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Structure–activity relationship of neuritogenic spongean acetylene alcohols, lembehynes

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Abstract—Two new long-chain acetylene alcohols named lembehynes B (**2**) and C (**3**) were isolated from an Indonesian marine sponge of *Haliclona* sp. Lembehynes B (**2**) and C (**3**), which have different types of long carbon-chain parts compared with that of lembehyne A (**1**), also exhibited neuritogenic activity against a neuroblastoma cell line, Neuro 2A. For structure–activity relationship study of lembehynes, analogue-I (**4**), analogue-II (**5**) and analogue-III (**6**), which have different types of long carbon-chain parts, were synthesized from suitable fatty acids. As a result of neurite outgrowth assay for these related compounds, it was revealed that the carbon-chain length is important for neuritogenic activity, while the unsaturated bonds in the long-chain part are not. On the other hand, analogue-IV (**7**) with 3*S* configuration showed much weaker activity than analogue-III (**6**) with 3*R* configuration and the same type of long carbon-chain part. This indicates the importance of the stereochemistry of the hydroxyl group at C-3 in lembehynes for neuritogenic activity.

1. Introduction

During neuronal development, precursor cells differentiate to functional matured cells under the control of various protein factors including neurotrophins. The differentiation program in neuroblastoma, which accounts for 15% of cancer deaths in children, is thought to be disrupted. Therefore, differentiation inducers of cells are expected to be new candidates for chemotherapy of various neuronal diseases. In fact, clinical trials are in progress to determine the efficacy of retinoids, which induce differentiation against various neuroblastoma cell lines, on differentiation therapy of cancers that resist surgery.[1,2]

In the course of our study of bioactive substances from marine organisms, we found a neuritogenic long-chain polyketide having a terminal 1-yn-3-ol moiety, lembehyne A (**1**), from an Indonesian marine sponge of *Haliclona* sp.[3] In our subsequent investigation, **1** was revealed to induce neuronal differentiation of a murine neuroblastoma cell line, Neuro 2A, not only morphologically but also functionally. Furthermore, the neuronal differentiation accompanied with G1 arrest of the cell cycle and the terminal 1-yn-3-ol moiety and the unsaturated long alkyl chain in **1** were required for the activity.[4] However, the importance of the 3*R* configuration at the carbinol carbon and the optimal length of the carbon-chain for neuronal differentiation-inducing activity were still unclear. Recently, we isolated analogous acetylene alcohols, lembehynes B (**2**) and C (**3**), which had different types of long carbon-chain parts compared with that of **1**. Furthermore, we synthesized four analogues of **1**, in which the stereostructure at C-3 or the long carbon-chain part in **1** was converted. In this paper, we describe the structure elucidation of **2** and **3**, the syntheses of the analogues, and the structure–activity relationship study using these related compounds.

2. Results and discussion

The MeOH extract of an Indonesian marine sponge of *Haliclona* sp. (dried, 100 g) was partitioned into an EtOAc–water mixture. The EtOAc-soluble portion was further partitioned into an *n*-hexane–90% aq. MeOH mixture. The *n*-hexane-soluble portion was fractionated by SiO_2 column chromatography and HPLC (ODS, $CHCl_3$/MeOH, then phenylated SiO_2, MeOH/H_2O) to furnish lembehynes B (**2**) and C (**3**).

The positive FAB MS of **2** gave a quasi-molecular ion [$(M+Na)^+$] peak at *m/z* 537 and the molecular formula of **2** was determined as $C_{36}H_{66}O$ by HR-positive FAB MS and NMR analysis. The IR and NMR spectra of **2** indicated the presence of a long alkyl chain (large signal at δ_H 1.30–1.26), a hydroxyl group (3312 cm^{-1}), two Z olefins (total 4H at δ_H 5.39–5.37; 4 carbon signals at δ_C 130.4–129.1 and 4 carbon signals at δ_C 27.4–27.3), and a terminal acetylenic

Keywords: neuronal differentiation; lembehynes; Neuro 2A; structure–activity relationship.

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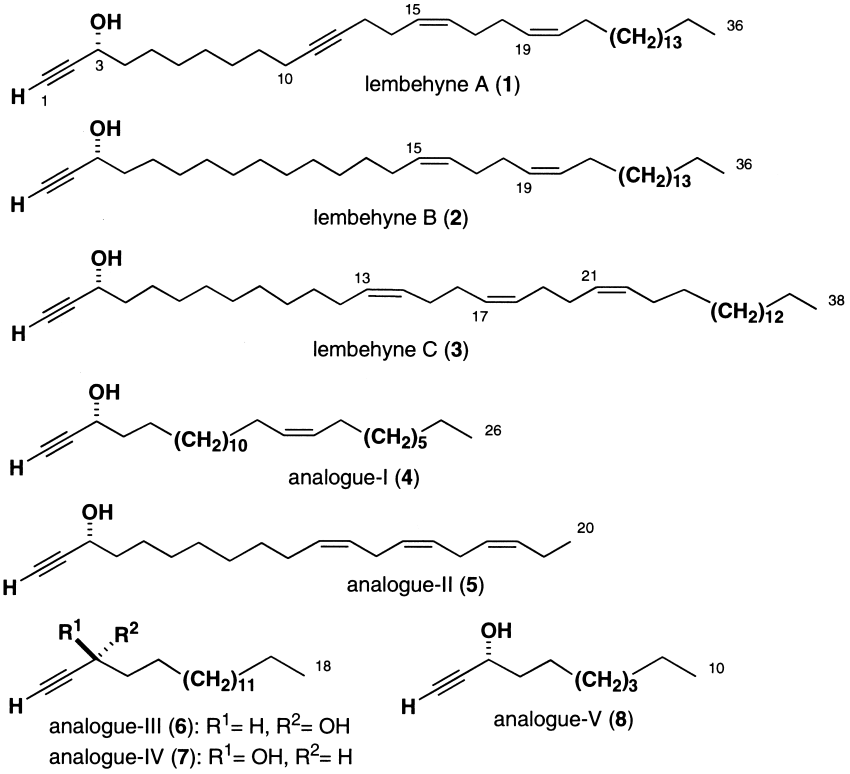


Chart 1.

group (δ_H 2.47, δ_C 85.0 and 72.8), which were similar to those of lembehyne A (**1**). The notable difference in the NMR spectra between **1** and **2** was the lack of the signals of the acetylenic carbon located in the middle part of **1**. The

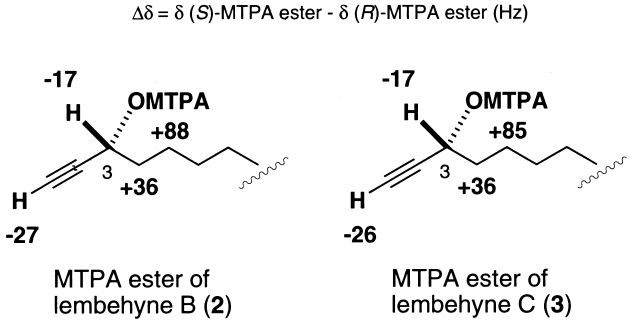


Figure 1. Application of the modified Mosher method.

2D NMR analysis of **2** allowed us to deduce the presence of the 1-yn-3-ol moiety and two double bonds combined by the ethylene unit in **2**. Ozonolysis of **2** followed by $NaBH_4$ treatment gave 1-heptadecanol, which was identified with an authentic sample by GC–MS. From this evidence, the plane structure of lembehyne B (**2**) was determined to be a tetrahydro analogue of lembehyne A (**1**).

The positive FAB MS of lembehyne C (**3**) gave a quasi-molecular ion [$(M+Na)^+$] peak at *m/z* 563, and the molecular formula of **3** was determined as $C_{38}H_{68}O$ by HR-positive FAB MS. The ^1H and ^{13}C NMR spectra of **3** were superimposable to those of **2** except for one more *Z* olefinic signal (total 6H at δ_H 5.38–5.34; 6 carbon signals at δ_C 130.4–129.1 and 6 carbon signals at δ_C 27.5–27.3). Each *Z* olefin has been shown to be combined by an ethylene unit by 2D NMR analysis. Ozonolysis of **3** followed by $NaBH_4$ treatment also gave 1-heptadecanol. From this evidence,

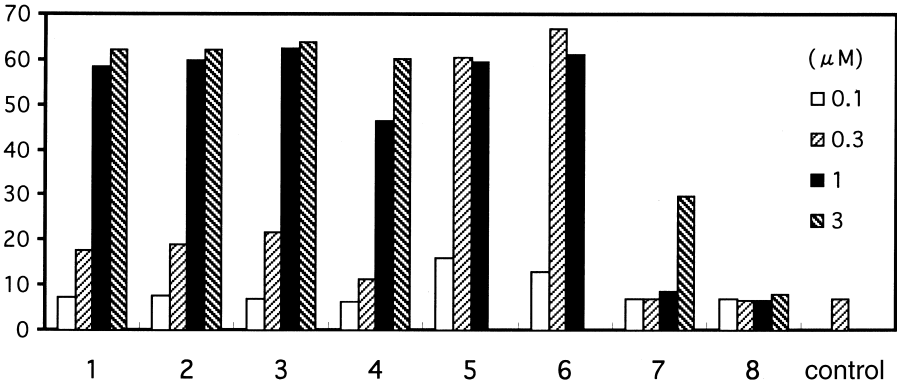
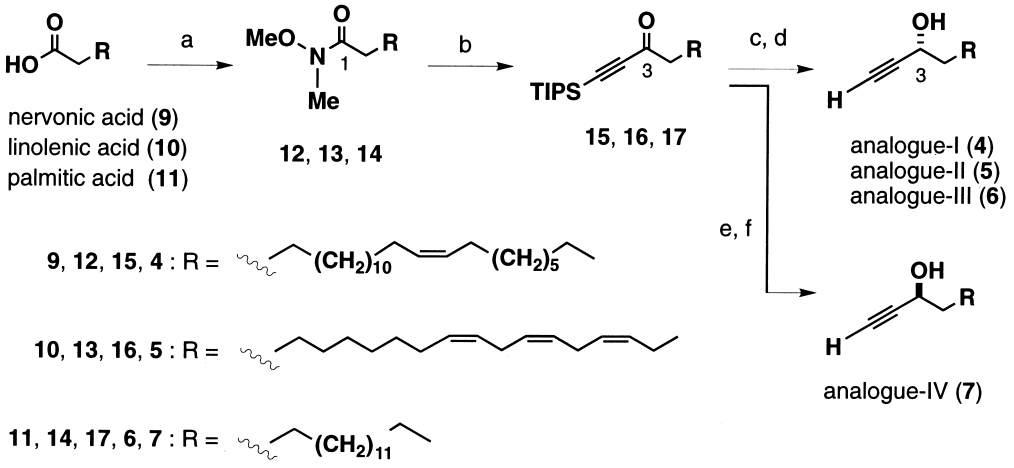


Figure 2. Quantification of neurites induced by lembehynes A (**1**), B (**2**), and C (**3**) and their analogues (**4**–**8**).



Scheme 1. *Reagents*: (a) MeONHMe·HCl, EDCI, DMAP, CH_2Cl_2, 90–94%, (b) Li (TIPS)acetylide, THF, 61–70%, (c) (*R*)-oxazaborolidine, BH_3–Me_2S, THF, (d) TBAF, THF, 63–71% (97–99% ee), two steps, (e) (*S*)-oxazaborolidine, BH_3–Me_2S, THF, (f) TBAF, THF, 79% (95% ee), two steps.

the plane structure of **3** was determined to be as shown in Chart 1. Furthermore, the absolute configuration at C-3 of both **2** and **3** was determined as *R* by application of the modified Mosher method[5] (Fig. 1). Lembehynes B (**2**) and C (**3**) showed neuritogenic activity against Neuro 2A at the same concentration as **1** (Fig. 2).

Next, we synthesized three analogues, which have different types of long carbon-chain parts compared with that of **1**, from various fatty acids (Scheme 1). Each fatty acid (**9**, **10** or **11**) was treated with *N*,*O*-dimethylhydroxylamine hydrochloride to furnish a Weinreb amide[6] (**12**, **13** or **14**) in 90–94% yields. Nucleophilic substitution of **12**, **13** or **14** with lithium (triisopropylsilyl (TIPS))acetylide provided an alkyne **15**, **16** or **17**, respectively, in 61–70% yield. Then, asymmetric reduction of **15**, **16** or **17** using (*R*)-oxazaborolidine[7,8] followed by deprotection of the TMS group provided analogue-I (**4**), analogue-II (**5**), and analogue-III (**6**) in 63–71% yield with high enantiomeric selectivity (97–99% ee), respectively. Among these analogues, analogue-II (**5**) having C20 chain length and analogue-III (**6**) having C18 chain length showed stronger neuritogenic activities against Neuro 2A cells than **1** at 0.3 μM concentration (Fig. 2). The neuritogenic activity of analogue-I (**4**) having C26 chain length was a little weaker than that of **1**. In the preceding study,[4,9] analogue-V (**8**) having C10 chain length was shown to be completely inactive. These results indicate that the carbon-chain length is important for neuritogenic activity of lembehynes, while the unsaturated bonds in the long-chain part are not. On the other hand, analogue-IV (**7**) with 3*S* configuration and C18 chain length was similarly synthesized from **17** using (*S*)-oxazaborolidine. Analogue-IV (**7**) showed much weaker activity than analogue-III (**6**) with 3*R* configuration and the same type of the long carbon-chain part. This indicates the importance of the stereochemistry of the hydroxyl group at C-3 in lembehynes for neuritogenic activity. Analogue-II (**5**) and analogue-III (**6**), which showed stronger neuritogenic activity than **1** and were simply synthesized from linolenic acid or palmitic acid, may be useful tools for analyzing the action mechanism involved in neuronal differentiation.

3. Experimental

3.1. Isolation of lembehynes B (2) and C (3) from a marine sponge of *Haliclona* sp.

The titled sponge (100 g, dry weight) collected in July, 1999 at Lembeh Island, Bitung, Indonesia, was extracted with MeOH (1 L) at room temperature three times. The residue obtained by evaporation of the solvent under reduced pressure was partitioned into an EtOAc–water mixture (1:1), and the EtOAc layer was evaporated to give the EtOAc-soluble portion (7 g). The EtOAc-soluble portion was further partitioned into an *n*-hexane–90% aq. MeOH mixture (1:1). The *n*-hexane layer was evaporated to give the *n*-hexane-soluble portion (2.8 g). The *n*-hexane-soluble portion (361 mg) was purified by SiO_2 column chromatography (eluted with *n*-hexane–EtOAc=8:1→3:1→EtOAc→MeOH) to give seven fractions (Fr. A–Fr. G). Next, the active Fr. C (68 mg) was purified by HPLC (Cosmosil $5C_{18}$-AR, 10ϕ×250 mm, $CHCl_3$–MeOH=1:4) to give a mixture of lembehynes B (**2**) and C (**3**). Finally, the mixture was separated by phenylated SiO_2 HPLC column (Cosmosil 5Ph, 10ϕ×250 mm, MeOH–H_2O=91:9) to isolate **2** (3 mg, 0.8% from the *n*-hexane ext.) and **3** (2.5 mg, 0.7% from the *n*-hexane ext.).

3.1.1. Lembehyne B (2). Colorless powder, $[\alpha]_D$=+0.4° (*c*=0.20, $CHCl_3$, 25°C). IR ν_{max} (KBr): 3312 cm^{-1}. FAB MS (nitrobenzyl-alcohol): *m*/*z* 537 $(M+Na)^+$. HR-FAB MS: *m*/*z* 537.5011: calcd for $C_{36}H_{66}ONa$. Found: *m*/*z* 537.4984. ^1H NMR (600 MHz, $CDCl_3$, δ): 5.39–5.37 (4H, m, H-15, 16, 19, 20), 4.38 (1H, td, *J*=6.9, 2.2 Hz, H-3), 2.47 (1H, d, *J*=2.2 Hz, H-1), 2.09 (4H, m, H-17, 18), 2.03 (4H, m, H-14, 21), 1.72 (2H, m, H-4), 1.47 (2H, m, H-5), 1.35–1.26 (ca. 44H, m), 0.89 (3H, t, *J*=6.9 Hz, H-36). ^{13}C NMR (150 MHz, $CDCl_3$, δ): 130.4–129.1 (4 signals for C-15, 16, 19, 20), 85.0 (C-2), 72.8 (C-1), 62.4 (C-3), 37.7 (C-4), 31.9 (C-34), 29.7–29.2 (C-6–13, 22–33), 27.4 (C-17, 18), 27.3 (C-14, 21), 25.0 (C-5), 22.7 (C-35), 14.1 (C-36).

3.1.2. Lembehyne C (3). Colorless powder, $[\alpha]_D$=+0.7° (*c*=0.21, $CHCl_3$, 25°C). IR ν_{max} (KBr): 3414 cm^{-1}. FAB

MS (nitrobenzyl-alcohol): *m/z* 563 $(M+Na)^+$. HR-FAB MS: *m/z* 563.5168: calcd for $C_{38}H_{68}ONa$. Found: *m/z* 563.5181. ^1H NMR (600 MHz, $CDCl_3$, δ): 5.40–5.38 (6H, m, H-13, 14, 17, 18, 21, 22), 4.38 (1H, td, *J*=6.9, 2.2 Hz, H-3), 2.47 (1H, d, *J*=2.2 Hz, H-1), 2.09 (8H, m, H-15, 16, 19, 20), 2.03 (4H, m, H-12, 23), 1.72 (2H, m, H-4), 1.47 (2H, m, H-5), 1.30–1.26 (ca. 40H, m), 0.89 (3H, t, *J*=6.9 Hz, H-38). ^{13}C NMR (150 MHz, $CDCl_3$, δ): 130.4–129.1 (6 signals for C-13, 14, 17, 18, 21, 22), 85.0 (C-2), 72.8 (C-1), 62.4 (C-3), 37.7 (C-4), 31.9 (C-36), 29.7–29.2 (C-6–11, 24–35), 27.5–27.3 (C-12, 15, 16, 19, 20, 23), 25.0 (C-5), 22.7 (C-37), 14.1 (C-38).

3.2. Preparation of (*S*)- or (*R*)-MTPA ester of lembehynes B (2) and C (3)

A CH_2Cl_2 (0.25 mL) solution of **2** or **3** (0.5 mg) was treated with (*S*)-(−)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid (MTPA) (1.2 mg), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (1.0 mg), and *N*,*N*-dimethylaminopyridine (DMAP) (0.4 mg). The reaction mixture was stirred at room temperature (25°C) for 1 h under an Ar atmosphere. The reaction was quenched by saturated aq. NH_4Cl and the whole was extracted with EtOAc. The EtOAc phase was washed by 5% HCl, saturated aq. $NaHCO_3$, and saturated aq. NaCl, then dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by SiO_2 HPLC (Cosmosil 5SL, 10ϕ×250 mm, *n*-hexane–EtOAc=19:1) to afford the (*S*)-MTPA ester **2a** (0.4 mg) or **3a** (0.5 mg). A CH_2Cl_2 (0.25 mL) solution of **2** or **3** (0.5 mg) was similarly treated with (*R*)-(+)-MTPA (1.2 mg), EDCI (1.0 mg), and DMAP (0.4 mg) to afford the (*R*)-MTPA ester **2b** (0.5 mg) or **3b** (0.3 mg), respectively.

3.2.1. (*S*)-MTPA ester 2a. Colorless powder, FAB MS: *m/z* 753 $(M+Na)^+$. HR-FAB MS: *m/z* 753.5410: calcd for $C_{46}H_{73}O_3F_3Na$. Found: 753.5388. ^1H NMR (500 MHz, $CDCl_3$, δ): 7.53 (2H, m, ArH), 7.42–7.39 (3H, m, ArH), 5.51 (1H, td, *J*=6.6, 1.9 Hz, H-3), 5.39–5.37 (4H, m, H-15, 16, 19, 20), 3.55 (3H, s, OMe), 2.49 (1H, d, *J*=1.9 Hz, H-1), 2.09 (4H, m, H-17, 18), 2.03 (4H, m, H-14, 21), 1.86 (2H, m, H-4), 1.45 (2H, m, H-5), 0.88 (3H, t, *J*=6.9 Hz, H-36).

3.2.2. (*S*)-MTPA ester 3a. Colorless powder, FAB MS: *m/z* 779 $(M+Na)^+$. HR-FAB MS: *m/z* 779.5566: calcd for $C_{48}H_{75}O_3F_3Na$. Found: 779.5592. ^1H NMR (500 MHz, $CDCl_3$, δ): 7.54 (2H, m, ArH), 7.41–7.39 (3H, m, ArH), 5.51 (1H, td, *J*=6.6, 2.2 Hz, H-3), 5.40–5.38 (6H, m, H-13, 14, 17, 18, 21, 22), 3.55 (3H, s, OMe), 2.49 (1H, d, *J*=2.2 Hz, H-1), 2.09 (8H, m, H-15, 16, 19, 20), 2.03 (4H, m, H-12, 23), 1.86 (2H, m, H-4), 1.45 (2H, m, H-5), 0.88 (3H, t, *J*=6.9 Hz, H-38).

3.2.3. (*R*)-MTPA ester 2b. Colorless powder, FAB MS: *m/z* 753 $(M+Na)^+$. HR-FAB MS: *m/z* 753.5410: calcd for $C_{46}H_{73}O_3F_3Na$. Found: 753.5378. ^1H NMR (500 MHz, $CDCl_3$, δ): 7.54 (2H, m, ArH), 7.41–7.38 (3H, m, ArH), 5.54 (1H, td, *J*=6.5, 1.9 Hz, H-3), 5.39–5.37 (4H, m, H-15, 16, 19, 20), 3.60 (3H, s, OMe), 2.53 (1H, d, *J*=1.9 Hz, H-1), 2.09 (4H, m, H-17, 18), 2.03 (4H, m, H-14, 21), 1.79 (2H, m, H-4), 1.32 (2H, m, H-5), 0.88 (3H, t, *J*=7.0 Hz, H-36).

3.2.4. (*R*)-MTPA ester 3b. Colorless powder, FAB MS: *m/z* 779 $(M+Na)^+$. HR-FAB MS: *m/z* 779.5566: calcd for $C_{48}H_{75}O_3F_3Na$. Found: 779.5546. ^1H NMR (500 MHz, $CDCl_3$, δ): 7.54 (2H, m, ArH), 7.41–7.39 (3H, m, ArH), 5.54 (1H, td, *J*=6.6, 2.2 Hz, H-3), 5.40–5.38 (6H, m, H-13, 14, 17, 18, 21, 22), 3.60 (3H, s, OMe), 2.53 (1H, d, *J*=2.2 Hz, H-1), 2.09 (8H, m, H-15, 16, 19, 20), 2.03 (4H, m, H-12, 23), 1.79 (2H, m, H-4), 1.32 (2H, m, H-5), 0.88 (3H, t, *J*=7.0 Hz, H-38).

3.3. Ozonolysis of lembehynes B (2) and C (3)

The CH_2Cl_2 solution (0.4 mL) of **2** or **3** (each 0.5 mg) was treated with ozone (O_3) for 1 min at −78°C, and then $NaBH_4$ (1.0 mg) was added. The reaction mixture was stirred for 1 h at room temperature (25°C). After filtration through cotton, the crude product was subjected to GC–MS analysis. GC–MS conditions: ionization, EI; column, DB-5 (0.25 mm ϕ×25 m); mobile phase, N_2; column temperature, initial 100°C and +5°C/min gradient for 36 min from 2 min after injection. The product was determined to be 1-heptadecanol [t_R=17.3 min, *m/z* 238 $(M-H_2O)^+$], which was identified with an authentic sample.

3.4. General procedure for the preparation of Weinreb amides (12–14)

A solution of nervonic acid (**9**), linolenic acid (**10**) or palmitic acid (**11**) in CH_2Cl_2 (0.18 mmol/mL) was treated with *N*,*O*-dimethylhydroxylamine hydrochloride (1.5 equiv. of **9**, **10** or **11**), EDCI (1.5 equiv. of **9**, **10** or **11**), and DMAP (1.5 equiv. of **9**, **10** or **11**) and stirred at room temperature (25°C) for 1 h, respectively. The reaction was quenched by saturated aq. NaCl and the whole was extracted with EtOAc. The EtOAc phase was washed by 5% HCl and saturated aq. NaCl, then dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by SiO_2 column chromatography to afford a Weinreb amide **12**, **13** or **14**, respectively.

3.4.1. Compound 12. 94% yield. Colorless oil, FAB MS: *m/z* 410 $(M+H)^+$. HR-FAB MS: *m/z* 410.3998: calcd for $C_{26}H_{52}NO_2$. Found: 410.3980. ^1H NMR (500 MHz, $CDCl_3$, δ): 5.35 (2H, m, H-15, 16), 3.68 (3H, s, OMe), 3.18 (3H, s, NMe), 2.41 (2H, t, *J*=7.3 Hz, H-2), 2.01 (4H, m, H-14, 17), 1.62 (2H, m, H-3), 0.88 (3H, t, *J*=6.9 Hz, H-24).

3.4.2. Compound 13. 90% yield. Colorless oil, FAB MS: *m/z* 322 $(M+H)^+$. HR-FAB MS: *m/z* 322.2746: calcd for $C_{20}H_{36}NO_2$. Found: 322.2731. ^1H NMR (500 MHz, $CDCl_3$, δ): 5.42–5.30 (6H, m, H-9, 10, 12, 13, 15, 16), 3.68 (3H, s, OMe), 3.17 (3H, s, NMe), 2.81 (4H, t, *J*=5.6 Hz, H-11, 14), 2.41 (2H, t, *J*=7.4 Hz, H-2), 2.04 (4H, m, H-8, 17), 1.62 (2H, m, H-3), 0.97 (3H, t, *J*=7.7 Hz, H-18).

3.4.3. Compound 14. 92% yield. Colorless powder, FAB MS: *m/z* 300 $(M+H)^+$. HR-FAB MS: *m/z* 300.2903: calcd for $C_{18}H_{38}NO_2$. Found: 300.2923. ^1H NMR (500 MHz, $CDCl_3$, δ): δ 3.67 (3H, s, OMe), 3.17 (3H, s, NMe), 2.40 (2H, t, *J*=7.6 Hz, H-2), 1.61 (2H, m, H-3), 0.87 (3H, t, *J*=7.0 Hz, H-16).

3.5. General procedure for the preparation for alkynes 15–17

To a solution of triisopropylsilyl acetylene (3 equiv. of **12**, **13** or **14**) in THF (0.2 mmol/mL) was added a hexane solution of *n*-BuLi (1.56 M in hexane, 2 equiv. of **12**, **13** or **14**) dropwise at −40°C. The solution was stirred for 1 h at −40 to 0°C. Subsequently, a solution of **12**, **13** or **14** in THF (0.84 mmol/mL) was added dropwise to the acetylide solution at −10°C under an Ar atmosphere. The reaction mixture was further stirred for 1 h at −10°C. The reaction was quenched by saturated aq. NH_4Cl and the whole was extracted with Et_2O. The Et_2O phase was washed by saturated aq. NaCl, then dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by SiO_2 column chromatography to afford an alkyne **15**, **16** or **17**, respectively.

3.5.1. Compound 15. 61% yield. Colorless oil, FAB MS: *m/z* 531 $(M+H)^+$. HR-FAB MS: *m/z* 531.4961: calcd for $C_{35}H_{67}OSi$. Found: 531.4968. ^1H NMR (500 MHz, $CDCl_3$, δ): 5.35 (2H, m, H-17, 18), 2.55 (2H, t, *J*=7.3 Hz, H-4), 2.01 (4H, m, H-16, 19), 1.70 (2H, m, H-5), 1.13–1.07 (21H, m, 1-TIPS), 0.88 (3H, t, *J*=6.7 Hz, H-26).

3.5.2. Compound 16. 70% yield. Colorless oil, FAB MS: *m/z* 443 $(M+H)^+$. HR-FAB MS: *m/z* 443.3709: calcd for $C_{29}H_{51}OSi$. Found: 443.3720. ^1H NMR (500 MHz, $CDCl_3$, δ): 5.42–5.30 (6H, m, H-11, 12, 14, 15, 17, 18), 2.81 (4H, t, *J*=5.5 Hz, H-13, 16), 2.55 (2H, t, *J*=7.3 Hz, H-4), 2.04 (4H, m, H-10, 19), 1.70 (2H, m, H-5), 1.15–1.05 (21H, m, 1-TIPS), 0.97 (3H, t, *J*=7.6 Hz, H-20).

3.5.3. Compound 17. 64% yield. Colorless powder, FAB MS: *m/z* 421 $(M+H)^+$. HR-FAB MS: *m/z* 421.3866: calcd for $C_{27}H_{53}OSi$. Found: 421.3854. ^1H NMR (500 MHz, $CDCl_3$, δ): 2.55 (2H, t, *J*=7.4 Hz, H-4), 1.69 (2H, m, H-5), 1.13–1.07 (21H, m, 1-TIPS), 0.88 (3H, t, *J*=7.0 Hz, H-18).

3.6. General procedure of asymmetric reduction followed by deprotection giving analogue-I (4), analogue-II (5), analogue-III (6), and analogue-IV (7)

A solution of **15**, **16** or **17** in THF (0.1 mmol/mL) was treated with (*R*)-2-methyl-CBS-oxazaborolidine (1 M in toluene, 2 equiv. of **15**, **16** or **17**) and borane–methyl sulfide complex (BMS) (2 M in THF, 5 equiv. of **15**, **16** or **17**) at −40°C. The reaction mixture was stirred at −40°C for 1 h under an Ar atmosphere. The reaction was quenched by MeOH and diluted with Et_2O. The Et_2O phase was washed by saturated aq. NH_4Cl, 5% aq. $NaHCO_3$ and saturated aq. NaCl, then dried over $MgSO_4$. After evaporation of the solvent, the crude compound was used on the next reaction without purification. To a solution of the crude compound in THF (0.1 mmol/mL) was added TBAF (1 M in THF, 1.2 equiv. of **15**, **16** or **17**) at 0°C, and then the whole was stirred at room temperature (25°C) for 6 h. The reaction was quenched by saturated aq. NaCl and the whole was extracted with Et_2O. The Et_2O phase was washed by saturated aq. NaCl, then dried over $MgSO_4$. After evaporation of the solvent, the residues were purified by SiO_2 column chromatography to afford analogue-I (**4**), analogue-II (**5**), and analogue-III (**6**). A solution of **17** in THF was similarly treated with (*S*)-2-methyl-CBS-oxazaborolidine and BMS to furnish analogue-V (**7**) with 3*S* configuration finally.

3.6.1. Compound 4. 71% yield for two steps (97% ee). Colorless powder, $[\alpha]_D$=+1.1° (*c*=0.55, $CHCl_3$, 25°C). FAB MS: *m/z* 399 $(M+Na)^+$. HR-FAB MS: *m/z* 399.3603: calcd for $C_{26}H_{48}ONa$. Found: 399.3626. ^1H NMR (500 MHz, $CDCl_3$, δ): 5.35 (2H, m, H-17, 18), 4.37 (1H, td, *J*=6.1, 1.8 Hz, H-3), 2.46 (1H, d, *J*=1.8 Hz, H-1), 2.01 (4H, m, H-16, 19), 1.72 (2H, m, H-4), 1.45 (2H, m, H-5), 0.88 (3H, t, *J*=6.7 Hz, H-26).

3.6.2. Compound 5. 63% yield for 2 steps (99% ee). Colorless oil, $[\alpha]_D$=+1.6° (*c*=1.36, $CHCl_3$, 25°C). FAB MS: *m/z* 311 $(M+Na)^+$. HR-FAB MS: *m/z* 311.2351: calcd for $C_{20}H_{32}ONa$. Found: 311.2349. ^1H NMR (500 MHz, $CDCl_3$, δ): 5.42–5.30 (6H, m, H-11, 12, 14, 15, 17, 18), 4.37 (1H, td, *J*=6.7, 2.1 Hz, H-3), 2.81 (4H, t, *J*=6.1 Hz, H-13, 16), 2.46 (1H, d, *J*=2.1 Hz, H-1), 2.06 (4H, m, H-10, 19), 1.71 (2H, m, H-4), 1.46 (2H, m, H-5), 0.98 (3H, t, *J*=7.6 Hz, H-20).

3.6.3. Compound 6. 71% yield for 2 steps (98% ee). Colorless powder, $[\alpha]_D$=+0.7° (*c*=0.24, $CHCl_3$, 25°C). FAB MS: *m/z* 289 $(M+Na)^+$. HR-FAB MS: *m/z* 289.2508: calcd for $C_{18}H_{34}ONa$. Found: 289.2493. ^1H NMR (500 MHz, $CDCl_3$, δ): 4.37 (1H, td, *J*=6.7, 1.8 Hz, H-3), 2.46 (1H, d, *J*=1.8 Hz), 1.70 (2H, m, H-4), 1.46 (2H, m, H-5), 0.88 (3H, t, *J*=7.0 Hz, H-18).

3.6.4. Compound 7. 79% yield for two steps (95% ee). Colorless powder, $[\alpha]_D$=−1.0° (*c*=0.48, $CHCl_3$, 25°C). FAB MS: *m/z* 289 $(M+Na)^+$. HR-FAB MS: *m/z* 289.2508: calcd for $C_{18}H_{34}ONa$. Found: 289.2511. ^1H NMR (500 MHz, $CDCl_3$, δ): 4.37 (1H, td, *J*=6.4, 1.8 Hz, H-3), 2.46 (1H, d, *J*=1.8 Hz), 1.71 (2H, m, H-4), 1.46 (2H, m, H-5), 0.88 (3H, t, *J*=7.0 Hz, H-18).

Enantiomeric excesses were determined by integral of the methoxyl proton in ^1H NMR spectra of the corresponding Mosher esters.

3.7. Assay for neuritogenic activity in Neuro 2A cells

Neuro 2A cells were grown in DMEM with 10% FBS. The cells were kept in an incubator at 37°C with 5% CO_2. The cells were plated on 24-well plates at a density of 1×10^4 per well with 1 mL of the culture medium. After 24 h cultivation, the medium was exchanged for fresh medium, and the testing sample as 10 μL of EtOH solution was added to each well. After 48 h incubation, morphological changes in the cells were observed under microscope. The cells that have longer processes than the diameter of the cell body were evaluated as neurite-bearing cells. The percentage of the cells with neurites in a particular culture was determined by counting 300 cells at least in the photomicrographs of the areas where the cell density was representative.

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