Synthesis and transformations of metallacycles. 45.* Cross-cyclomagnesiation of 1,2-dienes in the synthesis of 5*Z*,9*Z*-dienoic acids, efficient inhibitors of human topoisomerase I**

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An original method for the synthesis of natural and synthetic 5*Z*,9*Z*-dienoic acids with high selectivity (>98%) and ~50% yields was elaborated. The method is based on a new Cp_2TiCl_2-catalyzed cross-cyclomagnesiation reaction of terminal aliphatic and *O*-containing 1,2-dienes using Grignard reagents. The synthesized acids exhibited *in vitro* high inhibiting activity against human topoisomerase I.

Key words: cyclomagnesiation, 1,2-dienes, magnesacyclopentane, 5*Z*,9*Z*-dienoic acids, titanocene dichloride, topoisomerase I.

The interest to higher 5*Z*,9*Z*-dienoic acids is due to a wide range of their biological activity. These compounds belong to a group of low-molecular-weight bioregulators of the lipid nature. Some representatives of these acids exhibit antimalarial, antimicrobial, antitumor, and antiviral activity. Apart from that, it is known[2—5] that they can serve as inhibitors of one of the key cell cycle enzymes, topoisomerase I.

In nature, 5*Z*,9*Z*-dienoic acids with 16—34 carbon atoms are found in insignificant amounts in lipids of some sea sponge species and fruits of coniferous gymnosperms. Considerable contribution into the development of methods of isolation and identification and approaches to the synthesis of dienoic fatty acids was made by the research groups of C. Djerassi, N. Carballeira, Y. Sakagami, and others.[6—9] It should be noted that all the methods for the synthesis of 5*Z*,9*Z*-dienoic acids described in the literature[10,11] are multistep processes (5—12 steps), while the yields of the target compounds were only 0.5—15%.

Recently, we have elaborated a reaction of intermolecular (cross) cyclomagnesiation of *O*-, *N*-, *Si*-containing and aliphatic 1,2-dienes using available Grignard reagents in the presence of magnesium (acceptor of halide ions) and a Cp_2TiCl_2 catalyst. This method makes it possible to synthesize functionally substituted hydrocarbons containing a 1*Z*,5*Z*-dienoic group in one preparative step.[12—14] This approach was used[5,15] in the development of an original two-step method for the synthesis of higher natural and synthetic 5*Z*,9*Z*-dienoic acids with 14—24 carbon atoms in up to 60% yields.

In continuation of these studies, in the present work we report the results of the synthesis of natural 5*Z*,9*Z*-dienoic acids, *viz.*, (5*Z*,9*Z*)-5,9-heptacosa- and (5*Z*,9*Z*)-5,9-octacosadienoic acids, as well as the data on the application of this reaction for the preparation of earlier undescribed 5*Z*,9*Z*-dienoic acids containing a bulky steroid fragment.

We suggested that such unsaturated acids with bulky substituents in the hydrocarbon chain can exhibit high inhibiting activity toward human topoisomerase I.

First (Scheme 1), we carried out a Cp_2TiCl_2-catalyzed cross-cyclomagnesiation of terminal aliphatic allenes **1a**,**b** (1,2-eicosadiene, 1,2-heneicosadiene) with hepta-5,6-dien-1-ol tetrahydropyran ether **2** using EtMgBr, under conditions[5] selected earlier (**1a**,**b** : **2** : EtMgBr : Mg : [Ti] = = 10 : 12 : 40 : 32 : 0.5, Et_2O, 20—22 °C, 6 h). A subsequent hydrolysis of magnesacyclopentanes **3a,b** formed *in situ* led to *O*-containing dienes **4a**,**b** in 74 and 71% yields, respectively. However, because of the low solubility of pyran ethers **4a** and **4b** their direct oxidation with Jones reagent gave the target acids **6a,b** in the yields below 10%. To optimize the yield of (5*Z*,9*Z*)-5,9-heptacosa- (**6a**) and (5*Z*,9*Z*)-5,9-octacosadienoic (**6b**) acids, we removed the

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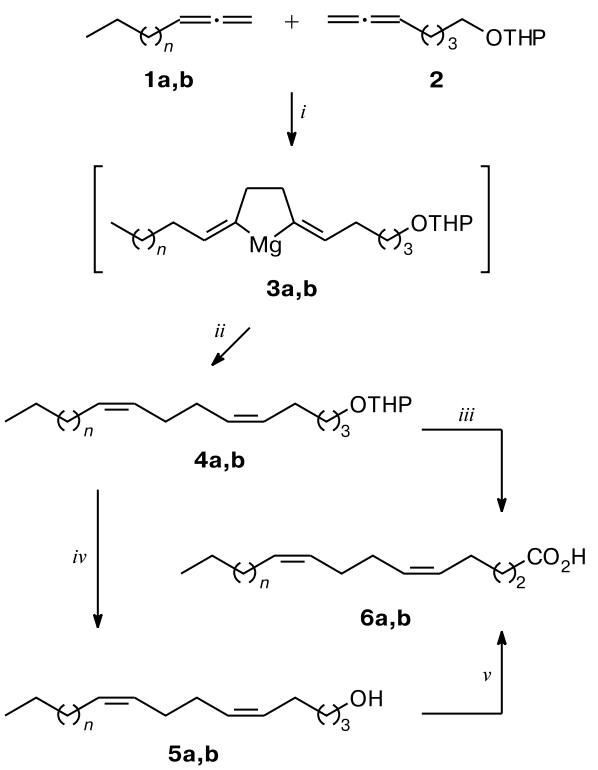
pyranyl protecting groups from hydroxy groups of ethers **4a**,**b**[16] and carried out the oxidation of alcohols **5a**,**b** with pyridinium chlorochromate (PDC) supported on aluminum oxide Al_2O_3.[17] This allowed us to synthesize the target acids **6a**,**b** in 47—50% yields (see Scheme 1).

Scheme 1



1: n = 15 (**a**), 16 (**b**)

Reagents and conditions: *i*. EtMgBr, Mg, Cp_2TiCl_2 (5 mol.%), Et_2O; *ii*. H_3O^+; *iii*. Jones reagent, acetone, CH_2Cl_2; *iv*. *p*-TsOH, MeOH, $CHCl_3$; *v*. PDC/Al_2O_3.

To study the scope of the cross-cyclomagnesiation reaction of 1,2-dienes, as well as to elaborate original methods and approaches to the synthesis of new 5*Z*,9*Z*-dienoic acid derivatives with the steroid framework, we carried out the cross-cyclomagnesiation of 1,2-diene cholesterol derivatives with hepta-5,6-dien-1-ol tetrahydropyran ether.

First, we synthesized earlier undescribed 1,2-dienes **9a**,**b** in two steps (Scheme 2). The reaction of cholesterol **7** with alkynols in the presence of montmorillonite K-10 upon reflux in chloroform over 7 days gave ethers **8a**,**b**.[18] They were involved into the Mannich reaction[19] with paraformaldehyde to obtain the target 1,2-dienes **9a**,**b**.

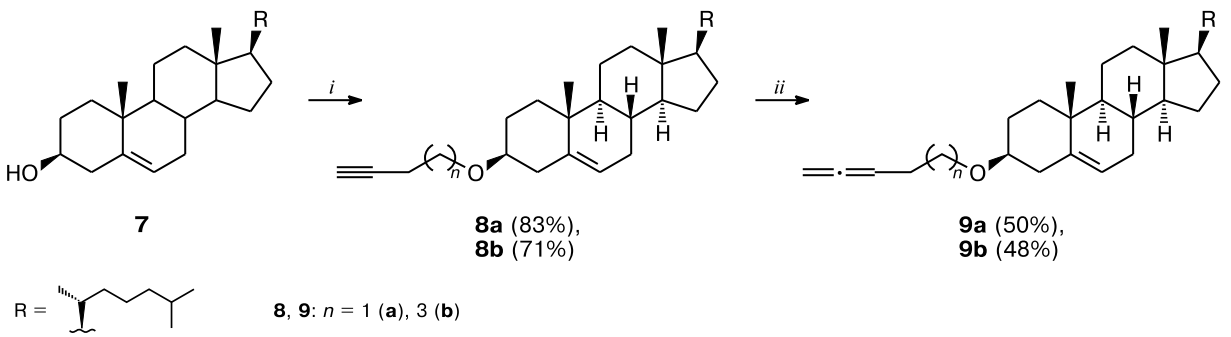
Cholesterol ethers **9a**,**b** in accordance with the planned strategy for the synthesis of 5*Z*,9*Z*-dienoic acids were involved into the cross-cyclomagnesiation reaction with hepta-5,6-dien-1-ol tetrahydropyran ether **2** using EtMgBr in the presence of magnesium metal and a Cp_2TiCl_2 catalyst (10 mol.%) under the following conditions **9a**,**b** : **2** : EtMgBr : Mg : [Ti] = 1 : 3 : 8 : 12 : 0.1, Et_2O, room temperature, 24 h. Dienes **11a**,**b** were obtained after the acid hydrolysis (Scheme 3). A direct oxidation of ethers **11a**,**b** with Jones reagent led to 5*Z*,9*Z*-dienoic acids **12a**,**b** in ~50% yields.[5]

The structures of compounds **11** and **12** were reliably inferred based on the 1D (^1H, ^{13}C, Dept 135), 2D (HSQC, HMBC, and ^1H—^1H COSY, NOESY) NMR experiments. The presence in the ^{13}C NMR spectra of the high-field signals for the allyl carbon atoms in the region of δ 27 indicates the *cis*-configuration of substituents at the double bonds in compounds **11** and **12**.

In the last years, significant attention is paid to the search of new inhibitors of enzymes synthesizing or modifying nucleic acids. One of the principal enzymes in this series is a DNA-dependent enzyme topoisomerase I, which catalyzes topological rearrangements of DNA and plays one of the leading roles in all the aspects of genome functioning.[2,3] Topoisomerases are considered as intracellular targets for chemotherapeutical agents, since these compounds, inhibiting topoisomerase I and II, cause damage of DNA molecules, forcing a programmed cell death.[2,3]

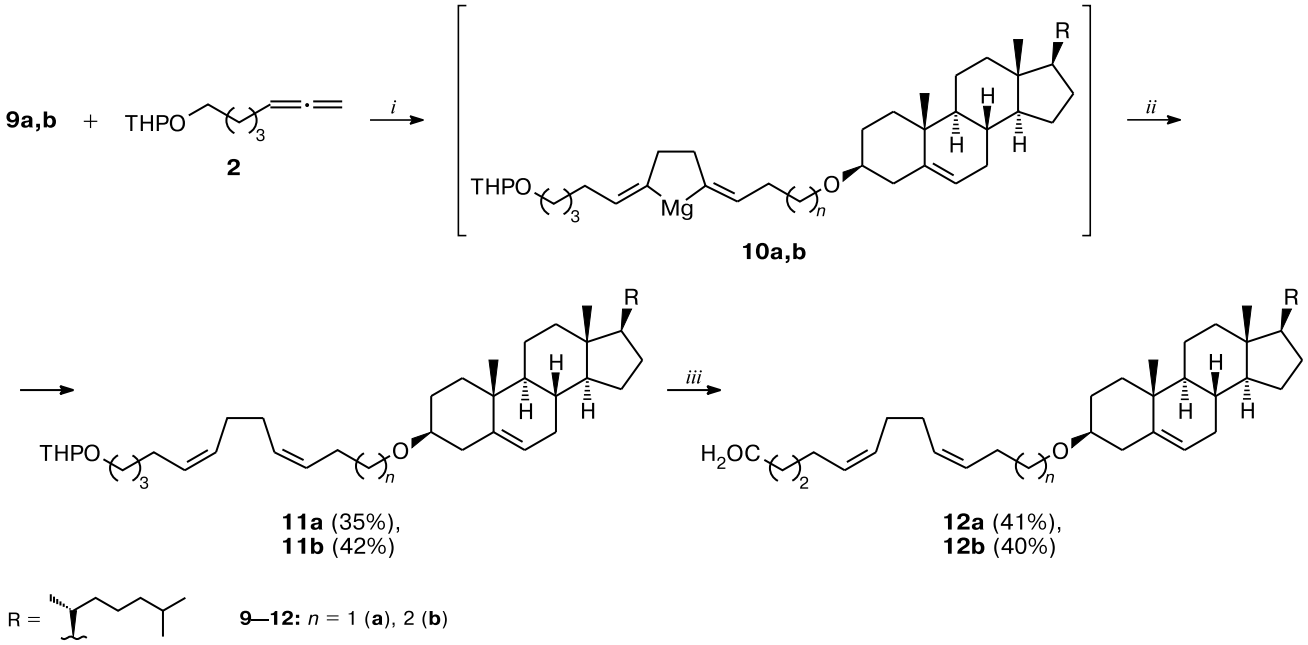
It is known that some types of tumor cell lines can contain the topoisomerase forms resistant to camptothe-

Scheme 2



Reagents and conditions. *i*. Montmorillonite K-10, $CHCl_3$, but-3-yn-1-ol (for **8a**) or hex-5-yn-1-ol (for **8b**); *ii*. paraformaldehyde, CuI, Pr^i_2NH, 1,4-dioxane, reflux.

Scheme 3



Reagents and conditions. *i*. EtMgBr, Mg, Cp_2TiCl_2 (10 mol.%), Et_2O; *ii*. H_3O^+; *iii*. Jones reagent, acetone, CH_2Cl_2.

cin derivatives widely used in medical practice. Such enzyme has been first described for the cell line CPT-K15 isolated from a patient with an acute lymphoblastic leukemia. It was also shown that topoisomerase I acquires such properties because of the replacement of aspartic acid with glycine at positions 533 and 583 of the protein molecule.[20] The Japanese researches found[6] that the synthesized by us aliphatic unsaturated acids **6a**,**b**, which were earlier isolated from Australian sponge *Amphimedon sp*., possess extremely high inhibiting activity against human topoisomerase I resistant to camptothecin and its synthetic derivatives, irinotecan and topotecan. The testing of acids **6a**,**b** on the inhibiting activity against topoisomerase I showed that IC_{50} for these acids is 0.86 and 1.3 mmol L^{-1}, respectively.[6]

In the next step of our studies, to compare inhibiting activity of natural 5*Z*,9*Z*-dienoic acid with that of synthetic 5*Z*,9*Z*-dienoic acids containing a steroid fragment, we studied the ability of acids **12a**,**b** to inhibit the DNA-dependent enzyme topoisomerase I *in vitro* in the supercoiled plasmid DNA relaxation reaction under standard conditions.

The results obtained in these experiments indicate that acids **12a**,**b** exhibit high inhibiting activity against topoisomerase I at the concentrations above 1 μmol L^{-1}.

In conclusion, a Cp_2TiCl_2-catalyzed cross-cyclomagnesiation of *O*-containing allenes with aliphatic 1,2-dienes using Grignard reagents was accomplished for the first time. An efficient approach to the synthesis of natural and synthetic 5*Z*,9*Z*-dienoic acids in high yields was developed. The compounds obtained were shown to possess antiviral, antitumor, and antiparasitic activity. For the first time, 5*Z*,9*Z*-dienoic acids containing a steroid fragment were found to inhibit *in vitro* human topoisomerase I in the concentration above 1 mmol L^{-1}, that opens prospects to the design of new highly efficient and selective antitumor agents of new generation based on 5*Z*,9*Z*-dienoic acids of different structure.

Experimental

Chromatographic analysis was carried out on a Shimadzu GC-9A instrument, a 2000×2-mm column, stationary phase Silicon SE-30 (5%) on Chromaton N-AW-HMDS (0.125—0.160 mm), carrier gas helium (30 mL min^{-1}), the temperature was programmed from 50 to 300 °C, at the heating rate of 8 °C min^{-1}. ^1H and ^{13}C NMR spectra were recorded on Bruker Avance-400 (100 MHz for ^{13}C and 400 MHz for ^1H) and Bruker Avance-500 (125 MHz for ^{13}C and 500 MHz for ^1H) spectrometers in $CDCl_3$, chemical shifts are given relative to Me_4Si. A Shimadzu GCMS-QP2010 Plus spectrometer was used in the work (a SLB-5ms glass capillary column, 60000×0.25 mm×0.25 m (Supelco, USA), temperature of the ion source 200 °C, energy of ionized electrons 70 eV). IR spectra were recorded on a Bruker VERTEX 70V IR Fourier-transform spectrometer in KBr pellets or solutions in $CHCl_3$. Elemental analysis of the samples was carried out on a Carlo Erba 1106 elemental analyzer. Sorbfil plates were used for TLC, eluent hexane—ethyl acetate, visualization with a solution of anisaldehide in acetic acid. Acros Organics silica gel (0.060—0.200 mm) was used for column chromatography. Reactions with organometallic compounds were

carried out under dry argon. Solvents were dried and used freshly distilled. Cholesterol alkynyl ethers **8a**,**b** were obtained according to the known procedure.[18] Compounds **6a**,**b** and **8a** were identified by the comparison with data reported in the literature.[18,21,22] Protection of allene alcohols by conversion to the corresponding ethers and removal of the THP-protecting groups were carried out according to the procedures described in the book.[23]

Studies *in vitro* of inhibiting activity of acids against human topoisomerase I. Inhibiting activity of 5*Z*,9*Z*-dienoic acids was determined using a Topoisomerase I Drug Screening Kit (TG-1018-2, Topogen, USA) (a test compound was introduced before the addition of the enzyme topoisomerase I). The relaxation reaction of the supercoiled DNA upon treatment with topoisomerase I was carried out as follows: a reaction mixture (20 mL) containing pHOT plasmid DNA (TopoGen, USA) (0.25 mg), recombinant topoisomerase I (TopoGen, USA) (1 act.un.), and a tested (5*Z*,9*Z*)-5,9-dienoic acid was incubated for 30 min in the buffer (35 m*M* Tris-HCl, pH 8.0; 72 m*M* KCl, 5 m*M* MgCl, 5 m*M* dithiothreitol, 5 m*M* spermidine, 0.01% bovine serum albumin) at 37 °C in a Biosan thermostat (Latvia). Alkaloid camptothecin (TopoGEN, USA) was used to control inhibiting effect on topoisomerase I. The reaction was finalized by the addition of sodium dodecylsulfate to the final concentration of 1%. After addition of a proteinase solution (5 mg mL^{-1}) (Sigma, USA) (1 : 10), the reaction mixture was additionally incubated for 15 min at 37 °C. A 0.1% solution of bromophenol blue (1 : 10) was added to the analyzed samples, which were subjected to electrophoresis in the absence of ethidium bromide. The reaction products were separated on a 1% agarose gel (3 V cm^{-1}) over 2—3 h. After the electrophoresis, the gels were treated with a solution of ethidium bromide (0.5 mg mL^{-1}). The gels were visualized under UV light on a BioRad Gel Doc EZ gel-documenting system (BioRad, USA). A possible effect of compounds under study on the supercoiled DNA was controlled by carrying out the reaction without topoisomerase I, the test compounds were used in the same concentrations as in the reaction with the enzyme.

Cross-cyclomagnesiation of hepta-5,6-dien-1-ol tetrahydropyran ether (2) with terminal 1,2-dienes 1a,b using EtMgBr in the presence of metallic Mg and a Cp_2TiCl_2 catalyst (general procedure). Diethyl ether (10 mL), compound **2** (10 mmol), the corresponding aliphatic 1,2-diene **1a,b** (12 mmol), EtMgBr (40 mmol, 1.5 *M* solution in Et_2O), Mg powder (32 mmol), and Cp_2TiCl_2 (0.5 mmol) were placed into a glass reactor under dry argon (~0 °C) with stirring. The temperature of the reaction mixture was elevated to ambient (20—22 °C). The reaction mixture was stirred for 6—8 h and treated with 5% aqueous HCl. The reaction products were extracted with diethyl ether, the extracts were dried with $MgSO_4$, the solvent was evaporated, the residue was subjected to chromatography on a column (SiO_2, eluent light petroleum ether—EtOAc (50 : 1)).

2-(Heptacosa-5*Z*,9*Z*-dien-1-yloxy)tetrahydro-2*H*-pyran (4a). The yield was 74%, R_f 0.46. Found (%): C, 80.37; H, 12.62. $C_{32}H_{60}O_2$. Calculated (%): C, 80.61; H, 12.68. MS (MALDI TOF), *m*/*z*: 476.8. ^{1}H NMR, δ: 0.89 (t, 3 H, CH_3, J = 7.2 Hz); 1.24—1.83 (m, 34 H, 17 CH_2); 2.01—2.06 (m, 8 H, 4 C$\underline{H}_2$CH=); 3.37—3.85 (m, 4 H, 2 CH_2O); 4.54 (t, 1 H, OCHO, J = 3.2 Hz); 5.34—5.37 (m, 4 H, 4 HC=). ^{13}C NMR, δ: 14.1 (C(27)); 19.6 (C(30)); 22.6 (C(26)); 25.5 (C(31)); 26.3 (C(3)); 27.0 (C(4)); 27.2 (C(2)); 27.3 (C(7)); 27.4 (C(8)); 28.9 (C(11)); 29.34, 29.37, 29.41, 29.57, 29.61, 29.65, 29.71 (6 C), 29.74 (C(12)—C(24)); 30.72 (C(29)); 31.8 (C(25)); 62.1 (C(32)); 67.3 (C(1)); 98.7 (C(28)); 129.0 (C(9)); 129.5 (C(6)); 129.9 (C(5)); 130.4 (C(10)).

2-(Octacosa-5*Z*,9*Z*-dien-1-yloxy)tetrahydro-2*H*-pyran (4b). The yield was 71%, R_f 0.47. Found (%): C, 80.42; H, 12.66. $C_{33}H_{62}O_2$. Calculated (%): C, 80.75; H, 12.73. MS (MALDI TOF), *m*/*z*: 490.8. ^{1}H NMR, δ: 0.89 (t, 3 H, CH_3, J = 7.2 Hz); 1.22—1.84 (m, 36 H, 18 CH_2); 2.01—2.07 (m, 8 H, 4 C$\underline{H}_2$CH=); 3.37—3.85 (m, 4 H, 2 CH_2O); 4.55 (t, 1 H, OCHO, J = 3.2 Hz); 5.33—5.37 (m, 4 H, 4 HC=). ^{13}C NMR, δ: 14.2 (C(28)); 19.6 (C(31)); 22.6 (C(27)); 25.5 (C(32)); 26.3 (C(3)); 27.0 (C(4)); 27.2 (C(2)); 27.3 (C(7)); 27.4 (C(8)); 28.9 (C(11)); 29.34, 29.36, 29.41, 29.58, 29.61, 29.66, 29.70 (7 C), 29.73 (C(12)—C(25)); 30.72 (C(30)); 31.8 (C(26)); 62.1 (C(33)); 67.3 (C(1)); 98.7 (C(29)); 129.0 (C(9)); 129.5 (C(6)); 129.9 (C(5)); 130.5 (C(10)).

Heptacosa-5*Z*,9*Z*-dien-1-ol (5a). The yield was 89%, R_f 0.38. Found (%): C, 82.21; H, 13.29. $C_{27}H_{52}O$. Calculated (%): C, 82.58; H, 13.35. MS (MALDI TOF), *m*/*z*: 392.7. ^{1}H NMR, δ: 0.91 (t, 3 H, CH_3, J = 7.2 Hz); 1.24—1.64 (m, 34 H, 17 CH_2); 2.02—2.10 (m, 8 H, 4 C$\underline{H}_2$CH=); 3.68 (t, 2 H, CH_2O, J = 7.2 Hz); 5.38—5.42 (m, 4 H, 4 HC=). ^{13}C NMR, δ: 14.1 (C(27)); 22.6 (C(26)); 26.3 (C(3)); 27.0 (C(4)); 27.3 (C(2)); 27.3 (C(7)); 27.4 (C(8)); 28.9 (C(11)); 29.34, 29.38, 29.57, 29.60, 29.69, 29.71 (6 C), 29.74, 32.2 (C(12)—C(24)); 31.8 (C(25)); 61.9 (C(1)); 129.0 (C(9)); 129.5 (C(6)); 129.9 (C(5)); 130.5 (C(10)).

Octacosa-5*Z*,9*Z*-dien-1-ol (5b). The yield was 88%, R_f 0.38. Found (%): C, 82.41; H, 13.33. $C_{28}H_{54}O$. Calculated (%): C, 82.68; H, 13.38. MS (MALDI TOF), *m*/*z*: 406.7. ^{1}H NMR, δ: 0.90 (t, 3 H, CH_3, J = 7.2 Hz); 1.24—1.62 (m, 36 H, 18 CH_2); 2.03—2.11 (m, 8 H, 4 C$\underline{H}_2$CH=); 3.67 (t, 2 H, CH_2O, J = 7.2 Hz); 5.38—5.41 (m, 4 H, 4 HC=). ^{13}C NMR, δ: 14.1 (C(28)); 22.7 (C(27)); 25.8 (C(3)); 27.0 (C(4)); 27.3 (C(2)); 27.4 (C(7)); 27.5 (C(8)); 29.0 (C(11)); 29.34, 29.37, 29.58, 29.61, 29.67, 29.71 (7 C), 29.74, 32.3 (C(12)—C(25)); 31.9 (C(26)); 62.9 (C(1)); 129.0 (C(9)); 129.7 (C(6)); 129.8 (C(5)); 130.5 (C(10)).

Synthesis of cholesterol acetylene derivatives 8a,b (general procedure). A mixture of but-3-yn-1-ol or hex-5-yn-1-ol (53 mmol), chloroform (50 mL), cholesterol (2 g, 4.91 mmol), and montmorillonite K-10 (4.5 g, activated at 120 °C over 12—14 h) was stirred for 7 days at 55 °C. The reaction mixture was filtered. After evaporation of the solvent *in vacuo*, the reaction product was purified by column chromatography (SiO_2, eluent hexane—EtOAc (30 : 1)).

(3b)-3-(But-3-yn-1-yloxy)cholest-5-ene (8a). A white crystalline compound. The yield was 83%. M.p. 38—40 °C. IR, ν/cm^{-1}: 730, 851, 971, 1100, 1347, 1362, 1429, 1463, 2152, 2859, 2927, 3259. ^{1}H NMR, δ: 0.69 (s, 3 H, H(18)); 0.88 (d, 6 H, H(26) and H(27), J = 6.5 Hz); 0.93 (d, 3 H, H(21), J = 6.5 Hz); 0.93—2.35 (m, 28 H, CH_2, CH); 1.01 (s, 3 H, H(19)); 1.96 (t, 1 H, C$\underline{H}$C$\equiv$CCH_2, J = 2.5 Hz); 2.45 (m, 2 H, CHC$\equiv$C$\underline{H}_2$); 3.18 (m, 1 H, H(3)); 3.60 (t, 2 H, CH_2O, J = 6.0 Hz); 5.35 (m, 1 H, H(6)). ^{13}C NMR, δ: 11.9 (C(18)), 18.8 (C(21)), 19.4 (C(19)), 20.3 (C(2´)), 21.1 (C(11)), 22.6 (C(26)), 22.8 (C(27)), 23.9 (C(23)), 24.3 (C(15)), 28.0 (C(25)), 28.3 (C(16)), 28.5 (C(2)), 31.9 (C(7)), 31.9 (C(8)), 35.8 (C(20)), 36.2 (C(22)), 36.9 (C(10)), 37.2 (C(1)), 39.1 (C(4)), 39.5 (C(24)), 39.8 (C(12)), 42.3 (C(13)), 50.2 (C(9)), 56.2 (C(17)), 56.8 (C(14)), 66.2 (C(1´)), 69.2 (C(4´)), 79.3 (C(3)), 81.4 (C(3´)), 121.5 (C(6)), 140.8 (C(5)).

(3b)-3-(Hex-5-yn-1-yloxy)cholest-5-ene (8b). A white crystalline compound. The yield was 71%. M.p. 45—47 °C. Found (%): C, 84.76; H, 11.64. $C_{33}H_{54}O$. Calculated (%): C, 84.91; H, 11.66. IR, ν/cm^{-1}: 733, 846, 968, 1108, 1347, 1365, 1437, 1466, 2158,

2866, 2932, 3267. ^{1}H NMR, δ: 0.69 (s, 3 H, H(18)); 0.88 (d, 6 H, H(26) and H(27), J = 6.5 Hz); 0.93 (d, 3 H, H(21), J = 6.5 Hz); 0.93—2.35 (m, 28 H, CH_2, CH); 1.01 (s, 3 H, H(19)); 1.62 (m, 2 H, CH_2); 1.68 (m, 2 H, CH_2); 1.95 (t, 1 H, C<u>H</u>CCH_2, J = 2.5 Hz); 2.23 (m, 2 H, CHCC<u>H_2</u>); 3.13 (m, 1 H, H(3)); 3.49 (t, 2 H, CH_2O, J = 6.0 Hz); 5.35 (m, 1 H, H(6)). ^{13}C NMR, δ: 11.9 (C(18)), 18.3 (C(4′)), 18.8 (C(21)), 19.4 (C(19)), 21.1 (C(11)), 22.6 (C(26)), 22.8 (C(27)), 23.9 (C(23)), 24.3 (C(15)), 25.3 (C(3′)), 28.0 (C(25)), 28.3 (C(16)), 28.5 (C(2)), 29.3 (C(2′)), 31.9 (C(7)), 31.9 (C(8)), 35.8 (C(20)), 36.2 (C(22)), 36.9 (C(10)), 37.3 (C(1)), 39.2 (C(4)), 39.5 (C(24)), 39.8 (C(12)), 42.3 (C(13)), 50.2 (C(9)), 56.2 (C(17)), 56.8 (C(14)), 67.3 (C(1′)), 68.4 (C(6′)), 79.0 (C(3)), 84.4 (C(5′)), 121.5 (C(6)), 141.0 (C(5)).

Synthesis of cholesterol 1,2-diene derivatives 9a,b (general procedure). Paraformaldehyde (79 mg), copper iodide (21 mg, 0.1 mmol), and diisopropylamine (0.28 mL, 2 mmol) were sequentially added in a solution of compound **8a** or **8b** in anhydrous dioxane (15 mL). The reaction mixture was refluxed with stirring for 24 h, treated with 2 *M* aqueous HCl, and extracted with diethyl ether. The organic layer was sequentially washed with aqueous $NaHCO_3$, water, saturated aqueous NaCl and dried with $MgSO_4$. After the solvent was evaporated *in vacuo*, the reaction product was isolated by column chromatography (SiO_2, eluent hexane—EtOAc (30 : 1)).

(3b)-3-(Penta-3,4-dien-1-yloxy)cholest-5-ene (9a). A white crystalline compound. The yield was 50%. M.p. 42—44 °C. Found (%): C, 84.65; H, 11.55. $C_{32}H_{52}O$. Calculated (%): C, 84.89; H, 11.58. IR, ν/cm^{-1}: 737, 841, 958, 1105, 1344, 1365, 1437, 1466, 1958, 2866, 2932. ^{1}H NMR, δ: 0.69 (s, 3 H, H(18)); 0.89 (d, 6 H, H(26) and H(27), J = 6.5 Hz); 0.93 (d, 3 H, H(21), J = 6.5 Hz); 0.93—2.38 (m, 28 H, CH_2, CH); 1.02 (s, 3 H, H(19)); 2.29 (m, 2 H, CHCCH_2C<u>H_2</u>); 3.18 (m, 1 H, H(3)); 3.56 (t, 2 H, C<u>H_2</u>O, J = 6.5 Hz); 4.69 (m, 2 H, C<u>H_2</u>CCH); 5.16 (m, 1 H, CH_2CC<u>H</u>); 5.36 (m, 1 H, H(6)). ^{13}C NMR, δ: 11.9 (C(18)), 18.7 (C(21)), 19.4 (C(19)), 21.1 (C(11)), 22.6 (C(26)), 22.8 (C(27)), 23.9 (C(23)), 24.3 (C(15)), 28.0 (C(25)), 28.3 (C(16)), 28.5 (C(2)), 29.2 (C(2′)), 31.9 (C(7)), 31.9 (C(8)), 35.8 (C(20)), 36.2 (C(22)), 36.9 (C(10)), 37.3 (C(1)), 39.2 (C(4)), 39.5 (C(24)), 39.8 (C(12)), 42.3 (C(13)), 50.2 (C(9)), 56.2 (C(17)), 56.8 (C(14)), 67.3 (C(1′)), 74.9 (C(5′)), 79.0 (C(3)), 86.8 (C(3′)), 121.5 (C(6)), 141.1 (C(5)), 208.9 (C(4′)).

(3b)-3-(Hepta-5,6-dien-1-yloxy)cholest-5-ene (9b). A white crystalline compound. The yield was 48%. M.p. 62—64 °C. Found (%): C, 84.70; H, 11.71. $C_{34}H_{56}O$. Calculated (%): C, 84.93; H, 11.74. IR, ν/cm^{-1}: 742, 762, 955, 1103, 1344, 1362, 1433, 1467, 1951, 2866, 2933. ^{1}H NMR, δ: 0.69 (s, 3 H, H(18)); 0.88 (d, 6 H, H(26) and H(27), J = 6.5 Hz); 0.93 (d, 3 H, H(21)), J = 6.5 Hz); 0.91—2.38 (m, 28 H, CH_2, CH); 1.02 (s, 3 H, H(19)); 1.49 (m, 2 H, CH_2); 1.62 (m, 2 H, CH_2); 2.05 (m, 2 H, $CHCCH_2CH_2$); 3.14 (m, 1 H, H(3)); 3.48 (t, 2 H, CH_2O, J = 6.5 Hz); 4.67 (m, 2 H, CH_2CCH); 5.11 (m, 1 H, CH_2CCH); 5.35 (m, 1 H, H(6)). ^{13}C NMR, δ: 11.9 (C(18)), 18.7 (C(21)), 19.4 (C(19)), 21.1 (C(11)), 22.6 (C(26)), 22.8 (C(27)), 23.9 ((23)), 24.3 (C(15)), 25.8 (C(3′)), 28.0 (C(25)), 28.1 (C(4′)), 28.3 (C(16)), 28.5 (C(2)), 29.6 (C(2′)), 31.9 (C(7)), 31.9 (C(8)), 35.8 (C(20)), 36.2 (C(22)), 36.9 (C(10)), 37.3 (C(1)), 39.2 (C(4)), 39.5 (C(24)), 39.8 (C(12)), 42.3 (C(13)), 50.2 (C(9)), 56.2 (C(17)), 56.8 (C(14)), 67.8 (C(1′)), 74.7 (C(7′)), 78.9 (C(3)), 89.9 (C(5′)), 121.4 (C(6)), 141.1 (C(5)), 208.6 (C(6′)).

Cross-cyclomagnesiation reaction of 1,2-diene derivatives 9a,b with 5,6-hepta-5,6-dien-1-ol tetrahydropyran ether (2) using EtMgBr in the presence of metallic Mg and a Cp_2TiCl_2 catalyst (general procedure). Anhydrous diethyl ether (10 mL), cholesterol 1,2-diene derivative **9a**,**b** (1.0 mmol), 5,6-hepta-5,6-dien-1-ol tetrahydropyran ether (**2**) (3.0 mmol), EtMgBr (8.0 mmol, 1.5 *M* solution in Et_2O), Mg powder (12.0 mmol), and Cp_2TiCl_2 (0.1 mmol) were sequentially placed into a glass reactor under dry argon at 0 °C with stirring. The reaction mixture was warmed-up to room temperature and stirred for 24 h, then treated with 5% aqueous solution of NH_4Cl. The reaction products were extracted with diethyl ether, dried with $MgSO_4$, and isolated by column chromatography (SiO_2, eluent hexane—EtOAc (35 : 1)).

(3b)-3-{[(3*Z*,7*Z*)-12-(Tetrahydro-2*H*-pyran-2-yloxy)dodeca-3,7-dien-1-yl]oxy}cholest-5-ene (11a). A colorless oily compound. The yield was 35%. Found (%): C, 80.92; H, 11.41. $C_{44}H_{74}O_3$. Calculated (%): C, 81.17; H, 11.46. IR, ν/cm^{-1}: 734, 805, 972, 1034, 1108, 1365, 1380, 1440, 1465, 2867, 2936. ^{1}H NMR, δ: 0.69 (s, 3 H, H(18)); 0.88 (d, 6 H, H(26) and H(27), J = 6.5 Hz); 0.93 (d, 3 H, H(21), J = 6.5 Hz); 0.95—2.37 (m, 28 H, CH_2, CH); 1.02 (s, 3 H, H(19)); 1.45 (m, 2 H, CH_2); 1.53 and 1.85 (m, 2 H, CH_2); 1.55 (m, 2 H, CH_2); 1.62 (m, 2 H, CH_2); 1.72 (m, 2 H, CH_2); 2.08 (m, 2 H, C<u>H_2</u>CH=); 2.11 (m, 4 H, C<u>H_2</u>CH=); 2.33 (m, 2 H, C<u>H_2</u>CH=); 3.16 (m, 1 H, H(3)); 3.41 and 3.76 (m, 2 H, CH_2O); 3.48 (m, 2 H, CH_2O); 3.51 and 3.88 (m, 2 H, CH_2O); 4.59 (m, 1 H, CH_2C<u>H</u>O); 5.35 (m, 1 H, H(6)); 5.46 (m, 1 H, HC=); 5.40 (m, 2 H, HC=); 5.43 (m, 1 H, HC=). ^{13}C NMR, δ: 11.9 (C(18)), 18.7 (C(21)), 19.4 (C(19)), 19.7 (C(4′)), 21.1 (C(11)), 22.6 (C(26)), 22.8 (C(27)), 23.8 (C(23)), 24.3 (C(15)), 25.5 (C(5I)), 26.4 (C(10′)), 27.1 (C(9′)), 27.3, 27.5 (C(5′), C(6′)), 28.0 (C(25)), 28.2 (C(16)), 28.5 (C(2)), 28.5 (C(2′)), 29.4 (C(11′)), 30.8 (C(3I)), 31.9 (C(7)), 31.9 (C(8)), 35.8 (C(20)), 36.2 (C(22)), 36.9 (C(10)), 37.3 (C(1)), 39.2 (C(4)), 39.5 (C(24)), 39.8 (C(12)), 42.3 (C(13)), 50.2 (C(9)), 56.2 (C(17)), 56.8 (C(14)), 62.3 (C(6I)), 67.5 (C(12′)), 67.6 (C(1′)), 79.0 (C(3)), 98.8 (C(2I)), 121.5 (C(6)), 126.02 (C(3′)), 129.3 (C(7′)), 130.1 (C(8′)), 131.2 (C(4′)), 141.1 (C(5)).

(3b)-3-{[(5*Z*,9*Z*)-14-(Tetrahydro-2*H*-pyran-2-yloxy)tetradeca-5,9-dien-1-yl]oxy}cholest-5-ene (11b). A colorless oily compound. The yield was: 42%. Found (%): C, 81.19; H, 11.49. $C_{46}H_{78}O_3$. Calculated (%): C, 81.36; H, 11.58. IR, ν/cm^{-1}: 736, 835, 985, 1034, 1112, 1361, 1384, 1436, 1465, 2867, 2940. ^{1}H NMR, δ: 0.69 (s, 3 H, H(18)); 0.88 (d, 6 H, H(26) and H(27), J = 6.5 Hz); 0.93 (d, 3 H, H(21), J = 6.5 Hz); 1.02 (s, 3 H, H(19)); 1.08—2.38 (m, 28 H, CH_2, CH); 1.44 (m, 4 H, CH_2); 1.54 and 1.85 (m, 2 H, CH_2); 1.55 (m, 2 H, CH_2); 1.59 (m, 2 H, CH_2); 1.60 (m, 2 H, CH_2); 1.72 (m, 2 H, CH_2); 2.08 (m, 4 H, C<u>H_2</u>CH=); 2.11 (m, 4 H, C<u>H_2</u>CH=); 3.15 (m, 1 H, H(3)); 3.40 and 3.76 (m, 2 H, C<u>H_2</u>O); 3.46 (m, 2 H, CH_2O, J = 7.5 Hz); 3.53 and 3.89 (m, 2 H, C<u>H_2</u>O); 4.59 (m, 1 H, CH_2C<u>H</u>O); 5.35 (m, 1 H, H(6)); 5.39 (m, 4 H, HC=). ^{13}C NMR, δ: 11.9 (C(18)), 18.7 (C(21)), 19.4 (C(19)), 19.7 (C(4I)), 21.1 (C(11)), 22.6 (C(26)), 22.8 (C(27)), 23.8 (C(23)), 24.3 (C(15)), 25.5 (C(5I)), 26.4 (C(3′)), 26.4 (C(12′)), 27.1 (C(11′), 27.1 (C(4′), 27.4 (C(7′), 27.4 (C(8′), 28.0 (C(25)), 28.2 (C(16)), 28.5 (C(2)), 29.4 (C(13′)), 29.8 (C(2′)), 30.8 (C(3′′)), 31.9 (C(7)), 31.9 (C(8)), 35.8 (C(20)), 36.2 (C(22)), 36.9 (C(10)), 37.3 (C(1)), 39.2 (C(4)), 39.5(C(24)), 39.8 (C(12)), 42.3 (C(13)), 50.2 (C(11)), 56.2 (C(17)), 56.8 (C(14)), 62.3 (C(6′′)), 67.5 (C(14′)), 67.9 (C(1′)), 78.9 (C(3)), 98.8 (C(2′′)), 121.4 (C(6)), 129.4 (C(5′)), 129.5 (C(9′)), 129.9 (C(10′)), 130.0 (C(6′)), 141.2 (C(5)).

Oxidation of compounds 11a,b by Jones reagent (general procedure). Jones reagent (0.5 mL) was added dropwise to a solution

of compound **11a**,**b** (0.5 mmol) in a mixture of acetone (12 mL) and CH_2Cl_2 (2 mL) at room temperature. The reaction mixture was stirred at room temperature for 1 h, then treated with water (5 mL), the solvents were evaporated *in vacuo*, the aqueous layer was extracted with diethyl ether (3×10 mL). The organic layer was dried with $MgSO_4$. After evaporation of the solvent, the reaction product was purified by column chromatography (SiO_2, eluent hexane—EtOAc (3 : 1)).

((5*Z*,9*Z*)-12-[(3b)-Cholest-5-en-3-yloxy]dodeca-5,9-dienoic acid (12a). A white solid compound. The yield was 51%. $[a]_D^{20}$ −13.8 (*c* 1.09, $CHCl_3$). Found (%): C, 80.49; H, 11.04. $C_{39}H_{64}O_3$. Calculated (%): C, 80.63; H, 11.10. IR, ν/cm^{-1}: 736, 801, 952, 1021, 1104, 1240, 1381, 1437, 1466, 1714, 2868, 2933. ^{1}H NMR, δ: 0.69 (s, 3 H, H(18)); 0.88 (d, 6 H, H(26) and H(27), J = 6.5 Hz); 0.93 (d, 3 H, H(21), J = 6.5 Hz); 1.02 (s, 3 H, H(19)); 1.08—2.38 (m, 28 H, CH_2, CH); 1.72 (m, 2 H, CH_2); 2.11 (m, 4 H, C$\underline{H}_2$CH=); 2.14 (m, 4 H, C$\underline{H}_2$CH=); 2.35 (m, 2 H, CH_2CH=); 2.37 (m, 2 H, C$\underline{H}_2$COOH); 3.21 (m, 1 H, H(3)); 3.49 (m, 2 H, CH_2O, J = 7.5 Hz); 5.35 (m, 1 H, HC=); 5.36 (m, 1 H, H(6)); 5.40 (m, 1 H, HC=); 5.47 (m, 2 H, HC=). ^{13}C NMR, δ: 11.9 (C(18)), 18.7 (C(21)), 19.4 (C(19)), 21.1 (C(11)), 22.6 (C(26)), 22.8 (C(27)), 23.8 (C(23)), 24.3 (C(15)), 24.6 (C(3´)), 26.4 (C(4´)), 27.4, 27.5 (C(8´), (C(7´)), 28.0 (C(25)), 28.3 (C(16)), 28.4 (C(11´)), 28.4 (C(2)), 31.9 (C(8)), 31.9 (C(7)), 33.2 (C(2´)), 35.8 (C(20)), 36.2 (C(22)), 36.9 (C(10)), 37.3 (C(1)), 39.1 (C(4)), 39.5(C(24)), 39.8 (C(12)), 42.3 (C(13)), 50.2 (C(11)), 56.2 (C(17)), 56.8 (C(14)), 67.7 (C(12´)), 79.2 (C(3)), 121.6 (C(6)), 126.0 (C(10´)), 128.8 (C(5´)), 130.4 (C(6´)), 131.1 (C(9´)), 140.9 (C(5)), 178.7 (C(1´)). MS (MALDI TOF), *m*/*z*: 603.039 $[M + Na]^+$. Calculated: 603.475.

(5*Z*,9*Z*)-14-[(3b)-Cholest-5-ene-3-yloxy]tetradeca-5,9-dienoic acid (12b). A white solid compound. The yield was 53%. $[a]_D^{20}$ −19.6 (*c* 1.39, $CHCl_3$). Found (%): C, 81.01; H, 11.21. $C_{41}H_{68}O_3$. Calculated (%): C, 80.86; H, 11.25. IR, ν/cm^{-1}: 738, 810, 972, 1080, 1113, 1255, 1386, 1431, 1476, 1721, 2872, 2932. ^{1}H NMR, δ: 0.69 (s, 3 H, H(18)); 0.88 (d, 6 H, H(26) and H(27), J = 6.5 Hz); 0.93 (d, 3 H, H(21), J = 6.5 Hz); 1.02 (s, 3 H, H(19)); 1.08—2.38 (m, 28 H, CH_2, CH); 1.43 (m, 2 H, CH_2); 1.59 (m, 2 H, CH_2); 1.71 (m, 2 H, CH_2); 2.09 (m, 4 H, C$\underline{H}_2$CH=); 2.13 (m, 4 H, C$\underline{H}_2$CH=); 2.36 (m, 2 H, C$\underline{H}_2$COOH); 3.16 (m, 1 H, H(3)); 3.49 (m, 2 H, CH_2O); 5.33 (m, 1 H, HC=); 5.35 (m, 1 H, H(6)); 5.38 (m, 2 H, HC=); 5.43 (m, 2 H, HC=). ^{13}C NMR, δ: 11.9 (C(18)), 18.7 (C(21)), 19.4 (C(19)), 21.1 (C(11)), 22.6 (C(26)), 22.8 (C(27)), 23.9 (C(23)), 24.3 (C(15)), 24.6 (C(3´)), 26.3 (C(12´)), 26.5 (C(4´)), 27.1 (C(11´)), 27.3, 27.5 (C(8´), C(7´)), 28.0 (C(25)), 28.3 (C(16)), 28.4 (C(2)), 29.7 (C(13´)), 31.9 (C(8)), 31.9 (C(7)), 33.3 (C(2´)), 35.8 (C(20)), 36.2 (C(22)), 36.9 (C(10)), 37.3 (C(1)), 39.1 (C(4)), 39.5 (C(24)), 39.8 (C(12)), 42.3 (C(13)), 50.2 (C(11)), 56.2 (C(17)), 56.8 (C(14)), 68.0 (C(14´)), 79.2 (C(3)), 121.5 (C(6)), 128.8 (C(5´)), 129.3 (C(10´)), 130.1 (C(9´)), 130.5 (C(6´)), 141.1 (C(5)), 179.0 (C(1´)). MS (MALDI TOF), *m*/*z*: 631.244 $[M + Na]^+$. Calculated: 631.507.

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