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Synthesis of Fluorescent Ring-Fused 2-Pyridone Peptidomimetics

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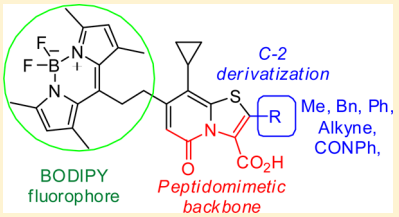
Supporting Information

ABSTRACT: Thiazolino fused 2-pyridone peptidomimetics are of significant biological importance due to their ability to interfere with adhesive fiber formation in uropathogenic *Escherichia coli* and oligomerization of amyloid fibers. We have developed an efficient synthetic route to fluorescent BODIPY analogues, with structural diversification from a key intermediate enabling introduction of C-2 substituents and late incorporation of the BODIPY moiety. A mild lithium halide mediated hydrolysis enabled preparation of peptidomimetic fluorophores with useful photophysical properties for further chemical biology applications.



Antibiotics represent one of the greatest innovations in medicine, but the emergence of widespread resistance has precipitated an urgent need for new therapeutic strategies. Peptidomimetic ring-fused 2-pyridones represent a promising class of compounds that can disrupt virulence in uropathogenic *Escherichia coli* (UPEC), a causative agent of urinary tract infection (UTI).[1] In their colonization of the urinary tract, a variety of extracellular fibers are utilized by UPEC in order to adhere to host cells.[2] The formation of one of the primary types of these adhesive fibers, type-1 pili, is disrupted by ring-fused 2-pyridones termed pilicides (**1**−**4**, Figure 1).[3]

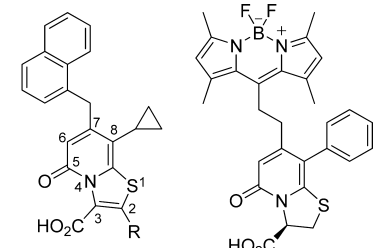


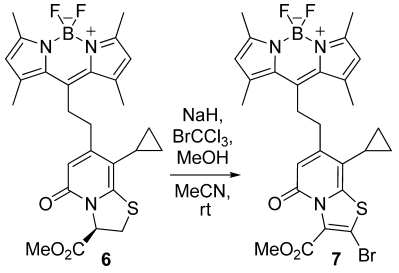
Figure 1. Representative peptidomimetic thiazolo 2-pyridones **1**−**4** and fluorescent analogue **5**. The IC_{50} refers to pili-dependent biofilm formation in the clinical UPEC isolate UTI89, while Φ_F refers to the quantum yields.[4,5]

Introducing bulkier substituents in the C-8 position produces analogues which inhibit formation of another type of fiber in UPEC, curli.[6] These functional amyloid fibers are employed by UPEC in forming biofilms, which are implicated in the pathogenesis of recurrent and serious UTIs.[6] Significantly, thiazolino 2-pyridone analogues also interfere with the oligomerization of neurodegenerative associated amyloid fibers.[7,8] These peptidomimetics are therefore of significant interest as chemical biology tools and for potential therapeutic applications.

Fluorescent probes are essential tools in gaining insight into cellular and bacterial environments,[9,10] and previously we developed dihydrothiazolo-fused 2-pyridones containing BODIPY fluorophores in the C-7 or C-8 position (e.g., **5**, Figure 1).[5] Initial evaluations revealed **5** possessed useful photophysical properties and promising efficacy in halting biofilm formation in the clinical UPEC strain UTI89.[5] We were interested in developing further BODIPY analogues that incorporated C-2 substituents, as these are known to improve activity against pili-dependent biofilm formation (**1**−**4**, Figure 1).[4] In this paper, we present an expedient synthesis to BODIPY containing ring-fused 2-pyridones, which allows introduction of diverse C-2 substituents through a key intermediate, and late introduction of the sensitive BODIPY moiety, thereby greatly increasing the overall efficiency.

Initially, we attempted to prepare the C-2 substituted BODIPY analogues from the previously synthesized methyl ester **6**,[5] using a one-pot procedure developed for this scaffold (Scheme 1).[11]

Scheme 1. Oxidation/Bromination of BODIPY Derivative 6



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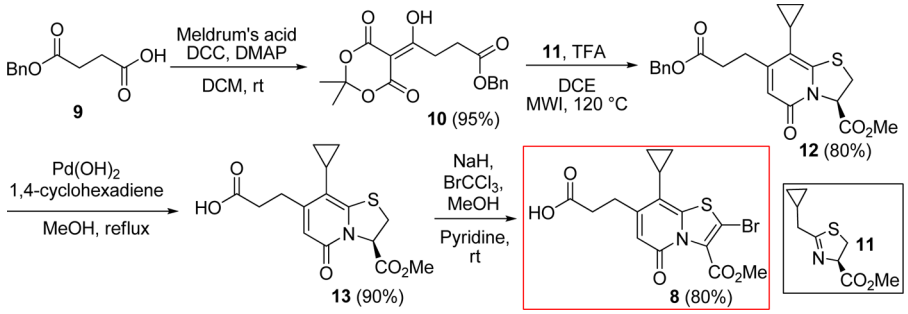
Scheme 2. Synthesis of Key 2-Bromo Substituted Intermediate 8

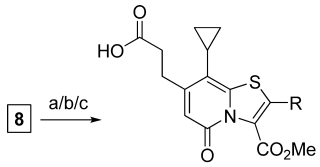


However, we were unable to obtain **7** in yields >20% due to the sensitivity of BODIPY to the basic conditions. This inherent vulnerability of BODIPY to both basic and acidic reaction conditions is well documented.[12−14] In addition to providing a substantial impediment to the scope and success of subsequent transformations, introducing the fluorophore at the outset of the synthesis was inefficient due to the low yields in generating **6** (11% over three steps from succinic anhydride).[5] We therefore envisaged preparing the C-2 substituted thiazolo 2-pyridones from a key intermediate containing a carboxylic acid substituent in the R-7 position to permit installation of the BODIPY moiety at a later stage, and a bromine to enable functionalization of the C-2 position (e.g., **8**, Scheme 2).

To access **8**, we prepared the acylated Meldrum's acid derivative **10** (Meldrum's acid, 2,2-dimethyl-1,3-dioxane-4,6-dione) from monobenzylated succinic ester **9**. Subsequent cyclocondensation of **10** with thiazoline **11** furnished the dihydrothiazolino 2-pyridone **12** in 80% yield.[15] While classical hydrogenation conditions to deprotect benzyl ester **12** proved sluggish, with only 50% conversion after 24 h with Pd/C or $Pd(OH)_2/C$ and 1 atm of hydrogen at rt in methanol, transfer hydrogenation with 1,4-cyclohexadiene and $Pd(OH)_2/C$ was more effective and provided the carboxylic acid **13** in 90% yield. We next attempted the one-pot oxidation/bromination of **13** using the previously developed conditions (3 equiv of NaH, 3 equiv of CCl_3Br, and 2 equiv of MeOH in CH_3CN),[11] but the poor solubility of **13** in acetonitrile prohibited success. Switching to THF or CH_2Cl_2/MeOH mixtures afforded no improvement as we noted hydrolysis of the methyl ester, while in DMF formylation occurred at C-6, in addition to the desired reaction. However, using pyridine as the reaction solvent overcame these issues and the key 2-bromothiazole intermediate **8** was obtained in 80% yield.

The structural diversification of **8** was then investigated, first by Suzuki−Miyaura cross-couplings. Applying the method utilized in the derivatization of **4** (benzylboronic acid pinacol ester, $Pd(OAc)_2$, KF, MeOH and microwave irradiation (MWI) at 100 °C, 10 min) afforded only limited conversion, so a catalyst screen was undertaken.[4] With the same procedure, no conversion was observed by LC-MS using $Pd(PPh_3)_4$, while [1,3-Bis(2,6-diisopropylphenyl)imidazol-2-ylidene](3-chloropyridyl)palladium(II) dichloride and 1,3-Bis(2,6-diisopropylphenyl)imidazolidene)(3-chloropyridyl)palladium(II) dichloride catalysts gave only dehalogenated **8**. Improved conversion was afforded by $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, albeit with a low isolated yield of 44% and significant dehalogenation. However, upon switching from KF to K_2CO_3 a dramatic increase in yield was obtained, with **14a** isolated in 81% yield with $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ and MWI for 12 min at 80 °C (Scheme 3).

Scheme 3. Scaffold Derivatization from 2-Bromo Substituted 8[a]



[a]Reagents and conditions: **14a−c**: (a) $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (10 mol %), $RB(OH)_2$/pinacol ester, K_2CO_3, MeOH, MWI, 80 °C, 12 min. **15**: (b) Phenylacetylene, $Pd(Ph_3P)_2Cl_2$ (5 mol %), CuI (10 mol %), K_2CO_3, MWI, 80 °C, 10 min, 80%. **16**: (c) (i) *n*-BuLi, −78 °C, 10 min; (ii) PhNCO, −70 °C, 15 min, 64%.

These conditions were also successful with phenyl and methyl boronic acids, with the respective cross-coupled products **14b** and **14c** each obtained in 79% yield. We next investigated the Sonogashira coupling of **8** with phenylacetylene: in analogy with the Suzuki coupling, our previously developed method was not successful ($Pd(Ph_3P)_2Cl_2$, CuI, and NEt_3 in DMF with MWI at 110 °C).[16] However on exchanging the base for K_2CO_3, the acetylene substituted **15** was obtained in 80% yield. Lithiation of the C-2 bromine was attempted next, with 2.0 equiv of *n*-BuLi at −78 °C followed by addition of phenyl isocyanate. Pleasingly, this gave phenylamide **16** featuring an extended peptidomimetic backbone in 64% yield. We noted that the reaction with the electrophile did not proceed unless a slight warming to −70 °C was allowed.

The 2-bromothiazole **8** proved to be an effective intermediate for structural elaboration, and we proceeded to introduce the BODIPY fluorophore, by conversion to the acyl chloride, condensation with pyrrole, and treatment of the dipyrromethene intermediate with $BF_3{\cdot}Et_2O$ and NEt_3.[17]

These conditions proved effective for all substrates examined, providing the BODIPY methyl esters **6** and **17a−17c** in good yields for this reaction; formation of the BODIPY moiety is known to be challenging, and these yields represent a >2-fold improvement over comparable BODIPY forming steps in our previous route[5] and compare favorably with the synthesis of other BODIPY derivatized small molecules.[18,19] We noted the C-3 stereocenter of dihydrothiazolo analogue ***rac*-6** was epimerized during BODIPY formation; however, both enantiomers in similar dihydrothiazolo analogues display comparable efficacy in pili-dependent UPEC biofilm assays.[20]

The carboxylic acid is an essential feature of biological activity in pilicides affecting UPEC virulence,[20] and the

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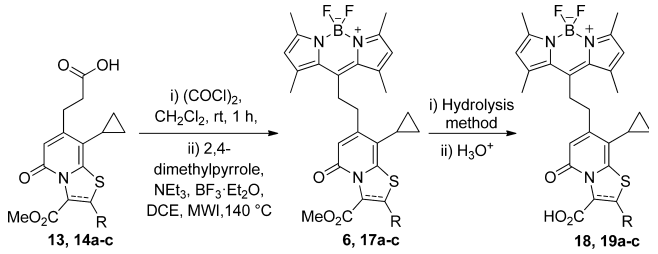
Table 1. Synthesis of New BODIPY Derivatives and Photophysical Characterization



substrate	bond C2−C3	R	BODIPY product (yield, %)	hydrolysis method	hydrolysis product (yield, %)	photophysical properties[a] λ_{abs} (nm)	λ_{fl} (nm)	τ (ns)	Φ_F (%)
13	single	H	***rac*-6** (41%)	LiOH, THF/MeOH, rt, 2 h	***rac*-18** (84%)	499	508	1.7	18
14a	double	Bn	**17a** (33%)	LiBr, DMF, MWI, 90 °C, 70 min	**19a** (65%)	500	509	3.8	2
14b	double	Ph	**17b** (39%)	LiI, DMF, MWI, 80 °C, 60 min	**19b** (68%)	500	509	3.4	4
14c	double	Me	**17c** (38%)	LiBr, DMF, MWI, 100 °C, 30 min	**19c** (55%)	499	508	3.7	3

[a]Measured in DMSO at concentrations of approximately 10^{-5} mol/L at 25 °C.

subsequent hydrolysis of the dihydrothiazolo ***rac*-6** proceeded smoothly using aqueous LiOH to afford carboxylate ***rac*-18** in 81% yield.[5] However, hydrolysis of the thiazolo BODIPY methyl esters **17a−c** proved challenging, and aqueous LiOH was ineffective. We consequently examined a range of hydrolysis conditions with **17a** as the substrate (Supporting Information, Supplementary Table 1) and identified lithium bromide halogenolysis as a mild and efficient method to achieve this transformation: with LiBr (50 equiv) in DMF and MWI at 90 °C for 70 min, the desired carboxylic acid **19a** was obtained in 65% yield. This method also proved effective for 2-methyl substituted **17c**, but less efficient for the 2-phenyl substituted **17b**, with only a 23% yield of the corresponding carboxylic acid **19b** obtained. Employing LiI as the halide source resulted in an increased yield of 68%. The LiI mediated cleavage of methyl esters in DMF has classically been applied to terpenoid derivatives, and to our knowledge this is the first time it has been utilized for BODIPY methyl esters.[21,22] Our initial evaluations with a range of BODIPY derivatives suggest it may be more generally applicable as a mild method for ester removal (Supporting Information, Supplementary Table 2).

Evaluation of the photophysical properties of the new derivatives revealed that the dihydrothiazolo analogue ***rac*-18** possessed the highest quantum yield (Φ_F = 18%, Table 1), with much lower values recorded for the thiazolo derivatives **19a−c**. All the compounds evaluated exhibited biphasic fluorescence relaxation (exemplified by Figure S1, Supporting Information), with average lifetimes ranging between 1.7 and 3.8 ns. If one assumes that the shortened lifetimes are due to dynamic quenching, and one uses the known radiative lifetime of BODIPY,[23] the expected quantum yields would range between 30 and 70%. However, the experimental quantum yields were considerably lower. Since the shapes of the recorded BODIPY absorption and fluorescence spectra were very similar to those of free BODIPY, it is unlikely that static quenching caused by self-aggregation of the studied BODIPY derivatives provided the principle relaxation pathway.[24] Instead, static *intramolecular* quenching was probably the dominating relaxation path. A similar pattern was observed with other solvents (CH_3Cl and 1,2-propanediol), which supports the probability of *intramolecular* quenching processes. We previously attributed the influence of the thiazolino sulfur to the low observed quantum yields, since replacement with oxygen or a sulfoxide gave BODIPY analogues with greatly improved quantum yields.[5] Oxidation to the thiazole and substitution in C-2 does not appear to negate this effect or provide photophysical improvements to the observed quantum yields. However, the quantum yield of ***rac*-18** improves considerably on the most interesting fluorescent analogues from our previous study and indicates the C-8 cyclopropyl may be beneficial to the photophysical properties over phenyl in this position (**5**, Figure 1).

In summary, we have developed an expedient synthetic route to synthesize highly substituted fluorescent dihydrothiazolo/thiazolo 2-pyridone peptidomimetics. Comparison with our previous route for preparation of dihydrothiazolo **6** confirms the improved efficacy of the new route (28% vs 11%; Supporting Information, Schemes S1 and S2). Elaboration from a key intermediate enabled transition metal catalyzed C−C bond formation in the C-2 position with sp, sp^2, and sp^3 coupling partners, while lithium−halogen exchange made introduction of a new C−N bond possible to extend the peptidomimetic backbone and develop important tools for further applications.

EXPERIMENTAL SECTION

General. All reagents and solvents were used as received from commercial suppliers, unless indicated otherwise. CH_2Cl_2 and THF were dried in a solvent drying system (drying agent: neutral alumina) and collected fresh prior to every reaction. Pyridine was dried over 4 Å molecular sieves. NaH was prewashed with pentane, dried under vacuum, and stored in a desiccator. Microwave reactions were performed using a Biotage Initiator microwave synthesizer in sealed vessels; temperatures were monitored by an internal IR probe. TLC was performed on aluminum backed silica gel plates (median pore size 60 Å, fluorescent indicator 254 nm) and detected with UV light at 254 nM. Column chromatography was performed using silica gel with an average particle diameter 50 μm (range 40−65 μm, pore diameter 53 Å), and eluents are given in brackets. Optical rotation was measured with a polarimeter at 25 °C at 589 nm. IR spectra were recorded on a spectrometer equipped with an ATR device. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz spectrometer at 298 K and calibrated by using the residual peak of the solvent as the internal standard ($CDCl_3$: δ_H = 7.26 ppm; δ_C = 77.16 ppm. DMSO-d_6: δ_H = 2.50 ppm; δ_C = 39.50 ppm). HRMS was performed by using a mass spectrometer with ESI-TOF (ES+); sodium formate was used as the calibration chemical. Compounds **9** and **11** were synthesized according to literature procedures.[25,26]

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4-(2,2-Dimethyl-4,6-dioxo-[1,3]dioxan-5-ylidene)-4-hydroxy-butyric Acid Benzyl Ester 10. Succinic acid monobenzyl ester (5 g, 24 mmol), 2,2-dimethyl-1,3-dioxane-4,6-dione (3.63 g, 25.2 mmol), and DMAP (4.7 g, 38.4 mmol) were dissolved in CH_2Cl_2 (125 mL), and the mixture was cooled to 0 °C. DCC (6.4 g, 31.2 mmol) was dissolved in CH_2Cl_2 (15 mL) and added dropwise to the solution. The reaction mixture was then left at rt overnight. The reaction was quenched with 6% aq. $KHSO_4$, and the resulting precipitate was filtered off. The filtrate was washed with 6% aq. $KHSO_4$ (3 × 80 mL) and brine (80 mL), dried over Na_2SO_4, filtered, and concentrated giving **9** (7.65 g, 95%) as a greenish viscous liquid and was used without further purification; R_f = 0.34 (CH_2Cl_2–MeOH, 97/3); IR (υ cm^{-1}) (neat) 3320, 2927, 2850, 1731, 1656, 1571, 1382, 1269, 1155; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 15.59 (br s, 1H), 7.36–7.32 (m, 5H), 5.13 (s, 2H), 3.46 (t, 2H, J = 6.6 Hz), 2.79 (t, 2H, J = 6.6 Hz), 1.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 195.5, 171.6, 170.7, 160.3, 135.6, 128.6 (2C), 128.4, 128.3 (2C), 105.2, 91.7, 66.8, 30.9, 28.8, 26.8 (2C). HRMS (EI): m/z: calcd for $C_{17}H_{18}O_7$: 357.0950 [M+Na]; found: 357.0961.

7-(2-Benzyloxycarbonylethyl)-8-cyclopropyl-5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Methyl Ester 12. **10** (1.6 g, 8.02 mmol) and **11** (6.1 g, 18.4 mmol) were dissolved in 1,2-dichloroethane (32 mL), and TFA (0.61 mL, 8.02 mmol) was added; the reaction mixture was then heated in a microwave oven at 120 °C for 140 s (the reaction was carried out in four different batches). The reaction mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ (aq), and the organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude material on purification by column chromatography (heptane/EtOAc from 4/1 to 1/4) afforded **12** as a brown oil (2.65 g, 80%); R_f = 0.52 (EtOAc); $[\alpha]_D^{20}$ = −197.8 (c 0.7, CH_2Cl_2); IR (υ cm^{-1}) (neat) 2954, 1736, 1655, 1578, 1490, 1354, 1210, 1172; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.34–7.27 (m, 5H), 6.05 (s, 1H), 5.51 (dd, 1H, J = 2.4, 8.4 Hz), 5.08 (s, 2H), 3.73 (s, 3H), 3.58 (dd, 1H, J = 8.4, 11.6 Hz), 3.42 (dd, 1H, J = 2.4, 11.6 Hz), 3.05–2.89 (m, 2H), 2.66–2.61 (m, 2H), 1.55–1.47 (m, 1H), 0.92–0.81 (m, 2H), 0.60–0.53 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 172.2, 168.7, 161.2, 156.3, 147.5, 135.8, 128.6 (2C), 128.3, 128.2 (2C), 113.4 (2C), 66.6, 62.8, 53.2, 32.9, 31.6, 27.6, 10.9, 7.7, 7.5; HRMS (EI): m/z: calcd for $C_{22}H_{23}NO_5S$: 436.1195 [M+Na]; found: 436.1204.

7-(2-Carboxy-ethyl)-8-cyclopropyl-5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Methyl Ester 13. To **12** (748 mg, 1.81 mmol) in 15 mL of methanol were added $Pd(OH)_2$/C (127 mg, 0.18 mmol) and 1,4-cyclohexadiene (2.57 mL, 27.2 mmol) while stirring. The mixture was refluxed at 65 °C for 12 h and then filtered on Celite. Purification of the crude material by column chromatography (CH_2Cl_2–MeOH 97/3 and CH_2Cl_2–MeOH–AcOH 95/5/1, from 1/0 to 0/1) afforded **13** as a white solid (525 mg, 90%); R_f = 0.38 (CH_2Cl_2–MeOH–AcOH, 95/5/1); $[\alpha]_D^{20}$ = −205 (c 0.5, CH_2Cl_2–MeOH 7:3); IR (υ cm^{-1}) (neat) 2962, 1753, 1714, 1631, 1553, 1495, 1345, 1285, 1207, 1190; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 6.11 (s, 1H), 5.56 (dd, 1H, J = 2.0, 8.8 Hz), 3.77 (s, 3H), 3.74 (dd, 1H, J = 8.8, 12.0 Hz), 3.52 (dd, 1H, J = 2.0, 11.8 Hz), 3.09–2.93 (m, 2H), 2.63–2.59 (m, 2H), 1.65–1.58 (m, 1H), 1.02–0.88 (m, 2H), 0.67–0.58 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 174.7, 168.6, 162.1, 158.1, 148.7, 114.9, 112.5, 63.1, 52.8, 32.7, 31.2, 27.6, 10.7, 7.4, 7.2; HRMS (EI): m/z: calcd for $C_{15}H_{17}NO_5S$: 346.0725 [M+Na]; found: 346.0750.

2-Bromo-7-(2-carboxy-ethyl)-8-cyclopropyl-5-oxo-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Methyl Ester 8. NaH (229 mg, 9.56 mmol, washed with *n*-pentane) was added to a stirred solution of **13** (773 mg, 2.39 mmol) dissolved in 18 mL of dry pyridine at 0 °C. After 10 min, $BrCCl_3$ (0.71 mL, 7.17 mmol) was added and the mixture was allowed to reach rt and stirred for an additional 10 min, followed by the addition of dry methanol (0.15 mL, 3.60 mmol). After 4 h of stirring at rt, the reaction was quenched by dropwise addition of 6% aq. $KHSO_4$. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate and acidified. The organic layer was washed with 6% aq. $KHSO_4$ (3×) and brine, dried over Na_2SO_4, filtered, and concentrated. Purification by column chromatography (CH_2Cl_2–MeOH 97/3 and CH_2Cl_2–MeOH–AcOH 95/5/1, from 1/0 to 0/1) gave **8** as a pale yellow solid (768 mg, 80%); R_f = 0.44 (CH_2Cl_2–MeOH–AcOH, 95/5/1); IR (υ cm^{-1}) (neat) 2949, 1747, 1714, 1629, 1541, 1474, 1432, 1330, 1247, 1168; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 6.27 (s, 1H), 3.94 (s, 3H), 3.14 (t, 2H, J = 7.6 Hz), 2.70 (t, 2H, J = 7.6 Hz), 1.89–1.82 (m, 1H), 1.14–1.08 (m, 2H), 0.78–0.69 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 175.9, 161.3, 159.8, 156.7, 148.8, 131.6, 115.1, 110.7, 106.0, 53.9, 33.9, 28.8, 11.6, 8.4 (2C); HRMS (EI): m/z: calcd for $C_{15}H_{14}BrNO_5S$: 421.9674 [M+Na]; found: 421.9678.

Typical Procedure for Suzuki Coupling. The procedure described for **14a** is representative for Suzuki–Miyaura couplings.

2-Benzyl-7-(2-carboxy-ethyl)-8-cyclopropyl-5-oxo-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Methyl Ester 14a. **8** (227 mg, 0.567 mmol), benzyl boronic acid pinacol ester (0.12 mL, 1.42 mmol), $Pd(dppf)_2Cl_2{\cdot}CH_2Cl_2$ (46.3 mg, 0.056 mmol), and K_2CO_3 (391.8 mg, 2.84 mmol) were dissolved in MeOH (8.5 mL), and the reaction heated by MWI at 80 °C for 12 min. The reaction mixture was diluted with ethyl acetate and acidified. The organic layer was washed with water, brine, dried over Na_2SO_4, filtered, and concentrated. Purification by column chromatography (CH_2Cl_2–MeOH 97/3 and CH_2Cl_2–MeOH–AcOH 95/5/1, from 1/0 to 0/1) gave **14a** as a pale yellow solid (189 mg, 81%); R_f = 0.41 (CH_2Cl_2–MeOH–AcOH, 95/5/1); IR (υ cm^{-1}) (neat) 2925, 1721, 1633, 1553, 1481, 1433, 1334, 1191, 1008; ^1H NMR (MeOD, 400 MHz) δ (ppm) 7.34–7.28 (m, 5H), 6.23 (s, 1H), 4.07 (s, 2H), 3.94 (s, 3H), 3.11 (t, 2H, J = 7.6 Hz), 2.66 (t, 2H, J = 7.6 Hz), 1.83–1.75 (m, 1H), 1.07–1.01 (m, 2H), 0.68–0.63 (m, 2H); ^{13}C NMR (MeOD, 100 MHz) δ (ppm) 174.6, 161.3, 159.2, 154.6, 147.5, 137.1, 132.7, 128.6 (2C), 128.4 (2C), 127.2, 126.4, 113.7, 108.9, 52.3, 32.8, 31.8, 27.5, 10.2, 7.1 (2C); HRMS (EI): m/z: calcd for $C_{22}H_{21}NO_5S$: 434.1038 [M+Na]; found: 434.1047.

7-(2-Carboxy-ethyl)-8-cyclopropyl-5-oxo-2-phenyl-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Methyl Ester 14b. Prepared according to the procedure for **14a** starting from **8** (45 mg, 0.11 mmol) and phenyl boronic acid (41 mg, 0.34 mmol) and purification by column chromatography (CH_2Cl_2–MeOH 97/3 and CH_2Cl_2–MeOH–AcOH 95/5/1, from 1/0 to 0/1) to give **14b** as a pale yellow solid (32 mg, 79%); R_f = 0.34 (CH_2Cl_2–MeOH–AcOH, 95/5/1); IR (υ cm^{-1}) (neat) 2954, 1734, 1710, 1629, 1545, 1474, 1429, 1335, 1269, 1171, 1024, 972; ^1H NMR (MeOD–$CDCl_3$, 400 MHz) δ (ppm) 7.56–7.53 (m, 2H), 7.49–7.43 (m, 3H), 6.25 (s, 1H), 3.86 (s, 3H), 3.13 (t, 2H, J = 7.8 Hz), 2.66 (t, 2H, J = 7.8 Hz), 1.86–1.78 (m, 1H), 1.13–1.07 (m, 2H), 0.75–0.69 (m, 2H)); ^{13}C NMR (MeOD–$CDCl_3$, 100 MHz) δ (ppm) 174.5, 161.8, 159.4, 154.3, 147.1, 130.2, 130.1, 129.2 (2C), 128.4 (2C), 128.2, 125.3, 113.4, 109.4, 53.1, 33.2, 27.7, 10.6, 7.7 (2C); HRMS (EI): m/z: calcd for $C_{21}H_{19}NO_5S$: 420.0882 [M+Na]; found: 420.0903.

7-(2-Carboxy-ethyl)-8-cyclopropyl-2-methyl-5-oxo-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Methyl Ester 14c. Prepared according to the procedure for **14a** starting from **8** (38 mg, 0.095 mmol) and methyl boronic acid (17 mg, 0.29 mmol) and purification by column chromatography (CH_2Cl_2–MeOH 97/3 and CH_2Cl_2–MeOH–AcOH 95/5/1, from 1/0 to 0/1) to give **14c** as a pale yellow solid (25 mg, 79%); R_f = 0.35 (CH_2Cl_2–MeOH–AcOH, 95/5/1); IR (υ cm^{-1}) (neat) 2949, 1726, 1625, 1541, 1476, 1432, 1337, 1251, 1175, 1061; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 6.37 (s, 1H), 3.94 (s, 3H), 3.15–3.08 (m, 2H), 2.74–2.67 (m, 2H), 2.37 (s, 3H), 1.81–1.72 (m, 1H), 1.11–1.01 (m, 2H), 0.71–0.63 (m, 2H); 13C NMR ($CDCl_3$, 100 MHz) δ (ppm) 175.5, 161.3, 159.0, 153.6, 146.9, 127.2, 127.0, 113.1, 109.8, 53.3, 33.1, 27.4, 11.9, 10.7, 7.9 (2C); HRMS (EI): m/z: calcd for $C_{16}H_{17}NO_5S$: 358.0725 [M+Na]; found: 358.0743.

7-(2-Carboxy-ethyl)-8-cyclopropyl-5-oxo-2-phenylethynyl-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Methyl Ester 15. A mixture of **8** (25 mg, 0.062 mmol), phenylacetylene (20 μL, 0.186 mmol), CuI (1.2 mg, 0.006 mmol), $Pd(Ph_3P)_2Cl_2$ (2.1 mg, 0.003 mmol), and K_2CO_3 (42.8 mg, 0.31 mmol) in DMF (1.2 mL) was heated by MWI at 80 °C for 10 min. The reaction mixture was diluted with CH_2Cl_2 and acidified. The organic layer was washed with brine,

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dried over Na_2SO_4, filtered, and concentrated. Purification by column chromatography (CH_2Cl_2–MeOH 97/3 and CH_2Cl_2–MeOH–AcOH 95/5/1, from 1/0 to 0/1) gave **15** as a yellow solid (23 mg, 80%); R_f = 0.42 (CH_2Cl_2–MeOH–AcOH, 95/5/1); IR (υ cm^{-1}) (neat) 2951, 1736, 1704, 1654, 1569, 1474, 1244, 1217, 1163, 1030; ^1H NMR (MeOD–$CDCl_3$, 400 MHz) δ (ppm) 7.50–7.48 (m, 2H), 7.41–7.35 (m, 3H), 6.23 (s, 1H), 3.98 (s, 3H), 3.11 (t, 2H, J = 7.6 Hz), 2.65 (t, 2H, J = 7.6 Hz), 1.82–1.75 (m, 1H), 1.13–1.06 (m, 2H), 0.73–0.68 (m, 2H); ^{13}C NMR (MeOD and $CDCl_3$, 100 MHz) δ (ppm) 175.1, 161.2, 159.5, 155.8, 146.9, 133.2, 132.4 (2C), 130.6, 129.2 (2C), 121.5, 114.1, 112.8, 110.3, 101.5, 76.3, 53.9, 33.7, 28.4, 11.2, 8.3 (2C); HRMS (EI): m/z: calcd for $C_{23}H_{19}NO_5S$: 444.0882 [M+Na]; found: 444.0897.

7-(2-Carboxy-ethyl)-8-cyclopropyl-5-oxo-2-phenylcarbamoyl-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Methyl Ester 16. **8** (100 mg, 0.25 mmol) was dissolved in THF (10 mL) and cooled to −78 °C. *n*-BuLi (0.5 mmol) was added dropwise, and the solution was allowed to warm to −70 °C and stirred for 10 min at that temperature. PhNCO (82 μL, 0.75 mmol) was added dropwise, and the mixture was stirred for 15 min at −70 °C. The reaction was quenched using 2% aq. $KHSO_4$ and diluted with CH_2Cl_2, and the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. Purification by column chromatography (CH_2Cl_2–MeOH 97/3 and CH_2Cl_2–MeOH–AcOH 95/5/1, from 1/0 to 0/1) gave **16** as a yellow solid (71 mg, 64%); R_f = 0.41 (CH_2Cl_2–MeOH–AcOH, 95/5/1); IR (υ cm^{-1}) (neat) 3250, 2954, 1740, 1712, 1672, 1635, 1603, 1544, 1472, 1441, 1324, 1246, 1214; ^1H NMR (MeOD–$CDCl_3$, 400 MHz) δ (ppm) 7.56 (d, 2H, J = 8.4 Hz), 7.34 (t, 2H, J = 8.0 Hz), 7.16 (t, 1H, J = 7.4 Hz), 6.24 (s, 1H), 3.98 (s, 3H), 3.12 (t, 2H, J = 7.8 Hz), 2.65 (t, 2H, J = 7.8 Hz), 1.83–1.75 (m, 1H), 1.14–1.08 (m, 2H), 0.74–0.69 (m, 2H); ^{13}C NMR (MeOD–$CDCl_3$, 100 MHz) δ (ppm) 174.4, 162.0, 159.6, 156.8, 155.8, 146.6, 136.8, 129.3 (2C), 125.5, 120.6 (2C), 113.4, 109.8, 53.8, 33.0, 27.8, 10.6, 7.8, 7.5; HRMS (EI): m/z: calcd for $C_{22}H_{20}N_2O_6S$: 463.0940 [M+Na]; found: 463.0948.

Typical Procedure for the Synthesis of C-2 Substituted BODIPY Esters *rac*-6 and 17a–c. The procedure described for **17a** is representative for **6** and **17b–c**.

2-Benzyl-8-cyclopropyl-7-(2-(1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene-8-yl)ethyl)-5-oxo-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Methyl Ester 17a. Oxalyl chloride (0.15 mL, 1.79 mmol) was added dropwise to **14a** (368 mg, 0.894 mmol) in CH_2Cl_2 (13 mL) at 0 °C, and the mixture was allowed to attain rt and stirred for 2 h. The solvent was evaporated off, and the residue was dissolved in dry DCE (13 mL). 2,4-Dimethyl-1*H*-pyrrole (0.64 mL, 6.26 mmol) was added and stirred for 40 min at rt followed by the addition of triethylamine (0.87 mL, 6.26 mmol) and $BF_3.Et_2O$ (0.79 mL, 6.26 mmol), and the mixture was heated by MWI at 140 °C for 90 min. The reaction mixture was diluted with CH_2Cl_2 and acidified. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. Purification by column chromatography (heptane/EtOAc from 1/0 to 3/2) gave **17a** as a reddish solid (180 mg, 33%); R_f = 0.28 (heptane/EtOAc, 1/1); IR (υ cm^{-1}) (neat) 1726, 1654, 1546, 1507, 1468, 1305, 1192, 1157, 1074, 1024, 972; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.37–7.27 (m, 5H), 6.35 (s, 1H), 6.06 (s, 2H), 4.03 (s, 2H), 4.01 (s, 3H), 3.33–3.29 (m, 2H), 3.06–3.01 (m, 2H), 2.53 (s, 6H), 2.33 (s, 6H), 1.60–1.52 (m, 1H), 0.97–0.89 (m, 2H), 0.54–0.49 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 161.4, 158.9, 154.6 (2C), 152.5, 146.9, 144.4, 140.3 (2C), 136.8, 131.4 (2C), 131.3, 129.0 (2C), 128.7 (2C), 127.6, 126.8, 121.9 (2C), 111.9, 108.3, 53.4, 33.0, 32.7, 25.9, 16.4 (2C), 14.6 (2C), 10.6, 8.1 (2C); HRMS (EI): m/z: calcd for $C_{34}H_{34}BF_2N_3O_3S$: 636.2280 [M+Na]; found: 636.2302.

8-Cyclopropyl-7-(2-(1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene-8-yl)ethyl)-5-oxo-2-phenyl-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Methyl Ester 17b. Prepared according to the procedure for **17a** starting from **14b** (28 mg, 0.07 mmol). Purification by column chromatography (heptane/EtOAc 1/0, to 3/2) gave **17b** as a reddish solid (16 mg, 39%); R_f = 0.33 (heptane/EtOAc, 1/1); IR (υ cm^{-1}) (neat) 2920, 1690, 1652, 1551, 1509, 1471, 1269, 1201, 1060, 975; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.60–7.57 (m, 2H), 7.47–7.44 (m, 3H), 6.39 (s, 1H), 6.08 (s, 2H), 3.94 (s, 3H), 3.37–3.32 (m, 2H), 3.11–3.06 (m, 2H), 2.54 (s, 6H), 2.36 (s, 6H), 1.69–1.65 (m, 1H), 1.05–1.02 (m, 2H), 0.65–0.62 (m, 2H)); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 161.7, 159.0, 154.6 (2C), 152.6, 146.6, 144.3, 140.3 (2C), 131.4, 130.1 (2C), 129.3, 129.3 (2C), 128.5 (2C), 125.5, 121.9 (2C), 111.7, 108.2, 53.5, 33.1, 25.9, 16.5 (2C), 14.6 (2C), 10.7, 8.2 (2C); HRMS (EI): m/z: calcd for $C_{33}H_{32}BF_2N_3O_3S$: 622.2123 [M+Na]; found: 622.2136.

8-Cyclopropyl-7-(2-(1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene-8-yl)ethyl)-2-methyl-5-oxo-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Methyl Ester 17c. Prepared according to the procedure for **17a** starting from **14c** (26 mg, 0.08 mmol). Purification by column chromatography (heptane/EtOAc 1/0 to 2/3) gave **17c** as a reddish solid (16 mg, 38%); R_f = 0.42 (heptane/EtOAc, 3/7); IR (υ cm^{-1}) (neat) 2924, 1735, 1655, 1553, 1508, 1474, 1311, 1205, 1061, 974; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 6.34 (s, 1H), 6.07 (s, 2H), 3.98 (s, 3H), 3.35–3.31 (m, 2H), 3.08–3.04 (m, 2H), 2.53 (s, 6H), 2.39 (s, 3H), 2.34 (s, 6H), 1.64–1.58 (m, 1H), 1.02–0.96 (m, 2H), 0.59–0.55 (m, 2H)); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 161.4, 158.7, 154.6 (2C), 152.3, 146.8, 144.4, 140.3 (2C), 131.4, 126.9, 126.8, 121.9 (2C), 111.7, 108.3, 53.3, 33.1, 25.9, 16.5 (2C), 14.6 (2C), 11.9, 10.6, 8.1 (2C); HRMS (EI): m/z: calcd for $C_{28}H_{30}BF_2N_3O_3S$: 560.1967 [M+Na]; found: 560.1982.

5-Oxo-8-cyclopropyl-7-(2-(1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene-8-yl)ethyl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid Methyl Ester *rac*-6. Prepared according to the procedure for **17a** starting from **13** (58 mg, 0.18 mmol). Purification by column chromatography (heptane/EtOAc, from 9/1 to 0/1) gave ***rac*-6** as a brick red solid (39 mg, 41%); R_f = 0.45 (EtOAc); IR (υ cm^{-1}) (neat) 1749, 1644, 1549, 1487, 1407, 1307, 1199, 1157, 1075; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 6.25 (s, 2H), 6.07 (s, 1H), 5.58 (dd, 1H, J = 2.4, 8.8 Hz), 3.80 (s, 3H), 3.65 (dd, 1H, J = 8.8, 12.0 Hz), 3.48 (dd, 1H, J = 2.4, 12.0 Hz), 3.29–3.22 (m, 2H), 2.97–2.90 (m, 2H), 2.52 (s, 6H), 2.33 (s, 6H), 1.49–1.42 (m, 1H), 0.92–0.80 (m, 2H), 0.57–0.47 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 168.4, 161.2, 155.8, 154.5, 147.2, 144.3, 140.3, 131.3, 121.8 (2C), 113.2, 111.8, 62.7, 53.2, 32.9, 31.5, 25.3, 16.3 (2C), 14.4 (2C), 10.8, 7.9, 7.5; HRMS (EI): m/z: calcd for $C_{27}H_{30}BF_2N_3O_3S$: 548.1967 [M+Na]; found: 548.1981.

Procedure for Hydrolysis. *Method A:* 1 M aqueous LiOH was added dropwise to a stirred solution of the substrate in THF (30 mL/mmol) at 0 °C. The solution was then allowed to reach room temperature and was continuously stirred for 12 h. The mixture was diluted with CH_2Cl_2 and acidified with 1 M HCl. The organic layer was dried over Na_2SO_4, filtered, and concentrated. Purification by column chromatography (CH_2Cl_2–MeOH 97/3 and CH_2Cl_2–MeOH–AcOH 90/8/2, from 1/0 to 0/1) gave the carboxylic acid.

Method B: LiBr (50.0 equiv) was added to the substrate in DMF (20 mL/mmol) at rt, and the mixture was heated by MWI at 90/100 °C. The reaction mixture was diluted with water, acidified, and extracted with CH_2Cl_2. The organic phase was washed with water, brine, dried over Na_2SO_4, filtered, and concentrated. The crude products were purified by column chromatography (CH_2Cl_2–MeOH 97/3 and CH_2Cl_2–MeOH–AcOH 95/5/1, from 1/0 to 0/1)

Method C: LiI (50.0 equiv) was added to the substrate in DMF (20 mL/mmol) at rt, and the mixture was heated by MWI for 90 min at 80 °C. The reaction mixture was diluted with water, acidified, and extracted with CH_2Cl_2. The organic phase was washed with water, brine, dried over Na_2SO_4, filtered, and concentrated. The crude products were purified by column chromatography (CH_2Cl_2–MeOH 97/3 and CH_2Cl_2–MeOH–AcOH 95/5/1, 1/0 to 0/1)

5-Oxo-8-cyclopropyl-7-(2-(1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene-8-yl)ethyl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid (*rac*-18). By following hydrolysis method A, ***rac*-6** (55 mg, 0.105 mmol) gave *rac*-**18** (45 mg, 88%) as a brick red solid; R_f = 0.60 (CH_2Cl_2–MeOH–AcOH, 90/8/2): IR (υ cm^{-1}) (neat) 1720, 1629, 1548, 1492, 1308, 1197, 1157, 1078, 982; ^1H NMR (DMSO, 400 MHz) δ (ppm) 6.24 (s, 2H), 6.17 (s, 1H), 5.39 (d, 1H, J = 9.2 Hz), 3.78 (dd, 1H, J = 9.4, 12.2 Hz), 3.49

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(d, 1H, J = 12.0 Hz), 3.33−3.18 (m, 2H), 3.01−2.82 (m, 2H), 2.41 (s, 6H), 2.35 (s, 6H), 1.60−1.52 (m, 1H), 0.84−0.74 (m, 2H), 0.55−0.41 (m, 2H). ^{13}C NMR (DMSO, 100 MHz) δ (ppm) 170.1, 160.6, 155.6, 154.1, 148.3, 145.9, 141.5, 131.3, 122.4 (2C), 112.1, 111.5, 62.9, 32.8, 31.7, 25.5, 16.2 (2C), 14.6 (2C), 10.9, 7.9, 7.7. HRMS (EI): m/z: calcd for $C_{26}H_{28}BF_2N_3O_3S$: 534.1810 [M+Na]; found: 534.1810.

2-Benzyl-8-cyclopropyl-7-(2-(1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene-8-yl)ethyl)-5-oxo-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid 19a. By following hydrolysis method B (MWI at 90 °C for 70 min), **17a** (35 mg, 0.057 mmol) gave **19a** (17 mg, 65%) as a reddish solid (yield based on recovered staring material); R_f = 0.36 (CH_2Cl_2−MeOH−AcOH, 95/5/1); IR (υ cm^{-1}) (neat) 2922, 1742, 1636, 1610, 1546, 1508, 1473, 1406, 1305, 1193, 1157, 1079, 974; ^{1}H NMR (DMSO, 400 MHz) δ (ppm) 7.38−7.24 (m, 5H), 6.33 (s, 1H), 6.26 (s, 2H), 4.09 (s, 2H), 3.37−3.32 (m, 2H), 3.06−3.01 (m, 2H), 2.43 (s, 6H), 2.37 (s, 6H), 1.71−1.65 (m, 1H), 0.90−0.84 (m, 2H), 0.53−0.48 (m, 2H); ^{13}C NMR (DMSO, 100 MHz) δ (ppm) 161.7, 158.2, 154.1 (2C), 152.6, 146.9, 145.8, 141.5 (2C), 138.2, 131.2 (2C), 129.3 (2C), 129.0 (2C), 127.6, 122.4 (2C), 111.5, 108.0, 32.8, 32.1, 25.8, 16.2 (2C), 14.6 (2C), 10.8, 8.0 (2C); HRMS (EI): m/z: calcd for $C_{33}H_{32}$ $BF_2N_3O_3S$: 622.2123 [M+Na]; found: 622.2151.

8-Cyclopropyl-7(2-(1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene-8-yl)ethyl)-5-oxo-2-phenyl-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid 19b. By following hydrolysis method C, **17b** (27 mg, 0.045 mmol) gave **19b** (13 mg, 68%) as a reddish solid (yield based on recovered starting material); R_f = 0.25 (CH_2Cl_2−MeOH−AcOH, 95/5/1); IR (υ cm^{-1}) (neat) 3300, 2923, 1705, 1636, 1549, 1508, 1466, 1308, 1197, 1157, 1061, 974; ^{1}H NMR (MeOD:$CDCl_3$, 400 MHz) δ (ppm) 7.64−7.61 (m, 2H), 7.44−7.40 (m, 3H), 6.37 (s, 1H), 6.08 (s, 2H), 3.38−3.34 (m, 2H), 3.13−3.08 (m, 2H), 2.49 (s, 6H), 2.35 (s, 6H), 1.72−1.65 (m, 1H), 1.05−1.01 (m, 2H), 0.65−0.61 (m, 2H)); ^{13}C NMR (MeOD−$CDCl_3$, 100 MHz) δ (ppm) 162.6, 159.4, 154.5 (2C), 152.9, 147.3, 144.4, 140.5 (2C), 131.4, 130.0 (2C), 129.2, 129.0 (2C), 128.5 (2C), 126.7, 122.0 (2C), 112.7, 107.8, 33.1, 25.9, 16.2 (2C), 14.2 (2C), 10.6, 8.1 (2C); HRMS (EI): m/z: calcd for $C_{32}H_{30}$ $BF_2N_3O_3S$: 608.1967 [M+Na]; found: 608.1988.

8-Cyclopropyl-7-(2-(1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene-8-yl)ethyl)-2-methyl-5-oxo-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic Acid 19c. By following hydrolysis method B (MWI at 100 °C for 30 min), **17c** (15 mg, 0.03 mmol) gave **19c** (7 mg, 55%) as a reddish solid (yield based on recovered starting material); R_f = 0.31 (CH_2Cl_2−MeOH−AcOH, 95/5/1); IR (υ cm^{-1}) (neat) 3302, 2924, 1587, 1551, 1508, 1474, 1406, 1309, 1200, 1155, 1087, 1061; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 6.64 (s, 1H), 6.09 (s, 2H), 3.39−3.35 (m, 2H), 3.17−3.13 (m, 2H), 2.85 (s, 3H), 2.54 (s, 6H), 2.35 (s, 6H), 1.72−1.65 (m, 1H), 1.15−1.09 (m, 2H), 0.66−0.61 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 161.1, 159.3, 154.9 (2C), 153.1, 147.4, 143.5, 140.1 (2C), 131.4, 129.5, 122.2 (2C), 116.5, 109.4, 32.8, 25.8, 17.4, 16.6 (2C), 14.5 (2C), 10.7, 8.7 (2C); HRMS (EI): m/z: calcd for $C_{27}H_{28}$ $BF_2N_3O_3S$: 546.1810 [M+Na]; found: 546.1822.

Photophysical Measurements. Absorption spectra were recorded on a UV−vis−NIR spectrophotometer. The fluorescence spectra of the reference compound and samples were recorded on a spectrometer equipped with polarizers at 25 °C. The compounds were dissolved in the solvent and heated to approximately 60 °C for 2 h. The samples were equilibrated to 25 °C over 1 h. Absorbance spectra were then recorded and compared to those obtained before heating. Thereafter fluorescence data were collected. The excitation wavelength (λ_{ex}) was 470 nm, and the fluorescence spectrum was collected in the range 480−600 nm. In order to reduce reabsorption the peak absorbance (@ $\lambda_{A,max}$) was kept below 0.08. The fluorescence quantum yield of a sample (Φ_s) was calculated from:

$$\Phi_s = \Phi_{ref}\frac{F_s(1-\exp[-A_{ref}(\lambda_{ex})\ln 10])n_s^2}{F_{ref}(1-\exp[-A_s(\lambda_{ex})\ln 10])n_{ref}^2}$$

Here A and F refers to the absorbance at the excitation wavelength, and the integrated fluorescence spectrum, respectively. The reported quantum yield of the reference substance sodium fluorescein is 93%.[27] The refractive index (n) for the reference and the samples were 1.333 (water) and 1.479 (DMSO). For fluorescence lifetimes, time-resolved fluorescence decays were measured by the time-correlated single photon-counting technique on a spectrometer. For excitation, a pulsed nano LED centered at 467 nm was used. Data were collected under the magic-angle condition. The fluorescence lifetimes were calculated by using a deconvolution method based on the Levenberg−Marquardt algorithm.[28] Decay data were fitted to a sum of exponential functions ($\sum a_i \exp(-t/\tau_i)$) and the average fluorescence lifetime calculated from:

$$\langle\tau\rangle = \sum_i a_i\cdot\tau_i^2 / \sum_i a_i\cdot\tau_i$$

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of all new compounds, Schemes S1 and S2 comparing old and new syntheses, Table S1 with examined hydrolysis conditions, Table S2 with LiI halogenolysis of BODIPY methyl esters, and Figure S1 showing biphasic relaxation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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