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Synthesis of Migrastatin Analogues as Inhibitors of Tumour Cell Migration: Exploring Structural Change in and on the Macrocyclic Ring

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Abstract: Migrastatin and isomigrastatin analogues have been synthesised in order to contribute to structure–activity studies on tumour cell migration inhibitors. These include macrocycles varying in ring size, functionality and alkene stereochemistry, as well as glucuronides. The synthesis work included application of the Saegusa–Ito reaction for regio- and stereoselective unsaturated macroketone formation, diastereoselective Brown allylation to generate 9-methylmigrastatin analogues and chelation-induced anomerisation to vary glucuronide configuration. Compounds were tested in vitro against both breast and pancreatic cancer cell lines and inhibition of tumour cell migration was observed in both wound-healing (scratch) and Boyden chamber assays. One unsaturated macroketone showed low affinity for a range of secondary drug targets, indicating it is at low risk of displaying adverse side effects.

Introduction

Tumour metastasis is a process in which cancer cells detach from a primary tumour, migrate and then colonize a distant organ and the process leads to mortality for cancer patients.[1] Small molecules that interfere with tumour cell migration[2] have significant potential as anti-metastatic drugs[3] or as tools[4] for the study of tumour cell migration. Migrastatin (**1**), is a natural product that inhibits tumour cell migration (μM range) in vitro. Truncated[5] or simpler analogues of migrastatin, such as **2**, **3**, **MGSTA-4**, and **MGSTA-5** (Figure 1), prepared in Danishefsky's laboratory,[6] have been reported to show orders of magnitude higher activity than migrastatin itself in vitro and both **3** and **MGSTA-5**[7] and other compounds[8] have demonstrated inhibitory activity in vivo. We have been engaged in the synthesis of migrastatin analogues[9] and recently through collaboration with Anderson and Nobis have shown that macroketone **MGSTA-5** inhibited E-cadherin dynamics in vivo, in a manner consistent with increased cell adhesion and reduced invasive potential.[10] It has been reported that the target for

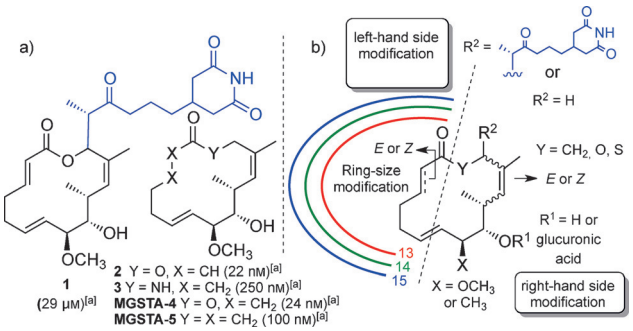

Figure 1. a) Migrastatin (**1**) and truncated analogues **2**–**3** IC_{50} values (in parentheses) in Boyden chamber assay against 4T1 mouse mammary cancer cells.[6] b) Modification of migrastatin core in this paper.

MGSTA-5 is fascin, which is recognized as being involved in cell motility and migration processes and is upregulated in many human tumours as well as in several cancer cell lines including breast,[11] pancreatic[12] and colon cancer cells.[13] Importantly, fascin is also involved in the chemotherapeutic resistance of breast[14] and colon[15] cancer cells. However, whether fascin is the target for **MGSTA-5** is still debated[16] and there is a belief that there may be other unidentified target(s) of migrastatin and its analogues.[16]

For these reasons the synthesis of analogues of migrastatin, including new analogues, continues to be of interest.[6–8,16–19] Here we report the preparation and migrastatin analogues shown in Figure 2. The modifications have included variation in macrocycle ring size and functionality within the ring as well as varying alkene geometry and glucuronidation. Included are

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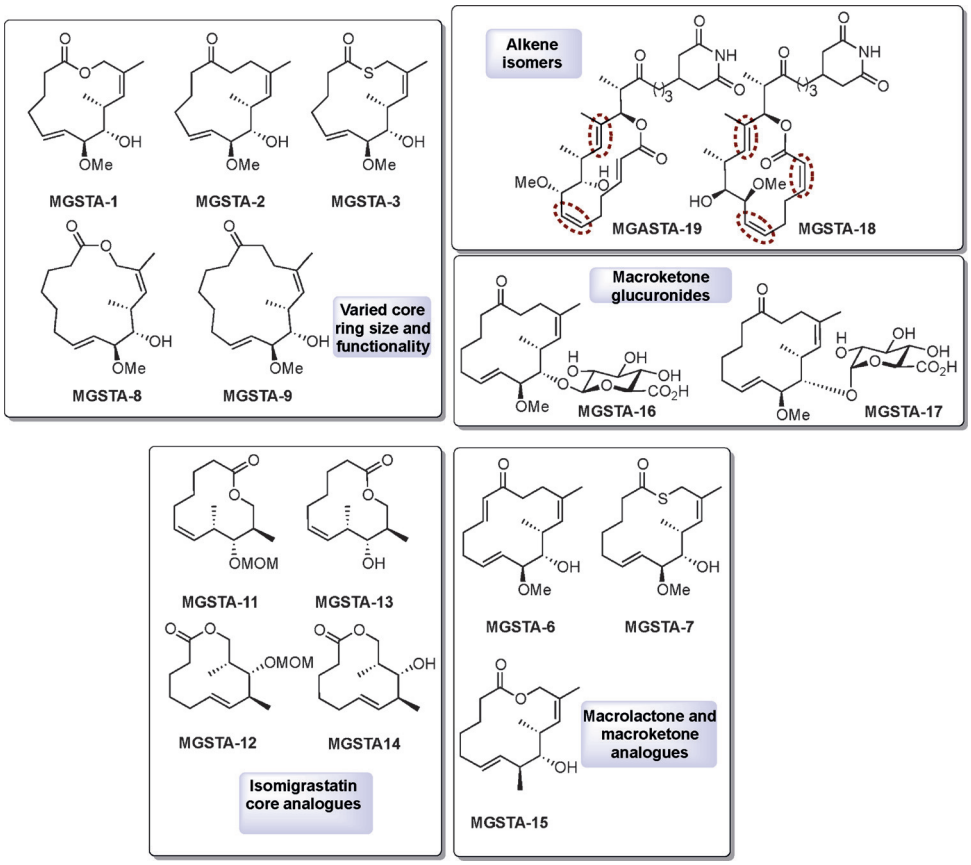


Figure 2. Migrastatin analogues prepared in this manuscript.

the syntheses of related isomigrastatin analogues. The synthesis of these various analogues will facilitate establishment of structure–activity relationships and in helping to try to identify more potent analogues. The activity of selected compounds in relevant bioassays was subsequently investigated and results of these are described. The project has also provided a good testing ground for synthetic methodology such as Brown allylation, the Saeguso–Ito reaction and Lewis acid promoted anomerisation.

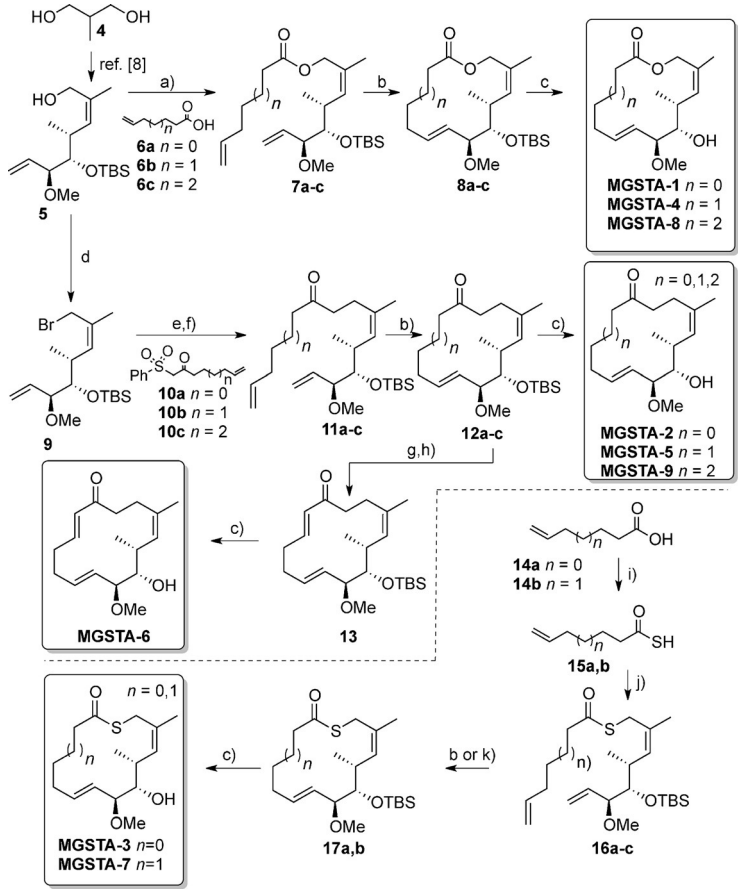
Results and Discussion

Modification of the macrocycle structure and ring size

The synthetic work began from intermediate **5**, which was prepared in ~two gram quantity using the route recently developed in our laboratory.[10] This intermediate was first used to prepare 13–15-membered ring structures based on macrolactones, macroketones and macrothiolactones. Firstly, the allylic alcohol **5** was coupled to carboxylic acids **6a–c** (Scheme 1) under Mitsunobu conditions and the esters formed were then treated with the Grubbs II metathesis catalyst to afford the macrolactones **8a–c** by ring-closing metathesis (RCM). Removal of the TBS group using HF·pyridine gave **MGSTA-4**[6] and its new 13- and 15-membered analogues **MGSTA-1** and **MGSTA-8**. Macroketones, were prepared by conversion of alcohol **6** into the allylic bromide **9** and subsequently reacting **9** with β-ketosulfones **10a–c** as previously described[6] to give **11a–c**. Ring-closing metathesis followed by TBS removal afforded **MGSTA-5**[6] and new analogues **MGSTA-2** and **MGSTA-9**. Macroketone **12b** was reacted with LHMDS and TMSCl to give an enolate which was then oxidized by Saegusa–Ito conditions to afford the α,β-unsaturated macroketone **13** which after desilylation gave **MGSTA-6**. Macrothiolactones were prepared by the Mitsunobu reaction of thioacids **15a–b** with the allylic alcohol **5**. Thioacids **15a** and **15b** were firstly readily prepared by treating the corresponding carboxylic acid with Lawesson's reagent under microwave irradiation.[20] The thioacids **15a–b** were not stable to silica gel and hence were instead purified by distillation under reduced pressure (Kugelrohr, $P = 50\times10^{-3}$ mbar, $T = 45\,^{\circ}C$, 2 h) or were used without distillation in the next step. Coupling of **15a–b** to **5** using Mitsunobu conditions, and subsequent RCM and TBS group removal afforded macrothiolactones **MGSTA-3** and **7**. The compounds **MGSTA-1** to **9**, were purified using flash chromatography (representative mobile phase: petroleum ether/ethyl acetate, 1:1). The purity of these macrocyclic agents could be increased by their distillation under reduced pressure (Kugelrohr, $P = 0.05$ mbar, $T = 90\,^{\circ}C$, distillation time from 2 h to 24 h).

Synthesis of isomigrastatin analogues

Isomigrastatin **18**, a natural product isolated from Streptomyces platensis[21] and a precursor to migrastatin, is a potent inhibitor of tumour cell migration.[17d] In view of this, we have used monoprotected diol **19**, readily available in four steps from **4**,[10] for the preparation of truncated isomigrastatins. Firstly the direct exchange of the OH in **19** for Br was attempted, but when **19** was treated with polymer-bound Ph_3P and CBr_4 in CH_2Cl_2, the methoxy group at C-4 migrated to the C-1 position giving allylic bromide **20** (68%), with no trace of the desired compound being found. Attempts to form the tosylate or chloride from **19** led to complex reaction mixtures. On the other hand, when **19** was coupled with carboxylic acid **6b** under Mitsunobu conditions, the desired ester **22** was isolated in a 57% yield together with a small amount of rearranged compound **21** (14%). With **22** in hand, RCM was next attempted. Unfortunately, reaction of **22** with the Grubbs II in toluene



Scheme 1. Synthesis of migrastatin core analogues: a) **6a**–**c**, Ph_3P, DIAD, $PhCH_3$, RT; **7a** (69%), **7c** (76%); b) Grubbs II catalyst, $PhCH_3$, reflux, **8a** (68%), **12a** (99%), **17a** (88%), **8c** (73%), **12c** (60%); c) HF·Py, THF, RT; **MGSTA-1** (61%), **MGSTA-2** (84%), **MGSTA-3** (85%), **MGSTA-6** (90%), **MGSTA-7** (63%), **MGSTA-8** (54%), **MGSTA-9** (82%); d) CBr_4, Ph_3P polymer-bound, CH_2Cl_2; e) **10a**–**c**, DBU, $PhCH_3$ then **9**, RT, **11a** (51%) **11c** (51%), from **9**; f) Na/Hg, MeOH, RT; g) TMSCl, LHMDS, THF, 0 °C, 2 h; h) $Pd(OAc)_2$, CH_3CN, RT, 2 h, 78% from **12b**; i) Lawesson's reagent, CH_2Cl_2, MW, 100 °C, 10 min; j) **15a**,**c**, Ph_3P, DIAD, $PhCH_3$, RT; **16a** (42%), **16b** (39%); k) Grubbs II catalyst, CH_2Cl_2, MW, 100 °C, 30 min, **17b** (49%). DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; DIAD = diisopropyl azodicarboxylate; LHMDS = lithium bis(trimethylsilyl)amide; TMSCl = chlorotrimethylsilane.

only gave a complex mixture (TLC evidence) and the desired product was not detected (MS and ^1H NMR spectroscopic evidence). The TBS group was removed from **22** and the secondary alcohol generated then protected as the MOM ether **23**. In this case, **23** readily cyclized using Grubbs II to afford macrocycle **24** (Scheme 2) as previously described.[22] A mechanistic proposal for formation of **20** and **21** is suggested in Scheme 2B.

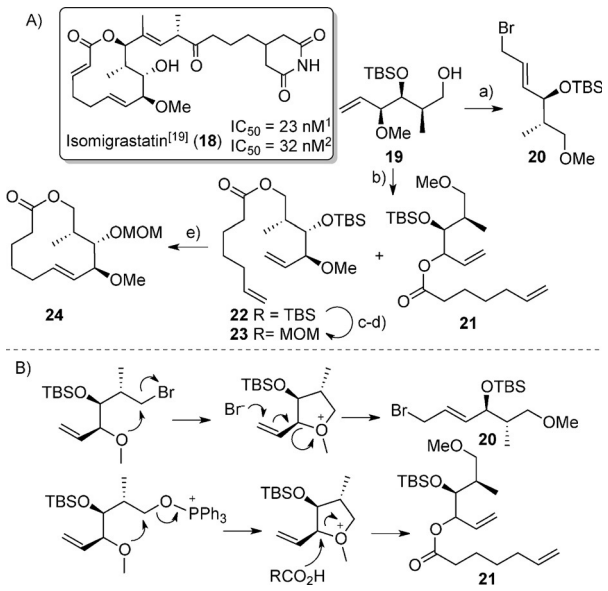
Synthesis of 9-methylmacrolactone analogues of migrastatin and isomigrastatin

Brown allylation was used as the key step to generate an analogue bearing a methyl group instead of methoxy group at C-9. Thus Brown allylation[23] of **25** (Scheme 3) with *cis*-butene and β-methoxydiisopinocampheylborane installed two contiguous stereocenters with subsequent removal of the benzoate group enabling diol **26** to be isolated in moderate yield; the relative configuration was determined by obtaining an X-ray crystal structure determination for **26**. Diol **26** was then protected as its di-*O*-TBS ether and subsequent treatment with 10% TsOH in MeOH removed the TBS group from the primary position to give the alcohol **27**. This alcohol **27** was oxidized with the Dess–Martin periodinane and the aldehyde obtained was then treated with the Ando phosphonate[17b,k,24] **35** to give **28**. Reduction of the ester group in **28** using DIBAL-H gave a primary alcohol and its subsequent Mitsunobu reaction gave **29**. However, RCM of **29**, under several conditions and reaction times, only gave complex reaction mixtures (TLC) with no trace of the desired product. Alcohol **32** was prepared with a view to resolving this problem and for application in isomigrastatin analogue synthesis. Since the presence of the TBS group was problematic in the attempted RCM with compound **22**, the secondary alcohol was protected as its MOM ether. Regioselective protection of the primary alcohol in **26** with a TBDPS group gave **30** and subsequent protection of its secondary alcohol as MOM ether afforded the orthogonally protected alkene **31**, which gave **32** after TBDPS removal.

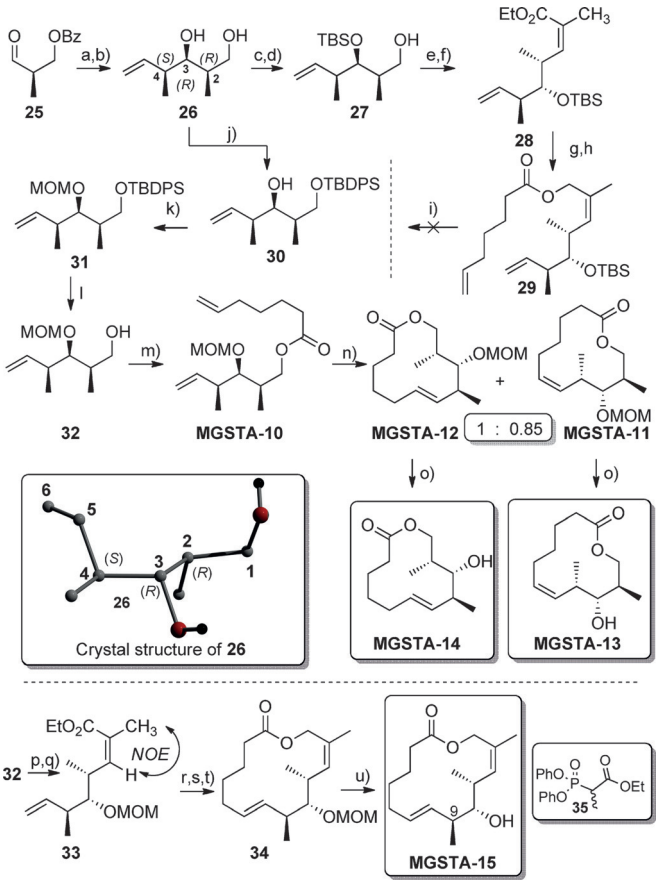
The Mitsunobu reaction of **32** gave **MGSTA-10**. Ring-closing metathesis of **MGSTA-10** afforded the *cis*- and *trans*-alkene-containing macrocycles **MGSTA-11** and **MGSTA-12** in a 1:0.85 ratio. The MOM protecting group was removed from both intermediates by using TMSBr to give truncated isomigrastatin analogues **MGSTA-13** and **MGSTA-14**. For the synthesis of migrastatin-core analogue **MGSTA-15** bearing the methyl group at C-9, the primary alcohol **32** was oxidized using the Dess–Martin periodinane, and then treated with the Ando phosphonate[17b,k,24] **35** giving the trisubstituted alkene **33**, with the *Z* configuration. The configuration of the newly formed double bond was supported by NOE spectroscopic experiments, for which NOEs were observed for **33** as indicated in Scheme 3. The reduction of **33** with DIBAL-H and subsequent esterification using Mitsunobu conditions gave the RCM precursor to **S6** (see Supporting Information for structure of **S6**). As for **29**, the RCM reaction of this precursor was unsuccessful and macrocyclic product could not be detected under several reaction conditions. Fortunately, addition of 1,4-benzoquinone[25] to the reaction mixture led to formation of desired **34** in modest yield, which after deprotection afforded the 9-methyl-macrolactone **MGSTA-15**.

Synthesis of migrastatin glucuronides

Glucuronides are an important class of phase 2 metabolites and glucuronidation in vivo of small molecules usually leads to compounds with higher water solubility, which are more easily excreted through the kidney.[26] Glucuronide metabolites are often biologically inactive but in some cases can display interesting bioactivity.[27,28] The 6-*O*-glucuronide of morphine (M6G)



Scheme 2. A) Synthesis of **24**: a) CBr_4, Ph_3P polymer-bound, CH_2Cl_2, RT; 1 h, 68%; b) **6b**, Ph_3P, DIAD, $PhCH_3$, RT; 57%; c) TBAF, THF, RT, 3 days, 60%; d) MOMCl, CH_2Cl_2, DIPEA, 0 °C to RT, 18 h, 60%; e) Grubbs II catalyst, $PhCH_3$, reflux; 31% $^1IC_{50}$ values using 4T1 mouse mammary adenocarcinoma cells. $^2IC_{50}$ values using MDA-MB-231 human breast adenocarcinoma cells.[17d] B) Proposed mechanism for formation of **20** and **21** under Mitsunobu and Appel conditions. TBAF = tetrabutylammonium fluoride; MOMCl = methoxymethyl chloride; DIPEA = *N*,*N*-diisopropylethylamine.

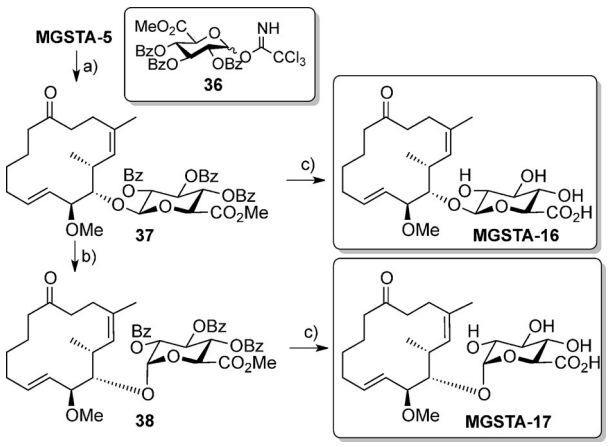


Scheme 3. Synthesis of methyl migrastatin core analogues: a) i) *cis*-Butene, *n*BuLi, THF, 45 min, −20 °C; ii) (+)-Ipc_2BOMe, 30 min, −78 °C; iii) BF_3-Et_2O, then **25**, 28 h; iv) NaOH 1 M, H_2O_2 30%, 16 h; b) K_2CO_3, MeOH, RT, 24 h, 32% from **25**; c) TBSOTf, DCM, DIPEA, 0 °C, 5 h; d) *p*-TSA, MeOH, 3 h 0 °C, 62% from **26**; e) DMP, CH_2Cl_2, RT, 18 h, 88%; f) **35**, THF, NaH, 0 °C, 1 h, then aldehyde obtained from oxidation of **27**, −78 °C to RT, 18 h, 85%; g) DIBAL, CH_2Cl_2, −78 °C, 10 min 63%; h) Ph_3P, DIAD, 6-heptenoic acid, RT, 2 h, 81%; i) Grubbs II catalyst, $PhCH_3$, reflux; j) TBDPSCl, imidazole, CH_2Cl_2, RT, 62%; k) MOMCl, DIPEA, CH_2Cl_2, RT, 48 h, 89%; l) TBAF 1 M, THF, RT, 18 h, 98%; m) Ph_3P, DIAD, 6-heptenoic acid, RT, 4 h, 88%; n) Grubbs II catalyst, $PhCH_3$, 1 h, heat at reflux, 33% **MGSTA-12** and 31% **MGSTA-11**; o) TMSBr, CH_2Cl_2, −20 °C, 2 h, 77% (**MGSTA-13**), 74% (**MGSTA-14**); p) DMP, CH_2Cl_2, pyridine, RT, 4 h, 70%; q) **35**, THF, NaH, 0 °C, 1 h, then aldehyde obtained from the oxidation of **32**, −78 °C to RT, 15 h, 73%; r) DIBAL, CH_2Cl_2, −78 °C, 15 min. 76%; s) Ph_3P, DIAD, 6-heptenoic acid, RT, 3 h, 78%; t) Grubbs II catalyst, $PhCH_3$, benzoquinone, 80 °C, 5 h, 13%; u) TMSBr, CH_2Cl_2, −20 °C, 3 h, 60%. DIBAL = diisobutylaluminium hydride; DMP = Dess–Martin periodinane; *p*-TSA = *p*-toluenesulfonic acid; Ipc_2BOMe = (+)-B-methoxydiisopinocampheylborane; TBSOTf = *tert*-butyldimethylsilyl trifluoromethanesulfonate; TBDPSCl = *tert*-butyl(chloro)diphenylsilane; TMSBr = bromotrimethylsilane.

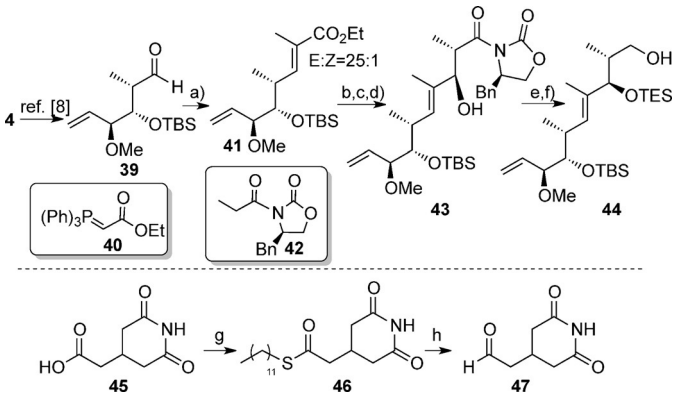
displays full agonist properties at the μ1-opioid receptor and appears to be more potent than morphine itself with fewer side effects.[26,29] In addition, the glucuronide of ezetimibe, a selective inhibitor of cholesterol absorption, is more active than ezetimibe itself.[26,30] The biological activity of thiocolchicoside, a myorelaxant used for painful muscle contraction, has been attributed to the 3-*O*-glucuronidated metabolite.[26] A glucuronide can be hydrolyzed through an enzymatic or non-enzymatic reaction to give the active aglycon[27] In this latter context glucuronidation has been used as for the preparation of SN-38 and taxol prodrugs.[27,31] The strategy relies on the fact that β-glucuronidase is generally overexpressed in tumour tissues. In view of these interesting properties of glucuronides and of the possibility that in vivo generation of glucuronides can occur we decided to prepare the glucuronide of **MGSTA-5**. Thus, **MGSTA-5**, which had been prepared on a 100 mg scale, was coupled with trichloroacetimidate **36**[32] using TMSOTf affording exclusively the protected β-glucuronide **37** (Scheme 4). Simultaneous removal of benzoate protective groups and hydrolysis of the methyl ester using aqueous NaOH gave the macroketone β-glucuronide **MGSTA-16**. In order to obtain the α-anomer of this glucuronide, we applied the chelation-induced anomerization,[33] which has been of interest in our laboratory[33d] for the synthesis of the α-glucuronide. Thus, glucuronide **37** was treated with $TiCl_4$ in $CDCl_3$ at 4 °C giving the α-glucuronide **38** as the only product. Deprotection and ester hydrolysis gave **MGSTA-17**.

Synthesis of glutarimide-containing analogues of migrastatin for which alkene geometry is varied

The preparation of **MGSTA-18** and **MGSTA-19**, containing the glutarimide side chain, for which olefin geometry is modified in the macrocyclic core was included in the synthetic study. Thus, diene precursors were prepared from freshly made aldehyde **39** (Scheme 5) by using Wittig reagent **40** to give the *E*-alkene **41** with high stereoselectivity (*E*/*Z* = 25:1); this reaction provided the isomer, which would enable preparation of migrastatin analogues with an *E*-alkene at C-3 in the macrocycle.



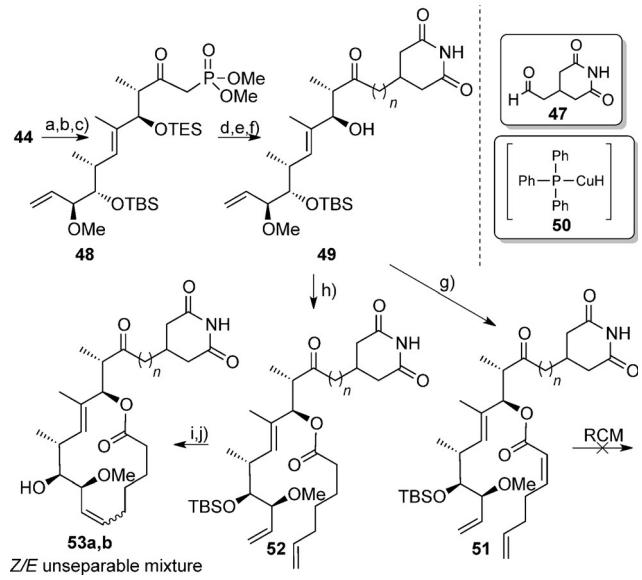
Scheme 4. Synthesis of **MGSTA-17** and **MGSTA-16**: a) **36** TMSOTf, MS (AW 300), CH_2Cl_2, −78 °C, 5 h, 60%; b) $TiCl_4$, $CDCl_3$, 4 °C, 69%; c) NaOH aq., MeOH, RT, 18 h, **MGSTA-17** (61%), **MGSTA-16** (73%). TMSOTf = Trimethylsilyl trifluoromethanesulfonate.



Scheme 5. Synthesis of **47**: a) **40**, $PhCH_3$, reflux, 22 h, 96%; b) DIBAL, CH_2Cl_2, −78 °C, 2 h, 84%; c) DMP, CH_2Cl_2, pyridine, 18 h, 92%; d) **42**, $MgCl_2$, Et_3N, TMSCl, RT, 48 h then TFA, MeOH; e) TESCl, CH_2Cl_2, imidazole, RT, 12 h, 88% over two steps; f) $LiBH_4$, MeOH, THF, RT, 1 h, 86%; g) *N*-methylimidazole, TsCl, MeCN then 1-dodecanthiol, 70 min 0 °C, 80%; h) Pd/C 10%, Et_3Si, acetone, RT, 1 h, 91%. TFA = Trifluoroacetic acid; TESCl = chlorotriethylsilane; TsCl = 4-toluenesulfonyl chloride.

The subsequent steps were similar to those used by Danishefsky and co-workers[6,17h] for their preparation of migrastatin. Thus, the reduction of the ester with DIBAL-H gave the corresponding allylic alcohol which was subsequently oxidized using the Dess–Martin reagent and the aldehyde generated was reacted with propionyl oxazolidinone **42** in the presence of $MgCl_2$, triethylamine and TMSCl to afford, after treatment with TFA, the desired anti-aldol product **43**. Protection of the hydroxy group as a TES ether, and reductive removal of the auxiliary gave primary alcohol **44** in good yield (Scheme 5). The glutarimide aldehyde **47** needed for the introduction of the glutarimide chain was prepared from **45** via thioester **46** (Scheme 5).[34]

With **44** in hand the preparation of **51** was next completed. Hence Dess–Martin oxidation of **44** gave the corresponding aldehyde, which was reacted with the anion generated by reaction of dimethyl methylphosphonate with butyl lithium to give

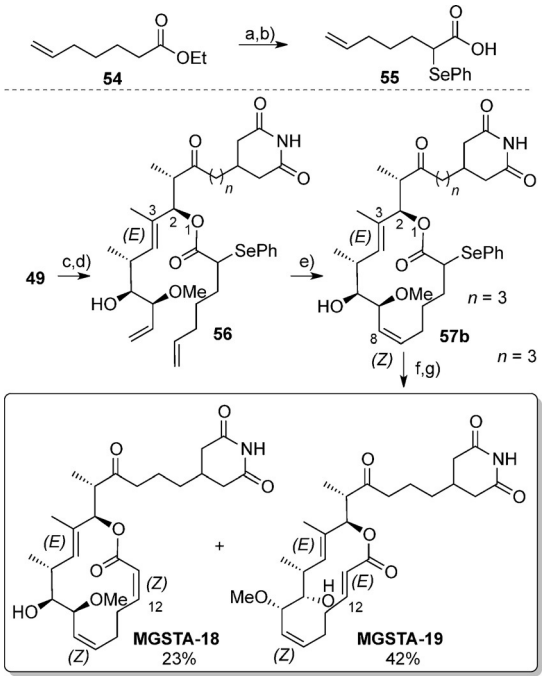


Scheme 6. Synthesis of **53a,b**: a) DMP, CH_2Cl_2, RT, 1.5 h; b) dimethyl methylphosphonate, BuLi, −78 to 0 °C, 15 min; c) DMP, CH_2Cl_2, RT, 1 h, 69% over three steps; d) **47**, LiCl, DBU, CH_3CN, 1 h, RT, 94%; e) **50**, $PhCH_3$, RT, 89%; f) AcOH, THF, H_2O, RT, 3 h, 97%; g) (*E*)-2,6-heptadienoic acid, 2,4,6-thrichlorobenzoyl chloride, DIPEA, pyridine, $PhCH_3$, 88%; h) 6-heptenoyl chloride, DMAP, CH_2Cl_2, RT, 75%; i) Grubbs II catalyst, $PhCH_3$, 1 h, reflux, 70%; j) HF·Py, THF, RT, 75%. DMAP = 4-Dimethylaminopyridine

an alcohol intermediate. Oxidation of this alcohol afforded phosphonate **48**. Then glutarimide aldehyde **47** was treated with **48** using the Masamune–Roush variant of the Horner–Wadsworth–Emmons reaction, giving the corresponding *E*-enone which was reduced with the Stryker reagent **50** and subsequent selective cleavage of TES protecting group gave alcohol **49** (Scheme 6). The synthesis of stereoisomers of migrastatin was possible with **49** in hand, as it had a trisubstituted alkene with the *E*-configuration (Scheme 6). Thus the modified Yamaguchi acylation from 2,6-heptadienoic acid gave **51**. However, the subsequent RCM reaction, attempted using both the Grubbs II and Hoveyda–Grubbs II catalysts, did not give rise to a ring-closed product. The coupling of **49** with 6-heptenoyl chloride, which gave **52** was carried out. Substrate **52** does not contain the unsaturated ester and in this case the RCM reaction succeeded and gave the ring-closed product as a mixture of *E*- and *Z*-stereoisomers (2:1), which were difficult to separate. Removal of the TBS group from the mixture was effectively achieved using HF/pyridine to give both **53a** and **53b**. The presence of both *Z*/*E* isomers in the mixture was deduced by alkene proton signals in the ^1H NMR spectrum of the mixture of **53a** and **53b** with coupling constants $J = 10.7$ (*Z*) and 15.2 Hz (*E*).

Encouraged that the presence of the *E*-alkene in **52** did not totally preclude macrocycle formation by RCM, we revised the approach to **MGSTA-18** and **MGSTA-19**. Danishefsky and his co-workers, in their total synthesis of isomigrastatin,[35] reported that RCM gave the 12-membered ring only when the alkene group adjacent to the carbonyl group was absent. They used an α-phenylselenide derivative and introduced the alkene adjacent to the carbonyl group after RCM. In order to obtain

MGSTA-18 and **MGSTA-19**, the selenium containing acid **55** was, therefore, first prepared from commercially available **54** in two steps. Yamaguchi acylation of **49** with racemic **55** and subsequent removal of the TBS group provided a mixture of diastereoisomers **56**. The RCM from **56** using the Grubbs II reagent gave a 1:2 mixture of *E*-isomer **57a** (not shown) and the *Z*-isomer **57b**. The polarity of **57a** and **57b** was similar on TLC, but it was possible to separate small amounts of **57a** and **57b** through repeated chromatographic purification steps. However, despite repeated chromatography it was not possible to obtain these compounds with high purity at the scale at which the reaction was carried out. Thus the oxidative deselenation of the *Z*-isomer **57b** was carried out with the material obtained and this gave the *E*-isomer **MGSTA-19** in 42% yield, along with 23% of the *Z*-isomer **MGSTA-18** (Scheme 7).



Scheme 7. Synthesis of **MGSTA-18** and **MGSTA-19**: a) BuLi, *i*Pr_2NH, Ph_2Se_2, THF; b) LiOH, THF/H_2O/MeOH, 86%; c) **55**, 2,4,6-trichlorobenzoyl chloride, DIPEA, $PhCH_3$, RT, 3 h then **49**, toluene, pyridine, RT, 4 days, 92%; d) HF·pyridine, pyridine, RT, 24 h, 61%; e) Grubbs II catalyst, $PhCH_3$, reflux; 5 min, 78%; f) *m*CPBA, CH_2Cl_2, −78 °C, 30 min then DIPEA, RT, 2 h, **MGSTA-18** (23%), **MGSTA-19** (42%). *m*CPBA = *meta*-chloroperoxybenzoic acid.

The structural assignment for **MGSTA-19** was supported by a typical α,β-unsaturated ester proton signal ($\delta = 6.83$) with *J* value typical of a *E*-alkene (16.0 Hz), whereas **MGSTA-18** had a signal at $\delta = 6.23$ and *J* value typical of an *Z*-alkene (11.1 Hz).

Biological evaluation of migrastatin-core analogues

With a variety of congeners now prepared, biological studies were next carried out with the capacity of compounds to inhibit tumor cell migration investigated using both wound healing and trans-well assays. Firstly, in order to eliminate the possibility that any decrease of migration was caused by an inhibition of cell proliferation or toxicity, rather than an interaction with the cell motility machinery, the effect of newly synthesized migrastatin-core analogues on three breast (MCF7, MCF7-Dox, MDA-MB361) and one pancreatic cancer (HPAC) cell lines were evaluated using the MTT assay. The newly synthesized analogues did not cause any toxicity or arrest of proliferation at the tested concentrations (1, 10 and 100 μM) (see the Supporting Information provided). Therefore, wound-healing assays were next carried out to address the effect on cell migration following exposure to compounds. The migrastatin analogues when given at the concentration of 10 μM strongly inhibited migration of the low-invasive MCF7[36] and doxorubicin resistant MCF7-Dox breast cancer cells, with average scratch closure of 14.6 and 14.9%, respectively compared to 100% of scratch closure in the controls.

Interestingly, treatment of MDA-MB361 breast cancer cells with migrastatin analogues resulted in the inhibition of cell migration with an average scratch closure of 42.0% compared to 100% scratch closure in the controls. MDA-MB361 are known to have poor cell–cell adhesion.[36] As a result, some migrastatin analogues were tested on the MDA-MB-231 cell line that displays a more invasive phenotype,[36] and with this in vitro model, the average scratch closure was 63.3% compared to 100% scratch closure in the controls. When the migrastatin analogues where tested on the pancreatic cancer cell line, HPAC, we noted an average of scratch closure of 33.5% (see the Supporting Information) compared to 100% scratch closure in the controls.

Next a selected number of the compounds tumour cell migration inhibitory activity was evaluated using the Boyden chamber assay as this is considered a superior model with which to conduct cell migration studies.[1] Eight of the compounds were selected based on considering macrocycle ring size and other structural features: compounds **MGSTA-13** and **MGSTA-14** were selected because of their relationship to the isomigrastatin macrocyclic core; **MGSTA-9** contains an extra carbon in the macrocyclic ring compared to migrastatin; **MGSTA-6** possesses an α,β-unsaturated carbonyl group that is present in migrastatin; **MGSTA-15** is the direct analogue of **MGSTA-4** with a methyl group instead of a methoxy at the 9 position; **MGSTA-16** and **17** are the glucuronides and **MGSTA-5** (Danishesky's macroketone), was selected for use as the internal control. As shown in Figures 3 and 4, the selected compounds inhibit cell migration in the less-invasive MCF7 cells in the nanomolar range. The only exception was **MGSTA-6**, which inhibited cell migration with considerably lower potency ($IC_{50} > 1$ μM) when compared with the macroketone **MGSTA-5** ($IC_{50} = 13$ nM). The glucuronides were found to be less potent than the macroketone against this cell type with the β-glucuronide more potent than its α-anomer. **MGSTA-5**,**6** and **14** were tested using the more invasive MDA-MB361 cell line. Interestingly, **MGSTA-6** ($IC_{50} = 410$ nM) was 2.4-fold more potent than **MGSTA-5** ($IC_{50} = 974$ nM) in this particular cell line, contrasting with the result for the MCF7 cells. In addition, the isomigrastatin-core analogue **MGSTA-14** strongly inhibited cell migration of MDA-MB361 cells ($IC_{50} = 301$ nM). **MGSTA-5**,**6**,**9**,**15**,**16** and **17** all strongly inhibited cell migration in HPAC cells; however, the

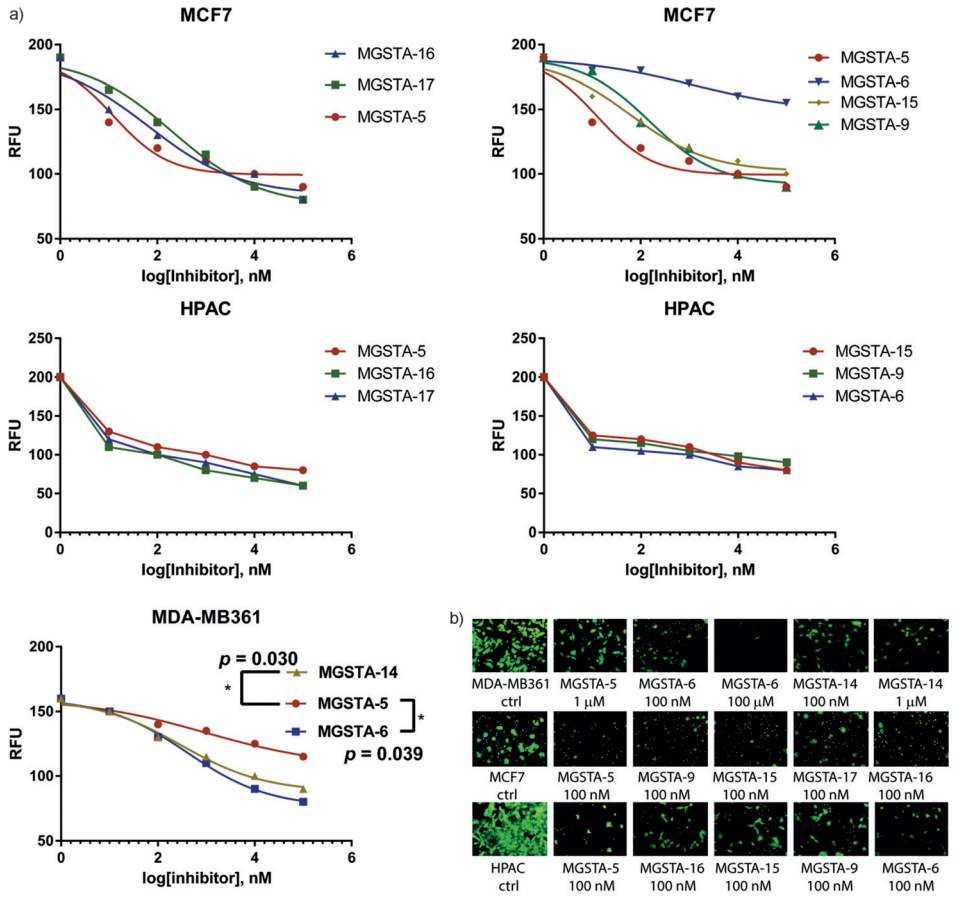


Figure 3. The effect of selected migrastatin analogues on human breast (MCF7, MDA-MB361) and pancreatic (HPAC) cancer cell lines. a) Quantification of migration of examined cell lines is presented as Relative Fluorescent Units (RFU). The treatment of cancer cells with selected migrastatin analogues at different concentrations, decreased cell migration through the membrane. b) Micrograph of the migrated MCF7, MDA-MB361 and HPAC cells taken with Olympus microsopy BX60 at 4× magnification.

	MCF7	MDA MB361
	IC_{50}	
MGSTA-13	64 nM	n.d.
MGSTA-14	n.d.	301 nM
MGSTA-15	61 nM	n.d.
MGSTA-9	153 nM	n.d.
MGSTA-6	> 1 μM	410 nM
MGSTA-5	13 nM	974 nM
MGSTA-17	205 nM	n.d.
MGSTA-16	66 nM	n.d.

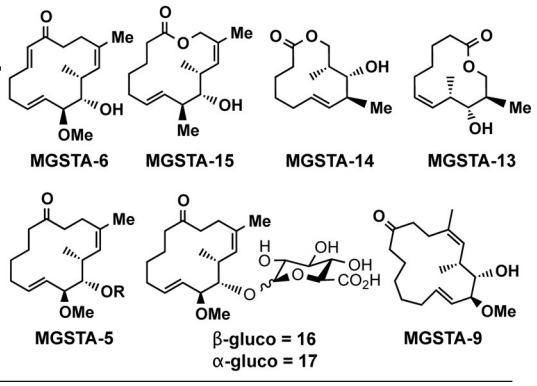


Figure 4. The effect of selected migrastatin analogues on human breast cancer cell lines MCF7 and MDA-MB36 assessed with Boyden chamber assay.

curve dose response was very wide and the IC_{50} could not be extrapolated.

Thus, **MGSTA-6** showed good cell-migration inhibition in highly metastatic MDA–MB361 but not in less invasive MCF7 cells. This apparent paradox could be related with cytoskeleton proteins targeted by migrastatin analogues[9,19] and with different molecular changes occurring during the complex event of metastasis in invasive and non-invasive cells. In a previous paper, we showed that **MGSTA-6** inhibited the formation and elongation of filopodia by interfering with fascin1-dependent cross-linking of actin filaments and subsequently leads to inhibition of stress fibre formation.[9] The importance of these cytoskeleton rearangements in cancer invasion indicates a correlation between high fascin expression and poor prognosis in human carcinomas.[37] Stress fibre formation is the last and crucial step of complex changes occurring in cytoskeleton of invasive cancer cell before its movement and it could tentatively explain the more pronounced biological activity of **MGSTA-6** in MDA-MB361 cells.

In vitro pharmacological profile of MGSTA-6

The pharmacological profile of a biologically active small molecule can be classified into primary (interaction with the intendent target) and secondary (interactions with different targets). Interaction with secondary targets can lead to adverse drug reaction (ADRs). From the drug discovery point of view, it is extremely important to identify the potential side effects as early as possible. Recently,[38] it has been suggested that drug candidates should be screened against a variety of targets (receptors, ion channels, enzymes and transporters). Compounds that interact with several of the above mentioned targets, are more prone to induce ADRs. Unsaturated macroketone **MGSTA-6** strongly inhibits cell migration in MDA-MB361 and HPAC cells (see above) and was earlier shown to interfere with the fascin1-dependent cross-linking of actin filaments leading to inhibition of stress fibre formation in canine mammary cancer cells.[9] This agent was, therefore, selected from the panel of agents for screening against 55 targets (receptors, ion channels

and transporters) known to be linked to ADRs. At the concentration of 10 μM, **MGSTA-6** only showed weak inhibition of two targets: adenosine receptor A_{2A} (27%) and prostanoid EP4 receptor (39%). It has been suggested[38] that compounds with a promiscuity index (percentage of targets giving more than 50% inhibition at 10 μM in a set of at least 50 targets) of more than 20% should be considered promiscuous and this has been linked to market withdrawal and clinical trial failure. Unsaturated macroketone **MGSTA-6**, with a promiscuity index between 0–5%, is a possible candidate as a tumour cell migration inhibitor with potentially low side effects. However, the pharmacological panel in this study does not include enzymes and further investigations would be necessary in order to confirm the selectivity of **MGSTA-6** (Table S1, Supporting Information).

Conclusion

The stereoselective synthesis of intermediates based on Brown allylation and alkoxyallylation has enabled the preparation of a series of migrastatin and isomigrastatin analogues. Chelation-induced anomerisation is compatible with the various functionalities found in the migrastatin macroketone skeleton and could be exploited to give macroketone glycosides for biological study. The Saeguso–Ito reaction enabled synthesis of MGSTA-6 in a regio- and stereoselective fashion. Compounds showed inhibitory activity when tested against breast and pancreatic cancer cell lines (MCF-7, MCF7-Dox, MDA-MB631, MDA-MB261, HPAC) using the wound healing assay and Boyden chamber assay (nM range). One inhibitor (**MGSTA-6**) has low activity against a variety of receptors, ion channels and transporters, which indicates that it, and possibly other migrastatin analogues, may have potential to inhibit tumour metastasis without adverse drug reactions.

Experimental Section

General

NMR spectra were recorded on a 500 MHz spectrometer at 30 °C. Chemical shifts are reported relative to internal Me_4Si in$CDCl_3$ (δ = 0.0 ppm) for ^1H and $CDCl_3$ (δ = 77.16 ppm) for ^{13}C at 30 °C, unless otherwise stated. ^{13}C signals were assigned with the aid of HSQC. Coupling constants are reported in Hertz. High-resolution mass spectra were measured by using an LC time-of-flight mass spectrometer and were measured in positive and/or negative mode as indicated. TLC was performed on aluminium sheets pre-coated with Silica Gel 60 (HF254, Merck Millipore) and spots were visualised by charring with vanillin solutions. Chromatography was carried out by using silica gel 60 (0.040–0.630 mm, E. Merck). Dichloromethane, tetrahydrofuran, MeOH and toluene were used as obtained from a PureSolv solvent purification system. Petroleum ether is the fraction with b.p. = 40–60 °C.

General procedure for TBS deprotection

To a stirred solution of TBS-protected macrocycle in THF (24 mM), HF·pyridine 70% (250 equiv) was added at RT. The resulting solution was stirred at RT for 18 h. After the careful addition of MeOTMS (350 equiv), the mixture was stirred for an additional 30 min and then the solvent was removed under reduced pressure and the residue was purified by flash chromatography (FC).

(6*E*,8*S*,9*S*,10*R*,11*Z*)-9-Hydroxy-8-methoxy-10,12-dimethyloxacyclotrideca-6,11-dien-2-one (MGSTA-1): Purification by FC (PE/EtOAc 10:1) afforded **MGSTA-1** (15.1 mg, 61%) as a white solid; $[\alpha]_D$ −13.0° (c = 0.03 in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ = 5.65–5.61 (m, 1H, H-11) 5.62–5.57 (dd, 1H, $J_{6,7trans}$ = 15.5, $J_{5,6}$ = 7.5 Hz, H-6), 5.26 (ddt, 1H, $J_{7,8}$ = 6.2, J = 1.4 Hz, H-7), 5.13 (dd, 1H, $J_{13,13'}$ = 12.1, $J_{Me,13}$ = 1.2 Hz, H-13), 3.82 (d, 1H, H-13′), 3.41–3.34 (m, 1H, H-8), 3.31–3.27 (m, 4H, O*CH*$_3$, H-9), 2.42–2.28 (m, 3H, *CH*$_2$, H-10), 2.27–2.12 (m, 2H, *CH*$_2$), 2.05–1.94 (m, 1H, *CH*$_2$), 1.82 (d, 3H, *CH*$_3$ at C-12), 1.77–1.68 (m, 1H, *CH*$_2$), 0.95 ppm (d, 3H, $J_{10,10Me}$ = 6.8 Hz, *CH*$_3$ at C-10); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 173.4 (*C*-2), 136.4 (*C*-11), 132.9 (*C*-6), 129.3 (*C*-7), 128.5 (*C*-12), 82.7 (*C*-8), 77.6 (*C*-9), 63.3 (*C*-13), 56.8 (O*CH*$_3$), 32.9 (*C*-10), 32.0 (*CH*$_2$), 31.7 (*CH*$_2$), 24.0 (*CH*$_3$ at C-12), 21.8 (*CH*$_2$), 13.0 ppm (*CH*$_3$ at C-10); HRMS-ESI: *m/z*: calcd for $C_{15}H_{24}O_4Na$: 291.1572; found: 291.1584.

(4*Z*,6*R*,7*S*,8*S*,9*E*)-7-Hydroxy-8-methoxy-4,6-dimethylcyclotrideca-4,9-dienone (MGSTA-2): Purification by FC (PE/EtOAc 10:1) afforded **MGSTA-2** (11.8 mg, 84%) as a yellow syrup; $[\alpha]_D$ 87.4 (*c* = 1.64 in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ = 5.62 (ddd, 1H, $J_{9,10trans}$ = 15.0, $J_{10,11}$ = 8.5, $J_{10,11'}$ = 5.9 Hz, H-10), 5.30 (d, 1H, $J_{5,6}$ = 10 Hz, H-5), 5.22 (ddt, 1H, $J_{8,9}$ = 7.6, J = 1.3 Hz, H-9), 3.37–3.34 (m, 1H, H-8), 3.31–3.29 (m, 4H, H-7 and O*CH*$_3$), 2.82 (s, 1H, OH), 2.64–2.59 (m, 1H, *CH*$_2$), 2.51–2.45 (m, 2H, *CH*$_2$), 2.43–2.25 (m, 4H, H-6, *CH*$_2$), 2.10–2.03 (m, 1H, *CH*$_2$), 1.91–1.81 (m, 2H, *CH*$_2$), 1.76–1.70 (m, 1H, *CH*$_2$), 1.70 (d, 3H, J = 1.3 Hz, *CH*$_3$ at C-4), 0.95 ppm (d, 3H, $J_{6,Me}$ = 6.9 Hz, *CH*$_3$ at C-6); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 210.7 (C-1), 135.7 (C-10), 133.9 (*Cq*), 130.7 (C-5), 129.5 (C-9), 83.9 (C-8), 77.8 (C-7), 56.4 (O*CH*$_3$), 42.5 (*CH*$_2$), 40.2 (*CH*$_2$), 32.9 (C-6), 31.9 (*CH*$_2$), 26.3 (*CH*$_2$), 23.6 (*CH*$_3$), 21.5 (*CH*$_2$), 13.9 ppm (*CH*$_3$); HRMS-ESI: *m/z*: calcd for $C_{16}H_{26}O_3Na$: 289.1780; found: 289.1772.

(6*E*,8*S*,9*S*,10*R*,11*Z*)-9-Hydroxy-8-methoxy-10,12-dimethylthiacyclotrideca-6,11-dien-2-one (MGSTA-3): Purification by FC (PE/EtOAc 15:1) afforded **MGSTA-3** (5.0 mg, 85%) as a yellow syrup; $[\alpha]_D$ = 1.6 (*c* = 0.46 in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ = 5.65–5.48 (m, 2H, H-6,10), 5.15–5.05 (dd, 1H, $J_{6,7trans}$ = 15.4, $J_{7,9}$ = 7.7 Hz, H-7), 4.21 (d, 1H, J = 13.4 Hz, H-13), 3.33–3.27 (m, 2H, H-7,8), 3.26 (s, 3H, OCH_3), 2.84 (s, 1H, OH), 2.78 (d, 1H, H-13′), 2.66 (ddd, 1H, J = 16.7, 7.0, 2.0 Hz, H-3), 2.57–2.51 (m, 1H, H-3′), 2.51–2.42 (m, 1H, H-5), 2.22 (m, 1H, H-4), 2.12 (m, 1H, H-10), 2.01–1.92 (m, 1H, H-5′), 1.76–1.74 (s, 3H, Me at C-12), 1.69–1.62 (m, 1H, H-4′), 0.97 ppm (d, 3H, $J_{10,Me}$ = 6.8 Hz, Me at C-10);^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.0 (C-2), 136.8 (C-6), 133.9 (C-10), 130.1 (C-11), 128.8 (C-7), 83.1 (C-8 or C-9), 75.0 (C-8 or C-9), 56.3 (OCH_3), 43.7 (C-3), 34.3 (C-5), 32.5 (C-10), 28.9 (C-13), 25.3 (CH_3 at C-12), 22.7 (C-4), 12.6 ppm (CH_3 at C-10); HRMS-ESI: calcd for $C_{15}H_{23}O_3S$: 283.1368; found: 283.1375.

(2*E*,6*E*,8*S*,9*S*,10*R*,11*Z*)-9-[(*tert*-Butyldimethylsilyl)oxy]-8-methoxy-10,12-dimethylcyclotetradeca-2,6,11-trien-1-one (13): To stirred **12b** (26.7 mg; 0.068 mmol) in THF (0.5 mL) at 0 °C, TMSCl (26 μL; 0.203 mmol) and LHMDS (203 μL; 0.203 mmol, 1 M in THF) were added dropwise. The resulting solution was stirred for 2 h at 0 °C, quenched with NH_4Cl and extracted with EtOAc. The organic layers were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was dissolved in CH_3CN (0.5 mL) and $Pd(OAc)_2$ (18 mg; 0.082 mmol) was added. The mixture was stirred for 3 h and then diluted with NaHCO and extracted with CH_2Cl_2. The organic layers were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. Purification by FC (PE/EtOAc 20:1) afforded **14** (17 mg: 64%) as a yellow syrup; $[\alpha]_D$ 42.9° (*c* = 1.2 in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ = 6.74 (dt, 1H, $J_{2,3}$ = 16.2, $J_{3,4}$ = 7.0 Hz, H-3), 5.93 (d, 1H H-2), 5.64 (ddd, 1H, $J_{6,7}$ = 15.4, J = 8.9, J = 4.7 Hz, H-6), 5.37–5.14 (m, 2H, H-7,11), 3.45–

3.27 (m, 2H, H-8,9), 3.21 (s, 3H, OCH_3), 2.70–2.31 (m, 7H, H-10, CH_2), 2.31–2.20 (m, 1H, CH_2), 2.05 (m, 1H, CH_2), 1.69 (d, 3H, J= 1.5 Hz, Me at C-12), 0.90 (s, 9H, $C(CH_3)_3$), 0.87 (d, 3H, J=6.6 Hz, Me at C-10), 0.04 (s, 3H, SiCH_3), 0.00 ppm (s, 3H, SiCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ=201.5 (C-1), 148.2 (C-3), 132.8 (C-6), 132.0, 132.0, 131.7, 131.4, 85.2 (C-8 or C-9), 79.0 (C-8 or C-9), 56.4 (OCH_3), 38.4 (CH_2), 34.5 (C-10), 32.4 (CH_2), 31.6 (CH_2), 30.7 (CH_2), 26.3 ($C(CH_3)_3$), 23.0 (Me at C-12), 18.8 ($C(CH_3)_3$), 12.7 (Me at C-10), −3.7 (SiCH_3), −4.9 ppm (SiCH_3); HRMS-ESI: *m/z*: calcd for $C_{23}H_{39}O_3Si$: 391.2668; found: 391.2678.

(2*E*,6*E*,8*S*,9*S*,10*R*,11*Z*)-9-Hydroxy-8-methoxy-10,12-dimethylcyclotetradeca-2,6,11-trienone (MGSTA-6): Purification by FC (PE/EtOAc 5:1) afforded **MGSTA-6** (7.6 mg, 90%) as a yellow syrup; $[\alpha]_D$ 92.2° (c=0.6 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ=6.73 (dt, 1H, $J_{2,3}$= 16.4, $J_{3,4}$=7.1 Hz, H-3), 5.93 (d, 1H, H-2), 5.69 (ddd, 1H, $J_{6,7}$=15.4, J=9.4, J=4.4 Hz, H-6), 5.37 (d, 1H, $J_{10,,11}$=9.9 Hz, H-11), 5.24 (ddd, 1H, $J_{7,8}$=8.4, J=1.4 Hz, H-7), 3.44 (appt, 1H, $J_{7,8}$=$J_{8,9}$=8.9 Hz, H-8), 3.34 (dd, 1H, J=1.3 Hz, H-9), 3.31 (s, 3H, OCH_3), 2.70 (s, 1H, OH), 2.64 (m, 1H, CH_2), 2.57–2.46 (m, 4H, H-10, CH_2), 2.42–2.22 (m, 3H, CH_2), 2.14 (m, 1H, CH_2), 1.72 (d, 3H, J=1.4 Hz, Me at C-12), 0.92 ppm (d, 3H, J=6.8 Hz, Me at C-10); ^{13}C NMR (125 MHz, $CDCl_3$): δ=201.3 (C-1), 147.8 (C-3), 134.9 (C-6), 132.9 (C-12), 131.7 (C-2), 130.9 (C-11), 130.5 (C-7), 84.1 (C-8), 77.2 (C-9), 56.6 (OCH_3), 38.4 (CH_2), 32.7 (C-10), 32.3 (CH_2), 31.3 (CH_2), 30.6 (CH_2), 22.9 (Me at C-12), 12.6 ppm (Me at C-10); HRMS-ESI: *m/z*: calcd for $C_{17}H_{25}O_3$: 277.1804; found: 277.1801.

(7*E*,9*S*,10*S*,11*R*,12*Z*)-10-Hydroxy-9-methoxy-11,13-dimethylthiacyclotetradeca-7,12-dien-2-one (MGSTA-7): Purification by FC (hexane/EtOAc 15:1) afforded **MGSTA-7** (12.8 mg, 63%) as a yellow syrup; $[\alpha]_D$ 93.2 (c=0.07 in $CHCl_3$);^{1}H NMR ($CDCl_3$, 500 MHz): δ= 5.80–5.70 (m, 1H, H-7), 5.58 (d, 1H, $J_{11,12}$=9.6 Hz, H-12), 5.28–5.20 (ddt, 1H, $J_{7,8}$=15.8, $J_{8,9}$=7.3, J=1.4 Hz, H-8), 3.72 (d, 1H, $J_{14,14'}$= 12.4 Hz, H-14), 3.43 (dd, 1H, $J_{9,10}$=9.0 Hz, H-9), 3.35 (dd, 1H, $J_{10,OH}$= 1.6 Hz, H-10), 3.30 (s, 3H, O*Me*), 3.18 (d, 1H, H-14′), 2.80 (br s, 1H, OH), 2.61 (m, 1H), 2.49–2.37 (m, 2H, H-11, CH_2), 2.22 (m, 1H), 2.02 (m, 1H), 1.77 (d, 3H, J=1.4 Hz, *Me* at C-13), 1.75–1.59 (m, 3H), 1.45–1.35 (m, 1H), 0.95 ppm (d, 3H, J=6.9 Hz, *Me* at C-11); ^{13}C NMR ($CDCl_3$, 125 MHz): δ=200.9 (C-2), 135.0 (C-7), 134.2 (C-12), 128.4 (C-13), 127.8 (C-8), 83.8 (C-9), 76.6 (C-10), 56.6 (OMe), 43.5 (CH_2), 32.9 (C-11), 31.1 (C-14), 29.6 (CH_2), 27.4 (CH_2), 25.6 (CH_2), 25.1 (Me at C-13), 12.8 ppm (Me at C-11); HRMS-ESI: *m/z*: calcd for $C_{16}H_{26}O_3NaS$: 321.1500; found: 321.1496.

(8*E*,10*S*,11*S*,12*R*,13*Z*)-11-Hydroxy-10-methoxy-12,14-dimethyloxacyclopentadeca-8,13-dien-2-one (MGSTA-8): Purification by FC (PE/EtOAc 10:1→5:1) afforded **MGSTA-8** (28.7 mg, 54%) as a white syrup; $[\alpha]_D$ 72° (c=1.7 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ=5.79 (dq, 1H, $J_{12,13}$=9.4, $J_{13,Me}$=1.6 Hz, H-13), 5.64 (ddd, 1H, $J_{8,9}$=15.3, $J_{8,7}$=8.9, $J_{8,7'}$=5.2 Hz, H-8), 5.16 (dd, 1H, $J_{9,10}$=8.0 Hz, H-9), 4.52 (d, 1H, $J_{15a,15b}$=11.8 Hz, H-15a), 4.27 (d, 1H, H-15b), 3.46–3.34 (m, 2H, H-10, H-12), 3.29 (s, 3H, OMe), 2.82 (t, 1H, $J_{OH,12}$=1.3 Hz, OH), 2.51 (q, 1H, J=7.4 Hz), 2.39–2.26 (m, 2H), 2.28–2.18 (m, 1H, H-7), 2.11–2.00 (m, 1H, H-7′), 1.81 (d, 3H, C-14*Me*), 1.74–1.55 (m, 2H), 1.50–1.33 (m, 2H, H-6,6′), 1.30–1.10 (m, 2H), 0.96 ppm (d, 3H, C-12*Me*); ^{13}C NMR($CDCl_3$, 125 MHz): δ=175.0 (C-2), 136.7 (C-8), 136.3 (C-13), 128.2 (C-14), 128.0 (C-9), 84.0 (C-11 or C-10), 76.0 (C-11 or C-10), 64.0 (C-15), 56.2 (OMe), 35.1 (CH_2), 32.9 (C-12), 32.0 (C-7), 27.6 (CH_2), 27.4 (C-6), 25.8 (CH_2), 23.5 (Me), 13.2 ppm (Me); HRMS-ESI: *m/z*: calcd for $C_{17}H_{28}O_4Na$: 319.1885; found: 319.1882.

(4*Z*,6*R*,7*S*,8*S*,9*E*)-7-Hydroxy-8-methoxy-4,6-dimethylcyclopentadeca-4,9-dienone (MGSTA-9): Purification by FC (PE/EtOAc 10:1) afforded **MGSTA-9** (11.8 mg, 82%) as a white syrup; $[\alpha]_D$ 87° (c= 0.9 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ=5.66 (ddd, 1H, J 14.7, 8.8, 5.2 Hz, H-10), 5.41 (d, 1H, $J_{5,6}$=9.6 Hz, H-5), 5.17 (dd, 1H, $J_{8,9}$= 7.3 Hz, H-9), 3.44–3.33 (m, 2H, H-7,8), 3.28 (s, 3H, OMe), 2.78 (d, 1H, $J_{7,OH}$=1.3 Hz, OH), 2.51–2.31 (m, 5H), 2.29–2.17 (m, 2H), 2.14–2.01 (m, 2H), 1.73–1.56 (m, 5H), 1.52–1.34 (m, 2H), 1.29–1.08 (m, 2H), 0.93 ppm (d, 3H, $J_{6,Me}$=6.8 Hz, C6*Me*); ^{13}C NMR ($CDCl_3$, 125 MHz): δ=213.0 (C-1), 136.5 (C-10), 133.4 (C-4), 129.8 (C-5), 128.1 (C-9), 84.2 (C-7 or C-8), 76.6 (C-7 or C-8), 56.3 (OMe), 43.1 (CH_2), 41.8 (CH_2), 32.7 (C-6), 31.8 (CH_2), 27.9 (CH_2), 27.8 (CH_2), 26.4 (CH_2), 25.7 (CH_2), 23.4 (Me at C-4), 13.0 ppm (Me at C-6). HRMS-ESI: *m/z*: calcd for $C_{18}H_{30}O_3Na$: 317.2093; found: 317.2087.

(2*R*,3*R*,4*S*)-2,4-Dimethylhex-5-ene-1,3-diol (26): To stirred *t*BuOK (1.63 g; 14.57 mmol) in THF (4 mL) at −45 °C *n*-butyllithium in heptane (9.1 mL, 14.57 mmol, 1.6 M) was added. The mixture was cooled to −78 °C and β-methoxydiisopinocampheylborane (5.5 g, 17.4 mmol) in THF (17 mL) was added via cannula. The mixture was stirred at −78 °C for an additional 1 h and boron trifluoride etherate (2.4 mL, 19.45 mmol) was added dropwise at −78 °C. Immediately, a solution of aldehyde **25** (3.94 g, 20.52 mmol) in THF (7 mL) was added, and the mixture was stirred at −78 °C for 18 h. Then aqueous NaOH (10.66 mL; 32 mmol, 3 M) was added followed by H_2O_2 (4.5 mL, 30%), and the biphasic mixture was allowed to warm to room temperature slowly and refluxed for 1 h. The solution was diluted with CH_2Cl_2 and washed with water. The organic layer was separated, and then dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was subjected to silica gel chromatography (PE/EtOAc, 1:6) affording a mixture of the title compound and pinenol during the workup (9.87 g) which can be used directly in the next step. The mixture (9.87 g) was dissolved in dry methanol (200 mL) and K_2CO_3 (2.32 g) was added. After stirring at room temperature for 24 h AcOH was added until pH 7 was reached. The solvent was removed under reduced pressure and the residue was purified by chromatography (PE/EtOAc 1:10→Et_2O) to afford **26** (940 mg; 32% from 25) as white crystal (needles). M.p.=175–181 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ=5.62 (ddd, 1H, $J_{5,6trans}$=17.1, $J_{5,6cis}$=10.3, $J_{4,5}$=8.7 Hz, H-5), 5.08–5.04 (m, 1H, H-6$_{trans}$), 4.98 (dd, 1H, J_{gem}=1.8 Hz, H-6$_{cis}$), 3.71 (dd, 1H, $J_{1,1'}$= 10.5, $J_{1,2}$=4.1 Hz, H-1), 3.66 (dd, 1H, $J_{1',2}$=5.5 Hz, H-1′), 3.56 (dd, 1H, $J_{3,4}$=9.1, $J_{2,3}$=2.4 Hz, H-3), 2.67 (br s, 2H, OH), 2.33–2.26 (m, 1H, H-4), 1.82 (dddd, 1H, $J_{2,Me}$=6.8 Hz, H-2), 1.09 (d, 3H, $J_{4,Me}$= 6.7 Hz, CH_3 at C-4), 0.93 ppm (d, 3H, CH_3 at C-2); ^{13}C NMR ($CDCl_3$, 125 MHz): δ=141.0 (C-5), 114.9 (C-6), 77.5 (C-3), 67.9 (C-1), 42.4 (C-4), 36.7 (C-2), 17.3 (CH_3 at C-4), 9.1 ppm (CH_3 at C-2); HRMS-ESI: *m/z*: calcd for $C_8H_{15}O_2Na$: 143.1072; found: 143.1071.

Ring-closing metathesis on MGSTA-10

To a stirred solution of **MGSTA-10** (100 mg; 0.33 mmol), in degassed $PhCH_3$ (650 mL) the Grubbs II catalyst (57 mg, 0.067 mmol) was added by cannula. The mixture was stirred at reflux for 60 min. The $PhCH_3$ was removed under reduced pressure. Purification of the residue by FC (EtOAc/Cy 1:30) afforded **MGSTA-11** (25 mg, 31%) and **MGSTA-12** (23.1 mg; 33%) as yellow syrups.

(9*S*,10*R*,11*R*,*Z*)-10-(Methoxymethoxy)-9,11-dimethyloxacyclododec-7-en-2-one (MGSTA-11): ^{1}H NMR ($CDCl_3$, 500 MHz): δ=5.48–5.40 (m, 1H, H-8), 5.34 (td, 1H, $J_{7,8}$=10.6, $J_{6,7}$=5.0 Hz, H-7), 4.67 (d, 1H, J=6.6 Hz, OCH_2O), 4.62 (d, 1H, J=6.6 Hz, OCH_2O), 4.38 (dd, 1H, $J_{12,12'}$=11.4, $J_{12,11}$=3.1 Hz, H-12), 3.89 (dd, 1H, $J_{11,12'}$=5.2 Hz, H-12′), 3.43–3.38 (m, 4H, H-10, OCH_3), 2.71 (dqd, 1H, $J_{8,9}$=9.9, $J_{9,Me}$= 6.8, $J_{9,10}$=2.9 Hz, H-9), 2.44 (ddd, 1H, J=12.7, 8.5, 3.9 Hz, CH_2), 2.33 (ddd, 1H, J=13.3, 8.9, 3.7 Hz, CH_2), 2.26–2.14 (m, 1H, CH_2), 2.14–2.02 (m, 1H, H-11), 1.91–1.75 (m, 2H, CH_2), 1.64 (dddt, 2H, J=20.6, 8.7, 5.8, 3.2 Hz, CH_2), 1.42–1.29 (m, 1H, CH_2), 1.11 (d, 3H, J=7.3 Hz, CH_3 at C-11), 0.98 ppm (d, 3H, J=6.8 Hz, H- CH_3 at C-9); ^{13}C NMR ($CDCl_3$, 125 MHz): δ=174.5 (C-2), 135.8 (C-8), 128.6 (C-7), 98.3

(OCH_2O), 85.6 (C-10), 67.4 (C-12), 56.3 (OCH_3), 36.2 (C-11), 34.6 (CH_2), 34.5 (C-9), 28.8 (CH_2), 25.9 (CH_2), 23.6 (CH_2), 15.1 (CH_3 at C-11), 14.6 ppm (CH_3 at C-9); HRMS-ESI: *m/z*: calcd for $C_{15}H_{26}O_4Na$: 293.1729; found: 293.1731.

(9*S*,10*R*,11*R*,*E*)-10-(Methoxymethoxy)-9,11-dimethyloxacyclododec-7-en-2-one (MGSTA-12): ^1H NMR ($CDCl_3$, 500 MHz): $\delta = 5.42$ (ddd, 1H, $J_{7,8} = 14.9$, 8.3, 6.3 Hz, H-7), 5.25 (dd, 1H, $J_{8,9} = 8.2$ Hz, H-8), 4.70–4.66 (m, 2H, OCH_2O), 4.47 (dd, 1H, $J_{12,12'} = 10.6$, $J_{11,12} = 2.9$ Hz, H-12), 3.54 (appt, 1H, $J_{12,12'} = J_{11,12'} = 10.7$ Hz, H-12′), 3.40 (s, 3H, OCH_3), 3.32 (d, 1H, $J = 10.1$ Hz, H-10), 2.46–2.38 (m,1H, CH_2), 2.17–2.06 (m, 3H, H-9, CH_2), 2.02–1.93 (m, 1H, H-11), 1.74–1.61 (m, 4H, CH_2), 1.20–1.12 (m, 1H, CH_2), 1.06 (d, 3H, $J = 6.8$ Hz, CH_3 at C-9), 0.89 ppm (d, 3H, $J = 7.3$ Hz, CH_3 at C-11); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 173.4$ (C-2), 135.5 (C-7), 131.4 (C-8), 98.7 (OCH_2O), 83.9 (C-10), 66.5 (C-12), 56.0 (OCH_3), 40.4 (C-9), 35.7 (C-11), 34.7 (CH_2), 30.4 (CH_2), 26.9 (CH_2), 22.6 (CH_2), 17.2 (CH_3 at C-9), 10.6 ppm (CH_3 at C-11); HRMS-ESI: *m/z*: calcd for $C_{15}H_{26}O_4Na$: 293.1729; found: 293.1732.

(9*S*,10*R*,11*R*,*Z*)-10-Hydroxy-9,11-dimethyloxacyclododec-7-en-2-one (MGSTA-13): To stirred **MGSTA-11** (28.1 mg; 0.104 mmol) in CH_2Cl_2 (5 mL), TMSBr (20.6 μL; 0.0156 mmol) was added at −20 °C. The mixture was stirred for 1 h. Satd $NaHCO_3$ was then added and the solution was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried and the solvent was removed under reduced pressure. Purification by FC (EtOAc/Cy 1:8) gave **MGSTA-13** (18.2 mg, 77%) as a colourless oil. $[\alpha]_D$ −20° ($c = 1.5$ in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): $\delta = 5.54$–5.47 (m, 1H, H-8), 5.39 (td, 1H, $J_{7,8} = 10.6$, $J_{6,7} = 5.3$ Hz, H-7), 4.47 (dd, 1H, $J_{12,12'} = 11.5$, $J_{12,11} = 3.4$ Hz, H-12), 3.82 (dd, 1H, $J_{11,12'} = 4.5$ Hz, H-12′), 3.54 (ddd, 1H, $J_{10,11} = 7.6$, $J_{10,OH} = 4.9$, $J_{9,10} = 2.2$ Hz, H-10), 2.73 (dqd, 1H, $J_{8,9} = 9.2$, $J_{9,Me} = 6.8$ Hz, H-9), 2.42 (ddd, 1H, $J = 12.9$, 8.8, 4.0 Hz, CH_2), 2.33 (ddd, 1H, $J = 13.1$, 8.8, 3.7 Hz, CH_2), 2.24–2.14 (m, 1H, CH_2), 2.01–1.93 (m, 1H, H-11), 1.91–1.84 (m, 1H, CH_2), 1.83–1.74 (m, 1H, CH_2), 1.70–1.63 (m, 2H, CH_2), 1.43 (d, 1H, OH), 1.37–1.28 (m, 1H, CH_2), 1.14 (d, 3H, $J_{11,Me} = 7.0$ Hz, CH_3 at C-11), 0.96 ppm (d, 3H, CH_3 at C-9);^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 174.6$ (C-2), 135.5 (C-8), 128.9 (C-7), 78.4 (C-10), 67.4 (C-12), 36.3 (C-11), 34.8 (CH_2), 33.8 (C-9), 28.8 (CH_2), 26.1 (CH_2), 23.7 (CH_2), 15.0 (CH_3 at C-11), 12.8 ppm (CH_3 at C-9); HRMS-ESI: *m/z*: calcd for $C_{15}H_{25}NO_3Na$: 290.1732; found: 290.1726.

(9*S*,10*R*,11*R*,*E*)-10-Hydroxy-9,11-dimethyloxacyclododec-7-en-2-one (MGSTA-14): To stirred **MGSTA-12** (23.4 mg; 0.087 mmol) in CH_2Cl_2 (6 mL), TMSBr (23 μL; 0.174 mmol) was added at −20 °C. The mixture was stirred for 2 h. Saturated $NaHCO_3$ was added and the solution was extracted with CH_2Cl_2 (3×10 mL) and the combined organic layers were dried and the solvent was removed under reduced pressure. Purification by FC (EtOAc/Cy 1:10) gave **MGSTA-14** (14.5 mg, 74%) as a colourless oil. $[\alpha]_D = 6.49°$ ($c = 0.99$ in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): $\delta = 5.44$ (ddd, 1H, $J_{7,8} = 14.6$, $J_{7,6} = 8.0$, $J_{7,6'} = 6.4$ Hz, H-7), 5.28 (dd, 1H, $J_{8,9} = 8.1$ Hz, H-8), 4.41 (dd, 1H, $J_{12,12'} = 10.7$, 2.8 Hz, H-12), 3.56 (t, 1H, H-12′), 3.43 (dd, 1H, $J = 9.7$, $J_{10,OH} = 6.4$ Hz, H-10), 2.44–2.36 (m, 1H, CH_2), 2.16–2.06 (m, 3H, H-9, CH_2), 2.03–1.96 (m, 1H, H-11), 1.74–1.62 (m, 4H, CH_2), 1.45 (d, 1H, OH), 1.20–1.12 (m, 1H, CH_2), 1.09 (d, 3H, $J_{9,Me} = 6.8$ Hz, CH_3 at C-9), 0.88 ppm (d, 3H, $J_{11,Me} = 7.3$ Hz, CH_3 at C-11); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 173.4$ (C-2), 135.3 (C-8), 131.2 (C-7), 76.3 (C-10), 66.6 (C-12), 39.9 (C-9), 35.9 (C-11), 34.9 (CH_2), 30.2 (CH_2), 27.4 (CH_2), 22.5 (CH_2), 16.8 (CH_3 at C-9), 10.0 ppm (CH_3 at C-11); HRMS-ESI: *m/z*: calcd for $C_{13}H_{22}O_3Na$: 249.1467; found: 249.1459.

(7*E*,9*S*,10*R*,11*R*,12*Z*)-10-Hydroxy-9,11,13-trimethyloxacyclotetradeca-7,12-dien-2-one (MGSTA-15): To a stirred solution of **34** (8.7 mg; 0.028 mmol) in CH_2Cl_2 (5 mL), TMSBr (5.5 μL; 0.042 mmol) was added at −15 °C. The mixture was stirred for 3 h. Saturated $NaHCO_3$ was added and the solution was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried and the solvent was removed under reduced pressure. Purification by FC (EtOAc/Cy 1:7) gave **MGSTA-15** (4.5 mg, 60%) as a colourless oil. $[\alpha]_D$ 37.6° ($c = 0.19$ in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): $\delta = 5.60$ (dt, 1H, $J_{11,12} = 10.2$, $J_{12,Me} = 1.4$ Hz, H-12), 5.46 (ddd, 1H, $J_{7,8} = 15.3$, $J = 7.8$, $J = 5.8$ Hz, H-7), 5.30 (ddt, 1H, $J = 8.6$, $J = 1.3$ Hz, H-8), 4.56 (dd, 1H, $J_{14,14'} = 13.1$, $J = 1.0$ Hz, H-14), 4.34 (d, 1H, H-14′), 3.24 (d, 1H, $J = 9.3$ Hz, H-10), 3.02–2.95 (m, 1H, H-11), 2.46–2.39 (m, 1H, CH_2), 2.30–2.18 (m, 2H, H-9, CH_2), 2.17–2.10 (m, 1H, CH_2), 1.98–1.90 (m, 1H, CH_2), 1.82–1.77 (m, 1H, CH_2), 1.76 (d, 3H, CH_3 at C-13), 1.64–1.57 (m, 1H, CH_2), 1.53–1.47 (m, 1H, CH_2), 1.41–1.32 (m, 1H CH_2), 1.09 (d, 3H, $J_{9,Me} = 6.7$ Hz, CH_3 at C-9), 0.92 ppm (d, 3H, $J_{11,Me} = 7.0$ Hz, CH_3 at C-11); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 174.0$ (C-2), 134.7 (C-12), 133.9 (C-8), 129.9 (C-7), 127.5 (C-13), 78.9 (C-10), 65.0 (C-14), 41.4 (C-9), 34.6 (C-11), 34.0 (CH_2), 30.0 (CH_2), 27.6 (CH_2), 23.5 (CH_2), 23.4 (CH_3 at C-13), 18.1 (CH_3 at C-9), 12.9 ppm (CH_3 at C-11); HRMS-ESI: *m/z*: calcd for $C_{16}H_{26}O_3Na$: 289.1780; found: 289.1775.

(4*Z*,6*R*,7*S*,8*S*,9*E*)-7-*O*-(Methyl-2′,3′,4′-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-4,6-dimethylcyclotetradeca-8-methoxy-4,9-dienone (37): To stirred macroketone **MGSTA-5** (5 mg, 18 μmol), the trichloroacetimidate 36 (24 mg, 36 μmol), and powdered molecular sieves 4 A (25 mg) in dry CH_2Cl_2 (0.5 mL) was cooled to −40 °C under nitrogen atmosphere. To this mixture was added 10% TMSOTf in CH_2Cl_2 (11 μL, 7.2 μmol) dropwise, and the mixture was stirred at −40 °C for 5 h, and then EtOAc was added (1 μL, 7.2 μmol). The mixture was filtered and concentrated. FC of the residue (PE/EtOAc 4:1) gave **37** (8 mg, 57%).$[\alpha]_D$ −9.4° ($c = 0.58$ in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): $\delta = 8.00$–8.00 (m, 2H, Ar*H*), 7.93–7.91 (m, 2H, Ar*H*), 7.88–7.81 (m, 2H, Ar*H*), 7.53–7.36 (m, 7H, Ar*H*), 7.31–7.28 (m, 2H, Ar*H*), 5.94 (appt, 1H, $J_{2',3'} = J_{3',4'} = 9.6$ Hz, H-3′), 5.69 (dd, 1H, $J_{3',4'} = J_{4',5'} = 9.7$ Hz, H-4′), 5.62–5.52 (m, 3H, H-2′, H-5,10), 5.23 (dd, 1H, $J_{9,10trans} = 15.7$, $J_{8,9} = 7.5$ Hz, H-9), 5.21 (d, 1H, $J_{1',2'} = 7.9$ Hz, H-1′), 4.27 (d, 1H, H-5′), 3.69 (s, 3H, O*Me*), 3.48–3.38 (m, 2H, H-7,8), 2.87 (s, 3H, O*Me*), 2.56–2.41 (m, 2H, H-6, CH_2), 2.40–2.30 (m 2H, CH_2), 2.28–2.06 (m, 5H, CH_2), 1.70 (d, 3H, $J = 1.5$ Hz, *Me* at C-4), 1.68–1.57 (m, 2H, CH_2), 1.52–1.45 (m, 2H, CH_2), 0.91 ppm (d, 3H, $J_{6,Me} = 6.8$ Hz, *Me* at C-6); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta =$ 212.3 (C-1), 167.6 (C=O), 165.9 (C=O), 165.4 (C=O), 165.0 (C=O), 134.5 (C=C), 133.5 (Ar*C*H), 133.4 (Ar*C*H), 133.2 (Ar*C*H), 132.0 (*Cq*), 130.5 (C=C), 130.0 (Ar*C*H), 130.0 (Ar*C*H), 129.9 (Ar*C*H), 129.8 (*Cq*), 129.4 (C-9), 129.1 (*Cq*), 129.1 (*Cq*), 128.6 (Ar*C*H), 128.5 (Ar*C*H), 128.4 (Ar*C*H), 101.5 (C-1′), 84.9 (C-7 or 8), 84.5 (C-7 or 8), 72.9 (C-5), 72.7 (C-3′), 72.1 (C-2′), 70.8 (C-4′), 56.5 (OCH_3), 52.9 (OCH_3), 42.3 (CH_2), 41.0 (CH_2), 33.2 (C-6), 30.5 (CH_2), 28.8 (CH_2), 27.2 (CH_2), 23.4 (CH_2), 23.2 (CH_3 at C-4), 13.5 ppm (CH_3 at C-6); HRMS-ESI: *m/z*: calcd for $C_{45}H_{50}O_{12}Na$: 805.3200; found: 805.3202.

(4*Z*,6*R*,7*S*,8*S*,9*E*)-7-*O*-(β-D-Glucopyranosyluronic acid)-4,6-dimethylcyclotetradeca-8-methoxy-4,9-dienone (MGSTA-16): To stirred **37** (12.7 mg; 16 μmol) in MeOH (1.2 mL), NaOH aq. 5% (48 μL, 59 μmol) was added. The resulting solution was stirred at RT for 20 h. A solution of 1 M AcOH was added until the pH was 7. The solvent was removed under reduced pressure and the residue was passed through silica gel 100 C18-reversed-phase column (H_2O to H_2O/MeOH 4:1 to H_2O/MeOH 2:1 to H_2O/MeOH 1:1 to H_2O/MeOH 1:2 to MeOH). The residue was subjected to vacuum distillation to remove the aromatic impurities and then passed again through silica gel 100 C18-reverse-phase silica (eluant, H_2O to H_2O/MeOH 4:1 to H_2O/MeOH 2:1 to H_2O/MeOH 1:1 to H_2O/MeOH 1:2 to MeOH) to give **MGSTA-16** (5.3 mg; 73%). $[\alpha]_D$ +5.32° ($c = 0.36$ in MeOH); ^1H NMR ($CDCl_3$, 500 MHz): 5.85 (ddd, 1H, $J_{9,10} = 15.0$, 9.6, 4.8 Hz, H-10), 5.44 (dd, 1H, $J_{8,9} = 9.0$ Hz, H-9), 5.37 (d, 1H, $J_{5,6} = 9.2$ Hz, H-5), 4.44 (d, 1H, $J_{1',2'} = 7.9$ Hz, H-1′), 3.85 (appt, 1H, H-8), 3.60 (d, 1H, $J_{4',5'} = 9.3$ Hz, H-5′), 3.56–3.46 (m, 3H, H-7,3′,4′), 3.37

(dd, 1H, $J_{2',3'}$ = 7.8 Hz, H-2′), 3.32 (s, 3H, OCH_3), 2.96 (m, 1H, CH_2), 2.69 (m, 1H, CH_2), 2.42 (m, 1H, CH_2), 2.35–2.16 (m, 6H, CH_2, H-6), 1.71–1.60 (m, 5H, Me at C-4, CH_2), 1.50 (m, 1H, CH_2), 1.36–1.25 (m, 1H, CH_2), 0.87 ppm (d, 3H, $J_{Me,6}$ = 6.7 Hz, Me at C-6); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 218.8 (C-1), 175.4 (C-6′), 137.2 (C-10), 133.1 (C-4), 130.5 (C-5), 127.0 (C-9), 104.3 (C-1′), 87.7 (CH), 84.4 (C-8), 76.9 (C-5′), 75.4 (CH), 73.8 (C-2′), 71.9 (CH), 55.5 (OCH_3), 40.4 (CH_2), 38.7 (CH_2), 32.9 (C-6), 29.1 (CH_2), 28.6 (CH_2), 24.7 (CH_2), 22.1 (Me at C-4), 20.5 (CH_2), 12.2 ppm (Me at C-6); HRMS-ESI: *m/z*: calcd for $C_{23}H_{36}O_9Na$: 479.2257; found: 479.2263.

(4*Z*,6*R*,7*S*,8*S*,9*E*)-7-*O*-(Methyl 2′,3′,4′-tri-*O*-benzoyl-α-D-glucopyranosyluronate)-4,6-dimethylcyclotetradeca-8-methoxy-4,9-dienone (38): To stirred **37** (20.8 mg; 27 μmol), in $CDCl_3$ (0.75 mL), $TiCl_4$ (66 μL; 54 μmol; 0.82 M in $CDCl_3$) was added. After storage for 12 h at 4 °C, the solution was diluted with CH_2Cl_2 and quenched with $NaHCO_3$. The organic layers were collected, dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. FC of the residue (PE/EtOAc 4:1) gave **38** (14.5 mg, 69%); $[\alpha]_D$ +33.4° (*c* = 0.75 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 8.05–7.99 (m, 2H, Ar*H*), 8.00–7.94 (m, 2H, Ar*H*), 7.92–7.85 (m, 2H, Ar*H*), 7.56–7.48 (m, 2H, Ar*H*), 7.47–7.35 (m, 5H, Ar*H*), 7.30 (m, 2H, Ar*H*), 6.27 (appt, 1H, $J_{2',3'} = J_{3',4'}$ = 9.9 Hz, H-3′), 5.71 (ddd, 1H, $J_{9,10}$ = 14.9, 8.4, 5.9 Hz, H-10), 5.58 (t, 1H, $J_{4',5'}$ = 10.0 Hz, H-4′), 5.45 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1′), 5.37 (dd, 1H, H-2′), 5.31 (dd, 1H, $J_{8,9}$ = 8.1 Hz, H-9), 5.21 (d, 1H, H-5′), 4.80 (d, 1H, $J_{5,6}$ = 9.6 Hz, H-5), 3.70 (appt, 1H, H-8), 3.67 (s, 3H, OCH_3), 3.56 (d, 1H, $J_{7,8}$ = 9.1 Hz, H-7), 3.27 (s, 3H, OCH_3), 2.63–2.57 (m, 1H, CH_2), 2.38–2.07 (m, 7H, H-6, CH_2), 1.98–1.93 (m, 1H, CH_2), 1.67–1.57 (m, 2H, CH_2), 1.48–1.38 (m, 2H, CH_2), 1.42 (d, 3H, $J_{Me,4}$ = 5.8 Hz, Me at C-4), 0.85 ppm (d, 3H, $J_{Me,6}$ = 6.6 Hz, Me at C-6); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 211.1 (C-1), 169.1 (C=O), 166.0 (C=O), 165.7 (C=O), 165.6 (C=O), 134.9 (C-10), 134.0 (Cq), 133.7 (ArCH), 133.4 (ArCH), 133.4 (ArCH), 130.1 (ArCH), 129.9 (ArCH), 129.9 (ArCH), 129.6 (C-5), 129.3 (Cq), 129.3 (Cq), 129.1 (C-9), 129.0 (Cq), 128.7 (ArCH), 128.6 (ArCH), 128.5 (ArCH), 97.3 (C-1′), 86.2 (C-7), 82.6 (C-8), 71.8 (C-2′), 70.9 (C-4′), 70.0 (C-3′), 68.7 (C-5′), 56.1 (OCH_3), 52.8 (OCH_3), 40.8 (CH_2), 40.3 (CH_2), 33.9 (C-6), 30.2 (CH_2), 29.0 (CH_2), 26.3 (CH_2), 22.8 (Me at C-4), 22.2 (CH_2), 12.8 ppm (Me at C-6); HRMS-ESI: *m/z*: calcd for $C_{45}H_{50}O_{12}Na$: 805.3200; found: 805.3212.

(4*Z*,6*R*,7*S*,8*S*,9*E*)-7-*O*-(α-D-Glucopyranosyluronic acid)-4,6-dimethylcyclotetradeca-8-methoxy-4,9-dienone (MGSTA-17): To stirred **38** (9 mg; 11.5 μmol) in MeOH (0.8 mL), NaOH aq. 5% (26.2 μL, 32.2 μmol) was added. The resulting solution was stirred at RT for 48 h. A solution of 1 M AcOH was added until pH 7 was reached. The solvent was removed under reduced pressure and the residue was passed through a silica gel 100 C18-reversed phase column (eluant, H_2O to H_2O/MeOH 4:1 to H_2O/MeOH 2:1 to H_2O/MeOH 1:1 to H_2O/MeOH 1:2 to MeOH) to give **MGSTA-17** (3.2 mg; 61%). $[\alpha]_D$ +63.2 (*c* = 0.032 in MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 5.81 (ddd, 1H, $J_{9,10}$ = 15.0, 10.1, 4.5 Hz, H-10), 5.46–5.39 (dd, 1H, $J_{8,9}$ = 9.4 Hz, H-9), 5.34 (dd, 1H, *J* = 9.9, 1.6 Hz, H-5), 5.01 (d, 1H, $J_{1',2'}$ = 3.9 Hz, H-1′), 4.39 (d, 1H, $J_{4',5'}$ = 10.2 Hz, H-5′), 3.81 (dd, 1H, $J_{2',3'}$ = 9.9, $J_{3',4'}$ = 8.9 Hz, H-3′) 3.74–3.71 (appt, 1H, H-8′), 3.56 (m, 2H, H-7,2′), 3.48 (dd, 1H, $J_{3',4'}$ = 9.0 Hz, H-4′), 3.29 (s, 3H, OCH_3), 3.12–3.05 (m, 1H, CH_2), 2.78–2.73 (m, 1H, CH_2), 2.58–2.52 (m, 1H, CH_2), 2.36–2.10 (m, 6H, H-6, CH_2), 1.72–1.62 (m, 2H, CH_2), 1.70 (d, 3H, *J* = 1.4 Hz, Me at C-4), 1.53–1.44 (m, 1H, CH_2), 1.27–1.13 (m, 1H, CH_2), 0.89 ppm (d, 3H, $J_{6,Me}$ = 6.7 Hz, Me at C-6); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 218.4 (C-1), 175.7 (C-6′), 136.8 (C-10), 133.4 (C-4), 130.6 (C-5), 127.3 (C-9), 99.2 (C-1′), 84.4 (C-7), 83.2 (C-8), 72.6 (C-3′), 71.9 (C-4′), 71.5 (C-2′), 71.3 (C-5′), 55.3 (OCH_3), 40.3 (CH_2), 37.8 (CH_2), 33.8 (C-6), 29.0 (CH_2), 28.7 (CH_2), 24.2 (CH_2), 22.0 (Me at C-4), 19.9 (CH_2), 12.0 ppm (Me at C-6); HRMS-ESI: calcd for $C_{23}H_{36}O_9Na$: 479.2257; found: 479.2256.

MGSTA-18 and MGSTA-19

To **57b** (21 mg, not fully pure, < 32.5 μmol,) at −78 °C in 2.5 mL CH_2Cl_2, *m*CPBA (≤ 77%, 7.6 mg, 33.9 μmol) in CH_2Cl_2 (2.5 mL) was added. After 30 min at −78 °C, TLC (PE/EtOAc 1:1.5) showed only baseline material. *i*Pr_2NEt (6.8 μL, 39 μmol) was then added and the mixture was allowed to warm to room temperature over 10 min. After 2 h at room temperature, TLC showed there still to have a baseline material, so more *i*Pr_2NEt (7 μL) was added. After 1 h the mixture was concentrated under reduced pressure and purified by chromatography on silica eluting with PE/EtOAc (1:1.5) to give **MGSTA-19** (4.7 mg) and **MGSTA-18** (8.5 mg) in 65% yield for 2 steps.

4-{(*S*)-5-[(2*R*,3*E*,5*R*,6*S*,7*S*,8*Z*,12*Z*)-6-Hydroxy-7-methoxy-3,5-dimethyl-14-oxooxacyclotetradeca-3,8,12-trien-2-yl]-4-oxohexyl}piperidine-2,6-dione (MGSTA-18): ^{1}H NMR (500 MHz): δ = 7.70 (br s, 1H), 6.23 (ddd, 1H, *J* = 11, 11, 6.5 Hz, CH=CHC=O, H-3), 5.78 (ddd, 1H, *J* = 11, 11, 5.3 Hz, H-8), 5.72 (d, 1H, *J* = 11.4 Hz, H-4), 5.55 (apt t, 1H, *J* = 10.5 Hz, H-7), 5.51 (d, 1H, *J* = 10 Hz, H-11), 5.39 (d, 1H, *J* = 11.0 Hz, CHOC=O), 3.79 (d, 1H, *J* = 10.1 Hz), 3.19 (s, 3H), 3.04 (t, 1H, *J* = 10.2 Hz, 10.2 Hz), 3.00 (dd, 1H, *J* = 11.1 Hz, 7.1 Hz), 2.80–2.68 (m, 4H), 2.52 (dt, 2H, *J* = 6.9, 6.8, 3.3 Hz), 2.30–2.19 (m, 2H), 2.17–2.06 (m, 3H), 1.96 (m, 1H), 1.63 (d, 3H, *J* = 1.0 Hz), 1.65–1.60 (m, 2H), 1.40–1.33 (m, 2H), 1.08 (d, 3H, *J* = 6.6 Hz), 0.97 ppm (d, 3H, *J* = 7.1 Hz); ^{13}C NMR (125 MHz): δ = 210.9 (C), 171.7 (C), 165.5 (C), 145.1 (CH), 139.6 (CH), 133.7 (CH), 129.5 (C), 127.0 (CH), 121.4 (CH), 81.7 (CH), 77.2 (CH), 73.1 (CH), 54.9 (CH_3), 46.6 (CH), 41.3 (CH_2), 37.7 (CH_2), 35.9 (CH), 34.2 (CH_2), 30.4 (CH), 29.8 (CH_2), 28.3 (CH_2), 20.1 (CH_2), 17.2 (CH_3), 13.4 (CH_3), 10.5 ppm (CH_3); ESI/MS$^-$ *m/z*: 512.26 [*M*+Na^+], 488.27 [*M*−H^-]; HRMS-ESI: *m/z*: calcd for $C_{27}H_{39}NO_7Na$: 512.2624; found: 512.2598.

4-{(*S*)-5-[(2*R*,3*E*,5*R*,6*S*,7*S*,8*Z*,12*E*)-6-Hydroxy-7-methoxy-3,5-dimethyl-14-oxooxacyclotetradeca-3,8,12-trien-2-yl]-4-oxohexyl}piperidine-2,6-dione (MGSTA-19): ^{1}H NMR (500 MHz): δ = 7.75 (br s, 1H, NH), 6.84 (ddd, 1H, *J* = 15.5, 6.0, 7.0 Hz, CH=CHC=O, H-3), 5.85 (d, 1H, *J* = 15.5 Hz, CH=CHC=O, H-2), 5.69 (ddd, 1H, *J* = 11, 11, 5.5 Hz, H-6), 5.59 (dd, 1H, *J* = 11, 9.5 Hz, H-7), 5.34 (d, 1H, *J* = 10.0 Hz, CH_3C=CH, H-11), 5.24 ppm (d, 1H, *J* = 10.4 Hz, CH–O–C=O, H-13); ^{13}C NMR (125 MHz): δ = 211.1 (C), 171.8 (C), 167.0 (C), 150.7 (CH), 136.2 (CH), 133.1 (CH), 131.6 (C), 127.8 (CH), 123.0 (CH), 82.7 (CH), 77.5 (CH), 74.8 (CH), 55.5 (CH_3), 47.2 (CH), 41.1 (CH_2), 37.73 (CH_2), 37.72 (CH_2), 35.6 (CH), 34.0 (CH_2), 30.4 (CH_2), 30.3 (CH), 29.3 (CH_2), 20.0 (CH_2), 17.6 (CH_3), 13.5 (CH_3), 11.4 ppm (CH_3); ESI/MS *m/z*: 512.26 [*M*+Na^+], 488.27 [*M*−H^-]; HRMS-ESI: *m/z*: calcd for $C_{27}H_{39}NO_7Na$: 512.2624; found: 512.2621.

Cell viability assay (MTT-assay)

Cell viability (metabolic activity of viable cells) was quantified by MTT assay. Cells were seeded onto 96-well plate (Nunc Inc., Denmark) at the concentration of 1×10^4 cells per well. When cells reached 60–70% confluence, the medium (DMEM) was replaced with medium containing 10% FBS and six various migrastatin analogues at the concentrations of: 1, 10, or 100 μM. The cultures were incubated for 24 h. Then, cells were incubated with 0.5 mg mL^{-1} tetrazolium salt MTT diluted in phenol red-free DMEM medium (Sigma Aldrich) for 4 h at 37 °C. To complete solubilization of the formazan crystals, 100 μL of DMSO (dimethyl sulfoxide, Sigma Aldrich) were added to each well. Cell viability was quantified by measuring photometric absorbance at 570 nm in a multi-well plate reader (Infinite 200 PRO Tecan™, TECAN, Switzerland). All the samples were examined in triplicate, each experiment was conducted three times (*n* = 9).

In vitro wound healing assay (scratch test)

The scratch assay was conducted to assess the influence of migrastatin analogues on MCF7, MCF7-Dox, MDA-MB361, MDA-MB231, HPAC cancer cell motility. The cells were seeded in a high density at 600 mm Petri dishes (Corning-Costar, Cambridge, USA). After 24 h, the medium was removed and replaced with medium (DMEM) containing 10% FBS and one migrastatin analogues or DMSO (control). The monolayer was wounded by scratching the surface: as uniformly and straight as possible with a pipette tip (1000 μL) at least three times. The images of cells invading the scratch were captured at indicated time points (0, 6 and 24 or 48 h where indicated) using phase-contrast microscopy (IX 70 Olympus Optical Co., Germany). The pictures were analyzed using a computer-assisted image analyzer (Olympus Microimage™ Image Analysis, software version 4.0 for Windows, USA). The migration rate was expressed as a percentage of scratch closure and was calculated as follows: % of scratch closure = a−b/a, in which (a) is a distance between edges of the wound, and (b) is the distance which remained cell-free during cell migration to close the wound.[39] The values are presented as means of three independent wound fields from three independent experiments ($n = 9$).

Trans-well migration assay

The Boyden chamber cell migration assay was performed using the BD Falcon™ FluoroBlock™ 24-Multiwell Insert Plates (8 micron pore size) (BD Biosciences, USA). Firstly, cells (1×10^5) were suspended in FBS free medium and added to the apical chambers of an insert plates (500 μL). Then 750 μL of chemoattractant (10% FBS) was added to the basal chambers. Migrastatin analogues or DMSO (as a control) were added to the medium in both chambers. For dose-dependent studies MGSTA analogues were used in the range of concentration from 0.01 to 100 μM. Migration assays were carried out for 18–20 h at standard culture conditions. Then, the medium was carefully removed from the apical chamber and the insert system was transferred into a second 24-well plate containing 500 μL of 2.5 $\mu g\,mL^{-1}$ Calcein AM in Hanks' Balanced Salt solution (HBSS). Plates were incubated for 1 hour at standard culture conditions. Then, the fluorescence of migrated cells was measured at excitation wavelength 485 nm and emission wavelength 530 nm using florescent plate reader with bottom reading capabilities Infinite 200 PRO Tecan™ (TECAN, Switzerland). All samples were assayed in triplicate, and each experiment was conducted three times ($n = 9$). For each experiment two negative controls were used: 1) cells grown as given above without adding chemoattractant to the medium and 2) medium added as described without cells. To determine fluorescence of cells that migrated through the membrane, plates were analysed using a fluorescence microscope (Olympus BX60, magnification x4).

In vitro pharmacology: Binding assays

Cell membrane homogenates are incubated with radioligand binding in the absence or presence of **MGSTA-6** in a buffer. Non specific binding is determined in the presence of a specific agonist or antagonist at the target. Following incubation, the samples are filtered rapidly under vacuum through glass fiber filters presoaked in a buffer and rinsed several times with an ice-cold buffer using a 96-sample cell harvester. The filters are dried then counted for radioactivity in a scintillation counter using a scintillation cocktail. The results are expressed as a percent inhibition of the control radioligand specific binding.[40]

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