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The facile synthesis of the 5*Z*,9*Z*-dienoic acids and their topoisomerase I inhibitory activity†

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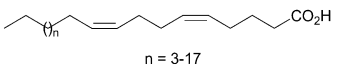
An original, effective approach to the synthesis of natural and synthetic 5*Z*,9*Z*-dienoic acids in high yields (61–67%) and with high selectivity (>98%) was developed. The approach is based on the use of the new intermolecular catalytic cross cyclomagnesiation of terminal aliphatic and oxygenated 1,2-dienes upon treatment with Grignard reagents in the presence of the Cp_2TiCl_2 catalyst. High activity of (5*Z*,9*Z*)-5,9-eicosadienoic acid as a human topoisomerase I inhibitor at concentrations above 0.1 μM was elucidated.

The DNA-dependent enzyme topoisomerase, which catalyzes the topological transformations of DNA and plays a key role in all aspects of genome functioning, is one of the most important enzymes that participate in the cell cycle. The introduction of single-stranded (by topoisomerase I) and double-stranded (topoisomerase II) cleavages followed by ligation to restore the integrity of the DNA molecule accounts for the mobility needed for conformational changes of DNA during template-directed synthesis and for chromosome mobility during mitosis. Topoisomerases are considered as cellular targets for chemotherapeutic agents, as these compounds prevent the DNA repair synthesis and, hence, cause accumulation of damaged DNA molecules and promote cell death.[1]

Currently, intensive search and selection of natural inhibitors of topoisomerase I are in progress, and new synthetic analogs and semisynthetic derivatives of known antitumor compounds able to change the catalytic activity of enzymes by stabilizing the DNA–protein complexes are being developed.[1a,b]

Great potential for solution and implementation of the above-indicated tasks is inherent in higher fatty acid derivatives containing two *cis* carbon–carbon double bonds in positions 5 and 9 of the hydrocarbon chain, which have been isolated earlier in minor amounts from sea sponges and conifer cones.[2]

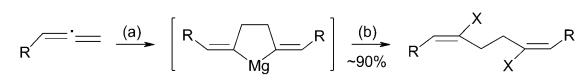


According to published data,[2] 5*Z*,9*Z*-dienoic fatty acids exhibit antimalarial, antimicrobial, and antiviral activities combined with low toxicity, which make this class of compounds fairly attractive for the development of modern pharmaceutical drugs.

Considerable contributions to the development of isolation and identification methods and approaches to the synthesis of dienoic fatty acids were made by research groups of N. Carballeira, Y. Sakagami, C. Djerassi, and others.[3] Note that synthetic routes to 5*Z*,9*Z*-dienoic acids reported in the literature comprise multiple steps (5–20) and give target compounds in only 0.5 to 15% yields;[4] moreover, in most cases, the reactions result in mixtures of stereoisomers, which seems to be the key issue that hampers further investigation and application of this class of compounds to design modern pharmaceutical drugs. Therefore, the development of new effective methods for the synthesis of 5*Z*,9*Z*-dienoic acids is an important and topical task.

Previously we demonstrated[5] that aliphatic 1,2-dienes react with EtMgBr in the presence of Mg (halide ion acceptor) and the Cp_2TiCl_2 catalyst to give 2,5-dialkylidenemagnesacyclopentanes, which undergo acid hydrolysis to be converted to symmetrical 1*Z*,5*Z*-dienes in ~90% yields and >98% stereoselectivity (Scheme 1).

Subsequently this approach was extended to N-containing allenes, which participated in the cyclomagnesiation to give symmetrical diamines containing a 1*Z*,5*Z*-diene group in up to 85% yields.[6]



[Ti] = Cp_2TiCl_2; (a): EtMgBr, Mg, [Ti]; (b): H_3O^+(D_3O^+)
R = alkyl, Ph, Bn, cyclo; X = H,D

Scheme 1 Catalytic cyclomagnesiation of aliphatic 1,2-dienes.

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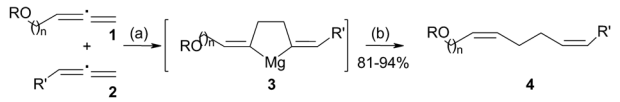
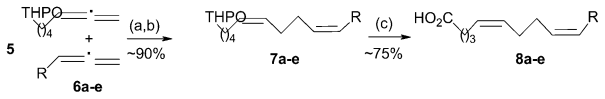


[Ti] = Cp_2TiCl_2; (a): EtMgBr, Mg, [Ti]; (b): H_3O^+
n = 2: R = THP, R' = C_6H_{13} (**a**); R = Bn, R' = C_6H_{13} (**b**); n = 3: R = THP, R' = $C_{12}H_{25}$ (**c**);
n = 4: R = THP, R' = C_4H_9 (**d**); R = THP, R' = CH_2Ph (**e**); n = 6: R = THP, R' = C_4H_9 (**f**).

Scheme 2 Intermolecular catalytic cross cyclomagnesiation of aliphatic and oxygenated 1,2-dienes.



(a): EtMgBr, Mg, [Ti]; (b): H_3O^+; (c) Jones oxidation.
[Ti] = Cp_2TiCl_2; R = C_6H_{13} (**a**); C_9H_{19} (**b**); $C_{10}H_{21}$ (**c**); $C_{12}H_{25}$ (**d**); $C_{14}H_{29}$ (**e**).

Scheme 3 New approach to the synthesis of 5*Z*,9*Z*-dienoic acids.

The developed reactions were used to prepare substituted 1,5-dienes of a specified structure, unsaturated macrocarbocycles, and carbo- and heterocyclic products.[5a–c,7]

This communication gives an account of the results we obtained while continuing our research into the cross cyclomagnesiation of oxygenated and aliphatic 1,2-dienes, in particular, those containing functional substituents, and application of these products in the synthesis of unsymmetrical natural and synthetic 5*Z*,9*Z*-dienoic acids.

Relying on the earlier results on cross cyclomagnesiation of N-containing and terminal aliphatic 1,2-dienes, we found that cross cyclomagnesiation of oxygenated 1,2-dienes (**1**) in which the OH group is protected by a pyranyl or a benzyl group with terminal alkyl-substituted 1,2-dienes (**2**) induced by EtMgBr in the presence of magnesium metal and the Cp_2TiCl_2 catalyst (**1** : **2** : EtMgBr : Mg : [Ti] = 10 : 12 : 40 : 32 : 0.5; Et_2O, 6 h, 20–22 °C) affords only unsymmetrical OMC (**3**) in >80% yields. After acid hydrolysis, these products were converted to unsymmetrical 1*Z*,5*Z*-diene ethers (**4**) (Scheme 2).

We found that oxygenated 1,2-dienes with unprotected hydroxyl groups or their trimethylsilyl ethers cannot be involved into cyclomagnesiation under the developed conditions.

The observed high chemoselectivity of formation of only the unsymmetrical magnesacyclopentane (**3**) is due to the fact that the reaction is carried out in diethyl ether in which, as was shown earlier,[5a,b] the intermolecular catalytic cyclomagnesiation of aliphatic terminal allenes does not occur. Under conditions we selected for the cross cyclomagnesiation of functionally substituted 1,2-dienes with aliphatic 1,2-dienes, the introduction of a slight excess of aliphatic 1,2-diene (**2**) completely suppresses the homo-cyclomagnesiation of 1,2-dienes, and, hence, the formation of symmetrical magnesacyclopentanes; under the reaction conditions, they are produced in ~2% yield.

The *cis*-configuration of the double bonds in the resulting 1,5-dienes can be derived from the high-field signals of the internal allylic carbon atoms at ~27 ppm present in the ^{13}C NMR spectrum, which attest to *cis*-interaction with the external allylic carbon atoms.[8]

The vicinal spin–spin coupling constants between the protons at C(3) and C(6) (d. ^3J = 11 Hz, t ^3J = 7 Hz) for the hydrolysis products (**4**) serve as evidence for the *cis*-arrangement of hydrogen atoms at the double bonds.[9]

The reaction rate decreases at 0 °C and yields of the products (**3**) do not exceed 70% for 10 h. Heating of the reaction mixture to 30–35 °C did not substantially influence yields of the cyclomagnesiation products (**3**).

The replacement of EtMgBr by other Grignard reagents, for example, EtMgCl, i-PrMgBr, or BuMgBr prepared in diethyl ether, does not affect the yield or selectivity of target OMC (**3**).

Subsequently, we assumed that the proposed approach to the synthesis of oxygenated dienes using cross cyclomagnesiation may serve as the basis for the development of an efficient approach to the synthesis of 5*Z*,9*Z*-dienoic acids.

According to the strategy we developed for the synthesis of unsaturated 1*Z*,5*Z*-ethers, the first step is the Cp_2TiCl_2-catalyzed cross cyclomagnesiation of terminal aliphatic allenes (**5**) with the tetrahydropyran ether of hepta-5,6-dien-1-ol (**6**) by means of EtMgBr under the above-described conditions. The subsequent hydrolysis of the reaction mixture affords oxygenated dienes (**7**). The subsequent oxidation of dienes (**7**) with the Jones reagent[10] (CrO_3–H_2SO_4) gives rise to the target (5*Z*,9*Z*)-5,9-dienoic acids (**8**) in 61–67% yields and with >98% stereoselectivity (Scheme 3).

In the next phase of investigations, we found an exceptionally high inhibitory activity of (5*Z*,9*Z*)-5,9-eicosadienoic acid (**8c**) with respect to human topoisomerase I.

The ability of (5*Z*,9*Z*)-5,9-eicosadienoic acid to inhibit the DNA-dependent enzyme topoisomerase I was studied *in vitro* in the relaxation of supercoiled plasmid DNA under standard conditions (Fig. 1).

The action of topoisomerase I induces relaxation of supercoiled plasmid DNA to give a set of topoisomers. Determination of the inhibition of topoisomerase I is based on the high sensitivity of the electrophoretic mobility of various forms of DNA (the original DNA and the DNA relaxed under the action of the enzyme) to differences in the duplex conformation, *i.e.*, either supercoiled or circular plasmid. The introduction of a

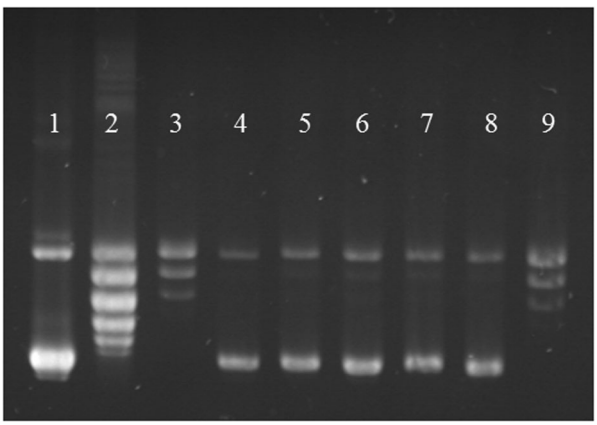


Fig. 1 Electropherogram of the products of relaxation of supercoiled plasmid DNA *in vitro* induced by topoisomerase I (Topogen, USA) in the presence of (5*Z*,9*Z*)-5,9-eicosadienoic acid. (1) Supercoiled plasmid DNA (pHOT1). (2) Relaxed DNA form (visualization of the set of topoisomers). (3) Negative control with DMSO (concentration 3%). (4) Relaxation reaction of plasmid DNA in the presence of camptothecin (10 μM). (5–9) Effect of different concentrations of (5*Z*,9*Z*)-5,9-eicosadienoic acid on the relaxation of plasmid DNA (5 – 0.75, 6 – 0.5, 7 – 0.25, 8 – 0.1, 9 – 0.01 μM).

compound that inhibits topoisomerase I disturbs the relaxation, and this may result in fewer number of topoisomers formed, higher fraction of the circular plasmid, and the presence of residual amounts of the supercoiled plasmid DNA.

The results presented in Fig. 1 indicate that the relaxation of supercoiled plasmid DNA with topoisomerase I activity (Topogen, USA) being inhibited by (5*Z*,9*Z*)-5,9-eicosadienoic acid (in this example, one enzyme unit is inhibited by 0.1 μM of the tested compound) results in a decrease in the residual amount of the supercoiled plasmid DNA and an increase in the number of topoisomers formed (lane 9) following a successive decrease in the concentration of the added compound from 0.75 to 0.01 μM. The effects of (5*Z*,9*Z*)-5,9-eicosadienoic acid and camptothecin on the enzyme in question were comparable; however, (5*Z*,9*Z*)-5,9-eicosadienoic acid inhibited the enzyme when present in a concentration of 0.1 μM, whereas camptothecin was topo-I-active in a concentration of 10 μM, and this was correlated with its inhibitory activity against various lines of tumor cells.[1d] Judging by the residual amount of supercoiled plasmid DNA, the presence of (5*Z*,9*Z*)-5,9-eicosadienoic acid in the reaction mixture, like the presence of camptothecin, hampers the relaxation of supercoiled DNA. A quite probable mechanism of inhibition of (5*Z*,9*Z*)-5,9-eicosadienoic acid is violation of recognition of DNA binding sites by the enzyme and/or induced local disruptions of the duplex conformation, which non-specifically retards the operation of topoisomerase I in the relaxation of supercoiled DNA. Also, (5*Z*,9*Z*)-5,9-docosadienoic acid (**8d**) completely inhibits topo I in 1 μM concentration.

Note that (5*Z*,9*Z*)-5,9-eicosadien-1-ol, which is the precursor of (5*Z*,9*Z*)-5,9-eicosadienoic acid, did not inhibit topoisomerase I even in concentrations above 500 μM.

Thus, cross cyclomagnesiation of oxygenated allenes with aliphatic 1,2-dienes was performed for the first time upon treatment with Grignard reagents in the presence of the Cp_2TiCl_2 catalyst. An efficient approach to the synthesis of natural and synthetic 5*Z*,9*Z*-dienoic acids in up to 67% yield was proposed, these acids exhibiting high inhibitory activity with respect to human topoisomerase I.

The developed reaction appears to have a broad synthetic scope, which would enable the preparation of quite a few dienoic acids with different positions of double bonds. The evaluation of the topo I-inhibitory activity and elucidation of the structure–activity relationships in the series of 5*Z*,9*Z*-dienoic acids and their semisynthetic derivatives and synthetic analogs would facilitate the targeted search for new active inhibitors of this enzyme that may potentially become pharmaceutical drugs, which is important for preclinical tests of antibiotics.

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