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# Steroid-based head-to-tail amphiphiles as effective iono- and protonophores

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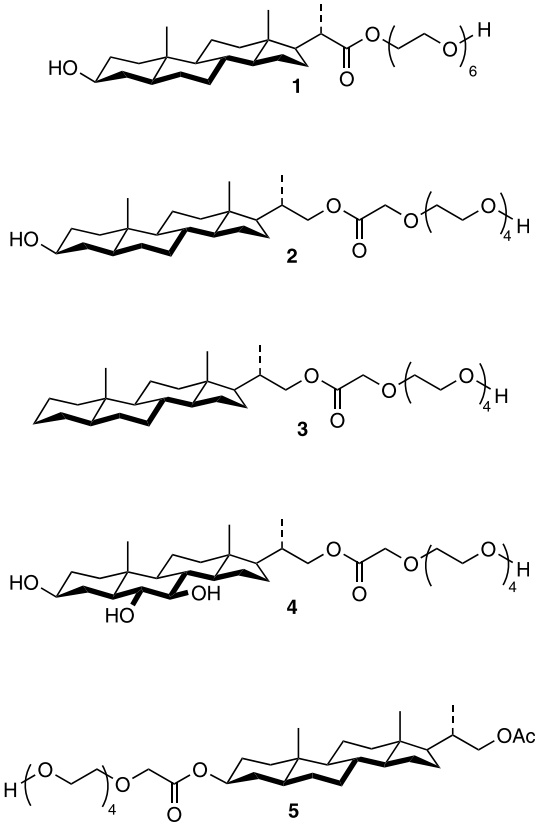
**Abstract**—The synthesis of five steroid-oligo(ethyleneglycol) conjugates (**1**–**5**) has been accomplished starting from commercially available *epi*-androsterone (**8**) and known 3β-[(*tert*-butyldiphenylsilyl)oxy]-5α-23,24-bisnorchol-16-en-6α,7β,22-triol (**27**). The synthetic strategy was based on a convergent approach including stereoselective C-17 side chains construction and standard coupling reactions. The activities of the head-to-tail amphiphiles, once incorporated in 95:5 egg PC/PG vesicular membranes, have been assessed by direct determination of transported species by NMR techniques ($^{23}Na^+$) and fluorescence spectroscopy ($H^+$). The sodium and proton transmembrane transport was compared to those evaluated for the polyene macrolide antibiotic amphotericin B and those shown by the known related $C_2$-symmetric sterol-polyether conjugates **6** and **7**.


## 1. Introduction

A large number of organisms control microbial growth through the biosynthesis of membrane-lytic compounds. Steroidal alkaloids, such as squalamine,[1] polyketides, such as amphotericin B,[2] and helical peptides, such as gramicidin,[3] are examples of secondary metabolites whose bactericidal and fungicidal activity is based on their transmembrane ion channel/pore formation.

Despite intense multidisciplinary efforts, the structural requirements for membrane permeabilization are still uncertain.[4] Evidence in the literature shows that the separation between the polar and non-polar domains along the major axis of the molecule (facially amphiphilic morphology)[5] is crucial for ion transport.[6]

In this paper, we wish to report the design, synthesis and the iono- and protonophoric properties of the structurally simple head-to-tail steroid-oligo(ethyleneglycol) conjugate amphiphiles **1**–**5** and the comparison of their activities with those exerted by the antibiotic amphotericin B and the related, known, $C_2$-symmetric **6** and **7**.[5d]

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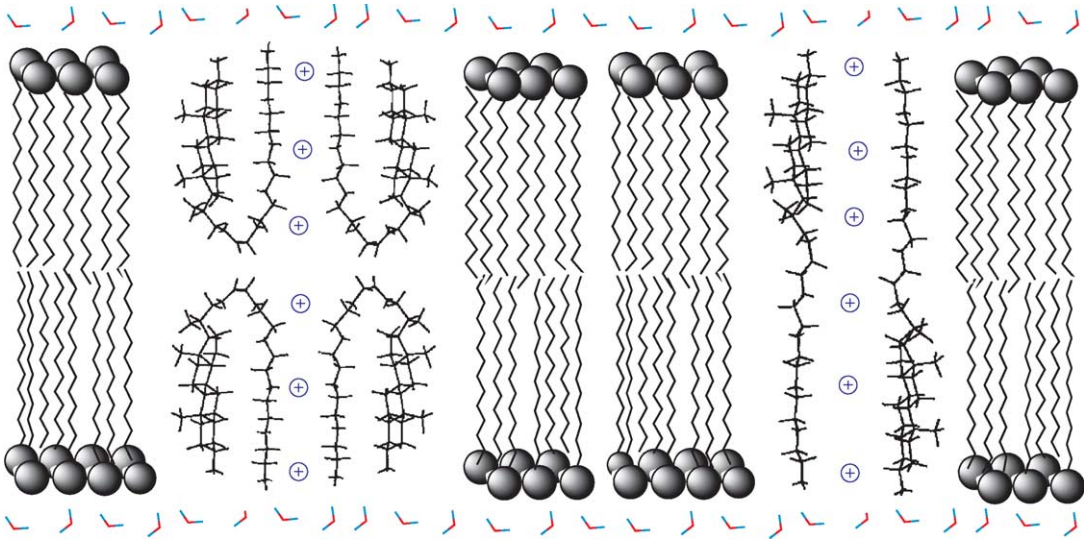
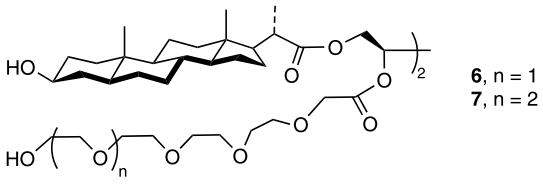


**Figure 1.** On the left, **2**, in the 'folded' conformation, constitutes a barrel-rosette[6] self-assembly and two half-channels aggregate in order to produce a contiguous pore across the bilayer.[9] On the right, a structurally simpler supramolecular alternate barrel-stave[6] assembly architecture is formed when **2** is in the 'extended' conformation.[10]




Conjugates **1**–**5** were designed considering that special cases of head-to-tail amphiphilicity[7] could induce the formation of membrane-active clusters similar to those shown by facially amphiphilic molecules, as shown in Figure 1.[8]

## 2. Results and discussion

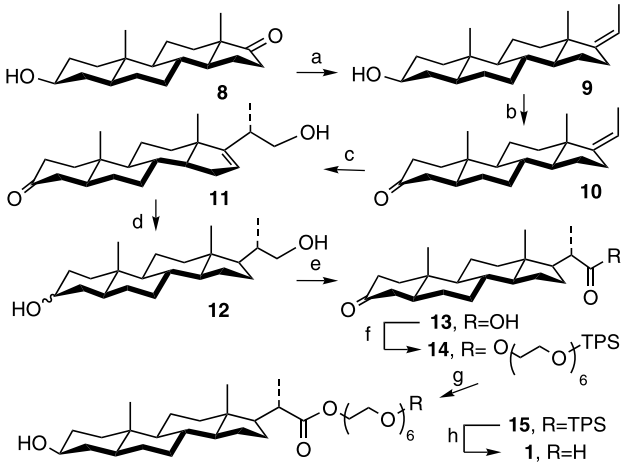
### 2.1. Synthesis and $Na^+$-transporting activities of 1 and 2

The synthesis of **1**, **2**, and that of the penta- and hexa(ethyleneglycol) side chains, is depicted in Schemes 1–3 and, in part, follows the procedure previously communicated for the construction of the $C_2$-symmetric sterol-polyether conjugates **6** and **7**.[5d]

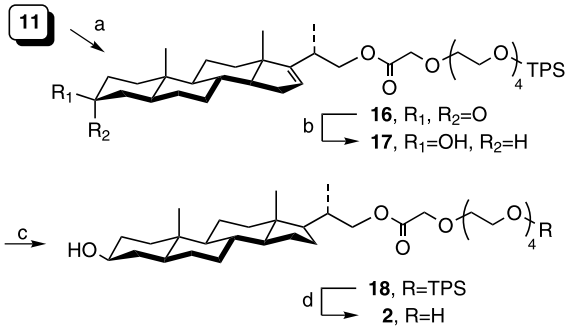
Amphiphile **1** was assembled using, as a key intermediate, the 3-oxo-5α-23,24-bisnorcholanic acid (**13**). This was obtained, in five steps and 43% overall yield, from commercially available *epi*-androsterone (**8**) and coupled with the mono-protected hexa(ethyleneglycol) **21** (see Scheme 3), in order to yield adduct **14**. Stereoselective $BH_3 \cdot SMe_2$-mediated C-3 carbonyl reduction and final deprotection with HF/pyridine, afforded target **1** in 7% overall yield (eight steps) from **8**.

The construction of the C-22 alcohol conjugate **2**, prototype of the latter reported amphiphiles **3**–**5**, proceeded through a shorter and higher yielding synthetic route.

Scheme 2 reports its elaboration, starting from 5α-23,24-

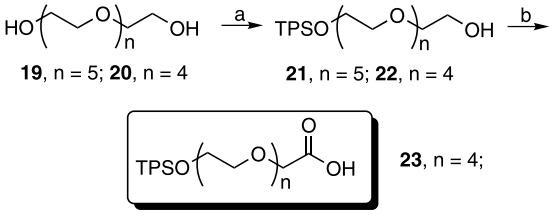



**Scheme 1.** Reagents and conditions: (a) $CH_3CH_2PPh_3Br$, *t*BuOK, THF, reflux, 78%; (b) PDC, $CH_2Cl_2$, 86%; (c) paraformaldehyde, $BF_3 \cdot OEt_2$, 0 °C, 98%; (d) $H_2$, $PtO_2$, EtOH, AcOEt; (e) Jones reagent, acetone/$CH_2Cl_2$, 65% for two steps; (f) **21**, EDC, DMAP, $CH_2Cl_2$, 35%; (g) $BH_3 \cdot SMe_2$, THF, 0 °C, 65%; (h) HF, Py, 0 °C, 73%.




**Scheme 2.** Reagents and conditions: (a) **23**, EDC, DMAP, $CH_2Cl_2$, 58%; (b) $BH_3 \cdot SMe_2$, THF, 0 °C, 97%; (c) $H_2$, Pt/C, EtOH; (d) HF, Py, 0 °C, 62% for two steps.

bisnorchol-16-en-22-ol-3-one (**11**), including the coupling with the acid **23** (see Scheme 3) and the final HF-induced desilylation. The desired amphiphile **2** was thus synthesized in 23% overall yield (seven steps), starting from *epi*-androsterone (**8**).




**Scheme 3.** Reagents and conditions: (a) TPSCl, DBU, $CH_2Cl_2$, 37% (for $n=5$), 39% (for $n=4$); (b) Jones reagent, acetone, 43%.

The synthesis of the two polar oligo(ethyleneglycol) heads, the previously cited **21** and **23**, started from hexa- and penta(ethyleneglycols) (**19** and **20**, respectively) and proceeded according to Scheme 3.

The ionophoric properties of **1** and **2** were investigated using a $^{23}Na^+$ NMR based assay.[11] The experimental kinetic profiles, compared with those previously reported for **6**, are shown in Figure 2.

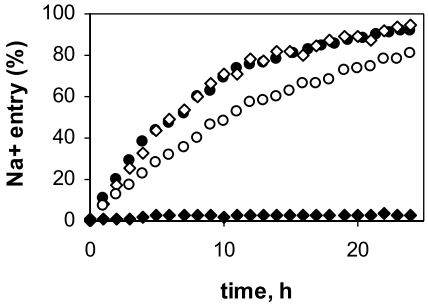



**Figure 2.** Kinetic profiles for the entry of $Na^+$ into 95:5 egg PC/PG vesicles contaning **1** (1.0%, ●), **2** (1.0%, ○), **6** (1.0%, ◇), and without additives (♦) at 25 °C. The concentration of steroid derivative is given in percent with respect to the total concentration of lipid. The total concentration of lipids was 10 mM.

Inspection of Figure 2 shows that compounds **1** and **2** behave as powerful ionophores. Surprisingly, they have a very similar activity to that found for the related, $C_2$-symmetric, **6**. Fitting of the data to a first order rate equation gives the apparent rate constants ($k_{obsd}$, $h^{-1}$) for the $Na^+$ entry process, which are 0.099 and 0.073 $h^{-1}$ for **1** and **2**, and 0.096 $h^{-1}$ for **6**, respectively. This means that, the preorganization in a dimeric structure (via covalent bond, as in **6**) seems unnecessary for the $Na^+$-transporting activity. In any case, the activities of the steroid derivatives compare well with that of the naturally occurring ionophore amphotericin B ($k_{obsd}=0.16\ h^{-1}$)[5b] underling the efficacy of these artificial ionophores.

## 2.2. Synthesis of 3–5 and $H^+$-transporting activities of 1–5

In recent years there has been intense research aimed at discovering new proton conductors.[12] The conversion of acquired energy (due to electron transfer or light harvesting) into a proton gradient, provides the energy for ATP synthesis and it is of fundamental importance for organisms from bacteria to man.[13] Most of the proton channels conduct $H^+$ ions by a hydrogen-bonded chain mechanism in which the proton hops from one molecule of water to the next (Grotthuss' mechanism or 'prototropic' transfer).[14] The whole process explains why proton permeability is much higher than that of other cations[15] and provides a tool to better understand the structural features of the membrane pores.

On the basis of these considerations, we decided to study the proton conductivities of **1** and **2** and compare them with those from **6** and **7** as shown in Figure 3.[16]

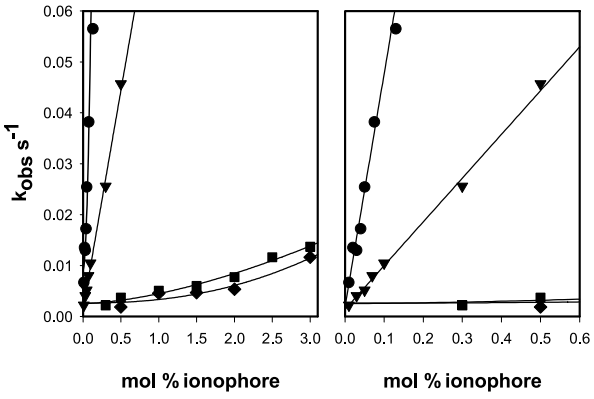



**Figure 3.** Plot of $k_{obsd}$ as a function of mol% of **1** (■), **2** (♦), **6** (▼) and **7** (●) for the $H^+$-transport. The two panels are the same graph with different *X*-axis.

This time the variation of the $H^+$-transport, in relation to the structure of the conjugate, is striking. Amphiphiles **1** and **2** show a similar activity, comparable with that of amphotericin B.[17] On the other hand, the $C_2$-symmetric sterol-polyether conjugates **6** and **7** show much higher activities. Interestingly, the shape of the kinetic profiles is different, being linear in the case of dimeric compounds **6** and **7** and showing an upward curvature in the case of the shorter analogs **1** and **2**, suggesting a different mechanism of action. Taking into account the length of the two molecular systems it seems likely that **6** and **7** act as a single molecule in stabilizing the continuous transmembrane row of molecules of water thus limiting its fluctuation and favoring the $H^+$-transport. On the contrary, in the case of **1** and **2** it seems that a less stable supramolecular assembly is formed, having a negative impact on the proton transport. These types of non-linear kinetic profiles are usually fitted with the Hill equation in order to determine the Hill coefficient *n*, indicative for the number of monomers needed to form an active supramolecular pore.[18] In the case of **1** and **2** we obtained *n* values close to 2, indicating that two monomers assemble in the membrane to form the active transmembrane species probably following a barrel-rosette or a barrel-stave model[6] (Fig. 4A and B). In any case, it is evident that this supramolecular pore is less stable with respect to the unimolecular one formed by the dimeric steroid derivatives and, as a consequence, the activity is remarkably lower.

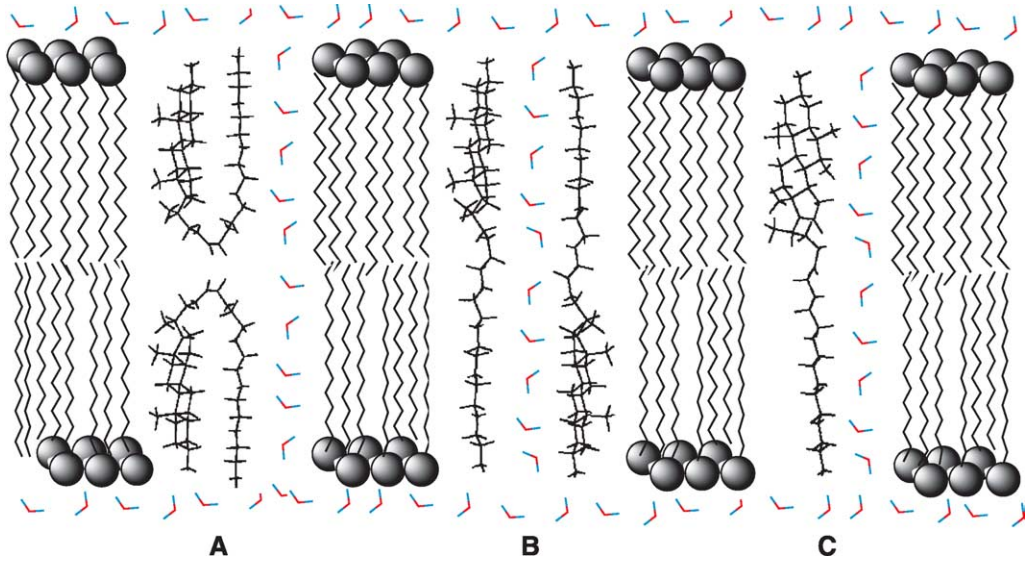
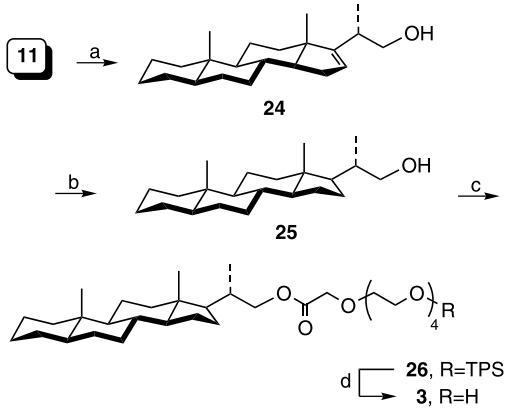



**Figure 4.** Proposed structure for the proton conducting pore formed by the different steroid derivatives.

In this context, we decided to vary the structure of our head-to-tail amphiphiles in order to stabilize the pore aggregate and, consequently, we designed the new derivatives **3**–**5**. In particular, compounds **3** and **4**, showing a different number of the hydroxyl groups on the tetracyclic nucleus, were conceived on the basis of theoretical studies correlating the polarity of the channel with the efficiency of proton transport.[19]

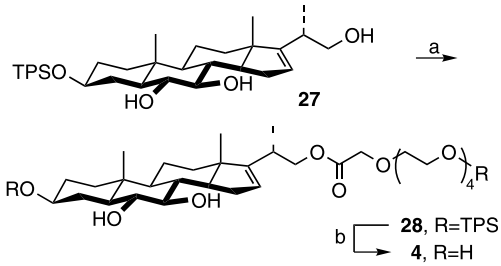
Compound **5**, in which the polar side chain was switched from C-22 to C-3, was designed in order to evaluate the effect of the oligo(ethyleneglycol) attachment (from the D to the A ring) on the proton transport.[20]

The synthesis of **3**, reported in Scheme 4, started with a C-3 Wolff-Kishner deoxygenation of the 5α-23,24-bisnorchol-16-en-22-ol-3-one (**11**). Its stereoselective hydrogenation, a coupling with acid **23** and desilylation, gave the expected target in 9% yield.



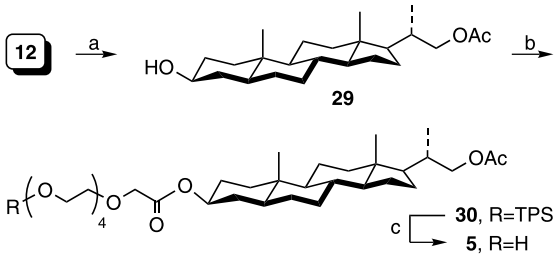

**Scheme 4.** Reagents and conditions: (a) $NH_2NH_2 \cdot H_2O$, KOH, $HOCH_2CH_2OH$, EtOH, 62%; (b) $H_2$, Pt/C, EtOH, 97%; (c) **23**, EDC, DMAP, $CH_2Cl_2$, 34%; (d) HF, Py, 0 °C, 44%.

The synthesis of **4** started from the known[5c] 3β-[(*tert*-butyldiphenylsilyl)oxy]-5α-23,24-bisnorchol-16-en-6α,7β,22-triol (**27**, Scheme 5). This was regioselectively acylated at C-22 with 1.1 equiv of **23**, to give conjugate **28**. Its desilylation, with HF/pyridine, afforded **4** in 16% overall yield (from **27**).




**Scheme 5.** Reagents and conditions: (a) **23**, EDC, DMAP, $CH_2Cl_2$, 31%; (b) HF, Py, 0 °C, 51%.

Compound **5** was synthesized in three steps and 34% overall yield, starting from the C-3 epimeric mixture **12**, according to Scheme 6. Regioselective acetylation on primary C-22 and subsequent silica gel purification, afforded 22-acetoxy-5α-23,24-bisnorcholan-6β-ol (**29**). The free hydroxyl at C-3 was coupled with the protected penta(ethyleneglycol) derivative **23** to yield conjugate **30**. Standard deprotection from the *tert*-butyldiphenylsilyl group afforded **5**.




**Scheme 6.** Reagents and conditions: (a) $Ac_2O$, Py, $CH_2Cl_2$, 50%; (b) **23**, EDC, DMAP, $CH_2Cl_2$, 82%; (c) HF, Py, 0 °C, 82%.

The protonophoric properties of the head-to-tail amphiphiles **3**–**5** and, for comparison, of steroid **1** are reported in Figure 5.

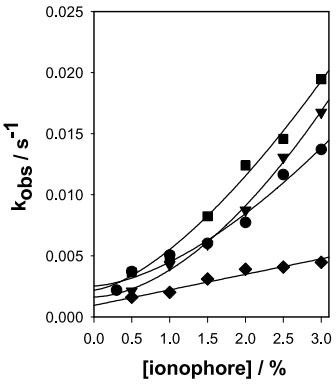



**Figure 5.** Plot of $k_{obsd}$ as a function of mol% of **1** (●), **3** (▼), **4** (♦), and **5** (■) for the $H^+$-transport.

Steroids **3** and **5** behave very similarly to **1**. Again we observe an upward curvature of the kinetic profiles and the fitting of the curves with the Hill equation gives *n* values close to 2. Therefore, these two amphiphiles seem to act in a way similar to **1** forming small supramolecular assemblies, which perturb the membrane permeability and the system is little sensitive to the structural variations. On the other side, compound **4** is less active, probably because of the higher hydrophilicity, but shows a linear dependence of the transport rate from the ionophore concentration suggesting the formation of a unimolecular pore. Due to the presence of the hydroxyl groups on the steroid nucleus, in the extended conformation, steroid **4** is able to span the membrane forming a continuous polar surface, which may interact with the transmembrane row of water molecules promoting the proton transport (Fig. 4C). As a consequence, it acts as a single molecule in a way similar to ionophores **6** and **7**. If this hypothesis is correct then we may speculate that a similar mode of insertion in the membrane should be valid also for the other monomeric steroid derivatives and, therefore, that reported in Figure 4B should be preferred to that of Figure 4A. However, further studies are necessary to confirm such a hypothesis.

## 3. Conclusions

The synthesis of five new head-to-tail steroid-oligo(ethyleneglycol) conjugates **1**–**5** has been accomplished from readily available starting materials. These amphiphiles, once incorporated in a 95:5 egg PC/PG vesicular membranes, showed ionophoric activities ($Na^+$ and $H^+$ transfer) comparable with those reported for the channel-forming antibiotic amphotericin B. Head-to-tail amphiphiles **1**–**5** represent the simplest steroid-based cation-conductors and establish a new class of prototypes for membrane permeabilization. Moreover, these studies have shown the importance of the molecular structure on the proton-transport ability of the steroid derivatives with the dimeric ionophores **6** and **7** being much more active than the monomeric analogs.

## 4. Experimental

### 4.1. General methods

All reactions were carried out under a dry argon atmosphere using freshly distilled and dried solvents, unless otherwise noted. Tetrahydrofuran (THF) was distilled from $LiAlH_4$. Toluene, methylene chloride, and diethyl ether were distilled from calcium hydride. Glassware was flame-dried (0.05 Torr) prior to use. When necessary, compounds were dried in vacuo over $P_2O_5$ or by azeotropic removal of water with toluene under reduced pressure. Starting materials and reagents purchased from commercial suppliers were generally used without purification. Reaction temperatures were measured externally; reactions were monitored by TLC on Merck silica gel plates (0.25 mm) and visualized by UV light and spraying with $H_2SO_4$–$Ce(SO_4)_2$, *p*-anisaldeyde-EtOH–$H_2SO_4$–AcOH solutions and drying. Flash cromatography was performed on Merck silica gel (60, particle size: 0.040–0.063 mm). Yields refer to chromatographically and spectroscopically ($^1H$ and $^{13}C$ NMR) pure materials. The NMR spectra were recorded at rt on a Bruker DRX 400 spectrometer ($^1H$ at 400 MHz, $^{13}C$ at 100 MHz) or on Bruker DRX 300 spectrometer ($^1H$ at 300 MHz, $^{13}C$ at 75 MHz). Chemical shifts are reported relative to the residual solvent peak ($CHCl_3$: $\delta=7.26$, $^{13}CDCl_3$: $\delta=77.0$). HR ESMS were performed on a Q-Star Applied Biosystem mass spectrometer. Optical rotations were measured with a JASCO DIP-1000 polarimeter.

### 4.2. Procedures for the synthesis of compounds described in Scheme 1

**4.2.1. Compound 9.** To a solution of ethyltriphenylphosphonium bromide (19.1 g, 51.6 mmol) in dry THF (50 ml), *t*BuOK (5.21 g, 46.5 mmol) was added. The resulting mixture was stirred at rt for 10 min, then a solution of *epi*-androsterone (5.00 g, 17.2 mmol) in dry THF (10 ml) was added. The reaction mixture was refluxed for 3 h, cooled to rt, quenched with water, concentrated under reduced pressure to remove the excess of THF and the aqueous layer was extracted with diethyl ether (3×20 ml). The combined organic phases were dried over $Na_2SO_4$, filtered and concentrated in vacuo to give a crude product, which was purified by flash chromatography (40–70% diethyl ether in petroleum ether) to afford **9** (4.1 g, 78%) as a white amorphous solid.

*Compound* **9**. $R_f=0.07$ (10% diethyl ether in petroleum ether). $[\alpha]_D$ +17.6 (*c* 2.0, $CHCl_3$).$^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$: 0.80 (3H, s, C*H*$_3$-18), 0.85 (3H, s, C*H*$_3$-19), 1.66 (3H, d, $J=7.1$ Hz, C*H*$_3$-21), 3.58 (1H, m, H-3), 5.09 (1H, q, $J=7.0$ Hz, H-20). $^{13}C$ NMR ($CDCl_3$, 400 MHz) $\delta$: 12.2, 13.0, 16.8, 21.34, 24.3, 28.6, 31.4, 31.8 (×2), 55.0, 35.4, 36.9 (×2), 37.1, 38.1, 44.7, 54.3, 56.1, 71.1, 113.1, 150.3. HRES-MS, *m*/*z*: 303.2643 (calcd 303.2688 for $C_{21}H_{35}O$) [$MH^+$].

**4.2.2. Compound 10.** To a solution of **9** (4.05 g, 1.34 mmol) in dry $CH_2Cl_2$ (200 ml) at rt, molecular sieves (powered, 4 Å, 7.5 g) and pyridinium dichromate (PDC, 7.66 g, 20.2 mmol) were added. The resulting suspension was stirred for 3 h, quenched with diethyl ether, filtered through

a pad of silica gel–$CaSO_4$ (w/w: 90/10) and concentrated in vacuo to give the crude product, which was purified by flash chromatography (0–30% ethyl acetate in petroleum ether) to afford **10** (3.45 g, 86%) as a white amorphous solid.

*Compound* **10**. $R_f$=0.8 (20% ethyl acetate in petroleum ether). $[\alpha]_D$ +45.8 (*c* 2.5, $CHCl_3$).$^1$H NMR ($CDCl_3$, 300 MHz) $\delta$: 0.84 (3H, s, C*H*$_3$-18), 0.97 (3H, s, C*H*$_3$-19), 1.59 (3H, d, *J*=7.2 Hz, C*H*$_3$-21), 5.06 (1H, q, *J*=7.0 Hz, H-20). $^{13}$C NMR ($CDCl_3$, 400 MHz) $\delta$: 11.2, 13.0, 16.7, 21.5, 24.2, 28.8, 31.2, 31.4, 34.8, 35.5, 36.9, 38.0, 38.3 (× 2), 44.5, 46.4, 53.7, 55.8, 113.3, 149.8, 211.4. HRES-MS, *m/z*: 301.2571 (calcd 301.2531 for $C_{21}H_{33}O$) [$MH^+$].

**4.2.3. Compound 11.** To a solution of **10** (1.69 g, 5.63 mmol) in dry $CH_2Cl_2$ (170 ml) at 0 °C, paraformaldehyde (0.93 g, 60.5 mmol) and $BF_3\cdot OEt_2$ (0.80 g, 0.56 mmol), were added. The resulting mixture was stirred at rt for 10 min, then quenched with water (30 ml), extracted with $CH_2Cl_2$ (3×30 ml), dried on $Na_2SO_4$ and concentrated in vacuo to give the crude product, which was purified by flash chromatography (40–70% diethyl ether in petroleum ether) to afford **11** (1.82 g, 98%) as a white amorphous solid.

*Compound* **11**. $R_f$=0.40 (30% ethyl acetate in petroleum ether). $[\alpha]_D$ +21.4 (*c* 1.9, $CHCl_3$). $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$: 0.77 (3H, s, C*H*$_3$-18), 0.99 (3H, d, *J*=7.1 Hz, C*H*$_3$-21), 1.00 (3H, s, C*H*$_3$-19), 3.49 (1H, dd, *J*=10.4, 6.3 Hz, H-22), 3.60 (1H, dd, *J*=10.4, 7.7 Hz, H-22′), 5.37 (1H, br s, H-16). $^{13}$C NMR ($CDCl_3$, 400 MHz) $\delta$: 12.0, 16.2, 18.8, 21.9, 24.5, 31.7, 32.2, 34.7, 35.4, 35.9, 36.7, 37.9, 38.7, 39.0, 44.6, 47.5, 55.0, 57.6, 67.1, 123.4, 157.5, 201.3. HRES-MS, *m/z*: 331.2682 (calcd 331.2637 for $C_{22}H_{35}O_2$) [$MH^+$].

**4.2.4. Compound 13.** To a solution of **11** (0.450 g, 1.36 mmol) in absolute ethanol (20 ml) and ethyl acetate (1 ml), palladium(II) oxide (0.025 g) was added. The flask was evacuated (20 Torr) and flushed with hydrogen three times. The reaction mixture was vigorously stirred under an atmosphere of hydrogen for 14 h, filtered and concentrated under reduced pressure to give the C-3 epimeric mixture **12** (0.340 g) as a white amorphous solid, which was used in the next step without further purification.

To a solution of crude **12** (0.340 g, 1.02 mmol) in acetone (22 ml) and $CH_2Cl_2$ (2 ml) at rt, Jones reagent (1.0 ml) was added dropwise. The reaction mixture was stirred at rt for 2 h, then quenched with water (5 ml), concentrated under reduced pressure to remove the excess of acetone and $CH_2Cl_2$, and the aqueous layer extracted with ethyl acetate (3×10 ml). The organic layer was dried over $Na_2SO_4$/$NaHCO_3$ and concentrated in vacuo to give **13** (0.305 g, 65%, two steps from **11**) as a white amorphous solid, which was used in the next step without further purification.

*Compound* **13**. $R_f$=0.45 (5% methanol in $CH_2Cl_2$). $[\alpha]_D$ +14.9 (*c* 1.0, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.69 (3H, s, C*H*$_3$-18), 1.00 (3H, s, C*H*$_3$-19), 1.23 (3H, d, *J*=7.0 Hz, C*H*$_3$-21), 10.0 (1H, br s, COO*H*). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 11.4, 12.2, 16.9, 21.3, 24.2, 27.2, 28.8, 31.5, 35.3, 35.6, 38.0, 38.4, 39.5, 42,3, 42.6, 44.6, 46.5, 52.4, 53.6, 55.8, 181.1, 212.2. HRES-MS, *m/z*: 347.2601 (calcd 347.2586 for $C_{22}H_{35}O_3$) [$MH^+$].

**4.2.5. Compound 14.** To a solution of crude **13** (0.29 g, 0.84 mmol) in $CH_2Cl_2$ (1 ml) at rt, DMAP (0.31 g, 2.54 mmol), a solution of **21** (0.49 g, 0.94 mmol) in $CH_2Cl_2$ (2 ml) and EDC (0.81 g, 4.26 mmol) were sequentially added. The reaction mixture was stirred for 16 h, quenched with water (5 ml) and extracted with ethyl acetate (10 ml). The organic layer was washed with a saturated solution of $NaHCO_3$, with water, dried over $Na_2SO_4$, filtered and concentrated in vacuo. The crude (0.86 g) was purified by flash chromatography (0–1% methanol in chloroform), to furnish **14** (0.25 g, 35%) as a white amorphous solid.

*Compound* **14**. $R_f$=0.81 (10% methanol in $CHCl_3$). $[\alpha]_D$ +8.6 (*c* 2.1, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.64 (3H, s, C*H*$_3$-18), 0.97 (3H, s, C*H*$_3$-19), 1.03 (9H, s, C(C*H*$_3$)$_3$), 1.17 (3H, d, *J*=7.0 Hz, C*H*$_3$-21), 3.62 (20H, m, O–(C*H*$_2$C*H*$_2$O)$_5$), 3.79 (2H, t, *J*=5.3 Hz, C*H*$_2$OTPS), 4.18 (2H, br t, *J*=4.8 Hz, C*H*$_2$OCOR), 7.40 (6H, m, Ar-*H*), 7.66 (4H, m, Ar-*H*).$^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 11.4, 12.2, 17.0, 18.8, 21.3, 24.1, 26.7 (×3), 27.0, 28.8, 31.5, 35.3, 35.6, 38.0, 38.4, 39.5, 42.4, 42.6, 44.6, 46.4, 52.8, 53.6, 55.7, 62.9, 63.3, 69.1, 70.5 (×8), 72.3, 127.5 (×4), 129.5 (×2), 133.6 (×2), 135.6 (×4), 176.7, 211.8. HRES-MS, *m/z*: 849.5311 (calcd 849.5337 for $C_{50}H_{77}O_9Si$) [$MH^+$].

**4.2.6. Compound 15.** To a solution of **14** (0.25 g, 0.29 mmol) in THF (5 ml) at 0 °C, $BH_3\cdot SMe_2$ (300 µl, 0.56 mmol) was added. The reaction mixture was stirred for 1.5 h at 0 °C, quenched with water (5 ml), concentrated in vacuo to remove the excess of THF and extracted with $CHCl_3$. The organic layer was washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo to give **15** (0.16 g, 65%) as a white amorphous solid, which was used without further purification.

*Compound* **15**. $R_f$=0.47 (10% methanol in $CH_2Cl_2$). $[\alpha]_D$ +2.3 (*c* 2.7, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.65 (3H, s, C*H*$_3$-18), 0.79 (3H, s, C*H*$_3$-19), 1.03 (9H, s, C(C*H*$_3$)$_3$), 1.17 (3H, d, *J*=7.0 Hz, C*H*$_3$-21), 2.42 (1H, m, *H*-20), 3.63 (21H, m, O–(C*H*$_2$C*H*$_2$O)$_5$– and *H*-3), 3.79 (2H, t, *J*=5.3 Hz, C*H*$_2$OTPS), 4.19 (2H, br t, *J*=4.7 Hz, C*H*$_2$OCOR), 7.38 (6H, m, Ar-*H*), 7.67 (4H, m, Ar-*H*). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 12.2 (×2), 17.0, 19.1, 21.1, 24.2, 26.8 (×3), 27.1, 28.6, 31.4, 32.0, 35.4 (×2), 36.9, 38.1, 39.7, 42.5, 42.6, 44.7, 52.8, 54.2, 55.9, 63.0, 63.4, 69.2, 70.5 (×8), 71.2, 72.4, 127.6 (×4), 129.6 (×2), 133.6 (×2), 135.6 (×4), 176.9. HRES-MS, *m/z*: 851.5560 (calcd 851.5493 for $C_{50}H_{79}O_9Si$) [$MH^+$].

**4.2.7. Compound 1.** To a solution of **15** (0.16 g, 0.18 mmol) in pyridine (0.5 ml) at 0 °C, a solution of 70% hydrofluoric acid in pyridine (70 µl, 2.44 mmol) was added. The reaction mixture was stirred for 1.5 h and concentrated under a stream of $N_2$. The residue was purified by flash chromatography (silica gel, 3% methanol in $CHCl_3$) to afford **1** (0.080 g, 73%) as a white amorphous solid.

*Compound* **1**: $R_f$=0.4 (10% methanol in $CH_2Cl_2$). $[\alpha]_D$ +3.3 (*c* 2.7, $CHCl_3$). $^1$H NMR.(400 MHz, $CDCl_3$) $\delta$: 0.63

(3H, s, C$H_3$-18), 0.77 (3H, s, C$H_3$-19), 1.14 (3H, d, $J$= 6.7 Hz, C$H_3$-21), 2.40 (1H, m, *H*-20), 3.62 (23H, m, O–(C$H_2$C$H_2$O)$_5$–, C$H_2$OH and *H*-3), 4.17 (2H, t, $J$= 4.7 Hz, C$H_2$OCOR). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 12.3 (×2), 17.0, 21.0, 24.2, 27.0, 28.6, 31.4, 31.9, 35.4 (×2), 36.9, 38.0, 39.7, 42.4, 42.6, 44.7, 52.8, 54.2, 56.0, 61.6, 62.9, 69.1, 70.2, 70.5 (×7), 71.1, 72.4, 176.8. HRES-MS, *m/z*: 613.4321 (calcd 613.4316 for $C_{34}H_{61}O_9$) [$MH^+$].

### 4.3. Procedures for the synthesis of compounds described in Scheme 2

**4.3.1. Compound 16.** To a solution of **11** (0.10 g, 0.30 mmol) in $CH_2Cl_2$ (1 ml) at rt, DMAP (0.11 g, 0.91 mmol), a solution of **23** (0.22 g, 0.45 mmol) in $CH_2Cl_2$ (2 ml) and EDC (0.29 g, 1.51 mmol) were sequentially added. The mixture was stirred for 16 h, quenched with water (5 ml) and extracted with ethyl acetate (5 ml). The organic layer was washed with a saturated solution of $NaHCO_3$, then water, dried over $Na_2SO_4$, filtered and concentrated in vacuo. The crude (0.30 g) was purified by flash chromatography (20–90% diethyl ether in petroleum ether) to furnish **16** (0.14 g, 58%) as a white amorphous solid.

*Compound* **16**. $R_f$=0.2 (20% diethyl ether in petroleum ether). $[\alpha]_D$ +19.5 (*c* 0.6, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.73 (3H, s, C$H_3$-18), 1.05 (15H, br s, C$H_3$-19, $CH_3$-21, (C$H_3$)$_3$Si–, overlapped), 3.57–3.69 (14H, m, (OC$H_2$C$H_2$O)$_3$C$H_2$, overlapped), 3.79 (2H, $J$=5.3 Hz, C$H_2$OTPS), 4.01 (1H, m, H-22), 4.11 (2H, br s, OCOC$H_2$O), 4.19 (1H, m, H′-22), 5.38 (1H, br s, H-16), 7.34–7.40 (6H, m, Ar-*H*), 7.66–7.68 (4H, m, Ar-*H*). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 11.4, 16.2, 18.6, 19.1, 21.2, 26.8 (×3), 28.8, 29.6, 31.4, 31.5, 34.0, 34.7, 35.8, 38.1, 38.3, 44.7, 46.8, 47.2, 54.4, 56.7, 63.4, 68.6, 68.7, 70.5 (×4), 70.7, 70.9, 72.4, 122.8, 127.6 (×4), 129.5 (×2), 133.6 (×2), 135.6 (×4), 156.5, 170.5, 212.0. HRES-MS, *m/z*: 803.4992 (calcd 803.4918 for $C_{48}H_{71}O_8Si$) [$MH^+$].

**4.3.2. Compound 17.** To a solution of **16** (0.134 g, 0.167 mmol) in THF (3 ml) at 0 °C, $BH_3 \cdot SMe_2$ (230 µl, 0.56 mmol) was added. The reaction mixture was stirred for 1 h at 0 °C, the reaction was quenched with water (3 ml), concentrated in vacuo to remove the excess of THF and extracted with $CHCl_3$. The organic layer was washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo to give **17** (0.131 g, 97%) as a white amorphous solid, which was used without further purification.

*Compound* **17**. $R_f$=0.2 (20% petroleum ether in diethyl ether). $[\alpha]_D$ +0.2 (*c* 2.0, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.74 (3H, s, C$H_3$-18), 0.83 (3H, s, C$H_3$-19), 1.03–1.04 (12H, br s, (C$H_3$)$_3$Si– and C$H_3$-21, overlapped), 2.44 (1H, m, *H*-20), 3.57–3.69 (15H, m, (OC$H_2$C$H_2$O)$_3$C$H_2$ and H-3 overlapped), 3.79 (2H, $J$=5.3 Hz,, C$H_2$OTPS), 4.01 (1H, m, H-22), 4.12 (2H, br s, OCOC$H_2$O), 4.19 (1H, m, H′-22), 5.38 (1H, s, H-16), 7.34–7.40 (6H, m, Ar-*H*), 7.66–7.68 (4H, m, Ar-*H*). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 12.3, 16.3, 18.6, 19.2, 21.1, 26.8 (×3), 28.6, 29.7, 31.2, 31.5, 31.9, 34.1, 34.9, 35.7, 36.8, 38.2, 45.1, 47.3, 55.0, 56.9, 63.4, 68.6, 68.7, 70.6 (×4), 70.7, 70.9, 71.3, 72.4, 122.8, 127.6 (×4), 129.6 (×2), 133.7 (×2), 135.6 (×4), 156.7, 170.5. HRES-MS, *m/z*: 805.5012 (calcd 805.5075 for $C_{48}H_{73}O_8Si$) [$MH^+$].

**4.3.3. Compounds 18 and 2.** To a solution of crude **17** (0.135 g, 0.168 mmol) in absolute ethanol (2 ml), Pt/C (5% w/w, 0.016 g) was added. The flask was evacuated (20 Torr) and flushed with hydrogen three times. The reaction mixture was stirred vigorously under hydrogen for 24 h, filtered through a pad of Celite, the Celite was washed with chloroform and the solvent concentrated in vacuo to afford **18** (0.128 g) as a white amorphous solid, which was used in the next step without further purification.

To a solution of crude **18** (0.128 g, 0.159 mmol) in pyridine (0.5 ml) at 0 °C, a solution of 70% hydrofluoric acid in pyridine (60 µl, 2.09 mmol) was added. The reaction mixture was stirred for 1.5 h and concentrated under a stream of $N_2$. The residue was purified by flash chromatography (silica gel, 3% methanol in $CHCl_3$) to afford **2** (0.056 g, 62% for two steps) as a white amorphous solid.

*Compound* **2**. $R_f$=0.1 (diethyl ether). $[\alpha]_D$ +10.4 (*c* 0.7 in $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.66 (3H, s, C$H_3$-18), 0.79 (3H, s, C$H_3$-19), 0.98 (3H, d, $J$=6.6 Hz, C$H_3$-21), 3.60 (3H, m, C$H_2$OH and H-3, overlapped), 3.64–3.74 (14H, m, (OC$H_2$C$H_2$O)$_3$C$H_2$–), 3.83 (1H, m, H-22), 4.12 (2H, s, COC$H_2$O), 4.13 (1H, m, H′-22). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 12.1, 12.3, 17.1, 21.2, 24.2, 27.6, 28.6, 31.5, 32.0, 35.5 (×2), 35.8, 36.9, 38.1, 39.8, 42.7, 44.8, 52.7, 54.3, 56.1, 61.7, 68.6, 69.8, 70.3, 70.5 (×4), 70.8, 71.3, 72.5, 170.7. HRES-MS, *m/z*: 569.4031 (calcd 569.4053 for $C_{32}H_{57}O_8$) [$MH^+$].

### 4.4. Procedures for the synthesis of compounds described in Scheme 3

**4.4.1. Compound 21.** To a solution of **19** (1.00 g, 3.54 mmol) in $CH_2Cl_2$ (10 ml) at rt, 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU, 0.82 ml, 5.30 mmol) and *tert*-butyldiphenylsilylchloride (TPS-Cl, 0.92 ml, 3.54 mmol) were sequentially added. The solution was stirred for 3 h, quenched with a solution of HCl (2 M, 6 ml) and extracted with $CH_2Cl_2$. The organic layer was washed with a saturated solution of $NaHCO_3$ (4 ml), dried over $Na_2SO_4$, filtered and concentrated in vacuo. The crude was purified by flash chromatography (1–2% methanol in $CH_2Cl_2$) to furnish **21** (0.69 g, 37%) as a colorless oil.

*Compound* **21**. $R_f$=0.6 (4% methanol in $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 1.04 (9H, s, (C$H_3$)$_3$Si–), 3.56–3.63 (20H, m, –C$H_2$(OC$H_2$C$H_2$O)$_4$C$H_2$), 3.68 (2H, m, C$H_2$OH), 3.79 (2H, d, $J$=5.3 Hz, C$H_2$OTPS), 7.34–7.40 (6H, m, Ar-*H*), 7.66–7.68 (4H, m, Ar-*H*). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 19.4, 27.0 (×3), 62.0, 63.7, 70.5, 70.9 (×7), 72.7, 72.8, 127.9 (×4), 129.8 (×2), 133.5 (×2), 135.9 (×4). HRES-MS, *m/z*: 521.2890 (calcd 521.2935 for $C_{28}H_{45}O_7Si$) [$MH^+$].

**4.4.2. Compound 22.** To a solution of **20** (5.00 g, 20.9 mmol) in $CH_2Cl_2$ (50 ml) at rt, DBU (4.69 ml, 31.3 mmol) and TPS-Cl (5.30 ml, 20.9 mmol) were sequentially added. The solution was stirred for 3 h, quenched with a solution of HCl (2 M, 30 ml) and extracted with $CH_2Cl_2$.

The organic layer was washed with a saturated solution of $NaHCO_3$ (20 ml), dried over $Na_2SO_4$, filtered and concentrated in vacuo. The crude was purified by flash chromatography (1–2% methanol in $CH_2Cl_2$) to furnish **22** (3.88 g, 39%) as a colorless oil.

*Compound* **22**. $R_f$=0.6 (4% methanol in $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 1.04 (3H, s, $(CH_3)_3Si$–), 3.56–3.63 (16H, m, $-CH_2(OCH_2CH_2O)_3CH_2$), 3.68 (2H, m, $CH_2OH$), 3.79 (2H, d, $J$=5.3 Hz, $CH_2$OTPS), 7.34–7.40 (6H, m, Ar-*H*), 7.66–7.68 (4H, m, Ar-*H*). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 19.1, 26.7 (×3), 61.9, 63.6, 70.5, 70.8 (×4), 70.9, 72.6, 72.8, 127.9 (×4), 129.8 (×2), 133.5 (×2), 135.9 (×4). HRES-MS, *m*/*z*: 477.2703 (calcd 477.2672 for $C_{26}H_{41}O_6Si$) [$MH^+$].

**4.4.3. Compound 23.** To a solution of **22** (1.40 g, 2.94 mmol) in acetone (30 ml) at rt, Jones reagent (2.2 ml) was added dropwise. The reaction mixture was stirred at rt for 0.5 h, then quenched with water (10 ml), concentrated under reduced pressure to remove the excess of acetone, extracted with ethyl acetate (3×15 ml). The organic layer was finally dried over $Na_2SO_4$/$NaHCO_3$ and concentrated in vacuo. The crude was purified by flash chromatography (2–3% methanol in $CH_2Cl_2$) to give **23** (0.63 g, 43%) as a colorless oil.

*Compound* **23**. $R_f$=0.4 (6% methanol in $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 1.05 (3H, s, $(CH_3)_3Si$–), 3.56–3.63 (14H, m, $-(OCH_2CH_2O)_3CH_2$), 3.79 (2H, d, $J$=5.3 Hz, $CH_2$OTPS), 4.13 (2H, m, $CH_2COOH$), 7.34–7.40 (6H, m, Ar-*H*), 7.66–7.68 (4H, m, Ar-*H*). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 19.1, 26.9 (×3), 63.7, 70.5, 70.6, 70.9 (×4), 71.3, 72.6, 127.9 (×4), 129.8 (×2), 133.5 (×2), 135.9 (×4), 172.0. HRES-MS, *m*/*z*: 491.2460 (calcd 491.2465 for $C_{26}H_{39}O_7Si$) [$MH^+$].

### 4.5. Procedures for the synthesis of compounds described in Scheme 4

**4.5.1. Compound 24.** To a suspension of **11** (0.243 g, 0.736 mmol) in dry di(ethylene)glycol (4 ml) and absolute ethanol (1 ml) at rt, potassium hydroxide (KOH, 0.177 g, 3.17 mmol) and hydrazine monohydrate ($NH_2NH_2\cdot H_2O$, 0.43 ml, 8.82 mmol) were added. The reaction mixture was stirred for 30 min at 110 °C, the temperature was then raised up to 200 °C, for 3 h. The reaction was quenched with water (4 ml) and the resulting mixture was extracted four times with dichloromethane. The organic layer was dried over $Na_2SO_4$, filtered and evaporated in vacuo, affording a crude that was purified by flash chromatography (silica gel, 20–30% diethyl ether in petroleum ether) to furnish **24** (0.144 g, 62%) as a white amorphous solid.

*Compound* **24**. $R_f$=0.6 (40% petroleum ether in diethyl ether). $[\alpha]_D$ +4.1 (*c* 1.0 in $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.76 (3H, s, $CH_3$-18), 0.79 (3H, s, $CH_3$-19), 1.00 (3H, d, $J$=6.9 Hz, $CH_3$-21), 2.35 (1H, m, H-20), 3.54 (2H, m, H-22), 5.39 (1H, s, H-16). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 12.2, 16.4, 18.1, 20.6, 22.1, 26.8, 28.9, 29.0, 31.1, 32.0, 34.2, 34.9, 35.3, 36.5, 38.5, 47.2, 47.3, 55.3, 57.4, 66.5, 122.9, 157.7. HRES-MS, *m*/*z*: 317.2822 (calcd 317.2844 for $C_{22}H_{37}O$) [$MH^+$].

**4.5.2. Compound 25.** To a solution of **24** (0.167 g, 0.528 mmol) in absolute ethanol (3 ml), Pt/C (5% w/w, 0.011 g) was added. The flask was evacuated (20 Torr) and flushed with hydrogen three times. The reaction mixture was vigorously stirred under hydrogen overnight, then filtered through a pad of Celite, the Celite washed with chloroform and the solvent concentrated in vacuo to afford **25** (0.141 g, 97%) as a white amorphous solid.

*Compound* **25**. $R_f$=0.6 (40% petroleum ether in diethyl ether). $[\alpha]_D$ +13.9 (*c* 0.7 in $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.67 (3H, s, $CH_3$-18), 0.77 (3H, s, $CH_3$-19), 1.03 (3H, d, $J$=6.8 Hz, $CH_3$-21), 3.35 (1H, dd, $J$=10.5, 7.0 Hz, H-22), 3.62 (1H, dd, $J$=10.5, 3.1 Hz, H′-22). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 12.1, 12.2, 16.7, 20.8, 22.1, 24.3, 26.8, 27.7, 29.0 (×2), 32.1, 35.5, 36.2, 38.6, 38.8, 39.9, 42.7, 47.0, 52.5, 54.7, 56.3, 68.0. HRES-MS, *m*/*z*: 319.2976 (calcd 319.3001 for $C_{22}H_{39}O$) [$MH^+$].

**4.5.3. Compound 26.** To a solution of **25** (0.033 g, 0.104 mmol) in $CH_2Cl_2$ (1 ml) at rt, DMAP (0.039 g, 0.032 mmol), a solution of **23** (0.104 g, 0.19 mmol) in $CH_2Cl_2$ (1 ml) and EDC (0.102 g, 0.53 mmol) were sequentially added. The reaction mixture was stirred for 16 h, quenched with water (5 ml) and extracted with ethyl acetate (10 ml). The organic layer was washed with a saturated solution of $NaHCO_3$, with water, dried over $Na_2SO_4$, filtered and concentrated in vacuo. The crude was purified by flash chromatography (silica gel, 50% diethyl ether in petroleum ether) to furnish **26** (0.028 g, 34%) as a white amorphous solid.

*Compound* **26**. $R_f$=0.3 (40% petroleum ether in diethyl ether). $[\alpha]_D$ +8.6 (*c* 1.4 in $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.66 (3H, s, $CH_3$-18), 0.77 (3H, s, $CH_3$-19), 0.99 (3H, d, $J$=6.5 Hz, $CH_3$-21), 1.04 (9H, s, $(CH_3)_3Si$–), 3.58–3.70 (14H, m, $O(CH_2CH_2)_3OCH_2$–), 3.81 (2H, m, $-CH_2$-OTPS), 3.85 (1H, m, H-22), 4.13 (2H, br s, $OCOCH_2O$) 4.14 (1H, m, H′-22) 7.34–7.40 (6H, m, Ar-*H*), 7.66–7.68 (4H, m, Ar-*H*). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 12.1 (×2), 17.1, 19.2, 20.8, 20.8, 22.2, 24.3, 26.8 (×4), 27.7, 29.0 (×2), 32.1, 35.6, 35.9, 38.7, 39.9, 42.8, 47.0, 52.8, 54.7, 56.3, 63.4, 68.6, 69.9, 70.6 (×5), 70.9, 72.4, 127.6 (×4), 129.6 (×2), 133.6 (×2), 135.6 (×4), 170.1. HRES-MS, *m*/*z*: 791.5302 (calcd 791.5282 for $C_{48}H_{75}O_7Si$) [$MH^+$].

**4.5.4. Compound 3.** To a solution of **26** (0.027 g, 0.034 mmol) in pyridine (200 µl) at 0 °C, a solution of 70% hydrofluoridric acid in pyridine (40 µl, 1.39 mmol) was added. The reaction mixture was stirred for 1.5 h and concentrated under a stream of $N_2$. The residue was purified by flash chromatography (silica gel, 0–5% methanol in $CH_2Cl_2$) to afford **3** (0.0082 g, 44%) as a white amorphous solid.

*Compound* **3**. $R_f$=0.1 (100% diethyl ether). $[\alpha]_D$ +24.7 (*c* 0.4, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.66 (3H, s, $CH_3$-18), 0.76 (3H, s, $CH_3$-19), 0.98 (3H, d, $J$=6.5 Hz, $CH_3$-21), 3.60 (2H, m, $OCH_2CH_2OH$), 3.66–3.77 (14H, m, $O(CH_2CH_2)_3OCH_2$–, overlapped), 3.84 (1H, m, H-22), 4.15 (2H, m, $OCOCH_2O$) 4.16 (1H, m, H′-22). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 12.4, 12.5, 17.4, 21.1, 22.5, 24.5, 27.1, 27.9, 29.3 (×2), 32.4, 35.8, 36.1, 36.5, 39.0, 40.2,

43.0, 47.3, 53.0, 55.0, 56.6, 62.0, 68.9, 70.2, 70.5, 70.8 (× 4), 71.2, 72.9, 171.0. HRES-MS, *m/z*: 553.4110 (calcd 553.4104 for $C_{32}H_{57}O_7$) [$MH^+$].

### 4.6. Procedures for the synthesis of compounds described in Scheme 5

**4.6.1. Compound 28.** To a solution of **27** (0.088 g, 0.146 mmol) in $CH_2Cl_2$ (0.5 ml) at rt, DMAP (0.055 g, 0.45 mmol), a solution of **23** (0.080 g, 0.163 mmol) in $CH_2Cl_2$ (1 ml) and EDC (0.140 g, 0.73 mmol) were sequentially added. The reaction mixture was stirred for 16 h, quenched with water (2 ml) and extracted with ethyl acetate (4 ml). The organic layer was washed with a saturated solution of $NaHCO_3$, with water, dried over $Na_2SO_4$, filtered and concentrated in vacuo. The crude was purified by flash chromatography (silica gel, 0–1% methanol in $CHCl_3$) to afford **28** (0.049 g, 31%) as a white amorphous solid.

*Compound* **28**. $R_f$=0.3 (5% methanol in $CHCl_3$). $[\alpha]_D$ +14.0 (*c* 1.7, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.74 (3H, s, C*H*$_3$-18), 0.87 (3H, s, C*H*$_3$-19), 1.02 (3H, d, *J*= 6.9 Hz, C*H*$_3$-21), 1.05 (9H, s, (C*H*$_3$)$_3$Si–), 2.48 (1H, m, H-20), 3.08 (1H, m, H-6 or H-7), 3.27 (1H, m, H-7 or H-6), 3.59–3.70 (15H, m, –(OC*H*$_2$C*H*$_2$O)$_3$C*H*$_2$– and H-3, overlapped), 3.81, (2H, m, –C*H*$_2$OTPS), 4.04 (1H, m, H-22), 4.13 (2H, br s, OCOC*H*$_2$O), 4.25 (1H, m, H′-22), 5.41 (1H, s, *H*-16), 7.34–7.40 (6H, m, Ar-*H*), 7.66–7.68 (4H, m, Ar-*H*). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 13.6, 16.1, 18.7, 19.2, 20.9, 26.8 (×3), 26.9 (×3), 31.3 (×2),, 32.2, 33.9, 34.5, 35.9, 37.1, 39.8, 47.6, 48.0, 52.2, 55.8, 63.4, 68.6, 68.7, 70.6 (×4), 70.7 (×2), 70.9, 72.4, 72.5, 74.8, 80.3, 123.5, 127.4 (×4), 127.6 (×4), 129.5 (×2), 129.6 (×2), 133.7 (×2), 134.6, 134.8, 135.6 (×4), 135.8 (×4), 155.4, 170.4; HRES-MS, *m/z*: 1077.6326 (calcd 1077.6307 for $C_{64}H_{93}O_{10}Si_2$) [$MH^+$].

**4.6.2. Compound 4.** To a solution of **28** (0.060 g, 0.056 mmol) in pyridine (0.3 ml) at 0 °C, a solution of 70% hydrofluoric acid in pyridine (200 µl, 7.00 mmol) was added. The reaction mixture was stirred for 1.5 h and concentrated under a stream of $N_2$. The residue was purified by flash chromatography (silica gel, 30–70% ethyl acetate in petroleum ether) to afford **4** (0.017 g, 51%) as a white amorphous solid.

*Compound* **4**. $R_f$=0.1 (8% methanol in $CHCl_3$). $[\alpha]_D$ +41.3 (*c* 0.8 in $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.75 (3H, s, C*H*$_3$-18), 0.87 (3H, s, C*H*$_3$-19), 1.04 (3H, d, *J*=6.8 Hz, C*H*$_3$-21), 2.46 (1H, m, H-20), 3.09 (1H, m, H-6 or H-7), 3.24 (1H, m, H-7 or H-6), 3.54 (1H, m, H-3), 3.58 (2H, m, C*H*$_2$OH), 3.67–3.71 (14H, m, –(OC*H*$_2$C*H*$_2$O)$_3$C*H*$_2$–), 3.99 (1H, dd, *J*=10.5, 7.9 Hz, H-22), 4.12 (2H, s, COC*H*$_2$O), 4.21 (1H, dd, *J*=10.5, 6.4 Hz, H′-22), 5.42 (1H, s, H-16). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 13.6, 16.1, 18.7, 21.0, 30.7, 31.4, 32.3, 33.9, 34.6, 35.9, 37.1, 39.8, 47.7, 48.1, 52.3, 55.9, 61.6, 68.6, 68.7, 70.2, 70.4 (×4), 70.8, 72.1, 72.6, 74.6, 80.1, 123.7, 155.3, 170.5. HRES-MS, *m/z*: 601.3948 (calcd 601.3952 for $C_{32}H_{57}O_{10}$) [$MH^+$].

### 4.7. Procedures for the synthesis of compounds described in Scheme 6

**4.7.1. Compound 29.** To a solution of **12** (0.600 g, 1.80 mmol) in dichloromethane (10 ml) at 0 °C, pyridine (5 ml) and acetic anhydride (0.5 ml) were sequentially added. The reaction mixture was allowed to warm to rt, stirred overnight and quenched with a solution of HCl (2 M, 3 ml). The aqueous layer was extracted three times with dichloromethane, the organic layer was dried over $Na_2SO_4$, filtered, evaporated in vacuo and purified by flash chromatography (silica gel, 10–15% ethyl acetate in petroleum ether) to furnish **29** (0.338 g, 50%) as a white amorphous solid.

*Compound* **29**. $R_f$=0.45 (30% ethyl acetate in petroleum ether). $[\alpha]_D$ +12.3 (*c* 2.5 in $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.65 (3H, s, C*H*$_3$-18), 0.78 (3H, s, C*H*$_3$-19), 0.97 (3H, d, *J*=6.6 Hz, C*H*$_3$-21), 2.03 (3H, s, C*H*$_3$CO–), 3.55 (1H, m, H-3), 3.75 (1H, dd, *J*=10.6, 7.6 Hz, H-22), 4.05 (1H, dd, *J*=10.6, 3.4 Hz, H′-22). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 12.0, 12.2, 17.0, 20.9, 21.2, 24.2, 27.6, 28.6, 31.4, 32.0, 35.4, 35.5, 35.7, 37.0, 38.1, 39.8, 42.7, 44.8, 52.8, 54.3, 56.1, 69.5, 71.2, 171.3. HRES-MS, *m/z*: 377.3019 (calcd 377.3056 for $C_{24}H_{41}O_3$) [$MH^+$].

**4.7.2. Compound 30.** To a solution of **29** (0.116 g, 0.308 mmol) in $CH_2Cl_2$ (1 ml) at rt, DMAP (0.123 g, 1.00 mmol), a solution of **23** (0.200 g, 0.407 mmol) in $CH_2Cl_2$ (1 ml) and EDC (0.321 g, 1.67 mmol) were sequentially added. The reaction mixture was stirred for 24 h, quenched with water (2 ml) and extracted with ethyl acetate (4 ml). The organic layer was washed with a saturated solution of $NaHCO_3$, with water, dried over $Na_2SO_4$, filtered and concentrated in vacuo. The crude was purified by flash chromatography (silica gel, 10–40% ethyl acetate in petroleum ether) to afford **30** (0.214 g, 82%) as a white amorphous solid.

*Compound* **30**. $R_f$=0.3 (30% ethyl acetate in petroleum ether). $[\alpha]_D$ +4.4 (*c*=1.1 in $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.67 (3H, s, C*H*$_3$-18), 0.81 (3H, C*H*$_3$-19), 1.00 (3H, d, *J*=6.7 Hz, C*H*$_3$-21), 1.04 (9H, s, (C*H*$_3$)$_3$Si–), 2.04 (3H, s, C*H*$_3$CO), 3.63–3.74 (14H, m, (OC*H*$_2$C*H*$_2$O)$_3$C*H*$_2$–), 3.76, (1H, m, H-22), 3.80 (2H, m, –C*H*$_2$OTPS), 4.07 (1H, m, H′-22), 4.09 (2H, br s, COC*H*$_2$O), 4.76 (1H, m, H-3), 7.34–7.41 (6H, m, Ar-*H*), 7.66–7.68 (4H, m, Ar-*H*). $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$: 12.0, 12.2, 17.0, 19.1, 20.9, 21.1, 24.2, 26.8 (×3), 27.4, 27.6, 28.5, 31.8, 33.9, 35.4 (×2), 35.7, 36.6, 39.7, 42.7, 44.5, 52.8, 54.1, 56.0, 63.4, 68.8, 69.4, 70.5(×4), 70.7, 70.8, 72.4, 74.2, 127.5 (×4), 129.5 (×2), 133.7 (×2), 135.5(×4), 169.9, 171.2. HRES-MS, *m/z*: 849.5341 (calcd 849.5337 for $C_{50}H_{77}O_9Si$) [$MH^+$].

**4.7.3. Compound 5.** To a solution of **30** (0.214 g, 0.252 mmol) in pyridine (0.4 ml) at 0 °C a solution of 70% hydrofluoric acid in pyridine (200 µl, 7.00 mmol) was added. The reaction mixture was stirred for 1.5 h and concentrated under a stream of $N_2$. The residue was purified by flash chromatography (silica gel, 30–70% ethyl acetate in petroleum ether) to afford **5** (0.126 g, 82%) as a white amorphous solid.

*Compound* **5**. $R_f$=0.1 (30% ethyl acetate in petroleum ether). $[\alpha]_D$ +5.2 (*c* 1.1 in $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.67 (3H, s, C*H*$_3$-18), 0.77 (3H, s, C*H*$_3$-19), 1.00 (3H, d, *J*=6.6 Hz, C*H*$_3$-21), 3.60 (2H, m, –C*H*$_2$OH), 3.63–3.74 (14H, m, (OC*H*$_2$C*H*$_2$O)$_3$C*H*$_2$–), 3.76, (1H, m, H-22), 4.07 (1H, m, H′-22), 4.09 (2H, br s, COC*H*$_2$O), 4.76 (1H, m, H-3). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta$: 12.0, 12.1, 17.0, 20.8, 21.1, 24.1, 27.3, 27.5, 28.4, 31.8, 33.8, 35.3 (×2), 35.6, 36.6, 39.6, 42.6, 44.5, 52.7, 54.0, 56.0, 61.6, 68.7, 69.4, 70.2, 70.4 (×4), 70.7, 72.4, 74.3, 169.9, 171.2. HRES-MS, *m*/*z*: 611.4148 (calcd 611.4159 for $C_{34}H_{59}O_9$) [$MH^+$].

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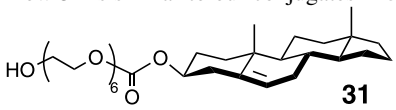
7. A case in which the polar head (the oligo(ethyleneglycol) moiety), matches the length of the lipophilic part (the sterol nucleus).
8. Molecular mechanic calculations (MM3) were performed in order to optimize the geometries of the ‘folded’ and the ‘extended’ conformations of **2**.
9. This is generally believed to be the active conformation of the poly(ethyleneglycol) derivative (see: Stadler, E.; Dedek, P.; Yamashita, K.; Regen, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 8975–8976. ). It is worth noting that on note 23 of the paper from Stadler et al., no ionophoric activity was found for 5-androsten-3β-(oxycarbonyl)-hexa(ethylenglycol) (**31**, 2% mol in egg PC vesicles) over a 20 h period. It is easy to recognize how **31** is similar to our conjugates **1**–**5**.




**31**

10. It must be noted that for ‘complex minimalist systems’ (see Ref. 6), such as those represented by monomeric steroids, this kind of structurally simple self-assembly motif (never proposed before), can be equally probable.
11. As previously described (see Ref. 5b), a solution of NaCl (75.0 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 (100.0 mM). Compounds **1** and **2** 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.
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16. The protonophoric activities were measured using an assay based on the response of the intravesicular pH-sensitive pyranine fluorophore. See: Clement, N. R.; Gould, J. M. *Biochemistry* **1981**, *20*, 1534–1538.
17. In our experimental conditions the observed rate constant in the presence of 1.5% of ionophore were: **1**, $k_{obsd}=5.0\times10^{-3}$ s$^{-1}$; **2**, $k_{obsd}=4.7\times10^{-3}$ s$^{-1}$, amphotericin B, $k_{obsd}=2.3\times10^{-3}$ s$^{-1}$.
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19. Theoretical studies showed that the size and polarity of the inner channel influences proton transport acting on the degree of proton solvatation. See: Wu, Y.; Voth, G. *Biophys. J.* **2003**, *85*, 864–875.
20. It is well known that in most of the membrane-active saponins and steroidal oligoglycosides the polar sugars are linked at C-3 and/or C-6. See: D’Auria, M. V.; Minale, L.; Riccio, R. *Chem. Rev.* **1993**, *93*, 1839–1895.