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Synthesis and in vitro evaluation of analogues of avocado-produced toxin (+)-(*R*)-persin in human breast cancer cells

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ABSTRACT

A structure–activity study of several new synthetic analogues of the avocado-produced toxin persin has been conducted, with compounds being evaluated for their cytostatic and pro-apoptotic effects in human breast cancer cells. A 4-pyridinyl derivative demonstrated activity comparable to that of the natural product, suggesting future directions for exploration of structure–activity relationships.

1. Introduction

Breast cancer is the most common cancer in women in both developed and undeveloped countries, and the second most frequent cause of cancer deaths, despite a major decline in breast cancer mortality in the past decade.[1] Current chemotherapy, predominantly for estrogen receptor-negative (ER−) cancers, includes the use of microtubule-interacting molecules such as paclitaxel and related taxanes, plant-derived compounds whose apoptosis-promoting abilities fuel much of the continuing interest in natural products for treatment of human cancers.[2,3] The susceptibility of cancer cells to such disruptors of intracellular cytoskeletal integrity is further emphasized by the varied structural classes of microtubule-targeting agents currently in clinical[4,5] and pre-clinical development,[6] and exemplified by the recent US and European approval of eribulin (E7389,[7] Halaven®) for the treatment of advanced metastatic breast cancer in women for whom anthracycline- and taxane-based therapies have not been successful, or suitable.[8] Eribulin affects microtubule dynamics in a unique fashion.[9] This property, coupled with the fact that it is a fully synthetic, structurally simplified yet more potent macrocyclic ketone analogue of the marine sponge natural product halichondrin B,[13] whose isolation and potent anti-cancer activity was reported 25 years ago,[10] serves to underline the importance of continued investigations of natural products to inspire and inform the development of new pharmaceuticals.

(+)-(*R*)-(12*Z*,15*Z*)-2-Hydroxy-4-oxohenicosa-12,15-dien-1-yl acetate (**1**) (Fig. 1), commonly called (+)-(*R*)-persin, is a novel polyketide ('acetogenin'), comprised of a β-hydroxy ketone system, flanked on one side by an acetate group, and on the other by a long, partially unsaturated hydrocarbon chain. It was originally isolated from the leaves of the avocado *Persea americana* Mill. (Lauraceae) by Japanese workers, who described its inhibition of the growth of *Bombyx mori* (silkworm) larvae, and deduced its chemical structure.[11] However, the absolute configuration about C-2 of **1** was not confirmed until a stereoselective synthesis two decades later.[12] Bioassay-driven fractionation analysis by Rodriguez-Saona et al. located **1** and the structurally related 2-alkylfurans ('avocadofurans': exemplified by **2** and originally reported by Kashman et al.[13] and Chang et al.[11]) in the idioblast oil cells of avocado fruit, and found **1** to deter feeding by the beet armyworm [*Spodoptera exigua* Hübner (Noctuidae)], a major phytophagous insect pest in agriculture.[14] These workers also reported the isolation and characterization from avocado idioblast oil cells of a compound they called 'isopersin' (**3**), in which the C-1 acetate and C-2 hydroxyl moieties are in opposite positions relative to their location in **1**, and which had no deterrent effect to feeding by *S. exigua*.[15] A solution of **3**

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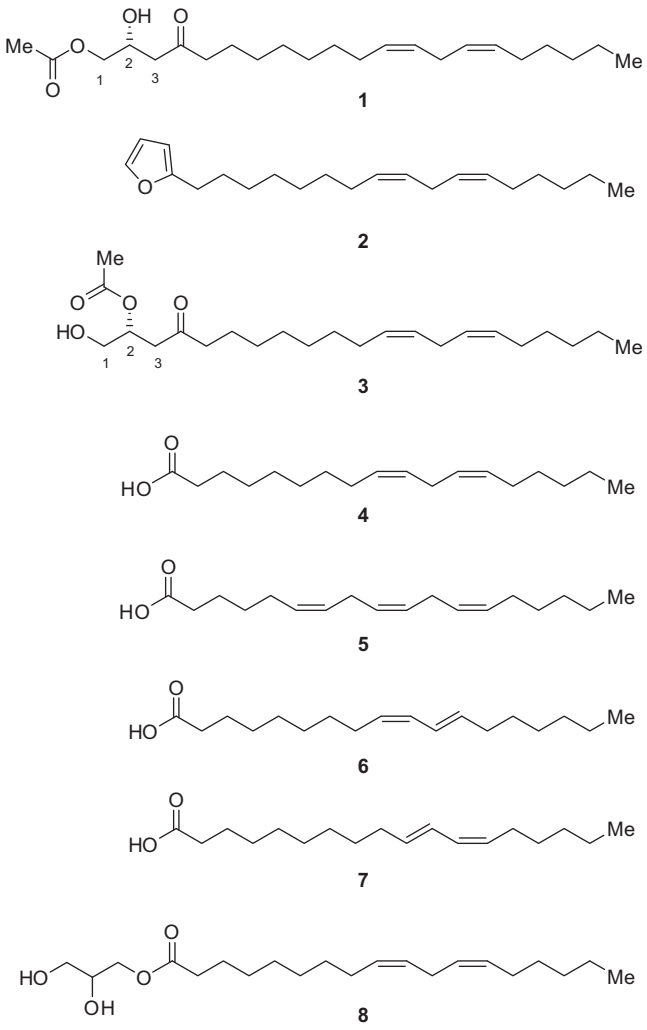


Figure 1. Structures of (+)-(*R*)-persin (**1**), a 2-alkyl furan derived from 1 (**2**), (*R*)-isopersin (**3**), linoleic acid (**4**), γ-linolenic acid (**5**), 9-*cis*-11-*trans*-linoleic acid (**6**), 10-*trans*-12-*cis*-linoleic acid (**7**), and a monoglyceride of linoleic acid (**8**).

in acetone at −20 °C was found to equilibrate to a mixture of **3** and **1** over several weeks, with the lack of prior reports of **3** being attributed to this instability. Analogous to **1**, compound **3** underwent dehydration to **2** in the presence of trace acid.[‡] The chemistry and biology of these molecules, and around forty others isolated from various parts of the avocado plant, have been reviewed.[16]

An Australian team reported in 1995[17] that the agalactia and non-infectious mastitis observed in lactating animals fed avocado leaves was due to **1** causing coagulative necrosis of the secretory epithelium. High doses were shown to cause myocardial fibrosis and necrosis in mice, accounting for the reported cardiotoxicity of ingested avocado leaves to animals. These researchers claimed the utilization of **1** (and side-chain analogues thereof) for the treatment of cancer (in particular breast cancer) in mammals, as well for the inhibition of lactation, in a patent of the same year.[18] The structure of **1** suggests it shares a common biosynthetic origin with polyunsaturated fatty acids linoleic acid (**4**) and γ-linolenic acid (**5**), and the conjugated linoleic acids (CLAs) exemplified by *cis*-9-*trans*-11-linoleic acid (**6**) and *trans*-10-*cis*-12-linoleic acid (**7**) (Fig. 1). Biologically, **4** and **5** directly affect ER levels in cells, thereby modifying cellular sensitivity to circulating steroid hormone levels.[19,20] In addition, significant positive effects have been observed in the dual treatment of primary ER+ breast cancers with **5** and the anti-estrogen, tamoxifen.[21] **5** has also been reported to synergize with the microtubule inhibitor vinorelbine, an alkaloid derived by semi-synthesis from alkaloids extracted from the rosy periwinkle (*Catharanthus roseus*), in the inhibition of breast cancer cell proliferation.[22] **6** and **7** are potent anti-cancer agents, particularly against breast cancer,[23,24] however the pro-carcinogenicity of **7** in mouse models[25] suggests its use in cancer chemopreventative strategies should be avoided.

It has been proposed[26] that the similarity of **1** to a monoglyceride of linoleic acid (**8**) may indicate it interferes with fundamental lipid metabolism processes within cells, thus accounting for its wide range of biological activities. The precise biosynthetic origins of **1** are unclear; whilst one might imagine its derivation from **2**, Carman and co-workers note[27,28] that there are as-yet no known enzymatic or natural chemical processes whereby the necessary extrusion of an oxygen atom (a 'reverse' of the Baeyer–Villiger oxidation) can be effected.[§]

We have previously described biological studies of **1**, demonstrating its selective induction of G_2-M cell-cycle arrest, concomitant with caspase-dependent apoptosis, in human breast cancer cells.[29] Observation of the intracellular morphological changes resulting from this G_2-M arrest, and other data, led us to hypothesize that **1** may interact with microtubules in a similar manner to paclitaxel, although this has yet to be conclusively established, and may be distinct from its activity in breast tissue. Further investigations revealed marked synergistic, pro-apoptotic cytotoxicity between **1** and the active metabolite of tamoxifen, 4-hydroxytamoxifen, in human breast cancer cells, which we found to depend on expression of the pro-apoptotic protein, Bim, and to be associated with an increase in de novo ceramide synthesis.[30] Crucially, **1** had no effect on normal breast epithelial cells and, in contrast with **4** and **5**, its anti-cancer effects are independent of the ER status of cells.[¶] Although sensitization to the effects of tamoxifen has been observed in ER− MDA-MB-231 cells treated with a celecoxib analogue,[31] and in ER+ MCF-7 cells treated with extracts of *Coptis japonica* (or the purified major alkaloid from the plant),[32] the ER-independent mode of action of **1** appears unique. These observations, and the specificity of **1** for the malignant phenotype, spurred our interest in synthesizing and examining the structure–activity relationships of a set of new analogues of **1**.

2. Chemistry

Synthesis of **1** used the methodology of MacLeod and Schäffeler,[12] whereby treatment of ketone **9** with a commercially available solution of (−)-*B*-chlorodiisopinocampheylborane [(−)-DIP-Cl][33] formed enol borinate **10**, which then underwent an enantioselective aldol reaction with volatile aldehyde **11** (obtained by ozonolysis of allyl acetate) to afford a reasonable yield of the natural product (Scheme 1). Comparison of the measured optical rotation value of the product ($[\alpha]_D^{25}$ = +10.78° (*c* 1.02, $CHCl_3$)) with that obtained by MacLeod and Schäffeler ($[\alpha]_D^{20}$ = +10.20° (*c* 1.00, $CHCl_3$))[12] confirmed the desired (*R*)-isomer had been formed. The close agreement between these values (despite their being measured at slightly different temperatures) suggests that the enantiomeric

[‡] This acid-lability of **1** and **3**, and the facility of acyl migration from C-2 to C-1 in **3**, illustrates the restrictions incumbent on any synthetic approaches to analogues of these compounds.

[§] The only literature example comprises a formal 'reverse Baeyer–Villiger', induced via a 3-step process, in a fully synthetic environment (Smith, III, A. B.; Foster, A. M.; Agosta, W. C. *J. Am. Chem. Soc.* **1972**, *94*, 5100).

[¶] It has been suggested (Ref. 29) that the apparent in vivo specificity shown by **1** for lactating mammarian tissue described in previous reports (Ref. 18) may be due to the lactation process itself i.e. the dynamic changes in lipid composition known to occur in lactating mammary epithelium could effect increased absorption/incorporation of **1** and its metabolites, generating the observed toxicity.

excess (*e.e.*) of the quantity of **1** obtained by us was approximately equal to the >90% *e.e.* of the literature material, which was calculated by chiral shift ^{1}H NMR analysis of **1** with Eu(hfc)$_3$.[12] Under the same conditions, reaction of **11** and 2-octanone (**12**) afforded the truncated-chain analogue (+)-(*R*)-hexylpersin (**13**).

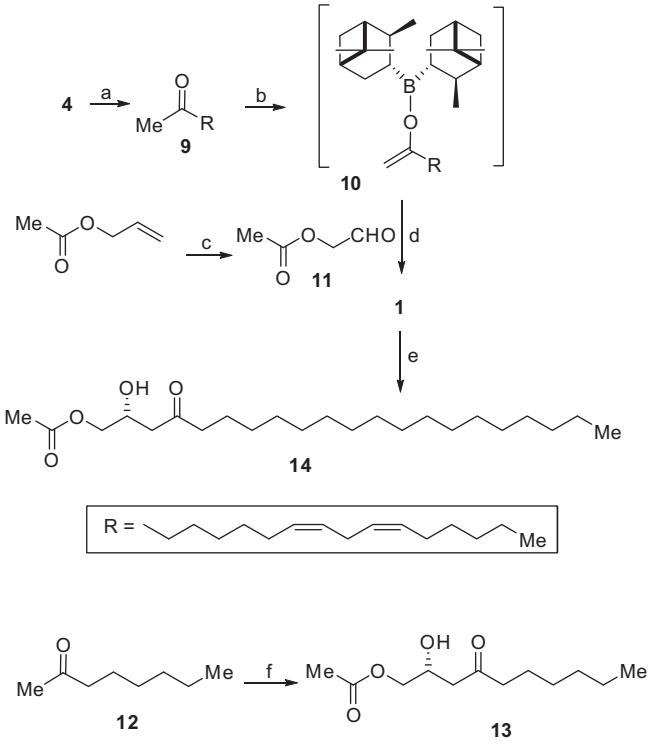
Subsequent reduction of **1** gave its fully saturated analogue (+)-(*R*)-tetrahydropersin (**14**). The isolation, from the avocado plant,[28] synthesis[34,27] and the equipotency[17,18] of **14** with **1** in anti-cancer and anti-lactation screens have been previously described. These workers also synthesized the enantiomeric (−)-(*S*)-persin, and, interestingly, found it to be devoid of the biological activity shown by **1**. A recent computational chemistry study suggests that this may be due to: (a) the enantiomers possessing different torsion angles in the (*Z*,*Z*)-1,4-pentadiene system; and (b) a possible anionic reaction intermediate of the (*S*)-enantiomer being incapable of favorable molecular orbital interactions with any electrophilic reactant.[35]

Attempts to access a more soluble analogue by transformation of **14** to a C-2 phosphate ester via standard procedures (e.g.,[36,37]) were fruitless, with only unreacted and/or degraded **14** being recovered in each case. This may be due to intramolecular hydrogen-bonding between the secondary β-hydroxyl group of **14** and the adjacent carbonyl oxygen rendering the former moiety unreactive (the presence of such an H-bond is supported by computational studies[‖]) and/or inherent instability of **14** to the phosphorylation conditions themselves.

Subsequently, following information [MacLeod, J. K., personal communication] that replacement of the acetate group of **1** with a benzoate group afforded a compound with only slightly less activity than **1**, we turned to accessing an alternative set of (*R*)-aryl persin analogues. Variation of the above route to **1**, utilizing appropriate aroyl aldehydes, was initially investigated as an approach to these. Unfortunately, although the enantioselective aldol reaction between **9** and 2-oxoethyl benzoate[38] afforded some of the desired phenyl analogue (**15**), it was inseparable (via column or preparative TLC chromatography) from large quantities of aldehyde-derived impurities, and/or most of **9** was recovered unreacted. Other substituted aryl aldehydes yielded similar results, so this approach was abandoned.

An alternative route to **15** was then devised, based upon the route to **1** described by Li and co-workers.[39] Thus, Horner–Wadsworth–Emmons olefination of methyl linoleate (**16**) with dimethyl methyl phosphonate, followed by condensation of the resulting adduct with glycoaldehyde dimer, yielded enone alcohol **17**. Treatment of **17** with phenylboronic acid and the quinine-thiourea catalyst **18** (obtained in a two-step process from quinine, as per Vakulya et al.[40]), followed by hydrogen peroxide, effected a stereospecific Michael addition of hydroxide to the β-carbon of the olefin to give key β,γ-diol **19** (Scheme 2). As expected, reaction of **19** with benzoyl chloride and pyridine furnished **15**, together with a significant quantity of *bis*-addition adduct **20**. Reaction of **19** with various appropriately functionalized aroyl chlorides successfully produced several other persin analogues (**21**–**25**). In these experiments, use of the more hindered 2,4,6-trimethylpyridine as a base, as prescribed by Li et al.[39] afforded a greater ratio of the desired mono-aroylated product **21** than bis-isomer **22** (c.f. yields of **15** vs **20**), but it is not known whether this is a general effect.

Comparison of the optical rotation of the catalyst **18** with the literature value ([α]$_D$ (29 °C) = −125.6° (*c* 1.06, CHCl$_3$); lit.[40] [α]$_D$ (25 °C) = −127.9° (*c* 0.50, CHCl$_3$)) showed it had the correct stereochemistry to afford the desired (+)-(*R*)-enantiomers, and this was supported by the measurement of the optical rotation of **15**, which was both positive and similar in magnitude to the literature value ([α]$_D$ (28 °C) = +9.35° (*c* 1.03, CHCl$_3$); lit.[39] [α]$_D$ (20 °C) = +10.70° (*c* 0.43, CHCl$_3$)). Formation of deshydroxy compound **23** was unexpected (only a trace amount of the desired 4-nitrophenyl product was isolated), but seems to be related to the presence of the 4-nitro substituent on the phenyl ring. It is possible that re-exposure of **23** to the stereoselective hydroxide addition conditions described above might yield the desired product, but this was not explored.

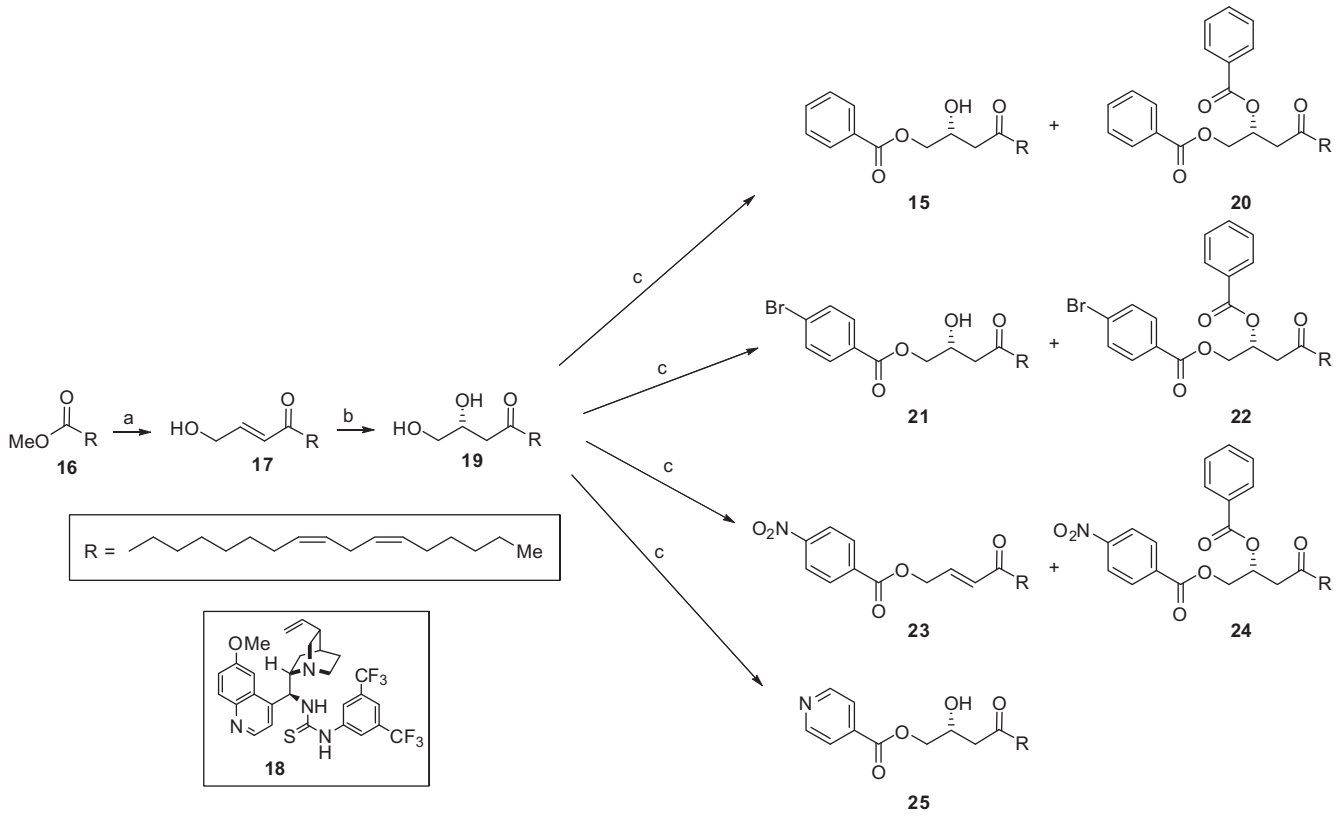


Scheme 1. Synthesis of (+)-(*R*)-persin (**1**) (+)-(*R*)-hexylpersin (**13**) and (+)-(*R*)-tetrahydropersin (**14**). Reagents and conditions: (a) MeLi, THF, 0 °C, 2 h (76%); (b) (−)-DIP-Cl (50–65 wt% in hexane), (*i*-Pr)$_2$NEt, CH$_2$Cl$_2$, −78 °C, 5 h; (c) (i) O$_3$, CH$_2$Cl$_2$, −78 °C; (ii) PPh$_3$, CH$_2$Cl$_2$, −78 °C, 3 h then −78 °C to rt, 12 h; (iii) Et$_2$O, −15 °C, 12 h; (d) (i) **11** (6.32 equiv), CH$_2$Cl$_2$, −78 °C, 1 h; then −15 °C, 19 h; (ii) 30% aq H$_2$O$_2$, MeOH: pH 7 buffer, 2 h (33% from **9**); (e) Pd/C, H$_2$, EtOAc, rt, 12–24 h (quant.). (f) (i) (−)-DIP-Cl (50–65 wt% in hexane), (*i*-Pr)$_2$NEt, CH$_2$Cl$_2$, −78 °C, 2.5 h; (ii) **11** (6.04 equiv), CH$_2$Cl$_2$, −78 °C, 68 h; (iii) 30% aq H$_2$O$_2$, MeOH: pH 7 buffer, 3.3 h (quant.).

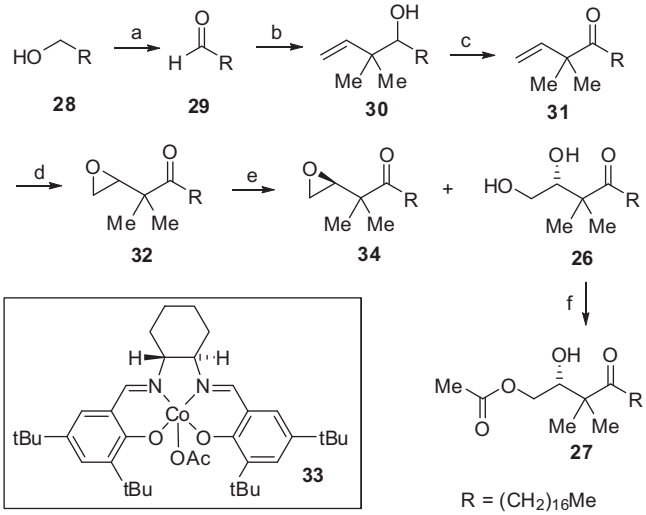
The fully saturated analogue **14** had proven similarly potent to **1** in our own and reported screens,[17] and Kashman et al.[13] and Carman et al.[26] have both suggested possible acid-catalyzed mechanisms by which avocado-derived β-hydroxy ketones such as (or similar to) **1** and **14** might cyclize to 2-alkylfurans (e.g., **2**, Fig. 1) which have also been isolated from the plant,[14,16] by a process involving loss of an α-hydrogen from C-3. We therefore reasoned that an analogue with a quaternary centre at C-3 might possess greater stability, and hence improved biological activity.[41] Thus, we devised a synthetic approach to **26**, the α-dimethyl-β,γ-diol analogue of **19**, which we envisaged could be elaborated to α-dimethyl-tetrahydropersin (**27**).

Octadecanol (**28**) was oxidized via the conditions of Easton et al.[42] to the corresponding aldehyde **29**, which was in turn reacted with an in situ-generated prenyl-indium species[43,44] to yield the key α-dimethyl-β,γ-unsaturated alcohol **30** (Scheme 3). Albright-Goldman oxidation[44,45] of **30** to ketone **31**, followed by epoxidation of the terminal olefin of **31**,[46] afforded α-dimethyl-β, γ-epoxide **32**. Crucially, by utilization of hydrolytic kinetic resolution (HKR)

[‖] As delineated by Mendoza-Wilson et al. (Ref. 35), the presence of a hydrogen bond in **1** is supported by: (a) the relatively downfield chemical shift for the 2-OH proton in the ^{1}H NMR spectrum of **1** (3.24 ppm); and (b) by the calculated distance between the 2-OH proton and the oxygen of the C-4 carbonyl group in the optimized aqueous phase structures (of both enantiomers) being 2.00 Å (correlating with the existence of a medium-to-weak-strength hydrogen bond).



Scheme 2. Synthesis of (*R*)-aryl-substituted persin analogues. Reagents and conditions: (a) (i) $MeP(O)(OMe)_2$, LDA, THF, −78 °C, 2 h; (ii) $[HC(O)CH_2OH]_2$, LiCl, $(i\text{-}Pr)_2NEt$, CH_3CN, rt, 2 h; (b) $PhB(OH)_2$, **18** (10 mol %), 4Å MS, CH_2Cl_2, rt, 56 h then H_2O_2, Na_2CO_3, rt, 15 min (71%); (c) benzoyl chlorides, pyridine, CH_2Cl_2, rt, 12–23 h.



Scheme 3. Route to (−)-(*R*)-α-dimethyl tetrahydropersin (**27**). Reagents and conditions: (a) PCC, CH_2Cl_2, rt (82%); (b) 1-bromo-3-methylbut-2-ene, In (powder), DMF, rt (22%); (c) Ac_2O, DMSO, rt, 46 h (96%); (d) Oxone®, $NaHCO_3$, acetone:H_2O (4:1), rt (75%); (e) **33** (2.7 mol %), H_2O, rt, 36 h [53%(**34**), 34%(**26**)]; (f) acetyl chloride, 2,4,6-trimethylpyridine, CH_2Cl_2, −78 °C to rt (40%).

methodology developed by Schaus et al.[47] as employed in a structurally analogous system by Liu et al.[46] and also by reference to the HKR conditions of others,[48–50] (*S*,*S*)-cobalt(III)-salen complex **33**[51] effected the transformation of racemic epoxide **32** into a ~1.7:1.0 mixture of epoxide **34** and desired α-dimethyl-β, γ-diol **26**. Treatment of **26** with acetyl chloride, under basic conditions analogous to those used for formation of **21**–**25**, provided **27** in fair yield. To our surprise, the optical rotation for **27** was negative ($[\alpha]_D^{28} = -13.66°$ (*c* 1.03, $CHCl_3$)), the significance of which will be discussed later.

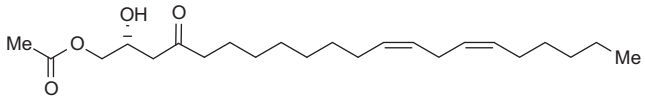
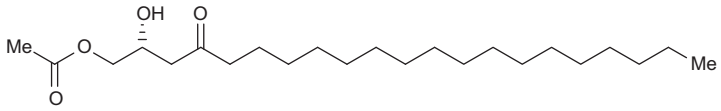
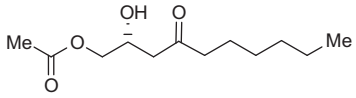
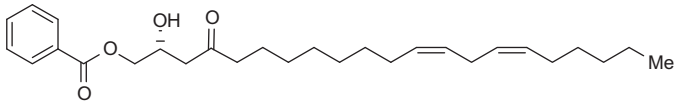
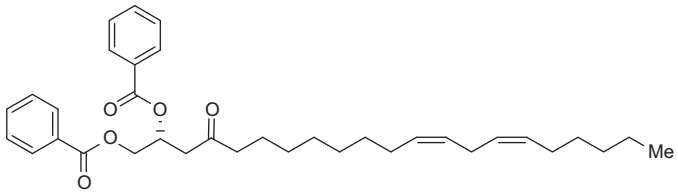
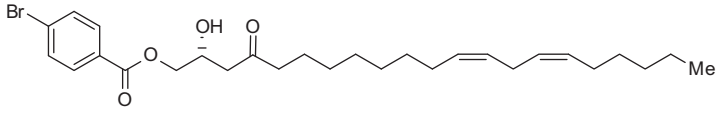
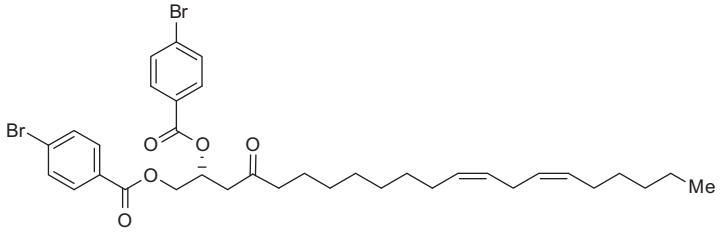
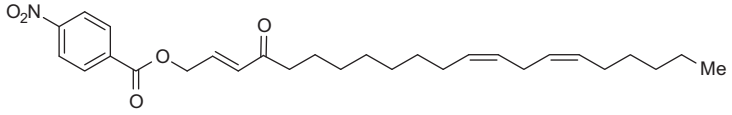
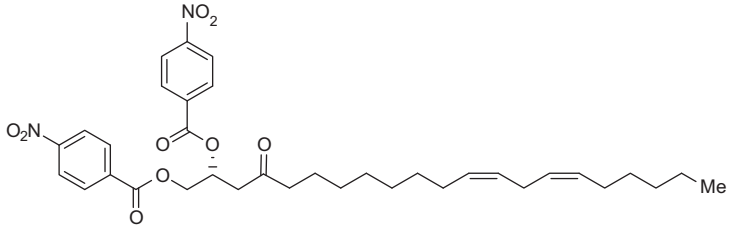
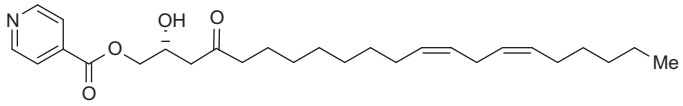
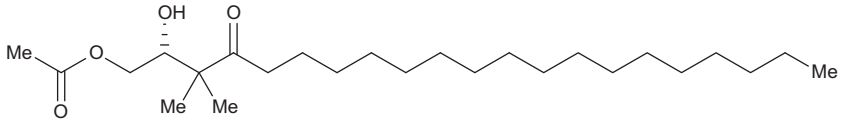
3. Biological results and discussion

Of the many biologically active compounds isolated from avocados,[14,16] (+)-(*R*)-persin (**1**) and, additionally, (+)-(*R*)-tetrahydropersin (**14**), have been determined as responsible for the toxicity observed in mammary and heart tissues of animals.[17] Having noted the structural similarities between **1** and the naturally occurring compounds linoleic acid (**4**), γ-linolenic acid (**5**), and CLAs **6** and **7** (Fig. 1), some of which are known to possess anti-cancer properties of their own,[21–24] we conducted and have previously reported investigations which revealed its ability to selectively induce G_2-M cell cycle arrest in human breast cancer cells.[29] Moreover, we found that **1** demonstrated synergistic pro-apoptotic cytotoxicity with the anti-estrogen, tamoxifen, in human breast cancer cells, which depended on expression of the pro-apoptotic protein BIM, and was associated with increased de novo ceramide synthesis.[30]

Given these observations, and the fact that **1** had no effect on normal breast cancer cells, nor any requirement for cells possessing a particular ER status,[30] we instigated the research project described herein, with the aim of finding a more potent analogue of **1**. The results of this work are collated in Tables 1 and 2.

All compounds were tested for efficacy over a 24 h period, and cell viability was determined by MTS assay. As can be seen in Table 1, the fully saturated natural product (+)-(*R*)-tetrahydropersin (**14**), which has been reported as being as active as **1** in anti-lactation screens,[17] indeed proved similarly potent, whilst the truncated-chain analogue **13** was devoid of activity within the studied concentration range. These data indicate that side-chain length is crucial to

Table 1
Inhibition of growth of ER+ human breast cancer cell line MCF-7 by 24 h treatment with (+)-(*R*)-persin (**1**) and analogues

Structure	No.	*c*log*P*[a]	tPSA[a]	IC_{50} (μM)
	**1**	6.8	63.6	15.1[b] ± 1.3[c]
	**14**	7.8	63.6	17.1 ± 1.7
	**13**	2.0	63.6	Inactive (>65)[d]
	**15**	8.5	63.6	27.0 ± 5.5
	**20**	10.9	69.7	Inactive (>27)[c]
	**21**	9.37	63.6	23.8 ± 2.2
	**22**	12.7	69.7	Inactive (>21)[c]
	**23**	9.1	95.2	Inactive (>32)[c]
	**24**	10.4	173.3	Inactive (>24)[c]
	**25**	7.1	76.0	20.1 ± 3.6
	**27**	8.7	63.6	29.0 ± 4.2

[a] *c*log*P* = calculated logarithm of the octanol/water partition coefficient, tPSA = topological polar surface area (calculated by ChemBioDraw Ultra v.12.0).
[b] c.f. IC_{50} = 21.5 ± 1.9 for **1** extracted from avocado leaves: this demonstrates the efficacy of a synthetic approach to **1**.
[c] Standard deviation.
[d] IC_{50} lies above the stated (highest tested) concentration (= 15 μg/mL): **1** is highly toxic to the majority of breast cancer cell lines studied at 10 μg/mL, thus, in order to identify any more analogues, 15 μg/mL was the maximum tested concentration.

Table 2
Inhibition of growth of human cancer cell lines by 24 h treatment with (+)-(*R*)-persin (**1**) and (+)-(*R*)-tetrahydropersin (**14**)[f]

Tissue	Cell line	SHR status[b]	p53 status	Bim status[c]	IC_{50}[a] (μM)	
					1	**14**
Breast	MCF-7	ER+	wt	+++	15.1 ± 1.3	18.2 ± 2.3
	T-47D	ER+	mut	+−−	30.3 ± 2.3	20.7 ± 3.2
	MDA-MB-468	ER−	mut	+++	25.0 ± 2.8	>39[d]
	MDA-MB-157	ER−	mut	+−−	12.8 ± 1.25	>39
	SkBr3	ER−	mut	+++	19.7 ± 1.3	>39
	Hs578T	ER−	mut	−−−	32.1 ± 2.3	>39
	MDA-MB-231	ER−	mut	−−−	>39	>39
	MCF-10A (n)[e]	ER−	wt	+−−	>39	>39
Ovarian	OVCAR3	n/a	mut	−−−	27.9 ± 4.5	>39
	IGROV-1	n/a	mut	+++	15.6 ± 3.6	>39
Prostrate	PC-3	AR−	mut	++−	30.0 ± 3.0	>39
	LNCaP	AR+	wt	+++	22.0 ± 1.8	>39

[a] Mean ± standard error of triplicate wells from at least three independent experiments.
[b] SHR = steroid hormone receptor.
[c] '+++' indicates expression of BimEL, BimL, and BimS isoforms, respectively.
[d] IC_{50} lies above the stated (highest tested) concentration (= 15 μg/mL: see Table 1 footnote).
[e] (n) = normal.
[f] The experiments summarised in Table 1 (a comparison of the effect of several different analogues on MCF-7 cells) and Table 2 (a comparison of the effect of two analogues on a panel of cell-lines, including MCF-7 cells) were performed independently, and the differing values for MCF-7 cells (although not actually statistically different from each other) may reflect small differences in batches of cell lines, sera or other biological variables.

the anti-breast cancer activity of **1**, whilst the presence of unsaturation is not. It is interesting to note, however, that short-chain α,β-unsaturated β′-hydroxy-, -acetoxy-, and -phenoxy-ketones have potency against malignant pleural mesothelioma cell lines, have been shown to potentiate the effects of paclitaxel in pancreatic ductal adenocarcinoma cells,[52,53] and, in addition, are efficacious against colon carcinoma, ovarian, breast and non small-cell lung cancer cell lines.[54]

The activity of **15** and **21**, whilst less than that of **1**, indicates there is a significant degree of lipophilic bulk-tolerance at the left-hand end of the molecule, with the more lipophilic and more electron-deficient bromophenyl compound **21** being slightly more potent. This may suggest an electronic-deficient aromatic system is more favored at this position. In contrast, bis-aroylated compounds **20**, **22** and **24**, and the enone **23**, were inactive, underscoring the importance of the β-hydroxyl moiety [and echoing the inactivity of **3** (Fig. 1), albeit in a different context[15]].

The comparable activity of pyridinyl compound **25** with that of **1** is noteworthy, and may be partly attributable to these compounds' similar lipophilicity. In addition, the greater polarity imparted by the presence of the N atom in the aromatic ring, as evidenced by its higher tPSA value (Table 1), presumably enhances the solubility of **25**. This suggests that future exploration of similar heteroaryl analogues of **1** could prove fruitful.

Finally, it can be seen that the dimethyl moiety on C-3 of **27** does not enhance its biological activity relative to **14**, as had been hoped. This may be due to steric constraints imposed on the β-hydroxy ketone system by the presence of an α-dimethyl group, and indicative of a role played by the α-CH_2 moiety in the in vitro mode of action of these compounds. However, as no data have been reported on the anti-cancer activity of alkyl furan **2** (Fig. 1) or its analogues (although one publication describes the inhibition of chemically induced carcinogenesis by 2-*n*-heptyl furan and a related alkyl thiophene[55]), this is not necessarily evidence of a 2-alkyl furan derived from **14** possessing biologically activity. It is nevertheless interesting to note that the activity of **27** is comparable to that of **15** and **21**, which shows that α-substitution of the β-hydroxyketone system is not wholly deleterious.

The negative optical rotation exhibited by **27** is intriguing, being in contrast to the positive optical rotations displayed by the other isomers of **1** described here.[56] However, given the reported inactivity of (−)-(*S*)-persin in lactating mammary glands,[17] the fact that **27** did display activity suggests the hydrolytic kinetic resolution of epoxide **32** catalyzed by **33** did afford the desired (*R*)-diol **26**, with the absolute configuration about C-2 being preserved in **27**. (The activity of (−)-(*S*)-persin against breast cancer cells has yet to be investigated, although it seems reasonable to propose that this unnatural isomer will be as inactive therein as it is in lactating mammary tissue).[††]

Accordingly, we hypothesize that the presence of the two methyl groups on C-3, adjacent to the chiral carbon, has profound effects on the configuration adopted by **27** in solution, leading to the observed optical rotation. This novel finding needs further investigation, but may be analogous to the large variability in optical rotation values observed in a set of tubulin-binding thiocolchicines, which is attributed to changes in biaryl dihedral angle resulting from different substituents on a position *beta* to a chiral carbon.[57] An even more striking example of substituent effects upon the optical rotation of otherwise identical molecules is seen in calyculins A and B, members of a family of natural products with significant anti-protein phosphatase and both anti- and pro-carcinogenic activities isolated from the sponge *Discodermia calyx*.[58] In this instance, a 70-unit difference in the optical rotation of the two compounds is observed, with the only structural difference between the molecules being the position of a –CN substituent on a double bond separated by nine carbons from the nearest asymmetric centre (of which there are 15). A computational analysis concluded that this remarkable variation is due to the differing degree of polarizability of the –CN group in each isomer, which influences the electronic character of the adjoining carbon–carbon double bond, and which may be amplified through the molecules' extended conjugated system.[59]

We have previously reported that the activity of **1** in breast cancer cells is not dependent on their ER status, and is specific for the malignant phenotype,[30] and this is supported by further data in Table 2. Firstly, the phenotypically normal mammary cell-line MCF-10A was non-responsive to both **1** and **14**, in keeping with prior observations.[30] Moreover, it can be seen that the presence of neither ER nor androgen receptor (AR) is necessary for the cytostatic effects of **1** to be observed, which suggests **1** and its

[††] In contrast to this inactivity of (*S*)-**1**, the orientation of the C-2 hydroxyl group in persenones A & B, α′,β′-unsaturated analogues of **1**, is reportedly not critical to their activity (Kim, O. K.; Murakami, A.; Nakamura, Y.; Kim, H. W. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 2500).

analogues may have efficacy in other cancers.[‡‡] The same trend cannot be said to hold for **14**, however, whose fully saturated side-chain appears to render it inactive in all but ER+ cells. The toxicity of **1** in Hs578 cells indicates Bim-independent cytostatic pathways may also be activated by this compound.

A recent study demonstrated that in a cell-line model of ER−/AR+ molecular apocrine breast cancer, MDA-MB-453, AR emulates the pro-proliferative DNA-binding and transcriptional regulation capabilities of ER.[60] The authors suggest that these findings add further weight to the concept of utilizing anti-androgens for the treatment of apocrine breast cancers, which do not respond well to therapy with ER antagonists (e.g., tamoxifen, aromatase inhibitors).[61] This thesis is supported by other new research, showing that the anti-androgen bicalutamide inhibits the growth of ER−/AR+/HER2+ tumors (derived from MDA-MB-453 xenografts) in mice, via inhibition of the androgen-stimulated HER2/HER3 signaling cascade.[62] Bicalutamide is currently under investigation for the treatment of metastatic ER−/AR+ breast cancer (NCT00468715), and these researchers note that the androgen-signaling- and HER2-dependence of the growth inhibition they observed may be critical to its efficacy in this setting. It would be interesting to examine the role **1** might play in this milieu, given the known ability of **1** to potentiate the pro-apoptotic effects of tamoxifen in both ER− and ER+ cells.[30]

4. Conclusion

Several analogues of the avocado-produced toxins (+)-(*R*)-persin (**1**) and (+)-(*R*)-tetrahydropersin (**14**) have been synthesized, and analyzed for their cytostatic and pro-apoptotic effects in human breast cancer cell lines. A number of structural features important and/or critical to the biological activity of such compounds have been revealed. Truncated-chain analogue **13** was inactive within the concentration range of interest, showing that the length of the side-chain was crucial for activity. Enone **23** and bis-aroylated compounds **20**, **22** and **24** were similarly inactive, indicating that a free β-hydroxyl group is essential. Phenyl (**15**) and 4-bromophenyl (**21**) analogues were less active than the natural products, but 4-pyridinyl derivative **25** demonstrated potency comparable to that of **1**, indicating heterocyclic analogues may be a fruitful area for future exploration. In contrast, the lower activity of α-dimethyl analogue **27** suggests the natural β-hydroxyketone system is important for the optimal anti-cancer activity of these compounds.

5. Experimental section

5.1. General procedures

All non-aqueous reactions were conducted in oven- or heat-gun-dried glassware under a positive pressure of dry nitrogen, and utilizing commercially available dry solvents, unless otherwise noted. 2,4,6-Trimethyl pyridine was distilled from CaH_2, and stored over 4 Å molecular sieves. pH 7 buffer was prepared from aqueous mixtures of NaH_2PO_4 and Na_2HPO_4. Melting points were determined on an Electrothermal IA9100 melting point apparatus, and are as read. ^{1}H NMR spectra were measured on a Bruker Avance 400 spectrometer at 400 MHz and are referenced to Me_4Si. Chemical shifts and coupling constants are recorded in units of parts per million (ppm) and hertz (Hz), respectively. High-resolution electron impact (HREIMS) mass spectra were determined on a VG-70SE mass spectrometer at nominal 5000 resolution. High-resolution electrospray ionization (HRESIMS) and atmospheric pressure chemical ionization (HRAPCIMS) mass spectra were determined on a Bruker micrOTOF-Q II mass spectrometer. Low-resolution atmospheric pressure chemical ionization (APCI) mass spectra were measured for organic solutions on a ThermoFinnigan Surveyor MSQ mass spectrometer, connected to a Gilson autosampler. Thin-layer chromatography was carried out on aluminium-backed silica gel plates (Merck 60 F_{254}), with visualization of components by UV light (254 and 365 nm), and by staining in basic aqueous permanganate and ethanolic phosphomolybdic acid solutions. Column chromatography was carried out on silica gel (Merck 230–400 mesh). Tested compounds were unanalyzable by standard HPLC techniques due to their lack of a suitable chromophore and/or instability, but were estimated as being >90–95% pure by ^{1}H NMR and TLC analysis. Optical rotation measurements were performed on a Schmidt and Haensch Polartronic NH8 polarimeter at 589.44 nm, and are referenced to a certified quartz cell. The absolute configurations of **1**,[12] **14**[28] and **15**[39] were established by comparison of their optical rotations with literature values. All other absolute configurations (**20**, **22**, **24** and **25**) were assigned by analogy, although a similar assumption cannot be made as to the polarity of optical rotation of these compounds. A slight modification of the literature method[12] was used for the synthesis of (+)-(*R*)-persin, as outlined below. Other known compounds were synthesized by reported methods, as indicated.

5.2. Synthesis

5.2.1. 2-Acetoxyacetaldehyde (11)

Two batches of allyl acetate (each of 13.00 mL, 119.46 mmol; total of 238.92 mmol) were ozonolysed according to the procedure of MacLeod & Schäffeler[12] and the resulting crude product mixtures combined. Column chromatography of this material on silica gel, eluting with 0–50% Et_2O:CH_2Cl_2, afforded **11** as a pale yellow oil (15.62 g, 64%), which was estimated as ~60% clean by ^{1}H NMR analysis, and whose primary ^{1}H NMR signals were consistent with the literature.[12] This was used immediately, without further purification: TLC R_f = 0.29 in 10% Et_2O:CH_2Cl_2.

5.2.2. (+)-(*R*)-(12*Z*,15*Z*)-2-Hydroxy-4-oxohenicosa-12,15-dien-1-yl acetate [(+)-(*R*)-persin] (1)

A solution of (−)-*B*-chlorodiisopinocampheylborane [(−)-DIP-Cl] (50–65 wt.% in hexanes—assumed to be 50 wt.%, 7.35 mL, 10.64 mmol, 2.00 equiv) was added to CH_2Cl_2 (5 mL), and the resulting solution cooled to −78 °C. To this solution (*i*-Pr)$_2$NEt (2.78 mL, 15.96 mmol, 3.00 equiv) was added dropwise, followed by a solution of linoleic ketone **9**[12] (1.48 g, 5.32 mmol, 1.00 equiv) in CH_2Cl_2 (10 mL), and the resulting mixture stirred in the dark at −78 °C for 195 min. At this point, further quantities of (−)-DIP-Cl (7.35 mL, 10.64 mmol, 2.00 equiv) and (*i*-Pr)$_2$NEt (2.78 mL, 15.96 mmol, 3.00 equiv) were added, and stirring was continued in the dark at −78 °C for 325 min. After this time, a solution of freshly columned aldehyde **11** (~60% clean, 5.72 g, 33.59 mmol, 6.32 equiv) in CH_2Cl_2 (19 mL) was added, and the resulting mixture stirred in the dark at −78 °C for 65 min, and then left sitting between −30 and −20 °C in a freezer in the dark for 19 h. The reaction mixture, held at −20 °C in a cooling-bath, was then quenched by addition of pH 7 buffer (50 mL), and the resulting mixture extracted with Et_2O (×4). The combined organic extracts were dried ($MgSO_4$), and then concentrated to dryness in vacuo. The residue was re-dissolved in MeOH (100 mL) and pH 7 buffer (30 mL), and cooled to 0 °C. 30% aq H_2O_2 (30 mL) was added, and resulting mixture stirred at rt in the dark for 2 h. The mixture was then poured into H_2O, and extracted with CH_2Cl_2 (×3). The combined organic extracts were washed sequentially with saturated aqueous $NaHCO_3$ and brine, and then dried

[‡‡] This finding illustrates that the cytostatic effects of **1** cannot be ascribed solely to dynamic changes in lipid composition occurring during lactation. It is interesting to speculate that **1** may behave as a less lipophilic analogue of linoleic acid (**4**), and thus deleteriously perturb levels of arachidonic acid and derived eicosanoids in rapidly replicating cancer cells.

($MgSO_4$), and finally concentrated to dryness in vacuo. The resulting colorless oil was purified by column chromatography on silica gel, eluting with 0–40% EtOAc/hexanes, to afford **1** (658 mg, 33%) as a colorless oil: ^{1}H NMR ($CDCl_3$): 5.48–5.24 (m, 4H), 4.38–4.24 (m, 1H), 4.11 (dd, J = 11.5, 4.0 Hz, 1H), 4.05 (dd, J = 11.5, 6.2 Hz, 1H), 3.11 (d, J = 3.9 Hz, 1H), 2.77 (distorted t, 2H), 2.65–2.60 (m, 2H), 2.44 (t, J = 7.4 Hz, 2H), 2.10 (s, 3H), 2.05 (app q, J = 13.6, 6.7 Hz, 4H), 1.65–1.50($m_{overlapping\ water\ peak}$, 2H), 1.43–1.22 (m, 14H), 0.89 (t, J = 6.9 Hz, 3H); HRESIMS calc. for $C_{23}H_{40}NaO_4$ $(M+Na)^+$ m/z 403.2819, found 403.2832; $[\alpha]_D^{25}$ = +10.78° (c 1.02, $CHCl_3$; lit. $[\alpha]_D^{20}$ = +10.20° (c 1.00, $CHCl_3$),[12] $[\alpha]_D^{22}$ = +10.70° (c 1.00, $CHCl_3$),[17] $[\alpha]_D^{22}$ = +11.98° (c 1.00, $CHCl_3$),[17] $[\alpha]_D^{24}$ = +11.30° (c 0.45, $CHCl_3$),[11] $[\alpha]_D^{23}$ +11.20° (c 1.00, $CHCl_3$),[63] $[\alpha]_D^{22}$ +10.50° (c 0.26, $CHCl_3$)[64]); TLC R_f = 0.20–0.30 (30% EtOAc/hexanes).

5.2.3. (*R*)-2-Hydroxy-4-oxodecyl acetate (13)

A solution of (−)-*B*-chlorodiisopinocampheylborane [(−)-DIP-Cl] (50–65 wt.% in hexanes—assumed to be 50 wt.%, 1.50 mL, 2.04 mmol, 2.00 equiv) was added to CH_2Cl_2 (1.5 mL), and the resulting solution cooled to −78 °C. To this solution (i-Pr)$_2$NEt (0.53 mL, 3.07 mmol, 3.00 equiv) was added dropwise, followed by a solution of 2-octanone (**12**) (0.16 mL, 1.02 mmol, 1.00 equiv) in CH_2Cl_2 (1.5 mL), and the resulting mixture stirred in the dark at −78 °C for 80 min. At this point, further quantities of (−)-DIP-Cl (1.50 mL, 2.04 mmol, 2.00 equiv) and (i-Pr)$_2$NEt (0.53 mL, 3.07 mmol, 3.00 equiv) were added, and stirring was continued in the dark at −78 °C for 85 min. After this time, a solution of freshly columned aldehyde **11**[12] (~50% clean, 1.261 g, 6.17 mmol, 6.04 equiv) in CH_2Cl_2 (10 mL) was added, and the resulting mixture stirred in the dark at −78 °C for 70 min, and then left sitting between −30 and −20 °C in a freezer in the dark for 68 h. The reaction mixture, held at −20 °C in a cooling-bath, was then quenched by addition of pH 7 buffer (10 mL), and the resulting mixture extracted with Et_2O (×4). The combined organic extracts were dried ($MgSO_4$), and then concentrated to dryness in vacuo. The residue was re-dissolved in MeOH (40 mL) and pH 7 buffer (15 mL), and cooled to 0 °C. 30% aq H_2O_2 (40 mL) was added, and resulting mixture stirred at rt in the dark for 2 h. The mixture was then poured into H_2O, and extracted with CH_2Cl_2 (×3). The combined organic extracts were washed sequentially with saturated aqueous $NaHCO_3$ and brine, and then dried ($MgSO_4$), and finally concentrated to dryness in vacuo. The resulting colorless oil was purified by column chromatography on silica gel, eluting with 0–40% EtOAc/hexanes, to afford **13** (303 mg, quantitative) as a colorless oil: ^{1}H NMR ($CDCl_3$): 4.35–4.25 (m, 1H), 4.11 (dd, J = 11.5, 4.1 Hz, 1H), 4.06 (dd, J = 11.5, 6.2 Hz, 1H), 3.12 (d, J = 3.9 Hz, 1H), 2.67–2.59 (m, 2H), 2.44 (t, J = 7.4 Hz, 2H), 2.10 (s, 3H), 1.65–1.51 ($m_{overlapping\ water\ peak}$, 2H), 1.37–1.22 (m, 6H), 0.96–0.82 (m, 3H); HRESIMS calcd for $C_{12}H_{22}NaO_4$ $(M+Na)^+$ m/z 253.1410, found 253.1423; TLC R_f = 0.20–0.30 (30% EtOAc/hexanes).

5.2.4. (+)-(*R*)-2-Hydroxy-4-oxohenicosyl acetate [(+)-(*R*)-tetrahydropersin] (14)

To a solution of **1** (160 mg, 0.42 mol) in EtOAc (150 mL) was added 10% Pd/C (~100 mg), and the resulting suspension was hydrogenated at 15 psi overnight. After this time, the mixture was filtered through a pad of Celite®, and concentrated to dryness in vacuo. Purification of the resulting white solid residue by column chromatography, eluting with 0–40% EtOAc/hexanes, and re-precipitation from acetone, afforded **14** (81 mg, 50%) as an amorphous white waxy solid: mp 71–72 °C (lit.[28] 68.5–70 °C); ^{1}H NMR ($CDCl_3$): 4.35–4.25 (m, 1H), 4.11 (dd, J = 11.5, 4.0 Hz, 1H), 4.05 (dd, J = 11.5, 6.2 Hz, 1H), 3.12 (d, J = 4.0 Hz, 1H), 2.62 (m, 2H), 2.44 (t, J = 7.4 Hz, 2H), 2.10 (s, 3H), 1.63–1.51 ($m_{overlapping\ water\ peak}$, 2H), 1.37–1.17 (m, 28H), 0.88 (t, J = 6.8 Hz, 3H); HRESIMS calcd for $C_{23}H_{44}NaO_4$ m/z $(M+Na)^+$ 407.3132, found 407.3160; $[\alpha]_D^{30}$ = +6.94° (c 0.87, $CHCl_3$; lit.[28] $[\alpha]_D^{30}$ = +13.8° (c 3.90, $CHCl_3$)); TLC R_f = 0.20–0.40 (30% EtOAc/hexanes).

5.2.5. (+)-(*R*)-(12*Z*,15*Z*)-2-hydroxy-4-oxohenicosa-12,15-dien-1-yl benzoate (15) and (*R*)-(12*Z*,15*Z*)-4-oxohenicosa-12,15-diene-1,2-diyl dibenzoate (20)

To a solution of β,γ-diol **19**[39] (381 mg, 1.13 mmol, 1.00 equiv) in CH_2Cl_2 (20 mL) was added pyridine (0.45 mL, 5.63 mmol, 5.00 equiv), and the resulting solution cooled to 0 °C. Benzoyl chloride (0.33 mL, 2.81 mmol, 2.50 equiv) was added slowly to this solution, and the resulting mixture allowed to stir for a further 5 min at 0 °C, and then allowed to warm to rt overnight, in the dark. After this time, the reaction mixture was cooled to 0 °C, quenched with saturated aqueous $NaHCO_3$, and allowed to warm to rt The mixture was extracted with CH_2Cl_2 (×5), and the combined extracts were washed with brine, dried ($MgSO_4$), and concentrated to dryness in vacuo. The resulting oil was purified by column chromatography on silica gel, eluting with 0–25% EtOAc/hexanes, to afford **20** (431 mg, 70%) as a colorless oil: ^{1}H NMR ($CDCl_3$): 8.06–7.96 (m, 4H), 7.60–7.51 (m, 2H), 7.46–7.38 (m, 4H), 5.89–5.79 (m, 1H), 5.44–5.28 (m, 4H), 4.64 (dd, J = 12.0, 3.7 Hz, 1H), 4.58 (dd, J = 12.0, 5.4 Hz, 1H), 3.20 (dd, J = 17.0, 6.5 Hz, 1H), 2.90 (dd, J = 17.0, 6.5 Hz, 1H), 2.76 (t, J = 6.2 Hz, 2H), 2.46 (t, J = 7.4 Hz, 2H), 2.10–1.96 (m, 2H), 1.67–1.51 ($m_{overlapping\ water\ peak}$, 4H), 1.41–1.18 (m, 14H), 0.88 (t, J = 6.9 Hz, 3H); HRESIMS calcd for m/z $C_{35}H_{46}NaO_5$ m/z $(M+Na)^+$ 569.3237; found 569.3253; TLC R_f = 0.41 (20% EtOAc/hexanes); and **15** (91 mg, 18%) as a low-melting crystalline solid: mp <37 °C; ^{1}H NMR ($CDCl_3$): 8.09–8.01 (m, 2H), 7.62–7.54 (m, 1H), 7.50–7.41 (m, 2H), 5.44–5.27 (m, 4H), 4.49–4.40 (m, 1H), 4.40–4.30 (m, 2H), 3.20 (br s, 1H), 2.77 (distorted t, 2H), 2.71 (d, J = 6.0 Hz, 2H), 2.46 (t, J = 7.4 Hz, 2H), 2.11–1.98 (m, 4H), 1.68–1.44 ($m_{overlapping\ water\ peak}$, 2H), 1.41–1.22 (m, 14H), 0.89 (t, J = 6.9 Hz, 3H); HRESIMS calcd for $C_{28}H_{42}NaO_4$ m/z $(M+Na)^+$ 465.2975, found 465.2987; $[\alpha]_D^{28}$ = +8.74° (c 1.03, $CHCl_3$; lit.[39] $[\alpha]_D^{20}$ = +10.70 (c 0.43, $CHCl_3$)); TLC R_f = 0.29 (20% EtOAc/hexanes).

5.2.6. (+)-(*R*)-(12*Z*,15*Z*)-2-hydroxy-4-oxohenicosa-12,15-dien-1-yl 4-bromobenzoate (21) and (*R*)-(12*Z*,15*Z*)-4-oxohenicosa-12,15-diene-1,2-diyl bis(4-bromobenzoate) (22)

A solution of β,γ-diol **19**[39] (412 mg, 1.22 mmol, 1.00 equiv) in CH_2Cl_2 (35 mL) was allowed to react with 2,4,6-trimethyl pyridine (0.80 mL, 6.09 mmol, 5.00 equiv) and 4-bromobenzoyl chloride (724 mg, 3.30 mmol, 2.71 equiv) for 23 h under the conditions outlined in Section 5.2.5. The resulting solid was purified by column chromatography on silica gel, eluting with 0–20% EtOAc/hexanes, to afford **22** (195 mg, 23%) as a colorless oil: ^{1}H NMR ($CDCl_3$): 7.95–7.77 (m, 4H), 7.67–7.49 (m, 4H), 5.86–5.77 (m, 1H), 5.43–5.27 (m, 4H), 4.62 (dd, J = 12.0, 3.5 Hz, 1H), 4.55 (dd, J = 12.0, 5.8 Hz, 1H), 2.99 (dd, J = 17.1, 6.5 Hz, 1H), 2.87 (dd, J = 17.1, 6.5 Hz, 1H), 2.76 (t, J = 6.0 Hz, 2H), 2.45 (t, J = 7.4 Hz, 2H), 2.10–1.80 (m, 4H), 1.65–1.52 ($m_{overlapping\ water\ peak}$, 2H), 1.44–1.17 (m, 14H), 0.89 (t, J = 6.9 Hz, 3H); HRESIMS calcd for $C_{35}H_{44}Br_2NaO_5$ $(M+Na)^+$ m/z 727.1431, 725.1448, found 727.1432, 725.1456; TLC R_f = 0.51 (20% EtOAc/hexanes); and **21** (386 mg, 61%) as a white feathery crystalline solid: mp 50–52 °C; ^{1}H NMR ($CDCl_3$): 7.91 (dt, J = 9.0, 2.1 Hz, 2H), 7.59 (dt, J = 9.0, 2.1 Hz, 2H), 5.44–5.27 (m, 4H), 4.49–4.38 (m, 1H), 4.38–4.28 (m, 2H), 3.20 (d, J = 4.0 Hz, 1H), 2.77 (t, J = 6.4 Hz, 2H), 2.69 (d, J = 6.0 Hz, 2H), 2.45 (t, J = 7.4 Hz, 2H), 2.05 (app q, J = 13.5, 6.7 Hz, 4H), 1.66–1.52 (m, 2H), 1.41–1.21 (m, 14H), 0.89 (t, J = 6.9 Hz, 3H); HRESIMS calcd for $C_{28}H_{42}BrO_4$ $(M+H)^+$ m/z 523.2242, 521.2261, found 523.2236, 521.2257; $[\alpha]_D^{28}$ = +3.77° (c 1.06, $CHCl_3$); TLC R_f = 0.25 (20% EtOAc/hexanes).

5.2.7. (2*E*,12*Z*,15*Z*)-4-oxohenicosa-2,12,15-trien-1-yl 4-nitro benzoate (23) and (*R*)-(12*Z*,15*Z*)-4-oxohenicosa-12,15-diene-1,2-diyl bis(4-nitrobenzoate) (24)

A solution of β,γ-diol **19**[39] (402 mg, 1.19 mmol, 1.00 equiv) in CH_2Cl_2 (40 mL) was allowed to react with 2,4,6-trimethyl pyridine (0.80 mL, 6.05 mmol, 5.00 equiv) and 4-nitrobenzoyl chloride (741 mg, 3.85 mmol, 3.24 equiv) for 16 h under the conditions outlined in Section 5.2.5. The resulting solid was purified by column chromatography on silica gel, eluting with 0–20% EtOAc/hexanes, to afford **23** (156 mg, 28%) as yellow semi-solid oil: ^{1}H NMR ($CDCl_3$): 8.32 (dt, *J* = 8.9, 2.1 Hz, 2H), 8.25 (dt, *J* = 8.9, 2.1 Hz, 2H), 6.89 (dt, *J* = 16.0, 4.9 Hz, 1H), 6.36 (dt, *J* = 16.0, 4.9 Hz, 1H), 5.43–5.28 (m, 4H), 5.05 (dd, *J* = 4.9, 1.8 Hz, 2H), 2.77 (t, *J* = 6.4 Hz, 2H), 2.58 (t, *J* = 7.4 Hz, 2H), 2.05 (app q, *J* = 13.6, 6.7 Hz, 4H), 1.68–1.58 (m, 2H), 1.42–1.23 (m, 14H), 0.89 (t, *J* = 6.9 Hz, 3H); HRESIMS calcd for $C_{28}H_{39}NNaO_5$ $(M+Na)^+$ *m/z* 492.2720, found 492.2712; TLC R_f = 0.45 (20% EtOAc/hexanes); and **24** (277 mg, 37%) as an amorphous greasy solid: ^{1}H NMR ($CDCl_3$): 8.31–8.24 (m, 4H), 8.20–8.12 (m, 4H), 5.94–5.87 (m, 1H), 5.43–5.28 (m, 4H), 4.72 (dd, *J* = 12.1, 3.2 Hz, 1H), 4.63 (dd, *J* = 12.1, 6.2 Hz, 1H), 3.05 (dd, *J* = 17.4, 6.6 Hz, 1H), 2.92 (dd, *J* = 17.4, 6.3 Hz, 1H), 2.76 (t, *J* = 6.1 Hz, 2H), 2.48 (t, *J* = 7.4 Hz, 2H), 2.11–1.98 (m, 4H), 1.66–1.52 ($m_{overlapping\ water\ peak}$, 2H), 1.41–1.22 (m, 14H), 0.88 (t, *J* = 6.8 Hz, 3H); HRESIMS calcd for $C_{35}H_{44}N_2NaO_9$ $(M+Na)^+$ *m/z* 659.2939, found 659.2936; TLC R_f = 0.30 (20% EtOAc/hexanes).

5.2.8. (*R*)-(12*Z*,15*Z*)-2-hydroxy-4-oxohenicosa-12,15-dien-1-yl isonicotinate (25)

A solution of β,γ-diol **19**[39] (666 mg, 1.97 mmol, 1.00 equiv) in CH_2Cl_2 (60 mL) and DMF (25 mL) was allowed to react with 2,4,6-trimethyl pyridine (1.95 mL, 14.76 mmol, 7.50 equiv) and isonicotinoyl chloride hydrochloride (876 mg, 4.92 mmol, 2.50 equiv) for 16 h under the conditions outlined in Section 5.2.5. After this time, the reaction mixture was cooled to 0 °C, quenched with saturated aqueous $NaHCO_3$, and allowed to warm to rt The mixture was extracted with CH_2Cl_2 (×5), and the combined extracts were washed with H_2O (×3), and the aqueous layer back-extracted with CH_2Cl_2 (×1). The combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated to dryness in vacuo. The resulting solid was purified by column chromatography on silica gel, eluting with 0–50% EtOAc/hexanes, to afford **25** (98 mg, 11%) as an off-white semi-solid oil: ^{1}H NMR ($CDCl_3$): 8.79 (app dd, *J* = 4.5, 1.5 Hz, 2H), 7.86 (app dd, *J* = 4.4, 1.7 Hz, 2H), 5.45–5.26 (m, 4H), 4.51–4.41 (m, 1H), 4.41–4.31 (m, 2H), 3.23 (d, *J* = 4.0 Hz, 1H), 2.77 (t, *J* = 6.3 Hz, 2H), 2.70 (d, *J* = 5.9 Hz, 2H), 2.46 (t, *J* = 7.4 Hz, 2H), 2.05 (app q, *J* = 13.6, 6.8 Hz, 4H), 1.66–1.50 ($m_{overlapping\ water\ peak}$, 2H), 1.42–1.22 (m, 14H), 0.89 (t, *J* = 6.9 Hz, 3H); HRESIMS calcd for $C_{27}H_{42}NO_4$ $(M+H)^+$ *m/z* 444.3108, found 444.3109; TLC R_f = 0.32 (50% EtOAc/hexanes).

5.2.9. 3,3-Dimethylhenicos-1-en-4-ol (30)

To a suspension of In powder (3.09 g, 26.91 mmol, 1.51 equiv) in DMF (80 mL) was added a mixture of prenyl bromide (3.11 mL, 26.67 mmol, 1.50 equiv) and aldehyde **29**[42] (4.77 g, 17.78 mmol, 1.00 equiv) in DMF (30 mL), resulting in bubbling and a slightly exothermic reaction. This mixture was stirred at rt for 19 h, and then diluted with EtOAc. The resulting suspension was filtered through a pad of Celite,® and the filtrate was diluted with 30% EtOAc/hexanes. This mixture was washed sequentially with 1 M HCl, saturated aqueous $NaHCO_3$, and brine, and then dried ($MgSO_4$). Removal of solvent in vacuo afforded an amorphous off-white solid, which was purified by column chromatography on silica gel, eluting with 0–10% Et_2O:hexanes, to give **30** (1.33 g, 22%, unoptimized): mp 34–36 °C; ^{1}H NMR ($CDCl_3$): 5.86 (dd, *J* = 17.5, 10.9 Hz, 1H), 5.13–5.00 (m, 2H), 3.24 (ddd, *J* = 10.0, 4.9, 1.5 Hz, 1H), 1.62–1.45 ($m_{overlapping\ water\ peak}$, 2H), 1.39 (d, *J* = 5.0 z, 1H), 1.36–1.16 (m, 30H), 1.00 (app d, *J* = 1.8 Hz, 6H), 0.88 (t, *J* = 6.8 Hz, 3H); HRESIMS calcd for $C_{23}H_{46}ONa$ $(M+Na)^+$ *m/z* 361.3441, found 361.3435; TLC R_f = 0.34 (10% Et_2O/hexanes).

5.2.10. 3,3-Dimethylhenicos-1-en-4-one (31)

A solution of alcohol **30** (1.25 g, 3.70 mmol, 1.00 equiv) in DMSO (70 mL) was treated with acetic anhydride (14.00 mL, 14.72 mL, 39.00 equiv), and the resulting mixture stirred at rt for 10 min. After this time, NEt_3 (14.00 mL, 99.84 mmol, 27.00 equiv) was added, and the resulting mixture was stirred at rt in the dark for 46 h. At this point, the reaction mixture was diluted first with EtOAc, and then with 30% EtOAc/hexanes, and the resulting solution washed sequentially with H_2O (×2) and brine, and then dried ($MgSO_4$). Solvent was removed in vacuo to give an odoriferous dark brown amorphous solid, which was purified by column chromatography on silica gel, eluting with 0–1% Et_2O/hexanes, to give **31** (1.20 g, 96%) as a semi-crystalline off-white solid. This material was estimated by ^{1}H NMR analysis to contain ~4% unreacted **30**, and was used without further purification: ^{1}H NMR ($CDCl_3$): 5.91 (dd, *J* = 17.5, 10.5 Hz, 1H), 5.15 (dd, *J* = 3.9, 0.8 Hz, 1H), 5.11 (dd, *J* = 2.9, 0.8 Hz, 1H), 2.43 (t, *J* = 7.4 Hz, 2H), 1.52 ($m_{overlapping\ water\ peak}$, 2H), 1.35–1.15 (m, 34H), 0.88 (t, *J* = 7.4 Hz, 3H); HRESIMS calcd for $C_{23}H_{44}NaO$ $(M+Na)^+$ *m/z* 359.3284, found 359.3293; TLC R_f = 0.75 (10% Et_2O:hexanes).

5.2.11. 2-Methyl-2-(oxiran-2-yl)icosan-3-one (32)

To a mixture of ketone **31** (1.19 g, 3.54 mmol, 1.00 equiv) and EDTA (5 mg) in acetone (71.6 mL) and H_2O (2.4 mL) at 0 °C was added, portionwise, a mixture of $NaHCO_3$ (1.67 g) and Oxone® (1.67 g, 5.31 mmol, 1.54 equiv), and the resulting suspension stirred at rt for 40 h. At this point, TLC analysis indicated that the reaction mixture was only partially complete, and so the reaction mixture was re-cooled to 0 °C, and a further, larger excess of Oxone® (25.89 g, 84.23 mmol, 23.82 equiv) and $NaHCO_3$ (25.89 g) was added, portionwise, together with proportional quantities of EDTA (62 mg) and acetone (50 mL). The resulting mixture was stirred vigorously at rt for another 24 h. After this time, TLC analysis of the reaction mixture indicated a significant amount of **31** remained unreacted, and so the reaction mixture was re-cooled to 0 °C, and more Oxone® (31.31 g, 101.867 mmol, 28.81 equiv) and $NaHCO_3$ (31.31 g) was added, portionwise, together with proportional quantities of EDTA (105 mg) and acetone (50 mL). The resulting mixture was stirred vigorously at rt for another 23 h and then, with only a trace of **31** being visible by TLC analysis of the reaction mixture, filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (×5), and the combined organic extracts were dried ($MgSO_4$) and concentrated to dryness in vacuo. The resulting pale yellow amorphous solid was purified by column chromatography on silica gel, eluting with 0–5% Et_2O:hexanes, to furnish **32** (941 mg, 75%) as an amorphous white solid: ^{1}H NMR ($CDCl_3$): 3.04 (dd, *J* = 4.0, 2.8 Hz, 1H), 2.74 (t, *J* = 4.3 Hz, 1H), 2.64 (dd, *J* = 4.6, 2.8 Hz, 1H), 2.60–2.50 (m, 2H), 1.62–1.52 ($m_{overlapping\ water\ peak}$, 2H), 1.27 (br s, 28H), 1.10 (s, 6H), 0.90 (t, *J* = 6.8 Hz, 3H); HRESIMS calcd for $C_{23}H_{44}NaO_2$ $(M+Na)^+$ *m/z* 375.3234, found 375.3231; TLC R_f = 0.16 (5% Et_2O:hexanes).

5.2.12. (+)-(*R*)-1,2-Dihydroxy-3,3-dimethylhenicosan-4-one (26)

Catalyst formation:[47,48,50] To a solution of (*S*,*S*)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminato(2$^-$)]cobalt(II) [(*S*,*S*)-*t*-Bu cobalt(II) salen] (42 mg, 0.07 mmol, 0.027 equiv) in CH_2Cl_2 (2 mL) exposed to air was added acetic acid (0.036 mL, 0.620 mmol, 0.238 equiv), generating an immediate color change of the solution from vermilion to dark brown. The solution was stirred at rt for 72 min, after which time solvent was removed in vacuo to give the desired (*S*,*S*)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminato(2$^-$)]cobalt(III) acetate [(*S*,*S*)-*t*-Bu cobalt(II) salen acetate] catalyst **33** as an amorphous brown solid.

Hydrolytic kinetic resolution:[47–50] Epoxide **32** (921 mg, 2.61 mmol, 1.00 equiv) was dissolved in THF (2.5 mL), and the resulting solution added to catalyst **33** (as prepared above). This mixture was cooled to 0 °C, and then treated dropwise with distilled H_2O (0.038 mL, 2.09 mmol, 0.80 equiv) over 1 min. The resulting solution was allowed to warm to rt and stirred thus for 100 h. After this time, the reaction mixture was diluted with THF and MeOH, and then concentrated to dryness in vacuo. The brown semi-solid residue was purified by column chromatography on silica gel, eluting with 0–30% EtOAc/hexanes, to afford recovered epoxide **34** (484 mg) and diol **26** (331 mg) as an amorphous white solid (APCI + MS $[M-H_2O]+m/z$ 354.0). The presence of the desired α-dimethyl-β,γ-diol system was confirmed by comparison of the ^{1}H NMR data of this material with that reported for an analogous system by Liu et al.,[46] and **26** was then used without further purification. HRESIMS calcd for $C_{23}H_{46}NaO_3$ $(M+Na)^+$ m/z 393.3339, found 393.3338; TLC R_f = 0.30 (30% EtOAc/hexanes).

5.2.13. (−)-(*R*)-2-hydroxy-3,3-dimethyl-4-oxohenicosyl acetate (27)

To a solution of α-dimethyl-β,γ-diol **26** (324 mg) in CH_2Cl_2 (35 mL) cooled to −78 °C were sequentially added 2,4,6-trimethyl pyridine (0.250 mL, 1.89 mmol, 2.16 equiv) and acetyl chloride (0.075 mL, 1.049 mmol, 1.20 equiv), and the resulting solution was stirred at rt for 60 h. After this time, the reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$, and then extracted with CH_2Cl_2 (×5). The combined organic extracts were washed with brine, dried ($MgSO_4$), and then concentrated to dryness in vacuo. The residue was purified by column chromatography on silica gel, eluting with 0–20% EtOAc/hexanes, to afford **27** (144 mg, 40%) as a white waxy solid: mp 41–44 °C; ^{1}H NMR ($CDCl_3$): 4.23 (dd, J = 11.2, 2.2 Hz, 1H), 4.02 (dd, J = 11.2, 8.0 Hz, 1H), 3.98–3.92 (m, 1H), 2.90 (d, J = 5.2 Hz, 1H), 2.57–2.43 (m, 2H), 2.08 (s, 3H), 1.60–1.50 ($m_{overlapping\ water\ peak}$, 2H), 1.26 (br s, 28H), 1.21 (s, 3H), 1.19 (s, 3H), 0.88 (t, J = 6.8 Hz, 3H); HRESIMS calcd for $C_{25}H_{48}NaO_4$ $(M+Na)^+$ m/z 435.3445, found 435.3439; $[\alpha]_D^{28} = -13.66°$ (c 1.03, $CHCl_3$); TLC R_f 0.33 (20% EtOAc/hexanes).

5.3. Biological methods

The panel of normal and breast cancer cell lines were all obtained from ATCC (American Type Culture Collection, Manassas, VA, USA). Characteristics of these cell lines have been described previously.[65] Human breast cancer cell lines were routinely maintained in RPMI-1640 supplemented with 5% fetal calf serum (FCS), 10 μg/mL insulin and 2.92 mg/mL glutamine under standard conditions. The phenotypically normal, human mammary epithelial cell line MCF 10A was maintained in DMEM supplemented with 5% horse serum, 20 ng/mL human recombinant EGF, 0.5 μg/mL hydrocortisone, 100 ng/mL cholera toxin, 10 μg/mL bovine insulin, 50 units/mL penicillin G, and 50 μg/mL streptomycin sulfate under standard conditions.

Cell viability was determined by MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay (Promega Corp., Madison, WI) following the manufacturer's instructions. Cells were plated in 96-well plates and treated in triplicate for 24 h with drug or vehicle. Cell viability was assessed by the percentage of absorbance of treated cells relative to that of solvent controls, using a wavelength of 490 nm on a spectrophotometer. IC_{50} values were calculated using linear interpolation.

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