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MediaChrom: Discovering a Class of Pyrimidoindolone-Based Polarity-Sensitive Dyes

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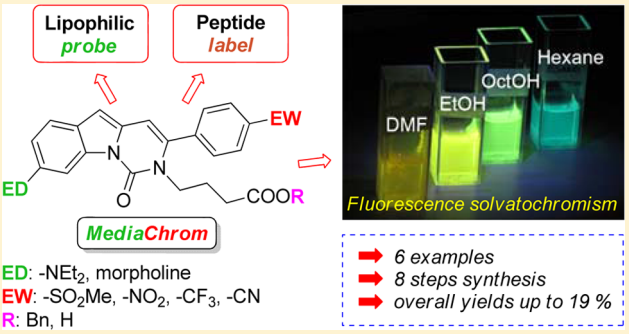
Supporting Information

ABSTRACT: A small library of six polarity-sensitive fluorescent dyes, nicknamed MediaChrom, was prepared. This class of dyes is characterized by a pyrimidoindolone core fitted out with a conjugated push–pull system and a carboxy linker for a conceivable coupling with biomolecules. The optimized eight-step synthetic strategy involves a highly chemo- and regioselective gold-catalyzed cycloisomerization reaction. The photophysical properties of MediaChrom dyes have been evaluated in-depth. In particular, the MediaChrom bearing a diethylamino as an electron-donating group and a trifluoromethyl as an electron-withdrawing group displays the most interesting and advantageous spectroscopic features (e.g., absorption and emission in the visible range and a good quantum yield). Promising results in terms of sensitivity have been obtained in vitro on this dye as a membrane/lipophilic probe and as a peptide fluorescent label.



INTRODUCTION

Modern biological research needs the continuous development of highly specific and sensitive fluorescent dyes for monitoring a wide range of molecular processes and events.[1] A particular class of dyes, called environment-sensitive dyes, is able to change their spectroscopic properties in response to the change of the chemical–physical features of their surroundings. Among them, the polarity-sensitive dyes[2] (also called solvatochromic dyes)[3] have the unique features of displaying a different emission maximum as a function of the polarity of the medium (i.e., solvent). This peculiarity makes polarity-sensitive dyes the ideal probes to monitor the local properties of particular cell districts as well as biomolecular interactions[4,5] (e.g., peptide–nucleic acid, protein–protein, and peptide–lipid interactions).

Two main classes of polarity-sensitive dyes are available, single-band and two-band solvatochromic dyes.[6,7] The former are the most used because the latter suffer from poor photostability.[8] Single-band solvatochromic dyes are usually characterized by a rigid aromatic backbone bearing conjugated electron acceptor and electron donor groups at the opposite sides. In these molecules, the dipole moment increases by electronic excitation due to an intramolecular charge transfer from the electron-donating (ED) to the electron-withdrawing (EW) group. If their excited states are stabilized by dipole–dipole or H-bonding interactions with the surrounding medium, these dyes exhibit a bathochromic effect of their emission spectrum in response to an increase in solvent polarity (positive solvatochromism).

Several polarity-sensitive dyes have been developed, but most of them are far from simultaneously meeting all of the optimal spectroscopic requirements for biological applications, such as a strong solvatochromism, absorption close to the visible range, large Stokes shift, high extinction coefficient, high quantum yield, and good photostability. Among the most common and commercially available polarity-sensitive dyes, Dansyl[9] and its derivatives absorb in the UV region (around 340 nm) and display low extinction coefficients as does DMAP[10] and its derivatives, as well.[11] Dapoxyl[12] and Prodan,[13] two of the best solvatochromic dyes available, display only a slightly red-shifted absorption (373 and 360 nm, respectively). Furthermore, Dapoxyl and its derivatives are characterized by low extinction coefficients. Anthradan,[14] a benzo homologue of Prodan, solved the problem of the absorption in the UV range (around 450 nm), but its brightness is compromised by a low extinction coefficient. The performances of Prodan-type solvatochromic dyes were recently strongly improved by the substitution of the polycyclic aromatic skeleton with a fluorene core.[15] NBD[16] displays an interesting

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red-shifted absorption (around 465 nm), but its solvatochromism is limited. Fluoroprobe,[17] probably the molecule with the highest solvatochromic effect to date, suffers from a marked blue-shifted absorption (around 310 nm), a low extinction coefficient, and a strong quenching of fluorescence in polar media. These examples highlight that the ideal polarity-sensitive dye has not yet been discovered. Besides the above-mentioned well-consolidated polarity-sensitive dyes, a number of new interesting solvatochromic D−π−A molecules have been recently published, testifying that the interest of the scientific community in this field is experiencing a dramatic growth.[18]

For many years, we have been interested in the development of new strategies for the synthesis and the functionalization of indoles and polycyclic indole-based heterocycles.[19] The barely investigated—but interesting—fluorescence properties of the pyrimidoindolone nucleus[20] prompt us to design a new class of polarity-sensitive dyes of general structure **I**, characterized by the presence of selected ED and EW groups in a proper conjugate position (Figure 1).

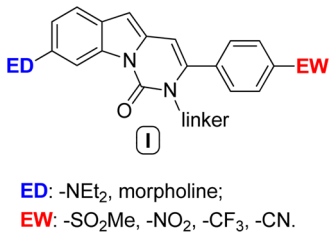


Figure 1. General formula of the planned pyrimidoindolone-based polarity-sensitive dyes.

Our ambitious goal was to obtain a small library of original pyrimidoindolone-based solvatochromic dyes with enhanced photophysical properties. The ED and EW groups were selected based on literature findings. The most used ED groups in push−pull solvatochromic dyes are secondary amines. When a weaker donating group, such as the alkoxy group, was used, the spectroscopic performance was, in general, worse.[21] Therefore, in our study we used diethylamino and morpholine groups. In particular, we preferred these amino groups with larger alkyl residues than the standard dimethylamino group because recent studies demonstrated that this modification is able to improve brightness, photostability, and quantum yield of the dye.[22] Different EW groups are present in most effective polarity-sensitive dyes, ranging from carbonyl to sulfonic, nitro, and cyano groups. We planned to investigate the effect of the latter three groups characterized by a strong mesomeric EW effect and the trifluoromethyl, a strong inductive EW group seldom encountered in this context.[23] Moreover, our synthetic strategy allows the functionalization of the lactam nitrogen with a suitable linker for a handy conjugation to biomolecules (Figure 1). After the synthesis, the photophysical properties of the new solvatochromic dyes were evaluated, and as a proof of concept, their applications as a membrane/lipophilic probe and as a peptide fluorescent label were briefly investigated. In this paper, we describe our results.

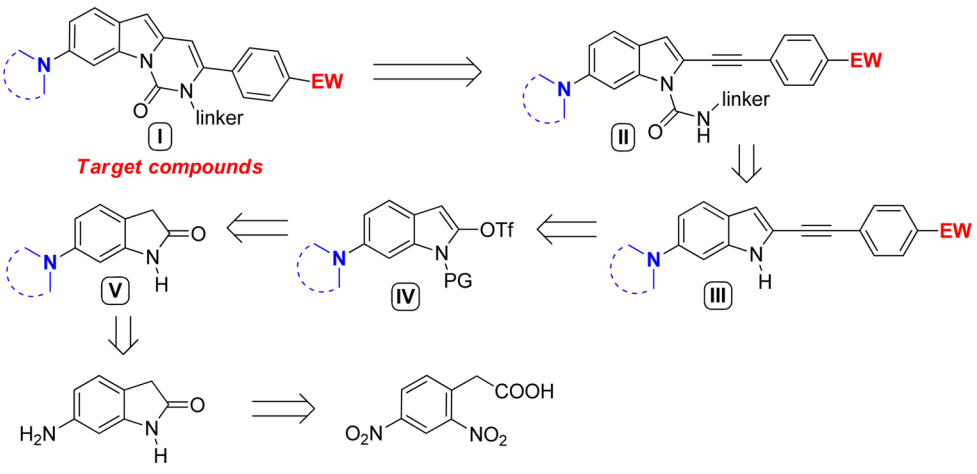
RESULTS AND DISCUSSION

Synthesis of the Pyrimidoindolone (MediaChrom) Library. We designed a retrosynthetic approach (Scheme 1), taking into account our experience with the transformation of indolin-2-ones (**V**) in the indole-2-triflates (**IV**), useful building blocks for the preparation of 2-alkynyl indoles (**III**).[24] The early synthetic steps have sound basis in the literature[25] but involve the synthesis of a number of unknown compounds. In particular, the synthesis and the reactivity of 6-amino-substituted indoles (**IV**) and indolinones (**V**) are seldom explored.[26] The target compounds (**I**) should be obtained from intermediates **III** by the aminocarbonylation of the indole nitrogen with phosgene and a suitable amine to give compound **II**, followed by a metal-catalyzed cycloisomerization (Scheme 1).

We started our study by trying to synthesize a properly substituted key intermediate **IV**, that is, the N-protected 6-dialkylaminoindole-2-triflate (**4**) (Scheme 2). 6-Aminoindolin-2-one **1** was prepared by the one-pot reduction/lactamization of 2,4-dinitrophenylacetic acid.[25] The following reductive amination[26] with acetaldehyde gave the corresponding 6-diethylaminoindolin-2-one **2** in 80% yield. Surprisingly, this approach broke down in the last step because every attempt to transform the N-protected 6-diethylaminoindolin-2-one **3** in the corresponding indole-2-triflate **4** was unsuccessful (see Supporting Information for details).

This drawback prompted us to plan an alternative synthetic strategy. Because iodine is a good leaving group as a triflate, we identified the N-protected 6-amino-2-iodoindoles **8** and **10** as suitable new key intermediates. They were obtained in four steps

Scheme 1. Retrosynthetic Approach



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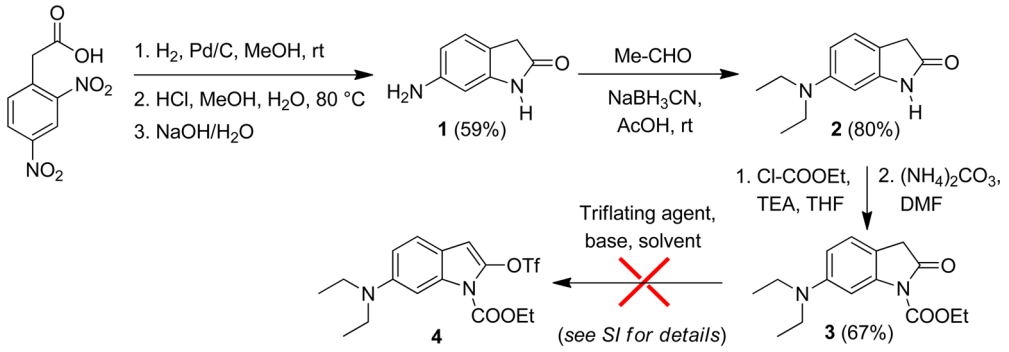
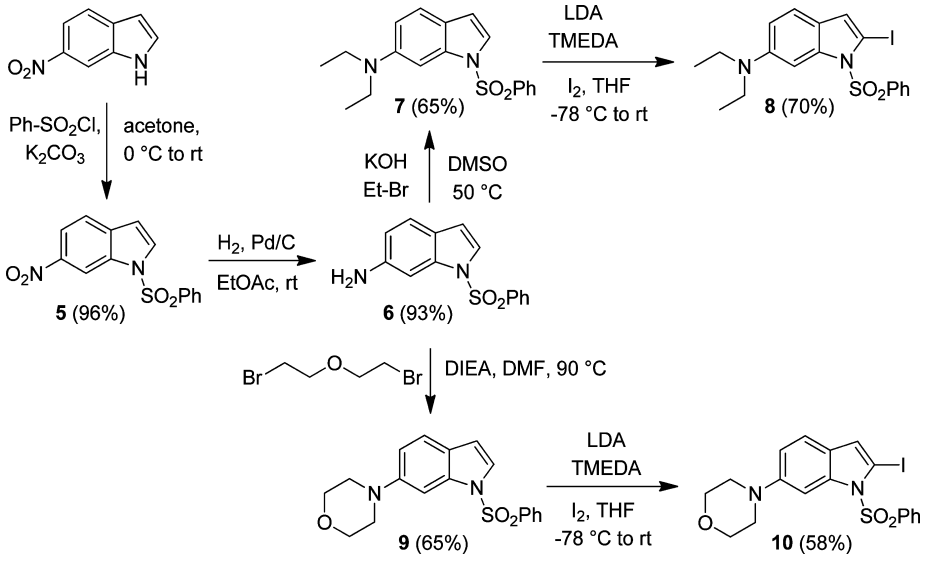
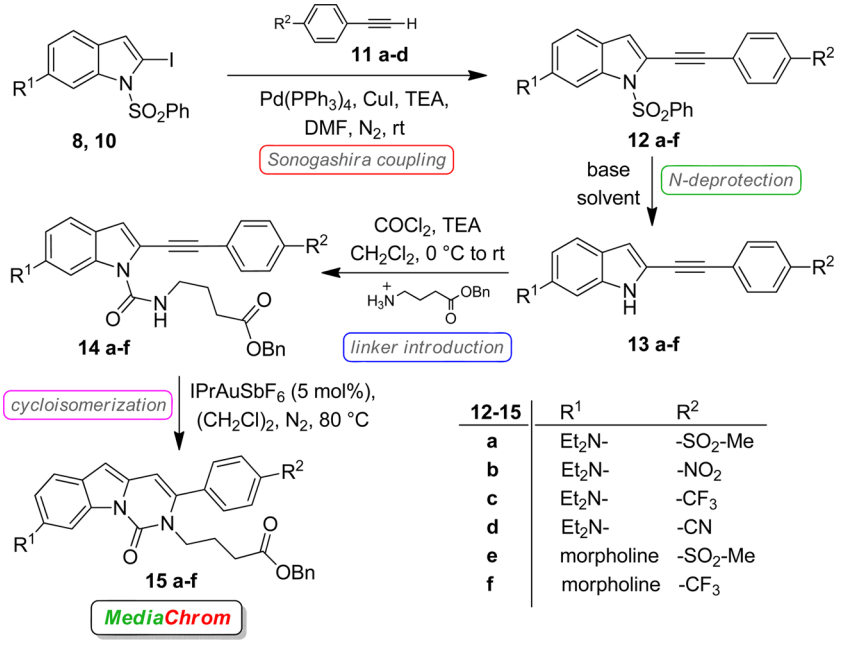
Scheme 2. Tentative Approach to Intermediate 4



Scheme 3. Alternative Strategy to 2-Iodinated Key Intermediates 8 and 10



Scheme 4. Synthesis of MediaChrom Dyes 15a–f



12-15	R^1	R^2
a	Et_2N-	-SO_2-Me
b	Et_2N-	-NO_2
c	Et_2N-	-CF_3
d	Et_2N-	-CN
e	morpholine	-SO_2-Me
f	morpholine	-CF_3

starting from cheap and commercially available 6-nitroindole (Scheme 3).

The nitrogen group of 6-nitroindole was protected with a benzenesulfonyl group to give the 1-benzenesulfonyl-6-nitro-

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Table 1. Synthesis of Compounds 12a−f and 13a−f

substrate	R^1	alkyne	R^2	**8**/**10** → **12**[a] time (h)	yield[b] (%)	**12** → **13**[c] method	time (h)	yield[b] (%)
8	Et_2N-	**11a**	-SO_2-Me	2	**12a** (90)	A	8	**13a** (96)
8	Et_2N-	**11b**	-NO_2	5	**12b** (52)	B	3	**13b** (57)
8	Et_2N-	**11c**	-CF_3	6	**12c** (55)	C	3	**13c** (87)
8	Et_2N-	**11d**	-CN	4	**12d** (79)	D	6	**13d** (52)
10	morpholine	**11a**	-SO_2-Me	16	**12e** (90)	A	4	**13e** (97)
10	morpholine	**11c**	-CF_3	24	**12f** (78)	B	5	**13f** (91)

[a]Reaction conditions: **8** or **10** (0.4 mmol) in DMF (1.6 mL), **11** (0.48 mmol), TEA (8 mmol), $Pd(PPh_3)_4$ (0.016 mmol), CuI (0.008 mmol), rt, N_2. [b]Pure isolated products. [c]Method A: **12a** or **12e** (0.4 mmol) in MeOH (8 mL), aq NaOH (2 M, 2.5 mL), reflux. Method B: **12b** or **12f** (0.4 mmol) in MeOH (24 mL), aq NaOH (6 M, 2.5 mL), reflux. Method C: **12c** (0.4 mmol) in MeOH (16 mL), aq NaOH (6 M, 0.8 mL), reflux. Method D: **12d** (0.4 mmol) in dioxane (3 mL), *t*-BuONa (0.8 mmol), 80 °C, N_2.

Table 2. Synthesis of Compounds 14a−f and 15a−f

substrate	R^1	R^2	**13** → **14**[a] time (h)	yield[b] (%)	**14** → **15**[c] time (h)	yield[c] (%)
13a	Et_2N-	-SO_2-Me	4	**14a** (70)	6	**15a** (53)
13b	Et_2N-	-NO_2	4	**14b** (70)	8	**15b** (45)
13c	Et_2N-	-CF_3	3	**14c** (53)	8	**15c** (78)
13d	Et_2N-	-CN	4	**14d** (46)	4.5	**15d** (78)
13e	morpholine	-SO_2-Me	24	**14e** (20)	4	**15e** (90)
13f	morpholine	-CF_3	3	**14f** (76)	2	**15f** (99)

[a]Reaction conditions: **13a−f** (0.2 mmol) in CH_2Cl_2 (2 mL), $COCl_2$ (0.4 mmol), TEA (0.8 mmol), 0 °C, N_2, 30 min, then *p*-toluenesulfonate salt of the benzyl 4-aminobutanoate (0.8 mmol) in CH_2Cl_2 (1 mL) and TEA (0.4 mmol) was added, rt. [b]Pure isolated products. [c]Reaction conditions: **14a−f** (0.1 mmol) in DCE (3.5 mL), $IPrAuSbF_6$ (0.005 mmol), 80 °C, N_2.

indole **5** in excellent yield.[27] The catalytic reduction of **5** in ethyl acetate[28] led to the 1-benzenesulfonyl-6-aminoindole **6** in 93% yield, along with a small amount of the corresponding 1-benzenesulfonyl-6-aminoindoline derived from the partial reduction of the C2−C3 indole double bond. The N,N-bisalkylation of **6** by reductive amination was ruled out because it was reported that, by using $NaBH_3CN$, the reduction of the indole C2−C3 double bond can occur.[29] Therefore, the N,N-bisalkylation of **6** was performed under basic conditions with ethyl bromide at 50 °C,[30] giving rise to the 1-benzenesulfonyl-6-(*N*,*N*-diethylamino)indole derivative **7** in 65% yield. As mentioned above, to modulate the properties of the ED group at position 6 of the indole, an alternative morpholine substituent was introduced by the reaction of **6** with bis(2-bromoethyl)ether in the presence of *N*,*N*-diisopropylethylamine to give the 1-benzenesulfonyl-6-(morpholin-1-yl)indole derivative **9** in 65% yield. Finally, the N-protected 6-amino-2-iodoindoles **8** and **10**[31] were obtained by well-customized iodination reactions[32] of compounds **7** and **9**, respectively.

Starting from key intermediates **8** and **10**, we obtained the library of target compounds **15**—nicknamed MediaChrom—in four steps (Scheme 4).

N-Protected 2-alkynyl-6-aminoindoles **12a−f** were obtained in fair to excellent yields (Table 1) by a typical Sonogashira cross-coupling reaction[33] with four different alkynes (**11a−d**) in the presence of $Pd(PPh_3)_4$, CuI, and TEA in DMF. Alkynes **11b−d** are commercially available, while **11a** was synthesized from the corresponding aryl halide by a Pd-catalyzed cross-coupling with TMS-acetylene followed by desilylation. Subsequent deprotection reactions of the indole nitrogen of compounds **12a−f** to give the corresponding 6-amino-2-alkynylindoles **13a−f** were performed under alkaline conditions, properly customized to overcome specific solubility (i.e., **12b**,**c**,**f**) or hydrolysis (i.e., **12d**) problems of the substrates (Table 1).

The insertion of the amido function at the nitrogen atom required for the final cyclization step—and the simultaneous introduction of a suitable linker for a potential connection with a biomolecule—involved the preparation of a proper spacer. Our idea was conceived to allow the easy insertion of different sized linkers. This molecular architecture calls for a versatile use of MediaChrom dyes as the fluorescent probe dye as well as fluorescent labels for biomacromolecules (e.g., peptides, proteins, or amino-modified oligonucleotides). At this stage, the four-carbon spacer derived from γ-aminobutyric acid (GABA) was chosen as the compromise model, with the aim to introduce in the molecule an unhindered linking point for an amine-containing biomolecule that was not too distant from the core of the dye.[34] Thus, the *p*-toluenesulfonate salt of the benzyl 4-aminobutanoate[35] was prepared by reaction of GABA with benzyl alcohol in the presence of *p*-TSA. This was reacted with substrates **13a−f** in the presence of phosgene and triethylamine, in dichloromethane at 0 °C, to give compounds **14a−f** in fair to good yields (Table 2).[36]

Finally, the cyclization to MediaChrom **15a−f** was accomplished by a gold-catalyzed reaction.[37] The gold-catalyzed intramolecular addition of an amide group on an alkyne shows chemoselectivity (O vs N) and regioselectivity (6-*endo* vs 5-*exo*) problems, due to the bidentate nature of both the nucleophile (amide) and the electrophile (alkyne).[38] In particular, it has been recently reported that the cycloisomerization of some scaffolds closely related to **14** afforded either N-cyclization or O-cyclization products depending on the nature of the counterion of the metal catalyst.[39] A brief screening of gold- and silver-based catalytic systems (see Supporting Information for details) resulted in the selection of 1,3-bis(diisopropylphenyl)imidazol-2-ylidene gold(I) hexafluoroantimonate ($IPrAuSbF_6$, 5 mol %) as the catalyst of choice, able to give the pyrimidoindolones **15a−f** in a chemo- and regiospecific fashion from fair to excellent yields (Table 2).

MediaChrom dyes **15** were designed with the linker protected as the benzyl ester to be easily deprotected, transforming the MediaChrom *dyes* **15** in the carboxy-free MediaChrom *labels* **15′**.

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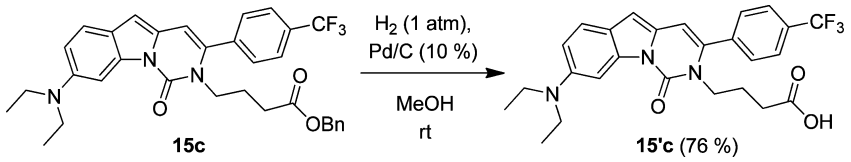
Scheme 5. Deprotection of the Carboxy Terminus of MediaChrom 15c



Because MediaChrom **15c** displayed the most interesting photophysical properties (see below), it was chosen as the lead compound to be transformed in the carboxy-free derivative **15′c** for biomolecular labeling. Debenzylation of the carboxy terminus was obtained by treatment of **15c** under heterogeneous reductive conditions (H_2, Pd/C) in methanol at rt to give **15′c** in 76% yield (Scheme 5).

The structure of product **15′c** was unequivocally confirmed by NOESY and HSQC experiments (see Supporting Information for details), giving insights indirectly on the entire library of MediaChrom dyes **15**.

Photophysical Evaluation of MediaChrom Dyes. The spectroscopic properties of MediaChrom dyes **15** were first characterized by collecting absorption spectra (Table 3). The well-known polarity-sensitive dye Prodan was chosen as the basis for comparison.

Table 3. Onsager Radius and Absorption Properties of MediaChrom Dyes 15a–f Compared to Prodan (20 μg/mL, ethanol)

dye	Onsager radius (Å)	$\lambda_{absorption}$ [a] (nm)	ε (mM^{-1} cm^{-1})
15a	5.7162	401	8.5
15b [b]	5.5919	456	7.6
15c	5.6943	393	13.8
15d	5.6234	409	15.4
15e [c]	5.6323	369	17.1
15f	5.6100	377	17.5
Prodan	4.3628 [d]	360 [d]	18.4 [d]

[a]As the wavelength of the most red-shifted peak. [b]C = 0.5 mg/mL. [c]Poor solubility. [d]From ref 13.

All MediaChrom dyes showed intense absorption peaks, spanning from 369 to 456 nm. MediaChrom **15b**, which is the dye with the most red-shifted absorption band, exhibits lower absorption intensity. In order to obtain acceptable quality spectra, a 25-fold more concentrated solution was prepared. The presence of the stronger EW group (nitro group) is responsible of the marked red shift, typically observed when the conjugation of the chromophoric system is increased. The comparison of the spectra of MediaChrom **15a** and **15c**, characterized by the presence of the same ED group ($-NMe_2$) but different EW groups ($-SO_2Me$ vs $-CF_3$), indicates that the presence of a mesyl resulted in a 8 nm red-shifted absorption peak, with respect to the trifluoromethyl group. A similar effect was observed by comparison of the absorption peaks of MediaChrom **15e** and **15f**, where the same ED group (morpholine) was introduced. In these dyes, the presence of the morpholine substituent causes a blue shift of the absorption peaks. MediaChrom **15d** seemed to be the most promising fluorophore for imaging applications since an interesting red-shifted absorption peak is accompanied by good absorption intensity (Table 3). In fact, the availability of a dye that can be excited in the visible range with many cheap and accessible light excitation sources represents a remarkable benefit.

Next, all dyes were characterized for their fluorescence solvatochromic properties by collecting fluorescence spectra at 20 °C in solvents at different polarities, such as hexane, *n*-octanol, ethanol, and DMF (Table 4). All MediaChrom dyes exhibited

Table 4. Solvatochromic Properties of MediaChrom Dyes 15a–f

	$\lambda_{emission}$ (nm) [a]					
solvent	**15a**	**15b**	**15c**	**15d**	**15e**	**15f**
hexane	525	594	490	525	512	482
n-octanol	585	–	525	585	568	514
ethanol	595	–	540	595	582	528
DMF	605	–	565	615	597	553

[a]Excitation wavelength; see Table 3.

fluorescence emissions in all solvents with a solvatochromic shift spanning over 90 nm from hexane to DMF. The only exception is **15b**, which showed significant fluorescence only in hexane (Table 4). This high sensitivity is comparable to that observed for many commercially available solvatochromic probes (e.g., Prodan, Laurdan, Dansyl).

For each MediaChrom dye, the integration of the emission peak in different solvents was calculated and normalized to the peak with the maximum intensity between them to evaluate the relative intensity of fluorescence emission in solvents with different polarity (Figure 2).

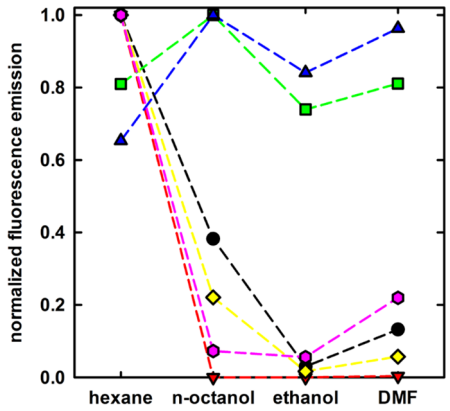


Figure 2. Normalized fluorescence emission peak of MediaChrom dyes at 1.6 μg/mL concentration in different solvents at 20 °C: **15a** (black circles), **15b** (red triangles), **15c** (green squares), **15d** (yellow diamonds), **15e** (magenta hexagon), and **15f** (blue triangles).

Figure 2 shows that only MediaChrom **15c** and **15f** preserve an almost constant fluorescence emission in all tested solvents over a wide range of polarity, together with a simultaneous large change in the Stokes shift. These properties make both of these molecules good candidates for several purposes, ranging from structural and dynamic studies on biological macromolecules to cell imaging applications. When absorption and emission

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Table 5. Comparison of the Absorbance and Fluorescence Properties of 15c and Prodan in Four Solvents with Different Polarities

	λ_{max} (abs), nm		ε (mM^{-1} cm^{-1})[a]		λ_{max} (fluor), nm[b]		QY[c] (%)	
solvent	Prodan	**15c**	Prodan	**15c**	Prodan	**15c**	Prodan	**15c**
hexane	346	396	36.8	14.6	412	480	60.4	51.5
n-octanol	362	397	33.6	14.0	473	522	59.9	52.4
ethanol	368	393	19.9	13.8	487	540	71.0	39.9
DMF	355	396	39.3	13.7	455	562	64.2	36.9

[a]Absorption spectra acquired at four different concentrations (**15c** from 139 to 13.9 μM, Prodan from 100 to 10 μM). [b]Excitation wavelength: 380 nm, [dye] = from 2.5 to 5 μM. [c]Quantum yield values were corrected for the solvent refractive index.

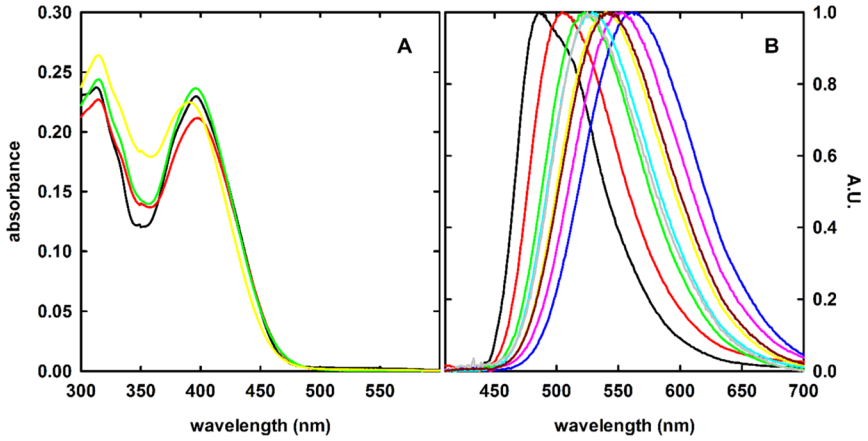

Figure 3. Absorption (A) and normalized fluorescence (B) spectra of **15c** in different solvents at 20 °C. **15c** absorbance spectra in hexane (black line), *n*-octanol (red line), DMF (green line), and methanol (yellow line). (B) **15c** normalized fluorescence spectra (excitation wavelength = 380 nm) in hexane (black line), triethylamine (red line), *n*-octanol (green line), chloroform (gray line), *n*-butanol (light blue line), ethanol (yellow line), methanol (brown line), acetone (magenta line), and DMF (blue line).

properties are combined, **15c** is considered to be the best candidate because it requires a red-shifted excitation with respect to **15f** (see Table 3). In fact, in applications such as fluorescent labeling for user-friendly detection kits or dyes for cell imaging, the possibility to work at longer wavelength guarantees a reduced biological tissue autoflorescence and scattering and/or decreased cell damage. For these reasons, we focused on MediaChrom **15c**, and its properties were compared to the properties of the commercial solvatochromic probe Prodan. The polarity-sensitive properties of **15c** were investigated by collecting absorbance and fluorescence spectra in solvents at different polarities. Fluorescence quantum yields were determined by taking Prodan in ethanol (quantum yield, QY = 71%) as a reference.[13] For spectroscopic measurements, solutions of the dyes at different concentrations (from 2.5 to 5 μM) were both excited at 380 nm, with an average excitation wavelength between the λ_{max} of absorbance of Prodan and MediaChrom **15c**. Results are reported in Table 5 and Figure 3.

The absorption spectra of **15c** in different solvents did not show significant differences (Figure 3A). In all investigated solvents, the most red-shifted absorption peak lies around 395 ± 2 mn. This property guarantees that **15c** can be excited at the same excitation wavelength (for example, with a common 405 nm diode laser), irrespective to the polarity of the solvent. Conversely, Prodan displays a pronounced difference in the maximum of absorbance depending on the nature of the solvent (from 346 to 368 nm), together with an unfavorable blue-shifted absorption maximum (ranging from −25 nm in ethanol to −50 nm in hexane, compared to **15c**). The absorption coefficients (ε) of **15c** in the four solvents tested are from 13.7 to 14.6 mM^{-1} cm^{-1}. In all solvents (except for ethanol), they are lower than those observed for Prodan. The fluorescence spectra of MediaChrom **15c** in solvents with different polarities show that the dye exhibits a Stokes shift from 84 nm in hexane to 166 nm in DMF (Table 5 and Figure 3B). This large solvatochromic shift (82 nm) suggests that the chromophore is able to detect even small polarity changes. Moreover, the emission maxima are also strongly red-shifted with respect of those of Prodan. Despite being lower than those of Prodan, the quantum yields of MediaChrom **15c** in all examined solvents are good.

To describe quantitatively the effects of the physical properties of the solvent on the fluorescent emission spectra of the dye, the Lippert-Mataga equation was used (see eq 2 in Experimental Section).[40] This correlation is based on the assumption that the solvent is a continuum in which the fluorophore is contained, and solvent-specific interactions are not considered. It can be approximated that the energy difference between the ground and the excited states is a property of the refractive index (n) and the dielectric constant (ε) of the solvents. In Figure 4, the orientational polarizability (Δf) is plotted against the Stokes shift (in cm^{-1}) for MediaChrom **15c** and compared to Prodan under the same experimental conditions.

For both fluorophores, a clear dependence of the Stokes shift on orientational polarizability of the solvent was observed. It is worth noting that the Stokes shifts for MediaChrom **15c** in protic solvents are weaker than those for aprotic solvents of similar polarity (Figure 4, red triangles vs black triangles), while Prodan displays exactly the opposite trend (Figure 4, red circles vs black circles). These phenomena are usually retraced to specific interactions of the fluorophore with the solvent. For example, in Prodan, this phenomenon was related to H-bonding of the protic solvents with the carbonyl group of the fluorophore.[14] The

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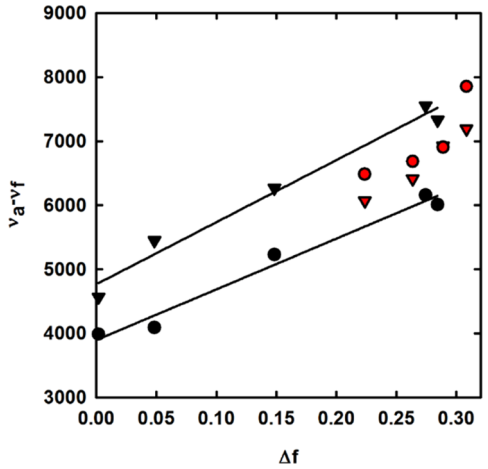

Figure 4. Lippert plot for MediaChrom **15c** (triangles) and Prodan (circles) in aprotic (black) and protic (red) solvents.

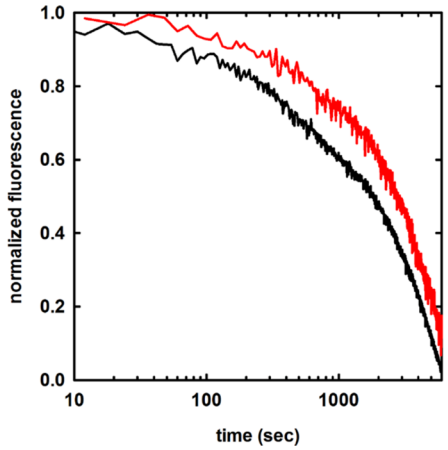

Figure 5. Photodegradation test for MediaChrom **15c** (red line) and Prodan (black line) in ethanol (c = 5 μM, excitation wavelength = 380 nm).

reverse effect observed in MediaChrom **15c** could be related to a particular interaction with the solvent, probably due to the presence of the uncommon trifluoromethyl group in the D-π−A system.

Since the Lippert-Mataga equation usually describes fluorophore behavior in aprotic solvents well, only data for both dyes in aprotic solvents were used to fit and determine the change in dipole moment upon excitation ($\mu^*-\mu$).

MediaChrom **15c** and Prodan Stokes shifts showed a well-defined dependence to orientational polarizability, considering all solvents. In particular, data obtained in aprotic solvents showed a good correlation, with a $\mu^*-\mu$ of 13.3 ± 0.6 and 8.1 ± 0.4 D, respectively. For all other MediaChrom dyes, which showed similar solvatochromic shifts, the dipole moment changes fall in the 13.0−13.4 D range (see Table S1 in Supporting Information). This finding demonstrates that, regardless of the substituent pattern, the charge transfer process is similar. Moreover, the differences in the Stokes shift suggest an effect of substituents on the non-emitting energy loss in the excited state.

To estimate the photostability of MediaChrom **15c**, we performed a photodegradation test. Ethanol solutions of **15c** and Prodan were prepared in two different quartz cuvettes and illuminated at 380 nm with a xenon lamp for 100 min, while the fluorescence signal was recorded as a function of time. The power density applied to the sample was 6.7 mW/cm^2. As depicted in Figure 5, MediaChrom **15c** decays significantly slower than Prodan, showing a halved emission after 2800 s, while the latter is after 1750 s.

Based on these results, the comparison between MediaChrom **15c** and Prodan properties suggests that, in spite of exhibiting a slightly reduced quantum yield, the former possesses photochemical properties comparable to those of the commercial dye Prodan, with the advantage of a strong, red-shifted absorption and fluorescence emission and an increased photostability.

Behavior of MediaChrom 15c in Dipalmitoylphosphatidylcholine (DPPC) Vesicles. Prodan dye is widely used for probing the membrane state, including transition phases and oxidation processes of phospholipid systems, due to its capability of penetrating in the lipophilic layer and sensing polarity changes.[41] In order to evaluate the potential of MediaChrom **15c** as a membrane probe, we collected fluorescence spectra of both Prodan and MediaChrom **15c** after their addition to a DPPC vescicles suspension at different temperatures. It has already been observed that an increase in temperature causes a change in the fluorescence spectrum of Prodan. This is due to the transition of the phospholipid system from the gel to the liquid phase that allows more water molecules to enter into the lipid phase.[42] The fluorescence spectra of MediaChrom **15c** embedded in DPPC vesicles showed an emission peak centered at 528 nm (Figure 6), very similar to that observed when it is dissolved in *n*-octanol (525 nm) at the same temperature (20 °C). This result suggests that the fluorophore lies in the lipid phase of DPPC vesicles, probing this region. The comparison of the behavior of MediaChrom **15c** and Prodan clearly demonstrates that the former is able to sense the transition phase of phospholipid systems similarly to Prodan, with the advantage of an emission wavelength red-shifted by 100 nm, significantly reducing scattering effects that are considerably high in micellar suspension as well as when cellular membranes are present.

MediaChrom 15′ as a Peptide Label. As described above, MediaChrom dyes **15** were designed with a benzyl ester to be easily deprotected, transforming the MediaChrom *dyes* **15** into carboxy-free MediaChrom *labels* **15′**.[43] This transformation makes these dyes suitable for an easy conjugation with proteins, peptides, or an amino-modified oligonucleotide. These labeled bioconjugates could thus be used for the detection of interactions involving a change of polarity of the system, such as the protein/DNA binding.

As a proof of concept, we selected the protein Cro, whose interaction with its consensus DNA sequence (O_R3) is known and widely investigated.[44] In particular, Cro is a 66 amino acids dimeric protein that plays a key role in the switch from the lysogenic to the lytic cycle in bacteriophage λ. Its interaction with DNA is mainly restricted to a small helix-turn-helix motif spanning from residue 15 to 38,[45] with a K_d of around 25 μM.[46] The wild-type DNA binding sequence (GQTKTAKDLGVYQSAINKAIHAG) was prepared by microwave-assisted solid-phase synthesis[47] and on resin labeled at the N-terminus with the MediaChrom **15′c** using standard protocols.[48]

The fluorescence spectra of **15′c**-labeled Cro:1 in the presence and in the absence of O_R3 consensus DNA sequence were recorded (Figure 7).

In the absence of a cognate DNA sequence, the labeled peptide **15′c**-Cro:1 displayed a maximum of fluorescence at 545 nm (Figure 7, black line). Upon addition of the O_R3 consensus

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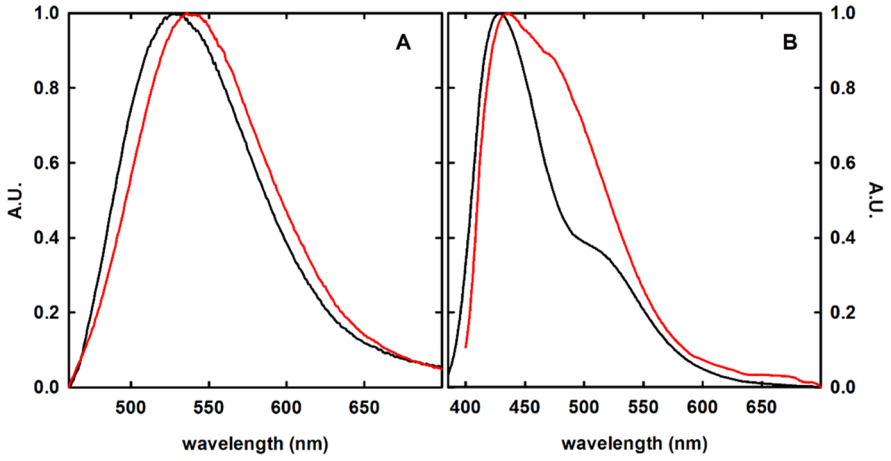

Figure 6. Fluorescence spectra of 1.6 μg/mL MediaChrom **15c** (A) and Prodan (B) in DPPC vesicles at different temperatures: 20 °C (black lines) and 37 °C (red lines). Excitation wavelength = 380 nm.

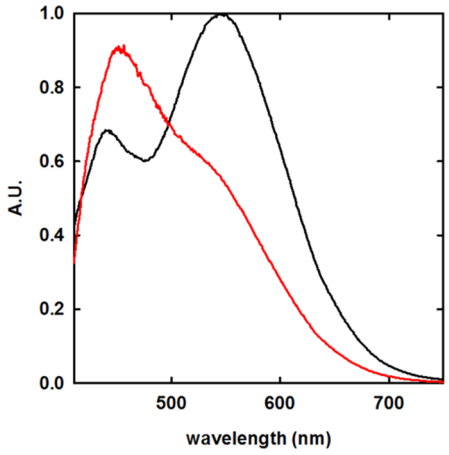

Figure 7. Normalized fluorescence spectra of peptide in the presence (red line) and in the absence (black line) of O_R3 consensus sequence (**15′c**-Cro:1 50 μM, O_R3 50 μM, PBS buffer, pH 7.4, 20 °C). Excitation wavelength = 380 nm.

sequence, a strong hypsochromic shift (93 nm) was observed (Figure 7, red line), thus indicating that the MediaChrom **15′c** sensitively detected the change in the polarity of the environment when the peptide interacts with the DNA sequence. The minor peak present in the **15′c**-Cro:1 spectrum and the shoulder in the peak of the **15′c**-Cro:1-O_R3 complex suggests that there is an equilibrium of two discrete peptide conformations, clearly signaled by the dye. The binding of O_R3 consensus sequence shifts the peptide conformational equilibrium from one state to the other.

CONCLUSIONS

We synthesized a six-membered library of polarity-sensitive fluorescent dyes called MediaChrom, characterized by a pyrimidoindolone skeleton endowed with a conjugated push–pull system. The modular synthesis involves eight steps starting from simple and commercially available materials, with overall yields up to 19%. An added value of MediaChrom dyes is the presence of a linker (whose length can be modulated) with a protected carboxy terminus that allows the handy conjugation with biomolecules, making possible the transformation of the *dyes* in useful fluorescent solvatochromic *labels*. All MediaChrom dyes display interesting photophysical profiles. Among them, MediaChrom **15c** shows the best features for biological applications: a high absorption coefficient in the visible range that is almost constant in solvents with different polarities, a wide solvatochromic effect combined with an almost constant fluorescence emission, a good QY, and a noteworthy photostability.[49] In general, MediaChrom **15c** displays some advantage compared to the well-known solvatochromic fluorophore Prodan. The photophysical features of **15c** made it competitive also with the more recently developed small size polarity-sensitive organic dyes, such as the structural analogues of Prodan based on anthracene[14] and fluorene[15] cores. For these reasons, MediaChrom **15c** was taken as the lead compound to test some conceivable applications. MediaChrom **15c** was demonstrated to be a versatile solvatochromic dye that can be used as a membrane/lipophilic probe, and its parent carboxy-free MediaChrom **15′c** was demonstrated to be a highly sensitive label for peptide tagging, useful for studying the interaction between peptides (or proteins) and other target biomolecules, such as DNA. Current efforts in our laboratories are now devoted to investigate some other biological applications of MediaChrom dyes in vivo, such as prokaryote/eukaryote cell stains and probes.

EXPERIMENTAL SECTION

General. Anhydrous solvents are commercially available and stored in a protected atmosphere of nitrogen. All the reactions that involve the use of reagents sensitive to oxygen or hydrolysis were carried out under nitrogen. The glassware was previously dried in an oven at 110 °C and set with cycles of vacuum and nitrogen. The chromatographic column separations were performed by a flash technique, using silica gel (pore size 60 Å, particle size 230–400 mesh). TLC Al foils with a fluorescent indicator (254 nm) were used for TLC analysis, and the detection was performed by irradiation with UV light (λ = 254 nm and/or 366 nm); ^{1}H NMR analyses were performed with 200 or 300 MHz spectrometers at rt. Spectra were referenced to residual chloroform (7.27 ppm, ^{1}H, 77.0 ppm, ^{13}C). The coupling constants (J) are expressed in hertz (Hz) and the chemical shifts (δ) in parts per million; ^{13}C NMR analysis were performed with the same instruments at 50.3 and 75.45 MHz. The attached proton test sequence (APT) was used to distinguish the methine and methyl carbon signals from those arising from methylene and quaternary carbon atoms. All ^{13}C NMR spectra were recorded with complete proton decoupling. The ^{1}H NMR signals of MediaChrom **15′c** described in the following have been attributed by correlation spectroscopy (COSY) and nuclear Overhauser effect spectroscopy (NOESY) techniques. Infrared spectra were recorded using discs of NaCl for liquid samples and KBr tablets for solid samples. The absorbance is reported in wavenumbers (cm^{-1}) with values between

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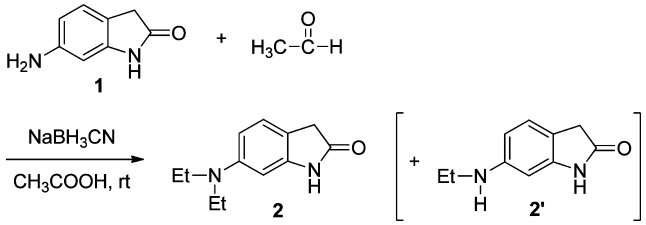
4000 and 400 cm^{-1}. Low-resolution MS spectra were recorded with electron impact source and electrospray/ion trap instruments, using a syringe pump device to directly inject sample solutions. The values are reported as mass-charge ratio, and the relative intensities of the most significant peaks are shown in brackets. The melting points of the solid products are uncorrected. UV−visible and fluorescence spectra were collected at 20 °C. 6-Aminoindolin-2-one (**1**) was prepared according to the literature.[25]

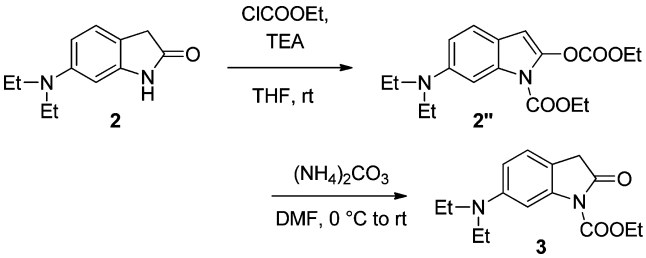
Synthesis of 6-(Diethylamino)indolin-2-one (2).



$NaBH_3CN$ (190 mg, 3.02 mmol) was added to a solution of 6-aminoindolin-2-one (**1**) (180 mg, 1.21 mmol) in glacial AcOH (2 mL) followed by acetaldehyde (0.48 mL, 373 mg, 8.47 mmol), and the mixture was stirred at rt for 24 h. The suspension was concentrated in vacuo, poured into H_2O (25 mL), and extracted with EtOAc (3 × 20 mL). The organic layers were collected, dried over Na_2SO_4, and filtered, and the solvent was removed at reduced pressure. The crude material was purified by flash chromatography over a silica gel column using CH_2Cl_2/MeOH (99:1) to afford the desired product **2** as a yellow solid (198 mg, 80%): mp 106−108 °C; R_f = 0.13 (silica gel, EtOAc/AcOH 3%), 0.31 (silica gel, CH_2Cl_2/MeOH 98:2); IR (KBr) ν_{max} = 3183, 2966, 2934, 1701, 1632, 1514, 1353, 1123, 1111, 770 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ = 1.04 (t, 6H, CH_3, J = 7.0 Hz), 3.20−3.30 (m, 6H, CH_2), 6.12 (d, 1H, CH, J = 2.3 Hz), 6.19 (dd, 1H, CH, J = 8.2, 2.3 Hz), 6.92 (d, 1H, CH, J = 8.2 Hz), 10.09 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50.3 MHz) δ = 177.8 (C═O), 148.1 (C_q), 145.5 (C_q), 125.5 (CH), 112.3 (C_q), 105.2 (CH), 94.4 (CH), 44.6 (CH_2), 35.7 (CH_2), 13.1 (CH_3); ESI-MS m/z (%) 205 $[M + 1]^+$ (100). Anal. Calcd for $C_{12}H_{16}N_2O$: C, 70.56; H, 7.90; N, 13.71. Found: C, 70.67; H, 7.98; N, 13.54.

In addition, a modest amount of the corresponding monoethylated derivative **2′** (6-(ethylamino)indolin-2-one) was obtained: yellow/orange solid (21 mg, 10%); mp 165−169 °C (decomp.); R_f = 0.40 (silica gel, EtOAc/AcOH 3%), 0.19 (silica gel, CH_2Cl_2/MeOH 98:2); IR (KBr) ν_{max} = 3371, 3193, 2965, 2917, 1693, 1628, 1519, 1335, 1193, 1172, 1110, 801 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ = 1.12 (t, 3H, CH_3, J = 7.0 Hz), 2.95 (dq, 2H, CH_2, J = 7.0, 5.1 Hz), 3.24 (s, 2H, CH_2), 5.46 (bt, 1H, NH, J = 5.1 Hz), 6.06−6.10 (m, 2H, CH), 6.84 (d, 1H, CH, J = 8.1 Hz), 10.09 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50.3 MHz) δ = 177.9 (C═O), 149.6 (C_q), 145.1 (C_q), 125.2 (CH), 112.6 (C_q), 105.2 (CH), 94.8 (CH), 38.2 (CH_2), 35.8 (CH_2), 15.0 (CH_3); ESI-MS m/z (%) 177 $[M + 1]^+$ (100). Anal. Calcd for $C_{10}H_{12}N_2O$: C, 68.16; H, 6.86; N, 15.90. Found: C, 67.96; H, 6.72; N, 15.73.

Synthesis of Ethyl 6-(Diethylamino)-2-oxoindoline-1-carboxylate (3).

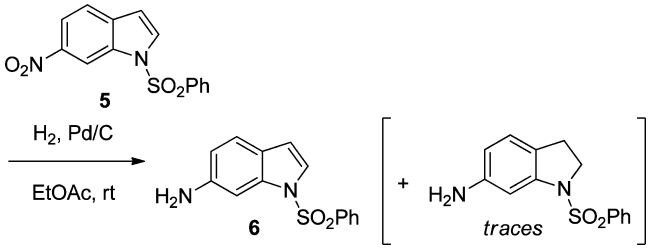


To a solution of 6-(diethylamino)indolin-2-one (**2**) (250 mg, 1.22 mmol) and TEA (0.39 mL, 285 mg, 2.81 mmol) in THF (6 mL) was added ethyl chlorocarbonate (0.26 mL, 292 mg, 2.69 mmol) dropwise. The temperature was kept below 30 °C during the addition. After being stirred for 4.0 h at rt, the solvent was evaporated. Water (5 mL) was added to the residue, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were collected, dried over Na_2SO_4, and filtered, and the solvent was removed at reduced pressure. The crude material was purified by flash chromatography over a silica gel column using Hex/EtOAc (90:10) to afford the intermediate N,O-diacylated product **2″** (ethyl 6-(diethylamino)-2-((ethoxycarbonyl)oxy)-*1H*-indole-1-carboxylate) as a yellow wax (404 mg, 95%), which darkened rapidly on exposure to air and light: mp 50−51 °C; R_f = 0.49 (silica gel, EtOAc/AcOH 3%), 0.51 (silica gel, Hex/EtOAc 80:20); IR (KBr) ν_{max} = 2976, 2934, 1779, 1739, 1615, 1502, 1378, 1326, 1275, 1239, 1133, 1102, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.18 (t, 6H, CH_3, J = 7.0 Hz), 1.36−1.47 (m, 6H, CH_3), 3.39 (q, 4H, CH_2, J = 7.0 Hz), 4.35 (q, 2H, CH_2, J = 7.0 Hz), 4.44 (q, 2H, CH_2, J = 7.0 Hz), 6.16 (s, 1H, CH), 6.71 (dd, 1H, CH, J = 8.7, 2.2 Hz), 7.29 (d, 1H, CH, J = 8.7 Hz), 7.51 (d, 1H, CH, J = 2.1 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 153.1 (C_q), 150.8 (C_q), 146.5 (C_q), 139.4 (C_q), 135.0 (C_q), 121.2 (CH), 116.7 (C_q), 110.8 (CH), 99.8 (CH), 97.2 (CH), 65.7 (CH_2), 63.2 (CH_2), 45.4 (CH_2), 14.4 (CH_3), 14.3 (CH_3), 12.7 (CH_3); ESI-MS m/z (%) 349 $[M + 1]^+$ (100). Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 62.35; H, 7.12; N, 7.82.

To a solution of N,O-diacylated product **2″** (375 mg, 1.08 mmol) in DMF (3 mL) was added finely powdered ammonium carbonate (103 mg, 1.08 mmol) at 0−5 °C. The mixture was stirred for 5.0 h at rt then poured into ice−water (20 mL) and extracted with EtOAc (3 × 20 mL). Combined organic phases were washed with water (100 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography over a silica gel column using Hex/EtOAc (90:10) to afford the desired product **3** as a yellow solid (212 mg, 71%), which darkened rapidly on exposure to air and light: mp 58−60 °C; R_f = 0.42 (silica gel, Hex/EtOAc 80:20); IR (KBr) ν_{max} = 2972, 2905, 1760, 1732, 1624, 1513, 1369, 1305, 1266, 1055, 767 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.17 (t, 6H, CH_3, J = 7.0 Hz), 1.44 (t, 3H, CH_3, J = 7.0 Hz), 3.37 (q, 4H, CH_2, J = 7.0 Hz), 3.57 (s, 2H, CH_2), 4.46 (q, 2H, CH_2, J = 7.0 Hz), 6.45 (dd, 1H, CH, J = 8.0, 2.2 Hz), 7.04 (d, 1H, CH, J = 8.0 Hz), 7.32 (d, 1H, CH, J = 2.2 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 174.4 (C_q), 151.4 (C_q), 148.3 (C_q), 142.2 (C_q), 124.8 (CH), 109.6 (C_q), 108.1 (CH), 99.8 (CH), 63.3 (CH_2), 45.0 (CH_2), 36.1 (CH_2), 14.5 (CH_3), 12.7 (CH_3); ESI-MS m/z (%) 277 $[M + 1]^+$ (100). Anal. Calcd for $C_{15}H_{20}N_2O_3$: C, 65.20; H, 7.30; N, 10.14. Found: C, 65.09; H, 7.24; N, 10.24.

Synthesis of 6-Nitro-1-(phenylsulfonyl)-1*H*-indole (5). $PhSO_2Cl$ (4.7 mL, 6.53 g, 37.00 mmol) was added dropwise at 0 °C to a mixture of 6-nitroindole (3.00 g, 18.50 mmol) and K_2CO_3 (6.39 g, 46.25 mmol) in acetone (66 mL). The reaction mixture was stirred at rt for 24 h. The residue was poured into H_2O (200 mL) and extracted with EtOAc (3 × 150 mL), washed with brine, and dried over Na_2SO_4. The solvent was removed at reduced pressure, and then the crude product was purified by flash chromatography over a silica gel column using Hex/EtOAc (from 90:10 to 1:1) to afford the desired product **5** as a pale yellow solid (5.37 g, 96%): mp 199−201 °C (decomp.); R_f = 0.24 (silica gel, Hex/EtOAc 80:20); IR (KBr) ν_{max} = 3117, 3107, 1593, 1508, 1371, 1342, 1144, 727 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 6.77 (d, 1H, C_3indole-H, J = 3.7 Hz), 7.46−7.58 (m, 3H, CH), 7.63 (d, 1H, CH, J = 8.8 Hz), 7.84 (d, 1H, C_2indole-H, J = 3.7 Hz), 7.94 (d, 2H, CH, J = 7.0 Hz), 8.14 (dd, 1H, CH, J = 8.8, 1.9 Hz), 8.91 (d, 1H, CH, J = 1.8 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 145.4 (C_q), 137.9 (C_q), 135.7 (C_q), 134.8 (CH), 133.8 (C_q), 131.4 (CH), 129.9 (CH), 127.2 (CH), 121.8 (CH), 118.9 (CH), 110.1 (CH), 109.0 (CH); ESI-MS m/z (%) 325 $[M + Na]^+$ (100), 303 $[M + 1]^+$ (15). Anal. Calcd for $C_{14}H_{10}N_2O_4S$: C, 55.62; H, 3.33; N, 9.27. Found: C, 55.39; H, 3.36; N, 8.95.

Synthesis of 6-Amino-1-(phenylsulfonyl)-1*H*-indole (6).



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To a solution of 6-nitro-1-(phenylsulfonyl)-1*H*-indole (**5**) (2.50 g, 8.27 mmol) in EtOAc (120 mL) was added Pd/C (10 wt %, 250 mg). The mixture was charged with hydrogen and stirred at rt for 7.0 h. The crude was filtered over Celite and washed with EtOAc. The solution was concentrated under reduced pressure. The crude product was purified by flash chromatography over a silica gel column using Hex/CH_2Cl_2/TEA (60:40:10) to afford the desired product (**6**) as a pale orange wax (2.09 g, 93%) (containing inseparable traces of a byproduct derived from the reduction of the C2−C3 indole double bond); R_f = 0.24 (silica gel, Hex/EtOAc 70:30); IR (NaCl) ν_{max} = 3384, 2927, 1622, 1359, 1172, 1120, 727 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 3.68 (bs, 2H, NH_2), 6.52 (d, 1H, C_3indole-H, J = 3.7 Hz), 6.62 (dd, 1H, CH, J = 8.4, 1.8 Hz), 7.24−7.56 (m, 6H, CH), 7.86 (d, 2H, CH, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 144.7 (C_q), 138.7 (C_q), 136.7 (C_q), 133.8 (CH), 129.4 (CH), 126.9 (CH), 124.2 (CH), 123.3 (C_q), 122.1 (CH), 113.2 (CH), 109.6 (CH), 99.6 (CH); ESI-MS m/z (%) 273 $[M + 1]^+$ (100), 295 $[M + Na]^+$ (50). Elemental analysis was not performed because the product contains traces of the indoline derivative.

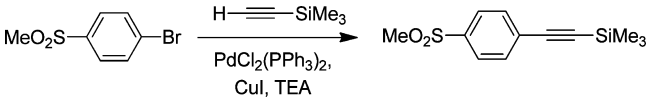
Synthesis of 6-(*N*,*N*-Diethylamino)-1-(phenylsulfonyl)-1*H*-indole (7). Under a nitrogen atmosphere, to a solution of 6-amino-1-(phenylsulfonyl)-1*H*-indole (**6**) (2.00 g, 7.34 mmol) in dry DMSO (30 mL) was added KOH 85% (969 mg, 14.68 mmol). The solution was stirred under rt, then ethyl bromide (2.2 mL, 3.20 g, 29.36 mmol) was added, and the reaction mixture was stirred at 50 °C. After 6.0 h, another 2 equiv of ethyl bromide was added, and the reaction mixture was stirred overnight at 50 °C until no more starting product was detected by TLC analysis. Upon completion, the reaction mixture was quenched by H_2O (400 mL) and extracted with EtOAc (3 × 300 mL). Combined organic phases were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography over a silica gel column using Hex/EtOAc (95:5) to afford the desired product **7** as a yellow solid (1.57 g, 65%) with traces of the corresponding monoethylated derivative (not isolated nor characterized): mp 78−80 °C; R_f = 0.46 (silica gel, Hex/EtOAc 80:20); IR (KBr) ν_{max} = 3413, 2971, 2930, 1622, 1501, 1359, 1173, 1119, 728 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.18 (t, 6H, CH_3, J = 7.0 Hz), 3.40 (q, 4H, CH_2, J = 7.0 Hz), 6.50 (d, 1H, C_3indole-H, J = 3.7 Hz), 6.67 (dd, 1H, CH, J = 8.8, 2.2 Hz), 7.27−7.52 (m, 6H, CH), 7.85 (d, 2H, CH, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 146.5 (C_q), 138.8 (C_q), 137.4 (C_q), 133.7 (CH), 129.3 (CH), 127.0 (CH), 123.6 (CH), 121.8 (CH), 121.0 (C_q), 110.8 (CH), 109.4 (CH), 96.8 (CH), 45.3 (CH_2), 12.7 (CH_3); ESI-MS m/z (%) 329 $[M + 1]^+$ (100), 351 $[M + Na]^+$ (20). Anal. Calcd for $C_{18}H_{20}N_2O_2S$: C, 65.83; H, 6.14; N, 8.53. Found: C, 65.55; H, 6.03; N, 8.53.

Synthesis of 6-(*N*,*N*-Diethylamino)-2-iodo-1-(phenylsulfonyl)-1*H*-indole (8). The lithium diisopropylamide (LDA) solution was freshly prepared as follows: under a nitrogen atmosphere, a solution of diisopropylamine (0.41 mL, 294 mg, 2.90 mmol) in dry THF (3.8 mL) was cooled to −78 °C and a solution of *n*-butyllithium (1.6 M in hexanes, 1.6 mL, 2.56 mmol) was added. The solution was stirred at −78 °C for 10 min, warmed to 0 °C, and stirred for 10 min, then cooled back to −78 °C. Under a nitrogen atmosphere, to a solution of 6-(*N*,*N*-diethylamino)-1-(phenylsulfonyl)-1*H*-indole (**7**) (700 mg, 2.13 mmol) and TMEDA (0.38 mL, 297 mg, 2.56 mmol) in dry THF (11 mL) was added the freshly prepared LDA solution via syringe over 10 min at −78 °C. After being stirred for 1.5 h at −78 °C, the yellow solution was treated dropwise over 30 min with a solution of iodine (541 mg, 2.13 mmol) in dry THF (3.8 mL), and the mixture was allowed to warm slowly to rt overnight. The reaction mixture was cooled to 0−5 °C, treated with 5% aqueous sodium thiosulfate (80 mL), and extracted with EtOAc (3 × 70 mL). Combined organic phases were washed again with sodium thiosulfate (200 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography over a silica gel column using Hex/EtOAc (98:2) to afford the desired product (**8**) as a pale brown wax (774 mg, 80%): R_f = 0.21 (silica gel, Hex/EtOAc 95:5); IR (NaCl) ν_{max} = 2970, 2928, 1615, 1503, 1373, 1197, 1173, 730 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.21 (t, 6H, CH_3, J = 7.0 Hz), 3.43 (q, 4H, CH_2, J = 7.0 Hz), 6.64 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.82 (s, 1H, C_3indole-H), 7.18 (d, 1H, CH, J = 8.8 Hz), 7.36−7.54 (m, 3H, CH), 7.58 (d, 1H, J = 2.2 Hz), 7.86 (d, 2H, CH, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 175.5 (C_q), 146.7 (C_q), 141.0 (C_q), 138.8 (C_q), 133.9 (CH), 129.2 (CH), 127.3 (CH), 125.0 (CH), 122.4 (C_q), 120.2 (CH), 110.9 (CH), 99.1 (CH), 45.3 (CH_2), 12.8 (CH_3); ESI-MS m/z (%) 455 $[M + 1]^+$ (100). Anal. Calcd for $C_{18}H_{19}IN_2O_2S$: C, 47.59; H, 4.22; N, 6.17. Found: C, 47.93; H, 4.41; N, 6.30.

Synthesis of 6-Morpholino-1-(phenylsulfonyl)-1*H*-indole (9). Under a nitrogen atmosphere, to a solution of 1-(phenylsulfonyl)-1*H*-indol-6-amine (**6**) (800 mg, 2.94 mmol) in dry DMF (12 mL) were added *N*,*N*-diisopropylethylamine (1.02 mL, 759 mg, 5.88 mmol) and bis(2-bromoethyl)ether (0.55 mL, 1.02 g, 4.41 mmol). The reaction mixture was stirred overnight at 90 °C until no more starting product was detected by TLC analysis. The residue was poured into saturated aqueous $NaHCO_3$ (200 mL) and extracted with EtOAc (3 × 150 mL), washed with brine, and dried over Na_2SO_4. The solvent was removed at reduced pressure, and then the crude product was purified by flash chromatography over a silica gel column using Hex/EtOAc (80:20) to afford the desired product (**9**) as a pale yellow solid (650 mg, 65%): mp 161−163 °C; R_f = 0.42 (silica gel, Hex/EtOAc 60:40); IR (KBr) ν_{max} = 3436, 3140, 2967, 2853, 1615, 1488, 1448, 1367, 1176, 1128, 724 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 3.20 (t, 4H, CH_2, J = 4.8 Hz), 3.91 (t, 4H, CH_2, J = 4.8 Hz), 6.56 (dd, 1H, C_3indole-H, J = 3.7, 0.7 Hz), 6.92 (dd, 1H, CH, J = 8.8, 2.2 Hz), 7.37−7.55 (m, 6H, CH), 7.82−7.88 (m, 2H, CH); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 149.7 (C_q), 138.6 (C_q), 136.5 (C_q), 133.9 (CH), 129.4 (CH), 126.9 (CH), 125.2 (CH), 124.5 (C_q), 121.9 (CH), 114.3 (CH), 109.4 (CH), 100.8 (CH), 67.1 (CH_2), 50.6 (CH_2); ESI-MS m/z (%) 343 $[M + 1]^+$ (100). Anal. Calcd for $C_{18}H_{18}N_2O_3S$: C, 63.14; H, 5.30; N, 8.18. Found: C, 62.88; H, 5.19; N, 8.00.

Synthesis of 2-Iodo-6-morpholino-1-(phenylsulfonyl)-1*H*-indole (10). The iodination reaction at position 2 was performed under the previously optimized reaction conditions (see the synthesis of compound **8**). Reagent **9** and product **10** are not separable through a standard chromatographic column (R_f = 0.13, silica gel, Hex/EtOAc 80:20). The yield (58%) was calculated via ^1H NMR (t_1 = 10 s) in the mixture of the two compounds obtained after a brief purification by flash chromatography over a silica gel column using Hex/EtOAc (80:20). Since the 2-iodinated indoles are quite unstable, the next Sonogashira coupling step was performed starting from this mixture; therefore, the iodurate compound (**10**) was not fully characterized.

Synthesis of 1-Ethynyl-4-(methylsulfonyl)benzene 11a.[50]



11a

Under a nitrogen atmosphere, to a solution of 1-bromo-4-(methylsulfonyl)benzene (500 mg, 2.13 mmol) in TEA (8.5 mL) were added ethynyltrimethylsilane (0.35 mL, 251 mg, 2.56 mmol) and *trans*-dichlorobis(triphenylphosphine)palladium(II) (30 mg, 0.04 mmol). The reaction was stirred at rt for 15 min, and then CuI (4 mg, 0.023 mmol) was added. The reaction mixture was stirred at 50 °C for 3.0 h until no more starting product was detected by TLC analysis. The solvent was then evaporated under reduced pressure, and the crude material was purified by flash chromatography over a silica gel column using Hex/EtOAc (85:15) to afford the desired trimethyl((4-(methylsulfonyl)phenyl)ethynyl)silane as a pale yellow solid (489 mg, 91%): mp 103−105 °C; R_f = 0.30 (silica gel, Hex/EtOAc 80:20); IR (KBr) ν_{max} = 2959, 2160, 1591, 1309, 1145, 866, 837, 534 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 0.26 (s, 9H, CH_3), 3.04 (s, 3H, CH_3), 7.62 (d, 2H, CH, J = 8.4 Hz), 7.87 (d, 2H, CH, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 140.1 (C_q), 132.9 (CH), 129.2 (C_q), 127.5 (CH), 103.1 (Csp), 99.4 (Csp), 44.7 (CH_3), 0.0 (CH_3); ESI-MS m/z (%) 275 $[M + Na]^+$ (100), 253 $[M + 1]^+$ (10). Anal. Calcd for $C_{12}H_{16}O_2SSi$: C, 57.10; H, 6.39. Found: C, 57.33; H, 6.42.

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To a stirred solution of trimethyl((4-(methylsulfonyl)phenyl)-ethynyl)silane (400 mg, 1.58 mmol) in MeOH (11 mL) was added K_2CO_3 (438 mg, 3.16 mmol). The reaction was stirred at rt for 3.0 h. The residue was poured into H_2O (100 mL) and extracted with CH_2Cl_2 (3 × 150 mL), washed with brine, and dried over Na_2SO_4. The solvent was removed at reduced pressure to give the desired product (**11a**) as an orange solid (279 mg, 98%): mp 100−101 °C; R_f = 0.42 (silica gel, Hex/EtOAc 70:30); IR (KBr) ν_{max} = 3242, 3018, 2106, 2923, 1590, 1300, 1146, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 3.05 (s, 3H, CH_3), 3.28 (s, 1H, CspH), 7.66 (d, 2H, CH, J = 8.4 Hz), 7.90 (d, 2H, CH, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 140.6 (C_q), 133.1 (CH), 128.2 (C_q), 127.6 (CH), 82.0 (Csp), 81.4 (Csp), 44.6 (CH_3); ESI-MS m/z (%) 203 $[M + Na]^+$ (100), 181 $[M + 1]^+$ (10). Anal. Calcd for $C_9H_8O_2S$: C, 59.98; H, 4.47. Found: C, 60.14; H, 4.56.

General Procedure for the Preparation of Compounds 12a−f. Under a nitrogen atmosphere, to a solution of *N,N*-diethyl-2-iodo-1-(phenylsulfonyl)-1*H*-indol-6-amine (**8**) (182 mg, 0.40 mmol) or 4-(2-iodo-1-(phenylsulfonyl)-1*H*-indol-6-yl)morpholine (**10**) (187 mg, 0.40 mmol) in dry DMF (1.6 mL) were added the appropriate alkyne (**11a−d**) (0.48 mmol), TEA (1.1 mL, 810 mg, 8.00 mmol), and tetrakis(triphenylphosphine)palladium(0) (18 mg, 0.016 mmol). The reaction was stirred at rt for 15 min, and then CuI (2 mg, 0.008 mmol) was added. The reaction mixture was stirred at rt until no more starting product was detected by TLC analysis. The reaction mixture was poured into H_2O (30 mL) and extracted with EtOAc (3 × 20 mL). The organic layers were collected, dried over Na_2SO_4, and filtered, and the solvent was removed at reduced pressure. The crude material was purified by flash chromatography over a silica gel column.

*N,N-Diethyl-2-((4-(methylsulfonyl)phenyl)ethynyl)-1-(phenylsulfonyl)-1H-indol-6-amine (**12a**).* Reaction time = 2.0 h. Eluent for chromatography: Hex/EtOAc (75:25). Yield 182 mg (90%). Brown solid: mp 168−170 °C (decomp.); R_f = 0.21 (silica gel, Hex/EtOAc 70:30); IR (KBr) ν_{max} = 3436, 2967, 2928, 2873, 2207, 1615, 1594, 1503, 1375, 1308, 1152, 1113, 588 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.24 (t, 6H, CH_3, J = 7.0 Hz), 3.08 (s, 3H, CH_3), 3.47 (q, 4H, CH_2, J = 7.0 Hz), 6.72 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.89 (s, 1H, C_3indole-H), 7.31−7.53 (m, 5H, CH), 7.76 (d, 2H, CH, J = 8.8 Hz), 7.90−7.96 (m, 4H, CH); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 148.0 (C_q), 139.8 (C_q), 139.6 (C_q), 139.0 (C_q), 133.9 (CH), 131.8 (CH), 129.3 (CH), 127.7 (CH), 127.0 (CH), 122.1 (CH), 119.6 (CH), 118.9 (C_q), 116.8 (C_q), 111.3 (CH), 96.8 (CH), 94.6 (Csp), 86.4 (Csp), 45.3 (CH_2), 44.7 (CH_3), 12.8 (CH_3) ppm (one C_q signal obscured); ESI-MS m/z (%) 529 $[M + Na]^+$ (100), 507 $[M + 1]^+$ (60). Anal. Calcd for $C_{27}H_{26}N_2O_4S_2$: C, 64.01; H, 5.17; N, 5.53. Found: C, 63.81; H, 5.12; N, 5.54.

*N,N-Diethyl-2-((4-nitrophenyl)ethynyl)-1-(phenylsulfonyl)-1H-indol-6-amine (**12b**).* Reaction time: 5.0 h. Eluent for chromatography: Hex/EtOAc (95:5). Yield 99 mg (52%). Brown/red solid: mp 149−151 °C; R_f = 0.30 (silica gel, Hex/EtOAc 80:20); IR (KBr) ν_{max} = 3436, 2968, 2925, 2870, 2201, 1618, 1593, 1504, 1375, 1334, 1278, 1170, 1104, 588 cm^{-1}; ^1H NMR ($CDCl_3$ 200 MHz) δ = 1.24 (t, 6H, CH_3, J = 7.0 Hz), 3.47 (q, 4H, CH_2, J = 7.0 Hz), 6.73 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.91 (s, 1H, C_3indole-H), 7.27−7.57 (m, 5H, CH), 7.72 (d, 2H, CH, J = 8.8 Hz), 7.92 (dd, 2H, CH, J = 7.0, 1.5 Hz), 8.24 (d, 2H, CH, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 148.1 (C_q), 147.0 (C_q), 139.9 (C_q), 139.0 (C_q), 133.9 (CH), 131.7 (CH), 130.4 (C_q), 129.3 (CH), 127.0 (CH), 124.0 (CH), 122.2 (CH), 119.9 (CH), 118.9 (C_q), 116.7 (C_q), 111.3 (CH), 96.7 (CH), 94.7 (Csp), 87.9 (Csp), 45.3 (CH_2), 12.8 (CH_3); ESI-MS m/z (%) 474 $[M + 1]^+$ (100), 496 $[M + Na]^+$ (80). Anal. Calcd for $C_{26}H_{23}N_3O_4S$: C, 65.94; H, 4.90; N, 8.87. Found: C, 66.13; H, 5.01; N, 8.76.

*N,N-Diethyl-1-(phenylsulfonyl)-2-((4-(trifluoromethyl)phenyl)-ethynyl)-1H-indol-6-amine (**12c**).* Reaction time: 6.0 h. Eluent for chromatography: Hex/CH_2Cl_2 (60:40). Yield 109 mg (55%). Dark red solid: mp 144.5−147.5 °C; R_f = 0.47 (silica gel, Hex/EtOAc 80:20), 0.19 (silica gel, Hex/CH_2Cl_2 60:40); IR (KBr) ν_{max} = 3435, 2975, 2936, 2213, 1614, 1506, 1368, 1322, 1172, 1120, 590 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.24 (t, 6H, CH_3, J = 7.0 Hz), 3.46 (q, 4H, CH_2, J = 7.0 Hz), 6.72 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.86 (s, 1H, C_3indole-H), 7.27−7.57 (m, 5H, CH), 7.60−7.78 (m, 4H, CH), 7.94 (dd, 2H, CH, J = 7.0, 1.5 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 147.8 (C_q), 139.7 (C_q), 139.1 (C_q), 133.9 (CH), 130.0 (q, $^2J_{C,F}$ = 33.0 Hz), 129.3 (CH), 127.3 (C_q), 127.2 (C_q),127.1 (CH), 125.6 (q, $^3J_{C,F}$ = 3.8 Hz), 124.2 (q, $^1J_{C,F}$ = 272.0 Hz), 121.9 (CH), 119.0 (CH), 117.1 (C_q), 111.3 (CH), 96.9 (CH), 94.8 (Csp), 84.6 (Csp), 45.2 (CH_2), 12.8 (CH_3); ESI-MS m/z (%) 496 $[M + 1]^+$ (100), 519 $[M + Na]^+$ (50). Anal. Calcd for $C_{27}H_{23}F_3N_2O_2S$: C, 65.31; H, 4.67; N, 5.64. Found: C, 65.36; H, 4.56; N, 5.71.

*4-((6-(Diethylamino)-1-(phenylsulfonyl)-1H-indol-2-yl)ethynyl)-benzonitrile (**12d**).* Reaction time: 4.0 h. Eluent for chromatography: Hex/EtOAc (90:10). Yield 143 mg (79%). Dark orange solid: mp 141.5−143.5 °C; R_f = 0.36 (silica gel, Hex/EtOAc 80:20); IR (KBr) ν_{max} = 3436, 2967, 2925, 2854, 2191, 1734, 1600, 1490, 1397, 1371, 1357, 1279, 1181, 1114, 587 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.24 (t, 6H, CH_3, J = 7.0 Hz), 3.46 (q, 4H, CH_2, J = 7.0 Hz), 6.72 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.88 (s, 1H, C_3indole-H), 7.30−7.56 (m, 5H, CH), 7.66 (bs, 4H, CH), 7.91 (d, 2H, CH, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 148.0 (C_q), 139.8 (C_q), 139.0 (C_q), 133.9 (CH), 132.3 (CH), 131.6 (CH), 129.3 (CH), 128.3 (C_q), 127.0 (CH), 122.1 (CH), 119.5 (CH), 118.9 (C_q), 116.8 (C_q), 111.4 (C_q), 111.3 (CH), 96.8 (CH), 94.7 (Csp), 86.8 (Csp), 45.3 (CH_2), 12.8 (CH_3) ppm (one C_q signal obscured); ESI-MS m/z (%) 314 $[M - SO_2Ph]^+$ (100), 454 $[M + 1]^+$ (40). Anal. Calcd for $C_{27}H_{23}N_3O_2S$: C, 71.50; H, 5.11; N, 9.26. Found: C, 71.24; H, 5.17; N, 9.18.

*4-(2-((4-(Methylsulfonyl)phenyl)ethynyl)-1-(phenylsulfonyl)-1H-indol-6-yl)morpholine (**12e**).* Reaction time: 16.0 h. Eluent for chromatography: Hex/EtOAc (60:40). Yield 187 mg (90%). Yellow solid: mp 193−195 °C (decomp.); R_f = 0.28 (silica gel, Hex/EtOAc 1:1); IR (KBr) ν_{max} = 3437, 2924, 2852, 2202, 1613, 1593, 1363, 1315, 1178, 1149, 1122, 763, 583 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 3.09 (s, 3H, CH_3), 3.29 (t, 4H, CH_2, J = 4.8 Hz), 3.94 (t, 4H, CH_2, J = 4.8 Hz), 6.93 (s, 1H, C_3indole-H), 7.06 (d, 1H, CH, J = 8.8 Hz), 7.33−7.58 (m, 4H, CH), 7.72−7.84 (m, 3H, CH), 7.87−8.01 (m, 4H, CH); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 140.2 (C_q), 138.8 (C_q), 138.7 (C_q), 138.5 (C_q), 134.3 (CH), 132.1 (CH), 129.5 (CH), 128.7 (C_q), 127.8 (CH), 127.0 (CH), 123.4 (C_q), 122.2 (CH), 119.2 (C_q), 118.6 (CH), 115.2 (CH), 102.1 (CH), 95.1 (Csp), 85.2 (Csp), 66.6 (CH_2), 50.8 (CH_2), 44.7 (CH_3); ESI-MS m/z (%) 521 $[M + 1]^+$ (100). Anal. Calcd for $C_{27}H_{24}N_2O_5S_2$: C, 62.29; H, 4.65; N, 5.38. Found: C, 62.07; H, 4.58; N, 5.45.

*4-(1-(Phenylsulfonyl)-2-((4-(trifluoromethyl)phenyl)ethynyl)-1H-indol-6-yl)morpholine (**12f**).* Reaction time: 24.0 h. Eluent for chromatography: toluene/EtOAc 5%. Yield 159 mg (78%). Yellow solid: mp 189−191 °C; R_f = 0.18 (silica gel, toluene/EtOAc 5%); IR (KBr) ν_{max} = 3436, 2960, 2853, 2210, 1612, 1450, 1377, 1320, 1189, 1117, 1066, 724, 588 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 3.28 (t, 4H, CH_2, J = 4.8 Hz), 3.93 (t, 4H, CH_2, J = 4.8 Hz), 6.90 (s, 1H, C_3indole-H), 6.97 (dd, 1H, CH, J = 8.8, 1.8 Hz), 7.35−7.78 (m, 9H, CH), 7.92 (m or d, 2H, CH, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 151.0 (C_q), 138.8 (C_q), 138.6 (C_q), 134.2 (CH), 131.7 (CH), 130.4 (q, $^2J_{C,F}$ = 33.0 Hz), 129.4 (CH), 127.0 (CH), 126.8 (C_q), 125.7 (q, $^3J_{C,F}$ = 3.8 Hz), 124.1 (q, $^1J_{C,F}$ = 272.0 Hz), 122.5 (C_q), 121.9 (CH), 118.9 (C_q), 118.4 (CH), 114.8 (CH), 101.2 (CH), 95.2 (Csp), 83.7 (Csp), 67.0 (CH_2), 50.1 (CH_2); ESI-MS m/z (%) 511 $[M + 1]^+$ (100). Anal. Calcd for $C_{27}H_{21}F_3N_2O_3S$: C, 63.52; H, 4.15; N, 5.49. Found: C, 63.63; H, 4.18; N, 5.54.

Deprotection of Indole Nitrogen: Preparation of Compounds 13a−f. *N,N-Diethyl-2-((4-(methylsulfonyl)phenyl)ethynyl)-1H-indol-6-amine (**13a**) (Method A).* A solution of aq NaOH 2 M (2.5 mL, 4.92 mmol) was added to a stirring solution of *N,N*-diethyl-2-((4-(methylsulfonyl)phenyl)ethynyl)-1-(phenylsulfonyl)-1*H*-indol-6-amine (**12a**) (203 mg, 0.40 mmol) in 8 mL of MeOH and heated to reflux at 85 °C under a nitrogen atmosphere. The reaction mixture was stirred 8.0 h until no more starting product was detected by TLC analysis. Methanol was removed at reduced pressure, and the crude product was poured into H_2O (25 mL) and extracted with EtOAc (4 × 20 mL), washed with brine, and dried over Na_2SO_4. The solvent was removed at reduced pressure, and then the residue was purified by flash chromatography over a silica gel column using Hex/EtOAc (70:30) to afford the desired product (**13a**) as an orange solid (141 mg, 96%): mp 131.5−133.5 °C (decomp.); R_f = 0.51 (silica gel, Hex/EtOAc 1:1); IR

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(KBr) ν_{max} = 3367, 2968, 2925, 2191, 1627, 1591, 1357, 1304, 1148, 1106, 1086, 959, 805, 762, 543 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.19 (t, 6H, CH_3, J = 7.0 Hz), 3.07 (s, 3H, CH_3), 3.40 (q, 4H, CH_2, J = 7.0 Hz), 6.55 (s, 1H, C_3indole-H), 6.72 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.79 (d, 1H, CH, J = 2.2 Hz), 7.42 (d, 1H, CH, J = 8.8 Hz), 7.64 (d, 2H, CH, J = 8.4 Hz), 7.91 (d, 2H, CH, J = 8.4 Hz), 7.96 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 146.7 (C_q), 139.3 (C_q), 139.2 (C_q), 131.7 (CH), 129.4 (C_q), 127.7 (CH), 121.8 (CH), 119.3 (C_q), 114.9 (C_q), 111.1 (CH), 110.3 (CH), 92.8 (CH), 91.2 (Csp), 87.7 (Csp), 45.2 (CH_2), 44.7 (CH_3), 12.9 (CH_3); ESI-MS m/z (%) 367 $[M + 1]^+$ (100). Anal. Calcd for $C_{21}H_{22}N_2O_2S$: C, 68.82; H, 6.05; N, 7.64. Found: C, 68.78; H, 6.00; N, 7.60.

*N,N-Diethyl-2-((4-nitrophenyl)ethynyl)-1H-indol-6-amine (**13b**) (Method B).* A solution of 6 M NaOH (2.5 mL, 14.76 mmol) was added to a stirring solution of *N,N*-diethyl-2-((4-nitrophenyl)ethynyl)-1-(phenylsulfonyl)-1*H*-indol-6-amine (**12b**) (190 mg, 0.40 mmol) in 24 mL of MeOH and heated to reflux at 85 °C under a nitrogen atmosphere. The reaction mixture was stirred 3.0 h until no more starting product was detected by TLC analysis. Methanol was removed at reduced pressure, and the crude product was poured into H_2O (25 mL) and extracted with EtOAc (4 × 20 mL), washed with brine, and dried over Na_2SO_4. The solvent was removed at reduced pressure, and then the residue was purified by flash chromatography over a silica gel column using Hex/EtOAc (85:15) to afford the desired product (**13b**) as a black-purple solid (76 mg, 57%): mp 131−133 °C; R_f = 0.28 (silica gel, Hex/EtOAc 70:30); IR (KBr) ν_{max} = 3430, 2964, 2921, 2851, 2187, 1739, 1631, 1506, 1334, 1103, 807 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.19 (t, 6H, CH_3, J = 7.0 Hz), 3.40 (q, 4H, CH_2, J = 7.0 Hz), 6.53 (s, 1H, C_3indole-H), 6.72 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.81 (d, 1H, CH, J = 2.2 Hz), 7.43 (d, 1H, CH, J = 8.8 Hz), 7.60 (d, 2H, CH, J = 8.8 Hz), 7.94 (bs, 1H, NH), 8.21 (d, 2H, CH, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 146.7, 139.3, 131.6, 130.4, 124.0, 122.0, 119.5, 115.0, 111.4, 110.4, 93.0, 91.5 (Csp), 89.2 (Csp), 45.4 (CH_2), 12.8 (CH_3) ppm (one signal obscured); ESI-MS m/z (%) 334 $[M + 1]^+$ (100). Anal. Calcd for $C_{20}H_{19}N_3O_2$: C, 72.05; H, 5.74; N, 12.60. Found: C, 71.85; H, 5.61; N, 12.45.

*N,N-Diethyl-2-((4-(trifluoromethyl)phenyl)ethynyl)-1H-indol-6-amine (**13c**) (Method C).* A solution of 6 M NaOH (0.8 mL, 4.92 mmol) was added to a stirring solution of *N,N*-diethyl-1-(phenylsulfonyl)-2-((4-(trifluoromethyl)phenyl)ethynyl)-1*H*-indol-6-amine (**12c**) (199 mg, 0.40 mmol) in 16 mL of MeOH and heated to reflux at 85 °C under a nitrogen atmosphere. The reaction mixture was stirred 3.0 h until no more starting product was detected by TLC analysis. Methanol was removed at reduced pressure, and the crude product was poured into H_2O (25 mL) and extracted with EtOAc (4 × 20 mL), washed with brine, and dried over Na_2SO_4. The solvent was removed at reduced pressure, and then the residue was purified by flash chromatography over a silica gel column using Hex/EtOAc (80:20) to afford the desired product (**13c**) as a pale brown solid (124 mg, 87%): mp 127−128.5 °C (decomp.); R_f = 0.16 (silica gel, Hex/EtOAc 80:20); IR (KBr) ν_{max} = 3436, 2980, 2942, 2204, 1630, 1612, 1324, 1162, 1125, 1105, 837, 808 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.19 (t, 6H, CH_3, J = 7.0 Hz), 3.40 (q, 4H, CH_2, J = 7.0 Hz), 6.58 (s, 1H, C_3indole-H), 6.75 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.79 (d, 1H, CH, J = 2.2 Hz), 7.45 (d, 1H, CH, J = 8.8 Hz), 7.62 (s, 4H, CH), 7.95 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.45 MHz) δ = 146.7 (C_q), 139.1 (C_q), 131.6 (CH), 129.8 (q, $^2J_{C,F}$ = 33.0 Hz), 127.4 (C_q), 125.7 (q, $^3J_{C,F}$ = 3.8 Hz), 124.4 (q, $^1J_{C,F}$ = 272.0 Hz), 121.9 (CH), 119.5 (C_q), 115.5 (C_q), 110.5 (CH), 110.4 (CH), 93.1 (CH), 91.4 (Csp), 85.9 (Csp), 45.4 (CH_2), 13.0 (CH_3); ESI-MS m/z (%) 357 $[M + 1]^+$ (100). Anal. Calcd for $C_{21}H_{19}F_3N_2$: C, 70.77; H, 5.37; N, 7.86. Found: C, 70.72; H, 5.31; N, 7.88.

*4-((6-(Diethylamino)-1H-indol-2-yl)ethynyl)benzonitrile (**13d**) (Method D).* Under a nitrogen atmosphere, an oven-dried screw-cap test tube was charged with 4-((6-(diethylamino)-1-(phenylsulfonyl)-1*H*-indol-2-yl)ethynyl)benzonitrile (**12d**) (181 mg, 0.40 mmol) and NaO*t*-Bu (77 mg, 0.80 mmol) and fitted with a septum. The test tube was evacuated and backfilled with nitrogen. The evacuation/backfill was repeated two additional times. Dioxane (3.0 mL) was added by syringe to rinse the side of the tube. The septum was replaced with a Teflon screw cap; the tube was sealed, and the mixture was stirred at 80 °C for 6.0 h, until no more starting product was detected by TLC analysis. After being cooled, the reaction mixture was evaporated almost to dryness and then quenched with H_2O (30 mL) and extracted with EtOAc (3 × 20 mL). The organic layers were collected, dried over Na_2SO_4, and filtered, and the solvent was removed at reduced pressure. The crude material was purified by flash chromatography over a silica gel column using Hex/EtOAc (from 90:10 to 80:20) to afford the desired product (**13d**) as a dark orange solid (65 mg, 52%): mp 108−110.5 °C; IR (KBr) ν_{max} = 3369, 2966, 2926, 2855, 2228, 2196, 1632, 1603, 1359, 1109, 838, 808 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ = 1.19 (t, 6H, CH_3, J = 7.0 Hz), 3.40 (q, 4H, CH_2, J = 7.0 Hz), 6.56 (s, 1H, C_3indole-H), 6.72 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.78 (d, 1H, CH, J = 2.2 Hz), 7.43 (d, 1H, CH, J = 8.8 Hz), 7.55 (d, 2H, CH, J = 8.4 Hz), 7.63 (d, 2H, CH, J = 8.4 Hz), 7.96 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$, 75.45 MHz) δ = 146.8 (C_q), 139.3 (C_q), 132.5 (CH), 131.7 (CH), 128.6 (C_q), 122.0 (CH), 119.5 (C_q), 119.0 (C_q), 115.2 (C_q), 111.3 (C_q), 111.2 (CH), 110.5 (CH), 93.1 (CH), 91.5 (Csp), 88.2 (Csp), 45.5 (CH_2), 13.0 (CH_3); ESI-MS m/z (%) 314 $[M + 1]^+$ (100). Anal. Calcd for $C_{21}H_{19}N_3$: C, 80.48; H, 6.11; N, 13.41. Found: C, 80.27; H, 5.98; N, 13.50.

*4-(2-((4-(Methylsulfonyl)phenyl)ethynyl)-1H-indol-6-yl)-morpholine (**13e**).* The reaction was performed according to Method A starting from **12e** (208 mg, 0.40 mmol). Reaction time: 4.0 h. The product obtained by the aqueous workup was sufficiently pure, and it was used in the following step without further purification. Yield 148 mg (97%). Yellow solid: mp 222−224.5 °C (decomp.); R_f = 0.15 (silica gel, Hex/EtOAc 1:1); IR (KBr) ν_{max} = 3430, 2925, 2859, 2199, 1624, 1591, 1299, 1145, 1122, 1107, 813 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ = 3.08 (t, 4H, CH_2, J = 4.8 Hz), 3.25 (s, 3H, CH_3), 3.75 (t, 4H, CH_2, J = 4.8 Hz), 6.72 (s, 1H, C_3indole-H), 6.78 (d, 1H, CH, J = 1.5 Hz), 6.86 (dd, 1H, CH, J = 8.8, 2.2 Hz), 7.40 (d, 1H, CH, J = 8.8 Hz), 7.75 (d, 2H, CH, J = 8.8 Hz), 7.95 (d, 2H, CH, J = 8.8 Hz), 11.46 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50.3 MHz) δ = 149.6 (C_q), 140.7 (C_q), 138.8 (C_q), 132.1 (CH), 128.2 (C_q), 128.1 (CH), 121.7 (C_q), 121.6 (CH), 116.2 (C_q), 113.1 (CH), 109.8 (CH), 97.0 (CH), 91.1 (Csp), 87.8 (Csp), 66.9 (CH_2), 50.4 (CH_2), 44.1 (CH_3); ESI-MS m/z (%) 381 $[M + 1]^+$ (100). Anal. Calcd for $C_{21}H_{20}N_2O_3S$: C, 66.29; H, 5.30; N, 7.36. Found: C, 66.20; H, 5.24; N, 7.37.

*4-(2-((4-(Trifluoromethyl)phenyl)ethynyl)-1H-indol-6-yl)-morpholine (**13f**).* The reaction was performed according to Method B starting from **12f** (204 mg, 0.40 mmol). Reaction time: 5.0 h. Eluent for chromatography: Hex/EtOAc (80:20). Yield 135 mg (91%). Gold yellow solid: mp 219−221 °C (decomp.); R_f = 0.25 (silica gel, Hex/EtOAc 70:30); IR (KBr) ν_{max} = 3436, 3179, 2918, 2849, 2217, 1623, 1613, 1320, 1260, 1176, 1125, 1063, 839 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ = 3.08 (t, 4H, CH_2, J = 4.8 Hz), 3.75 (t, 4H, CH_2, J = 4.8 Hz), 6.72 (s, 1H, C_3indole-H), 6.76 (d, 1H, CH, J = 1.7 Hz), 6.86 (dd, 1H, CH, J = 8.8, 1.9 Hz), 7.40 (d, 1H, CH, J = 8.8 Hz), 7.72 (d, 2H, CH, J = 8.8 Hz), 7.78 (d, 2H, CH, J = 8.8 Hz), 11.44 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50.3 MHz) δ = 149.6 (C_q), 138.7 (C_q), 132.2 (CH), 129.0 (q, $^2J_{C,F}$ = 33.0 Hz), 127.3 (C_q), 126.4 (q, $^3J_{C,F}$ = 3.8 Hz), 124.7 (q, $^1J_{C,F}$ = 272.0 Hz), 121.7 (C_q), 121.5 (CH), 116.3 (C_q), 113.1 (CH), 109.6 (CH), 97.0 (CH), 91.0 (Csp), 86.9 (Csp), 66.9 (CH_2), 50.4 (CH_2); ESI-MS m/z (%) 371 $[M + 1]^+$ (100). Anal. Calcd for $C_{21}H_{17}F_3N_2O$: C, 68.10; H, 4.63; N, 7.56. Found: C, 68.17; H, 4.52; N, 7.61.

General Procedure for the Linker Introduction. Preparation of compounds 14a−f. Under a nitrogen atmosphere, to a solution of **13a−f** (0.20 mmol) in dry CH_2Cl_2 (2.1 mL) at 0 °C was added TEA (0.11 mL, 81 mg, 0.80 mmol) followed by $COCl_2$ (20 wt % in toluene, 1.9 M in toluene, 0.21 mL, 0.40 mmol). The resulting mixture was stirred at 0 °C for 30 min, then the *p*-toluenesulfonate salt of the benzyl 4-aminobutanoate amine linker was added. The amine was previously liberated by dissolving the *p*-toluenesulfonate salt (292 mg, 0.80 mmol) in dry CH_2Cl_2 (1.1 mL) and adding TEA (0.055 mL, 40 mg, 0.40 mmol) at rt. The reaction mixture was stirred at 0 °C for 1.0 h and then stirred at rt until no more starting product was detected by TLC analysis. The reaction mixture was poured into 0.1 M HCl (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were collected, dried over Na_2SO_4, and filtered, and the solvent was removed at reduced pressure. The crude material was purified by flash chromatography over a silica gel column.

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*Benzyl 4-(6-(Diethylamino)-2-((4-(methylsulfonyl)phenyl)-ethynyl)-1H-indole-1-carboxamido)butanoate (**14a**).* Reaction time: 4.0 h. Eluent for chromatography: CH_2Cl_2/EtOAc (90:10). Yield 82 mg (70%). Bright yellow solid: mp 154−156 °C; R_f = 0.45 (silica gel, Hex/EtOAc 1:1); IR (KBr) ν_{max} = 3436, 3355, 2966, 2928, 2190, 1723, 1677, 1617, 1524, 1501, 1309, 1279, 1146, 1136, 812, 758 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.21 (t, 6H, CH_3, J = 7.0 Hz), 2.03 (quint, 2H, CH_2, J = 7.0 Hz), 2.51 (t, 2H, CH_2, J = 7.3 Hz), 3.06 (s, 3H, CH_3), 3.44 (q, 4H, CH_2, J = 7.0 Hz), 3.57 (q, 2H, CH_2, J = 7.0 Hz), 5.08 (s, 2H, CH_2), 6.75 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.90 (bt, 1H, NH, J = 5.5 Hz), 6.98 (s, 1H, C_3indole-H), 7.27−7.37 (m, 6H, CH), 7.66 (d, 2H, CH, J = 8.4 Hz), 7.68 (s, 1H, CH), 7.92 (d, 2H, CH, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 173.0 (C═O), 152.6 (C_q), 148.1 (C_q), 140.4 (C_q), 140.1 (C_q), 136.0 (C_q), 131.7 (CH), 128.8 (CH), 128.5 (CH), 128.4 (CH), 128.3 (C_q), 127.9 (CH), 121.6 (CH), 118.9 (CH), 118.3 (C_q), 113.2 (C_q), 111.0 (CH), 97.9 (CH), 95.9 (Csp), 86.9 (Csp), 66.7 (CH_2), 45.1 (CH_2), 44.7 (CH_3), 40.4 (CH_2), 31.8 (CH_2), 25.3 (CH_2), 12.9 (CH_3); ESI-MS m/z (%) 586 $[M + 1]^+$ (100). Anal. Calcd for $C_{33}H_{35}N_3O_5S$: C, 67.67; H, 6.02; N, 7.17. Found: C, 67.53; H, 5.84; N, 7.01.

*Benzyl 4-(6-(Diethylamino)-2-((4-nitrophenyl)ethynyl)-1H-indole-1-carboxamido)butanoate (**14b**).* Reaction time: 4.0 h. Eluent for chromatography: CH_2Cl_2/EtOAc (96:4). Yield 77 mg (70%). Black-purple solid: mp 152−154.5 °C; R_f = 0.33 (silica gel, Hex/EtOAc 70:30); IR (KBr) ν_{max} = 3435, 3336, 2965, 2924, 2866, 2185, 1733, 1676, 1615, 1588, 1529, 1509, 1332, 1279, 1149, 1096, 824 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ = 1.21 (t, 6H, CH_3, J = 7.0 Hz), 2.04 (quint, 2H, CH_2, J = 7.0 Hz), 2.51 (t, 2H, CH_2, J = 7.0 Hz), 3.44 (q, 4H, CH_2, J = 7.0 Hz), 3.58 (q, 2H, CH_2, J = 7.0 Hz), 5.08 (s, 2H, CH_2), 6.75 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.89 (bt, 1H, NH, J = 5.5 Hz), 6.99 (s, 1H, C_3indole-H), 7.31−7.38 (m, 6H, CH), 7.61 (d, 2H, CH, J = 8.8 Hz), 7.65 (s, 1H, CH), 8.19 (d, 2H, CH, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 75.45 MHz) δ = 173.1 (C═O), 152.7 (C_q), 148.4 (C_q), 147.4 (C_q), 140.6 (C_q), 136.1 (C_q), 131.7 (CH), 129.4 (C_q), 129.0 (CH), 128.7 (CH), 128.5 (CH), 124.4 (CH), 121.9 (CH), 119.3 (CH), 118.5 (C_q), 113.3 (C_q), 111.2 (CH), 97.9 (CH), 96.2 (Csp), 88.4 (Csp), 66.9 (CH_2), 45.3 (CH_2), 40.6 (CH_2), 31.9 (CH_2), 25.4 (CH_2), 13.0 (CH_3); ESI-MS m/z (%) 553 $[M + 1]^+$ (100). Anal. Calcd for $C_{32}H_{32}N_4O_5$: C, 69.55; H, 5.84; N, 10.14. Found: C, 69.34; H, 5.90; N, 10.04.

*Benzyl 4-(6-(Diethylamino)-2-((4-(trifluoromethyl)phenyl)-ethynyl)-1H-indole-1-carboxamido)butanoate (**14c**).* Reaction time: 3.0 h. Eluent for chromatography: CH_2Cl_2/EtOAc (97:3). Yield 61 mg (53%). Bright yellow solid: mp 114−116.5 °C; R_f = 0.47 (silica gel, Hex/EtOAc 80:20); IR (KBr) ν_{max} = 3436, 3337, 2970, 2927, 2871, 2195, 1735, 1674, 1612, 1500, 1321, 1115, 1099 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.21 (t, 6H, CH_3, J = 7.0 Hz), 2.02 (quint, 2H, CH_2, J = 7.0 Hz), 2.50 (t, 2H, CH_2, J = 7.3 Hz), 3.44 (q, 4H, CH_2, J = 7.0 Hz), 3.56 (q, 2H, CH_2, J = 7.3 Hz), 5.07 (s, 2H, CH_2), 6.75 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.95 (s, 1H, C_3indole-H), 7.02 (bt, 1H, NH, J = 5.5 Hz), 7.27−7.37 (m, 6H, CH), 7.59 (s, 4H, CH), 7.71 (d, 1H, CH, J = 2.1 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 172.9 (C═O), 152.7 (C_q), 148.0 (C_q), 140.2 (C_q), 136.0 (C_q), 131.4 (CH), 130.5 (q, $^2J_{C,F}$ = 33.0 Hz), 128.8 (CH), 128.5 (CH), 128.4 (CH), 126.2 (C_q), 125.8 (q, $^3J_{C,F}$ = 3.8 Hz), 124.0 (q, $^1J_{C,F}$ = 272.0 Hz), 121.5 (CH), 118.4 (C_q), 118.3 (CH), 113.4 (C_q), 110.9 (CH), 98.2 (CH), 96.1 (Csp), 85.1 (Csp), 66.6 (CH_2), 45.1 (CH_2), 40.4 (CH_2), 31.8 (CH_2), 25.3 (CH_2), 12.8 (CH_3); ESI-MS m/z (%) 576 $[M + 1]^+$ (100). Anal. Calcd for $C_{33}H_{32}F_3N_3O_3$: C, 68.86; H, 5.60; N, 7.30. Found: C, 68.75; H, 5.48; N, 7.22.

*Benzyl 4-(2-((4-Cyanophenyl)ethynyl)-6-(diethylamino)-1H-indole-1-carboxamido)butanoate (**14d**).* Reaction time: 4.0 h. Eluent for chromatography: CH_2Cl_2/EtOAc (97:3). Yield 49 mg (46%). Yellow solid: mp 163.5−165.5 °C; R_f = 0.12 (silica gel, Hex/EtOAc 80:20), 0.32 (silica gel, CH_2Cl_2/EtOAc 95:5); IR (KBr) ν_{max} = 3436, 3326, 2966, 2937, 2893, 2223, 2187, 1736, 1669, 1600, 1536, 1494, 1351, 1164, 1145, 1096, 819 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 1.21 (t, 6H, CH_3, J = 7.0 Hz), 2.02 (quint, 2H, CH_2, J = 7.0 Hz), 2.50 (t, 2H, CH_2, J = 7.3 Hz), 3.44 (q, 4H, CH_2, J = 7.0 Hz), 3.56 (q, 2H, CH_2, J = 7.0 Hz), 5.08 (s, 2H, CH_2), 6.75 (dd, 1H, CH, J = 8.8, 2.2 Hz), 6.91 (bt, 1H, NH, J = 5.5 Hz), 6.96 (s, 1H, C_3indole-H), 7.27−7.37 (m, 6H, CH), 7.57 (s, 4H, CH), 7.67 (d, 1H, CH, J = 2.2 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 173.0 (C═O), 152.6 (C_q), 148.1 (C_q), 140.4 (C_q), 136.0 (C_q), 132.5 (CH), 131.4 (CH), 128.8 (CH), 128.6 (CH), 128.4 (CH), 127.2 (C_q), 121.6 (CH), 118.8 (CH), 118.6 (C_q), 118.4 (C_q), 113.2 (C_q), 111.9 (C_q), 111.0 (CH), 97.8 (CH), 96.0 (Csp), 87.2 (Csp), 66.7 (CH_2), 45.1 (CH_2), 40.4 (CH_2), 31.8 (CH_2), 25.3 (CH_2), 12.9 (CH_3); ESI-MS m/z (%) 533 $[M + 1]^+$ (100). Anal. Calcd for $C_{33}H_{32}N_4O_3$: C, 74.41; H, 6.06; N, 10.52. Found: C, 74.51; H, 6.19; N, 10.39.

*Benzyl 4-(6-Morpholino-2-((4-(trifluoromethyl)phenyl)ethynyl)-1H-indole-1-carboxamido)butanoate (**14e**).* Reaction time: 24.0 h. Eluent for chromatography: CH_2Cl_2/acetone (92:8). Yield 24 mg (20%). Bright yellow solid: mp 179.5−181 °C; R_f = 0.36 (silica gel, Hex/EtOAc 60:40), 0.18 (silica gel, CH_2Cl_2/acetone 92:8); IR (KBr) ν_{max} = 3318, 2963, 2921, 2853, 2197, 1740, 1674, 1617, 1531, 1309, 1141, 759 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 2.03 (quint, 2H, CH_2, J = 7.0 Hz), 2.51 (t, 2H, CH_2, J = 7.3 Hz), 3.07 (s, 3H, CH_3), 3.26 (t, 4H, CH_2, J = 4.8 Hz), 3.57 (q, 2H, CH_2, J = 7.0 Hz), 3.88 (t, 4H, CH_2, J = 4.8 Hz), 5.08 (s, 2H, CH_2), 6.96−7.02 (m, 3H, 2CH+1NH), 7.31 (s, 5H, CH), 7.44 (d, 1H, CH, J = 8.8 Hz), 7.69 (d, 2H, CH, J = 8.6 Hz), 7.93−7.95 (m, 1H, CH), 7.94 (d, 2H, CH, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 172.9 (C═O), 152.3 (C_q), 140.62 (C_q), 140.59 (C_q), 139.2 (C_q), 135.9 (C_q), 132.0 (CH), 128.8 (CH), 128.5 (CH), 128.4 (CH), 128.0 (CH), 127.8 (C_q), 121.8 (C_q), 121.5 (CH), 118.0 (CH), 115.1 (C_q), 114.5 (CH), 102.4 (CH), 96.0 (Csp), 85.8 (Csp), 67.0 (CH_2), 66.7 (CH_2), 50.4 (CH_2), 44.6 (CH_3), 40.5 (CH_2), 31.8 (CH_2), 25.2 (CH_2); ESI-MS m/z (%) 600 $[M + 1]^+$ (100). Anal. Calcd for $C_{33}H_{33}N_3O_6S$: C, 66.09; H, 5.55; N, 7.01. Found: C, 66.13; H, 5.51; N, 7.38.

*Benzyl 4-(6-Morpholino-2-((4-(trifluoromethyl)phenyl)ethynyl)-1H-indole-1-carboxamido)butanoate (**14f**).* Reaction time: 3.0 h. Eluent for chromatography: CH_2Cl_2/EtOAc (95:5). Yield 90 mg (76%). Yellow solid: mp 137−138.5 °C; R_f = 0.19 (silica gel, Hex/EtOAc 70:30), 0.22 (silica gel, CH_2Cl_2/EtOAc 90:10); IR (KBr) ν_{max} = 3325, 2951, 2927, 2855, 2199, 1728, 1670, 1613, 1542, 1321, 1117, 1066 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ = 2.02 (quint, 2H, CH_2, J = 7.0 Hz), 2.50 (t, 2H, CH_2, J = 7.3 Hz), 3.25 (t, 4H, CH_2, J = 4.8 Hz), 3.56 (q, 2H, CH_2, J = 7.0 Hz), 3.88 (t, 4H, CH_2, J = 4.8 Hz), 5.07 (s, 2H, CH_2), 6.94−6.99 (m, 2H, CH), 7.05 (bt, 1H, NH, J = 5.5 Hz), 7.31−7.32 (m, 5H, CH), 7.43 (d, 1H, CH, J = 8.8 Hz), 7.61 (s, 4H, CH), 7.92 (s, 1H, CH); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ = 172.9 (C═O), 152.4 (C_q), 151.0 (C_q), 139.1 (C_q), 136.0 (C_q), 131.6 (CH), 130.9 (q, $^2J_{C,F}$ = 33.0 Hz), 128.8 (CH), 128.5 (CH), 128.4 (CH), 125.9 (q, $^3J_{C,F}$ = 3.8 Hz), 125.8 (C_q), 124.1 (q, $^1J_{C,F}$ = 272.0 Hz), 121.7 (C_q), 121.3 (CH), 117.6 (CH), 115.2 (C_q), 114.4 (CH), 102.5 (CH), 96.3 (Csp), 84.2 (Csp), 67.1 (CH_2), 66.6 (CH_2), 50.3 (CH_2), 40.5 (CH_2), 31.8 (CH_2), 25.2 (CH_2); ESI-MS m/z (%) 590 $[M + 1]^+$ (100). Anal. Calcd for $C_{33}H_{30}F_3N_3O_4$: C, 67.22; H, 5.13; N, 7.13. Found: C, 67.30; H, 5.17; N, 7.44.

General Procedure for the Au-Catalyzed Cyclization Reactions. Preparation of Compounds 15a−f. Under a nitrogen atmosphere, to a solution of **14a−f** (0.10 mmol) in dry DCE (3.5 mL) was added the catalyst IPrAuSbF$_6$ (4.3 mg, 0.005 mmol). The reaction mixture was heated at 80 °C until no more starting product was detected by TLC. The reaction mixture was evaporated to dryness and the crude purified by flash chromatography over a silica gel column.

*Benzyl 4-(8-(Diethylamino)-3-(4-(methylsulfonyl)phenyl)-1-oxopyrimido[1,6-a]indol-2(1H)-yl)butanoate (**15a**).* Reaction time: 6.0 h. Eluent for chromatography: CH_2Cl_2/EtOAc (90:10). Yield 31 mg (53%). Yellow solid: mp 150−152 °C; R_f = 0.43 (silica gel, Hex/EtOAc 1:1), 0.18 (silica gel, CH_2Cl_2/EtOAc 90:10); IR (KBr) ν_{max} = 3436, 2966, 2925, 2870, 1732, 1683, 1613, 1498, 1359, 1315, 1151, 1119, 955, 774 cm^{-1}; ^1H NMR (C_6D_6, 200 MHz) δ = 1.02 (t, 6H, CH_3, J = 7.0 Hz), 1.57 (quint, 2H, CH_2, J = 7.0 Hz), 1.84 (t, 2H, CH_2, J = 7.0 Hz), 2.29 (s, 3H, CH_3), 3.18 (q, 4H, CH_2, J = 7.0 Hz), 3.57 (t, 2H, CH_2, J = 7.0 Hz), 4.72 (s, 2H, CH_2), 5.71 (s, 1H, CH), 6.33 (s, 1H, CH), 6.86 (d, 2H, CH, J = 8.4 Hz), 7.04−7.12 (m, 6H, CH), 7.55 (d, 1H, CH, J = 8.4 Hz), 7.69 (d, 2H, CH, J = 8.4 Hz), 8.65 (s, 1H, CH); ^{13}C NMR (C_6D_6, 50.3 MHz) δ = 171.7 (C═O), 149.4 (C_q), 141.5 (C_q), 140.4 (C_q), 136.3 (C_q), 136.2 (C_q), 131.8 (C_q), 129.6 (CH), 128.5 (CH), 128.1 (CH), 127.6 (CH), 122.0 (C_q), 120.5 (CH), 112.6 (CH), 102.7 (CH), 100.1 (CH), 98.9 (CH), 66.1 (CH_2), 45.2 (CH_2), 44.4 (CH_2), 43.6 (CH_3), 30.9

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(CH_2), 24.2 (CH_2), 12.7 (CH_3) ppm (one CH overlapped and two C_q obscured by the solvent); ESI-MS m/z (%) 586 $[M + 1]^+$ (100). Anal. Calcd for $C_{33}H_{35}N_3O_5S$: C, 67.67; H, 6.02; N, 7.17. Found: C, 68.02; H, 6.16; N, 7.47.

*Benzyl 4-(8-(Diethylamino)-3-(4-nitrophenyl)-1-oxopyrimido[1,6-a]indol-2(1H)-yl)butanoate (**15b**).* Reaction time: 8.0 h. Eluent for chromatography: CH_2Cl_2/EtOAc (95:5). Yield 25 mg (45%). Black-purple solid: mp 113−115 °C; R_f = 0.15 (silica gel, CH_2Cl_2/EtOAc 95:5); IR (KBr) ν_{max} = 3436, 2970, 2926, 1736, 1682, 1592, 1519, 1337, 1280, 1173, 823, 758 cm^{-1}; ^1H NMR (C_6D_6, 300 MHz) δ = 1.01 (t, 6H, CH_3, J = 7.0 Hz), 1.56 (quint, 2H, CH_2, J = 7.0 Hz), 1.87 (t, 2H, CH_2, J = 7.0 Hz), 3.17 (q, 4H, CH_2, J = 7.0 Hz), 3.55 (t, 2H, CH_2, J = 7.0 Hz), 4.72 (s, 2H, CH_2), 5.65 (s, 1H, CH), 6.34 (s, 1H, CH), 6.65 (d, 2H, CH, J = 8.8 Hz), 6.89 (dd, 1H, CH, J = 8.8, 2.2 Hz), 7.02−7.12 (m, 5H, CH), 7.55 (d, 1H, CH, J = 8.8 Hz), 7.73 (d, 2H, CH, J = 8.8 Hz), 8.63 (d, 1H, CH, J = 2.2 Hz); ^{13}C NMR (C_6D_6, 75.45 MHz) δ = 172.0 (C═O), 149.6 (C_q), 148.0 (C_q), 146.2 (C_q), 141.6 (C_q), 136.7 (C_q), 136.6 (C_q), 136.0 (C_q), 131.9 (C_q), 129.7 (CH), 128.8 (CH), 128.7 (CH), 123.8 (CH), 122.2 (C_q), 120.9 (CH), 112.9 (CH), 103.2 (CH), 100.4 (CH), 100.3 (CH), 99.6 (CH), 66.4 (CH_2), 45.5 (CH_2), 44.8 (CH_2), 31.2 (CH_2), 24.6 (CH_2), 13.0 (CH_3); ESI-MS m/z (%) 553 $[M + 1]^+$ (100). Anal. Calcd for $C_{32}H_{32}N_4O_5$: C, 69.55; H, 5.84; N, 10.14. Found: C, 69.68; H, 5.98; N, 10.31.

*Benzyl 4-(8-(Diethylamino)-1-oxo-3-(4-(trifluoromethyl)phenyl)-pyrimido[1,6-a]indol-2(1H)-yl)butanoate (**15c**).* Reaction time: 8.0 h. Eluent for chromatography: CH_2Cl_2/EtOAc (95:5). Yield 45 mg (78%). Bright yellow oil: R_f = 0.10 (silica gel, CH_2Cl_2/EtOAc 95:5); IR (NaCl) ν_{max} = 3368, 2971, 2930, 1738, 1683, 1616, 1498, 1415, 1360, 1324, 1281, 1167, 1126, 1068, 1017, 851, 752 cm^{-1}; ^1H NMR (C_6D_6, 300 MHz) δ = 1.17 (t, 6H, CH_3, J = 7.0 Hz), 1.75 (quint, 2H, CH_2, J = 7.0 Hz), 2.01 (t, 2H, CH_2, J = 7.0 Hz), 3.34 (q, 4H, CH_2, J = 7.0 Hz), 3.73 (t, 2H, CH_2, J = 7.0 Hz), 4.90 (s, 2H, CH_2), 5.86 (s, 1H, CH), 6.47 (s, 1H, CH), 6.99 (d, 2H, CH, J = 8.0 Hz), 7.05 (dd, 1H, CH, J = 8.8, 2.2 Hz), 7.03−7.12 (m, 5H, CH), 7.39 (d, 2H, CH, J = 8.0 Hz), 7.69 (d, 1H, CH, J = 8.8 Hz), 8.79 (d, 1H, CH, J = 2.2 Hz); ^{13}C NMR (C_6D_6, 75.45 MHz) δ = 172.0 (C═O), 149.7 (C_q), 146.1 (C_q), 139.5 (C_q), 136.8 (C_q), 136.7 (C_q), 136.6 (C_q), 132.2 (C_q), 130.7 (q, $^2J_{C,F}$ = 33.0 Hz), 129.7 (CH), 128.8 (CH), 128.7 (CH), 128.5 (CH), 125.7 (q, $^3J_{C,F}$ = 3.8 Hz), 124.8 (q, $^1J_{C,F}$ = 272.0 Hz), 122.3 (C_q), 120.7 (CH), 112.9 (CH), 102.7 (CH), 100.6 (CH), 98.9 (CH), 66.3 (CH_2), 45.5 (CH_2), 44.7 (CH_2), 31.3 (CH_2), 24.6 (CH_2), 13.0 (CH_3); ESI-MS m/z (%) 576 $[M + 1]^+$ (75). Anal. Calcd for $C_{33}H_{32}F_3N_3O_3$: C, 68.86; H, 5.60; N, 7.30. Found: C, 69.02; H, 5.74; N, 7.45.

*Benzyl 4-(3-(4-Cyanophenyl)-8-(diethylamino)-1-oxopyrimido-[1,6-a]indol-2(1H)-yl)butanoate (**15d**).* Reaction time: 4.5 h. Eluent for chromatography: CH_2Cl_2/EtOAc (95:5). Yield 42 mg (78%). Orange oil: R_f = 0.24 (silica gel, CH_2Cl_2/EtOAc 90:10); IR (NaCl) ν_{max} = 3359, 2968, 2929, 2229, 1729, 1680, 1607, 1495, 1414, 1350, 1282, 1167, 1119, 1018, 839, 752 cm^{-1}; ^1H NMR (C_6D_6, 200 MHz) δ = 1.01 (t, 6H, CH_3, J = 7.0 Hz), 1.55 (quint, 2H, CH_2, J = 7.0 Hz), 1.85 (t, 2H, CH_2, J = 7.0 Hz), 3.17 (q, 4H, CH_2, J = 7.0 Hz), 3.52 (t, 2H, CH_2, J = 7.0 Hz), 4.74 (s, 2H, CH_2), 5.61 (s, 1H,CH), 6.32 (s, 1H,CH), 6.60 (d, 2H, CH, J = 8.4 Hz), 6.88 (d, 2H, CH, J = 8.4 Hz), 7.02−7.12 (m, 6H, CH), 7.54 (d, 1H, CH, J = 8.8 Hz), 8.62 (s, 1H, CH); ^{13}C NMR (C_6D_6, 50.3 MHz) δ = 171.6 (C═O), 149.3 (C_q), 145.9 (C_q), 139.4 (C_q), 136.3 (C_q), 136.1 (C_q), 131.9 (CH), 131.7 (C_q), 129.2 (CH), 128.4 (CH), 127.9 (CH), 121.9 (C_q), 120.5 (CH), 118.2 (C_q), 112.6 (CH), 102.7 (CH), 100.1 (CH), 99.0 (CH), 66.1 (CH_2), 45.2 (CH_2), 44.4 (CH_2), 31.0 (CH_2), 24.3 (CH_2), 12.7 (CH_3) ppm (one CH overlapped and one C_q obscured by the solvent); ESI-MS m/z (%) 533 $[M + 1]^+$ (100). Anal. Calcd for $C_{33}H_{32}N_4O_3$: C, 74.41; H, 6.06; N, 10.52. Found: C, 74.25; H, 5.94; N, 10.58.

*Benzyl 4-(3-(4-(Methylsulfonyl)phenyl)-8-morpholino-1-oxopyrimido[1,6-a]indol-2(1H)-yl)butanoate (**15e**).* Reaction time: 4.0 h. Eluent for chromatography: CH_2Cl_2/EtOAc (90:10). Yield 54 mg (90%). Bright yellow solid: mp 67−68 °C; R_f = 0.18 (silica gel, CH_2Cl_2/EtOAc 90:10); IR (KBr) ν_{max} = 3449, 2958, 2922, 2851, 1734, 1684, 1636, 1487, 1450, 1410, 1313, 1152, 1122, 958, 775 cm^{-1}; ^1H NMR (C_6D_6, 200 MHz) δ = 1.60 (quint, 2H, CH_2, J = 7.0 Hz), 1.88 (t, 2H, CH_2, J = 7.0 Hz), 2.31 (s, 3H, CH_3), 2.95 (t, 4H, CH_2, J = 4.8 Hz), 3.54−3.58 (m, 6H, CH_2), 4.72 (s, 2H, CH_2), 5.73 (s, 1H, CH), 6.32 (s, 1H, CH), 6.89 (d, 2H, CH, J = 8.3 Hz), 6.95−7.11 (m, 6H, CH), 7.54 (d, 1H, CH, J = 8.8 Hz), 7.71 (d, 2H, CH, J = 8.3 Hz), 8.74 (d, 1H, CH, J = 2.1 Hz); ^{13}C NMR (C_6D_6, 50.3 MHz) δ = 171.7 (C═O), 149.1 (C_q), 141.8 (C_q), 140.2 (C_q), 137.0 (C_q), 136.3 (C_q), 135.3 (C_q), 132.9 (C_q), 129.7 (CH), 128.5 (CH), 128.2 (CH), 127.6 (CH), 124.7 (C_q), 120.2 (CH), 115.6 (CH), 103.6 (CH), 102.3 (CH), 98.6 (CH), 66.9 (CH_2), 66.1 (CH_2), 50.7 (CH_2), 44.6 (CH_2), 43.6 (CH_3), 30.9 (CH_2), 24.3 (CH_2) ppm (one CH overlapped and one C_q obscured by the solvent); ESI-MS m/z (%) 600 $[M + 1]^+$ (100). Anal. Calcd for $C_{33}H_{33}N_3O_6S$: C, 66.09; H, 5.55; N, 7.01. Found: C, 66.01; H, 5.64; N, 7.20.

*Benzyl 4-(8-Morpholino-1-oxo-3-(4-(trifluoromethyl)phenyl)-pyrimido[1,6-a]indol-2(1H)-yl)butanoate (**15f**).* Reaction time: 2.0 h. Eluent for chromatography: CH_2Cl_2/EtOAc (95:5). Yield 58 mg (99%). Bright yellow oil: R_f = 0.37 (silica gel, CH_2Cl_2/EtOAc 90:10); IR (NaCl) ν_{max} = 3368, 2961, 2919, 2853, 1738, 1683, 1616, 1486, 1450, 1413, 1324, 1168, 1123, 1068, 1017, 851, 752 cm^{-1}; ^1H NMR (C_6D_6, 200 MHz) δ = 1.61 (quint, 2H, CH_2, J = 7.0 Hz), 1.88 (t, 2H, CH_2, J = 7.0 Hz), 2.96 (t, 4H, CH_2, J = 4.8 Hz), 3.54−3.61 (m, 6H, CH_2), 4.73 (s, 2H, CH_2), 5.69 (s, 1H, CH), 6.32 (s, 1H, CH), 6.82 (d, 2H, CH, J = 8.1 Hz), 6.95−7.12 (m, 6H, CH), 7.24 (d, 2H, CH, J = 8.0 Hz), 7.55 (d, 1H, CH, J = 8.8 Hz), 8.77 (d, 1H, CH, J = 2.2 Hz); ^{13}C NMR (C_6D_6, 50.3 MHz) δ = 171.7 (C═O), 149.2 (C_q), 149.1 (C_q), 139.0 (C_q), 137.3 (C_q), 136.3 (C_q), 135.3 (C_q), 133.0 (C_q), 130.7 (q, $^2J_{C,F}$ = 33.0 Hz), 129.4 (CH), 128.5 (CH), 128.2 (CH), 128.1 (CH), 125.4 (q, $^3J_{C,F}$ = 3.8 Hz), 124.7 (C_q), 123.6 (q, $^1J_{C,F}$ = 272.0 Hz), 120.1 (CH), 115.6 (CH), 103.7 (CH), 102.1 (CH), 98.3 (CH), 66.9 (CH_2), 66.1 (CH_2), 50.7 (CH_2), 44.5 (CH_2), 31.0 (CH_2), 24.4 (CH_2); ESI-MS m/z (%) 613 $[M + Na]^+$ (100), 590 $[M + 1]^+$ (75). Anal. Calcd for $C_{33}H_{30}F_3N_3O_4$: C, 67.22; H, 5.13; N, 7.13. Found: C, 67.36; H, 5.22; N, 7.28.

Preparation of 4-(8-(Diethylamino)-1-oxo-3-(4-(trifluoromethyl)phenyl)pyrimido[1,6-*a*]indol-2(1*H*)-yl)butanoic acid (15′c). To a solution of **15c** (150 mg, 0.26 mmol) in MeOH (50 mL) was added Pd/C (10 wt %, 15 mg). The mixture was charged with hydrogen and stirred at rt for 2.0 h. The crude was filtered over Celite and washed with MeOH. The reaction mixture was evaporated to dryness and the crude purified by flash chromatography over a silica gel column using Hex/EtOAc (1:1) to afford the desired product (**15′c**) as an orange-yellow solid (96 mg, 76%): mp 148−150.5 °C (decomp.); R_f = 0.11 (silica gel, Hex/EtOAc 1:1); IR (KBr) ν_{max} = 3437, 2964, 2925, 2854, 1717, 1675, 1616, 1496, 1409, 1368, 1320, 1130, 1116, 1066, 1017, 831, 755 cm^{-1}; ^1H NMR (acetone-*d*, 300 MHz) δ = 1.21 (t, 6H, CH_3, J = 7.0 Hz), 1.85 (t, 2H, CH_2, J = 7.3 Hz), 2.20 (t, 2H, CH_2, J = 7.3 Hz), 3.48 (q, 4H, CH_2, J = 7.0 Hz), 3.94 (t, 2H, CH_2, J = 7.3 Hz), 6.41 (s, 1H, CH), 6.45 (s, 1H, CH), 6.93 (dd, 1H, CH, J = 8.8, 2.6 Hz), 7.47 (d, 1H, CH, J = 8.8 Hz), 7.79−7.89 (m, 4H, CH), 8.12 (d, 1H, CH, J = 2.2 Hz); ^{13}C NMR (acetone-*d*, 75.45 MHz) δ = 173.1 (C═O), 149.4 (C_q), 145.4 (C_q), 139.8 (C_q), 137.4 (C_q), 135.7 (C_q), 132.1 (C_q), 130.9 (q, $^2J_{C,F}$ = 33.0 Hz), 130.0 (CH), 125.7 (q, $^3J_{C,F}$ = 3.8 Hz), 124.7 (q, $^1J_{C,F}$ = 272.0 Hz), 121.8 (C_q), 120.3 (CH), 112.3 (CH), 102.3 (CH), 99.6 (CH), 98.3 (CH), 45.1 (CH_2), 44.7 (CH_2), 30.7 (CH_2), 29.7 (CH_2), 24.3 (CH_2), 12.3 (CH_3); ESI-MS m/z (%) 486 $[M + 1]^+$ (100). Anal. Calcd for $C_{26}H_{26}F_3N_3O_3$: C, 64.32; H, 5.40; N, 8.66. Found: C, 64.70; H, 5.68; N, 8.29.

Absorption Spectroscopy. MediaChrom stock solutions (5 mg/mL) were prepared by dissolving lyophilized powders in DMSO, while Prodan stock solution was prepared in ethanol. To collect absorption spectra, 2 μL of stock solutions was layered in a vial, and DMSO or ethanol was evaporated by a vacuum concentrator. Five hundred microliters of different solvents was then added and transferred to the cuvette to obtain a final dye concentration of 0.02 mg/mL. The cuvette was kept protected from light. Extinction coefficients were determined by acquiring absorption spectra at four different concentrations (MediaChrom **15c** from 139 to 13.9 μM, Prodan from 100 to 10 μM) in solvents with different polarity (hexane, *n*-octanol, ethanol, DMF).

Fluorescence Spectroscopy. Dye stock solution was diluted in ethanol to obtain a 0.2 mg/mL solution that was further diluted in ethanol at different concentrations (from 2.5 to 5 μM). The fluorescence intensity values were recorded by exciting the samples at a 380 nm

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wavelength. Fluorescence quantum yields were determined by taking Prodan in ethanol (QY = 71%) as a reference, using an excitation wavelength of 380 nm. The quantum yield values were corrected for the solvent refractive index.

Fluorophore Characterization. Onsager cavity radii were calculated for MediaChrom dyes and Prodan by applying the following eq 1:[51]

$$a = \sqrt[3]{\left(\frac{3M}{4\pi\delta N_A}\right)} \quad (1)$$

where M is the molecular weight of the fluorophore, NA is Avogadro's number, and δ is the compound density. Dipole moment changes upon excitation were assessed through the general solvent effects described by the Lippert-Mataga equation (eq 2):[40]

$$\overline{\upsilon}_a - \overline{\upsilon}_f = \frac{2}{hc}\left(\frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}\right)\frac{(\mu^* - \mu)^2}{a^3} + \text{const} \quad (2)$$

where $\overline{\upsilon}_a$ and $\overline{\upsilon}_f$ are the wavenumbers in cm^{-1} of absorption and emission peaks, h is Planck's constant, c is the speed of light, a is the Onsager cavity radius, and μ^* and μ are the dipole moment of the molecule in the excited and ground state, respectively.

Photodegradation Test. In the photodegradation tests, a 5 μM solution in ethanol of a given dye in a quartz cuvette (path length 0.5 cm) was illuminated at 380 nm by the light of a xenon lamp of a spectrofluorometer (excitation slits open to 8 nm, emission slits open to 1 nm). During the time of illumination (100 min), the fluorescence signal was recorded as a function of time. A THORLABS power meter (PM100USB) was used to measure the energy on the sample, corresponding to 1.01 mW on an area of 15 mm^2 for this experimental setup.

Dipalmitoylphosphatidylcholine Vesicle Preparation. DPPC vesicles were prepared by sonication for 5 min a 1 mg/mL DPPC solution in double distilled water.

Peptide Synthesis. The peptide Cro:1 (GQTKTAKDLGVYQSA-INKAIHAG) was prepared by microwave-assisted solid-phase synthesis[47] based on Fmoc chemistry on Fmoc-Rink-amide resin (0.57 molar equiv/g substitution), using a 5-fold molar excess of 0.2 M Fmoc-protected amino acids dissolved in *N*-methylpyrrolidinone and using HOBT/HBTU/DIEA (5:5:10 equiv) as activators. Coupling reactions were performed for 5 min at 40 W with a maximum temperature of 75 °C. Deprotection was performed in two stages using 20% piperidine in dimethylformamide (5 and 10 min each).

On-Resin Peptide Labeling with MediaChrom 15′c. The labeling of Cro:1 was performed on resin, using 2 equiv of **5′c** and HOBT/HBTU/DIEA (2:2:4 equiv) as activators.[48] The coupling reaction was performed for 3 h in the dark under vigorous shaking. Cleavage from the resin was performed using 10 mL of reagent K (trifluoroacetic acid/phenol/water/thioanisole/1,2-ethanedithiol; 82.5:5:5:5:2.5) for 180 min. Following cleavage, the labeled peptide was precipitated and washed using ice-cold anhydrous ethyl ether. The peptide was purified by RP-HPLC using a gradient elution of 5−70% solvent B (solvent A: water/acetonitrile/trifluoroacetic acid 95:5:0.1; solvent B: water/acetonitrile/trifluoroacetic acid 5:95:0.1) over 20 min at a flow rate of 20 mL/min^{-1}. The purified peptide was freeze-dried and stored in the dark at 0 °C. The identity and purity of the labeled peptide was confirmed by ESI-MS. **15′c**-Cro:1, M = 2837.9; ESI-MS m/z (%) 1419.8 $[(M + 2)/2]^+$ (100), 946.9 $[(M + 3)/3]^+$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b02066.

Attempts to obtain compound **4**; choice of the catalyst for the Au-catalyzed cycloisomerization; table of the change in the dipole moments of MediaChrom **15a−f**; table comparing fluorescent emission peaks of MediaChrom **15c** and **15′c**; copies of chromatogram and mass spectra of **15′c**-Cro:1; ^1H and ^{13}C NMR spectra of all new compounds; and COSY, NOESY, and HSQC of **15′c** (PDF)

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Notes

The authors declare no competing financial interest.

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