Development of highly sensitive and selective molecules for detection of spermidine and spermine†

Daisuke Tanima,[a] Yoko Imamura,[a] Takeo Kawabata[a] and Kazunori Tsubaki*[b]

Received 18th May 2009, Accepted 21st July 2009
First published as an Advance Article on the web 9th September 2009
DOI: 10.1039/b909682e

To establish an effective and concise procedure for determining the concentrations of spermidine and spermine, several functional molecules based on phenolphthalein and two crown loops were constructed. Host **5** with a dimethylamino group showed excellent selectivity for spermidine and spermine among other biogenic amines and high sensitivity compared with basic host **1**.

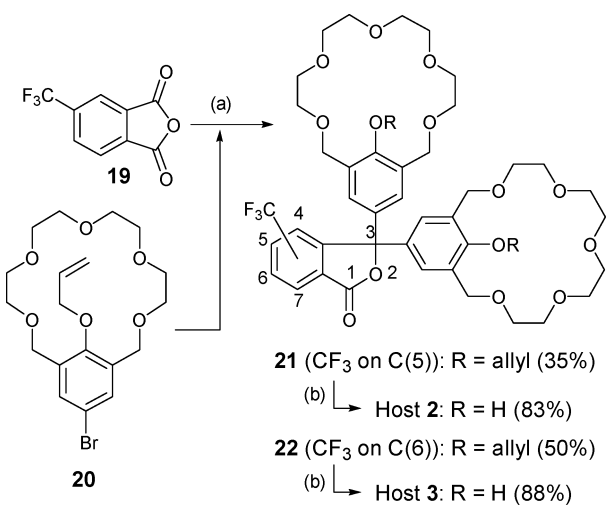
Introduction

Spermidine and spermine, which are biogenic polyamines, are widely found in vivo, especially in rapidly growing tissues, and play important roles in vital activity.[1] Recently, diacetylspermidine and diacetylspermine (metabolites of spermidine and spermine) in urine have been used as useful tumor markers.[2] One might assume that if the levels of these amines in urine have risen, then the levels in tissue or blood may also have risen. However, elaborate procedures such as deproteination, centrifugation, fluorescent derivatization and HPLC analysis have been required to determine the concentrations of spermidine and spermine in tissue or blood.[3] Therefore, the development of an effective and concise procedure is needed and this method should be suitable for clinical use.

We have investigated colorimetric recognition based on phenolphthalein derivatives with two crown ethers.[4,5] Through these studies, we have achieved the colorimetric recognition of spermidine and spermine from among other linear triamines using basic host **1** (Fig. 1).[5b] Host **1** can trap spermidine through a three-point interaction: two interactions between the crown phenol moieties of host **1** and the terminal amines of spermidine and one interaction between the carboxylate of **1** and the inner amine group of spermidine. We have also found that there are two types of complex (colored carboxylate form and colorless lactone form) between host **1** and spermidine and the position of the equilibrium between them determines the strength of the color (Fig. 2). The sensitivity of host **1** toward spermidine is too low to apply this system in clinical practice. Hence, we have been trying to develop more sensitive host molecules by applying the following two concepts: (1) improvement of the binding constant between the host and target amines as well as displacement of the equilibrium toward the colored complex through the introduction of an electron-withdrawing group on the benzolactone ring, and (2) conversion of the host molecules from a colorimetric response to a fluorescence response through expansion of the benzolactone ring to a polycyclic aromatic lactone ring. In this paper, we report the development of host molecules **2–8** highly sensitive toward spermidine (**9**) and spermine (**10**) among other biogenic amines (**11–16**) (Fig. 1).

Results and discussion

The synthetic route to host molecules **2** and **3** is outlined in Scheme 1. Crown ether **20** [6] was treated with *t*-BuLi and allowed to react with 5-trifluoromethylphthalic anhydride (**19**)[7] to give **21** and its regioisomer **22** in respective yields of 35% and 50%. The phenolic allyl group of **21** was removed by $Pd(PPh_3)_4$ and sodium borohydride to give host **2** in 83% yield.[8] Host **3** was synthesized from **22** by a similar procedure (88% yield). Colorless crystals of **21** were precipitated from methanol in a form that included two methanols, and the structure of **21** was clarified by X-ray analysis.† Although part of the crown ring was disordered, the trifluoromethyl group on the benzolactone ring was determined to be at the 5-position (Fig. S1†). Other host molecules **4–8** were constructed in a similar manner (see ESI†).



Scheme 1 Synthetic route to hosts **2** and **3**. Reagents: (a) *t*-BuLi, (b) $Pd(PPh_3)_4$, $NaBH_4$.

The sensitivities and functions of all hosts **1–8** toward spermidine (**9**) and spermine (**10**) were screened by the naked eye with the

[a]*Institute for Chemical Research, Kyoto University. Gokasho, Uji, Kyoto 611-0011, Japan*
[b]*Graduate School of Life and Environmental Science, Kyoto Prefectural University. Shimogamo, Sakyo-ku, Kyoto 606-8522, Japan. E-mail: tsubaki@kpu.ac.jp*

† Electronic supplementary information (ESI) available: Fig. S1–S4; experimental details; ^{1}H NMR spectra for new compounds. CCDC reference number 669857. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b909682e

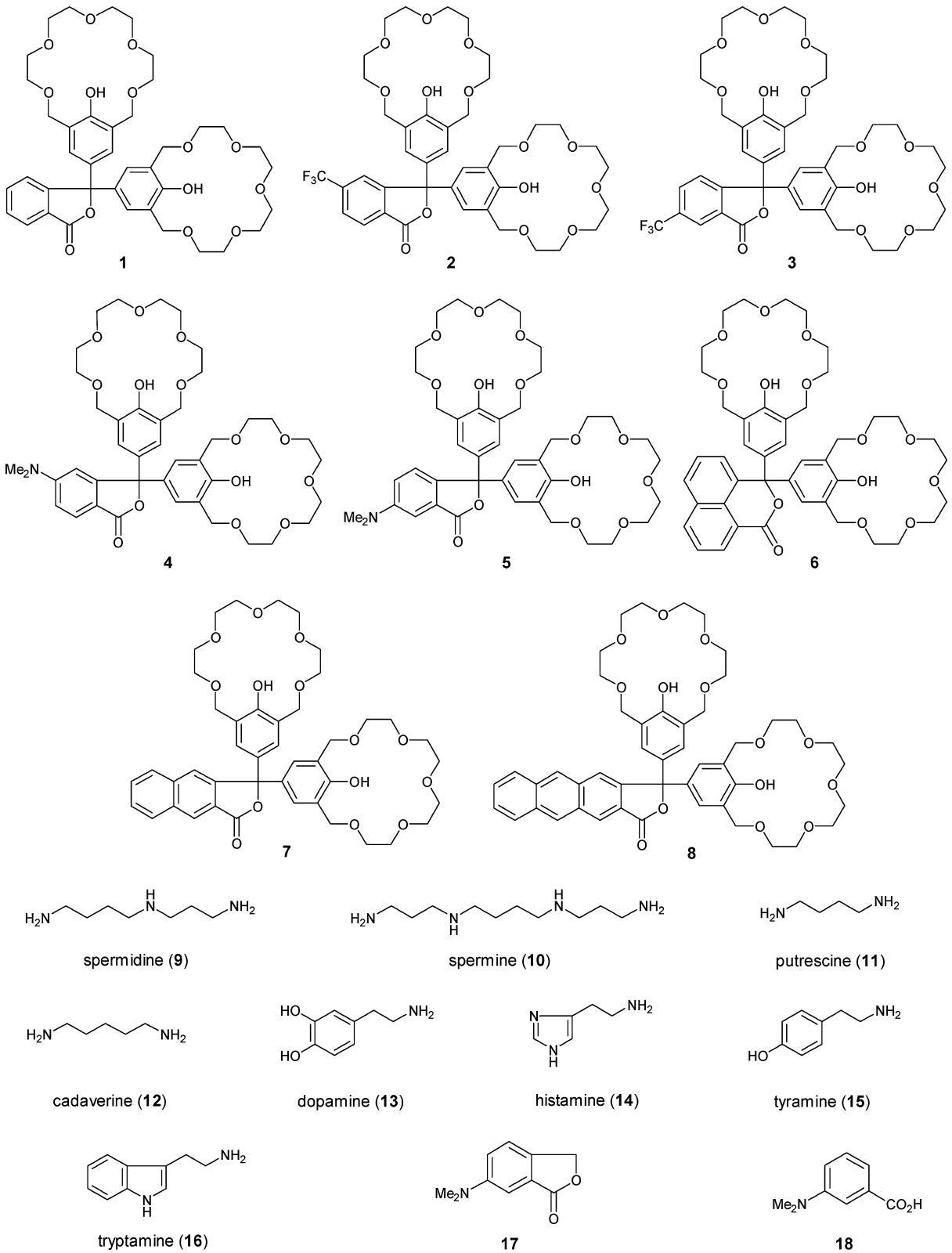


Fig. 1 Hosts, guests and related compounds.

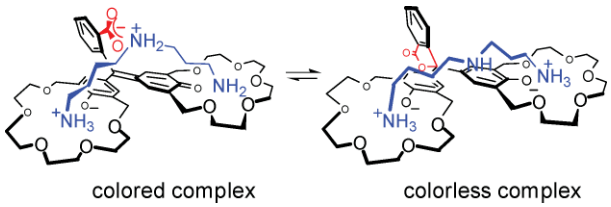


Fig. 2 Equilibrium between colored and colorless complexes.

host (2.0×10^{-4} M) and guest (2.0×10^{-4} M) mixed under methanol conditions. Pictures of hosts **1–8** with **9** (Fig. 3a) and **10** (Fig. 3b) are shown along with their UV–vis spectra (Fig. 3c–d).

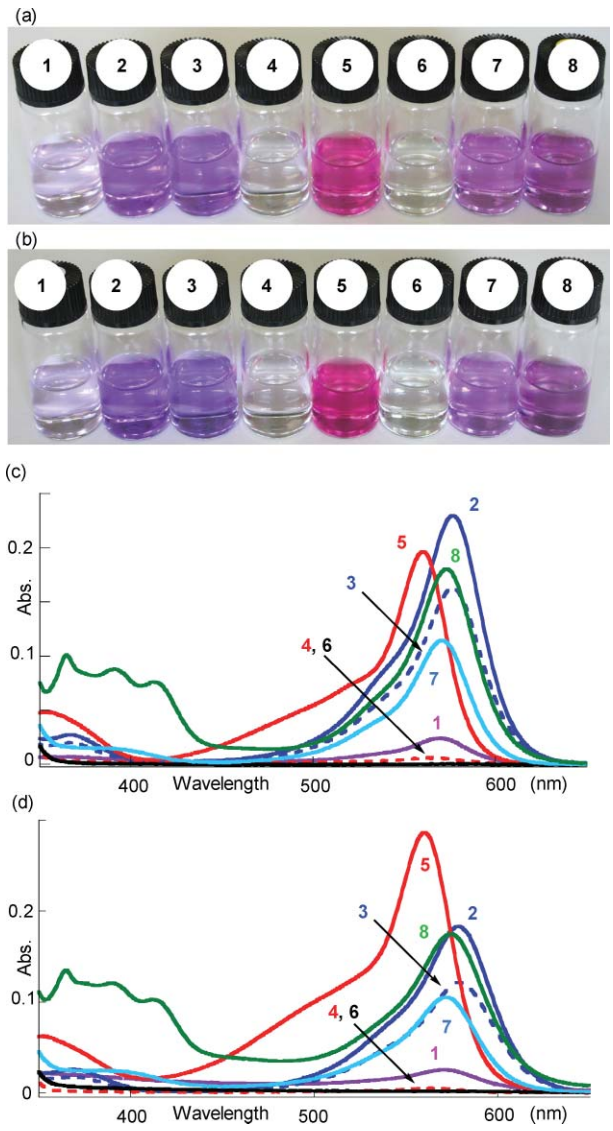


Fig. 3 Colorimetric recognition of hosts **1–8** with spermidine (**9**) or spermine (**10**). (a) and (b) color development by hosts **1–8** with spermidine **9** and spermine **10**, respectively. (c) and (d) UV–vis spectra of hosts **1–8** with **9** and **10**, respectively. Conditions: [host **1–8**] = [amine **9–10**] = 2.0×10^{-4} M, 20 °C, light path length for UV measurement = 0.1 cm.

Compared with host **1**, hosts **4** and **6** were inferior with regard to coloration ability with spermidine (**9**) and spermine (**10**). In contrast, hosts **2**, **3**, **5**, **7** and **8** showed higher sensitivity for the guests. Among the trifluoromethyl-substituted hosts **2** and **3**, host **2** was selected for its high sensitivity and coloration ability against the amines. When the equilibrium between colored complex and colorless complex (as mentioned above) is taken into consideration, it is reasonable for host **2** to have high sensitivity for the amines, since the carboxylate derived from ring-opening of the benzolactone should be stabilized by the trifluoromethyl group at its *para* position. In the case of host **8**, the carboxylate should be stabilized by a larger conjugated anthracene group. Why does host **5**, which has an electron-donating dimethylamino group at the position *meta* to the carbonyl group, show such high sensitivity, comparable to those of hosts **2** and **8**? This sensitivity may be due to the possibility that an additional rhodamine-type resonance plays a crucial role along with normal resonance between two phenols (Fig. 4). In the case of host **4**, a dimethylamino group at the position *para* to the carbonyl group destabilized the carboxylate anion, so that coloration was weaker than that of basic host **1** (Fig. 3a–b).

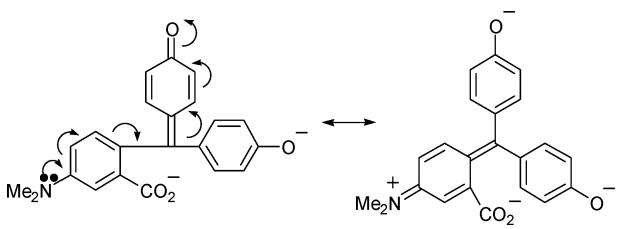


Fig. 4 Stabilization of a colored dianion by a newly formed rhodamine–type conjugated system. The crown ether moieties are omitted for clarity.

After the host/guest ratio (= 1/1) was determined by a Job's plot between host **5** with spermidine (**9**) and spermine (**10**) (Fig. S2†), the association constants (*Ka*) between hosts **2**, **5**, **7**, and **8** and spermidine or spermine, as well as molar absorption coefficients (ε), were determined by UV–vis titration and analyzed by the nonlinear least square method.[9] The results are summarized in Table 1.

The binding constant and ε for basic host **1** with spermidine (**9**) are about 790 and 5000, respectively. The binding constants between hosts **2**, **5**, **7** and **8** with **9** are almost the same or slightly greater than that of host **1** with **9**. However, the molar absorption coefficients (ε) of the hosts were up to about 15 times greater than that of host **1**. These large molar absorption coefficients of the hosts were found to be a major cause of their high sensitivities. We defined a sensitivity index (*SI*) to conveniently reflect the coloration ability with the amines as follows: $SI = (Ka \times \varepsilon)/(Ka_1 \times \varepsilon_1)$, where Ka_1 and ε_1 indicate the association constant of host **1** with the amine and its molar absorption coefficient (ε), respectively. While hosts **2**, **5** and **8** showed almost comparable abilities, host **7** showed only modest ability (*SI* = 5–6) and we removed it from further consideration.

To narrow the candidates, the detection sensitivities of hosts **2**, **5** and **8** against spermidine (**9**) using saturated solutions in methanol were examined (Fig. S3†). Since hosts **2**, **5** and **8** have almost comparable binding constants and ε values, the solubility of the hosts in methanol was an important factor in their selection. Host **5** was eventually selected as the most promising candidate and investigated further.

Table 1 Binding constants (*Ka*) of complexes of hosts with spermidine (**9**) or spermine (**10**) in methanol at 25 °C

host	*Ka* (**9**)[f]	ε (**9**)	*SI*[g]	*Ka* (**10**)[f]	ε (**10**)	*SI*[g]
1[a]	790 ± 60	5000 ± 200	1.0	3600 ± 230	1900 ± 30	1.0
2[b]	1410 ± 130	42000 ± 1800	15.0	3930 ± 330	18100 ± 500	10.4
5[c]	860 ± 30	46300 ± 900	10.1	2570 ± 110	31300 ± 500	11.8
7[d]	1210 ± 30	19900 ± 200	6.1	3420 ± 340	10100 ± 300	5.1
8[e]	1450 ± 50	26500 ± 400	9.7	2930 ± 160	16500 ± 300	7.1

Conditions:[a] $[\mathbf{1}]_0 = 4.0 \times 10^{-4}$ M. [b] $[\mathbf{2}]_0 = 4.0 \times 10^{-5}$ M for **9** and 8.0×10^{-5} M for **10**. [c] $[\mathbf{5}]_0 = 5.0 \times 10^{-5}$ M. [d] $[\mathbf{7}]_0 = 2.0 \times 10^{-4}$ M for **9** and 1.0×10^{-4} M for **10**. [e] $[\mathbf{8}]_0 = 5.0 \times 10^{-5}$ M. [f] *Ka* (**9**) and *Ka* (**10**) are association constants for **9** and **10**, respectively. [g] $SI = (Ka \times \varepsilon)/(Ka_1 \times \varepsilon_1)$.

The selectivity and sensitivity of host **5** toward spermidine (**9**) and spermine (**10**) were very high compared to those toward other biogenic amines (**11–16**) (Fig. 5b), and we can clearly detect these amines from among other biogenic amines by the naked eye (Fig. 5a). As shown in Fig. 5a, clear coloration was observed for the combination of host **5** and polyamine **9** or **10**, whereas almost no coloration was found for other biogenic amines. Fig. 5c shows the selectivity of host **5** toward **9** in the presence of large amounts of tryptamine (**16**) and cadaverine (**12**). Thus, **16** (1eq.–10eq.) was added in a stepwise manner to a solution of host **5**, then **12** (1eq.–10eq.) was added to the solution, and finally **9** was superadded and the absorbance at 560 nm was monitored (Fig. 5c). Host **5** was inactive against **16** (10 eq.) and **12** (10 eq.). On the other hand, host **5** showed color in response to **9**. Thus, host **5** can be used to discriminate **9** from among other interfering substances.

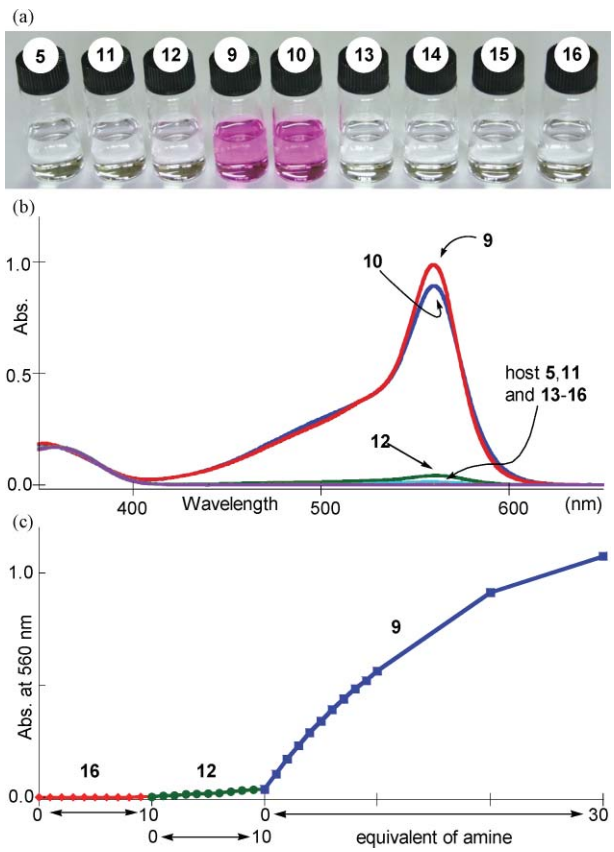


Fig. 5 Naked-eye recognition of spermidine (**9**) and spermine (**10**) among biogenic amines (**11–16**) using host **5**. (a) Photograph of color development by host **5** with amines (**9–16**). (b) UV–vis spectra of host **5** with amines (**9–16**). Conditions: [host **5**] = 8.0×10^{-5} M, [**9–16**] = 4.0×10^{-4} M, [*N*-ethylpiperidine] = 4.0×10^{-3} M, 25 °C, methanol, light path length for UV measurement = 1 cm. (c) Change in color of host **5** with three kinds of biogenic amines. Conditions: [host **5**] = 5.0×10^{-5} M, $[\text{amine}]_0 = 5.0 \times 10^{-3}$ M, 25 °C, methanol, light path length for UV measurement = 1 cm.

Furthermore, both host **8**, which has an anthracene moiety, and host **5** show a fluorescent response (Fig. 6). Recently, Kapiuk *et al.* reported the fluorescent property of 6-dimethylamino phthalide (**17**) based on twisted intramolecular charge transfer,[10] and therefore the fluorescence of host **5** may be due to twisted intramolecular charge transfer of the 6-dimethylamino phthalide skeleton.

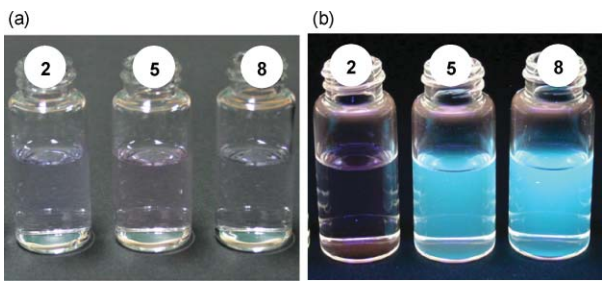


Fig. 6 Photographs of hosts **2**, **5** and **8** under (a) natural light and (b) UV light. Conditions: [host] = 5.0×10^{-6} M, 25 °C, methanol.

In general, fluorescence is more sensitive than absorption. Hence, the relationship between coloration and fluorescence intensity was investigated using hosts **5** and **8** with spermidine (**9**) (Fig. 7).

In both measurements, the behavior of host **5** with **9** was similar to that of host **8** with **9**. Thus, as **9** was added to the host solution, the absorbance at around 560 nm increased, while the fluorescence at around 490 nm decreased. To determine how host **5** affected the fluorescence spectra, 6-dimethylamino phthalide (**17**) and 3-dimethylaminobenzoic acid (**18**) were prepared. Using these molecules, we confirmed the following findings (see Fig. S4†): (1) *N*-ethylpiperidine (100 eq.), which does not interact with the phenol crown of host **5**, did not affect any fluorescence of host **5** (Fig. S4a); (2) even in the presence of **9** (100 eq.), no change in the fluorescence spectra of **17** was observed (Fig. S4b); (3) the fluorescence of **18**, which should act as a mimic of the ring-opened form of host **5**, increased with the addition of either **9** or sodium hydroxide (Fig. S4c,d). These results indicated that (1) the lone pair of the amine nitrogen did not act as an intermolecular quencher, and (2) structural conversion from the benzolactone form of host **5** to the carboxylate form also did not affect the fluorescence intensity. While we cannot clearly explain

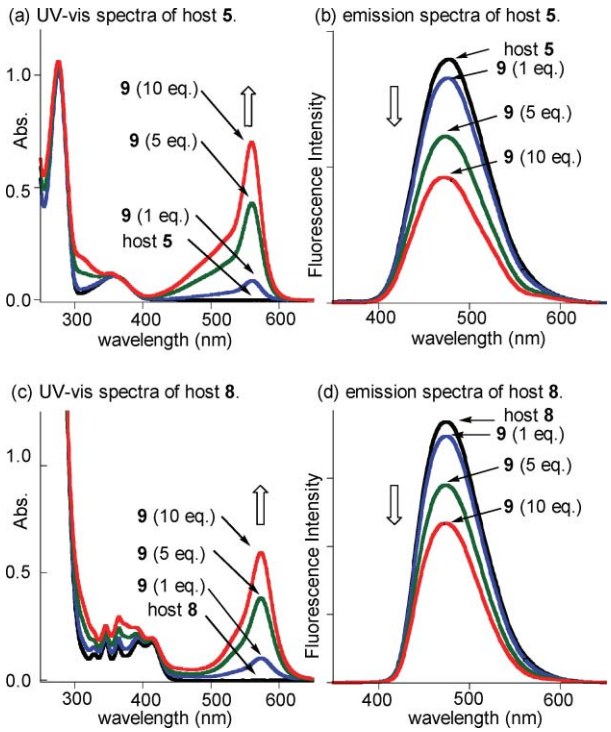


Fig. 7 UV and fluorescence spectra of hosts **5** and **8** in the presence of spermidine (**9**). Conditions: [host **5**] = [host **8**] = 5.0×10^{-5} M, [**9**] = 5, 25, 50×10^{-5} M, respectively. 25 °C, methanol. λ_{ex} = 276 nm.

the quenching of the fluorescence of host **5** with the addition of **9**, we speculate that the two conjugated crown phenol parts of host **5** generated from benzolactone-opening might have acted as an effective intramolecular quencher and consequently increased the absorbance, whereas fluorescence decreased with the addition of **9** to host **5**.

Conclusions

In summary, by introducing an appropriate substituent to the benzolactone ring of mother skeleton **1** or by extending an aromatic ring system, we have developed highly sensitive and selective chemosensors toward spermidine and spermine. In particular, host **5**, which has a dimethylamino group at the position *meta* to the lactone carbonyl group, was selected as a promising candidate and showed excellent functions. Prior to clinical use, further improvements in sensitivity will be required. If the fluorescent behavior of host **5** could be changed from On-Off type to Off-On type, a useful and practical sensor for the detection of spermidine and spermine could be synthesized. Further research on this point is currently in progress.

Experimental

Melting points are uncorrected. Nuclear magnetic resonance (NMR) spectra were taken at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR, with chemical shifts being reported as d ppm from tetramethylsilane as an internal standard. FT-IR, UV, FL and CD spectra were obtained on a JASCO FT/IR-4200, JASCO V-550, JASCO FP-750 and JASCO J-720W, respectively.

X-Ray crystallographic analysis of [21·2MeOH]

The intensity data were collected on a RIGAKU Saturn70 CCD(system) with VariMax Mo Optic using MoKα radiation (λ = 0.71070 Å). Single crystals suitable for X-ray analysis were obtained by slow recrystallization from MeOH at room temperature. A colorless crystal (0.10 × 0.10 × 0.02 mm^3) of [**21**·2MeOH] was mounted on a glass fiber. The structure was solved by a direct method (SIR-97[11]) and refined by full-matrix least-squares procedures on F^2 for all reflections (SHELXL-97[12]). Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 669857.† Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; E-mail: deposit@ccdc.cam.ac.uk.).

Crystal data for **21**·2MeOH: $C_{49}H_{65}F_3O_{16}$, M = 967.01, T = 103(2) K, monoclinic, a = 16.2859(2), b = 18.5760(4), c = 17.0941(3) Å, b = 107.1049(8), V = 4942.68(15) Å^3, space group $P21/a$ (#14), Z = 4, D = 1.300 g/cm^3, Mo-Ka (λ = 0.71070 Å, T = 93 K), measured reflections = 42593. Structure solution by SIR-97, refinement by full-matrix least-squares using all reflections (SHELXL-97), R = 0.0475 [$|Fo| > 2r(Fo)$], Rw = 0.119 (all reflections), GOF = 1.061.

Compounds 21 and 22

A solution of **20** (1.5 g, 3.45 mmol) in dry THF (25 ml) was added dropwise to a solution of *t*-BuLi (1.18 M pentane solution; 4.13 ml, 4.87 mmol) under an Ar atmosphere at −78 °C. After 25 min stirring, a THF (10 ml) solution of **19** (300 mg, 1.39 mmol) was added dropwise. The resultant solution was stirred at −78 °C for 2 hours, and then allowed to warm to room temperature with stirring for 5 hours. To the reaction mixture was added 1 M *aq.* HCl. After 3 hours stirring, the reaction mixture was poured into water and then extracted twice with EtOAc. The combined organic layers were successively washed with saturated *aq.* $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to dryness. The residue was purified by flash column chromatography (eluent; *n*-hexane-EtOAc, 5:1 → 3:1) to give **21** (445 mg, 35%, white foam) and **22** (623 mg, 50%, white foam).

21. White powder; R_f = 0.58 (EtOAc only); mp. 104.5–105 °C (from MeOH); IR (KBr) 2869, 1777, 1472, 1352, 1330, 1235, 1120 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.83 (s, 1H), 7.20 (s, 1H), 6.24–6.12 (m, 2H), 5.50 (dd, J = 17.6, 2.0 Hz, 2H), 5.22 (dd, J = 10.4, 2.0 Hz, 2H), 4.93 (d, J = 5.2 Hz, 4H), 4.492 (ABq, $\Delta\upsilon$ = 315 Hz, J_{AB} = 10.4 Hz, 4H), 4.488 (ABq, $\Delta\upsilon$ = 312 Hz, J_{AB} = 10.4 Hz, 4H), 3.67–3.32 (m, 32H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 168.2, 159.5, 152.7, 136.2, 136.0 (q, $^2J_{C\text{-}F}$ = 33 Hz), 134.2, 132.9, 132.8, 130.9, 130.7, 126.9, 126.8 (q, $^3J_{C\text{-}F}$ = 3.3 Hz), 123.3 (q, $^1J_{C\text{-}F}$ = 274 Hz), 121.3 (q, $^3J_{C\text{-}F}$ = 3.3 Hz), 115.1, 91.4, 70.79, 70.77, 70.26, 70.25, 70.15, 70.13, 69.1, 68.8, 68.7; MS (FAB+, NBA) *m/z* (rel. intensity) = 941 [(M+K)$^+$, 10], 925 [(M+Na)$^+$, 100], 903 [(M+H)$^+$, 10]; HRMS (FAB+, NBA) Calcd for $C_{47}H_{58}F_3O_{14}$ (M+H)$^+$ 903.3779, Found 903.3794; *Anal.* Calcd for $C_{47}H_{58}F_3O_{14}$: C, 62.52; H, 6.36. Found: C, 62.38; H, 6.36.

22. White foam; R_f = 0.48 (EtOAc only); IR (KBr) 2871, 1777, 1631, 1475, 1352, 1334, 1236, 1123, 1026 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.98 (dd, J = 8.0, 1.2 Hz, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.21 (dd, J = 10.0, 2.4 Hz 4H), 6.25–6.12 (m, 2H), 5.51 (dd, J = 17.6, 2.0 Hz, 2H), 5.21 (dd, J = 10.4, 2.2 Hz, 2H), 4.94 (d, J = 5.2 Hz, 4H), 4.49 (ABq, $\Delta\upsilon$ = 300 Hz, J_{AB} = 10.4 Hz, 4H), 4.48 (ABq, $\Delta\upsilon$ = 304 Hz, J_{AB} = 10.4 Hz, 4H), 3.70–3.35 (m, 32H);^{13}C-NMR (100 MHz, $CDCl_3$) δ 168.3, 159.5, 155.2, 136.3, 134.1, 132.9, 132.2 (q, $^2J_{C\text{-}F}$ = 33 Hz), 131.2 (q, $^3J_{C\text{-}F}$ = 4.2 Hz), 130.9, 130.6, 126.3, 125.2, 123.5 (q, $^3J_{C\text{-}F}$ = 4.2 Hz), 123.4 (q, $^1J_{C\text{-}F}$ = 274 Hz), 115.0, 91.5, 70.8, 70.25, 70.23, 70.17, 70.16, 69.0, 68.9; MS (FAB+, NBA) m/z (rel. intensity) = 941 [(M+K)$^+$, 10], 925 [(M+Na)$^+$, 100], 903 [(M+H)$^+$, 10]; HRMS (FAB+, NBA) Calcd for $C_{47}H_{58}F_3O_{14}$ (M+H)$^+$ 903.3779, Found 903.3794; *Anal.* Calcd for $C_{47}H_{58}F_3O_{14}$: C, 62.52; H, 6.36. Found: C, 62.24; H, 6.42.

Host 2

To a solution of **21** (700 mg, 0.775 mmol) and $Pd(PPh_3)_4$ (35.8 mg, 0.031 mmol) in MeOH (15 ml), under an Ar atmosphere, was added $NaBH_4$ (41 mg, 1.16 mmol). The solution color changed from pale yellow to deep purple within a few minutes in keeping with generation of gas (propene). After stirring at room temperature for 25 min, 1 M *aq.* HCl was added. The reaction mixture was poured into water, and extracted three times with $CHCl_3$. The combined organic layers were successively washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to dryness. The residue was purified by flash column chromatography (eluent; $CHCl_3$:MeOH:AcOH, 20:1:0.3) to give crude host **2** (709 mg). The crude product was further purified by recycling preparative HPLC (Japan Analytical Industry Co., Ltd. LC-908) with connected JAIGEL-1H (20 × 600 mm) and JAIGEL-2H (20 × 600 mm) under the conditions of 3.5 ml/min of flow rate with $CHCl_3$ detected by UV (254 nm) and RI (refractive index) to afford host **2** (532 mg, 83%) as white solid.

Host 2. White solid; R_f = 0.17 (EtOAc-MeOH, 10:1)/R_f = 0.40 ($CHCl_3$-MeOH, 10:1); mp. 86–87 °C (from EtOAc); IR (KBr) 3427, 2873, 1770, 1616, 1486, 1330, 1241, 1172, 1121 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 2H, -O<u>H</u>), 8.04 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.74 (s, 1H), 7.03 (s, 4H), 4.58 (AB, $\Delta\upsilon$ = 0 Hz, J_{AB} = 10.4 Hz, 8H), 3.80–3.56 (m, 32H);^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.2, 168.5, 156.6, 152.9, 135.8 (q, $^2J_{C\text{-}F}$ = 33 Hz), 130.7, 128.73, 128.67, 126.7, 126.6 (q, $^3J_{C\text{-}F}$ = 3.3 Hz), 123.3 (q, $^1J_{C\text{-}F}$ = 274 Hz), 121.3 (q, $^3J_{C\text{-}F}$ = 3.3 Hz), 92.1, 70.6, 70.4, 70.3, 70.0, 69.4, 60.4; MS (FAB+, NBA) m/z (rel. intensity) = 883 [(M+Na+K−H)$^+$, 10], 867 [(M+2Na−H)$^+$, 100], 845 [(M+Na)$^+$, 40]; HRMS (FAB+, Glycerol) Calcd for $C_{41}H_{50}F_3O_{14}$ (M+H)$^+$ 823.3153, Found 823.3169; *Anal.* Calcd for $C_{47}H_{58}F_3O_{14}\cdot 1.0\ H_2O$: C, 58.57; H, 6.11. Found: C, 58.66; H, 5.91.

Host 3

Host **3** was similarly prepared from compound **22**.

Host 3. Pale yellow foam; R_f = 0.10 (EtOAc-MeOH, 10:1)/R_f = 0.34 ($CHCl_3$-MeOH, 10:1); IR (KBr) 3363, 2871, 1771, 1631, 1487, 1356, 1334, 1237, 1171, 1124 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 2H, -O<u>H</u>), 8.16 (s, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.64 (d, J = 8.4 Hz, 1H), 7.28 (s, 4H), 4.58 (AB, $\Delta\upsilon$ = 0 Hz, J_{AB} = 10.8 Hz, 8H), 4.12 (q, J = 7.2 Hz, 3/2H, $CH_3COO\underline{CH_2}CH_3$ × 0.75), 3.75–3.58 (m, 32H), 2.64 (s, 9/4H, $\underline{CH_3}COOCH_2CH_3$ × 0.75), 1.26 (t, J = 7.2 Hz, 9/4H, $CH_3COOCH_2\underline{CH_3}$ × 0.75); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 168.4, 156.5, 155.5, 132.0 (q, $^2J_{C\text{-}F}$ = 33.9 Hz), 130.7 (q, $^3J_{C\text{-}F}$ = 3.3 Hz), 128.5, 126.3, 125.1, 124.8, 123.362 (q, $^3J_{C\text{-}F}$ = 3.3 Hz), 123.358 (q, $^1J_{C\text{-}F}$ = 274 Hz), 92.1, 70.7, 70.4, 70.3, 70.2, 70.0, 69.5; MS (FAB+, NBA) m/z (rel. intensity) = 861 [(M+K)$^+$, 15], 845 [(M+Na)$^+$, 100], 823 [(M+H)$^+$, 40]; HRMS (FAB+, NBA) Calcd for $C_{41}H_{50}F_3O_{14}$ (M+H)$^+$ 823.3153, Found 823.3152; *Anal.* Calcd for $C_{47}H_{58}F_3O_{14}\cdot 0.75$ EtOAc: C, 59.45; H, 6.24. Found: C, 59.26; H, 6.03.

Acknowledgements

We thank Prof. Norihiro Tokitoh and Prof. Takahiro Sasamori (Kyoto Univ.) for performing the X-ray structural analysis of **21**, and Prof. Yasuhisa Kuroda (Kyoto Institute of Technology) for a gift of SPANA software. This study was partly supported by KAKENHI (No. 18390003), Foundation for Pharmaceutical Sciences, Japan, and Promoting Technological Seeds from JSPS.

References

1 (*a*) C. W. Tabor and H. Tabor, *Ann. Rev. Biochem.*, 1984, **53**, 749–790; (*b*) A. E. Pegg, *Cancer Res.*, 1988, **48**, 759–774; (*c*) K. Igarashi and K. Kashiwagi, *Biochem. Biophys. Res. Commun.*, 2000, **271**, 559–564; (*d*) K. Igarashi and K. Kashiwagi, *J. Biochem.*, 2006, **139**, 11–16.
2 (*a*) K. Hiramatsu, M. Sugimoto, S. Kamei, M. Hoshino, K. Kinoshita, K. Iwasaki and M. Kawakita, *J. Biochem.*, 1995, **117**, 107–112; (*b*) M. Kawakita and K. Hiramatsu, *J. Biochem.*, 2006, **139**, 315–322.
3 (*a*) K. Igarashi, K. Kashiwagi, H. Hamasaki, A. Miura, T. Kakegawa, S. Hirose and S. Matsuzaki, *J. Bacteriol.*, 1986, **166**, 128–134; (*b*) M. Y. Khuhawar and G. A. Qureshi, *J. Chromatogr., B: Biomed. Sci. Appl.*, 2001, **764**, 385–407; (*c*) H. Yoshida, H. Harada, Y. Nakano, H. Nohta, J. Ishida and M. Yamaguchi, *Biomed. Chromatogr.*, 2004, **18**, 687–693.
4 (*a*) For recent colorimetric recognitions, see: S. M. Butterfield, A. Hennig and S. Matile, *Org. Biomol. Chem.*, 2009, **7**, 1784–1792; (*b*) R. M. Duke, J. E. O'Brien, T. McCabe and T. Gunnlaugsson, *Org. Biomol. Chem.*, 2008, **6**, 4089–4092.
5 (*a*) K. Fuji, K. Tsubaki, K. Tanaka, N. Hayashi, T. Otsubo and T. Kinoshita, *J. Am. Chem. Soc.*, 1999, **121**, 3807–3808; (*b*) K. Tsubaki, N. Hayashi, M. Nuruzzaman, T. Kusumoto and K. Fuji, *Org. Lett.*, 2001, **3**, 4067–4069; (*c*) K. Tsubaki, T. Kusumoto, N. Hayashi, M. Nuruzzaman and K. Fuji, *Org. Lett.*, 2002, **4**, 2313–2316; (*d*) K. Tsubaki, M. Nuruzzaman, T. Kusumoto, N. Hayashi, B.-G. Wang and K. Fuji, *Org. Lett.*, 2001, **3**, 4071–4073; (*e*) K. Tsubaki, D. Tanima, M. Nuruzzaman, T. Kusumoto, K. Fuji and T. Kawabata, *J. Org. Chem.*, 2005, **70**, 4609–4616; (*f*) K. Tsubaki, D. Tanima, T. Sasamori, N. Tokitoh and T. Kawabata, *Tetrahedron Lett.*, 2007, **48**, 2135–2138.
6 J. van Gent, E. J. R. Sudhölter, P. V. Lambeck, T. J. A. Popma, G. J. Gerritsma and D. N. Reinhoudt, *J. Chem. Soc., Chem. Commun.*, 1988, 893–895.
7 G. Pawlowski and M. Hanack, *Synth. Commun.*, 1981, **11**, 351–363.
8 (*a*) R. Beugelmans, S. Bourdet, A. Bigot and J. Zhu, *Tetrahedron Lett.*, 1994, **35**, 4349–4350; (*b*) R. Beugelmans, L. Neuville, M. Bois-Choussy, J. Chastanet and Z. Zhu, *Tetrahedron Lett.*, 1995, **36**, 3129–3132.
9 Calculated with SPANA as a software developed by Prof. Y. Kuroda, Kyoto Institute of Technology (JAPAN).
10 J. Karpiuk, Y. N. Svartsov and J. Nowacki, *Phys. Chem. Chem. Phys.*, 2005, **7**, 4070–4081.
11 A. Altomare, M. C. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori and R. Spagna, *J. Appl. Crystallogr.*, 1999, **32**, 115–119.
12 G. M. Sheldrick, 1997 *SHELX-97, Program for the Refinement of Crystal Structures*, University of Göttingen, Germany.

