We report the synthesis of hydroxyethyl tetrathiatriarylmethyl radicals OX063 and its deuterated analogue OX071 for biomedical EPR applications.
We report the synthesis of hydroxyethyl tetrathiaatriarylmethyl radicals OX063 and its deuterated analogue OX071 for biomedical EPR applications.

Soluble organic radicals such as tetrathiaatriarylmethyls (TAM, trityl) or nitroxides have been used extensively for biomedical electron paramagnetic resonance (EPR) and dynamic nuclear hyperpolarization (DNP). The in vivo applications of nitroxide radicals are hampered by their fast bio-reduction, leading to an EPR-silent hydroxylamine. In addition, their broad linewidths and hyperfine couplings with the nitrogen nucleus \( I = 1 \) of the nitroxide fragment decreases the analytical sensitivity and performance as polarizing agents. The second class of spin probes widely used, tetrathiaatriarylmethyl radicals, has been first reported by Nycomed Innovation in the late 90’s. They chemically modified Gomberg’s trityl radical (Fig. 1) with the aim to avoid hyperfine splitting, increase the stability and provide water solubility. The two most popular structures are the Finland trityl (FT) and its more hydrophilic analogue OX063. Those radicals exhibit unmatched properties, such as a single-line EPR spectrum, ultra-narrow linewidth (<200 mG) and water solubility. The publication in the scientific literature in 2002 by Reddy et al. of the synthesis of Finland trityl enabled the creation of a wide variety of Finland-based structures, highly hydrophilic fragments such as PEGs, polyamidoamines, and dextran, conjugated. The high molecular weight of those probes decreases their spin density and tissue perfusion and none of them have been used beyond their initial proof of concept.

On the other hand, OX063 shows a high hydrophilicity due to twelve additional alcohol functions, preventing interactions with biomacromolecules, allowing for a systemic delivery of the probe. Unfortunately, the synthesis of OX063 has not been reported in the scientific literature, its synthesis remained elusive and is commercially available at a very high cost ($>10,000/g). In order to circumvent the limitations of FT-based structures, highly hydrophilic fragments such as PEGs, polyamidoamines, and dextran, were conjugated. The high molecular weight of those probes decreases their spin density and tissue perfusion and none of them have been used beyond their initial proof of concept.

Recently, a hybrid trityl radical possessing only one hydroxylated aryl group has been reported. However, to date, OX063 remains the sole spin probe used upon systemic delivery. Hereby we report the synthesis of OX063 and its partially deuterated analogue OX063-d24, also named OX071.

The synthesis starts with the construction of the protected aryl moiety 4 (Scheme 1). The condensation of dimethyl acetonocarboxylate with the 1,2,4,5-tetrathienobenzene generated in situ leads to thiokeatal 2, recovered by a simple filtration. Next, the four methyl esters of 2 were reduced using 4.5 equivalents of LiAlH4 and the resulting alcohols were protected with tert-butyl groups using isobutene and triflic acid as a source of tert-butyl cation. The synthesis of 4 only requires...
one column chromatography purification and can be performed on multiple gram-scale.

Scheme 1 Synthesis of the protected key intermediate 4.

Our initial attempt to generate the trityl alcohol 6 upon three successive additions of the aryllithium generated from the direct deprotonation of 4 using n-BuLi to diethyl carbonate, as classically performed for the synthesis of FT.\textsuperscript{24} (Scheme 2, path A), failed to provide any amount of trityl alcohol. Unreacted material, mixed with unidentified compounds, were recovered.

The use of other aryllithium reagents (sec-BuLi, tert-BuLi) or other solvents (THF, n-hexane) did not result in any improvement. We hypothesized that the incomplete liithiation of 4 was responsible for this result, as unreacted aryllithium reagent could react with the diethyl carbonate or open the thioetal after nucleophilic attack on the sulfur.\textsuperscript{16, 25} In order to quantitatively form the desired aryllithium of 7, we thought to use a halogen-metal exchange reaction and undertook the synthesis of the iodinated derivative 5. To avoid the possible attack of the base on the sulfur,\textsuperscript{16, 25} we used the more sterically hindered LiTMP. The treatment of 4 with 2.5 equivalents of LiTMP at -78°C, followed by the addition of iodide, resulted in the formation of the mono-iodide 5 in an excellent yield. Indeed, less than 5% of diiodinated derivative was formed (Scheme 2, path B).

Scheme 2 Synthesis of the trityl alcohol 7.

The aryl iodide 5 was then treated at -78°C with sec-BuLi in n-hexane to generate the corresponding aryllithium, followed by a slow addition of diethyl carbonate at room temperature to yield the trityl alcohol 6 in 80% yield. It is worth noting that the use of n-BuLi in diethyl ether did not result in the formation of the desired trityl 6 (Table 1, entry 1), as the deiodinated compound 4 was recovered together with unidentified compounds. The use of methyl chloroformate as an electrophile resulted in even more degradation (entry 2). A similar result was obtained in THF, with the exception of the formation of 40% of the butylated aryl analogue of 4 (entry 3).\textsuperscript{26} The use of sec-BuLi in THF prevented the formation of the butylated compound but did not result in the formation of the trityl alcohol 6 (entry 4). We found that only the use of the non-coordinating solvent n-hexane led to an efficient formation of trityl 6 in 80% yield (entry 5).

Table 1 Reaction conditions for the conversion of iodide 5 to trityl alcohol 6.

| Entry | Base | Solvent | Electrophile | 6 (%) |
|-------|------|---------|--------------|-------|
| 1     | n-BuLi | Et,O    | CO(OMe)\textsubscript{2} | 0     |
| 2     | n-BuLi | Et,O    | MeCOCI       | 0     |
| 3     | n-BuLi | THF     | CO(OMe)\textsubscript{2} | 0°    |
| 4     | s-BuLi | THF     | CO(OMe)\textsubscript{2} | 0     |
| 5     | s-BuLi | n-hexane | CO(OMe)\textsubscript{2} | 80%   |

a Base added at -78°C, stirred for 15 min, then warmed to room temperature, then the electrophile was added slowly over 3h.
b Butylated aryl analogue formed.

c The use of methyl chloroformate as an electrophile resulted in even more degradation.

The introduction of carbonyl groups onto the trityl 6 was achieved by treatment with an excess of sec-BuLi (15 eq.) in anhydrous TMEDA at -30°C, followed by bubbling of carbon dioxide. Interestingly, the treatment of 6 with 15 equivalents of tert-BuLi and TMEDA in benzene, followed by its addition to a solution of diethyl carbonate, as performed for the synthesis of FT,\textsuperscript{24} did not afford any esterified trityl, as the starting material was recovered (Table 2, entry 1). The same results were obtained in n-hexane, THF or diethyl ether (entries 2-4). When TMEDA was used as a solvent under similar conditions, a complex mixture of the starting material (8%) mono-(37%), di-(43%) and triester (7%) mixed with unidentified compounds (5%) was obtained, as determined by HPLC-MS, indicating that the deprotonation only occurred in TMEDA. Surprisingly, when diethyl carbonate was replaced by gaseous carbon dioxide, a clean mixture of triacid (70%) and diacid (30%) trityl alcohol was obtained. The mixture was then esterified using iodomethane and sodium carbonate in DMF in order to allow a large-scale purification. 7 was obtained in 60% yield after purification on silica gel.

Table 2 Reaction conditions for the conversion of trityl alcohol 6 to triester 7.

| Entry | Base | Solvent | Additive | Electrophile | 7 (%) |
|-------|------|---------|----------|--------------|-------|
| 1\textsuperscript{a} | t-BuLi | C\textsubscript{6}H\textsubscript{6} | TMEDA\textsuperscript{4} | CO(OMe)\textsubscript{2} | 0     |
| 2\textsuperscript{b} | t-BuLi | n-hexane | TMEDA\textsuperscript{4} | CO(OMe)\textsubscript{2} | 0     |
| 3\textsuperscript{b} | t-BuLi | THF     | TMEDA\textsuperscript{4} | CO(OMe)\textsubscript{2} | 0     |
| 4\textsuperscript{b} | s-BuLi | Et,O    | TMEDA\textsuperscript{4} | CO(OMe)\textsubscript{2} | 0     |
| 5\textsuperscript{b} | s-BuLi | TMEDA   | -        | CO(OMe)\textsubscript{2} | 7     |
| 6\textsuperscript{c} | s-BuLi | TMEDA   | -        | CO\textsubscript{2} | 60°   |

a Base (15 eq.) was added at room temperature, stirred for 2h, then added to a solution of 30 eq. electrophile at room temperature and stirred for 1h.
b Base (15 eq.) was added at -30°C, stirred for 2h, then added to a solution of 30 eq. electrophile at room temperature and stirred for 1h.
c Base (15 eq.) was added at -30°C, stirred for 2h, then CO\textsubscript{2} was bubbled for 30 min at -30°C and 30 min at room temperature.
d 15 eq.
e Isolated yield after esterification.
The next step was the deprotection of the 12 alcohol groups (Scheme 3). The fully protected trityl alcohol 7 was heated at 45°C for 90 minutes in formic acid, leading to a quantitative conversion of the tert-butyl ethers to formyl esters. Then, the trityl cation was generated using triflic acid and subsequently reduced to the radical by tin chloride (II). Finally, the esters were hydrolysed using sodium hydroxide, leading to OX063, isolated in 91% yield over the three steps.

Scheme 3 Conversion from 7 to OX063.

OX063 EPR spectrum (50 µM) in PBS (10 mM, pH 7.4) recorded at X-band under nitrogen exhibits a single line pattern with a peak-to-peak linewidth of 160 mG (Fig. 2), which is consistent with the reported value.27

Fig. 2 X-band EPR spectrum of OX063 (50 µM) in deoxygenated PBS (10 mM, pH 7.4)

The partial deuteration of the 12 methylene groups adjacent to the thioketals of OX063 (Fig. 3) leads to a narrowing of the linewidth to 80 mG.27 A narrower linewidth increases the oxygen sensitivity and leads to a higher signal-to-noise ratio, which is of primary importance for in vivo applications.

Fig. 3 Structure of OX071.

The synthesis of OX071 was achieved by exchange of the enolizable hydrogens of the intermediate 2 with CH₃OD/CH₃ONa in THF. The deuterated compound 2-d₈ was isolated in 85% yield without any purification (Scheme 4). OX071 was synthesized from 2-d₈ using the same procedures as OX063.

Scheme 4 Deuteration of 2.

Conclusions

We have developed an efficient synthesis of hydrophilic trityl radicals OX063 and its deuterated analogue OX071. Our synthetic protocol involves 7 steps and 4 chromatography columns and leads to OX063 with a total yield of 10%. This development will allow for the in vivo measurement of pO₂ by EPRI and OMRI upon systemic delivery and for DNP applications. Moreover, our synthetic strategy will allow for the synthesis of new derivatives with extended functional sensitivity, such as phosphonated analogues for concurrent pO₂, pH, and inorganic phosphate (Pi) measurement or new DNP agents and non-metallic contrast agents for MRI.28

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Conflicts of interest

There are no conflicts to declare.

Notes and references

1. WO9839277, US5530140.
2. T. J. Reddy, T. Iwama, H. J. Halpern and V. H. Rawal, J. Org. Chem., 2002, 67, 4635-4639.
3. J. H. Ardenkjær-Larsen, I. Laursen, I. Leunbach, G. Ehnholm, L. G. Wistrand, J. S. Petersson and K. Golman, J. Mag. Reson., 1998, 133, 1-12.
4. B. Driesschaert, V. Marchand, P. Levêque, B. Gallez and J. Marchand-Brynaert, Chem. Commun., 2012, 48, 4049-4051.
5. V. Marchand, P. Levêque, B. Driesschaert, J. Marchand-Brynaert and B. Gallez, Mag. Reson. Med., 2017, 77, 2438-2443.
6. I. Dhimitruka, A. A. Bobko, T. D. Eubank, D. A. Komarov and V. V. Khramtsov, JACS, 2013, 135, 5904-5910.
7. A. A. Bobko, T. D. Eubank, B. Driesschaert, I. Dhimitruka, J. Evans, R. Mohammad, E. E. Tchekneva, M. M. Dikov and V. V. Khramtsov, Sci. Rep., 2017, 7, 41233.
8. Y. Liu, Y. Song, A. Rockenbauer, J. Sun, C. Hemann, F. A. Villamena and J. L. Zweier, *J. Org. Chem.*, 2011, **76**, 3853-3860.

9. Y. Liu, F. A. Villamena, A. Rockenbauer and J. L. Zweier, *Chem. Commun.*, 2010, **46**, 628-630.

10. G. Y. Shevelev, E. L. Gulyak, A. A. Lomzov, A. A. Kuzhelev, O. A. Krumkacheva, M. S. Kupryushkin, V. M. Tormyshev, M. V. Fedin, E. G. Bagryanskaya and D. V. Pyshnyi, *J. Phys. Chem. B*, 2018, **122**, 137-143.

11. Z. Yang, Y. Liu, P. Borbat, J. L. Zweier, J. H. Freed and W. L. Hubbell, JACS, 2012, **134**, 9950-9952.

12. G. Mathies, M. A. Caporini, V. K. Michaelis, Y. Liu, K.-N. Hu, D. Mance, J. L. Zweier, M. Rosay, M. Baldus and R. G. Griffin, *Angew. Chem. Int. Ed.*, 2015, **54**, 11770-11774.

13. Y. Song, Y. Liu, W. Liu, F. A. Villamena and J. L. Zweier, *RSC Adv.*, 2014, **4**, 47649-47656.

14. A. A. Gorodetskii, T. D. Eubank, B. Driesschaert, M. Poncelet, E. Ellis, V. V. Krhamtsov and A. A. Bobko, *Sci. Rep.*, 2019, **9**, 12093.

15. B. Epel, M. C. Maggio, E. D. Barth, R. C. Miller, C. A. Pelizzari, M. Krzykawska-Serda, S. V. Sundramoorthy, B. Aydogan, R. R. Weichselbaum, V. M. Tormyshev and H. J. Halpern, *Int. J. Radiat. Oncol. Biol. Phys.*, 2019, **103**, 977-984.

16. Y. Qu, Y. Li, X. Tan, W. Zhai, G. Han, J. Hou, G. Liu, Y. Song and Y. Liu, *Chem. Eur. J.*, 2019, **25**, 7888-7895.

17. M. Serda, Y.-K. Wu, E. D. Barth, H. J. Halpern and V. H. Rawal, *Chem. Res. Tox.*, 2016, **29**, 2153-2156.

18. Y. song, Y. Liu, C. Hemann, F. A. Villamena and J. L. Zweier, *J. Org. Chem.*, 2013, **78**, 1371-1376.

19. W. Liu, J. Nie, X. Tan, H. Liu, N. Yu, G. Han, Y. Zhu, F. A. Villamena, Y. Song, J. L. Zweier and Y. Liu, *J. Org. Chem.*, 2017, **82**, 588-596.

20. B. Driesschaert, A. A. Bobko, T. D. Eubank, A. Samouilov, V. V. Krhamtsov and J. L. Zweier, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 1742-1744.

21. B. Driesschaert, P. Levêque, B. Galiez and J. Marchand-Brynaert, *Tet. Lett.*, 2013, **54**, 5924-5926.

22. Y. Liu, F. A. Villamena and J. L. Zweier, *Chem. Commun.*, 2008, 4336-4338.

23. M. Poncelet, B. Driesschaert, O. Tseytlin, M. Tseytlin, T. D. Eubank and V. V. Krhamtsov, *Bioorg. Med. Chem. Lett.*, 2019, **29**, 1756-1760.

24. I. Dhimitruka, M. Velayutham, A. A. Bobko, V. V. Krhamtsov, F. A. Villamena, C. M. Hadad and J. L. Zweier, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 6801-6805.

25. H. Hintz, A. Vanas, D. Klose, G. Jeschke and A. Godt, *J. Org. Chem.*, 2019, **84**, 3304-3320.

26. R. E. Merrill and E. Negishi, *J. Org. Chem.*, 1974, **39**, 3452-3453.

27. B. Epel and H. J. Halpern, in *Methods in Enzymology*, eds. P. Z. Qin and K. Warncke, Academic Press, 2015, vol. 564, pp. 501-527.

28. H. V. T. Nguyen, A. Detappe, N. M. Gallagher, H. Zhang, P. Harvey, C. Yan, C. Mathieu, M. R. Golder, Y. Jiang, M. F. Ottaviani, A. Jasanoff, A. Rajca, I. Ghobrial, P. P. Ghorooghchian and J. A. Johnson, *ACS Nano*, 2018, **12**, 11343-11354.
7-8 steps

OX063

OX063-d_{24}
Electronic Supplementary Information

Synthesis of hydroxyethyl tetrathiaatriaryl methyl 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 SQD2 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-Tetra-tert-butylthiobenzene was purchased from Atomax (China). 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(1,3)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 flame-dried 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 solution, now yellow and heterogeneous, is filtered and the solid 2 was washed with 300 mL methanol until it becomes 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 [C₂₀H₂₂O₈S₄+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 (2-d₈)

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.

^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, 64.9, 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 (3-d₈)
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. The solution was filtered and the solid was filtered and washed with 1 L of methanol. The solid was dissolved in 1 L of 1M hydrochloric acid and heated to 100°C until all the big chunks of solid dis-aggregate. 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 [C₁₆H₂₂O₄S₄+H]⁺ 407.0474 m/z, found 407.0500 m/z.

3-d₈ was synthesized using the same procedure, with a 62% yield.

¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 3.57 (s, 8H), 4.23 (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 (4-d₈)

The tetraalcohol 3 (9 g, 21.7 mmol) was suspended in 500 mL of an isobutene solution in THF (15% v/v) in a 1 L flame-dried flask. Triflic acid (6 mL, 65.2 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 3 mL triflic acid (32.6 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 insoluble polyisobutene, which could strongly decrease the flow rate during chromatography.
\[^1\text{H} \text{NMR} \ (400 \text{ MHz, CDCl}_3) \ \delta \ (\text{ppm}): \ 1.17 \ (\text{s, } 36\text{H}), 2.33 \ (t, J=6.8 \text{ Hz, } 8\text{H}), 3.56 \ (t, J=6.8 \text{ Hz, } 8\text{H}), 6.95 \ (s, 2\text{H}).\]

\[^{13}\text{C} \text{ NMR} \ (100 \text{ MHz, CDCl}_3) \ \delta \ (\text{ppm}): 27.6, 41.2, 58.6, 71.5, 73.2, 116.3, 135.4.\]

HRMS (ESI) calcd for \([\text{C}_{32}\text{H}_{54}\text{O}_4\text{S}_4]^+\) 630.2905 m/z, found 630.2971 m/z.

4-d\(_8\) was synthesized using the same procedure, with a 64% yield.

\[^1\text{H} \text{NMR} \ (400 \text{ MHz, CDCl}_3) \ \delta \ (\text{ppm}): 1.17 \ (\text{s, } 36\text{H}), 3.55 \ (s, 8\text{H}), 6.95 \ (s, 2\text{H}).\]

\[^{13}\text{C} \text{ NMR} \ (100 \text{ MHz, CDCl}_3) \ \delta \ (\text{ppm}): 27.6, 58.6, 56.7, 73.2, 116.3, 135.4.\]

HRMS (ESI) calcd for \([\text{C}_{32}\text{H}_{46}\text{D}_8\text{O}_4\text{S}_4]^+\) 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-d-ethyl))-4-iodo-benzo[1,2-d:4,5-d']bis(1,3)dithiole (5-d\(_8\))

\[
\begin{align*}
\text{R} = \text{H, 4} \\
\text{R} = \text{D, 4-d\(_8\)}
\end{align*}
\]

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 Na\(_2\)S\(_2\)O\(_3\) and the reaction was stirred 10 min until the dark color disappears. The aryl iodide 5 was extracted twice with 200 mL of ethyl acetate. The combined organic phases were dried over MgSO\(_4\) 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.*

\[^1\text{H} \text{NMR} \ (400 \text{ MHz, CDCl}_3) \ \delta \ (\text{ppm}): 1.18 \ (\text{s, } 36\text{H}), 2.34 \ (t, J=6.8 \text{ Hz, } 8\text{H}), 3.56 \ (t, J=6.8 \text{ Hz, } 8\text{H}), 6.86 \ (s, 2\text{H}).\]

\[^{13}\text{C} \text{ NMR} \ (100 \text{ MHz, CDCl}_3) \ \delta \ (\text{ppm}): 27.6, 41.5, 58.6, 68.8, 73.2, 81.6, 115.5, 132.9, 141.5.\]

HRMS (ESI) calcd for \([\text{C}_{32}\text{H}_{55}\text{IO}_4\text{S}_4]^+\) 756.1871 m/z, found 756.1913 m/z.
5-\(d_8\) was synthesized in a similar way, with a 98% yield.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) (ppm): 1.15 (s, 36H), 3.53 (s, 8H), 6.84 (s, 2H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) (ppm): 27.6, 58.3, 68.8, 73.1, 81.6, 115.4, 132.8, 141.5.

HRMS (ESI) calcd for \([\text{C}_{32}\text{H}_{45}\text{D}_8\text{IO}_4\text{S}_4]^+\) 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)

The aryl iodide 5 (5 g, 6.61 mmol) was dissolved in degassed, 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 MgSO\(_4\) 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.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) (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).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) (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 \([\text{C}_{97}\text{H}_{160}\text{O}_{13}\text{S}_{12}]^-\) 1916.8507 m/z, found 1916.8442 m/z.

6-\(d_{24}\) was synthesized in a similar way, with a 71% yield.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) (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).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) (ppm): 27.6, 27.7, 58.0, 58.1, 58.8, 59.4, 68.7, 68.9, 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 \([\text{C}_{97}\text{H}_{136}\text{D}_{24}\text{O}_{13}\text{S}_{12}]^-\) 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(1-t-butoxy-2,2-d2-ethyl))benzo[1,2-d:4,5-d']bis(1,3)dithio-4-yl)methanol (7-d24)

The trityl alcohol 6 (100 mg, 0.047 mmol) was dissolved in 1 mL of degassed, 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 turning to 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 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 (18 µL, 0.282 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 washed with 10 mL water. 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).

1H 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 [C₁₀₃H₁₆₆O₁₉S₁₂]- 2090.8672 m/z, found 2090.8596 m/z.

7-d24 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, 138.3, 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-4-yl)methyl (OX063)
Tris(8-hydroxycarbonyl-2,2,6,6-Tetra(1-t-butoxy-2,2-d2-ethyl))benzo[1,2-d:4,5-d’]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. 350 µL of triflic acid (1.34 mmol, 20 eq.) was added, the solution turned deep green-blue 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 with 0.1% TFA. The solvents were removed under reduced pressure to afford OX063 in 91% yield (240 mg).

HRMS (ESI) calcd for [C52H63O18S12]+ 1359.0663 m/z, found 1359.0670 m/z.

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

HRMS (ESI) calcd for [C52H39D24O18S12]− 1383.2169 m/z, found 1383.1993 m/z.
3. NMR spectra
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 follows: 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.

Gradient 1, flow rate 1.5 mL/min

| 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 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

\[
\begin{align*}
\text{6} & \quad \xrightarrow{1. 15 \text{ eq. sec-BuLi}} \quad \text{TMEDA} \quad \xrightarrow{2. \text{ CO}_2} \\
\end{align*}
\]

ketone
Crude

COOH 2X
H 1X

+/- BuO

Metro, Na₂CO₃
DMF

Crude

COO(Me) 2X
COOH 1X

H 1X
Synthesis of OX063, gradient 2 (8) and 3 (OX063)

7 \xrightarrow{\text{HCOOH}} 8

1. \text{CF}_3\text{SO}_3\text{H, ACN}
2. \text{SnCl}_2
3. \text{NaOH}

OX063
UV spectra extracted from chromatograms
5. HRMS data
