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Proceeding Paper

Development of New Effective Methods for the Synthesis of Lembehynes A–C Exhibiting Cytotoxic and Neuritogenic Activity †

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Abstract: The report presents data on our studies on the preparation of the precursor lembehyne A and the complete stereoselective synthesis of natural lembehynes B and C, which have cytotoxic and neuritogenic activity. All methods and approaches to the synthesis of the above-mentioned lembehynes presented in the report are based on the use of the catalytic cross-cyclomagnesiation of 1,2-dienes (Dzhemilev reaction) at the key stage of the synthesis.

Keywords: Lembehynes A–C; 1Z,5Z-dienes; cross-cyclomagnesiation; anti-cancer and neuritogenic activity

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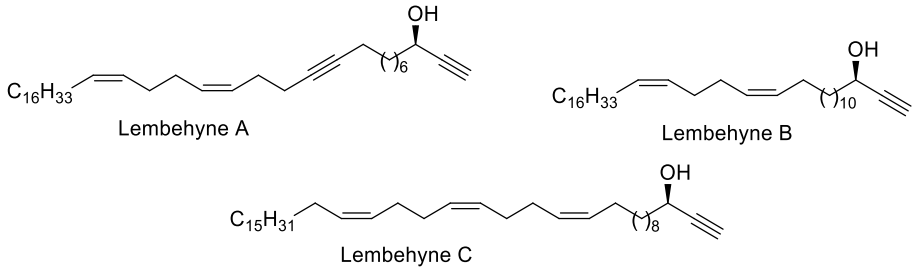
1. Introduction

To date, a wide range of unique molecules have been isolated from marine organisms, and some of them are used as drug candidates and reagents for biomedical research [1–4]. Sponges have been recognized as the richest sources of biologically active compounds among marine invertebrates, and their metabolites are diverse in structure, many have antibacterial, antiviral, antifungal, antimalarial, anthelmintic, immunosuppressive, muscle relaxant and anti-inflammatory activity. Currently, there is a steady increase in the drug resistance of microorganisms, so the search and development of new pharmaceuticals is the most important task. Some compounds derived from sea sponges are undergoing preclinical and clinical trials as agents against cancer, microbial infections, and inflammation; however, the development of drugs based on them is very difficult due to the low content of the corresponding compounds in sponge tissues [5–8].

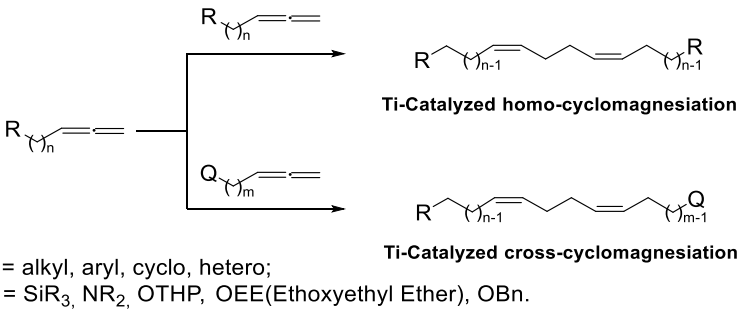
Among the wide variety of compounds found in sea sponges, acetylenic alcohols lembehynes A, B, C are of particular interest. Lembehyne A has been shown to exhibit neuritogenic activity against pheochromacetoma PC12 and neuroblastoma Neuro 2A cells. These data allow us to consider natural acetylenic alcohols as the basis for the development of modern drugs for the treatment of such neurodegenerative diseases as Alzheimer's disease, Parkinson's disease, and Huntington's chorea. Meanwhile, the inaccessibility and lack of effective methods for the synthesis of natural lembehynes are a significant constraint for a detailed study of their properties and the full disclosure of their biomedical potential [6–12].



An analysis of the structure of lembehynes and known approaches to their complete synthesis showed that the most complex and multistage stage is the stage of stereoselective formation of the 1Z,5Z diene fragment in lembehyne molecules. Thus, during the implementation of the complete 11-stage synthesis of lembehyne A, 6 stages were spent to obtain the key synthon, 4Z,8Z-pentacose-4,8-dien-1-ol.

Based on the literature data, the most common and popular approaches to the formation of the 1Z,5Z-diene fragment are methods based on the use of the Wittig reaction, alkene metathesis, alkyne alkylation, and stereoselective reduction of 1,5-diynes.

Previously, we developed new Ti-catalyzed reactions of homo- and cross-cyclomagnesiation of 1,2-dienes using Grignard reagents (Scheme 1), which were successfully used in the strictly stereoselective synthesis of 1Z,5Z-dienes of a given structure, giant macrocarbocycles, as well as natural 5Z,9Z-dienic acids, which exhibit antitumor activity according to the following scheme [13–25].

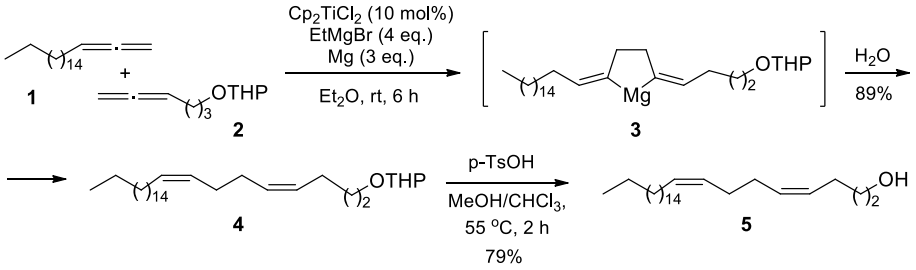


Scheme 1. Ti-catalyzed homo- and cross-cyclomagnesiation of 1,2-dienes.

2. Results and Discussion

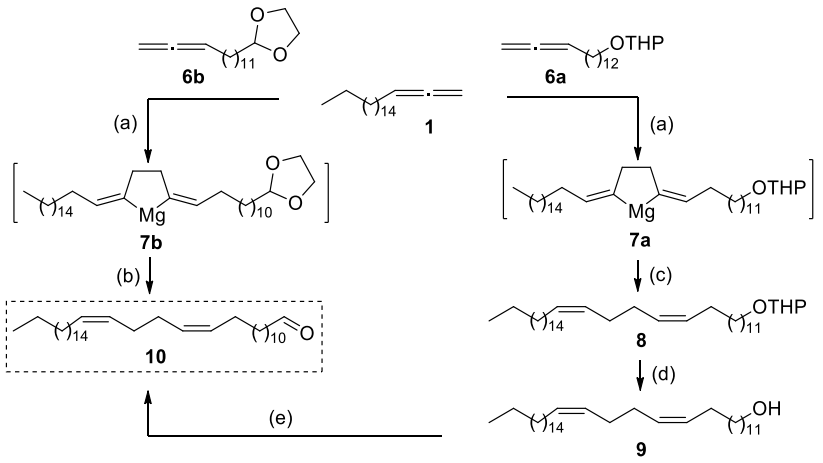
Based on our previous results [13–16], we put forward the idea of the possibility of using the cross-cyclomagnesiation reaction of O-containing and aliphatic allenes in the synthesis of neuritogenic acetylenic alcohols, lembehynes A, B and C.

We have carried out the synthesis of the key monomer of lembehyne A, (4Z,8Z)-pentacose-4,8-dien-1-ol, by the reaction of cross-cyclomagnesiation of 1,2-nonadecadiene **(1)** with 4,5-hexadienol tetrahydropyran ester **(2)** with EtMgBr in the presence of metallic Mg (a halogen ion acceptor) and a Cp_2TiCl_2 catalyst (10 mol %) to give the intermediate magnesacyclopentane **(3)**, the subsequent hydrolysis of which gives (4Z,8Z)-pentacose-4,8-dien-1-ol tetrahydropyran ester **(4)** with a yield of 89% (Scheme 2). Refluxing of tetrahydropyran ester 4 in a MeOH–CHCl3 (1:1) mixture in the presence of p-TsOH leads to the target (4Z,8Z)-pentacose-4,8-dien-1-ol **(5)** with a total yield of ~70%. As a result, unsaturated alcohol **(5)** can later be used in the complete synthesis of lembehyne A.



Scheme 2. Short synthesis of (4Z,8Z)-pentacose-4,8-dien-1-ol **(5)**-a key synthon in the preparation of lembehyne A.

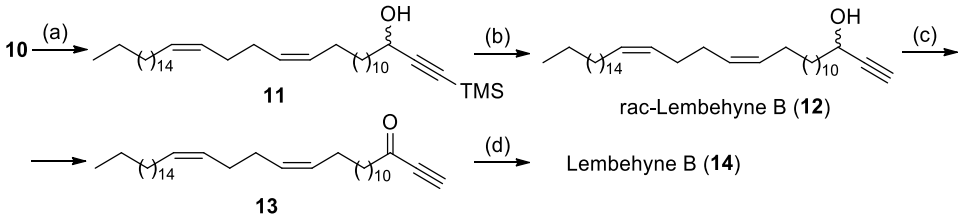
As a follow-up to these studies, an original scheme for the complete stereoselective synthesis of natural lembehyne B was developed for the first time catalyst Cp_2TiCl_2 (10 mol %) to give magnesacyclopentane **(7a)**, hydrolysis of which gives (13Z,17Z)-tetraconta-13,17-dienol tetrahydropyran ester **(8)** in 88% yield. Successive reactions of deprotection of tetrahydropyranyl protection and oxidation of unsaturated alcohol **(9)** with Dess-Martin periodinane led to the key monomer (13Z,17Z)-tetraconta-13,17-dienal **(10)** in ~64% yield in two stages (Scheme 3).



Scheme 3. Ti-catalyzed cross-cyclomagnetization of aliphatic and O-containing 1,2-dienes in the synthesis of (13Z,17Z)-tetraconta-13,17-dienal (**10**). (a) EtMgBr, Mg, Cp_2TiCl_2, Et_2O, rt; (b) H^+; (c) p-TsOH, $MeOH/CHCl_3$, 77%; (d) Dess-Martin periodinane, THP, rt, 83%.

We have also developed an alternative approach to the synthesis of the key monomer in the preparation of lembehyne B. Cross-cyclomagnesiation of 1,2-nonadecadiene **(1)** with using EtMgBr in the presence of metallic Mg (halogen ion acceptor) and catalytic amounts of Cp_2TiCl_2 (10 mol.%) (**1**:**6b**:EtMgBr:Mg:[Ti] = 12:10:30:20:0.1, Et2O, 20–22 °C, 7h) leads to magnesacyclopentane **(7b)**, acid hydrolysis of which gives (13Z,17Z)-tetraconta-13,17-dienal **(10)** in a single preparative step in ~77% yield (Scheme 3).

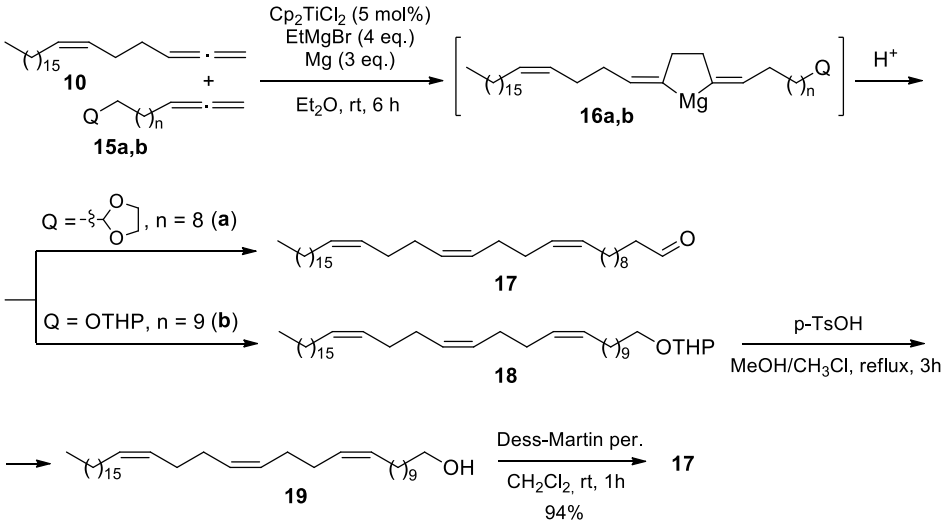
Thus, the yield of (13Z,17Z)-tetracont-13,17-dienal was 64% by the first route and 77% by the second. At the final stage in the complete synthesis of lembehyne B, aldehyde **(10)** was reacted with lithium trimethylsilylacetylenide, which was preliminarily prepared by the reaction of equimolar amounts of trimethylsilylacetylene with n-BuLi in THF. After keeping the reaction mixture for 3 days at room temperature, silane **(11)** was obtained with a yield of 90%. Removal of trimethylsilyl protection with tetrabutylammonium fluoride (TBAF) in THF in 4 hours allows to obtain racemic lembehyne B **(14)** in almost quantitative yield (Scheme 4).



Scheme 4. Synthesis of natural lembehyne B. (a) Lithiumtrimethylsilylacetylenide, THF, rt, 90%; (b) TBAF, THF, rt, 99%; (c) Dess-Martinperiodinane, THF, rt, 86%; (d) Alpine-borane, THF, rt, 84%.

Subsequently, starting from racemic lembehyne B, we synthesized its natural stereoisomer with the 3R configuration of the hydroxyl group at the C-3 carbon atom. Thus, oxidation of alkynol **(12)** with Dess-Martin periodinane in CH_2Cl_2 at room temperature for 1 hour yielded (15Z,19Z)-hexaconta-15,19-dien-1-yl-3-one **(13)** with a yield of 86% (Scheme 4). Stereoselective reduction of ketone **(13)** was carried out using the reagent B-3-pinanyl-9-borabicyclo[3.3.1]nonane (Alpine-borane), previously prepared from (+)-α-pinene (98%ee) and 9-borabicyclo[3.3.1]nonane. As a result, lembehyne B **(14)** was obtained with a yield of 84% and an enantiomeric purity (95%ee).

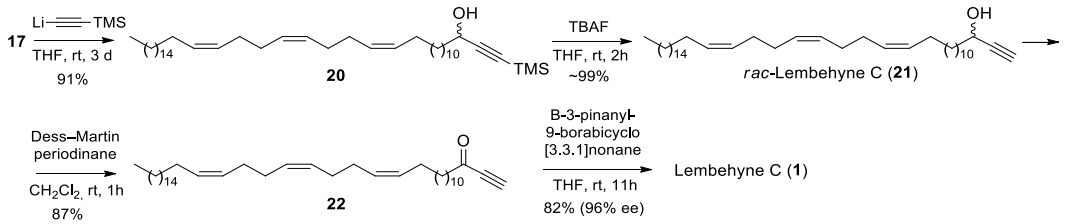
We were the first to carry out the complete synthesis of racemic (±)lembehyne C and stereoisomerically pure (ee) lembehyne using the cross-cyclomagnesiation reaction of tricose-1,2,6Z-triene with 2-dodeca-10,11-diene-1-yl-1,3-dioxalane or 11,12-tridecadien-1-ol ester with EtMgBr in the presence of a Cp_2TiCl_2 catalyst. First, by the reaction of intermolecular cross-cyclomagnesiation of (6Z)-tricose-1,2,6-triene **(10)** with 2-dodeca-10,11-dien-1-yl-1,3-dioxolane **(15a)** or tetrahydropyran ether 11, 12-tridecadienol **(15b)** with EtMgBr in the presence of Mg (powder) and catalytic amounts of Cp_2TiCl_2 (**10**:**15a(15b)**:EtMgBr:Mg:[Ti] = 12:10:36:24:0.1, Et2O, 20–22 °C, 10 h), which, after acid hydrolysis of magnesacyclopentanes **(16a,b)** formed in situ, gave aldehyde **(17)** and tetrahydropyran ester **(18)** containing 1Z,5Z,9Z-diene fragments in 81 and 89% yields, respectively. Ether **(18)** was converted into aldehyde **(17)** in ~64% yield by successive reactions of deprotection of tetrahydropyranyl protection and oxidation with Dess-Martin reagent (Scheme 5), which indicates the preference for the synthesis of aldehyde **(17)** via dioxalane **(15a)**.



Scheme 5. (*Z*,*Z*,*Z*)-Stereoselective Synthesis of Hexaconta-11Z,13Z,17Z-trienal **(17)**.

The interaction of hexaconta-11Z,13Z,17Z-triene-1-al **(17)** with lithium trimethylsilylacetylenide, preliminarily synthesized by the reaction of equimolar amounts of trimethylsilylacetylene and n-BuLi in THF, in 3 days at room temperature led to the preparation

of silane **(20)** with a yield of 91%. Removal of the terminal trimethylsilyl group by treatment of the silane **(20)** with trimethylbutylammonium fluoride (TBAF) in THF for 4 hours leads directly to racemic lembehyne C **(21)** in almost quantitative yield (Scheme 6).



Scheme 6. Total Synthesis of Natural Lembehyne C **(1).**

Racemic lembehyne C **(21)** was oxidized with Dess-Martin periodinane (CH_2Cl_2, r.t., 1 h) to the corresponding ketone **(22)** in 87% yield (Scheme 6). At the final stage of the synthesis using the reagent B-3-pinanyl-9-borabicyclo[3.3.1]nonane (Alpine-borane),8 preliminarily prepared from (+)-α-pinene (98%ee) and 9-borabicyclo[3.3.1]nonane, stereoselective reduction of ketone **(22)** to natural lembehyine C **(1)** was realized in 82% yield and enantiomeric purity (96%ee).

The reactions developed by us made it possible to synthesize natural lembehynes B, C and the key precursor of lembehyne A. For the synthesized natural lembehynes B, C, apoptosis-inducing activity against five tumor cell lines Jurkat, K562, U937, HeLa and HEK293 and neuritogenic activity against cell cultures were studied in detail. PC12, PC9 and Neuro2A.

Experimental section, 1H and 13C NMR spectra and general procedure for all synthesized compounds are presented in previously published articles [17–22].

3. Conclusions

Thus, for the first time, we synthesized natural lembechins B and C and a precursor of lembehyne A using the cross-cyclomagnesiation reaction of aliphatic and O-containing 1,2-dienes catalyzed by Cp_2TiCl_2 at the key stage of the synthesis, and also studied their antitumor activity using modern methods of flow cytometry and multiplex analysis.

Author Contributions: Conceptualization, U.M.D. and L.U.D.; methodology, A.A.M.; validation, E.K.M., resources, E.K.M.; data curation, U.M.D.; writing—original draft preparation, E.K.M., A.A.M.; writing—review and editing, U.M.D. and L.U.D.; visualization, E.K.M.; supervision, U.M.D.; project administration, A.A.M.; funding acquisition, A.A.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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