Design and Synthesis of Pironetin Analogue/Combretastatin A-4 Hybrids and Evaluation of Their Cytotoxic Activity

ARTICLE *in* EUROPEAN JOURNAL OF ORGANIC CHEMISTRY · APRIL 2014

Impact Factor: 3.07 · DOI: 10.1002/ejoc.201301725

7 AUTHORS, INCLUDING:

Concepción Vilanova
Universitat Jaume I
8 PUBLICATIONS **7** CITATIONS
SEE PROFILE

Santi Diaz
Universitat Jaume I
48 PUBLICATIONS **241** CITATIONS
SEE PROFILE

Eva Falomir
Universitat Jaume I
86 PUBLICATIONS **1,012** CITATIONS
SEE PROFILE

EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201301725

Design and Synthesis of Pironetin Analogue/Combretastatin A-4 Hybrids and Evaluation of Their Cytotoxic Activity

Sandra Torijano-Gutiérrez,[a] Concepción Vilanova,[a] Santiago Díaz-Oltra,[a] Juan Murga,*[a] Eva Falomir,[a] Miguel Carda,[a] and J. Alberto Marco*[b]

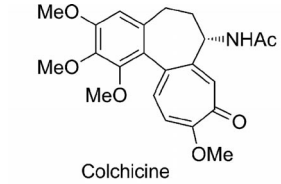
Keywords: Medicinal chemistry / Drug design / Cytotoxicity / Anticancer agents / Structure–activity relationships

We describe the preparation of a series of hybrid molecules containing a combretastatin A-4 moiety and a pironetin analogue fragment linked by an ester spacer of variable length. The cytotoxic activities of these compounds have been measured and the relationship between the structure and cytotoxicity is discussed. Some of the tested compounds showed cytotoxicity values of the same order of magnitude as those of the parental molecules, combretastatin A-4 and pironetin, and were less toxic than the latter for normal cells.

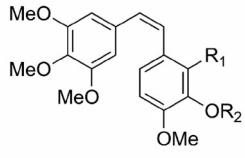
Introduction

It is widely known that cancer, one of the leading causes of death in developed countries,[1] may be induced by a plethora of both external and internal factors, including genetic mutations. Accordingly, many types of therapeutic attack have been investigated.[2,3] 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.[4] As a matter of fact, many such drugs act through their 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. Microtubules are composed of a protein named tubulin, the functional form of which is a heterodimer formed through noncovalent binding of two monomeric constituents, 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.[5] Any molecule that influences microtubule instability will also influence the cell division process, not only of normal cells but also of tumoral cells. Therefore, it is not surprising that tubulin-binding molecules (TBMs) constitute a very important class of anticancer agents.

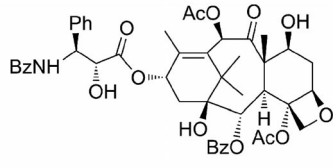
TBMs may be divided into two broad categories, those that bind to α-tubulin and those that bind to β-tubulin. The latter group is presently by far the most numerous and contains products that cause either disruption or stabilization of microtubules. Among the drugs that belong to this group, the well-known colchicine[6] and the combretastatins[7] (Figure 1) exert their effects by causing disruption of microtubules. In contrast, another important representative of the same group, paclitaxel, was the first described tubulin-binding drug that was found to stabilize microtubules.[8] Even though they display opposite effects, these drugs are



Colchicine



Combretastatin A-1 R_1 = OH R_2 = H
Combretastatin A-4 R_1 = R_2 = H



Paclitaxel

Figure 1. Structures of some natural products reported to selectively bind to β-tubulin.

[a] Departamento de Química Inorgánica y Orgánica, Univ. Jaume I,
12071 Castellón, Spain
E-mail: mcarda@qio.uji.es
http://www.sinorg.uji.es/index.htm
[b] Departamento de Química Orgánica, Univ. de Valencia,
50, c/D. Moliner, 46100 Burjassot, Spain
E-mail: alberto.marco@uv.es
http://www.uv.es/~marcoj/

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201301725.

known to bind to β-tubulin, albeit to different sites within this protein subunit.[9–11]

The number of products that bind to α-tubulin is very small, with the naturally occurring 5,6-dihydro-α-pyrone pironetin being the first reported example,[12] followed, a short time later, by the peptide-like hemiasterlin family[13] (Figure 2). Pironetin is a potent inhibitor of tubulin assembly and has been found to arrest cell cycle progression in the G2/M phase.[14]

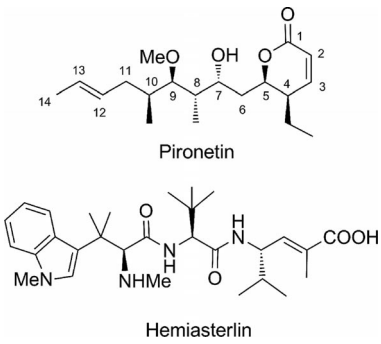


Figure 2. Structures of two natural products reported to selectively bind to α-tubulin.

Some structure-activity relationship (SAR) studies on pironetin have been reported. 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, are essential for biological activity. The presence of a (7*R*)-hydroxyl group also seems to be relevant. It has been suggested, although not yet conclusively demonstrated, that the Lys352 residue of the α-tubulin chain adds in a Michael fashion to the conjugated double bond of pironetin, thus forming a covalent bond with C-3 of the dihydropyrone ring (Figure 3). It has also 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.[14]

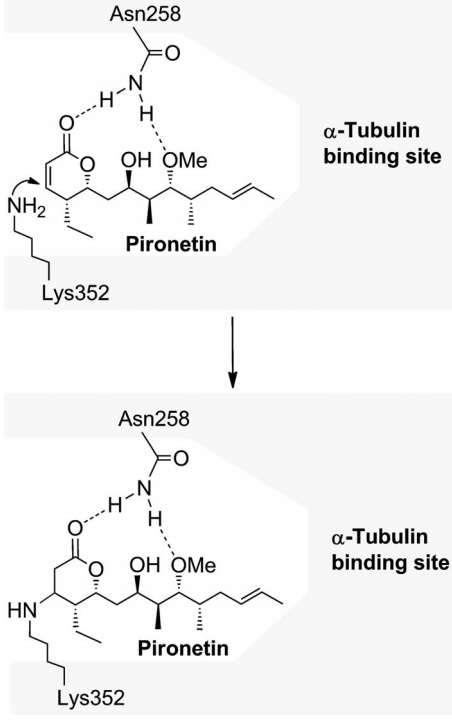


Figure 3. Schematic model suggested for the covalent union of pironetin to its binding site at the α-tubulin surface.

The emergence of resistances to existing drugs has led to a continuous need to develop new bioactive compounds that overcome such problems. Even though first observed in the case of antibiotics,[15] resistances have been reported to therapies with various types of anticancer agents.[16] The investigation of new compounds with such biological properties therefore constitutes an important goal in chemistry and pharmacology.

Concept and Design of Hybrid Tubulin-Binding Ligands

As a member of the small group of TBMs that bind to α-tubulin, pironetin constitutes a pharmacologically interesting target. Not surprisingly, appreciable numbers of total syntheses of this natural compound have appeared in the literature.[17] To develop SAR studies based upon the pironetin framework, two years ago[5a] we designed a simplified model structure in which all elements that had not yet proven to be essential for biological activity were removed. The target structures **I/II** are schematically shown in Figure 4. The elements that were maintained were 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**), to assess its influence on 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 stereocenters 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.[5a]

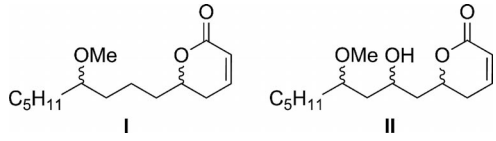


Figure 4. General structures of simplified pironetin analogues of the first generation.[5a]

The cytotoxic activity of these analogues and their interactions with tubulin were subsequently investigated. It was found, on one hand, that analogues **I/II** were cytotoxic in the low micromolar range, about three orders of magnitude less active than the parent molecule. On the other hand, we also found that they behave in the same way as pironetin, share the mechanism of action of the natural compound, and compete for the same binding site in α-tubulin. As observed for the parent compound, they also lead to disruption of the microtubule network.[5a]

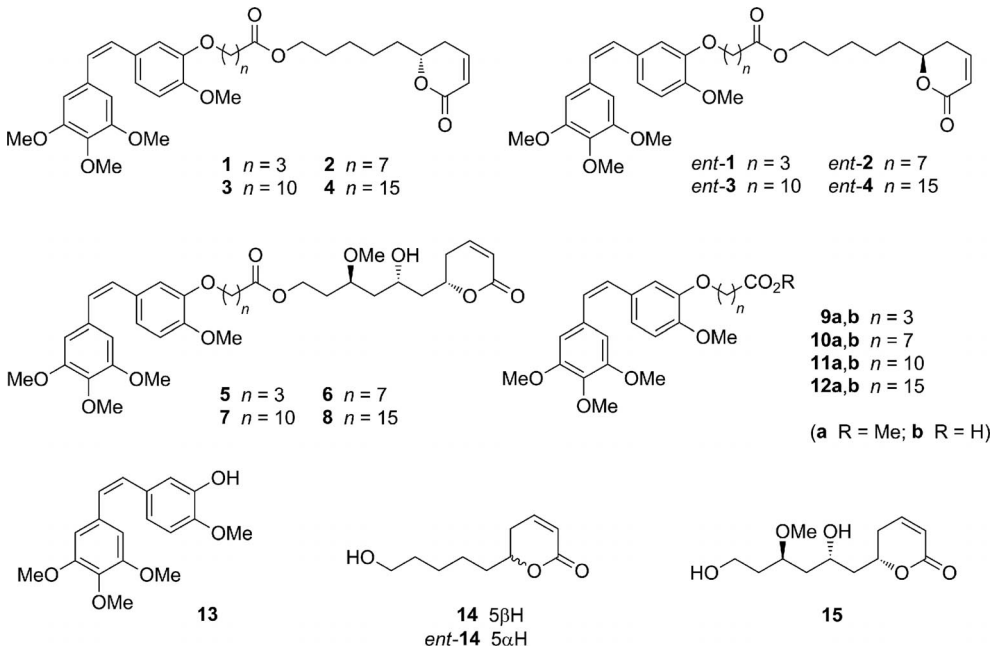
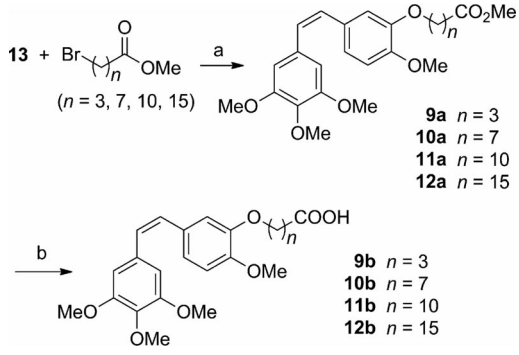


Figure 5. Structures of the pironetin analogue/combretastatin A-4 hybrids used in this study (**1**/**8** and *ent*-**1**/*ent*-**4**) and of their synthetic precursors (**9**–**15**), including combretastatin A-4 (**13**).

With the aim of extending the scope of our project, we wanted to prepare cytotoxic TBMs with a dual ability to bind to either α- or β-tubulin and exert a microtubule-destabilizing effect. Since these properties are specifically exhibited by pironetin (binds to α-tubulin) and combretastatin A-4 (binds to β-tubulin), respectively, we decided to prepare compounds with a hybrid structure such as **1**–**8** (Figure 5). These molecules contain a moiety of combretastatin A-4 (itself numbered as **13** in Figure 5) and another of the simplified pironetin type (**14**, *ent*-**14** and **15**), connected, in turn, by a spacer of variable length. Some of these molecules (**1**–**4** and *ent*-**1** to *ent*-**4**) have been prepared in both antipodal forms.[18]



Scheme 1. Synthesis of combretastatin A-4 derivatives **9a**–**12a** and **9b**–**12b**: *Reagents and conditions:* (a) K_2CO_3, DMF, room temp., 24 h (**9a**: 92%; **10a**: 92%; **11a**: 91%; **12a**: 90%); (b) aq. NaOH, MeOH, room temp., 18 h (**9b**: 80%; **10b**: 78%; **11b**: 76%; **12b**: 75%).

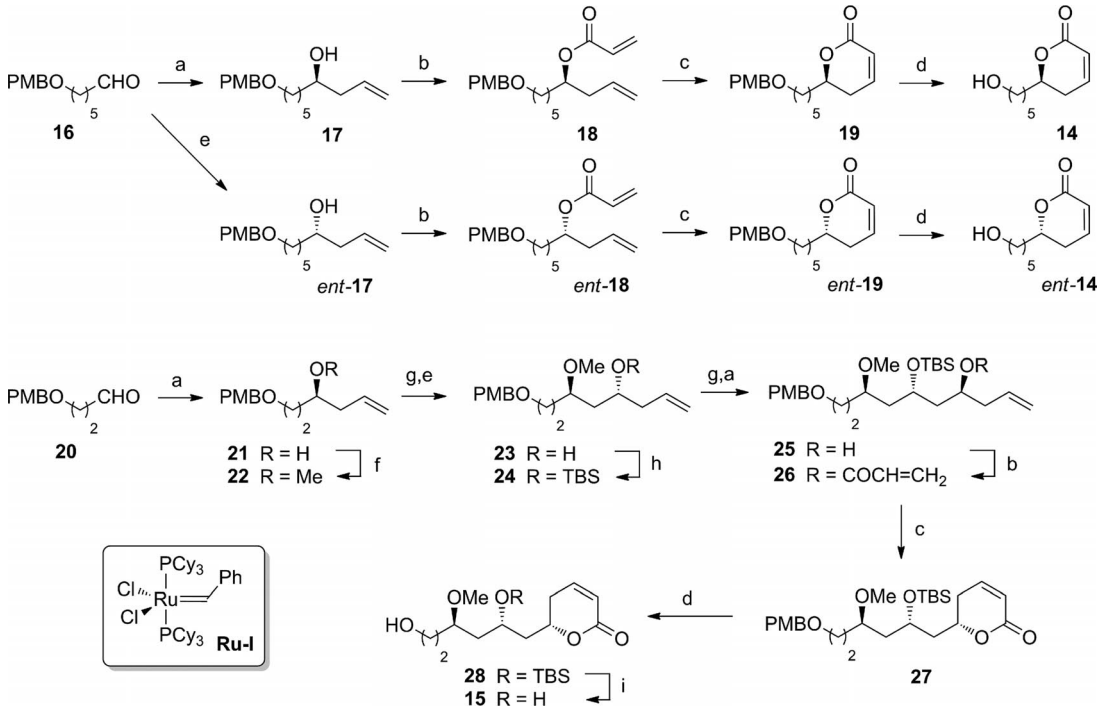
Results and Discussion

Chemical Results

Combretastatin A-4 (**13**) was prepared according to a published procedure.[19] Its *O*-alkyl derivatives **9a**–**12a** were prepared by means of *O*-alkylation with four commercially available ω-bromo esters (Scheme 1). Saponification of esters **9a**–**12a** gave the corresponding acids **9b**–**12b**.

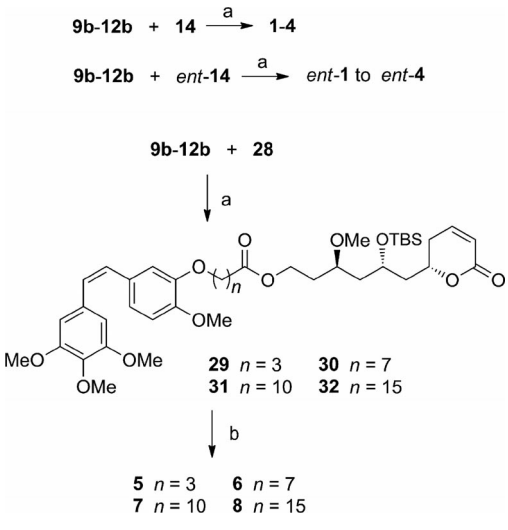
The synthesis of the pironetin fragments **14**, *ent*-**14** and **15** is depicted in Scheme 2 and was performed according to the general concept used in previous papers.[5a,5b] Thus, Brown′s asymmetric allylation[20] of known aldehyde **16**[21] afforded homoallyl alcohol **17**. The required chiral allylborane was prepared through reaction of allylmagnesium bromide with commercially available (–)-diisopinocampheylboron chloride [(–)-Ipc_2BCl]. Reaction of **17** with acryloyl chloride at low temperature gave acrylate **18**, which was subjected to ring-closing metathesis (RCM)[22] in the presence of Grubbs first-generation catalyst **Ru-I**. This furnished dihydropyrone **19**, deprotection of which provided **14** in good yield. Dihydropyrone *ent*-**14** was prepared in the same way, except that (+)-Ipc_2BCl was used as the chiral boron reagent.

Dihydropyrone **15** was not connected as such with the combretastatin A-4 fragment, but as its *O*-silyl derivative **28** (Scheme 2), which was also prepared according to the previous methodology. Brown′s asymmetric allylation of known aldehyde **20**[23] furnished alcohol **21**, which was then converted into methyl ether **22**. The latter was then sequentially subjected to a two-step oxidative cleavage of the olefinic bond, followed by Brown's asymmetric allylation to yield **23**. Silylation of the hydroxyl group of **23** and repetition of the previous sequence gave alcohol **25**, which was



Scheme 2. Synthesis of pironetin fragments **14**, *ent*-**14** and **15**. *Reagents and conditions:* (a) (–)-Ipc$_2$BCl, allylMgBr, Et$_2$O, –78 °C, 1 h, then addition of the aldehyde, 1 h, –78 °C (**17**: 83%, *er* 96:4; **21**: 85%, *er* 94:6; **25**, 78% overall yield from **24**, *dr* 76:24); (b) CH$_2$=CHCOCl, CH$_2$Cl$_2$, *i*Pr$_2$NEt, –78 °C, 30 min (**18**: 94%; *ent*-**18**: 90%; **26**: 91%); (c) 10% cat. **Ru-I**, CH$_2$Cl$_2$, Δ, 2 h (**19**: 81%; *ent*-**19**: 80%; **27**: 83%); (d) DDQ, wet CH$_2$Cl$_2$, 30 min (**14**: 69%; *ent*-**14**: 67%; **28**: 74%); (e) (+)-Ipc$_2$BCl, allylMgBr, Et$_2$O, –78 °C, 1 h, then addition of the aldehyde, 1 h, –78 °C (*ent*-**17**: 89%, *er* 96:4; **23**: 90% overall yield from **22**, *dr* 86:14); (f) NaH, THF, 0 °C, then MeI, room temp., overnight (88%); (g) 1. OsO$_4$, NMO, aq. *t*BuOH, THF, room temp., overnight. 2. Pb(OAc)$_4$, CH$_2$Cl$_2$, 0 °C; (h) TBSOTf, 2,6-lut, 0 °C, 1 h (80%); (i) PPTS (cat.), MeOH, Δ, overnight (85%). Acronyms and abbreviations: Ipc, isopinocampheyl; DDQ, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone; TBS, *tert*-butyldimethylsilyl; Tf, trifluoromethanesulfonyl; NMO, *N*-methylmorpholine *N*-oxide; 2,6-lut, 2,6-lutidine; PPTS, pyridinium *p*-toluenesulfonate.

esterified with acryloyl chloride. The resulting acrylate **26** was subjected to ring-closing metathesis to **27**, which was then oxidatively deprotected[24] to **28**. Desilylation of **28** gave **15**, which was also used in the biological evaluations.



Scheme 3. Synthesis of hybrid molecules **1**–**4**, *ent*-**1** to *ent*-**4**, and **5**–**8**. *Reagents and conditions:* (a) **9b**–**12b**, 2,4,6-trichlorobenzoyl chloride, Et$_3$N, room temp., then addition of the alcohol component, room temp., 2 h (**1**: 38%; **2**: 39%; **3**: 37%; **4**: 35%; *ent*-**1**: 42%; *ent*-**2**: 43%; *ent*-**3**: 38%; *ent*-**4**: 40%; **29**: 41%; **30**: 40%; **31**: 40%; **32**: 36%); (b) PPTS (cat.), MeOH, Δ, overnight (**5**: 86%; **6**: 85%; **7**: 83%; **8**: 81%).

Dihydropyrones **1**–**8**, including *ent*-**1** to *ent*-**4**, were prepared by esterification of acids **9b**–**12b** with dihydropyrones **14**, *ent*-**14** and **28** by means of the Yamaguchi method[25] (Scheme 3). In the case of compounds **5**–**8**, an additional desilylation step was required.

When the synthesis of all aforementioned hybrid molecules was complete, the compounds were investigated in relation to their cytotoxic activity towards two types of tumoral lines and one normal cell line.

Biological Results

Cellular Effects of the Compounds; Cytotoxicity Values

We investigated the cytotoxicity of the hybrid molecules **1**–**4** and their respective enantiomers (*ent*-**1** to *ent*-**4**), **5**–**8**, as well as combretastatin A-4 derivatives **9a**–**12a** and **9b**–**12b**. Pironetin analogues **14**, *ent*-**14** and **15** were also tested. Cytotoxicity assays were performed as described in the Experimental Section by using two types of tumoral cells, the human colon adenocarcinoma HT-29 and the breast adenocarcinoma MCF-7 cell lines. In addition, one normal cell line, the human embryonic kidney cell line (HEK-293),[26] was employed in the assays for comparison. Cytotoxicity

values, expressed as the compound concentration [μmol/L] that causes 50% inhibition of cell growth (IC_{50}), are shown in Table 1. The observed values are, in most cases, in the low to medium micromolar range. Compounds *ent*-**1**, **3**, **7**, **10a**, **11a**, **12a** and **12b** showed the highest cytotoxicities for the HT-29 cell line, with IC_{50} values that were not very different (in some cases even lower) than those of combretastatin A-4 and pironetin for this particular cell line. In the case of the MCF-7 cell line, the lowest IC_{50} values were shown by compounds **7**, **9b**, **10a**, **11a**, **11b**, **12a**, **12b** and **15**. But a further aspect that is also worth mentioning is the fact that some of the synthetic compounds were found to be much more toxic for tumoral cells than for normal cells, which is clearly a desirable feature. This can be better appreciated with the α and β coefficients, obtained by dividing the IC_{50} values of the normal cell line (HEK-293) by those of one of the tumoral cell lines (see footnote in Table 1). The higher the value of either the α or β coefficient, the higher the therapeutic safety margin of the compound in the corresponding cell line. Thus, combretastatin A-4 itself shows good values of both coefficients, most particularly in the case of the MCF-7 cell line. Pironetin shows a similarly good value for the HT-29 cell line (α), albeit less so for the MCF-7 cell line. Among the compounds with a high cytotoxicity (low IC_{50} values), *ent*-**1**, **7**, **11a**, **12a** and **12b** are particularly worth mentioning because they exhibit high values for both α and β coefficients. Compounds **3** showed good selectivity only in the case of the HT-29 line ($\alpha > 30$), whereas compounds **11b** and **15** showed good selectivity in the specific case of the MCF-7 line ($\beta > 60$).

Table 1. Cytotoxicity of pironetin analogue/combretastatin A-4 hybrids **1**–**8** and *ent*-**1** to *ent*-**4**, combretastatin A-4 derivatives **9a**–**12a** and **9b**–**12b**, and pyrones **14**, *ent*-**14** and **15**.[a]

	HT-29	MCF-7	HEK-293	α[b],[d]	β[c],[d]
CoA4	4.2 ± 0.5	1 ± 0.2	25 ± 3	5.9	25
Pironetin	8 ± 2	18 ± 3	46 ± 6	5.7	2.5
1	12 ± 2	52 ± 10	39 ± 4	3.2	0.7
2	32 ± 5	> 300	80 ± 14	2.5	< 0.3
3	*10 ± 1*	> 300	> 300	*> 30*	–
4	107 ± 5	> 300	> 300	> 2.8	–
ent-**1**	*3.5 ± 0.9*	*17 ± 2*	*114 ± 5*	*32*	*6.7*
ent-**2**	19 ± 6	> 300	> 300	> 15	–
ent-**3**	38 ± 3	69 ± 7	59 ± 10	1.6	0.8
ent-**4**	64 ± 7	> 300	72 ± 3	1.1	< 0.3
5	15.4 ± 0.5	31 ± 1	22 ± 2	1.4	0.7
6	44 ± 5	> 300	> 300	> 6.8	–
7	*2.9 ± 0.2*	*5.6 ± 0.1*	*22 ± 2*	*7.6*	*3.9*
8	50.8 ± 0.6	21 ± 4	81 ± 10	1.6	3.8
9a	60.1 ± 0.5	10.4 ± 0.5	121 ± 5	2	12
9b	50 ± 2	2.9 ± 0.2	67 ± 2	1.4	23
10a	3.5 ± 0.3	9.6 ± 0.2	9.6 ± 0.3	2.7	1
10b	16 ± 2	17 ± 4	> 300	> 19	> 18
11a	*8 ± 1*	*3 ± 0.6*	*> 300*	*> 37*	*> 100*
11b	103 ± 6	*5 ± 0.9*	> 300	> 30	*> 60*
12a	*5 ± 1*	*9.6 ± 0.2*	*> 300*	*> 60*	*> 30*
12b	*1.9 ± 0.3*	*4.4 ± 0.8*	*39 ± 5*	*20*	*8.8*
14	109 ± 20	> 300	35 ± 7	1.9	< 0.7
ent-**14**	81 ± 12	109 ± 8	119 ± 16	1.5	1.1
15	25.4 ± 0.2	*2.5 ± 0.4*	> 300	> 12	*> 120*
9a+*ent*-**14**	41 ± 3	23.9 ± 0.9	49 ± 7	1.2	2
11a+*ent*-**14**	33 ± 3	52 ± 5	72 ± 5	2.2	1.4
9a+15	25 ± 2	32.2 ± 0.5	22 ± 2	0.9	0.7
11a+15	56 ± 4	7.1 ± 0.1	10 ± 3	0.2	1.4

[a] IC_{50} values are expressed as the compound concentration (μmol/L or μM) that causes 50% inhibition of cell growth and are the average (± s.d.) of three different measurements performed as described in the Material and Methods section. The values refer to the hybrid molecules but also include their monomeric precursors, equimolecular mixtures of some of these and the parent natural compounds combretastatin A-4 (CoA4) and pironetin. Compounds with both high cytotoxicity towards one or both tumoral cell lines and low cytotoxicity towards the normal cell line have been highlighted. [b] $\alpha = IC_{50}$ (HEK-293)/IC_{50} (HT-29). [c] $\beta = IC_{50}$ (HEK-293)/IC_{50} (MCF-7). [d] Values of α and β have been rounded to one decimal place.

The relationship between the structures and the observed cytotoxicity deserves comment. In the case of two subsets of hybrid molecules[27] where there are differences in the carbon chain length (e.g., **1**–**4** and **5**–**8**), the cytotoxicity reaches a maximum for compounds **3** and **7**, which have a ten-carbon chain in the combretastatin A-4 segment (the cytotoxicities of the compounds of the enantiomeric series, *ent*-**1** to *ent*-**4**, are not very different to those of **1**–**4**, although the maximum cytotoxicity is found here for *ent*-**1**, the compound with the three-carbon chain). It is difficult to advance an explanation for these observations because we do not yet know whether the compounds are interacting with tubulin at the pironetin site (α-tubulin) or at the combretastatin A-4 site (β-tubulin). Although the distance between the pironetin and the combretastatin A-4 binding sites is not yet known with certainty, a cursory examination of the available X-ray data of tubulin[10d] indicates that the carbon chains of our compounds are not long enough to permit simultaneous binding to both sites. Thus, the variation of the cytotoxicity with the length of the carbon chain may be related to differential interactions with hydrophobic zones on the tubulin surface. In any case, we have not tried to prepare derivatives with longer chains because this would give highly hydrophobic compounds with a too low solubility.

Although more research in this direction is still needed, we compared the cytotoxicities of some of the hybrid compounds with the cumulative effect of their separate "monomeric" precursors. In the case of the HT-29 cell line, an equimolecular mixture of **9a** and *ent*-**14**, the precursors of *ent*-**1**, show higher IC_{50} values, thus less cytotoxicity, than that of the latter compound alone. The same result is observed with an equimolecular mixture of **11a** and **15**, the precursors of **7**. It is not known whether this indicates a preferential interaction of the hybrid molecules at one or the other binding site, or even the existence of some type of synergy. In contrast, equimolecular mixtures of **11** and *ent*-**14** (the precursors of *ent*-**3**), and of **9a** and **15** (the precursors of *ent*-**5**), show similar IC_{50} values to those of the hybrid molecules.

As regards the MCF-7 cell line, the cytotoxicities of the hybrid molecules are similar to those of their precursors and to those of pironetin itself, but markedly lower than those of combretastatin A-4. This may indicate that these hybrid molecules preferentially interact with the tubulin of such cells at the pironetin site. More definitive answers will

require an accurate knowledge of the geometry and location of the pironetin binding site.

We have also tested the cytotoxicity of the synthetic intermediates used for the preparation of the aforementioned hybrid molecules. As regards combretastatin A-4 derivatives **9**–**12a/b**, a dependence of the cytotoxicity on the carbon chain length is also observed (Table 1). For the methyl esters (**9a**–**12a**), a marked increase in the cytotoxicity is observed upon going from the compound with the three-carbon chain (**9a**) to those having longer chains (**10a**–**12a**), with IC_{50} values of the latter three being very similar (with both tumoral cell lines) and comparable to those of combretastatin A-4 itself. As for the free acids (**9b**–**12b**), the cytotoxicities vary in a rather erratic way and no systematic correlation is perceived between the IC_{50} values and chain length. Furthermore, marked differences were also observed in some cases between the IC_{50} values for the two tumoral cell lines.

The cytotoxicities of the three pironetin analogues **14**, *ent*-**14** and **15** were also measured. Compound **14** and its enantiomer, which contain a single stereocenter, represent the most simplified model possible for pironetin. From the results presented in Table 1, it is evident that **14** and *ent*-**14** show a very low cytotoxicity, much below of that observed for the hybrid molecules (**1**–**3**, and *ent*-**1** to *ent*-**3**) bearing these fragments (exceptions to this are **4** and *ent*-**4**, which show very high IC_{50} values, perhaps because of solubility problems). Although theses conclusions are still provisory, this seems to point to **1**–**3** and their enantiomers owing their cytotoxicity to interactions with tubulin through the combretastatin A-4 end. As regards compound **15**, it has three stereocenters and corresponds more closely to the pironetin models we have been investigating in recent years.[5] The results listed in Table 1 show that its cytotoxicity for the two tumoral cells is indeed much higher than those of **14**/*ent*-**14**, particularly in the case of the MCF-7 cell line. From the hybrid molecules **5**–**8** having this structural fragment, the highest cytotoxicity is shown by **7**, the molecule that displays the ten-carbon chain, as commented above. It is also worth noting that the IC_{50} value of **7** with the HT-29 cell line is much lower than the IC_{50} values of both **11b** and **15**, its two precursor components. This suggests that hydrolysis of **7** into **11b** and **15** is not taking place within the cell.

Conclusion

We have prepared a set of synthetic hybrid molecules containing a combretastatin A-4 moiety and a fragment that is structurally related to the natural product pironetin. Some of these molecules have been synthesized in both enantiomeric forms. Their cytotoxic action (IC_{50} values) towards a normal (HEK-293) and two tumoral (HT-29 and MCF-7) cell lines has then been measured. Whereas most of the synthetic derivatives proved to be cytotoxic towards at least one of the two tumoral cell lines, some of them showed cytotoxic activities of the same order as the natural compounds pironetin and combretastatin A-4. More interesting was the fact that, for some of the compounds, the normal/tumoral cytotoxicity ratio was markedly higher than in the case of the two aforementioned natural products, that is, they proved comparatively less cytotoxic towards normal cells (these compounds are highlighted in Table 1). This may make these compounds of pharmacological interest.

Experimental Section

Chemical Procedures

General Experimental Procedures: $^1H/^{13}C$ NMR spectra were recorded at 500/125 MHz in $CDCl_3$ solution at 25 °C. The signals of the deuterated solvent ($CDCl_3$) were taken as reference (the singlet at δ = 7.25 ppm for ^1H NMR spectroscopic data and the triplet centered at δ = 77.00 ppm for ^{13}C NMR spectroscopic data). Carbon atom types (C, CH, CH_2, CH_3) were determined with the DEPT pulse sequence. High-resolution mass spectra were run by the electrospray technique (ESMS) in either the positive or the negative ion mode. IR data are given only for compounds with significant functions (OH, C=O). Optical rotations were measured at 25 °C. Reactions that required an inert atmosphere were carried out under N_2 with flame-dried glassware. Et_2O and THF were freshly distilled from sodium/benzophenone ketyl and transferred by using a syringe. Dichloromethane was freshly distilled from CaH_2. Tertiary amines were freshly distilled from KOH. Toluene was freshly distilled from sodium wire. Commercially available reagents were used as received. Unless detailed otherwise, "workup" means pouring the reaction mixture into brine, followed by extraction with the solvent indicated in parenthesis. If the reaction medium was acidic, an additional washing with 5% aq. $NaHCO_3$ was performed. If the reaction medium was basic, an additional washing with aq. NH_4Cl was performed. Following washing with brine, drying over anhydrous Na_2SO_4, and elimination of the solvent under reduced pressure, purification was carried out by chromatography on a silica gel column (60–200 μm) and elution with the indicated solvent mixture. Where solutions were filtered through a Celite pad, the pad was additionally washed with the same solvent used, and the washings were incorporated with the main organic layer. All operations (including chromatographic separations) involving combretastatin A-4 and its derivatives were performed with minimum exposure to light.

Methyl (*Z*)-4-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]butanoate (9a): Combretastatin A-4 **13** (3.16 g, 10 mmol) was dissolved under N_2 in anhydrous DMF (150 mL), treated with K_2CO_3 (3.3 g, 20 mmol) and stirred for 1 h at room temperature. After addition of methyl 4-bromobutanoate (2.7 g, 15 mmol), the reaction mixture was further stirred at room temperature for 24 h. Workup (extraction with Et_2O) was followed by column chromatography of the oily residue on silica gel (hexane/EtOAc, 9:1) to afford **9a** (3.83 g, 92%) as an oil. ^1H NMR (500 MHz): δ = 6.84 (dd, J = 8.3, 1.8 Hz, 1 H), 6.81 (d, J = 1.8 Hz, 1 H), 6.75 (d, J = 8.3 Hz, 1 H), 6.50 (s, 2 H), 6.47 (d, J = 12 Hz, 1 H), 6.42 (d, J = 12 Hz, 1 H), 3.84 (t, J = 6.2 Hz, 2 H), 3.82 (s, 3 H), 3.81 (s, 3 H), 3.68 (s, 6 H), 3.65 (s, 3 H), 2.46 (t, J = 7.3 Hz, 2 H), 2.03 (br. quint, $J \approx$ 6.7 Hz, 2 H) ppm. ^{13}C NMR (125 MHz): δ = 173.4, 152.8 (×2), 148.7, 147.7, 137.1, 132.8, 129.8 (C), 129.6, 128.8, 122.1, 113.9, 111.4, 106.0 (×2) (CH), 67.7, 30.4, 24.4 (CH_2), 60.7, 55.9 (×3), 51.4 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1737 (C=O) cm^{-1}. HRMS (ESI): *m/z* calcd. for $C_{23}H_{28}NaO_7$ [M + Na^+] 439.1728; found 439.1733.

Methyl (*Z*)-8-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]octanoate (10a): Obtained as an oil in 92% yield from **13** and methyl 8-bromooctanoate under the same conditions used for the synthesis of **9a**. ^{1}H NMR (500 MHz): δ = 6.83 (dd, J = 8.2, 1.8 Hz, 1 H), 6.82 (d, J = 1.8 Hz, 1 H), 6.75 (d, J = 8.2 Hz, 1 H), 6.51 (s, 2 H), 6.48 (d, J = 12 Hz, 1 H), 6.42 (d, J = 12 Hz, 1 H), 3.82 (s, 3 H), 3.81 (s, 3 H), 3.78 (t, J = 6.7 Hz, 2 H), 3.69 (s, 6 H), 3.65 (s, 3 H), 2.29 (t, J = 7.5 Hz, 2 H), 1.70 (br. quint, $J \approx$ 7 Hz, 2 H), 1.62 (m, 2 H), 1.40–1.30 (br. m, 6 H) ppm. ^{13}C NMR (125 MHz): δ = 174.1, 152.8 (×2), 148.7, 148.0, 137.1, 132.9, 129.8 (C), 129.6, 128.7, 121.8, 113.5, 111.3, 106.0 (×2) (CH), 68.7, 34.0, 29.0 (×2), 28.9, 25.7, 24.8 (CH_2), 60.7, 55.9 (×3), 51.3 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1737 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{27}H_{36}NaO_7$ [M + Na$^+$] 495.2359; found 495.2359.

Methyl (*Z*)-11-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]undecanoate (11a): Obtained in 91% yield from **13** and methyl 11-bromoundecanoate under the same conditions used for the synthesis of **9a**. Solid; m.p. 39–40 °C. ^{1}H NMR (500 MHz): δ = 6.85–6.80 (m, 2 H), 6.75 (d, J = 8.2 Hz, 1 H), 6.51 (s, 2 H), 6.48 (d, J = 12 Hz, 1 H), 6.42 (d, J = 12 Hz, 1 H), 3.82 (s, 3 H), 3.81 (s, 3 H), 3.78 (t, J = 6.7 Hz, 2 H), 3.69 (s, 6 H), 3.65 (s, 3 H), 2.29 (t, J = 7.5 Hz, 2 H), 1.71 (br. quint, $J \approx$ 7 Hz, 2 H), 1.60 (m, 2 H), 1.40–1.25 (br. m, 12 H) ppm. ^{13}C NMR (125 MHz): δ = 174.1, 152.8 (×2), 148.7, 148.0, 137.1, 132.9, 129.8 (C), 129.7, 128.6, 121.8, 113.5, 111.3, 106.0 (×2) (CH), 68.7, 34.0, 29.4, 29.3, 29.2, 29.1, 29.0, 28.9, 25.8, 24.8 (CH_2), 60.7, 55.9 (×3), 51.3 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1737 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{30}H_{42}NaO_7$ [M + Na$^+$] 537.2828; found 537.2826.

Methyl (*Z*)-16-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]hexadecanoate (12a): Obtained in 90% yield from **13** and methyl 16-bromohexadecanoate under the same conditions used for the synthesis of **9a**. Solid; m.p. 60–61 °C. ^{1}H NMR (500 MHz): δ = 6.80–6.75 (m, 2 H), 6.70 (d, J = 8.5 Hz, 1 H), 6.47 (s, 2 H), 6.43 (d, J = 12 Hz, 1 H), 6.37 (d, J = 12 Hz, 1 H), 3.77 (s, 3 H), 3.76 (s, 3 H), 3.73 (t, J = 6.8 Hz, 2 H), 3.64 (s, 6 H), 3.60 (s, 3 H), 2.24 (t, J = 7.5 Hz, 2 H), 1.68 (br. quint, $J \approx$ 7 Hz, 2 H), 1.56 (m, 2 H), 1.35–1.20 (br. m, 22 H) ppm. ^{13}C NMR (125 MHz): δ = 173.9, 152.7 (×2), 148.5, 147.8, 137.0, 132.7, 129.6 (C), 129.5, 128.4, 121.6, 113.3, 111.1, 105.8 (×2) (CH), 68.6, 33.8, 29.4–28.8 (eleven partially overlapped peaks), 25.7, 24.7 (CH_2), 60.5, 55.7 (×3), 51.0 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1739 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{35}H_{52}NaO_7$ [M + Na$^+$] 607.3610; found 607.3625.

(*Z*)-4-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]butanoic Acid (9b): Methyl ester **9a** (3.33 g, 8 mmol) was dissolved in MeOH (400 mL) at room temperature. After addition of a 4 M aqueous solution of NaOH (25 mL, 100 mmol), the reaction mixture was stirred for 24 h at room temperature. Workup (extraction with Et_2O) was followed by column chromatography of the oily residue on silica gel (hexane/EtOAc, 9:1) to afford acid **9b** (2.57 g, 80%) as an oil. ^{1}H NMR (500 MHz): δ = 6.87 (dd, J = 8.3, 1.8 Hz, 1 H), 6.82 (d, J = 1.8 Hz, 1 H), 6.77 (d, J = 8.3 Hz, 1 H), 6.52 (s, 2 H), 6.49 (d, J = 12 Hz, 1 H), 6.45 (d, J = 12 Hz, 1 H), 3.86 (t, J = 6.2 Hz, 2 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.70 (s, 6 H), 2.54 (t, J = 7.3 Hz, 2 H), 2.06 (br. quint, $J \approx$ 6.7 Hz, 2 H) (OH signal not detected) ppm. ^{13}C NMR (125 MHz): δ = 178.0, 153.0 (×2), 148.8, 147.7, 137.1, 133.0, 129.9 (C), 129.7, 128.8, 122.4, 114.0, 111.4, 106.0 (×2) (CH), 67.7, 30.4, 24.3 (CH_2), 60.9, 55.9 (×3) (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 3500–2500 (br., OH), 1710 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{22}H_{25}O_7$ [M – H]$^-$ 401.1606; found 401.1600.

(*Z*)-8-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]octanoic Acid (10b): Obtained in 78% yield as an oil from **10a** under the same conditions used for the synthesis of **9b**. ^{1}H NMR (500 MHz): δ = 6.87 (dd, J = 8.2, 1.8 Hz, 1 H), 6.86 (d, J = 1.8 Hz, 1 H), 6.79 (d, J = 8.2 Hz, 1 H), 6.55 (s, 2 H), 6.52 (d, J = 12 Hz, 1 H), 6.47 (d, J = 12 Hz, 1 H), 3.87 (s, 3 H), 3.86 (s, 3 H), 3.82 (t, J = 6.7 Hz, 2 H), 3.73 (s, 6 H), 2.38 (t, J = 7.5 Hz, 2 H), 1.75 (br. quint, $J \approx$ 7 Hz, 2 H), 1.66 (m, 2 H), 1.45–1.35 (br. m, 6 H) (OH signal not detected) ppm. ^{13}C NMR (125 MHz): δ = 179.5, 152.9 (×2), 148.7, 148.0, 137.1, 132.9, 129.9 (C), 129.7, 128.7, 121.9, 113.5, 111.4, 106.0 (×2) (CH), 68.7, 34.0, 29.0, 28.9 (×2), 25.7, 24.6 (CH_2), 60.8, 55.9 (×3) (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 3500–2500 (br., OH), 1711 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{26}H_{34}O_7$-H [M – H]$^-$ 457.2226; found 457.2231.

(*Z*)-11-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]undecanoic Acid (11b): Obtained in 76% yield as an oil from **11a** under the same conditions used for the synthesis of **9b**. ^{1}H NMR (500 MHz): δ = 6.85 (dd, J = 8.1, 1.8 Hz, 1 H), 6.84 (d, J = 1.8 Hz, 1 H), 6.77 (d, J = 8.1 Hz, 1 H), 6.53 (s, 2 H), 6.50 (d, J = 12 Hz, 1 H), 6.44 (d, J = 12 Hz, 1 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.80 (t, J = 6.8 Hz, 2 H), 3.70 (s, 6 H), 2.35 (t, J = 7.5 Hz, 2 H), 1.73 (br. quint, $J \approx$ 7 Hz, 2 H), 1.64 (m, 2 H), 1.40–1.25 (br. m, 12 H) (OH signal not detected) ppm. ^{13}C NMR (125 MHz): δ = 179.5, 152.9 (×2), 148.7, 148.0, 137.1, 132.9, 129.9 (C), 129.8, 128.7, 121.9, 113.5, 111.4, 106.0 (×2) (CH), 68.8, 34.0, 29.4, 29.3 (×2), 29.2, 29.0 (×2), 25.8, 24.7 (CH_2), 60.8, 55.9 (×3) (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 3500–2500 (br., OH), 1709 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{29}H_{40}O_7$-H [M – H]$^-$ 499.2696; found 499.2696.

(*Z*)-16-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]hexadecanoic Acid (12b): Obtained in 75% yield from **12a** under the same conditions used for the synthesis of **9b**. Solid; m.p. 88–89 °C. ^{1}H NMR (500 MHz): δ = 6.85 (m, 2 H), 6.77 (d, J = 8.1 Hz, 1 H), 6.53 (s, 2 H), 6.50 (d, J = 12 Hz, 1 H), 6.45 (d, J = 12 Hz, 1 H), 3.85 (s, 3 H), 3.84 (s, 3 H), 3.81 (t, J = 6.8 Hz, 2 H), 3.71 (s, 6 H), 2.36 (t, J = 7.5 Hz, 2 H), 1.74 (br. quint, $J \approx$ 7 Hz, 2 H), 1.64 (m, 2 H), 1.40–1.25 (br. m, 22 H) (OH signal not detected) ppm. ^{13}C NMR (125 MHz): δ = 179.2, 152.9 (×2), 148.7, 148.1, 137.2, 132.9, 129.9 (C), 129.8, 128.7, 121.9, 113.5, 111.4, 106.0 (×2) (CH), 68.9, 34.0, 29.6–29.0 (eleven partially overlapped peaks), 25.9, 24.7 (CH_2), 60.8, 55.9 (×3) (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 3500–2500 (br., OH), 1710 (C=O) cm^{-1}. HRMS (ESI: *m*/*z* calcd. for $C_{34}H_{50}O_7$-H [M – H]$^-$ 569.3478; found 569.3478.

(*R*)-9-(4-Methoxybenzyloxy)non-1-en-4-ol (17): Allylmagnesium bromide (1M in Et_2O, 15 mL, 15 mmol) was added dropwise under N_2 by using a syringe to a cooled solution of (–)-Ipc_2BCl (5.77 g, ca. 18 mmol) in anhydrous Et_2O (75 mL) (dry ice-acetone bath). After finishing the addition, the dry ice-acetone bath was replaced by an ice bath, and the mixture was stirred for 1 h. The solution was allowed to stand, whereby precipitation of magnesium chloride took place. The supernatant solution was carefully transferred to another flask by using a cannula. After cooling the flask to –78 °C, a solution of aldehyde **16**[21] (2.83 g, 12 mmol) in anhydrous Et_2O (35 mL) was added dropwise by using a syringe. The resulting solution was further stirred at –78 °C for 2 h, then the reaction mixture was quenched through addition of phosphate pH 7 buffer solution (15 mL), MeOH (15 mL) and 30% H_2O_2 (7 mL). After stirring for 30 min, the mixture was poured onto satd. aq. $NaHCO_3$ and worked up (extraction with Et_2O). The residue was subjected to careful column chromatography on silica gel (hexane, then hexane/EtOAc, 7:3) to afford **17** (2.87 g, 86%) as an oil. The enantiomeric ratio was found to be 96:4 by means of NMR analysis of the corresponding Mosher ester. $[\alpha]_D$ = +3.1 (c = 1.15, $CHCl_3$). ^{1}H NMR (500 MHz): δ = 7.26 (br. d, $J \approx$ 8 Hz, 2 H), 6.88 (d, $J \approx$ 8 Hz, 2 H), 5.83 (m, 1 H), 5.15–5.05 (m, 2 H), 4.44 (s, 2 H), 3.80 (s, 3 H), 3.63

(m, 1 H), 3.45 (m, 2 H), 2.30 (m, 1 H), 2.15 (m, 1 H), 1.70–1.60 (br. m, 3 H), 1.50–1.30 (br. m, 6 H) ppm. ^{13}C NMR (125 MHz): δ = 158.7, 130.7 (C), 134.8, 129.2 (×2), 113.7 (×2), 70.6 (CH), 118.0, 72.5, 70.0, 41.9, 36.8, 29.7, 26.2, 25.5 (CH_2), 55.2 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 3430 (br., OH) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{17}H_{26}NaO_3$ [M + Na]$^+$ 301.1780; found 301.1777.

(*S*)-9-(4-Methoxybenzyloxy)non-1-en-4-ol (*ent*-17): Obtained with similar yield and *er* through the same procedure as above except that the chiral reagent (+)-Ipc_2BCl was used. $[\alpha]_D$ = –2.9 (*c* = 1.65, $CHCl_3$). Spectral data as for **17**.

(*R*)-9-(4-Methoxybenzyloxy)non-1-en-4-yl Acrylate (18): Alcohol **17** (1.4 g, 5 mmol) was dissolved under N_2 in anhydrous CH_2Cl_2 (100 mL), then cooled to –78 °C and treated sequentially with ethyl diisopropylamine (2.6 mL, 15 mmol) and acryloyl chloride (800 µL, 10 mmol). The reaction mixture was stirred for 1 h at –78 °C and then worked up (extraction with CH_2Cl_2). Column chromatography on silica gel (hexane/EtOAc, 7:3) afforded **18** (1.56 g, 94%) as an oil. $[\alpha]_D$ = +12.5 (*c* = 0.22, $CHCl_3$). ^{1}H NMR (500 MHz): δ = 7.25 (br. d, *J* ≈ 8 Hz, 2 H), 6.87 (d, *J* ≈ 8 Hz, 2 H), 6.38 (dd, *J* = 17.5, 1.5 Hz, 1 H), 6.10 (dd, *J* = 17.5, 10 Hz, 1 H), 5.80–5.70 (m, 2 H), 5.10–4.95 (m, 3 H), 4.41 (s, 2 H), 3.79 (s, 3 H), 3.42 (t, *J* = 6.8 Hz, 2 H), 2.40–2.30 (m, 2 H), 1.65–1.50 (br. m, 4 H), 1.40–1.25 (br. m, 4 H) ppm. ^{13}C NMR (125 MHz): δ = 165.8, 159.0, 130.7 (C), 133.6, 130.3, 129.2 (×2), 113.7 (×2), 73.4 (CH), 128.8, 117.6, 72.5, 69.9, 38.5, 33.5, 29.5, 26.0, 25.1 (CH_2), 55.2 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1718 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{20}H_{28}NaO_4$ [M + Na]$^+$ 355.1885; found 355.1880.

(*S*)-9-(4-Methoxybenzyloxy)non-1-en-4-yl Acrylate (*ent*-18): Obtained from *ent*-**17** as an oil in 90% yield by using the same procedure used for the preparation of **18**. $[\alpha]_D$ = –10.8 (*c* = 0.2, $CHCl_3$). Spectral data as for **18**.

(6*R*)-6-[5-(4-Methoxybenzyloxy)pentyl]-5,6-dihydro-2*H*-pyran-2-one (19): Diolefin **18** (1.33 g, 4 mmol) was dissolved under N_2 in anhydrous, degassed CH_2Cl_2 (500 mL) and treated with Grubbs ruthenium catalyst **Ru-I** (320 mg, ca. 0.4 mmol). The mixture was stirred and heated at reflux until consumption of the starting material (ca. 2–3 h; reaction monitored by TLC). DMSO was then added (1 mL) and the mixture was further stirred overnight at room temperature. Solvent removal under reduced pressure and column chromatography of the residue on silica gel (hexanes/EtOAc, 7:3) furnished dihydropyrone **19** (987 mg, 81%) as an oil. $[\alpha]_D$ = –49.7 (*c* = 1.1, $CHCl_3$). ^{1}H NMR (500 MHz): δ = 7.25 (br. d, *J* ≈ 8 Hz, 2 H), 6.87 (m, 3 H), 6.00 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.41 (s, 2 H), 4.40 (m, 1 H), 3.80 (s, 3 H), 3.44 (t, *J* = 6.5 Hz, 2 H), 2.35–2.25 (m, 2 H), 1.80 (m, 1 H), 1.70–1.35 (br. m, 7 H) ppm. ^{13}C NMR (125 MHz): δ = 164.5, 159.1, 130.7 (C), 145.0, 129.2 (×2), 121.4, 113.7 (×2), 77.8 (CH), 72.5, 69.8, 34.8, 29.6, 29.3, 26.0, 24.6 (CH_2), 55.2 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1719 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{18}H_{24}NaO_4$ [M + Na]$^+$ 327.1572; found 327.1576.

(6*S*)-6-[5-(4-Methoxybenzyloxy)pentyl]-5,6-dihydro-2*H*-pyran-2-one (*ent*-19): Obtained from *ent*-**18** as an oil in 80% yield by using the same procedure used for the preparation of **19**. $[\alpha]_D$ = +21.8 (*c* = 0.15, $CHCl_3$). Spectral data as for **19**.

(6*R*)-6-(5-Hydroxypentyl)-5,6-dihydro-2*H*-pyran-2-one (14): PMB derivative **19** (152 mg, 0.5 mmol) was dissolved in a CH_2Cl_2/H_2O mixture (20:1, 10 mL) and treated with DDQ (160 mg, ca. 0.7 mmol). The mixture was then stirred at room temperature until consumption of the starting material (45–60 min; reaction monitored by TLC). Workup (extraction with CH_2Cl_2) was followed by column chromatography of the oily residue on silica gel (hexanes/EtOAc, from 1:1 to 1:3) to provided **19** (64 mg, 69%) as an oil. $[\alpha]_D$ = –87.1 (*c* = 0.89, $CHCl_3$). ^{1}H NMR (500 MHz): δ = 6.88 (m, 1 H), 6.02 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.43 (m, 1 H), 3.65 (t, *J* = 6.6 Hz, 2 H), 2.40–2.30 (m, 2 H), 1.68 (m, 1 H), 1.70–1.35 (br. m, 8 H) ppm. ^{13}C NMR (125 MHz): δ = 164.6 (C), 145.0, 121.4, 77.8 (CH), 62.7, 34.8, 32.5, 29.4, 25.5, 24.6 (CH_2) ppm. IR: $\tilde{\nu}_{max}$ = 1712 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{10}H_{17}O_3$ [M + H]$^+$ 185.1178; found 185.1176.

(6*S*)-6-(5-Hydroxypentyl)-5,6-dihydro-2*H*-pyran-2-one (*ent*-14): Obtained from *ent*-**19** in 67% yield as an oil by using the same procedure as for the preparation of **14**. $[\alpha]_D$ = +84.1 (*c* = 1.3, $CHCl_3$). Spectral data as for **14**.

(*S*)-1-(4-Methoxybenzyloxy)hex-5-en-3-ol (21): Obtained with 85% yield and 94:6 *er* as an oil by using the same procedure used for the preparation of **17**. $[\alpha]_D$ = –3.6 (*c* = 1.6, $CHCl_3$). ^{1}H NMR (500 MHz): δ = 7.27 (br. d, *J* = 8.3 Hz, 2 H), 6.89 (d, *J* = 8.3 Hz, 2 H), 5.83 (ddt, *J* = 17, 10.3, 7.3 Hz, 1 H), 5.15–5.05 (m, 2 H), 4.47 (s, 2 H), 3.85 (m, 1 H), 3.81 (s, 3 H), 3.68 (m, 1 H), 3.61 (m, 1 H), 2.90 (br. s, 1 H, OH), 2.24 (t, *J* = 6.6 Hz, 2 H), 1.80–1.70 (br. m, 2 H) ppm. ^{13}C NMR (125 MHz): δ = 159.2, 130.1 (C), 134.8, 129.2 (×2), 113.8 (×2), 70.3 (CH), 117.4, 72.8, 68.5, 41.9, 35.8 (CH_2), 55.2 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 3460 (br., OH) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{14}H_{20}NaO_3$ [M + Na]$^+$ 259.1310; found 259.1313.

(*S*)-1-Methoxy-4-[(3-methoxyhex-5-en-1-yloxy)methyl]benzene (22): Sodium hydride (60% slurry in mineral oil, amount equivalent to 20 mmol) was washed two times under N_2 with anhydrous hexane and once with anhydrous THF. Then, THF (75 mL) was added and the suspension was cooled in an ice bath. Alcohol **21** (2.36 g, 10 mmol) was then dissolved in anhydrous THF (25 mL) and added dropwise to the sodium hydride suspension. The mixture was then allowed to reach room temperature and methyl iodide (1.87 mL, ca. 30 mmol) was added in one portion and the mixture was stirred overnight at room temperature. Workup (Et_2O) was followed by column chromatography on silica gel (hexanes/EtOAc, 9:1) to afford **22** (2.2 g, 88%) as an oil. $[\alpha]_D$ = +20.4 (*c* = 1.4, $CHCl_3$). ^{1}H NMR (500 MHz): δ = 7.30 (br. d, *J* = 8.3 Hz, 2 H), 6.92 (d, *J* = 8.3 Hz, 2 H), 5.86 (ddt, *J* = 17, 10.3, 7.2 Hz, 1 H), 5.15–5.10 (m, 2 H), 4.48 (s, 2 H), 3.83 (s, 3 H), 3.65–3.55 (m, 2 H), 3.45 (m, 1 H), 3.38 (s, 3 H), 2.30 (t, *J* = 6.6 Hz, 2 H), 1.85–1.75 (br. m, 2 H) ppm. ^{13}C NMR (125 MHz): δ = 159.1, 130.6 (C), 134.5, 129.1 (×2), 113.6 (×2), 77.4 (CH), 116.9, 72.5, 66.5, 37.8, 33.8 (CH_2), 56.6, 55.1 (CH_3) ppm. HRMS (ESI): *m*/*z* calcd. for $C_{15}H_{22}NaO_3$ [M + Na]$^+$ 273.1467; found 273.1459.

(4*R*,6*R*)-6-Methoxy-8-(4-methoxybenzyloxy)oct-1-en-4-ol (23): Olefin **22** (1.5 g, ca. 6 mmol) was dissolved in a THF/*t*BuOH/H_2O mixture (4:10:1, 120 mL), and the solution was treated with NMO (820 mg, 7 mmol) and 4% aq. OsO_4 (1 mL, ca. 0.15 mmol). The reaction mixture was then stirred overnight at room temperature and quenched by the addition of solid Na_2SO_3 (7.5 g), followed by additional stirring for 30 min. Extraction with EtOAc was followed by desiccation of the organic layer on anhydrous $MgSO_4$ and solvent removal under reduced pressure. The crude 1,2-diol was then dissolved in anhydrous CH_2Cl_2 (75 mL), cooled in an ice bath and treated with $Pb(OAc)_4$ (3.1 g, 7 mmol). The reaction mixture was then stirred at 0 °C until consumption of the starting material (ca. 2–3 h; reaction monitored by TLC). The reaction was then quenched by addition of solid Na_2CO_3 (6 g), and the mixture was stirred for 1 h. Filtration through Celite and solvent removal under reduced pressure gave a crude aldehyde, which was used without purification in the next step.

Allylmagnesium bromide (1M in Et_2O, 8 mL, 8 mmol) was added dropwise under N_2 by using a syringe to a cooled solution of (+)-Ipc_2BCl (3.2 g, ca. 10 mmol) in anhydrous Et_2O (50 mL) (dry ice-

acetone bath). After finishing the addition, the dry ice-acetone bath was replaced by an ice bath, and the mixture was stirred for 1 h. The solution was allowed to stand, whereby precipitation of magnesium chloride took place. The supernatant solution was carefully transferred to another flask by using a cannula. After cooling this flask at –78 °C, a solution of the crude aldehyde from above dissolved in anhydrous Et_2O (15 mL) was added dropwise by using a syringe. The resulting solution was further stirred at –78 °C for 2 h. The reaction mixture was quenched through addition of phosphate pH 7 buffer solution (40 mL), MeOH (40 mL) and 30% H_2O_2 (20 mL). After stirring for 30 min, the mixture was poured onto satd. aq. $NaHCO_3$ and worked up (extraction with Et_2O). The residue was subjected to careful column chromatography on silica gel (hexane/EtOAc, 4:1) to afford **23** (1.58 g, 90% overall from **22**) as an 86:14 mixture of diastereoisomers, which was used as such in the next step. An aliquot was purified for analytical purposes: oil. $[\alpha]_D$ = –2.2 (*c* = 1.3, $CHCl_3$). ^1H NMR (500 MHz): δ = 7.28 (br. d, *J* = 8.3 Hz, 2 H), 6.90 (d, *J* = 8.3 Hz, 2 H), 5.84 (ddt, *J* = 17, 10.3, 7.2 Hz, 1 H), 5.15–5.10 (m, 2 H), 4.45 (s, 2 H), 3.94 (m, 1 H), 3.81 (s, 3 H), 3.67 (br. qd, *J* ≈ 6.5, 4 Hz, 1 H), 3.60–3.50 (m, 2 H), 3.38 (s, 3 H), 3.00 (br. s, 1 H, OH), 2.30–2.20 (m, 2 H), 1.95 (dq, *J* = 14, 6.3 Hz, 1 H), 1.80 (m, 1 H), 1.71 (ddd, *J* = 14, 9.5, 4 Hz, 1 H), 1.60 (ddd, *J* = 14, 7, 3 Hz, 1 H) ppm. ^{13}C NMR (125 MHz): δ = 159.1, 130.4 (C), 134.8, 129.1 (×2), 113.7 (×2), 76.6, 67.9 (CH), 117.4, 72.6, 66.4, 42.2, 39.2, 33.5 (CH_2), 57.0, 55.1 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 3450 (br., OH) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{17}H_{26}NaO_4$ $[M + Na]^+$ 317.1729; found 317.1723.

(4*R*,6*R*)-4-(*tert*-Butyldimethylsilyloxy)-6-methoxy-8-(4-methoxybenzyloxy)oct-1-ene (24): Alcohol **23** (1.18 g, 4 mmol) was dissolved under N_2 in anhydrous CH_2Cl_2 (30 mL), cooled in an ice bath, 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 2 h at 0 °C and worked up (extraction with CH_2Cl_2). Column chromatography on silica gel (hexane/EtOAc, 9:1) afforded **24** (1.3 g, 80%) as an oil. $[\alpha]_D$ = –25.6 (*c* = 1.5, $CHCl_3$). ^1H NMR (500 MHz): δ = 7.28 (br. d, *J* = 8.3 Hz, 2 H), 6.90 (d, *J* = 8.3 Hz, 2 H), 5.84 (ddt, *J* = 17, 10.3, 7.2 Hz, 1 H), 5.10–5.05 (m, 2 H), 4.45 (s, 2 H), 3.95 (m, 1 H), 3.82 (s, 3 H), 3.60–3.50 (m, 3 H), 3.36 (s, 3 H), 2.30–2.20 (br. m, 2 H), 1.86 (dq, *J* = 14, 6.3 Hz, 1 H), 1.77 (m, 1 H), 1.64 (ddd, *J* = 14, 8.8, 3.5 Hz, 1 H), 1.52 (ddd, *J* = 14, 8.8, 3.5 Hz, 1 H), 0.92 (s, 9 H), 0.10 (s, 3 H), 0.09 (s, 3 H) ppm. ^{13}C NMR (125 MHz): δ = 159.1, 130.6, 18.1 (C), 134.8, 129.2 (×2), 113.7 (×2), 74.9, 68.5 (CH), 117.0, 72.7, 66.5, 42.6, 42.0, 33.6 (CH_2), 56.0, 55.2, 25.9 (×3), –4.1, –4.7 (CH_3) ppm. HRMS (ESI): *m*/*z* calcd. for $C_{23}H_{40}NaO_4Si$ $[M + Na]^+$ 431.2594; found 431.2591.

(4*S*,6*R*,8*R*)-6-(*tert*-Butyldimethylsilyloxy)-8-methoxy-10-(4-methoxybenzyloxy)dec-1-en-4-ol (25): Obtained (75% of a 76:24 mixture of diastereoisomers) from olefin **24** under the same conditions used for the preparation of **23**: oil. $[\alpha]_D$ = –25.7 (*c* = 2.5, $CHCl_3$). ^1H NMR (500 MHz): δ = 7.26 (br. d, *J* = 8.3 Hz, 2 H), 6.88 (d, *J* = 8.3 Hz, 2 H), 5.84 (ddt, *J* = 17, 10.3, 7.2 Hz, 1 H), 5.15–5.05 (m, 2 H), 4.43 (s, 2 H), 4.17 (m, 1 H), 4.03 (m, 1 H), 3.80 (s, 3 H), 3.55–3.45 (m, 3 H), 3.42 (m, 1 H), 3.30 (s, 3 H), 2.30–2.15 (br. m, 2 H), 1.86 (dq, *J* = 14, 6.5 Hz, 1 H), 1.80–1.65 (br. m, 4 H), 1.57 (ddd, *J* = 14, 4, 2.5 Hz, 1 H), 0.90 (s, 9 H), 0.11 (s, 3 H), 0.09 (s, 3 H) ppm. ^{13}C NMR (125 MHz): δ = 159.1, 130.4, 17.8 (C), 134.8, 129.1 (×2), 113.7 (×2), 75.0, 69.0, 67.8 (CH), 117.1, 72.7, 66.3, 42.3, 41.8, 41.1, 33.4 (CH_2), 55.9, 55.1, 25.8 (×3), –4.5, –4.9 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 3460 (br., OH) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{25}H_{44}NaO_5Si$ $[M + Na]^+$ 475.2856; found 475.2851.

(4*S*,6*S*,8*R*)-6-(*tert*-Butyldimethylsilyloxy)-8-methoxy-10-(4-methoxybenzyloxy)dec-1-en-4-yl Acrylate (26): Obtained in 91% yield from **25** under the same conditions used for the preparation of **18**. $[\alpha]_D$ = +16.9 (*c* = 1.75, $CHCl_3$). ^1H NMR (500 MHz): δ = 7.24 (br. d, *J* = 8.3 Hz, 2 H), 6.85 (d, *J* = 8.3 Hz, 2 H), 6.34 (dd, *J* = 17.5, 1.5 Hz, 1 H), 6.07 (dd, *J* = 17.5, 10.5 Hz, 1 H), 5.80–5.70 (m, 2 H), 5.10–5.05 (m, 3 H), 4.41 (br. s, 2 H), 3.85 (m, 1 H), 3.80 (s, 3 H), 3.55–3.40 (br. m, 3 H), 3.30 (s, 3 H), 2.40–2.30 (br. m, 2 H), 1.85–1.65 (br. m, 5 H), 1.57 (ddd, *J* = 14, 6.5, 4.5 Hz, 1 H), 0.87 (s, 9 H), 0.04 (s, 3 H), 0.03 (s, 3 H) ppm. ^{13}C NMR (125 MHz): δ = 165.6, 159.1, 130.6, 18.0 (C), 133.3, 130.3, 129.2 (×2), 113.7 (×2), 75.2, 71.1, 66.8 (CH), 128.9, 117.1, 72.7, 66.5, 43.0, 41.8, 39.0, 33.9 (CH_2), 56.1, 55.2, 25.9 (×3), –4.3, –4.4 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1723 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{28}H_{46}NaO_6Si$ $[M + Na]^+$ 529.2961; found 529.2963.

(6*S*)-6-{(2*S*,4*R*)-[2-(*tert*-Butyldimethylsilyloxy)-4-methoxy-6-(4-methoxybenzyloxy)hexyl]}-5,6-dihydro-2*H*-pyran-2-one (27): Obtained from **26** in 83% yield by using the same procedure used for the preparation of **19**: oil. $[\alpha]_D$ = –14 (*c* = 1, $CHCl_3$). ^1H NMR (500 MHz): δ = 7.25 (br. d, *J* = 8.3 Hz, 2 H), 6.88 (m, 3 H), 6.00 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.58 (m, 1 H), 4.42 (s, 2 H), 4.09 (m, 1 H), 3.78 (s, 3 H), 3.55–3.45 (m, 2 H), 3.40 (m, 1 H), 3.30 (s, 3 H), 2.30 (m, 2 H), 2.00 (ddd, *J* = 14, 9.3, 3.5 Hz, 1 H), 1.85–1.60 (br. m, 5 H), 0.88 (s, 9 H), 0.07 (s, 3 H), 0.06 (s, 3 H) ppm. ^{13}C NMR (125 MHz): δ = 164.1, 159.1, 130.5, 18.0 (C), 145.1, 129.2 (×2), 121.4, 113.7 (×2), 75.2, 74.4, 65.9 (CH), 72.6, 66.3, 43.3, 43.2, 33.9, 29.9 (CH_2), 56.1, 55.2, 25.9 (×3), –4.4, –4.5 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1728 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{26}H_{42}NaO_6Si$ $[M + Na]^+$ 501.2648; found 501.2653.

(6*S*)-6-{(2*S*,4*R*)-[2-(*tert*-Butyldimethylsilyloxy)-6-hydroxy-4-methoxyhexyl]}-5,6-dihydro-2*H*-pyran-2-one (28): Obtained from **27** in 74% yield by using the same procedure as for the preparation of **14**: amorphous solid; $[\alpha]_D$ = –2.2 (*c* = 1, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.89 (dt, *J* = 9.5, 4.5 Hz, 1 H), 6.01 (dt, *J* = 9.5, 1.8 Hz, 1 H), 4.59 (m, 1 H), 4.08 (m, 1 H), 3.80–3.70 (m, 2 H), 3.47 (m, 1 H), 3.34 (s, 3 H), 2.40 (br. s, 1 H, OH), 2.33 (m, 2 H), 1.98 (ddd, *J* = 14, 9.6, 3.3 Hz, 1 H), 1.90–1.75 (br. m, 2 H), 1.70–1.60 (br. m, 3 H), 0.88 (s, 9 H), 0.09 (s, 3 H), 0.07 (s, 3 H) ppm. ^{13}C NMR (125 MHz): δ = 164.1, 18.0 (C), 145.2, 121.4, 77.2, 74.4, 65.7 (CH), 60.1, 43.0, 42.6, 35.7, 29.9 (CH_2), 56.2, 25.8 (×3), –4.4, –4.6 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1720 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{18}H_{34}NaO_5Si$ $[M + Na]^+$ 381.2073; found 381.2064.

(6*S*)-6-[(2*S*,4*R*)-2,6-Dihydroxy-4-methoxyhexyl]-5,6-dihydro-2*H*-pyran-2-one (15): Silylated compound **28** (72 mg, 0.2 mmol) was dissolved in MeOH (8 mL) and treated with PPTS (10 mg, 0.04 mmol) and water (0.1 mL). The mixture was then heated at reflux for 18 h, cooled, and neutralized by addition of solid $NaHCO_3$ (10 mg). After filtering, the solution was evaporated under reduced pressure, and the oily residue was subjected to column chromatography on silica gel (hexane/EtOAc, 1:1) to provide **15** (42 mg, 85%) as an oil; $[\alpha]_D$ = –7.1 (*c* = 0.71, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.89 (ddd, *J* = 9.8, 5.5, 3.5 Hz, 1 H), 6.01 (dt, *J* = 9.8, 1.5 Hz, 1 H), 4.72 (m, 1 H), 4.23 (tt, *J* = 9.5, 2.5 Hz, 1 H), 3.80–3.70 (br. m, 3 H), 3.45 (br. s, 1 H, OH), 3.39 (s, 3 H), 2.60 (br. s, 1 H, OH), 2.40–2.35 (m, 2 H), 1.95–1.60 (br. m, 6 H) ppm. ^{13}C NMR (125 MHz): δ = 164.5 (C), 145.4, 121.3, 77.9, 75.0, 64.7 (CH), 59.8, 42.8, 39.8, 35.5, 30.0 (CH_2), 56.8 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 3400 (br., OH), 1710 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{12}H_{20}NaO_5Si$ $[M + Na]^+$ 267.1208; found 267.1209.

5-[(2*R*)-6-Oxo-3,6-dihydro-2*H*-pyran-2-yl]pentyl (*Z*)-4-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]butanoate (1): Acid **9b** (104 mg, 0.25 mmol) was dissolved under N_2 in anhydrous THF (5 mL) and treated sequentially with Et_3N (105 µL, 0.75 mmol) and 2,4,6-trichlorobenzoyl chloride (80 µL, ca. 0.5 mmol). The mixture was

then stirred at room temperature for 3 h. Subsequently, a solution of alcohol **14** (41 mg, 0.22 mmol) and DMAP (61 mg, 0.5 mmol) in anhydrous THF (2 mL) was added dropwise. The mixture was then further stirred at room temperature for 2 h. Workup (extraction with Et_2O) was followed by column chromatography of the oily residue on silica gel (hexane/EtOAc, 3:2) to provide ester **1** (54 mg, 38% based on **14**) as an amorphous solid. $[\alpha]_D$ = +30.9 (*c* = 1.88, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.90–6.80 (m, 3 H), 6.76 (d, *J* = 8.3 Hz, 1 H), 6.52 (s, 2 H), 6.49 (d, *J* = 12 Hz, 1 H), 6.44 (d, *J* = 12 Hz, 1 H), 6.01 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.40 (m, 1 H), 4.07 (t, *J* = 6.6 Hz, 2 H), 3.86 (t, *J* = 6.4 Hz, 2 H), 3.83 (s, 6 H), 3.70 (s, 6 H), 2.48 (t, *J* = 7.3 Hz, 2 H), 2.32 (m, 2 H), 2.05 (br. quint, *J* ≈ 7 Hz, 2 H), 1.80 (m, 1 H), 1.70–1.35 (br. m, 7 H) ppm. ^{13}C NMR (125 MHz): δ = 173.1, 164.4, 152.9 (×2), 148.7, 147.8, 137.2, 132.9, 129.9 (C), 144.9, 129.6, 128.9, 122.2, 121.5, 114.0, 111.5, 106.0 (×2), 77.8 (CH), 67.8, 64.3, 34.7, 30.7, 29.4, 28.5, 25.7, 24.5 (×2) (CH_2), 60.8, 55.9 (×3) (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1731 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{32}H_{40}NaO_9$ [M + Na^+] 591.2570; found 591.2570.

5-[(2*S*)-6-Oxo-3,6-dihydro-2*H*-pyran-2-yl]pentyl (*Z*)-4-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]butanoate (*ent*-1): Obtained in 42% yield from **9b** and *ent*-**14** under the same conditions used for the preparation of **1**: amorphous solid. $[\alpha]_D$ = –25 (*c* = 1.74, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1726 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{32}H_{40}NaO_9$ [M + Na^+] 591.2570; found 591.2567. NMR spectroscopic data as for **1**.

5-[(2*R*)-6-Oxo-3,6-dihydro-2*H*-pyran-2-yl]pentyl (*Z*)-8-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]octanoate (2): Obtained in 39% yield from **10b** and **14** under the same conditions used for the preparation of **1**: amorphous solid. $[\alpha]_D$ = +24.9 (*c* = 2, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.85–6.80 (m, 3 H), 6.75 (d, *J* = 8.3 Hz, 1 H), 6.51 (s, 2 H), 6.48 (d, *J* = 12 Hz, 1 H), 6.42 (d, *J* = 12 Hz, 1 H), 6.00 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.40 (m, 1 H), 4.05 (t, *J* = 6.6 Hz, 2 H), 3.82 (s, 3 H), 3.81 (s, 3 H), 3.78 (t, *J* = 6.8 Hz, 2 H), 3.69 (s, 6 H), 2.35–2.25 (m, 4 H), 1.80 (m, 1 H), 1.75–1.30 (br. m, 17 H) ppm. ^{13}C NMR (125 MHz): δ = 173.7, 164.3, 152.9 (×2), 148.6, 147.9, 137.2, 132.9, 129.9 (C), 144.9, 129.7, 128.7, 121.8, 121.4, 113.5, 111.3, 106.0 (×2), 77.7 (CH), 68.7, 64.0, 34.7, 34.2, 29.3 (×2), 29.0 (×2), 28.5, 25.7, 25.6, 24.8, 24.4 (CH_2), 60.8, 55.9 (×3) (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1726 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{36}H_{48}NaO_9$ [M + Na^+] 647.3196; found 647.3196.

5-[(2*S*)-6-Oxo-3,6-dihydro-2*H*-pyran-2-yl]pentyl (*Z*)-8-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]octanoate (*ent*-2): Obtained in 43% yield from **10b** and *ent*-**14** under the same conditions used for the preparation of **1**: amorphous solid. $[\alpha]_D$ = –25.4 (*c* = 1.81, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1726 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{36}H_{48}NaO_9$ [M + Na^+] 647.3196; found 647.3193. NMR spectroscopic data as for **2**.

5-[(2*R*)-6-Oxo-3,6-dihydro-2*H*-pyran-2-yl]pentyl (*Z*)-11-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]undecanoate (3): Obtained in 37% yield from **11b** and **14** under the same conditions used for the preparation of **1**: amorphous solid. $[\alpha]_D$ = +21.1 (*c* = 1.3, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.90–6.80 (m, 3 H), 6.74 (d, *J* = 8.3 Hz, 1 H), 6.52 (s, 2 H), 6.50 (d, *J* = 12 Hz, 1 H), 6.44 (d, *J* = 12 Hz, 1 H), 6.02 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.41 (m, 1 H), 4.07 (t, *J* = 6.6 Hz, 2 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.80 (t, *J* = 6.8 Hz, 2 H), 3.70 (s, 6 H), 2.35–2.25 (m, 4 H), 1.80 (m, 1 H), 1.75–1.55 (br. m, 9 H), 1.50–1.25 (br. m, 14 H) ppm. ^{13}C NMR (125 MHz): δ = 173.9, 164.4, 152.9 (×2), 148.7, 148.0, 137.2, 132.9, 129.9 (C), 144.9, 129.7, 128.7, 121.8, 121.5, 113.5, 111.4, 106.0 (×2), 77.7 (CH), 68.8, 64.0, 34.7, 34.3, 29.4–29.0 (seven partially overlapped signals), 28.5, 25.9, 25.7, 24.9, 24.5 (CH_2), 60.8, 55.9 (×3) (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1721 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{39}H_{54}NaO_9$ [M + Na^+] 689.3666; found 689.3665.

5-[(2*S*)-6-Oxo-3,6-dihydro-2*H*-pyran-2-yl]pentyl (*Z*)-11-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]undecanoate (*ent*-3): Obtained in 38% yield from **11b** and *ent*-**14** under the same conditions used for the preparation of **1**: amorphous solid. $[\alpha]_D$ = –20.3 (*c* = 1.86, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1718 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{39}H_{54}NaO_9$ [M + Na^+] 689.3666; found 689.3671. NMR spectroscopic data as for **3**.

5-[(2*R*)-6-Oxo-3,6-dihydro-2*H*-pyran-2-yl]pentyl (*Z*)-16-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]hexadecanoate (4): Obtained in 35% yield from **12b** and **14** under the same conditions used for the preparation of **1**: amorphous solid. $[\alpha]_D$ = +17.4 (*c* = 2, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.85–6.80 (m, 3 H), 6.74 (d, *J* = 8.3 Hz, 1 H), 6.50 (s, 2 H), 6.47 (d, *J* = 12 Hz, 1 H), 6.41 (d, *J* = 12 Hz, 1 H), 6.00 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.39 (m, 1 H), 4.04 (t, *J* = 6.6 Hz, 2 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.77 (t, *J* = 6.8 Hz, 2 H), 3.68 (s, 6 H), 2.35–2.25 (m, 4 H), 1.80–1.50 (br. m, 10 H), 1.45–1.20 (br. m, 24 H) ppm. ^{13}C NMR (125 MHz): δ = 173.9, 164.4, 152.9 (×2), 148.7, 148.0, 137.1, 132.9, 129.9 (C), 144.9, 129.7, 128.7, 121.8, 121.5, 113.5, 111.3, 106.0 (×2), 77.7 (CH), 68.8, 64.0, 34.7, 34.3, 29.6–29.0 (twelve partially overlapped signals), 28.5, 25.9, 25.7, 24.9, 24.5 (CH_2), 60.8, 55.9 (×3) (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1726 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{44}H_{64}NaO_9$ [M + Na^+] 759.4448; found 759.4456.

5-[(2*S*)-6-Oxo-3,6-dihydro-2*H*-pyran-2-yl]pentyl (*Z*)-16-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phen-oxy]hexadecanoate (*ent*-4): Obtained in 40% yield from **12b** and *ent*-**14** under the same conditions used for the preparation of **1**: amorphous solid. $[\alpha]_D$ = –21.2 (*c* = 1.68, $CHCl_3$). IR: $\tilde{\nu}_{max}$ = 1726 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{44}H_{64}NaO_9$ [M + Na^+] 759.4448; found 759.4456. NMR spectroscopic data as for **4**.

(3*R*,5*S*)-5-(*tert*-Butyldimethylsilyloxy)-3-methoxy-6-[(*S*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]hexyl 4-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]Butanoate (29): Obtained in 41% yield from **9b** and **28** under the same conditions used for the preparation of **1**: amorphous solid. $[\alpha]_D$ = –24.5 (*c* = 0.2, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.90–6.80 (m, 3 H), 6.76 (d, *J* = 8.2 Hz, 1 H), 6.50 (s, 2 H), 6.48 (d, *J* = 12 Hz, 1 H), 6.43 (d, *J* = 12 Hz, 1 H), 6.00 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.58 (m, 1 H), 4.20–4.10 (m, 3 H), 3.87 (t, *J* = 6 Hz, 2 H), 3.83 (s, 6 H), 3.70 (s, 6 H), 3.39 (m, 1 H), 3.30 (s, 3 H), 2.48 (t, *J* = 7.3 Hz, 2 H), 2.32 (m, 2 H), 2.10–1.95 (br. m, 3 H), 1.82 (m, 2 H), 1.75–1.55 (br. m, 3 H), 0.88 (s, 9 H), 0.10 (s, 3 H), 0.08 (s, 3 H) ppm. ^{13}C NMR (125 MHz): δ = 173.0, 164.2, 152.9 (×2), 148.8, 148.0, 137.2, 132.9, 130.0, 18.0 (C), 145.1, 129.6, 128.9, 122.2, 121.5, 114.1, 111.5, 106.0 (×2), 74.8, 74.4, 65.9 (CH), 67.8, 61.1, 43.3, 43.2, 32.7, 30.7, 30.0, 24.5 (CH_2), 60.8, 56.1, 55.9 (×3), 25.9 (×3), –4.4, –4.5 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1729 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{40}H_{58}NaO_{11}Si$ [M + Na^+] 765.3646; found 765.3639.

(3*R*,5*S*)-5-(*tert*-Butyldimethylsilyloxy)-3-methoxy-6-[(*S*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]hexyl 8-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]octanoate (30): Obtained in 40% yield from **10b** and **28** under the same conditions used for the preparation of **1**: amorphous solid. $[\alpha]_D$ = –7.2 (*c* = 0.8, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.90–6.80 (m, 3 H), 6.76 (d, *J* = 8 Hz, 1 H), 6.52 (s, 2 H), 6.50 (d, *J* = 12 Hz, 1 H), 6.44 (d, *J* = 12 Hz, 1 H), 6.02 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.58 (m, 1 H), 4.20–4.10 (m, 3 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.78 (t, *J* = 6.6 Hz, 2 H), 3.70 (s, 6 H), 3.40 (m, 1 H), 3.31 (s, 3 H), 2.35–2.25 (br. m, 4 H), 2.00 (ddd, *J* = 14, 9.3, 3.5 Hz, 1 H), 1.85–1.55 (br. m, 9 H), 1.45–1.30 (br. m, 6 H), 0.89 (s, 9 H), 0.10 (s, 3 H), 0.08 (s, 3 H) ppm. ^{13}C NMR (125 MHz): δ

= 173.7, 164.2, 152.9 (×2), 148.6, 148.0, 137.0, 132.9, 129.8, 18.0 (C), 145.1, 129.7, 128.7, 121.8, 121.4, 113.4, 111.2, 105.9 (×2), 74.8, 74.4, 65.8 (CH), 68.7, 60.7, 43.3, 43.2, 34.3, 32.7, 30.0, 29.0 (×3), 25.8, 24.8 (CH_2), 60.8, 56.1, 55.9 (×3), 25.9 (×3), –4.4, –4.5 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1728 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{44}H_{66}NaO_{11}Si$ [M + Na^+] 821.4272; found 821.4277.

(3*R*,5*S*)-5-(*tert*-Butyldimethylsilyloxy)-3-methoxy-6-[(*S*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]hexyl 11-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]undecanoate (31): Obtained in 40% yield from **11b** and **28** under the same conditions used for the preparation of **1**: amorphous solid. $[\alpha]_D$ = –8.3 (*c* = 1.23, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.90–6.80 (m, 3 H), 6.76 (d, *J* = 8.1 Hz, 1 H), 6.52 (s, 2 H), 6.50 (d, *J* = 12 Hz, 1 H), 6.44 (d, *J* = 12 Hz, 1 H), 6.02 (dt, *J* = 9.8, 1.5 Hz, 1 H), 4.58 (m, 1 H), 4.20–4.10 (m, 3 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.79 (t, *J* = 6.7 Hz, 2 H), 3.70 (s, 6 H), 3.40 (m, 1 H), 3.31 (s, 3 H), 2.35–2.25 (br. m, 4 H), 2.00 (ddd, *J* = 14, 9.3, 3.5 Hz, 1 H), 1.82 (m, 2 H), 1.75–1.60 (br. m, 7 H), 1.40–1.25 (br. m, 12 H), 0.88 (s, 9 H), 0.10 (s, 3 H), 0.08 (s, 3 H) ppm. ^{13}C NMR (125 MHz): δ = 173.7, 164.2, 152.9 (×2), 148.7, 148.0, 137.0, 132.9, 129.8, 18.0 (C), 145.1, 129.7, 128.7, 121.8, 121.4, 113.5, 111.4, 105.9 (×2), 74.9, 74.4, 65.9 (CH), 68.8, 60.8, 43.3, 43.2, 34.3, 32.7, 30.0, 29.5–29.0 (six partially overlapped signals), 25.9, 24.8 (CH_2), 60.7, 56.1, 56.0 (×3), 25.8 (×3), –4.4, –4.5 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1732 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{47}H_{72}NaO_{11}Si$ [M + Na^+] 863.4742; found 863.4744.

(3*R*,5*S*)-5-(*tert*-Butyldimethylsilyloxy)-3-methoxy-6-[(*S*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]hexyl 16-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]hexadecanoate (32): Obtained in 36% yield from **12b** and **28** under the same conditions used for the preparation of **1**: amorphous solid. $[\alpha]_D$ = –2.2 (*c* = 0.62, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.90–6.80 (m, 3 H), 6.77 (d, *J* = 8.1 Hz, 1 H), 6.52 (s, 2 H), 6.50 (d, *J* = 12 Hz, 1 H), 6.44 (d, *J* = 12 Hz, 1 H), 6.02 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.58 (m, 1 H), 4.20–4.10 (m, 3 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.80 (t, *J* = 6.6 Hz, 2 H), 3.70 (s, 6 H), 3.40 (m, 1 H), 3.32 (s, 3 H), 2.35–2.25 (br. m, 4 H), 2.00 (ddd, *J* = 14, 9.5, 3.7 Hz, 1 H), 1.85 (m, 2 H), 1.75–1.55 (br. m, 7 H), 1.40–1.25 (br. m, 22 H), 0.89 (s, 9 H), 0.10 (s, 3 H), 0.08 (s, 3 H) ppm. ^{13}C NMR (125 MHz): δ = 173.7, 164.2, 152.9 (×2), 148.7, 148.1, 137.1, 132.9, 129.9, 18.0 (C), 145.1, 129.8, 128.7, 121.8, 121.5, 113.5, 111.4, 106.0 (×2), 74.9, 74.5, 65.9 (CH), 68.8, 60.7, 43.3, 43.2, 34.3, 32.7, 30.0, 29.7–29.0 (eleven partially overlapped signals), 25.9, 24.9 (CH_2), 60.8, 56.1, 56.0 (×3), 25.8 (×3), –4.4, –4.5 (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1733 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{52}H_{82}NaO_{11}Si$ [M + Na^+] 933.5524; found 933.5533.

(3*R*,5*S*)-5-Hydroxy-3-methoxy-6-[(*S*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]hexyl 4-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]butanoate (5): Obtained in 86% yield from **29** under the same conditions used for the preparation of **15**: oil. $[\alpha]_D$ = –0.9 (*c* = 0.79, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.90–6.80 (m, 3 H), 6.77 (d, *J* = 8.2 Hz, 1 H), 6.52 (s, 2 H), 6.49 (d, *J* = 12 Hz, 1 H), 6.45 (d, *J* = 12 Hz, 1 H), 6.02 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.74 (m, 1 H), 4.26 (m, 1 H), 4.18 (m, 2 H), 3.88 (t, *J* = 6 Hz, 2 H), 3.83 (s, 6 H), 3.71 (s, 6 H), 3.60 (m, 1 H), 3.37 (s, 3 H), 3.10 (br. s, 1 H, OH), 2.50 (t, *J* = 7.5 Hz, 2 H), 2.37 (m, 2 H), 2.10–1.95 (br. m, 2 H), 1.90–1.65 (br. m, 3 H), 1.65–1.45 (br. m, 3 H) ppm. ^{13}C NMR (125 MHz): δ = 173.1, 164.3, 152.9 (×2), 148.8, 147.8, 137.2, 132.9, 130.0 (C), 145.1, 129.6, 128.9, 122.2, 121.5, 114.1, 111.5, 106.0 (×2), 76.5, 74.9, 64.5 (CH), 67.8, 61.2, 43.0, 39.6, 32.3, 30.7, 30.1, 24.5 (CH_2), 60.8, 57.1, 55.9 (×3) (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 3470 (br., OH), 1726 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{34}H_{44}NaO_{11}$ [M + Na^+] 651.2781; found 651.2777.

(3*R*,5*S*)-5-Hydroxy-3-methoxy-6-[(*S*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]hexyl 8-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]octanoate (6): Obtained in 85% yield from **30** under the same conditions used for the preparation of **15**: oil. $[\alpha]_D$ = –6.3 (*c* = 0.54, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.90–6.80 (m, 3 H), 6.77 (d, *J* = 8.4 Hz, 1 H), 6.52 (s, 2 H), 6.50 (d, *J* = 12 Hz, 1 H), 6.44 (d, *J* = 12 Hz, 1 H), 6.02 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.74 (m, 1 H), 4.28 (br. t, *J* ≈ 9 Hz, 1 H), 4.17 (t, *J* = 6.5 Hz, 2 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.80 (t, *J* = 6.8 Hz, 2 H), 3.71 (s, 6 H), 3.60 (m, 1 H), 3.37 (s, 3 H), 3.20 (br. s, 1 H, OH), 2.38 (m, 2 H), 2.30 (t, *J* = 7.5 Hz, 2 H), 2.00 (m, 1 H), 1.90–1.50 (br. m, 7 H), 1.45–1.20 (br. m, 8 H) ppm. ^{13}C NMR (125 MHz): δ = 173.7, 164.3, 152.9 (×2), 148.7, 148.0, 137.2, 132.9, 129.9 (C), 145.1, 129.7, 128.7, 121.9, 121.4, 113.6, 111.4, 106.0 (×2), 76.5, 74.9, 64.5 (CH), 68.8, 61.0, 43.0, 39.7, 34.3, 32.4, 30.0, 29.7, 29.1, 29.0, 25.8, 24.8 (CH_2), 60.9, 57.1, 55.9 (×3) (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 3500 (br., OH), 1726 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{38}H_{52}NaO_{11}$ [M + Na^+] 707.3407; found 707.3408.

(3*R*,5*S*)-5-Hydroxy-3-methoxy-6-[(*S*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]hexyl 11-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]undecanoate (7): Obtained in 83% yield from **31** under the same conditions used for the preparation of **15**: oil. $[\alpha]_D$ = –4.2 (*c* = 1.1, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.90–6.80 (m, 3 H), 6.77 (d, *J* = 8 Hz, 1 H), 6.52 (s, 2 H), 6.50 (d, *J* = 12 Hz, 1 H), 6.44 (d, *J* = 12 Hz, 1 H), 6.02 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.75 (m, 1 H), 4.28 (br. t, *J* = 9.5 Hz, 1 H), 4.17 (t, *J* = 6.6 Hz, 2 H), 3.85 (s, 3 H), 3.83 (s, 3 H), 3.80 (t, *J* = 6.8 Hz, 2 H), 3.71 (s, 6 H), 3.60 (m, 1 H), 3.38 (s, 3 H), 3.10 (br. s, 1 H, OH), 2.38 (m, 2 H), 2.30 (t, *J* = 7.5 Hz, 2 H), 2.00 (m, 1 H), 1.90–1.55 (br. m, 9 H), 1.40–1.20 (br. m, 12 H) ppm. ^{13}C NMR (125 MHz): δ = 173.8, 164.3, 152.9 (×2), 148.7, 148.0, 137.2, 132.9, 129.9 (C), 145.1, 129.7, 128.7, 121.9, 121.4, 113.6, 111.4, 106.0 (×2), 76.6, 74.9, 64.5 (CH), 68.8, 61.0, 43.0, 39.6, 34.3, 32.4, 30.0, 29.5–29.0 (six partially overlapped signals), 25.9, 25.0 (CH_2), 60.9, 57.1, 56.0 (×3) (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 1726 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{41}H_{58}NaO_{11}$ [M + Na^+] 749.3877; found 749.3871.

(3*R*,5*S*)-5-Hydroxy-3-methoxy-6-[(*S*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]hexyl 16-[2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy]hexadecanoate (8): Obtained in 81% yield from **32** under the same conditions used for the preparation of **15**: oil. $[\alpha]_D$ = –2 (*c* = 0.88, $CHCl_3$). ^1H NMR (500 MHz): δ = 6.90–6.80 (m, 3 H), 6.77 (d, *J* = 8 Hz, 1 H), 6.52 (s, 2 H), 6.50 (d, *J* = 12 Hz, 1 H), 6.45 (d, *J* = 12 Hz, 1 H), 6.02 (br. d, *J* ≈ 9.8 Hz, 1 H), 4.75 (m, 1 H), 4.28 (br. t, *J* ≈ 9 Hz, 1 H), 4.17 (t, *J* = 6.6 Hz, 2 H), 3.85 (s, 3 H), 3.84 (s, 3 H), 3.81 (t, *J* = 6.8 Hz, 2 H), 3.71 (s, 6 H), 3.60 (m, 1 H), 3.38 (s, 3 H), 3.10 (br. s, 1 H, OH), 2.38 (m, 2 H), 2.30 (t, *J* = 7.5 Hz, 2 H), 2.00 (m, 1 H), 1.90–1.50 (br. m, 11 H), 1.40–1.20 (br. m, 20 H) ppm. ^{13}C NMR (125 MHz): δ = 173.9, 164.3, 152.9 (×2), 148.7, 148.1, 137.2, 133.0, 129.9 (C), 145.1, 129.8, 128.8, 121.9, 121.4, 113.6, 111.4, 106.0 (×2), 76.6, 74.9, 64.5 (CH), 68.9, 61.0, 43.0, 39.6, 34.3, 32.4, 30.0, 29.7–29.0 (eleven partially overlapped signals), 25.9, 25.0 (CH_2), 60.9, 57.1, 55.9 (×3) (CH_3) ppm. IR: $\tilde{\nu}_{max}$ = 3470 (br., OH), 1726 (C=O) cm^{-1}. HRMS (ESI): *m*/*z* calcd. for $C_{46}H_{68}NaO_{11}$ [M + Na^+] 819.4659; found 819.4679.

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 IU/mL), streptomycin (50 μg/mL) and amphoterycin (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 described previously.[28] Approximately 5×10^3 cells of HT-29, MCF-7 or HEK-293 cells in a total volume of 100 μL of their respective growth media were incubated with serial dilutions of the tested compounds. After 3 d incubation (37 °C, 5% CO_2 in a humid atmosphere), 10 μL MTT (5 mg/mL in PBS) was added to each well and the plate was incubated for a further 4 h (37 °C). The resulting formazan was dissolved in 0.04 N HCl/2-propanol (150 μL) and read at 550 nm. All determinations were carried out in triplicate.

Supporting Information (see footnote on the first page of this article): Copies of ^1H and ^{13}C NMR spectra of all new compounds.

Acknowledgments

Financial support was granted by the Spanish Government (Ministerio de Economía y Competitividad (MEC), grant numbers CTQ2008-02800 and CTQ2011-27560), by the Consellería d'Empresa, Universitat i Ciencia de la Generalitat Valenciana (grant numbers PROMETEO/2013/027 and ACOMP09/113) and by the Universitat Jaume I, Castellón, Spain (grant numbers P1-1B-2008-14 and PI-1B-2011-37). S. T.-G. thanks the Generalitat Valenciana for a predoctoral fellowship of the Santiago Grisolía program. C. V. thanks the Ministerio de Economía y Competitividad for a predoctoral fellowship of the FPI program. J. A. M. thanks the COST Action CM0804 for aid in establishing cooperations with other research groups.

[1] M. Garcia, A. Jemal, E. M. Ward, M. M. Center, Y. Hao, R. L. Siegel, M. J. Thun, *Global Cancer Facts & Figures 2007*, American Cancer Society, Atlanta, GA, **2007**.

[2] a) D. Hanahan, R. A. Weinberg, *Cell* **2000**, *100*, 57–70; b) M. R. Stratton, P. J. Campbell, P. A. Futreal, *Nature* **2009**, *458*, 719–724; c) D. Hanahan, R. A. Weinberg, *Cell* **2011**, *144*, 646–674.

[3] a) F. T. Boyle, G. F. Costello, *Chem. Soc. Rev.* **1998**, *27*, 251–261; b) J. B. Gibbs, *Science* **2000**, *287*, 1969–1973.

[4] a) L. Z. Penn, *Curr. Opin. Invest. Drugs* **2001**, *2*, 684–692; b) B. Zhou, Z.-L. Liu, *Pure Appl. Chem.* **2005**, *77*, 1887–1903; c) H.-J. Park, H.-J. Jung, K.-T. Lee, J. Choi, *Nat. Prod. Sci.* **2006**, *12*, 175–192; d) L. Portt, G. Norman, C. Clapp, M. Greenwood, M. T. Greenwood, *Biochim. Biophys. Acta* **2011**, *1813*, 238–259; e) F. Torres-Andón, B. Fadeel, *Acc. Chem. Res.* **2013**, *46*, 733–742.

[5] For additional comments on tubulin structure and function, see our previous papers in this area, see: a) J. A. Marco, J. García-Pla, M. Carda, J. Murga, E. Falomir, C. Trigili, S. Notararigo, J. F. Díaz, I. Barasoain, *Eur. J. Med. Chem.* **2011**, *46*, 1630–1637; b) M. Carda, J. Murga, S. Díaz-Oltra, J. García-Pla, J. Paños, E. Falomir, C. Trigili, J. F. Díaz, I. Barasoain, J. A. Marco, *Eur. J. Org. Chem.* **2013**, 1116–1123; c) M. Carda, J. Murga, J. Paños, C. A. Angulo-Pachón, J. García-Pla, S. Díaz-Oltra, J. A. Marco, C. Trigili, M. Redondo-Horcajo, J. F. Díaz, I. Barasoain, *Curr. Med. Chem.* **2013**, *20*, 1173–1182; d) 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, J. A. Marco, *Org. Biomol. Chem.* **2013**, *11*, 5809–5826.

[6] J. Chen, T. Liu, X. Dong, Y. Hu, *Mini-Rev. Med. Chem.* **2009**, *9*, 1174–1190.

[7] a) A. Cirla, J. Mann, *Nat. Prod. Rep.* **2003**, *20*, 558–564; b) V. Srivastava, A. S. Negi, J. K. Kumar, M. M. Gupta, S. P. S. Khanuja, *Bioorg. Med. Chem.* **2005**, *13*, 5892–5908; c) G. U. Dachs, A. J. Steele, C. Coralli, C. Kanthou, A. C. Brooks, S. P. Gunningham, M. J. Currie, A. I. Watson, B. A. Robinson, G. M. Tozer, *BMC Cancer* **2006**, *6*, 280–290; d) G. C. Tron, T. Pirali, G. Sorba, F. Pagliai, S. Busacca, A. A. Genazzani, *J. Med. Chem.* **2006**, *49*, 3033–3044; e) Y. Shan, J. Zhang, Z. Liu, M. Wang, Y. Dong, *Curr. Med. Chem.* **2011**, *18*, 523–538.

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

[9] a) M. A. Jordan, *Curr. Med. Chem. Anticancer Drugs* **2002**, *2*, 1–17; b) M. Abal, J. M. Andreu, I. Barasoain, *Curr. Cancer Drug Targets* **2003**, *3*, 193–203.

[10] a) J. J. Correia, S. Lobert, *Curr. Pharm. Des.* **2001**, *7*, 1213–1228; b) J. Jiménez-Barbero, F. Amat-Guerri, J. P. Snyder, *Curr. Med. Chem. Anticancer Drugs* **2002**, *2*, 91–122; c) J. F. Díaz, J. M. Andreu, J. Jiménez-Barbero, *Top. Curr. Chem.* **2009**, *286*, 121–149; d) B. Gigant, A. Cormier, A. Dorléans, R. B. G. Ravelli, M. Knossow, *Top. Curr. Chem.* **2009**, *286*, 259–278; e) E. M. Daly, R. E. Taylor, *Curr. Chem. Biol.* **2009**, *3*, 367–379.

[11] a) V. M. Sánchez-Pedregal, C. Griesinger, *Top. Curr. Chem.* **2009**, *286*, 151–208; b) J. H. Nettles, K. H. Downing, *Top. Curr. Chem.* **2009**, *286*, 209–257; c) M. Botta, S. Forli, M. Magnani, F. Manetti, *Top. Curr. Chem.* **2009**, *286*, 279–328.

[12] F. Sarabia, M. García-Castro, A. Sánchez-Ruiz, *Curr. Bioact. Compd.* **2006**, *2*, 269–299.

[13] H. J. Anderson, J. E. Coleman, R. J. Andersen, M. Roberge, *Cancer Chemother. Pharmacol.* **1997**, *39*, 223–226.

[14] a) M. Kondoh, T. Usui, S. Kobayashi, K. Tsuchiya, K. Nishikawa, T. Nishikiori, T. Mayumi, H. Osada, *Cancer Lett.* **1998**, *126*, 29–32; b) M. Kondoh, T. Usui, T. Nishikiori, T. Mayumi, H. Osada, *Biochem. J.* **1999**, *340*, 411–416; c) H. Watanabe, H. Watanabe, T. Usui, M. Kondoh, H. Osada, T. Kitahara, *J. Antibiot.* **2000**, *53*, 540–545; d) T. Usui, H. Watanabe, H. Nakayama, Y. Tada, N. Kanoh, M. Kondoh, T. Asao, K. Takio, H. Watanabe, K. Nishikawa, T. Kitahara, H. Osada, *Chem. Biol.* **2004**, *11*, 799–806.

[15] a) L. M. Rossi, P. Rangasamy, J. Zhang, X.-Q. Qiu, G. Y. Wu, *J. Pharm. Sci.* **2007**, *97*, 1060–1070; b) P. Courvalin, *J. Int. Med. Res.* **2008**, *264*, 4–16; c) M. Gualtieri, F. Baneres-Roquet, P. Villain-Guillot, M. Pugniere, J.-P. Leonetti, *Curr. Med. Chem.* **2009**, *16*, 390–393.

[16] a) M. Kavallaris, *Nat. Rev. Cancer* **2010**, *10*, 194–204; b) C. Holohan, S. Van Schaeybroeck, D. B. Longley, P. G. Johnston, *Nat. Rev. Cancer* **2013**, *13*, 714–726.

[17] a) K. Yasui, Y. Tamura, K. Nakatani, K. Kawada, M. Ohtani, *J. Org. Chem.* **1995**, *60*, 7567–7574; b) M. K. Gurjar, J. T. Henri Jr., D. S. Bose, A. V. R. Rao, *Tetrahedron Lett.* **1996**, *37*, 6615–6618; c) N. Chida, M. Yoshinaga, T. Tobe, S. Ogawa, *Chem. Commun.* **1997**, 1043–1044; d) H. Watanabe, H. Watanabe, M. Bando, M. Kido, T. Kitahara, *Tetrahedron* **1999**, *55*, 9755–9776; e) G. E. Keck, C. E. Knutson, S. A. Wiles, *Org. Lett.* **2001**, *3*, 707–710; f) L. C. Dias, L. G. de Oliveira, M. A. de Sousa, *Org. Lett.* **2003**, *5*, 265–268; g) X. Shen, A. S. Wasmuth, J. Zhao, C. Zhu, S. G. Nelson, *J. Am. Chem. Soc.* **2006**, *128*, 7438–7439; h) D. Enders, S. Dhulut, D. Steinbusch, A. Herrbach, *Chem. Eur. J.* **2007**, *13*, 3942–3949; i) C. Bressy, J.-P. Vors, S. Hillebrand, S. Arseniyadis, J. Cossy, *Angew. Chem.* **2008**, *120*, 10291; *Angew. Chem. Int. Ed.* **2008**, *47*, 10137–10140; j) M. T. Crimmins, A.-M. R. Dechert, *Org. Lett.* **2009**, *11*, 1635–1638.

[18] S. Torijano-Gutiérrez, *Ph. D. Thesis*, University Jaume I, **2013**.

[19] K. Gaukroger, J. A. Hadfield, L. A. Hepworth, N. J. Lawrence, A. T. McGown, *J. Org. Chem.* **2001**, *66*, 8135–8138.

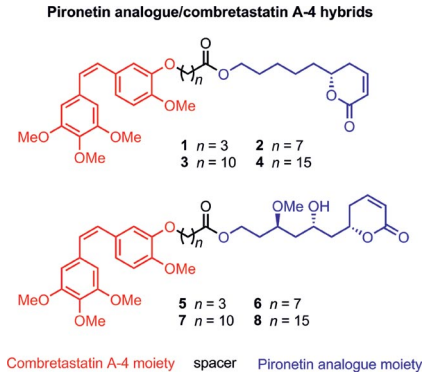
[20] a) P. V. Ramachandran, G.-M. Chen, H. C. Brown, *Tetrahedron Lett.* **1997**, *38*, 2417–2420; b) for pinane-based versatile "allyl" boranes, see:P. V. Ramachandran, *Aldrichim. Acta* **2002**, *35*, 23–35.

[21] D. Tripathi, S. K. Pandey, P. Kumar, *Tetrahedron* **2009**, *65*, 2226–2231.
[22] For general reviews on metathesis, some with particular emphasis in RCM, see: a) A. Fürstner, *Angew. Chem.* **2000**, *112*, 3140; *Angew. Chem. Int. Ed.* **2000**, *39*, 3012–3043; b) L. Jafarpour, S. P. Nolan, *Adv. Organomet. Chem.* **2000**, *46*, 181–222; c) T. M. Trnka, R. H. Grubbs, *Acc. Chem. Res.* **2001**, *34*, 18–29; d) *Handbook of Metathesis* (Ed.: R. H. Grubbs), Wiley-VCH, Weinheim, Germany, **2003**; e) R. H. Grubbs, *Tetrahedron* **2004**, *60*, 7117–7140; f) D. Astruc, *New J. Chem.* **2005**, *29*, 42–56; g) A. H. Hoveyda, A. R. Zhugralin, *Nature* **2007**, *450*, 243–251; h) *Metathesis in Natural Product Synthesis* (Eds.: J. Cossy, S. Arseniyadis, C. Meyer), Wiley-VCH, Weinheim, Germany, **2010**.
[23] R. D. Cink, C. J. Forsyth, *J. Org. Chem.* **1997**, *62*, 5672–5673.
[24] K. Horita, T. Yoshioka, T. Tanaka, Y. Oikawa, O. Yonemitsu, *Tetrahedron* **1986**, *42*, 3021–3028.
[25] J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, M. Yamaguchi, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989–1993.
[26] N. Arden, M. J. Betenbaugh, *Trends Biotechnol.* **2004**, *22*, 174–180.
[27] For some selected reviews on the interest and utility of hybrid molecules in medicine and pharmacology, see: a) S. Hanessian, *ChemMedChem* **2006**, *1*, 1300–1330; b) B. Meunier, *Acc. Chem. Res.* **2008**, *41*, 69–77; c) M. Decker, *Curr. Med. Chem.* **2011**, *18*, 1464–1475.
[28] S. Rodríguez-Nieto, M. A. Medina, A. R. Quesada, *Anticancer Res.* **2001**, *21*, 3457–3460.

Received: November 16, 2013
Published Online: ■

Drug Design

The preparation of a series of hybrid molecules containing a combretastatin A-4 moiety and a pironetin analogue fragment linked by a spacer of variable length is described. The relationship between structure and cytotoxicity is discussed. Cytotoxicity values of some compounds were similar to those of the parent molecules, combretastatin A-4 and pironetin, and some were less toxic than the latter for normal cells.



S. Torijano-Gutiérrez, C. Vilanova, S. Díaz-Oltra, J. Murga,* E. Falomir, M. Carda, J. A. Marco* 1–14

Design and Synthesis of Pironetin Analogue/Combretastatin A-4 Hybrids and Evaluation of Their Cytotoxic Activity

Keywords: Medicinal chemistry / Drug design / Cytotoxicity / Anticancer agents / Structure–activity relationships

