Bioorganic & Medicinal Chemistry Letters 23 (2013) 1302–1305

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and micellar mimic properties of bile acid trimers

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ARTICLE INFO

Article history:
Received 18 October 2012
Revised 13 December 2012
Accepted 28 December 2012
Available online 12 January 2013

Keywords:
Bile acid
Trimer
Extraction

ABSTRACT

Two fan-shaped bile acid trimers have been synthesized via Cu^{I}-catalyzed azide–alkyne cycloaddition (CuAAC) 'click chemistry', and their extraction experiments of cresol red sodium (CR) and pyrene were investigated in the polar and non-polar solvents, respectively. The transmission electron microscopy (TEM) results showed that the homogenous hollow capsules formed with the diameter size range of 40–70 nm in a solution of water and acetone. Thus the amphiphilicity of fan-shaped bile acid trimers might be used as the promising candidate in biological and drug delivery applications.

Bile acid, a class of natural compounds, exhibits a series of interesting properties, such as unique facial amphiphilicity, bioactivity, biocompatibility and multifunctional groups with well-controlled chemistry (Fig. 1).[1–5] Currently, constructing supramolecular architectures by self-assembly of molecules spontaneously is a subject of great interest in various areas from chemistry, biology to materials science,[6–10] and amphiphile is one of the most useful building blocks to fabricate supramolecular architectures with controlled structure and functions on the basis of non-covalent interactions such as van der Waals force, hydrogen bonding, and π–π interaction.[11–13] Among the self-assembly amphiphilic architectures, bile acid has been considered as a versatile platform for the construction of environmentally responsive amphiphiles and functional supramolecular systems.

Till now, various polymers and oligomers based on bile acid have been prepared for their potential biological and pharmaceutical applications in drug delivery vehicles,[14–27] molecular containers,[28–31] non-polymeric hydrogelators,[32,33] and chemosensors.[34] For example, Zhu and co-workers[35] synthesized cholic acid-contained oligomers which were able to form hydrophobic pockets to solubilize pyrene in polar media, and recognize metal ions because of its 1,2,3-triazole groups; Zhao et al.[31] have successfully used calixarene and bile acid to form 'molecular basket' oligomers for binding various guests. At the same time, our previous work showed that triazine ring is a nice scaffold for synthesizing oligomers containing functional groups,[36–38] and it has also been focused on more and more in assembly properties recently.[39–41] As a continuation, two fan-shaped bile acid trimers (Fig. 2), their

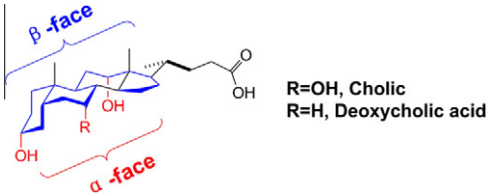

Figure 1. Structure of bile acids (cholic acid and deoxycholic acid).

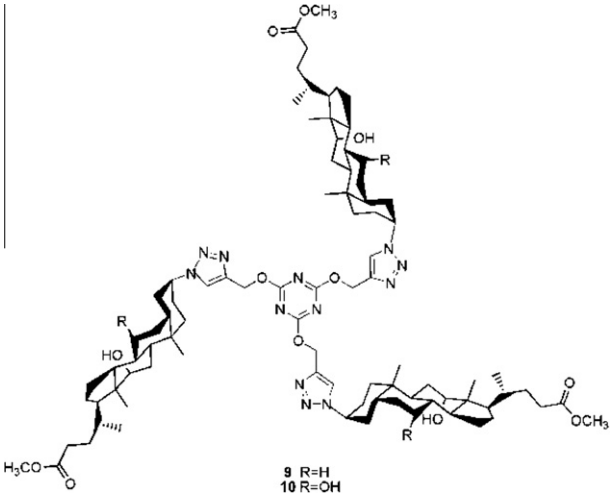

Figure 2. Structure of two fan-shaped molecules containing three bile acid units.

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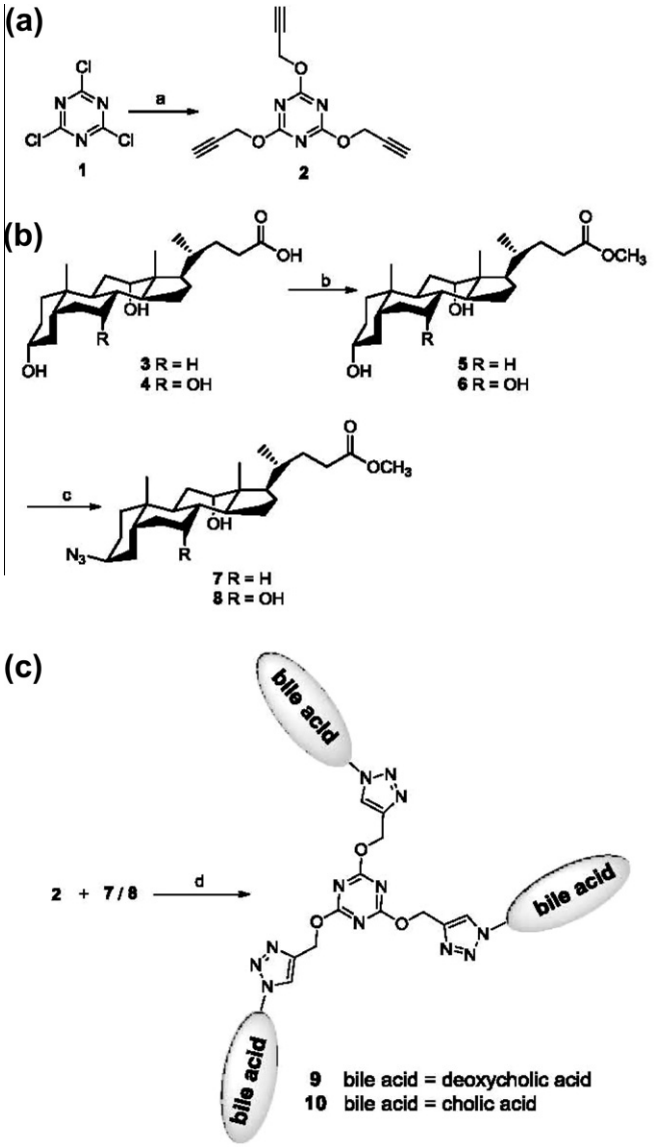
http://dx.doi.org/10.1016/j.bmcl.2012.12.102

extraction experiments and self-assembly behavior are reported in this Letter. In these molecules, triazine ring, 1,2,3-triazole and bile acid are considered as the core, the linker and the functional ends, respectively. The 1,2,3-triazole group are not only formed easily via Cu^{I}-catalyzed azide–alkyne cycloaddition (CuAAC) 'click chemistry',[42–44] but also often found in biologically active compounds and π-conjugated functional materials, as well as its great potential applications in supramolecular chemistry.[45–48]

The synthesis route for bile acid trimers **9** and **10** was shown in Scheme 1. Tosylation on hydroxyl group of the corresponding bile acid methyl ester followed by treatment with sodium azide in DMF gave the desired azides **7** and **8** (Scheme 1b).[46] On the other hand, 2,4,6-tris(prop-2-ynyloxy)-1,3,5-triazine **2** was obtained through treating 2-propynyl alcohol in the presence of potassium hydroxide (Scheme 1a).[36] Synthesis of the fan-shaped bile acid–triazole trimers was described in Scheme 1c as the following procedure:[49] a solution of azide and **2** (molar ratio = 3:1), 5 mol % $CuSO_4{\cdot}5H_2O$ and 20 mol % sodium ascorbate in *t*-BuOH–CH_3OH–H_2O (10:2:1) was stirred at 65 °C for 6 h. The pure compounds were obtained after column chromatographic purification with 75% and 83% yields, respectively.



Scheme 1. Synthesis of (a) triazine terminal alkynes, (b) steroidal azides, and (c) bile acid trimers. Reagents and conditions: (a) 2-propynyl alcohol, KOH, THF, rt; (b) HCl, CH_3OH, rt; (c) (i) TsCl, pyridine, rt, (ii) NaN_3, DMF, 60 °C; (d) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, *t*-BuOH/CH_3OH/H_2O (10:2:1), 65 °C.

Due to the facial amphiphilicity and flexible conformation of these two molecules, their conformation can be changed by solvents' polarity. In non-polar solvents, the molecules utilize the hydrophilic faces of cholates to bind hydrophilic molecules, while in polar solvents the molecules employ the hydrophobic faces of the cholates to bind hydrophobic guests.[50] So we investigated the solubilization of cresol red sodium (CR, a hydrophilic dye) by fan-shaped molecules in chloroform based on solid–liquid extraction measurement (Fig. 3).[51] Obviously, the amount of extracted CR increased linearly with the concentration of fan-shaped trimers (Fig. 3a), and compound **10** led to better encapsulation than **9**, which may result from possessing more hydroxyl groups of **10** compared to trimer **9** (Fig. 3b). Meanwhile, in a polar environment, the trimers were expected to bind hydrophobic guests by its micelle like conformer. So the solubilization of pyrene was investigated by adding trimer **10** in methanol/water (4:1). As shown in Figure 4, UV–vis absorption of pyrene increased with the concentration of trimer **10**, indicating the fan-shaped trimer **10** could promote the solubilization of pyrene in polar solvent.

In order to confirm the amphiphilicity and flexible conformation of the trimers, **10** was used to investigate the self-assembly images by transmission electron microscopy (TEM). At a volume fraction of water (20%) in acetone, homogenous hollow capsules with the diameter size range of 40–70 nm formed (Fig. 5), which further explains why trimer **10** could be used to promote the solubility of polar/non-polar molecules in the non-polar/polar solvents.

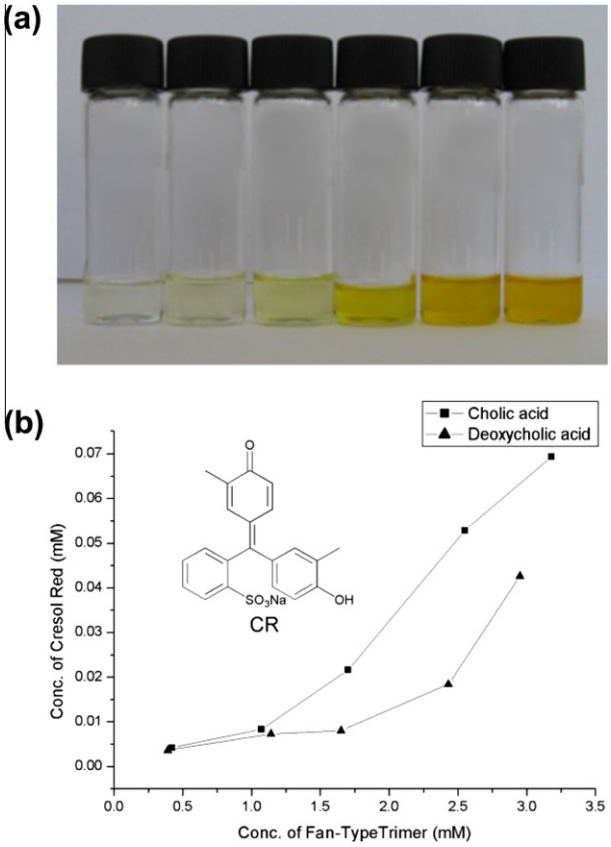


Figure 3. (a) Extraction of CR by increasing concentrations of **10**. Left to right: [**10**] = 0, 0.4, 1.1, 1.7, 2.5, 3.2 mM; (b) solubilization of CR (inset) by trimers **9** and **10**.

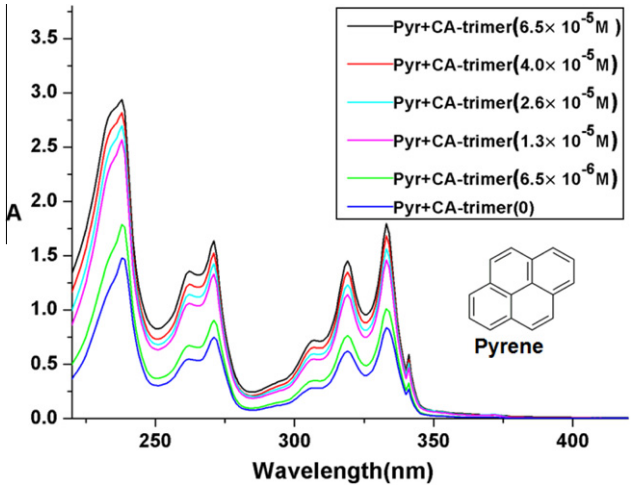


Figure 4. UV–vis absorption spectra of pyrene extracted by increasing concentrations of **10** (in CH_3OH/H_2O = 4).

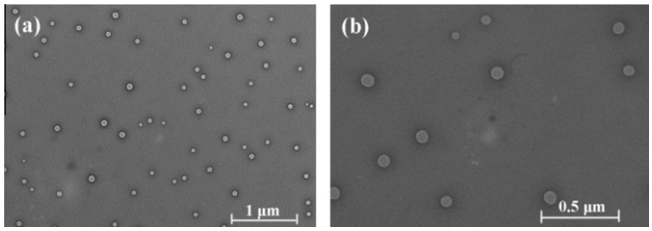


Figure 5. TEM images of the supramolecular aggregation of **10** in water/acetone (v/v = 1/4) (2 mg/mL).

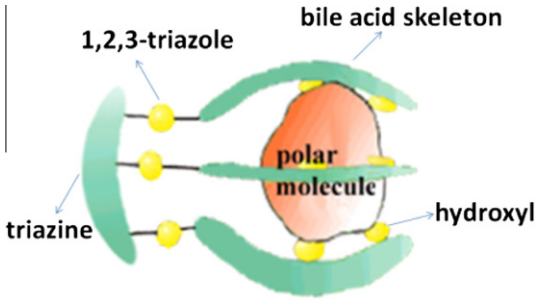


Figure 6. Assumption mode of the trimer's amphiphilic conformation in non-polar solvent systems.

Based on the extraction experiments of cresol red sodium (CR) and pyrene in polar and non-polar solvent systems, as well as the facial amphiphilicity of bile acid molecules, a possible assumption mode of these two trimers' amphiphilic conformation was showed in Figure 6. In this model, bile acid skeleton and hydroxyl can evert to wrap different guests depending on the polarity of the environment, and 1,2,3-triazole linking the triazine core is used to make the eversion more easily.

In summary, two fan-shaped molecules containing three bile acid units were synthesized via CuAAC 'click chemistry', and their extraction and the assembly properties were investigated in polar and non-polar solvents, respectively. The results showed that the amphiphilicity of these trimers might be used as the promising candidate in biological and drug delivery applications.

Acknowledgment

The project is supported by NSFC (No. 21172130) and NBRPC (973 Program, No. 2012CB821600).

Supplementary data

Supplementary data [52–54] (^{1}H NMR, ^{13}C NMR and MS spectra of compounds **2**, **5**, **6**–**10**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.102.

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52. Data for compound **2** see Ref. 36.
53. General procedure for synthesis bile acid methyl ester (**5**, **6**) and azide (**7**, **8**) see Ref. 46. *Compound* ***5***: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.68 (s, 3H, 18-CH_3), 0.91 (s, 3H, 19-CH_3), 0.96 (d, 3H, J = 5.8 Hz, 21-CH_3), 3.61 (m, 1H, 3-CH), 3.66 (s, 3H, 24-CO_2CH_3), 3.98 (m, 1H, 12-CH); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.7, 17.2, 23.1, 23.6, 26.1, 27.1, 27.4, 28.6, 30.8, 31.0, 33.6, 34.1, 35.1, 35.2, 35.9, 36.4, 42.0, 46.4, 47.2, 48.2, 51.4, 71.7, 73.1, 174.7; ESI-MS(+): m/z = 429.5 $[M+Na]^+$; *Compound* ***6***: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.68 (s, 3H, 18-CH_3), 0.89 (s, 3H, 19-CH_3), 0.98 (d, 3H, J = 5.8 Hz, 21-CH_3), 3.47 (m, 1H, 3-CH), 3.66 (s, 3H, 24-CO_2CH_3), 3.84 (m, 1H, 7-CH), 3.96 (m, 1H, 12-CH); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.5, 17.3, 22.5, 23.2, 26.4, 27.5, 28.1, 30.4, 30.9, 31.1, 34.6, 34.8, 35.3, 39.5, 41.5, 41.6, 46.4, 47.0, 51.5, 68.5, 71.9, 73.1, 76.1, 174.8; ESI-MS(+): m/z = 445.8 $[M+Na]^+$, 461.5 $[M+K]^+$; *Compound* ***7***: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.68 (s, 3H, 18-CH_3), 0.94 (s, 3H, 19-CH_3), 0.97 (d, 3H, J = 5.8 Hz, 21-CH_3), 3.65 (s, 3H, 24-CO_2CH_3), 3.94 (m, 3H, 3-CH), 3.98 (m, 1H, 12-CH); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.5, 17.1, 23.3, 23.4, 24.1, 25.9, 26.3, 27.1, 28.7, 29.7, 30.0, 30.7, 30.8, 33.04, 34.3, 34.9, 35.6, 37.1, 46.3, 47.1, 48.1, 51.3, 58.6, 72.9, 174.5; ESI-MS(+): m/z = 454.0 $[M+Na]^+$; *Compound* ***8***: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.69 (s, 3H, 18-CH_3), 0.93 (s, 3H, 19-CH_3), 0.97 (d, 3H, J = 5.8 Hz, 21-CH_3), 3.66 (s, 3H, 24-CO_2CH_3), 3.87 (m, 3H, 3-H), 3.90 (m, 1H, 7-CH), 3.98 (m, 1H, 12-CH); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.5, 17.3, 22.8, 23.2, 24.5, 26.1, 27.4, 28.4, 29.6, 30.5, 30.8, 31.0, 33.0, 34.1, 35.1, 35.2, 36.7, 39.4, 41.9, 46.5, 47.2, 51.5, 58.7, 68.4, 72.9, 76.6, 174.7; ESI-MS(+): m/z = 470.3 $[M+Na]^+$; ESI-MS(−): m/z = 483.6 $[M+Cl]^-$.
54. *General procedure for synthesis trimer* ***9*** *and* ***10***: A solution of azide (0.6 mmol) and 2, 4, 6-tris (prop-2-ynyloxy)-1,3,5-triazine **2** (0.2 mmol) in *t*-BuOH–CH_3OH (18 mL, 5:1 v/v) was stirred at 65 °C for 15 min. Then, $CuSO_4{\cdot}5H_2O$ (5 mol % in 0.5 mL of H_2O) and sodium ascorbate (20 mol % in 0.5 mL of H_2O) were added. The reaction mixture was stirred at 65 °C for 6 h, and then the solvent was evaporated under reduced pressure to afford a crude product. The crude was dissolved in CH_2Cl_2, washed with saturated brine, dried with magnesium sulfate and filtered, followed by the removal of the solvent under vacuum. Finally, the crude product was purified by column chromatography on silica gel to obtain pure fan-shaped bile acid trimers with 75% and 83% yields. *Compound* ***9***: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.63 (s, 3 × 3H, 18-CH_3), 0.84 (s, 3 × 3H, 19-CH_3), 0.92 (d, 3 × 3H, J = 5.8 Hz, 21-CH_3), 3.60 (s, 3 × 3H, 24-CO_2CH_3), 3.96 (m, 3 × 1H, 12-CH), 4.61 (m, 3 × 1H, 3-CH), 5.52 (s, 3 × 2H, OCH_2), 7.78 (s, 3 × 1H, triazole-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.8, 17.4, 23.6, 23.7, 24.8, 25.9, 26.4, 27.5, 28.9, 29.8, 30.6, 30.9, 31.1, 33.6, 34.4, 35.2, 35.8, 37.3, 46.6, 47.3, 48.3, 51.6, 57.0, 61.8, 73.0, 123.2, 141.6, 172.8, 174.8; ESI-MS (+): m/z = 1560.4 $[M+Na]^+$, 1576.8 $[M+K]^+$; ESI-MS (−): m/z = 1572.5 $[M+Cl]^-$; HRMS(ESI): m/z $[M+H]^+$ calcd for $C_{87}H_{132}N_{12}O_{12}$: 1538.0166; found: 1538.0160; *Compound* ***10***: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.65 (s, 3 × 3H, 18-CH_3), 0.82 (s, 3 × 3H, 19-CH_3), 0.95 (d, 3 × 3H, 21-CH_3), 3.61 (s, 3 × 3H, 24-CO_2CH_3), 3.86 (m, 3 × 1H, 7-CH), 3.96 (m, 3 × 1H, 12-CH), 4.54 (m, 3 × 1H, 3-CH), 5.53 (s, 3 × 2H, OCH_2), 7.84 (s, 3 × 1H, triazole-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.6, 14.2, 17.4, 21.1, 22.8, 23.4, 23.5, 26.6, 27.7, 28.5, 30.7, 31.0, 31.2, 32.5, 34.1, 35.0, 35.4, 36.9, 39.4, 41.8, 46.6, 47.3, 51.6, 57.1, 60.5, 68.2, 73.1, 76.7, 123.7, 141.6, 172.8, 174.8; ESI-MS (+): m/z = 1606.9 $[M+Na]^+$, 1624.7 $[M+K]^+$; ESI-MS (−): m/z = 1585.2 $[M-H]^-$; HRMS(ESI): m/z $[M+H]^+$ calcd for $C_{87}H_{132}N_{12}O_{15}$: 1586.0013; found: 1586.0008.