Rigid Hydrophilic Structures for Improved Properties of Conjugated Polymers and Nitrotyrosine Sensing in Water
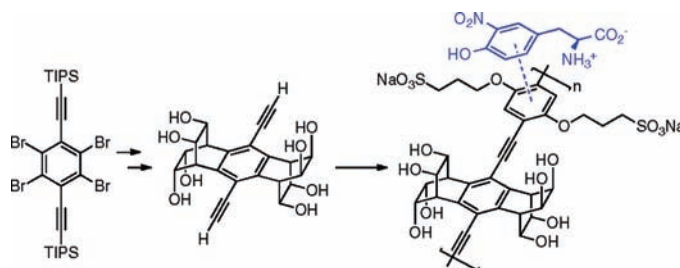
Brett VanVeller, Koji Miki, and Timothy M. Swager*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

tswager@mit.edu

ABSTRACT

The efficient synthesis of a hydrophilic monomer bearing a three-dimensional noncompliant array of hydroxyl groups is described that prevents water-driven excimer features of hydrophobic poly(*p*-phenylene ethynylene) backbones. Sensitivity of the polymer to 3-nitrotyrosine is also discussed.

Fluorescent conjugated polyelectrolytes (CPEs) are made water-soluble by pendant ionic functionalities along the polymer backbone. CPEs have seen application in a variety of chemical and biological sensing schemes[1] because the intra- and interchain migration of excitons along the polymer backbone results in the amplification of fluorescence signals relative to small-molecule fluorophores.[1a,2] However, the rigid hydrophobic main chains of CPEs are prone to interpolymer $\pi-\pi$ interactions leading to broad, ill-defined emission features and reduced luminescence.[3] Supramolecular and macromolecular approaches to mitigate these effects have been developed that effectively isolate polymer chains from each other.[4,5] However, these strategies can result in large interpolymer separation that can reduce energy transfer between chains, which is the basis of some transduction schemes.[1b]

The three-dimensional, noncompliant structure of iptycene-type monomers (**2**, Figure 1) has been shown by our laboratory to prevent strong quenching interactions between polymer chains while still promoting productive interpolymer

energy transfer necessary for amplification.[6] We hypothesized that monomer **1**, with its three-dimensional hydrophilic architecture, might impart the same properties to water-soluble polymers as **2** does in nonpolar media.
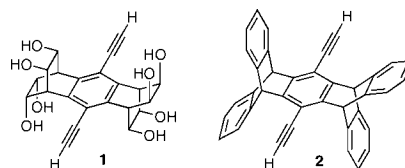

Figure 1. Three-dimensional monomers.

The synthesis of **1** began with *p*-bromanil (**3**); double addition of TIPS acetylide to **3** followed by SnCl$_2$ reductive aromatization provided **4**.[7] We envisioned that the three-dimensional carbon framework might be established in one step via a double aryne-cycloaddition

protocol.[8] In the event, addition of *n*-BuLi to dienophile precursor **4** at −45 °C produces a benzyne intermediate after initial lithium−halogen exchange. This putative dienophile underwent [4 + 2] cycloaddition with **5**[9] to provide a mixture of **6-anti** and **6-syn** cycloaddition products in a ratio of 1.7:1. The reaction was evaluated under a variety of solvent and temperature conditions (a more detailed discussion can be found in Supporting Information), but the ratio of isomers varied only slightly. The isomers were not easily separated, so the mixture was carried on to the next step; however, sufficient quantities of **6-anti** could be isolated to confirm the stereochemistry by X-ray crystallography (Scheme 1). The structure of

Scheme 1. Synthesis of the Carbon Framework
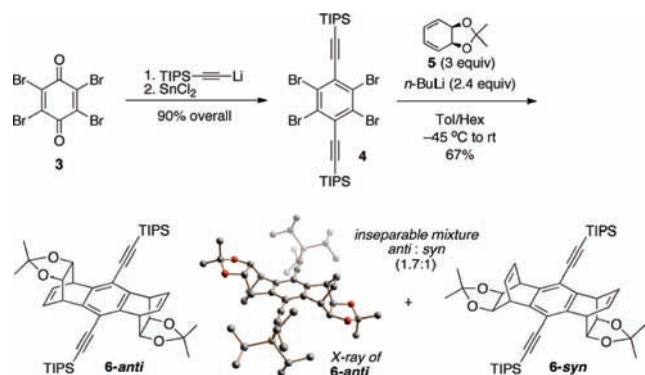
6-syn was determined from a dioxo-diosmium bridged species isolated in a subsequent step (*vide infra* Scheme

Scheme 2. Dihydroxylation
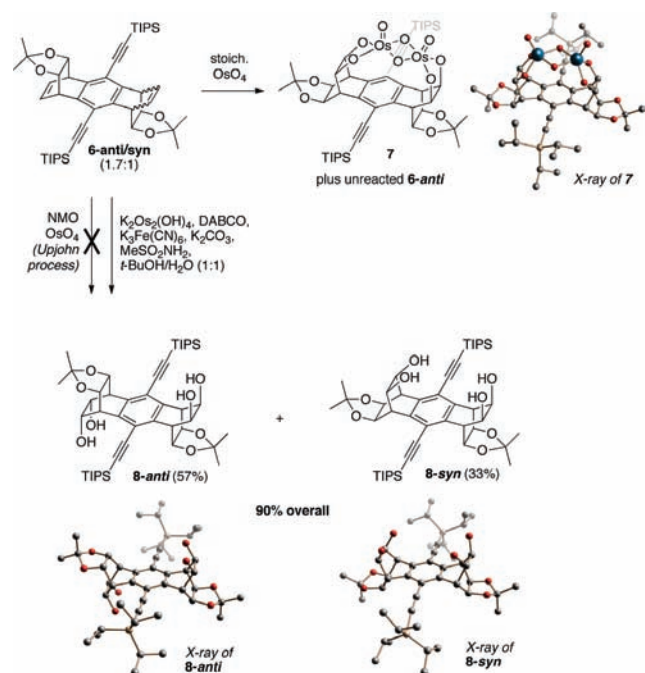
2). Despite a modest yield, this procedure creates considerable complexity in a single step. Further, this transformation can be performed on multigram scales (>5 g).

Modeling studies suggested that the double dihydroxylation of **6** would occur on the less hindered face of the alkene in **6-anti/syn** to produce the corresponding isomers **8-anti** and **8-syn** (Scheme 2). Unfortuantely, all attempts with a variety of NMO-mediated osmium tetroxide dihydroxylation conditions (Upjohn process)[10,11] showed no conversion of starting material. However, ^{1}H NMR analyses of the crude reaction mixtures revealed the amount of **6-syn** diminished relative to **6-anti** with increasing OsO_4 loadings. To investigate this observation further, a reaction using a stoichiometric amount of OsO_4 was performed, and **7** was obtained. While dioxo-diosmium type species are known,[12]

(1) (a) Thomas, S. W.; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339. (b) Satrijo, A.; Swager, T. M. *J. Am. Chem. Soc.* **2007**, *129*, 16020. (c) Wosnick, J. H.; Mello, C. M.; Swager, T. M. *J. Am. Chem. Soc.* **2005**, *127*, 3400. (d) Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10954. (e) Wang, D.; Gong, X.; Heeger, P. S.; Rininsland, F.; Bazan, G. C.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 49. (f) Wang, S.; Gaylord, B. S.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 5446. (g) Kumaraswamy, S.; Bergstedt, T.; Shi, X.; Rininsland, F.; Kushon, S.; Xia, W.; Ley, K.; Achyuthan, K.; McBranch, D.; Whitten, D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7511. (h) Phillips, R. L.; Miranda, O. R.; You, C. C.; Rotello, V. M.; Bunz, U. H. F. *Angew. Chem., Int. Ed.* **2008**, *47*, 2590. (i) You, C. C.; Miranda, O. R.; Gider, B.; Ghosh, P. S.; Kim, I. B.; Erdogan, B.; Krovi, S. A.; Bunz, U. H. F.; Rotello, V. M. *Nat. Nanotechnol.* **2007**, *2*, 318. (j) Yang, C. J.; Pinto, M.; Schanze, K.; Tan, W. *Angew. Chem., Int. Ed.* **2005**, *44*, 2572. (k) Pinto, M. R.; Schanze, K. S. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7505. (l) Xu, H.; Wu, H.; Huang, F.; Song, S.; Li, W.; Cao, Y.; Fan, C. *Nucleic Acids Res.* **2005**, *33*, e83. (m) Ho, H. A.; Najari, A.; Leclerc, M. *Acc. Chem. Res.* **2008**, *41*, 168. (n) Ho, H. A.; Boissinot, M.; Bergeron, M. G.; Corbeil, G.; Doré, K.; Boudreau, D.; Leclerc, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 1548. (o) Nilsson, K. P. R.; Inganäs, O. *Nat. Mater.* **2003**, *2*, 419. (p) Sigurdson, C. J.; Nilsson, K. P. R.; Hornemann, S.; Manco, G.; Polymenidou, M.; Schwarz, P.; Leclerc, M.; Hammarström, P.; Wüthrich, K.; Aguzzi, A. *Nat. Methods* **2007**, *4*, 1023. (q) Li, C.; Numata, M.; Takeuchi, M.; Shinkai, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 6371. (r) Feng, F.; Wang, H.; Han, L.; Wang, S. *J. Am. Chem. Soc.* **2008**, *130*, 11338. (s) Feng, F.; Tang, Y.; Wang, S.; Li, Y.; Zhu, D. *Angew. Chem., Int. Ed.* **2007**, *46*, 7882. (t) Tang, Y.; Feng, F.; He, F.; Wang, S.; Li, Y.; Zhu, D. *J. Am. Chem. Soc.* **2006**, *128*, 14972. (u) An, L.; Liu, L.; Wang, S.; Bazan, G. C. *Angew. Chem., Int. Ed.* **2009**, *48*, 4372.

(2) Swager, T. M. *Acc. Chem. Res.* **1998**, *31*, 201.

(3) (a) Tan, C.; Pinto, M. R.; Schanze, K. S. *Chem. Commun.* **2002**, 446. (b) Tan, C.; Atas, E.; Müller, J. G.; Pinto, M. R.; Kleiman, V. D.; Schanze, K. S. *J. Am. Chem. Soc.* **2004**, *126*, 13685. (c) Chen, L.; McBranch, D. W.; Wang, H. L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12287.

(4) For a review, see: Frampton, M. J.; Anderson, H. L. *Angew. Chem., Int. Ed.* **2007**, *46*, 1028.

(5) (a) Lee, K.; Cho, J. C.; DeHeck, J.; Kim, J. *Chem. Commun.* **2006**, 1983. (b) Khan, A.; Müller, S.; Hecht, S. *Chem. Commun.* **2005**, 584. (c) Jiang, D. L.; Choi, C. K.; Honda, K.; Li, W. S.; Yuzawa, T.; Aida, T. *J. Am. Chem. Soc.* **2004**, *126*, 12084. (d) Kuroda, K.; Swager, T. M. *Chem. Commun.* **2003**, 26. (e) Terao, J.; Tsuda, S.; Tanaka, Y.; Okoshi, K.; Fujihara, T.; Tsuji, Y.; Kambe, N. *J. Am. Chem. Soc.* **2009**, *131*, 16004. (f) Terao, J.; Tanaka, Y.; Tsuda, S.; Kambe, N.; Taniguchi, M.; Kawai, T.; Saeki, A.; Seki, S. *J. Am. Chem. Soc.* **2009**, *131*, 18046.

(6) Swager, T. M. *Acc. Chem. Res.* **2008**, *41*, 1181.

(7) Bowles, D. M.; Palmer, G. J.; Landis, C. A.; Scott, J. L.; Anthony, J. E. *Tetrahedron* **2001**, *57*, 3753.

(8) (a) Hart, H.; Lai, C.; Nwokogu, G. C.; Shamouilian, S. *Tetrahedron* **1987**, *43*, 5203. (b) Chen, C. L.; Sparks, S. M.; Martin, S. F. *J. Am. Chem. Soc.* **2006**, *128*, 13696.

(9) (a) Baran, A.; Seçen, H.; Balci, M. *Synthesis* **2003**, 1500. (b) Yang, N. C. C.; Chen, M. J.; Chen, P. *J. Am. Chem. Soc.* **1984**, *106*, 7310.

(10) VanRheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, *17*, 1973.

(11) Dupau, P.; Epple, R.; Thomas, A. A.; Fokin, V. V.; Sharpless, K. B. *Adv. Synth. Catal.* **2002**, *344*, 421.

the *cis*-dioxo variant **7** demonstrates remarkable stability in a variety of solvents and can be purified by silica gel chromatography. The steric bulk surrounding the osmium in this system likely prevents reoxidation by NMO, thus trapping the osmium catalyst and halting the catalytic cycle.

These observations led us to examine the Sharpless asymmetric dihydroxylation (SAD), where conditions allow for hydrolysis of the osmate ester before reoxidation.[13] After some modification of the SAD conditions (see Supporting Information), separable isomers of **8-anti** and **8-syn** were isolated in excellent yield and the geometry of dihydroxylation was confirmed by X-ray crystallography (Scheme 2).

Global deprotection of **8-anti** (see Supporting Information for **8-syn**) with BCl_3 to remove the acetonide groups followed by TBAF to remove the TIPS groups provided **1-anti** (Scheme 3). Unfortunately, homopolymerization of **1-anti**

Scheme 3. Deprotection and Polymerization
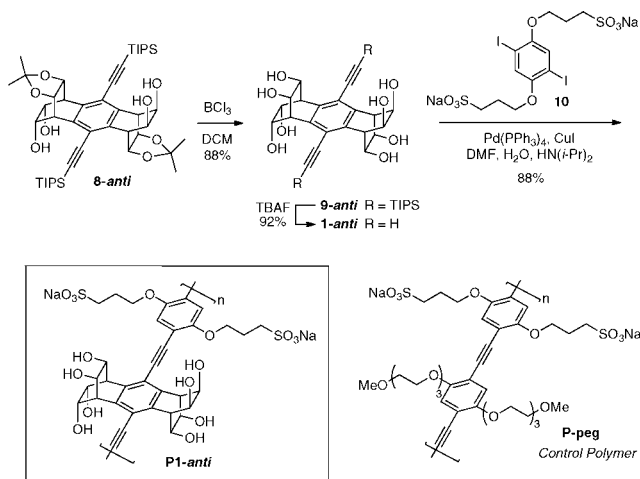
using palladium-mediated oxidative homocoupling of acetylenes[14] was not possible, and only insoluble material could be isolated.

Attempts to polymerize diol protected versions of **1-anti** and then deprotect after polymerization also failed to yield water-soluble homopolymers. These observations suggest the rigid structure of **1-anti** may not provide enough favorable entropic interactions with water to produce a soluble homopolymer. Copolymerization, however, with the sulfonated diiodide **10**[3a,15] by Sonogashira polymerization produced

high molecular weight ($M_n = 14,500$, PDI = 1.7, DP = 18) and highly water-soluble (>8 mg/mL) polymers in good yield.

The immediate effects of incorporating a monomer such as **1-anti** with its noncompliant three-dimensional architecture can be seen in Figure 2. The emission characteristics of
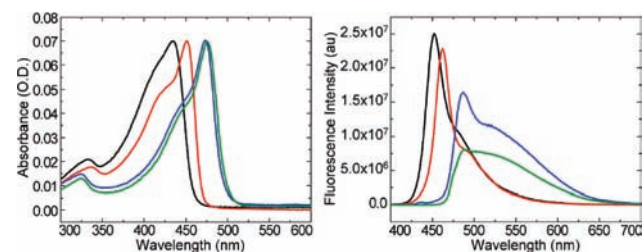
Figure 2. UV−vis absorbance and fluorescence spectra of **P1-anti** in water (black), **P1-anti** in 1X PBS (red), **P-peg** in water (blue), and **P-peg** in 1X PBS (green).

P1-anti in comparison to an analogous control polymer (**P-peg**)[5a] lacking a deaggregating structure[16] is evident by the sharper, well-defined emission features. Further, sensing of biological analytes will likely not take place in pure water but in higher ionic strength solutions, such as phosphate-buffered saline (PBS). Indeed, **P1-anti** shows the same sharp emission features in 1X PBS with a quantum yield within the error of measurement (Table 1). The control polymer,

Table 1. Peak Analysis for Figure 2

	Φ_F (%)	peak height[a]	fwhm[a,b]	ratio[c]
P1-anti, water	19	41.81	32.50	1.30
P1-anti, 1X PBS	17	36.99	24.82	1.50
P-peg, water	19	22.10	92.47	0.24
P-peg, 1X PBS	12	10.86	100.29	0.11
P1-anti, water/glycerol[d]	49	143.7	28.48	5.04
P1-anti, water/methanol[d]	50	149.9	29.51	5.08

[a] Arbitrary units. [b] fwhm = full width at half-maximum. [c] Ratio = (peak height):(fwhm). [d] At 1:1.

however, shows lower energy emission features, consistent with the formation of low-energy emissive traps arising from interpolymer $\pi-\pi$ interactions. Peak analysis data in Table 1 shows numerically what is visually represented in Figure 2. The presence of **1-anti** (**P1-anti**) produces distinctly sharper fluorescence signals with visible vibrational features. The blue-shifted absorbance and emission of **P1-anti** relative to the control is due to a lower HOMO level resulting from the absence of conjugated oxygen lone pairs in **1-anti**.

Interestingly, despite the sharper emission peaks of **P1-anti**, there is not an observed increase in quantum yield often seen

(12) (a) Phillips, F. L.; Skapski, A. C. *J. Chem. Soc., Dalton Trans.* **1975**, 2586. (b) Casey, C. P. *J. Chem. Soc., Chem. Commun.* **1983**, 126. (c) Cartwright, B. A.; Griffith, W. P.; Schröder, M.; Skapski, A. C. *J. Chem. Soc., Chem. Commun.* **1978**, 853. (d) Cleare, M. J.; Hydes, P. C.; Griffith, W. P.; Wright, M. J. *J. Chem. Soc,. Dalton Trans.* **1977**, 941. (e) Marzilli, L. G.; Hanson, B. E.; Kistenmacher, T. J.; Epps, L. A.; Stewart, R. C. *Inorg. Chem.* **1976**, *15*, 1661.

(13) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.

(14) Williams, V. E.; Swager, T. M. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 4669.

(15) Chen, W.; Joly, A. G.; Malm, J. O.; Bovin, J. O.; Wang, S. *J. Phys. Chem. B* **2003**, *107*, 6544.

(16) CPE copolymers with **2** are not readily soluble in water; see: Zheng, J.; Swager, T. M. *Chem. Commun.* **2004**, 2798.

when bulky side chains are used. This may indicate that **P1-*anti*** is still in an aggregated form. Indeed, more solubilizing water/alcohol solvent mixtures show higher quantum yields relative to pure water (Table 1), indicating a decrease in aggregation (see Supporting Information for spectra).

To further understand the nature of **P1-*anti*** in solution, Stern−Volmer quenching experiments were performed with 3-nitrotyrosine, a modified tyrosine residue associated with over 50 disease states (Figure 3).[17] A greater quenching
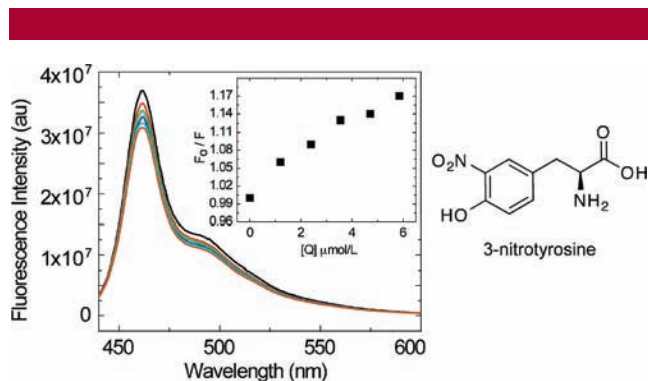

Figure 3. Fluorescence spectra for the addition of micromolar quanitites of 3-nitrotyrosine to **P1-*anti*** in 1X PBS. Stern−Volmer plot (inset), $K_{SV} = 27{,}737$.

constant can be expected for more aggregated polymers because of increased interchain exciton migration and transfer to the electron-deficient nitroaromatic. Indeed, a K_{SV} of 27,737 was found in PBS and the K_{SV} decreased for better solvents (4,774 in water and 1,232 in water/methanol),

indicating a decrease in aggreagation with better solvents. Quenching experiments performed with **P-peg** as control showed no response, presumably because the aggregated emissive states of the polymer provide lower energy states than the LUMO of 3-nitrotyrosine (see Suporting Information for spectra).

In summary, we have developed an expedient synthesis of monomer **1-*anti*** (and **1-*syn***, see Supporting Information). Further, the hydrophilic three-dimensional structure of **1-*anti*** was shown to promote spectral purity with decreased lower energy excimer emission in both water and PBS despite an aggregated state. Further interchain energy migration was shown through Stern−Volmer analysis, and work toward applying interpolymer energy transfer for further sensing applications is underway.

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

OL1001768

(17) Neumann, H.; Hazen, J. L.; Weinstein, J.; Mehl, R. A.; Chin, J. W. *J. Am. Chem. Soc.* **2008**, *130*, 4028, and references therein.