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An acyclic phane receptor with a pair of disulfonaphthalene arms recognizing 2,3-*trans*-gallate-type catechins in water

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ABSTRACT

In order to develop a receptor molecule for recognizing differences in catechin structures, complexation between catechins and a water-soluble acyclic phane composed of an isophthalate and two aminodisulfonaphthalenes was investigated with ^{1}H NMR spectroscopy. The phane receptor formed 1:1 complexes with the catechins and showed preferential binding ability for the 2,3-*trans*-gallate-type catechin. The binding studies demonstrated the length of naphthalene framework required to form a hydrophobic environment for the complexation.

1. Introduction

Catechins, found in the leaves and buds of the tea plant (*Camellia sinensis*), are polyphenolic compounds that exhibit a variety of physiologically modulating effects.[1] The strength of these bioactivities is closely related to the chemical structures of the catechin molecules. The major catechins in green tea can be classified into four categories by the existence of a galloyl group on the oxygen atom at the C3 position and the relative stereochemistry between the C2 and C3 positions: (1) 2,3-*cis*-gallate-type, (2) 2,3-*trans*-gallate-type, (3) 2,3-*cis*-non-gallate-type, and (4) 2,3-*trans*-non-gallate-type. Typical structures are illustrated in Figure 1. Generally, the gallate-type catechins (**1** and **2**) show higher activities than the non-gallate-type catechins (**3** and **4**).[2]

A supramolecular approach to recognizing of differences in chemical structures is attractive. Previously, we reported that a simple water-soluble acyclic phane receptor can distinguish differences in the structures of the catechin analogs.[3] Receptor **5** forms 1:1 complexes with the catechins, and shows excellent binding ability for the 2,3-*trans*-gallate-type catechins. On the other hand, when the receptor molecule is applied to functionalized self-assembled monolayers[4] or column packing materials, it must be stable in a wide variety of chemical environments because of the various analytical and maintenance conditions used by catechin recognizing apparatuses equipped with the materials. However, receptor **5** is not stable in a wide pH range, because the three benzene rings of **5** are linked by ester groups. Consequently, in

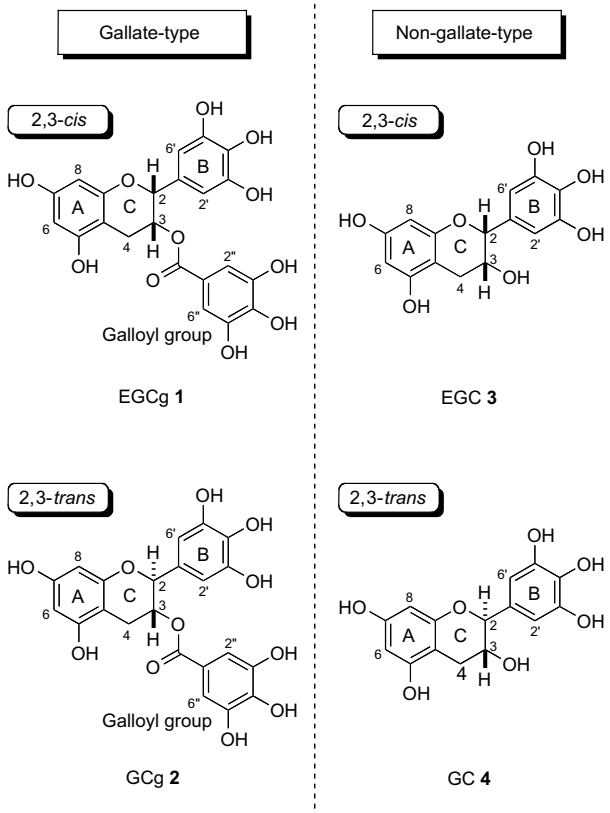

Figure 1. Structures of the catechins examined.

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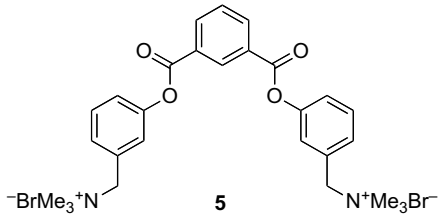
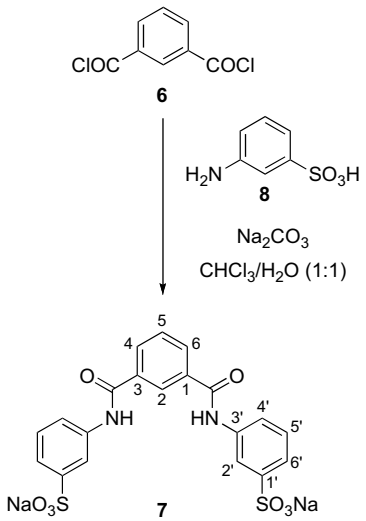
order to increase the potential application of this compound, we sought to develop receptor molecules exhibiting increased chemical stability. In this paper, complexation of three water-soluble acyclic phanes with three aromatic rings linked by amide groups, with the four catechins (EGCg **1**, GCg **2**, EGC **3**, and GC **4**) is investigated by NMR, and the requirements for recognizing the chemical structures of the catechins are discussed.



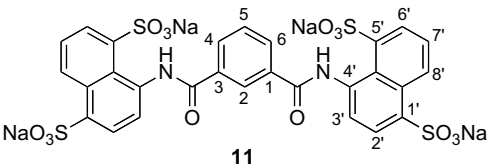
2. Results and discussion

Synthesis of an analog in which the ester groups of **5** are replaced by amide groups did not succeeded due to the insolubility and low reactivity of the synthetic intermediates. Therefore, for synthetic convenience, the receptor **7** with sulfonic acid groups as the hydrophilic groups was prepared by modification of the synthetic procedure for the analogs (Scheme 1).[5] Although the ionic motif in the receptor molecule was switched from positively charged ammonium to negatively charged sulfonate under neutral conditions, the logic of the receptor design based on hydrophobic interactions between the catechins and the receptor in aqueous solution is the same in both **5** and **7**. The binding ability of receptor **7** for the catechins (**1**, **2**, **3**, and **4**) was evaluated by ^{1}H NMR experiments. Figure 2 shows the chemical shift changes ($\Delta\delta_{obs}^{cat}$) in each proton of the catechins against the total concentration of the receptor **7** under neutral and acidic conditions (the deuterium oxide solutions were buffered at pD 7.0 with NaH_2PO_4/Na_2HPO_4 and at pD 1.7 with DCl/KCl, respectively).[6] Although these $\Delta\delta_{obs}^{cat}$ values were small, all of the ^{1}H NMR signals of the catechins shifted upfield with increasing concentration of **7**. To investigate the stoichiometric ratios of the complexes between **7** and the catechins, Job's plot experiments were performed using ^{1}H NMR chemical shift changes of the catechin C2′(6′)–H, which revealed that the ratios were 1:1 in every case.[7] The standard binding isotherm for the formation of a 1:1 complex was applied to the ^{1}H NMR titration plots in Figure 2 to obtain the binding constants (Table 1).[8] The binding constants under acidic conditions were larger by 20–200% than those under neutral conditions as **7** became more hydrophobic due to an increase in the SO_3H form.[9] However, the binding modes would not necessarily differ between neutral and acidic conditions, because the profile of the chemical shift changes of both the catechins and receptor **7** is similar under both conditions (Figs. 2 and 3). Interestingly, unlike receptor **5**, the binding ability of receptor **7** for the catechins was low. Moreover, receptor **7** is not able to recognize the differences in the chemical structures of the catechins, such as the presence of the galloyl group and the relative stereochemistry between the C2 and C3 positions. These results might be due to the property differences between the sulfonic acid group and the quaternary ammonium group and between the amide and ester groups, which result in different conformations between **7** and **5**. However, the depth of the binding site also appears to be an important factor. The quaternary nitrogen atom of **5** is attached to the benzene ring via the benzyl methylene, making the length of the hydrophobic portion 0.379–0.429 nm (Fig. 4a).[10] On the other hand, the corresponding length of **7** is only 0.242–0.279 nm (Fig. 4b), creating a shallower binding site.



Scheme 1. Preparation of receptor **7**.

Therefore, to increase the depth of the binding site of the amide-type receptor, compound **9** with two naphthalene frameworks as the arms was prepared (Scheme 2).[11,12] The length of the hydrophobic moiety in the arm of **9** is 0.421–0.487 nm (Fig. 4c). Binding between **9** and the catechins was investigated by ^{1}H NMR experiments. Figure 5 shows the chemical shift changes ($\Delta\delta_{obs}^{cat}$) in each proton of the catechins against the total concentration of receptor **9** under neutral and acidic conditions. All the ^{1}H NMR signals of the catechins shifted upfield with increasing concentration of receptor **9** and $\Delta\delta_{obs}^{cat}$ values were larger than those in **7**. Job's plot experiments performed using ^{1}H NMR chemical shift changes of the catechin C2′(6′)–H revealed that the stoichiometric ratios of the complexes were 1:1 in every case. The binding constants of **9** for the catechins were calculated by the analysis of the 1:1 complex using the plots in Figure 5. As shown in Table 2, the binding constants of **9** were larger than those of **7**, and were also larger under acidic conditions by 15–30% than under neutral conditions. Judging from the profile of the chemical shift changes of the catechins and receptor **9** (Figs. 5 and 6), the binding modes would be similar under both these conditions. The chemical shift changes in the protons of **9** shown in Figure 6, especially the slight changes of C2′–H between the two hydrophilic groups, suggest that the binding occurs in the more hydrophobic and inner part of the binding site, which is surrounded by the three aromatic rings. As expected, the binding behavior of receptor **9** for the catechin molecules was similar to that of receptor **5**: higher affinities for the gallate-type catechins (**1** and **2**) than the non-gallate-type catechins (**3** and **4**) and the 2,3-*trans*-gallate-type catechin (**2**) were preferred over the 2,3-*cis*-gallate-type catechin (**1**). However, the same effects are not necessarily obtained with other naphthalene arms. For example, receptor **11** exhibited weak catechin binding and recognition (Table 3).[13] This is likely due to insufficient length of the hydrophobic moieties in the binding site (the length of the hydrophobic moiety in the arm of **11** is 0.245–0.279 nm as illustrated in Fig. 4d). These results demonstrate that the binding and recognition abilities of **9** for the catechins are due to increased length of the hydrophobic moiety of the binding site.



To obtain additional information about the complexation between receptor **9** and the gallate-type catechins, NOESY

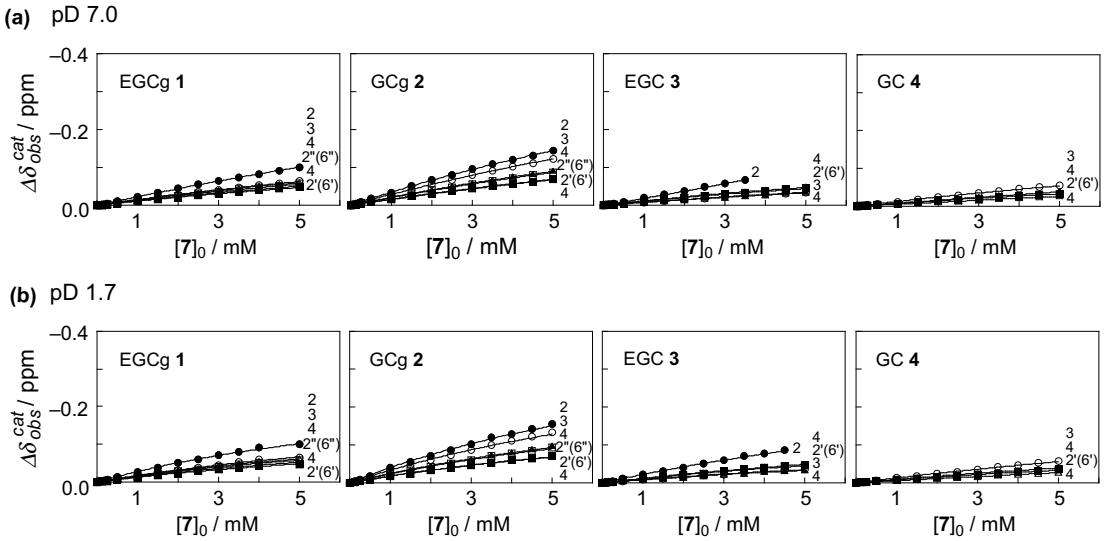


Figure 2. ^{1}H NMR titration curves for receptor **7** and the catechins in D_2O at 300 K. $\Delta\delta_{obs}^{cat}$ is the difference between the observed ^{1}H NMR chemical shift of the catechins with and without the receptor. [**7**]$_0$ is the total concentration of receptor **7**. The numbers in the graphs identify the protons of the catechins according to their attached carbon number. Some data points are not plotted in the graphs because their ^{1}H NMR signals were hidden under the residual proton signal of D_2O. The protons on the A-ring (C6–H and C8–H) were not detected due to deuterium substitution.

Table 1
Binding constants (M^{-1}) of the complex between receptor **7** and the catechins at 300 K in D_2O

Catechin protons	pD 7.0				pD 1.7			
	EGCg **1**	GCg **2**	EGC **3**	GC **4**	EGCg **1**	GCg **2**	EGC **3**	GC **4**
C2–H	31±5	57±3	—[a]	—[a]	125±20	105±16	64±17	—[a]
C3–H	56±14	47±3	54±14	61±15	89±18	97±16	77±17	49±13
C4–H	60±14	52±3	51±14	21±9	116±20	100±16	66±16	43±13
C4–H	72±14	41±4	66±16	22±11	122±21	99±18	65±15	27±12
C2′+6′-H	52±14	50±4	58±14	50±14	97±18	97±16	68±15	64±15
C2″+6″-H	67±15	47±3			114±20	100±16		
Mean	56±13	49±3	57±14	38±12	110±19	99±16	68±16	46±13

[a] These binding constants could not be determined, because the ^{1}H NMR signals were hidden under the residual proton signal of D_2O.

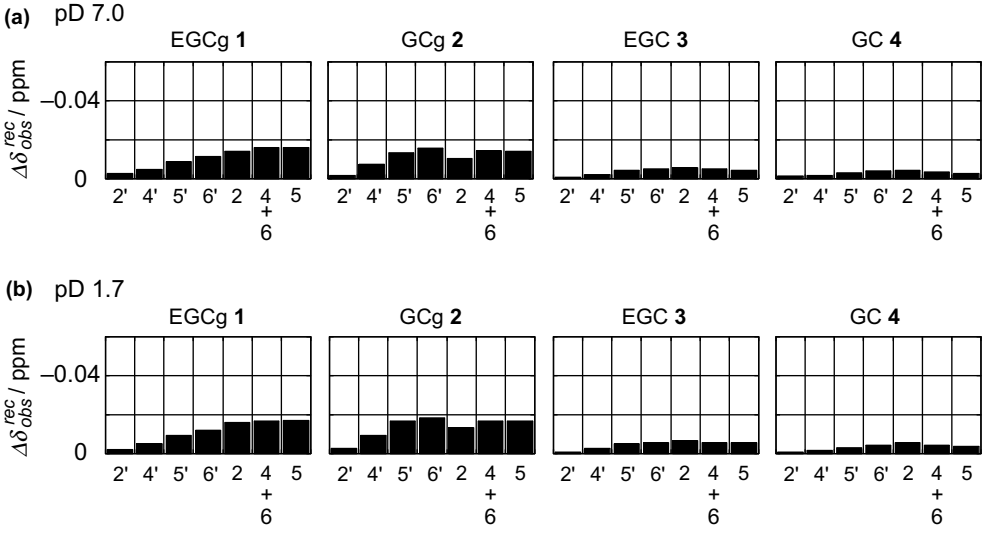


Figure 3. ^{1}H NMR chemical shift changes in receptor **7** (1.00 mM) by addition of the catechins (1.00 mM) in D_2O at 300 K. $\Delta\delta_{obs}^{rec}$ is the difference between the observed ^{1}H NMR chemical shifts of the receptor with and without the catechins. The *x*-axis identifies the protons of **7** according to their attached carbon number.

experiments were carried out. A spectrum in aqueous solution including **9** and EGCg **1** showed intermolecular cross peaks between the protons of the B-ring of **1** and the protons of the naphthalene rings of **9**, between the protons of the galloyl group of **1** and the protons of the naphthalene rings of **9**, between the protons of the B-ring of **1** and the protons of the benzene ring of **9**, and between the protons of the galloyl group of **1** and the protons of the benzene ring of **9** (Fig. 7a). Furthermore, intermolecular cross peaks were also observed between C2–H of **1** and C4′–H of **9**, between C4–H of **1** and C5′–H of **9**, and between C4–H of **1** and C8′–H of **9**.

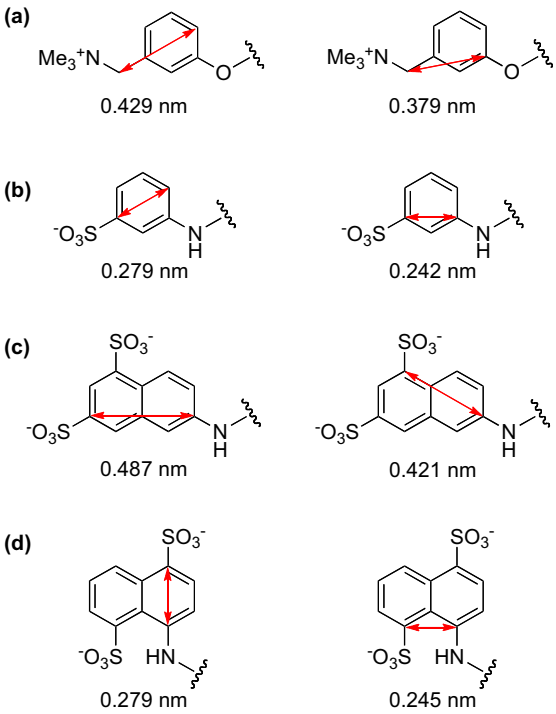
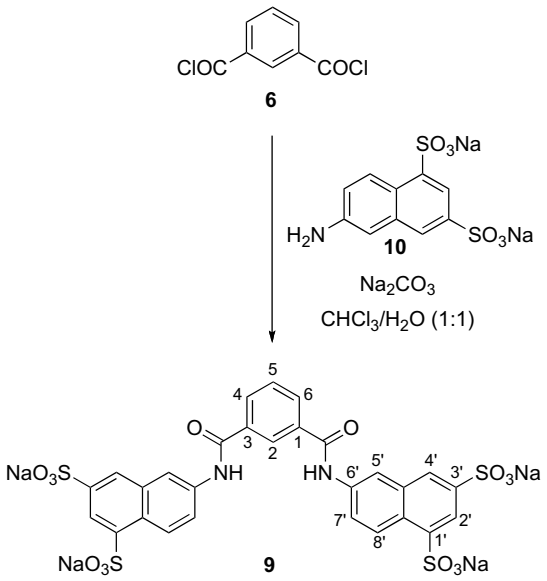


Figure 4. Lengths of the hydrophobic moieties in the arms of the receptors. (a) Receptor **5**, (b) receptor **7**, (c) receptor **9**, and (d) receptor **11**.



Scheme 2. Preparation of receptor **9**.

These results suggest that plural complex structures exist between **9** and **1**. On the other hand, a NOESY spectrum in aqueous solution including **9** and GCg **2** showed intermolecular cross peaks mainly between the protons of the B-ring of **2** and the protons of the naphthalene rings of **9**, between the protons of the galloyl group of **2** and the protons of the naphthalene rings of **9** (Fig. 7b). Similar NOESY results were observed between **5** and EGCg **1** and between **5** and GCg **2**.[3] However, in the case of the complexation between **9** and **2**, an intermolecular cross peak was also observed between C2″(6″)–H of **2** and C4(6)–H of **9**, suggesting that there is more than one major complex structure.

In this study, water-soluble acyclic phane receptors for the 2,3-*trans*-gallate-type catechin with higher chemical stability than the ester-type receptor **5** were examined. As a result, the amide-type receptor **9** with a pair of disulfonaphthalene arms was revealed to be well suited for the purpose. The depth of the binding site formed required utilizing the maximum length of the naphthalene framework in order to achieve full catechin recognition. Receptor **9** is stable in a wide pH range.[14] In the future, it is expected that the structure of **9** will be utilized as a practical receptor in recognizing apparatuses for the 2,3-*trans*-gallate-type catechins.

3. Experimental

3.1. General

The preparation of **7**, **9**, and **11** was performed in Kjeldahl shape flasks (short neck type) with a polyethylene stopper made a few holes by a needle under an air atmosphere. All reactions were magnetically stirred. Solutions were concentrated by rotary evaporation at 30 °C.

Special reagent-grade solvents and reagents were used without further purification. All catechins, the aromatic compounds (**6**, **8**, **10**, and **12**) were obtained from commercial suppliers.

Proton and carbon-13 nuclear magnetic resonance (NMR) spectra were recorded on a 500 MHz spectrometer. Chemical shifts in ^{1}H NMR spectra are reported on the parts per million scale, and are referenced to tetramethylsilane (δ=0.0000 ppm) in carbon tetrachloride as an external standard, which was inserted into an NMR tube (ϕ=5 mm) with a coaxial cell. Chemical shifts in ^{13}C NMR spectra are reported on the parts per million scale and are referenced to the carbon resonances of carbon tetrachloride (δ=96.1 ppm) as an external standard, which was inserted into an NMR tube (ϕ=5 mm) with a coaxial cell. ^{1}H NMR data are represented as follows: chemical shift, integration, multiplicity (s=singlet, d=doublet, t=triplet, m=multiplet, and br=broad), and coupling constant in hertz. The proton signals of the compounds **7**, **9**, and **11** were assigned by Double Quantum Filtered COSY (DQF-COSY), NOESY, HMQC, and HMBC spectra. Hydrogen multiplicity information was obtained from DEPT and HMQC spectra. Infrared (IR) spectra were recorded on an FT/IR spectrometer. High-resolution mass spectra (HRMS) were obtained at the Center for Instrumental Analysis, Hokkaido University.

3.2. Synthesis of the receptors (7, 9, and 11)

*3.2.1. Disodium 3,3′-[1,3-phenylenebis(carbonylimino)]bisbenzenesulfonate (**7**).* A chloroform solution (48 mL) of isophthaloyl chloride **6** (2.00 g, 9.85 mmol) and 2.00 M sodium carbonate aqueous solution (9.96 mL) were added to an aqueous solution (48 mL) of 3-aminobenzenesulfonic acid **8** (3.42 g, 19.7 mmol) at 0 °C. The solution was stirred at 25 °C for 20 h. The formed precipitate was separated by Kiriyama funnel, and then washed with dichloromethane. The residual solvent was removed in vacuo to give **7** (4.22 g, 82%) as a white solid: mp (**7** did not change in appearance up to 300 °C); ^{1}H NMR (500 MHz, D_2O) δ 8.21 (1H, br s, C2–*H*), 7.94 (2H, dd, *J*=1.6, 8.0 Hz, C4(6)–*H*), 7.80 (1H, t, *J*=1.9 Hz, C2′–*H*), 7.61 (2H, ddd, *J*=1.0, 1.9, 8.0 Hz, C6′–*H*), 7.55 (1H, t, *J*=8.0 Hz, C5–*H*), 7.49 (2H, ddd, *J*=1.0, 1.9, 8.0 Hz, C4′–*H*), and 7.41 (2H, t, *J*=8.0 Hz, C5′–*H*); ^{13}C NMR (125 MHz, D_2O) δ 167.9 (C), 143.0 (C), 137.2 (C), 133.9 (C), 130.9 (CH), 129.6 (CH), 129.2 (CH), 126.4 (CH), 124.6 (CH), 122.1 (CH), and 118.6 (CH); IR (KBr) 3435, 1680, 1542, 1190, and 1046 cm^{-1}; HR-FABMS, Calcd for $C_{20}H_{14}N_2NaO_8S_2$ ([M−Na]$^-$), 497.0095, found 497.0107.

*3.2.2. Tetrasodium 6,6′-[1,3-phenylenebis(carbonylimino)]bis-1,3-naphthalenedisulfonate (**9**).* A chloroform solution (24 mL) of isophthaloyl chloride **6** (1.00 g, 4.93 mmol) and 2.00 M sodium carbonate aqueous solution (2.49 mL) were added to an aqueous

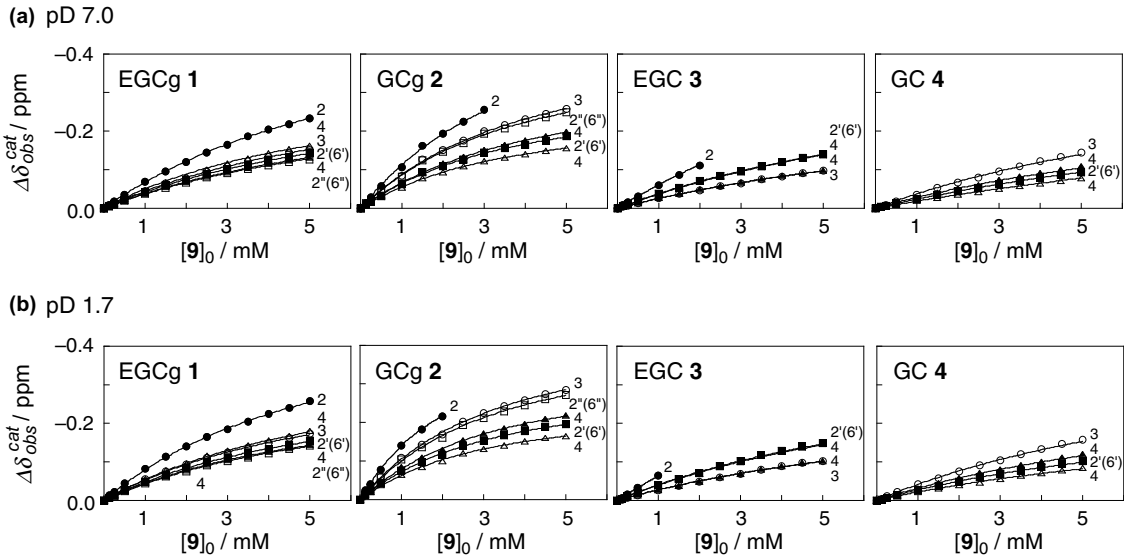


Figure 5. ^{1}H NMR titration curves for receptor **9** and the catechins in D_2O at 300 K. $\Delta\delta_{obs}^{cat}$ is the difference between the observed ^{1}H NMR chemical shift of the catechins with and without the receptor. $[\mathbf{9}]_0$ is the total concentration of receptor **9**. The numbers in the graphs identify the protons of the catechins according to their attached carbon number. Some data points are not plotted in the graphs, because their ^{1}H NMR signals were hidden under the residual proton signal of D_2O. The protons on the A-ring (C6–H and C8–H) were not detected due to deuterium substitution.

Table 2
Binding constants (M^{-1}) of the complex between receptor **9** and the catechins at 300 K in D_2O

Catechin protons	pD 7.0				pD 1.7			
	EGCg **1**	GCg **2**	EGC **3**	GC **4**	EGCg **1**	GCg **2**	EGC **3**	GC **4**
C2–H	155±5	300±49	110±36	—[a]	215±9	—[a]	—[a]	—[a]
C3–H	158±6	297±16	97±4	108±16	212±10	396±22	117±16	119±14
C4–H	154±5	282±15	99±4	111±20	209±10	394±24	118±16	121±15
C4–H	203±26	351±18	105±3	50±4	198±8	445±27	123±15	106±16
C2′+6′-H	163±5	331±18	114±3	76±4	212±10	400±22	128±15	126±14
C2″+6″-H	154±5	324±17			195±10	400±22		
Mean	165±9	314±22	105±10	86±11	207±10	407±23	122±16	118±15

[a] These binding constants could not be determined, because the ^{1}H NMR signals were hidden under the residual proton signal of D_2O.

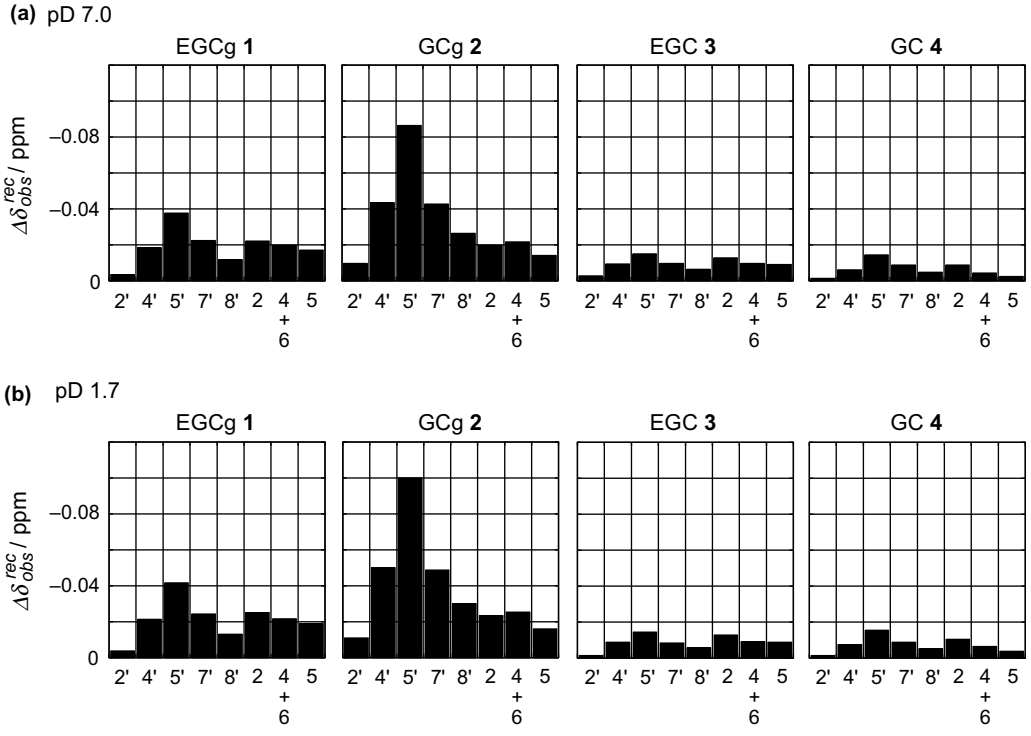


Figure 6. ^{1}H NMR chemical shift changes in receptor **9** (1.00 mM) by addition of the catechins (1.00 mM) in D_2O at 300 K. $\Delta\delta_{obs}^{rec}$ is the difference between the observed ^{1}H NMR chemical shifts of the receptor with and without the catechins. The *x*-axis identifies the protons of **9** according to their attached carbon number.

Table 3
Binding constants (M^{-1}) of the complex between receptor **11** and the catechins at 300 K in D_2O

Catechin protons	pD 7.0				pD 1.7			
	EGCg **1**	GCg **2**	EGC **3**	GC **4**	EGCg **1**	GCg **2**	EGC **3**	GC **4**
C2–H	114±14	133±10	—[a]	—[a]	129±14	188±16	—[a]	—[a]
C3–H	82±6	153±14	59±14	42±5	136±17	160±6	50±14	66±16
C4–H	128±16	161±15	62±16	50±7	131±14	154±7	68±14	60±14
C4–H	130±21	155±15	63±14	47±9	97±7	159±6	67±16	61±15
C2′+6′-H	119±15	160±15	49±14	46±13	129±14	165±8	67±16	63±15
C2″+6″-H	121±18	159±14			144±22	155±6		
Mean	116±15	153±14	58±14	46±9	128±15	164±8	63±15	62±15

[a] These binding constants could not be determined, because the ^{1}H NMR signals were hidden under the residual proton signal of D_2O.

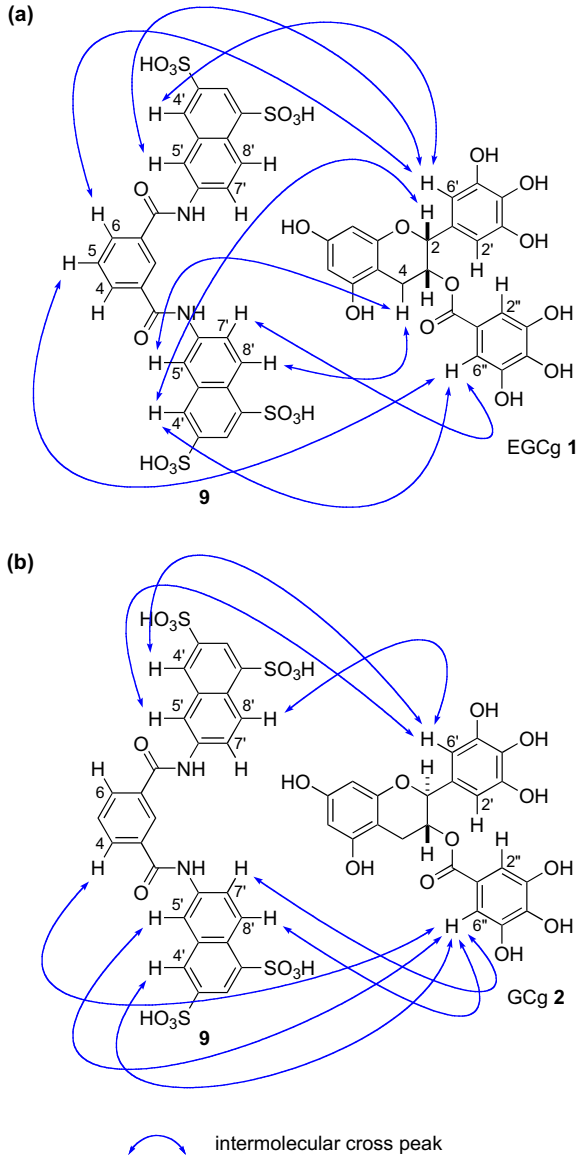


Figure 7. Intermolecular cross peaks in the NOESY spectra.

solution (24 mL) of disodium 6-amino-1,3-naphthalenedisulfonate **10** (3.42 g, 9.86 mmol) at 0 °C. The solution was stirred at 25 °C for 15 h. The aqueous layer was separated, and the solvent was removed in vacuo. The aqueous solution of the residue was acidified to less than pH 1 with 1 M hydrochloric acid. This solution was passed through 50 mL of Amberlite (IR120B H AG) in a glass column. The eluant was concentrated in vacuo. The residue was dissolved in methanol, and the solvent was removed in vacuo. This process was repeated six times to give the residue (3.42 g). 2.00 M sodium carbonate aqueous solution (4.65 mL) was added to an aqueous solution of this residue, and the resulting solution was lyophilized to give **9** (3.65 g, 90%) as a pale yellow-white solid: mp (**9** did not change in appearance up to 300 °C); ^{1}H NMR (500 MHz, D_2O) δ 8.53 (2H, d, J=9.2 Hz, C8′–*H*), 8.35 (2H, m, C4′–*H*), 8.31 (1H, t, J=1.9 Hz, C2–*H*), 8.27 (2H, d, J=2.2 Hz, C5′–*H*), 8.18 (2H, d, J=1.9 Hz, C2′–*H*), 8.01 (2H, dd, J=1.9, 8.0 Hz, C4(6)–*H*), 7.80 (2H, dd, J=2.2, 9.2 Hz, C7′–*H*), and 7.59 (1H, t, J=8.0 Hz, C5–*H*); ^{13}C NMR (125 MHz, D_2O) δ 168.1 (C), 139.2 (C), 138.9 (C), 136.2 (C), 133.9 (C), 133.6 (C), 130.8 (CH), 129.2 (CH), 128.9 (CH), 126.4 (CH), 126.2 (C), 126.1 (CH), 124.4 (CH), 121.4 (CH), and 120.3 (CH); IR (KBr) 3440, 1668, 1543, 1195, and 1041 cm^{-1}; HR-ESI-MS, Calcd for $C_{28}H_{16}N_2Na_3O_{14}S_4$ ([M−Na]$^-$), 800.91829, found 800.91520.

*3.2.3. Tetrasodium 4,4′-[1,3-phenylenebis(carbonylimino)]bis-1,5-naphthalenedisulfonate (**11**).* A chloroform solution (24 mL) of isophthaloyl chloride **6** (1.00 g, 4.93 mmol) and 2.00 M sodium carbonate aqueous solution (4.98 mL) were added to an aqueous solution (24 mL) of sodium 4-amino-1,5-naphthalenedisulfonate **12** (3.21 g, 9.86 mmol) at 0 °C. The solution was stirred at 25 °C for 16 h. The aqueous layer was separated, and the solvent was removed in vacuo. The aqueous solution of the residue was acidified to less than pH 1 with 1 M hydrochloric acid. This solution was passed through 50 mL of Amberlite (IR120B H AG) in a glass column. The eluant was concentrated in vacuo. The residue was dissolved in methanol, and the solvent was removed in vacuo. This process was repeated six times to give the residue (3.60 g). 2.00 M sodium carbonate aqueous solution (4.88 mL) was added to an aqueous solution of this residue, and the resulting solution was lyophilized to give **11** (3.95 g, 97%) as a white solid: mp (**11** did not change in appearance up to 300 °C); ^{1}H NMR (500 MHz, D_2O) δ 8.82 (2H, dd, J=1.3, 8.6 Hz, C8′–*H*), 8.43 (1H, br s, C2–*H*), 8.30 (2H, dd, J=1.3, 7.5 Hz, C6′–*H*), 8.14 (2H, d, J=8.0 Hz, C2′–*H*), 8.11 (2H, br d, J=7.8 Hz, C4(6)–*H*), 7.79 (2H, d, J=8.0 Hz, C3′–*H*), 7.62 (1H, t, J=7.8 Hz, C5–*H*), and 7.59 (2H, dd, J=7.5, 8.6 Hz, C7′–*H*); ^{13}C NMR (125 MHz, D_2O) δ 168.8 (C), 137.75 (C), 137.67 (C), 134.3 (C), 134.2 (C), 131.2 (CH), 131.0 (C), 130.0 (CH), 129.4 (CH), 129.2 (CH), 127.1 (CH), 126.5 (CH), 126.2 (CH), 125.9 (CH), and 124.8 (C); IR (KBr) 3452, 1653, 1532, 1189, and 1040 cm^{-1}; HR-ESI-MS, Calcd for $C_{28}H_{16}N_2Na_3O_{14}S_4$ ([M−Na]$^-$), 800.91829, found 800.91485.

3.3. ^{1}H NMR titration experiments

Stock solutions of the catechins (5.50 mM) and the receptors (6.11 mM) in deuterium oxide buffered at pD 7.0 with NaH_2PO_4/Na_2HPO_4 or at pD 1.7 with DCl/KCl were prepared separately. For each combination between the catechins and the receptors, 13 NMR tubes were filled separately with different concentrations of the receptor at 0.000, 0.125, 0.250, 0.500, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00 mM against a constant concentration of the

catechin (1.00 mM) in a 0.550 mL total solution for each. ^{1}H NMR spectra were obtained for each tube at 300 K. The chemical shift changes ($\Delta\delta_{obs}^{cat}$) in each proton of the catechins were plotted against the total concentration of the receptors. The fitting curves for the plots were calculated by nonlinear least-squares regression procedures according to Eq. 1 using KaleidaGraph (Synergy Software) to obtain the binding constants (Eq. 1 is the standard binding isotherm for the formation of a 1:1 complex). In Eq. 1, $[R]_0$ and $[C]_0$ are the total concentrations of the receptors and the catechins, respectively, K_b is a binding constant, and $\Delta\delta_{11}^{cat}$ is the difference between the ^{1}H NMR chemical shift of the catechins forming the 1:1 complex with the receptors (δ_{11}^{cat}) and the free catechins ($\delta_{obs}^{cat(\mathrm{free})}$), i.e., $\Delta\delta_{11}^{cat} = \delta_{11}^{cat} - \delta_{obs}^{cat(\mathrm{free})}$.

$$\Delta\delta_{obs}^{cat} = \Delta\delta_{11}^{cat}\left[1 + K_b[C]_0 + K_b[R]_0 - \left\{(1 + K_b[C]_0 + K_b[R]_0)^2 - 4K_b^2[C]_0[R]_0\right\}^{1/2}\right] \Big/ 2K_b[R]_0 \quad (1)$$

3.4. Chemical shift changes in the protons of the receptors (7 and 9) with the addition of the catechins

Stock solutions of each receptor (2.00 mM) and each catechin (2.00 mM) in D_2O buffered at pD 7.0 with NaH_2PO_4/Na_2HPO_4 or at pD 1.7 with DCl/KCl were prepared separately. The NMR tubes including only receptors and including both the receptors and the catechins were prepared with 1.00 mM of the receptors and 1.00 mM of the catechins for a 0.550 mL total solution. ^{1}H NMR spectra were obtained for each tube at 300 K.

3.5. NOESY experiments

A solution in D_2O buffered by DCl/KCl (0.600 mL) including the receptor **9** (1.20 mmol) and EGCg **1** or GCg **2** (1.20 mmol each) was prepared. NOESY spectra using pulsed field gradients were acquired at 300 K under the following conditions: relaxation delay of 1.0 s, mixing time of 100 ms, 128 scans, 512×512 data points. The data were processed with a sine-bell window function.

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Supplementary data

A Job's plot experimental procedure. Job's plot curves (Figs. S1 and S2). ^{1}H NMR titration curves for receptor **11** and the catechins (Fig. S3). NOESY spectra in aqueous solutions including **1** and **9**. A NOESY spectrum in aqueous solutions including **2** and **9**. This material is available via the Internet. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.07.071.

References and notes

1. Anti-carcinogenic activity: (a) Lambert, J. D.; Yang, Y. C.; Lin-Shiau, S. Y. *Mutat. Res.* **2003**, *523*, 201–208; (b) Ahmad, N.; Cheng, P.; Mukhtar, H. *Biochem. Biophys. Res. Commun.* **2000**, *275*, 328–334; (c) Cao, Y.; Cao, R. *Nature* **1999**, *398*, 381–382; (d) Suganuma, M.; Okabe, S.; Sueoka, N.; Sueoka, E.; Matsuyama, S.; Imai, K.; Nakachi, K.; Fujiki, H. *Mutat. Res.* **1999**, *428*, 339–344; (e) Lin, J. K.; Liang, Y. C.; Lin-Shiau, S. Y. *Biochem. Pharmacol.* **1999**, 911–915; (f) Kurada, Y.; Hara, Y. *Mutat. Res.* **1999**, *436*, 69–97; (g) Wang, Z.-N.; Hong, J.-Y.; Huang, M.-T.; Reuhl, K. R.; Conney, A. H.; Yang, C. S. *Cancer Res.* **1995**, *52*, 1943–1947; Antimetastatic activity: (a) Maeda-Yamamoto, M.; Kawahara, H.; Tahara, N.; Tsuji, K.; Hara, Y.; Isemura, M. *J. Agric. Food Chem.* **1999**, *47*, 2350–2354; (b) Sazuka, M.; Imazawa, H.; Shoji, Y.; Mita, T.; Hara, Y.; Isemura, M. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1504–1506; (c) Sazuka, M.; Murakami, S.; Isemura, M.; Satoh, K.; Nukiwa, T. *Cancer Lett.* **1995**, *98*, 27–31; (d) Isemura, M.; Suzuki, Y.; Satoh, K.; Narumi, K.; Motomiya, M. *Cell Biol. Int.* **1993**, *17*, 559–564; Anti-oxidative activity: (a) Hashimoto, F.; Ono, M.; Masuoka, C.; Ito, Y.; Sakata, Y.; Shimizu, K.; Nonaka, G.; Nishioka, I.; Nohara, T. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 396–401; (b) Kimura, M.; Umegaki, K.; Kasuya, Y.; Sugisawa, A.; Higuchi, M. *Eur. J. Clin. Nutr.* **2002**, *56*, 1186–1193; (c) Bors, W.; Saran, M. *Free Radical Res. Commun.* **1987**, *2*, 289–294; (d) Okuda, T.; Kimura, Y.; Yoshida, T.; Hatano, T.; Okuda, H.; Arichi, S. *Chem. Pharm. Bull.* **1983**, *32*, 1625–1631; Anti-hypertensive activity: (a) Yokozawa, T.; Okura, H.; Sakanaka, S.; Ishigaki, S.; Kim, M. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 855–858; Anti-hypercholesterolemic: (a) Murase, T.; Nagasawa, A.; Suzuki, J.; Hase, T.; Tokimitsu, I. *Int. J. Obes. Relat. Metab. Disord.* **2002**, *26*, 1459–1464; (b) Masumoto, N.; Okushio, K.; Hara, Y. *J. Nutr. Sci. Vitaminol.* **1998**, *44*, 337–342; (c) Chisaka, T.; Matsuda, H.; Kubomura, Y.; Mochizuka, M.; Yamahara, J.; Fujimura, H. *Chem. Pharm. Bull.* **1988**, *36*, 227–233; Anti-bacterial activity: (a) Fukai, K.; Ishigami, T.; Hara, Y. *Agric. Biol. Chem.* **1991**, *55*, 1895–1897; Anti-dental caries activity: (a) Sakanaka, S.; Shimura, N.; Masumi, M.; Kim, M.; Yamamoto, T. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 592–594; (b) Sakanaka, S.; Sato, T.; Kim, M.; Taniguchi, M.; Yamamoto, T. *Agric. Biol. Chem.* **1990**, *54*, 2925–2929; (c) Hattori, M.; Kusumoto, I.; Namba, T.; Ishigami, T.; Hara, Y. *Chem. Pharm. Bull.* **1990**, *38*, 717–720; Intestinal flora amelioration activity: (a) Okubo, T.; Ishihara, N.; Okura, A.; Serit, M.; Kim, M.; Yamamoto, T.; Mitsuoka, T. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 588–591.
2. (a) Lee, S. M.; Kim, C. W.; Kim, J. K.; Shin, H. J.; Baik, J. H. *Lipids* **2008**, *43*, 419–429; (b) Okabe, S.; Suganuma, M.; Hayashi, M.; Sueoka, E.; Komori, A.; Fujiki, H. *Jpn. J. Cancer Res.* **1997**, *88*, 639–643; (c) Tezuka, M.; Suzuki, H.; Suzuki, Y.; Hara, Y.; Okada, S. *Jpn. J. Toxicol. Environ. Health* **1997**, *43*, 311–315; (d) Ishikawa, T.; Suzukawa, M.; Ito, T.; Yoshida, H.; Ayaori, M.; Nishiwaki, M.; Yonemura, A.; Hara, Y.; Nakamura, H. *Am. J. Clin. Nutr.* **1997**, *66*, 261–266; (e) Miura, S.; Watanabe, J.; Tomita, T.; Sano, M.; Tomita, I. *Biol. Pharm. Bull.* **1994**, *17*, 1567–1572; (f) Hara, Y.; Watanabe, M. *Nippon Shokuhin Kogyo Gakkaishi* **1989**, *36*, 951–955.
3. Hayashi, N.; Ujihara, T. *J. Org. Chem.* **2008**, *73*, 4848–4854.
4. Love, J. C.; Estroff, L. A.; Kriebel, J. K.; Nuzzo, R. G.; Whitesides, G. M. *Chem. Rev.* **2005**, *105*, 1103–1169.
5. Aoki, M.; Nishikawa, N.; Wu, Y. Patent WO 97/19916, 1997.
6. The NaH_2PO_4/Na_2HPO_4–D_2O buffer was prepared by diluting a solution of 0.20 M Na_2HPO_4–D_2O (18.75 mL) and 0.20 M NaH_2PO_4–D_2O (31.25 mL) to 100 mL with D_2O. A pH meter in the buffer solution at 22 °C measured a pH of 6.6. The DCl/KCl–D_2O buffer was prepared by diluting a solution of 0.20 M DCl–D_2O (12.8 mL) and 0.20 M KCl–D_2O (37.2 mL) to 100 mL with D_2O. A pH meter in the buffer solution at 22 °C measured a pH of 1.3. The pH values were converted into the pD values by the following equation: pD=pH + 0.40 (Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188–190).
7. Connors, K. A. *Binding Constants*; Wiley-Interscience: New York, NY, 1987; pp 24–28.
8. Hirose, K. *J. Inclusion Phenom. Macrocycl. Chem.* **2001**, *39*, 193–209.
9. An accurate ratio cannot be calculated, because the pK_a value of the sulfonic acid group of **7** is unclear. If the value is assumed to be 3.70, which is the pK_a value of 3-aminobenzenesulfonic acid **8** (*The Merck Index*, 13th ed.; Merck & Company: New Jersey, NJ, 2001;), the ratio of the SO_3H form is 99.6% at pH 1.30 (pD 1.70). However, the actual ratio might be lower than this calculated ratio, because the pK_a value of **7** will be smaller than that of **8** on account of the acylated amino group of **7**. On the other hand, the ratio of the SO_3^- form at pH 6.60 (pD 7.00) is probably more than 99.9%.
10. The length between the atoms was estimated by ChemBio3D 11.0 (CambridgeSoft).
11. Suzuki, M. Jpn. Kokai Tokkyo Koho JP 2004292322, 2004.
12. The analogs with one sulfonic acid group on one naphthalene ring do not have sufficient solubility in water.
13. Wong, M. F.; Huang, P. P.; Brinkworth, R. I.; Yashiro, M.; Mohan, P.; Fairlie, D. P.; Baba, M.; Verma, S. *Eur. J. Med. Chem.* **1996**, *31*, 249–255.
14. Receptor **9** was stable under basic conditions (in 0.1 M sodium hydroxide aqueous solution at room temperature) as well as under acidic and neutral conditions described in the text.