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Synthesis and Spectral Properties of Cholesterol- and FTY720-Containing Boron Dipyrromethene Dyes

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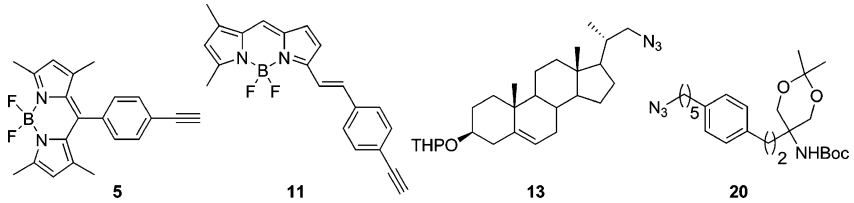

Two analogues (**1**, **2**) of free cholesterol and one analogue (**3**) of the immunosuppressive sphingolipid FTY720 containing a boron dipyrromethene chromophore (BODIPY) were synthesized. The synthetic routes involved preparation of boron dipyrromethene moieties (**5**, **11**), bearing a phenylethynyl group at different positions of the chromophore, and lipids (**13**, **20**) bearing an azido group. The dye was tethered to the lipid via a 1,2,3-triazole in the linker by the click reaction. Analogues derived from **11** [in which an (*E*)-styrylethynyl moiety is bonded to C-5 of BODIPY] exhibited a marked red shift (∼70−80 nm) compared with those derived from **5** (in which a phenylethynyl moiety is bonded to C-8 of BODIPY).

Introduction

The 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene fluorophore, better known as BODIPY, possesses many distinctive and desirable properties: it is relatively hydrophobic and has a high molar absorption coefficient and fluorescence quantum yield.[1] The photochemical and chemical stabilities of the boron dipyrromethene chromophore are higher than those of many other dyes, and the extent of π-electron conjugation can be modified by introduction of substituents, affording red-shifted BODIPY derivatives. BODIPY-linked reagents have been used in a wide range of applications, for example, as fluorescent switches[2] and chemosensors for protons,[3] metal ions,[4] nitric oxide,[5] toxins,[6] and peroxyl radicals.[7] Biochemical applications of BODIPY include bioconjugates with proteins,[8] DNA,[9] and carbohydrates.[10] Because BODIPY is relatively hydrophobic, its conjugates with lipids can partition efficiently into biomembranes.[8,11] Indeed, BODIPY derivatives of many lipids, including fatty acids,[12] triglycerides,[13] sphingolipids,[14] phospholipids,[15] and glycolipids,[16] have been prepared and used for biological studies. When the local concentration of the probe increases,

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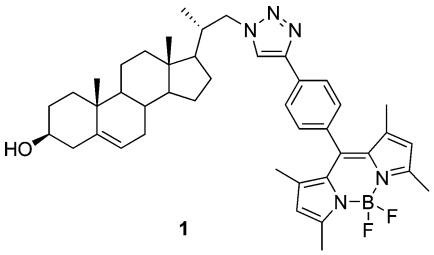
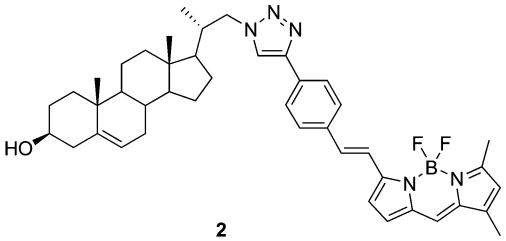


1

(linked via C-8 of BODIPY)



2

(linked via C-5 of BODIPY)

FIGURE 1. Compounds **1** and **2**.

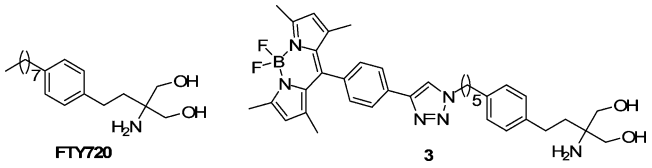


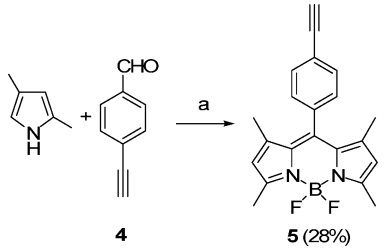
FIGURE 2. FTY720 and compound **3**.

the characteristic green emission of the boron dipyrromethene chromophore is red-shifted via excimer formation, permitting visualization of probe accumulation within subcellular compartments of living cells by fluorescence microscopy.[16c,d]

We recently reported the synthesis of BODIPY-modified cholesterol analogues[17] and found that one of the analogues can partition into liquid-ordered domains of model membranes.[18] The spectroscopic and membrane properties of this BODIPY−cholesterol analogue and the commercially available 7-nitrobenz-2-oxa-1,3-diazolyl (NBD)−cholesterol have been summarized in a recent review.[19] We now describe the preparation of two new red-shifted BODIPY conjugates of free cholesterol, compounds **1** and **2** (Figure 1).

We also report the introduction of the BODIPY chromophore into the synthetic sphingosine analogue known as FTY720 (Figure 2). FTY720, which was discovered during the optimization of the natural product myriocin (which inhibits the first enzyme in the sphingomyelin biosynthetic pathway),[20] has attracted a great deal of attention in the past decade because it is a new immunosuppressant with a unique mechanism of action. FTY720-phosphate [(*S*)-FTY720-P], formed in vivo via sphingosine kinases 1 and 2,[21] is an agonist for several G-protein-coupled sphingosine 1-phosphate (S1P) receptors.[22] FTY720-P exhibits immunosuppressive activity by redirecting the trafficking of circulating lymphocytes. FTY720 has been in phase III clinical trials as an immunosuppresant for organ transplants and recently entered into a clinical trial for the treatment of multiple sclerosis.[23] An FTY720 derivative bearing the relatively hydrophilic NBD fluorophore has been synthesized and used to study the metabolism and mechanism of action of FTY720.[24] However, since the NBD conjugates induce an "upside down orientation in the membrane",[25] we directed our attention to the more lipophilic BODIPY conjugates. We report herein the synthesis of compound **3** (Figure 2), which contains a BODIPY fluorophore in the alkyl side chain and retains the intact hydrophilic head group of FTY720.

SCHEME 1. Synthesis of BODIPY-Acetylene Derivative 5[a]



4 **5** (28%)

[a] Reagents and conditions: (a) (i) TFA, CH_2Cl_2, rt, overnight; (ii) *p*-chloranil, rt, 30 min; (iii) $BF_3{\cdot}OEt_2$, NEt_3, rt, 6 h.

The key step in these syntheses is the 1,3-dipolar cycloaddition between an alkyne and an azide, which was first studied and reviewed by Huisgen et al.[26] The conventional procedure of 1,3-dipolar cycloaddition of an alkyne and an azide usually leads to a 1,2,3-triazole as a mixture of two regioisomers, which has limited the application of this reaction. The discovery that a catalytic amount of Cu(I) allows the 1,3-dipolar cycloaddition reaction to proceed under milder conditions with high regiospecificity[27] spurred interest in using this reaction in various fields ranging from medicinal chemistry to material science.[28] This reaction is often referred to as the "click" reaction.[29] We have applied the click reaction to prepare BODIPY conjugates of lipids, compounds **1**−**3**.

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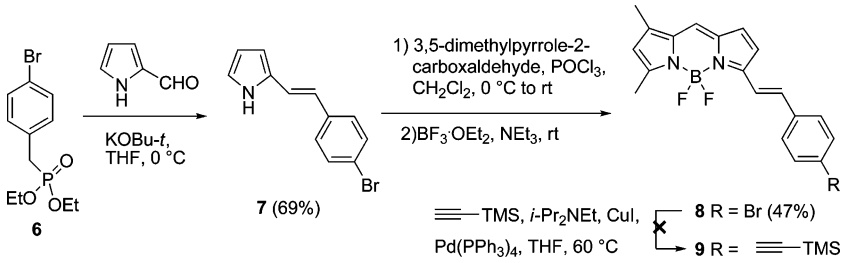
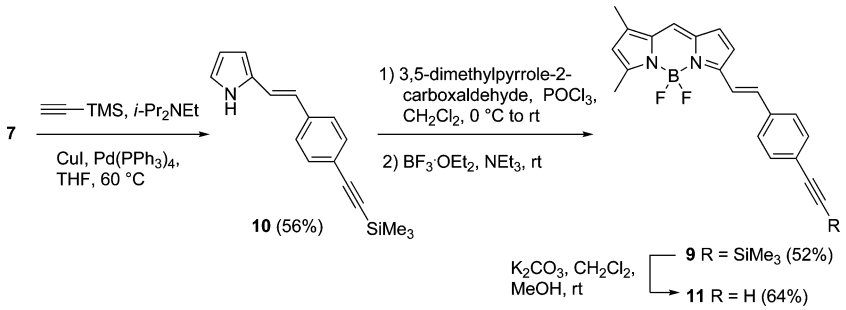
SCHEME 2. De Novo Synthesis of BODIPY with Extended Conjugation



SCHEME 3. Synthesis of BODIPY-Acetylene 11 with Extended Conjugation



Results and Discussion

Synthesis of BODIPY-Substituted Alkynes. The synthesis of BODIPY lipid conjugates by click chemistry involved the preparation of BODIPY derivatives bearing a terminal acetylene group. We prepared two such derivatives with different emission wavelengths. Scheme 1 outlines the preparation of the first alkynyl-BODIPY derivative. Condensation of 4-ethynylbenzaldehyde (**4**) with 2,4-dimethylpyrrole in the presence of a catalytic amount of TFA, followed by oxidation with *p*-chloranil and chelation with $BF_3{\cdot}OEt_2$ in the presence of NEt_3, gave compound **5** in 28% overall yield.

We explored a variety of routes to the second alkynyl-linked BODIPY derivative. Generally, there are three routes for synthesizing BODIPY analogues with extended conjugation: (1) de novo synthesis from a pyrrole derivative bearing a substituent with external conjugation,[30] (2) condensation of the 3- and/or 5-methyl group of BODIPY with an aldehyde,[31] and (3) a transition metal-catalyzed coupling reaction of BODIPY via a halide substituent[32] or direct activation of a C−H bond.[33] Our initial attempt to prepare an alkynyl-linked BODIPY with extended conjugation through condensation of 4,4-difluoro-1,3,5,7-tetramethyl-2,6,8-triethyl-4-bora-3a,4a-diaza-*s*-indacene[34] with **4** by following reported procedures[31] failed, probably because of the instability of **4** under the relatively harsh reaction conditions (toluene/acetic acid/piperidine, reflux). We observed the total consumption of **4** by TLC (hexane/ethyl acetate 6:1) during the reaction and the complete recovery of the diaza-*s*-indacene starting material after workup. We then turned to the synthesis of a TMS-substituted terminal alkyne as outlined in Scheme 2. A Wittig reaction of **6**[35] with pyrrole-2-carboxaldehyde, with potassium *tert*-butoxide as the base, furnished **7**[36] in 69% yield. After **7** underwent condensation[37] with 3,5-dimethylpyrrole-2-carboxaldehyde in the presence of $POCl_3$, chelation with $BF_3{\cdot}OEt_2$ afforded BODIPY analogue **8** in 47% yield. Unfortunately, the Sonogashira reaction for conversion of bromide **8** to alkyne **9** was unsuccessful, producing many unidentified products. After reversing the reaction sequences and performing the Sonogashira reaction on bromide **7**, we were able to obtain **10** in 56% yield. The synthesis of **9** was accomplished by condensation of **10** with 3,5-dimethylpyrrole-2-carboxaldehyde, followed by chelation with $BF_3{\cdot}OEt_2$ in 52% overall yield (Scheme 3). The TMS group in **9** was easily removed by treating **9** with potassium carbonate in a mixture of methanol and dichloromethane to give **11** in 64% yield.

Synthesis of BODIPY−Cholesterol Conjugates. We next explored the use of the two alkynyl-substituted BODIPY derivatives (**5** and **11**) in preparing fluorescent cholesterol conjugates. Known alcohol **12**[17] was converted by the conventional three-step sequence of mesylate to bromide to azide **13** in 85% overall yield (Scheme 4). BODIPY cholesterol conjugate **1** was obtained in 91% yield after deprotection of **14**, which was prepared in 59% yield via a click reaction between **5** and **13** catalyzed by CuI in DMSO. A similar route was used to prepare BODIPY-cholesterol conjugate **2**, which has a longer emission wavelength than **1**, in 52% overall yield (Scheme 5).

Synthesis of BODIPY−FTY720 Conjugate 3. The synthesis of a BODIPY−FTY720 conjugate started with the Wittig reaction of **6** and aldehyde **16**,[38] with potassium *tert*-butoxide as the base. The resulting bromide **17** was subjected to a

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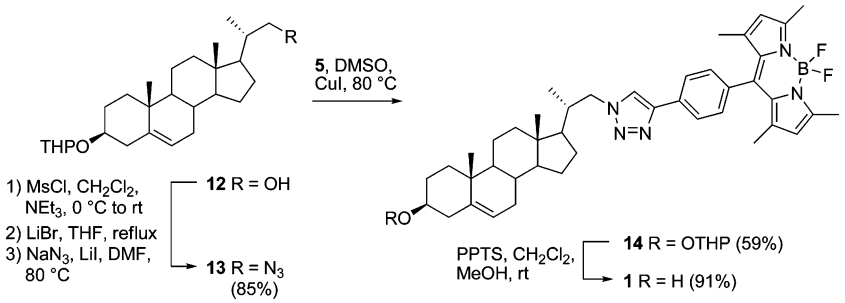
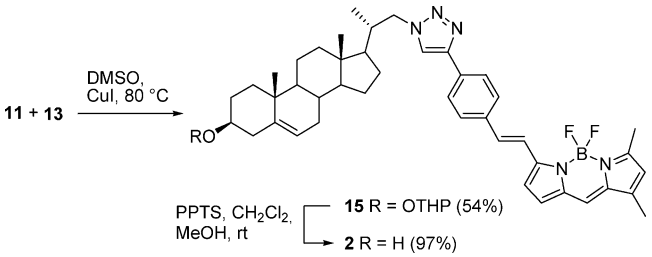
SCHEME 4. Synthesis of BODIPY−Cholesterol Conjugate 1



SCHEME 5. Synthesis of BODIPY−Cholesterol Conjugate 2



Sonogashira reaction with 5-benzyloxy-1-pentyne[39] to give **18** in 53% yield. The double and triple bonds were hydrogenated and the benzyl group was removed by hydrogenolysis with palladium hydroxide in methanol. The ketal protecting group was also partially removed under these conditions, but it was easily reinstalled by treating the resulting mixture with 2,2-dimethoxypropane in the presence of CSA to give **19** in 72% yield. After conversion of the hydroxy group in **19** to the mesylate and treatment with NaN_3 in DMF, azide **20** was obtained in 67% yield. The click reaction of **20** with **5**, catalyzed by CuI in DMF at room temperature, gave protected BODIPY−FTY720 conjugate **21**. Deprotection of **21** proved to be troublesome, although it has been reported that a NHBoc group can be removed with 4 M HCl in dioxane in the presence of a BODIPY moiety.[14] When we treated **21** with proton acids, including HCl, trifluoroacetic acid, and *p*-toluenesulfonic acid, the ketal was removed but the Boc group remained intact under mild conditions. Harsher conditions destroyed the BF_2 chelate of the BODIPY fluorophore. We then reasoned that since the BODIPY fluorophore is formed by chelation in the presence of $BF_3{\cdot}OEt_2$, and that $BF_3{\cdot}OEt_2$ has been reported to deprotect a NHBoc group under quite mild conditions,[40] this Lewis acid might remove the NHBoc and ketal groups in one pot. Indeed, treatment of **21** with $BF_3{\cdot}OEt_2$ in the presence of 4 Å molecular sieves in dichloromethane at 0 °C gave BODIPY−FTY720 conjugate **3** in 61% yield (Scheme 6).

Photospectroscopic Properties. Spectroscopic data are presented in Table 1, and the emission spectra are shown in Figure 3. All compounds exhibited the characteristic absorption and emission patterns of the BODIPY fluorophore, with high extinction coefficients and small Stokes shifts. The absorption and emission maxima were red-shifted in chloroform (dielectric constant 5.5) compared with ethanol (dielectric constant 24.3), and the molar extinction coefficient (ϵ) was higher. Note that for compounds **1**, **3**, and **21**, which have a phenyl substituent at the 8-position of the BODIPY moiety, the absorption and emission wavelengths are very similar to those of the parent 1,3,5,7-tetramethyl-BODIPY chromophore. The phenyl group at the 8-position is almost orthogonal to the BODIPY plane[41] because of steric hindrance imposed by the methyl groups at the 1- and 7-positions and thus does not contribute to the conjugation of the BODIPY fluorophore. The introduction of extended conjugation at the 5-position of the BODIPY moiety, however, did cause a significant red shift (~70 nm) in the absorption and emission wavelengths compared with the cor-

SCHEME 6. Synthesis of BODIPY−FTY720 Conjugate 3

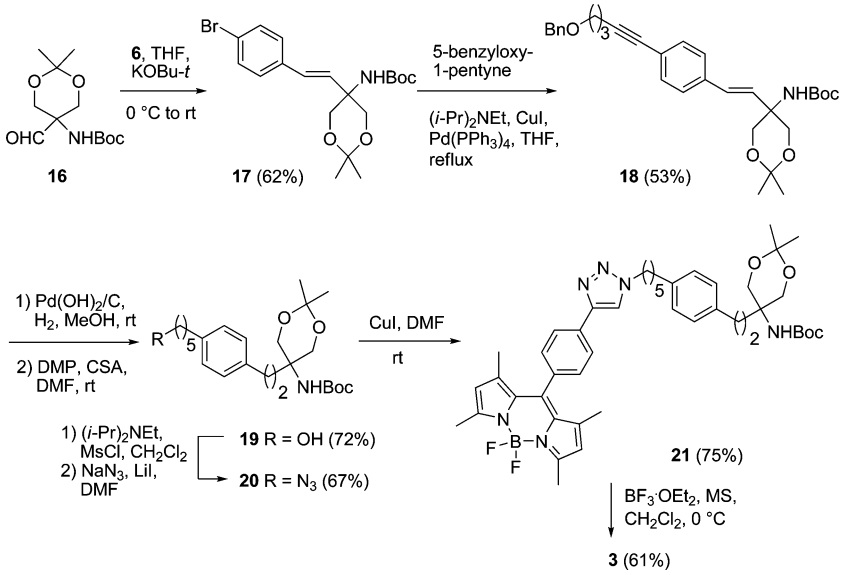


TABLE 1. Spectroscopic Data in Ethanol and Chloroform[a]

compd	λ_{max}[b] (abs), nm	ϵ, M^{-1} cm^{-1}	λ_{em},[c] nm
1	499	110 000	508
2	569 (578)	80 000 (89 000)	583 (591)
3	500 (502)	72 000 (85 000)	508 (512)
8	562	118 000	572
9	568 (577)	104 000 (117 000)	579 (585)
11	567 (572)	114 000 (119 000)	574 (580)
15	568	76 000	577
21	495 (502)	75 000 (85 000)	500 (515)

[a] Parentheses indicate data obtained with compounds dissolved in chloroform. [b] Absorption spectra were recorded at 6.25×10^{-6} M at 25 °C. [c] Emission spectra were recorded at 1.0×10^{-7} M.

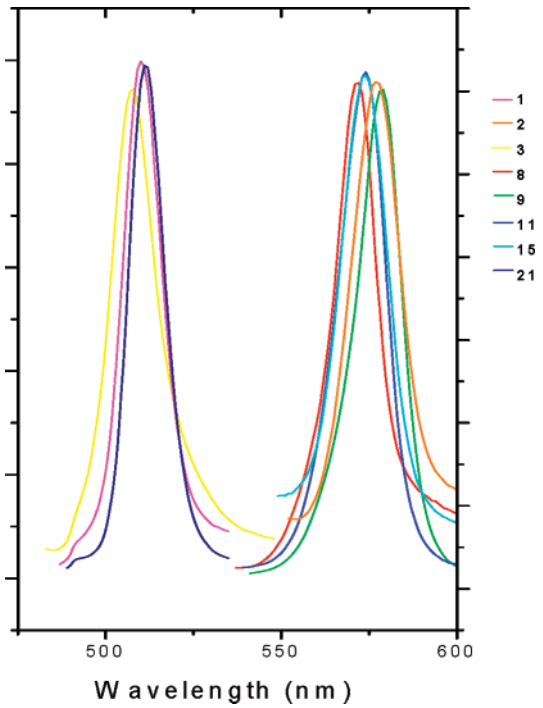


FIGURE 3. Emission spectra of compounds **1**−**3**, **8**, **9**, **11**, **15**, and **21** in EtOH. Spectra were recorded at 1.0×10^{-7} M at 25 °C; an excitation wavelength of 365 nm was used.

responding 5-methyl-substituted chromophore, in good agreement with previous reports.[31]

Conclusion

We have described the syntheses of two acetylenic-linked BODIPY fluorescent dyes (**5** and **11**) with different absorption and emission wavelengths. Lipids **13** and **20**, which bear terminal azido groups, were covalently derivatized with one of these dyes to prepare BODIPY-conjugated cholesterol analogues **1** and **2** and FTY720 analogue **3** by utilizing the click reaction. As these new analogues exhibited intense absorption and strong fluorescence, they can serve as probes for monitoring the effects of cholesterol and FTY720 in model membranes and cells and for donor−acceptor resonance energy transfer studies. Their excellent luminescent properties make it possible to visualize the distribution and transport of these lipids in living cells by fluorescence microscopy.

Experimental Section

4,4-Difluoro-8-(4-ethynylphenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (5). To a solution of 4-ethynylbenzaldehyde (0.13 g, 1.0 mmol, compound **4**) and 2,4-dimethylpyrrole (0.22 g, 2.3 mmol) in CH_2Cl_2 (50 mL) was added trifluoroacetic acid (7.6 μL, 0.1 mmol) under N_2. After the mixture was stirred at room temperature overnight, a solution of *p*-chloranil (0.246 g, 1.0 mmol) in CH_2Cl_2 (10 mL) was added. The mixture was stirred at room temperature for an additional 30 min. BF_3·OEt_2 (2.60 g, 18.6 mmol) and NEt_3 (1.7 g, 17.0 mmol) were added, followed by stirring at room temperature for 6 h. The reaction mixture was washed with water (4 × 60 mL) and dried (Na_2SO_4). The solvent was removed under vacuum and the residue was purified by chromatography (hexanes/EtOAc 20:1 to 5:1) to give compound **5** (98.8 mg, 28%). ^{1}H NMR δ 7.63 (d, 2H, J = 7.8 Hz), 7.27 (d, 2H, J = 7.8 Hz), 5.99 (s, 2H), 3.18 (s, 1H), 2.55 (s, 6H), 1.40 (s, 6H); ^{13}C NMR δ 155.8, 143.0, 140.6, 135.6, 132.9, 131.1, 128.2, 122.9, 121.4, 82.9, 78.6, 14.62, 14.58; ^{19}F NMR δ −146.2 (m). HRMS *m/z* calcd for $C_{21}H_{20}BF_2N_2$ (MH^+), 349.1682; found, 349.1689.

4,4-Difluoro-5-[(*E*)-(4-bromophenyl)ethenyl]-1,3-dimethyl-4-bora-3a,4a-diaza-*s*-indacene (8). A solution of 3,5-dimethylpyrrole-2-carboxaldehyde (0.12 g, 1.0 mmol) and **7**[35] (0.248 g, 1.0 mmol) in CH_2Cl_2 (15 mL) was cooled to 0 °C. After a solution of $POCl_3$ (0.15 g, 1.0 mmol) in CH_2Cl_2 (1 mL) was added with caution, the mixture was stirred at 0 °C for 1 h and then at room temperature overnight. BF_3·OEt_2 (0.52 mL, 4.0 mmol) and *N*,*N*-diisopropylethylamine (0.70 mL, 4.0 mmol) were added sequentially at 0 °C, and the resulting mixture was stirred at room temperature for 6 h. The solution was washed with water (3 × 20 mL), dried (Na_2SO_4), and concentrated under vacuum. Compound **8** (0.19 g, 47%) was obtained by flash chromatography (hexanes/EtOAc 50:1 to 5:1). ^{1}H NMR δ 7.60 (d, 1H, J = 16.0 Hz), 7.50−7.43 (m, 4H), 7.18 (d, 1H, J = 16.0 Hz), 7.06 (s, 1H), 6.94 (d, 1H, J = 4.4 Hz), 6.84 (d, 1H, J = 4.4 Hz), 6.13 (s, 1H), 2.60 (s, 3H), 2.26 (s, 3H); ^{13}C NMR δ 160.0, 152.7, 143.3, 135.5, 134.8, 133.9, 131.9, 128.7, 128.1, 122.7, 122.2, 120.4, 119.9, 115.2, 15.1, 11.4; ^{19}F NMR δ −143.0 (m). HRMS *m/z* calcd for $C_{19}H_{17}BBrF_2N_2$ (MH^+), 401.0631; found, 401.0632.

4,4-Difluoro-1,3-dimethyl-5-[(*E*)-(4-trimethylsilylethynylphenyl)ethenyl]-4-bora-3a,4a-diaza-*s*-indacene (9). Synthesis of compound **9** (294 mg, 52%) was accomplished via the procedure for compound **8**, with **10** (362 mg, 1.35 mmol) as the starting material. ^{1}H NMR δ 7.61 (d, 1H, J = 16.4 Hz), 7.50 (d, 2H, J = 8.4 Hz), 7.44 (d, 2H, J = 8.4 Hz), 7.22 (d, 1H, J = 16.4 Hz), 7.03 (s, 1H), 6.92 (d, 1H, J = 4.4 Hz), 6.83 (d, 1H, J = 4.4 Hz), 6.12 (s, 1H), 2.60 (s, 3H), 2.25 (s, 3H), 0.26 (s, 9H); ^{13}C NMR δ 159.7, 152.8, 143.1, 136.6, 135.5, 134.9, 134.4, 132.3, 128.1, 127.1, 123.2, 122.1, 120.3, 120.0, 115.3, 105.1, 95.9, 15.0, 11.3, −0.06; ^{19}F NMR δ −142.9 (m). HRMS *m/z* calcd for $C_{24}H_{26}BF_2N_2Si$ (MH^+), 419.1920; found, 419.1928.

2-[(*E*)-(4-Trimethylsilylethynylphenyl)ethenyl]pyrrole (10). To a solution of trimethylsilylacetylene (0.98 g, 10 mmol), **7** (0.50 g, 2.0 mmol), and *N*,*N*-diisopropylethylamine (4 mL, 22.9 mmol) in THF (12 mL) were added CuI (58 mg, 0.30 mmol) and tetrakis(triphenylphosphine)palladium (0.36 g, 0.3 mmol) under N_2 at room temperature. After the mixture was stirred at 60 °C overnight under N_2 and then cooled to room temperature, it was passed through a short pad of silica gel to remove insoluble salts or byproducts. The filtrate was concentrated under vacuum, and the crude product was purified by chromatography (hexanes/EtOAc 20:1 to 5:1) to give

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10 (0.30 g, 56%). ^{1}H NMR δ 8.28 (br s, 1H), 7.40 (d, 2H, J = 8.4 Hz), 7.30 (d, 2H, J = 8.4 Hz), 6.92 (d, 1H, J = 16.4 Hz), 6.76 (m, 1H), 6.55 (d, 1H, J = 16.4 Hz), 6.36 (m, 1H), 6.24 (m, 1H), 0.25 (s, 9H); ^{13}C NMR δ 137.3, 132.2, 130.5, 125.5, 122.4, 121.2, 119.8, 119.6, 110.1, 109.7, 105.3, 94.8, −0.02. HRMS *m/z* calcd for $C_{17}H_{20}NSi$ (MH^+), 266.1359; found, 266.1356.

4,4-Difluoro-1,3-dimethyl-5-[(*E*)-(4-ethynylphenyl)ethenyl]-4-bora-3a,4a-diaza-*s*-indacene (11). Compound **9** (42 mg, 0.10 mmol) was dissolved in a mixture of CH_2Cl_2 (5 mL) and MeOH (5 mL). After addition of K_2CO_3 (41 mg, 0.30 mmol), the mixture was stirred at room temperature under N_2 until the disappearance of **9** (∼4 h, monitored by TLC, hexanes/EtOAc 5:1). The solvent was removed under vacuum. The residue was distributed in CH_2Cl_2 (20 mL) and water (10 mL), and then neutralized with acetic acid. The organic layer was separated, washed with water (3 × 10 mL), dried (Na_2SO_4), and concentrated to give a blue crude product. Compound **11** (22.1 mg, 64%) was obtained by chromatographic purification with gradient elution (hexanes/EtOAc 20:1 to 5:1). ^{1}H NMR δ 7.63 (d, 1H, J = 16.4 Hz), 7.54 (d, 1H, J = 8.4 Hz), 7.48 (d, 1H, J = 8.4 Hz), 7.22 (d, 1H, J = 16.4 Hz), 7.06 (s, 1H), 6.94 (d, 1H, J = 4.0 Hz), 6.85 (d, 1H, J = 4.0 Hz), 6.13 (s, 1H), 3.17 (s, 1H), 2.61 (s, 3H), 2.26 (s, 3H); ^{13}C NMR δ 160.0, 152.6, 143.2, 137.0, 135.6, 134.9, 134.2, 132.5, 128.1, 127.1, 122.2, 122.1, 120.4, 120.3, 115.3, 83.7, 78.6, 15.1, 11.4; ^{19}F NMR −143.0 (m). HRMS *m/z* calcd for $C_{21}H_{18}BF_2N_2$ (MH^+), 347.1526; found, 347.1528.

22-Azido-3β-tetrahydropyranyloxy-23,24-bisnorchol-5-ene (13). A solution of alcohol **12** (2.2 g, 5.3 mmol) and NEt_3 (5.3 g, 53 mmol) in CH_2Cl_2 (150 mL) was cooled to 0 °C. Methanesulfonyl chloride (3.0 g, 26.4 mmol) was added dropwise. After the addition was complete, the mixture was stirred at 0 °C for 3 h and then at room temperature overnight. The solution was washed with water (3 × 100 mL), dried, and concentrated to give a viscous oil, which solidified after storage at low temperature. To the solution of the mesylate in THF (100 mL) was added lithium bromide (0.92 g, 10.6 mmol). The mixture was heated at reflux for 1 day and then concentrated to give a white solid, which was distributed in CH_2Cl_2 (100 mL) and water (100 mL). The organic layer was washed with water (2 × 100 mL), dried over Na_2SO_4, and concentrated to give the corresponding bromide (2.48 g, 98% from **12**). ^{1}H NMR δ 5.34 (m, 1H), 4.71 (m, 1H), 3.91 (m, 1H), 3.60−3.40 (m, 2H), 3.37−3.32 (m, 2H), 2.44−0.88 (m, 33H), 0.70 (s, 3H); ^{13}C NMR δ 140.9, 140.7, 121.3, 121.2, 96.8, 96.7, 75.79, 75.77, 62.7, 62.6, 56.2, 53.6, 49.91, 49.88, 43.4, 42.2, 40.1, 39.3, 38.6, 37.6, 37.3, 37.1, 36.61, 36.57, 31.8, 31.7, 31.2, 29.5, 27.8, 27.4, 25.4, 24.1, 20.9, 19.93, 19.88, 19.2, 18.6, 12.1.

The above bromide (0.30 g, 0.63 mmol), sodium azide (0.122 g, 1.88 mmol), and a catalytic amount of lithium iodide (10 mg, 75 μmol) were mixed in dry DMF (10 mL). The mixture was heated at 80 °C for 18 h and then cooled to room temperature. Water (30 mL) was added, and the precipitate was collected by filtration. The solid was washed with water (4 × 5 mL) and then dried to give **13** (0.235 g, 85%). ^{1}H NMR δ 5.34 (m, 1H), 4.72 (m, 1H), 3.92 (m, 1H), 3.57−3.45 (m, 2H), 3.37 (dd, 1H, J = 12.0, 3.2 Hz), 3.04 (dd, 1H, J = 12.0, 7.2 Hz), 2.38−0.88 (m, 33H), 0.69 (s, 3H); ^{13}C NMR δ 141.1, 140.9, 121.4, 97.0, 96.8, 76.0, 62.9, 58.0, 56.5, 53.2, 50.1, 42.5, 39.5, 38.7, 37.4, 37.2, 36.9, 36.8, 31.9, 31.3, 27.9, 25.5, 24.3, 21.0, 20.1, 19.4, 17.8, 11.9. HRMS *m/z* calcd for $C_{27}H_{44}NO_2$ ([M − N_2 + H]$^+$), 414.3366; found, 414.3354.

3β-Tetrahydropyranyloxy-22-{4-[4-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacen-8-yl)phenyl]-1,2,3-triazol-1-yl}-23,24-bisnorchol-5-ene (14). A mixture of **5** (16.8 mg, 48 μmol), **13** (21.3 mg, 48 μmol), and CuI (0.9 mg, 4.8 μmol) in DMSO (3 mL) was stirred at 80 °C until the disappearance of alkyne **5** (∼5 h). Water (10 mL) was added, and the mixture was extracted with Et_2O (4 × 20 mL). The ether layers were combined, washed with water (2 × 20 mL), and dried (Na_2SO_4). The solvent was removed under vacuum, and the residue was purified by chromatography (hexanes/EtOAc 10:1 to 2:1) to give **14** (22.4 mg, 59%). ^{1}H NMR δ 7.99 (d, 2H, J = 8.0 Hz), 7.81 (s, 1H), 7.35 (d, 2H, J = 8.0 Hz), 5.99 (s, 2H), 5.35 (m, 1H), 4.72 (m, 1H), 4.45 (dd, 1H, J = 13.6, 3.2 Hz), 4.14 (dd, 1H, J = 13.6, 9.2 Hz), 3.95−3.88 (m, 1H), 3.58−3.43 (m, 2H), 2.56 (s, 6H), 2.40−0.82 (m, 39H), 0.76 (s, 3H); ^{13}C NMR δ 155.6, 146.8, 143.1, 141.3, 141.1, 141.0, 134.7, 131.5, 131.4, 128.6, 126.3, 121.3, 120.5, 97.0, 96.9, 76.0, 62.9, 56.5, 56.3, 53.7, 50.1, 42.7, 39.6, 38.8, 38.1, 36.78, 36.75, 31.9, 31.3, 29.69, 29.65, 29.4, 28.2, 28.0, 25.5, 24.4, 22.7, 21.0, 20.1, 19.4, 17.2, 14.64, 14.59, 14.1, 12.0; ^{19}F NMR δ −146.2 (m). HRMS *m/z* calcd for $C_{48}H_{63}BF_2N_5O_2$ (MH^+), 790.5037; found, 790.5042.

22-{4-[4-(4,4-Difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacen-8-yl)phenyl]-1,2,3-triazol-1-yl}-23,24-bisnorchol-5-en-3β-ol (1). To a solution of **14** (25.0 mg, 31.7 μmol) in a mixture of CH_2Cl_2 (2 mL) and MeOH (6 mL) was added PPTS (2.0 mg, 8.0 μmol). After being stirred at room temperature overnight, the mixture was concentrated and redistributed between CH_2Cl_2 (20 mL) and water (10 mL). The organic layer was washed with water (2 × 10 mL) and dried (Na_2SO_4). The solvent was removed under vacuum, and the residue was purified by chromatography (hexanes/EtOAc 4:1 to 2:1) to give **14** (8.8 mg) and **1** (13.2 mg, 91% based on reacted **14**). ^{1}H NMR δ 7.99 (d, 2H, J = 8.4 Hz), 7.81 (s, 1H), 7.35 (d, 2H, J = 8.4 Hz), 5.99 (s, 2H), 5.36 (m, 1H), 4.45 (dd, 1H, J = 13.6, 4.0 Hz), 4.14 (dd, 1H, J = 13.6, 8.8 Hz), 3.58−3.48 (m, 1H), 2.56 (s, 6H), 2.48−0.82 (m, 33H), 0.76 (s, 3H); ^{13}C NMR δ 155.6, 146.8, 143.1, 141.3, 140.8, 134.7, 131.5, 131.4, 128.6, 126.3, 121.5, 121.3, 120.5, 71.7, 56.5, 56.2, 53.7, 50.0, 42.7, 42.2, 39.5, 38.0, 37.2, 36.5, 31.9, 31.8, 31.6, 29.7, 29.6, 29.3, 28.2, 24.4, 22.7, 21.0, 20.3, 19.4, 17.2, 14.62, 14.57, 14.1, 12.0; ^{19}F NMR δ −146.1 (m). HRMS *m/z* calcd for $C_{43}H_{55}BF_2N_5O$ (MH^+), 706.4462; found, 706.4464.

3β-Tetrahydropyranyloxy-22-{4-[4-((*E*)-(4,4-difluoro-1,3-dimethyl-4-bora-3a,4a-diaza-*s*-indacen-5-yl)ethenyl)phenyl]-1,2,3-triazol-1-yl}-23,24-bisnorchol-5-ene (15). Compound **15** (10.2 mg, 54%) was prepared from **11** (8.8 mg, 25.5 μmol), **13** (10.0 mg, 22.3 μmol), CuI (1.0 mg, 5.3 μmol), and DMF (3 mL) by a procedure similar to that used to prepare compound **14**. ^{1}H NMR δ 7.85 (d, 2H, J = 8.8 Hz), 7.76 (s, 1H), 7.68 (s, 1H), 7.65 (d, 2H, J = 8.8 Hz), 7.31 (s, 1H), 7.06 (s, 1H), 6.97 (d, 1H, J = 4.4 Hz), 6.88 (d, 1H, J = 4.4 Hz), 6.13 (s, 1H), 5.36 (t, 1H, J = 6.0 Hz), 4.74−4.70 (m, 1H), 4.43 (dd, 1H, J = 13.6, 4.0 Hz), 4.12 (dd, 1H, J = 13.6, 9.6 Hz), 3.95−3.88 (m, 1H), 3.57−3.44 (m, 2H), 2.62 (s, 3H), 2.27 (s, 3H), 2.39−0.80 (m, 33H), 0.75 (s, 3H); ^{13}C NMR δ 159.3, 153.3, 147.1, 142.7, 140.9, 136.2, 135.3, 135.1, 134.9, 131.0, 128.2, 127.9, 125.9, 122.0, 121.4, 120.3, 120.2, 119.2, 115.3, 97.0, 96.9, 75.9, 62.9, 56.4, 56.2, 53.7, 50.0, 42.7, 40.2, 39.5, 38.7, 38.0, 37.4, 36.75, 36.71, 31.8, 31.3, 29.68, 29.64, 28.2, 25.5, 24.4, 22.7, 21.0, 20.1, 20.0, 19.4, 17.1, 15.0, 14.1, 11.9, 11.4, 11.3; ^{19}F NMR δ −143.1 (m). HRMS *m/z* calcd for $C_{48}H_{61}BF_2N_5O_2$ (MH^+), 788.4881; found, 788.4888.

22-{4-[4-((*E*)-(4,4-Difluoro-1,3-dimethyl-4-bora-3a,4a-diaza-*s*-indacen-5-yl)ethenyl)phenyl]-1,2,3-triazol-1-yl}-23,24-bisnorchol-5-en-3β-ol (2). Compound **2** was synthesized in 97% yield via the procedure to prepare **1**. ^{1}H NMR δ 7.85 (d, 2H, J = 8.0 Hz), 7.75 (s, 1H), 7.68 (s, 1H), 7.65 (d, 2H, J = 8.0 Hz), 7.31 (s, 1H), 7.06 (s, 1H), 6.96 (d, 1H, J = 4.0 Hz), 6.88 (d, 1H, J = 4.0 Hz), 6.13 (s, 1H), 5.38−5.33 (m, 1H), 4.43 (dd, 1H, J = 13.6, 3.6 Hz), 4.12 (dd, 1H, J = 13.6, 8.8 Hz), 3.58−3.48 (m, 1H), 2.62 (s, 3H), 2.27 (s, 3H), 2.37−0.81 (m, 27H), 0.76 (s, 3H); ^{13}C NMR δ 159.3, 153.3, 147.1, 142.8, 140.7, 136.2, 135.3, 135.1, 134.9, 131.0, 128.3, 127.9, 125.9, 122.0, 121.5, 120.3, 120.2, 119.2, 115.3, 71.7, 56.4, 56.2, 53.7, 49.9, 42.7, 39.5, 38.0, 37.2, 36.4, 31.9, 31.6, 29.7, 24.4, 22.7, 21.0, 19.4, 17.1, 15.0, 14.1, 11.9, 11.3; ^{19}F NMR δ −143.1 (m). HRMS *m/z* calcd for $C_{43}H_{53}BF_2N_5O$ (MH^+), 704.4306; found, 704.4289.

5-*tert*-Butoxycarbonylamino-5-[(*E*)-(4-bromophenyl)ethenyl]-2,2-dimethyl-1,3-dioxane (17). To a solution of aldehyde **16** (0.82g, 3.14 mmol) and phosphonate **6** (1.45 g, 4.7 mmol) in dry THF (10 mL) was added potassium *tert*-butoxide (1.58 g, 14.1 mmol) slowly at 0 °C. After the mixture was stirred at 0 °C for 3

h and at room temperature overnight, ice−water (20 mL) was added and the suspension was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was washed with water (2 × 20 mL) and brine (2 × 20 mL), dried (Na_2SO_4), and concentrated under vacuum. The residue was purified by chromatography with gradient elution (hexanes/EtOAc 20:1 to 8:1) to give **17** (0.80 g, 62%). ^{1}H NMR δ 7.37 (d, 2H, J = 8.4 Hz), 7.19 (d, 2H, J = 8.4 Hz), 6.46 (d, 1H, J = 16.4 Hz), 6.21 (d, 1H, J = 16.4 Hz), 5.42 (s, 1H), 3.97 (d, 2H, J = 11.2 Hz), 3.88 (d, 2H, J = 11.2 Hz), 1.50−1.39 (m, 15H); ^{13}C NMR δ 154.5, 135.3, 131.2, 129.0, 128.7, 127.6, 121.1, 97.9, 79.1, 65.6, 52.7, 28.1, 27.0, 19.5. HRMS *m/z* calcd for $C_{19}H_{27}BrNO_4$ (MH^+), 412.1118; found, 412.1120.

5-*tert*-Butoxycarbonylamino-5-{(*E*)-[4-(5-benzyloxy-pent-1-ynyl)phenyl]ethenyl}-2,2-dimethyl-1,3-dioxane (18). To a solution of **17** (0.21 g, 0.51 mmol) and 5-benzyloxy-1-pentyne (133 mg, 0.77 mmol) in *N*,*N*-diisopropylethylamine (1 mL, 5.7 mmol) and dry THF (5 mL) were added CuI (9.7 mg, 51 μmol) and tetrakis-(triphenylphosphine)palladium(0) (29.5 mg, 25.5 μmol) under nitrogen. The mixture was heated at reflux under nitrogen for 1 day and then was cooled to room temperature and filtered through a short pad of silica gel to remove insoluble and very polar components in the mixture. The filtrate was concentrated and dissolved in CH_2Cl_2 (50 mL). The solution was washed with water (2 × 20 mL) and brine (2 × 20 mL) and dried over Na_2SO_4. The solvent was removed under vacuum, and the residue was purified by column chromatography (hexanes/EtOAc 20:1 to 6:1) to give **18** (0.14 g, 53%). ^{1}H NMR δ 7.43−7.19 (m, 4H), 6.49 (d, 1H, J = 16.4 Hz), 6.21 (d, 1H, J = 16.4 Hz), 5.43 (s, 1H), 4.50 (s, 2H), 3.98 (d, 2H, J = 11.2 Hz), 3.87 (d, 2H, J = 11.2 Hz), 3.59 (t, 2H, J = 6.0 Hz), 2.53 (t, 2H, J = 6.8 Hz), 1.89 (m, 2H), 1.45 (s, 15H); ^{13}C NMR δ 154.4, 138.1, 135.5, 131.3, 129.3, 127.9, 127.2, 127.1, 125.9, 122.8, 97.8, 90.1, 80.6, 79.0, 72.5, 68.3, 65.6, 52.7, 28.5, 28.0, 27.1, 19.3, 15.9. HRMS *m/z* calcd for $C_{31}H_{39}NNaO_5$ (MNa^+), 528.2720; found, 528.2727.

5-*tert*-Butoxycarbonylamino-5-{2-[4-(5-hydroxyl-1-pentyl)-phenyl]ethyl}-2,2 -dimethyl-1,3-dioxane (19). Palladium hydroxide on carbon (20%) (36 mg, 0.05 mmol) was added to a solution of **18** (0.361 g, 0.71 mmol) in MeOH (8 mL). Hydrogen was bubbled through the suspension with stirring until **18** was completely consumed and converted to a polar substance (monitored by TLC, hexanes/EtOAc 2:1). The mixture was filtered through a short pad of silica gel and concentrated under vacuum. To a solution of the resulting yellow oil in dry DMF (5 mL) were added 2,2-dimethoxypropane (90.5 mg, 0.84 mmol) and camphorsulfonic acid (10 mg, 0.043 mmol). After the mixture was stirred at room temperature for 1 day, it was diluted with saturated aqueous $NaHCO_3$ solution (15 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layer was washed sequentially with saturated aqueous $NaHCO_3$ solution (2 × 20 mL), water (2 × 20 mL), and brine (20 mL) and was then dried (Na_2SO_4). The solvent was removed under vacuum, and the residue was purified by chromatography (hexanes/EtOAc 8:1 to 2:1) to give **19** (217 mg, 72%). ^{1}H NMR δ 7.15−7.05 (m, 4H), 5.09 (s, 1H), 3.89 (d, 2H, J = 11.6 Hz), 3.67 (d, 2H, J = 11.6 Hz), 3.60 (t, 2H, J = 6.8 Hz), 2.60−2.50 (m, 4H), 2.40 (br s, 1H), 1.96 (t, 2H, J = 8.0 Hz), 1.65−1.36 (m, 21H); ^{13}C NMR δ 154.7, 139.9, 139.0, 128.2, 128.0, 98.2, 79.1, 66.1, 62.4, 51.5, 35.5, 33.5, 32.4, 31.2, 28.4, 28.2, 27.2, 25.3, 19.7. HRMS *m/z* calcd for $C_{24}H_{40}NO_5$ (MH^+), 422.2901; found, 422.2904.

5-*tert*-Butoxycarbonylamino-5-{2-[4-(5-azido-1-pentyl)phenyl]-ethyl}-2,2-dimethyl-1,3-dioxane (20). A solution of **19** (168 mg, 0.40 mmol) and *N*,*N*-diisopropylethylamine (155 mg, 1.2 mmol) in CH_2Cl_2 (10 mL) was cooled to 0 °C. Methanesulfonyl chloride (66 mg, 0.60 mmol) was added, and the mixture was stirred at 0 °C for 2 h and then at room temperature overnight. The mixture was washed with saturated aqueous $NaHCO_3$ solution (2 × 10 mL), water (2 × 10 mL), and brine (2 × 10 mL) and then dried (Na_2SO_4). After the solvent was removed, the residue was dissolved in dry DMF (5 mL), and lithium iodide (7 mg, 48 μmol) and sodium azide (78 mg, 1.2 mmol) were added. The reaction mixture was stirred at 80 °C for 18 h and then was cooled to room temperature. Water (15 mL) was added, and the suspension was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layer was washed with water (3 × 30 mL) and brine (2 × 20 mL), dried (Na_2SO_4), and concentrated under vacuum. The residue was purified by chromatography (hexanes/EtOAc 4:1) to give **20** (119 mg, 67%). ^{1}H NMR δ 7.14−7.04 (m, 4H), 5.02 (s, 1H), 3.89 (d, 2H, J = 11.6 Hz), 3.67 (d, 2H, J = 11.6 Hz), 3.24 (t, 2H, J = 7.2 Hz), 2.60−2.50 (m, 4H), 1.97 (t, 2H, J = 8.0 Hz), 1.66−1.56 (m, 4H), 1.47 (s, 9H), 1.43 (s, 3H), 1.41 (s, 3H), 1.40−1.36 (m, 2H); ^{13}C NMR δ 154.7, 139.7, 139.2, 128.3, 128.2, 98.2, 79.1, 66.2, 51.6, 51.2, 35.2, 33.5, 30.9, 28.6, 28.5, 28.3, 27.3, 26.2, 19.6. HRMS *m/z* calcd for $C_{24}H_{38}N_4NaO_4$ (MNa^+), 469.2785; found, 469.2786.

5-*tert*-Butoxycarbonylamino-5-{2-[4-(5-(4-(4-(4,4-difluoro-1,3,5,7-tetramethyl-3a,4a-diaza-*s*-indacen-8-yl)phenyl)-1,2,3-triazol-1-yl)pentyl)phenyl]ethyl}-2, 2-dimethyl-1,3-dioxane (21). To a solution of **20** (22.3 mg, 50 μmol) and **5** (17.4 mg, 50 μmol) in dry DMF (1 mL) was added CuI (1 mg, 5 μmol). The mixture was stirred at room temperature until the starting materials are completely consumed (monitored by TLC, hexanes/EtOAc 2:1). Water (5 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was washed with water (2 × 20 mL) and brine (2 × 20 mL) and dried over Na_2SO_4. After the solvent was removed under vacuum, the residue was purified by chromatography (hexanes/EtOAc 4:1 to 2:1) to afford **21** (29.8 mg, 75%). ^{1}H NMR δ 7.99 (d, 2H, J = 8.0 Hz), 7.84 (s, 1H), 7.35 (d, 2H, J = 8.0 Hz), 7.13−7.04 (m, 4H), 5.99 (s, 2H), 5.00 (br s, 1H), 4.42 (t, 2H, J = 6.8 Hz), 3.90 (d, 2H, J = 11.6 Hz), 3.68 (d, 2H, J = 11.6 Hz), 2.56 (s, 6H), 2.63−2.49 (m, 4H), 2.03−1.93 (m, 4H), 1.71−1.63 (m, 2H), 1.47 (s, 9H), 1.44 (s, 6H), 1.43 (s, 3H), 1.42 (s, 3H); ^{13}C NMR δ 155.5, 154.8, 146.9, 143.0, 141.2, 139.5, 139.4, 134.6, 131.4, 131.3, 128.5, 128.4, 128.3, 126.2, 121.2, 119.8, 98.3, 66.3, 51.6, 50.4, 35.1, 33.6, 30.8, 30.3, 28.6, 28.4, 27.4, 26.1, 19.6, 14.6; ^{19}F NMR δ −146.1 (m). HRMS *m/z* calcd for $C_{45}H_{57}BF_2N_6NaO_4$ (MNa^+), 817.4395; found, 817.4401.

2-Amino-2-{2-[4-(5-(4-(4-(4,4-difluoro-1,3,5,7-tetramethyl-3a,-4a-diaza-*s*-indacen-8-yl)phenyl)-1,2,3-triazol-1-yl)pentyl)phenyl]-ethyl}propane-1,3-diol (3). To a cold solution (0 °C) of **21** (40 mg, 50.3 μmol) in dry CH_2Cl_2 (10 mL) were added 4 Å molecular sieves (0.40 g) and BF_3·OEt_2 (0.18 g, 1.27 mmol) with vigorous stirring. The mixture was stirred at 0 °C, and the reaction was monitored by TLC (hexanes/EtOAc 2:1). Upon the disappearance of **21** (∼5 h), the reaction was quenched by adding saturated aqueous $NaHCO_3$ solution (10 mL). After the mixture was stirred for 30 min, CH_2Cl_2 (30 mL) was added. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ solution (20 mL), water (2 × 20 mL), and brine (2 × 20 mL). The solution was dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by chromatography (CH_2Cl_2/MeOH 9:1) to give **3** (20.1 mg, 61%). ^{1}H NMR δ 7.96 (d, 2H, J = 8.4 Hz), 7.84 (s, 1H), 7.34 (d, 2H, J = 8.4 Hz), 7.11−7.01 (m, 4H), 5.98 (s, 2H), 4.39 (t, 2H, J = 7.2 Hz), 3.85−3.57 (m, 4H), 3.56−3.01 (br s, 4H), 2.61−2.51 (m, 4H), 2.55 (s, 6H), 2.00−1.92 (m, 2H), 1.70−1.56 (m, 2H), 1.43 (s, 6H), 1.46−1.05 (m, 4H); ^{13}C NMR δ 155.6, 147.0, 143.0, 141.2, 139.8, 138.6, 134.7, 131.4, 128.6, 128.5, 128.3, 126.3, 122.5, 121.3, 119.9, 50.4, 35.1, 30.8, 30.3, 29.7, 29.4, 26.0, 14.6, 14.1; ^{19}F NMR δ −146.1 (m). HRMS *m/z* calcd for $C_{37}H_{46}BF_2N_6O_2$ (MH^+), 655.3737; found, 655.3746.

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Supporting Information Available: Copies of ^{1}H, ^{13}C, and ^{19}F NMR spectra for all new compounds described herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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