

## SUPPORTING INFORMATION

### Efficient Asymmetric Synthesis of Lamivudine via Enzymatic Dynamic Kinetic Resolution

*Lei Hu, Fredrik Schauffelberger, Yan Zhang and Olof Ramström\**

KTH - Royal Institute of Technology, Department of Chemistry,  
Teknikringen 30, S-10044 Stockholm, Sweden; Email: ramstrom@kth.se

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**General:** All commercially available starting materials were of reagent grade and used as received. Subtilisin Carlsberg was purchased from Sigma-Aldrich (Art. No: P5380). <sup>1</sup>H NMR and <sup>13</sup>C NMR data were recorded on a Bruker Avance DMX 500 at 500 MHz. Chemical shifts are reported as  $\delta$  values (ppm) with CDCl<sub>3</sub> (<sup>1</sup>H NMR  $\delta$  7.26, <sup>13</sup>C NMR  $\delta$  77.16) as internal standard. *J* values are given in Hertz (Hz). Thin layer chromatography (TLC) was performed on pre-coated Cromatofolios AL Silica gel 60 F<sub>254</sub> (Merck, Darmstadt, Germany), visualized with UV-detection. Flash column chromatography was performed on silica gel 60, 0.040-0.063 mm (SDS). Analytical high performance liquid chromatography (HPLC) with chiral stationary phases was performed using an HP-Agilent 1110 series controller, equipped with a Daicel chiralpak OJ (4.6x250 mm, 20  $\mu$ m) column. Solvents for HPLC use were of spectrometric grade.

**Immobilization of subtilisin Carlsberg:** To a solution of octyl  $\beta$ -D-glucopyranoside (25 mg) and Brij 56 (25 mg) in phosphate buffer (0.5 M, pH 7.2, 10 mL) was added subtilisin Carlsberg (100 mg). The mixture was rapidly frozen with liquid nitrogen and lyophilized overnight.

#### **((2R)-5-acetoxy-1,3-oxathiolan-2-yl)methyl benzoate (9a)<sup>2</sup>**

Compound **8** (85.4 mg, 0.52 mmol), 1,4-dithiane-2,5-diol **6** (47.5 mg, 0.31 mmol), triethylamine (72.5  $\mu$ L, 0.52 mmol), and phenyl acetate (196  $\mu$ L, 1.56 mmol) in dry THF (4 mL) were added to a sealed-cap vial containing surfactant-treated subtilisin Carlsberg (80 mg) together with 4Å molecular sieves (120 mg). The reaction mixture was cooled to 4 °C. After 2 days, the mixture was filtered and washed with saturated NH<sub>4</sub>Cl and brine, after which the mixture was dried over MgSO<sub>4</sub> and the solvent removed under vacuum. The crude product was purified by column chromatography (hexane:EtOAc = 8:1) affording compound **9a** as a clear oil (130.5 mg, 0.46 mmol, 89%), 82% *ee*, determined by HPLC analysis (Chiral OJ,  $\lambda$ = 254 nm, Hex:<sup>i</sup>PrOH = 9:1, 0.5 mL/min; Colorless oil; <sup>1</sup>H NMR (500 Hz, CDCl<sub>3</sub>)  $\delta$  = 8.05 (2H, d, *J* = 8.0 Hz), 7.58 (1H, t, *J* = 7.13 Hz), 7.45 (2H, t, *J* = 7.57 Hz), 6.72 (1H, d, *J* = 4.12 Hz), 5.68 (1H, dd, *J* = 4.96, 1.53 Hz), 4.53 (2H, m), 3.20 (2H, m), 2.12 (3H, s); <sup>13</sup>C NMR (125 Hz, CDCl<sub>3</sub>)  $\delta$  = 169.2, 166.0, 133.29, 129.8, 128.4, 99.2, 83.3, 66.1, 37.6, 21.1.

#### **((2S)-5-acetoxy-1,3-oxathiolan-2-yl)methyl benzoate (9b)<sup>2</sup>**

Following the procedure for compound **9a**, the reaction of compound **8** (8.5 mg, 0.05 mmol) and **6** with TEA (7  $\mu$ L, 0.05 mmol), phenyl acetate (19  $\mu$ L, 0.15 mmol), and CAL B (20 mg) in toluene (0.5 mL) afforded compound **9b** (11.7 mg, 83%), 84% *ee*, determined by HPLC analysis (Chiral OJ,  $\lambda$ = 254 nm, Hex:<sup>i</sup>PrOH = 9:1, 0.5 mL/min. The NMR data were the same as compound **9a**, which was the enantiomer of compound **9b**.

#### **((2R,5S)-5-(4-acetamido-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolan-2-yl)methyl benzoate (10)<sup>2</sup>**

Silylated *N*<sup>4</sup>-acetylcytosine was generated *in situ* by stirring *N*<sup>4</sup>-acetylcytosine (55.4 mg, 0.362 mmol) with hexamethyldisilazane (HMDS, 1.0 mL, 4.8 mmol) at reflux (135 °C) under argon until all solid had dissolved (approximately 3 h). The solution was slowly cooled to rt, and HMDS was removed under anhydrous conditions. The system was evacuated five times with argon, and dried under HV for 30 minutes. Meanwhile, compound **9** (63.2 mg, 0.224 mmol) was dissolved in anhydrous acetonitrile (6 mL) and stirred with pre-activated 3Å molecular sieves for 30 minutes under N<sub>2</sub>. The solution was transferred via syringe to the dried silylated *N*<sup>4</sup>-acetylcytosine, and the resulting solution was cooled to 0 °C. TMSI (70.0  $\mu$ L, 0.504 mmol) was added dropwise over 15 minutes and the resulting yellow solution was stirred under argon at 0 °C for 1 h. The reaction was quenched by addition of a mixture of ethyl acetate (10 mL) and aqueous NaHCO<sub>3</sub> solution (5 wt%, 6 mL) and the resulting two-phase system was stirred at rt for 15 minutes. The mixture was further diluted with ethyl acetate (10 mL) and the organic phase was washed with saturated aqueous NaHCO<sub>3</sub> solution (15 mL), distilled H<sub>2</sub>O (2 x 15 mL) and brine (2 x 15 mL). Drying over MgSO<sub>4</sub>, filtration and concentration *in vacuo* yielded the crude product mixture as a yellow solid. Purification by column chromatography (EtOAc : methanol = 5 : 1) allowed for separation of the diastereoisomers to yield the *cis* (34.4 mg, 0.113 mmol, 51%) isomers (**10**) as white solids. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  = 8.23 (1H, d, *J* = 7.54 Hz), 8.07 (1H, d, *J* = 8.32 Hz), 7.64 (1H, t, *J* = 9.26 Hz), 7.52 (1H, t, *J* = 7.79 Hz), 7.32 (1H, d, *J* = 8.13 Hz), 6.36 (1H, dd, *J* = 5.31, 2.76 Hz), 5.53 (1H, dd, *J* = 4.21, 2.98 Hz), 4.82 (2H, m), 3.67 (1H, dd, *J* = 12.71, 5.39 Hz), 3.26 (1H, dd, *J* = 12.69, 2.69 Hz), 2.25 (3H, s); <sup>13</sup>C NMR  $\delta$  = 170.3, 165.9, 162.6, 154.7, 144.7, 133.8, 129.7, 129.1, 128.7, 96.2, 97.8, 85.5, 63.5, 39.3, 29.7, 25.0.

#### **4-amino-1-((2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl)pyrimidin-2(1H)-one (Lamivudine 1a)**

To a solution of compound **10** (19 mg, 0.05 mmol) in methanol (0.5 mL), K<sub>2</sub>CO<sub>3</sub> (6.9 mg, 0.05 mmol) was added and the mixture stirred at rt under N<sub>2</sub> for 14 h. The mixture was diluted with methanol, filtered and concentrated. Purification by column chromatography (EtOAc:methanol = 4:1), yielded compound **1a** (10.2 mg, 0.045 mmol, 89%). <sup>1</sup>H NMR (500 Hz, D<sub>2</sub>O)  $\delta$  = 7.96 (1H, dd, *J* = 7.58 Hz), 6.29 (1H, dd, *J* = 5.54, 4.33 Hz), 5.99 (1H, d, *J* = 7.57 Hz), 5.32 (1H, dd, *J* = 4.44, 3.40 Hz), 3.91 (2H, m), 3.53 (1H, dd, *J* = 12.26, 5.65 Hz), 3.18 (1H, dd, *J* = 12.26, 4.23 Hz). <sup>13</sup>C NMR (125 Hz, D<sub>2</sub>O)  $\delta$  = 164.8, 155.8, 140.3, 94.5, 85.8, 84.5, 60.7, 35.3.

### **((2S)-5-acetoxy-1,3-oxathiolan-2-yl)methyl acetate (7a)<sup>1</sup>**

Following the procedure for compound **9a**, the reaction of compound **5** (60.1 mg, 0.50 mmol) and **6** (45.7 mg, 0.30 mmol) with CAL B (20 mg), TEA (70  $\mu$ L, 0.50 mmol), phenyl acetate (188  $\mu$ L, 1.5 mmol) in toluene (4 mL) afforded compound **7a** (101.3 mg, 92%), 83% *ee*, determined by HPLC analysis (Chiral OJ,  $\lambda$ = 210 nm, Hex:<sup>i</sup>PrOH = 9:1, 0.5 mL/min. <sup>1</sup>H NMR (500 Hz, CDCl<sub>3</sub>)  $\delta$  = 6.67 (1H, d, *J* = 4.15 Hz), 5.53 (1H, dd, *J* = 6.38, 3.97 Hz), 4.20-4.32 (2H, m), 3.10-3.34 (2H, m), 2.09 (3H, s). <sup>13</sup>C NMR (125 Hz, CDCl<sub>3</sub>)  $\delta$  = 168.1, 167.3, 96.8, 80.7, 63.4, 35.1, 18.8, 18.4.

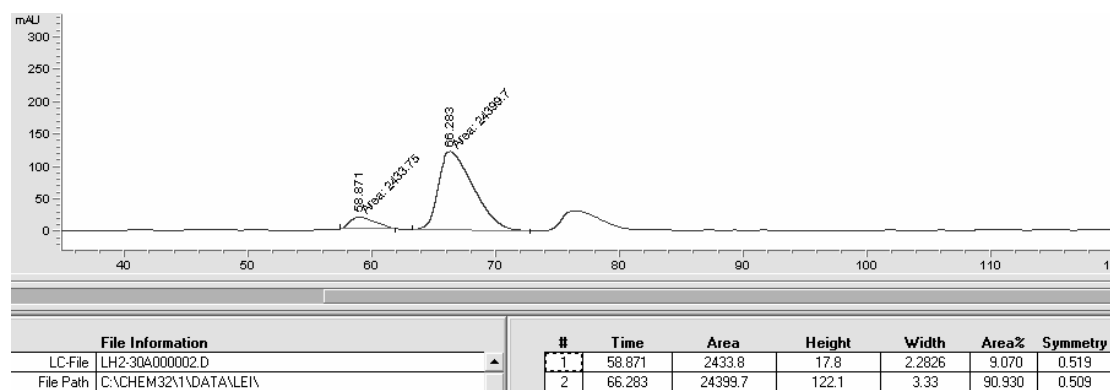
### **((2R)-5-acetoxy-1,3-oxathiolan-2-yl)methyl acetate (7b)<sup>1</sup>**

Following the procedure for compound **9a**, the reaction of compound **5** (6 mg, 0.05 mmol) and **6** (4.6 mg, 0.03 mmol) with STS (10 mg), TEA (7  $\mu$ L, 0.05 mmol), phenyl acetate (19  $\mu$ L, 0.15 mmol) in toluene (0.5 mL) afforded compound **7b** (9.6 mg, 87%), 64% *ee*, determined by HPLC analysis (Chiral OJ,  $\lambda$ = 210 nm, Hex:<sup>i</sup>PrOH = 9:1, 0.5 mL/min. The NMR data were the same as compound **7a**, which was the enantiomer of compound **7b**.

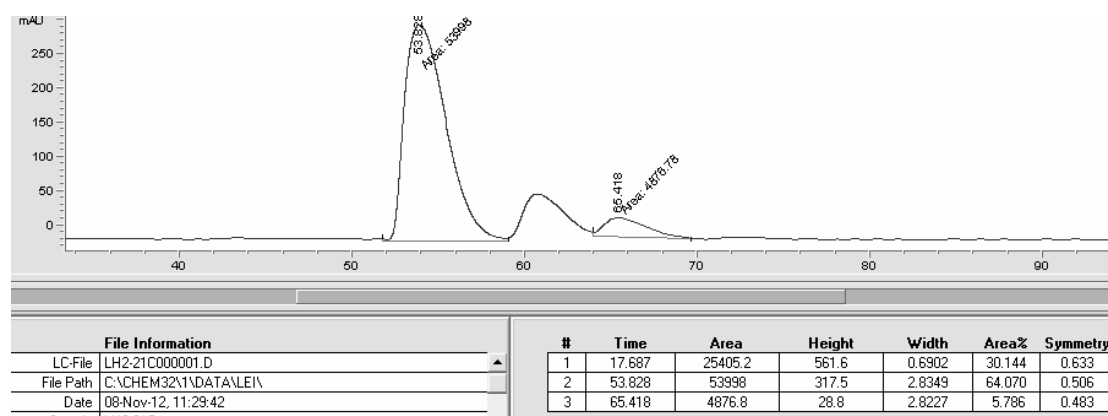
### **4-amino-1-((2S,5R)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl)pyrimidin-2(1H)-one (1b)**

Silylated *N*<sup>4</sup>-acetylcytosine was generated *in situ* by stirring *N*<sup>4</sup>-acetylcytosine (64.3 mg, 0.42 mmol) with hexamethyldisilazane (HMDS, 868  $\mu$ L, 4.1 mmol) at reflux (135 °C) under argon until all solid had dissolved (approximately 3 h). The solution was slowly cooled to rt, and HMDS was removed under anhydrous conditions. The system was evacuated five times with argon, and dried under HV for 30 minutes. Meanwhile, compound **7a** (62 mg, 0.28 mmol) was dissolved in anhydrous acetonitrile (6 mL) and stirred with pre-activated 3Å molecular sieves for 30 minutes under N<sub>2</sub>. The solution was transferred via syringe to the dried silylated *N*<sup>4</sup>-acetylcytosine, and the resulting solution was cooled to 0 °C. TMSI (70.0  $\mu$ L, 0.504 mmol) was added dropwise over 15 minutes and the resulting yellow solution was stirred under argon at 0 °C for 1 h. The reaction was quenched by addition of a mixture of ethyl acetate (10 mL) and aqueous NaHCO<sub>3</sub> solution (5 wt%, 6 mL) and the resulting two-phase system was stirred at rt for 15 minutes. The mixture was further diluted with ethyl acetate (10 mL) and the organic phase was washed with saturated aqueous NaHCO<sub>3</sub> solution (15 mL), distilled H<sub>2</sub>O (2 x 15 mL) and brine (2 x 15 mL). Drying over MgSO<sub>4</sub>, filtration and concentration *in vacuo* yielded the crude product mixture as a yellow solid (65 mg). The solid was dissolved in methanol (2 mL), and K<sub>2</sub>CO<sub>3</sub> (24 mg, 0.17 mmol) was added. The mixture was stirred at rt overnight. The mixture was diluted with methanol, filtered and concentrated. Purification by column chromatography (EtOAc:methanol = 4:1), yielded compound **1a** (26 mg, 40% for two steps). <sup>1</sup>H NMR (500 Hz, DMSO)  $\delta$  = 7.82 (1H, d, *J* = 7.48 Hz), 7.21 (1H, d, *J* = 33.29 Hz), 6.21 (1H, t, *J* = 5.65 Hz), 5.73 (1H, d, *J* = 6.93 Hz), 5.32 (1H, t, *J* = 5.72 Hz), 5.17 (1H, t, *J* = 4.95 Hz), 3.69-3.75 (1H, m), 3.41 (1H, dd, *J* = 12.29, 4.02 Hz), 3.04 (1H, dd, *J* = 11.58, 4.73 Hz). <sup>13</sup>C NMR (125 Hz, DMSO)  $\delta$  = 165.6, 154.6, 140.9, 93.9, 86.5, 85.7, 62.8, 36.2.

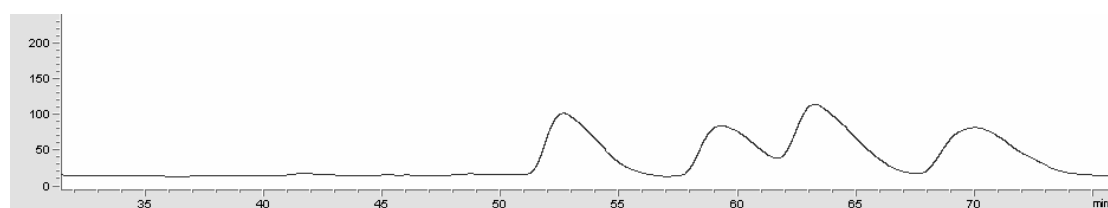
### **Chiral HPLC of compound 9a**



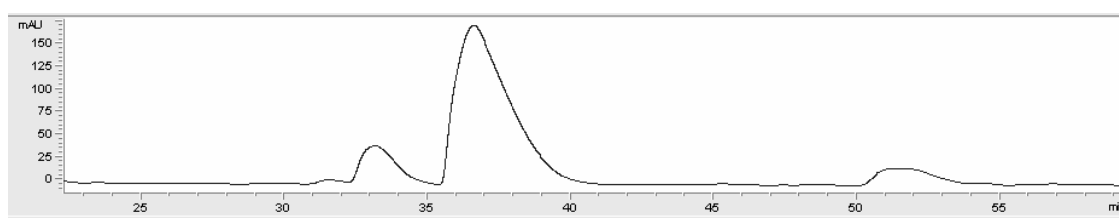
### Chiral HPLC of compound 9b



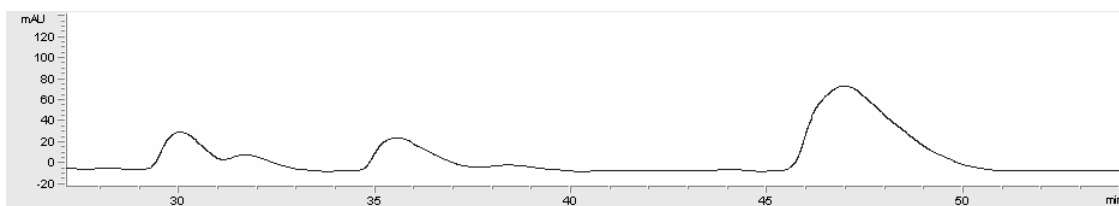
### Chiral HPLC of racemic compound 9



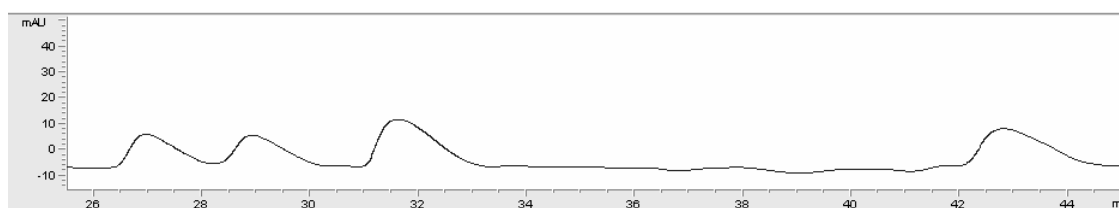
### Chiral HPLC of compound 7a



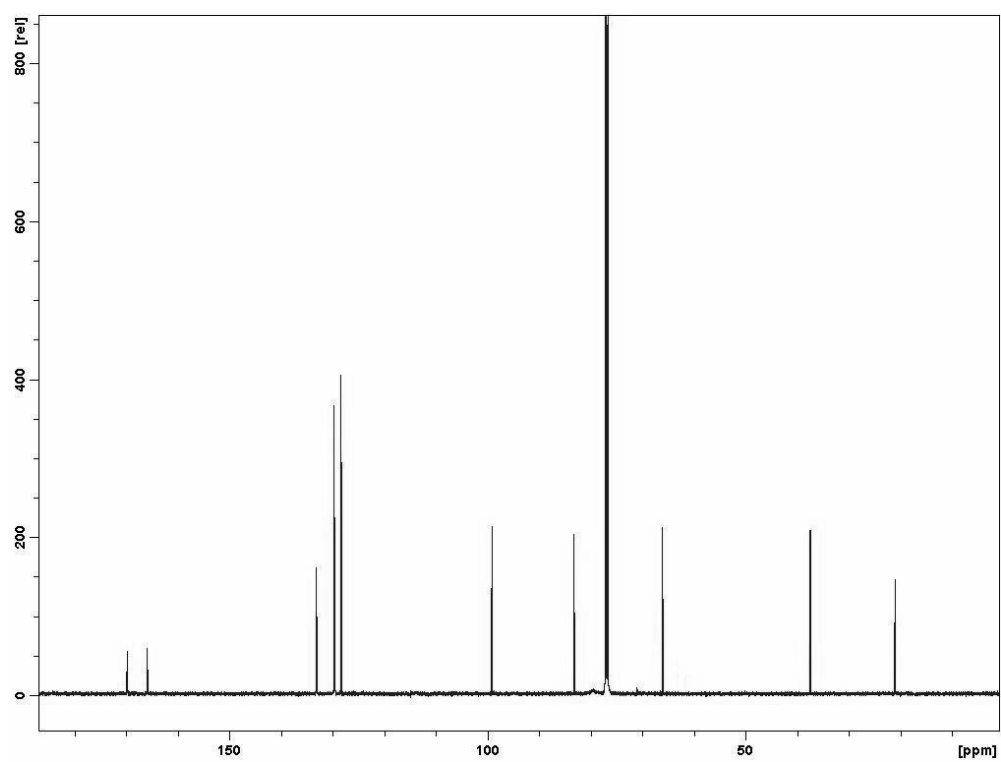
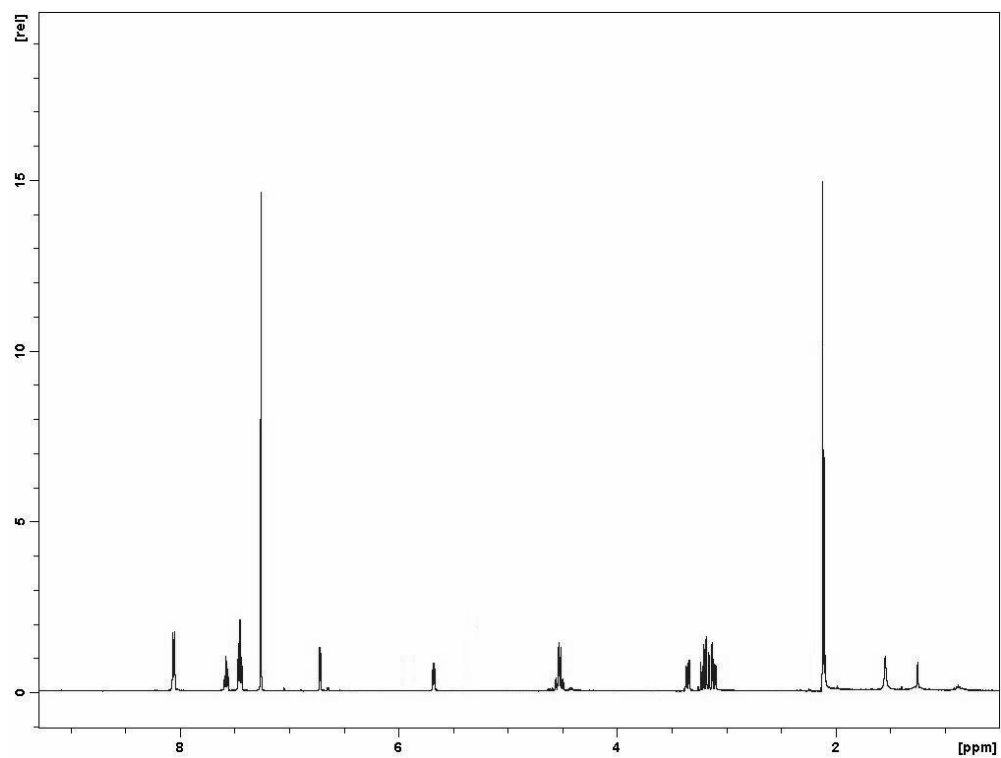
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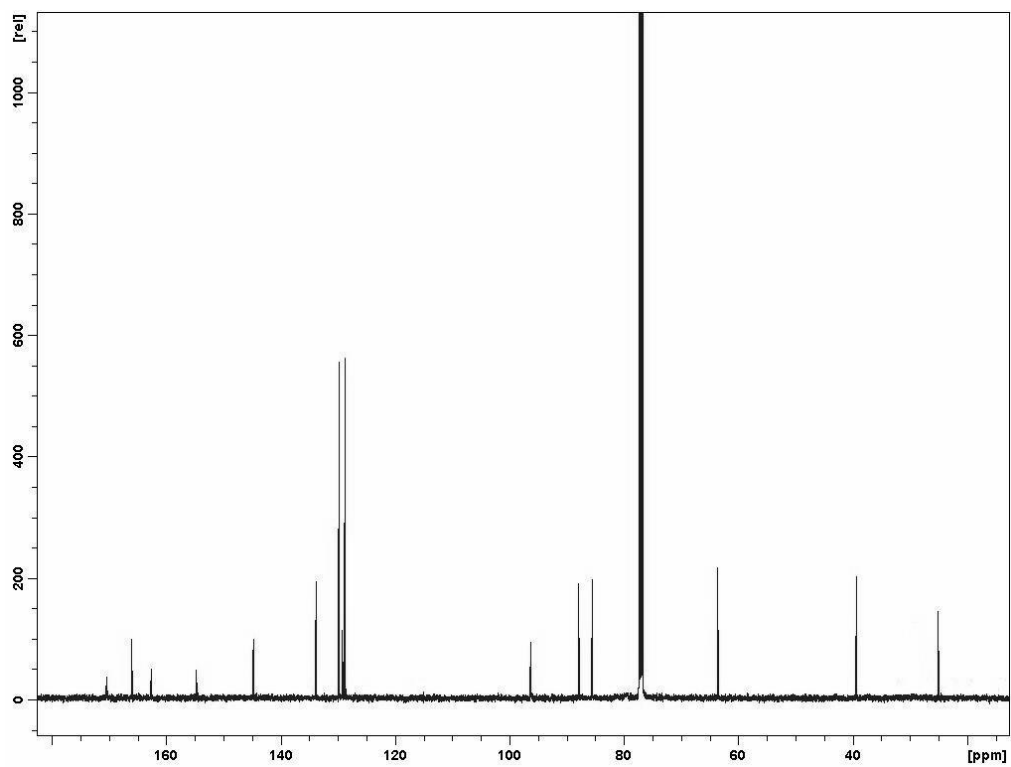
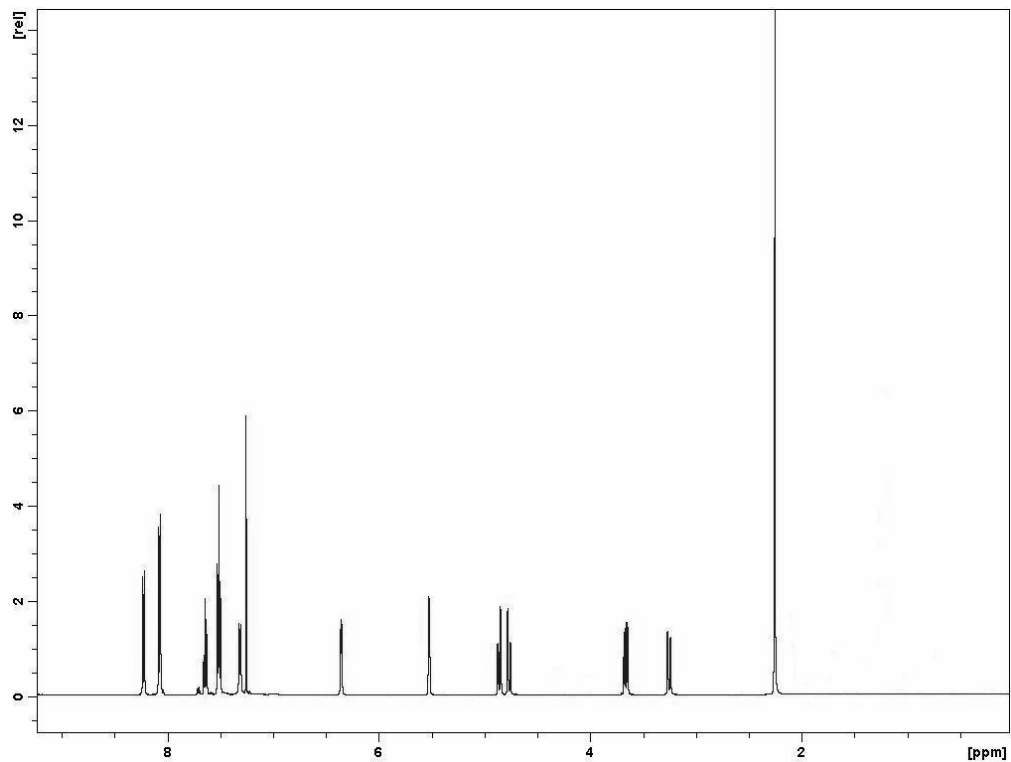
### Chiral HPLC of racemic compound 7



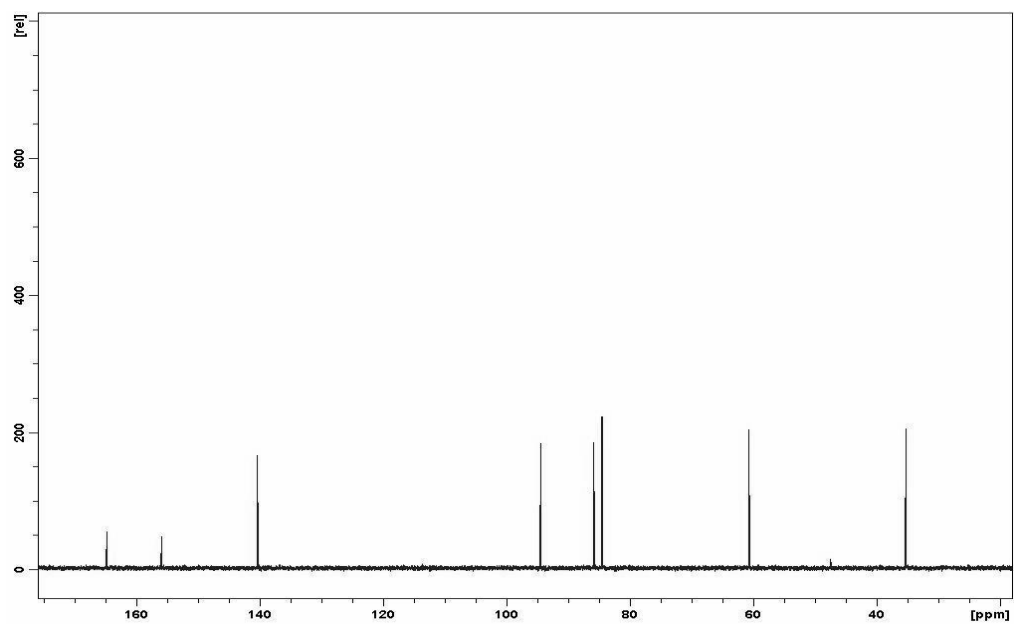
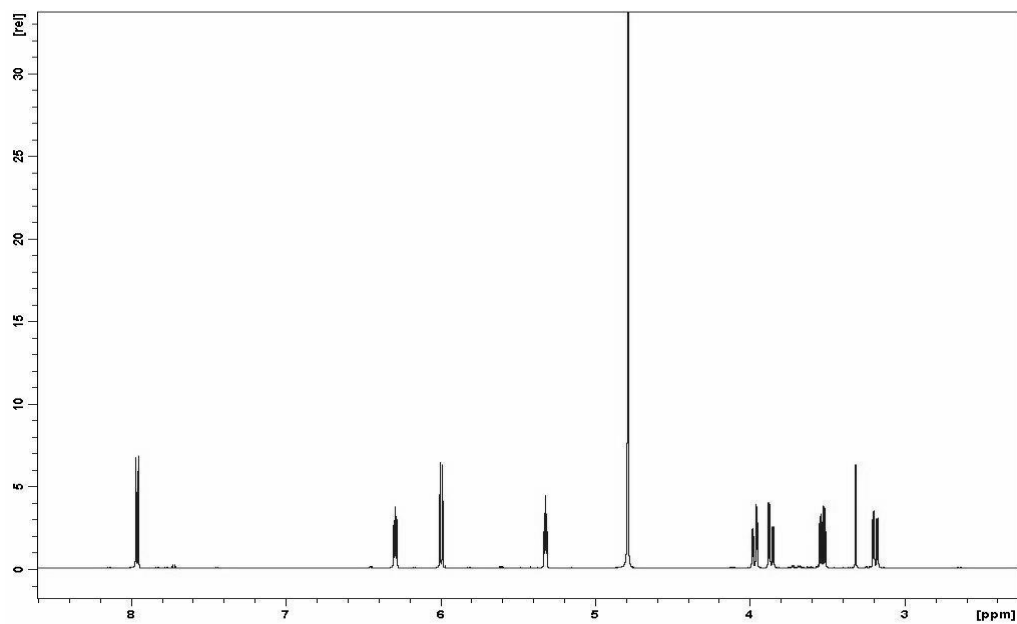
**$^1\text{H}$  NMR and  $^{13}\text{C}$  NMR of 9**



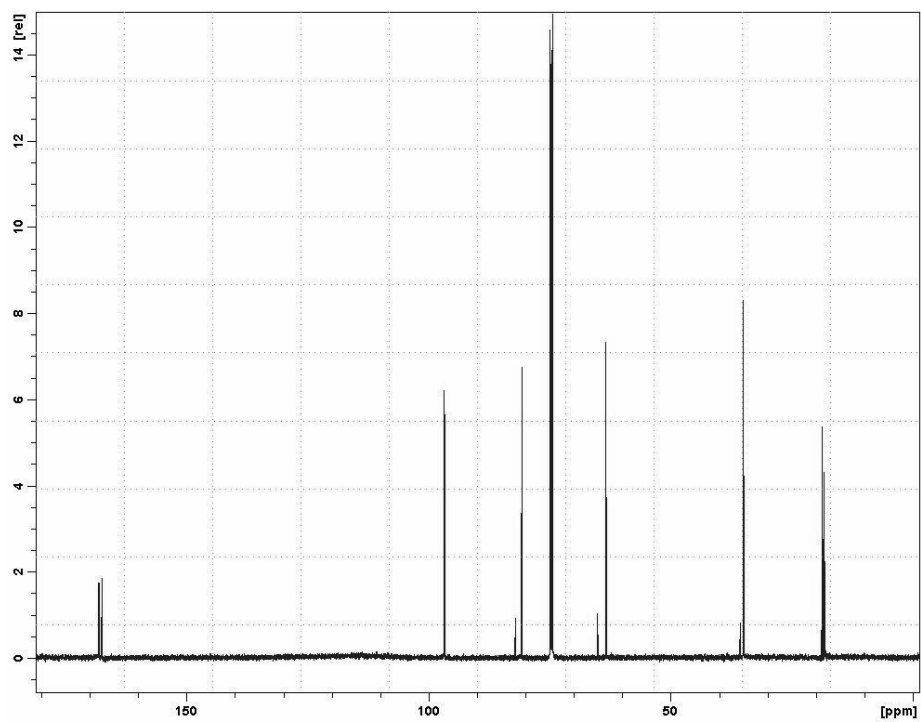
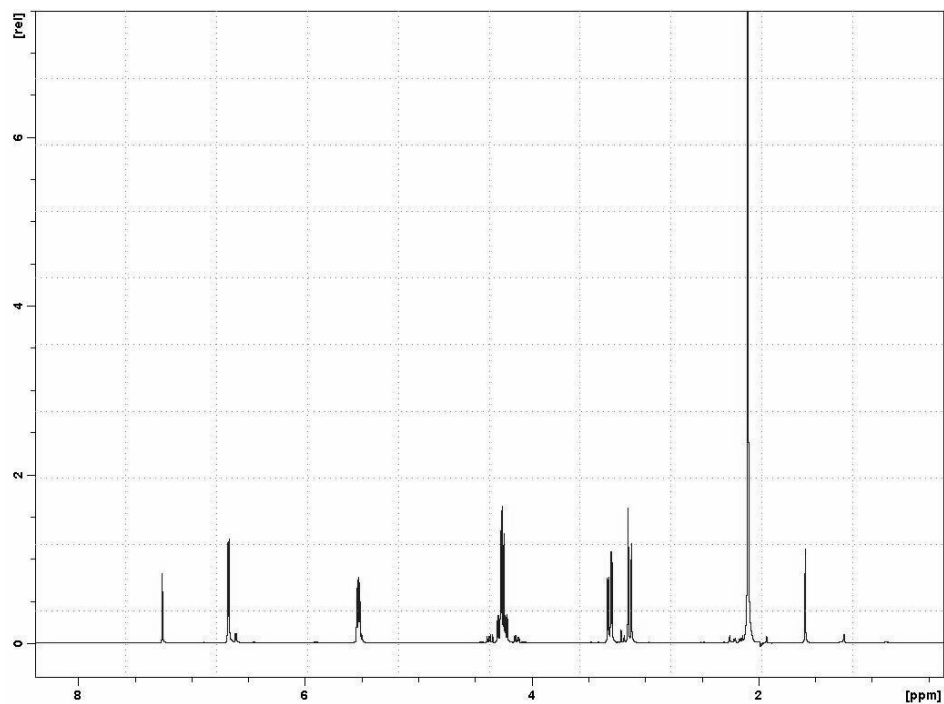
**$^1\text{H}$  NMR and  $^{13}\text{C}$  NMR of 10**



### $^1\text{H}$ NMR and $^{13}\text{C}$ NMR of 1a

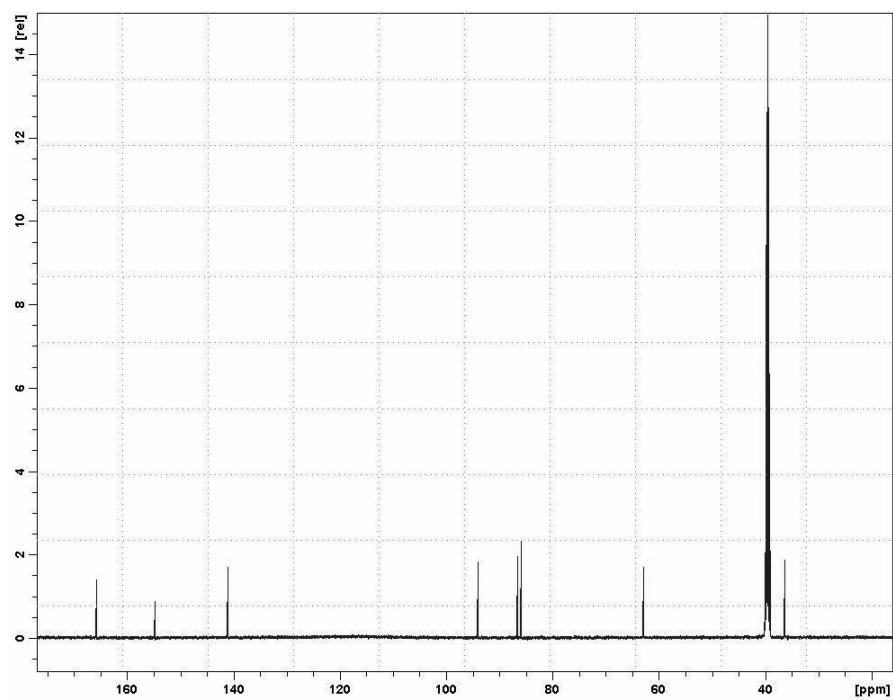
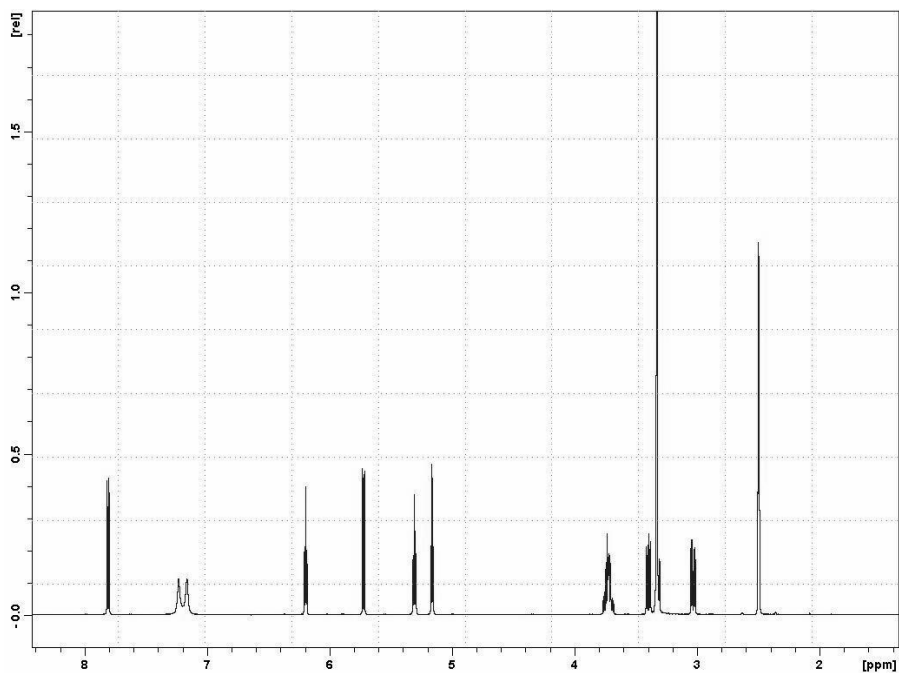


**$^1\text{H}$  NMR and  $^{13}\text{C}$  NMR of 7**





### $^1\text{H}$ NMR and $^{13}\text{C}$ NMR of 1b



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1. Hoong, L. K.; Luise E. Strange, L. E.; Liotta, D. C.; Koszalka, G. W.; Burns, C. L.; Schinazi, R. F. *J. Org. Chem.* 1992. **57**. 5563.
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