Supplementary Information (SI) for Nanoscale.
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Supporting Information

Macroscopic chiral symmetry breaking in gelation of Fmoc-amino acids. Homochiral selective secondary nucleation promoted by the choice of solvent or stirring

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1. General methods

Chemicals and solvents were purchased from TCI, Watanabe Chemical Industries, Nacalai Tesque or Kanto Chemical and used as received. Electronic absorption spectra were recorded on a JASCO model V-650DS spectrophotometer. Circular dichroism (CD) spectra were recorded on a JASCO Type J-820 spectropolarimeter by using a quartz cell with the optical length of 1 or 0.1 mm. Dynamic light scattering (DLS) data were obtained on a Malvern Panalytical model Zetasizer Nano ZS. Analytical chiral HPLC was carried out on a JASCO model PU-2080 Plus equipped with a variable-wavelength UV/Vis detector UV-2070 Plus and a DAICEL CHIRALPAK® IA-3 (250 x 2.1 mm), using a hexane/EtOH/trifluoroacteic acid mixture (90/10/0.1, in vol.) as an eluent at a flow rate of 0.13 or 0.20 mL min^{-1}. Scanning electron microscopy (SEM) was performed on a Hitachi S-4800 operating at 10 kV. A dried sample placed on a silicon wafer was coated with Pt and subjected to SEM observation.

2. Preparation of racemic mixtures

2.1. Racemic mixture of 2 (rac-2)

rac-**2**: L-**2** (TCI; 1.00 g, 2.58 mmol) and D-**2** (TCI; 1.00 g, 2.58 mmol) were dissolved in hot MeOH (21 mL) and filtered through a PTFE filter. To the filtrate was added distilled water (6 mL) and the mixture was left at 25 °C for 2 h. The resultant precipitate separated by filtration with a PTFE filter was dried under reduced pressure to leave rac-**2** as white solid (1.80 g). D/L ratio in the resultant solid was evaluated to be 50/50 by means of chiral HPLC (Fig. S3a).

2.2. Racemic mixture of 3 (rac-3)

rac-**3**: L-**3** (Watanabe Chemical Industries; 0.76 g, 1.79 mmol) and D-**3** (Watanabe Chemical Industries; 0.76 g, 1.79 mmol) were dissolved in hot EtOH (40 mL) and the solution was left at 25 °C for 1 h. To the solution was added distilled water (50 mL) and the mixture was left at 25 °C for 3 days. The resultant precipitate separated by filtration with a PTFE filter was dried under reduced pressure to leave rac-**3** as white solid (1.41 g). D/L ratio in the resultant solid was evaluated to be 50/50 by means of chiral HPLC (Fig. S3b).

3. Gelation

While the reported examples of gelation of Fmoc-amino acids were mostly attempted by using neutral buffers[1,2] or gradual neutralization of an alkaline media,[3] the former approach was chosen in this work as the latter uses optically active glucono-δ-lactone for the neutralization. Typically, **2** in phosphate buffer (PB, 50 mM, pH = 7.4) was sonicated for 5–10 minutes and the resultant suspension was heated at 70 °C. Alternatively, to a DMSO solution of **2** or **3** was added PB to make PB/DMSO ratio as 9/1 in vol., and the resultant heterogeneous mixture was heated at 70 °C. The homogeneous solution thus prepared was filtered with a PTFE syringe filter (pore size: 0.20 μm) and divided into multiple portions, individual of which (1.5 mL) was then kept in a vial at 20 °C. For the stirring-triggered gelation, the filtered solution of **2** or **3** was stirred with a stirring tip gently (50–100 rpm) at 20 °C for 2 h and then successively kept at 20 °C without stirring. To minimize the effects of stirring direction, four among eight samples were stirred for 1 h in clockwise (cw) and then for further 1 h in counterclockwise (ccw) directions, while the other four were treated in opposite order.

Stereochemistry	**Conditions**	**Result**
L- or D-**2**	15.5 mM, PB/DMSO (9/1, in vol.)	Gel
L- or D-**2**	5.2 mM, PB	Weak Gel
L- or D-**3**	30.9 mM, PB/DMSO (9/1, in vol.)	Gel
L-**2** rich (L/D = 2/1)	15.5 mM, PB/DMSO (9/1, in vol.)	Gel
L-**2** rich (L/D = 2/1)	5.2 mM, PB	Weak Gel
L-**3** rich (L/D = 2/1)	30.9 mM, PB/DMSO (9/1, in vol.)	Gel
rac-**2**	15.5 mM, PB/DMSO (9/1, in vol.)	Weak Gel
rac-**2**	5.2 mM, PB	Sol–Gel Mixture
rac-**3**	30.9 mM, PB/DMSO (9/1, in vol.)	Weak Gel

Table S1. Summary of the gelation of L-, D- and rac-**2** and **3**.

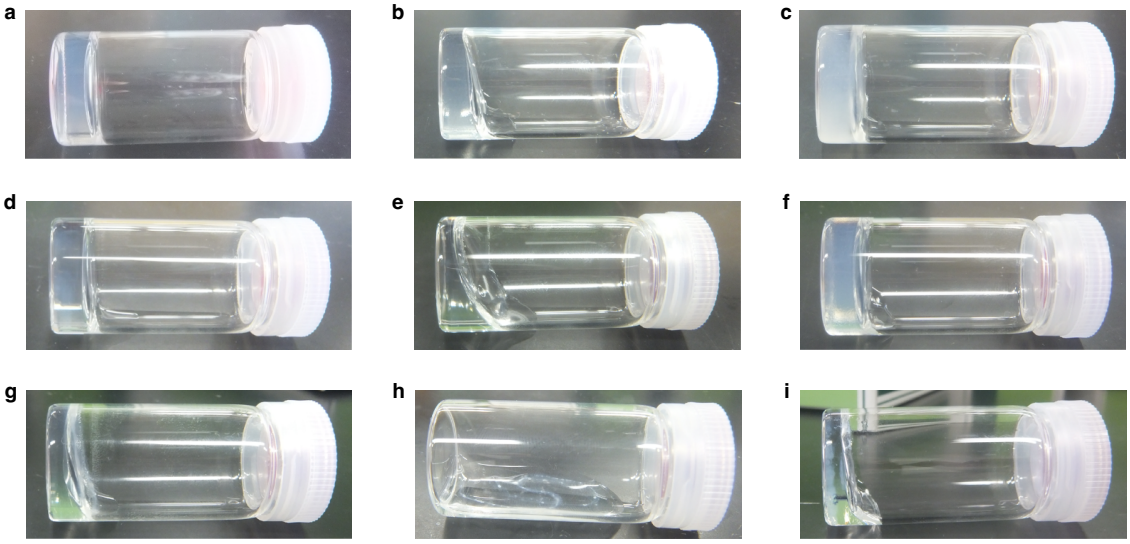

Fig. S1. Appearance of gels and sol–gel mixtures of **2** or **3** prepared from a) L-**2** in PB/DMSO, b) L-**2** in PB, c) L-**3** in PB/DMSO, d) L-**2** rich mixture (L/D = 2/1) in PB/DMSO, e) L-**2** rich mixture (L/D = 2/1) in PB, f) L-**3** rich mixture (L/D = 2/1) in PB/DMSO, g) rac-**2** in PB/DMSO, h) rac-**2** in PB, i) rac-**3** in PB/DMSO.

4. Scanning electron microscopy

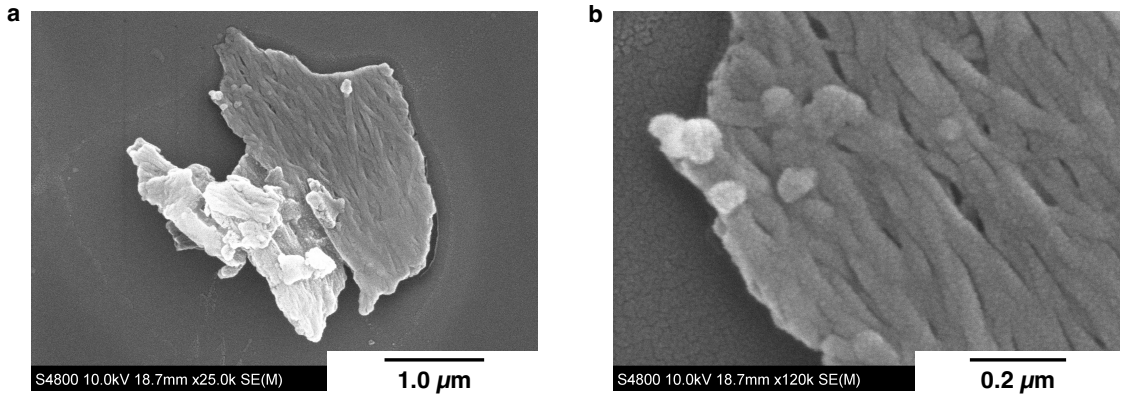

Fig. S2. SEM images of rac-**2** xerogels prepared by freeze-drying of the gel in PB.

5. Chiral HPLC

Typically, a gel or sol–gel mixture of **2** or **3** in a vial was subjected to the suction filtration and the residue on the PTFE filter (pore size: 0.50 μm) was dried overnight under air. Then the filter was dipped in an EtOH/trifluoroacetic acid mixture (100/0.1, in vol.) to afford a homogeneous solution, an aliquot of which was diluted with hexane and subjected to the analysis. **2** or **3** in a filtered solution was extracted with ethyl acetate. After the evaporation of the solvent, the residue dissolved in the eluent was also subjected the analysis.

Column: DAICEL CHIRALPAK® IA-3 (250 x 2.1 mm)

Eluent: hexane/EtOH/trifluoroacetic acid mixture (90/10/0.1, in vol.)

Flow rate: 0.13 (Figs. S8 and 15a,c,d) or 0.20 mL/min

Wavelength: 255 nm

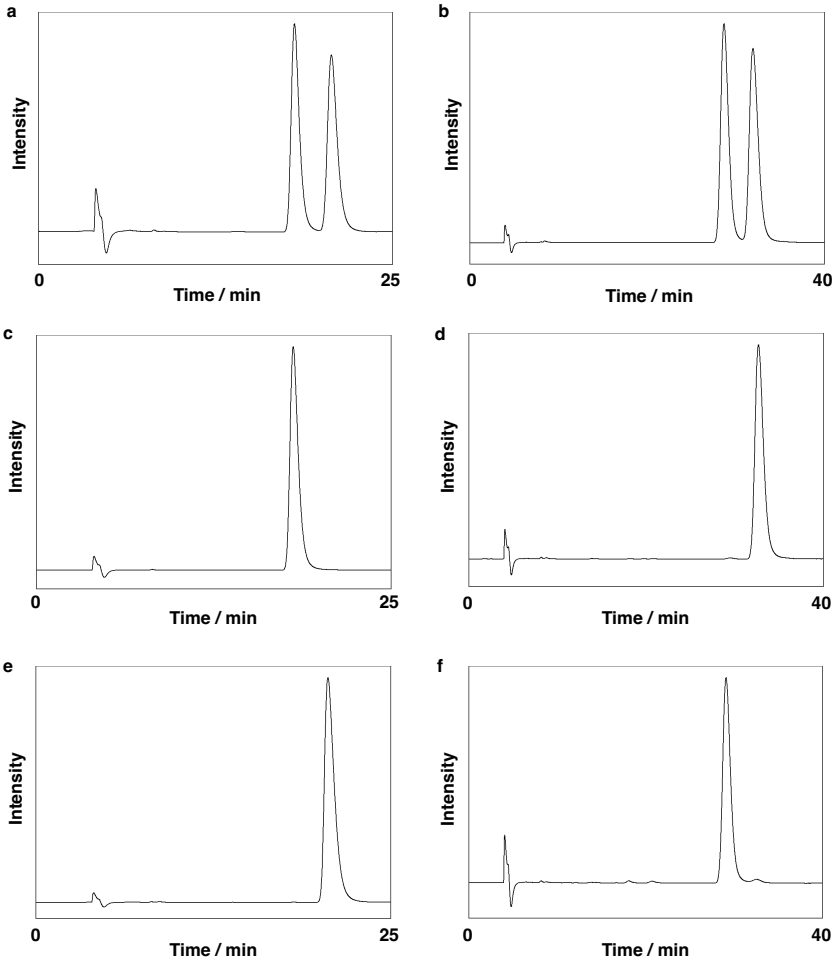

Fig. S3. Chiral HPLC chromatograms of a) rac-**2**, b) rac-**3**, c) L-**2**, d) L-**3**, e) D-**2** and f) D-**3**.

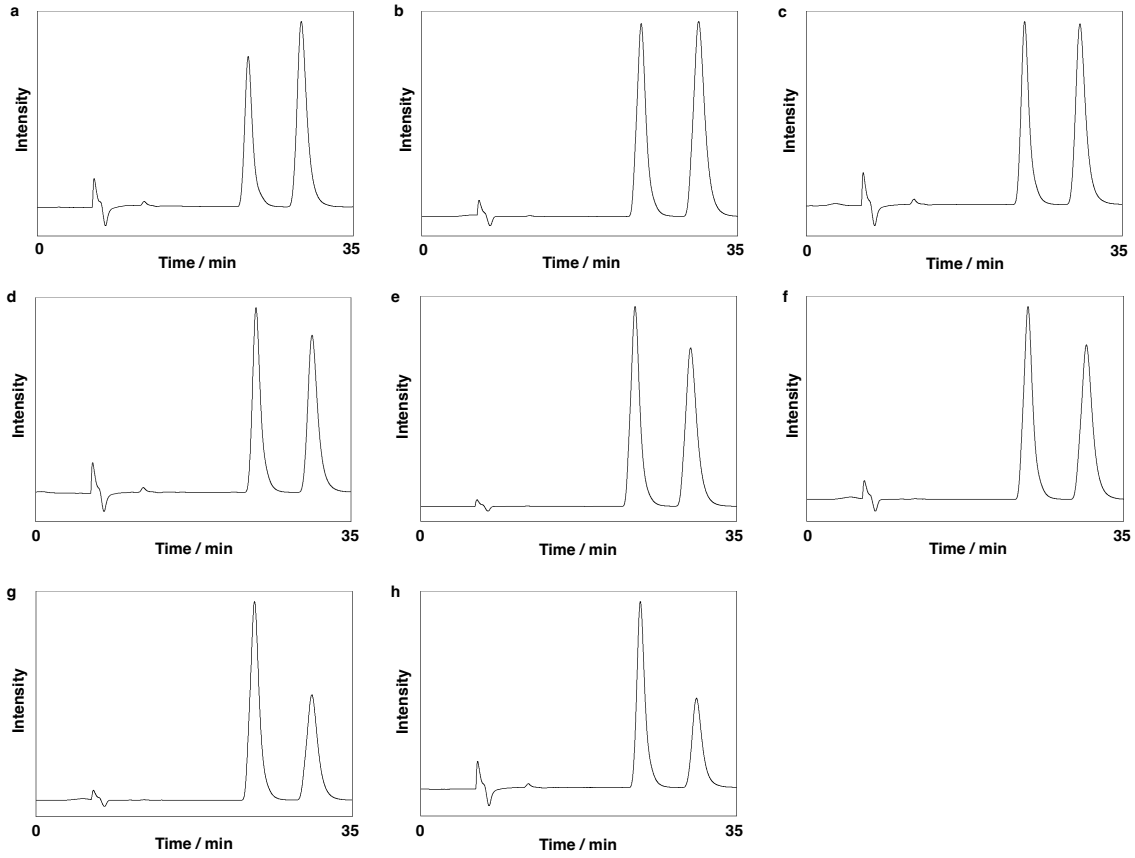

Fig. S4. Chiral HPLC chromatograms of gels obtained from eight divided portions of a common PB/DMSO (9/1, in vol.) solution of rac-**2** in a single experiment. [L-**2**] = [D-**2**] = 7.8 mM.

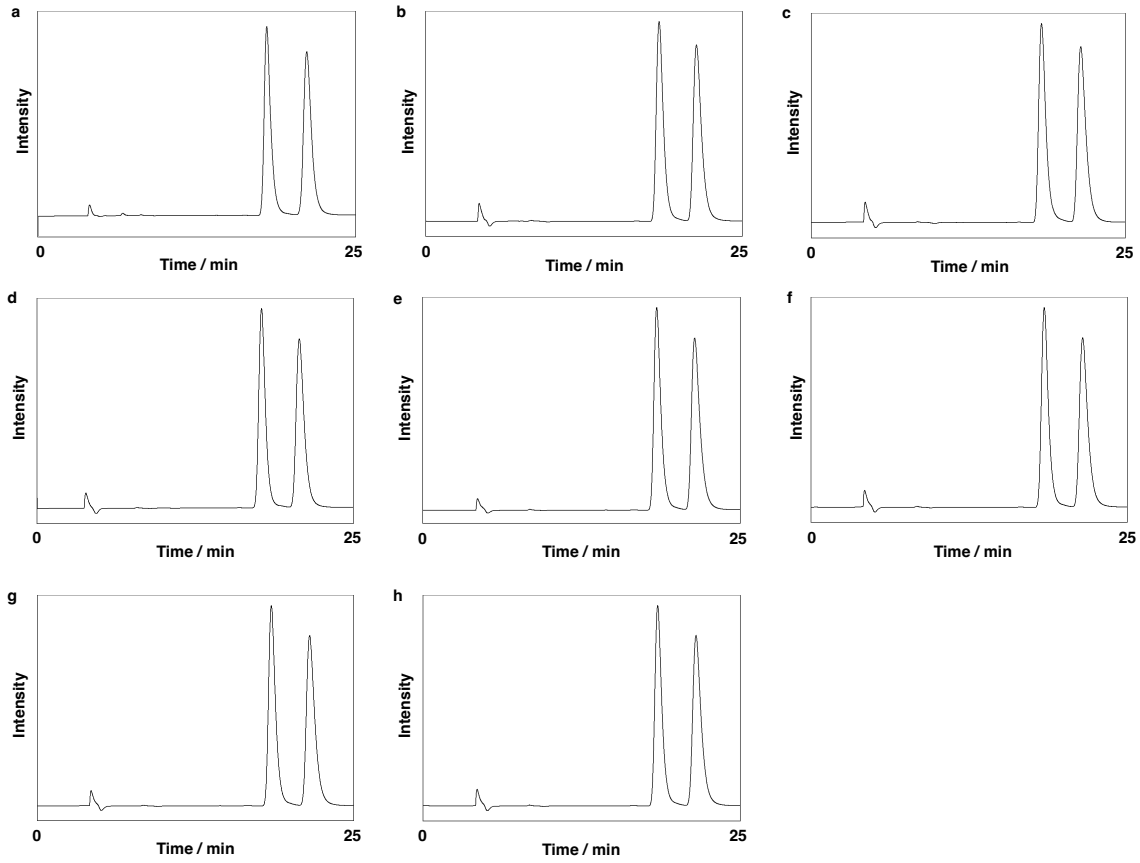

Fig. S5. Chiral HPLC chromatograms of gels obtained from eight divided portions of a common PB solution of rac-**2** in a single experiment. [L-**2**] = [D-**2**] = 2.6 mM.

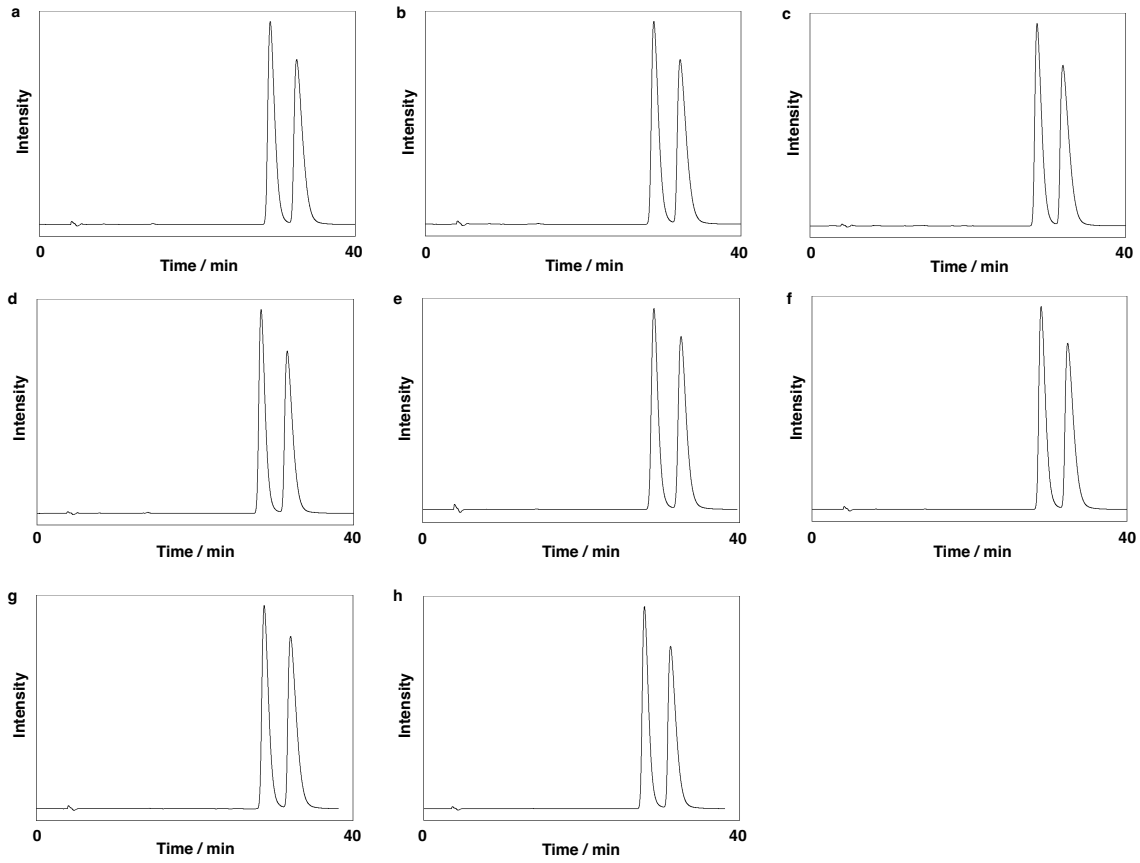

Fig. S6. Chiral HPLC chromatograms of gels obtained from eight divided portions of a common PB/DMSO (9/1, in vol.) solution of rac-**3** in a single experiment. [L-**3**] = [D-**3**] = 15.5 mM.

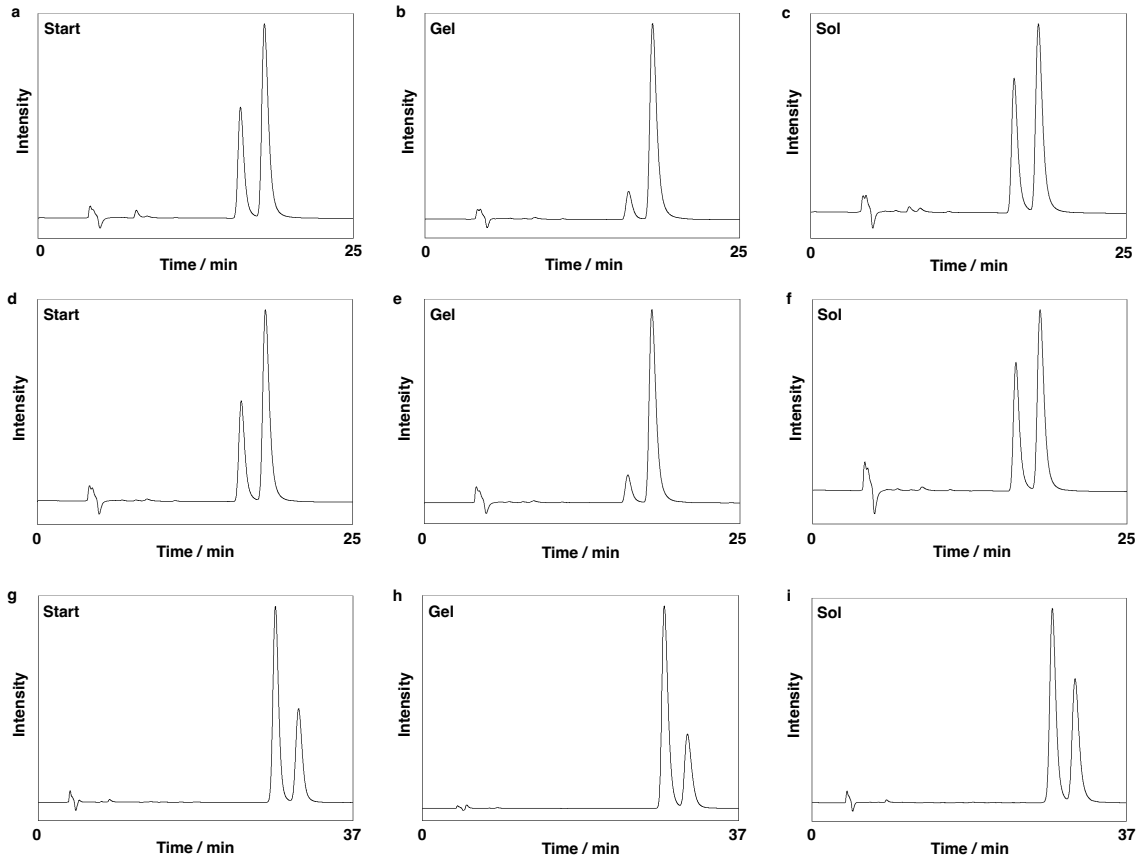

Fig. S7. Chiral HPLC chromatograms of L-rich starting mixtures of L- and D-enantiomers as well as gels and sols obtained from them; a) starting mixture, b) gel and c) sol for **2** ([**2**] = 15.5 mM) in PB/DMSO (9/1, in vol.), d) starting mixture, e) gel and f) sol for **2** ([**2**] = 5.2 mM) in PB, g) starting mixture, h) gel and i) sol for **3** ([**3**] = 30.9 mM) in PB/DMSO (9/1, in vol.).

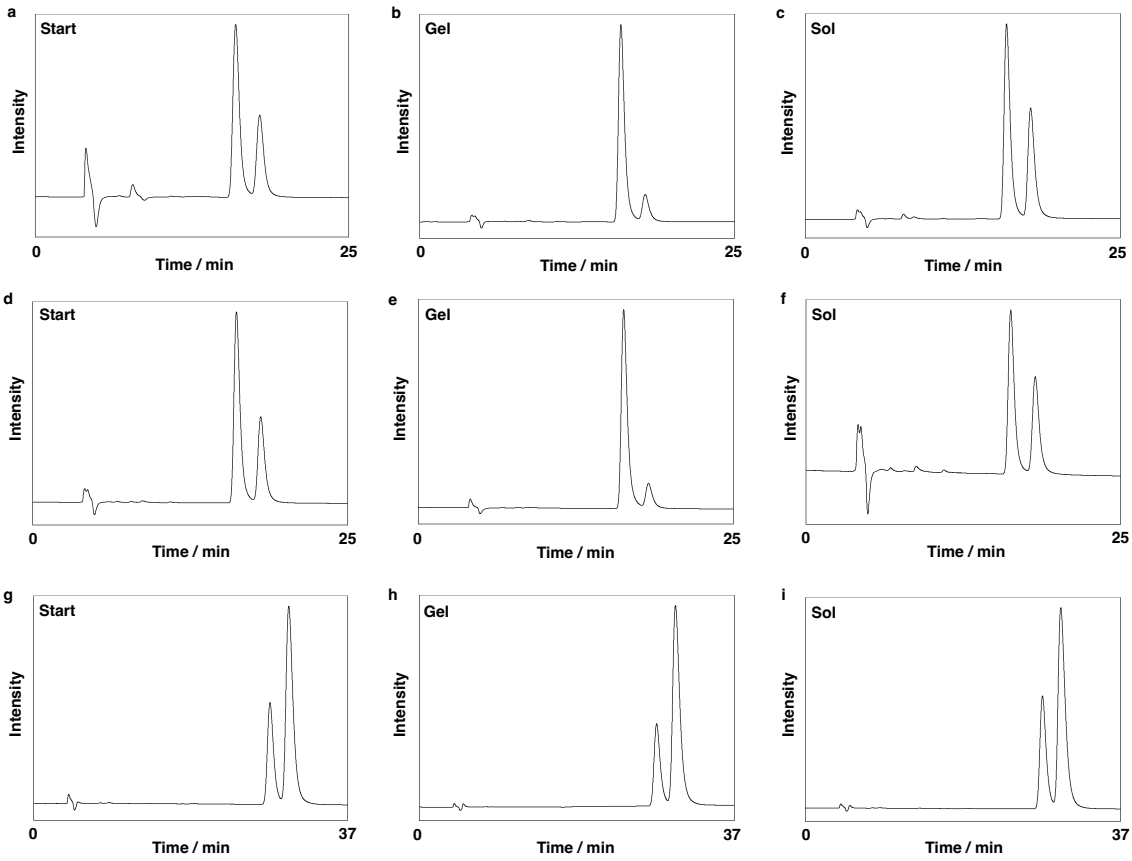

Fig. S8. Chiral HPLC chromatograms of D-rich starting mixtures of L- and D-enantiomers as well as gels and sols obtained from them; a) starting mixture, b) gel and c) sol for **2** ([**2**] = 15.5 mM) in PB/DMSO (9/1, in vol.), d) starting mixture, e) gel and f) sol for **2** ([**2**] = 5.2 mM) in PB, g) starting mixture, h) gel and i) sol for **3** ([**3**] = 30.9 mM) in PB/DMSO (9/1, in vol.).

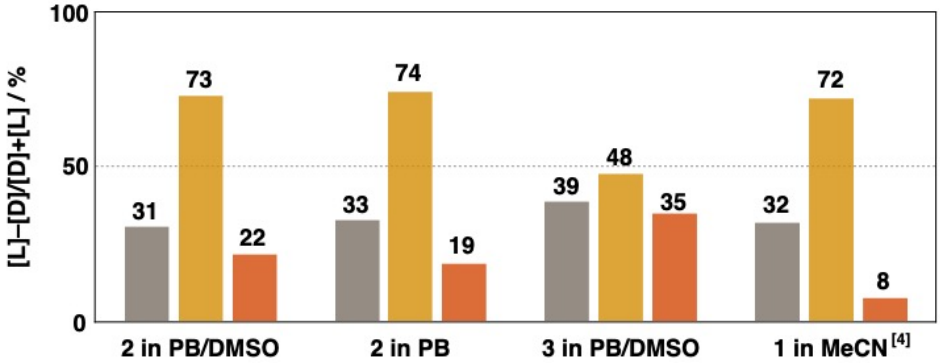

Fig. S9. Enantiomeric excess (ee) values of **1**–**3** in starting mixtures (gray), gels (ocher) and sols (dark orange) obtained from L-rich mixtures of L- and D-enantiomers. Results for **1** in MeCN reported in reference S4 are included for comparison.

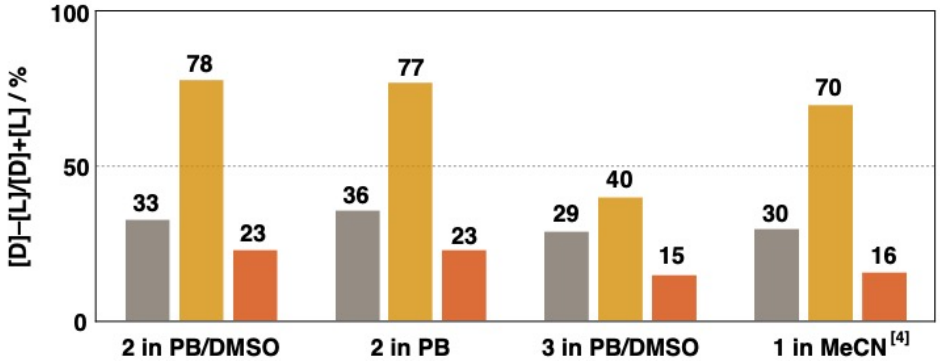

Fig. S10. Enantiomeric excess (ee) values of **1**–**3** in starting mixtures (gray), gels (ocher) and sols (dark orange) obtained from D-rich mixtures of L- and D-enantiomers. Blue line represents the ee value for the starting mixtures. Results for **1** in MeCN reported in reference S4 are included for comparison.

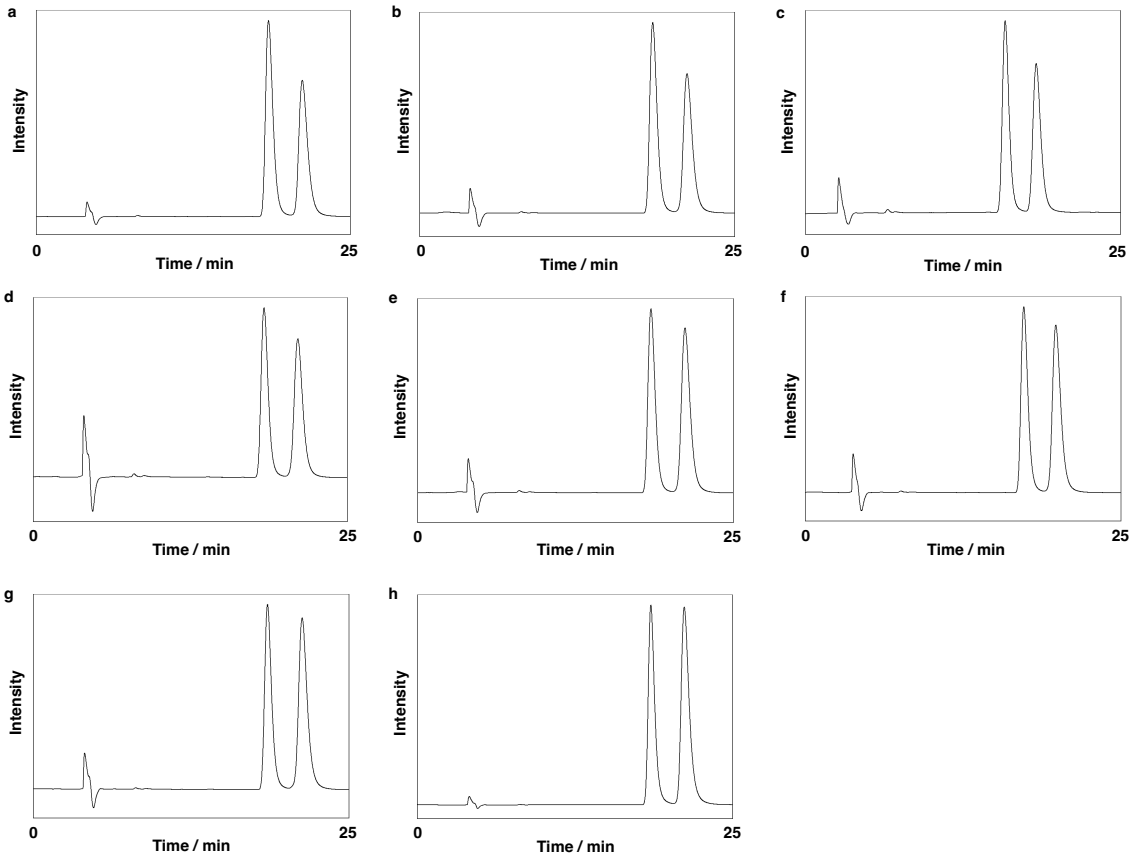

Fig. S11. Chiral HPLC chromatograms of gels obtained from eight divided portions of a common PB/DMSO (9/1, in vol.) solution of rac-**2** in a single experiment with stirring. [L-**2**] = [D-**2**] = 6.5 mM.

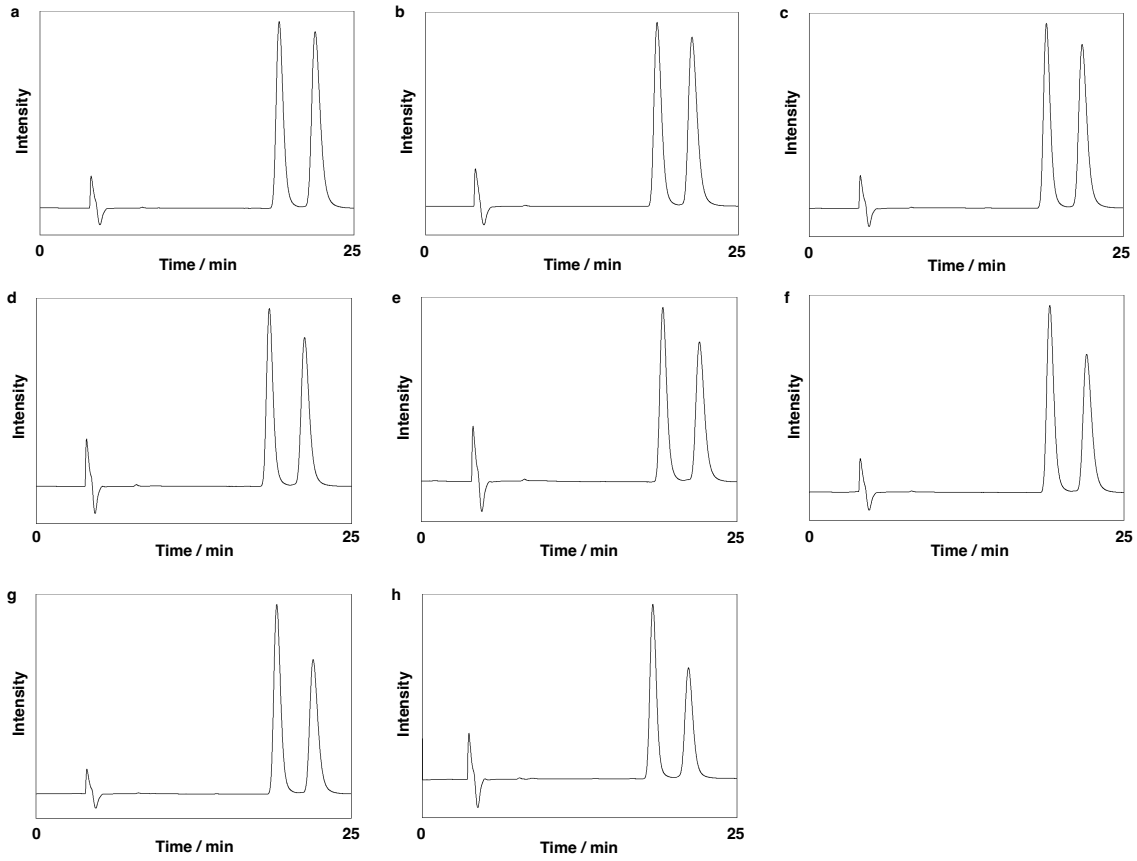

Fig. S12. Chiral HPLC chromatograms of gels obtained from eight divided portions of a common PB solution of rac-**2** in a single experiment with stirring. [L-**2**] = [D-**2**] = 2.2 mM.

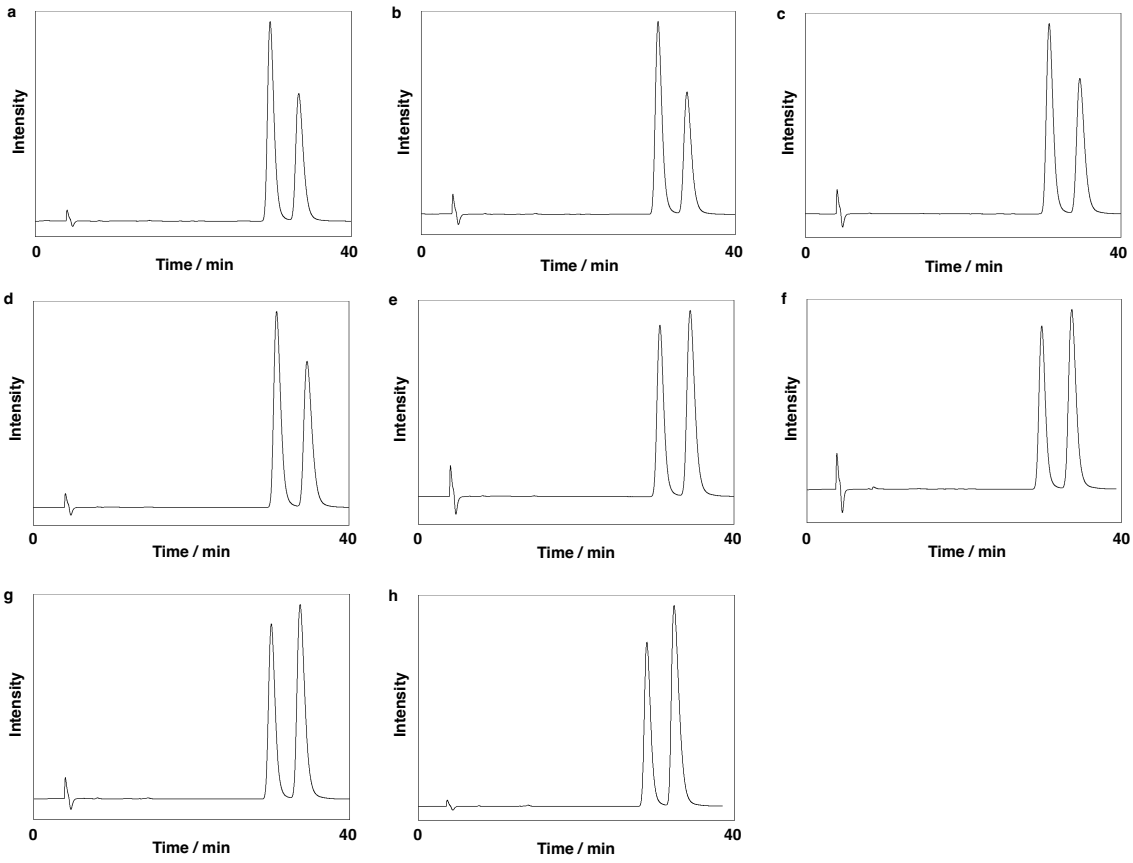

Fig. S13. Chiral HPLC chromatograms of gels obtained from eight divided portions of a common PB/DMSO (9/1, in vol.) solution of rac-**3** in a single experiment with stirring. [L-**3**] = [D-**3**] = 13.1 mM.

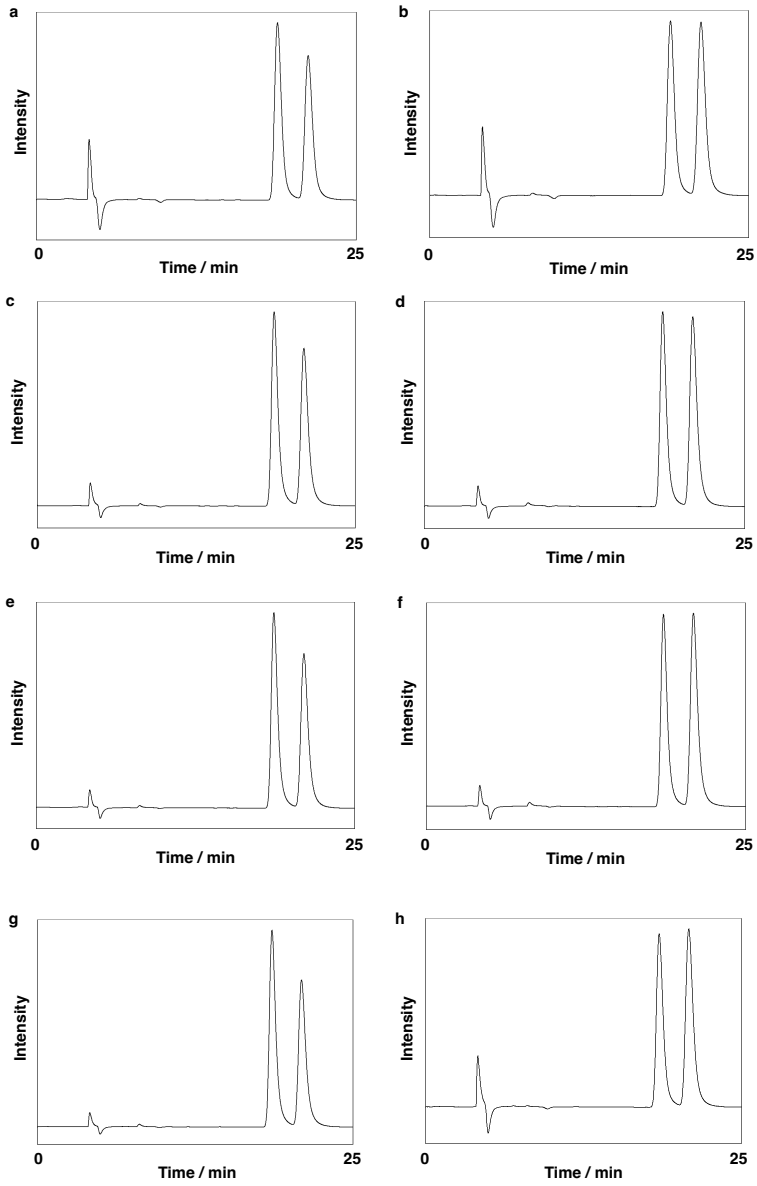

Fig. S14. Chiral HPLC chromatograms of the seed gels enriched in a) L-**2** and b) D-**2** as well as second gels as obtained by adding c) the L-rich seed gel, d) the D-rich seed gel, e,g) the precursor suspension for the L-rich seed gel, f,h) the precursor suspension for the D-rich seed gel to rac-**2** in PB ([L-**2**] = [D-**2**] = 2.2 mM). The gels for c–f were obtained by standing, while those for g and h were obtained with a stirring step.

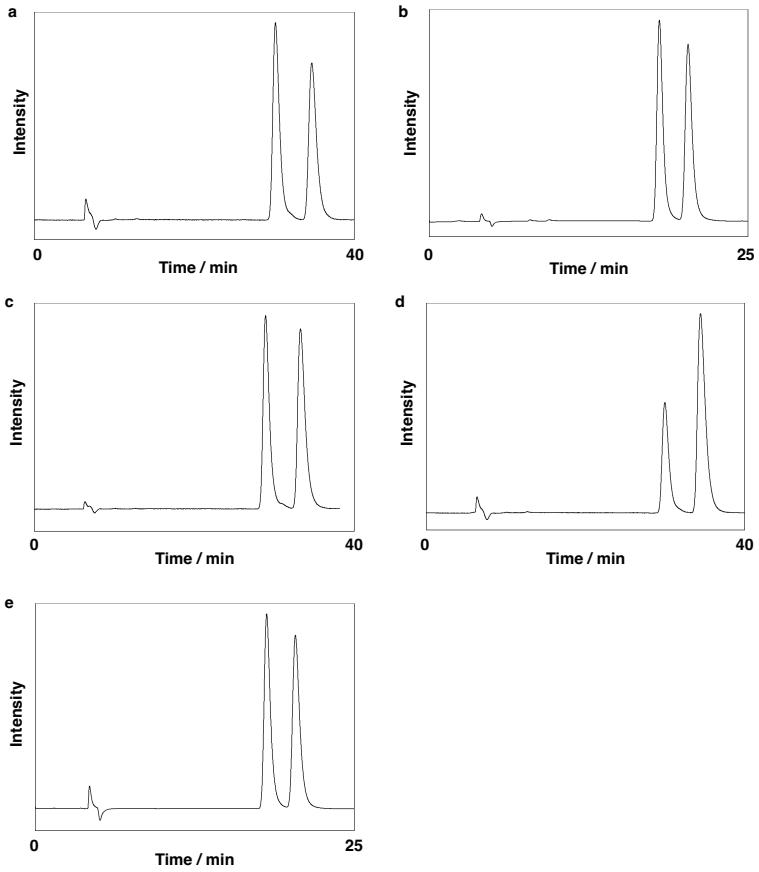

Fig. S15. Chiral HPLC chromatograms of the gels of **2** for the comparison to their CD spectra in Fig. S17b. a–d) gels prepared from rac-**2** in PB/DMSO (9/1, in vol.), [L-**2**] = [D-**2**] = 7.8 mM. e) a gel prepared from rac-**2** in PB, [L-**2**] = [D-**2**] = 2.6 mM.

6. Circular dichroism spectroscopy

Typically, a gel or suspension was transferred from the vial to a quartz plate with a dip of 0.1 mm by using a GILSON model microman E pipet, where, in the case of a sol–gel mixture, gel located at the bottom of the vial was taken. No re-dispersion or another additional treatment of the sample was performed. After covering with a flat quartz plate, the sample sandwiched with the two quartz plates was subjected to the measurement.

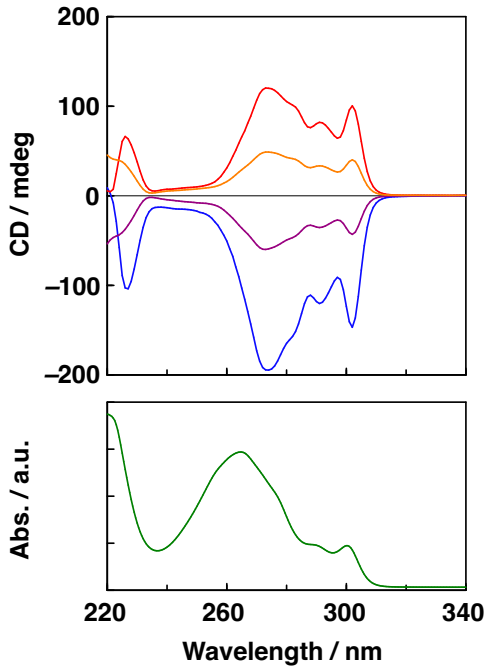

Fig. S16. CD spectra of gels prepared from L-**2** in PB (orange) or PB/DMSO (9/1, in vol. red) and those from D-**2** in PB (purple) or PB/DMSO (9/1, in vol. blue) at 20 °C, together with the absorption spectrum (green) of the gel from L-**2** in PB/DMSO (9/1, in vol.). [**2**] = 7.8 and 15.5 mM in PB and PB/DMSO, respectively.

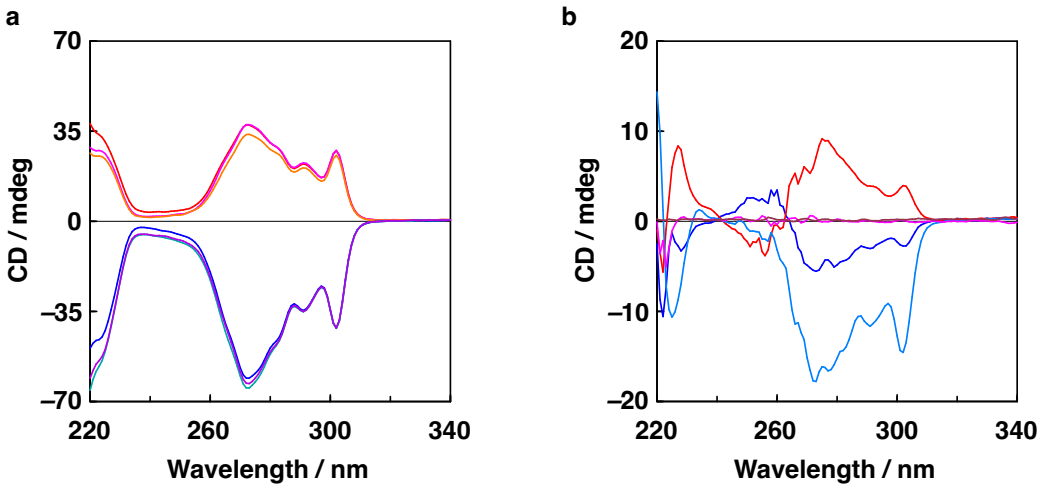

Fig. S17. a) Angle-dependent CD spectra of L- (red, pink and orange) and D-**2** (blue, blue green and purple) gels obtained in PB. The spectra colored in pink and orange were measured with 90° cw and ccw rotations of the sample, respectively, from the standard position (red). Similarly, CD spectra colored in blue (standard), blue green (cw) and purple (ccw) were measured. b) CD spectra of gels enriched in L-**2** (red, 3.7% ee, Fig. S15a) or D-**2** (blue, 4.6% ee, Fig. S15c and light blue, 35.5% ee, Fig. S15d) and those with no detectable enrichment (pink, Fig. S15b and brown, Fig. S15e, <1 % ee). The corresponding chiral HPLC chromatograms are shown in Fig. S15.

7. Dynamic light scattering

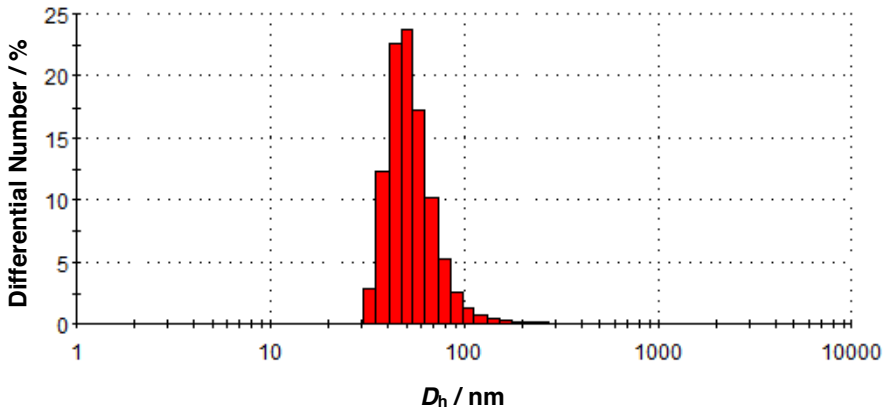

Fig. S18. DLS profile of rac-**2** in PB after stirring for 2 h at 20 °C.

8. References

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S4. K. Tashiro, T. Takei, A. M. Fracaroli, H. Ohtsu, M. Kawano and D. Hashizume, *Chem Asian J.*, 2022, **17**, e202200230.