Synthesis and Biological Evaluation of Simplified Pironetin Analogues with Modifications in the Side Chain and the Lactone Ring

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The preparation of several new analogues of the natural dihydropyrone pironetin is described. They differ from the natural product mainly in the nature of the side chain and the lactone ring. Their cytotoxic activity has been measured. In addition, their interaction with tubulin and their ability to inhibit the secretion of the vascular endothelial growth factor (VEGF) and the expression of angiogenesis and telomerase-related genes have been determined. Some of the compounds have been found active in some of these biological properties.

Introduction

It is widely known that cancer, one leading cause of death in developed countries, may be induced by a plethora of both external and internal factors, including genetic mutations. Accordingly, a number of types of therapeutic attack has been investigated.[1] One of these involves the use of cytotoxic drugs, which exert their effect in many cases by means of inducing various mechanisms of cell death.[2] As a matter of fact, many of such drugs owe this property to interaction with the microtubule network. Microtubules are dynamic polymers that play a central role in a number of cellular processes, most particularly cell division, as they are key constituents of the mitotic spindle.[3] Microtubules are constituted of a protein named tubulin, the functional form of which, and the most abundant component, is a heterodimer formed through non-covalent binding of two monomeric constituents, called α- and β-tubulin. For cell division to occur in a normal way, microtubules must be in a constant state of formation and disruption, a process named microtubule dynamic instability.[4] Molecules which influence microtubule instability will also influence the cell division process, not only of normal cells but also of tumoral ones. Therefore, it is not surprising that tubulin-binding molecules (TBMs) constitute a very important class of anticancer agents.[5]

TBM are able to interfere with microtubule assembly and functions, either by causing disruption of the microtubules or else through their stabilization. Most of the hitherto described active drugs are natural products or derivatives thereof.[6] Major drugs can already be found on the market and many other promising compounds are in clinical trials.

TBM may be divided in two broad categories, those that bind to α-tubulin and those that bind to β-tubulin. The latter group is presently by far the most numerous and contains products which cause either disruption[7] or stabilization[8] of microtubules. The number of products that bind to α-tubulin is, however, very small,[9] the naturally occurring 5,6-dihydro-α-pyrone pironetin (Figure 1) being the first-reported example. Pironetin is a potent inhibitor of tubulin assembly and has been found to arrest cell cycle progression in the G2/M phase.[10] This feature has motivated a number of groups to undertake total syntheses of this natural compound.[11] It is worth mentioning here that 5,6-dihydro-α-pyrones constitute an ample group of natural products endowed with a broad variety of pharmacologically useful properties, most likely related to the presence of the Michael acceptor moiety in the pyrone ring.[12]

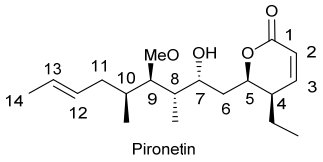


Figure 1 Structure of pironetin, a highly cytotoxic natural pyrone.

Some structure-activity (SAR) studies on pironetin have been reported.[10] These studies have shown that the presence of the conjugated double bond in the lactone ring and of the hydroxyl group at C-9, either free or methylated, are essential for the biological activity.[9] The epoxidation of the C12=C13 double bond has been shown to cause a decrease in the activity but this may perhaps represent a negative feature of the oxirane ring, rather than a strict need of this C=C bond.

As a member of the up to now small group of products that bind to α-tubulin, pironetin constitutes a pharmacologically interesting target. Thus, a key purpose of our research is the preparation of pironetin analogues that retain a substantial proportion of the biological activity of the natural metabolite while displaying a more simplified structure. In order to develop SAR studies based upon the pironetin framework, we designed several years ago[13] a simplified model structure where all elements that had not yet proven to be essential for the biological activity were removed. The target structures **I**/**II** are schematically shown in Figure 2. The elements that were maintained are the conjugated dihydropyrone ring and the side chain with the methoxy group at C-9. The hydroxyl group at C-7 was removed in some substrates (**I**) and retained in others (**II**), in order to see its influence on the activity. All alkyl pendants (methyl groups at C-8 and C-10, ethyl at C-4) and the isolated C12−C13 double bond were removed. The

configurations of the two/three remaining stereocentres were then varied in a systematic way. Thus, all four possible stereoisomers with general constitution **I**, with no hydroxyl group at C-7, were prepared. Likewise, all eight stereoisomers exhibiting general structure **II**, with a hydroxyl group at C-7, were synthesized. Subsequently, the cytotoxic activity of these analogues and their interactions with tubulin were investigated.[13]

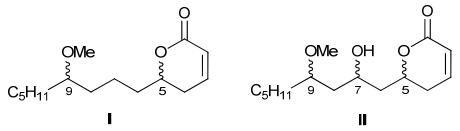


Figure 2 General structures of the first generation of simplified pironetin analogues (ref. 13).

In continuation of this line of research, we concentrated our attention on the importance of the alkyl pendants in the pironetin molecule for the biological properties of the natural compound. In line with this reasoning, we prepared the six pironetin analogues **III**-**VIII** (Fig. 3). In all these compounds, the configurations at the oxygenated carbons C-5, C-7 and C-9 are as in natural pironetin. With respect to general structure **II** (Fig. 2), compounds **III** and **IV** contain an additional methyl residue at C-10 with either configuration, whereas in compounds **V** and **VI**, the extra methyl pendant is allocated at C-8. Finally, compounds **VII** and **VIII** display an extra alkyl residue (methyl or ethyl) at C-4, in both cases with the same configuration as in natural pironetin.[14]

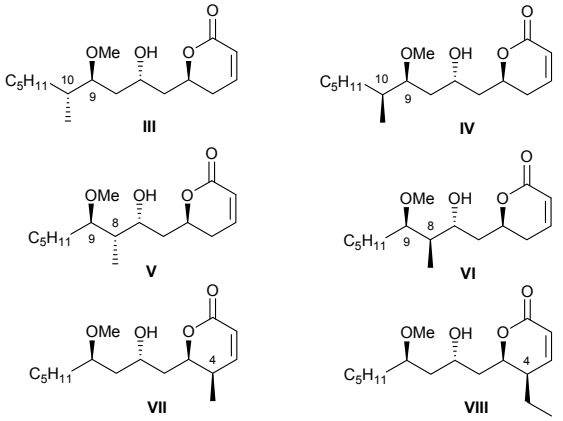


Figure 3 Structures **III**-**VIII** of the second series of pironetin analogues (ref. 13).

The cytotoxic activities of pironetin analogues **III**-**VIII** were then investigated. Most compounds proved cytotoxic in the low micromolar range, therefore about two-three orders of magnitude less active than pironetin itself.[14] These results suggest that all alkyl pendants are important for the full biological activity, this being most likely due to the fact that the alkyl groups restrict the conformational mobility of the molecule and reduce the number of available conformations.[15,16] This in turn makes more probable that the molecule adopts a shape that fits better into the active site of α-tubulin.

In view of these results, we decided to prepare a new group of pironetin analogues with a higher degree of alkylation in the side chain but still retaining a simplified structure. Figure 4 shows the eight compounds we have prepared and evaluated for their biological properties.

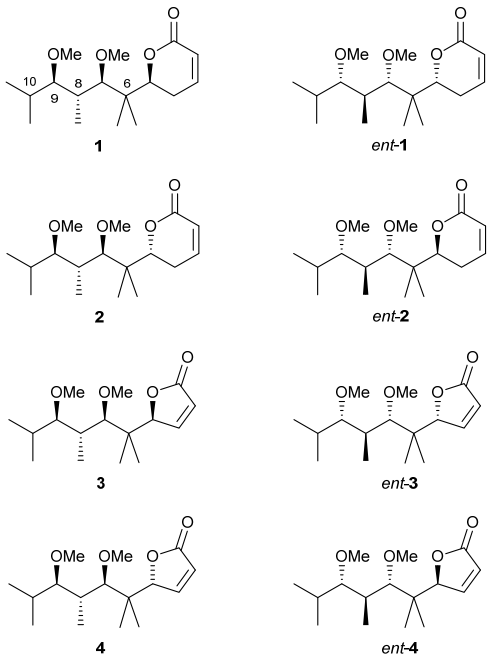


Figure 4 Structures of compounds of the third series of pironetin analogues (this work).

In comparison to pironetin, pyrones **1**, *ent*-**1**, **2** and *ent*-**2** in Fig. 4 display a shorter carbon chain, two stereocentres less (C-4 and C-10 in pironetin numbering) and an additional gem-dimethyl moiety (at C-6 in pironetin numbering). Furthermore, and in order to investigate the importance of the lactone ring size, analogues **3**, *ent*-**3**, **4** and *ent*-**4** having a furanone system were also prepared.

In recent times, we have not limited our biological investigations on bioactive molecules to solely measurements of their cytotoxic activity, expressed as IC_{50} values. Indeed, while mechanisms of anticancer activity are often related to interference with microtubule assembly and functions, other mechanisms may also be operative. In most solid tumors, for example, angiogenesis is an important process for tumor growth and metastasis. Many different mediators are involved in this process, including the vascular endothelial growth factor (VEGF), which has been shown to play a critical role in pathological angiogenesis.[17]

Another relevant mechanism in cancer genesis is related to the role of the chromosomal telomers. Most cancer cells exhibit telomerase activity. The latter mantains the length of the telomeres, thus preserving genomic stability.[18] Telomerase is a ribonucleoprotein composed of two main subunits which, in the case of human beings, are called human telomerase RNA (hTR) and human telomerase protein (hTERT). Many studies have demonstrated that interference in the expression of the *hTERT* gene can efficiently inhibit the growth and tumorigenicity of cancer cells, as the *hTERT* gene is a rate-

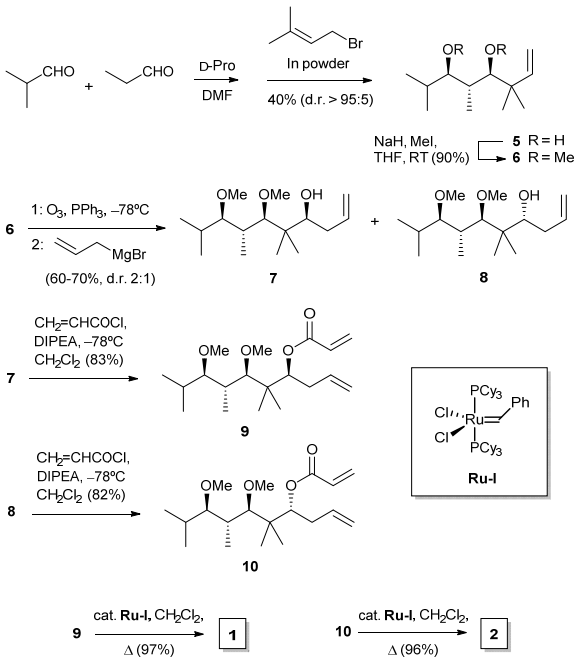
limiting factor in telomerase synthesis and activity. Equally important is the *c-Myc* gene, which has been found to be amplified in various types of human cancers. The result of the expression of this gene, the c-Myc protein, is a transcriptional factor with an important role in cell proliferation, differentiation, invasion and adhesion of tumor cells.[19] It is also involved in the activation of *hTERT* gene transcription.

Since on one hand tumoral cell secretion of VEGF is an important factor in metastasis and, on the other hand, telomerase is responsible for the inmortality of tumoral cells, the potential multiple ability[20] of some compounds to perturb microtubule dynamics and, at the same time, to inhibit VEGF secretion by tumoral cells and the expression of the *VEGF*, *hTERT* and *c-Myc* genes was considered a goal worth pursuing. For that reason, we have also included the last types of biological activities in our unvestigation of the general pharmacological profile of our compounds.

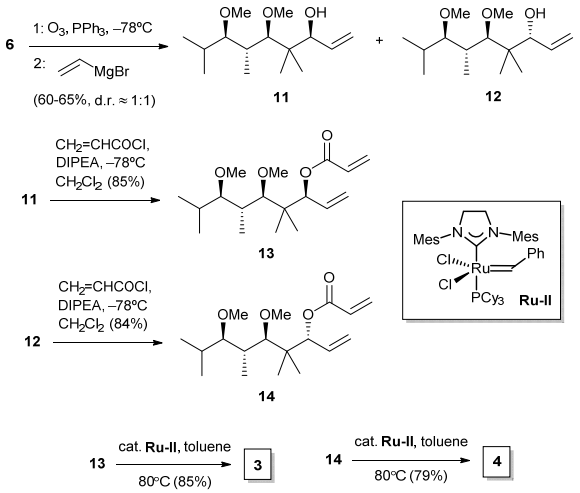
Results and discussion

Synthesis of compounds **1-4** and their enantiomers

For our purposes, we aimed at performing a simple synthetic sequence in which stereochemical complexity is rapidly achieved through a convergent methodology. Thus, the synthesis of compounds **1**-**4** was carried out as depicted in Scheme 1. Creation of chirality was achieved by means of an adaptation of a published organocatalytic procedure.[21] Thus, propionaldehyde and isobutyraldehyde were allowed to react in DMF in the presence of D-proline. This gave a crossed aldol product which was subjected *in situ* to Barbier-type, indium-mediated prenylation to yield diol **5** in fair yield and high enantio- and diastereoselectivity.[21] Methylation to **6** was followed by ozonolysis to yield an unstable intermediate aldehyde which, without isolation, was allowed to react with allylmagnesium bromide in THF. This sequence furnished a mixture of diastereoisomeric alcohols **7** and **8** (d.r. 2:1), which proved amenable to chromatographic separation. Both compounds were then esterified with acryloyl chloride, and the resulting acrylates, **9** and **10**, were subjected to ruthenium-catalyzed ring-closing metathesis[22] to afford the target dihydropyranones **1** and **2**, respectively. Their enantiomers *ent*-**1** and *ent*-**2** were obtained by means of an identical synthetic sequence with the only difference of using L-proline as the organocatalyst (see Experimental).

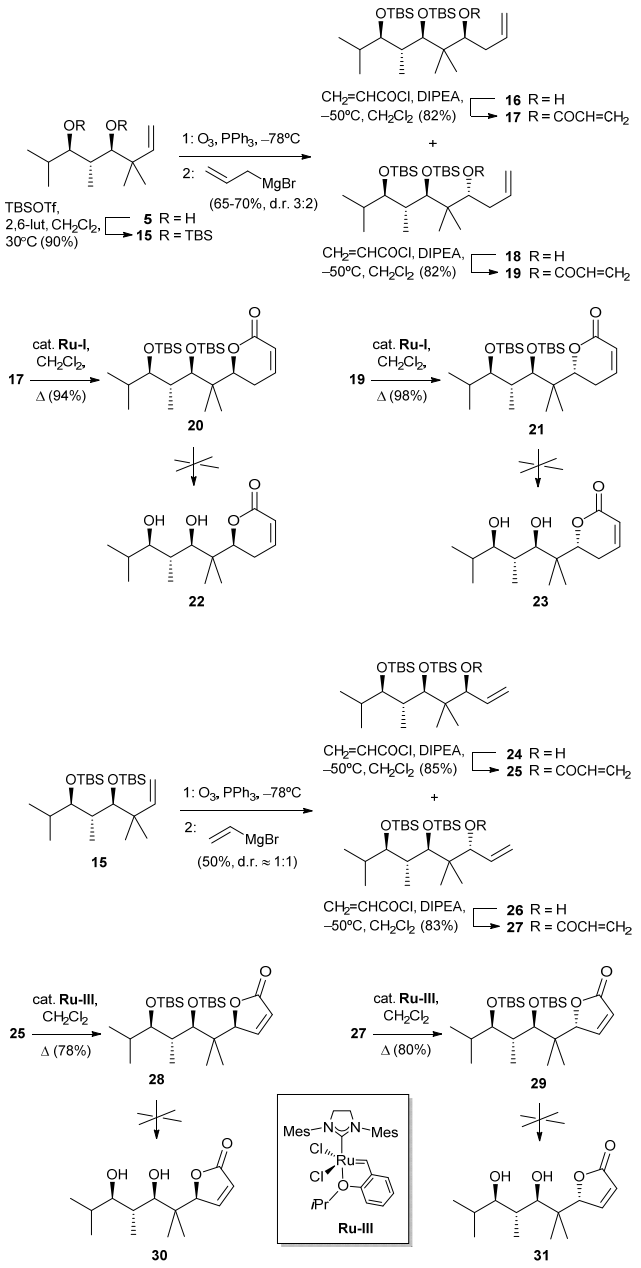


Scheme 1 Synthesis of dihydropyranones **1** and **2**. Abbreviations: D-Pro, D-proline; DIPEA, ethyl *N,N*-di*iso*propylamine.



Scheme 2 Synthesis of furanones **3** and **4**.

Furanones **3** and **4** were prepared by means of a similar reaction sequence starting from olefin **6** (Scheme 2). Thus, the latter compound was subjected to ozonolysis followed by treatment of the crude unstable aldehyde with vinylmagnesium chloride to yield alcohols **11** and **12**. These were then separated and subjected to esterification to acrylates **13** and **14**, respectively. Ring-closing metathesis of the latter compounds required the use of a second generation Grubbs ruthenium catalyst[22] in hot toluene as the solvent, and provided the target furanones **3** and **4**. Their enantiomers *ent*-**3** and *ent*-**4** were obtained from *ent*-**6** alongside the same reaction sequence (see Experimental).



Scheme 3 Attempts at the synthesis of dihydropyranones **22** and **23** and furanones **30** and **31**. Abbreviation: 2,6-lut, 2,6-lutidine.

In order to check the influence of having hydroxy instead of methoxy groups in the side chain (pironetin and compounds in Fig. 3 have one methoxy group and one hydroxyl group), we also tried to prepare analogues of compounds **1**-**4** with two hydroxy groups. To that purpose, diol **5** was doubly silylated to **15**, and the latter subjected to the same ozonolysis/allylation or alternatively ozonolysis/vinylation sequence to yield the diastereoisomeric pairs **16**/**18** and **24**/**26**, respectively (Scheme 3). After esterification with acryloyl chloride to **17**/**19** and **25**/**27**, ring-closing metathesis using in this case a Hoveyda-Grubbs-type ruthenium catalyst[22] afforded **20**/**21** and **28**/**29**, respectively. Unfortunately, all attempts at desilylation of the latter compounds under many different conditions to the desired lactones **22**, **23**, **30** and **31** only led to either no reaction, decomposition or formation of complex inseparable mixtures.[23]

Biological properties of pironetin analogues **1**-**4** and their enantiomers

Cellular effects of the compounds. We have determined the IC_{50} values for pironetin analogues **1**-**4**, and *ent*-**1**/*ent*-**4** on five tumoral cell lines: human colorectal adenocarcinoma HT-29 and HTC-116, human breast adenocarcinoma MCF-7, human cervical cancer HeLa and human promyelocytic leukemia HL-60, and compared these values with that of pironetin. Table 1 shows the cytotoxicity values for pironetin (nM) and pironetin analogues expressed as the compound concentration (μM) that causes 50% inhibition of cell growth (IC_{50}).

Table 1. IC_{50} values (μM)

Comp	HT-29	HTC-116	MCF-7	HeLa	HL-60
Piron.	7.1± 0,4 nM	8.3± 0.5 nM	6.8± 0,6 nM	9.2± 0.8 nM	12.6± 0.9 nM
1	4.2 ± 0.4	30 ± 1	22.25 ± 0.18	38 ± 3	3.4 ± 0.7
2	28.5 ± 0.5	62 ± 2	21 ± 2	60.5 ± 0.3	4.0 ± 0.5
3	>100	>100	>100	95 ± 5	>100
4	>100	>100	>100	>100	>100
***ent*1**	47 ± 2	62.0 ± 0.6	50 ± 2	54.2 ± 0.4	33 ± 7
***ent*-2**	5.9 ± 0.8	36 ± 1	12.9 ± 0.9	53.8 ± 0.1	1.38 ± 0.15
***ent*-3**	>100	>100	>100	>100	>100
***ent*-4**	>100	>100	>100	>100	>100

[a]IC_{50} values (μM for **1**-**4**, and *ent*-**1**/*ent*-**4** and nM for pironetin) are the mean ± standard error of three independent experiments.

The first conclusion that can be drawn from the IC_{50} values is that furanones **3** and **4** and their respective enantiomers *ent*-**3** and *ent*-**4** show almost no cytotoxicity. Pyranones did prove cytotoxic in the low micromolar range, thus about two-three orders of magnitude less active than pironetin itself. Among pyranones the most active compounds are **1** and *ent*-**2**. These two compounds share a common structural feature, the configuration of the lactone stereocenter, which is the same as in pironetin.

Effect of pironetin derivatives on the in vitro microtubule assembly. As pyranone derivatives showed to be cytotoxic we selected them to study their effect on the in vitro *microtubule assembly*. Figure 5 shows the effects of pyranones **1**, **2**, *ent*-**1** and *ent*-**2** on the microtubule formation studied by time-resolved turbidity measurements. Blue line shows the effects on the

microtubule assembly when 25 µM of tubulin was reacted in the presence of 20 mM sodium phosphate (NaPi), 10 mM $MgCl_2$, 1 mM EGTA, 3.4 M glycerol and 0.1 mM of GTP at pH=6.5. It can be seen that the nucleation phase takes aproximately 18 min. Then elongation phase starts and after 20 min the steady state is reached. When tubulin assembly is carried out in the presence of 27.5 µM of paclitaxel, absorbance is immediately increased (orange line) which is in accordance with the behaviour of a drug that promotes tubulin polymerization. Conversely, when tubulin assembly is carried out in the presence of 27.5 µM of pironetin, no absorbance is measured throughout the reaction time (green line), which is in accordance with the behaviour of a drug that inhibits tubulin polymerization. When tubulin assembly is carried out in the presence of 27.5 µM of each pyranone the dinamic of microtubule formation is quite similar to the one in the ausence of any compound (blue line). It can be inferred from these experiments that, in contrast to pironetin itself, pyranones **1**, **2**, *ent*-**1** and *ent*-**2** have little influence on the process of tubulin polymerization.

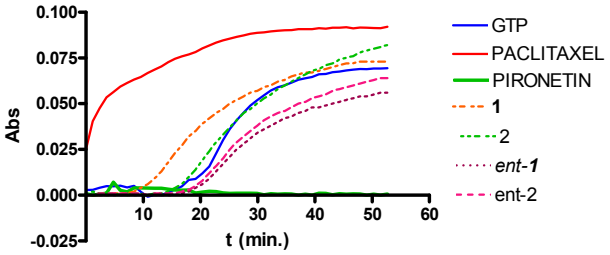


Figure 5.. Effects of colchicine, pironetine and compounds **1**, **2**, *ent*-**1** and *ent*-**2,** as well as paclitaxel and pironetin, on the *in vitro* microtubule assembly. The lines in the figure show the turbidimetric time course of polymerization of tubulin in the presence of GTP, and in the presence of 27.5 µM of each of the indicated compound.

Effect of pyranone derivatives on the hTERT, c-Myc and *VEGF gene inhibition and on the VEGF protein secretion.* We have also studied the ability of pyranones **1**, **2**, *ent*-**1** and *ent*-**2** to downregulate the expression of the *hTERT* and *c-Myc* genes, both involved in telomerase activity, and the expression of the *VEGF* gene and its associated protein VEGF, both involved in angiogenic activity. The expression of *hTERT*, *c-Myc* and *VEGF* genes were measured upon reverse transcription quantitative PCR (RT-qPCR) analysis on HT-29 tumoral cells. The VEGF protein production was determined with the ELISA procedure and corresponds mainly to the lighter VEGFA-165 isoform that is secreted to the culture medium. Table 2 shows expression percentage of the *hTERT*, *c-Myc* and *VEGF* genes after 48 h of incubation of HT-29 cells. Table 2 also shows VEGF protein secretion percentage from HT-29 cells determined after 72 h of incubation of HT-29 cells.

Table 2. Percentages of gene expression and VEGF protein secretion

Comp.	Concent.	*hTERT*[a] (%)	*c-Myc*[a] (%)	*VEGF*[a] (%)	VEGF protein[a] (%)
1	5 µM	49 ± 4	18 ± 1	45 ± 5	29 ± 4
2	25 µM	42 ± 7	25 ± 2	26 ± 1	36 ± 2
ent-**1**	25 µM	59 ± 8	36.5 ± 1.5	36 ± 2	63 ± 7
ent-**2**	5 µM	39 ± 5	25 ± 4	19.0 ±0.2	76 ± 4

[a]At least three measurements were performed in each case.

As regards the inhibition of the *hTERT* and *c-Myc* genes, compounds **1** and *ent*-**2** are the most active ones, especially if one considers that the concentration of these two compounds is five times lower than that of compounds **2** and *ent*-**1**. Particularly appealing is the activity of compound **1** on the inhibition in the expression of the *c-Myc* gene, which is decreased to 18% of the control value. Regarding the *VEGF* gene expression compound *ent*-**2** shows the greatest inhibition. However, this high decrease in gene expression is not accompanied by a similar decrease in VEGF protein secretion as compound *ent*-**2** downregulates protein secretion by only 24%. In this sense, the most active compound is pyranone **1** which downregulates VEGF protein secretion by 71%.

Summary

Pironetin analogues **1**-**4** and their enantiomers were synthesized with the aim at exploring the influence of the alkyl pendants as well as their stereochemistry and lactone ring size in their biological activity. Pyranones showed to be cytotoxic at micromolar level while furanones showed no cytotoxicity. Among pyranones the most cytotoxic were **1** and *ent*-**2**, which have the same configuration at the lactone stereocenter as pironetin. The influence of pyranones in tubulin polymerization was also measured but, in contrast to pironetin, they seem to have little influence in the tubulin polymerization process. It thus seems that removal of the methyl pendants at the side chain by a *gem*-dimethylated pattern causes a strong decrease in the interaction of the compounds with tubulin. As regards the inhibition of the *c-Myc* and *VEGF* genes, pyranones **1** and *ent*-**2** proved to be the most active compounds with **1** showing the strongest inhibition of VEGF protein secretion.

Experimental

Chemical procedures

NMR spectra were recorded at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) in $CDCl_3$ solution at 25 °C, with the solvent signals as internal reference. ^{13}C NMR signal multiplicities were determined with the APT pulse sequence. Mass spectra were run in the electrospray (ESMS) mode. IR data, which were measured as films on NaCl plates (oils) or as KBr pellets (solids), are given only when relevant functions (C=O, OH) are present. Optical rotations were measured at 25 °C. Reactions which required an inert atmosphere were carried out under dry N_2 with flame-dried glassware. Commercial reagents were used as received. THF and Et_2O were freshly distilled from sodium-benzophenone ketyl. Dichloromethane was freshly distilled from CaH_2. Toluene was freshly distilled from sodium wire. Tertiary amines were freshly distilled from KOH.

(3*R*,4*R*,5*R*)-2,4,6,6-Tetramethyloct-7-ene-3,5-diol (**5**). The following reaction conditions should be strictly adhered to, with particular attention to the words highlighted in *italics*: D-proline (230 mg, 2 mmol) was dissolved under N_2 in dry DMF (2 mL) and placed in an ice bath. After stirring for 5 minutes, *freshly distilled* isobutyraldehyde (1.82 mL, 20 mmol) was added

followed by dry DMF (2.5 mL). In a separate flask, *freshly distilled* propionaldehyde (722 μL, 10 mmol) was dissolved in dry DMF (7 mL). The resulting solution was ice-cooled and added dropwise to the isobutyraldehyde solution by means of syringe pump at a rate of 0.10 mL/h (the needle of the pump should be just below the surface of the liquid). *Caution: higher addition rates give rise to diminished yields!* When all the propionaldehyde solution had been added, the reaction mixture was stirred at the same temperature for 15 h. Subsequently, prenyl bromide (2.31 mL, 20 mmol) was added dropwise (ca. 10 min.) followed by sodium iodide (1.65 g, 11 mmol) and powdered metallic indium (1.27 g, 11 mmol). The mixture was then *very vigorously* stirred for 5 min. at 0 ºC. After allowing the mixture to reach room temperature, water (11 mL) was added and the stirring was continued for 48 h. The reaction mixture was then poured onto saturated ammonium chloride and carefully extracted with EtOAc (*caution, emulsions may be formed!*). The organic layers were then dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The resulting oil was carefully chromatographed on silica gel (hexane-EtOAc, from 95:5 to 80:20). This yielded diol **5** (801 mg, 40% based on propionaldehyde) as off-white crystals (from Et_2O-$CHCl_3$): mp 76-77 ºC (from Et_2O-CH_2Cl_2), $[\alpha]_D$ +17.6 (*c* 1; $CHCl_3$). Spectral data were consistent with those published[17] (see Electronic Supplementary Information).

The procedure described above represents the maximum scale at which we were able to obtain reasonable yields. Attempts at increasing the scale only led to a decrease in the yield.

The procedure was repeated under the same conditions with L-proline to yield *ent*-**5**: $[\alpha]_D$ −18.1 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **5**.

The stereostructures of **5** and *ent*-**5** have been secured by means of an X-ray diffraction analysis.[24]

(4*R*,5*R*,6*R*)-4,6-Dimethoxy-3,3,5,7-tetramethyloct-1-ene (6). Sodium hydride (60% slurry in mineral oil, amount equivalent to 16 mmol) was washed two times under N_2 with dry hexane and once with dry THF. Then, THF (50 mL) was added and the suspension was cooled in an ice bath. Alcohol **5** (801 mg, 4 mmol) was then dissolved in dry THF (10 mL) and added dropwise to the sodium hydride suspension. The mixture was then allowed to reach room temperature. Subsequently, methyl iodide (1.25 mL, 20 mmol) was added dropwise and the mixture was stirred for 24 h at room temperature. The reaction mixture was then poured onto saturated ammonium chloride and extracted several times with Et_2O. The organic layers were then dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The resulting oil was carefully chromatographed on silica gel (hexane-EtOAc, 95:5). This yielded **6** (822 mg, 90%): oil, $[\alpha]_D$ +1.8 (*c* 1; $CHCl_3$); ^{1}H NMR δ 5.99 (1H, dd, *J* = 17.5, 11 Hz), 4.98 (1H, dd, *J* = 17.5, 1.5 Hz), 4.94 (1H, dd, *J* = 11, 1.5 Hz), 3.44 (3H, s), 3.38 (3H, s), 3.06 (1H, dd, *J* = 6, 3.5 Hz), 2.88 (1H, d, *J* = 5 Hz), 1.98 (1H, m), 1.88 (1H, br m), 1.08 (3H, s), 1.07 (3H, s), 0.99 (3H, d, *J* = 7.5 Hz), 0.93 (3H, d, *J* = 7 Hz), 0.90 (3H, d, *J* = 7 Hz); ^{13}C NMR δ 43.2 (C), 146.6, 91.6, 86.9, 38.8, 30.3 (CH), 110.8 (CH_2), 61.4, 59.5, 25.7, 23.3, 22.0, 17.1, 16.4 (CH_3); HR ESMS *m/z* 251.1991 ($M+Na^+$), calcd. for $C_{14}H_{28}NaO_2$, 251.1987.

(*ent*-**6**): oil, $[\alpha]_D$ −1.8 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **6**.

(4*S*,6*R*,7*R*,8*R*)-6,8-Dimethoxy-5,5,7,9-tetramethyldec-1-en-4-ol (7) and **(4*R*,6*R*,7*R*,8*R*)-6,8-dimethoxy-5,5,7,9-tetramethyldec-1-en-4-ol (8)**. Olefin **6** (685 mg, 3 mmol) was dissolved in dry CH_2Cl_2 (60 mL) and cooled to −78°C. A stream of ozone-oxygen was bubbled through the solution until persistence of the bluish color. Dry N_2 was then bubbled through the solution for 10 min. at the same temperature. After addition of PPh_3 (1.18 g, 4.5 mmol), the solution was left to stir at room temperature for 2 h. Solvent removal under reduced pressure gave a solid material, which was put on the top of a short silica gel pad and rapidly washed with hexane-EtOAc 9:1. After removal of volatiles under reduced pressure, the crude oily aldehyde was then directly used as such in the next allylation step (for weight calculations, the yield of the ozonolysis step was assumed to be quantitative).

The oily material from above was dissolved under N_2 in dry THF (15 mL) and cooled in an ice bath. After this, a 1 M solution of allylmagnesium bromide in THF (4 mL, 4 mmol) was added dropwise, and the mixture was allowed to reach room temperature, followed by stirring for 3 h (TLC monitoring). The reaction mixture was then poured onto saturated ammonium chloride and extracted several times with Et_2O. The organic layers were then dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The resulting oil was the subjected to a slow and careful chromatography on silica gel (hexane-Et_2O, 9:1) to yield **7** (367 mg, 45%) and **8** (182 g, 22%).

(**7**): oil, $[\alpha]_D$ −23.3 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 3400 (br, OH); ^{1}H NMR δ 5.90 (1H, ddt, *J* = 17, 10.5, 7 Hz), 5.01 (1H, dm, *J* ~ 17 Hz), 4.94 (1H, dm, *J* ~ 10.5 Hz), 4.90 (1H, br d, *J* ~ 3 Hz, OH), 3.68 (1H, dt, *J* = 10, 3 Hz), 3.45 (6H, s), 3.02 (1H, dd, *J* = 10, 1 Hz), 2.74 (1H, d, *J* = 2.5 Hz), 2.38 (1H, m), 2.14 (1H, m), 2.04 (1H, m), 1.81 (1H, m), 0.99 (3H, d, *J* = 7 Hz), 0.90 (3H, d, *J* = 7.5 Hz), 0.89 (3H, s), 0.87 (3H, s), 0.80 (3H, d, *J* = 7 Hz); ^{13}C NMR δ 43.4 (C), 137.9, 97.1, 87.3, 73.0, 36.2, 30.3 (CH), 115.1, 35.9 (CH_2), 62.0, 60.3, 25.5, 22.2, 21.1, 17.2, 14.5 (CH_3); HR ESMS *m/z* 273.2429 ($M+H^+$), calcd. for $C_{16}H_{33}O_3$, 273.2430.

(*ent*-**7**): oil, $[\alpha]_D$ +21.5 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **7**.

(**8**): oil, $[\alpha]_D$ +9.5 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 3480 (br, OH); ^{1}H NMR δ 5.94 (1H, ddt, *J* = 17, 10.5, 7 Hz), 5.08 (1H, dm, *J* ~ 17 Hz), 5.04 (1H, dm, *J* ~ 10.5 Hz), 3.70 (2H, m), 3.47 (3H, s), 3.41 (3H, s), 3.02 (2H, m), 2.22 (1H, m), 2.10 (2H, m), 1.90 (1H, m), 1.03 (3H, d, *J* = 7.5 Hz), 0.99 (3H, s), 0.98 (3H, d, *J* = 7 Hz), 0.90 (3H, d, *J* = 7 Hz), 0.88 (3H, s); ^{13}C NMR δ 42.9 (C), 137.4, 95.1, 86.8, 75.7, 37.0, 30.5 (CH), 115.9, 36.7 (CH_2), 61.9, 59.9, 22.5, 21.7, 21.0, 19.7, 16.0 (CH_3); HR ESMS *m/z* 273.2430 ($M+H^+$), calcd. for $C_{16}H_{33}O_3$, 273.2430.

(*ent*-**8**): oil, $[\alpha]_D$ −11.8 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **8**.

(4*S*,6*R*,7*R*,8*R*)-6,8-Dimethoxy-5,5,7,9-tetramethyldec-1-en-4-yl acrylate (**9**) and **(4*R*,6*R*,7*R*,8*R*)-6,8-dimethoxy-5,5,7,9-tetramethyldec-1-en-4-yl acrylate** (**10**). Alcohol **7** or **8** (82 mg, 0.3 mmol) was dissolved under N_2 in dry CH_2Cl_2 (5 mL),

cooled to −78°C and treated sequentially with ethyl *N,N*-di*iso*propylamine (160 μL, 0.9 mmol) and acryloyl chloride (50 μL, 0.6 mmol). The reaction mixture was then stirred for 3 h at −78°C. The reaction mixture was then poured onto saturated ammonium chloride and extracted several times with CH_2Cl_2. The organic layers were then dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The resulting oil was the subjected in each case to chromatography on silica gel (hexane-EtOAc, 98:2) to afford **9** (81 mg, 83%) and **10** (80 mg, 82%), respectively.

(**9**): oil, $[\alpha]_D$ +6.8 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1724 (C=O); ^{1}H NMR δ 6.38 (1H, dd, *J* = 17.5, 1.5 Hz), 6.10 (1H, dd, *J* = 17.5, 10.5 Hz), 5.79 (1H, dd, *J* = 10.5, 1.5 Hz), 5.75 (1H, m), 5.20 (1H, dd, *J* = 10, 2.5 Hz), 5.02 (1H, br dd, *J* = 17, 1.5 Hz), 4.97 (1H, br dd, *J* ~ 10, 1.5 Hz), 3.43 (3H, s), 3.39 (3H, s), 3.05-3.00 (2H, m), 2.59 (1H, m), 2.24 (1H, m), 2.04 (1H, d quint, *J* = 7, 4 Hz), 1.90 (1H, d quint, *J* = 7, 2.5 Hz), 1.02 (3H, d, *J* = 7.5 Hz), 0.98 (3H, d, *J* = 7.5 Hz), 0.96 (3H, s), 0.95 (3H, s), 0.90 (3H, d, *J* = 7 Hz); ^{13}C NMR δ 165.8, 43.6 (C), 135.6, 128.9, 90.2, 86.4, 77.7, 37.5, 30.5 (CH), 130.1, 116.7, 35.5 (CH_2), 60.9, 59.7, 21.7, 20.8, 20.3, 18.8, 16.1 (CH_3); HR ESMS *m/z* 349.2359 ($M+Na^+$), calcd. for $C_{19}H_{34}NaO_4$, 349.2355.

(*ent*-**9**): oil, $[\alpha]_D$ −9.1 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **9**.

(**10**): oil, $[\alpha]_D$ −22.8 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1726 (C=O); ^{1}H NMR δ 6.38 (1H, dd, *J* = 17.5, 1.5 Hz), 6.11 (1H, dd, *J* = 17.5, 10.5 Hz), 5.80 (1H, dd, *J* = 10.5, 1.5 Hz), 5.76 (1H, m), 5.23 (1H, dd, *J* = 10, 3 Hz), 5.01 (1H, br dd, *J* = 17, 1.5 Hz), 4.97 (1H, br dd, *J* ~ 10, 1.5 Hz), 3.39 (3H, s), 3.33 (3H, s), 3.02 (1H, dd, *J* = 8.5, 2 Hz), 2.94 (1H, d, *J* = 3.5 Hz), 2.45 (1H, m), 2.24 (1H, m), 1.98 (1H, d quint, *J* = 6.5, 3.5 Hz), 1.89 (1H, d quint, *J* = 7, 2.5 Hz), 1.02 (3H, d, *J* = 7 Hz), 0.97 (3H, d, *J* = 7 Hz), 0.96 (3H, s), 0.94 (3H, s), 0.88 (3H, d, *J* = 6.5 Hz); ^{13}C NMR δ 165.8, 43.4 (C), 135.5, 129.1, 88.3, 86.2, 76.8, 37.6, 30.5 (CH), 130.1, 116.8, 35.1 (CH_2), 60.7, 59.8, 21.7, 19.1, 19.0, 18.5, 15.6 (CH_3); HR ESMS *m/z* 349.2360 ($M+Na^+$), calcd. for $C_{19}H_{34}NaO_4$, 349.2355.

(*ent*-**10**): oil, $[\alpha]_D$ +17.8 (*c* 1; $CHCl_3$).

(6*S*)-[(3*R*,4*R*,5*R*)-3,5-Dimethoxy-2,4,6-trimethylheptan-2-yl]-5,6-dihydro-2*H*-pyran-2-one (1) and **(6*R*)-[(3*R*,4*R*,5*R*)-3,5-dimethoxy-2,4,6-trimethylheptan-2-yl]-5,6-dihydro-2*H*-pyran-2-one (2).** Diolefin **9** or **10** (65 mg, 0.2 mmol) was dissolved under N_2 in dry, degassed CH_2Cl_2 (20 mL) and treated with Grubbs first-generation ruthenium catalyst **Ru-I** (16 mg, ca. 0.02 mmol). The mixture was heated at reflux until consumption of the starting material (2-3 h, TLC monitoring!). Removal of volatiles under reduced pressure and column chromatography of the residue on silica gel (hexane-EtOAc 9:1) furnished the desired metathesis products **1** (57 mg, 97%) and **2** (56 mg, 96%), respectively.

(**1**): oil, $[\alpha]_D$ −78.2 (*c* 1.05; $CHCl_3$); IR ν_{max} (cm^{-1}): 1725 (C=O); ^{1}H NMR δ 6.92 (1H, ddd, *J* = 9.5, 6.5, 2.5 Hz), 6.00 (1H, dd, *J* = 9.5, 2 Hz), 4.38 (1H, dd, *J* = 12.5, 3.5 Hz), 3.42 (3H, s), 3.39 (3H, s), 3.20 (1H, d, *J* = 3 Hz), 3.00 (1H, dd, *J* = 8, 3 Hz), 2.49 (1H, ddt, *J* = 18, 12.5, 2.5 Hz), 2.36 (1H, ddd, *J* = 18, 6.5, 3.5 Hz), 2.00-1.85 (2H, m), 1.02 (3H, d, *J* = 7 Hz), 0.97 (3H, d, *J* = 7 Hz), 0.91 (3H, s), 0.86 (3H, d, *J* = 7 Hz), 0.85 (3H, s); ^{13}C NMR δ 164.9, 42.7 (C), 146.3, 121.1, 89.6, 86.3, 82.7, 37.3, 30.4 (CH), 25.4 (CH_2), 61.1, 59.8, 21.7, 20.3, 19.8, 19.1, 15.7 (CH_3); HR ESMS *m/z* 321.2040 ($M+Na^+$), calcd. for $C_{17}H_{30}NaO_4$, 321.2042.

(*ent*-**1**): oil, $[\alpha]_D$ +71.4 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **1**.

(**2**): off-white solid, mp 67-69 ºC (from Et_2O-CH_2Cl_2), $[\alpha]_D$ −10.6 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1727 (C=O); ^{1}H NMR δ 6.93 (1H, ddd, *J* = 9.5, 6.5, 2 Hz), 6.00 (1H, dd, *J* = 9.5, 2 Hz), 4.58 (1H, dd, *J* = 13, 3.5 Hz), 3.43 (3H, s), 3.37 (4H overall, an OMe singlet overlapping an one-proton signal), 2.99 (1H, dd, *J* = 8, 2.5 Hz), 2.37 (1H, ddt, *J* = 18, 13, 2.5 Hz), 2.25 (1H, ddd, *J* = 18, 6.5, 3.5 Hz), 1.90-1.80 (2H, m), 1.02 (3H, d, *J* = 7 Hz), 0.97 (3H, d, *J* = 7 Hz), 0.91 (3H, s), 0.86 (3H, d, *J* = 7 Hz), 0.85 (3H, s); ^{13}C NMR δ 164.8, 42.5 (C), 146.1, 121.1, 87.4, 86.2, 81.1, 37.2, 30.4 (CH), 24.4 (CH_2), 61.1, 59.8, 21.7, 19.8, 18.2, 17.4, 15.5 (CH_3); HR ESMS *m/z* 321.2040 ($M+Na^+$), calcd. for $C_{17}H_{30}NaO_4$, 321.2042.

(*ent*-**2**): off-white solid, $[\alpha]_D$ +6.1 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **2**.

The stereostructures of **2** and *ent*-**2** have been secured by means of an X-ray diffraction analysis.[24]

(3*S*,5*R*,6*R*,7*R*)-5,7-Dimethoxy-4,4,6,8-tetramethylnon-1-en-3-ol (11) and **(3*R*,5*R*,6*R*,7*R*)-5,7-dimethoxy-4,4,6,8-tetramethylnon-1-en-3-ol (12)**. Olefin **6** (685 mg, 3 mmol) was dissolved in dry CH_2Cl_2 (60 mL) and cooled to −78°C. A stream of ozone-oxygen was bubbled through the solution until persistence of the bluish color. Dry N_2 was then bubbled through the solution for 10 min. at the same temperature. After addition of PPh_3 (1.18 g, 4.5 mmol), the solution was left to stir at room temperature for 2 h. Solvent removal under reduced pressure gave a solid material, which was put on the top of a short silica gel pad and rapidly washed with hexane-EtOAc 9:1. After removal of volatiles under reduced pressure, the crude oily aldehyde was then directly used as such in the next allylation step (for weight calculations, the yield of the ozonolysis step was assumed to be quantitative).

The oily material from above was dissolved under N_2 in dry THF (15 mL) and cooled in an ice bath. After this, a 1.6 M solution of vinylmagnesium chloride in THF (2.5 mL, 4 mmol) was added dropwise, and the mixture was allowed to reach room temperature, followed by stirring for 2 h (TLC monitoring). The reaction mixture was then poured onto saturated ammonium chloride and extracted several times with Et_2O. The organic layers were then dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The resulting oil was the subjected to a slow and careful chromatography on silica gel (hexane-Et_2O, from 98:2 to 95:5) to yield **11** (255 mg, 33%) and **12** (240 mg, 31%).

(**11**): oil, $[\alpha]_D$ −26.4 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 3380 (br, OH); ^{1}H NMR δ 5.85 (1H, ddd, *J* = 17, 10.5, 6.5 Hz), 5.22 (1H, br d, *J* ~ 17 Hz), 5.20 (1H, br s, OH), 5.09 (1H, br d, *J* ~ 10.5 Hz), 4.17 (1H, m), 3.47 (6H, s), 3.05 (1H, dd, *J* = 10, 1.5 Hz), 2.80 (1H, br d, *J* ~ 3 Hz), 2.38 (1H, m), 1.83 (1H, m), 1.00 (3H, s), 0.91 (3H, d, *J* = 7.5 Hz), 0.88 (3H, s), 0.85 (3H, s), 0.81 (3H, d, *J* = 7 Hz); ^{13}C NMR δ 43.1 (C), 138.0, 96.5, 87.2, 75.1, 36.2, 30.3 (CH), 115.3 (CH_2), 61.9, 60.3, 25.6, 22.0, 21.1, 17.1, 14.5 (CH_3); HR ESMS *m/z* 281.2094 ($M+Na^+$), calcd. for $C_{15}H_{30}NaO_3$, 281.2093.

(*ent*-**11**): oil, $[\alpha]_D$ +22.4 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **11**.

(**12**): oil, $[\alpha]_D$ +17.9 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 3450 (br, OH); ^1H NMR δ 5.90 (1H, ddd, *J* = 17, 10.5, 6 Hz), 5.27 (1H, br d, *J* ~ 17 Hz), 5.15 (1H, br d, *J* ~ 10.5 Hz), 4.11 (1H, m), 3.95 (1H, br d, *J* ~ 4 Hz, OH), 3.45 (3H, s), 3.41 (3H, s), 3.08 (1H, d, *J* = 4 Hz), 3.01 (1H, d, *J* = 8.5, 2 Hz), 2.06 (1H, m), 1.89 (1H, m), 1.03 (3H, d, *J* = 7 Hz), 0.98 (3H, d, *J* = 7 Hz), 0.96 (3H, s), 0.89 (3H, s), 0.88 (3H, d, *J* = 7 Hz); ^{13}C NMR δ 42.6 (C), 138.0, 94.4, 86.7, 78.4, 37.1, 30.4 (CH), 115.9 (CH_2), 61.4, 60.0, 22.3, 21.6, 21.2, 19.6, 15.6 (CH_3); HR ESMS *m/z* 281.2095 ($M+Na^+$), calcd. for $C_{15}H_{30}NaO_3$, 281.2093.

(*ent*-**12**): oil, $[\alpha]_D$ −16.9 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **12**.

(3*S*,5*R*,6*R*,7*R*)-5,7-Dimethoxy-4,4,6,8-tetramethylnon-1-en-3-yl acrylate (**13**) and **(3*R*,5*R*,6*R*,7*R*)-5,7-dimethoxy-4,4,6,8-tetramethylnon-1-en-3-yl acrylate** (**14**). Alcohol **11** or **12** were subjected to esterification with acryloyl chloride under the same conditions used for the preparation of **9** and **10**. In this way, acrylates **13** (85%) and **14** (84%) were obtained.
(**13**): oil, $[\alpha]_D$ −20.7 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1728 (C=O); ^1H NMR δ 6.42 (1H, dd, *J* = 17.5, 1.5 Hz), 6.15 (1H, dd, *J* = 17.5, 10.5 Hz), 5.90 (1H, ddd, *J* = 17.5, 10.5, 7 Hz), 5.83 (1H, dd, *J* = 10.5, 1.5 Hz), 5.37 (1H, br d, *J* ~ 7 Hz), 5.30-5.20 (2H, m), 3.41 (3H, s), 3.40 (3H, s), 3.07 (1H, d, *J* = 3.5 Hz), 3.03 (1H, dd, *J* = 8, 2 Hz), 2.00 (1H, d quint, *J* = 7, 3.5 Hz), 1.88 (1H, d quint, *J* = 7, 2.5 Hz), 1.03 (3H, d, *J* = 8 Hz), 1.02 (3H, s), 0.95 (3H, d, *J* = 8 Hz), 0.94 (3H, s), 0.88 (3H, d, *J* = 6.5 Hz); ^{13}C NMR δ 165.3, 43.5 (C), 133.8, 128.9, 88.9, 86.2, 79.7, 37.5, 30.5 (CH), 130.4, 118.2 (CH_2), 60.4, 59.8, 21.7, 19.8 (x 2), 18.6, 15.7 (CH_3); HR ESMS *m/z* 335.2201 ($M+Na^+$), calcd. for $C_{18}H_{32}NaO_4$, 335.2198.

(*ent*-**13**): oil, $[\alpha]_D$ +20.8 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **13**.

(**14**): oil, $[\alpha]_D$ +16.7 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1729 (C=O); ^1H NMR δ 6.42 (1H, dd, *J* = 17.5, 1.5 Hz), 6.16 (1H, dd, *J* = 17.5, 10.5 Hz), 5.90-5.80 (2H, m), 5.42 (1H, br d, *J* ~ 7 Hz), 5.30-5.20 (2H, m), 3.41 (3H, s), 3.31 (3H, s), 3.06 (1H, d, *J* = 3 Hz), 3.03 (1H, dd, *J* = 8, 2 Hz), 1.93 (1H, d quint, *J* = 7, 3.5 Hz), 1.85 (1H, d quint, *J* = 7, 2.5 Hz), 1.00 (3H, d, *J* = 7 Hz), 0.94 (3H, d, *J* = 7.5 Hz), 0.91 (3H, s), 0.89 (3H, s), 0.84 (3H, d, *J* = 7 Hz); ^{13}C NMR δ 165.2, 42.8 (C), 133.4, 128.9, 87.7, 86.2, 78.6, 37.5, 30.4 (CH), 130.3, 118.3 (CH_2), 60.5, 59.8, 21.6, 18.7, 18.6 (x 2), 15.5 (CH_3); HR ESMS *m/z* 335.2198 ($M+Na^+$), calcd. for $C_{18}H_{32}NaO_4$, 335.2198.

(*ent*-**14**): oil, $[\alpha]_D$ −17.2 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **14**.

(5*S*)-[(3*R*,4*R*,5*R*)-3,5-Dimethoxy-2,4,6-trimethylheptan-2-yl]furan-2(5*H*)-one (3) and **(5*R*)-[(3*R*,4*R*,5*R*)-3,5-dimethoxy-2,4,6-trimethylheptan-2-yl]furan-2(5*H*)-one (4).** Diolefin **13** or **14** (62 mg, 0.2 mmol) was dissolved under N_2 in dry, degassed toluene (20 mL) and treated with Grubbs second-generation ruthenium catalyst **Ru-II** (17 mg, ca. 0.02 mmol). The mixture was then heated at 80 ºC for 36 h. An addditional amount of ruthenium catalyst (10 mg) was added and the heating was continued until consumption of the starting material (ca. 3 d overall, TLC monitoring!). Removal of volatiles under reduced pressure and column chromatography of the residue on silica gel (hexane-EtOAc 9:1) furnished the desired metathesis products **3** (48 mg, 85%) and **4** (45 mg, 79%), respectively.
(**3**): oil, $[\alpha]_D$ −98.3 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1758 (C=O); ^1H NMR δ 7.61 (1H, dd, *J* = 6, 1.5 Hz), 6.01 (1H, dd, *J* = 6, 2 Hz), 5.00 (1H, dd, *J* = 2, 1.5 Hz), 3.39 (3H, s), 3.31 (3H, s), 2.99 (1H, dd, *J* = 9.5, 2.5 Hz), 2.94 (1H, d, *J* = 3.5 Hz), 2.00 (1H, m), 1.89 (1H, m), 1.07 (3H, s), 1.04 (3H, d, *J* = 7.5 Hz), 0.99 (3H, d, *J* = 7.5 Hz), 0.94 (3H, s), 0.86 (3H, d, *J* = 7 Hz); ^{13}C NMR δ 173.6, 44.3 (C), 157.3, 119.4, 89.6, 88.8, 86.1, 37.3, 30.4 (CH), 60.3, 60.0, 21.4, 21.1, 20.8, 19.0, 15.3 (CH_3); HR ESMS *m/z* 307.1882 ($M+Na^+$), calcd. for $C_{16}H_{28}NaO_4$, 307.1885.

(*ent*-**3**): off-white solid, $[\alpha]_D$ +91 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **3**.

(**4**): off-white solid, mp 57-59 ºC (from Et_2O-CH_2Cl_2), $[\alpha]_D$ +47 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1759 (C=O); ^1H NMR δ 7.50 (1H, dd, *J* = 6, 1.5 Hz), 6.10 (1H, dd, *J* = 6, 2 Hz), 5.17 (1H, dd, *J* = 2, 1.5 Hz), 3.47 (3H, s), 3.37 (3H, s), 3.22 (1H, d, *J* = 4 Hz), 2.97 (1H, dd, *J* = 8, 2.5 Hz), 1.90-1.80 (2H, m), 1.00 (3H, d, *J* = 7 Hz), 0.97 (3H, s), 0.93 (3H, d, *J* = 7 Hz), 0.84 (3H, d, *J* = 7 Hz), 0.69 (3H, s); ^{13}C NMR δ 173.2, 43.5 (C), 155.8, 122.2, 88.9, 87.8, 86.1, 37.2, 30.4 (CH), 60.8, 59.8, 21.5, 19.4, 17.9, 17.6, 15.3 (CH_3); HR ESMS *m/z* 307.1888 ($M+Na^+$), calcd. for $C_{16}H_{28}NaO_4$, 307.1885.

(*ent*-**4**): off-white solid, $[\alpha]_D$ −47.4 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **4**.

The stereostructures of **4** and *ent*-**4** have been secured by means of an X-ray diffraction analysis.[24]

(5*R*,6*R*,7*R*)-5-Isopropyl-2,2,3,3,6,9,9,10,10-nonamethyl-7-(2-methylbut-3-en-2-yl)-4,8-dioxa-3,9-disilaundecane (15). Alcohol **5** (800 mg, 4 mmol) was dissolved under N_2 in dry CH_2Cl_2 (25 mL) and treated sequentially with 2,6-lutidine (1.4 mL, 12 mmol) and TBSOTf (2.1 mL, 9 mmol). The reaction mixture was then stirred for 12 h at 30 ºC. The reaction mixture was then poured onto saturated ammonium chloride and extracted several times with CH_2Cl_2. The organic layers were then dried over anhydrous Mg_2SO_4, filtered and evaporated under reduced pressure. The resulting oil was then subjected to column chromatography on silica gel (hexane) to yield **15** (1.55 g, 90%): oil, $[\alpha]_D$ +4.9 (*c* 1; $CHCl_3$); ^1H NMR δ 6.20 (1H, dd, *J* = 17.5, 11 Hz), 5.00 (1H, br d, *J* ~ 17.5 Hz), 4.96 (1H, dd, *J* ~ 11 Hz), 3.90 (1H, d, *J* = 7.5 Hz), 3.75 (1H, d, *J* = 1 Hz), 2.07 (1H, br quint, *J* ~ 7 Hz), 1.94 (1H, br quint, *J* ~ 7 Hz), 1.12 (3H, s), 1.10 (3H, s), 0.99 (3H, d overlapped), 0.98 (9H, s), 0.96 (9H, s), 0.91 (3H, d, *J* = 7 Hz), 0.88 (3H, d, *J* = 7 Hz), 0.16 (3H, s), 0.12 (3H, s), 0.09 (3H, s), 0.05 (3H, s); ^{13}C NMR δ 42.8, 18.6, 18.5 (C), 146.5, 80.7, 76.5, 45.2, 31.0 (CH), 110.0 (CH_2), 26.7, 26.3 (x 3), 26.2 (x 3), 25.8, 21.0, 16.2, 13.5, –2.9, –3.0, –4.3, –4.4 (CH_3); HR ESMS *m/z* 451.3408 ($M+Na^+$), calcd. for $C_{24}H_{52}NaO_2Si_2$, 451.3404.

(*ent*-**15**): oil, $[\alpha]_D$ −6.6 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **15**.

(4*S*,6*R*,7*R*,8*R*)-6,8-Bis(*tert*-butyldimethylsilyloxy)-5,5,7,9-tetramethyldec-1-en-4-ol (16) and **(4*R*,6*R*,7*R*,8*R*)-6,8-bis(*tert*-butyldimethylsilyloxy)-5,5,7,9-tetramethyldec-1-en-4-ol (18)**. Olefin **15** (1.29 g, 3 mmol) was dissolved in dry CH_2Cl_2 (60 mL) and cooled to −78°C. A stream of ozone-oxygen was bubbled through the solution until persistence of the bluish color. Dry N_2 was then bubbled through the solution for 10 min. at the same temperature. After addition of PPh_3 (1.18 g, 4.5 mmol), the solution was left to stir at room temperature for 2 h. Solvent removal under reduced pressure gave a solid material, which was put on the top of a short silica gel pad and rapidly washed with hexane-Et_2O 99:1. After removal of volatiles under reduced pressure, the crude oily aldehyde was then directly used as such in the next allylation step (for weight calculations, the yield of the ozonolysis step was assumed to be quantitative).

The oily material from above was dissolved under N_2 in dry THF (15 mL) and cooled in an ice bath. After this, a 1 M solution of allylmagnesium bromide in THF (4 mL, 4 mmol) was added dropwise, and the mixture was allowed to reach room temperature, followed by stirring for 3 h (TLC monitoring). The reaction mixture was then poured onto saturated ammonium chloride and extracted several times with Et_2O. The organic layers were then dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The resulting oil was the subjected to a slow and careful chromatography on silica gel (hexane-Et_2O, 99:1) to yield **16** (540 mg, 38%) and **18** (369 mg, 26%).

(**16**): oil, $[\alpha]_D$ +5.5 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 3480 (br, OH); ^1H NMR δ 5.85 (1H, ddt, *J* = 17, 10.5, 7 Hz), 5.20-5.15 (2H, m), 3.92 (1H, d, *J* = 6 Hz), 3.87 (1H, d, *J* = 2 Hz), 3.65 (1H, d, *J* = 10 Hz), 2.44 (1H, m), 2.20 (1H, m), 2.15-2.05 (2H, m), 1.95 (1H, br s, OH), 1.07 (3H, d, *J* = 7 Hz), 0.99 (3H, s), 0.95 (9H, s), 0.94 (3H, s), 0.93 (9H, s), 0.91 (3H, d, *J* = 7 Hz), 0.89 (3H, d, *J* = 7 Hz), 0.16 (3H, s), 0.11 (3H, s), 0.10 (3H, s), 0.08 (3H, s); ^{13}C NMR δ 43.5, 18.8, 18.6 (C), 136.2, 80.7, 77.1, 75.1, 43.4, 30.8 (CH), 118.2, 37.1 (CH_2), 26.5 (x 3), 26.3 (x 3), 22.0, 21.1, 20.3, 16.8, 14.3, –2.2, –3.3, –4.3, –4.4 (CH_3); HR ESMS *m/z* 495.3667 ($M+Na^+$), calcd. for $C_{26}H_{56}NaO_3Si_2$, 495.3666.

(*ent*-**16**): oil, $[\alpha]_D$ −6.1 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **16**.

(**18**): oil, $[\alpha]_D$ +23.6 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 3470 (br, OH); ^1H NMR δ 5.93 (1H, ddt, *J* = 17, 10.5, 7 Hz), 5.09 (1H, dm, *J* ~ 17 Hz), 5.05 (1H, dm, *J* ~ 10.5 Hz), 4.35 (1H, br s, OH), 4.00 (1H, dd, *J* = 10, 1.5 Hz), 3.92 (1H, d, *J* = 2 Hz), 3.86 (1H, dd, *J* = 7.5, 1 Hz), 2.18 (1H, m), 2.10-2.00 (3H, m), 1.14 (3H, d, *J* = 7 Hz), 1.04 (3H, s), 0.92 (24H, br s), 0.88 (3H, d, *J* = 7 Hz), 0.15 (3H, s), 0.13 (3H, s), 0.12 (3H, s), 0.09 (3H, s); ^{13}C NMR δ 42.6, 18.8, 18.3 (C), 137.0, 83.7, 78.1, 76.1, 45.0, 31.5 (CH), 116.3, 36.9 (CH_2), 26.4 (x 3), 26.2 (x 3), 23.1, 21.8, 20.5, 16.4, 13.7, –3.0, –3.4, –4.3 (x 2) (CH_3); HR ESMS *m/z* 495.3666 ($M+Na^+$), calcd. for $C_{26}H_{56}NaO_3Si_2$, 495.3666.

(*ent*-**18**): oil, $[\alpha]_D$ −16.6 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **18**.

(4*S*,6*R*,7*R*,8*R*)-6,8-Bis(*tert*-butyldimethylsilyloxy)-5,5,7,9-tetramethyldec-1-en-4-yl acrylate (**17**) and **(4*R*,6*R*,7*R*,8*R*)-6,8-bis(*tert*-butyldimethylsilyloxy)-5,5,7,9-tetramethyldec-1-en-4-yl acrylate** (**19**). Alcohol **16** or **18** (142 mg, 0.3 mmol) was dissolved under N_2 in dry CH_2Cl_2 (5 mL), cooled to −78 °C and treated sequentially with ethyl *N,N*-di*iso*propylamine (160 μL, 0.9 mmol) and acryloyl chloride (50 μL, 0.6 mmol). The reaction mixture was then stirred for 3 h at −50°C. The reaction mixture was then poured onto saturated ammonium chloride and extracted several times with CH_2Cl_2. The organic layers were then dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The resulting oil was the subjected in each case to chromatography on silica gel (hexane-Et_2O, 98:2) to afford, respectively, **17** (130 mg, 82%) and **19** (130 mg, 82%), respectively.

(**17**): oil, $[\alpha]_D$ +10.9 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1727 (C=O); ^1H NMR δ 6.37 (1H, dd, *J* = 17.3, 1.5 Hz), 6.10 (1H, dd, *J* = 17.3, 10.5 Hz), 5.80 (1H, dd, *J* = 10.5, 1.5 Hz), 5.74 (1H, dddd, *J* = 17, 10, 8, 6 Hz), 5.30 (1H, dd, *J* = 10.5, 2.5 Hz), 5.03 (1H, dd, *J* = 17, 1.5 Hz), 4.98 (1H, dd, *J* = 10, 1.5 Hz), 3.90 (1H, dd, *J* = 6.3, 1.5 Hz), 3.77 (1H, d, *J* = 2.2 Hz), 2.68 (1H, m), 2.30-2.15 (2H, m), 2.00 (1H, d quint, *J* = 7.5, 2.2 Hz), 1.07 (3H, d, *J* = 7.5 Hz), 1.04 (3H, s), 0.99 (3H, s), 0.96 (9H, s), 0.93 (9H, s), 0.92 (3H, d, *J* = 7 Hz), 0.90 (3H, d, *J* = 7 Hz), 0.15 (3H, s), 0.13 (6H, s), 0.10 (3H, s); ^{13}C NMR δ 165.7, 43.9, 18.7, 18.6 (C), 135.2, 128.8, 79.7, 77.4, 77.1, 43.9, 31.1 (CH), 130.1, 117.0, 35.8 (CH_2), 26.5 (x 3), 26.2 (x 3), 22.2, 21.4, 20.4, 17.2, 14.6, –2.5, –3.4, –4.2, –4.3 (CH_3); HR ESMS *m/z* 549.3774 ($M+Na^+$), calcd. for $C_{29}H_{58}NaO_3Si_2$, 549.3771.

(*ent*-**17**): oil, $[\alpha]_D$ −6.3 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **17**.

(**19**): oil, $[\alpha]_D$ +9.1 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1728 (C=O); ^1H NMR δ 6.40 (1H, dd, *J* = 17.3, 1.5 Hz), 6.13 (1H, dd, *J* = 17.3, 10.5 Hz), 5.81 (1H, dd, *J* = 10.5, 1.5 Hz), 5.72 (1H, ddt, *J* = 17, 10, 7 Hz), 5.21 (1H, dd, *J* = 10, 2.5 Hz), 5.03 (1H, dd, *J* = 17, 1.5 Hz), 4.99 (1H, dd, *J* = 10, 1.5 Hz), 4.05 (1H, d, *J* = 5 Hz), 3.63 (1H, d, *J* = 1.5 Hz), 2.34 (1H, hept, *J* = 7 Hz), 2.30-2.20 (2H, m), 1.92 (1H, m), 1.13 (3H, d, *J* = 7.5 Hz), 1.03 (3H, s), 0.97 (3H, s), 0.96 (9H, s), 0.94 (9H, s), 0.89 (3H, d, *J* = 7 Hz), 0.88 (3H, d, *J* = 7 Hz), 0.17 (3H, s), 0.16 (3H, s), 0.15 (3H, s), 0.11 (3H, s); ^{13}C NMR δ 165.7, 43.7, 19.0, 18.4 (C), 134.3, 128.8, 81.5, 76.9, 75.7, 42.9, 30.4 (CH), 130.3, 117.5, 34.8 (CH_2), 26.6 (x 3), 26.1 (x 3), 22.9, 22.6, 20.1, 17.2, 14.8, –1.9, –3.7, –4.3, –4.7 (CH_3); HR ESMS *m/z* 549.3774 ($M+Na^+$), calcd. for $C_{29}H_{58}NaO_3Si_2$, 549.3771.

(*ent*-**19**): oil, $[\alpha]_D$ −8.1 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **19**.

(6*S*)-[(3*R*,4*R*,5*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-2,4,6-trimethylheptan-2-yl]-5,6-dihydro-2*H*-pyran-2-one (20) and **(6*R*)-[(3*R*,4*R*,5*R*)-3,5-bis(*tert*-butyldimethylsilyloxy)-2,4,6-trimethylheptan-2-yl]-5,6-dihydro-2*H*-pyran-2-one (21).** Diolefin **17** or **19** (105 mg, 0.2 mmol) was dissolved under N_2 in dry, degassed toluene (20 mL) and treated with ruthenium catalyst **Ru-I** (16 mg, ca. 0.02 mmol). The mixture was heated at reflux until consumption of the starting material (ca. 4 h, TLC monitoring!). Removal of volatiles under reduced pressure and column chromatography of the residue on silica gel (hexane-Et_2O 9:1) furnished the desired metathesis products **20** (94 mg, 94%) and **21** (98 mg, 98%),

respectively.

(**20**): off-white solid, mp 134-135 ºC (from Et_2O-CH_2Cl_2), $[\alpha]_D$ –30.5 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1731 (C=O); ^1H NMR δ 6.92 (1H, ddd, *J* = 9.5, 6.3, 2.2 Hz), 6.01 (1H, dd, *J* = 9.5, 2 Hz), 4.55 (1H, dd, *J* = 12.2, 4 Hz), 3.90 (1H, d, *J* = 2.5 Hz), 3.80 (1H, dd, *J* = 5.5, 1 Hz), 2.50-2.35 (2H, m), 2.20 (1H, br quint, *J* ~ 7 Hz), 2.05 (1H, m), 1.12 (3H, s), 1.08 (3H, d, *J* = 7 Hz), 1.00 (3H, s), 0.93 (9H, s), 0.90 (9H, s), 0.89 (3H, d, *J* = 7 Hz), 0.88 (3H, d, *J* = 7 Hz), 0.14 (3H, s), 0.09 (3H, s), 0.04 (3H, s), 0.02 (3H, s); ^{13}C NMR δ 164.4, 42.8, 18.8, 18.5 (C), 145.6, 121.3, 82.3, 79.3, 77.1, 43.4, 30.9 (CH), 26.4 (x 3), 26.1 (x 3), 25.5, 22.1, 21.8, 19.8, 17.2, 14.4, –2.4, –3.5, –4.3, –4.4 (CH_3); HR ESMS *m/z* 521.3451 ($M+Na^+$), calcd. for $C_{27}H_{54}NaO_4Si_2$, 521.3458.

(*ent*-**20**): oil, $[\alpha]_D$ +31.1 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **20**.

The stereostructures of **20** and *ent*-**20** have been secured by means of an X-ray diffraction analysis.[24]

(**21**): oil, $[\alpha]_D$ +16.8 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1737 (C=O); ^1H NMR δ 6.89 (1H, ddd, *J* = 9.5, 6.5, 2 Hz), 5.98 (1H, dd, *J* = 9.5, 2 Hz), 4.45 (1H, dd, *J* = 13, 3.5 Hz), 4.09 (1H, d, *J* = 2.5 Hz), 3.88 (1H, dd, *J* = 5.5, 1 Hz), 2.33 (1H, ddt, *J* = 18, 13, 2.5 Hz), 2.25-2.15 (2H, m), 1.93 (1H, m), 1.08 (3H, d, *J* = 7.5 Hz), 1.02 (3H, s), 0.92 (3H, s), 0.89 (9H, s), 0.86 (9H, s, overlapping two methyl doublets), 0.12 (3H, s), 0.05 (3H, s), 0.03 (3H, s), 0.005t (3H, s), ^{13}C NMR δ 164.4, 42.7, 18.8, 18.4 (C), 145.6, 121.2, 80.6, 76.8, 76.3, 43.5, 30.6 (CH), 26.4 (x 3), 26.1 (x 3), 24.0, 22.2, 19.7, 18.6, 17.0, 14.4, –2.3, –3.6, –4.5, –4.8 (CH_3); HR ESMS *m/z* 521.3456 ($M+Na^+$), calcd. for $C_{27}H_{54}NaO_4Si_2$, 521.3458.

(*ent*-**21**): oil, $[\alpha]_D$ −18.2 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **21**.

(3*S*,5*R*,6*R*,7*R*)-5,7-bis(*tert*-Butyldimethylsilyloxy)-4,4,6,8-tetramethylnon-1-en-3-ol (24) and **(3*R*,5*R*,6*R*,7*R*)-5,7-bis(*tert*-butyldimethylsilyloxy)-4,4,6,8-tetramethylnon-1-en-3-ol (26)**. Olefin **15** was subjected to the same sequence of ozonolysis followed by addition of vinylmagnesium chloride performed with **6**. Work-up and careful chromatography on silica gel (hexane-Et_2O, 99:1) afforded **24** (26%) and **26** (24%).

(**24**): oil, $[\alpha]_D$ –15 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 3460 (br, OH); ^1H NMR δ 5.95 (1H, ddd, *J* = 17, 10, 6.5 Hz), 5.26 (1H, br dt, *J* ~ 17, 1.5 Hz), 5.20 (1H, br dt, *J* ~ 10, 1.5 Hz), 4.14 (1H, dt, *J* = 6.5, 1.5 Hz), 4.01 (1H, d, *J* = 2.5 Hz), 3.89 (1H, dd, *J* = 10, 1.5 Hz), 2.50 (1H, br s, OH), 2.10 (2H, m), 1.05 (3H, d, *J* = 7 Hz), 0.99 (3H, s), 0.94 (9H, s), 0.93 (9H, s, overlapping a methyl doublet), 0.90 (3H, s), 0.89 (3H, d, *J* = 7 Hz), 0.17 (3H, s), 0.14 (6H, s), 0.09 (3H, s); ^{13}C NMR δ 43.4, 18.7, 18.6 (C), 138.0, 80.3, 79.6, 77.8, 44.7, 31.3 (CH), 116.7 (CH_2), 26.3 (x 6), 21.1, 21.0, 19.9, 16.6, 13.8, –2.5, –3.0, –4.3 (x 2) (CH_3); HR ESMS *m/z* 481.3513 ($M+Na^+$), calcd. for $C_{25}H_{54}NaO_3Si_2$, 481.3509.

(*ent*-**24**): oil, $[\alpha]_D$ +19.3 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **24**.

(**26**): oil, $[\alpha]_D$ +8.9 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 3450 (br, OH); ^1H NMR δ 5.84 (1H, ddd, *J* = 17, 10.5, 6.5 Hz), 5.26 (1H, br ddd, *J* ~ 17, 2, 1.5 Hz), 5.15 (1H, br ddd, *J* ~ 10, 2, 1.5 Hz), 4.70 (1H, br s, OH), 4.46 (1H, d, *J* = 6.5 Hz), 4.01 (1H, d, *J* = 2.2 Hz), 3.87 (1H, dd, *J* = 8.2, 1.5 Hz), 2.08 (1H, d quint, *J* = 8, 2 Hz), 2.02 (1H, d quint, *J* = 7, 1.5 Hz), 1.16 (3H, d, *J* = 7.5 Hz), 1.04 (3H, s), 0.95 (3H, d, *J* = 7 Hz), 0.94 (9H, s), 0.93 (9H, s), 0.91 (3H, s), 0.90 (3H, d, *J* = 7 Hz), 0.16 (3H, s), 0.15 (3H, s), 0.14 (3H, s), 0.10 (3H, s); ^{13}C NMR δ 42.3, 18.8, 18.3 (C), 137.9, 83.1, 78.5, 78.2, 45.3, 31.6 (CH), 116.6 (CH_2), 26.4 (x 3), 26.1 (x 3), 23.2, 22.2, 20.2, 16.3, 13.3, –2.8, –3.4, –4.3, –4.4 (CH_3); HR ESMS *m/z* 481.3506 ($M+Na^+$), calcd. for $C_{25}H_{54}NaO_3Si_2$, 481.3509.

(*ent*-**26**): oil, $[\alpha]_D$ −8.1 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **26**.

(3*S*,5*R*,6*R*,7*R*)-5,7-Bis(*tert*-butyldimethylsilyloxy)-4,4,6,8-tetramethylnon-1-en-3-yl acrylate (**25**) and **(3*R*,5*R*,6*R*,7*R*)-5,7-bis(*tert*-butyldimethylsilyloxy)-4,4,6,8-tetramethylnon-1-en-3-yl acrylate** (**27**). Alcohol **24** or **26** were subjected to esterification with acryloyl chloride under the same conditions as for the preparation of **17** and **19**. Work-up and column chromatography on silica gel (hexane-Et_2O, 98:2) furnished respectively, **25** (85%) and **27** (83%).

(**25**): oil, $[\alpha]_D$ –14.2 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1732 (C=O); ^1H NMR δ 6.40 (1H, dd, *J* = 17.3, 1.5 Hz), 6.14 (1H, dd, *J* = 17.3, 10.5 Hz), 5.92 (1H, ddd, *J* = 17.3, 10.5, 6.2 Hz), 5.82 (1H, dd, *J* = 10.5, 1.5 Hz), 5.43 (1H, d, *J* = 6.2 Hz), 5.30-5.20 (2H, m), 3.93 (1H, d, *J* = 5.5 Hz), 3.78 (1H, d, *J* = 2 Hz), 2.25 (1H, hept, *J* = 7 Hz), 1.93 (1H, m), 1.06 (3H, d, *J* = 7 Hz), 1.05 (3H, s), 1.00 (3H, s), 0.96 (9H, s), 0.92 (9H, s), 0.91 (3H, d, *J* = 7 Hz), 0.89 (3H, d, *J* = 7 Hz), 0.15 (3H, s), 0.13 (3H, s), 0.12 (3H, s), 0.10 (3H, s); ^{13}C NMR δ 164.9, 43.3, 18.9, 18.5 (C), 133.7, 128.7, 79.7, 78.9, 76.7, 43.4, 30.9 (CH), 130.3, 118.2 (CH_2), 26.5 (x 3), 26.2 (x 3), 22.0 (x 2), 20.1, 17.3, 14.7, –2.2, –3.6, –4.2, –4.4 (CH_3); HR ESMS *m/z* 535.3618 ($M+Na^+$), calcd. for $C_{28}H_{56}NaO_4Si_2$, 535.3615.

(*ent*-**25**): oil, $[\alpha]_D$ +13.3 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **25**.

(**27**): oil, $[\alpha]_D$ +26.5 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1731 (C=O); ^1H NMR δ 6.38 (1H, dd, *J* = 17.3, 1.5 Hz), 6.15 (1H, dd, *J* = 17.3, 10.5 Hz), 5.83 (1H, dd, *J* = 10.5, 1.5 Hz), 5.77 (1H, ddd, *J* = 17.3, 10.5, 6.2 Hz), 5.37 (1H, d, *J* = 6.2 Hz), 5.30-5.20 (2H, m), 3.97 (1H, d, *J* = 6 Hz), 3.70 (1H, d, *J* = 2 Hz), 2.28 (1H, hept, *J* = 7 Hz), 2.00 (1H, m), 1.09 (3H, d, *J* = 7 Hz), 1.04 (3H, s), 0.99 (3H, s), 0.96 (9H, s), 0.93 (9H, s), 0.90 (3H, d, *J* = 7 Hz), 0.89 (3H, d, *J* = 7 Hz), 0.13 (6H, s), 0.11 (3H, s), 0.06 (3H, s); ^{13}C NMR δ 165.3, 43.3, 19.0, 18.5 (C), 132.9, 128.9, 79.4, 78.7, 76.2, 43.5, 30.5 (CH), 130.4, 119.1 (CH_2), 26.6 (x 3), 26.1 (x 3), 22.6, 21.6, 19.4, 17.2, 14.5, –2.2, –3.5, –4.5, –4.8 (CH_3); HR ESMS *m/z* 535.3613 ($M+Na^+$), calcd. for $C_{28}H_{56}NaO_4Si_2$, 535.3615.

(*ent*-**27**): oil, $[\alpha]_D$ –28.4 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **27**.

The stereostructure of *ent*-**27** has been secured by means of an X-ray diffraction analysis.[24]

(5*S*)-[(3*R*,4*R*,5*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-2,4,6-trimethylheptan-2-yl]furan-2(5*H*)-one (28) and **(5*R*)-[(3*R*,4*R*,5*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-2,4,6-trimethylheptan-2-yl]furan-2(5*H*)-one (29).** Diolefin **25** or **27** (102 mg, 0.2 mmol) was dissolved under N_2 in dry, degassed toluene (25 mL) and treated with Hoveyda-Grubbs

ruthenium catalyst **Ru-III** (12 mg, ca. 0.02 mmol). The mixture was heated at 80 ºC until consumption of the starting material (ca. 4 h, TLC monitoring!). Removal of volatiles under reduced pressure and column chromatography of the residue on silica gel (hexane-Et_2O 9:1) furnished the desired metathesis products **28** (75 mg, 78%) and **29** (77 mg, 80%), respectively.

(**28**): oil, $[\alpha]_D$ –42 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1763 (C=O); ^{1}H NMR δ 7.62 (1H, dd, *J* = 6, 1.5 Hz), 6.12 (1H, dd, *J* = 6, 2 Hz), 5.25 (1H, dd, *J* = 2, 1.5 Hz), 3.95 (1H, d, *J* = 2.5 Hz), 3.80 (1H, dd, *J* = 7.5, 2 Hz), 2.08 (2H, m), 1.08 (3H, d, *J* = 7.5 Hz), 1.05 (3H, s), 0.95 (3H, d, *J* = 7 Hz), 0.93 (9H, s), 0.92 (9H, s), 0.94 (3H, s), 0.90 (3H, d, *J* = 7 Hz), 0.15 (3H, s), 0.11 (3H, s), 0.10 (3H, s), 0.07 (3H, s); ^{13}C NMR δ 173.1, 44.7, 18.7, 18.5 (C), 155.8, 121.8, 87.7, 78.3, 77.8, 45.3, 31.5 (CH), 26.3 (x 3), 26.2 (x 3), 22.4, 20.3 (x 2), 16.8, 13.8, –2.8, –3.1, –4.3, –4.4 (CH_3); HR ESMS *m/z* 507.3296 ($M+Na^+$), calcd. for $C_{26}H_{52}NaO_4Si_2$, 507.3302.

(*ent*-**28**): oil, $[\alpha]_D$ +44.5 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **28**.

(**29**): oil, $[\alpha]_D$ +34.5 (*c* 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1763 (C=O); ^{1}H NMR δ 7.46 (1H, dd, *J* = 6, 1.5 Hz), 6.15 (1H, dd, *J* = 6, 2.2 Hz), 5.10 (1H, dd, *J* = 2.2, 1.5 Hz), 4.14 (1H, d, *J* = 2.5 Hz), 3.80 (1H, dd, *J* = 7, 1.5 Hz), 2.13 (1H, d quint, *J* = 7, 1.5 Hz), 1.98 (1H, d quint, *J* = 7.5, 2.5 Hz), 1.09 (3H, s), 1.07 (3H, d, *J* = 7.5 Hz), 0.92 (3H, d, *J* = 7 Hz), 0.91 (9H, s), 0.90 (9H, s), 0.89 (3H, d, *J* = 7 Hz), 0.82 (3H, s), 0.17 (3H, s), 0.13 (3H, s), 0.12 (3H, s), 0.05 (3H, s); ^{13}C NMR δ 173.0, 44.4, 18.8, 18.7 (C), 155.0, 123.1, 87.8, 77.5, 77.0, 45.0, 31.5 (CH), 26.5 (x 3), 26.4 (x 3), 21.2, 20.2, 18.1, 17.2, 14.1, –2.6, –3.1, –4.1, –4.3 (CH_3); HR ESMS *m/z* 507.3304 ($M+Na^+$), calcd. for $C_{26}H_{52}NaO_4Si_2$, 507.3302.

(*ent*-**29**): oil, $[\alpha]_D$ –36.6 (*c* 1; $CHCl_3$). Physical and spectral data identical to those of **29**.

Biological procedures

Cell culture

Cell culture media were purchased from Gibco (Grand Island, NY, USA). Fetal bovine serum (FBS) was a product of Harlan-Seralab (Belton, U.K.). Supplements and other chemicals not listed in this section were obtained from Sigma Chemicals Co. (St. Louis, Mo., USA). Plastics for cell culture were supplied by Thermo Scientific™ BioLite. All tested compounds were dissolved in DMSO at a concentration of 10 µg/mL and stored at –20°C until use.

Cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) containing glucose (1 g/L), glutamine (2 mM), penicillin (50 U/mL), streptomycin (50 µg/mL) and amphotericin B (1.25 µg/mL), supplemented with 10% FBS.

Cytotoxicity assays

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, MO) dye reduction assay in 96-well microplates was used, as previously described.[25] Some 5 x 10^3 cells of HT-29, HTC-116, MCF-7 and HL-60 and 2.5 x 10^3 cells of HEK-293 and Hela cells in a total volume of 100 µL of their respective growth media were incubated with serial dilutions of the tested compounds. After 2 days of incubation (37 °C, 5% CO_2 in a humid atmosphere), 10 µl of MTT (5 mg/ml in PBS) were added to each well and the plate was incubated for further 4 h (37 °C). The resulting formazan was dissolved in 150 µL of 0.04 N HCl/2-propanol and read at 550 nm. All determinations were carried out in triplicate.

Tubulin polymerization

Tubulin polymerization was carried out in a 96 well plate. In each well 50 µL of a solution of 25 µM of tubulin in GAB buffer was added to 50 µL of 27.5 µM solution of the corresponding compounds in GAB buffer (20 mM sodium phosphate, 10 mM $MgCl_2$, 1 mM EGTA, 30% glycerol) and 0.1 mM GTP at pH = 6.5. Then, the plate was incubated at 37 ºC in Multiskan (R) and absorbance at 340 nM was registered every 30 seconds during 2 hours.

ELISA analysis

HT-29 cells at 70–80% confluence were collected and 1.5 x 10^5 cells were placed in a six well plate in 1.5 mL of medium. After 24h, cells were incubated with the corresponding compounds for 72 h. Culture supernatants were collected and VEGF secreted by HT-29 cells was determined using Invitrogen Human Vascular Endothelial Growth Factor ELISA Kit according to the manufacturer's instructions.

RT-qPCR analysis

HT-29 cells at 70–80% confluence were collected and 1.5 x 10^5 cells were placed in a six well plate in 1.5 mL of medium. After 24h, cells were incubated with the corresponding compounds for 72 h. Cells were collected and the total cellular RNA from HT-29 cells was isolated using Ambion RNA extraction Kit according to the manufacturer's instructions. The cDNA was synthesized by MMLV-RT with 1–21 µg of extracted RNA and oligo(dT)15 according to the manufacturer's instructions.

Genes were amplified by use of a thermal cycler and StepOnePlus ™ Taqman ® probes. TaqMan ® Gene Expression Master Mix Fast containing the appropriate buffer for the amplification conditions, dNTPs, thermostable DNA polymerase enzyme and a passive reference probe was used. To amplify each of the genes the predesigned primers were used and sold by Life Technologies TaqMan ® Gene Expression Assays, Hs99999903-m1 (β-actin), Hs00900055-m1 (VEGF), Hs00972646-m1 (hTERT) y Hs00153408-m1 (c-Myc).

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References

1 D. Hanahan and R. A. Weinberg, *Cell*, 2011, **144**, 646-674.
2 F. Torres-Andón and B. Fadeel, *Acc. Chem. Res*., 2013, **46**, 733-742.
3 T. Fojo (Ed.), *The Role of Microtubules in Cell Biology, Neurobiology and Oncology*, Humana Press, Totowa, NJ, 2008.
4 G. M. Alushin, G. C. Lander, E. H. Kellogg, R. Zhang, D. Baker, E. Nogales, *Cell*, 2014, **157**, 1117-1129.
5 (a) T. Beckers and S. Mahboobi, *Drugs Fut*., 2003, **28**, 767-785. (b) J. A. Hadfield, S. Ducki, N. Hirst and A. T. McGown, *Progr. Cell Cycle Res.*, 2003, **5**, 309-325. (c) M. A. Jordan and L. Wilson, *Nat.*

Rev. Cancer, 2004, **4**, 253-265. (d) S.-H. Chen and J. Hong, *Drugs Fut*., 2006, **31**, 123-150. (e) E. Pasquier and M. Kavallaris, *IUBMB Life*, 2008, **60**, 165-170. (f) P. Singh, K. Rathinasamy, R. Mohan and D. Panda, *IUBMB Life*, 2008, **60**, 368-375. (g) P. G. Morris and M. N. Fornier, *Clin. Cancer Res.*, 2008, **14**, 7167-7172. (h) E. A. Perez, *Mol. Cancer Ther*., 2009, **8**, 2086-2095. (i) S. M. Chen, L.-H. Meng and J. Ding, *Expert Opin. Invest. Drugs*, 2010, **19**, 329-343. (j) D. Calligaris, P. Verdier-Pinard, F. Devred, C. Villard, D. Braguer and D. Lafitte, *Cell. Mol. Life Sci.*, 2010, **67**, 1089-1104.

6 (a) K.-H. Altmann and J. Gertsch, *Nat. Prod. Rep*., 2007, **24**, 327-357. (b) D. G. I. Kingston, *J. Nat. Prod*., 2009, **72**, 507-515.

7 J. Chen, T. Liu, X. Dong and Y. Hu, *Mini-Rev. Med. Chem*., 2009, **9**, 1174-1190.

8 Y. Fu, S. Li, Y. Zu, G. Yang, Z. Yang, M. Luo, S. Jiang, M. Wink and T. Efferth, *Curr. Med. Chem*., 2009, **16**, 3966-3985.

9 F. Sarabia, M. García-Castro and A. Sánchez-Ruiz, *Curr. Bioact. Comp*., 2006, **2**, 269-299.

10 T. Usui, H. Watanabe, H. Nakayama, Y. Tada, N. Kanoh, M. Kondoh, T. Asao, K. Takio, H. Watanabe, K. Nishikawa, T. Kitahara and H. Osada, *Chem. & Biol*., 2004, **11**, 799-806.

11 (a) K. Yasui, Y. Tamura, K. Nakatani, K. Kawada and M. Ohtani, *J. Org. Chem*., 1995, **60**, 7567-7574. (b) M. K. Gurjar, J. T. Henri, Jr., D. S. Bose and A. V. R. Rao, *Tetrahedron Lett*., 1996, **37**, 6615-6618. (c) N. Chida, M. Yoshinaga, T. Tobe and S. Ogawa, *Chem. Comm*., 1997, 1043-1044. (d) H. Watanabe, H. Watanabe, M. Bando, M. Kido and T. Kitahara, *Tetrahedron*, 1999, **55**, 9755-9776. (e) G. E. Keck, C. E. Knutson and S. A. Wiles, *Org. Lett*., 2001, **3**, 707-710. (f) L. C. Dias, L. G. de Oliveira and M. A. de Sousa, *Org. Lett*., 2003, **5**, 265-268. (g) X. Shen, A. S. Wasmuth, J. Zhao, C. Zhu and S. G. Nelson, *J. Am. Chem. Soc*., 2006, **128**, 7438-7439. (h) D. Enders, S. Dhulut, D. Steinbusch and A. Herrbach, *Chem. Eur. J*., 2007, **13**, 3942-3949. (i) C. Bressy, J.-P. Vors, S. Hillebrand, S. Arseniyadis and J. Cossy, *Angew. Chem. Int. Ed*., 2008, **47**, 10137-10140. (j) M. T. Crimmins and A.-M. R. Dechert, *Org. Lett*., 2009, **11**, 1635-1638.

12 For two comprehensive, recent reviews, see: (a) J. A. Marco, M. Carda, J. Murga and E. Falomir, *Tetrahedron*, 2007, **63**, 2929-2958. (b) J. A. Marco and M. Carda, Recent advances in the field of naturally occurring 5,6-dihydropyran-2-ones, in *Natural Lactones and Lactams. Synthesis, Occurrence and Biological Activity*, T. Janecki (Ed.), Wiley-VCH, 2014, pp. 51-100. For pironetin tubulin binding site: (a) J. Yang, Y. Wang, T. Wang, J. Jiang, C. H. Botting, H. Liu, Q. Chen, J. Yang, J. H. Naismith, X. Zun and L. Chen, *Nature Communicantions*, 2016, **7**, doi:10.1038/ncomms12103. (b) A. E Prota,. J. Setter, A. B Waight, K. Bargsten, J. Murga, J. F. Diaz and M. O. Steinmetz, *Journal of Molecular Biology*, 2016, doi: 10.1016/j.jmb.2016.06.023.

13 J. A. Marco, J. García-Pla, M. Carda, J. Murga, E. Falomir, C. Trigili, S. Notararigo, J. F. Díaz and I. Barasoain, *Eur. J. Med. Chem*., 2011, **46**, 1630-1637.

14 J. Paños, S. Díaz-Oltra, M. Sánchez-Peris, J. García-Pla, J. Murga, E. Falomir, M. Carda, M. Redondo-Horcajo, J. F. Díaz, I. Barasoain and J. A. Marco, *Org. Biomol. Chem*. 2013, **11**, 5809-5826.

15 R. W. Hoffmann, *Chem. Rev*., 1989, **89**, 1841-1860.

16 For two interesting reviews on the conformational aspects of open-chain carbon backbones, see: (a) R. W. Hoffmann, M. Stahl, U. Schopfer and G. Frenking, *Chem. Eur. J*., 1998, **4**, 559-566. (b) R. W. Hoffmann, *Angew. Chem. Int. Ed*., 2000, **39**, 2054-2070.

17 J. Welti, S. Loges, S. Dimmeler and P. Carmeliet, *J. Clin. Invest.* 2013, **123**, 3190-3200.

18 C. Yinnan and Z. Yanmin Z., *Pharmacol. Ther*., 2016, **163**, 24-47.

19 (a) H. Huang, H. Weng, H. Zhou and L. Qu, *Curr. Pharm. Des*., 2014, **20**, 6543-6554. (b) B.-J. Chen, Y.-L. Wu, Y. Tanaka and W. Zhang, *Int. J. Biol. Sci.*, 2014, **10**, 1084-1096.

20 This corresponds to the concept of polypharmacology: J.-U. Peters, *J. Med. Chem*. 2013, **56**, 8955-8971.

21 S. Källström, A. Erkkilä, P. M. Pihko, R. Sjöholm, R. Sillanpää and R. Leino, *Synlett*, 2005, 751-756. In order to work efficiently at a much higher scale than in the original paper, the experimental procedure had to be carried out as described in the Experimental.

22 For recent reviews on metathesis, see: (a) J. Cossy, S. Arseniyadis and C. Meyer (Eds.), *Metathesis in Natural Product Synthesis*, Wiley-VCH, Weinheim, 2010. (b) A. H. Hoveyda, *J. Org. Chem*., 2014, **79**, 4763-4792.

23 In some cases, products from intramolecular Michael addition of one hydroxyl group in the side chain to the conjugated C=C bond were detected.

24 The crystal structures have been deposited at the Cambridge Crystallographic Data Centre and allocated the following deposition numbers: **2** (CCDC-1431101), *ent*-**2** (CCDC-1431108), **4** (CCDC-1431102), *ent*-**4** (CCDC-1431106), **5** (CCDC-1431104), *ent*-**5** (CCDC-1431105), **20** (CCDC-1431103), *ent*-**20** (CCDC-1431107) and *ent*-**27** (CCDC-1431109).

25 S. Rodríguez-Nieto, M. A. Medina and A. R. Quesada, *Anticancer Res*. 2001, **21**, 3457-3460.