Electronic Supplementary Information

Fluorescent α-Cyclodextrin as a Chemosensor for Halomethanes

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Figure S4  Estimated structure of the α-CD/CBr4 complex.
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 ($\delta = 4.70$) or MeOH ($\delta = 3.34$) 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$_3$aq. : 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$_2$O, 500 MHz): $\delta$ 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).

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

(1)
Table S1  Binding constants ($K_b$) and $\Delta I_{max}/I_0$ of NC0αCD for halomethanes and alcohols.

<table>
<thead>
<tr>
<th>Guest</th>
<th>$K_b$</th>
<th>$\Delta I_{max}/I_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH</td>
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<tr>
<td>iPrOH</td>
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<tr>
<td>CH$_2$Cl$_2$</td>
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<tr>
<td>CHCl$_3$</td>
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<tr>
<td>CCl$_4$</td>
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<tr>
<td>CH$_2$Br$_2$</td>
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<td>CHBr$_3$</td>
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<tr>
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<tr>
<td>CH$I_3$</td>
<td>19700</td>
<td>0.414</td>
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**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 NC0εCD on the concentration of halomethanes; (a) CH$_2$Cl$_2$, (b) CHCl$_3$, (c) CCl$_4$, (d) CH$_2$Br$_2$, (e) CHBr$_3$, (f) CBr$_4$, (g) CH$_2$I$_2$, (h) CHI$_3$. 

S4
3. Estimation of a Stable Structure of the \( \alpha \)-CD/CBr\(_4\) Complex

3.1 Molecular Mechanics Calculation Procedure

To elucidate a plausible structure for the \( \alpha \)-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 \( \alpha \)-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 \( \alpha \)-CD cavity, a stable structure similar to Fig 4S was obtained.
3.2 Estimated Structure of the $\alpha$-CD/CBr$_4$ Complex

a) view from the secondary hydroxy side

![Diagram showing the estimated structure from the secondary hydroxy side.]

b) side view

![Diagram showing the estimated structure from the side view.]

**Figure S4** Estimated Structure of the $\alpha$-CD/CBr$_4$ Complex.
3.3 Distance between Two Atoms

<table>
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<th>H-3</th>
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<th>O(3)</th>
<th>OH(2)</th>
<th>OH(3)</th>
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<td>4.5585</td>
<td>4.2351</td>
<td>4.6059</td>
<td>3.8097</td>
</tr>
</tbody>
</table>

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