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Synthesis of Novel Macrolactam and Macroketone Analogues of Migrastatin from D-Glucal and Comparison with Macrolactone and Acyclic Analogues: A Dorrigocin A Congener Is a Potent Inhibitor of Gastric Cancer Cell Migration

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Novel macrolactam and macroketone analogues of the migrastatin macrolide core have been synthesised from tri-*O*-acetyl-D-glucal in order to facilitate structure-activity studies. The Horner olefination, followed by ring-closing metathesis were key steps in the synthesis of the macroketone. The ability of the macroketone and macrolactam derivatives to inhibit the migration of gastric tumour cells as determined using a transwell migration assay were compared with macrolactone analogues and dorrigocin A analogues. One dorrigocin A congener was the most potent inhibitor of gastric cancer cell migration.

Introduction

Cell migration is involved in physiological[1] and pathological processes. The latter includes tumour angiogenesis, cancer cell invasion and metastasis, and therefore selective inhibitors have potential as new therapies for cancer. Migrastatin (**1**),[2] a natural product derived from isomigrastatin, has shown selective inhibition of tumour cell migration.[3] The synthesis of a number of analogues of **1** by the Danishefsky group led to the identification of analogues, such as **3**, that are more potent than migrastatin in migration assays in vitro.[4] The macrolactam **4a** and macroketone **4b**, close structural analogues to **3** (Figure 1) prepared also by the Danishefsky group, inhibit the metastasis of highly metastatic mammary carcinoma cells in mouse models.[5] They also inhibit metastasis of breast cancer cells, prostate cancer cells, and colon cancer cells but not normal mammary-gland epithelial cells, fibroblasts or leukocytes, indicating they are specific inhibitors of tumour metastasis. More recently, quinic acid based macrolides, which possibly have structural relationships to isomigrastatin, have been synthesized that inhibit murine 4T1 breast tumour cell migration in vitro.[6] Another natural product, derived from isomigrastatin is dorrigocin A (**2**), which is related to migrastatin by hydrolysis of the lactone and isomerisation of one alkene group. Dorrigocin A displays interesting biological properties, inhibiting the carboxymethyltransferase involved in Ras processing[7] and reversing the morphology of ras-transformed NIH/3T3 cells.[8] The synthesis of the C-1 to C-13 fragment of 2,3-dihydrodorrigocin A was achieved,[9] but the biological evaluation of this fragment or other related fragments has not been described. Recently, novel analogues of migrastatin, **5** and **6**, were prepared from D-glucal, a precursor that also facilitated the preparation of novel analogues of dorrigocin A, **7**–**9**. The novel analogue **5** differs from the migrastatin macrolide core **3** in that it lacks the methyl substituent at C-12, contains an acetoxy group at C-10 with opposite configuration to that in the natural product, and the configuration of the methoxy group at C-8 is inverted. The analogue **6** differs from **5** in that it contains a hydroxy group at C-10 and has the same configuration at C-8 as **3**. The effects of modifications from C-6 to C-12 in the macrocyclic scaffold have not been explored apart from at C-9, which were investigated by Danishefsky and co-workers.[4] Acyclic derivative **9** is analogous to the C-1 to C-13 fragment of dorrigocin A, the major differences being that there is no C-2 alkene, that **9** lacks the methyl group at C-12 and contains a hydroxy group rather than a methyl group at C-10, the hydroxy group having opposite configuration to that in **2**.[4] Compounds **8** and **9** are protected variants of **7**. Preliminary biological evaluation of **5**–**9** indicated that these compounds were less potent than **3** and **4** as inhibitors of breast tumour (4T1) cell migration,

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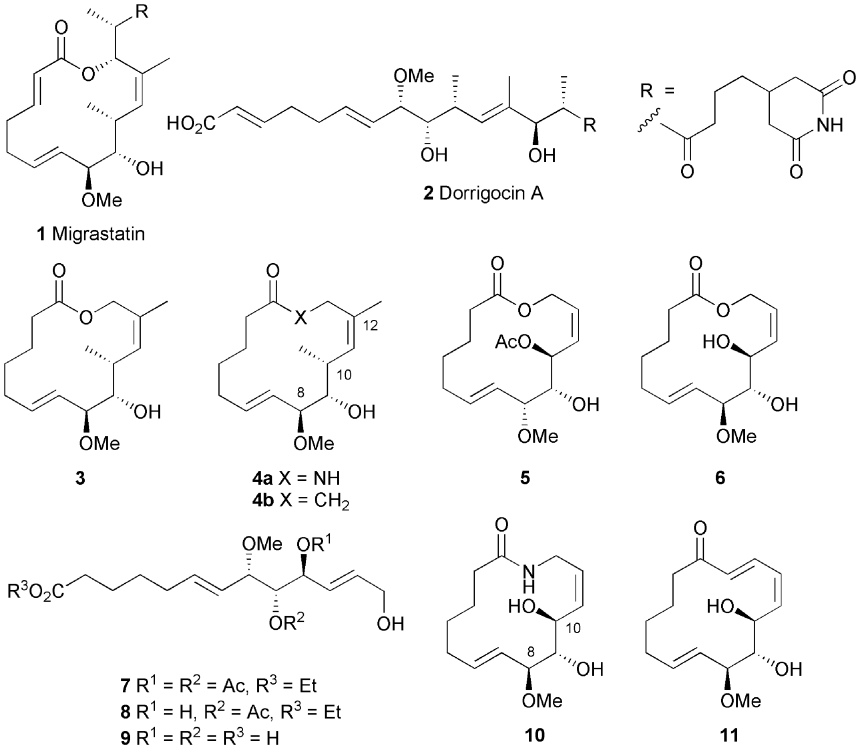
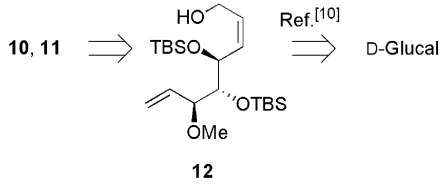


Figure 1. Structures of migrastatin, dorrigocin A and analogues.

although the dorrigocin A analogues **7**–**9** had similar potency to the migrastatin analogues **5** and **6**. Herein we describe the synthesis of the novel macrolactam and macroketone derivatives **10** and **11** from D-glucal in order to further contribute to the structure-activity studies.[10] The macroketone synthesized herein is different to those macroketone analogues synthesized previously in that it can be considered a macrocyclic dienone. Evaluation of **5**–**11** as inhibitors of migration of gastric cancer cells showed that the dorrigocin A analogue **9** is the more potent inhibitor.

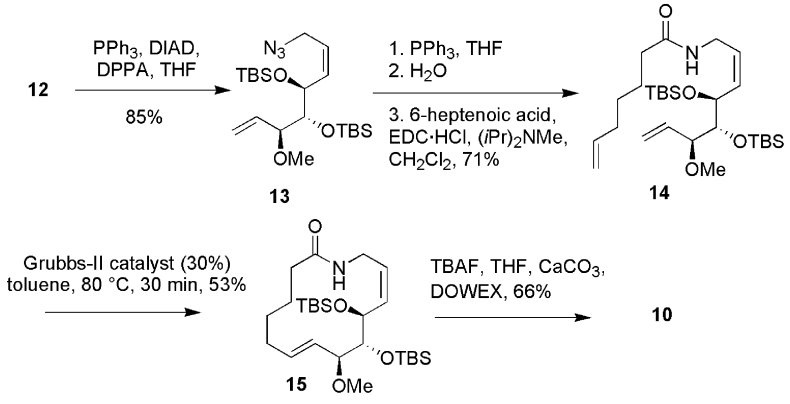
Results and Discussion

The acyclic compound **12**, obtained from D-glucal previously[10] was proposed as the key intermediate for the preparation of both **10** and **11** (Scheme 1).



Scheme 1. Retrosynthetic analysis of macrolide precursors from D-glucal.

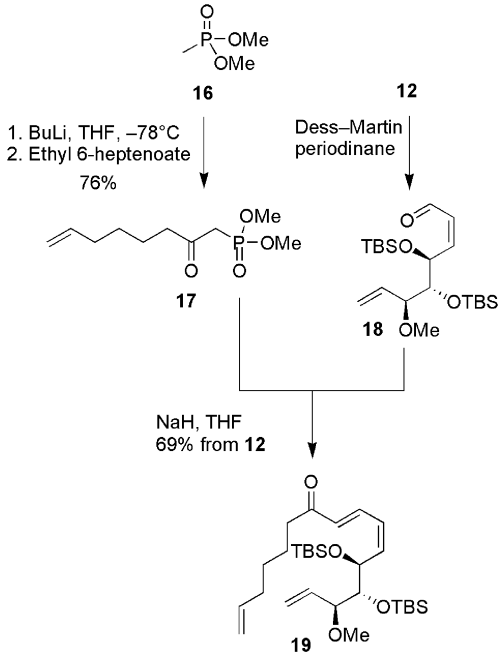
The reaction of **12**,[10] with diphenylphosphoryl azide in the presence of triphenylphosphane and diisopropylazodicarboxylate (DIAD) led to the exchange of the free hydroxy group for an azide group and gave **13** in 85% yield (Scheme 2). The azide **13** was reduced using the Staudinger



Scheme 2. Synthesis of **10**.

reaction, and the resulting amine was coupled to 6-heptenoic acid using EDC in the presence of a base to give **14**. The ring-closing metathesis (RCM)[11] of **14** gave **15** in 53% yield using the Grubbs 2nd-generation catalyst in toluene at 80 °C after 30 min. The yields are lower than observed during the synthesis of the related macrolactone derivative **6**,[10] possibly due to the more polar amide group deactivating the catalyst somewhat. Other conditions for the RCM reaction were attempted, but these conditions were most suitable; increased or reduced reaction times led to lower yields. The increased steric bulk in the environment of the (*Z*)-alkene provided by the TBS groups in **14** is considered important in blocking metathesis processes[12] at this alkene.[10] The TBS groups were finally removed from **15** to give **16** (66%) using TBAF/THF as recently described by Kaburagi and Kishi.[13]

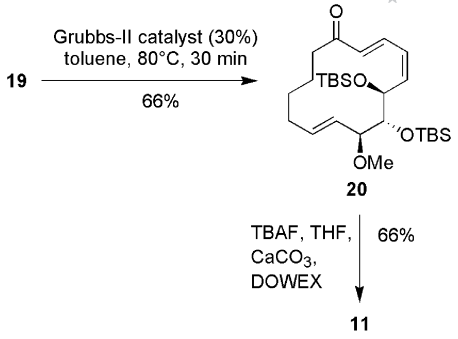
Next, the synthesis of the macroketone **11** was carried out. First of all, the β-oxophosphonate **17** was prepared in 76% yield by the treatment of **16** with butyllithium at –78 °C and subsequent reaction of the resulting anion with ethyl 6-heptenoate. The Horner reaction[14] of **17** with aldehyde **18**, freshly prepared by the oxidation of **12** using the Dess–Martin reagent, gave the (*E*,*Z*)-diene **19** (69%) from **12**. The aldehyde **18** was not purified before carrying out the olefination reaction (Scheme 3).



Scheme 3. Synthesis of **19**.

The ring-closing metathesis of **19** using the Grubbs 2nd-generation catalyst in toluene at 80 °C after 30 min gave the macrolactone **20** in 66% yield. The TBS groups were removed from **20** to give the desired macroketone **11** (Scheme 4) using TBAF/THF as described above.[13]

The newly synthesised compounds **10** and **11** were evaluated for their effects on proliferation and migration of gastric tumour cells, and these compounds were compared with the macrolactones and dorrigocin A analogues synthesised previously (**5**–**9**). None of the compounds **5**–**11** inhibited proliferation (MTS assay, Promega Corp) of gastric cancer cells. Conversely, a number of migrastatin and dorrigocin A analogues inhibited migration of AGS gastric cancer cells in a trans-well cellular locomotion assay and the results are summarised in Table 1; all compounds were tested at concentrations of 10 nM to 50 μM. Compounds **5**, **7** and **9** showed a dose-dependant inhibition of migration with the dorrigocin analogue **9** being the more potent compound at concentrations below 1 μM. Unlike **5** and **7**, dorrigocin A analogue **9** did not inhibit migration at concentrations above 1 μM. The macrolactam **10** was active only at 1 μM where it caused 50% inhibition of migration; macroketone **11** was also active only at a concentration of 1 μM where it inhibited migration by 38%. Compounds **6** and **8** were inactive at all concentrations. The reasons for the various effects observed (e.g. why **8** is inactive, whereas both **7** and **9** are active, or why dorrigocin derivative **9** is the most potent) are as yet unclear and will require further detailed pharmacological investigation.



Scheme 4. Synthesis of macroketone **11**.

Table 1. Inhibition of the migration of AGS gastric cancer cells.

Compound	IC_{50} [μM]
5	17
6	not active
7	29
8	not active
9	0.032[a]
10	50% at 1 μM[a]
11	38% at 1 μM[a]

[a] Compounds **9**–**11** were inactive at concentrations >1 μM.

Conclusion

The synthesis of novel macrolactam and macroketone analogues of migrastatin have been completed from D-glucal. The overall yield of these derivatives is 1.5% after 17 or 18 steps. The biological evaluation of these compounds as well as previously synthesized macrolactones and dorrigocin A analogues show that the compounds inhibit AGS gastric cancer cell migration. One novel dorrigocin A derivative was a potent inhibitor (IC_{50} = 32 nM) of mi-

gration of these tumour cells, indicating that dorrigocin A analogues have potential as *anti*-metastatic agents and that they could be evaluated in parallel to macrolactone derivatives. Further work to establish the biological mechanism of action, with a view to verifying if dorrigocin A and migrastatin derivatives have similar modes of action are underway and will be reported in due course.

Experimental Section

General Experimental Conditions: The general experimental conditions were the same as those described previously.[10]

(2*Z*,4*S*,5*R*,6*R*)-4,5-Bis(*tert*-butyldimethylsilanyloxy)-6-methoxyocta-2,7-diene 1-Azide (13): A mixture of the alcohol **12** (80 mg, 0.192 mmol) and triphenylphosphane (76 mg, 0.29 mmol) in THF (5 mL) was stirred at 0 °C, and diisopropyl azodicarboxylate (55 μL, 0.28 mmol) was then added dropwise. After 20 min, diphenylphosphoryl azide (50 μL, 0.232 mmol) was added, and the mixture was stirred at room temp. After 5 h, the reaction was quenched with solid NH_4Cl and the mixture then extracted with EtOAc (3 × 15 mL). The organic layers were combined, dried ($MgSO_4$), and the solvent was removed under diminished pressure. Chromatography of the residue (EtOAc/cyclohexane, 1:99; R_f = 0.25) gave **13** as colourless oil (72 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.75 (ddt, $J_{3,2}$ = 11.1, $J_{3,4}$ = 9.5, $J_{3,1a}$ = $J_{3,1b}$ = 1.3 Hz, 1 H, 3-H), 5.69 (ddd, $J_{7,8b}$ = 17.4, $J_{7,8a}$ = 10.3, $J_{7,6}$ = 8.0 Hz, 1 H, 7-H), 5.52 (dt, $J_{2,3}$ = 11.3, $J_{2,1}$ = 7.0 Hz, 1 H, 2-H), 5.29 (dd, $J_{8a,7}$ = 10.3, $J_{8a,8b}$ = 1.6 Hz, 1 H, 8a-H), 5.23 (dd, $J_{8b,7}$ = 17.4, $J_{8a,8b}$ = 0.9 Hz, 1 H, 8b-H), 4.42 (dd, $J_{4,3}$ = 9.4, $J_{4,5}$ = 2.9 Hz, 1 H, 4-H), 3.92 (ddd, $J_{1a,1b}$ = 14.2, $J_{1a,2}$ = 7.8, $J_{1a,3}$ = 1.1 Hz, 1 H, 1a-H), 3.76 (ddd, $J_{1b,1a}$ = 14.3, $J_{1b,2}$ = 6.2, $J_{1b,3}$ = 1.3 Hz, 1 H, 1b-H), 3.67 (dd, $J_{5,6}$ = 6.4, $J_{5,4}$ = 3.3 Hz, 1 H, 5-H), 3.41 (br. t, $J_{6,7}$ = $J_{6,5}$ = 7.2 Hz, 1 H, 6-H), 3.23 (s, 3 H, OCH_3), 0.90, 0.89, 0.87 [3 s, 18 H, $C(CH_3)_3$], 0.07, 0.06, 0.05, 0.03 (4 s, 12 H, $SiCH_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 135.4 (C-7), 134.5 (C-3), 123.7 (C-2), 118.9 (C-8), 84.1 (C-6), 79.4 (C-5), 69.1 (C-4), 56.3 (OCH_3), 48.4 (C-1), 26.1, 25.9 [2 C(CH_3)$_3$], 18.2 [C(CH_3)$_3$], –4.2, –4.2, –4.3, –4.7 (4 SiCH_3) ppm. IR (thin film): $\tilde{\nu}_{max}$ = 2955, 2930, 2888, 2858, 2098, 1472, 1254, 1148, 1078, 835, 777 cm^{-1}. HR-ESMS: calcd. for $C_{21}H_{43}O_3N_3Si_2Na$ [M + Na]$^+$ 464.2741, found 464.2758. $[\alpha]_D$ = +34.3 (c = 0.85, $CHCl_3$).

(2*Z*,4*S*,5*R*,6*R*)-*N*-[4,5-bis(*tert*-butyldimethylsilyloxy)octa-6-methoxy-2,7-dien-1-yl]hept-6-enamide (14): To **13** (50 mg, 0.11 mmol) in THF (8 mL), triphenylphosphane (66 mg, 0.25 mmol) was added, and the resulting mixture was stirred at 70 °C for 3 h, and then water (100 μL) was added. After 30 min, the reaction mixture was extracted with EtOAc (3 × 15 mL), the organic layers were combined, washed with brine, dried ($MgSO_4$), and the solvent was removed under diminished pressure to give a residual oil. To a solution of this oil in CH_2Cl_2 (10 mL) 6-heptenoic acid (30 μL, 0.22 mmol), diisopropyl(methyl)amine (120 μL, 0.69 mmol) and EDC·HCl (41 mg, 0.21 mmol) were added. After 24 h, the solvent was removed under diminished pressure, and the residue was purified chromatography (EtOAc/cyclohexane, 2:8; R_f = 0.15) to afford **14** as a colourless oil (42 mg, 71%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.79 (ddt, J = 17.0, J = 10.2, J = 6.8 Hz, 1 H, CH), 5.73 (ddd, $J_{7,8b}$ = 17.9, $J_{7,8a}$ = 10.0, $J_{7,6}$ = 8.0 Hz, 1 H, 7-H), 5.63 (t, $J_{3,2}$ = $J_{3,4}$ = 10.3 Hz, 1 H, 3-H), 5.58 (s, 1 H, NH), 5.46 (dt, $J_{2,3}$ = 11.2, $J_{2,1}$ = 7.0 Hz, 1 H, 2-H), 5.28 (d, $J_{8a,7}$ = 10.3 Hz, 1 H, 8a-H), 5.23 (d, $J_{8b,7}$ = 17.3 Hz, 1 H, 8b-H), 5.00 (d, J = 17.1 Hz, 1 H, $CH_2CH{=}CHH_E$), 4.94 (d, J = 10.2 Hz, 1 H, $CH_2CH{=}CHH_Z$), 4.54 (dd, $J_{4,3}$ = 9.5, $J_{4,5}$ = 4.0 Hz, 1 H, 4-H), 3.87 (m, 2 H, 1-H), 3.65 (dd, $J_{5,6}$ = 5.4, $J_{5,4}$ = 4.2 Hz, 1 H, 5-H), 3.48 (dd, $J_{6,7}$ = 7.3, $J_{6,5}$ = 6.2 Hz, 1 H, 6-H), 3.24 (s, 3 H, OCH_3), 2.16 (t, J = 7.6 Hz, 2 H, CH_2), 2.06 (m, 2 H, CH_2), 1.65 (m, 2 H, CH_2), 1.42 (m, 2 H, CH_2), 0.90, 0.87 [2 s, 18 H, 2 C(CH_3)$_3$], 0.07, 0.06, 0.04, 0.01 (4 s, 12 H, 4 $SiCH_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.6 (C=O), 138.5 (CH), 135.7 (C-7), 133.8 (C-3), 126.2 (C-2), 118.8 (C-8), 114.6 (CH_2), 83.9 (C-6), 79.3 (C-5), 68.7 (C-4), 56.3 (OCH_3), 37.4 (C-1), 36.5 (CH_2), 33.5 (CH_2), 28.6 (CH_2), 26.1, 25.9, [2 C(CH_3)$_3$], 25.2 (CH_2), 18.4, 18.2 [2 C(CH_3)$_3$], –4.0, –4.2, –4.2, –4.6 (4 $SiCH_3$) ppm. IR(thin film): $\tilde{\nu}_{max}$ = 3291, 3078, 2953, 2931, 2894, 2857, 1642, 1549, 1472, 1251, 1148, 1075, 835, 777 cm^{-1}. HR-ESMS: calcd. for $C_{28}H_{56}NO_4Si_2$ [M + H]$^+$ 526.3748, found 526.3723. $[\alpha]_D$ = +4.2 (c = 0.85, $CHCl_3$).

(7*E*,9*R*,10*R*,11*S*,12*Z*)-10,11-Bis(*tert*-butyldimethylsilyloxy)-9-methoxy-1-azacyclotetradeca-7,12-dien-2-one (15): A solution of **14** (18 mg, 34 mmol) in anhydrous toluene (100 mL) was thoroughly degassed and heated at 80 °C. The Grubbs 2nd-generation catalyst (8.3 mg, 9.8 mmol) was dissolved in dry toluene and added through a cannula, and the mixture was heated at 80 °C for 30 min and then filtered through silica and the solvent removed under diminished pressure. Chromatography (EtOAc/cyclohexane, 7:3; R_f = 0.45) gave **15** as a colourless oil (9 mg, 53%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.36 (br. s, 1 H, NH), 5.82 (dt, $J_{6,7}$ = 15.0, $J_{6,5}$ = 7.4 Hz, 1 H, 6-H), 5.70–5.50 (overlapping signals, 3 H, 7-H, 11-H, 12-H), 4.71 (dd, $J_{10,11}$ = 8.9, $J_{10,9}$ = 6.5 Hz, 1 H, 10-H), 3.96 (dt, $J_{13a,13b}$ = 15.5, $J_{13a,12}$ = 4.5 Hz, 1 H, 13a-H), 3.83 (dd, $J_{9,10}$ = 6.4, $J_{9,8}$ = 4.3 Hz, 1 H, 9-H), 3.78 (dt, $J_{13b,13a}$ = 15.7, $J_{13b,12}$ = 5.3 Hz, 1 H, 13b-H), 3.67 (dd, $J_{8,7}$ = 8.2, $J_{8,9}$ = 4.2 Hz, 1 H, 8-H), 3.27 (s, 3 H, OCH_3), 2.33 (t, $J_{2,3}$ = 7.1 Hz, 2 H, 2-H), 2.17 (m, 2 H, 5-H), 1.71 (m, 2 H, 3-H), 1.51 (m, 2 H, 4-H), 0.90, 0.87 [2 s, 18 H, 2 C(CH_3)$_3$], 0.13, 0.10, 0.06, 0.03 (4 s, 12 H, 4 SiCH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.6 (C=O), 136.2 (C-6), 135.5 (C-7), 127.5 (C-11), 125.1 (C-12), 85.8 (C-8), 76.9 (C-9), 68.3 (C-10), 56.5 (OCH_3), 37.6 (C-13), 36.0 (C-2), 31.3 (C-5), 26.6 (C-4), 26.0, 25.9 [2 C(CH_3)$_3$], 24.5 (C-3), 18.3, 18.2 [2 C(CH_3)$_3$], –4.0, –4.3, –4.6 (4 $SiCH_3$) ppm. IR (thin film): $\tilde{\nu}_{max}$ = 3446, 3295, 2953, 2928, 2886, 2856, 1637, 1252, 1096, 1071, 835, 776 cm^{-1}. HR-ESMS: calcd. for $C_{26}H_{52}NO_4Si_2$ [M + H]$^+$ 498.3435, found 498.3417. $[\alpha]_D$ = –26 (c = 0.40, $CHCl_3$).

(7*E*,9*S*,10*R*,11*S*,12*Z*)-10,11-Dihydroxy-9-methoxy-1-azacyclotetradeca-7,12-dien-2-one (10): To a solution of **15** (25 mg, 0.05 mmol) in THF (2 mL), TBAF (150 μL of a 1 M solution) was added, and the resulting mixture was stirred at room temp. for 30 min. Calcium carbonate (100 mg), Dowex 50WX8-100 (300 mg) and MeOH (1 mL) were then added to the mixture, and stirring was continued for a further 30 min. Dilution with EtOAc (15 mL) was followed by filtration of the mixture through silica, and the solvent was removed under diminished pressure. Chromatography (CH_2Cl_2/MeOH, 100:0 to 97:3) gave **10** as a colourless oil (9.0 mg, 66%); R_f = 0.20 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.75–5.85 (overlapping signals, 2 H, 7-H, 13-H), 5.73 (t, $J_{12,13}$ = $J_{12,11}$ = 10.0 Hz, 1 H, 12-H), 5.36 (dd, $J_{8,7}$ = 15.6, $J_{8,9}$ = 7.8 Hz, 1 H, 8-H), 4.50 (dd, $J_{11,12}$ = 8.5, $J_{11,10}$ = 5.8 Hz, 1 H, 11-H), 3.87 (dd, $J_{14a,14b}$ = 15.2, $J_{14a,13}$ = 7.6 Hz, 1 H, 14a-H), 3.77 (dd, $J_{14b,14a}$ = 15.2, $J_{14b,13}$ = 6.4 Hz, 1 H, 14b-H), 3.65–3.70 (m, 2 H, 9-H, 10-H), 3.28 (s, 3 H, OCH_3), 2.00–2.20 (m, 4 H, 3-H, 6-H), 1.60–1.65 (m, 2 H, 4-H), 1.50–1.57 (m, 1 H, 5a-H), 1.35–1.45 (m, 1 H, 5b-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.8 (C=O), 137.2 (C-7), 132.5 (C-12), 127.9 (C-13), 126.3 (C-8), 84.7 (C-9), 73.0 (C-10), 67.1 (C-11), 56.3 (OCH_3), 36.9 (C-14), 35.5 (C-3), 30.3 (C-6), 27.0 (C-5), 23.6 (C-4) ppm. IR (thin film): $\tilde{\nu}_{max}$ = 3428, 2937, 1633,

1449, 1078, 1034 cm^{-1}. HR-ESMS: calcd. for $C_{14}H_{24}NO_4$ [M + H]$^+$ 270.1705, found 270.1700. $[a]_D$ = –18 (c = 0.40, CH_3OH).

(3*S*,4*R*,5*S*,6*Z*,8*E*)-4,5-Bis(*tert*-butyldimethylsilyloxy)-3-methoxyhexadeca-1,6,8,15-tetraen-10-one (19): To a solution of **12** (45 mg, 0.11 mmol) in CH_2Cl_2 (4 mL) Dess–Martin periodinane (65 mg, 0.15 mmol) was added, and the resulting mixture was stirred at room temp for 2 h. Sodium thiosulfate and $NaHCO_3$ were added, and the mixture was then extracted with EtOAc (3 × 15 mL). The organic layers were combined, dried ($MgSO_4$), and the solvent was removed under diminished pressure to give aldehyde **18** as a colourless oil (45 mg). At the same time, butyllithium (2.0 mL, 3.2 mmol) was added to a solution of dimethyl methylphosphonate (474 μL, 4.37 mmol) in THF (5 mL) at –78 °C, and ethyl 6-heptenoate (230 μL, 1.31 mmol) was added after 15 min. The resulting mixture was stirred at 0 °C for 15 min. The reaction was quenched by the addition of NH_4Cl and the mixture extracted with EtOAc (3 × 15 mL). The combined organic layers were dried ($MgSO_4$), and the solvent was removed under diminished pressure. Chromatography of the residue (EtOAc/cyclohexane, 3:7; R_f = 0.35) gave the phosphonate **17** as a yellow oil (234 mg, 76%). To a solution of **17** (138 mg, 0.59 mmol) in THF (7 mL) at 0 °C, sodium hydride (18.5 mg, 0.46 mmol) was added, and the resulting mixture was stirred at 0 °C for 30 min. The freshly prepared aldehyde **18** (45 mg) in THF (3 mL) was then added through a cannula and stirring continued at room temp. for 20 h. The reaction was quenched by the addition of NH_4Cl and the mixture extracted with Et_2O (3 × 15 mL). The combined organic layers were dried ($MgSO_4$), and the solvent was removed under diminished pressure. Chromatography of the residue (EtOAc/cyclohexane, 1:19; R_f = 0.30) gave the title compound **19** as a colourless oil (39 mg, 69%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36 (ddd, $J_{8,9}$ = 15.5, $J_{8,7}$ = 11.5, $J_{8,6}$ = 0.9 Hz, 1 H, 8-H), 6.15–6.10 (m, 2 H, 9-H, 7-H), 5.96 (t, $J_{6,7}$ = $J_{6,5}$ = 10.3 Hz, 1 H, 6-H), 5.80 (ddt, $J_{15,16a}$ = 16.9, $J_{15,16b}$ = 10.2, $J_{15,14}$ = 6.7 Hz, 1 H, 15-H), 5.66 (ddd, $J_{2,1a}$ = 17.3, $J_{2,1b}$ = 10.3, $J_{2,3}$ = 8.3 Hz, 1 H, 2-H), 5.32 (dd, $J_{1b,2}$ = 10.4, $J_{1b,1a}$ = 1.9 Hz, 1 H, 1b-H), 5.15 (dd, $J_{1a,2}$ = 17.3, $J_{1a,1b}$ = 1.8 Hz, 1 H, 1a-H), 5.00 (dd, $J_{16a,15}$ = 17.1, $J_{16a,16b}$ = 3.6 Hz, 1 H, 16a-H), 4.95 (dd, $J_{16b,15}$ = 10.2, $J_{16b,16a}$ = 2.2 Hz, 1 H, 16b-H), 4.69 (dd, $J_{5,6}$ = 9.5, $J_{5,4}$ = 3.0 Hz, 1 H, 5-H), 3.75 (dd, $J_{4,3}$ = 6.9, $J_{4,5}$ = 2.9 Hz, 1 H, 4-H), 3.34 (dd, $J_{3,2}$ = 8.2, $J_{3,4}$ = 7.0 Hz, 1 H, 3-H), 3.21 (s, 3 H, OCH_3), 2.56 (t, $J_{11a,12}$ = 7.3 Hz, 1 H, 11a-H), 2.55 (t, $J_{11b,12}$ = 4.2 Hz, 1 H, 11b-H), 2.07 (m, 2 H, 14-H), 1.65 (m, 2 H, 12-H), 1.43 (m, 2 H, 13-H), 0.89, 0.87 [2 s, 18 H, 2 C(CH_3)$_3$], 0.08, 0.07, 0.04, 0.00 (4 s, 12 H, 4 SiCH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 200.5 (C=O), 140.4 (C-15), 137.6 (C-8), 135.0 (C-2), 130.9 (C-9, C-7), 127.3 (C-6), 119.7 (C-1), 114.6 (C-16), 84.4 (C-3), 79.6 (C-4), 69.4 (C-5), 56.2 (OCH_3), 40.3 (C-11), 33.6 (C-14), 28.6 (C-13), 26.1, 25.9 [2 C(CH_3)$_3$], 23.8 (C-12), 18.4, 18.2 [2 *C*(CH_3)$_3$], –4.2, –4.4, –4.6 (4 SiCH_3) ppm. IR (thin film): $\tilde{\nu}_{max}$ = 3078, 2954, 2929, 2888, 2857, 1668, 1604, 1472, 1253, 1150, 1076, 835, 777 cm^{-1}. HR-ESMS: calcd. for $C_{29}H_{55}O_4Si_2Na$ [M + H]$^+$ 523.3639, found 523.3625. $[a]_D$ = +30.9 (c = 0.5, $CHCl_3$).

(2*E*,4*Z*,6*S*,7*R*,8*S*,9*Z*)-6,7-Bis(*tert*-butyldimethylsilyloxy)-8-methoxycyclotetradeca-2,4,9-triene-1-one (20): A solution of the tetraene **19** (8 mg, 15 μmol) in dry toluene (40 mL) was thoroughly degassed and then heated to 80 °C. The Grubbs 2nd-generation catalyst (4.1 mg, 4.8 μmol) was dissolved in dry toluene and then added to the flask containing **19** through a cannula and the mixture heated at 80 °C for 30 min. The solution was then filtered through silica gel and the solvent removed under diminished pressure. Chromatography of the residue (EtOAc/cyclohexane, 1:9; R_f = 0.20) gave **20** as a colourless oil (5.0 mg, 66%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.30 (dd, $J_{3,2}$ = 16.4, $J_{3,4}$ = 11.4 Hz, 1 H, 3-H), 6.19 (t, $J_{4,5}$ = $J_{4,3}$ = 11.0 Hz, 1 H, 4-H), 6.02 (t, $J_{5,4}$ = $J_{5,6}$ = 10.2 Hz, 1 H, 5-H), 5.91 (d, $J_{2,3}$ = 16.2 Hz, 1 H, 2-H), 5.54 (ddd, $J_{10,9}$ = 15.4, $J_{10,11a}$ = 9.0, $J_{10,11b}$ = 4.0 Hz, 1 H, 10-H), 5.37 (dd, $J_{9,10}$ = 15.7, $J_{9,8}$ = 8.9 Hz, 1 H, 9-H), 4.68 (dd, $J_{6,5}$ = 9.4, $J_{6,7}$ = 2.3 Hz, 1 H, 6-H), 3.83 (dd, $J_{7,8}$ = 8.1, $J_{7,6}$ = 2.5 Hz, 1 H, 7-H), 3.18 (s/m, 3/1 H, OCH_3/8-H), 2.88 (m, 1 H, 14a-H), 2.38–2.11 (m, 3 H, 14b-H, 11-H), 1.80–1.75 (m, 2 H, 13-H), 1.60–1.55 (m, 2 H, 12-H), 0.90, 0.89, 0.87 [3 s, 18 H, 3 C(CH_3)$_3$], 0.09, 0.08, 0.05, 0.02 (4 s, 12 H, 4 SiCH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 203.2 (C=O), 140.0 (C-5, C-3), 137.3 (C-10), 130.6 (C-2), 127.3 (C-9), 126.8 (C-4), 84.8 (C-8), 79.0 (C-7), 69.2 (C-6), 55.6 (OCH_3), 38.2 (C-14), 29.7 (C-11), 27.0 (C-12), 26.0, 25.9 [2 C(*C*H_3)$_3$], 24.9 (C-13), 18.5, 18.3 [2 *C*(CH_3)$_3$], –4.3, –4.4, –4.6 (4 SiCH_3) ppm. IR (thin film): $\tilde{\nu}_{max}$ = 2955, 2928, 2855, 1660, 1638, 1253, 1143, 1075, 835, 777 cm^{-1}. HR-ESMS: calcd. for $C_{27}H_{51}O_4Si_2$ [M + H]$^+$ 495.3326, found 495.3342. $[a]_D$ = +8.0 (c = 0.3, $CHCl_3$).

(2*E*,4*Z*,6*S*,7*R*,8*S*,9*Z*)-6,7-Dihydroxy-8-methoxycyclotetradeca-2,4,9-trien-1-one (11): To **20** (14.0 mg, 0.028 mmol) in THF (1.5 mL) was added TBAF (110 μL of a 1.0 M solution in THF), and the resulting mixture was stirred at room temp. for 15 min. Calcium carbonate (70 mg), Dowex 50WX8-100 (200 mg) and MeOH (1 mL) were then added, and the resulting mixture was stirred at room temp. for 30 min. Ethyl acetate (15 mL) was then added and the mixture then filtered through silica, and the solvent was removed under diminished pressure. Chromatography of the residue (EtOAc/cyclohexane, 7:3; R_f = 0.20) gave **11** as a colourless oil (5.0 mg, 66%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.33 (dd, $J_{3,2}$ = 16.2, $J_{3,4}$ = 11.2 Hz, 1 H, 3-H), 6.35 (t, $J_{4,5}$ = $J_{4,3}$ = 11.0 Hz, 1 H, 4-H), 6.02 (t, $J_{5,4}$ = $J_{5,6}$ = 10.2 Hz, 1 H, 5-H), 5.95 (d, $J_{2,3}$ = 16.1 Hz, 1 H, 2-H), 5.57 (ddd, $J_{10,9}$ = 15.4, $J_{10,11a}$ = 9.8, $J_{10,11b}$ = 4.0 Hz, 1 H, 10-H), 5.38 (ddd, $J_{9,10}$ = 15.5, $J_{9,8}$ = 8.8, $J_{9,11b}$ = 1.4 Hz, 1 H, 9-H), 4.74 (br. d, $J_{6,5}$ = 8.8 Hz, 1 H, 6-H), 3.86 (dd, $J_{7,8}$ = 8.1, $J_{7,6}$ = 4.3 Hz, 1 H, 7-H), 3.44 (t, $J_{8,9}$ = $J_{8,7}$ = 8.4 Hz, 1 H, 8-H), 2.93 (ddd, $J_{14a,14b}$ = 13.2, $J_{14a,13a}$ = 7.3, $J_{14a,13b}$ = 6.0 Hz, 1 H, 14a-H), 2.84 (br. s, 1 H, OH), 2.54 (br. s, 1 H, OH), 2.35–2.25 (m, 2 H, 14b-H, 11a-H), 2.15–2.05 (m, 1 H, 11b-H), 1.80–1.75 (m, 2 H, 2 13-H), 1.60–1.55 (m, 2 H, 2 12-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 203.0 (C=O), 139.7 (C-10), 139.3 (C-3), 136.8 (C-5), 131.2 (C-2), 129.5 (C-4), 126.4 (C-9), 84.1 (C-8), 75.2 (C-7), 68.0 (C-6), 56.2 (OCH_3), 39.2 (C-14), 30.7 (C-11), 26.6 (C-12), 25.1 (C-13) ppm. IR (thin film): $\tilde{\nu}_{max}$ = 2926, 2855, 1640, 1279, 1086, 1037, 976 cm^{-1}. HR-ESMS: calcd. for $C_{15}H_{22}O_4Na$ [M + Na]$^+$ 289.1416, found: 289.1403.

Cell Proliferation Assay: For the investigation of tumour cell proliferation, 5 × 10^4 AGS gastric cancer cells were added to each well of a 96-well plate and grown in the absence of migrastatin analogues for 24 h in Ham's F12 media. Analogues were dissolved in DMSO at 5 mM and added to the cells at the following concentrations: 10 nM, 100 nM, 1 μM and 50 μM in 100 μL media. Control samples were included to which no analogues were added. All treatments were repeated in triplicate. Following a 24 h incubation, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) (Promega Corp.) (20 μL) was added to each well and incubated under standard growth conditions of 37 °C and 5% CO_2 for 3 h.[15] Absorbance at 492 nm was detected using a SpectraMax-M5e (Molecular Devices) plate reader.

In Vitro Migration Assay: Cellular locomotion was assessed using a 96-well fluorescent trans-well assay (ECM510, Millipore Corp.).[16] Briefly, 1 × 10^5 AGS gastric cancer cells were seeded into each upper chamber containing 1% fetal bovine serum (FBS) in Ham's F12 media with or without the migrastatin analogues at the following concentrations: 10 nM, 100 nM, 1 μM and 50 μM. All treatments were

repeated in triplicate. Cells were allowed to migrate through 8 μM pores into a lower chamber containing 20% FBS with or without the analogues at the same concentrations in Ham's F12 media for 24 h. Migratory cells were lysed, stained and quantitated fluorescently using a SpectraMax-M5 plate reader (Molecular Devices Corp.).

Statistical Analysis: Mean values are expressed, obtained from triplicate experiments. Statistical significance, defined as $p < 0.05$, was detected using a students t-test.

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