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General Synthesis of Persistent Trityl Radicals for EPR Imaging of Biological Systems

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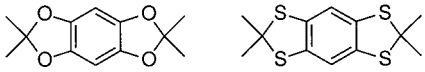
In this paper we describe the syntheses of the tetraoxygenated triarylmethyl (trityl) radical **14** and the tetrathiatriarylmethyl (trityl) radicals **15** and **16**. The syntheses include new and improved preparations of the key intermediate compounds **1** and **2**. The new route to compound **2** is noteworthy for its efficiency and its avoidance of the highly toxic compound phosgene as well as the isolation of the air-sensitive 1,2,4,5-benzenetetrathiol.

Introduction

Molecular oxygen plays a key role in cell metabolism. Diseases such as cancer, myocardial infarction, stroke, and pulmonary toxicity are closely related to abnormal concentrations of molecular oxygen in a human body. Despite its importance, it has proven difficult to conveniently measure molecular oxygen concentrations in living tissues much less map them.[1] Measuring and mapping of molecular oxygen concentrations in biological systems are important for understanding the role of oxygen and, eventually, to develop a clinical diagnostic to individuate patient treatments. Electron paramagnetic resonance (EPR) spectroscopy has been shown to be an effective method for measuring the spatial distribution of free radicals in biological systems (in vivo EPR imaging, or EPRI), in which spin traps such as nitrones are used to enhance imaging.[2] However, in vivo EPR imaging of molecular oxygen concentrations has been hampered by the lack of suitable spin labels, although some progress has been made with deuterated nitroxides[3] and with slurries of finely ground glucose chars.[4] Recently triarylmethyl (trityl) radicals have been developed as image-enhancing agents for such a purpose.[5] Shown below are the building blocks (compounds **1** and **2**)



1 **2**

required for the preparation of two related trityl radicals. The trityl radical derived from sulfur derivative **2** is reported to be more useful than that from the oxygen analogue **1** for in vivo EPR imaging. These species demonstrate great advantages such as enhanced stability in biological systems, excellent water solubility (in the cases of carboxylate derivatives), and narrow single signals in EPR.[6] Application of trityls as image-enhancing

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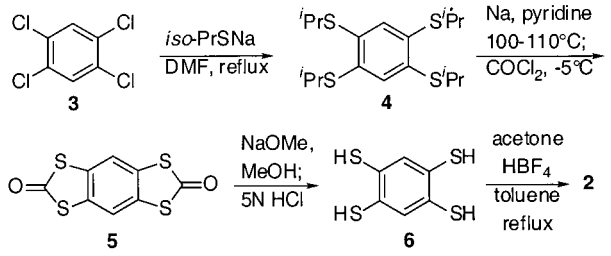
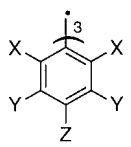
SCHEME 1



agents is still in its infancy, and further progress is limited by synthetic access to these and related compounds. The only reports on the syntheses of trityls useful for EPRI have appeared in the patent literature.[5]

In connection with a project associated with EPR studies of biological systems, we require access to trityl radicals that are persistent and water soluble and have a relatively sharp signal. These requirements are satisfied by symmetrical trityl radicals that can be represented by the general structure **A**. The syntheses of these

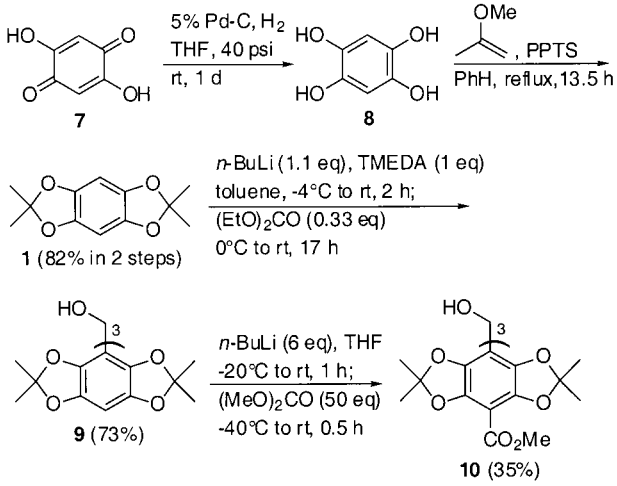


A

and related trityl radicals have been described in the patent literature by workers from Nycomed Innovation AB. These workers had recognized that placement of heteroatoms in these systems would eliminate hydrogens in close proximity to the radical species, and consequently the broadening of the EPR signal caused by coupling of the radical electron with the hydrogen nuclear spins. These heteroatom-containing trityls not only gave a sharp EPR signal, but also were more stable to oxidants and reductants in biological systems, presumably because the lone pair electrons on the heteroatom, especially sulfur, shielded the radical species.

Although the preparations of **1** and **2** have been described in the patent literature, we have found these syntheses to allow room for improvement. For example, the patented procedure for the preparation of the tetrathiaacetonide **2** requires four steps from tetrachlorobenzene **3** (Scheme 1).[5] Compound **3** is reacted with sodium isopropylthiolate[7−9] to afford tetraisopropylthiobenzene **4**. The isopropyl groups are reductively dealkylated, and the resulting thiolate then reacted with the highly toxic reagent phosgene to afford bisthiocarbonate **5**.[9] Alkaline hydrolysis of the bisthiocarbonate followed by acidic workup affords the highly air sensitive benzene-1,2,4,5-tetrathiol (**6**), which is then converted to the acetonide under acid catalysis. Tetrathiol **6** has also been prepared by conversion of **4** into 1,2,4,5-tetramethylthiobenzene followed by treatment with sodium in refluxing ammonia followed by acidic workup (three steps from **3**).[8] Finally,

SCHEME 2



a one-step synthesis of 1,2,4,5-tetramethylthiobenzene has been reported, but the procedure calls for the use of the considerably more expensive tetrafluorobenzene rather than tetrachlorobenzene **3**.[8,10] In this paper, we describe convenient and economical syntheses of the tetraoxygenated acetonide and tetrathiaacetonide, **1** and **2**, respectively. These key intermediates have been transformed to the trityl alcohols, from which were generated the trityl radicals **14**−**16**.

The initial effort in this project was directed at the synthesis of the oxygenated trityl alcohol **10** (Scheme 2).[5a] Tetrahydroxybenzene **8**[11] was easily prepared by Pd-catalyzed hydrogenation of dihydroxybenzoquinone **7**. Direct concentration of the reaction mixture, without removal of the Pd−C catalyst, afforded a quantitative yield of compound **8**, which was of sufficiently high purity for use in the next step. Treatment of crude **8** with 2-methoxypropene in the presence of PPTS catalyst under reflux in benzene afforded bisacetonide **1** in 82% overall yield for the two steps from **7**.[5a] Acetonide **1** was deprotonated by addition 1.1 equiv of *n*-BuLi and 1.1 equiv of TMEDA in toluene at −4 °C followed by allowing the solution to rise to ambient temperature (2 h total). The resultant aryl anion was treated with 0.33 equiv of diethyl carbonate at 0 °C, and then stirred at room temperature for 17 h to provide trityl alcohol **9** in 73% yield.[12] Finally, the three ester groups were introduced by treatment of **9** with 6 equiv of *n*-BuLi in THF followed by rapid addition of a large excess of dimethyl carbonate to provide **10** in 35% yield.[13]

Having completed the synthesis of the oxygenated trityl alcohol, the effort was then directed at the prepara-

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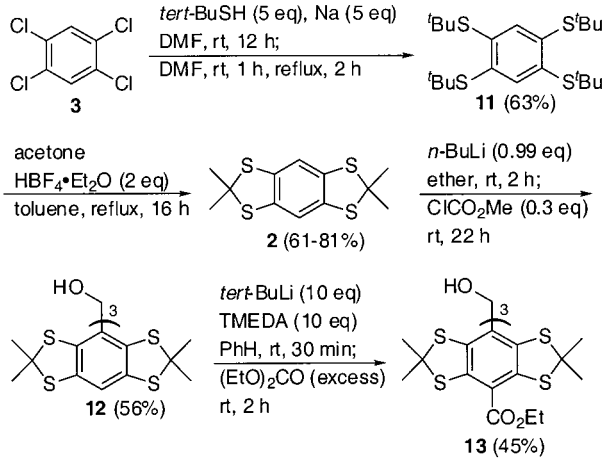
(12) The patent procedure afforded trityl alcohol **9** in the same yield (73%). See ref 5a.

SCHEME 3

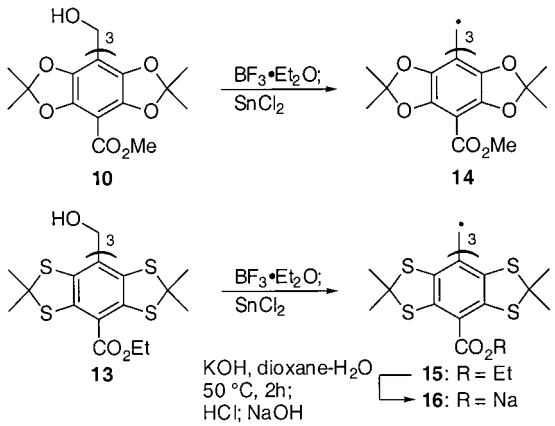


tion of the sulfur analogue, trityl alcohol **13** (Scheme 3).[5b,d] Our route to the key tetrathiaacetonide **2** envisioned the use of tetra-*tert*-butylthiobenzene **11** as an equivalent of the required tetrathiol. Treatment of tetrachlorobenzene **3** with *tert*-butylthiolate in DMF and heating the resulting mixture to reflux afforded tetra-*tert*-butylthiobenzene **11** in 63% yield. Compound **11** was then converted directly to the desired thioacetonide **2** by condensation with acetone in the presence of HBF_4. The acetonide was obtained in 81% yield by column chromatography or, even more conveniently, in 61% yield by simple trituration of the crude product. This new route to thioacetal **2** represents a considerable improvement over the reported method.[5a] The present sequence is not only shorter (by two steps), simpler, and higher yielding than the previous route, but also avoids the use of phosgene and the generation of the highly air sensitive benzenetetrathiol **6**. Treatment of **2** with 1 equiv of *n*-BuLi followed by 0.3 equiv of methyl chloroformate afforded trityl alcohol **12** in 56% yield (based on methyl chloroformate). Following the earlier method,[5b,d] compound **12** was deprotonated with *tert*-BuLi and TMEDA and the resultant anion treated with excess diethyl carbonate to afford triester **13** in 45% yield. The overall synthesis of trityl alcohol **13** requires only four steps from tetrachlorobenzene **3**.

We next examined methods for the reductive deoxygenation of the trityl alcohol intermediates to the corresponding trityl radicals (Scheme 4).[5] Radical **14** was generated by treatment of trityl alcohol **10** with $BF_3{\cdot}Et_2O$ followed by $SnCl_2$. The reaction solution turned a deep blue-green, indicating the formation of the radical. The presence of the radical **14** was confirmed using an EPR spectrometer, and the spectrum shown in Figure 1 was obtained. Trityl alcohol **13** was similarly converted to the corresponding radical **15**, and the ester group were hydrolyzed using KOH to afford the water-soluble tricarboxylate **16**. As with the oxygenated radical **14**, the thia-substituted radicals **15** and **16** were also deep green-blue in solution. Examination of the EPR spectrum of the aqueous solution of **16** showed a clean single signal

(13) Trityl alcohol **10** was prepared in two steps by formylation (*n*-BuLi and DMF) of **9** followed by MnO_2 oxidation of the formyl group in MeOH. No experimental details for the formylation reaction were described; see ref 5a.

SCHEME 4



(line width 90 mG), which confirmed the existence of trityl radical species.

In summary, we have described the syntheses of trityl radicals **14**−**16**. In the course of this work, we have developed concise and convenient syntheses of the requisite bisacetal and bisthioacetal compounds, **1** and **2**, respectively. The preparation of **2** is noteworthy as it avoids two problematic issues with the patented procedure to these compounds: (a) use of the highly toxic reagent phosgene and (b) generation of the highly air sensitive 1,2,4,5-benzenetetrathiol. Trityl radical precursors **10** and **13** were synthesized, and generation of trityl radicals **14**−**16** was confirmed by EPR spectrum.

Experimental Section

2,2,6,6-Tetramethylbenzo[1,2-*d*;4,5-*d*′]bis[1,3]dioxole (1). A mixture of 2,5-dihydroxy-1,4-benzoquinone (15.0 g, 107 mmol) and 5% Pd−C (200 mg) in THF (200 mL) was shaken in a Parr hydrogenation apparatus at room temperature under 40 psi of H_2 for 1 d. The resultant reaction mixture was concentrated in vacuo to give crude 1,2,4,5-tetrahydroxybenzene in quantitative yield. The unpurified 1,2,4,5-tertahydroxybenzene was placed in a round-bottomed flask fitted with a Dean−Stark apparatus, and 2-methoxypropene (31 mL, 0.32 mol), pyridinium *p*-toluenesulfonate (PPTS; 1.88 g, 7.5 mmol), and benzene (450 mL) were added. The mixture was heated to reflux for 3.5 h, during which time 25 mL of benzene was collected in the Dean−Stark trap. A second portion of 2-methoxypropene (25 mL, 0.26 mol) was added and heating continued for 10 h. After cooling, the reaction mixture was washed with 20% aqueous KOH (2 × 50 mL) and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product obtained was determined to be quite pure by ^{1}H NMR. Purification by recrystallization from petroleum ether gave 14.78 g (first crop, 6.78 g; second crop, 8.00 g) of the title compound as colorless to slightly yellow crystals. The mother liquid was concentrated and sublimed to give another 4.60 g of pure **1**. The total yield was 19.38 g (82%): mp 119−122 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.63 (s, 12H), 6.31 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.5, 92.8, 117.8, 140.4; IR ($CHCl_3$) 2989, 1478, 1375, 1320, 1247, 1210, 1136, 975, 872, 828, 732 cm^{-1}.

Tris(2,2,6,6-tetramethylbenzo[1,2-*d*;4,5-*d*′]bis[1,3]dioxol-4-yl)methanol (9). Method A. To a stirred, chilled (−4 °C, bath temp) solution of **1** (1.50 g, 6.75 mmol) and TMEDA (1.0 mL, 6.63 mmol) in toluene (12 mL) was added 2.6 M *n*-BuLi in hexanes (2.86 mL, 7.44 mmol). After the mixture was stirred for 30 min, the cold bath was removed, and stirring was continued for an additional 1.5 h at room temperature. The resultant suspension was cooled to 0 °C, treated dropwise with diethyl carbonate (270 μL, 2.23 mmol), then allowed to reach

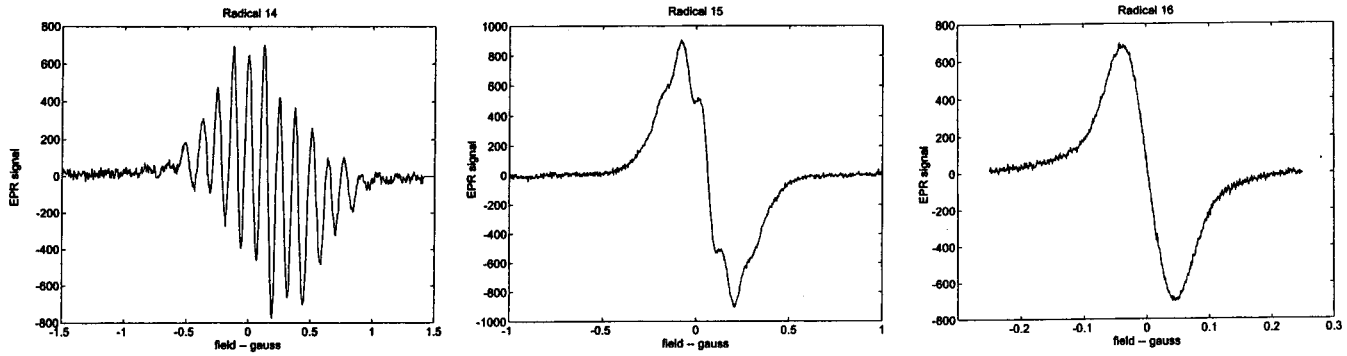


FIGURE 1. EPR spectra of trityl radicals **14**−**16**. All widths and HFS in EPR are given in gauss. Radical **14**: EPR (THF), *g* value ∼2.003, Lorentzian line width (HWHM) 0.0436979. Radical **15**: EPR (pyridine), *g* value ∼2.003, Lorentzian line width (HWHM) 0.0247558. Radical **16**: EPR (H_2O) *g* value ∼2.003, Lorentzian line width (HWHM) 0.03322091, peak to peak width 84.1 mG.

room temperature gradually, and stirred for another 17 h. The reaction mixture was poured into saturated aqueous NH_4Cl (20 mL), and the crude product was extracted with CH_2Cl_2 (2 × 50 mL). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting solid was triturated with petroleum ether (4 × 5 mL) to give 1.13 g (73%) of the title compound as a yellow powder.

Method B.[5a] To a stirred, chilled (−20 °C, bath temp) solution of **1** (5.06 g, 22.8 mmol) in THF (90 mL) was added 2.5 M *n*-BuLi in hexanes (10.0 mL, 25 mmol). The cold bath was removed, and the yellow solution was stirred at room temperature for 1 h. The solution was cooled again to −20 °C, and dimethyl carbonate (634 μL, 7.52 mmol) was added dropwise. The resulting orange solution was allowed to reach room temperature, and stirred for 8 h. The reaction mixture was poured into 2% aqueous AcOH (150 mL), and the crude product was extracted with ether (2 × 100 mL). The extracts were washed with water (2 × 30 mL), dried over Na_2SO_4, and concentrated in vacuo to afford an orange solid. Trituration of the crude product with petroleum ether gave 3.66 g (70%) of the trityl alcohol **9** as a yellow powder: mp > 220 °C (gradually turned black, dec); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.48 (s, 36H), 4.27 (s, 1H), 6.26 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.5, 72.8, 91.7, 112.7, 116.8, 139.1, 140.2; IR ($CHCl_3$) 3555, 2996, 2938, 1445, 1368, 1147, 986, 831, 736 cm^{-1}.

Tris(8-methoxycarbonyl-2,2,6,6-tetramethylbenzo[1,2-*d*;4,5-*d*′]bis[1,3]dioxol-4-yl)methanol (10). A stirred, chilled (−20 °C, bath) suspension of the trityl alcohol **9** (693 mg, 1.0 mmol) in THF (15 mL) was treated dropwise with 2.5 M *n*-BuLi in hexanes (2.4 mL, 6 mmol). The cold bath was removed, and the resulting orange solution was stirred at room temperature for 30 min. The resulting brown pasty mixture was cooled again to −40 °C, and dimethyl carbonate (4.21 mL, 50 mmol) was added in one portion. The resulting dark brown solution was allowed to reach room temperature and stirred for 30 min. Saturated aqueous KH_2PO_4 (20 mL) was added, and the crude product was extracted with ether (2 × 20 mL). The extracts were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (elution with ethyl acetate/petroleum ether, 1:1) to give 303 mg (35%) of the title compound as a yellow powder: mp > 260 °C (gradually turned black, dec); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.53 (s, 36H), 3.88 (s, 9H), 4.33 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.5, 51.8, 72.7, 99.1, 115.1, 118.3, 139.2, 140.5, 163.5; IR ($CHCl_3$) 3548, 2989, 1717, 1431, 1283, 1206, 1022, 1011, 725 cm^{-1}.

1,2,4,5-Tetra-*tert*-butylthiobenzene (11). To a stirred, degassed solution of 2-methyl-2-propanethiol (65 mL, 0.58 mol) in dry DMF (290 mL) was added small pieces of sodium (13.3 g, 0.58 mol) at 0 °C. The mixture was allowed to reach room temperature and stirred overnight. 1,2,4,5-Tetrachlorobenzene (25.0 g, 116 mmol) was added at once, and the resulting mixture was heated to a gentle reflux for 2 h. After being cooled to room temperature, the reaction mixture was poured over ice (300 g). The precipitate was removed by filtration, washed with water, and dried to give 31.3 g (63%) of the title compound as an off-white powder: mp 145−153 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 36H), 7.95 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 31.3, 48.2, 139.3, 144.7; IR ($CHCl_3$) 2952, 1453, 1416, 1158, 1111, 1052, 883, 725 cm^{-1}.

2,2,6,6-Tetramethylbenzo[1,2-*d*;4,5-*d*′]bis[1,3]dithiole (2). To a suspension of **11** (8.62 g, 20 mmol) in toluene (80 mL) was added acetone (8 mL) followed by 54% HBF_4 in ether (4.12 mL, 40 mmol) at room temperature. The mixture was stirred at room temperature for 4 h and then heated to reflux for 16 h. After being cooled to room temperature, the resulting black mixture was treated with saturated aqueous $NaHCO_3$ (80 mL), and the crude product was extracted with ether (2 × 80 mL). The extracts were dried over $MgSO_4$ and concentrated in vacuo. The main byproduct of the reaction, *p*-*tert*-butyltoluene, was also removed under high vacuum at this stage. The resulting pale yellow solid was triturated with MeOH and petroleum ether to give 3.51 g (61%) of the title compound as an off-white powder: mp 145−147 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.88 (s, 12H), 7.02 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 31.3, 65.7, 116.8, 135.7; IR ($CHCl_3$) 2993, 2960, 1449, 1364, 1324, 1166, 1089, 846, 728 cm^{-1}.

Tris(2,2,6,6-tetramethylbenzo[1,2-*d*;4,5-*d*′]bis[1,3]dithiol-4-yl)methanol (12). To a stirred suspension of **2** (2.86 g, 10 mmol) in ether (70 mL) was added 2.6 M *n*-BuLi in hexanes (3.8 mL, 9.9 mmol) dropwise at room temperature. After the mixture was stirred at room temperature for 2 h, $ClCO_2Me$ (231 μL, 3.0 mmol) was added dropwise over 40 min. The resulting orange mixture was stirred for 1.5 d. Saturated aqueous KH_2PO_4 (40 mL) was added, and the resulting mixture was stirred until the yellow precipitate disappeared. The organic layer was separated, and the aqueous layer was extracted with ether (70 mL). The combined organic layers were dried over $MgSO_4$, and concentrated in vacuo. The orange residue was crystallized with 5% MeCN in THF (5 mL) to give a yellow powder containing THF. The yellow powder was dissolved in benzene and concentrated in vacuo to give 1.61 g (56%) of the title compound as a yellow powder that was a ca. 1:1 mixture of **12**/benzene: mp > 290 °C (gradually turned black, dec); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.68 (s, 9H), 1.72 (s, 9H), 1.80 (s, 9H), 1.82 (s, 9H), 6.23 (s, 1H), 7.17 (s, 3H), 7.36 (s, ca. 6H, benzene); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.6, 29.1, 32.2, 34.8, 63.3, 64.0, 83.6, 118.1, 128.3 (benzene), 131.8, 137.2, 137.8, 138.3, 139.2; IR ($CHCl_3$) 3360, 2960, 2923, 1453, 1361, 1144, 728 cm^{-1}.

Tris(8-methoxycarbonyl-2,2,6,6-tetramethylbenzo[1,2-*d*;4,5-*d*′]bis[1,3]dithiol-4-yl)methanol (13).[5b,d] To a stirred solution of **12** (193 mg, 0.2 mmol) and TMEDA (302 μL, 2.0 mmol) in benzene (2.4 mL) was added 1.7 M *n*-BuLi in pentane

(1.18 mL, 2.0 mmol) dropwise at room temperature at 5−10 °C. After being stirred for 30 min, the resultant solution was added to a solution of diethyl carbonate (1.26 mL, 10.4 mmol) in benzene (1.2 mL) that was maintained at 5−10 °C. After the mixture was stirred for 2 h, saturated aqueous KH_2PO_4 was added. The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The red residue was crystallized from MeCN (4 mL) to give 98 mg (45%) of the title compound as orange crystals that contained a small amount of inseparable impurities: mp > 270 °C (gradually turned black, dec); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.46 (t, J = 7 Hz, 9H), 1.66 (s, 18H), 1.75 (s, 9H), 1.77 (s, 9H), 4.44 (m, 6H), 6.78 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 28.6, 29.2, 31.8, 33.8, 60.8, 60.9, 62.3, 84.3, 121.3, 133.9, 139.2, 140.3, 141.4, 141.8, 166.1; IR ($CHCl_3$) 3335, 2970, 1699, 1239, 1217, 1018, 728 cm^{-1}.

Generation of Tris(8-methoxycarbonyl-2,2,6,6-tetramethylbenzo[1,2-*d*;4,5-*d*′]bis[1,3]dioxol-4-yl)methyl (14). To a stirred solution of **10** (17 mg, 0.02 mmol) in CH_2Cl_2 (4 mL) was added BF_3·Et_2O (20 μL, 0.16 mmol) dropwise at room temperature. After being stirred for 30 min, the resulting dark red solution was treated with a solution of $SnCl_2$ (6.4 mg, 0.034 mmol) in THF (0.8 mL). The mixture turned dark green-blue. After 10 min, saturated aqueous KH_2PO_4 was added. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo to give 15.2 mg of the crude trityl radical as a dark green-blue solid. The crude trityl radical was dissolved in THF, and EPR spectrum was measured.

Generation of Tris(8-carboxyl-2,2,6,6-tetramethylbenzo-[1,2-*d*;4,5-*d*′]bis[1,3]dithiol-4-yl)methyl Sodium Salt (16). To a stirred solution of **13** (22 mg, 0.02 mmol) in CH_2Cl_2 (4 mL) was added BF_3·Et_2O (20 μL, 0.16 mmol) dropwise at room temperature. After being stirred for 1 h, the resulting dark green-blue solution was treated with a solution of $SnCl_2$ (6.4 mg, 0.034 mmol) in THF (0.8 mL). After 10 min, saturated aqueous KH_2PO_4 was added. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo to give 21 mg of the crude trityl radical **15** as an orange-brown solid. The crude solid was dissolved in dioxane (0.4 mL), and 1 M aqueous KOH (0.2 mL) was added. The resultant dark orange-brown solution was heated at 50 °C for 2 h. After being cooled to room temperature, the reaction mixture was diluted with water, and washed twice with ether. The aqueous layer was acidified with 1 M HCl, and the resultant orange brown precipitate was extracted with ether. The ether layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in 0.1 M aqueous NaOH (0.6 mL), and concentrated in vacuo to give 18.7 mg of the title compound as a dark green-yellow solid. The crude trityl radical was dissolved in water, and the EPR spectrum was measured: line width 84 mG.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra (compounds **1**, **2**, **9**−**13**) and EPR spectra (compounds **14**−**16**). This material is available free of charge via the Internet at http://pubs.acs.org.

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