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Synthesis of hydroxyethyl tetrathiatriarylmethyl radicals OX063 and OX071†

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

Soluble organic radicals such as tetrathiatriarylmethyls (TAM, trityl) or nitroxides have been used extensively for biomedical electron paramagnetic resonance (EPR) and dynamic nuclear polarization (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 decrease the analytical sensitivity and performance as polarizing agents. The second class of spin probes widely used, tetrathiatriarylmethyl radicals, was 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**.[1] Those radicals exhibit unmatched properties, such as a single-line EPR spectrum, ultra-narrow linewidth (<200 mG) and water solubility.[2,3] The publication in the scientific literature in 2002 by Reddy *et al.*[2] of the synthesis of Finland trityl enabled the synthesis of a wide variety of Finland-based structures for biomedical EPR applications. **FT**-based trityls showing sensitivities to physiological parameters, such as pO_2, *p*H, inorganic phosphate,[4–7] thiol concentration[8] or redox status[9] have been reported. **FT**-based spin labels of biomacromolecules have allowed for distance measurements in DNA[10] or proteins.[11] Finally, high performance **FT**-nitroxide biradical polarizing agents have been developed.[12] All of these structural modifications took place at the *para* position of the trityl scaffold, which is the only position that can be easily modified.

Despite their widespread uses, the lipophilic core of **FT**-based molecules is responsible for their aggregation at low pH and hydrophobic interactions with plasma biomacromolecules (*e.g.* albumin[13]), resulting in a broadening of the EPR line. For this reason, *in vivo* applications are limited to intra-tissue deliveries only.[6,7,14]

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.[15] Unfortunately, the synthesis of **OX063** has not been reported in the scientific literature and its synthesis remained elusive[16] although being commercially available at a very high cost (>$10,000 per g).[17] In order to circumvent the limitations of **FT**-based structures, highly hydrophilic fragments such as PEGs,[18,19] polypeptides,[20,21] polyamidoamines,[22] and dextrans,[23] were conjugated. The high molecular weight of those probes decreases their spin density and tissue perfusion and none of them have been used beyond their initial proof of concept. Recently, a hybrid trityl radical possessing only one hydroxylated aryl group has been reported.[16] However, to date, **OX063** remains the sole spin probe used upon systemic delivery. Hereby we report the synthesis of **OX063** and its partially deuterated analogue **OX063-d$_{24}$**, also named **OX071**.

The synthesis starts with the construction of the protected aryl moiety **4** (Scheme 1). The condensation of dimethyl acetonedicarboxylate with the 1,2,4,5-tetrathiobenzene generated *in situ* leads to thioketal **2**, recovered by a simple filtration. Next, the four methyl esters of **2** were reduced using 4.5 equivalents of $LiAlH_4$ and the resulting alcohols were protected with *tert*-butyl groups using isobutene and triflic acid as a source of *tert*-butyl

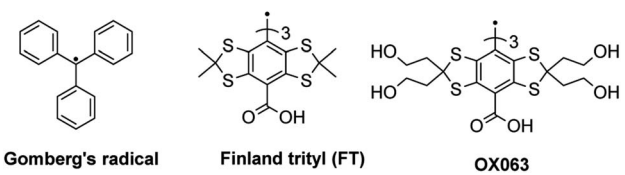

Fig. 1 Structures of Gomberg's trityl, Finland trityl (FT) and OX063.

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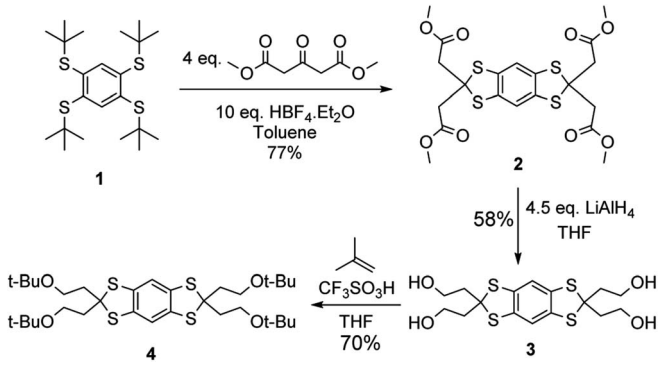


Scheme 1 Synthesis of the protected key intermediate **4**.

cation. The synthesis of **4** only requires one column chromatography purification and can be performed on multiple gram-scale.

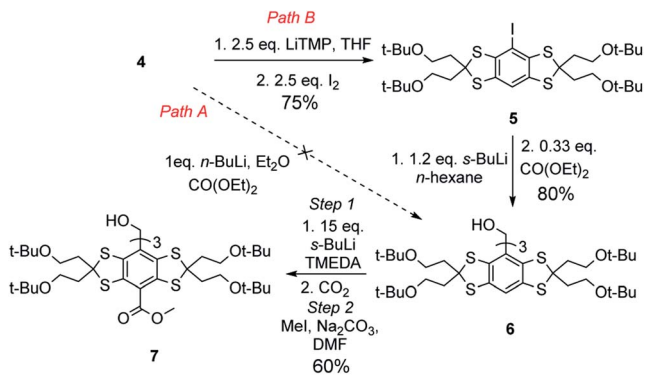
Our initial attempt to generate the trityl alcohol **6** upon three successive additions of the aryllithium generated from the direct deprotonation of **4** using *n*-BuLi to diethyl carbonate, as classically performed for the synthesis of **FT**[2,24] (Scheme 2, path A), failed to provide any amount of trityl alcohol. Unreacted material, mixed with unidentified compounds was recovered. The use of other alkyllithium reagents (*sec*-BuLi, *tert*-BuLi) or other solvents (THF, *n*-hexane) did not result in any improvement. We hypothesized that the incomplete lithiation of **4** was responsible for this result, as unreacted alkyllithium reagent could react with the diethyl carbonate or open the thioketal after nucleophilic attack on the sulfur.[16,25] In order to quantitatively form the desired aryllithium of **4**, 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,[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 iodine, 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).

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).[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).

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**,[2,24] 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 alcohols was obtained. The carboxylic acids were then esterified from the mixture 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.

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 tritylium cation was generated using triflic acid and subsequently reduced to radical by tin chloride(II). Finally, the esters were hydrolysed using sodium hydroxide, leading to **OX063**, isolated in 91% yield over the three steps.

OX063 EPR spectrum (50 μM) in PBS (10 mM, pH 7.4) recorded at X-band under nitrogen exhibits a single line pattern



Scheme 2 Synthesis of the trityl alcohol **7**.

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

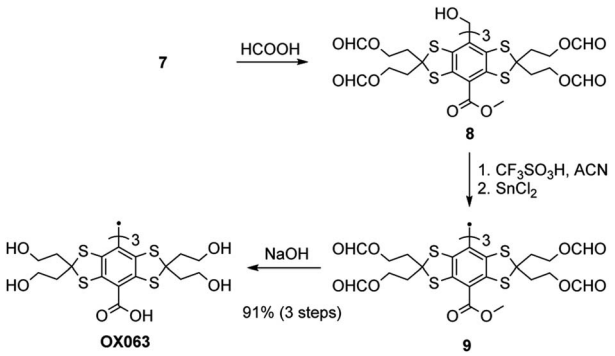
Entry[a]	Base	Solvent	Electrophile	**6** (%)
1	*n*-BuLi	Et_2O	$CO(OEt)_2$	0
2	*n*-BuLi	Et_2O	$ClCO_2Me$	0
3	*n*-BuLi	THF	$CO(OEt)_2$	0[b]
4	*s*-BuLi	THF	$CO(OEt)_2$	0
5	*s*-BuLi	*n*-Hexane	$CO(OEt)_2$	80%

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

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

Entry	Base	Solvent	Additive	Electrophile	7 (%)
1[a]	*t*-BuLi	C_6H_6	TMEDA[d]	$CO(OEt)_2$	0
2[b]	*t*-BuLi	*n*-Hexane	TMEDA[d]	$CO(OEt)_2$	0
3[b]	*t*-BuLi	THF	TMEDA[d]	$CO(OEt)_2$	0
4[b]	*s*-BuLi	Et_2O	TMEDA[d]	$CO(OEt)_2$	0
5[b]	*s*-BuLi	TMEDA	—	$CO(OEt)_2$	7
6[c]	*s*-BuLi	TMEDA	—	CO_2	60[e]

[a] Base (15 eq.) was added at room temperature, stirred for 2 h, then added to a solution of 30 eq. electrophile at room temperature and stirred for 1 h. [b] Base (15 eq.) was added at −30 °C, stirred for 2 h, then added to a solution of 30 eq. electrophile at room temperature and stirred for 1 h. [c] Base (15 eq.) was added at −30 °C, stirred for 2 h, then CO_2 was bubbled for 30 min at −30 °C and 30 min at room temperature. [d] 15 eq. [e] Isolated yield after esterification.



Scheme 3 Conversion from **7** to **OX063**.

with a peak-to-peak linewidth of 160 mG (Fig. 2), which is consistent with the reported value.[27]

The partial deuteration of the 12 methylene groups adjacent to the thioketals of **OX063** leads to **OX071** (Fig. 3), with a sharper linewidth.[27] Indeed, unresolved splitting with hydrogen nuclei is responsible for an inhomogeneous broadening of the EPR line of **OX063**. The deuteration of **OX063** decreases this inhomogeneous broadening due to the lower magnetic moment of the deuterium nucleus. A narrower linewidth increases the

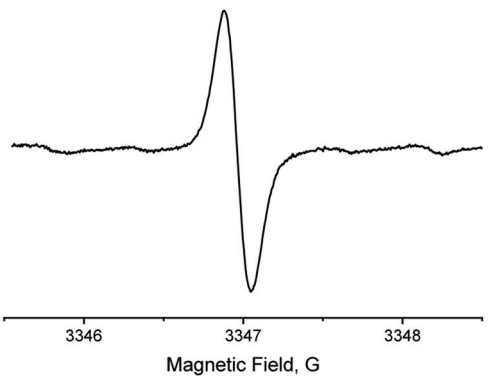


Fig. 2 X-band EPR spectrum of **OX063** (50 μM) in deoxygenated PBS (10 mM, pH = 7.4).

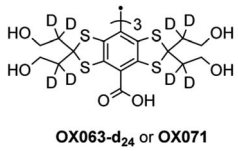
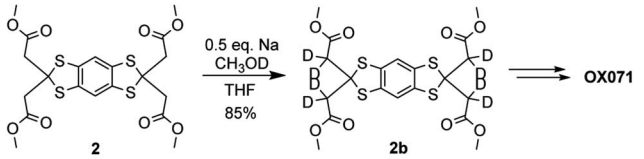


Fig. 3 Structure of **OX071**.



Scheme 4 Deuteration of **2**.

oxygen sensitivity and leads to a higher signal-to-noise ratio, which is of primary importance for *in vivo* applications.

The synthesis of **OX071** was achieved by exchange of the enolizable hydrogens of the intermediate **2** with CH_3OD/CH_3ONa in THF. The deuterated compound **2b** was isolated in 85% yield without any purification (Scheme 4). **OX071** was synthesized from **2b** using the same procedures as **OX063**. The EPR spectrum of **OX071** exhibits a single line pattern with a peak-to-peak linewidth of 80 mG (see ESI†), consistent with the value reported in the literature.[27]

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 pO_2 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 pO_2, *p*H, and inorganic phosphate (Pi) measurement or new DNP agents and non-metallic contrast agents for MRI.[28]

Conflicts of interest

There are no conflicts to declare.

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