Electronic Supplementary Information

Fluorescent α-Cyclodextrin as a Chemosensor for Halomethanes

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Contents

1. Synthesis of NC0αCD

1.1. Materials

α-Cyclodextrin was kindly donated by Nihon Shokuhin Kako Co., Ltd., and was used without further purification. Reagents were purchased from Sigma-Aldrich Co., Tokyo Kasei Kogyo Co., Ltd., and Wako Pure Chemical Industries, Ltd, and were used without further purification. Deuterium oxide for NMR measurements was obtained from Merck Co.

1.2. Measurements

Reverse phase HPLC was performed using a HITACHI HPLC system comprised of a HITACHI L-7100 Intelligent Pump, HITACHI D-7500 Chromato-Integrator and HITACHI L-7400 UV-VIS Detector. ^{1}H NMR spectra were measured on a Varian VXR 500S spectrometer (500 MHz). HDO (δ = 4.70) or MeOH (δ = 3.34) was used as an internal standard. Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed on a SHIMADZU KRATOS KOMPACT MALDI III mass spectrometer using α-cyano-4-hydroxycinnamic acid as a matrix. Thin-layer chromatography (TLC; n-butanol : ethanol : water = 5:4:3, and conc. NH_3aq. : ethyl acetate : 2-propanol : water = 1:3:5:4) was carried out with silica gel F_{254} (Merck Co.).

1.3. NC0αCD

4-Chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Cl, 616 mg, 3.09 mmol) was added to a DMF (25 ml)/methanol (25 ml) solution containing triethylamine (156.2 mg, 1.54 mmol) and mono-6-deoxy-6-amino-α-cyclodextrin (300 mg, 0.309 mmol). The reaction mixture was stirred overnight at room temperature. After concentration of solvents, ethanol (100 ml) was added to the flask. The precipitates were collected and dried in vacuo overnight, giving 327 mg of crude product. This crude product was purified by reverse phase HPLC, and the final product was obtained as a yellow powder (266 mg, 75.9%). ^{1}H NMR (D_2O, 500 MHz): δ 3.25-4.18 (m, 36H, H-2, H-3, H-4, H-5, H-6), 4.85-5.10 (m, 6H, H-1), 6.35 (d, 1H, aromatic), 8.23 (bs, 1H, aromatic). MALDI-TOF MS: m/z 1157.5 (calcd for $[M+Na]^+$, 1157.3).

2. Evaluation of Binding Affinities of NC0αCD

2.1. General

Absorption spectra were measured on a SHIMADZU UV-Visible spectrophotometer UV-3100. Fluorescence spectra were measured on a SHIMADZU fluorescence spectrophotometer RF-5300PC. Distilled water and methanol used as solvents for spectroscopy were special fluorometry grade (Uvasol) from Kanto Chemicals.

The absorbance and fluorescence of the complexes of **NC0αCD** with a guest were measured via an absorption and fluorescence spectrophotometer equipped with a thermostated cell compartment maintained at 25 °C. All measurements were performed with a quartz cuvette.

The concentration of the stock solutions of the host, **NC0αCD**, in 0.1 M pH 7.0 phosphate buffer was 5 x 10^{-6} M. The concentration of stock solution of each guest in methanol differs in order to make the final concentration in a cuvette suitable for titration analysis.

2.2 Fluorescence Titration Procedure

First, the absorbance and fluorescence spectra of **NC0αCD** (3 ml, 5 x 10^{-6} M) with no guest added were measured. Then, a solution of guest in methanol (0.5 or 1.0 μL) was added to the solution of **NC0αCD** (3 ml, 5 x 10^{-6} M) in the quartz cuvette with stirring. After stirring in 3 min., the absorbance and fluorescence spectra were measured. This procedure was repeated until the total amount of the guest solution was 6 μL.

2.3 Determination of Binding Constants of NC0αCD for Guests

The binding constants (K_b) were obtained with the aid of non-linear least-square curve fitting analysis for the dependence of fluorescence intensity variation on the guest concentration.

The plot of $\Delta I/I_0$ versus the guest concentration can be fitted to an equation for a 1:1 host-guest complex (Eq. 1).

$$\Delta I / I_0 = \frac{\Delta I_{\max} / I_0 \left\{ \left([\mathrm{H}]_0 + [\mathrm{G}]_0 + 1/K_b\right) - \sqrt{\left([\mathrm{H}]_0 + [\mathrm{G}]_0 + 1/K_b\right)^2 - 4[\mathrm{H}]_0[\mathrm{G}]_0} \right\}}{2[\mathrm{H}]_0} \qquad (1)$$

Table S1 Binding constants (K_b) and $\Delta I_{max}/I_0$ of **NC0αCD** for halomethanes and alcohols.

Guest	K_b	$\Delta I_{max}/I_0$
EtOH	15.9	1.32
*i*PrOH	25.9	1.36
CH_2Cl_2	257	0.443
$CHCl_3$	332	0.777
CCl_4	956	0.959
CH_2Br_2	612	0.446
$CHBr_3$	1040	0.932
CBr_4	7520	0.874
CH_2I_2	4580	0.373
CHI_3	19700	0.414

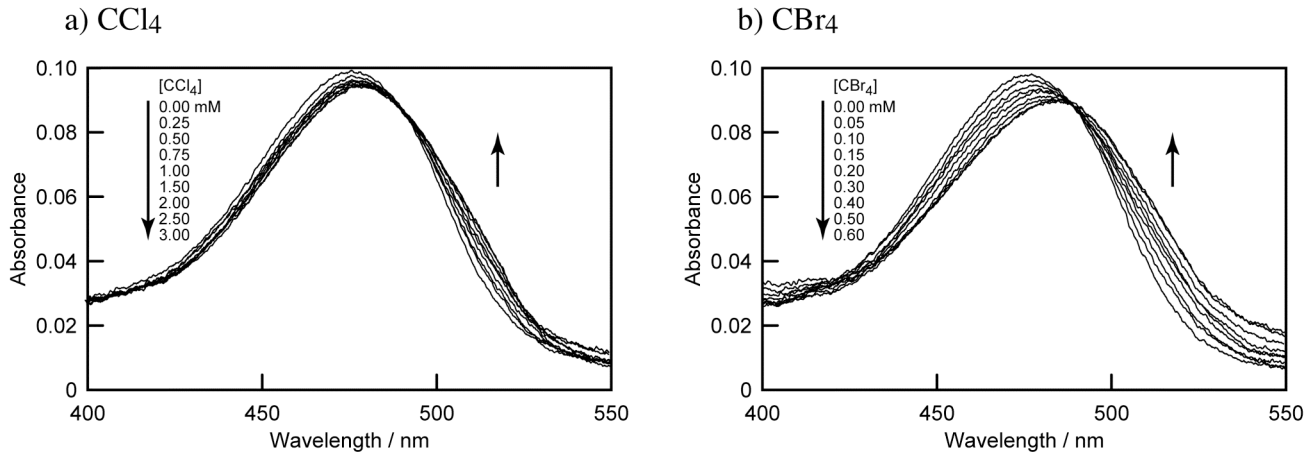


Figure S1 Absorption spectra of **NC0αCD** (5 x 10^{-6} M) in the presence of various concentrations of (a) CCl_4 and (b) CBr_4 in phosphate buffer (0.1 M, pH 7.0) at 25 °C;
(a) [CCl_4] = 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0 x 10^{-3} M, (b) [CBr_4] = 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0 x 10^{-4} M.

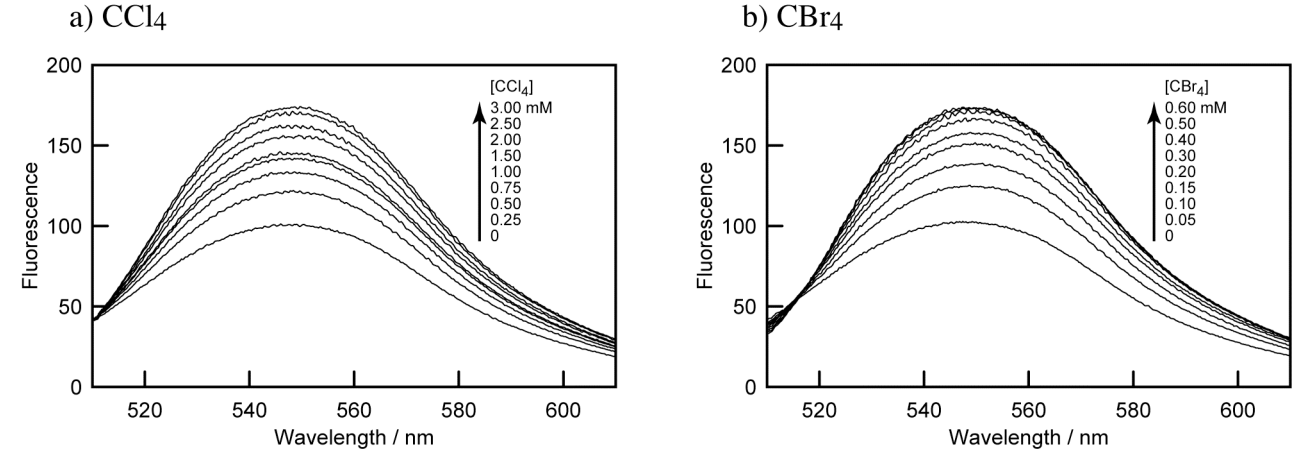


Figure S2 Fluorescence spectra of **NC0αCD** (5 x 10^{-6} M) in the presence of various concentrations of (a) CCl_4 and (b) CBr_4 in phosphate buffer (0.1 M, pH 7.0) at 25 °C;
(a) [CCl_4] = 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0 x 10^{-3} M, (b) [CBr_4] = 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0 x 10^{-4} M.

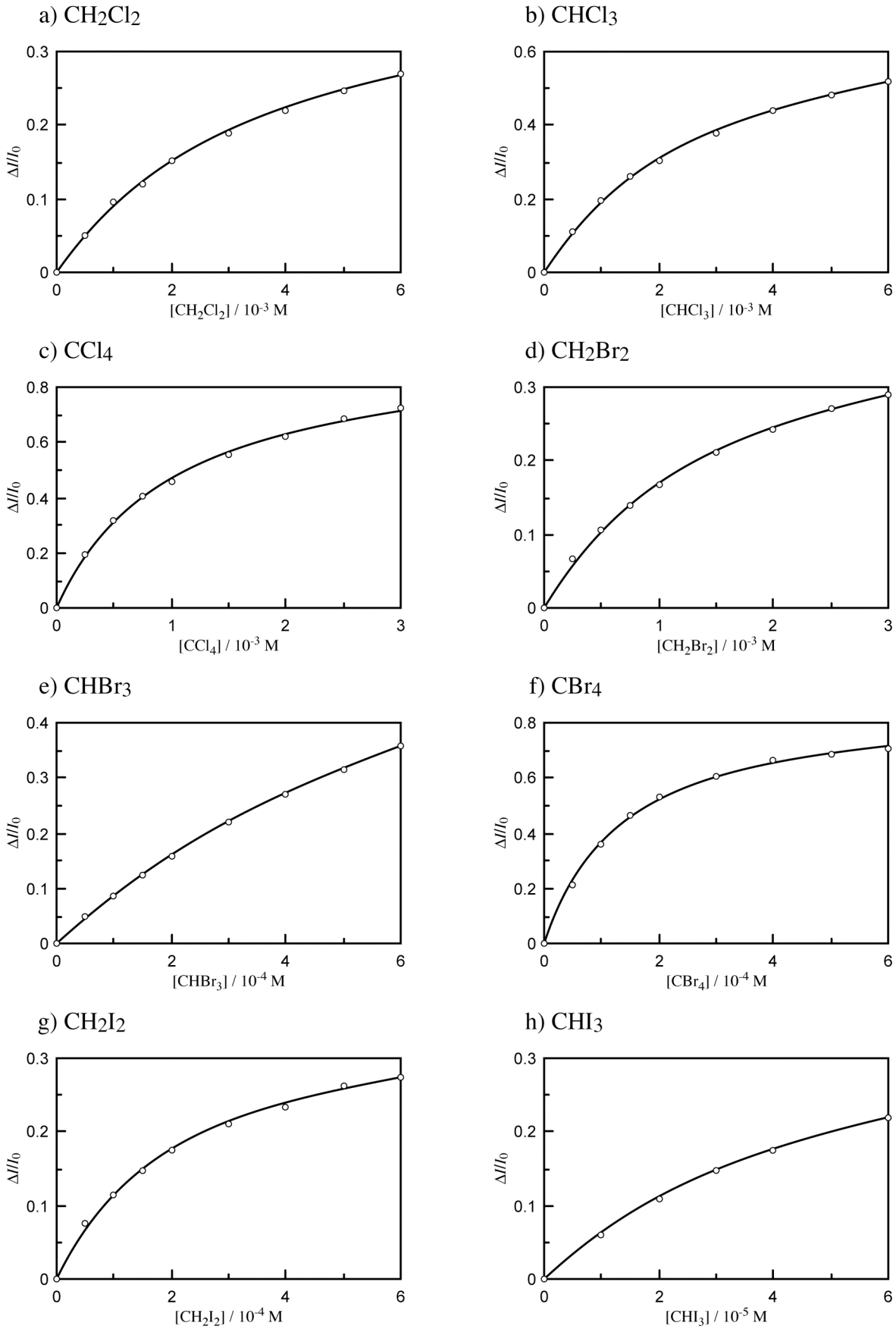


Figure S3 Curve-fitting analysis for the dependence of $\Delta I/I_0$ of **NC0αCD** on the concentration of halomethanes; (a) CH_2Cl_2, (b) $CHCl_3$, (c) CCl_4, (d) CH_2Br_2, (e) $CHBr_3$, (f) CBr_4, (g) CH_2I_2, (h) CHI_3.

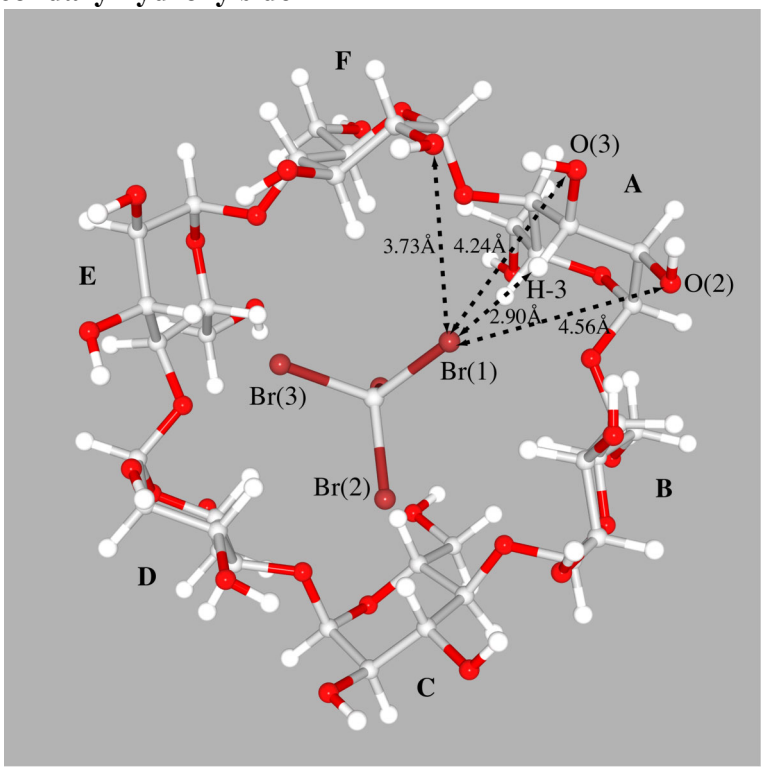
3. Estimation of a Stable Structure of the α-CD/CBr_4 Complex

3.1 Molecular Mechanics Calculation Procedure

To elucidate a plausible structure for the α-CD/CBr_4 complex, molecular mechanics calculations were performed using ChemBio3D Ultra 11.0 (CambridgeSoft Corporation, 2008) software with a modified Allinger's MM2 force field. The guest, CBr_4, was initially placed near the secondary hydroxy side of the α-CD, and energy minimization of the complex was carried out. Several different initial positions for the CBr_4 were tried for energy minimization, and almost the same stable structure was obtained in each case. Even if CBr_4 was initially placed in the α-CD cavity, a stable structure similar to Fig 4S was obtained.

3.2 Estimated Structure of the α-CD/CBr_4 Complex

a) view from the secondary hydroxy side



b) side view

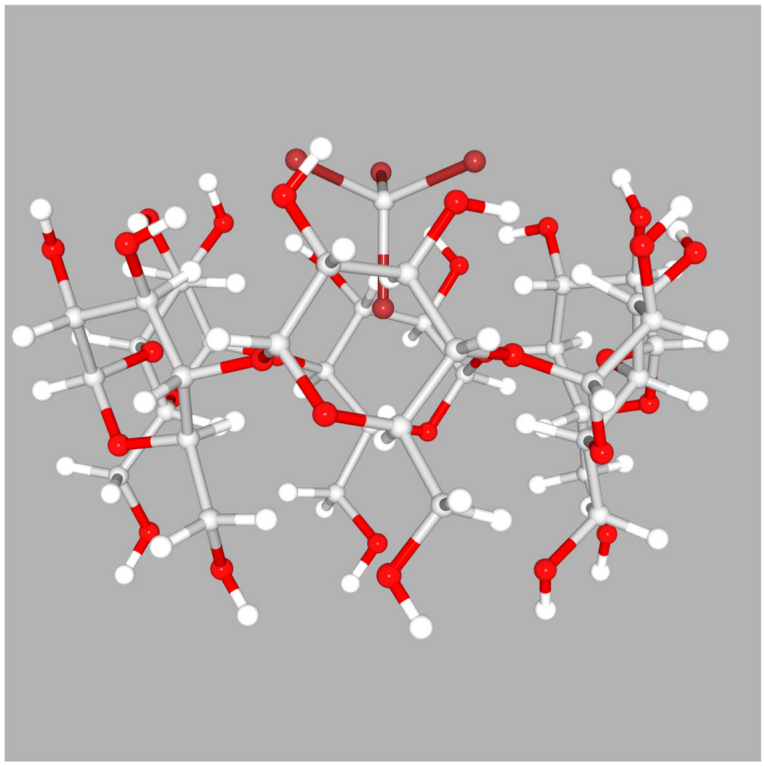


Figure S4 Estimated Structure of the α-CD/CBr_4 Complex.

3.3 Distance between Two Atoms

Table S2 Distance between a bromine atom on CBr_4 and an atom on α-CD in the estimated complex[a].

	H-3	O(2)	O(3)	OH(2)	OH(3)
Glucose(F)-Br(1)	3. 4753	3. 7312	4. 4907	3. 6414	4. 8342
Glucose(A)-Br(1)	2. 8987	4. 5585	4. 2351	4. 6059	3. 8097
Glucose(B)-Br(2)	3. 4734	3. 7311	4. 4906	3. 6412	4. 8342
Glucose(C)-Br(2)	2. 8987	4. 5586	4. 2352	4. 6060	3. 8098
Glucose(D)-Br(3)	3. 4734	3. 7312	4. 4906	3. 6412	4. 8342
Glucose(E)-Br(3)	2. 8986	4. 5585	4. 2351	4. 6059	3. 8097

[a] The distances were measured with ChemBio3D Ultra 11.0.
Distances in red number are shown in **Figure S4**.