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BODIPY-Based Hydroxyaryl Derivatives as Fluorescent pH Probes

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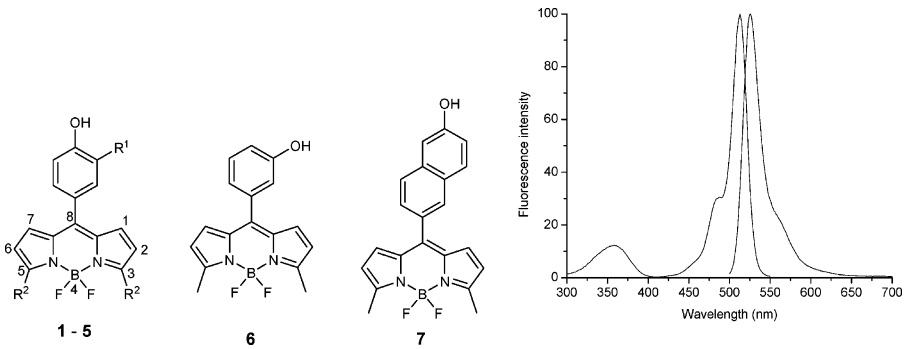

Seven new 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes with phenolic or naphtholic subunits on position 8 and with substituents having different electron driving forces on positions 3 and 5 were synthesized. Their absorption and steady-state fluorescence properties were investigated as a function of solvent. The novel compounds, with the exception of 4,4-difluoro-8-(4-hydroxyphenyl)-3,5-bis-(4-methoxyphenyl)-4-bora-3a,4a-diaza-*s*-indacene, are characterized by absorption maxima in the range 493−515 nm and small (400−600 cm^{-1}) Stokes shifts. The exceptional dye has absorption maxima in the 570−580 nm region and fluorescence emission maxima around 610−620 nm, depending on the solvent. In aqueous solution, the dyes show a large fluorescent enhancement upon increasing the acidity of the solution. They can be used in aqueous solution as fluorescent pH probes excitable with visible light, with pK_a values ranging from 7.5 to 9.3, depending on the substitution pattern on positions 3, 5, and 8.

Introduction

Measurement of pH by fluorescence-based techniques is well established for both imaging and sensing applications. It offers significant advantages over other techniques due to its generally nondestructive character, high sensitivity and specificity, and the wide range of indicator dyes available.[1] In recent years, a lot of work has been focused on the synthesis[2] of 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (borondipyrromethene, BDP, or BODIPY[3])-based fluorescent probes and their application[4] as selective and efficient sensors of ionic species. BDP dyes have many valuable qualities such as high photostability, high fluorescence quantum yields, and relatively high absorption coefficients. Furthermore, they are photoexcitable with visible light, have narrow emission bandwidths with high peak intensities, and are amenable to structural modification.[5] BDP-based fluorophores have been applied as pH sensors in organic solvents or aqueous−organic mixed media.[6−8] Boradiazaindacenes bearing phenolic,[6] dialkylaminophenyl,[7] and calix[4]-

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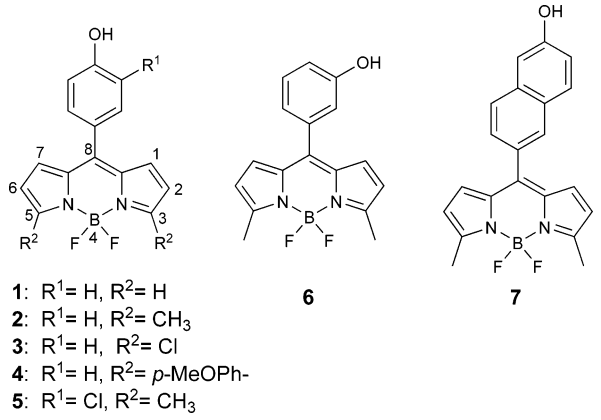

FIGURE 1. BODIPY-based pH probes.

arene[8] subunits at position 8 all showed deprotonation/protonation dependent fluorescence off/on switching. The phenolic, *N*,*N*-dialkylaniline, and calix[4]arene derivatives sense the alkaline, the acidic, and the near neutral pH range, respectively. The low emission intensity of the phenolate[6,8] or uncharged dialkylaniline[7] forms has been attributed to charge transfer from the phenolate or uncharged *N*,*N*-dialkylaniline donors to the excited-state indacene acceptor moiety.

The present study is directed toward the synthesis and fluorimetric characterization of BDP fluorophores that can probe pH changes by large associated changes in the emission intensity around 510 nm and possess pK_a values close to the physiological pH range. Seven 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene dyes with phenolic or naphtholic subunits at position 8 have been investigated (compounds **1**−**7** in Figure 1). The BDP compounds have no substituents at the 1 and 7 positions, whereas the 3 and 5 positions are substituted with groups having different electron driving forces to study the effect on pK_a and fluorescence quantum yields.

Results and Discussion

We synthesized compounds that are 3,5-unsubstituted (**1**), 3,5-disubstituted with methyl (**2**, **5**, **6**, and **7**) and *p*-methoxyphenyl (**4**) groups (electron-donating functionalities), and 3,5-disubstituted with chlorine atoms (**3**) (inductively electron-withdrawing group). The syntheses of **1** and **3** (Scheme 1) were carried out through an existing procedure[2] starting from 4-hydroxybenzaldehyde (**8**). The needed dipyrromethane **9** was prepared according to a published procedure[9] by condensation of aldehyde **8** with neat excess pyrrole catalyzed by trifluoroacetic acid (TFA) at room temperature. Dipyrromethane **9** was purified, and one portion was oxidized by *p*-chloranil to dipyrromethene **10**. Compound **10** was separated, followed by reaction with triethylamine (TEA) and BF_3-OEt_2 in refluxing toluene to afford compound **1**. The other portion of dipyrromethane **9** was chlorinated with 2 equiv of *N*-chlorosuccinimide (NCS) at −78 °C in tetrahydrofuran (THF) according to the procedure described for the halogenation of pyrrole[10] to afford dipyrromethane **11**.

For the synthesis of dye **2** (Scheme 2), we first prepared 5-(4-hydroxyphenyl)-1,9-dimethyl dipyrromethene **13** through a known procedure[2] by condensing aldehyde **8** with 2 equiv of 2-methylpyrrole (**12**) in CH_2Cl_2 at room temperature in the presence of TFA as catalyst, followed by oxidation with *p*-chloranil. Product **13** was further reacted with TEA and BF_3-OEt_2 to afford compound **2**. The BDP derivatives **5**, **6**, and **7** were also synthesized by the same procedure[2] by condensing 2-methylpyrrole (**12**)[11] with 3-chloro-4-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, and 6-hydroxy-2-naphthaldehyde,[12] respectively. Compound **4** was synthesized similarly[2] from aldehyde **8** and 2-(4-methoxyphenyl) pyrrole.[2c]

The purified and characterized (^{1}H, ^{13}C NMR, mass spectra) new BDP compounds were dissolved in several solvents to study their spectroscopic properties (Table 1). The shortest wavelength absorption (or excitation) and emission were observed for the 3,5-unsubstituted compound **1**, which also had the lowest fluorescence quantum yields ϕ_f. The 3,5-dimethylBDP compounds **2**, **5**, **6**, and **7** all have their maxima of excitation (505−514 nm) and emission (517−530 nm) within a very narrow wavelength range in the four solvents. The influence of the 8-substituent on the absorption and emission maxima of the 3,5-dimethylBDP probes is minimal, even for the 8-naphthol derivative **7**. 3,5-DichloroBDP compound **3** has excitation (506−515 nm) and emission (517−527 nm) maxima similar to those of the 3,5-dimethylBDP compounds **2**, **5**, **6**, and **7**. As compared to the other probes, compound **4** with *p*-methoxyphenyl substituents in positions 3 and 5 shows a clear shift in the excitation (570−581 nm) and emission (612−618 nm) maxima toward longer wavelength, along with an increase of ϕ_f. This is due to the increasing conjugation of the π-system of the chromophore. While the Stokes shifts for all other compounds are small ($\Delta\bar{\nu} = 370-650$ cm^{-1}), derivative **4** has relatively large Stokes shifts (1030−1380 cm^{-1}). Compound **4** is not soluble in water, so further study in aqueous solution is not feasible. For all compounds, the excitation and emission maxima shift to the red wavelength region with decreasing solvent polarity, indicating the more polar character of the ground state. The most bathochromic excitation and emission is found in toluene as solvent. Concurrently, the fluorescence quantum yields decrease with increasing solvent polarity. The highest ϕ_f values are measured in toluene solution, the second

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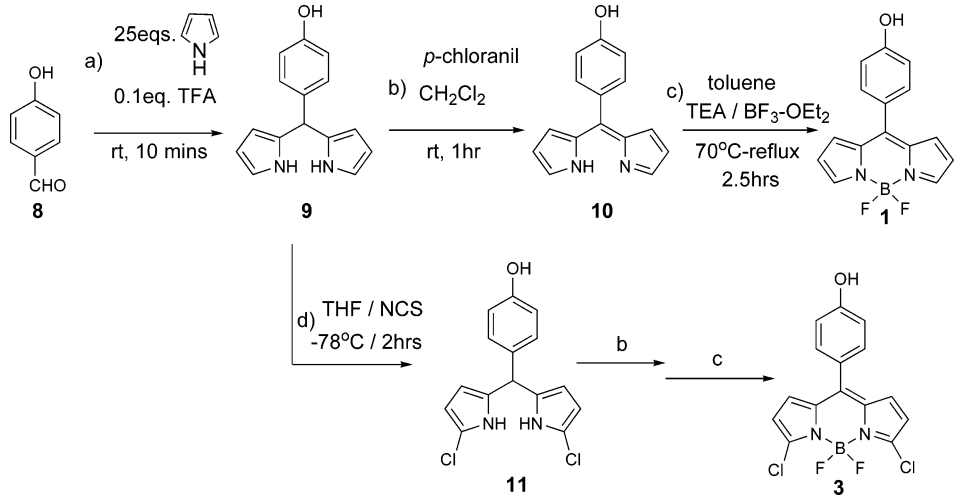
SCHEME 1. Synthesis of 1 and 3



SCHEME 2. Synthesis of 2

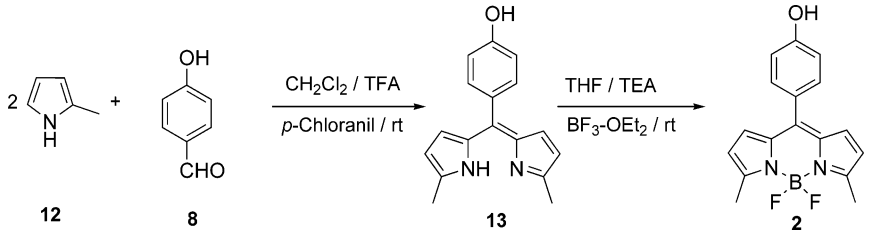


TABLE 1. Fluorescence Characteristics of the BDP Dyes in Different Solvents

BDP	solvent	λ_{Abs} (max/nm)	λ_{em} (max/nm)	λ_{ex} (max/nm)	$\Delta_{max}\upsilon$ (cm^{-1})	ϕ_f
1	MeOH	493	508	494	599	0.015
	MeCN	493	508	493	599	0.023
	cyclohexane	499	512	499	509	0.043
	toluene	501	518	501	655	0.081
2	MeOH	505	517	505	460	0.28
	MeCN	505	517	505	496	0.24
	cyclohexane	510	521	510	414	0.45
	toluene	511	524	511	486	0.63
3	MeOH	506	517	506	420	0.014
	MeCN	506	518	506	458	0.089
	cyclohexane	513	523	513	373	0.33
	toluene	515	527	515	442	0.63
4	MeOH	570	612	570	1204	0.57
	MeCN	567	615	567	1377	0.48
	toluene	581	618	581	1031	0.68
5	MeOH	507	520	507	493	0.16
	MeCN	507	521	508	530	0.20
	cyclohexane	513	526	513	482	0.28
	toluene	515	529	514	514	0.56
6	MeOH	508	521	507	491	0.19
	MeCN	507	521	507	530	0.17
	cyclohexane	512	525	512	484	0.25
	toluene	514	528	513	516	0.44
7	MeOH	508	522	508	528	0.025
	MeCN	507	524	507	640	0.058
	cyclohexane	512	527	512	556	0.17
	toluene	514	530	513	587	0.26

highest in cyclohexane. The position of the hydroxyl and 3,5-dimethylBDP functionalities on the benzene ring in dyes **2** (*para*) and **6** (*meta*) has no significant influence on the spectral properties and fluorescence quantum yields. 3,5-DichloroBDP compound **3** shows the largest increase of ϕ_f when going from protic polar methanol to apolar toluene. The molar absorption coefficients for most of the synthesized BDP derivatives are high and lie in the 60 000−110 000 M^{-1} cm^{-1} range.

The acidity constants K_a of probes **1**−**3** and **5**−**7** were determined in aqueous nonbuffered solution by fluorimetric titration as a function of pH using the fluorescence excitation and/or emission spectra. Nonlinear curve-fitting of eq 1 to the fluorescence data F recorded as a function of pH yielded values of K_a, the fluorescence signals F_{min} and F_{max} at minimal and maximal $[H^+]$, respectively, and n (the stoichiometry of H^+ binding to the phenolate or naphtholate form of the probe). Fitting eq 1 to the steady-state fluorescence data F with n, K_a, F_{min}, and F_{max} as freely adjustable parameters always gave values of n close to 1, indicating that one proton is bound per phenoxide or naphthoxide anion. Therefore, n was kept fixed at 1 in the final curve fittings, from which the estimated values of K_a, F_{min}, and F_{max} are reported here.

$$F = \frac{F_{max}[H^+]^n + F_{min}K_a}{K_a + [H^+]^n} \quad (1)$$

As examples, the absorption, emission, and excitation spectra of **5** are shown in Figure 2 as a function of pH. The absorption spectra (Figure 2a) show a small shift of the maximum (from 505 to 502 nm) with increasing pH and a clear isosbestic point around 392 nm. The fluorescence emission (Figure 2b) and excitation (Figure 2c) intensities increase at lower pH values. There is no significant shift of the absorption (or excitation) and emission maxima as a function of pH (Table 2). Figure 3 is a representative example of the fit of eq 1 with $n = 1$ to the fluorescence emission data of **5** as a function of $[H^+]$.

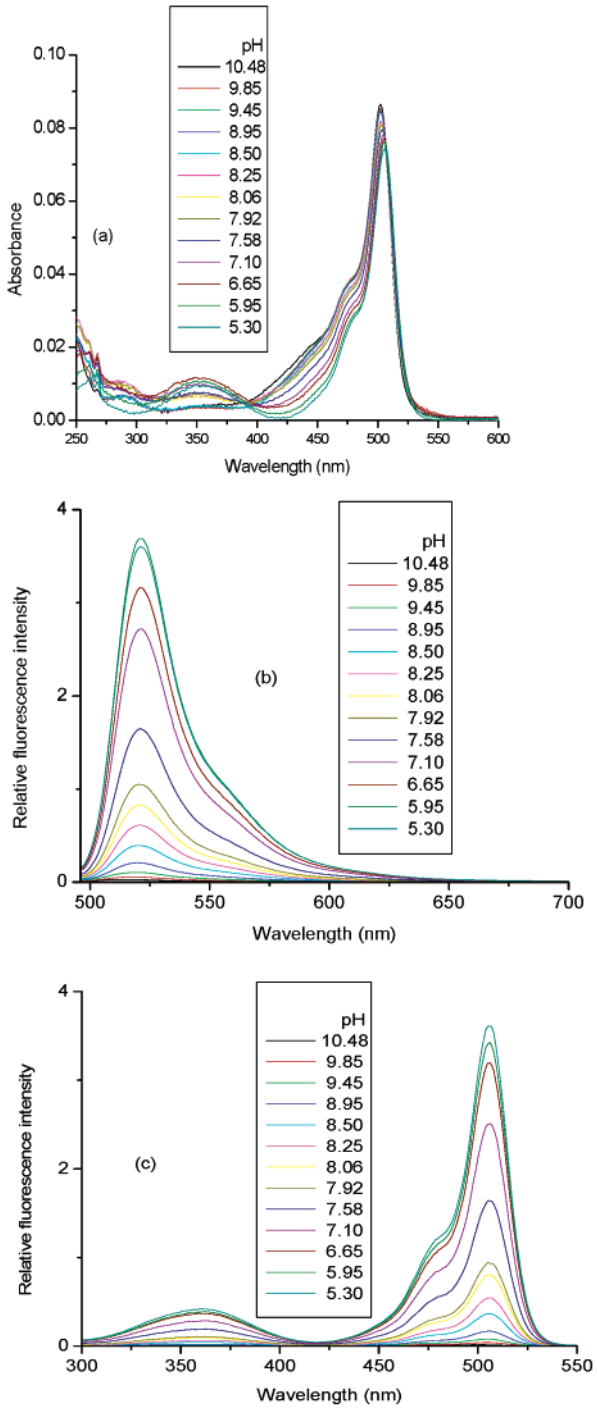


FIGURE 2. (a) Absorption spectra of compound **5** in aqueous nonbuffered solution as a function of pH. (b) Corresponding fluorescence emission spectra (λ_{ex} = 488 nm) and (c) fluorescence excitation spectra (λ_{em} = 560 nm).

The pK_a of the phenol derivatives **1**, **2**, **3**, **5**, and **6** can be tuned by varying the substituents on BDP. When phenol (with a pK_a of 10.00)[13] is substituted at the *para* position with the BDP moiety (compound **1**), the pK_a drops to 8.69. When the α-positions to the nitrogen atoms are substituted with the (inductively) electron-donating methyl group (in compound **2**), the pK_a of the phenolic

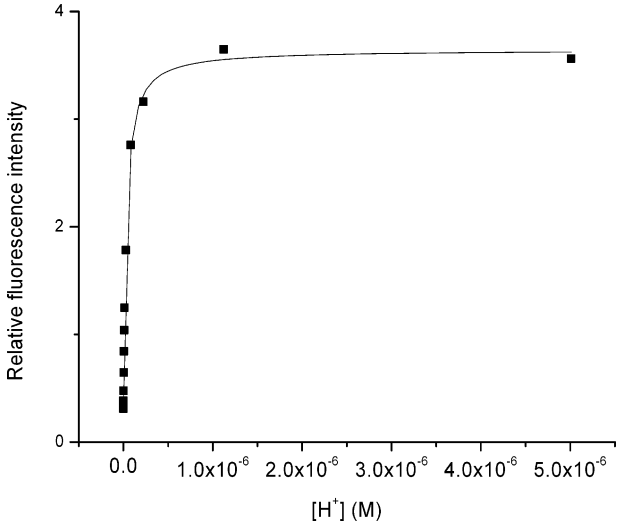


FIGURE 3. The solid line represents the best fit of eq 1 with n = 1 to the fluorimetric titration data of dye **5** obtained from the emission spectra of Figure 2b (λ_{ex} = 488 nm, λ_{em} = 520 nm).

group is 8.75. In contrast, the 3,5-dichloroBDP substituent (in compound **3**) decreases the pK_a to 8.41.

These observed effects are in line with the electron-withdrawing (or releasing) power of the substituents in α-position to the nitrogen atoms (Table 2). When phenol is substituted in the *meta* position with 3,5-dimethylBDP (in compound **6**), the pK_a is 9.34. When *o*-chlorophenol (with a pK_a of 8.53 [13]) is substituted at the *para* position with 3,5-dimethylBDP as in dye **5**, the pK_a is reduced to 7.49. In contrast to the phenol derivatives, the pK_a of 2-naphthol (~9.5)[14,15] is hardly affected by the boradiazaindacene moiety in compound **7**. Also, the fluorescence excitation and emission spectra of compound **7** are those of 3,5-dimethylBDP. Probe **5**, where a chlorine atom is introduced in the *ortho* position to the OH functionality in the phenyl ring, has the most favorable pK_a value for intracellular pH measurements. The high photostability, the capability of using visible light excitation (necessary to avoid autofluorescence of most biological samples), the pK_a suitable for near-neutral pH measurements, the solubility in aqueous solution, the negligible sensitivity of pK_a to low ionic strength (see below), the high molar absorption coefficients, and the high fluorescence enhancement factor F_{max}/F_{min} make molecule **5** an interesting lead compound for designing biologically useful fluorescent off/on pH probes.

To study the influence of buffer and added salt on the steady-state fluorescence, we recorded pH-dependent fluorescence emission and excitation spectra of dye **5** under various conditions. First, the steady-state fluorescence spectra of compound **5** were measured in the presence of different concentrations of MOPS (3-[*N*-morpholino]propanesulfonic acid) buffer but in the absence of added salt. Second, measurements in the absence and presence of various concentrations of MOPS buffer were performed in the presence of 0.1 M KCl. The pK_a values obtained are compiled in Table 3, indicating that

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TABLE 2. Photophysical Properties of the BDP Derivatives in Aqueous Nonbuffered Solution[a]

BDP	$\lambda_{abs-acid}/\lambda_{abs-base}$ (max/nm)	$\lambda_{em-acid}/\lambda_{em-base}$ (max/nm)	ϕ_{f-acid} (pH)	ϕ_{f-base} (pH)	pK_a	F_{max}/F_{min}
1	493/489	510/510	0.017 (7.10)	0.0007 (10.12)	8.69 ± 0.02	40
2	505/501	510/509	0.12 (7.15)	0.007 (9.95)	8.75 ± 0.05	25
3	508/503	521/523	0.008 (6.50)	0.0008 (10.00)	8.41 ± 0.03	8
5	505/502	521/519	0.09 (5.30)	0.0007 (10.48)	7.49 ± 0.03	130
6	507/506	523/523	0.11 (7.45)	0.009 (10.55)	9.34 ± 0.04	35
7	506/506	525/526	0.02 (6.56)	0.003 (10.98)	9.20 ± 0.03	6

[a] K_a, F_{max}, and F_{min} values were determined by fitting eq 1 with $n = 1$ to the fluorescence data. The fluorescence quantum yields ϕ_{f-base} and ϕ_{f-acid} were determined at, respectively, the highest and lowest pH values (between parentheses) used in the fluorimetric titration.

TABLE 3. pK_a Values of Dye 5 Determined by Direct Fluorimetric Titration in the Absence/Presence of MOPS Buffer and Added Salt (KCl)[a]

C (M)	ionic strength (M)	pK_a
0 M MOPS + 0 M KCl	0	7.49 ± 0.03
0.01 M MOPS + 0 M KCl	0.02	7.45 ± 0.03
0.05 M MOPS + 0 M KCl	0.10	7.41 ± 0.05
0 M MOPS + 0.1 M KCl	0.10	7.48 ± 0.03
0.05 M MOPS + 0.1 M KCl	0.20	7.35 ± 0.05

[a] The values of the ground-state acidity constants K_a measured from emission spectra ($\lambda_{ex} = 488$ nm and $\lambda_{em} = 520$, 530 nm) and excitation spectra ($\lambda_{em} = 560$ nm and $\lambda_{ex} = 506$, 516 nm) are in excellent agreement. The ionic strength values were calculated for solutions at 20 °C characterized by pH 7.20 (equal to the pK_a of MOPS).

the ground-state acidity constants of **5** are nearly insensitive to added salt and/or MOPS buffer in the concentration range studied. As the effects of added salt and buffer are very small, pH probe **5** has the additional advantage of a negligible sensitivity to low ionic strength.

Conclusion

We synthesized seven new 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene dyes (**1**−**7**) with phenolic or naphtholic subunits (Figure 1). Their absorption and steady-state fluorescence properties were investigated as a function of solvent. Compounds **1**−**3** and **5**−**7** are characterized by absorption maxima in the range 493−515 nm and small (400−600 cm^{-1}) Stokes shifts. Fluorescent dye **4** has absorption maxima in the 570−580 nm region and fluorescence emission maxima around 610−620 nm, depending on the solvent. In aqueous solution, compounds **1**−**3** and **5**−**7** show a large fluorescent enhancement upon increasing the acidity of the solution. They can be used in aqueous solution as fluorescent pH probes excitable with visible light, with pK_a values ranging from 7.5 to 9.3, depending on the substitution pattern on positions 3, 5, and 8.

Experimental Section

General Procedure for the Preparation of Phenolic BODIPYs 1, 3, and 4. A toluene solution of 1 equiv of dipyrromethene was purged with argon. To the solution was added 10 equiv of triethylamine. This solution was heated at 70 °C for 0.5 h. Next, 15 equiv of BF_3-OEt_2 was added and the reaction was heated at reflux temperature for 2 h. To the cooled reaction mixture was added a 1 M aqueous solution of sodium hydroxide. The layers were separated. The water layer was brought to pH ∼5−6 by addition of HCl and extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate, and the solvent was removed by distillation. The residue was chromatographed on silica, starting elution with CH_2Cl_2 and gradually increasing the polarity by adding EtOAc (for **1**, up to 100% (v) EtOAc; for **3**, up to 50% EtOAc; for **4**, up to 5% EtOAc).

4,4-Difluoro-8-(4-hydroxyphenyl)-4-bora-3a,4a-diaza-*s*-indacene (1). Yield: 64%. Orange crystals mp 153 °C; recrystallized from chloroform/cyclohexane. ^{1}H NMR ($CDCl_3$): δ 6.15 (broad s, 1H, OH), 6.56 (m, 2H), 6.97 (d, 2H, J = 8.8 Hz), 6.99 (m, 2H), 7.47 (d, 2H, J = 8.8 Hz), 7.93 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 116.0 (d), 118.8 (d), 126.6 (s), 131.9 (d), 133.0 (d), 135.2 (s), 143.8 (d), 147.9 (s), 159.1 (s). LRMS (EI, 70 eV): *m/z* (%) 285 (M$^+$, 20), 284 (M$^+$, 100), 283 (M$^+$, 80), 263 (20), 198 (30), 155 (50), 121 (40), 91 (100). HRMS calcd. M$^+$ for $C_{15}H_{11}BF_2N_2O$ 284.09325, found 284.09342.

4,4-Difluoro-8-(4-hydroxyphenyl)-3,5-dichloro-4-bora-3a,4a-diaza-*s*-indacene (3). Yield: 47%. Red crystals mp 224 °C; recrystallized from chloroform/cyclohexane. ^{1}H NMR ($CDCl_3$): δ 5.77 (broad s, 1H, OH), 6.45 (d, 2H, J = 4.4 Hz), 6.89 (d, 2H, J = 4.4 Hz), 6.99 (d, 2H, J = 8.8 Hz), 7.42 (d, 2H, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$): δ 116.3 (d), 119.0 (d), 127.7 (s), 132.0 (d), 132.9 (d), 134.0 (s), 144.3 (s), 159.6 (s), 172.7 (s). LRMS (EI, 70 eV): *m/z* (%) 354 (M$^+$, 5), 353 (M$^+$, 5), 352 (M$^+$, 15), 317 (15), 304 (15), 269 (40), 232 (40), 198 (35), 155 (60), 121 (40), 91 (100). HRMS calcd. M$^+$ for $C_{15}H_9BCl_2F_2N_2O$ 352.01530, found 352.01521.

4,4-Difluoro-8-(4-hydroxyphenyl)-3,5-bis-(4-methoxyphenyl)-4-bora-3a,4a-diaza-*s*-indacene (4). Yield: 30%. Deep blue crystals; mp 250 °C; recrystallized from chloroform/cyclohexane. ^{1}H NMR ($CDCl_3$): δ 3.86 (s, 6H, OCH_3), 6.62 (d, 2H, J = 4.4 Hz), 6.89 (d, 2H, J = 4.4 Hz), 6.97 (d, 4H, J = 8.8 Hz), 6.99 (d, 2H, J = 8.8 Hz), 7.49 (d, 2H, J = 8.8 Hz), 7.88 (d, 4H, J = 8.8 Hz), OH is not seen. ^{13}C NMR ($CDCl_3$): δ 55.7 (q), 114.2 (d), 115.7 (d), 120.7 (d), 125.7 (s), 127.5 (s), 130.7 (d), 131.4 (d), 131.59 (s), 131.55 (s), 132.8 (d), 151.9 (s), 157.9 (s), 161.0 (s). LRMS (EI, 70 eV): *m/z* (%) 497 (M$^+$, 30), 496 (M$^+$, 100), 495 (M$^+$, 20), 481 (35), 480 (5), 206 (50), 170 (40). HRMS calcd. M$^+$ for $C_{29}H_{23}BF_2N_2O_3$ 496.17698, found 496.17685.

Synthesis of 4,4-Difluoro-3,5-dimethyl-8-(4-hydroxyphenyl)-4-bora-3a,4a-diaza-*s*-indacene (2). 100 mg (0.38 mmol) of compound **13** was dissolved in 60 mL of absolute THF purged with argon. To the solution was added 1.5 mL of absolute triethylamine, and the mixture was stirred for 0.5 h at room temperature under argon atmosphere. 1.5 mL of BF_3-OEt_2 was added dropwise through a syringe, and the mixture was stirred for 6−7 h (sometimes overnight) at room temperature. A deep green fluorescence was observed in the mixture. The solvent was evaporated, and the crude product was purified by column chromatography over silica gel with pure ethyl acetate to afford a red powder, which was recrystallized from hexane/chloroform to yield 37 mg (31%) of red crystals, mp 156−157 °C. ^{1}H NMR ($CDCl_3$): δ 2.66 (s, 6H), 6.27 (d, 2H, J = 4.3 Hz), 6.74 (d, 2H, J = 4.3 Hz), 6.91 (d, 2H, J = 8.1 Hz), 7.38 (d, 2H, J = 8.1 Hz). ^{13}C NMR ($CDCl_3$): δ 15.3, 115.6, 119.6, 130.7, 132.6, 134.9, 143.0, 157.4, 158.0, 167.4. LRMS (EI, 70 eV): *m/z* (%) 312 (M$^+$ 100); 311 (M$^+$ 61), 292(67), 291 (60). HRMS calcd. M$^+$ for $C_{17}H_{15}BF_2N_2O$ 312.12455, found 312.12423.

Indicators **5**, **6**, and **7** were prepared by the same procedure as for **2**.

4,4-Difluoro-3,5-dimethyl-8-(3-chloro-4-hydroxyphenyl)-4-bora-3a,4a-diaza-*s*-indacene (5). Yield 42%. Red crystals; mp 180−181 °C; recrystallized from chloroform/cyclohexane. ^{1}H NMR ($CDCl_3$): δ 2.66 (s, 6H), 6.29 (d, 2H, J = 4.4 Hz), 6.73 (d, 2H, J = 4.4 Hz), 7.12 (d, 1H, J = 8.7 Hz), 7.35 (dd, 1H, J = 8.0 Hz, J = 2.2 Hz), 7.51 (d, 1H, J = 2.2 Hz). ^{13}C NMR ($CDCl_3$): δ 15.3, 27.3, 116.5, 119.9, 120.4, 127.9, 130.5, 131.2, 131.2, 134.7, 153.4, 158.2. LRMS (EI, 70 eV): *m/z* (%) 348 (M^+ 68), 346 (M^+ 100), 327 (54), 326 (97), 325 (91). HRMS calcd. M^+ for $C_{17}H_{14}BClF_2N_2O$ 346.08558, found 346.08510.

4,4-Difluoro-3,5-dimethyl-8-(3-hydroxyphenyl)-4-bora-3a,4a-diaza-*s*-indacene (6). Yield 52%. Shiny green crystals; mp > 300 °C recrystallized from chloroform/cyclohexane. ^{1}H NMR ($CDCl_3$): δ 2.66 (s, 6H), 6.26 (d, 2H, J = 4.4 Hz), 6.75 (d, 2H, J = 4.4 Hz), 6.97−7.07 (m, 3H), 7.32 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 15.3, 117.3, 117.7, 119.8, 123.5, 129.9, 130.8, 134.8, 135.9, 155.6, 158.1. LRMS (EI, 70 eV): *m/z* (%) 312 (M^+ 100), 311 (M^+ 60), 292 (68), 291 (63). HRMS calcd. M^+ for $C_{17}H_{15}BF_2N_2O$ 312.12455, found 312.12328.

4,4-Difluoro-3,5-dimethyl-8-(6-hydroxynaphth-2-yl)-4-bora-3a,4a-diaza-*s*-indacene (7). Yield 31%. Red crystals; mp 223−224 °C; recrystallized from chloroform/cyclohexane. ^{1}H NMR ($CDCl_3$): δ 2.68 (s, 6H), 6.28 (d, 2H, J = 4.1 Hz), 6.76 (d, 2H, J = 4.1 Hz), 7.18−7.24 (m, 2H), 7.55 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz), 7.75−7.84 (m, 2H), 7.92 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 15.3, 47.2, 109.8, 119.3, 119.7, 126.7, 128.4, 128.8, 129.8, 130.7, 130.9, 135.1, 135.6, 155.2, 157.7. LRMS (EI, 70 eV): *m/z* (%) 363 (M^+ 20), 362 (M^+ 100), 361 (M^+ 45), 342 (45), 341 (30). HRMS calcd. M^+ for $C_{21}H_{17}BF_2N_2O$ 362.14020, found 362.14059.

Materials. All solvents were spectroscopic grade and were used without further purification. Potassium chloride (KCl, 99.999%) and MOPS (3-[*N*-morpholino]propanesulfonic acid, >99.5%) were used as such. The chemicals used for the synthesis were of the best grade available and were used as received. Dichloromethane (p.a.) was dried over molecular sieves. Boron trifluoride etherate (BF_3−OEt_2) was ca. 48% BF_3.

Steady-State Spectroscopy. The absorption measurements were performed on a Perkin-Elmer Lambda 40 UV/vis spectrophotometer. Corrected steady-state excitation and emission spectra were recorded on a SPEX Fluorolog. For the determination of the relative fluorescence quantum yields (ϕ_f), only dilute solutions with an absorbance below 0.1 at the excitation wavelength λ_{ex} were used. Rhodamin 6G in H_2O (λ_{ex} = 488 nm, ϕ_f = 0.76) and/or Cresyl Violet (λ_{ex} = 546 nm, ϕ_f = 0.55) in methanol were used as fluorescence standard.[16] The quantum efficiencies of fluorescence in this work were obtained from average values of multiple (generally four) independent measurements. Correction for the refractive index was applied. All spectra were recorded at 20 °C.

Determination of K_a via Fluorimetric Titration. To determine the acidity constants K_a of **1**−**3** and **5**−**7**, these probes were dissolved in 1.0 M NaOH solution. Next, 0.1 and 0.01 M solutions of HCl and/or NaOH were used to adjust the pH of the solutions. All of the measurements were done at 20 °C on nonbuffered solutions, unless specified otherwise.

Determination of K_a of Compound 5 in the Presence/Absence of MOPS Buffer/KCl. An appropriate amount of dye **5** in 1.0 M NaOH solution (with a final absorbance below 0.1 at the excitation wavelength λ_{ex} after dilution), 0 or 2 or 10 mL of 0.1 M MOPS, and 0 or 1 mL of 2.0 M KCl were pipetted into a 20 mL measuring flask. The mixture was diluted to 20 mL with Milli-Q water. A few microliters of 0.1 and 0.01 M solutions of HCl and/or NaOH were used to adjust the pH of the solutions. All of the measurements were done at 20 °C.

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Supporting Information Available: All experimental procedures and spectroscopic characterization (^{1}H and ^{13}C NMR, mass spectra) of compounds **1**−**7** and some intermediates; fluorescence excitation and emission spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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