Bright, Red Single-Molecule Emitters: Synthesis and Properties of Environmentally Sensitive Dicyanomethylenedihydrofuran (DCDHF) Fluorophores with Bisaromatic Conjugation

Zhikuan Lu,[†] Na Liu,[†] Samuel J. Lord,[‡] Scott D. Bunge,[†] W. E. Moerner,[‡] and Robert J. Twieg*,[†]

Department of Chemistry, Kent State University, Kent, Ohio 44242-0001, and Department of Chemistry, Stanford University, Stanford, California 94305-5080

Received June 30, 2008. Revised Manuscript Received December 12, 2008

A group of new fluorescent dye materials for single-molecule imaging applications, comprised of an amine donor, a π-system consisting of phenyl and thiophene rings, and a dicyanomethylenedihydrofuran acceptor group have been synthesized. Relative to comparable single-ring compounds, these doubly aromatic conjugated fluorophores have red-shifted absorption and emission, usually accompanied by significantly increased quantum yields. Solvatochromism studies indicate that the photophysical properties of these dyes are sensitive to the solvent polarity and environmental rigidity. Photophysical studies demonstrate that these DCDHF dye materials are strong single-molecule emitters and the total number of detected photons per molecule is among the highest observed thus far for this family of fluorophores.

Introduction

Fluorescence imaging of single molecules is a powerful technique in which characteristics of the structure and dynamics of complex condensed materials are revealed that are otherwise hidden in ensemble measurements.[1] The successful imaging of individual molecules requires strong and stable fluorescent emitters that can be detected at the single-copy level. We have demonstrated that the family of molecules comprised of a dicyanomethylenedihydrofuran (DCDHF) acceptor and a dialkylamino donor linked by a π-conjugated unit are well-suited for this purpose.[2] The DCDHF acceptor group was first described by Melikian and co-workers,[3] and this group soon found extensive applications in electro-optic materials[4] and photorefractive materials.[5] The original DCDHF fluorophores that evolved from the photorefractive materials had a single benzene or thiophene ring (see Figure 1, molecules **1** and **2**) as the conjugated bridge. These compounds are pumped at a wavelength of 488 nm; however, at this wavelength, autofluorescence occurs in typical biological systems and emission from single fluorophores is not easily observed. As a result, fluorophore materials with red-shifted absorption and emission are highly desired; specifically, autofluorescence is significantly reduced at pumping wavelengths longer than 514 or 532 nm, beyond

* Author to whom correspondence should be addressed. E-mail: rtwieg@kent.edu.

[†] Department of Chemistry, Kent State University.

[‡] Department of Chemistry, Stanford University.

(1) (a) Moerner, W. E.; Orrit, M. *Science* **1999**, *283*, 1670–1676. (b) Weiss, S. *Science* **1999**, *283*, 1676–1683.

(2) (a) Willets, K. A.; Nishimura, S. Y.; Schuck, P. J.; Twieg, R. J.; Moerner, W. E. *Acc. Chem. Res.* **2005**, *38*, 549–556. (b) Willets, K. A.; Ostroverkhova, O.; He, M.; Twieg, R. J.; Moerner, W. E. *J. Am. Chem. Soc.* **2003**, *125*, 1174–1175. (c) Twieg, R.; Wang, H.; Lu, Z.; Kim, S. Y.; Lord, S.; Nishimura, S.; Schuck, P. J.; Willets, K. A.; Moerner, W. E. *Nonlinear Opt., Quantum Opt.* **2005**, *34*, 241–246. (d) Schuck, P. J.; Willets, K. A.; Fromm, D. P.; Twieg, R. J.; Moerner, W. E. *Chem. Phys.* **2005**, *318*, 7–11. (e) Willets, K. A.; Callis, P. R.; Moerner, W. E. *J. Phys. Chem. B* **2004**, *108*, 10465–10473. (f) Nishimura, S.; Lord, S.; Willets, K.; Moerner, W. E.; He, M.; Lu, Z.; Twieg, R. J. *J. Phys. Chem. B* **2006**, *110*, 8151–8157. (g) Wang, H; Lu, Z.; Lord, S. J.; Willets, K. A.; Bertke, J. A.; Bunge, S. D.; Moerner, W. E.; Twieg, R. J. *Tetrahedron* **2007**, *63*, 103–114. (h) Wang, H.; Lu, Z.; Lord, S. J.; Moerner, W. E.; Twieg, R. J. *Tetrahedron Lett.* **2007**, *48* (19), 3471–3474.

(3) Melikian, G.; Rouessac, F. P.; Alexandre, C. *Synth. Commun.* **1995**, *25*, 3045–3051.

(4) For example: (a) Zhang, C.; Ren, A. S.; Wang, F.; Zhu, J. S.; Dalton, L. R.; Woodford, J. N.; Wang, C. H. *Chem. Mater.* **1999**, *11*, 1966–1968. (b) Zhang, C.; Dalton, L. R.; Oh, M. C.; Zhang, H.; Steier, W. H. *Chem. Mater.* **2001**, *13*, 3043–3050. (c) Gopalan, P.; Katz, H. E.; McGee, D. J.; Erben, C.; Zielinski, T.; Bousquet, D.; Muller, D.; Grazul, J.; Olsson, Y. *J. Am. Chem. Soc.* **2004**, *126*, 1741–1747. (d) He, M. Q.; Leslie, T.; Garner, S.; DeRosa, M.; Cites, J. *J. Phys. Chem. B* **2004**, *108*, 8731–8736. (e) Liu, S.; Haller, M. A.; Ma, H.; Dalton, L. R.; Jang, S. H.; Jen, A. K. Y. *Adv. Mater.* **2003**, *15*, 603–607. (f) Qiu, L.; Shen, Y. Q.; Hao, J. M.; Zhai, J. F.; Zu, F. H.; Zhang, T.; Zhao, Y. X.; Clays, K.; Persoons, A. *J. Mater. Sci.* **2004**, *39*, 2335–2340. (g) Sullivan, P. A.; Rommel, H.; Liao, Y.; Olbricht, B. C.; Akelaitis, A. J. P.; Firestone, K. A.; Kang, J. W.; Luo, J. D.; Davies, J. A.; Choi, D. H.; Eichinger, B. E.; Reid, P. J.; Chen, A. T.; Jen, A. K. Y.; Robinson, B. H.; Dalton, L. R. *J. Am. Chem. Soc.* **2007**, *129*, 7523–7530. (h) Beverina, L.; Fu, J.; Leclercq, A.; Zojer, E.; Pacher, P.; Barlow, S.; Van Stryland, E. W.; Hagan, D. J.; Bredas, J. L.; Marder, S. R. *J. Am. Chem. Soc.* **2005**, *127*, 7282–7283. (i) Bouit, P. A.; Wetzel, G.; Berginc, G.; Loiseaux, B.; Toupet, L.; Feneyrou, P.; Bretonniere, Y.; Kamada, K.; Maury, O.; Andraud, C. *Chem. Mater.* **2007**, *19*, 5325–5335.

(5) (a) Wright, D.; Gubler, U.; Roh, Y.; Moerner, W. E.; He, M.; Twieg, R. J. *Appl. Phys. Lett.* **2001**, *79*, 4274–4276. (b) Ostroverkhova, O.; Wright, D.; Gubler, U.; Moerner, W. E.; He, M.; Sastre-Santos, A.; Twieg, R. J. *Adv. Funct. Mater.* **2002**, *12*, 621–629. (c) He, M.; Twieg, R. J.; Gubler, U.; Wright, D.; Moerner, W. E. *Chem. Mater.* **2003**, *15*, 1156–1164. (d) You, W.; Cao, S. K.; Hou, Z. J.; Yu, L. P. *Macromolecules* **2003**, *36*, 7014–7019. (e) Hayden, L. M.; Sinyukov, A. M.; Leahy, M. R.; French, J.; Lindahl, P.; Herman, W. N.; Twieg, R. J.; He, M. *J. Polym. Sci., Part B* **2003**, *41*, 2492–2500. (f) Ostroverkhova, O.; He, M.; Twieg, R. J.; Moerner, W. E. *Chem. Phys. Chem.* **2003**, *4*, 732–744. (g) Ostroverkhova, O.; Moerner, W. E.; He, M.; Twieg, R. J. *Appl. Phys. Lett.* **2003**, *82*, 3602–3604. (h) You, W.; Hou, Z. J.; Yu, L. P. *Adv. Mater.* **2004**, *16*, 356–360. (i) Hou, Z. J.; You, W.; Yu, L. P. *Appl. Phys. Lett.* **2004**, *85*, 5221–5223.

10.1021/cm801783f CCC: $40.75
Published on Web 02/05/2009

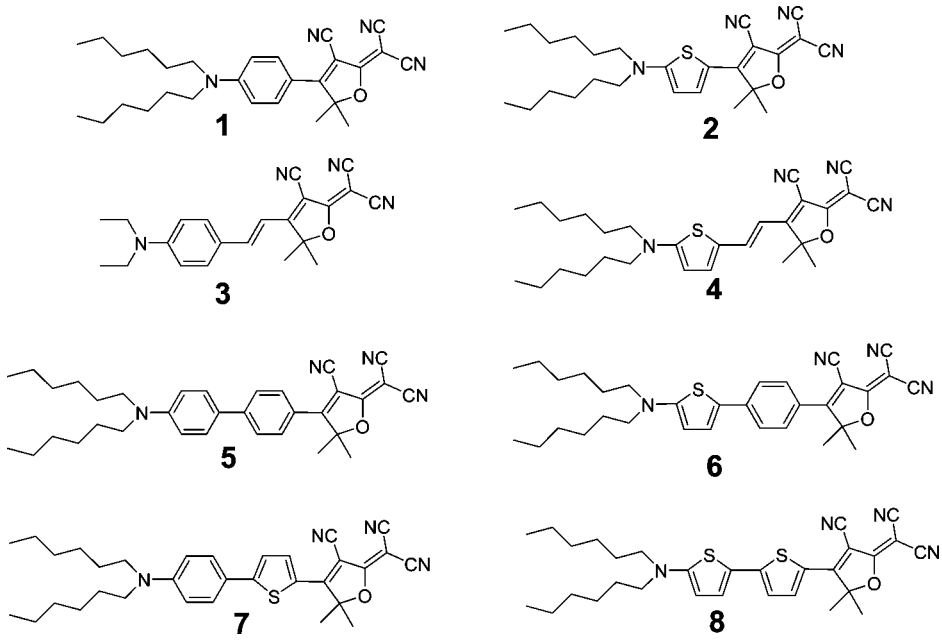


Figure 1. Structures of DCDHF chromophores. Compounds **1**−**4** are typical of those examined previously, whereas the set of new bisaromatic compounds (**5**−**8**) are described here in detail.

the wavelength where flavins absorb.[6] The introduction of an olefin unit, as is usually employed in electro-optic chromophores, red-shifts the absorption very effectively but the fluorescence quantum yield is significantly reduced, probably because of the introduction of nonradiative processes that involve rotations about the olefins (see Figure 1, molecules **3** and **4**). This loss of quantum yield prompted us to explore an alternative extension of conjugation via the addition of aromatic rings such as phenyl and thiophene. This approach complements our earlier successful push to longer wavelengths using fused rings.[7] We proposed that DCDHFs with only oligoaromatic conjugation would have the desired red shift but with a smaller reduction of quantum yields. The more extensively conjugated bisaromatic systems (**5**, **6**, **7**, and **8** in Figure 1) are expected to show a bathochromic shift of the absorption and emission band. In addition, a trisaromatic dye and two bisaromatic dyes with an olefin unit were also synthesized for comparison.

The conjugated donor−π-bridge−acceptor push−pull chromophores with π-bridges of phenyl and thiophene rings and their combinations are widely recognized for applications in materials science, because of their interesting and useful optoelectronic properties.[8] Extension of the conjugation unit from one aromatic ring to two aromatic rings leads to a bathochromic shift for their charge-transfer electronic absorption. For example, a biphenyl unit has been broadly applied to optoelectronic material synthesis.[9] Chromophores with a benzo-1,3-dithiol-2-ylidene donor and dicyanomethylene acceptor with a 4,4′-biphenyl instead of a 1,4-benzene push the absorption from the visible region to the near-infrared region.[10] Because the thiophene ring has less resonance energy than benzene, it permits more-effective π-electron delocalization.[11] Bithiophene provides a pronounced red shift, compared to biphenyl, which is indicative of enhanced electron transmission from the donor to the acceptor.[12]

Here, we describe the synthetic aspects as well as spectroscopic properties—including absorption, fluorescence, solvatochromism, and single-molecule imaging properties—of DCDHF dyes with an extended π-system comprised of combinations of benzene and thiophene rings. Most of these

(6) Harms, G. S.; Cognet, L.; Lommerse, P. H. M.; Blab, G. A.; Schmidt, T. *Biophys. J.* **2001**, *80*, 2396–2408.
(7) (a) Lord, S. J.; Lu, Z.; Wang, H.; Willets, K. A.; Schuck, P. J.; Lee, H. D.; Nishimura, S. Y.; Twieg, R. J.; Moerner, W. E. *J. Phys. Chem. A* **2007**, *111* (37), 8934–8941. (b) Lu, Z; Lord, S. J.; Wang, H.; Moerner, W. E.; Twieg, R. J. *J. Org. Chem.* **2006**, *71*, 9651–9657.
(8) For example: (a) Nesterov, E. E.; Skoch, J.; Hyman, B. T.; Klunk, W. E.; Bacskai, B. J.; Swager, T. M. *Angew. Chem.—Int. Ed.* **2005**, *44*, 5452–5456. (b) Aoki, S.; Kagata, D.; Shiro, M.; Takeda, K.; Kimura, E. *J. Am. Chem. Soc.* **2004**, *126*, 13377–13390.
(9) (a) Cheng, L. T.; Tam, W.; Marder, S. R.; Stiegman, A. E.; Rikken, G.; Spangler, C. W. *J. Phys. Chem.* **1991**, *95*, 10643–10652. (b) Goodbrand, H. B.; Hu, N. X. *J. Org. Chem.* **1999**, *64*, 670–674. (c) Lim, S. F.; Friend, R. H.; Rees, I. D.; Li, J.; Ma, Y. G.; Robinson, K.; Holmes, A. B.; Hennebicq, E.; Beljonne, D.; Cacialli, F. *Adv. Funct. Mater.* **2005**, *15*, 981–988. (d) Goodall, W.; Wild, K.; Arm, K. J.; Williams, J. A. G. *J. Chem. Soc.—Perkin Trans.* **2002**, *2*, 1669–1681.
(10) Inoue, S.; Aso, Y.; Otsubo, T. *Chem. Commun.* **1997**, 1105–1106.
(11) (a) Dirk, C. W.; Katz, H. E.; Schilling, M. L.; King, L. A. *Chem. Mater.* **1990**, *2*, 700–705. (b) Jen, A. K. Y.; Rao, V. P.; Wong, K. Y.; Drost, K. J. *J. Chem. Soc.—Chem. Commun.* **1993**, 90–92. (c) Raimundo, J. M.; Blanchard, P.; Gallego-Planas, N.; Mercier, N.; Ledoux-Rak, I.; Hierle, R.; Roncali, J. *J. Org. Chem.* **2002**, *67*, 205–218. (d) Moylan, C. R.; McNelis, B. J.; Nathan, L. C.; Marques, M. A.; Hermstad, E. L.; Brichler, B. A. *J. Org. Chem.* **2004**, *69*, 8239–8243. (e) Jia, W. L.; Bai, D. R.; McCormick, T.; Liu, Q. D.; Motala, M.; Wang, R. Y.; Seward, C.; Tao, Y.; Wang, S. M. *Chem.—Eur. J.* **2004**, *10*, 994–1006.
(12) (a) Effenberger, F.; Wurthner, F.; Steybe, F. *J. Org. Chem.* **1995**, *60*, 2082–2091. (b) Bedworth, P. V.; Cai, Y. M.; Jen, A.; Marder, S. R. *J. Org. Chem.* **1996**, *61*, 2242–2246. (c) Steybe, F.; Effenberger, F.; Gubler, U.; Bosshard, C.; Gunter, P. *Tetrahedron* **1998**, *54*, 8469–8480. (d) Milian, B.; Orti, E.; Hernandez, V.; Navarrete, J. T. L.; Otsubo, T. *J. Phys. Chem. B* **2003**, *107*, 12175–12183. (e) Raposo, M. M. A.; Fonseca, A. M. C.; Kirsch, G. *Tetrahedron* **2004**, *60*, 4071–4078. (f) Tabet, A.; Hartmann, H. *Synthesis* **2005**, 610–616. (g) Takahashi, T.; Takimiya, K.; Otsubo, T.; Aso, Y. *Org. Lett.* **2005**, *7*, 4313–4316.

compounds have the desired combination of enhanced quantum yields with absorption and fluorescence at longer wavelengths that very effectively overcome the background autofluorescence that is observed in many biological samples. On the single-molecule level, this new group of dyes includes some of the brightest and most long-lived among the DCDHF class of single-molecule emitters. For many of these dyes in PMMA, single copies emit brightly for tens of seconds to minutes under typical epifluorescence imaging conditions (even without oxygen scavengers), emitting several millions of photons before photobleaching. Moreover, as with other DCDHFs studied,[2b] these fluorophores seldom exhibit blinking on the time scale of our experiments (integration time = 100 ms). These DCDHF fluorophores compare favorably with other excellent single-molecule emitters, such as rhodamines and perylene diimides, which emit millions of photons;[13] terrylene diimides can emit tens or even hundreds of millions of photons, but face significant solubility hurdles.[14] Overall, these new DCDHF dyes exhibit excellent promise as bright, long-lived red emitters, which is necessary criterion for single-molecule experiments in the cellular environment.

Experimental Section

Bulk Spectroscopy. Solutions were prepared in pure solvents, and bulk absorption and emission spectra were obtained using a Perkin-Elmer Model Lambda 19 UV−vis spectrometer and a SPEX Model Fluromax-2 fluorimeter, respectively. Quantum yields were measured against standards with known quantum yields and corrected for differences in optical density and solvent refractive index.[15] Bulk samples in poly(methyl methacrylate) (PMMA; T_g = 105 °C, MW = 75 000 g/mol, atactic, polydispersity of ∼2.8, from PolySciences, Inc.) were prepared by spin-casting 20% (w/w) solutions of PMMA in toluene, doped with a small volume of highly concentrated dye solution, onto clean glass slides. These colored films were placed in the spectrometer and fluorimeter to obtain quantitative absorption and emission spectra, respectively.

Single-Molecule Imaging. Single-molecule samples were prepared by spin-casting 1% (w/w) solutions of PMMA in toluene, doped with nanomolar fluorophore concentrations, onto clean glass coverslips. After drying, these samples were studied using an inverted microscope (Nikon, Model Diaphot 200) in an epifluorescence configuration.[16] Samples were illuminated using a continuous-wave 532-nm laser (Spectra-Physics, Model Millenia); the intensity at the sample was 0.5−3.5 kW/cm^2. The emission was collected through a 100×, 1.4 N.A. Nikon microscope objective, filtered to remove scattered excitation light, and imaged onto a back-illuminated frame-transfer Si CCD camera (Roper Scientific, Model MicroMax) with an integration time of 100 ms.

Crystal Structures and Calculations. To perform X-ray crystallography, crystals of **5**, **6**, and **21** were mounted onto a thin glass fiber from a pool of Fluorolube and immediately placed under a low-temperature N_2 stream on a diffractometer (Bruker, Model AXS). The radiation used was graphite monochromatized Mo Kα radiation (λ = 0.7107 Å). The lattice parameters were optimized from a least-squares calculation on carefully centered reflections. Lattice determination, data collection, structure refinement, scaling, and data reduction were conducted using a software package (APEX2, Version 1.0-27).

Each structure was solved using direct methods. This procedure yielded the number of C, N, and O atoms. Subsequent Fourier synthesis yielded the remaining atom positions. The H atoms were fixed in positions of ideal geometry and refined within the XSHELL software. These idealized H atoms had their isotropic temperature factors fixed at values 1.2 or 1.5 greater than the equivalent isotropic (U) of the C atoms to which they were bonded. The final refinement of each compound included anisotropic thermal parameters on all nonhydrogen atoms. Data collection parameters are listed in Table S1 in the Supporting Information.

The Gaussian 03 program[17] was used to calculate the optimized ground-state geometries for molecules **1**−**8** and **21**. To determine the best level of theory and optimal basis set for these molecules, we performed a series of systematic tests. We used our measured crystal structure of molecule **6** as a starting structure and performed ground-state geometry optimizations using Hartree-Fock (HF), BLYP, and B3LYP; for each level of theory, we used 3-21G and 6-31G(d) basis sets. We also ran calculations using more-elaborate basis sets (e.g., 6-31+G(d)) and/or higher levels of theory (e.g., second-order Møller−Plesset (MP2)), but found only modest increases in accuracy at very high costs. HF theory, using either basis set, produces a structure that is qualitatively incorrect: the dihedral angles between the phenyl and thiophene rings are off by ∼40°. Density functional theory (DFT) performed much better, and BLYP and B3LYP structures were all qualitatively comparable to each other and the known crystal structure. To quantify the errors, we compared each of the bond lengths, bond angles, and dihedral angles for each of optimized structures directly with the corresponding values in the known crystal structure, using a home-written program. When ignoring errors in hydrogen dihedral angles—which should have minimal effect on the photophysics of the molecules—we determined that BLYP/6-31G outperformed the other viable combinations of the basis set and the level of theory; therefore, we used this combination to calculate the optimized geometries of molecules **1**−**8** and **21**. In cases where there were conformers, we report the structures with the lowest calculated energy.

Synthetic Procedures. The preparative details for some of the compounds are found in the Supporting Information. This is especially the case where the method is described herein as "by a similar procedure".

*4-Bromo-4′-(N,N-dihexylamino)biphenyl (**11a**).* 4-Amino-4′-bromobiphenyl (4.55 g, 0.018 mol), 1-bromohexane (10.59 g, 9.1 mL, 0.064 mol, 3.5 equiv), potassium carbonate (7.60 g, 0.055 mol, 3

(13) (a) Soper, S. A.; Nutter, H. L.; Keller, R. A.; Davis, L. M.; Shera, E. B. *Photochem. Photobiol.* **1993**, *57*, 972–977. (b) Margineanu, A.; Hofkens, J.; Cotlet, M.; Habuchi, S.; Stefan, A.; Qu, J.; Kohl, C.; Mullen, K.; Vercammen, J.; Engelborghs, Y.; Gensch, T.; Deschryver, F. C. *J. Phys. Chem. B* **2004**, *108*, 12242–12251.

(14) Jung, C.; Müller, B. K.; Lamb, D. C.; Nolde, F.; Müllen, K.; Brauchle, C. *J. Am. Chem. Soc.* **2006**, *128*, 5283–5291.

(15) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Springer: New York, 2006.

(16) Moerner, W. E.; Fromm, D. P. *Rev. Sci. Instrum.* **2003**, *74*, 3597–3619.

(17) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N. ; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C. ; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03, Revision C.01*; Gaussian, Inc.: Wallingford, CT, 2004.

equiv), and dimethyl formamide (DMF) (40 mL) were combined in a 200-mL round-bottom flask that was equipped with a stirbar. The mixture was stirred under nitrogen at 110 °C for 10 h. The reaction was cooled to room temperature and water (100 mL) was added to the reaction mixture. The product was extracted with hexane (200 mL) twice. The organic layers were combined and washed twice with water (100 mL), and then the combined organic layers were dried over magnesium sulfate. The organic layer was adsorbed on silica gel, placed at the top of a silica gel column, and eluted with hexane. The desired product was obtained as a white solid (4.82 g, 63% yield). Mp 67 °C. IR (neat): 2955, 2925, 2854, 1604, 1525, 1482, 804 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.55−7.40 (m, 6H), 6.73 (d, $J = 8.8$ Hz, 2H), 3.34 (t, $J = 7.6$ Hz, 4H), 1.67−1.62 (m, 4H), 1.40−1.30 (m, 12H), 0.96 (t, $J = 6.8$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.8, 140.3, 131.7, 127.7, 127.6, 126.3, 119.6, 111.8, 51.1, 31.8, 27.3, 26.9, 22.8, 14.1. Anal. Calcd for $C_{24}H_{34}BrN$: C, 69.22; H, 8.23; N, 3.36. Found: C, 69.41; H, 8.24; N, 3.48.

*1-(4′-Dihexylamino-biphenyl-4-yl)-2-hydroxy-2-methyl-propan-1-one (**12a**).* Magnesium turnings (0.17 g, 7.0 mmol) and 4-bromo-4′-(*N*,*N*-dihexylamino)biphenyl (2.08 g, 5.0 mmol) were combined, under nitrogen, in a dry, 100-mL two-neck round-bottom flask that was equipped with a stirbar. Anhydrous tetrahydrofuran (THF) (10 mL) was added with stirring to the mixture and then 1,2-dibromoethane (4 drops) was added. After the reaction was initiated, another portion of anhydrous THF (20 mL) was added to the mixture by syringe. The reaction mixture was heated to reflux for 3 h. The mixture then was cooled to room temperature and 2-methyl-2-trimethylsilanyloxy-propionitrile (1.60 g, 10.0 mmol) was added. The reaction was reheated to reflux overnight and then cooled to room temperature. Hydrochloric acid (6 N, 5 mL) was added to the reaction mixture, and it was stirred at room temperature for 3 h. Solid sodium bicarbonate was added to the reaction mixture until it was neutral. The reaction mixture was extracted with EtOAc (100 mL), and the crude product was adsorbed on silica gel, placed at the top of a silica column, and eluted (pure hexane to hexane/EtOAc = 10/1) to give the desired product as a green−yellow liquid (1.70 g, 80% yield). IR (neat): 3460, 2954, 2927, 2856, 1659, 1611, 1530, 1497, 1165 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, $J = 8.4$ Hz, 2H), 7.66 (d, $J = 8.4$ Hz, 2H), 7.56 (d, $J = 8.8$ Hz, 2H), 6.73 (d, $J = 9.2$ Hz, 2H), 4.35 (br s, 1H), 3.34 (t, $J = 8.0$ Hz, 4H), 1.70 (s, 6H), 1.64−1.62 (m, 4H), 1.39−1.36 (m, 12H), 0.95−0.92 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.7, 148.4, 146.0, 130.6, 128.1, 127.4, 125.4, 115.5, 111.9, 111.8, 51.1, 31.7, 28.7, 27.3, 26.9, 22.7, 14.1. HRMS *m/z* Calcd. for $C_{28}H_{41}NO_2$ (M+H): 424.3215. Found: 424.3206.

*3-Cyano-2-dicyanomethylen-5,5-dimethyl-4-[(4′-dihexyl-amino-biphenyl)-4-yl]-2,5-dihydrofuran (**5**).* 1-(4′-Dihexylamino-biphenyl-4-yl)-2-hydroxy-2-methyl-propan-1-one (1.40 g, 0.0033 mol), malononitrile (1.59 g, 0.024 mol), pyridine (20 mL), and acetic acid (4 drops) were combined in a 200-mL round-bottom flask that was equipped with a stirbar. The reaction mixture was stirred at room temperature for 48 h. The reaction mixture was poured into 400 mL of ice water and vigorously stirred. The precipitate was filtered out and recrystallized from dichloromethane and methanol to give purple crystals (0.60 g, 35% yield). Mp 189 °C. IR (neat): 2952, 2925, 2856, 2225 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.97 (ddd, $J = 8.0$, 2.4, 2.0 Hz, 2H), 7.78 (ddd, $J = 8.0$, 2.4, 2.0 Hz, 2H), 7.60 (ddd, $J = 9.0$, 3.1, 2.0 Hz, 2H), 6.74 (ddd, $J = 9.0$, 3.1, 2.0 Hz, 2H), 3.36 (t, $J = 7.6$ Hz, 4H), 1.90 (s, 6H), 1.66−1.63 (m, 4H), 1.39−1.36 (m, 12H), 0.94 (t, $J = 6.8$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.0, 175.8, 149.2, 147.4, 129.7, 128.2, 126.2, 124.0, 123.4, 111.9, 111.5, 110.9, 98.7, 98.3, 57.6, 51.1, 31.7, 27.3, 27.0, 26.8, 22.7, 14.1. UV−vis(CH_2Cl_2): λ_{max} = 535 nm, $\varepsilon_{max} = 2.84 \times 10^4$ L mol^{-1} cm^{-1}. Anal. Calcd for $C_{34}H_{40}N_4O$: C, 78.42; H, 7.74; N, 10.76. Found: C, 78.33; H, 7.47; N, 10.93.

*4-(4-Bromophenyl)-4-oxo-butyric acid dihexylamide (**9**).* 4-(4-Bromophenyl)-4-oxobutanoic acid (12.9 g, 50 mmol), triethylamine (24 mL, 170 mmol), and THF (200 mL) were placed in a 500-mL round-bottom flask. The flask was cooled to between −40 °C and −50 °C. Ethyl chloroformate (6.1 g, 56 mmol) was added to the stirred solution via syringe over a period of 10 min. The reaction mixture was stirred for 40 min and the temperature was allowed to rise to −20 °C. Dihexylamine (10.2 g, 55 mmol) was added and the reaction was stirred overnight at room temperature. Hexane (400 mL) was added to the reaction flask and stirred for an additional hour. The precipitate was filtered off, and the solution was washed with 5% hydrochloric acid (HCl) and brine and then dried over anhydrous $MgSO_4$. Solvent was removed by rotary evaporation and the residue was purified by silica gel flash chromatography (hexane/EtOAc = 4/1) to give the amide (17.2 g, 81% yield). IR (neat): 2955, 2927, 2857, 1687, 1640 cm^{-1}; ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.89 (ddd, $J = 8.6$, 2.4, 1.9 Hz, 2H), 7.60 (ddd, $J = 8.6$, 2.4, 1.9 Hz, 2H), 3.33−3.26 (m, 6H), 2.77 (t, $J = 6.5$ Hz, 2H), 1.68−1.58 (m, 2H), 1.57−1.46 (m, 2H), 1.38−1.22 (m, 12H), 0.95−0.85 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.4, 170.8, 135.7, 131.8, 129.7, 128.1, 48.0, 46.2, 33.8, 31.62, 31.56, 28.9, 27.8, 27.2, 26.7, 26.6, 22.62, 22.60, 14.04, 14.02. HRMS *m/z* Calcd. for $C_{22}H_{34}BrNO_2$ (M+Na): 446.1671. Found: 446.1693.

*2-Dihexylamino-5-(4-bromophenyl)-thiophene (**11b**).* A mixture of 4-(4-bromophenyl)-4-oxobutyric acid dihexylamide (7.4 g, 17.4 mmol) and Lawesson's reagent (12.0 g, 29.7 mmol) in 200 mL of toluene was refluxed for 4 h. The mixture was cooled and 2 M Na_2CO_3 (30 mL) was added to the flask and stirred overnight. The organic layer was separated and the aqueous layer was extracted with hexane. The combined organic layers were washed with water and brine and then dried over anhydrous $MgSO_4$. Solvent was removed with a rotary evaporator and the residue was purified using flash chromatography on a silica gel with hexane as the eluent. The product was obtained as yellow oil (6.34 g, 86% yield). IR (neat): 2953, 2926, 2855, 1500 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.41 (ddd, $J = 8.6$, 2.5, 1.0 Hz, 2H), 7.32 (ddd, $J = 8.6$, 2.5, 1.0 Hz, 2H), 7.03 (d, $J = 4.0$ Hz, 1H), 5.79 (d, $J = 4.0$ Hz, 1H), 3.25 (t, $J = 7.6$ Hz, 4H), 1.70−1.61 (m, 4H), 1.40−1.32 (m, 12H), 0.94 (t, $J = 6.9$ Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.9, 134.5, 131.6, 125.3, 124.5, 123.5, 118.3, 101.6, 53.7, 31.7, 27.0, 26.8, 22.7, 14.1. HRMS *m/z* Calcd. for $C_{22}H_{32}BrNS$ (M+H): 422.1517. Found: 422.1519.

*[4-(5-Dihexylaminothien-2-yl)phenyl)]-2-hydroxy-2-methyl-propan-1-one (**12b**).* 2-Dihexylamino-5-(4-bromophenyl)-thiophene (2.1 g, 5.0 mmol) and 15 mL anhydrous THF were placed in a 200-mL round-bottom flask. The mixture was cooled to −78 °C, and 2.4 mL (6.0 mmol) of *n*-BuLi (in hexane) (2.5 M) was added to this stirred mixture over 10 min. The resulting mixture was stirred for an additional hour at −78 °C and then 2-methyl-2-trimethyl-silyloxypropionitrile (1.57 g, 10.0 mmol) was added to the resulting mixture via a syringe over 10 min. After the addition was complete, the reaction was allowed to warm to room temperature and then stirred for an additional 12 h at room temperature. The reaction was quenched with 50 mL of water and 10 mL of 6 M HCl, stirred overnight, and then extracted with ethyl acetate. The organic layer was washed with water and brine and then dried with anhydrous $MgSO_4$. Solvent was removed with a rotary evaporator, and the residue was purified by silica gel chromatography (hexane/EtOAc = 20/1) to give an orange oil (1.79 g, 83% yield). IR (neat): 3451, 2954, 2927, 2856, 1655, 1595, 1505, 1483, 1445, 1162, 1066 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.00 (ddd, $J = 8.6$, 2.2, 1.9 Hz,

2H), 7.48 (ddd, J = 8.6, 2.2, 1.9 Hz, 2H), 7.33 (d, J = 4.1 Hz, 1H), 5.82 (d, J = 4.1 Hz, 1H), 4.20 (br, s, 1H), 3.29 (t, J = 7.6 Hz, 4H), 1.67 (s, 6H), 1.71−1.62 (m, 4H), 1.38−1.31 (m, 12H), 0.93 (t, J = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.9, 159.6, 140.2, 130.7, 125.8, 124.7, 124.0, 122.8, 102.3, 75.8, 53.7, 31.6, 28.6, 27.1, 26.7, 22.5, 13.8. HRMS *m/z* Calcd. for $C_{26}H_{39}NO_2S$ (M+H): 430.2780. Found: 430.2768.

*3-Cyano-2-dicyanomethylen-5,5-dimethyl-4-[4-(5-dihexylaminothien-2-yl)phenyl]-2,5-dihydrofuran (**6**).* The title compound was synthesized using a procedure that was similar to that used for compound **5**. It was given as green-gold crystals (1.66 g, 73% yield). Mp 129.7−131.0 °C. IR (neat): 3011, 3079, 2955, 2924, 2854, 2224 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (ddd, J = 9.0, 2.4, 1.9 Hz, 2H), 7.50 (ddd, J = 9.0, 2.4, 1.9 Hz, 2H), 7.39 (d, J = 4.3 Hz, 1H), 5.91 (d, J = 4.3 Hz, 1H), 3.34 (t, J = 7.7 Hz, 4H), 1.86 (s, 6H), 1.74−1.64 (m, 4H), 1.41−1.31 (m, 12H), 0.93 (t, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.2, 174.1, 161.8, 142.1, 130.2, 129.3, 123.1, 122.4, 121.4, 112.4, 112.2, 111.4, 103.2, 98.1, 95.4, 56.2, 53.8, 31.6, 27.3, 27.1, 26.7, 22.6, 14.0. APCI-MS *m/z* 527.0. UV−vis (CH_2Cl_2): λ_{max} = 625 nm, ε_{max} = 5.54 × 10^4 L cm^{-1} mol^{-1}. Anal. Calcd for $C_{32}H_{38}N_4OS$: C, 72.97; H, 7.27; N, 10.64; S, 6.09. Found: C, 72.85; H, 6.94; N, 10.94; S, 6.15.

*Dihexyl-(4-thiophen-2-yl-phenyl)-amine (**11c**).* Magnesium turnings (2.92 g, 0.12 mol), anhydrous THF (10 mL), and 1,2-dibromoethane (two drops) were combined in a dry 500-mL two-neck round-bottom flask that was equipped with a stirbar. Next, 2-bromothiophene (16.3 g, 0.10 mol) in THF (60 mL) was added via an addition funnel during 30 min under nitrogen. After refluxing for 1 h, the reaction was cooled to 0 °C, and dry $ZnCl_2$ (20.44 g, 0.15 mol) in anhydrous THF (200 mL) was added to the reaction mixture through a cannula. After all of the $ZnCl_2$ had been added, the reaction was stirred at room temperature for another 10 min, and then $Pd(PPh_3)_4$ (2.85 g, 0.0024 mol, 2 mol %) and 4-bromo-*N*,*N*-dihexylaniline (27.23 g, 0.08 mol) were added to the reaction mixture and the reaction was heated to reflux for 14 h. The reaction was cooled to room temperature and all of the salt was removed from the THF solution through suction filtration. The solid was washed several times with hexane and ether. The filtrate and washing solution were combined and adsorbed on silica gel, placed at the top of a silica column, and eluted (hexane/ether = 20/1) to give the product as a light yellow liquid (19.0 g, 69% yield). IR (neat): 2954, 2926, 2853, 1609, 1538, 1505, 1146, 805 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (ddd, J = 8.77, 3.20, 2.05 Hz, 2H), 7.16−7.14 (m, 2H), 7.06−7.03 (m, 1H), 6.65 (ddd, J = 8.77, 3.20, 2.05 Hz, 2H), 3.30 (t, J = 7.76 Hz, 4H), 1.64−1.56 (m, 4H), 1.45−1.31 (m, 12H), 0.94 (t, J = 6.70 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.6, 145.5, 127.8, 127.1, 122.4, 121.6, 120.5, 111.7, 51.1, 31.8, 27.3, 26.9, 22.7, 14.1; HRMS *m/z* Calcd. for $C_{22}H_{33}NS$ (M+H): 344.2412. Found: 344.2425.

*3-Cyano-2-dicyanomethylen-5,5-dimethyl-4-[5-(4-dihexylaminophenyl)thien-2-yl]-2,5-dihydrofuran (**7**) [from nitrile **10**].* The title compound was synthesized in two steps, similar to the synthesis of compounds **12b** and **5**, respectively. It was given as a blue solid (46 mg, 2.8% yield for two steps). Mp 131.9−133.6 °C. IR (neat): 2953, 2923, 2853, 2224, 1603, 1582, 1550 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (d, J = 4.5 Hz, 1H), 7.60 (d, J = 9.1 Hz, 2H), 7.38 (d, J = 4.5 Hz, 1H), 6.67 (d, J = 9.1 Hz, 2H), 3.36 (t, J = 7.7 Hz, 4H), 1.90 (s, 6H), 1.66−1.58 (m, 4H), 1.40−1.32 (m, 12H), 0.93 (t, J = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4, 166.9, 160.5, 150.3, 138.3, 128.5, 124.7, 122.9, 118.3, 112.6, 112.3, 111.7, 97.1, 91.5, 55.0, 51.2, 31.7, 27.5, 27.4, 26.7, 22.7, 14.0. APCI-MS *m/z* 526.9. UV−vis (CH_2Cl_2): λ_{max} = 599 nm, ε_{max} = 6.11 × 10^4 L cm^{-1} mol^{-1}. Anal. Calcd for $C_{32}H_{38}N_4OS$: C, 72.97; H, 7.27; N, 10.64; S, 6.09. Found: C, 72.56; H, 7.09; N, 10.86; S, 6.12.

*N,N-Dihexyl-4-oxo-4-thiophen-2-yl-butylamide (**14**).* The title compound was synthesized using a procedure similar to that used for compound **9**. The amide was given with 90% yield (34.0 g). IR (neat): 2955, 2927, 2857, 1639, 1519, 1459, 1416 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (dd, J = 3.8, 1.2 Hz, 1H), 7.62 (dd, J = 5.0, 1.2 Hz, 1H), 7.14 (dd, J = 5.0, 3.8 Hz, 1H), 3.34−3.27 (m, 6H), 2.77 (t, J = 6.7 Hz, 2H), 1.66−1.58 (m, 2H), 1.55−1.47 (m, 2H), 1.37−1.24 (m, 12H), 0.94−85 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 192.3, 170.8, 144.1, 133.4, 132.0, 128.1, 48.0, 46.2, 34.5, 31.6, 31.55, 29.0, 27.8, 27.2, 26.7, 26.6, 22.6, (2C) 14.04, 14.01. HRMS *m/z* Calcd. for $C_{20}H_{33}NO_2S$ (M+Na): 374.2130. Found: 374.2129.

*5-N,N-Dihexylamino-2,2′-bithiophene (**11d**).* The title compound was synthesized using a procedure that was similar to that used for compound **11b**. It was given as a yellow oil (26.0 g, 77% yield). IR (neat): 2954, 2926, 2855, 1556, 1514, 1493, 1451 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.07 (dd, J = 4.8, 1.5 Hz, 1H), 6.99−6.94 (m, 2H), 6.90 (d, J = 3.9 Hz, 1H), 5.75 (d, J = 3.9 Hz, 2H), 3.25 (t, J = 7.5 Hz, 4H), 1.70−1.62 (m, 4H), 1.44−1.28 (m, 12H), 0.97 (t, J = 6.8 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.3, 139.3, 127.7, 124.0, 121.8, 120.7, 120.0, 101.5, 53.9, 31.9, 27.2, 27.0, 22.9, 14.3. HRMS *m/z* Calcd. for $C_{20}H_{31}NS_2$ (M+H): 350.1976. Found: 350.1983.

*3-Cyano-2-dicyanomethylen-5,5-dimethyl-4-[5′-(dihexylamino)-2,2′-bithien-5-yl]-2,5-dihydrofuran (**8**) [from nitrile **10**].* The title compound was synthesized in two steps, using procedures similar to those used in the synthesis of compounds **12b** and **5**, respectively. It was given as green crystals (0.12 g, 2.5% yield for two steps). Mp 191.2−192.8 °C. IR (neat): 2952, 2928, 2854, 2222, 1574, 1544 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, J = 4.6 Hz, 1H), 7.36 (d, J = 4.5 Hz, 1H), 7.05 (d, J = 4.6 Hz, 1H), 5.93 (d, J = 4.5 Hz, 1H), 3.35 (t, J = 7.7 Hz, 4H), 1.86 (s, 6H), 1.74−1.65 (m, 4H), 1.40−1.32 (m, 12H), 0.93 (t, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.6, 165.1, 163.3, 154.2, 138.4, 132.1, 122.4, 121.8, 116.4, 113.1, 112.9, 112.2, 104.1, 96.5, 54.1, 31.5, 27.4, 27.0, 26.6, 22.6, 14.0. APCI-MS *m/z* 532.2. UV−vis (CH_2Cl_2): λ_{max} = 671 nm, ε_{max} = 10.5 × 10^4 L cm^{-1} mol^{-1}. Anal. Calcd for $C_{30}H_{36}N_4OS_2$: C, 67.63; H, 6.81; N, 10.52; S, 12.04. Found: C, 67.46; H, 6.60; N, 10.64; S, 12.31.

*2-Hydroxy-N-methoxy-2,N-dimethyl-propioamide (**17**).* 1,1′-Carbonyldiimidazole (4.21 g, 26 mmol), in portions over 10 min, was added to a solution of 2-hydroxyisobutyric acid (**16**) (2.08 g, 20 mmol) in 40 mL of dry dichloromethane. After the final addition, the solution was stirred for 15 min, then *N*,*O*-dimethylhydroxylamine hydrochloride (2.54 g, 26 mmol) was added in one portion. The reaction was allowed to stir at room temperature overnight. Diethyl ether (50 mL) was added and, after stirring for 5 min, the clear solution was decanted. The residue was stirred again with diethyl ether (40 mL), which was decanted off. The combined organic solution was washed with saturated sodium bicarbonate and brine and then dried over anhydrous $MgSO_4$. The crude product was purified by flash chromatography (hexane/EtOAc = 5/1) to give 2-hydroxy-*N*-methoxy-2,*N*-dimethyl-propionamide as a colorless liquid (1.83 g, 73% yield). IR (neat): 3468, 2989, 2943, 1744 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.70 (s, 3H), 3.25 (s, 3H), 1.44 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.2, 72.1, 61.0, 33.6, 26.5. HRMS *m/z* Calcd. for $C_6H_{13}NO_3$ (M+Na): 170.0793. Found: 170.0793.

*N-Methoxy-2,N-dimethyl-2-trimethylsilanyloxy-propionamide (**18**).* Chlorotrimethylsilane (1.6 mL, 12.5 mmol) was added dropwise, while chilling in an ice-water bath, to a solution of 2-hydroxy-*N*-methoxy-2,*N*-dimethyl-propionamide (1.5 g, 10.2

mmol) in pyridine (0.98 g, 12.4 mmol). The resulting mixture was stirred at room temperature for 8 h. The mixture then was poured into a saturated sodium bicarbonate solution (100 mL) and ethyl ether (100 mL). After stirring for 1 h, the mixture was separated, washed with brine, and dried over $MgSO_4$. After evaporation of the solvent, the residue was distilled under vacuum to give product as a clear liquid (1.8 g, 80% yield). IR (neat): 2957, 1658, 1462, 1032 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 3.73 (s, 3H, OCH_3), 3.35 (s, 3H, NCH_3), 1.52 (s, 6H, $C(CH_3)_2$), 0.19 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.2, 76.6, 60.4, 28.2, 26.5, 2.2. HRMS *m/z* Calcd. for $C_9H_{21}NO_3Si$ (M+Na): 242.1188. Found: 242.1185.

*1-(5′-Dihexylamino-[2,2′]bithiophenyl-5-yl)-2-hydroxy-2-methyl-propan-1-one (**12d**) [from the Weinreb amide (**18**)].* (5-*N*,*N*-Dihexylamino)-2,2′-thiophene (1.47 g, 4.2 mmol) and 15 mL of anhydrous THF were placed in a 100-mL round-bottom flask. The mixture was cooled to −78 °C, and 1.93 mL (4.84 mmol) of *n*-BuLi in hexane (2.5 M) was added to this stirred mixture over a period of 5 min. The resulting mixture was stirred for an additional hour at −78 °C and then at room temperature for 10 min. Subsequently, the reaction mixture was cooled to −78 °C and *N*-methoxy-2,*N*-dimethyl-2-trimethylsilanyloxy-propionamide (**18**) (1.11 g, 5.04 mmol) was added, using a syringe, over a period of 5 min. After the addition was complete, the temperature was allowed to increase to room temperature over a period of 3 h and the reaction mixture was stirred for an additional 6 h at room temperature. The reaction was quenched with 5% HCl (10 mL) and the mixture was stirred for 5 h and extracted with dichloromethane. The organic layer was washed with water and brine and then dried with anhydrous $MgSO_4$. Solvent was removed with a rotary evaporator, and the residue was purified using flash column chromatography (hexane/EtOAc = 5/1); 1-(5′-dihexylamino-[2,2′]bithiophenyl-5-yl)-2-hydroxy-2-methyl-propan-1-one was obtained as yellow oil (1.73 g, 94% yield). IR (neat): 3442, 2954, 2927, 2856, 1619, 1551, 1513, 1485, 1419, 1048 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, J = 4.1 Hz, 1H), 7.12 (d, J = 4.1 Hz, 1H), 6.89 (d, J = 4.1 Hz, 1H), 5.76 (d, J = 4.1 Hz, 1H), 4.11 (br, s, 1H), 3.27 (t, J = 7.6 Hz, 4H), 1.70−1.60 (m, 10H), 1.38−1.31 (m, 12H), 0.91 (t, J = 6.7 Hz, 6H). ^{13}C NMR (100 Hz, $CDCl_3$): δ 195.6, 159.6, 149.4, 135.8, 132.7, 127.5, 120.1, 117.5, 101.6, 75.6, 53.7, 31.6, 29.0, 27.0, 26.7, 22.6, 14.0. HRMS *m/z* Calcd. for $C_{24}H_{37}NO_2S_2$ (M+Na): 458.2163. Found: 458.2172.

*3-Cyano-2-dicyanomethylen-5,5-dimethyl-4-[5′-(dihexyl-amino)-2,2′-bithien-5-yl]-2,5-dihydrofuran (**8**).* The title compound was synthesized in a similar procedure as compound **5**. It was obtained as a green solid (0.90 g, 47% yield).

*1-[5-(4-Dihexylamino-phenyl)-thiophen-2-yl]-2-hydroxy-2-methyl-propan-1-one (**12c**) [from the Weinreb Amide (**18**)].* The title compound was synthesized using a procedure similar to that used for compound **12d**, from the Weinreb amide (**18**). It was obtained as an oil (1.90 g, 86%). IR(neat): 3446, 2955, 2927, 2857, 1739, 1603, 1366 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, J = 4.1 Hz, 1H), 7.54 (ddd, J = 9.0, 3.1, 2.1 Hz, 2H), 7.18 (d, J = 4.1 Hz, 1H), 6.65 (ddd, J = 9.0, 3.1, 2.1 Hz, 2H), 4.14(br, s, 1H), 3.22 (t, J = 7.7 Hz, 4H), 1.67 (s, 6H), 1.67−1.60 (m, 4H), 1.39−1.32 (m, 12H), 0.95 (t, J = 6.7 Hz, 6H). ^{13}C NMR (100 Hz, $CDCl_3$): δ 196.2, 155.6, 148.9, 135.8, 134.5, 127.6, 121.1, 119.8, 111.5, 75.7, 51.1, 31.7, 29.0, 27.2, 26.8, 22.7, 14.1. APCI *m/z* 430.5. HRMS *m/z* Calcd. for $C_{26}H_{39}NO_2S$ (M+Na): 452.2599. Found: 452.2603.

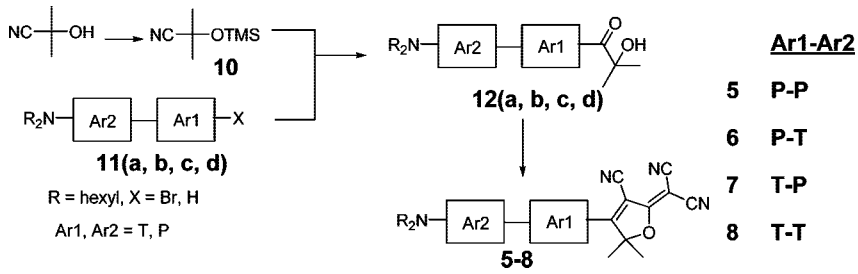
*3-Cyano-2-dicyanomethylen-5,5-dimethyl-4-[5-(4-dihexyl-aminophenyl)thien-2-yl]-2,5-dihydrofuran (**7**).* The title compound was synthesized using a procedure similar to that used for compound **5**. It was obtained as a purple solid (1.0 g, 51% yield).

*{4-[5-(4-Bromophenyl)-thiophen-2-yl]-phenyl}-dihexyl-amine (**19**).* Dihexyl-(4-thiophen-2-yl-phenyl)-amine (3.44 g, 10 mmol) and anhydrous THF (35 mL) were combined in a dry 200-mL two-neck round-bottom flask that was equipped with a stirbar. The mixture then was cooled to −78 °C. After stirring for ∼5 min, *n*-BuLi (2.5 M in hexane, 0.77 g, 4.8 mL, 12 mmol) was added. The mixture was stirred at −78 °C under nitrogen for 20 min, then the temperature was increased slowly over 30 min to room temperature. The reaction mixture was cooled to −78 °C again, and dry $ZnCl_2$ (2.05 g, 15 mmol) in THF (15 mL) was added to the reaction mixture. After ∼5 min, the reaction mixture was slowly increased to room temperature. After another 10 min, 1,4-dibromobenzene (11.8 g, 50 mmol, 5 equiv) and $Pd(PPh_3)_4$ (0.23 g, 0.2 mmol, 2 mol %) were added. The reaction mixture was stirred under nitrogen overnight. The reaction mixture was diluted with hexane, washed with water, and dried with anhydrous $MgSO_4$. After the solvent was removed, the residue was adsorbed on silica gel, placed at the top of a silica column, and eluted (hexane) to give a yellow solid crude product, which was recrystallized from 1-propanol to give the desired product as a pure yellow solid (3.10 g, 63% yield). Mp 90 °C. IR (neat): 2955, 2927, 2857, 2361, 1607 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.51−7.46 (m, 6H), 7.25 (d, J = 3.8 Hz, 1H), 7.10 (d, J = 3.8 Hz, 1H), 6.66 (ddd, J = 8.9, 3.1, 2.2 Hz, 2H), 3.31 (t, J = 7.7 Hz, 4H), 1.64−1.61 (m, 4H), 1.41−1.32 (m, 12H), 0.94 (t, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.8, 145.6, 139.4, 133.7, 131.9, 126.9, 126.8, 124.3, 121.4, 121.2, 120.5, 111.6, 51.1, 31.8, 27.3, 26.9, 22.7, 14.1; Anal. Calcd for $C_{28}H_{36}BrNS$: C, 67.45; H, 7.28; N, 2.81; S, 6.43. Found: C, 67.58; H, 6.99; N, 2.96; S, 6.16.

*2-(3-Cyano-4-{4-[5-(4-dihexylamino-phenyl)-thiophen-2-yl]-phenyl}−5,5-dimethyl-5H-furan-2-ylidene)-malononitrile (**21**).* The title compound was synthesized by two steps, using methods similar to those employed for the synthesis of compounds **12b** and **5**, respectively. It was obtained as purple crystals (0.25 g, 9.2% yield for two steps). Mp 178 °C. IR (neat): 2954, 2922, 2854, 2224 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.94 (ddd, J = 8.8, 2.3, 2.0 Hz, 2H), 7.79 (ddd, J = 8.8, 2.3, 2.0 Hz, 2H), 7.52 (ddd, J = 9.0, 3.0, 2.0 Hz, 2H), 7.50 (d, J = 4.0 Hz, 1H), 7.20 (d, J = 4.0 Hz, 1H), 6.67 (ddd, J = 9.0, 3.0, 2.0 Hz, 2H,), 3.33 (t, 4H), 1.89 (s, 6H), 1.69−1.57 (m, 4H), 1.41−1.31 (m, 12H), 0.88 (t, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.5, 175.4, 149.2, 148.4, 140.8, 137.7, 129.7, 127.4, 127.1, 125.6, 124.3, 122.0, 120.4, 111.7, 111.6, 111.2, 110.7, 99.1, 98.7, 58.1, 51.1, 31.7, 27.3, 26.9, 26.8, 22.7, 14.1; UV−vis (CH_2Cl_2): λ_{max}= 555 nm, $\varepsilon_{max} = 2.6 \times 10^4$ L mol^{-1} cm^{-1}. Anal. Calcd for $C_{38}H_{42}N_4OS$: C, 75.71; H, 7.02; N, 9.29; S, 5.32. Found: C, 75.82; H, 6.89; N, 9.44; S, 5.40.

*5-(4-Dihexylamino-phenyl)-thiophene-2-carbaldehyde (**22**).* 2-(4-*N*,*N*-Dihexylaminophenyl)thiophene (1.03 g, 3.0 mmol) and 15 mL of anhydrous THF were combined in a 200-mL round-bottom flask. The mixture was cooled to −78 °C, and 1.44 mL (3.6 mmol) of *n*-BuLi in hexane (2.5 M) was added to this stirred mixture over 10 min. The resulting mixture was stirred for an additional hour at −78 °C and then at room temperature for 10 min. Subsequently, the reaction mixture was cooled to −78 °C and anhydrous DMF (459 mg, 6.0 mmol) was added to the resulting mixture via a syringe as quick as possible. After the addition was complete, the temperature was allowed to increase to room temperature over a period of 3 h and the reaction mixture was stirred for an additional 24 h at room temperature. The reaction was quenched with 30 mL of water and 1 mL of 6 M HCl and extracted with dichloromethane. The organic layer was washed with water and brine and then dried with anhydrous $MgSO_4$. Solvent was removed with a rotary evaporator and the residue was purified by silica gel chromatography (dichloromethane) to give the desired product as oil (0.97 g,

Scheme 1. A General Strategy for Synthesis of DCDHF Chromophores with Two Aromatic Rings between the Donor and Acceptor Units



87% yield). IR (neat): 2954, 2926, 2855, 2791, 2740, 1656, 1601, 1440 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 9.83 (s, 1H), 7.68 (d, J = 4.0 Hz, 1H), 7.55 (ddd, J = 9.1, 3.1, 2.1 Hz, 2H), 7.23 (d, J = 4.0 Hz, 1H), 6.65 (ddd, J = 9.1, 3.1, 2.1 Hz, 2H), 3.32 (t, J = 7.7 Hz, 4H), 1.66−1.57 (m, 4H), 1.41−1.25 (m, 12H), 0.94 (t, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.4, 156.4, 149.0, 139.7, 138.2, 127.7, 121.1, 119.7, 111.5, 51.1, 31.7, 27.2, 26.8, 22.7, 14.1. HRMS *m/z* Calcd. for $C_{23}H_{33}NOS$(M+H): 372.2361. Found: 372.2369.

*1-(3-Cyano-2-dicyanomethylen-5,5-dimethyl-2,5-dihydrofuran-4-yl)-2-{5-[4-(N,N-dihexylaminophenyl)]thien-2-yl}ethene (**24**).* A mixture of 5-(4-dihexylaminophenyl)-thiophene-2-carbaldehyde (557 mg, 1.5 mmol) and 2-dicyanomethylen-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (301 mg, 1.4 mmol) was dissolved in 15 mL of dry pyridine. After the addition of three drops of acetic acid, the mixture was stirred at room temperature for 48 h. The reaction mixture was transferred to a separatory funnel and 150 mL of ethyl acetate was added. The solution was washed with 5% HCl, water, and brine and then dried over anhydrous $MgSO_4$. Solvent was removed with a rotary evaporator and the residue was purified by silica gel chromatography with hexane and hexane/ethyl acetate mixture as the eluent to give a green solid, which was recrystallized from dichloromethane/methanol to give the desired product as green crystals (610 mg, 77% yield). Mp. 185.7−186.7 °C. IR (neat): 3093, 2957, 2925, 2857, 2225, 1592 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, J = 15.5 Hz, 1H), 7.55 (d, J = 9.1 Hz, 2H), 7.45 (d, J = 4.1 Hz, 1H), 7.26 (d, J = 4.1 Hz, 1H), 6.66 (d, J = 9.1 Hz, 2H), 6.56 (d, J = 15.5 Hz, 1H), 3.35 (t, J = 7.8 Hz, 4H), 1.77 (s, 6H), 1.65−1.59 (m, 4H), 1.40−1.33 (m, 12H), 0.93 (t, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.0, 173.2, 156.6, 149.6, 139.9, 138.5, 136.5, 127.9, 122.6, 119.4, 112.5, 111.72, 111.67, 111.3, 110.8, 96.9, 95.1, 55.4, 51.1, 31.7, 27.3, 26.8, 26.6, 22.7, 14.1. UV−vis (CH_2Cl_2): λ_{max} = 658 nm, ε_{max} = 5.37 × 10^4 L cm^{-1} mol^{-1}. Anal. Calcd for $C_{34}H_{40}N_4OS$: C, 73.88; H, 7.29; N, 10.14; S, 5.80. Found: C, 73.62, H, 7.42; N, 9.99; S, 5.93.

*5′-Dihexylamino-[2,2′]-bithiophenyl-5-carbaldehyde (**23**).* The title compound was synthesized using a procedure similar to that used for compound **22**. It was given as an oil (1.21 g, 93% yield). IR (neat): 2953, 2926, 2856, 1651, 1518, 1424, 1042 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 9.75 (s, 1H), 7.57 (d, J = 4.1 Hz, 1H), 7.12 (d, J = 4.1, 1H), 6.92 (d, J = 4.1 Hz, 1H), 5.76 (d, J = 4.1 Hz, 1H), 3.27 (t, J = 7.6 Hz, 4H), 1.71−1.62 (m, 4H), 1.40−1.29 (m, 12H), 0.92 (t, J = 6.9 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.7, 159.9, 150.0, 138.2, 138.0, 127.9, 120.0, 117.4, 101.6, 53.7, 31.6, 27.0, 26.7, 22.6, 14.0. HRMS *m/z* Calcd. for $C_{21}H_{31}NOS_2$ (M+H): 378.1925. Found: 378.1935.

*1-(3-Cyano-2-dicyanomethylen-5,5-dimethyl-2,5-dihydrofuran-4-yl)-2-[5′-(N,N-dihexylamino)-2,2′-bithien-5-yl]ethene (**25**).* The title compound was synthesized using a procedure similar to that used for compound **24**. It was given as green crystals (0.62 g, 63% yield). Mp 194.0−196.1 °C. IR (neat): 2927, 2856, 2222, 1593 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, J = 15.2 Hz, 1H), 7.36 (d, J = 4.3 Hz, 1H), 7.24 (d, J = 4.3 Hz, 1H), 6.95 (d, J = 4.3 Hz, 1H), 6.34 (d, J = 15.2 Hz, 1H), 5.89 (d, J = 4.3 Hz, 1H), 3.33 (t, J = 7.7 Hz, 4H), 1.77 (s, 6H), 1.71−1.62 (m, 4H), 1.40−1.33 (m, 12H), 0.93 (t, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.3, 172.4, 162.1, 150.9, 139.5, 139.0, 135.1, 130.5, 121.9, 117.7, 113.0, 112.3, 111.9, 109.2, 103.6, 96.4, 92.6, 54.0, 53.7, 31.6, 27.1, 26.7, 26.66, 22.6, 14.0. UV−vis (CH_2Cl_2): λ_{max} = 761 nm, ε_{max} = 7.74 × 10^4 L cm^{-1} mol^{-1}. Anal. Calcd for $C_{32}H_{38}N_4OS_2$: C, 68.78; H, 6.85; N, 10.03; S, 11.48. Found: C, 68.73; H, 6.71; N, 10.02; S, 11.62.

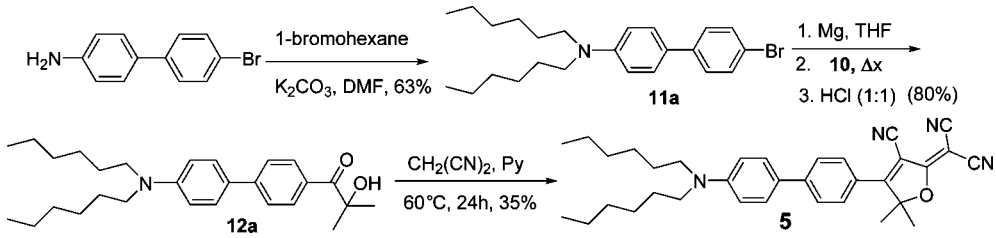
Results and Discussion

Synthesis. Much of the general synthetic strategy for the DCDHF chromophores with multiple rings and without a vinyl link (seen in Scheme 1) follows from our previous procedures.[2g,18] The first step is to make the dialkylamino-bisaromatic compounds **11a**−**11d**, which are converted to organometallic derivatives and then reacted with nitrile **10** (the TMS-protected acetone cyanohydrin), leading to the α-ketols **12a**−**12d** after hydrolysis of the imine intermediate. Knoevenagel condensation between the α-ketols **12a**−**12d** and malononitrile directly provides the target DCDHF fluorophores **5**−**8**. Subsequently, we describe separately the preparation of these individual bisaromatic DCDHF compounds with Ar1−Ar2 = P−P, P−T, T−P, and T−T. (The naming scheme specifies the π system: Ar1 is the π unit closest to DCDHF acceptor, Ar2 is the π unit one away from the acceptor, and so on. The π units are denoted as "P" (for phenylene), "V" (for vinyl), and "T" (for thiophene).)[19] In particular, we discuss a problem involving the thienyllithium reaction with **10** at the trimethylsilyl group predominating over the cyano group and the development of a solution using a Weinreb amide **18** to make the thienyl α-ketols much more efficiently.

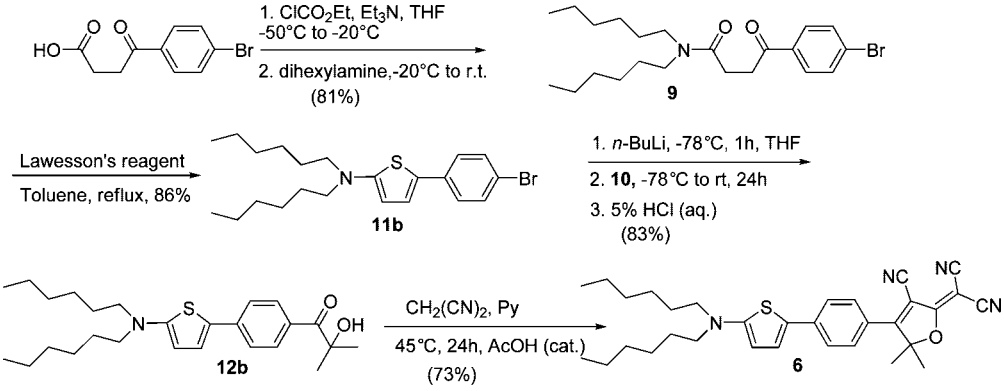
Scheme 2 depicts compound **5** (Ar1−Ar2 = P−P), which was prepared starting with the nitration of 4-bromobiphenyl with fuming nitric acid to give 4-bromo-4′-nitrobiphenyl, which was then reduced to 4-amino-4′-bromobiphenyl.[20] The 4-amino-4′-bromobiphenyl was *N*,*N*-dialkylated and treated with magnesium in anhydrous THF, and the resulting Grignard reagent then was quenched with nitrile **10** to afford,

(18) He, M.; Twieg, R. J.; Ostroverkhova, O.; Gubler, U.; Wright, D.; Moerner, W. E. *Proc. SPIE* **2002**, *4802*, 9–20.
(19) Lord, S. J.; Conley, N. R.; Lee, H.-L. D.; Nishimura, S. Y.; Pomerantz, A. K.; Willets, K. A.; Lu, Z.; Wang, H.; Liu, N.; Samuel, R.; Weber, R.; Semyonov, A.; He, M.; Twieg, R. J.; Moerner, W. E. *ChemPhysChem* **2009**, *10*, 55−65.
(20) Lemenovskii, D. A.; Makarov, M. V.; Dyadchenko, V. P.; Bruce, A. E.; Bruce, M. R. M.; Larkin, S. A.; Averkiev, B. B.; Starikova, Z. A.; Antipin, M. Y. *Russ. Chem. Bull.* **2003**, *52*, 607–615.

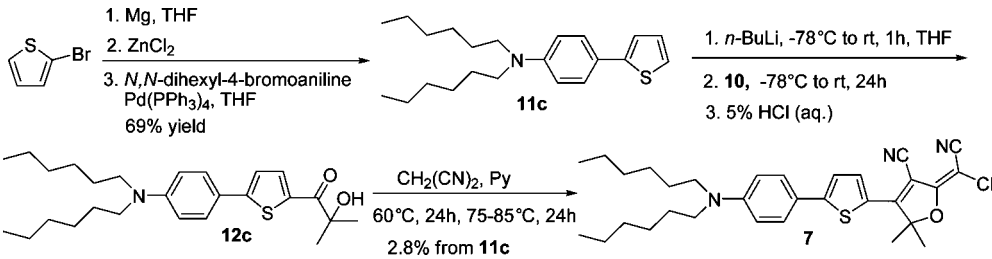
Scheme 2. Synthesis of DCDHF Dye 5 with Ar1−Ar2 = P−P



Scheme 3. Synthesis of DCDHF Dye 6 with Ar1−Ar2 = P−T



Scheme 4. Synthesis of DCDHF Dye 7 with Ar1−Ar2 = T−P



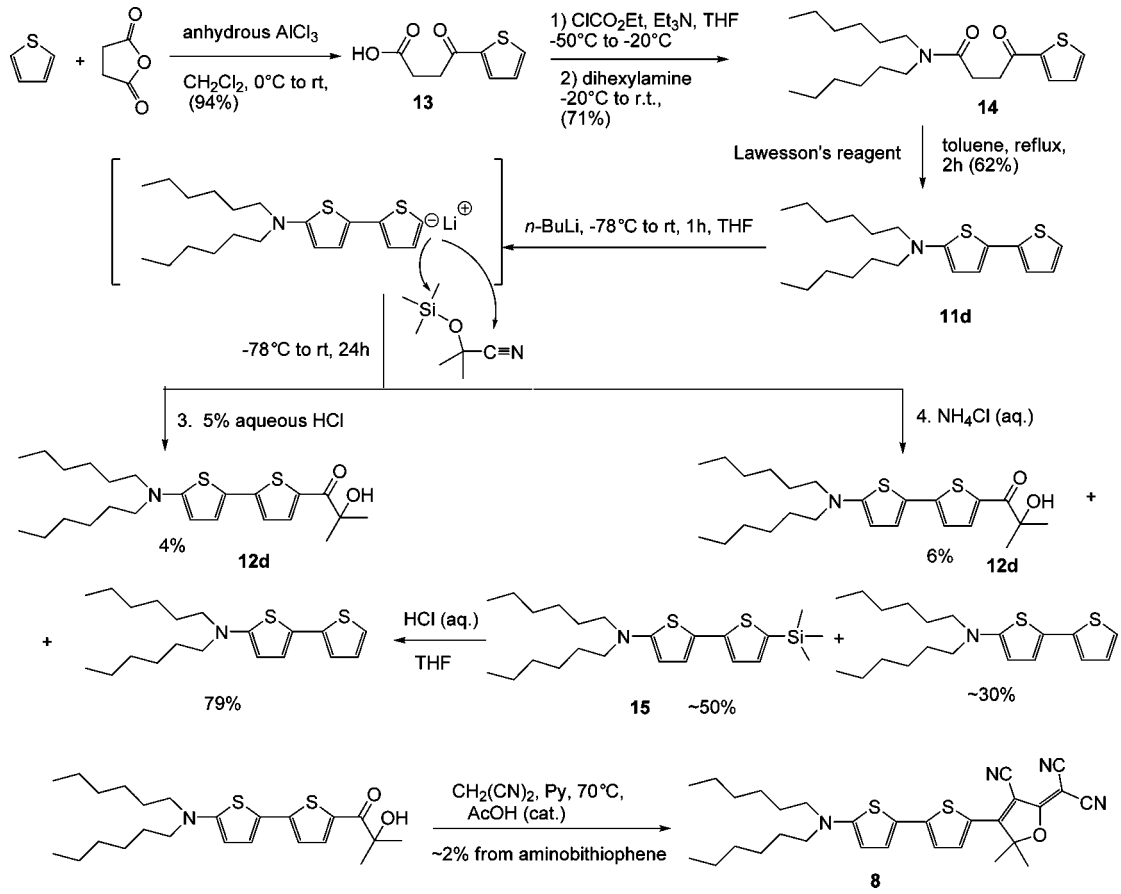
after hydrolysis of the imine, the α-ketol, which was then converted to DCDHF dye **5** by treatment with malononitrile in pyridine.

Scheme 3 depicts compound **6** (Ar1−Ar2 = P−T) features a combination of a thiophene and a phenyl ring with the thiophene ring on the donor side and the phenyl ring linked to the acceptor group. The synthesis starts with the Friedel−Crafts acylation of bromobenzene with succinic anhydride, using a modified version of the procedure reported by Seed and co-workers,[21] to give 4-(bromothien-2-yl)-4-oxobutanoic acid. Conversion of the carboxylic acid to the amide **9** used the method of Kozikowski et al.,[22] and the resulting γ-ketoamide was cyclized with Lawesson's reagent in toluene.[23] We found that 1.0 equiv of Lawesson's reagent gave only a low yield of the thiophene, whereas more than 1.5 equiv of Lawesson's reagent was required for the cyclization reaction to proceed in good yield. Lithium−halogen exchange of 2-dihexylamino-5-(4-bromophenyl)-thiophene (**11b**) was accomplished with *n*-BuLi and the resulting lithium reagent was then trapped with nitrile **10** to afford the α-ketol (**12b**), which was converted to DCDHF dye **6** with Ar1−Ar2 = P−T by treatment with malononitrile in pyridine.

Scheme 4 depicts molecule **7** (Ar1−Ar2 = T−P), which, again, is a combination of phenyl and thiophene rings but, in this case, with reversed order (having phenyl on the donor side and thiophene directly linked to the acceptor). To synthesize **7**, 2-bromothiophene was reacted with magnesium in THF to give a Grignard reagent, which was treated with anhydrous zinc chloride and then subjected to a palladium-catalyzed Negishi coupling with *N*,*N*-dihexyl-4-bromoaniline to give **11c**.[24] Next, **11c** was lithiated with *n*-BuLi at −78 °C and then warmed to 10 °C over 1 h, quenched with nitrile **10**, and hydrolyzed with aqueous HCl to afford a mixture containing α-ketol **12c**, which was not immediately purified after hydrolysis, because of low yield. The crude α-ketol was directly used to run the DCDHF ring formation reaction to give a low yield of **7**. This problem of low yield is common to the syntheses of all α-ketols with the acceptor on the thiophene side, and a solution for this problem is discussed in detail later.

(21) Sonpatki, V. M.; Herbert, M. R.; Sandvoss, L. M.; Seed, A. J. *J. Org. Chem.* **2001**, *66*, 7283–7286.
(22) Kozikowski, A. P.; Ma, D.; Brewer, J.; Sun, S.; Costa, E.; Romeo, E.; Guidotti, A. *J. Med. Chem.* **1993**, *36*, 2908–2920.
(23) Raposo, M. M. M.; Kirsch, G. *Heterocycles* **2001**, *55*, 1487–1498.
(24) (a) Negishi, E.; King, A. O.; Okukado, N. *J. Org. Chem.* **1977**, *42*, 1821–1823. (b) Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem.−Int. Ed.* **2005**, *44*, 4442–4489.

Scheme 5. Synthesis of DCDHF Dye 8 with Ar1−Ar2 = T−T (the Side Reactions of Metallated 11d, Leading to Byproducts and/or the Return of Starting Material, Are Also Shown)



The synthesis of chromophore **8** (Ar1−Ar2 = T−T) with a thiophene−thiophene π-system is shown in Scheme 5. It began with a Friedel−Crafts acylation reaction of thiophene with succinic anhydride to afford keto-acid **13**.[25] Keto-acid **13** was treated with ethyl chloroformate at approximately −45 °C to give a mixed anhydride and dihexylamine was added to the same flask to give amide **14**, which was subsequently reacted with Lawesson's reagent to give bithiophene **11d**, using procedures similar to those used for the synthesis of **6**. Dialkylaminobithiophene **11d** was then deprotonated with *n*-BuLi and the organolithium derivative was subsequently trapped with nitrile **10** and hydrolyzed with 5% HCl to afford α-ketol **12d**. The crude α-ketol **12d** was not further purified but directly reacted with malononitrile to give DCDHF dye **8** but in only 2.5% yield from **11d**.

The low yields of both **7** and **8**, which share the common feature of a thiophene linked directly to the acceptor, prompted us to further explore the problems involved in their preparation, in which the lithiated thiophene is trapped with the TMS-protected acetone cyanohydrin. We carefully examined the conversion of **11d** to **12d**, and we determined that, after our usual workup with HCl, most of the starting bithiophene was recovered and that only a low yield of α-ketol **12d** was obtained. We initially assumed that this outcome was due to a failure to form the lithiated thiophene, or the failure of the lithiated thiophene to react with the TMS-protected acetone cyanohydrin, or possibly due to instability of the α-ketol **12d** (if formed).[26] However, and much to our surprise, when the reaction mixture was worked up with ammonium chloride solution instead, ∼50% of the dihexyl-(5′-trimethylsilanyl-[2,2]bithiophenyl-5-yl)-amine (**15**) was isolated. This product results from thienyllithium attack on **10** at the trimethylsilyl group, strongly predominating over attack at the cyano group. Such an attack of an organolithium reagent on a trimethylsilyl group is uncommon but has been documented in the literature. For example, phenyl lithium reacts with ethoxytrimethylsilane in ethereal solution to give phenyltrimethylsilane in 72% yield.[27] Therefore, in the case at hand, the formation of **15** was obscured because the reaction was worked up with the ordinary HCl under which conditions **15** would be protiodesilylated to **11d**. To confirm this assumption, a THF solution of **15** (itself isolated after a mild ammonium chloride workup) was treated with 5% HCl at room temperature and the trimethylsilyl group was indeed removed in a protiodesilylation reaction, leading cleanly to aminobithiophene **11d**.

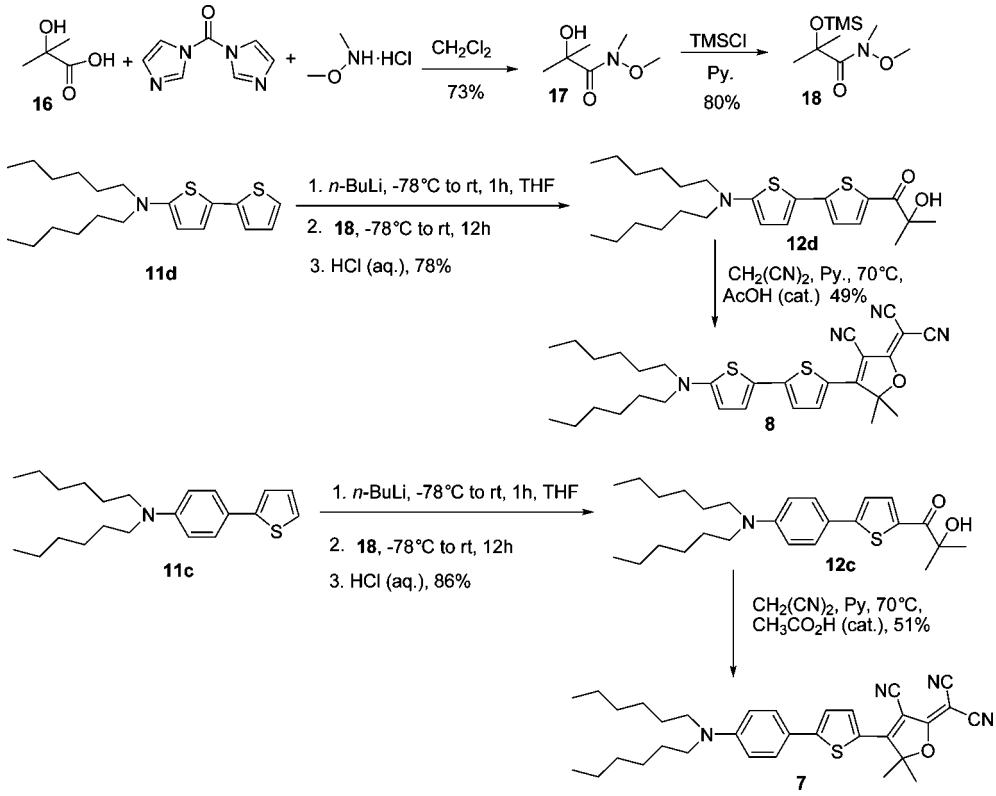
Because of the unfavorable result for the reaction requiring thienyllithium attack on the cyano functionality of **10**, an alternative electrophile for the formation of the α-hydroxyketone is desired. The Weinreb amide is widely used for the acylation reaction of organometallics, because it will

(25) Cauquil-Caubere, I.; Kamenka, J. M. *Eur. J. Med. Chem.* **1998**, *33*, 867–877.

(26) Liska, R. *Heterocycles* **2001**, *55*, 1475–1486.

(27) Gilman, H.; Ingham, R. K.; Smith, A. G. *J. Org. Chem.* **1953**, *18*, 1743–1749.

Scheme 6. Synthesis of the T−P Dye 7 and T−T Dye 8 Using the Weinreb Intermediate 18



stabilize the organometallic intermediate formed by chelation to prevent tertiary alcohol formation.[28] Thus, Weinreb amide **18** was proposed to replace the TMS-protected acetone cyanohydrin **10** (see Scheme 6). The synthesis of **18** began with the reaction of 2-hydroxyisobutyric acid with 1,1′-carbonyldiimidazole, followed by the addition of HN(OMe)-Me·HCl to afford the alcohol-containing Weinreb amide **17**.[29] The hydroxyl functionality in **17** was protected with TMS by reacting **17** with trimethylsilylchloride in pyridine to produce the final Weinreb amide **18**. Now, **11d** was deprotonated with *n*-BuLi and the organolithium derivative was subsequently trapped with the Weinreb amide **18** and hydrolyzed with 5% HCl to afford α-ketol **12d**, which was now isolated in a much-improved 78% yield. The ring formation of α-ketol **12d** with malononitrile in pyridine at 70 °C now gave DCDHF dye **8** in 49% yield and the overall yield from **11d** to dye **8** was 38%. A comparable good result was also obtained when the Weinreb amide **18** was used for the synthesis of DCDHF dye **7** with the thiophene directly linked to the DCDHF and the α-ketol **12c** was isolated in 86% yield. The corresponding ring formation reaction gave 51% yield, and the overall yield from **11c** to dye **7** was 44%. Therefore, the Weinreb amide derivative **18** is much more reactive and useful than the TMS-protected acetone cyanohydrin **10** originally used for creation of the α-hydroxyketone needed as a DCDHF intermediate, particularly in the case of less-reactive nucleophiles such as thiophene-derived anions.

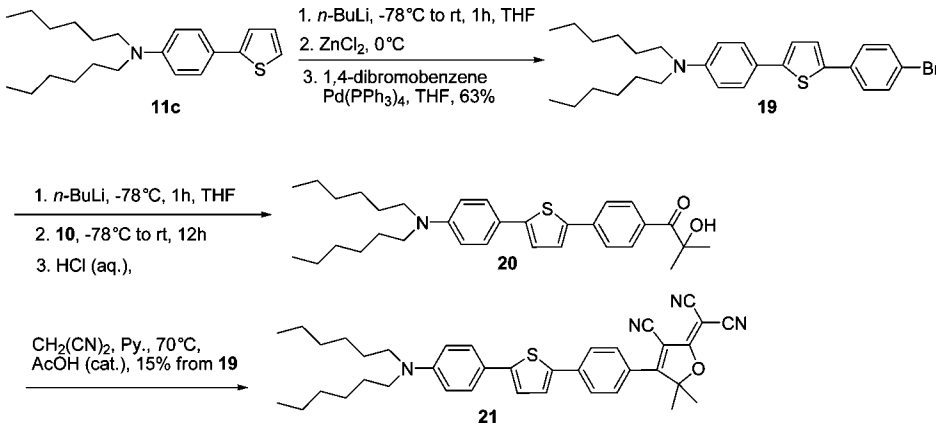
In addition to the set of four new DCDHF dye materials just discussed, we have also synthesized a three-ring system and a pair of two-ring compounds extended with an alkene. DCDHF dye **21** with a P−T−P conjugation between the donor and the acceptor unit was prepared to examine the influence of the additional aromatic unit on the photophysical properties (see Scheme 7). To synthesize **21**, biaromatic **11c** was deprotonated with *n*-BuLi in THF and subsequently treated with anhydrous zinc chloride, the organozinc reagent was then subjected to a palladium-catalyzed Negishi coupling with large excess of 1,4-dibromobenzene to give the bromine-terminated triaromatic **19**.[24] Bromide **19** was lithiated with *n*-BuLi, quenched with nitrile **10** and hydrolyzed with 6 N HCl to afford a mixture containing α-ketol **20**, which was not further purified after hydrolysis. The crude α-hydroxyketone was used directly in the DCDHF ring formation reaction to give **21** in 15% yield from **19**.

The synthesis of the additional two vinylogous dyes with V−T−P structure (**24**) and the V−T−T structure (**25**) is presented in Scheme 8. Both are synthesized by converting the requisite dialkylamino bisaromatic compound **11c** and **11d** to their corresponding aldehydes **22** and **23**, which were subsequently converted to **24** and **25** by condensing the aldehydes **22** and **23** with 2-dicyanomethylen-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran, respectively.[2g]

Absorption Spectra. The absorption and other photophysical properties of DCDHFs **1**−**8**, **21**, **24**, and **25** are all summarized in Table 1. All these dyes have the same dialkylamine donor and the same DCDHF acceptor and differ only in the π-conjugation linkage that is present. Thus, differences in the longest wavelength absorption band (the charge-transfer band) reflect only the composition of the long axis π-conjugation bridge of the molecules. A bathochromic shift results from addition of a second ring, either a thiophene

(28) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815–3818.
(29) Garg, N. K.; Caspi, D. D.; Stoltz, B. M. *J. Am. Chem. Soc.* **2005**, *127*, 5970–5978.

Scheme 7. Synthesis of DCDHF Fluorophore 21 with a Three-Ring P−T−P Linkage



Scheme 8. Synthesis of Two DCDHF Fluorophores (24 and 25) with V−Ar1−Ar2 Linkages

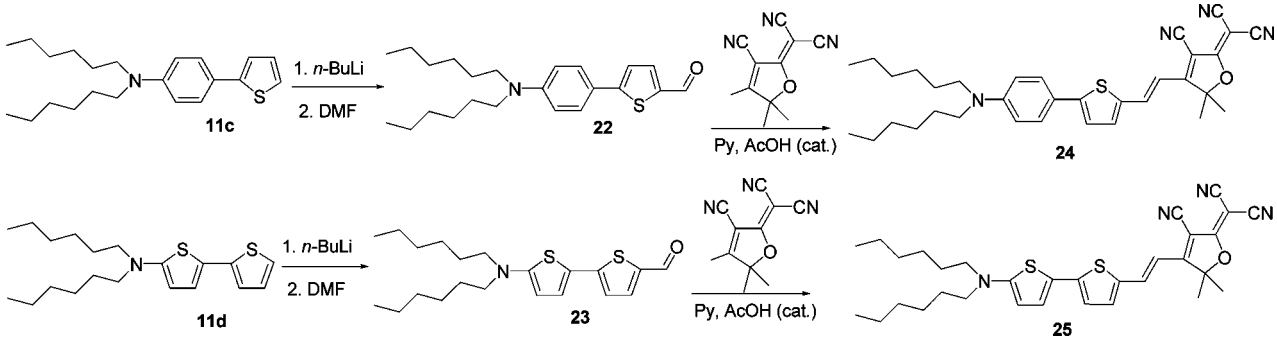


Table 1. Summary of the Photophysical Properties of DCDHF Chromophores 1−8, 21, 24, and 25[a]

	DCDHF−(π)−NR_2	ε_{max} (M^{-1} cm^{-1})[b]	λ_{abs} (nm)[b]	λ_{em} (nm)[b]	Stokes shift (cm^{-1})[b]	λ_{ex} (nm)[b]	Φ_F toluene [PMMA]	$N_{tot,emitted}$[c]
1	P	71 000	486	505	774	470	0.044[d] [0.92]	2.4×10^6
2	T	100 000	514	528	516	488	0.11	$\sim 9.1 \times 10^5$
3	V−P	45 500	562	603	1210	532	0.02 [0.39]	$\sim 1.9 \times 10^6$
4	V−T	114 000	614	646	807	594	0.02	$\sim 2.3 \times 10^5$
5	P−P	31 000	506	623	3711	488	0.82	4.5×10^6
6	P−T	44 000	591	663	1838	532	0.21	6.4×10^6
7	T−P	22 000	575	631	1543	532	0.74	2.9×10^6
8	T−T	71 800	634	679	1045	600	0.50	$> 2.3 \times 10^5$[e]
21	P−T−P	28 000	541	709	4380	488	0.34	9.1×10^5
24	V−T−P	47 300	611	723	2535	570	0.07	$> 5.4 \times 10^5$[e]
25	V−T−T	49 800	708	779	1287	600	0.13	$> 3.8 \times 10^4$[e]

[a] Photostability values $N_{tot,emitted}$ will be discussed in the following single-molecule section. [b] All the absorption and emissions were measured in toluene. [c] For comparison, $N_{tot,emitted}$ for Rhodamine 6G = 1.4×10^6 photons per molecule (bulk measurement in PMMA). [d] The quantum yield value reported before (10%) is less accurate and has been corrected in this paper. [e] These values were determined from bulk measurements of the dyes doped in PMMA films, excited at 633 nm. They are lower limits because the calculation uses the quantum yields in toluene, which are lower than those in PMMA; if the (correct) higher PMMA quantum yields were easily measurable, the $N_{tot,emitted}$ values would be higher (in a linear fashion).

or benzene, to the parent DCDHF **1** or **2**. The absorption spectra of the set of four Ar1−Ar2 DCDHF chromophores **5**−**8** in toluene are shown in Figure 2. The addition of a benzene ring to the single phenyl linkage in **1** led to a bathochromic shift of 20 nm (**5**), whereas a thiophene ring addition induced a shift of 105 nm (**6**) or 89 nm (**7**), depending on the position of the new ring. Similarly, an addition of a second phenyl ring to the thiophene linkage dye **2** led to a bathochromic shift of 77 nm (**6**) or 61 nm (**7**) and an additional thiophene ring for dye **2** gave a 120-nm (**8**) bathochromic shift. Overall, the most effective conjugation was observed with two thiophene rings. Comparison of the absorption maxima of the different DCDHF derivatives indicates that the charge-transfer properties can be effectively tuned by iterations of the π-bridge.

The P−P linkage (**5**) shows diminished π-overlap efficiency, because of a confinement of π-electrons associated with the high aromatic stabilization energy of the benzene ring and the potential barriers arising from the steric hindrance of the *ortho* hydrogens that inhibit the coplanarity between the two phenyl rings. In its X-ray structure, the dihedral angle (dr_2 in Table 2) between the two aromatic (phenyl) rings is −23.0°, which is larger than that between the thiophene and the phenyl ring in compound **6** (8.1°). It is well-known that the absorption wavelength increases as the number of phenyl units in the push−pull system increases, but this effect saturates (usually with the biphenyls or terphenyls); after saturation, adding more phenyl units eventually causes a hypsochromic shift.[30] Adding a third phenyl ring does indeed cause a hypsochromic shift: the

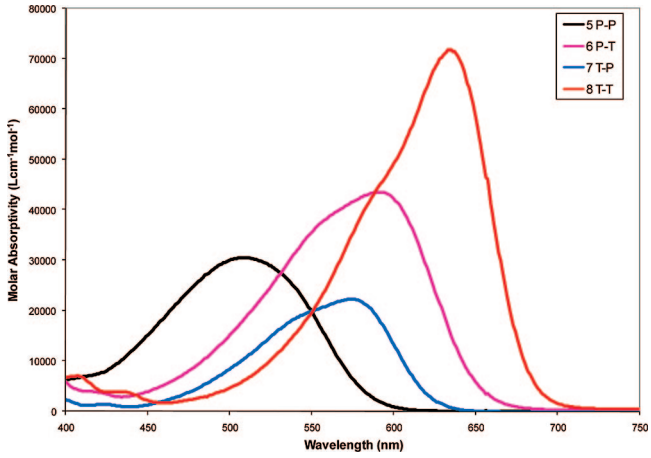
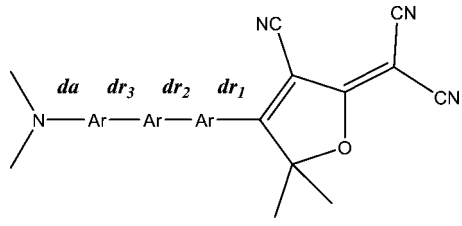


Figure 2. Visible range absorption spectra of DCDHF bisaromatic dyes **5**−**8** in toluene.

Table 2. Calculated Torsion Angles between the Carbon off the Amine and the Average Plane of the Ring Adjacent to the Amine (*da*), between the DCDHF and the Adjacent Ring (dr_1), between the First Ring and its Adjacent Ring (dr_2), and between the Second and Third Rings (dr_3), where Applicable[a]



	da	dr_1	dr_2	dr_3
1	0.5	9.7		
2	8.5	0.9		
3	−0.1	0.0	0.0	
4	−170.5	−0.3	−0.2	
5	1.3 [−7.8]	13.9 [−2.2]	−27.2 [−23.0]	
6	10.0 [−3.2]	7.5 [13.2]	−0.1 [8.1]	
7	0.7	1.5	−11.6	
8	−167.9	−0.4	−179.4	
21	3.6 [1.1]	−3.1 [1.0]	−172.2 [−162.3]	162.9 [−168.5]

[a] Values in brackets are taken from available X-ray structures.

trisaromatic P−T−P dye **21** has an additional phenyl ring, compared to the T−P dye **7**, and the absorption maximum of **21** is 541 nm, 34 nm to the blue of **7**. This blue shift occurs because the additional phenyl ring disturbs the charge transfer, because the entire molecule deviates from a planar structure, as shown from the dihedral angle (dr_3) between the thiophene and the phenyl ring ($dr_3 = -168.5°$; see Table 2). In contrast, the introduction of thiophene in the π-conjugated system tolerates more thiophene units before the absorption maxima saturates. This has been explained by the reduced aromaticity of the thiophene ring and the relative ease of coplanarity for adjacent thiophene rings.[9a] As such, it is no surprise to observe that the absorption maxima of the P−T **6** and T−P **7** dyes fall in the range of the absorption maxima between the P−P **5** and the T−T **8** dyes. To some extent, P−T **6** has better electronic push−pull character than T−P **7** (the Stokes shift of the former is 295 cm^{-1} higher), which can be explained from the calculated dihedral angles (dr_2) between the phenyl and the thiophene ring of these

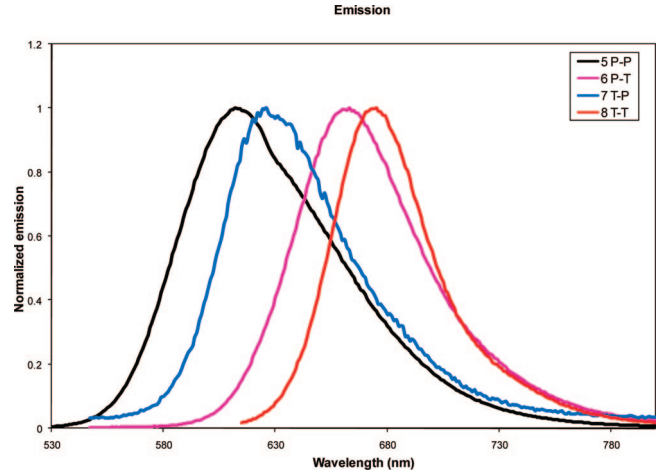


Figure 3. Normalized emission spectra of DCDHF fluorophores **5**−**8** in toluene.

two molecules (see Table 2). In the T−T **8** dye, the *trans* conformation between two thiophene rings produces little steric interaction of the hydrogens at the β,β' position; therefore, a preferred coplanar structure is possible.[31] This is also consistent with dihedral angle calculation of this molecule in Table 2. Finally, and in contrast to the addition of a third aromatic ring, dyes **24** and **25** (with an additional vinyl unit on the T−P dye **7** and the T−T dye **8** between the aromatic core and the acceptor) continue to shift the absorption to the red significantly.

Dihedral angles from both of the available X-ray results and model calculations indicate that the rings are twisted, although the calculations may not always capture the correct direction or exact magnitude. Errors in calculating values for *da* may be due to the use of a dimethylamino donor group in the calculation model, rather than the dihexyl amino group found in the actual molecules. This analysis shows that the dihedral angle between aromatic rings (dr_2) is a better indicator of the degree of charge-transfer character than that between DCDHF ring and the adjacent ring (dr_1). Although these dr_2 values for each compound from X-ray results and model calculations are different, they have the same trends, with P−P **5** having the largest dihedral angle and P−T **6** having the smallest value.

Emission Spectra. The emission maxima, Stokes shift, and quantum yield of the DCDHF dyes measured in toluene are summarized in Table 1, while Figure 3 shows the normalized emission spectra of the four Ar1−Ar2 DCDHF fluorophores **5**−**8** in toluene. The addition of an olefin to the single aromatic ring dye clearly provides a significant bathochromic shift, which is accomplished with a sacrifice of quantum yield (in solution) (Table 1). In contrast, the introduction of the second aromatic ring, particularly a thiophene ring, affords not only a red shift but also increased quantum yield (Φ_F) in toluene, compared with the original dyes with a single benzene or thiophene π-bridge. For example, the addition of a benzene ring to the single phenyl linkage provides a small bathochromic shift of 22 nm from

(30) Meier, H. *Angew. Chem.−Int. Ed.* **2005**, *44*, 2482–2506.

(31) (a) Effenberger, F.; Würthner, F. *Angew. Chem.−Int. Ed.* **1993**, *32*, 719–721. (b) Garcia, P.; Pernaut, J. M.; Hapiot, P.; Wintgens, V.; Valat, P.; Garnier, F.; Delabouglise, D. *J. Phys. Chem.* **1993**, *97*, 513–516.

486 nm for **1** to 506 nm for **5**, but this is accompanied by an order-of-magnitude increase in quantum yield (4.4% to 82%). If the second ring is a thiophene, both the absorption wavelength and quantum yield are further enhanced. The T−P **7** dye has the highest quantum yield (74%) and the T−T **8** dye has the longest absorption wavelength and offers five times the quantum yield (50%), compared to the single thiophene dye **2** (11%). The trisaromatic **21** has a longer wavelength emission maximum than any of the bisaromatic dyes (**5**−**8**), whereas its absorption maximum wavelength is less than any of the thiophene-containing dyes (**6**−**8**); this triaromatic possesses the highest Stokes shift (4380 cm^{-1}) among all the DCDHF dyes studied here. The bisaromatic-vinyl compounds **24** and **25** have longer wavelength emission maxima and higher quantum yields (7% for **24**, 13% for **25**), compared with V−P **3** (2%) and V−T **4** (2%), but the quantum yields are still much lower than their corresponding bisaromatic counterparts (**7** and **8**, respectively).

Interestingly, the P−P **5** dye has the highest Stokes shift among all the bisaromatic dyes. The Stokes shift provides information about the excited state, and it is quite possible that flattening (reduction of torsion angles) of the excited-state structure has a role in this phenomenon. In the ground state, there exists a significant torsion angle between the two phenyl rings that prevents the effective conjugation and results in a short-wavelength absorption. The very large Stokes shift is indicative of a large excited-state dipole moment and the change in electronic structure due to molecular flattening.[32] The high Stokes shift for the trisaromatic **21** may also be due to flattening in the excited state.

Solvatochromism. These Ar−Ar dye materials exhibit strong solvatochromism. Because the DCDHF dyes are donor−acceptor fluorophores, the dipole moment of the molecule is expected to increase upon excitation and charge transfer. The influence of the solvent polarity on the Stokes shift was explored with the Lippert−Mataga equation (eq 4), in which the fluorophore is modeled as a dipole located in a cavity with radius of a in a continuous solvent−dipole environment. The polarity of the solvent is approximated using the orientation polarizability Δf, which represents how easily solvent molecules rearrange around a dipole in a continuous medium.[15]

Most light-induced charge transfers further increase the charge separation in a molecule. When a charge-transfer fluorophore is excited from the ground state to the excited state, the solvent dipole can reorient around the excited-state dipole moment. This solvent relaxation stabilizes the structure and reduces the energy of the excited state, which is an effect that becomes larger as the solvent polarity is increased. Thus, the emission will be red-shifted in more-polar solvents. The Lippert−Mataga equation is

$$\bar{\nu}_A - \bar{\nu}_F = \frac{2}{hc}\Delta f\left[\frac{(\mu_E - \mu_G)^2}{a^3}\right] + \text{constant} \qquad (1)$$

where

$$\Delta f = \frac{\varepsilon_r - 1}{2\varepsilon_r + 1} - \frac{n^2 - 1}{2n^2 + 1}$$

$\bar{\nu}_A$ and $\bar{\nu}_F$ are the respective wavenumbers of the absorption and emission, and μ_G and μ_E are the ground- and excited-state dipole moments, respectively. The refractive index is given by n, ε_r is the relative low-frequency dielectric constant of the solvent, h is Planck's constant, and c is the speed of light.

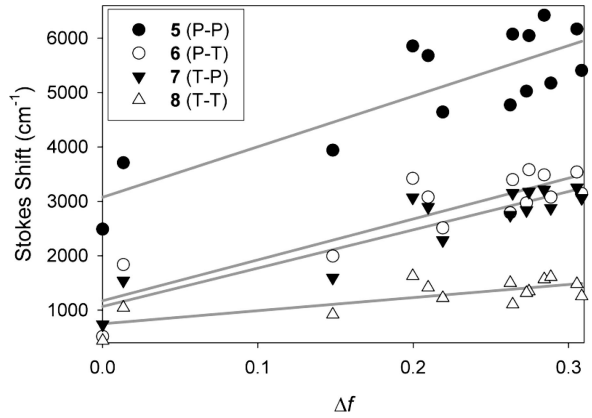


Figure 4. Lippert−Mataga plots of molecules **5**−**8**. The slopes for the fits are 9290 ($R^2 = 0.70$), 7517 ($R^2 = 0.77$), 7047 ($R^2 = 0.83$), and 2416 cm^{-1} ($R^2 = 0.56$), respectively. The steeper the slope, the greater the excited-state charge transfer and solvatochromism is exhibited.

Plots of the Stokes shift, as a function of the solvent Δf, for **5**−**8** are shown in Figure 4. The absorption and emission wavelengths of Ar1−Ar2 dyes **5**−**8** in solvents with a range of Δf (orientation polarizability) values are found in a table in the Supporting Information. The slopes for the fits are 9290, 7517, 7047, and 2416 cm^{-1}, respectively. The previously reported highest slope value for a DCDHF is 7757 cm^{-1} for DCDHF-A, which is a red-emitting fluorophore with an anthracene core.[7a] The Lippert−Mataga slope for the phenyl DCDHF (**1**) is only 1588 cm^{-1}. The steeper the slope, the greater the change in dipole moment upon photoexcitation (i.e., $\mu_E - \mu_G$) and the more sensitive the solvatochromism that is exhibited. The significant solvatochromism of the bisaromatic DCDHFs is not surprising, because the extended conjugation allows for more charge separation upon charge transfer in the excited state. Such large Stokes shifts can be usefully applied in practice, using emission filters with longer wavelength cutoff, further reducing the background. Moreover, it is possible to use the changes in emission wavelength to probe local (i.e., nanometer-scale) polarity.

Single-Molecule Imaging. In the context of optical imaging, one rigorous test of the utility of a fluorophore is its ability to be successfully imaged at the single-molecule level, which requires strong fluorescence, weak coupling with dark states, and photostability. These bisaromatic DCDHF fluorophores are strong single-molecule emitters: single copies of **6** in PMMA are easily visible in an epifluorescence image of a typical sample (see Figure 5). To better characterize the quality of these single-molecule emitters using one simple parameter, we recorded the distribution of the number of photons detected from single fluorophores before photobleaching and converted this value to the total number of

(32) (a) Klock, A. M.; Rettig, W.; Hofkens, J.; Vandamme, M.; Deschryver, F. C. *J. Photochem. Photobiol. A* **1995**, *85*, 11–21. (b) Swiatkowski, G.; Menzel, R.; Rapp, W. *J. Lumin.* **1987**, *37*, 183–189. (c) Berlman, I. B. *J. Phys. Chem.* **1970**, *74*, 3085–3093.

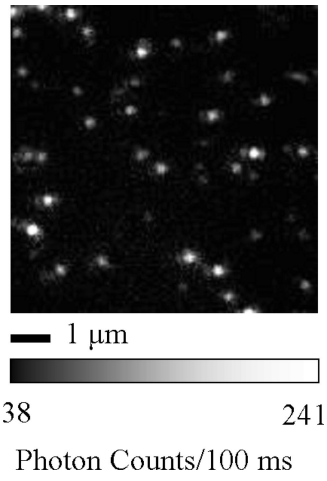


Figure 5. Epifluorescence image of single copies of DCDHF fluorophore **6** in PMMA.

photons emitted ($N_{tot,emitted}$), using the known losses in our collection system. The photon collection efficiency of our setup is

$$D = \eta_Q F_{coll} F_{opt} F_{filter}$$

which is the product of the camera quantum efficiency (η_Q), the angular collection factor determined by the objective NA (F_{coll}), the transmission factor through the microscope optics (F_{opt}), and the transmission factor through the various filters (F_{filter}). At the emission wavelengths, $\eta_Q = 85\%$ for our camera. The maximum possible F_{coll} for our setup is 38% for a single dipole emitter aligned horizontally;[33] we measured F_{opt} for our setup to be 50%, and we measured F_{filter} to be 50%−65% for the different emission ranges. After histogramming a single-molecule emitted photon distribution, a fit to one or two exponential decays yielded the average number of photons.

Values for the total number of photons detected and the total number of photons emitted ($N_{tot,emitted}$) are reported in Table 1. (Some of the $N_{tot,emitted}$ values in Table 1 are measured from bulk samples in PMMA. This measurement used bleaching curves and the rate of photon absorption to estimate the total photons emitted. The equation for this calculation can be found in ref 7a.) The $N_{tot,emitted}$ values for these derivatives are all high; in fact, some are higher than those for Rhodamine 6G (1.9×10^6 photons emitted per molecule),[13a] which is a demonstrably good single-molecule fluorophore. These red bisaromatic DCDHF emits millions of photons, without requiring rigorous removal of oxygen from the sample,[34] and therefore offer a potentially useful tool for high-resolution measurements of location or dynamics within living cells.

Conclusions

A group of new fluorescent dye materials for single-molecule imaging applications that contain a dicyanomethylenedihydrofurans (DCDHFs) acceptor group and a combination of phenyl and thiophene π-conjugation have been synthesized. The synthesis of some thiophene-containing fluorophores required the development of some improved synthetic methodology to obtain useful yields. These fluorophores with two aromatic rings providing conjugation linkage have their absorption red-shifted, generally exhibit significantly increased quantum yields, and show increased resistance to photobleaching in many cases. The thiophene−thiophene combination provided the longest absorption wavelength, whereas the phenyl−thiophene gave the best quantum yield. Single-molecule analysis reveals that bisaromatic DCDHFs include some of the brightest and longest-life emitters in this class of fluorophores studied so far. These red emitters are easily imaged and observed for minutes before photobleaching, making them attractive for applications in single-molecule biological and cellular studies.

Acknowledgment. Support from DOE (DG-FG02-04ER63777) and the Ohio Board of Regents is acknowledged. This work was supported in part by the National Institutes of Health through the NIH Roadmap for Medical Research, Grant No. P20-HG003638-04.

Supporting Information Available: X-ray data table; thermal ellipsoid plots for compounds **5**, **6**, and **21**; detailed synthesis procedures for compounds **6**, **7**, **8**, **11d**, **12c**, **14**, **21**, **23**, **25**; and standard one-dimensional (1D) ^{1}H NMR and ^{13}C NMR spectra for all new compounds mentioned in this paper. This material is available free of charge via the Internet at http://pubs.acs.org.

CM801783F

(33) Zander, C., Enderlein, J, Keller, R. A., Eds. *Single-Molecule Detection in Solution: Methods and Applications*; Wiley−VCH: Berlin, 2002.

(34) Aitken, C. E.; Marshall, A. R.; Puglisi, J. *Biophys. J.* **2008**, *94*, 1826–1835.