Supporting Information

The axial chirality hidden in vitamin D and its application in cocrystal prediction

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EXPERIMENTAL SECTIOIN

Materials. Vitamin D_3 (**A**) and cholesterol (**C**) were obtained from Zhejiang Garden Biochemical High-Tech Co., LTD. Calcifediol monohydrate (**B**), and cholestanol (**D**) were bought from J&K Chemical Ltd, with greater than 99% purity. All analytical grade solvents were purchased from Sinopharm Chemical Reagent Co., Ltd and used without further purification.

Preparation of BC. A mixture of **B** (41.6 mg, 0.1 mmol) and **C** (38.6 mg, 0.1 mmol) was dissolved in 3 mL acetonitrile in a sealed flask, and stirred at room temperature for 8 h. The resulting solution was filtrated and evaporated slowly at 4 °C. Crystals grew within a few days. The colorless needle-shaped crystals of **BC** were recovered by filtration.

Preparation of BD. A mixture of **B** (41.6 mg, 0.1 mmol) and **D** (38.8 mg, 0.1 mmol) was dissolved in 5 mL Acetonitrile by ultrasound. The resulting solution was filtrated and evaporated slowly in a desiccator at 25 °C. Crystals grew within a few days. The colorless needle-shaped crystals of **BD** were recovered by filtration.

Powder X-ray diffraction (PXRD). PXRD patterns were obtained using a Bruker D8 Advance X-ray diffractometer (Cu Kα radiation). Voltage and current of the generator was set to 40 kV and 40 mA, respectively. Data over the range 3−40° 2θ were collected with a scan rate of 5°/min at ambient temperature. The data were imaged and integrated with RINT Rapid and peaks were analyzed with Jade 6.0 from Rigaku. Calibration of the instrument was performed using a corindon standard (Bruker AXS Korund-probe).

Single-crystal X-ray diffraction (SCXRD). X-ray diffractions of all single crystals were carried out on a Bruker Apex II CCD diffractometer using Cu Kα radiation (λ = 1.54178 Å) and Mo Kα radiation (λ = 0.71073 Å) for **BC** and **BD**, respectively. The collected data integration and reduction were processed with SAINT software,[1] and multi-scan absorption corrections were performed using the SADABS program.[2] The structures were solved by direct methods using SHELXTL[3] and were refined on F^2 by the full-matrix least-squares technique using the SHELXL-2014 program package.[4] All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms of O-H and N-H were found from a Fourier difference map, and refined with a fixed distance of 0.86 (0.01) Å and isotropic displacement parameters of 1.50 times U_{eq} of the parent atoms. The remaining hydrogen atoms were placed in calculated positions and refined with a riding model with distance of 0.95 Å (sp^2) and 0.98 Å (sp^3) with isotropic displacement parameters set to 1.20 (sp^2) and 1.50 (sp^3) times U_{eq} of the parent atom. Crystallographic data in cif format have been deposited in the Cambridge Crystallographic Data Center, CCDC No. 1977141-1977142. Crystallographic data and refinement details are summarized in Table S1.

Thermogravimetric analysis (TGA). Thermogravimetric analysis was carried out on Netzsch TG 209F3 equipment. Samples were placed in open aluminum oxide pans and heated at 10 °C min^{-1} to 400 °C. Nitrogen was used as purge gas at 20 mL min^{-1}.

Differential scanning calorimetry (DSC). DSC experiments were performed on a DSC TA Q2000 instrument under a nitrogen gas flow of 50 $\mathrm{mL \cdot min^{-1}}$ purge. Ground samples weighing 1−3 mg were heated in sealed aluminum pans at a heating rate 10 $\mathrm{°C \cdot min^{-1}}$. Two-point calibration using indium and tin was carried out to check the temperature axis and heat flow of the equipment.

Solid-state electronic circular dichroism (ECD). Solid-state ECD spectroscopy was carried out on BRIGHTTIME chirascan equipment by Disk from. A mixture of samples and inert medium (KBr) was pressed into a transparent circular sheet. Data over the range 400-190 nm were collected with a scan rate of 50-80 $\mathrm{nm \cdot min^{-1}}$ at ambient temperature. ECD (KBr) λ_{max} (mdeg), 0.1 mg of **A** (polymorph B) 100 mg KBr: 269 (–0.71), 207 (+ 9.18). ECD (KBr) λ_{max} (mdeg), 0.1 mg of **AC** in 100 mg KBr: 264 (+0.99), 220 (–9.41).

ECD titration. ECD titration is a titrimetric method for determination of binding contants. **C** was gradually add to the 0.1 mg/mL solution of **A** or **B**, the changes of the absorption of **A** or **B** upon the concentration of **C** were record by a ECD spectrophotometer.

Computational Details Geometry optimizations were calculated at the B3LYP/6-31G(d) level in Gaussian09.[5] ECD calculations were carried out by using time dependent density functional theory (TDDFT) at the same functional and basis set. The ECD spectra of conformers were obtained by using SpecDis 1.62.[6]

Job's plot. Job's plot experiments were performed on BRIGHTTIME chirascan instrument. In this method, the total molar concentration of the two binding partners (eg **A** and **C**, **B** and **C**, or **B** and **D**) are held constant, but their mole fractions are varied. By plotting the difference of the ECD signal of a solution of host between the ligand added or not added against the mole fraction of ligand, the maximum on the plot corresponds to the stoichiometric ratio of the two species. The total molar concentrations of **A** and **C** are 2.1×10^{-3} mol/L, **B** and **C** are 1×10^{-2} mol/L.

Reference

[1] G.M. Sheldrick, SAINT and XPREP, 5.1 ed.; Siemens Industrial Automation Inc.: Madison, WI, 1995.

[2] SADABS, Empirical Absorption Correction Program; University of Göttingen: Göttingen, Germany, 1997.

[3] G.M. Sheldrick, SHELXTL Reference Manual: Version 5.1; Bruker AXS: Madison, WI, 1997.
[4] G. M. Sheldrick, *Acta Crystallogr. C*, 2015, **71**, 3-8.
[5] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, Jr. J. A. Montgomery, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, Gaussian 03, Revision C.02. 2004, Gaussian Inc., Wallingford CT.
[6] X. F. Wang, F. F. Liu, Z. Zhu, Q. Q. Fang, S. J. Qu, W. Zhu, L. Yang, J. P. Zuo and C. H. Tan, *Fitoterapia*, 2019, **133**, 96-101.

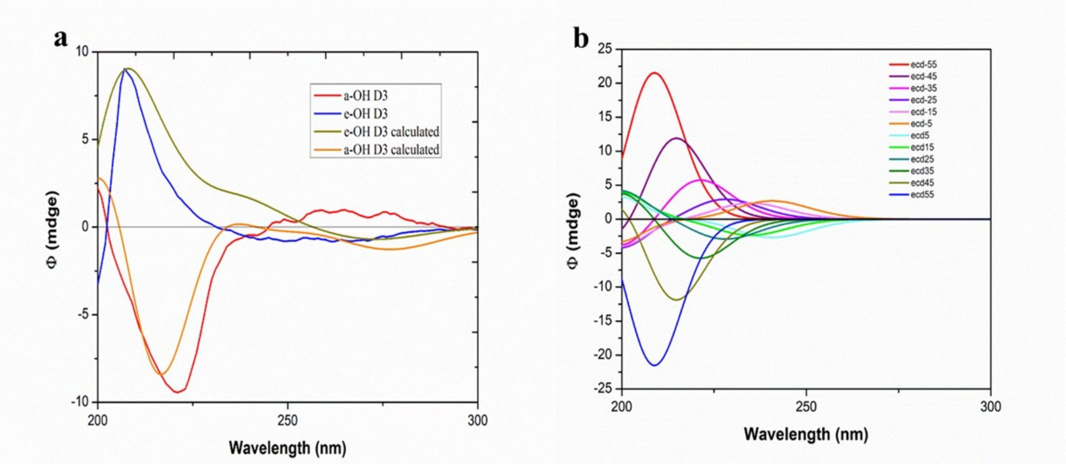

Figure S1. Experimental and calculated ECD spectra. **a**, Solid-state ECD spectra of two D_3 conformers recorded as KBr disk and TDDFT calculated ECD spectra (B3LYP/631G(d)) using X-ray geometry as input. **b**, simulated ECD spectra of 1,2-dimethylenehexane (B3LYP/631G(d)).

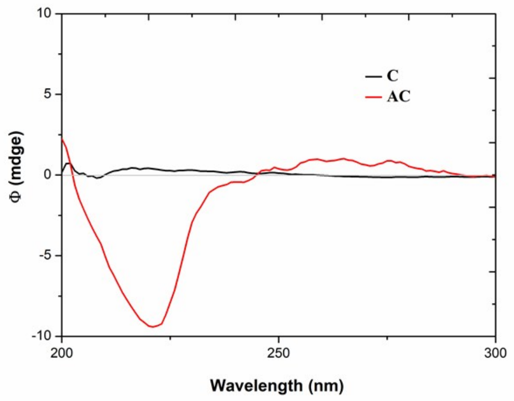

Figure S2. Solid-state ECD spectrum of cholesterol compared with that of **AC**.

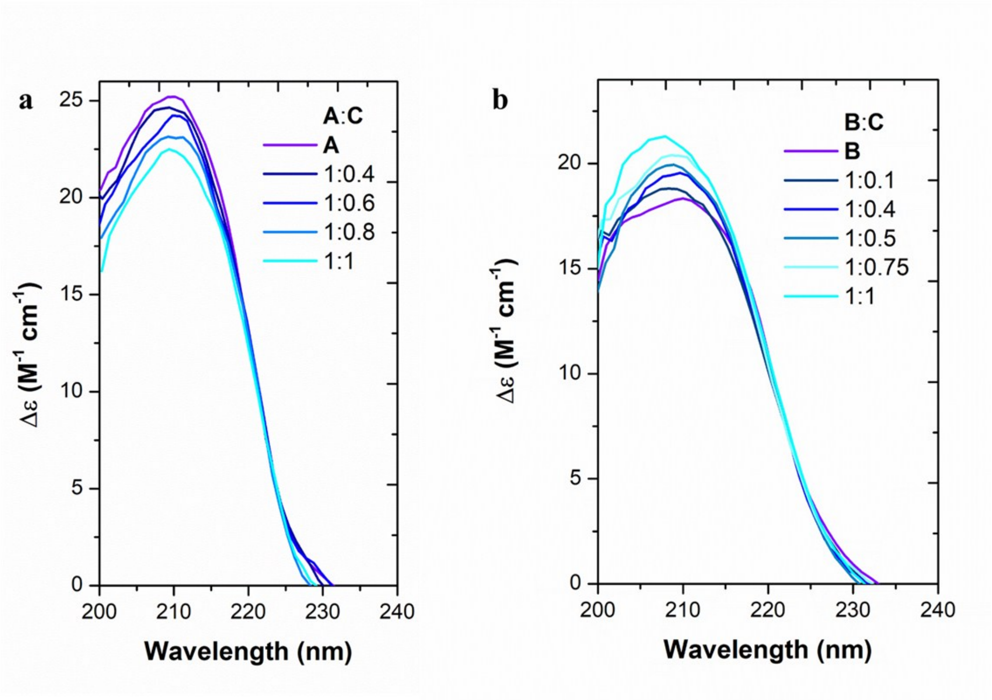

Figure S3. The peak value at 210 nm. **a**, decrease with increasing ratios of cholesterol in acetonitrile solution. **b**, increase with increasing ratios of cholesterol in acetonitrile solution. The inset depicts the ratio of **A** to **C**.

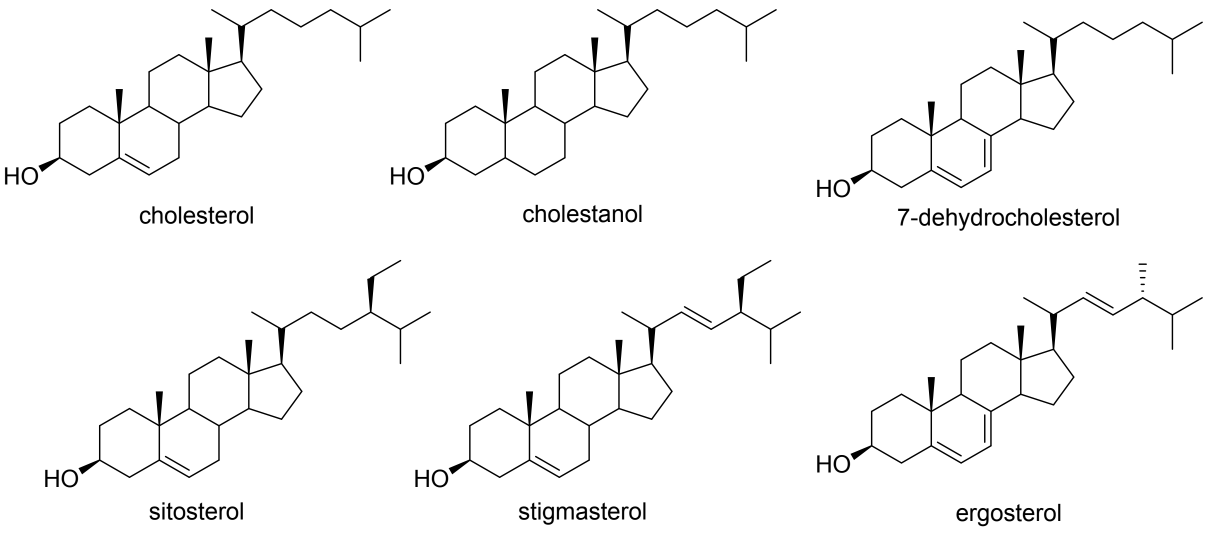

Figure S4. Chemical structures of steroids for **B** cocrystal screening.

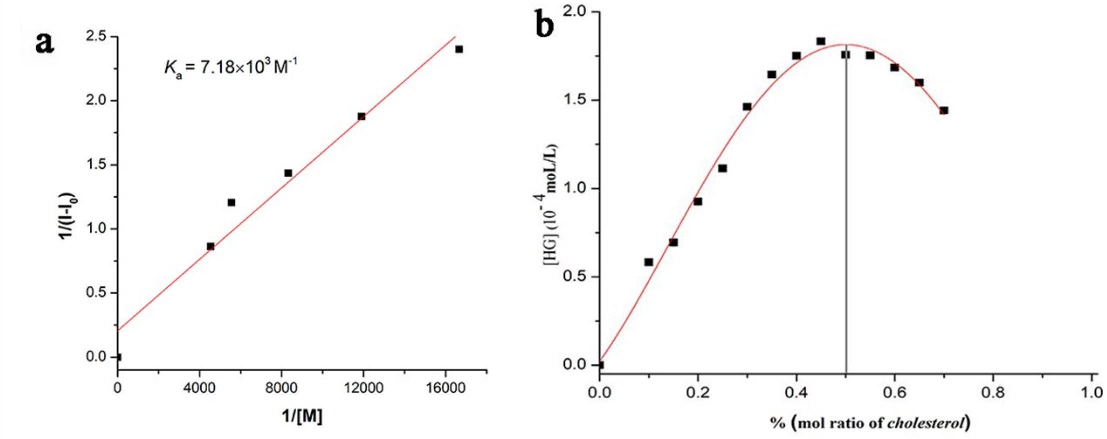

Figure S5. Solution ECD spectra of **B** in solution. **a**, titration experiment, K_a calculated at 210 nm in acetonitrile at 293 K. The red lines show the best fitting. **b**, Job's plot under the same conditions as the titration experiment.

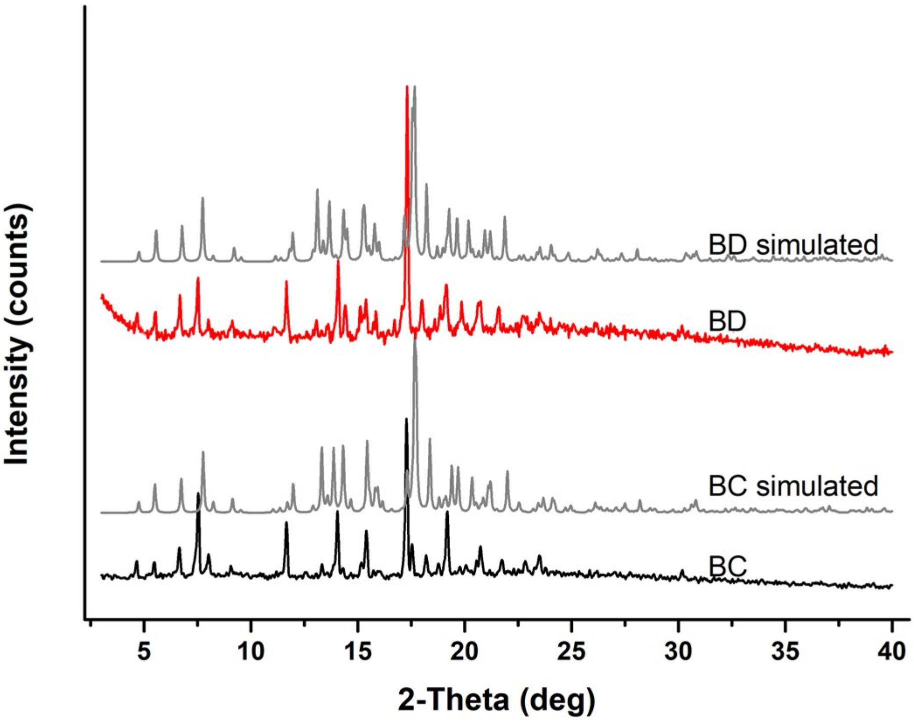

Figure S6. PXRD patterns of **BC** and **BD**.

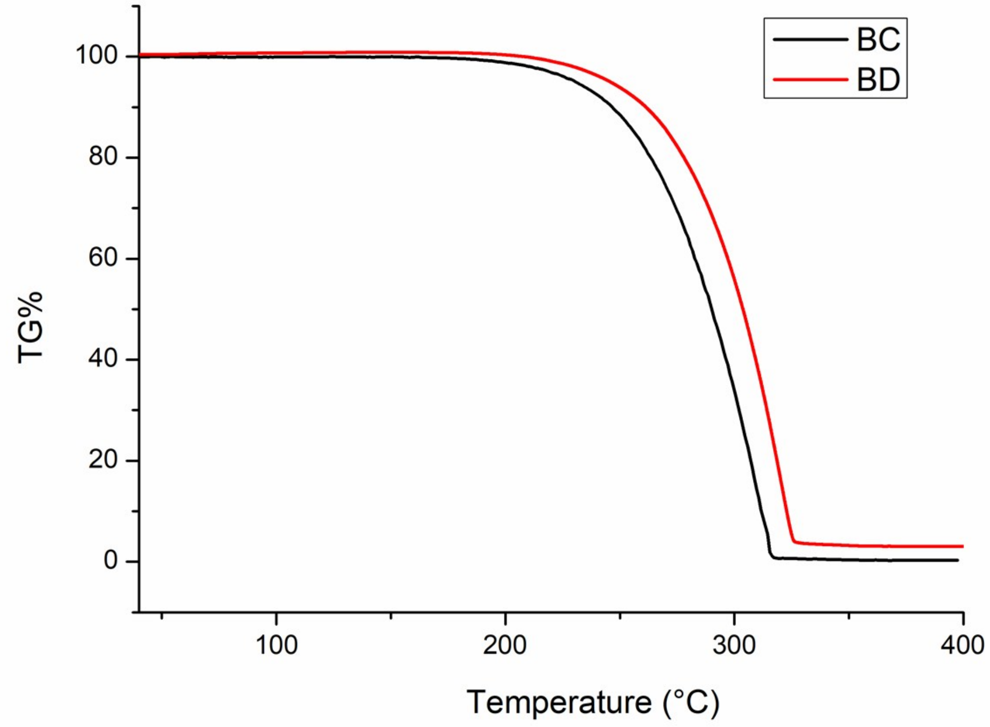

Figure S7. TGA curves of **BC** and **BD**.

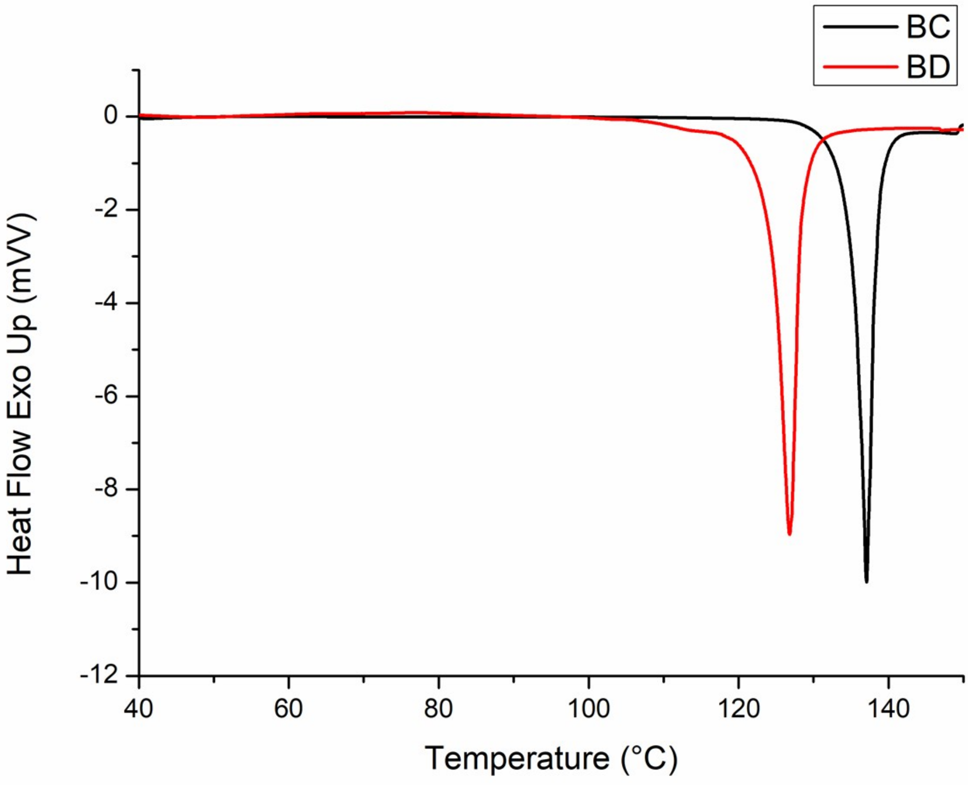

Figure S8. DSC curves of **BC** and **BD**.

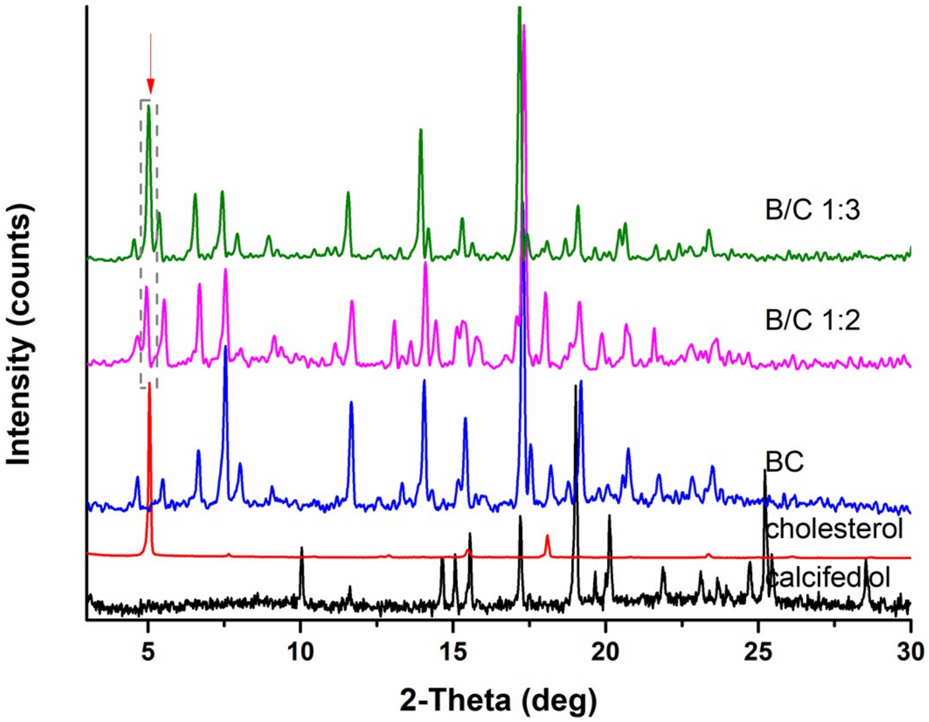

Figure S9. XRPD patterns of **BC** and results of excess of coformer for cocrystallization.

Table S1 Crystallographic Data for cocrystals.

	AC[a]	**BC**	**BD**
Formula	$C_{54}H_{90}O_2$	$C_{54}H_{90}O_3$	$C_{54}H_{92}O_3$
Formula weight	771.26	787.25	789.27
Crystal system	monoclinic	orthorhombic	orthorhombic
Space group	C_2	$P2_12_12_1$	$P2_12_12_1$
Temperature (K)	100(2)	173	170
a (Å)	33.5853(15)	6.79080(10)	6.9042(8)
b (Å)	6.6602(3)	22.7451(4)	22.803(3)
c (Å)	21.7816(10)	32.0260(7)	31.694(4)
α (°)	90	90	90
β (°)	99.059(2)	90	90
γ (°)	90	90	90
Cell volume (Å^3)	4811.4(4)	4946.65(16)	4989.8(11)
Calc. density (g/cm^3)	1.062	1.057	1.051
Z	4	4	4
Z'	1	1	1
λ	0.71073	1.54178	0.71073
S	1.035	1.031	1.031
R_1	0.053	0.053	0.068
R_{int}	0.029	0.038	0.077
wR_2	0.125	0.134	0.150
CCDC no.	942948	1977141	1977142

[a] *Data reported in ChemComm,2014, 50, 855-858*

[b] *Z* represents the number of cocrystal formula in a unit cell

Table S2 Hydrogen bonding distances and angles for **BC** and **BD**

	D−H···A	H···A (Å)	D···A (Å)	∠D−H···A (°)	Symmetry code
BC	O_1−H_1···O_3	1.88	2.803(4)	173	1/2+x,1/2-y,1-z
	O_2−H_2···O_1	1.97	2.804(3)	174	3/2-x,1-y,-1/2+z
	O_3−H_3···O_2	1.93	2.763(3)	174	2-x,-1/2+y,1/2-z
BD	O_1−H_1···O_3	1.97	2.825(5)	177	1-x,1/2+y,1/2-z
	O_2−H_2···O_1	1.96	2.834(5)	176	1/2-x,1-y,1/2+z
	O_3−H_3···O_2	1.90	2.789(5)	169	-1/2+x,1/2-y,1-z