

Supplementary Information

Immobilization of *Pseudomonas fluorescens* lipase on silk fibroin spheres: An alternative protocol for the enantioselective synthesis of halohydrins

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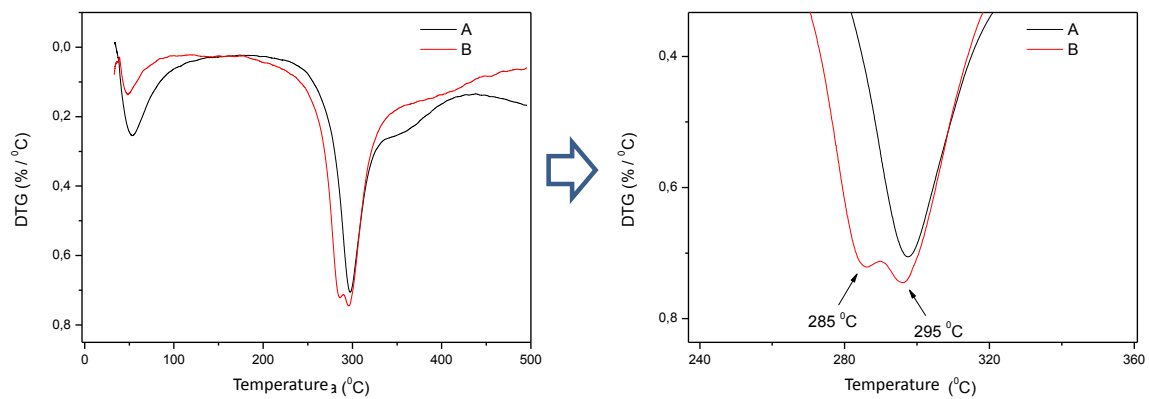
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Optical rotations of the products obtained from of *P. fluorescens* lipase on silk fibroin spheres.

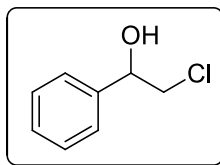
Compounds	$[\alpha]_D^T$	$[\alpha]_D^T$
	Experimental	Literature
(R)-1a	$[\alpha]_D^{26} - 39.2$ (c 1.0, CHCl ₃) ^a	$[\alpha]_D^{20} - 45.1$ (c 1.0, CHCl ₃) ¹
(R)-1b	$[\alpha]_D^{26} - 16.2$ (c 0.8, CHCl ₃) ^a	$[\alpha]_D^{20} - 39.5$ (c 0.5, CHCl ₃) ²
(R)-1c	$[\alpha]_D^{26} - 10.8$ (c 1.0, CHCl ₃) ^a	$[\alpha]_D^{20} - 31.8$ (c 2.85, CHCl ₃) ³
(R)-1d	$[\alpha]_D^{26} - 31.2$ (c 1.1, CHCl ₃) ^a	$[\alpha]_D^{20} - 59.5$ (c 1.0, CHCl ₃) ¹
(S)-2a	$[\alpha]_D^{26} + 82.4$ (c 1.5, CHCl ₃) ^a	$[\alpha]_D^{20} + 77.7$ (c 1.0, EtOAc) ⁴
(S)-2b	$[\alpha]_D^{26} + 30.4$ (c 0.5, CHCl ₃) ^a	-
(S)-2c	$[\alpha]_D^{26} + 26.7$ (c 2, CHCl ₃) ^a	$[\alpha]_D^{25} + 56.6$ (c 3.4, CHCl ₃) ³
(S)-2d	$[\alpha]_D^{26} + 44.7$ (c 1.0, CHCl ₃) ^a	$[\alpha]_D^{25} + 38.6$ (c 1.15, CHCl ₃) ⁵
(S)-3a	$[\alpha]_D^{26} + 13.6$ (c 0.4, CHCl ₃) ^a	$[\alpha]_D^{24} + 25.1$ (c 1.0, CHCl ₃) ⁶
(S)-3b	$[\alpha]_D^{26} + 11.8$ (c 0.6, CHCl ₃) ^a	$[\alpha]_D^{24} + 14.6$ (c 1.0, CHCl ₃) ⁶
(S)-3c	$[\alpha]_D^{25} + 10.3$ (c 0.6, CHCl ₃) ^a	$[\alpha]_D^{24} + 24.9$ (c 1.0, CHCl ₃) ⁶
(S)-4	$[\alpha]_D^{25} + 2.5$ (c 0.4, CHCl ₃) ^b	-

^aThe optical rotation was measured in CHCl₃ on a Perkin-Elmer 241 polarimeter equipped with a lamp Na-589nm. ^bThe optical rotation was measured on a JASCO in CHCl₃ P2000 polarimeter equipped with a 589nm-lamp Na.

Thermogravimetric curves of samples: (--) Silk fibroin sphere; (—) Immobilized lipase on silk fibroin sphere.

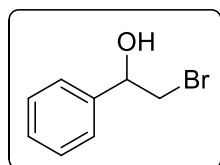


2-chloro-1-phenylethanol (1a):



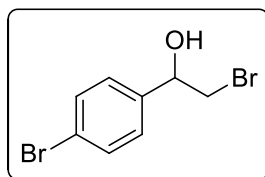
C_8H_9ClO , $156.03 \text{ g.mol}^{-1}$. Yellow oil; 1H NMR (400 MHz, $CDCl_3$ ppm) δ : 3.71 (dd, $J = 11.7$ and 4.6 Hz, 1H), 3.78 (dd, $J = 11.7$ and 8.0 Hz, 1H), 5.95 (dd, $J = 8.0$ and 4.6 Hz, 1H), 7.37 – 7.32 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 50.9, 74, 126, 128.4, 128.6, 139.9; MS (EI, 70 eV) m/z (%): 156 (11), 107 (100), 79 (77), 51 (17); IR ν_{max} (cm^{-1}): 3404, 2956, 1494, 1064, 725.

2-bromo-1-phenylethanol (1b):



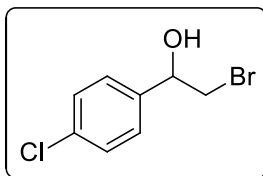
C_8H_9BrO , $199.98 \text{ g.mol}^{-1}$. Yellow oil; 1H NMR (400 MHz, $CDCl_3$ ppm) δ : 1.57 (s, 1H), 3.48 (dd, $J = 10.5$ and 8.8 Hz, 1H), 3.60 (dd, $J = 10.5$ and 3.4 Hz, 1H), 4.89 (dd, $J = 8.8$ and 3.2 Hz, 1H), 7.25-7.46 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$ ppm) δ : 39.9, 73.1, 127.3, 128.8, 134.2, 138.7; MS (EI, 70 eV) m/z (%): 200 (4), 107 (100), 79 (28), 51 (12); IR ν_{max} (cm^{-1}): 3398, 2954, 2926, 1429, 1454, 1377, 1068, 759.

2-bromo-1-(4-bromophenyl)ethanol (1c):



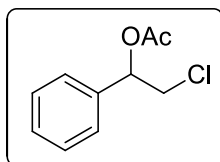
$C_8H_8Br_2O$, 277.9 g.mol^{-1} . White solid, m.p.: $59 \text{ }^\circ C$ (lit.⁷ 69-71); 1H NMR (400 MHz, $CDCl_3$ ppm) δ : 3.49 (dd, $J = 10.8$ Hz, 1H), 3.61 (dd, $J = 10.5$, 4 Hz, 1H), 4.91 (m, 1H), 7.27 (d, $J = 8$ Hz), 7.51 (d, $J = 8$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$ ppm) δ : 39.9, 73, 122.3, 127.6, 131.7, 139.2; MS (EI, 70 eV) m/z (%): 280 (8), 185 (100), 157 (23), 77 (75), 51 (20); IR ν_{max} (cm^{-1}): 3398, 2954, 2926, 1492, 1454, 1068, 759, 700.

2-bromo-1-(4-chlorophenyl)ethanol (1d):



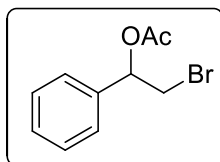
C_8H_8BrClO , $235.51 \text{ g}\cdot\text{mol}^{-1}$. White solid, m.p.: $59 \text{ }^\circ\text{C}$ (lit.⁸ $61\text{-}62 \text{ }^\circ\text{C}$); ^1H NMR (400 MHz, CDCl_3 ppm) δ : 3.50 (dd, $J = 10.4$ and 8.8 Hz, 1H), 3.61 (dd, $J = 10.5$ and 3.4 Hz, 1H), 4.91 (dt, $J = 8.6$ and 3.1 , 1H), 7.28–7.39 (m, 4H); ^{13}C NMR (100 MHz, CDCl_3 ppm) δ : 138.7, 128.8, 127.3, 126.8, 73.0, 39.9; MS (EI, 70 eV) m/z (%): 236 (6), 141 (100), 113 (20), 77 (54), 51 (8); IR ν_{max} (cm^{-1}): 3390, 2960, 2865, 1597, 1429, 1091, 833, 727, 702.

2-chloro-1-phenylethyl acetate (2a):



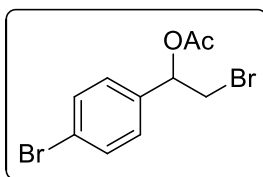
$C_{10}H_{11}ClO_2$, $198.04 \text{ g}\cdot\text{mol}^{-1}$. White solid, m.p.: $47 \text{ }^\circ\text{C}$; ^1H NMR (500 MHz, CDCl_3 ppm) δ : 3,71 (dd, $J = 11,7$ and $4,6$ Hz, 1 H), 3,78 (dd, $J = 11,7$ and 8 Hz, 1 H), 5,95 (dd, $J = 8,4$ and $4,9$ Hz, 1 H), 7,48 - 7,30 (m, 5 H) ppm; ^{13}C NMR (125 MHz, CDCl_3 ppm) δ : 20,98, 46,50, 75,0, 126,6, 128,7, 128,8, 137,1, 169,8; MS (EI, 70 eV) m/z (%): 198 (3), 162 (15), 102 (12), 77 (23), 43 (100); IR ν_{max} (cm^{-1}): 2960, 2929, 1747, 1373, 1230, 1024, 761, 689.

2-bromo-1-phenylethyl acetate (2b):



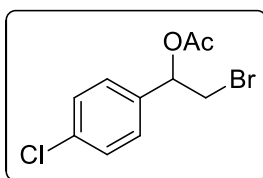
$C_{10}H_{11}BrO_2$, $241.99 \text{ g}\cdot\text{mol}^{-1}$. Colorless oil; ^1H NMR (500 MHz, CDCl_3 ppm) δ : 7.40 – 7.28 (m, 1H), 5.05 (dd, $J = 7.5$ and 6.2 Hz, 1H), 4.46 – 4.39 (m, 2H), 2.05 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3) δ (ppm): 169.7, 136.6, 131.9, 128.3, 122.9, 74.1, 33.7, 20.9; MS (EI, 70 eV) m/z (%): 242 (8), 198 (16), 141 (32), 103 (36), 77 (78), 40 (100); IR ν_{max} (cm^{-1}): 2964, 1745, 1489, 1236, 758.

2-bromo-1-(4-bromophenyl)ethyl acetate (2c):



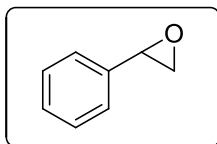
$C_{10}H_{10}Br_2O_2$, 322.00 $g \cdot mol^{-1}$. Colorless oil; 1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.49 (d, $J = 8.5$ Hz, 2H), 7.25 – 7.21 (m, 2H), 5.90 (dd, $J = 7.6$ and 5.1 Hz, 1H), 3.60 (dd, $J = 10.8$ and 7.7 Hz, 1H), 3.54 (dd, $J = 10.8$ and 5.1 Hz, 1H), 2.12 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 169.7, 136.6, 131.9, 128.3, 122.9, 74.1, 33.7, 20.9; MS (EI, 70 eV) m/z (%): 322 (0.3), 242 (15), 200 (31), 183 (26), 157 (6), 102 (21), 77 (23), 43 (100); IR ν_{max} (cm^{-1}): 2962, 2926, 1745, 1489, 1371, 1236, 1070, 1012, 821, 723.

2-bromo-1-(4-chlorophenyl)ethyl acetate (2d):



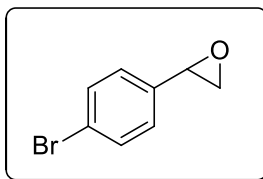
$C_{10}H_{10}BrClO_2$, 277.94 $g \cdot mol^{-1}$. Colorless oil; 1H NMR (500 MHz, $CDCl_3$ ppm) δ : 7.34 (d, $J = 8.6$ Hz, 2H), 7.28 (d, $J = 8.6$ Hz, 2H), 5.92 (dd, $J = 7.7$ and 5.0 Hz, 1H), 3.60 (dd, $J = 10.8$ and 7.7 Hz, 1H), 3.54 (dd, $J = 10.8$ and 5.0 Hz, 1H), 2.12 (d, $J = 2.4$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ ppm) δ : 169.66, 136.12, 134.70, 128.90, 128.00, 126.54, 74.07, 33.83, 20.89; MS (IE, 70 eV) m/z (%): 278 (0.15), 196 (20), 154 (45), 137 (40), 103 (14), 77 (17), 43 (100); IR ν_{max} (cm^{-1}): 3032, 2964, 1745, 1492, 1373, 1238, 1091, 827.

2-phenyloxirane (3a):



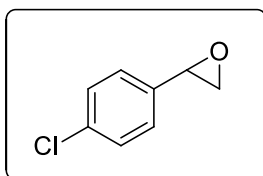
C_8H_8O , 120.06 $g \cdot mol^{-1}$. Colorless oil; NMR 1H (400 MHz, $CDCl_3$ ppm) δ : 2.82 (dd, $J = 5.5$ and 2.6 Hz, 1H), 3.17 (dd, $J = 5.5$ and 4.1 Hz, 1H), 3.88 (dd, $J = 4.0$ and 2.6 Hz, 1H), 7.55 – 7.13 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 137.6 (s), 128.5, 128.1, 125.5, 52.3, 51.1; MS (EI, 70 eV) m/z (%): 120 (43), 119 (67), 91 (100), 77 (10), 51 (20); IR ν_{max} (cm^{-1}): 2913, 1495, 1453, 876, 760.

2-(4-bromophenyl)oxirane (3b):



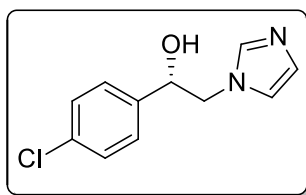
C_8H_7BrO , 197.07 $g \cdot mol^{-1}$. Colorless oil; 1H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.75 (dd, $J = 5.4$ and 2.5 Hz, 1H); 3.14 (dd, $J = 5.3$ and 4.2 Hz, 1H), 3.84 – 3.81 (m, 1H), 7.15 (d, $J = 8.4$ Hz, 2H), 7.47 (d, $J = 8.4$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 51.2, 58.8, 122, 127, 131, 137; MS (EI, 70 eV) m/z (%): 198 (13), 169 (20), 119 (55), 89 (100), 77 (7), 63 (32); IR ν_{max} (cm^{-1}): 2958, 1745, 1517, 1230, 1026.

2-(4-chlorophenyl)oxirane (3c):



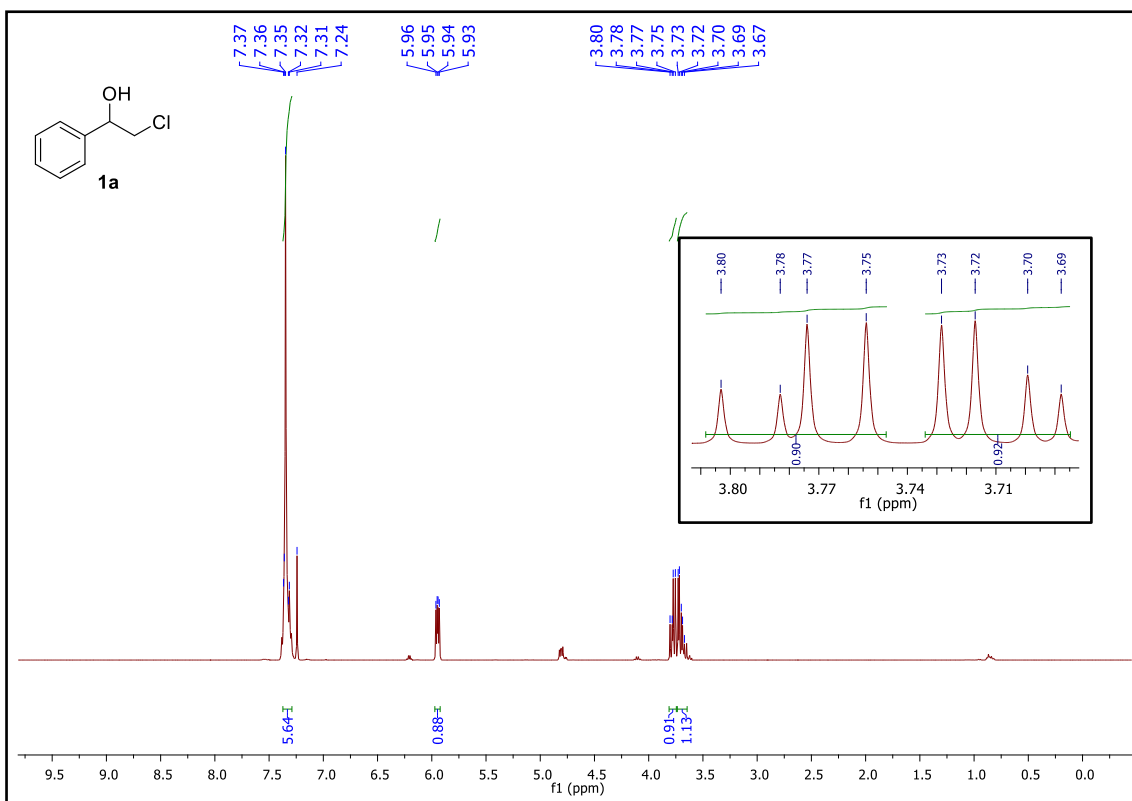
C_8H_7ClO , 154.07 $g \cdot mol^{-1}$. Colorless oil. 1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.30 (d, $J = 8.5$ Hz, 2H), 7.19 (d, $J = 8.5$ Hz, 2H), 3.82 (dd, $J = 3.7$ and 2.8 Hz, 1H), 3.13 (dd, $J = 5.4$ and 4.1 Hz, 1H), 2.73 (dd, $J = 5.4$ and 2.5 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 136.1, 133.9, 128.7, 126.8, 51.7, 51.2 ppm; MS (EI, 70 eV) m/z (%): 154 (23), 119 (69), 89 (100), 63 (20); IR ν_{max} (cm^{-1}): 2927, 2854, 1215, 759.

(S)-1-(4-chlorophenyl)-2-(1H-1,2,3-triazol-1-yl)ethanol (4):

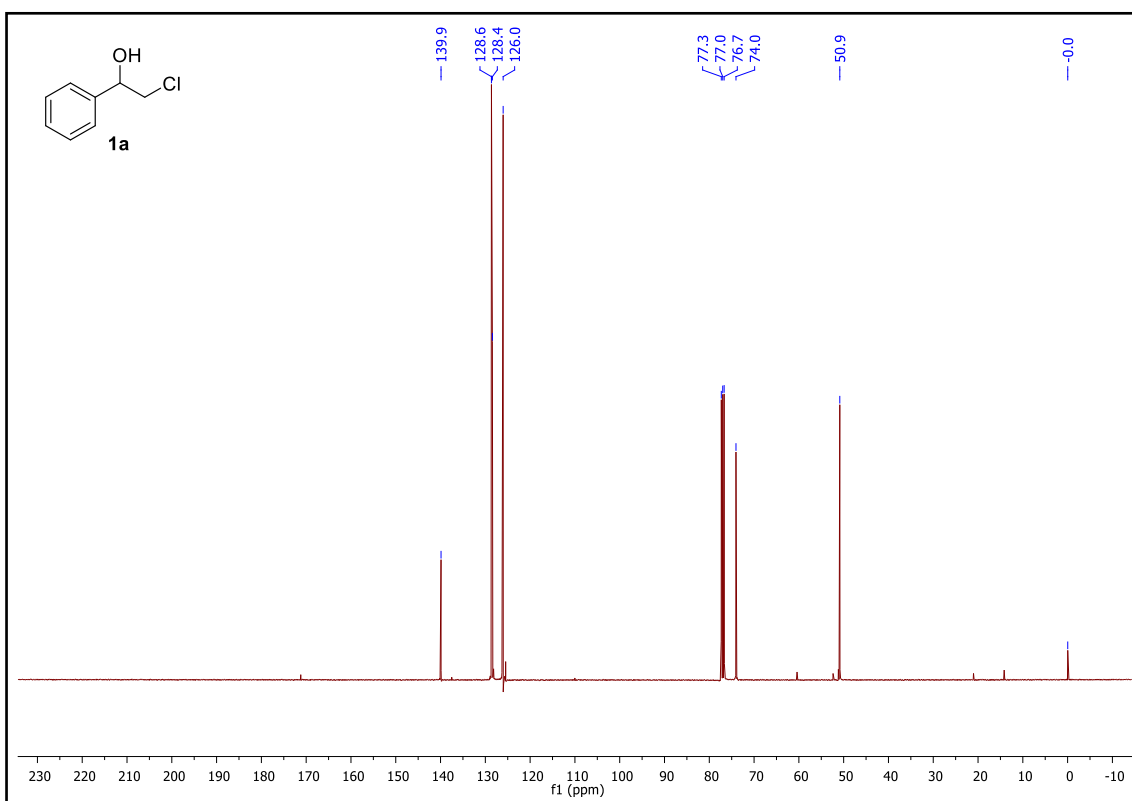


$C_{11}H_{11}ClN_2O$, 222.06 $g \cdot mol^{-1}$. White solid, m.p.: 185-186 °C (lit.⁸ 160-164); 1H NMR (400 MHz, CD_3OD) δ (ppm): 4.14 (dd, $J = 14.1$ and 6.9 Hz, 1H), 4.21 (dd, $J = 14.1$ and 4.4 Hz, 1H), 7.46 (s, 1H), 4.91 (dd, $J = 6.9$ and 4.5 Hz, 1H), 7.03 (t, $J = 1.3$ Hz, 1H), 6.88 (s, 1H), 7.34-7.22 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ (ppm): 141.8, 139, 134.5, 129.4, 128.7, 128.4, 121.5, 73.4, 55; MS (EI, 70 eV) m/z (%): 222 (5), 141 (19), 113 (13), 82 (100), 77 (40), 51 (7); IR ν_{max} (cm^{-1}) = 3116, 2929, 2856, 1514, 1408, 1072, 748, 661.

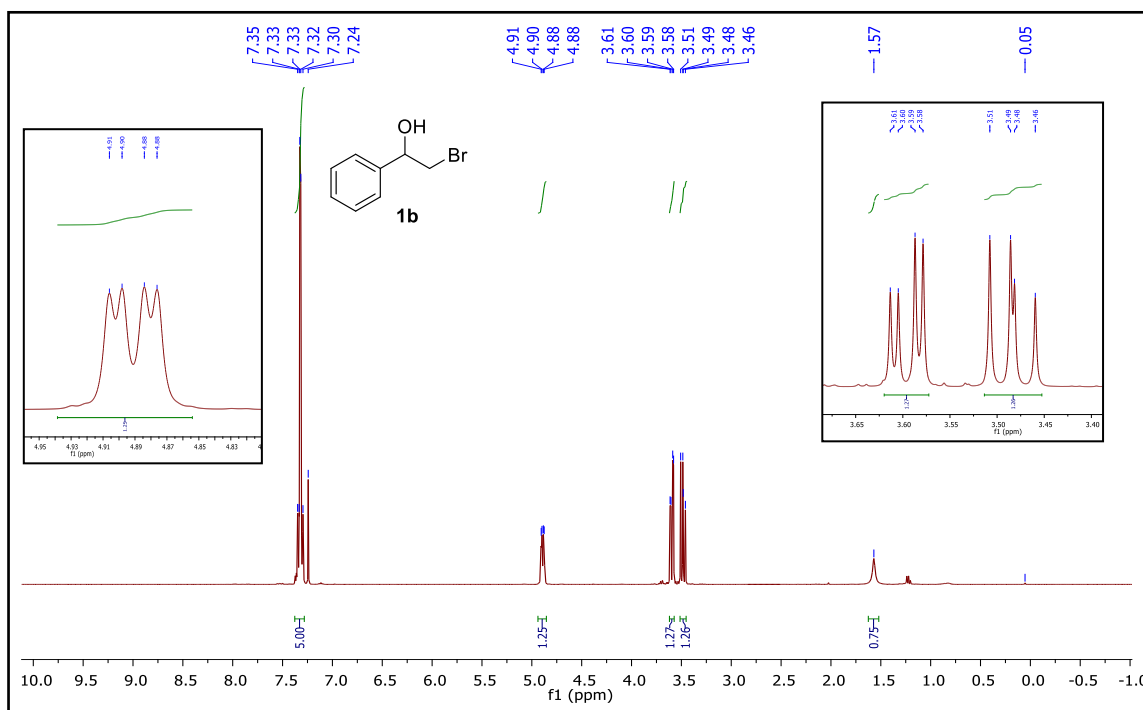
^1H NMR (500 MHz, CDCl_3) of 2-chloro-1-phenylethanol (**1a**)



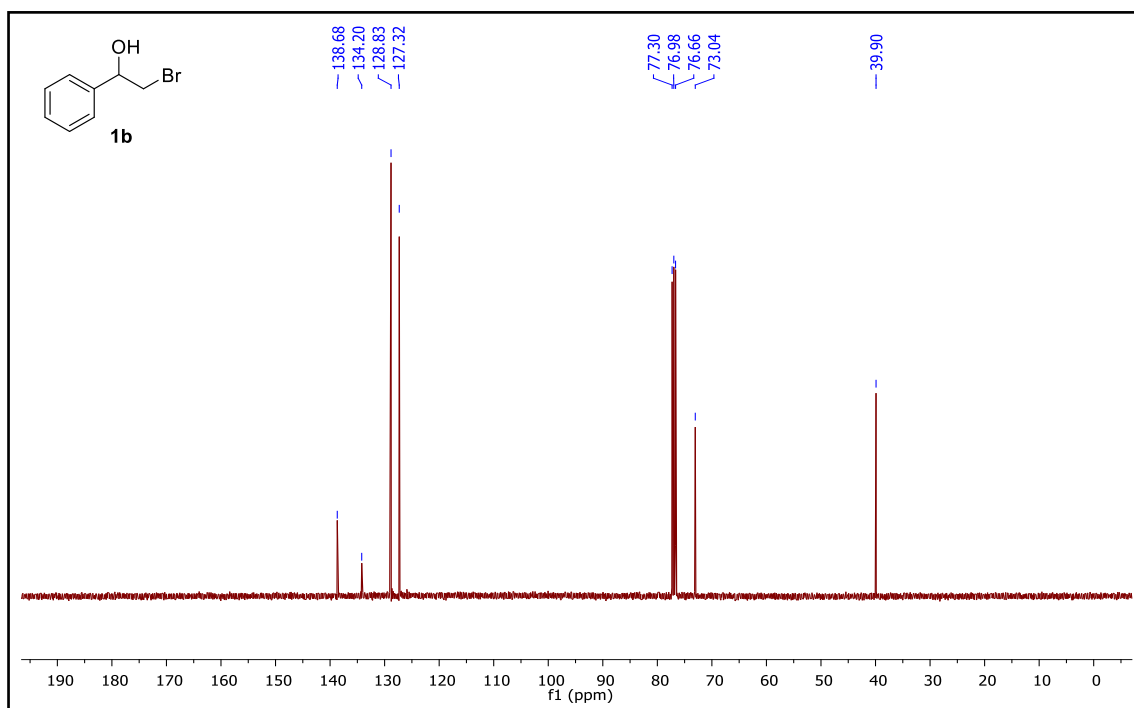
^{13}C NMR (125 MHz, CDCl_3) of 2-chloro-1-phenylethanol (**1a**)



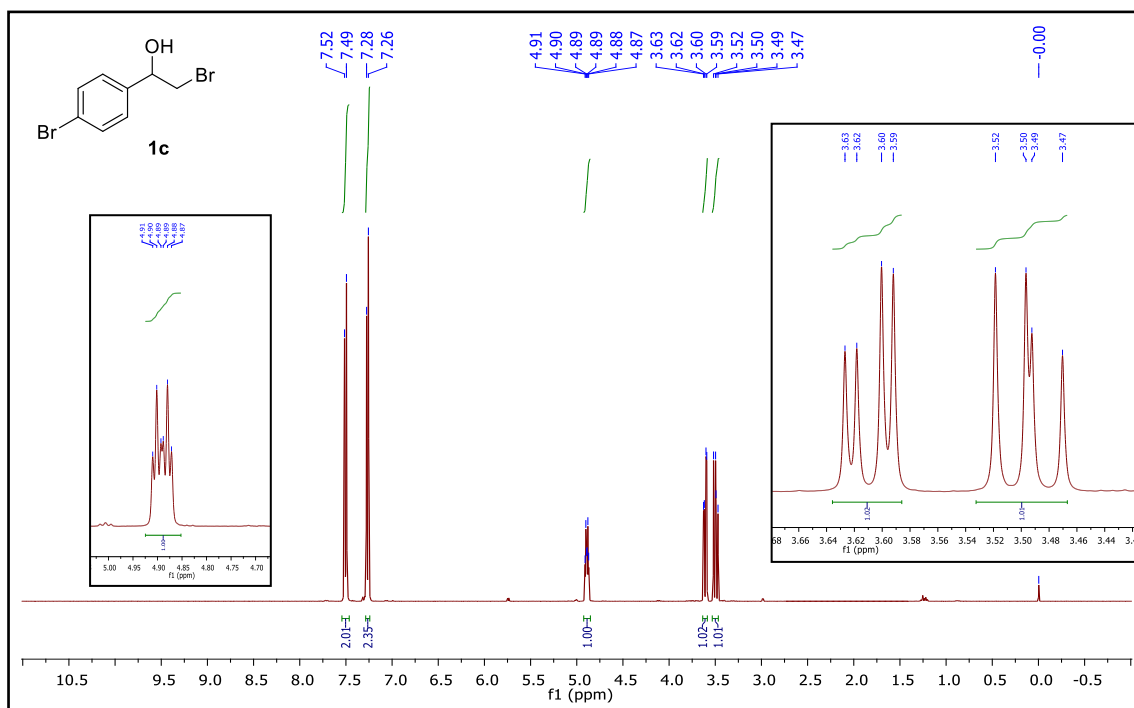
^1H NMR (400 MHz, CDCl_3) of 2-bromo-1-phenylethanol (**1b**)



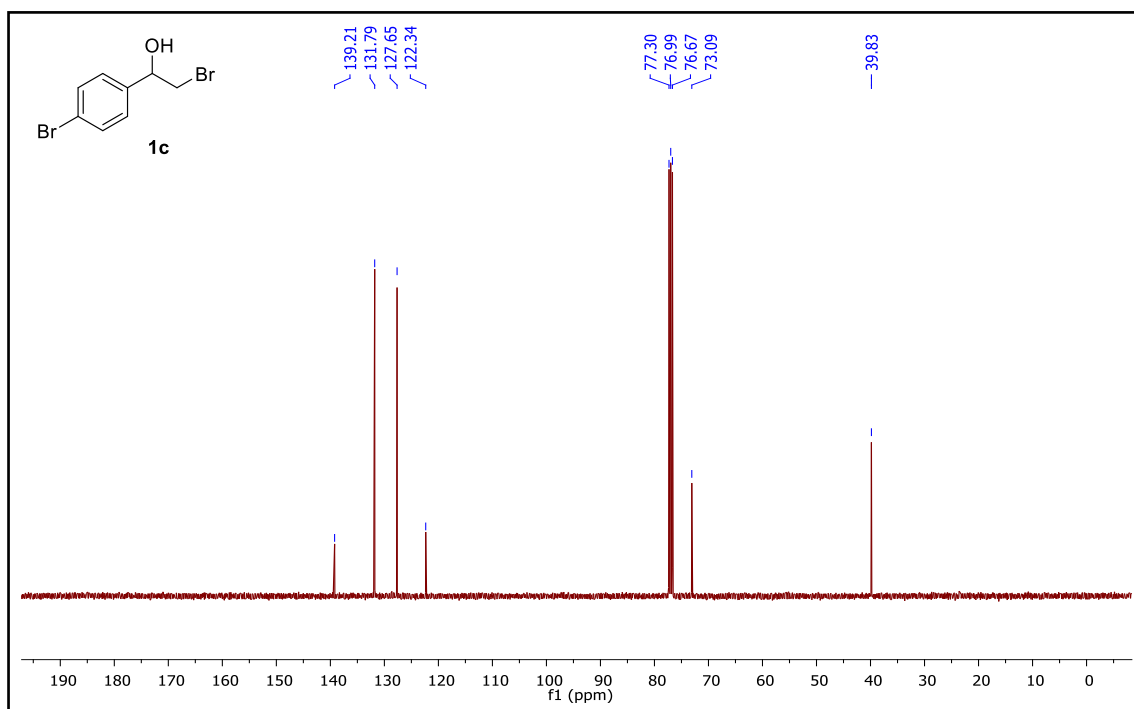
^{13}C NMR (100 MHz, CDCl_3) of 2-bromo-1-phenylethanol (**1b**)



^1H NMR (400 MHz, CDCl_3) of 2-bromo-1-(4-bromophenyl)ethanol (**1c**).



^{13}C NMR (100 MHz, CDCl_3) of 2-bromo-1-(4-bromophenyl)ethanol (**1c**).



¹H NMR (400 MHz, CDCl₃) of 2-bromo-1-(4-chlorophenyl)ethanol (**1d**)

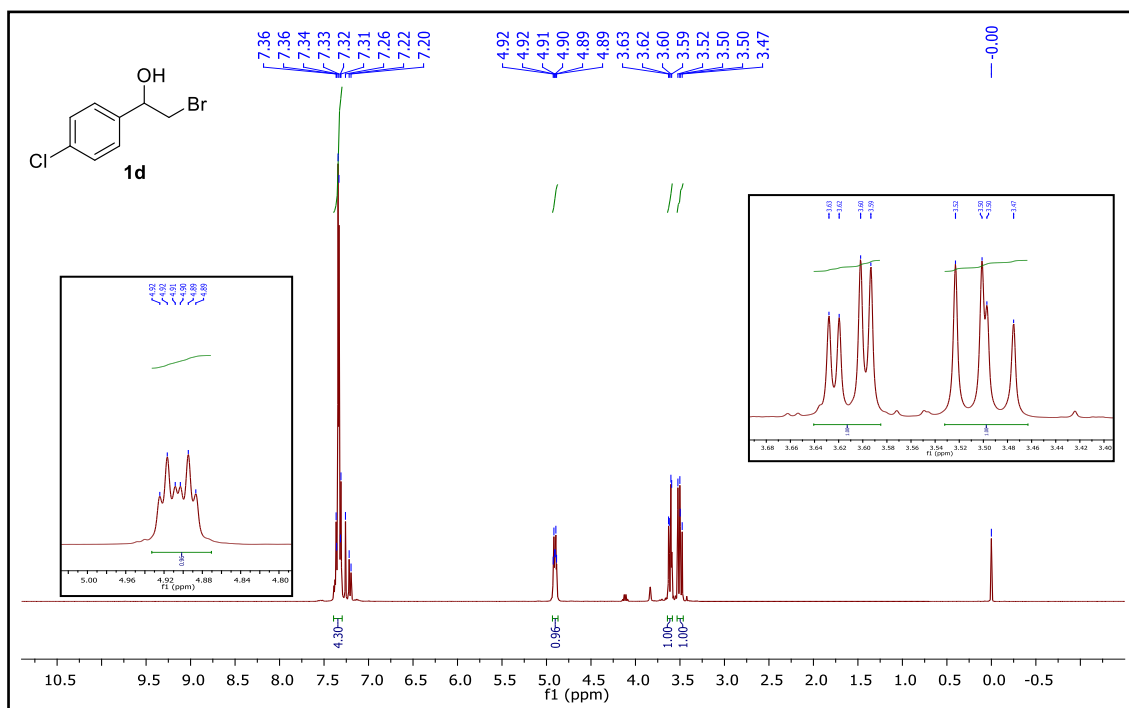
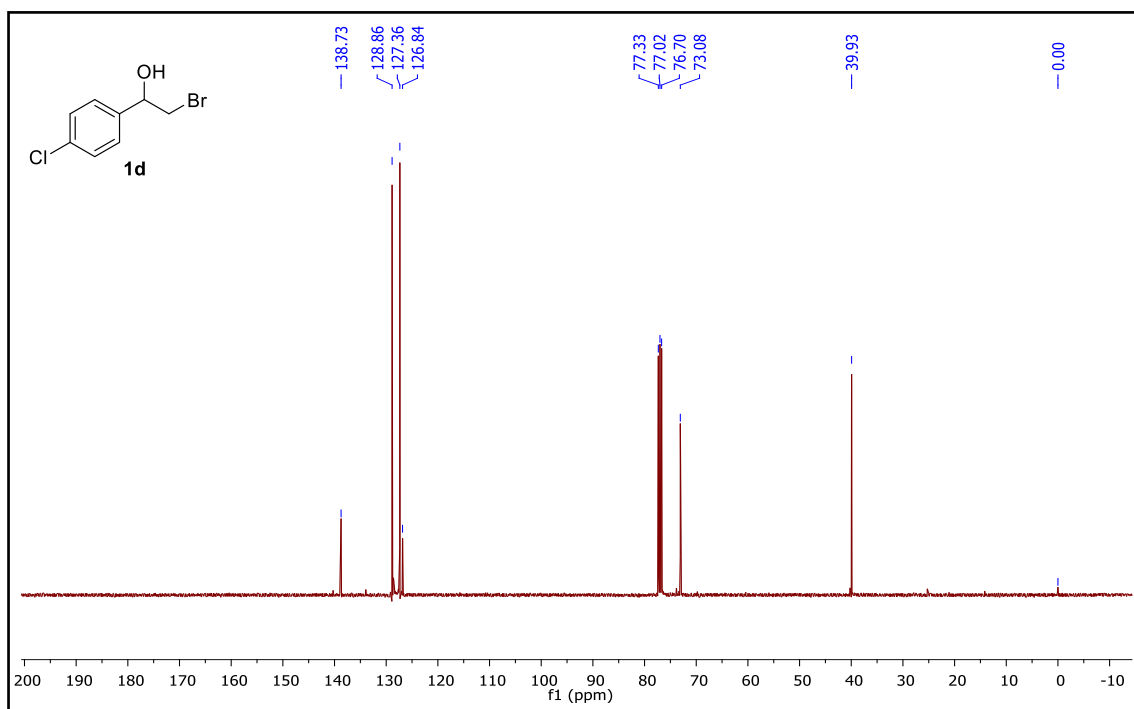
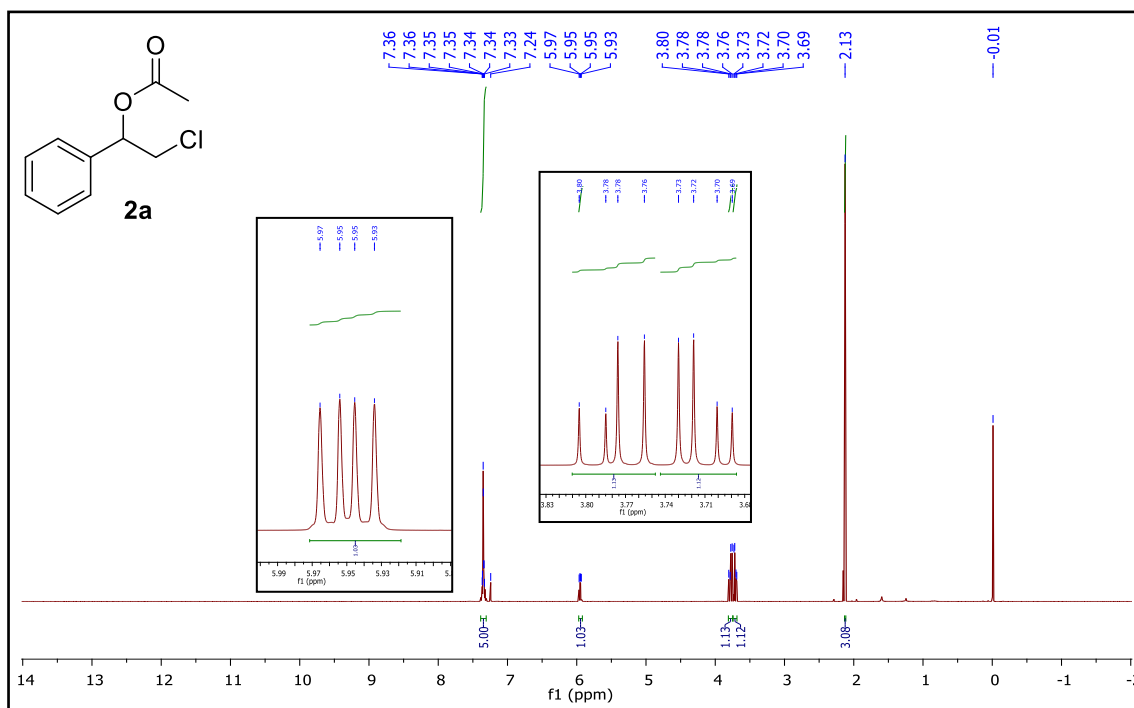


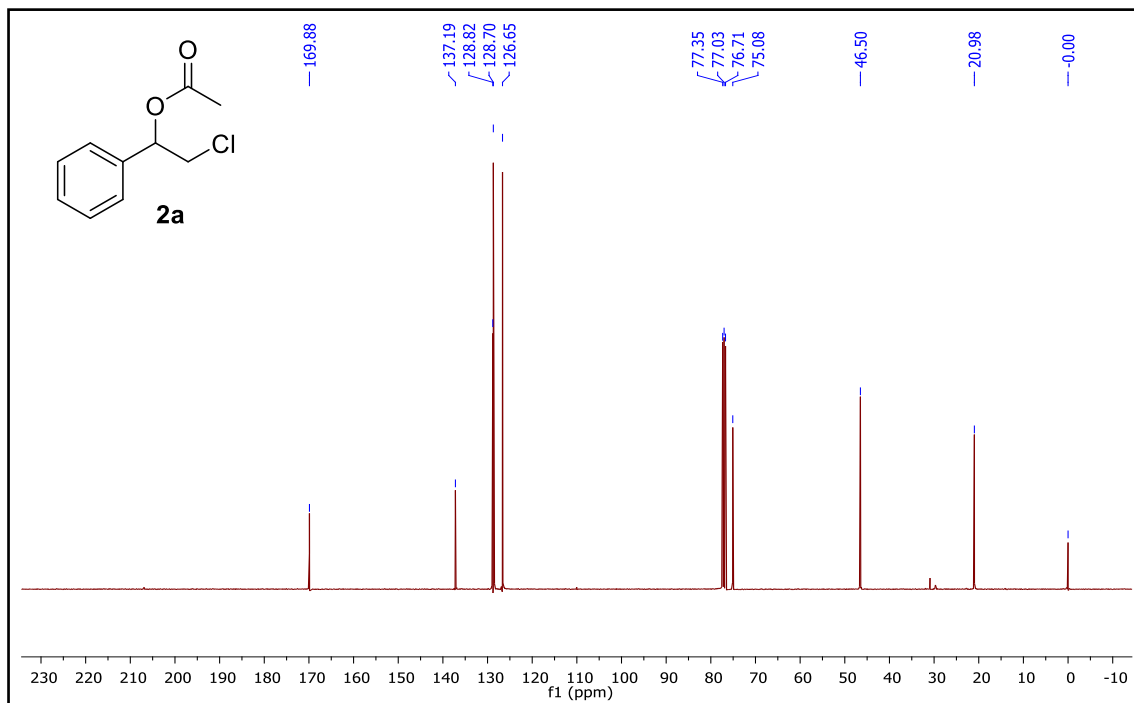
Fig. S19. ¹³C NMR (100 MHz, CDCl₃) of 2-bromo-1-(4-chlorophenyl)ethanol (**1d**)



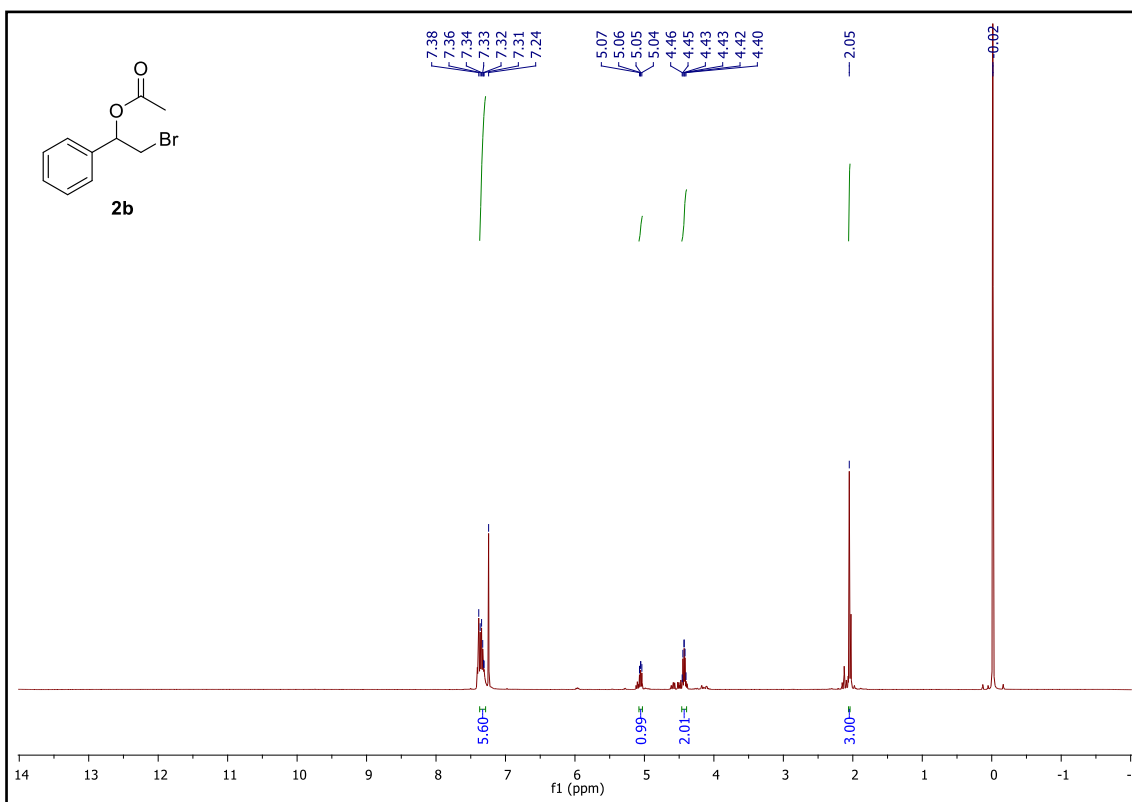
^1H NMR (500 MHz, CDCl_3) of 2-chloro-1-phenylethyl acetate (**2a**).



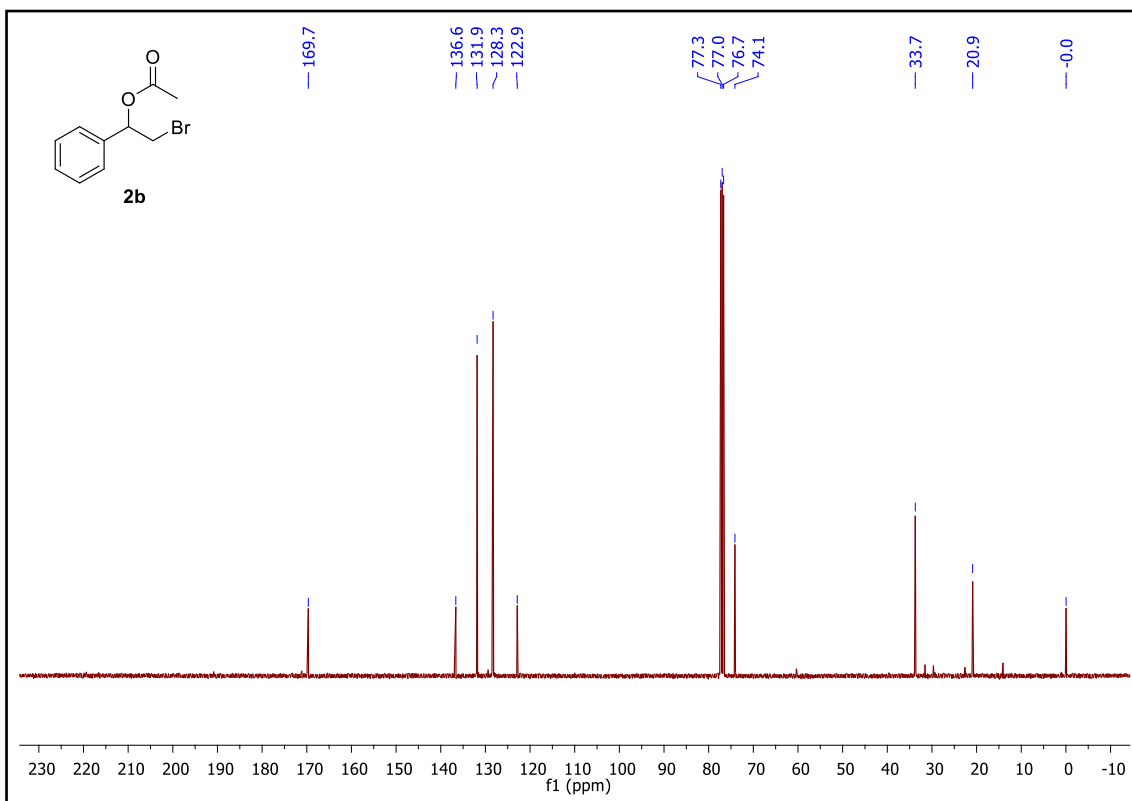
^{13}C NMR (125 MHz, CDCl_3) of 2-chloro-1-phenylethyl acetate (**2a**).



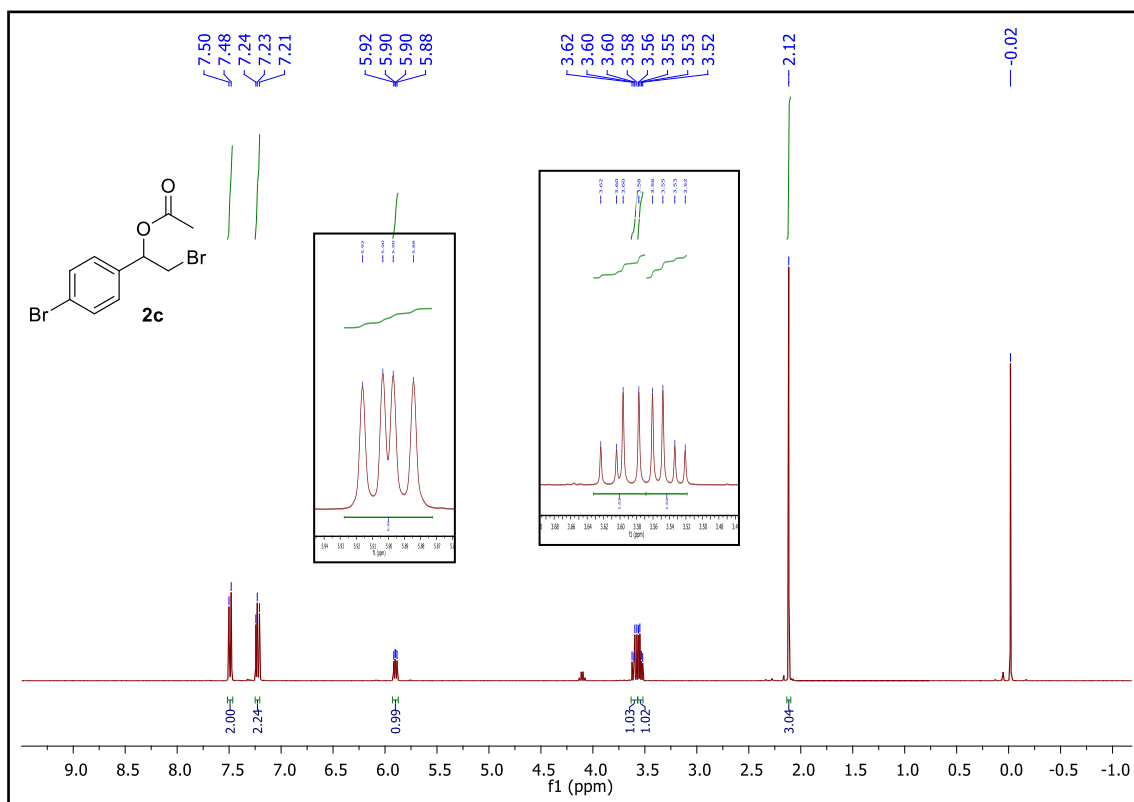
^1H NMR (500 MHz, CDCl_3) of 2-bromo-1-phenylethyl acetate (**2b**).



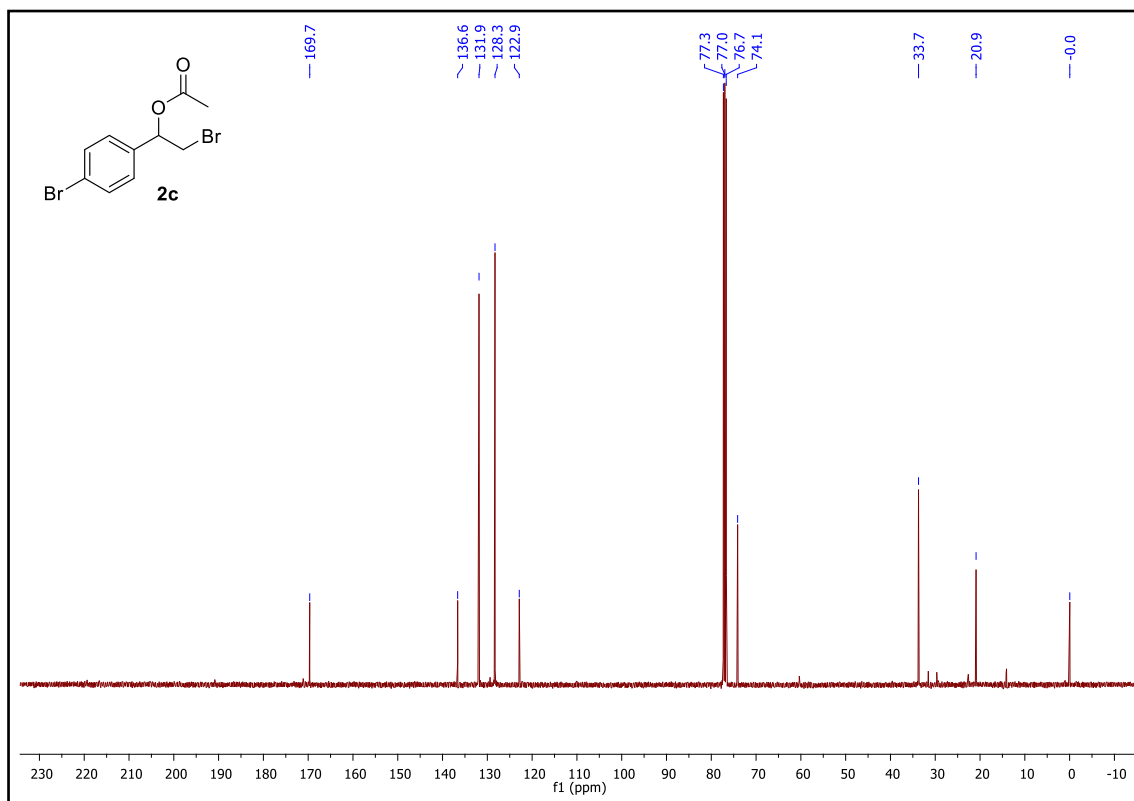
^{13}C NMR (125 MHz, CDCl_3) of 2-bromo-1-phenylethyl acetate (**2b**).



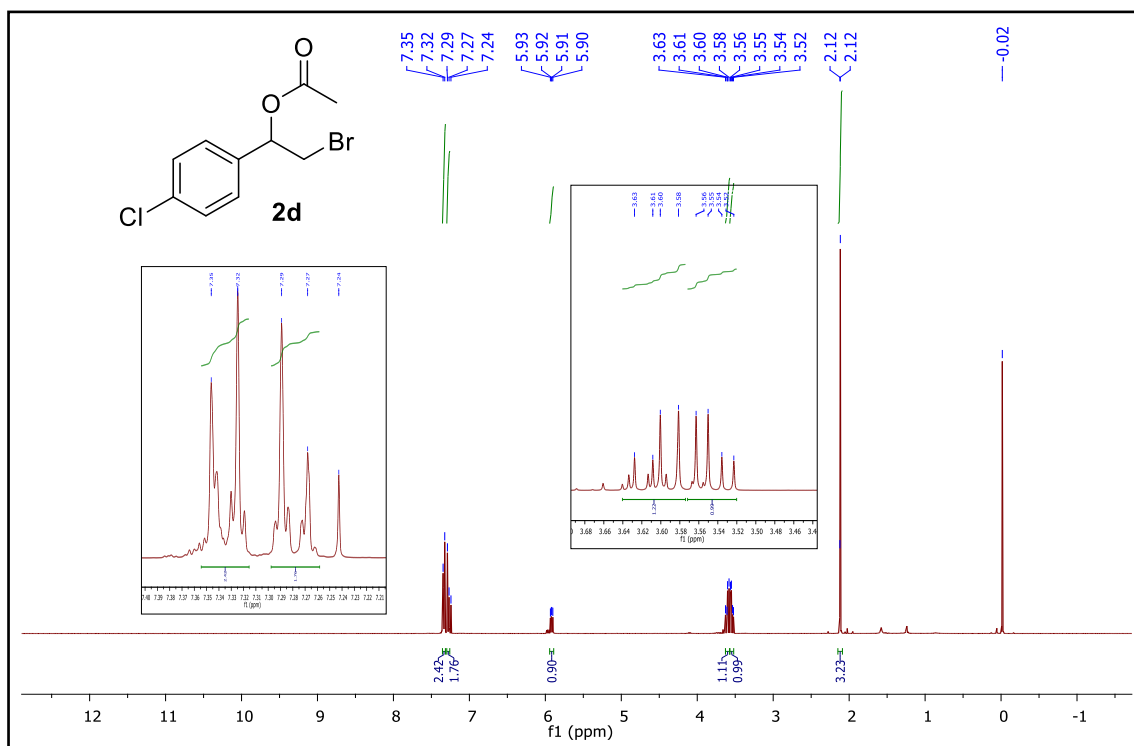
^1H NMR (400 MHz, CDCl_3) of 2-bromo-1-(4-bromophenyl)ethyl acetate (**2c**).



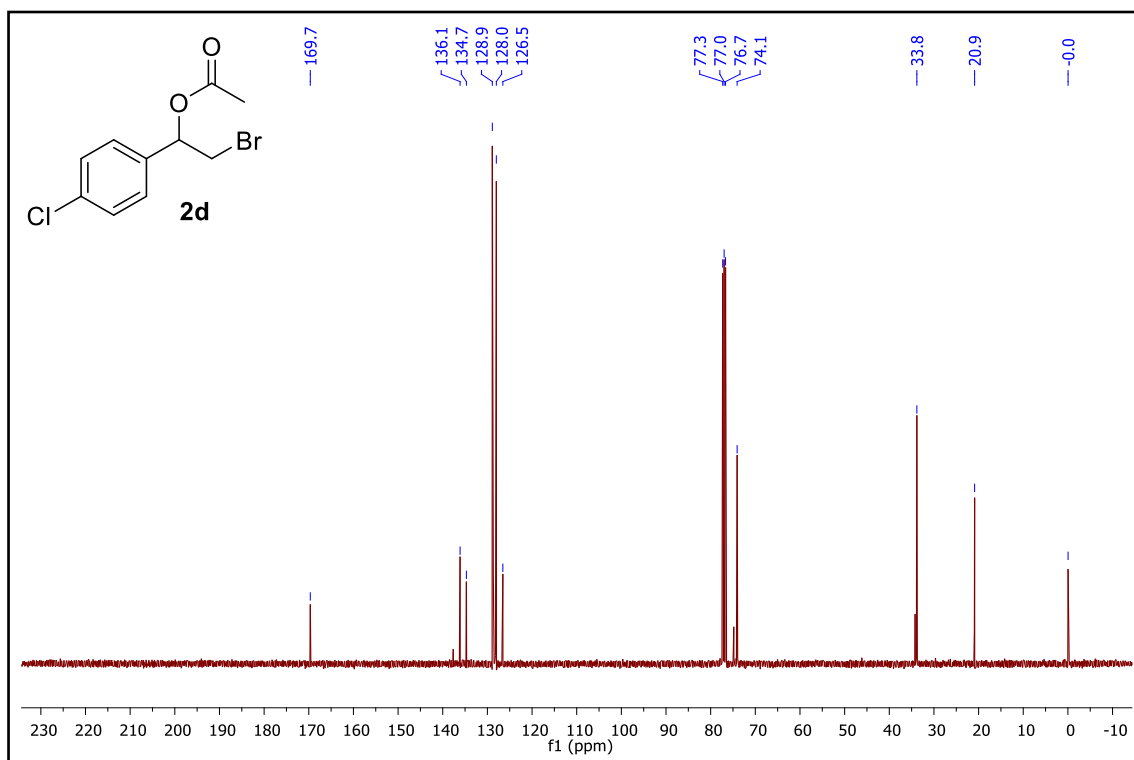
^{13}C NMR (100 MHz, CDCl_3) of 2-bromo-1-(4-bromophenyl)ethyl acetate (**2c**).



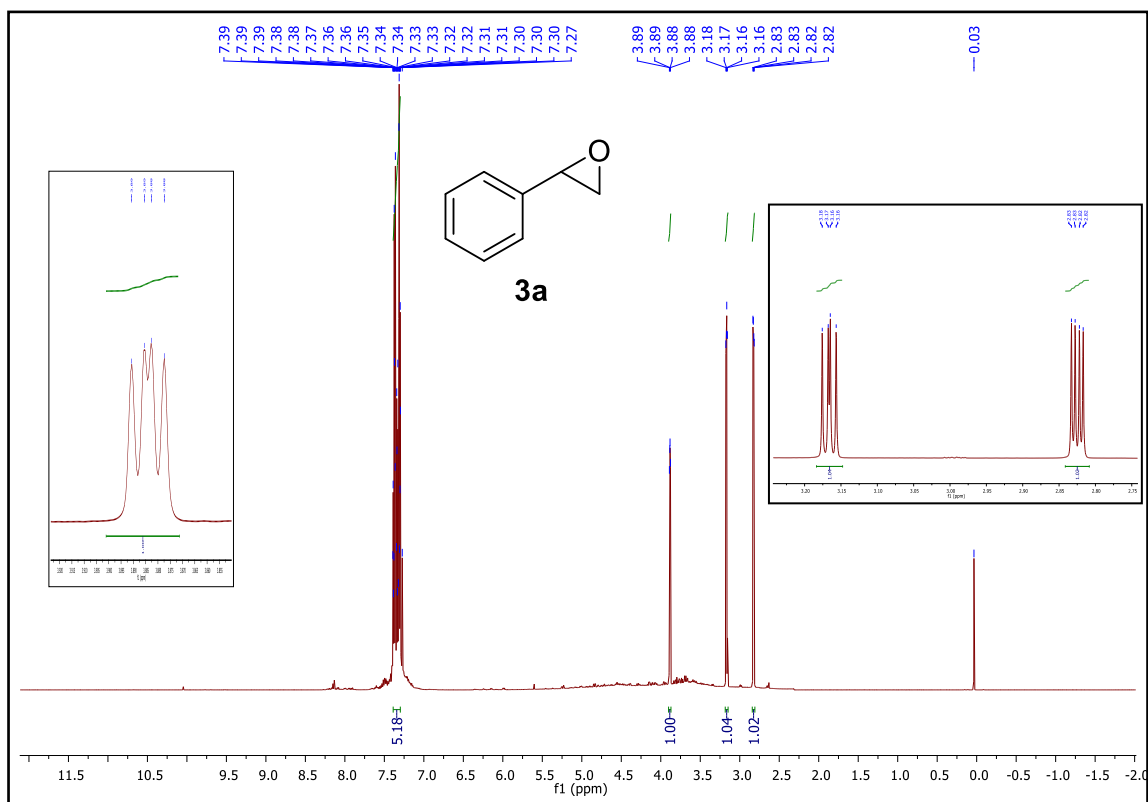
^1H NMR (400 MHz, CDCl_3) of 2-bromo-1-(4-chlorophenyl)ethyl acetate (**2d**).



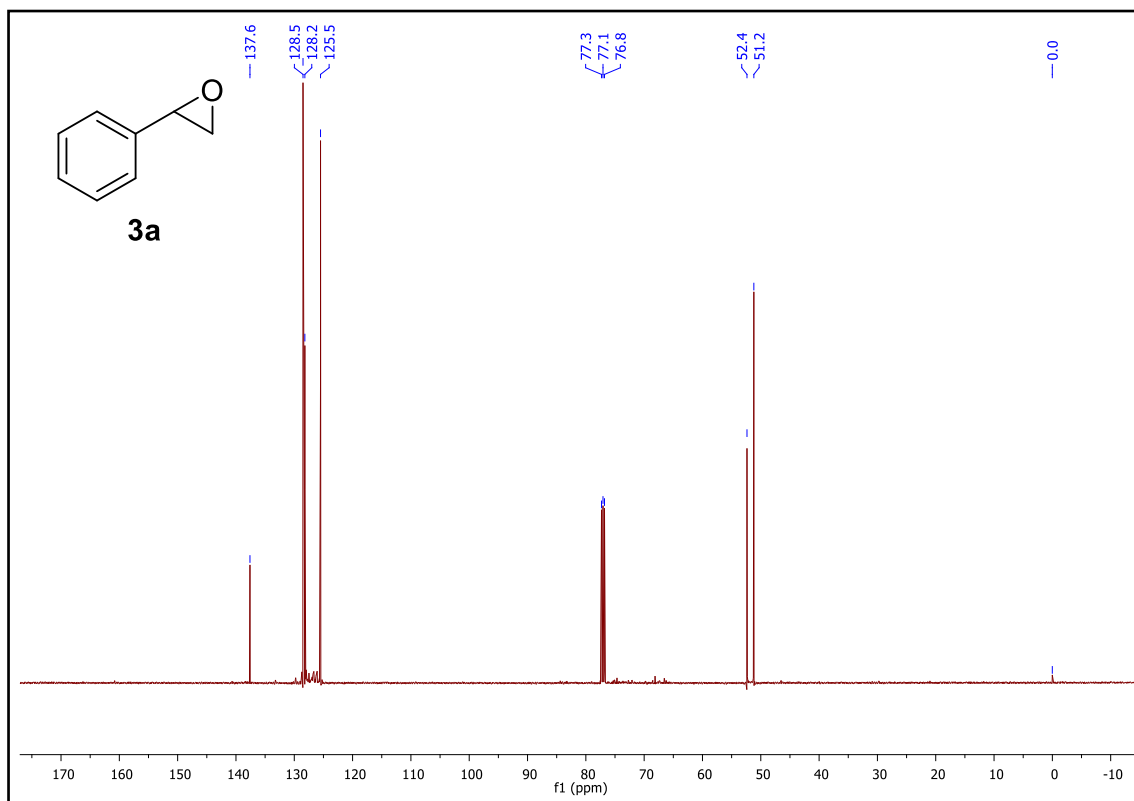
^{13}C NMR (100 MHz, CDCl_3) of 2-bromo-1-(4-chlorophenyl)ethyl acetate (**2d**).



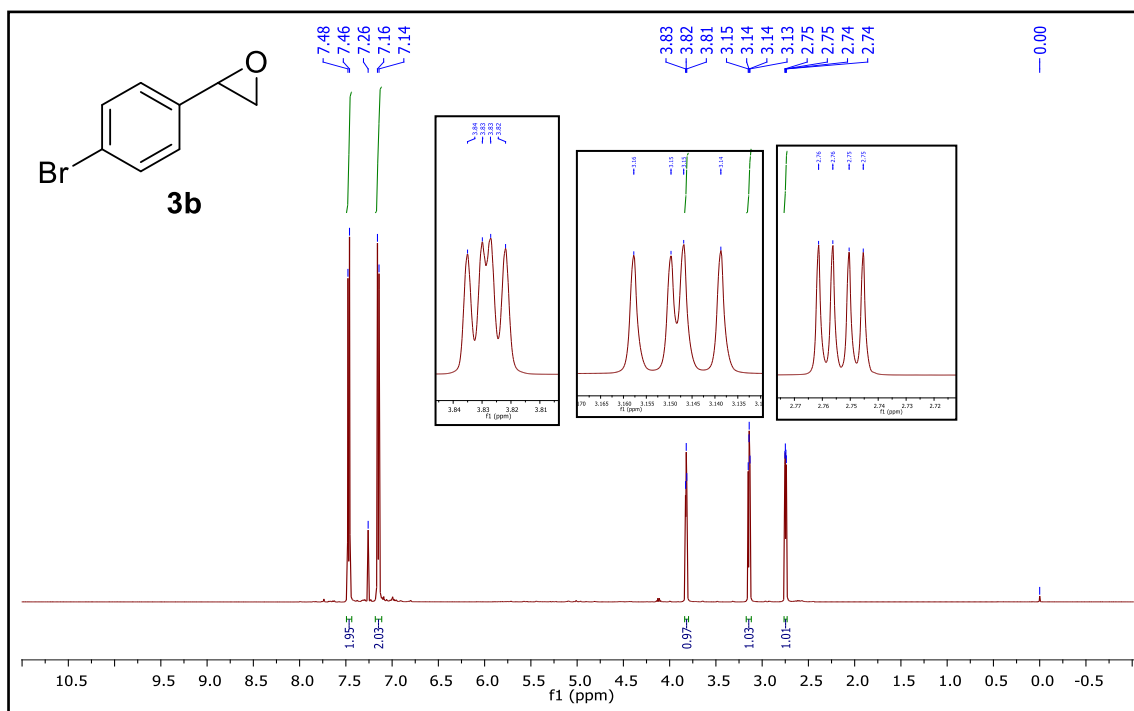
^1H NMR (400 MHz, CDCl_3) of 2-phenyloxirane (**3a**)



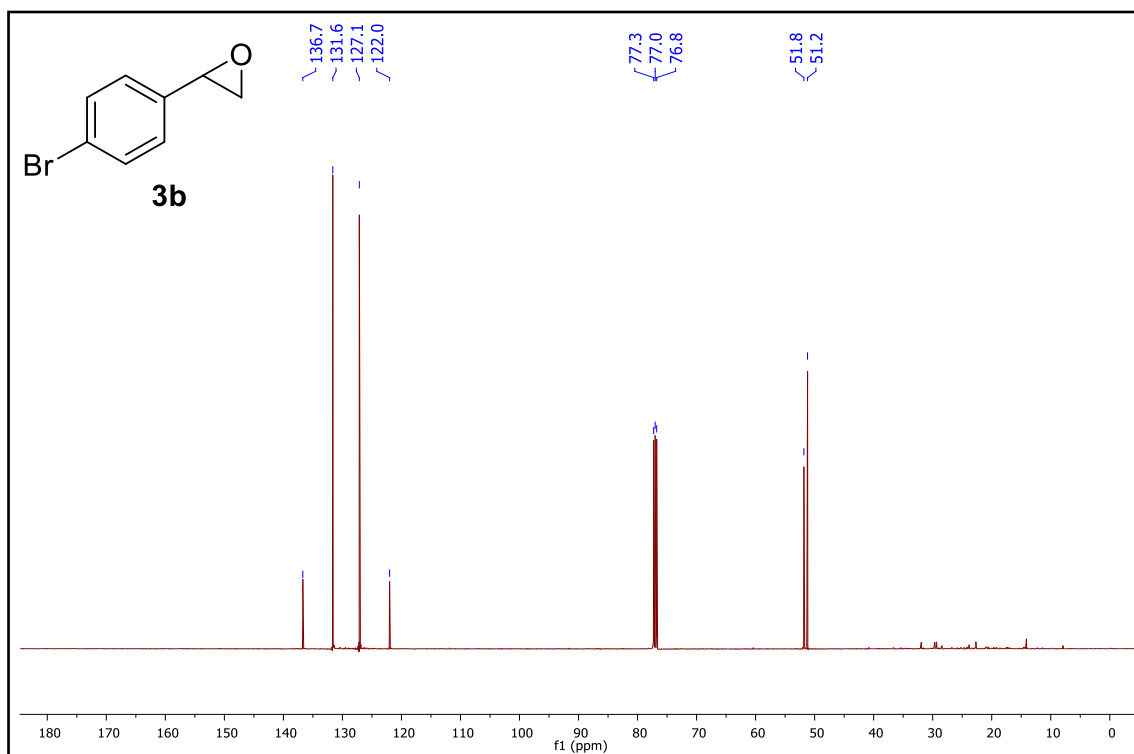
^{13}C NMR (100 MHz, CDCl_3) of 2-phenyloxirane (**3a**)



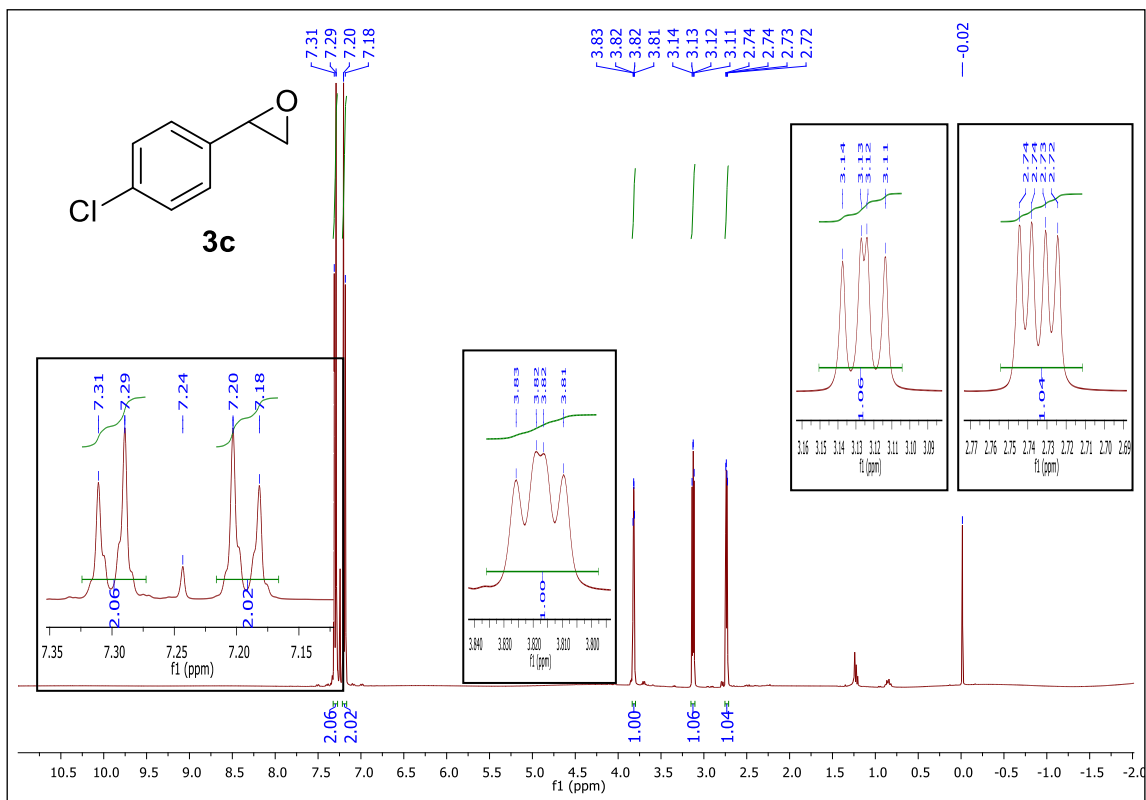
¹H NMR (400 MHz, CDCl₃) of 2-(4-bromophenyl)oxirane (**3b**)



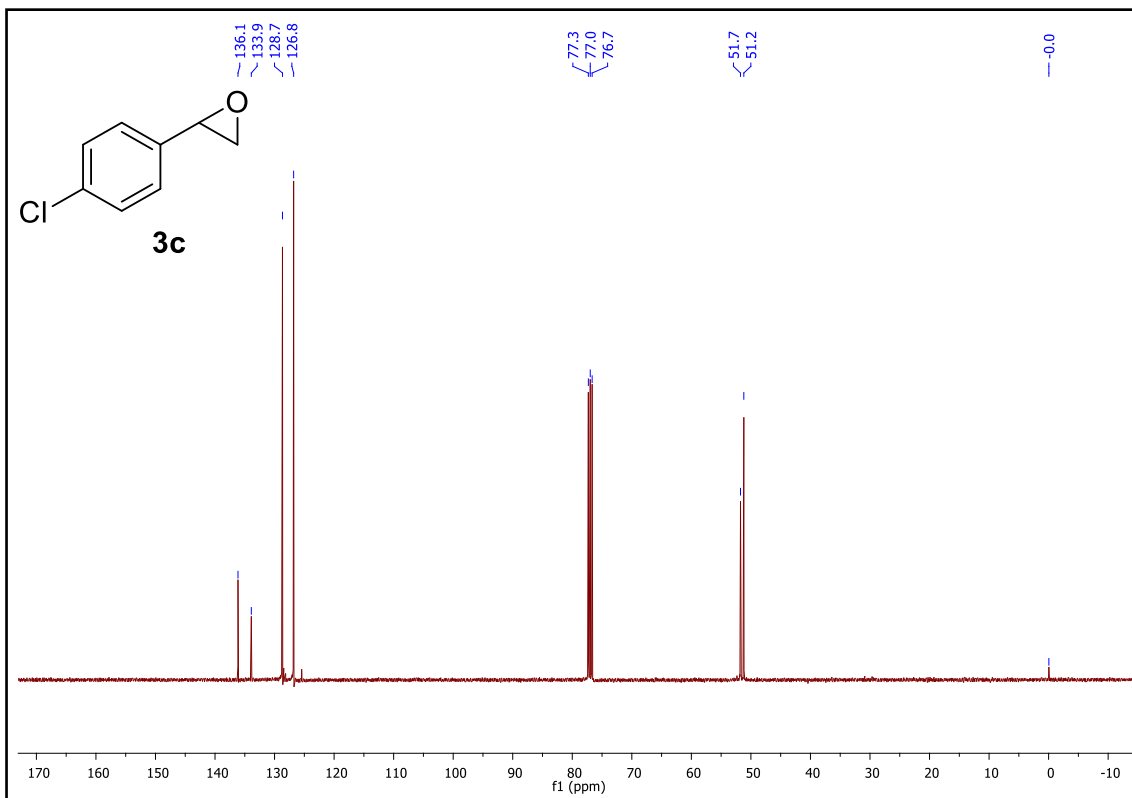
¹³C NMR (100 MHz, CDCl₃) of 2-(4-bromophenyl)oxirane (**3b**)



^1H NMR (400 MHz, CDCl_3) of 2-(4-chlorophenyl)oxirane (**3c**)



^{13}C NMR (100 MHz, CDCl_3) of 2-(4-chlorophenyl)oxirane (**3c**)



^1H NMR (400 MHz, CD_3OD) of (*S*)-1-(4-chlorophenyl)-2-(1H-1,3-triazol-1-yl)ethanol

(4)

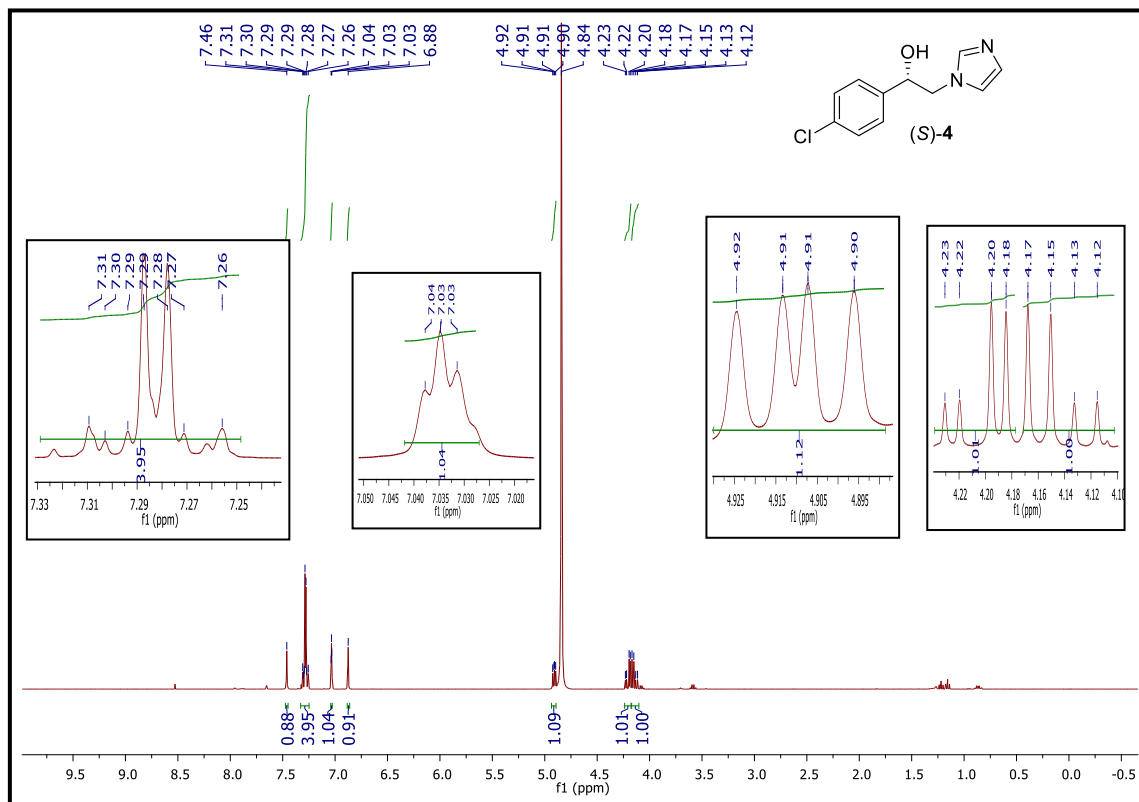
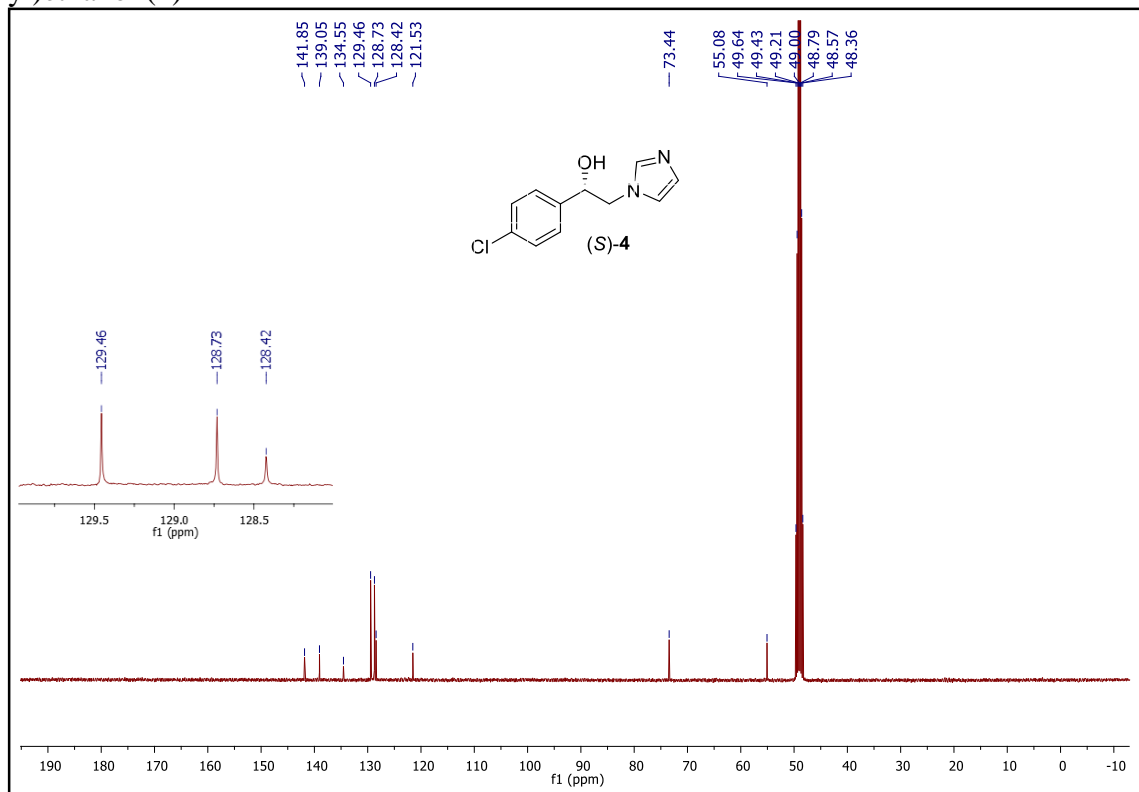
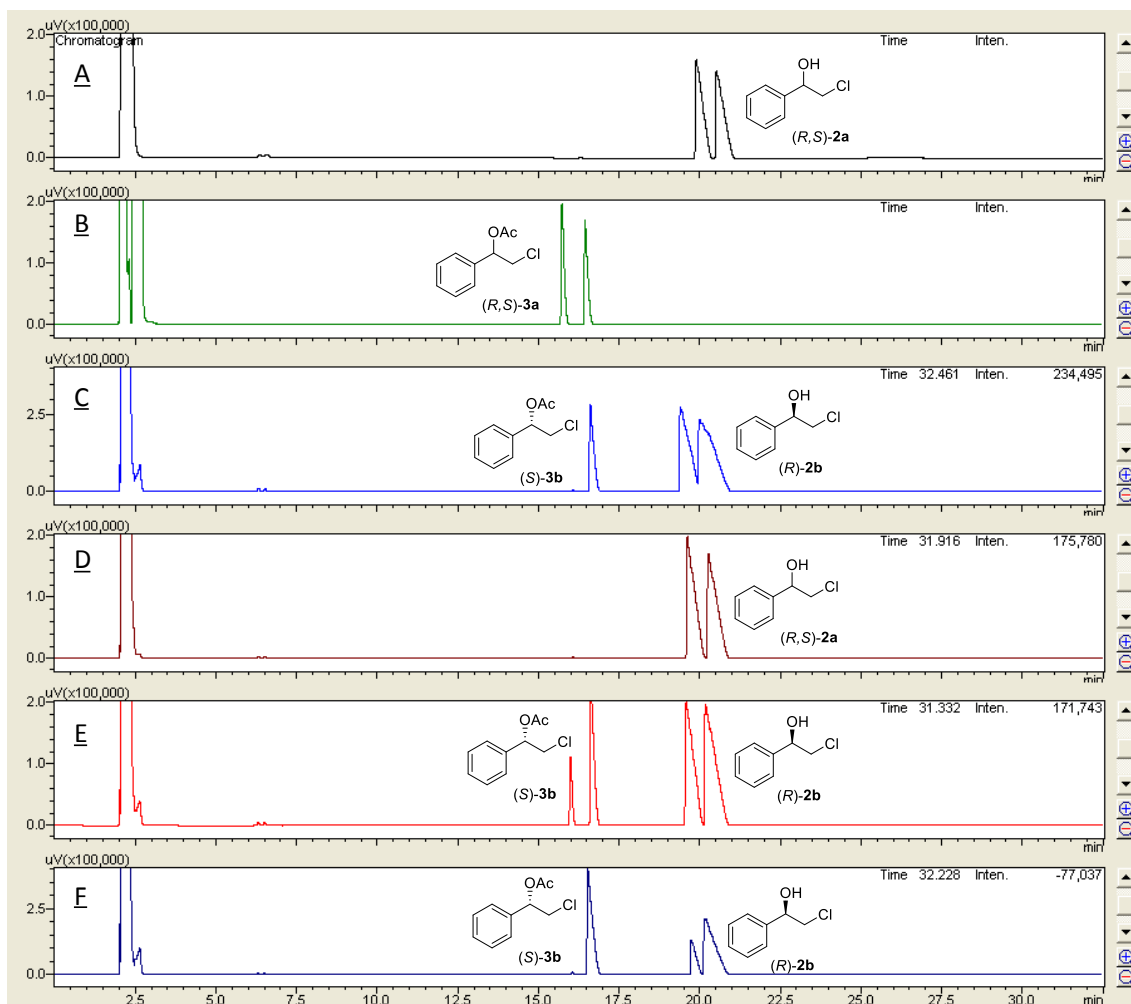


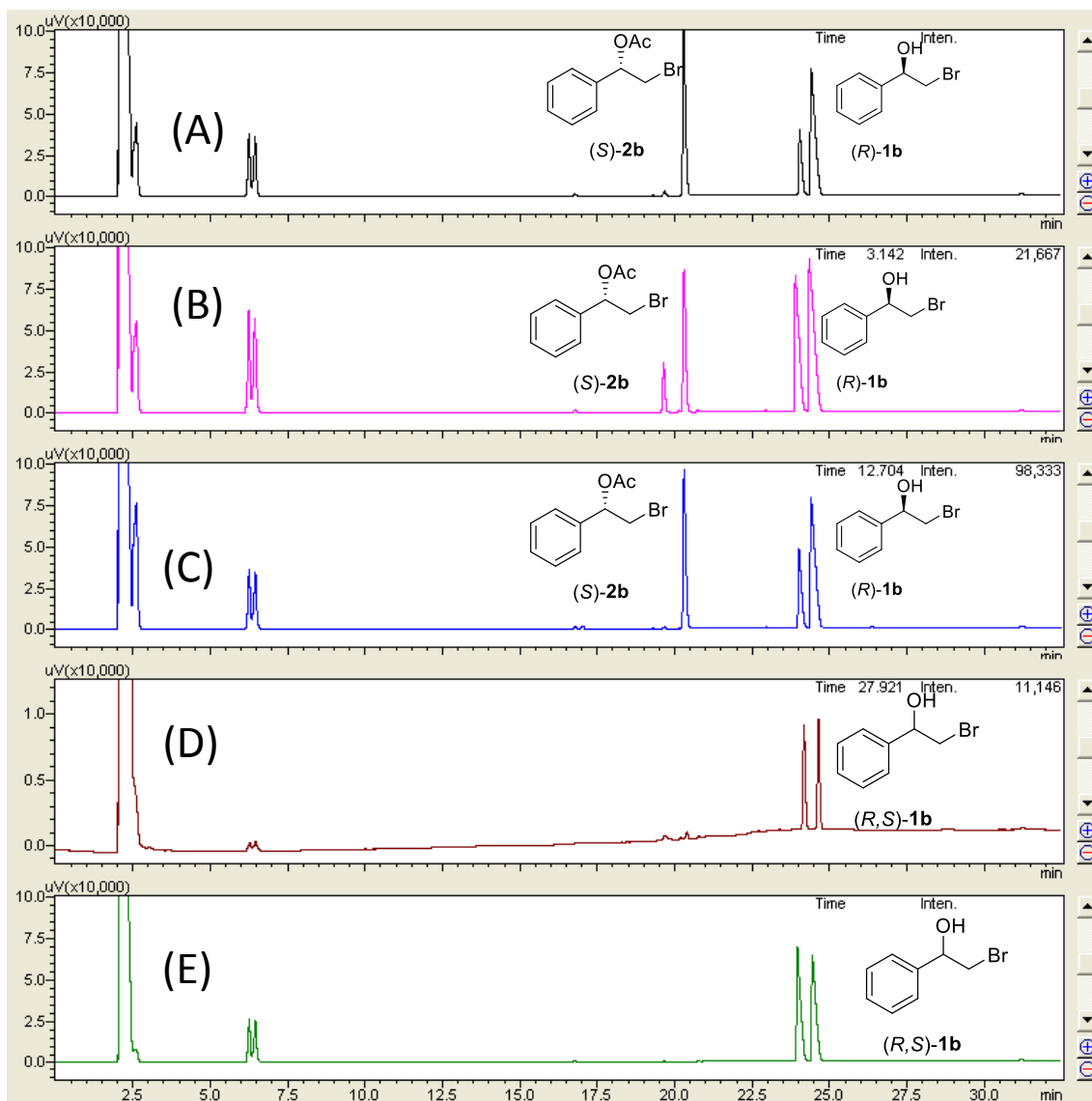
Fig. S51. ^{13}C NMR (100 MHz, CD_3OD) of (*S*)-1-(4-chlorophenyl)-2-(1H-1,3-triazol-1-yl)ethanol (4)



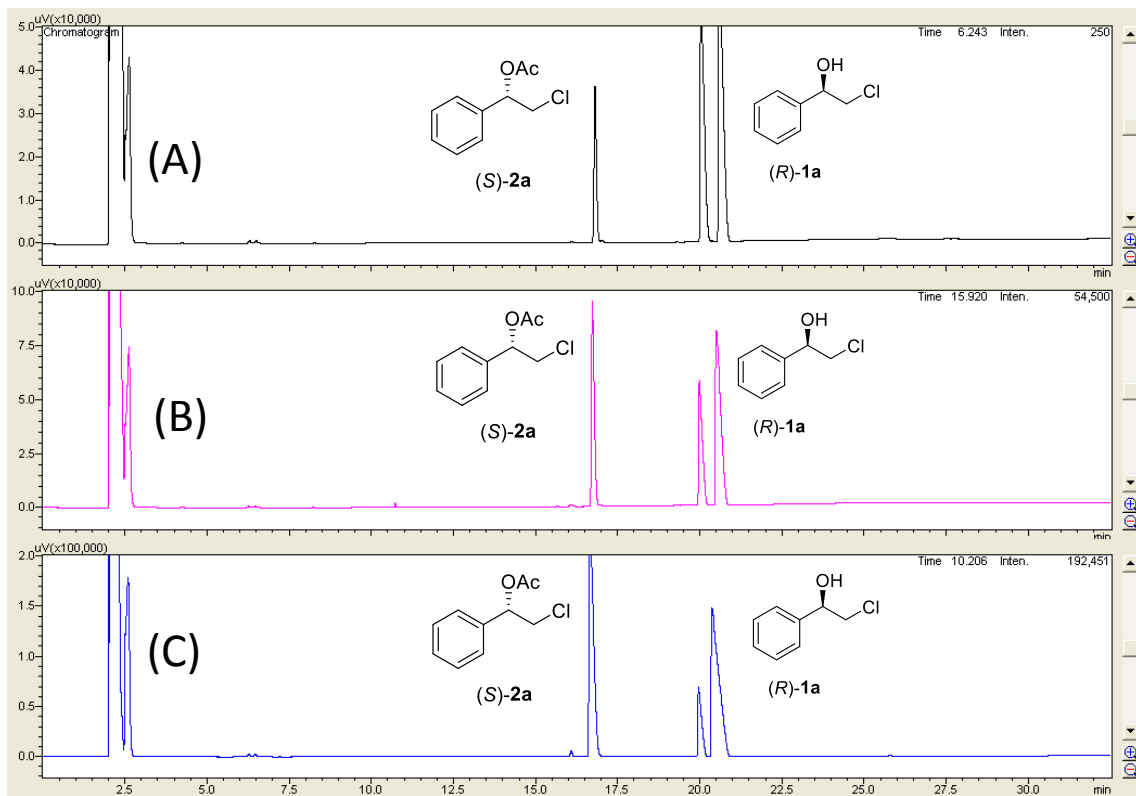
GC-FID Chromatograms: (A) Standard of (*R,S*)-2-chloro-1-phenylethanol **1a**. (B) Standard of (*R,S*)-2-chloro-1-phenylethyl acetate **2a**. (C) Kinetic resolution of (*S*)-**2a** and (*R*)-**1a** by lipase from *C. cylindracea* (D) *R. niveus* (E) *A. niger* (F) *P. fluorescens* after 24 h of reaction on 32 °C, 130 rpm.



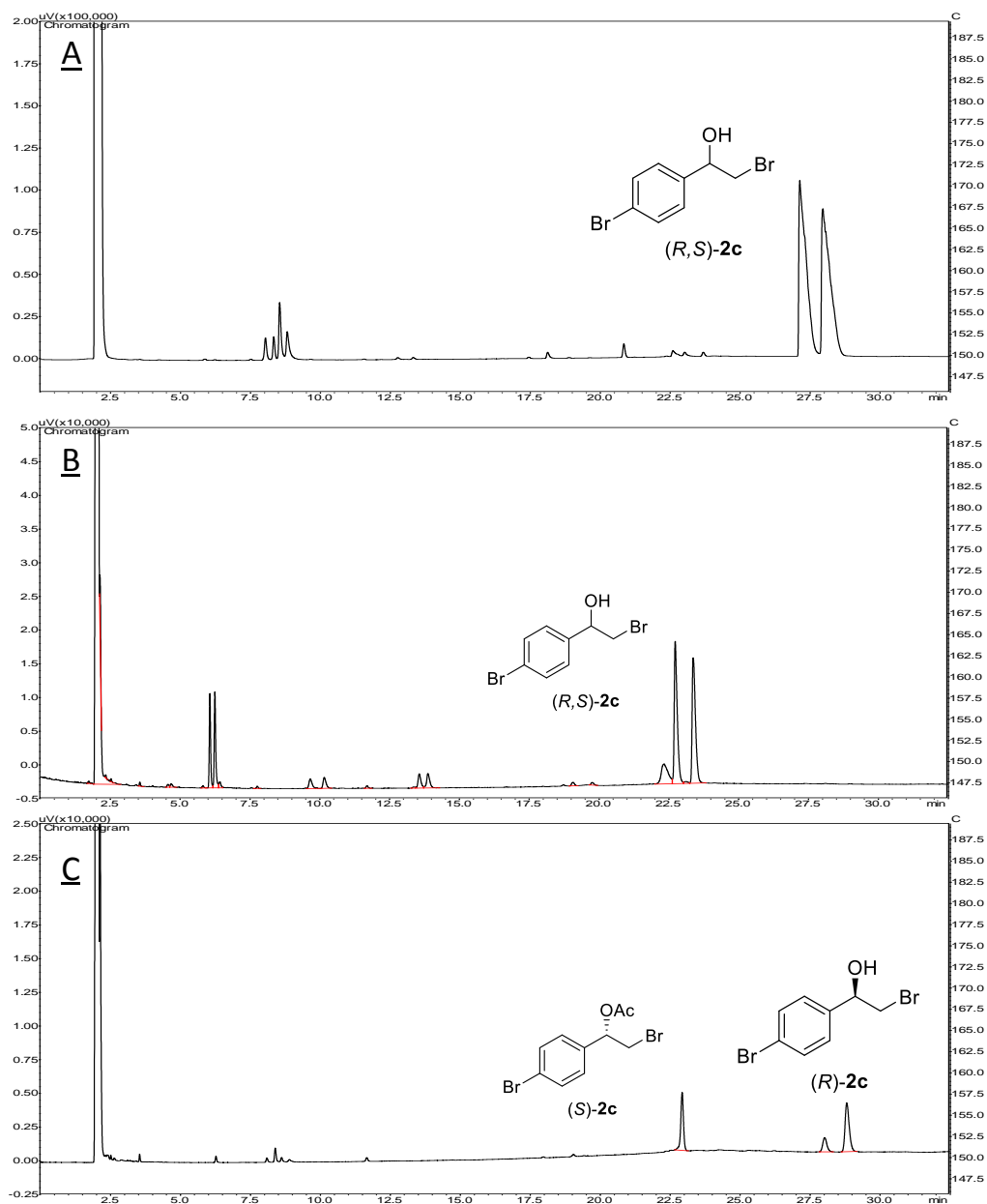
GC-FID Chromatograms: Kinetic resolution of (*S*)-**2b** and (*R*)-**1b** by lipase from (A) *P. fluorescens* (B) *C. cylindracea* (C) *R. niveus* (D) *A. niger* after 24 h of reaction on 32 °C, 130 rpm. (F) Standard of (*R,S*)-2-chloro-1-phenylethanol **1b**



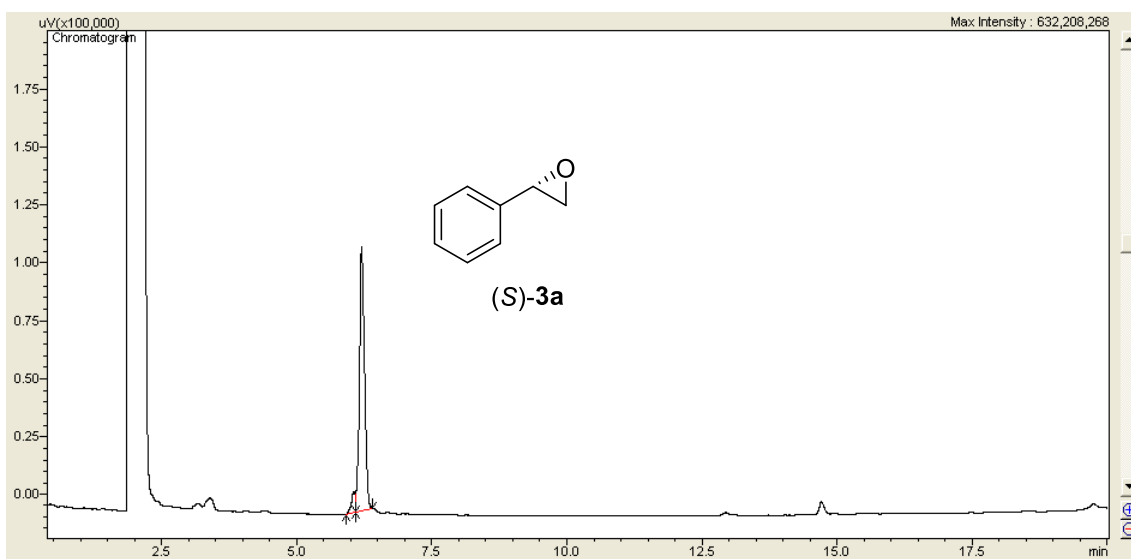
GC-FID Chromatograms: Kinetic resolution of (*R,S*)-**2a** by FS-*LPf* in different concentrations (a) 50 mg (24h, 32°C, 130 rpm); (b) 150 mg (24h, 32°C, 130 rpm); (c) 100 mg (48h, 32°C, 130 rpm).



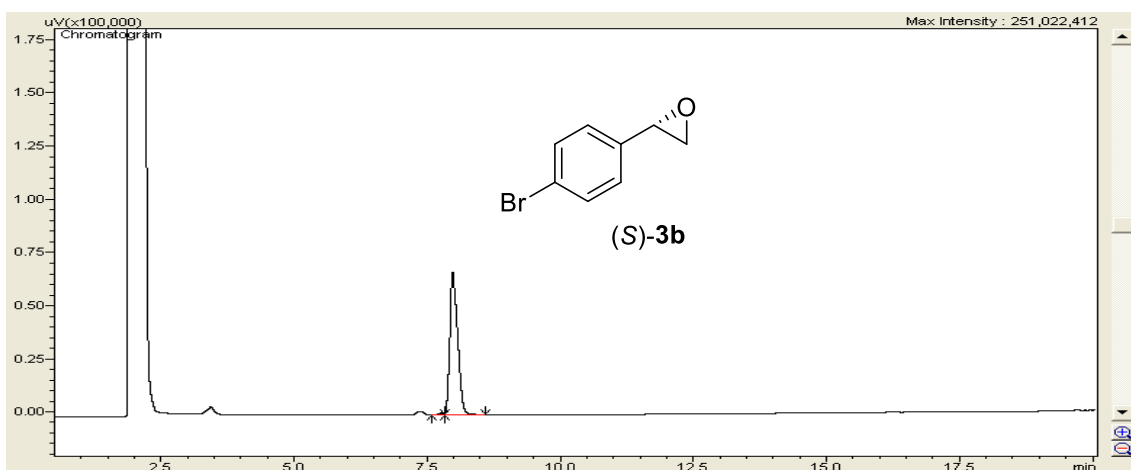
GC-FID Chromatograms: (A) Standard of (*R,S*)-**1c**. (B) Standard of acetate (*R,S*)-**2c**. (C) Kinetic resolution of (*S*)-**2c** and (*R*)-**1c** by FS-LPF after 24 h of reaction (32 °C, 130 rpm).



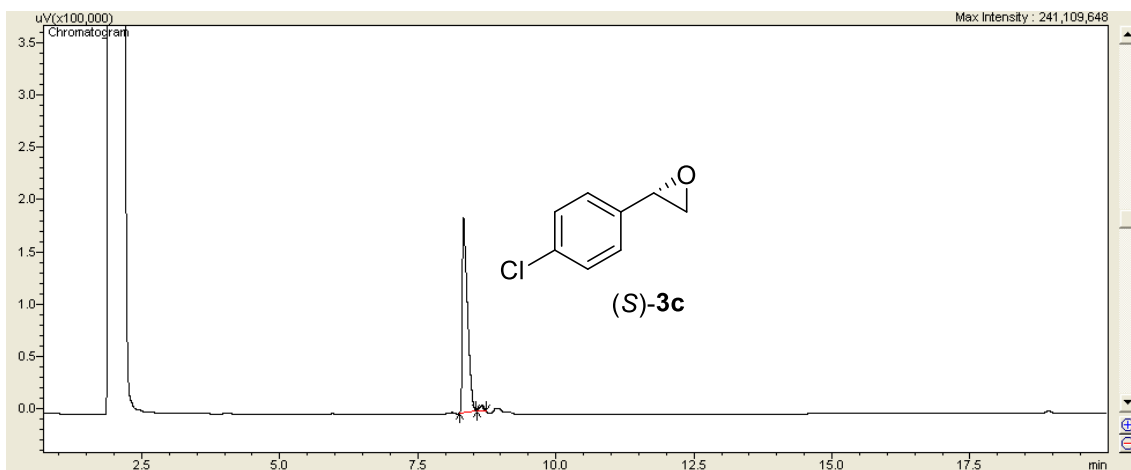
GC-FID Chromatograms: Epoxide (*S*)-**3a**.



GC-FID Chromatograms: Epoxide (*S*)-**3b**.



GC-FID Chromatograms: (A) Standard of (*R,S*)-**3c**.



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