Special Collection of Papers

This article is dedicated to Professor Satoshi Ōmura in celebration of his 2015 Nobel Prize.

Regular Article

N-Methylniphatyne A, a New 3-Alkylpyridine Alkaloid as an Inhibitor of the Cancer Cells Adapted to Nutrient Starvation, from an Indonesian Marine Sponge of *Xestospongia* sp.

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In the course of searching for selective growth inhibitors of the cancer cells adapted to nutrient starvation, a new 3-alkylpyridine alkaloid named *N*-methylniphatyne A (1) was isolated from an Indonesian marine sponge of *Xestospongia* sp. The chemical structure of 1 was determined on the basis of the spectroscopic analysis and comparison with the synthesized 1 and its analogues. Compound 1 showed the cytotoxic activity against PANC-1 cells under the condition of glucose starvation with IC_{50} value of 16 μM, whereas no growth-inhibition was observed up to 100 μM under the general culture conditions.

Key words *N*-methylniphatyne A; marine sponge; cancer; nutrient starvation

Tumor contains hypoxic and nutrient-starved regions due to the abnormal cell proliferation coupled with defective formation of vasculature structurally and functionally.[1] In addition, the cancer cells that have adapted to these environments in tumors are generally thought to stimulate the pathological progression of cancer by promoting tumor growth, angiogenesis, metastasis, and drug resistance.[2,3] Therefore, compounds that selectively inhibit the growth of cancer cells under these conditions should have potential for anti-cancer drugs. So far, we have established a screening system searching for hypoxia-selective growth inhibitors against cancer cells and have isolated furospinosulin-1 (furanosesterterpene)[4] and dictyoceratins-A and -C (sesquiterpene phenols)[5] from the Indonesian marine sponge of *Dactylospongia elegans*. Moreover, we have shown the validity of these compounds as new drug leads, through the analytical studies of target molecules and the structure–activity relationships (SAR) studies using the synthesized analogue compounds.[6–8)]

From the recent biological studies of the cancer cells adapted to the nutrient starvation, the activation of phosphoinositide 3-kinase (PI3k)/v-akt murine thymoma viral oncogene homolog 1 (Akt)/mammalian target of rapamycin (mTOR) signaling pathway and the unfolded protein response (UPR) such as induction of glucose-related protein 78 (GRP78) were found to be important for the adaptation of cancer cells to nutrient starvation, and these processes have attracted attention as drug targets for cancer chemotherapy.[9,10] Kigamicin D, a polycyclic xanthone isolated from the culture broth of *Amycolatopsis* sp., inhibited activation of Akt signaling and showed selective cytotoxic activity against pancreatic cancer cells under the condition of nutrient starvation compared with general culture conditions.[11] Two coumarins [kayeassamins (from *Kayea assamica*)[12] and angelmarin (from *Angelica pubescens*)[13]] and a lignan [arctigenin (from *Arctium lappa*)[14]] have been reported to show the preferential cytotoxic activity under the conditions of nutrient starvation, while showing no activity in the general culture conditions. Moreover, Momose *et al.* have reported that the inhibitors of mitochondrial functions such as rotenone (complex I inhibitor), atpenin A5 (complex II inhibitor), antimycin A (complex III inhibitor), and leucinostatin A (complex V inhibitor), exhibited the preferential growth inhibitory activity against the cancer cells adapted to the nutrient-starved conditions.[15] Following this background, we established a screening system to search selective growth inhibitors against the cancer cells adapted to the nutrient-starved conditions by utilizing the glucose-deficient culture medium. As a result of screening from the marine medicinal resources and bioassay-guided separation, a new 3-alkylpyridine alkaloid named *N*-methylniphatyne A (**1**) was isolated from an Indonesian marine sponge of *Xestospongia* sp. In this paper, the isolation, structure elucidation and biological activity of *N*-methylniphatyne A (**1**) are presented.

Results and Discussion

The MeOH extract of the dried marine sponge (200 g) of *Xestospongia* sp., which was collected in 2004 at Indonesia, showed the selective cytotoxic activity against the human pancreatic carcinoma PANC-1 cells adapted to nutrient starvation by cultivating in the Glucose Deficient Medium. On the guidance of bioassay, the MeOH extract (27 g) was partitioned into a water–EtOAc mixture. The active EtOAc soluble portion (2 g) was fractionated by SiO_2 column chromatography, octa-

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decyl silica (ODS) column chromatography, and ODS HPLC to afford *N*-methylniphatyne A (**1**, 43 mg, 0.16% yield from the MeOH extract) (Fig. 1).

N-Methylniphatyne A (**1**) was obtained as an oil. The electrospray ionization time-of-flight mass spectrometry (ESI-TOF MS) of **1** showed a quasi-molecular ion peak $[M+Na]^+$ at *m/z* 381, and the molecular formula was determined as $C_{23}H_{38}N_2O$ by high-resolution (HR)-ESI-TOF MS analysis. The ^{1}H- and ^{13}C-NMR signals for the aromatic region [δ_H 8.84 (br s, H-2 and H-6), 7.49 (d, J=7.6 Hz, H-4) and 7.21 (dd, J=7.6 and 4.9 Hz, H-5); δ_C 149.7 (C-2), 147.0 (C-6), 138.0 (C-3), 135.9 (C-4) and 123.3 (C-5)] suggested the presence of 3-alkylpyridine unit (Table 1). The UV absorption maximum at 260 nm also implied the presence of pyridine chromophore. The ^{13}C-NMR signals at δ_C 80.1 (C-15) and 80.2 (C-16) indicated the presence of an internal alkyne moiety. Two pairs of characteristic ^{1}H/^{13}C-NMR signals observed at δ_H 3.50 (3H, s)/δ_C 60.0 and δ_H 2.55 (3H, s)/δ_C 45.3 suggested the presence of a *N*-methoxy-*N*-methyl moiety. In addition, the detailed analysis of correlation spectroscopy (^{1}H–^{1}H COSY) and heteronuclear multiple bond coherence (HMBC) spectra of **1** allowed three partial structures (A, B and C) as shown in Fig. 2. Although no additional connectivities were obtained from the NMR analysis because of the overlap of the NMR signals of the methylene region, these data suggested that the partial structure C was linked to the partial structures A and B through the methylene units as shown in Fig. 2. In order to determine the position of the alkyne moiety, ESI-TOF-MS/MS experiment was carried out. As shown in Fig. 3, the fragmentations observed at *m/z* 214, 202, and 190 implied the position of the alkyne moiety to be at C-15 and C-16. The structure of *N*-methylniphatyne A (**1**) was therefore speculated as shown in Fig. 1.

A number of 3-alkylpyridine alkaloids have been isolated from the marine sponges of several genera such as *Xestospon-*

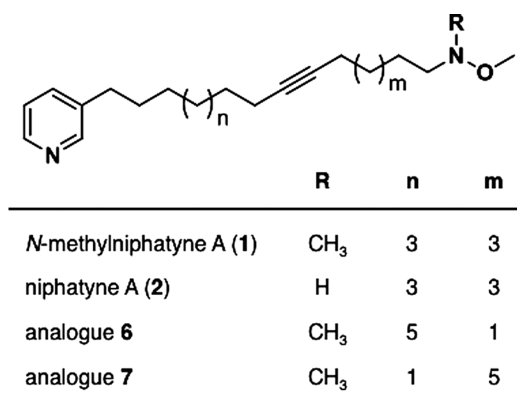


Fig. 1. Chemical Structures of *N*-Methylniphatyne A (**1**) and Its Related Compounds

Table 1. ^{1}H- and ^{13}C-NMR Data for *N*-Methylniphatyne A (**1**)

Position	*N*-Methylniphatyne A (**1**)	
	δ_C $^{a)}$	δ_H $^{b)}$
2	149.7	8.44 (br s)
3	138.0	
4	135.9	7.49 (d, 7.6 Hz)
5	123.3	7.21 (dd, 7.6, 4.9 Hz)
6	147.0	8.44 (br s)
7	33.0	2.59 (t, 7.8 Hz)
8	31.1	1.60 (m)
9	29.0 or 29.3	1.32 (m)
10	29.0 or 29.3	1.27–1.40
11	29.0 or 29.3	1.27–1.40
12	28.8$^{c)}$	1.34 (m)$^{c)}$
13	29.1$^{c)}$	1.46 (m)$^{c)}$
14	18.7$^{c)}$	2.13 (m)$^{c)}$
15	80.1	
16	80.2	
17	18.7$^{c)}$	2.13 (m)$^{c)}$
18	29.1$^{c)}$	1.47 (m)$^{c)}$
19	28.7$^{c)}$	1.34 (m)$^{c)}$
20	27.0	1.33 (m)
21	27.2	1.54 (m)
22	60.9	2.59 (t, 7.8 Hz)
NCH_3	45.3	2.55 (s)
OCH_3	60.0	3.50 (s)

a) ^{13}C-NMR: δ_C (ppm), (150 MHz, $CDCl_3$). *b)* ^{1}H-NMR: δ_H (ppm, J in Hz), (600 MHz, $CDCl_3$). *c)* These signals were overlapped, respectively.

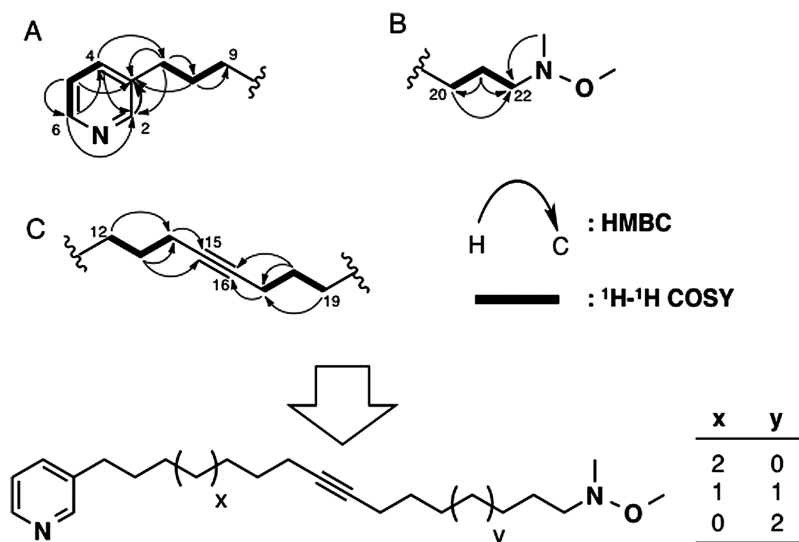


Fig. 2. ^{1}H–^{1}H COSY and HMBC Correlations for *N*-Methylniphatyne A (**1**)

gia,[16)] *Niphates*[17–19)] and *Amphimedon*.[16,20,21)] Most of them have a long aliphatic chain with a various nitrogen-containing terminus. In addition, the cytotoxic activity and the antimicrobial activity have been reported for this class of compounds so far.[16–21)] Quiñoà and Crews have isolated niphatyne A (**2**, Fig. 1), which was structurally similar to **1**, from a Fijian marine sponge of *Niphates* sp., and the cytotoxic activity of **2** against murine lymphoma P388 cells has been reported.[17)] Niphatyne A (**2**) has a methoxylamine terminus in the side chain, whereas the terminus structure of *N*-methylniphatyne A (**1**) is *N*-methoxy-*N*-methyl group.

We then engaged in the total synthesis of *N*-methylniphatyne A (**1**), for the confirmation of the deduced chemical structure of **1** and for the sustainable supply of **1**. We anticipated that *N*-methylniphatyne A (**1**) could be easily elaborated through the coupling between lithium acetylide derived from an aminoalkyne and a pyridylalkyl bromide, as depicted in Chart 1. Aminoalkyne **4a** (m=3) was prepared from 8-bromo-1-octyne (**3a**)[22)] and *N*,*O*-dimethylhydroxylamine hydrochloride in the presence of Et_4NI and K_2CO_3. Pyridylalkyl bromide **5a** (n=3), another fragment, was synthesized through the reported procedure.[23)] Then, the lithium acetylide derived from **4a** was reacted with bromide **5a** to provide a desired *N*-methylniphatyne A (**1**). All of the spectral data of the synthetic **1** were identical with those of the natural product, indicating that the chemical structure of **1** was as deduced (Fig. 1).

Continuously, two related congeners (**6** and **7**) of the alkyne position, possessing the same chain length, were also prepared for the comparison of their spectral and biological properties with those of natural product. Thus, the similar procedure using two fragments with the different chain length (**4b,c, 5b,c**) afforded two analogues **6** (m=1, n=5) and **7** (m=5, n=1). The ^{1}H-NMR spectra and MS/MS fragmentation patterns of two analogues (**6** and **7**) were obviously different with those of the natural and synthetic **1** (Figs. 3, 4).

N-Methylniphatyne A (**1**) showed the cytotoxic activity against the PANC-1 cells adapted to nutrient starvation by

Fig. 3. ESI-TOF-MS/MS Analysis for *N*-Methylniphatyne A (**1**)

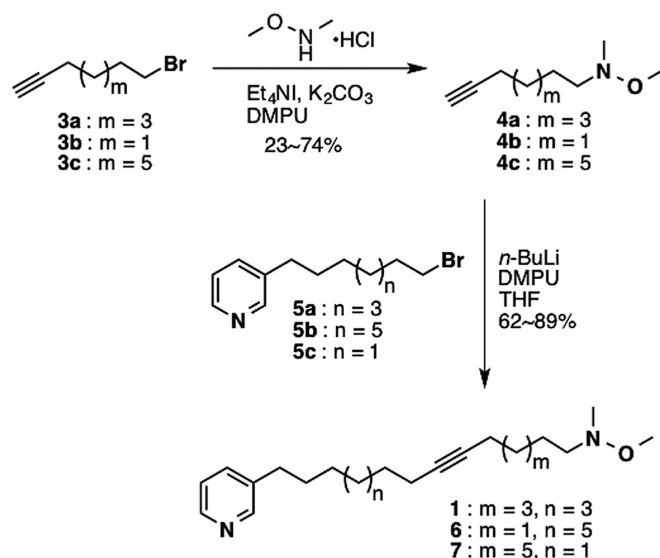
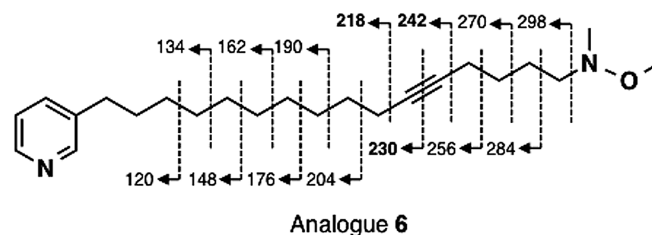


Chart 1. Syntheses of *N*-Methylniphatyne A (**1**) and Two Analogues **6** and **7**

Table 2. Growth Inhibitory Activity of Compounds on the PANC-1 Cells under Both Glucose-Deficient Conditions and General Culture Conditions

	IC$_{50}$ (μM)		S.I.[c)]
	Glucose (−)[a)]	Glucose (+)[b)]	
Natural *N*-methylniphatyne A (**1**)	16	>100	>6.3
Synthetic *N*-methylniphatyne A (**1**)	17	>100	>5.8
Analogue **6**	18	>100	>5.5
Analogue **7**	8.8	>100	>11.3
Antimycin A[d)]	0.003	>0.1	>33

a) Conditions of glucose-deficient medium. *b*) Conditions of general glucose medium. *c*) Selective index. *d*) Compound for positive control.



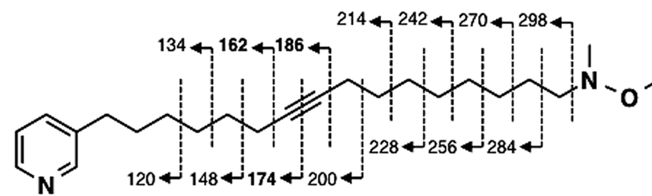


Fig. 4. ESI-TOF-MS/MS Analysis for **6** and **7**

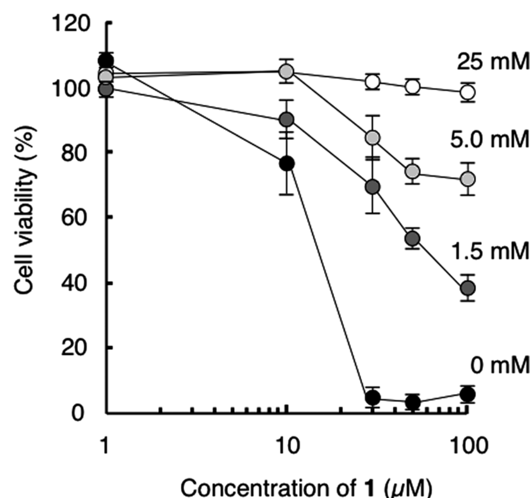


Fig. 5. Effect of Glucose Concentration on the Cytotoxic Activity of *N*-Methylniphatyne A (**1**) against PANC-1 Cells

cultivating in the Glucose Deficient Medium with IC_{50} value of 16 μM, whereas compound **1** did not show the cytotoxic activity up to 100 μM in the General Glucose Medium. The selective index (S.I.) value was estimated to be more than 6.3 (Table 2). In addition, the cytotoxic activity of compound **1** depended on the concentration of glucose as shown in Fig. 5. These data indicated that *N*-methylniphatyne A (**1**) was selective growth inhibitor against the PANC-1 cells adapted to nutrient starvation.

The analogue **6** showed the similar growth-inhibitory activity, while the analogue **7** showed the slightly potent activity. These results suggested that the position of the alkyne moiety was not important for the cytotoxic activity. The further studies such as detail mode of action, target molecule, and *in vivo* efficacy are currently underway.

Conclusion

We isolated a new 3-alkylpyridine alkaloid named *N*-methylniphatyne A (**1**) from an Indonesian marine sponge of *Xestospongia* sp. on the basis of the constructed bioassay-guided separation. Compound **1** exhibited the preferential growth-inhibitory activity to the PANC-1 cells under the glucose-deficient conditions, and the activity of compound **1** depended on the glucose concentration. Preliminary SAR study using the synthesized analogues revealed that the position of the alkyne moiety did not affect the growth-inhibitory activity. *N*-Methylniphatyne A (**1**), which could be easily supplied by synthetic method, is expected to be a promising drug lead for anti-cancer drugs that target the cancer cells adapted to nutrient starvation.

Experimental

General Experimental Procedures NMR spectra, referenced to tetramethylsilane (TMS), were measured on a JEOL ECA-500 (^{1}H: 500 MHz, ^{13}C: 125 MHz) and an Agilent NMR system (^{1}H: 600 MHz, ^{13}C: 150 MHz). ESI-TOF-MS was recorded on a Q-Tof Ultima (Waters Co., MA, U.S.A.). IR spectra were obtained with a JASCO FT/IR-5300 (KBr pellets). UV spectra were obtained with UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan). Column chromatography was performed on Silica gel BW-200 (Fuji Silysia, Aichi, Japan), Cosmosil ODS (75C_{18}-OPN, Nacalai Tesque, Kyoto, Japan), and Cosmosil 5C_{18}-MS-II (10 mm i.d. × 250 mm, Nacalai Tesque). TLC analysis was carried out by silica gel 60F_{254} (Merck Chemical, Darmstadt, Germany). HPLC was performed by Hitachi High Sensitivity Series system (UV-detector: L-4000H), respectively.

Cell Culture and Bioassay Human pancreatic carcinoma PANC-1 cells was maintained in the Dulbecco's modified Eagle's medium (DMEM) supplemented with heat-inactivated 10% fetal bovine serum (FBS) and kanamycin (50 μg/mL) in a humidified atmosphere of 5% CO_2 at 37°C. In the case of the condition of nutrient starvation, PANC-1 cells was cultured in the Glucose Deficient Medium [Basal Medium (25 mM *N*-(2-hydroxyethyl) piperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) supplemented with 6.4 g/L NaCl, 700 mg/L $NaHCO_3$, 400 mg/L KCl, 265 mg/L $CaCl_2 \cdot 2H_2O$, 200 mg/L $MgSO_4 \cdot 7H_2O$, 125 mg/L NaH_2PO_4, 0.1 mg/L $Fe(NO_3) \cdot 9H_2O$, 15 mg/L Phenol red, 10 mL/L MEM vitamin solution (X100) (GIBCO, Carlsbad, CA, U.S.A.), 200 mmol/L L-glutamine solution (GIBCO), 50 mg/L kanamycin) containing 10% dialyzed FBS]. The General Glucose Medium [Basal Medium supplemented with 10% FBS and 2.0 g/L glucose (final 25 mM)] was also used for bioassay as the general culture conditions to compare the activity of the sample under the conditions of nutrient starvation.

PANC-1 cells (1×10^4 cells/100 μL in 96 well plastic plate) were pre-incubated in the DMEM supplement with 10% FBS for 24 h. The medium was then replaced with either General Glucose Medium or Glucose Deficient Medium adapting to the nutrient starvation. After 12 h incubation, serial diluted samples were added, and the plates were incubated for an additional 12 h in a humidified atmosphere of 5% CO_2 at 37°C. The cell proliferation was detected by WST-8 colorimetric reagent. The IC_{50} value was determined by linear interpolation from the growth inhibition curve. We assessed the selectivity of anti-proliferative activity (S.I.) on the basis of the difference of IC_{50} values in the General Glucose Medium and the Glucose Deficient Medium.

Extraction and Isolation of Active Compounds The dried marine sponge of *Xestospongia* sp. (200 g), which was collected in 2004 at Java Island, Indonesia, was extracted with MeOH. On the guidance of bioassay, the MeOH extract [27 g, IC_{50} (Glucose Deficient Medium)=0.8 μg/mL, IC_{50} (General Glucose Medium)=>3.0 μg/mL] was partitioned into a water–EtOAc mixture (1 : 1). The active EtOAc soluble portion [7.5 g, IC_{50} (Glucose Deficient Medium)=0.1 μg/mL, IC_{50} (General Glucose Medium)=>3.0 μg/mL] was further fractionated by SiO_2 gel column chromatography [$CHCl_3$–MeOH–H_2O (lower phase)] to give eight fractions (Frs. 1–8). The active Fr. 1 [1.4 g, IC_{50} (Glucose Deficient Medium)=0.2 μg/mL, IC_{50} (General Glucose Medium)=3.0 μg/mL] was then separated by ODS column chromatography (MeOH–H_2O) to obtain six fractions (Frs. 1-1–1-6). As a result of bioassay, the fr. 1-2 (173 mg, IC_{50} (Glucose Deficient Medium)=3.0 μg/mL, IC_{50} (General Glucose Medium)=>10 μg/mL) and the fr. 1-3 (528 mg, IC_{50} (Glucose Deficient Medium)=3.0 μg/mL, IC_{50} (General Glucose Medium)=>10 μg/mL) showed the cytotoxic activity against PANC-1 cell in the Glucose Deficient Medium selectively. Then, the NMR spectra of both active fractions showed that the structurally similar constituents were contained in the both active fractions. Therefore, we continued the further purification of active substance from fr. 1-3. The fr. 1-3 (528 mg) was then further purified by ODS HPLC (Cosmosil MS-II, CH_3CN–H2O=8 : 2) to afford *N*-methylniphatyne A (**1**) (43 mg, 0.16% yield from the MeOH extract) (Fig. 1).

N-Methylniphatyne A (**1**) An oil; High resolution ESI-TOF-MS *m/z*: 381.2882 (Calcd for $C_{23}H_{38}N_2ONa$: 381.2901). IR (KBr) cm^{-1}: 2930, 1570. UV λ_{max} (MeOH) nm (log ε): 206 (3.76), 260 (3.52). ^{1}H-NMR (600 MHz, $CDCl_3$, δ_H), ^{13}C-NMR (150 MHz, $CDCl_3$, δ_C) spectra: as shown in Table 1.

N,O-Dimethyl-*N*-(oct-7-yn-1-yl)hydroxylamine (**4a**)

K_2CO_3 (498 mg, 3.60 mmol), *N,O*-dimethylhydroxylamine hydrochloride (351 mg, 3.60 mmol) and Et_4NI (35 mg, 0.14 mmol) were succesively added to a solution of 8-bromooct-1-yne (**3a**, 280 mg, 1.48 mmol)[22] in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU, 3 mL), and the reaction mixture was stirred at room temperature (r.t.) for 3 h and at 60°C for 9 h. Sat. NH_4Cl aq. was added to the reaction mixture, and the whole mixture was extracted with Et_2O. The combined organic layer was washed with brine and dried (Na_2SO_4). Removal of the solvent under reduced pressure to

give a crude product, which was purified by SiO_2 column chromatography (hexane–Et_2O=5 : 1) to give **4a** (183 mg, 74%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.50 (3H, s), 2.58 (2H, br s), 2.55 (3H, s), 2.17 (2H, td, *J*=7.1, 2.6 Hz), 1.92 (1H, t, *J*=2.6 Hz), 1.58–1.49 (4H, m), 1.45–1.32 (4H, m). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 84.6, 68.1, 60.8, 60.0, 45.2, 28.6, 28.3, 27.2, 26.9, 18.3. IR (KBr) cm^{-1}: 3308, 2937, 2859, 1464, 1048. ESI-TOF-MS *m/z*: 170 $(M+H)^+$. HR-ESI-TOF-MS *m/z*: 170.1540 (Calcd for $C_{10}H_{20}NO$: 170.1545).

N-(Hex-5-yn-1-yl)-*N*,*O*-dimethylhydroxylamine (**4b**)

Using the same procedure as that for **4a**, compound **4b** (55.7 mg, 23%) was obtained from 6-bromohex-1-yne (**3b**)[24] (273 mg, 1.70 mmol).

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 3.47 (3H, s), 2.57 (2H, br s), 2.52 (3H, s), 2.15 (2H, td, *J*=7.1, 2.6 Hz), 1.91 (1H, t, *J*=2.6 Hz), 1.55–1.45 (4H, m), 1.40–1.22 (8H, m). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 84.6, 68.0, 60.9, 60.0, 45.2, 29.4, 29.0, 28.6, 28.4, 27.3, 27.2, 18.3. IR (KBr) cm^{-1}: 3312, 2927, 1459, 1047. ESI-TOF-MS *m/z*: 142 $(M+H)^+$. HR-ESI-TOF-MS *m/z*: 142.1234 (Calcd for $C_8H_{16}NO$: 142.1232).

N-(Dec-9-yn-1-yl)-*N*,*O*-dimethylhydroxylamine (**4c**)

Using the same procedure as that for **4a**, compound **4c** (169 mg, 56%) was obtained from 10-bromodec-1-yne (**3c**)[25] (333 mg, 1.53 mmol).

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 3.48 (3H, s), 2.56 (2H, br s), 2.53 (3H, s), 2.17 (2H, td, *J*=7.1, 2.6 Hz), 1.90 (1H, t, *J*=2.6 Hz), 1.70–1.50 (4H, m). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 84.3, 68.4, 60.2, 60.1, 45.2, 26.4, 26.3, 18.3. IR (KBr) cm^{-1}: 3310, 2934, 2856, 1462, 1049. ESI-TOF-MS *m/z*: 198 $(M+H)^+$. HR-ESI-TOF-MS *m/z*: 198.1860 (Calcd for $C_{12}H_{24}NO$: 198.1858).

3-(10-Bromodecyl)pyridine (**5b**)

n-BuLi (1.63 M in hexane, 4.0 mL, 6.52 mmol) was added to a solution of *i*Pr_2NH (0.62 mL, 4.4 mmol) in tetrahydrofuran (THF) (5 mL) at 0°C, and the whole mixture was stirred for 30 min at 0°C. 3-Picoline (0.39 mL, 4.02 mmol) was added to the mixture at −78°C, and the whole mixture was stirred for 20 min. Then, 1,9-dibromononane (2.43 mL, 12 mmol) was added to the mixture, and the whole mixture was gradually warmed to r.t. with stirring overnight. Sat. $NaHCO_3$ aq. was added to the reaction mixture, and the whole mixture was extracted with AcOEt. The combined organic layer was washed with brine and dried (Na_2SO_4). Removal of the solvent under reduced pressure to give a crude product, which was purified by SiO_2 column chromatography (hexane–AcOEt=8 : 1) to give **5b** (388 mg, 33%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.35–8.30 (2H, m), 7.39 (1H, d, *J*=7.7 Hz), 7.10 (1H, dd, *J*=7.7, 4.9 Hz), 3.30 (2H, t, *J*=6.9 Hz), 2.50 (2H, t, *J*=7.7 Hz), 1.74 (1H, quint, *J*=7.1 Hz), 1.53–1.48 (2H, m), 1.35–1.12 (12H, m). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 149.6, 146.8, 137.6, 135.5, 123.0, 33.8, 32.7, 32.5, 30.8, 29.11, 29.10, 29.07, 28.8, 28.4, 27.9. IR (KBr) cm^{-1}: 2926, 2854, 1574, 1422, 1026. ESI-TOF-MS *m/z*: 298 $(M+H)^+$. HR-ESI-TOF-MS *m/z*: 298.1163 (Calcd for $C_{15}H_{25}NBr$: 298.1170).

3-(8-Bromooctyl)pyridine (**5a**) and 3-(6-Bromohexyl)pyridine (**5c**)

These compounds were prepared according to a reported procedure, from 1,7-dibromoheptane and 1,5-dibromopentane, respectively.[23] All the spectral data of the compounds were identical to the reported ones.

N-Methylniphatyne A (**1**)

n-BuLi (1.63 M in hexane, 0.21 mL, 0.34 mmol) was added to a solution of **4a** (37.8 mg, 0.22 mmol) in THF (0.5 mL) at −15°C, and the whole mixture was stirred for 30 min at −15°C. **5a** (21.7 mg, 0.08 mmol) in DMPU (0.5 mL) was added to the mixture *via* cannula at −15°C, and the whole mixture was gradually warmed to r.t. with stirring overnight. H_2O was added to the reaction mixture, and the whole mixture was extracted with AcOEt. The combined organic layer was washed with brine and dried (Na_2SO_4). Removal of the solvent under reduced pressure to give a crude product, which was purified by SiO_2 column chromatography (hexane–AcOEt=2 : 1) to give **1** (25.6 mg, 89%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.42 (2H, br s), 7.47 (1H, d, *J*=8.0 Hz), 7.19 (1H, dd, *J*=8.0, 4.9 Hz), 3.49 (3H, s), 2.58 (4H, t, *J*=7.7 Hz), 2.54 (3H, s), 2.12 (4H, q-like), 1.65–1.22 (20H, m). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 149.8, 147.1, 137.9, 135.8, 123.2, 80.2, 80.1, 60.9, 60.0, 45.3, 33.0, 31.1, 29.3, 29.1, 29.0, 28.8, 28.7, 27.2, 26.9, 18.68, 18.65. IR (KBr) cm^{-1}: 2930, 2855, 1461, 1261, 1048. ESI-TOF-MS *m/z*: 381 $(M+Na)^+$. HR-ESI-TOF-MS *m/z*: 381.2887 (Calcd for $C_{23}H_{38}N_2ONa$: 381.2882).

13-Yne Analogue (**6**)

Using the same procedure as that for **1**, compound **6** (19.7 mg, 62%) was obtained from **4b** (51.8 mg, 0.26 mmol) and **5b** (21.2 mg, 0.088 mmol).

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.44 (2H, br s), 7.48 (1H, d, *J*=8.0 Hz), 7.21 (1H, t-like), 3.50 (3H, s), 2.60 (4H, t, *J*=7.7 Hz), 2.55 (3H, s), 2.13 (4H, t-like), 1.61 (2H, quint, *J*=7.6 Hz), 1.53–1.22 (18H, m). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 149.8, 147.1, 137.9, 135.8, 123.3, 80.3, 80.0, 60.9, 60.0, 45.3, 32.9, 31.0, 29.4, 29.11, 29.06, 29.0, 28.8, 28.6, 28.5, 27.4, 27.3, 18.71, 18.67. IR (KBr) cm^{-1}: 2929, 2855, 1462, 1260, 1049. ESI-TOF-MS *m/z*: 359 $(M+H)^+$. HR-ESI-TOF-MS *m/z*: 359.3054 (Calcd for $C_{23}H_{39}N_2O$: 359.3062).

17-Yne Analogue (**7**)

Using the same procedure as that for **1**, compound **7** (11.7 mg, 66%) was obtained from **4c** (21.2 mg, 0.15 mmol) and **5c** (14.9 mg, 0.05 mmol).

^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.48 (2H, br s), 7.50 (1H, d, *J*=7.5 Hz), 7.23 (1H, dd, *J*=7.5, 5.2 Hz), 3.51 (3H, s), 2.60 (4H, t, *J*=7.7 Hz), 2.56 (3H, s), 2.17 (2H, t, *J*=6.9 Hz), 2.12 (2H, t, *J*=6.9 Hz), 1.70–1.40 (8H, m), 1.35–1.22 (12H, m). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 149.8, 147.0, 138.0, 135.8, 123.2, 80.5, 79.7, 60.4, 60.0, 45.2, 33.0, 31.1, 29.7, 29.5, 29.3, 29.10, 29.09, 28.8, 26.9, 26.5, 18.70, 18.66. IR (KBr) cm^{-1}: 2927, 2854, 1459, 1260, 1047. ESI-TOF-MS *m/z*: 381 $(M+Na)^+$. HR-ESI-TOF-MS *m/z*: 381.2886 (Calcd for $C_{23}H_{38}N_2ONa$: 381.2882).

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Conflict of Interest The authors declare no conflict of interest.

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