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## 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  $\text{Cp}_2\text{TiCl}_2$ . 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.

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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.<sup>1</sup> 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.<sup>2</sup>

Recently,<sup>3</sup> Kobayashi and co-workers isolated, from the Indonesian marine sponge *Haliclona* sp., micro quantities (10–15 mg kg<sup>-1</sup>) of lembehynes 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.<sup>4</sup> Hence, lembehynes 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 lembehynes hamper detailed investi-

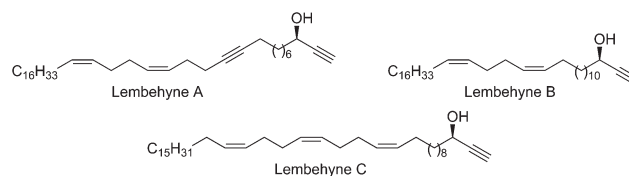


Fig. 1 Structures of lembehynes A–C.

gation of the properties and preclude taking full advantage of the biomedical potential of these compounds.<sup>5</sup>

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

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

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 1*Z*,5*Z*-dienes of a specified structure, giant macrocarbocycles, and natural 5*Z*,9*Z*-dienoic acids exhibiting antitumor activities.<sup>7</sup>

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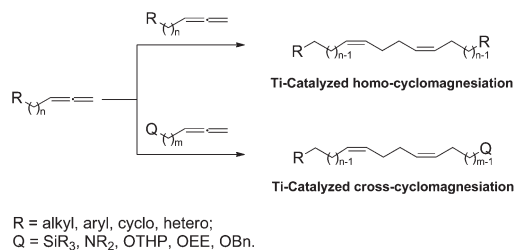
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Scheme 1 Ti-Catalyzed homo- and cross-cyclomagnesiation.

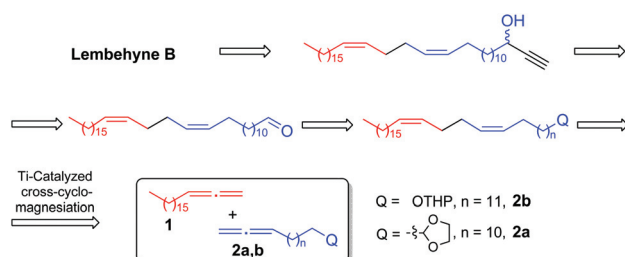
product accumulation and *in vitro* testing for the antitumor activity on Jurkat, HL-60, and K562 cell lines using flow cytometry.

## Results and discussion

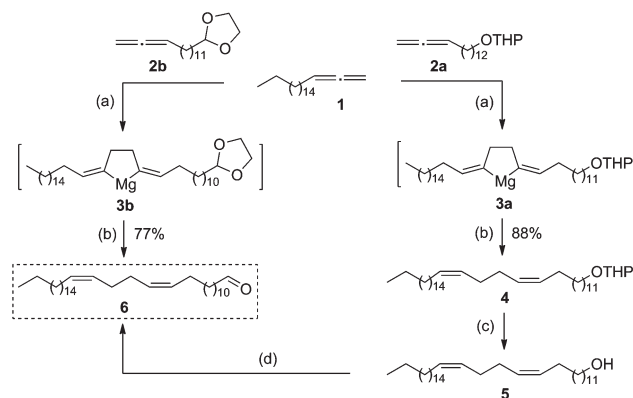
By retrosynthetic analysis of the lembhehne B structure, we identified the key monomer: (13*Z*,17*Z*)-tetraconta-13,17-dienal, which is accessible through cross-magnesiation of 1,2-nona-decadiene (**1**) with the tetrahydropyran ether of 13,14-penta-decadienol (**2a**) or 2-tetradeca-12,13-dien-1-yl-1,3-dioxolane (**2b**) (Scheme 2). The final steps are intended for the formation of the terminal propargyl moiety to obtain racemic lembhehne 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 Cp<sub>2</sub>TiCl<sub>2</sub> catalyst (10 mol%). The reaction proceeded *via* magnesacyclopentane **3a**, which was hydrolyzed to give (13*Z*,17*Z*)-tetraconta-13,17-dienol tetrahydropyran ether (**4**) in 88% yield (Scheme 3). Successive tetrahydropyran deprotection<sup>8</sup> and Dess–Martin periodinane oxidation of unsaturated alcohol **5**<sup>9</sup> yielded the target (13*Z*,17*Z*)-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 Cp<sub>2</sub>TiCl<sub>2</sub> (10 mol%) (**1** : **2b** : EtMgBr : Mg : [Ti] = 12 : 10 : 30 : 20 : 0.1, Et<sub>2</sub>O, 20–22 °C, 7 h) furnishes magnesacyclopentane **3b**, which is acid-hydrolyzed to afford (13*Z*,17*Z*)-tetraconta-13,17-dienal (**6**) in ~77% yield in one preparative step (Scheme 3).



Scheme 2 Retrosynthetic analysis of lembhehne B.



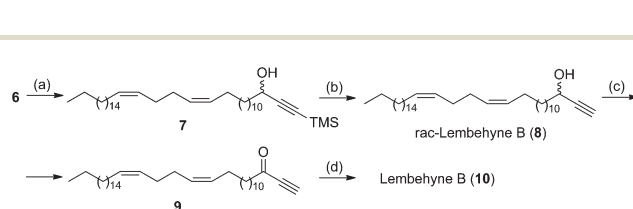
Scheme 3 Ti-Catalyzed cross-cyclomagnesiation of aliphatic and oxygenated 1,2-dienes in the synthesis of (13*Z*,17*Z*)-tetraconta-13,17-dienal (**6**). Reagents and condition: (a) EtMgBr, Mg, Cp<sub>2</sub>TiCl<sub>2</sub>, Et<sub>2</sub>O, rt; (b) H<sup>+</sup>; (c) *p*-TsOH, MeOH/CHCl<sub>3</sub>,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

Thus, the yield of (13*Z*,17*Z*)-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 trimethylsilylacetylide,<sup>10</sup> 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 lembhehne B (**8**) in nearly quantitative yield (Scheme 4).

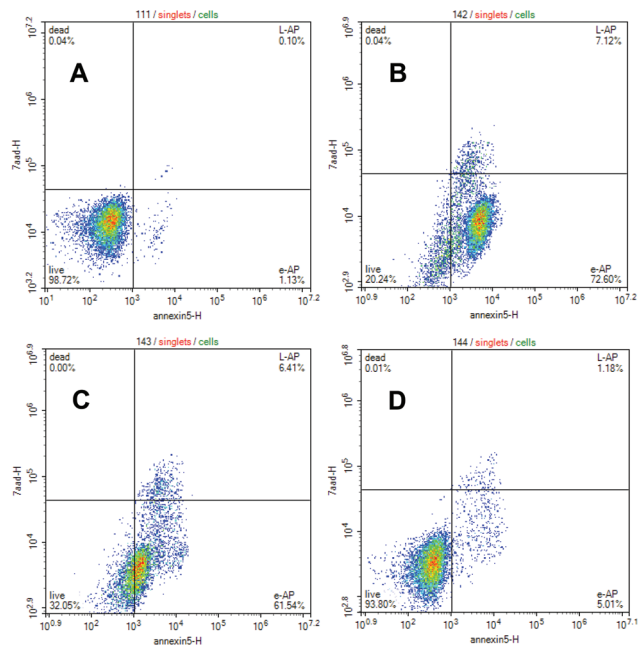
Using racemic lembhehne B, we synthesized its natural stereomer with the 3*R*-configuration of the hydroxy group at the C-3 carbon atom. First, Dess–Martin periodinane oxidation of alkynol **8** in CH<sub>2</sub>Cl<sub>2</sub> at room temperature gave (15*Z*,19*Z*)-hexaconta-15,19-dien-1-yl-3-one (**9**) 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,<sup>11</sup> prepared beforehand from (+)- $\alpha$ -pinene (98% ee) and 9-borabicyclo[3.3.1]nonane. This gave lembhehne 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.<sup>2</sup> Meanwhile, no data on the antitumor activity of natural lembhehynes are available



Scheme 4 Synthesis of lembhehne B. Reagents and condition: (a) lithium trimethylsilylacetylide, 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 lembhehne B (**10**) were double-stained with annexin V/PI and analyzed by flow cytometry. (A) Control; (B) **10**, 2  $\mu$ M; (C) **10**, 1  $\mu$ M; (D) **10**, 0.5  $\mu$ M.

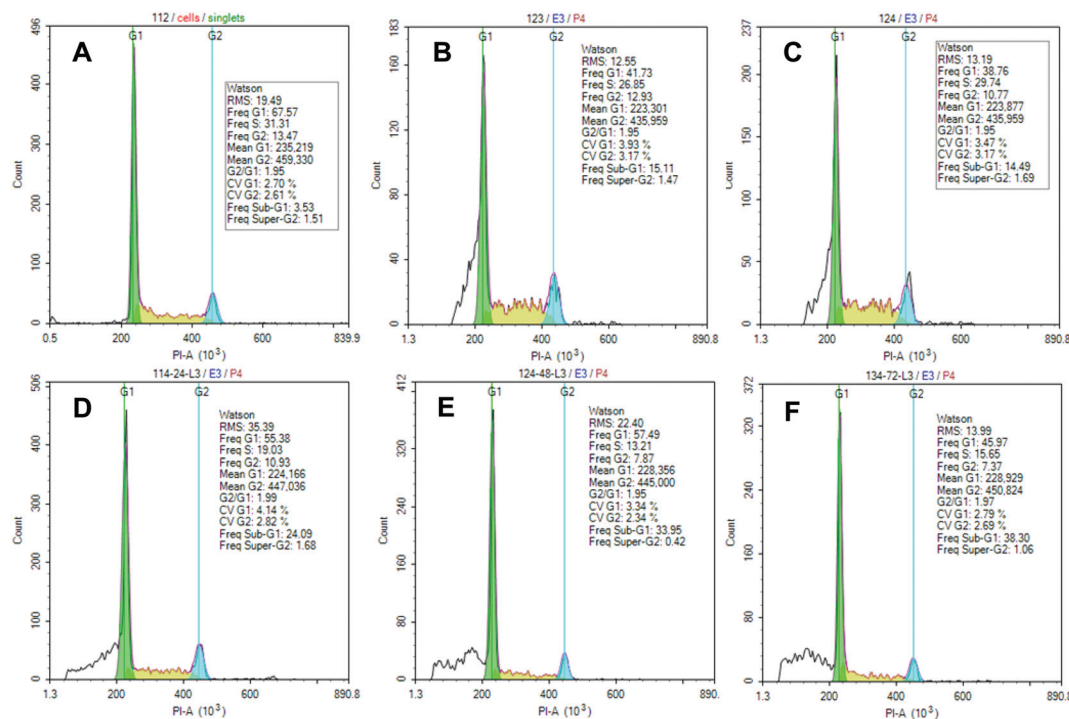
from the literature. Therefore, we performed the first *in vitro* tests of lembhehne B for the antitumor activity on leukemia cells (Jurkat, HL-60 and K562). The tests included determination of  $IC_{50}$ , study of cell viability and effect on the cell cycle by means of flow cytometry.

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

The *in vitro* cytotoxic activity of lembhehne B against Jurkat, HL-60, and K562 human leukemia cells was studied using Guava ViaCount (Millipore). Lembhehne B in concentrations of 0.5 to 4  $\mu$ 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  $\mu$ M), whereas for Jurkat and HL-60, the  $IC_{50}$  values were 2  $\mu$ M and 2.2  $\mu$ M, respectively.

The lembhehne 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 lembhehne 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 lembhehne 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  $\mu$ 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), lembhehne 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  $\mu$ L, (C) **10**, 1  $\mu$ L. Lower row: (**10**, 2  $\mu$ L): (D) 24 h, (E) 48 h, (F) 72 h.



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

## Conclusions

Thus, an original stereoselective method for the synthesis of lembeyne 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 lembeynes 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 lembeyne 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 lembeynes and their analogues for performing, in particular, full-scale pharmacological investigations of the biological activity and structure–activity relationships.

## Experimental section

### General methods

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were obtained using a Bruker Ascend 500 spectrometer in  $\text{CDCl}_3$  operating at 500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$  and Bruker AVANCE 400 spectrometer in  $\text{CDCl}_3$  operating at 400 MHz for  $^1\text{H}$  and 100 MHz for  $^{13}\text{C}$ . Mass spectra were obtained with a MALDI TOF/TOF spectrometer in a  $\alpha$ -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 NovoCyte™ 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 procedures.<sup>7g,10,12,13</sup>

### 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  $\text{NaHCO}_3$ , water, and brine and a drying with anhydrous  $\text{MgSO}_4$ . 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  $\text{C}_{20}\text{H}_{36}\text{O}_2$  (%): C, 77.87; H, 11.76. IR (film)  $\nu_{\text{max}}$  2928, 2851, 1463, 1379, 1354, 1301, 1248, 1108, 1068, 815, 722  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 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).  $^{13}\text{C}$  NMR (100.62 MHz,  $\text{CDCl}_3$ )  $\delta$ : 208.5 (C), 98.8 (CH), 90.0 (CH), 74.4 ( $\text{CH}_2$ ), 67.6 ( $\text{CH}_2$ ), 62.2 ( $\text{CH}_2$ ), 30.8 ( $\text{CH}_2$ ), 29.8 ( $\text{CH}_2$ ), 29.6 (signals of 4C,  $\text{CH}_2$ ), 29.5 ( $\text{CH}_2$ ), 29.4 ( $\text{CH}_2$ ), 29.1 ( $\text{CH}_2$ ), 29.1 ( $\text{CH}_2$ ), 28.3 ( $\text{CH}_2$ ), 26.2 ( $\text{CH}_2$ ), 25.5 ( $\text{CH}_2$ ), 19.7 ( $\text{CH}_2$ ).

### 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 $\text{Cp}_2\text{TiCl}_2$ 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  $\text{Et}_2\text{O}$ ), Mg powder (0.29 g, 12.0 mmol) and  $\text{Cp}_2\text{TiCl}_2$  (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  $\text{NH}_4\text{Cl}$  in  $\text{H}_2\text{O}$  (20 mL) and extracted with diethyl ether ( $2 \times 100$  mL). The combined organic phases were dried over  $\text{MgSO}_4$ , filtered, and the solvents were removed under reduced pressure. Silica gel column chromatography (hexane/ $\text{EtOAc}$  (35/1)) of the residue gave compound **5** (1.98 g, 88%) as a pale yellow oily liquid.

**2-[(13Z,17Z)-Tetraconta-13,17-dien-1-yloxy]tetrahydro-2H-pyran (4).** MS (MALDI-TOF),  $m/z$ : 574  $[\text{M}]^+$ . Found (%): C, 81.29; H, 12.94. Calc. for  $\text{C}_{39}\text{H}_{74}\text{O}_2$  (%): C, 81.46; H, 12.97. IR (film)  $\nu_{\text{max}}$  2929, 2853, 1465, 1384, 1360, 1303, 1256, 1110, 1075, 815, 722  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 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).  $^{13}\text{C}$  NMR (100.62 MHz,  $\text{CDCl}_3$ )  $\delta$ : 130.3 (2C, CH), 129.1 (2C, CH), 98.8 (CH), 67.7 ( $\text{CH}_2$ ), 62.2 ( $\text{CH}_2$ ), 31.9 ( $\text{CH}_2$ ), 30.8 ( $\text{CH}_2$ ), 29.8–29.3 (signals of 21C,  $\text{CH}_2$ ), 27.4 (2C,  $\text{CH}_2$ ), 27.3 (2C,  $\text{CH}_2$ ), 26.3 ( $\text{CH}_2$ ), 25.5 ( $\text{CH}_2$ ), 22.7 ( $\text{CH}_2$ ), 19.7 ( $\text{CH}_2$ ), 14.1 ( $\text{CH}_3$ ).

### THP-deprotection of ether (4) was carried out with *p*-TsOH in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ using known method<sup>8</sup>

**(13Z,17Z)-Tetraconta-13,17-dien-1-ol (5).** Yield 77%. MS (MALDI-TOF),  $m/z$ : 490  $[\text{M}]^+$ . Found (%): C, 83.02; H, 13.57. Calc. for  $\text{C}_{34}\text{H}_{66}\text{O}$  (%): C, 83.19; H, 13.55.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 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).  $^{13}\text{C}$  NMR (100.62 MHz,  $\text{CDCl}_3$ )  $\delta$ : 130.4 (2C, CH), 129.2 (2C, CH), 63.1 ( $\text{CH}_2$ ), 32.8 ( $\text{CH}_2$ ), 31.9 ( $\text{CH}_2$ ), 29.8–29.3 (signals of 19C,  $\text{CH}_2$ ), 27.4 (2C,  $\text{CH}_2$ ), 27.2 (2C,  $\text{CH}_2$ ), 25.7 ( $\text{CH}_2$ ), 22.7 ( $\text{CH}_2$ ), 19.7 ( $\text{CH}_2$ ), 14.1 ( $\text{CH}_3$ ).

#### The oxidation of the alcohol (5) with Dess–Martin periodinane was carried out according known procedure<sup>9</sup>

(13Z,17Z)-Tetraconta-13,17-dien-1-ol (6). Yield 83%. MS (MALDI-TOF),  $m/z$ : 488  $[\text{M}]^+$ . Found (%): C, 83.39; H, 13.16. Calc. for  $\text{C}_{34}\text{H}_{64}\text{O}$  (%): C, 83.53; H, 13.20.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.91 (3H, t,  $\text{CH}_3$ ,  $J = 6$  Hz), 1.23–1.48 (46H, m,  $\text{CH}_2$ ), 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,  $\text{O}=\text{CH}$ ).  $^{13}\text{C}$  NMR (100.62 MHz,  $\text{CDCl}_3$ )  $\delta$ : 202.9 (CH), 130.4 (CH), 129.1 (CH), 43.9 ( $\text{CH}_2$ ), 31.9 ( $\text{CH}_2$ ), 29.2–29.7 (signals of 22C,  $\text{CH}_2$ ), 27.4 (2C,  $\text{CH}_2$ ), 27.3 (2C,  $\text{CH}_2$ ), 22.7 ( $\text{CH}_2$ ), 22.1 ( $\text{CH}_2$ ), 14.1 ( $\text{CH}_3$ ).

#### 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 $\text{Cp}_2\text{TiCl}_2$ 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  $\text{Et}_2\text{O}$ ), Mg powder (0.48 g, 20.0 mmol) and  $\text{Cp}_2\text{TiCl}_2$  (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 10% solution of HCl in  $\text{H}_2\text{O}$  (20 mL) and extracted with diethyl ether ( $2 \times 100$  mL). The combined organic phases were dried over  $\text{MgSO}_4$ , filtered, and the solvents were removed under reduced pressure. Silica gel column chromatography (hexane/ $\text{EtOAc}$  (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  $n$ -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  $\text{NH}_4\text{Cl}$  in  $\text{H}_2\text{O}$  (20 mL) and extracted with diethyl ether ( $2 \times 100$  mL). The combined organic phases were dried over  $\text{MgSO}_4$ , 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  $[\text{M}]^+$ . Found (%): C, 79.62; H, 12.66. Calc. for  $\text{C}_{39}\text{H}_{74}\text{OSi}$  (%): C, 79.79; H, 12.70.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.19 (9H, s,  $\text{CH}_3$ ), 0.90 (3H, t,  $\text{CH}_3$ ,  $J = 6$  Hz), 1.25–1.72 (49H, m,  $\text{CH}_2$ ), 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,  $=\text{CH}$ ).  $^{13}\text{C}$  NMR (100.62 MHz,  $\text{CDCl}_3$ )  $\delta$ : 130.4 (CH), 129.2 (CH),

106.9 (C), 89.3 (CH), 62.9 (CH), 37.7 ( $\text{CH}_2$ ), 31.9 ( $\text{CH}_2$ ), 29.2–29.8 (signals of 22C,  $\text{CH}_2$ ), 27.4 (2C,  $\text{CH}_2$ ), 27.3 (2C,  $\text{CH}_2$ ), 25.1 ( $\text{CH}_2$ ), 22.7 ( $\text{CH}_2$ ), 14.1 ( $\text{CH}_3$ ),  $-0.11$  (3C,  $\text{CH}_3$ ).

#### Procedure for preparation of *rac*-lembehynes 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 ( $2 \times 50$  mL). The combined organic phases were dried over  $\text{MgSO}_4$ , 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 according<sup>9</sup>

(15Z,19Z)-Hexaconta-15,19-dien-1-yl-3-one (9). Yield 86%. MS (MALDI-TOF),  $m/z$ : 512  $[\text{M}]^+$ . Found (%): C, 84.09; H, 12.55. Calc. for  $\text{C}_{36}\text{H}_{64}\text{O}$  (%): C, 84.30; H, 12.58.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.90 (3H, t,  $\text{CH}_3$ ,  $J = 6$  Hz), 1.23–1.48 (44H, m,  $\text{CH}_2$ ), 1.64–1.72 (2H, m,  $\text{CH}_2$ ), 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,  $\text{CH}$ ), 5.39–5.42 (4H, m,  $=\text{CH}$ ).  $^{13}\text{C}$  NMR (100.62 MHz,  $\text{CDCl}_3$ )  $\delta$ : 187.5 (C), 130.4 (CH), 130.3 (CH), 129.1 (2C, CH), 81.5 (C), 78.2 (CH), 45.5 ( $\text{CH}_2$ ), 31.9 ( $\text{CH}_2$ ), 29.3–29.7 (signals of 19C,  $\text{CH}_2$ ), 28.9 ( $\text{CH}_2$ ), 27.4 (2C,  $\text{CH}_2$ ), 27.3 (2C,  $\text{CH}_2$ ), 22.7 ( $\text{CH}_2$ ), 23.8 ( $\text{CH}_2$ ), 14.1 ( $\text{CH}_3$ ).

#### The stereoselective reduction of ketone 9 with *B*-3-pinanyl-9-borabicyclo[3.3.1]nonane (Alpine-borane reagent) was carried out according procedure<sup>11</sup>

The spectral data of compound 10 were identical to previously published for lembehynes B.<sup>3c</sup>

Lembehynes B (10). Yield 49% (95% ee).  $[\alpha]_D^{25} +0.43$  ( $c$  0.3,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.90 (3H, t,  $\text{CH}_3$ ,  $J = 7$  Hz), 1.23–1.54 (44H, m,  $\text{CH}_2$ ), 1.71–1.75 (2H, m,  $\text{CH}_2$ ), 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,  $=\text{CH}$ ).  $^{13}\text{C}$  NMR (100.62 MHz,  $\text{CDCl}_3$ )  $\delta$ : 130.4 (2C, CH), 129.2 (2C, CH), 85.0 (C), 72.8 (CH), 62.4 (CH), 37.7 ( $\text{CH}_2$ ), 31.9 ( $\text{CH}_2$ ), 29.3–29.7 (signals of 19C,  $\text{CH}_2$ ), 29.2 ( $\text{CH}_2$ ), 27.4 (2C,  $\text{CH}_2$ ), 27.3 (2C,  $\text{CH}_2$ ), 25.0 ( $\text{CH}_2$ ), 22.7 ( $\text{CH}_2$ ), 14.1 ( $\text{CH}_3$ ).

## 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%  $\text{CO}_2$  atmosphere. Testing compounds were 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-2H-tetrazolium bromide) colorimetric assay.<sup>14</sup> The IC<sub>50</sub> 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% (IC<sub>50</sub>) and the standard error (SE) of IC<sub>50</sub> 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 Nexin reagent (Millipore, Bedford, MA, USA) was added to 5 × 10<sup>5</sup> 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 NovoCyte™ 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 × 10<sup>5</sup> 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 NovoCyte™ 2000 FlowCytometry System (ACEA).

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