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Phosphorylated 1,6-diphenyl-1,3,5-hexatriene

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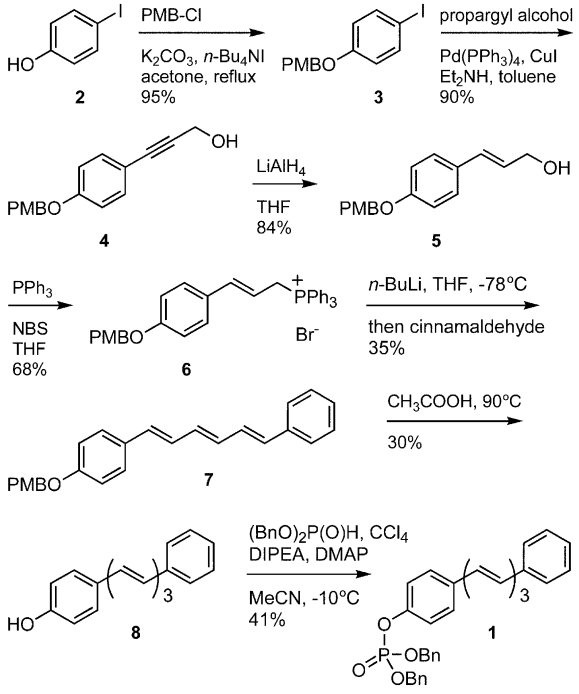
Abstract—A lipophilic dye consisting of a (1*E*,3*E*,5*E*)-1,6-diphenyl-1,3,5-hexatriene (DPH) fluorophore attached to a phosphate diester was prepared, and its fluorescence behavior in different solvent systems and in a liposomal membrane bilayer was examined. The key step in the synthesis of the functionalized end of the dye is a Sonogashira coupling of protected iodophenol with propargyl alcohol; the remaining phenyl ring and double bonds of the all-*trans* polyene core arise from a Wittig reaction with *trans*-cinnamaldehyde. Like DPH itself, the emission intensity of its phosphorylated derivative is quenched in polar media.

1. Introduction

The phosphorylation of tyrosine residues of membrane-spanning kinase enzymes, to give cytosolic phosphotyrosine (pTyr) units, is an early step in the transmission of biochemical signals from the outside to the inside of cells.[1,2] Artificial mimics of pTyr can serve to probe and/or inhibit the protein–protein recognition events that are required for cell signaling, and therefore such mimics show promise in the treatment of diseases that arise when signaling goes awry.[3–6] We reasoned that direct attachment of a phosphate moiety to one of the phenyl rings of fluorescent (1*E*,3*E*,5*E*)-1,6-diphenyl-1,3,5-hexatriene (DPH) would yield a pTyr model with the ability to report on its binding state, as the emission behavior of DPH is known to be sensitive to the polarizability of its local environment.[7,8] Furthermore, a DPH-based system would be expected to localize in lipid bilayers,[9–14] thereby modeling a key aspect of the native tyrosine kinase enzymes themselves. This paper addresses the feasibility of using DPH as a pTyr scaffold, both from a synthetic standpoint, and by quantifying the effects of phosphate substitution on the photophysical behavior of the fluorophore.

The synthesis of phosphorylated DPH **1** is shown in Scheme 1. Commercially available 4-iodophenol **2** was protected as the *p*-methoxybenzyl (PMB) ether, then the resultant iodide **3** was coupled to propargyl alcohol under Sonogashira conditions.[15] Excellent yields (>99%) for these first two steps were also achieved using *tert*-butyldimethylsilyl as the -OH protecting group.[16] Treatment of alkyne **4** with $LiAlH_4$ in THF at reflux slowly effected reduction to allylic alcohol **5**, the *E* stereochemistry of which was confirmed by ^1H NMR spectrometry ($J_{CH=CH} = 15.9$ Hz). To convert alcohol **5** to phosphonium salt **6**, the syntheses of precursor cinnamyl halides were attempted first. Both iodination (I_2,



Scheme 1.

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PPh_3, imidazole, CH_3CN) and bromination (CBr_4, PPh_3, CH_2Cl_2) gave products that were too unstable to be isolated. As an alternative, direct synthesis of **6** from **5** using *N*-bromosuccinimide (NBS) and excess PPh_3 did not require isolation of the presumed cinnamyl bromide intermediate.[17,18]

The central C=C bond of the DPH moiety was installed via a Wittig reaction[19,20] between the ylide formed from **6** and *trans*-cinnamaldehyde, giving dye **7** as a yellow solid with an intense blue fluorescence on ordinary silica gel TLC plates ($\lambda_{excit} = 365$ nm). Although yields for this step were never greater than 40%, the product was isolated in analytically pure form simply by filtering it from the reaction medium and washing to remove inorganic salts and $Ph_3P=O$. Subsequent removal of the PMB group of **7** proved to be problematic. Oxidative cleavage using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[21] in CH_2Cl_2–H_2O (20:1, v:v) at ambient temperature was incomplete after two days, while catalytic hydrogenolysis (5% Pd/C, H_2, EtOH)[22] gave only non-fluorescent products, presumably arising from hydrogenation of the triene unit. Disappearance of fluorescence was observed with other deprotection methods, as well.[23,24] Ultimately, phenol **8** was formed in 30% yield by treating **7** with excess acetic acid at 90 °C.[25] Attachment of a dibenzylphosphate ester[26] to **8** provided desired product **1** in 41% yield after column chromatography. Consistent with the structure of **1**, all but one of its ^{13}C NMR signals appear between 124 and 142 ppm, in the region expected for sp^2-hybridized carbon atoms.

The presence of the phosphate group in **1** has a modest influence on the photophysical properties of the diphenylhexatriene chromophore. As shown in Table 1, dye **1** displays three absorption peaks in benzene, dichloromethane, and methanol, with λ_{max} appearing at 362, 359, and 353 nm, respectively. Like DPH, the luminescence intensity of **1** is sensitive to solvent polarizability/polarity. When excited at 360 nm, the fluorescence quantum yield of **1** is about six times greater in C_6H_6 than in CH_3OH.[27] As expected, quenching is also observed when adding water to ethanol solutions of **1**. In 4:1 (v:v) CH_3CH_2OH–H_2O, the integrated emission intensity of **1** from 370–600 nm is approximately 15% of that observed in neat ethanol. For DPH under the same conditions, this value is 22%. When incorporated into liposomes of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) in aqueous phosphate buffer solution,[28] the electronic absorption and fluorescence emission spectra of **1** resemble those acquired in CH_2Cl_2 (Fig. 1). This suggests that the fluorophore localizes within the hydrophobic portion of the bilayer. Analogues of **1** bearing deprotected, anionic phosphate would presumably have a greater likelihood of residing near the polar head groups of the membrane.

Table 1. Photophysical data for **1**

Solvent	λ_{max} (abs.), nm	λ_{max} (em.), nm	Φ_F
Benzene	347, 362, 382	428	0.20
Dichloromethane	343, 359, 379	431	0.13
Methanol	337, 353, 372	431	0.032
DMPC/aq. phosphate[a]	343, 360, 379	429	Not determined

[a] Liposomes prepared from 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, containing 1 mol% **1**.

2. Experimental

2.1. 4-Iodo-1-[(4-methoxyphenyl)methoxy]benzene (3)

4-Iodophenol **2** (10.84 g, 49.27 mmol), K_2CO_3 (7.49 g, 54.2 mmol), and a catalytic amount of tetrabutylammonium iodide were combined in 120 mL of

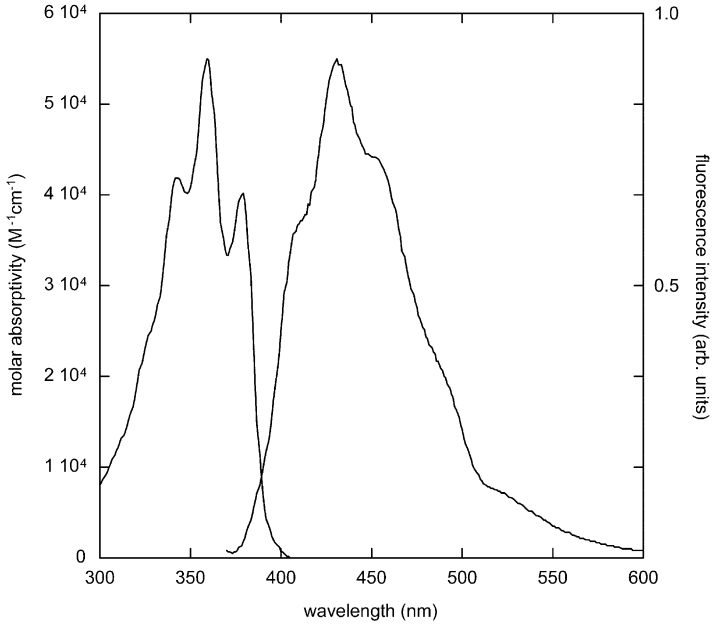

Figure 1. Absorption and emission spectra of **1** in aerated CH_2Cl_2 at 23 °C. $\lambda_{excit} = 360$ nm.

acetone. With stirring, 4-methoxybenzyl chloride (8.00 mL, 59.0 mmol) was then added dropwise at rt. After the addition was completed, the solution was heated to reflux for 48 h, during which time a white precipitate formed. The mixture was cooled to rt. The solid was collected by suction filtration, washed with water, and dried under vacuum to afford 15.89 g (95%) of **3**. Mp 138–139 °C; ^{1}H NMR ($CDCl_3$): δ 7.54 (d, 2H), 7.33 (d, 2H), 6.91 (d, 2H), 6.72 (d, 2H), 4.94 (s, 2H), 3.81 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 159.8, 158.9, 138.4, 129.4, 117.6, 114.3, 83.1, 70.1, 55.6. Anal. calcd for $C_{14}H_{13}IO_2$: C 49.43, H 3.85; found: C 49.35, H 3.87.

2.2. 3-{4-[(4-Methoxyphenyl)methoxy]phenyl}prop-2-yn-1-ol (4)

Protected iodide **3** (6.44 g, 18.9 mmol), tetrakis(triphenylphosphine)palladium(0) (0.67 g, 0.58 mmol), and copper(I) iodide (0.72 g, 3.8 mmol) were combined in 120 mL of toluene, and the mixture was stirred for 2 h at rt. The reaction flask was cooled in an ice bath, then a solution of diethylamine (13.9 mL, 134 mmol) and propargyl alcohol (5.00 mL, 85.9 mmol) was added dropwise. The reaction was stirred for 2 h and was then allowed to warm to rt for a further 10 h. Saturated aqueous NH_4Cl (100 mL) was added, the layers were separated, and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and filtered. The filtrate was evaporated, and the crude product was recrystallized from diethyl ether–hexanes (1:1, v:v) to afford 4.55 g (90%) of **4** as a light yellow solid. Mp 120–121 °C; ^{1}H NMR ($CDCl_3$): δ 7.38 (m, 4H), 6.92 (m, 4H), 4.99 (s, 2H), 4.48 (s, 2H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 159.8, 159.5, 133.1, 129.6, 128.7, 115.2, 115.1, 114.2, 86.0, 85.9, 70.1, 55.6, 51.9. Anal. calcd for $C_{17}H_{16}O_3$: C 76.10, H 6.01; found: C 75.88, H 5.90.

2.3. (2*E*)-3-{4-[(4-Methoxyphenyl)methoxy]phenyl}prop-2-en-1-ol (5)

With stirring, a solution of alkyne **4** (3.11 g, 11.6 mmol) in THF (30 mL) was added dropwise to a suspension of lithium aluminum hydride (0.88 g, 23 mmol) in 30 mL of THF at 0 °C. After 2 h, the temperature was raised to reflux for 48 h. Ice water was cautiously added, and the mixture was filtered through a pad of Celite®. The filtrate was dried over $MgSO_4$ and evaporated under reduced pressure. Recrystallization from diethyl ether-hexanes (1:1, v:v) gave 2.65 g (84%) of light yellow **5**. Mp 141–142 °C; ^{1}H NMR ($CDCl_3$): δ 7.33 (m, 4H), 6.92 (d, 4H), 6.55 (d, 1H), 6.25 (m, 1H), 4.99 (s, 2H), 4.30 (d, 2H), 3.82 (s, 3H), 1.59 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 159.7, 158.8, 131.2, 129.8, 129.4, 129.1, 127.9, 126.5, 115.2, 114.2, 70.1, 64.2, 55.5. Anal. calcd for $C_{17}H_{18}O_3$: C 75.53, H 6.71; found: C 75.63, H 6.56.

2.4. (2*E*)-3-{4-[(4-Methoxyphenyl)methoxy]phenyl}prop-2-en-1-triphenylphosphonium bromide (6)

A solution of allylic alcohol **5** (5.41 g, 20.0 mmol), triphenylphosphine (13.08 g, 49.9 mmol), and *N*-bromosuccinimide (4.26 g, 23.9 mmol) in 300 mL of THF was heated to reflux in the dark for 24 h. Upon cooling to rt, the product precipitated. It was collected by filtration and washed with ice-cold THF to afford 8.00 g (68%) of **6**. Mp 168 °C (dec); ^{1}H NMR ($CDCl_3$): δ 7.90–7.13 (m, 23H), 7.08 (d, 2H), 6.69 (d, 2H), 6.50 (dd, 1H), 5.85 (dd, 1H), 5.00 (d, 2H), 4.94 (s, 2H), 4.30 (s, 2H), 3.80 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 159.5, 159.1, 139.8, 135.0, 134.1, 130.4, 129.2, 128.8, 128.7, 127.9, 118.6, 118.0, 115.0, 114.0, 111.0, 70.0, 55.3. Anal. calcd for $C_{35}H_{32}BrO_2P{\cdot}1/3H_2O$: C 69.89, H 5.47; found: C 69.86, H 5.63.

2.5. (1*E*,3*E*,5*E*)-1-{4-[(4-Methoxyphenyl)methoxy]phenyl}-6-phenylhexa-1,3,5-triene (7)

A suspension of phosphonium salt **6** (7.76 g, 13.0 mmol) in 200 mL of THF at −78 °C was treated dropwise with *n*-BuLi (9.77 mL of a 1.6 M solution, 15.6 mmol). After 30 min, the flask was wrapped in aluminum foil to exclude light, and *trans*-cinnamaldehyde (1.64 mL, 13.0 mmol) was added dropwise. The mixture was allowed to warm to rt over 24 h. The crude product was collected by filtration and washed with water then diethyl ether. Drying under vacuum afforded 1.68 g (35%) of functionalized DPH **7** as a yellow solid. Mp 205 °C (dec); ^{1}H NMR ($CDCl_3$): δ 7.50–6.40 (m, 13H; m, 6H), 5.00 (s, 2H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 135.2, 134.4, 134.3, 134.1, 132.7, 132.6, 132.2, 130.6, 130.5, 129.5, 129.4, 128.9, 127.8, 127.6, 127.4, 126.5, 115.3, 114.2, 70.1, 55.5; UV–vis (CH_2Cl_2) λ_{max} (ε M^{-1} cm^{-1}) 352 (sh, ~52,000), 366 (63,000), 384 (45,000). Anal. calcd for $C_{26}H_{24}O_2$: C 84.75, H 6.57; found: C 84.84, H 6.46.

2.6. (1*E*,3*E*,5*E*)-4-(6-Phenylhexa-1,3,5-trienyl)phenol (8)

Compound **7** (1.96 g, 5.32 mmol) and acetic acid (350 mL) were heated to 90 °C for 72 h in the dark. The acetic acid was evaporated under reduced pressure, and the residue was partitioned between CH_2Cl_2 and water. The organic layer was washed with saturated aqueous $NaHCO_3$ then brine, and was dried over Na_2SO_4. Evaporation of the filtrate gave 0.43 g (30%) of **8** as a light yellow solid. Mp 190 °C (dec); ^{1}H NMR ($CDCl_3$): δ 7.42–6.48 (m, 9H; m, 6H); ^{13}C NMR ($CDCl_3$): δ 158.0, 137.9, 135.1, 133.5, 132.6, 132.1, 130.2, 129.4, 128.9, 128.5, 128.0, 126.9, 116.3. Anal. calcd for $C_{18}H_{16}O{\cdot}1/2H_2O$: C 84.01, H 6.66; found: C 83.76, H 6.34.

2.7. Phosphoric acid dibenzyl ester (1*E*,3*E*,5*E*)-4-(6-phenylhexa-1,3,5-trienyl)phenyl ester (1)

A solution of phenol **8** (0.20 g, 0.80 mmol) in 30 mL of CH_3CN was cooled to −10 °C in an ice/salt bath. Carbon tetrachloride (0.58 mL, 6.1 mmol) was then added. After 10 min, the stirred mixture was treated dropwise with *N,N*-diisopropylethylamine (0.46 mL, 2.7 mmol) via syringe, followed by 4-(dimethylamino)pyridine (0.0157 g, 0.13 mmol) and dibenzyl phosphite (0.42 mL, 1.9 mmol). The reaction was stirred at −10 °C for 20 h, then 0.5 M aqueous Na_2HPO_4 (10 mL) was added. The mixture was warmed to rt and extracted with EtOAc. The organic extracts were washed with water and brine, then were dried over $MgSO_4$. Filtration and evaporation of the filtrate gave the crude product, which was

purified by flash column chromatography on silica gel using CH_2Cl_2 as the eluent. A total of 0.17 g (41%) of **1** was obtained as a yellow solid. Mp 99–102 °C; ^1H NMR ($CDCl_3$): δ 7.44–6.54 (m, 25H; m, 6H), 7.33 (d, 2H), 5.14 (s, 4H); ^{13}C NMR ($CDCl_3$): δ 141.6, 139.3, 138.0, 137.4, 136.8, 135.1, 133.6, 133.0, 132.8, 132.6, 132.5, 132.1, 132.0, 131.5, 131.4, 130.2, 124.2, 124.1, 74.4; UV–vis (CH_2Cl_2) λ_{max} (ε M^{-1} cm^{-1}) 343 (41,000), 359 (55,000), 379 (40,000). Anal. calcd for $C_{32}H_{29}O_4P$: C 75.58, H 5.75; found: C 75.48, H 5.98.

2.8. Liposome preparation

A stock solution of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was prepared in absolute ethanol such that the concentration was 0.040 M. Dye **1** was also dissolved in ethanol, to a concentration of 0.0035 M. Aliquots of the lipid solution (50 μL) and dye solution (5.0 μL) were combined and slowly injected into 15 mL of stirring phosphate buffer (comprised of 0.026 M KH_2PO_4 and 0.041 M Na_2HPO_4 in water). The resulting mixture was sonicated at 40 °C for 5 min, and was used immediately. For samples used in fluorescence measurements, an electronic absorption spectrum was acquired to ensure that the OD was less than 0.1.

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