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Synthesis and Antioxidant Activity of [60]Fullerene–BHT Conjugates**

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Abstract: Fullerene derivatives incorporating one or two 3,5-di-*tert*-butyl-4-hydroxyphenyl groups were synthesized by 1,3-dipolar cycloaddition of azomethine ylides to C_{60}. The O–H bond dissociation enthalpies (BDEs) of these compounds were estimated by studying, by means of EPR spectroscopy, the equilibration of each of these phenols and 2,6-di-*tert*-butyl-4-methylphenol (BHT) with the corresponding phenoxyl radicals. The antioxidant activity of the investigated phenols was also determined by measuring the rate constants for their reaction with peroxyl radicals in controlled autoxidation experiments and compared to that recorded under identical experimental settings for [60]fullerene itself and unlinked BHT. The results indicate that linking of the BHT structure to C_{60} does not substantially alter the thermochemistry and kinetics of its reaction with peroxyl radicals, but such adducts may behave as interesting bimodal radical scavengers. The inherent rate constant for trapping of peroxyl radicals by C_{60} per se ($k_{inh} = 3.1 \pm 1.1 \times 10^2\,M^{-1}s^{-1}$) indicates that, contrary to previous reports, [60]fullerene is an extremely weak chain-breaking antioxidant.

Keywords: antioxidants · EPR spectroscopy · fullerenes · radical reactions · radicals

Introduction

Oxidative damage plays a significant pathological role in human diseases such as cancer and age-related neurodegenerative diseases.[1,2] This oxidative damage, mainly due to free radicals and reactive oxygen species (ROS), can be prevented by antioxidants. It is generally accepted that diets rich in food containing antioxidants help to reduce oxidative damage in humans.[3,4] In recent years, it was shown that fullerene derivatives can be used as protective drugs against neurodegenerative diseases related to oxidative stress.[5–8] This observation is directly related to the fact that fullerenes and their organic derivatives can trap several radicals per molecule; these compounds may be regarded as "radical sponges".[9,10]

This makes [60]fullerene an interesting lead structure in the development of novel radical-scavenging compounds with specific functionalities. For instance, it was recently shown that a number of water-soluble fullerene derivatives behave as potent ROS scavengers in cell cultures and can protect human skin keratinocytes from UV irradiation and oxidative damage by *tert*-butyl hydroperoxide.[11]

Recently, some of us described the synthesis of new fullerene derivatives bearing structural moieties with known antioxidant activity (e.g., flavonoids).[12] Here we report the synthesis and antioxidant activity of four C_{60} derivatives incorporating one or two aryl groups structurally related to BHT,[13] a phenolic antioxidant widely used in the food industry.[14,15]

The aim of this project is to develop phenolic antioxidants with special functionalities, such as limited diffusion in polymeric matrices and biomembranes, or extended reactivity toward alkyl radicals,[16] and to investigate possible variations in the antioxidant behavior of the BHT-like structure as a consequence of its conjugation with [60]fullerene, particularly a synergistic effect between the two type of antioxidants (the [60]fullerene moiety and the phenolic antioxidant).

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[**] BHT = 2,6-Di-*tert*-butyl-4-methylphenol.

To evaluate the antioxidant activity of these compounds we measured two physicochemical parameters which are believed to be crucial in determining the ease with which phenolic antioxidants reduce the rate of oxidation of oxidizable substrates, that is, the bond dissociation enthalpy (BDE) of the O–H bond[19] broken during the inhibition reaction and the rate constant k_{inh} for the reaction of the antioxidant with peroxyl radicals.[20,21]

Results and Discussion

Synthesis: [60]Fullerene derivatives **1**–**4** were obtained in low to moderate yields (16–58%) by 1,3-dipolar cycloaddition reactions of C_{60} with azomethine ylides generated in situ in refluxing toluene, as already described for similar systems.[12] Products were purified by flash chromatography on silica with toluene/cyclohexane.

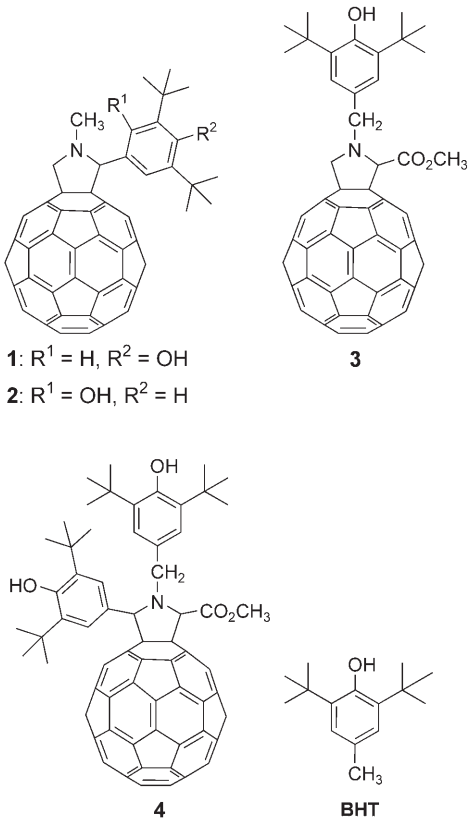
Fullerene derivatives **1** and **2** were obtained from the reaction of the commercially available aldehydes **5** and **6**, respectively, with *N*-methylglycine and C_{60} (Scheme 1). While compound **1** was obtained in 58% yield, its isomer **2** was isolated in only 16% yield. This difference reflects the lower reactivity of 2-hydroxybenzaldehyde derivative **6**, presumably due to intramolecular hydrogen bonding.

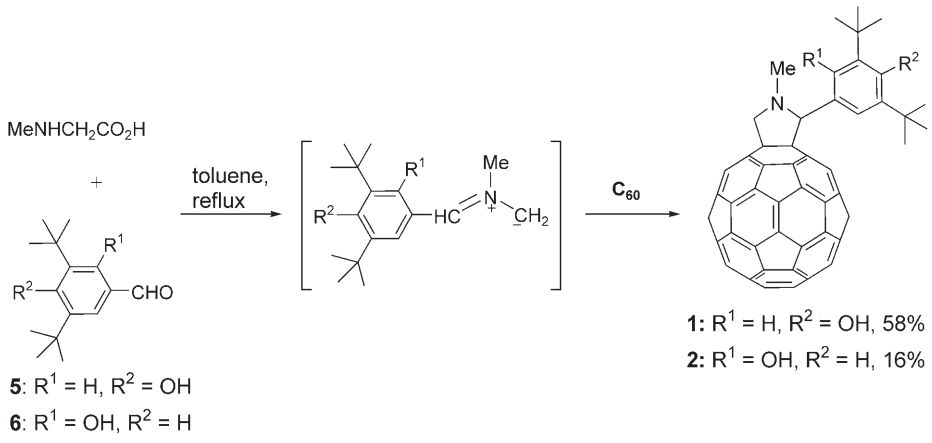
The mass spectra of compounds **1** and **2** show the $[M+H]^+$ ion (*m*/z 982) and the NMR spectra confirm the expected structures. The main difference in the ^{1}H NMR spectra of the two isomers is the position of the OH proton signal, which appears at $\delta = 4.85$ ppm in **1**, but at $\delta = 11.34$ ppm in **2** due to intramolecular hydrogen bonding with the nitrogen atom of the pyrrolidine ring. This intramolecular hydrogen bonding hampers rotation around the C2–Ar bond at room temperature.[22] This is confirmed by the well-resolved signals corresponding to H-4 and H-6 of the aryl group, which appear as an AB spin system at $\delta = 7.11$–7.13 ppm with $J = 2.4$ Hz.

In the ^{1}H NMR spectrum of **1** the signal corresponding to the *ortho* protons of the aryl group is a broadened singlet, indicative of restricted rotation of this aryl group. This effect has already been observed in similar compounds.[23,24] Synthesis of **3** and **4** required use of glycine derivative **8**, which was prepared in two steps by reductive amination of aldehyde **5** with glycine methyl ester in 90% overall yield (Scheme 2). The structures of **7** and **8** were confirmed by ^{1}H and ^{13}C NMR and MS.

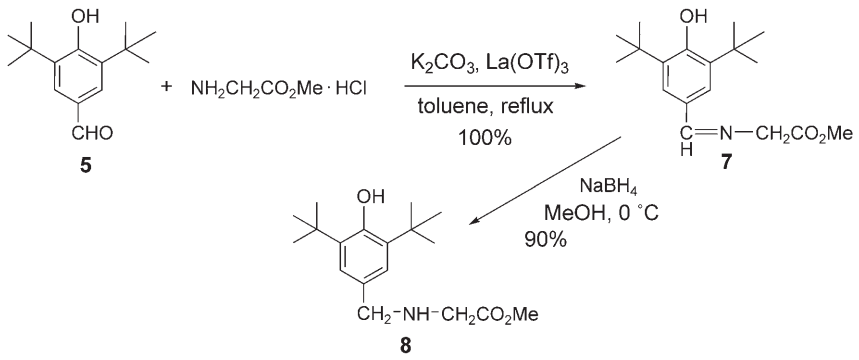
Compound **3** was obtained in reasonable yield by reaction of glycine derivative **8**, paraformaldehyde and C_{60} in refluxing toluene (Scheme 3). Compound **4** was obtained under similar conditions from aldehyde **5** with $La(OTf)_3$ as catalyst. In the absence of the catalyst the reaction does not occur. Although formation of two adducts **4** (with *cis* or *trans* configuration) could be expected, only one diastereoisomer was detected. The absence of correlation between pyrrolidine protons H-2 and H-5 in the NOESY spectrum of **4** presumably indicates that these protons are in a *trans* configuration.

In both **3** and **4**, the NCH_2 protons are diastereotopic and geminal coupling is observed in the ^{1}H NMR spectra. While the resonances of these protons appear as a AB system ($J = 13.5$ Hz) in **3**, in **4** they appear as two doublets ($J = 13.9$ Hz).

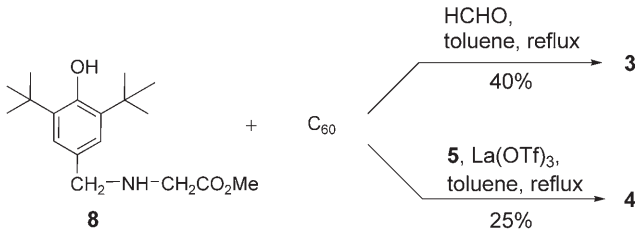




Scheme 1.



Scheme 2.



Scheme 3.

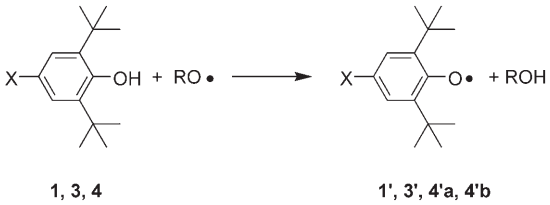
The signals of the *ortho* protons of the substituted benzyl group in compounds **3** and **4** appear as sharp singlets at $\delta =$ 7.42 and 7.52 ppm, respectively. In contrast, and as observed for **1** and **2**, in the ^{1}H NMR spectrum of compound **4** the resonances of the *ortho* protons of the aryl group at the 5-position of the pyrrolidine ring appear as a broad singlet due to restricted rotation of the aryl group. In the ^{13}C NMR spectra of compounds **3** and **4** the signal corresponding to the ester carbonyl group appears at $\delta = 170.5$ and 172.1 ppm, respectively.

EPR spectra of the free radicals obtained from 1–4: Radicals were produced at room temperature inside the cavity of an EPR spectrometer by reaction of **1–4** with alkoxyl radicals generated photolytically from di-*tert*-butyl peroxide in deoxygenated benzene solution [Eq. (1)]. In the case of **1**, **3**, and **4** highly persistent radicals were obtained whose EPR spectra showed good signal-to-noise ratio and *g* factors of 2.0046–2.0047 (see Table 1 for spectral parameters). When solutions of **2** were photolyzed the only signal observed was a broad singlet centered at $g =$ 2.0024 with a peak-to-peak line width of 1.9 G. On the basis of the small *g* factor and the line width, we attribute this singlet to a persistent radical adduct to the [60]fullerene moiety. Similar spectra were observed by Krusic et al.[25] when photolyzing toluene or benzene solutions of C_{60} in the presence of a variety of radical precursors and attributed to C_{60}–radical adducts. The same singlet, superimposed on the spectrum of the phenoxyl radical, was also observed on prolonged photolysis of solutions of **3**. The reason why no phenoxyl radical from **2** could be detected is likely a combination of two factors, that is, its low persistency due to the absence of a second *tert*-butyl substituent *ortho* to the radical oxygen atom, and the very limited reactivity of the OH group toward hydrogen abstraction due to intramolecular hydrogen bonding with the pyrrolidine nitrogen atom.

Table 1. EPR spectral parameters for the phenoxyl radicals obtained by abstraction of the OH hydrogen atom from **1**, **3**, and **4**.

Phenol	Radical	$a(2H_m)$ [G]	a(N) [G]	a(other) [G]	g factor
1	**1′**	1.85	0.80	4.80 (H-2)[a]	2.0046
3	**3′**	1.80	1.80	11.20 (2H)[b,c]	2.0047
				12.20 (2H)[b,c]	
	4′a	1.84	1.53	7.60 (H-2)[a]	2.0046
	4′b	1.76	1.40	1.74 (H-5)[a]	2.0046

[a] Hyperfine splitting (hfs) constants at the pyrrolidine ring protons. [b] Average value of the hfs constants at the two diastereotopic benzyl protons. [c] Only the two outer multiplets were observed due to selective line-broadening effects.



(1)

Photolysis of **4** in the presence of the peroxide gave rise to an EPR pattern consisting of the superimposition of spectra due to two different species arising from the H abstraction from the OH group on the substituted benzyl group (**4′a**) and from the OH group on aryl ring directly bonded to the pyrrolidine ring (**4′b**). The observed EPR spectrum and its computer simulation are shown in Figure 1.

Interestingly, in the spectra of both radicals **3′** and **4′a**, the central line of the expected 1:2:1 triplet due to the benzyl protons was not observed, while the separation between the two outer lines (ca. 22–24 G) was twice as large as the hyperfine splitting constant of the methyl protons in the radical from BHT (11.2 G).[19] This means that central triplet line is strongly broadened by spin-relaxation effects. A similar behavior was previously observed in the EPR spectra of the phenoxyl radicals obtained from 2,6-di-*tert*-butylphenols substituted at the 4-position with unsymmetrical *N*,*N*′-dialkylaminomethyl groups $CH_2N(R)R'$ and attributed to the magnetic inequivalence of the benzyl protons, which are diastereotopic due to slow inversion at nitrogen on the EPR timescale.[26] Since the same explanation can also be given in the present case, we tried to accelerate the rate of interconversion between the benzyl proton splittings to reach the

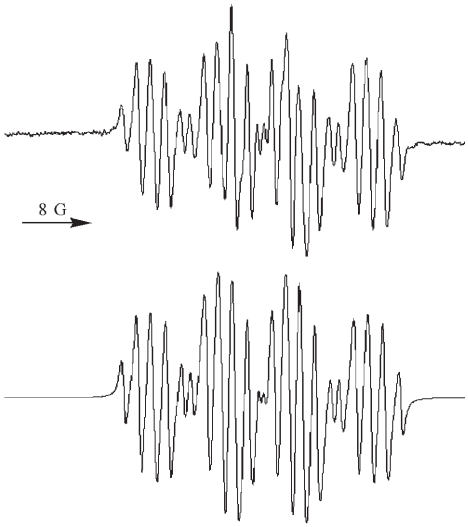


Figure 1. Room-temperature experimental (top) and simulated (bottom) EPR spectrum observed under continuous irradiation of a deoxygenated benzene solution of **4** containing di-*tert*-butyl peroxide The outer multiplets are due to radical **4′a** (see text for additional details), and the central part of the spectrum is due to **4′b**.

fast-exchange region where the selective line-broadening effect might disappear.[27] Thus, the sample temperature was increased to the boiling point of benzene, but no substantial changes in the EPR spectrum were detected.

The remarkable difference between the hyperfine splittings at the pyrrolidine nuclei (both nitrogen and protons) in the similar phenoxyl radicals **1′** and **4′b** is also noteworthy. This is likely due to different geometry of the two radicals arising from the very strong steric crowding in **4′**.

O–H bond dissociation enthalpies (BDE): To measure the O–H BDEs of the title compounds we used the EPR radical equilibration technique, which, among the various experimental methods for the determination of bond strengths, seems to guarantee the best accuracy at present.[19,28] For this purpose we measured the equilibrium constant K_e for hydrogen-atom transfer between BHT as reference phenol Ar′OH (revised BDE value 79.9 kcal mol^{-1}[29]), one of the phenols **1**, **3**, and **4** (ArOH), and the corresponding phenoxyl radicals [Eq. (2)] generated under continuous photolysis in deoxygenated benzene at room temperature (25 °C).

$$\mathrm{ArOH} + \mathrm{Ar'O^{\cdot}} \rightleftharpoons \mathrm{ArO^{\cdot}} + \mathrm{Ar'OH} \qquad (2)$$

Experiments were performed on benzene solutions with the highest concentration of the [60]fullerene derivatives compatible with their low solubility (ca. 10^{-3} M). The persistence of the related phenoxyl radicals guarantees achievement of equilibrium, despite the relatively low concentration of their precursors. In the calculation of K_e, the initial concentrations of ArOH and Ar′OH were used, while the relative radical concentrations were determined by means of EPR spectroscopy. The BDEs for ArOH were calculated, under the assumption that the entropic term can be neglected,[27] by means of Equation (3) from K_e and the BDE of BHT.

$$\mathrm{BDE(ArO{-}H)} \approx \mathrm{BDE(Ar'O{-}H)} - RT\ln(K_e) \qquad (3)$$

Measurements were repeated under different light intensity to check the constancy of K_e. The BDEs obtained in benzene solution (Table 2) show that all the C_{60}-containing phenols are characterized by O–H bond strengths very close to that of their precursor BHT. This suggests that the [60]fullerene moiety is not substantially involved in the hydrogen transfer reaction [Eq. (2)]. Indeed, closer inspection of the data in Table 2 reveals that the C_{60}-containing substituent slightly increases the O–H BDE, perhaps due to the weak electron-withdrawing behavior expected for [60]fullerene.

Table 2. O–H BDEs for **1**–**4** measured at room temperature in benzene containing 10% di-*tert*-butyl peroxide, rate constants k_{inh} for their reaction with peroxyl radicals in cumene at 30 °C, and number of radicals n trapped by each antioxidant molecule.

Compound	BDE [kcal mol^{-1}]	k_{inh} [10^3 M^{-1} s^{-1}]	n
1	80.1 ± 0.1	7.3 ± 1.1	2
2	–	0.40 ± 0.10	n.d.
3	80.3 ± 0.2	8.2 ± 1.1	2
4	80.4 ± 0.3[a]	6.7 ± 1.3	4
	80.3 ± 0.3[b]		
BHT	79.9 ± 0.1[c]	10.7 ± 1.0	2
[60]fullerene	–	0.31 ± 0.11	n.d.

[a] For the OH group linked to the substituted benzyl group (radical **4′a**). [b] For the OH group linked to the aryl ring directly bonded to the pyrrolidine ring (radical **4′b**). [c] Recalculated from the data of ref. [15] on the basis of the revision of the O–H BDE of reference phenol tBu$_3$C$_6$H$_2$OH from 81.2 to 80.1 kcal mol^{-1}.[30]

Inhibition rate constants: The rate constants k_{inh} for reaction of **1**, **3**, and **4** with peroxyl radicals were determined by studying the inhibition of the thermally initiated autoxidation of cumene.[21] Cumene was chosen because of its lower oxidizability, which magnifies the antioxidant behavior of a given compound and allows the antioxidant activity of moderately effective inhibitors to be more easily differentiated.

The reaction was followed by monitoring the oxygen consumption (Figure 2) during the autoxidation with an automatically recording gas-absorption apparatus, built in our laboratory and described previously,[32] which uses as detector a commercial differential pressure transducer. The reactions [Eqs. (4)–(9)], initiated by the thermal decomposition of 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN), were carried out at 30 °C under controlled conditions in air-saturated solutions of cumene, both in the absence and in the presence of each antioxidant; BHT was used as reference chain breaking inhibitor.

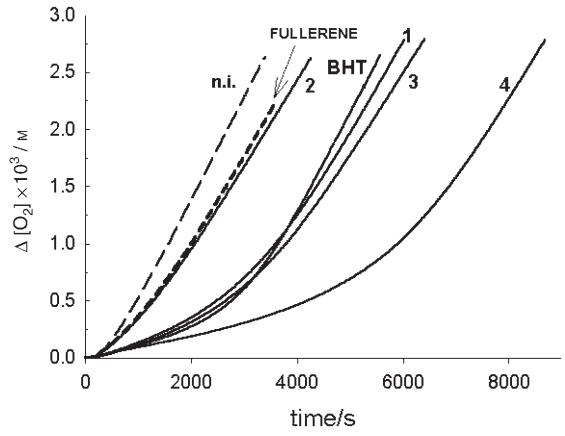


Figure 2. Oxygen consumption observed during the autoxidation of cumene initiated by AMVN (5×10^{-3} M) at 30 °C in the absence of any antioxidant (not inhibited, n.i.) and in the presence of BHT, [60]fullerene, or each of the four investigated derivatives (5.0×10^{-6} M).

$$\text{Initiator}\xrightarrow{R_i}\text{R}^{\cdot} \quad (4)$$

$$\text{R}^{\cdot} + \text{O}_2 \rightarrow \text{ROO}^{\cdot} \quad (5)$$

$$\text{ROO}^{\cdot} + \text{RH}\xrightarrow{k_p}\text{ROOH} + \text{R}^{\cdot} \quad (6)$$

$$\text{ROO}^{\cdot} + \text{ROO}^{\cdot}\xrightarrow{2k_t}\text{products} \quad (7)$$

$$\text{ROO}^{\cdot} + \text{ArOH}\xrightarrow{k_{inh}}\text{ROOH} + \text{ArO}^{\cdot} \quad (8)$$

$$\text{ROO}^{\cdot} + \text{ArO}^{\cdot} \rightarrow \text{products} \quad (9)$$

The inhibition rate constant k_{inh} [Eq. (8)] of each compound was determined by means of a kinetic treatment[32] consisting of measuring the initial rates of oxidation of cumene both in the presence ($-\text{d}[\text{O}_2]/\text{d}t = R_{ox}$) and absence ($(-\text{d}[\text{O}_2]/\text{d}t)_0 = R_{ox,0}$) of antioxidant, ArOH, and calculating k_{inh} from these data by means of Equation (10).

$$\frac{R_{ox,0}}{R_{ox}} - \frac{R_{ox}}{R_{ox,0}} = \frac{nk_{inh}[\text{AH}]_0}{\sqrt{2k_tR_i}} \quad (10)$$

This equation allows evaluation of k_{inh} even when the inhibition and termination [Eq. (7)] rates are comparable. The use of Equation (10) requires knowledge of the initiation rate R_i, which was determined in preliminary experiments as described in the Experimental Section, and the termination constant $2k_t$ for the self-combination of cumylperoxyl radicals. Unfortunately, the value of this constant, measured by following the decay of the cumylperoxyl radicals, shows considerable variations with cumene concentration.[33] This is due to the irreversible decomposition of the tetroxide, formed in reaction (7), to give molecular oxygen and two caged alkoxyl radicals, 10% of which combine to give the corresponding peroxide, while the greater portion (ca. 90%) escapes into the reaction medium and undergoes reactions typical of the RO$^{\cdot}$ radical such as hydrogen-atom abstraction and β scission.[21]

The true value of $2k_t$ for cumylperoxyl radicals was obtained by an indirect procedure by measuring the ratio $k_{inh}/\sqrt{2k_t}$ [Eq. (10)] during the initiated oxidation of cumene inhibited by BHT. Under these conditions the formation of tetroxide from cumylperoxyl radicals is almost negligible, since most of them terminate by reacting with the antioxidant [Eqs. (8) and (9)]. Then, a very accurate determination of the value of BHT was made by using styrene as oxidizable substrate and analyzing the data obtained at different antioxidant concentrations with the method proposed by Darley-Usmar et al.[34] The resulting k_{inh} value of $1.1\times10^4\,\text{M}^{-1}\,\text{s}^{-1}$ is only slightly different from that determined for BHT by Ingold et al. ($1.4\times10^4\,\text{M}^{-1}\,\text{s}^{-1}$).[20] By inserting the inhibition rate constant in the ratio $k_{inh}/\sqrt{2k_t}$ experimentally determined as $50.2\,\text{M}^{-1/2}\,\text{s}^{-1/2}$, the $2k_t$ value at 30 °C was obtained as $4.6\times10^4\,\text{M}^{-1}\,\text{s}^{-1}$.[35]

A second determination of $2k_t$ for cumene at 30 °C was made by following, by EPR spectroscopy, the decay of the signal due to the cumylperoxyl radical in pure cumene. Under these conditions (cumene concentration 7.2 M) the fragmentation of cumyloxyl radicals to acetophenone and methyl radicals ($k_f = 1.23\times10^6\,\text{s}^{-1}$)[36] is 15 times slower than hydrogen-atom abstraction from cumene ($k_H = 2.6\times10^6\,\text{M}^{-1}\,\text{s}^{-1}$)[36] and thus the measured termination rate constant is expected to be very close to the true value. This determination led to a $2k_t$ value of $4.5\times10^4\,\text{M}^{-1}\,\text{s}^{-1}$, which is surprisingly similar to that obtained from autoxidation experiments.

The term n represents the stoichiometric coefficient, that is, the number of peroxyl radicals trapped by each antioxidant molecule, and can be determined from Equation (11) by measuring the length of the induction period τ during which the rate of oxygen consumption in the inhibited autoxidation of cumene is strongly reduced. The results of these determinations are reported in Table 2.

$$n = \frac{R_i\tau}{[\text{AH}]} \quad (11)$$

From Figure 2 it can be inferred that **1** and **3** show, during the inhibition period, moderate antioxidant activity, comparable to that of BHT. In fact, the inhibition rate constants are only slightly lower than that of BHT, that is, the presence of the [60]fullerene group does not improve the radical-scavenging ability of BHT. Phenol **2**, on the other hand, is practically devoid of any antioxidant activity, presumably due to formation of an intramolecular hydrogen bond between the phenolic OH group and the nitrogen atom of the pyrrolidine ring ($\delta(\text{OH}) = 11.34$ ppm in the ^{1}H NMR spectrum), which leads to a large increase in O–H bond strength. Compound **4**, although characterized by a k_{inh} value similar to those of **1** and **3**, shows an antioxidant efficacy lasting twice as long as that of the other phenols. This can be easily explained in terms of the presence of two radical-scavenging units.

Careful examination of Figure 2 shows that the slopes of the oxygen-consumption plots for **1**, **3**, and **4**, after the end

of the inhibition period, are not exactly parallel to that observed with BHT. This might suggest that these compounds, even when no more phenolic hydrogen atoms are present in solution, still retain a weak scavenging ability for peroxyl radicals, conceivably due to the C_{60} group per se. An investigation by Hwang et al.[37] attributes to C_{60} an antioxidant activity in liposomes higher than that of α-tocopherol, based on the relative ability of a series of antioxidants (at the same concentration) to prevent pH changes in the aqueous phase internal to the liposomes during the generation of hydroxyl or superoxide radicals. The authors, who were themselves surprised at this result, suggest that this is due to the multiple binding sites (30 double bonds) available on C_{60} to accept several peroxyl radicals per molecule, as opposed to α-tocopherol, which is able to quench "only" two peroxyl units. Although the ability of [60]fullerene to add several *tert*-butylpexoxyl radicals to form stable multiple peroxides has been proven by NMR spectroscopy,[38] it should be kept in mind that the antioxidant ability of a given compound does not merely reflect the stoichiometry of its reaction with peroxyl radical, but it is mainly due to the rate of such reaction. For this reason we measured the antioxidant activity of unsubstituted [60]fullerene under the same experimental conditions employed for its derivatives **1**–**4**. The resulting rate constant for peroxyl radical trapping ($k_{inh} = 313\ M^{-1}s^{-1}$) is not significantly different from the value recorded for compound **2** (see errors in Table 2) and indicates that, contrary to previous reports, [60]fullerene itself is an extremely weak chain-breaking antioxidant. When this value is divided by 30, that is, the number of double bonds in the C_{60} structure, its "intrinsic" reactivity ($k \approx 10\ M^{-1}s^{-1}$) with peroxyl radical is not greater than that of styrene ($k \approx 41\ M^{-1}s^{-1}$).[21] The reason why [60]fullerene behaves as a poor antioxidant rather than propagating the oxidation chain like styrene is the lack of reactivity of the resulting extensively conjugated carbon-centered radical. In this respect, C_{60} shows a reactivity quite similar to that of other polyenes such as β-carotene.[39,40]

Thus, the rate of reaction of [60]fullerene itself, or the [60]fullerene moiety in the investigated derivatives, with peroxyl radicals does not justify further development of C_{60}-linked phenols. However, the C_{60} unit is known to be an extremely efficient trap for alkyl radicals that outperforms BHT nearly 3000-fold and even α-tocopherol 23-fold, as can be judged by comparison of their rate constants of reaction at room temperature.[16] Due to the high reactivity of carbon-centered radicals with molecular oxygen, this additional radical-scavenging ability would be relevant only at low partial pressures of oxygen, for example, in vivo under hypoxic conditions or in bulk polymers, where oxygen diffusion is limited.

Conclusion

Three different types of [60]fullero[*c*]pyrrolidines bearing one or two 3,5-di-*tert*-butyl-4-hydroxyphenyl groups were synthesized and their antioxidant activities determined. The experimental results suggest that the introduction of the [60]fullerene moieties affects only marginally the overall rate of reaction with peroxyl radicals with respect to the simpler precursor BHT. The lower rates of inhibition correlate nicely with the corresponding slightly higher O–H BDE values.[41]

Although these compounds behave as moderately efficient antioxidants under aerobic conditions, an additional radical-scavenging mode, that is, efficient trapping of alkyl radicals, is expected to be contributed by the C_{60} group under hypoxic conditions, and this would make these phenolic C_{60} derivatives interesting bimodal radical scavengers that could find interesting applications in pharmaceuticals and polymer chemistry (e.g., as polymerization inhibitors and antioxidants).[42] In these areas the bulky C_{60} unit could also contribute additional properties to these adducts, for example, limited mobility or preferential location in some biological compartment.

Experimental Section

^1H and ^{13}C NMR spectra were recorded on Bruker Avance 300 or 500 spectrometers at 300 or 500 MHz and 75 or 125 MHz, respectively. $CDCl_3$ and $CDCl_3/CS_2$ were used as solvents, and TMS was used as internal reference. Mass spectra and HRMS were recorded on VG AutoSpec Q and M mass spectrometer. *m*-Nitrobenzyl alcohol was used as matrix for FAB^+ mass spectrometry. Melting points were determined with a Reichert Thermovar electric instrument and are uncorrected. Flash chromatography was carried out with silica gel 0.032–0.063 mm.

Synthesis of C_{60} derivatives 1 and 2: General procedure: A solution of C_{60} (70 mg, 9.7×10^{-5} mol), *N*-methylglycine (43 mg, 5 equiv), and aldehyde **5** or **6** (24 mg, 1 equiv) in toluene (60 mL), was heated at reflux under N_2 for 6 h. The mixture was concentrated and purified by flash chromatography with a gradient of cyclohexane/toluene as eluent. The first fraction was unconsumed C_{60}, and the second the monoadduct. Compounds **1** and **2** were crystallized from CS_2/chloroform.

1-Methyl-2-(3,5-bis-*tert*-butyl-4-hydroxyphenyl)[60]fullero[*c*]pyrrolidine (1): M.p. >310 °C. ^1H NMR (500 MHz, $CDCl_3/CS_2$): $\delta = 1.36$ (s, 18H; *t*Bu), 2.79 (s, 3H; NCH_3), 4.20 (d, $J = 9.3$ Hz, 1H; H-5), 4.85 (s, 1H; OH), 4.89 (d, $J = 9.3$ Hz, 1H; H-5), 5.05 (s, 1H; H-2), 7.46 ppm (br s, 2H; ArH); ^{13}C NMR (125 MHz, $CDCl_3/CS_2$): $\delta = 30.2$ ($C(CH_3)_3$), 34.1 ($C(CH_3)_3$), 39.8, 68.6, 69.8, 77.6, 83.8, 125.9, 127.1, 135.6, 135.7, 136.3, 139.0, 139.4, 139.8, 140.0, 141.3, 141.4, 141.5, 141.7, 141.8, 141.90, 141.97, 142.0, 142.1, 142.4, 142.5, 142.8, 143.0, 144.2, 144.5, 144.6, 144.9, 145.0, 145.16, 145.23, 145.3, 145.4, 145.6, 145.7, 145.88, 145.92, 146.0, 146.1, 146.4, 146.7, 147.1, 153.4, 153.6, 153.9, 154.2, 156.2 ppm; MS (FAB^+): *m*/z: 982 $[M+H]^+$, 720 $[C_{60}^{\bullet+}]$; HRMS (ESI): *m*/z calcd for $C_{77}H_{28}NO$ $[M+H]^+$: 982.2165, found: 982.2174.

1-Methyl-2-(3,5-bis-*tert*-butyl-2-hydroxyphenyl)[60]fullero[*c*]pyrrolidine (2): M.p. >310 °C. ^1H NMR (300 MHz, $CDCl_3/CS_2$): $\delta = 1.18$ (s, 9H; *t*Bu), 1.31 (s, 9H; *t*Bu), 3.06 (s, 3H; NCH_3), 4.28 (d, $J = 9.5$ Hz, 1H; H-5), 5.06 (d, $J = 9.5$ Hz, 1H; H-5), 5.07 (s, 1H; H-2), 7.12 and 7.13 (AB, $J = 2.4$ Hz, 2H; ArH), 11.34 ppm (s, 1H; OH); ^{13}C NMR (75 MHz, $CDCl_3/CS_2$): $\delta =$ 29.0, 31.3 ($C(CH_3)_3$), 33.6, 34.6 ($C(CH_3)_3$), 40.1, 68.0, 69.4, 76.8, 84.5, 117.9, 123.7, 124.2, 135.1, 136.23, 136.27, 136.7, 138.6, 139.3, 139.7, 139.8, 140.16, 140.23, 141.05, 141.09, 141.2, 141.4, 141.6, 141.69, 141.73, 141.76, 141.81, 141.87, 141.91, 142.15, 142.22, 142.4, 143.96, 143.97, 144.2, 144.69, 144.77, 144.84, 144.86, 145.1, 145.27, 145.28, 145.46, 145.51, 145.55, 145.68, 145.79, 145.84, 145.9, 146.1, 146.2, 146.9, 147.0, 151.4, 152.5, 152.6, 152.9, 154.6 ppm; MS (FAB^+): *m*/z: 982 $[M+H]^+$, 720 $[C_{60}^{\bullet+}]$; HRMS (ESI): *m*/z calcd for $C_{77}H_{28}NO$ $[M+H]^+$: 982.2165, found: 982.2180.

Synthesis of C_{60} derivatives 3 and 4: General procedure: a solution of C_{60} (70 mg, 9.7×10^{-5} mol), glycine derivative **8** (36 mg, 1.2 equiv), and paraformaldehyde (5.9 mg, 2 equiv) or aldehyde **5** (35 mg, 1.5 equiv) in toluene (60 mL) was heated at reflux under N_2 for 6 h. For the synthesis of compound **4**, La(OTf)$_3$ (5.7 mg, 0.1 equiv) was also added. The mixture was concentrated and purified by flash chromatography with a gradient of cyclohexane/toluene as eluent. The first fraction was unconsumed C_{60}, and the second the monoadduct. Compounds **3** and **4** were crystallized from chloroform/methanol.

Methyl 1-(3,5-bis-*tert*-butyl-4-hydroxyphenylmethyl)[60]fullero[*c*]pyrrolidine-2-carboxylate (3): M.p. 147–149 °C. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=$ 1.53 (s, 18H; *t*Bu), 3.89 (s, 3H; OCH_3), 4.29 and 4.49 (AB, $J=13.5$ Hz, 2H; NCH_2Ar), 4.35 (d, $J=9.4$ Hz, 1H; H-5), 4.98 (d, $J=9.4$ Hz, 1H; H-5), 5.06 (s, 1H), 5.29 (s, 1H), 7.42 ppm (s, 2H; ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=30.4$ ($C(CH_3)_3$), 34.4 ($C(CH_3)_3$), 52.3, 55.3, 64.2, 69.3, 72.8, 75.6, 126.0, 126.5, 130.1, 135.6, 136.0, 136.2, 136.5, 137.7, 139.7, 139.8, 140.2, 140.3, 141.8, 141.9, 141.97, 142.01, 142.08, 142.11, 142.2, 142.3, 142.62, 142.64, 142.7, 143.0, 144.4, 144.48, 144.54, 144.7, 145.26, 145.27, 145.30, 145.36, 145.40, 145.5, 145.6, 145.7, 145.8, 145.9, 146.04, 146.06, 146.2, 146.3, 146.4, 147.3, 147.4, 151.3, 153.4, 153.6, 154.7, 154.8, 170.5 ppm (C=O); MS (FAB$^+$): *m*/z: 1040 $[M+H]^+$, 720 $[C_{60}^{\cdot+}]$; HRMS (ESI): *m*/z calcd for $C_{79}H_{30}NO_3$ $[M+H]^+$: 1040.2220, found: 1040.2200.

Methyl 5-(3,5-bis-*tert*-butyl-4-hydroxyphenyl)-1-(3,5-bis-*tert*-butyl-4-hydroxyphenylmethyl)[60]fullero[*c*]pyrrolidine-2-carboxylate (4): M.p. >310 °C. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.37$ (s, 18H; 5-(Ar*t*Bu)), 1.51 (s, 18H; 1-(Ar*t*Bu)), 3.89 (s, 3H; OCH_3), 4.03 (d, $J=13.9$ Hz, 1H; NCH_2Ar), 4.62 (d, $J=13.9$ Hz, 1H; NCH_2Ar), 5.17 (s, 1H; 5-ArO*H*), 5.23 (s, 1H; 1-ArO*H*), 5.55 (s, 1H; H-2), 6.57 (s, 1H; H-5), 7.48 (brs, 1H; 5-ArH), 7.52 (s, 2H; 1-ArH), 7.92 ppm (brs, 1H; 5-ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=30.40$ (5-ArC(CH_3)$_3$), 30.44 (1-ArC(CH_3)$_3$), 34.44 (5-ArC(CH_3)$_3$), 34.48 (1-ArC(CH_3)$_3$), 51.3 (CH_2Ar), 51.6 (OCH_3), 70.3 (C_{60}-sp^3), 73.2 (C-2), 76.7 (C-5 and C_{60}-sp^3), 124.9, 127.3, 128.8, 134.9, 135.9, 136.0, 136.1, 136.2, 137.5, 139.1, 139.7, 139.8, 141.46, 141.52, 141.55, 141.9, 142.97, 142.05, 142.15, 142.18, 142.3, 142.55, 142.61, 142.7, 142.9, 145.05, 145.17, 145.20, 145.23, 145.36, 145.42, 145.47, 145.50, 145.53, 145.79, 145.86, 145.90, 145.92, 146.05, 146.08, 146.14, 146.4, 146.6, 147.0, 147.3, 147.4, 151.0, 151.6, 152.4, 153.1, 153.66, 153.75, 155.15, 156.15, 172.1 ppm (C=O); MS (FAB$^+$): *m*/z: 1244 $[M+H]^+$, 720 $[C_{60}^{\cdot+}]$; HRMS (ESI): *m*/z calcd for $C_{93}H_{50}NO_4$ $[M+H]^+$:1244.3734, found: 1244.3745.

Synthesis of glycine derivative 8: Glycine methyl ester hydrochloride (154 mg, 3 equiv), K_2CO_3 (170 mg, 3 equiv), and La(OTf)$_3$ (24 mg, 0.1 equiv) were added to a solution of aldehyde **1** (100 mg, 0.41 mmol) in anhydrous toluene (40 mL). The reaction mixture was refluxed under nitrogen atmosphere for 14 h. The mixture was cooled to room temperature and filtered, and the solvent evaporated to afford imine **7** as a yellow solid (125 mg, 100% yield). M.p. 178–180 °C. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.46$ (s, 18H; *t*Bu), 3.77 (s, 3H; OCH_3), 3.37 (s, 2H; α-CH_2), 5.54 (s, 1H; OH), 7.59 (s, 2H; ArH), 8.20 ppm (s, 1H; N=C*H*Ar); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=30.2$ ($C(CH_3)_3$), 34.3 ($C(CH_3)_3$), 52.0, 61.9, 125.8, 127.6, 136.1, 156.8, 166.2, 171.0 ppm; MS (EI$^+$): *m*/z (%): 305 (26) $[M^{\cdot+}]$, 290 (27), 246 (12), 234 (36), 219 (100), 203 (7).

Imine **7** (125 mg, 0.41 mmol) was dissolved in anhydrous methanol (10 mL), the solution cooled to 0 °C, and $NaBH_4$ (47 mg, 3 equiv) added. The mixture was stirred for 15 min under nitrogen atmosphere at 0 °C. Acetic acid (0.8 mL) was then added and the solvent was evaporated. The resulting residue was dissolved in chloroform (60 mL) and washed with water (2×25 mL). The organic phase was dried (Na_2SO_4) and the solvent was evaporated to afford compound **8** (113 mg, 90% yield). M.p. 72–74 °C. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.43$ (s, 18H; *t*Bu), 3.45 (s, 2H; α-CH_2), 3.70 (s, 2H; NCH_2Ar), 3.73 (s, 3H; OCH_3), 5.15 (s, 1H; OH), 7.11 ppm (s, 2H; ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=30.3$ (C-(CH_3)$_3$), 34.3 ($C(CH_3)_3$), 50.0, 51.7, 53.7, 125.1, 129.9, 135.8, 152.9, 173.0 ppm; MS (EI+): *m*/z (%): 307 (21) $[M^{\cdot+}]$, 292 (8), 250 (23), 248 (4), 234 (78), 219 (100), 203 (16); elemental analysis (%) calcd for $C_{18}H_{29}NO_3$: C 70.32, H 9.51, N 4.56; found: C 70.42, H 9.24, N 4.84.

Kinetic measurements: The rate constants for the reaction of the title compounds with peroxyl radicals were measured by following the autoxidation of pure cumene at 303 K with AMVN (5×10^{-3} M) as initiator. The reaction was performed in a oxygen-uptake apparatus built in our laboratories and based on a Validyne DP15 differential-pressure transducer, which has been previously described in detail.[31] The entire apparatus was immersed in a thermostatically controlled bath which ensured a constant temperature within ±0.1 °C.

In a typical experiment, air-saturated cumene containing the antioxidant was equilibrated with the reference solution containing an excess of α-tocopherol (1×10^{-3} to 1×10^{-2} M) in the same solvent at 30 °C. After equilibration, a concentrated chlorobenzene solution of AMVN was injected into both the reference and sample flasks, and the oxygen consumption in the sample was measured, after calibration of the apparatus, from the differential pressure recorded with time between the two channels. This instrumental setting allowed the N_2 production and the oxygen consumption due to decomposition of the azo initiator to already be subtracted from the measured reaction rates. The antioxidant concentration was kept constant for all measurements (5.0×10^{-6} M) in order to compare more easily their behavior. Initiation rates R_i were determined for each condition in preliminary experiments by the inhibitor method with α-tocopherol as reference antioxidant: $R_i=2[\alpha\text{-TOH}]/\tau$.[21]

EPR and thermochemical measurements: Deoxygenated benzene solutions containing the phenols (0.01–0.001 M), and di-*tert*-butyl peroxide (10 vol%) were sealed under nitrogen in a suprasil quartz EPR tube. The sample was inserted at room temperature into the cavity of an EPR spectrometer, and photolyzed with the unfiltered light from a 500 W high-pressure mercury lamp. The temperature was controlled with a standard variable-temperature accessory and was monitored before and after each run with a copper–constantan thermocouple.

The EPR spectra were recorded on a Bruker ESP 300 spectrometer equipped with a Hewlett Packard 5350B microwave frequency counter for the determination of the *g* factors, which were corrected with respect to that of perylene radical cation in concentrated H_2SO_4 ($g=2.00258$).

For mixtures of BHT and one of the investigated phenols, the molar ratio of the two equilibrating radicals was obtained from the EPR spectra and used to determine the equilibrium constant K_1. Spectra were recorded a few seconds after starting irradiation to avoid significant consumption of the phenols during the course of the experiment.

Relative radical concentrations were determined by comparison of the digitized experimental spectra with computer-simulated ones, as previously described.[19]

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[1] E. O. Hileman, J. Liu, M. Albitar, M. J. Keating, P. Huang, *Cancer Chemother. Pharmacol.* **2004**, *53*, 209–219.
[2] N. Durany, G. Münch, T. Michel, P. Riederer, *Eur. Arch. Psychiatry Clin. Neurosci.* **1999**, *249*, III/68-III/73.
[3] V. Calabrese, G. Scapagnini, C. Colombrita, A. Ravagna, G. Pennisi, A. M. G. Stella, F. Galli, D. A. Butterfield, *Amino Acids* **2003**, *25*, 437–444.
[4] İ. Gülcin, M. Oktay, Ö. İ. Küfrevioğlu, A. J. Aslan, *J. Ethnopharmacol.* **2002**, *79*, 325–329.
[5] L. L. Dugan, E. G. Lovett, K. L. Quick, J. Lotharious, T. T. Lin, K. L. O' Malley, *Parkinsonism Relat. Disord.* **2001**, *7*, 243–246.
[6] S. S. Huang, S. K. Tsai, C. L. Chih, L.-Y Chiang, H. M. Hsieh, C. M. Teng, M. C. Tsai, *Free Radical Biol. Med.* **2001**, *30*, 643–649.

[7] D. Monti, L. Moretti, S. Salvioli, E. Straface, W. Malorni, R. Pellicciari, G. Schettini, M. Bisaglia, C. Pincelli, C. Fumelli, M. Bonafé, C. Franceschi, *Biochem. Biophys. Res. Commun.* **2000**, *277*, 711–717.
[8] L. L. Dugan, D. M. Turetsky, C. Du, D. Lobner, M. Wheeler, C. R. Almli, C. K.-F Shen, T.-Y. Luh, D. W. Choi, T. S. Lin, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 9434–9439.
[9] P. J. Krusic, E. Wassermann, P. N. Keizer, J. R. Morton, K. F. Preston, *Science* **1991**, *254*, 1183–1185.
[10] J. R. Morton, F. Negri, K. F. Preston, *Acc. Chem. Res.* **1998**, *31*, 63–69.
[11] L. Xiao, H. Takada, K. Maeda, M. Haramoto, M. Nobuhiko, *Biomed. Pharmacother.* **2005**, *59*, 351–358.
[12] M. D. L. De la Torre, A. G. P. Rodrigues, A. C. Tomé, A. M. S. Silva, J. A. S. Cavaleiro, *Tetrahedron* **2004**, *60*, 3581–3592.
[13] BHT is the abbreviated name of butylated hydroxytoluene (2,6-di-*tert*-butyl-4-methylphenol).
[14] N. Nenadis, I. Zafiropoulon, M. Tsimidou, *Food Chem.* **2003**, *28*, 403–407.
[15] İ. Gülcin, M. E. Büyükokuroğlu, Ö. İ. Küfrevioğlu, M. J. Oktay, *J. Ethnopharmacol.* **2003**, *86*, 51–58.
[16] For instance, the rate constant for the reaction (addition) of benzyl radical with [60]fullerene was reported as $1.4\times10^{7}\,\text{M}^{-1}\text{s}^{-1}$ at 298 K,[17] which can be compared to the value of $4.8\times10^{3}\,\text{M}^{-1}\text{s}^{-1}$ measured at the same temperature for hydrogen abstraction from BHT by neophyl radical.[18]
[17] M. Walbiner, H. Fischer, *J. Phys. Chem.* **1993**, *97*, 4880–4881.
[18] P. Franchi, M. Lucarini, G. F. Pedulli, L. Valgimigli, B. Lunelli, *J. Am. Chem. Soc.* **1999**, *121*, 507–514.
[19] M. Lucarini, P. Pedrielli, G. F. Pedulli, S. Cabiddu, C. Fattuoni, *J. Org. Chem.* **1996**, *61*, 9259–9263.
[20] G. W. Burton, T. Doba, E. J. Gabe, L. Hughes, F. L. Lee, L. Prasad, K. U. Ingold, *J. Am. Chem. Soc.* **1985**, *107*, 7053–7065.
[21] J. A. Howard in *Free Radicals, Vol. 2* (Ed.: J. K. Kochi), Wiley-Interscience, New York, **1975**, Chapter 12.
[22] Restricted rotation in other (*ortho*-substituted phenylpyrrolidino)[60]fullerene derivatives was studied recently: F. Ajamaa, T. M. F. Duarte, C. Bourgogne, M. Holler, P. W. Fowler, J.-F. Nierengarten, *Eur. J. Org. Chem.* **2005**, *17*, 3766–3774.
[23] J.-F. Eckert, J.-F. Nicoud; J.-F. Nierengarten, S.-G. Liu, L. Echegoyen, F. Barigelletti, N. Amaroli, L. Ouali, V. Krasnikov, G. Hadzijoannou, *J. Am. Chem. Soc.* **2000**, *122*, 7467–7479.
[24] P. De la Cruz, A. De la Hoz, L. M. Font, F. Langa, M. C. Pérez-Rodríguez, *Tetrahedron Lett.* **1998**, *39*, 6053–6056.
[25] P. J. Krusic, E. Wasserman, B. A. Parkinson, B. Malone, E. R. Holler, Jr., P. N. Keizer, J. R. Morton, K. F. Preston, *J. Am. Chem. Soc.* **1991**, *113*, 6274–6275.
[26] V. Fischer, K. Z. Scheffler, *Naturforsch. A* **1983**, *38*, 68–73.
[27] P. Franchi, M. Lucarini, G. F. Pedulli, E. Bandini, *Chem. Commun.* **2002**, 560–561.
[28] G. Brigati, M. Lucarini, V. Mugnaini, G. F. Pedulli, *J. Org. Chem.* **2002**, *67*, 4828–4832.
[29] All the BDE values determined in benzene solution by means of the EPR radical equilibration technique,[19] based on the O–H BDE of 2,4,6-tri-*tert*-butylphenol determined many years earlier by Mahoney et al.[30] using calorimetric measurements, must be downscaled by 1.1 kcal mol^{-1} due to the revision of the heat of formation of (*E*)-azobenzene.[31]
[30] L. R. Mahoney, F. C. Ferris, M. A. DaRooge, *J. Am. Chem. Soc.* **1969**, *91*, 3883.
[31] P. Mulder, H.-G. Korth, D. A. Pratt, G. A. DiLabio, L. Valgimigli, G. F. Pedulli, K. U. Ingold, *J. Phys. Chem. A* **2005**, *109*, 2647–2655.
[32] R. Amorati, G. F. Pedulli, L. Valgimigli, O. A. Attanasi, P. Filippone, C. Fiorucci, R. Saladino, *J. Chem. Soc. Perkin Trans. 2* **2001**, 2142–2146.
[33] M. Lucarini, G. F. Pedulli, L. Valgimigli, *J. Org. Chem.* **1998**, *63*, 4497–4499.
[34] V. M. Darley-Usmar, A. Hersey, L. G. Garland, *Biochem. Pharmacol.* **1989**, *38*, 1465.
[35] From the measured oxidizability of cumene ($1.72\times10^{-3}\,\text{M}^{-1/2}\text{s}^{-1/2}$) at 30 °C the propagation rate constant k_p [Eq. (6)] can be derived as $0.37\,\text{M}^{-1}\text{s}^{-1}$.
[36] A. Baignée, J. A. Howard, J. C. Scaiano, L. C. Steward, *J. Am. Chem. Soc.* **1983**, *105*, 3883.
[37] I. C. Wang, L. A. Tai, D. D. Lee, P. P. Kanakamma, C. K.-F. Shen, T.-Y. Luh, C. H. Cheng, K. C. Hwang, *J. Med. Chem.* **1999**, *42*, 4614–4620.
[38] L. Gan, S. Huang, X. Zhang, A. Zhang, B. Cheng, H. Cheng, X. Li, G. Shang, *J. Am. Chem. Soc.* **2002**, *124*, 13384–13385.
[39] G. W. Burton, K. U. Ingold, *Science* **1984**, *224*, 569–573.
[40] A. El-Agamey, D. J. McGarvey, *J. Am. Chem. Soc.* **2003**, *125*, 3330–3340.
[41] Despite the fact that the BDE differences among the investigated compounds are of the same magnitude as the experimental errors, it should be considered that these differences were obtained with the same reference phenol (BHT) and thus reflect the actual BDE hierarchic sequence.
[42] E. B. Zeinalov, G. Koβmehl, *Polym. Degrad. Stab.* **2001**, *71*, 197–202.

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