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Proximate Stochastic Chiral Symmetry Breaking is Mechanically Tunable: Formation of Enantiomeric Hetero-double-helices and Aggregates from Racemic Oxymethylenehelicene Oligomers

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General method Elemental analyses were conducted with Yanaco CHN CORDER MT-6. Circular dichroism (CD) and UV-vis spectra were measured on a JASCO J-720 spectropolarimeter. The path length 0.0102 cm of quartz round cell were used. Dynamic light scattering (DLS) determined at 173° scattering angle was observed by a Zetasizer Nano S. Atomic force microscope (AFM) images were recorded under ambient conditions using a Digital Instrument Multimode Nanoscope IIIa operating in the tapping mode regime. Micro-fabricated silicon cantilever tips (OMCL-AC160TS-C2) were used.

1. Materials

The compounds (*P*)-**1** and (*M*)-**1** were synthesized as described previously.[1]
(*P*)-**1**: Anal. ($C_{261}H_{294}O_{30}$) Calcd for: H, 7.58; C, 80.15%. Found: H, 7.78; C, 80.17%.

2. Chiral symmetry breaking using racemic mixtures

<Chiral symmetry breaking experiment>

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total 0.5 mM) was prepared. (*P*)-**1** (2.93 mg, 7.5×10^{-4} mmol) and (*M*)-**1** (2.93 mg, 7.5×10^{-4} mmol) were weighed in a cylindrical glass vial (diameter, 21 mm) by a microbalance within error of 0.5%. The samples were dissolved in trifluoromethylbenzene (3.0 mL, total 0.5 mM) by heating to 90 °C for 10 min. Then, the solution was cooled to 25 °C, and mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm at 25 °C. Then, CD and UV-vis spectra were obtained (Figure 3).

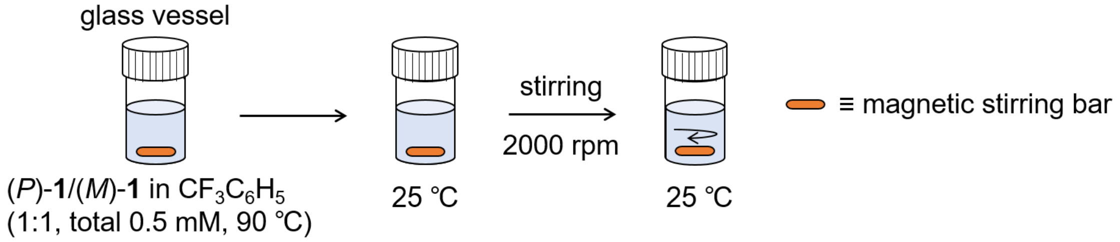


Figure S1. Procedure of chiral symmetry breaking experiment by mechanical stirring.

Chiral symmetry breaking of (*P*)-**1**/(*M*)-**1** (1:1) was conducted with a different lot of sample to confirm reproducibility.

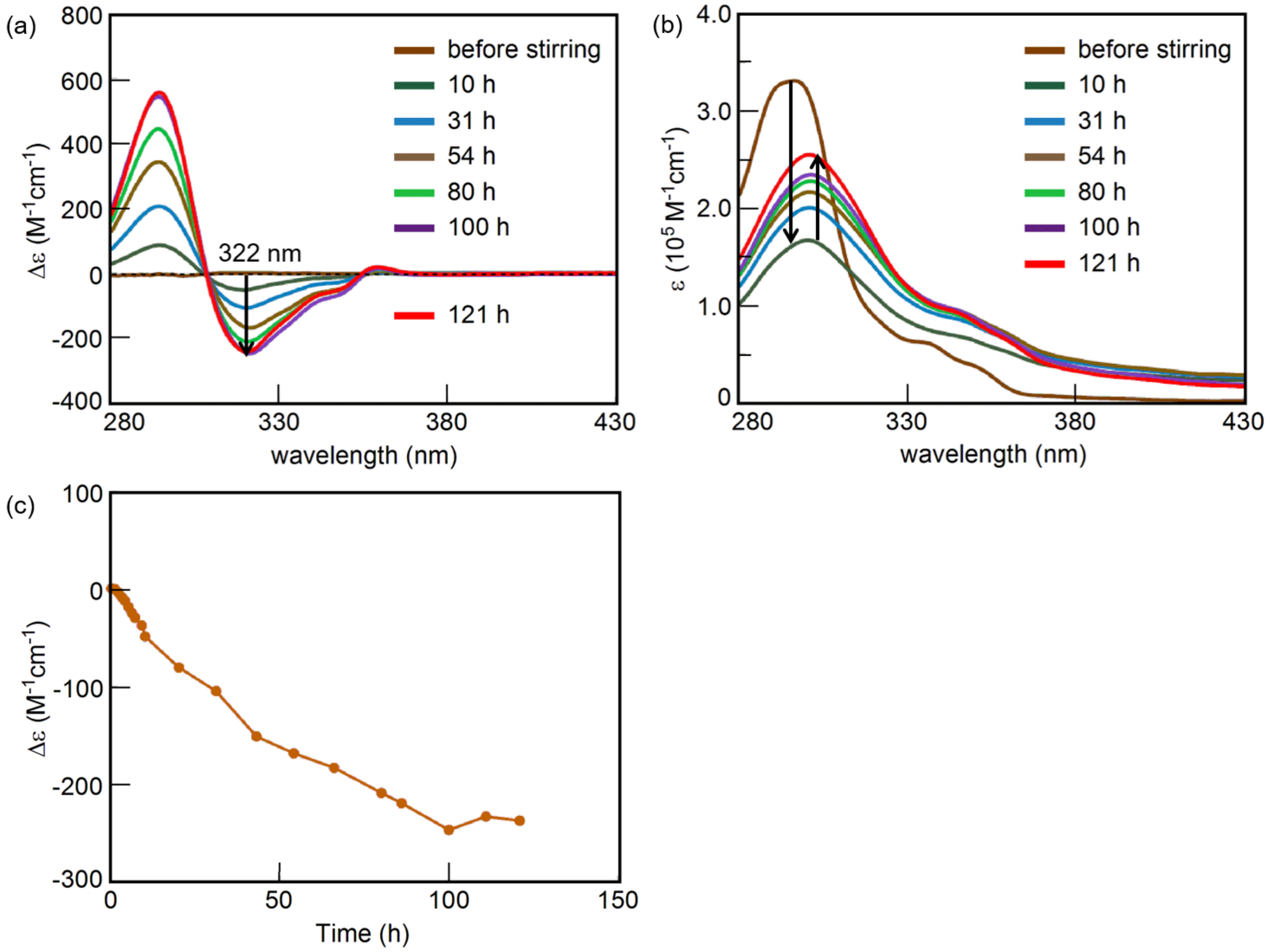


Figure S2. CD spetra (a), UV-vis spectra (b), and the Δε (322 nm)/time profiles (c) of a 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total 0.5 mM) at 25 °C by mechanical stirring experiment. Lines are drawn to connect the points.

<DLS analysis>

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.5 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (1.47 mg, 3.8×10^{-4} mmol) and (*M*)-**1** (1.47 mg, 3.8×10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). The solution was cooled to 25 °C. A part of the solution was transferred to a cell, and DLS measurements were conducted giving an average diameter of 2.3 nm. Then, the rest of solution was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm at 25 °C for 40 h. The solution was transferred to a cell, and DLS measurements were conducted giving an average diameter of 106 nm.

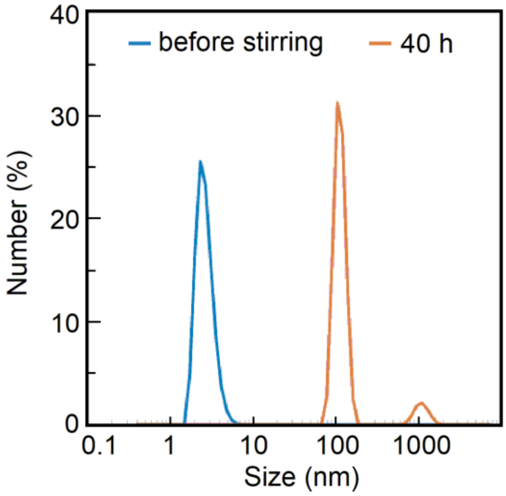


Figure S3. Size distributions of a 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total 0.5 mM) obtained by DLS experiments.

<AFM analysis>

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (0.978 mg, 2.5 × 10^{-4} mmol) and (*M*)-**1** (0.978 mg, 2.5 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). The solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm. After mechanical stirring for 100 h, a part of the solution was diluted 10-fold, immediately dropped on a mica plate, and the plate was spin coated at the rate of 2000 rpm for 30 sec. Then, AFM analysis was conducted.

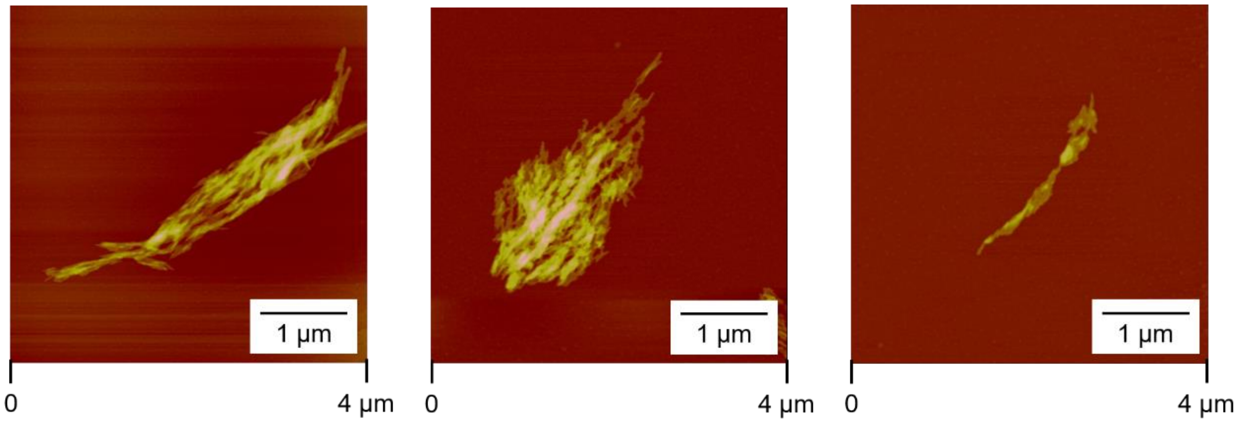


Figure S4. AFM images (height mode) of a 50:50 mixture of (*P*)-**1**/(*M*)-**1** obtained from trifluoromethylbenzene solution (total 0.5 mM) after mechanically stirring for 100 h at 25 °C.

<Kinetics>

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (3.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (2.93 mg, 7.5 × 10^{-4} mmol) and (*M*)-**1** (2.93 mg, 7.5 × 10^{-4} mmol) with heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 21 mm). The solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm. After mechanical stirring for a certain period of time, a part of the solution was taken, and analyzed by CD and UV-vis. The sample was returned to the reaction mixture, and stirring was continued.

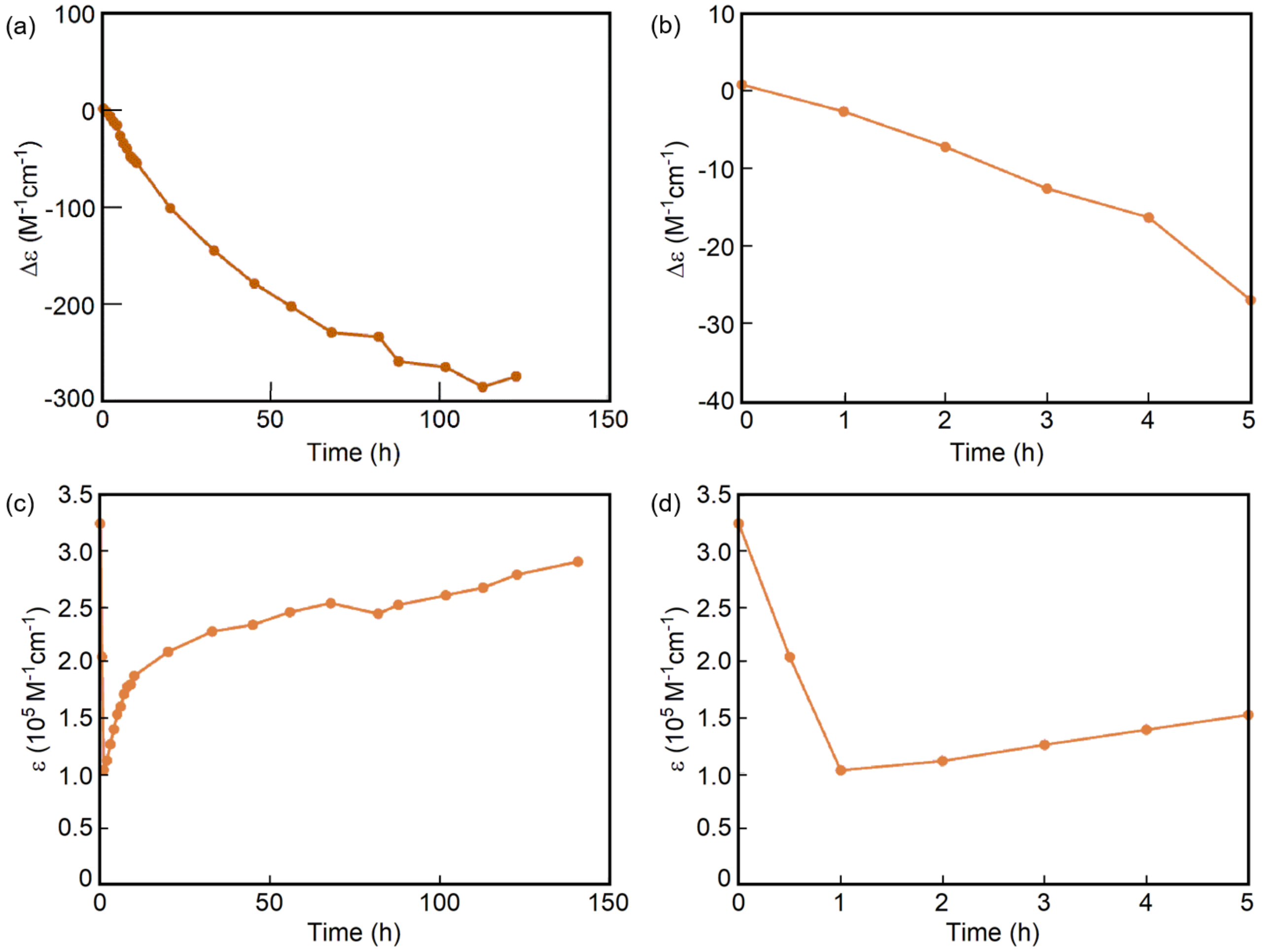


Figure S5. Kinetic analysis of a 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total, 0.5 mM) at 25 °C by stirring experiment shown by Δε at 322 nm (a) and ε at 294 nm (c). (b) and (d) are expansions of (a) and (c), respectively. Lines are drawn to connect the points.

<Effect of stirring rate>

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (2.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (1.96 mg, 5.0 × 10^{-4} mmol) and (*M*)-**1** (1.96 mg, 5.0 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). The solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 1000 and 3000 rpm for 10 h, during which the process was monitored by Δε at 322 nm.

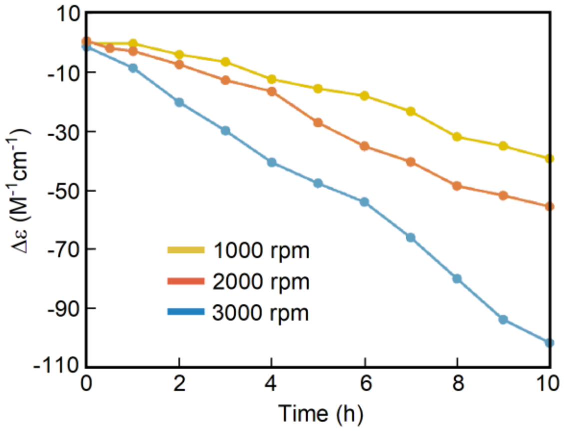
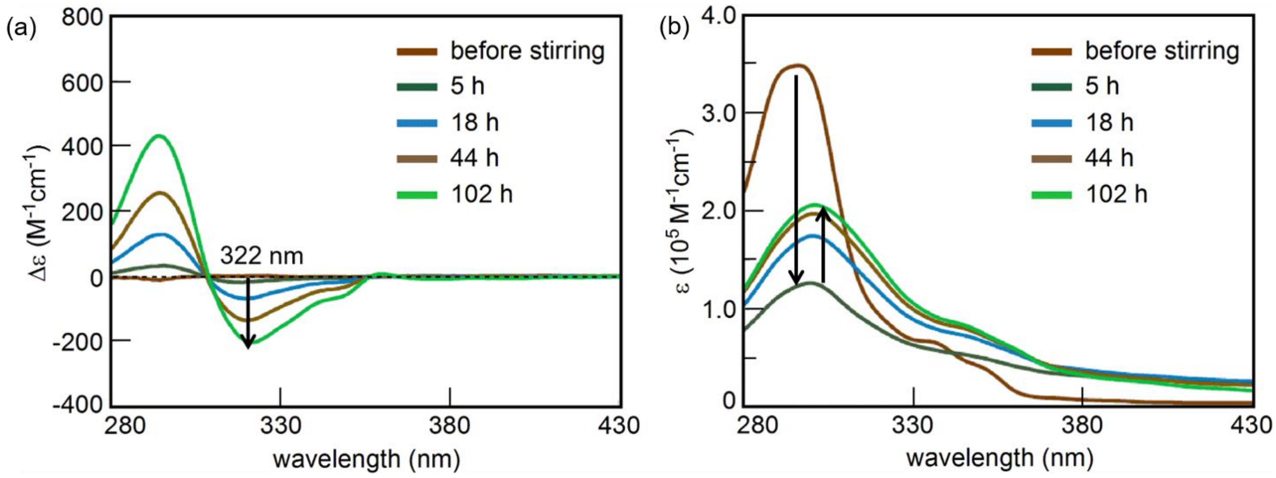


Figure S6. Kinetic analysis of a 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total 0.5 mM) at 25 °C by stirring experiments at the rate of 1000, 2000, and 3000 rpm, which are shown by Δε (322 nm)/time profiles. Lines are drawn to connect the points.

<Counter-clockwise rotation stirring>

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (3.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (2.93 mg, 7.5 × 10^{-4} mmol) and (*M*)-**1** (2.93 mg, 7.5 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 21 mm). The solution was cooled to 25 °C, and was mechanically stirred in counter-clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 102 h.



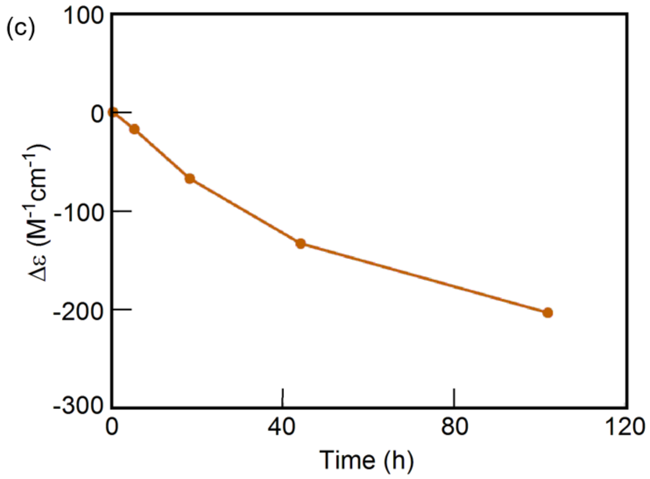


Figure S7. CD spectra (a), UV-vis (b) spectra, and the Δε (322 nm)/time profiles (c) of a 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total, 0.5 mM) at 25 °C by counter-clockwise rotation stirring experiment. Lines are drawn to connect the points.

<Experiment without stirring>

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (2.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (1.96 mg, 5.0 × 10^{-4} mmol) and (*M*)-**1** (1.96 mg, 5.0 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). The solution was cooled to 25 °C, allowed to settle for 144 and 265 h, and then CD and UV-vis analyses were conducted.

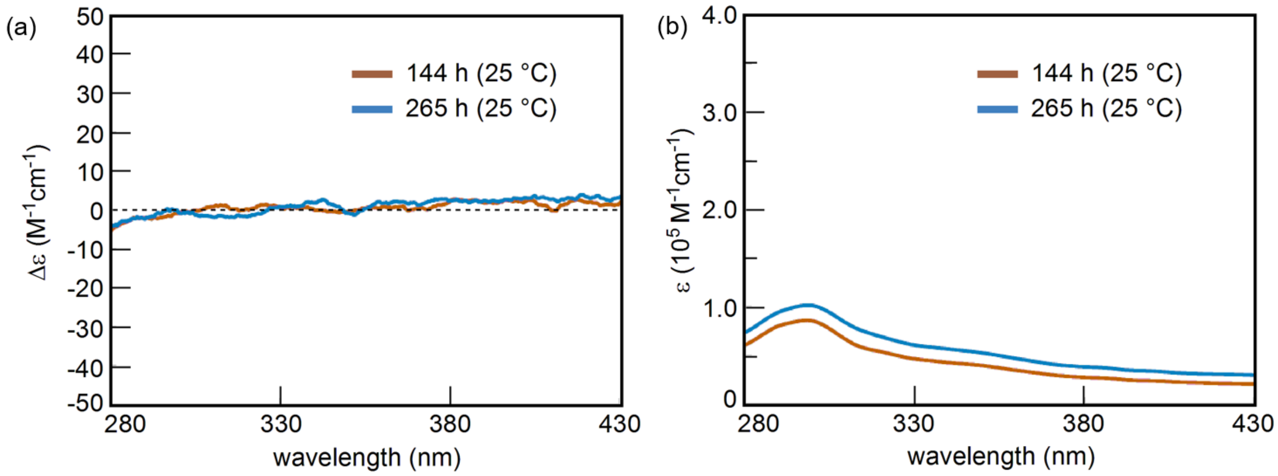


Figure S8. CD spectra (a) and UV-vis (b) spectra of a 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total 0.5 mM) at 25 °C without mechanical stirring.

<Concentration at 0.25 mM>

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.25 mM) was prepared by dissolving (*P*)-**1** (0.489 mg, 1.25 × 10^{-4} mmol) and (*M*)-**1** (0.489 mg, 1.25 × 10^{-4} mmol) with heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). The solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 54 h.

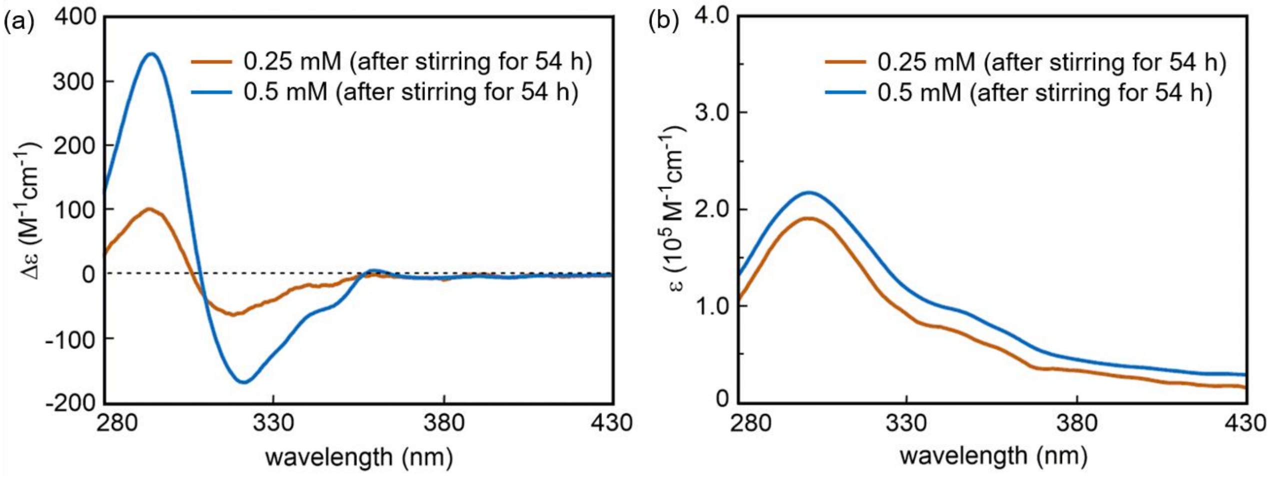


Figure S9. CD spectra (a) and UV-vis (b) spectra of a 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total 0.25 and 0.5 mM) at 25 °C by mechanical stirring experiment.

<Effect of vessel materials>

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (0.978 mg, 2.5 × 10^{-4} mmol) and (*M*)-**1** (0.978 mg, 2.5 × 10^{-4} mmol) with heating at 90 °C for 10 min in a cylindrical PFA (tetrafluoroethylene-perfluoroalkyl vinyl ether copolymer) vessel (diameter, 17 mm). The solution was heated at 90 °C for 10 min, cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 85 h. Then, CD and UV-vis analyses were conducted. The same experiments were conducted in polyethylene vessel and glass vessel (diameter, 12 mm) for 88 h and 85 h, respectively (Figure 4a).

<Effect of added glass beads>

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (3.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (2.93 mg, 7.5 × 10^{-4} mmol) and (*M*)-**1** (2.93 mg, 7.5 × 10^{-4} mmol) with heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 21 mm). Then, the solution was cooled to 25 °C, and glass beads (diameter, 0.5 mm; weight, 0.5 g) were added. The mixture was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 75 h. Then, CD and UV-vis analyses were conducted (Figure 4b).

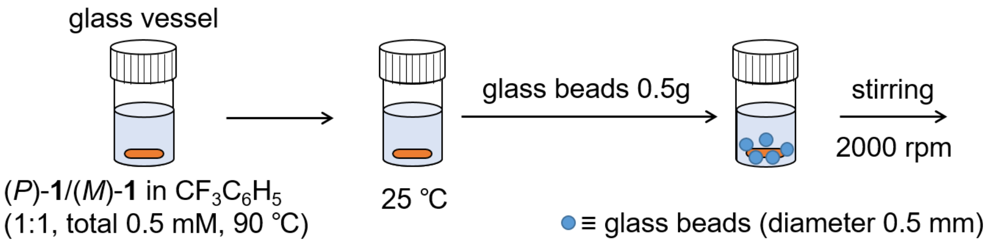


Figure S10. Procedure of chiral symmetry breaking experiment by mechanical stirring in the presence of glass beads.

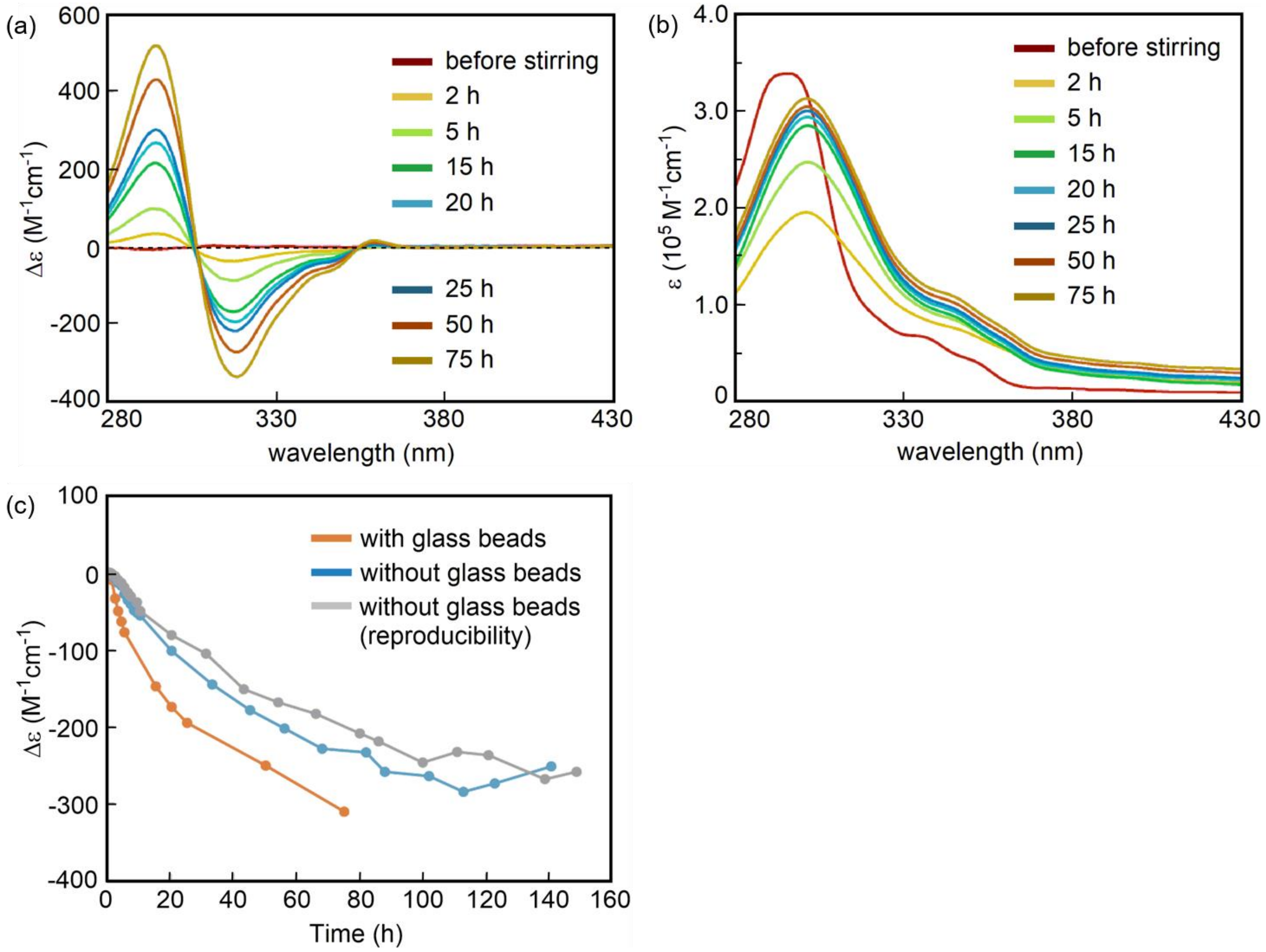


Figure S11. CD spectra (a), UV-vis spectra (b), and Δε (322 nm)/time profiles (c) of a 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total 0.5 mM) at 25 °C by stirring experiment in the presence of glass beads. Lines are drawn to connect the points.

<Centrifusion experiment>

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (0.978 mg, 2.5×10^{-4} mmol) and (*M*)-**1** (0.978 mg, 2.5×10^{-4} mmol) by heating at 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). Then, the solution was cooled to 25 °C, and centrifuged at 10000 rpm for 5 h. Then, CD and UV-vis analyses were conducted.

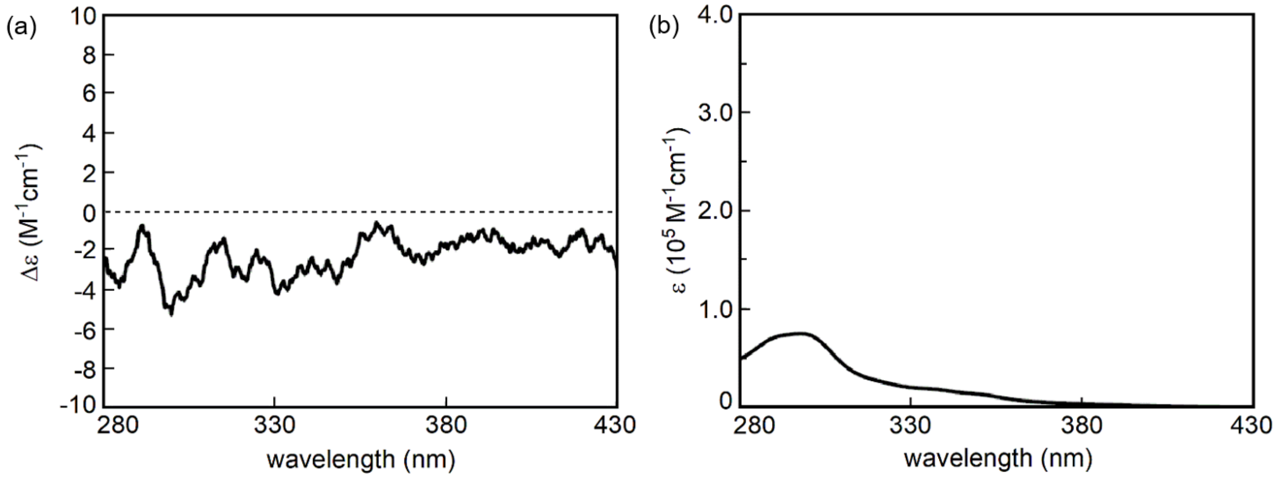


Figure S12. CD spectrum (a) and UV-vis spectrum (b) of a 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total 0.5 mM) at 25 °C by centrifusion experiment.

<Effect of linear dichroism (LD)>

Effect of linear dichroism (LD) was not observed in the CD analysis by rotation of sample.

Round quartz cell experiment: A trifluoromethylbenzene solution (2.0 mL) of (*P*)-**1**/(*M*)-**1** (1:1) (total concentration, 0.5 mM) was prepared by dissolving (*P*)-**1** (1.95 mg, 5.0×10^{-4} mmol) and (*M*)-**1** (1.95 mg, 5.0×10^{-4} mmol) in a cylindorical glass vial (diameter, 12 mm), and was heated at 90 °C for 3 min. Then, the solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm. After mechanical stirring for 50 h, a part of the solution was transferred to a quartz cell. CD spectra were obtained as the cell was rotated at 45 degree intervals (Figures S29a).

Quartz plate experiment: The same sample solution prepared as above was dropped on a quartz plate and dried under ambient condition. CD spectra were obtained as the quartz plate was rotated at 45 degree intervals (Figures S29b).

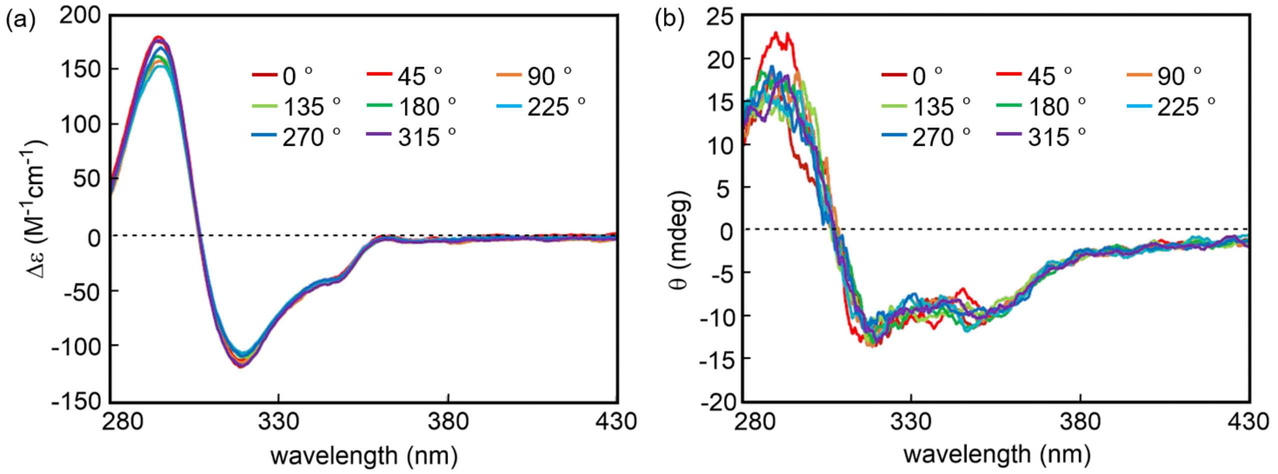


Figure S13. Experiment on the LD effect shown by CD spectra of (*P*)-**1**/(*M*)-**1** (trifluoromethylbenzene; total 0.5 mM) after stirring at the rate of 2000 rpm for 50 h. CD spectra of (a) sample solution in a quartz cell rotated at 45 degree intervals; (b) sample spin-coated on a quartz plate rotated at 45 degree intervals.

3. Seeding experiment

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (0.978 mg, 2.5 × 10^{-4} mmol) and (*M*)-**1** (0.978 mg, 2.5 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). Then, a part of the solution (0.2 mL) was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 73 h (solution I). Then, the solution I (0.2 mL) was mixed with the rest of solution containing a 50:50 mixture of random-coil (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (0.8 mL, total 0.5 mM). The resulted mixture was allowed to settle for 50 min, and CD and UV-vis analyses were conducted.

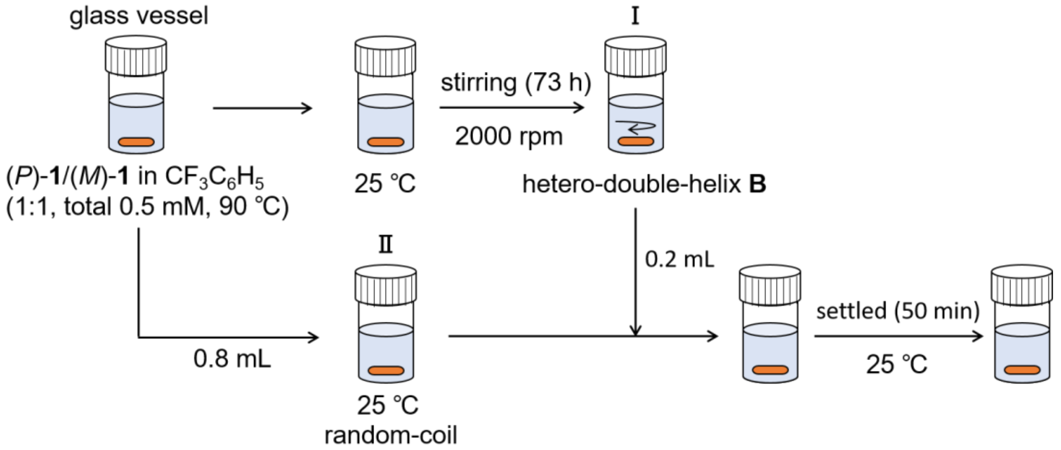


Figure S14. Procedure of seeding experiment.

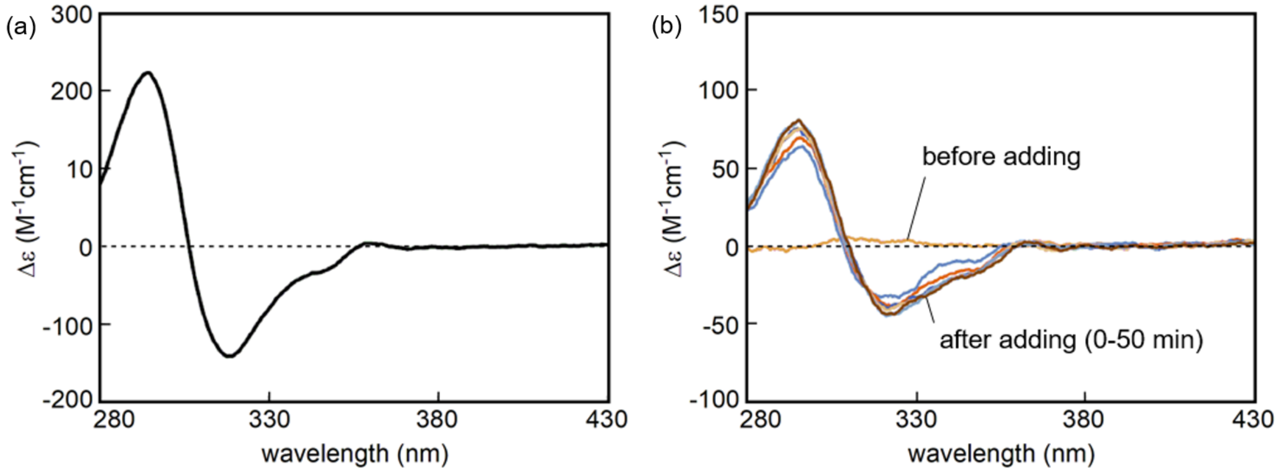


Figure S15. CD spectra of a 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total, 0.5 mM) at 25 °C after stirring for 73 h (solution I) (a) and after mixed with random-coil solution II (b).

4. Repeated experiments of racemic mixtures

A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (0.978 mg, 2.5×10^{-4} mmol) and (*M*)-**1** (0.978 mg, 2.5×10^{-4} mmol) by heating at 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). Then, the solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 100 h. Then, CD and UV-vis analyses were conducted. The same lot and three different lots of samples (Table S1) were subjected to the experiment 10 times.

Table S1. The amounts of (*P*)-**1** and (*M*)-**1** used in repeated experiments of racemic mixtures. 10 samples were prepared from three different lots of samples. (*P*)-**1** and (*M*)-**1** were weighed in a vial, and were dissolved in trifluoromethylbenzene (1.0 mL, total 5.0 mM).

		Weight (mg)				Weight (mg)	
Lot	Exp.	(*P*)-**1**	(*M*)-**1**	Lot	Exp.	(*P*)-**1**	(*M*)-**1**
1	1st	0.9757	0.9776	2	2nd	0.9781	0.9765
1	2nd	0.9790	0.9787	2	3rd	0.9781	0.9766
1	3rd	0.9963	0.9777	3	1st	0.9792	0.9786
1	4th	0.9795	0.9784	3	2nd	0.9765	0.9801
2	1st	0.9771	0.9771	3	3rd	0.9790	0.9796

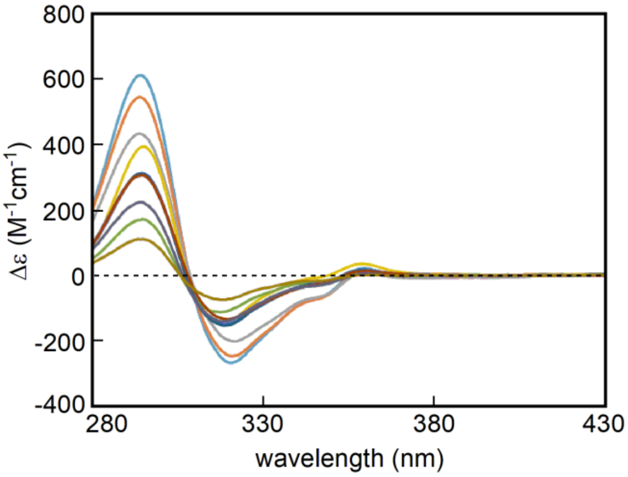


Figure S16. CD spectra of 50:50 mixtures of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total 0.5 mM) at 25 °C after stirring for 100 h. CD spectra of 10 experiments are shown.

5. Different ratio experiment

<Chiral symmetry breaking>

Different ratio experiments were conducted using the procedure described in 2. Chiral symmetry breaking, in which the molar fraction of (*P*)-**1** and (*M*)-**1** was changed between 40:60 and 60:40 using different lots of samples (Table S2).

Table S2. The amounts of (*P*)-**1** and (*M*)-**1** used in the experiments with different molar fraction of (*P*)-**1**/(*M*)-**1** mixtures. (*P*)-**1** and (*M*)-**1** were weighed in a vial, and were dissolved in trifluoromethylbenzene (1.0 mL, total 5.0 mM).

		Weight (mg)				Weight (mg)	
(*P*)-**1**/(*M*)-**1**	Exp.	(*P*)-**1**	(*M*)-**1**	(*P*)-**1**/(*M*)-**1**	Exp.	(*P*)-**1**	(*M*)-**1**
40:60	1st	0.7846	1.1773	48:52	2nd	0.9359	1.0196
45:55	1st	0.8816	1.0777	48:52	3rd	0.9388	1.0139
45:55	2nd	0.8793	1.0767	49:51	1st	0.9574	0.9981
45:55	3rd	0.8795	1.0754	49:51	2nd	0.9606	0.9960
45:55	4th	0.8818	1.0753	49:51	3rd	0.9569	0.9970
46:54	1st	0.8991	1.0532	51:49	1st	0.9993	0.9569
47:53	1st	0.9211	1.0353	53:47	1st	1.0403	0.9197
47:53	2nd	0.9180	1.0354	55:45	1st	1.0726	0.8791
47:53	3rd	0.9183	1.0362	60:40	1st	1.1758	0.7812
48:52	1st	0.9383	1.0132				

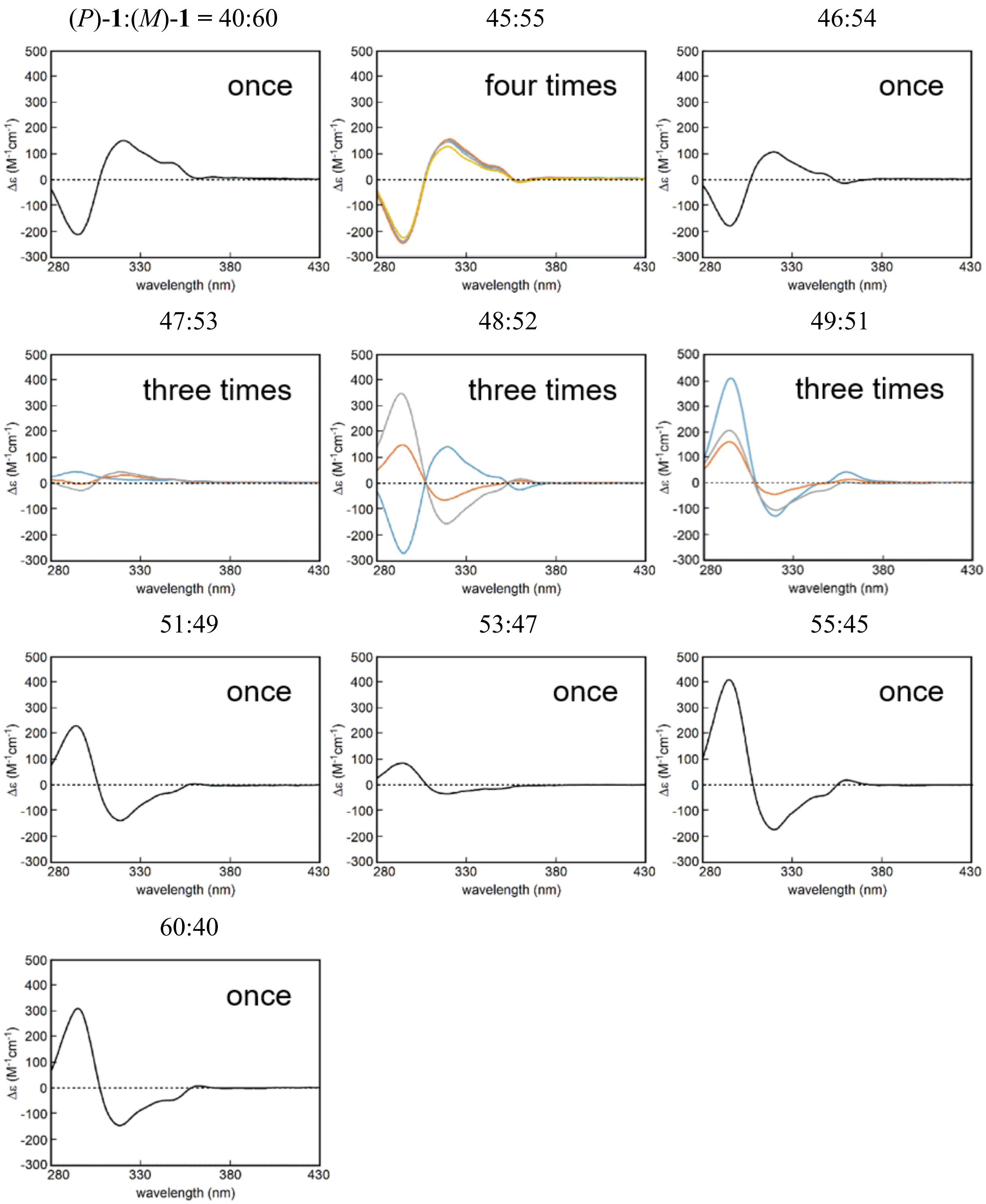


Figure S17. Different ratio experiments. CD spectra of mixtures of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total, 0.5 mM) at 25 °C after stirring for 100 h. Experiments were repeated as noted in spectra.

6. Stochastic chiral symmetry breaking by mixing experiments

<Mixing of hetero-double-helix B (55:45) and random-coil (45:55)>

A 55:45 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (1.08 mg, 2.75 × 10^{-4} mmol) and (*M*)-**1** (0.880 mg, 2.25 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). Then, the solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 50 h (solution I). A 45:55 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (0.880 mg, 2.25 × 10^{-4} mmol) and (*M*)-**1** (1.08 mg, 2.75 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm) (solution II). A part of solution I (0.5 mL) and a part of solution II (0.5 mL) were mixed, and mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 45 h. Then, CD and UV-vis analyses were conducted (Figure 6a).

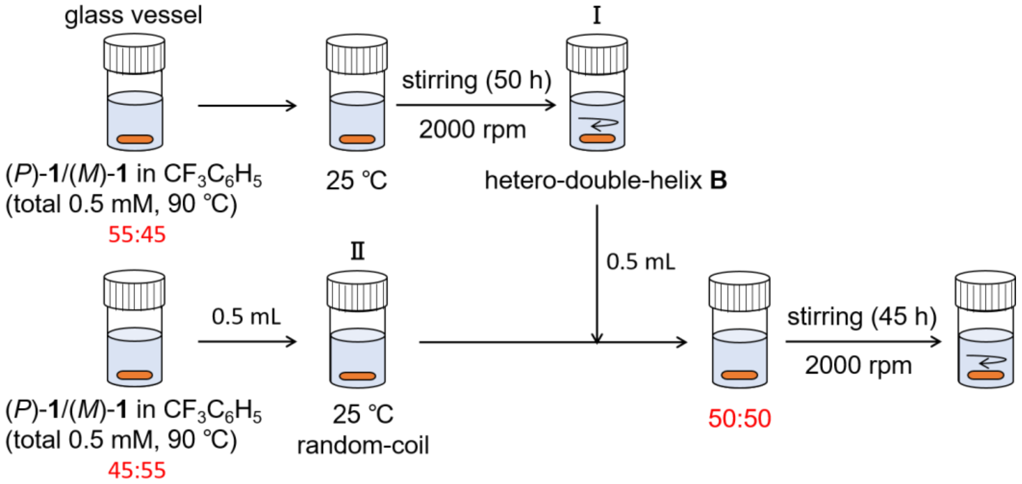


Figure S18. Procedure of mixing experiment of hetero-double-helix **B** (55:45) and random-coil (45:55).

<Mixing of hetero-double-helix ent-B (45:55) and random-coil (55:45)>

A 45:55 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (0.880 mg, 2.25 × 10^{-4} mmol) and (*M*)-**1** (1.08 mg, 2.75 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). Then, the solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 70 h (solution I). A 55:45 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (1.08 mg, 2.75 × 10^{-4} mmol) and (*M*)-**1** (0.880 mg, 2.25 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm) (solution II). A part of solution I (0.5 mL) and a part of solution II (0.5 mL) were mixed, and mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 22 h. Then, CD UV-vis analyses were conducted (Figure 6c).

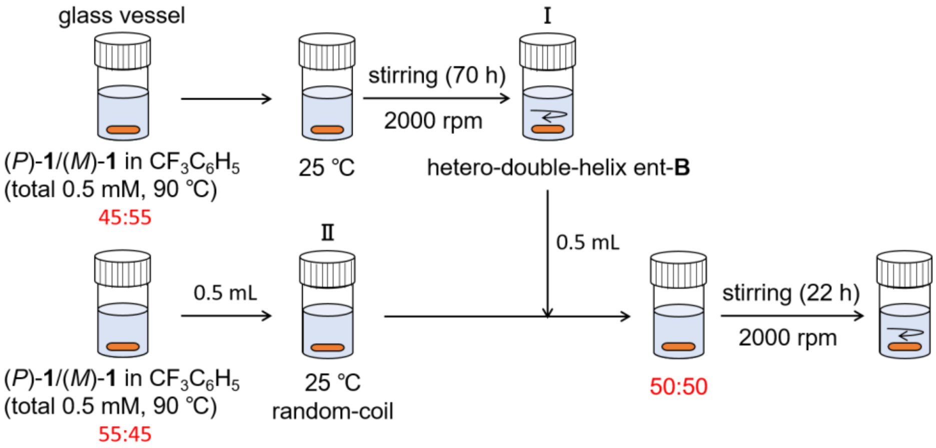


Figure S19. Procedure of mixing experiment of hetero-double-helix ent-**B** (45:55) and random-coil (55:45).

<Mixing hetero-double-helix B (55:45) and random-coil (50:50)>

A 55:45 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (1.08 mg, 2.75×10^{-4} mmol) and (*M*)-**1** (0.880 mg, 2.25×10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). Then, the solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 30 h (solution I). A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (0.978 mg, 2.5×10^{-4} mmol) and (*M*)-**1** (0.978 mg, 2.5×10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm) (solution II). A part of solution I (0.2 mL) and a part of solution II (0.8 mL) were mixed, and mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 25 h. Then, CD and UV-vis analyses were conducted.

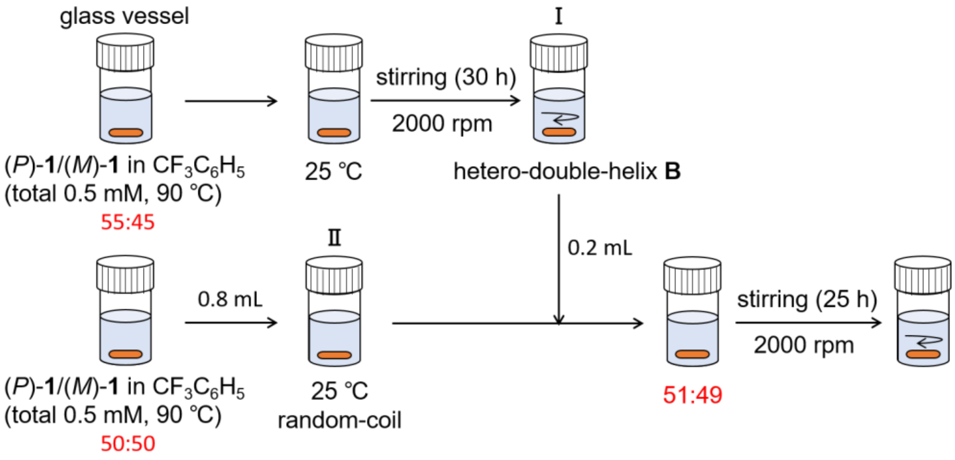


Figure S20. Procedure of mixing experiment of hetero-double-helix **B** (55:45) and random-coil (50:50).

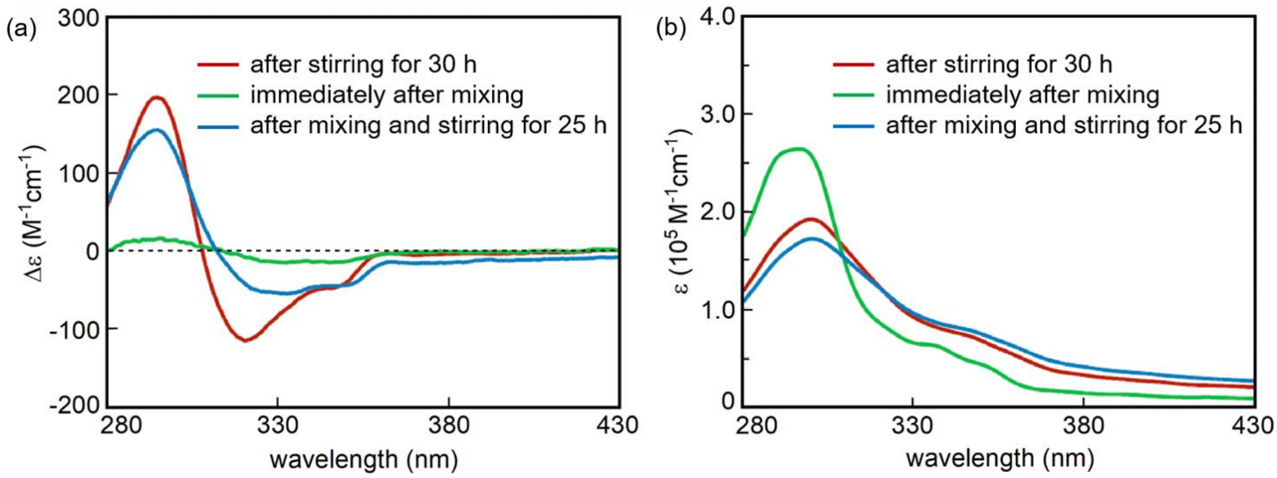


Figure S21. CD spectra (a) and UV-vis (b) spectra of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total, 0.5 mM) at 25 °C by mixing experiment of hetero-double-helix **B** (55:45) and random-coil (50:50).

<Mixing hetero-double-helix ent-B (45:55) and random-coil (50:50)>

A 45:55 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (0.880 mg, 2.25 × 10^{-4} mmol) and (*M*)-**1** (1.08 mg, 2.75 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). Then, the solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 24 h (solution I). A 50:50 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.0 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (0.978 mg, 2.5 × 10^{-4} mmol) and (*M*)-**1** (0.978 mg, 2.5 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm) (solution II). A part of solution I (0.2 mL) and a part of solution II (0.8 mL) were mixed, and mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 24 h. Then, CD and UV-vis analyses were conducted.

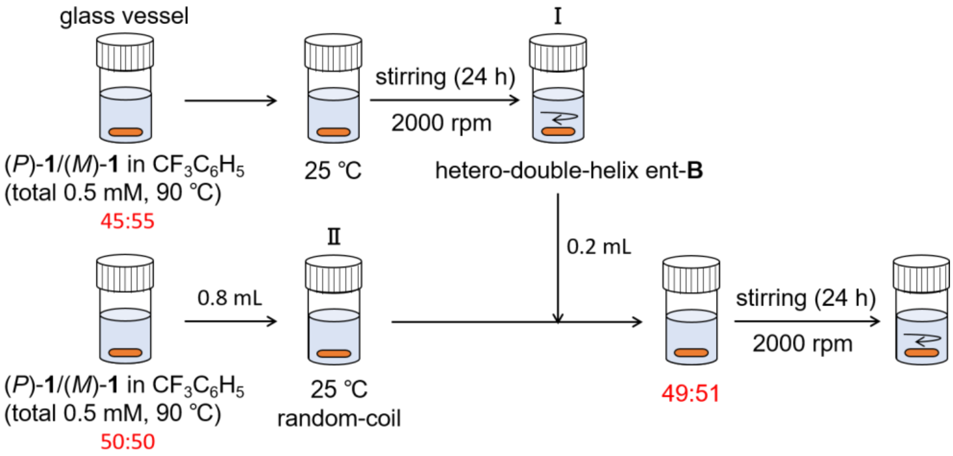


Figure S22. Procedure of mixing experiment of hetero-double-helix ent-**B** (45:55) and random-coil (50:50).

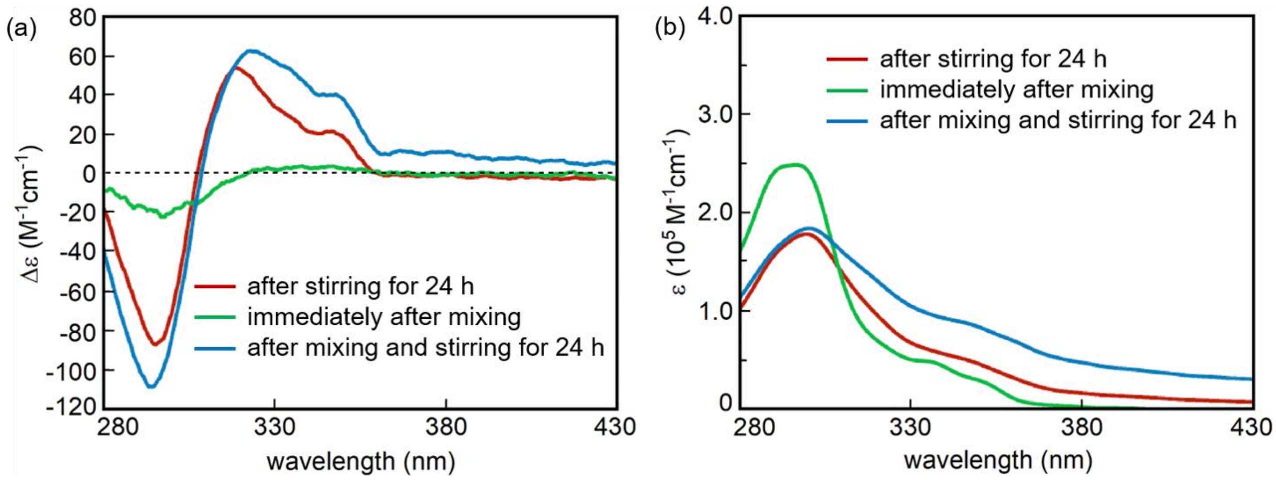


Figure S23. CD spectra (a) and UV-vis spectra (b) of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total, 0.5 mM) at 25 °C by mixing experiment of hetero-double-helix ent-**B** (45:55) and random-coil (50:50).

7. Stop-stirring experiment

(*P*)-**1** (4.89 mg, 1.25 × 10^{-3} mmol) and (*M*)-**1** (4.89 mg, 1.25 × 10^{-3} mmol) were weighed in a cylindrical glass vial (diameter, 21 mm) by a microbalance within error of 0.5%. The samples were dissolved in trifluoromethylbenzene (5.0 mL) by heating to 90 °C for 10 min to afford a sample solution (total 0.5 mM). Then, the solution was cooled to 25 °C, and mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm at 25 °C for 5 h, during which $\Delta\varepsilon$ at 322 nm was monitored every hour. Stirring was stopped for 5 h, during which $\Delta\varepsilon$ at 322 nm was monitored every hour. The same procedures were repeated for the next 20 h (Figure 7).

8. Proximate stochastic chiral symmetry breaking by mixing experiments

<Mixing of hetero-double-helix B (55:45) and homo-double-helix (*M*)-1>

A solution of (*M*)-**1** in trifluoromethylbenzene (0.50 mL, 0.5 mM) was prepared by dissolving (*M*)-**1** (0.978 mg, 2.5 × 10^{-4} mmol) by heating to 90 °C for 10 min and cooled to 0 °C (solution I). A 55:45 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.5 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (1.61 mg, 4.13 × 10^{-4} mmol) and (*M*)-**1** (1.32 mg, 3.38 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). Then, the solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 24 h (solution II). A part of solution I (0.1 mL) and a part of solution II (1.0 mL) were mixed, and mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 20 h. Then, CD and UV-vis analyses were conducted (Figures 8a and 8b).

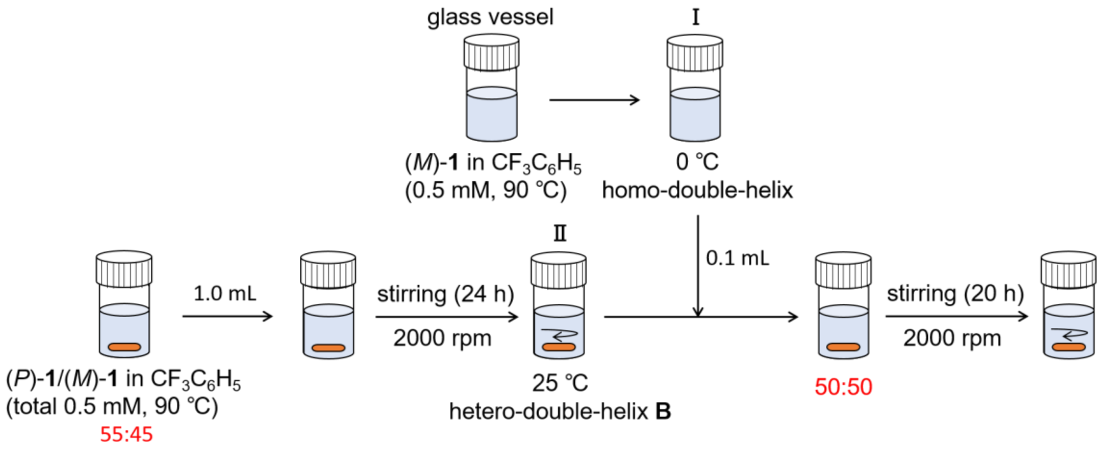


Figure S24. Procedure of mixing experiment of hetero-double-helix **B** (55:45) and homo-double-helix (*M*)-**1**.

<Mixing of hetero-double-helix B (55:45) and random-coil (*M*)-1>

A solution of (*M*)-**1** in trifluoromethylbenzene (0.50 mL, 0.5 mM) was prepared by dissolving (*M*)-**1** (0.978 mg, 2.5×10^{-4} mmol) by heating to 90 °C for 10 min and cooled to 60 °C (solution I). A 55:45 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.5 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (1.61 mg, 4.13×10^{-4} mmol) and (*M*)-**1** (1.32 mg, 3.38×10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). Then, the solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 20 h (solution II). A part of solution I (0.1 mL) and a part of solution II (1.0 mL) were mixed, and mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 20 h. Then, CD and UV-vis analyses were conducted.

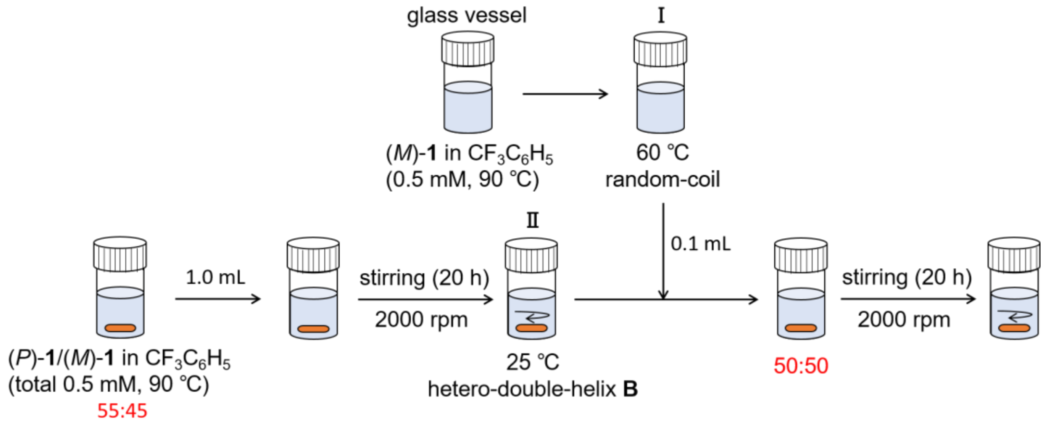


Figure S25. Procedure of mixing experiment of hetero-double-helix **B** (55:45) and random-coil (*M*)-**1**.

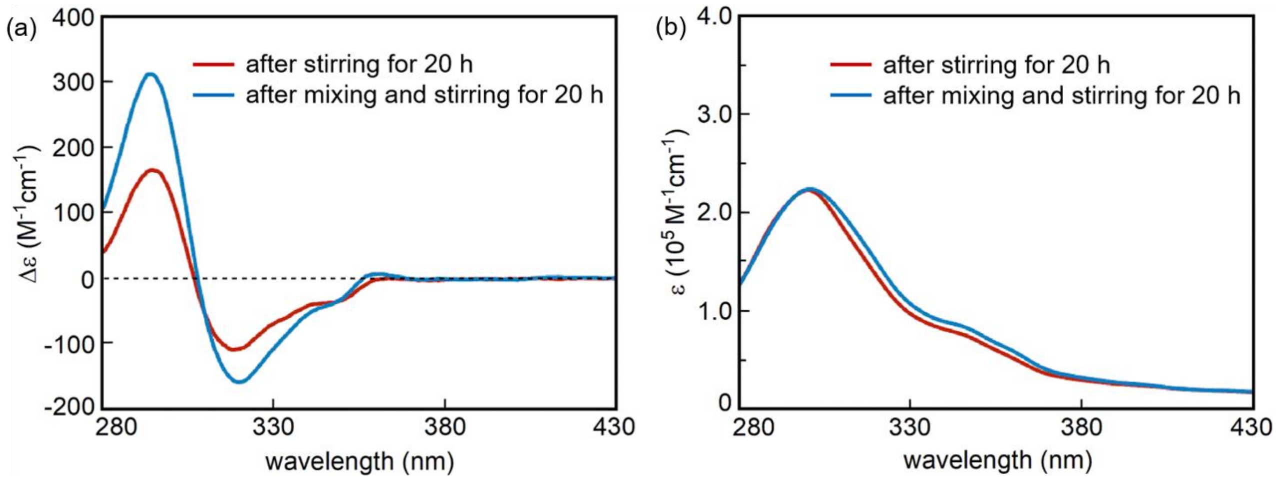


Figure S26. CD spectra (a) and UV-vis spectra (b) of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total 0.5 mM) at 25 °C formed by mixing experiment of hetero-double-helix **B** (55:45) and random-coil (*M*)-**1**.

<Mixing of hetero-double-helix B (45:55) and homo-double-helix (*P*)-1>

A solution of (*P*)-**1** in trifluoromethylbenzene (0.50 mL, 0.5 mM) was prepared by dissolving (*P*)-**1** (0.978 mg, 2.5 × 10^{-4} mmol) with heating to 90 °C for 10 min and cooled to 0 °C (solution I). A 45:55 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.5 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (1.32 mg, 3.38 × 10^{-4} mmol) and (*M*)-**1** (1.61 mg, 4.13 × 10^{-4} mmol) with heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). Then, the solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 25 h (solution II). A part of solution I (0.1 mL) and a part of solution II (1.0 mL) were mixed, and mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 40 h. Then, CD and UV-vis analyses were conducted, in which CD spectrum inverted from that of solution I (Figures 8c and 8d).

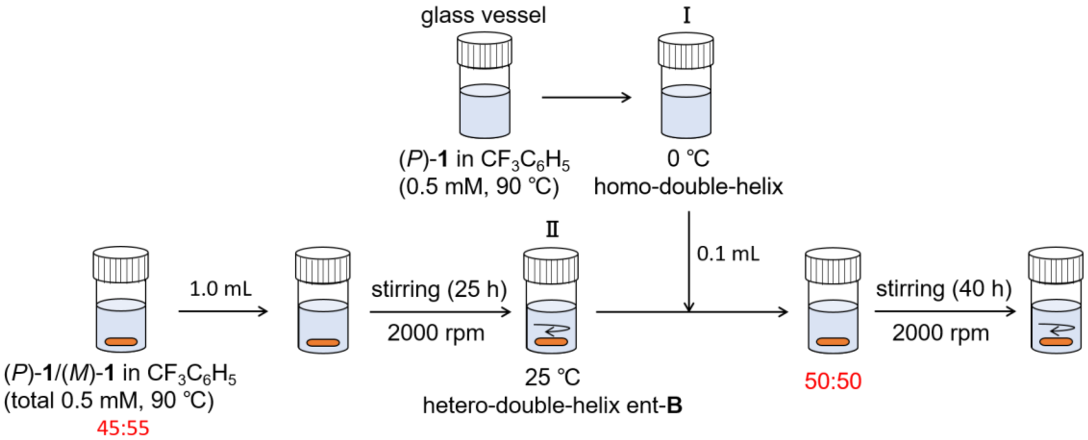


Figure S27. Procedure of mixing experiment of hetero-double-helix **B** (45:55) and homo-double-helix (*P*)-**1**.

<Mixing of hetero-double-helix B (45:55) and random-coil (*P*)-1>

A solution of (*P*)-**1** in trifluoromethylbenzene (0.50 mL, 0.5 mM) was prepared by dissolving (*P*)-**1** (0.978 mg, 2.5 × 10^{-4} mmol) with heating to 90 °C for 10 min and cooled to 60 °C (solution I). A 45:55 mixture of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (1.5 mL, total 0.5 mM) was prepared by dissolving (*P*)-**1** (1.32 mg, 3.38 × 10^{-4} mmol) and (*M*)-**1** (1.61 mg, 4.13 × 10^{-4} mmol) by heating to 90 °C for 10 min in a cylindrical glass vial (diameter, 12 mm). Then, the solution was cooled to 25 °C, and was mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 20 h (solution II). A part of solution I (0.1 mL) and a part of solution II (1.0 mL) were mixed, and mechanically stirred in clockwise direction with an oval-shaped Teflon magnetic stirring bar (weight, 0.40 g) at the rate of 2000 rpm for 40 h. Then, CD and UV-vis analyses were conducted, in which CD spectrum inverted from that of solution I.

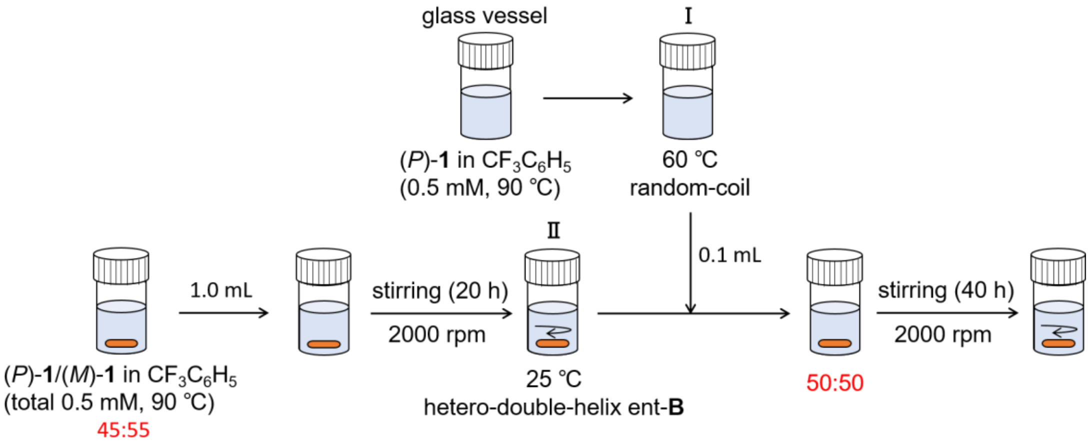


Figure S28. Procedure of mixing experiment of hetero-double-helix **B** (45:55) and random-coil (*P*)-**1**.

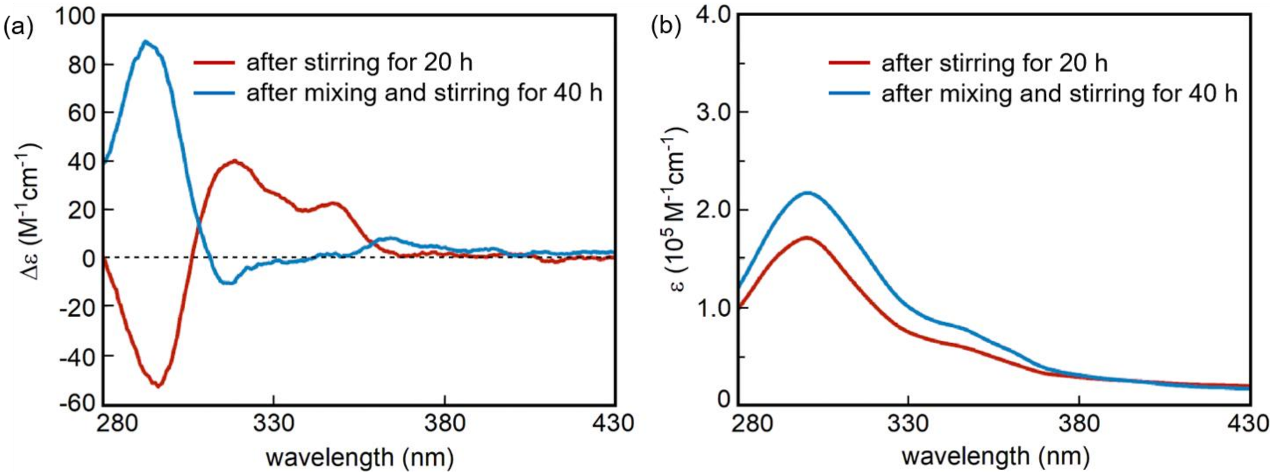


Figure S29. CD spectra (a) and UV-vis spectra (b) of (*P*)-**1**/(*M*)-**1** in trifluoromethylbenzene (total 0.5 mM) at 25 °C formed by mixing experiment of hetero-double-helix ent-**B** (45:55) and random-coil (*P*)-**1**.

9. References

1. Y. Kushida, T. Sawato, M. Shigeno, N. Saito, M. Yamaguchi, *Chem. Eur. J.* **2017**, *23*, 327-333.