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Synthesis and spectroscopic studies of new bile acid derivatives linked by a 1,2,3-triazole ring

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This work is dedicated to the memory of Professor Elżbieta Wyrzykiewicz (1935–2011).

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ABSTRACT

A novel method for the synthesis of cholic acid derivatives has been developed using 'click chemistry'. Intermolecular 1,3-dipolar cycloaddition of the propargyl ester and azide groups of 3α-azidoacetoxy-7α,12α-diformyloxy-5β-cholan-24-oate gave a new dimer and oligomer linked by a 1,2,3-triazole ring. The structures of the products were confirmed by spectral (^{1}H NMR, ^{13}C NMR, and FT-IR) analysis, mass spectrometry and PM5 semiempirical methods. Estimation of the pharmacotherapeutic potential has been accomplished for the synthesized compounds on the basis of Prediction of Activity Spectra for Substances (PASSs).

Steroids are compounds of natural origin and play very important roles in biological systems. Compounds of this family are the main sex hormones in mammals (e.g., testosterone and estrogens), they are constituents of the cell membrane in eukaryotes (e.g., cholesterol), and they play important functions in regulating metabolism (e.g., bile acids and vitamin D).[1] Especially important compounds are bile acids and their derivatives. These compounds are attractive for synthetic chemists because they have a large, rigid, and curved skeleton. Moreover, they have chemically different polar hydroxy groups and amphiphilic properties. An additional advantage of these compounds is their ready availability and low cost. Bile acids play an important role in supramolecular chemistry and pharmacology.[2] Steroids themselves have been used as building blocks for the design and construction of new molecular receptors that are capable of recognition of guest molecules of diverse chemical nature.[3] Steroid dimers can be used for the synthesis of macrocyclic compounds which may function as artificial receptors.[4] Some derivatives of bile acids are very good organogelators.[5]

'Click chemistry' is a relatively new trend in modern organic synthesis and was developed by Sharpless and coworkers. It includes a broad spectrum of carbon–heteroatom bond forming reactions that fulfils specified requirements such as high efficiency and selectivity, simple reaction conditions and easy product

Table 1
PA (Probability 'to be Active') values for predicted biological activity of compounds **4**–**7**.

Compound	Focal predicted activity (PA >0.7)
4	Dimethylaniline monooxygenase (*N*-oxide-forming) inhibitor (0.895)
	1-Acylglycerol-3-phosphate *O*-acyltransferase inhibitor (0.866)
	Dextranase inhibitor (0.862)
	Squalene-hopene cyclase inhibitor (0.843)
	Peptidoglycan glycosyltransferase inhibitor (0.833)
	Acylglycerol lipase inhibitor (0.849)
	Hypercholesterolemic (0.822)
	N-(long-chain-acyl)ethanolamine deacylase inhibitor (0.816)
	Alkenylglycerophosphoethanolamine hydrolase inhibitor (0.803)
	Cholesterol synthesis inhibitor (0.788)
	Vitamin-K-epoxide reductase (warfarin-insensitive) inhibitor (0.773)
	Signal peptidase I inhibitor (0.769)
	trans-Octaprenyltranstransferase inhibitor (0.765)
	Plasmanylethanolamine desaturase inhibitor (0.733)
	Phospholipase D inhibitor (0.722)
	Ceramide glucosyltransferase inhibitor (0.727)
	Nephrotoxic (0.727)
	Glucan endo-1,3-β-D-glucosidase inhibitor (0.725)
	Hepatotoxic (0.739)
5	1-Acylglycerol-3-phosphate *O*-acyltransferase inhibitor (0.827)
	Squalene-hopene cyclase inhibitor (0.797)
6	1-Acylglycerol-3-phosphate *O*-acyltransferase inhibitor (0.810)
	Squalene-hopene cyclase inhibitor (0.787)
7	1-Acylglycerol-3-phosphate *O*-acyltransferase inhibitor (0.737)
	Squalene-hopene cyclase inhibitor (0.703)

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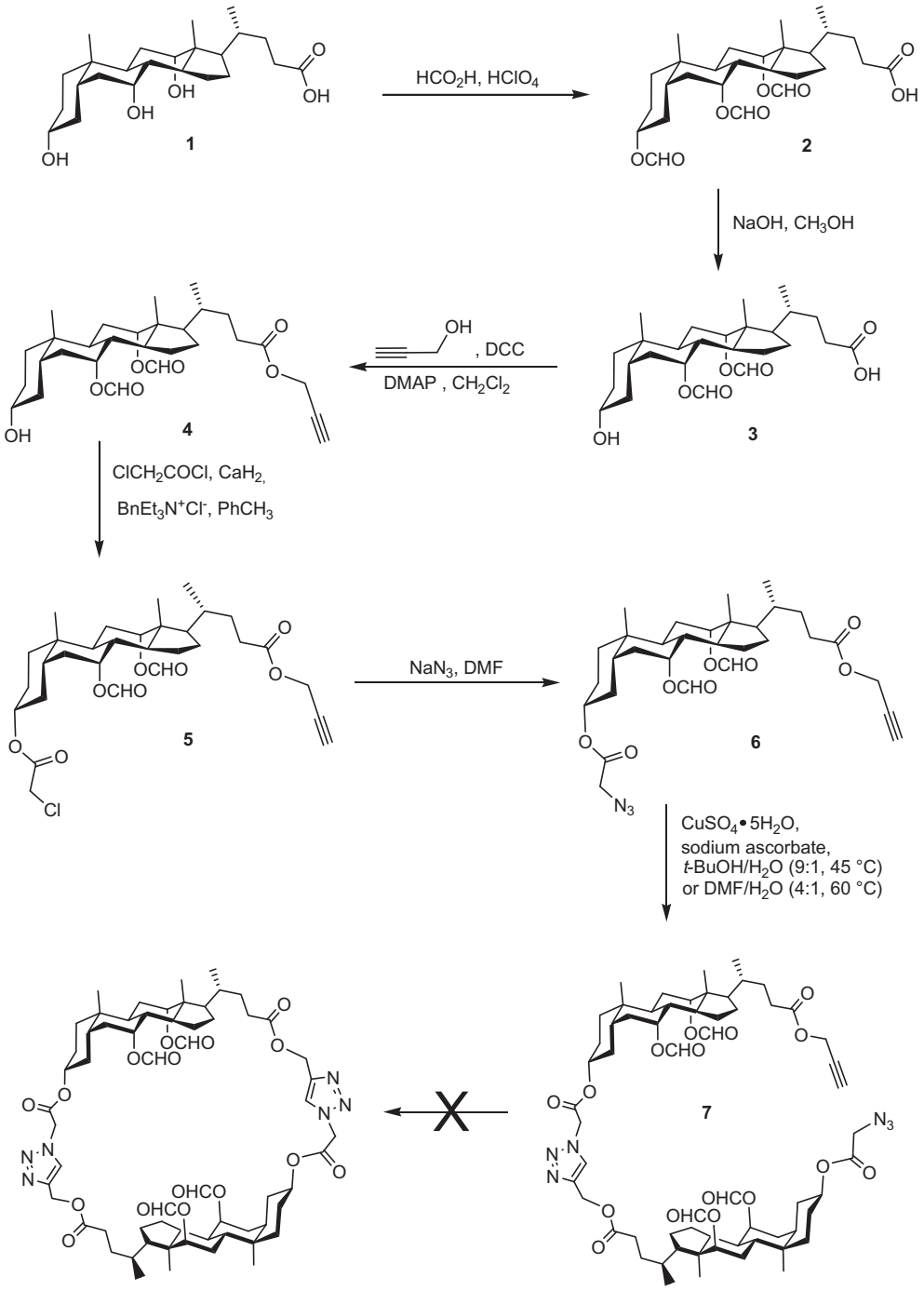

Scheme 1. Synthesis of dimer 7.

isolation.[6] The products of 'click' reactions are stable to various solvents, including water.[7] The copper(I)-catalyzed 1,3-dipolar cycloaddition (the Huisgen reaction) between azides and terminal alkynes is regarded as an important example of the 'click' reaction. It is a convenient, efficient, and selective method for the synthesis of 1,2,3-triazoles which are very stable to hydrolysis, reductive, and oxidative conditions and metabolic degradation. Moreover, 1,4-disubstitued 1,2,3-triazoles show the ability to participate in hydrogen bonds and dipole interactions.[8] Due to the above-mentioned advantages the Huisgen cycloaddition has found many applications in modern organic chemistry including for example: drug discovery,[9] materials science[10] and the synthesis of natural product derivatives.[11] The Cu(I)-catalyzed 'click' reaction is an extremely useful method for obtaining new 1,2,3-triazole derivatives of bile acids.[12]

Potential pharmacological actions of compounds synthesized have been found on the basis of computer-aided drug discovery approaches with the Prediction of Activity Spectra for Substances (PASSs) program. It is based on a robust analysis of structure–activity relationships in a heterogeneous training set currently including about 60,000 biologically active compounds from different chemical series with about 4500 types of biological activity. Since only the structural formula of the chemical compound is necessary to obtain a PASS prediction, this approach can be used at earliest stages of an

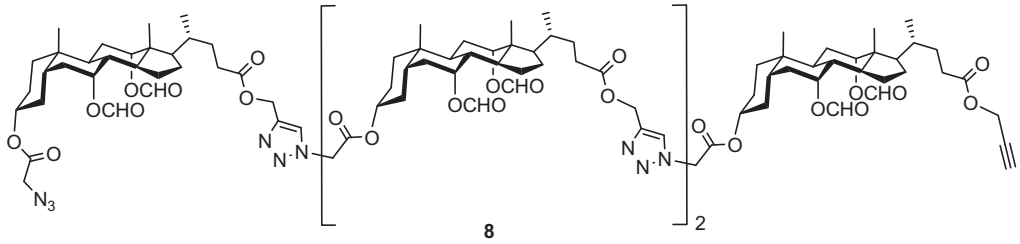

Figure 1. The structure of the oligomeric compound **8**.

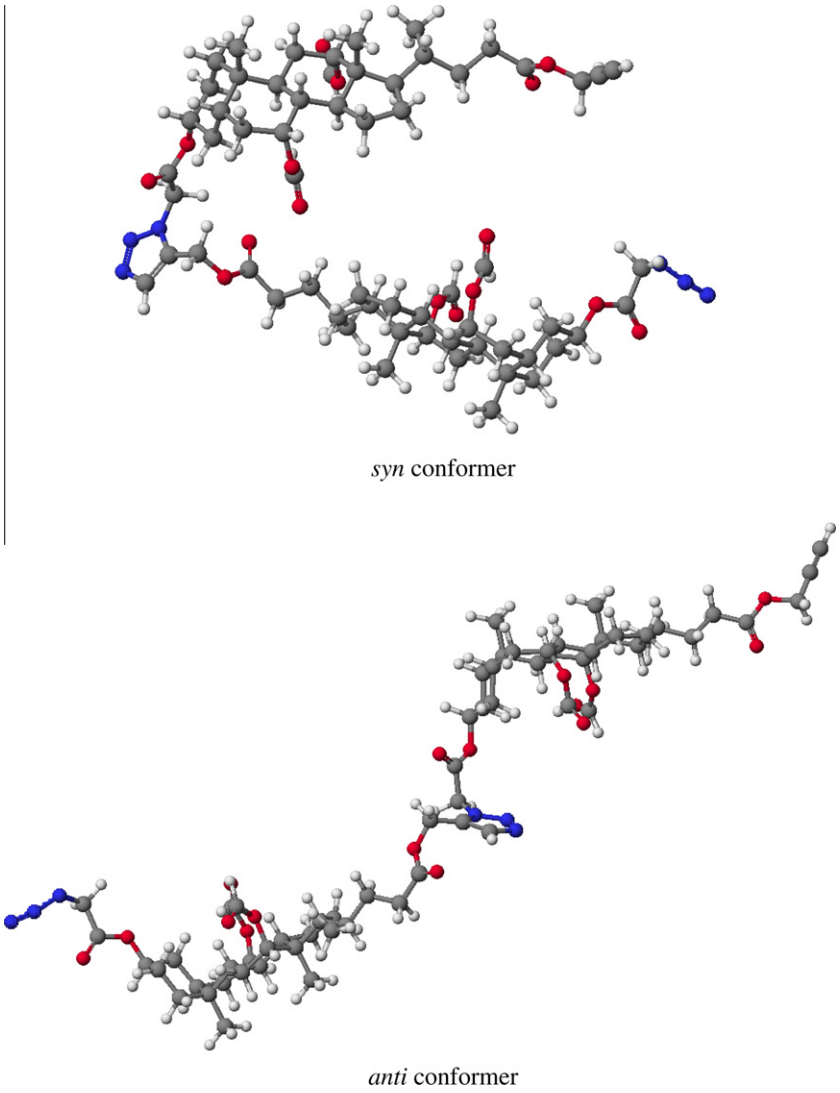

Figure 2. The *syn* and *anti* conformations of dimer **7**.

investigation. There are many examples of the successful use of the PASS approach leading to new pharmacological agents.[13]

This Letter reports the synthesis and physicochemical properties of new cholic acid derivatives linked by a 1,2,3-triazole ring. The structures of all the compounds obtained were determined from their ^{1}H and ^{13}C NMR, FT-IR, EI-MS, ESI-MS, and MALDI-TOF spectra. Moreover, PM5 calculations were performed on compound **7**. Additionally, analyses of the biological prediction activity spectra for the new dimer prepared herein are good examples of in silico studies of chemical compounds.

The biological activity spectra were predicted with PASS for four compounds **4**–**7** synthesized. We also selected the types of activity that were predicted for a potential compound with the highest probability (focal activities), (Table 1). According to these data the most frequently predicted types of biological activity are inhibition of: 1-acylglycerol-3-phosphate *O*-acyltransferase, squalene-hopene cyclase, peptidoglycan glycosyltransferase, acylglycerol lipase, hypercholesterolemic, *N*-(long-chain-acyl)ethanolamine deacylase, alkenylglycerophosphoethanolamine hydrolase, and cholesterol synthesis.

The synthesis of the cholic acid dimer **7** linked by a 1,2,3-triazole ring is shown in Scheme 1. The structure of the oligomeric compound **8** is shown in Figure 1.

Compound **2** was prepared from cholic acid **1** according to the literature procedure.[14] The ^{1}H NMR spectrum showed three characteristic signals at 8.02, 8.10, and 8.16 ppm assigned to the –OCHO groups.[14]

Alcohol **3** was obtained by a selective hydrolysis of the 3α-OCHO group of compound **2**. Our attempts to carry out the reaction according to the literature procedure failed.[14] Carrying out the hydrolysis in acetone in the presence of 0.2 or 0.1 M NaOH aqueous solutions led to mixtures containing small amounts of product, but containing mostly unreacted substrate. Changing the solvent to methanol and using 0.1 M aqueous NaOH gave compound **3** in high yield. The ^{1}H NMR spectrum confirmed the loss of the signal at 8.02 ppm assigned to the 3α-OCHO group and the appearance of a signal at 3.51 ppm due to the 3β-H proton.

The synthesis of compound **4** presented difficulties as well. Esterification in the presence of propargyl alcohol and a catalytic amount of *p*-toluenesulfonic acid caused hydrolysis of the –OCHO groups.[15] Compound **4** was synthesized in the presence of DCC, DMAP, and propargyl alcohol in good yield.[16] The structure of the product obtained was confirmed by ^{1}H NMR spectroscopy which showed two characteristic signals at 2.46 and 4.61 ppm assigned to the –C≡CH and –O–CH_2 protons of the propargyl group.

Compound **5** was easily obtained from ester **4** in high yield by reaction with chloroacetyl chloride in the presence of CaH_2 and triethylbenzylammonium chloride. The structure of the product was confirmed by ^{1}H NMR spectroscopy which showed a characteristic signal at 4.03 ppm assigned to the –CH_2–Cl group.

Compound **5** was transformed into azide **6** via a substitution reaction carried out in DMF in the presence of NaN_3. The ^{1}H NMR spectrum showed a signal at 3.85 ppm protons of the –CH_2–N_3 group.

Freshly obtained compound **6** was used as a substrate in the 'click' reaction in the presence of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate. Application of two different mixtures of solvents: *t*-BuOH/H_2O (9:1) and DMF/H_2O (4:1) gave the same results. The mixture of unreacted substrate **6**, acyclic dimer **7** and oligomeric compound **8** was obtained and separated by column chromatography.

The ^{1}H NMR spectrum of compound **7** in $CDCl_3$ showed a characteristic, diagnostic singlet at 7.73 ppm assigned to the triazole ring. Moreover, the spectrum also showed singlets in the range 8.11–8.18 ppm assigned to the protons of the four –OCHO groups. Three diagnostic singlets for compound **7** at 3.85, 5.12, and 5.22 ppm were assigned to the –CH_2–N_3, –CH_2–C-triazole ring, and –CH_2–N-triazole ring. The methylene doublet resonance at 4.67 ppm was assigned to the –O–CH_2.

The oligomerization reaction occurred unexpectedly and spontaneously at reduced temperature without additional external factors. The ^{1}H NMR spectrum of tetramer **8** in $CDCl_3$ showed singlets at 7.73–7.70 ppm assigned to the three protons of the triazole rings. Moreover, the **8** exhibited singlets in the range 8.17–8.08 ppm assigned to the protons of the eight –OCHO groups. The diagnostic singlet at 3.85 ppm and triplet at 2.47 ppm were assigned to the –CH_2–N_3 and –C≡CH protons, respectively.

The ^{13}C NMR and FT-IR spectra of compounds **4**–**7** were in agreement with the assigned structures.

PM5 semiempirical calculations were preformed using the WinMopac 2003 program. Compound **7** can exist in both *syn* and *anti* conformations as shown in Figure 2. In solution the equilibrium between the two conformers is expected to depend on the polarity of the solvent. The *anti* conformer is almost a linear structure. The final heat of formation for the *anti* conformer is −617.9462 kcal/mol, and that of the *syn* conformer is −620.6547 kcal/mol. The oligomeric compound **8** is formed preferentially because it has a lower final heat of formation (−1287.0911 kcal/mol) than cyclic compound (−664.5813 kcal/mol). Furthermore, the presence of additional groups –OCHO in positions 7α and 12α of the steroid ring causes steric hindrance and increases the repulsive interaction between two steroid units.

In conclusion, five new compounds, propargyl 7α,12α-diformyloxy-3α-hydroxy-5β-cholan-24-oate (**4**), propargyl 3α-chloroacetoxy-7α,12α-diformyloxy-5β-cholan-24-oate (**5**), propargyl 3α-azidoacetoxy-7α,12α-diformyloxy-5β-cholan-24-oate (**6**), dimer (**7**), and tetramer (**8**) linked by 1,2,3-triazole ring were prepared from cholic acid.[17,18]

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Supplementary data

Supplementary data (General procedures.) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.11.027.

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17. Compound **7** – ^{1}H NMR (300 MHz, $CDCl_3$, TMS, ppm): δ ^{1}H NMR: 8.18 (s, 1H, –OCHO), 8.15 (s, 1H, –OCHO), 8.13 (s, 1H, –OCHO), 8.11 (s, 1H, –OCHO), 7.73 (s, 1H, H-triazole ring), 5.24 (s, 2H, 12β-H), 5.22 (s, 2H, $–CH_2–$N-triazole ring), 5.12 (s, 2H, $–CH_2–$C–triazole ring), 5.07 (s, 2H, 7β-H), 4.70 (m, 1H, 3β-H), 4.69 (m, 1H, 3β-H), 4.67 (d, 2H, J = 2.4, $O–CH_2$), 3.85 (s, 2H, $–CH_2–N_3$), 2.47 (t, 1H, –C≡CH), 2.39–1.05 (m, 44H, steroid skeleton), 0.94 (s, 6H, CH_3-19), 0.85 (d, 3H, J = 6.1, CH_3-21), 0.83 (d, 3H, J = 6.1, CH_3-21), 0.76 (s, 3H, CH_3-18), 0.73 (s, 3H, CH_3-18). ^{13}C NMR (75 MHz, $CDCl_3$, TMS, ppm): 173.79 (C-24), 173.07 (C-24′), 167.68 (CO_2), 165.58 (C′O_2), 160.53 (12-OCHO, 7-OCHO), 143.30 (–C-triazole ring), 125.09 (–HC-triazole ring), 76.57 (–C≡), 75.72 (C-12), 74.75 (≡CH), 70.63 (C-3), 51.81 ($O–CH_2$), 50.97 (O–C′H_2), 50.53 (CH_2N_3), 47.20 (C-17), 45.02 (C-13), 42.96 (C-14), 40.80 (C-5), 37.71 (C-4), 37.71 (C-8), 34.68 (C-20), 34.49 (C-20′), 34.43 (C-1), 34.28 (C-10), 31.35 (C-6), 30.91 (C-22)*, 30.81 (C-23)*, 30.45 (C-9), 28.57 (C-16), 27.15 (C-2), 26.56 (C-11), 25.57 (C-15), 22.77 (C-19), 22.31 (C-19′), 17.46 (C-21), 12.12 (C-18). FT-IR (KBr, cm^{-1}): 3271 ν(≡C–H), 2941 ν(C–H), 2117 ν(C≡C), 1718 ν(C=O). ESI-MS (m/z): 1193.1 $[M+Na]^+$, 1209.1 $[M+K]^+$. MALDI-MS (m/z, matrix 2,5-DHB, standard β-CD): 1210.5 $[M+K]^+$, 1325.5 $[M+matrix]^+$, 1364.6 $[M+matrix+K]^+$, 1213.4 $[M+matrix-4\times CO]^-$. Anal. Calcd for $C_{62}H_{86}N_6O_{16}$: C, 63.57; H 7.40, N, 7.17. Found: C, 63.61; H, 7.33, N, 7.25.
*These signals may be interchanged.
18. Compound **8** – ^{1}H NMR (300 MHz, $CDCl_3$, TMS, ppm): δ 8.17 (s, 1H, –OCHO), 8.15 (s, 1H, –OCHO), 8.13 (s, 2H, –OCHO), 8.11 (s, 2H, –OCHO), 8.09 (s, 1H, –OCHO), 8.08 (s, 1H, –OCHO), 7.73 (s, 1H, H-triazole ring), 7.72 (s, 1H, H-triazole ring), 7.70 (s, 1H, H-triazole ring), 5.23 (s, 2H, 12β-H), 5.19 (s, 2H, 12β-H), 5.17 (s, 6H, $–CH_2–$N-triazole ring), 5.16 (s, 4H, $–CH_2–$C-triazole ring), 5.13 (s, 2H, $–CH_2–$C-triazole ring), 5.07 (s, 4H, 7β-H), 4.67 (m, 1H, 3β-H), 4.66 (m, 3H, 3β-H), 4.67 (d, 2H, $O-CH_2$), 3.85 (s, 2H, $–CH_2–N_3$), 2.47 (t, 1H, –C≡CH), 2.39–1.05 (m, 96H, steroid skeleton), 0.95 (s, 3H, CH_3-19), 0.94 (s, 3H, CH_3-19), 0.93 (s, 3H, CH_3-19), 0.92 (s, 3H, CH_3-19), 0.88 (d, 3H, CH_3-21), 0.85 (d, 6H, CH_3-21), 0.83 (d, 3H, CH_3-21), 0.77 (s, 3H, CH_3-18), 0.75 (s, 3H, CH_3-18), 0.74 (s, 3H, CH_3-18), 0.73 (s, 3H, CH_3-18). FT-IR (KBr, cm^{-1}): 3274 ν(≡C–H), 2941 ν(C–H), 2111 ν(C≡C), 1723 ν(C=O). ESI-MS (m/z): 2363.7 [M of tetramer+Na]$^+$. MALDI-MS (m/z, matrix 2,5-DHB, standard β-CD): 1194.3 [M of dimer+Na]$^+$, 1210.1 [M of dimer+K]$^+$, 1778.7 [M of trimer+Na]$^+$, 2363.5 [M of tetramer+Na]$^+$.