Electronic Supplementary Information

Synthesis of hydroxyethyl tetrathiatriarylmethyl radicals OX063 and OX071

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1. General Information

HPLC analyses were performed on a Waters Alliance e2695 separation module, equipped with a 2998 PDA detector and a SOD2 Mass Detector. Separations were carried out using a Waters XBridge BEH C18 4.6 mm x 50 mm, 2.5 µm column. NMR spectra were recorded on a Jeol ECZ 400S NMR spectrometer and data was processed with MestReNova 14. EPR spectra were recorded using an X-band ELEXSYS E580 EPR spectrometer. EPR acquisition parameters were as follows: microwave power, 0.15 mW; modulation amplitude, 0.12 G for OX063 and 0.07 G for OX071; modulation frequency, 30 kHz; sweep width, 3 G; sweep time, 30.72 s; conversion time, 30.00 ms, number of points, 1024. Teflon tubes with a diameter of 1.14 mm and wall thickness of 60 µm (Zeus, Inc., USA) were filled with 50 µL of a 50 µM solution of trityl and nitrogen was flushed to remove oxygen. HRMS spectra were recorded using a Thermofisher Scientific Q Exactive Mass Spectrometer with an Electron Spray Ionization (ESI) source. Purifications were carried out on a Teledyne CombiFlash Rf+ purificator. All solvents were purchased from Fisher Scientific. All commercially available reagents were used as received without further purification. 1,2,4,5-Tetratert-butylthiobenzene was purchased from Atomax (China). Isobutene 15% v/v was purchased from TCI. THF and DMF were purified on an Inert Pure Solv Solvent Purification system from Innovative Technologies, Inc. All reactions were carried out in flame-dried glassware and with degassed and anhydrous solvents. Cryogenic conditions were maintained using a Julabo FT 901 immersion cooler.

2. Procedures



Benzo[1,2-d:4,5-d']bis(l,3)dithiole-2,2,6,6-tetraacetic acid methyl ester (2)

1,2,4,5-Tetra-*tert*-butylthiobenzene **1** (123 g, 0.286 mol) was dissolved in 3 L of toluene in a flamedried round-bottom flask. Methyl acetonedicarboxylate (200 g, 1.14 mol, 4 eq.) was added and the mixture was flushed with argon. HBF₄.Et₂O (54% w/w, 390 mL, 2.85 mol, 10 eq.) was added and the reaction was stirred vigorously overnight at room temperature. The yellow and heterogeneous solution was filtered and the solid **2** washed with 300 mL methanol until it became white. After drying under vacuum, 114 g of **2** was obtained as a white powder (77% yield).

Note: this is important to maintain a vigorous stirring of the biphasic mixture throughout the reaction.

¹H NMR (400 MHz, CDCl₃) δ (ppm): 3.50 (s, 8H), 3.69 (s, 12H), 6.96 (s, 2H). ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 43.4, 52.1, 65.0, 116.3, 134.7, 170.3. HRMS (ESI) calcd for $[C_{20}H_{22}O_8S_4+H]^+$ 519.0276 m/z, found 519.0226 m/z.

Benzo[1,2-d:4,5-d']bis(l,3)dithiole-2,2,6,6-tetraacetic-2-d2-acid methyl ester (2b)



A flame-dried flask was charged with 80 mL anhydrous THF and equipped with a condenser. 134 mL of CH₃OD were added then 26.8 g of tetraester **2** (51.7 mmol) were added. Metallic sodium (594 mg, 25.8 mmol, 0.5 eq.) was added and the reaction was heated to reflux overnight. The solvent was removed under reduced pressure then the solid was dissolved in 100 mL of dichloromethane and washed with 100 mL water. The organic phase was dried over MgSO₄ and the solvent evaporated under reduced pressure to afford 23.1 g of the title compound as a white solid with 85% yield. From ¹H NMR, the deuteration percentage was determined to be 80-85%.

¹H NMR (400 MHz, CDCl₃) δ (ppm): 3.70 (s, 12H), 6.98 (s, 2H).
¹³C NMR (100 MHz, CDCl₃) δ (ppm): 43.4, 52.0, 64.6, 64.7, 64.7, 64.9, 116.2, 134.6, 170.1.
HRMS (ESI) calcd for [C₂₀H₁₄D₈O₈S₄]⁺ 526.0700 m/z, found 526.0710 m/z.

2,2,6,6-Tetra(hydroxyethyl)benzo[1,2-d:4,5-d']bis(1,3)dithiole (3) 2,2,6,6-Tetra(2-(1-hydroxy-2,2-d2-ethyl))benzo[1,2-d:4,5-d']bis(1,3)dithiole (3b)



Tetraester 2 (100 g, 0.193 mol) was dissolved in 4 L of anhydrous THF. LiAlH₄ (33 g, 0.869 mol, 4.5 eq.) was added by portions to avoid excessive heating of the reaction (less than 60°C). The reaction was stirred overnight at room temperature and then carefully quenched with 100 mL of methanol. 1 L of methanol, followed by 400 mL of water and 1200 mL of methanol, were added successively. The solution was filtered and the remaining solid was washed with 1 L of methanol. The combined organic phases were evaporated, then the solid was dissolved in 1 L of 1M hydrochloric acid and heated to 100°C until all the big chunks of solid disaggregate. The solution was cooled down in an ice bath and filtered. The solid was washed successively with 3x200 mL of water and then 2x200 mL of cold methanol. The remaining white solid was dried under vacuum to afford 46 g of **3** (58% yield).

¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 2.23 (t, *J*=6.8 Hz, 8H), 3.58 (t, *J*=6.8 Hz, 8H), 4.70 (s, 4H), 7.20 (s, 2H). ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 43.1, 58.0, 70.6, 116.0, 135.1. HRMS (ESI) calcd for $[C_{16}H_{22}O_4S_4+H]^+$ 407.0474 m/z, found 407.0500 m/z.

3b was synthesized using the same procedure, with a 62% yield.

¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 3.57 (s, 8H), 4.47 (broad s, 4H), 7.20 (s, 2H).
¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 57.8, 70.3, 70.3, 116.0, 135.0.
HRMS (ESI) calcd for [C₁₆H₁₄D₈O₄S₄+H]⁺ 415.0976 m/z, found 415.1017 m/z.

2,2,6,6-Tetra(t-butoxyethyl)benzo[1,2-d:4,5-d']bis(1,3)dithiole (4) 2,2,6,6-Tetra(2-(1-t-butoxy-2,2-d₂-ethyl))benzo[1,2-d:4,5-d']bis(1,3)dithiole (4b)



The tetraalcohol **3** (9 g, 22.2 mmol) was suspended in 500 mL of an isobutene solution in THF (15% v/v from TCI) in a 1 L flame-dried flask. Triflic acid (5.9 mL, 66.2 mmol, 3 eq.) was added until all solids were dissolved (ca. 20 min) and the solution turned from pale yellow to light pink. An additional 2.9 mL triflic acid (33.1 mmol, 1.5 eq.) was added. The reaction was stirred for 1h30 at room temperature. Solid sodium bicarbonate was slowly added to the solution until the end of formation of CO₂ bubbles, leading to a light orange solution. The solids were filtered off from the solution and the solution was evaporated under reduced pressure. Flash chromatography using 2-10% ethyl acetate in hexanes afforded 9.8 g of the title compound as a white solid (70% yield). *Note: it is important to quench the reaction after max. 2h in order to limit the formation of polyisobutene, which could strongly decrease the flow rate during chromatography.*

¹H NMR (400 MHz, CDCl₃) δ (ppm): 1.17 (s, 36H), 2.33 (t, *J*=6.8 Hz, 8H), 3.56 (t, *J*=6.8 Hz, 8H), 6.95 (s, 2H).
¹³C NMR (100 MHz, CDCl₃) δ (ppm): 27.6, 41.2, 58.7, 71.5, 73.2, 116.3, 135.4.
HRMS (ESI) calcd for [C₃₂H₅₄O₄S₄]⁺ 630.2905 m/z, found 630.2971 m/z.

4b was synthesized using the same procedure, with a 64% yield.

¹**H** NMR (400 MHz, CDCl₃) δ (ppm): 1.17 (s, 36H), 3.55 (s, 8H), 6.95 (s, 2H). ¹³**C** NMR (100 MHz, CDCl₃) δ (ppm): 27.6, 58.6, 56.7, 73.2, 116.3, 135.4. HRMS (ESI) calcd for $[C_{32}H_{46}D_8O_4S_4]^+$ 638.3407 m/z, found 638.3381 m/z.

2,2,6,6-Tetra(t-butoxyethyl)-4-iodo-benzo[1,2-d:4,5-d']bis(1,3)dithiole (5) 2,2,6,6-Tetra(2-(1-t-butoxy-2,2-d2-ethyl))-4-iodo-benzo[1,2-d:4,5-d']bis(1,3)dithiole (5b)



2,2,6,6-Tetramethylpiperidine (6.75 mL, 39.7 mmol, 2.5 eq.) was dissolved in 60 mL of dry degassed THF and cooled down to -50° C. A solution of *n*-BuLi (15.8 mL, 2.5M, 39.7 mmol, 2.5 eq.) was added. The mixture was stirred 10 minutes at -50° C and 10 minutes at room temperature. The aryl **4** (10 g, 15.87 mmol) was dissolved in 300 mL of dry THF and cooled down to -78° C. The formed LiTMP solution was transferred using a cannula into the compound **4** solution and the reaction was stirred 3h at -78° C. Iodine (10 g, 39.7 mmol, 2.5 eq.) was added, the resulting solution was brought to room temperature and stirred for one hour, turning dark brown to dark red. The reaction was quenched by 200 mL of a saturated solution Na₂S₂O₃ and the reaction was stirred 10 min until the dark color disappeared. The aryl iodide **5** was extracted twice with 200 mL of ethyl acetate. The combined organic phases were dried over MgSO₄ and evaporated under reduced pressure. Flash chromatography using 0-10% ethyl acetate in hexanes afforded 9.1 g of pure iodide **5** as a yellow thick oil (75% yield).

Note: over time, the thick oil may slowly crystallize.

¹**H NMR (400 MHz, CDCl₃) δ (ppm)**: 1.18 (s, 36H), 2.34 (t, *J*=6.8 Hz, 8H), 3.56 (t, *J*=6.8 Hz, 8H), 6.86 (s, 2H).

¹³C NMR (100 MHz, CDCl₃) δ (ppm): 27.6, 41.5, 58.6, 68.8, 73.2, 81.6, 115.5, 132.9, 141.5. HRMS (ESI) calcd for [C₃₂H₅₃IO₄S₄]⁺ 756.1871 m/z, found 756.1913 m/z. **5b** was synthesized in a similar way, with a 98% yield.

¹H NMR (400 MHz, CDCl₃) δ (ppm): 1.15 (s, 36H), 3.53 (s, 8H), 6.84 (s, 2H).
 ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 27.6, 58.3, 73.1, 81.6, 115.4, 132.8, 141.5.
 HRMS (ESI) calcd for [C₃₂H₄₅D₈IO₄S₄]⁺ 764.2373 m/z, found 764.2352 m/z.

 $Tris(2,2,6,6-tetra(t-butoxyethyl)benzo[1,2-d:4,5-d']bis(1,3)dithio-4-yl)methanol (6) Tris(2,2,6,6-tetra(2-(1-t-butoxy-2,2-d_2-ethyl))benzo[1,2-d:4,5-d']bis(1,3)dithio-4-yl)methanol (6b)$



The aryl iodide **5** (5 g, 6.61 mmol) was dissolved in 200 mL of <u>degassed</u>, anhydrous *n*-hexane. The solution was cooled to -78° C then a solution of *sec*-BuLi (1.4M, 5.66 mL, 1.2 eq.) was added and stirred 15 minutes at the same temperature, then brought to room temperature, leading to a light yellow and heterogeneous solution. A solution of diethyl carbonate (257 µL, 2.18 mmol, 0.33 eq.) in 5 mL anhydrous *n*-hexane was added dropwise over 3h using a syringe pump, turning the cloudy solution into a yellow-orange solution. After stirring overnight, reaction was quenched with 50 mL of a 1M solution of ammonium chloride. The product was extracted twice with 100 mL of dichloromethane. The combined organic phases were dried over MgSO₄ and evaporated under reduced pressure. Flash chromatography using 0-10% ethyl acetate in hexanes afforded 3.37 g of the pure trityl alcohol **6** as a yellow foam (80% yield).

Notes: during the addition of the carbonate, the solution turns green then yellow. The orange color indicates the presence of the diaryl ketone.

¹H NMR (400 MHz, CDCl₃) δ (ppm): 1.02 (s, 27H), 1.10 (s, 27H), 1.14 (s, 27H), 1.16 (s, 27H), 2.11-2.45 (m, 24H), 3.33-3.54 (m, 24H), 6.55 (s, 1H), 7.05 (s, 3H).
¹³C NMR (100 MHz, CDCl₃) δ (ppm): 27.5, 27.6, 38.5, 39.0, 40.1, 42.9, 58.1, 58.2, 58.9, 59.5, 69.0, 70.6, 72.7, 72.8, 73.0, 73.2, 83.5, 117.2, 132.2, 137.4, 137.5, 137.6, 138.8.
HRMS (ESI) calcd for [C₉₇H₁₆₀O₁₃S₁₂]⁻ 1916.8507 m/z, found 1916.8442 m/z.

6b was synthesized in a similar way, with a 71% yield.

¹H NMR (400 MHz, CDCl₃) δ (ppm): 1.04 (s, 27H), 1.11 (s, 27H), 1.15 (s, 27H), 1.17 (s, 27H), 3.33-3.54 (m, 24H), 6.55 (s, 1H), 7.06 (s, 3H).
¹³C NMR (100 MHz, CDCl₃) δ (ppm): 27.6, 27.7, 58.0, 58.1, 58.8, 59.4, 68.7, 68.7, 70.3, 72.7, 72.8, 73.0, 73.1, 73.2, 83.6, 117.3, 132.2, 137.4, 137.6, 137.6, 138.8.
HRMS (ESI) calcd for [C₉₇H₁₃₆D₂₄O₁₃S₁₂]⁻ 1941.0014 m/z, found 1940.9824 m/z.

 $Tris(8-methoxycarbonyl-2,2,6,6-Tetra(t-butoxyethyl)benzo[1,2-d:4,5-d']bis(1,3)dithio-4-yl)methanol (7) Tris(8-methoxycarbonyl-2,2,6,6-Tetra(2-(1-t-butoxy-2,2-d_2-ethyl))benzo[1,2-d:4,5-d']bis(1,3)dithio-4-yl)methanol (7b)$



The trityl alcohol **6** (100 mg, 0.052 mmol) was dissolved in 1 mL of <u>degassed</u>, anhydrous TMEDA (ca. 0.1M) then cooled down to -30°C. *sec*-BuLi (1.4M, 0.58 mL, 15 eq.) was added and the solution was stirred 2 hours at -30°C. The color of the solution turned yellow to green when adding the base and slowly turned brown over the 2 hours. The mixture was diluted with 1 mL of anhydrous TMEDA, then CO₂ was bubbled through the solution for 30 min at -30°C and for an additional 30 min at room temperature, the solution slowly turned orange. HPLC monitoring shows the conversion of the starting material to a mixture of diacid and triacid in the 3 to 7 ratio. Solvents were evaporated under reduced pressure, then 10 mL of diethyl ether and 10 mL of 1M HCl were added. The layers were separated and the organic phase was dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was dissolved in 2 mL anhydrous DMF, iodomethane (19.5 μ L, 0.312 mmol, 6 eq.) and anhydrous Na₂CO₃ (100 mg) were added. The reaction was stirred for an hour at 50°C. 10 mL of ethyl acetate were added and the organic phase was dried over MgSO₄ and the solvent was removed under reduced pressure. Separation of the diester and the triester was carried out by flash chromatography using 0-20% ethyl acetate in hexanes to afford 65 mg of the triester **7** (60% yield).

¹**H NMR (400 MHz, CDCl₃) δ (ppm):** 1.01 (s, 27H), 1.11 (s, 54H), 1.17 (s, 27H), 2.13-2.38 (m), 3.26-3.56 (m, 24H), 3.88 (s, 9H), 7.07 (s, 1H).

¹³C NMR (100 MHz, CDCl₃) δ (ppm): 27.5, 27.6, 27.6, 27.7, 29.8, 38.6, 39.1, 40.3, 42.8, 52.1, 57.9, 58.0, 58.8, 59.2, 66.9, 67.6, 72.8, 72.8, 73.1, 73.1, 84.3, 120.8, 134.4, 138.8, 138.9, 141.7, 142.3, 166.5.

HRMS (ESI) calcd for $[C_{103}H_{166}O_{19}S_{12}]^{-}$ 2090.8672 m/z, found 2090.8596 m/z.

7b was synthesized using the same procedure, with a 63% yield.

¹H NMR (400 MHz, CDCl₃) δ (ppm): 1.00 (s, 27H), 1.10 (s, 54H), 1.16 (s, 27H), 1.16 (s, 27H), 3.33-3.54 (m, 24H), 3.87 (s, 9H), 7.05 (s, 1H).
¹³C NMR (100 MHz, CDCl₃) δ (ppm): 27.4, 27.5, 27.7, 52.05, 57.7, 57.8, 57.9, 58.6, 59.1, 72.7, 72.8, 73.0, 73.1, 84.3, 120.8, 134.3, 138.8, 138.9, 141.7, 142.3, 166.5.
HRMS (ESI) calcd for [C₁₀₃H₁₄₂D₂₄O₁₉S₁₂]⁻ 2115.0178 m/z, found 2114.9949 m/z.

Tris(8-hydroxycarbonyl-2,2,6,6-Tetra(t-butoxyethyl)benzo[1,2-d:4,5-d']bis(1,3)dithio-4yl)methyl (OX063) Tris(8-hydroxycarbonyl-2,2,6,6-Tetra(2-(1-t-butoxy-2,2-d2-ethyl))benzo[1,2-d:4,5d']bis(1,3)dithio-4-yl)methyl (OX071)



The triester trityl alcohol 7 (412 mg, 0.194 mmol) was dissolved in 5 mL formic acid and heated at 45°C for 90 minutes. The HPLC-MS shows a complete deprotection and subsequent esterification of the 12 alcohols moieties into formyl esters (m/z= 1712). The solvent was then evaporated under reduced pressure, then the residue was dissolved in 3 mL anhydrous acetonitrile under argon. 342 µL of triflic acid (3.88 mmol, 20 eq.) was added, the solution turned deep greenblue and the reaction was stirred 30 min at room temperature. 38 mg of SnCl₂ (0.2 mmol, 1.05 eq.) dissolved in 1 mL of anhydrous THF were added and the reaction was stirred for an additional 30 min. HPLC-MS shows the conversion of the trityl alcohol to the radical. 4.6 mL of a solution of phosphate and NaCl (1 g of NaH₂PO₄ and 0.4 g of NaCl) was added. 4.6 mL of ethyl acetate was added and the mixture was stirred for 5 min. The phases were separated, the aqueous layer was extracted with 3 mL of ethyl acetate and organic phases were combined and evaporated. The residue was dissolved in 10 mL of a 2.5M solution of NaOH and stirred under argon at 55°C overnight. The hydrolysis of all esters (formyl and methyl) was verified by HPLC. The pH was adjusted to 1 with HCl 1.5M and the solution was loaded on a Hypersep C18 cartridge (3 cm diameter, 3 cm length). The cartridge was washed with water containing 0.1% of TFA to remove all the salts and the compound was recovered with 50% MeOH in water with 0.1% TFA. The solvents were removed under reduced pressure to afford OX063 in 91% yield (240 mg).

HRMS (ESI) calcd for $[C_{52}H_{63}O_{18}S_{12}]^+$ 1359.0663 m/z, found 1359.0670 m/z.

OX071 was synthesized using the same procedure, with a 90% yield.

HRMS (ESI) calcd for $[C_{52}H_{39}D_{24}O_{18}S_{12}]^{-1383.2169}$ m/z, found 1383.1993 m/z.

3. NMR spectra









S12





S14







































4. HPLC analyses

In this section, relevant chromatograms (such as crude mixtures and purified compounds) have been added to help with the reproduction of the protocols.

Gradient conditions were as follow: Solvent A was water, solvent B acetonitrile, solvent C water containing 1% of trifluoroacetic acid, and solvent D was methanol; column temperature, 40°C; UV detection from 210 to 800 nm.

Time (min)	Solvent A (%)	Solvent B (%)	Solvent C (%)	Solvent D (%)	
0	80	10	10	0	
5	0	90	10	0	
6	0	100	0	0	
11	0	100	0	0	

Gradient 1, flow rate 1.5 mL/min

Gradient 2, flow rate 1.5 mL/min

Time (min)	Solvent A (%)	Solvent B (%)	Solvent C (%)	Solvent D (%)
0	80	0	10	10
4	0	0	10	90
5	0	0	0	100
12	0	0	0	100

Gradient 3, flow rate 1.5 mL/min

Time (min)	Solvent A (%)	Solvent B (%)	Solvent C (%)	Solvent D (%)
0	80	10	10	0
1	80	10	10	0
6	0	90	10	0
7	0	90	10	0

Synthesis of 4, gradient 1



Synthesis of 5, gradient 1



Synthesis of 6, gradient 2



Synthesis of 7, gradient 2







Synthesis of OX063, gradient 2 (8) and 3 (OX063)





UV spectra extracted from chromatograms





5. HRMS data









T: FTMS - p ESI Full ms [1700.0000-2100.0000]



T: FTMS - p ESI Full ms [1800.0000-2200.0000]



S45

T: FTMS - p ESIFull ms [1800.0000-2200.0000]



6. EPR spectrum of OX071



X-band EPR spectrum of OX071 (50 $\mu\text{M})$ in deoxygenated PBS (10 mM, pH=7.4)