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Microwave-assisted synthesis of amphiphilic spin probes

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ABSTRACT

New amphiphilic nitroxide spin probes have been synthesized. The key reaction is based on microwave-assisted epoxide ring opening with amines as nucleophiles using calcium trifluoromethanesulfonate as a catalyst. High yields, in short reaction times, were obtained without any detectable nitroxide decomposition.

Lipids and proteins are not distributed randomly in cell membranes but are organized locally into lipid rafts or domains depending on the interactions between them.[1] The membrane domain structure therefore has a strong impact on the conformation and consequent function of certain proteins involved in transmembrane signal transduction.[2] Apoptosis, or programmed cell death, for example, depends strongly on transmembrane signalling pathways which influence significantly the membrane domain structure when triggered.[3] Electron paramagnetic resonance (EPR) spectroscopy and spin labelling is the method of choice for studying such structural and dynamic changes in biological membranes.[4]

We focused our attention on long-chain amino-alcohol derivatives, such as 1-(cyclohexylamino)hexadecan-2-ol, described as inducers of apoptosis in some cancer cell lines. These molecules contain a hydroxyethylamine (HEA) linker in their structures.[5] To study their influence on cancer cell membranes and mechanisms of triggering apoptosis we have attempted to prepare spin labelled analogs with comparable biological activities to those of the parent compounds. Different synthetic approaches for the preparation of HEA-containing fragments have been described. The most straightforward route appears to be via ring opening of the appropriate epoxide by an amine.[6] Recently, we reported that calcium trifluoro methanesulfonate ($Ca(OTf)_2$) is one of the best catalysts for epoxide ring opening with amino acids.[7] We have now applied microwave irradiation for this process, with superior yields and shorter reaction times than previously published methods.[8]

Combining our knowledge of nitroxide spin-probe synthesis and epoxide ring opening, a series of spin labelled long-chain amino-alcohols **5a**–**m** (Scheme 1) were synthesized from various amines (**1**, **4a**–**c**) and epoxides (**2a**–**m**, **3**).[7–10] The reaction was optimized with respect to reaction time, temperature and power of the microwave reactor. Calcium trifluoromethanesulfonate was used as the catalyst for epoxide ring opening because of the low nucleophilicity of the amino group.[7,8] While the exact mechanism of the reaction is not known, Sova et al. showed that microwaves do not replace the catalyst.[8] The reaction was carried out in a quartz microwave reactor vessel using dioxane as the solvent, which is known to be almost transparent to microwaves.[11] Water can behave as a strong nucleophile and reacts with the epoxide ring, so anhydrous dioxane must be used.

In a typical procedure, 1.1 mmol of the mono-substituted amine, 1 mmol of epoxide and 0.5 mmol of $Ca(OTf)_2$ were suspended in 6 mL of anhydrous dioxane and the reaction mixture was stirred for 20 min at 140 °C and 2–3 bar pressure in a microwave reactor.[12] After cooling to ambient temperature with compressed air, the catalyst was removed by filtration and the solvent evaporated under reduced pressure, giving a crude product which was purified with flash or circular chromatography using dichloromethane/methanol (15/1) as eluent.

The starting epoxides (**2a**–**m**, **3**), amines (**1**, **4a**–**c**), products (**5a**–**m**, **7a**–**c**) and final yields are listed in Table 1. The obvious side products of the reaction were tertiary amines (**6b**–**l**, **8a**–**c**) which formed in less than 20% yield and were isolated as a mixture of diastereoisomers (Table 1). Tertiary amine side products formed from racemic epoxides contain two stereogenic centres: two optically active products and the nonchiral *meso* form are possible due to molecular symmetry. The new spin probes were fully

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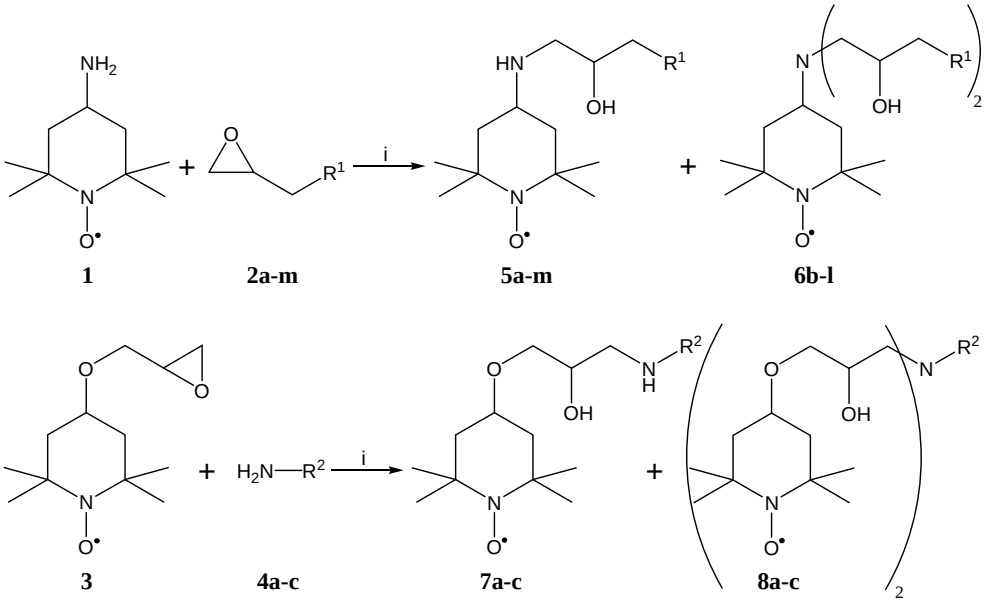

Scheme 1. Synthesis of amphiphilic spin probes. Reagents and conditions: (i) $Ca(OTf)_2$, microwave irradiation, dioxane.

Table 1
Amphiphilic spin probes **5a–m** and **7a–c** produced according to Scheme 1

Entry	Amine (R^2)	Epoxide (R^1)	Product (R^1 or R^2)	Yield[a] (%)	Side product (R^1 or R^2)	Yield[a] (%)
1	**1**[15–17]	$(CH_2)_2CH_3$ (**2a**)	$(CH_2)_2CH_3$ (**5a**)	85	n.i.[b]	n.d.[b]
2	**1**	$(CH_2)_4CH_3$ (**2b**)	$(CH_2)_4CH_3$ (**5b**)	77	$(CH_2)_4CH_3$ (**6b**)	8
3	**1**	$(CH_2)_6CH_3$ (**2c**)	$(CH_2)_6CH_3$ (**5c**)	76	n.i.	n.d.
4	**1**	$(CH_2)_8CH_3$ (**2d**)	$(CH_2)_8CH_3$ (**5d**)[18]	85	$(CH_2)_8CH_3$ (**6d**)	8
5	**1**	$(CH_2)_{10}CH_3$ (**2e**)	$(CH_2)_{10}CH_3$ (**5e**)	77	$(CH_2)_{10}CH_3$ (**6e**)	6
6	**1**	$(CH_2)_{12}CH_3$ (**2f**)	$(CH_2)_{12}CH_3$ (**5f**)	76	$(CH_2)_{12}CH_3$ (**6f**)	9
7	**1**	$(CH_2)_{14}CH_3$ (**2g**)	$(CH_2)_{14}CH_3$ (**5g**)	68	$(CH_2)_{14}CH_3$ (**6g**)	12
8	**1**	$(CH_2)_{16}CH_3$ (**2h**)	$(CH_2)_{16}CH_3$ (**5h**)	50	$(CH_2)_{16}CH_3$ (**6h**)	10
9	**1**	$O(CH_2)_7CH_3$ (**2i**)	$O(CH_2)_7CH_3$ (**5i**)[19]	74	$O(CH_2)_7CH_3$ (**6i**)	17
10	**1**	$O(CH_2)_{11}CH_3$ (**2j**)	$O(CH_2)_{11}CH_3$ (**5j**)	69	$O(CH_2)_{11}CH_3$ (**6j**)	18
11	**1**	$O(CH_2)_{13}CH_3$ (**2k**)	$O(CH_2)_{13}CH_3$ (**5k**)	79	$O(CH_2)_{13}CH_3$ (**6k**)	14
12	**1**	$O(CH_2)_{17}CH_3$ (**2l**)	$O(CH_2)_{17}CH_3$ (**5l**)	75	$O(CH_2)_{17}CH_3$ (**6l**)	11
13	**1**	O-Naphthalene (**2m**)	O-Naphthalene (**5m**)[20]	91	n.i.	n.d.
14	$(CH_2)_{13}CH_3$ (**4a**)	**3**[22,23]	$(CH_2)_{13}CH_3$ (**7a**)	67	$(CH_2)_{13}CH_3$ (**8a**)	15
15	$(CH_2)_{15}CH_3$ (**4b**)	**3**	$(CH_2)_{15}CH_3$ (**7b**)[21]	79	$(CH_2)_{15}CH_3$ (**8b**)	18
16	$(CH_2)_{17}CH_3$ (**4c**)	**3**	$(CH_2)_{17}CH_3$ (**7c**)	75	$(CH_2)_{17}CH_3$ (**8c**)	14

[a] Refers to yields of isolated racemic or diastereoisomeric mixtures following flash or circular chromatography.
[b] Side product was not isolated (n.i.) from the reaction mixture and the yield was not determined (n.d.).

characterized by IR, MS and NMR spectroscopy.[13] Applying this methodology, a spin-labelled analogue of propanolol (**5m**) was obtained in a very short reaction time, and in excellent yield (91%) compared to the reported procedure (36%).[14]

Preliminary EPR studies were conducted in ethanol and liposomes. The red line spectrum shows a typical EPR signal of the solution in which no aggregation occurs (Fig. 1). The black line spectrum is a typical membrane EPR spectrum composed of contributions from labels in at least two different environments. Spin probes with longer lipophilic alkyl chains can anchor into a lipid bilayer to a greater extent, and the isotropic part of the EPR spectrum consequently decreases.

In conclusion, a direct and efficient synthetic method is presented for the preparation of amphiphilic spin probes containing a piperidine-type nitroxide, a HEA linker and a variety of long alkyl chains. The epoxide ring opening reaction was performed in high yields, without any detectable nitroxide decomposition, in a microwave reactor using calcium trifluoromethanesulfonate as the catalyst. Spin probes with a single lipophilic alkyl chain easily anchor

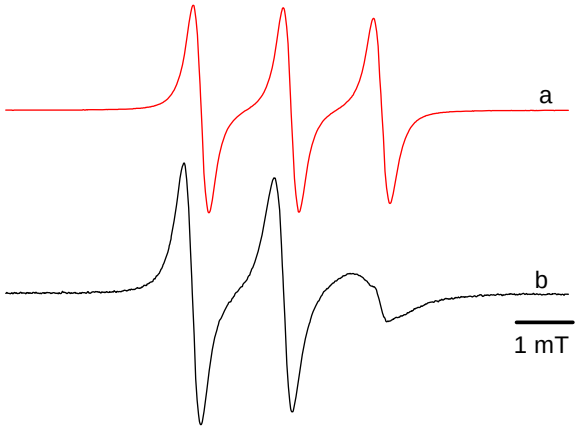

Figure 1. EPR spectra of **5i** dissolved in (a) ethanol and (b) liposome suspension, both at a final concentration of 4.0×10^{-4} M and room temperature.[24]

into lipid bilayers of biological membranes such as liposomes, and they can be used in membrane studies as reporter molecules, especially at the lipid water interface.

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18. *4-(2-Hydroxydodecylamino)-2,2,6,6-tetramethyl-1-oxylpiperidine* (**5d**): Red-orange oil; R_f ($CHCl_3$/MeOH, 9/1): 0.14; MS (EI) *m*/*z*: 356 $(MH)^+$; HRMS Calcd for $C_{21}H_{44}N_2O_2$ *m*/*z*: 356.3403 $(M+1)^+$, found 356.3370; IR (KBr, cm^{-1}): 3436, 2923, 2851, 1653, 1559, 1467, 1364, 1242, 1179, 1079, 1049, 913; EPR a_N (ethanol, mT): 1.6065. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 0.91 (t, 3H, *J* = 6.6 Hz, CH_3), 1.25–1.33 (m, 16H, 8 × CH_2), 1.42–1.46 (m, 2H, CH–C*H*$_2$–CH_2), 2.43 (dd, 1H, *J* = 9.3 Hz, *J* = 11.9 Hz, NH–C*H*$_2$–CH), 2.43 (dd, 1H, *J* = 9.3 Hz, *J* = 11.9 Hz, NH–C*H*$_2$–CH), 3.54–3.62 (m, 1H, CH), 5.26 (br s, 1H, –OH), piperidine ring: 1.16, 1.20 (2s, 12H, *cis*-, *trans*-2,6-CH_3), 1.39–1.47 (m, 2H, *cis*-3,5-H, CH_2), 1.80–1.90 (m, 2H, *trans*-3,5-H, CH_2), 2.80–2.88 (m, 1H, CH).
19. *4-(3-(Octyloxy)-2-hydroxypropylamino)-2,2,6,6-tetramethyl-1-oxylpiperidine* (**5i**): Red-orange oil; R_f ($CHCl_3$/MeOH, 9/1): 0.24; MS (EI) *m*/*z*: 358 $(MH)^+$; HRMS Calcd for $C_{20}H_{42}N_2O_3$ *m*/*z*: 358.3195 $(M+1)^+$, found 358.3200; IR (KBr, cm^{-1}): 3456, 2884, 1651, 1464, 1380, 1361, 1281, 1249, 1174, 1128, 1032, 961; EPR a_N (ethanol, mT): 1.6043. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 0.90 (t, 3H, *J* = 6.7 Hz, CH_3), 1.24–1.38 (m, 10H, 5 × CH_2), 1.54–1.61 (m, 2H, O–CH_2–C*H*$_2$), 2.07 (s, 1H, NH), 2.63 (dd, 1H, *J* = 8.1 Hz, *J* = 11.9 Hz, NH–C*H*$_2$–CH), 2.78 (dd, 1H, *J* = 3.6 Hz, *J* = 11.8 Hz NH–C*H*$_2$–CH), 3.45 (dd, 2H, *J* = 5.9 Hz, *J* = 12.3 Hz O–C*H*$_2$–CH_2), 3.47 (t, 2H, *J* = 6.6 Hz O–C*H*$_2$–CH_2), 3.81–3.88 (m, 1H, O–CH_2–C*H*), 5.28 (s, 1H, –OH), piperidine ring: 1.16, 1.20 (2s, 12H, *cis*-, *trans*-2,6-CH_3), 1.29–1.38 (m, 2H, *cis*-3,5-H, CH_2), 1.82–1.87 (m, 2H, *trans*-3,5-H, CH_2), 2.78–2.88 (m, 1H, CH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) = 13.97 (CH_3), 22.51, 32.07, 29.30, 29.46, 29.13, 31.68 (6 × CH_2), 71.62 (CH_2–O), 73.51, 68.84, 59.13 (O–CH_2–CH(OH)–CH_2–NH), piperidine ring: 20.09, 25.98 (4Me, *cis*-, *trans*-2,6-Me), 45.78, 45.58 (2 × CH_2), 48.41 (CH), 49.50 (*tert*-C).
20. *4-(2-Hydroxy-3-(naphthalen-1-yloxy)propylamino)-2,2,6,6-tetramethyl-1-oxylpiperidine* (**5m**)[14]: Red-orange oil; R_f ($CHCl_3$/MeOH, 9/1): 0.38; MS (EI): *m*/*z*: 372 $(MH)^+$; IR (KBr, cm^{-1}): 3419, 2932, 1628, 1579, 1508, 1459, 1399, 1269, 1178, 1101, 1068, 1030, 994; EPR: a_N (ethanol, mT): 1.6028 ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 2.08 (s, 1H, NH) 2.94 (dd, 1H, *J* = 7.5 Hz, *J* = 12.2 Hz, NH–C*H*$_2$–CH), 3.10 (dd, 1H, *J* = 3.3 Hz, *J* = 12.1 Hz, NH–C*H*$_2$–CH), 3.73 (s, 1H, N–OH), 4.12–4.17 (m, 1H, CH_2–C*H*–CH_2), 4.21–4.30 (m, 2H, O–C*H*$_2$–CH), 5.31 (s, 1H, –OH), 7.36–7.41 (m, 2H, H-Ar), 7.46–7.55 (m, 3H, H-Ar), 7.81–7.85 (m, 1H, H-Ar), 8.27-8.30 (m, 1H, H-Ar), piperidine ring: 1.16, 1.22 (2s, 12H, *cis*-, *trans*-2,6-CH_3), 1.33–1.42 (m, 2H, *cis*-3,5-H, CH_2), 1.92 (dd, 2H, *J* = 3.2 Hz, *J* = 12.5 Hz, 2H, *trans*-3,5-H, CH_2), 2.91–3.00 (m, 1H, CH).
21. *4-(3-(Hexadecylamino)-2-hydroxypropoxy)-2,2,6,6-tetramethyl-1-oxylpiperidine* (**7b**): Red-orange oil; R_f ($CHCl_3$/MeOH, 9/1): 0.31; MS (EI) *m*/*z*: 470.5 $(MH)^+$; HRMS Calcd for $C_{28}H_{58}N_2O_3$ *m*/*z*: 470.4447 $(M+1)^+$, found 470.4460; IR (KBr, cm^{-1}): 3452, 2928, 2849, 1636, 1468, 1380, 1364, 1247, 1168, 1110, 1031 901; EPR a_N (ethanol, mT): 1.6002. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 0.88 (t, 3H, *J* = 6.7 Hz, CH_3), 1.21–1.25 (m, 26H, 13 × CH_2), 1.65–1.67 (m, 2H, NH–CH_2–C*H*$_2$), 2.01 (s, 1H, NH), 2.91–2.98 (m, 1H, NH–C*H*$_2$–CH), 3.06 (dd, 1H, *J* = 3.3 Hz, *J* = 12.4 Hz NH–C*H*$_2$–CH), 3.46–3.51 (m, 1H, O–C*H*$_2$–CH_2), 3.55–3.67 (m, 1H, O–C*H*$_2$–CH), 3.97–4.00 (m, 1H, O–C*H*$_2$–CH), 4.07–4.14 (m, 1H, CH), 5.28 (s, 1H, –OH), piperidine ring: 1.16, 1.21 (2s, 12H, *cis*-, *trans*-2,6-CH_3), 1.40–1.49 (m, 2H, *cis*-3,5-H, CH_2), 1.90-1.95 (m, 2H, *trans*-3,5-H, CH_2), 2.81–2.93 (m, 1H, CH).
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24. Liposomes were prepared from soybean phosphatidylcholine (Phospholipon® 80) and PBS buffer by the lipid film method and a final lipid concentration of 50 mg/mL. Labelling: a stock solution of **5i** in ethanol (10^{-3} M) was placed in a test tube. The ethanol was removed under reduced pressure, resulting in a film of **5i** on the test tube wall. Liposomes were added to the film, and the test tube was shaken for 30 min.