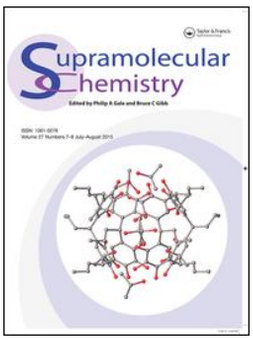

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Design, synthesis and application of new bile acid ligands with 1,2,3-triazole ring

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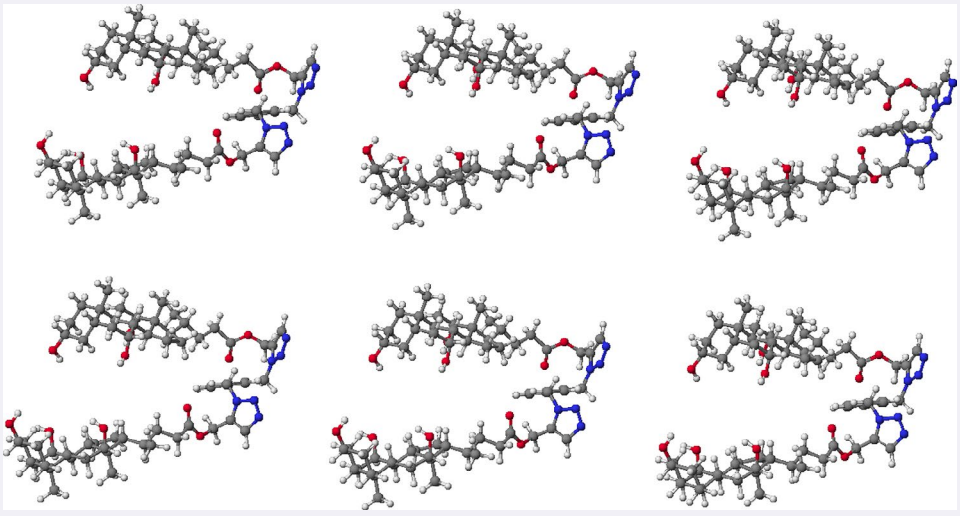
ABSTRACT

New dimers have been obtained from propargyl ester of bile acids and α,α′-diazide-*m*-xylene by intermolecular 1,3-dipolar cycloaddition. These compounds have been used as ligands to form intermolecular hydrogen bonds with various aromatic acids. The structures of all products were confirmed by spectroscopic (^{1}H NMR, ^{13}C NMR and FT-IR) analysis, mass spectrometry (ESI, MALDI) and PM5 semiempirical methods.



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Bile acids; click chemistry; 1,2,3-triazole ring, hydrogen bonds

1. Introduction

The major metabolites of cholesterol are polyhydroxylated steroidal acids commonly known as bile acids. These compounds are conjugated with taurine or glycine in the liver, forming bile salts. Bile acids reduce the surface tension of solutions and activate digestive enzymes (*1–5*). They possess a rigid, large, curved skeleton where two rings A/B have a *cis* geometry. Bile acids have chemically different polar hydroxyl groups 3α; 3α,7α; 3α,12α; or 3α,7α,12α which are responsible to some extent for their amphiphilic properties. All of these attributes make bile acids and their modified derivatives very interesting starting materials for the synthesis of macrocyclic molecular dimers, molecular tweezers or cholaphanes (*6–9*). On the other hand, bile acid dimers can be used for the synthesis of macrocyclic compounds as artificial receptors with good organogeling properties (*10–17*).

The modification of bile acids by the 'click chemistry' allows to synthesise novel conjugates with a variety of applications (*18*). K. B. Sharpless was the first to describe copper-catalysed azide–alkyne cycloaddition (CuAAC). It is one of the most important reactions in organic, material and polymer chemistry, and especially in bioconjugation reactions (*18, 19*). The 'click chemistry' creates a broad spectrum of rings with carbon–heteroatom bonds. These simple reaction conditions are characterised by high selectivity and productivity as well as an easy way of product isolation. Products are resistant to metabolic processes. They are also stable in various solvents, including water (*19–22*). Especially important is the 1,3-dipolar cycloaddition between terminal alkynes and azides in the presence of copper(I) catalyst known as Huisgen reaction (*23, 24*). The copper(I)-catalysed 'click' reaction is an immensely useful synthetic way to obtaining 1,2,3-triazole derivatives

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of bile acids (*23–33*). The regioselective of cycloaddition reactions depends on catalysts and temperatures. The 1,5-disubstituted 1,2,3-triazoles are formed in the presence of catalysts containing ruthenium(II); on the other hand, 1,4-disubstituted 1,2,3-triazoles are obtained in the presence of catalysts containing copper(I) (*34, 35*). These five-membered heterocyclic rings can form intra- and intermolecular hydrogen bonds together with dipole–dipole interactions with an increase in the solubility of conjugates and facilitate binding of molecular targets (*36*).

2. Experimental

2.1. Chemicals and instruments

General. The NMR spectra were measured with a Spectrometer NMR Varian Mercury 300 MHz (Oxford, UK), operating at 300.07 and 75.4614 MHz for ^{1}H and ^{13}C, respectively. Typical conditions for the proton spectra were as follows: pulse width 32°, acquisition time 5 s, FT size 32 K and digital resolution 0.3 Hz per point, and for the carbon spectra, pulse width 60°, FT size 60 K and digital resolution 0.6 Hz per point, the number of scans varied from 1200 to 10,000 per spectrum. The ^{13}C and ^{1}H chemical shifts were measured in $CDCl_3$ and DMSO-d_6 relative to an internal standard of TMS. Infrared spectra were recorded in the KBr pellets or film using a FT-IR Bruker IFS 66 spectrometer (Karlsruhe, Germany). The ESI (electron spray ionisation) mass spectra were recorded on a Waters/Micromass (Manchester, UK) ZQ mass spectrometer equipped with a Harvard Apparatus (Saint Laurent, Canada), syringe pump. The sample solutions were prepared in methanol at the concentration of approximately 10^{-5} M. The standard ESI-MS mass spectra were recorded at the cone voltage of 90 V. PM5 semiempirical calculations were performed using the WinMopac 2003 program. The MALDI (matrix-assisted laser desorption/ionisation) mass spectra were recorded on a Waters Maldi Q-Tof Premiere. The sample solutions were prepared in methanol at the concentration of approximately 10^{-5} M. The matrix was 2,5-dihydroxybenzoic acid (gentisic acid), and the standard was β-cyclodextrin (*m/z* 1157.3218).

2.2. Preparation of ligands (11–15)

Procedure for compound (**11**): Propargyl lithocholate (330 mg, 0.8 mmol) was dissolved in a mixture of *t*-butanol/methanol (6 mL, 5:1), and 1,3-di(azidomethyl)benzene (75 mg, 0.4 mmol) was added. Next, to the homogenous mixture were added $CuSO_4{\cdot}5H_2O$ (3 mg, 3 mol%) and sodium ascorbate (9 mg, 20 mol%) in water (0.3 mL). The reaction mixture was heated at 60 °C for 5 h and then extracted with chloroform, washed with brine and dried over anhydrous Na_2SO_4. The crude compound was purified by column chromatography on silica gel using $CHCl_3$/AcOEt (50:1) as an eluent.

Procedure for compound (**12**): Propargyl deoxycholate (345 mg, 0.8 mmol) was dissolved in a mixture of *t*-butanol/methanol (6 mL, 5:1), and 1,3-di(azidomethyl)benzene (75 mg, 0.4 mmol) was added. Next, to the homogenous mixture were added $CuSO_4{\cdot}5H_2O$ (3 mg, 3 mol%) and sodium ascorbate (9 mg, 20 mol%) in water (0.3 mL). The reaction mixture was heated at 60 °C for 5 h and then extracted with chloroform, washed with brine and dried over anhydrous Na_2SO_4. The crude compound was purified by column chromatography on silica gel using $CHCl_3$/AcOEt (10:1) as an eluent.

Procedure for compound (**13**): Propargyl cholate (360 mg, 0.8 mmol) dissolved in a mixture of *t*-butanol/methanol (6 mL, 5:1), and 1,3-di(azidomethyl)benzene (75 mg, 0.4 mmol) was added to. Next, to the homogenous mixture were added $CuSO_4{\cdot}5H_2O$ (3 mg, 3 mol%) and sodium ascorbate (9 mg, 20 mol%) in water (0.3 mL). The reaction mixture was heated at 60 °C for 5 h and then extracted with chloroform, washed with brine and dried over anhydrous Na_2SO_4. The crude compound was purified by column chromatography on silica gel using $CHCl_3$/MeOH (20:1) as an eluent.

Procedure for compound (**14**): Compound (**9**) (330 mg, 0.62 mmol) was dissolved in a mixture of *t*-butanol/methanol (6 mL, 5:1). Then, corresponding amounts of 1,3-di(azidomethyl)benzene were added (58 mg, 0.31 mmol). Next, to the homogenous mixture were added $CuSO_4{\cdot}5H_2O$ (3 mg, 3 mol%) and sodium ascorbate (9 mg, 20 mol%) in water (0.3 mL). The reaction mixture was heated at 60 °C for 8 h and then extracted with chloroform, washed with brine and dried over anhydrous Na_2SO_4. The crude compound was purified by column chromatography on silica gel using $CHCl_3$/AcOEt (5:1) as an eluent.

Procedure for compound (**15**): Compound (**10**) (500 mg, 0.91 mmol) was dissolved in a mixture of *t*-butanol/methanol (6 mL, 5:1). Then, corresponding amounts of 1,3-di(azidomethyl)benzene were added (85 mg, 0.45 mmol). Next, to the homogenous mixture were added $CuSO_4{\cdot}5H_2O$ (3 mg, 3 mol%) and sodium ascorbate (9 mg, 20 mol%) in water (0.3 mL). The reaction mixture was heated at 60 °C for 8 h and then extracted with chloroform, washed with brine and dried over anhydrous Na_2SO_4. The crude compound was purified by column chromatography on silica gel using $CHCl_3$/AcOEt (5:1) as an eluent.

Compound (**11**). Yield 70%. (280 mg).m.p. 145–149 °C. FT-IR (KBr) ν_{max}: 3414, 2931, 2862, 1737, 1449, 1161, 753. ^{1}H NMR ($CDCl_3$): δ 7.55 (s, 2H, CH-triazole ring), 7.27–7.22 (m, 4H, Ar–H), 5.51 (s, 4H, Ph–CH_2–triazole ring), 5.19 (s, 4H, O–CH_2–triazole ring), 3.67–3.57 (m, 2H, 3β–H), 0.91 (s,

6H, CH_3–19), 0.87 (d, *J* = 5.1 Hz, 6H, CH_3–21), 0.61 (s, 6H, CH_3–18). ^{1}H NMR (DMSO-d_6): δ 8.18 (s, 2H, CH-triazole ring), 7.34–7.25 (m, 4H, Ar–H), 5.58 (s, 4H, Ph–CH_2–triazole ring), 5.10 (s, 4H, O–CH_2–triazole ring), 4.45 (d, *J* = 4.5 Hz, 2H, 3α–OH), 3.48–3.37 (m, 2H, 3β–H), 0.87 (s, 6H, CH_3–19), 0.84 (d, *J* = 6.3 Hz, 6H, CH_3–21), 0.57 (s, 6H, CH_3–18). ^{13}C NMR ($CDCl_3$): δ 174.11, 143.58, 135.58, 130.01, 128.33, 127.54, 123.69, 71.79, 57.38, 56.46, 55.88, 53.71, 42.69, 42.05, 40.39, 40.13, 36.42, 35.80, 35.29, 34.54, 31.06, 30.82, 30.52, 28.16, 27.16, 26.38, 24.16, 23.34, 20.78, 18.21, 11.99. ^{13}C NMR (DMSO-d_6): δ 173.02, 172.94, 142.17, 136.48, 129.20, 127.78, 127.59, 124.80, 69.82, 56.91, 55.99, 55.43, 52.49, 42.22, 41.49, 39.93, 39.78, 39.63, 39.22, 38.94, 38.67, 36.26, 35.33, 35.12, 34.69, 34.17, 30.49, 30.41, 30.35, 27.64, 26.87, 26.12, 23.79, 23.24, 20.37, 18.01, 11.77. HRMS (MALDI) (*m/z*): calcd for $C_{62}H_{92}N_6O_6Cl$ [M + Cl]: 1051.6766; found: 1051.6735.

Compound (**12**). Yield 58% (255 mg), m.p. 99–105 °C. FT-IR (KBr) ν_{max}: 3423, 2935, 2862, 1734, 1448, 1164, 754. ^{1}H NMR ($CDCl_3$): δ 7.55 (s, 2H, CH-triazole ring), 7.27–7.22 (m, 4H, Ar–H), 5.52 (s, 4H, Ph–CH_2–triazole ring), 5.19 (s, 4H, O–CH_2–triazole ring), 3.95 (s, 2H, 12β–H), 3.66–3.55 (m, 2H, 3β–H), 0.91 (s, 6H, CH_3–19), 0.92 (d, *J* = 8.1 Hz, 6H, CH_3–21), 0.65 (s, 6H, CH_3–18). ^{1}H NMR (DMSO-d_6): δ 8.18 (s, 2H, CH-triazole ring), 7.43–7.40 (m, 4H, Ar–H), 5.58 (s, 4H, Ph–CH_2–triazole ring), 5.10 (s, 4H, O–CH_2–triazole ring), 4.46 (d, *J* = 4.3 Hz, 2H, 3α–OH), 4.18 (d, *J* = 4.1 Hz, 2H, 12α–OH), 3.77 (d, *J* = 3.5 Hz, 2H, 12β–H), 3.41–3.28 (m, 2H, 3β–H), 0.88 (d, *J* = 6.3 Hz, 6H, CH_3–21), 0.84 (s, 6H, CH_3–19), 0.55 (s, 6H, CH_3–18). ^{13}C NMR ($CDCl_3$): δ 174.07, 143.58, 135.59, 130.01, 128.37, 127.54, 123.69, 76.58, 73.03, 71.71, 57.41, 53.72, 48.24, 47.13, 46.45, 42.04, 36.39, 35.97, 35.22, 35.12, 34.08, 33.60, 31.09, 30.77, 30.45, 29.66, 28.65, 27.45, 27.09, 26.11, 23.62, 23.12, 17.23, 12.66. ^{13}C NMR (DMSO-d_6): δ 172.99, 142.20, 136.46, 129.21, 127.75, 127.60, 124.78, 70.94, 69.90, 56.90, 52.50, 47.40, 46.11, 45.95, 41.57, 39.78, 39.23, 38.95, 38.67, 36.26, 35.60, 35.11, 34.83, 33.77, 32.88, 30.55, 30.20, 28.54, 27.09, 26.95, 26.05, 23.44, 23.05, 16.80, 12.33. HRMS (MALDI) (*m/z*): calcd for $C_{62}H_{92}N_6O_8Cl$ [M + Cl]: 1083.6665; found: 1083.6647.

Compound (**13**). Yield 40% (172 mg), m.p. 108–115 °C. FT-IR (KBr) ν_{max}: 3420, 2930, 1734, 1464, 1168, 753. ^{1}H NMR ($CDCl_3$): δ 7.58 (s, 2H, CH-triazole ring), 7.27–7.20 (m, 4H, Ar–H), 5.52 (s, 4H, Ph–CH_2–triazole ring), 5.19 (s, 4H, O–CH_2–triazole ring), 3.93 (s, 2H, 12β–H), 3.83 (s, 2H, 7β–H), 3.51–3.20 (m, 2H, 3β–H), 0.92 (d, *J* = 5.7 Hz, 6H, CH_3–21), 0.87 (s, 6H, CH_3–19), 0.64 (s, 6H, CH_3–18). ^{1}H NMR (DMSO-d_6): 8.18 (s, 2H, CH-triazole ring), 7.40–7.25 (m, 4H, Ar–H), 5.58 (s, 4H, Ph–CH_2–triazole ring), 5.10 (s, 4H, O–CH_2–triazole ring), 4.31 (d, *J* = 4.4 Hz, 2H, 3α–OH), 4.10 (d, *J* = 3.5 Hz, 2H, 12α–OH), 4.00 (d, *J* = 3.4 Hz, 2H, 7α–OH), 3.75 (d, *J* = 3.17 Hz, 2H, 12β–H), 3.61 (s, 2H, 7β–H), 3.23–3.14 (m, 2H, 3β–H), 0.81 (s, 6H, CH_3–19), 0.89 (d, *J* = 6.2 Hz, 6H, CH_3–21), 0.55 (s, 6H, CH_3–18). ^{13}C NMR ($CDCl_3$): δ 174.22, 143.53, 135.60, 129.99, 128.38, 127.58, 123.80, 72.94, 71.84, 68.37, 57.46, 53.71, 46.75, 46.39, 41.65, 41.47, 39.47, 35.27, 34.73, 31.05, 30.77, 30.38, 28.17, 27.54, 26.37, 23.19, 22.46, 17.23, 12.41. ^{13}C NMR (DMSO-d_6): δ 173.03, 142.21, 136.45, 129.22, 127.75, 127.62, 124.77, 79.12, 70.95, 70.40, 66.21, 56.91, 52.51, 46.04, 45.72, 41.49, 41.32, 39.78, 39.22, 38.95, 38.67, 35.28, 34.92, 34.84, 34.35, 30.62, 30.55, 30.37, 28.47, 27.20, 26.17, 22.75, 22.58, 16.83, 12.23. HRMS (MALDI) (*m/z*): calcd for $C_{62}H_{92}N_6O_{10}Cl$ [M + Cl]: 1115.6564; found: 1115.6549.

Compound (**14**). Yield 42% (162 mg), m.p. 145–149 °C. FT-IR (KBr) ν_{max}: 3430, 2932, 1744, 1451, 1227, 1163, 754. ^{1}H NMR ($CDCl_3$): δ 7,54 (s, 2H, CH-triazole ring), 7.33–7.20 (m, 4H, Ar–H), 5.50 (s, 4H, Ph–CH_2-triazole ring), 5.19 (s, 4H, O–CH_2–triazole ring), 4.88–4.69 (m, 2H, 3β–H), 3.80 (s, 4H, 3α–CH_2–Br), 0.93 (s, 6H, CH_3–19), 0.87 (d, *J* = 6.1 Hz, 6H, CH_3–21), 0.62 (s, 6H, CH_3–18). ^{13}C NMR ($CDCl_3$): δ 174.09, 166.74, 143.55, 135.59, 130.00, 129.00, 128.34, 127.55, 123.71, 57.38, 56.41, 55.92, 53.71, 42.70, 41.86, 41.22, 40.39, 40.06, 35.74, 35.28, 34.89, 34.55, 32.01, 31.90, 31.06, 30.81, 30.48, 29.67, 28.16, 26.95, 26.40, 26.36, 26.27, 24.14, 23.27, 20.81, 18.27, 12.0. ESI-MS (*m/z*): 1157.3 $[C_{66}H_{96}N_6O_8 + CH_3OH + K]^+$.

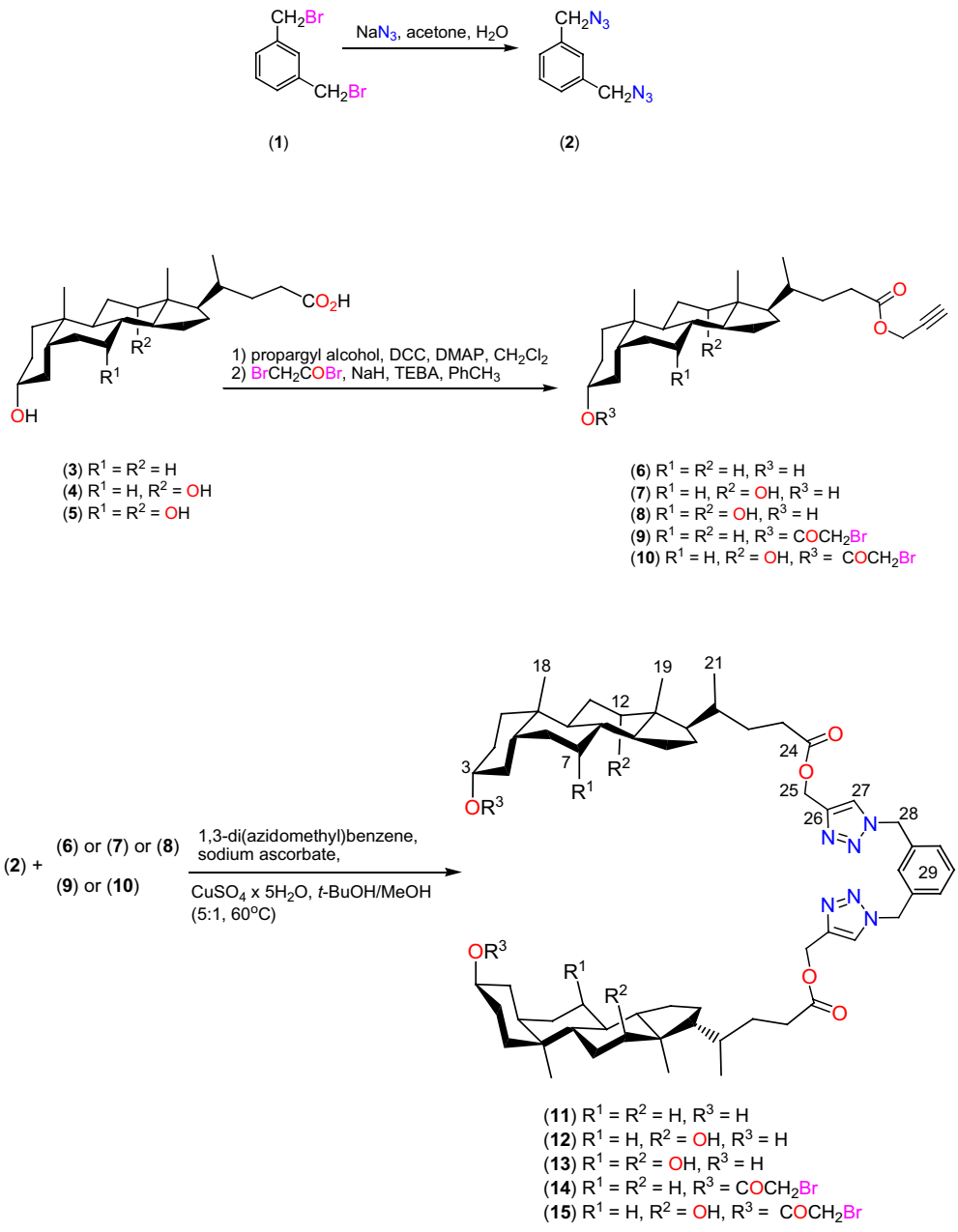
Compound (**15**). Yield 39% (225 mg), 151–154 °C. FT-IR (KBr) ν_{max}: 3424, 2926, 1734, 1449, 1285, 1158, 754. ^{1}H NMR ($CDCl_3$): δ 7,54 (s, 2H, CH-triazole ring), 7.33– 7.22 (m, 4H, Ar–H), 5.50 (s, 4H, Ph–CH_2-triazole ring), 5.18 (s, 4H, O–CH_2–triazole ring), 5.14 (s, 2H, 12β–H), 4.89–4.71 (m, 2H, 3β–H), 3.84 (s, 4H, 3α–CH_2–Br), 0.92 (s, 6H, CH_3–19), 0.78 (d, *J* = 6.1 Hz, 6H, CH_3–21), 0.72 (s, 6H, CH_3–18). ^{13}C NMR ($CDCl_3$): δ 173.92, 166.70, 166.54, 143.42, 135.57, 129.99, 129.68, 128.99, 128.53, 128.34, 128.18, 127.93, 127.56, 123.75, 57.38, 54.29, 53.91, 53.70, 49.24, 49.15, 47.21, 45.11, 41.68, 41.19, 35.55, 34.70, 34.61, 34.24, 34.03, 33.98, 31.89, 31.77, 30.97, 30.55, 29.66, 27.31, 26.75, 26.35, 26.20, 25.99, 25.85, 25.49, 25.28, 23.39, 22.87, 17.40, 12.27. ESI-MS (*m/z*): 1135,3 $[C_{66}H_{96}N_6O_{10} + H]^+$, 1154,3 $[C_{66}H_{96}N_6O_{10} + H + Na]^+$.

2.3. Preparation of adducts of dimers (11–13)

Sample of compound (**11**) (10 mg, 0.0098 mmol) was dissolved in acetonitrile. Then, phthalic acid (1.63 mg, 0.0098 mmol) was added in acetonitrile. The mixture was evaporated to dryness.

Sample of compound (**11**) (10 mg, 0.0098 mmol) was dissolved in acetonitrile. Then, terephthalic acid (1.63 mg, 0.0098 mmol) was added in acetonitrile. The mixture was evaporated to dryness.

Sample of compound (**11**) (10 mg, 0.0098 mmol) was dissolved in acetonitrile. Then, *p*-aminobenzoic acid



Scheme 1. (Colour online) Synthesis of 1,3-di(azidomethyl)benzene (**2**), propargyl esters of bile acids (**6–10**) and dimers of bile acids derivatives (**11–15**) linked by 1,2,3-triazole ring.

(1.35 mg, 0.0098 mmol) was added in acetonitrile. The mixture was evaporated to dryness.

Sample of compound (**12**) (10 mg, 0.0095 mmol) was dissolved in acetonitrile. Then, phthalic acid (1.57 mg, 0.0095 mmol) was added in acetonitrile. The mixture was evaporated to dryness.

Sample of compound (**12**) (10 mg, 0.0095 mmol) was dissolved in acetonitrile. Then, terephthalic acid (1.57 mg, 0.0095 mmol) was added in acetonitrile. The mixture was evaporated to dryness.

Sample of compound (**12**) (10 mg, 0.0095 mmol) was dissolved in acetonitrile. Then, *p*-aminobenzoic acid (1.3 mg, 0.0095 mmol) was added in acetonitrile. The mixture was evaporated to dryness.

Sample of compound (**13**) (10 mg, 0.0093 mmol) was dissolved in acetonitrile. Then, phthalic acid (1.54 mg, 0.0093 mmol) was added in acetonitrile. The mixture was evaporated to dryness.

Sample of compound (**13**) (10 mg, 0.0093 mmol) was dissolved in acetonitrile. Then, terephthalic acid (1.54 mg, 0.0093 mmol) was added in acetonitrile. The mixture was evaporated to dryness.

Sample of compound (**13**) (10 mg, 0.0093 mmol) was dissolved in acetonitrile. Then, *p*-aminobenzoic acid

Table 1. ^{1}H NMR and ^{13}C NMR chemical shifts (ppm) of compounds (**11–13**) in $CDCl_3$.

	Compound					
	11		**12**		**13**	
No. of atom	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
3	3.67–3.57	71.79	3.66–3.55	71.71	3.51–3.20	71.84
7	–	–	–	–	3.83	68.39
12	–	–	3.95	73.03	3.93	72.94
18	0.61	11.99	0.65	12.66	0.64	12.41
19	0.91	20.78	0.91	23.12	0.87	22.46
21	0.87	18.21	0.92	17.23	0.92	17.23
24	–	174.11	–	174.07	–	174.22
25	5.19	42.69	5.19	42.04	5.19	41.49
26	–	143.58	–	143.58	–	143.53
27	7.55	123.69	7.55	123.69	7.58	123.80
28	5.51	53.71	5.52	53.72	5.52	53.71
29	7.27–7.22	135.58	7.27–7.22	130.01	7.27–7.20	129.99
		130.01		128.37		128.38
		128.33		127.54		127.58

Table 2. ^{1}H NMR and ^{13}C NMR chemical shifts (ppm) of compounds (**11–13**) in DMSO-d_6.

	Compound					
	11		**12**		**13**	
No. of atom	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
3	3.48–3.37	69.82	3.41–3.28	69.90	3.23–3.14	66.21
7	–	–	–	–	3.61	70.40
12	–	–	3.77	70.94	3.75	70.95
18	0.57	11.77	0.55	12.33	0.55	12.23
19	0.87	23.34	0.84	23.05	0.81	22.75
21	0.84	18.01	0.88	16.80	0.89	16.83
24	–	172.94	–	172.99	–	173.03
25	5.10	42.22	5.10	41.57	5.10	41.32
26	–	142.17	–	142.20	–	142.21
27	8.18	124.80	8.18	124.78	8.18	124.77
28	5.58	52.49	5.58	52.50	5.58	52.51
29	7.34–7.25	136.48	7.43–7.40	136.46	7.40–7.25	136.45
		129.70		129.21		129.22
		127.78		127.60		127.75
3OH	4.45	–	4.46	–	4.31	–
7OH	–	–	–	–	4.00	–
12OH	–	–	4.18	–	4.10	–

Table 3. The differences Δ (ppm) between the respective chemical shifts.

	Compound					
	11		**12**		**13**	
No. of atom	Δ ^{1}H	Δ ^{13}C	Δ ^{1}H	Δ ^{13}C	Δ ^{1}H	Δ ^{13}C
3	0.20	1.97	0.26	1.81	0.17	5.63
7	–	–	–	–	0.22	–2.01
12	–	–	0.18	2.09	0.18	1.99
18	0.04	0.22	0.10	0.33	0.09	0.18
19	0.04	–2.56	0.07	0.07	0.06	–0.29
21	0.03	0.20	0.04	0.43	0.03	0.40
24	–	1.17	–	1.08	–	1.19
25	0.09	0.47	0.09	0.47	0.09	0.17
26	–	1.41	–	1.38	–	1.32
27	–0.63	–1.11	–0.63	–1.09	–0.60	–0.97
28	–0.07	1.22	–0.06	1.22	–0.06	1.20
29	–0.05	–0.90	–0.17	–6.45	–0.09	–6.46
		0.31		–0.84		–0.84
		0.55		–0.06		–0.17

Notes.
Δ^{1}H = δ(**11** in $CDCl_3$) – δ(**11** in DMSOd_6); **Δ^{13}C** = δ(**11** in $CDCl_3$) – δ(**11** in DMSOd_6).
Δ^{1}H = δ(**12** in $CDCl_3$) – δ(**12** in DMSOd_6); **Δ^{13}C** = δ(**12** in $CDCl_3$) – δ(**12** in DMSOd_6).
Δ^{1}H = δ(**13** in $CDCl_3$) – δ(**13** in DMSOd_6); **Δ^{13}C** = δ(**13** in $CDCl_3$) – δ(**13** in DMSOd_6).

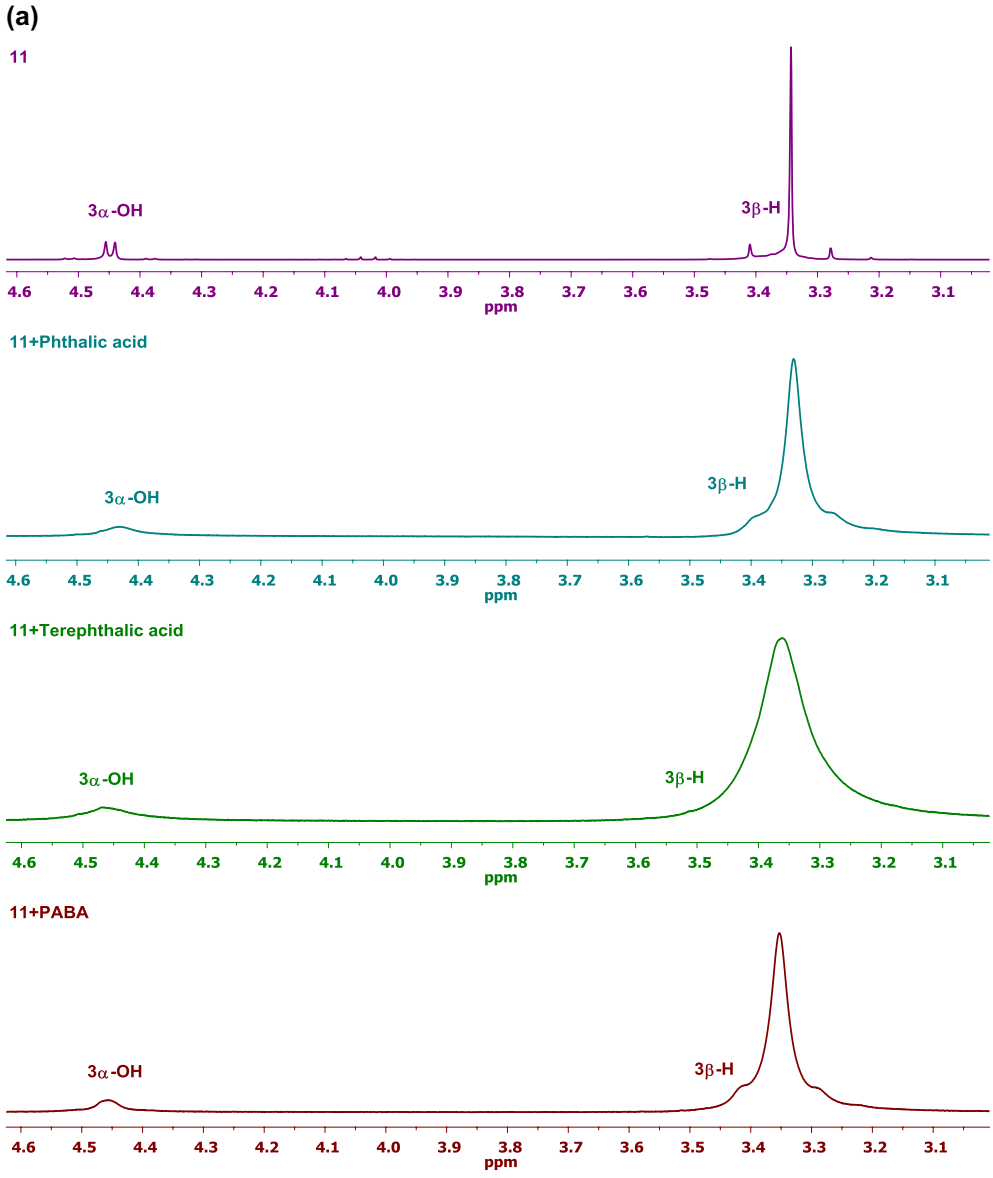


Figure 1a. (Colour online) ^{1}H NMR in 4.5–3.0 ppm region of dimers (**11–13**) and its adducts with phthalic acid, terephthalic and PABA in DMSO-d_6.

(1.27 mg, 0.00933 mmol)] was added in acetonitrile. The mixture was evaporated to dryness.

2.4. Semiempirical calculations

The optimised geometries, natural bond orbital charge distributions and the heats of formation (HOF) of the free ligand as well as adducts were calculated by the PM5 suite of programs (*37–39*). PM5 semiempirical calculations were performed using the WinMopac 2003 program.

3. Results and discussion

In our previous work, we described intermolecular 1,3-dipolar cycloaddition of the propargyl ester of bile acids and azide groups of 1,3,5-tris(azidomethyl)benzene and a quasi-podands with 1,2,3-triazole rings (*40*). Encouraged by these works, in this paper we describe the application of the CuAAC reaction for the synthesis of 1,2,3-triazoles containing bile acid, as well as the application of the previously unknown dimers in the literature to adduct formation with 1,2-benzenedicarboxylic acid (phthalic acid), 1,4-benzenedicarboxylic acid (terephthalic acid) and 4-aminobenzoic acid (PABA, vitamin B_{10}).

The synthetic route to prepare 1,3-di(azidomethyl) benzene (**2**), propargyl esters of bile acids (**6–8**) and their bromoacetoxy derivatives (**9**,**10**) and conjugates (**11–15**) is shown in Scheme 1. The structures of all synthesised conjugates (**11–15**) as well as adducts of (**11–13**) with

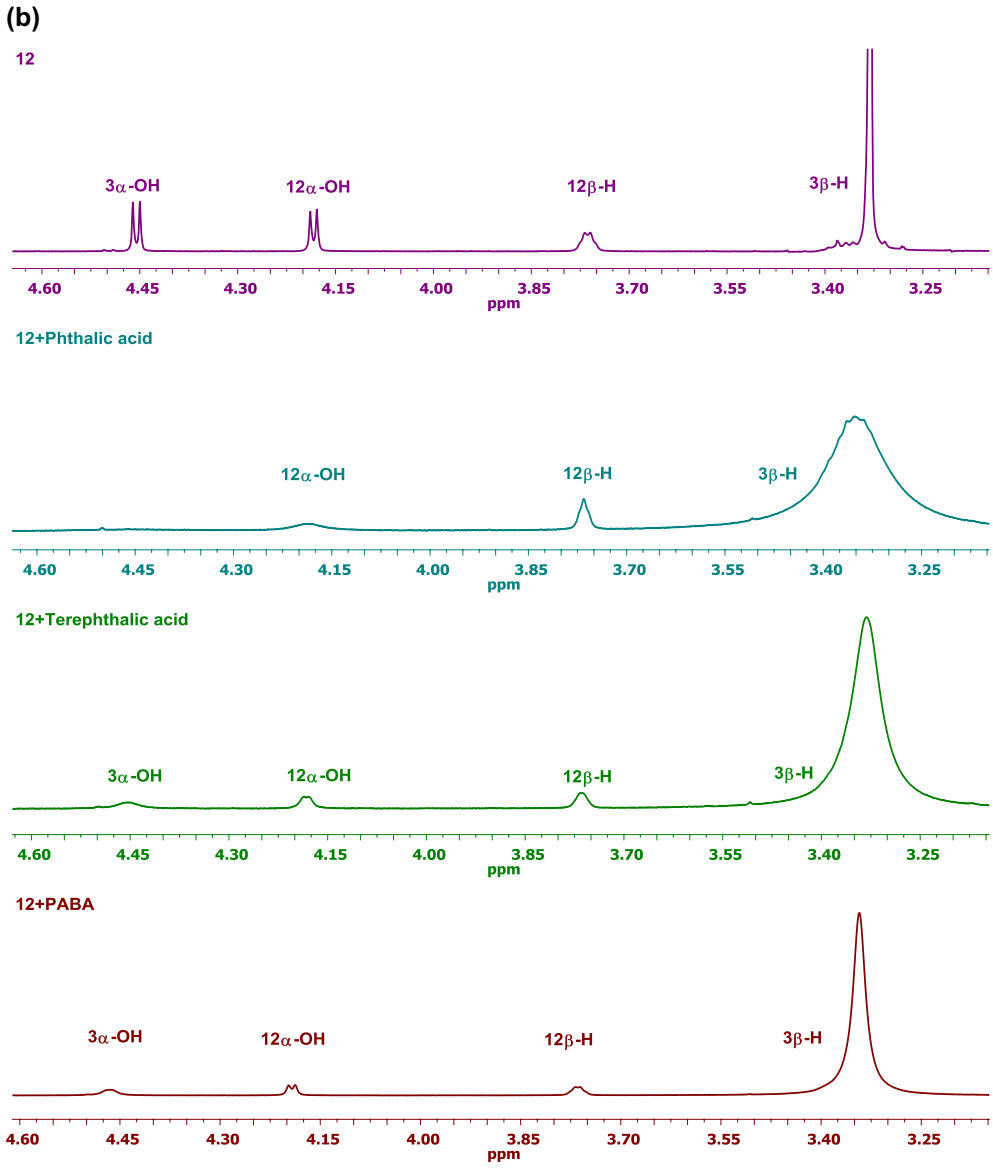


Figure 1b. (*Continued*). ^{1}H NMR in 4.5–3.0 ppm region of dimers (**12**) and its adducts with phthalic acid, terephthalic and PABA in DMSO-d_6.

1,2-benzenedicarboxylic acid, 1,4-benzenedicarboxylic acid and 4-aminobenzoic acid were determined from their ^{1}H and ^{13}C NMR, FT-IR and MALDI-MS spectra. Moreover, PM5 calculation methods were performed for all compounds (*37–39*).

3.1. Synthesis studies

The 1,3-di(azidomethyl)benzene (**2**), propargyl esters of bile acids (**6–8**) and bromoacetoxy derivatives of propargyl esters of bile acids (**9**,**10**) were prepared according to the literature procedures (*41–43*). The bile acid esters were obtained with high yields (95–99%). The propargyl 3α-bromoacetoxy-5β-cholan-24-oate (**9**) and propargyl 3α-bromoacetoxy-12α-hydroxy-5β-cholan-24-oate (**10**) were synthesised by the reaction of corresponding bile acid propargyl esters with bromoacetic acid bromide in anhydrous toluene with sodium hydride and TEBA. This one-pot reaction leads to bile acid derivatives with 53 and 47% yield, respectively. The azide (**2**) and propargyl esters of bile acids (**6–8**) and its derivatives (**9**,**10**) were used as a substrate in the 'click' reaction in the presence of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate. Application of mixtures of solvents: *t*-BuOH/MeOH (5:1) gave very good results. The mixture of products (**11–15**) was obtained and separated by column chromatography. Additionally, conjugate (**13**) has been studied as a ligand in the formation of hydrogen bonds with the 1,2-benzenedicarboxylic acid, 1,4-benzenedicarboxylic acid and 4-aminobenzoic acid.

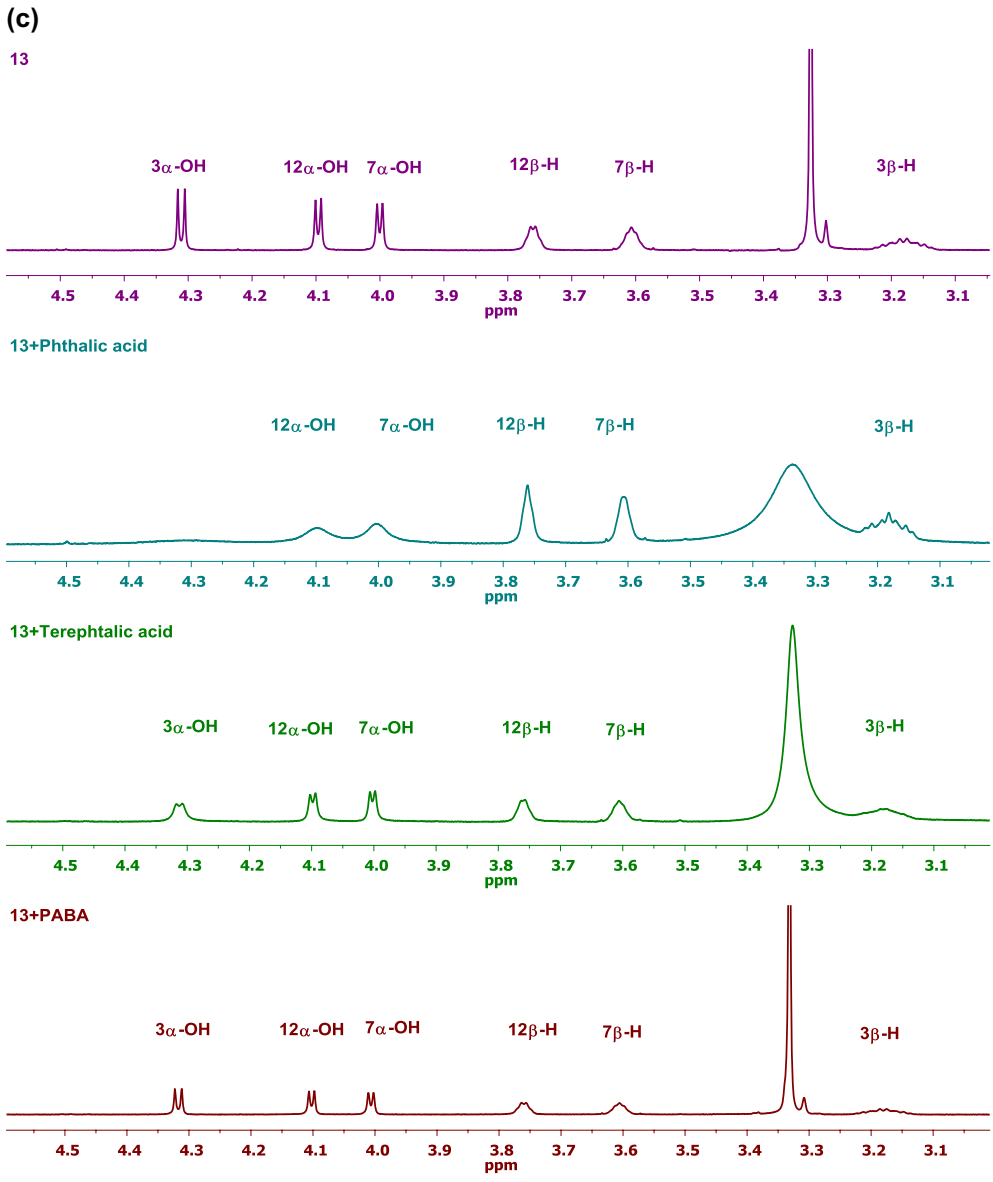


Figure 1c. (*Continued*). ^{1}H NMR in 4.5–3.0 ppm region of dimers (**13**) and its adducts with phthalic acid, terephthalic and PABA in DMSO-d_6.

3.2. NMR studies

The ^{1}H and ^{13}C NMR data of compounds (**11–13**) are shown in Tables 1 and 2, respectively. The differences Δ (ppm) between the respective chemical shifts observed in the spectra of the dimers in chloroform and DMSO-d_6 are shown in Table 3.

In the ^{1}H NMR spectrum of compounds (**11–13**) in both solutions, the signals of the benzene ring protons are found in the range of 7.43–7.20 ppm. The proton signals of the triazole ring C(27)H arise as a singlet at about 7.55 and 8.18 ppm, assigned in $CDCl_3$ and DMSO-d_6, respectively. The protons of methylene group C(25)H are located in a very similar range at 5.19 ppm. The ^{1}H NMR spectra of compounds (**11–13**) show characteristic multiplets in the range of 3.68–3.14 ppm assigned to the 3β–H protons of the steroid skeleton and two hydrogen singlets ranging from 0.65 to 0.55 and 0.91 to 0.81 and characteristic doublets at 0.92–0.84 ppm assigned to CH3–18, CH3–19 and CH3–21, respectively. In the ^{1}H NMR spectrum in DMSO-d_6 of (**11–13**), the protons of 3α–OH hydroxyl groups are found as a doublet at 4.45, 4.46 and 4.31 ppm, respectively. On the other hand, protons of 12α–OH in (**12**) and (**13**) as well as 7α–OH in (**13**) are located at 4.18, 4.10 and 4.00 ppm, respectively. The ^{1}H NMR spectra of compounds (**14**) and (**15**) show characteristic singlets in the range of 3.84–3.80 ppm for the protons of the 3α-$OCOCH_2Br$ group. This is consistent with our previous studies of bromoacetyl-substituted derivatives of bile acids (*43*).

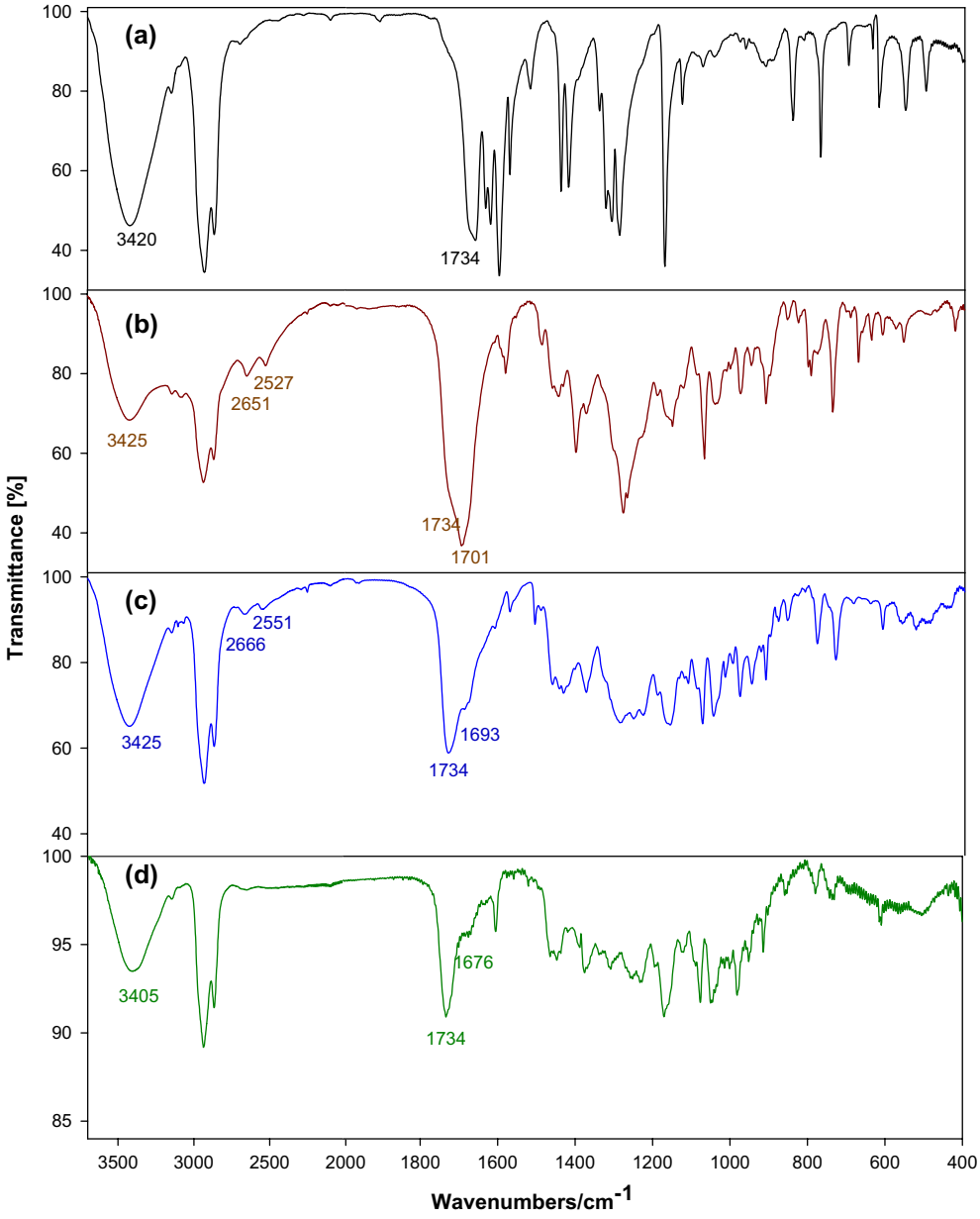


Figure 2. (Colour online) The FT-IR spectra of dimer (**11**) (**a**) and for adduct of dimer (**11**) with phthalic acid (**b**), terephthalic acid (**c**), and PABA (**d**).

The adducts were obtained from the corresponding dimer of bile acids (**11–13**) and aromatic acids (phthalic acid, terephthalic acid and PABA). The ^{1}H NMR spectra of obtained adducts were performed in DMSO-d_6. In Figure 1 are shown the ^{1}H NMR spectrum of ligands **11**, **12** and **13** and their adducts.

A characteristic feature of these spectra is the broadening of the signals 3β-H and 12β-H and broadening and flattening of signals 3α-OH and 12α-OH. For dimer **13**, it was demonstrated that the greatest change in the ^{1}H NMR spectrum is shown in its adduct with phthalic acid. Signals 7α-OH and 12α-OH were substantially flattened, and signal 3α-OH disappeared. This is due to the participation of these groups in the formation of hydrogen bonds. The probability of hydrogen bond formation is much higher at the *ortho* position. Introduction of the bromoacetyl group in C(3)α position of the steroid skeleton (**14**) and (**15**) prevented the formation of adduct with hydrogen bonds. It suggests that only hydroxyl groups form hydrogen bonds, without the participation of nitrogen atoms of the triazole ring. On the other hand, the protons of carboxyl groups in phthalic and terephthalic acid as well as PABA in ^{1}H NMR spectra are at about 13 and 12 ppm, respectively. They create a broad signal. However, in the spectra of the adducts, we can see the complete flattening of the signals in these areas.

Table 4. Heat of formation (HOF) [kcal/mol] of guest molecules and dimers as well as its adducts.

Substrates	HOF [kcal/mol]	ΔHOF [kcal/mol]
Phthalic acid	−154.2706	–
Terephthalic acid	−157.2468	–
PABA	−69.1173	–
11	−286.5609	–
12	−371.0931	–
13	−449.7501	–
14	−365.9395	–
15	−450.8937	–
Adducts	HOF [kcal/mol]	ΔHOF [kcal/mol]
11 + Phthalic acid	−448.0196	−7.1881
11 + Terephthalic acid	−445.0589	−1.2512
11 + PABA	−363.3503	−7.6721
12 + Phthalic acid	−531.5170	−6.1533
12 + Terephthalic acid	−528.0278	0.3121
12 + PABA	−447.3165	−7.1061
13 + Phthalic acid	−617.5121	−13.4914
13 + Terephthalic acid	−615.3107	−8.3135
13 + PABA	−530.4993	−11.6319

Notes.
$\Delta HOF = HOF_{(Adduct\ \mathbf{11})} - [HOF_{(Dimer\ \mathbf{11})} + HOF_{(Phthalic\ acid)}]$.
$\Delta HOF = HOF_{(Adduct\ \mathbf{11})} - [HOF_{(Dimer\ \mathbf{11})} + HOF_{(Terephthalic\ acid)}]$.
$\Delta HOF = HOF_{(Adduct\ \mathbf{11})} - [HOF_{(Dimer\ \mathbf{11})} + HOF_{(PABA)}]$.
$\Delta HOF = HOF_{(Adduct\ \mathbf{12})} - [HOF_{(Dimer\ \mathbf{12})} + HOF_{(Phthalic\ acid)}]$.
$\Delta HOF = HOF_{(Adduct\ \mathbf{12})} - [HOF_{(Dimer\ \mathbf{12})} + HOF_{(Terephthalic\ acid)}]$.
$\Delta HOF = HOF_{(Adduct\ \mathbf{12})} - [HOF_{(Dimer\ \mathbf{12})} + HOF_{(PABA)}]$.
$\Delta HOF = HOF_{(Adduct\ \mathbf{13})} - [HOF_{(Dimer\ \mathbf{13})} + HOF_{(Phthalic\ acid)}]$.
$\Delta HOF = HOF_{(Adduct\ \mathbf{13})} - [HOF_{(Dimer\ \mathbf{13})} + HOF_{(Terephthalic\ acid)}]$.
$\Delta HOF = HOF_{(Adduct\ \mathbf{13})} - [HOF_{(Dimer\ \mathbf{13})} + HOF_{(PABA)}]$.

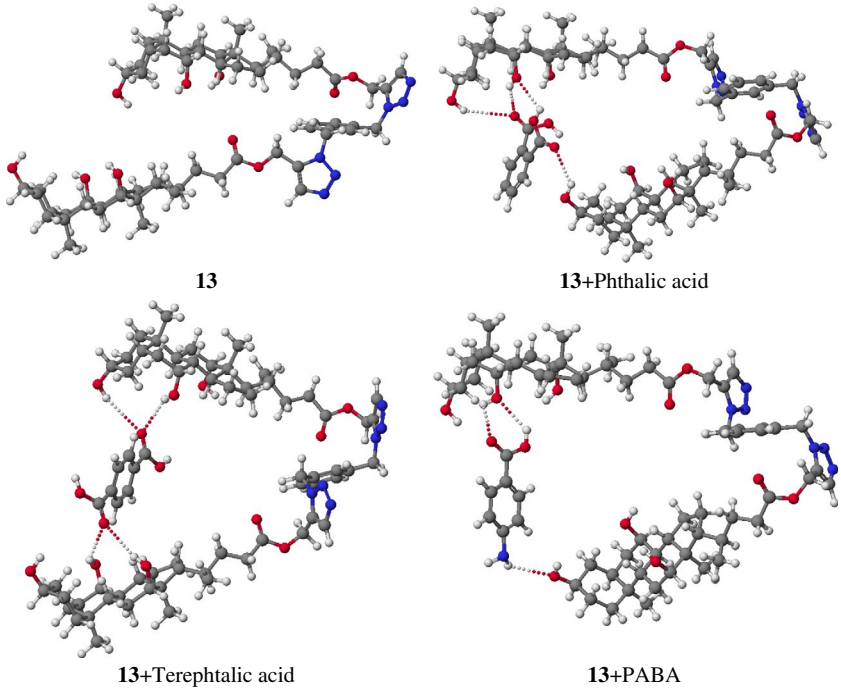


Figure 3. (Colour online) Molecular models of representative dimer (**13**) and its adducts with phthalic and terephthalic acid and PABA calculated by PM5 method.

The visible differences in chemical shifts of the signals in the ^{13}C NMR spectra of dimers in chloroform and in DMSO-d_6 solutions appear for carbon atoms in steroid skeleton: C(3), C(21), C(24). In the case of dimers (**12**) and (**13**), differences in chemical shifts of signals C(7) and C(12) were also seen. Smaller differences in chemical shifts of carbons were observed for C(25), C(26) and C(27). This demonstrates the greater involvement in the interactions of the hydroxyl groups of the steroid skeleton with solvent.

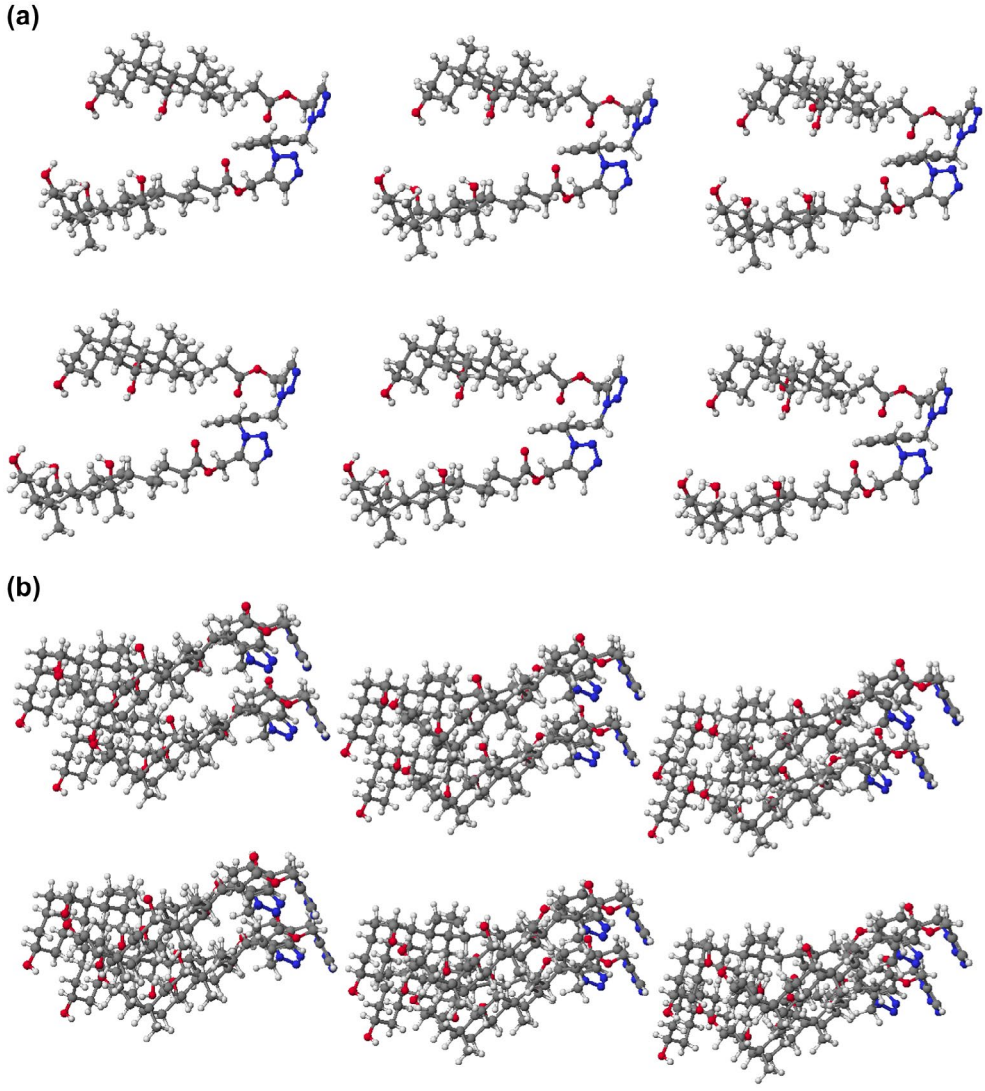


Figure 4. (Colour online) Molecular models of conjugates (**13**) calculated by PM5 method six molecules (**a**) sand channel formed by twelve molecules (**b**).

3.3. FT-IR studies

The FT-IR spectra of dimer (**11**) and for adduct of dimer (**11**) with phthalic acid, terephthalic, and PABA are shown in Figure 2. The intense broad absorption in the 3500–3300 cm^{-1} region corresponds to the overlapping νOH and νNH stretching vibrations. In adducts, this band is broadened and shifted to lower frequencies due to intra- and intermolecular H-bonding of OH groups (Figure 2). The most prominent feature of FT-IR spectra are bands in carbonyl region. The carboxylic acids usually absorb in the region of range 1725–1700 cm^{-1}, while esters absorb somewhat at a higher frequency range of 1750–1735 cm^{-1}. The presence of H-bonding in the molecule causes the additional shift of these bands: 1734 cm^{-1} for dimer (**11**) (Figure 2(a)), 1734 and 1701 cm^{-1} for its adducts with phthalic acid, 1734 and 1693 cm^{-1} with terephthalic acid and 1734 and 1676 cm^{-1} with PABA (Figure 2 (b)–(d)).

The shape of the spectra is a proof of the formation of the H-bonds. The νOH frequency calculated by PM5 method in adduct of dimer (**11**) with phthalic acid, terephthalic acid and PABA exhibits a linear relationship for hydrogen bond length: dimer (**11**) with phthalic acid O···O = 4.06; 3.64 and 3.29 Å, dimer (**11**) with terephthalic acid O···O = 3.87; 3.79; 3.57 and 3.32 Å and dimer (**11**) with PABA O···O = 2.96 Å, respectively.

3.4. Semiempirical studies

PM5 semiempirical calculations were performed using the WinMopac 2003 program. The final HOF [kcal/mol] for phthalic acid, terephthalic acid, PABA and dimers (**11–15**) as well as adducts of dimers (**11**), (**12**) and (**13**) are presented in Table 4. Representative compounds (**13**) and its adducts are shown in Figure 3.

In the case of HOF of dimers (**11–13**), a clear correlation is shown such that with the increase in the number of free hydroxyl groups in steroid skeletons, the value of HOF decreases. It can be also caused by difficulty to form intramolecular hydrogen bonds. In turn, the dimers (**14**) and (**15**) with a lower HOF are deoxycholic acid derivatives. This fact can be additionally explained by the greater stability of bromoester groups in isolated molecule. On the other hand, the HOF of all adducts is lower than the HOF of corresponding dimers. This is due to the formation of intermolecular hydrogen bonds, which was confirmed by ^{1}H NMR spectrum analysis and FT-IR. Besides hydrogen bonding, these adducts may be stabilised by electrostatic interactions that arise from the number of hydroxyl groups in the bile acid molecule which is related with a corresponding geometry of the molecule.

The spatial arrangement and interaction of the conjugate **13** are shown in Figure 4. The final heat of formation is –2737.5566 kcal/mol (Figure 4(a)), and the distances between the benzene rings in vertical and between the triazole rings and atom C(3) steroid skeleton in horizontal are 19.1 and 8.9 Å, respectively. In contrast, the heat of formation of supramolecular compound is –5481.8837 kcal/mol (Figure 4(b)) . The molecules are arranged to form specific kind of channel, through which ions or neutral molecules can be transferred. Compensation charges occur only through intermolecular electrostatic interaction. This is a very good confirmation of the conclusion that interactions reduce HOF.

4. Conclusion

In summary, three new dimers of bile acids linked with 1,2,3-triazole ring (**11–13**) and two dimers of bromoacetyl-substituted derivatives of bile acids (**14**), (**15**) were prepared from propargyl esters of bile acids and 1,3-di(azidomethyl)benzene in *t*-butanol/methanol mixture in the presence of sodium ascorbate and $CuSO_4 \cdot 5H_2O$ at 60 °C. These dimers linked with 1,2,3-triazole ring were characterised by spectroscopic (FT-IR, NMR) and molecular structure (PM5) methods. It has been shown that the hydroxyl group participates in the formation of hydrogen bonds with phthalic acid, terephthalic acid and PABA and forms adducts.

Supplemental material

Supplemental data for this paper can be accessed online here: http://dx.doi.org/10.1080/10610278.2016.1175568

Disclosure statement

No potential conflict of interest was reported by the authors.

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