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Hydroxyl-Terminated Dendritic Oligomers from Bile Acids: Synthesis and Properties

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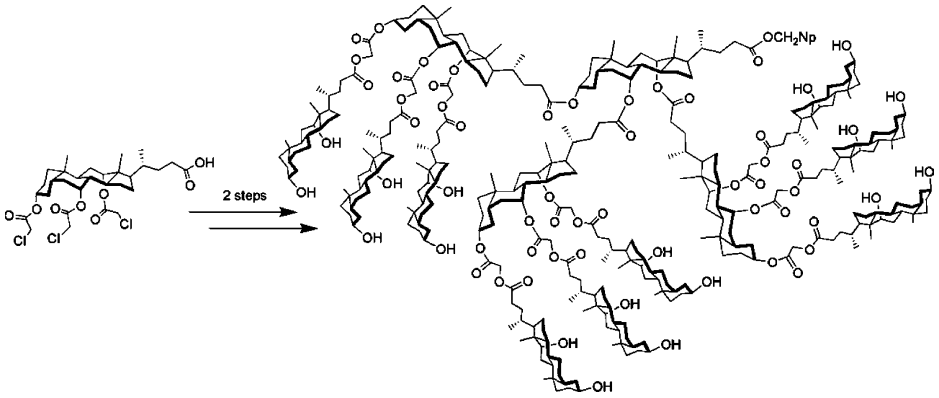

The high reactivity of the chloroacetyl group has been exploited for the synthesis of bile acid based first and second generation dendrons with multiple hydroxyl groups. The synthesis involves only a few steps and avoids the use of protecting groups for the terminal hydroxyl groups. These dendritic structures with facially amphiphilic bile acid backbones on the periphery were able to solubilize cresol red, a hydrophilic dye, in a nonpolar solvent. HPLC analysis of the dendrons suggests that hydrophobicity increases with increase in oligomer size, but in each generation, the dendrons with a higher degree of branching are less hydrophobic.

Introduction

Dendrimers are well-defined, hyperbranched macromolecules that are prepared by highly controlled iterative methodologies.[1] The ability to modulate the size, molecular weight, chemical functionalities, and the position and the number of functional groups in dendrimers make them promising candidates for diverse applications.[2] Since the first report of poly(propyleneimine) dendrimers by Vögtle et al. in 1978,[3] numerous dendritic architectures have been developed. Of particular interest are dendrimers with amphiphilic moieties that can exhibit micelle-like properties in solution and have potential applications in molecular encapsulation, drug delivery, catalysis, and nanoscopic transport.[4] The use of amphiphilic dendrimers as *unimolecular micelles* was first demonstrated in 1991 by Newkome et al,[5] who synthesized a dendrimer with an aliphatic core and charged carboxylate groups at the periphery. The micellar behavior of this dendrimer in aqueous solution was established through its ability to encapsulate hydrophobic dyes. Meijer and co-workers developed the first *inverted* dendritic unimolecular

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micelle by modification of polar poly(propyleneimine) dendrimers with apolar alkyl chains.[6] These dendrimers were able to encapsulate hydrophilic dyes such as Rose Bengal in organic media. Following these pioneering contributions, several other examples of novel amphiphilic dendritic systems have been reported.[7]

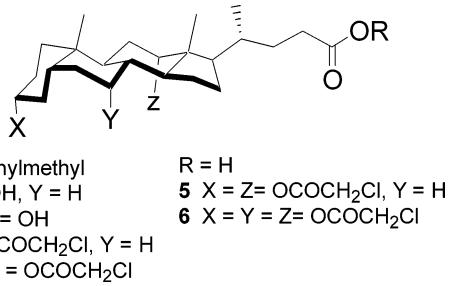
Bile acids are natural products consisting of a facially amphiphilic steroid nucleus and a polar side chain. In the salt form, they act as emulsifiers, assisting in the solubilization and absorption of fats and lipids in the small intestine. It is now well recognized that besides their biological importance, these molecules are useful starting materials in designing novel structures with potential applications in supramolecular science.[8] The synthesis of bile acid derived dendritic structures was first reported by us, followed by a recent contribution by Kolehmainen et al.[9] The dendrons we reported earlier had acetate-protected bile acid backbones at the periphery. Because of the unique facial amphiphilicity of bile acids, we reasoned that it would be interesting to design and synthesize dendrons with end groups resembling monomeric bile acid units and examine their potential amphiphilic properties. In this work, we report the synthesis of dendrons with glycolate linkers and facially amphiphilic bile acid backbones on the periphery. We also provide evidence that such molecules can exhibit reverse micellar characteristics in an organic solvent.

Results and Discussions

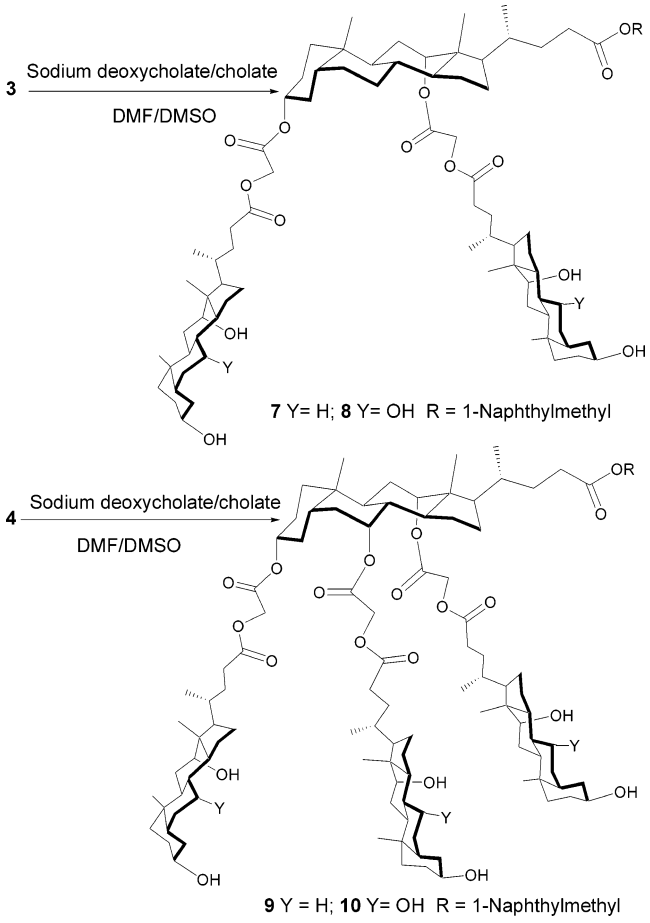
Synthesis. The construction of a bile acid based dendritic light-harvesting system was recently accomplished by us using per(chloroacetylated) bile acid based dendrons, by conveniently displacing all of the chlorides with naproxen units.[10] We further explored the possibility of directly coupling bile salts (deoxycholate/cholate) to chloroacetate-functionalized bile acid monomers and dendrons to generate dendritic structures with multiple hydroxyl groups. For the generation of reactive monomer units, the carboxyl groups of deoxycholic and cholic acid were first protected as 1-naphthylmethyl esters.[9,11] Compounds **1** and **2** (Chart 1) were subsequently chloroacetylated using $ClCH_2COCl$ in the presence of CaH_2 and $PhCH_2Et_3N^+Cl^-$ in refluxing toluene to generate compounds **3** (86%) and **4** (75%), respectively (Chart 1). Bis(chloroacetylated) monomer **3** on reaction with a slight excess of sodium deoxycholate/cholate generated the desired trimers **7** (86%) and **8** (87%), respectively. Tetramers **9** (76%) and **10** (66%) were readily synthesized from tris-(chloroacetylated) monomer **4** (Scheme 1) in an analogous manner. Thus by adopting a simple synthetic route the first generation dendrons with varying numbers of bile acid units and hydroxyl groups were synthesized in good yields. Moreover, this strategy eliminates the need for protecting groups for the terminal hydroxyls, which would have required additional steps to obtain the desired dendrons.

The same strategy was subsequently extended for the synthesis of second generation dendrons. Compounds **5** and **6**

CHART 1



SCHEME 1. Synthesis of First Generation Dendrons 7−10



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(11) The 1-naphthylmethyl group was employed for easier identification of the dendrons in the purification steps, and also for HPLC and NMR analysis.

SCHEME 2. Synthesis of Second Generation Dendrons 13 and 14

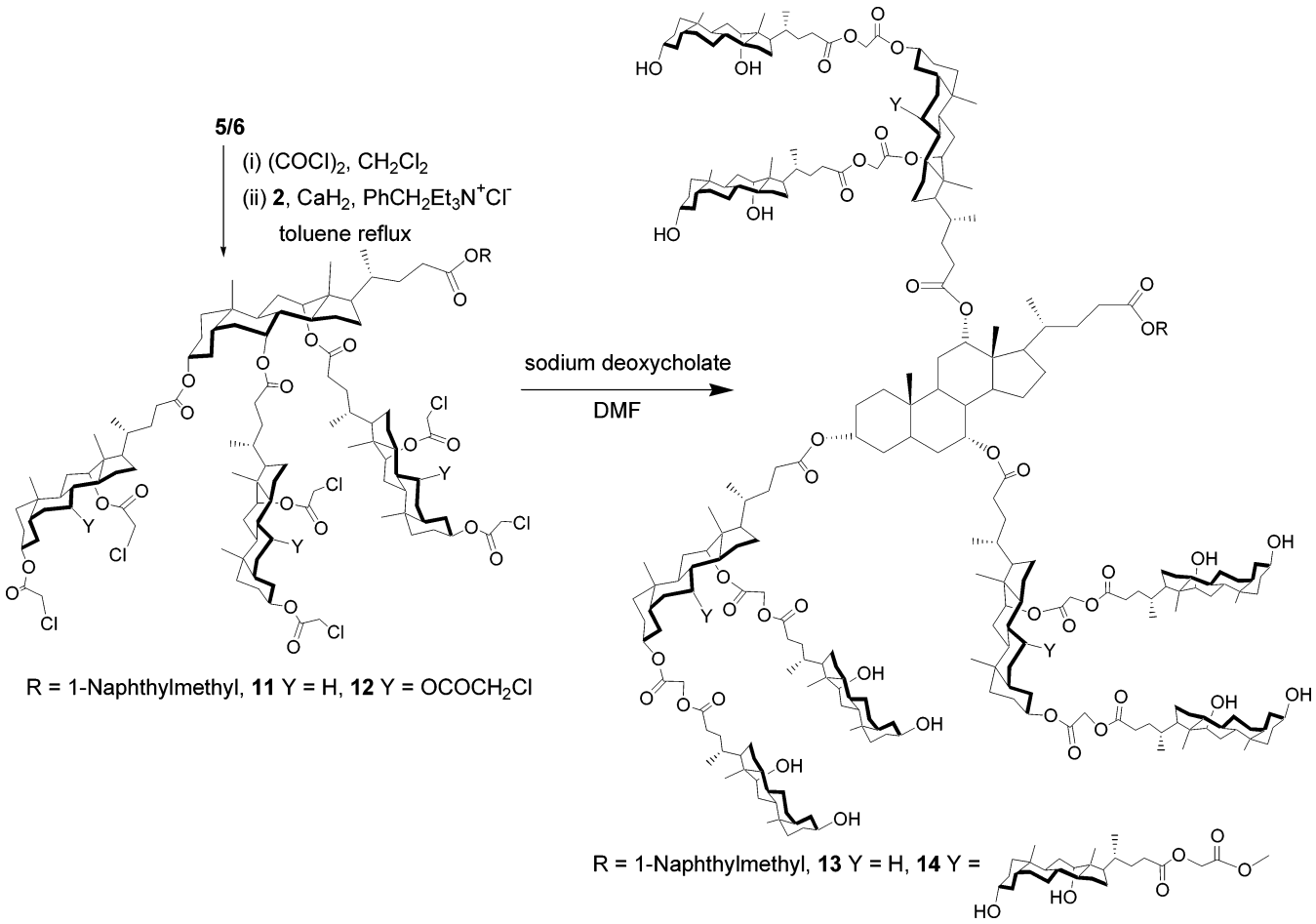

TABLE 1. HPLC Data of Bile Acid Based Dendrons

compd	solvent system	t_R (min)	t_R (min) (85% MeOH/15% THF)	k' (85% MeOH/15% THF)
7	85% MeOH/15% THF	7.5	7.5	1.53
8	MeOH	14.0	5.46	0.83
9	90% MeOH/10% THF	9.7	6.45	1.17
10	MeOH	11.0	4.77	0.6
13	80% MeOH/20% THF	16.9	55.01	17.52
14	80% MeOH/20% THF	9.0	22.96	6.73

with chloroacetyl and free carboxylic acid groups were synthesized from deoxycholic and cholic acids, respectively, by reaction with $(ClCH_2CO)_2O$ in pyridine (Chart 1). Compound **5** was then converted to its acid chloride and reacted with **2** following the Oppenauer protocol[12] to generate **11** (66%) with six functionalizable chloroacetate groups (Scheme 2). Tetramer **11** was then reacted with an excess of sodium deoxycholate to generate decamer **13** (37%). In an analogous manner tetramer **12** (67%) was synthesized starting from **6** and **2** and was subsequently reacted with sodium deoxycholate to furnish tridecamer **14** (35%) (Scheme 2). The lower yields of **13** and **14** are mainly due to irreversible adsorption on silica during purification. All of the dendritic structures were characterized by IR, ^1H and ^{13}C NMR, MALDI-TOF/ESI-MS, and HPLC.

HPLC Analysis. All dendrons were examined by HPLC using a C-18 reverse phase column, which provided information about their purity and polarity/lipophilicity. The compounds were detected by monitoring the absorbance at 280 nm (naphthylmethyl absorption), and a single peak was observed in each case (see Table 1 for retention times). Reverse phase HPLC has been used for the determination of relative hydrophobicities of different bile acids/salts (capacity factor/elution time was directly correlated to the hydrophobicity/lipophilicity of the eluted compound).[13] Here the dendrons were investigated using an isocratic flow of 85% MeOH/15% THF for a comparison of their *relative* lipophilicities (Figure 1 showing an overlay of the chromatograms). The capacity factors (k') showed (Table 1) that the order of lipophilicity was **13** $\gg$ **14** $\gg$ **7** > **9** > **8** > **10**. It was observed that lipophilicity of the dendrons increased with an increase in the oligomer size (increasing capacity factors). Interestingly, with increasing number of branching units, in a given generation, the capacity factor decreased, suggesting a decrease in lipophilicity. For example, the capacity factor of dendron **7** is 1.53 in 85% MeOH/15% THF, whereas that of dendron **9** is 1.17. Also, the elution time of the dendrons is highly sensitive to the polarity of the

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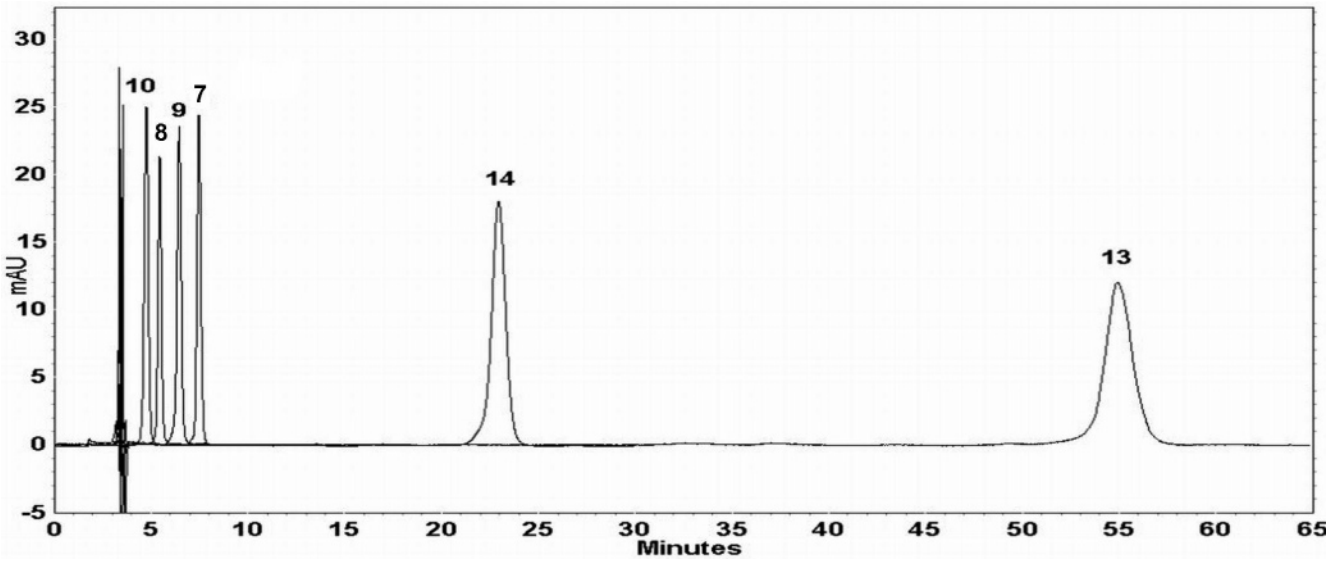

FIGURE 1. Overlay of the HPLC profiles of the dendrons in 85% MeOH/15% THF.

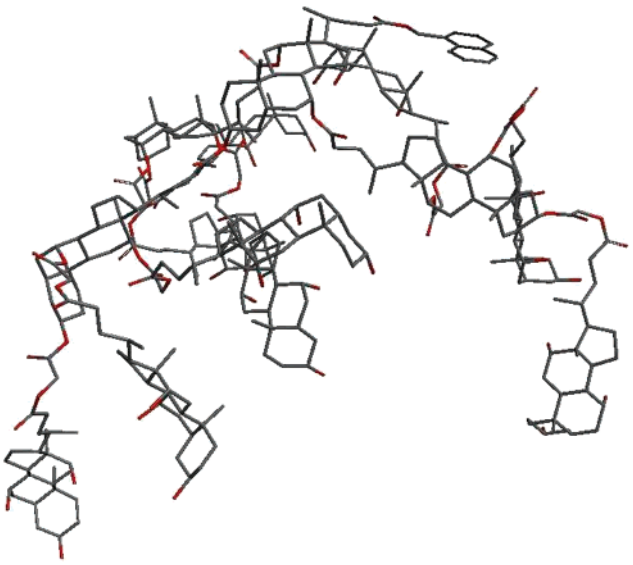

FIGURE 2. Representation of one of the several possible conformations of **14**. H atoms are omitted for clarity.

eluent, particularly for **13** and **14**. A change of eluent from 80% MeOH/20% THF to 85% MeOH/15% THF increased the elution time of **14** by 14 min, and that of **13** increased by 38.1 min.

Molecular Modeling. Structural optimization of the dendritic molecules in vacuum was performed using molecular mechanics method to get information about size and shape.[14] Modeling suggests that these molecules have dimensions of the order of 2.0−4.0 nm. One such possible conformation of **14** is shown in Figure 2.

Dye Solubilization Studies. The dendritic structures were readily soluble in THF and chloroform. These molecules are conformationally flexible and have facially amphiphilic peripheral branching units. We reasoned, therefore, that they can adopt a reverse-micelle-like conformation in such nonpolar solvents, with the hydrophobic faces turned outward (toward the solvent).[15] Hence, such dendrons are interesting candidates for solubilizing hydrophilic substances in nonpolar solvents. We investigated the extraction of the monosodium salt of cresol red (CR), a hydrophilic dye (inset, Figure 3) in chloroform. The solubilization of CR by the dendrons in $CHCl_3$ was carried out by solid−liquid extraction protocol.

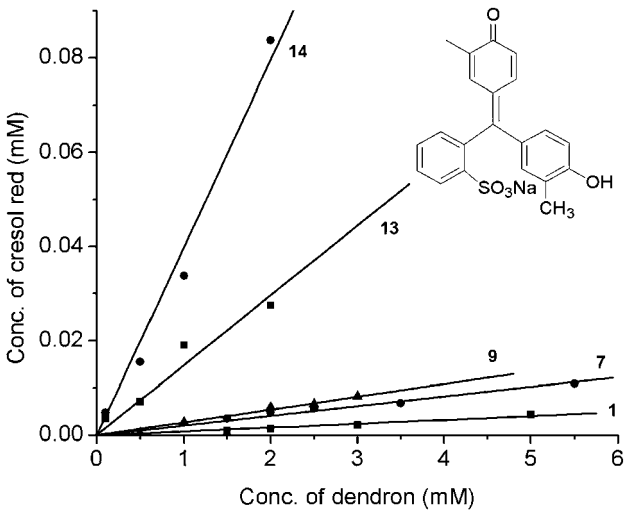

FIGURE 3. Solubilization of CR (inset) by dendrons **7**, **9**, **13**, and **14** and monomer **1**. Straight lines are line fitting of the experimental data.

The dye was observed to have a negligible solubility in chloroform in the absence of any additive. The dendritic structures solubilized the dye to varying extents. We first studied the extraction ability of dendrons **7**, **9**, **13**, and **14**, which have deoxycholate units at the periphery. In general, the amount of dye solubilized increased linearly with the concentration of dendrons. Compounds **7** and **9** showed much less extraction as compared to **13** and **14** (Figure 3). A control experiment performed with monomer **1** showed poor dissolution of the dye (0.8 μM per mM of monomer). The relative solubilization of the dye by the dendritic compounds when compared to the

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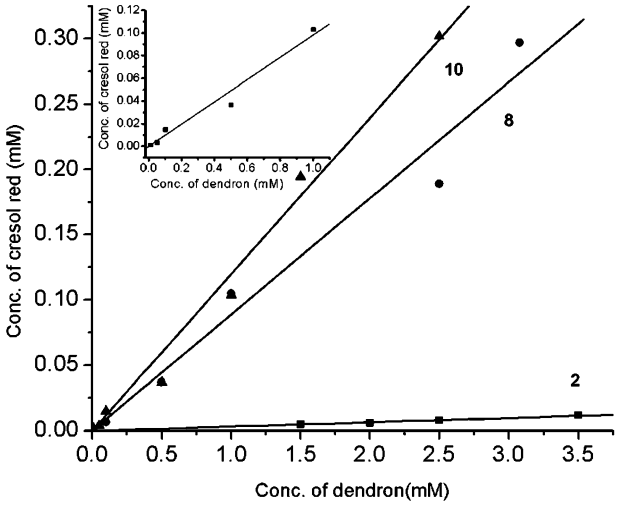

FIGURE 4. Solubilization of CR by dendrons **8** and **10** and monomer **2**. Inset shows the linear increase in solubilization of the dye at 0.01−1 mM of dendron **10**.

monomeric analogue exhibited an interesting pattern. Although the first generation dendrons showed only a slight increase compared to the monomer, the second generation dendrons exhibited much better dye uptake (Figure 3). Dendrons **13** and **14** showed 20- and 50-fold increase, respectively, in the solubilization of CR. These results suggest that the dendritic branching in **13** and **14** led to better encapsulation of the dye.

We then investigated the degree of dye uptake by the dendrons with cholate as branching units (**8** and **10**) using **2** as control. The concentration of the extracted CR in the organic layer increased linearly with the concentration of the dendrons, and efficient solubilization of the dye (relative to monomer **2**) was observed (Figure 4). Interestingly, the extraction efficiency of cholate-based dendrons was much higher than that of deoxycholate counterparts **7** and **9**. This efficient dye solubilization by the dendrons with cholate branching units indicates that the 7-OH probably induces an additional hydrogen-bonding with CR, which may result in stronger binding of the dye to **8** and **10**. Also, it is known that cholic acid is more facially amphiphilic as compared to deoxycholic acid because of the additional 7-OH. Hence, the reverse-micelle-like conformations are probably more stable in the case of dendrons with cholate branching units, which leads to increased solubilization of the dye.

Conclusion

We demonstrate that using simple chemical transformations it is possible to design highly flexible dendritic structures with bile acid both in the core and in branching units possessing multiple hydroxyl groups at the periphery. These dendrons exhibited the ability to solubilize a hydrophilic dye in a nonpolar solvent, suggesting that such molecules can be further explored as encapsulating agents in organic media. The dendritic structures are composed of biocompatible building blocks and easily hydrolyzable ester bonds, which also makes them attractive candidates for biological and drug delivery applications. Moreover, these molecules can be further functionalized by modification of the peripheral hydroxyl groups.

Experimental Section

Dye Solubilization Studies. A solution of known concentration of the dendron in $CHCl_3$ (1 mL) was mixed with solid CR (15 mg) and stirred for 24 h at 24.5 °C (±0.5). The resulting solution was diluted to 2 mL in the case of **1**, **2**, **7**, **9**, **13**, and **14** and 5 mL in the case of **8** and **10**. All solutions were filtered through 0.5 μm PTFE membrane filters. The degree of dye uptake was monitored by UV−vis spectroscopy. The concentration of cresol red in $CHCl_3$ (in the presence of dendron/monomer) was calculated using the molar extinction coefficient (14700) at 420 nm in 1% EtOH/$CHCl_3$. In the presence of the bile acid monomer/dendron the absorption maxima of the dye was observed at 414 ± 2 nm.

Synthesis. Compounds **1** and **2** were synthesized following reported procedures.[9]

1-Naphthylmethyl 3α,12α-Bis(chloroacetyloxy)-5β-cholan-24-oate, 3. To a solution of compound **1** (0.50 g, 0.94 mmol) in toluene (4 mL) were added CaH_2 (0.177 g, 4.2 mmol), $PhCH_2Et_3N^+Cl^-$ (0.068 g, 0.3 mmol), and $ClCH_2COCl$ (0.25 mL, 3.14 mmol). After the reaction mixture was refluxed for 3 h, it was cooled, diluted with $CHCl_3$ (5 mL), and filtered through Celite. The crude product obtained after removal of solvent was chromatographed on silica gel using 10% EtOAc/hexanes to yield 0.56 g (86%) of compound **3** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.01 (d, 1H, J = 7.5 Hz), 7.91−7.82 (m, 2H), 7.59−7.43 (m, 4H), 5.59 (d, 1H, J = 12.3 Hz), 5.54 (d, 1H, J = 12.3 Hz) 5.15 (s, 1H), 4.83−4.75 (m, 1H), 4.03 (s, 4H), 2.44−2.21 (m, 2H), 1.91−0.97 (m), 0.91 (s, 3H); 0.78 (d, 3H, J = 5.7 Hz), 0.66 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.0, 166.8, 166.6, 133.7, 131.7, 131.5, 129.3, 128.7, 127.6, 126.5, 126.0, 125.3, 123.6, 78.0, 76.3, 64.4, 49.3, 47.4, 45.1, 41.7, 41.2, 41.1, 35.6, 34.62, 34.59, 34.3, 34.0, 31.9, 31.2, 30.8, 27.3, 26.8, 26.3, 25.8, 25.5, 23.4, 22.9, 17.5, 12.3. IR (film, cm^{-1}): 2950, 2870, 1733, 1291, 1172, 971, 793. HRMS-ESI: m/z calcd for $C_{39}H_{50}Cl_2O_6 + Na^+$ 707.288, obsd 707.285.

Trimer (4 OH, COOCH$_2$Np), 7. To a solution of compound **3** (0.1 g, 0.15 mmol) in DMF (2 mL) was added sodium deoxycholate (0.18 g, 0.44 mmol), and the reaction mixture was stirred at 60 °C for 7 h. The reaction mixture was then cooled, poured into water (30 mL), and extracted with $CHCl_3$ (50 mL). The organic layer was washed with H_2O (30 mL) and aqueous $NaHCO_3$ solution (30 mL). The crude product obtained after removal of the solvent was chromatographed on silica gel using 2−3% EtOH/$CHCl_3$ to yield 0.18 g (86%) of compound **7** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.02 (d, 1H, J = 8.4 Hz), 7.90−7.84 (m, 2H), 7.58−7.43 (m, 5H), 5.57−5.53 (unresolved doublets, 2H), 5.15 (s, 1H), 4.78−4.52 (m, 5H), 3.98 (s, 2H), 3.64−3.57 (m, 2H), 2.51−2.31 (m, 6H), 1.84−0.97 (m), 0.99−0.65 (angular methyls). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.0, 173.6, 173.1, 167.4, 167.2, 133.6, 131.6, 131.5, 129.2, 128.6, 127.4, 126.5, 125.8, 125.2, 123.5, 75.5, 73.0, 71.6, 64.3, 60.8, 60.7, 49.3, 48.1, 47.5, 47.1, 47.0, 46.4, 45.0, 42.0, 41.7, 36.3, 35.9, 35.5, 35.2, 35.1, 34.6, 34.3, 34.1, 33.95, 33.5, 32.0, 31.2, 30.8, 30.7, 30.3, 28.5, 27.5, 27.1, 26.8, 26.7, 26.2, 26.1, 25.9, 25.5, 23.6, 23.3, 23.1, 22.9, 17.3, 17.24, 17.17, 12.6, 12.2. IR (film, cm^{-1}): 3401, 2939, 2864, 1741, 1153, 1041. LRMS-ESI: m/z calcd for $C_{87}H_{128}O_{14} + Na^+$ 1419.9, obsd 1420. $[\alpha]^{25}_D$ +56.9 (c 1.18, $CHCl_3$). Anal. Calcd for $C_{87}H_{128}O_{14}$: C 74.75, H 9.23. Found: C 74.68, H 9.07.

Trimer (6 OH, COOCH$_2$Np), 8. To a solution of compound **3** (0.20 g, 0.30 mmol) in DMSO (2 mL) was added sodium cholate (0.38 g, 0.89 mmol), and the reaction mixture was stirred at 60 °C for 7 h. The reaction mixture was then cooled, poured into water (50 mL), and extracted with EtOAc (70 mL). The organic layer was washed with H_2O (50 mL) and aqueous $NaHCO_3$ solution (70 mL). The crude product obtained after removal of the solvent was chromatographed on silica gel using 6−11% EtOH/$CHCl_3$ to yield 0.47 g (87%) of compound **8** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.01 (d, 1H, J = 7.6 Hz,), 7.88−7.83 (m, 2H), 7.56−7.42 (m, 4H), 5.59 (d, 1H, J = 12.6 Hz), 5.55 (d, 1H, J = 12.9 Hz), 5.12 (s, 1H), 4.75 (m, 1H), 4.66−4.51 (m, 4H), 3.96 (s, 2H), 3.83 (s, 2H), 3.42 (m, 2H), 2.47−0.99 (m), 0.94−0.63 (angular methyls). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.0, 173.7, 173.2, 167.5, 167.3, 133.7, 131.6, 131.5, 129.2, 128.6, 127.4, 126.5, 125.9, 125.2, 123.5, 77.2, 75.6, 73.0, 71.8, 68.3, 64.4, 60.9, 49.3, 47.5,

46.8, 46.7, 46.4, 46.3, 45.0, 41.7, 41.4, 39.40, 39.38, 35.5, 35.33, 35.26, 35.23, 35.20, 34.73, 34.70, 34.63, 34.59, 34.21, 34.17, 34.0, 32.0, 31.2, 30.9, 30.8, 30.73, 30.71, 30.65, 30.62, 30.60, 30.57, 30.29, 30.25, 30.2, 28.13, 28.06, 28.0, 27.52, 27.48, 27.46, 27.2, 26.76, 26.75, 26.2, 25.97, 25.9, 25.8, 25.42, 25.41, 23.34, 23.27, 23.23, 23.19, 22.9, 22.4, 17.3, 17.2, 12.4, 12.2. IR (film, cm^{-1}): 3394, 2937, 2867, 1741, 1153, 737. LRMS-ESI: *m*/*z* calcd for $C_{87}H_{128}O_{16} + Na^+$ 1451.9, $C_{87}H_{128}O_{16} + K^+$ 1467.9, obsd 1452.2, 1468.2. Anal. Calcd for $C_{87}H_{128}O_{16}$: C 73.08, H 9.02. Found: C 72.79, H 8.69. $[\alpha]^{25}_D$ +49.1 (*c* 1.63, $CHCl_3$).

1-Naphthylmethyl 3α,7α,12α-Tris(chloroacetyloxy)-5β-cholan-24-oate, 4. The procedure for the synthesis of **3** was followed. From **2** (0.997 g, 1.82 mmol) was obtained 1.06 g (75%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.01 (d, 1H, J = 7.8 Hz), 7.91−7.85 (m, 2H), 7.55−7.42 (m, 4H), 5.59 (d, 1H, J = 12.1 Hz), 5.54 (d, 1H, J = 12.3 Hz), 5.16 (s, 1H), 5.02 (s, 1H), 4.67 (m, 1H), 4.11−4.02 (m, 6H), 2.43−1.02 (m), 0.93 (s, 3H), 0.79 (d, 3H, J = 5.4 Hz), 0.66 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.8, 166.8, 166.5, 166.2, 133.7, 131.6, 131.5, 129.3, 128.7, 127.6, 126.5, 125.9, 125.2, 123.50, 77.2, 75.8, 73.0, 64.4, 47.2, 45.1, 42.8, 41.14, 41.06, 40.5, 37.8, 34.44, 34.41, 34.33, 34.31, 34.2, 31.12, 31.07, 30.6, 28.4, 27.0, 26.4, 25.1, 22.8, 22.2, 17.4, 11.9. IR (film, cm^{-1}): 2953.4, 2871, 1731.7, 1291.1, 1186, 1005. LRMS-ESI: *m*/*z* calcd for $C_{41}H_{51}Cl_3O_8 + Na^+$ 799.3, $C_{41}H_{51}Cl_3O_8 + K^+$ 815.2, obsd 799, 815. Anal. Calcd for $C_{41}H_{51}Cl_3O_8$: C 63.28, H 6.67. Found: C 63.32, H 6.66.

Tetramer (6 OH, $COOCH_2Np$), 9. From **4** (0.10 g, 0.13 mmol) was obtained 0.16 g (66%) of the title compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.00 (d, 1H, J = 7.6 Hz), 7.88−7.83 (m, 2H), 7.56−7.42 (m, 4H), 5.59 (d, 1H, J = 12.1 Hz), 5.55 (d, 1H, J = 12.3 Hz), 5.08 (s, 1H), 4.98 (s, 1H), 4.77−4.45 (m, 7H), 3.95 (s, 3H), 3.80 (s, 3H), 3.40 (m, 3H), 2.47−0.971 (m), 0.90−0.63 (angular methyls). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.9, 173.52, 173.45, 173.3, 167.5, 167.2, 133.8, 131.7, 129.2, 128.7, 127.4, 126.6, 126.0, 125.3, 123.6, 75.3, 73.1, 72.2, 71.8, 64.4, 61.0, 60.80, 60.75, 48.2, 47.5, 47.3, 47.2, 46.6, 45.3, 43.3, 42.2, 40.8, 37.9, 36.5, 36.1, 35.4, 35.3, 35.2, 34.7, 34.6, 34.3, 34.2, 33.7, 31.4, 31.2, 31.1, 31.0, 30.8, 30.6, 28.9, 28.7, 27.5, 27.2, 27.1, 26.6, 26.2, 25.5, 23.7, 23.2, 22.7, 22.5, 17.4, 12.7, 12.1. IR (film, cm^{-1}): 3441, 2938, 2865, 1742, 1449, 1382, 1151, 1043. MALDI-TOF: *m*/*z* calcd for $C_{113}H_{168}O_{20} + Na^+$ 1869.5, $C_{113}H_{168}O_{20} + K^+$ 1885.6, obsd 1868.8, 1884.1. Anal. Calcd for $C_{113}H_{168}O_{20}$: C 73.5, H 9.17. Found: C 73.47, H 8.89. $[\alpha]^{25}_D$ +55.1 (*c* 1.11, $CHCl_3$).

Tetramer (9-OH, $COOCH_2Np$), 10. In an analogous manner **4** (0.29 g, 0.37 mmol) yielded 0.54 g (76%) of the title compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.00 (d, J = 8 Hz, 1H), 7.88−7.83 (m, 2H), 7.56−7.42 (m, 4H), 5.59 (d, 1H, J = 12.1 Hz), 5.54 (d, 1H, J = 12.3 Hz), 5.13 (s, 1H), 4.99 (s, 1H), 4.69−4.53 (m, 7H), 3.97 (s, 3H), 3.58 (m, 3H), 2.48−0.97 (m), 0.90−0.63 (angular methyls). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.9, 173.2, 167.5, 167.4, 167.3, 133.8, 131.7, 129.2, 128.7, 127.4, 126.5, 126.0, 125.3, 123.6, 73.2, 73.0, 72.1, 72.03, 71.95, 68.4, 64.4, 61.3, 60.9, 60.8, 47.5, 47.1, 47.0, 46.52, 46.48, 45.3, 43.2, 41.6, 40.7, 39.7, 39.6, 37.9, 35.5, 34.8, 34.7, 34.6, 34.4, 34.3, 31.4, 31.2, 31.0, 30.9, 30.4, 30.8, 28.7, 28.2, 27.7, 27.1, 26.4, 25.2, 23.3, 22.7, 22.5, 17.4, 17.2, 12.5, 12.0. IR (film, cm^{-1}): 3421, 2938, 2868, 1742, 1466, 1383, 1294, 1150, 1076. MALDI-TOF MS: *m*/*z* calcd for $C_{113}H_{168}O_{23} + Na^+$ 1917.5, $C_{113}H_{168}O_{23} + K^+$ 1933.6, obsd 1915.1, 1930.2. $[\alpha]^{25}_D$ +48.6 (*c* 1.06, $CHCl_3$).

3α,12α-Bis(chloroacetyloxy)-5β-cholanic Acid, 5. To an ice-cooled suspension of deoxycholic acid (0.56 g, 1.43 mmol) in pyridine (2.5 mL) was added chloroacetic anhydride (0.74 g, 4.33 mmol), and the mixture was stirred for 45 min at 0 °C. The reaction mixture was poured into 10% HCl (30 mL) and extracted with EtOAc (50 mL). The organic layer was washed with 10% HCl (50 mL), water (40 mL), and aqueous $NaHCO_3$ solution (40 mL) and dried over anhydrous Na_2SO_4. The crude product obtained after evaporation of the solvent was chromatographed on silica gel using 3−5% EtOAc/$CHCl_3$ to yield 0.58 g (75%) of compound **5** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.20 (s, 1H), 4.79 (m, 1H), 4.08 (s, 2H), 4.04 (s, 2H), 2.45−2.19 (m, 2H), 1.96−0.98 (m), 0.92 (s, 3H), 0.83 (d, 3H, J = 6.3 Hz), 0.75 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 180.1, 166.8, 166.6, 78.0, 76.3, 49.3, 47.4, 45.1, 41.7, 41.2, 41.1, 35.5, 34.6, 34.5, 34.3, 34.0, 31.9, 30.8, 30.5, 27.3, 26.7, 26.3, 25.8, 25.5, 23.3, 22.9, 17.4, 12.3. IR (film, cm^{-1}): 2945, 1728, 1296, 1184. LRMS-ESI: *m*/*z* calcd for $C_{28}H_{42}Cl_2O_6 + Na^+$ 567.2, $C_{28}H_{42}Cl_2O_6 + K^+$ 583.2, obsd 567, 583. Anal. Calcd for $C_{28}H_{42}Cl_2O_6$: C 61.65, H 7.76. Found: C 61.80, H, 7.82.

Tetramer (6 Cl, $COOCH_2Np$), 11. To a solution of compound **5** (0.49 g, 0.91 mmol) in toluene (4 mL) was added oxalyl chloride (0.13 g, 1.49 mmol) followed by DMF (6 μL), and the reaction mixture was stirred at room temperature for 1 h. Volatiles were removed under vacuum, and to the crude acid chloride were added compound **2** (0.088 g, 0.154 mmol), CaH_2 (0.037 g, 5.8 mmol), and $PhCH_2Et_3N^+Cl^-$ (0.013 g, 0.40 mmol). The reaction mixture was refluxed for 48 h, cooled, and filtered through Celite, and the crude product obtained after removal of the solvent was chromatographed on silica gel using 3−4% EtOAc/$CHCl_3$ to yield 0.24 g (66%) of compound **11** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.0 (d, 1H, J = 7.6 Hz), 7.9−7.84 (m, 2H), 7.55−7.43 (m, 4H), 5.59 (s, 2H), 5.18 (s, 3H), 5.05 (s, 1H), 4.90 (s, 1H), 4.79 (m, 3H), 4.55 (m, 1H), 4.08−4.04 (m, 12H), 2.31−1.09 (m), 1.04−0.64 (angular methyls). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 173.7, 173.5, 173.3, 173.1, 166.7, 166.5, 166.40, 166.36, 133.6, 131.5, 131.4, 129.2, 128.7, 127.4, 126.5, 125.9, 125.2, 123.4, 77.9, 77.2, 76.2, 73.9, 64.4, 49.2, 47.7, 47.6, 47.4, 45.11, 45.08, 45.0, 43.3, 41.6, 41.1, 40.8, 37.7, 35.5, 34.9, 34.7, 34.6, 34.5, 34.2, 33.9, 31.9, 31.62, 31.60, 31.5, 31.43, 31.42, 31.3, 31.1, 30.89, 30.87, 30.8, 28.7, 27.3, 27.22, 27.19, 26.7, 26.3, 25.8, 25.49, 25.45, 25.3, 23.4, 22.8, 22.5, 17.54, 17.49, 17.40, 17.35, 12.3, 12.0. IR (film, cm^{-1}): 2946, 2868, 1732, 1290, 1174, 737. ESI-MS: *m*/*z* calcd for $C_{119}H_{168}Cl_6O_{20} + Na^+$ 2150, $C_{119}H_{168}Cl_6O_{20} + K^+$ 2165.99, obsd 2151, 2166. Anal. Calcd for $C_{119}H_{168}Cl_6O_{20}$: C 67.06, H 7.95. Found: C 67.01, H, 8.10. $[\alpha]^{25}_D$ +104.3 (*c* 0.94, $CHCl_3$).

Decamer (12 OH, $COOCH_2Np$), 13. To a solution of compound **11** (0.041 g, 0.020 mmol) in DMF (2 mL) was added sodium deoxycholate (0.097 g, 0.39 mmol), and the reaction mixture was stirred at 60 °C for 24 h. DMF was removed under high vacuum, and the residue was extracted with 1:1 MeOH/$CHCl_3$ (40 mL). The solution was washed with aqueous $NaHCO_3$ (20 mL) and the lower organic layer was dried over anhydrous Na_2SO_4. The crude product obtained after removal of solvent was purified by preparative thin-layer chromatography (silica) using 9% EtOH/$CHCl_3$ to yield 0.030 g (37%) of compound **13** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.00 (d, 1H, J = 8.1 Hz), 7.91−7.84 (m, 2H)), 7.55−7.43 (m, 5H), 5.57 (unresolved doublets, 2H), 5.18 (s, 3H), 5.04 (s, 1H), 4.90 (s, 1H), 4.77−4.51 (m, 16H), 3.98 (s, 6H), 3.59 (m, 6H), 2.45−2.18 (m), 1.83−0.99 (m), 0.90−0.63 (angular methyls). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 173.6, 173.2, 167.5, 167.2, 133.8, 131.6, 131.5, 129.2, 128.8, 127.4, 126.5, 125.9, 125.3, 123.5, 77.2, 75.6, 73.1, 71.7, 60.9, 49.4, 48.7, 47.6, 47.2, 46.5, 45.1, 42.1, 41.8, 41.0, 36.4, 36.0, 35.6, 35.3, 34.7, 34.4, 34.1, 33.6, 31.6, 31.3, 29.3, 28.6, 27.6, 27.2, 26.8, 26.2, 25.6, 23.7, 23.1, 23.0, 22.63, 17.45, 17.42, 17.4, 17.3, 12.7, 12.4. IR (film, cm^{-1}): 3413, 2935, 2865, 1741, 1153, 738. MALDI-TOF MS: *m*/*z* calcd for $C_{263}H_{402}O_{44} + Na^+$ 4290.97, obsd 4293.9. Anal. Calcd for $C_{263}H_{402}O_{44}$: C 74.01, H 9.49. Found: C 73.58, H, 9.38. $[\alpha]^{25}_D$ + 56.2 (*c* 0.9, $CHCl_3$).

3α,7α,12α-Tris(chloroacetyloxy)-5β-cholanic Acid, 6. The procedure for the synthesis of **5** was followed. From cholic acid (0.80 g, 1.97 mmol) was obtained 1 g (79%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.21 (s, 1H), 5.04 (s, 1H), 4.67 (m, 1H), 4.13−4.03 (6H), 2.43−1.05 (m), 0.95 (s, 3H), 0.85 (d, 3H, J = 6.3 Hz), 0.76 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 179.8, 166.8, 166.6, 77.3, 75.8, 73.1, 47.2, 45.1, 42.8, 41.2, 41.1, 40.5, 37.9, 34.5, 34.4, 34.3, 34.2, 31.1, 30.7, 30.4, 28.5, 27.1, 26.4, 25.1, 22.8, 22.3, 17.5, 12.0. IR (film, cm^{-1}): 3436, 2954, 2872, 1732, 1293, 1189, 1006, 969, 789. LRMS-ESI: *m*/*z* calcd

for $C_{30}H_{43}Cl_3O_8 + Na^+$ 659.23, obsd 659. Anal. Calcd for $C_{30}H_{43}Cl_3O_8$: C 56.48, H 6.79. Found: C 56.55, H, 6.77.

Tetramer (9 Cl, $COOCH_2Np$), 12. The procedure for the synthesis of **11** was followed. From **6** (1.65 g, 2.59 mmol) and **2** (0.25 g, 0.454 mmol) was obtained 0.62 g (67%) of the title compound as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.00 (d, $J = 7.8$ Hz), 7.9−7.85 (m, 2H), 7.53 −7.46 (m, 4H), 5.59 (d, 1H, $J = 12.3$ Hz), 5.54 (d, 1H, $J = 12.6$ Hz), 5.20 (s, 3H), 5.04 (s, 4H), 4.89 (s, 1H), 4.67 (m, 1H), 4.54 (m, 1H), 4.11−4.03 (m, 18H), 2.34−1.05 (m), 0.94−0.64 (angular methyls). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.7, 173.4, 173.2, 173.0, 166.8, 166.5, 166.2, 133.8, 131.7, 129.3, 128.8, 127.5, 126.6, 126.0, 125.3, 123.6, 75.9, 75.3, 74.1, 73.1, 70.6, 64.4, 47.7, 47.63, 47.58, 47.5, 45.3, 45.2, 43.4, 42.9, 41.2, 40.7, 38.0, 37.9, 35.1, 34.6, 34.4, 31.6, 31.3, 31.0, 30.81, 30.76, 30.6, 29.7, 28.9, 28.6, 27.2, 27.1, 26.49, 25.52, 25.3, 22.9, 22.6, 22.3, 17.7, 17.6, 17.5, 12.1. IR (film, cm^{-1}): 2950, 1729, 1469, 1288, 1176. MALDI-TOF MS: *m*/*z* calcd for $C_{143}H_{171}Cl_9O_{26} + Na^+$ 2431.7, $C_{143}H_{171}Cl_9O_{26} + K^+$ 2447.9, obsd 2431.7, 2447.7. Anal. Calcd for $C_{143}H_{171}Cl_9O_{26}$: C 62.33, H 7.16. Found: C 61.98, H 7.25. $[\alpha]^{25}_D$ +78.3 (*c* 0.83, $CHCl_3$)

Tridecamer (18 OH, $COOCH_2Np$), 14. The procedure for the synthesis of **13** was followed. From **12** (0.05 g, 0.02 mmol) was obtained 0.030 g (35%) of the title compound as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.0 (d, 1H, $J = 7.5$ Hz), 7.89−7.84 (m, 2H), 7.62−4.43 (m), 5.57 (unresolved doublets, 2H), 5.17 (s, 3H), 5.04−5.00 (m, 4H), 4.90 (s, 1H), 4.72−4.57 (m, 22H), 3.98 (s, 9H), 3.58 (s, 9H), 2.45−2.35 (m), 1.82 0.98 (m), 0.90−0.62 (angular methyls). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.8, 173.6, 173.53, 173.45, 173.4, 173.3, 173.2, 167.5, 167.4, 167.3, 167.2, 133.72, 131.60, 131.55, 129.2, 128.7, 127.4, 126.5, 126.0, 125.3, 123.5, 77.2, 75.2, 73.1, 73.02, 72.95, 71.7, 64.4, 61.01, 60.8, 48.0, 47.2, 47.1, 46.5, 45.3, 45.1, 42.1, 41.0, 37.8, 36.4, 35.5, 35.3, 35.0, 34.7, 34.5, 34.4, 34.3, 34.1, 33.5, 31.3, 31.1, 31.0, 30.9, 30.7, 29.1, 29.8, 28.6, 27.6, 27.2, 26.5, 26.2, 25.5, 23.8, 23.1, 22.7, 22.6, 22.5, 17.4, 17.31, 17.29, 17.2, 12.69, 12.66, 12.20, 12.17. IR (film, cm^{-1}): 3397, 2938, 2865, 1742, 1150, 1041, 735. MALDI-TOF MS: *m*/*z* calcd for $C_{341}H_{522}O_{62} + Na^+$ 5636.8, obsd 5642.5. $[\alpha]^{25}_D + 53.1$ (*c* 1.13, $CHCl_3$).

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Supporting Information Available: General experimental methods and MALDI-TOF spectrum of compound **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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