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Rigid-Core Fluorescent Oligothiophene-*S*,*S*-dioxide Isothiocyanates. Synthesis, Optical Characterization, and Conjugation to Monoclonal Antibodies

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In this paper we report the synthesis of a new class of fluorescent thiophene-based isothiocyanates containing a 3,5-disubstituted dithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide moiety as the rigid core, using the palladium-catalyzed cross-coupling reaction of aryl stannanes with aryl bromides (Stille coupling). By changing the molecular structure through the progressive addition of thienylene or phenylene units, light emission from blue to orange was obtained. Photoluminescence quantum yields ranged from 0.65 to 0.90 for blue and green light emitters to 0.10−0.35 for yellow and orange ones. Optically and chemically stable fluorescent bioconjugates were prepared by spontaneous reaction of the isothiocyanates with monoclonal antibodies anti-CD3 and anti-CD8 in slightly basic solutions.

Introduction

Today, there is great interest in fluorescent organic compounds because of their potential in very different fields, from electroluminescent devices and flat panel display technology to biolabeling and measurements of cells by flow cytometry or microscopy.[1]

We have recently reported not only that thiophene oligomers exhibit great potential as active layers in thin-film electroluminescent devices and lasers[2a,b] but that they can also be modified in such a way as to become attractive, photostable, fluorescent markers for biopolymers.[2c,d]

Fluorescence detection allows many clinical and research applications aimed to identify specific populations of cells and monitor metabolic processes.[3] It is now largely used in monitoring antigen−antibody interactions, in nucleic acid labeling, in genomic studies, and in hystological tests.[3] Furthermore, immunochemical methods and fluorescence detection techniques are becoming more and more important in environmental studies.[5]

Currently, fluorescence techniques are limited by the chemical nature of fluorescent markers. Commercially available fluorophores are either small organic molecules with great membrane permeability or high-weight natural compounds obtained from microalgae or even mixtures of compounds emitting light through a complex pattern of intermolecular interactions, all requiring a different chemistry to bind biomolecules.[3,4]

The growing importance of fluorescence detection techniques has prompted the search for new classes of compounds with improved optical properties and tunable fluorescence frequencies that are easy to functionalize, to allow the standardization of labeling procedures and make multilabeling experiments more accessible. Recently, several studies have been reported on modified fluoresceins[6] and cyanines,[7] antracene and squaraine derivatives,[8,9] conjugated polymers,[10] and probes able to detect ions that play a metabolic role.[11]

On these grounds, we have started research work aimed at synthesizing new classes of thiophene-based

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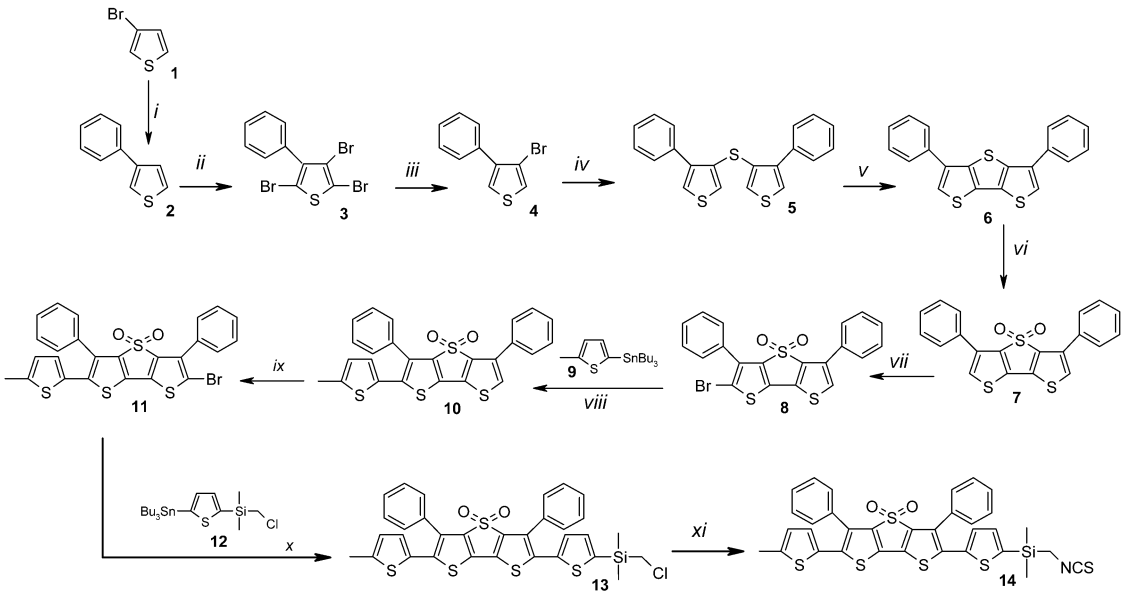
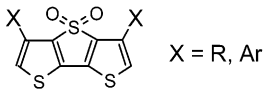
SCHEME 1. Synthesis of Isothiocyanate 14 Starting from Commercial 3-Bromothiophene[a]



[a] Reagents and conditions: (i) phenylmagnesium bromide, Et_2O; (ii) (1) NBS, CH_2Cl_2/CH_3COOH; (2) Br_2, CH_2Cl_2; (iii) *n*-BuLi, Et_2O; (iv) *n*-BuLi, SCl_2, Et_2O; (v) *n*-BuLi, $CuCl_2$, Et_2O; (vi) mCPBA, CH_2Cl_2; (vii) NBS, DMF; (viii) **9**, $Pd(AsPh_3)_4$, toluene; (ix) NBS, CH_2Cl_2/CH_3COOH; (x) **12**, $Pd(PPh_3)_4$, toluene; (xi) NaSCN, acetone.

fluorescent markers. Our strategy consists of building thiophene-based structures containing the isothiocyanate functionality, −N=C=S, capable of forming a stable linkage with biomolecules containing $-NH_2$ amino groups.[3] In experiments with bovine serum albumin and monoclonal antibodies we have been able to demonstrate that oligothiophene isothiocyanates bind spontaneously to proteins and form very stable fluorescent bioconjugates that display unaltered biological activity.[2c]

In this paper we report the synthesis of oligothiophene isothiocyanates containing a 3,5-disubstituted dithieno-[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide moiety (DTTO), which



DTTO

has been demonstrated to be a very efficient light emitter.[12a] By means of the Stille coupling,[13] we have synthesized oligomers containing the DTTO moiety and functionalized with the NCS group at one or both terminal positions. The size of the oligomers was increased by the systematic addition of thienylene or phenylene rings, to tune the fluorescence frequency of the molecule.

Finally, we show that the newly synthesized isothiocyanates react spontaneously with monoclonal antibodies, forming bioconjugates that are chemically and optically stable in phosphate buffer solutions.

Results

(I) Synthesis of Rigid-Core Oligothiophene-*S,S*-Dioxide Isothiocyanates. The synthetic patterns followed for the preparation of rigid-core isothiocyanates via the Stille coupling are shown in Schemes 1−3.

Scheme 1 reports the synthesis of 3,5-diphenyldithieno-[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (DTTOPh, **7**), starting from commercial 3-bromothiophene, and isothiocyanate **14** bearing this moiety as the inner rigid core.

Polybrominated thiophene **3** was prepared in two steps. First, the α positions were brominated using *N*-bromosuccinimide in CH_2Cl_2/CH_3COOH, and then the β position was brominated using Br_2 in CH_2Cl_2. Subsequent treatment with BuLi in ethyl ether led to 3-bromo-4-phenylthiophene **4** in good yield. This procedure was found to be much less expensive than the direct introduction of the phenyl group in one of the β positions through the Grignard reaction on 3,4-dibromothiophene.[14] Also, disulfide **5** was not prepared according to well-established procedures,[12,15a] but was synthesized in a more expedient way by the action of SCl_2 on **4**. The subsequent steps of dithienothiophene formation (v−ix, Scheme 1), namely, selective sulfur oxidation, selective bromination at one of the terminal positions, and cross-coupling of the bromo derivative with thienyl stannanes occurred with yields that were lower than those obtained in the preparation of 3,5-dimethyldithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (**15**, DTTOMe) and its derivatives.[12a] Probably, this was the result of both the electronic and steric effects of the phenyl substituents on the different reaction steps. In particular, for steric reasons, the rate of the Stille coupling in the presence of the bulky phenyl groups of DTTOPh must be lower than in the presence of the methyl substituents of DTTOMe. Low cross-coupling rates favor the self-coupling of the reagents and bromine−

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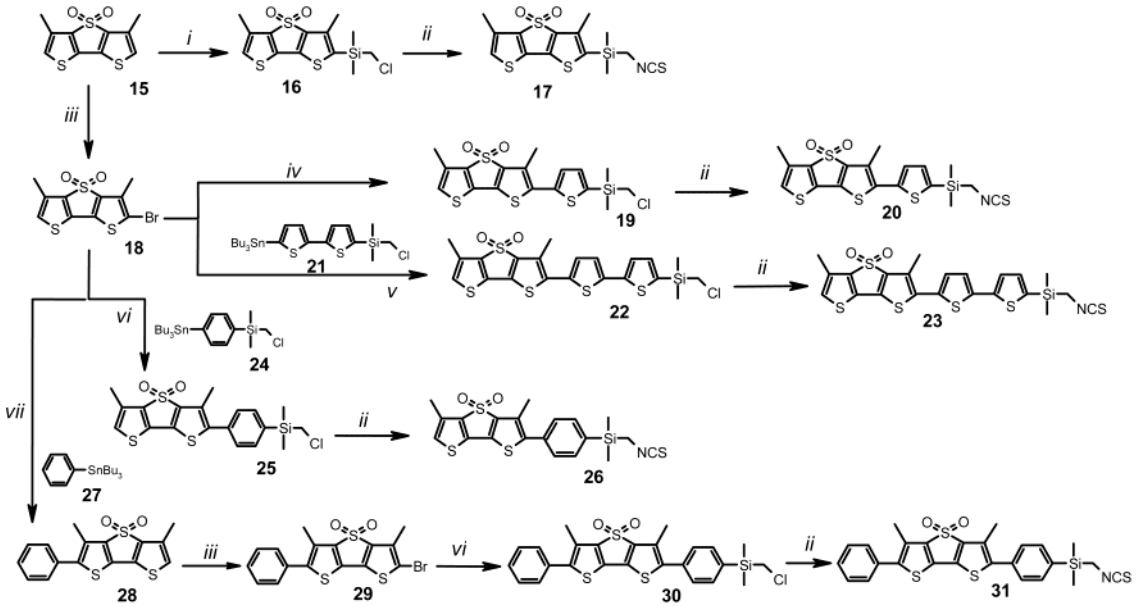
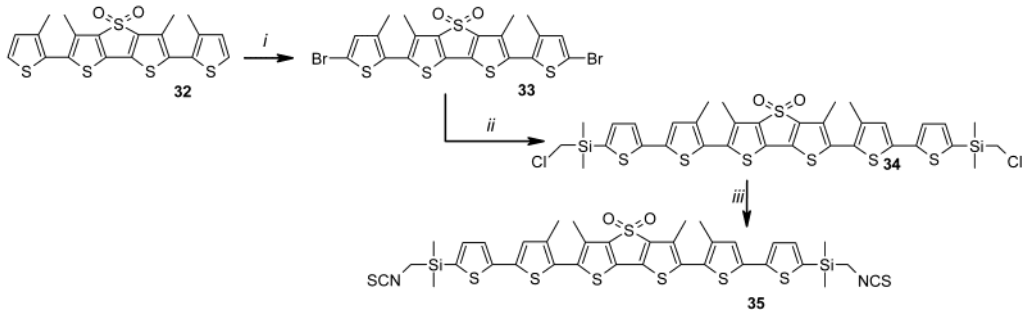
SCHEME 2. Synthesis of Isothiocyanates 17, 20, 23, 26, and 31 Starting from Dithienothiophene 15[a]



[a] Reagents and conditions: (i) LDA, $ClSi(CH_3)_2CH_2Cl$, THF; (ii) NaSCN, acetone; (iii) NBS, CH_2Cl_2/CH_3COOH; (iv) **12**, $Pd(AsPh_3)_4$, toluene; (v) **21**, $Pd(AsPh_3)_4$, toluene; (vi) **24**, $Pd(AsPh_3)_4$, toluene; (vii) **27**, $Pd(AsPh_3)_4$, toluene.

SCHEME 3. Synthesis of Isothiocyanate 35 from Tetramer 32[12a,a]



[a] Reagents and conditions: (i) NBS, CH_2Cl_2; (ii) **12**, $Pd(AsPh_3)_4$, toluene; (iii) NaSCN, acetone.

tin exchange reactions,[16] leading to a variety of undesired byproducts, lowering the yields, and making more difficult the purification of the targeted oligomer.

Scheme 2 shows the synthesis of isothiocyanates **17**, **20**, **23**, **26**, and **31** containing DTTOMe[12a] as the rigid core.

Compound **17**, an intensely blue fluorescent compound, was obtained by direct functionalization of DTTOMe with chloro(chloromethyl)dimethylsilane following selective lithiation at one of the terminal positions and subsequent conversion of chlorine to NCS using sodium thiocyanate in acetone. The other isothiocyanates were obtained via the Stille coupling of mono- or dibrominated rigid cores with the appropriate thienyl or phenyl stannanes followed by conversion of chlorine to the isothiocyanate functionality.

The conversion to −NCS of the chlorine atom of $-Si(CH_3)_2CH_2Cl$ attached to a phenyl group, for the preparation of compounds **26** and **31**, occurred in rather low yields (48% and 55%, respectively, compared to 98% and 88% conversion in compounds **20** and **23)**.

Scheme 3 shows the synthesis of compound **35**, containing the isothiocyanate functionality at both terminal positions, obtained from the highly fluorescent tetramer **32**.[12a]

The formation of dibromo derivative **33** was almost quantitative, and the subsequent Stille coupling occurred in good yield, as well as the conversion of the terminal chlorine to −NCS at both positions.

(II) Optical Properties. Table 1 shows the molar extinction coefficients (ϵ), the maximum absorption and emission wavelengths (λ_{max} and λ_{PL}), and the photoluminescence quantum efficiencies (PLQEs) of the newly synthesized isothiocyanates together with those of **32** and **15**, used as reference compounds. Table 2 shows the effect of solvent changes on the optical properties of isothiocyanates **31** and **35**. Figure 1 shows the fluorescence colors of the newly synthesized isothiocyanates under excitation with a UV lamp at $\lambda_{exc} = 364$ nm.

Table 1 shows that the λ_{max} values of the isothiocyanates vary within a range of 70 nm, and the λ_{PL} values within a range of 115 nm, with the fluorescence colors spanning from deep blue to orange (Figure 1).

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TABLE 1. Molar Extinction Coefficients (ϵ, cm^{-1} mol^{-1}), Maximum Absorption (λ_{max}, nm) and Emission (λ_{PL}, nm) Wavelengths, and Photoluminescence Quantum Efficiencies (PLQEs, %) of Rigid-Core Isothiocyanates 14, 17, 20, 23, 26, 31, and 35 and of the Reference Compounds 15 and 32

compd	ϵ	λ_{max}	λ_{PL}	$\Delta(\lambda_{PL} - \lambda_{max})$	PLQE
17	8050	368	451	83 (5000)[a]	0.65
26	15400	383	483	100 (5320)	0.70
31	23400	405	505	100 (4920)	0.90
20	12700	401	514	113 (5480)	0.35
14	18500	438	540	102 (4480)	0.20
23	21900	425	550	125 (5160)	0.10
35	12100	422	566	144 (6030)	0.10
15[b]	7290	364	457	93 (5300)	0.77[b,c]
32[b]	16900	400	545	145 (6600)	0.85[b,c]

[a] Values in parentheses are in cm^{-1}. [b] Reference 12a. [c] Absolute values measured using an integrating sphere.

TABLE 2. Effect of Solvent Changes on the Optical Properties of Isothiocyanates 31 and 35

compd	solvent	ϵ[a]	λ_{max}[b]	λ_{PL}[b]	$\Delta(\lambda_{PL} - \lambda_{max})$
31	CH_2Cl_2	22700	402	505	103 (5070)[c]
	CarbTween[d]	90700	402	511	109 (5310)
	PBS[e]	48700	402	504	102 (5030)
35	CH_2Cl_2	22700	421	573	151 (6300)
	CarbTween[d]	17700	435	591	156 (6070)
	PBS[e]	18400	435	591	156 (6070)

[a] In cm^{-1} mol^{-1}. [b] In nm. [c] Values in parentheses are in cm^{-1}. [d] Carbonate (0.5 M) buffer solution containing 0.05% Tween 20, pH 9.5. [e] Phosphate buffer solution, pH 7.2.

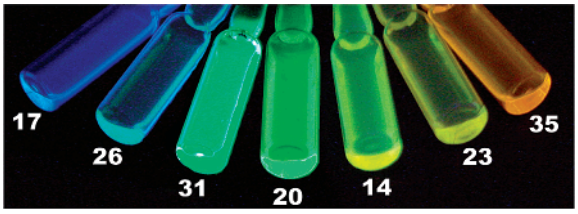

FIGURE 1. Fluorescence of 10^{-5} M solutions of rigid-core isothiocyanates under excitation with a UV lamp at $\lambda_{exc} = 364$ nm.

The molar extinction coefficient increases as does the oligomer size, with the highest value, 23400 cm^{-1} mol^{-1}, being that of isothiocyanate **31**. The differences between absorption and emission wavelengths are quite large for all compounds, being in the range 4480−6030 cm^{-1}. As an example, Figure 2 shows the normalized absorption and emission spectra of isothiocyanates **31** (top) and **35** (bottom).

The PLQE values of isothiocyanates **17** and **26** were measured using **15** as the reference compound, whereas the reference for **31**, **20**, **14**, **23**, and **35** was compound **32**. For both **15** and **32**, the absolute photoluminescence efficiency values, measured by using an integrating sphere, were indeed available[12a] (Table 1).

Although the measurements of PLQE values are always affected by large experimental errors,[17] the trend of PLQEs in Table 1 shows clearly that, as the emission wavelength increases, the photoluminescence quantum yield decreases.

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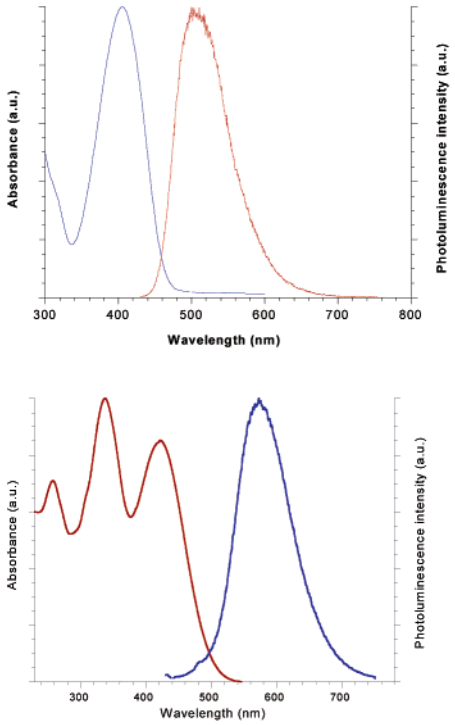

FIGURE 2. Normalized absorption and photoluminescence spectra of isothiocyanates **31** (top) and **35** (bottom), 10^{-5} M in methylene chloride.

Oligothiophene-*S,S*-dioxides are amphiphilic compounds, and their optical properties are more sensitive to solvent variations than those of conventional thiophene oligomers.[18] High sensitivity to solvent is also displayed by the isothiocyanates, as shown by the results reported in Table 2 for **31** and **35**.

The comparison of the optical characterististics of **31** and **35** in an organic solvent such as methylene chloride with those in aqueous solvents such as CarbTween and PBS shows that there is a remarkable increase in molar extinction coefficients in aqueous solvents, whereas the λ_{max} and λ_{PL} values of both compounds are affected to a much lesser extent. Indeed, while absorption and emission wavelengths of **31** are the same, within experimental error in all solvents, those of **35** are increased by no more than 14−18 nm in aqueous solvents.

(III) Conjugation with Monoclonal Antibodies. The newly synthesized isothiocyanates were conjugated to either anti-CD3 (purified from mouse ascitic fluid) or anti-CD8 antibodies.

Anti-CD3 and anti-CD8 are IgG1 isotype-specific antibodies which react with 30/32 kDa and 22−30 kDa CD8 and CD3 antigens, respectively, expressed on human T cells having cytotoxic activity.[19] Both are used for diagnostic purposes in flow cytometry and immunohistochemistry, and their conjugates with fluorescein isothiocyanate (isomer 1, FITC) are commercially available.

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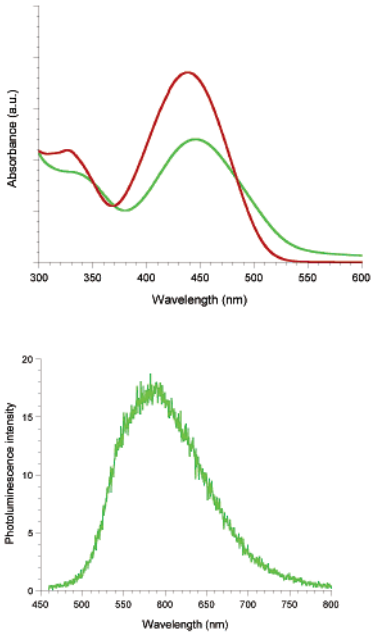

FIGURE 3. Top: Absorption spectrum of isothiocyanate **14** alone (red trace) and bound to antibody anti-CD3 (green trace) in PBS (pH 7.2). Bottom: Photoluminescence spectrum of the bioconjugate.

The conjugation was carried out according to standard modalities, by dissolving the isothiocyanates in anhydrous dimethyl sulfoxide and then reacting them with the antibodies in carbonate buffer solution (pH 9.5). The mixtures were incubated for 3 h at ambient temperature, and then the bioconjugates were separated from the unbound fluorophores by filtration through a desalting column that enabled the transfer of pure bioconjugate in PBS solution (pH 7.2). Appropriate tests (indirect method on flow cytometry using a second-step antibody conjugated with FITC) showed that the biological activity of the antibodies was entirely preserved in all cases. The lifetimes of the solutions of bioconjugates in PBS remained unaltered for many weeks, the limit being that of the stability of the antibody. Values of the fluorophore to protein molar ratios up to 30:1 were attained without precipitation of the antibody.

Figure 3 shows the absorption spectra of isothiocyanate **14** alone (red trace) and conjugated to antibody anti-CD3 (green trace). It is seen that the absorption spectrum of the bioconjugate shows only some broadening compared to the absorption spectrum of unbound **14**. A similar broadening of the absorption band of the bioconjugates (small for **17** and large for **35**), relative to that of unbound fluorophores, was observed for all oligothiophene isothiocyanates,[2c] including compound **31**, whose bionconjugate with antibody anti-CD8 is described in ref 2d.

Figure 4 shows the photoluminescence spectrum of the conjugate of isothiocyanate **35** with antibody anti-CD8 (red trace) compared to the photoluminescence spectrum (green trace) of the same antibody bound to FITC.[3] The fluorescence quantum efficiency and the molar extinction coefficient of FITC, which emits an intense green light upon photoexcitation, are 0.9 and 80000 cm^{-1} mol^{-1},[3] respectively, and are then much higher than those of the orange-emitting isothiocyanate 35 reported in Table 1. Both bioconjugates were prepared by the same modality,

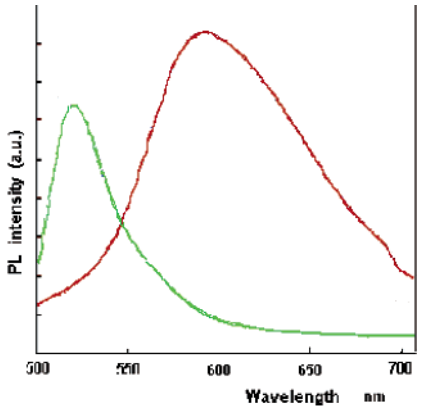

FIGURE 4. Photoluminescence spectrum of the bioconjugate of isothiocyanate **35** with antibody anti-CD8 in a 15:1 molar ratio (red trace) compared to that of a 20 times more diluted solution of the bioconjugate of FITC with the same antibody in a 6:1 molar ratio (green trace).

i.e., in the experimental conditions described above. The photoluminescence spectra were obtained by irradiation at the maximum absorption wavelength of the fluorophores.

To obtain the spectra of Figure 4, with comparable photoluminescence intensities for the two bioconjugates, it was necessary (a) to use a solution with a fluorophore to antibody molar ratio that was 6:1 for FITC and 15:1 for **35** and (b) to have a solution of FITC/antibody anti-CD8 conjugate that was 20 times more diluted than that of the **35**/anti-CD8 antibody bioconjugate. As shown in the figure, the photoluminescence spectrum of the bioconjugate of **35** with anti-CD8 antibody is markedly broader than that of the bioconjugate of FITC.

Discussion

The use of thiophene oligomers as fluorescent markers for biopolymers is recent, and the synthesis of only a few oligothiophene isothiocyanates has been reported so far.[2c]

The data presented in this paper confirm that our strategy of introducing the −NCS functionality into the aromatic skeleton of a preformed oligomer by means of commercial chloro(chloromethyl)dimethylsilane[2c] is very useful and allows for a large variety of fluorescent oligothiophene isothiocyanates to be prepared.

Chloro(chloromethyl)dimethylsilane can be directly incorporated into the molecular skeleton at one of the terminal positions following lithiation, as in the case of compound **17**, or by means of the Stille coupling with a thienyl or phenyl stannane already functionalized with the $-Si(CH_3)_2CH_2Cl$ moiety, as, for example, in the case of compounds **14** (Scheme 1) and **31** (Scheme 2). Also, it can be incorporated at both the terminal positions, as in compound **35** (Scheme 3). Doubly functionalized oligomers are easier to prepare than those containing the −NCS group at only one of the terminal positions; thus, they are more convenient in terms of reaction yields, purification procedures, and overall costs.

When the $-Si(CH_3)_2CH_2Cl$ group is bound to a thienyl ring, the conversion of chlorine into −NCS occurs in good yield upon treatment with NaSCN in acetone. However, when it is bound to a phenyl ring, the conversion is much more difficult, as in the case of compounds **26** and **31**.

Probably, this is due to the strongly stabilizing *para* position of the $-Si(CH_3)_2CH_2Cl$ group in these compounds, and future work should take into account phenyl derivatives bearing the silylated group in the *meta* position to increase the yields.

The versatility of the Stille reaction for the formation of the aryl−aryl bond,[16] combined with the possibility of introducing the isothiocyanate functionality in the last formation steps of the molecular skeleton, allows for a great variety of fluorescent isothiocyanates to be prepared. Those reported in this paper—fluorescing from deep blue to orange (Figure 1)—are only a few of the many which could be synthesized using the rigid dithienothiophene core DTTO.

The PLQEs of the isothiocyanates of Table 1 are up to 2 orders of magnitude greater than those containing one nonrigid thienyl-*S*,*S*-dioxide moiety.[2c,12a,b]

The PLQE value of isothiocyanate **31** (0.9) is close to that of FITC, the most studied and currently used fluorescent marker in diagnostics.

Compound **31** emits an intense green light similar to that of FITC. However, contrary to FITC, whose fluorescence decay time is only a few minutes,[3] the fluorescence of **31** is persistent. For example, solutions maintained without any caution in the presence of air and light, at ambient temperature, display unaltered optical characteristics after one year.

Isothiocyanates **17** and **26**, which emit blue light, are also characterized by high photoluminescence efficiencies (0.65 and 0.70), whereas the other isothiocyanates, emitting yellow and orange light, display much lower fluorescence efficiencies (from 0.10 to 0.35).

The decrease of PLQE on increasing fluorescence frequencies is a commonly observed trend in organic and inorganic fluorescent compounds. In the specific case of oligothiophene isothiocyanates this is probably due to a variety of different effects whose investigation is beyond the aim of the present study. Nevertheless, we can speculate that increased exciton delocalization and increased conformational freedom (the oligomers emitting yellow and orange light are the longer ones) should favor competitive nonradiative processes—probably intramolecular, such as internal conversion (IC) and intersystem crossing (ISC)[18,21]—and reduce the overall quantum efficiency.

In the past few years the development of monoclonal antibodies has greatly increased the capability of detecting cells in situ for specific antigens in many applications, including cancer detection. Monoclonal antibodies marked with fluorescent compounds are detected by flow cytometry, a technique that allows the number of cells to be measured by their light-scattering characteristics and their phenotype through fluorescence measurements. This application provides information on the cell size and morphology of intracellular and superficial antigens, and many other important biochemical and clinical parameters.

The conjugation of oligothiophene isothiocyanates to monoclonal antibodies occurs spontaneously at basic pH by reaction of the $-N=C=S$ functionality with the ϵ-NH_2 groups of the lysine residues to form thioureas ($-NH-C(S)-NH-$).[2c] This is also the case for the isothiocyanates described in this paper.

The bionconjugates formed by the isothiocyanates of Schemes 1−3 show absorption spectra that are similar to those of the unbound markers except for some broadening of the low-energy band (see, for example, Figure 3), more or less accentuated depending on the molecular structure of the marker. The fact that the smallest broadening is observed for the shorter isothiocyanate, **17**, while the largest is observed for the longer one, **35**, suggests that the effect might be due to an increased number of conformations of the marker once it is bound to the antibody.

Isothiocyanate **35** was synthesized to check whether the greater proximity of the fluorophore to the antibody, caused by the attachment to the antibody with both ends of the molecule, would lead to fluorescence quenching or would alter the biological activity of the antibody. We found that the biological activity of the anti-CD8 antibody marked with **35** remained unaltered and that the resulting bioconjugate gave rise to an intense, orange, fluorescence signal, which is shown in Figure 4.

To gain a better insight into the efficiency of this marker, we have compared the photoluminescence spectrum of the **35**/anti-CD8 antibody conjugate with the photoluminesce spectrum of the bioconjugate of FITC with the same antibody, prepared using parallel modalities.

Figure 4 shows that it is possible to obtain photoluminescence spectra of comparable intensities. This was achieved by diluting the solution of the conjugate with FITC 20 times relative to that of **35**. Moreover, the fluorophore:antibody molar ratio was 6:1 for FITC and 15:1 for **35**. Considering the differences in fluorescence frequency and quantum yield between **35** and FITC, we believe that the comparison of Figure 4 is very encouraging.

Figure 4 shows also that the **35**/anti-CD8 antibody conjugate displays a photoluminescence signal which is significantly broader than that of the corresponding bioconjugate with FITC. A narrow photoluminescence signal is important for multilabeling experiments, and clearly, the synthesis of oligothiophene isothiocyanates requires further tailoring to achieve more appropriate signal widths.

On the other hand, oligothiophene isothiocyanates are just beginning to be studied as fluorescent markers for biopolymers, and the overall data reported in this paper indicate that their level of efficiency can rapidly be improved by way of chemical synthesis.

Nevertheless, even at the present level of efficiency oligothiophene isothiocyanates are of great interest for their optical stability and color tunability.

Conclusion

The oligothiophene-*S*,*S*-dioxide isothiocyanates, whose synthesis and conjugation to monoclonal antibodies are reported in this paper, represent an improvement with respect to the oligothiophene isothiocyanates already

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described. Our results confirm the great potential of oligothiophene isothiocyanates as fluorescent markers, owing to their good optical properties, chemical and optical stability, easy color tunability, and facile modalities to bind biomolecules.

Experimental Section

Synthesis of Materials. Organic solvents were dried by standard procedures. Analytical thin-layer chromatography (TLC) was carried out using 0.2 mm sheets of silica gel 60 F_{254} or 0.2 mm sheets of aluminum oxide 60 F_{254} neutral. Preparative thin-layer chromatography (PLC) was performed using glass plates precoated with silica gel 60 F_{254} with a layer thickness of 1 mm. Preparative column chromatography was performed on glass columns of different sizes packed with silica gel 60 (particle sizes 0.040−0.063 mm) or aluminum oxide 90 standardized (particle sizes 0.063−0.200 mm). Petroleum ether refers to the fraction of bp 40−70 °C. The reactions that allow the introduction of the isothiocyanate group were performed in 2.5−5−10 mL conical V-Vials made from Wheaton-33 low extractable borosilicate glass. 2-Methylthiophene, tetrakis(triphenylphosphine)palladium(0), tributyltin chloride, diisopropylamine, bromine, phenylmagnesium bromide, [1,3-bis(diphenylphosphino)propane]nickel(II) chloride, *n*-butyllithium, *N*-bromosuccinimide, copper(II) dichloride, 3-bromothiophene, 1,4-dibromobenzene, sulfur dichloride, sodium thiocyanate, (chloromethyl)dimethylchlorosilane, and 3-chloroperbenzoic acid were commercial compounds. $Pd(AsPh_3)_4$ was prepared in situ from commercial tris(dibenzylideneacetone)dipalladium(0)−chloroform adduct and triphenylarsine.[22] Compounds **2**,[14] **4**,[23] **12**[2d], **15**,[15] **21**[2d], and **32**[15] have already been described.

The synthesis and the characterization of compounds **3**−**4**, **8**−**11**, **18**, **24**, **28**−**29**, and **33** is reported in the Supporting Information.

3-Phenyl-4-[(4-phenylthien-3-yl)thio]thiophene (5). *n*-Butyllithium (5.65 mL, 14.12 mmol, 2.5 M in hexane) was added over 15 min to a solution of **4** (3.07 g, 12.84 mmol) in 20 mL of dry ether at −70 °C. After 1 h sulfur dichloride (0.66 g, 0.41 mL, 6.42 mmol) was added dropwise. The mixture was allowed to warm to room temperature and stirred overnight before quenching with water. The product was extracted with ether (2 × 25 mL) and CH_2Cl_2 (2 × 25 mL). The combined organic layers were dried over anhydrous sodium sulfate, and then the solvent was removed by rotary evaporation. The residue was purified by flash chromatography (aluminum oxide; pentane/ethyl acetate, 9:1) to afford 1.18 g (yield 52%) of the title product as a faint yellow solid: mp 82−83 °C; MS *m/e* 350 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 230 nm; ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 7.42 (m, 4H), 7.33 (m, 6H), 7.25 (d, ^3J = 3.6 Hz, 2H), 7.01 (d, ^3J = 3.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 142.88, 135.45, 130.53, 128.66, 128.09, 127.44, 126.83, 123 80.

3,5-Diphenyldithieno[3,2-*b*:2′,3′-*d*]thiophene (6). *n*-Butyllithium (1.50 mL, 3.74 mmol, 2.5 M in hexane) was added over 15 min to a solution of **5** (0.66 g, 1.87 mmol) in 10 mL of dry ether at room temperature. The mixture was refluxed for 2 h and then was added via cannula to a solution of copper(II) dichloride (0.55 g, 4.11 mmol) in 5 mL of dry ether at 0 °C. The mixture was stirred overnight at room temperature before quenching with water. The product was extracted with ether (3 × 25 mL) and CH_2Cl_2 (1 × 25 mL), and the organic extracts were dried over sodium sulfate. The solvent was removed by rotary evaporation, and the residue was purified by flash chromatography (aluminum oxide; pentane/ethyl acetate, 8:2) to provide 0.31 g (yield 48%) of the title product as a yellow powder: mp 153−154 °C; MS *m/e* 348 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 317 nm; ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 7.79 (m, 4H), 7.50 (m, 4H), 7.48 (s, 2H), 7.39 (m, 2H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 139.44, 135.87, 134.50, 131.43, 129.06, 127.92, 126.57, 121.27.

3,5-Diphenyldithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (7). A 15 mL solution of 3-chloroperbenzoic acid (0.67 g, 3.86 mmol) previously dried over magnesium sulfate was added dropwise to a solution of **6** (0.31 g, 0.90 mmol) in 15 mL of dichloromethane. The mixture was stirred at room temperature overnight before washing sequentially with 10% KOH_{aq}, 10% $NaHCO_{3\,aq}$, and brine. The organic layer was dried over sodium sulfate, and the solvent was removed in vacuo. The crude product was purified by flash chromatography (aluminum oxide; pentane/ethyl acetate, 8:2) to afford 0.16 g (46% yield) of the title compound as a deep yellow solid: mp > 250 °C; MS *m/e* 380 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 371 nm; FTIR (neat) ν_{SO_2} 1301, 1150 cm^{-1}; ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 7.82 (m, 4H), 7.46 (m, 4H), 7.39 (m, 2H), 7.35 (s, 2H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 141.12, 139.15, 137.01, 131.90, 129.18, 128.90, 127.78, 124.21.

2-(5-Methylthien-2-yl)-3,5-diphenyl-6-[5-dimethyl(chloromethyl)silylthien-2-yl]dithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (13). A 67 mg (0.12 mmol) sample of **11** and 57 mg (0.12 mmol) of **12**[2c] were added to a 5 mL toluene solution containing 14 mg (0.012 mmol) of tetrakis(triphenylphosphine)palladium(0). The mixture was refluxed for 6 h. After evaporation of toluene the residue was purified by flash chromatography (silica gel; pentane/ethyl acetate/dichloromethane, 8:1:1) to give 57 mg (yield 72%) of the title product as an amorphous orange powder: mp > 250 °C; MS *m/e* 664 ($M^{\bullet+}$); ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 7.52 (m, 4H), 7.38 (m, 6H), 7.13 (d, ^3J = 3.6 Hz, 1H), 7.02 (d, ^3J = 3.6 Hz 1H), 6.83 (d, ^3J = 3.6 Hz, 1H), 6.61 (m, 1H), 2.89 (s, 2H), 2.41 (s, 3H), 0.417 (s, 6H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 142.96, 142.78, 142.46, 140.19, 137.83, 137.29, 135.87, 135.76, 133.82, 133.04, 133.01, 131.91, 131.57, 130.99, 130.89, 129.84, 129.75, 129.12, 128.88, 128.88, 128.75, 128.71, 127.70, 125.78, 29.69, 15.32, −3.56.

2-(5-Methylthien-2-yl)-3,5-diphenyl-6-[5-(dimethyl(isothiocyanatomethyl)silyl)thien-2-yl]dithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (14). A 2.5 mL conical Wheaton V-Vial was charged with **13** (42 mg, 0.063 mmol) and sodium thiocyanate (51 mg, 0.63 mmol) in distilled acetone (2.0 mL). The mixture was vigorously stirred at 200 °C for 5 h. After being cooled at room temperature, the crude material was filtered through a silica gel plug to remove the excess sodium salt and then was purified by preparative thin-layer chromatography to provide 24 mg (yield 55%) of the title product as a polycrystalline orange solid: mp > 250 °C; MS *m/e* 687 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 438 nm; FTIR (neat) ν_{NCS} 2150, ν_{SO_2} 1313, 1145 cm^{-1}; ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 7.52 (m, 4H), 7.38 (m, 6H), 7.13 (d, ^3J = 3.6 Hz, 1H), 7.05 (d, ^3J = 3.6 Hz, 1H), 6.83 (d, ^3J = 3.6 Hz, 1H), 6.61 (m, 1H), 2.49 (s, 2H), 2.41 (m, 3H), 0.472 (s, 6H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 142.96, 142.78, 142.54, 140.95, 137.46, 136.34, 136.19, 135.33, 134.16, 133.30, 133.06, 131.79, 131.54, 130.95, 130.76, 129.84, 129.75, 129.12, 128.93, 128.82, 128.74, 127.74, 125.81, 113.79, 18.59, 15.35, −2.65. Anal. Calcd for $C_{33}H_{25}NO_2S_6Si$ (688.04): C, 57.61; H, 3.66. Found: C, 57.48; H, 3.55.

2-Dimethyl(chloromethyl)silyl-3,5-dimethyldithieno-[2,3-*d*:3′,2′-*b*]thiophene-4,4-dioxide (16). *n*-Butyllithium (0.14 mL, 0.34 mmol, 2.5 M in hexane) was added dropwise at −78 °C to a solution of diisopropylamine (34 mg, 0.066 mL, 0.34 mmol) in THF (2 mL). The mixture was allowed to warm to 0 °C for 15 min and recooled to −78 °C. The above solution was added dropwise to a mixture of **15**[12a] (80 mg, 0.31 mmol) and (chloromethyl)dimethylchlorosilane (48 mg, 0.044 mL, 0.34 mmol) in dry THF (10 mL) at −78 °C via cannula. The solution was stirred at room temperature for 1 h and then heated to reflux for 2 h before quenching with water. The aqueous layer was extracted with ether, and the organic extracts were sequentially washed with saturated $NaHCO_{3\,aq}$, brine, and water. After the organic extracts were dried over sodium sulfate, the solvent was removed by rotary evaporation, and

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the residue was purified by column chromatography (SiO_2; petroleum ether/dichloromethane, 8:2) to provide 39 mg (yield 35%) of the title product as a yellow solid: mp 123−125 °C; MS *m/e* 362 ($M^{\bullet+}$); ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 6.89 (q, ^3J = 1.1 Hz, 1H), 2.98 (s, 2H), 2.47 (s, 3H), 2.41 (d, ^3J = 1.1 Hz, 3H), 0.50 (s, 6H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 144.97, 143.19, 140.27, 139.88, 135.46, 135.21, 133.12, 125.15, 29.48, 14.18, 13.09, −3.37.

2-Dimethyl(isothiocyanatomethyl)silyl-3,5-dimethyldithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (17). A mixture of **16** (30 mg, 0.08 mmol) and sodium thiocyanate (65 mg, 0.80 mmol) in distilled acetone (1.5 mL) was placed in a 2.5 mL conical Wheaton V-Vial. The mixture was vigorously stirred at 200 °C for 30 min and then cooled to room temperature. A 10 mL portion of dry ether was added, and the mixture was filtered through a silica gel plug to provide 26 mg (yield 81%) of the title product as a thick off-white liquid: MS *m/e* 385 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 368 nm; FTIR (neat) ν_{NCS} 2150, ν_{SO_2} 1034, 1152 cm^{-1}; ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 6.92 (q, ^4J = 1.2 Hz, 1H), 2.53 (s, 2H), 2.48 (s, 3H), 2.42 (d, ^4J = 1.2 Hz, 3H), 0.57 (s, 6H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 145.45, 143.67, 141.10, 140.64, 135.42, 133.88, 133.48, 125.84, 113.69, 29.94, 14.51, 13.35, −2.10. Anal. Calcd for $C_{14}H_{15}NO_2S_4Si$ (385.62): C, 43.61; H, 3.92. Found: C, 44.78; H, 3.79.

Dimethyl(chloromethyl)silylthien-2-yl]-3,5-dimethyldithieno[2,3-*d*:3′,2′-*b*]thiophene-4,4-dioxide (19). To a flame-dried flask charged with a 5 mL toluene solution containing 0.011 mmol of $Pd(Ph_3As)_4$ formed in situ was added **18** (75 mg, 0.22 mmol), then the mixture was heated to reflux, and **12**[2c] (105 mg, 0.22 mmol) was slowly added by syringe. After this addition the reflux was continued for 4 h, then the solvent was removed by rotary evaporation, and the residue was purified by column chromatography (silica gel; petroleum ether/dichloromethane/ethyl acetate, 7:2:1) to afford 65 mg (yield 67%) of the title product as a yellow solid: mp 156−158 °C; MS *m/e* 444 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 403 nm; FTIR (neat) ν_{SO_2} 1294, 1145 cm^{-1}; ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 7.29 (d, ^3J = 3.2 Hz 1H), 7.24 (d, ^3J = 3.2 Hz 1H), 6.90 (q, ^4J = 1.2 Hz, 1H), 2.97 (s, 2H), 2.53 (s, 3H), 2.42 (d, ^4J = 1.2 Hz, 3H), 0.49 (s, 6H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 143.83, 141.93, 140.20, 137.52, 135.97, 135.76, 135.57, 133.107, 132.61, 128.82, 127.94, 125.01, 30.24, 13.13, 12.74, −3.49.

2-[5-Dimethyl(isothiocyanatomethyl)silylthien-2-yl]-3,5-dimethyldithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (20). A mixture of **19** (60 mg, 0.13 mmol) and sodium thiocyanate (109 mg, 1.35 mmol) in distilled acetone (3 mL) was placed in a 5 mL conical Wheaton V-Vial. The mixture was vigorously stirred at 200 °C for 1 h before being cooled to room temperature. A 10 mL portion of dry ether was added, and then the mixture was filtered through a silica gel plug to provide 62 mg (yield 98%) of the title product as a yellow-brown solid: mp 134−136°C; MS *m/e* 467 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 401 nm; FTIR (neat) ν_{NCS} 2150, ν_{SO_2} 1032, 1146 cm^{-1}; ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 7.29 (d, ^3J = 3.2 Hz 1H), 7.25 (d, ^3J = 3.2 Hz 1H), 6.91 (q, ^4J = 1.2 Hz, 1H), 2.54 (s, 3H), 2.51 (s, 2H), 2.43 (d, ^4J = 1.2 Hz, 3H), 0.55 (s, 6H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 143.84, 142.01, 140.91, 136.41, 136.02, 135.63, 135.12, 133.14, 132.88, 129.11, 128.10, 125.17, 113.81, 29.68, 13.13, 12.77, −2.58. Anal. Calcd for $C_{18}H_{17}NO_2S_5Si$ (467.75): C, 46.22; H, 3.66. Found: C, 46.31; H, 3.74.

2-[(5-(5-Dimethyl(chloromethyl)silyl)thien-2-yl)thien-2-yl]-3,5-dimethyldithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (22). 18 (67 mg, 0.20 mmol) in 2 mL of toluene was added to a 4 mL toluene solution containing 0.02 mmol of $Pd(Ph_3As)_4$ prepared in situ. The mixture was warmed to reflux, and then **21**[2c] (112 mg, 0.20 mmol) dissolved in 2 mL of toluene was added dropwise. After this addition the reflux was continued for 16 h. After the mixture was cooled at room temperature, the solvent was removed by rotary evaporation, and then the crude material dissolved in 10 mL of dichloromethane was filtered through Celite to remove the palladium residue. After evaporation of the solvent, the resulting solid was washed with ethanol and hexane to provide 70 mg (yield 67%) of an amorphous red powder: mp 178−180 °C °C; MS *m/e* 526 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 424 nm; FTIR (neat) ν_{SO_2} 1300, 1146 cm^{-1}; ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 7.26 (d, ^3J = 3.6 Hz, 1H), 7.23 (d, ^3J = 3.6 Hz, 1H), 7.14 (d, ^3J = 3.6 Hz, 1H), 7.05 (d, ^3J = 3.6 Hz, 1H), 6.88 (q, ^4J = 1.2 Hz, 1H), 2.96 (s, 2H), 2.54 (s, 3H), 2.42 (d, ^4J = 1.2 Hz, 3H), 0.48 (s, 6H) ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 143.81, 142.30, 141.81, 138.17, 136.26, 135.65, 135.60, 135.50, 133.12, 133.00, 132.19, 128.52, 127.24, 125.46, 125.04, 124.46, 30.33, 13.09, 12.80, −3.54.

2-[(5-(5-Dimethyl(isothiocyanatomethyl)silyl)thien-2-yl)thien-2-yl]-3,5-dimethyldithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (23). A 10 mL conical Wheaton V-Vial was charged with **22** (63 mg, 0.12 mmol) and sodium thiocyanate (194 mg, 2.40 mmol) in distilled acetone (9 mL). The mixture was vigorously stirred at 200 °C for 2 h. After being cooled at room temperature, the crude material was filtered through a silica gel plug to remove the excess sodium salt. The solvent was removed by rotary evaporation, and the residue was washed with ether before recrystallization from toluene to provide 58 mg (yield 88%) of the title product as an amorphous red solid: mp 174−176 °C; MS *m/e* 549 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 425 nm; FTIR (neat) ν_{NCS} 2146, ν_{SO_2} 1297, 1144 cm^{-1}; ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 7.30 (d, ^3J = 3.6 Hz, 1H), 7.24 (d, ^3J = 3.6 Hz, 1H), 7.18 (d, ^3J = 3.6 Hz, 1H), 7.08 (d, ^3J = 3.6 Hz, 1H), 6.91 (q, ^4J = 1.2 Hz, 1H), 2.55 (s, 3H), 2.50 (s, 2H), 2.43 (d, ^4J = 1.2 Hz, 3H), 0.54 (s, 6H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 143.97, 143.12, 142.04, 137.93, 136.76, 135.67, 135.43, 134.11, 133.51,133.14, 132.45, 128.86, 127.43, 125.67, 125.08, 124.81, 113.86, 18.71, 13.11, 12.78, −2.58. Anal. Calcd for $C_{22}H_{19}NO_2S_6Si$ (549.87): C, 48.06; H, 3.48. Found: C, 47.89; H, 3.55.

2-[(4-Dimethyl(chloromethyl)silyl)phenyl]-3,5-dimethyldithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (25). 18 (137 mg, 0.41 mmol) dissolved in toluene (10 mL) was added to a 10 mL toluene solution containing 0.02 mmol of tetrakis-(triphenylarsine)palladium(0) prepared in situ. The reaction was heated to reflux, and then **24** (194 mg, 0.41 mmol) was added dropwise by syringe. After this addition the reflux was continued for 4 h. The solvent was removed by rotary evaporation, and the remaining residue was purified by column chromatography (SiO_2; hexane/dichloromethane, 8:2) to provide 40 mg (yield 22%) of the title compound as a medium yellow powder: mp 177−179 °C; MS *m/e* 438 ($M^{\bullet+}$); ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 7.63 (d, ^3J = 8 Hz, 2H), 7.43 (d, ^3J = 8 Hz, 2H), 6.90 (s, 1H), 2.98 (s, 2H), 2.48 (s, 3H), 2.44 (s, 3H), 0.46 (s, 6H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 143.74, 142.43, 141.93, 136.93, 136.02, 134.39, 133.82, 133.23, 133.09, 128.63, 128.16, 124.79, 30.09, 13.15, 12.30, −4.51.

2-[(4-Dimethyl(isothiocyanatomethyl)silyl)phenyl]-3,5-dimethyldithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (26). A 2.5 mL conical Wheaton V-Vial was charged with **25** (36 mg, 0.08 mmol) and sodium thiocyanate (66 mg, 0.82 mmol) in distilled acetone (1.5 mL). The mixture was vigorously stirred at 200 °C for 4 h. After being cooled at room temperature, the crude material was poured into 10 mL of ether and filtered through a silica plug to remove the excess sodium salt. The solvent was evaporated, and the crude product was recrystallized from 2-propanol to afford 18 mg (yield 48%) of the title product as orange microcrystals: mp 156−157 °C; MS *m/e* 461 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 384 nm; FTIR (neat) ν_{NCS} 2150, ν_{SO_2} 1303, 1145 cm^{-1}; ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 7.59 (d, ^3J = 8 Hz, 2H), 7.46 (d, ^3J = 8 Hz, 2H), 6.90 (q, ^4J = 1.2 Hz, 1H), 2.52 (s, 2H), 2.48 (s, 3H), 2.44 (d, ^4J = 1.2 Hz, 3H), 0.51 (s, 6H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 143.77, 142.02, 141.99, 135.90, 135.47, 134.38, 134.22, 133.40, 133.10, 128.82, 128.38, 124.92, 114.03, 18.19, 13.13, 12.31, −3.67. Anal. Calcd for $C_{21}H_{19}NO_2S_4Si$ (461.72): C, 52.03; H, 4.15. Found: C, 52.16; H, 4.28.

2-Phenyl-3,5-dimethyl-6-[(4-dimethyl(chlromethyl)silyl)phenyl]dithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (30). To a 5 mL toluene solution containing 0.006 mmol of $Pd(Ph_3$-

$As)_4$ prepared in situ was added 50 mg (0.12 mmol) of **29** dissolved in 10 mL of toluene. The mixture was warmed to reflux, and then **24** was slowly added by syringe. After this addition the reflux was continued for 3 h, and then the solvent was removed by rotary evaporation. The resulting residue was purified by column chromatography (SiO_2; hexane/dichloromethane, 8:2) to afford 26 mg (yield 43%) of the title compound as a bright yellow solid: mp 127−129 °C; MS *m/e* 514 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 405 nm; ^1H NMR ($CDCl_3$, TMS/ppm) δ 7.64 (d, $^3J = 8$ Hz, 2H), 7.44 (m, 7H), 2.99 (s, 2H), 2.50 (s, 3H), 2.48 (s, 3H), 0.46 (s, 6H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 142.95, 142.88, 136.88, 134.38, 133.85, 133.40, 133.04, 132.57, 128.98, 128.85, 128.63, 128.59, 128.34, 128.12, 30.09, 12.36, 12.26, −4.52.

2-Phenyl-3,5-dimethyl-6-[(4-dimethyl(isothiocyanatomethyl)silyl)phenyl]dithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (31). A 2.5 mL conical Wheaton V-Vial was charged with **30** (21 mg, 0.041 mmol) and sodium thiocyanate (33 mg, 0.41 mmol) in 2.5 mL of distilled acetone. The mixture was vigorously stirred at 200 °C for 2 h. After being cooled at room temperature, the crude material was filtered through a silica gel plug to remove the excess sodium salt. The solvent was removed by rotary evaporation. No further purification was necessary to afford 12 mg (yield 55%) of the title compound as a yellow solid: mp 126−128 °C; MS *m/e* 537 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 405 nm; FTIR (neat) ν_{SO_2} 1144, 1302, ν_{NCS} 2149 cm^{-1}; ^1H NMR ($CDCl_3$, TMS/ppm) δ 7.60 (d, $^3J = 8$ Hz, 2H), 7.45 (m, 7H), 2.52 (s, 2H), 2.50 (s, 3H), 2.48 (s, 3H), 0.52 (s, 6H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 143.34, 143.20, 142.27, 135.68, 134.68, 134.47, 133.85, 133.18, 132.77, 129.24, 129.10, 129.05, 128.92, 128.62, 114.28, 18.45, 12.505, −3.42. Anal. Calcd for $C_{26}H_{23}NO_2S_4Si$ (537.82): C, 58.07; H, 4.31. Found: C, 57.99; H, 4.27.

2,6-Di[3-methyl-5-(5-dimethyl(chloromethyl)silyl)thien-2-yl)]-3,5-dimethyldithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (34). To a 10 mL toluene solution containing 0.05 mmol of tetrakis(triphenylarsine)palladium(0) prepared in situ was added **33** (0.25 g, 0.41 mmol) in 10 mL of toluene. The mixture was warmed to reflux, and then **12** (0.39 g, 0.82 mmol) in 4 mL of toluene was added dropwise. After this addition the reflux was continued for 5 h. The solvent was removed by rotary evaporation, and the remaining residue was purified by flash chromatography (SiO_2; pentane/ethyl acetate/dichloromethane, 90:5:5) to provide 0.21 g (yield 62%) of the title compound as an amorphous red-orange powder: mp > 250 °C; MS *m/e* 826 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 422 nm; FTIR (neat) ν_{SO_2} 1309, 1145 cm^{-1}; ^1H NMR ($CDCl_3$, TMS/ppm) δ 7.25 (d, $^3J = 3.6$ Hz, 2H), 7.23 (d, $^3J = 3.6$ Hz, 2H), 7.06 (s, 2H), 2.97 (s, 4H), 2.40 (s, 6H), 2.22 (s, 6H), 0.49 (s, 12H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 142.35, 142.04, 138.88, 137.68, 136.10, 135.33, 134.32, 134.08, 131.46, 127.09, 125.75, 125.33, 30.49, 15.17, 12.51, −3.32.

2,6-Di[3-methyl-5-(5-dimethyl(isothiocyanatomethyl)-silyl)thien-2-yl)thien-2-yl]-3,5-dimethyldithieno[3,2-*b*:2′,3′-*d*]thiophene-4,4-dioxide (35). A 10 mL conical Wheaton V-Vial was charged with **34** (0.12 g, 0.15 mmol) and sodium thiocyanate (0.12 g, 1.50 mmol) in distilled acetone (9 mL). The mixture was vigorously stirred at 200 °C for 2 h. After being cooled at room temperature, the crude material was filtered through a silica gel plug to remove the excess sodium salt. The solvent was removed by rotary evaporation, and the residue was purified by preparative thin-layer chromatography to provide 0.93 g (yield 71%) of the title product as an amorphous red-orange solid: mp > 250 °C; MS *m/e* 870 ($M^{\bullet+}$); λ_{max} (CH_2Cl_2) 422 nm; FTIR (neat) ν_{NCS} 2150, ν_{SO_2} 1308, 1144 cm^{-1}; ^1H NMR ($CDCl_3$, TMS/ppm) δ 7.26 (d, $^3J = 3.2$ Hz, 2H), 7.22 (d, $^3J = 4$ Hz, 2H), 7.07 (s, 2H), 2.51 (s, 4H), 2.40 (s, 6H), 2.23 (s, 6H), 0.55 (s, 12H); ^{13}C NMR ($CDCl_3$, TMS/ppm) δ 143.06, 142.05, 138.94, 137.29, 136.58, 134.33, 133.96, 133.76, 131.50, 127.32, 126.03, 125.45, 113.84, 29.77, 15.15, 12.49, −2.41. Anal. Calcd for $C_{36}H_{34}N_2O_2S_9Si_2$ (871.43): C, 49.62; H, 3.93. Found: C, 49.72; H, 4.07.

Bioconjugation Modalities. Monoclonal antibodies anti-CD3 and anti-CD8 were first concentrated via ultrafiltration under nitrogen on a micrometer membrane with a 10kDa cutoff. Then they were transferred to a 0.05 M carbonate buffer solution containing 0.05% Tween 20 (pH 9.5), with the use of a buffer exchange column of Sephadex G25 equilibrated with 5 bed volumes of the same buffer. Fractions of 0.5 mL were collected. To these solutions aliquots of oligothiophene-*S*,*S*-dioxide isothiocyanates dissolved in DMSO (concentration 10 mg/mL) were added in the amount needed to reach the desired protein:fluorophore molar ratios. The solutions were incubated for 3 h at room temperature with stirring. Finally, the conjugates were chromatographed on a desalting 1.0 mL GH25 column in PBS (pH 7.4).

Absorption and Photoluminescence Measurements. Absorption and photoluminescence spectra were recorded using 10^{-5}−10^{-6} M solutions in CH_2Cl_2 or aqueous solvents (absorbance 0.1−0.2). The excitation wavelengths were those of the maximum absorption wavelengths of the fluorophores.

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Supporting Information Available: Synthesis and characterization of compounds **3**−**4**, **8**−**11**, **18**, **28**−**29**, and **33** and NMR spectra of compounds **9** and **24**. This material is available free of charge via the Internet at http://pubs.acs.org.

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