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Synthesis and biological evaluation of truncated α-tubulin-binding pironetin analogues lacking alkyl pendants in the side chain or the dihydropyrone ring†

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The preparation of several new truncated analogues of the natural dihydropyrone pironetin is described. They differ from the natural product mainly in the suppression of some of the alkyl pendants in either the side chain or the dihydropyrone ring. Their cytotoxic activity and their interactions with tubulin have been investigated. It has been found that all analogues are cytotoxic towards two either sensitive or resistant tumoral cell lines with similar IC_{50} values in each case, thus strongly suggesting that, like natural pironetin, they also display a covalent mechanism of action. Their cytotoxicity is, however, lower than that of the parent compound. This indicates that all alkyl pendants are necessary for the full biological activity, with the ethyl group at C-4 seemingly being particularly relevant. Most likely, the alkyl groups cause a restriction in the conformational mobility of the molecule and reduce the number of available conformations. This makes it more probable that the molecule preferentially adopts a shape which fits better into the binding point in α-tubulin.

Introduction

Microtubules are dynamic polymers which play a central role in a number of cellular processes, particularly cell division, as they are key constituents of the mitotic spindle.[1] Their shape can be described as hollow tubes of about 25 nm external diameter composed of a protein named tubulin. The functional form of this protein is a heterodimer formed in turn through the non-covalent binding of two monomeric constituents. These are two very similar polypeptides of about 450 amino acid residues, called α- and β-tubulin.[2] For cell division to occur in a normal way, microtubules must be in a constant state of formation and disruption, a process named microtubule dynamics in which GTP hydrolysis into GDP plays a key role.[3]

It is easy to understand that any molecule which exerts some type of action on microtubule dynamics will be able to influence the cell division process not only of normal cells but also of tumoral ones. Since such an influence may be exerted by molecules that bind to any of the tubulin components, it is not surprising that tubulin-binding molecules (TBM) constitute a most important class of anticancer agents.[4] TBM are able to interfere with microtubule assembly and functions, either by causing disruption of the microtubules or through their stabilization. In both cases, this results in mitotic arrest of eukaryotic cells and subsequent cell death. Most of the hitherto described active drugs are natural products or derivatives thereof.[5] Major drugs can already be found on the market and many other promising compounds are in clinical trials.[4,5]

TBM may be divided into 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 or stabilization of microtubules. Among the drugs that belong to this group, the venerable alkaloid colchicine[6] exerts its effects by causing disruption of microtubules. In contrast, another renowned representative of the same group, paclitaxel, was the first-described tubulin-interacting drug that was found to stabilize microtubules.[7] In spite of the fact that they exert opposite effects on the mitotic spindle, both drugs are known to bind to β-tubulin, even though to different sites within this protein.

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†Electronic supplementary information (ESI) available: Additional experimental procedures and tabulated spectral data of all synthetic intermediates. Graphical NMR spectra of all new compounds (five PDF files). CCDC 917056. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3ob40854j

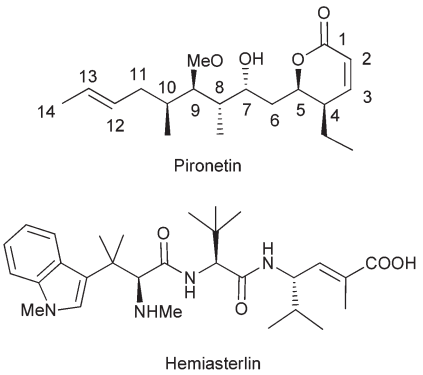


Fig. 1 Structures of two natural products reported to selectively bind to α-tubulin.

The mechanisms of action[8] of many of these TBM and the molecular aspects[9] of their interactions with tubulin have been studied using a broad palette of methods.[10]

The number of products that bind to α-tubulin is very small,[11] the naturally occurring 5,6-dihydro-α-pyrone pironetin (Fig. 1) being the first-reported example, followed a short time later by the peptide-like hemiasterlin family.[12] Pironetin is a potent inhibitor of tubulin assembly and has been found to arrest cell cycle progression in the G2/M phase.[13] This feature has motivated a number of groups to undertake total syntheses of this natural compound.[14] Some synthetic and biological studies on modified variants of pironetin have recently been published.[15]

Some structure–activity (SAR) studies on pironetin have been reported.[13] These studies have shown that the presence of the conjugated C2═C3 double bond and of the hydroxyl group at C-9, either free or methylated, is essential for the biological activity. The presence of a (7*R*)-hydroxyl group also seems to be relevant.[13] The epoxidation of the C12═C13 double bond has been shown to cause a decrease in the activity[13a,b] but this may perhaps represent a negative feature of the oxirane ring, rather than a strict need of this C═C bond. No data are available about the importance of the remaining structural features.[15] It has been proposed that the Lys352 residue of the α-tubulin chain adds in a Michael fashion to the conjugated double bond of pironetin, therefore forming a covalent bond with C-3 of the dihydropyrone ring (Fig. 2). In addition, it has been suggested that the Asn258 residue of α-tubulin holds the pironetin molecule through two hydrogen bonds to the dihydropyrone carbonyl and the methoxyl oxygen atoms.[13]

The appearance of resistances to existing drugs has led to a continuous need for developing new bioactive compounds that overcome such problems. Even though first observed in the case of antibiotics, resistances have also been reported to TBM.[4c,e,h,16] The investigation of new members of this compound class therefore constitutes an important goal in chemistry and pharmacology. 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 aim of our

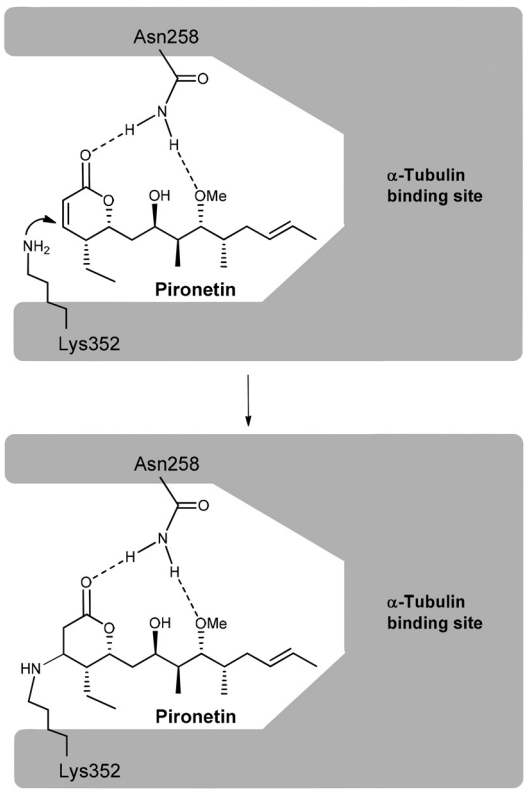


Fig. 2 Schematic model of the covalent union of pironetin to its binding site at the α-tubulin surface.

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. Although pironetin is not an extremely complex molecule, a total synthesis will be lengthy enough to make preparation on a large scale difficult. Our investigation aims at establishing which elements of the pironetin molecule are essential for its activity and, if possible, at achieving an improvement of this activity.

In order to develop SAR studies based upon the pironetin framework, we designed two years ago[17] 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 Fig. 3. 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,

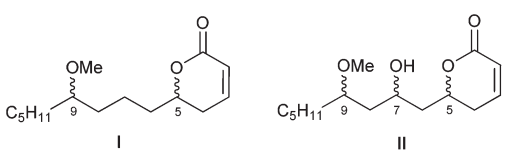


Fig. 3 General structures of simplified pironetin analogues of the first generation (ref. 17).

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 the general constitution **I**, with no hydroxyl group at C-7, were prepared. Likewise, all eight stereoisomers exhibiting the general structure **II**, with a hydroxyl group at C-7, were synthesized.[17]

The cytotoxic activity of these analogues and their interactions with tubulin were subsequently investigated. For the measurement of the cytotoxic activity, the ovarian carcinoma cell types A2780 (sensitive to chemotherapy) and A2780AD (resistant to chemotherapy) were used. It was found on the one hand that analogues **I**/**II** were cytotoxic in the low micromolar range, thus much less active than the parent molecule.[17] On the other hand, we also found that they behaved in the same way as pironetin in that they killed both sensitive and resistant cells with similar IC_{50} values. This indicates that these compounds are not substrates for the P-glycoprotein[18] that resistant cells overexpress in order to pump out cytotoxic compounds, a feature expected for compounds which act through a covalent mechanism of action.[19] The general conclusion was that the simplified pironetin analogues **I**/**II** share the mechanism of action of the natural compound and compete for the same binding site to α-tubulin, leading to disruption of the microtubule network. Furthermore, it is worth mentioning that variations in the configurations of the three stereocentres (C-5, C-7, C-9) did not translate into significant differences in the biological activity.[17]

In continuation of this line of research, we have now investigated the importance of the alkyl pendants in the pironetin molecule for the biological properties of the natural compound. In line with this reasoning, we have prepared the six pironetin analogues **1–6** (Fig. 4). 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. 3), compounds **1** and **2** contain an additional methyl residue at C-10 with either configuration, whereas in compounds **3** and **4**, the extra methyl pendant is allocated at C-8. Finally, compounds **5** and **6** display an extra alkyl residue (methyl or ethyl) at C-4, in both cases with the same configuration as in natural pironetin.

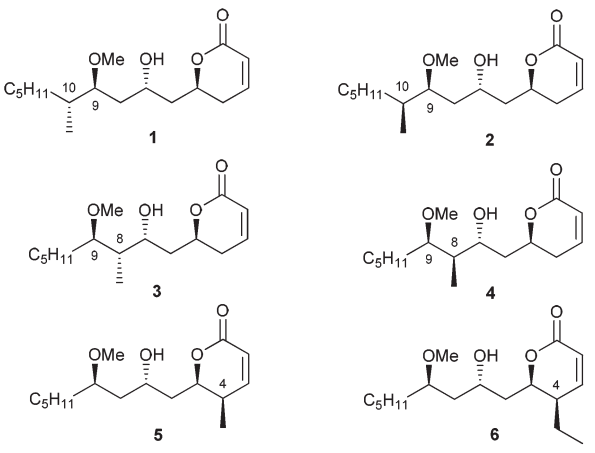


Fig. 4 Structures **1–6** of the new series of pironetin analogues.

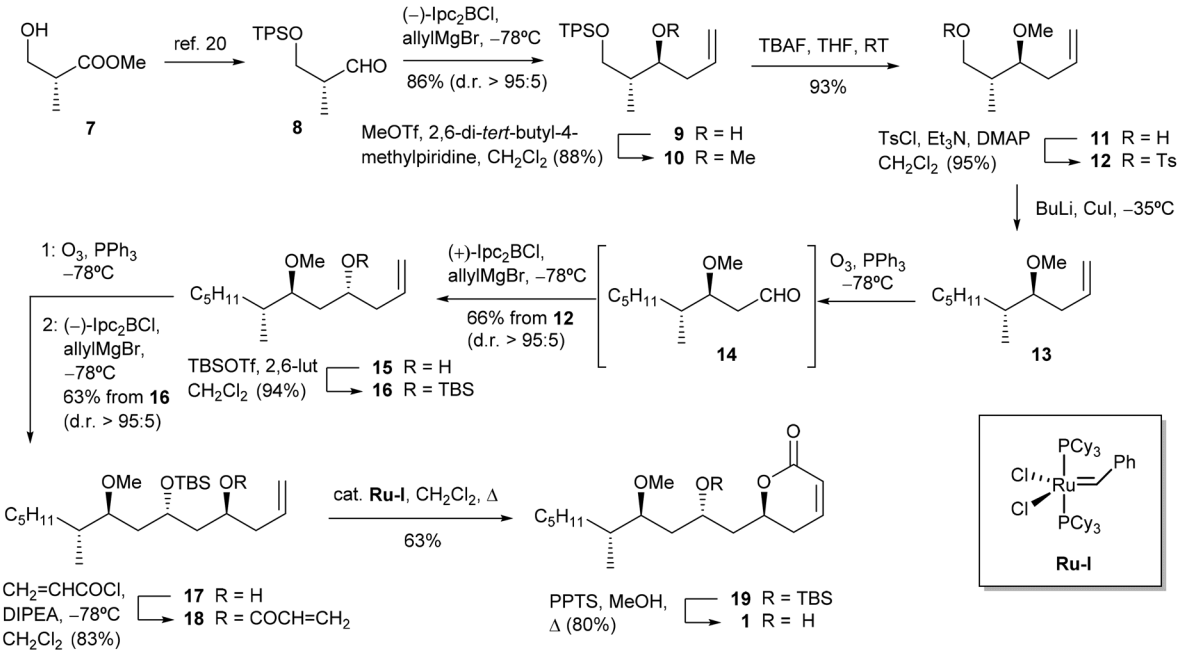
Results and discussion

Synthesis of compounds 1–6

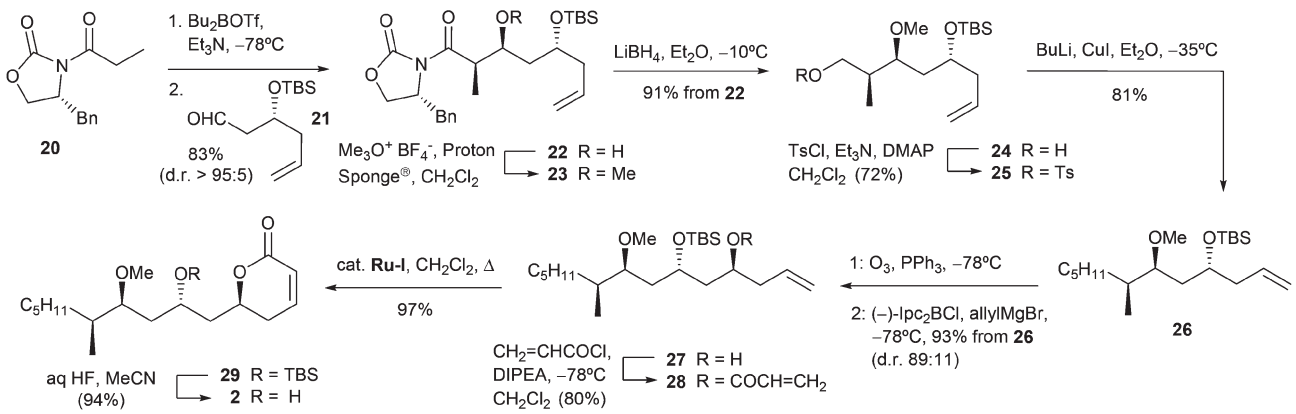
The synthesis of dihydropyrones **1–6** followed in part the general strategy based on iterative ozonolysis/allylation sequences[17] used for the preparation of **I**/**II** (Fig. 3). However, the presence of the extra methyl or ethyl-bearing stereocentres required the inclusion of additional elements in the strategy. Scheme 1, for instance, depicts the synthesis of dihydropyrone **1**. The chiral starting material was the commercially available Roche ester **7**, which was first converted into the known aldehyde **8** *via* a reported procedure.[20] Asymmetric allylation of **8** by means of Brown's methodology[21] using the reagent combination (−)-Ipc_2BCl/allylMgBr gave alcohol **9**, which was then methylated to **10** with methyl triflate and a bulky amine (2,6-di-*tert*-butyl-4-methylpyridine).[22] Desilylation of **10** followed by tosylation of the alcohol function afforded tosylate **12**, which was then coupled with a butylcuprate reagent[23] to yield the very volatile olefin **13**. Ozonolysis of **13** gave the unstable aldehyde **14**, which was not purified but immediately subjected in crude form to asymmetric Brown allylation, this time using (+)-Ipc_2BCl/allylMgBr. Careful chromatographic purification of the reaction product furnished alcohol **15** as a single stereoisomer in 66% overall yield from tosylate **12**. After *O*-silylation of **15**, the ozonolysis/allylation sequence was repeated to give homoallyl alcohol **17**, which was then treated with acryloyl chloride to yield ester **18**. The latter was then subjected to ring-closing metathesis[24] with ruthenium first-generation catalyst **Ru-I** to give **19**, desilylation of which afforded the target molecule **1**.

We then tried to prepare dihydropyrone **2** through the same strategy used in the case of **1** but with the antipode of **7** as the chiral starting compound. However, we met unanticipated problems with the olefin counterpart of **13** (Scheme 1). Its volatility was much higher than that of its diastereoisomer, with low yields in its preparation being the consequence. In view of this, we took recourse to a different strategy, depicted in Scheme 2, where chirality was generated with the aid of an asymmetric aldol reaction.

The *syn* relationship of the substituents at C-10 and C-9 in dihydropyrone **2** led us to select the Evans aldol methodology[25] for the preparation of this fragment of the molecule. Thus, the *Z* boron enolate generated from the commercially available *N*-propionyl oxazolidinone **20** was allowed to react with the known chiral aldehyde **21**.[26] This yielded aldol adduct **22** with good yield as well as excellent diastereoselectivity. Methylation of **22** with trimethyloxonium tetrafluoroborate and Proton Sponge® as a base[22b] furnished compound **23**, the stereostructure of which was confirmed by means of an X-ray diffraction analysis (see ESI†). Reductive cleavage of the chiral auxiliary with $LiBH_4$ was followed by tosylation of the primary alcohol and coupling of the tosylate as above with the butylcuprate



Scheme 1 Synthesis of dihydropyrone **1**. Abbreviations: DMAP, 4-(*N*,*N*-dimethylamino)pyridine; Ipc, isopinocampheyl; TBAF, tetra-*n*-butylammonium fluoride; TBS, *tert*-butyldimethylsilyl; TPS, *tert*-butyldiphenylsilyl; Tf, trifluoromethanesulfonyl; Ts, *p*-toluenesulfonyl; PPTS, pyridinium *p*-toluenesulfonate; Cy, cyclohexyl; 2,6-lut, 2,6-lutidine; DIPEA, ethyl *N*,*N*-diisopropylamine.

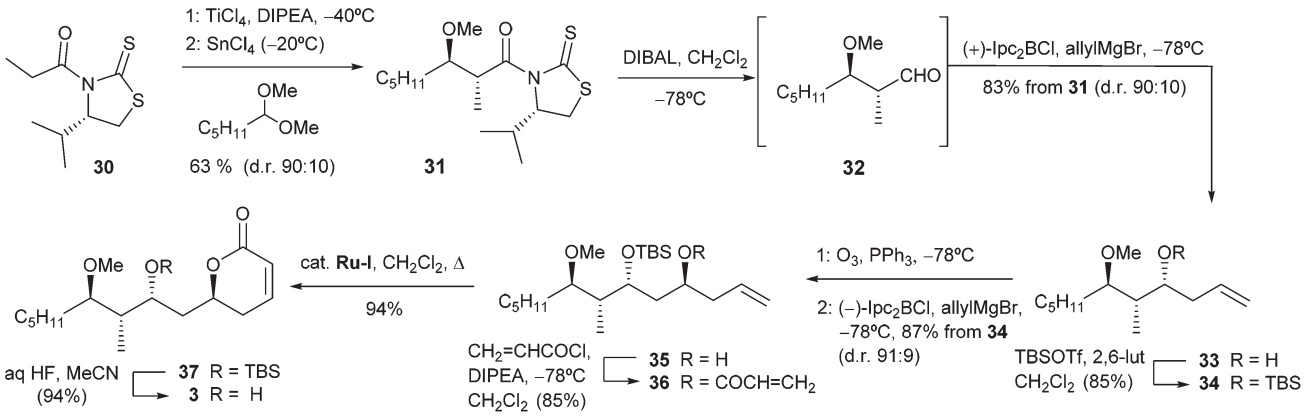


Scheme 2 Synthesis of dihydropyrone **2**. Abbreviation: Bn, benzyl.

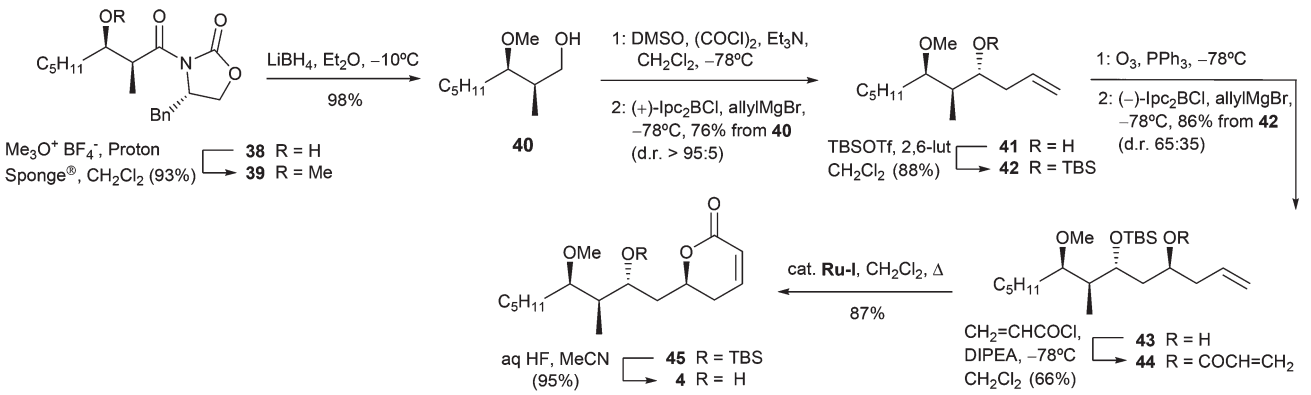
reagent to yield olefin **26.** The ozonolysis of **26** followed by asymmetric allylation of the intermediate aldehyde (not depicted in Scheme 2) gave alcohol **27**, which was then subjected to esterification to acrylate **28.** Ruthenium-catalyzed ring-closing metathesis of **28** furnished **29**, which was subsequently desilylated to the desired **2.**

The *anti* relationship of the substituents at C-9 and C-8 in dihydropyrone **3** led us to select the acetal variant[27] of the Crimmins aldol methodology[28] for the preparation of this fragment of the molecule. Thus, hexanal dimethylacetal[29] (Scheme 3) was allowed to react with the titanium enolate of *N*-propionyl thiazolidinethione **30**[30] to yield adduct **31** with good diastereoselectivity (d.r. 90 : 10). Reductive cleavage of the chiral auxiliary gave the intermediate aldehyde **32**, which was used in crude form in the asymmetric Brown allylation to yield homoallyl alcohol **33**, subsequently silylated to **34.** As in the previously discussed syntheses, an ozonolysis/asymmetric allylation sequence was performed on **34** to furnish alcohol **35**, which was esterified to acrylate **36.** Ruthenium-catalyzed ring-closing metathesis of **36** furnished **37**, which was then desilylated to the target molecule **3.**

In compound **4**, the *syn* relationship of the substituents at C-9 and C-8 led us to consider again an aldol reaction with a chiral auxiliary of the Crimmins type. However, while the aldol reaction worked in a satisfactory way, we were unable to perform the *O*-methylation of the resulting aldol. We then decided to switch to a chiral auxiliary of the Evans type. Thus, the known Evans aldol adduct **38**[31] was methylated to yield **39** (Scheme 4). Reductive cleavage of the chiral auxiliary afforded the primary alcohol **40**, which was then oxidized with the



Scheme 3 Synthesis of dihydropyrone **3**. Abbreviation: DIBAL, diisobutylaluminum hydride.



Scheme 4 Synthesis of dihydropyrone **4**. Abbreviation: DMSO, dimethylsulfoxide.

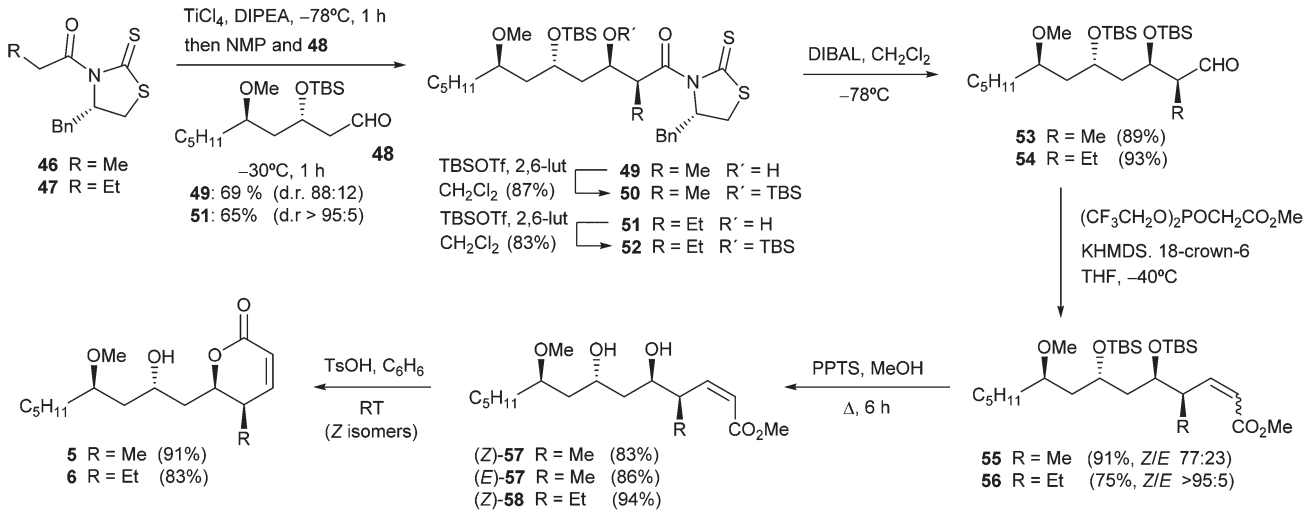
Swern procedure[32] to the corresponding aldehyde (not depicted in Scheme 4). The latter was subjected to asymmetric Brown allylation to give homoallyl alcohol **41**, which was then silylated to **42**. Ozonolysis of **42** followed by asymmetric allylation of the intermediate aldehyde (not depicted in Scheme 4) proceeded with good yield but medium diastereoselectivity (d.r. 65 : 35). The major stereoisomer **43** was esterified to acrylate **44**. Ruthenium-catalyzed ring-closing metathesis of **44** furnished **45**, which was then desilylated to the target molecule **4**.

For the synthesis of dihydropyrones **5** and **6** we made again use of Crimmins aldol methodology.[28] The reaction sequence was essentially identical for both compounds (Scheme 5). Thus, the titanium enolate of *N*-propionyl thiazolidinethione **46**[33] was allowed to react with the known[17] chiral aldehyde **48** to give adduct **49** with good diastereoselectivity. Silylation of the hydroxy group in **49** to yield **50** followed by reductive cleavage of the chiral auxiliary provided aldehyde **53**. Olefination of **53** was performed using the Still–Gennari methodology[34] and yielded conjugated ester **55** with good overall yield and acceptable *Z*/*E* diastereoselectivity. Heating *Z*-**55** in acidic methanol at reflux caused cleavage of the two silyl groups but not lactone ring closure. Forcing the reaction conditions led to intramolecular Michael addition of one hydroxyl group to the conjugated olefinic bond. However, isolation of the desilylated product **57** followed by acidic treatment in benzene at room temperature gave the desired **5**.

Dihydropyrone **6** was obtained through an analogous reaction sequence starting from *N*-butyryl thiazolidinethione **47**.[35]

Biological properties of pironetin analogues

Cellular effects of the compounds. We have determined the IC_{50} values for pironetin analogues **1–6**, as well as for synthetic intermediates (*E*)-**57**, (*Z*)-**57** and (*Z*)-**58**, and compared these values with that of pironetin on both A2780 and A2780AD human ovary carcinoma cells (Table 1). While pironetin was active at the nanomolar range, the activities of the pironetin analogues here under study were in the micromolar range, that is, they are around three orders of magnitude less active. The most cytotoxic compounds against both A2780 and multiresistant A2780AD cells were, in this order, **6**, **1** and (*E*)-**57** and, to a somewhat lesser extent, **3** and (*Z*)-**58**. Compounds **4**, **2** and (*Z*)-**57** were clearly less active, whereas **5** did not display a noticeable activity. As shown in Table 1, pironetin and most of the investigated compounds are able to overcome the resistance of the A2780AD cell line due to efflux mediated by the P-glycoprotein[18] and show comparable IC_{50} values for the resistant and parental cell lines. As already commented, this is



Scheme 5 Synthesis of dihydropyrones **5** and **6**. Abbreviations: NMP, *N*-methylpyrrolidone; KHMDS, potassium hexamethyldisilazide.

Table 1 Effects of pironetin analogues and synthetic intermediates on the growth of A2780 and A2780AD (MDR overexpressing P-glycoprotein) ovarian carcinoma cells

Compound	IC_{50}[a] (A2780)	IC_{50}[a] (A2780AD)	R/S[b]
Pironetin	0.009 ± 0.002	0.008 ± 0.001	0.8
1	25 ± 2	23 ± 1	0.9
2	51 ± 1	45 ± 3	0.9
3	32 ± 6	34 ± 1	1.1
4	43 ± 2	37 ± 4	0.9
5	>200	>200	—
6	10 ± 2	16 ± 1	1.6
(*E*)-**57**	18 ± 3	18.4 ± 1	1
(*Z*)-**57**	85 ± 12	188 ± 107	2.2
(*Z*)-**58**	32 ± 8	42 ± 6	1.3

[a] IC_{50} values (μM) are the mean ± standard error of three independent experiments. [b] Resistance index (relative resistance, obtained dividing the IC_{50} of the resistant cell line by that of the parental A2780 cell line).

a feature expected for compounds which act through a covalent mechanism of action.[19]

In order to study the effect of the aforementioned pironetin analogues on the microtubule cytoskeleton, we incubated cells in the presence of these ligands for 24 hours (Fig. 5). Pironetin at 50 nM concentration completely depleted cytoplasmic microtubules (B and inset): cells are arrested in the prometaphase[13a,b] and type IV mitotic spindles are observed,[36] with the chromosomes being arranged in a ball of condensed DNA with no microtubules. When using 50 μM **1**, a reduction in the number of microtubules and the presence of type III mitotic spindles were observed, with a ball of condensed DNA enclosing one or more star shaped aggregates of microtubules being present in the preparations. Shrinking of the nucleus occurred in some cells (C and inset). With higher concentrations of this ligand (100 μM and 200 μM), a great cytotoxic effect, extensive cell death and nucleus shrinking was observed (results not depicted in Fig. 5).

Ligands **3** and **4** at 100 μM concentration induced some depolymerization of cytoplasmic microtubules and type III mitotic spindles (D, E and insets). The most cytotoxic of all tested ligands, compound **6** (25 μM) induced extensive microtubule depletion and type IV mitotic spindles (F and inset). Compound (*E*)-**57**, the second most cytotoxic ligand, at 50 μM concentration induced microtubule depletion and both type III and IV mitotic spindles (G and inset). Finally, ligand (*Z*)-**58** (100 μM) induces microtubule depolymerization and type III mitotic spindles (H and inset). In the presence of ligands **2**, **5** and (*Z*)-**57** at 100 μM (results not depicted in Fig. 5), the array of microtubules looked like in control cells (A).

We next studied whether the aforementioned ligands were capable of blocking cells in the G2/M phase of the cell cycle of A549, as other microtubule inhibitors do. We incubated these cells for 20 hours in the presence of the different ligands or the drug vehicle (Fig. 6 and Table 2). Pironetin at 50 nM concentration almost completely arrested cells in the G2/M phase and, interestingly, so did **6** although at the micromolar level (25 μM). Ligand (*E*)-**57** (25 μM) also caused arrest at the G2/M but to a somewhat lower level than **6**. Ligands **1** (50 μM), and (*Z*)-**58** (100 μM) caused arrest to a much lower level whereas ligands **2–5** and (*Z*)-**57**, all 100 μM, left the cell cycle practically unaltered as compared with the control. Table 2 shows the percentage of cells in each phase of the cell cycle at the indicated ligand concentration. As commented above, pironetin (50 nM), **6** (25 μM) and (*E*)-**57** (25 μM) show the strongest effects with 97%, 86% and 70%, respectively, of the cells being in the G2/M phase.

Tubulin assembly. The critical concentration of purified tubulin required for assembly was determined in GAB in the presence of a large excess (100 μM) of dihydropyrones **1–6** and synthetic intermediates (*E*)-**57**, (*Z*)-**57** and (*Z*)-**58** (Table 3). Docetaxel is included in the Table as it is known to be a microtubule-stabilizer agent, as shown by its low CrC value, and acts therefore as a contrasting (positive) control element. As shown in the Table, the concentration of tubulin required to produce

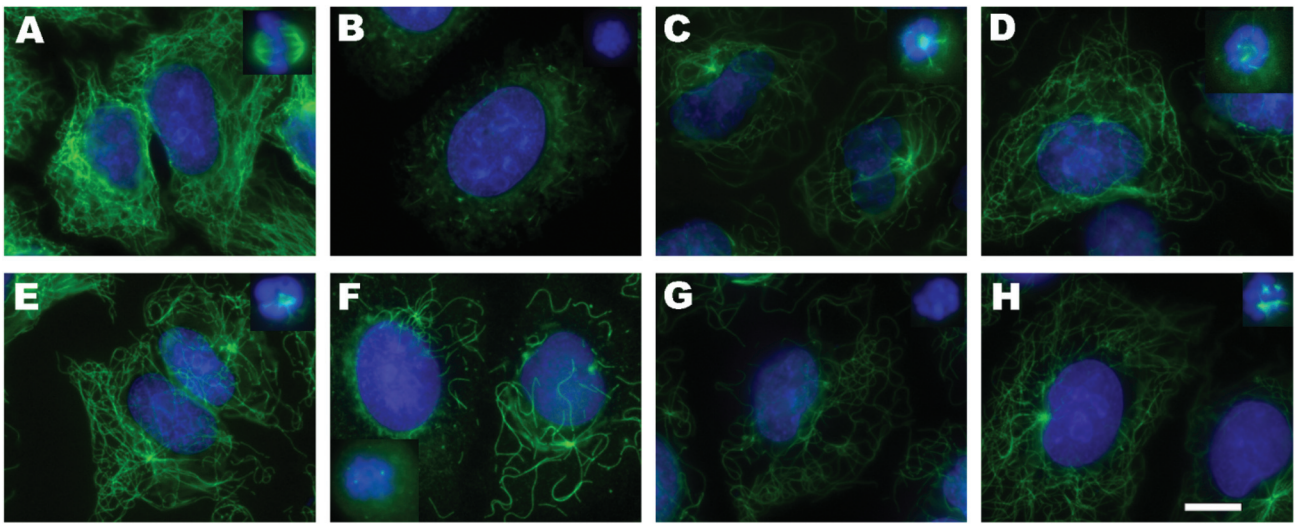


Fig. 5 Effect of pironetin analogues **1**, **3**, **4**, **6**, (*E*)-**57** and (*Z*)-**58** as compared to the parental molecule pironetin on the microtubule network and nucleus morphology. A549 cells were incubated for 24 hours with either drug vehicle DMSO (A), 50 nM pironetin (B), 50 μM **1** (C), 100 μM **3** (D), 100 μM **4** (E), 25 μM **6** (F), 50 μM (*E*)-**57** (G) and 100 μM (*Z*)-**58** (H). Microtubules are stained with α-tubulin antibodies, whereas DNA was stained with Hoechst 33342. Insets (A–H) are mitotic spindles from the same preparation. The scale bar in H represents 10 μm. All panels have the same magnification.

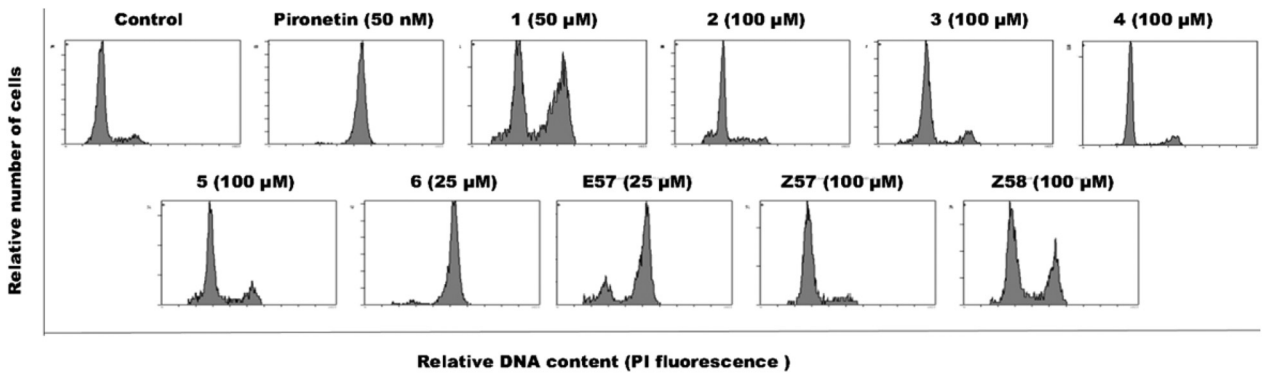


Fig. 6 Cell cycle histograms of A549 lung carcinoma cells untreated or treated with pironetin analogues **1–6** and synthetic intermediates (*E*)-**57**, (*Z*)-**57** and (*Z*)-**58**. The lowest ligand concentration that induces maximal arrest in the G2/M phase is depicted.

Table 2 Cell cycle distribution of A549 cells treated with compounds **1–6**, (*E*)-**57**, (*Z*)-**57** and (*Z*)-**58**[a]

Ligand	Sub G1	G0/G1	S	G2/M
Control	3	83	7	7
Pironetin	0.5	1	1.5	97
1	2	33	17	48
2	7	77	6	10
3	3	71	15	11
4	2	71	14	13
5	5	70	11	14
6	2	9	3	86
(*E*)-**57**	2	22	6	70
(*Z*)-**57**	4	78	7	11
(*Z*)-**58**	1	46	4	36

[a] Cells were incubated for 20 hours with the respective ligand at the concentration indicated in Fig. 6. Numbers in the table are percentages (%) of cells in each phase of the cell cycle. The sub-G1 peaks are presumably apoptotic cells.

Table 3 Critical concentration values of tubulin for microtubule assembly induced by pironetin analogues **1–6** and intermediates (*E*)-**57**, (*Z*)-**57** and (*Z*)-**58** (ligand concentrations used are 25 μM for docetaxel and 100 μM for the remaining compounds)

Compound	CrC[a] (μM)
DMSO	3.30
Docetaxel	0.58 ± 0.46
Pironetin	>15
1	3.23 ± 0.90
2	3.98 ± 0.15
3	3.75 ± 0.49
4	4.48 ± 1.32
5	3.86 ± 0.21
6	4.04 ± 0.56
(*E*)-**57**	3.91 ± 0.70
(*Z*)-**57**	4.96 ± 0.54
(*Z*)-**58**	3.18 ± 0.42

[a] CrC values are the mean ± standard error of at least three independent experiments.

assembly (critical concentration[37]) oscillate between 3.3 μM in the absence of ligands (DMSO) and 4.96 μM in the presence of (*Z*)-**57**, the most active of these compounds as regards this particular property. The observed increase of the critical concentration required indicates that most compounds in the Table are also able to inhibit the assembly of tubulin. This is that expected for a pironetin-like structure and has already been observed in previous pironetin analogues prepared by us.[17]

The highest *in vitro* activities were shown, in this order, by compounds (*Z*)-**57**, **4**, **6**, **2** and (*E*)-**57**, followed by the remaining ligands. It is noteworthy that molecules without the dihydropyrone ring such as (*Z*)-**57** and (*E*)-**57** still retain a significant percentage of this microtubule-destabilizing activity. This likely suggests that the long side chain is still able to interact with the pironetin binding site, even in the absence of the dihydropyrone ring. However, since ligand **6** has been shown to be much more cytotoxic than (*Z*)-**57**, **4**, **2** and (*E*)-**57**, it appears that the *in vitro* effect expressed in Table 2 does not correlate well with cellular results such as the IC_{50} values (Table 1) or the cell cycle (Fig. 6), which are determined *in vivo*. This may possibly indicate that the various chemical modifications performed in the pironetin molecule have a significant effect in the transport of the compounds through the cell membrane.

The differences in activity between the compounds discussed here are not easy to explain. Compound **2**, which has the same configuration in its stereocentres as natural pironetin, displays a lower cytotoxicity than its C-10 epimer **1** (Table 1), as well as a much lower ability to arrest cells at the G2/M phase (Table 2). In contrast, **2** shows a higher ability to inhibit tubulin assembly (Table 3). Compounds **3** and **4**, epimeric at C-8, behave in almost the same way except for the ability to inhibit tubulin assembly, which is much higher in **4**. The most surprising case is that of compounds **5** and **6**. A mere replacement of an ethyl group at C-4 (as in **6**) by a methyl group (as in **5**) gives rise to a tremendous decrease in cytotoxicity (Table 1) and in the ability to inhibit tubulin assembly. This is specially surprising in view of the fact that the pironetin analogue lacking the alkyl group at C-4 is also much more cytotoxic than **5** even though less than **6**.[17] The comparatively high cytotoxicity of dihydropyrone **6** and conjugated ester (*E*)-**57** are coherent with the fact that they are the compounds which cause an effect on the microtubule network more similar to pironetin (Fig. 5) and also a complete or extensive arrest of the cell cycle at the G2/M phase (Fig. 6 and Table 2).

Summary

Pironetin analogues **1–6** were synthesized with the aim at exploring the influence of the alkyl pendants of the parent molecule in its biological activity. Most compounds proved cytotoxic in the low micromolar range against both non-resistant and multidrug resistant P-glycoprotein overexpressing, ovarian carcinoma cell lines, similar IC_{50} values being found in both cell lines. Thus, most of the aforementioned compounds are able to inhibit microtubule assembly, both *in vitro* and in cell cultures, therefore sharing the same general mechanism of action of tubulin assembly inhibition by the natural dihydropyrone pironetin.

The results described above suggest that all alkyl pendants are necessary for the full biological activity, perhaps with a certain emphasis on the role of ethyl group at C-4. This is most likely due to the fact that the alkyl groups restrict the conformational mobility of the molecule and reduce the number of available conformations.[38,39] This further makes it more probable that the molecule adopts a shape which fits better into the binding point in α-tubulin. The preparation and biological evaluation of further advanced pironetin analogues, including those having hybrid structures, is currently under way in our laboratory.

Experimental

Chemical procedures

NMR spectra were recorded at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) in a $CDCl_3$ solution at 25 °C, if not otherwise indicated, with the solvent signals as the internal reference. ^{13}C NMR signal multiplicities were determined with the DEPT 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 (all except those involving water in the reaction medium) 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. Unless detailed otherwise, "standard work-up" means pouring the reaction mixture into brine, followed by extraction with the solvent indicated in parentheses. If the reaction medium was acidic, an additional washing of the organic layer with 5% aq. $NaHCO_3$ was performed. If the reaction medium was basic, an additional washing with aq. NH_4Cl was performed. Where solutions were filtered through a Celite pad, the pad was additionally washed with the same solvent used, and the washings were incorporated into the main organic layer. The latter was dried over anhydrous Na_2SO_4 and the solvent was eliminated under reduced pressure. Column chromatography of the residue on a silica gel column (60–200 μm) was performed with elution with the indicated solvent mixtures.

General reaction conditions. They are given below for reactions which were repeated two or more times. Reactions that are used only once are described together with the compound they originate from. Compounds are described in numerically increasing order.

Asymmetric allylboration. Allylmagnesium bromide (commercial 1 M solution in Et_2O, 10 mL, 10 mmol) was added dropwise under N_2 *via* a syringe to a solution of (+)- or (−)-Ipc_2BCl (3.85 g, 12 mmol) in dry Et_2O (50 mL) cooled in a dry ice-acetone bath. After replacing the latter by an ice bath, the mixture was stirred for 1 h. The solution was then allowed to stand, which caused precipitation of magnesium chloride. The supernatant solution was then carefully transferred to another flask *via* canula. After cooling this flask at −78 °C, a solution of the appropriate aldehyde (8 mmol) in dry Et_2O (25 mL) was added dropwise *via* a syringe. The resulting solution was further stirred at the same temp. for 1 h. The reaction mixture was then quenched through the addition of a phosphate pH 7 buffer solution (50 mL), MeOH (50 mL) and 30% H_2O_2 (25 mL). After stirring for 30 min, the mixture was poured onto satd. aq. $NaHCO_3$ and subjected to standard work-up (Et_2O). Column chromatography on silica gel (hexanes–EtOAc mixtures) afforded the desired homoallylic alcohol. Compounds **9**, **33** and **41** were prepared in this way using in each case the appropriate enantiomer of Ipc_2BCl (yields and diastereomeric ratios are indicated in the corresponding schemes).

Ozonolysis/asymmetric allylation sequence. The appropriate olefin (10 mmol) was dissolved in dry CH_2Cl_2 (100 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 (5.25 g, 20 mmol), the solution was left to stir at room temperature for 2 h. Solvent removal under reduced pressure gave a solid material, which was stirred three times under pentane (3 × 25 mL). The residual insoluble solid (Ph_3PO) was discarded, and the organic phase was evaporated under reduced pressure to yield the crude aldehyde as a colorless oil, which was used as such in the asymmetric allylation as described above (for weight calculations, the yield of the ozonolysis step was assumed to be quantitative). Compounds **15**, **17**, **27**, **35** and **43** were prepared in this way (yields and diastereomeric ratios are indicated in the corresponding Schemes).

Silylation with TBSOTf. The appropriate alcohol (4 mmol) was dissolved under N_2 in dry CH_2Cl_2 (20 mL) and treated sequentially with 2,6-lutidine (700 μL, 6 mmol) and TBSOTf (1.15 mL, 5 mmol). The reaction mixture was then stirred for 1–2 h at room temperature until consumption of the starting material (TLC monitoring). Standard work-up (CH_2Cl_2) and column chromatography on silica gel (hexanes–EtOAc mixtures) afforded the desired silylated derivative. Compounds **16**, **34**, **42**, **50** and **52** were prepared in this way (yields are indicated in the corresponding Schemes).

Acylation with acryloyl chloride. The appropriate alcohol (5 mmol) was dissolved under N_2 in dry CH_2Cl_2 (40 mL), cooled to −78 °C and treated sequentially with ethyl *N*,*N*-diisopropylamine (2.6 mL, 15 mmol) and acryloyl chloride (810 μL, 10 mmol). The reaction mixture was then stirred for 2 h at −78 °C. Standard work-up (CH_2Cl_2). Column chromatography on silica gel (hexanes–EtOAc mixtures) afforded the desired ester. Compounds **18**, **28**, **36** and **44** were prepared in this way (yields are indicated in the corresponding Schemes).

Ring-closing metathesis with ruthenium catalyst Ru-I. The appropriate diolefin (1 mmol) was dissolved under N_2 in dry, degassed CH_2Cl_2 (100 mL) and treated with Grubbs first-generation ruthenium catalyst **Ru-I** (82 mg, 0.1 mmol). The mixture was heated at reflux until consumption of the starting material (2–3 h, TLC monitoring!). Solvent removal under reduced pressure and column chromatography of the residue on silica gel (hexanes–EtOAc mixtures) furnished the desired metathesis product. Compounds **19**, **29**, **37** and **45** were prepared in this way (yields are indicated in the corresponding Schemes).

Desilylation. (A) With PPTS/MeOH: the silylated compound (0.6 mmol) was dissolved in MeOH (30 mL) and treated with PPTS (30 mg, 0.12 mmol) and water (0.3 mL). The mixture was then heated at reflux for 18 h, cooled and neutralized by addition of solid $NaHCO_3$. After filtering, the solution was evaporated under reduced pressure, and the oily residue was subjected to column chromatography on silica gel (hexanes–EtOAc mixtures). This provided the desired hydroxy compound. Compounds **1**, (*Z*)-**57**, (*E*)-**57** and **58** were prepared in this way (yields are indicated in the corresponding Schemes). (B) With aq. HF/MeCN: the silylated compound (0.1 mmol) was dissolved in MeCN (4 mL) and treated with 48% HF (36 μL, 1 mmol). The mixture was then stirred at room temperature for 1.5 h. Standard work-up (EtOAc) and column chromatography on silica gel (hexanes–EtOAc mixtures) furnished the desired hydroxy compound. Compounds **2**, **3** and **4** were prepared in this way (yields are indicated in the corresponding Schemes).

Alcohol tosylation. A solution of the alcohol (10 mmol) in CH_2Cl_2 (150 mL) was treated under N_2 with DMAP (12 mg, 0.1 mmol), Et_3N (7 mL, 50 mmol) and TsCl (5.72 g, 30 mmol). The mixture was then stirred at room temperature for 18 h. Standard work-up (CH_2Cl_2) and column chromatography on silica gel (hexanes–EtOAc mixtures) afforded the desired tosylate. Compounds **12** and **25** were prepared in this way (yields are indicated in the corresponding Schemes).

Cross-coupling of a tosylate with lithium dibutylcuprate. Copper(I) iodide (3.81 g, 20 mmol) was placed in a flask and carefully desiccated by means of gentle heating under reduced pressure. Then, it was suspended under N_2 in dry Et_2O (40 mL), cooled to −35 °C and treated with *n*BuLi (commercial 1.6 M solution in hexane, 25 mL, 40 mmol). The mixture was stirred at the same temperature for 30 minutes. The appropriate tosylate (10 mmol) was dissolved in dry Et_2O (50 mL) and added dropwise under N_2 to the cuprate solution, followed by stirring for 1 h under the same conditions. Standard work-up (Et_2O) and column chromatography on silica gel (hexanes–Et_2O mixtures) provided the desired coupling product. Compounds **13** and **26** were prepared in this way (yields are indicated in the corresponding Schemes).

Caution: In the case of **13**, evaporations have to be performed under a not too low pressure in order to avoid losses due to its marked volatility. Therefore, crude **13** contains variable amounts of solvent and was used as such in the next step

(a small sample was purified for analytical purposes). For this reason, the yield given in Scheme 1 refers to the overall conversion of **12** into **15** (*via* **13** and the similarly volatile aldehyde **14**, for which the same caution has to be observed).

Alcohol methylation. (A) With methyl triflate:[22] a solution of the alcohol (10 mmol) in dry CH_2Cl_2 (150 mL) was treated under N_2 at room temperature with 2,6-di-*tert*-butyl-4-methylpiridine (6.16 g, 30 mmol) and MeOTf (3.4 mL, 30 mmol). The mixture was then stirred at reflux until consumption of the starting material (12–18 h, TLC monitoring). Standard work-up (extraction with CH_2Cl_2) and column chromatography on silica gel (hexanes–EtOAc, 19 : 1) gave the desired *O*-methyl derivative. Compound **10** was prepared in this way in 88% yield. (B) With Meerwein salt:[22b] a solution of the alcohol (2 mmol) in dry CH_2Cl_2 (10 mL) was treated under N_2 at room temperature first with a solution of Proton Sponge® (2.14 g, 10 mmol) in dry CH_2Cl_2 (8 mL) and then with a solution of trimethyloxonium tetrafluoroborate (1.48 g, 10 mmol) in dry CH_2Cl_2 (8 mL). The reaction mixture was protected from light and stirred at room temperature for 48 h. Standard work-up (CH_2Cl_2) and column chromatography on silica gel (hexanes–EtOAc mixtures) gave the desired *O*-methyl derivative. Compounds **23** and **39** were prepared in this way (yields are indicated in the corresponding Schemes).

Hydride reductions. (A) With $LiBH_4$:[22b] a solution of the compound to be reduced (2 mmol) in dry Et_2O (10 mL) was cooled to −10 °C and treated with EtOH (140 μL, 2.4 mmol) and then with $LiBH_4$ (commercial 2 M solution in THF, 1.2 mL, 2.4 mmol). The reaction mixture was stirred at the same temperature for 1 h. Standard work-up (Et_2O) and column chromatography on silica gel (hexanes–EtOAc mixtures) gave the desired product (a primary alcohol). Compounds **24** and **40** were prepared in this way (yields are indicated in the corresponding Schemes). (B) With DIBAL: a solution of the compound to be reduced (2 mmol) in dry CH_2Cl_2 (20 mL) was cooled to −78 °C and treated with DIBAL (commercial 1 M solution in hexane, 4 mL, 4 mmol). The reaction mixture was stirred at the same temperature for 30 min. Standard work-up (CH_2Cl_2) and column chromatography on silica gel (hexanes–EtOAc mixtures) gave the desired product (an aldehyde). Compounds **32**, **53** and **54** were prepared in this way (yields are indicated in the corresponding Schemes).

(6*S*)-6-[(2*R*,4*S*,5*S*)-2-Hydroxy-4-methoxy-5-methyldecyl]-5,6-dihydro-2*H*-pyran-2-one (1). Oil, $[\alpha]_D$ −70.4 (*c* 0.7; $CHCl_3$); IR ν_{max} (cm^{-1}): 3450 (br, OH), 1716 (C=O); ^1H NMR δ 6.88 (1H, ddd, *J* = 10, 5.8, 2.8 Hz), 6.01 (1H, br dd, *J* = 10, 1.5 Hz), 4.73 (1H, m), 4.20 (1H, tt, *J* = 9, 3 Hz), 3.34 (3H, s), 3.30 (1H, ddd, *J* = 8, 5.6, 3 Hz), 3.00 (1H, br s, OH), 2.45–2.30 (2H, m), 1.90–1.80 (2H, m), 1.71 (1H, ddd, *J* = 14, 9.8, 3.5 Hz), 1.61 (1H, ddd, *J* = 14.5, 8.3, 3 Hz), 1.54 (1H, ddd, *J* = 14.5, 8.3, 3 Hz), 1.35–1.10 (8H, br m), 0.88 (3H, t, *J* = 6.8 Hz), 0.82 (3H, d, *J* = 6.8 Hz); ^{13}C NMR δ 164.4 (C), 145.2, 121.4, 82.6, 75.3, 65.1, 33.9 (CH), 42.8, 35.5, 33.1, 32.1, 30.0, 27.1, 22.6 (CH_2), 56.7, 14.0, 13.9 (CH_3); HR ESMS *m*/*z* 321.2040 (M + Na^+), calcd for $C_{17}H_{30}NaO_4$, 321.2042.

(6*S*)-6-[(2*R*,4*S*,5*S*)-2-Hydroxy-4-methoxy-5-methyldecyl]-5,6-dihydro-2*H*-pyran-2-one (2). Oil, $[\alpha]_D$ −34.2 (*c* 0.65; $CHCl_3$); IR ν_{max} (cm^{-1}): 3450 (br, OH), 1712 (C=O); ^1H NMR δ 6.88 (1H, ddd, *J* = 10, 5, 3.5 Hz), 6.02 (1H, ddd, *J* = 10, 2.5, 1.5 Hz), 4.73 (1H, m), 4.20 (1H, m), 3.37 (3H, s), 3.30 (1H, ddd, *J* = 8, 5.3, 3.5 Hz), 3.00 (1H, br s, OH), 2.38 (2H, m), 1.90 (1H, ddd, *J* = 14, 8.8, 3 Hz), 1.80 (1H, m), 1.75–1.40 (5H, br m), 1.35–1.25 (6H, br m), 0.89 (3H, t, *J* = 6.8 Hz), 0.88 (3H, d, *J* = 7 Hz); ^{13}C NMR δ 164.3 (C), 145.2, 121.4, 83.3, 75.3, 65.3, 42.7 (CH), 36.3, 34.5, 32.2, 31.2, 30.1, 27.1, 22.6 (CH_2), 57.3, 15.8, 14.1 (CH_3); HR ESMS *m*/*z* 321.2038 (M + Na^+), calcd for $C_{17}H_{30}NaO_4$, 321.2042.

(6*S*)-6-[(2*R*,3*S*,4*R*)-2-Hydroxy-4-methoxy-3-methylnonyl]-5,6-dihydro-2*H*-pyran-2-one (3). Oil, $[\alpha]_D$ −74 (*c* 0.24; $CHCl_3$); IR ν_{max} (cm^{-1}): 3480 (br, OH), 1717 (C=O); ^1H NMR δ 6.88 (1H, ddd, *J* = 10, 6.3, 2.7 Hz), 6.02 (1H, ddd, *J* = 10, 2.5, 1 Hz), 4.70 (1H, m), 4.20 (1H, m), 3.45 (1H, br s, OH), 3.37 (3H, s), 3.22 (1H, br q, *J* ∼ 5.5 Hz), 2.44 (1H, dddd, *J* = 18.5, 6, 4.5, 1 Hz), 2.35 (1H, ddt, *J* = 18.5, 11.5, 2.5 Hz), 1.80–1.55 (5H, br m), 1.35–1.25 (6H, br m), 0.98 (3H, d, *J* = 7.4 Hz), 0.90 (3H, t, *J* = 6.8 Hz); ^{13}C NMR δ 164.5 (C), 145.2, 121.4, 86.5, 75.7, 67.5, 39.9 (CH), 40.0, 32.0, 30.8, 30.3, 24.8, 22.6 (CH_2), 58.0, 14.0, 12.1 (CH_3); HR ESMS *m*/*z* 307.1882 (M + Na^+), calcd for $C_{16}H_{28}NaO_4$, 307.1885.

(6*S*)-6-[(2*R*,3*R*,4*R*)-2-Hydroxy-4-methoxy-3-methylnonyl]-5,6-dihydro-2*H*-pyran-2-one (4). Oil, $[\alpha]_D$ −75.1 (*c* 0.9; $CHCl_3$); IR ν_{max} (cm^{-1}): 3460 (br, OH), 1719 (C=O); ^1H NMR δ 6.88 (1H, ddd, *J* = 10, 5.8, 2.8 Hz), 6.00 (1H, dt, *J* = 10, 1 Hz), 4.76 (1H, m), 4.00 (1H, br s, OH), 3.94 (1H, td, *J* = 8, 2.5 Hz), 3.37 (3H, s), 3.29 (1H, m), 2.45–2.30 (2H, m), 1.96 (1H, br dd, *J* ∼ 14, 9 Hz), 1.82 (1H, m), 1.65–1.55 (2H, m), 1.50–1.40 (2H, m), 1.35–1.20 (5H, br m), 0.89 (3H, t, *J* = 6.8 Hz), 0.85 (3H, d, *J* = 7.3 Hz); ^{13}C NMR δ 164.5 (C), 145.2, 121.4, 85.6, 75.1, 70.0, 38.8 (CH), 41.4, 31.8, 30.3, 29.2, 26.1, 22.6 (CH_2), 57.2, 14.0, 12.6 (CH_3); HR ESMS *m*/*z* 285.2067 (M + H^+), calcd for $C_{16}H_{29}O_4$, 285.2066.

(5*R*,6*R*)-6-[(2*S*,4*R*)-2-Hydroxy-4-methoxynonyl]-5-methyl-5,6-dihydro-2*H*-pyran-2-one (5). A solution of ester *Z*-**57** (32 mg, 0.1 mmol) and *p*-toluenesulfonic acid (3 mg, *ca.* 0.02 mmol) in 3 mL of dry benzene was stirred at room temperature until consumption of the starting material (2–3 h, TLC monitoring). Solvent removal under reduced pressure was performed followed by column chromatography of the residue on silica gel (hexanes–EtOAc, 1 : 1) to yield **5** (13 mg, 91%): oil, $[\alpha]_D$ −32.4 (*c* 1.3; $CHCl_3$); IR ν_{max} (cm^{-1}): 3440 (br, OH), 1713 (C=O); ^1H NMR δ 6.96 (1H, dd, *J* = 9.8, 6.5 Hz), 5.96 (1H, br d, *J* ∼ 9.8 Hz), 4.78 (1H, dt, *J* = 7, 3 Hz), 4.25 (1H, br t, *J* ∼ 8.5 Hz), 3.49 (1H, m), 3.37 (3H, s), 2.39 (1H, m), 1.84 (1H, ddd, *J* = 14, 10, 2.7 Hz), 1.78 (1H, ddd, *J* = 14, 9.5, 3.5 Hz), 1.75–1.45 (5H, br m), 1.40–1.20 (6H, br m), 1.06 (3H, d, *J* = 6.8 Hz), 0.91 (3H, t, *J* = 6.8 Hz); ^{13}C NMR δ 164.7 (C), 151.8, 120.0, 79.9, 76.9, 64.8, 32.8 (CH), 39.7, 39.2, 32.7, 31.9, 25.3, 22.7 (CH_2), 56.6, 14.0, 11.5 (CH_3); HR ESMS *m*/*z* 307.1885 (M + Na^+), calcd for $C_{16}H_{28}NaO_4$, 307.1885.

(5*R*,6*R*)-5-Ethyl-6-[(2*S*,4*R*)-2-hydroxy-4-methoxynonyl]-5,6-dihydro-2*H*-pyran-2-one (6). Compound **6** was obtained from ester *Z*-**58** in 83% yield under the same conditions used to prepare **5**: oil, $[\alpha]_D$ −196 (*c* 0.6; $CHCl_3$); IR ν_{max} (cm^{-1}): 3450

(br, OH), 1717 (C=O); ^{1}H NMR δ 7.00 (1H, dd, J = 9.8, 5.8 Hz), 6.02 (1H, dd, J = 9.8, 1 Hz), 4.78 (1H, dt, J = 10, 3 Hz), 4.22 (1H, br t, $J \sim$ 4.5 Hz), 3.48 (1H, m), 3.36 (4H, s, overlapping a broad OH signal), 2.27 (1H, m), 1.86 (1H, ddd, J = 14, 10.3, 2.5 Hz), 1.76 (1H, ddd, J = 14, 8.8, 3.5 Hz), 1.70–1.45 (6H, br m), 1.40–1.20 (6H, br m), 0.97 (3H, t, J = 7.5 Hz), 0.90 (3H, t, J = 7 Hz); ^{13}C NMR δ 164.7 (C), 150.6, 120.8, 79.9, 77.2, 64.8, 39.0 (CH), 39.3, 39.2, 32.7, 31.9, 25.3, 22.6, 20.8 (CH_2), 56.6, 14.0, 11.0 (CH_3); HR ESMS m/z 321.2038 (M + Na^+), calcd for $C_{17}H_{30}NaO_4$, 321.2042.

(2*R*,3*S*)-1-(*tert*-Butyldiphenylsilyloxy)-2-methylhex-5-en-3-ol (9). Oil: $[\alpha]_D$ −9.1 (c 1.3; $CHCl_3$); IR ν_{max} 3450 (br, OH) (cm^{-1}); ^{1}H NMR δ 7.70 (4H, m), 7.45–7.40 (6H, br m), 5.94 (1H, ddt, J = 17, 10.3, 7), 5.15–5.10 (2H, m), 3.79 (1H, dd, J = 10.2, 4.5 Hz), 3.71 (1H, td, J = 7.5, 4 Hz), 3.66 (1H, dd, J = 10.2, 6.8 Hz), 3.40 (1H, br s, OH), 2.38 (1H, m), 2.25–2.20 (1H, m), 1.84 (1H, m), 1.08 (9H, s), 0.88 (3H, d, J = 7.3 Hz); ^{13}C NMR δ 133.0 (×2), 19.2 (C), 135.7 (×2), 135.6 (×2), 135.3, 129.8 (×2), 127.7 (×4), 75.1, 39.6 (CH), 117.2, 68.5, 39.4 (CH_2), 26.9 (×3), 13.4 (CH_3); HR ESMS m/z 391.2072 (M + Na^+), calcd for $C_{23}H_{32}NaO_2Si$, 391.2069.

***tert*-Butyl [(2*R*,3*S*)-3-methoxy-2-methylhex-5-enyloxy] diphenylsilane (10).** Oil, $[\alpha]_D$ +6 (c 1.25; $CHCl_3$); ^{1}H NMR δ 7.70 (4H, m), 7.45–7.40 (6H, br m), 5.87 (1H, ddt, J = 17, 10.3, 7), 5.15–5.05 (2H, m), 3.72 (1H, dd, J = 10, 6 Hz), 3.63 (1H, dd, J = 10, 5.5 Hz), 3.35 (1H, m), 3.33 (3H, s), 2.35–2.30 (1H, m), 2.20–2.15 (1H, m), 1.97 (1H, apparent heptuplet, $J \sim$ 6.2 Hz), 1.08 (9H, s), 0.94 (3H, d, J = 7 Hz); ^{13}C NMR δ 134.0, 133.9, 19.4 (C), 135.7 (×2), 135.6 (×2), 135.4, 129.6, 129.5, 127.6 (×4), 81.6, 38.2 (CH), 116.5, 65.7, 34.3 (CH_2), 57.3, 26.9 (×3), 12.7 (CH_3); HR ESMS m/z 405.2229 (M + Na^+), calcd for $C_{24}H_{34}NaO_2Si$, 405.2226.

(2*R*,3*S*)-3-Methoxy-2-methylhex-5-en-1-ol (11). Compound **10** (7.65 g, 20 mmol) was dissolved in THF (250 mL) and treated with TBAF trihydrate (7.57 g, 24 mmol). The mixture was stirred for 24 h at room temperature. Standard work-up (EtOAc) and column chromatography on silica gel (hexanes–EtOAc, 4 : 1) gave alcohol **11** (2.25 g, 78%): oil, $[\alpha]_D$ +51.9 (c 1.1; $CHCl_3$); IR ν_{max} (cm^{-1}): 3400 (br, OH); ^{1}H NMR (CD_3OD) δ 5.75 (1H, ddt, J = 17, 10.3, 7), 5.10–5.00 (2H, m), 3.55 (1H, dd, J = 10.7, 6.3 Hz), 3.43 (1H, dd, J = 10.7, 5.5 Hz), 3.31 (3H, s), 3.18 (1H, td, J = 6.5, 4.5 Hz), 2.40–2.25 (1H, m), 2.20–2.10 (1H, m), 1.81 (1H, m), 0.88 (3H, d, J = 7 Hz) (OH signal not detected); ^{13}C NMR (CD_3OD) δ 136.2, 83.8, 39.5 (CH), 117.2, 65.2, 35.5 (CH_2), 57.7, 13.3 (CH_3); HR ESMS m/z 167.1045 (M + Na^+), calcd for $C_8H_{16}NaO_2$, 167.1048.

(2*R*,3*S*)-3-Methoxy-2-methylhex-5-enyl *p*-toluenesulfonate (12). Oil, $[\alpha]_D$ +28.8 (c 1.3; $CHCl_3$); ^{1}H NMR δ 7.80 (2H, br d, $J \sim$ 8 Hz), 7.35 (2H, br d, $J \sim$ 8 Hz), 5.75 (1H, ddt, J = 17, 10.3, 7), 5.10–5.05 (2H, m), 4.05 (2H, m), 3.24 (3H, s), 3.10 (1H, m), 2.45 (3H, s), 2.32 (1H, m), 2.16 (1H, m), 1.94 (1H, m), 0.92 (3H, d, J = 7 Hz); ^{13}C NMR δ 144.6, 133.1 (C), 133.7, 129.7 (×2), 127.9 (×2), 80.7, 36.0 (CH), 117.4, 72.4, 34.2 (CH_2), 57.3, 21.5, 13.2 (CH_3); HR ESMS m/z 321.1140 (M + Na^+), calcd for $C_{15}H_{22}NaO_4S$, 321.1137.

(4*S*,5*R*)-4-Methoxy-5-methyldec-1-ene (13). Oil, $[\alpha]_D$ +8.2 (c 1.5; $CHCl_3$); ^{1}H NMR δ 5.86 (1H, ddt, J = 17, 10.3, 7), 5.07 (1H. br dd, J = 17, 1.5 Hz), 5.03 (1H, br dd, J = 10.3, 1 Hz), 3.34 (3H, s), 3.03 (1H, dt, J = 7.2, 4.8 Hz), 2.25–2.15 (2H, m), 1.70 (1H, m), 1.40–1.10 (8H, br m), 0.89 (3H, t, J = 7.2 Hz), 0.86 (3H, d, J = 6.8 Hz); ^{13}C NMR δ 136.0, 85.1, 35.2 (CH), 116.2, 34.4, 32.5, 32.2, 27.1, 22.7 (CH_2), 57.3, 14.8, 14.1 (CH_3); HR ESMS m/z 207.1733 (M + Na^+), calcd for $C_{12}H_{24}NaO$, 207.1725.

(4*R*,6*S*,7*R*)-6-Methoxy-7-methyldodec-1-en-4-ol (15). Oil, $[\alpha]_D$ −24 (c 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 3425 (br, OH); ^{1}H NMR δ 5.82 (1H, ddt, J = 17, 10.3, 7), 5.15–5.10 (2H, m), 3.90 (1H, m), 3.37 (3H, s), 3.35 (1H, m, overlapped), 2.50 (1H, br s, OH), 2.25 (2H, br t, $J \sim$ 6.5 Hz), 1.85 (1H, m), 1.58 (1H, ddd, J = 14.5, 8.8, 2.5 Hz), 1.50 (1H, ddd, J = 14.5, 8.8, 2.5 Hz), 1.40–1.10 (8H, br m), 0.89 (3H, t, J = 7.2 Hz), 0.83 (3H, d, J = 6.8 Hz); ^{13}C NMR δ 135.0, 82.3, 68.1, 34.2 (CH), 117.5, 42.3, 35.1, 33.1, 32.1, 27.1, 22.6 (CH_2), 57.0, 14.0, 13.9 (CH_3); HR ESMS m/z 251.1990 (M + Na^+), calcd for $C_{14}H_{28}NaO_2$, 251.1987.

***tert*-Butyl [(4*R*,6*S*,7*R*)-6-methoxy-7-methyldodec-1-en-4-yloxy] dimethylsilane (16).** Oil, $[\alpha]_D$ −61.8 (c 1; $CHCl_3$); ^{1}H NMR δ 5.82 (1H, ddt, J = 17, 10.3, 7), 5.05–5.00 (2H, m), 3.94 (1H, m), 3.30 (3H, s), 3.29 (1H, m, overlapped), 2.25 (2H, br t, $J \sim$ 6 Hz), 1.87 (1H, m), 1.45–1.10 (10H, br m), 0.91 (9H), 0.90 (3H, t, J = 6.8 Hz), 0.80 (3H, d, J = 6.8 Hz), 0.09 (6H, s); ^{13}C NMR δ 18.1 (C), 134.9, 80.5, 68.7, 33.2 (CH), 116.9, 43.1, 36.6, 33.3, 32.2, 27.4, 22.6 (CH_2), 56.1, 26.0 (×3), 14.1, 13.5, −4.0, −4.7 (CH_3); HR ESMS m/z 365.2862 (M + Na^+), calcd for $C_{20}H_{42}NaO_2Si$, 365.2852.

(4*S*,6*R*,8*S*,9*R*)-6-(*tert*-Butyldimethylsilyloxy)-8-methoxy-9-methyltetradec-1-en-4-ol (17). Oil, $[\alpha]_D$ −28.6 (c 1.2; $CHCl_3$); IR ν_{max} (cm^{-1}): 3450 (br, OH); ^{1}H NMR δ 5.83 (1H, ddt, J = 17, 10.3, 7), 5.10–5.05 (2H, m), 4.16 (1H, apparent sextuplet, $J \sim$ 4 Hz), 4.03 (1H, m), 3.27 (3H, s), 3.20 (1H, br dt, $J \sim$ 10, 2.5 Hz), 2.25–2.15 (2H, m), 1.88 (1H, m), 1.71 (1H, ddd, J = 14, 10.5, 4 Hz), 1.64 (1H, ddd, J = 14, 8.5, 1.5 Hz), 1.56 (1H, dt, J = 14, 2.7 Hz), 1.47 (1H, ddd, J = 14, 10.5, 3.7 Hz), 1.40–1.10 (9H, br m), 0.89 (12H, br s, overlapping a methyl triplet), 0.80 (3H, d, J = 6.8 Hz), 0.12 (3H, s), 0.09 (3H, s); ^{13}C NMR δ 17.9 (C), 135.0, 80.8, 69.7, 68.1, 33.0 (CH), 117.0, 42.4, 42.3, 35.9, 33.2, 32.1, 27.4, 22.6 (CH_2), 55.9, 25.9 (×3), 14.0, 13.2, −4.2, −4.9 (CH_3); HR ESMS m/z 409.3112 (M + Na^+), calcd for $C_{22}H_{46}NaO_3Si$, 409.3114.

(4*S*,6*R*,8*S*,9*R*)-6-(*tert*-Butyldimethylsilyloxy)-8-methoxy-9-methyltetradec-1-en-4-yl acrylate (18). Oil, $[\alpha]_D$ −7.6 (c 0.8; $CHCl_3$); IR ν_{max} (cm^{-1}): 1726 (C=O); ^{1}H NMR δ 6.38 (1H, dd, J = 17.5, 1.5 Hz), 6.10 (1H, dd, J = 17.5, 10.5 Hz), 5.80–5.70 (2H, m), 5.10–5.00 (3H, m), 3.87 (1H, apparent quintuplet, $J \sim$ 6 Hz), 3.30 (3H, s), 3.21 (1H, br dt, $J \sim$ 8, 4 Hz), 2.45–2.35 (2H, m), 1.90–1.80 (2H, m), 1.73 (1H, ddd, J = 14, 6.6, 4.5 Hz), 1.50–1.45 (2H, m), 1.40–1.10 (8H, br m), 0.89 (12H, br s, overlapping a methyl triplet), 0.83 (3H, d, J = 6.8 Hz), 0.06 (3H, s), 0.05 (3H, s); ^{13}C NMR δ 165.6, 18.0 (C), 133.4, 129.0, 81.1, 71.2, 67.5, 33.6 (CH), 130.2, 117.9, 42.2, 39.0, 37.9, 33.0, 32.2, 27.4, 22.7 (CH_2), 56.2, 26.0 (×3), 14.0, 13.7, −4.2, −4.3 (CH_3); HR ESMS m/z 463.3401 (M + H^+), calcd for $C_{25}H_{49}O_4Si$, 463.3400.

(6*S*)-6-[(2*R*,4*S*,5*R*)-2-(*tert*-Butyldimethylsilyloxy)-4-methoxy-5-methyldecyl]-5,6-dihydro-2*H*-pyran-2-one (19). Oil, $[\alpha]_D$ −40 (c 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1732 (C=O); ^{1}H NMR δ 6.86 (1H,

ddd, J = 10, 5, 3.5 Hz), 6.00 (1H, br d, $J \sim 10$ Hz), 4.56 (1H, m), 4.04 (1H, m), 3.27 (3H, s), 3.16 (1H, dt, J = 9, 3 Hz), 2.35–2.25 (2H, m), 2.06 (1H, ddd, J = 14, 9, 4 Hz), 1.82 (1H, m), 1.64 (1H, ddd, J = 14, 9, 4 Hz), 1.50 (1H, ddd, J = 14, 6, 2.5 Hz), 1.44 (1H, ddd, J = 14, 6, 4 Hz), 1.35–1.10 (8H, br m), 0.88 (3H, t, J = 6.8 Hz), 0.87 (9H, br s), 0.79 (3H, d, J = 6.8 Hz), 0.07 (3H, s), 0.05 (3H, s); ^{13}C NMR δ 164.3, 18.0 (C), 145.1, 121.4, 81.3, 74.6, 66.8, 33.4 (CH), 43.6, 38.2, 33.1, 32.1, 30.0, 27.3, 22.6 (CH_2), 56.1, 25.9 (×3), 14.1, 13.5, −4.4, −4.5 (CH_3); HR ESMS m/z 435.2907 (M + Na^+), calcd for $C_{23}H_{44}NaO_4Si$, 435.2907.

(4*R*)-4-Benzyl-3-[(2*R*,3*S*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-3-hydroxy-2-methyloct-7-enoyl]oxazolidin-2-one (22). A solution of oxazolidinone **20** (1.17 g, 5 mmol) in CH_2Cl_2 (15 mL) was treated under N_2 with triethyl amine (1.4 mL, 10 mmol). The mixture was cooled to 0 °C and treated with Bu_2BOTf (commercial 1 M solution in CH_2Cl_2, 7.5 mL, 7.5 mmol), followed by stirring at 0 °C for 1 h and then at −78 °C for 30 min. After this time, a solution of aldehyde **21** (3.42 g, 15 mmol) in CH_2Cl_2 (15 mL) was added. The stirring was maintained for 2 h under the same conditions. The reaction was quenched by addition of buffer pH 7 (30 mL) and MeOH (30 mL), followed by 30% H_2O_2 (15 mL). After allowing the mixture to reach room temperature, standard work-up (CH_2Cl_2) and column chromatography on silica gel (hexanes–EtOAc, 9 : 1, then 4 : 1) yielded compound **22** (1.91 g, 83%): oil, $[\alpha]_D$ −89.6 (c 1.1; $CHCl_3$); IR ν_{max} (cm^{-1}): 3530 (br, OH), 1781, 1702 (C=O); ^{1}H NMR δ 7.35–7.15 (5H, br m), 5.80 (1H, ddt, J = 17.3, 10.2, 7 Hz), 5.10–5.05 (2H, m), 4.70 (1H, m), 4.30–4.15 (3H, m), 4.05 (1H, m), 3.76 (1H, qd, J = 7, 3.8 Hz). 3.40 (1H, s, OH), 3.28 (1H, m), 2.78 (1H, dd, J = 13, 9.5 Hz), 2.35–2.30 (2H, m), 1.70–1.65 (1H, m), 1.55–1.50 (1H, m), 1.25 (3H, d, J = 7 Hz), 0.88 (9H, s), 0.10 (3H, s), 0.08 (3H, s); ^{13}C NMR δ 176.6, 153.1, 135.2, 18.0 (C), 134.5, 129.4 (×2), 128.9 (×2), 127.3, 70.0, 68.3, 55.2, 41.6 (CH), 117.4, 66.1, 43.0, 39.1, 37.7 (CH_2), 25.8 (×3), 11.2, −4.5, −4.8 (CH_3); HR ESMS m/z 484.2492 (M + Na^+). Calcd for $C_{25}H_{39}NNaO_5Si$, 484.2495.

(4*R*)-4-Benzyl-3-[(2*R*,3*S*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-3-methoxy-2-methyloct-7-enoyl]oxazolidin-2-one (23). Solid, mp 60–61 °C (slow evaporation from MeCN), $[\alpha]_D$ −22.9 (c 0.7; $CHCl_3$); IR ν_{max} (cm^{-1}): 1781, 1702 (C=O); ^{1}H NMR δ 7.30–7.15 (5H, br m), 5.77 (1H, ddt, J = 17.3, 10.2, 7 Hz), 5.05–5.00 (2H, m), 4.59 (1H, m), 4.20 (1H, qd, J = 6.8, 5 Hz), 4.09 (2H, m), 3.85 (1H, m), 3.52 (1H, dt, J = 7.7, 4.5 Hz). 3.36 (3H, s), 3.25 (1H, br dd, $J \sim 13$, 3 Hz), 2.74 (1H, dd, J = 13, 9.5 Hz), 2.22 (2H, br t, $J \sim 5.7$ Hz), 1.58 (2H, m), 1.15 (3H, d, J = 7 Hz), 0.86 (9H, s), 0.06 (3H, s), 0.05 (3H, s); ^{13}C NMR δ 175.0, 153.1, 135.3, 18.0 (C), 134.5, 129.4 (×2), 128.8 (×2), 127.2, 80.0, 68.7, 55.8, 39.5 (CH), 117.1, 65.9, 42.6, 39.0, 37.7 (CH_2), 57.7, 25.9 (×3), 12.9, −3.9, −4.8 (CH_3); HR ESMS m/z 476.2834 (M + H^+). Calcd for $C_{26}H_{42}NO_5Si$, 476.2832.

(2*S*,3*S*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-3-methoxy-2-methyloct-7-en-1-ol (24). Oil, $[\alpha]_D$ −39.5 (c 1.4; $CHCl_3$); IR ν_{max} (cm^{-1}): 3425 (br, OH); ^{1}H NMR δ 5.82 (1H, ddt, J = 17, 10, 7 Hz), 5.10–5.00 (2H, m), 3.92 (1H, m), 3.68 (1H, dd, J = 10.8, 8.5 Hz), 3.51 (1H, dd, J = 10.8, 5 Hz), 3.45 (1H, dt, J = 9.2, 3 Hz), 3.40 (3H, s), 2.30–2.20 (3H, m), 2.70 (1H, br s, OH), 1.62 (1H, ddd, J = 14, 9.2, 3.2 Hz), 1.51 (1H, ddd, J = 14, 8.8, 3 Hz), 0.90 (9H, s), 0.82 (3H, d, J = 7 Hz), 0.09 (3H, s), 0.08 (3H, s); ^{13}C NMR δ 18.1 (C), 134.5, 82.1, 68.8, 42.7 (CH), 117.2, 65.9, 36.8, 35.2 (CH_2), 57.1, 25.9 (×3), 12.6, −3.9, −4.6 (CH_3); HR ESMS m/z 303.2358 (M + H^+). Calcd for $C_{16}H_{35}O_3Si$, 303.2355.

(2*S*,3*S*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-3-methoxy-2-methyloct-7-enyl *p*-toluenesulfonate (25). Oil, $[\alpha]_D$ −59.9 (c 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1363, 1180 (SO_2); ^{1}H NMR δ 7.80 (2H, br d, $J \sim 8$ Hz), 7.32 (2H, br d, $J \sim 8$ Hz), 5.79 (1H, ddt, J = 17, 10, 7 Hz), 5.05–5.00 (2H, m), 4.10 (1H, dd, J = 9.3, 6 Hz), 3.84 (2H, m), 3.33 (1H, m), 3.22 (3H, s), 2.43 (3H, s), 2.20 (2H, m), 2.15–2.10 (1H, m), 1.45–1.35 (2H, m), 0.86 (12H, s, overlapping a methyl doublet), 0.06 (3H, s), 0.05 (3H, s); ^{13}C NMR δ 144.6, 133.3, 18.0 (C), 134.3 (×2), 129.8, 127.8 (×2), 78.1, 71.9, 37.7 (CH), 117.2, 68.8, 42.4, 35.1 (CH_2), 57.2, 25.9 (×3), 21.5, 11.8, −4.0, −4.7 (CH_3); HR ESMS m/z 479.2255 (M + H^+). Calcd for $C_{23}H_{40}O_5SSi$, 479.2263.

***tert*-Butyl [(4*R*,6*S*,7*S*)-6-methoxy-7-methyldodec-1-en-4-yloxy] dimethylsilane (26).** Oil, $[\alpha]_D$ −61.5 (c 1.1; $CHCl_3$); ^{1}H NMR δ 5.83 (1H, ddt, J = 17, 10, 7 Hz), 5.10–5.00 (2H, m), 3.91 (1H, m), 3.32 (3H, s), 3.24 (1H, dt, J = 8.2, 3.6 Hz), 2.25–2.20 (2H, m), 1.77 (1H, m), 1.50–1.20 (10H, br m), 0.91 (12H, s, overlapping a methyl triplet), 0.83 (3H, d, J = 7 Hz), 0.08 (6H, s); ^{13}C NMR δ 18.1 (C), 134.8, 81.6, 69.0, 37.6 (CH), 116.9, 42.9, 34.2, 32.2, 30.9, 27.4, 22.7 (CH_2), 56.7, 25.9 (×3), 15.5, 14.1, −4.0, −4.6 (CH_3); HR ESMS m/z 343.3025 (M + H^+). Calcd for $C_{20}H_{43}O_2Si$, 343.3032.

(4*S*,6*R*,8*S*,9*S*)-6-(*tert*-Butyldimethylsilyloxy)-8-methoxy-9-methyltetradec-1-en-4-ol (27). Oil, $[\alpha]_D$ −36.5 (c 0.95; $CHCl_3$); IR ν_{max} (cm^{-1}): 3450 (br, OH); ^{1}H NMR δ 5.84 (1H, ddt, J = 17, 10, 7 Hz), 5.15–5.05 (2H, m), 4.15 (1H, m), 4.05 (1H, m), 3.50 (1H, br s, OH), 3.31 (3H, s), 3.14 (1H, dt, J = 9, 3.3 Hz), 2.30–2.15 (2H, m), 1.80–1.20 (13H, br m), 0.91 (12H, s, overlapping a methyl triplet), 0.85 (3H, d, J = 7 Hz), 0.13 (3H, s), 0.10 (3H, s); ^{13}C NMR δ 17.9 (C), 135.1, 81.9, 69.8, 68.1, 33.8 (CH), 117.2, 42.4, 42.1, 36.7, 32.2, 30.7, 27.4, 22.7 (CH_2), 56.5, 25.9 (×3), 15.5, 14.1, −4.3, −4.8 (CH_3); HR ESMS m/z 387.3298 (M + H^+), calcd for $C_{22}H_{47}O_3Si$, 387.3294.

(4*S*,6*R*,8*S*,9*S*)-6-(*tert*-Butyldimethylsilyloxy)-8-methoxy-9-methyltetradec-1-en-4-yl acrylate (28). Oil, $[\alpha]_D$ −5.7 (c 0.9; $CHCl_3$); IR ν_{max} (cm^{-1}): 1727 (C=O); ^{1}H NMR δ 6.36 (1H, dd, J = 17.3, 1.5 Hz), 6.10 (1H, dd, J = 17.3, 10.4 Hz), 5.85–5.70 (2H, m), 5.10–5.05 (3H, m), 3.83 (1H, apparent quintuplet, $J \sim 6$ Hz), 3.30 (3H, s), 3.15 (1H, br td, $J \sim 6.2$, 3.5 Hz), 2.45–2.30 (2H, m), 1.83 (1H, ddd, J = 14, 8.4, 5 Hz), 1.75–1.65 (2H, m), 1.52 (2H, t, J = 6 Hz), 1.45–1.10 (8H, br m), 0.89 (12H, br s, overlapping a 3H triplet), 0.83 (3H, d, J = 7 Hz), 0.05 (3H, s), 0.03 (3H, s); ^{13}C NMR δ 165.5, 18.0 (C), 133.4, 129.0, 81.9, 71.2, 67.5, 34.4 (CH), 130.2, 117.9, 42.1, 39.0, 38.8, 32.2, 31.2, 27.4, 22.6 (CH_2), 56.8, 25.9 (×3), 15.3, 14.1, −4.3, −4.4 (CH_3); HR ESMS m/z 463.3221 (M + Na^+), calcd for $C_{25}H_{48}NaO_4Si$, 463.3220.

(6*S*)-6-[(2*R*,4*S*,5*S*)-2-(*tert*-Butyldimethylsilyloxy)-4-methoxy-5-methyldecyl]-5,6-dihydro-2*H*-pyran-2-one (29). Oil, $[\alpha]_D$ −51.8 (c 1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1732 (C=O); ^{1}H NMR δ 6.88 (1H, ddd, J = 10, 4.5, 3.5 Hz), 6.03 (1H, dt, J = 10, 3.5 Hz), 4.60 (1H, m), 4.05 (1H, m), 3.32 (3H, s), 3.14 (1H, dt, J = 8, 3.5 Hz),

2.35–2.30 (2H, m), 2.05 (1H, ddd, J = 14, 9, 4 Hz), 1.80–1.40 (6H, br m), 1.35–1.20 (6H, br m), 0.89 (12H, br s, overlapping a 3H triplet), 0.85 (3H, d, J = 7 Hz), 0.10 (3H, s), 0.08 (3H, s); ^{13}C NMR δ 164.3, 18.0 (C), 145.2, 121.5, 82.3, 74.6, 66.8, 34.3 (CH), 43.5, 39.1, 32.2, 31.0, 30.0, 27.4, 22.6 (CH_2), 56.8, 25.9 (×3), 15.4, 14.1, −4.3, −4.4 (CH_3); HR ESMS m/z 435.2902 (M + Na^+), calcd for $C_{23}H_{44}NaO_4Si$, 435.2907.

(2*R*,3*R*)-1-[(4*S*)-4-Isopropyl-2-thioxothiazolidin-3-yl]-3-methoxy-2-methyloctan-1-one (31). A solution of *N*-propionyl thiazolidinethione **30** (4.35 g, 20 mmol) in dry CH_2Cl_2 (175 mL) was cooled under N_2 to 0 °C and treated dropwise with $TiCl_4$ (2.4 mL, 22 mmol). The mixture was then cooled to −78 °C followed by dropwise addition of DIPEA (3.83 mL, 22 mmol). The temperature of the mixture was then allowed to reach −40 °C, followed by stirring for 2 h. Recooling to −78 °C was followed by dropwise sequential addition of a solution of hexanal dimethyl acetal (1.73 mL, 10 mmol) in dry CH_2Cl_2 (5 mL) and then $SnCl_4$ (1.17 mL, 10 mmol). Stirring was continued for 15 min at −78 °C and then for 4 h at −20 °C. Standard work-up (CH_2Cl_2) and column chromatography on silica gel (hexanes–EtOAc, 4 : 1) afforded compound **31** (2.1 g, 63%) obtained as a 90 : 10 mixture of diastereoisomers. For analytical purposes, a small sample of pure **31** could be prepared *via* a careful column chromatography: oil, $[\alpha]_D$ +174.2 (c 1.5; $CHCl_3$); IR ν_{max} (cm^{-1}): 1697 (C═O); ^{1}H NMR δ 5.28 (1H, m), 5.02 (1H, apparent quintuplet, $J \sim 7$ Hz), 3.63 (1H, td, J = 8.2, 3.5 Hz), 3.44 (1H, dd, J = 11.5, 8.5 Hz), 3.30 (3H, s), 2.98 (1H, dd, J = 11.5, 1.7 Hz), 2.30 (1H, m), 1.60–1.20 (8H, br m), 1.08 (3H, d, J = 6.8 Hz), 1.06 (3H, d, J = 7 Hz), 0.98 (3H, d, J = 7 Hz), 0.89 (3H, t, J = 7 Hz); ^{13}C NMR δ 202.5, 177.1 (C), 82.5, 71.8, 42.0, 30.6 (CH), 32.1, 29.8, 29.1, 24.3, 22.6 (CH_2), 57.5, 19.1, 17.1, 14.1, 13.2 (CH_3); HR ESMS m/z 354.1541 (M + Na^+), calcd for $C_{16}H_{29}NNaO_2S_2$, 354.1537.

(4*R*,5*S*,6*R*)-6-Methoxy-5-methylundec-1-en-4-ol (33). Prepared from **31** (90 : 10 mixture of diastereoisomers) *via* **32** and obtained also as a 90 : 10 diastereoisomeric mixture, which was used as such in the next step: oil; IR ν_{max} (cm^{-1}) 3470 (br, OH); ^{1}H NMR (signals from the major diastereoisomer) δ 5.80 (1H, ddt, J = 17, 10, 7 Hz), 5.10–5.00 (2H, m), 3.92 (1H, td, J = 6.8, 1.5 Hz), 3.38 (3H, s), 3.18 (1H, br q, $J \sim 5.5$ Hz), 3.15 (1H, br s, OH), 2.25 (1H, m), 2.12 (1H, m), 1.70–1.45 (4H, br m), 1.35–1.20 (6H, br m), 0.94 (3H, d, J = 7 Hz), 0.88 (3H, t, J = 6.8 Hz); ^{13}C NMR (signals from the major diastereoisomer) δ 135.5, 85.6, 70.5, 39.0 (CH), 116.5, 38.7, 32.0, 30.6, 24.6, 22.5 (CH_2), 57.8, 13.9, 10.8 (CH_3); HR ESMS m/z 237.1835 (M + Na^+), calcd for $C_{13}H_{26}NaO_2$, 237.1831.

***tert*-Butyl [(4*R*,5*R*,6*R*)-6-methoxy-5-methylundec-1-en-4-yloxy] dimethylsilane (34).** Oil, $[\alpha]_D$ −25.4 (c 0.96; $CHCl_3$); ^{1}H NMR δ 5.80 (1H, ddt, J = 17, 10, 7 Hz), 5.10–5.00 (2H, m), 3.97 (1H, td, J = 6.5, 3.3 Hz), 3.28 (3H, s), 3.16 (1H, m), 2.30–2.20 (2H, m), 1.80–1.70 (1H, m), 1.60–1.20 (8H, br m), 0.90 (12H, br s, overlapping a 3H triplet), 0.80 (3H, d, J = 7 Hz), 0.07 (3H, s), 0.06 (3H, s); ^{13}C NMR δ 18.2 (C), 135.1, 81.0, 71.3, 40.3 (CH), 116.7, 38.9, 32.3, 29.3, 23.9, 22.7 (CH_2), 56.0, 26.0 (×3), 14.1, 9.0, −3.8, −4.7 (CH_3); HR ESMS m/z 329.2876 (M + H^+), calcd for $C_{19}H_{41}O_2Si$, 329.2876.

(4*S*,6*R*,7*R*,8*R*)-6-(*tert*-Butyldimethylsilyloxy)-8-methoxy-7-methyltridec-1-en-4-ol (35). Oil, $[\alpha]_D$ +2.8 (c 1.5; $CHCl_3$); IR ν_{max} (cm^{-1}): 3450 (br, OH); ^{1}H NMR δ 5.83 (1H, ddt, J = 17, 10.2, 7 Hz), 5.20–5.10 (2H, m), 4.00–3.90 (2H, m), 3.31 (3H, s), 3.08 (1H, m), 3.00 (1H, br s, OH), 2.30–2.20 (2H, m), 2.00 (1H, apparent sextuplet, $J \sim 6.5$ Hz), 1.62 (2H, m), 1.45–1.20 (8H, br m), 0.90 (15H, br s, overlapping a methyl triplet and a methyl doublet), 0.12 (3H, s), 0.09 (3H, s); ^{13}C NMR δ 18.1 (C), 134.8, 81.2, 72.1, 68.2, 42.4 (CH), 117.7, 40.7, 39.4, 32.1, 29.2, 24.6, 22.7 (CH_2), 56.4, 26.0 (×3), 14.1, 10.4, −4.1, −4.6 (CH_3); HR ESMS m/z 395.2950 (M + Na^+), calcd for $C_{21}H_{44}NaO_3Si$, 395.2957.

(4*S*,6*R*,7*R*,8*R*)-6-(*tert*-Butyldimethylsilyloxy)-8-methoxy-7-methyltridec-1-en-4-yl acrylate (36). Oil, $[\alpha]_D$ −7.8 (c 0.2; $CHCl_3$); IR ν_{max} (cm^{-1}): 1727 (C═O); ^{1}H NMR δ 6.38 (1H, dd, J = 17, 1.5 Hz), 6.10 (1H, dd, J = 17, 10.4 Hz), 5.80–5.70 (2H, m), 5.15–5.05 (2H, m), 4.98 (1H, m), 4.06 (1H, br t, $J \sim 6.5$ Hz), 3.26 (3H, s), 3.14 (1H, m), 2.40–2.30 (2H, m), 1.90–1.55 (4H, br m), 1.40–1.20 (7H, br m), 0.88 (12H, br s, overlapping a methyl triplet), 0.80 (3H, d, J = 6.8 Hz), 0.05 (3H, s), 0.04 (3H, s); ^{13}C NMR δ 165.7, 18.2 (C), 133.4, 128.9, 80.7, 71.5, 68.6, 40.5 (CH), 130.3, 118.0, 39.3, 38.6, 32.3, 29.3, 23.4, 22.7 (CH_2), 55.8, 26.0 (×3), 14.1, 9.0, −4.1, −4.5 (CH_3); HR ESMS m/z 449.3067 (M + Na^+), calcd for $C_{24}H_{46}NaO_4Si$, 449.3063.

(6*S*)-6-[(2*R*,3*R*,4*R*)-2-(*tert*-Butyldimethylsilyloxy)-4-methoxy-3-methylnonyl]-5,6-dihydro-2*H*-pyran-2-one (37). Oil, $[\alpha]_D$ −28.3 (c 0.7; $CHCl_3$); IR ν_{max} (cm^{-1}): 1732 (C═O); ^{1}H NMR δ 6.88 (1H, m), 6.02 (1H, br d, $J \sim 9.8$ Hz), 4.49 (1H, m), 4.22 (1H, m), 3.30 (3H, s), 3.17 (1H, m), 2.40–2.30 (2H, m), 1.97 (1H, ddd, J = 14, 8, 5.8 Hz), 1.73 (1H, ddd, J = 14, 7.5, 5 Hz), 1.65–1.50 (2H, br m), 1.50–1.20 (7H, br m), 0.89 (12H, br s, overlapping a methyl triplet), 0.82 (3H, d, J = 7.3 Hz), 0.09 (3H, s), 0.07 (3H, s); ^{13}C NMR δ 164.2, 18.2 (C), 145.0, 121.5, 80.7, 74.9, 67.8, 41.0 (CH), 40.9, 32.2, 29.8, 29.5, 23.6, 22.7 (CH_2), 56.1, 26.0 (×3), 14.1, 9.7, −4.1, −4.3 (CH_3); HR ESMS m/z 421.2753 (M + Na^+), calcd for $C_{22}H_{42}NaO_4Si$, 421.2750.

(4*S*)-4-Benzyl-3-[(2*S*,3*R*)-3-methoxy-2-methyloctanoyl] oxazolidin-2-one (39). Oil, $[\alpha]_D$ +63.4 (c 0.56; $CHCl_3$); IR ν_{max} (cm^{-1}): 1782, 1698 (C═O); ^{1}H NMR δ 7.35–7.20 (5H, br m), 4.62 (1H, m), 4.20–4.10 (2H, m), 4.02 (1H, apparent quintuplet, $J \sim 6.5$ Hz), 3.40 (1H, br q, $J \sim 6$ Hz), 3.35 (3H, s), 3.27 (1H, br dd, $J \sim$ 13.2, 3 Hz), 2.76 (1H, dd, J = 13.2, 9.8 Hz), 1.50–1.40 (3H, br m), 1.35–1.25 (6H, br m), 1.23 (3H, d, J = 7 Hz), 0.87 (3H, t, J = 6.8 Hz); ^{13}C NMR δ 175.2, 152.9, 135.2 (C), 129.2 (×2), 128.7 (×2), 127.1, 82.4, 55.5, 40.9 (CH), 65.8, 37.5, 31.7, 31.6, 25.2, 22.4 (CH_2), 58.0, 13.8, 12.4 (CH_3); HR ESMS m/z 348.2173 (M + H^+). Calcd for $C_{20}H_{30}NO_4$, 348.2175.

(2*R*,3*R*)-3-Methoxy-2-methyloctan-1-ol (40). Oil: $[\alpha]_D$ −6.6 (c 1.19; $CHCl_3$); IR ν_{max} (cm^{-1}): 3410 (br, OH); ^{1}H NMR δ 3.66 (1H, dd, J = 10.6, 7.5 Hz), 3.55 (1H, dd, J = 10.7, 4.9 Hz), 3.37 (3H, s), 3.24 (1H, m), 2.60 (1H, br s, OH), 2.00 (1H, m), 1.54 (1H, m), 1.40 (2H, m), 1.35–1.25 (5H, br m), 0.89 (3H, t, J = 7 Hz), 0.86 (3H, d, J = 7 Hz); ^{13}C NMR δ 85.0. 36.6 (CH), 66.3, 32.0, 29.7, 25.9, 22.6 (CH_2), 57.6, 14.0, 11.5 (CH_3); HR ESMS m/z 197.1519 (M + Na^+), calcd for $C_{10}H_{22}NaO_2$, 197.1517.

(4*R*,5*R*,6*R*)-6-Methoxy-5-methylundec-1-en-4-ol (41). A solution of DMSO (4.26 mL, 60 mmol) in dry CH_2Cl_2 (60 mL) was cooled under N_2 to −78 °C and treated with oxalyl chloride (2.54 mL, 30 mmol). After stirring at the same temperature for 5 min, a solution of alcohol **40** (24 mmol) in CH_2Cl_2 (20 mL) was added dropwise. The mixture was then stirred at −78 °C for a further 15 min. After addition of triethyl amine (16.8 mL, 120 mmol), the mixture was stirred for 5 min at −78 °C and then for 20 min at 0 °C. Standard work-up (CH_2Cl_2) afforded an aldehyde which was used in crude form in the subsequent allylation step (see the conditions above) to yield **41**: oil, $[\alpha]_D$ −2.6 (*c* 1.61; $CHCl_3$); IR ν_{max} (cm^{-1}): 3470 (br, OH); ^1H NMR δ 5.86 (1H, ddt, *J* = 17, 10.3, 7 Hz), 5.10–5.00 (2H, m), 3.70 (1H, br s, OH), 3.62 (1H, td, *J* = 7.8, 4.5 Hz), 3.33 (3H, s), 3.30 (1H, m), 2.30 (1H, m), 2.12 (1H, m), 1.78 (1H, qd, *J* = 7.3, 3 Hz), 1.55 (1H, m), 1.40–1.30 (2H, m), 1.30–1.20 (5H, br m), 0.85 (3H, t, *J* = 6.8 Hz), 0.80 (3H, d, *J* = 7.3 Hz); ^{13}C NMR δ 135.1, 84.4, 73.2, 38.0 (CH), 116.8, 39.8, 31.8, 29.5, 25.9, 22.5 (CH_2), 57.2, 13.9, 11.9 (CH_3); HR ESMS *m*/*z* 215.2013 (M + H^+), calcd for $C_{13}H_{27}O_2$, 215.2011.

***tert*-Butyl [(4*R*,5*S*,6*R*)-6-methoxy-5-methylundec-1-en-4-yloxy] dimethylsilane (42).** Oil, $[\alpha]_D$ −26.9 (*c* 0.93; $CHCl_3$); ^1H NMR δ 5.88 (1H, ddt, *J* = 17, 10.3, 7 Hz), 5.05–5.00 (2H, m), 3.75 (1H, br q, *J* ∼ 5.5 Hz), 3.33 (3H, s), 3.28 (1H, m), 2.30–2.20 (2H, m), 1.65 (1H, m), 1.55 (1H, m), 1.43 (1H, m), 1.35–1.20 (6H, br m), 0.91 (12H, br s, overlapping a 3H triplet), 0.85 (3H, d, *J* = 6.8 Hz), 0.07 (3H, s), 0.06 (3H, s); ^{13}C NMR δ 18.2 (C), 135.4, 81.1, 73.2, 41.6 (CH), 116.5, 38.0, 32.1, 30.8, 25.4, 22.7 (CH_2), 57.2, 26.0 (×3), 14.1, 9.0, −4.0, −4.6 (CH_3); HR ESMS *m*/*z* 329.2872 (M + H^+), calcd for $C_{19}H_{41}O_2Si$, 329.2876.

(4*S*,6*R*,7*S*,8*R*)-6-(*tert*-Butyldimethylsilyloxy)-8-methoxy-7-methyltridec-1-en-4-ol (43). Major stereoisomer formed in the ozonolysis/allylation of **42** (for configurational assignment, see ESI†): oil, $[\alpha]_D$ −1.6 (*c* 0.36; $CHCl_3$); IR ν_{max} (cm^{-1}): 3480 (br, OH); ^1H NMR δ 5.83 (1H, ddt, *J* = 17, 10.2, 7 Hz), 5.15–5.05 (2H, m), 4.03 (1H, td, *J* = 6.6, 3 Hz), 3.95 (1H, m), 3.31 (3H, s), 3.22 (1H, dt, *J* = 7.5, 4.5 Hz), 2.90 (1H, br s, OH), 2.22 (2H, m), 1.84 (1H, m), 1.70–1.40 (4H, br m), 1.35–1.20 (6H, br m), 0.91 (12H, br s, overlapping a 3H triplet), 0.85 (3H, d, *J* = 6.8 Hz), 0.13 (3H, s), 0.10 (3H, s); ^{13}C NMR δ 18.0 (C), 135.0, 81.0, 72.0, 67.9, 42.6 (CH), 117.4, 41.3, 38.2, 32.1, 30.2, 25.2, 22.7 (CH_2), 56.7, 25.9 (×3), 14.0, 9.0, −4.2, −4.8 (CH_3); HR ESMS *m*/*z* 373.3139 (M + H^+), calcd for $C_{21}H_{45}O_3Si$, 373.3138.

(4*S*,6*R*,7*S*,8*R*)-6-(*tert*-Butyldimethylsilyloxy)-8-methoxy-7-methyltridec-1-en-4-yl acrylate (44). Oil, $[\alpha]_D$ +32 (*c* 0.61; $CHCl_3$); IR ν_{max} (cm^{-1}): 1726 (C═O); ^1H NMR δ 6.36 (1H, br d, *J* ∼ 17 Hz), 6.09 (1H, dd, *J* = 17, 10.3 Hz), 5.80–5.70 (2H, m), 5.10–5.00 (3H, m), 3.76 (1H, m), 3.32 (3H, s), 2.92 (1H, br q, *J* ∼ 5.5 Hz), 2.40 (2H, br t, *J* ∼ 6.3 Hz), 1.78 (1H, m), 1.62 (2H, m), 1.51 (1H, m), 1.43 (1H, m), 1.35–1.20 (6H, br m), 0.92 (3H, d, *J* = 6.8 Hz), 0.88 (12H, br s, overlapping a 3H triplet), 0.01 (3H, s), 0.00 (3H, s); ^{13}C NMR δ 165.6, 18.0 (C), 133.4, 129.0, 83.1, 71.6, 70.2, 42.5 (CH), 130.1, 117.8, 39.3, 36.3, 32.2, 30.5, 24.3, 22.7 (CH_2), 57.4, 25.9 (×3), 14.0, 8.7, −4.3, −4.9 (CH_3); HR ESMS *m*/*z* 427.3248 (M + H^+), calcd for $C_{24}H_{47}O_4Si$, 427.3244.

(6*S*)-6-[(2*R*,3*S*,4*R*)-2-(*tert*-Butyldimethylsilyloxy)-4-methoxy-3-methylnonyl]-5,6-dihydro-2*H*-pyran-2-one (45). Oil, $[\alpha]_D$ −4.2 (*c* 0.45; $CHCl_3$); IR ν_{max} (cm^{-1}): 1730 (C═O); ^1H NMR δ 6.87 (1H, dt, *J* = 9.7, 4.5 Hz), 6.00 (1H, d, *J* = 9.7 Hz), 4.56 (1H, m), 4.06 (1H, br d, *J* ∼ 9 Hz), 3.28 (3H, s), 2.96 (1H, br q, *J* ∼ 5.5 Hz), 2.30 (2H, m), 1.80 (2H, m), 1.60–1.40 (3H, br m), 1.35–1.20 (6H, br m), 0.86 (3H, d, *J* = 6.8 Hz), 0.84 (12H, br s, overlapping a 3H triplet), 0.05 (3H, s), 0.03 (3H, s); ^{13}C NMR δ 164.3, 18.0 (C), 145.3, 121.4, 82.7, 74.7, 69.9, 42.3 (CH), 37.5, 31.9, 30.7, 30.2, 24.9, 22.6 (CH_2), 57.1, 25.8 (×3), 14.0, 7.4, −4.5, −4.8 (CH_3); HR ESMS *m*/*z* 399.2930 (M + H^+), calcd for $C_{22}H_{43}O_4Si$, 399.2931.

(2*S*,3*R*,5*R*,7*R*)-1-[(4*S*)-4-Benzyl-2-thioxothiazolidin-3-yl]-5-(*tert*-butyldimethylsilyloxy)-3-hydroxy-7-methoxy-2-methyldodecan-1-one (49). A solution of thiazolidinethione **46** (1.6 g, 6 mmol) in dry CH_2Cl_2 (60 mL) was cooled under N_2 to −78 °C and treated dropwise with $TiCl_4$ (690 μL, 6.3 mmol). After stirring for 15 min, the mixture was treated dropwise with DIPEA (1.15 mL, 6.6 mmol). The stirring was then kept for a further 45 min. Addition of *N*-methylpyrrolidone (1.16 mL, 12 mmol) was followed by stirring for 15 min, addition of a solution of aldehyde **48** (2.09 g, 6.6 mmol) in dry CH_2Cl_2 (25 mL) and further stirring for 1 h at −30 °C. Standard work-up (CH_2Cl_2) and column chromatography on silica gel (hexanes–EtOAc, 9 : 1) afforded aldol adduct **49** (2.41 g, 69%) as an 88 : 12 mixture of diastereoisomers. These could then be separated with a second careful chromatography on silica gel (hexanes–EtOAc, 19 : 1). Data are given for the major diastereoisomer: oil, $[\alpha]_D$ +88.6 (*c* 1.3; $CHCl_3$); IR ν_{max} (cm^{-1}): 3470 (br, OH), 1697 (C═O); ^1H NMR δ 7.30–7.20 (5H, br m), 5.24 (1H, m), 4.53 (1H, m), 4.30 (1H, m), 4.15 (1H, m), 3.65 (1H, s, OH), 3.35 (1H, dd, *J* = 11.2, 6.8 Hz), 3.29 (3H, s), 3.30–3.20 (2H, m), 3.03 (1H, dd, *J* = 13.2, 10.7 Hz), 2.85 (1H, d, *J* = 11.7 Hz), 1.80 (1H, m), 1.70 (2H, m), 1.55–1.40 (3H, br m), 1.35–1.20 (6H, br m), 1.23 (3H, d, *J* = 6.3 Hz), 0.88 (12H, br s, overlapping a 3H triplet), 0.11 (3H, s), 0.08 (3H, s); ^{13}C NMR δ 201.2, 177.1, 136.6, 17.9 (C), 129.4 (×2), 128.9 (×2), 127.1, 77.2, 69.9, 69.3, 69.0, 44.4 (CH), 40.8, 39.7, 36.7, 32.7, 32.1, 32.0, 24.2, 22.6 (CH_2), 55.6, 25.8 (×3), 14.0, 11.1, −4.4, −4.9 (CH_3); HR ESMS *m*/*z* 604.2926 (M + Na^+), calcd for $C_{30}H_{51}NNaO_4S_2Si$, 604.2926.

(2*S*,3*R*,5*S*,7*R*)-1-[(4*S*)-4-Benzyl-2-thioxothiazolidin-3-yl]-3,5-bis(*tert*-butyldimethylsilyloxy)-7-methoxy-2-methyldodecan-1-one (50). Oil, $[\alpha]_D$ +99.1 (*c* 1.15; $CHCl_3$); IR ν_{max} (cm^{-1}): 1702 (C═O); ^1H NMR δ 7.30–7.20 (5H, br m), 5.20 (1H, m), 4.55 (1H, m), 4.02 (1H, apparent q, *J* ∼ 5.5 Hz), 3.83 (1H, apparent quintuplet, *J* ∼ 6.2 Hz), 3.27 (3H, overlapped m), 3.25 (3H, s), 3.00 (1H, dd, *J* = 12.7, 10.7 Hz), 2.83 (1H, d, *J* = 11.3 Hz), 1.80 (1H, m), 1.70–1.65 (2H, m), 1.50–1.40 (3H, m), 1.35–1.20 (6H, br m), 1.20 (3H, d, *J* = 6.4 Hz), 0.88 (12H, br s, overlapping a 3H triplet), 0.86 (9H, s), 0.08 (3H, s), 0.07 (6H, s), 0.03 (3H, s); ^{13}C NMR δ 200.8, 176.5, 136.8, 18.1, 18.0 (C), 129.5 (×2), 128.9 (×2), 127.2, 77.5, 72.1, 69.5, 67.4, 45.1 (CH), 44.7, 43.0, 36.5, 33.2, 32.2, 32.0, 24.5, 22.7 (CH_2), 56.0, 26.0 (×3), 25.9 (×3), 14.1, 13.1, −3.5, −3.6, −4.1, −4.4 (CH_3); HR ESMS *m*/*z* 718.3798 (M + Na^+), calcd for $C_{36}H_{65}NNaO_4S_2Si_2$, 718.3791.

(2*S*,3*R*,5*R*,7*R*)-1-[(4*S*)-4-Benzyl-2-thioxothiazolidin-3-yl]-5-(*tert*-butyldimethylsilyloxy)-2-ethyl-3-hydroxy-7-methoxydodecan-1-one (51). Obtained in 65% yield as a single diastereoisomer through reaction of thiazolidinethione **47** with aldehyde **48** under the same conditions as for the preparation of **49**: Oil, $[\alpha]_D$ +77.5 (*c* 1.15; $CHCl_3$); IR ν_{max} (cm^{-1}): 1697 (C═O); ^1H NMR δ 7.35–7.25 (5H, br m), 5.32 (1H, m), 4.89 (1H, dt, *J* = 8.8, 5 Hz), 4.31 (1H, br dd, *J* ~ 9.3, 5 Hz), 4.20 (1H, apparent sextuplet, *J* ~ 4 Hz), 3.70 (1H, br s, OH), 3.34 (1H, br dd, *J* ~ 11.3, 7 Hz), 3.31 (3H, s), 3.30–3.25 (2H, m), 3.08 (1H, dd, *J* = 13, 10.6 Hz), 2.85 (1H, d, *J* = 11.2 Hz), 2.00–1.85 (2H, m), 1.75–1.60 (3H, br m), 1.60–1.40 (3H, br m), 1.40–1.25 (6H, br m), 1.00 (3H, t, *J* = 7.5 Hz), 0.88 (12H, s, overlapping one methyl triplet), 0.12 (3H, s), 0.10 (3H, s); ^{13}C NMR δ 201.7, 176.1, 136.7, 17.9 (C), 129.5 (×2), 128.9 (×2), 127.2, 77.2, 70.0, 69.4, 69.3, 50.3 (CH), 40.7, 39.1, 37.0, 32.7, 32.1, 32.0, 24.3, 22.7, 20.4 (CH_2), 55.6, 25.9 (×3), 14.1, 11.8, −4.4, −4.8 (CH_3); HR ESMS *m*/*z* 618.3087 (M + Na^+), calcd for $C_{31}H_{53}NNaO_4S_2Si$, 618.3083.

(2*S*,3*R*,5*S*,7*R*)-1-[(4*S*)-4-Benzyl-2-thioxothiazolidin-3-yl]-3,5-bis(*tert*-butyldimethylsilyloxy)-2-ethyl-7-methoxydodecan-1-one (52). Oil, $[\alpha]_D$ +47.3 (*c* 1.5; $CHCl_3$); IR ν_{max} (cm^{-1}): 1702 (C═O); ^1H NMR δ 7.35–7.25 (5H, br m), 5.25 (1H, m), 4.70 (1H, td, *J* = 6.5, 4.4 Hz), 4.07 (1H, dt, *J* = 7.5, 4 Hz), 3.87 (1H, apparent quintuplet, *J* ~ 6.2 Hz), 3.31 (3H, s), 3.29 (3H, overlapped m), 3.06 (1H, dd, *J* = 13, 10.7 Hz), 2.85 (1H, d, *J* = 11.3 Hz), 1.94 (1H, apparent heptuplet, *J* ~ 6 Hz), 1.85 (1H, ddd, *J* = 14, 7.3, 4 Hz), 1.80–1.65 (2H, br m), 1.65–1.45 (4H, br m), 1.40–1.25 (6H, br m), 1.00 (3H, t, *J* = 7.5 Hz), 0.90, 0.88 (21H, 2 × s, overlapping one methyl triplet), 0.11 (6H, s), 0.10 (3H, s), 0.07 (3H, s); ^{13}C NMR δ 201.1, 175.8, 136.8, 18.1, 18.0 (C), 129.5 (×2), 128.9 (×2), 127.2, 77.7, 70.9, 69.5, 67.6, 51.1 (CH), 43.8, 43.0, 36.5, 33.3, 32.1, 31.8, 24.5, 22.7, 21.8 (CH_2), 56.1, 26.0 (×3), 25.9 (×3), 14.1, 11.6, −3.6 (×2), −4.1, −4.3 (CH_3); HR ESMS *m*/*z* 732.3955 (M + Na^+), calcd for $C_{37}H_{67}NNaO_4S_2Si_2$, 732.3948.

(2*S*,3*R*,5*S*,7*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-7-methoxy-2-methyldodecanal (53). Oil: $[\alpha]_D$ +13.8 (*c* 1.2; $CHCl_3$); IR ν_{max} (cm^{-1}): 1732 (C═O); ^1H NMR δ 9.75 (1H, br s), 4.26 (1H, td, *J* = 6.3, 2.5 Hz), 3.83 (1H, m), 3.30 (3H, s), 3.29 (1H, m, overlapped by the 3H singlet), 2.45 (1H, qd, *J* = 6.8, 2.5 Hz), 1.75 (1H, m), 1.65–1.55 (2H, m), 1.55–1.50 (2H, m), 1.45–1.40 (1H, m), 1.35–1.20 (6H, br m), 1.08 (3H, d, *J* = 6.8 Hz), 0.90 (12H, br s, overlapping a methyl triplet), 0.86 (9H, s), 0.10 (6H, s), 0.09 (3H, s), 0.05 (3H, s); ^{13}C NMR δ 18.1, 18.0 (C), 204.8, 77.5, 69.4, 67.4, 51.2 (CH), 43.3, 42.8, 33.1, 32.1, 24.5, 22.7 (CH_2), 56.0, 25.9 (×3), 25.8 (×3), 14.1, 7.2, −3.8, −4.0, −4.1, −4.6 (CH_3); HR ESMS *m*/*z* 511.3621 (M + Na^+), calcd for $C_{26}H_{56}NaO_4Si_2$, 511.3615.

(2*S*,3*R*,5*S*,7*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-2-ethyl-7-methoxydodecanal (54). Oil: $[\alpha]_D$ +26.3 (*c* 1.3; $CHCl_3$); IR ν_{max} (cm^{-1}): 1727 (C═O); ^1H NMR δ 9.80 (1H, d, *J* = 1.5 Hz), 4.18 (1H, m), 3.84 (1H, apparent quintuplet, *J* ~ 6 Hz), 3.29 (3H, s), 3.24 (1H, m), 2.30 (1H, m), 1.80 (1H, m), 1.70 (1H, m), 1.65–1.60 (2H, m), 1.55–1.35 (4H, br m), 1.35–1.20 (6H, br m), 0.96 (3H, t, *J* = 7.5 Hz), 0.90, 0.88 (21H, 2 × s, overlapping one methyl triplet), 0.11 (3H, s), 0.09 (6H, s), 0.08 (3H, s); ^{13}C NMR δ 18.1, 18.0 (C), 205.2, 77.6, 69.8, 67.6, 59.1 (CH), 43.1, 42.8, 33.2, 32.1, 24.5, 22.7, 17.2 (CH_2), 56.1, 26.0 (×3), 25.8 (×3), 14.1, 12.4, −3.8, −3.9, −4.2, −4.4 (CH_3); HR ESMS *m*/*z* 503.3956 (M + H^+), calcd for $C_{27}H_{59}O_4Si_2$, 503.3952.

Methyl (*Z* + *E*)-(4*R*,5*R*,7*S*,9*R*)-5,7-bis(*tert*-butyldimethylsilyloxy)-9-methoxy-4-methyltetradec-2-enoate (55). A solution of phosphonate $(CF_3CH_2O)_2POCH_2COOMe$ (423 μL, 2 mmol) and 18-crown-6 (1.58 g, 6 mmol) in dry THF (10 mL) was cooled to −40 °C and treated dropwise under N_2 with KHMDS (commercial 0.5 M solution in toluene, 4 mL, 2 mmol). The mixture was then stirred for 1 h at the same temperature. After this, a solution of aldehyde **53** (489 mg, 1 mmol) in dry THF (8 mL) was added dropwise. The mixture was stirred for 3.5 h at −40 °C. Standard work-up (EtOAc) afforded enoate **55** as a ~77 : 23 mixture of *Z*/*E* diastereoisomers. A careful column chromatography on silica gel (hexanes–EtOAc, 49 : 1) permitted the separation of both compounds and furnished (*Z*)-**55** (379 mg, 70%) and (*E*)-**55** (117 mg, 21%):

(*Z*)-**55**: oil, $[\alpha]_D$ −31.8 (*c* 1.75; $CHCl_3$); IR ν_{max} (cm^{-1}): 1723 (C═O); ^1H NMR δ 6.21 (1H, dd, *J* = 11.5, 10 Hz), 5.77 (1H, br d, *J* ~ 11.5 Hz), 3.88 (1H, m), 3.74 (1H, m), 3.69 (3H, s), 3.57 (1H, m), 3.32 (1H, m, overlapped by the OMe singlet), 3.30 (3H, s), 1.80–1.65 (2H, m), 1.60–1.40 (4H, br m), 1.35–1.20 (6H, br m), 1.00 (3H, d, *J* = 6.8 Hz), 0.89, 0.87 (21H, 2 × s, overlapping a methyl triplet), 0.09 (3H, s), 0.08 (3H, s), 0.06 (3H, s), 0.02 (3H, s); ^{13}C NMR δ 166.5, 18.1, 18.0 (C), 153.4, 118.4, 77.5, 72.6, 67.5, 37.7 (CH), 43.8, 42.7, 33.5, 32.1, 24.6, 22.7 (CH_2), 55.8, 51.0, 25.9 (×3), 25.8 (×3), 14.1, 14.0, −3.8, −4.0 (×2), −4.4 (CH_3); HR ESMS *m*/*z* 567.3871 (M + Na^+), calcd for $C_{29}H_{60}NaO_5Si_2$, 567.3877.

(*E*)-**55**: oil, $[\alpha]_D$ +31.4 (*c* 0.6; $CHCl_3$); IR ν_{max} (cm^{-1}): 1729 (C═O); ^1H NMR δ 7.05 (1H, dd, *J* = 16, 6.6 Hz), 5.81 (1H, dd, *J* ~ 16, 1.5 Hz), 3.90–3.80 (2H, m), 3.72 (3H, s), 3.28 (3H, s), 3.26 (1H, m), 2.46 (1H, m), 1.65–1.55 (2H, m), 1.55–1.40 (4H, br m), 1.35–1.20 (6H, br m), 1.03 (3H, d, *J* = 6.8 Hz), 0.89 (21H, s, overlapping a methyl triplet), 0.09 (3H, s), 0.08 (3H, s), 0.07 (3H, s), 0.04 (3H, s); ^{13}C NMR δ 167.1, 18.1 (×2) (C), 152.2, 120.5, 77.5, 72.7, 67.9, 41.7 (CH), 43.0, 42.9, 33.2, 32.1, 24.5, 22.7 (CH_2), 56.0, 51.3, 25.9 (×6), 14.1, 13.0, −3.7, −3.8, −4.3 (×2) (CH_3); HR ESMS *m*/*z* 567.3880 (M + Na^+), calcd for $C_{29}H_{60}NaO_5Si_2$, 567.3877.

Methyl (4*R*,5*R*,7*S*,9*R*,*Z*)-5,7-bis(*tert*-butyldimethylsilyloxy)-4-ethyl-9-methoxytetradec-2-enoate (56). Obtained in 75% yield and >95 : 5 *Z*/*E* stereoselectivity by means of olefination of aldehyde **54** under the same conditions used for the preparation of **55**: oil, $[\alpha]_D$ −65.2 (*c* 1.1; $CHCl_3$); IR ν_{max} (cm^{-1}): 1729 (C═O); ^1H NMR δ 6.13 (1H, t, *J* = 11.3 Hz), 5.85 (1H, br d, *J* ~ 11.3 Hz), 3.90 (1H, m), 3.80 (1H, m), 3.70 (3H, s), 3.49 (1H, m), 3.31 (3H, s), 3.30 (1H, m, overlapped by the OMe singlet), 1.80–1.65 (2H, m), 1.65–1.55 (2H, m), 1.55–1.40 (4H, br m), 1.35–1.20 (6H, br m), 0.90, 0.88 (24H, 2 × s, overlapping two methyl triplets), 0.10 (6H, s), 0.08 (3H, s), 0.04 (3H, s); ^{13}C NMR δ 166.6, 18.1 (×2) (C), 152.2, 120.2, 77.5, 72.3, 67.6, 45.0 (CH), 43.7, 42.8, 33.5, 32.1, 24.6, 22.7, 22.2 (CH_2), 55.8, 51.0, 25.9 (×6), 14.1, 11.8, −3.8, −3.9 (×2), −4.3 (CH_3); HR ESMS *m*/*z* 581.4032 (M + Na^+), calcd for $C_{30}H_{62}NaO_5Si_2$, 581.4033.

Methyl (4*R*,5*R*,7*R*,9*R*,*Z*)-5,7-dihydroxy-9-methoxy-4-methyltetradec-2-enoate (*Z*-57). Oil, $[\alpha]_D$ −42.2 (*c* 1.2; $CHCl_3$); IR ν_{max}

(cm^{-1}): 3420 (br, OH), 1723 (C=O); ^1H NMR δ 6.10 (1H, dd, J = 11.7, 10.3 Hz), 5.77 (1H, br d, $J \sim 11.7$ Hz), 4.26 (1H, m), 3.87 (1H, m), 3.72 (3H, s), 3.62 (1H, m), 3.50 (1H, br s, OH), 3.47 (1H, m), 3.36 (3H, s), 3.20 (1H, br s, OH), 1.84 (1H, ddd, J = 14.5, 9.8, 3.8 Hz), 1.70–1.55 (5H, br m), 1.55–1.40 (2H, br m), 1.10 (3H, d, J = 6.8 Hz), 0.90 (3H, t, J = 6.8 Hz); ^{13}C NMR δ 167.1 (C), 152.3, 119.4, 79.9, 72.7, 66.6, 38.7 (CH), 40.0, 38.8, 32.8, 32.0, 25.3, 22.6 (CH_2), 56.8, 51.2, 16.0, 14.0 (CH_3); HR ESMS m/z 339.2150 ($M + Na^+$), calcd for $C_{17}H_{32}NaO_5$, 339.2147.

Methyl (4*R*,5*R*,7*R*,9*R*,*E*)-5,7-dihydroxy-9-methoxy-4-methyltetradec-2-enoate (*E*-57). Oil, $[\alpha]_D$ +39.8 (c 0.1; $CHCl_3$); IR ν_{max} (cm^{-1}): 3430 (br, OH), 1727 (C=O); ^1H NMR δ 6.93 (1H, dd, J = 15.8, 8.3 Hz), 5.86 (1H, dd, J = 15.8, 1.5 Hz), 4.26 (1H, m), 3.87 (1H, m), 3.73 (3H, s), 3.60 (1H, br s, OH), 3.46 (1H, m), 3.36 (3H, s), 3.00 (1H, br s, OH), 2.47 (1H, m), 1.88 (1H, ddd, J = 14.5, 9.5, 3.8 Hz), 1.70–1.65 (1H, m), 1.60–1.40 (5H, br m), 1.35–1.20 (5H, br m), 1.13 (3H, d, J = 6.8 Hz), 0.89 (3H, t, J = 7 Hz); ^{13}C NMR δ 167.0 (C), 151.3, 121.2, 80.1, 71.7, 66.6, 38.0 (CH), 42.9, 40.1, 32.5, 32.0, 25.3, 22.6 (CH_2), 56.8, 51.5, 15.1, 14.0 (CH_3); HR ESMS m/z 339.2142 ($M + Na^+$), calcd for $C_{17}H_{32}NaO_5$, 339.2147.

Methyl (4*R*,5*R*,7*R*,9*R*,*Z*)-4-ethyl-5,7-dihydroxy-9-methoxy-tetradec-2-enoate (58). Oil, $[\alpha]_D$ −33 (c 1.15; $CHCl_3$); IR ν_{max} (cm^{-1}): 3420 (br, OH), 1722 (C=O); ^1H NMR δ 6.00 (1H, dd, J = 11.7, 9.8 Hz), 5.95 (1H, br d, $J \sim 11.7$ Hz), 4.26 (1H, m), 3.94 (1H, m), 3.73 (3H, s), 3.55–3.40 (3H, br m, overlapping one OH signal), 3.36 (4H, s, overlapping one OH signal), 1.85–1.75 (2H, m), 1.70–1.45 (8H, br m), 1.40–1.20 (8H, br m), 0.89 (6H, two overlapped triplets); ^{13}C NMR δ 167.4 (C), 151.2, 121.4, 79.9, 72.0, 66.6, 46.0 (CH), 39.7, 38.9, 32.8, 32.0, 25.3, 24.0, 22.6 (CH_2), 56.8, 51.3, 14.0, 11.8 (CH_3); HR ESMS m/z 353.2302 ($M + Na^+$), calcd for $C_{18}H_{34}NaO_5$, 353.2304.

Biological procedures

Cell culture

Human A549 non-small lung carcinoma cells were continuously maintained in RPMI-1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 40 μg mL^{-1} gentamycin, 100 IU mL^{-1} penicillin and 100 μg mL^{-1} streptomycin.[19b] Human ovarian carcinoma A2780 (parental cell line) and A2780AD (multidrug resistant cell line overexpressing P-glycoproteins) were cultured as above with the addition of 0.25 unit per mL of bovine insulin.

Cytotoxicity assays

A2780 and A2780AD cells were seeded in 96 well plates at a density of 15 000 cells in 0.08 mL per well. On the following day, the cells were exposed to 0.02 mL serial dilutions of ligands for 48 hours, after which time a modified MTT assay[40] was performed in order to determine viable cells. For this purpose, 20 μL of 2.5 mg mL^{-1} of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well, incubated for 4 h at 37 °C, and then treated with 0.1 mL MTT solubilizer (10% SDS, 45% dimethylformamide, pH 5.5). Plates were again incubated overnight at 37 °C in order to solubilize the blue formazan precipitate before measuring the absorbance at 595/690 nm in an automated Multiscan microplate reader. Control wells containing medium without cells were used as blanks. MTT response is expressed as a percentage of the control (untreated) cells. The IC_{50} was calculated from the log–dose response curves.

Indirect immunofluorescence

A549 cells were plated at a density of 150 000 cells per mL onto 24 well tissue culture plates containing 12 mm round coverslips, cultured overnight and then treated with ligands at different concentrations or with drug vehicle (DMSO) for 24 hours. Residual DMSO was less than 0.5%. Attached cells were permeabilized with Triton X-100 and fixed with 3.7% formaldehyde, as previously described.[41] Cytoskeletons were incubated with DM1A monoclonal antibody reacting with α-tubulin, washed twice and incubated with FITC goat anti-mouse immunoglobulins. The coverslips were washed with 1 μg mL^{-1} Hoechst 33342 in order to stain the chromatin. After washing, the samples were examined and photographed using a Zeiss Axioplan epifluorescence microscope. The images were recorded with a Hamamatsu 4742-95 cooled CCD camera.

Cell cycle analysis

Progression through the cell cycle was assessed by flow cytometry DNA determination with propidium iodide. Cells (150 000 per mL) were incubated with several concentrations of the drugs for 24 hours. The cells were fixed with 70% ethanol, treated with RNase and stained with propidium iodide as previously described.[42] The analysis was performed with a Coulter Epics XL flow cytometer.

Effects of ligands on microtubule assembly

The effect of the pironetin analogues in the assembly of purified tubulin was determined by incubating 20 μM of purified tubulin at 37 °C for 30 minutes in GAB (glycerol assembling buffer, 3.4 M glycerol, 10 mM sodium phosphate, 1 mM EGTA, 1 mM GTP, 6 mM $MgCl_2$ at pH 6.5) in the presence of 25 μM docetaxel, 100 μM of an appropriate analogue or 2 μL DMSO (vehicle). In this buffer, tubulin can assemble without ligand with a critical concentration of about 3.3 μM.[43] The polymers were sedimented at 90 000g for 20 minutes in a TLA 100 rotor, preequilibrated at 37 °C, in a Beckman Optima TLX ultracentrifuge. The supernatants were carefully removed by pipetting, and the pellets were resuspended in 10 mM phosphate, 1% SDS, pH 7.0. The pellets and the supernatants were diluted 1 : 10 in the same buffer, and their concentrations were fluorimetrically measured employing a Fluorolog 3 spectrofluorimeter (excitation wavelength 285 nm, emission wavelength 320 nm using slits of 2 and 5 nm, respectively). Tubulin concentration standard curves were constructed for each experiment, using spectrophotometrically measured concentrations of purified tubulin. The critical concentration for tubulin

assembly[37] in the presence of the ligands was calculated as described.[42]

Authors' contribution

J. Paños, S. Díaz-Oltra, M. Sánchez-Peris and Jorge García-Pla were involved at different times in various phases of the chemical work. The work will be a part of the PhD thesis of three of them.

Juan Murga, Eva Falomir and Miguel Carda were involved in guiding the aforementioned people (as PhD supervisors).

M. Redondo-Horcajo, J. F. Díaz and I. Barasoain were involved in the biological work, which was done in Madrid.

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