Supporting Information

Outer-surface adduct formation and selective separation of *cis*-type gallated and *cis*-type non-gallated catechins using cucurbit[7]uril

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Experimental

1. Materials

(−)-Epigallocatechin-3-*O*-gallate (EGCg), (−)-epicatechin-3-*O*-gallate (ECg), (−)-epigallocatechin (EGC), and (−)-epicatechin (EC) were purchased from BLD Pharm. Deuterium oxide (D_2O, 99.8%) was obtained from Eurisotop. Phosphoric acid (H_3PO_4) and methanol (MeOH) for HPLC were purchased from FUJIFILM Wako Pure Chemical Corporation. All reagents were used as received without further purification. Cucurbit[7]uril (CB[7]) and cucurbit[5]uril (CB[5]) were synthesized and purified as described by Bardelang et al.[1]

2. ^{1}H NMR experiments

NMR experiments were performed using a JMN-ECZ600R spectrometer (JEOL, Japan) operating at 600 MHz. The spectra were recorded at 298 K in D_2O. ^{1}H NMR spectra were obtained using a spectral width of 9,000 Hz and acquisition parameters of 16, 256, or 512 scans. Data processing and spectral analysis were performed using Delta NMR software version 6.1.0 (JEOL USA, Inc.). The residue peak HOD (4.65 ppm) was used as the standard in D_2O.

3. UV measurements

UV spectroscopy was performed using a UVmini-1240 spectrophotometer (SHIMADZU CORPORATION, Japan) at 298 K. Absorbance measurements were recorded at the following wavelengths: 273.4 nm for EGCg, 276.2 nm for ECg, 270.2 nm for EGC, and 278.2 nm for EC.

4. HPLC experiments

HPLC was performed using an LC-20A pump coupled with an SPD-10AV UV–visible detector (SHIMADZU CORPORATION, Japan) and a reverse-phase InertSustain C30 column (250 mm × 4.6 mm I.D., GL Sciences Inc., Japan). At 220 nm, *cis*-type catechins were detected. The analysis used a linear gradient system with H_3PO_4 (0.1% v/v) and MeOH, eluting at a flow rate of 0.5 mL/min at 313 K (CTO-10A Column Oven, SHIMADZU CORPORATION, Japan). The injection volume for all analyses was 10 μL. The gradient elution was as follows: 0–20 min: MeOH increased linearly from 10% to 50%; 20–30 min: 50% H_3PO_4 (0.1% v/v) and 50% MeOH; and 30–40 min: 90% H_3PO_4 (0.1% v/v) and 10% MeOH.

5. Calculation of the residue rate of *cis*-type catechins in a solution containing a specific type of *cis*-type catechin and either CB[7] or CB[5] based on UV measurements

5-1. CB[7]

An aqueous solution (2 mL) containing 0.5 mM *cis*-type catechins (EGCg, ECg, EGC, or EC) and varying concentrations of CB[7] (0, 0.25, 0.5, 1, 2, or 3 mM) was incubated at a constant temperature (278, 283, 288, 293, or 298 K) for 3 h. The mixture was then centrifuged at 12,000 rpm for 15 min. The supernatant (1 mL) was diluted with water (3 mL) and subjected to UV measurement.

5-2. CB[5]

A 2.5 M HCl solution (2 mL) containing 0.5 mM *cis*-type catechins (EGCg, ECg, EGC, or EC) and varying concentrations of CB[5] (0, 0.25, 0.5, 1, 2, or 3 mM) was incubated at a constant temperature (278, 283, 288, 293, or 298 K) for 3 h. The mixture was then centrifuged at 12,000 rpm for 15 min. The supernatant (1 mL) was diluted with 2.5 M HCl solution (3 mL) and subjected to UV measurement.

6. Determination of the mass and composition of the precipitate arising from the solution containing a specific type of *cis*-type catechins and either CB[7] or CB[5]

The remaining solution (1 mL) from sections 5-1 and 5-2, which was not used for calculating the residue rate of *cis*-type catechins, was filtered through a polytetrafluoroethylene membrane filter (pore size: 0.5 μm, ADVANTEC Toyo Kaisha, Ltd.) to collect the precipitate. The precipitate was dried under reduced pressure overnight, and its mass was measured using a Cubis® MCA6.6S-2S01-M microbalance (Sartorius AG, Germany). For further analysis, the precipitates were dissolved in D_2O for CB[7] or in a 0.9% w/v NaCl D_2O solution for CB[5], and their compositions were determined by ^1H NMR measurements.

7. Selective separation of *cis*-type gallated catechins and *cis*-type non-gallated catechins using CB[7]

A 400 μL aqueous solution containing 0.25 mM EGCg, ECg, EGC, and EC, along with 2 mM CB[7], was maintained at a constant temperature of 293 K for 3 h. The solution was then centrifuged at 12,000 rpm for 15 min. The resulting supernatant (20 μL) was diluted with 180 μL of an H_2O/MeOH (9:1, v/v) mixture and subjected to HPLC analysis.

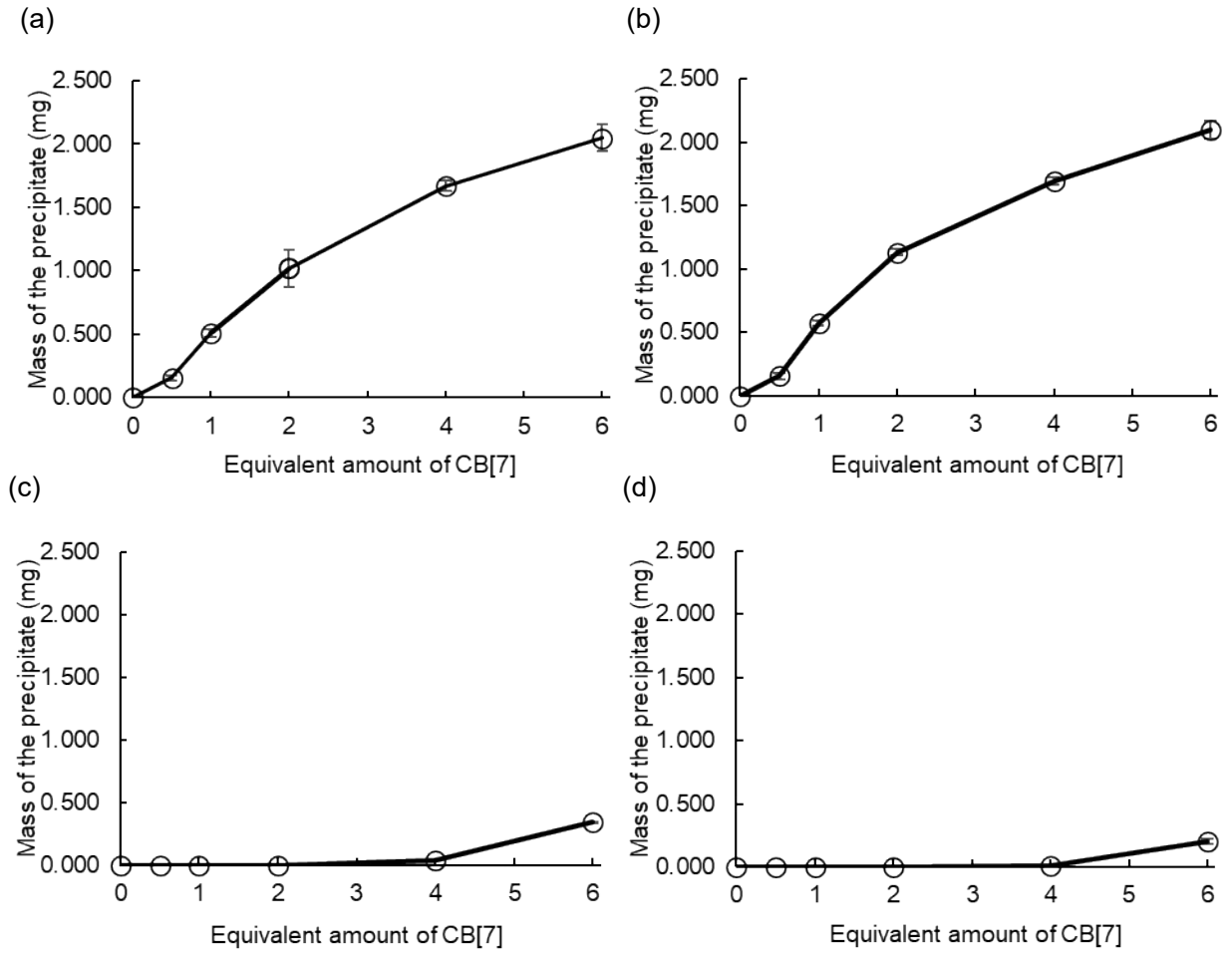


Fig. S1 The mass of the precipitate obtained from filtering these solutions increased with the equivalents of CB[7] for all *cis*-type catechins (n = 3). (a) EGCg, (b) ECg, (c) EGC, and (d) EC

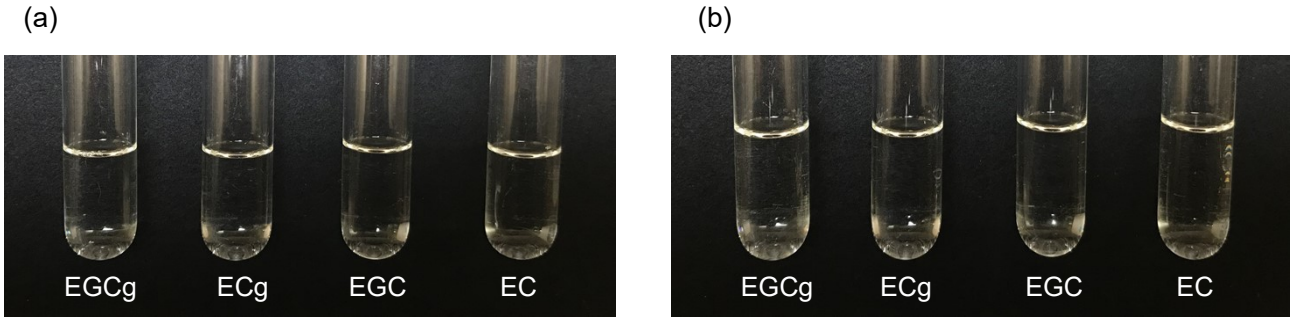
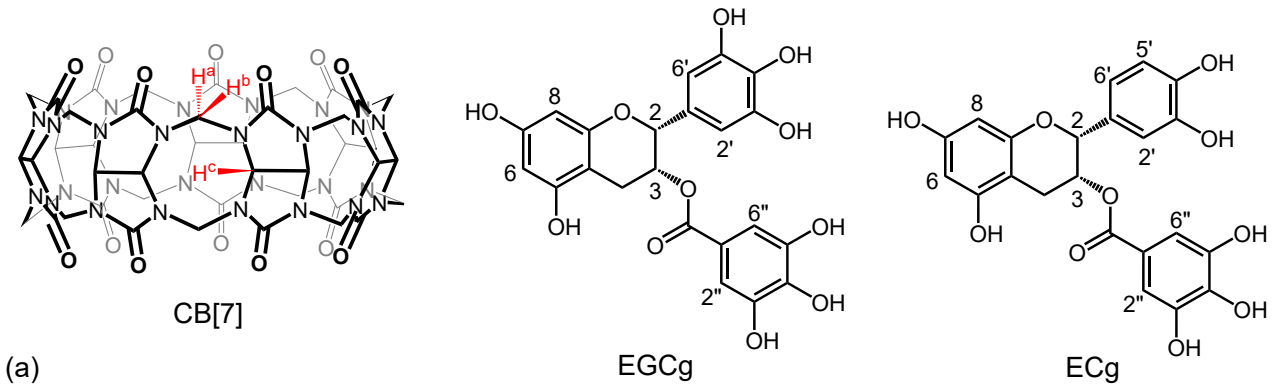
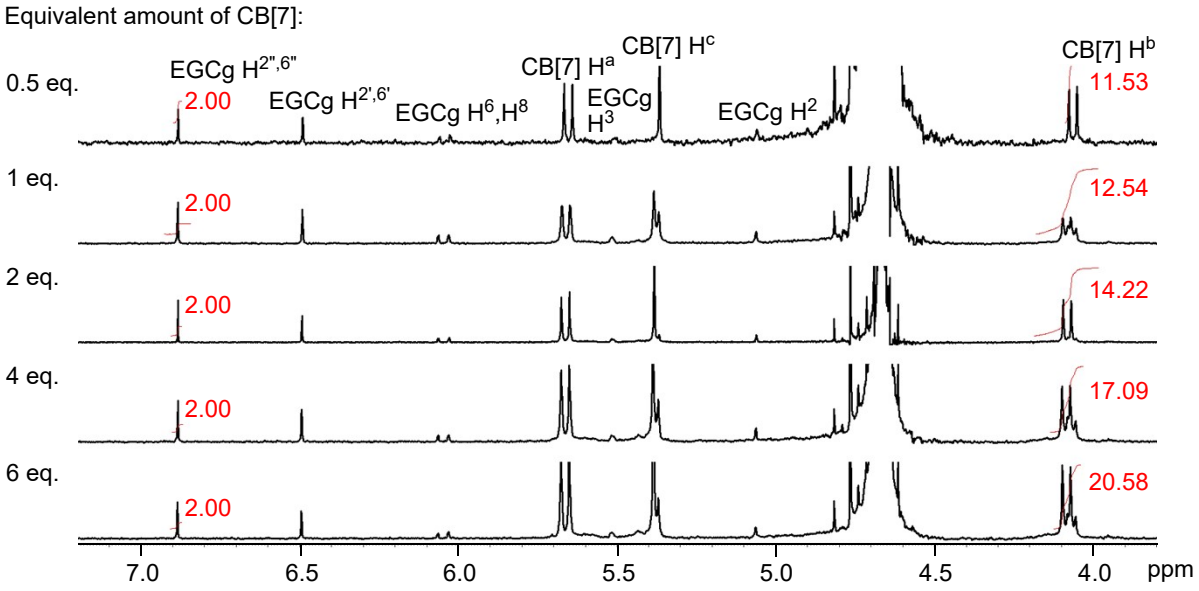


Fig. S2 Appearance of the aqueous solution containing *cis*-type catechins (0.5 mM) at 293 K. (a) pH4, (b) pH5.



(a)



(b)

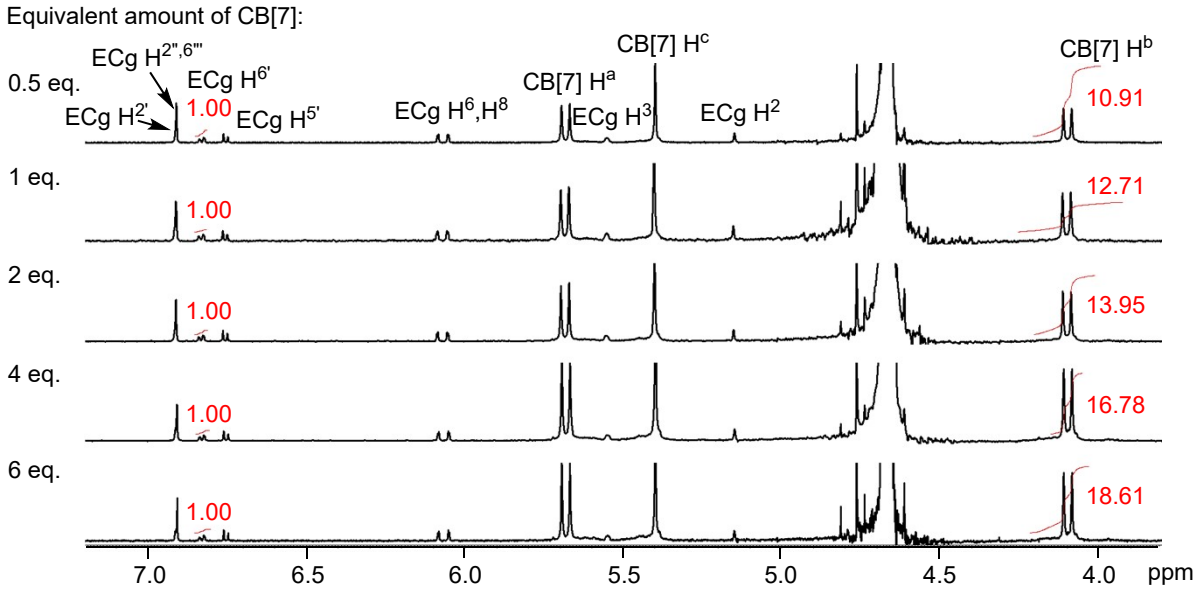


Fig. S3 ^{1}H NMR spectra of the precipitates formed from mixing *cis*-type gallated catechins (constant concentration: 0.5 mM) with 0.5–6 equivalents of CB[7]. (a) EGCg, (b) ECg. The red numbers represent the integral values.

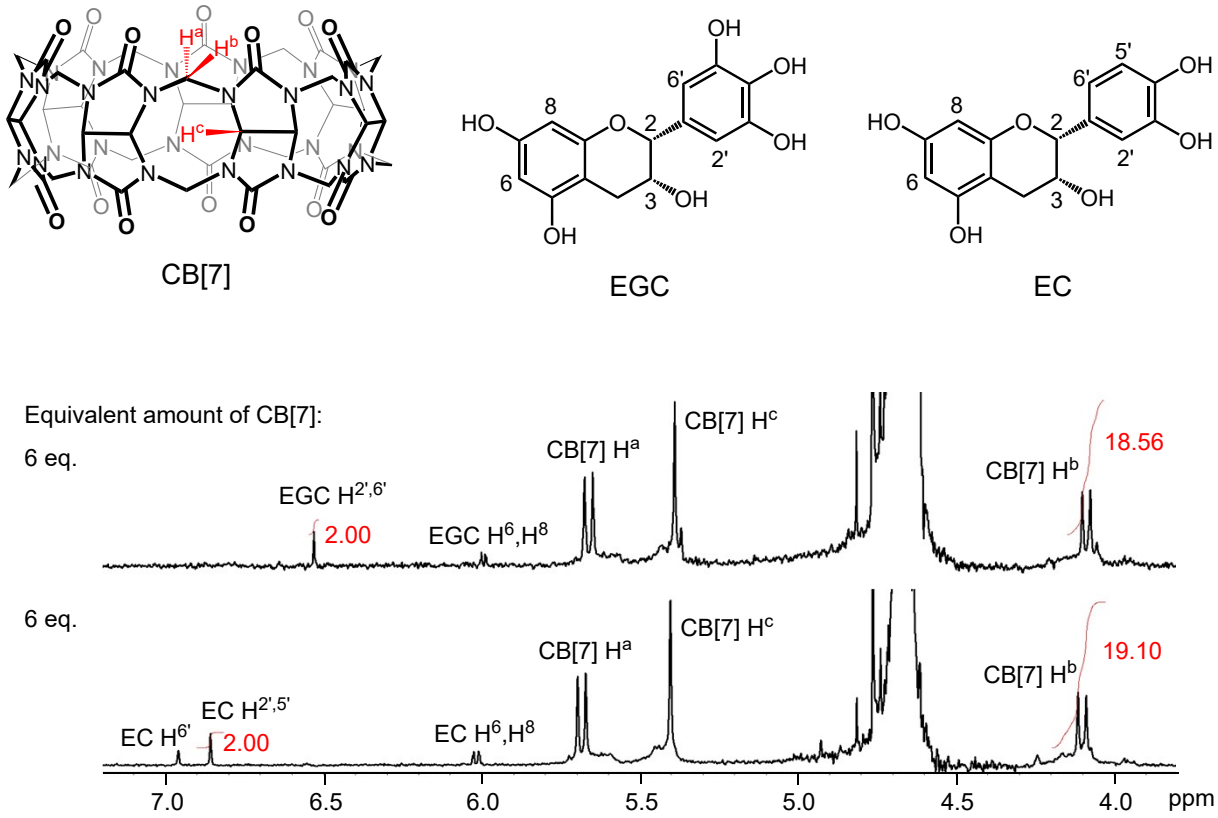


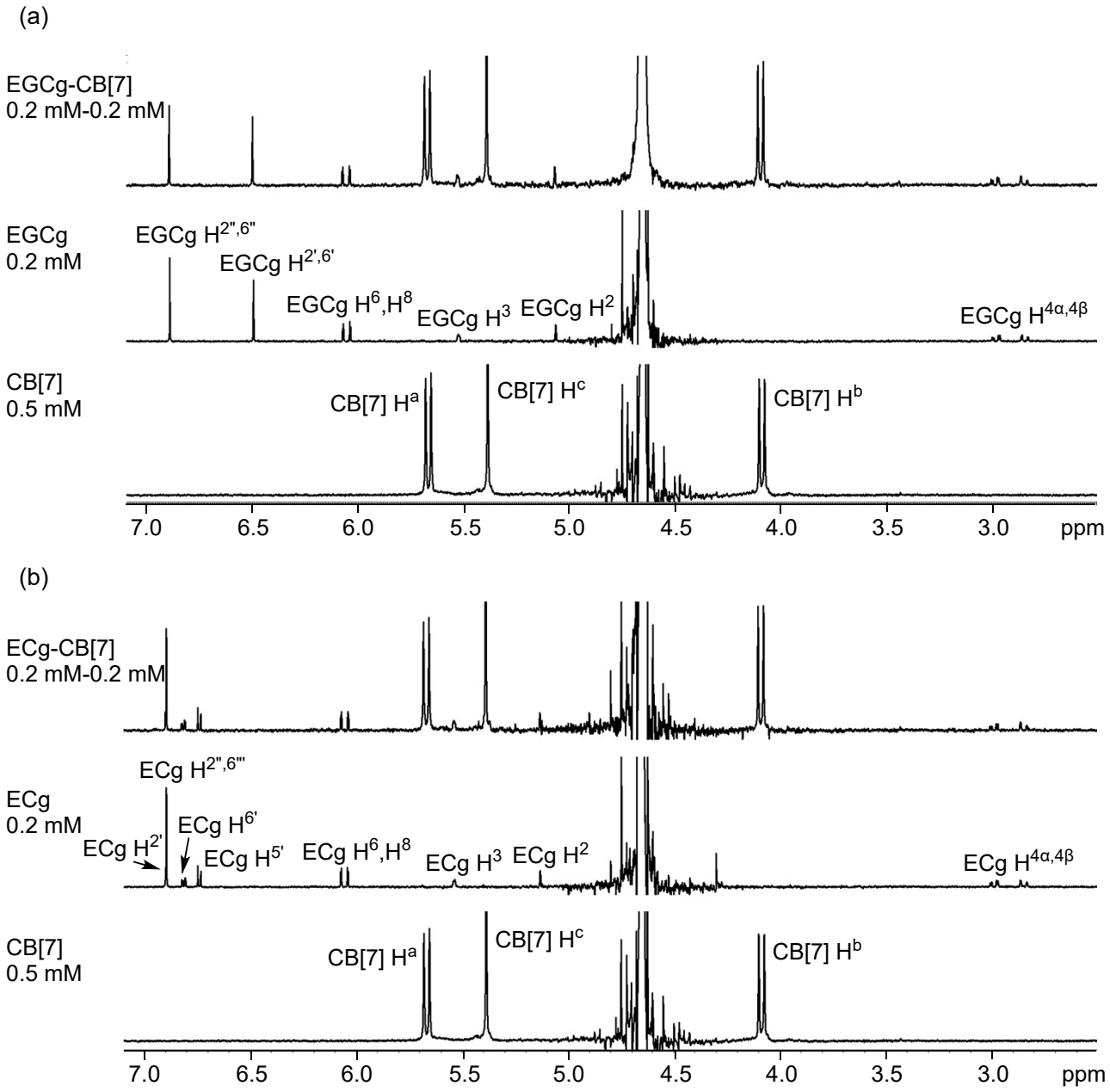
Fig. S4 ^{1}H NMR spectra of the precipitates formed from mixing *cis*-type non-gallated catechins (constant concentration: 0.5 mM) with 6 equivalents of CB[7]. (a) EGC, (b) EC. The red numbers represent the integral values.

Table S1 Molar ratio of CB[7] to *cis*-type catechins in the precipitate

Equivalent amount of CB[7]	Molar ratio of CB[7] to *cis*-type catechins			
	EGCg	ECg	EGC	EC
0.5	0.82	0.78	–	–
1	0.90	0.91	–	–
2	1.02	1.00	–	–
4	1.22	1.20	–	–
6	1.47	1.33	1.33	1.36

The molar ratio of CB[7] to *cis*-type catechins in the precipitate was calculated using the following equation.

Molar ratio of CB[7] to *cis*-type catechins = Integral value of CB[7] H^{b}/14


(a)
EGCg-CB[7]
0.2 mM-0.2 mM
EGCg
0.2 mM
EGCg H2",6"
EGCg H2',6'
EGCg H6,H8
EGCg H3
EGCg H2
EGCg H4α,4β
CB[7]
0.5 mM
CB[7] Ha
CB[7] Hc
CB[7] Hb
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm
(b)
ECg-CB[7]
0.2 mM-0.2 mM
ECg
0.2 mM
ECg H2",6'''
ECg H2'
ECg H6'
ECg H5'
ECg H6,H8
ECg H3
ECg H2
ECg H4α,4β
CB[7]
0.5 mM
CB[7] Ha
CB[7] Hc
CB[7] Hb
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm

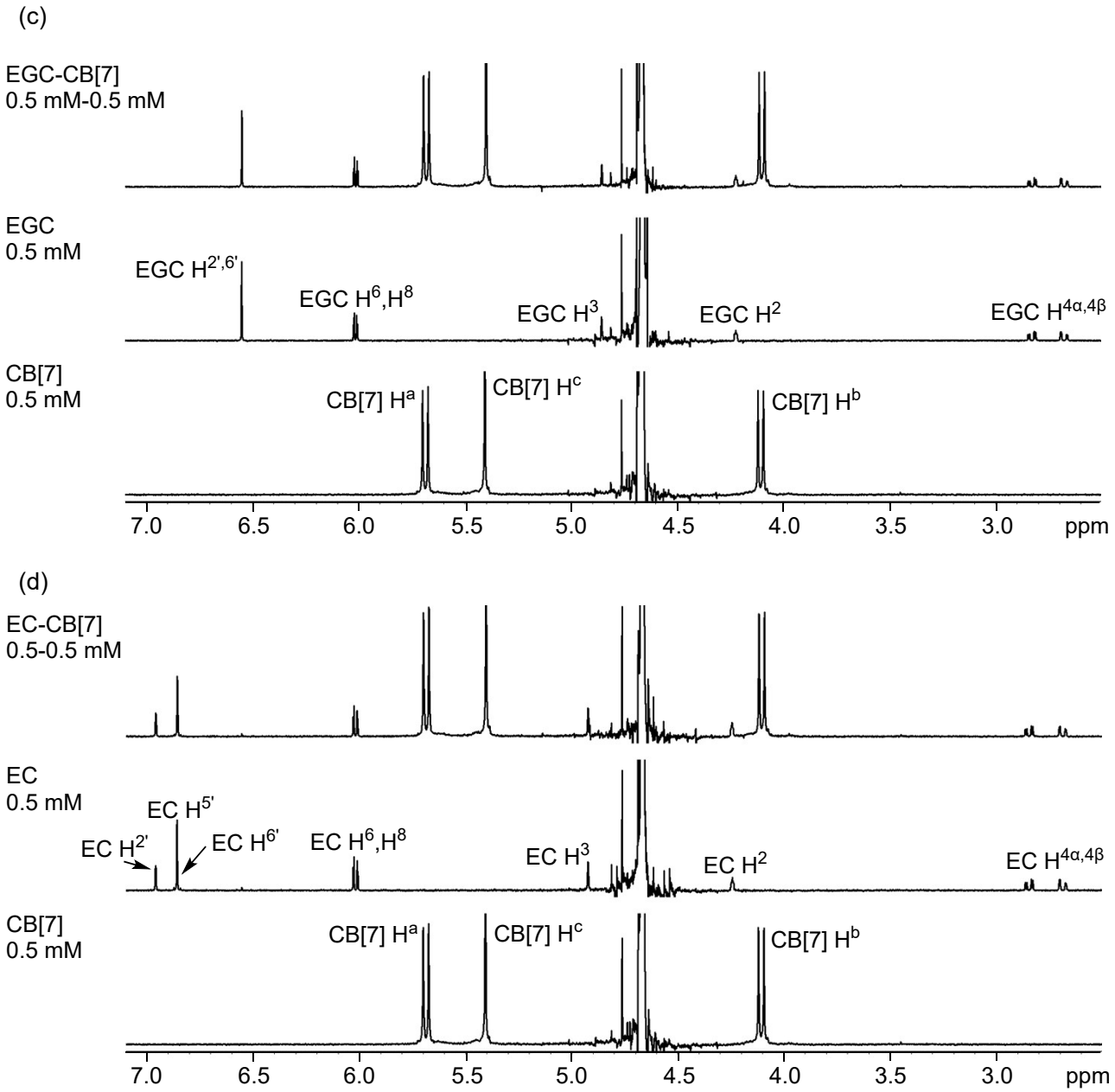


Fig. S5 ^{1}H NMR spectra of *cis*-type catechins and CB[7], *cis*-type catechins, and CB[7]. (a) EGCg, (b) ECg, (c) EGC, and (d) EC.

Since precipitation occurred in the 0.5 mM CB[7] and 0.5 mM EGCg solution or the 0.5 mM CB[7] and 0.5 mM ECg solution, their supernatants were used as the measurement solutions. The concentrations of EGCg, ECg and CB[7] in these solutions were calculated to be 0.2 mM by ^{1}H NMR. Since EGCg and ECg cause self-association, which causes a shift change of proton signal, the concentrations of EGCg and ECg alone were set at 0.2 mM. The proton signal of CB[7] does not change at any concentration, so the concentration of CB[7] alone was set at 0.5 mM.

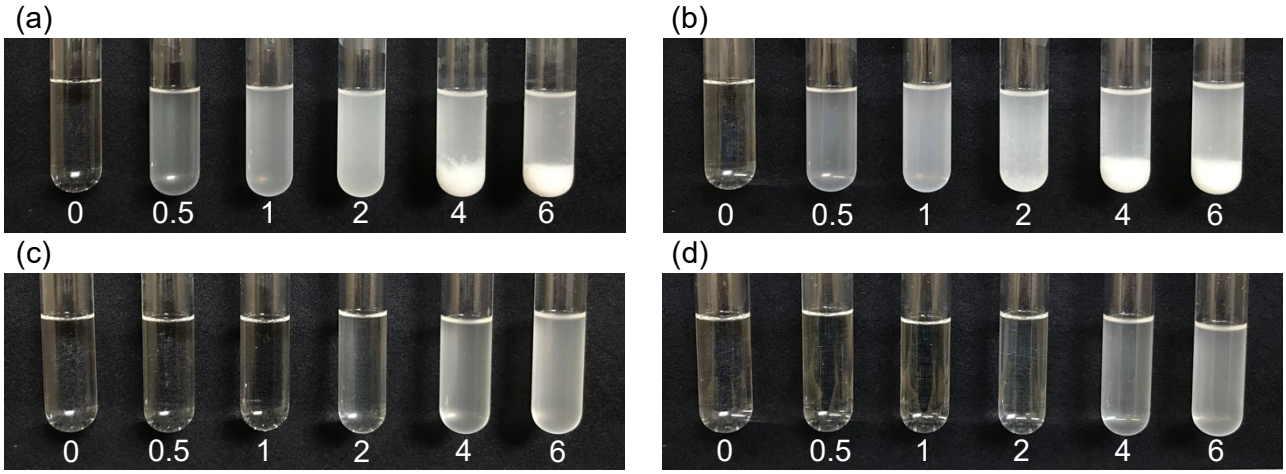


Fig. S6 Appearance of the aqueous solution containing *cis*-type catechins mixed with CB[5] at 293 K. (a) EGCg and CB[5], (b) ECg and CB[5], (c) EGC and CB[5], (d) EC and CB[5]. The numbers in the images indicate the equivalents of CB[5] relative to the *cis*-type catechins.

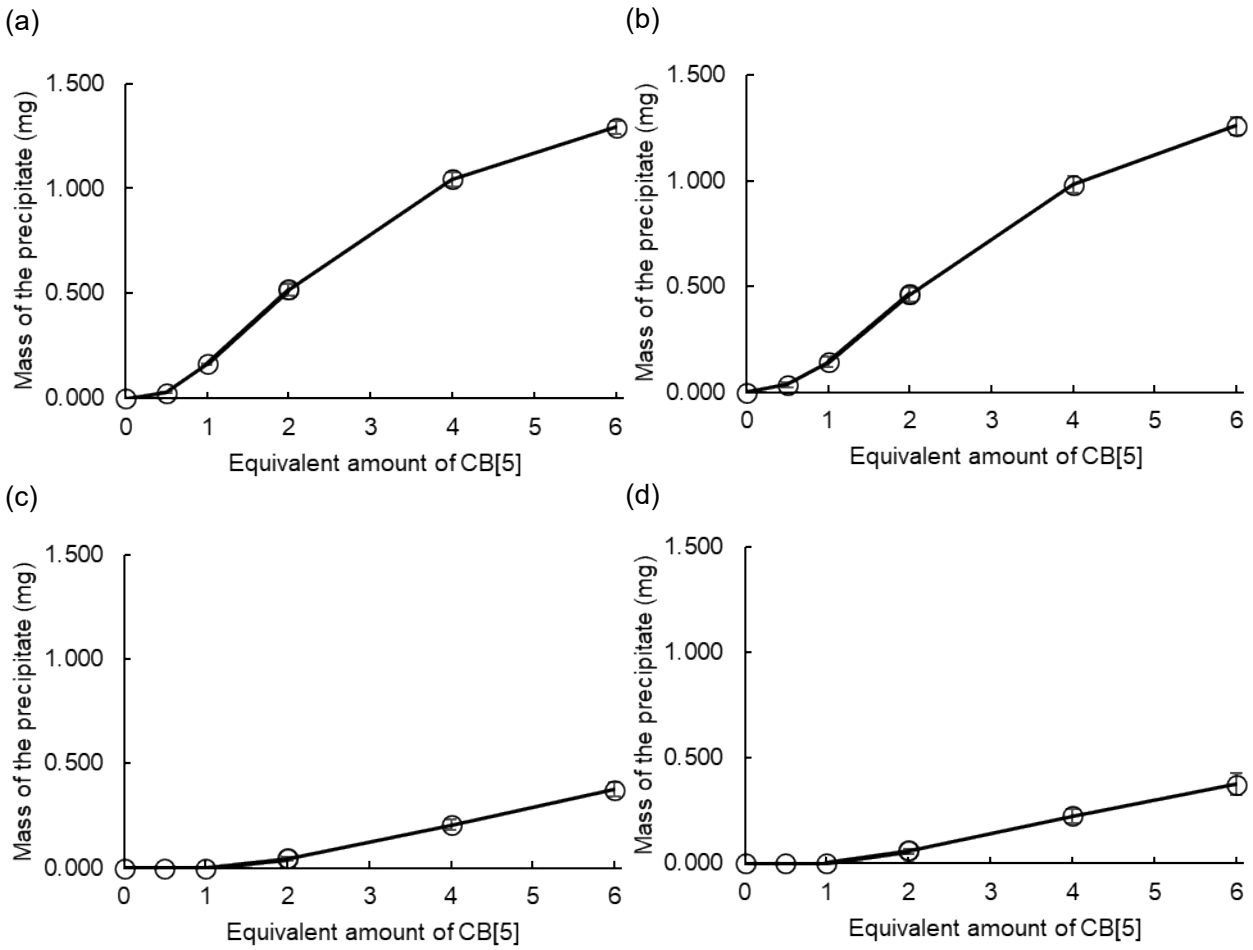
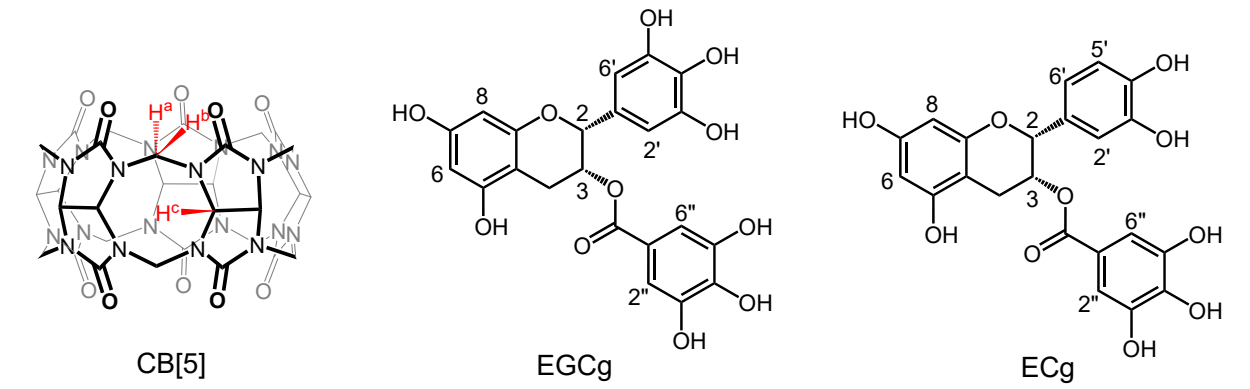
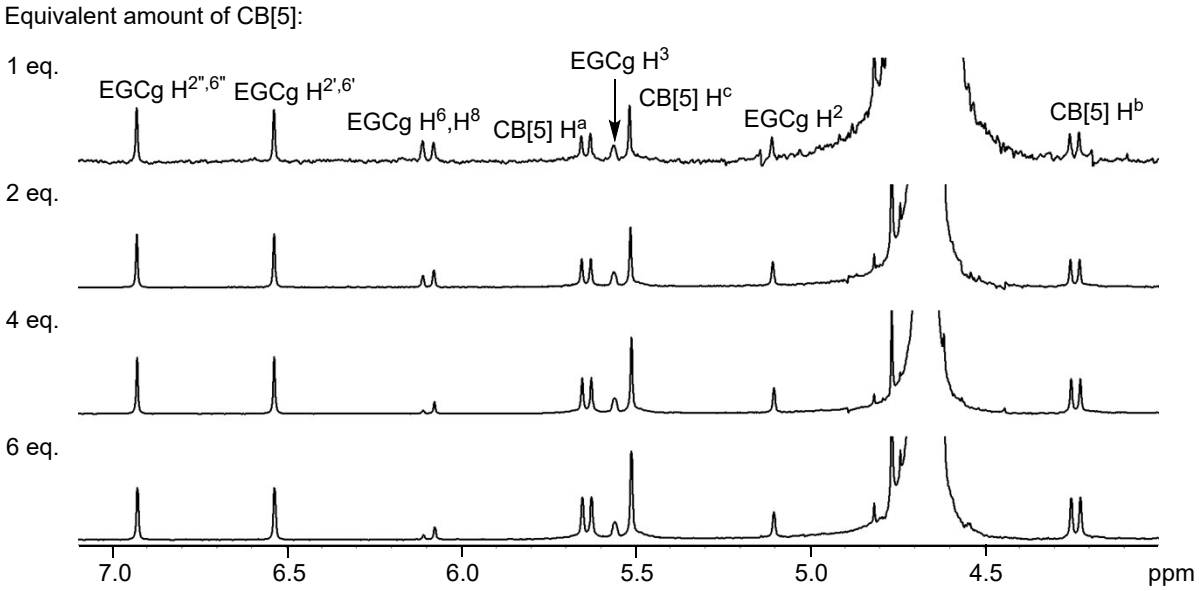


Fig. S7 The mass of the precipitate obtained from filtering these solutions increased with the equivalent amount of CB[5] added for all *cis*-type catechins (n = 3). (a) EGCg, (b) ECg, (c) EGC, and (d) EC



(a)



(b)

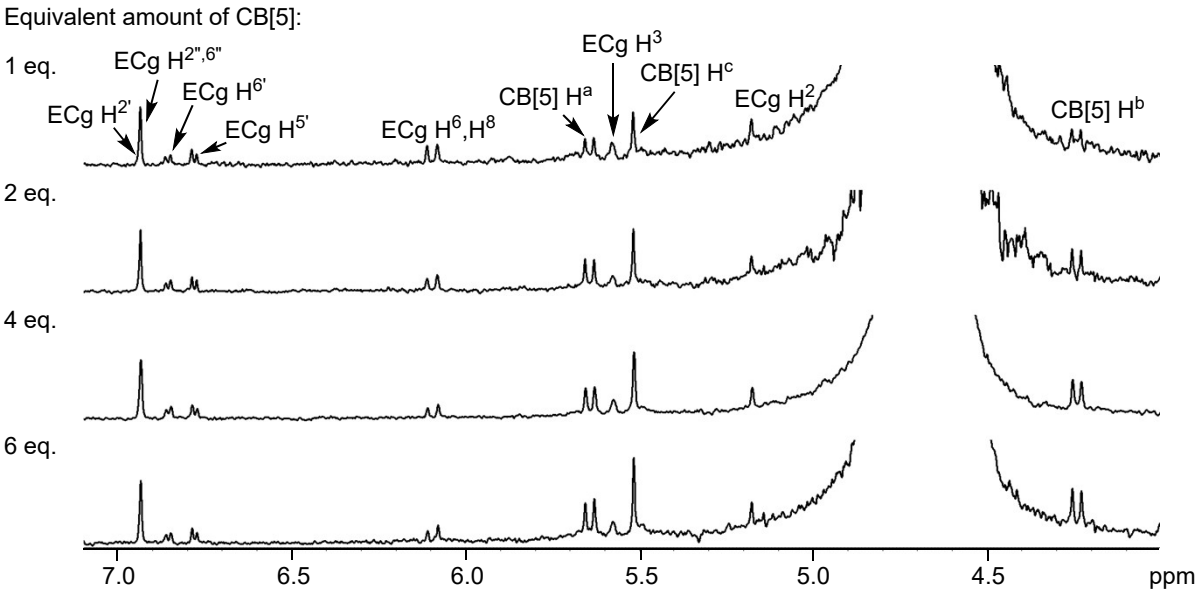
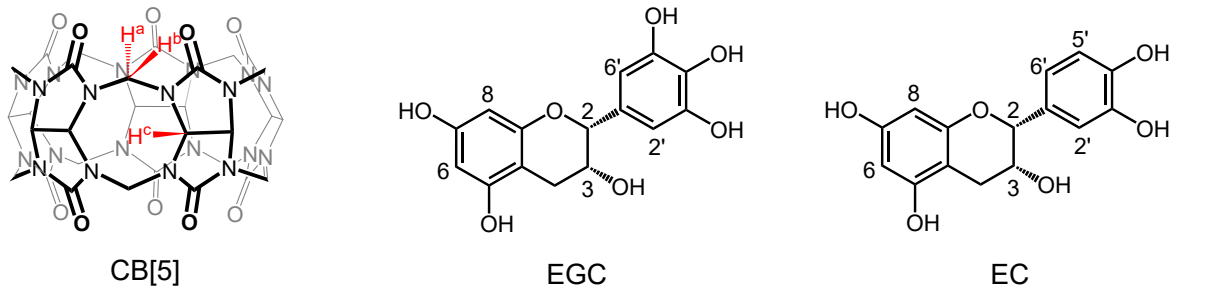
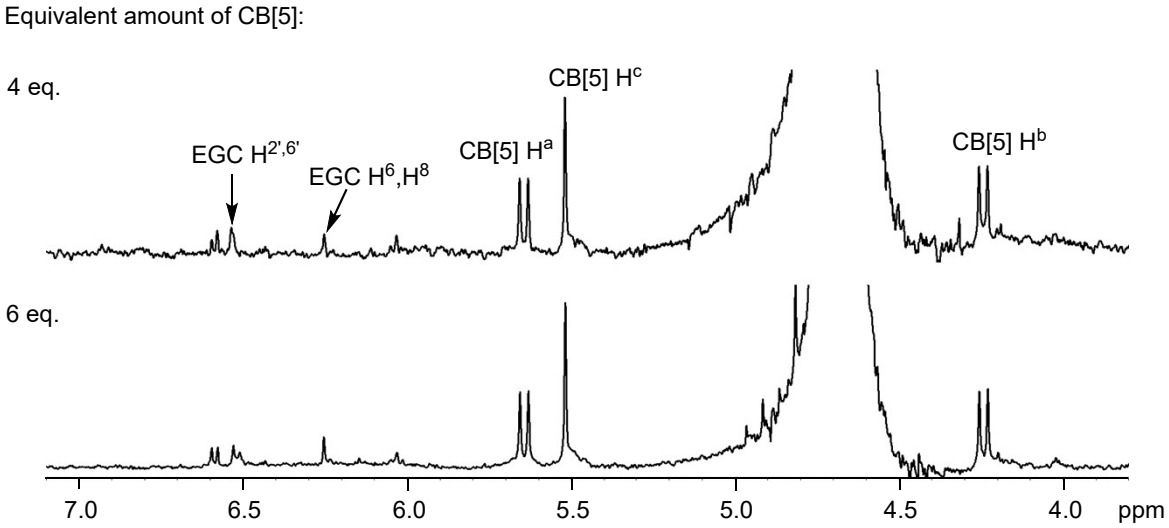


Fig. S8 ^{1}H NMR spectra of the precipitates formed by mixing *cis*-type gallated catechins (constant concentration: 0.5 mM) with 1–6 equivalents of CB[5]. (a) EGCg, (b) ECg



(a)



(b)

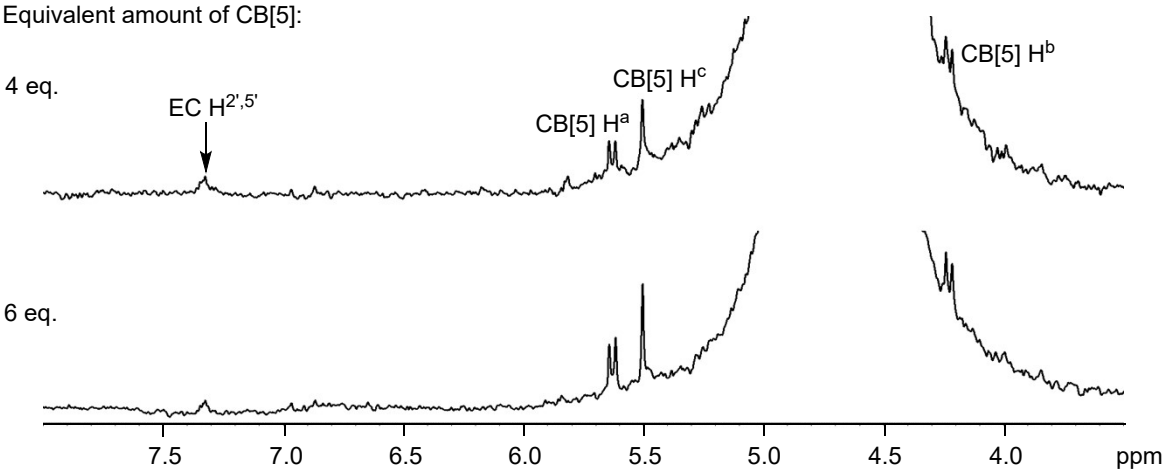


Fig. S9 ^{1}H NMR spectra of the precipitates formed by mixing *cis*-type non-gallated catechins (constant concentration: 0.5 mM) with 4–6 equivalents of CB[5]. (a) EGC, (b) EC

Table S2 Residue rate of EGCg with increasing equivalents of CB[7] at a constant EGCg concentration (0.5 mM) at 278, 283, 288, 293, and 298 K (n = 3)

Temperature (K)	Equivalents of CB[7]	Residue rate of EGCg (%)			Average (%)	Standard error (%)
		No. 1	No. 2	No. 3		
298	0	100.0	100.0	100.0	100.0	0.0
	0.5	91.9	92.2	88.5	90.9	2.0
	1	66.8	68.9	63.8	66.5	2.6
	2	31.3	33.3	30.8	31.8	1.3
	4	10.4	11.6	12.0	11.3	0.8
	6	7.3	6.7	9.3	7.8	1.4
293	0	100.0	100.0	100.0	100.0	0.0
	0.5	79.0	76.9	77.3	77.7	1.1
	1	51.0	49.7	49.0	49.9	1.0
	2	21.5	21.3	19.8	20.8	0.9
	4	7.5	6.3	7.5	7.1	0.7
	6	5.6	5.8	4.7	5.4	0.6
288	0	100.0	100.0	100.0	100.0	0.0
	0.5	69.3	66.7	67.6	67.9	1.3
	1	42.1	37.1	38.9	39.4	2.5
	2	13.6	13.8	13.8	13.8	0.1
	4	5.8	5.2	4.8	5.2	0.5
	6	3.1	3.2	5.0	3.8	1.0
283	0	100.0	100.0	100.0	100.0	0.0
	0.5	62.5	61.7	61.2	61.8	0.7
	1	33.4	33.7	32.2	33.1	0.8
	2	12.0	10.2	12.3	11.5	1.2
	4	5.4	4.2	4.2	4.6	0.7
	6	4.5	3.5	4.6	4.2	0.6
278	0	100.0	100.0	100.0	100.0	0.0
	0.5	51.1	51.4	53.7	52.1	1.4
	1	23.0	22.2	22.8	22.7	0.5
	2	5.4	8.0	5.7	6.4	1.4
	4	2.4	4.9	2.5	3.3	1.4
	6	2.7	1.5	2.6	2.3	0.7

Table S3 Residue rate of ECg with increasing equivalents of CB[7] at a constant ECg concentration (0.5 mM) at 278, 283, 288, 293, and 298 K (n = 3)

Temperature (K)	Equivalents of CB[7]	Residue rate of ECg (%)			Average (%)	Standard error (%)
		No. 1	No. 2	No. 3		
298	0	100.0	100.0	100.0	100.0	0.0
	0.5	84.1	84.2	84.4	84.2	0.1
	1	60.4	62.3	61.1	61.3	1.0
	2	30.3	30.6	31.5	30.8	0.6
	4	11.4	11.4	11.7	11.5	0.1
	6	6.7	6.7	6.3	6.6	0.3
293	0	100.0	100.0	100.0	100.0	0.0
	0.5	72.9	71.5	72.6	72.4	0.7
	1	48.3	48.0	45.7	47.3	1.4
	2	20.8	20.3	20.7	20.6	0.3
	4	7.8	7.6	8.2	7.9	0.3
	6	4.8	4.7	6.1	5.2	0.8
288	0	100.0	100.0	100.0	100.0	0.0
	0.5	65.3	66.8	65.5	65.8	0.8
	1	39.7	39.5	38.1	39.1	0.8
	2	15.9	14.3	13.9	14.7	1.1
	4	5.0	4.3	3.7	4.3	0.7
	6	4.5	2.8	2.5	3.2	1.1
283	0	100.0	100.0	100.0	100.0	0.0
	0.5	55.1	53.0	58.8	55.6	2.9
	1	29.1	27.1	31.3	29.2	2.1
	2	9.7	9.4	12.0	10.4	1.4
	4	4.0	2.6	4.6	3.7	1.0
	6	3.8	1.8	3.0	2.9	1.0
278	0	100.0	100.0	100.0	100.0	0.0
	0.5	52.9	51.6	49.2	51.2	1.9
	1	27.5	26.6	22.6	25.6	2.6
	2	10.4	7.5	6.1	8.0	2.2
	4	3.9	2.7	2.5	3.1	0.8
	6	2.9	1.5	2.6	2.3	0.7

Table S4 Residue rate of EGC with increasing equivalents of CB[7] at a constant EGC concentration (0.5 mM) at 278, 283, 288, 293, and 298 K (n = 3)

Temperature (K)	Equivalents of CB[7]	Residue rate of EGC (%)			Average (%)	Standard error (%)
		No. 1	No. 2	No. 3		
298	0	100.0	100.0	100.0	100.0	0.0
	0.5	98.1	95.6	97.6	97.1	1.3
	1	100.7	96.4	99.3	98.8	2.2
	2	99.9	96.7	96.9	97.8	1.8
	4	97.3	97.6	96.9	97.3	0.4
	6	90.6	89.7	90.3	90.2	0.5
293	0	100.0	100.0	100.0	100.0	0.0
	0.5	96.2	98.8	98.8	97.9	1.5
	1	97.2	101.8	98.6	99.2	2.4
	2	97.8	97.6	99.0	98.1	0.8
	4	93.8	94.1	92.6	93.5	0.8
	6	80.3	81.3	80.4	80.7	0.5
288	0	100.0	100.0	100.0	100.0	0.0
	0.5	98.9	96.5	98.6	98.0	1.3
	1	101.3	98.0	99.0	99.4	1.7
	2	93.9	96.7	95.9	95.5	1.4
	4	81.9	85.8	84.0	83.9	2.0
	6	68.4	70.8	66.1	68.4	2.4
283	0	100.0	100.0	100.0	100.0	0.0
	0.5	104.5	97.9	98.1	100.2	3.7
	1	99.8	96.2	98.7	98.2	1.9
	2	95.7	89.9	94.3	93.3	3.0
	4	79.1	70.0	75.7	74.9	4.6
	6	55.8	54.8	58.7	56.4	2.0
278	0	100.0	100.0	100.0	100.0	0.0
	0.5	97.2	96.4	96.5	96.7	0.5
	1	95.1	94.5	93.7	94.5	0.7
	2	87.9	82.3	84.9	85.0	2.8
	4	66.2	58.9	63.6	62.9	3.7
	6	50.2	42.9	48.4	47.1	3.8

Table S5 Residue rate of EC with increasing equivalents of CB[7] at a constant EC concentration (0.5 mM) at 278, 283, 288, 293, and 298 K (n = 3)

Temperature (K)	Equivalents of CB[7]	Residue rate of EC (%)			Average (%)	Standard error (%)
		No. 1	No. 2	No. 3		
298	0	100.0	100.0	100.0	100.0	0.0
	0.5	97.6	102.1	100.2	100.0	2.2
	1	97.0	102.2	97.5	98.9	2.9
	2	97.1	97.7	97.5	97.5	0.3
	4	96.6	97.7	96.8	97.0	0.6
	6	93.5	94.4	93.8	93.9	0.5
293	0	100.0	100.0	100.0	100.0	0.0
	0.5	101.1	94.6	99.1	98.3	3.3
	1	100.0	95.0	97.9	97.6	2.5
	2	98.9	95.7	97.8	97.5	1.6
	4	94.9	94.8	96.0	95.2	0.7
	6	85.9	86.7	87.7	86.8	0.9
288	0	100.0	100.0	100.0	100.0	0.0
	0.5	99.2	97.4	98.5	98.4	0.9
	1	98.6	96.2	97.7	97.5	1.2
	2	98.2	96.1	96.9	97.1	1.1
	4	92.6	89.0	89.4	90.3	2.0
	6	78.1	77.2	76.4	77.2	0.9
283	0	100.0	100.0	100.0	100.0	0.0
	0.5	98.2	98.4	99.2	98.6	0.6
	1	97.4	98.2	98.0	97.9	0.4
	2	95.2	95.5	95.5	95.4	0.1
	4	81.2	80.7	80.0	80.6	0.6
	6	65.2	66.4	64.1	65.2	1.2
278	0	100.0	100.0	100.0	100.0	0.0
	0.5	100.6	95.0	96.5	97.4	2.9
	1	96.0	93.4	93.6	94.3	1.5
	2	88.9	87.0	87.6	87.8	1.0
	4	67.1	67.1	69.7	68.0	1.5
	6	50.9	50.9	52.6	51.5	1.0

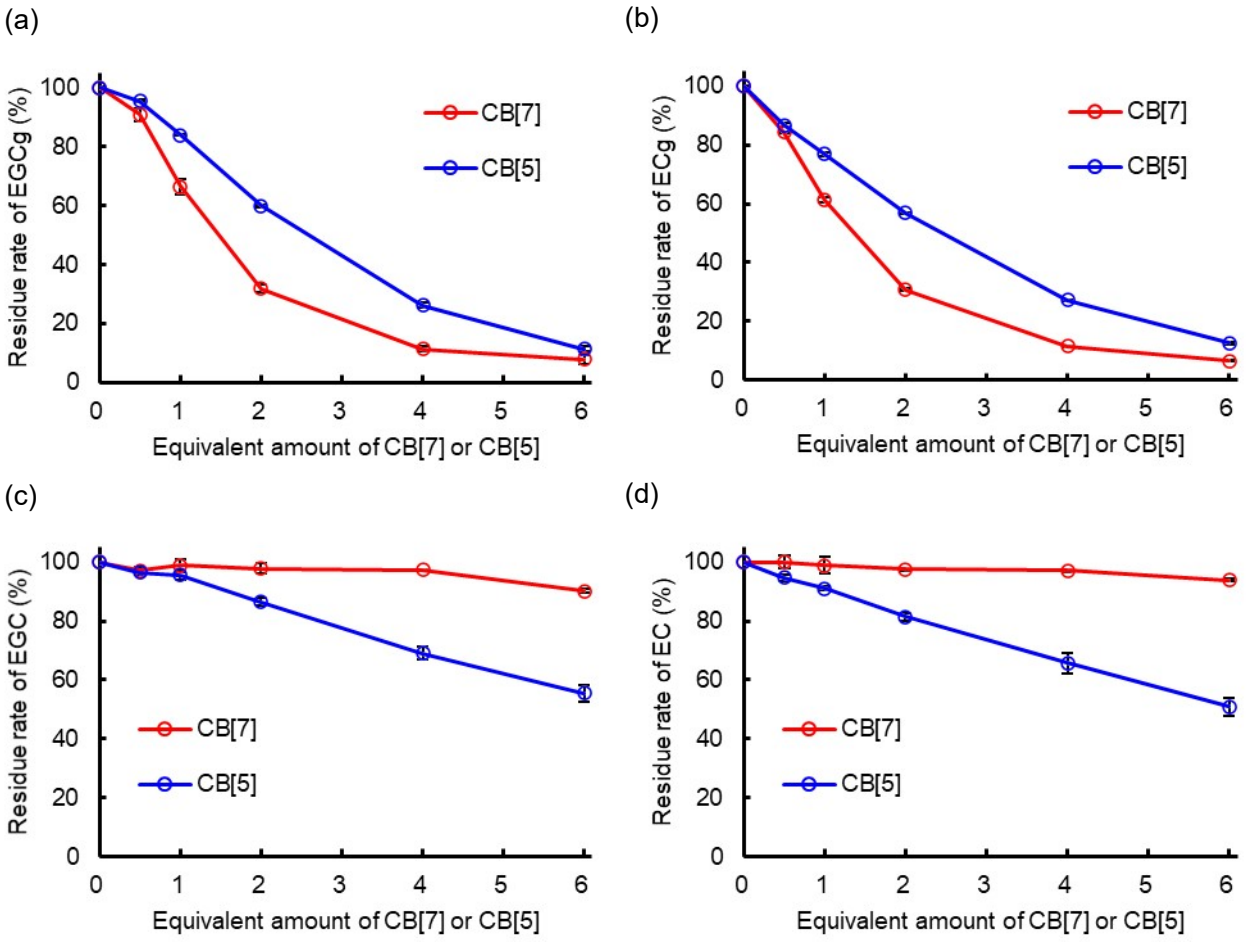


Fig. S10 The mass of the precipitate obtained from filtering these solutions increased with the equivalent amount of CB[5] added for all *cis*-type catechins at 293 K (n = 3). (a) EGCg, (b) ECg, (c) EGC, and (d) EC

Table S6 Difference in residue rates between *cis*-type gallated and *cis*-type non-gallated catechins

Temperature (K)	Equivalents of CB[7]	Difference in residue rates between *cis*-type gallated and *cis*-type non-gallated catechins (%)				Average (%)
		EGC − EGCg	EC − EGCg	EGC − ECg	EC − ECg	
298	0.5	6.2	9.1	12.9	15.8	11.0
	1	32.4	32.5	37.5	37.6	35.0
	2	66.0	65.7	67.0	66.6	66.3
	4	85.9	85.7	85.8	85.5	85.7
	6	82.5	86.2	83.7	87.4	84.9
293	0.5	20.2	23.4	25.6	25.9	23.8
	1	49.2	50.0	51.9	50.3	50.4
	2	77.3	78.0	77.5	76.8	77.4
	4	86.4	87.8	85.6	87.4	86.8
	6	75.3	80.5	75.5	81.5	78.2
288	0.5	30.1	30.5	32.1	32.5	31.3
	1	60.1	58.1	60.3	58.4	59.2
	2	81.7	83.3	80.8	82.4	82.1
	4	78.7	85.1	79.6	86.0	82.3
	6	64.7	73.5	65.2	74.0	69.3
283	0.5	38.4	36.8	44.5	43.0	40.7
	1	65.1	64.7	69.1	68.7	66.9
	2	81.8	83.9	82.9	85.0	83.4
	4	70.3	76.0	71.2	76.9	73.6
	6	52.2	61.0	53.5	62.3	57.3
278	0.5	44.6	45.3	45.4	46.1	45.4
	1	71.8	71.7	68.9	68.8	70.3
	2	78.7	81.4	77.0	79.8	79.2
	4	59.6	64.7	59.8	64.9	62.3
	6	44.8	49.2	44.8	49.2	47.0

The yellow highlights denote the top three conditions showing the greatest differences in residue rates between *cis*-type gallated and *cis*-type non-gallated catechins.

“Average (%)” was calculated using the following equation:

Average (%) =([EGC − EGCg] + [EC – EGCg] + [EGC – ECg] + [EC − ECg])/4

The difference in residue rates between *cis*-type gallated and *cis*-type non-gallated catechins was calculated using the following equation:

Difference in residue rates = Residue rates of *cis*-type non-gallated catechins − Residue rates of *cis*-type gallated catechins

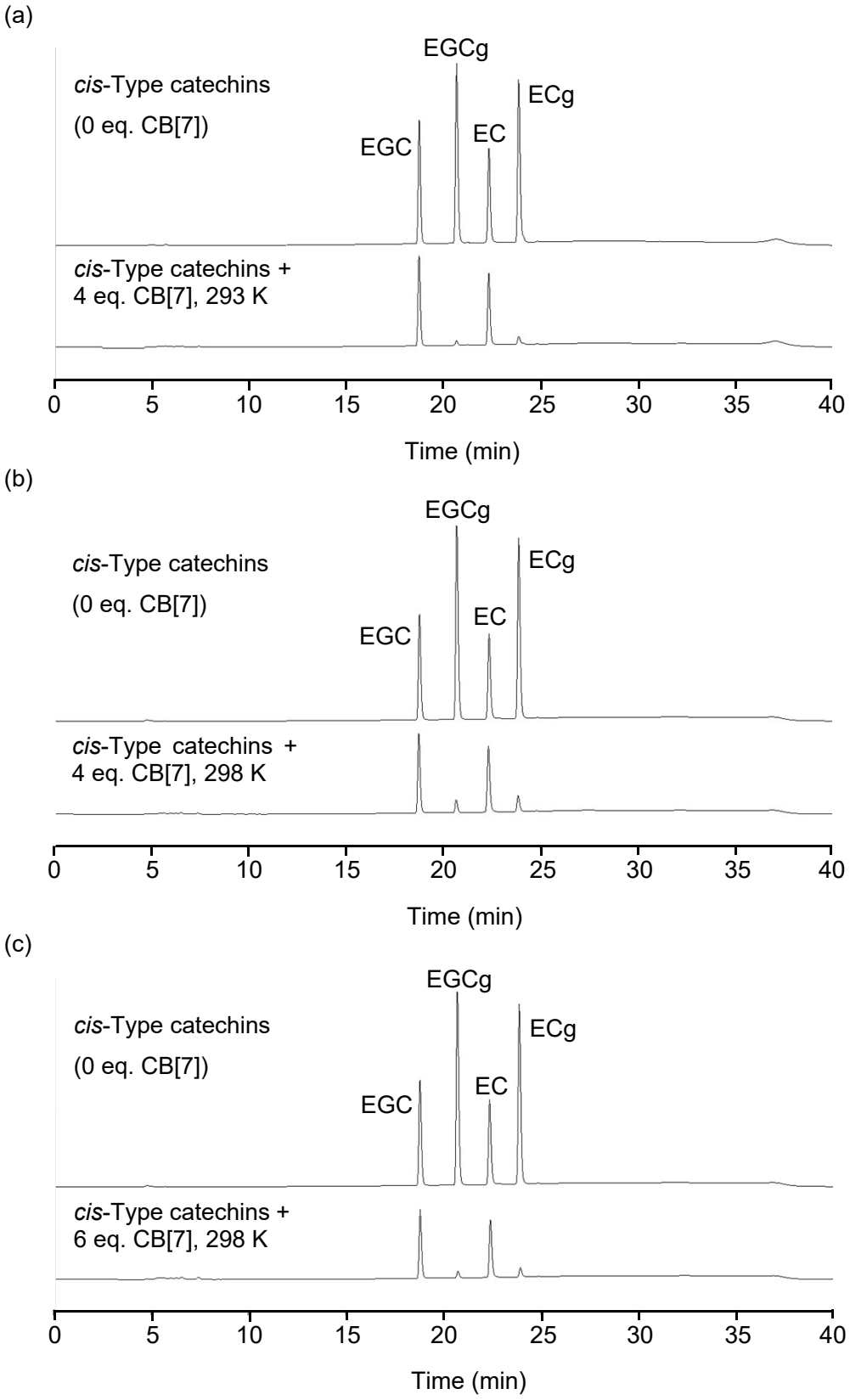


Fig. S11 HPLC chromatograms of *cis*-type catechins solution before and after the addition of CB[7] (a) 293 K and 4 eq. CB[7], (b) 298 K and 4 eq. CB[7], and (c) 298 K and 6 eq. CB[7]

Table S7 HPLC peak area values of *cis*-type catechins in the solution before and after the addition of CB[7] (n = 3)

Condition	*cis*-Type catechins	HPLC Peak area values		
		No. 1	No. 2	No. 3
0 eq. CB[7]	EGCg	764289.9	757374.3	813108.0
	ECg	725571.9	711904.1	759647.9
	EGC	455378.5	436450.8	435002.5
	EC	367309.2	375997.7	373015.5
(ⅰ) 4 eq. CB[7], 293 K	EGCg	25689.5	19250.8	22922.9
	ECg	54662.2	39710.4	44617.7
	EGC	360394.3	334526.3	330390.2
	EC	307789.7	288618.5	282496.5
(ⅱ) 4 eq. CB[7], 298 K	EGCg	58392.5	54169.4	57364.1
	ECg	72195.6	71881.7	69957.9
	EGC	317584.9	325432.2	307841.8
	EC	307789.7	288618.5	282496.5
(ⅲ) 6 eq. CB[7], 298 K	EGCg	30152.0	31367.2	31831.8
	ECg	40921.5	47123.5	46648.1
	EGC	271879.0	267606.1	276181.8
	EC	259787.7	264594.7	264594.7

Table S8 Residue rates of *cis*-type catechins in the solution after adding CB[7] to a constant concentration of EGCg, ECg, EGC, and EC (each at 0.25 mM) (n = 3)

cis-Type catechins		Residue rates in the solution (%)		
		(i) 293 K, 4 eq.	(ii) 298 K, 4 eq.	(iii) 298 K, 6 eq.
cis-Type gallated catechins	EGCg	2.9 ± 0.4	7.3 ± 0.3	4.0 ± 0.1
	ECg	6.3 ± 1.1	9.8 ± 0.5	6.1 ± 0.5
cis-Type non-gallated catechin	EGC	77.2 ± 1.7	71.7 ± 2.5	61.5 ± 1.9
	EC	78.8 ± 4.4	77.1 ± 1.6	70.0 ± 1.5

Residue rates of *cis*-type catechins in the mixture solution were calculated using the following equation.

Residue rates of *cis*-type catechins = (HPLC peak area value of *cis*-type catechins with adding CB[7] / HPLC peak area value of *cis*-type catechins (0 eq. CB[7])) × 100

Reference

1 D. Bardelang, K. A. Udachin, D. M. Leek, J. C. Margeson, G. Chan, C. I. Ratcliffe and J. A. Ripmeester, *Cryst. Growth Des*., 2011, **11**, 5598.