Electronic Supplementary Information for

Synthesis, Physico-Chemical and Biological Properties of Amphiphilic Amino Acid Conjugates of Nitroxides

Grégory Durand, a,* Fanny Choteau, a Robert A. Prosak, b Antal Rockenbauer, c Frederick A. Villamena b,d,* and Bernard Pucci a

aLaboratoire de Chimie BioOrganique et des Systèmes Moléculaires Vectoriels, Faculté des Sciences, Université d’Avignon et des Pays de Vaucluse, 33 Rue Louis Pasteur, 84000 Avignon, France
bDepartment of Pharmacology, dCenter for Biomedical EPR Spectroscopy and Imaging, The Davis Heart and Lung Research Institute, College of Medicine, The Ohio State University, Columbus, OH 43210
cChemical Research Center, Institute of Structural Chemistry, H-1025 Budapest, Pusztaszeri 59, Hungary

gregory.durand@univ-avignon.fr   frederick.villamena@osumc.edu

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Material, general procedure and instrumentation for the synthesis

Synthesis. All reagents were used as purchased from Sigma-Aldrich or Acros except for amino acids which were purchased from Iris Biotech. 1H,1H,2H,2H-perfluorooctyl iodide which was from Elf Atochem and were used without further purification. All solvents were distilled and dried according to standard procedures. TLC analyses were performed on sheets precoated with silica gel 60F254 (Merck). Compound detection was achieved either by exposure to ultraviolet light (254 nm), by spraying with a 5% sulphuric acid solution in ethanol or 2% ninhydrin solution in ethanol and then heating at ~150°C. Flash chromatography purifications were carried out on Merck silica gel Gerduran Si 60 (40-63 µm). Size exclusion chromatography purifications were carried out on Sephadex LH20 resin (Amersham Biosciences). UV spectra were recorded on a Varian CARY 100. Melting points were measured on an Electrothermal IA9100 apparatus and have not been corrected. Optical rotations were measured at 25°C on a Perkin Elmer MC 241 polarimeter. The 1H, 13C, 19F and DEPT NMR sequences were recorded on a Bruker AC-250 spectrometer and were performed at 250, 62.86 and 235 MHz for 1H, 13C and 19F experiments, respectively. Chemical shifts are given in ppm relative to the solvent residual peak as a heteronuclear reference for 1H and 13C. Abbreviations used for signal patterns are: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublet. Mass spectra were recorded on a Qstar Elite (Applied Biosystems SCIEX) for MS ESI+ experiments and on a 3200 Qtrap (Applied Biosystems SCIEX) for HR-MS ESI+ experiments.

Experimental procedures for 10, 11, 13 and 14

N-(2,3,4,6,2′,3′,4′,6′-O-acetyl-lactobionyl)-β-(4-amidoTEMPO)-L-aspartyl-1H,1H,2H,2H-perfluorooctylamide (10). At 0°C, compound 923 (0.3 g, 2.48 10⁻⁴ mol) was dissolved in a TFA/CH₂Cl₂ 4:6 (v/v) mixture. After 2 h of being stirred, the solution was concentrated under vacuum to give the corresponding acid derivative as a white powder (0.252 g, 2.18 10⁻⁴ mol, 88%). The acid derivative (0.240 g, 2.08 10⁻⁴ mol), EDC (0.075 g, 3.91 10⁻⁴ mol), 4-AT (0.024 g, 4.24 10⁻⁴ mol) and a catalytic amount of HOBt were dissolved in dry CH₂Cl₂ with DIEA (pH = 8-9) under argon. The mixture was stirred for 28 h at room temperature and then the solvent was evaporated under vacuum. The crude mixture was purified by flash chromatography (EtOAc/cyclohexane 7:3 v/v) followed by size exclusion chromatography (CH₂Cl₂/MeOH 1:1 v/v) to give compound 10 (0.162 g, 1.24 10⁻⁴ mol, 60%) as an orange powder. Rf 0.56 (EtOAc). mp 66-67°C (decomposition). λmax(CH₂Cl₂)/nm 229. MS (ESI+, m/z) 1308.4 [(M+H)+], 1325.4 [(M+Na)+], 1346.4 [(M+K)+].

N-Lactobionyl-β-(4-amidoTEMPO)-L-aspartyl-1H,1H,2H,2H-perfluorooctylamide (11). Compound 10 (0.150 g, 1.15 10⁻⁴ mol) was dissolved under argon in MeOH and a catalytic amount of sodium methoxide was added. The mixture was stirred for 4 h and HCl IN was added dropwise to neutralize the solution. The solvent was evaporated under vacuum and the crude mixture was purified by size exclusion chromatography (MeOH) to give compound 11 (0.073 g, 9.89 10⁻⁵ mol, 86%) as an orange powder. Rf 0.55
(EtOAc/MeOH/H₂O 7:2:1 v/v/v). mp 78-79°C (decomposition). $\left[\alpha\right]_{D}^{25} +18.2$ (c 0.1 in MeOH). $\lambda_{\text{max}}$(MeOH)/nm 229. HR-MS (ESI+, m/z) calcd for C$_{33}$H$_{49}$N$_4$O$_{14}$F$_{13}$ [(M+H)$^+$]: 972.3032, found 972.3030.

$N$-(2,3,4,6,2',3',4',6'-O-acetyl-lactobionyl)-$N$$^\varepsilon$-(carboxyproxyl)-L-Lysinyl-$1H,1H,2H,2H$-perfluorooctylamide (13). At 0°C, compound 12 (0.3 g, 2.30 $10^{-4}$ mol) was dissolved in ethanol/acetic acid 99:1 (v/v) and 0.018 g of 10% Pd/C was portion wise added under stirring. The reaction mixture was submitted to a hydrogen atmosphere for 12 h (8 bars). After filtration of the catalyst through a pad of Celite, the solvent was evaporated under vacuum to give the corresponding amino derivative (0.263 g, 2.26 $10^{-4}$ mol). The resulting amino (0.250 g, 2.14 $10^{-4}$ mol), EDC (0.049 g, 2.57 $10^{-3}$ mol), 3-CP (0.048 g, 2.57 $10^{-5}$ mol) and a catalytic amount of HOBT were dissolved in dry CH$_2$Cl$_2$ with DIEA (pH = 8-9) under argon. The mixture was stirred for 18 h at room temperature and the solvent was evaporated under vacuum. The crude mixture was purified by flash chromatography (EtOAc) and by size exclusion chromatography (CH$_2$Cl$_2$/MeOH 1:1 v/v) to give compound 13 (0.082 g, 6.14 $10^{-5}$ mol, 28%) as a yellow powder. $R_f$ 0.58 (EtOAc).

$N$-lactobionyl-$N$$^\varepsilon$-(carboxyproxyl)-L-Lysinyl-$1H,1H,2H,2H$-perfluorooctylamide (14). Compound 13 (0.070g, 5.24 $10^{-5}$ mol) was dissolved under argon in MeOH and a catalytic amount of sodium methoxide was added. The mixture was stirred for 4 h and HCl 1N was added dropwise to neutralize the solution. The solvent was evaporated under vacuum and the crude mixture was purified by size exclusion chromatography (MeOH), to give the compound 14 (0.046g, 4.6 $10^{-5}$ mol, 88%) as a yellow powder. $R_f$ 0.51 (EtOAc/MeOH/H$_2$O 7:2:1 v/v/v). mp 108.5-109.5°C (decomposition). $\left[\alpha\right]_{D}^{25} +11.3$ (c 0.1 in MeOH). $\lambda_{\text{max}}$(MeOH)/nm 203. HR-MS (ESI+, m/z) calcd for C$_{35}$H$_{56}$N$_4$O$_{14}$F$_{13}$+$\square$[(M+H)$^+$]$: 1000.3345, found 1000.3353; calcd for C$_{35}$H$_{56}$N$_5$O$_{14}$F$_{13}$+$\square$[(M+Na)$^+$]: 1017.3611, found 1017.3621.

Material and general procedure for cell culturing

All reagents or materials were purchased and used without further purification. Bovine aortic endothelial cells (BAEC) were purchased from Cell Systems (Kirkland, WA). Cells were cultured in 75 cm$^2$ tissue culture flasks using Dulbecco's modified eagle medium with 4.5 g/L D-glucose and supplemented with 10% fetal bovine serum, 4 mM L-glutamine, 2.5 mg/L endothelial cell growth supplement, and 1% non-essential amino acids (Gibco) in the absence of antibiotics at 37°C in a humidified atmosphere of 5% CO$_2$ and 20% O$_2$. The medium was changed every 2-3 days and cells were sub-cultured once they reached 90-95% confluence.
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**Figure S26.** Reduction and restoration of the nitroxide EPR signals by ascorbic acid and potassium ferricyanide. 25 µM of nitroxide was measured by EPR (see experimental for details) over a 30 min period and the signal intensity was found to be stable. 250 µM ascorbic acid was added and the EPR spectra were taken again for 30 min. 250 µM K$_3$[Fe(CN)$_6$] was then added to the sample with ascorbic acid and the EPR spectra was taken again for another 30 min. The y-axis corresponds to the relative intensity compared to the 25 µM of nitroxide. n=2.
**Figure S27.** Relative EPR signal intensities of extracellular nitroxides after 1 h and 24 h incubation in the presence and absence of BAEC. EPR spectra were again taken after addition of 250 µM K₃[Fe(CN)₆]. Plots are relative to the signal intensity of 25 µM nitroxide in DMEM normalized to 100%. In this figure is also shown the result of lysed cells one hour after being incubated with 4-AT. An EPR signal consistent with the presence of 4-AT compound was observed with a relative intensity of 7.5% while the extracellular concentration is about 50%.