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Daxabe – A Xanthene-Based Fluorescent Sensor for 3,5-Dinitrobenzoic Acid and Anions

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A new set of receptors based on a dimethylxanthene skeleton has been synthesized. Owing to its suitable oxyanion hole structure, these receptors can associate with carboxylic acids. The combination of this skeleton with a fluorescent unit such as dansyl allows the detection of small amounts of carboxylic acids by making use of fluorescent techniques, such that the response of daxabe depends on the nature of the guest. Anions such as halogenides are also suitable guests for daxabe and can be associated. This receptor also afforded good results in the extraction of zwitterionic amino acids from the aqueous to the organic layer in the presence of 18-crown-6 ether.

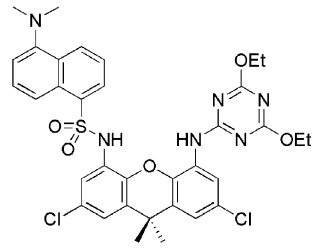
Introduction

Fluorescent sensors are very sensitive analytic tools, and their chemistry has recently been reviewed.[1–3] Most work dealing with this kind of sensor reports the detection of cations[4–15] or anions.[16–29] To date, sensors for neutral compounds have had little success since, with a few exceptions,[30–34] neutral compounds are more difficult to bind.

Among the exceptions for neutral guests are carboxylic acids, due to the strong H bonds these guests can forge with amines with a suitable pK_a. Carboxylic acids are promising analytes for detection with fluorescent sensors.

Results and Discussion

A first carboxylic acid sensor, daxacyan (**1**), is shown in Figure 1.[35] This compound shows good quenching of dansyl fluorescence in the presence of 3,5-dinitrobenzoic acid. The detection of very small amounts of dinitrobenzoic acid was difficult, because the association constant (K_a) of 8.5×10^3 M^{-1} was relatively low. For easier detection of 3,5-dinitrobenzoic acid, a larger K_a was desirable.



1
(daxacyan)

Figure 1. Structure of daxacyan (**1**), a previously synthesized molecular receptor.

A good explanation for the low K_a between carboxylic acids and receptor **1** could be the poor tuning between the carboxylic acid and the pK_a of the receptor amino group. In order to look for a better match, we prepared a series of molecular receptors for carboxylic acids with different basicities of the receptor amines.

To facilitate the study of the K_a values by means of NMR techniques, we replaced the dansyl group by an acetamide. Molecular models showed that the acetamide methyl group should lie in the anisotropic shielding cone of aromatic guests, making the measurement of complex formation easier. The preparation of **5**–**9** is depicted in Scheme 1.

We used 3-nitrobenzoic acid as the guest owing to its suitable solubility in chloroform and carried out competitive titrations between the different receptors to assess their relative K_a values. Absolute values can be deduced from the

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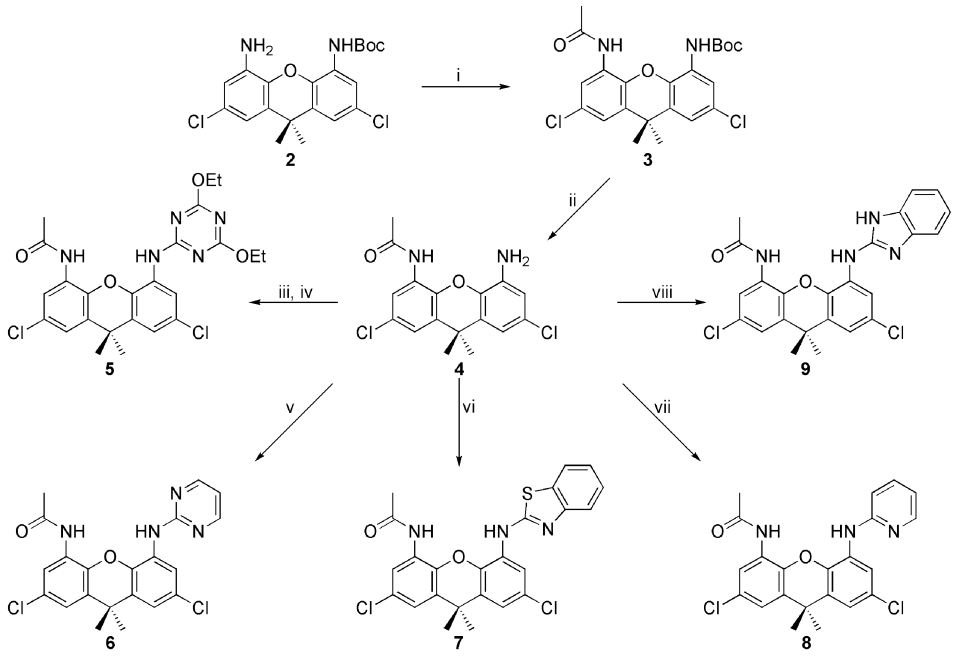


Scheme 1. Synthesis of the receptors with carboxamides **5**–**9**. Reagents and conditions: i) Ac_2O ii) trifluoroacetic acid iii) 2,4,6-trichloro-1,3,5-triazine, CH_2Cl_2 iv) ethanol, THF, H_2SO_4 v) 2-chloropyrimidine, Δ vi) 2-chlorobenzo[*d*]thiazole, Δ vii) 2-bromopyridine, Δ viii) 2-chloro-1*H*-benzo[*d*]imidazole, dyglime, Δ.

$K_a = 1.4 \times 10^3$ M^{-1} measured for receptor **5** and 3-nitrobenzoic acid using the standard NMR procedure.[36] The results are shown in Table 1.

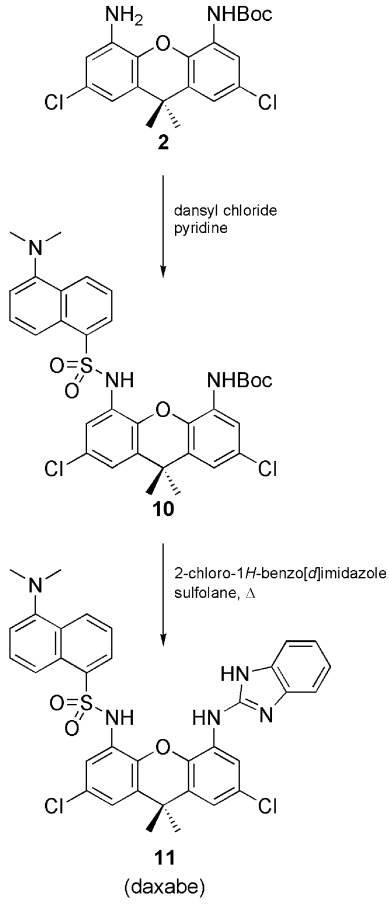
Table 1. K_a values of receptors **5**–**9** with 3-nitrobenzoic acid in deuterochloroform at 298 K. The K_a of receptor **5** was measured by direct NMR titration. The K_a of receptors **6**–**9** were measured by competitive experiments.

Entry	Receptor	K_a [M^{-1}]
1	**5**	1.4×10^3
2	**6**	2.1×10^3
3	**7**	5.9×10^3
4	**8**	2.0×10^4
5	**9**	1.6×10^5

As shown in Table 1, the cyanuric acid derivative was the worst choice for acid association. The presence of the three nitrogen atoms in the aromatic ring strongly reduces the nitrogen basicity. The strong effect of the basicity of the heterocyclic nitrogens can also be observed in five-membered rings. The combined effect of the three nitrogens in the aminobenzimidazole provides the best K_a values.

Since aminobenzimidazole seems to be the most appropriate group for carboxylic acid association, receptor **11** was prepared as shown in Scheme 2. Daxabe was chosen as a reasonable name for sensor **11** since it is made up of a dansyl group, a xanthene skeleton and a benzimidazole unit.

The NMR spectra of daxabe (**11**) changed with the concentration, indicating the presence of a self-aggregate in the



Scheme 2. Synthesis of daxabe (**11**).

solution. The plot of the chemical shifts versus concentration allowed a dimerization constant (K_d) of 6.3×10^2 M^{-1} to be calculated. Corey–Pauling–Koltun (CPK) models showed that two reasonable structures for this dimer can be proposed, as seen in Figure 2, which also shows the most representative $\Delta\delta$ induced in the protons due to dimer formation.

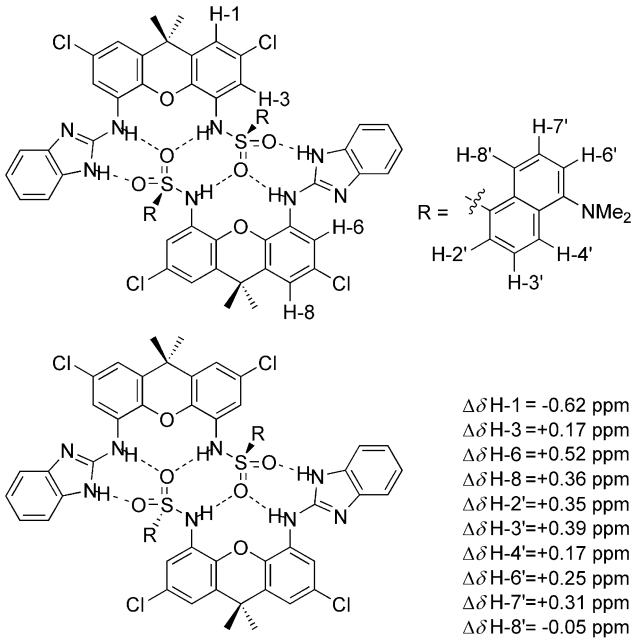


Figure 2. Structure of the two possible dimers of daxabe (**11**).

Six H bonds stabilize the dimer structure. Nevertheless, CPK models showed that the fit was not very good, and hence, the K_d was not very large. The induced chemical shifts observed cannot be easily explained on the basis of a single dimeric structure. Both geometries are probably in equilibrium in solution, resulting in the dansyl groups pointing toward or away from the molecular plane.

We selected several carboxylic acids for study of their K_a values with daxabe (Table 2).

Table 2. K_a values of daxabe (**11**) with carboxylic acids in deuterochloroform at 298 K. The K_a for the binding of acetic acid was determined by direct ^{1}H NMR titration. The K_a for binding of 3,5-dinitrobenzoic acid was determined by fluorescent titration. Remaining K_a values were measured by competitive experiments.

Entry	Carboxylic acid	K_a [M^{-1}]
1	acetic acid	6.2×10^3
2	4-methylbenzoic acid	1.4×10^4
3	3-nitrobenzoic acid	1.7×10^5
4	3,5-dinitrobenzoic acid	4.8×10^5

Acetic acid showed the lowest K_a, but as the acidity of the guest increased, the K_a values increased, with the highest value for 3,5-dinitrobenzoic acid. Complex formation with 3,5-dinitrobenzoic acid can easily be observed owing to the strong shielding of the guest *ortho* protons, the signal of which moved from 9.30 ppm in the free form to 9.00 ppm in the complex. The shielding cone of the dansyl group may be responsible for this effect.

The fluorescence of daxabe complexes with carboxylic acids in chloroform was interesting. We carried out fluorescent assays in chloroform, at 293 K, using 348 nm as the excitation wavelength and daxabe at 1.67×10^{-5} M. Free daxabe mainly displayed a typical fluorescence for the dansyl group at approximately 516 nm. The fluorescence emission spectrum of daxabe was enhanced (15–25%) upon complexation with carboxylic acids such as acetic, decanoic or benzoic acid.

The behaviour of other carboxylic acids, such as α-naphthoic acid or nitrobenzoic acids, proved to be more curious. The intrinsic emission fluorescence of α-naphthoic acid at 360 nm was quenched upon addition of daxabe. Figure 3 (a) presents the titration curves of α-naphthoic acid with daxabe. As seen from the Figure, the α-naphthoic acid fluorescence (360 nm) decreased upon acid binding to daxabe, while further increasing the daxabe concentration led to an enhancement of the dansyl fluorescence of **11** (500 nm). Naphthoic acid fluorescence quenching was probably due

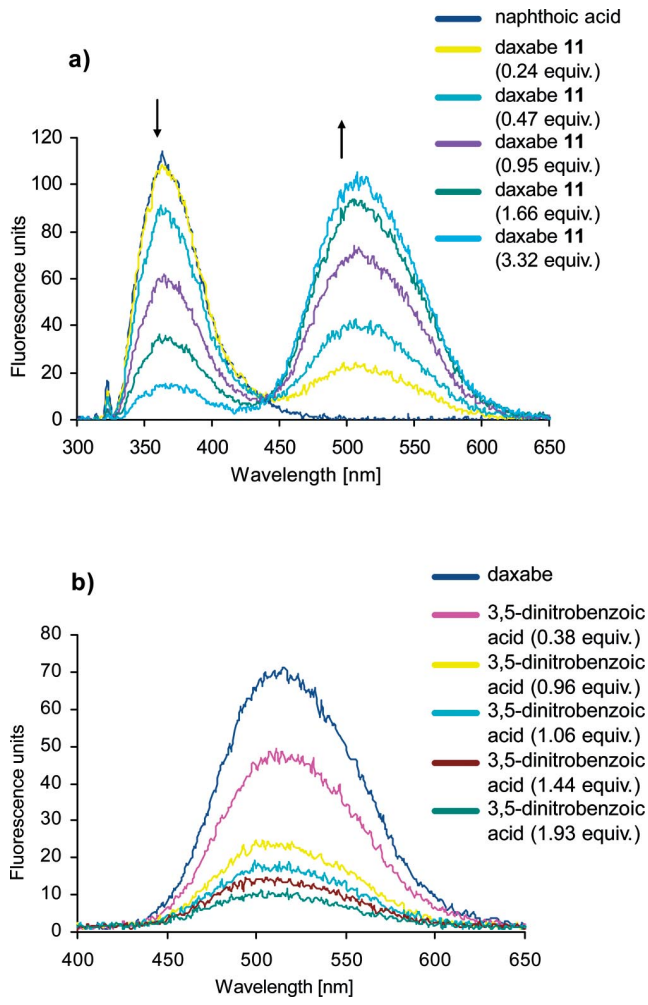


Figure 3. (a) Fluorescence changes in α-naphthoic acid (360 nm, 10^{-5} M) and daxabe (**11**) (500 nm) in chloroform at 293 K upon addition of increasing amounts of daxabe (0.24–3.32 equiv.). (b) Fluorescent titrations of daxabe (500 nm, 10^{-5} M) upon the addition of 3,5-dinitrobenzoic acid (0.38–1.93 equiv.) in chloroform at 293 K.

to energy transfer from the naphthalene to the dansyl ring.

On the other hand, nitrobenzoic acids strongly quenched daxabe fluorescence (500 nm), with 3,5-dinitrobenzoic acid showing the strongest effect (Figure 3, b). Electron transfer from the excited dansyl orbital to the nitro group would probably be responsible for this result. 3,5-dinitrobenzoic acid is an important industrial by-product,[37] and small amounts of 3,5-dinitrobenzoic acid can readily be detected with this receptor.

We calculated the possible geometry of the complex between daxabe (**11**) and 3,5-dinitrobenzoic acid using a semiempirical (AM1)[38] method implemented in the GAUSSIAN98W program (Figure 4).[39]

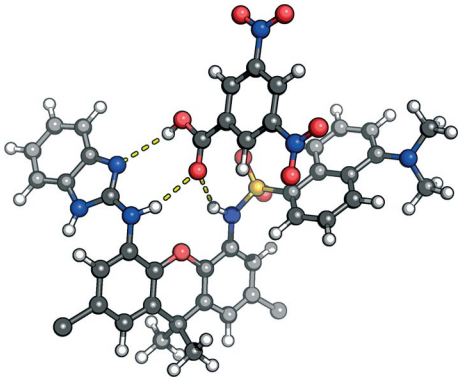


Figure 4. Structure of the complex between daxabe (**11**) and 3,5-dinitrobenzoic acid obtained from modelling studies.

In the final analysis, the ground-state conformation of **11** was not ideal for carboxylic acid association. During the titrations, large shifts occurred in the NMR spectra for the H-6 signal (from 8.76 ppm in the free form to 7.16 ppm in the complex with acetic acid), showing the change in the conformation of the benzimidazole unit (Figure 5), which in the complex with the carboxylic acid no longer forms the intramolecular H bond with H-6.

The geometry of **11** is, therefore, ideal for associating anions, forming three linear H bonds with these guests. We tested several anions, and their effect on dansyl fluorescence is shown in Figure 6.

Basic anions such as hydroxide, fluoride and acetate were able to abstract the sulfonamide proton, yielding large fluorescence quenching; non-basic anions only increased the fluorescence to a moderate extent, with the exception of iodide, which reduced the fluorescence by 20% (Figure 6). In this case, a photo-induced electron transfer (PET) process could be involved due to the reducing character of iodide.

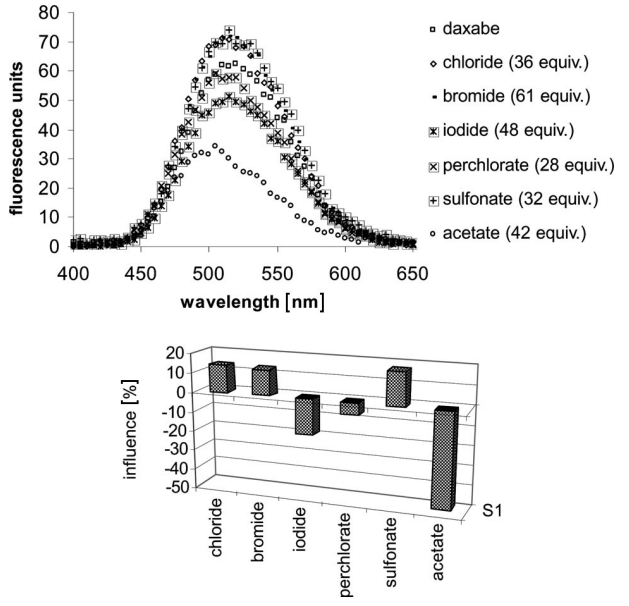


Figure 6. Fluorescence properties of daxabe (**11**) (500 nm, 10^{-5} M) in chloroform at 293 K in the presence of different anions.

Another interesting way to analyse anions was by making use of the daxabe/3,5-dinitrobenzoic acid system previously mentioned. This system showed a small degree of fluorescence due to the quenching of the dansyl fluorescence of **11** in the presence of nitro groups. Anions such as chloride were able to break the 3,5-dinitrobenzoic acid complex, yielding large increases in the receptor fluorescence, as shown in Figure 7. Owing to the high stability of

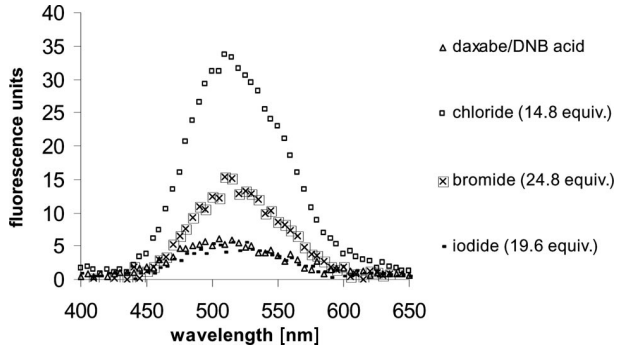


Figure 7. Fluorescent properties of the daxabe/3,5-dinitrobenzoic acid complex in the presence of halogenides.

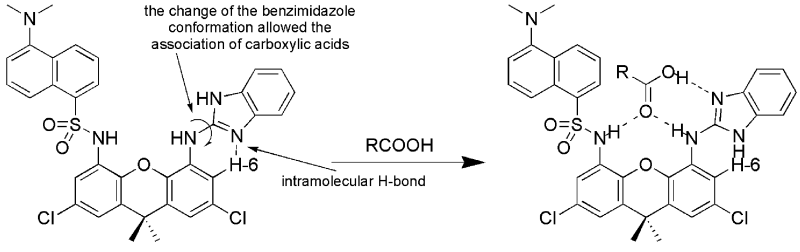


Figure 5. Change of the daxabe (**11**) conformation to complex with carboxylic acids.

the initial complex in chloroform, bromide only showed a small effect, and iodide was essentially unable to regenerate receptor fluorescence.

Daxabe was also highly efficient at extracting zwitterionic amino acids from aqueous solution into the chloroform phase. The presence of 18-crown-6 ether (1.5 equiv. relative to receptor **11**) was necessary for extraction to take place. We carried out amino acid extraction experiments at 1.0×10^{-2} M receptor **11** concentration and established the amino acid concentration in the chloroform phase through NMR integration. Since we used saturated water solutions of the amino acids, it was not necessary to establish their concentration. Neither daxabe itself nor the crown ether alone were effective at extracting the amino acids. The results with several amino acids are shown in Table 3. We obtained no changes in the receptor fluorescence, and when the complex with 3,5-dinitrobenzoic acid was used, extraction did not take place since this guest blocked the cleft.

Table 3. Amount of complex (%) with respect to daxabe (**11**) in the amino acid extraction experiments from the aqueous layer to chloroform, in the presence of daxabe (10^{-2} M) and 18-crown-6 (1.5×10^{-2} M) at 293 K.

Entry	Amino acid	Amount of complex [%]
1	alanine	100
2	glycine	68
3	leucine	100
4	phenylalanine	75
5	phenylglycine	60
6	serine	69

Conclusions

In conclusion, daxabe is a versatile receptor. It is able to associate neutral compounds such as carboxylic acids and anionic compounds such as chloride or bromide. The changes in the fluorescence intensity of its dansyl group in the presence of suitable guests make it a promising sensor.

Experimental Section

General Methods: IR spectra were recorded with a Nicolet IR100 or with a Bonem MB-100FT IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at room temperature with Bruker WP-200-SY, Varian Mercury VS. 2000 or Bruker Advance DRX spectrometers in deuterated chloroform (unless otherwise stated). *J* values are reported in Hz, and chemical shifts are reported in ppm with the solvent signal as an internal standard. Mass spectra were recorded with an Applied Biosystems QSTAR XL. Fluorescence spectra were collected at 293 K using a Shimadzu RF-5301PC series spectrophotometer.

Materials

tert*-Butyl 5-Acetamido-2,7-dichloro-9,9-dimethyl-9*H*-xanthen-4-yl-carbamate (3):** Compound **2** (20.0 g, 48.9 mmol) was dissolved in acetic anhydride (100 mL). After 10 min, the solution was added dropwise to stirred water (250 mL) over 30 min. The mixture was extracted with ethyl acetate (3×100 mL), and the combined organic phases were dried with Na_2SO_4 and concentrated to dryness. The crude product was purified by crystallization from THF/water to give the title **3** (17.0 g, 77%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.18 (s, 1 H, NH), 7.79 [s (broad), 2 H, H-3, H-6], 7.47 (s, 1 H, NH), 7.12 (d, J = 2 Hz, 1 H, H-8), 7.05 (d, J = 2 Hz, 1 H, H-1), 2.22 [s, 3 H, -C(O)CH*$_3$**], 1.55 (s, 6 H, ***CH*$_3$**-C-***CH*$_3$**), 1.48 [s, 9 H, C***(CH*$_3$*)*$_3$**] ppm. IR: $\tilde{\nu}$ = 3400, 3280, 1551, 1402, 1320, 1232, 1057, 906, 763 cm^{-1}. MS: *m*/*z* = 473.113 [M + Na]$^+$.

N*-(5-Amino-2,7-dichloro-9,9-dimethyl-9*H*-xanthen-4-yl)acetamide (4):** Compound **3** (17.0 g, 37.7 mmol) was dissolved in TFA (40.0 mL), and the progress of the reaction was monitored by TLC using CH_2Cl_2 as the eluent. Once the reaction was finished, the mixture was added to a stirred solution of aqueous sodium carbonate (4% w/w, 250 mL) and extracted with ethyl acetate (150 mL). The layers were separated, and the organic layer was washed with aqueous sodium carbonate (4% w/w) until the pH was basic. The organic layer was then dried with Na_2SO_4, and the solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/diethyl ether, 4:1) affording **4** (78%, 10.3 g). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.99 (d, J = 2 Hz, 1 H, H-3), 7.49 (s, 1 H, NH), 7.12 (d, J = 2 Hz, 1 H, H-1), 6.77 (d, J = 2 Hz, 1 H, H-6), 6.64 (d, J = 2 Hz, 1 H, H-8), 2.13 [s, 3 H, -C(O)CH*$_3$**], 1.57 (s, 6 H, ***CH*$_3$**-C-***CH*$_3$**) ppm. IR: $\tilde{\nu}$ = 3286, 1657, 1625, 1527, 1250, 1200, 846, 723 cm^{-1}. MS: *m*/*z* = 351.066 [M + H]$^+$, 373.049 [M + Na]$^+$.

N*-[2,7-Dichloro-5-(4,6-diethoxy-1,3,5-triazin-2-ylamino)-9,9-dimethyl-9*H*-xanthen-4-yl]acetamide (5):** 2,4,6-Trichloro-1,3,5-triazine (1.2 g, 6.4 mmol) was added to a solution of **4** (2.0 g, 5.7 mmol) in CH_2Cl_2 (20.0 mL). The solution was stirred for 5 min, water (20.0 mL) was then added, and stirring was continued for an additional 14 h. The layers were separated, and the organic layer was dried with Na_2SO_4 and concentrated to dryness to leave an intermediate compound (2.5 g, 89% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.98 (d, J = 2 Hz, 1 H, H-6), 7.91 (d, J = 2 Hz, 1 H, H-3), 7.16 (d, J = 2 Hz, 1 H, H-8), 7.02 (d, J = 2 Hz, 1 H, H-1), 2.04 [s, 3 H, -C(O)CH*$_3$**], 1.59 (s, 6 H, ***CH*$_3$**-C-***CH*$_3$**) ppm. IR: $\tilde{\nu}$ = 3334, 1682, 1609, 1519, 1350, 1200, 1130, 1052, 823, 745 cm^{-1}. MS: *m*/*z* = 520.000 [M + Na]$^+$.

This compound (2.5 g, 5.1 mmol) was dissolved in a stirred solution of THF (25.0 mL) and absolute ethanol (25.0 mL), and the mixture was cooled in a water bath. Concentrated H_2SO_4 (5.0 mL) was then slowly added, and the reaction mixture was stirred at room temperature for 14 h. The solution was added to an aqueous sodium carbonate solution (4% w/w, 100 mL) and extracted with ethyl acetate. The organic layer was dried with Na_2SO_4, and the solvent was evaporated to dryness. Compound **5** was purified by chromatography with CH_2Cl_2 as the eluent, affording **5** (2.2 g, 83%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.11 (d, J = 2 Hz, 1 H, H-6), 7.74 (d, J = 2 Hz, 1 H, H-3), 7.13 [s (broad), 2 H, H-1, H-8], 4.42 (q, J = 6 Hz, 4 H, -O***CH*$_2$**CH$_3$), 2.28 [s, 3 H, -C(O)***CH*$_3$**], 1.59 (s, 6 H, ***CH*$_3$**-C-***CH*$_3$**), 1.40 (t, J = 6 Hz, 6 H, -OCH$_2$***CH*$_3$**) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.9 (2×Ar***C***), 168.9 [***C(O)***], 166.5 (Ar***C***), 139.3 (Ar***C***), 138.6 Ar***C***), 131.2 (Ar***C***), 131.0 (Ar***C***), 128.7 (Ar***C***), 128.4 (Ar***C***), 126.8 (2×Ar***C***), 121.0 (Ar***CH***), 121.0 (Ar***CH***), 120.9 (Ar***CH***), 120.6 (Ar***CH***), 63.89 (2×***CH*$_2$*)*, 35.0 (***C***), 31.5 (2×***CH*$_3$*)*, 24.3 (***CH*$_3$*)*, 14.2 (2×***CH*$_3$*)* ppm. IR: $\tilde{\nu}$ = 3300, 1670, 1600, 1560, 1216, 1150, 1050 cm^{-1}. MS: *m*/*z* = 518.131 [M + H]$^+$, 540.116 [M + Na]$^+$.

***N*-[2,7-Dichloro-9,9-dimethyl-5-(pyrimidin-2-ylamino)-9*H*-xanthen-4-yl]acetamide (6):** Compound **4** (200 mg, 0.6 mmol) and 2-chloropyrimidine (500 mg, 4.4 mmol) were heated together at 170 °C for 2 h. The reaction was monitored by TLC with CH_2Cl_2 as the eluent. The reaction mixture was added to water (20.0 mL) and ethyl

acetate (30.0 mL). The organic layer was dried with Na_2SO_4, and the organic solvents were evaporated to dryness. Compound **6** was purified by chromatography with CH_2Cl_2 as the eluent, affording **6** (127 mg, 53%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.43 (d, J = 4 Hz, 2 H, H-4′, H-6′), 8.21 (d, J = 2 Hz, 1 H, H-3), 8.09 (d, J = 2 Hz, 1 H, H-6), 7.13 (d, J = 2 Hz, 1 H, H-8), 7.07 (d, J = 2 Hz, 1 H, H-1), 6.78 (t, J = 4 Hz, 1 H, H-5′), 2.24 [s, 3 H, -C(O)***CH₃***], 1.59 (s, 6 H, ***CH₃***-C-***CH₃***) ppm. IR: $\tilde{\nu}$ = 3396, 3247, 1664, 1620, 1586, 1514, 1286, 1209, 846, 723 cm^{-1}. MS: m/z = 429.089 [M + H]$^+$, 451.070 [M + Na]$^+$.

N*-[5-(Benzo[*d*]thiazol-2-ylamino)-2,7-dichloro-9,9-dimethyl-9*H*-xanthen-4-yl]acetamide (7):** Compound **4** (200 mg, 0.6 mmol) and 2-chlorobenzo[*d*]thiazole (400 mg, 3.0 mmol) were heated together at 130 °C for 1 h. The reaction was monitored by TLC with CH_2Cl_2 as the eluent. The crude reaction mixture was purified by chromatography (CH_2Cl_2) to afford **7** (83%, 225 mg). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.27 (s, 1 H, NH), 7.94 (d, J = 2 Hz, 1 H, H-3), 7.82 (d, J = 2 Hz, 1 H, H-6), 7.55 (dd, ^1J = 8, ^2J = 4 Hz, 2 H, H-4′, H-7′), 7.29 (dd, ^1J = 8, ^2J = 4 Hz, 2 H, H-5′, H-6′), 7.18 (d, J = 2 Hz, 1 H, H-1), 7.15 (d, J = 2 Hz, 1 H, H-8), 7.11 (s, 1 H, NH), 2.10 [s, 3 H, -C(O)CH₃***], 1.56 (s, 6 H, ***CH₃***-C-***CH₃***) ppm. IR: $\tilde{\nu}$ = 3240, 1657, 1630, 1612, 1550, 1164, 1129, 846, 749, 723 cm^{-1}. MS: m/z = 484.065 [M + H]$^+$, 506.044 [M + Na]$^+$.

N*-[2,7-Dichloro-9,9-dimethyl-5-(pyridin-2-ylamino)-9*H*-xanthen-4-yl]acetamide (8):** Compound **4** (200 mg, 0.6 mmol) and 2-bromopyridine (0.56 mL, 5.9 mmol) were heated together at 160 °C for 2 h. The reaction was monitored by TLC (CH_2Cl_2/diethyl ether, 4:1). The solution was then cooled to room temperature and added to 2 N HCl and ice (20.0 mL). The reaction mixture was extracted with ethyl acetate (20.0 mL), dried with Na_2SO_4 and concentrated to dryness. The crude material was purified by chromatography on silica gel (CH_2Cl_2/diethyl ether, 4:1) providing title **8** (94 mg, 39% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.07 [s (broad), 2 H, H-3, H-6′], 7.60 (t, J = 6 Hz, 1 H, H-4′), 7.43 (d, J = 2 Hz, 1 H, H-6), 7.07 (d, J = 2 Hz, 2 H, H-1, H-8), 6.99 (d, J = 6 Hz, 1 H, H-3′), 6.81 (t, J = 6 Hz, 1 H, H-5′), 2.07 [s, 3 H, -C(O)CH₃***], 1.59 (s, 6 H, ***CH₃***-C-***CH₃***) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 168.5 [***C*(*O*)**], 156.0 (Ar***C***), 148.2 (Ar***CH***), 141.1 (Ar***C***), 138.2 (Ar***CH***), 137.9 (Ar***C***), 132.0 (Ar***C***), 130.7 (Ar***C***), 129.2 (Ar***C***), 128.7 (2 × Ar***C***), 127.0 (Ar***C***), 121.4 (Ar***CH***), 121.2 (Ar***CH***), 120.5 (Ar***CH***), 119.4 (Ar***CH***), 115.7 (Ar***CH***), 108.4 (Ar***CH***), 35.0 (***C***), 31.6 (2 × ***CH₃***), 24.3 (***CH₃***) ppm. IR: $\tilde{\nu}$ = 3390, 1664, 1630, 1540, 1216, 833, 768, 730 cm^{-1}. MS: m/z = 428.091 [M + H]$^+$, 450.066 [M + Na]$^+$.

N*-[5-(1*H*-Benzo[*d*]imidazol-2-ylamino)-2,7-dichloro-9,9-dimethyl-9*H*-xanthen-4-yl]acetamide (9):** Compound **4** (200 mg, 0.6 mmol) and sublimated 2-chloro-1*H*-benzo[*d*]imidazole (200 mg, 1.3 mmol) were suspended in diglyme (1.0 mL). The reaction mixture was heated at 160 °C for about 1 h until TLC (CH_2Cl_2/ethyl acetate, 1:1) revealed the absence of the starting material. The reaction mixture was added to water (20.0 mL) and extracted with ethyl acetate (20.0 mL). The organic layer was dried with Na_2SO_4, and the solvents were evaporated to dryness. The crude product was further purified by column chromatography on silica gel (CH_2Cl_2/ethyl acetate, 1:1) to give **9** (204 mg, 78%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.89 (d, J = 2 Hz, 1 H, H-3), 7.38 (d, J = 2 Hz, 1 H, H-6), 7.27 (m, 2 H, H-4′, H-7′), 7.15 (m, 2 H, H-5′, H-6′), 6.96 (d, J = 2 Hz, 1 H, H-8), 6.91 (d, J = 2 Hz, 1 H, H-1), 2.08 [s, 3 H, -C(O)CH₃***], 1.43 (s, 6 H, ***CH₃***-C-***CH₃***) ppm. IR: $\tilde{\nu}$ = 3221, 1657, 1620, 1566, 1540, 1250, 1203, 833, 736 cm^{-1}. MS: m/z = 467.101 [M + H]$^+$.

tert*-Butyl 2,7-Dichloro-5-[5-(dimethylamino)naphthalene-1-sulfonamido]-9,9-dimethyl-9*H*-xanthen-4-ylcarbamate (10):** Compound **2** (6.9 g, 16.9 mmol) and dansyl chloride (4.6 g, 17.1 mmol) were dissolved in pyridine (10.0 mL). The reaction was monitored by TLC with CH_2Cl_2 as the eluent. After 30 min, the reaction mixture was added to HCl (2 N, 50.0 mL) and extracted with ethyl acetate (100 mL). The organic phase was separated, dried with Na_2SO_4 and concentrated under vacuum. The crude product was further purified by column chromatography on silica gel (CH_2Cl_2) to give the **10** (9.0 g, 83%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.51 (d, J = 6 Hz, 1 H, H-4′), 8.31 (d, J = 6 Hz, 1 H, H-2′), 8.26 (d, J = 6 Hz, 1 H, H-8′), 8.02 [s (broad), 1 H, H-3], 7.49 (t, J = 6 Hz, 1 H, H-3′), 7.37 (t, J = 6 Hz, 1 H, H-7′), 7.35 (d, J = 2 Hz, 1 H, H-6), 7.12 (d, J = 2 Hz, 1 H, H-1), 7.05 (d, J = 6 Hz, 1 H, H-6′), 6.89 (d, J = 2 Hz, 1 H, H-8), 2.79 (s, 6 H, ***CH₃-*N*-***CH₃***), 1.63 [s, 9 H, C***(CH₃)₃***], 1.39 (s, 6 H, ***CH₃***-C-***CH₃***) ppm. IR: $\tilde{\nu}$ = 3442, 3300, 1623, 1551, 1364, 1222, 1174, 1121, 912, 750 cm^{-1}. MS: m/z = 664.155 [M + Na]$^+$.

Daxabe (11): Compound **10** (600 mg, 0.9 mmol) and sublimated 2-chloro-1*H*-benzo[*d*]imidazole (400 mg, 2.6 mmol) were suspended in sulfolane (1.0 mL). Oxygen was evacuated, and the reaction mixture was heated at 130 °C for 1 h. The reaction was monitored by TLC (CH_2Cl_2/diethyl ether, 4:1). The reaction mixture was added to aqueous sodium carbonate (4% w/v, 50 mL) and ethyl acetate (50 mL). The organic layer was dried with Na_2SO_4, and the organic solvents were evaporated to dryness. The crude material was purified by chromatography on silica gel (CH_2Cl_2/diethyl ether, 4:1) providing daxabe **11** (498 mg, 82%). ^{1}H NMR (200 MHz, $CDCl_3$/CD_3OD, 10:1): δ = 8.24 (d, J = 6 Hz, 1 H, H-4′), 8.20 (d, J = 2 Hz, 1 H, H-6), 8.17 (d, J = 2 Hz, 1 H, H-2′), 8.00 (d, J = 6 Hz, 1 H, H-8′), 7.42 [s (broad), 2 H, H-4′′, H-7′′], 7.31 (d, J = 2 Hz, 1 H, H-3), 7.15 [s (broad), 2 H, H-5′′, H-6′′], 7.13 (t, J = 6 Hz, 1 H, H-3′), 7.05 (d, J = 2 Hz, 1 H, H-1), 7.03 (t, J = 2 Hz, 1 H, H-7′), 6.81 (d, J = 6 Hz, 1 H, H-6′), 6.78 (d, J = 2 Hz, 1 H, H-8), 2.65 (s, 6 H, ***CH₃***-*N*-***CH₃***), 1.37 (s, 6 H, ***CH₃***-C-***CH₃***) ppm. ^{13}C NMR (400 MHz, $CDCl_3$/CD_3OD, 10:1): δ = 151.3 (Ar***C***, C-1′), 149.2 (Ar***C***, C-5′), 141.0 (Ar***C***, C-1′′), 136.4 (Ar***C***, C-7), 134.6 (Ar***C***, C-2), 131.4 (Ar***C***), 130.6 (Ar***CH***, C-4′), 129.9 (Ar***C***), 129.5 (Ar***C***, C-2), 129.5 (Ar***CH***, C-2′), 128.9 (Ar***C***), 128.7 (Ar***C***), 128.3 (Ar***C***), 127.7 (Ar***CH***, C-7′), 125.6 (Ar***C***, C-9), 123.0 (Ar***CH***, C-3′), 122.8 2 × Ar***CH***, C-4′′, C-7′′), 122.7 (2 × Ar***CH***, C-5′′, C-6′′), 121.5 (2 × Ar***CH***, C-1, C-3), 118.4 (Ar***CH***, C-8′), 117.7 (Ar***CH***, C-6′), 115.8 (Ar***CH***, C-6), 115.0 (Ar***CH***, C-8), 45.1 (2 × ***CH₃***, ***CH₃***N***CH₃***), 31.6 (2 × ***CH₃***, ***CH₃***C***CH₃***) ppm. IR: $\tilde{\nu}$ = 1735, 1618, 1566, 1333, 1250, 1216, 1158, 1015, 742 cm^{-1}. MS: m/z = 658.145 [M + H]$^+$, 680.119 [M + Na]$^+$.

Supporting Information (see also the footnote on the first page of this article): Daxabe spectra (^{1}H NMR, ^{13}C NMR, IR and MS), daxabe **11** dimerization titration, absolute association titrations of daxabe with acetic acid and 3,5-dinitrobenzoic acid, fluorescence responses of the halogenides with the daxabe and **11**/3,5-dinitrobenzoic acid systems, ^{1}H NMR and ^{13}C NMR of **5** and **8**, optimized cartesian coordinates for the complex between daxabe and 3,5-dinitrobenzoic acid.

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