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Design, synthesis, and micellar properties of bile acid dimers and oligomers linked with a 1,2,3-triazole ring

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Abstract—1,3-Dipolar cycloaddition of propargyl esters of bile acids to azide group attached at different positions of bile acids gave dimers, trimer, and tetramer linked with 1,2,3-triazole. These dimeric and oligomeric structures were able to solubilize hydrophilic dye—cresol red, in nonpolar solvent.

1. Introduction

Bile acids are versatile building blocks for the design and synthesis of macrocyclic and open chain supramolecular hosts.[1] Bile acids and their derivatives are also important compounds from the pharmaceutical point of view, as has been reviewed recently by Virtanen and Kolehmainen.[2] Bile acids are a valuable group of compounds due to their large, rigid, and curved steroidal skeletons, chemically different hydroxy groups, enantiomeric purities, and their unique amphiphilicity, together with their availability and low cost. Some bile acid derivatives have been able to act as organogelators,[3–6] which are important compounds in the field of nanotechnology. Bile acids in their crystal lattice are known to show inclusion complexation of *N*-nitrosamine and *trans*-azobenzene.[7] In their dimeric form, bile acids or their derivatives show inclusion of methanol,[8a] carbohydrate,[8b] perylene without micelle formation,[8c] and DNA.[8d] Such dimers also act as artificial ionophores and are found to be potential receptors for neutral molecules or metal cations.[9] Recently, we have synthesized bile acid dimers having antifungal and antiproliferative activities.[10] The configuration of dendritic structure and steroidal moieties in the same molecule might give rise to potential molecular assemblies with many interesting nano-scale applications, including organ-targeted drug carriers, artificial ion channels, and molecular switches. Maitra et al. reported the first bile acid-based chiral dendrons,[11] through the use of acetoxy-functionalized cholic acid and deoxycholic acid as starting materials in the preparation of a heptamer, a nonamer, and a decamer. Such molecules exhibited reverse micellar characteristics in an organic solvent and were able to encapsulate hydrophobic and hydrophilic dyes.[12]

1,2,3-Triazole moieties are attractive connecting units, since they are stable to metabolic degradation and are capable of hydrogen bonding, which can be favorable in binding of biomolecular targets and for solubility.[13] There is a recent review on the use of 1,2,3-triazole as a linker in supramolecular chemistry, drug candidates, and materials science.[14]

For the synthesis of 1,2,3-triazoles, cycloaddition of azides and alkynes is a useful transformation.[15] In continuation of our work on bile acids[16] we have reported the synthesis of bile acid dimers linked with a 1,2,3-triazole ring at C-3, C-11, and C-24 positions using 'click chemistry'.[16a] As an extension of this work we have now synthesized bile acid dimers containing 1,2,3-triazole at C-3α, C-3β, and C-24 positions, trimeric, and tetrameric compounds having small linkages and studied their micellar properties using hydrophilic dye cresol red (CR).

2. Results and discussion

In our earlier work we reported the synthesis of dimers linked with 1,2,3-triazole at C-3α, and C-24 position of deoxycholic acid and C-11 position of cholic acid using 1,3-dipolar cycloaddition in *t*-BuOH/H_2O using Cu(I) as a catalyst.[16a] Now we wish to report the synthesis of bile acid dimers **13**–**18**, a trimer **23**, and a tetramer **24** using microwave assisted click reaction, having a 1,2,3-triazole linker and facially amphiphilic bile acid backbone on

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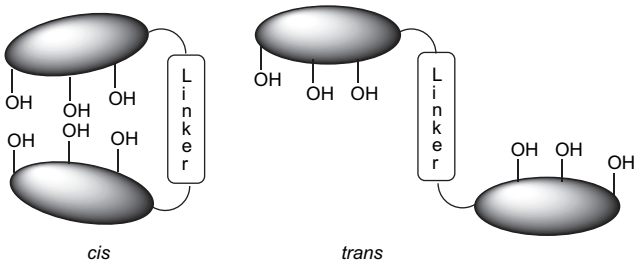


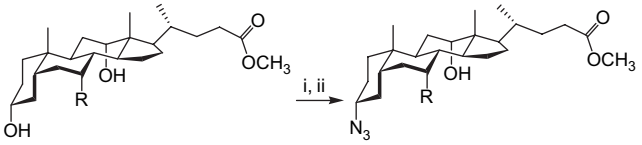
Figure 1.

periphery. Steroidal trimeric, tetrameric, and dendritic compounds are known[12] to show encapsulation of hydrophilic and hydrophobic dyes. In the trimer two bile acid units are attached to the α face (C-3α and C-12α) while in the tetramer three bile acid units are attached to the α face (C-3α, C-7α, and C-12α) of rigid steroidal unit. This may form a cage-like structure, which can easily adopt a reverse micelle-like conformation in polar and nonpolar solvents and encapsulation of hydrophobic and hydrophilic dyes may take place. In dimers, both cis and trans forms are possible (Fig. 1) due to the flexible side chain. Now we have found that the dimers **13**–**18** having a 1,2,3-triazole ring also encapsulate hydrophilic dye cresol red in nonpolar solvent. This clearly shows that these dimers adopt a cis-conformation in nonpolar solvent and then hydrogen bonding helps them to encapsulate the dye. Our new trimeric compound **23** and tetrameric compound **24** are also found to encapsulate hydrophilic cresol red in nonpolar solvent.

2.1. Synthesis of terminal azides

Terminal azides **5**, **6**, **7**, and **8** were synthesized according to our earlier report[16b] from methyl deoxycholate **3** and methyl cholate **4** (Fig. 2).

Methyl 3α-azido-12α-hydroxy-5β-cholan-24-oate **9** and methyl 3α-azido-7α,12α-dihydroxy-5β-cholan-24-oate **10** were synthesized from compounds **3** and **4** by Mitsunobu reaction using methane sulfonic acid.[17] The resulting C-3β mesyloxy compounds were used without purification for further nucleophilic substitution with sodium azide in DMF to get compounds **9** and **10** (Scheme 1). These compounds showed the characteristic azide absorbance at 2100 cm^{-1}. In ^1H NMR, C-3α proton of **9** and **10** are multiplets at δ 3.33 and 3.19 ppm, respectively. These compounds were fully characterized by ^1H NMR, ^{13}C NMR, mass spectrometry, and elemental analysis.



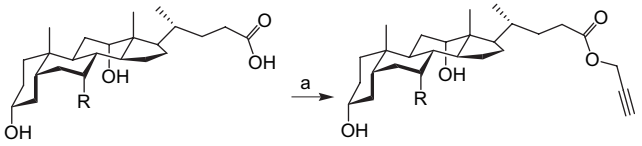
3 R = H
4 R = OH

9 R = H
10 R = OH

Scheme 1. Reagents and conditions: (i) Ph_3P, Et_3N, $MeSO_3H$, DEAD, THF, 45 °C, 24 h; (ii) NaN_3, DMF, 60–65 °C, 24 h, 75–78% (overall in two steps).

2.2. Synthesis of terminal alkynes

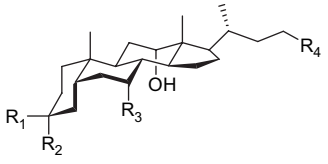
Terminal alkynes were prepared by esterification of bile acids **1** and **2** using an excess of propargyl alcohol and a catalytic amount of *para*-toluene sulfonic acid to get compounds **11** (96%) and **12** (95%) (Scheme 2).[16a] Compounds **11** and **12** show a characteristic acetylenic CH absorption at 3300 cm^{-1} in IR. In both the esters acetylenic proton was identified as triplet at δ 2.47 ppm and OCH_2 as doublet at δ 4.68 ppm in ^1H NMR. These compounds were fully characterized by ^1H NMR, ^{13}C NMR, mass spectrometry, and elemental analysis.



1 R = H
2 R = OH

11 R = H
12 R = OH

Scheme 2. Reagents and conditions: (a) PTSA (10 mol %), propargyl alcohol (5–10 mL), 55–60 °C, 7 h, **11** (96%), **12** (95%).

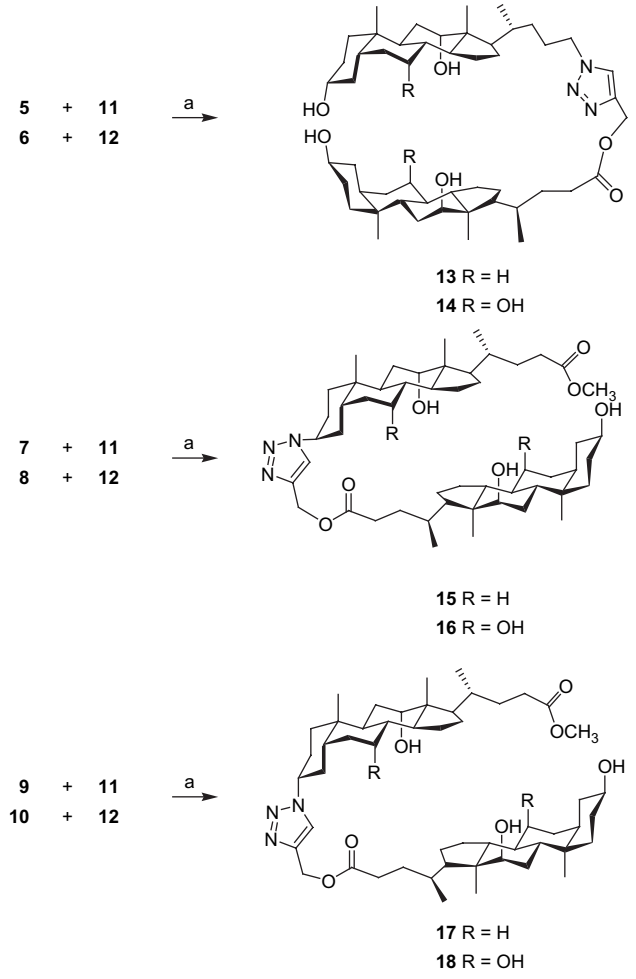


1 R_1 = H	R_2 = OH	R_3 = H	R_4 = COOH	Deoxycholic acid
2 R_1 = H	R_2 = OH	R_3 = OH	R_4 = COOH	Cholic acid
3 R_1 = H	R_2 = OH	R_3 = H	R_4 = $COOCH_3$	Methyl 3α,12α–dihydroxy-5β–cholan-24-oate
4 R_1 = H	R_2 = OH	R_3 = OH	R_4 = $COOCH_3$	Methyl 3α,7α,12α–trihydroxy-5β–cholan-24-oate
5 R_1 = H	R_2 = OH	R_3 = H	R_4 = N_3	3α,12α–Dihydroxy-24-azido-5β–cholane
6 R_1 = H	R_2 = OH	R_3 = OH	R_4 = N_3	3α,7α,12α–Trihydroxy-24-azido-5β–cholane
7 R_1 = N_3	R_2 = H	R_3 = H	R_4 = $COOCH_3$	Methyl 3β–azido–12α–hydroxy-5β–cholan-24-oate
8 R_1 = N_3	R_2 = H	R_3 = OH	R_4 = $COOCH_3$	Methyl 3β–azido–7α,12α–dihydroxy-5β–cholan-24-oate
9 R_1 = H	R_2 = N_3	R_3 = H	R_4 = $COOCH_3$	Methyl 3α–azido–12α–hydroxy-5β–cholan-24-oate
10 R_1 = H	R_2 = N_3	R_3 = OH	R_4 = $COOCH_3$	Methyl 3α–azido–7α,12α–dihydroxy-5β–cholan-24-oate

Figure 2.

2.3. Synthesis of dimeric compounds

1,3-Dipolar cycloaddition reaction of terminal alkyne **11** and **12** with C-24 azido compounds **5** and **6** was attempted under previously reported conditions[16a] with copper sulfate and sodium ascorbate in *t*-BuOH/H_2O. This reaction gave dimers **13** and **14** in good yields but needed long reaction times (3–12 h). However, when the reaction was carried out under microwave irradiation in DMF/H_2O, it gave the dimers **13** and **14** in 5 min (Scheme 3) in 90–95% yield. Under similar conditions, reaction of C-3β azido compounds **7** and **8** or C-3α azido compounds **9** and **10** with propargyl esters **11** and **12** gave dimers **15–18** in 90–96% yield. Formation of 1,2,3-triazole in all dimers was confirmed by the appearance of aromatic proton at δ 7.6–7.8 ppm in ^{1}H NMR and appearance of carbons at around 123 and 142 ppm in ^{13}C NMR. All the dimeric compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, and mass spectrometry and elemental analysis.



Scheme 3. Reagents and conditions: (a) $CuSO_4 \cdot 5H_2O$ (5 mol %), Sodium ascorbate (40 mol %), DMF/H_2O (4:1), microwave, 5 min, 90–96%.

2.4. Synthesis of oligomeric compounds

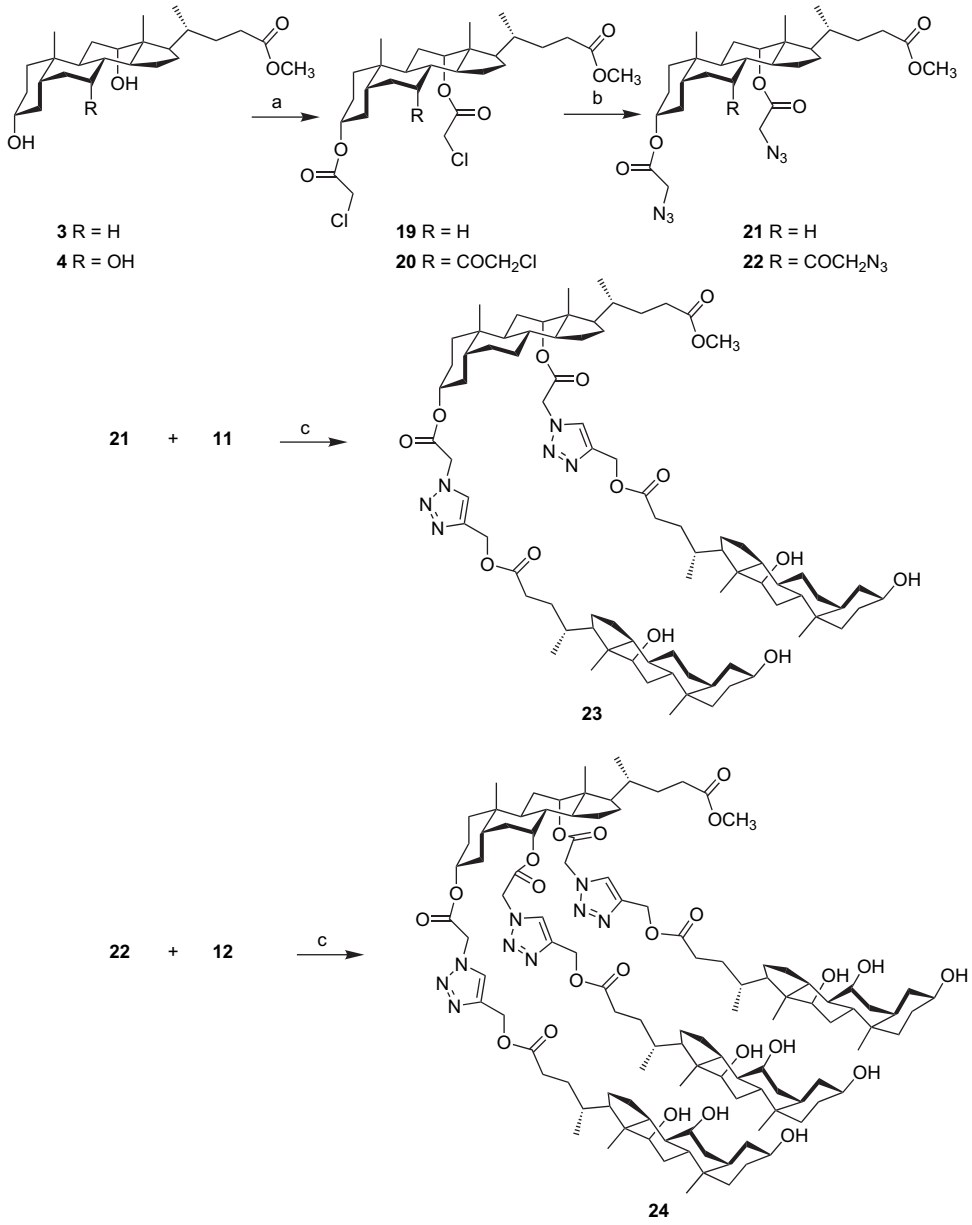
For the synthesis of trimer **23** and tetramer **24**, compounds **3** and **4** were treated with chloroacetyl chloride in the presence of CaH_2 and tetrabutylammonium bromide (TBAB) in refluxing toluene to form bis (chloroacetylated) compound **19** and tris (chloroacetylated) compound **20**, respectively (Scheme 4).

Formation of these compounds was confirmed from the downfield shift of protons at C-3, C-12, and C-7 (in case of **20**). These two compounds, on further reaction with sodium azide in dry DMF at 75 °C for 12 h, afforded methyl 3,12-bis(azidoacetoxy)-5β-cholan-24-oate **21** and methyl 3,7,12-tris(azidoacetoxy)-5β-cholan-24-oate **22**. Conversion of all the chloro atoms to azido was confirmed by LC–MS and also by little upfield shift of all the protons attached to azido groups. Cycloaddition reaction of **21** with propargyl deoxycholate **11** gave trimeric compound **23**. Similarly compound **22** on cycloaddition reaction with propargyl cholate **12** gave tetramer **24** having nine hydroxyl groups in a single molecule at the periphery. Both trimer **23** and tetramer **24** were fully characterized by ^{1}H NMR, ^{13}C NMR, and mass spectrometry. Thus by using a simple synthetic route, trimer and tetramer with varying numbers of bile acid units and hydroxyl groups were synthesized in good yields.

2.5. Dye solubilization study

The newly synthesized dimers **13–18** and oligomers **23** and **24** are soluble in chloroform and methanol. There are some recent reports[12] that bile acid dendrons can adopt a reverse micelle-like conformation in nonpolar solvent with the hydrophobic face turned outward (toward the solvent). Here, we have carried out a study of the extraction of the sodium salt of cresol red dye (hydrophilic dye) in nonpolar solvent, chloroform. The solubilization of dye by dimers, trimer, and tetramer was carried out by solid liquid extraction protocol.[11b] We found that dimeric compounds of methyl cholate as well as methyl deoxycholate show dye solubilization, which increases linearly with an increase in the concentration of dimer (Fig. 3).

It is observed that dye solubilization by methyl deoxycholate **3** in which C-7 OH group was absent showed poor encapsulation of cresol red and in dimeric forms **13**, **15**, and **17**, there was only a 2- to 5-fold increase in the encapsulation (Fig. 3A). On the other hand, dimers of cholic acid **14**, **16**, and **18** (Fig. 3B), which has one additional OH group at C-7 position of its monomeric unit methyl cholate **4**, show better encapsulation, C-3β (**16**) and C-3α (**18**) dimers show 10-fold increase in dye solublization while C-24 dimer shows a 13-fold increase in dye solubilization compared with the monomeric unit. The same experiments were carried out with trimer **23** and tetramer **24**. Interestingly, tetramer **24** shows a 45-fold increase in the encapsulation of cresol red in comparison with methyl cholate, C-24 dimer of cholic acid **14** shows more encapsulation as compared to trimer **23** (Fig. 3C). Visual expression of these results confirm the same order of dye extraction: tetramer **24**>C-24 cholic dimer **14**>trimer **23** (Fig. 3D). This may be because of the peripheral cholate units in tetramer. In C-24 dimer **14** there are two additional C-7 α hydroxy groups, which induce additional hydrogen bonding with cresol red. The extraction efficiencies of cholate-based



Scheme 4. Reagents and conditions: (a) CaH_2, $ClCH_2COCl$, tetrabutylammonium bromide (TBAB), toluene (reflux), 3 h, 78–86%; (b) NaN_3, DMF, 75 °C, 12 h, 79–82%; (c) $CuSO_4 \cdot 5H_2O$ (5 mol %), sodium ascorbate (40 mol %), DMF/H_2O (4:1), microwave, 5 min, **23** (85%), **24** (82%).

dimers and oligomers were found to be much better than that of deoxycholate based dimers and oligomers.

3. Conclusion

New steroidal dimers and oligomers with varying numbers of bile acid units have been synthesized in very good yields with 1,2,3-triazole as linker using click chemistry. All these molecules exhibited reverse micellar characteristics in nonpolar solvent. Cholic acid-based molecules were able to encapsulate greater amounts of hydrophilic dye than deoxycholate-based molecules. This is the first report to show that dimeric compounds are also able to encapsulate hydrophilic dye in nonpolar solvent, which may be due to the formation of the cis-conformation in the presence of dye which helps hydrogen bonding. There may be additional hydrogen bonding due to the 1,2,3-triazole ring.[13b]

4. Experimental

4.1. General experimental techniques and apparatus

TLC was performed on precoated silica gel F-254 plates (0.25 mm; E. Merck), and product(s) and starting material(s) were detected by either viewing under UV light or treating with an ethanolic solution of phosphomolybdic acid or anisaldehyde spray followed by heating. Column chromatography was performed on silica gel (100–200 mesh, Merck). Optical rotations were obtained on Bellingham and Stanley ADP-220 polarimeter. Specific rotations ($[\alpha]_D$) are reported in deg/dm, and the concentration (c) is given in g/100 mL in the specified solvent. Infrared spectra were recorded in $CHCl_3$ as a solvent, on Shimadzu 8400 series FTIR instrument. UV absorbances were recorded on Perkin–Elmer Lambda 35 UV/vis spectrometer. ^{1}H NMR spectra

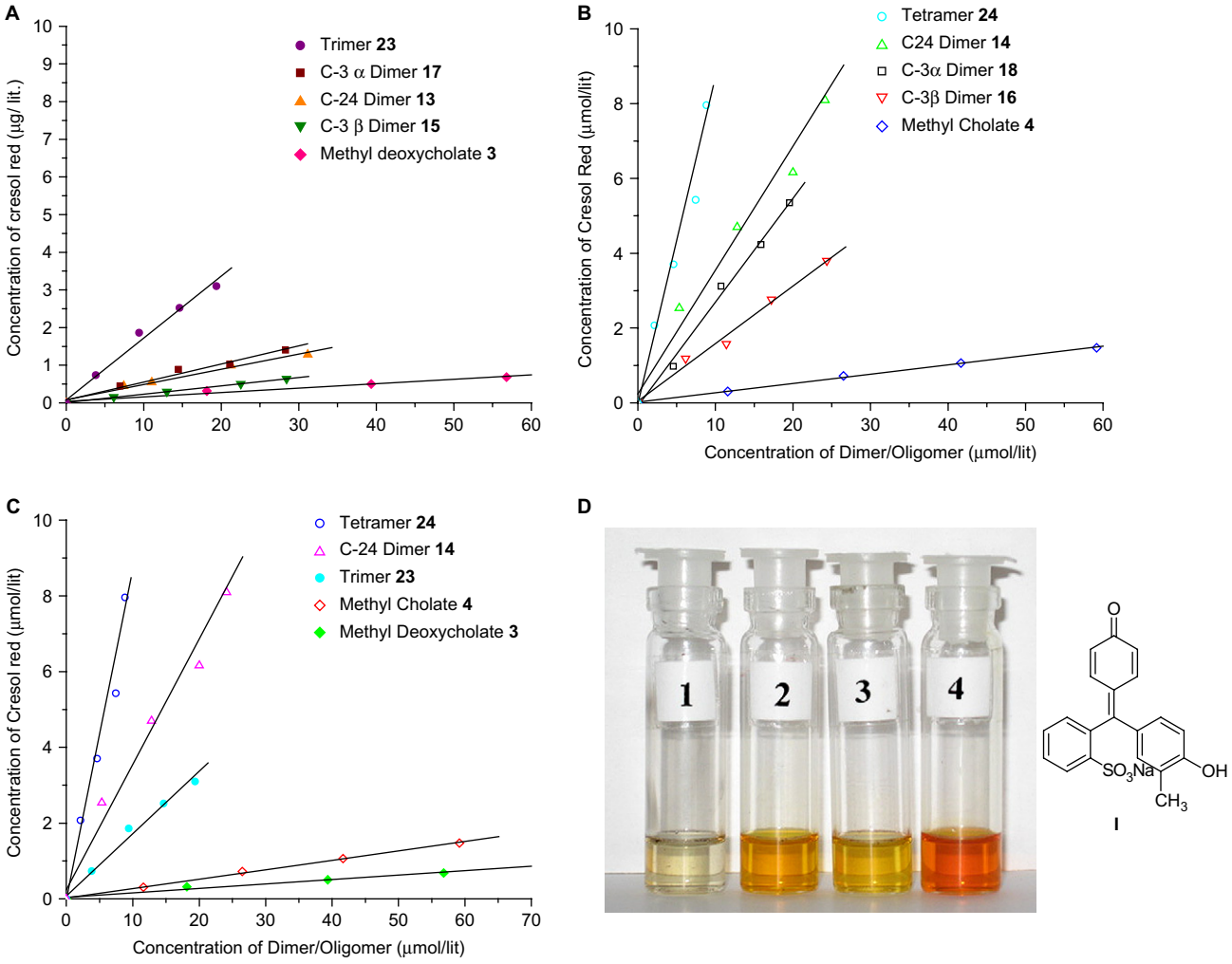


Figure 3. Solubilization of cresol red (CR) **I** in chloroform by: (A) trimer **23**, dimers **13**, **15**, and **17**, and monomer **3**; (B) tetramer **24**, dimers **14**, **16**, and **18**, and monomer **4**; (C) tetramer **24**, C-24 dimer **14**, trimer **23**, and monomers **3** and **4**. Straight lines are linear fit lines of experimental data; (D) cresol red sodium salt solubilization in chloroform having a ratio of chloroform/cresol red/oligomer (1 mL:5 mg:5 mg); each sample was filtered through 0.5 µm nylon membrane after 12 h. Vial 1 has no additive, vial 2 has C-24 dimer **14**, vial 3 has trimer **23**, and vial 4 has tetramer **24**.

were recorded on a Bruker AC-200 and 400 spectrometers at 200.13 and 400.13 MHz and ^{13}C NMR spectra were recorded on a Bruker AC-200 at 50.32 MHz. The chemical shifts are given in parts per million relative to tetramethylsilane or $CDCl_3$. Mass spectra were recorded on LC-MS/MS-TOF API QSTAR PULSAR spectrometer, samples introduced by infusion method using electrospray ionization technique. Elemental analyses were performed using CHNS-O EA 1108-elemental analyser, Carloerba Instrument (Italy) or Elementar Vario EL (Germany) and were within ±0.4% of calculated values. Melting points were determined on a Thermonik Campbell melting point apparatus and are uncorrected. Microwave irradiation was carried out in an open glass vessel using a domestic microwave oven (800 W, BPL-make). Standard workup: after extraction of all the reactions, the organic extracts were washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuum.

4.2. Synthesis of terminal azides

To a solution of **3** or **4** (1 mmol), and triphenylphosphine (0.865 g, 3.3 mmol), in dry THF (20 mL) at 0 °C, triethylamine (0.3 mL, 2 mmol) and methane sulfonic acid (0.14 mL, 2.1 mmol) were added. The mixture was warmed to 45 °C and diethylazodicarboxylate (DEAD) (0.6 mL, 3.1 mmol) was added dropwise with stirring. Reaction was continued for 24 h, after which the solvent was removed under reduced pressure and the residue was purified by column chromatography (5% EtOAc/hexane) to give C-3β mesyloxy compounds contaminated with DEAD residues. This material was redissolved in dry DMF (10 mL) and solid sodium azide (0.325 g, 5 mmol) was added and stirring was continued at 60–65 °C for 24 h. The reaction mixture was allowed to cool to room temperature and it was then poured into ice-cold water (30 mL) and was extracted with EtOAc (3×50 mL). The organic extract was washed with cold water (3×50 mL), followed by brine (25 mL), and it was dried over Na_2SO_4. Solvent was evaporated under reduced pressure to afford crude products. Purification of the crude products by column chromatography on silica gel (10% EtOAc/hexane) produced compounds **9** or **10** as white solids.

4.2.1. Methyl 3α-azido-12α-hydroxy-5β-cholan-24-oate (9). White solid, yield: 75% (overall in two steps); mp 82 °C; $[\alpha]_D^{25}$ +49.8 ($CHCl_3$, *c* 0.44); IR (cm^{-1}): 3332,

2094, 1730; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H, CH_3-18), 0.93 (s, 3H, CH_3-19), 0.97 (d, J=5.9 Hz, 3H, CH_3-21), 3.33 (m, 1H, CH-3), 3.67 (s, 3H), 3.98 (br s, 1H, CH-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.7, 17.1, 23.0, 23.5, 25.9, 26.6, 26.9, 27.3, 28.6, 30.8, 30.9, 32.3, 35.5, 34.0, 34.9, 35.3, 35.8, 42.2, 46.4, 47.1, 48.0, 51.3, 61.1, 72.8, 174.5; Anal. Calcd for $C_{25}H_{41}N_3O_3$: C, 69.57; H, 9.57; N, 9.74. Found: C, 69.23; H, 9.53; N, 9.56; MS (LC–MS) *m/z*: 454.64 (M+23 for Na).

4.2.2. Methyl 3α-azido-7α,12α-dihydroxy-5β-cholan-24-oate (10). White solid, yield: 78% (overall in two steps); mp 107–109 °C (lit.[17b] 109–110 °C); $[\alpha]_D^{25}$ +37.2 ($CHCl_3$, *c* 0.86); IR (cm^{-1}): 3470, 2093, 1730; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.69 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.97 (d, J=6.3 Hz, 3H, CH_3-21), 3.16 (m, 1H, CH-3), 3.67 (s, 3H), 3.86 (br s, 1H, CH-7), 3.98 (br s, 1H, CH-12); ^{13}C NMR (50 MHz, $CDCl_3$): δ 12.3, 17.2, 22.3, 23.1, 26.3, 26.7, 27.4, 28.0, 30.7, 30.9, 34.7, 35.3, 35.4, 39.1, 41.6, 46.4, 47.1, 51.4, 61.2, 68.2, 73.0, 174.8; Anal. Calcd for $C_{25}H_{41}N_3O_4$: C, 67.08; H, 9.23; N, 9.39. Found: C, 66.86; H, 9.48; N, 9.25; MS (LC–MS) *m/z*: 470.54 (M+23 for Na).

4.3. Synthesis of terminal acetylenes

To a solution of **1** or **2** (5 mmol) in propargyl alcohol (5–10 mL), a catalytic amount (10 mol %) of *para*-toluene sulfonic acid (PTSA) was added. The reaction mixture was then heated at 55–60 °C for 7 h. It was then poured on crushed ice and extracted with EtOAc (3×25 mL). The extract was washed with water (3×25 mL), brine (25 mL), and dried over Na_2SO_4. Solvent was evaporated under reduced pressure to afford crude products. Purification of the crude products by column chromatography on silica gel (2% MeOH/CH_2Cl_2) produced compound **11** or **12** as white solid.

4.3.1. Propargyl 3α,12α-dihydroxy-5β-cholan-24-oate (11). White solid, yield: 96%; mp 160–161 °C; $[\alpha]_D^{25}$ +43.5 ($CHCl_3$, *c* 2.2); IR (cm^{-1}): 3430, 3305, 1730; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.67 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.97 (d, J=5.9 Hz, 3H, CH_3-21), 2.47 (t, J=2.2 Hz, 1H), 3.60 (m, 1H, CH-3), 3.98 (br s, 1H, CH-12), 4.68 (d, J=2.20 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.6, 17.1, 22.9, 23.6, 26.0, 27.1, 27.4, 28.5, 30.2, 30.7, 30.9, 33.5, 34.0, 35.1, 35.2, 35.9, 36.3, 42.0, 46.4, 47.1, 48.1, 51.6, 71.5, 72.9, 74.6, 77.7, 173.2; Anal. Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.18; H, 10.02; MS (LC–MS) *m/z*: 453.14 (M+23 for Na).

4.3.2. Propargyl 3α,7α,12α-trihydroxy-5β-cholan-24-oate (12). White solid, yield: 95%; mp 112–114 °C; $[\alpha]_D^{25}$ +26.15 ($CHCl_3$, *c* 1.3); IR (cm^{-1}): 3404, 3307, 1737; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.68 (s, 3H, CH_3-18), 0.89 (s, 3H, CH_3-19), 0.98 (d, J=5.8 Hz, 3H, CH_3-21), 2.47 (t, J=2.5 Hz, 1H), 3.45 (m, 1H, CH-3), 3.85 (br s, 1H, CH-7), 3.97 (br s, 1H, CH-12), 4.68 (d, J=2.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.3, 17.2, 22.3, 23.1, 26.1, 27.4, 28.0, 29.6, 30.2, 30.6, 30.9, 34.7, 35.1, 39.3, 41.4, 46.3, 46.8, 51.6, 68.3, 71.7, 73.0, 74.7, 77.7, 173.4; Anal. Calcd for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.25; H, 9.57; MS (LC–MS) *m/z*: 469.15 (M+23 for Na).

4.4. Synthesis of dimeric compounds

*General procedure for cycloaddition (**13–18**).* The alkyne **11** or **12** (1 equiv) and the azides **5–10** (1.3 equiv) were dissolved in DMF/H_2O 4:1 (10 mL). To this solution $CuSO_4 \cdot 5H_2O$ (5 mol %) and sodium ascorbate (40 mol %) were added. The reaction mixture was placed in a domestic microwave reactor and irradiated for 5 min at 415 W. The reaction mixture was cooled, ice was added, and it was then extracted with EtOAc. The extract was washed with water and brine. Solvent was evaporated under reduced pressure and crude product was purified by column chromatography on silica gel using 5% MeOH/CH_2Cl_2 system to obtain dimers **13–18** (90–96%).

4.4.1. Dimeric compound (14). White solid, yield: 93%; mp 148–150 °C; $[\alpha]_D^{25}$ +33.2 ($CHCl_3$, *c* 0.73); IR (cm^{-1}): 3396, 1728; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.64 (s, 3H), 0.66 (s, 3H), 0.88 (s, 6H), 0.96–0.98 (6H), 3.41 (m, 2H), 3.82 (br s, 2H) 3.93 (br s, 2H), 4.34 (br s, 2H), 5.12–5.31 (dd, J=12.5 and 24.0 Hz, 2H, OCH_2), 7.66 (s, 1H, triazole H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.0, 16.8, 17.1, 22.1, 22.8, 25.9, 26.5, 27.2, 27.7, 29.3, 29.6, 30.5, 30.9, 32.1, 34.2, 34.4, 34.9, 35.0, 38.9, 39.0, 41.2, 46.0, 46.4, 46.5, 47.9, 48.2, 48.4, 48.6, 48.8, 49.0, 49.2, 50.6, 54.8, 57.0, 68.0, 71.3, 72.7, 123.6, 142.4, 174.1; Anal. Calcd for $C_{51}H_{83}N_3O_8$: C, 70.71; H, 9.66; N, 4.85. Found: C, 70.35; H, 10.01; N, 4.63; MS (LC–MS) *m/z*: 867.4 (M+1), 889.4 (M+23 for Na).

4.4.2. Dimeric compound (15). White solid, yield: 94%; mp 119–120 °C; $[\alpha]_D^{25}$ +42.1 ($CHCl_3$, *c* 1.47); IR (cm^{-1}): 3402, 1728; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.65 (s, 3H), 0.69 (s, 3H), 0.90 (s, 6H), 0.96–0.99 (m, 6H), 3.62 (m, 1H), 3.67 (s, 3H, OCH_3), 3.96 (br s, 1H), 4.02 (s, 1H), 4.68 (br s, 1H), 5.23 (s, 2H), 7.71 (s, 1H, triazole H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.4, 12.5, 16.9, 17.0, 22.9, 23.3, 23.4, 23.5, 24.5, 25.6, 25.9, 26.1, 26.9, 27.2, 28.4, 28.5, 29.5, 30.3, 30.5, 30.6, 30.8, 30.9, 33.2, 33.8, 34.1, 34.8, 34.9, 35.0, 35.5, 35.7, 36.0, 37.0, 41.8, 46.2, 46.9, 47.9, 51.3, 56.7, 57.3, 71.2, 72.6, 123.0, 142.0, 174.0, 174.5; Anal. Calcd for $C_{52}H_{83}N_3O_7$: C, 72.43; H, 9.70; N, 4.87. Found: C, 72.10; H, 9.58; N, 4.72; MS (LC–MS) *m/z*: 863.8 (M+1), 885.8 (M+23 for Na).

4.4.3. Dimeric compound (16). White solid, yield: 90%; mp 138–141 °C; $[\alpha]_D^{25}$ +28.3 ($CHCl_3$, *c* 1.27); IR (cm^{-1}): 3402, 1728; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.63 (s, 3H), 0.68 (s, 3H), 0.87 (s, 6H), 0.96–1.00 (6H), 3.26 (br s, OH), 3.46 (br s, 1H), 3.66 (s, 3H, OCH_3), 3.84–3.99 (4H), 4.72 (br s, 1H), 5.22 (s, 2H), 7.79 (s, 1H, triazole H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 12.2, 17.0, 17.0, 22.2, 22.5, 23.0, 24.5, 26.1, 26.2, 27.3, 28.0, 28.1, 30.9, 32.3, 33.8, 34.5, 34.7, 35.0, 36.6, 39.2, 41.3, 46.1, 46.2, 46.7, 51.3, 56.8, 57.4, 67.9, 68.0, 71.5, 72.7, 123.1, 141.9, 174.1, 174.5; Anal. Calcd for $C_{52}H_{83}N_3O_9$: C, 69.84; H, 9.36; N, 4.70. Found: C, 69.56; H, 8.98; N, 4.82; MS (LC–MS) *m/z*: 895.54 (M+1), 917.57 (M+23 for Na).

4.4.4. Dimeric compound (18). White solid, yield: 92%; mp 147–149 °C; $[\alpha]_D^{25}$ +38.5 ($CHCl_3$, *c* 0.83); IR (cm^{-1}): 3433, 1731; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.63 (s, 3H), 0.69 (s, 3H), 0.86–0.97 (12H), 3.53 (br s, 1H+OH), 3.65 (s, 3H,

OCH_3), 3.84–3.86 (br s, 2H), 3.94 (br s, 1H), 4.00 (br s, 1H), 4.34 (m, 1H), 5.15 (br s, 2H), 7.83 (s, 1H, triazole H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.2, 12.3, 17.1, 22.3, 22.3, 22.5, 23.0, 26.1, 26.4, 27.4, 28.0, 29.5, 30.0, 30.5, 30.7, 30.8, 30.9, 34.2, 34.4, 34.5, 34.7, 35.2, 35.6, 36.2, 39.3, 41.4, 41.6, 41.9, 46.2, 46.3, 46.4, 46.7, 46.9, 51.4, 57.4, 61.1, 67.7, 67.8, 68.2, 71.6, 72.8, 121.8, 141.9, 174.2, 174.8; Anal. Calcd for $C_{52}H_{83}N_3O_9$: C, 69.84; H, 9.36; N, 4.70. Found: C, 69.93; H, 9.42; N, 4.61; MS (LC–MS) *m/z*: 916.94 (M+23 for Na).

We have already reported the spectral data of compounds **13** and **17**.[16a]

4.5. Synthesis of compounds 19 and 20

To a solution of compound **3** or **4** (1 mmol) in toluene (10 mL) were added CaH_2 (0.189 g, 4.5 mmol), tetrabutylammonium bromide (0.106 g, 0.33 mmol), and $ClCH_2COCl$ (0.26 mL, 3.3 mmol). The reaction mixture was refluxed for 3 h, cooled, and it was then extracted with EtOAc (3×25 mL). The extract was washed with water and brine. Solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica gel (10% EtOAc/hexane) to yield compounds **19** or **20**.

4.5.1. Methyl 3α,7α-bis(chloroacetoxy)-5β-cholan-24-oate (19). White solid, yield: 86%; mp 120 °C; $[\alpha]_D^{25}$ +81.2 ($CHCl_3$, *c* 1.78); IR (cm^{-1}): 1733; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.75 (s, 3H, CH_3-18), 0.82 (d, *J*=6.1 Hz, 3H, CH_3-21), 0.92 (s, 3H, CH_3-19), 3.66 (s, 3H), 4.03 (s, 2H), 4.08 (s, 2H), 4.79 (m, 1H), 5.19 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.1, 17.3, 22.8, 23.2, 25.4, 25.7, 26.2, 26.6, 27.2, 30.6, 30.7, 31.8, 33.8, 34.1, 34.4, 34.5, 35.4, 41.0, 41.1, 41.6, 45.0, 47.2, 49.2, 51.4, 76.2, 77.9, 166.4, 166.6, 174.4; Anal. Calcd for $C_{29}H_{44}Cl_2O_6$: C, 62.25; H, 7.93. Found: C, 62.08; H, 8.15; MS (LC–MS) *m/z*: 559.56 (M+1), 561.57 (M+1), 581.55 (M+23 for Na), 583.55 (M+23 for Na).

4.5.2. Methyl 3α,7α,12α-tris(chloroacetoxy)-5β-cholan-24-oate (20). White solid, yield: 78%; mp 51–52 °C; $[\alpha]_D^{25}$ +69.9 ($CHCl_3$, *c* 1.09); IR (cm^{-1}): 1737, 1731; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.76 (s, 3H, CH_3-18), 0.84 (d, *J*=6.1 Hz, 3H, CH_3-21), 0.94 (s, 3H, CH_3-19), 3.66 (s, 3H), 4.03 (s, 2H), 4.07 (d, *J*=2.2 Hz, 2H, geminal coupling), 4.10 (s, 2H), 4.67 (m, 1H), 5.05 (br s, 1H) 5.20 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 11.9, 17.3, 22.1, 22.7, 25.0, 26.3, 26.9, 28.3 30.5, 30.6, 31.0, 34.1, 34.2, 34.3, 34.4, 37.7, 40.4, 40.9, 41.0, 42.7, 45.0, 47.0, 51.3, 72.9, 75.7, 77.1, 166.1, 166.4, 166.6, 174.2; Anal. Calcd for $C_{31}H_{45}Cl_3O_8$: C, 57.10; H, 6.96. Found: C, 56.87; H, 6.79; MS (LC–MS) *m/z*: 675.48 (M+23 for Na), 677.48 (M+23 for Na).

4.6. Synthesis of compounds 21 and 22

To a solution of **19** or **20** (1 mmol) in dry DMF (10 mL) sodium azide (0.390 g, 6 mmol) was added and stirring was continued at 75 °C for 12 h. The reaction mixture was allowed to cool to room temperature. It was then poured into ice-cold water (30 mL) and extracted with EtOAc. The organic extract was washed with cold water and brine. Solvent was evaporated under reduced pressure to afford crude product, which on purification by column chromatography on silica gel (10% EtOAc/hexane) produced pure compound **21** or **22**.

4.6.1. Methyl 3α,7α-bis(azidoacetoxy)-5β-cholan-24-oate (21). White solid, yield: 82%; mp 93–95 °C; $[\alpha]_D^{25}$ +89.8 ($CHCl_3$, *c* 2.56); IR (cm^{-1}): 2108, 1743, 1718; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.76 (s, 3H, CH_3-18), 0.82 (d, *J*=6.1 Hz, 3H, CH_3-21), 0.93 (s, 3H, CH_3-19), 3.66 (s, 3H), 3.84 (s, 2H), 3.89 (d, *J*=1.9 Hz, 2H, geminal coupling), 4.83 (m, 1H), 5.26 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.1, 17.4, 22.7, 23.2, 25.5, 25.6, 26.3, 26.5, 27.1, 30.5, 30.7, 31.7, 33.8, 34.1, 34.4, 34.5, 35.3, 41.5, 44.9, 47.4, 49.3, 50.3, 50.7, 51.3, 75.7, 77.7, 167.3, 167.6, 174.3; Anal. Calcd for $C_{29}H_{44}N_6O_6$: C, 60.82; H, 7.74; N, 14.67. Found: C, 60.98; H, 7.53; N, 14.58; MS (LC–MS) *m/z*: 595.60 (M+23 for Na).

4.6.2. Methyl 3α,7α,12α-tris(azidoacetoxy)-5β-cholan-24-oate (22). Gum, yield: 79%; $[\alpha]_D^{25}$ +71.29 ($CHCl_3$, *c* 1.01); IR (cm^{-1}): 2108, 1739; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.77 (s, 3H, CH_3-18), 0.82 (d, *J*=6.2 Hz, 3H, CH_3-21), 0.96 (s, 3H, CH_3-19), 3.66 (s, 3H, OCH_3), 3.84 (br s, 4H), 3.89 (d, *J*=3.8 Hz, 2H, geminal coupling), 4.71 (m, 1H), 5.08 (br s, 1H), 5.27 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 11.7, 17.2, 22.0, 22.4, 25.1, 26.2, 26.7, 28.6, 30.3, 30.4, 30.8, 33.9, 34.0, 34.2, 37.4, 40.2, 43.0, 44.8, 47.0, 50.1, 50.4, 50.5, 51.2, 72.5, 75.1, 76.8, 167.2, 167.3, 167.4, 174.0; Anal. Calcd for $C_{31}H_{45}N_9O_8$: C, 55.43; H, 6.75; N, 18.77. Found: C, 55.05; H, 6.38; N, 18.39; MS (LC–MS) *m/z*: 694.76 (M+23 for Na).

4.7. Trimeric compound (23)

Trimer **23** was synthesized from azide **21** (1 equiv) and terminal alkyne **11** (2.2 equiv) using general procedure and purification of the crude product by column chromatography on silica gel (5% MeOH/CH_2Cl_2) gave pure trimer **23**.

White solid, yield: 85%; mp 139–142 °C; $[\alpha]_D^{25}$ +69.3 ($CHCl_3$, *c* 0.66); IR (cm^{-1}): 3498, 1737, 1731; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.65–0.68 (br s, 6H), 0.72 (br s, 3H), 0.81–0.94 (m, 18H,), 3.60 (m, 2H), 3.68 (s, 3H, OCH_3), 3.96 (br s, 2H), 4.78 (m, 1H, CH-3), 5.20–5.28 (m, 9H), 7.82 (br s, 1H, Triazole H), 7.90 (br s, 1H, Triazole H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 12.2, 12.6, 12.7, 17.2, 17.7, 22.7, 23.1, 23.3, 23.6, 25.3, 25.9, 26.1, 26.6, 27.1, 27.2, 27.5, 28.6, 30.4, 30.7, 30.9, 31.1, 31.7, 31.8, 33.6, 33.9, 34.0, 35.5, 34.6, 35.1, 35.2, 35.4, 36.0, 36.4, 41.5, 41.8, 42.1, 45.1, 46.5, 47.1, 47.2, 47.4, 48.2, 49.3, 51.2, 51.3, 51.5, 57.3, 57.4, 71.7, 73.0, 74.0, 77.2, 78.5, 125.2, 125.4, 143.2, 143.3, 165.2, 165.9, 174.0, 174.1, 174.6; MALDI-TOF *m/z*: 1457.06 (M+23 for Na) calcd for $C_{83}H_{128}N_6O_{14}$, 1433.94.

4.8. Tetrameric compound (24)

Tetramer **24** was synthesized from azide **22** (1 equiv) and terminal alkyne **12** (3.3 equiv) using general procedure to give crude compound, which was purified by column chromatography on silica gel (5% MeOH/CH_2Cl_2) to give pure tetramer **24**.

White solid, yield: 82%; mp 164–168 °C; $[\alpha]_D^{25}$ +45.6 ($CHCl_3$, *c* 0.48); IR (cm^{-1}): 3388, 1735, 1730; ^1H NMR ($CDCl_3$, 500 MHz): δ 0.62–0.77 (br s, 12H), 0.85–0.93 (m, 24H,), 3.39 (br s, 3H), 3.68 (s, 3H, OCH_3), 3.80 (br s, 3H), 3.92 (br s, 3H), 4.58 (m, 1H, CH-3), 5.03 (br s, 1H), 5.10 (br s, 1H), 5.19–5.60 (m, 12H), 8.00–8.01 (br s, 3H, Triazole H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 11.9, 12.4, 14.1, 17.2, 17.5, 20.9, 22.3, 22.4, 22.8, 23.2, 25.1, 26.4, 26.5, 27.0, 27.5, 28.1, 28.4, 30.3, 30.6, 30.7, 30.9, 31.0, 31.2, 34.2, 34.3, 34.5, 34.6, 34.7, 35.1, 35.4, 37.8, 39.5, 40.6, 41.5, 41.6, 42.5, 45.2, 46.4, 46.5, 46.6, 46.7, 47.3, 51.2, 51.5, 57.1, 57.3, 57.4, 57.5, 60.3, 68.3, 71.8, 72.9, 73.2, 76.2, 77.67, 125.5, 125.7, 125.8, 143.0, 143.1, 143.2, 165.5, 165.8, 166.2, 171.0, 174.1, 174.2, 174.4; MALDI-TOF *m/z*: 2035.41 (M+23 for Na) calcd for $C_{112}H_{171}N_9O_{23}$, 2011.60.

4.9. Dye solubilization study

In four different vials, 4.1–26.0 mg of dimer (4.58–28.44 μM)/oligomers (2.14–19.39 μM) were dissolved in chloroform (1 mL each). Cresol red sodium salt (5.0 mg) was added to each vial, the vials were sealed, and the mixture was stirred at 22–28 °C for 12 h. The resulting solution was diluted to 5 mL chloroform. All solutions were filtered through 0.5 μm PTFE membrane filters in a round bottom flask. Chloroform was evaporated completely; the residue was dissolved in methanol and diluted with methanol to 10 mL. From this stock solution, 1 mL was diluted to 100 mL with methanol and the absorption spectra were recorded at 420 nm on UV–vis spectrometer. The concentration of cresol red in methanol (in the presence of monomer, dimer, and oligomer) was calculated using the molar extinction coefficient (15,812) at 420 nm in methanol. The same experiment was carried out without adding dimer/oligomer for blank reading.

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References and notes

1. (a) Davis, A. P. *Chem. Soc. Rev.* **1993**, 243–253; (b) Li, Y.; Dias, J. R. *Chem. Rev.* **1997**, *97*, 283–304; (c) Wallimann, P.; Marti, T.; Fürer, A.; Diederich, F. *Chem. Rev.* **1997**, *97*, 1567–1608; (d) Kolehmainen, E.; Tamminen, J. *Molecules* **2001**, *6*, 21–46; (e) Davis, A. P. *Coord. Chem. Rev.* **2006**, *250*, 2939–2951.
2. Virtanen, E.; Kolehmainen, E. *Eur. J. Org. Chem.* **2004**, 3385–3399.
3. Maitra, U.; Mukhopadhyay, S.; Sarkar, A.; Rao, P.; Indi, S. S. *Angew. Chem., Int. Ed.* **2001**, *40*, 2281–2283.
4. Willemen, H. M.; Vermonden, T.; Marcelis, A. T. M.; Sudhölter, E. J. R. *Eur. J. Org. Chem.* **2001**, 2329–2335.
5. Willemen, H. M.; Vermonden, T.; Marcelis, A. T. M.; Sudhölter, E. J. R. *Langmuir* **2002**, *18*, 7102–7106.
6. Valkonen, A.; Lahtinen, M.; Virtanen, E.; Kaikkonen, S.; Kolehmainen, E. *Biosens. Bioelectron.* **2004**, *20*, 1233–1241.
7. (a) Gdaniec, M.; Milewska, M. J.; Polonski, T. *Angew. Chem., Int. Ed.* **1999**, *38*, 392–395; (b) Szyrszyng, M.; Nowak, E.; Gdaniec, M.; Milewska, M. J.; Polonski, T. *Tetrahedron: Asymmetry* **2004**, *15*, 3257–3261.
8. (a) Burrows, C. J.; Sauter, R. A. *J. Inclusion Phenom.* **1987**, *5*, 117–121; (b) Kinneary, J. F.; Roy, T. M.; Albert, J. S.; Yoon, H.; Wagler, T. R.; Shen, L.; Burrows, C. J. *J. Inclusion Phenom.* **1989**, *7*, 155–168; (c) McKenna, J.; McKenna, J. M.; Thornthwaite, D. W. *J. Chem. Soc., Chem. Commun.* **1977**, 809–811; (d) Hsieh, H. P.; Muller, J. G.; Burrows, C. J. *J. Am. Chem. Soc.* **1994**, *116*, 12077–12078.
9. (a) Goto, C.; Yamamura, M.; Satake, A.; Kobuke, Y. *J. Am. Chem. Soc.* **2001**, *123*, 12152–12159; (b) Riccardis, F. D.; Filippo, M. D.; Garrisi, D.; Izzo, I.; Mancin, F.; Pasquato, L.; Scrimin, P.; Tecilla, P. *Chem. Commun.* **2002**, 3066–3067; (c) Joachimiak, R.; Paryzek, Z. *J. Inclusion Phenom. Macrocycl. Chem.* **2004**, *49*, 127–132.
10. Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Bhat, M. K.; Nahar, P. B.; Deshpande, M. V. *J. Med. Chem.* **2004**, *47*, 1591–1594.
11. (a) Balasubramanian, R.; Rao, P.; Maitra, U. *Chem. Commun.* **1999**, 2353–2354; (b) Balasubramanian, R.; Maitra, U. *J. Org. Chem.* **2001**, *66*, 3035–3040.
12. (a) Vijayalakshmi, N.; Maitra, U. *J. Org. Chem.* **2006**, *71*, 768–774; (b) Ghosh, S.; Maitra, U. *Org. Lett.* **2006**, *8*, 399–402.
13. (a) Dalvie, D. K.; Kalgutkar, A. S.; Khojasteh-Bakht, S. C.; Obach, R. S.; O'Donnell, J. P. *Chem. Res. Toxicol.* **2002**, *15*, 269–299; (b) Horne, W. S.; Yadav, M. K.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 15366–15367.
14. Gil, M. V.; Arévalo, M. J.; López, Ó. *Synthesis* **2007**, *11*, 1589–1620.
15. (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599; (b) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064.
16. (a) Aher, N. G.; Pore, V. S. *Synlett* **2005**, 2155–2158; (b) Pore, V. S.; Aher, N. G.; Kumar, M.; Shukla, P. K. *Tetrahedron* **2006**, *62*, 11178–11186; (c) Hazra, B. G.; Pore, V. S.; Dey, S. K.; Datta, S.; Darokar, M. P.; Saikia, D.; Khanuja, S. P. S.; Thakur, A. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 773–777; (d) Salunke, D. B.; Hazra, B. G.; Gonnade, R. G.; Bhadbhade, M. M.; Pore, V. S. *Tetrahedron* **2005**, *61*, 3605–3612; (e) Salunke, D. B.; Ravi, D. S.; Pore, V. S.; Mitra, D.; Hazra, B. G. *J. Med. Chem.* **2006**, *49*, 2652–2655.
17. (a) Davis, A. P.; Perez-Payan, M. N. *Synlett* **1999**, 991–993; (b) Lawless, L. J.; Blackburn, A. G.; Ayling, A. J.; Pérez-Payán, M. N.; Davis, A. P. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1329–1341.