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Supplementary Material Available: ^{13}C NMR spectra of compounds **1–5** and APT spectrum of compound **3** (6 pages). Ordering information is given on any current masthead page.

Naphthyl Dioxetane Phosphates: Synthesis of Novel Substrates for Enzymatic Chemiluminescent Assays

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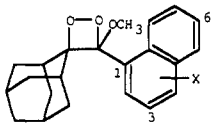
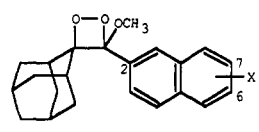
We wish to report the first synthesis of enzyme-cleavable naphthyl 1,2-dioxetane phosphates **2a–d** designed for use in bioassay systems. Although several literature accounts



2a: X=3-OPO3Na2
2b: X=6-OPO3Na2

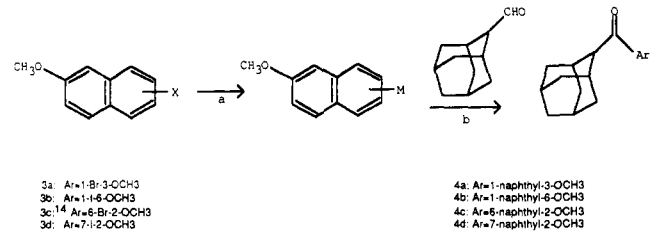


2c: X=6-OPO3Na2
2d: X=7-OPO3Na2

give chemiluminescent properties of related phenyl and xanthenyl dioxetane phosphates, at this time no corresponding syntheses have been described.[1,2] These compounds, unlike luminol, acridinium esters, and other common chemiluminescent systems,[3] operate as a direct light source in the presence of alkaline phosphatase, requiring no additional reagents for generating chemiluminescence. Recent work in our laboratory, using a similar phenyl 1,2-dioxetane phosphate (disodium 3-(4-methoxyspiro-[1,2-dioxetane-3,2′-tricyclo[3.3.1.1^{3,7}]decan]-4-yl)phenyl phosphate, AMPPD), demonstrates that incorporating these substrates into enzyme-linked immunoassays or DNA probe protocols generates a chemiluminescent signal proportional to the concentration of an alkaline phosphatase label. Amplification of the signal by efficient enzyme turnover provides very high detection sensitivity.[4] Such sensitivity offers an attractive alternative to radioisotopic methods.

A general synthesis for aryl 1,2-dioxetane phosphates must accommodate limitations in synthetic methods imposed by the inherent instability of a peroxidic bond confined to a small, four-membered ring. Consequently, it is desirable to introduce the labile dioxetane function late in the synthesis, preferably as the last synthetic step. The key intermediate, from which the dioxetane will be generated, depends on the choice of dioxetane synthesis. Dioxetanes are commonly prepared by one of two routes. The Kopecky method, in which β-bromo hydroperoxides are cyclized,[5] is limited to small-scale synthesis since the use of concentrated hydrogen peroxide is required and decomposition of the unstable hydroperoxide intermediates may occur during purification even when low temperatures are used. An alternative method depends on oxygenation of electron-rich olefins with singlet oxygen produced by photosensitization or by nonphotochemical reactions.[6] One disadvantage of the singlet oxygen approach is that other oxidative processes may compete, such as ene reactions or 2 + 4 cycloadditions. For our synthesis, we chose to photooxygenate electron-rich enol ether

Scheme I[a]

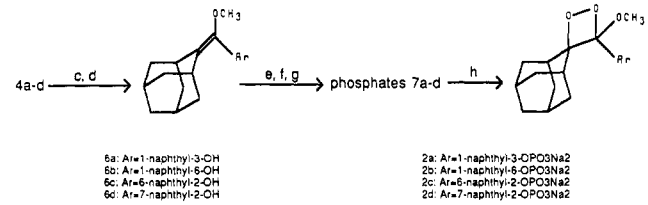


3a: Ar=1-Br-3-OCH3
3b: Ar=1-I-6-OCH3
3c:[14] Ar=6-Br-2-OCH3
3d: Ar=7-I-2-OCH3

4a: Ar=1-naphthyl-3-OCH3
4b: Ar=1-naphthyl-6-OCH3
4c: Ar=6-naphthyl-2-OCH3
4d: Ar=7-naphthyl-2-OCH3

[a] (a) nBuLi, Et_2O; (b) H^+ workup, Jones oxidation.

Scheme II[a]



5a: Ar=1-naphthyl-3-OH
5b: Ar=1-naphthyl-6-OH
5c: Ar=6-naphthyl-2-OH
5d: Ar=7-naphthyl-2-OH

2a: Ar=1-naphthyl-3-OPO3Na2
2b: Ar=1-naphthyl-6-OPO3Na2
2c: Ar=6-naphthyl-2-OPO3Na2
2d: Ar=7-naphthyl-2-OPO3Na2

[a] (c) tBuOK, $(MeO)_2SO_2$, DMSO; (d) NaSEt, DMF, reflux; (e) NaH, 2-chloro-2-oxo-1,3,2-dioxaphospholane, DMF; (f) NaCN, DMF; (g) 7 M NH_4OH, Na_2CO_3; (h) $^1O_2$, TPP, $CHCl_3$, 10 °C.

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(2) A. P. Schaap has reported photooxygenation of an aryl enol ether acetate to the corresponding 1,2-dioxetane acetate (see compound **1c**, 6-acetoxy(methoxytricyclo[3.3.1.1^{3,7}]dec-2-ylidenemethyl)-2-naphthalene in *Tetrahedron Lett.* **1987**, *28*, 935–938). Schaap also reported a similar photooxygenation of an aryl alkene phosphate to the related 1,2-dioxetane phosphate (see compound **1**, pyridinium 3-(tricyclo[3.3.1.1^{3,7}]dec-2-ylidenemethyl)-9,9′-xanthenyl phosphate in *Tetrahedron Lett.* **1987**, *28*, 1159–1162). After an extensive search, we have not found any literature examples of photooxygenation of aryl enol ether phosphates to 1,2-dioxetane phosphates or syntheses of the aryl enol ether acetate and aryl alkene phosphate.

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phosphates **7a–d**, since these intermediates are not susceptible to competing ene reactions, having only unreactive allylic bridgehead protons. Examples of photooxygenation of structurally similar compounds have been reported in the literature,[7] and preliminary model studies using a naphthyl enol ether acetate successfully yielded the desired dioxetane as the major product.[8] Furthermore, this route seemed amenable to future scale up.

We also discovered that the intermediate enol ethers are readily accessible by regiospecific O-alkylation of adamantyl aryl ketones **4a–d** in polar aprotic solvent. Classically, enol ethers are synthesized by acid-catalyzed thermolysis of acetals.[9] However, it was thought that steric hindrance from the bulky adamantyl group might significantly hamper ketalization of compounds **4a–d**, rendering thermolysis a low-yield route. Unfortunately, newer methods do not have broad application or require special reagents.[10] An enol ether approach using metal ions, e.g., the Boord reaction of β-halo acetals, is particularly unattractive since any trace metal carried through the synthesis could ultimately compromise the stability of the dioxetanes. Although alkylation of enolate anions usually leads to C-alkylation,[11] we speculated that the hindered nature of the α-adamantyl carbon of ketones **4a–d** might work to our advantage, favoring preferential O-alkylation. To our considerable satisfaction, O-alkylation of the enolate occurred almost exclusively when using potassium *tert*-butoxide and dimethyl sulfate in dimethyl sulfoxide (DMSO). Only ketone **4b** showed traces of C-alkylation.

Results and Discussion

Isomeric naphthalene dioxetanes **2a–d** were synthesized by following the general procedure described below for disodium 7-(4-methoxyspiro[1,2-dioxetane-3,2′-tricyclo-[3.3.1.1^{3,7}]decan]-4-yl)-2-naphthalenyl phosphate (**2d**) (Schemes I and II). Two possible routes offered immediate access to the carbon framework: nucleophilic attack of metalated adamantane on naphthaldehydes or, conversely, nucleophilic attack of metalated naphthalenes on adamantane-2-carboxaldehyde.[12] The latter approach appeared to be more feasible since it avoided the problematic use of adamantyllithium or adamantylmagnesium bromide. To this end, methoxynaphthyl halides, having the regiochemistry desired in the final dioxetane fluorophore, were synthesized according to literature procedures via Sandmeyer intermediates.[13] Coupling the corresponding lithiated naphthalenes with adamantane-2-carboxaldehyde proceeded in acceptable yields. For example, 2-lithio-7-methoxynaphthalene, generated from naphthalene iodide **3d**, reacted rapidly with adamantane-2-carboxaldehyde in anhydrous THF at 0 °C. Jones oxidation of the intermediate alcohol gave naphthyl ketone **4d** in 42% yield, based on the initial weight of 2-iodo-7-methoxynaphthalene. Deprotonation of **4d** in DMSO with potassium *tert*-butoxide at 50 °C, followed by addition of dimethyl sulfate, resulted in exclusive O-alkylation of the enolate to generate enol ether **5d**. ^{1}H NMR data confirmed the enol ether structure with a new methyl singlet appearing at 3.31 ppm and broad singlets for the adjacent bridgehead protons appearing at 2.69 ppm and 3.30 ppm. Subsequent demethylation of the aryl methyl ether with sodium thioethoxide in refluxing DMF cleanly yielded naphthol **6d** while preserving the acid-sensitive enol ether functionality.[15] A one-pot phosphorylation, nucleophilic ring opening, and β-elimination sequence, employing the method of Thuong and Chabrier,[16] provided the water soluble enol ether phosphate **7d**. Ion exchange with a pyridinium sulfonate resin converted the disodium phosphate salt to the more solvent-soluble monopyridinium phosphate. Photooxygenation in a homogeneous chloroform solution at 10 °C, using 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine (TPP) as a sensitizer, afforded **2d** in 69% yield after in situ reversion to the disodium salt during reverse-phase HPLC purification. Dioxetane formation was monitored by light emission from the product spot upon heating the TLC plate and confirmed by chemiluminescence of the substrate upon exposure to alkaline phosphatase. The ^{1}H NMR spectrum for **2b** showed characteristic upfield doublets at 0.68 and 0.89 ppm, which correspond to the β adamantane protons, shielded by the proximate aromatic ring. The purified dioxetane was im-

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(13) 1-Bromo-3-hydroxynaphthalene was synthesized according to the method of Newman, M. S.; Sankaran, V.; Olson, D. R. *J. Am. Chem. Soc.* **1976**, *98*, 3237–3242. 1-Amino-6-hydroxynaphthalene was synthesized by Bucherer amination of 2,5-dihydroxynaphthalene in the more reactive α position (66% yield, mp 180 °C, lit. value 186.8°, Brown, W. F.; Hebden, J. C.; Withrow, J. R. *J. Am. Chem. Soc.* **1929**, *51*, 1766–1769). The naphthol was then methylated in DMF with dimethyl sulfate, using NaH as the base, to give 91% yield of 1-amino-6-methoxynaphthalene, mp 64 °C (lit. values have been reported as 64 °C [Campbell; LaForge; Campbell *J. Org. Chem.* **1949**, *14*, 346–354] and as 74 °C [Butenandt; Schramm. *Ber.* **1935**, *68*, 2083–2091]). Spectral data for 1-amino-6-methoxynaphthalene: IR ($CHCl_3$, cm^{-1}) 3468, 3445, 3393, 3000, 1707, 1623, 1447, 1362, 1271, 1225; ^{1}H NMR ($CDCl_3$, ppm) 3.89 (3 H, s), 4.05 (2 H, br s), 6.62 (1 H, d, J = 7.03 Hz), 7.11 (2 H, m), 7.22 (1 H, d), 7.24 (1 H, d), 7.69 (1 H, d, J = 9.96 Hz). Bucherer reaction with 2,7-dihydroxynaphthalene afforded a mixture of 2-amino-7-hydroxynaphthalene (46%, mp 186–188 °C) and 2,7-diaminonaphthalene. Spectral data for 2-amino-7-hydroxynaphthalene: IR (Nujol, cm^{-1}) 3401, 3323, 3313, 1629, 1512, 1301, 1216, 883, 831; ^{1}H NMR (d_6-DMSO, ppm) 5.26 (2 H, br s), 6.585 (1 H, s), 6.64 (1 H, dd, J = 8.00, 2.33 Hz), 6.66 (1 H, dd, J = 7.96, 2.14 Hz), 6.71 (1 H, d, J = 1.96 Hz), 7.40 (1 H, d, J = 8.54 Hz), 7.42 (1 H, d, J = 8.55 Hz), 9.33 (1 H, br s); mass spectrum (EI, 70 eV), exact mass calcd for $C_{10}H_9NO$ 159.0685, found 159.0687. Methylation under standard conditions gave 2-amino-7-methoxynaphthalene in 97% yield (mp 157 °C). Spectral data: IR (Nujol, cm^{-1}) 3403, 3318, 1628, 1511, 1213, 1028, 871, 826; ^{1}H NMR ($CDCl_3$, ppm) 3.84 (2 H, br s), 3.90 (3 H, s), 6.73–7.00 (4 H, m), 7.54–7.60 (2 H); Mass spectrum (EI, 70 eV), exact mass calcd for $C_{11}H_{11}NO$ 173.0841, found 173.0841.

(14) The 6-bromo-2-methoxynaphthalene, upon conversion to the Grignard reagent in refluxing THF, reacted with 1-cyanoadamantane at reflux overnight. Acidic hydrolysis of the imine afforded the 6-naphthyl-2-methoxy ketone **4c**. See: van Leusen, A. M.; van Leusen, D. *Synth Commun.* **1978**, *8*, 397–401. Kidwell, R. L.; Murphy, M.; Darling, S. D. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, pp 918–921.

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Table I. Experimental Data for Dioxetanes and Corresponding Synthetic Intermediates

compd.	yield, %	mp, °C	IR (cm^{-1})	^1H NMR (ppm, $CDCl_3$)	^{31}P NMR[a] (ppm, D_2O)
4a	60[b]	95	1672 (C=O)	1.56–2.02 (12 H), 2.36 (2 H), 3.32 (1 H), 3.93 (3 H), 7.19–8.11 (6 H)	
4b	47[b]	97	1674 (C=O)	1.57–2.04 (12 H), 2.38 (2 H), 3.37 (1 H), 3.93 (3 H), 7.16–8.18 (6 H)	
4c	75[c]	174	1667 (C=O)	1.56–2.12 (12 H), 2.36 (2 H), 3.58 (1 H), 3.94 (3 H), 7.13–8.26 (6 H)	
4d	65[b]	116	1674 (C=O)	1.59–2.13 (12 H), 2.39 (2 H), 3.59 (1 H), 3.94 (3 H), 7.23–8.24 (6 H)	
5a	80	159		1.62–2.01 (12 H), 2.17 (1 H), 3.25 (3 H), 3.42 (1 H), 3.95 (3 H), 7.06–8.05 (6 H)	
5b	100	114		1.58–1.96 (12 H), 2.10 (1 H), 3.20 (3 H), 3.39 (1 H), 3.91 (3 H), 7.13–8.02 (6 H)	
5c	94	79		1.81–1.99 (12 H), 2.69 (1 H), 3.31 (1 H), 3.32 (3 H), 3.92 (3 H), 7.13–7.72 (6 H)	
5d		oil		1.80–1.98 (12 H), 2.69 (1 H), 3.30 (1 H), 3.31 (3 H), 3.91 (3 H), 7.10–7.72 (6 H)	
6a	100	oil	3583 (OH), 3290 (OH)	1.62–1.99 (12 H), 2.15 (1 H), 3.26 (3 H), 3.41 (1 H), 7.06–8.04 (6 H)	
6b	75	166	3595 (OH), 3300 (OH)	1.58–1.96 (12 H), 2.11 (1 H), 3.21 (3 H), 3.39 (1 H), 7.08–8.02 (6 H)	
6c	89		3590 (OH), 3310 (OH)	1.80–1.97 (12 H), 2.68 (1 H), 3.30 (1 H), 3.32 (3 H), 7.10–7.72 (6 H)	
6d	78[d]	oil	3590 (OH), 3320 (OH)	1.82–2.08 (12 H), 2.72 (1 H), 3.33 (1 H), 3.37 (3 H), 5.81 (1 H), 7.12–7.77 (6 H)	
7a	55	204		1.46–1.86 (13 H), 3.10 (1 H), 3.17 (3 H), 7.17–7.86 (6 H)[e]	0.825
7b	63	220		1.47–1.84 (12 H), 1.87 (1 H), 3.10 (1 H), 3.15 (3 H), 7.23–7.86 (6 H)[e]	1.008
7c	46	228		1.59–1.80 (12 H), 2.41 (1 H), 2.99 (1 H), 3.18 (3 H), 7.23–7.70 (6 H)[e]	1.12
7d	28	211		1.60–1.83 (12 H), 2.46 (1 H), 3.02 (1 H), 3.22 (3 H), 7.20–7.72 (6 H)[e]	0.99
2a	100			0.40 (1 H), 0.90 (1 H), 1.31–1.71 (9 H), 1.97 (1 H), 2.04 (1 H), 2.87 (1 H), 3.05 (3 H), 7.30–8.40 (6 H)[f]	1.011
2b	96			0.39 (1 H), 0.86 (1 H), 1.31–1.68 (9 H), 1.95 (2 H), 2.88 (1 H), 3.02 (3 H), 7.28–8.40 (6 H)[f]	1.051
2c	57			0.65 (1 H), 0.92 (1 H), 1.30–1.91 (10 H), 2.04 (1 H), 2.74 (1 H), 3.03 (3 H), 7.26–7.96 (6 H)[f]	
2d	69			0.68 (1 H), 0.89 (d, 1 H), 1.25–1.67 (10 H), 2.025 (1 H), 2.74 (1 H), 2.97 (3 H), 7.15–7.99 (6 H)[f]	1.229

[a] ^{31}P NMR data are relative to an 85% H_3PO_4 standard. [b] Yield is for a two-step coupling and oxidation sequence. [c] Yield is for a two-step Grignard coupling and imine hydrolysis sequence. [d] Yield is for a two-step O-alkylation and aryl demethylation sequence. [e] Solvent = D_2O. [f] NMR spectra are of a lyophilized 1:1 Na_2CO_3/dioxetane powder in D_2O.

Table II. Chemiluminescence Parameters for Naphthalene Dioxetanes

	2a (1,3)	**2b** (1,6)	**2c** (2,6)	**2d** (2,7)
emission λ_{max}[a] (nm)	488	560	463	550
$t_{1/2}$ of anion[b] (h:m:s)	00:12:00	00:01:30	00:00:09	00:23:00
detection limits for alkaline phosphatase[c]	5.0×10^{-14}M	1.5×10^{-12}M	2.0×10^{-11}M	3.0×10^{-13}M

[a] Chemiluminescent emission spectra were generated by scanning a solution of 1.2 mM dioxetane and 2.481×10^{-10}M alkaline phosphatase in 0.05 M Na_2CO_3 five times at 0.5 s/nm. [b] Anioni half-lives were calculated from light emission decay curves recorded on a Turner TD-20e luminometer using 0.05 M Na_2CO_3/1 mM $MgCl_2$ solutions of 0.004 mM dioxetane dephosphorylated with 9.26×10^{-10} M alkaline phosphatase at pH 9.5. [c] Enzyme detection experiments were performed in duplicate by injecting 300 μL of 0.4 mM dioxetane (0.05 M Na_2CO_3/1 mM $MgCl_2$, pH 9.5) into 100 μL of carbonate buffer containing alkaline phosphatase at a known concentration. Light readings were taken at 30-s intervals during steady state emission on a Berthold Clinulumat LB 952T luminometer.

mediately lyophilized and stored at −20 °C in a 1:1 Na_2CO_3/dioxetane matrix. Table I lists experimental data for all naphthyl dioxetanes and their corresponding synthetic intermediates.

Chemiluminescent properties of the products formed upon enzymatic dephosphorylation of dioxetanes **2a–d** vary substantially (Table II). Dioxetanes **2a** and **2c**, having 1,3- and 2,6-substitution, luminesced at 488 and 463 nm, respectively. In marked contrast, dioxetanes **2b** and **2d**, having 1,6- and 2,7-substitution, emitted in the green region at 560 and 550 nm. Anion half-lives, calculated from light emission decay curves upon rapid dephosphorylation in the presence of high alkaline phosphatase concentration, ranged from 9 s for **2b** to 23 min for **2d**. Chemiluminescent emission from dioxetanes **2a–d** was measured as a function of alkaline phosphatase concentration. Amounts of 10^{-15} to 10^{-17} mol of enzyme in 1 mL of solution gave easily detectable signals. Still greater sensitivity is obtained by using protein and polymeric agents for signal enhancement. Detailed results will be reported in a separate publication.

Conclusion

We have developed a general synthesis for 3-aryl-1,2-dioxetane phosphates. This method features regiospecific O-alkylation of hindered adamantyl aryl enolates to generate enol ethers. These enol ethers, bearing naphthoxy substituents, are phosphorylated and subsequently converted to 1,2-dioxetanes by photosensitized oxygenation. Upon dephosphorylation in alkaline media, the resultant naphthoate ions undergo chemiluminescent decomposition, exhibiting a range of emission wavelengths and anion stabilities.

Experimental Section

General. Commercial reagents were used as obtained without further purification. Baker silica gels (60–200 mesh for gram scale and 230–400 mesh for milligram scale) were used for flash chromatography. ^{31}P NMR spectra were reported in parts per million relative to a phosphoric acid standard. High resolution mass spectral analyses were run by J. L. Kachinski at Johns Hopkins University and elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Syntheses of naphthalene dioxetanes **2a**, **2b**, and **2c** were carried out following the procedure described above for dioxetane **2d**. Yields, melting points (uncorrected), and spectral data are summarized below for isolated intermediates.

2-Iodo-7-methoxynaphthalene (3d).[17] Diazotization of 2-amino-7-methoxynaphthalene (17.02 g, 0.098 mol) in 20% aqueous

(17) To our knowledge, the synthesis and properties of 2-iodo-7-methoxynaphthalene have not been reported in the chemical literature.

HCl (81.4 mL, 0.197 mol of HCl) proceeded upon addition of $NaNO_2$ (7.11 g, 0.103 mol) in 65 mL of H_2O at 0 °C. The reaction was stirred for 1 h, adding a small amount of EtOAc to reduce foaming as needed. A solution of K_2CO_3 (20.41 g, 0.148 mol), diethylamine (15.3 mL, 0.148 mol), and H_2O (60 mL) was added to the cooled diazonium salt to form the *N,N*-diethyltriazene.[18] After dissolving the newly formed naphthalene triazene in 30 mL of ethyl ether, followed by stirring for 35 min at low temperature, the reaction solution was poured into a separatory funnel and the aqueous layer was drawn off. The ether layer was washed with H_2O, dried over Na_2SO_4, and evaporated under reduced pressure with low heat behind a shield, giving an orange oil. The crude triazene was used immediately without purification in the next reaction.

2-(3′,3′-Diethyltriazeno)-7-methoxynaphthalene, dissolved in 120 mL dry CH_3CN, was added dropwise (1 drop/4 s) to a vigorously stirred slurry of ground KI (49.12 g, 0.296 mol), Amberlyst XN-1010 resin (37.3 g), and ground molecular sieves in dry DMF/CH_3CN (50 (50 mL/150 mL) mL) at 70 °C overnight behind a shield. Upon complete conversion of the triazene to the iodide, the cooled solution was carefully decanted into a separatory funnel, rinsing the resin with two EtOAc washes. The organic material was partitioned with EtOAc and evaporated.

The crude reaction mixture was dissolved in CH_3CN and partitioned with hexanes until all desired iodide had been removed from the CH_3CN layer. The hexanes solution was evaporated to yield, upon addition of petroleum ether, 10.36 g (33.1%) of 2-iodo-7-methoxynaphthalene as acrid orange crystals, mp 116 °C. IR ($CHCl_3$, cm^{-1}): 3005, 1625, 1504, 1458, 1394, 1359, 1250, 1176, 1126, 1034, 958, 916, 886, 841, 631. ^1H NMR (ppm): 3.90 (3 H, s), 6.99 (1 H, d, J = 2.44 Hz), 7.13 (1 H, dd, J = 8.9, 2.5 Hz), 7.47 (1 H, d, J = 8.47 Hz), 7.56 (1 H, d, J = 8.46 Hz), 7.67 (1 H, d, J = 9.0 Hz), 8.12 (1 H, s). Mass spectrum (EI, 70 eV): exact mass calcd for $C_{11}H_9IO$ 283.9698, found 283.9701.

7-Methoxy-2-(tricyclo[3.3.1.1^{3,7}]dec-2-ylhydroxymethyl)naphthalene (4d′). Lithiation of 2-iodo-7-methoxynaphthalene (1.84 g, 6.48 mmol) in 23 mL of anhydrous ether proceeded cleanly upon addition of *n*-BuLi (1.6 M, 4.85 mL) at 0 °C under argon. Dropwise addition of freshly prepared adamantane-2-carboxaldehyde (1.395 g, 8.51 mmol), dissolved in anhydrous ether (7 mL), to the naphthyllithium solution immediately yielded the coupled lithium alkoxide. After being warmed to room temperature, the reaction was quenched by partitioning between EtOAc/H_2O, followed by two EtOAc washes of the aqueous layer to completely recover the polar alcohols. The crude oil was chromatographed on silica gel, eluting with 15% EtOAc/hexanes, to give 1.69 g (81.1%) of a light yellow foam. Mass spectrum (EI, 70 eV): exact mass calcd for $C_{22}H_{26}O_2$ 322.1934, found 322.1939.

Tricyclo[3.3.1.1^{3,7}]dec-2-yl 7-Methoxynaphth-2-yl Ketone (4d). 7-Methoxy-2-(tricyclo[3.3.1.1^{3,7}]dec-2-ylhydroxymethyl)naphthalene (2.0 g, 6.21 mmol) was dissolved in 13 mL of acetone and oxidized by dropwise addition of Jones reagent (approximately 2 mL) at 0 °C until analytical TLC indicated complete conversion to the ketone. Any excess Jones reagent was quenched with 0.5 mL of 2-propanol. The reaction mixture was directly filtered through a short silica gel plug and the column was washed well with EtOAC. After the EtOAc solution was evaporated to half the volume, the reaction mixture was washed with dilute brine three times and with saturated bicarbonate solution twice. The aqueous layers were then back extracted with EtOAc. The crude ketone was purified on a silica gel column, eluting with 10% EtOAc/hexanes, to give 1.59 g (80.1%) of the desired ketone as an oil. A small amount of the oil sublimed to give, after trituration with acetone, a fine white powder, mp 116 °C. IR ($CHCl_3$, cm^{-1}): 2908, 2846, 1674 (C=O), 1629, 1605, 1466, 1272, 1257, 1179, 1132, 1126, 1105, 1035, 844. ^1H NMR (ppm): 1.59–2.13 (12 H, m), 2.39 (2 H, s), 3.59 (1 H, s), 3.94 (3 H, s), 7.23 (1 H, s), 7.245 (1 H, d, J – 3.61 Hz), 7.74 (1 H, d, J = 3.17 Hz), 7.76 (1 H, d, J = 8.30 Hz), 7.80 (1 H, d, J = 8.36 Hz), 8.24 (1 H, s). Mass spectrum (EI, 70 eV): exact mass calcd for $C_{22}H_{24}O_2$ 320.1777, found 320.1789.

7-Methoxy-2-(methoxytricyclo[3.3.1.1^{3,7}]dec-2-ylidenemethyl)naphthalene (5d). Anion formation from tricyclo[3.3.1.1^{3,7}]dec-2-yl 7-methoxynaphth-2-yl ketone (2.17 g, 6.79 mmol) occurred at 50 °C under argon in the presence of excess potassium *tert*-butoxide (1.54 g, 13.75 mmol) with dry DMSO (15 mL) as the solvent. After being stirred for 15 min, addition of dimethyl sulfate (0.65 mL, 6.88 mmol) gave O-methylation; the reaction was completed upon a second addition of 0.65 mL $(MeO_2)SO_2$. Analytical TLC indicated that a trace of starting ketone remained. The cooled solution was partitioned between CH_2Cl_2/H_2O followed by two CH_2Cl_2 washes of the aqueous layer to completely recover the enol ether. Pumping the oily solution on high vacuum with gentle warming removed most of the DMSO. The oil was then flashed through a short silica gel column, eluting with 4% triethylamine/hexanes. IR ($CHCl_3$, cm^{-1}): 2905, 2840, 1624, 1600, 1504, 1458, 1441, 1389, 1269, 1246, 1168, 1121, 1095, 1085, 1078, 1030, 840. ^1H NMR (ppm): 1.8–1.98 (12 H, m), 2.69 (1 H, br s), 3.30 (1 H, br s), 3.31 (3 H, s), 3.91 (3 H, s), 7.10 (1 H, s), 7.13 (1 H, m), 7.29 (1 H, dd, J = 8.3, 1.5 Hz), 7.64 (1 H, s), 7.71 (1 H, d), 7.72 (1 H, d, J = 8.06 Hz). Mass spectrum (EI, 70 eV): exact mass calcd for $C_{23}H_{26}O_2$ 334.1934, found 334.1937.

7-Hydroxy-2-(methoxytricyclo[3.3.1.1^{3,7}]dec-2-ylidenemethyl)naphthalene (6d). 7-Methoxy-2-(methoxytricyclo[3.3.1.1^{3,7}]dec-2-ylidenemethyl)naphthalene (2.27 g, 6.79 mmol) was added to sodium thioethoxide (1.2 equiv, 8.08 mmol) in dry DMF (4 mL), and the solution was refluxed under argon for 40 h, deprotecting the aromatic methoxy moiety to the naphthol. Excess sodium thioethoxide (1.15 equiv, 7.8 mmol) was added in three portions during the reflux to push demethylation to completion. The cooled naphthoate solution was partitioned between EtOAc and minimal H_2O. Traces of naphthol in the aqueous layer were recovered with two EtOAc washes, after salting the aqueous layer with NaCl. Purification on a silica gel column, eluting with 25% EtOAc/hexanes/4% triethylamine, yielded 1.70 g of enol ether naphthol as an oil (78.2% in two steps). IR ($CHCl_3$, cm^{-1}): 3590 (OH), 3320 (OH), 2901, 2840, 1621, 1504, 1442, 1250, 1076, 840. ^1H NMR (ppm): 1.822–2.076 (12 H, m), 2.716 (1 H, s), 3.333 (1 H, s), 3.368 (3 H, s), 5.81 (1 H, br s), 7.12 (1 H, dd, J = 8.79, 2.25 Hz), 7.19 (1 H, d, J = 2.25 Hz), 7.31 (1 H, dd, J = 8.47, 1.44 Hz), 7.645 (1 H, s), 7.745 (1 H, s), 7.766 (1 H, s). Mass spectrum (EI, 70 eV): exact mass calcd for $C_{22}H_{24}O_2$ 320.1777, found 320.1786.

Disodium 7-(Methoxytricyclo[3.3.1.1^{3,7}]dec-2-ylidenemethyl)-2-naphthalenyl Phosphate (7d). Sodium hydride (50% in mineral oil, 240 mg, 6.0 mmol) was added under an argon atmosphere to 7-hydroxy-2-(methoxytricyclo[3.3.1.1^{3,7}]dec-2-ylidenemethyl)naphthalene (1.45 g, 4.53 mmol) dissolved in sieve-dried DMF (15 mL). The solution was stirred for 10 min at room temperature to allow complete sodium naphthoxide formation and then cooled to 0 °C, at which time 0.54 mL (5.87 mmol) of 2-chloro-2-oxo-1,3,2-dioxaphospholane (Fluka) was added dropwise to the suspension. The reaction mixture was slowly warmed to room temperature over 15 min to insure formation of 2-[[7-(methoxytricyclo[3.3.1.1^{3,7}]dec-2-ylidenemethyl)-2-naphthalenyl]oxy]-1,3,2-dioxaphospholane 2-oxide. Vacuum-dried sodium cyanide (648 mg, 13.2 mmol) was added as a powder, under argon, followed by stirring at room temperature for 1.5 h to effect in situ ring opening of the cyclic phosphate ester. Upon completion of the reaction by TLC analysis (silica gel, 10% EtOAc/hexanes and 30% MeOH/EtOAc), the solvent was stripped in vacuo while being warmed very gently. The crude sodium 2-cyanomethyl 7-(methoxytricyclo[3.3.1.1^{3,7}]dec-2-ylidenemethyl)-2-naphthalenyl phosphate was dissolved in 7 M NH_4OH (10 mL) and stirred for 48 h at 40 °C. As the reaction proceeded, the product precipitated as a white gum. The aqueous solution and gum were lyophilized to a light brown powder after adding 564 mg (6.7 mmol) of $NaHCO_3$ and allowing some of the NH_3 to evaporate. The freeze-dried powder was then dissolved in acetonitrile, precipitated, and collected as flocculent, yellow crystals upon cooling. Upon reducing the filtrate volume and cooling the solution, another crop of crystals was obtained. This filtrate reduction and crystal collection cycle was repeated three more times. The dried crystal cakes were combined and purified by preparative HPLC, using a CH_3CN/H_2O gradient through a polystyrene column (PLRP-S, Polymer Laboratories). The product fractions were lyophilized to yield 572 mg (28%) of disodium 7-(methoxytricyclo[3.3.1.1^{3,7}]-dec-2-ylidenemethyl)-2-naphthalenyl phosphate as a white, fluffy powder, which decomposed at 210–213 °C. ^1H NMR (D_2O, ppm): 1.60–1.83 (12

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H, m), 2.46 (1 H, d, J = 0.97 Hz), 3.02 (1 H, br s), 3.22 (3 H, s), 7.20 (1 H, d, J = 8.43 Hz), 7.29 (1 H, d, J = 9.28 Hz), 7.51 (1 H, s), 7.65 (1 H, s), 7.72 (2 H, m). ^{31}P NMR (D_2O, 85% H_3PO_4 std, ppm): 0.99 (1 P).

Anal. Calcd for $C_{22}H_{23}Na_2PO_5 \cdot 1.5H_2O$: C, 56.06; H, 5.56; P, 6.57. Found: C, 56.11; H, 5.49; P, 6.44.

Disodium 7-(4-Methoxyspiro[1,2-dioxetane-3,2′-tricyclo-[3.3.1.1^{3,7}]decan]-4-yl)-2-naphthalenyl Phosphate (2d). A solution of disodium 7-(methoxytricyclo[3.3.1.1^{3,7}]dec-2-ylidenemethyl)-2-naphthalenyl phosphate (which was converted to the monopyridinium salt by passing the disodium phosphate as an aqueous solution through a pyridinium sulfonate Amberlite 120 plus resin followed by lyophilization [53.1 mg, 0.111 mmol]) and 5,10,15,20-tetraphenyl-21H,23H-porphine (TPP, 20 μL of a 2% solution in $CHCl_3$ by weight) in $CHCl_3$ (10 mL) was irradiated with a 250-W, high pressure sodium lamp at 10 °C while passing a stream of oxygen through the solution. A 5-mil piece of Kapton polyimide film (DuPont) placed between the lamp and the reaction mixture filtered out unwanted UV radiation. Analytical HPLC (UV detector at 230 nm) showed complete dioxetane formation upon irradiating 5 min. After evaporation of the chloroform at 0 °C, the residue was dissolved in ice water in the presence of 15 mg of Na_2CO_3 (0.14 mmol), passed through a 0.45-μm filter, and separated by preparative HPLC on a polystyrene column with an acetonitrile/0.1% Na_2CO_3 (w/v) gradient. The fractions were frozen and lyophilized at 0 °C, yielding 85.5 mg total weight of a white fluffy powder, consisting of approximately 49.0 mg of Na_2CO_3 and 36.5 mg of dioxetane (69% yield). TLC of the product exhibited blue chemiluminescence by thermal decomposition upon heating. Enzymatic cleavage of the phosphate induced decomposition with light emission at 550 nm in 0.05 M Na_2CO_3/1 mM $MgCl_2$ solutions at pH 9.5. ^{1}H NMR (D_2O, ppm): 0.681 (1 H, d), 0.890 (1 H, d), 1.25–1.67 (10 H, m), 2.025 (1 H, br s), 2.740 (1 H, br s), 2.971 (3 H, br s), 7.15 (1 H, very br s), 7.31 (1 H, d, J = 9.28 Hz), 7.525 (1 H, br s), 7.65 (2 H, d, J = 8.6 Hz), 7.99 (1 H, very br s). ^{31}P NMR (D_2O, 85% H_3PO_4 std, ppm): 1.229 (1 P).

Acknowledgment. We thank Dr. Fritz Berthold for use of the LB95T AutoClinilumat Luminometer and Dr. Kurt Loening for assistance with nomenclature.

Registry No. **2a**, 130199-37-0; **2b**, 130199-38-1; **2c**, 130199-39-2; **2d**, 124951-97-9; **3a**, 5111-34-2; **3b**, 63469-49-8; **3c**, 5111-65-9; **3d**, 66240-21-9; **4a**, 130199-29-0; **4b**, 128542-63-2; **4c**, 124955-91-5; **4d**, 128542-60-9; **4d′**, 128542-60-9; **5a**, 130219-47-5; **5b**, 130199-30-3; **5c**, 124991-65-7; **5d**, 128557-00-6; **6a**, 130199-31-4; **6b**, 130199-32-5; **6c**, 110371-06-7; **6d**, 130199-33-6; **7a**, 130199-34-7; **7b**, 130199-35-8; **7c**, 130199-36-9; **7d**, 124951-98-0; TPP, 917-23-7; 2-adamantanecarboxaldehyde, 39750-93-1; alkaline phosphatase, 9001-78-9; 2-amino-7-methoxynaphthalene, 92287-46-2; 1-cyanoadamantane, 23074-42-2.

Supplementary Material Available: IR, ^{1}H NMR, and ^{31}P NMR (where applicable) spectral data for dioxetane phosphates **2a–c** and their corresponding synthetic intermediates (6 pages). Ordering information is given on any current masthead page.

Protonation of N,N',N''-Triphenyl-1,3,5-triaminobenzenes: Stable σ-Complexes

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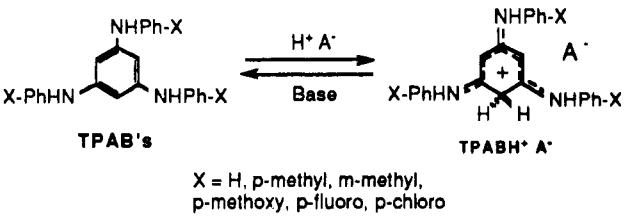
Cationic σ-complexes of aromatics are of considerable interest, most notably because of their role as intermediates in electrophilic aromatic substitution reactions.[1]

Scheme I. Protonation of TPAB's



X = H, p-methyl, m-methyl, p-methoxy, p-fluoro, p-chloro

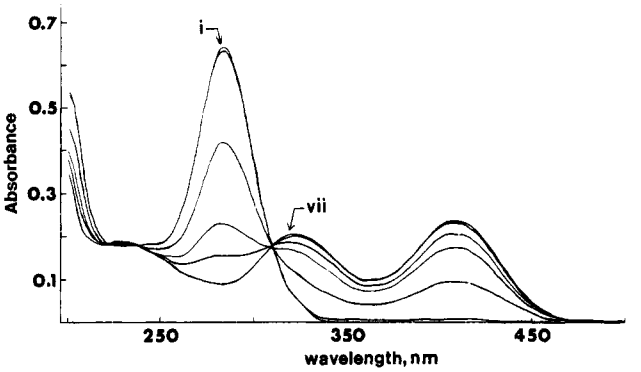


Figure 1. Spectrometric titration for nonsubstituted TPAB in 90% methanol/water at 25.0 °C, μ = 0.5 ($NaClO_4$), [TPAB] = 1.0×10^{-5} M. pH (−log [H^+]) values decrease from (i) to (vii) as follows: (i) 6.52, (ii) 4.35, (iii) 3.08, (iv) 2.38, (v) 1.76, (vi) 0.76, (vii) 0.26.

Generation of cationic σ-complexes usually takes place under severe chemical conditions and they are seldom stable enough to be isolated.[2] It has been known for some time that 1,3,5-triaminobenzene can be protonated at an aromatic carbon in solutions of intermediate pH, but instability precludes the isolation and detailed characterization of the σ-complex.[3,4] It was later shown that certain N-hexaalkylated 1,3,5-triaminobenzenes (HTAB's), the foremost of which is 1,3,5-tripyrrolidinobenzene, can form stable σ-complexes on protonation[5] and their behavior, structure, and reactions have been extensively explored.[6] Although studies of HTAB's have been fruitful, they are not very flexible with regards to introduction of functional groups that can influence the electronic structure of the molecules. It has been suggested that N-monosubstituted analogues of HTAB's will not exhibit formation of stable σ-complexes because they can easily deprotonate at nitrogen to form nonbenzenoid, tautomeric imine species.[7] However, we have recently discovered that N,N',N''-triphenyl-1,3,5-triaminobenzene[8] and its substituted analogues (TPAB's, Scheme I) undergo protonation at an aromatic ring carbon to form stable, isolable cationic σ-complexes and the pK_a's of their conjugate acids are sensitive to substituent effects.

UV–vis spectroscopy was used to investigate protonation equilibria of TPAB's in solutions of 0.5 M sodium perchlorate in 90% methanol/water.[9,10] Solutions of TPAB's

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