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On the importance of the pore inner cavity for the ionophoric activity of 1,3-*alternate* calix[4]arene/steroid conjugates

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Abstract—1,3-*Alternate* calix[4]arenes, decorated with four nonpolar 'all-trans' tetracyclic nuclei and cation-stabilizing β-methoxyethoxy appendages, were synthesized from commercially available starting materials and through straightforward functional groups transformations/couplings. Their Na^+-transport activities, when compared with those exerted by the known conformationally-rigidified 1,3-*alternate* calix[4]-arene AB/cis cholic acid conjugates, suggest that the cation conductance is related to the morphology of the pendant steroids.

1. Introduction

In living organisms, ion transport is mediated by integral membrane proteins that span from the internal to the external surface of the lipid bilayer. The intimate chemical details of the cation transport in biological systems still remain elusive,[1] but details of the conductance mechanisms are emerging thanks to the structure/function relationship analysis of natural and artificial transporters.[2]

In this context, we have recently demonstrated[3] that in conformationally-rigidified 1,3-*alternate* (i.e., **1**, Fig. 1) and *cone* calix[4]arene/hydroxycholanic derivatives, the observed unimolecular ion transport is dependent on the length of the channel.

In this paper we try to assess how the presence of a pre-organized pore cavity influences the cation transport process. This is a crucial issue for the design of artificial ion channels.

With the idea of clarifying the structural requisites useful for the ionophoric properties, we embarked on the synthesis of two conformationally immobilized 1,3-*alternate* calix[4] arene/all-trans steroid conjugates **2** and **3** (Fig. 1), and compared their ionophoric activity with that of the powerful Na^+-transporter **1**,[3] showing a 'folded' AB-cis-cholane framework.

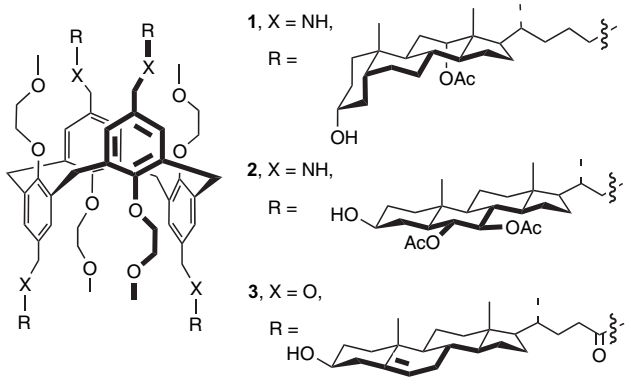

Figure 1. Calix[4]arenes conjugates **1**–**3**.

Compound **2** was designed in order to have approximately the same length as **1** (35±2 Å), an AB-trans ring junction and two equatorial acetoxy groups, useful for transient cation stabilization, lying in the same plane of the tetracyclic nucleus.

In conjugate **3**, the steroid moiety was simplified to the *flat* (20*R*)-3β-hydroxy-chol-5-enoate residue, showing a slightly longer side chain (estimated overall length of **3**: 39±2 Å)[4] and no oxygenated substituents, apart from the hydroxy group at C-3.

The absence of a pre-existing inner cavity in the N- and O-linked calix[4]arene/sterol **2** and **3** is suggested by molecular modeling studies.[5] Figure 2 displays the extended lowest energy conformations of conjugates **1**–**3**. In the case of the 'folded' AB-cis framework, as in **1**, the presence of an

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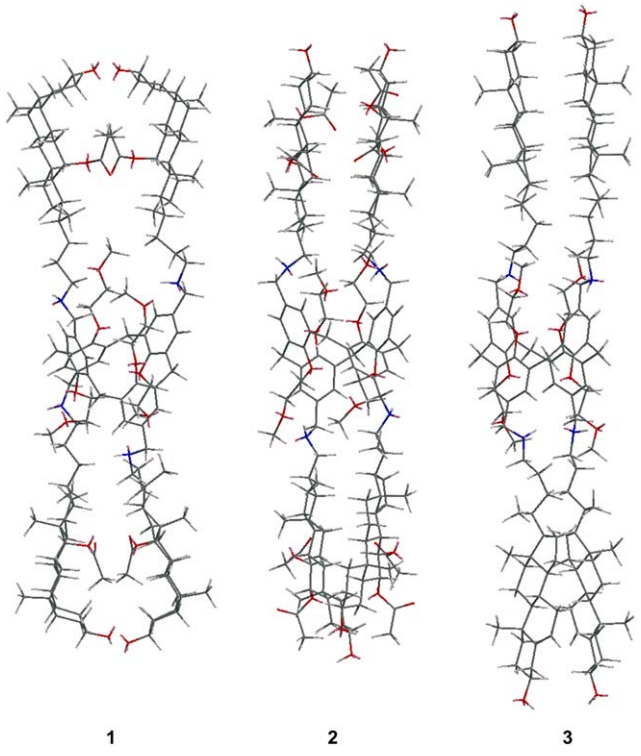


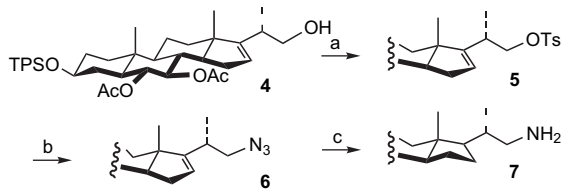
Figure 2. Energy-minimized structures obtained by molecular modeling of compounds **1–3** in their extended conformations.

intramolecular hollow is evident. In derivatives **2** and **3**, Van der Waals forces stabilize the interactions of the facing all-trans steroid pairs, packing the nonpolar appendages in a more 'compact' morphology.

2. Results and discussion

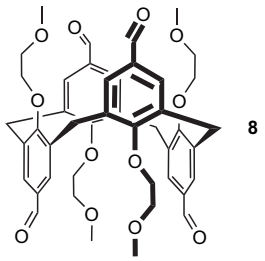
2.1. Synthesis of conjugate 2

The 1,3-*alternate* calix[4]arene/6α,7β-diacetoxy-5α-23,24-bisnorcholan-3β-ol-22-amino derivative was synthesized starting from the known[6] (20*S*)-6α,7β-diacetoxy-3β-[(*tert*-butyldiphenylsilyl)oxy]-5α-23,24-bisnorchol-16-en-22-ol (**4**, Scheme 1). This was first tosylated[7] at the primary hydroxy group and then substituted in the presence of sodium azide[8] to yield **6**. The C-22 azide was subsequently reduced,[9] with molecular hydrogen and Adam's catalyst, to give, in good yield and high stereoselectivity,[10] the saturated (20*S*)-6α,7β-diacetoxy-3β-[(*tert*-butyldiphenylsilyl)oxy]-22-amino-5α-23,24-bisnorcholane (**7**).

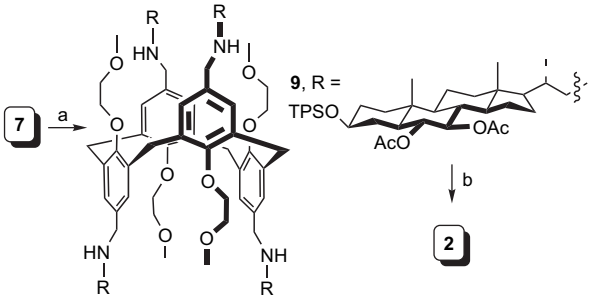


Scheme 1. Reagents and conditions: (a) TsCl, Py, CH_2Cl_2, 91%; (b) NaN_3, DMF, 60 °C, 96% and (c) H_2, PtO_2, EtOH, 79%.

With the aminosteroid **7** in our hands we performed the well described $NaBH(OAc)_3$-mediated reductive amination[11] in the presence of the known conformationally immobilized



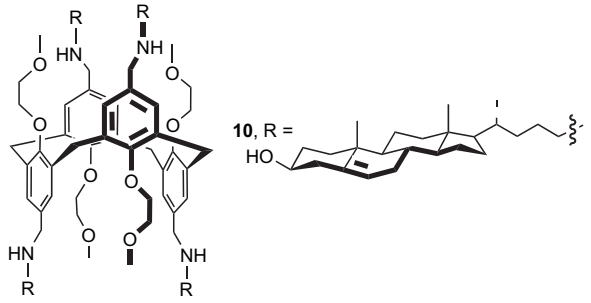
calix[4]arene-1,3-*alt* tetra-aldehyde **8**[12,3] and, to our delight, we isolated, after chromatographic purification, the tetra-adduct **9** in a satisfying 83% yield (Scheme 2). HF/pyridine-mediated *tert*-butyldiphenylsilyl deprotection, gave the expected target **2**.



Scheme 2. Reagents and conditions: (a) **8**, AcOH, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, 83% and (b) HF, Py, 48%.

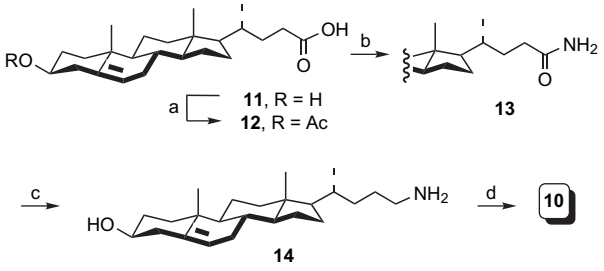
2.2. Attempted synthesis of conjugate 10

Having secured the first 1,3-*alternate* calix[4]arene/all-trans steroid derivative, we turned our attention to the synthesis of the Δ^5-conjugate **10**.



Its supposedly easier convergent synthesis (Scheme 3), started from a C-3 acetylation of the commercially available cholenic acid (**11**) to give the (20*R*)-3β-acetoxy-chol-5-enic acid (**12**). This was transformed into the carboxamide **13** (99% yield), using, as condensing agent, the diphenylphosphorylazide (DPPA)[13] and then reduced to the required (20*R*)-24-amino-chol-5-ene-3β-ol (**14**) with $LiAlH_4$. This time we failed to synthesize the requested tetra-amino calix[4]arene **10** using the $NaBH(OAc)_3$-mediated reductive amination conditions obtaining, instead, a complex mixture containing the mono and the bis-adducts (ESI-MS analysis). In order to improve the efficiency of the coupling reaction we decided to change the reaction conditions. In particular, considering the low solubility of the amine **14** in halogenated solvents, we turned to the more polar solvent DMF and, in a subsequent experiment, we increased the number of

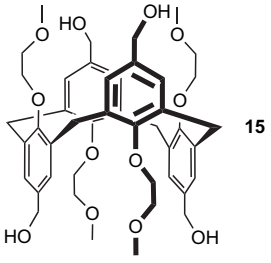
equivalents of the steroid primary amine (see Section 4). Unfortunately, none of the attempted reactions gave the desired tetra-aminated calix[4]arene **10**. We invariably observed the formation of a complex mixture of lower adducts (ESI-MS analysis, see Section 4) and no trace of the required **10**.



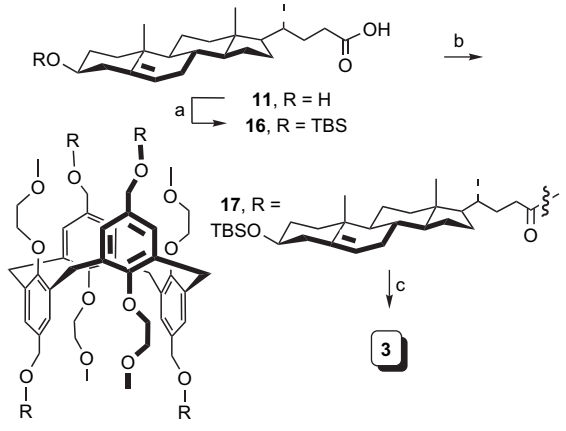
Scheme 3. Reagents and conditions: (a) Ac_2O, Py, CH_2Cl_2; (b) DPPA, NH_4Cl, NEt_3, DMF, 0 °C, 99%, for two steps; (c) $LiAlH_4$, THF, reflux, 54% and (d) **8**, AcOH, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$ or DMF (see Section 4).

2.3. Synthesis of conjugate 3

To overcome the difficulties posed by the reductive amination, we decided to link the two coupling partners (1,3-*alternate* calix[4]arene and the all-trans sterol) via an ester bond. Having the cholenic acid ready for use, we focused our attention to the reduction of the tetra-aldehyde **8**, in order to obtain the tetra-alcohol **15**. The conformationally restricted 1,3-*alternate* calix[4]arene tetraol **15** was thus easily formed through $NaBH_4$-induced carbonyl reduction of the tetra-aldehyde **8**.



The sterol partner used in the EDC-mediated condensation reaction was the 3β-[(*tert*-butyldimethylsilyl)oxy]-chol-5-enic acid (**16**), easily synthesized in two steps from the commercially available cholenic acid (**11**, Scheme 4).



Scheme 4. Reagents and conditions: (a) TBSCl, imidazole, DMF, 0 °C then K_2CO_3, MeOH, THF, H_2O, 79%; (b) EDC, DMAP, CH_2Cl_2, 0 °C, 46% and (c) HF (48% in H_2O), THF, quant.

The esterification reaction went smoothly and gave, after chromatographic purification, the pure silylated tetra-adduct **17** (46% yield). Finally, HF-mediated desilylation, produced the C_4 symmetric target **3**.

2.4. Na^+-transporting activities of conjugates 2 and 3

To investigate the ionophoric properties of conjugates **2** and **3** we studied their ability to promote the transport of Na^+ across a lipid bilayer using a $^{23}Na^+$ NMR based methodology.[14] Figure 3 shows that conjugates **2** and **3** behave as poor ionophores: in the presence of 1% of each of them (percent of steroids with respect to the total concentration of lipid, curve ○ for **2** and ● for **3**), and after more than 13 h, the amount of Na^+ entering the internal vesicular compartment was only slightly higher than in the absence of any additive (curve ◆). On the contrary, as previously reported,[3] conjugate **1** efficiently induces the transport of Na^+ across the lipid bilayer and its transmembrane gradient is fully discharged in almost 12 h (curve ■).[15]

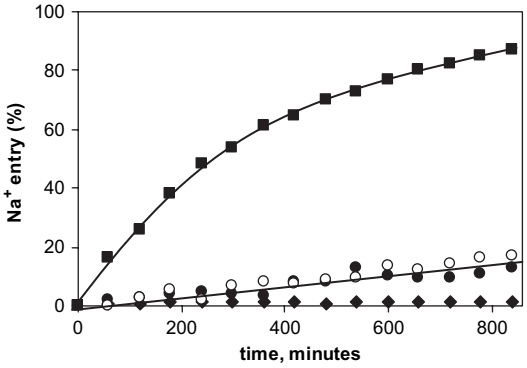


Figure 3. Kinetic profiles for the entry of Na^+ into 95:5 egg PC/PG vesicles containing **1** (1%, ■), **2** (1%, ○), and **3** (1%, ●), and without additives (◆) at 25 °C. The total concentration of lipid was 10 mM.

3. Conclusions

The results of this endeavor clarify the relevance of an extended cavity throughout the pore for the transport of Na^+ across a lipid bilayer. The two newly prepared 1,3-*alternate* calix[4]arene/steroid conjugates characterized by the presence of all-trans junctions behave as poor ionophores regardless to the presence of the acetoxy moieties. As suggested by the molecular modeling studies, in these conjugates the 'flat' steroid nucleus adopts a compact packing hampering the cation transport. On the other hand, the 'folded' AB-cis geometry of the steroid appendages present in **1** favors the formation of an inner cavity, which appears essential for the transport process.

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), visualized by UV light or with H_2SO_4–$Ce(SO_4)_2$ or ninhydrin solutions. Flash chromatography 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 or, when indicated, at 80 °C or 100 °C on a Bruker DRX 400 (^1H at 400 MHz, ^{13}C at 100 MHz) and a Bruker DRX 300 (^1H at 300 MHz, ^{13}C at 75 MHz) spectrometers. Chemical shifts are reported relative to the residual solvent peak ($CHCl_3$: δ=7.26, $^{13}CDCl_3$: δ=77.0; $C_2H_2Cl_4$: δ=5.80, $^{13}C_2D_2Cl_4$ (TCDE): δ=72.1). HRES-MS was performed on a Q-Star Applied Biosystem mass spectrometer. Optical rotations were measured with a JASCO DIP-1000 polarimeter.

4.1.1. Compound 5. To a solution of the alcohol **4** (0.100 g, 0.145 mmol) in CH_2Cl_2 (1.5 ml), pyridine (0.035 ml, 0.435 mmol) and *p*-toluenesulfonylchloride (0.055 g, 0.290 mmol) were added and the reaction mixture was stirred overnight. Pyridine (0.023 ml, 0.29 mmol) and *p*-toluenesulfonylchloride (0.042 g, 0.217 mmol) were further added. After 24 h the reaction was treated with a saturated solution of NH_4Cl (4 ml), extracted with CH_2Cl_2 (3×5 ml), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was flash-chromatographed (10–40% ethyl acetate in petroleum ether) to give **5** (0.111 g, 91%) as a white amorphous solid.

5: $[\alpha]_D$ +17.3 (*c* 2.4, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.67 (3H, s, C*H*$_3$-18), 0.94 (3H, s, C*H*$_3$-19), 0.97 (3H, d, *J*=6.9 Hz, C*H*$_3$-21), 1.03 (9H, s, (*CH*$_3$)$_3$CSi–), 1.87 (3H, s, COC*H*$_3$), 1.96 (3H, s, COC*H*$_3$), 2.38 (1H, m, H-20), 2.44 (3H, s, C*H*$_3$(Ts)), 3.53 (1H, m, H-3), 3.83 (1H, br t, *J*=9.4 Hz, H-22), 3.98 (1H, dd, *J*=9.4, 5.9 Hz, H′-22), 4.65 (1H, br t, *J*=9.5 Hz, H-6 or H-7), 4.79 (1H, br t, *J*=9.5 Hz, H-6 or H-7), 5.20 (1H, br s, H-16), 7.36 (8H, m, Ar*H* and Ar*H*(Ts)), 7.63 (4H, m, Ar*H*), 7.65 (2H, d, *J*=7.2 Hz, Ar*H*(Ts)). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 13.3, 15.9, 18.3, 19.0, 20.6, 20.9, 21.3, 21.5, 26.9 (×3), 31.0, 31.5, 32.0, 32.1, 34.1, 35.9, 36.8, 37.8, 46.4, 47.8, 52.0, 54.6, 71.9, 73.8, 74.2, 77.5, 123.6, 127.4 (×4), 127.8 (×2), 129.5 (×2), 129.7 (×2), 133.2, 134.5, 134.6, 135.7 (×4), 144.5, 154.2, 170.5, 170.6. HRES-MS, *m*/*z*: 841.4135 (calcd 841.4169 for $C_{49}H_{65}O_8SSi$) [MH^+].

4.1.2. Compound 6. To a solution of the tosylate **5** (0.111 g, 0.132 mmol) in dry DMF (1 ml), sodium azide (0.043 g, 0.661 mmol) was added and the reaction mixture was stirred at 60 °C for 22 h. The reaction was quenched with brine (2 ml) and then extracted with Et_2O (3×4 ml). The combined organic phases were dried over K_2CO_3, filtered, and concentrated in vacuo. The residue was flash-chromatographed (10–30% ethyl acetate in petroleum ether) to give **6** (0.090 g, 96%) as a white amorphous solid.

6: $[\alpha]_D$ +9.2 (*c* 1.7, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.77 (3H, s, C*H*$_3$-18), 0.96 (3H, s, C*H*$_3$-19), 1.03 (12H, s, (*CH*$_3$)$_3$CSi– and C*H*$_3$-21), 1.87 (3H, s, COC*H*$_3$), 1.97 (3H, s, COC*H*$_3$), 2.32 (1H, m, H-20), 3.16 (1H, dd, *J*=12.0, 7.7 Hz, H-22), 3.35 (1H, dd, *J*=12.0, 5.9 Hz, H′-22), 3.54 (1H, m, H-3), 4.68 (1H, br t, *J*=9.5 Hz, H-6 or H-7), 4.80 (1H, br t, *J*=9.5 Hz, H-6 or H-7), 5.34 (1H, br s, H-16), 7.37 (6H, m, Ar*H*), 7.64 (4H, m, Ar*H*).^{13}C NMR ($CDCl_3$, 100 MHz) δ: 13.3, 15.9, 19.0, 19.6, 20.6, 21.0, 21.4, 26.9 (×3), 31.1, 32.0, 32.1, 32.3, 34.4, 36.0, 36.8, 37.9, 46.4, 48.0, 52.1, 54.8, 56.8, 72.0, 74.2, 77.6, 123.1, 127.4 (×4), 129.5 (×2), 134.6 (×2), 135.7 (×4), 155.8, 170.5, 170.7. HRES-MS, *m*/*z*: 712.4119 (calcd 712.4146 for $C_{42}H_{58}N_3O_5Si$) [MH^+].

4.1.3. Compound 7. To a solution of the azide **6** (0.069 g, 0.097 mmol) in ethyl acetate (1.0 ml), PtO_2 (0.007 g) was added and the reaction mixture was stirred under hydrogen atmosphere for 48 h. The catalyst was then filtered off on a short pad of Celite™ washing with ethyl acetate. The solvent was removed under reduced pressure and the crude product was flash-chromatographed (10–20% methanol in chloroform) to give **7** (0.053 g, 79%) as a white amorphous solid.

7: $[\alpha]_D$ +11.8 (*c* 1.6, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.65 (3H, s, C*H*$_3$-18), 0.92 (3H, s, C*H*$_3$-19), 0.97 (3H, d, *J*=6.9 Hz, C*H*$_3$-21), 1.03 (9H, s, (*CH*$_3$)$_3$CSi–), 1.86 (3H, s, COC*H*$_3$), 1.93 (3H, s, COC*H*$_3$), 2.38 (1H, dd, *J*=11.9, 7.2 Hz, H-22 or H′-22), 2.71 (1H, br d, *J*=11.9 Hz, H-22 or H′-22), 3.51 (1H, m, H-3), 4.62 (1H, br t, *J*=9.5 Hz, H-6 or H-7), 4.75 (1H, br t, *J*=9.5 Hz, H-6 or H-7), 7.37 (6H, m, Ar*H*), 7.63 (4H, m, Ar*H*).^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.0, 13.3, 16.9, 19.1, 20.7, 21.2, 21.5, 24.8, 26.9 (×3), 28.0, 31.1, 32.1, 35.7, 37.0, 39.0 (×2), 39.3, 43.6, 46.1, 46.9, 51.7, 52.4, 54.6, 72.0, 74.5, 77.9, 127.5 (×4), 129.5 (×2), 134.5 (×2), 135.7 (×4), 170.6, 170.9. HRES-MS, *m*/*z*: 688.4377 (calcd 688.4397 for $C_{42}H_{62}NO_5Si$) [MH^+].

4.1.4. Compound 9. To a solution of aldehyde **8** (0.051 g, 0.066 mmol) and **7** (0.454 g, 0.660 mmol) in 1,2-dichloroethane (2.5 ml) $NaBH(OAc)_3$ (0.084 g, 0.396 mmol) and AcOH (15 µl, 0.264 mmol) were added. The reaction mixture was stirred at rt overnight, quenched with 1 M NaOH solution and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The crude residue was flash-chromatographed (5–20% of methanol in dichloromethane) to give **9** (0.190 g, 83%) as a white amorphous solid.

9: $[\alpha]_D$ +12.2 (*c* 1.0, $CHCl_3$). ^1H NMR (TCDE, 100 °C, 400 MHz) δ: 0.52 (12H, s, C*H*$_3$-18), 0.77 (24H, m, C*H*$_3$-19 and C*H*$_3$-21 overlapped), 0.91 (36H, s, (*CH*$_3$)$_3$CSi–), 1.69 (12H, s, COC*H*$_3$), 1.75 (12H, s, COC*H*$_3$), 2.46 (8H, br m, C*H*$_2$NH), 3.16 (12H, br s, OC*H*$_3$), 3.16–3.67 (36H, m, ArOC*H*$_2$C*H*$_2$OCH$_3$, ArC*H*$_2$Ar, NHC*H*$_2$Ar and H-3 overlapped), 4.48 (4H, m, H-6 or H-7), 4.61 (4H, m, H-6 or H-7), 7.07 (8H, br s, Ar*H*), 7.17–7.24 (24H, m, Ar*H*(TPS)), 7.50 (16H, m, Ar*H*(TPS)). ^{13}C NMR (TCDE, 80 °C, 100 MHz) δ: 10.2 (×4), 11.5 (×4), 16.3 (×4), 17.2 (×4), 18.7 (×4), 19.5 (×4), 19.6 (×4), 22.9 (×4), 25.3 (×12), 25.8 (br, ×4), 27.8 (×4), 29.4 (×4), 30.5 (×4), 32.0 (br, ×4), 33.9 (×4), 35.4 (×4), 37.4 (×8), 42.0 (×4), 44.6 (×4), 48.0 (br, ×4), 50.1 (×4), 51.0 (×8), 52.8 (×4), 57.0 (×4), 68.9 (br,

×4), 69.8 (×4), 70.4 (×4), 72.7 (×4), 76.2 (×4), 121.0 (br, ×4), 125.7 (×16), 127.6 (×8), 130.2 (br, ×8), 132.4 (br, ×8), 133.0 (×4), 133.1 (×4), 133.9 (×16), 155.4 (br, ×4), 168.4 (×4), 168.6 (×4). ES-MS, *m/z*: 3455.0501 (calcd 3455.0703 for $C_{212}H_{293}N_4O_{28}Si_4$) [$MH^+$].

4.1.5. Compound 2. To a solution of **9** (0.064 g, 0.018 mmol) in pyridine (0.5 ml) at 0 °C, a solution of 70% hydrofluoric acid in pyridine (0.3 ml) was added. The reaction mixture was stirred overnight and concentrated under a stream of N_2. The residue was purified by flash chromatography (silica gel, 8–20% methanol in $CHCl_3$) to afford **2** (0.022 g, 48%) as a white amorphous solid.

2: R_f=0.4 (10% methanol in CH_2Cl_2). $[\alpha]_D$ +25.1 (*c* 1.0, MeOH). ^{1}H NMR (TCDE, 80 °C, 400 MHz) δ: 0.56 (12H, s, C*H*$_3$-18), 0.77 (24H, m, C*H*$_3$-19 and C*H*$_3$-21 overlapped), 1.79 (12H, s, COC*H*$_3$), 1.82 (12H, s, COC*H*$_3$), 2.67 (8H, br m, C*H*$_2$NH), 3.23 (12H, br s, OC*H*$_3$), 3.35–3.98 (36H, m, ArOC*H*$_2$C*H*$_2$OCH$_3$, ArC*H*$_2$Ar, NHC*H*$_2$Ar and H-3 overlapped), 4.57 (4H, m, H-6 or H-7), 4.68 (4H, m, H-6 or H-7), 7.07 (8H, br s, Ar*H*), 7.17–7.24 (24H, m, Ar*H*(TPS)), 7.50 (16H, m, Ar*H*(TPS)). ^{13}C NMR (TCDE, 80 °C, 100 MHz) δ: 10.2 (×4), 11.5 (×4), 15.2 (×4), 18.9 (×4), 19.6 (×8), 22.9 (×4), 25.7 (×4), 27.8 (×4), 29.3 (×4), 30.5 (×4), 31.9 (×4), 34.0 (×4), 35.3 (×4), 37.4 (×8), 42.2 (×4), 44.6 (×4), 48.0 (br, ×4), 50.1 (×4), 50.8 (×8), 52.7 (×4), 56.8 (×4), 68.8 (×4), 70.0 (×8), 72.8 (×4), 76.1 (×4), 121.0 (br, ×4), 129.8 (×8), 132.6 (×8), 155.4 (×4), 168.6 (×8). ES-MS, *m/z*: 2502.7001 (calcd 2502.5992 for $C_{148}H_{221}N_4O_{28}$) [MH^+].

4.1.6. Compound 13. To a solution of cholenic acid (0.288 g, 0.77 mmol) in dry CH_2Cl_2 (20 ml), pyridine (1.00 ml, 12.3 mmol), DMAP (0.005 g, 0.04 mmol), and Ac_2O (0.145 ml, 1.54 mmol) were added. The resulting mixture was stirred for 3 h, diluted with CH_2Cl_2 (10 ml), quenched with 1 M HCl (10 ml) and extracted with CH_2Cl_2 (2×10 ml). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo to give a crude product, which was used in the next step without further purification. To a solution of the crude compound in DMF (10 ml) at 0 °C, diphenylphosphorylazide (DPPA, 0.40, 1.85 mol), NH_4Cl (0.099 g, 1.85 mol), and Et_3N (0.054 ml, 3.85 mmol) were sequentially added. The mixture was stirred at −10 °C overnight, diluted with ethyl acetate (10 ml), treated with a saturated solution of NH_4Cl (10 ml) and extracted with ethyl acetate (2×15 ml). The combined organic phases were washed with a solution of $NaHCO_3$ (10% in water), brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was flash-chromatographed (2–10% ethyl acetate in petroleum ether) to give **13** (0.317 g, 99%, for two steps) as a white amorphous solid.

13: $[\alpha]_D$ −27.9 (*c* 1.0, $CHCl_3$). ^{1}H NMR (CD_3OD, 300 MHz) δ: 0.76 (3H, s, C*H*$_3$-18), 1.01 (3H, d, *J*=6.7 Hz, C*H*$_3$-21), 1.08 (3H, s, C*H*$_3$-19), 2.05 (3H, s, C*H*$_3$CO), 4.56 (1H, m, H-3), 5.42 (1H, br d, *J*=4.8 Hz, H-6). ^{13}C NMR (CD_3OD, 100 MHz) δ: 12.3, 18.9, 19.7, 21.2, 22.1, 25.3, 28.8, 29.2, 33.0, 33.2 (×2), 33.5, 36.9, 37.7, 38.2, 39.1, 41.0, 43.5, 51.5, 57.2, 58.0, 75.4, 123.6, 141.0, 172.4, 179.8. HRES-MS, *m/z*: 416.3209 (calcd 416.3165 for $C_{26}H_{42}NO_3$) [MH^+].

4.1.7. Compound 14. To a solution of **13** (0.387 g, 0.93 mmol) in THF, $LiAlH_4$ (1 M in THF, 2.3 mmol) was added. The reaction mixture was refluxed at 65 °C for 3 h, then water (0.1 ml) and NaOH (15% solution of in water, 0.1 ml) were added. The resulting mixture was vigorously stirred, filtered under reduced pressure washed with 30% CH_3OH in ethyl acetate. The solvent was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was flash-chromatographed (2–25% methanol in CH_2Cl_2/CH_3COOH 99:1) to give **13** (0.180 g, 54%) as a white amorphous solid.

14: $[\alpha]_D$ −12.8 (*c* 1.0, MeOH). ^{1}H NMR (CD_3OD, 300 MHz) δ: 0.76 (3H, s, C*H*$_3$-18), 1.01 (3H, d, *J*=6.7 Hz, C*H*$_3$-21), 1.05 (3H, s, C*H*$_3$-19), 2.90 (3H, s, C*H*$_2$NH$_2$), 3.43 (1H, m, H-3), 5.36 (1H, br d, *J*=4.8 Hz, H-6). ^{13}C NMR (CD_3OD, 100 MHz) δ: 12.3, 19.1, 19.9, 22.1, 25.3 (×2), 29.2, 32.3, 33.0, 33.2, 33.8, 36.8, 37.6, 38.5, 41.2 (×2), 43.0, 43.5, 51.6, 57.2, 58.1, 72.4, 122.4, 142.2. HRES-MS, *m/z*: 360.3285 (calcd 360.3266 for $C_{24}H_{42}NO$) [MH^+].

4.1.8. First attempted synthesis of 10. To a solution of amine **14** (0.105 g, 0.29 mmol) in 1,2-dichloroethane (1 ml), aldehyde **8** (0.038 g, 0.049 mmol), $NaBH(OAc)_3$ (0.062 g, 0.29 mmol), and AcOH (11 μl, 0.20 mmol), were added. The reaction mixture was stirred at rt overnight, quenched with 1 N NaOH solution and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The crude residue was flash-chromatographed (2–30% methanol in dichloromethane) to give a complex mixture containing the mono and the bis-adduct. ES-MS, *m/z*: 1111.6 (mono-adduct); *m/z*: 1455.0 (bis-adduct). Mass peaks ratio: 4:1.

4.1.9. Second attempted synthesis of 10. To a solution of amine **14** (0.105 g, 0.29 mmol) in 1,2-DMF (1 ml), aldehyde **8** (0.038 g, 0.049 mmol), $NaBH(OAc)_3$ (0.062 g, 0.29 mmol), and AcOH (11 μl, 0.20 mmol), were added. The reaction mixture was stirred at rt overnight, quenched with 1 M NaOH solution and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude residue was flash-chromatographed (2–30% methanol in dichloromethane) to give a complex mixture containing the mono and the bis-adduct. ES-MS, *m/z*: 1111.6 (mono-adduct); *m/z*: 1455.0 (bis-adduct). Mass peaks ratio: 3:1.

4.1.10. Third attempted synthesis of 10. To a solution of amine **14** (0.217 g, 0.60 mmol) in DMF (4 ml), aldehyde **8** (0.061 g, 0.079 mmol), $NaBH(OAc)_3$ (0.127 g, 0.60 mmol), and AcOH (22 μl, 0.39 mmol) were added. The reaction mixture was stirred at rt overnight, quenched with 1 N NaOH solution and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude residue was flash-chromatographed (2–30% of methanol in dichloromethane) to give a complex mixture containing the mono and the bis-adduct. ES-MS, *m/z*: 1111.6 (mono-adduct); *m/z*: 1455.0 (bis-adduct). Mass peaks ratio: 3:1.

4.1.11. Compound 15. To a solution of aldehyde **8** (0.143 g, 0.19 mmol) in EtOH (2 ml) at 0 °C, $NaBH_4$ (0.032 g, 0.84 mmol), was added. The resulting mixture was stirred

at rt for 2 h, quenched with HCl (10% solution in water, 5 ml) and concentrated under reduced pressure to remove the excess of EtOH. The aqueous layer was extracted with ethyl acetate (3×10 ml) and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo to give a residue, which was purified by flash chromatography (2–15% MeOH in EtOAc) to afford **15** (0.112 g, 76%) as a white amorphous solid.

15: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.50 (12H, s, CH_3O–), 3.67 (8H, s, Ar–CH_2–Ar), 3.71 (8H, m, –OCH$_2$CH_2O–), 3.91 (8H, m, –OCH_2CH$_2$O–), 4.45 (8H, s, –CH_2OH), 7.05 (8H, s, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 35.7 (×4), 58.7 (×4), 65.3 (×4), 71.5 (×4), 72.0 (×4), 130.3 (×8), 133.4 (×8), 135.0 (×4), 155.3 (×4). HRES-MS, *m*/*z*: 777.3811 (calcd 777.3850 for $C_{44}H_{57}O_{12}$) [MH$^+$].

4.1.12. Compound 16. To a solution of **11** (0.612 g, 1.63 mmol) in DMF (14 ml) at 0 °C, imidazole (0.888 g, 13.04 mmol), 4-dimethylaminopyridine (DMAP, 0.398 g, 3.26 mmol), and *tert*-butyldimethylsilyl chloride (TBSCl, 0.737 g, 4.89 mmol), were added. The resulting mixture was stirred at rt overnight, then quenched with HCl (1 M, 10 ml), extracted with ethyl acetate (3×30 ml), washed with brine, dried on Na_2SO_4, and concentrated in vacuo to give the crude, which was used in the next step without further purification. To a solution of the crude material in MeOH (14 ml) and THF (14 ml), a solution of K_2CO_3 (10% w/w, 7 ml) in water, was added. The resulting mixture was stirred at rt for 2 h then concentrated under reduced pressure to remove the excess of THF and MeOH. A saturated solution of NaCl was added and the aqueous layer was acidified with HCl (5%) to pH 3. The mixture was extracted with ethyl acetate (3×40 ml), dried on Na_2SO_4 and concentrated in vacuo to give pure **16** (0.630 g, 79%) as a white amorphous solid.

16: $[\alpha]_D$ −29.3 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.06 (6H, s, (CH_3)$_2$CSi–), 0.67 (3H, s, CH_3-18), 0.88 (9H, s, (CH_3)$_3$Si–), 0.93 (3H, d, *J*=6.7 Hz, CH_3-21), 0.99 (3H, s, CH_3-19), 3.48 (1H, m, H-3), 5.31 (1H, br s, H-6), 10.30 (1H, br s, COO*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: −4 (×2), 11.9, 18.3 (×2), 19.4, 21.0, 24.2, 25.9 (×3), 28.1, 30.8, 31.1, 31.9, 32.0, 35.3 (×2), 36.5, 37.4, 39.7, 42.4, 42.8, 50.1, 55.7, 56.7, 72.6, 121.1, 141.5, 180.6. HRES-MS, *m*/*z*: 489.3711 (calcd 489.3764 for $C_{30}H_{53}O_3Si$) [MH$^+$].

4.1.13. Compound 17. To a solution of **16** (0.41 g, 0.84 mmol) in dry CH_2Cl_2 (8 ml) at 0 °C, DMAP (0.048 g, 0.39 mmol), a solution of **15** (0.11 g, 0.14 mmol) in dry CH_2Cl_2 (2 ml) and EDC (0.17 g, 0.90 mmol) were sequentially added. The reaction mixture was stirred for 16 h, quenched with water (5 ml) and extracted with ethyl acetate (20 ml×3). The organic layer was washed with a saturated solution of $NaHCO_3$ and water, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude was purified by flash chromatography (1–25% of a solution of 1% AcOH in EtOAc in petroleum ether), to furnish **17** (0.172 g, 46%) as a white amorphous solid and 0.060 g of a mixture containing **16** and **17** in a 6:1 ratio.

17: $[\alpha]_D$ −20.2 (*c* 0.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.06 (24H, s, (CH_3)$_2$CSi–), 0.67 (12H, s, CH_3-18), 0.89 (36H, s, (CH_3)$_3$Si–), 0.92 (12H, d, *J*=6.7 Hz, CH_3-21), 0.99 (12H, s, CH_3-19), 3.44 (12H, s, CH_3O–), 3.45 (4H, m, H-3), 3.54 (8H, s, Ar–CH_2–Ar), 3.63 (8H, m, –OCH$_2$CH_2O–), 3.83 (8H, m, –OCH_2CH$_2$O–), 4.92 (8H, br s, –CH_2OCO), 5.31 (1H, br s, H-6), 7.08 (8 H, s, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: −4.6 (×8), 11.8 (×4), 18.2 (×4), 18.3 (×4), 19.4 (×4), 21.0 (×4), 24.2 (×4), 25.9 (×12), 28.1 (×4), 30.9 (×4), 31.3 (×4), 31.9 (×8), 32.0 (×4), 34.8 (×4), 35.3 (×4), 36.5 (×4), 37.3 (×4), 39.7 (×4), 42.3 (×4), 42.8 (×4), 50.1 (×4), 55.8 (×4), 56.7 (×4), 58.8 (×4), 66.2 (×4), 71.2 (×4), 71.9 (×4), 72.6 (×4), 121.1 (×4), 128.9 (×4), 130.5 (×8), 133.3 (×8), 141.5 (×4), 155.7 (×4), 174.0 (×4). ES-MS, *m*/*z*: 2680.6845 (2680.7990 calcd for $C_{164}H_{256}NaO_{20}Si_4$) [MNa$^+$].

4.1.14. Compound 3. To a solution of **17** (0.098 g, 0.0368 mmol) in THF (1.0 ml) at 0 °C, a solution of 48% hydrofluoric acid in water (40 µl, 2.21 mmol) was added. The reaction mixture was stirred overnight then concentrated under a stream of N_2. The residue was purified by flash chromatography (silica gel, 1–10% methanol in $CHCl_3$) to afford **3** (0.081 g, quant.) as a white amorphous solid.

3: $[\alpha]_D$ −23.1 (*c* 0.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.65 (12H, s, CH_3-18), 0.91 (12H, d, *J*=6.7 Hz, CH_3-21), 0.99 (12H, s, CH_3-19), 3.45 (12H, s, CH_3O–), 3.50 (4H, m, H-3), 3.53 (8H, s, Ar–CH_2–Ar), 3.61 (8H, m, –OCH$_2$CH_2O–), 3.81 (8H, m, –OCH_2CH$_2$O–), 4.90 (4H, d, *J*=12.1 Hz, –CH*H*OCO), 4.93 (4H, d, *J*=12.1 Hz, –C*H*HOCO), 5.33 (1H, br d, *J*=4.8 Hz, H-6), 7.07 (8H, s, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 11.8 (×4), 18.3 (×4), 19.4 (×4), 21.0 (×4), 24.2 (×4), 28.1 (×4), 30.9 (×4), 31.6 (×4), 31.8 (×12), 34.9 (×4), 35.3 (×4), 36.4 (×4), 37.2 (×4), 39.7 (×4), 42.2, (×4), 42.3 (×4), 50.0 (×4), 55.8 (×4), 56.7 (×4), 58.8 (×4), 66.2 (×4), 71.2 (×4), 71.6 (×4), 71.8 (×4), 121.5 (×4), 128.9 (×4), 130.5 (×8), 133.3 (×8), 140.8 (×4), 155.7 (×4), 174.0 (×4). ES-MS, *m*/*z*: 2224.4679 (2224.4531 calcd for $C_{140}H_{200}NaO_{20}$) [MNa$^+$].

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