

Near-Infrared Fluorophores Containing
Benzo[*c*]heterocycle Subunits

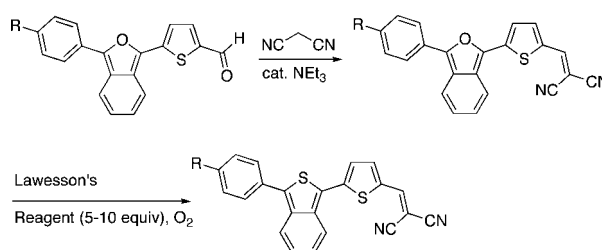
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ABSTRACT



The syntheses and spectroscopic properties of eight new push–pull-type near-infrared fluorophores that contain either isobenzofuran or isothianaphthene subunits are presented. The isobenzofuran dyes demonstrate significantly red-shifted absorption compared with their isothianaphthene counterparts, which is attributed to isobenzofuran's more potent pro-quinoidal character.

In vivo near-infrared (NIR) fluorescence imaging is rapidly emerging as a powerful diagnostic method.¹ In the NIR region (650–900 nm) biological chromophores exhibit low absorption and autofluorescence,² thus allowing photons to pass through the tissue and rendering this technique relatively noninvasive. With applications such as vascular mapping of the heart³ and brain⁴ and visualization of various pathologies including tumors,⁵ atherosclerosis,⁶ and β -amyloid plaques,⁷ the demand for new NIR fluorescent contrast agents is ever increasing.

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Our group recently reported the synthesis and *in vivo* imaging properties of NIAD-4 (**1**), NIM-1 (**2**), and NIM-2 (**3**), all donor–acceptor type dyes (Figure 1).^{7a,8} In the case of **2** and **3**, it was found that the incorporation of benzo[*c*]heterocycle isothianaphthene (ITN) into the conjugated bridge red-shifted both the absorption and emission substantially. Herein we explore the use of benzo[*c*]heterocycle isobenzofuran (IBF) in place of ITN as a molecular component in this class of dyes.

Isothianaphthene has a relatively long history as a component in functional materials, with examples in fluorophores,⁹ OLE-Ds,¹⁰ photovoltaics,¹¹ and low band gap polymers, of which polyisothianaphthene (PITN)¹² is the progenitor. The smaller band gap of these ITN-containing materials over similar

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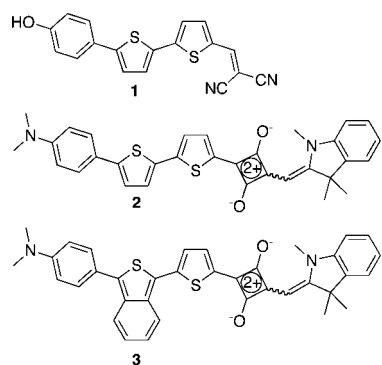


Figure 1. Structures of NIAD-4, NIM-1, and NIM-2.

thiophene-containing materials is a result of the increased contribution of the quinoid resonance structure (Figure 2) due

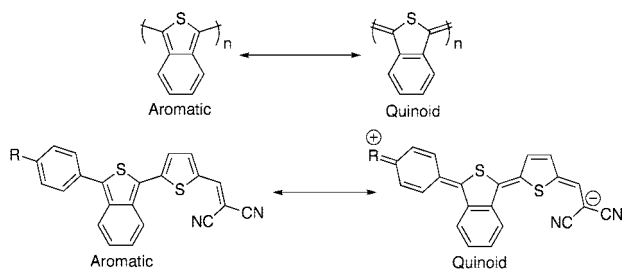


Figure 2. Aromatic and quinoid resonance structures of PITN and ITN donor-acceptor dyes.

to the stabilization achieved by aromatization of the benzene ring.¹³

In contrast, only a few examples¹⁴ of functional materials containing isobenzofuran are reported in the literature, presumably because of its reduced stability.¹⁵ For this reason, we predicted that IBF would prove even more pro-quinoidal and that its incorporation into donor-acceptor dyes would further reduce the band gap of these materials.

To test this principal, we synthesized a series of IBF and ITN dyes similar to NIAD-4 (Scheme 1). Four different electron-donating (R) groups, H-, MeO-, HO-, and Me₂N-, were incorporated in order to span a range of wavelengths. Appropriately substituted aryl-lactones **5a-d** were either purchased or synthesized via acid-mediated condensation¹⁶ of 2-carboxybenzaldehyde and substituted benzenes. In the case of the phenol group (R = OH), TiPS protection was employed. Initially, the IBF framework was constructed with

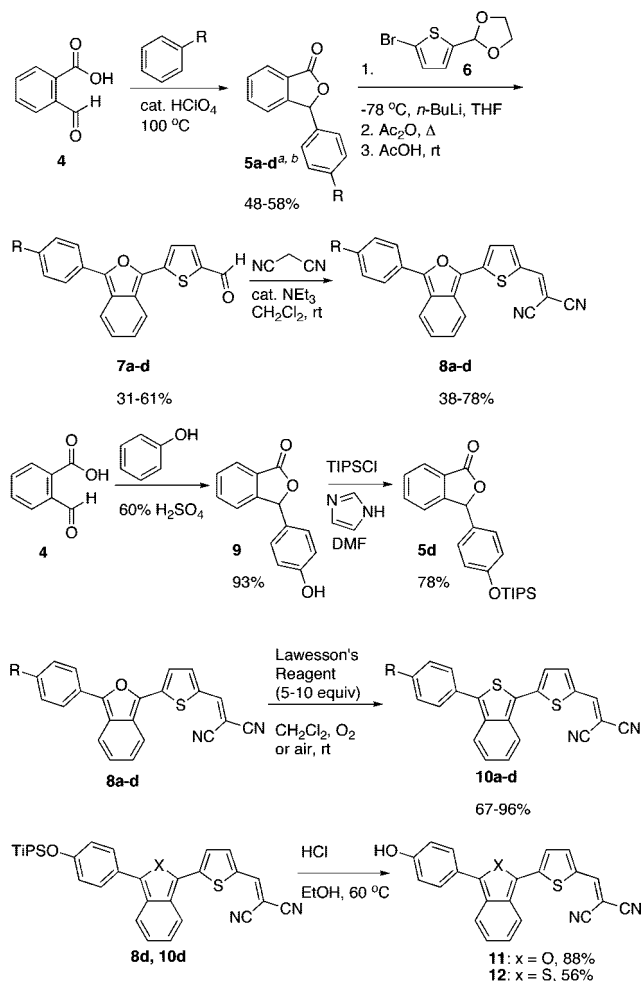
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Scheme 1. Synthesis of IBF and ITN Dyes



^aa: R = H-. b: R = MeO-. c: R = Me₂N-. d: R = TIPSO-. ^b5a is commercially available, 5d synthesized as above.

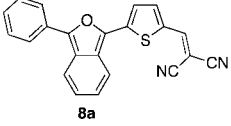
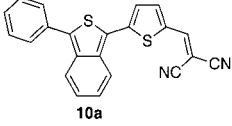
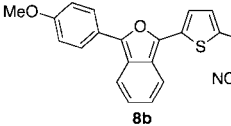
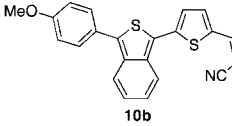
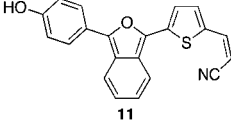
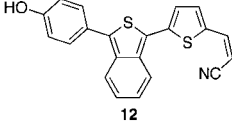
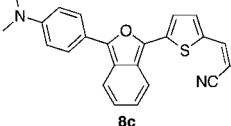
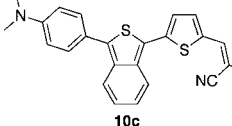
a free 5 position on the thiophene, which was subsequently lithiated and then formylated with DMF.

However, these unsubstituted intermediate compounds proved relatively difficult to isolate and purify, resulting in poor yields for the formylation. Exploiting the acetal-protected aldehydes allowed us to bypass these troublesome intermediates. Protected aldehydes **7a-d** were produced in one step by reaction of the lactones with the lithio-derivative of **6**¹⁷ followed by dehydration with acetic anhydride and deprotection with aqueous acetic acid. Knoevenagel condensation of the aldehydes with malonitrile installed the dicyanomethylene electron-withdrawing group to afford dyes **8a-d** in moderate yields. Deprotection of **8d** was achieved under acidic conditions as fluoride was found to revert the dicyanovinyl group to the aldehyde.

To access the ITN counterparts to dyes **10a-d**, we decided to explore methods by which the IBF dyes could be converted directly. It was found that such a transformation could be accomplished with good yields, in one pot, using a

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Table 1. Spectroscopic Properties of IBF and ITN Dyes^a

IBF dye	λ_{max} abs (nm)	λ_{max} em (nm)	Φ^b	ITN dye	λ_{max} abs (nm)	λ_{max} em (nm)	Φ^b
 8a	556 ^c 578 ^d	658 ^c 635 ^d	.077 ^c .075 ^d	 10a	525 ^c 544 ^d	637 ^c 629 ^d	.061 ^c .079 ^d
 8b	578 ^c 599 ^d	686 ^c 675 ^d	.078 ^c .16 ^d	 10b	537 ^c 559 ^d	672 ^c 663 ^d	.046 ^c .099 ^d
 11	584 ^c 600 ^d	696 ^c 679 ^d	.049 ^c .12 ^d	 12	545 ^c 559 ^d	684 ^c 667 ^d	.023 ^c .071 ^d
 8c	637 ^c 656 ^d	790 ^c 762 ^d	.0055 ^c .046 ^d	 10c	579 ^c 604 ^d	785 ^c 743 ^d	.0015 ^c .021 ^d

^a Spectra available in Supporting Information. ^b Quantum yields calculated by comparison to standards; see Supporting Information. ^c MeOH as solvent. ^d CHCl₃ as solvent.

5–10-fold excess of Lawesson's reagent in the presence of air or anhydrous oxygen. A possible mechanism includes the formation of an endoperoxide upon reaction of the IBF and oxygen, which in turn rearranges into a diketone¹⁸ followed by conversion of the diketone to the dithioketone with Lawesson's reagent and cyclization to form the ITN.¹⁹ In the case of **8c**, the dicyanovinyl group was cleaved during the procedure, presumably to a thioaldehyde by Lawesson's reagent,²⁰ and thus treatment with excess malonitrile and base was necessary to obtain **10c**. Dye **10d** was deprotected under acid conditions in a similar manner as **8d**.

The spectroscopic properties of **8a–c**, **10a–c**, **11**, and **12** are listed in Table 1. In all cases, the IBF dyes exhibited markedly red-shifted absorption from the corresponding ITN moieties, thus demonstrating the narrower band gaps of the IBF compounds. The degree of this effect increases as the efficacy of the electron-donating group increases. The degree

of red shift in the emission proved only modest, especially in the case of **8c** and **10c**, indicating that perhaps the quinoid resonance structure stabilization does not factor as heavily into the excited-state energies of these molecules.

In conclusion, we have demonstrated the greater effectiveness of isobenzofuran over isothianaphthene as a red-shifting component in donor–acceptor-type dyes, an effect attributable to the great pro-quinoidal nature of IBF. Currently, these compounds are being screened as NIR contrast agents for biomedical applications, and future investigations will explore the use alternate electron-withdrawing groups and the incorporation of different heterocycles into this fascinating class of compounds.

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Supporting Information Available: Experimental procedures and characterization data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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