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Introduction

The Global Ocean occupies more than 70% of the Earth's surface; therefore, flora and fauna inhabiting the Ocean are considered as important sources of biologically active compounds that could serve as the base for the development of modern pharmaceutical agents for treating cancer, neurodegenerative diseases, viral and bacterial infections, and other dangerous human diseases.¹ During the last decade alone, about ten thousand new compounds were isolated from marine organisms; some of these compounds are already under Phase I or II clinical trials.²

Recently,³ Kobayashi and co-workers isolated, from the Indonesian marine sponge *Haliclona* sp., micro quantities (10–15 mg kg⁻¹) of lembdehynes A–C – acetylenic alcohols (Fig. 1), efficient small-molecule analogues of proteins belonging to the family of neurotrophins, which perform an important function of maintaining the vitality of neurons.⁴ Hence, lembdehynes are considered as potential pharmaceutical drugs for treating neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's chorea.

The poor accessibility and the lack of efficient methods for the synthesis of natural lembdehynes hamper detailed investi-

The first total synthesis of the marine acetylenic alcohol, lembehyne B – a selective inducer of early apoptosis in leukemia cancer cells[†]

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The communication reports a new stereoselective method for the synthesis of a natural acetylenic alcohol, lembehyne B. The key stage of the process uses new cross-cyclomagnesiation reaction of aliphatic and oxygenated 1,2-dienes with Grignard reagents in the presence of a catalytic amount of Cp₂TiCl₂. A study of the cytotoxic properties of lembehyne B on tumor cell lines using flow cytometry demonstrated that this is a selective inducer of early apoptosis of the Jurkat, HL-60 and K562 cell cultures and hypodiploid (sub-G1) sub-population inducer in cell cycle studies for all cell lines used.

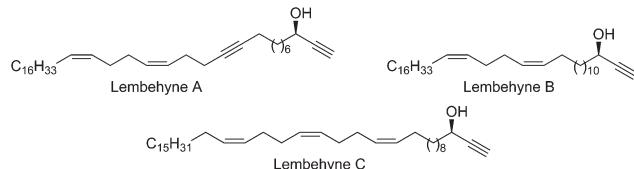


Fig. 1 Structures of lembdehynes A–C.

gation of the properties and preclude taking full advantage of the biomedical potential of these compounds.⁵

Analysis of the lembehyne structure demonstrated that stereoselective formation of the 1Z,5Z-diene moiety present in the lembehyne molecule is the most complicated and multi-step part.⁵ Indeed, in the 11-step total synthesis of lembehyne A, six steps were spent for the preparation of the key synthon, 4Z,8Z-pentacosa-4,8-dien-1-ol.⁵

Proceeding from the published data, the most popular approaches to the formation of the 1Z,5Z-diene moiety are based on the Wittig reaction, alkene metathesis, alkylation of alkynes, and stereoselective reduction of 1,5-dynes.⁶

Previously, we developed Ti-catalyzed homo- and cross-cyclomagnesiation reactions of 1,2-dienes with Grignard reagents (Scheme 1), which were successfully used in the strictly stereoselective syntheses of 1Z,5Z-dienes of a specified structure, giant macrocyclics, and natural 5Z,9Z-dienoic acids exhibiting antitumor activities.⁷

In view of the foregoing, in this paper we report the development of a preparative stereoselective synthesis of lembehyne B using cross-cyclomagnesiation of aliphatic and oxygenated 1,2-dienes in the key stage of the synthesis,

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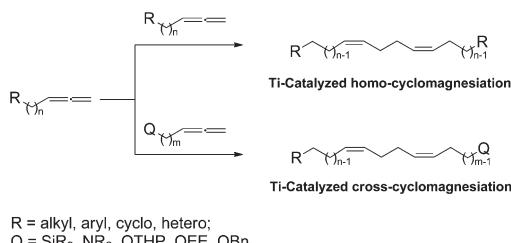
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Scheme 1 Ti-Catalyzed homo- and cross-cyclomagnesiation.

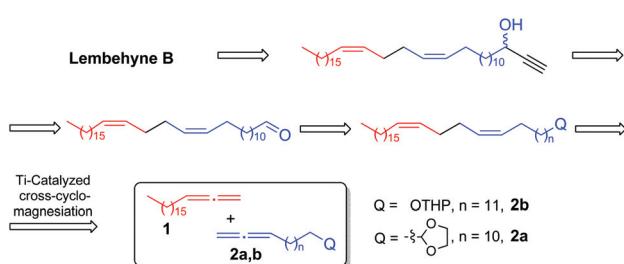
product accumulation and *in vitro* testing for the antitumor activity on Jurkat, HL-60, and K562 cell lines using flow cytometry.

Results and discussion

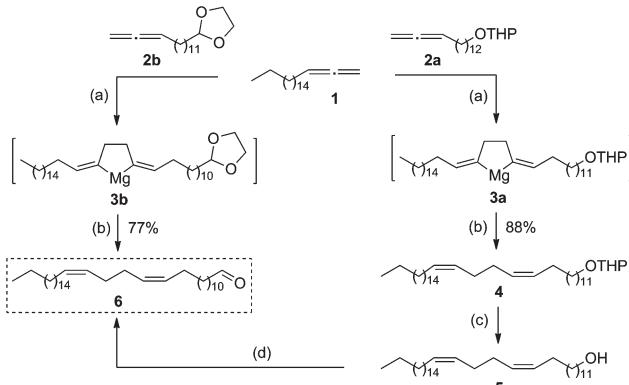
By retrosynthetic analysis of the lembehyne B structure, we identified the key monomer: (13Z,17Z)-tetraconta-13,17-dienal, which is accessible through cross-magnesiation of 1,2-nonadecadiene (**1**) with the tetrahydropyran ether of 13,14-pentadecadienol (**2a**) or 2-tetradeca-12,13-dien-1-yl-1,3-dioxolane (**2b**) (Scheme 2). The final steps are intended for the formation of the terminal propargyl moiety to obtain racemic lembehyne B and hydroxy group oxidation followed by stereoselective reduction (Scheme 2).

In accordance with the developed strategy, 1,2-nonadecadiene (**1**) and 13,14-pentadecadienol tetrahydropyran ether (**2a**) were first subjected to cross-cyclomagnesiation on treatment with EtMgBr in the presence of Mg metal and Cp₂TiCl₂ catalyst (10 mol%). The reaction proceeded *via* magnesacyclopentane **3a**, which was hydrolyzed to give (13Z,17Z)-tetraconta-13,17-dienol tetrahydropyran ether (**4**) in 88% yield (Scheme 3). Successive tetrahydropyran deprotection⁸ and Dess–Martin periodinane oxidation of unsaturated alcohol **5**⁹ yielded the target (13Z,17Z)-tetraconta-13,17-dienal (**6**) in ~64% yield over two steps.

In turn, cross-magnesiation of 1,2-nonadecadiene (**1**) and 2-tetradeca-12,13-dien-1-yl-1,3-dioxolane (**2b**) with EtMgBr in the presence of Mg metal (halide ion acceptor) and a catalytic amount of Cp₂TiCl₂ (10 mol%) (**1**:**2b**:EtMgBr:Mg:[Ti] = 12:10:30:20:0.1, Et₂O, 20–22 °C, 7 h) furnishes magnesacyclopentane **3b**, which is acid-hydrolyzed to afford (13Z,17Z)-tetraconta-13,17-dienal (**6**) in ~77% yield in one preparative step (Scheme 3).



Scheme 2 Retrosynthetic analysis of lembehyne B.

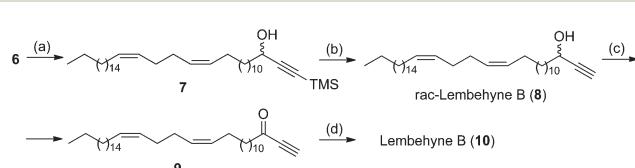
Scheme 3 Ti-Catalyzed cross-cyclomagnesiation of aliphatic and oxygenated 1,2-dienes in the synthesis of (13Z,17Z)-tetraconta-13,17-dienal (**6**). Reagents and condition: (a) EtMgBr, Mg, Cp₂TiCl₂, Et₂O, rt; (b) H⁺; (c) p-TsOH, MeOH/CHCl₃, 77%; (d) Dess–Martin periodinane, THP, rt, 83%.

Thus, the yield of (13Z,17Z)-tetraconta-13,17-dienal was 64% according to the former route and 77% according to the latter one.

At the final step, aldehyde **6** was made to react with lithium trimethylsilylacetylenide,¹⁰ prepared beforehand by the reaction of equimolar amounts of trimethylsilylacetylene and *n*-BuLi in THF, the reaction affording silane **7** in 90% yield over a period of 3 days at room temperature. The trimethylsilyl deprotection on treatment with tetrabutylammonium fluoride (TBAF) in THF over a period of 4 h furnished racemic lembehyne B (**8**) in nearly quantitative yield (Scheme 4).

Using racemic lembehyne B, we synthesized its natural stereomer with the 3*R*-configuration of the hydroxy group at the C-3 carbon atom. First, Dess–Martin periodinane oxidation of alkynol **8** in CH₂Cl₂ at room temperature gave (15Z,19Z)-hexaconta-15,19-dien-1-yl-3-one (**9**) in 86% yield over a period of 1 h (Scheme 4). The stereoselective reduction of ketone **9** was performed using the *B*-3-pinanyl-9-borabicyclo[3.3.1]nonane (Alpine-borane) reagent,¹¹ prepared beforehand from (+)- α -pinene (98% ee) and 9-borabicyclo[3.3.1]nonane. This gave lembehyne B (**10**) with enantiomeric excess of 95% in 84% yield.

During the last 10–15 years, several hundred natural alkynes, exhibiting high antitumor activities, were isolated from macro- and microalgae, freshwater- and marine cyanobacteria, and other marine organisms.² Meanwhile, no data on the antitumor activity of natural lembehynes are available



Scheme 4 Synthesis of lembehyne B. Reagents and condition: (a) lithium trimethylsilylacetylenide, THF, rt, 90%; (b) TBAF, THF, rt, 99%; (c) Dess–Martin periodinane, THF, rt, 86%; (d) Alpine-borane, THF, rt, 84%.



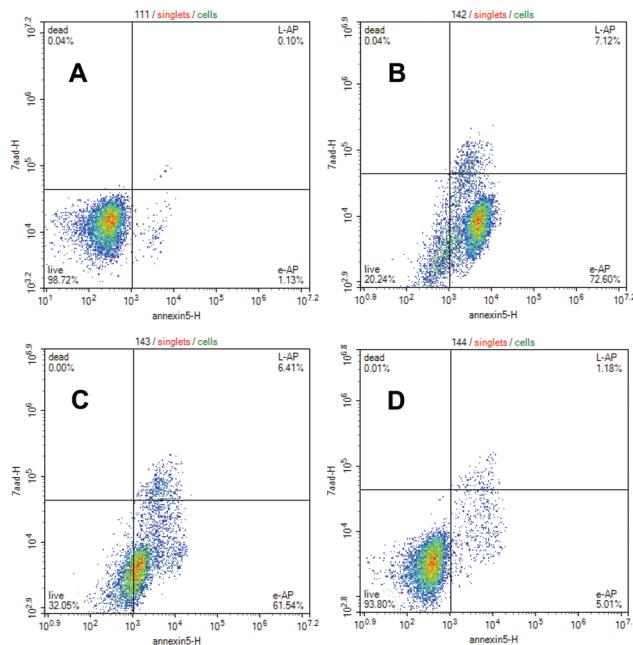


Fig. 2 Jurkat cells treated with different concentrations of lembayne B (**10**) were double-stained with annexine V/PI and analyzed by flow cytometry. (A) Control; (B) **10**, 2 μ M; (C) **10**, 1 μ M; (D) **10**, 0.5 μ M.

from the literature. Therefore, we performed the first *in vitro* tests of lembayne B for the antitumor activity on leukemia cells (Jurkat, HL-60 and K562). The tests included determination of IC_{50} , study of cell viability and effect on the cell cycle by means of flow cytometry.

The quantitative and qualitative analyses of cell viability, cell cycle, and apoptosis-inducing activity of lembayne B were performed using the Guava Nixin Reagent and Guava Cell Cycle (Millipore).

The *in vitro* cytotoxic activity of lembayne B against Jurkat, HL-60, and K562 human leukemia cells was studied using Guava ViaCount (Millipore). Lembayne B in concentrations of 0.5 to 4 μ M exhibited a clear-cut cytotoxic effect against all types of cancer cells we studied. However, the highest IC_{50} value was found for K562 cells (3 μ M), whereas for Jurkat and HL-60, the IC_{50} values were 2 μ M and 2.2 μ M, respectively.

The lembayne B-induced apoptosis in Jurkat, HL-60, and K562 cell cultures was estimated by detection of phosphatidylserine externalization on the plasmatic membrane after treatment of cell cultures with the test compound. It is noteworthy that the effect of lembayne on the induction of apoptosis is more pronounced in Jurkat cells than in other cells, which is consistent with high cytotoxicity of the compound against this cell line. As can be seen from Fig. 2, exposure of Jurkat tumor cell culture to lembayne B results in a considerable dose-dependent increase in the amount of apoptotic cells occurring at early stage of apoptosis. The highest percentage of early apoptosis (72.6%) is observed at 2 μ M concentration of the test compounds. In particular, as shown in Fig. 2, for Jurkat cells, both early and late stages of apoptosis are enhanced as compared with HL60 ($P \leq 0.0001$) or K562 ($P \leq 0.0001$).

According to cell cycle data determined by Guava Cell Cycle Reagent (Fig. 3), lembayne B is a potent inducer of hypodiploid cell population (sub-G1 phase) in all three cell lines after the appropriate treatment with the test compounds. No

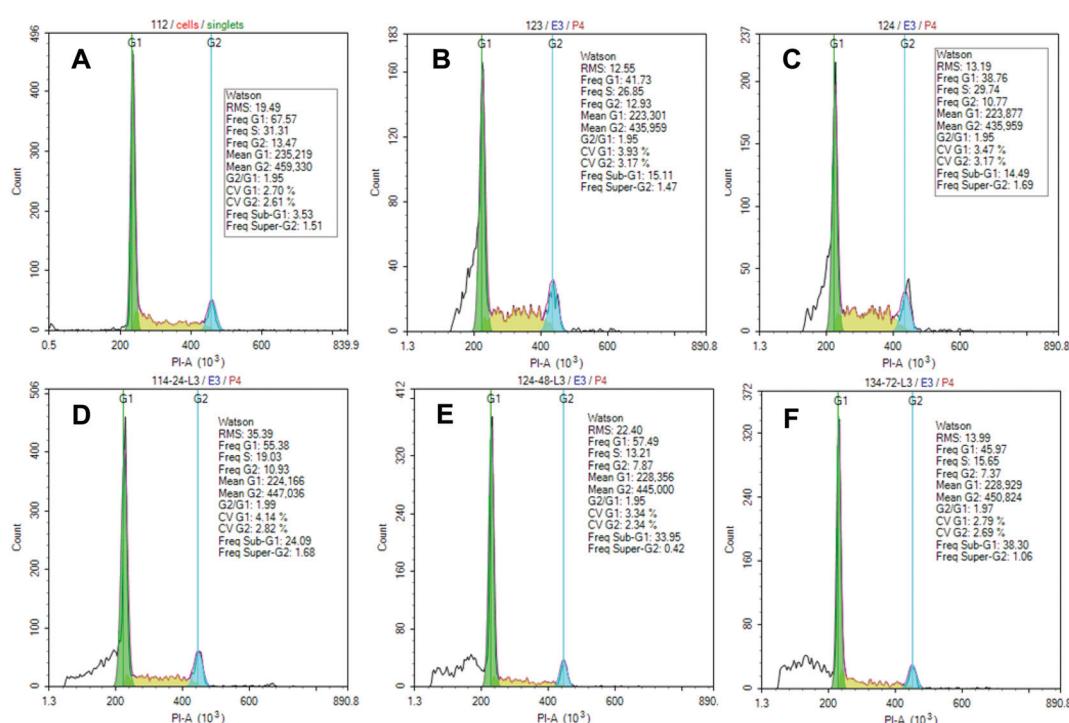


Fig. 3 Cell cycle phases for Jurkat cell line. Upper row: (A) control, (B) **10**, 2 μ L, (C) **10**, 1 μ L. Lower row: (**10**, 2 μ L): (D) 24 h, (E) 48 h, (F) 72 h.



significant differences were identified between cell-cycle distribution of cell subpopulations for lembehyne B-treated Jurkat, HL-60, and K562 cells.

Conclusions

Thus, an original stereoselective method for the synthesis of lembehyne B was developed for the first time. The key stage of the synthesis was based on the new reaction that we developed, namely, Ti-catalyzed cross-cyclomagnesiation of oxygenated aliphatic 1,2-dienes with Grignard reagents. We believe that this method bears huge synthetic potential for the preparation of stereochemically pure lembehynes and their analogues by varying the structure of the starting 1,2-dienes. By means of flow cytometry, it was shown for the first time that lembehyne B is a selective early apoptosis inducer for the Jurkat, HL-60, and K562 cell cultures. Currently, active research along this line is in progress in order to implement stereoselective methods for the synthesis of the whole range of natural lembehynes and their analogues for performing, in particular, full-scale pharmacological investigations of the biological activity and structure-activity relationships.

Experimental section

General methods

¹H and ¹³C NMR spectra were obtained using a Bruker Ascend 500 spectrometer in CDCl₃ operating at 500 MHz for ¹H and 125 MHz for ¹³C and Bruker AVANCE 400 spectrometer in CDCl₃ operating at 400 MHz for ¹H and 100 MHz for ¹³C. Mass spectra were obtained with a MALDI TOF/TOF spectrometer in a α -cyano-4-hydroxycinnamic acid matrix. Elemental analyses were measured on a 1106 Carlo Erba apparatus. Individuality and purity of the synthesized compounds were controlled using TLC on Sorbfil plates; anisic aldehyde in acetic acid was used as a developer. Column chromatography was carried out on Acrus silica gel (0.060–0.200 mm). Flow cytometry studies were obtained using a NovoCyteTM 2000 FlowCytometry System (ACEA). All solvents were dried (1,4-dioxane, tetrahydrofuran, diethyl ether over Na) and freshly distilled before use. All reactions were carried out under a dry argon atmosphere. Aliphatic and oxygenated 1,2-dienes have been synthesized according to procedures.^{7g,10,12,13}

Methods of synthesis and characterization data of all compounds

Synthesis of 2-(pentadeca-13,14-dien-1-yloxy)tetrahydro-2H-pyran (2a). Paraformaldehyde (2.6 g), copper iodide (3.4 g, 17.8 mmol), and dicyclohexylamine (14.0 mL, 70.4 mmol) were sequentially added to a solution of 2-(tetradec-13-yn-1-yloxy)tetrahydro-2H-pyran (10.0 g, 34.0 mmol) in anhydrous dioxane (120 mL). The resulting mixture was heated under reflux for 24 h. The addition of 2 M HCl (50 mL) and extraction with diethyl ether was followed by an extraction of the organic layer

with NaHCO₃, water, and brine and a drying with anhydrous MgSO₄. The solvent was evaporated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 30/1) to afford 2-(pentadeca-13,14-dien-1-yloxy)tetrahydro-2H-pyran (2a) (8.1 g, 78%) as colorless liquid.

2-(Pentadeca-13,14-dien-1-yloxy)tetrahydro-2H-pyran (2a).

Found (%): C, 77.69; H, 11.73. Calc. for C₂₀H₃₆O₂ (%): C, 77.87; H, 11.76. IR (film) ν_{max} 2928, 2851, 1463, 1379, 1354, 1301, 1248, 1108, 1068, 815, 722 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ : 1.24–1.42 (18H, m), 1.49–1.62 (6H, m), 1.71 (1H, m), 1.83 (1H, m), 1.99 (2H, m), 3.38 (1H, m), 3.49 (1H, m), 3.73 (1H, m), 3.87 (1H, m), 4.58 (1H, m), 4.64 (2H, m), 5.08 (1H, m). ¹³C NMR (100.62 MHz, CDCl₃) δ : 208.5 (C), 98.8 (CH), 90.0 (CH), 74.4 (CH₂), 67.6 (CH₂), 62.2 (CH₂), 30.8 (CH₂), 29.8 (CH₂), 29.6 (signals of 4C, CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.1 (CH₂), 29.1 (CH₂), 28.3 (CH₂), 26.2 (CH₂), 25.5 (CH₂), 19.7 (CH₂).

Cross-cyclomagnesiation of nonadeca-1,2-diene (1) and 2-(pentadeca-13,14-dien-1-yloxy)tetrahydro-2H-pyran (2a) with EtMgBr in the presence of Mg metal and Cp₂TiCl₂ catalyst

Diethyl ether (30 mL), nonadeca-1,2-diene (1) (1.27 g, 4.8 mmol), 2-(pentadeca-13,14-dien-1-yloxy)tetrahydro-2H-pyran (2a) (1.23 g, 4.0 mmol), EtMgBr (16.0 mmol) (as 1.5 M solution in Et₂O), Mg powder (0.29 g, 12.0 mmol) and Cp₂TiCl₂ (0.1 g, 0.4 mmol) were placed in a glass reactor with stirring under argon (\sim 0 °C). The reaction mixture was warmed-up to room temperature (20–22 °C) and stirred for 6 h. The reaction mixture was treated with a 5% solution of NH₄Cl in H₂O (20 mL) and extracted with diethyl ether (2 \times 100 mL). The combined organic phases were dried over MgSO₄, filtered, and the solvents were removed under reduced pressure. Silica gel column chromatography (hexane/EtOAc (35/1)) of the residue gave compound 5 (1.98 g, 88%) as a pale yellow oily liquid.

2-[*(13Z,17Z*)-Tetraonta-13,17-dien-1-yloxy]tetrahydro-2H-pyran (4). MS (MALDI-TOF), *m/z*: 574 [M]⁺. Found (%): C, 81.29; H, 12.94. Calc. for C₃₉H₇₄O₂ (%): C, 81.46; H, 12.97. IR (film) ν_{max} 2929, 2853, 1465, 1384, 1360, 1303, 1256, 1110, 1075, 815, 722 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ : 0.90 (3H, t, *J* = 6 Hz), 1.25–1.41 (46H, m), 1.52–1.63 (6H, m), 1.72–1.73 (1H, m), 1.84–1.86 (1H, m), 2.03–2.09 (8H, m), 3.36–3.42 (1H, m), 3.48–3.54 (1H, m), 3.72–3.77 (1H, m), 3.86–3.91 (1H, m), 4.58–4.61 (1H, m), 5.34–5.43 (4H, m). ¹³C NMR (100.62 MHz, CDCl₃) δ : 130.3 (2C, CH), 129.1 (2C, CH), 98.8 (CH), 67.7 (CH₂), 62.2 (CH₂), 31.9 (CH₂), 30.8 (CH₂), 29.8–29.3 (signals of 21C, CH₂), 27.4 (2C, CH₂), 27.3 (2C, CH₂), 26.3 (CH₂), 25.5 (CH₂), 22.7 (CH₂), 19.7 (CH₂), 14.1 (CH₃).

THP-deprotection of ether (4) was carried out with *p*-TsOH in CH₂Cl₂/MeOH using known method⁸

(13Z,17Z)-Tetraonta-13,17-dien-1-ol (5). Yield 77%. MS (MALDI-TOF), *m/z*: 490 [M]⁺. Found (%): C, 83.02; H, 13.57. Calc. for C₃₄H₆₆O (%): C, 83.19; H, 13.55. ¹H NMR (400 MHz, CDCl₃) δ : 0.90 (3H, t, *J* = 6 Hz), 1.25–1.42 (46H, m), 1.52–1.63 (3H, m), 2.03–2.09 (8H, m), 3.64–3.67 (2H, m), 5.35–5.45

(4H, m). ^{13}C NMR (100.62 MHz, CDCl_3) δ : 130.4 (2C, CH), 129.2 (2C, CH), 63.1 (CH_2), 32.8 (CH_2), 31.9 (CH_2), 29.8–29.3 (signals of 19C, CH_2), 27.4 (2C, CH_2), 27.2 (2C, CH_2), 25.7 (CH_2), 22.7 (CH_2), 19.7 (CH_2), 14.1 (CH_3).

The oxidation of the alcohol (5) with Dess–Martin periodinane was carried out according known procedure⁹

(13Z,17Z)-Tetraconta-13,17-dien-1-al (6). Yield 83%. MS (MALDI-TOF), m/z : 488 [M]⁺. Found (%): C, 83.39; H, 13.16. Calc. for $\text{C}_{34}\text{H}_{64}\text{O}$ (%): C, 83.53; H, 13.20. ^1H NMR (400 MHz, CDCl_3) δ : 0.91 (3H, t, CH_3 , J = 6 Hz), 1.23–1.48 (46H, m, CH_2), 1.59–1.66 (3H, m), 2.04–2.10 (8H, m, $=\text{CH}-\text{CH}_2$), 2.42–2.45 (2H, m, $\text{O}=\text{CH}-\text{CH}_2$), 5.39–5.40 (4H, m, $=\text{CH}$), 9.78–9.79 (1H, m, $\text{O}=\text{CH}$). ^{13}C NMR (100.62 MHz, CDCl_3) δ : 202.9 (CH), 130.4 (CH), 129.1 (CH), 43.9 (CH_2), 31.9 (CH_2), 29.2–29.7 (signals of 22C, CH_2), 27.4 (2C, CH_2), 27.3 (2C, CH_2), 22.7 (CH_2), 22.1 (CH_2), 14.1 (CH_3).

Cross-cyclomagnesiation of nonadeca-1,2-diene (1) and 2-tetradeca-12,13-dien-1-yl-1,3-dioxolane (2b) with EtMgBr in the presence of Mg metal and Cp_2TiCl_2 catalyst

Diethyl ether (30 mL), nonadeca-1,2-diene (1) (1.27 g, 4.8 mmol), 2-tetradeca-12,13-dien-1-yl-1,3-dioxolane (2b) (1.06 g, 4.0 mmol), EtMgBr (30.0 mmol) (as 1.5 M solution in Et_2O), Mg powder (0.48 g, 20.0 mmol) and Cp_2TiCl_2 (0.1 g, 0.4 mmol) were placed in a glass reactor with stirring under argon (~ 0 °C). The reaction mixture was warmed-up to room temperature (20–22 °C) and stirred for 6 h. The reaction mixture was treated with a 10% solution of HCl in H_2O (20 mL) and extracted with diethyl ether (2 × 100 mL). The combined organic phases were dried over MgSO_4 , filtered, and the solvents were removed under reduced pressure. Silica gel column chromatography (hexane/EtOAc (35/1)) of the residue gave dienal 6 (1.50 g, 77%) as a pale yellow oily liquid.

Procedure for preparation of alkyne (7)

To a solution of trimethylsilyl acetylene 0.58 g (6 mmol) in THF (10 ml) was added dropwise a solution of 4 ml $n\text{-BuLi}$ (1.5 M in hexane) at –40 °C. The solution was stirred for 1 h at –40 to 0 °C. Then the solution was added dropwise to THF solution of 1.5 g (3.08 mmol) dienal (7) at –10 °C. The reaction mixture was warmed-up to room temperature (20–22 °C) and stirred for 3 days. The reaction mixture was treated with a 5% solution of NH_4Cl in H_2O (20 mL) and extracted with diethyl ether (2 × 100 mL). The combined organic phases were dried over MgSO_4 , filtered, and the solvents were removed under reduced pressure. Silica gel column chromatography of the residue gave compound 7 (1.63 g, 90%) as a pale yellow oily liquid.

(15Z,19Z)-1-(Trimethylsilyl)hexatriaconta-15,19-dien-1-yn-3-ol (7). MS (MALDI-TOF), m/z : 587 [M]⁺. Found (%): C, 79.62; H, 12.66. Calc. for $\text{C}_{33}\text{H}_{74}\text{OSi}$ (%): C, 79.79; H, 12.70. ^1H NMR (400 MHz, CDCl_3) δ : 0.19 (9H, s, CH_3), 0.90 (3H, t, CH_3 , J = 6 Hz), 1.25–1.72 (49H, m, CH_2), 2.04–2.11 (8H, m, $=\text{CH}-\text{CH}_2$), 4.37 (1H, t, $\text{HO}-\text{CH}$, J = 5 Hz), 5.38–5.41 (4H, m, $=\text{CH}$). ^{13}C NMR (100.62 MHz, CDCl_3) δ : 130.4 (CH), 129.2 (CH),

106.9 (C), 89.3 (CH), 62.9 (CH), 37.7 (CH_2), 31.9 (CH_2), 29.2–29.8 (signals of 22C, CH_2), 27.4 (2C, CH_2), 27.3 (2C, CH_2), 25.1 (CH_2), 22.7 (CH_2), 14.1 (CH_3), –0.11 (3C, CH_3).

Procedure for preparation of *rac*-lembheyne B (8)

To a solution of alkyne (7) 1.17 g (2 mmol) in THF (10 ml) was added TBAF (1 M in THF, 1.2 equiv.) at 0 °C, then the solution was stirred for 4 h at room temperature. The reaction mixture was treated with saturated aq. NaCl and extracted with diethyl ether (2 × 50 mL). The combined organic phases were dried over MgSO_4 , filtered, and the solvents were removed under reduced pressure. Silica gel column chromatography of the residue gave compound 8 (1.07 g, 99%) as a colorless waxy solid.

The oxidation of the alcohol (8) with Dess–Martin periodinane was carried out according⁹

(15Z,19Z)-Hexaconta-15,19-dien-1-yl-3-one (9). Yield 86%. MS (MALDI-TOF), m/z : 512 [M]⁺. Found (%): C, 84.09; H, 12.55. Calc. for $\text{C}_{36}\text{H}_{64}\text{O}$ (%): C, 84.30; H, 12.58. ^1H NMR (400 MHz, CDCl_3) δ : 0.90 (3H, t, CH_3 , J = 6 Hz), 1.23–1.48 (44H, m, CH_2), 1.64–1.72 (2H, m, CH_2), 2.04–2.10 (8H, m, $=\text{CH}-\text{CH}_2$), 2.58–2.62 (2H, m, $\text{O}=\text{CH}-\text{CH}_2$), 3.22 (1H, s, CH), 5.39–5.42 (4H, m, $=\text{CH}$). ^{13}C NMR (100.62 MHz, CDCl_3) δ : 187.5 (C), 130.4 (CH), 130.3 (CH), 129.1 (2C, CH), 81.5 (C), 78.2 (CH), 45.5 (CH_2), 31.9 (CH_2), 29.3–29.7 (signals of 19C, CH_2), 28.9 (CH_2), 27.4 (2C, CH_2), 27.3 (2C, CH_2), 22.7 (CH_2), 23.8 (CH_2), 14.1 (CH_3).

The stereoselective reduction of ketone 9 with *B*-3-pinanyl-9-borabicyclo[3.3.1]nonane (Alpine-borane reagent) was carried out according procedure¹¹

The spectral data of compound 10 were identical to previously published for lembheyne B.^{3c}

Lembheyne B (10). Yield 49% (95% ee). $[\alpha]_{\text{D}}^{25} +0.43$ (*c* 0.3, CHCl_3). ^1H NMR (400 MHz, CDCl_3) δ : 0.90 (3H, t, CH_3 , J = 7 Hz), 1.23–1.54 (44H, m, CH_2), 1.71–1.75 (2H, m, CH_2), 2.04–2.10 (8H, m, $=\text{CH}-\text{CH}_2$), 4.39 (1H, td, J = 7.0, 2.0 Hz), 5.39–5.41 (4H, m, $=\text{CH}$). ^{13}C NMR (100.62 MHz, CDCl_3) δ : 130.4 (2C, CH), 129.2 (2C, CH), 85.0 (C), 72.8 (CH), 62.4 (CH), 37.7 (CH_2), 31.9 (CH_2), 29.3–29.7 (signals of 19C, CH_2), 29.2 (CH_2), 27.4 (2C, CH_2), 27.3 (2C, CH_2), 25.0 (CH_2), 22.7 (CH_2), 14.1 (CH_3).

Biology

Cell culture

Human leukemia cells Jurkat, HL-60, K562 were obtained from the HPA Culture Collections (UK). The cell lines were cultured in RPMI (Gibco BRL) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin solution at 37 °C in a humidified incubator under a 5% CO_2 atmosphere. Testing compounds was dissolved in DMSO and diluted with the tissue culture medium before use.



Viability and apoptosis

In vitro cytotoxicity was assessed using a standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) colorimetric assay.¹⁴ The IC₅₀ value was determined from plots of % viability against the dose of the compound added. The percentage of cell growth inhibition was calculated as 100 minus (mean cell culture optical density in the test/mean cell culture optical density in the control) multiplied by 100. The value obtained for the first three wells without addition of the test compound (control triplet), which was measured in parallel for each test compound, was taken to be 100%. The mean value and the error of mean were calculated separately for each concentration of the test compound. The results were used to plot the cell viability (%) *versus* the test compound concentration, and the dose inhibiting the cell viability by 50% (IC₅₀) and the standard error (SE) of IC₅₀ were calculated using the GraphPad Prism 7.0 software (GraphPad Inc., USA).

Apoptosis was determined by flow cytometric analysis of Annexin V and 7-aminoactinomycin D staining. Briefly, 200 µl of Guava Annexin reagent (Millipore, Bedford, MA, USA) was added to 5 × 10⁵ cells in 200 µl, and the cells were incubated with the reagent for 20 min at room temperature in the dark. At the end of incubation, the cells were analyzed on NovoCyteTM 2000 FlowCytometry System (ACEA).

Cell cycle analysis

Cell cycle was analyzed using the method of propidium iodide staining. Briefly, cells were plated in 24-well round bottom plates at a density 10 × 10⁵ cells per well, centrifuged at 450g for 5 minutes, and fixed with ice-cold 70% ethanol for 24 hour at 0 °C. Cells were then washed with PBS and incubated with 250 µl of Guava Cell Cycle Reagent (Millipore) for 30 minutes at room temperature in the dark. Samples were analyzed on NovoCyteTM 2000 FlowCytometry System (ACEA).

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