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Synthesis of unsymmetrical spin-labelled bolaamphiphiles

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ABSTRACT

This work presents the efficient synthesis of a novel spin-labelled bolaamphiphile bearing sugar and cationic polar heads. Preliminary ESR studies were conduced both in CH_2Cl_2 and water and have confirmed that this methodology can be used to study the transversal rotation (flip–flop) dynamic of membrane involving such bolaamphiphiles.

Bipolar lipids found in archaebacterial membranes, generally termed bolaamphiphiles or bolas,[1] induce increased stability in membranes exposed to environments such as acidic conditions, high temperatures, high salt concentrations and/or absence of oxygen. Several approaches to synthetic bipolar lipids have been performed in order to mimic archaeal membranes.[2–8] The flip–flop, that is, the transversal rotation of lipid units in the membrane is one of the fundamental processes involved in the membrane dynamics (Fig. 1).[9] Generally, the flip–flop from the exovesicular to the endovesicular membrane surface (and vice versa) is a relatively slow process, which is due to the high energy barrier in transferring the polar amphiphilic heads through the lipophilic membrane.[10,11]

Flip–flop phenomenon can be involved in cell membrane transport mechanisms[12] and its rate in this case is strongly dependent on polar head-group composition and less dependent on the alkyl chain length.[13] Recently, the transbilayer flip–flop of early intermediates in the glycosylphosphatidylinositol (GPI) biosynthetic pathway has been demonstrated using novel fluorescent GPI probes and a biochemical reconstitution approach.[14] The ESR spectroscopy is also well-adapted for the study of such dynamic as described previously for monopolar lipids.[11] Few studies have been performed on the flip–flop dynamics of bolas[4,5,15] but to our knowledge no data are available on unsymmetrical bolas.

Our research group has already studied the self-organisation of unsymmetrical bolaamphiphiles such as **1** (Fig. 2).[7] Indeed **1** self-assembles into different shapes (discs, vesicles) depending on the temperature and we assume that the transition between these two types of organisation is due to flip–flop dynamics within the membrane. To point out this kind of dynamics we describe herein the synthesis of a spin-labelled analogue of **1** (**2**) (Fig. 2).

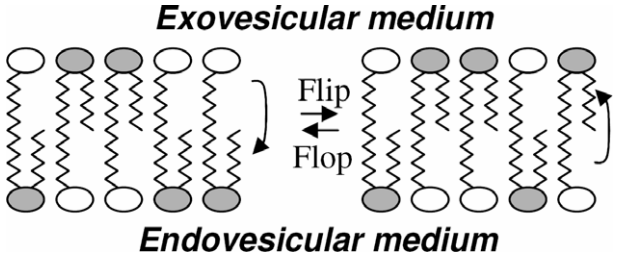

Figure 1. Flip–flop phenomenon of bolas.

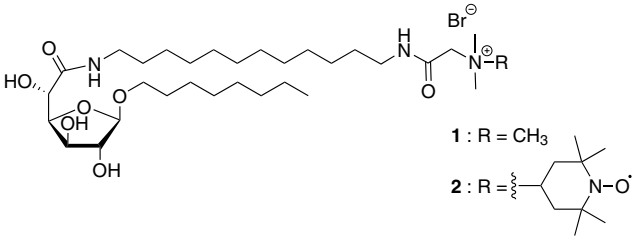

Figure 2. Non-labelled (**1**) and labelled bolas (**2**).

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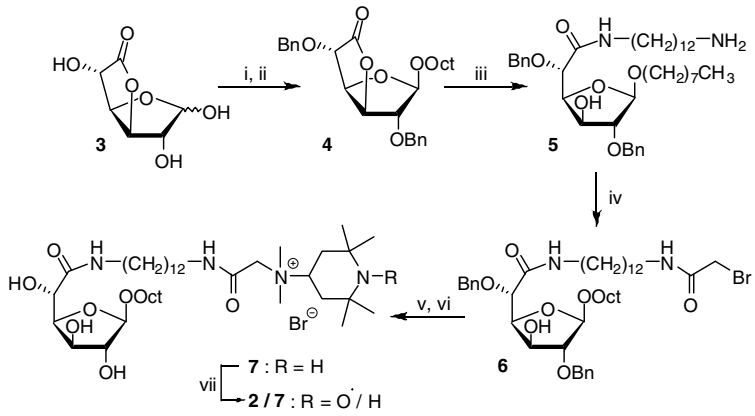
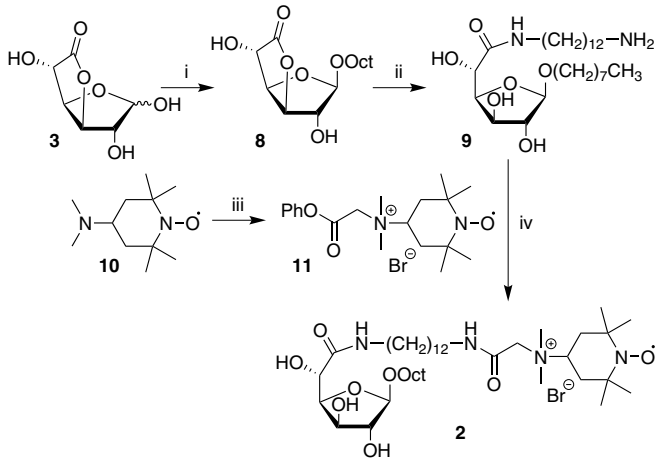


Scheme 1. First strategy for the preparation of labelled bola **2**. Reagents and conditions: (i) 1-octanol, $BF_3{\cdot}Et_2O$, THF, reflux, 70%; (ii) Ag_2O, BnBr, CH_2Cl_2, 70%; (iii) 1,12-diaminododecane, MeOH, 60%; (iv) $BrC(O)CH_2Br$, pyridine, 65%; (v) 4-dimethylamino-2,2,6,6-tetramethylpiperidine, DMF, 59%; (vi) H_2, Pd/C, MeOH, 70%; (vii) oxone, acetone, $n Bu_4NHSO_4$, CH_2Cl_2, phosphate buffer pH 7, 76%.



Scheme 2. Efficient synthesis of pure-labelled bola **2**. Reagents and conditions: (i) 1-octanol, $BF_3{\cdot}Et_2O$, THF, reflux, 70%; (ii) 1,12-diaminododecane (1.5 equiv), MeOH, 70%; (iii) $BrCH_2COOPh$ (1.0 equiv), THF, 75%; (iv) **11** (1.5 equiv), DMF, rt, 50%.

Bolaamphiphile **2** has been synthesised according to two strategies which involve the introduction of a spin-active nitroxide either in a final step (oxidation) (Scheme 1) or through a readily available nitroxide piperidine (Scheme 2).

The first procedure involves a strategy similar to the one already-described for bola **1**.[7] D-Glucofuranurono-6,3-lactone **3** was converted into the corresponding octyl-β-D-glucofuranosiduronо-6,3-lactone. Protection of the free hydroxyl groups was performed under mild conditions by reaction of the glycoside with Ag_2O and BnBr in CH_2Cl_2 in order to prevent the ring lactone opening. Careful treatment of the protected lactone **4** with 1.5 equiv of 1,12-diaminododecane at room temperature in MeOH provided the glucofuranosiduronamide **5** resulting from the monoacylation of the diamine in 60% yield (the separable diacylated product was formed in 18% yield). *N*-Acylation of amine **5** was performed with bromoacetyl bromide in pyridine. The protection of the 5-OH as a benzyloxy group found its importance at this stage as it inhibited the competitive *O*-acylation at this position, observed with the unprotected counterpart. Quaternarisation of 4-dimethylamino-2,2,6,6-tetramethyl-piperidine with the alkyl bromide derivative **6** in DMF resulted in a clean reaction at the tertiary amine group leading to the corresponding ammonium derivative **7** after hydrogenolysis of the benzyloxy groups. The conversion of the secondary amine to the corresponding nitroxide **2** under biphasic conditions (CH_2Cl_2/phosphate buffer) using acetone, oxone and phase-transfer catalyst Bu_4NHSO_4 led to a mixture of nitroxide **2** and amine **7**. Unfortunately, neither a complete oxidation of **7** nor an efficient separation of **2** from the remaining amine **7** could be performed, leading to a mixture of non-labelled and spin-labelled products in 76% yield after chromatography on silica gel and gel filtration on Sephadex G-10.

To circumvent the troublesome drawback of the precedent strategy, we then considered a more convergent synthetic pathway involving this time a readily available pure-labelled piperidine nitroxide (Scheme 2). Unprotected octyl-β-D-glucofuranosiduronо-6,3-lactone **8** was converted without protection to the glucofuranosiduronamide **9** by a condensation of 1,12-diaminododecane in MeOH (70% yield).[7] A nucleophilic substitution between amine **10**[16,17] and phenyl bromoacetate furnished the nitroxide ammonium **11**,[18] which was enough reactive to induce, in a next step, the acylation of the glucofuranosiduronamide **9** in DMF (50% yield).

Attempts with other activated esters, instead of the phenyl derivative, such as more reactive 2,2,2-trifluoroethyl,[19] and more stable 3,3′-dimethoxyphenyl ester did not improve the yield of the final step. This strategy, removing protection (benzylation) and deprotection (hydrogenolysis) steps, has afforded, in moderate overall yield but pure-labelled bolaamphiphile **2**[20] from D-glucofuranurono-6,3-lactone **3** (25%, 3 steps).

Preliminary ESR studies were conduced in CH_2Cl_2 and in water (c = 10 mg/mL). The plain-line spectrum shows a narrow signal from the bolaamphiphile **2** dispersed in CH_2Cl_2, where no aggregation occurs (Fig. 3). The triplet observed in the ESR spectrum is due to the nitroxide signal with the characteristic constants a = 15.55 G

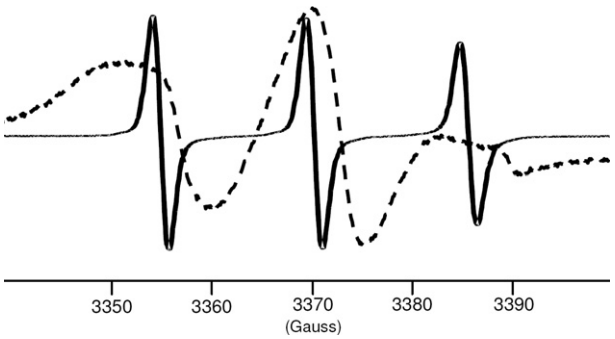


Figure 3. ESR spectra of spin-labelled bolaamphiphile **2**; in CH_2Cl_2 (plain line); in H_2O (dashed line). (All the experiments were performed at room temperature.)

and $g = 2.00585$. The dashed-line ESR spectrum is much broader and shows the signal of bolaamphiphile **2** molecules in H_2O (Fig. 3), where a self-organisation can occur. The widening of the signal comes from the superposition of signals of nitroxide from different environments.

In conclusion, we have synthesised a pure-labelled unsymmetrical bolaamphiphile bearing sugar and cationic polar heads. Note that phenyl ester **11** would be easily used for the preparation of other cationic-labelled molecules. The preliminary ESR measurements have confirmed its potential for the characterisation of flip–flop dynamic of such bolas in water. A full physicochemical characterisation of the self-organised aggregates is now under investigation and ESR kinetic studies would provide useful information on the membrane dynamic of relevant systems.

Acknowledgements

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18. *For the synthesis of* **11**: To a solution of **10** (439 mg, 2.20 mmol, 1.0 equiv) in anhydrous THF (10 mL) was added phenyl bromoacetate (444 mg, 2.20 mmol, 1.0 equiv) under nitrogen atmosphere. The reaction mixture was stirred for 8 h at rt and Et_2O (20 mL) was added. The resulting suspension was filtered and extensively washed with Et_2O to afford **11** as an orange solid (675 mg, 1.63 mmol, 75%). Mp = 185 °C; IR ν (cm^{-1}): 1770, 1377; HRMS calcd for $C_{19}H_{30}N_2O_3$: 334.2251; found, 334.2250.
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20. *For the synthesis of bolaamphiphile* **2**: To a solution of **9** (100 mg, 0.20 mmol, 1.0 equiv) in anhydrous DMF (1 mL) was added **11** (88 mg, 0.22 mmol, 1.1 equiv) under nitrogen atmosphere. The reaction mixture was stirred at 30 °C for 3 days and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc/*i*-PrOH/H_2O/NH_4OH: 6:3:1:0.5) to afford **2** as an orange solid (83 mg, 0.10 mmol, 50%). $R_f = 0.2$ (EtOAc/*i*-PrOH/H_2O/NH_4OH: 6:3:1:0.5); IR ν (cm^{-1}): 3330, 1674, 1548; ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm): 14.4, 26.9, 27.8–30.5, 32.7, 40.2, 40.8, 58.1, 69.3, 71.8, 77.1, 81.3, 83.7, 109.7, 163.5, 173.8; HMRS calcd for $C_{39}H_{76}N_4O_8$: 728.5663; found, 728.5661.