

Regenerable Thiophenolic Radical-Trapping Antioxidants

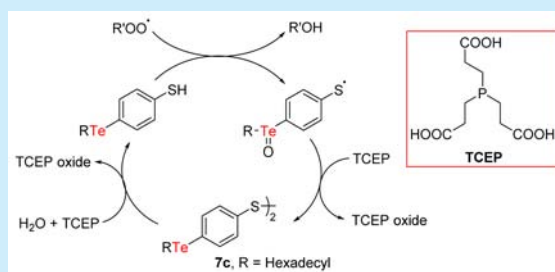
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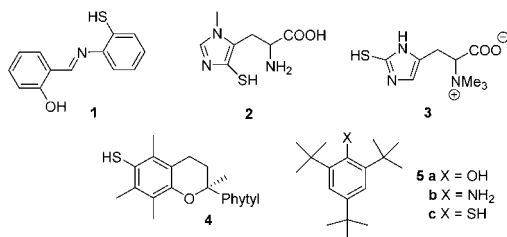
Supporting Information

ABSTRACT: Diphenyl disulfides carrying alkyltelluro groups in the *o*-, *m*-, and *p*-positions were prepared using ortho-lithiation and lithium halogen exchange reactions. The novel antioxidants showed only minimal inhibitory effect on the azo-initiated peroxidation of linoleic acid in chlorobenzene until reduced to the corresponding thiophenols by tris(2-carboxyethyl)phosphine (TCEP). The best in situ generated thiophenol (from **7c**) under these conditions quenched peroxy radicals more efficiently than α -tocopherol with an almost 3-fold increase in inhibition time.



As compared with phenols, their sulfur counterparts, the thiophenols, would seem attractive as radical trapping antioxidants. The S–H bond dissociation enthalpy (BDE) in benzenethiol (79.1 kcal/mol^{1a}) is considerably lower than the O–H BDE in hydroxybenzene (89.9 kcal/mol^{1b}). According to both theory² and experimentation,³ thiophenol is also more reactive than phenol toward peroxy radicals.

Some arenethiol- and heteroarenethiol derivatives have been shown to function as antioxidants in biological systems. Due to its anti-inflammatory properties, salicylideneamino-2-thiophenol (**1**) has been used as a cure for pain, fever, and rheumatism.^{4,5} It is also known to stimulate bone formation.⁶



The heteroaromatic imidazol-4-thiol family of natural products known as the ovothiols (represented by **2**) and less polar derivatives thereof^{7–9} have been found to act as biological antioxidants. Compounds of this sort could scavenge lipid peroxy radicals almost as efficiently as α -tocopherol (α -T) and vitamin C.¹⁰ The natural product ergothioneine (**3**), which is a derivative of imidazol-2-thiol, has also demonstrated numerous antioxidative and cytoprotective capabilities.^{11,12} 6-Mercapto- α -tocopherol (**4**), a sulfur analog of α -T, has been described in the early literature,^{13,14} but little is known about its antioxidative properties.

Whereas phenolic compounds are the most common radical trapping stabilizers for natural and man-made organic materials, aromatic thiols are rarely used. The unpleasant odor of many compounds lacking polar substituents would of course preclude

most stabilizer applications, but there are also chemical problems associated with the use of arenethiols as radical trapping antioxidants. Some 50 years ago,¹⁵ Ingold and co-workers compared the capacity of 2,4,6-tri-*tert*-butylphenol (**5a**), -aniline (**5b**), and -thiophenol (**5c**) to inhibit autoxidation of cumene. All compounds **5** could quench peroxy radicals by formal H atom transfer. However, whereas the resulting phenoxyl and phenylaminy radicals can quench a second peroxy radical, the phenylthiyl radicals disappear by rapid recombination ($k = 5 \times 10^7 \text{ M}^{-1} \text{ s}^{-116}$) to form a diaryl disulfide. The stoichiometric factor *n*, the number of peroxy radicals that could be destroyed per antioxidant molecule, was therefore only 0.95 for **5c**.

We have tried for some time to find novel antioxidants that could outperform the traditional phenols and aromatic amines when it comes to radical trapping activity and regenerability.^{17,18} In this work, we found that alkyltelluro groups have a remarkable effect. For example, introducing an octyltelluro group next to the OH in phenol caused a ca. 4 orders of magnitude increase in the reactivity toward peroxy radicals. Furthermore, this modification of the structure also improved the regenerability of the antioxidant in the lipid phase by aqueous phase co-antioxidants such as *N*-acetylcysteine (NAC).

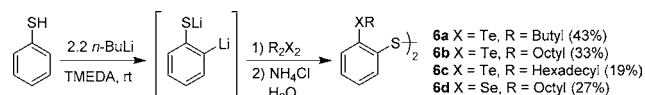
We were curious to see how alkyltelluro substitution in thiophenol would affect its radical trapping activity. For comparison, some of the corresponding alkylseleno compounds were also prepared. Obviously, we could expect that these compounds would be readily converted into inactive “dormant” diaryl disulfide antioxidants, but we were challenged by the perspective to find a suitable co-antioxidant that, “on demand”, could make them come alive again. We herein describe the synthesis of the target antioxidants as well as their radical trapping activity and regenerability in a simple two-phase peroxidation model designed to mimic a biological membrane.

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For the preparation of ortho-functionalized compounds, we decided to take advantage of the directing effect of an arenethiolate ion in lithiation.¹⁹ Thiophenol was therefore treated with 2.2 equiv of *n*-butyllithium in the presence of tetramethylethylenediamine (TMEDA). Addition of dibutyl-, dioctyl-, dihexadecyl ditelluride and dioctyl diselenide, respectively, to the in situ prepared solution of 2,*S*-dilithiated thiophenol afforded diphenyl disulfides **6a–d** in modest yields (19–43%) after acidic workup and chromatographic purification (Scheme 1). Obviously, the ortho-functionalized

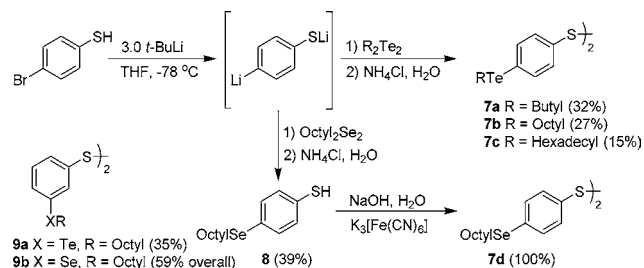
Scheme 1. Synthesis of Compounds **6a–d**



thiophenols initially formed are oxidized to the corresponding disulfides during workup.

For the functionalization of thiophenol in the para- and meta-positions, we relied on lithium halogen exchange.^{17c} 4-Bromothiophenol upon treatment with 3 equiv of *tert*-butyllithium and then dibutyl-, dioctyl-, and dihexadecyl ditelluride, respectively, returned disulfides **7a–c**, again in low (15–32%) isolated yields (Scheme 2).

Scheme 2. Synthesis of Compounds **7a–d** and **9a–b**



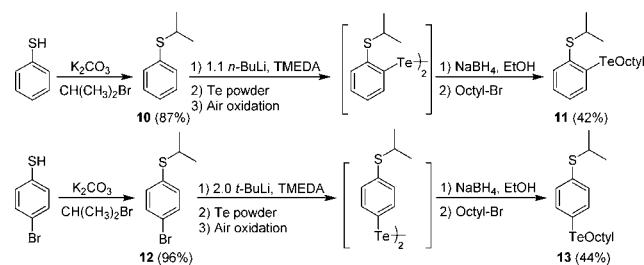
Surprisingly, when we used a similar protocol for the preparation of the corresponding octylseleno-derivative **7d**, 4-(octylseleno)thiophenol **8** was isolated in 39% yield. Oxidation with potassium ferricyanide under basic conditions afforded bis-4-(octylseleno)phenyl disulfide in quantitative yield. Compounds **9**, functionalized with octyltelluro (**9a**, 35% yield) and octylseleno groups (**9b**, 59% overall yield) in the meta-position, were also prepared by lithium halogen exchange using 3-bromothiophenol as a starting material.

For reference purposes, to see the effect of the aromatic sulfhydryl group, we also prepared two *S*-isopropylated octyltelluro-functionalized thiophenols **11** and **13**. *S*-Isopropylated thiophenol (**10**) was ortho-lithiated, and elemental tellurium allowed insertion into the carbon lithium bond. After air oxidation, the resulting crude ditelluride was reduced with sodium borohydride and the arenetelluroolate formed alkylated with octyl bromide (Scheme 3).

S-Isopropylated 4-bromothiophenol (**12**) was subjected to lithium halogen exchange and then converted to **13** using a similar protocol as described for the preparation of compound **11**.

The radical trapping capacity and regenerability of our novel thiophenol-derived antioxidants were studied in a two-phase model.²⁰ In the lower chlorobenzene layer, containing the antioxidant to be evaluated, peroxidation of linoleic acid was ongoing, initiated by azo-bis-dimethylvaleronitrile.

Scheme 3. Synthesis of Compounds **11** and **13**



The conjugated diene formed as a result of this process was monitored by HPLC with UV detection at 234 nm. The upper aqueous layer contained a water-soluble co-antioxidant that could hopefully regenerate the active antioxidant in the organic phase. By following the increase in conjugated diene with time, information about the radical quenching activity and regenerability of the antioxidant could be obtained. Usually, two stages could be distinguished in the peroxidation traces (Figure 1).

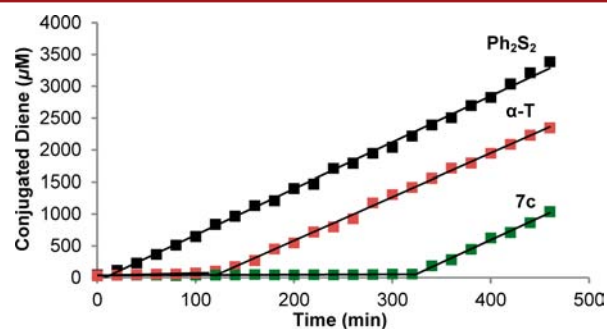


Figure 1. Peroxidation traces (conjugated diene concentration vs time) recorded with compounds **7c**, Ph_2S_2 , and $\alpha\text{-T}$ ($40 \mu\text{M}$) as antioxidants in the chlorobenzene layer in the presence of TCEP (0.5 mM) in the aqueous phase.

During the first inhibited phase, most of the peroxy radicals were quenched by the antioxidant and the concentration of the conjugated diene increased only slowly with time (R_{inh}). Then, in a second phase, the diene concentration with time increased suddenly to a higher constant value, R_{uninh} , which was very similar to the one recorded in the absence of any antioxidant in the chlorobenzene layer. The cross-point of the inhibited and uninhibited lines was calculated and reported as the inhibition time, T_{inh} , of the antioxidant.

Compounds **6**, **7**, **9**, **11**, and **13** were first evaluated in the absence of any co-antioxidant in the aqueous phase. Diphenyl disulfide (Ph_2S_2) and $\alpha\text{-T}$ were also included for reference purposes (Table 1). Except for $\alpha\text{-T}$ ($R_{\text{inh}} = 28 \mu\text{M/h}$ and $T_{\text{inh}} = 109 \text{ min}$), none of the compounds tested could inhibit peroxidation. Rather, they acted as poor retarders of peroxidation and no inhibition time could be recorded.

Previously, we have found that the water-soluble thiol NAC could significantly prolong the T_{inh} for many chalcogen-containing antioxidants.¹⁷ The thiol probably serves to reduce the chalcogen from the tetravalent to the divalent state. It was our hope that NAC, via thiol exchange with diaryl disulfides (eq 1), would also serve to increase the concentration of aromatic thiol in the lipid phase. All compounds were therefore

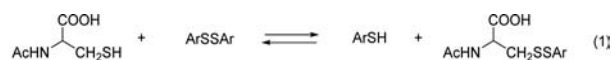


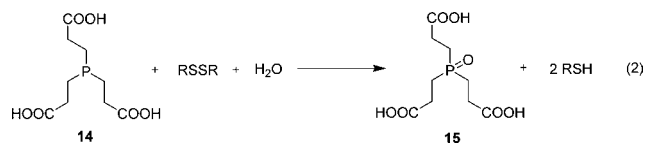
Table 1. Inhibited Rates of Conjugated Diene Formation (R_{inh}) and Inhibition Times (T_{inh}) with TCEP (0.5 mM), NAC (1.0 mM), and without Co-antioxidants

AO ^a	with TCEP		with NAC		without co-antioxidant	
	R_{inh}^b ($\mu\text{M}/\text{h}$)	T_{inh}^c (min)	R_{inh}^b ($\mu\text{M}/\text{h}$)	T_{inh}^c (min)	R_{inh}^b ($\mu\text{M}/\text{h}$)	T_{inh}^c (min)
6a	9.7 \pm 1	150 \pm 5	43 \pm 3	66 \pm 3	437	0
6b	8.2 \pm 2	148 \pm 0	32 \pm 3	73 \pm 6	333	0
6c	7.3 \pm 1	156 \pm 5	19 \pm 2	84 \pm 1	318	0
6d	307	0	300	0	371	0
7a	3.9 \pm 0	284 \pm 7	19 \pm 3	132 \pm 3	358	0
7b	3.7 \pm 1	304 \pm 4	20 \pm 1	146 \pm 3	321	0
7c	2.3 \pm 1	324 \pm 5	15 \pm 1	170 \pm 4	342	0
7d	304	0	343	0	385	0
9a	6.9 \pm 1	164 \pm 2	20 \pm 1	88 \pm 7	367	0
9b	338	0	371	0	436	0
11 ^d	81 \pm 2	37 \pm 2	72 \pm 2	78 \pm 4	390	0
13 ^d	118 \pm 5	32 \pm 3	23 \pm 1	101 \pm 3	388	0
Ph ₂ S ₂	431	0	372	0	421	0
α -T	21 \pm 2	123 \pm 7	25 \pm 1	97 \pm 5	28 \pm 2	109 \pm 2

^aAO = Antioxidant. 40 μM was used in the assay. ^bRate of peroxidation during the inhibited phase. Uninhibited rate is 453 $\mu\text{M}/\text{h}$ with TCEP, 425 $\mu\text{M}/\text{h}$ with NAC, and 479 $\mu\text{M}/\text{h}$ without antioxidant and co-antioxidant. Errors correspond to \pm SD for triplicates. ^cInhibited phase of peroxidation. Reactions were monitored for 460 min. Errors correspond to \pm SD for triplicates. ^d80 μM of antioxidant was used in the assay.

tested again in the presence of NAC (1 mM). Considerable improvement was seen. Some of the catalysts (6c, 7a–c, 9a) could now match α -T when it comes to the inhibited rate of peroxidation and inhibition time. As noted before, NAC did not show any inhibiting effect on peroxidation when tested alone in a control experiment and it did not serve to extend the T_{inh} for α -T. In a similar vein, all catalysts were also tested in the presence of 0.5 mM of the water-soluble dithiol dithiothreitol (DTT), acidified with acetic acid to the same pH (3.3; R_{inh} and T_{inh} showed some pH-dependence).

However, the R_{inh} and T_{inh} data recorded were almost a blueprint of those obtained with NAC (Table S1 in the Supporting Information). It may be that the thiol exchange of NAC and DTT with the diaryl disulfides is not favorable enough that the compounds could exert their full antioxidant capacity. Therefore, a better water-soluble disulfide reducing agent was sought. It occurred to us that tris(2-carboxyethyl)-phosphine (TCEP, 14) could be a useful co-antioxidant in our system. It is known to reduce disulfides as shown in eq 2.²¹

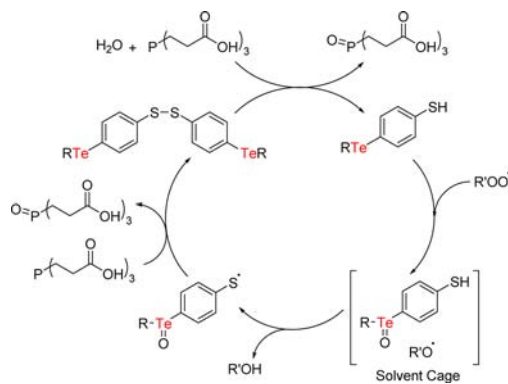


Interestingly, when TCEP hydrochloride (0.5 mM; pH = 3.27) was added to the aqueous phase, R_{inh} was notably reduced and T_{inh} was prolonged for most of the antioxidants tested. This would indicate efficient reduction of diaryl disulfide and facile regeneration of the antioxidant under the conditions of the assay. Whenever the alkyl part of the alkyltelluro moiety was varied in a systematic way (disulfides 6 and 7) the performance increased slightly with increasing chain length/lipophilicity (butyl < octyl < hexadecyl). Overall, the *p*-alkyltelluro functionalized disulfides 7a–c showed lower R_{inh} and longer T_{inh} than their *o*-substituted counterparts 6. The antioxidant activity of the only *m*-alkyltelluro functionalized derivative 9a prepared was in between those of the corresponding *o*- and *p*-derivatives. The best antioxidant, *p*-disubstituted diphenyl

disulfide 7c, inhibited peroxidation for 324 min with R_{inh} as low as 2.3 $\mu\text{M}/\text{h}$ (see Figure 1). In contrast, none of the analogous alkylseleno substituted diphenyl disulfides 6d, 7d, and 9b or diphenyl disulfide could act as radical trapping agents in the two-phase model. Apparently, tellurium is a vital constituent in these antioxidants.

We postulate below that alkyltelluro thiophenols are the active antioxidants generated in situ under the conditions of the two-phase model. It was therefore of interest to study the performance of some *S*-alkylated thiophenols. In the presence of NAC, *S*-isopropylated compound 13 (at 80 μM) showed the same antioxidant characteristics as α -T (at 40 μM). The performance of the corresponding *o*-disubstituted compound 11 was not so impressive. Although an inhibited phase of peroxidation could be distinguished for both 11 and 13, the R_{inh} and T_{inh} values were not near those recorded for the corresponding disulfides 6b and 7b (at 40 μM). It may be that these compounds quench peroxy radicals by donating electrons from tellurium. In the absence of an aqueous-phase reducing agent they are likely to be oxidized to the corresponding inactive telluroxides by residual amounts of linoleic acid hydroperoxide always present in linoleic acid. Our proposed catalytic antioxidant mechanism is shown in Scheme 4.

Scheme 4. Proposed Catalytic Mechanism for Peroxyl Radical Trapping by Bis-4-(alkyltelluro)phenyl Disulfides in the Presence of TCEP



TCEP-induced reduction of disulfide will produce alkyltelluro thiophenol which quenches peroxy radicals by transfer of oxygen to tellurium.¹⁸ Then, in a solvent cage, the resulting alkoxy radical will abstract a hydrogen atom from the thiophenol. Recombination of thiyl radicals will follow, accompanied by reduction of tetravalent tellurium (shown as a telluroxide) to reform the disulfide antioxidant. In fact, it is known that trivalent phosphorus compounds could act as reducing agents toward telluroxides.²² No attempt was made to isolate intermediates proposed in Scheme 4.

We were also curious to see the performance of our catalysts in the presence of varying (0.0625, 0.125, 0.25, 0.5, and 1.0 mM) amounts of TCEP (Table S2 in the Supporting Information). As shown in Figure 2, T_{inh} increased linearly with

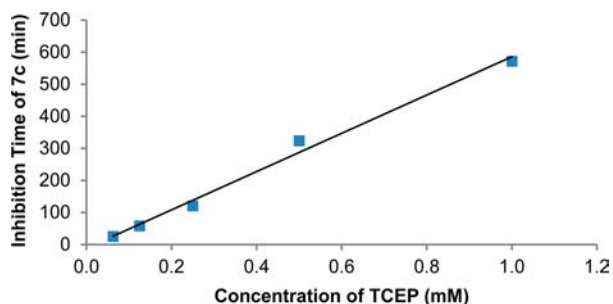


Figure 2. Inhibition time of compound 7c versus concentration of TCEP.

the concentration of the co-antioxidant. On the other hand, the corresponding R_{inh} values decreased to reach a constant value above 0.5 mM TCEP. Obviously, at the lower concentrations of TCEP, the thiol concentration in the chlorobenzene phase is so low that the peroxy radicals cannot be efficiently quenched.

In conclusion, we have shown that (alkyltelluro)thiophenols generated in situ in a lipid phase can quench peroxy radicals more efficiently than α -T. The tris(2-carboxyethyl)phosphine used to bring about disulfide to thiol reduction has an additional role: it reduces the telluroxide form of the catalyst to the corresponding telluride and thus allows for antioxidant regeneration and a catalytic mode of action. What makes these antioxidants unique is the possibility to keep them turned off under nonreducing conditions and, whenever needed, make them come alive. It remains to be seen if this capability can be exploited in biological systems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03169.

Experimental section including spectral data for all compounds prepared (PDF)

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Notes

The authors declare no competing financial interest.

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