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Targeted Synthesis and Antitumor Activity In Vitro Macrodiolides Containing 1Z,5Z-Diene and 1,3-Diyne Moieties †

Ilgiz Islamov *, Adelya Yusupova, Lilya U. Dzhemileva and Usein Dzhemilev

Institute of Petrochemistry and Catalysis of RAS, 450075 Ufa, Russia; adelyusupova97@mail.ru (A.Y.); Dzhemilev@mail.ru (L.U.D.); Dzhemilev@anrb.ru (U.D.)

* Correspondence: iislamovi@gmail.com

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Abstract: Efficient methods have been developed for synthesizing previously unknown macrodiolides incorporating 1Z,5Z-diene and 1,3-diyne moieties in 54–84% yields and with >98% stereoselectivity by means of the intermolecular cyclocondesation of (7Z,11Z)-octadeca-7,11-dienedioic acid with α,ω-diols catalyzed by hafnium triflate $Hf(OTf)_4$ as well as via the oxidative coupling of α,ω-diynes obtained by the esterification of (7Z,11Z)-octadeca-7,11-dienedioic acid with alkynols. The synthesized macrodiolides exhibit cytotoxic activity toward the Jurkat, K562, U937, HL-60, HeLa, and Hek293 cell lines in vitro.

Keywords: macrodiolides; 1,5-dienoic compounds; 1,3-diynes; 1,2-dienes; cyclomagnesiation; homogeneous catalysis

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1. Introduction

Macrocyclic compounds are widespread in nature and have a huge range of useful properties; therefore, they are the object of close attention from researchers. A large number of macrocycles are currently used in pharmaceuticals, materials science, supramolecular, and medicinal chemistry. Drugs based on macrolactones are highly effective and, at the same time, are considered one of the safest groups of antibacterial drugs. They do not have a high toxic effect on organs and tissues, and less often, compared to many other antibiotics, cause allergic reactions [1,2].

In this regard, there is increasing interest in the synthesis of new polyfunctional macrolactones that contain various pharmacophore groups in the structure as well as in the study of their biological properties. One of the active pharmacophore groups is the 1,3-diyne fragment, which is found in the structure of a large number of natural biologically active compounds with antitumor, anti-HIV, antifungal, antibacterial, and antiviral activity [3–9].

Natural 1,3-diyne macrolactones, which exhibit high biological activity are well known. For example, new macrocyclic lactones, Ivorenolide A and Ivorenolide B, containing a 1,3-diyne fragment in their structure were isolated from trees of the genus *Khaya Ivorenesis A*. by Yue et al. in 2012 [10,11]. Crude extracts of the stem bark of this tree are used in traditional medicine to treat malaria and other tropical diseases. Biological studies have revealed the anti-plasmodial and anti-inflammatory properties of these extracts. Recently, biological studies of isolated macrocycles have demonstrated high immunosuppressive activity and the surprisingly high inhibition of Con A-induced T-cell proliferation [12–15].

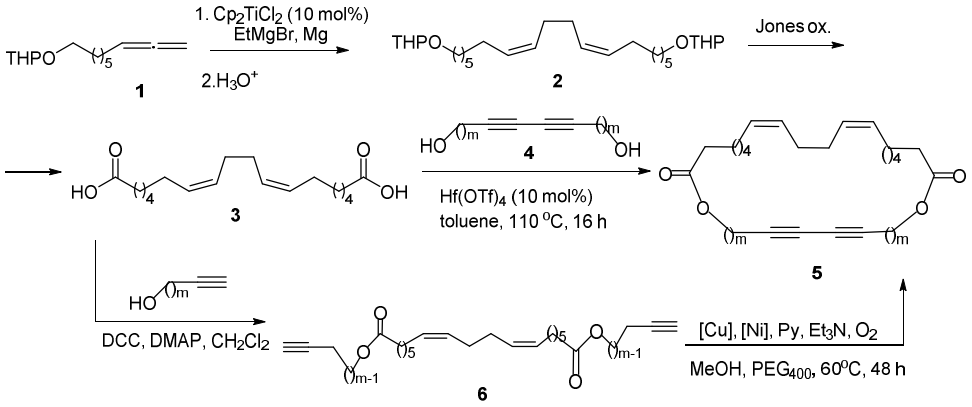
In view of the above and as a continuation of our research on the development of original methods for the synthesis of biologically active macrocyclic compounds [16–18], within the framework of this work, we put forward the possibility of obtaining new

macrodiolides containing a 1,3-diyne fragment in their structure as well as a 1Z,5Z-diene group.

2. Results and Discussion

Recently, we developed two original methods for the synthesis of macrodiolides containing 1Z,5Z-diene and 1,3-diyne moieties in their structure with yields of 55-79% and stereoselectivity >98%. We found that synthesized unsaturated macrolactones exhibit high cytotoxic activity against the Jurkat, K562, U937, HL-60, and Hek293 cell lines in vitro [19].

In this work, we present data on the synthesis of new macrodiolides that we obtained for the first time according to the scheme below (Scheme 1).



[Cu] = $CuCl_2$;
[Ni] = $Ni(NO)_3 \cdot 6H_2O$;
m = 1-4

Scheme 1. Synthesis of macrodiolides containing 1Z,5Z-diene and 1,3-diyne moieties.

The key precursor, (7Z,11Z)-octadeca-7,11-dienedioic acid **3**, was synthesized according to the previously developed scheme in three stages using the original catalytic homo-cyclomagnesiation reaction of O-containing 1,2-dienes (Dzhemilev reaction), obtaining a total yield of 47% and stereoselectivity >98% [18]. The target macrolactones were obtained by means of the Hf-catalyzed cyclocondensation of acid **3** with 1,3-diyne α,ω-diols **4**, obtaining yields of 54–72%. In addition, an alternative two-step approach for the preparation of macrodiolides using an intramolecular oxidative coupling reaction with a total yield of 67–84% is shown (Scheme 1).

A preliminary assessment of the cytotoxicity of the obtained macrocyclic compounds against the Jurkat, K562, Hek293, HeLa, and U937 cell lines and fibroblasts in vitro was carried out, and IC50 was determined via flow cytometry using Guava ViaCount reagent kits (Millipore). The macrodiolides that were synthesized were found to exhibit cytotoxic activity toward the Jurkat, K562, U937, HL-60, and Hek293 cell lines in vitro (IC50 = 0.05–0.76 μM).

Currently, at the Laboratory of Molecular Design and Biological Screening of Candidate Substances for the Pharmaceutical Industry at the Institute of Petrochemistry and Catalysis of RAS, more detailed studies on the antitumor activity of the synthesized macrodiolides are being carried out to study the effect of this class of compounds on the cell cycle and their ability to induce apoptosis.

3. Materials and Methods

All reactions were carried out in an inert atmosphere. The ethereal and aromatic solvents were dried over Na. Commercial 2-propyn-1-ol, 3-butyn-1-ol, 4-pentyn-1-ol, 5-hexyn-1-ol, $Hf(OTf)_4$, and Cp_2TiCl_2 (Aldrich) were used without preliminary purification. (7Z,11Z)-octadeca-7,11-dienedioic acid **3** was prepared from oct-7-yn-1-ol using a method

reported by [18]. One- (^{1}H, ^{13}C) and two-dimensional heteronuclear (HSQC, HMBC) NMR spectra were recorded in $CDCl_3$ using the Bruker Avance-400 ((400.13 MHz (^{1}H), 100.62 MHz (^{13}C)) and Bruker Ascend-500 ((500 MHz (^{1}H), 125 MHz (^{13}C)). IR spectra were recorded on a Bruker VERTEX 70V using KBr discs covering the range of 400–4000 cm^{-1}. Mass spectra were obtained on a MALDI TOF/TOF spectrometer in a sinapic acid matrix. The mass spectra were obtained on an UltraFlex III TOF/TOF (Bruker Daltonik GmbH, Fahrenheitstrasse 4 28359 Bremen, Germany) operating in linear (TOF) and reflection (TOF/TOF) positive and negative ion modes. S_8 and DCTB (trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenyliden]malononitrile) were used as the matrix.

General synthesis procedure for macrodiolides:

Method 1. (7*Z*,11*Z*)-octadeca-7,11-dienedioic acid **3** (0.2 mmol, 1.0 equiv.) and diol (0.2 mmol, 1.0 equiv.) were dissolved in toluene (40 mL, 5 mM). Then, $Hf(OTf)_4$ (0.02 mmol, 0.1 equiv.) was added to the solution, and the reaction mixture was heated to 110 °C. The reaction mixture was stirred at this temperature for 16–18 h. After cooling to room temperature, silica gel (~1 mL) was added, and the slurry was concentrated under reduced pressure and purified by means of column chromatography (elution with petroleum ether/EtOAc (15/1)) to ensure that the the desired product was a colorless oil.

Method 2. $CuCl_2$ (5.0 mg, 0.44 mmol, 25 mol.%) and $Ni(NO_3)_2{\cdot}6H_2O$ (8.5 mg, 0.44 mmol, 25 mol.%) were added to a vial with a stirring bar. Polyethylene glycol 400 (3.05 mL), triethylamine (0.046 mL, 0.33 mmol, 3 equiv.), and pyridine (0.046 mL, 0.55 mmol, 5 equiv.) were added, and the mixture was stirred at room temperature for 15 min or until the metals were solubilized. Diyne (**6**) (0.11 mmol) was added to the homogenous mixture as a methanol solution (1.5 mL) in one portion. Oxygen was bubbled in the solution for 5 min, and the vial was then closed with a screw cap. The reaction was warmed to 60 °C and monitored for the consumption of the starting material (oxygen was bubbled again through the solution every 12 h) by TLC. When the starting material was completely consumed (TLC), the reaction was cooled to room temperature, and the crude mixture was loaded directly on a silica column. Purification by means of chromatography (elution with petroleum ether/EtOAc (15/1)) was carried out to determine that the desired product was a colorless oil.

(15*Z*,19*Z*)-1,8-dioxacyclohexacosa-15,19-dien-3,5-diyne-9,26-dione **5a**

Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 5.59–5.26 (4H, m, =CH), 4.76 (4H, s, O-CH_2), 2.42–2.32 (4H, m, CH_2), 2.23–1.95 (8H, m), 1.77–1.69 (4H, m, CH_2), 1.37–1.32 (m, 8H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 172.6, 130.8, 128.6, 76.8, 73.7, 51.6, 32.8, 28.6, 27.1, 26.8, 26.0, 24.5). IR (ν/cm^{-1}): 1735 (C=O), 1238, 1155 (C–O). HRMS (MALDI TOF) $[M]^-$ calcd. for $C_{24}H_{32}O_4$ 384.2301; Found 384.2309. Yield (method 1/method 2): 54%/62%.

(17*Z*,21*Z*)-1,10-dioxacyclooctacosa-17,21-dien-4,6-diyne-11,28-dione **5b**

Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 5.58–5.25 (4H, m, =CH), 4.16 (4H, t, J = 5.4 Hz, O-CH_2), 2.60 (4H, t, J = 5.4 Hz, CH_2), 2.34 (4H, t, J = 7.3 Hz, CH_2), 2.22–1.94 (8H, m, CH_2), 1.83–1.67 (4H, m, CH_2), 1.37–1.32 (m, 8H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 170.4, 130.2, 128.9, 74.1, 66.5, 61.6, 33.4, 28.5, 27.3, 26.9, 26.4, 24.7, 19.8. IR (ν/cm^{-1}): 1741 (C=O), 1245, 1165 (C–O). HRMS (MALDI TOF) $[M]^-$ calcd. for $C_{26}H_{36}O_4$ 412.2614; Found 412.2618. Yield (method 1/method 2): 59%/66%.

(19*Z*,23*Z*)-1,12-dioxacyclotriaconta-19,23-dien-5,7-diyne-13,30-dione **5c**

Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 5.58–5.25 (4H, m, =CH), 4.16 (4H, t, J = 5.4 Hz, O-CH_2), 2.60 (4H, t, J = 5.4 Hz, CH_2), 2.44–2.33 (8H, m), 2.22–1.96 (8H, m, CH_2), 1.89–1.71 (4H, m, CH_2), 1.37–1.32 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 173.4, 130.2, 128.9, 76.1, 66.1, 62.6, 33.5, 28.3, 27.3, 27.1, 26.7, 26.4, 24.6, 16.8. IR (ν/cm^{-1}): 1733 (C=O), 1240, 1170 (C–O). HRMS (MALDI TOF) $[M]^-$ calcd. for $C_{28}H_{40}O_4$ 440.2927; Found 440.2919. Yield (method 1/method 2): 65%/72%.

(21*Z*,25*Z*)-1,14-dioxacyclodotriaconta-21,25-dien-6,8-diyne-15,32-dione **5d**

Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 5.56–5.29 (4H, m, =CH), 4.10 (4H, t, J = 5.4 Hz, O-CH_2), 2.41–2.31 (8H, m), 2.21–2.01 (8H, m, CH_2), 1.87–1.51 (12H, m,

CH_2), 1.39–1.31 (m, 8H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 173.6, 130.4, 128.9, 76.7, 65.9, 63.6, 33.8, 28.4, 27.6, 27.2, 26.8, 26.6, 25.2, 24.7, 18.9. IR (ν/cm^{-1}): 1727 (C=O), 1239, 1177 (C–O). HRMS (MALDI TOF) $[M]^-$ calcd. for $C_{30}H_{44}O_4$ 468.3240; Found 468.3247. Yield (method 1/method 2): 75%/84%.

4. Conclusions

Previously undescribed biologically active macrodiolides were synthesized, and good yields and high stereoselectivity (>98%) were achieved. Preliminary studies of the anti-tumor activity of the synthesized macrocyclic compounds have shown high cytotoxicity against the c Jurkat, K562, U937, HL-60, HeLa, and Hek293 cells lines in vitro.

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