Design and Synthesis of New Bile Acid–Sterol Conjugates Linked *via* 1,2,3-Triazole Ring

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A new steroid conjugates have been obtained from bile acids and sterol derivatives using 'click chemistry'. Intermolecular 1,3-dipolar cycloaddition of the propargyl ester of bile acids (lithocholic, deoxycholic, and cholic acid) and azide derivatives of sterols (ergosterol and cholesterol) gave a new bile acid–sterol conjugates linked with a 1,2,3-triazole ring. The structures of all products were confirmed by spectroscopic (^{1}H- and ^{13}C-NMR, and FT-IR) analyses, mass spectrometry (ESI-MS), and *in silico* biological activity evaluation methods (PASS), as well as PM5 semiempirical methods.

Introduction. – Natural products are compounds produced by living organisms. This large group of compounds also includes steroids. All steroids are modified triterpenoids with the tetracyclic ring system of lanosterol. The sterols are constituents of the cell membrane in eukaryotes, the main sex hormones in mammals and plants control activities and modification of the structural characteristics of the tissue [1]. Bile acids and vitamin D play important roles in regulating metabolism [2]. Cholesterol is an important component of plasma membrane, the mitochondrial outer membrane, and endoplasmic reticulum. The ester form of cholesterol stabilizes and stiffens a protein–lipid membrane. This sterol is also present in significant concentrations in the brain and nervous tissue, and it is the biosynthetic precursor of other steroids [3]. In turn, ergosterol is the main sterol in fungi and yeasts. It fulfils two main objectives – a sparking function and a bulk membrane function. Moreover, ergosterol is a biological precursor to vitamin D_2 (ergocalciferol) [4]. Bile acids are polyhydroxylated steroidal acids, which are major metabolites of cholesterol. Bile acids are characterized by a large, rigid, and curved skeleton, as well as chemically different polar OH groups and amphiphilic properties. The primary bile acids – chenodeoxycholic and cholic acids are synthesized from cholesterol in the liver in many enzymatic steps. Subsequently, they are transformed into the secondary bile acids such as ursodeoxycholic, deoxycholic, and lithocholic acids [5–9]. The combination of these two important groups of steroids by the 1,2,3-triazole ring allows the synthesis of novel conjugates with a variety of applications [10]. Synthesis of new steroid conjugates entails the possibility of receiving more compounds with high biological activity [11].

The use of 'click chemistry' to develop new steroid conjugates is very attractive from the pharmacological and biological point of view. Moreover, application of 'click chemistry' creates a broad spectrum of rings with carbon-heteroatom bonds. Reactions fulfil specified requirements such as high productivity and selectivity, simple reaction conditions, an easy way to product isolation and its stability in various solvents,

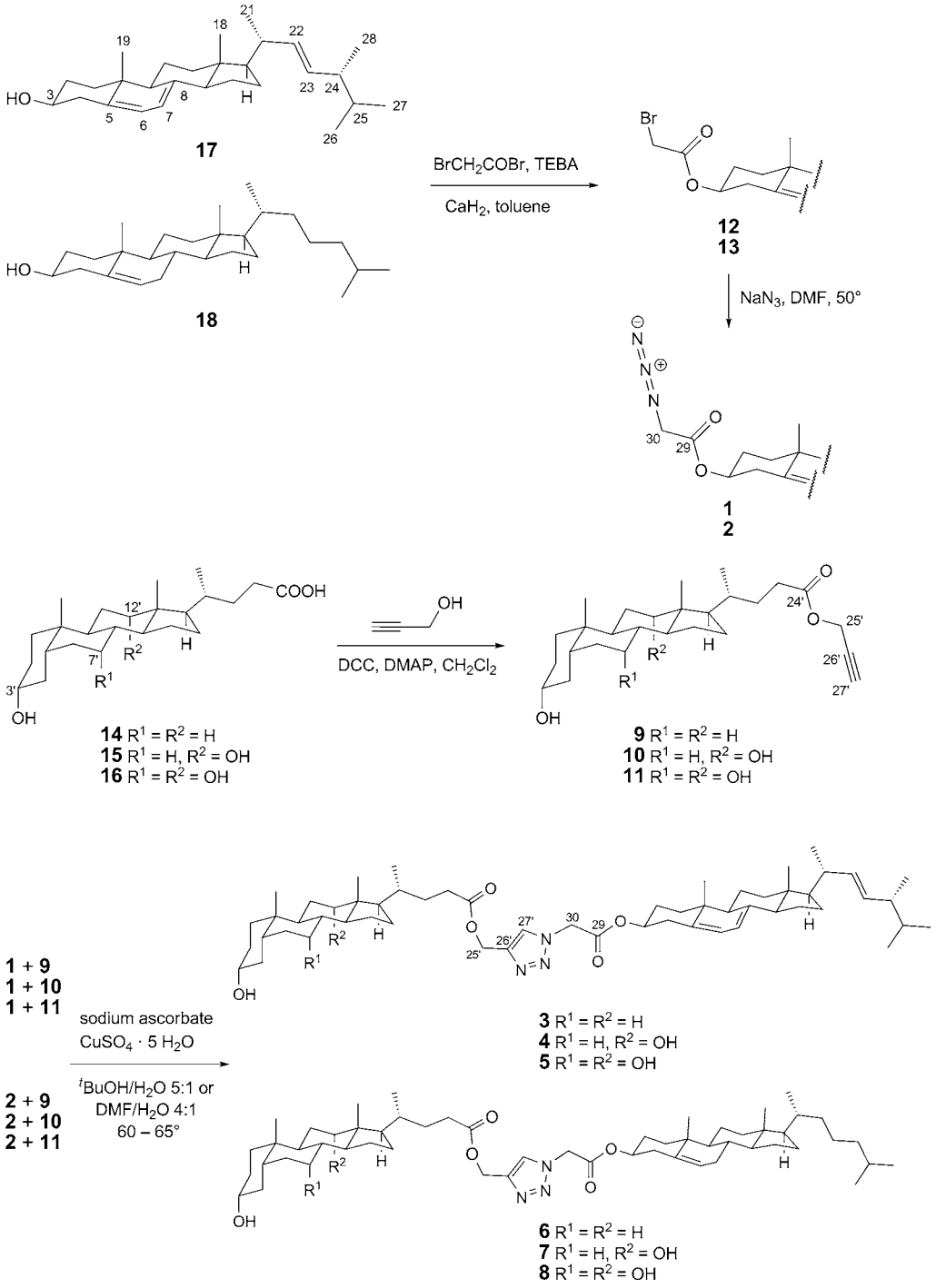
including H_2O [12][13]. Especially important is the *Huisgen* reaction (1,3-dipolar cycloaddition). It occurs between terminal alkynes and azides in the presence of Cu^I catalyst. It is a very convenient and simple method for the synthesis of 1,2,3-triazoles. Furthermore, different catalysts and temperatures are the cause of regioselectivity of cycloaddition reactions, and lead to the formation of 1,4- or 1,5-disubstituted 1,2,3-triazoles [14][15]. The 1,2,3-triazole rings are resistant to oxidation and reduction, as well as hydrolysis conditions of metabolic degradation. Moreover, 1,4-disubstitued 1,2,3-triazoles can interact with surrounding molecules *via* H-bonds and dipole–dipole interactions [16].

Results and Discussion. – To the best of our knowledge, no work has been published on the synthesis or the physicochemical properties of bile acid–sterol conjugates linked by a 1,2,3-triazole ring. This is the first report in literature on the mixed steroid conjugates linked *via* a 1,2,3-triazole ring. This work reports the synthesis and physicochemical properties of new bile acid–sterol conjugates linked with a 1,2,3-triazole ring of propargyl esters of bile acid and ster-3β-yl 2-azido-acetates. The structures of ergoster-3β-yl 2-azidoacetate (**1**) and cholester-3β-yl 2-azidoacetate (**2**) were confirmed by ^{1}H- and ^{13}C-NMR, and FT-IR analysis, as well as ESI-MS. The structures of all synthesized conjugates **3**–**8** were determined on the basis of their ^{1}H- and ^{13}C-NMR, FT-IR, and ESI-MS spectra. Moreover, PM5-calculation methods were performed for all compounds [17]. The syntheses of substrates **1**, **2**, and **9**–**11**, as well as conjugates **3**–**8**, are shown in the *Scheme*.

The ergoster-3β-yl 2-bromoacetate (**12**) and cholester-3β-yl 2-bromoacetate (**13**), as well as propargyl esters of bile acids **9**–**11**, were prepared according to the literature procedures [18]. The ster-3β-yl 2-bromoacetates, as well as bile acid esters, were obtained with high yields (85–94%). The ergoster-3β-yl 2-azidoacetate (**1**) and cholester-3β-yl 2-azidoacetate (**2**) were synthesized in the reaction of corresponding ster-3β-yl 2-bromoacetate with NaN_3 in DMF at 50°. This one-pot reaction leads to azide derivatives in 95 and 93% yield, respectively. The azides **1** or **2** and propargyl esters of bile acids **9**–**11** were used as a substrate in the 'click' reaction in the presence of $CuSO_4 \cdot 5\,H_2O$ and sodium ascorbate. Application of two different mixtures of solvents ^{t}BuOH/H_2O (5:1) and DMF/H_2O (4:1) gave the same results. A mixture of products **3**–**8** were obtained and separated by column chromatography. Particular attention should be paid to the derivatives of ergosterols, **3**–**5**, because they can undergo photochemical transformation.

Compounds **12** and **13** were transformed into azides **1** and **2** *via* a substitution reaction carried out in DMF in the presence of NaN_3. The ^{1}H-NMR spectra showed a signal of the N_3–CH_2 H-atoms at δ(H) 3.84 ppm in both compounds. The ^{1}H-NMR spectra of compounds **1** and **2** show characteristic *multiplet*s in the range of δ(H) 4.93–4.70 ppm assigned to H_α–C(3) of the steroid skeleton, and two *singlet*s at δ(H) 0.83 ppm for **1** and 0.68 ppm for **2**, as well as *singlet*s at δ(H) 1.02–1.01 ppm, and characteristic *doublet*s at δ(H) 0.93–0.92 ppm assigned to Me(18), Me(19), and Me(21), respectively. The ergoster-3β-yl 2-azidoacetate (**1**) showed *multiplet*s in the range of δ(H) 5.41–5.19 ppm assigned to H–C(7), H–C(22), and H–C(23) H-atoms. On the other hand, H–C(6) H-atoms characteristic for cholesterol and ergosterol derivatives gave a signal in the range of δ(H) 5.41–5.19 ppm. The *doublet*s appeared in

Scheme


17
18
BrCH2COBr, TEBA
CaH2, toluene
12
13
NaN3, DMF, 50°
1
2
14 R1 = R2 = H
15 R1 = H, R2 = OH
16 R1 = R2 = OH
DCC, DMAP, CH2Cl2
9 R1 = R2 = H
10 R1 = H, R2 = OH
11 R1 = R2 = OH
1 + 9
1 + 10
1 + 11
2 + 9
2 + 10
2 + 11
sodium ascorbate
CuSO4 · 5 H2O
tBuOH/H2O 5:1 or
DMF/H2O 4:1
60 – 65°
3 R1 = R2 = H
4 R1 = H, R2 = OH
5 R1 = R2 = OH
6 R1 = R2 = H
7 R1 = H, R2 = OH
8 R1 = R2 = OH

the δ(H) 0.86–0.83 ppm region for Me(26) and Me(27) of the both azides. Furthermore, for compound **5**, a *doublet* appeared at δ(H) 1.04 ppm assigned to Me(28).

The ^{1}H-NMR spectra of compounds **3**–**5** and **6**–**8** in $CDCl_3$ showed characteristic *multiplet*s in the range of δ(H) 4.95–4.64 and 3.68–3.33 ppm assigned to H_α–C(3) and H_β–C(3) H-atoms of the steroid skeletons, respectively (*Fig. 1*). In the ^{1}H-NMR spectra of compounds **5** and **8**, characteristic *singlet*s at δ(H) 3.84 ppm for H_β–C(7), as well as signals in the range of δ(H) 3.96–3.94 ppm due to H_β–C(12) for compounds **4**, **5**, **7**, and **8** were observed [19]. Two *singlet*s in the range of δ(H) 0.66–0.62 and 0.97–0.90 ppm, and characteristic *doublet*s in the range of δ(H) 0.97–0.84 ppm are assigned to Me(18), Me(19), and Me(21) for bile acid part, respectively. However, for the sterol part, these Me groups are located at δ(H) 0.83 ppm for **3**–**5** and 0.68 ppm for **6**–**8** (Me(18)), as well as in the ranges of δ(H) 1.07–0.98 (Me(19)) and 0.97–0.84 ppm (Me(21)). Moreover, very characteristic *triplet*s appeared at δ(H) 0.83 ppm for Me(26), as well as Me(27) of ergosterols **3**–**5**, and *multiplet*s in the region of δ(H) 0.96–0.84 ppm of cholesterol **6**–**8** substituted derivatives. Furthermore, for conjugates **3**–**5**, a *multiplet* appeared at δ(H) 1.07–0.98 ppm assigned to Me(28). The *multiplet*s arised from overlapping signals of other Me groups. Ergosterol derivatives showed *multiplet*s at δ(H) 5.36–5.17 ppm assigned to H–C(7), H–C(22), and H–C(23). In turn, characteristic H–C(6) of cholesterol and ergosterol gave a signal in the range of δ(H) 5.39–5.17 ppm.

The ^{13}C-NMR spectra of compounds **1** and **2**, as well as **3**–**8** in $CDCl_3$, showed signals at δ(C) 15.83–15.80 ppm (for **1**, **3**–**5**), 11.84–11.82 ppm (for **2**, **6**–**8**), 18.25–18.22 ppm (for **1**, **3**–**5**), 19.27–19.24 ppm (for **2**, **6**–**8**), 19.64–19.61 ppm (for **1**, **3**–**5**), and 18.69–18.67 ppm (for **2**, **6**–**8**), which were assigned to Me(18), Me(19), and Me(21) of ergosterol and cholesterol parts, respectively. In the case of bile acid parts, the signals of C-atoms of Me(18), Me(19), and Me(21) groups were situated in the ranges of δ(C) 12.67–11.97, 23.61–23.12, and 18.23–17.25 ppm, respectively. The following characteristic shifts of Me groups were present in the sterol side chain: Me(26) and Me(27) are positioned in the range of δ(C) 19.97–19.94 ppm (for **1**, **3**–**5**) and 22.80–22.77 ppm, 22.55–22.52 ppm (for **2**, **6**–**8**), and Me(28) (only for **1**, **3**–**5**) in the range of δ(C) 17.65–17.62 ppm. Analytical differences in the ^{13}C-NMR spectra of the Me groups are shown in *Fig. 2*.

In the ^{13}C-NMR spectra of compounds **1** and **2**, the signals of the CH_2 of N_3–CH_2 group appeared at δ(C) 47.97 and 49.95 ppm, respectively. On the other hand, C(29)=O groups resonated at δ(C) 167.81 (**1**) and 167.68 (**2**) ppm. The signals of C(3)–O for **1** and **2** appeared at δ(C) 75.31 and 75.87 ppm, respectively. The C-atoms of the C(24′)OO–CH_2(25′)–triazole ring unit in **3**–**8** resonated in the range of δ(C) 174.16–174.00 ppm (COO) and 57.39–57.35 ppm (CH_2). However, the C-atoms of the CH_2(30)–C(29)OO unit resonated in the range of δ(C) 165.67–165.44 ppm (COO) and 49.92–47.22 ppm (CH_2). The C-atoms of the triazole ring are located at δ(C) 143.49–143.29 and 125.96–125.10 ppm, and are assigned to C(26′) = C(27′)–N, respectively. The important signals of C(3)–O (sterol part) and C(3′)–O (bile acid part) appeared at δ(C) 77.20–77.17 and 71.90–71.77 ppm, respectively.

The FT-IR spectra of ergoster-3β-yl 2-azidoacetate (**1**) and cholester-3β-yl 2-azidoacetate (**2**) showed a very strong band at 2110 and 2108 cm^{-1} associated with the

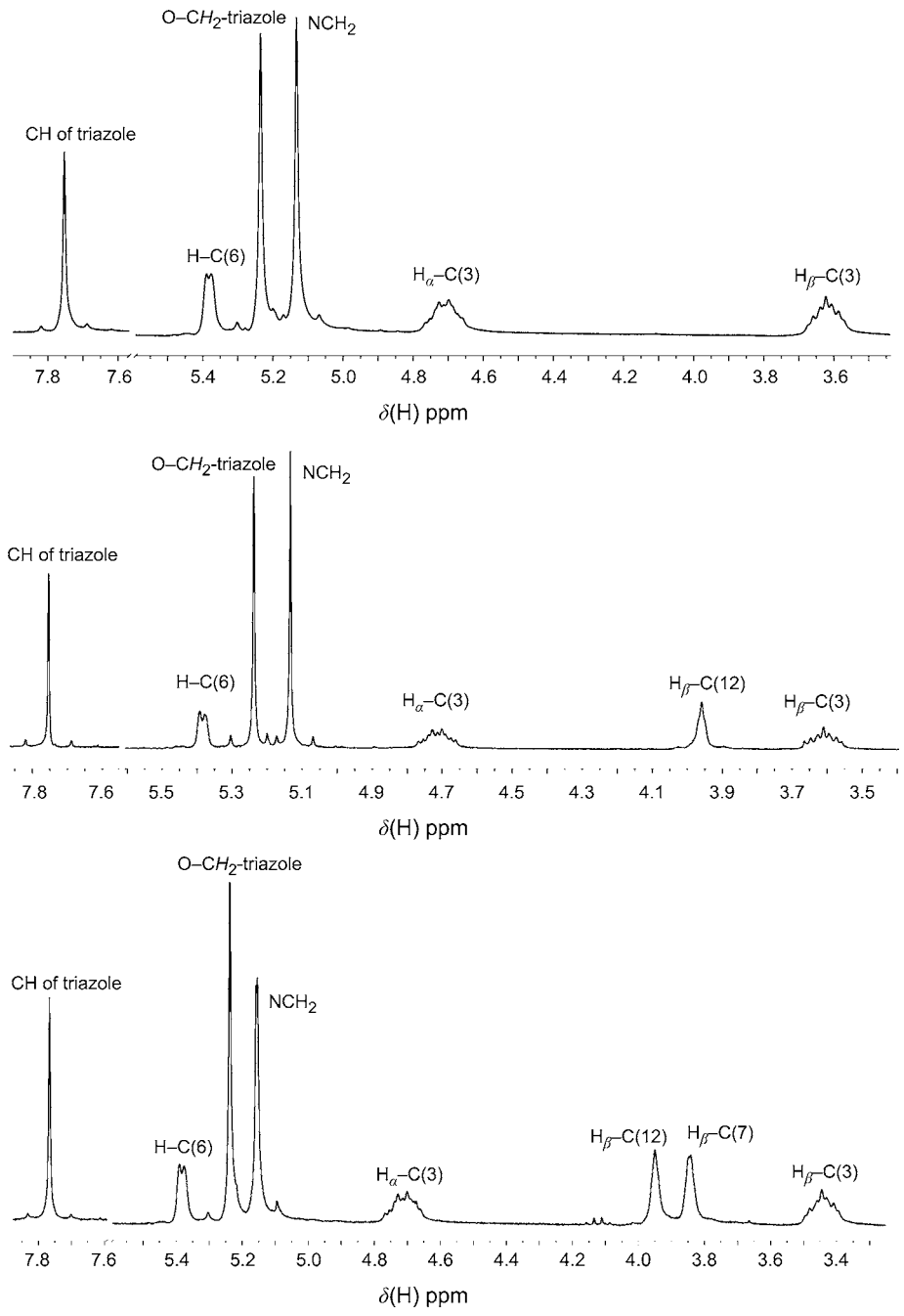


Fig. 1. *^{1}H-NMR Spectra in the region of 7.80–3.40 ppm for the most characteristic signals of compounds* **6**–**8**

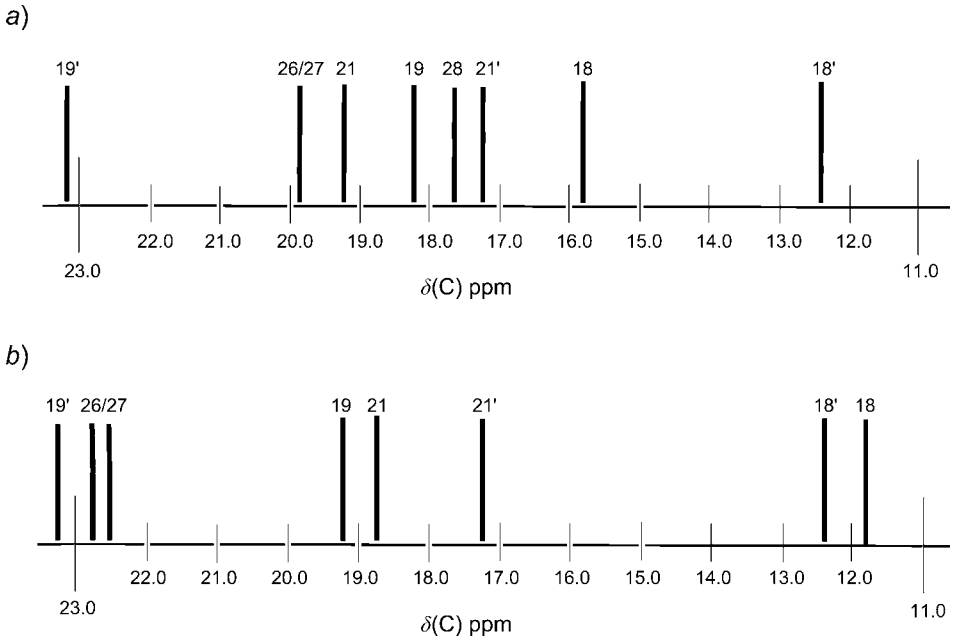


Fig. 2. *Analytical differences of Me groups of conjugates* **5** (*a*) *and* **8** (*b*) *in the corresponding* ^{13}*C-NMR spectra*

presence of $\nu(N^-{=}N^+{=}N{-})$ groups. These bands disappeared in the spectra of the conjugates. In *Fig. 3*, the FT-IR spectra of **6**–**8** are shown. The steroid skeleton itself, being a saturated hydrocarbon, is not a source of many useful IR features. Any vibrational bands due to C–C bonds were very weak and were lost among others in the fingerprint region. Stretching vibrations of C–H bonds merged into one broad band, for conjugate structure, between 2980 and 2860 cm^{-1}. Rather weak but sharper bands at 3152, 3154, and 3161 cm^{-1} were characteristic for the stretching vibrations of C–H bonds at olefinic positions for **6**, **7**, and **8**, respectively (*Fig. 4*,*a*).

The bending vibrations τ_{C-H} are featured as intense band between 758 and 756 cm^{-1} (*Fig. 3*). The most characteristic in the FT-IR spectra of **6**, **7**, and **8** are bands at 3397, 3404, and 3397 cm^{-1} assigned to stretching vibrations of the OH groups, respectively (*Fig. 4*,*a*). The position and intensity of these bands depend on the degree of the association of the conjugates. These bands indicated a complex structure according to the involvement of the OH groups in the intermolecular H-bonds. The similar effect is observed for the $\nu_{C=O}$ ester group of **6**, **7**, and **8** which are at 1740, 1739, and 1739 cm^{-1}, respectively (*Fig. 4*,*b*). The C=C strong bands were rather weak for conjugated steroids, and they were observed at 1669, 1671, and 1669 cm^{-1}, respectively (*Fig. 4*,*b*). All investigated esters of the sterols were characterized additionally by two strong C–O vibrations, giving a distinctive total of three strong bands, which generally dominate the spectrum [19]. The OC–O strong band was found near 1216 cm^{-1} for **6**–**8** and the OCO–C strong band occurred in the range of 1170–1164 cm^{-1}, close to the ν_{C-O} of alcohols, but with enhanced intensity.

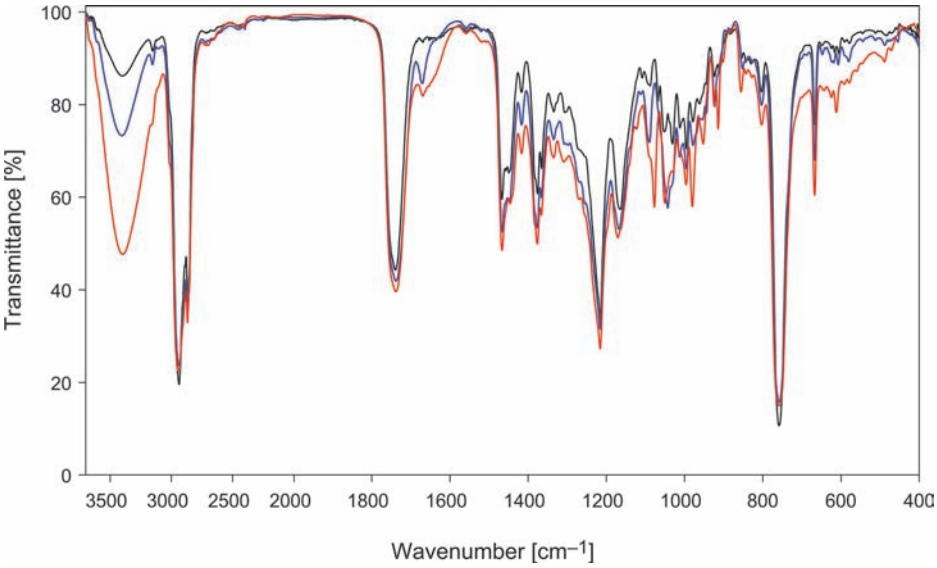


Fig. 3. *FT-IR Spectra of conjugates* **6** (black), **7** (blue), *and* **8** (red)

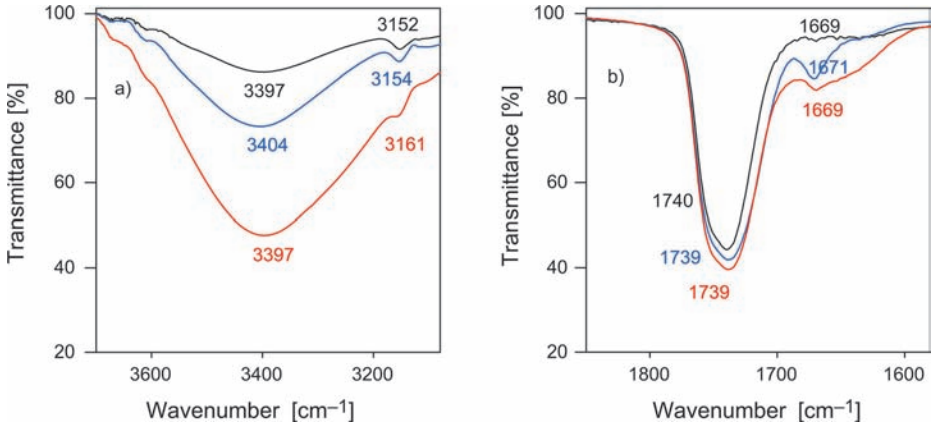


Fig. 4. *FT-IR Spectra of conjugates* **6** (black), **7** (blue), *and* **8** (red) *in the region of 3600–3100 cm*$^{-1}$ (*a*) *and 1800–1600 cm*$^{-1}$ (*b*)

All ESI-MS spectra were recorded in MeOH. In the case of the ergoster-3β-yl 2-azidoacetate (**1**) and cholester-3β-yl 2-azidoacetate (**2**), the characteristic mass fragmentation of azides, *i.e.*, elimination of neutral N_2 or HN_3 molecules, was not observed. For both compounds, the corresponding *pseudo*-molecular-ion peaks were observed at *m*/*z* 538 and 528 ($[M + MeCOO]^-$) for **1** and **2**, respectively. In addition, the mass spectra of both compounds showed more complex and more complicated ions, *e.g.*, $[C_{29}H_{47}N_3O_2 + HCOOH + MeOH + H_2O + Na]^+$ or $[C_{29}H_{47}N_3O_2 + AcOH + K]^+$

for cholester-3β-yl 2-azidoacetate. In all cases, the molecular ions were present, which were associated with a proton, alkali metals (Li^+, Na^+, K^+), as well as AcOH (for **7**) or HCOOH (for **8**), in the positive-ion-mode ESI-MS. In the negative-ion-mode ESI-MS, molecular ions were present, which were associated with anion halides (Cl^- or Br^-) or anions of HCOOH and AcOH. In *Fig. 5*, the ESI-MS spectra of conjugate **7** are shown. In the positive-ion-mode ESI-MS of this conjugate, the ion peaks were observed at *m*/*z* 960.70 (38%, $[M + AcOH + H]^+$), 939.63 (30%, $[M + K]^+$), 922.66 (100%, $[M + Na]^+$), and 900.68 (15%, $[M + H]^+$). The negative-ion-mode ESI-MS showed the ion peaks at *m*/*z* 952.65 (38%, $[M + H_2O + Cl]^-$) and 934.64 (100%, $[M + Cl]^-$).

PM5 Semiempirical calculations were performed using the WinMopac 2003 program. The final heats of formation (HOF; [kcal/mol]) for compounds **3**–**8** are presented in the *Table*. The molecular models of representative compounds **5** and **8** are shown in *Fig. 6*.

The lowest HOF values were observed for cholic acid derivatives **5** and **8**, where the OH groups facilitate the formation of intramolecular H-bonds and stable host–guest complexes. These complexes may be stabilized by H-bonding or electrostatic interactions that arise from the OH groups in the bile acid molecule. The HOF value decreases with the increasing number of OH groups in the steroid skeleton. On the other hand, it is noteworthy that the derivatives, **3**–**5**, with the ergosterol part have higher HOF values than those, **6**–**8**, with the cholesterol part. For cholesterol–bile acid conjugates **6**–**8**, the HOF values are the lowest, because there is only one C=C bond, in contrast to ergosterol–bile acid conjugates **3**–**5**, having three C=C bonds, which increase the reactivity of the molecule, thereby rising up values of HOF.

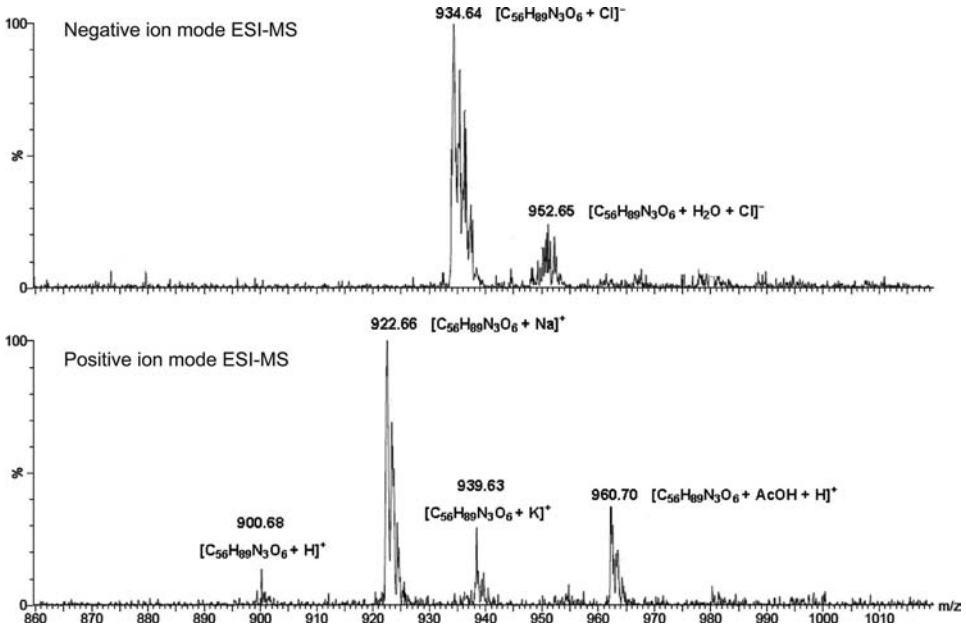


Fig. 5. *ESI-MS Spectrum of conjugate* **7**

Table. *Heat of Formation* (HOF; [kcal/mol]) *of Compounds* **3**–**8** *and* **14**–**16**

Compound	HOF	ΔHOF
3	– 293.4293	– 57.2708
4	– 335.6714	– 57.2098
5	– 375.3243	– 57.1558
6	– 336.4458	– 100.2873
7	– 378.6827	– 100.2211
8	– 418.3992	– 100.2307
14	– 236.1585	–
15	– 278.4616	–
16	– 318.1685	–

$\Delta HOF = HOF_{(\mathbf{3}\text{–}\mathbf{8})} - HOF_{\text{bile acids }(\mathbf{14}\text{–}\mathbf{16})}$

The spatial arrangement and interaction of the conjugate **8** is shown in *Fig. 7*. The final heat of formation is – 2277.1838 kcal/mol, and the distances between the C(19)–C(19′) and C(25)–C(25′) atoms of two conjugates are 6.92 and 6.29 Å. Compensation charges occur only through intermolecular electrostatic interaction. This is a very good confirmation of the conclusion that interactions reduce HOF.

Potential pharmacological activities of the synthesized compounds have been determined on the basis of a computer-aided drug discovery approach with *in silico* Prediction of Activity Spectra for Substances (PASSs) program [20]. In previous works were presented and discussed *in silico* studies of steroid conjugates with different long chain amines, as well as linked triazole rings [21]. In this article, the biological activity spectra were predicted for all six synthesized conjugates **3**–**8** with PASS. Additionally, the types of activity, which were predicted for a potential compound with the highest probability (focal activities), have also been selected. They are presented in *Fig. 8*. According to these data, the most frequently predicted types of biological activity are the inhibitors glyceryl-ether monooxygenase, oxidoreductase, and alcohol *O*-acetyltransferase, CYP3A4 and CYP3A substrates for ergosterol derivatives **3**–**5**, as well as cholesterol antagonist, antihypercholesterolemic, hypolipemic activities for cholesterol derivatives **6**–**8**.

Conclusions. – In summary, six new conjugates, **3**–**8**, of bile acids and sterols linked with a 1,2,3-triazole ring were prepared from propargyl esters of bile acids and ster-3β-yl 2-azidoacetates in a mixture of ^{t}BuOH (or DMF)/H_2O in the presence of sodium ascorbate and $CuSO_4 \cdot 5\ H_2O$ at 65°. Moreover, two new azido derivatives of sterols – ergoster-3β-yl 2-azidoacetate (**1**) and cholester-3β-yl 2-azidoacetate (**2**) were prepared from ergoster-3β-yl 2-bromoacetate and cholester-3β-yl 2-bromoacetate with NaN_3 in acetone. These new conjugates linked with a 1,2,3-triazole ring were characterized by spectroscopic and molecular structure methods. Additionally, their biological properties were tested *in silico* by the PASS method. The results obtained by the PASS method of the identification of the prospective pharmacological properties of **3**–**8** exhibit the possibility of finding new pharmacological agents from this class of compounds. These new conjugates modified by a triazole ring can be used in the molecular recognition, supramolecular chemistry, and in pharmacology [7–11]. In addition, they could find

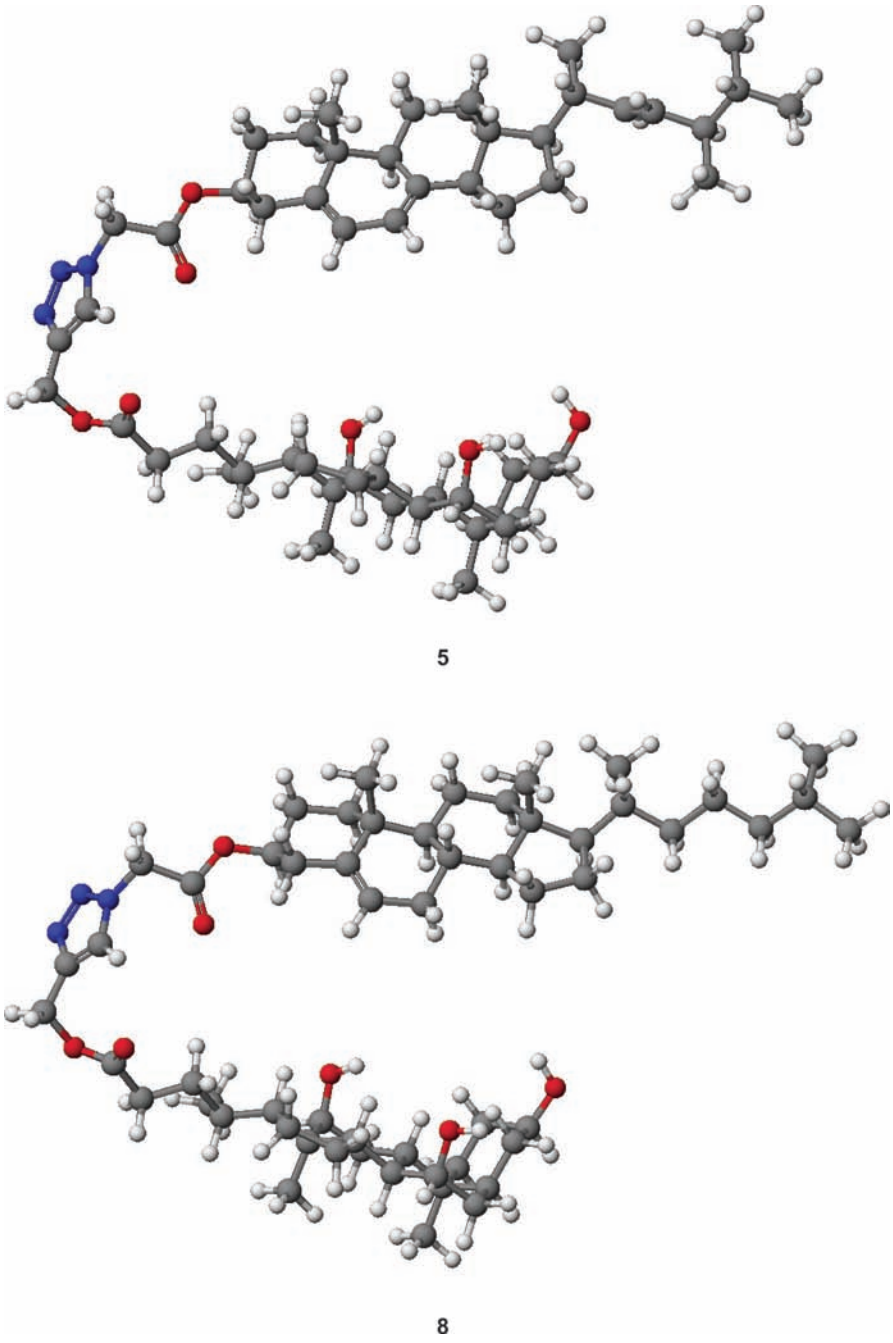


Fig. 6. *Molecular models of representative compounds* **5** *and* **8** *calculated by the PM5 method*

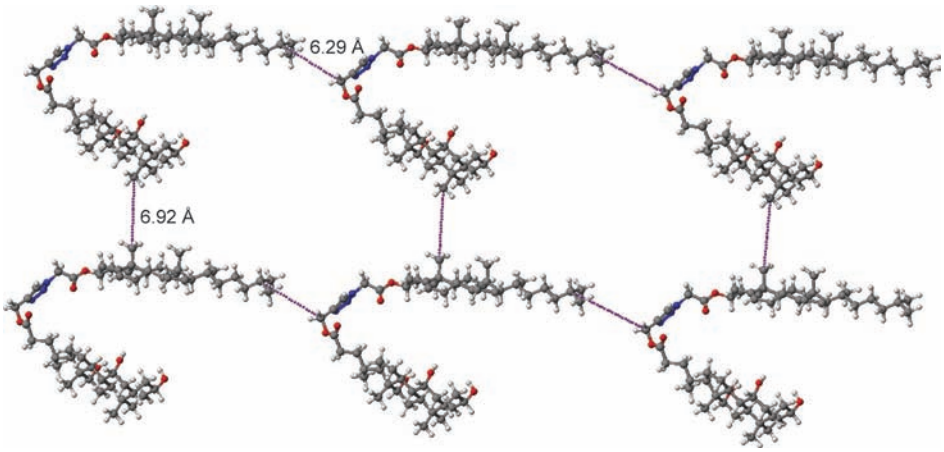


Fig. 7. *Molecular models of conjugates* **8** *calculated by the PM5 method*

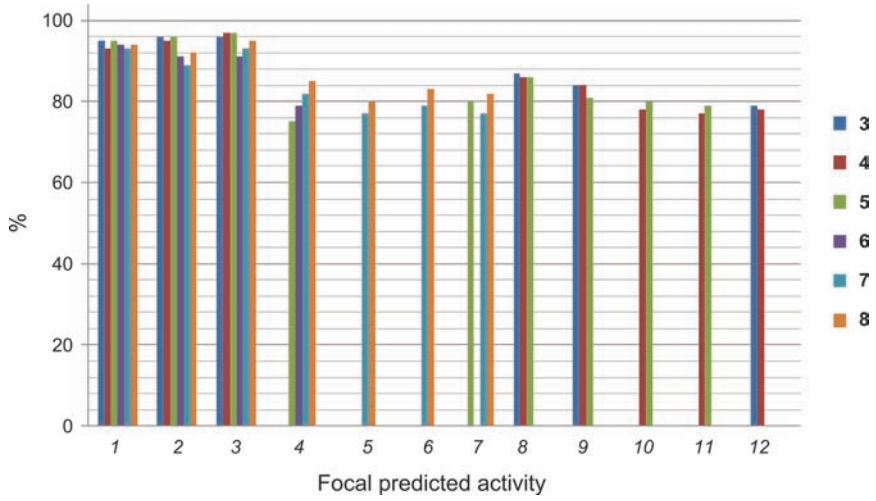


Fig. 8. *PA* (Probability 'to be Active') *values for predicted biological activities of compounds* **3**–**8**. *1*, Cholesterol antagonist; *2*, antihypercholesterolemic activity; *3*, hypolipemic activity; *4*, proliferative diseases treatment; *5*, CYP7 inhibitor; *6*, acylcarnitine hydrolase inhibitor; *7*, glyceryl-ether monooxygenase inhibitor; *8*, alcohol *O*-acetyltransferase inhibitor; *9*, caspase 3 stimulant; *10*, CYP3A4 substrate; *11*, CYP3A substrate; *12*, oxidoreductase inhibitor.

applications as artificial receptors [8][9], good organogelators [11], as well as novel drugs [12]. All of the obtained compounds contain functionalized groups which allow further modifications.

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Experimental Part

General. IR Spectra: *Bruker IFS 66* FT-IR spectrometer (Karlsruhe, Germany); in KBr pellets or film; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Varian Mercury 300 MHz* spectrometer (Oxford, UK), operating at 300.07 and 75.4614 for ^1H and ^{13}C, resp.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. Typical conditions for the H-atom spectra: pulse width 32°, acquisition time 5 s, FT size 32 K and digital resolution 0.3 Hz per point; and for the C-atom 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. ESI-MS: *Waters*/*Micromass* (Manchester, UK) ZQ mass spectrometer equipped with a *Harvard Apparatus* (Saint Laurent, Canada), syringe pump; in *m*/*z*. The sample solns. were prepared in MeOH at the concentration of *ca.* 10^{-5} M. The standard ESI-MS mass spectra were recorded at the cone voltage 90 V.

General Procedure for Compounds **1** *and* **2**. Ergoster-3β-yl 2-bromoacetate (**12**; 500 mg, 0.97 mmol) or cholester-3β-yl 2-bromoacetate (**13**; 500 mg, 0.99 mmol) was dissolved in 15 ml of DMF. Then, NaN_3 (189 mg, 2.92 mmol, or 193 mg, 2.97 mmol) was added, the mixture was heated at 50° for 4 h. DMF was evaporated, extracted with toluene, washed with brine, and dried (Na_2SO_4).

Ergoster-3β-yl 2-Azidoacetate (**1**). Yield 95%. Colorless glassy oil. IR (KBr): 2956, 2110, 1740, 1456, 1366, 1298, 1220. ^1H-NMR: 5.41–5.19 (*m*, H–C(6,7,22,23)); 4.93–4.78 (*m*, H_α–C(3)); 3.84 (*s*, CH_2N_3); 1.04 (*d*, *J* = 6.6, Me(28)); 1.01 (*s*, Me(19)); 0.93 (*d*, *J* = 6.9, Me(21)); 0.83 (*t*, *J* = 5.8, Me(18,26,27)). ^{13}C-NMR: 167.81; 140.10; 135.47; 132.08; 128.43; 123.30; 118.08; 75.31; 57.05; 55.95; 55.69; 54.51; 50.52; 47.97; 44.78; 44.48; 42.81; 40.76; 39.47; 38.89; 36.84; 36.68; 36.47; 35.00; 34.04; 33.08; 27.60; 26.46; 25.12; 21.80; 20.99; 19.97; 19.64; 18.25; 17.65; 15.83. ESI-MS: 538 ($[C_{30}H_{45}N_3O_2 + MeCO_2]^-$), 524 ($[C_{30}H_{45}N_3O_2 + HCO_2]^-$), 449 ($[C_{30}H_{45}N_3O_2 - HNO]^-$), 434 ($[C_{30}H_{45}N_3O_2 - N_2 - OH]^+$).

Cholester-3β-yl 2-Azidoacetate (**2**). Yield 93%. Colorless glassy oil. IR (KBr): 2939, 2107, 1794, 1671, 1468, 1283, 1214. ^1H-NMR: 5.40 (*d*, *J* = 5.5, H–C(6)); 4.74–4.70 (*m*, H_α–C(3)); 3.84 (*s*, CH_2N_3); 1.02 (*s*, Me(19)); 0.92 (*d*, *J* = 6.6, Me(21)); 0.86 (*dd*, *J* = 6.6, 1.8, Me(26,27)); 0.68 (*s*, Me(18)). ^{13}C-NMR: 167.68; 139.11; 123.13; 75.87; 56.64; 56.10; 50.50; 49.95; 42.28; 39.67; 39.49; 37.97; 36.86; 36.53; 36.15; 35.77; 31.87; 31.81; 28.20; 28.00; 27.69; 24.26; 23.81; 22.80; 22.55; 21.01; 19.27; 18.69; 11.84. ESI-MS: 528 ($[C_{29}H_{47}N_3O_2 + MeCO_2]^-$), 438 ($[C_{29}H_{47}N_3O_2 + HNO]^-$), 588 ($[C_{29}H_{47}N_3O_2 + HCO_2H + MeOH + H_2O + Na]^+$), 568 ($[C_{29}H_{47}N_3O_2 + MeCO_2H + K]^+$).

Representative Procedure for the Synthesis of Compound **6**. Propargyl lithocholate (50 mg, 0.12 mmol) was dissolved in 6 ml of a mixture of *t*BuOH/MeOH (5:1) or DMF/H_2O (4:1), and then, cholester-3β-yl 2-azidoacetate (**2**; 57.8 mg, 0.12 mmol) was added. The mixture was heated at 60–65° for 30 min. To the homogenous soln., $CuSO_4 \cdot 5\,H_2O$ (3 mg, 3 mol-%) and sodium ascorbate (9 mg, 20 mol-%) in H_2O (0.3 ml) were added. The mixture was heated at 60–65° for 3 h, and then, extracted with $CHCl_3$ (10 ml), washed with brine (15 ml), and dried (Na_2SO_4). Evaporation of the solvent and separation of the residue on silica gel ($CHCl_3$/hexane for **3** and **6**, $CHCl_3$/AcOEt for **4** and **7**, AcOEt for **5** and **8**).

*{1-[2-(Ergosta-5,7,22-trien-3-yloxy)-2-oxoethyl]-1*H*-1,2,3-triazol-4-yl}methyl 3α-Hydroxycholan-24-oate* (**3**). Oil. Yield 83%. IR (film): 3395, 3154, 2942, 2872, 1745, 1661, 1223, 1173, 755. ^1H-NMR: 7.75 (*s*, 1 H of triazole); 5.35–5.18 (*m*, 6 H, O–CH_2-triazole, H–C(6,7,22,23)); 5.13 (*s*, triazole–CH_2–CO); 4.92–4.79 (*m*, H_α–C(3)); 3.67–3.57 (*m*, H_β–C(3)); 1.05–1.01 (*m*, Me(28,19)); 0.94–0.90 (*m*, Me(21,19′,21′)); 0.83 (*t*, *J* = 5.8, Me(18,26,27)); 0.62 (*s*, Me(18′)). ^{13}C-NMR: 174.11; 165.56; 143.40; 139.93; 135.43; 132.08; 128.19; 125.11; 123.31; 118.12; 77.20; 71.83; 57.36; 57.02; 56.45; 55.89; 51.03; 47.92; 44.76; 42.84; 42.79; 42.70; 42.06; 40.72; 40.39; 40.12; 38.87; 36.82; 36.66; 36.42; 35.81; 35.56; 35.31; 34.94; 34.54; 33.93; 33.06; 31.08; 30.84; 30.52; 29.68; 28.18; 27.16; 26.40; 25.09; 24.18; 23.35; 21.79; 20.98; 20.79; 19.96; 19.63; 18.23; 17.64; 15.81; 11.99. ESI-MS: 953 ($[C_{57}H_{87}N_3O_5 + MeCO_2]^-$), 917 ($[C_{57}H_{87}N_3O_5 + Na]^+$), 901 ($[C_{57}H_{87}N_3O_5 + Li]^+$).

*{1-[2-(Ergosta-5,7,22-trien-3-yloxy)-2-oxoethyl]-1*H*-1,2,3-triazol-4-yl}methyl 3α,12α-Dihydroxycholan-24-oate* (**4**). Yield 91%. Oil. IR (film): 3390, 3159, 2940, 2875, 1746, 1658, 1225, 1176, 758. ^1H-NMR: 7.75 (*s*, 1 H of triazole); 5.36–5.17 (*m*, 6 H, O–CH_2-triazole ring, H–C(6,7,22,23)); 5.13 (*s*, triazole–CH_2–CO); 4.92–4.71 (*m*, H_α–C(3)); 3.96 (*s*, H_β–C(12)); 3.67–3.50 (*m*, H_β–C(3)); 1.07–0.99 (*m*, Me(28,19)); 0.97–0.92 (*m*, Me(21,19′,21′)); 0.83 (*t*, *J* = 5.7, Me(18,26,27)); 0.65 (*s*, Me(18′)). ^{13}C-NMR: 174.05; 165.50; 143.49; 139.84; 135.30; 132.14; 128.16; 125.13; 123.33; 118.11; 77.17; 73.07; 71.78; 57.37;

51.03; 48.22; 47.22; 46.55; 42.84; 42.05; 36.39; 35.99; 35.19; 35.04; 34.08; 33.63; 33.05; 31.09; 30.73; 30.45; 28.64; 27.41; 27.09; 26.10; 23.61; 23.12; 19.94; 19.61; 18.22; 17.62; 17.26; 15.80; 12.67. ESI-MS: 1049 ($[C_{57}H_{87}N_3O_6 + MeCO_2H + Br]^-$), 989 ($[C_{57}H_{87}N_3O_6 + Br]^-$), 949 ($[C_{57}H_{87}N_3O_6 + K]^+$), 933 ($[C_{57}H_{87}N_3O_6 + Na]^+$).

*{1-[2-(Ergosta-5,7,22-trien-3-yloxy)-2-oxoethyl]-1*H*-1,2,3-triazol-4-yl}methyl 3α,7α,12α-Trihydroxycholan-24-oate* (**5**). Yield 74%. Oil. IR (film): 3396, 3158, 2941, 2872, 1748, 1660, 1225, 1174, 753. ^{1}H-NMR: 7.77 (*s*, 1 H of triazole); 5.36–5.18 (*m*, 6 H, O–CH_2-triazole ring, H–C(6,7,22,23)); 5.15 (br. *s*, triazole–CH_2–CO); 4.95–4.66 (*m*, H_α–C(3)); 3.94 (*s*, H_β–C(12)); 3.84 (*s*, H_β–C(7)); 3.51–3.33 (*m*, H_β–C(3)); 1.06–0.98 (*m*, Me(28,19)); 0.97–0.90 (*m*, Me(21,19′,21′)); 0.88 (*s*, 1 H); 0.83 (*t*, *J* = 5.7, Me(18,26,27)); 0.65 (*s*, Me(18′)). ^{13}C-NMR: 174.16; 165.67; 143.29; 139.95; 135.29; 132.08; 128.21; 125.26; 123.32; 118.11; 77.20; 72.92; 71.87; 68.40; 57.38; 57.02; 55.92; 51.02; 47.92; 46.83; 46.42; 44.76; 44.42; 43.48; 42.83; 42.79; 41.70; 41.44; 40.72; 39.64; 39.50; 38.86; 36.81; 36.65; 36.41; 35.55; 35.25; 35.15; 34.93; 34.69; 34.56; 33.92; 33.05; 32.29; 31.03; 30.67; 30.44; 29.67; 28.18; 27.83; 27.46; 26.45; 25.08; 23.17; 22.46; 21.78; 21.15; 20.97; 19.94; 19.62; 18.23; 17.63; 17.25; 15.81; 12.41. ESI-MS: 961 ($[C_{57}H_{87}N_3O_7 + Cl]^-$), 933 ($[C_{57}H_{87}N_3O_7 + Li]^+$).

*{1-[2-(Cholest-5-en-3-yloxy)-2-oxoethyl]-1*H*-1,2,3-triazol-4-yl}methyl 3α-Hydroxycholan-24-oate* (**6**). Yield 97%. Oil. IR (film): 3397, 3152, 2937, 2867, 1740, 1669, 1216, 1164, 758. ^{1}H-NMR: 7.75 (*s*, 1 H of triazole); 5.38 (*d*, *J* = 5.5, H–C(6)); 5.24 (*s*, O–CH_2-triazole); 5.13 (*s*, triazole–CH_2–CO); 4.77–4.66 (*m*, H_α–C(3)); 3.67–3.57 (*m*, H_β–C(3)); 1.02 (*s*, Me(19)); 0.92 (*s*, Me(19′)); 0.91–0.85 (*m*, Me(21,21′,26,27)); 0.68 (*s*, Me(18)); 0.62 (*s*, Me(18′)). ^{13}C-NMR: 174.08; 165.44; 143.37; 138.86; 125.11; 123.30; 77.20; 71.79; 57.35; 56.61; 56.43; 56.43; 56.07; 55.88; 51.00; 49.91; 42.68; 42.26; 42.05; 40.37; 40.11; 39.64; 39.47; 37.84; 36.78; 36.49; 36.41; 36.13; 35.80; 35.74; 35.29; 34.53; 31.85; 31.77; 31.07; 30.82; 30.51; 28.16; 27.97; 27.58; 27.16; 26.38; 24.23; 24.17; 23.78; 23.34; 22.78; 22.53; 20.98; 20.78; 19.23; 18.68; 18.21; 11.97; 11.82. ESI-MS: 965 ($[C_{56}H_{89}N_3O_5 + Br]^-$), 944 ($[C_{56}H_{89}N_3O_5 + MeCO_2]^-$), 924 ($[C_{56}H_{89}N_3O_5 + K]^+$), 907 ($[C_{56}H_{89}N_3O_5 + Na]^+$), 885 ($[C_{56}H_{89}N_3O_5 + H]^+$).

*{1-[2-(Cholest-5-en-3-yloxy)-2-oxoethyl]-1*H*-1,2,3-triazol-4-yl}methyl 3α,12α-Dihydroxycholan-24-oate* (**7**). Yield 91%. Oil. IR (film): 3404, 3154, 2936, 2867, 1739, 1671, 1215, 1168, 758. ^{1}H-NMR: 7.75 (*s*, 1 H of triazole); 5.39 (*d*, *J* = 4.6, H–C(6)); 5.24 (*s*, O–CH_2–triazole); 5.13 (*s*, triazole–CH_2–CO); 4.74–4.69 (*m*, H_α–C(3)); 3.96 (br. *s*, H_β–C(12)); 3.68–3.48 (*m*, H_β–C(3)); 1.02 (*s*, Me(19)); 0.95–0.84 (*m*, Me(21,21′,26,27)); 0.91 (*s*, Me(19′)); 0.68 (*s*, Me(18)); 0.65 (*s*, Me(18′)). ^{13}C-NMR: 174.00; 165.45; 143.34; 138.86; 125.10; 123.30; 77.17; 73.04; 71.77; 57.37; 56.61; 56.07; 51.00; 49.91; 48.21; 47.22; 46.44; 42.26; 42.03; 39.64; 39.46; 37.84; 36.78; 36.49; 36.40; 36.13; 35.99; 35.74; 35.16; 35.00; 34.07; 33.63; 31.84; 31.77; 31.19; 31.08; 30.71; 30.47; 29.66; 28.64; 28.17; 27.97; 27.58; 27.38; 27.08; 26.08; 24.23; 23.78; 23.59; 23.12; 22.77; 22.52; 20.98; 19.23; 18.67; 17.26; 12.67; 11.82. ESI-MS: 953 ($[C_{56}H_{89}N_3O_6 + H_2O + Cl]^-$), 935 ($[C_{56}H_{89}N_3O_6 + Cl]^-$), 961 ($[C_{56}H_{89}N_3O_6 + MeCO_2H + H]^+$), 940 ($[C_{56}H_{89}N_3O_6 + K]^+$), 923 ($[C_{56}H_{89}N_3O_6 + Na]^+$), 901 ($[C_{56}H_{89}N_3O_7 + H]^+$).

*{1-[2-(Cholest-5-en-3-yloxy)-2-oxoethyl]-1*H*-1,2,3-triazol-4-yl}methyl 3α,7α,12α-Trihydroxycholan-24-oate* (**8**). Yield 78%. Oil. IR (film): 3396, 3161, 2939, 2868, 1739, 1669, 1216, 1170, 758. ^{1}H-NMR: 7.77 (*s*, 1 H of triazole); 5.38 (*d*, *J* = 4.2, H–C(6)); 5.24 (*s*, O–CH_2–triazole); 5.15 (*s*, triazole–CH_2–CO); 4.78–4.64 (*m*, H_α–C(3)); 3.95 (*s*, H_β–C(12)); 3.84 (*s*, H_β–C(7)); 3.57–3.36 (*m*, H_β–C(3)); 1.02 (*s*, Me(19)); 0.96–0.85 (*m*, Me(21′,21,26,27)); 0.92 (*s*, Me(19′)); 0.68 (*s*, Me(18)); 0.66 (*s*, Me(18′)). ^{13}C-NMR: 174.08; 165.53; 143.29; 138.89; 125.24; 123.31; 77.19; 72.84; 71.90; 68.34; 57.39; 56.62; 56.08; 51.01; 49.91; 46.87; 46.46; 42.27; 41.86; 41.43; 39.71; 39.65; 39.55; 39.47; 37.84; 36.78; 36.50; 36.14; 35.75; 35.19; 35.07; 34.65; 34.49; 31.85; 31.77; 31.00; 30.62; 30.55; 29.67; 28.27; 28.18; 27.98; 27.59; 27.43; 26.62; 24.24; 23.79; 23.13; 22.79; 22.53; 20.99; 19.24; 18.68; 17.25; 12.44; 11.82. ESI-MS: 996 ($[C_{56}H_{89}N_3O_7 + Br]^-$), 961 ($[C_{56}H_{89}N_3O_7 + HCO_2]^-$), 962 ($[C_{56}H_{89}N_3O_7 + HCO_2H]^+$), 955 ($[C_{56}H_{89}N_3O_7 + K]^+$), 939 ($[C_{56}H_{89}N_3O_6 + Na]^+$).

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