Electronic Supplementary Information for

Novel Chiral Recognition Beyond the Limitation Due to the Law of Mass Action: Highly Enantioselective Chiral Sensing Based on Non-Linear Response in Phase Transition Events

Keiji Hirose, Yuka Yachi, and Yoshito Tobe

Division of Frontier Materials Science, Graduate School of Engineering Science, Osaka University, 1-3 Machikaneyama, Toyonaka, Osaka 560-8531, Japan

Contents

1. General
2. Synthetic Procedures of (S,S)-1
   2-1. Synthesis of (S,S)-4 from (S,S)-2
   2-2. Synthesis of (S,S)-1
   2-3. ¹H-NMR Spectrum of (S,S)-1
   2-4. H-H COSY Spectra of (S,S)-1
3. Synthetic Procedures of Reference Copolymer (S,S)-8
   3-1. Synthesis of (S,S)-8 from (S,S)-7
   3-2. ¹H-NMR Spectrum of (S,S)-8
4. Determination of Binding Constants of (S,S)-1
   4-1. with (R)-NEA·HCl
   4-2. with (S)-NEA·HCl
5. Phase Separation Experiments
   5-1. Temperature Dependence of the Transmittance at 500 nm of (S,S)-1 with NEA·HCl
   5-2. Temperature Dependence of the Transmittance at 500 nm of (S,S)-1 with PGO·HCl
   5-3. Temperature Dependence of the Transmittance at 500 nm of (S,S)-1 with PEA·HCl
6. References
1. General

$^1$H NMR spectra were recorded with a JEOL GSX-270, a Varian Mercury 300, or a JEOL AL-400 spectrometer for solutions in CDCl$_3$ with SiMe$_4$ as an internal standard and $J$ values are given in Hz. $^{13}$C NMR spectra were recorded at 75.5 MHz with a JEOL GSX-270 spectrometer and chloroform ($\delta$ C 77.0) was used as a chemical shift reference. IR spectra were measured on a JASCO FT/IR-410 spectrophotometer. Elemental analyses were performed on a Perkin–Elmer 2400II analyzer. Melting points were measured with a hot-stage apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-40 polarimeter and [$\alpha$] values are given in units of $10^{-1}$ deg cm$^2$ g$^{-1}$. MS spectra were recorded with a JEOL JMS-700 spectrometer. Column chromatography and TLC were performed with Merck silica gel 60 (70–230 mesh ASTM) and Merck silica gel 60 F$\text{254}$, respectively. Preparative GPC separation was undertaken with a JAI LC-908 chromatograph using 600 mm×20 mm JAIGEL-1H and 2H GPC columns with CHCl$_3$ as an eluent. All reagents were obtained from commercial suppliers and used as received.

![Scheme S1. Synthesis of Chiral Sensor (S,S)-1](image-url)
2. Synthetic Procedures of (S,S)-I

2-1. Synthesis of (S,S)-4 from (S,S)-2

A mixture of (S,S)-2 (1.66 g, 3.18 mmol)\(^1\) and SOCl\(_2\) (0.450 mL, 6.30 mmol) was stirred for 2 h under reflux. After the removal of SOCl\(_2\) under reduced pressure, the obtained yellow residue was diluted with acetone (40 mL). NaN\(_3\) (760 mg, 11.7 mmol) was added into the solution. The mixture was stirred for 1 h at room temperature. The reaction mixture was filtered and the filtrate was concentrated. The residue was dissolved in toluene (130 mL) and a 50 % aqueous NaOH solution (70 mL) was added. The resulted mixture was refluxed overnight. After separation of the organic phase, the aqueous phase was extracted with CH\(_2\)Cl\(_2\). Chromatography on silica gel (hexane:ethyl acetate) followed by recrystallization from reaction mixture was extracted with CH\(_2\)Cl\(_2\) and concentrated. The residue was dissolved in toluene (130 mL) and an aqueous NaOH solution (70 mL) was added. The mixture was refluxed for 2 h under reduced pressure. After being dried over anhydrous MgSO\(_4\), the removal of the solvent gave amine (S,S)-3 (1.48 g) as a colorless solid which was unstable in air: mp 154.0–155.0 °C. Therefore (S,S)-3 was employed for the next amidation reaction without further purification: \(^1\)H NMR (300 MHz, CDCl\(_3\), 30 °C) \(\delta\) 7.34-7.26 (m, 10H), 6.62 (s, 2H), 4.66 and 4.41 (AB, \(J\) = 11 Hz, 4H), 4.53 (dd, \(J\) = 2.1, 8.4 Hz, 2H), 4.15 (s, 3H), 3.72-3.43 (m, 12H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\), 30 °C) \(\delta\) 150.4, 142.8, 139.0, 131.1, 128.4, 127.8, 126.8, 118.4, 81.7, 74.5, 70.1, 69.2, 68.6, 65.0; IR (neat) 3419, 3350, 3061, 3028, 2868, 2359, 1668, 1551, 1484, 1452, 1437, 1360, 1343, 1347, 1220, 1091, 1000, 959, 882, 800, 763, 704, 635, 522 cm\(^{-1}\); MS (FAB) \(m/z\) 516.2 (M+Na\(^+\)), HRMS (FAB) \(m/z\) calculated for C\(_{29}\)H\(_{32}\)O\(_2\)Na (M+Na\(^+\)) 516.2362, found 516.2365; [\(\alpha\)]\(^{23}\)_D\(^{\circ}\) = +121 (c 1.03, CHCl\(_3\)).

To a solution of (S,S)-3 (1.48 g) in CH\(_2\)Cl\(_2\) (40 mL) was added slowly acryloyl chloride (250 mL, 3.00 mmol) with ice-cooling. Then, the mixture was stirred for 1.5 h at ambient temperature. The reaction mixture was extracted with CH\(_2\)Cl\(_2\). The combined extracts were washed with aqueous NaOH solution and dried over anhydrous MgSO\(_4\). Chromatography on silica gel (hexane:ethyl acetate) followed by recrystallization from chloroform:hexane gave (S,S)-4 (1.09 g, 63% yield from (S,S)-2) as a colorless solid: mp 185.0–186.0 °C; \(^1\)H NMR (300 MHz, CDCl\(_3\), 30 °C) \(\delta\) 7.54 (s, 2H), 7.33-7.26 (m, 10H), 6.39 (AB, \(J\) = 16.8 Hz, 1H), 6.22 (ABX, \(J\) = 16.8, 9.9 Hz, 1H), 5.72 (AX, \(J\) = 9.9 Hz, 1H), 4.71 and 4.45 (AB, \(J\) = 10.5 Hz, 4H), 4.53 (dd, \(J\) = 2.1, 8.7 Hz, 2H), 4.25 (s, 3H), 3.71-3.41 (m, 12H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\), 30 °C) \(\delta\) 165.6, 155.9, 138.9, 133.0, 132.2, 131.2, 128.4, 127.9, 127.4, 126.9, 123.5, 81.7, 74.4, 70.7, 69.0, 68.3, 65.1; IR (KBr) 3502, 3028, 2868, 2359, 1668, 1608, 1551, 1484, 1452, 1437, 1360, 1343, 1247, 1220, 1091, 1000, 959, 882, 800, 763, 704, 635, 522 cm\(^{-1}\); MS (FAB) \(m/z\) 570.2 (M+Na\(^+\)), HRMS (FAB) \(m/z\) calculated for C\(_{32}\)H\(_{35}\)O\(_2\)Na (M+Na\(^+\)) 570.2468, found 570.2488; [\(\alpha\)]\(^{23}\)_D\(^{\circ}\) = +108 (c 1.08, CHCl\(_3\)); Anal. Calcd for C\(_{32}\)H\(_{35}\)O\(_2\)Na: C, 70.18; H, 6.81; N, 2.56. found: C, 70.33; H, 6.56; N, 2.67.
2-2. Synthesis of (S,S)-1

A solution of azobisisobutyronitrile (AIBN) (14.6 mg, 0.0889 mmol) in DMF (10 mL) as degassed by bubbling nitrogen for 10 min and a similarly degassed solution of (S,S)-4 (243 mg, 0.443 mmol), N-isopropylacrylamide (NIPAM, 5) (951 mg, 8.40 mmol), and 2-dodecylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid (RAFT CTA, 6) (42.0 mg, 0.116 mmol) in DMF (2.1 mL) were mixed at 60 ºC under nitrogen, and the mixture was heated at 70 ºC with stirring for 24 h. The copolymer dissolved in DMF was chromatographed by preparative GPC. Removal of the solvent of a high molecular weight fraction (retention time: 31-47 min at a flow rate of 3.8 mL/min) gave yellow solid. The solid was dissolved in acetone, and then purified by reprecipitation by adding to cold diethyl ether. (S,S)-1 (875 mg) was collected by filtration as a colorless powder: $^1$H NMR (300 MHz, CDCl$_3$, 30 ºC) $\delta$ 7.31 (m, broad), 6.21 (s, broad), 4.67 (s), 4.53 (d, $J$= 10.8 Hz), 4.43 (s), 4.00 (s), 3.78 (s), 3.72-3.42 (m), 2.27-1.13 (m); $M_n$ = 15000, $M_w/M_n$ = 1.14, SCE with DMF containing 0.01 M LiBr at 40 ºC, flow rate 0.06 mL/min.$^3$

2-3. $^1$H-NMR Spectrum of (S,S)-1

![Figure S1](image)

**Figure S1.** $^1$H NMR spectrum of (S,S)-1 (400 MHz, CDCl$_3$, 30 ºC).
Figure S2. Partial $^1$H NMR spectra (400 MHz) of monomer (S,S)-4 (a), 5 (b), and copolymer (S,S)-1 (c) in CDCl$_3$ at 30 °C.

The $^1$H NMR of (S,S)-1 and monomers (S,S)-4 and 5 are shown in Figure S2 with signal assignments. The signals of the polymer are significantly broadened. The assignments of the signals were carried out using information obtained from H-H COSY spectra shown in Figure S3. The content of the crown ether monomer unit in the polymer was estimated to be 4 mol % based on the integration of the signals for H$_8$ and H$_i$. 

S5
Figure S3. Partial H-H COSY spectra of (S,S)-1 (270 MHz, CDCl₃, 30 °C).
3. Synthetic Procedures of Reference Copolymer (S,S)-8

3-1. Synthesis of (S,S)-8 from (S,S)-7

![Scheme S2. Synthesis of Reference Copolymer (S,S)-8](image_url)

A solution of AIBN (14.6 mg, 0.0889 mmol) in DMF (10 mL) was degassed by bubbling nitrogen for 10 min and a similarly solution of N-phenylacrylamide (7) (65.0 mg, 0.442 mmol), NIPAM 5 (950 mg, 8.40 mmol), and RAFT CTA 6 (42.0 mg, 0.114 mmol) in DMF (2.1 mL) were mixed at 60 ºC under nitrogen, and the mixture was heated at 70 ºC with stirring for 36 h. The copolymer dissolved in DMF was purified by reprecipitation by addition to cold diethyl ether. (S,S)-6 (618 mg) was collected by filtration as a colorless solid: 

\[ \text{H NMR (300 MHz, CDCl}_3, 30 ^\circ\text{C)} \delta 7.61 (m), 7.06 (m), 6.41 (s, broad), 3.99 (s), 2.23-1.13 (m); M_n = 21000, M_w/M_n = 1.06, SCE with DMF containing 0.01 M LiBr at 40 ºC, flow rate 0.06 mL/min. \]

3-2. \( ^1\text{H-NMR Spectrum of (S,S)-8} \)

![Figure S4. \( ^1\text{H NMR spectrum of (S,S)-8 (400 MHz, CDCl}_3, 30 ^\circ\text{C)} \).](image_url)
4. Determination of Binding Constants of (S,S)-1

4-1. with (R)-NEA·HCl

A solution of host (S,S)-1 (10.0 mg, 3.07 mM of crown ether monomer unit) and a solution of guest (R)-NEA·HCl (145.4 mM) each in D$_2$O were prepared. An initial $^1$H NMR spectrum of (S,S)-1 was recorded at 5 ºC. Samples were made by adding each 35, 35, 60, 50, 50, 55, 55, 50, 50, 50 µL of the guest solutions to 660 µL solution of (S,S)-1. Then, spectra of these samples were recorded at the same temperature. The binding constant of (S,S)-1 with (R)-NEA·HCl was calculated by a non-linear least-squares method to be 6.4 ± 2.7 M$^{-1}$. The relevant data and the $^1$H NMR titration curve, respectively are shown in Table S1 and Figure S5, respectively.
Table S1. Tabulated $^1$H NMR Titration Tata of (S,S)-I with (R)-NEA·HCl in D$_2$O at 278 K, Calculated Binding Constant, and Calculated Chemical Shifts of the Complex.

<table>
<thead>
<tr>
<th>$[H_i]$ (mM) $^a$</th>
<th>$[G_i]$ (mM) $^b$</th>
<th>$[G_i] / [H_i]$ $^c$</th>
<th>$\delta$ (ppm) $^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0.0031</td>
<td>0.0000</td>
<td>0.0</td>
<td>7.010</td>
</tr>
<tr>
<td>2 0.0029</td>
<td>0.0073</td>
<td>2.5</td>
<td>6.992</td>
</tr>
<tr>
<td>3 0.0028</td>
<td>0.0139</td>
<td>5.0</td>
<td>6.987</td>
</tr>
<tr>
<td>4 0.0026</td>
<td>0.0239</td>
<td>9.3</td>
<td>6.975</td>
</tr>
<tr>
<td>5 0.0024</td>
<td>0.0312</td>
<td>12.9</td>
<td>6.959</td>
</tr>
<tr>
<td>6 0.0023</td>
<td>0.0376</td>
<td>16.5</td>
<td>6.952</td>
</tr>
<tr>
<td>7 0.0021</td>
<td>0.0439</td>
<td>20.5</td>
<td>6.950</td>
</tr>
<tr>
<td>8 0.0020</td>
<td>0.0494</td>
<td>24.4</td>
<td>6.939</td>
</tr>
<tr>
<td>9 0.0019</td>
<td>0.0540</td>
<td>28.0</td>
<td>6.940</td>
</tr>
<tr>
<td>10 0.0018</td>
<td>0.0582</td>
<td>31.6</td>
<td>6.933</td>
</tr>
<tr>
<td>11 0.0018</td>
<td>0.0620</td>
<td>35.2</td>
<td>6.929</td>
</tr>
<tr>
<td>12 0.0017</td>
<td>0.0654</td>
<td>38.8</td>
<td>6.924</td>
</tr>
</tbody>
</table>

$^a$ Total concentration of crown ether unit of (S,S)-I
$^b$ Total concentration of (R)-NEA·HCl.
$^c$ The ratio of crown ether unit of (S,S)-I over (R) -NEA·HCl.
$^d$ Observed chemical shifts of one of the phenyl protons of (S,S)-I.

\[ \delta_{\text{comp}} = 6.727 \]
\[ K = 6.4 \pm 2.7 \]

Figure S5. $^1$H NMR Titration Curve for the Complexation of (S,S)-I with (R) -NEA·HCl at 278 K.
4-1. with (S)-NEA·HCl

A solution of host (S,S)-1 (10.0 mg, 3.07 mM of crown ether monomer unit) and a solution of guest (S)-NEA·HCl (145.1 mM) each in D$_2$O were prepared. An initial $^1$H NMR spectrum of (S,S)-1 was recorded at 5 ºC. Samples were made by adding each 35, 35, 60, 50, 50, 55, 55, 50, 50, and 50 $\mu$L of the guest solutions to 660 $\mu$L solution of (S,S)-1. Then, spectra of these samples were recorded at the same temperature. The binding constant of (S,S)-1 with (S)-NEA·HCl was calculated by a non-linear least-squares method to be <1 M$^{-1}$. The relevant data and the $^1$H NMR titration curve, respectively are shown in Table S2 and Figure S6, respectively.
Table S2. Tabulated $^1$H NMR Titration Data of (S,S)-I with (S)-NEA-HCl in D$_2$O at 278 K, Calculated Binding Constant, and Calculated Chemical Shift of the Complex.

<table>
<thead>
<tr>
<th>$[G]_r$ (mM)</th>
<th>$[H]_i$ (mM)</th>
<th>$[G]_r / [H]_i$</th>
<th>$\delta$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0031</td>
<td>0.0000</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>0.0029</td>
<td>0.0081</td>
<td>2.8</td>
</tr>
<tr>
<td>3</td>
<td>0.0027</td>
<td>0.0154</td>
<td>5.6</td>
</tr>
<tr>
<td>4</td>
<td>0.0025</td>
<td>0.0262</td>
<td>10.4</td>
</tr>
<tr>
<td>5</td>
<td>0.0024</td>
<td>0.0339</td>
<td>14.4</td>
</tr>
<tr>
<td>6</td>
<td>0.0022</td>
<td>0.0407</td>
<td>18.4</td>
</tr>
<tr>
<td>7</td>
<td>0.0021</td>
<td>0.0472</td>
<td>22.8</td>
</tr>
<tr>
<td>8</td>
<td>0.0019</td>
<td>0.0530</td>
<td>27.3</td>
</tr>
<tr>
<td>9</td>
<td>0.0018</td>
<td>0.0577</td>
<td>31.3</td>
</tr>
<tr>
<td>10</td>
<td>0.0018</td>
<td>0.0620</td>
<td>35.3</td>
</tr>
<tr>
<td>11</td>
<td>0.0017</td>
<td>0.0658</td>
<td>39.3</td>
</tr>
<tr>
<td>12</td>
<td>0.0016</td>
<td>0.0693</td>
<td>43.3</td>
</tr>
</tbody>
</table>

$\delta_{comp}=4.079$

$K < 1$

---

$^a$ Total concentration of crown ether unit of (S,S)-I

$^b$ Total concentration of (S)-NEA-HCl.

$^c$ The ratio of crown ether unit of (S,S)-I over (S)-NEA-HCl.

$^d$ Observed chemical shifts of one of the phenyl protons of (S,S)-I.

Figure S6. $^1$H NMR Titration Curve for the Complexation of (S,S)-I with (S)-NEA-HCl at 278 K.
5. Phase Separation Experiments

A solution of host chiral sensor [(S,S)-1] (0.2 wt% of crown ether monomer unit) and a solution of guest (R)-NEA·HCl each in H₂O were prepared. The host (S,S)-1 solution (2.0 mL) was placed in a sample cell. Then, the phase transition was traced by monitoring the transmittance of a 500 nm light beam through a 1 cm sample cell at different temperatures on a specially constructed spectrophotometer (JASCO V-550). The rates of heating up and of cooling down of the sample cells were adjusted at 1 °C/min.

5-1. Temperature Dependence of the Transmittance at 500 nm of (S,S)-1 with NEA·HCl

![Figure S7](image)

**Figure S7.** Temperature dependence of the transmittance at 500 nm of 0.2 wt% aqueous solution of (S,S)-1 with 6.0 equivalent of (S)-NEA·HCl (red) and that with 6.0 equivalent of (R)-NEA·HCl (blue).
5-2. Temperature Dependence of the Transmittance at 500 nm of (S,S)-1 with PGO·HCl

**Figure S8.** Temperature dependence of the transmittance at 500 nm of 0.2 wt% aqueous solution of (S,S)-1 with 6.0 equivalent of (S)-PGO·HCl (red) and that with 6.0 equivalent of (R)-PGO·HCl (blue).
5-3. Temperature Dependence of the Transmittance at 500 nm of (S,S)-1 with PEA·HCl

Figure S9. Temperature dependence of the transmittance at 500 nm of 0.2 wt% aqueous solution of (S,S)-1 with 6.0 equivalent of (S)-PEA·HCl (red) and that with 6.0 equivalent of (R)-PEA·HCl (blue).
6. References