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C_2-symmetrical sterol–polyether conjugates as highly efficient synthetic ionophores

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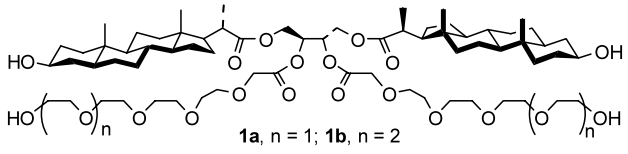
Abstract—A new class of artificial ionophores has been rationally designed and synthesized linking to a tetrafunctionalized L-treitol spacer two rigid hydrophobic 3β-hydroxy-5α-23,24-bisnorcholanic units and two flexible hydrophilic oligo(ethylene glycol) chains. Compounds **1a** and **1b** were incorporated into phospholipid vesicles and shown to facilitate Na^+-transport.

The bio- and pharmacological implication of synthetic channel-forming molecules is, currently, a focal point of attention.[1] Their activities give insight into the membranes' permeability mechanisms and may provide new chemical tools useful to circumvent drug resistance,[2] acting as ionic nano-devices[3] or able to interfere with the cellular transduction of the electrical signal.[4] Tremendous efforts have gone into chemical studies of peptide-based ion-channels[5] but alternative systems have also been explored.[6]

Recently, we have succeeded in constructing an artificial non peptide ionophore[7] inspired by membrane-interfering natural polyhydroxysteroids derivatives.[8] The active species were suggested to be supramolecular aggregates in which the steroidal amphipatic framework preorganizes into the membrane, promoting the formation of hydrophilic pores through the lipid bilayer.

However, because the synthetic pathway to the C_2-symmetric polyhydroxylated steroid dimer derivative was elaborate (16 steps)[9] and not amenable to a modular approach, we sought alternative sterol-based targets whose synthesis would be more convenient and whose structural features could be easily controlled in order to tune membrane interaction and activity.

With these ideas in mind we designed the two C_2-symmetric sterol–oligo(ethylene glycol) conjugates prototypes **1a** and **1b**.



1a, n = 1; **1b**, n = 2

Figure 1 shows their three main structural elements: a L-treitol-based central 'core', lying near the bilayer midplane, two hydrophobic steroidal 'wall' units and two partially hydrophilic oligo(ethylene glycol) moieties,[10] radiating from the alditol.

The 'wall' units should provide, with their 40 Å long extended conformation,[11] structural control to the transmembrane aggregate and drag the polar polyether chains into the bilayer. These last should point toward the hydrophilic face of a nearest-neighbor identical molecule, inducing a localized increase in membrane hydrophilicity. The four distal hydroxy polar 'head' groups should enforce the correct orientation of the molecule through the phospholipid bilayer.

Keywords: ionophore; steroid; amphiphiles.
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In this communication we report the synthesis and the Na^+ ionophoric activity of compounds **1a** and **1b** across a lipid bilayer using a $^{23}Na^+$ NMR based assay.[7]

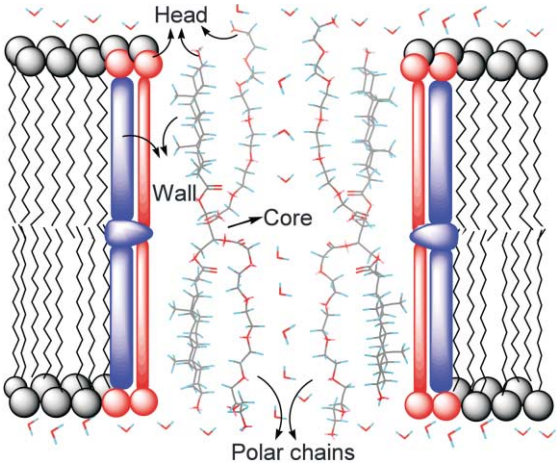
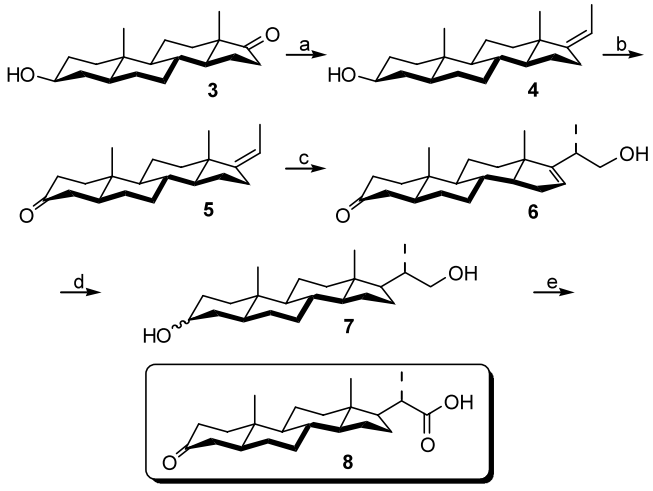
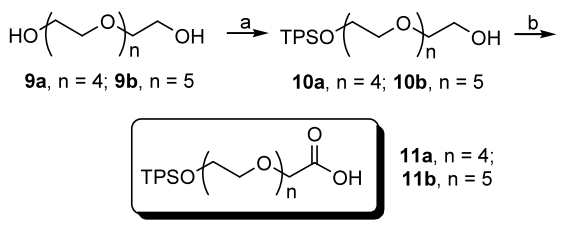


Figure 1. Design proposal for **1b** inserted into a phospholipid bilayer.



Scheme 1. *Reagents and conditions*: (a) 3 equiv. of $EtP(Ph)_3Br$, 2.7 equiv. of *t*-BuOK, THF, reflux, 3 h, 83%; (b) 1.5 equiv. of PDC, *m.s.*, CH_2Cl_2, rt, 2 h, 86%; (c) 5.5 equiv. of paraformaldehyde, 0.1 equiv. of $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt, 0.1 h, 98%; (d) H_2, Pt/C, EtOH, 1 h; (e) Jones reagent, acetone, rt, 2 h, 90% (two steps).



Scheme 2. *Reagents and conditions*: (a) 1.8 equiv. of DBU, 1.0 equiv. of TPS–Cl, CH_2Cl_2, 3 h, 40%; (b) Jones reagent, acetone, 1 h, 62%.

The starting material for the synthesis of the sterol–polyether conjugates **1a** and **1b** was commercially available *epi*-androsterone (**3**, Scheme 1). This was converted into the (*Z*)-17(20)-ethylidene steroid **4**[12] in 83% yield by means of a highly stereoselective Wittig coupling.[13] C-3 PDC oxidation[14] and a stereospecific ene reaction of the ketone **5** with paraformaldehyde in the presence of catalytic amount of boron trifluoride-diethyl ether,[15] afforded 5α-23,24-bisnorchol-16-en-22-ol-3-one (**6**) in 84% yield. The omoallyl alcohol **6** was stereoselectively hydrogenated at Δ^{16} to give a 9:1 mixture of 3β- and 3α-alcohols, both showing a 17β side chain. Jones oxidation[16] of the crude reaction mixture yielded the expected 3-oxo-5α-23,24-bisnorcholanic acid **8** in good overall yields (63%, five steps).

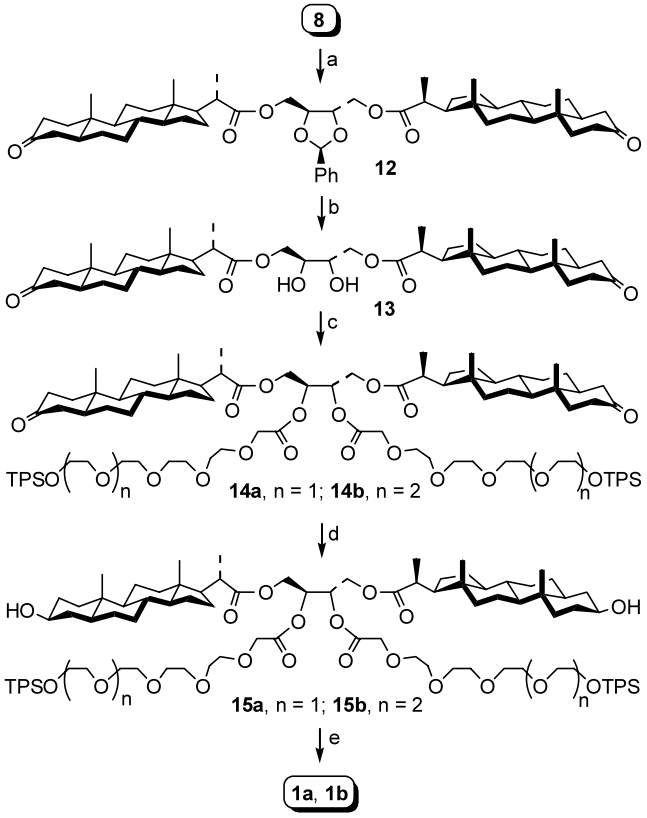
The synthesis of the two polar oligo(ethylene glycol) moieties started from penta- and hexa(ethylene glycols) **9a** and **9b**. They were easily transformed in the monoprotected carboxyl derivatives **11a** and **11b**, according the two-step synthetic sequence reported in Scheme 2.

Assembling of bisnorcholanic acid derivative **8** and monoprotected derivatives **11a**–**11b**, with the commercially available C-2,3 *bis*-protected (–)-2,3-*O*-benzylidene-L-treitol, by two subsequent double condensations, made use of the 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) hydrochloride.[17]

The first double esterification reaction between the protected treitol and the steroid **8**, gave the non-symmetric adduct **12** (Scheme 3).

C_2-symmetry was established by removal of the benzylidene protecting group, through Pd-mediated reductive debenzylation. **13** was thus formed in quantitative yield. A second EDC-mediated double condensation of the free C-2,3 treitol hydroxy groups with the oligo-(ethylene glycol) acid derivatives **11a** and **11b**, gave the bis-silyl protected adducts **14a** and **14b**. BH_3 induced[18] stereoselective reduction of the C-3 carbonyl, gave the β-alcohols **15a**,**b** in good yields. Primary 'head-group' desilylation with HF/pyridine[19] furnished target compounds **1a**[20] and **1b**.[21]

The ionophoric properties of the target compounds were investigated using a $^{23}Na^+$ NMR based assay.[7] In brief, a solution of NaCl (75 mM) plus a membrane-impermeable paramagnetic shift reagent ($DyCl_3$–tripolyphosphate complex, 4.0 mM) were added to a 95:5 egg phosphatidylcholine (PC) and egg phosphatidylglycerol (PG) dispersion (100 nm diameter, large unilamellar vesicles) prepared in aqueous LiCl (100 mM). Compounds **1a** and **1b** were incorporated in the lipid mixture before the formation of vesicles which were then prepared by extrusion through polycarbonate filters with a 100 nm pore diameter. Because the shift reagent is confined in the external bulk aqueous phase, the Na^+ entering the vesicular compartment appears as a separate (unshifted) resonance and integration of internal Na^+ signal, as a function of time, yields the kinetic profiles shown in Figure 2.



Scheme 3. *Reagents and conditions*: (a) 3.0 equiv. of DMAP, 1.0 equiv. of (−)-2,3-*O*-benzyliden-L-threitol, 3.0 equiv. of EDC, CH_2Cl_2, 48 h, 55%; (b) H_2, Pd/C, AcOEt, rt overnight, quant; (c) 10 equiv. of DMAP, 9 equiv. of EDC, 2 equiv. of **11a** or **11b**, CH_2Cl_2, 48 h, 72%; (d) 2 equiv. of $BH_3{\cdot}SMe_2$, THF, 1 h, 70%; (e) HF, Py, 2 h, 70%.

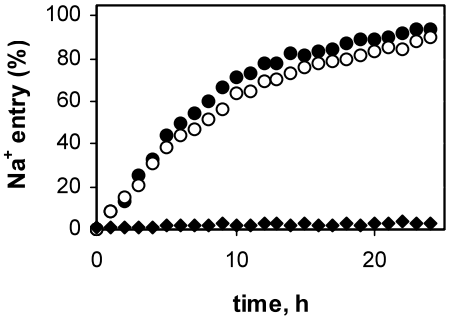


Figure 2. Kinetic profiles for the entry of Na^+ into 95:5 egg PC/PG vesicles containing **1a** (1.0%, ●), **1b** (1%, ○), and without additives (◆) at 25°C. The concentration of steroid derivative is given in percent with respect to the total concentration of lipid. The total concentration of lipids was 10 mM.

Inspection of Figure 2 shows that compounds **1a** and **1b** behave as powerful ionophores with very similar activity. Fitting of the data with a first order rate equation gives the apparent rate constants (k_{obs}, h^{-1}) for the Na^+ entry process which are 0.096 h^{-1} and 0.093 h^{-1} for **1a** and **1b**, respectively. Thus, the different length of the oligo(ethylene glycol) moieties is not relevant in determining the ionophoric activity. In the case of compound **1a** we have also investigated the activity/concentration dependence. The kinetic profile is shown in Figure 3.

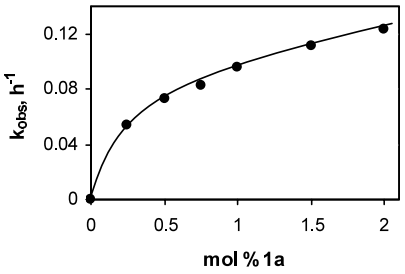


Figure 3. Plot of k_{obs} for Na^+ permeation as a function of mol% of **1a**.

Increasing the concentration of **1a** the rate of Na^+ entry increases showing a saturation behavior which can probably be attributed to a limited solubility of the ionophore in the lipid bilayer.[22] In any case, the observed activities are comparable to those of a previously reported polyhydroxysteroid derivative[7] and, more importantly, also to that of the natural occurring ionophore amphotericin B, which is often taken as the reference compound.[23]

In conclusion the present communication reports on a convenient approach towards the enantiospecific synthesis of C_2-symmetric sterol–oligo(ethylene glycol) derivatives with high ionophoric activity. These results may be considered as the starting point for a new class of easily accessible sterol-polyether-conjugate dimers with promising properties of altering the permeability of biological membranes and potentially useful as antibacterial drugs.

Acknowledgements

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20. **1a**: $[\alpha]_D$ −2.2 (*c* 0.7, CH_3OH); ^1H NMR (400.13 MHz, $CDCl_3$) δ 0.66 (6H, s, CH_3-18), 0.80 (6H, s, CH_3-19), 1.16 (6H, d, $J=6.8$ Hz CH_3-21), 2.40 (2H, m, H-20), 3.57–3.72 (34 H, m, -OCH_2CH_2O-, -OCH_2CH_2OH, H-3 overlapped), 4.04 (2H, dd, $J=11.7$, 3.8 Hz, -$CO_2CHHCHOR$), 4.13 (2H, d, $J=16.7$ Hz, -CO_2CHHO-), 4.18 (2H, d, $J=16.7$ Hz, -CO_2CHHO-), 4.36 (2H, bd, $J=11.7$ Hz, -$CO_2CHHCHOR$), 5.36 (2H, bs, -CO_2CH_2CHOR); ^{13}C NMR (100.06 MHz, $CDCl_3$), δ 12.2 (×2), 17.0, 21.1, 24.3, 27.3, 28.6, 31.4, 31.9, 35.4 (×2), 36.9, 38.1, 39.7, 42.5, 42.7, 44.7, 52.7, 54.2, 56.0, 61.2, 61.7, 68.2, 69.7, 70.2, 70.5 (×4), 70.9, 71.2, 72.5, 169.4, 176.1; ESMS *m/z* 1251 [MH^+]; calcd for $C_{68}H_{114}O_{20}$: C, 65.25; H, 9.18; Found: C, 65.24; H, 9.17.
21. **1b**: $[\alpha]_D$ +1.3 (*c* 1.0, $CHCl_3$); ^1H NMR (400.13 MHz, $CDCl_3$) δ 0.66 (6H, s, CH_3-18), 0.80 (6H, s, CH_3-19), 1.18 (6H, d, $J=6.8$ Hz CH_3-21), 2.40 (2H, m, H-20), 3.57–3.75 (42H, m, -OCH_2CH_2O-, -OCH_2CH_2OH, H-3 overlapped), 4.04 (2H, dd, $J=11.7$, 3.8 Hz, -$CO_2CHHCHOR$), 4.13 (2H, d, $J=16.7$ Hz, -CO_2CHHO-), 4.18 (2H, d, $J=16.7$ Hz, -CO_2CHHO-), 4.34 (2H, bd, $J=11.7$ Hz, -$CO_2CHHCHOR$), 5.36 (2H, bs, -CO_2CH_2CHOR); ^{13}C NMR (100.06 MHz, $CDCl_3$), δ 12.2 (×2), 16.9, 21.1, 24.2, 27.2, 28.5, 31.4, 31.9, 35.4 (×2), 36.9, 38.0, 39.7, 42.5, 42.6, 44.7, 52.6, 54.2, 55.9, 61.2, 61.6, 68.1, 69.6, 70.1, 70.4 (×4), 70.5 (×2), 70.9, 71.2, 72.5, 169.4, 176.0; ESMS *m/z* 1339 [MH^+]; calcd for $C_{72}H_{122}O_{22}$: C, 64.55; H. 9.18; Found: C, 64.60; H, 9.20.
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