Electronic Supplementary Information to:

Right-handed 2/1 Helical Arrangement of Benzene Molecules in Cholic Acid Crystal Established by Experimental and Theoretical Circular Dichroism Spectroscopy

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Experimental

Materials. Cholic acid (CA, 98%, TCI Co., Ltd.), benzene (CR, Kanto Chemistry Co., Inc), 1butanol (AR, Wako Pure Chemical Industries, Ltd.), d_4 -methanol (99.8%, Acros), and liquid paraffin (CR, Kanto Chemistry Co., Inc) were used as obtained.

Measurements. ¹H NMR spectra were taken on a JEOL JNM-EC400 spectrometer (400 MHz for ¹H measurement). CD and Linear dichroism (LD) spectra were recorded on a JASCO-820 spectrometer equipped with a diffuse reflection detecting system. Thermal gravity analysis (TGA) was taken on a Rigaku Thermo Plus TG8120 analyzer at a heating rate of 10 K/min in nitrogen atmosphere. Optical microscopic photographs were taken using a Nikon Eclipse E600 POL microscope. Peak resolution by Gaussian curve fitting of CD spectra was conducted using an Origin Pro 2015J software package.

Procedure of including benzene in cholic acid crystal.^{1,2} In a 20 mL vial, a mixture of CA (500 mg, 1.22 mmol) and 1-butanol (5 mL) was heated to clear, and then filtered to another warm 20 mL vial. After the solution was cooled to room temperature, benzene (5 mL, 4.4 g, 56 mmol) was added, and the consequent solution was allowed to settle overnight at 20 °C. Inclusion crystals were obtained by collecting the solid and removed the adhering solvent and benzene on the surface. The crystal sample was ground using a ceramic mortar to fine powder.

Diffuse reflectance CD/LD spectral measurements. CA and benzene-CA crystals were ground carefully to powder in order to minimize the contributions of LD and obtain true CD spectra (Fig. S13). The finely ground crystal powder sample was included in a cell with a quartz window. The CD and LD spectra were obtained by averaging those recorded at four (90 ° interval) different cell orientations (angles) with the cell quartz window positioned vertically to the incident light beam for measurement (Figs. S3, S5, S7, and S9). Absorbance spectra were obtained using the CD spectrometer.

Transmittance UV/CD/LD spectral measurements using nujol mulls. The finely ground crystal powder (30 mg) was mixed with 2 mL of liquid paraffin, and the mixture was vigorously stirred using a spatula to lead to a roughly homogeneous suspension (nujol mull). The obtained nujol mull was drop-casted onto a quartz glass plate (1 cm x 2 cm x 1 mm (thickness)) and then was covered with another quartz plate. Air bubbles were squeezed out from the sandwiched cell by manually pressing the two glass plates toward each other. Pure liquid paraffin was used as the control sample to obtain blank spectra. The UV, CD, and LD spectra were obtained by averaging those recorded at four (90 $^{\circ}$ interval) different cell orientations (angles) with the cell

quartz window positioned vertically to the incident light beam for measurement (Figs. S14, S16, S18, and S20). The CD spectra of the finely ground samples taken by transmittance mode well matched the spectral of the corresponding samples taken by diffuse reflectance mode (Figs. S22 and S23).

Theoretical Calculation

The intrinsic rotational strengths were calculated using Gaussian 03 package.³ 120 singlet excited states of two benzene molecules arranged in the 2/1 helix were considered in the time dependent density functional theory (TDDFT) framework. The Grimme's functional (B97D)⁴ and 6-31 G** basis sets were employed, after a geometry optimization at the same level. Rotatory strengths are reported in the usual c.g.s. units of 10^{-40} esu.cm.erg/Gauss. The contribution of the hydroxyl and carboxyl groups belonging to cholic acid were taken into account by adding methanol and formic acid molecules to the two benzenes. Those groups were inserted in the same position occurring in two cholic acid units. 120 singlet excited states of the former molecular framework were calculated consistently with the ECD calculation described for the two benzene molecules. The rotational strength R_i associated with an electronic transition $0 \rightarrow 1$ at frequency v_i can be estimated from quantum-mechanics from the dipole-length or the dipole-velocity formalism as follows:^{5,6}

$$Ri(length) = Im(\mu_{0i} \cdot m_{i0}) \tag{1}$$

$$Ri(velocity) = \frac{-eh}{4\pi^2 m v_i} Im(\nabla_{0i} \cdot m_{i0})$$
(2)

Where μ_{0i} is the electric dipole transition moment and \mathbf{m}_{io} the magnetic dipole transition moment. No significant differences between dipole-velocity and dipole length formalism were observed. The calculations of the ECD spectra at a given wavelength, λ were done assuming Gaussian bands with 1000 cm⁻¹ full width at half-height (from 150 nm to 350 nm) for all transitions centered in a given excitation wavelength. A factor of 2.278 was applied during the conversion of rotational strength and $\Delta \varepsilon$ values, as reported in references.^{7,8}

The calculations of electronic UV spectra were done in the same way, assuming the factor 2.8710^4 accounting for the conversion between oscillator strengths and the molar extinction coefficients.⁹



Fig. S1. ¹H NMR spectrum of the benzene-CA crystal recorded in d_4 -methanol [400 MHz, ambient temperature].

Note: ¹H NMR spectrum shows three characteristic peaks at 3.80, 3.97 and 7.34 ppm respectively, among which the first two peaks are separately assigned to two protons of CA, while the last one is ascribed to six protons of benzene. The integrated area ratio of these three peaks is 1:1:5.2.



Fig. S2. TGA profiles of the benzene-CA crystal recorded at a heating rate of 10 K/min. The green and blue lines refer to TG loss and heat flow, respectively.



Fig. S3. Raw CD spectra of finely ground benzene-CA crystal recorded at different orientations at every 90° of the sample within a plane vertical to the incident light beam.



Fig. S4. Averaged CD spectra of finely ground benzene-CA crystal.



Fig. S5. Raw LD spectra of finely ground benzene-CA crystal recorded at different orientations at every 90° of the sample within a plane vertical to the incident light beam.



Fig. S6. Averaged LD spectra of finely ground benzene-CA.



Fig. S7. Raw CD spectra of finely ground CA crystal recorded at different orientations at every 90° of the sample within a plane vertical to the incident light beam.



Fig. S8. Averaged CD spectra of finely ground CA crystal.



Fig. S9. Raw CD spectra of as-obtained benzene-CA crystal recorded at different orientations at every 90° of the sample within a plane vertical to the incident light beam.



Fig. S10. Averaged CD spectra of as-obtained benzene-CA crystal.



Fig. S11. Raw LD spectra of as-obtained benzene-CA crystal recorded at different orientations at every 90° of the sample within a plane vertical to the incident light beam.



Fig. S12. Averaged LD spectra of as-obtained benzene-CA crystal.

Fig. S13. Microscopic photograph of finely ground benzene-CA crystal (a), and as-obtained benzene-CA crystal (b).

Fig. S14. Raw CD spectra of benzene-CA crystal in nujol recorded at different orientations at every 90° of the sample within a plane vertical to the incident light beam.

Fig. S15. Averaged CD spectra of benzene-CA crystal in nujol.

Fig. S16. Raw LD spectra of benzene-CA crystal in nujol recorded at different orientations at every 90° of the sample within a plane vertical to the incident light beam.

Fig. S17. Averaged LD spectra of benzene-CA crystal in nujol.

Fig. S18. Raw CD spectra of CA crystal in nujol recorded at different orientations at every 90° of the sample within a plane vertical to the incident light beam.

Fig. S19. Averaged CD spectra of CA crystal in nujol.

Fig. S20. Raw LD spectra of CA crystal in nujol recorded at different orientations at every 90° of the sample within a plane vertical to the incident light beam.

Fig. S21. Averaged LD spectra of benzene-CA crystal in nujol.

Fig. S22. Comparison between normalized CD spectra of Benzene-CA crystal measured by transmittance mode using nujol mull (red) and by diffuse reflectance mode (blue).

Fig. S23. Comparison between normalized CD spectra of CA crystal measured by transmittance mode (red) and by diffuse reflectance mode (blue).

Fig. S24. Theoretical spectrum of the benzene-CA crystal model (Fig. 2D) (a), experimental spectrum of benzene-CA crystal (b) and theoretical spectrum of the two benzenes model (Fig. 2C) (c).

Fig. S25. Calculated CD spectra of CA, one benzene with CA groups, two benzenes with CA groups, and four benzene with CA groups.

Fig. S26. Theoretical CD spectrum of two CA molecules model (Fig. 2B) (a) and experimental spectrum of pure CA crystal (b) [panel **A**], and theoretical spectrum of the benzene-CA crystal model (Fig. 2D) (c) and experimental spectrum of benzene-CA crystal (d) [panel **B**] in $\Delta \epsilon$ unit for intensity.

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Fig. S27. (a) Solvent accessible surface of the crystals visualized by the Mercury Software,¹⁰⁻¹³ whose shape tightly fits (b) the molecular arrangement of the included benzene molecules.

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