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Synthesis of Tris-hydroxymethyl-Based Nitrone Derivatives with Highly Reactive Nitronyl Carbon

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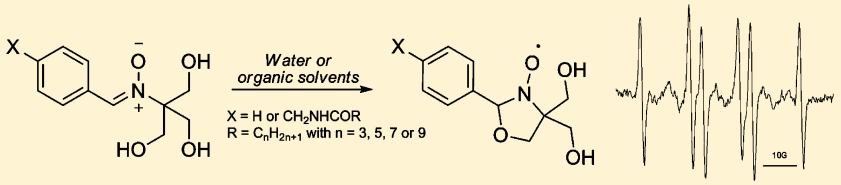
Supporting Information



ABSTRACT: A novel series of α-phenyl-*N*-*tert*-butyl nitrone derivatives, bearing a hydrophobic chain on the aromatic ring and three hydroxyl functions on the *tert*-butyl group, was synthesized through a short and convenient synthetic route based on a one-pot reduction/condensation of tris(hydroxymethyl)nitromethane with a benzaldehyde derivative. Because of the presence of hydroxyl functions on the *tert*-butyl group, an intramolecular Forrester−Hepburn reaction leading to the formation of an oxazolidine-*N*-oxyl compound was observed by electron paramagnetic resonance (EPR). The mechanism of cyclization was further studied by computational methods showing that intramolecular hydrogen bonding and high positive charge on the nitronyl carbon could facilitate the nucleophilic addition of a hydroxyl group onto the nitronyl carbon. At high nitrone concentrations, a second paramagnetic species, very likely formed by intermolecular nucleophilic addition of two nitrone molecules, was also observed but to a lesser extent. In addition, theoretical data confirmed that the intramolecular reaction is much more favored than the intermolecular one. These nitrones were also found to efficiently trap carbon-centered radicals, but complex spectra were observed due to the presence of oxazolidine-*N*-oxyl derivatives.

INTRODUCTION

Oxidative stress was defined as *a disturbance in the prooxidant−antioxidant balance in favor of the former, leading to potential damage*, and it has become clear that an overproduction of prooxidant molecules, also termed reactive oxygen and nitrogen species (ROS/RNS), is associated with several pathologies such as neurodegenerative diseases, cancers, and ischemia−reperfusion syndromes to name a few.[1] Consequently, any agent that can trap ROS/RNS to form more stable adducts, and as a result can alter the course of diseases progression, may be useful as a novel therapeutic approach.

Of particular interest are nitrone spin-traps, which undergo addition reaction with free radicals, making them a popular analytical reagent for the identification of short-lived radicals using electron paramagnetic resonance (EPR) spectroscopy.[2,3] The spin-trapping technique relies on the addition of a transient radical to a diamagnetic spin-trap (usually a nitrone or a nitroso compound) to yield a longer lived paramagnetic spin adduct (a nitroxide) that can be detected by conventional EPR spectroscopy. Analyzing the EPR spectrum obtained gives information on the addend structure.

The linear nitrone, α-phenyl-*N*-*tert*-butylnitrone (PBN), and its derivatives have also been widely used as antioxidant agents in several biological models including protection against ischemia-reperfusion injuries, stroke, hearing loss, as well as increasing life span of mice and rats.[4,5] Further studies on stroke demonstrated the superiority of the disulfonate PBN derivative (disodium 4-[(*tert*-butylimino)methyl]benzene-1,3-disulfonate *N*-oxide), also referred to as NXY-059, compared to PBN.[6] Moreover, experimental evidence suggest that the pharmacological activity of linear nitrones involves the inhibition of signal transduction and gene induction processes that lead to apoptosis and therefore is not solely due to their radical trapping capability.[4,7]

With the expectation that amphiphilic compounds possessing a hydrophilic polar head and a lipophilic alkyl chain would

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exhibit improved bioavailability and membrane permeability, our work has been devoted to the design of nitrone derivatives with an amphiphilic character.[8−11] Their biological evaluations have shown that the amphiphilicity is a key feature in determining bioactivity and protection against the oxidative toxicity in vitro and in vivo with the LPBNH15 exhibiting the highest antioxidant activity (Figure 1).[12] However, one of its

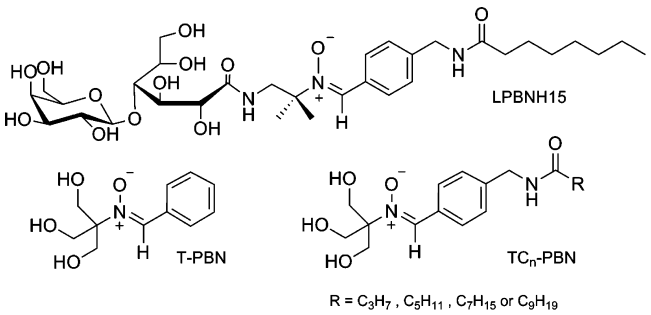


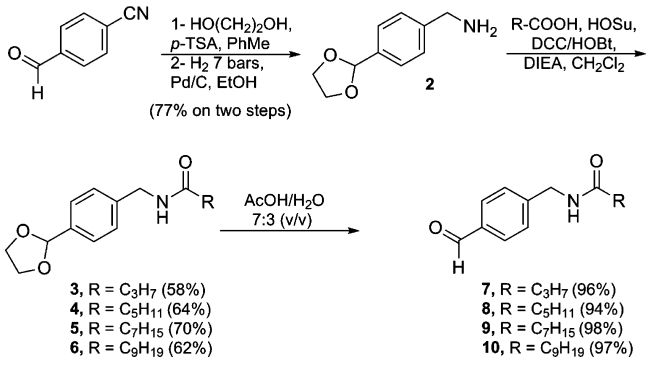
Figure 1. Chemical structures of LPBNH15 and the new TC$_n$-PBN series.

limitations as a potential therapeutic agent may come from its relatively long synthesis that requires 12 steps. Consequently, the present report deals with the design of a novel series of amphiphilic PBN derivatives whose synthesis has been significantly shortened allowing gram-scale preparation of pure nitrones (Figure 1). These newly designed nitrones are comprised of a hydrophobic chain grafted onto the aromatic ring and three hydroxyl functions grafted onto the *tert*-butyl group of the PBN. X-band EPR experiments were performed and demonstrated a unique reactivity for this series of tris-hydroxymethyl-based spin-traps due to the presence of hydroxyl functions on the *tert*-butyl group. Finally, these findings were rationalized with the use of computational methods.

RESULTS AND DISCUSSION

Synthesis. *Synthesis of Hydrophobic Benzaldehyde Derivatives.* 4-Cyanobenzaldehyde was used as starting material for the synthesis of the hydrophobic benzaldehyde derivatives following our previous procedure (Scheme 1).[13,14]

Scheme 1. Synthesis of the Hydrophobic Benzaldehyde Derivatives 7−10



The carbonyl group was first protected as a dioxolane, and then reduction of the cyano group by catalytic hydrogenation (10% Pd/C) in EtOH was carried out affording the benzylamine **2** in 77% yield in two steps (Scheme 1). It has to be noted that LiAlH$_4$ was previously used for the reduction of the cyano group, but we found out in this work that reduction by catalytic hydrogenation could lead to similar yields in a much simpler procedure, and therefore, this latter method was preferred. In parallel, the four carboxylic acids, namely butanoic, hexanoic, octanoic, and decanoic acid, were activated in the presence of *N*-hydroxysuccinimide (HO-Su), *N*,*N*-dicyclohexylcarbodiimide (DCC), and a catalytic amount of 1-hydroxybenzotriazole (HOBt) in dry CH_2Cl_2. The resulting crude mixtures were directly added to benzylamine **2** in the presence of DIEA in dry CH_2Cl_2 to give compounds **3**−**6** in good yields. Then, dioxolane group removal was achieved in a 7:3 v/v AcOH/H_2O mixture to give the benzaldehyde derivatives **7**−**10** in quantitative yields.

Synthesis of T-PBN. Several general methods have been described for the preparation of nitrone compounds.[15] Migrdichian demonstrated[16] the first example of alkylation of oxime, and despite its lack of regioselectivity, this method has been used successfully for the synthesis of substituted nitrones.[17] Among the other methods, one can cite the oxidation of imines or secondary aliphatic amines,[18,19] the condensation between a carbonyl group and a hydroxylamine, and the in situ reduction of nitro compounds in the presence of an aldehyde or a ketone.[20]

In an attempt at optimizing the formation of the nitronyl group, we carried out preliminary experiments with the commercially available benzaldehyde (Scheme 2). The synthesis of T-PBN by reduction of tris(hydroxymethyl)-nitromethane (also called 2-hydroxymethyl-2-nitro-1,3-propanediol) to the hydroxylamine derivative followed by condensation with benzaldehyde had previously been reported by Janzen and Zawalski; however, the overall yield was only 22%.[21] Therefore, the oxidation of an imine was the first method we tested following the procedure by Bernotas et al.[18] The imino compound **11** was obtained by reaction of benzaldehyde with tris(hydroxymethyl)aminomethane in refluxing toluene in a Dean−Stark apparatus. Because of its instability, the imino compound was not purified, and the crude compound was directly oxidized by hydrogen peroxide 30% in the presence of sodium tungstate catalyst according to the procedure described by Murahashi et al.[19] Under these conditions, the degradation of the imino compound was the predominant reaction, and no nitrone formation was detected. This is in agreement with the findings of Bernotas et al., who found that direct oxidation of benzazepines to nitrones under similar reaction conditions was unsuccessful.[22] We overcame this problem by using a two-step procedure consisting of first reducing the imine **11** before oxidizing the resulting amino compound into its nitronyl form. Reduction using $NaBH_4$ in methanol or in ethanol led to compound **12** after purification by chromatography on silica gel but in low yield (32% and 26%, respectively, in two steps from benzaldehyde). On the contrary, reduction by catalytic hydrogenation in the presence of Pd/C was found to be more efficient, yielding compound **12** in good yield. The last step involved oxidation of the amino group and this was achieved using the procedure described by Murahashi et al.[19] To optimize the yield of nitrone formation, several conditions were used and data are presented in Scheme 2. The yields of oxidation remained below 40%, but the best conditions appeared to be 2 equiv of H_2O_2 at 0 °C, leading to nitrone **13** (also called T-PBN) in 36% yield. Therefore, the overall yield of T-PBN formation using that synthetic strategy was 24%, similar to that of Janzen and Zawalski (22%).[21]

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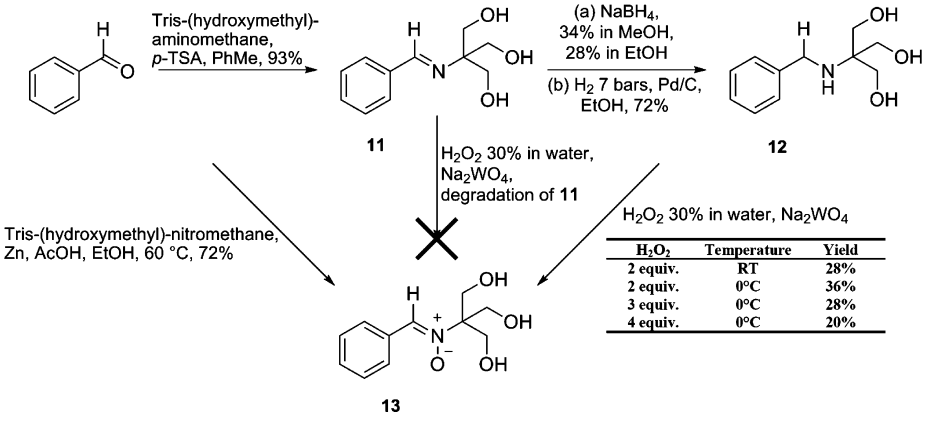
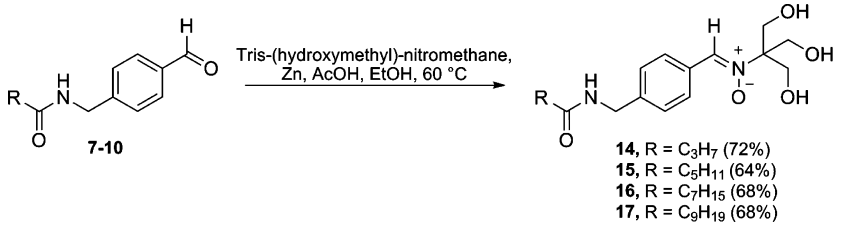
Scheme 2. Synthesis of T-PBN 13



H_2O_2	Temperature	Yield
2 equiv.	RT	28%
2 equiv.	0°C	36%
3 equiv.	0°C	28%
4 equiv.	0°C	20%

Scheme 3. Synthesis of TC_n-PBN Compounds 14−17



The mildest and the most selective route to prepare nitrones is the condensation of *N*-alkylhydroxylamines with carbonyl compounds.[15] Nevertheless, the drawback of this method is that only a few hydroxylamines are commercially available, and their preparation is often not straightforward. Moreover, during the coupling reaction between the aldehyde and the hydroxylamine, the oxidation of this latter can also occur, producing nitroso side products. To improve the formation of the nitronyl function, we next focused our attention on a one-pot method which involves in situ reduction of tris(hydroxymethyl)-nitromethane in the presence of a benzaldehyde derivative. As a wide range of nitro compounds are commercially available; this method has the advantage of being broad and general and usually gives high yields.[20] For instance, Gautheron-Chapoulaud et al. demonstrated that the in situ zinc mediated reduction of nitro compounds in the presence of sugar constitutes a direct and efficient method to synthesize a wide range of glycosylated nitrones.[23] Following this strategy, 2 equiv of tris(hydroxymethyl)nitromethane and benzaldehyde were solubilized in ethanol in the presence of 6 equiv of acetic acid. Zinc dust (4 equiv) was added at 0 °C, and then the reaction was heated at 60 °C for 20 h. After purification by chromatography and two successive crystallizations from ethyl acetate/*n*-hexane, pure T-PBN was obtained in 72% yield, which is much higher than the procedure used by Janzen and Zawalsky.[21]

Synthesis of Tris-hydroxymethyl-Based Nitrones. Having demonstrated the superiority of the second synthetic strategy, we next used this latter for the preparation of the TC_n-PBN series. Condensation of tris(hydroxymethyl)nitromethane with the four aldehyde derivatives **7−10** was done in the presence of zinc and acetic acid in ethanol (Scheme 3). Owing to the possible oxidation of hydroxylamine by free radical processes, the reaction was carried out in the dark and under argon. Addition of molecular sieves was also very useful in increasing the kinetics of the reaction. After chromatography on silica gel followed by two successive crystallizations from ethanol, TC_n-PBN compounds **14−17** were obtained in good yields ranging from 64% to 72%.

Physical−Chemical Properties. *Partition Coefficients.* The relative lipophilicities (log k'_W) of nitrones were measured by a HPLC technique, and as shown in Figure 2, log k'_W values

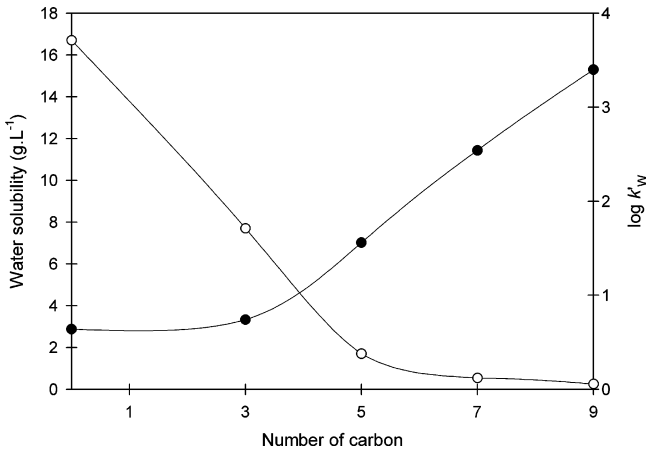


Figure 2. Water solubility (○) and lipophilicity (●) of TC_n-PBN compounds (n = 3, 5, 7, and 9) and T-PBN (n = 0).

are increased with the number of carbon of the alkyl chain, demonstrating that for a given polar head this parameter is linearly correlated to the length of the tail. Moreover, TC_7-PBN and LPBNH15[12] which have the same alkyl chain but a different polar head exhibited comparable hydrophobic properties with log k'_W values of 2.54 and 2.86, respectively. Such a result strongly confirms that the volume and the nature of the polar group have a low impact on the log k'_w value, in perfect agreement with our previous observations.[8,24]

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Solubility. The solubility of the nitrones in water was also determined, and the data are in agreement with the partition coefficient values, the longer the chain, the lower the solubility. However, when comparing these values with that of PBN (26.5 g/L),[12] despite the presence of a tris(hydroxymethyl)-aminomethane-based polar head, none of the TC_n-PBN nor the T-PBN exhibited a higher solubility than PBN. This suggests that the formation of hydrogen bonds between hydroxyl groups is likely responsible for the low water solubility of this series. In agreement with this, we have recently demonstrated that tris(hydroxymethyl)-based acrylamide monomers with a related structure to that of the TC_n-PBN polar head were able to form hydrogen bondings.[25]

Spin-Trapping. Prior to any spin-trapping assay, the supernatant of a saturated aqueous solution of T-PBN obtained after heating a heterogeneous mixture of 34 mg of T-PBN in 1 mL of water (0.15 mol·L^{-1}, that is ∼2× the solubility limit in water) was analyzed by EPR leading to the signal shown in Figure 3a. Simulation of this signal revealed the presence of two

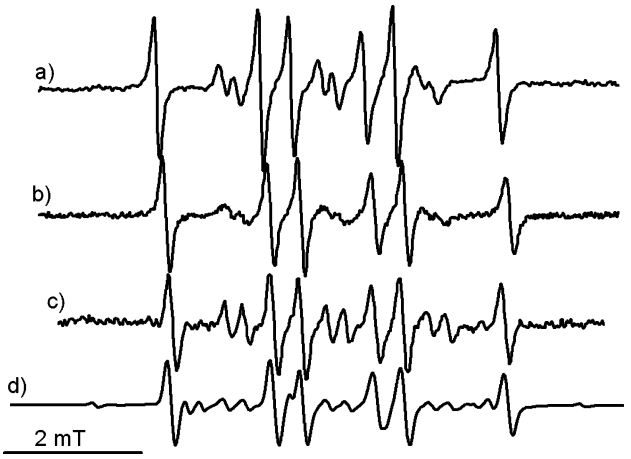

Figure 3. EPR spectra of various nitroxides derived from T-PBN: (a) signal recorded from the supernatant of a saturated aqueous T-PBN solution; (b) signal recorded after generating $^{\bullet}OH$ using a standard Fenton system in the presence of 0.008 mol·L^{-1} T-PBN in phosphate buffer; (c) signal recorded after generating $^{\bullet}CH_3$ using a standard Fenton system in phosphate buffer/DMSO (80/20) in the presence of 0.008 mol·L^{-1} T-PBN; (d) signal recorded after generating $^{\bullet}CH_3$ using a concentrated Fenton system in phosphate buffer/DMSO (80/20) in the presence of 0.15 mol·L^{-1} T-PBN.

nitroxides, both showing a six-line spectrum due to hyperfine couplings of the unpaired electron with nitrogen and β-hydrogen nuclei. In the major signal (a_N = 1.52 mT and a_H = 1.97 mT, ∼75%), the hyperfine coupling constant (hfcc) with the β-hydrogen was too high for being consistent with a PBN-type spin adduct,[26] but surprisingly, in agreement more with a five-membered cyclic nitroxide. This signal could be assigned to the oxazolidine-*N*-oxyl compound **18**, which very likely originates from intramolecular nucleophilic addition of a hydroxyl group onto the nitronyl carbon followed by fast autoxidation of the resulting hydroxylamine, the so-called Forrester-Hepburn mechanism (see Scheme 4a).[27] The possible formation of such cyclic nitroxides from hydroxy-PBN compounds was first proposed by Janzen and Zawalski in the late 1970s,[21] but to the best of our knowledge this has remained unproven. On the basis of its EPR parameters, the minor species could be assigned to an alkoxy-adduct of a PBN-type nitrone (a_N = 1.44 mT and a_H = 0.18 mT, ∼25%).[26] Though the identification of this second species remains hypothetical, it could likely correspond to adduct **23**, obtained after bimolecular nucleophilic addition of two nitrone molecules, a mechanism largely favored by the high nitrone concentrations used (see Scheme 4b). An EPR analysis of a less concentrated T-PBN solution (0.008 mol·L^{-1}, ∼10× below the solubility limit in water) showed the presence of the sole nitroxide **18**, confirming that the formation of **23** occurs only at high nitrone concentrations.

Similar results were obtained from EPR analysis of T-PBN solutions prepared in dimethylformamide (DMF), a solvent that favors nucleophilic addition processes.[28] Using 0.15 mol·L^{-1} solution of T-PBN in DMF, a mixture of nitroxide **18** (a_N = 1.48 mT and a_H = 1.98 mT, ∼80%) and of nitroxide **23** (a_N = 1.40 mT and a_H = 0.17 mT, ∼20%) was observed, while only nitroxide **18** was detected when 0.008 mol·L^{-1} solution was used. It is also worth noting that **18** was the only species observed when T-PBN was dissolved in methanol (a_N = 1.51 mT and a_H = 1.98 mT), whatever the nitrone concentration. Very similar observations were made with nitrones **14**−**17** solubilized in methanol and DMF and with nitrone **14** solubilized in water. The EPR parameters of such nitroxides **18**−**27** thus formed are reported in Table 1.

An estimation of the proportion of compound **18** in T-PBN solutions (0.01−0.05 mol·L^{-1}) was determined by using 3-carboxyproxyl (3-CP) as an internal reference and by comparing the area of the signal of 3-CP with that of compound **18**. Whatever the solvent, the concentration of the oxazolidine-*N*-oxyl compound **18** was found lower than 0.2%, i.e., 0.16% in H_2O, 0.15% in DMF and 0.10% in MeOH. The same observations were made with TC_5-PBN solutions, and the concentration of nitroxide **20** was found to be 0.25% in H_2O, 0.27% in DMF, and 0.22% in MeOH. Worth noting is that no increase of the oxazolidine-*N*-oxyl concentration was observed over a period of 2 h. This suggests that a rapid equilibrium is reached during the very first minutes of mixing, and then the concentration of the cyclic nitroxide remains constant. The stability of T-PBN (0.075 mol·L^{-1}) in organic solvents was also surveyed by TLC and ^1H NMR, and after 24 h of stirring at room temperature, no significant degradation of T-PBN was observed either in methanol or DMF (data not shown).

Because of its sensitivity and the structural information it can provide, electrospray mass spectrometry (ESI-MS) was employed to study the reactivity of T-PBN in water, methanol, and DMF. Unfortunately, tandem mass spectrometry experiments did not allow us to discriminate the nitrone **13** from the oxazolidine-*N*-oxyl compound **18**. Indeed, ESI-MS generally allows the observation of the hydroxylamine form obtained after reduction of the nitroxide compound rather than the paramagnetic nitroxide itself.[29,30] Therefore, since the cyclic hydroxylamine derived from nitroxide **18** and the parent T-PBN are isomers, they would be MS-detected at the same *m*/*z* value. An aqueous solution of T-PBN (0.035 mol·L^{-1}) was prepared and EPR analyzed before being submitted to ESI-MS showing the presence of nitroxides **18** and **23**. MS analysis of this solution led to the detection of a main signal at *m*/*z* 226.1 (Figure S28, Supporting Information), which could be assigned either to the protonated cyclic hydroxylamine or to the protonated nitrone (or both). Moreover, the main *m*/*z* 122.1 product ion generated upon dissociation of the *m*/*z* 226.1 ion (Figure S28, Supporting Information) could be accounted for by considering either the reduced form of **18** or the nitrone **13**

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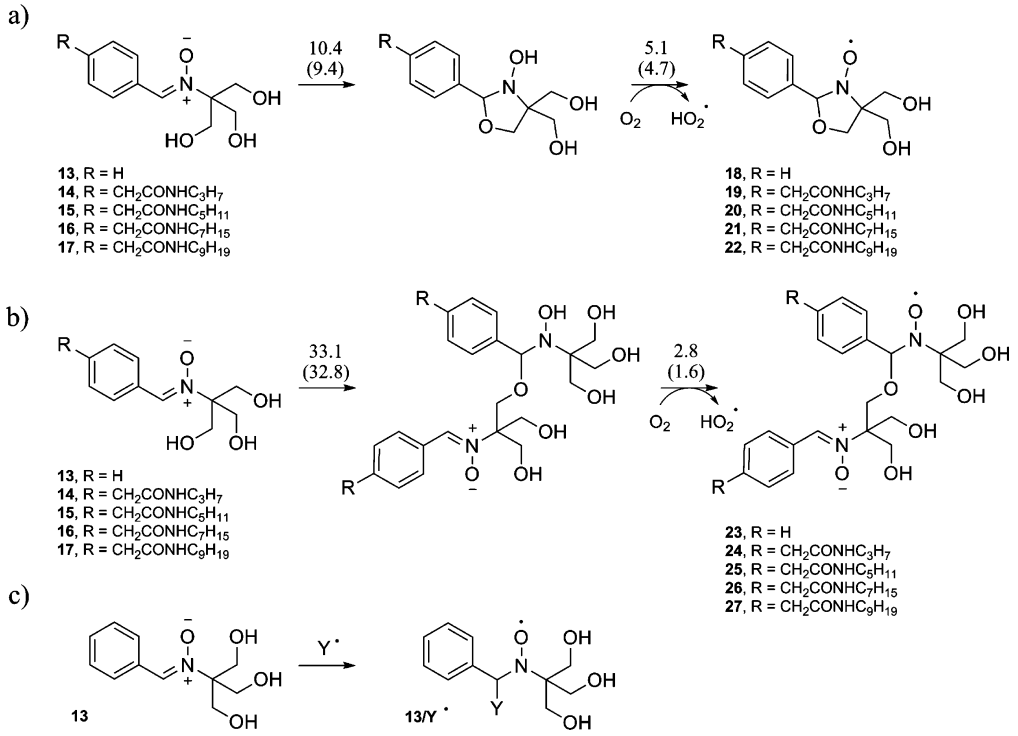
Scheme 4. (a) Mechanisms Proposed for the Cyclization Reaction Leading to the T-PBN and TC$_n$-PBN-Derived Nitroxides 18−22; (b) Mechanism Proposed for the Bimolecular Reaction Leading to the Formation of Nitroxides 23−27; (c) Spin-Trapping of a Free Radical $^{\bullet}$Y by the Nitrone 13 Yielding the Spin Adduct 13/$^{\bullet}$Y[a]



[a]Also shown are the free energies of reaction (ΔG_{298K}, in kcal/mol) in water and in DMSO (in parentheses) for compound **13** (R = H) at the PCM/B3LYP/6-31G+(d,p)// B3LYP/6-31G(d) level of theory.

as the precursor ion. Indeed, this unique dissociation pathway consists of the release of 2-(hydroxymethyl)prop-1-ene-1,3-diol (104 Da), whose formation can be envisaged upon activation of both isomers. The study of the reactivity of nitrone **15** led to similar conclusions (Figure S29, Supporting Information), with the detection of a signal at *m*/*z* 353.2 in the MS mode and two successive dissociation reactions (i.e., loss of 104 Da to yield *m*/*z* 249.2, followed by elimination of *N*-pentylformamide to produce *m*/*z* 134.1). As for the second species that could arise from bimolecular nucleophilic addition of two nitrone molecules, its concentration was much too low for MS detection regardless of the nitrone studied and the nature of the solvent. It has to be noted that the same results were obtained after EPR and MS analysis of solutions of nitrones **13** and **15** either in DMF or methanol.

Despite the tendency of nitrones **13**−**17** to form oxazolidine-*N*-oxyl compounds, their spin-trapping properties were nevertheless surveyed with a series of free radicals. In order to simplify the notation, the spin adduct obtained after addition of a radical $^{\bullet}$Y on a nitrone **X** will be noted **X**/$^{\bullet}$Y, as indicated in Scheme 4c. We first studied the spin-trapping properties of T-PBN at 0.008 mol·L^{-1}. EPR analysis of the medium always revealed the presence of the cyclic nitroxide **18**, regardless of the radical generating system. Moreover, we found that the concentration of nitroxide **18** remained constant during the spin-trapping experiments, which suggests that its rapid formation is not reversed by the spin-trapping reaction. This species was the only radical detected when $O_2^{\bullet-}$ was produced by the xanthine/xanthine oxidase system in pH 7.2 phosphate buffer. When $^{\bullet}$OH was generated using a standard Fenton system, the EPR signal given in Figure 3b was obtained. Its simulation showed another spin adduct present other than nitroxide **18** whose EPR signal consists of six lines (a_N = 1.46 mT and a_H = 0.21 mT) which could possibly be assigned to the **13**/$^{\bullet}$OH adduct; however, it could also correspond to a carbon-centered radical adduct **13**/$^{\bullet}$C, since $^{\bullet}$OH radicals are known to react with alcohols by hydrogen abstraction on the α-carbon.[31,32] Therefore, in the presence of **13**, $^{\bullet}$OH could generate a carbon-centered radical that could subsequently be trapped by the nitrone. In the next step, $^{\bullet}CH_2OH$ and $^{\bullet}CH_3$ were produced by generating $^{\bullet}$OH in the presence of methanol and DMSO, respectively. In each case, a minor six line EPR signal corresponding to either **13**/$^{\bullet}CH_2OH$ or **13**/$^{\bullet}CH_3$ was detected other than that of nitroxide **18**. To illustrate this, the EPR signal obtained when $^{\bullet}CH_3$ was produced in the presence of T-PBN is shown in Figure 3c. More complex signals were observed when the same experiments were performed by raising the nitrone concentration to 0.15 mol·L^{-1} and by generating twice more $^{\bullet}$OH (Figure 3d). It appears that many radical reactions could occur between T-PBN and $^{\bullet}$OH, yielding a complex mixture of several radical species. With nitrones **14**-**17**, the spin-trapping experiments were limited to $^{\bullet}CH_3$ and $^{\bullet}CH_2OH$ radicals owing to solubility issues. As previously observed, the signals of the various spin adducts were detected in addition to those of cyclic nitroxides **19**−**22**, and very complex spectra were systematically observed when higher amounts of $^{\bullet}$OH and nitrone were used. All of the EPR data collected for the various nitroxides detected are listed in Table 1. It should be noted that very large amounts of DMSO were used as $^{\bullet}$OH scavenger, which could result in the formation of an oxygen-centered radical derived from DMSO beside $^{\bullet}CH_3$. Consequently, there is uncertainty in the

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Table 1. EPR Hyperfine Coupling Constants for Various Nitroxides Derived from Nitrones 13−17

	Solvent					
	Water		MeOH		DMF	
nitroxides	a_N (mT)	a_H (mT)	a_N (mT)	a_H (mT)	a_N (mT)	a_H (mT)
18[a]	1.52	1.97	1.51	1.98	1.48	1.98
19[a]	1.50	1.84	1.49	1.84	1.46	1.83
20[a]			1.49	1.83	1.46	1.83
21[a]			1.49	1.84	1.46	1.83
22[a]			1.49	1.84	1.46	1.83
23[a]	1.44	0.18				
23−27					1.40	0.17

spin adducts	solvent	a_N (mT)	a_H (mT)
13/•OH or **13**/•C[b]	water	1.46	0.21
PBN/•OH[c]	water	1.53−1.57	0.27−0.29
13/•CH_3[b]	water/DMSO (80/20)	1.48	0.26
PBN/•CH_3[c]	water	1.65−1.66	0.35−0.37
13/•CH_2OH	water/MeOH (80/20)	1.44	0.25
PBN/•CH_2OH[c]	water	1.59−1.69	0.38−0.40
14/•CH_3[b]	water/DMSO (40/60)	1.49	0.28
14/•CH_2OH[b]	water/MeOH (40/60)	1.48	0.24
15/•CH_3[b]	water/DMSO (30/70)	1.48	0.29
15/•CH_2OH[b]	water/MeOH (30/70)	1.47	0.25
16/•CH_3[b]	water/DMSO (30/70)	1.47	0.27
16/•CH_2OH[b]	water/MeOH (30/70)	1.46	0.25
17/•CH_3[b]	water/DMSO (25/75)	1.47	0.26
17/•CH_2OH[b]	water/MeOH (25/75)	1.45	0.25

[a] g value: 2.0062. [b] g value: 2.0061. [c] Parameters obtained from the following Web site: http://www.chm.bris.ac.uk/stdb/.

identification of specific spin adducts observed by EPR in the presence of **14−17**, but the values a_N of 0.26−0.29 mT obtained correspond to carbon-centered radical adducts in general. Regardless, all these results pointed out that the presence of several hydroxymethyl groups yields a particular reactivity of **13−17** that notably led to the formation of cyclic nitroxides or to complex mixtures of paramagnetic species in the presence of •OH.

Computational Studies. The thermodynamics of nitrone cyclization to form the hydroxylamine and its subsequent oxidation to nitroxide were calculated for T-PBN at the PCM/B3LYP/6-31G+(d,p)//B3LYP/6-31G(d) level of theory in water and in DMSO (Scheme 4). In water, results indicate that intramolecular nucleophilic addition of one alcohol group to the nitronyl-C to form the oxazolidine−hydroxylamine is endoergic by 10.4 kcal/mol but its subsequent oxidation to nitroxide by oxygen to form hydroperoxyl radical is less endoergic at 5.1 kcal/mol (Scheme 4a). This indicates that the formation of the oxazolidine-*N*-oxyl compound **18** is probable considering that nucleophilic addition of superoxide to DMPO was previously calculated to be endoergic as well by 11.9 kcal/mol at the same level of theory in water.[33] Intermolecular nucleophilic addition is more endoergic with ΔG_{298K} of 33.1 kcal/mol indicating that in aqueous solution, the predominant hydroxylamine would result from intramolecular OH addition onto the nitrone (Scheme 4b). However, the oxidation of the resulting alkoxyhydroxylamine is highly favorable similar to the oxazolidine-*N*-oxyl formation with ΔG_{298K} = 2.8 kcal/mol. In DMSO, intra- and intermolecular nucleophilic addition as well as the oxidation process are only slightly less endoergic by <1 kcal/mol than in water. Therefore, the thermodynamics of nitroxide formation is in good agreement with the experimental observations which suggest that the intramolecular mechanism is more favored than the intermolecular one, the latter being observed only at high nitrone concentrations either in DMF or water with ∼20 and ∼25% molar ratio, respectively. The favorability of intramolecular cyclization could also be driven by the intramolecular H-bonding that can facilitate the nucleophilic addition of the alcohol onto the nitronyl carbon.

As shown in Figure 4, the various H-bond motifs favor two conformations in which one of the hydroxylic hydrogen H-bonds to the nitronyl oxygen (Figure 4a and 4b). On the contrary, the conformation that shows no H-bonding with the nitronyl-O but three H-bonding between the three hydroxyl groups is the least favorable (Figure 4c). Also worth noting is that the two favored conformers gave much higher positive charge density on the nitronyl-C, the site of nucleophilic addition, with values in water of 0.044 and 0.035 e, while the least favored one gave charge density of 0.025 e (Table 2). In comparison, that of PBN is significantly lower (0.009 e).[34] These findings strongly suggest that H-bonding with the nitronyl-O results in a higher charge density on the nitronyl-C,

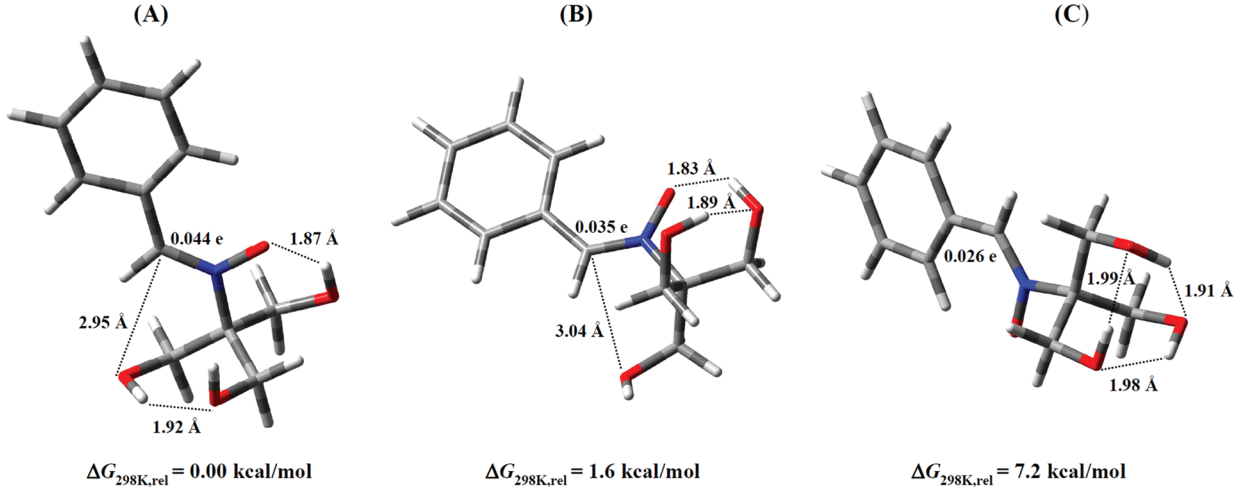

Figure 4. Various conformations of T-PBN (**13**) (in gas phase) showing the H-bond distances as well as the relative free energies and charge densities on the nitronyl-C at the PCM(water)/B3LYP/6-31G+(d,p)//B3LYP/6-31G(d) level of theory.

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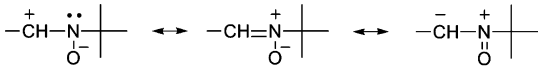
Table 2. Nitronyl-C and Nitronyl-H Charge Densities of the Nitronyl Group of PBN and T-PBN[a]

compd	nitronyl-C	nitronyl-H
PBN	0.009	0.253
	(0.010)	(0.248)
T-PBN (**A**)	0.044	0.266
	(0.044)	(0.264)
T-PBN (**B**)	0.035	0.265
	(0.035)	(0.253)
T-PBN (**C**)	0.026	0.255
	(0.025)	(0.250)

[a]NBO charges were determined from a natural population analysis at the PCM/B3LYP/6-31G+(d,p)//B3LYP/6-31G(d) level of theory in water and in DMSO (in parentheses).

therefore increasing the nitronyl reactivity toward nucleophilic additions, which in turn could facilitate cyclization reactions. The effect of H-bonding on the reactivity of nitronyl group had been previously demonstrated with amide-substituted nitrones[33,35] and was also observed upon protonation of the nitronyl-O.[36]

The effect of H-bonding on the nitronyl atoms charge densities was also investigated using ^{1}H NMR spectroscopy. We previously demonstrated a good correlation between the charge densities of the nitronyl group of para-substitued-PBN compounds with the β-hydrogen shifts, the chemical shift increasing linearly with the nitronyl-H charge density.[34] As shown in Figures S30 and S31, Supporting Information, the nitronyl ^{1}H and ^{13}C chemical shifts of T-PBN were measured in several solvents but no significant dependence to the solvent polarity parameter was observed. In DMSO-d_6, the chemical shift of the β-hydrogen of T-PBN was upfield shifted (7.60 ppm), which is characteristic of a shielding of the methine proton, while in $CDCl_3$, the peak of resonance was downfield shifted (7.89 ppm). Surprisingly, the opposite trend was observed as for PBN with β-hydrogen shifts of 7.85 and 7.53 ppm, respectively in DMSO-d_6 and $CDCl_3$, in agreement with the literature. Indeed, Janzen et al. had previously demonstrated the nitronyl ^{1}H chemical shift sensitivity to solvent of PBN based on the equation: $\delta = 0.022 \times E_{T(30)} + 6.7045$, and the conclusion was that polar solvents could stabilize the resonance form of PBN having the greatest phase separation (i.e., form (1)).[37]



As shown in Table 2, higher nitronyl-H charge densities were observed for the two conformers involving H-bonding with the nitronyl-O, with NBO charges densities in water of 0.266 e for (**A**) and 0.265 e for (**B**) while, conformer (**C**) exhibited a very close value (0.255 e) to that of PBN (0.253 e); the same trend was observed in DMSO. Therefore the upfield shift of the T-PBN methine proton observed in DMSO-d_6 suggests that T-PBN may adopt a particular conformation in which intermolecular H-bonds with solvent molecules may be occurring leading to a lower positive nitronyl-H charge density. On the contrary, the highest β-hydrogen chemical shift in $CDCl_3$ indicates that T-PBN may adopt a conformation for which the nitronyl-H charge density is the highest, in agreement with the fact that no H-bond with the solvent molecules is expected in such a nonpolar solvent.

Finally, the nitronyl-C charge density of conformer (**A**) (0.044 e) is unprecedented for linear nitrones since this value is in the same range as that of the highly reactive cyclic nitrones EMPO (0.040 e) and DEPMPO (0.043 e) and only slightly lower than that of the lead AMPO (0.060).[33] This suggests that the novel T-PBN series may exhibit a high reactivity for the trapping of free radicals, but because of that intrinsic reactivity and the presence of three hydroxyl groups, the Forrester–Hepburn mechanism is unfortunately more favored. Although the Forrester-Hepburn mechanism has been well documented in the literature over the past four decades, we report here a unique and specific intramolecular Forrester-Hepburn mechanism of *N-tert*-butyl-substituted PBN compounds, which was only suggested once in the literature.[21] To understand and overcome this uncontrolled reactivity, a thorough investigation of the reactivity of this *tris*-hydroxymethyl-PBN series as well as the development of mono- and dihydroxylated PBN derivatives are underway. Moreover, since the synthetic strategy of this novel series of amphiphilic nitrones is simple and straightforward allowing gram-scale synthesis of pure compounds, their potential pharmacological interest will be investigated in the near future.

CONCLUSION

A novel series of amphiphilic PBN derivatives, bearing both a hydrophobic chain on the aromatic ring and three hydroxyl functions on the *tert*-butyl group, was synthesized through a short and convenient synthetic route based on a one-pot method which involves in situ reduction of tris-(hydroxymethyl)nitromethane in the presence of a benzaldehyde compound. The water solubility of this series was found to be significantly affected by the length of the alkyl chain, the longer the chain, the lower the solubility, and an opposite trend was obviously observed as for the lipophilicity of the compounds. The relatively low solubility of these derivatives when compared to that of PBN may come from the formation of intramolecular H-bonds between the hydroxyl groups, which was supported by theoretical data obtained at the PCM/B3LYP/6-31G+(d,p)//B3LYP/6-31G(d) level of theory. Surprisingly, an intramolecular Forrester–Hepburn reaction leading to the formation of a cyclic nitroxide was observed by EPR when these compounds were dissolved in water or in organic solvents. Theoretical data showed that H-bonding between one hydroxyl function and the nitronyl oxygen gave much higher positive charge density on the nitronyl carbon, therefore increasing the nitronyl group reactivity toward nucleophilic additions, which in turn could facilitate cyclization reactions. At high nitrone concentration, a second paramagnetic species, very likely formed by intermolecular nucleophilic addition of two nitrone molecules, was also observed but to a lesser extent. The free energies of reaction of both mechanisms were computationally determined and were in full agreement with the experimental observations for the favorability of formation of adducts via Forrester–Hepburn mechanism. Despite the tendency of these tris-hydroxymethyl-based nitrones to form nitroxides, they were also found to trap carbon-centered radicals. Finally, the very high nitronyl-C charge density of T-PBN when compared to PBN suggests that this novel series of compounds may exhibit intrinsic high reactivity for the trapping of free radicals. Therefore, in the development of novel linear nitrone compounds with improved trapping capability, the importance of H-bonding with the nitronyl group will have to be taken into account. Finally, since

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gram-scale amounts of tris-hydroxymethyl-based nitrones are now available, their protective activities will be carefully investigated in the near future in in vitro and in vivo biological models of oxidative-mediated diseases.

EXPERIMENTAL SECTION

Synthesis. All reagents were from commercial sources and were used as received. All solvents were distilled and dried according to standard procedures. TLC analysis was performed on aluminum sheets coated with silica gel (40−63 μm). Compound detection was achieved either by exposure to UV light (254 nm) and by spraying a 5% sulfuric acid solution in ethanol or a 2% ninhydrin solution in ethanol and then by heating at ∼150 °C. Flash chromatography was carried out on silica gel (40−63 μm). Size-exclusion chromatography was carried out on hydroxypropylated cross-linked dextran. UV spectra were recorded on UV/vis spectrometer equipped with a double-compartment quartz cell of 10-mm length. Melting points have not been corrected. The ^{1}H and ^{13}C NMR spectra were recorded at 250 and 62.86 MHz, respectively. Chemical shifts are given in ppm relative to the solvent residual peak as a heteronuclear reference for ^{1}H and ^{13}C. Abbreviations used for signal patterns are: s, singlet; d, doublet; t, triplet; q, quartet; sext, sextuplet; m, multiplet; dd, doublet of doublet. Exact mass was obtained by electrospray ionization in positive mode $(M + H)^+$.

4-(1,3-Dioxacyclopent-2-yl)benzylamine (2).[14] Under stirring and at 0 °C, 4-cyano-1,3-dioxacyclopent-2-ylbenzyl (1.00 g, 5.8×10^{-3} mol) was dissolved in a 99:1 (v/v) ethanol/acetic acid mixture, and 0.46 g of 10% Pd/C was portionwise added under stirring. The reaction mixture was submitted to a hydrogen atmosphere for 12 h (8 bar), then the crude mixture was filtered off through a pad of Celite and the solvent was evaporated under vacuum to give compound **2** (0.98 g, 5.5×10^{-3} mol, 96%) as a yellow oil. Compound **2** was directly used in the next step without further purification. The spectral data of compound **2** were in agreement with those reported by Ouari et al.[14]

***N*-[4-(1,3-Dioxacyclopent-2-yl)benzyl]butanamide (3).** Under stirring, butanoic acid (1.33 g, 15.1×10^{-3} mol), HO-Su (2.09 g, 18.1×10^{-3} mol), and DCC (3.74 g, 18.2×10^{-3} mol) were dissolved in dry CH_2Cl_2 at room temperature. After 6 h of being stirred, the reaction mixture was filtered off, and the solvent was removed under vacuum. The resulting crude ester compound (2.25 g, 12.1×10^{-3} mol) and compound **2** (1.80 g, 10.1×10^{-3} mol) were dissolved in dry CH_2Cl_2 with DIEA (pH = 8−9). The mixture was stirred for 20 h at room temperature, and the solvent was removed under vacuum. The crude mixture was purified by flash chromatography (EtOAc/cyclohexane 2:8 v/v) to give compound **3** (1.40 g, 3.8×10^{-3} mol, 58%) as a white powder: R_f 0.32 (EtOAc/cyclohexane 6:4 v/v); mp 86.4−87.6 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.47 (2H, d, J = 8.1 Hz), 7.31 (2H, d, J = 8.1 Hz), 5.81 (2H, m), 4.46 (2H, d, J = 5.7 Hz), 4.10 (4H, m), 2.20 (2H, t, J = 7.4 Hz), 1.70 (2H, sext, J = 7.4 Hz), 0.97 (3H, t, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 62.86 MHz) δ 172.8 (CO), 139.5, 137.2 (C), 127.9, 126.8 (CH), 103.5 (CH-O), 65.3, 43.3, 38.7, 19.2 (CH_2), 13.8 (CH_3). UV (CH_2Cl_2) λ_{max} 230 nm; HR-MS (ESI+, m/z) calcd for $C_{14}H_{20}NO_3$ $[(M + H)^+]$ 250.1443, found 250.1443.

***N*-[4-(1,3-Dioxacyclopent-2-yl)benzyl]hexanamide (4).** The synthetic procedure was essentially the same as for compound **3**. Hexanoic acid (2.00 g, 17.2×10^{-3} mol), HO-Su (2.37 g, 20.6×10^{-3} mol), and DCC (4.25 g, 20.6×10^{-3} mol) were used as starting materials. The resulting crude ester compound (2.10 g, 9.9×10^{-3} mol) and compound **2** (1.60 g, 9.0×10^{-3} mol) were used for the reaction, and after purification by flash chromatography (EtOAc/cyclohexane 2:8 v/v) compound **4** (1.60 g, 4.3×10^{-3} mol, 64%) was obtained as a white powder: R_f 0.44 (EtOAc/cyclohexane 6:4 v/v); mp 73.5−74.3 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.48 (2H, d, J = 8.2 Hz), 7.31 (2H, d, J = 8.2 Hz), 5.82 (2H, m), 4.46 (2H, d, J = 5.7 Hz), 4.11 (4H, m), 2.22 (2H, t, J = 7.6 Hz), 1.69 (2H, m), 1.32 (4H, m), 0.91 (3H, t, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 62.86 MHz) δ 172.9 (CO), 139.5, 137.2 (C), 127.9, 126.8 (CH), 103.5 (CH−O), 65.3, 43.3, 36.8, 31.8, 25.4, 22.4 (CH_2), 14.0 (CH_3); UV (CH_2Cl_2) λ_{max} 230 nm; HR-MS (ESI+, m/z) calcd for $C_{16}H_{24}NO_3$ $[(M + H)^+]$ 278.1762, found 278.1756.

***N*-[4-(1,3-Dioxacyclopent-2-yl)benzyl]octanamide (5).** The synthetic procedure was essentially the same as for compound **3**. Octanoic acid (2.18 g, 15.1×10^{-3} mol), HO-Su (2.09 g, 18.2×10^{-3} mol), and DCC (3.74 g, 18.2×10^{-3} mol) were used as starting materials. The resulting crude ester compound (2.92 g, 12.1×10^{-3} mol) and compound **2** (1.80 g, 10.1×10^{-3} mol) were used for the reaction, and after purification by flash chromatography (EtOAc/cyclohexane 2:8 v/v) compound **5** (2.19 g, 7.2×10^{-3} mol, 70%) was obtained as a white powder: *Rf* 0.48 (EtOAc/cyclohexane 6:4 v/v); mp 77.2−78.4 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.47 (2H, d, J = 8.1 Hz), 7.31 (2H, d, J = 8.1 Hz), 5.82 (2H, m), 4.46 (2H, d, J = 5.7 Hz), 4.09 (4H, m), 2.22 (2H, t, J = 7.5 Hz), 1.66 (2H, m), 1.30 (8H, m), 0.90 (3H, m); ^{13}C NMR ($CDCl_3$, 62.86 MHz) δ 173.0 (CO), 139.8, 137.1 (C), 127.8, 126.6 (CH), 103.5 (CH-O), 65.3, 43.3, 36.8, 31.8, 29.4, 29.0, 25.8, 22.6 (CH_2), 13.8 (CH_3). The spectral data of compound **5** were in agreement with those reported by Durand et al.[12]

***N*-[4-(1,3-Dioxacyclopent-2-yl)benzyl]decanamide (6).** The synthetic procedure was essentially the same as for compound **3**. Decanoic acid (5.00 g, 29.0×10^{-3} mol), HO-Su (4.00 g, 38.8×10^{-3} mol), and DCC (7.17 g, 34.8×10^{-3} mol) were used as starting materials. The resulting crude ester compound (5.44 g, 20.2×10^{-3} mol) and compound **2** (3.00 g, 16.8×10^{-3} mol) were used for the reaction, and after purification by flash chromatography (EtOAc/cyclohexane 3:7 v/v) compound **6** (3.40 g, 10.2×10^{-3} mol, 62%) was obtained as a white powder: R_f 0.42 (EtOAc/cyclohexane 5:5 v/v); mp 80.1−80.9 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.47 (2H, d, J = 8.1 Hz), 7.31 (2H, d, J = 8.1 Hz), 5.82 (2H, m), 4.46 (2H, d, J = 5.7 Hz), 4.10 (4H, m), 2.22 (2H, t, J = 7.5 Hz), 1.66 (2H, m), 1.28 (12H, m), 0.90 (3H, m); ^{13}C NMR ($CDCl_3$, 62.86 MHz) δ 173.0 (CO), 139.5, 137.2 (C), 127.9, 126.9 (CH), 103.5 (CH-O), 65.3, 43.3, 36.8, 31.9, 29.5, 29.4, 29.3, 25.8, 25.6, 22.7 (CH_2), 14.1 (CH_3); UV (CH_2Cl_2) λ_{max} 230 nm; HR-MS (ESI+, m/z) calcd for $C_{20}H_{32}NO_3$ $[(M + H)^+]$ 334.2376, found 334.2382.

***N*-(4-Formylbenzyl)butanamide (7).** Under stirring, compound **3** (0.90 g, 3.6×10^{-3} mol) was dissolved in a 7:3 (v/v) $AcOH/H_2O$ mixture. After one night of stirring, 30 mL of EtOAc was added, and the organic layer was successively washed with $NaHCO_3$ saturated solution and brine, dried over Na_2SO_4, and evaporated under vacuum to give compound **7** (0.72 g, 3.5×10^{-3} mol, 96%) as a white powder: R_f 0.29 (EtOAc/cyclohexane 6:4 v/v); mp 72.2−73.0 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.97 (1H, s), 7.84 (2H, d, J = 8.1 Hz), 7.43 (2H, d, J = 8.1 Hz), 6.31 (1H, m), 4.51 (2H, d, J = 6.0 Hz), 2.25 (2H, t, J = 7.4 Hz), 1.70 (2H, sext, J = 7.4 Hz), 0.96 (3H, t, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 62.86 MHz) δ 191.9 (CHO), 173.4 (CO), 145.6, 135.6 (C), 130.1, 128.1 (CH), 43.2, 38.5, 19.2 (CH_2), 13.8 (CH_3); UV (CH_2Cl_2) λ_{max} 256 nm; HR-MS (ESI+, m/z) calcd for $C_{12}H_{16}NO_2$ $[(M + H)^+]$ 206.1173, found 206.1181.

***N*-(4-Formylbenzyl)hexanamide (8).** The synthetic procedure was essentially the same as for compound **7**. From compound **4** (1.12 g, 4.1×10^{-3} mol), compound **8** (0.90 g, 3.9×10^{-3} mol, 94%) was obtained as a white powder: R_f 0.34 (EtOAc/cyclohexane 6:4 v/v); mp 59.2−59.8 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.99 (1H, s), 7.84 (2H, d, J = 8.2 Hz), 7.43 (2H, d, J = 8.2 Hz), 6.18 (1H, m), 4.52 (2H, d, J = 6.0 Hz), 2.26 (2H, t, J = 7.6 Hz), 1.68 (2H, m), 1.34 (4H,m), 0.91 (3H, t, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 62.86 MHz) δ 192.1 (CHO), 174.0 (CO), 145.7, 135.4 (C), 130.1, 128.0 (CH), 43.1 (CH_2), 36.5, 31.4, 25.4, 22.3 (CH_2), 13.9 (CH_3); UV (CH_2Cl_2) λ_{max} 256 nm; HR-MS (ESI+, m/z) calcd for $C_{14}H_{20}NO_2$ $[(M + H)^+]$ 234.1488, found 234.1494.

***N*-(4-Formylbenzyl)octanamide (9).** The synthetic procedure was essentially the same as for compound **7**. From compound **5** (2.18 g, 7.1×10^{-3} mol), compound **9** (1.82 g, 7.0×10^{-3} mol, 98%) was obtained as a white powder: R_f 0.40 (EtOAc/cyclohexane 6:4 v/v); mp 62.5−63.5 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 10.0 (1H, s), 7.86 (2H, d, J = 8.1 Hz), 7.45 (2H, d, J = 8.1 Hz), 6.06 (1H, m), 4.54 (2H, d, J = 6.0 Hz), 2.27 (2H, t, J = 7.6 Hz), 1.68 (2H, m), 1.30 (8H, m), 0.89 (3H, t, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 62.86 MHz) δ 192.9 (CHO), 173.0 (CO), 139.7, 137.0 (C), 127.9, 126.8 (CH), 43.3 (CH_2), 36.8,

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31.7, 29.3, 29.0, 25.8, 22.6 (CH_2), 14.1 (CH_3). The spectral data of compound **5** were in agreement with those reported by Durand et al.[12]

***N*-(4-Formylbenzyl)decanamide (10).** The synthetic procedure was essentially the same as for compound **7**. From compound **6** (1.78 g, 5.3×10^{-3} mol), compound **10** (1.49 g, 5.1×10^{-3} mol, 97%) was obtained as a white powder: R_f 0.46 (EtOAc/cyclohexane 5:5 v/v); mp 69.0−69.8 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 10.0 (1H, s), 7.85 (2H, d, J = 8.2 Hz), 7.45 (2H, d, J = 8.2 Hz), 6.08 (1H, m), 4.54 (2H, d, J = 6.0 Hz), 2.27 (2H, t, J = 7.6 Hz), 1.68 (2H, m), 1.28 (12H, m), 0.90 (3H, m); ^{13}C NMR ($CDCl_3$, 62.86 MHz) δ 191.9 (CHO), 173.3 (CO), 145.6, 135.6 (C), 130.1, 128.1 (CH), 43.2 (CH_2), 36.7, 31.9, 29.5, 29.4, 29.3, 25.8, 25.6, 22.7 (CH_2), 14.1 (CH_3); UV (CH_2Cl_2) λ_{max} 256 nm; HR-MS (ESI+, m/z) calcd for $C_{18}H_{28}NO_2$ [$(M + H)^+$] 290.2118, found 290.2120.

***α*-Phenyl-*N*-(2-hydroxymethyl-1,3-dihydroxy-2-propyl)-imine (11).** Under stirring, benzaldehyde (6.00 g, 56.5×10^{-3} mol) and tris(hydroxymethyl)aminomethane (8.22 g, 67.8×10^{-3} mol) were dissolved in toluene, and the mixture was heated at reflux using a Dean−Stark apparatus. After 6 h of being refluxed, the reaction mixture was cooled and filtered, and the solvent was removed under vacuum to give compound **11** (11.0 g, 52.5×10^{-3} mol, 93%) as a white oil, which was used without further purification.

***α*-Phenyl-*N*-(2-hydroxymethyl-1,3-dihydroxy-2-propyl)-amine (12).** Under stirring and at 0 °C, compound **11** (2.00 g, 9.6×10^{-3} mol) was dissolved in a 99:1 (v/v) ethanol/acetic acid mixture, and 0.57 g of 10% Pd/C was portionwise added. The reaction mixture was submitted to a hydrogen atmosphere for 4 h (8 bar), and then the crude mixture was filtered off through a pad of Celite and the solvent was removed under vacuum. The crude mixture was purified by flash chromatography (EtOAc/methanol 9:1 v/v) to give compound **12** (1.46 g, 6.9×10^{-3} mol, 72%) as a white powder: R_f 0.39 (EtOAc/methanol 8:2 v/v); mp 86.4−87.6 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.36−7.21 (5H, m), 4.34 (4H, m), 3.73 (2H, s), 3.41 (6H, s); ^{13}C NMR ($CDCl_3$, 62.86 MHz) δ 142.3 (C), 128.6, 128.5, 126.9 (CH), 61.6 (CH_2), 60.5 (C), 45.9 (CH_2); UV (MeOH) λ_{max} 212 nm; HR-MS (ESI+, m/z) calcd for $C_{11}H_{18}NO_3$ [$(M + H)^+$] 212.1286, found 212.1287.

***α*-Phenyl-*N*-(2-hydroxymethyl-1,3-dihydroxy-2-propyl)-nitrone (13). (a) First Synthetic Strategy.** Under stirring and argon atmosphere, compound **12** (0.80 g, 3.8×10^{-3} mol) was dissolved in EtOH, and then Na_2WO_4 (0.038 g, $1.15.10^{-3}$ mol) dissolved in 1 mL of water was added to the mixture. The mixture was cooled to 0 °C, and H_2O_2 30% in water (0.78 g, $6.9.10^{-3}$ mol) was added dropwise, and then the mixture was stirred at room temperature for 18 h. The solvent was removed under vacuum, and the crude mixture was purified by flash chromatography (EtOAc) followed by two successive crystallizations from EtOAc/*n*-hexane to give compound **13** (0.160 g, 7.10^{-4} mol, 36%) as a white powder. **(b) Second Synthetic Strategy.** Under stirring and argon atmosphere, benzaldehyde (1.00 g, 9.4×10^{-3} mol), tris(hydroxymethyl)nitromethane (2.84 g, 18.9×10^{-3} mol), and AcOH (3.23 mL, 56.5×10^{-3} mol) were dissolved in EtOH. The mixture was cooled to 0 °C, and then zinc powder (2.45 g, 37.7×10^{-3} mol) was slowly added in order to keep the temperature at 15 °C. The mixture was stirred at room temperature for a couple of minutes and then heated at 60 °C in the dark for 10 h in the presence of molecular sieves (4 Å). The reaction mixture was filtered off through a pad of Celite, and the solvent was removed under vacuum. The crude mixture was purified by flash chromatography (EtOAc) followed by two successive crystallizations from EtOAc/*n*-hexane to give compound **13** (1.52 g, 6.7×10^{-3} mol, 72%) as a white powder: R_f 0.28 (EtOAc/methanol 9.5:0.5 v/v); mp 83.4−84.8 °C; ^{1}H NMR (DMSO, 250 MHz) δ 8.36 (2H, m), 7.60 (1H, s), 7.43 (3H, m), 4.94 (3H, t, J = 5.1 Hz), 3.82 (6H, d, J = 5.1 Hz); ^{13}C NMR (DMSO, 62.86 MHz) δ 133.7 (C), 131.7 (CH), 130.2, 129.3, 128.6 (CH), 80.4 (C), 60.4 (CH_2); UV (MeOH) λ_{max} 295 nm; MS (ESI+, m/z) 248.1 [M + Na]$^+$, 226.1 [M + H]$^+$. The spectral data of compound **13** were in agreement with those reported by Janzen and Zawalski except for the melting point that was found to be 89−91 °C.[21]

***α*-(4-Butanamidomethyl)phenyl-*N*-(2-hydroxymethyl-1,3-dihydroxy-2-propyl)nitrone (14).** Under stirring and argon atmosphere, compound **7** (0.80 g, 3.9×10^{-3} mol), tris(hydroxymethyl)-nitromethane (1.17 g, 7.8×10^{-3} mol), and AcOH (1.33 mL, 23.34×10^{-3} mol) were dissolved in EtOH. The mixture was cooled to 0 °C, and then zinc powder (1.00 g, 15.6×10^{-3} mol) was slowly added in order to keep the temperature at 15 °C. The mixture was stirred at room temperature for a couple of minutes and then heated at 60 °C in the dark for 10 h in the presence of molecular sieves (4 Å). The reaction mixture was filtered off through a pad of Celite, and the solvent was removed under vacuum. The crude mixture was purified by flash chromatography (EtOAc/methanol 9:1 v/v) followed by two successive crystallizations from ethanol to give compound **14** (0.90 g, 2.8×10^{-3} mol, 72%) as a white powder: R_f 0.40 (EtOAc/methanol 8:2 v/v); mp 160.2−160.6 °C; ^{1}H NMR (DMSO, 250 MHz) δ 8.35 (1H, t, J = 6.0 Hz), 8.30 (2H, d, J = 8.0 Hz), 7.57 (1H, s), 7.29 (2H, d, J = 8.0 Hz), 4.94 (3H, t, J = 5.5 Hz), 4.30 (2H, d, J = 6.0 Hz), 3.81 (6H, d, J = 5.5 Hz), 2.14 (2H, t, J = 7.2 Hz), 1.56 (2H, sext, J = 7.2 Hz), 0.87 (3H, t, J = 7.2 Hz); ^{13}C NMR (DMSO, 62.86 MHz) δ 172.5 (CO), 142.1 (C), 133.6 (CH), 130.2 (C), 129.3, 127.2 (CH), 80.3 (C), 60.4, 43.2, 37.8, 19.2 (CH_2), 14.1 (CH_3); UV (MeOH) λ_{max} 299 nm; HR-MS (ESI+, m/z) calcd for $C_{16}H_{25}N_2O_5$ [$(M + H)^+$] 325.1758, found 325.1760.

***α*-(4-Hexanamidomethyl)phenyl-*N*-(2-hydroxymethyl-1,3-dihydroxy-2-propyl)nitrone (15).** The synthetic procedure was essentially the same as for compound **14**. Compound **8** (0.60 g, 2.6×10^{-3} mol), tris(hydroxymethyl)nitromethane (0.78 g, 5.1×10^{-3} mol), AcOH (1.18 mL, 20.6×10^{-3} mol), and zinc powder (0.89 g, 13.7×10^{-3} mol) were used as starting materials. The crude mixture was purified by flash chromatography (EtOAc/methanol 9:1 v/v) followed by two successive crystallizations from ethanol to give compound **15** (0.58 g, 1.6×10^{-3} mol, 64%) as a white powder: R_f 0.44 (EtOAc/methanol 8:2 v/v); mp 137.6−138.2 °C; ^{1}H NMR (DMSO, 250 MHz) δ 8.35 (1H, t, J = 5.9 Hz), 8.30 (2H, d, J = 8.0 Hz), 7.57 (1H, s), 7.29 (2H, d, J = 8.0 Hz), 4.94 (3H, t, J = 5.4 Hz), 4.30 (2H, d, J = 5.9 Hz), 3.81 (6H, d, J = 5.4 Hz), 2.14 (2H, t, J = 7.2 Hz), 1.56 (2H, m), 1.27 (4H, m), 0.87 (3H, t, J = 7.2 Hz); ^{13}C NMR (DMSO, 62.86 MHz) δ 172.5 (CO), 142.1 (C), 133.6 (CH), 130.2 (C), 129.3, 127.2 (CH), 80.3 (C), 60.4, 43.2, 35.8, 31.3, 25.5, 22.3 (CH_2), 14.4 (CH_3); UV (MeOH) λ_{max} 299 nm; HR-MS (ESI+, m/z) calcd for $C_{18}H_{29}N_2O_5$ [$(M + H)^+$] 353.2057, found 353.2057.

***α*-(4-Octanamidomethyl)phenyl-*N*-(2-hydroxymethyl-1,3-dihydroxy-2-propyl)nitrone (16).** The synthetic procedure was essentially the same as for compound **14**. Compound **9** (0.80 g, 3.1×10^{-3} mol), tris(hydroxymethyl)nitromethane (0.92 g, 6.1×10^{-3} mol), AcOH (1.05 mL, 18.4×10^{-3} mol), and zinc powder (0.80 g, 12.2×10^{-3} mol) were used as starting materials. The crude mixture was purified by flash chromatography (EtOAc/methanol 9:1 v/v) followed by two successive crystallizations from ethanol to give compound **16** (0.78 g, 2.1×10^{-3} mol, 68%) as a white powder: R_f 0.36 (EtOAc/methanol 9:1 v/v); mp 144.6−145.4 °C; ^{1}H NMR (DMSO, 250 MHz) δ 8.38 (1H, t, J = 6.0 Hz), 8.29 (2H, d, J = 8.4 Hz), 7.58 (1H, s), 7.28 (2H, d, J = 8.4 Hz), 4.96 (3H, t, J = 5.5 Hz), 4.29 (2H, d, J = 6.0 Hz), 3.81 (6H, d, J = 5.5 Hz), 2.15 (2H, t, J = 7.3 Hz), 1.52 (2H, m), 1.25 (8H, m), 0.87 (3H, t, J = 7.1 Hz); ^{13}C NMR (DMSO, 62.86 MHz) δ 172.8 (CO), 142.1 (C), 133.6 (CH), 130.2 (C), 129.3, 127.2 (CH), 80.2 (C), 60.4, 42.3, 35.8, 31.7, 29.1, 28.9, 25.8, 22.5 (CH_2), 14.4 (CH_3); UV (MeOH) λ_{max} 299 nm; HR-MS (ESI+, m/z) calcd for $C_{20}H_{33}N_2O_5$ [$(M + H)^+$] 381.2384, found 381.2384.

***α*-(4-Decanamidomethyl)phenyl-*N*-(2-hydroxymethyl-1,3-dihydroxy-2-propyl)nitrone (17).** The synthetic procedure was essentially the same as for compound **14**. Compound **10** (1.20 g, 4.2×10^{-3} mol), tris(hydroxymethyl)nitromethane (1.26 g, 8.3×10^{-3} mol), AcOH (1.43 mL, 25.0×10^{-3} mol), and zinc powder (1.08 g, 16.6×10^{-3} mol) were used as starting materials. The crude mixture was purified by flash chromatography (EtOAc/methanol 9:1 v/v) followed by two successive crystallizations from ethanol to give compound **17** (1.16 g, 2.8×10^{-3} mol, 68%) as a white powder: R_f 0.40 (EtOAc/methanol 9:1 v/v); mp 150.8−151.6 °C; ^{1}H NMR (DMSO, 250 MHz) δ 8.38 (1H, t, J = 6.0 Hz), 8.30 (2H, d, J = 8.3 Hz), 7.57 (1H, s), 7.28 (2H, d, J = 8.3 Hz), 4.97 (3H, t, J = 5.5 Hz), 4.29 (2H, d, J =

6.0 Hz), 3.80 (6H, d, J = 5.5 Hz), 2.15 (2H, t, J = 7.3 Hz), 1.54 (2H, m), 1.25 (12H, m), 0.87 (3H, t, J = 6.8 Hz); ^{13}C NMR (DMSO, 62.86 MHz) δ 172.7 (CO), 142.0 (C), 133.5 (CH), 130.2 (C), 129.3, 127.2 (CH), 80.3 (C), 60.4, 43.2, 35.8, 31.8, 29.4, 29.2, 29.2, 29.1, 25.8, 22.6 (CH_2), 14.5 (CH_3); UV (MeOH) λ_{max} 299 nm; HR-MS (ESI+, m/z) calcd for $C_{22}H_{37}N_2O_5$ [$(M + H)^+$] 409.2697, found 409.2690.

Determination of log k'_W Values. Compounds were dissolved in MeOH at 1.0 mg/mL and were injected onto a Microsorb C18 reversed-phase column (250 mm × 4.6 mm, 5 μm). The compounds were eluted at various MeOH and water ratios (9:1 to 3:7 v/v) using a flow rate of 0.8 mL/min. The column temperature was 25 °C, and the UV detector wavelength was λ = 298 nm. Linear regression analysis were performed on three data points for TC_5-PBN (from 6:4 to 4:6; r^2 = 0.9998) and TC_7-PBN (from 8:2 to 6:4; r^2 = 1) and four points for T-PBN (from 7:3 to 3:7; r^2 = 0.9988), TC_3-PBN (from 6:4 to 3:7; r^2 = 0.9981) and for TC_9-PBN (from 9:1 to 6:4; r^2 = 0.9986). The log k' values were calculated by using the equation: $\log k' = \log((t - t_0)/t_0)$, where t is the retention time of the nitrone and t_0 is the elution time of MeOH, which is not retained on the column.

Electron Paramagnetic Resonance and Spin-Trapping Experiments. A saturated solution of T-PBN was first prepared at 0.15 mol·L^{-1} (~2× the solubility limit in water). The supernatant of that solution was used for the analysis and was referred to as the concentrated solution. In other experiments, a 0.008 mol·L^{-1} nitrone aqueous solution was prepared, and it was referred to as the low concentrated solution. The concentrations of **18** and **20** were evaluated in water, methanol, and DMF by simulation of the EPR signal recorded from a solution containing the nitrone **13** or **15** (0.01−0.05 mol·L^{-1}, depending on the nitrone and the solvent) in the presence of 3-carboxyproxyl (3-CP, 0.4 mmol·L^{-1}) used here as an internal standard. In the various spin-trapping experiments, the free radicals were produced in 10 mmol·L^{-1} phosphate buffer at pH 7.2, in the presence of various amounts of methanol or DMSO, and mostly using a 0.008 mol·L^{-1} nitrone concentration, though this parameter could be varied in between 0.004 mol·L^{-1} and 0.15 mol·L^{-1}. High nitrone concentrations were obtained by dissolving the spin trap in DMF or DMSO before adding phosphate buffer and the radical generator. The superoxide generating system employed used 0.4 mmol·L^{-1} xanthine and 0.1 unit·mL^{-1} xanthine oxidase. Hydroxyl radical was produced by a standard Fenton system consisting of 0.1% H_2O_2, 1 mmol·L^{-1} ethylenediaminetetraacetic acid (EDTA), and 1 mmol·L^{-1} $FeSO_4$. For the concentrated Fenton system, the concentrations of $FeSO_4$ and H_2O_2 were doubled. Carbon-centered radicals $^{\bullet}CH_3$ and $^{\bullet}CH_2OH$ were generated by adding DMSO (20−75%) or methanol (20−75%), respectively, to the standard Fenton system described above. EPR assays were carried out in capillary tubes and the spectra were recorded at room temperature (20−22 °C) on a continuous wave X-band Bruker EMX spectrometer, equipped with an NMR gaussmeter for magnetic field calibration in order to evaluate g values. The following conditions were used: modulation frequency, 100 kHz; non saturating microwave power, 1−20 mW; modulation amplitude, 0.1−0.15 mT; receiver gain, 10^5−10^6; time constant, 1.28−655 ms; scan time, 60−180 s. Simulations were carried out using the EPR Software WinSim2002.[38]

Mass Spectrometry. High-resolution MS and MS/MS experiments were performed using a QStar Elite (AB Sciex) mass spectrometer equipped with an electrospray ionization source operated in the positive mode. The capillary voltage was set at +5500 V and the cone voltage at +70 V. In this hybrid instrument, ions were measured using an orthogonal acceleration time-of-flight (oa-TOF) mass analyzer. A quadrupole was used for selection of precursor ions to be further submitted to collision-induced dissociation (CID) in MS/MS experiments. Air was used as the nebulizing gas (10 psi) while nitrogen was used as the curtain gas (20 psi) as well as the collision gas. Collision energy was set according to the experiments. Samples were prepared in water, methanol or DMF and kept at room temperature. Nitrone solutions were prepared as described in the EPR and spin trapping experiments section. After 1−2 h, an aliquot was submitted to an EPR analysis while the rest of the sample was diluted in methanol (1/10) and then in a methanol solution of ammonium acetate (3 mM) using a dilution factor of $1/10^3$. Diluted sample solutions were immediately introduced in the ionization source at a 5 μL·min^{-1} flow rate using a syringe pump.

Computational Methods. All calculations were performed at the Ohio Supercomputer Center. Density functional theory[39,40] was used in this study to determine the optimized geometry, vibrational frequencies, and single-point energy of all stationary points[41−43] using Gaussian 03.[44] The effect of aqueous solvation was also investigated using the polarizable continuum model (PCM).[45−47] Single-point energies were obtained at the B3LYP/6-31+G** level based on the optimized B3LYP/6-31G* geometries. Charge densities were obtained from a natural population analysis (NPA)[48] at the single point PCM/B3LYP/6-31+G** level. These calculations used six Cartesian d functions. Stationary points for nitrones and their respective adducts have zero imaginary vibrational frequency as derived from a vibrational frequency analysis (B3LYP/6-31G*). A scaling factor of 0.981 was used for the zero-point vibrational energy (ZPE) corrections for the B3LYP/6-31G* level.[49] Spin contamination for all of the stationary point of the radical structures was negligible, i.e., $\langle S^2 \rangle$ = 0.75.

■ ASSOCIATED CONTENT

Supporting Information

Complete ref 41; ^{1}H NMR spectra of compounds **3**, **4**, **6**−**8**, **10**, **12**, and **14**−**17**; ^{13}C NMR spectra of compounds **3**−**6**, **12**, and **14**−**17**; HRMS spectra of compounds **14**−**17**; HPLC chromatograms of compounds **14**−**17**; plot of the nitronyl ^{1}H and ^{13}C NMR chemical shift of T-PBN vs the solvent polarity parameter; ESI MS/MS spectrum of T-PBN and TC_5−PBN; thermodynamic parameters of the nitrones and nitroxides in aqueous and DMSO phases at the PCM/B3LYP/6-31+g**//B3LYP/6-31g* level of theory; Cartesian coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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