Preparation and Cancer Cell Invasion Inhibitory Effects of C_{16}-Alkynic Fatty Acids

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Five C_{16}-alkynic fatty acids (2—6) were prepared and examined their inhibitory effects on cancer cell invasion. It has been found that hexadeca-6,8,10-triynoic acid (5) and hexadeca-8,10,12-triynoic acid (6) exhibit similar potent inhibitory activities with that of octadeca-8,10,12-triynoic acid (1) which was isolated from *Scurrula atropurpurea* (Loranthaceae).

Key words C_{16}-alkynic fatty acid; cancer cell invasion inhibitory effect; anticancer; hexadeca-6,8,10-triynoic acid; hexadeca-8,10,12-triynoic acid

The whole plant of *Scurrula atropurpurea* (BL.) DANS. (Loranthaceae), a parasitic plant on the tea plant *Thea sinensis* L., has been traditionally used for the treatment of cancer in Java Island, Indonesia. In our previous paper,[1] we reported isolation of six C_{18}-fatty acids [(*Z*)-9-octadecenoic acid, (*Z*,*Z*)-octadeca-9,12-dienoic acid, (*Z*,*Z*,*Z*)-octadeca-9,12,15-trienoic acid, octadeca-8,10-diynoic acid, (*Z*)-octadec-12-ene-8,10-diynoic acid and octadeca-8,10,12-triynoic acid], besides two xanthines, two flavonol glycosides, a monoterpene glucoside, a lignan glycoside, and four flavanes. Among those C_{18}-fatty acids, octadeca-8,10,12-triynoic acid (**1**) showed the most potent inhibitory effect (99.4% inhibition at 10 μg/ml) on cancer cell invasion through a rat mesothelium monolayer by using MM1 cell line isolated from rat ascites hepatoma AH130 cells.[2] Furthermore, it was found that the rise of number of unsaturation function in the fatty acids seems to strengthen the inhibitory activity.

On the other hand, we examined cancer cell invasion inhibitory effects of four saturated fatty acids, namely myristic acid (C_{14}), palmitic acid (C_{16}), stearic acid (C_{18}), and eicosanoic acid (C_{20}). Among them, palmitic acid (C_{16}) showed much stronger activity (46.8% inhibition at 10 μg/ml) than myristic acid (31.5%), stearic acid (29.5%) and eicosanoic acid (20.5%) at the same concentration.

Therefore, we here describe a simple preparation route for five C_{16}-alkynic fatty acids [hexadec-8-ynoic acid (**2**), hexadec-10-ynoic acid (**3**), hexadeca-8,10-diynoic acid (**4**), hexadeca-6,8,10-triynoic acid (**5**) and hexadeca-8,10,12-triynoic acid (**6**)], in order to compare their inhibitory effects with that of octadeca-8,10,12-triynoic acid (**1**) isolated from *Scurrula atropurpurea*.

Preparation of C_{16}-Alkynic Fatty Acids (2—6) Among the C_{16}-alkynic fatty acids (**2**—**6**), hexadec-8-ynoic acid (**2**) was synthesized by Levine *et al.*[3] using alkylation of 7-bromoheptanoic acid with 1-nonyne, and hexadec-10-ynoic acid (**3**) was reported by Arsequell *et al.*[4] as a intermediate for the synthesis of cyclopropane fatty acids. Also hexadeca-8,10-diynoic acid (**4**) was synthesized by Gunstone and Sykes[5] using a coupling reaction of 1-bromohept-1-yne with 8-nonynoic acid. However, so far, the preparation of hexadeca-6,8,10-triynoic acid (**5**) and hexadeca-8,10,12-triynoic acid (**6**) have not yet been reported. We tried to prepare the C_{16}-alkynic fatty acids (**2**—**6**) by combination of known simple reactions as shown in Fig. 1.

A condensation of propargyl alcohol (**7**) and appropriate 1-bromoalkanes (**8** or **11**) by treatment with *n*-BuLi and potassium 3-aminopropylamide (KAPA)[6] afforded non-8-ynol (**9**)[7] and undec-10-ynol (**12**)[7] in 80% and 78% yield, respectively. Then a coupling reaction of **9** and **12** with proper 1-bromoalkanes (**10** or **13**) by *n*-BuLi treatment and subsequent CrO_3 oxidation[8] furnished hexadec-8-ynoic acid (**2**) and hexadec-10-ynoic acid (**3**) in moderate yields.

Hexadeca-8,10-diynoic acid (**4**) was prepared by use of the above-mentioned non-8-ynol (**9**). A coupling reaction of 1-iodo-1-heptyne (**15**), which was prepared from 1-heptyne (**14**), with **9** by treatment with CuI and pyrrolidine[9] and subsequent CrO_3 oxidation furnished hexadeca-8,10-diynoic acid (**4**) in 57% yield from **14**.

A coupling of propargyl alcohol (**7**) and 1-bromobutane (**16**) by treatment with *n*-BuLi and KAPA afforded hept-6-ynol (**17**)[7,10] in 78% yield. Then, **17** was converted into nona-6,8-diynol (**20**)[10] *via* 10-hydroxy-10-methylundeca-6,8-diynol (**19**) by the similar procedure reported by Nakanishi *et al.*[10] Finally, **20** was coupled with 1-iodo-1-heptyne (**15**) and oxidized by chromic acid to provide hexadeca-6,8,10-triynoic acid (**5**) in a moderate yield.

Undeca-8,10-diynol (**21**) was prepared from **9** and 3-methyl-1-pentyn-3-ol (**18**) through the procedures reported by Zeni *et al.*[11] **21** was coupled with 1-iodo-1-pentyne (**22**) in the presence of CuI and pyrrolidine followed by CrO_3 oxidation to afford hexadeca-8,10,12-triynoic acid (**6**) in a moderate yield.

Cancer Cell Invasion Inhibitory Effects of C_{16}-Alkynic Fatty Acids (2—6) As a result of the assay as shown in Table 1, five synthetic C_{16}-alkynic fatty acids (**2**—**6**) exhibited stronger inhibitory effects on cancer cell invasion than palmitic acid and palmitoleic acid. Especially, two triyne derivatives (**5**, **6**) showed potent inhibitory activities over 95% at a concentration of 10 μg/ml. Those activity were similar with that of the C_{18}-trialkynic fatty acid, octadeca-8,10,12-triynoic acid (**1**)[1] which had been isolated from *Scurrula atropurpurea* (Loranthaceae). Furthermore, the concentration-dependent behavior of the triyne derivatives (**5**, **6**) were examined at 5 μg/ml and 2.5 μg/ml, which indicating those C_{16}-

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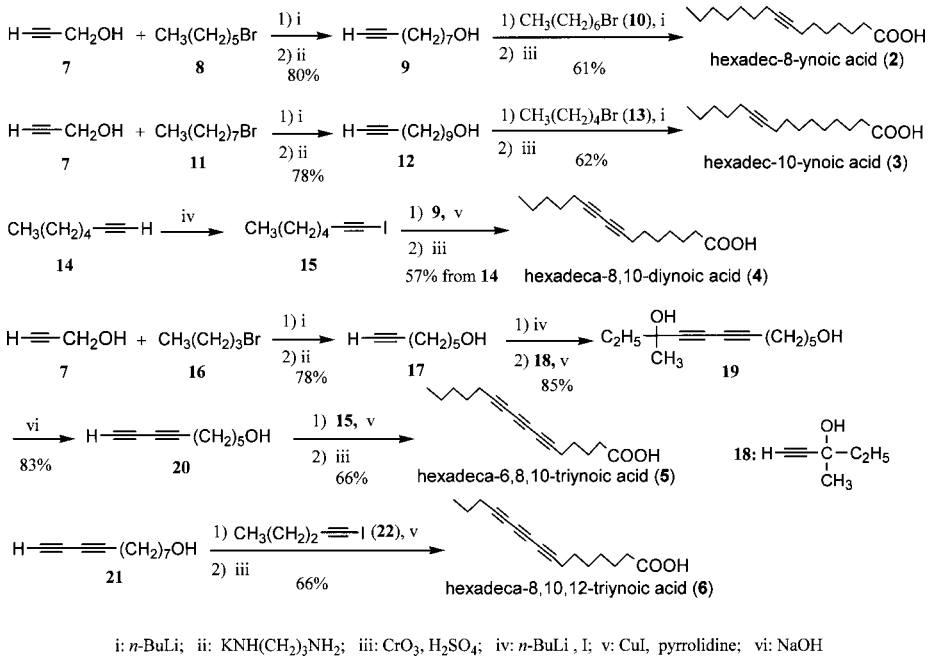

i: *n*-BuLi; ii: $KNH(CH_2)_3NH_2$; iii: CrO_3, H_2SO_4; iv: *n*-BuLi , I; v: CuI, pyrrolidine; vi: NaOH

Fig. 1. Preparation of C_{16}-Alkynic Fatty Acids (**2**—**6**)

Table 1. Cancer Cell Invasion Inhibitory Effects of C_{16}-Fatty Acid Derivatives

Compounds	Concentration (μg/ml)	Inhibitory activity (%)
Palmitic acid	10	46.8
Palmitoleic acid	10	49.7
Hexadec-8-ynoic acid (**2**)	10	82.4
Hexadec-10-ynoic acid (**3**)	10	77.2
Hexadeca-8,10-diynoic acid (**4**)	10	85.6
Hexadeca-6,8,10-triynoic acid (**5**)	10 5 2.5	95.7 85.4 50.3
Hexadeca-8,10,12-triynoic acid (**6**)	10 5 2.5	98.7 90.7 60.5
Octadeca-8,10,12-triynoic acid (**1**)	10 5 2.5	99.4 94.9 45.6
(−)-Epigallocatechin-3-*O*-gallate (EGCG)	10 5 2.5	82.8 59.7 40.1

alkynic fatty acids showed over 50% inhibitory effects even at 2.5 μg/ml.

Although no drug possessing cancer cell invasion inhibitory activity has been produced, (−)-epigallocatechin-3-*O*-gallate (EGCG, 82.8% at 10 μg/ml inhibition, 59.7% inhibition at 5 μg/ml, 40.1% inhibition at 2.5 μg/ml),[1] genistein (10 μg/ml; 80.5% inhibition, 5 μg/ml; 64.0% inhibition, 2.5 μg/ml; 55.7% inhibition)[12] and ginsenoside Rg_3 (25 μg/ml; 98.8% inhibition)[13] have so far been reported as natural occurring materials showing the inhibitory activity.

The present work has indicated that the C_{18}-triyne fatty acid (**1**) and the C_{16}-triyne fatty acids (**5**, **6**) are potent cancer cell invasion inhibitory materials in spite of the simple chemical structures.

It should be noted that the C_{18}- and C_{16}-alkynic fatty acids (**1**—**6**) show no cytotoxity to the cancer cells used in the present assay.[1,2]

Experimental

The instruments used to obtain physical data and experimental conditions for chromatography were the same as in our previous paper.[1]

Myristic acid, palmitic acid, stearic acid, eicosanoic acid and palmitoleic acid were purchased from Wako Pure Chemical Industries, Ltd.

Non-8-ynol (9) To a solution of propargyl alcohol (**7**, 1.96 g, 35.0 mmol) in tetrahydrofuran (THF, 32 ml) and hexamethylphosphoric triamide (HMPA, 18 ml) was added *n*-BuLi (1.6 M in hexane, 43.8 ml, 70.0 mmol) at −78 °C. After the reaction temperature allowed to reach at −30 °C, 1-bromohexane (**8**, 6.36 g, 38.5 mmol) was added to the mixture and stirred at room temperature for 12 h. The reaction mixture was treated with aqueous saturated NH_4Cl and extracted with Et_2O. The Et_2O extract was washed with brine and dried over $MgSO_4$. Removal of the solvent gave a product (6.8 g), which was purified by silica gel column chromatography (SiO_2 200 g, hexane : EtOAc=5 : 1) to afford non-2-ynol (4.17 g, 29.8 mmol), which was treated with the KAPA reagent[6] [prepared from 1,3-diaminopropane (100 ml), Li (1.40 g) and potassium *t*-butoxide (13.4 g)]. After stirring at room temperature for 30 min, the reaction mixture was poured into ice-water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with 5% aqueous HCl and brine, then dried over $MgSO_4$. Removal of the solvent gave a product (4.20 g), which was purified by silica gel column chromatography (SiO_2 150 g, hexane : EtOAc=3 : 1) to give non-8-ynol (**9**, 3.94 g, 28.1 mmol, 80% yield from **7**), which physicochemical properties were identical with those in the literature.[7]

Hexadec-8-ynoic Acid (2) To a solution of **9** (280 mg, 2.0 mmol) in THF (2.0 ml) and HMPA (1.0 ml) was added *n*-BuLi (1.6 M in hexane, 2.5 ml, 4.0 mmol) at −78 °C and the reaction temperature allowed to −30 °C. Then, 1-bromoheptane (**10**, 394 mg, 2.2 mmol) was added to the mixture at −30 °C and stirred at room temperature for 12 h. After treating the reaction mixture with aqueous saturated NH_4Cl, the whole was extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (604 mg), which was purified by silica gel column chromatography (SiO_2 60 g, hexane : EtOAc=5 : 1) to furnish hexadec-8-ynol (400 mg, 1.68 mmol). To a solution of hexadec-8-ynol (400 mg, 1.68 mmol) in acetone (4.0 ml) was added 1.7 ml of the chromic acid reagent[8] [prepared from CrO_3 (10 g), H_2SO_4 (16 g) and H_2O (50 ml)] and the whole was stirred at −10 °C for 15 min. The reaction mixture was treated with 2-propanol and extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (311 mg). Purification of the product by silica gel column chromatography (SiO_2 20 g, hexane : EtOAc=3 : 1) afforded hexadec-8-ynoic acid (**2**, 304 mg, 1.21 mmol, 61% yield from **9**). Physicochemical properties of **2** are given here, since they have not been reported in the literature.[3]

2: An amorphous solid. mp 34—35 °C (from MeOH). IR (film) cm^{-1}: 3300—2500 (br), 1710. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.20—1.55 (16H), 1.65 (2H, qu, J=7.4 Hz), 2.10—2.20 (4H), 2.35 (2H, t, J=7.4 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δc: 180.0, 80.5, 79.9, 34.0, 31.8, 29.2, 28.9, 28.8, 28.8, 28.6, 28.4, 24.6, 22.6, 18.8, 18.7, 14.1. EI-MS *m/z* (%): 252 (M^+, 0.01), 67 (100). High-resolution EI-MS *m/z*: Calcd for $C_{16}H_{28}O_2$: 252.2089. Found: 252.2073 [M^+].

Undec-10-ynol (12) To a solution of **7** (840 mg, 15.0 mmol) in THF (14 ml) and HMPA (7.0 ml) was added *n*-BuLi (1.6 M in hexane, 18.8 ml, 30.0 mmol) at −78 °C and the reaction temperature allowed to −30 °C. 1-Bromooctane (**11**, 3.18 g, 16.5 mmol) was added to the mixture and stirred at room temperature for 12 h. The reaction mixture was poured into aqueous saturated NH_4Cl and the whole was extracted with Et_2O. The Et_2O extract was worked up in the usual manner to give a product (3.0 g). Purification of the product by silica gel column chromatography (SiO_2 150 g, hexane : EtOAc=5 : 1) afforded undec-2-ynol (2.12 g, 12.6 mmol), which was treated with the KAPA reagent[6] [prepared from 1,3-diaminopropane (40 ml), Li (560 mg, 80 mmol) and potassium *t*-butoxide (5.4 g, 50 mmol)]. After stirring at room temperature for 30 min, the reaction mixture was poured into ice-water and extracted with $CHCl_3$. The $CHCl_3$ extract was worked up in the usual manner to give a product (2.5 g). Purification of the product by silica gel column chromatography (SiO_2 100 g, hexane : EtOAc=7 : 2) afforded undec-10-ynol (**12**, 1.97 g, 11.7 mmol, 78% yield from **7**), which physicochemical properties were identical with those in the literature.[7]

Hexadec-10-ynoic Acid (3) *n*-BuLi (1.6 M in hexane, 2.5 ml, 4.0 mmol) was added to a solution of **12** (336 mg, 2.0 mmol) in THF (2.0 ml) and HMPA (1.0 ml) at −78 °C and the reaction temperature allowed to −30 °C. 1-Bromopentane (**13**, 332 mg, 2.2 mmol) was added to the mixture and stirred at room temperature for 12 h. The reaction mixture was poured into aqueous saturated NH_4Cl and extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (740 mg). Purification of the product by silica gel column chromatography (SiO_2 40 g, hexane : EtOAc=5 : 1) gave hexadec-10-ynol (390 mg, 1.64 mmol). The chromic acid reagent[8] (1.7 ml, 3.4 mmol) was added to the solution of hexadec-10-ynol (390 mg) in acetone (3.5 ml) and the whole was stirred at −10 °C for 30 min. The reaction mixture was treated with 2-propanol and extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (376 mg). Purification of the product by silica gel column chromatography (SiO_2 30 g, hexane : EtOAc=3 : 1) gave hexadec-10-ynoic acid (**3**, 310 mg, 1.23 mmol, 62% yield from **12**), which physicochemical properties were identical with those in the literature.[4]

Hexadeca-8,10-diynoic Acid (4) To a solution of 1-pentyne (**14**, 960 mg, 10.0 mmol) in THF (8.0 ml) was added *n*-BuLi (1.6 M in hexane, 6.25 ml, 10.0 mmol) at −78 °C. Then, iodine (2.79 g, 11.0 mmol) in THF (5 ml) was added to the reaction mixture at −30 °C and stirred at room temperature for 30 min. The reaction mixture was poured into aqueous saturated NH_4Cl and the whole was extracted with Et_2O. The Et_2O extract was washed with aqueous $Na_2S_2O_3$ and brine, and dried over $MgSO_4$. Removal of the solvent furnished 1-iodopent-1-yne (**15**, 2.15 g). CuI (47.5 mg, 0.25 mmol) was added to a solution of **9** (350 mg, 2.5 mmol) and **15** (610 mg) in pyrrolidine (4.0 ml), and the whole mixture was stirred at room temperature for 30 min. The reaction mixture was treated with aqueous saturated NH_4Cl and extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (620 mg). Purification of the product by silica gel column chromatography (SiO_2 100 g, hexane : EtOAc=5 : 1) afforded hexadeca-8,10-diynol (503 mg, 2.15 mmol). To a solution of hexadeca-8,10-diynol (400 mg, 1.71 mmol) in acetone (4.0 ml) was added the chromic acid reagent[8] (1.70 ml, 3.4 mmol) and the whole was stirred at −10 °C for 15 min, then treated with 2-propanol and extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (430 mg). Purification of the product by silica gel column chromatography (SiO_2 30 g, hexane : EtOAc=5 : 2) and HPLC (Wakosil 5 SIL, hexane : EtOAc=4 : 1) afforded hexadeca-8,10-diynoic acid (**4**, 317 mg, 1.28 mmol, 57% from **14**). ^{1}H- and ^{13}C-NMR data of **4** are given here, since the spectra have not been reported in the literature.[5]

4: ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.89 (3H, t, J=7.1 Hz), 1.25—1.42 (8H), 1.47—1.55 (4H), 1.64 (2H, quintet, J=7.3 Hz), 2.22—2.27 (4H), 2.35 (2H, t, J=7.4 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δc: 179.7, 77.7, 68.3, 65.4, 65.2, 33.9, 31.0, 28.5, 28.4, 28.1, 28.0, 24.5, 22.2, 19.2, 19.1, 13.9.

Hept-6-ynol (17) *n*-BuLi (1.6 M in hexane, 15.6 ml, 25.0 mmol) was added to a solution of **7** (700 mg, 12.5 mmol) in THF (10 ml) and HMPA (6.5 ml) at −78 °C and then the reaction temperature allowed to −30 °C. 1-Bromobutane (**16**, 1.89 g, 13.8 mmol) was added to the solution and stirred at room temperature. After 12 h, the reaction mixture was poured into aqueous saturated NH_4Cl and the whole was extracted with Et_2O. The Et_2O extract was worked up in the usual manner to give a product (2.5 g). Purification of the product by silica gel column chromatography (SiO_2 150 g, hexane : EtOAc=8 : 1) gave hept-2-ynol (1.18 g, 10.5 mmol), which was treated with the KAPA reagent[6] [prepared from 1,3-diaminopropane (35 ml), Li (294 mg, 42.0 mmol) and potassium *t*-butoxide (4.60 g, 41.1 mmol)]. The whole mixture was stirred at room temperature for 1 h. The mixture was poured into ice-water and the whole was extracted with $CHCl_3$. The $CHCl_3$ extract was worked up in the usual manner to afford a product (1.24 g). Purification of the product by silica gel column chromatography (SiO_2 50 g, hexane :

EtOAc=9 : 1) afforded hept-6-ynol (**17**, 1.09 g, 9.73 mmol, 78% yield from **7**).

To a solution of **17** (900 mg, 8.0 mmol) in THF (6.0 ml) was added *n*-BuLi (1.6 M in hexane, 10.0 ml, 16.0 mmol) at −78 °C. I_2 (2.24 g, 8.8 mmol) in THF (3 ml) was added to the mixture at −30 °C and stirred at room temperature for 30 min. The reaction mixture was treated with aqueous saturated NH_4Cl and extracted with Et_2O. The Et_2O extract was worked up in the usual manner to give 7-iodohept-6-ynol (1.83 g). To a solution of 7-iodohept-6-ynol (1.83 g) and 3-methyl-1-pentyn-3-ol (**18**, 967 mg, 9.6 mmol) in pyrrolidine (8.0 ml) was added CuI (152 mg, 0.80 mmol) and the whole was stirred at room temperature for 30 min. The reaction mixture was treated with aqueous saturated NH_4Cl and the whole was extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (2.23 g), which was purified by silica gel column chromatography (SiO_2 200 g, hexane : EtOAc=2 : 1) to afford 10-hydroxy-10-methyldodeca-6,8-diynol (**19**, 1.60 g, 6.8 mmol, 85% yield from **17**).

19: Colorless oil. IR (film) cm^{-1}: 3600—3100 (br), 2253. UV λ_{max} (EtOH) nm (ε): 214 (327), 228 (327), 240 (333), 254 (216), 282 (38). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.03 (3H, t, J=7.4 Hz), 1.47 (3H, s), 1.40—1.63 (7H), 1.70 (2H, ddd, J=2.1, 7.4, 14.9 Hz), 2.31 (2H, t, J=6.8 Hz), 3.65 (2H, t, J=6.3 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δc: 81.2, 79.2, 69.1, 68.3, 64.6, 62.7, 36.4, 32.1, 29.1, 28.0, 25.0, 19.3, 8.9. EI-MS *m/z* (%): 190 (0.2), 179 (19), 91 (100). High-resolution EI-MS *m/z*: Calcd for $C_{13}H_{18}O$: 190.1358, and for $C_{11}H_{15}O_2$: 179.1072. Found: 190.1366 [M^+-H_2O] and 179.1091 [$M^+-C_2H_5$].

Nona-6,8-diynol (20) To a solution of **19** (700 mg, 3.37 mmol) in xylene (4.0 ml) was added NaOH (40 mg, 1.00 mmol) and the whole was stirred under reflux for 10 min. After cooling, the mixture was poured into ice-water and the whole was extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (809 mg). Purification of the product by silica gel column chromatography (SiO_2 100 g, hexane : EtOAc=2 : 1) and HPLC (Wakosil 5 SIL, hexane : EtOAc=4 : 1) afforded nona-6,8-diynol (**20**, 380 mg, 2.79 mmol, 83% yield), which physicochemical properties were identical with those in the literature.[10]

Hexadeca-6,8,10-triynoic Acid (5) To a solution of **15** (100 mg) and **20** (50 mg, 0.368 mmol) in pyrrolidine (1.0 ml) was added CuI (7.0 mg, 0.037 mmol) and the whole was stirred at room temperature for 30 min. The reaction mixture was treated with aqueous saturated NH_4Cl and extracted with EtOAc. The EtOAc extract was worked up in the usual manner to afford a product (122 mg), which was purified by silica gel column chromatography (SiO_2 10 g, hexane : EtOAc=2 : 1) to give hexadeca-6,8,10-triynol (81 mg, 0.352 mmol). To a solution of hexadeca-6,8,10-triynol (81 mg) in acetone (1.0 ml) was added the chromic acid reagent[8] (0.350 ml, 0.70 mmol) and stirred at −10 °C for 15 min. The reaction mixture was treated with 2-propanol and extracted with EtOAc. The EtOAc phase was worked up in the usual manner to give a product (76 mg). Purification of the product by silica gel column chromatography (SiO_2 10 g, $CHCl_3$: MeOH=30 : 1) and HPLC (Wakosil 5 SIL, hexane : EtOAc=4 : 1) afforded hexadeca-6,8,10-triynoic acid (**5**, 59 mg, 0.242 mmol, 66% yield from **20**).

5: Colorless needles. mp 86—88 °C (from ether). IR (film) cm^{-1}: 3300—2500 (br), 2216, 1697. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.90 (3H, t, J=7.1 Hz), 1.22—1.40 (4H), 1.45—1.68 (4H), 1.68—1.80 (2H), 2.28 (2H, t, J=7.1 Hz), 2.33 (2H, t, J=7.0 Hz), 2.38 (2H, t, J=7.2 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δc: 178.7, 79.6, 78.2, 66.2, 65.6, 60.7, 60.2, 33.3, 31.0, 27.8, 27.4, 23.8, 22.7, 19.4, 19.2, 13.9. EI-MS *m/z* (%): 244 (M^+, 3), 128 (100). High-resolution EI-MS *m/z*: Calcd for $C_{16}H_{20}O_2$: 244.1463. Found: 244.1471 [M^+].

Hexadeca-8,10,12-triynoic Acid (6) To a solution of undeca-8,10-diynol (**21**, 50 mg, 0.305 mmol) and 1-iodopent-1-yne (**22**, 71 mg) in pyrrolidine (1.0 ml) was added CuI (5.8 mg, 0.031 mmol) and the whole was stirred at room temperature for 30 min. The reaction mixture was treated with aqueous saturated NH_4Cl and extracted with EtOAc. The EtOAc extract was washed with brine and dried over $MgSO_4$. Removal of the solvent gave hexadeca-8,10,12-triynol (85 mg), which was purified by silica gel column chromatography (SiO_2 10 g, hexane : EtOAc=2 : 1) to afford hexadeca-8,10,12-triynol (63 mg, 0.27 mmol). To a solution of hexadeca-8,10,12-triynol (63 mg, 0.27 mmol) in acetone (1.0 ml) was added the chromic acid reagent[8] (0.28 ml, 0.55 mmol) and stirred at −10 °C for 15 min. The solution mixture was treated with 2-propanol and extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (73 mg). Purification of the product by silica gel column chromatography (SiO_2 10 g, $CHCl_3$: MeOH=30 : 1) and HPLC (Wakosil 5 SIL, hexane : EtOAc=4 : 1) afforded hexadeca-8,10,12-triynoic acid (**6**, 49 mg, 0.201 mmol, 66% yield from **21**).

6: Colorless needles. mp 70—72 °C (from ether). IR (film) cm^{-1}: 3300—2500 (br), 2216, 1695. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.99 (3H, t, J=7.3 Hz), 1.30—1.47 (4H), 1.50—1.65 (6H), 2.26 (2H, t, J=7.0 Hz), 2.29 (2H, t, J=7.0 Hz), 2.36 (2H, t, J=7.4 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δc: 179.1, 79.3, 79.0, 65.9, 65.8, 60.5, 60.3, 33.8, 28.5, 28.4, 27.8, 24.5, 21.6, 21.4, 19.3, 13.5. EI-MS *m/z* (%): 244 (M^+, 2.4), 128 (100). High-resolution EI-MS *m/z*: Calcd for $C_{16}H_{20}O_2$ 244.1463. Found: 244.1462 [M^+].

The detail of invasion assay procedure was described in our previous paper.[1]

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