Tetrahedron Letters 50 (2009) 1897–1901

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Fluorinated squaraine as near-IR label with improved properties for the labeling of oligonucleotides

Brice-Loïc Renard, Yves Aubert, Ulysse Asseline *

Centre de Biophysique Moléculaire, CNRS UPR 4301, Affiliated to the University of Orléans and to INSERM, Rue Charles Sadron, 45071 Orléans Cedex 02, France

ARTICLE INFO

Article history:
Received 9 January 2009
Revised 29 January 2009
Accepted 3 February 2009
Available online 8 February 2009

Keywords:
Fluorinated squaraine
Near-infrared label
Squaraine–oligonucleotide conjugates

ABSTRACT

A new squaraine dye with fluorinated benzothiazole rings was synthesized. This new label possesses improved photophysical properties and chemical stability as compared to the corresponding non-fluorinated and the dicyanosquaraines. These squaraines were used for the labeling of a series of oligonucleotides with various sequences, lengths, and chemistries. The conjugates involving the fluorinated squaraine possess the best properties: emission wavelength >670 nm, high quantum yields (0.27–0.39).

1. Introduction

The visualization of biomolecules in vivo can provide information about their location, kinetics, and function. Among these applications, the selective detection of specific DNA and RNA sequences can be achieved by using oligonucleotide probes complementary to the target sequence and containing a reporter group that can be monitored using fluorescence spectroscopy.[1] The development of oligonucleotide-based therapeutic strategies can also take advantage of the possibility to track the labeled oligonucleotides during cell internalisation and cell trafficking toward their targets. These applications require the use of labels that emit in the near-infrared (NIR) range because beyond 600 nm the autofluorescence of biological samples decreases.[2] Numerous long-wavelength labels belonging to rhodamine,[3] BODIPY,[4] oxazine,[5] and cyanine dyes[6] have been reported, and all have both associated advantages and disadvantages depending on the intended applications. The synthesis and spectral characterization of other NIR labels, derived from squaric acid, and their modification for covalent attachment to biomolecules such as cholesterol, sugar and proteins have also been reported.[7,8] To date, despite suitable spectroscopic properties, none of these compounds have been used for the labeling of oligonucleotides. We report now the synthesis and the photophysical properties of a series of five oligonucleotides, with different sequences, lengths, and chemistries, labeled with squaraine dyes based on benzothiazole. Since it has been previously reported that the photophysical properties of fluorinated fluorescein[3a] and thiazole orange[9] are improved as compared to those of the unsubstituted ones, we have tested the influence of fluorination on the properties of the squaraine dyes and the corresponding conjugates (Figs. 1 and 2).

2. Results and discussion

For the labeling of the oligonucleotides, we chose to use squaraines derived from benzothiazole (Scheme 1) and to proceed via the post-synthetic coupling strategy. One benzothiazole ring was methylated,[10] and the second was alkylated with a diiodohexyl linker in order to react the squaraines with a thiophosphate group

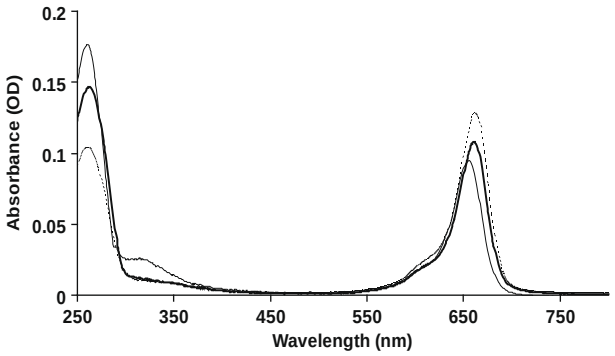

Figure 1. UV–visible absorption spectra of 1 μM solutions of the conjugates $\mathbf{10}_{\mathbf{SQFBt}}$ (dotted lines), $\mathbf{12}_{\mathbf{SQFBt}}$ (bold line), and $\mathbf{13}_{\mathbf{SQFBt}}$ (plain line) in 10 mM cacodylate buffer, pH 7, containing 100 mM NaCl recorded at 20 °C between $\lambda = 250$ and $\lambda = 800$ nm.

* Corresponding author. Fax: +33 2 38 63 15 17.
E-mail address: ulysse.asseline@cnrs-orleans.fr (U. Asseline).

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doi:10.1016/j.tetlet.2009.02.029

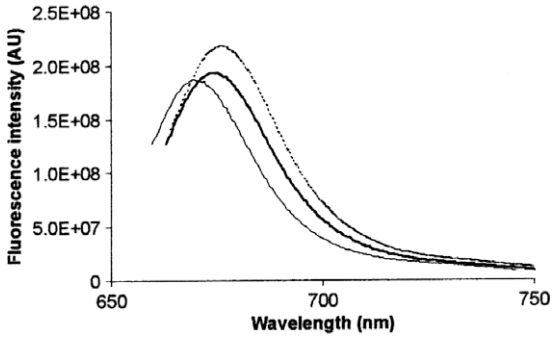
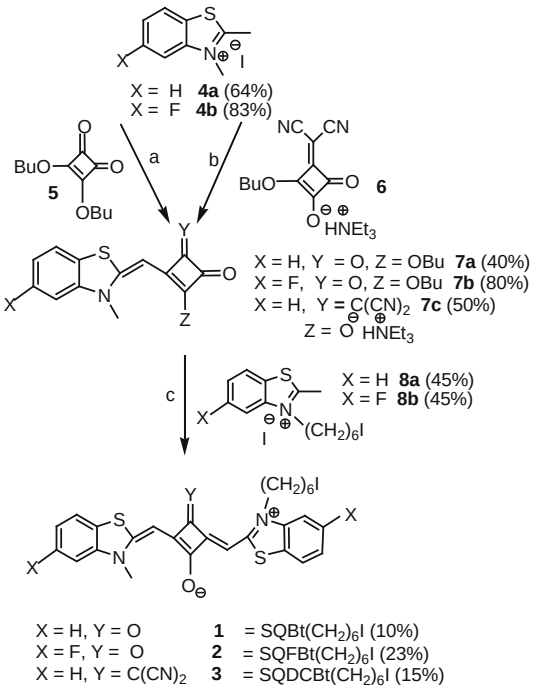


Figure 2. Fluorescence emission spectra of 1 μM solutions of the conjugates $\mathbf{10}_{\mathbf{SQFBt}}$ (dotted lines), $\mathbf{12}_{\mathbf{SQFBt}}$ (bold line), and $\mathbf{13}_{\mathbf{SQFBt}}$ (plain line) in 10 mM cacodylate buffer, pH 7, containing 100 mM NaCl recorded at 20 °C between $\lambda = 650$ and $\lambda = 750$ nm.



Scheme 1. Synthesis of the squaraine dyes **1**–**3**. Reagents and conditions: (a) EtOH, TEA, 65 °C, 90 min; (b) EtOH, TEA, 65 °C, 60 min; (c) butanol, toluene, 120 °C, 10 h, or 100 °C, 8 h for X = H, Y = $C(CN)_2$.

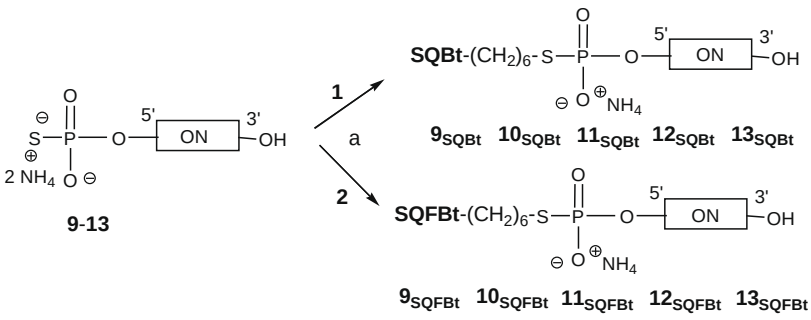
incorporated at the 5′-end of the oligonucleotides to form a phosphothiolodiester linkage (Scheme 2).[11]

The synthesis of the squaraine dyes **1**–**3** was performed via a multi-step procedure (Scheme 1). The dibutyl ester of the squaric acid **5** was first reacted with the methylated benzothiazole derivative[12] **4a** or with the corresponding fluorinated derivative **4b**, obtained by reaction of iodomethane excess with either benzothiazole or fluorobenzothiazole moieties, to give hemisquaraines **7a** and **7b**. The latter were then reacted with the benzothiazole nuclei involving the iodohexyl linker attached to the nitrogen atom of **8a** and **8b,** obtained by reaction of the benzothiazole and the fluorobenzothiazole with 1,6-diiodohexane, to give the squaraines **1** and **2** in moderate yields. For the synthesis of squaraine **3** involving a dicyanomethylene group on the squaric acid, we chose a similar strategy. First, the monobutyl ester of the dicyanomethyl squaric acid **6** was obtained following a reported procedure.[8a] The ester was reacted with the methylated benzothiazole **4a** to give hemisquaraine **7c**. The dicyanomethylated squaraine **3** was then obtained by reaction of **7c** with the benzothiazole linker derivative **8a**. All the compounds were characterized by NMR and mass spectrometry analysis (See Refs. 13–15 and Supplementary data).

In order to test the influence of the sequence, length, and chemistry of the oligonucleotide on the properties of the squaraine-oligonucleotide conjugates, we used five different sequences (Table 1). The 5′-thiophosphorylation was performed by our previously published procedure.[16] For the labeling step, different procedures were tested, either in methanol solutions of crown ethers to solubilize the oligonucleotides or in different mixtures of bicarbonate buffer and dimethylformamide, in order to increase the yields in conjugates. The coupling of squaraines **1** and **2** was successful with the five oligonucleotides **9**–**13** (Scheme 2 and Table 1). The corresponding conjugates $\mathbf{9}_{\mathbf{SQBt}}$–$\mathbf{13}_{\mathbf{SQBt}}$ and $\mathbf{9}_{\mathbf{SQFBt}}$–$\mathbf{13}_{\mathbf{SQFBt}}$ were obtained in yields ranging from 3% to 24% after purification by reversed-phase chromatography. The presence of the fluorine atoms on the benzothiazole nuclei led to conjugates with slightly increased retention times. Their masses were confirmed by mass spectrometry analysis. The preparation of the conjugates of the dicyanomethylated squaraine **3** failed despite the various conditions used. This may be due to the propensity of the dye to form aggregates and to its lack of stability under the reaction conditions used.

The dyes **1**–**3** were studied by absorption and fluorescence spectroscopy. The presence of the fluorine atoms (compound **2**) induced a red shift of 6 nm in the absorption spectra ($\lambda = 662$ vs 656 nm for **1**) in EtOH and ($\lambda = 675$ vs 669 nm) in chloroform. The presence of the dicyanomethyl group on the squaric acid induced a more pronounced red shift (+25 nm) for compound **3**. The ε values were high ($\geqslant 200{,}000\ M^{-1}\ cm^{-1}$) as were the quantum yields (~0. 44 for **1** and **2** and 0.35 for **3** in $CHCl_3$).

When the solutions were stored at 4 °C, no change in emission intensity was observed over the course of 15 h. At 20 °C, a 3%



Scheme 2. Synthesis of the conjugates labeled with **1** and **2**. Reagents and conditions: (a) 2.5% aqueous bicarbonate buffer, pH 9 (or pH 7 for **2**), (0.15 mL). The mixture was stirred for 4–10 h at rt.

Table 1
Characterizations for conjugates

ONs	Sequences	Rt (min) HPLC	Mass analysis		Yield (%)
9	$^{5'}p$(S)TTTTCTTTTC$^{3'}$	11 min 37 s	3044.00	3044.06	70
10	$^{5'}p$(S)ATTTGGAACC$^{3'}$	9 min 41 s	3123.08	3122.85	65
11	$^{5'}p$(S)TTCTCCCCCGCTTA$^{3'}$	12 min 40 s	4221.77	4221.70	50
12	$^{5'}p$(S)CCGCTTAATACTGA$^{3'}$	10 min 43 s	4318.87	4318.94	58
13	$^{5'}p$(S)(U-2′OMe)$^{3'}_{20}$	12 min 50 s	6438.97	6438.40	66
$\mathbf{9}_{\mathbf{SQBt}}$	SQBt-$(CH_2)_6$-$^{5'}$S*p*TTTTCTTTTC$^{3'}$	27 min 06 s	3519.63	3519.03	6
$\mathbf{10}_{\mathbf{SQBt}}$	SQBt-$(CH_2)_6$-$^{5'}$S*p*ATTTGGAACC$^{3'}$	27 min 10 s	4694.39	4695.55	5
$\mathbf{11}_{\mathbf{SQBt}}$	SQBt-$(CH_2)_6$-$^{5'}$S*p*TTCTCCCCCGCTTA$^{3'}$	26 min 22 s	4694.39	4695.55	6
$\mathbf{12}_{\mathbf{SQBt}}$	SQBt-$(CH_2)_6$-$^{5'}$S*p*CCGCTTAATACTGA$^{3'}$	26 min 33 s	4791.48	4791.56	8
$\mathbf{13}_{\mathbf{SQBt}}$	SQBt-$(CH_2)_6$-$^{5'}$S*p*(U-2′OMe)$^{3'}_{20}$	27 min 31 s	6910.59	6912.38	7
$\mathbf{9}_{\mathbf{SQFBt}}$	SQFBt-$(CH_2)_6$-$^{5'}$S*p*TTTTCTTTTC$^{3'}$	28 min 03 s	3553.62	3554.90	24
$\mathbf{10}_{\mathbf{SQFBt}}$	SQFBt-$(CH_2)_6$-$^{5'}$S*p*ATTTGGAACC$^{3'}$	27 min 34 s	3630.68	3629.30	4
$\mathbf{11}_{\mathbf{SQFBt}}$	SQFBt-$(CH_2)_6$-$^{5'}$S*p*TTCTCCCCCGCTTA$^{3'}$	26 min 59 s	4730.37	4729.64	3
$\mathbf{12}_{\mathbf{SQFBt}}$	SQFBt-$(CH_2)_6$-$^{5'}$S*p*CCGCTTAATACTGA$^{3'}$	27 min 58 s	4827.46	4827.99	5
$\mathbf{13}_{\mathbf{SQFBt}}$	SQFBt-$(CH_2)_6$-$^{5'}$S*p*(U-2′OMe)$^{3'}_{20}$	27 min 58 s	6946.57	6946.70	8

ONs: 5′-thiophosphorylated oligonucleotides and oligonucleotide-squaraine conjugates. Retention times obtained by reversed-phase chromatography analyses for the 5′-thiophosphorylated ONs **9**–**13** and the corresponding conjugates performed on a Lichrospher RP 18 (5 μm) column (125 mm × 4 mm) from Merck using a linear gradient of CH_3CN (5–38.5% over 45 min) in 0.1 M aqueous ammonium acetate, pH 7, with a flow rate of 1 mL/min. Mass analysis data for the 5′-thiophosphorylated ONs **9**–**13** and the corresponding conjugates. Yields for the 5-thiophosphorylated oligonucleotides and conjugates.

decrease was observed for **1** and only 2% for **2** over the same period of time. At 37 °C, no real change was observed over 4 h for **1** and **2**.

The absorption spectra of conjugates **9**–**13** were recorded between $\lambda = 250$ and $\lambda = 800$ nm. In all cases, the spectra contain an absorption band in the UV range corresponding to the absorbance of the oligonucleotide and the squaraine, while the absorption band in the visible range corresponds to that of the squaraine. The λ_{max} values of both the UV and visible absorption bands are given in Table 2. The intensity ratios of the UV–visible bands are also different depending on the squaraine considered (Table 2). A comparison of the two series of conjugates showed that for every squaraine, the λ_{max} visible was slightly different depending on the sequence, suggesting interactions between the squaraines and the oligonucleotides. The molar extinction coefficient values (ε) for conjugates $\mathbf{9}_{\mathbf{SQBt}}$, and $\mathbf{9}_{\mathbf{SQFBt}}$ were determined by titration of their solutions with the complementary sequence $^{5'}$GGGAAA AGAAAATTT$^{3'}$ at 4 °C. For the conjugates of **10**–**13** the ε values at 260 nm were approximated as the sum of the ε values of the corresponding oligonucleotides, determined using the nearest neighbor model,[17] and of the squaraines deducted from those of conjugates $\mathbf{9}_{\mathbf{SQBt}}$ and $\mathbf{9}_{\mathbf{SQFBt}}$.

A comparison of the emission spectra of the two series of conjugates clearly indicated similar emission shapes with λ_{max} emission around 670 nm depending on the oligonucleotide sequences. However, different fluorescence intensities were observed. The presence of the fluorine atoms induced intensity increases by about 50%. All the conjugates exhibited high quantum yields (Table 2) with the highest values (0.27–0.39) for those involving the fluorinated squaraine **2**.

The photostability of the squaraine linker derivatives **1**–**2** and of oligonucleotide-squaraine conjugates $\mathbf{9}_{\mathbf{SQBt}}$, $\mathbf{9}_{\mathbf{SQFBt}}$, $\mathbf{10}_{\mathbf{SQBt}}$, and $\mathbf{10}_{\mathbf{SQFBt}}$ was investigated and compared to those of the corresponding conjugates of the well-known dyes thiazole orange $\mathbf{9}_{\mathbf{TO}}$[18] and Indocyanine Green analogue[6i,19] $\mathbf{9}_{\mathbf{Cy7mh}}$ involving the dyes attached via the same linker (See Supplementary data). For the photostability measurements, 1 μM solutions were prepared in chloroform or ethanol for compounds **1**–**2** and in a 10 mM cacodylate buffer, pH 7, containing 100 mM NaCl for the conjugates $\mathbf{9}_{\mathbf{SQBt}}$, $\mathbf{9}_{\mathbf{SQFBt}}$, $\mathbf{10}_{\mathbf{SQBt}}$ and $\mathbf{10}_{\mathbf{SQFBt}}$, $\mathbf{9}_{\mathbf{TO}}$ and $\mathbf{9}_{\mathbf{Cy7mh}}$. Ten successive emission spectra were recorded at room temperature with the same solutions. No changes in the emission were observed for **2** and only 5% for **1** in ethanol. The same experiment performed with the conjugates indicated a 10% emission decrease for the conjugates involving the fluorinated cyanine versus 25% for the non-fluorinated ones. Under the same conditions, the emission of conjugates $\mathbf{9}_{\mathbf{TO}}$ and $\mathbf{9}_{\mathbf{Cy7mh}}$ remained unchanged.

Solutions of the conjugates $\mathbf{9}_{\mathbf{SQBt}}$ and $\mathbf{9}_{\mathbf{SQFBt}}$ in a 10 mM cacodylate buffer, pH 7, containing 100 mM NaCl were stored at different

Table 2
Absorption and fluorescence data for conjugates

Conjugates	Absorption				Fluorescence			
					Conjugates			Conjugates
	λ_{UVmax} (nm)	ε (M^{-1} cm^{-1})	λ_{VISmax} (nm)	ε (M^{-1} cm^{-1})	λ_{ex} (nm)	λ_{em} (nm)	I_f	ϕ
$\mathbf{9}_{\mathbf{SQBt}}$	268	87,100	654	114,300	654	668	111.48	0.24 (0.16 EtOH)
$\mathbf{10}_{\mathbf{SQBt}}$	260	105,100	656	128,400	656	670	137.05	0.26
$\mathbf{11}_{\mathbf{SQBt}}$	265	122,700	659	102,200	659	671	103.36	0.24
$\mathbf{12}_{\mathbf{SQBt}}$	262	140,600	657	108,600	657	671	100.00	0.23
$\mathbf{13}_{\mathbf{SQBt}}$	259	169,600	649	100,600	649	666	61.83	0.17
$\mathbf{9}_{\mathbf{SQFBt}}$	266	85,500	661	122,000	661	673	165.34	0.32 (0.20 EtOH)
$\mathbf{10}_{\mathbf{SQFBt}}$	260	103,500	662	126,600	662	676	177.56	0.37
$\mathbf{11}_{\mathbf{SQFBt}}$	266	121,100	666	119,300	666	678	176.33	0.39
$\mathbf{12}_{\mathbf{SQFBt}}$	262	139,000	661	114,300	661	675	160.00	0.35
$\mathbf{13}_{\mathbf{SQFBt}}$	260	168,000	655	102,000	665	671	110.27	0.27

Absorption and emission spectra were recorded in a 10 mM sodium phosphate buffer, pH 7, containing 140 mM KCl and 5 mM $MgCl_2$ at 20 °C. Concentrations were 1 μM in conjugates. The values in parenthesis correspond to the ϕ of the squaraines **1** and **2** determined in ethanol.
Molar absorption coefficients (ε) were determined experimentally for the conjugates $\mathbf{9}_{\mathbf{SQBt}}$ and $\mathbf{9}_{\mathbf{SQFBt}}$. The ε values at $\lambda = 260$ nm for the other conjugates were the sum of the ε values for the oligonucleotides and the squaraines.

temperatures. While the emission of conjugate $\mathbf{9_{SQBt}}$ was nearly undetectable after 4 h at 20 °C or 1 h at 37 °C, that of $\mathbf{9_{SQFBt}}$ was 70% of its initial value after 4 h at 20 °C and was 75% after 1 h at 37 °C. The fluorescence decrease observed at 20 °C was similar to that obtained with the conjugate $\mathbf{9_{Cy7mh}}$, while that of $\mathbf{9_{TO}}$ was unchanged.

The influence of the pH was also tested. The emission spectra of solutions of conjugates $\mathbf{9_{SQBt}}$, $\mathbf{9_{SQFBt}}$, $\mathbf{10_{SQBt}}$, and $\mathbf{10_{SQFBt}}$, were recorded in aqueous buffer with pH ranging from 5 to 8. The results indicated nearly equivalent values at any pH for the conjugates involving the fluorinated squaraine, while the conjugate derived from the non-fluorinated squaraine was not stable at pH below 7.

The presence of the squaraines induced a weak stability increase of the complexes formed by the conjugates $\mathbf{9_{SQBt}}$ and $\mathbf{9_{SQFBt}}$ with their single-stranded $^{5'}$GGGAAAAGAAAATTT$^{3'}$and double-stranded $^{5'}$GCCACTTTTTAAAAGAAAAGGGG$^{3'}$/$^{3'}$CGGTGAAAAATTT TCTTTTCCCC$^{5'}$ DNA targets. Upon hybridization at 20 °C, the fluorescence emission of the $\mathbf{9_{SQBt}}$ and $\mathbf{9_{SQFBt}}$ decreased by 28% and 9% in the presence of the single-stranded target and by 8% and 42% in the presence of the double-stranded target.

3. Conclusion

We have reported the synthesis of three squaraine dyes based on benzothiazole moiety with wavelength emissions beyond 670 nm. Two of them have been successfully used for the labeling of a series of five oligonucleotides with different sequences, lengths, and chemistries. The oligonucleotides labeled with the squaraines exhibited high fluorescent emissions, about 60 and 30-fold those of the corresponding oligonucleotides labeled with the well-known dyes thiazole orange and Indocyanine Green analogue. As previously reported for thiazole orange, the fluorination of the benzothiazole heterocycle of the squaraine led to an improvement in photostability. This photostability increase was also observed with the labeled oligonucleotides. The presence of the fluorine atom on the label induced a strong chemical stability increase, between pH 5 and 8, that reached that of the corresponding thiazole orange labeled oligonucleotide. These properties make the fluorinated squaraine dyes very promising tools for the labeling of oligonucleotides.

Acknowledgments

We thank the Region Centre for a doctoral fellowship to B.-L.R., the «Plateforme de Spectrometrie de masse» of the «Centre de Biophysique Moléculaire» for analysis facilities, G. Gabant for running the mass spectrometers, and H. Meudal for recording the NMR spectra.

Supplementary data

The Supplementary data section includes detailed experimental conditions and characterization data for compounds **4a**, **4b**, **6**, **7a**, **7b**, **7c**, **8a**, **8b** and **Cy7mh** and $\mathbf{9_{Cy7mh}}$. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.02.029.

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13. *4-[N-(6-iodohexyl)-1,3-benzothiazoliumyl-2-methylene]-2-(N-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenemethyl)-3-oxo-1-cyclobutene-1-olate* **1**: Compound **7a** (380 mg, 1 equiv, 1.20 mmol) was added to a solution of **8a** (587 mg, 1 equiv, 1.20 mmol) in a butanol/toluene (1:1, v/v) mixture (15 mL). The mixture was stirred at 120 °C for 10 h. After removal of the solvent by evaporation, the residue was purified on a silica gel column using a MeOH gradient (0–5%) in CH_2Cl_2, then on preparative silica gel plates using a CH_2Cl_2/MeOH mixture (96:4, v/v) as eluent to give a dark blue solid 42 mg. Yield: 12%. TLC: R_f (CH_2Cl_2/MeOH 8/2) = 0.45. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ (ppm) = 7.61 (t, 2H, J = 7.8 Hz, H_{Ar}), 7.47 (m, 2H, H_{Ar}), 7.32 (m, 4H, H_{Ar}), 6.39 (s, 2H, =CH), 4.18 (m, 2H, $^+$N–CH_2–), 3.75 (s, 3H, N–CH_3), 3.24 (t, J = 6.9 Hz, 2H, –CH_2I), 1.88 (m, 4H, 2 CH_2–), 1.56 (m, 4H, 2 CH_2–). ^{13}C NMR (126 MHz, $CDCl_3$, TMS): δ (ppm) = 6.8, 26.1, 27.4, 30.4, 33.0, 33.4, 46.2, 85.6, 111.4, 111.5, 122.3, 122.4, 124.1, 124.2, 127.2, 127.3, 128.7, 128.9, 141.2, 141.7, 159.9, 160.1. Maldi-Tof-MS: m/z, $C_{27}H_{25}O_2N_2S_2I$ calcd 600.55, found 600 (M^+).
14. *4-[5-Fluoro-N-(6-iodohexyl)-1,3-benzothiazoliumyl-2-methylene]-2-(5-fluoro-N-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenemethyl)-3-oxo-1- cyclobutene-1-olate* **2**: Compound **7b** (440 mg, 1 equiv, 1.32 mmol) was added to a solution of **8b** (668 mg, 1 equiv, 1.32 mmol) in a butanol/toluene (1:1, v/v) mixture (15 mL). The mixture was stirred at 120 °C for 10 h. After removal of the solvent by evaporation, the residue was purified on a silica gel column using a MeOH gradient (0–3%) in CH_2Cl_2, then on preparative silica gel plates using a CH_2Cl_2/MeOH mixture (96:4, v/v) as eluent to give a dark blue solid 91 mg. Yield: 23%. TLC: R_f (CH_2Cl_2/MeOH 8/2) = 0.45. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ (ppm) = 7.47 (m, 2H, H_{Ar}), 6.96 (m, 2H, H_{Ar}), 6.89 (m, 2H, H_{Ar}), 5.91 (s, 2H, =CH), 4.06 (t, 2H, J = 7.8 Hz, $^+$N–CH_2–), 3.64 (s, 3H, N–CH_3), 3.23 (t, J = 6.9 Hz, 2H, –CH_2I), 1.86 (m, 4H, 2 CH_2–), 1.52 (m, 4H, 2 CH_2–). ^{13}C NMR (126 MHz, $CDCl_3$, TMS): δ (ppm) = 6.7, 26.1, 27.3, 30.4, 33.2, 33.3, 46.6, 86.5 (d, J = 14.7 Hz), 99.4 (d, J_{C-F} = 28.1 Hz), 99.6 (J_{C-F} = 28.1 Hz), 111.7 (t, J_{C-F} = 23.1 Hz), 123.2 (t, J_{C-F} = 10.5 Hz), 161.0, 162.7 (d, J_{C-F} = 245.7 Hz). Maldi-Tof-MS: m/z, $C_{27}H_{23}O_2N_2S_2F_2I$ calcd 636.52, found 637.27 (M^+).
15. *3-Dicyanomethylene-4-[N-(6-iodohexyl)-1,3-benzothiazoliumyl-2-methylene]-2-(N-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenemethyl)-1-cyclobutene-1-olate* **3**: Compound **7c** (270 mg, 1 equiv, 0.66 mmol) was added to a solution of **8a** (362 mg, 1.2 equiv, 0.802 mmol) in EtOH (10 mL), and the mixture was heated to 85 °C for 8 h under stirring. After removal of the solvent by evaporation, the residue was purified on a silica gel column using a MeOH gradient (0–5%) in CH_2Cl_2, then on preparative silica gel plates using a CH_2Cl_2/MeOH mixture (98:2, v/v) as eluent to give a turquoise blue solid. Yield: 15%. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ (ppm) = 7.57 (t, 2H, J = 7.5 Hz, H_{Ar}), 7.41 (d t, 2H, J = 7.5 Hz, J = 8 Hz, H_{Ar}), 7.24 (q, 2H, J = 7 Hz, H_{Ar}), 7.17 (m, 2H, H_{Ar}), 5.92 (s, 2H, =CH), 4.12 (t, 2H, J = 7.5 Hz, –$^+$N–CH_2–), 3.68 (s, 3H, N–CH_3), 3.23 (t, J = 7 Hz, 2H, –CH_2I), 1.88 (m, 4H, 2 CH_2–), 1.52 (m, 4H, 2 CH_2–). ^{13}C NMR (126 MHz, $CDCl_3$, TMS): δ (ppm) = 7.1, 26.0, 27.6, 29.9, 30.3, 33.3, 46.9, 87.3, 112.0, 112.1,

119.1, 122.5, 122.6, 124.9. 127.7. Maldi-Tof-MS: *m/z*, $C_{30}H_{25}N_4OS_2I$ calcd 648.59, found 648.02 (M^+). TLC: R_f (CH_2Cl_2/MeOH 9/1) = 0.75.
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