optical rotations were measured with a Perkin-Elmer 241 digital polarimeter with a 1 dm cell. Analytical RP-HPLC was performed in Waters (996 Photo diode array detector) chromatography system using a chromolith column (0.4 x 10 cm, flow rate 3 mL.min<sup>-1</sup>). Compounds were detected by UV absorption at 560 nm. Preparative RP-HPLC was performed with a Waters Delta Prep LC4000 (Waters 486 Tunable Absorbance Detector, detection by UV,  $\lambda$  = 560 nm) using a Waters prepak cartridge 500 g column (RP-C18 20 mm, 300 Å pore size), flow rate 100 mL.min<sup>-1</sup> (gradient 1 %.min<sup>-1</sup> CH<sub>3</sub>CN). Mass spectra were provided by the “Service of Mass Spectrometry” of the Department of Chemistry and Biochemistry.

**(S)-6-Bromohexane-1,2-diol (S)-3.** A solution of AD-mix- $\alpha$  (15.70 g) in 1:1 *t*-BuOH/water (100 mL) was stirred at RT until both phases were clear and then was cooled at 0°C. 6-Bromo-1-hexene (1.5 mL, 11.21 mmol) was added slowly and the slurry stirred at 0°C for 2.5 days, monitoring the reaction by TLC. Solid Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> (16.83 g) was added at 0°C, and the resulting reaction mixture was allowed to reach RT and stirred at this temperature for 1.5 h. The mixture was extracted with EtOAc (4 x 150 mL), dried over MgSO<sub>4</sub>. The solvent was evaporated *in vacuo*. The crude residue was purified by flash chromatography (hexane/EtOAc, 1:1 to 1:2), yielding (S)-**3** (1.95 g, 88.1 %, 83.3 % ee) as a colorless oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 4.06 (s, 2H), 3.65 – 3.56 (m, 2H), 3.42 – 3.34 (m, 3H), 1.87 – 1.81 (m, 2H), 1.63 – 1.32 (m, 4H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$

= 72.08, 66.63, 33.79, 32.65, 32.09, 24.29.  $[\alpha]_{\text{D}}^{20} = -3.6$  ( $c = 0.40$ , MeOH). HRMS(ESI-TOF-MS): calc. for (C<sub>6</sub>H<sub>13</sub>BrO<sub>2</sub>Na) [M+Na<sup>+</sup>]: 219.9996, found: 218.0004.

**(R)-6-Bromohexane-1,2-diol (R)-3.** The procedure for (S)-**3** was repeated using AD-mix- $\beta$  (15.70 g) to give (R)-**3** (1.80 g, 81.4%, 83.5% ee) as a colorless oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta = 3.85$  (s, 2H), 3.67 – 3.58 (m, 2H), 3.43 – 3.36 (m, 3H), 1.89 – 1.82 (m, 2H), 1.62 – 1.40 (m, 4H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta = 72.11$ , 66.68, 33.80, 32.68, 32.13, 24.32.  $[\alpha]_{\text{D}}^{20} = +4.3$  ( $c = 0.43$ , MeOH). HRMS(ESI-TOF-MS): calc. for (C<sub>6</sub>H<sub>13</sub>BrO<sub>2</sub>Na) [M+Na<sup>+</sup>]: 218.9996, found: 219.0002.

**(S)-((S)-6-bromo-2-hydroxyhexyl) 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (S)-3 MTPA ester.** (S)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid (114 mg, 0.49 mmol, 1.0 equiv) was stirred in dry DCM (5.0 mL) with oxalyl chloride (87  $\mu$ L, 0.98 mmol, 2.0 equiv) and pyridine (83  $\mu$ L, 0.98 mmol, 2.0 equiv) under argon atmosphere. After 1 h, the solvent is removed *in vacuo*, and dried under high-vacuum for 2 h. To the acyl chloride thus formed was added a solution of diol (S)-**2** (105.1 mg, 0.53 mmol, 1.0 equiv) and collidine (77  $\mu$ L, 0.59 mmol, 1.1 equiv) in dry DCM (0.8 mL) and stirred for 10 h. Then DCM (5.0 mL) was added, and the solution was washed with 2.0 mL of 0.1 M aq. H<sub>3</sub>PO<sub>4</sub>. The organic layer is dried over MgSO<sub>4</sub>. The solvent was removed *in vacuo*. The crude residue was purified by flash chromatography (hexane/EtOAc, 7:3), yielding (S)-**3** MTPA ester (64 mg, 29%, dr = 0.91:10) as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta = 7.55 - 7.41$  (m, 5H), 4.39 – 4.17 (m, 2H), 3.92 – 3.88 (m, 1H), 3.57 – 3.56 (m, 3H), 3.41 – 3.38 (m, 2H), 1.90 – 1.47 (m, 7H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta = 166.69$ , 132.23, 129.92, 128.66, 127.41, 124.84, 121.97, 69.96, 69.33, 55.63, 44.80, 33.48, 32.55, 32.26, 24.10, 22.83. <sup>19</sup>F NMR (100 MHz, CDCl<sub>3</sub>):  $\delta = -71.89$  (s, 0.9F),  $-71.92$  (s, 10F) relative values of diastereomers.  $[\alpha]_{\text{D}}^{20} = +38.3$  ( $c = 0.60$ , CHCl<sub>3</sub>).

**(S)-((R)-6-bromo-2-hydroxyhexyl) 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (R)-3**

**MTPA ester.** The procedure with (S)-**3** was applied starting with (S)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid (93.2 mg, 0.40 mmol, 1.0 equiv), oxalyl chloride (71  $\mu$ L, 0.80 mmol, 2.0 equiv) and pyridine (68  $\mu$ L, 0.80 mmol, 2.0 equiv), and reaction with (R)-**2** (70.0 mg, 0.36 mmol, 1.0 equiv) and collidine (63  $\mu$ L, 0.48 mmol, 1.3 equiv) in dry DCM (0.6 mL). Purification by flash chromatography (hexane/EtOAc, 7:3) gave (R)-**3** MTPA ester (75 mg, 50 %, dr = 10:0.9) as colorless oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 7.53 – 7.41 (m, 5H), 4.36 (dd,  $J$  = 11.2 Hz, 3.2 Hz, 1H), 4.19 (dd,  $J$  = 11.2 Hz, 7.3 Hz, 1H), 3.91 – 3.86 (m, 1H), 3.56 (m, 3H), 3.41 – 3.38 (m, 2H), 1.97 (s, 1H), 1.88 – 1.47 (m, 6H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 166.72, 132.21, 129.90, 128.66, 127.39 – 127.38 (same C, diastereomer), 124.82, 121.95, 70.03, 69.32, 55.61 (2 signals, same C, diastereomer), 44.79, 33.48, 32.53, 32.27, 24.05, 22.77.  $^{19}\text{F}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  = -71.90 (s, 10F), -71.93 (s, 0.9F) relative values of diastereomers.  $[\alpha]_{\text{D}}^{20}$  = +33.7 ( $c$  = 0.55,  $\text{CHCl}_3$ )

**(S)-3-(5,6-Dihydroxyhexyloxy)benzaldehyde (S)-4.** A solution of 3-hydroxybenzaldehyde (1.62 g, 13.24 mmol, 1.5 equiv) in dry DMF (7.5 mL) was stirred. At 0°C, NaH (0.58 g, 55 % dispersion in oil, 13.24 mmol, 1.5 equiv) was added, and the mixture was stirred for 30 min. At 25°C, (S)-**3** (1.74 g, 8.83 mmol, 1.0 equiv) solubilized in 1.5 mL of dry DMF was slowly added. After addition, the reaction mixture was stirred at 80°C for 2.5 days. Then, at 25°C, water (50 mL) was added, and the mixture was extracted with EtOAc (4 x 50 mL). Organic layers are combined and dried over  $\text{MgSO}_4$ . The solvent was evaporated *in vacuo*. The crude residue was purified by flash chromatography (hexane/EtOAc, 3:7) to give (S)-**4** (1.90 g, 90.1 %) as a pale yellow oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{CN}$ ):  $\delta$  = 9.94 (s, 1H), 7.48 – 7.46 (m, 2H), 7.38 – 7.37 (m, 1H), 7.24 – 7.18 (m, 1H), 4.06 – 4.02 (m, 2H), 3.58 – 3.50 (m, 1H), 3.47 – 3.42 (dd,  $J$  = 10.9, 4.0 Hz, 1H), 3.33 – 3.27 (dd,  $J$  = 10.9, 7.0 Hz, 1H), 1.84 – 1.34 (m, 6H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 192.38,

159.66, 137.80, 130.11, 123.47, 122.01, 112.86, 72.15, 68.12, 66.80, 32.80, 29.16, 22.23.  $[\alpha]_{\text{D}}^{20} = -3.3$  ( $c = 1.00$ , MeOH). HRMS (EI-MS): calc. for (C<sub>13</sub>H<sub>18</sub>O<sub>4</sub>) [M]: 238.1205, found: 238.1206.

**(R)-3-(5,6-Dihydroxyhexyloxy)benzaldehyde (R)-4.** The procedure for (S)-4 was applied starting with 3-hydroxybenzaldehyde (1.49 g, 12.18 mmol, 1.5 equiv), NaH (0.53 g, 55 % dispersion in oil, 12.18 mmol, 1.5 equiv), (R)-3 (1.60 g, 8.12 mmol, 1.0 equiv) solubilized in 1.5 mL of dry DMF was slowly added. After addition, the reaction mixture was stirred at 80°C for 2.5 days. Then, at 25°C, water (50 mL) was added, and the mixture was extracted with EtOAc (4 x 50 mL). Organic layers are combined and dried over MgSO<sub>4</sub>. The solvent was evaporated *in vacuo*. The crude residue was purified by flash chromatography (hexane/EtOAc, 3:7), yielding (R)-4 (1.77 g, 91.4 %) as a pale yellow oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 9.97 (s, 1H), 7.45 – 7.38 (m, 3H), 7.19 – 7.15 (m, 1H), 4.05 – 4.01 (m, 2H), 3.76 – 3.65 (m, 3H), 3.49 – 3.43 (m, 1H), 1.87 – 1.49 (m, 8H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 192.38, 159.73, 137.90, 130.17, 123.59, 122.09, 112.85, 72.19, 68.15, 66.90, 32.89, 29.21, 22.29.  $[\alpha]_{\text{D}}^{20} = +3.5$  ( $c = 1.00$ , MeOH). HRMS (EI-MS): calc. for (C<sub>13</sub>H<sub>18</sub>O<sub>4</sub>) [M]: 238.1205, found: 238.1204.

**(S)-6-(3-Formylphenoxy)-2-hydroxyhexyl acetate (S)-C2.** Diol (S)-4 (99.8 mg, 0.42 mmol, 1.0 equiv) was stirred in dry DCM (2.0 mL) with collidine (83 μL, 0.63 mmol, 1.5 equiv) at 25°C. At –60°C, Ac<sub>2</sub>O (52 μL, 0.54 mmol, 1.3 equiv) was added dropwise. The reaction mixture was allowed to reach RT while stirring overnight. The mixture was then poured into 10 mL of DCM and washed with 1.5 mL of a 0.1 M H<sub>3</sub>PO<sub>4</sub> aqueous solution. The aqueous layer was extracted with DCM (2 x 5 mL). Organic layers are combined and dried over MgSO<sub>4</sub>. The solvent was removed *in vacuo*. The crude residue was purified by flash chromatography (hexane/EtOAc, 3:2), yielding (S)-C2 (42 mg, 36 %) as colorless oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 9.97 (s, 1H), 7.46 – 7.37 (m, 3H), 7.20 – 7.15 (m, 1H), 4.19 – 3.85 (m, 5H), 2.39 (broad s, 1H), 2.11 (s, 3H), 1.88 – 1.56 (m, 6H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 192.31, 171.33, 159.73, 137.94, 130.18, 123.57, 122.08, 112.89,

69.95, 68.84, 68.11, 33.09, 29.16, 22.18, 21.00.  $[\alpha]_{\text{D}}^{20} = +3.6$  ( $c = 0.85$ ,  $\text{CHCl}_3$ ). HRMS (ESI-MS): calc. for  $(\text{C}_{15}\text{H}_{20}\text{O}_5\text{Na})$   $[\text{M}+\text{Na}^+]$ : 303.1208, found: 303.1209.

**(R)-6-(3-Formylphenoxy)-2-hydroxyhexyl acetate (R)-C2.** The procedure for (S)-C2 was applied using diol (R)-4 (103.9 mg, 0.44 mmol, 1.0 equiv), dry pyridine (2.0 mL), and  $\text{Ac}_2\text{O}$  (54  $\mu\text{L}$ , 0.57 mmol, 1.3 equiv). Purification by flash chromatography (hexane/EtOAc, 6.5:3.5) gave (R)-C2 (54 mg, 44 %) as colorless oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta = 9.97$  (s, 1H), 7.46 – 7.38 (m, 3H), 7.20 – 7.15 (m, 1H), 4.20 – 3.85 (m, 5H), 2.65 (broad s, 1H), 2.11 (s, 3H), 1.89 – 1.56 (m, 6H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta = 192.17$ , 171.19, 159.59, 137.81, 130.03, 123.43, 121.94, 112.75, 69.81, 68.70, 67.97, 32.95, 29.01, 22.03, 20.87.  $[\alpha]_{\text{D}}^{20} = -3.6$  ( $c = 0.60$ ,  $\text{CHCl}_3$ ). HRMS (EI-MS): calc. for  $(\text{C}_{15}\text{H}_{20}\text{O}_5)$   $[\text{M}]$ : 280.1311, found: 280.1310.

**(S)-6-(3-Formylphenoxy)-2-hydroxyhexyl butyrate (S)-C4.** The procedure for (S)-C2 was applied with (S)-4 (107.2 mg, 0.45 mmol, 1.0 equiv) and butyryl chloride (61  $\mu\text{L}$ , 0.58 mmol, 1.3 equiv). Purification by flash chromatography (hexane/EtOAc, 3:2) gave (S)-C4 (81.5 mg, 59 %) as colorless oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta = 9.96$  (s, 1H), 7.45 – 7.36 (m, 3H), 7.18 – 7.14 (m, 1H), 4.19 – 3.84 (m, 5H), 2.94 (broad s, 1H), 2.35 – 2.30 (m, 2H), 1.89 – 1.55 (m, 8H), 0.97 – 0.92 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta = 192.30$ , 173.97, 159.70, 137.89, 130.13, 123.49, 122.03, 112.90, 69.97, 68.54, 68.08, 36.16, 33.09, 29.12, 22.13, 18.52, 13.74.  $[\alpha]_{\text{D}}^{20} = +3.2$  ( $c = 0.55$ ,  $\text{CHCl}_3$ ). HRMS (ESI-MS): calc. for  $(\text{C}_{17}\text{H}_{24}\text{O}_5\text{Na})$   $[\text{M}+\text{Na}^+]$ : 331.1521, found: 331.1534.

**(R)-6-(3-Formylphenoxy)-2-hydroxyhexyl butyrate (R)-C4.** The procedure for (S)-C2 was applied with (R)-4 (101.6 mg, 0.43 mmol, 1.0 equiv) and butyryl chloride (58  $\mu\text{L}$ , 0.55 mmol, 1.3 equiv). Purification by flash chromatography (hexane/EtOAc, 7:3) gave (R)-C4 (104.5 mg, 79.5 %) as a colorless oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta = 9.93$  (s, 1H), 7.42 – 7.34 (m, 3H), 7.16 – 7.12 (m, 1H), 4.17 – 3.83 (m, 5H), 3.05 (broad s, 1H), 2.28 – 2.33 (m, 2H), 1.84 – 1.53 (m, 8H), 0.95 – 0.90 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta = 192.32$ , 173.95, 159.62, 137.79, 130.07,

123.44, 121.97, 112.84, 69.83, 68.45, 68.01, 36.09, 33.04, 29.06, 22.08, 18.45, 13.69.  $[\alpha]_{\text{D}}^{20} = -2.9$  ( $c = 0.50$ ,  $\text{CHCl}_3$ ). HRMS (EI-MS): calc. for  $(\text{C}_{17}\text{H}_{24}\text{O}_5)$  [M]: 308.1324, found: 308.1625.

**(S)-6-(3-Formylphenoxy)-2-hydroxyhexyl hexanoate (S)-C6.** The procedure for (S)-C2 was applied with (S)-4 (108.1 mg, 0.45 mmol, 1.0 equiv) and hexanoyl chloride (81  $\mu\text{L}$ , 0.59 mmol, 1.3 equiv). Purification by flash chromatography (hexane/EtOAc, 7.5:3) gave (S)-C6 (100 mg, 65.4 %) as colorless oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta = 9.95$  (s, 1H), 7.44 – 7.36 (m, 3H), 7.17 – 7.13 (m, 1H), 4.18 – 3.83 (m, 5H), 2.42 (s, 1H), 2.36 – 2.33 (m, 2H), 1.87 – 1.55 (m, 8H), 1.27 – 1.28 (m, 4H), 0.90 – 0.86 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta = 192.33$ , 174.17, 159.68, 137.87, 130.14, 123.53, 122.05, 112.85, 69.96, 68.59, 68.07, 34.26, 33.09, 31.38, 29.13, 24.72, 22.40, 22.15, 14.00.  $[\alpha]_{\text{D}}^{20} = +2.2$  ( $c = 1.00$ ,  $\text{CHCl}_3$ ). HRMS (ESI-MS): calc. for  $(\text{C}_{19}\text{H}_{28}\text{O}_5\text{Na})$   $[\text{M}+\text{Na}^+]$ : 359.1834, found: 359.1821.

**(R)-6-(3-Formylphenoxy)-2-hydroxyhexyl hexanoate (R)-C6.** The procedure for (S)-C2 was applied with (R)-4 (100.2 mg, 0.42 mmol, 1.0 equiv) and hexanoyl chloride (76  $\mu\text{L}$ , 0.55 mmol, 1.3 equiv). Purification by flash chromatography (hexane/EtOAc, 7.5:3) gave (R)-C6 (111 mg, 78.5 %) as colorless oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta = 9.96$  (s, 1H), 7.44 – 7.37 (m, 3H), 7.18 – 7.14 (m, 1H), 4.19 – 3.84 (m, 5H), 3.09 (broad s, 1H), 2.37 – 2.32 (m, 2H), 1.87 – 1.27 (m, 12H), 0.91 – 0.86 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta = 192.31$ , 174.17, 159.71, 137.90, 130.13, 123.51, 122.04, 112.91, 69.98, 68.58, 68.09, 34.27, 33.10, 31.39, 29.14, 24.72, 22.39, 22.15, 13.69.  $[\alpha]_{\text{D}}^{20} = -2.9$  ( $c = 1.00$ ,  $\text{CHCl}_3$ ). HRMS (EI-MS): calc. for  $(\text{C}_{19}\text{H}_{28}\text{O}_5)$  [M]: 336.1937, found: 336.1938.

**(S)-6-(3-Formylphenoxy)-2-hydroxyhexyl octanoate (S)-C8.** The procedure for (S)-C2 was applied with (S)-4 (106.6 mg, 0.45 mmol, 1.0 equiv) and octanoyl chloride (100  $\mu\text{L}$ , 0.58 mmol, 1.3 equiv). Purification by flash chromatography (hexane/EtOAc, 8:2) gave (S)-C8 (92.4 mg, 56.7 %) as colorless oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta = 9.96$  (s, 1H), 7.44 – 7.36 (m, 3H), 7.18 –

7.14 (m, 1H), 4.19 – 3.85 (m, 5H), 3.74 (broad s, 1H), 2.37 – 2.32 (m, 2H), 1.84 – 1.56 (m, 8H), 1.28 (broad s, 8H), 0.89 – 0.85 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta = 192.32, 174.19, 159.71, 137.90, 130.13, 123.51, 122.05, 112.91, 70.00, 68.57, 68.09, 34.32, 33.10, 31.74, 29.19, 29.14, 29.00, 25.05, 22.68, 22.15, 14.15$ .  $[\alpha]_{\text{D}}^{20} = +3.2$  ( $c = 1.00, \text{CHCl}_3$ ). HRMS (EI-MS): calc. for  $(\text{C}_{21}\text{H}_{32}\text{O}_5)$  [M]: 364.2250, found: 364.2254.

**(R)-6-(3-Formylphenoxy)-2-hydroxyhexyl octanoate (R)-C8.** The procedure for (S)-C2 was applied with (R)-4 (99.5 mg, 0.42 mmol, 1.0 equiv) and octanoyl chloride (76  $\mu\text{L}$ , 0.55 mmol, 1.3 equiv). Purification by flash chromatography (hexane/EtOAc, 8:2.5) gave (R)-C8 (119 mg, 78.5 %) as colorless oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta = 9.88$  (s, 1H), 7.37 – 7.29 (m, 3H), 7.10 – 7.06 (m, 1H), 4.11 – 3.77 (m, 5H), 3.29 (broad s, 1H), 2.29 – 2.25 (m, 2H), 1.79 – 1.20 (m, 16H), 0.82 – 0.77 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta = 192.29, 174.15, 159.67, 137.86, 130.10, 123.46, 122.00, 112.90, 69.92, 68.53, 68.06, 34.27, 33.08, 31.71, 29.16, 29.11, 28.97, 25.01, 22.65, 22.12, 14.11$ .  $[\alpha]_{\text{D}}^{20} = -3.0$  ( $c = 1.00, \text{CHCl}_3$ ). HRMS (EI-MS): calc. for  $(\text{C}_{21}\text{H}_{32}\text{O}_5)$  [M]: 364.2250, found: 364.2248.

**(S)-6-(3-Formylphenoxy)-2-hydroxyhexyl decanoate (S)-C10.** The procedure for (S)-C2 was applied with (S)-4 (101.3 mg, 0.43 mmol, 1.0 equiv) and decanoyl chloride (113  $\mu\text{L}$ , 0.55 mmol, 1.3 equiv). Purification by flash chromatography (hexane/EtOAc, 9:1.5) gave (S)-C10 (92 mg, 55 %) as a waxy solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta = 9.96$  (s, 1H), 7.45 – 7.37 (m, 3H), 7.18 – 7.14 (m, 1H), 4.19 – 3.84 (m, 5H), 2.85 (broad s, 1H), 2.37 – 2.32 (m, 2H), 1.87 – 1.55 (m, 8H), 1.25 (broad s, 12H), 0.89 – 0.85 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta = 192.30, 174.18, 159.71, 137.92, 130.14, 123.52, 122.05, 112.91, 70.00, 68.59, 68.10, 34.32, 33.11, 31.96, 29.52, 29.35$  (2C), 29.25, 29.16, 25.06, 22.76, 22.16, 14.20.  $[\alpha]_{\text{D}}^{20} = +2.7$  ( $c = 0.80, \text{CHCl}_3$ ). HRMS (EI-MS): calc. for  $(\text{C}_{23}\text{H}_{36}\text{O}_5)$  [M]: 392.2563, found: 392.2582.



**(R)-6-(3-Formylphenoxy)-2-hydroxyhexyl decanoate (R)-C10.** The procedure for (*S*)-C2 was applied with (*R*)-4 (100.0 mg, 0.42 mmol, 1.0 equiv) and decanoyl chloride (111  $\mu$ L, 0.55 mmol, 1.3 equiv). Purification by flash chromatography (hexane/EtOAc, 9:1.5) gave (*R*)-C10 (99 mg, 60 %) as a waxy solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 9.96 (s, 1H), 7.45 – 7.37 (m, 3H), 7.16 – 7.14 (m, 1H), 4.19 – 3.84 (m, 5H), 2.83 (broad s, 1H), 2.37 – 2.32 (m, 2H), 1.86 – 1.56 (m, 8H), 1.25 (broad s, 12H), 0.89 – 0.85 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 192.29, 174.17, 159.71, 137.92, 130.14, 123.52, 122.05, 112.91, 70.00, 68.59, 68.10, 34.32, 33.11, 31.96, 29.52, 29.35 (2C), 29.25, 29.16, 25.06, 22.76, 22.16, 14.20.  $[\alpha]_{\text{D}}^{20} = -2.6$  ( $c = 1.00$ ,  $\text{CHCl}_3$ ). HRMS (ESI-MS): calc. for ( $\text{C}_{23}\text{H}_{36}\text{O}_5\text{Na}$ ) [ $\text{M}+\text{Na}^+$ ]: 415.2460, found: 415.2473.

**(S)-6-(3-Formylphenoxy)-2-hydroxyhexyl dodecanoate (S)-C12.** The procedure for (*S*)-C2 was applied with (*S*)-4 (100.1 mg, 0.42 mmol, 1.0 equiv) and dodecanoyl chloride (130  $\mu$ L, 0.55 mmol, 1.3 equiv). Purification by flash chromatography (hexane/EtOAc, 9:2) gave (*R*)-C12 (115 mg, 65.2 %) as a waxy solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 9.97 (s, 1H), 7.45 – 7.37 (m, 3H), 7.18 – 7.14 (m, 1H), 4.20 – 3.84 (m, 5H), 2.37 – 2.32 (m, 2H), 1.96 (broad s, 1H), 1.87 – 1.55 (m, 8H), 1.25 (broad s, 16H), 0.90 – 0.85 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 192.29, 174.19, 159.76, 137.98, 130.18, 123.57, 122.09, 112.91, 70.08, 68.66, 68.14, 34.37, 33.14, 32.05, 29.74 (2C), 29.60, 29.48, 29.40, 29.30, 29.20, 25.11, 22.83, 22.21, 14.25.  $[\alpha]_{\text{D}}^{20} = +2.3$  ( $c = 1.00$ ,  $\text{CHCl}_3$ ). HRMS (EI-MS): calc. for ( $\text{C}_{25}\text{H}_{40}\text{O}_5$ ) [ $\text{M}$ ]: 420.2876, found: 420.2883.

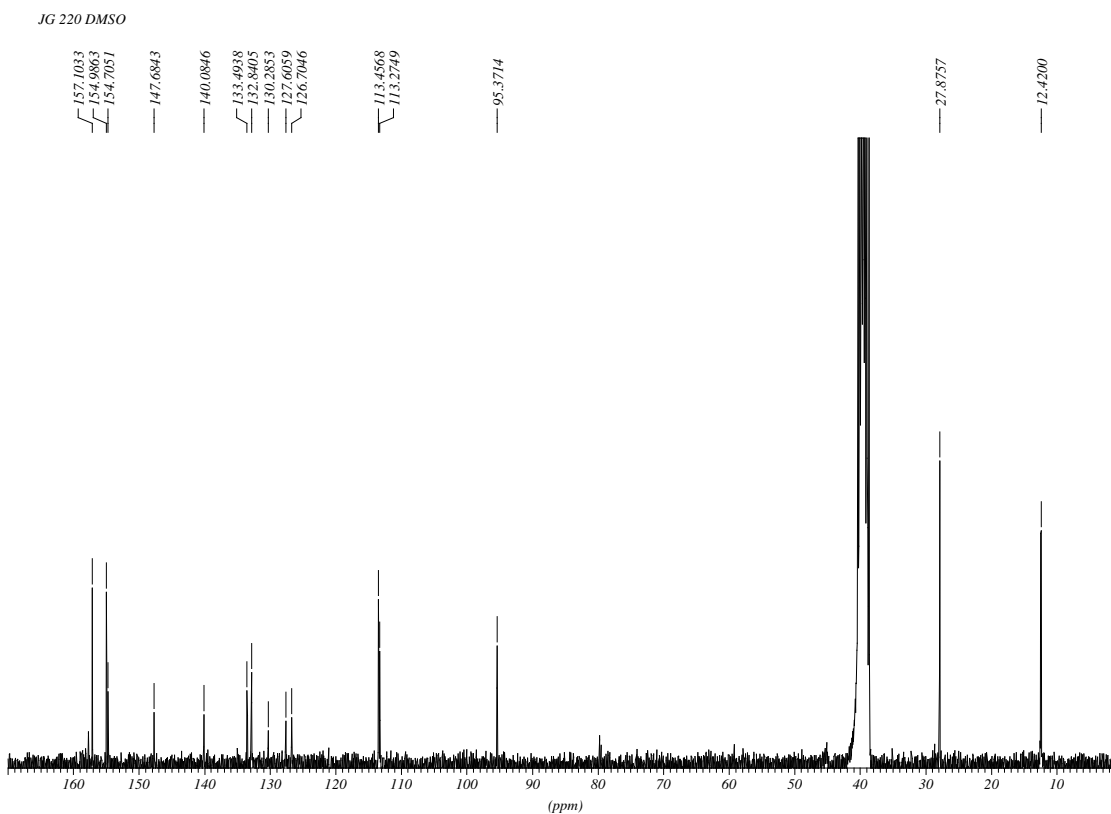
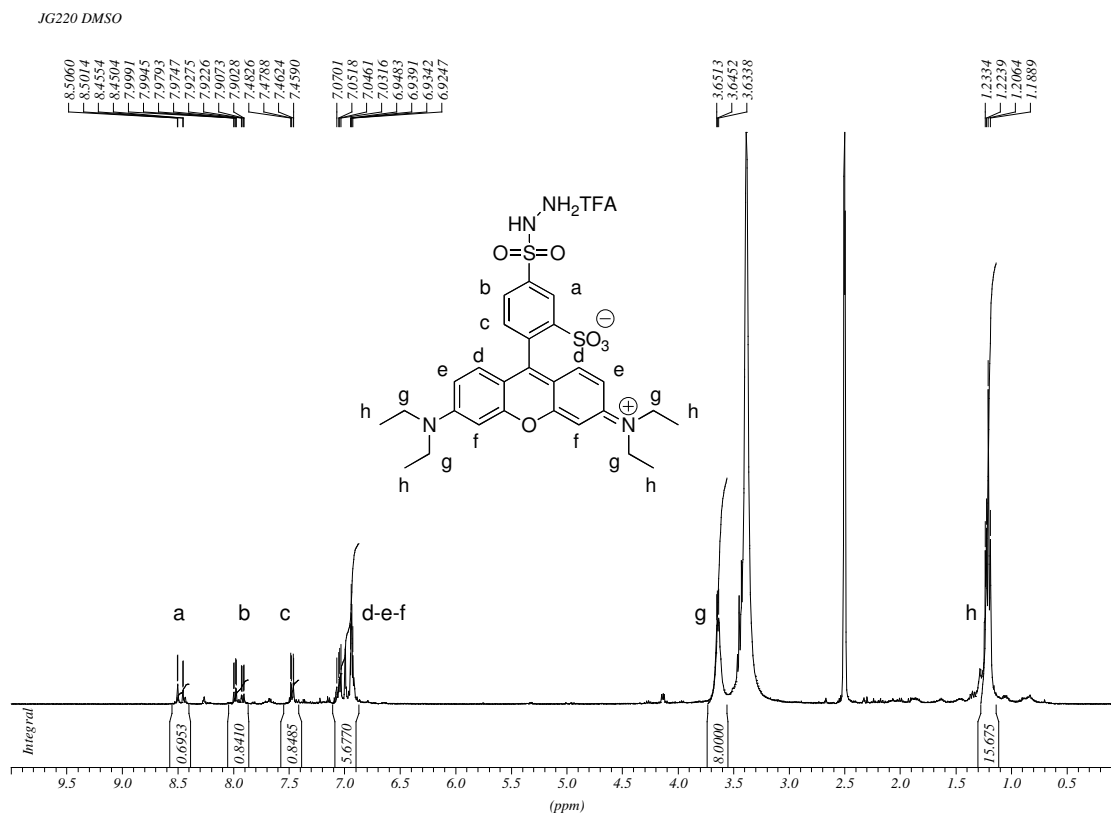
**(R)-6-(3-Formylphenoxy)-2-hydroxyhexyl dodecanoate (R)-C12.** The procedure for (*S*)-C2 was applied with (*R*)-4 (104.2 mg, 0.44 mmol, 1.0 equiv) and dodecanoyl chloride (135  $\mu$ L, 0.57 mmol, 1.3 equiv). Purification by flash chromatography (hexane/EtOAc, 9:2) gave (*R*)-C12 (109 mg, 59.1 %) as a waxy solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 9.98 (s, 1H), 7.46 – 7.38 (m, 3H), 7.21 – 7.14 (m, 1H), 4.21 – 3.85 (m, 5H), 2.38 – 2.33 (m, 2H), 2.06 (broad s, 1H), 1.89 – 1.56 (m, 8H), 1.26 (broad s, 16H), 0.91 – 0.86 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 192.29, 174.18, 159.76, 137.98, 130.18, 123.57, 122.09, 112.91, 70.08, 68.66, 68.14, 34.37, 33.14, 32.05, 29.74 (2C), 29.60,

29.48, 29.41, 29.30, 29.20, 25.11, 22.83, 22.21, 14.25.  $[\alpha]_{\text{D}}^{20} = -1.9$  ( $c = 0.60$ ,  $\text{CHCl}_3$ ). HRMS (ESI-MS): calc. for ( $\text{C}_{25}\text{H}_{40}\text{O}_5$ )  $[\text{M}]$ : 420.2876, found: 420.2876.

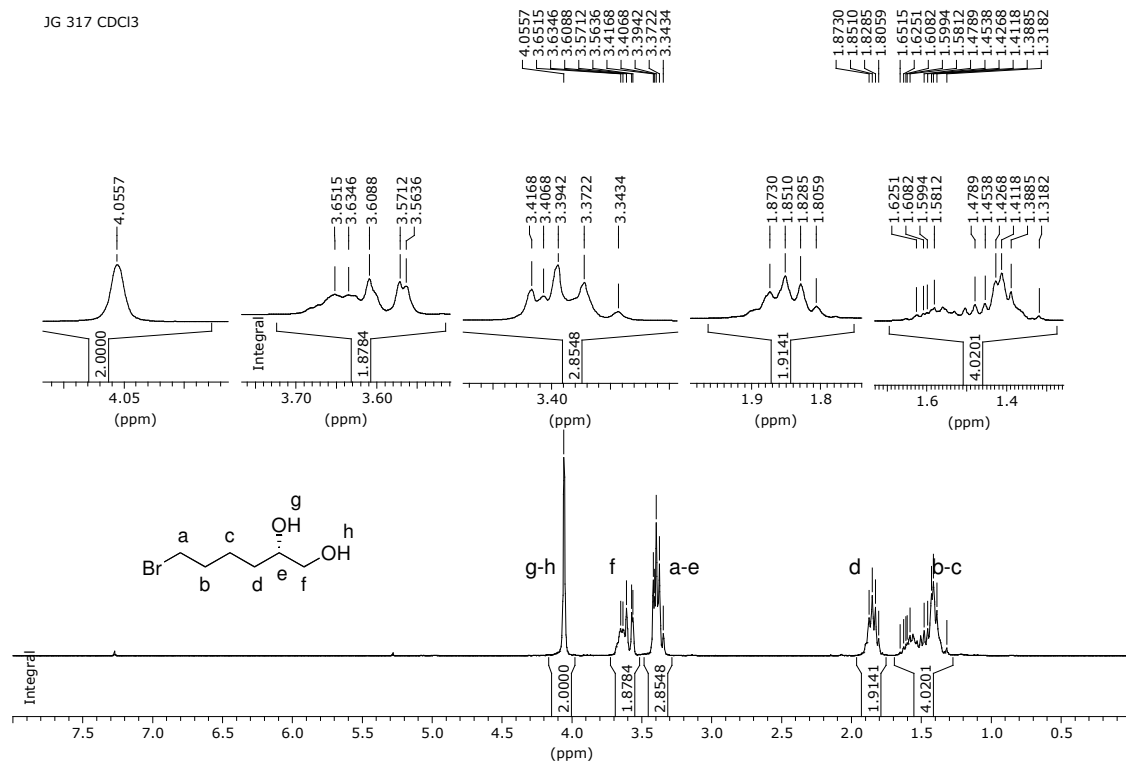
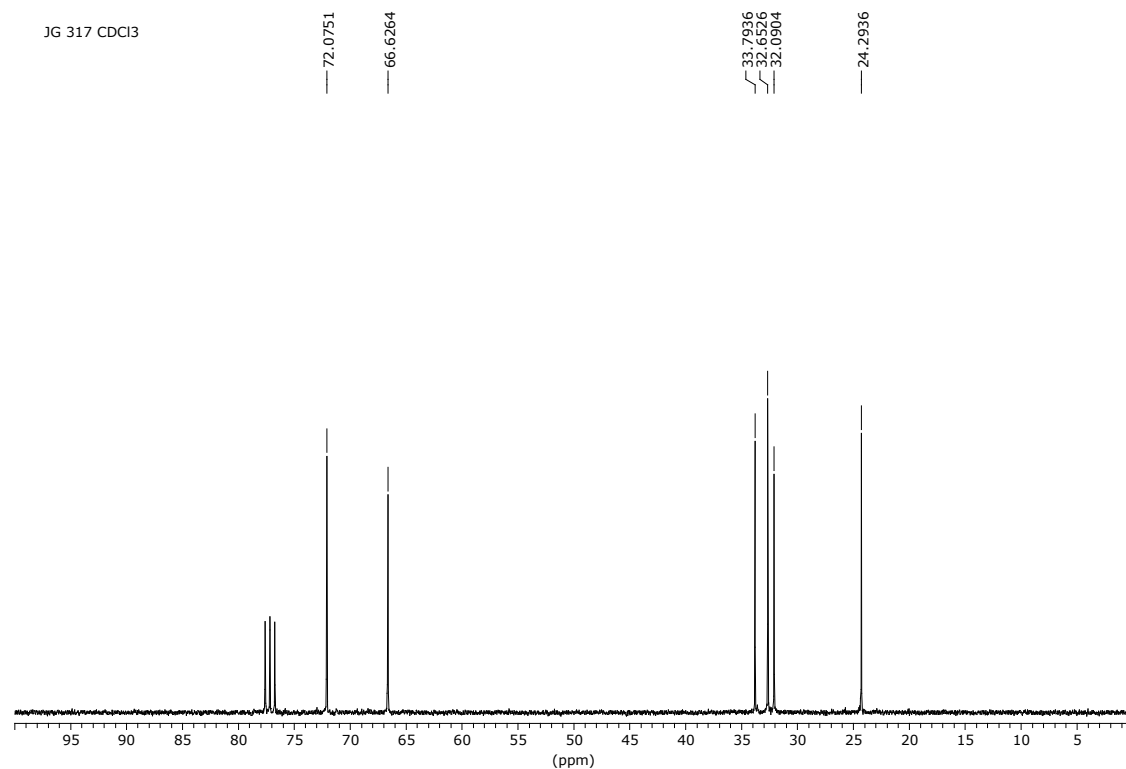
**5-(*N'*-Boc-hydrazinosulfonyl)-rhodamine B (6).** Sulforhodamine B acid chloride **5** (20.0 mg, 34.5  $\mu\text{mol}$ , 1 equiv), *t*-butyl carbazate (4.6 mg, 34.5  $\mu\text{mol}$ , 1 equiv) and 4-DMAP (4.2 mg, 34.5  $\mu\text{mol}$ , 1 equiv) were dissolved in DMF (2.0 mL) under argon atmosphere. After completion of the reaction (TLC), the solvent was removed under high-vacuum. The crude is purified by preparative RP-HPLC, yielding **5** (9.4 mg, 40 %) of a purple solid.  $^1\text{H}$  NMR (300 MHz, Acetone- $d_6$ ):  $\delta = 8.68$  (d,  $J = 1.7$  Hz, 1H), 7.99 – 7.96 (m, 1H), 7.41 (d,  $J = 8.1$  Hz, 1H), 7.26 (d,  $J = 9.6$  Hz, 2H), 6.98 (dd,  $J = 9.4$  Hz, 2.4 Hz, 2H), 6.86 (d,  $J = 2.4$  Hz, 2H), 3.81 – 3.67 (m, 8H), 1.36 – 1.28 (m, 21H).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta = 157.44$ , 156.83 (2C), 154.71 (2C), 154.43, 147.42, 139.81, 133.22, 132.57 (2C), 129.99, 127.33, 126.43, 113.18 (2C), 113.00 (2C), 95.10 (2C), 79.49, 27.60 (4C), 24.82 (3C), 12.15 (4C). HRMS (ESI-MS): calc. for ( $\text{C}_{32}\text{H}_{41}\text{N}_4\text{O}_8\text{S}_2$ )  $[\text{M}+\text{H}^+]$ : 673.2365, found: 673. 2364.

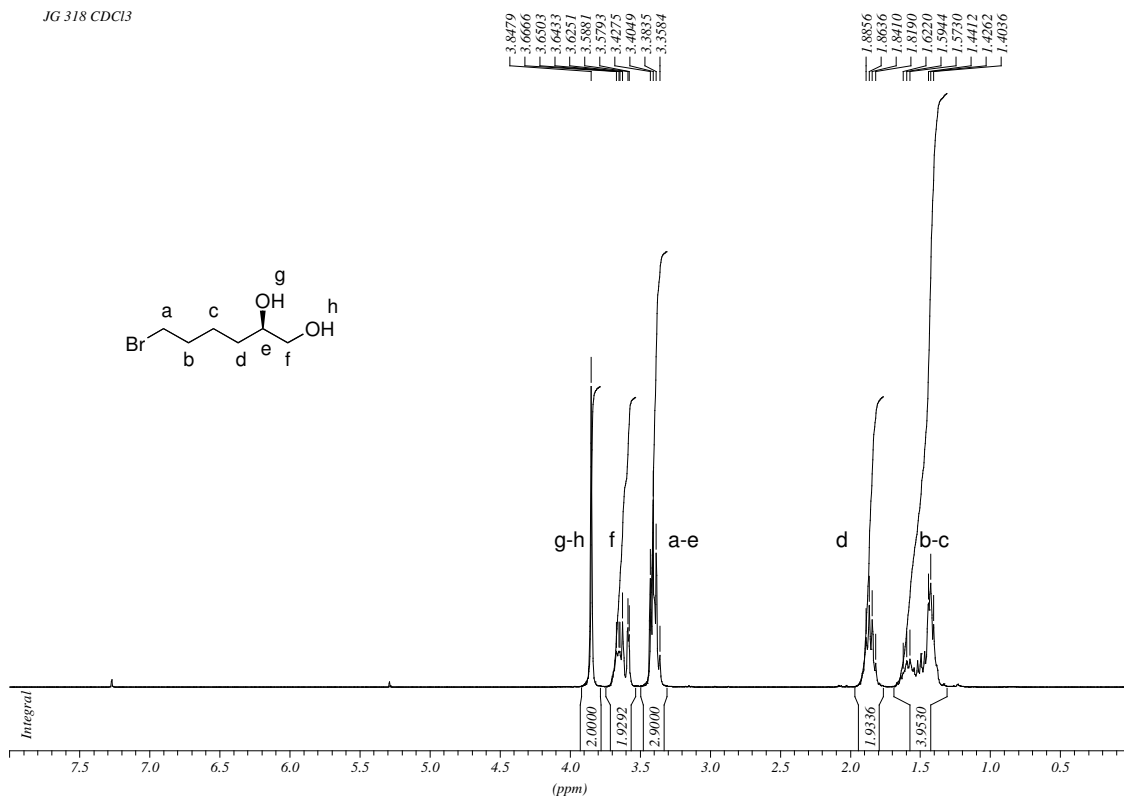
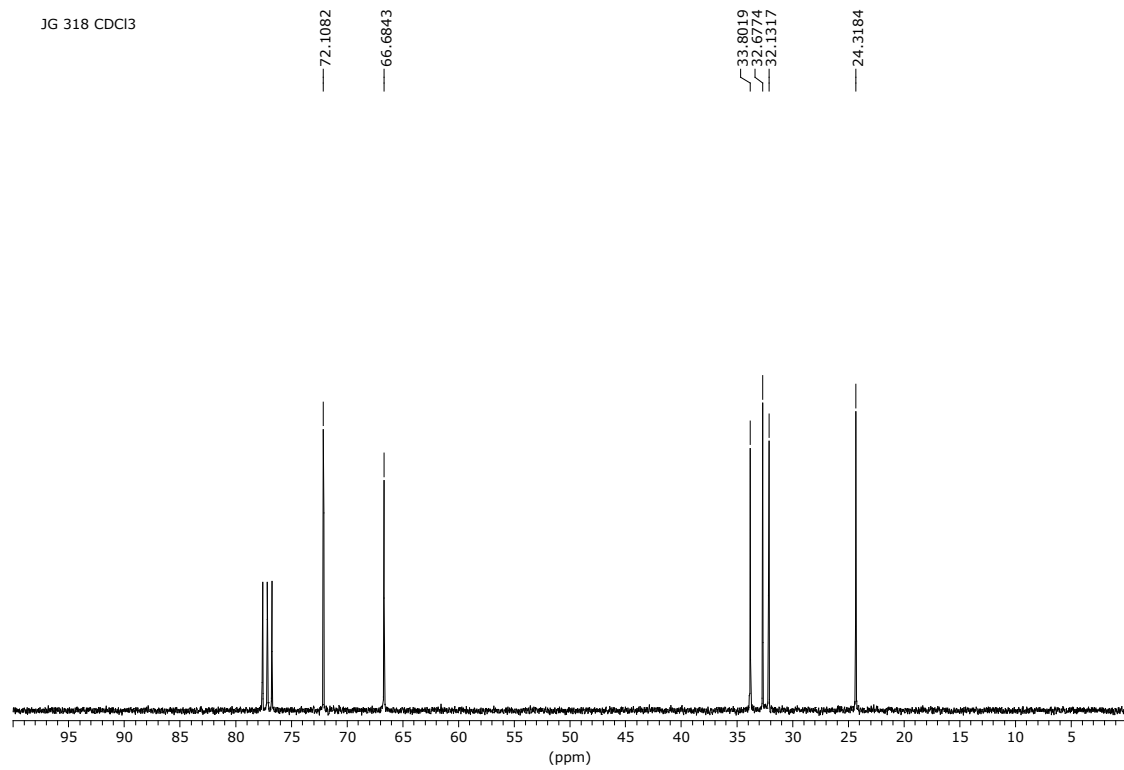
**5-Hydrazinosulfonyl-rhodamine B (1).** The Boc protected compound **5** (9.4 mg, 14.0  $\mu\text{mol}$ ) was stirred at 25°C in 0.5 mL of a DCM/TFA mixture (0.5 mL). The deprotection was monitored by RP-HPLC. After completion of the reaction, the solvent and the excess of acid are removed *in vacuo*, yielding **1** (7.7 mg, 100.0 %) as a purple solid.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta = 8.50$  (d,  $J = 1.8$  Hz, 1H), 8.46 – 7.90 (m, 1H), 7.48 – 7.46 (m, 1H), 7.07 – 6.92 (m, 6H), 3.65 – 3.63 (m, 8H), 1.23 – 1.19 (m, 12H).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta = 157.10$  (2C), 154.99 (2C), 154.71, 147.69, 140.08, 133.49, 132.84 (2C), 130.29, 127.61, 126.70, 113.46 (2C), 113.27 (2C), 95.37 (2C), 27.88 (4C), 12.42 (4C). HRMS (ESI-MS): calc. for ( $\text{C}_{27}\text{H}_{33}\text{N}_4\text{O}_6\text{S}_2$ )  $[\text{M}]$ : 573.1841, found: 573.1864.

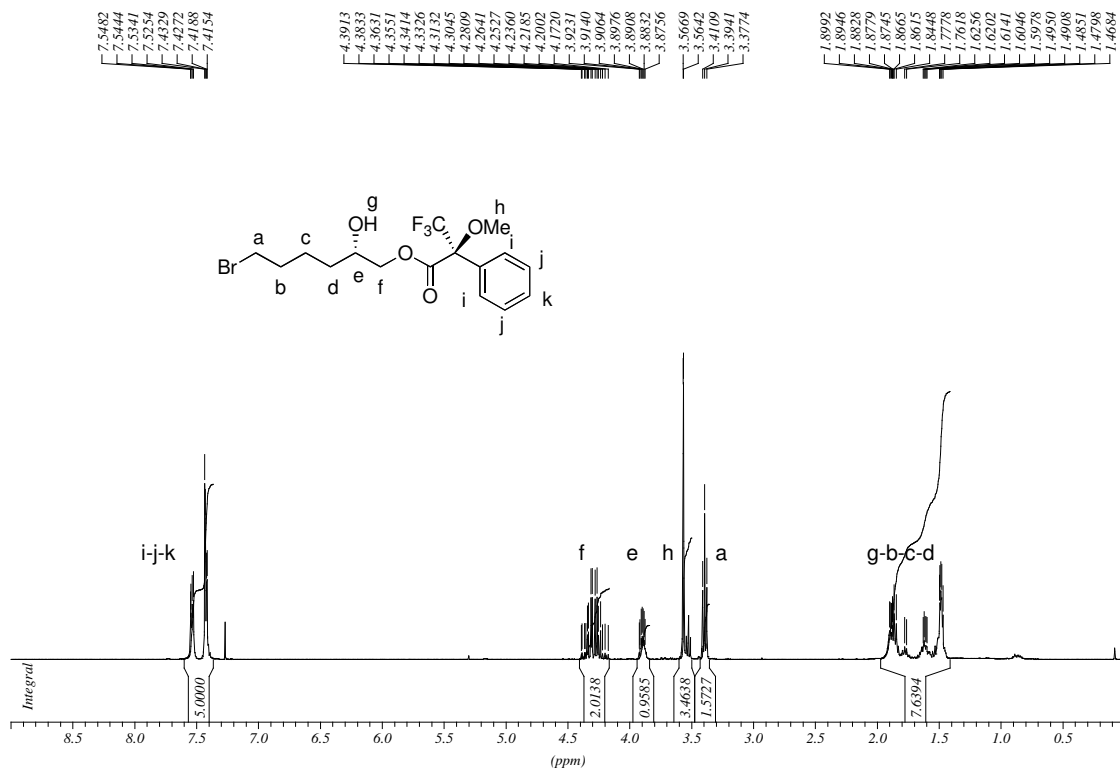
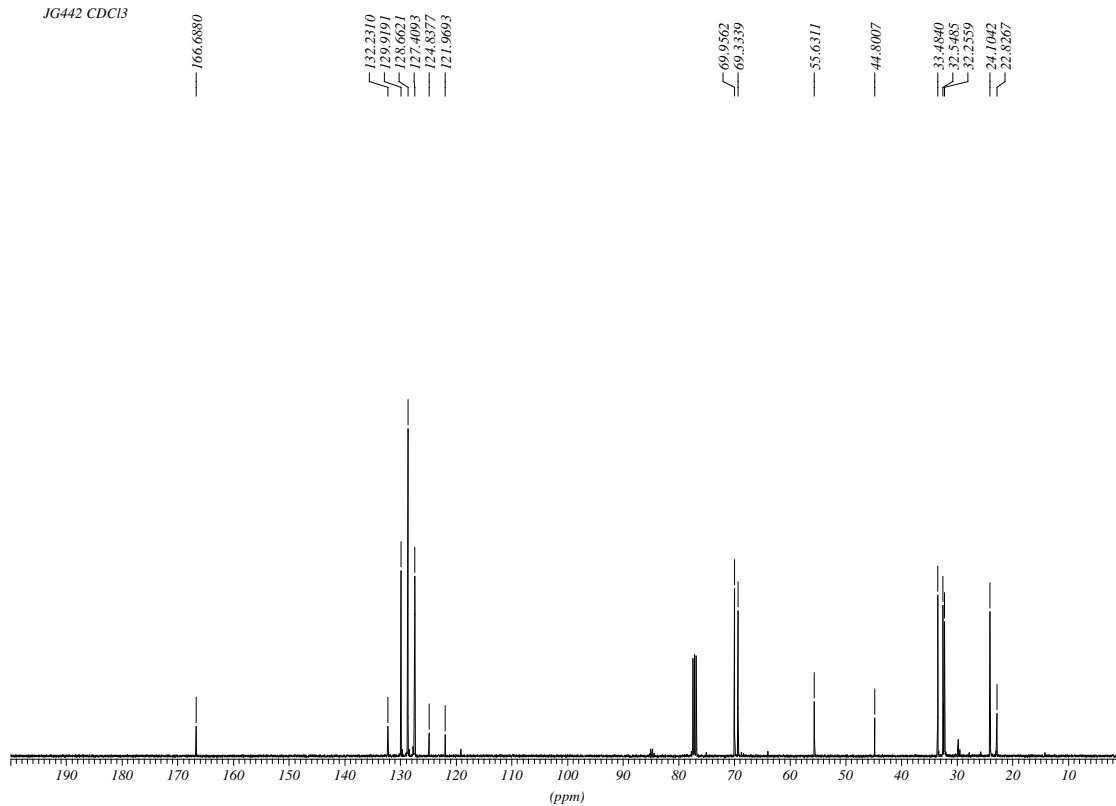
## 5-Hydrazinosulfonyl-rhodamine B (1)



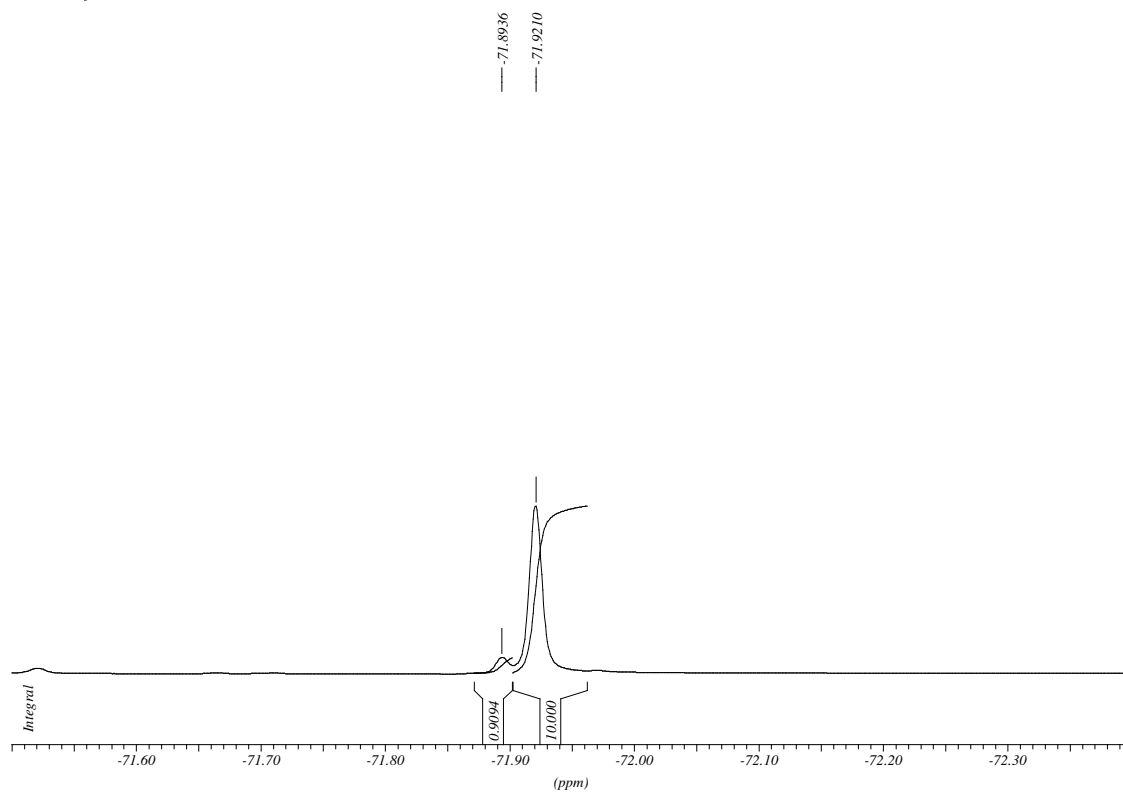


**(S)-6-Bromohexane-1,2-diol (S)-3**JG 317 CDCl<sub>3</sub>JG 317 CDCl<sub>3</sub>

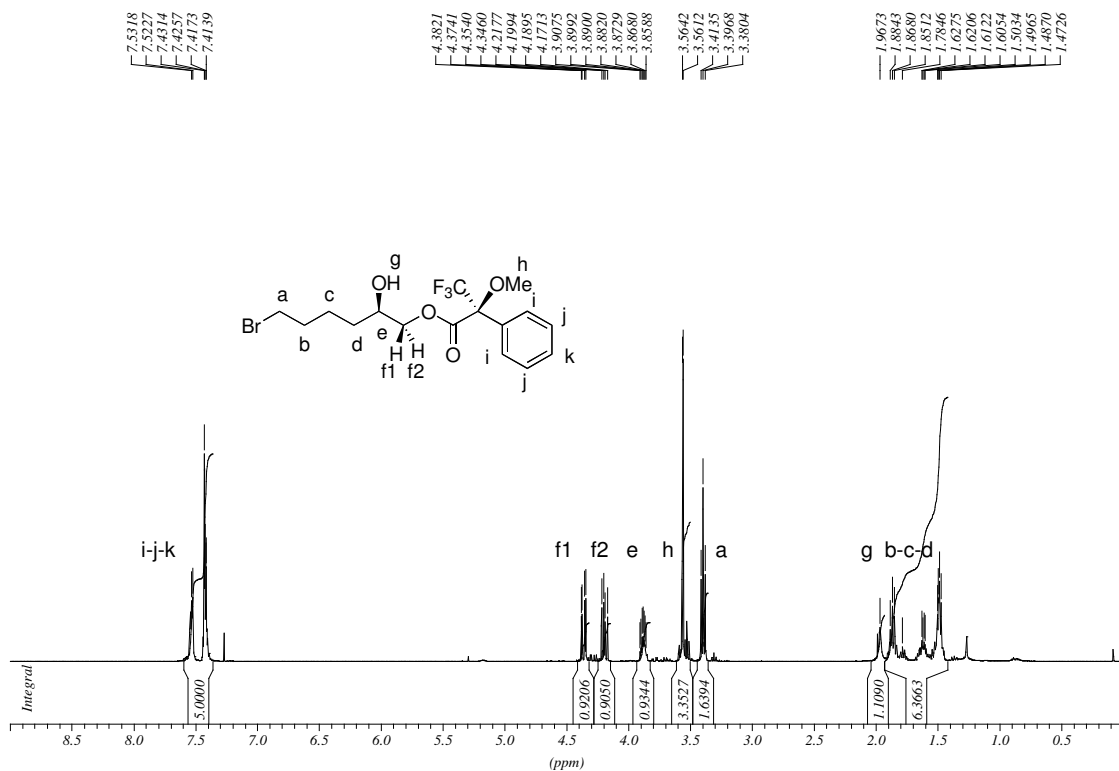
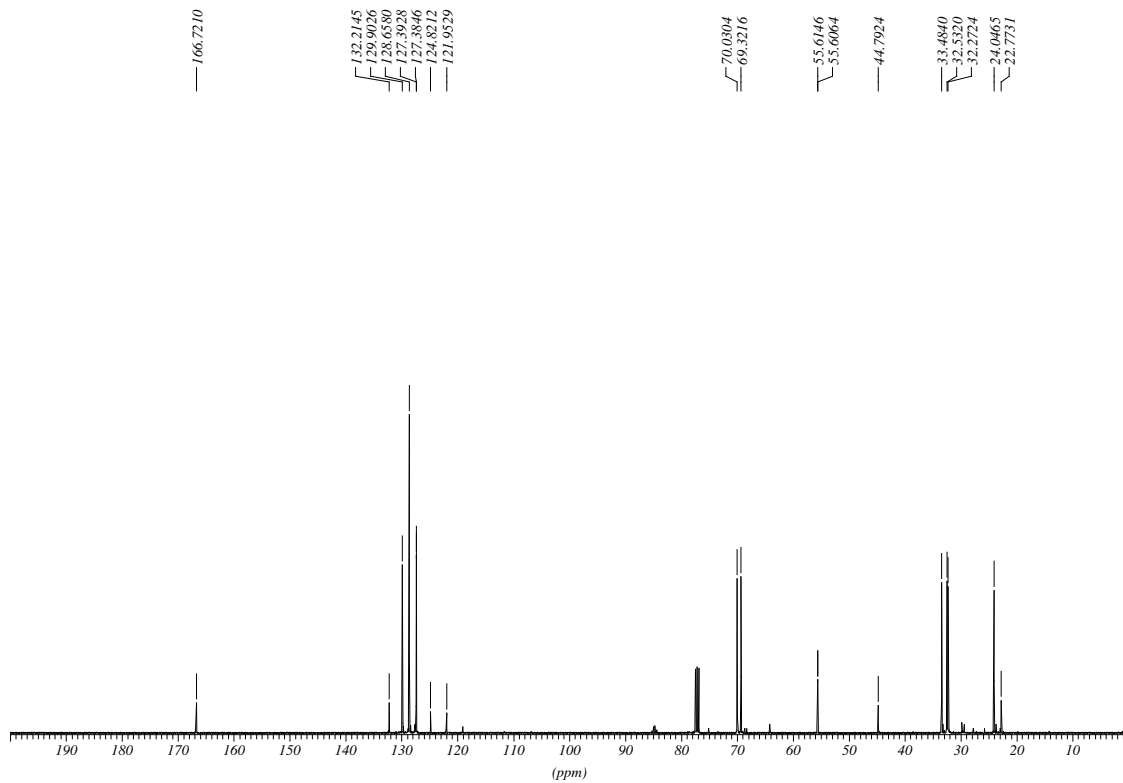
**(R)-6-Bromohexane-1,2-diol (R)-3**JG 318 CDCl<sub>3</sub>JG 318 CDCl<sub>3</sub>

**(S)-((S)-6-bromo-2-hydroxyhexyl) 3,3,3-trifluoro-2-methoxy-phenylpropanoate**JG442 CDCl<sub>3</sub>JG442 CDCl<sub>3</sub>

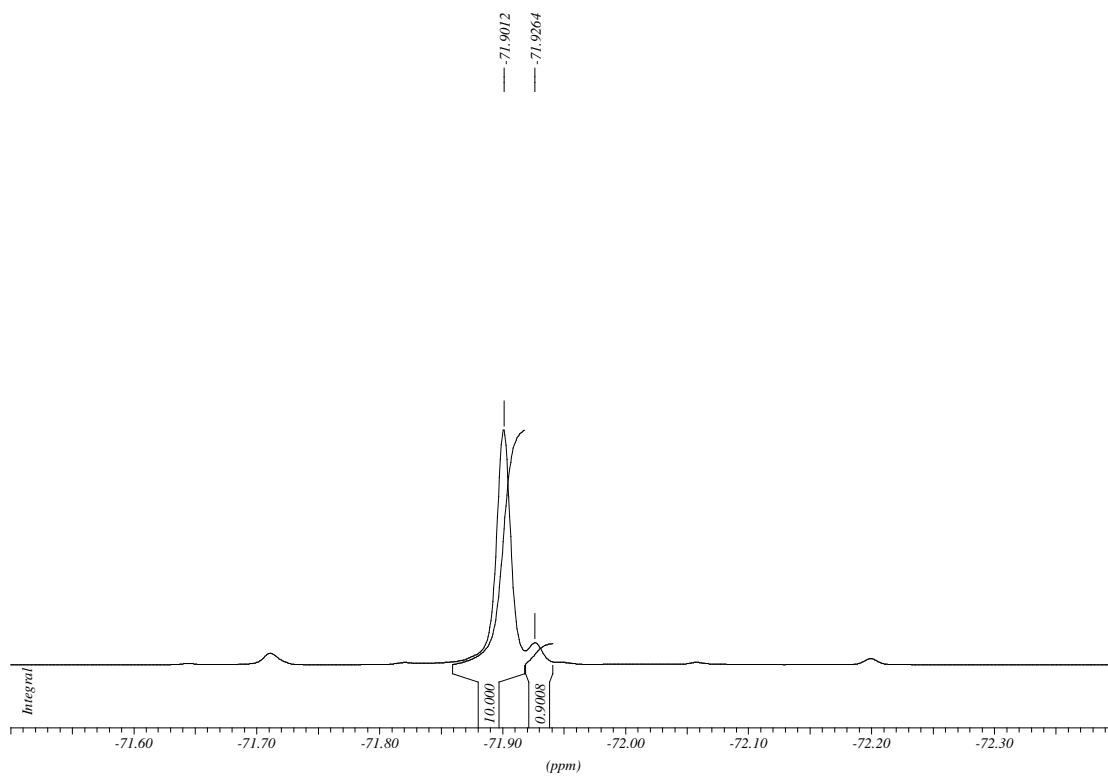
JG442 f19 CDCl3

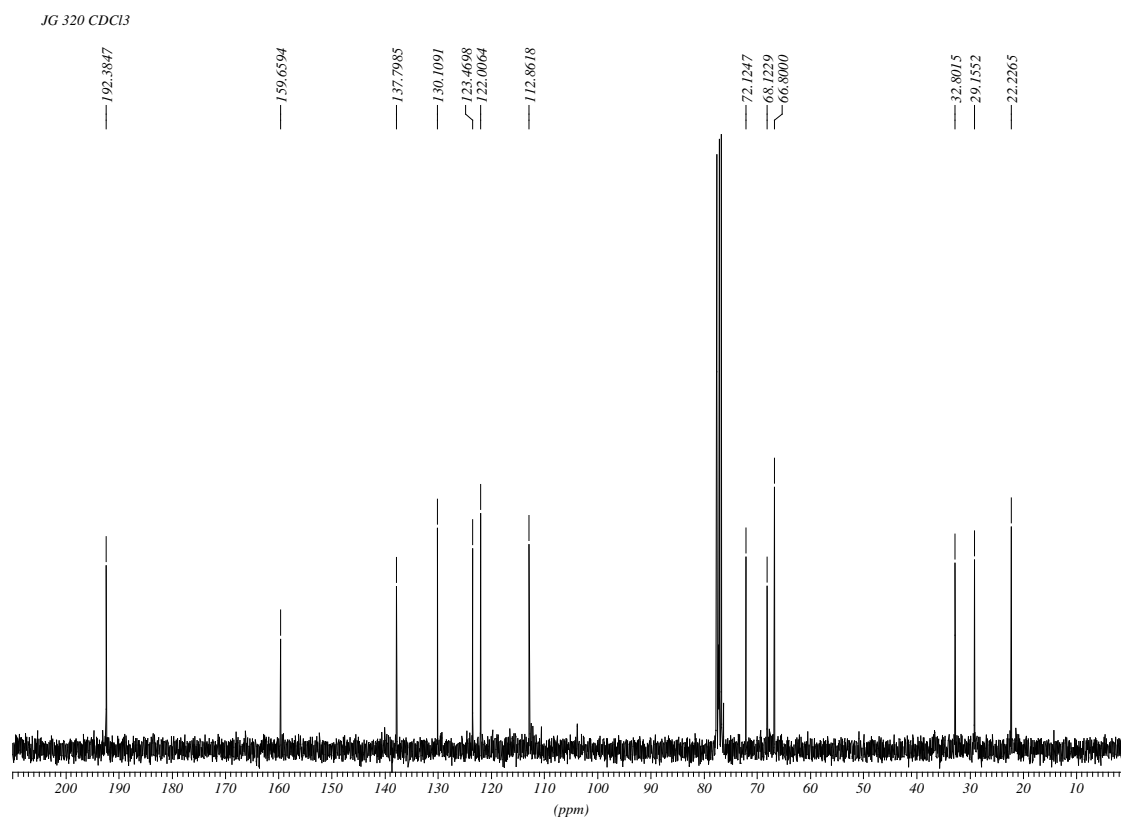
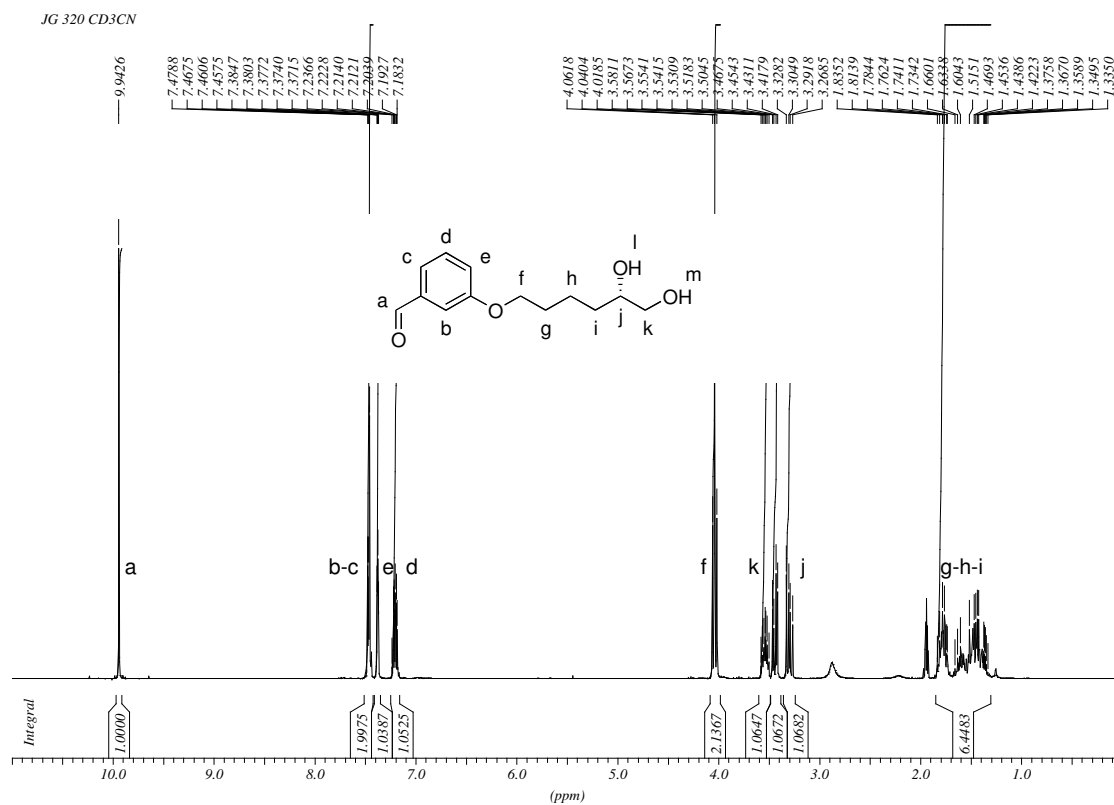


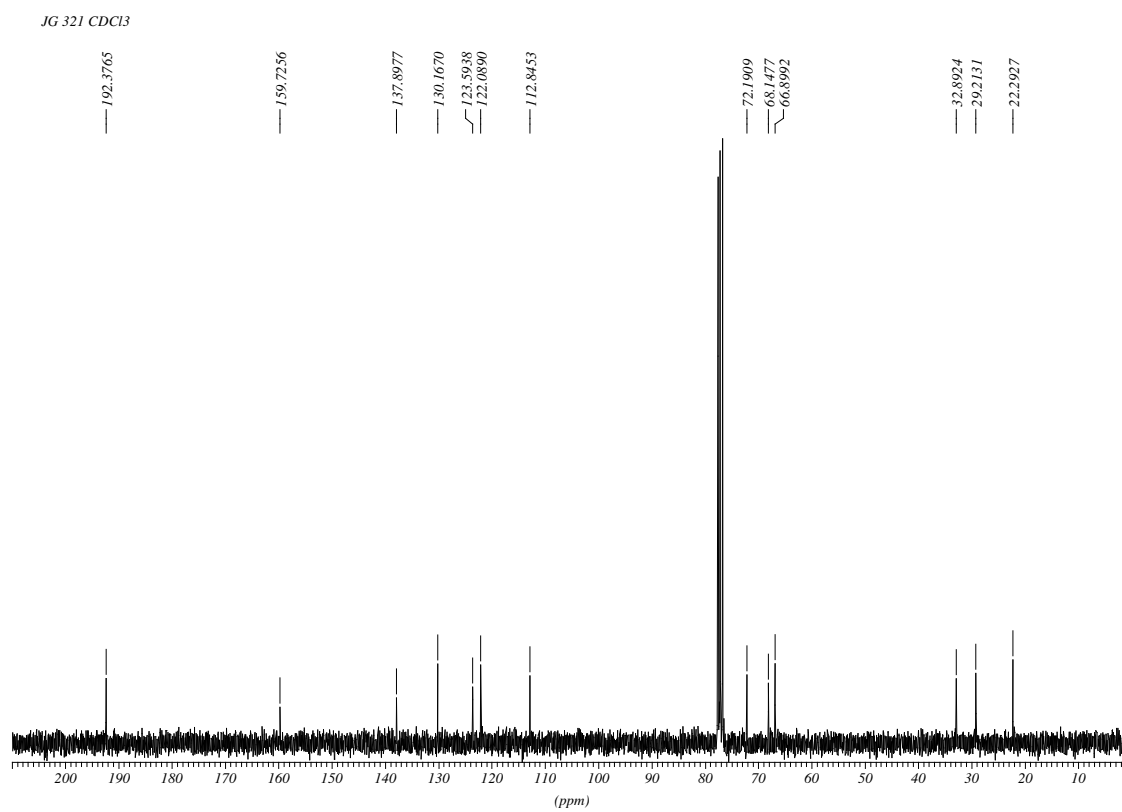
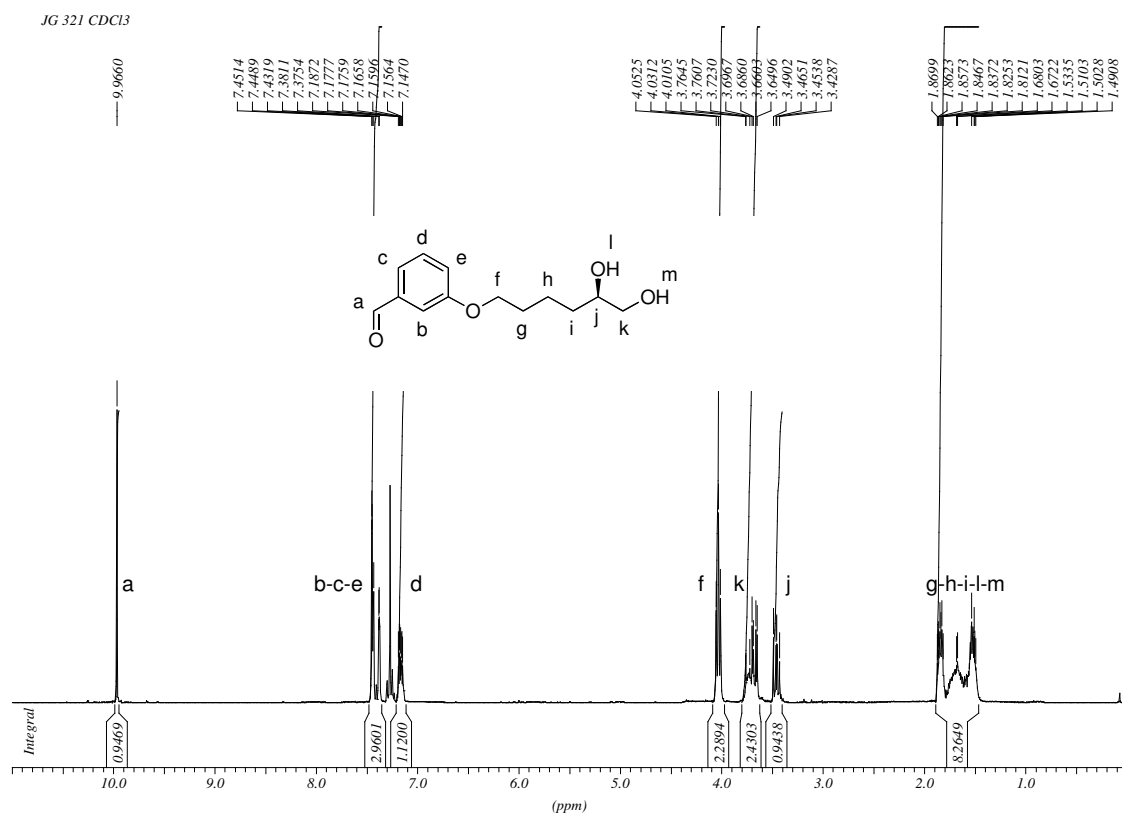


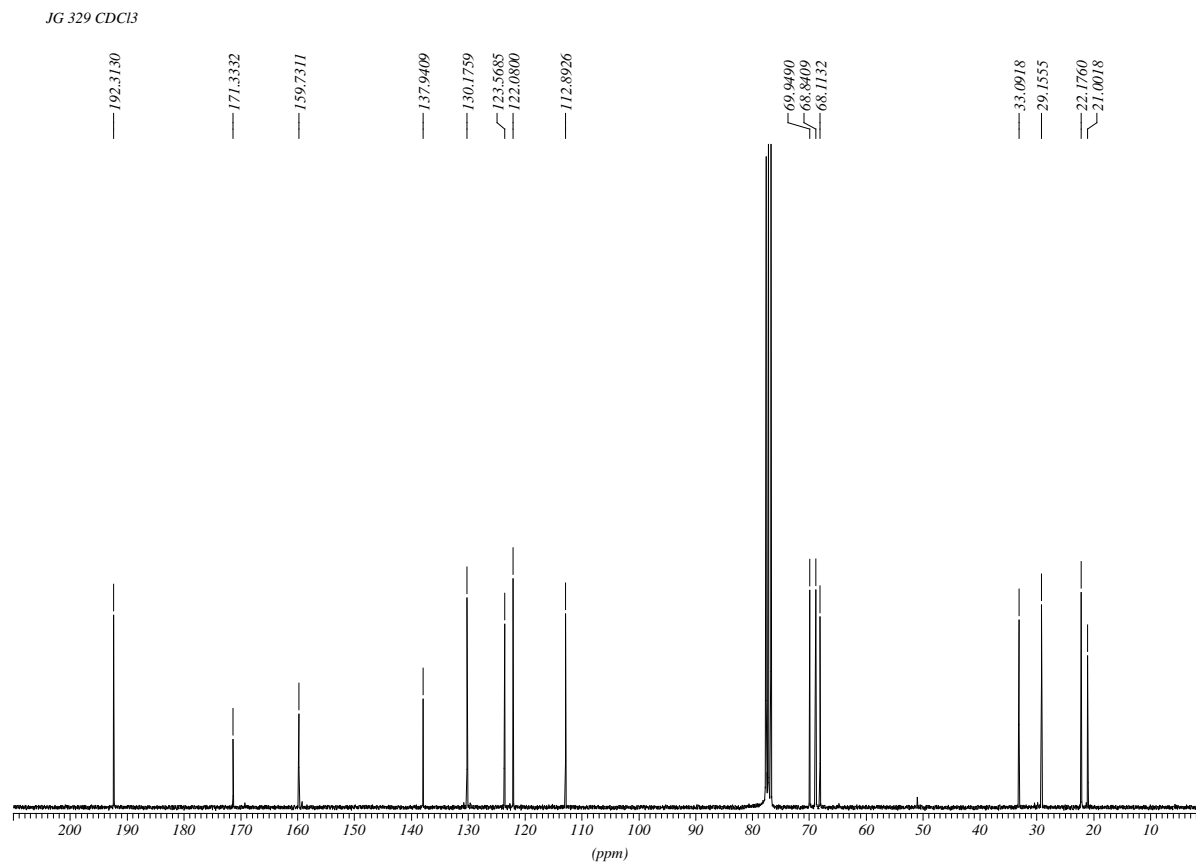
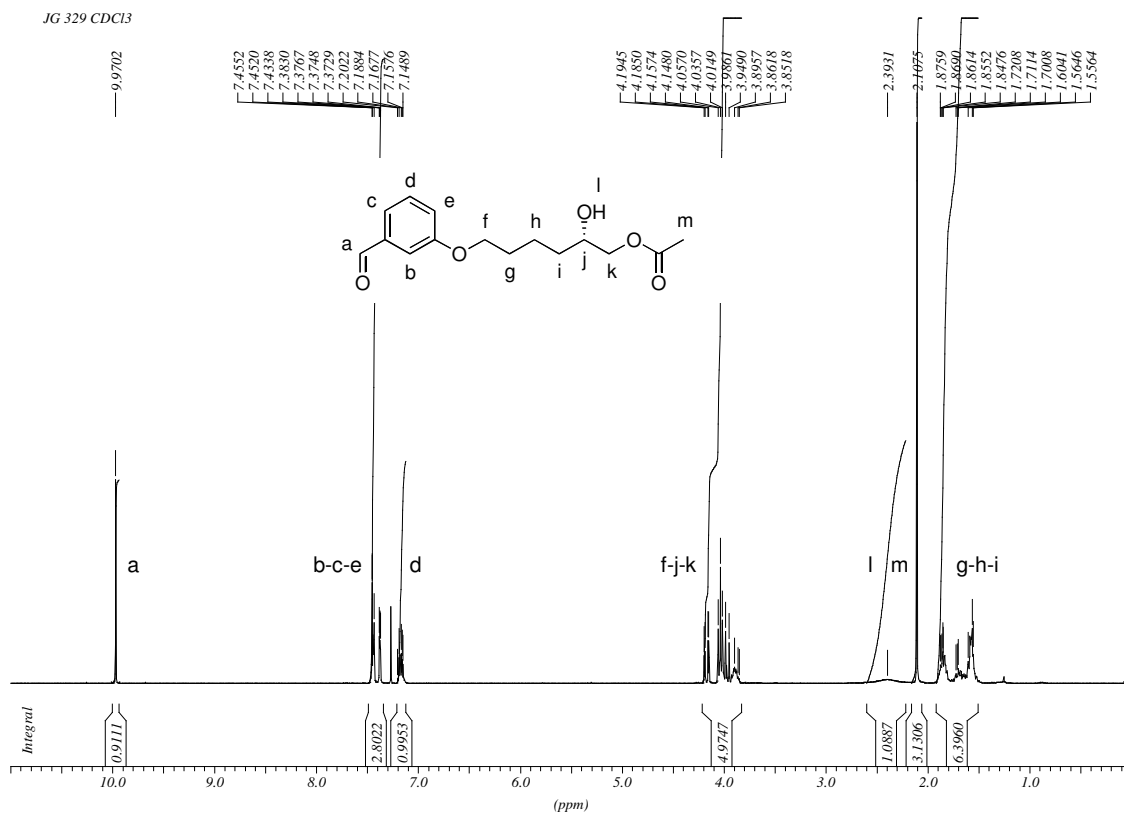
**(S)-((R)-6-bromo-2-hydroxyhexyl) 3,3,3-trifluoro-2-methoxy-phenylpropanoate 55b**JG441 CDCl<sub>3</sub>JG441 CDCl<sub>3</sub>

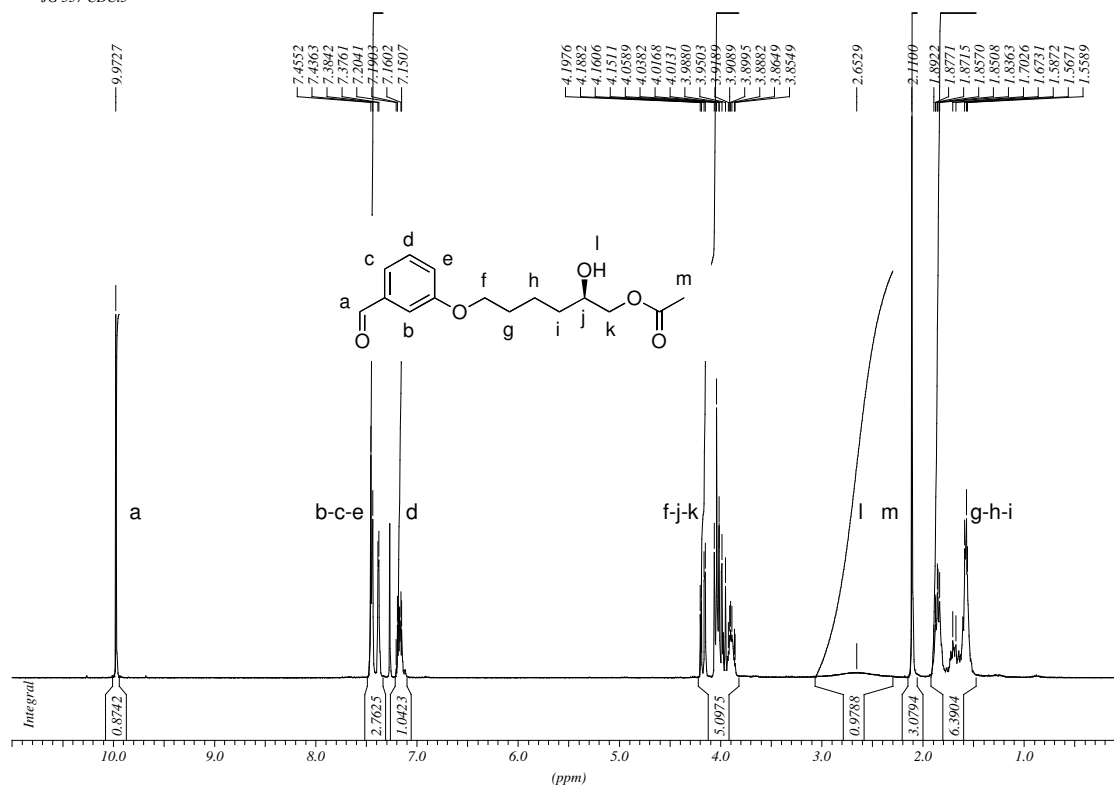
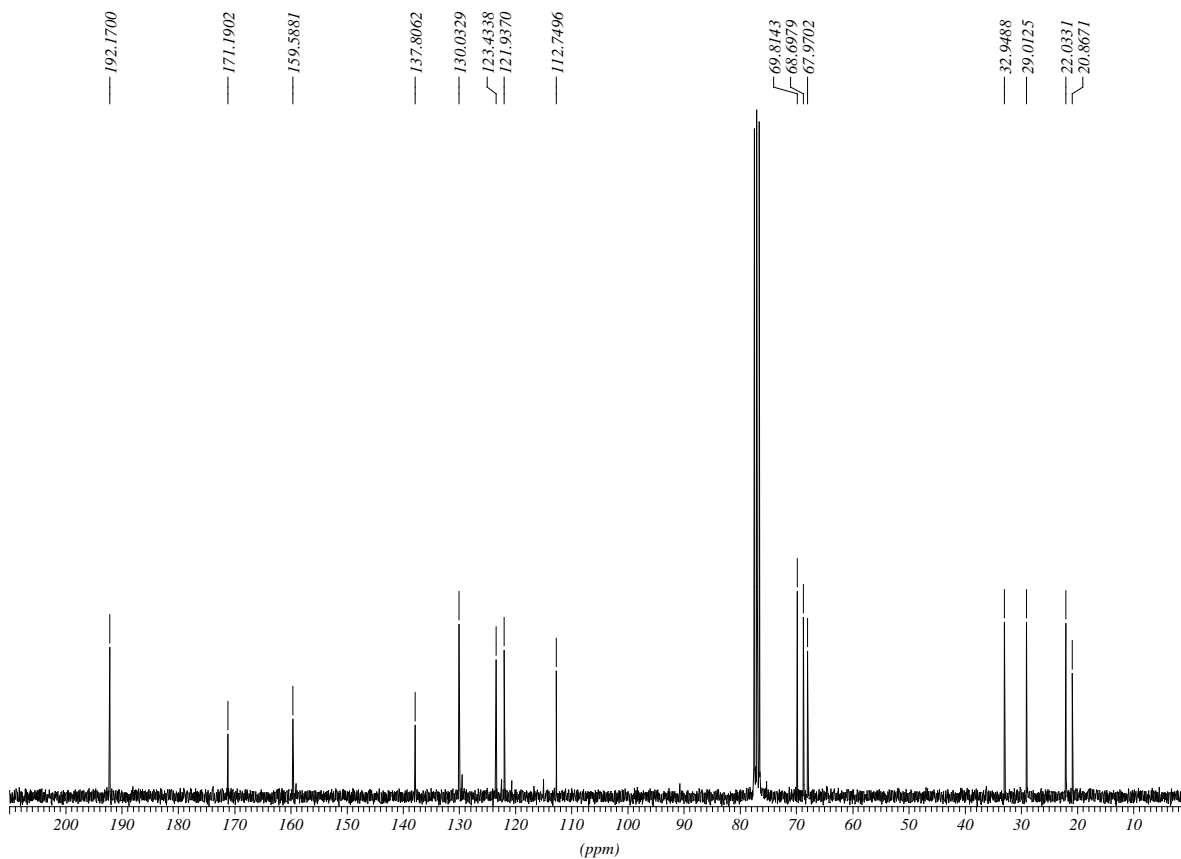
JG441 f19 CDCl3

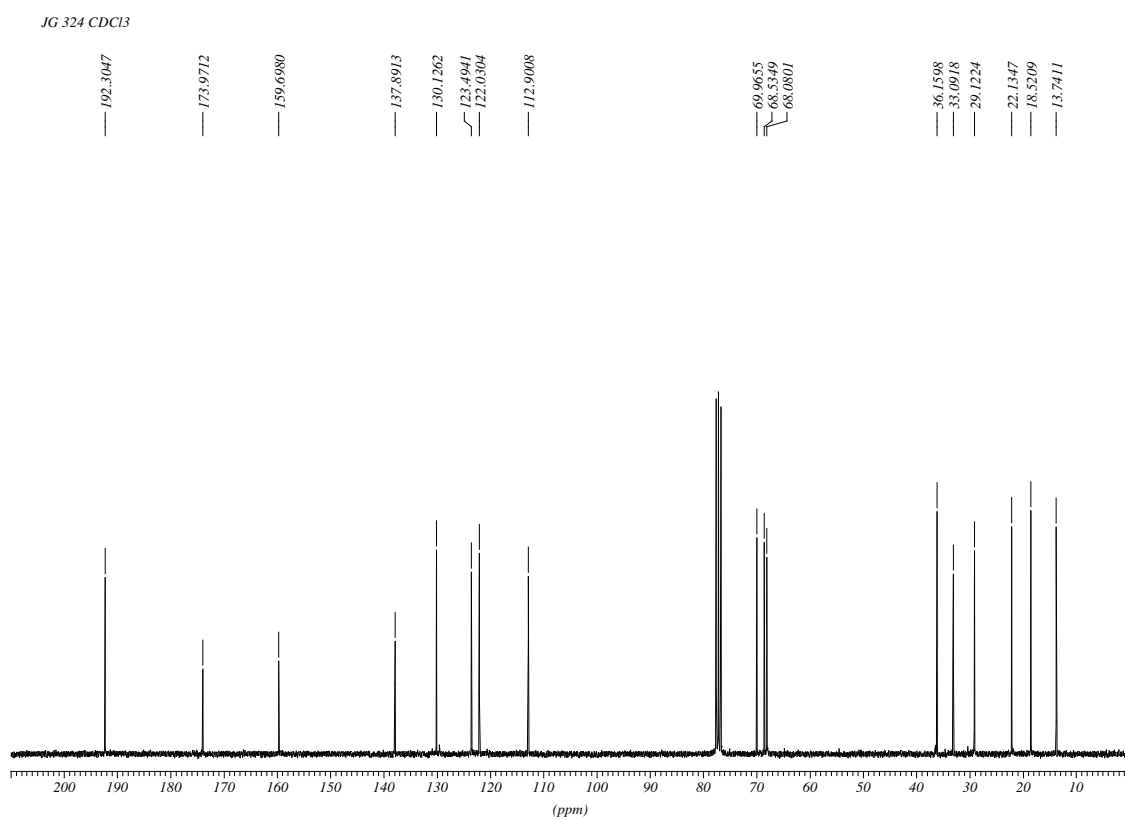
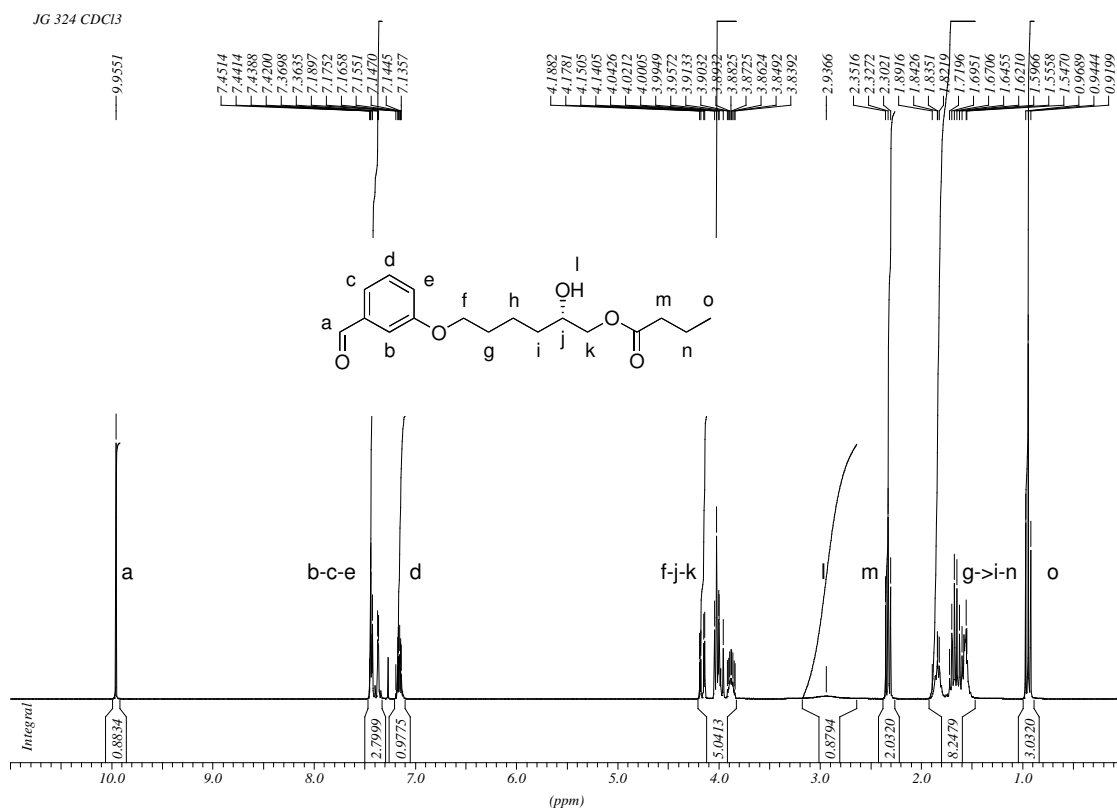


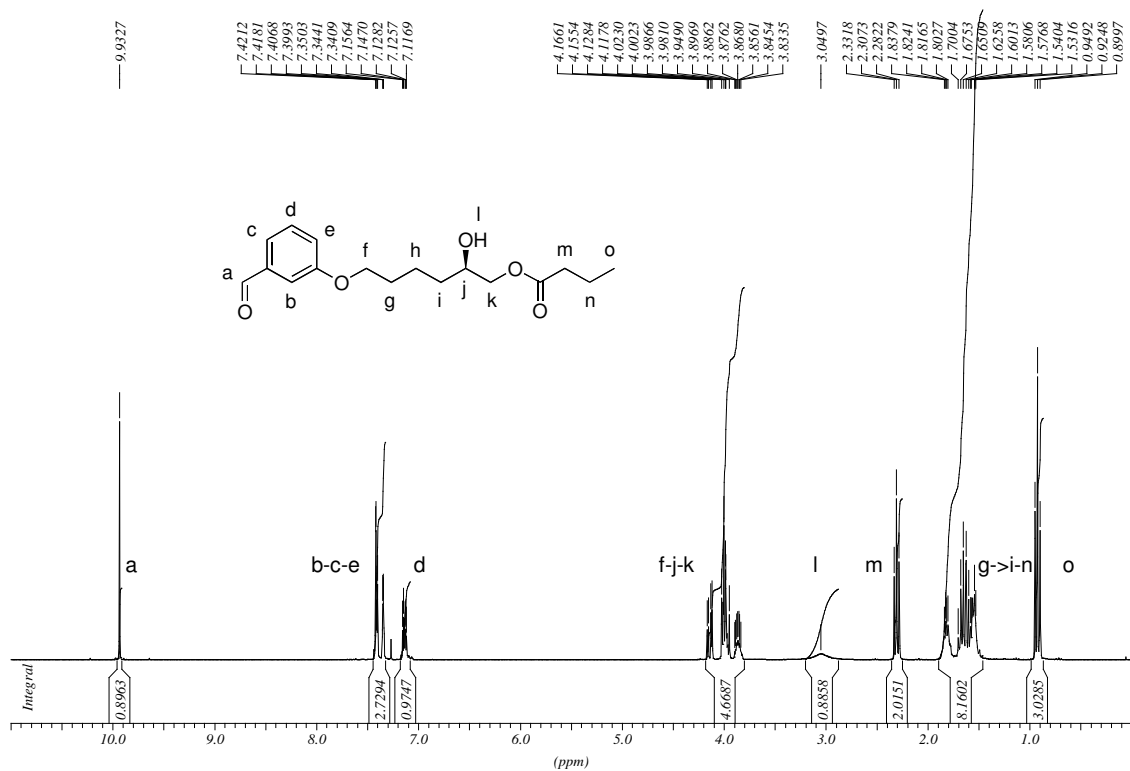
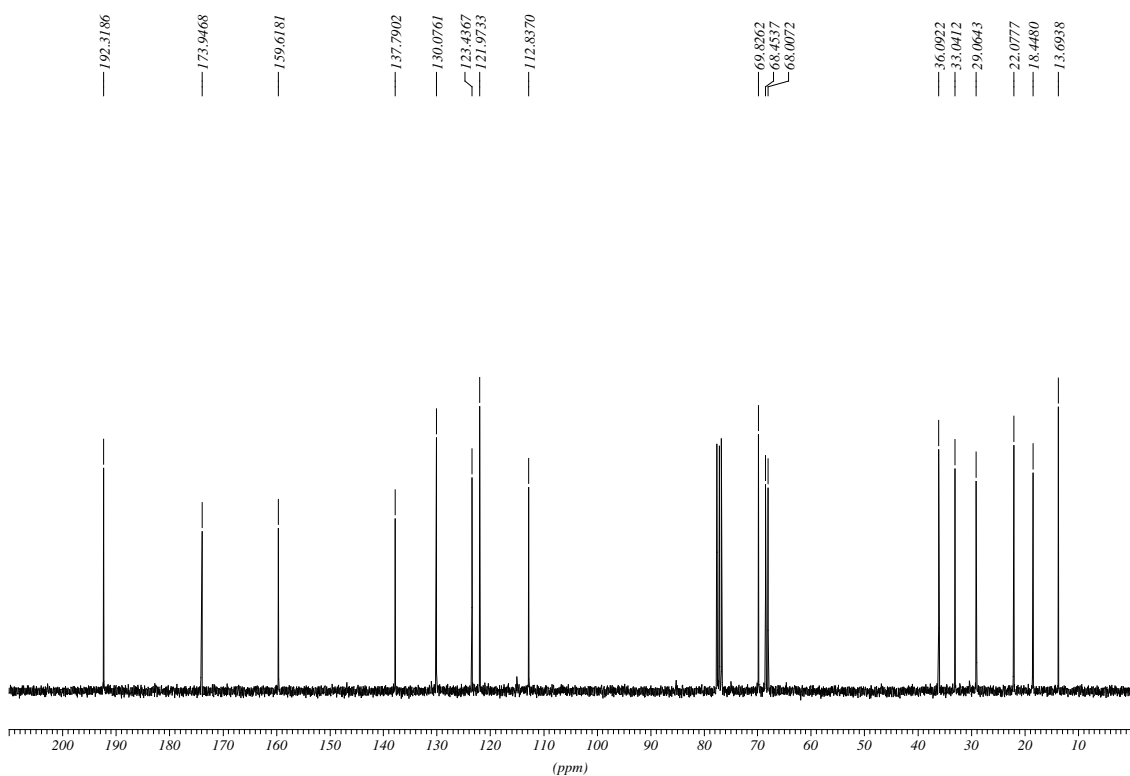
**(S)-3-(5,6-Dihydroxyhexyloxy)benzaldehyde (S)-4**

**(R)-3-(5,6-Dihydroxyhexyloxy)benzaldehyde (R)-4**

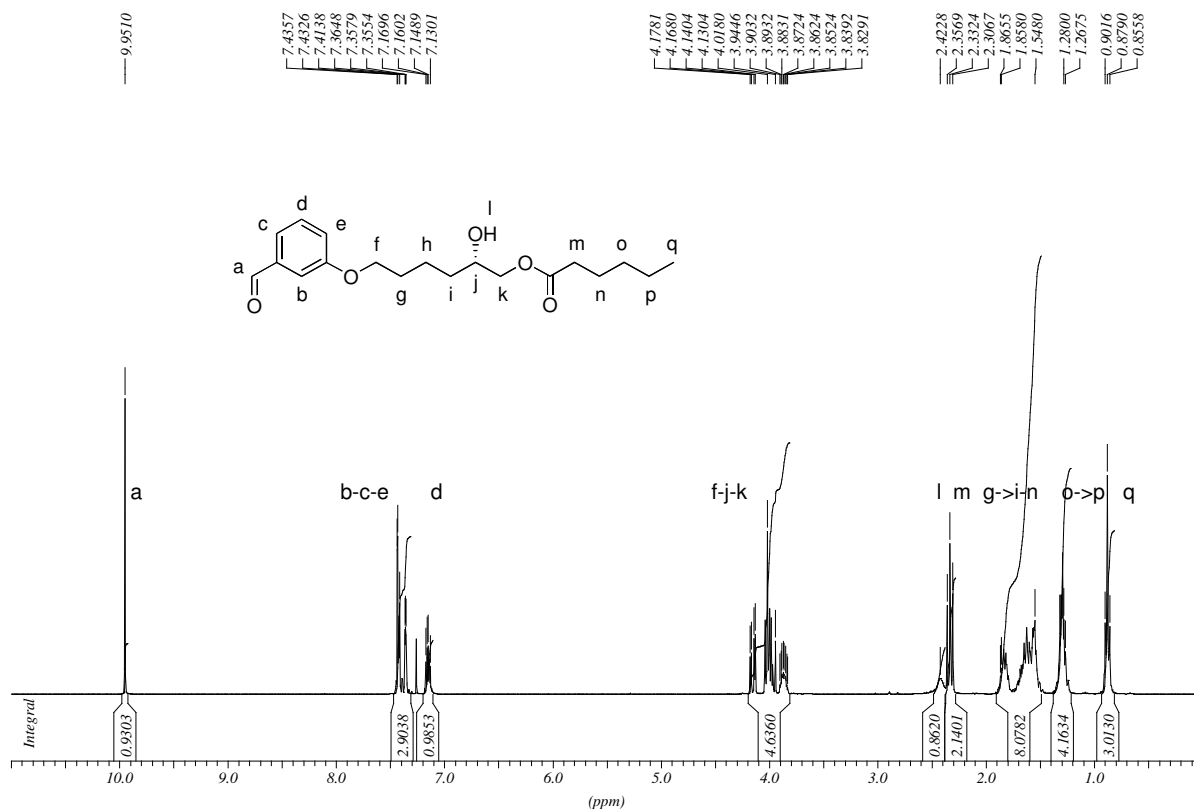
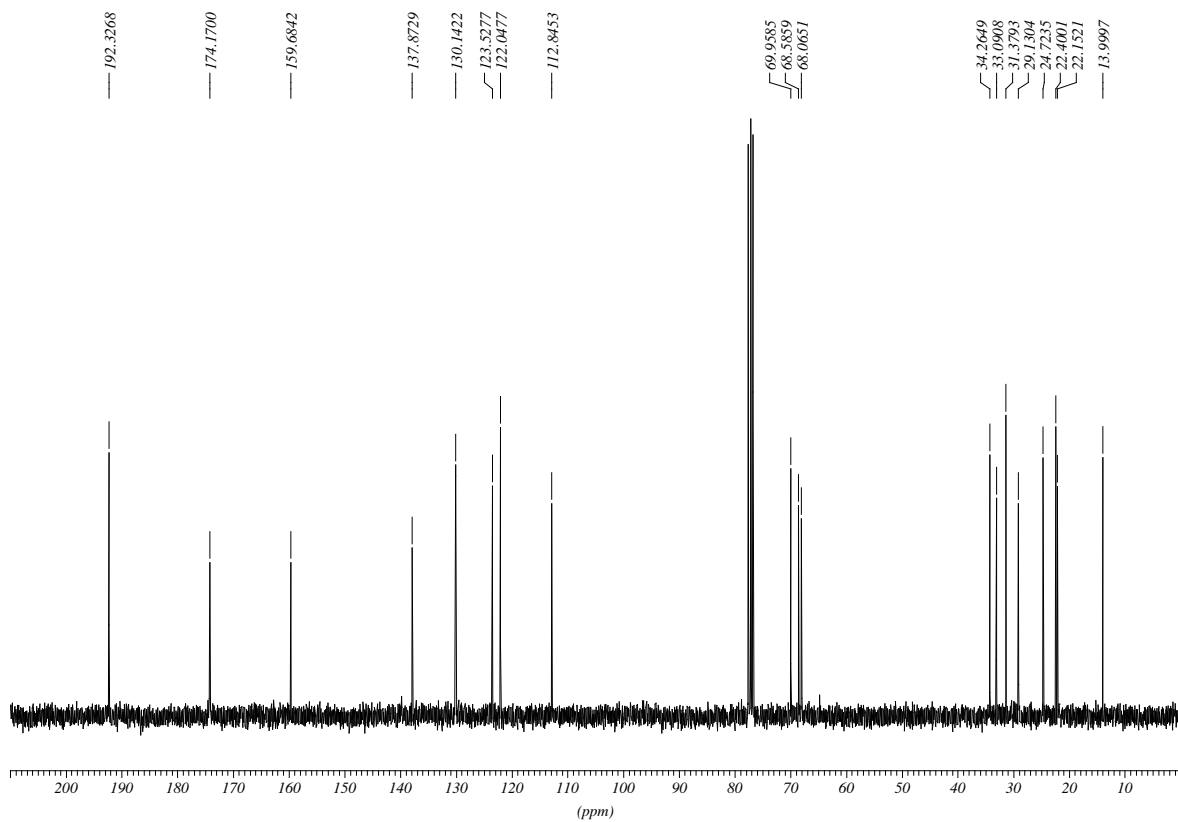
**(S)-6-(3-Formylphenoxy)-2-hydroxyhexyl acetate (S)-C2**

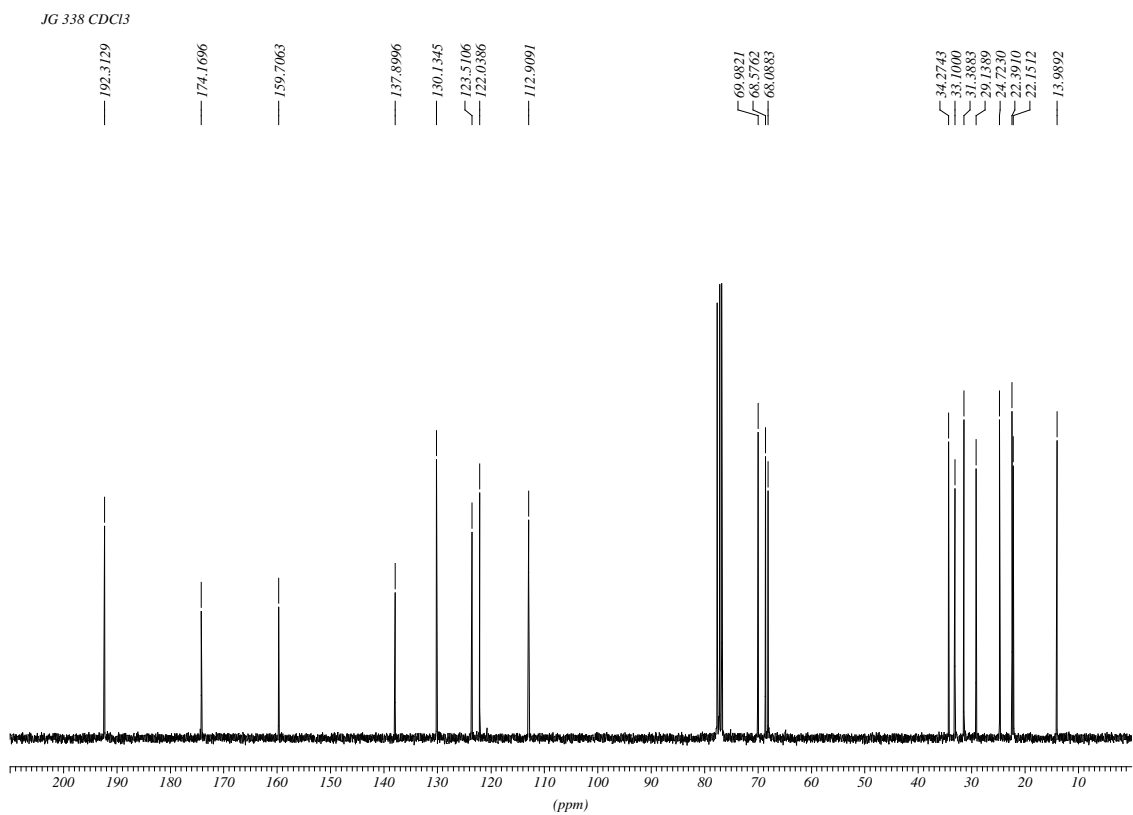
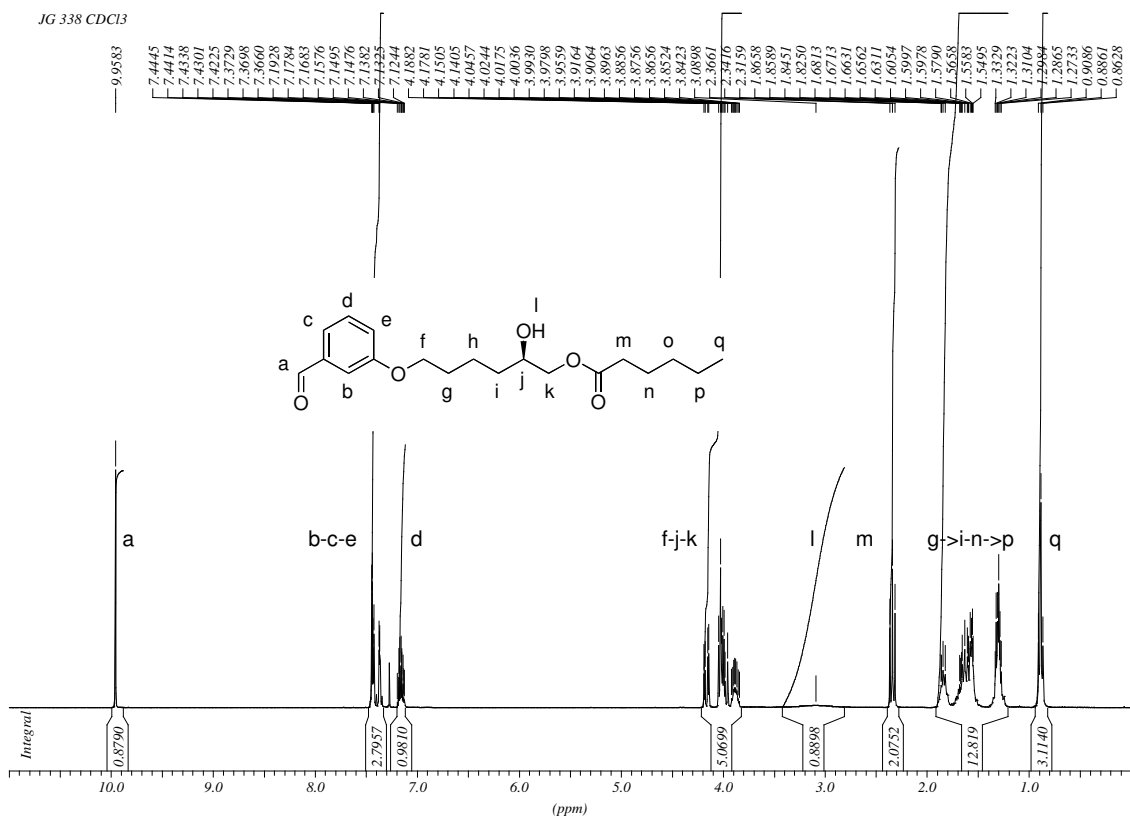
**(R)-6-(3-Formylphenoxy)-2-hydroxyhexylacetate (R)-C2**JG 337 CDCl<sub>3</sub>JG 337 CDCl<sub>3</sub>

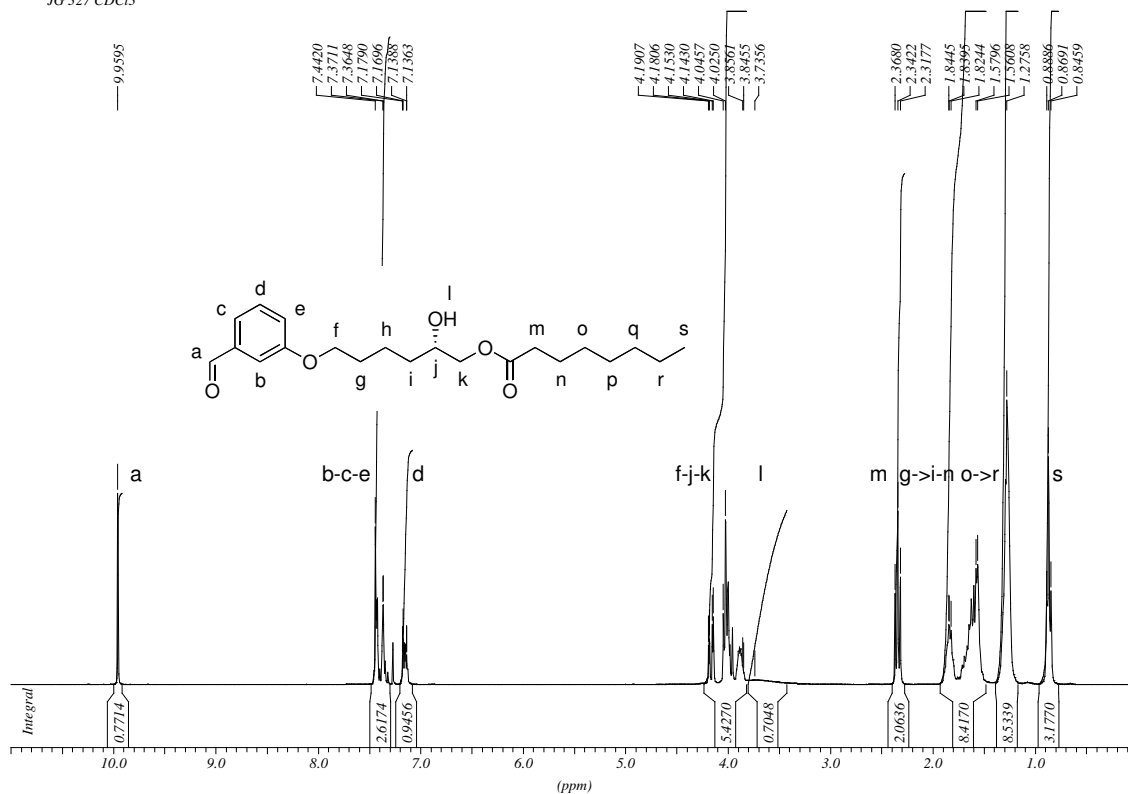
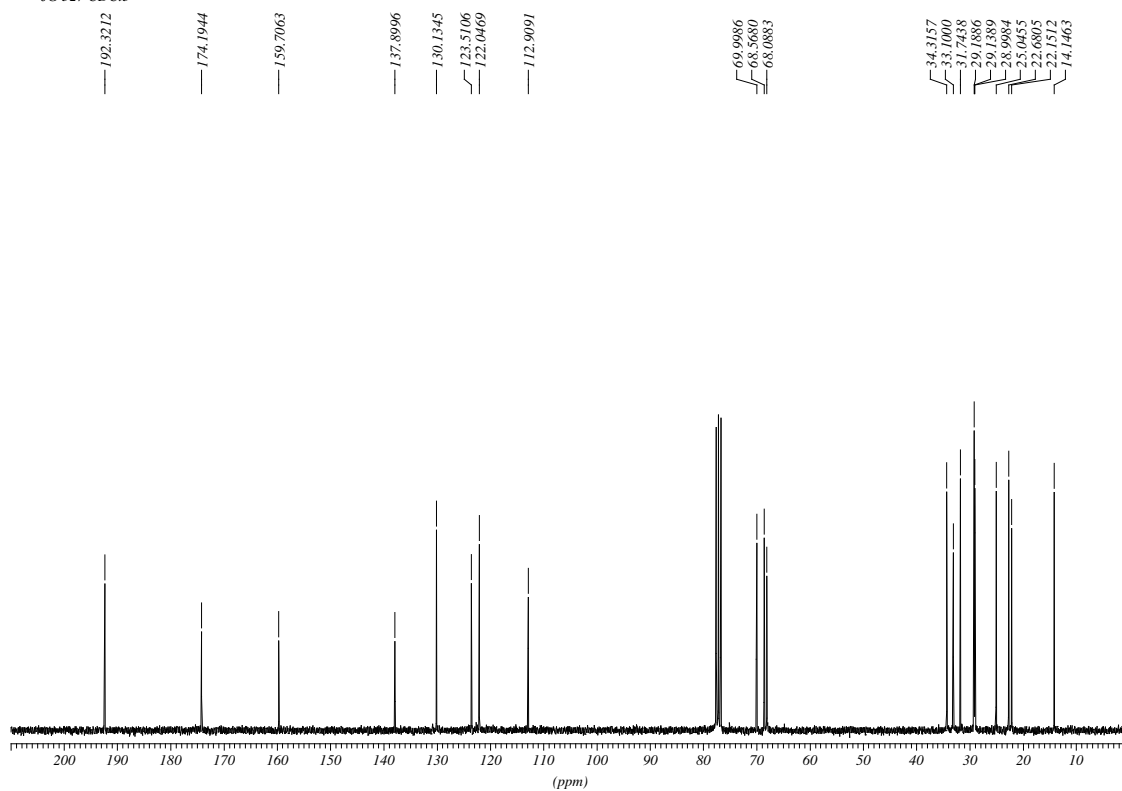
**(S)-6-(3-Formylphenoxy)-2-hydroxyhexyl butyrate (S)-C4**

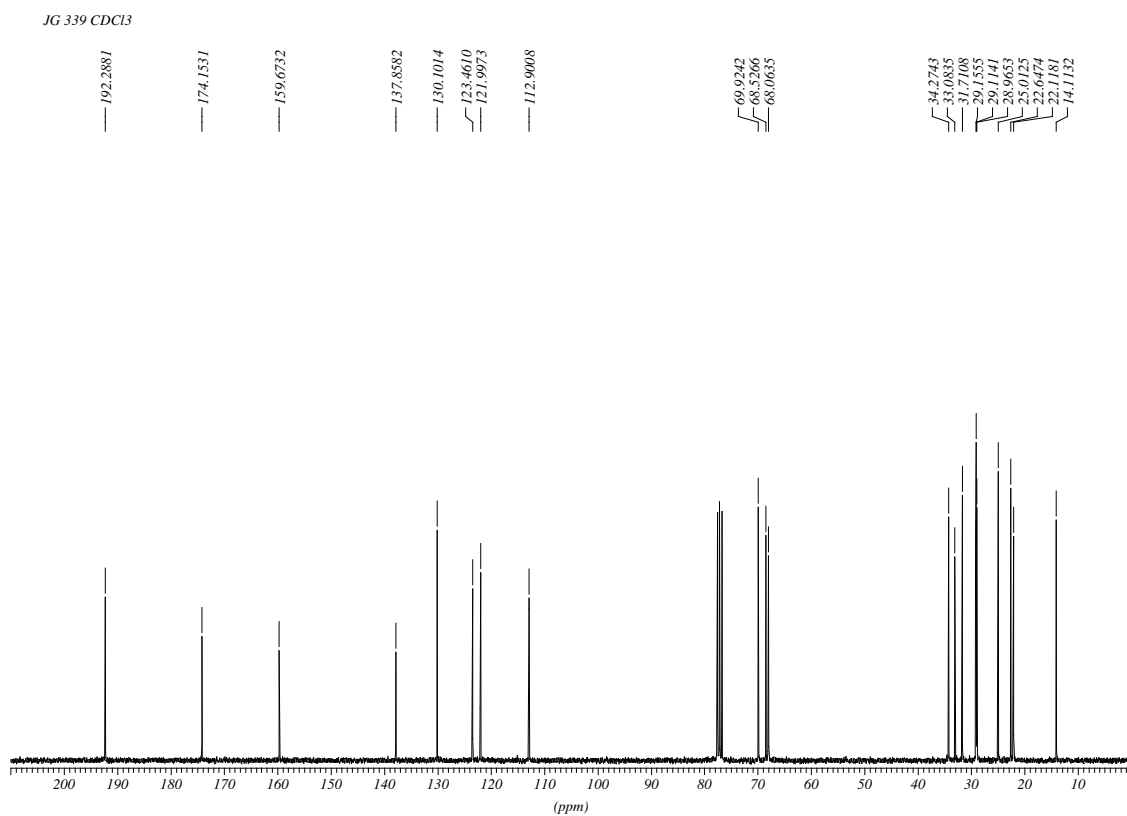
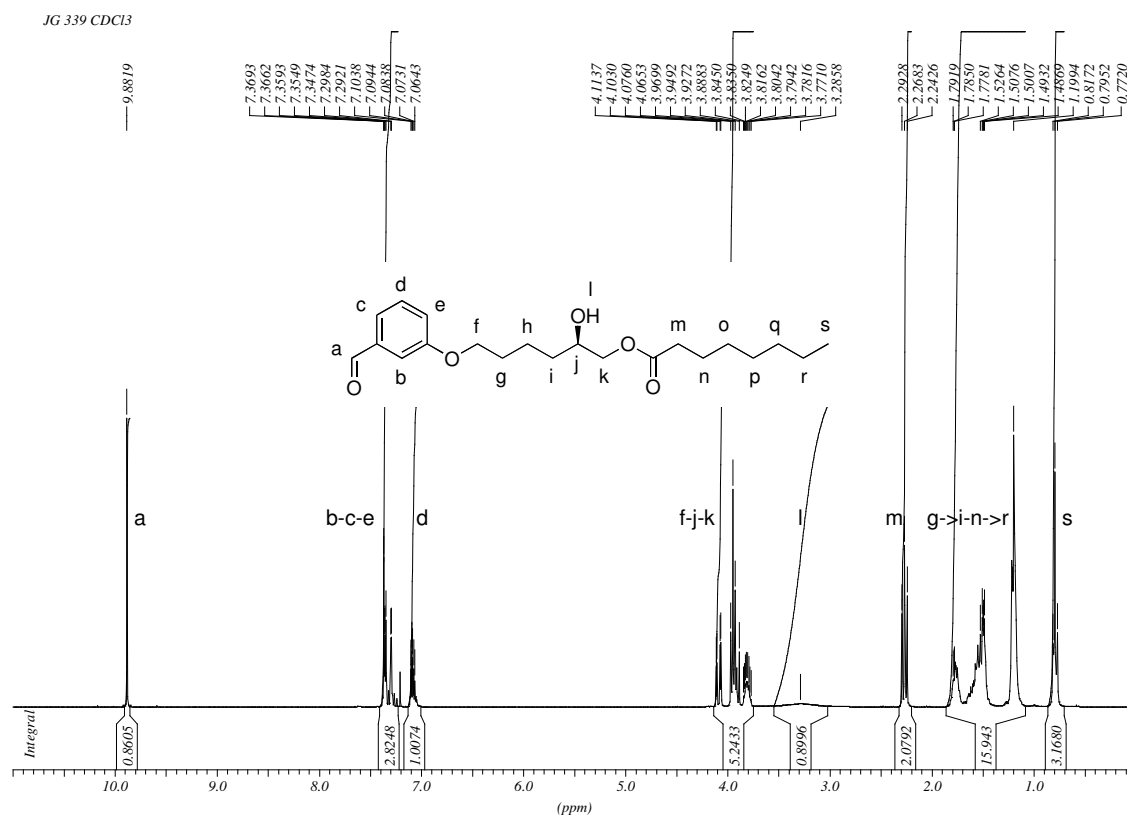
**(R)-6-(3-Formylphenoxy)-2-hydroxyhexyl butyrate (R)-C4**JG 330 CDCl<sub>3</sub>JG 330 CDCl<sub>3</sub>

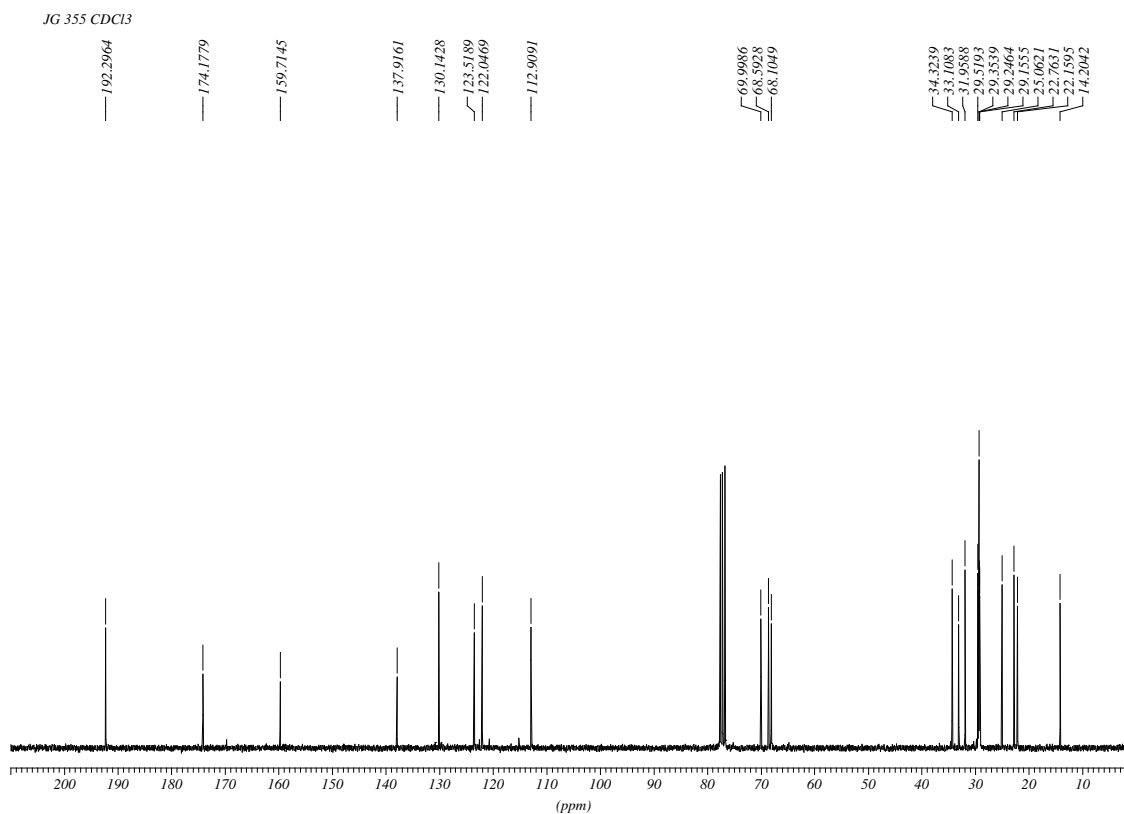
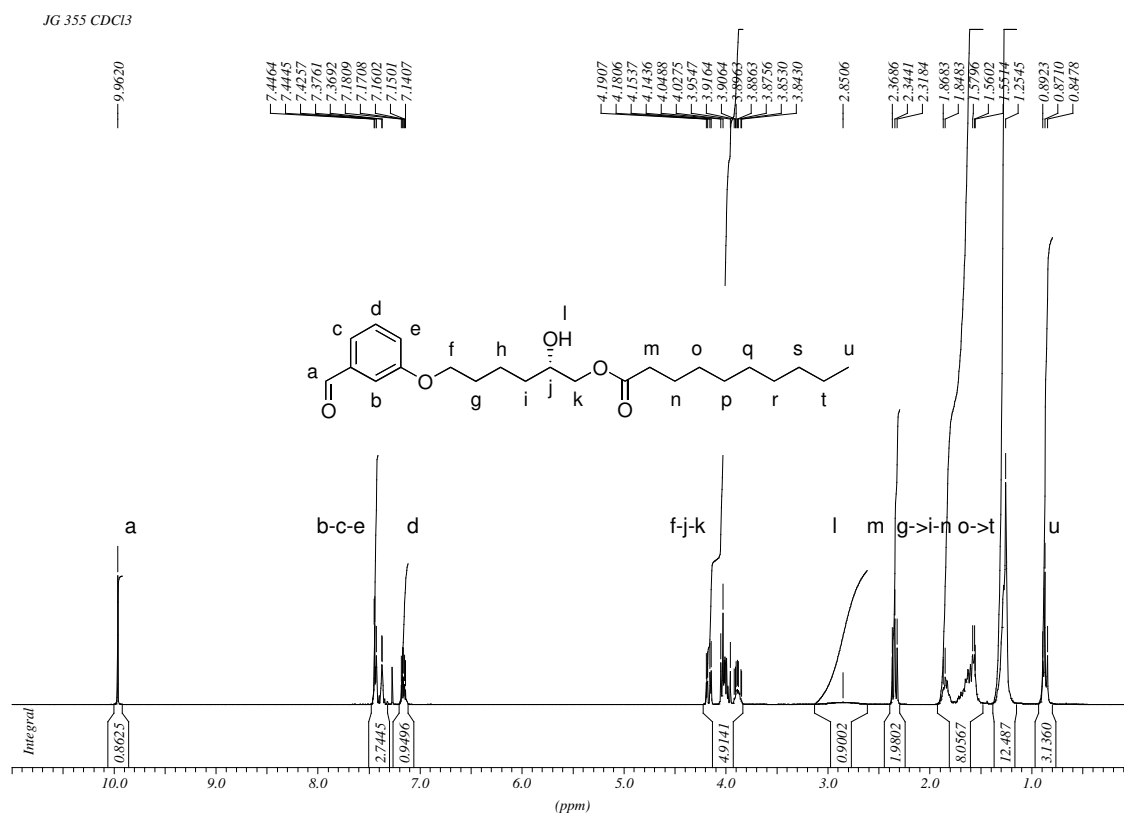


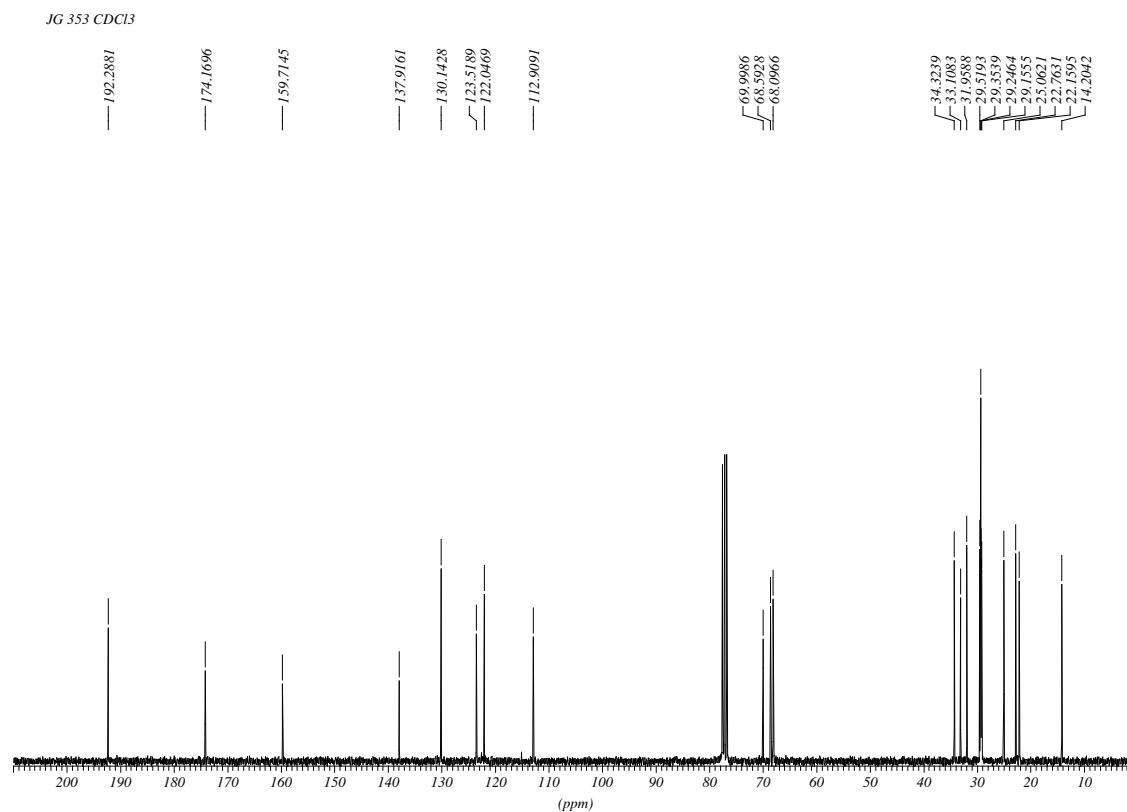
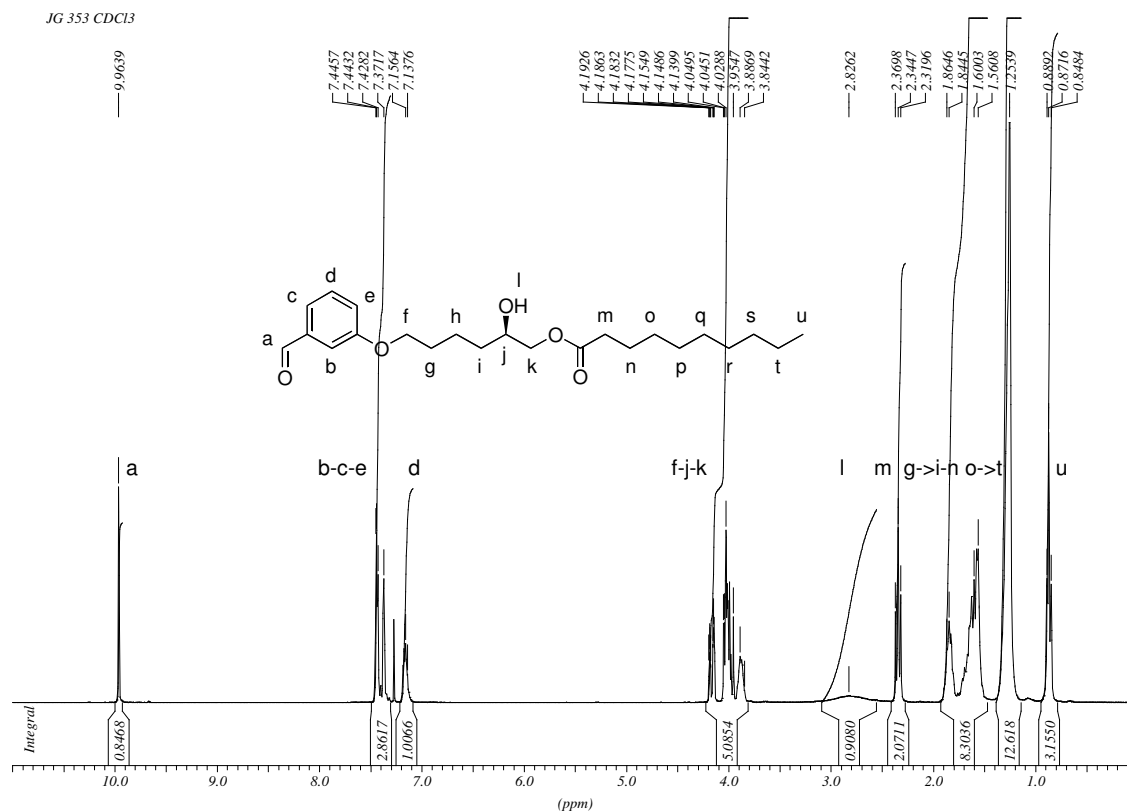
**(S)-6-(3-Formylphenoxy)-2-hydroxyhexyl hexanoate (S)-C6**JG 326 CDCl<sub>3</sub>JG 326 CDCl<sub>3</sub>

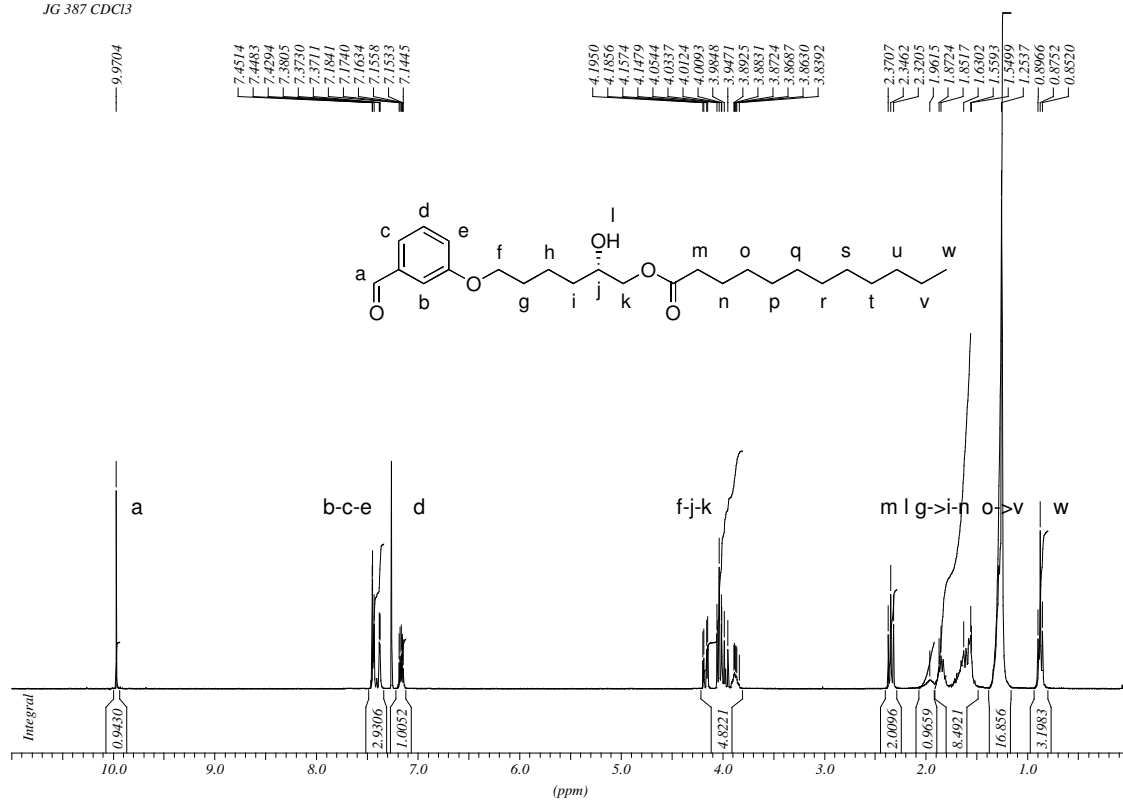
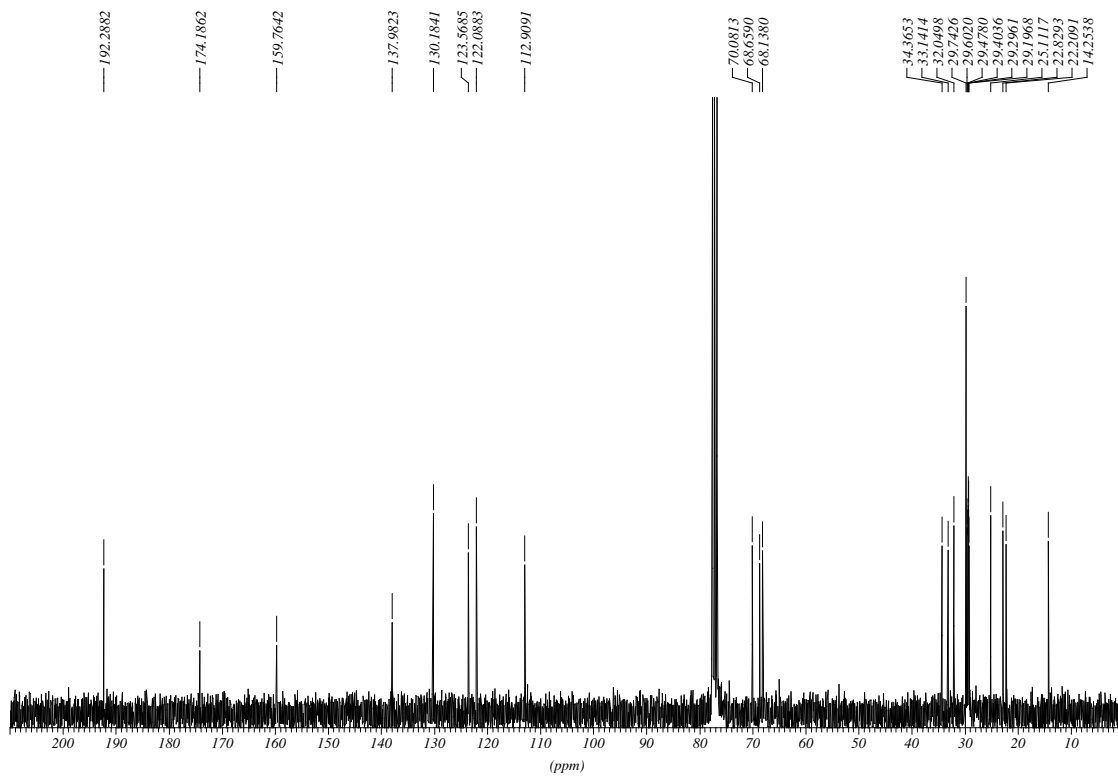
**(R)-6-(3-Formylphenoxy)-2-hydroxyhexyl hexanoate (R)-C6**

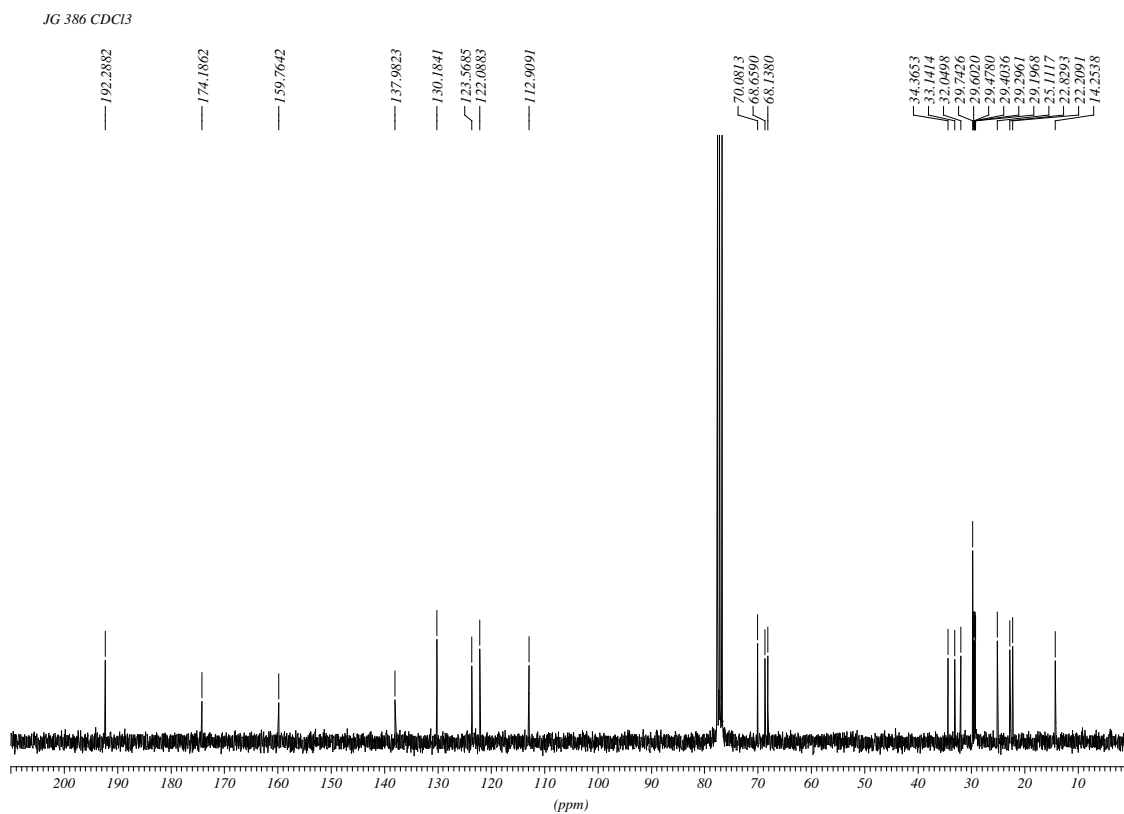
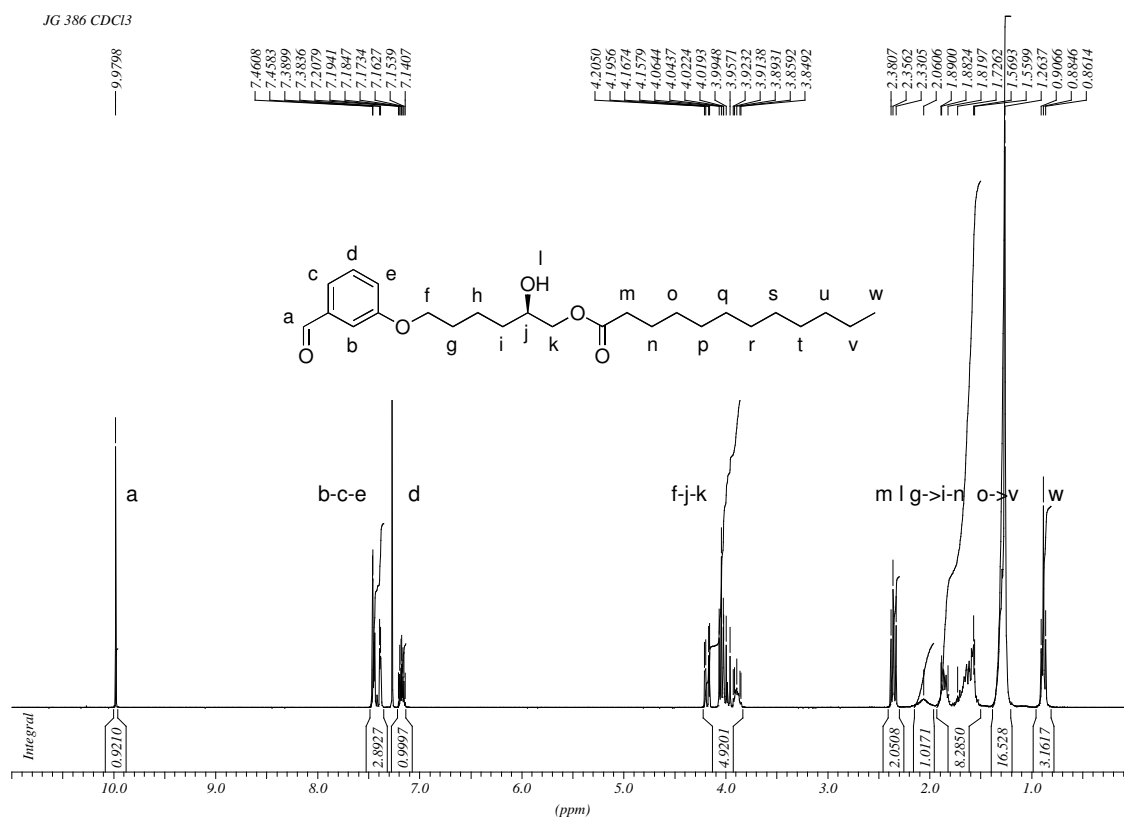
**(S)-6-(3-Formylphenoxy)-2-hydroxyhexyl octanoate (S)-C8**JG.327 CDCl<sub>3</sub>JG.327 CDCl<sub>3</sub>

**(R)-6-(3-Formylphenoxy)-2-hydroxyhexyl octanoate (R)-C8**

**(S)-6-(3-Formylphenoxy)-2-hydroxyhexyl decanoate (S)-C10**

**(R)-6-(3-Formylphenoxy)-2-hydroxyhexyl decanoate (R)-C10**

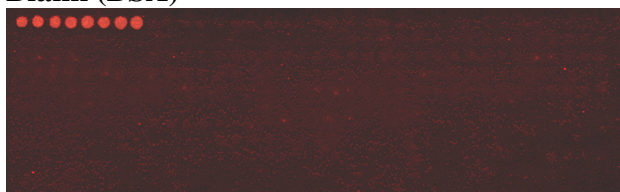
**(S)-6-(3-Formylphenoxy)-2-hydroxyhexyl dodecanoate (S)-C12**JG 387 CDCl<sub>3</sub>JG 387 CDCl<sub>3</sub>

**(R)-6-(3-Formylphenoxy)-2-hydroxyhexyl dodecanoate (R)-C12**

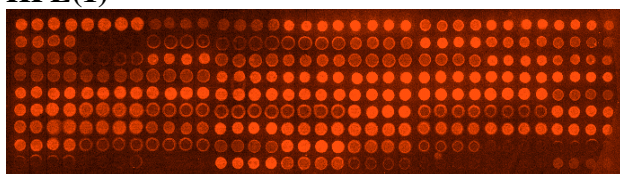


## Microarray images.

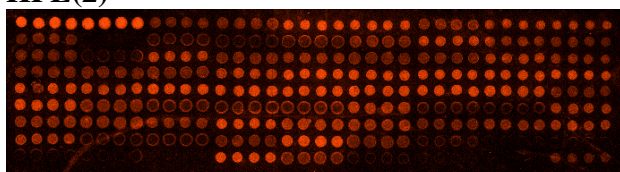
Blank (BSA)



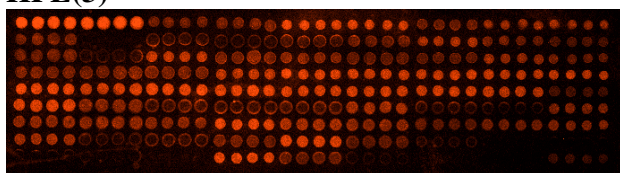
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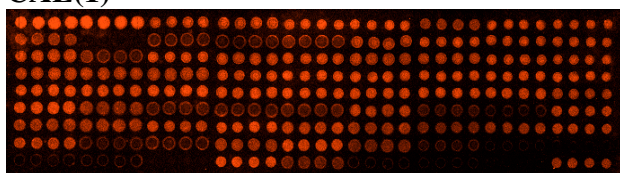
HPL(2)



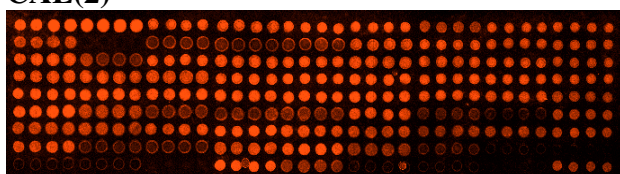
HPL(3)



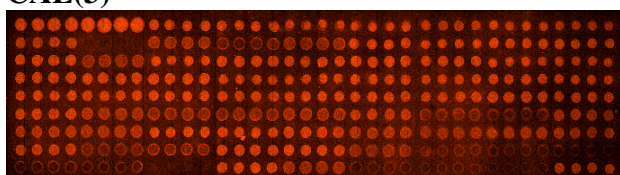
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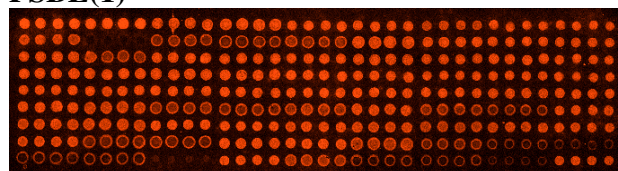
CAL(2)



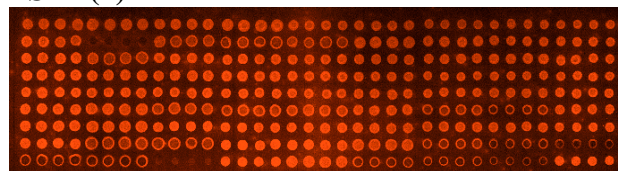
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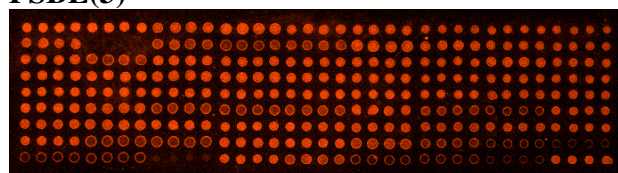
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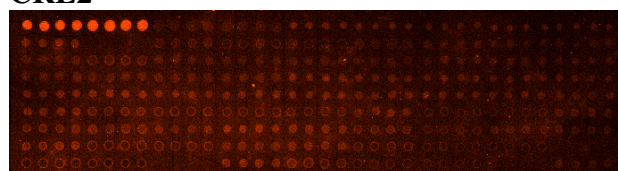
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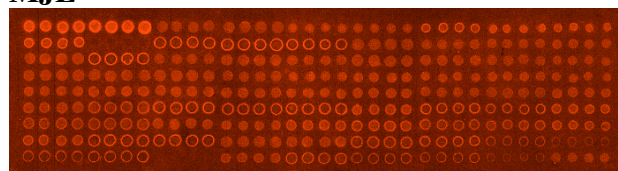
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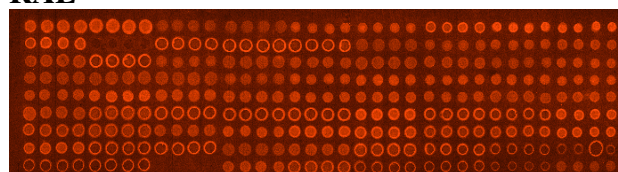
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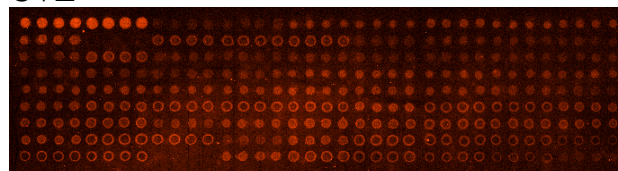
MJL



RAL



CVL



PSL1

