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MediaChrom: discovering a class of pyrimidoindolone based polarity-sensitive dyes

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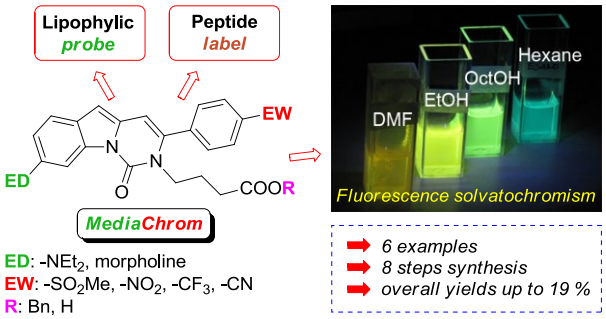


ABSTRACT: A small library of six polarity-sensitive fluorescent dyes, nicknamed MediaChrom, was prepared. This class of dyes is characterized by a pyrimidoindolone core fitted out with a conjugated push-pull system, and a carboxy-linker for a conceivable coupling with biomolecules. The optimized eight-step synthetic strategy involves a highly chemo- and regio-selective gold catalyzed cycloisomerization reaction. The photophysical properties of MediaChrom dyes have been in-depth evaluated. In particular, the MediaChrom bearing a diethylamino as electron-donating group and a trifluoromethyl as 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 membrane/lipophilic probe and as peptide fluorescent label.

INTRODUCTION

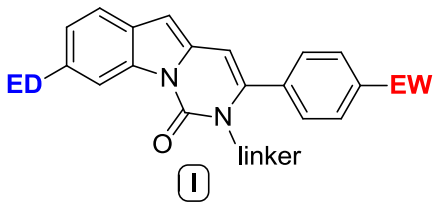
The modern biological research needs a continuous development of highly specific and sensitive fluorescent dyes for monitoring a wide range of molecular processes and events.[1] A particular class of dyes, called environment-sensitive dyes, is able to change their spectroscopical properties in response to the change of 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 to meet simultaneously all the optimal spectroscopic requirements for biological applications, *i.e.*, 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 well as DMAP[10] and its derivatives.[11] Dapoxyl[12] and Prodan,[13] two of the best solvatochromic dyes available, display only a slightly red-shifted absorption (373 nm 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 red-shifted absorption (around 465 nm), but its solvatochromism is limited. Fluoroprobe,[17] probably the molecule with 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 is not yet been discovered. Beside 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]

Since 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).



ED: -NEt_2, morpholine;
EW: -SO_2Me, -NO_2, -CF_3, -CN.

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

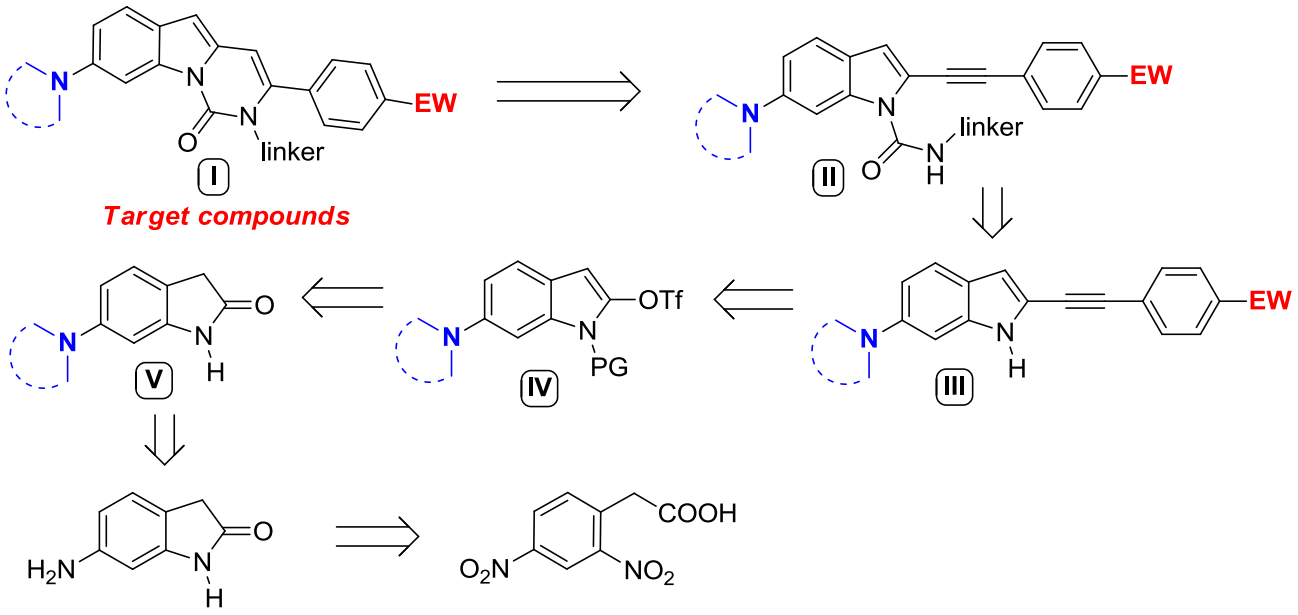
Our ambitious goal was to obtain a small library of original pyrimidoindolone-based solvatochromic dyes with enhanced photophysical properties. The ED and EW groups were selected based on literature findings. The most used ED groups in push-pull solvatochromic dye are secondary amines. When a weaker donating group, such as the alkoxy group, has been used the spectroscopical performance was in general worse.[21] Therefore, in our study we privileged the use of diethylammino 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 QY 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 EWG 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 proof of concept, their applications as a membrane/lipophilic probe and as peptide fluorescent label were briefly investigated. In this paper we describe our results.

RESULTS and DISCUSSION

Synthesis of the pyrimidoindolones (MediaChrom) library

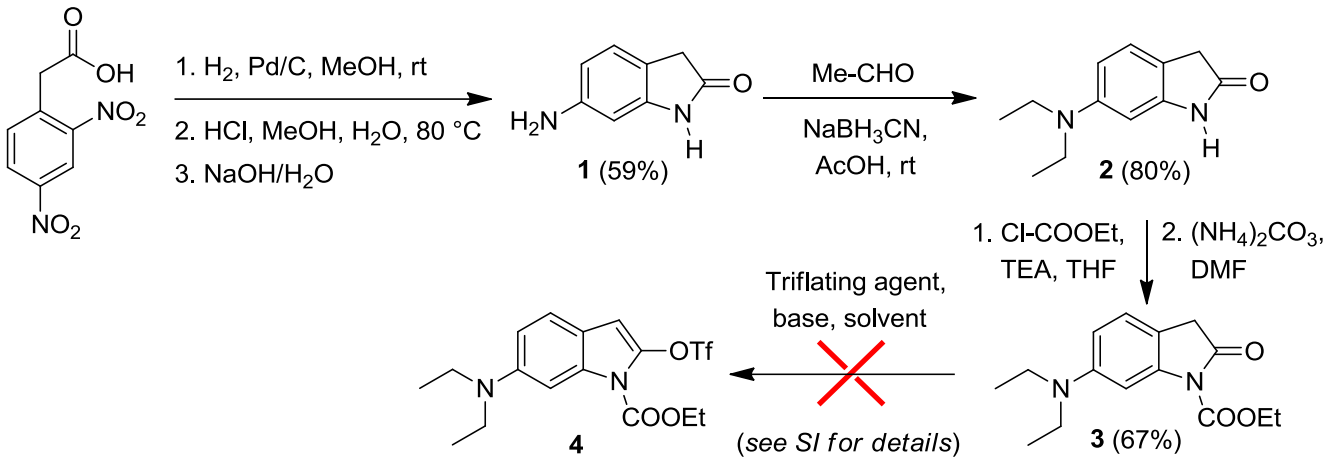
We designed a first retrosynthetic approach (Scheme 1) taking in mind our experience in the transformation of indolin-2-ones (**V**) in the indole-2-triflates (**IV**), useful building blocks for the preparation of 2-alkynyl indoles (**III**).[24] The early synthetic steps have sound bases in the literature,[25] but involve the synthesis of a number on unknown compounds. In particular the synthesis and the reactivity of 6-amino substituted indoles (**IV**) and indolinones (**V**), is seldom explored.[26] The target compounds (**I**) should be finally 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).



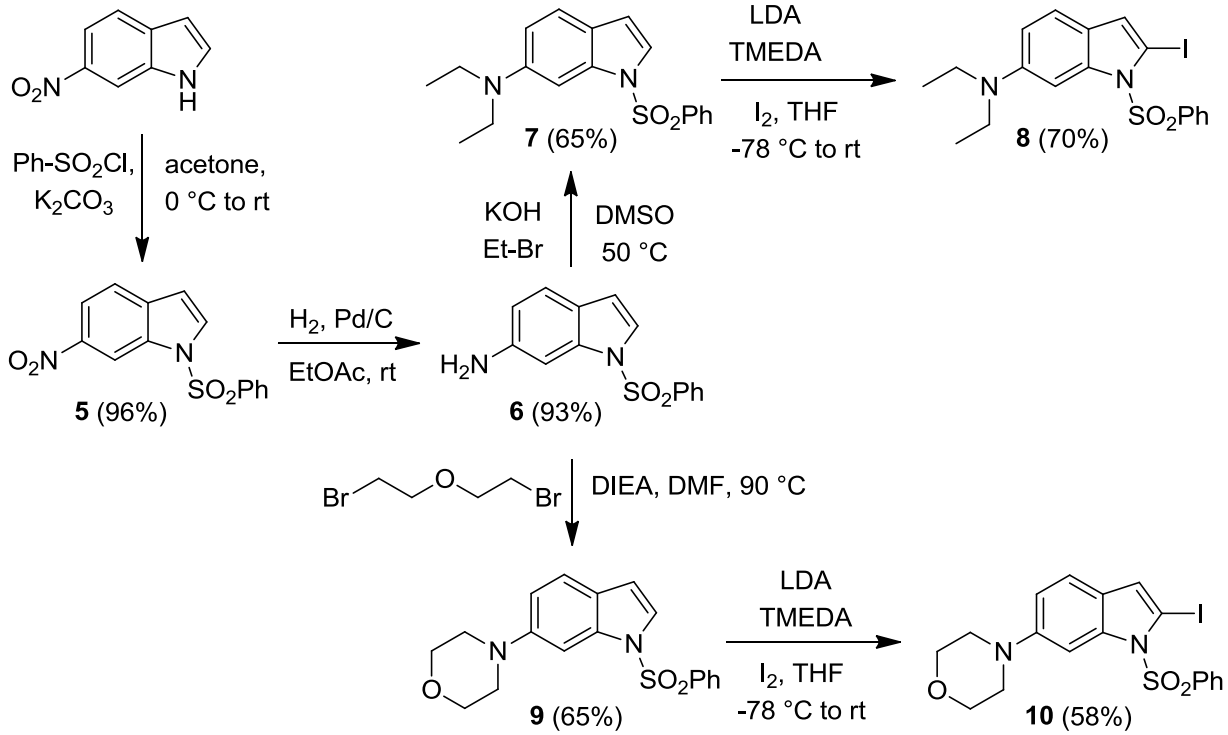
Scheme 1: Retrosynthetic approach.

We started our study trying to synthesize a properly substituted key intermediate **IV**, i.e., 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-diethylamino indolin-2-one **3** in the corresponding indole-2-triflate **4** was unsuccessful (see SI for details).



Scheme 2: Tentative approach to intermediate **4.**

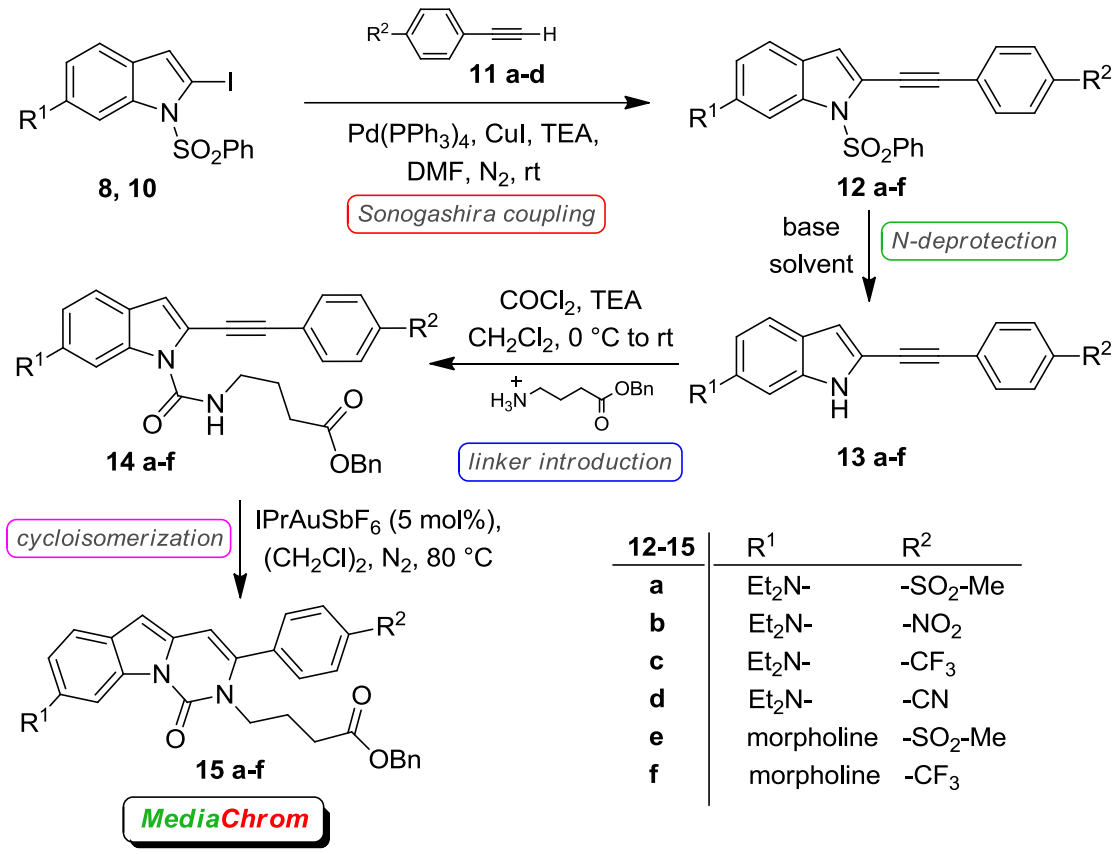
This drawback prompted us to plan an alternative synthetic strategy. As iodine is a good leaving group as triflate, we identify the *N*-protected 6-amino-2-iodoindoles **8** and **10** as suitable new key intermediates. They were obtained in four steps starting from cheap and commercially available 6-nitroindole (Scheme 3).



Scheme 3. Alternative strategy to 2-iodinated key intermediates **8** and **10**.

The nitrogen group of 6-nitroindole was protected with a benzenesulfonyl group to give the 1-benzenesulfonyl-6-nitroindole **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-bis*-alkylation of **6** by reductive amination was ruled-out because it was reported that using $NaBH_3CN$ the reduction of indole C2-C3 double bond can occur.[29] Therefore, the *N,N-bis*-alkylation of **6** was performed under basic condition with ethyl bromide at 50 °C,[30] giving rise to the 1-benzenesulfonyl-6-(*N,N*-diethylamino)-indole derivative **7** in 65 % yields. As mentioned above, to modulate the properties of the EDG in 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*-diisopropyl-ethylamine 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**, the library of target compounds **15** – nicknamed MediaChrom – was obtained in four steps, starting from 2-iodoindoles **8** and **10** (Scheme 4).



Scheme 4: Synthesis of MediaChrom dyes **15a-f**.

N-Protected 2-alkynyl-6-aminoindoles **12 a-f** were obtained from fair to excellent yields (Table 1) by a typical Sonogashira cross-coupling reaction[33] with four different alkynes (**11 a-d**) in the presence of $Pd(PPh_3)_4$, CuI, TEA in DMF. Alkynes **11 b-d** are commercially available, while **1a** was synthesized from the corresponding aryl halide by a Pd-catalyzed cross-coupling with TMS-acetylene followed by desilylation (see SI for details). The subsequent deprotection reactions of the indole nitrogen of compounds **12 a-f** to give the corresponding 6-amino-2-alkynylindoles **13 a-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).

Table 1: Synthesis of compounds **12 a-f** and **13 a-f**.

Sub.	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), NaOH aq. (2 M, 2.5 mL), reflux. Method **B**: **12b** or **12f** (0.4 mmol) in MeOH (24 mL), NaOH aq. (6 M, 2.5 mL), reflux. Method **C**: **12c** (0.4 mmol) in MeOH (16 mL), NaOH aq. (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.

The insertion of the amido function at the nitrogen atom required for the final cyclization step – and the simultaneous introduction of the 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 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 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 **13 a-f** in the presence of phosgene and triethylamine, in dichloromethane at 0° C to give the compounds **14 a-f** in fair to good yields (Table 2).[36]

Finally, the cyclization to MediaChrom **15 a-f** was accomplished by a gold catalyzed reaction.[37] The gold catalyzed intramolecular addition of an amide group on an alkyne can show chemo- (O *vs* N) and regio- (6-*endo vs* 5-*exo*) selectivity problems, due to the bidentate nature of both the nucleophile (amide) and

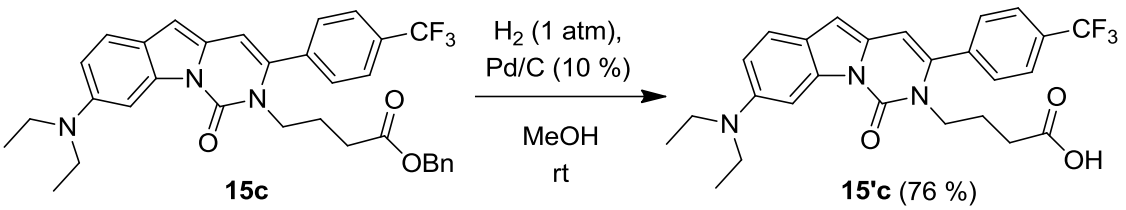
the electrophile (alkyne).[38] In particular, it has been recently reported that the cycloisomerization of some scaffolds closely related to **14**, afforded either *N*-cyclization or *O*-cyclization products depending from the nature of the counterion of the metal catalyst.[39] A brief screening of gold and silver based catalytic systems (see SI 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 **15 a-f** in a chemo- and regio-specific fashion from fair to excellent yields (Table 2).

Table 2: Synthesis of compounds **14 a-f** and **15 a-f**.

Sub.	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: **13 a-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), TEA (0.4 mmol) was added, rt. [b] Pure isolated products. [c] Reaction conditions: **14 a-f** (0.1 mmol) in DCE (3.5 mL), $IPrAuSbF_6$ (0.005 mmol), 80 °C, N_2.

MediaChrom dyes **15** were designed with the linker protected as benzyl ester, to be easily deprotected so transforming the MediaChrom *dyes* **15** in the carboxy-free MediaChrom *labels* **15'**. Since the MediaChrom **15c** displayed the most interesting photophysical proprieties (see below), it was chosen as lead compound to be transformed in the carboxy-free derivative **15'c** for biomolecular labelling. The 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).



Scheme 5: Deprotection of carboxy-terminus of MediaChrom **15c**.

The structure of product **15'c** was unequivocally confirmed by NOESY and HSQC experiments (see supporting information for details) giving indirectly insights 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 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] ref. 13.

All MediaChrom dyes showed intense absorption peaks, spanning from 369 nm to 456 nm. MediaChrom **15b**, which is the dye with the most red-shifted absorption band, exhibits the lower absorption intensity. In order to obtain acceptable quality spectra a 25-fold more concentrated solution was prepared. The

presence of the stronger EWG (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 EDG (-NMe_2) but different EWGs (-SO_2Me *vs* –CF_3), indicates that the presence of a mesyl resulted in a 8 nm red-shifted absorption peak, with respect to trifluoromethyl group. A similar effect was observed by comparison of the absorption peaks of MediaChrom **15e** and **15f**, where the same EDG (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 a 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 represent a remarkable benefit.

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

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

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).

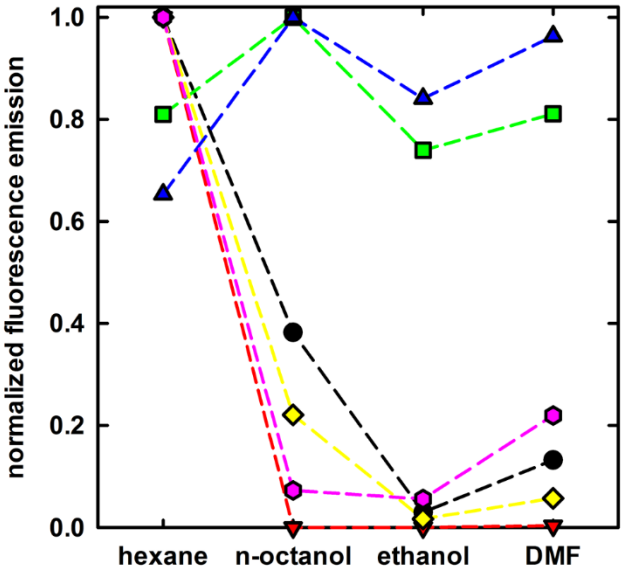


Figure 2: Normalized fluorescence emission peak of MediaChrom dyes at 1.6 μg/mL concentration in different solvent, at 20°C. **15a** (black circles), **15b** (red triangles-down), **15c** (green squares), **15d** (yellow diamonds), **15e** (magenta hexagon) and **15f** (blue triangles-up).

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 these molecules good candidates for several purposes, ranging from structural and dynamic studies on biological macromolecules to cell imaging applications Combining absorption and emission properties, **15c** is considered the best candidate, since it requires a red-shifted excitation respect to **15f** (see Table 3). In fact, in applications such as fluorescent labeling for user-friendly detection kits or dye for cell imaging, the possibility to work at longer wavelength guarantees a reduced biological tissues auto-florescence and scattering, and/or a 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 µM to 5 µM) were both excited at 380 nm, an average excitation wavelength between the λ_{max} of absorbance of Prodan and MediaChrom **15c**. Results are reported in Table 5 and Figure 3.

Table 5: Comparison of the absorbance and fluorescence properties of **15c** and Prodan in four solvents with different polarities.

Solvent	λ_{max} *(abs), nm*		ε *(mM^{-1} cm^{-1})* [a]		λ_{max} *(fluo), nm*[b]		*Q.Y.*[c]	
	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 µM to 13.9 µM, Prodan from 100 µM to 10 µM); [b] excitation wavelength: 380 nm, [dye] = from 2.5 µM to 5 µM; [c] quantum yield values were corrected for the solvent refractive index.

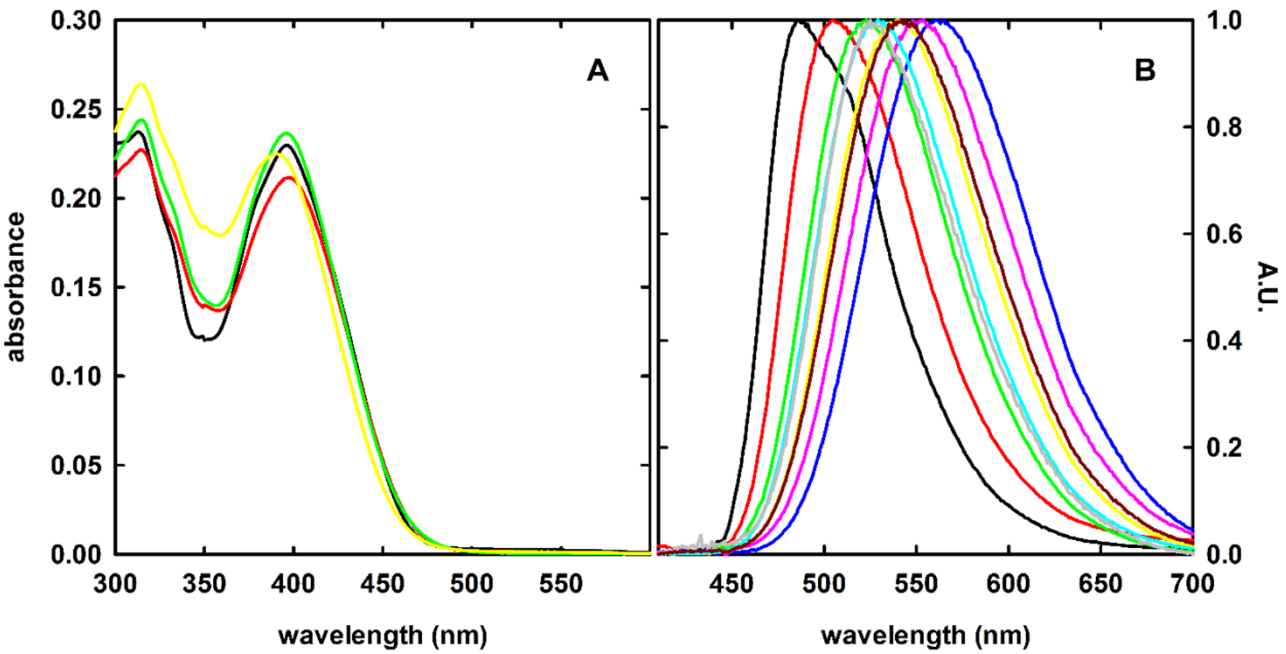


Figure 3: Absorption (A) and normalized fluorescence (B) spectra of **15c** in different solvents at 20°C. **15c** absorbance spectra in hexane (black line), *n*-octanol (red line), DMF (green line) and methanol (yellow line). B): **15c** normalized fluorescence spectra (excitation wavelength = 380 nm)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

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 nm 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, Figure 3-B). This large solvatochromic shift (82 nm) suggests that the chromophore is able to detect even small polarity changes. Moreover, also the emission maxima are strongly red shifted with respect of those of Prodan. Despite 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, 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

Stokes shift (in cm^{-1}) for MediaChrom **15c** and compared to Prodan under the same experimental conditions.

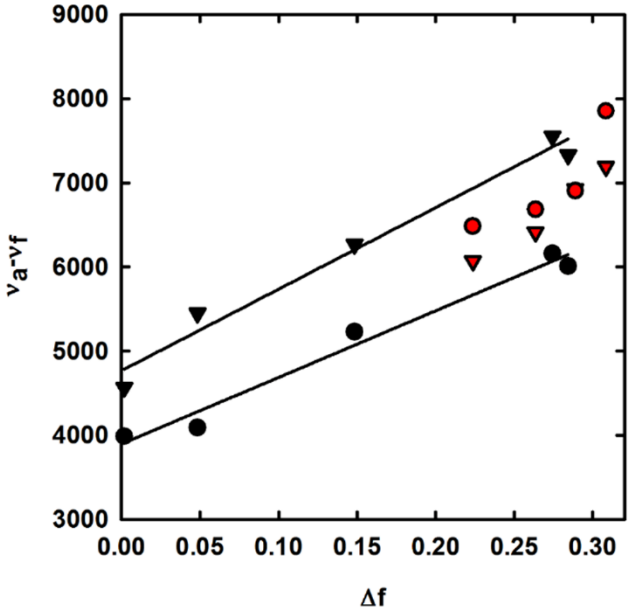


Figure 4: Lippert plot for MediaChrom **15c** (triangles-down) and Prodan (circles) in aprotic (black) and protic (red) solvents.

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 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 been related to H-bonding of the protic solvents with the carbonyl group of the fluorophore.[14] The 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 in the D-π-A system.

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

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 (μ^*-μ) of 13.3 ± 0.6 and 8.1 ± 0.4 Debye, respectively. For all other MediaChrom dyes, which showed similar solvatochromic shifts, the dipole moment changes fall in the 13.0-13.4 Debye range (see table S1 in SI). This finding demonstrates that, regardless the substituent pattern, the charge transfer process is similar. Moreover, the differences in the Stokes shift suggests an effect of substituents on the non-emitting energy loss in the excited state.

To estimate the photostability of MediaChrom **15c**, we made a photodegradation test. Ethanol solutions of **15c** and Prodan were prepared in two different quartz cuvette 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 seconds, while the latter after 1750 seconds.

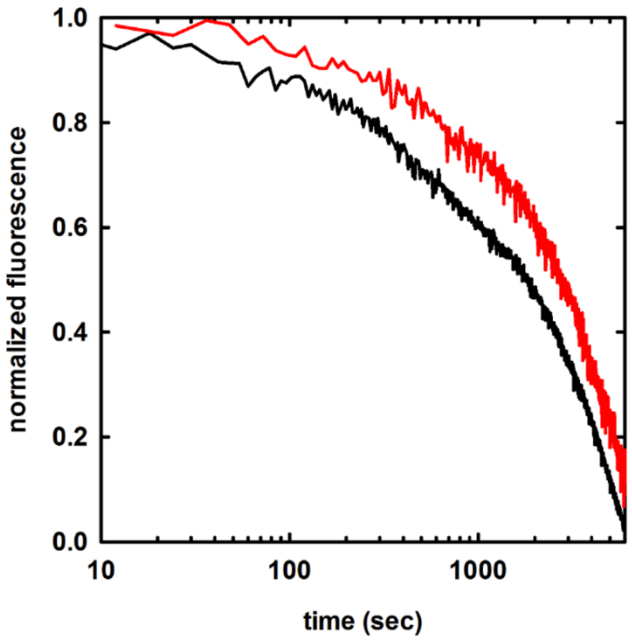


Figure 5: Photodegradation test for MediaChrom **15c** (red line) and Prodan (black line) in ethanol (c = 5 μM, excitation wavelength = 380 nm).

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 with 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 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 vesicles suspension at different temperatures. It has been already observed that an increase in temperature cause 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 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.

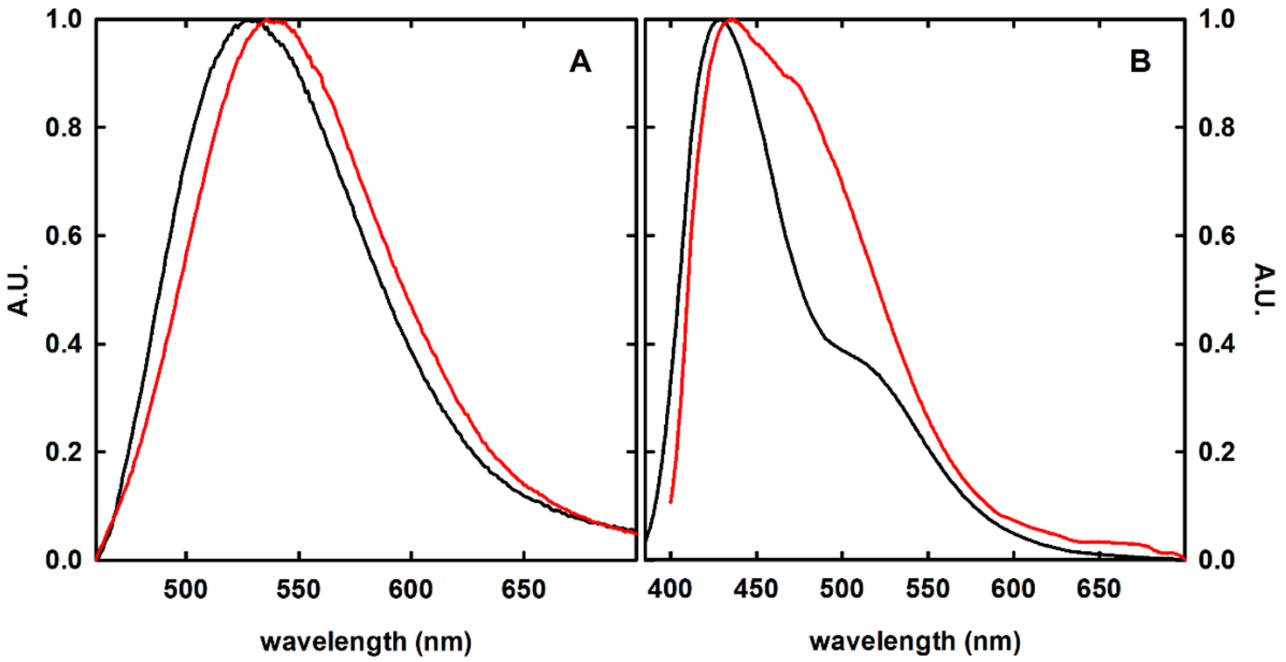


Figure 6: Fluorescence spectra of 1.6 μg/mL MediaChrom **15c** (A) and Prodan (B) in DPPC vesicles at different temperatures: 20°C (black lines) and 37°C (red lines). Excitation wavelength = 380 nm.

MediaChrom 15' as peptide labels

As described above, MediaChrom dyes **15** were designed with a benzyl ester, to be easily deprotected transforming the MediaChrom *dyes* **15** in the 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 bio-conjugates could be thus used for the detection of interaction 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, which plays a key role in the switch from lysogenic to 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 Kd of around 25 μM.[46] The wild-type DNA binding sequence (GQTKTAKDLGVYQSAINKAIHAG) was

prepared by microwave-assisted solid phase synthesis[47] and on resin labelled 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).

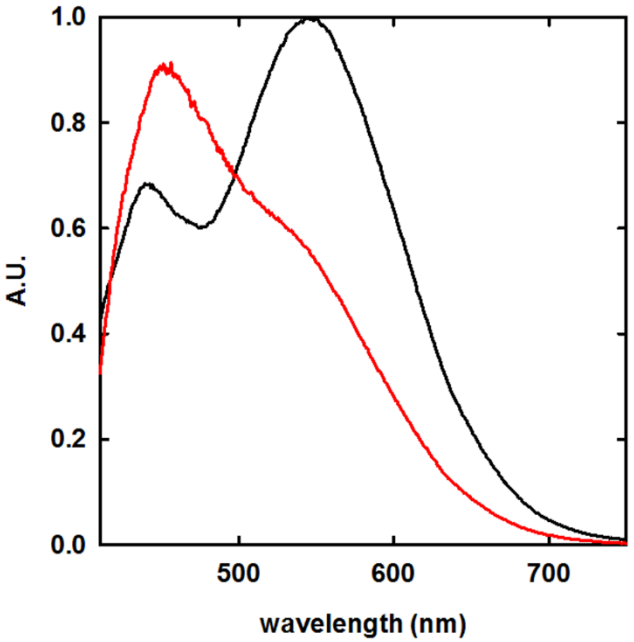


Figure 7: Normalized fluorescence spectra of peptide in the presence (red line) and in the absence (black line) of O_R3 consensus sequence (**15'c**-Cro:1 50 μM, O_R3 50 μM, PBS buffer, pH 7.4, 20°C). Excitation wavelength = 380 nm.

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 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 in 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.

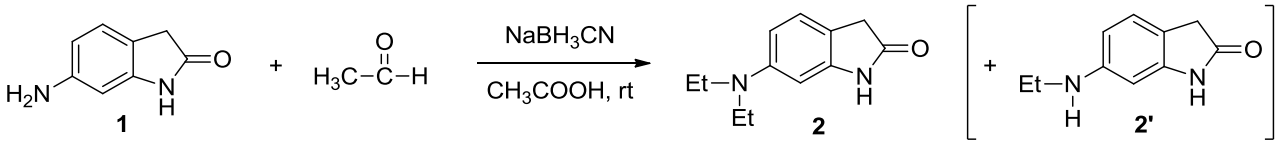
CONCLUSIONS

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 add 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 profile. Among them, MediaChrom **15c** shows the best features for biological applications: a high absorption coefficient in the visible range 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 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 take as lead compound to test some conceivable applications. MediaChrom **15c** demonstrated to be a versatile solvatochromic dye that can be used as a membrane/lipophilic probe as well as its parent carboxy-free MediaChrom **15'c** demonstrated to be a highly sensitive label for peptide tagging, useful for study 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 *in vivo* of MediaChrom dyes, e.g., 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 Alu foils with 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 MHz 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), the chemical shifts (δ) in ppm. ^{13}C NMR analysis were performed with the same instruments at 50.3 and 75.45 MHz. Attached Proton Test (APT) sequence 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 4000 and 400 cm^{-1}. Low-resolution MS spectra were recorded with electron impact source and electrospray/ion trap equipped instrument, 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).

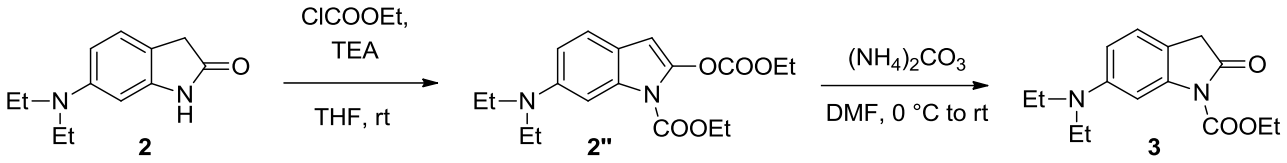


$NaBH_3CN$ (190 mg, 3.02 mmol) was added to a solution of 6-aminoindolin-2-one (**1**) (180 mg, 1.21 mmol) in glacial AcOH (2 mL) followed by acetaldehyde (0.48 mL, 373 mg, 8.47 mmol), and the mixture was stirred at rt for 24 h. The suspension was concentrated in vacuo, poured into H_2O (25 mL) and extracted with EtOAc (3 × 20 mL). The organic layers were collected, dried over Na_2SO_4, 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 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). 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.

Beside, a modest amount of the corresponding mono-ethylated derivative **2'** (6-(ethylamino)indolin-2-one) was obtained: yellow/orange solid (21 mg, 10%). Mp 165–169 °C (with decomposition). 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). 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).



2 **2''** **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 stirring 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, 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 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). 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.

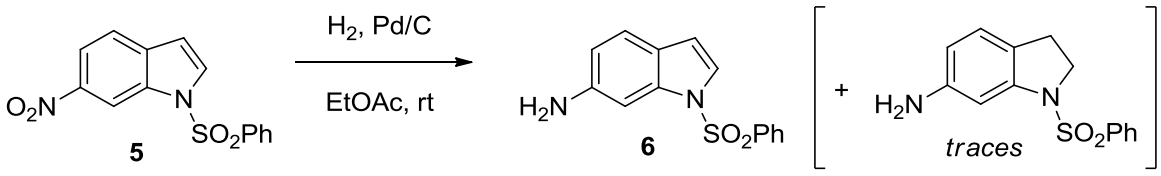
To a solution of *N*,*O*-diacylated product **2''** (375 mg, 1.08 mmol) in DMF (3 mL) was added finely powdered ammonium carbonate (103 mg, 1.08 mmol) at 0-5 °C. The mixture was stirred for 5.0 h at rt

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 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). 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)-*1H*-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 for 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 pale yellow solid (5.37 g, 96%). Mp 199–201 °C (dec.). 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). 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)-*1H*-indole (6).



To a solution of 6-nitro-1-(phenylsulfonyl)-*1H*-indole (**5**) (2.50 g, 8.27 mmol) in EtOAc (120 mL), Pd/C 10% (10% weight, 250 mg) was added. 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 pale orange wax (2.09 g, 93%) (containing inseparable traces of a by-product 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). Elem. Anal. was not performed because the product contains traces of the indoline derivative.

Synthesis of 6-(*N,N*-diethylamino)-1-(phenylsulfonyl)-*1H*-indole (7). Under a nitrogen atmosphere, to a solution of 6-amino-1-(phenylsulfonyl)-*1H*-indole (**6**) (2.00 g, 7.34 mmol) in dry DMSO (30 mL), KOH 85% (969 mg, 14.68 mmol) was added. 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 other 2 equivalents of ethyl bromide were added and the reaction mixture was stirred overnight at 50 °C until no more starting product was detectable by TLC analysis. Upon finished 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 yellow solid (1.57 g, 65%) beside traces of the corresponding monoethylated derivative (not isolated neither 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). 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)-*1H*-indole (8). The 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)-*1H*-indole (**7**) (700 mg, 2.13 mmol) and TMEDA (0.38 mL, 297 mg, 2.56 mmol) in dry THF (11 mL), the LDA solution freshly prepared was added 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

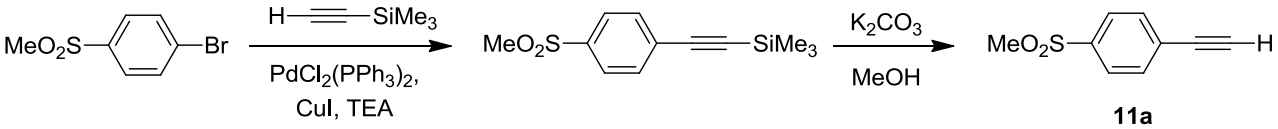
Hex/EtOAc (98:2) to afford the desired product (**8**) as pale brown wax (774 mg, 80%). R_f = 0.21 (silica gel, Hex/EtOAc 95:5). IR (NaCl): ν_{max} = 2970, 2928, 1615, 1503, 1373, 1197, 1173, 730 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ = 1.21 (t, 6H, CH_3, J = 7.0 Hz), 3.43 (q, 4H, CH_2, J = 7.0 Hz), 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). 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)-*1H*-indole (9). Under a nitrogen atmosphere, to a solution of 1-(phenylsulfonyl)-*1H*-indol-6-amine (**6**) (800 mg, 2.94 mmol) in dry DMF (12 mL), *N,N*-diisopropyl-ethylamine (1.02 mL, 759 mg, 5.88 mmol) and bis-(2-bromoethyl)ether (0.55 mL, 1.02 g, 4.41 mmol) were added. The reaction mixture was stirred overnight at 90 °C until no more starting product was detectable by TLC analysis. The residue was poured into sat. 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 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). 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)-*1H*-indole (10). The iodination reaction at the position 2 was performed under the previously optimized reaction conditions (see the synthesis of

compound **8**). The reagent **9** and the 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 ^{1}H-NMR (t_1 = 10s) on 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), ethynyltrimethylsilane (0.35 mL, 251 mg, 2.56 mmol) and *trans*-dichlorobis(triphenylphosphine)palladium(II) (30 mg, 0.04 mmol) were added. 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 detectable 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 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}. ^{1}H 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). Calcd for $C_{12}H_{16}O_2SSi$: C, 57.10; H, 6.39; found: C, 57.33; H, 6.42.

To a stirred solution of trimethyl((4-(methylsulfonyl)phenyl)ethynyl)silane (400 mg, 1.58 mmol) in MeOH (11 mL), K_2CO_3 (438 mg, 3.16 mmol) was added. 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 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). 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)-*1H*-indol-6-amine (**8**) (182 mg, 0.40 mmol) or 4-(2-iodo-1-(phenylsulfonyl)-*1H*-indol-6-yl)morpholine (**10**) (187 mg, 0.40 mmol) in dry DMF (1.6 mL), the appropriate alkyne (**11a-d**) (0.48 mmol), TEA (1.1 mL, 810 mg, 8.00 mmol) and tetrakis(triphenylphospine) palladium(0) (18 mg, 0.016 mmol) were added. 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 detectable 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, 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 (dec.). 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). 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). 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). 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). 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 (dec.). $R_f = 0.28$ (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). 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). 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 NaOH aq. 2M (2.5 mL, 4.92 mmol) was added to a stirring solution of *N,N*-diethyl-2-((4-(methylsulfonyl)phenyl)ethynyl)-1-(phenylsulfonyl)-*1H*-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 detectable 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 orange solid (141 mg, 96%). Mp 131.5–133.5 °C (dec.). R_f = 0.51 (silica gel, Hex/EtOAc 1:1). IR (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). 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 NaOH 6M (2.5 mL, 14.76 mmol) was added to a stirring solution of *N,N*-diethyl-2-((4-nitrophenyl)ethynyl)-1-(phenylsulfonyl)-*1H*-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 detectable 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 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}. ^{1}H 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). 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 NaOH 6M (0.8 mL, 4.92 mmol) was added to a stirring solution of *N,N*-diethyl-1-(phenylsulfonyl)-2-((4-(trifluoromethyl)phenyl)ethynyl)-*1H*-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 detectable 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 pale brown solid (124 mg, 87%). Mp 127–128.5 °C (dec.). 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). 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)-*1H*-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 detectable by TLC analysis. After cooling, the reaction mixture was evaporated almost to dryness then quenched with H_2O (30 mL) and extracted with EtOAc (3 × 20 mL). The organic layers were collected, dried over Na_2SO_4, 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 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). 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 work-up 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 (dec.). 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}. ^{1}H 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). 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 (dec.). 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}. ^{1}H 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). 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% by weight 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 detectable by TLC analysis. The reaction mixture was poured into HCl 0.1M (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were collected, dried over Na_2SO_4, filtered and the solvent was removed at reduced pressure. The crude material was purified by flash chromatography over a silica gel column.

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}. ^{1}H 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). 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). 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). 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). 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). 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}. ^{1}H 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). 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) the catalyst $IPrAuSbF_6$ (4.3 mg, 0.005 mmol) was added. The reaction mixture was heated at 80 °C until no more starting product was detectable 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(1*H*)-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 (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). 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(1*H*)-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). 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(1*H*)-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). 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(1*H*)-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). 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(1*H*)-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). 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(1*H*)-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). 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), Pd/C 10% (10% weight, 15 mg) was added. 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 orange-yellow solid (96 mg, 76%). Mp 148–150.5 °C (dec.). 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). 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 were layered in a vial and DMSO or ethanol were evaporated

by a vacuum concentrator. 500 µL of different solvents were 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 µM to 13.9 µM, Prodan from 100 µM 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 µM to 5 µM). The fluorescence intensity values were recorded exciting the samples at 380 nm wavelength. Fluorescence quantum yields were determined by taking Prodan in ethanol (quantum yield, 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 equation (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 the Avogadro's number and δ the compound density. Dipole moments changes upon excitation were assessed through the general solvent effects described by the Lippert-Mataga equation (2)[40]

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

Where $\overline{\upsilon_a}$ and $\overline{\upsilon_f}$ are the wavenumbers in cm^{-1} of absorption and emission peaks, h is the Planck's constant, c is the speed of light, a 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 photodegradation tests, a 5 µM solution in ethanol of a given dye in a quartz cuvette (pathlength 0.5 cm) was illuminated at 380 nm by the light of a Xenon lamp of a

spectrofluorimeter (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 experiment setup.

Dipalmitoylphosphatidylcholine (DPPC) vesicles preparation. DPPC vesicles were prepared by sonication for 5 minutes a 1 mg/mL DPPC solution in double distilled water.

Peptide synthesis. The peptide Cro:1 (GQTKTAKDLGVYQSAINKAIHAG) was prepared by microwave-assisted solid phase synthesis[47] based on Fmoc chemistry on Fmoc-Rinkamide resin (0.57 meq/g-1 substitution), using a fivefold molar excess of 0.2 M Fmoc-protected amino acids dissolved in *N*-methylpyrrolidinone, and using HOBT/HBTU/DIEA (5:5:10 eq) 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 labelling with MediaChrom 15'c. The labelling of Cro:1 was performed on resin, using 2 eq of **5'c** and HOBT/HBTU/DIEA (2:2:4 eq) as activators.[48] The coupling reaction was performed for 3 hours 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 labelled 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 labelled 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

Attempts to obtain compound **4**. Synthesis of alkyne **11a**. Choice of the catalyst for the Au-catalyzed cycloisomerization. Table of the change in the dipole moments of MediaChrom **15a-f**. Calculation of the distance between electron donors and acceptors groups in Mediachrom **15c** and Prodan. Table of comparison between fluorescent emission peaks of MediaChrom **15c** and **15'c**. Copies of chromatogram and mass spectra of **15'c-Cro:1**. ^{1}H and ^{13}C NMR spectra of all new compounds. COSY, NOESY, HSQC of Compound **15'c**. This material is available free of charge via the internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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