Synthesis and Preliminary Biological Evaluations of Ionic and Nonionic Amphiphilic α-Phenyl-*N*-*tert*-butylnitrone Derivatives

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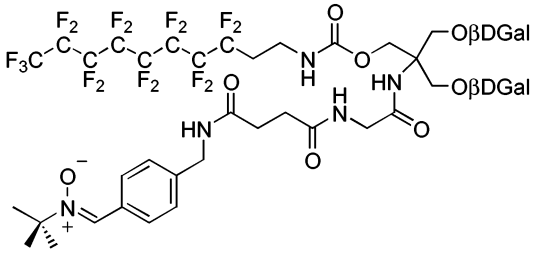
In this study we report the synthesis of a series of new amphiphilic compounds derived from α-phenyl-*N*-*tert*-butylnitrone (PBN). The nitrone function was fitted into the core of the molecule between its polar and apolar goups. The polar head consisted of a lactobionamide, an ammonium, or a carboxylate group. The hydrophobic part consisted of a hydro- or a perfluorocarbon chain. The hydrophobic chain was linked to the *tert*-butyl group of the PBN derivatives using an urethane, a thioether, or an amide bond. The impact of these different parameters on the hydrophilic lipophilic balance of these compounds and their spin trap activity were studied. The various ESR measurements indicated that the aromatic and *tert*-butyl functional groups of PBN did not affect its spin trap properties. Moreover, these compounds were found to increase the viability of cultured human skin fibroblasts harboring the neurogenic ataxia retinitis pigmentosa mutation and presenting a severe ATPase deficiency.

Introduction

Free radicals and reactive oxygen species (ROS) are thought to be involved in a number of chronic and constitutive diseases, including notably ischemia-reperfusion syndrome, Friedreich's ataxia, atherosclerosis, Alzheimer's and Parkinson's diseases, and cellular aging.[1,2] ROS have also been shown to act as signals for the apoptosis process.[3] Nitrone-based free radical traps can protect cells from ROS of both endogenous or exogenous origin. The frequently used nitrone, α-phenyl-*N*-*tert*-butylnitrone (PBN), has promising pharmacological properties and possesses broad neuroprotective activity.[4,5] The exact mechanism of the PBN effect has not yet been fully elucidated. It was first hypothesized that the beneficial activity of PBN was related to its ability to trap radicals forming stable adducts. However, according to Floyd et al.,[4] the free radical trapping activity might not represent the primary action of this compound, but it may rather interfere with cellular signal transduction processes as yet undetermined.

Because of their promising pharmacological effects, the control of nitrone bioavailability represents a great challenge. So far, high doses of PBN are required to display significant protective activity. It can be predicted, however, that derivatives of PBN will differ significantly in their ability to cross the blood−brain barrier (BBB). Accordingly, PBN has been shown to have a greater capacity to cross the BBB compared to its water-soluble analogue α-pyridyl-1-oxide-*N*-*tert*-butylnitrone. Thomas et al.[6] showed that amphipatic spin traps cross membranes better than PBN and can afford protection from free radicals in both the cytosol and cell membranes. To improve the bioavailability of PBN and to modify its hydrophilic lipophilic balance, Janzen et al.,[7] AstraZeneca,[8] and Dhainaut et al.[9] modified either the aromatic or *N*-terminal moieties of PBN.

Scheme 1. Structure of Compound **A**



We recently synthesized a glycosylated amphiphilic PBN (compound **A**)[10] by grafting its aromatic group onto the polar head of a Tris-derived perfluorocarbon amphiphile (Scheme 1). Whereas PBN showed no protective activity against superoxide-triggered apoptosis in vitro, this compound readily diminished superoxide dismutase (SOD) production and apoptosis in cultured skin fibroblasts with an isolated complex V deficiency of the respiratory chain, caused by the neurogenic ataxia retinitis pigmentosa (NARP) mutation.[11] Although this compound exhibited a very high antiapoptotic activity, its synthesis was long and tedious to carry out. Moreover, structural modifications of this compound to potentially enhance its biological activity appear particularly delicate. To allow a more detailed study of the structural and biological properties of PBN derivatives while taking these observations into account, we synthesized a new family of PBN-derived amphiphilic

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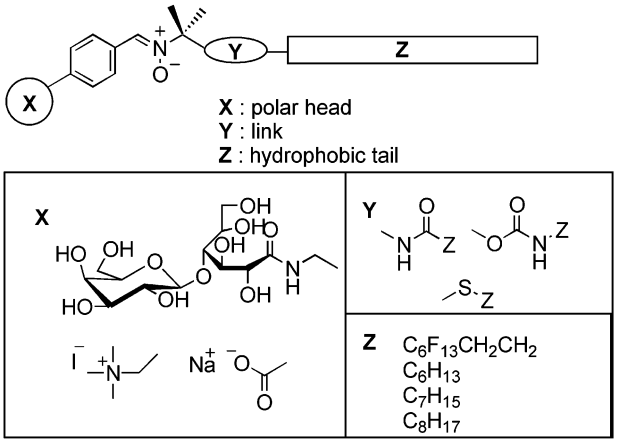
Scheme 2. General Structure of Amphiphilic PBN



compounds and performed a preliminary evaluation of their physicochemical and biological properties.[12]

To decrease the number of preparation steps, the nitrone function was inserted into the heart of the molecule (Scheme 2). It bridged the polar head grafted onto the aromatic function and the hydrophobic moiety bound to the PBN *tert*-butyl group. The convergent chemical pathway chosen is easy to perform and furthermore allows modification of the nature, the length, and the type of the hydrophobic moiety grafted, as well as the ionic or nonionic character of the polar head.

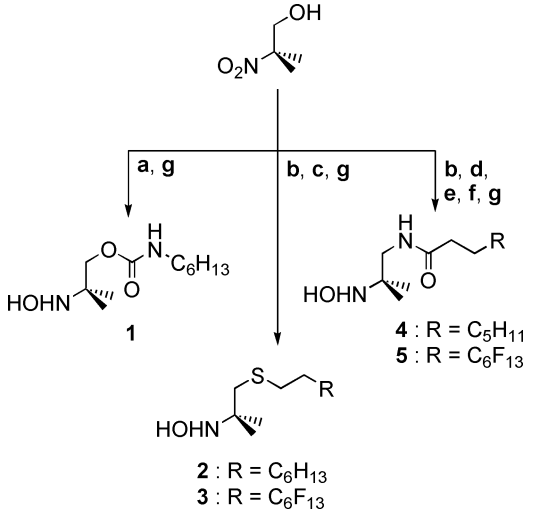
The polar head, which provides water solubility to the molecule, was linked to the PBN aromatic cycle in the para position. This motif was either an ionic (ammonium or carboxylate) or nonionic polyhydroxylated group. In the latter case, the introduction of a polar head derived from lactobionamide allowed us, on one hand, to solve the problem of the ionic strength of the solutions and, on the other, to strongly limit the toxicity of these compounds.

To ensure that this new nitrone family had an amphiphilic character, either a hydro- or perfluorocarbon chain of variable length was linked to the PBN-derived *tert*-butyl group, using a thioether, an amide, or a carbamate bond. The ester bond was removed because of its rapid hydrolysis under the preparation conditions of the polar head moiety (as illustrated by the hydrolysis of sugar-protective acetyl groups under Zemplen conditions). Considering the very high stability of ether bonds, we preferred to introduce a thioether link that could more easily be oxidized in vivo.

The hydrophobic chain length was limited to six to eight carbon atoms in order to ensure high water solubility and to maintain amphiphilic character. It has been well-established that water solubility is affected by the length of the chain, while the molecule's ability to cross cell membranes depends strongly on the hydrophilic lipophilic balance (HLB) of the active principle.[13−15] With less than six carbon atoms, the amphiphilic property is predictably lost. For these reasons, we also grafted perfluoroalkyl chains onto the molecule because the hydrophobic contribution of a difluoromethylene group is roughly 1.8 times higher than that of methylene.[16] Furthermore, most of the perfluoroalkyl surfactants exhibit a toxicity and a detergent power much lower than their hydrocarbon analogues.[17,18]

The impact of these various structural modifications on spin trap activity, HLB, cytotoxicity, membrane cell crossing ability, and the capacity of these amphiphilic nitrones to enhance the cell viability of cultured skin fibroblasts constitutively releasing increased ROS is reported in this paper.

Scheme 3. Synthesis of Hydrophobic Parts[a]



[a] Reagents: (a) $C_6H_{13}NCO$, toluene, DABCO, Δ, 100%; (b) TsCl, pyridine, CH_2Cl_2, 83%; (c) $C_8H_{17}SH$, *t*-BuOK, DMF, 97% or $C_6F_{13}CH_2CH_2SH$, MeONa, DMF, 58%; (d) NaN_3, DMF, 20 °C, 85%; (e) $P(Ph)_3$, THF, 20 °C, then 2 N NaOH, 87%; (f) $C_7H_{15}COCl$, TEA, DMF, −15 °C, 77% or $C_6F_{13}CH_2CH_2COOH$, DCC, HOBT, CH_2Cl_2, 20 °C, 90%; (g) 4 equiv of SmI_2, THF/MeOH 2:1 v/v, 50−100%.

Results and Discussion

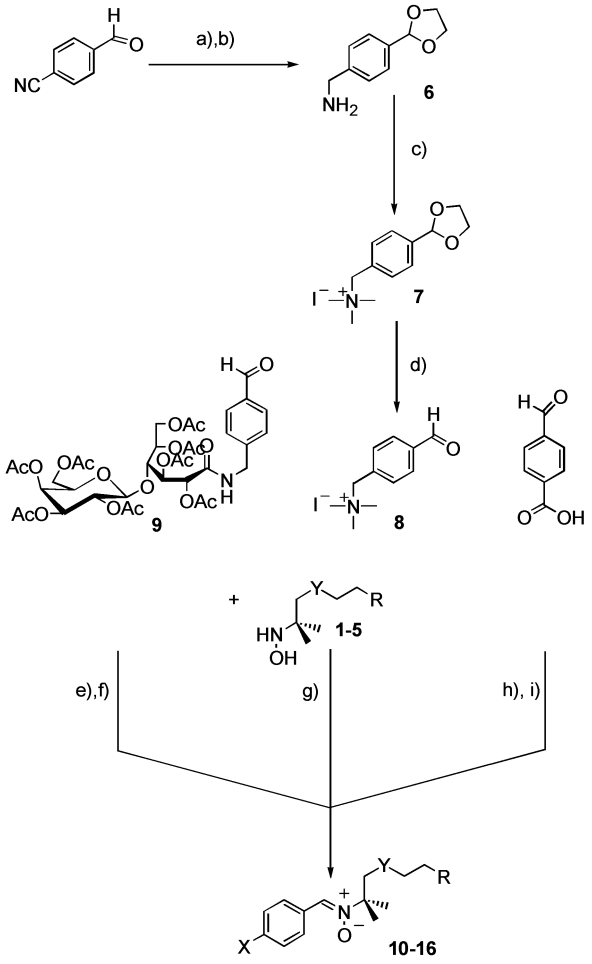
Synthesis. The chemical structures of most of these new antioxidants have been already mentioned,[12] and their syntheses can be summarized as follows.

The insertion of PBN into the core of the molecule required the grafting of functional groups onto both its *tert*-butyl and its aromatic functions. 2-Methyl-2-nitropropanol was therefore used as starting material. In the first step, an alkyl chain was grafted onto 2-methyl-2-nitropropanol through a carbamate, an amide-type bond, or a thioether-type bond. The nitro group of the resulting compound was then selectively reduced to hydroxylamine residues **1**−**5** with Kagan reagent[19] under mild conditions according to the method of Kende and Mendoza[20] (Scheme 3).

The nonionic hydrophilic head of the amphiphilic PBN consisted of a lactobionamide group grafted onto the para position of the benzaldehyde.[21] The linking of the aldehyde function of this hydrophilic aromatic moiety to the hydroxylamine groups **1**−**5** led to the final molecules **10**−**14** (Scheme 4). It should be noted that the Zemplen reaction hydrolysis in basic medium led to the formation of small quantities of deacetylated aldehyde. Therefore, after deacetylation, all glycosylated nitrones were purified by C18 reverse-phase HPLC (eluent methanol/water) and were obtained as a pure white powder without a residual ESR signal.

Concerning the anionic compound **15** (carboxylate), it was obtained by coupling 4-carboxybenzaldehyde with hydroxylamine **2** in ethanol in the dark under argon atmosphere. The reaction was complete after 3 days at 60 °C. The compound was purified by flash chromatography and recrystallization in methanol/ether. Carboxylic acid was neutralized by dropwise addition of a

Scheme 4. Synthesis of Amphiphilic Nitrones **10**−**16**[a]



10 : X = CH_2Lactobionamide ; Y = S ; R = C_6H_{13}
11 : X = CH_2Lactobionamide ; Y = S ; R = C_6F_{13}
12 : X = CH_2Lactobionamide ; Y = NHCO ; R = C_5H_{11}
13 : X = CH_2Lactobionamide ; Y = NHCO ; R = C_6F_{13}
14 : X = CH_2Lactobionamide ; Y = OCONH ; R = C_4H_9
15 : X = COONa ; Y = S ; R = C_6H_{13}
16 : X = $NMe_3^+I^-$; Y = S ; R = C_6F_{13}

[a] Reagents: (a) ethylene glycol, TsOH, toluene, Δ; (b) $AlLiH_4$, ether, 73% in two steps; (c) CH_3I, Bu_3N, DMF, 70%; (d) CH_3COOH/H_2O, 100%; (e) THF, 4 Å molecular sieves, argon, dark, 60 °C, 50−78%; (f) MeONa catalytic, MeOH, quantitative yield; (g) pyridine, 4 Å molecular sieves, argon, dark, 80 °C, 57%; (h) ethanol, 4 Å molecular sieves, argon, dark, 60 °C, 52%; (i) 0.05 N NaOH, quantitative yield.

solution of 0.05 N sodium hydroxide to produce the carboxylate nitrone **15**.

To obtain the quaternary ammonium polar head, 4-[1,3]dioxolan-2-yl-benzylamine **6** was permethylated according to a protocol developed by Sommer et al.,[22,23] using an excess of methyl iodide and 2 equiv of tributylamine in DMF. Subsequent addition of ethyl acetate yielded pure ammonium **7** as a white precipitate. Benzaldehyde derivative **8** was regenerated by hydrolysis of ethylene ketal with a mixture of acetic acid and water. Cationic compound **16** was then synthesized by reacting ammonium benzaldehyde **8** with perfluorohydroxylamine **3** in pyridine at 80 °C. Under these polar conditions, the reaction was fast (1 day) and the ammonium derivative **16** was obtained (57% yield) following recrystallization in methanol/ether.

All these PBN-derived amphiphilic compounds were then fully characterized by NMR and mass spectroscopy (see Experimental Section).

Table 1. Physicochemical Data of Glycosylated Nitrones

nitrones	Y	R	X	cmc (mM)	γ_{cmc} (mN/m)	log k'_W
10	S	C_6H_{13}	lacto	0.41	30	4.52
11	S	C_6F_{13}	lacto	0.035	20	5.71
12	NHCO	C_5H_{11}	lacto	*a*	*a*	2.76
13	NHCO	C_6F_{13}	lacto	0.213	20	4.65
14	OCONH	C_4H_9	lacto	*a*	*a*	2.66
PBN						1.64
A				0.016[b]	28[b]	5.70

[a] No cmc and no limit surface tension measurable. [b] See ref 10.

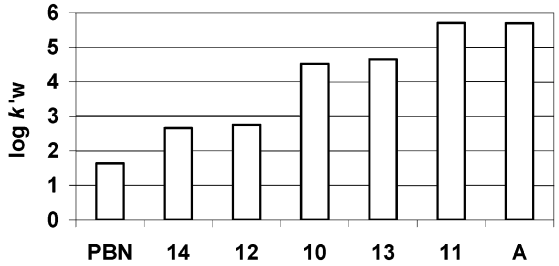


Figure 1. Evaluation of the relative lipophilicity of the nitrone compounds.

Physicochemical Measurements. All amphiphilic PBNs obtained were freely soluble in water. To specify their surfactant activity, we determined their critical micellar concentration (cmc) by using surface tension measurements (Table 1). In agreement with the literature,[16] the perfluoroalkyl chain sharply decreased the cmc and the surface tension at the cmc (γ_{cmc}) of these compounds. The perfluorocarbon compound **11** had a cmc 10 times lower than its hydrocarbon analogue **10**. Furthermore, with a hydrocarbon chain comprising less than eight carbon atoms, compounds **12** and **14** exhibited no cmc. Considering the respective cmc values of compound **10** (cmc = 0.41 mM) and of *n*-octyl glucoside (cmc = 14.5 mM), both bearing the same hydrophobic chain, it can be assumed that the hydrophobic portion of compound **10** included both the sulfur atom and the gem-dimethyl group. This hypothesis was strengthened by the marked difference between the cmc values of perfluoro compounds **11** (cmc = 0.035 mM) and **13** (cmc = 0.213 mM). The thioether bond apparently increased the hydrophobicity, while the amide bond conferred a marked hydrophilic character on the molecule.

The relative lipophilicities (log k'_W) of all of these compounds were measured by a rapid and well-established chromatographic technique (Table 1 and Figure 1).[24,25] Compound **A** and PBN were also included for the sake of comparison. The values obtained were in good agreement with data resulting from superficial tension measurements. The spin traps bearing a perfluorocarbon chain linked through a thioether bond were the most hydrophobic compounds. We also noticed that fluoro compounds **11** and **A** exhibited comparable hydrophobic properties (their log k'_W were almost identical), although their perfluorocarbon chain lengths were different. This result confirmed the contribution of both the thioether bond and the gem-dimethyl group to the lipophilicity of the molecule. The bond between the hydrophobic chain and the nitrone moiety thus sharply modifies the HLB of the compounds without affecting

Table 2. Hyperfine Splitting Constant for Radical Adducts of Some Amphiphilic Nitrones in Aqueous Media

	$^{\bullet}COO^{-}$ adduct		$^{\bullet}CH_3$ adduct	
nitrones	a_H (mT)	a_N (mT)	a_H (mT)	a_N (mT)
PBN	0.460[a]	1.590[a]	0.368[b]	1.651[b]
A	0.461	1.580	*c*	*c*
12	0.380	1.510	0.388	1.553
10	0.434	1.528	0.394	1.514
11	0.446	1.527	0.385	1.510
15	0.416	1.525	0.373	1.503

[a] See ref 26 and references therein. [b] See ref 27 and references therein. [c] Not determinated.

the water solubility of this new family of amphiphilic nitrones. Finally, our measurements confirmed the effectiveness of the perfluorocarbon chain compared to a hydrocarbon analogue in providing high hydrophobicity to particular molecules.

To estimate their spin-trapping properties, the amphiphilic nitrones **10**−**12** and **15** were also used to perform preliminary trapping studies of $^{\bullet}CH_3$ and $^{\bullet}CO_2^{-}$. The radicals were generated in a phosphate buffer (pH 7.0) solution of the nitrone (100 mM) by a Fenton reaction in the presence of dimethyl sulfoxide (DMSO) and sodium formate, respectively. Despite the presence of the bulky hydrophilic and hydrophobic groups, all of the nitrones tested yielded an easily detectable concentration of the corresponding spin adducts (Table 2). As we previously described with the glycosylated amphiphilic PBN **A**,[10] the electron spin resonance (ESR) signals obtained with **10**−**12** and **15** were composed of the superimposition of the isotropic and anisotropic spectra of the investigated spin adduct. The isotropic and anisotropic spectra corresponded to the free spin adduct and to the spin adduct partly immobilized within the micelles, respectively.[10] When dioxane was added to break the organization of these amphiphilic nitrones, only the isotropic spectrum was observed (Figure 2).

The ratio between the anisotropic and isotropic spectra was greater for nitrones with low cmc. Thus, the methyl spin adduct of **11** (cmc = 0.035 mM) is strongly immobilized (Figure 2a), while for **12** (cmc = 0.213 mM), both the free and immobilized spin adducts were observed (Figure 2c).

Preliminary Biological Experiments. We then investigated the ability of these different compounds to trap ROS overproduced in cultured human skin fibroblasts harboring the NARP mutation and presenting an ATPase deficiency. The endogenous ROS production associated with the NARP mutation in these fibroblasts readily triggers apoptosis and represents a potential target for the treatment of this disease.[11] We found that the ROS neutralization afforded by the nitrone compounds enhanced cell viability. In addition, since ROS production occurs within mitochondria, it follows that the protection afforded by these spin traps is highly dependent on their ability to cross cell membranes and on their cytoplasmic distribution.

When cultured in glucose-rich culture medium, no significant differences can be observed between the fate of ATPase-deficient and control fibroblasts. When galactose (selective medium), which enters glycolysis slowly compared with glucose, is substituted for glucose, we observed a decrease in cell viability, as measured by the MTT test.[28] Cells were exposed to increasing concentrations (100 and 200 μM) of the different nitrone compounds for 48 and 72 h in the selective medium for respiratory competent cells. The results obtained with the MTT assay are presented in Figure 3. After 48 and 72 h of incubation in the selective medium, we observed a strong reduction in cell viability. No compound showed a positive and statistical effect on cell survival at either of the concentrations used after 48 h of incubation. After 72 h of incubation at 100 μM or only 48 h of incubation at 200 μM, even nitrones **10**, **14**, and **15**, bearing either a hydrocarbon chain or a ionic polar head, were cytotoxic. PBN did not exhibit any protective effect regardless of the concentration used. However, the perfluorinated nitrones showed no cytotoxic effect under any of the conditions used. The high toxicity of ionic derivatives **15** and **16**, probably due to their ionic structures, precluded their biological assay.

Only compound **A**, as previously described,[11] restored a high rate of cell proliferation at 100 and 200 μM after 72 h in the selective medium. Furthermore, the most hydrophobic nitrone **11** (100 and 200 μM) restored a partial proliferation after 72 h of incubation compared to cells cultured in the normal medium.

Conclusion

The result obtained with our new amphiphilic spin traps clearly shows that the amphiphilic character is the most important parameter determining the biological efficiency of these spin traps. Their respective hydrophobic properties probably favored their transport through cell membranes and their distribution in the cytoplasm. However, the amphiphilic character of compounds **10** and **14**, bearing a hydrocarbon chain, resulted in a detergent effect probably responsible for the cytotoxicity observed. This problem presumably did not occur with PBN-derived perfluoroalkyl compounds, such as **11**, because of the strong hydrophobic and lipophobic behavior of the perfluorocarbon chains. Indeed, it is now well-accepted that perfluoroalkyl surfactants do not exhibit noticeable detergent effect on cells (in an appropriate concentration range), which could explain the lack of cytotoxicity of spin traps **A** and **11**, thus reinforcing their biological interest.

It is worth noting that the best biological results were obtained with compounds **A** and **11**, which have similar log k'_W values. However, compound **A** was more efficient at restoring the viability of ATPase-deficient fibroblasts. Thus, if it is true that the hydrophobic character plays a significant role in the biological activity of these compounds, one has also to take into account the positioning of the nitrone function within the molecules to explain their differences in effectiveness. The lateral presentation of the nitrone function seems to be more suitable for efficient ROS spin trapping, in contrast to an insertion within the heart of the molecule. The synthesis of a new family of PBN-derived amphiphilic compounds is currently underway in our lab to take into account this observation.

Experimental Section

General. The progress of the reactions and the homogeneity of the compounds were monitored by thin-layer chromatography (TLC Merck 254). Compound detection was achieved by exposure to UV light (254 nm), by spraying a 5% sulfuric acid solution in methanol or 5% ninhydrin solution in ethanol (in

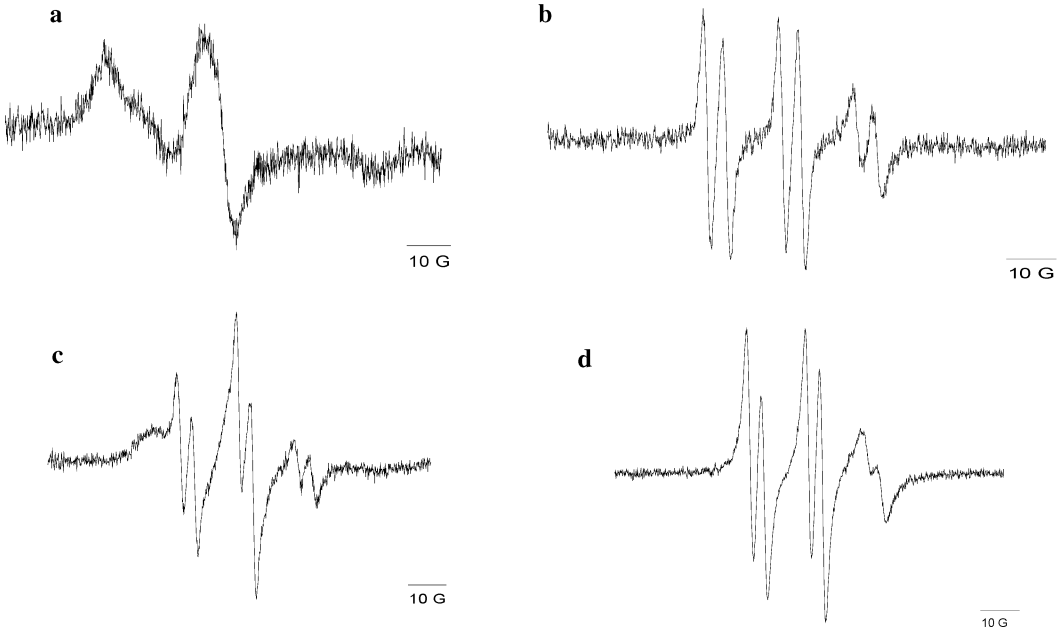


Figure 2. ESR signals observed during the trapping of $^{\bullet}CH_3$ with **11** and **12** (100 mM) in phosphate buffer (pH 7.0): (a) with **11**; (b) same as (a) 25 min after addition of dioxane (60%), $a_N = 1.51$ mT, $a_H = 0.38$ mT; (c) with **12**; (d) same as (c) 25 min after addition of dioxane (20%), $a_N = 1.55$ mT, $a_H = 0.39$ mT.

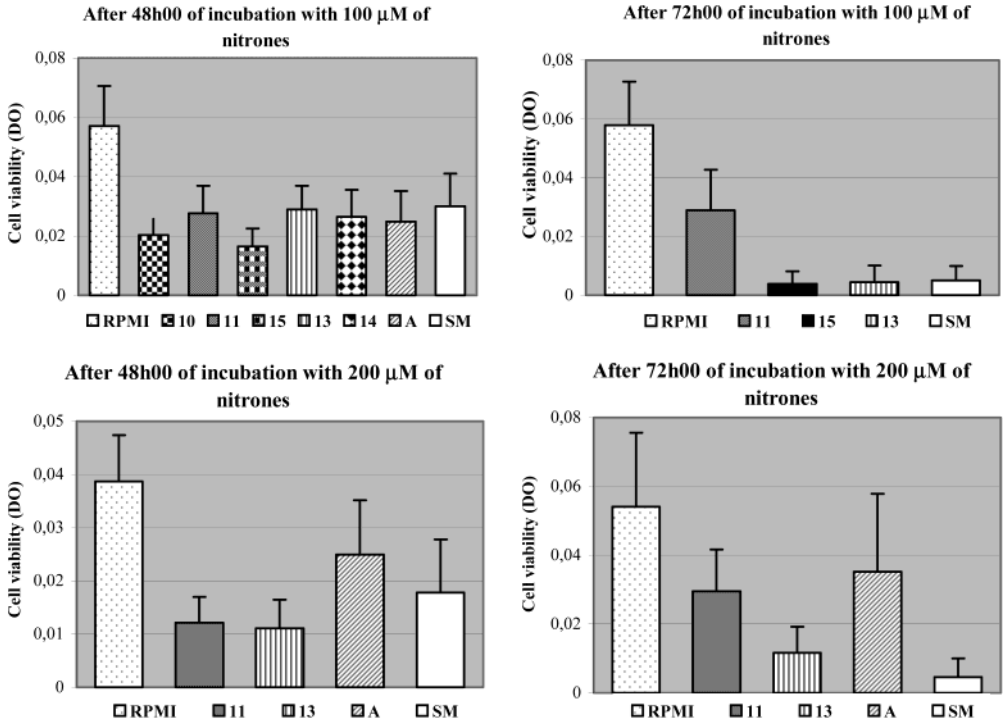


Figure 3. Effect of amphiphilic nitrones on cell viability of CV fibroblasts with NARP mutation. Cell viability with MTT test was measured as described in Experimental Section after 48 and 72 h of incubation with the indicated concentrations of nitrones. Values are the mean ±SD obtained from triplicated experiments on NARP fibroblasts in selective medium.

order to detect the amine-containing compound), and then heating at 150 °C. Kagan's reagent was prepared according to the Kagan's procedure[19] by using samarium powder (99.9%) from Strem Chemicals and 1,2-diiodoethane from Aldrich. Purification was performed by flash column chromatography over silica gel 200−400 mesh (Merck 60). Melting points were measured on an electrothermal machine and have not been corrected. The ^1H and ^{13}C NMR spectra were recorded using a Brucker AC 250 spectrometer. Chemical shifts are given in ppm relative to tetramethylsilane using the deuterium signal of the solvent ($CDCl_3$) as a heteronuclear reference for ^1H and ^{13}C. Mass spectra were recorded on a DX 300 JEOL apparatus. ESR spectra were recorded on Brucker ESP 300 spectrometer equipped with an NMR gauss meter for magnetic field calibration. Reactions were carried out under anhydrous conditions under argon. All the solvents were distilled and dried according to standard procedures. HPLC purifications were performed on a Varian Microsorb C18 column (5 μm, 21.4 mm × 250 mm i.d.) by using a mixture of methanol and water at a flow rate of 12 or 16 mL/min with UV detection at 278 nm. All solvents were removed in a vacuum. Nitrones were obtained in white powder form after lyophilization.

Cell Culture and MTT Assays. Fibroblast cultures were established from a skin biopsy from two controls and one

patient harboring the NARP mutation. Cells were grown in RPMi 1640 (Life technologies SARL, Cergy-Pontoise, France) supplemented with glutamax (446 mg/L), 10% undialyzed fetal calf serum, 100 μg/mL streptomycin, 100 IU/mL penicillin, 200 μM uridine, and 2.5 mM sodium pyruvate. For the cytotoxicity test, the cells were seeded at a density of 3000 cells per well in microplates at 37 °C under 5% of CO_2. After 24 h, the cells were exposed to increasing concentrations of different chemical compounds for 48 and 72 h in the selective medium for respiratory-competent cells (RPMI 1640 medium without glucose). For the sake of comparison, all studies were carried out on cells harvested after a similar number of population doublings.

The MTT test is a colorimetric method for determining the number of viable cells in the proliferation and cytotoxicity assays. The microplate wells were incubated with 20 μL of an MTT solution (5 mg/mL in PBS) for 1 h at 37 °C. An amount of 200 μL of isopropyl alcohol was then added to extract the MTT formazan, and the absorbance of each well was read by an automatic microplate reader at 540 nm.

Determination of log k'_W Values.[24,25] Compounds were dissolved in MeOH at 0.5 mg/mL, injected onto a Microsorb C18 column (250 mm × 4.6 mm, 5 μm), and eluted with various mixtures of MeOH and water (9:1 to 6:4 v/v, three concentrations percompound). The flow rate was 0.8 mL/min. The column temperature was 27 °C, and the UV detector wavelength was $\lambda = 298$ nm. The log k' values were calculated by using the equation

$$\log k' = \log\left(\frac{t - t_0}{t_0}\right)$$

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. Linear regression analysis ($r^2 > 0.999$) was performed on the three data points for each nitrone, and the resulting line was extrapolated to 100% aqueous to give the log k'_W values listed in Table 1 and Figure 3.

(4-[1,3]Dioxolan-2-ylbenzyl)trimethylammonium Iodide (7). A solution of 4-[1,3]dioxolan-2-ylbenzylamine[21] **6** (1.25 g, 7 mmol) and tributylamine (2.58 g, 14 mmol) in DMF (4 mL) was transferred to a tube. The reaction mixture was cooled at 0 °C, and methyl iodide (5.2 g, 35 mmol) was added slowly. The tube was sealed, and the mixture was stirred at room temperature for 20 h. The reaction mixture was added to a solution of ethyl acetate (100 mL). The precipitate was filtered off and washed two times with ether. Compound **7** was obtained pure as a white powder (1.70 g, 4.9 mmol) in 70% yield. Mp 196.4−198.3 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.59 (s, 4H), 5.80 (s, 1H), 4.61 (s, 2H), 4.2−3.9 (m, 4H), 3.06 (s, 9H). ^{13}C NMR (62.86 MHz, DMSO-d_6): δ 140.6, 133.3, 129.6, 127.5, 102.7, 67.6, 65.4, 52.2.

(4-Formylbenzyl)trimethylammonium Iodide (8). Compound **7** (0.38 g, 1.08 mmol) was dissolved in a 50% aqueous solution of acetic acid (10 mL). The mixture was stirred for 12 h and concentrated in vacuo. Compound **8** (0.34 g, 1.08 mmol) was obtained pure after crystallization in an ethanol/ether solution as a pale-yellow powder in a quantitative yield. Mp 204.8−205.4 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 10.12 (s, 1H), 8.06 (d, $J = 8$ Hz, 2H), 7.80 (d, $J = 8$ Hz, 2H), 4.69 (s, 2H), 3.09 (s, 9H). ^{13}C NMR (62.86 MHz, DMSO-d_6): δ 193. 4, 137.6, 134.8, 134.1, 130.2, 67.2, 52.5.

***N*-[4-(Octa-*O*-acetyllactobionamidomethylene)benzylidene]-*N*-(1,1-dimethyl-3-thia)undecylamine *N*-Oxide (10a).** *N*-(4-Formylbenzyl)octa-*O*-acetyllactobionamide **9**[21] (0.500 g, 0.62 mmol) was dissolved in anhydrous THF (10 mL) under argon. A solution of hydroxylamine **2** (0.100 g, 0.43 mmol) in THF (2 mL) and molecular sieves (4 Å) were added, and the reaction mixture was heated at 60 °C in the dark under argon for 8 days. Every 2 days, hydroxylamine **2** (0.050 g, 0.22 mmol) and a small amount of molecular sieves (4 Å) were added. The reaction mixture was filtered over Celite, and the filtrate was concentrated in vacuo. The residue was purified through column flash chromatography (SiO_2, 30% cyclohexane in ethyl acetate) and gel chromatography (sephadex LH-20, 1:1 v/v methanol/methylene chloride). Acetylated nitrone **10a** (0.313 g, 0.30 mmol) was obtained pure as a white foam in 50% yield. Mp 70 °C (dec). $[\alpha]_D$ +17.8° (*c* 1, CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.24 (d, $J = 8.1$ Hz, 2H), 7.49 (s, 1H), 7.25 (d, $J = 8.1$ Hz, 2H), 6.57 (m, 1H), 5.67 (d, $J = 6.6$ Hz, 1H), 5.60 (dd, $J = 3.8$ and 5.8 Hz, 1H), 5.37 (d, $J = 3$ Hz, 1H), 5.18 (dd, $J = 7.7$ and 10.3 Hz, 1H), 5.08 (m, 1H), 4.98 (dd, $J = 3.4$ and 10.4 Hz, 1H), 4.65 (d, $J = 7.9$ Hz, 1H), 4.62−4.45 (m, 2H), 4.42−4.30 (m, 2H), 4.23−3.98 (m, 3H), 3.90 (t, $J = 6.5$ Hz, 1H), 3.00 (s, 2H), 2.41 (t, $J = 7.25$ Hz, 2H), 2.13, 2.05, 2.02, 2.01, 1.98, 1.95 (6s, 24H), 1.61 (s, 6H), 1.43 (m, 2H), 1.3−1.1 (m, 10H), 0.82 (t, $J = 6.6$ Hz, 3H). ^{13}C NMR (62.86 MHz, $CDCl_3$): δ 170.4, 170.0, 170.0, 169.9, 169.7, 169.7, 169.5, 169.2, 167.1 (CO), 139.8 (C), 131.0 (CH), 130.1 (C), 129.2, 127.5, 101.7, 77.3 (CH), 73.4 (C), 71.7, 70.9, 69.7, 69.1, 68.9, 66.8 (CH), 61.6, 60.9, 42.9, 42.4, 33.3, 31.7, 29.9, 29.0, 29.0, 28.6 (CH_2), 25.7 (CH_3), 22.5 (CH_2), 20.8, 20.7, 20.6, 20.6, 20.6, 20.5, 20.5, 20.4, 14.0 (CH_3). MS (FAB, *m*/*z*): 1049 [$(M + Na)^+$, 10%], 1027 [$(M + H)^+$, 6%], 331 [$(C_{14}H_{19}O_9)^+$, 25%], 201 [$(C_{12}H_{25}S)^+$, 73%].

***N*-[4-(Lactobionamidomethylene)benzylidene]-*N*-(1,1-dimethyl-3-thia)undecylamine *N*-Oxide (10).** Acetylated nitrone **10a** (0.111 g, 0.108 mmol) was dissolved in anhydrous methanol (10 mL) under argon. A catalytic amount of sodium methoxide was added, and the mixture was stirred for 1 h. A 95% ethanol solution (10 mL) was added, and the solution was concentrated in vacuo. Deacetylated nitrone **10** (0.072 g, 0.104 mmol) was obtained as a white foam in 96% yield with a residual amount of deacetylated compound **9**. RP-HPLC: $t_R = 11.4$ min (flow rate of 12 mL/min, linear gradient of MeOH/H_2O from 70:30 to 85:15 v/v in 5 min, then isocratic period with MeOH/H_2O at 85:15 v/v for 10 min). Mp 115 °C (dec). $[\alpha]_D$ +17.2 (*c* 0.25, CH_3OH). UV (MeOH, nm): $\lambda_{max} = 298.8$. ^{1}H NMR (250 MHz, CD_3OD): δ 8.28 (d, $J = 8.25$ Hz, 2H), 7.82 (s, 1H,), 7.42 (d, $J = 8.25$ Hz, 2H), 4.65−4.35 (m, 4H), 4.25 (m, 1H), 4.00−3.35 (m, 10H), 3.01 (s, 2H), 2.43 (t, $J = 7.3$ Hz, 2H), 1.61 (s, 6H), 1.44 (m, 2H), 1.30−1.10 (m, 10H), 0.87 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (62.86 MHz, CD_3OD): δ 175.3 (CO), 143.4 (C), 136.0, 131.1 (CH), 130.6 (C), 128.3 (CH), 105.8, 83.3, 77.2 (CH), 74.8 (C), 74.6, 74.1, 73.2, 72.8, 72.5, 70.4 (CH), 63.8, 62.7, 43.5, 43.0, 34.2, 32.9, 31.0, 30.3, 30.2, 29.7 (CH_2), 26.0 (CH_3), 23.7 (CH_2), 14.4 (CH_3). MS (FAB, *m*/*z*): 729 [$(M + K)^+$, 1.5%], 713 [$(M + Na)^+$ 2.5%], 513 [$(C_{20}H_{29}N_2O_{12} + Na)^+$, 2.5%], 201 [$(C_{12}H_{25}S)^+$, 65%], 179 [$(C_6H_{11}O_6)^-$, 44%]. Anal. ($C_{48}H_{70}N_2O_{20}S\cdot 3H_2O$) C, N, S. H: calcd, 8.12; found, 7.66.

***N*-[4-(Octa-*O*-acetyllactobionamidomethylene)-benzylidene]-*N*-(1,1-dimethyl-3-thia-5-perfluorohexyl)-pentylamine *N*-Oxide (11a)**. The synthetic reaction was essentially the same as for compound **10a.** From compound **9** (0.800 g, 0.98 mmol) and hydroxylamine **3** (2.22 g, 4.64 mmol), acetylated nitrone **11a** (0.970 g, 0.77 mmol) was obtained pure as a white foam in 78% yield. Mp 85 °C (dec). $[\alpha]_D$ +13.0° (*c* 1, CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.27 (d, $J = 8.1$ Hz, 2H), 7.54 (s, 1H), 7.3 (d, $J = 8.1$ Hz, 2H), 6.55 (m, 1H), 5.8−3.8 (m, 15H), 3.09 (s, 2H), 2.67 (m, 2H), 2.45−2.25 (m, 2H), 2.16, 2.15, 2.09, 2.08, 2.05, 2.04, 2.02, 1.98 (8s, 24H), 1.66 (s, 6H). ^{13}C NMR (62.86 MHz, $CDCl_3$): δ 170.5, 170.5, 170.2, 170.1, 169.8, 169.8, 169.6, 169.3, 167.2 (CO), 140.1 (C), 131.5 (CH), 130.0 (C), 129.4, 127.7, 101.8, 77.3 (CH), 73.2 (C), 71.7, 71.0, 69.8, 69.2, 69.0, 66.8 (CH), 61.7, 60.9, 43.1, 42.1, 32.3 (CH_2), 25.9 (CH_3), 23.5 (CH_2), 20.9, 20.8, 20.7, 20.7, 20.6, 20.5 (6s, CH_3). ^{19}F NMR (235 MHz, $CDCl_3$): δ −81.0, −114.6, −122.2, −123.2, −123.7, −126.4. FTIR (KBr, cm^{-1}): ν 3396, 1755, 1228. MS (FAB, *m*/*z*): 1283 [$(M + Na)^+$, 0.5%], 1261 [$(M + H)^+$, 1.5%], 435 [$(C_{12}H_{12}F_{13}S)^+$, 40%], 331 [$(C_{14}H_{19}O_9)^+$, 14%].

***N*-[4-(Lactobionamidomethylene)benzylidene]-*N*-(1,1-dimethyl-3-thia-5-perfluorohexyl)pentylamine *N*-Oxide (11).** Following the same procedure as for compound **10**, deacetylated nitrone **11** was obtained as a white foam in 98% yield with a residual amount of deacetylated compound **9**. RP-HPLC: $t_R = 11.8$ min (flow rate of 12 mL/min, linear gradient of MeOH/H_2O from 70:30 to 90:10 v/v in 5 min and from 90:10 to 100:0 v/v in 5 min, then isocratic period with MeOH/H_2O of 100:0 v/v for 5 min. Mp 130 °C (dec). $[\alpha]_D$ +11.4 (*c* 0.25,

CH_3OH). UV (MeOH, nm): $\lambda_{max} = 298.8$. ^1H NMR (250 MHz, CD_3OD): δ 8.30 (d, $J = 8.3$ Hz, 2H), 7.84 (s, 1H), 7.42 (d, $J = 8.3$ Hz, 2H), 4.6−4.3 (m, 4H), 4.20 (m, 1H), 4.00−3.35 (m, 10H), 3.10 (s, 2H), 2.63 (m, 2H), 2.50−2.20 (m, 2H), 1.62 (s, 6H). ^{13}C NMR (62.86 MHz, CD_3OD): δ 175.4 (CO), 143.5 (C), 136.1, 131.1 (CH), 130.6 (C), 128.3, 105.8, 83.3, 77.2 (CH), 74.8 (C), 74.5, 74.0, 73.2, 72.8, 72.6, 70.4 (CH), 63.8, 62.7, 43.5, 42.6, 33.3 (CH_2), 26.0 (CH_3), 24.3 (CH_2−S). ^{19}F NMR (235 MHz, CD_3OD): δ −82.0, −115.0, −122.6,−123.6, −124.1, −127.0. MS (FAB, *m*/*z*): 947 [$(M + Na)^+$, 2%], 513 [$(C_{20}H_{29}N_2O_{12} + Na)^+$, 2%], 435 [$(C_{12}H_{12}F_{13}S)^+$, 21%]. Anal. ($C_{32}H_{41}F_{13}N_2O_{12}S\cdot 3.5H_2O$) C, N, S. H: calcd, 4.90; found, 4.36.

***N*-[4-(Octa-*O*-acetyllactobionamidomethylene)benzylidene]-*N*-[1,1-dimethyl-2-(*N*-octanoyl)amido]ethylamine *N*-Oxide (12a).** The synthetic reaction was essentially the same as for compound **10a.** From compound **9** (0.600 g, 0.74 mmol) and hydroxylamine **4** (1.6 g, 6.97 mmol), acetylated nitrone **12a** (0.475 g, 0.46 mmol) was obtained pure as a white foam in 63% yield. Mp 70 °C (dec). $[\alpha]_D$ + 14.6° (*c* 1, CH_2Cl_2). ^1H NMR (250 MHz, $CDCl_3$): δ 8.22 (d, $J = 8.1$ Hz, 2H), 7.47 (s, 1H), 7.32 (d, $J = 8.1$ Hz, 2H), 6.63 (m, 2H), 5.64 (d, $J = 6.0$ Hz, 1H), 5.59 (dd, $J = 3.4$ and 6.4 Hz, 1H), 5.35 (d, $J = 3.4$ Hz, 1H), 5.16 (dd, $J = 7.9$ and 10.2 Hz, 1H), 5.07 (m, 1H), 4.96 (dd, $J = 3.4$ and 10.4 Hz, 1H), 4.62 (d, $J = 7.7$ Hz, 1H), 4.60−4.47 (m, 2H), 4.46−4.27 (m, 2H), 4.23−3.95 (m, 3H), 3.85 (t, $J = 6.6$ Hz, 1H), 3.67 (d, $J = 6.4$ Hz, 2H), 2.20−2.10 (m, 8H), 2.07, 2.04, 2.03, 2.01, 1.97 (6s, 16H), 1.68−1.52 (m, 8H), 1.35−1.18 (m, 8H), 0.84 (t, $J = 6.4$ Hz, 3H). ^{13}C NMR (62.86 MHz, $CDCl_3$): δ 173.6, 170.5, 170.1, 170.0, 169.8, 169.7, 169.6, 169.3, 167.2 (CO), 140.3 (C), 131.2 (CH), 129.8 (C), 129.4, 127.7, 101.8, 77.5 (CH), 73.5 (C), 71.6, 71.0, 69.8, 69.2, 69.0, 66.8 (CH), 61.7, 60.9, 47.0, 42.9, 36.8, 31.6, 29.2, 28.9, 25.8 (CH_2), 25.0 (CH_3), 22.5 (CH_2), 20.8, 20.8, 20.7, 20.6, 20.5, 20.5, 14.0 (CH_3). FTIR (KBr, cm^{-1}): ν 3394, 1753. MS (FAB, *m*/*z*): 1047 [$(M + Na)^+$, 1%], 1025 [$(M + H)^+$, 4%], 331 [$(C_{14}H_{19}O_9)^+$, 8%], 198 [$(C_{12}H_{24}NO)^+$, 37%].

***N*-[4-(Lactobionamidomethylene)benzylidene]-*N*-[1,1-dimethyl-2-(*N*-octanoyl)amido]ethylamine *N*-Oxide (12).** Following the same procedure as for compound **10**, deacetylated nitrone **12** was obtained as a white foam in 95% yield with a residual amount of deacetylated compound **9**. RP-HPLC: $t_R = 8.7$ min (flow rate of 16 mL/min, linear gradient of MeOH/H_2O from 57:43 to 67:33 v/v in 2 min, from 67:33 to 71:29 v/v in 2 min, then isocratic period with MeOH/H_2O of 71:29 v/v for 8 min. Mp 160 °C (dec). $[\alpha]_D$ +15.1° (*c* 0.25, CH_3OH). UV (MeOH, nm): $\lambda_{max} = 298.8$. ^1H NMR (250 MHz, CD_3OD): δ 8.28 (d, $J = 8.55$ Hz, 2H), 7.82 (s, 1H), 7.42 (d, $J = 8.55$ Hz, 2H), 4.65−4.45 (m, 4H), 4.26 (m, 1H), 4.00−3.40 (m, 12H), 2.15 (t, $J = 7.25$ Hz, 2H), 1.53 (s, 8H), 1.23 (m, 8H), 0.85 (t, $J = 6.6$ Hz, 3H). ^{13}C NMR (62.86 MHz, CD_3OD): δ 176.8, 175.4 (CO), 143.5 (C), 136.0, 131.1 (CH), 130.6 (C), 128.3, 105.8, 83.3, 77.2 (CH), 75.0 (C), 74.8, 74.1, 73.2, 72.8, 72.6, 70.4 (CH), 63.8, 62.7, 47.4, 43.5, 37.1, 32.9, 30.2, 30.1, 27.1 (CH_2), 24.7 (CH_3), 23.7 (CH_2), 14.4 (CH_3). MS (FAB, *m*/*z*): 710 [$(M + Na)^+$, 4%], 688 [$(M + H)^+$, 1.5%], 198 [$(C_{12}H_{12}NO)^+$, 52%], 686 [$(M - H)^-$, 2.5%]. Anal. ($C_{32}H_{53}N_3O_{13}\cdot 1H_2O$) C, H, N.

***N*-[4-(Octa-*O*-acetyllactobionamidomethylene)benzylidene]-*N*-[1,1-dimethyl-2-(*N*-2*H*,2*H*,3*H*,3*H*-perfluorononanoyl)amido]ethylamine *N*-Oxide (13a).** The synthetic reaction was essentially the same as for compound **10a**. From compound **9** (0.610 g, 0.75 mmol) and hydroxylamine **5** (1.31 g, 2.73 mmol), acetylated nitrone **13a** (0.564 g, 0.44 mmol) was obtained pure as a white foam in 60% yield. Mp 95 °C (dec). ^1H NMR (250 MHz, $CDCl_3$): δ 8.21 (d, $J = 8.1$ Hz, 2H), 7.49 (s, 1H), 7.31 (d, $J = 8.1$ Hz, 2H), 6.95 (t, $J = 6$ Hz, 1H), 6.76 (t, $J = 6$ Hz, 1H), 5.45−3.80 (m, 15H), 3.69 (d, $J = 6.0$ Hz, 2H), 2.70−2.35 (m, 4H), 2.17, 2.16, 2.08, 2.07, 2.06, 2.05, 2.04, 1.98 (8s, 24H), 1.60 (s, 6H). ^{13}C NMR (62.86 MHz, $CDCl_3$): δ 170.6, 170.4, 170.2, 170.1, 170.0 169.8, 169.7, 169.3, 167.3 (CO), 140.6 (C), 131.5 (CH), 129.8 (C), 129.4, 127.7, 101.9, 77.5 (CH), 73.4 (C), 71.7, 71.0, 70.0, 69.3, 69.1, 66.9 (CH), 61.8, 60.9, 47.3, 43.1 (CH_2), 27.4−26.1 (m, CH_2), 24.9 (CH_3), 20.9, 20.8, 20.7, 20.7, 20.6, 20.5 (CH_3). ^{19}F NMR (235 MHz, $CDCl_3$): δ −81.1, −115.0, −122.3,−123.2, −123.9, −126.5. MS (FAB, *m*/*z*): 1295 [$(M + Na)^+$, 3.5%], 1273 [$(M + H)^+$, 1.5%], 446 [$(C_{13}H_{13}F_{13}NO)^+$, 24%], 331 [$(C_{14}H_{19}O_9)^+$, 10%].

***N*-[4-(Lactobionamidomethylene)benzylidene]-*N*-[1,1-dimethyl-2-(*N*-2*H*,2*H*,3*H*,3*H*-perfluorononanoyl)amido]ethylamine *N*-Oxide (13).** Following the same procedure as for compound **10**, deacetylated nitrone **13** was obtained as a white foam in 95% yield with a residual amount of deacetylated compound **9**. RP-HPLC: $t_R = 9.8$ min (flow rate of 16 mL/min, linear gradient of MeOH/H_2O from 70:30 to 80:20 v/v in 5 min, from 80:20 to 82:18 v/v in 3 min, then isocratic period with MeOH/H_2O of 82:18 v/v for 10 min. Mp 150 °C (dec). $[\alpha]_D$ +14.4° (*c* 0.25, CH_3OH). UV (MeOH, nm): $\lambda_{max} = 299.0$. ^1H (250 MHz, CD_3OD): δ 8.33 (d, $J = 8.4$ Hz, 2H), 7.86 (s, 1H), 7.47 (d, $J = 8.5$ Hz, 2H), 4.65−4.45 (m, 4H), 4.3 (m, 1H), 4.05−3.87 (m, 2H), 3.87−3.66 (m, 7H), 3.66−3.45 (m, 3H), 2.55−2.40 (m, 4H), 1.59 (s, 6H). ^{13}C NMR (62.86 MHz, CD_3OD): δ 174.0, 171.9 (CO), 142.1 (C), 134.6, 129.7 (CH), 129.2 (C), 126.9, 104.4, 82.0, 75.8 (CH), 73.5 (C), 73.4, 72.7, 71.8, 71.4, 71.2, 69.0 (CH), 62.4, 61.3, 46.3, 42.1, 26.0 (CH_2), 23.3 (CH_3). ^{19}F NMR (235 MHz, CD_3OD): δ −82.1, −115.3, −122.6,−123.6, −124.3, −127.0. MS (FAB, *m*/*z*): 446 [$(C_{13}H_{13}F_{13}NO)^+$, 21%], 375 [$(C_9H_4F_{13}O)^+$, 8%]. Anal. ($C_{33}H_{42}F_{13}N_3O_{13}\cdot 1H_2O$) C, H, N.

***N*-[4-(Octa-*O*-acetyllactobionamidomethylene)benzylidene]-*N*-[1,1-dimethyl-2-((hexylamino)carbonyl)oxy]ethylamine *N*-Oxide (14a).** The synthetic reaction was essentially the same as for compound **10a**. From compound **9** (0.895 g, 1.1 mmol) and hydroxylamine **1** (1.18 g, 5.03 mmol), acetylated nitrone **14a** (0.580 g, 0.56 mmol) was obtained pure as a white foam in 52% yield. Mp 75 °C (dec). ^1H NMR (250 MHz, $CDCl_3$): δ 8.26 (d, $J = 7.7$ Hz, 2H), 7.49 (s, 1H), 7.30 (d, $J = 8.1$ Hz, 2H), 6.60 (m, 1H), 5.60 (m, 2H), 5.36 (m, 1H), 5.30−4.90 (m, 3H), 4.85 (m, 1H), 4.75−4.25 (m, 7H), 4.23−3.75 (m, 4H), 3.13 (q, $J = 6.4$ Hz, 2H), 2.16, 2.08, 2.05, 2.04, 2.03,1.98 (6s, 24H), 1.59 (s, 6H), 1.55−1.30 (m, 8H), 0.87 (m, 3H). ^{13}C NMR (62.86 MHz, $CDCl_3$): δ 170.3, 170.1, 160.9, 169.8, 169.8, 169.7, 169.3, 167.2, 155.6 (CO), 140.0 (C), 131.0 (CH), 130.2 (C), 129.3, 127.7, 101.8, 77.3 (CH), 72.7 (C), 71.6, 71.0, 69.8, 69.2, 69.0 (CH), 68.2 (CH_2), 66.8 (CH), 61.7, 60.9, 43.0, 41.1, 31.4, 29.8 (CH_2), 26.4 (CH_3), 23.6, 22.5 (CH_2), 20.9, 20.8, 20.7, 20.6, 20.6, 20.5, 14.0 (CH_3). FTIR (KBr, cm^{-1}): ν 3392, 1751. MS (FAB, *m*/*z*): 1049 [$(M + Na)^+$, 9.5%], 1027 [$(M + H)^+$, 11%], 331 [$(C_{14}H_{19}O_9)^+$, 22%], 200 [$(C_{13}H_{22}NO_2)^+$, 80%].

***N*-[4-(Lactobionamidomethylene)benzylidene]-*N*-[1,1-dimethyl-2-((hexylamino)carbonyl)oxy]ethylamine *N*-Oxide (14).** Following the same procedure as for compound **10**, deacetylated nitrone **14** was obtained as a white foam in 96% yield with a residual amount of deacetylated compound **9**. RP-HPLC: $t_R = 8.2$ min (flow rate of 16 mL/min, linear gradient of MeOH/H_2O from 55:45 to 65:35 v/v in 2 min, from 65:35 to 68:32 v/v in 2 min, then isocratic period with MeOH/H_2O of 68:32 v/v for 8 min). Mp 135 °C (dec). $[\alpha]_D$ +17.6 (*c* 0.25, CH_3OH). UV (MeOH, nm): $\lambda_{max} = 298.0$. ^1H NMR (250 MHz, CD_3OD): δ 8.26 (d, $J = 8.1$ Hz, 2H), 7.82 (s, 1H), 7.42 (d, $J = 8.55$ Hz, 2H), 4.65−4.38 (m, 4H), 4.34 (s, 2H), 4.26 (m, 1H), 4.00−3.40 (m, 10H), 3.01 (t, $J = 6.8$ Hz, 2H), 1.58 (s, 6H), 1.45−1.15 (m, 8H), 0.86 (t, $J = 6.6$ Hz, 3H). ^{13}C NMR (62.86 MHz, CD_3OD): δ 175.4, 163.4 (CO), 143.6 (C), 136.3, 131.1 (CH), 130.5 (C), 128.3, 105.8, 83.3, 77.2 (CH), 74.8 (C), 73.8, 73.2, 72.8, 72.6, 72.3, 70.4 (CH), 69.2, 63.8, 62.7, 43.5, 41.8, 32.6, 30.8, 30.2, 27.5 (CH_2), 23.6, 14.4 (CH_3). MS (FAB, *m*/*z*): 728 [$(M + K)^+$, 2%], 712 [$(M + Na)^+$, 17%], 690 [$(M + H)^+$, 3%], 513 [$(C_{11}H_{22}N_2O_{12} + Na)^+$, 24%], 200 [$(C_{11}H_{22}N_2O_2)^+$, 17.5%]. Anal. ($C_{31}H_{51}N_3O_{14}\cdot 0.5H_2O$) C, H, N.

4-Carboxybenzylidene-*N*-(1,1-dimethyl-3-thia)undecylamine *N*-Oxide (15). 4-Carboxybenzaldehyde (1.18 g, 0.79 mmol) and molecular sieves (4 Å) were dissolved in anhydrous ethanol (5 mL) under argon. The reaction mixture was heated at 60 °C, and hydroxylamine **2** (0.07 g, 0.3 mmol) dissolved in anhydrous ethanol (5 mL) was added. The reaction mixture was heated at 60 °C in the dark under argon for 72 h. Every 2 days, hydroxylamine **2** (0.1 g, 0.42 mmol) and a small amount of molecular sieves (4 Å) were added. The mixture was filtered on Celite. The filtrate was concentrated in vacuo, and the residue was purified by flash chromatography (SiO_2, 30−40%

ethyl acetate in cyclohexane) and recrystallized (ethyl acetate/hexane) to afford compound **15** (0.15 g, 0.41 mmol) as a white powder in 52% yield. Mp 105.0−107.8 °C. UV (MeOH, nm): λ_{max} = 307.2. ^{1}H NMR (250 MHz, CD_3OD): δ 8.43 (m, J = 8.25 Hz, 2H), 8.08 (m, J = 8.25 Hz, 2H), 7.95 (s, 1H), 3.05 (s, 2H), 2.45 (t, J = 7.3 Hz, 2H), 1.65 (s, 6H), 1.55−1.00 (m, 12H), 0.87 (t, J = 6.65 Hz, 3H). ^{13}C NMR (62.86 MHz, CD_3OD): δ 169.2 (CO), 135.8, 134.8, 133.8 (C, CH), 130.7, 130.4 (CH), 75.4 (C), 43.3, 34.2, 32.9, 31.1, 30.3, 29.8 (CH_2), 26.0 (CH_3), 23.7 (CH_2), 14.4 (CH_3). FTIR (KBr, cm^{-1}): ν 3450, 1682. MS (FAB, *m/z*): 388 [$(M + Na)^+$, 58%], 366 [$(M + H)^+$, 35%], 234 [$(C_{12}H_{20}NOS)^+$, 61%], 201 [$(C_{12}H_{25}S)^+$, 70%]. Anal. ($C_{20}H_{31}NO_3S$) H, N, S. C: calcd, 65.72; found, 63.94.

***N*-[4-(Trimethylammonium iodide methylene)benzylidene]-*N*-(1,1-dimethyl-3-thia-5-perfluorohexyl)pentylamine *N*-Oxide (16).** (4-Formylbenzyl)trimethylammonium iodide **8** (0.250 g, 0.82 mmol), hydroxylamine **3** (0.480 g, 1.02 mmol), and molecular sieves (4 Å) were dissolved in anhydrous pyridine (5 mL) under argon. The reaction mixture was heated at 80 °C in the dark for 42 h. The mixture was concentrated in vacuo, and the solid residue was crystallized in MeOH/Et_2O to afford the pure compound **16** (0.350 g, 0.47 mmol) as a white powder in 57% yield. Mp 170.9−173.1 °C. UV (MeOH, nm): λ_{max} = 304.0. ^{1}H NMR (250 MHz, CD_3OD): δ 8.54 (d, J = 8.4 Hz, 2H), 8.03 (s, 1H), 7.71 (d, J = 8.4 Hz, 2H), 4.65 (s, 2H), 3.18 (s, 11H), 2.7 (m, 2H), 2.45 (m, 2H), 1.71 (s, 6H). ^{13}C NMR (62.86 MHz, CD_3OD): δ 133.3 (C), 132.8, 132.7 (CH), 129.8 (C), 129.7 (CH), 73.9 (C), 68.5 (CH_2), 51.9, 51.9, 51.8 (CH_3), 41.3, 31.9 (CH_2), 24.6 (CH_3), 23.0 (CH_2). ^{19}F NMR (235 MHz, CD_3OD): δ −82.3, −115.2, −112.9, −123.9, −124.3, −127.3. MS (FAB, *m/z*): 1510 [$(2M + H)^+$, 0.5%], 1381 [$(2M - I)^+$, 5%], 755 [$(M + H)^+$, 2.5%], 627 [$(M - I)^+$, 100%], 435 [$(C_{12}H_{12}F_{13}S)^+$, 100%], 201 [$(C_{12}H_{25}S)^+$, 70%]. HRMS calcd for $C_{23}H_{29}F_{13}IN_2OS$ ([M + H]$^+$): 755.0838. Found: 755.0851.

Supporting Information Available: Chemical data of compounds **1**−**5**, ^{1}H, ^{13}C, and ^{19}F NMR spectra of acetylated nitrone **13a**, ^{1}H and ^{13}C spectra of deacetylated nitrone **13**, HMQC, DEPT COSY H,H sequences of nitrone **13**, and analytical RP-HPLC chromatograms of nitrones **10**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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