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Synthesis of hydrophilic and amphiphilic spin probes

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Electron paramagnetic resonance spectroscopy (EPR), and especially spin labelling are widely used spectroscopic methods [1–4]. The applicability of spin labelling depends on the use of properly designed spin probes which are mostly stable free radicals of the nitroxide type [5–10]. They are important molecular tools for studying the structure and dynamics of complex systems. In many studies the spin probe is distributed between different phases of a sample. The specificity of partitioning is achieved by specifically designed spin probes [11]. Hydrophilic environments are routinely studied [12–14] using a range of water soluble nitroxides containing permanent ionic [1] or ionogenic groups, or neutral polar group(s) [7]. Similarly, many lipophilic spin probes have been developed and used in different membrane studies [1].

In a two phase (water and lipophilic phases) system many phenomena can be better understood if are known the structural and dynamical properties of the system close to the interphase surface, as well as how the dynamic properties of the phases are interrelated. For this, EPR spectroscopy and the application of amphiphilic nitroxide spin probes is the method of choice. In this communication we present the synthesis of one new and neutral hydrophilic and three amphiphilic spin probes which should be useful for studying the water phase very close to the membrane surface [15].

Synthesis of neutral spin probes containing one or more sugar moieties has been already described [7, 16]. However, the preparative procedures are complex and multi-step. In a search for an efficient and a simple approach for preparing hydrophilic and amphiphilic nitroxides we studied the reaction of D-gluconic acid lactone (**1**) with 1-oxyl-2,2,6,6-tetramethyl-4-aminopiperidine (4-tempamine, **2**).

Lactone (**1**) reacts with amine (**2**) giving an interesting neutral hydrophilic nitroxide compound **3** (Scheme). Refluxing **1** and **2** in THF for one week gave **3** in a low yield (up to 20%) with a lot of side products. Using a solvent with a higher boiling point resulted in even more side products. By adding sodium heptanoate as a catalyst

Scheme

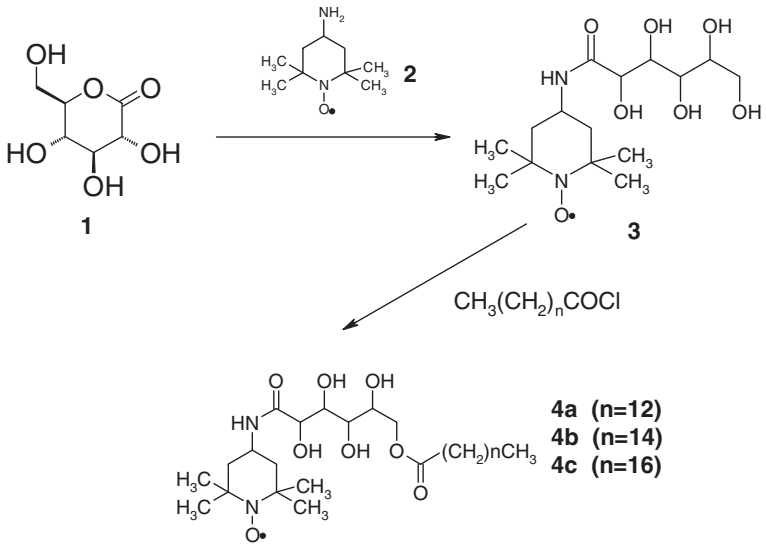

Table: Yields, melting points, molecular formula, MS peaks and retention factors of compounds 4a–c

Comp.	Yield[a] (%)	M.p. (°C)	Molecular formula	MS (FAB)	TLC retention factor[b]
4a (n = 12)	71.3	41–43	$C_{29}H_{55}N_2O_8$	561 [M + 2]	0.33
4b (n = 14)	68.2	45–47	$C_{31}H_{59}N_2O_8$	589 [M + 2]	0.31
4c (n = 16)	75.1	52–54	$C_{33}H_{63}N_2O_8$	517 [M + 2]	0.28
IR(KBr) **4a**, cm^{-1}	3382 (–OH), 2927, 2854, 1726 (CO–O), 1634 (CO–NH), 1549, 1466, 1379, 1323, 1245, 1180, 1090, 1044, 721.				
IR(KBr) **4b**, cm^{-1}	3448 (–OH), 2918, 2850, 1741 (CO–O), 1655 (CO–NH), 1544, 1468, 1179, 1112, 778, 562.				
IR(KBr) **4c**, cm^{-1}	3449 (–OH), 2918, 1752 (CO–O), 1656 (CO–NH), 1544, 1467, 1376, 1218, 1044, 779, 541				

[a] yields after isolation
[b] TLC were developed using mobile phase $CH_3Cl : CH_3OH = 3 : 1$

according to the procedure described [17], the yield after isolation of compound **3** was around 50% with a minimal amount of side products. Compound **3** appears as light red crystals after crystallization from absolute ethanol and is eatily soluble in water which permits the preparation of very concentrated solutions.

In order to anchor **3** into a membrane with the nitroxide group in the region of around 1,4 nm above the membrane surface, we introduced a lipophilic moiety into **3** by esterifying the primary hydroxyl group with acylchloride in pyridine. Under the conditions described the amount of side products (di- and tri-esters) is minimal, however, chromatographic purification is necessary to remove higher esters and unreacted acylchloride. Compounds **4a–c** are solid light orange compounds, slightly soluble in water and soluble in organic solvents such as chloroform, ether etc.

Experimental

1. Apparatus

Melting points were determined on a Kofler hot stage microscope (Reichert, Co.) and are uncorrected. IR spectra were recorded in KBr on a Perkin Elmer FTIR 1600 spectrometer. Mass spectra were recorded on an AutospecQ (Micromass, MS Centre, Jožef Stefan Institute).

EPR spectra of nitroxide solutions were measured on a BRUKER X-band CW-ESP spectrometer ESP 300E (EPR Centre, Jožef Stefan Institute) at room temperature in a glass capillary (1 mm inner diameter) and at 20 mW microwave power. Elemental analyses were performed on a Perkin Elmer 240 C microanalyzer (Faculty of Chemistry and Chemical Technology, University of Ljubljana), all compounds gave results in a acceptable range.

2. N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2,3,4,5,6-pentahydroxyhexanamide (3)

Lactone **1** (3.92 g, 0.022 mol), amine **2** (3.43 g, 0.02 mol), and sodium heptanoate (1.98 g, 0.013 mol) were suspended in THF (200 ml) and refluxed for 24 h. THF was removed under reduced pressure. The solid residue was dissolved in a minimal amount of water, and adjusted to pH 5 with 1 M HCl. The water phase was extracted with diethyl ether to remove heptanoic acid, and than neutralized with solid K_2CO_3. Anhydrous Na_2SO_4 was than added to give a semisolid which was washed with hot EtOH until the EtOH extract was colourless. The EtOH was removed and the red solid was crystallized from absolute EtOH. Yield of red needles (m.p. 145–147 °C) was 46.4%, R_f($CHCl_3$/MeOH 3 : 1) = 0.58. IR (KBr): ν = 3391, 2975, 2936, 1655, 1539, 1466, 1379, 1329, 1243, 1137, 1070, 961, 873, 784, 673 cm^{-1}. MS (FAB): 351 (M + 2). a_N (water) = 1,708 mT.

3. Esters of N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2,3,4,5,6-pentahydroxyhexa-namide (4a–c)

General procedure: Compound **3** (350 mg, 1.00 mmol) was dissolved in 3.5 ml of dry pyridine and cooled on ice. Acid chloride (1.05 mmol) dissolved in CH_2Cl_2 (7 ml) was added dropwise to the stirred cooled solution over 30 min. After the addition was completed the solution was stirred on ice for 2 h, then left over night at room temperature. To the reaction mixture ethyl acetate (10 ml) was added and 10% citric acid to pH 4. The acidic mixture was extracted with ethyl acetate until the organic phase was colourless. The latter was dried with anhydrous Na_2SO_4. After removing ethyl acetate, the red viscous residue, which still contained some acid chloride, was applied to a silica gel column and eluted, first with diethyl ether (removing acid chloride) and then with chloroform: isopropanol = 6 : 1. Yields, melting points, MS data, R_f values and IR data are presented in the Table. For **4a, 4b** and **4c** a_N (ether) = 1.543 mT.

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