Synthesis of a Glycolipidic Amphiphilic Nitrone as a New Spin Trap

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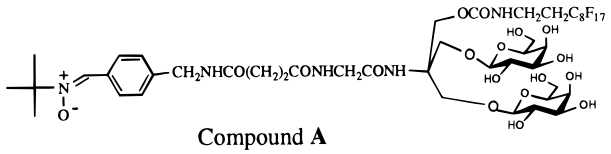
Received November 30, 1998

The synthesis of a new amphiphilic nitrone, **A**, derived from a digalactosyl tris(hydroxymethyl)-aminomethane bearing a perfluorocarbon chain is described. **A** exhibited a surfactant behavior (cmc = 1.6×10^{-5} mol/L), and the specific recognition of the galactosyl moiety grafted on **A** by the KbCWL1 membrane lectin was established. Preliminary experiments showed that **A** was able to trap free radicals in aqueous media, the shape of the observed ESR spectra being strongly dependent upon the nature of the trapped free radical.

The spin trapping technique coupled with ESR has emerged as one of the most useful tools to investigate the implication of short-lived free radicals in several clinical disorders.[1] Nitrones are the most appropriate spin traps used in biological systems,[2] and among them α-phenyl-*N*-*tert*-butylnitrone (PBN)[3] and the 5,5-dimethylpyrroline *N*-oxide (DMPO)[4] are still the most popular.

In a biological milieu, free radicals can decay by enzymatic or chemical reactions and their concentrations always remain very small. This is particularly true in the case of the superoxide radical in the presence of superoxide dismutase (SOD) or for the hydroxyl radical which reacts quickly with most of the biocomponents. To improve the efficiency of the spin trapping in biological systems, control of the delivery of the spin trap on the spot of the radical event is very important. To ensure a suitable pharmacomodulation, one of the possibilities is to graft the trapping agent on a natural or a synthetic carrier. This carrier could modify the hydro- or lipophilicity of the active principle and thus would modulate its membrane crossing ability. Furthermore, it could ensure specific cell targeting via its functionalization with ligands such as antibodies, peptides, or carbohydrates.[5]

Scheme 1



Compound **A**

As part of our program dealing with the vectorization and the cell targeting of spin traps,[6] we designed a new glycosidic amphiphilic nitrone, **A**, derived from PBN (Scheme 1). The nitronyl group grafted to the tris amino group by a peptidic spacer arm will ensure the spin trapping function, and the hydrophilic galactosyl groups should achieve targeting via specific recognition by membrane lectins. After iv or ip injections into rats, amphiphilic fluorocarbon telomers derived from tris(hydroxymethyl)aminomethane (Tris) and bearing perfluorocarbon chain were detected in all organs.[7] Furthermore, in vitro, these compounds easily cross the plasma membrane,[8] and it is thus reasonable to think that **A** can cross cell membranes. We report hereafter the synthesis of **A**, and some preliminary results on its trapping ability and its recognition by specific membrane lectins.

The synthesis of **A** was performed as reported in Scheme 2. The glycine derivative **1** was obtained by reaction of Z-protected glycine with Tris in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) as a coupling reagent in refluxing ethanol. Acetalization of two hydroxyl functions of the Tris group was carried out by treatment with 2,2-dimethoxypropane in acetonitrile to yield **1** in 75% yield. The perfluorocarbon chain was then introduced by reacting **1** with the corresponding perfluorocarbon isocyanate in the presence of DABCO followed by hydrolysis of the acetal by Montmorillonite K10 resin,[9] leading to **2** in 81% yield. Glycosylation of

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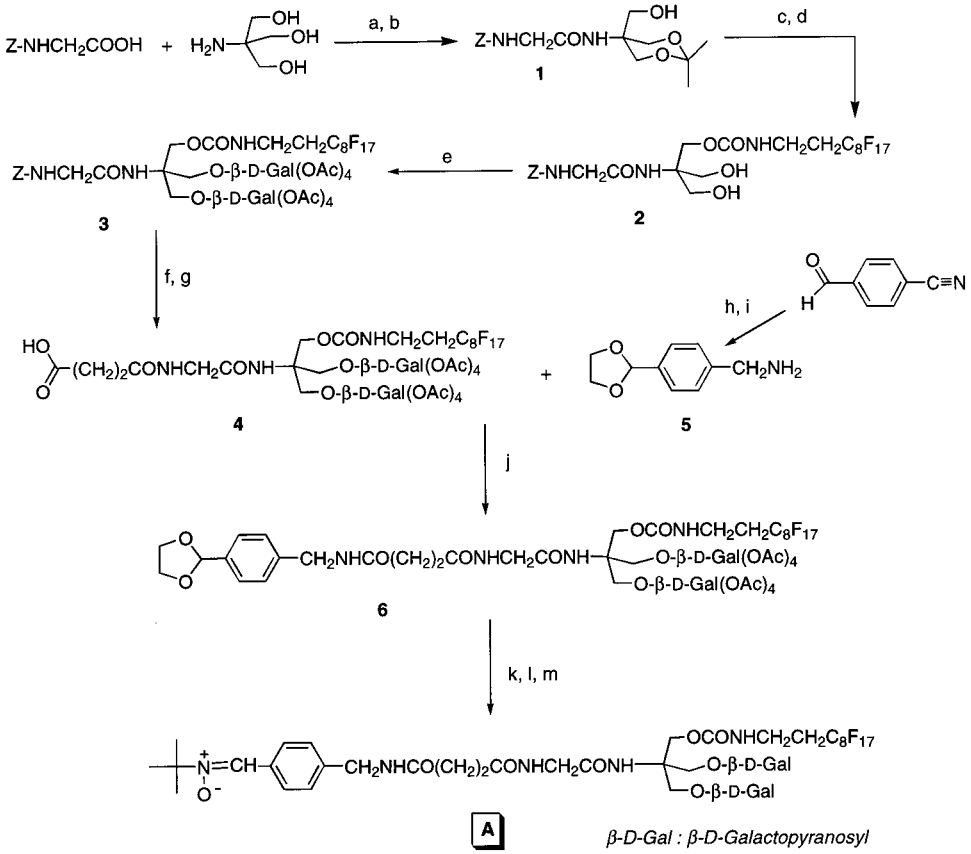
10.1021/jo982343+ CCC: $18.00
Published on Web 04/24/1999

Scheme 2[a]



[a] (a) EEDQ, EtOH, reflux, 75%; (b) 2,2-dimethoxypropane, cat. *p*-TsOH, CH_3CN, rt, 85%; (c) $C_8F_{17}(CH_2)_2N{=}C{=}O$, DABCO, toluene, reflux, 90%; (d) Montmorillonite K10, CH_2Cl_2, rt, 90%; (e) tetraacetobromogalactose, $Hg(CN)_2$, ultrasound, CH_3CN, 65%; (f) H_2, Pd−C, MeOH, 98%; (g) succinic anhydride, pyridine, rt, 94%; (h) ethylene glycol, cat. *p*-TsOH, toluene, reflux, 90%; (i) $AlLiH_4$, Et_2O, 0 °C, 78%; (j) DCC, HOBT, CH_2Cl_2, rt, 90%; (k) CH_3CHO, cat. *p*-TsOH, CH_2Cl_2, rt, 85%; (l) $(CH_3)_3CNHOH$, 3 Å molecular sieves, THF, argon, 45 °C, 52%; (m) MeONa, MeOH, Amberlite IRC 50S resin, rt, 95%.

the tris hydroxyl functions by 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in the presence of mercuric cyanide under ultrasonic activation led to compound **3**.[10] Under these conditions, the bisglycosylation was stereoselective and afforded the bis β-D-galactopyranoside derivative **3** in 65% yield after column chromatography. The Z-amine protecting group was then removed by hydrogenolysis (10% Pd/C) in MeOH, and the resulting amine was treated with 2 equiv of succinic anhydride in pyridine to give **4** in a good yield (92%). On the other hand, protection of the carbonyl group of 4-cyanobenzaldehyde as its ethylene ketal (90%) and reduction of the cyano group with $AlLiH_4$ in Et_2O (78%) afforded the benzylamine **5** which was then reacted with the tensioactive moiety **4**. The amide **6** was obtained in a good yield (90%) by the reaction of **4** with **5** in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) and a catalytic amount of 1-hydroxybenzotriazole (HOBT) in dry CH_2Cl_2 at room temperature. Then transacetalization with a large excess of acetaldehyde regenerated under mild conditions the benzaldehyde derivative in 85%. Condensation of the aldehyde with *N*-*tert*-butylhydroxylamine in dry THF introduced the nitrone function.[11] Owing to the possible degradation of hydroxylamine by oxidation followed by free radical processes, the reaction was carried out in the dark and under an inert atmosphere. Purification on silica gel and on Sephadex LH-20 resin, eluting with EtOH:CH_2Cl_2 (1:1), yielded the pure nitrone as a white foam (55%) without residual ESR signal. Removal of the *O*-acetyl protecting groups using MeONa/MeOH followed by purification on Sephadex G-25 resin (distilled water as eluant) afforded the β-D-galactopyranoside nitrone **A**.

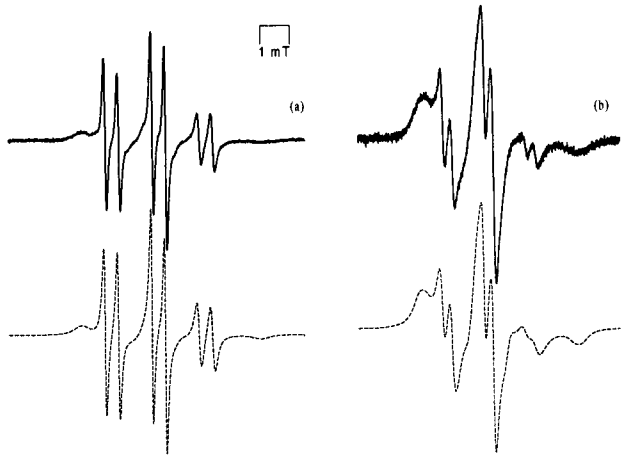


Figure 1. Observed (continuous line) and simulated (broken line) ESR spectra after hydroxyl radical generation with H_2O_2−$FeSO_4$−EDTA system in phosphate buffer at pH 7 in the presence of nitrone **A** and (a) 2 mM sodium formate, (b) 10% methanol.

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As expected, compound **A** shows a surfactant behavior, with a critical micellar concentration (cmc) of 1.6 10^{-5} mol/L and a surface tension of 28 mN/m.

Preliminary spin trapping experiments with **A** were performed, and the shape of the ESR spectra was strongly dependent upon the nature of the trapped radical. The radicals $^{\bullet}COO^{-},Na^{+}$ or $^{\bullet}CH_2OH$ were generated in phosphate buffer (pH 7) by a Fenton system with sodium formate or methanol, respectively, in the presence of **A** (100 mM). The resulting ESR spectra are shown in Figure 1. The use of ^{13}C-labeled sodium formate or methanol unambiguously supported the nature of the **A**$-CO_2^{-},Na^{+}$ and **A**$-CH_2OH$ spin adducts. The spectra were satisfactorily simulated by assuming that they correspond to the superimposition of the spectra of a spin adduct differently immobilized in two distinct environments. When dioxane was added to break the organization of the amphiphilic nitrone **A**, the percentage of the spectrum corresponding to the less immobilized spin adduct increased significantly.

To determine the lectin recognition ability of **A**, its inhibiting property was measured by using classical yeast flocculation processes involving lectins.[6,12] The yeast used was *Kluyveromyces bulgaricus* and involved the galactose specific lectin Kb CWL1. The affinity of nitrone **A** for Kb CWL1 was higher than that of free galactose. This recognition feature could lead to specific spin trap distribution in biological systems, and work is now in progress to determine the distribution and localization of nitrone **A** in cell culture.

Acknowledgment. We wish to thank Pr. Roger Bonaly and Dr. Joel Coulon from the University of Nancy for their collaboration.

Supporting Information Available: Experimental procedures including synthesis and characterization of all new compounds reported herein. This material is available free of charge via the Internet at http://pubs.acs.org.

JO982343+

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