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Vitamin D receptor agonist/histone deacetylase inhibitor molecular hybrids

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ABSTRACT

Incorporation of zinc-binding groups into the side-chain of 1α,25-dihydroxyvitamin D_3 (1,25D) fully bifunctional hybrid molecules which act both as vitamin D receptor agonists and histone deacetylase inhibitors. These bifunctional hybrids display in vitro antiproliferative activity against the AT84 squamous carcinoma cell line while lacking the in vivo hypercalcemic effects of 1,25D.

1. Introduction

The hormonal form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1,25D; **1**; Fig. 1) has long been known for its central role in controlling calcium homeostasis. A broad range of accumulating data has provided evidence that 1,25D also has cancer chemopreventive properties, and that it functions as a key regulator of innate and adaptive immune responses.[1–3] 1,25D signals by binding to the vitamin D receptor (VDR), a member of the nuclear receptor family of ligand-regulated transcription factors.[1,4] As the therapeutic activity of 1,25D is limited by its capacity to induce hypercalcemia, numerous laboratories have developed analogs of 1,25D that retain VDR agonism but minimize its calcemic actions.[5]

Histone deacetylases (HDACs), along with the complementary histone acetyl transferases (HATs), regulate the acetylation states of histones and several other nuclear and non-nuclear proteins such as tubulin and HSP90. HDACs and HATs act as modulators of gene transcription by controlling DNA–histone interactions in the nucleosome and through regulation of components of the transcription machinery. Inhibitors of HDACs (HDACi), including trichostatin A (TSA, **2**; Fig. 1) and suberoylanilide hydroxamic acid (SAHA, **3**; Fig. 1), have been investigated as anti-cancer agents. Like VDR agonists, HDACi induce cell cycle arrest, cellular differentiation and/or apoptosis.[6–8] The potential of HDACi's as therapeutics is underscored by the recent approval of SAHA, under the trade name Zolinza, for treatment of cutaneous T-cell lymphoma.[9]

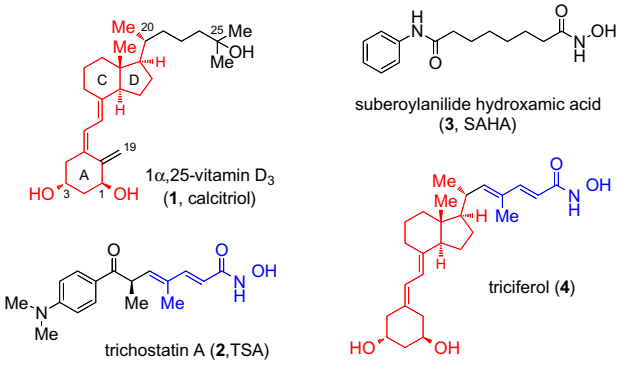

Figure 1. Structures of 1,25D, HDACi's and triciferol.

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We have been interested in developing bifunctional 1,25D analogs that combine VDR agonism with inhibition of histone deacetylase (HDAC) activity,[10] as both 1,25D and HDACs are modulators of gene transcription. Notably, combinatorial effects of TSA and 1,25D have been demonstrated on the proliferation of 1,25D-resistant cancer cells.[11] We have previously described the design, synthesis and biochemical characterization of triciferol (**4**, Fig 1), a molecule that combines agonism for the VDR with HDACi activity and displays enhanced cytostatic and cytotoxic activity relative to 1,25D.[10] The design of triciferol takes into account the need for a bifunctional molecule to fit entirely within the ligand binding pocket of the VDR. Simply linking 1,25D to TSA would create a molecule which would be too large to act as a VDR agonist. Thus, the dienylhydroxamic acid, which provides affinity for the catalytic zinc of HDAC active sites, was incorporated in the side-chain, replacing the normal 25-OH of 1,25D.

In extending the scope of 1,25D/HDACi hybrids, we sought to employ saturated, SAHA-like side-chains and, more importantly, to incorporate a variety of terminal zinc-binding groups (ZBGs). While hydroxamic acids are the most common ZBG in HDACi's, there is considerable interest in development of non-hydroxamate HDACi's, and a wide variety of alternative ZBGs have been reported, most notably the *ortho*-aminoanilides.[12,13] In the context of a 1,25D/HDACi hybrid, it was not clear whether other ZBGs, particularly the large *ortho*-aminoanilides, could be incorporated within the binding site of VDR. In this paper, we report the synthesis a series of 1,25D/HDACi hybrids incorporating a wide variety of ZBGs and show that there is indeed significant structural latitude in the types of molecules that function effectively in this context.

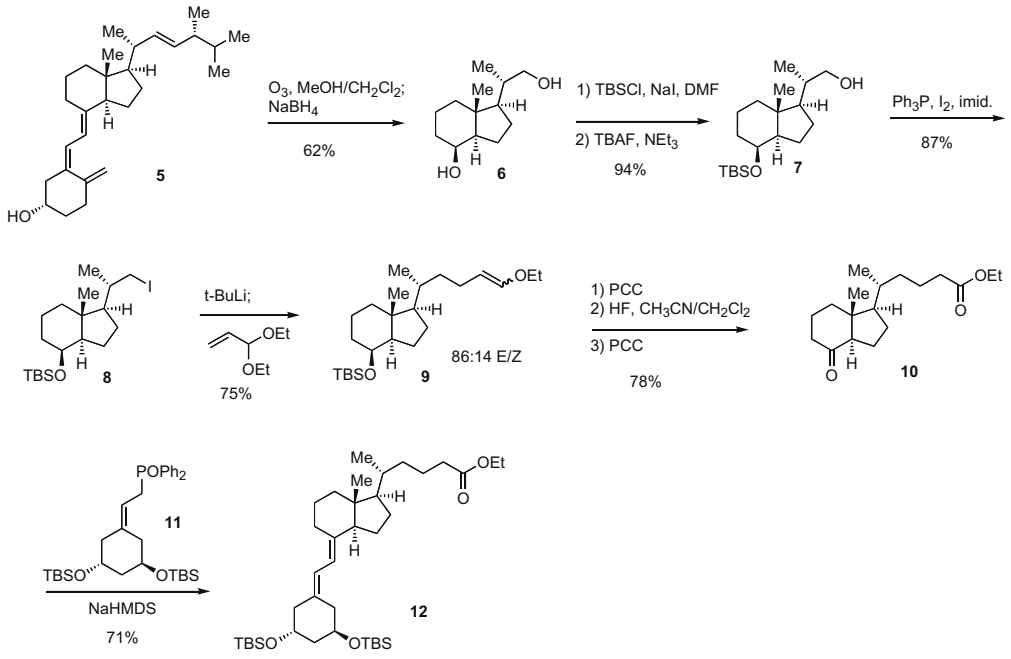
2. Results

2.1. Chemistry

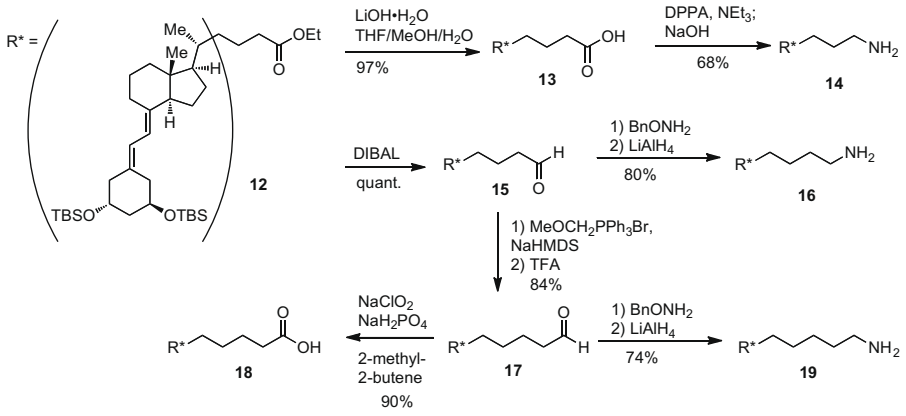
The synthesis of the hybrids makes use of a common precursor, **12**, which may be prepared in nine steps from commercially available vitamin D_2 (**5**, Scheme 1). Exhaustive ozonolysis of **5** followed by a reductive workup with sodium borohydride furnished the Inhoffen-Lythgoe diol **6** in 62% yield.[14] Disilylation with TBSCl followed by selective deprotection of the primary TBS group with TBAF afforded alcohol **7** in 94% yield. To set the stage for side-chain elongation, primary alcohol **7** was converted to the corresponding iodide **8**. In our hands, conversion of iodide **8** to either a cuprate or organozinc reagent followed by conjugate addition to acrylate derivatives was problematic.[15,16] However, metal–halogen exchange with *t*-BuLi followed by addition to acrolein diethylacetal cleanly effected an S_N2' displacement to form enol ether **9** in a reliable 75% yield.[17] An oxidation/deprotection sequence furnished ketone **10** which subsequently underwent clean Horner coupling with A-ring diphenylphosphine oxide (**11**) to furnish common precursor **12** as a single geometrical isomer.[18]

Ester **12** could be converted to a series of immediate hybrid precursors (Scheme 2). Simple saponification afforded acid **13** in high yield. Homologation by one carbon could be achieved by reduction of **12** with DIBAL to aldehyde **15** followed by Wittig olefination with methoxymethylene triphenylphosphorane. Hydrolysis of the resulting enol ether with TFA afforded aldehyde **17** which could be oxidized to acid **18** in high yield. Primary amines **14**, **16** and **19** could be generated, respectively, from **13** via Curtius rearrangement using DPPA or from **15** and **17** via reductive amination of the corresponding oximes.

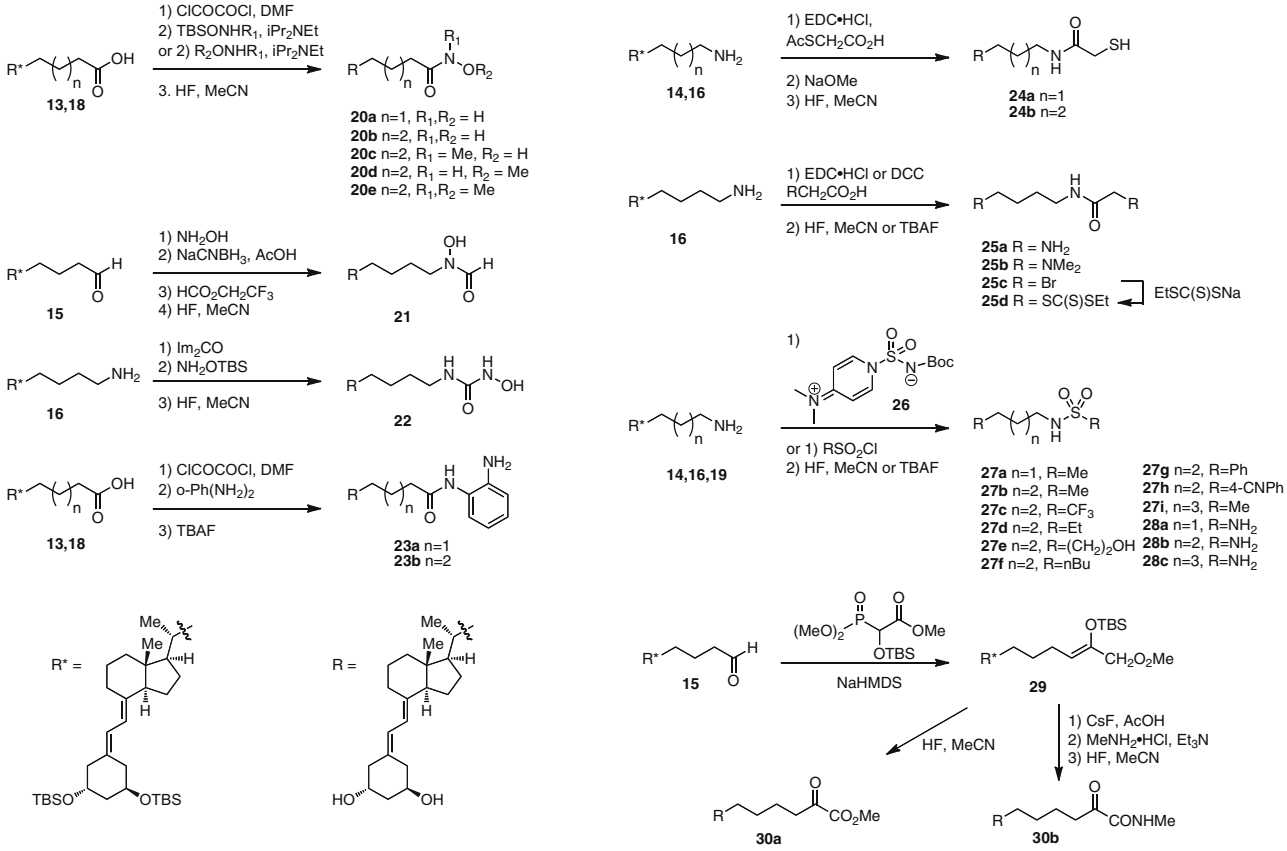
As shown in Scheme 3, the precursors prepared above were used to generate a wide variety of hybrids. Acids **13** and **18** were transformed to their corresponding acid chlorides and then coupled with either $TBSONH_2$, or substituted versions thereof, to generate, after global removal of silicon protecting groups with HF in acetonitrile, a series of hydroxamic acid and alkylhydroxamate hybrids. An *N*-hydroxyformamide (**21**) was also be prepared by treatment of aldehyde **15** with hydroxylamine, reduction of the resulting oxime with sodium cyanoborohydride and acylation with trifluoroethyl formate. Additionally, an *N*-hydroxyurea (**22**) was easily prepared by treatment of amine **16** with carbonyl diimidazole followed by addition of $TBSONH_2$. *ortho*-Aminoanilides **23a**,**b** were prepared by coupling acid chlorides derived from **13** and **18** with 1,2-diaminobenzene. Amines **14** and **16** could also be acylated with *S*-acetylthioglycolic acid, bromoacetic acid, Boc-glycine



Scheme 1. Synthesis of a common intermediate for hybrids.



Scheme 2. Synthesis of hybrid intermediates.



Scheme 3. Synthesis of VDR agonist/HDACi hybrids.

and *N*,*N*-dimethylglycine to afford other amide based hybrids. The intermediate bromoacetamide above, prior to silicon protecting group removal, could be further transformed into a trithiocarbonate (**25d**) by addition of sodium ethyl trithiocarbonate (from ethane thiol, NaHMDS and CS_2). A series of sulfonamides and sulfamides were prepared easily by reaction of amines **14**, **16** and **19** with alkyl and arylsulfonyl chlorides and sulfamidating agent **26**, respectively. Finally, α-ketoester and amide **30a** and **30b** were formed through a Horner–Wadsworth–Emmons olefination of aldehyde **15** with a silyloxy substituted phosphonate ester followed by either full deprotection or partial deprotection followed by aminolysis and final desilylation.

2.2. Testing of bifunctional activities of hybrids

Compound function was screened using a series of well-defined biochemical and cell-based assays to test for VDR agonism and HDAC inhibition. Results of these screens are summarized in Table 1. Compounds were initially tested at 1 μM for their capacity to induce expression of the *CYP24* gene (Table 1 and Fig. 2A and B). *CYP24* encodes the enzyme that initiates catabolism of **1**,[1] and its expression is exquisitely sensitive to VDR agonism. Hydroxamic acid hybrids **20a** and **20b**, which differ only by one methylene unit, displayed essentially identical potency, similar to that of **4**, in induction of *CYP24* expression (Fig. 2A). Moreover, peak *CYP24*

Table 1
Biochemical assessment of 1,25D/HDACi hybrids

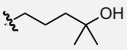
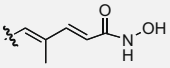
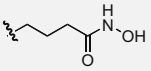
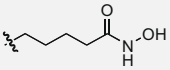
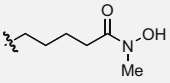
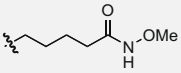
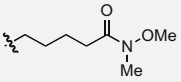
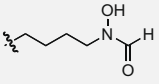
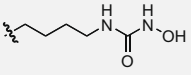
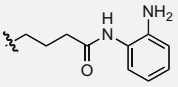
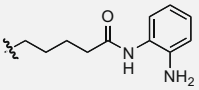
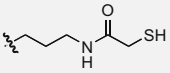
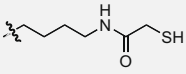
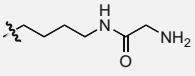
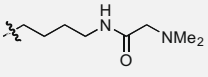
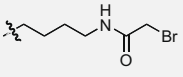
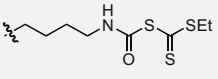
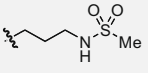
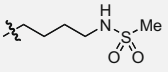
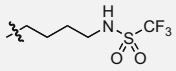
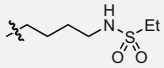
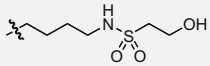
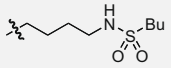
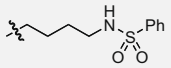
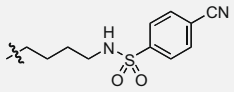
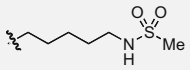
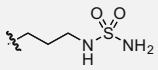
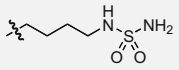
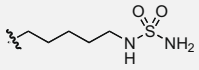
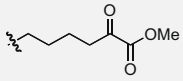
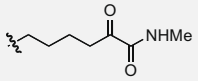
Compound	Structure	VDR agonism		HDAC activity					Hypercalcemia
		CYP24	FP (nM)	Tubulin	Histone	HDAC2 (μM)	HDAC3 (μM)	HDAC6 (μM)	
1		+++	13	–	–				+++
3	SAHA	–	–	+++	+++	0.21	0.15	0.35	
4		+++	87	++	+	10.4	13.3	0.58	–
20a		+++		–	+		3.2	9.3	
20b		++	248	++	+	16.5	14.5	3	–
20c		++		–	–				
20d		+++		+	–				
20e		+++		–	–				
21		+++		–	+				
22		–/+		–	–				
23a		++	524	++	++		460	330	–
23b		++	185	++	++	189	104	81.1	–
24a		+		–	+				
24b		++	36	++	+	96.3	32.3	1.75	–
25a		–/+		–	–				
25b		–/+		–	+				
25c		–		–	+				
25d		+++		–	–				
27a		+++	213	++	+/–		730	71.3	
27b		+++	321	+	++		718	191	–

Table 1 (*continued*)

Compound	Structure	VDR agonism		HDAC activity					Hypercalcemia
		CYP24	FP (nM)	Tubulin	Histone	HDAC2 (μM)	HDAC3 (μM)	HDAC6 (μM)	
27c		++	14	+	++		695	153	
27d		++		+	–				
27e		++		+	–/+				
27f		+++	29	–	–		725	101	–
27g		+++	15	++	++		508	293	–
27h		+++		–/+	+				
27i		+++		–/+	+				
28a		+++		+	–				
28b		++	280	+	++		470	50.4	
28c		+	196	++	+		605	31.6	
30a		+++		–	+/–				
30b		+++		+	+	90.4	36	64	

VDR agonism was assessed by RT-PCR analysis of CYP24 induction in SCC4 cells and direct binding to VDR was assessed using a fluorescence polarization (FP) assay. HDAC activity in SCC4 cells was assessed by measuring induction of tubulin and histone hyperacetylation using Western blot analysis. Direct inhibition of purified HDACs was measured using a fluorometric assay. Hypercalcemia assessed in mice at 240 or 1200 pmol/day.

levels induced by **20a** and **20b** were within a factor of two of that induced by **1**. Assaying other hybrids with wide-ranging ZBG's including *ortho*-aminoanilides **23a**,**b**, thioglycolate amides **24a**,**b**, sulfonamides **27a–i**, sulfamides **28a–c** and α-ketocarbonyls **30a**,**b**, showed moderate to strong agonism for the VDR. Conversely, amides **25a**,**b**,**c** and *N*-hydroxyurea **22** exhibited little or no VDR agonism and were not tested further.

Direct binding to and affinity for the VDR of selected bifunctional compounds (see below for assessment of HDACi activity) was tested using a fluorescence polarization (FP) assay.[10] Not surprisingly, FP assays revealed a range of affinities for the VDR, although IC_{50} values in FP assays were generally within a factor of 20–25 of that of **1** (Fig. 2C; Table 1). This includes *ortho*-aminoanilide **23b**, indicating a surprising tolerance for larger groups in the vicinity of the normal 25-OH binding region. Notably, four compounds, sulfonamides **27c**, **27f**, and **27g** and the thioglycolate amide **24b**, displayed affinities for the VDR in FP assays that were within a factor of 3 of the native ligand. The high affinities of **27f** and **27g** for the VDR were supported by the observation that they induced *CYP24* with a potency that was at least as high as that of **1** (Fig. 2B). These data confirm the broad latitude available in modifying the functionality of chain terminal groups in analogs of **1** without markedly sacrificing affinity for the VDR.

As in our previous study,[10] HDACi activity was initially assessed by screening the capacity of compounds at 1 μM concentration to induce acetylation of histones and tubulin (Table 1, Fig. 2D). The potential function of hydroxamic acids **20a** and **20b** as HDACi's was tested initially by assessing of levels of acetylated tubulin and histones H3 and H4 in SCC4 cells by Western blotting of extracts of vehicle- or compound-treated cells, using **2** and **4** as positive controls. Treatment for 24 h with **20a** and **20b** induced modest increases in histone acetylation, similar to results previously obtained with **4**.[10] Remarkably, while **20b** induced robust hyperacetylation of tubulin, **20a**, which is shorter by only one methylene unit, consistently had no effect (Fig. 2D). In addition to simple hydroxamic acids, *N*-methyl, *O*-methyl and *N*,*O*-dimethyl hydroxamates **20c**, **20d** and **20e** were also screened. While all three compounds displayed moderate VDR agonism (Table 1), their HDACi activity was weak, as **20c** and **20d** only very poorly induced tubulin and/or histone hyperacetylation while **20e** was completely inactive. Among hybrids with other ZBG's, *ortho*-aminoanilides **23a** and **23b**, and benzenesulfonamides and **27g** all induced substantial

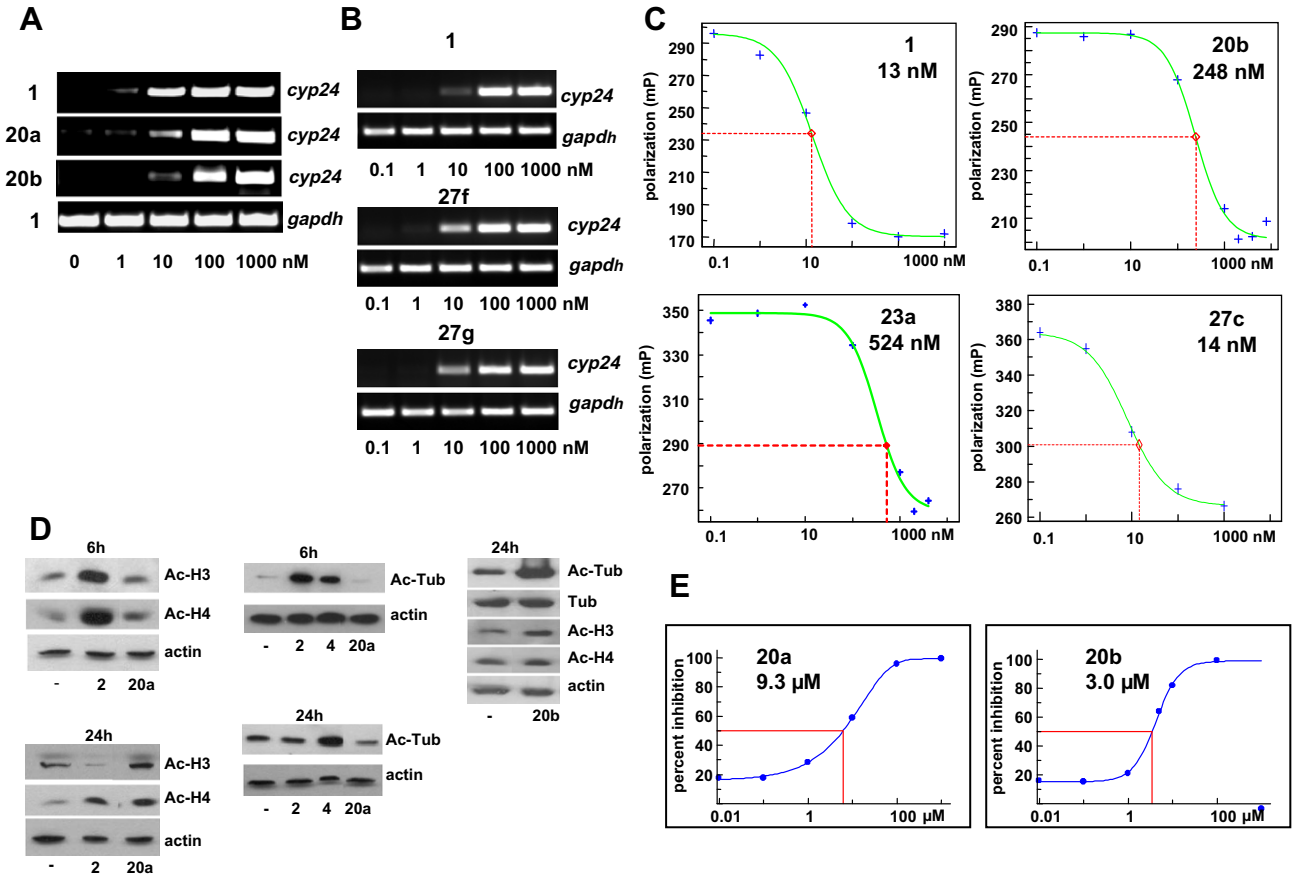


Figure 2. Representative VDR agonist and HDACi activities of hybrids. (A and B) Dose–response analysis of induction of *CYP24* mRNA expression by 1,25D (**1**) and hybrids **20a** and **20b** (A) and hybrids **27f** and **27g** (B). (C) Hybrids bind directly to the VDR ligand binding domain. Fluorescence polarization competition assays comparing displacement of a fluorescent tracer from the VDR ligand binding domain are shown. (mP, milli-Polarization units.) Estimated IC_{50}s for 1,25D and hybrids are indicated in the figure. (D) Western blotting analysis of induction of histone H3, histone H4 or tubulin acetylation after 6 or 24 h of treatment with TSA (**2**) or hybrids **4**, **20a** or **20b**, as indicated. (E) Inhibition of isolated HDAC6 by hybrids **20a** and **20b** as measured using a fluorometric substrate.

tubulin and histone hyperacetylation while others were more effective at inducing either tubulin (**24b**, **27a**, **28c**) or histone (**27b**, **27c**) hyperacetylation.

HDACi activities of a series of bifunctional compounds that induced histone and/or tubulin hyperacetylation at 1 μM concentration as assessed by Western blotting were further tested in a standard fluorometric assay using purified HDACs (Fig. 2E).[19,20] Consistent with its striking effects on tubulin, which is deacetylated primarily by HDAC6,[21] **4** inhibited purified HDAC6 deacetylase activity with sub-micromolar potency. The IC_{50} for **4** was very similar to that of positive control **3**. However, whereas **3** inhibited all three HDACs tested with very similar potencies, the IC_{50}'s of **4** for HDACs 2 and 3 were 20- to almost 100-fold higher than those for HDAC6, suggesting that the secosteroidal 'cap' group of **4** confers some degree of HDAC specificity on the compound. In agreement with these findings, we found that **20b**, a saturated side-chain analog of **4**, inhibited purified HDAC6 about fivefold more potently than HDACs 2 or 3 (Table 1). Several other compounds were tested in fluorometric assays and those found to have modest HDAC6i activity (IC_{50}: 10–100 μM) were *ortho*-aminoanilide **23b**, sulfonamide **27a**, sulfamides **28b** and **c** and α-ketoamide **30b**. As with the hydroxamic acids, the general trend among the alternative ZBG's was that they were more potent inhibitors of HDAC6, although hydroxamic acid **20a** and α-ketoamide **30b** were notable exceptions to this trend (Table 1). The most potent non-hydroxamate HDACi was thioglycolate **24b**, which displayed an IC_{50}'s of 1.75 μM, 32.3 μM and 96.3 μM against HDAC6, -3 and -2, respectively.

2.3. Lack of hypercalcemia in mice treated with hybrid compounds

As hypercalcemia is the dose-limiting toxicity associated with 1,25D treatment, **4** and seven selected second-generation compounds were compared with 1,25D for their capacity to induce hypercalcemia in mice (summarized in Table 1). Compounds chosen had a range of affinities for the VDR and a variety of terminal zinc-binding groups. Mice fed a normal calcium diet were infused using minipumps with **1** (12 or 24 pmol/day) or hybrids (240 or 1200 pmol/day) over 6–7 days. Elevated concentrations of hybrid were chosen in part because, based on the literature,[22,23] we expected they would not induce hypercalcemia as readily as **1**, but also to account for the lower affinity for the VDR of some hybrids. As expected, animals infused with 12 pmol/day **1** were hypercalcemic after 6–7 days of treatment (2.6–3.1 mM Ca vs 2.0–2.1 mM Ca for controls), and all animals treated with a 24 pmol/day dose were hypercalcemic within three days (2.9–3.4 mM Ca). In contrast, all animals infused with 240 or 1200 pmol/day of hybrid compounds appeared healthy (not shown). Moreover, most showed no sign of hypercalcemia after 6–7 days (1.9–2.3 mM Ca). This includes animals infused with compounds **24b** and **27g**, which have 1,25D- or near-1,25D-like affinities for the VDR as judged by FP assays and *CYP24* induction. Only hydroxamic acid hybrid **20a** showed

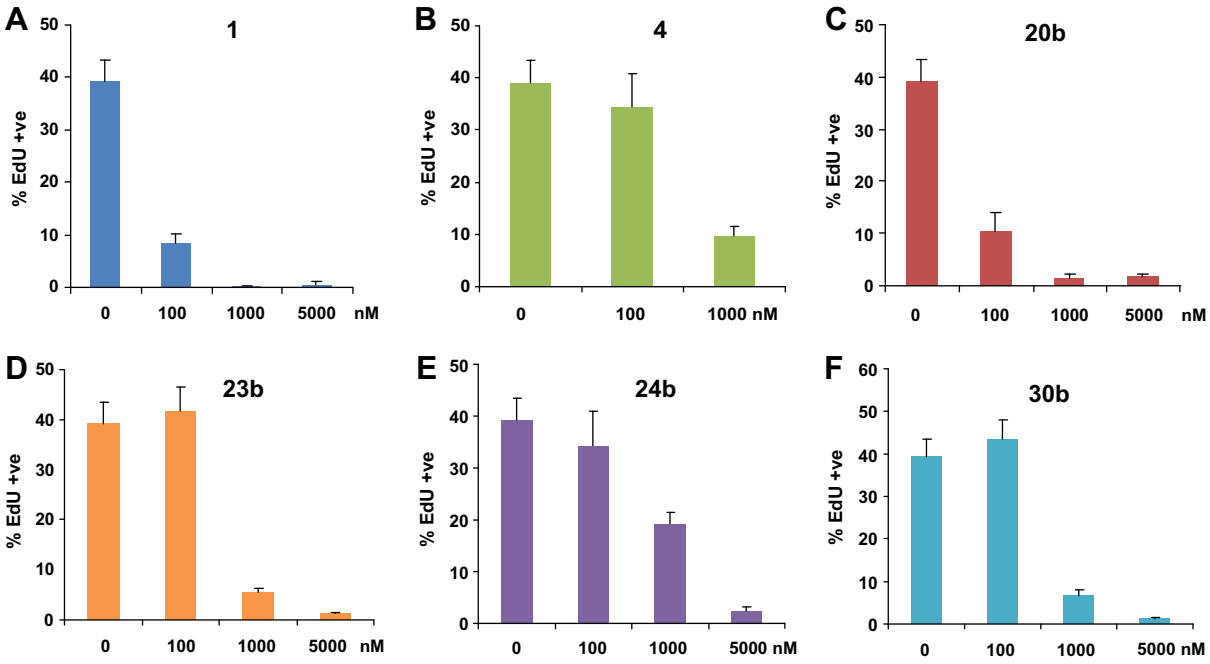


Figure 3. Analysis of the antiproliferative activities of selected hybrids. Murine AT84 head and neck squamous carcinoma cells were cultured under the recommended conditions and treated for 48 h with increasing doses, as indicated, of **1** (A) or hybrids **4** (B), **20b** (C), **23b** (D), **24b** (E) or **30b** (F). Cell proliferation was monitored using an EdU incorporation assay.

any significant level of elevated calcium levels (2.3–2.5 mM at 1200 pmol/day), but this is still significantly lower than observed with **1** and at a 100-fold higher concentration. Thus, all compounds tested lacked the dose-limiting toxicity associated with treatment with **1**.

2.4. Antiproliferative activity of hybrid compounds

Selected compounds were further tested by analyzing their capacity to inhibit the proliferation of the AT84 squamous carcinoma cell line. AT84 cells were chosen because they are sensitive to **1** in vitro and in vivo in tumor allografts.[24] This model is of interest for longer-term testing of compounds as, although **1** partially inhibits AT84 tumor growth in vivo, its efficacy is limited by hypercalcemia.[24] We tested a selection of bifunctional compounds (Fig. 3) that combined a range of potencies as VDR agonists with relatively potent HDACi activity in fluorometric assays. These included hydroxamates **4** and **20b**, *ortho*-aminoanilide **23b**, thioglycolate **24b**, and α-ketoamide **30b**. Remarkably, although **4** functioned comparably or better than **1** in previous cell growth assays,[10] its potency was markedly weaker than that of **1** in AT84 cells (Fig. 3A and B). In contrast, hydroxamate **20b** was a potent inhibitor of AT84 proliferation (Fig. 3C) even though it is a ~20-fold less potent VDR agonist than **1** (Table 1). Compounds **23b**, **24b**, and **30b** also markedly inhibited AT84 cell division, although at micromolar concentrations (Fig. 3D–F).

3. Discussion

The results above show that there is significant structural latitude in the creation of hybrid molecules possessing VDR agonism and HDACi activity, but that variation in chain length and ZBG has significant effects on activity and selectivity. We found a remarkable latitude in the types of ZBG's that may be incorporated into the side-chain while retaining VDR agonism.[25] Various *ortho*-aminoanilides, thioglycolates, α-ketoesters and amides, sulfonamides, sulfamides, and α-trithiocarbonyl amide **25d** all showed moderate to strong VDR agonism at 1 μM. Direct binding studies revealed that some of these compounds even displayed 1,25D-like affinity for the VDR. The VDR agonism of *ortho*-aminoanilides **23a** and **23b** is most striking, indicating a remarkable capacity of the VDR binding site to accommodate this large group. While aromatic rings have been incorporated in the side-chain of a few VDR agonists,[26] they are more commonly found in antagonists, particularly when appended to lactam rings.[27] Most of the successful hybrids containing alternative ZBG's also possessed HDACi activity in the micromolar range. In general, the hybrids were more potent toward HDAC6 than HDAC3, suggesting some induction of selectivity for this isoform by the secosteroidal vitamin D core.

One notable compound was the thioglycolate amide hybrid **24b**, which was a potent VDR agonist, and functioned with low micromolar potency as an HDAC6 inhibitor; its HDAC6i activity was within a factor of 5 of that of **3** (Table 1). Consistent with data for other secosteroidal hybrids, **24b** inhibited HDAC6 18.5- and >50-fold more potently than HDAC3 and HDAC2, respectively. Previous studies have suggested that thioglycolates (or mercaptoamides) are bidentate chelators of zinc ions and have shown that thioglycolate analogs of **3** could be developed with low micromolar HDACi activity when measured against HDACs present in HeLa cell extracts.[28]

We found that side-chain length was a critical parameter in controlling HDACi activity of some hybrid compounds. Hybrids **20a** and **20b**, which retain similar VDR agonist activity, possess saturated side-chains with **20a** being shorter by one methylene unit. Both compounds modestly induced histone hyperacetylation. However, whereas **20b** induced strong tubulin hyperacetylation in living cells, **20a** was inactive under the same conditions. Not unexpectedly, *N*- and *O*-alkylhydroxamates **20c–e** displayed significantly reduced HDAC activity, and inverting the hydroxamic acid to form an *N*-hydroxyformate resulted in minimal HDACi activity.

We noted apparent discrepancies between the data obtained from fluorometric assays with purified enzymes and those from analysis of protein acetylation in intact cells. For example, saturated hydroxamic acid **20b**, but not **20a**, induced robust tubulin

hyperacetylation in cells. Tubulin deacetylation is known to be mediated by HDAC6.[29] While **20a** was found to be a stronger inhibitor of HDAC3 relative to HDAC6, one of only two cases of reversed selectivity in all hybrids tested, the HDAC6 inhibition by **20a** was only a factor of three weaker than **20b**. Moreover, **20a** was more potent in the fluorescence assay than several other hybrids which did induce significant tubulin hyperacetylation such as **23a**, **23b**, and **27g** (Table 1; Fig. 2E). In particular, the latter three compounds, which were selected for the fluorometric assay due to their ability to induce strong histone and tubulin hyperacetylation in cells at 1 μM, inhibited activity of purified HDACs 2, 3 or 6 with relatively low potencies (IC_{50}s generally >100 μM). There are several possible explanations for these observations, including the fact that HDACs function as components of multiprotein complexes subject to multiple posttranslational modifications,[30] which may alter their conformation and susceptibility to inhibition relative to isolated enzyme. In addition, only two Class I HDACs were assayed in fluorometric assays, and these may not correspond to isoforms (e.g., HDAC1, HDAC8) controlling histone acetylation in SCC4 cells.[30] Finally, the HDAC6 inhibition assay is further complicated by the fact that it possesses two separate catalytic domains[29] making it difficult to directly correlate the fluorometric assay with activity in cells.

There is also some discrepancy between the binding potency of some of the hybrids with their observed affects on *CYP24* induction. Notably, hybrids **24b** and **27c** displace fluorescent ligand from the VDR at concentrations 10-fold lower than, for example, sulfonamides **27a** and **27b**, yet the latter were more potent inducers of *CYP24* induction. Similar results have been noted for other vitamin D analogs[31] and often reflect the capacity of different ligand–VDR complexes to release co-repressors and recruit co-activators for gene transcription in a manner which is reflective of the conformation of the ligand–receptor complex and not linearly correlated to binding affinity.

Selected hybrids tested showed antiproliferative activity against AT84 cells, with hydroxamic acid **20b**, *ortho*-aminoanilide **23b** and α-ketoamide **30b** showing the highest potency. While none of the compounds tested were more potent than **1**, it is important to note that none of them induced hypercalcemia even at concentrations that were 100-fold higher than a limiting dose of **1** (Table 1). In follow-up studies it will therefore be of interest to determine whether they display a greater therapeutic range than **1** in inhibiting AT84 tumor growth in vivo.

In conclusion, we have demonstrated that it is possible to create a wide range of bifunctional molecules which possess VDR agonism and HDACi activity. The structural latitude is significant with a wide variety of ZBGs amenable to incorporation into the side-chain of vitamin D-like secosteroids. Importantly several of these molecules function as antiproliferative agents against AT84 cells in vitro while possessing minimal hypercalcemic activity in vivo.

4. Experimental section

4.1. Synthesis of hybrid molecules

4.1.1. General experimental

MeCN, toluene and CH_2Cl_2 were distilled from CaH_2 under argon. THF and Et_2O were distilled from sodium metal/benzophenone ketyl under argon. All other commercial solvents and reagents were used as received from the Aldrich Chemical Company, Fischer Scientific Ltd, EMD Chemicals Inc., Strem or BDH. All glassware was flame dried and allowed to cool under a stream of dry argon. Silica gel (60 Å, 230–400 mesh) used in flash column chromatography was obtained from Silicycle and was used as received. Analytical thin-layer chromatography (TLC) was performed on pre-coated silica gel plates (Ultra Pure Silica Gel Plates purchased from Silicycle), visualized with a Spectroline UV_{254} lamp, and stained with a 20% phosphomolybdic acid in ethanol solution, or a basic solution of $KMnO_4$. Solvent systems associated with R_f values and flash column chromatography are reported as percent by volume values.

^1H and ^{13}C NMR, recorded at 300 MHz and 75 MHz, respectively, were performed on a Varian Mercury 300 spectrometer. ^1H and ^{13}C NMR, recorded at 400 MHz and 100 MHz, respectively, were performed on a Varian Mercury 400 spectrometer. Proton chemical shifts were internally referenced to the residual proton resonance in $CDCl_3$ (δ 7.26 ppm), CD_3OD (δ 3.31 ppm), CD_3CN (δ 1.94 ppm), or DMSO-d_6 (δ 2.50 ppm). Carbon chemical shifts were internally referenced to the deuterated solvent signals in $CDCl_3$ (δ 77.2 ppm), CD_3OD (δ 49.0 ppm), CD_3CN (δ 118.3 ppm and 1.3 ppm) or DMSO-d_6 (δ 39.5 ppm). FT-IR spectra were recorded on a Nicolet Avatar 360 ESP spectrometer with samples loaded as neat films on NaCl plates. References following compound names indicate literature articles where ^1H and ^{13}C NMR data have previously been reported.

4.1.1.1. Lythgoe-Inhoffen diol (6). A flame-dried 100 mL three-necked flask was charged sequentially with 28 mg (0.33 mmol, 0.06 equiv) of $NaHCO_3$, 20 mL of anhydrous MeOH, 60 mL of anhydrous CH_2Cl_2, and 2.0 g (5.12 mmol, 1 equiv) of ergocalciferol **5**. The solution was cooled to −78 °C and treated with O_3 until a blue color appears. The solution was subsequently flushed with Ar for 10–15 min until the blue color faded. Solid sodium borohydride (1.68 g, 44.55 mmol, 8.7 equiv) was added portionwise over a period of 10 min at −78 °C until complete disappearance of starting material was observed by TLC. The reaction mixture was warmed to 0 °C and stirred for 3 h. After being stirred for an additional 30 min at room temperature, the mixture was quenched with 1 N HCl (10 mL), extracted with EtOAc (3 × 50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by silica gel chromatography (30% EtOAc in hexanes) afforded 670 mg (3.17 mmol) of Lythgoe-Inhoffen diol **6** in 62% yield as a white solid. R_f = 0.5 (50% EtOAc in hexanes); Mp 108–110 °C (lit.[14] mp 109–110 °C); ^1H NMR (400 MHz, $CDCl_3$) δ 4.08 (1H, br s), 3.63 (1H, dd, J = 10.4, 2.8 Hz), 3.37 (1H, J = 10.0, 6.8 Hz), 1.98 (1H, d, J = 12.8 Hz), 1.90–1.75 (3H, m), 1.60–1.40 (5H, m), 1.38–1.29 (4H, m), 1.22–1.13 (2H, m), 1.02 (3H, d, J = 6.8 Hz), 0.95 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 69.1, 67.7, 52.9, 52.3, 41.8, 40.2, 38.2, 33.5, 26.6, 22.5, 17.4, 16.6, 13.5; IR (KBr) ν 3621, 3464, 3017, 2943 cm^{-1}.

4.1.1.2. (*S*)-2-((1*R*,3a*R*,4*S*,7a*R*)-4-(*tert*-Butyldimethylsilyloxy)-7a-methyloctahydro-1*H*-inden-1-yl)propan-1-ol (7). To a solution of Lythgoe-Inhoffen diol **6** (2.017 g, 9.5 mmol, 1 equiv) in 60 mL of dry DMF under argon atmosphere was added *tert*-butyldimethylsilylchloride (5.71 g, 38 mmol, 4 equiv) followed by NEt_3 (5.54 mL, 42.7 mmol, 4.5 equiv) and sodium iodide (5.69 g, 38 mmol, 4 equiv). The reaction mixture was refluxed for 30 min and then cooled and quenched with H_2O (10 mL) and concentrated in vacuo. The residue was dissolved in EtOAc (100 mL) and washed with H_2O (2 × 30 mL), the aqueous portion was extracted with EtOAc (30 mL) and the combined organic layers were washed with brine (30 mL), dried ($MgSO_4$), filtered, concentrated by rotary evaporation, and immediately purified by silica gel chromatography (10% EtOAc in hexanes) to afford 4.013 g (9.12 mmol) of bis-silylated diol intermediate in 96% yield. In a flame-dried round bottom flask under argon, 4.013 g (9.12 mmol) of the bis-silylated diol was dissolved in 80 mL of anhydrous THF and 4 mL of NEt_3. To this stirring solution was added TBAF (10.9 mL of a 1 M solution in THF, 10.9 mmol, 1.15 equiv). The resulting reaction mixture was stirred at room temperature for 3 h, concentrated in vacuo, and purified by silica gel column chromatography (20% EtOAc in hexanes) to af-

ford 2.91 g (8.94 mmol) of the desired alcohol **7** in 94% yield. R_f = 0.5 (20% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.08 (1H, br s), 3.57 (1H, dd, J = 9.8, 3.0 Hz), 3.26 (1H, dd, J = 9.0, 7.8 Hz), 1.99 (1H, d, J = 13.6 Hz), 1.87–1.75 (3H, m), 1.58–1.40 (5H, m), 1.35–1.28 (3H, m), 1.40–1.09 (2H, m), 0.97 (3H, d, J = 6.8), 0.94 (3H, s), 0.89 (9H, s), 0.025 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 69.3, 67.7, 53.2, 52.3, 41.8, 40.2, 38.5, 33.6, 26.6, 26.0, 22.6, 18.4, 17.4, 16.8, 13.6, −5.3, −5.4; IR (KBr) ν 3300, 1470, 1250, 1160 cm^{-1}.

4.1.1.3. (*S*)-2-((1*R*,3a*R*,4*S*,7a*R*)-4-(*tert*-Butyldimethylsilyloxy)-7a-methyloctahydro-1*H*-inden-1-yl)-1-iodopropane (8). I_2 (9.34 g, 36.8 mmol, 4.6 equiv) was added portionwise to an ice cooled solution of PPh_3 (4.82 g, 18.4 mmol, 2.3 equiv) and imidazole (3.26 g, 48.0 mmol, 6 equiv) in 300 mL of CH_2Cl_2. The cooled mixture, which become heterogeneous after 5 min, was stirred for 35 min and treated with a solution of the primary alcohol **7** (2.61 g, 8 mmol, 1 equiv) in 100 mL of CH_2Cl_2 during 30 min and then the mixture was stirred at room temperature for 4 h. The mixture was quenched with a 2.5% solution of Na_2SO_3 (30 mL). The organic layer was washed with H_2O (30 mL), brine (30 mL), dried ($MgSO_4$), concentrated in vacuo, and then purified by silica gel column chromatography (5% EtOAc in hexanes) to afford 3.14 g (6.96 mmol) of iodide **8** in 87% yield as a white solid. R_f = 0.6 (5% EtOAc in hexanes); Mp 40–41 °C (lit.[32] mp 41–42 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99 (1H, s), 3.32 (1H, d, J = 9.6 Hz), 3.17 (1H, dd, J = 9.6, 5.2 Hz), 1.90 (1H, d, J = 12.8 Hz), 1.84–1.76 (2H, m), 1.66 (1H, d, J = 13.6 Hz), 1.59–1.54 (1H, m), 1.40–1.08 (8H, m), 0.98 (3H, d, J = 5.2 Hz), 0.94 (3H, s), 0.88 (9H, s), −0.01 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 69.3, 56.0, 52.7, 42.1, 40.3, 36.4, 34.3, 26.6, 25.8, 22.9, 21.7, 20.7, 18.0, 17.6, 14.6, −4.8, −5.2; IR (KBr) ν 2932, 2857, 1462, 1375, 1253, 1160, 1084, 1032, 836, 774 cm^{-1}.

4.1.1.4. (*S*)-5-((1*R*,3a*R*,4*S*,7a*R*)-4-(*tert*-Butyldimethylsilyloxy)-7a-methyloctahydro-1*H*-inden-1-yl)-1-ethoyhex-1-ene (9). In a flame-dried round bottom flask under argon atmosphere, **8** (678 mg, 1.5 mmol, 1 equiv) was dissolved in dry Et_2O (3 mL), the solution was cooled to −78 °C, then *t*-BuLi (1.42 mL of a 2.22 M solution in hexanes, 3.15 mmol, 2.1 equiv) was slowly added (ca. 1 h). The solution was stirred at −78 °C for 1 h, then warmed to 0 °C for 5 min and then recooled to −78 °C. A solution of acrolein acetal (251 μL, 1.65 mmol, 1.1 equiv) in dry Et_2O (2 mL) was slowly added via cannula to the carbanion solution and the mixture was warmed to room temperature. Stirring was continued for 30 min then the solution was quenched with satd NH_4Cl (10 mL). Extraction with Et_2O (3 × 20 mL) afforded an organic phase that was washed with H_2O (20 mL), brine (20 mL) and dried ($MgSO_4$), concentrated in vacuo, and then purified by silica gel column chromatography (hexanes then 1% Et_2O in hexanes) to afford 444 mg (1.12 mmol) of the enol ether as a mixture 86/14 of *E* and *Z* in 75% yield. R_f = 0.2 (hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ (**9** *E*): 6.21 (1H, d, J = 12.4 Hz), 4.74 (1H, dt, J = 12.4, 6.2 Hz), 3.98 (1H, s), 3.69 (2H, q, J = 6.8 Hz), 1.95 (2H, br d, J = 12.4 Hz), 1.81–1.73 (3H, m), 1.65 (1H, br d, J = 13.2 Hz), 1.60–1.51 (1H, m), 1.42–1.29 (10H, m), 1.27–1.00 (3H, m), 0.95–0.80 (15H, m), 0.00 (3H, s), −0.01 (3H, m); (**9** *Z*): 5.90 (1H, d, J = 9.2 Hz), 4.30 (1H, dd, J = 9.2, 7.5 Hz), 3.98 (1H, br s), 3.77 (2H, q, J = 7.5 Hz), 1.95 (2H, br d, J = 12.4 Hz), 1.81–1.73 (3H, m), 1.65 (1H, br d, J = 13.2 Hz), 1.60–1.51 (1H, m), 1.42–1.29 (10H, m), 1.27–1.00 (3H, m), 0.95–0.80 (15H, m), 0.00 (3H, s), −0.01 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.6, 104.8, 69.5, 64.5, 56.7, 53.0, 42.1, 40.7, 37.0, 34.8, 34.4, 27.3, 25.8, 24.5, 23.0, 18.5, 18.0, 17.7, 14.8, 13.7, −4.8, −5.2; IR (KBr) ν 2931, 2856, 1652, 1469, 1374, 1251, 1165, 1083, 1023, 977, 924, 836, 774 cm^{-1}; HRMS (ESI): *m/z* calcd for [(M+Na)$^+$] = 417.3165, found = 417.3155.

4.1.1.5. (*R*)-Ethyl-5-((1*R*,3a*R*,7a*R*)-7a-methyl-4-oxo-octahydro-1*H*-inden-1-yl)hexanoate (10). PCC (546 mg, 2.52 mmol, 2 equiv) was added to a stirred solution of **9** (500 mg, 1.26 mmol) and Celite (600 mg) in CH_2Cl_2 (20 mL). The solution was stirred for 3 h, and then the mixture was filtered on a silica pad. The precipitate was washed with CH_2Cl_2 (3 × 20 mL) then the combined organic fractions were concentrated in vacuo to provide the crude ester as a colorless oil. The oil was diluted in CH_2Cl_2 (10 mL) and CH_3CN (10 mL) and a 48% solution of HF (1 mL) was added. The solution was stirred for 24 h at room temperature. The reaction mixture was cautionly quenched by the addition of a satd solution of $NaHCO_3$ until no effervescence was observed. The solution was extracted with CH_2Cl_2 (3 × 20 mL), the combined organic layers were then washed with H_20 (20 mL) and brine (20 mL), then dried ($MgSO_4$). The solution was concentrated in vacuo, then diluted in CH_2Cl_2 (20 mL), Celite was added (500 mg) followed by PCC (546 mg, 2.52 mmol, 2 equiv). After stirring for 3 h at room temperature, the reaction mixture was filtered on a silica pad, the precipitate was washed with CH_2Cl_2 (3 × 20 mL) and the solution was concentrated in vacuo. The residual oil was purified by silica gel column chromatography (15% EtOAc in hexanes) to afford 363 mg (1.23 mmol) of **10** in 78% yield. R_f = 0.2 (15% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.08 (1H, q, J = 7.2 Hz), 2.46 (1H, dd, J = 11.6, 7.6 Hz), 2.30–2.12 (4H, m), 2.07 (1 h, d, J = 13.6 Hz), 2.02–1.76 (4H, m), 1.75–1.60 (2H, m), 1.60–1.31 (3H, m), 1.21 (3H, t, J = 6.8 Hz), 0.93 (3H, d, J = 6.4 Hz), 0.84 (3H, t, J = 6.8 Hz), 0.59 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.2, 173.9, 62.1, 60.4, 56.5, 50.1, 41.1, 39.1, 35.4, 31.8, 27.6, 25.5, 24.2, 22.8, 21.6, 19.2, 14.4, 12.6; IR (KBr) ν 2957, 2874, 1710, 1704, 1463, 1377, 1239, 1179, 1099, 1037 cm^{-1}; HRMS (ESI): *m/z* calcd for [(M+Na)$^+$] = 319.2093, found = 319.2086.

4.1.1.6. (*R*)-Ethyl-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-bis(*tert*-butyldimethylsilyloxy)cyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexanoate (12). In a flame-dried round bottom flask under argon atmosphere at −78 °C, NaHMDS (514 μL of a 1 M solution in THF, 0.514 mmol, 1.05 equiv) was added to a solution of phosphine oxide **11** (307 mg, 0.539 mmol, 1.1 equiv) in dry THF (6 mL). The reaction vessel was suspended above the ice bath for 5 min, then recooled to −78 °C. To this solution was added via cannula over a period of 5 min a solution of **10** (144 mg, 0.490 mmol, 1 equiv) in dry THF (2 mL). The reaction was left to stir at −78 °C for 2 h then warmed to room temperature for 1 h, and quenched with satd NH_4Cl (10 mL). The layers were separated and the aqueous layer extracted with EtOAc (2 × 25 mL). The organic layers were combined and extracted with satd NH_4Cl (2 × 10 mL), H_2O (10 mL) and brine (10 mL), then dried ($MgSO_4$), and concentrated in vacuo. Purified by silica gel column chromatography (20% EtOAc in hexanes) afforded 247 mg (0.382 mmol) of **12** in 71% yield. R_f = 0.6 (20% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.16 (1H, d, J = 11.2 Hz), 5.80 (1H, d, J = 11.2 Hz), 4.20–4.00 (4H, m), 2.80 (1H, d, J = 11.6 Hz), 2.45–2.18 (4H, m), 2.09 (1H, dd, J = 12.8, 8.0 Hz), 1.98 (2H, d, J = 10.0 Hz), 1.80–1.25 (15H, m), 1.25 (3H, t, J = 6.8 Hz), 0.93 (3H, d, J = 6.4 Hz), 0.86 (9H, s), 0.85 (9H, s), 0.52 (3H, s), 0.05 (12H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 140.7, 133.6, 121.7, 116.1, 68.1, 67.9, 68.1, 60.1, 56.2, 46.0, 45.6, 43.7, 40.6, 36.7, 35.9, 35.3, 34.7, 28.7, 27.7, 25.9, 25.8, 23.4, 22.2, 21.6, 18.7, 18.13, 18.09, 14.3, 12.0, −4.7, −4.76, −4.83, −4.9; HRMS (ESI): *m/z* calcd for [(M+Na)$^+$] = 669.4710, found = 669.4744.

4.1.1.7. (5*S*)-5-((1*R*,3*R*,7*E*,17β)-1,3-Bis[*tert*-butyl(dimethyl)silyloxy]-9,10-secoestra-5,7-dien-17-yl)hexanoic acid (13). LiOH. H_2O was added to a stirred solution of ester **12** (128 mg, 0.197 mmol, 1 equiv) inn THF (2.5 mL), MeOH (0.75 mL) and H_2O (0.75 mmol). The solution was refluxed for 3 h, then cooled to room temperature and diluted with EtOAc (20 mL), then quenched

with a 1 M solution of HCl (10 mL). The aqueous layer was extracted with EtOAc (2 × 10 mL) and then the combined organic fractions were washed with H_2O (10 mL), brine (10 mL) then dried (Na_2SO_4). The solution was concentrated in vacuo to provide the acid **13** in 97% yield without further purification (118 mg, 0.191 mmol). R_f = 0.6 (40% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.16 (1H, d, J = 10.8 Hz), 5.81 (1H, d, J = 10.8 Hz), 4.15–4.01 (2H, m), 2.81 (1H, br d, J = 11.6 Hz), 2.42–2.21 (5H, m), 2.12–1.05 (14H, m), 0.94 (3H, d, J = 7.2 Hz), 0.87 (9H, s), 0.86 (9H, s), 0.53 (3H, s), 0.04 (12H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.0, 140.7, 133.7, 121.7, 116.1, 68.1, 68.0, 56.2, 46.0, 45.6, 43.7, 40.5, 36.7, 35.9, 35.3, 34.5, 28.7, 27.7, 25.9, 25.8, 23.4, 22.2, 21.3, 18.7, 18.2, 18.1, 12.0, −4.6, −4.7, −4.8, −4.9; IR (KBr) ν 3435 (br), 2951, 1637, 1459, 1247, 1086, 835, 780, 668 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 641.4397, found = 641.4383.

4.1.1.8. (*R*)-4-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)cyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)pentan-1-amine (14). To a solution of carboxylic acid **13** (102 mg, 0.165 mmol, 1 equiv) in 1 mL of toluene was added via syringe Et_3N (25 μL, 0.181 mmol, 1.1 equiv), followed by DPPA (35 μL, 0.165 mmol, 1 equiv). The solution was stirred at room temperature for 30 min then heated to reflux overnight and then concentrated under reduced pressure. The crude isocyanate was treated with 2 N NaOH (1.3 mL) and THF (3.3 mL) then stirred for 20 min at room temperature. The resulting solution was extracted with CH_2Cl_2 (2 × 10 mL), and the organic extract was washed with brine and then dried ($MgSO_4$). The solution was concentrated in vacuo then purified by silica gel column chromatography (10/10/80, Et_3N/MeOH/EtOAc) to obtain the amine **14** in 68% yield (66 mg, 0.111 mmol). R_f = 0.35 (10/10/80, Et_3N/MeOH/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14 (1H, d, J = 11.2 Hz), 5.79 (1H, d, J = 11.2 Hz), 4.11–4.01 (2H, m), 2.79 (1H, d, J = 12.0 Hz), 2.71–2.52 (2H, m), 2.42–2.30 (52H, m), 2.24 (1H, d, J = 12.4 Hz), 2.01–1.72 (5H, m), 1.71–0.98 (13H, m), 0.92 (3H, d, J = 8.0 Hz), 0.85 (9H, s), 0.84 (9H, s), 0.51 (3H, s), 0.04 (12H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.7, 133.6, 121.7, 116.1, 68.1, 67.9, 56.4, 56.2, 46.0, 45.6, 43.7, 42.4, 40.6, 36.7, 36.0, 33.0, 29.7, 28.7, 27.7, 25.85, 25.83, 23.4, 22.2, 18.8, 18.13, 18.09, 12.0, −4.67, −4.77, −4.84, −4.9; IR (KBr) ν 3350 (br), 2950, 2856, 1468, 1377, 1253, 1088, 836, 775 cm^{-1}; HRMS (ESI): m/z calcd for [(M+H)$^+$] = 590.4789, found = 590.4792.

4.1.1.9. (5*S*)-5-((1*R*,3*R*,7*E*,17β)-1,3-Bis[*tert*-butyl(dimethyl)silyloxy]-9,10-secoestra-5,7-dien-17-yl)hexanal (15). DIBALH (172 μL of a 1 M solution in toluene, 0.172 mmol, 1.1 equiv) was added dropwise to a stirred solution of ester **12** (101 mg, 0.157 mmol, 1 equiv) in dry CH_2Cl_2 (1.5 mL) under Ar at −78 °C. The solution was stirred at −78 °C for 1 h and warmed up to room temperature. CH_2Cl_2 (10 mL) was added to the reaction mixture, then H_2O (0.16 mL) followed by 15% NaOH (0.16 mL) then more H_2O (0.48 mL). The solution was stirred for 30 min then $MgSO_4$ was added to the mixture. The solution was filtered, the precipitate washed with CH_2Cl_2 (5 mL) and the combined organic fractions were concentrated in vacuo to provide the aldehyde **15** in quantitative yield without further purification (95 mg, 0.157 mmol). R_f = 0.6 (CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (1H, s), 6.16 (1H, d, J = 12.0 Hz), 5.81 (1H, d, J = 12.0 Hz), 4.40–4.43 (2H, m), 2.80 (1H, dd, J = 11.0, 3.1 Hz), 2.43–2.34 (4H, m), 2.24 (1H, br d, J = 13.6 Hz), 2.89 (1H, dd, J = 12.8, 8.4 Hz), 2.05–1.85 (2H, m), 1.85–1.20 (15H, m), 1.20–1.05 (1H, m), 0.94 (3H, d, J = 6.0 Hz), 0.87 (9H, s), 0.86 (9H, s), 0.53 (3H, s), 0.05 (12H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.9, 140.6, 133.7, 121.7, 116.2, 68.1, 67.9, 56.22, 56.20, 46.0, 45.6, 44.3, 43.7, 40.5, 36.7, 36.0, 35.4, 28.7, 27.7, 25.87, 25.84, 23.4, 22.2, 18.7, 18.15, 18.10, 12.0, −4.6, −4.7, −4.8, −4.9; IR (KBr) ν 2950, 2883, 2856, 1728, 1468, 1377, 1361, 1253, 1087, 1025, 836, 775 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 625.4448, found = 625.4434.

4.1.1.10. (*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)cyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexan-1-amine (16). A solution of aldehyde **15** (23 mg, 0.038 mmol, 1 equiv), *O*-benzylhydroxylamine hydrochloride (6.1 mg, 0.038 mmol, 1 equiv) and sodium acetate (3.1 mg, 0.03 mmol, 1 equiv) in 3 mL of toluene was refluxed for 30 min. The mixture was then concentrated and loaded directly onto silica gel. The oximes were isolated by silica gel column chromatography (CH_2Cl_2) as a mixture of *E* and *Z* compounds. In a flame-dried round bottom flask under argon atmosphere, a solution of the oximes in Et_2O (1 mL) was added to a suspension of $LiAlH_4$ (4.3 mg, 0.114 mmol, 3 equiv) in Et_2O (1 mL) at 0 °C. The solution was stirred at room temperature for 4 h then cooled to 0 °C and quenched by addition of H_2O (5 μL), then 15% NaOH (5 μL) and then H_2O (15 μL). The mixture was diluted with Et_2O (10 mL), washed with water (5 mL), satd $NaHCO_3$ (5 mL), brine (5 mL) and dried (Na_2SO_4). The solution was concentrated in vacuo then purified by silica gel column chromatography (10/10/80, Et_3N/MeOH/EtOAc) to provide 18 mg (0.030 mmol) of the amine **16** in 80% yield. R_f = 0.35 (10/10/80, Et_3N/MeOH/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.16 (1H, d, J = 11.1 Hz), 5.81 (1H, d, J = 11.1 Hz), 4.12–4.01 (2H, m), 2.80 (1H, d, J = 10.5 Hz), 2.75–2.62 (2H, m), 2.44–2.31 (2H, m), 2.25 (1H, d, J = 11.1 Hz), 2.10 (1H, dd, J = 12.6, 7.8 Hz), 2.04–1.92 (2H, m), 1.91–1.73 (2H, m), 1.70–0.98 (17H, m), 0.91 (3H, d, J = 6.3 Hz), 0.87 (9H, s), 0.86 (9H, s), 0.53 (3H, s), 0.05 (12H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.1, 133.9, 122.0, 116.3, 68.3, 68.2, 56.7, 56.5, 46.2, 45.9, 43.9, 40.8, 37.0, 36.3, 36.0, 28.9, 28.0, 27.9, 26.10, 26.08, 23.6, 22.5, 19.0, 18.4, 18.3, 12.3, −4.4, −4.5, −4.6, −4.7; IR (KBr) ν 2930, 2857, 1469, 1377, 1362, 1253, 1085, 836, 775 cm^{-1}; HRMS (ESI): m/z calcd for [(M+H)$^+$] = 604.4945, found = 604.4948.

4.1.1.11. (6*S*)-6-((1*R*,3*R*,7*E*,17β)-1,3-Bis[*tert*-butyl(dimethyl)silyloxy]-9,10-secoestra-5,7-dien-17-yl)heptanal (17). In a flame-dried round bottom flask under argon atmosphere, NaHMDS (1.0 mL of a 1 M solution in THF, 1.0 mmol, 10 equiv) was added to a solution of (methoxymethyl)-triphenylphosphonium chloride (360 mg, 1.0 mmol, 10 equiv) in dry THF (10 mL) at −78 °C. The reaction vessel was suspended above the ice bath for 30 min, then recooled to −78 °C. To this solution was added via cannula over a period of 5 min a solution of **15** (60 mg, 0.100 mmol, 1 equiv) in dry THF (1 mL). The reaction was left to stir at −78 °C for 1 h then warmed to 0 °C for 4 h, and quenched with satd NH_4Cl (25 mL). The layers were separated and the aqueous layer extracted with EtOAc (2 × 25 mL). The organic layers were combined and extracted with satd NH_4Cl (2 × 25 mL), H_2O (25 mL) and brine (25 mL), then dried ($MgSO_4$), and concentrated in vacuo. The oil was then purified by silica gel column chromatography (5% EtOAc in hexanes, R_f = 0.6) to provide 53 mg of a mixture 7/3 of *E*/*Z* enol ethers. The enol ethers mixture was dissolved in a solution of $CHCl_3$ (1 mL), distilled H_2O (0.5 mL) and TFA (0.15 mL), and cooled to 0 °C. The reaction was stirred at 0 °C for approx. 30 min and monitored by TLC until complete consumption of the starting material, the reaction was quenched with satd $NaHCO_3$ (5 mL). CH_2Cl_2 (10 mL) was added to the mixture, the layers were separated and the aqueous layer extracted with CH_2Cl_2 (2 × 10 mL). The organic layers were combined and washed with satd $NaHCO_3$ (2 × 25 mL), distilled H_2O (25 mL) and brine (25 mL), then dried ($MgSO_4$), and concentrated in vacuo to give the crude product. **17** was isolated via FCC (10% EtOAc in hexanes) as a clear oil in 84% yield (51.8 mg, 0.084 mmol). R_f = 0.2 (10% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (1H, t, J = 1.6 Hz), 6.16 (1H, d, J = 10.0 Hz), 5.81 (1H, d, J = 10.0 Hz), 4.12–4.02 (2H, m), 2.81 (1H, d, J = 11.6 Hz), 2.43 (2H,

t, J = 7.2 Hz), 2.37 (2H, dd, J = 12.4, 5.2 Hz), 2.25 (1H, d, J = 11.6 Hz), 2.10 (1H, dd, J = 12.6, 8.2 Hz), 2.04–1.93 (2H, m), 1.93–1.75 (2H, m), 1.70–1.45 (8H, m), 1.45–1.15 (7H, m), 1.15–1.00 (1H, m), 0.91 (3H, d, J = 6.0 Hz), 0.87 (9H, s), 0.86 (9H, s), 0.53 (3H, s), 0.05 (12H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.3, 141.0, 133.9, 121.9, 116.4, 68.3, 68.2, 56.6, 56.5, 46.3, 45.9, 44.3, 43.9, 40.8, 37.0, 36.2, 35.8, 28.9, 27.9, 26.11, 26.08, 25.9, 23.6, 22.8, 22.5, 19.0, 18.4, 18.3, 12.3, −4.4, −4.5, −4.6, −4.7; IR (KBr) ν 2949, 2884, 2856, 1728, 1468, 1252, 1052, 1025, 960, 920, 837, 776 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 639.4605, found = 639.4599.

4.1.1.12. (6*S*)-6-((1*R*,3*R*,7*E*,17β)-1,3-Bis[*tert*-butyl(dimethyl)silyl-oxy]-9,10-secoestra-5,7-dien-17-yl)heptanoic acid (18). A freshly prepared solution of $NaClO_2$ (30 mg, 0.33 mmol, 3 equiv) and NaH_2PO_4 (76 mg, 0.55 mmol, 5 equiv) in H_2O (2 mL) was added to a stirred solution of aldehyde **17** (69 mg, 0.11 mmol, 1 equiv) and 2-methyl-2-butene (0.5 mL) in *t*BuOH (2 mL) and the mixture was stirred vigorously at room temperature for 1 h. H_2O (15 mL) was then added, and the mixture was extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with brine (10 mL) and dried (Na_2SO_4). The solution was concentrated in vacuo then the oil was purified by silica gel column chromatography (19/1/80 EtOAc/AcOH/hexanes) to afford 65 mg (0.10 mmol) of acid **18** in 90% yield. R_f = 0.6 (40% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.16 (1H, d, J = 11.0 Hz), 5.81 (1H, d, J = 11.0 Hz), 4.12–4.01 (2H, m), 2.80 (1H, d, J = 11.6 Hz), 2.44–2.31 (4H, m), 2.25 (1H, d, J = 13.6 Hz), 2.10 (1H, dd, J = 12.8, 8.4 Hz), 2.03–1.94 (2H, m), 1.93–1.83 (1H, m), 1.83–1.75 (1H, m), 1.69–1.46 (9H, m), 1.45–1.34 (3H, m), 1.33–1.18 (4H, m), 1.12–1.00 (1H, m), 0.91 (3H, d, J = 6.0 Hz), 0.87 (9H, s), 0.86 (9H, m), 0.53 (3H, s), 0.05 (12H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.9, 141.0, 133.9, 121.9, 116.4, 68.4, 68.2, 56.7, 56.5, 46.2, 45.9, 43.9, 40.8, 37.0, 36.2, 35.7, 34.3, 28.9, 27.9, 26.11, 26.08, 25.9, 25.4, 23.7, 22.5, 19.0, 18.4, 18.3, 12.3, −4.4, −4.5, −4.6, −4.7; IR (KBr) ν 2950, 2857, 1710, 1468, 1253, 1088, 920, 836, 775 cm^{-1}; HRMS (ESI): m/z calcd for [(M−H)$^-$] = 631.4578, found = 631.4578.

4.1.1.13. (*R*)-6-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)cyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)heptan-1-amine (19). This was prepared from aldehyde **17** by following the same procedure described for **16**. The reagents used were as follows: **17** (30 mg, 0.048 mmol, 1 equiv), *O*-benzylhydroxylamine hydrochloride (7.7 mg, 0.048 mmol, 1 equiv), sodium acetate (4.9 mg, 0.03 mmol, 1 equiv) and $AlLiH_4$ (5.4 mg, 0.144 mmol, 3 equiv). This afforded 22 mg (0.035 mmol) of amine **19** in 74% yield. R_f = 0.35 (10/10/80, Et_3N/MeOH/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.16 (1H, d, J = 10.8 Hz), 5.81 (1H, d, J = 10.8 Hz), 4.15–4.01 (2H, m), 3.34 (1H, t, J = 7.2 Hz), 2.80 (1H, d, J = 12.0 Hz), 2.70 (1H, d, J = 7.2 Hz), 2.42–2.30 (2H, m), 2.25 (1H, d, J = 12.4 Hz), 2.10 (1H, dd, J = 12.4, 8.0 Hz), 2.02–1.71 (5H, m), 1.70–0.98 (17H, m), 0.91 (3H, d, J = 6.0 Hz), 0.87 (9H, s), 0.86 (9H, s), 0.53 (3H, s), 0.05 (12H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.9, 133.6, 121.7, 116.1, 68.1, 67.9, 56.5, 56.3, 46.0, 45.6, 43.7, 40.6, 36.7, 36.1, 28.7, 27.7, 27.4, 25.9, 25.8, 23.4, 22.2, 18.8, 18.15, 18.10, 12.0, −4.7, −4.6, −4.8, −4.9; IR (KBr) ν 2930, 2855, 1468, 1377, 1252, 1086, 836, 775 cm^{-1}; HRMS (ESI): m/z calcd for [(M+H)$^+$] = 618.5102, found = 618.5089.

4.1.1.14. (*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-*1*-inden-1-yl)-*N*-hydroxyhexanamide (20a). In a flame-dried round bottom flask under argon atmosphere, oxalylchloride (12.9 μL, 0.153 mmol, 1.5 equiv) was added to a solution of acid **13** (63 mg, 0.102 mmol, 1 equiv) in dry CH_2Cl_2 (2 mL) and dry DMF (1 μL) at 0 °C then the solution was left to stir at room temperature for 1 h and recooled to 0 °C. A solution of DIPEA (53.3 μL, 0.306, 3 equiv) and *O*-*tert*-butyldimethylsilylhydroxylamine (30 mg, 0.204 mmol, 2 equiv) in CH_2Cl_2 (1 mL) was added to the reaction mixture. The solution was left to stir at 0 °C for 1 h then at room temperature for 1 h. A solution of citric acid (10 mL, 1 M) was then added, and the mixture was extracted with EtOAc (3 × 10 mL) and the combined organic layers were washed with H_2O (10 mL), brine (10 mL) and dried ($MgSO_4$). The solution was concentrated in vacuo, then the crude material was dissolved in CH_2Cl_2 (1 mL) and CH_3CN (1 mL). A 48% solution of HF (70 μL, 2.04 mmol, 20 equiv) was added and the solution was stirred at room temperature for 2 h. The mixture was quenched cautionly by the addition of satd $NaHCO_3$ until no effervescence was observed then acidified with a 1 M aq solution of citric acid (5 mL). The solution was extracted with CH_2Cl_2 (3 × 5 mL) then the combined organic layers were washed with H_2O (5 mL), brine (5 mL), dried ($MgSO_4$) and then concentrated in vacuo. The residual oil was purified by octadecyl-functionalized silica gel column chromatography (100% H_2O to 100% MeOH) to afford the hydroxamic acid **20a** in 68% yield. R_f = 0.30 (88/10/2 CH_2Cl_2/MeOH/CH_3COOH); ^{1}H NMR (400 MHz, CD_3OD) δ 6.22 (1H, d, J = 11.0 Hz), 5.89 (1H, d, J = 11.0 Hz), 4.08–3.92 (2H, m), 2.83 (1H, br d, J = 12.4 Hz), 2.59 (1H, br d, J = 13.2 Hz), 2.52–1.04 (27H, m), 0.97 (3H, d, J = 3.2 Hz), 0.58 (3H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 176.6, 140.9, 132.7, 122.3, 116.0, 66.8, 66.5, 56.6, 56.3, 45.6, 44.2, 41.5, 40.7, 36.5, 36.1, 35.3, 34.2, 28.7, 27.5, 23.4, 22.1, 21.6, 18.1, 11.3; IR (KBr) ν 3390 (br), 2941, 2870, 1709, 1439, 1376, 1212, 1046 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 428.2777, found = 428.2769.

4.1.1.15. (*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)-*N*-hydroxyheptanamide (20b). This was prepared from acid **18** by following the same procedure described for **20a**. The reagents used were as follows: acid **18** (30 mg, 0.047 mmol, 1 equiv), oxalylchloride (5.9 μL, 0.070 mmol, 1.5 equiv), DIPEA (24.5 μL, 0.141, 3 equiv) and *O*-*tert*-butyldimethylsilylhydroxylamine (14 mg, 0.094 mmol, 2 equiv). Compound **20b** was purified by octadecyl-functionalized silica gel column chromatography (100% H_2O to 100% MeOH) to afford 13 mg (0.032 mmol) of the hydroxamic acid **20b** in 67% yield. R_f = 0.30 (88/10/2 CH_2Cl_2/MeOH/CH_3COOH); ^{1}H NMR (400 MHz, CD_3OD) δ 6.21 (1H, d, J = 11.0 Hz), 5.88 (1H, d, J = 11.0 Hz), 4.08–3.93 (2H, m), 2.83 (1H, d, J = 11.6), 2.59 (1H, dd, J = 13.2, 3.2 Hz), 2.41 (1H, dd, J = 13.2, 3.2 Hz), 2.18–2.12 (2H, m), 2.12–2.05 (2H, m), 2.05–1.98 (2H, m), 1.98–1.89 (1H, m), 1.89–1.80 (1H, m), 1.80–1.71 (1H, m), 1.71–1.15 (17H, m), 1.15–1.03 (1H, m), 0.95 (3H, d, J = 6.0 Hz), 0.57 (3H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 171.9, 140.9, 132.7, 122.2, 116.0, 66.8, 66.5, 56.7, 56.3, 45.6, 44.2, 41.5, 40.7, 36.4, 36.2, 35.6, 32.7, 28.6, 27.5, 26.1, 25.5, 23.3, 22.1, 18.1, 11.2; IR (KBr) ν 3370 (br), 2937, 2868, 1650, 1456, 1046 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 442.2933, found = 442.2925.

4.1.1.16. (*R*)-6-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)-*N*-hydroxy-*N*-methylheptanamide (20c). This was prepared from acid **18** by following the same procedure described for **20a**. The reagents used were as follows: acid **18** (6.5 mg, 0.010 mmol, 1 equiv), oxalylchloride (1.2 μL, 0.014 mmol, 1.5 equiv), DIPEA (1.6 μL, 0.027, 3 equiv) and *O*-*tert*-butyldimethylsilyl-*N*-methyl hydroxylamine (2.26 μL, 0.018 mmol, 2 equiv). Compound **20c** was purified by octadecyl-functionalized silica gel column chromatography (100% H_2O to 100% MeOH) to afford 2.2 mg (0.005 mmol) of the hydroxamic acid **20c** in 51% yield. R_f = 0.40 (88/10/2 CH_2Cl_2/MeOH/CH_3COOH); ^{1}H NMR (400 MHz, CD_3OD) δ 6.21 (1H, d, J = 11.2 Hz), 5.88 (1H, d, J = 11.2 Hz), 4.08–3.92 (2H, m), 3.19 (3H, s), 2.83 (1H, d, J = 12.4 Hz), 2.59 (1H, dd, J = 13.2, 4.0 Hz), 2.52–2.35 (3H, m), 2.26-2.12 (2H, m), 2.08–1.18 (19H, m), 1.15–1.02 (1H, m), 0.95 (3H, d, J = 6.0 Hz), 0.57 (3H, s); ^{13}C NMR (75 MHz,

CDCl3) δ 174.8, 140.7, 132.4, 122.0, 115.7, 66.5, 66.3, 56.5, 56.1, 45.4, 43.9, 41.2, 40.5, 36.2, 35.9, 35.4, 34.8, 31.7, 28.4, 27.3, 25.6, 24.9, 23.1, 21.8, 17.9, 11.0; IR (KBr) ν 3343 (br), 2934, 2848, 1620, 1455, 1200, 1045, 668 cm^{-1}; HRMS (ESI): m/z calcd for $[(M+Na)^+]$ = 456.3090, found = 456.3089.

4.1.1.17. (*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)-*N*-methoxyheptanamide (20d). This was prepared from acid **18** by following the same procedure described for **20a**. The reagents used were as follows: acid **18** (23 mg, 0.036 mmol, 1 equiv), oxalylchloride (4.5 μL, 0.054 mmol, 1.5 equiv), DIPEA (225.1 μL, 0.144, 4 equiv) and *O*-methyl hydroxylamine hydrochloride (6 mg, 0.072 mmol, 2 equiv). Compound **20d** was purified by octadecyl-functionalized silica gel column chromatography (100% H_2O to 100% MeOH) to afford 12.5 mg (0.029 mmol) of the hydroxamic acid **20d** in 80% yield. R_f = 0.4 (1/9/90 AcOH/MeOH/CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (1H, br s), 6.31 (1H, d, J = 11.2 Hz), 5.85 (1H, d, J = 11.2 Hz), 4.17–4.09 (1H, m), 4.09–4.01 (1H, m), 3.77 (3H, m), 2.79 (1H, d, J = 12.0), 2.74 (1H, dd, J = 13.6, 3.6 Hz), 2.41 (1H, dd, J = 13.2, 3.2 Hz), 2.48 (1H, d, J = 13.6 Hz), 2.28–2.17 (2H, m), 2.03–1.75 (5H, m), 1.73–1.15 (16H, m), 1.12–1.00 (1H, m), 0.91 (3H, d, J = 6.0 Hz), 0.53 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 143.1, 131.1, 123.8, 115.2, 67.4, 67.2, 60.4, 56.4, 56.3, 45.8, 44.6, 42.1, 40.4, 37.2, 35.9, 35.5, 28.9, 27.6, 25.7, 23.5, 22.3, 21.1, 18.8, 14.2, 12.1; IR (KBr) ν 3342 (br), 2931, 2867, 1651, 1437, 1048, 975, 734 cm^{-1}; HRMS (ESI): *m/z* calcd for $[(M+H)^+]$ = 434.3270, found = 434.3270.

4.1.1.18. (*R*)-6-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyloctahydro-1*H*-inden-1-yl)-*N*-methoxy-*N*-methylheptanamide (20e). This was prepared from acid **18** by following the same procedure described for **20a**. The reagents used were as follows: acid **18** (6.5 mg, 0.010 mmol, 1 equiv), oxalylchloride (1.3 μL, 0.015 mmol, 1.5 equiv), DIPEA (11 μL, 0.060, 6 equiv) and *N,O*-dimethylhydroxylamine hydrochloride (4 mg, 0.040 mmol, 4 equiv). Compound **20e** was purified by silica gel column chromatography (50% EtOAc in hexanes) to afford 3.3 mg (0.0074 mmol) of the hydroxamic acid **20e** in 74% yield. R_f = 0.3 (50% EtOAc in hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 11.2 Hz), 5.84 (1H, d, J = 11.2 Hz), 4.11 (1H, br s), 4.04 (1H, br s), 3.68 (3H, m), 3.17 (3H, m), 2.84–2.79 (2H, m), 2.47 (1H, d, J = 13.2 Hz), 2.41 (2H, t, J = 7.2 Hz), 2.27–2.15 (2H, m), 2.04–1.84 (5H, m), 1.83–1.74 (1H, m), 1.73–1.16 (14H, m), 1.12–1.02 (2H, m), 0.91 (3H, d, J = 6.0 Hz), 0.53 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 143.4, 131.3, 124.1, 115.5, 67.6, 67.5, 61.4, 56.7, 56.5, 46.0, 44.9, 42.4, 40.7, 37.4, 36.2, 35.8, 32.2, 29.2, 27.8, 26.2, 25.3, 23.8, 22.5, 19.0, 12.2; IR (KBr) ν 3402 (br), 2938, 2876, 1645, 1440, 1380, 1049, 994, 732 cm^{-1}; HRMS (ESI): *m/z* calcd for $[(M+Na)^+]$ = 470.3241, found = 470.3241.

4.1.1.19. *N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexyl)-*N*-hydroxyformamide (21). A solution of aldehyde **15** (30 mg, 0.050 mmol, 1 equiv), hydroxylamine (9.2 μL of a 50% wt solution in water, 0.50 mmol, 10 equiv) in 4 mL of toluene was refluxed for 30 min. The mixture was then concentrated and the crude residue was dissolved in AcOH/THF (2:1, 2 mL) then cooled to 0 °C. $NaBH_3CN$ (6 mg, 0.10 mmol, 2 equiv) was added portionwise. The mixture was stirred at 0 °C for 1 h then 0.5 M NaOH was slowly added until alkaline pH. The solution was extracted with CH_2Cl_2 (3 × 10 mL), the combined organic layers were washed with brine (10 mL) then dried (Na_2SO_4). The solution was concentrated and the hydroxylamine was obtained in quantitative yield without further purification. The hydroxylamine was dissolved in THF (1 mL) then trifluoroethylformate (7.5 mg of a 86% wt solution in formic acid, 0.50 mmol, 10 equiv) was added and the mixture was refluxed for 4 h. The solution was concentrated in vacuo, then the crude material was dissolved in CH_2Cl_2 (1 mL) and CH_3CN (1 mL). A 48% solution of HF (34 μL, 1 mmol, 20 equiv) was added and the solution was stirred at room temperature for 2 h. The mixture was quenched cautionly by the addition of a satd $NaHCO_3$ until no effervescence was observed. The solution was extracted with CH_2Cl_2 (3 × 5 mL) then the combined organic layers were washed with H_2O (5 mL), brine (5 mL), dried ($MgSO_4$) and then concentrated in vacuo. The oil was purified by octadecyl-functionalized silica gel column chromatography (100% H_2O to 100% MeOH) to afford 8.2 mg (0.019 mmol) of **21** in 40% yield. ^1H NMR (400 MHz, $CDCl_3$) δ 6.16 (1H, d, J = 11.0 Hz), 5.81 (1H, d, J = 11.0 Hz), 4.13–4.01 (2H, m), 2.94 (2H, t, J = 6.8 Hz), 2.81 (1H, d, J = 11.6 Hz), 2.44–2.33 (2H, m), 2.25 (1H, d, J = 13.2 Hz), 2.10 (1H, dd, J = 12.4, 8.4 Hz), 2.04–1.94 (2H, m), 1.93–1.75 (2H, m), 1.70–1.15 (15H, m), 1.12–0.98 (1H, m), 0.92 (3H, d, J = 6.4 Hz), 0.87 (9H, s), 0.86 (9H, s), 0.53 (3H, s), 0.05 (12H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.0, 133.9, 122.0, 116.4, 68.3, 68.2, 56.7, 56.5, 54.2, 46.2, 45.9, 43.9, 40.8, 37.0, 36.3, 36.0, 28.9, 27.6, 26.10, 26.08, 23.9, 23.6, 22.5, 19.0, 18.4, 18.3, 12.3, −4.4, −4.5, −4.6, −4.7; IR (KBr) ν 2931, 2857, 1468, 1253, 1087, 1052, 1026, 906, 835, 805, 775, 735 cm^{-1}; HRMS (ESI): *m/z* calcd for $[(M+H)^+]$ = 420.3114, found = 420.3106.

4.1.1.20. 1-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexyl)-3-hydroxyurea (22). A solution of amine **16** (13.1 mg, 0.022 mmol, 1 equiv) in CH_2Cl_2 (130 μL) was added dropwise to a stirred solution of 1,1-carbonyldiimidazole (25.6 mg, 0.22 mmol, 10 equiv) in THF (50 μL) under argon cooled to 0 °C. The reaction mixture was stirred 16 h at room temperature, then *O-tert*-butyldimethylsilylhydroxylamine (95 mg, 0.66 mmol, 30 equiv) was added to the mixture, the solution was then allowed to stir a further 16 h. The reaction mixture was diluted with EtOAc (5 mL), washed with 0.1 M HCl (2 × 5 mL), water (5 mL), brine (5 mL) and then dried (Na_2SO_4). The solution was then concentrated in vacuo to provide an oil which was purified by silica gel column chromatography (50% EtOAc in hexanes, R_f = 0.2) to give the *O-tert*-butyldimethylsilylhydroxyurea. The product was then directly submitted to deprotection: the oil was dissolved in CH_2Cl_2 (100 μL) and CH_3CN (100 μL) then a 48% solution of HF was added (two drops) and the solution was stirred overnight. The mixture was quenched cautionly by the addition of satd $NaHCO_3$ until no effervescence was observed then acidified with a 1 M aq solution of citric acid (5 mL). The solution was extracted with CH_2Cl_2 (3 × 5 mL) then the combined organic layers were washed with H_2O (5 mL), brine (5 mL), dried ($MgSO_4$) and then concentrated in vacuo. The oil was purified by octadecyl-functionalized silica gel column chromatography (100% H_2O to 100% MeOH) to afford the hydroxyurea **22** in 44% yield (4.2 mg, 0.009 mmol). R_f = 0.2 (88/10/2 CH_2Cl_2/MeOH/CH_3COOH); ^1H NMR (400 MHz, CD_3OD) δ 6.21 (1H, d, J = 11.4 Hz), 5.88 (1H, d, J = 11.4 Hz), 4.08–4.01 (1H, m), 4.00–3.93 (1H, m), 3.18 (2H, t, J = 6.8 Hz), 2.83 (1H, d, J = 9.6 Hz), 2.59 (1H, d, J = 6.0 Hz), 2.41 (1H, d, J = 10.4 Hz), 2.25–2.12 (2H, m), 2.06–1.98 (2H, m), 1.97–1.18 (17H, m), 1.15–1.04 (1H, m), 0.95 (3H, d, J = 6.0 Hz), 0.57 (3H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 170.9, 140.9, 132.7, 122.2, 115.9, 66.8, 66.5, 56.7, 56.3, 45.6, 44.2, 41.4, 40.7, 39.9, 36.4, 36.2, 35.6, 30.5, 28.6, 27.5, 23.3, 22.1, 18.1, 11.2; (KBr) ν 3312 (br), 2937, 2841, 1651, 1457, 1049, 735 cm^{-1}; HRMS (ESI): *m/z* calcd for $[(M+Na)^+]$ = 457.3042, found = 457.3042.

4.1.1.21. (*R*)-*N*-(2-Aminophenyl)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexanamide (23a). HBTU (4.6 mg, 0.012 mmol, 1.1 equiv) was added to a solution of acid **13** (6.8 mg, 0.011 mmol, 1 equiv), 1,2-phenylenediamine (1.2 mg, 0.011 mmol, 1 equiv), HOBt (7.4 mg, 0.055 mmol, 5 equiv) and DIPEA (5.7 μL, 0.033 mmol, 3 equiv) in DMF (110 μL). The mixture was stirred at room temperature for 1 h, then diluted with EtOAc (10 mL). The organic solution was washed with satd $NaHCO_3$ (5 mL), water (5 mL), brine (5 mL) and dried (Na_2SO_4). The solution was filtered, concentrated, and the oil was purified by silica gel column chromatography (50/45/5 EtOAc/hexanes/Et_3N, R_f = 0.5) to afford 7 mg (0.010 mmol) of protected *o*-aminoanilide. The product was dissolved under argon in THF (0.5 mL), then TBAF (40 μL of a 1 M solution in THF, 0.040 mmol, 4 equiv) and Et_3N (5.6 μL, 0.040 mmol, 4 equiv) were added and the mixture was stirred for 48 h. The solution was concentrated in vacuo and loaded directly onto silica gel. Compound **23a** was purified by octadecyl-functionalized silica gel column chromatography (100% H_2O to 100% MeOH) to afford 2.9 mg (0.006 mmol) of **23a** in 55% yield. R_f = 0.33 (90/10 EtOAc/Et_3N); ^{1}H NMR (500 MHz, CD_3OD) δ 7.07 (1H, d, J = 8.0 Hz), 7.02 (1H, t, J = 7.6 Hz), 6.84 (1H, d, J = 8.0 Hz), 6.71 (1H, t, J = 7.5 Hz), 6.22 (1H, d, J = 11.0 Hz), 5.89 (1H, d, J = 11.0 Hz), 4.09–4.01 (1H, m), 4.00–3.96 (1H, m), 2.84 (1H, d, J = 12.0 Hz), 2.60 (1H, d, J = 13.5 Hz), 2.46–2.31 (3H, m), 2.29–2.11 (3H, m), 2.09–1.91 (4H, m), 1.88–1.73 (4H, m), 1.72–1.12 (9H, m), 1.00 (3H, d, J = 7.0 Hz), 0.59 (3H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 174.0, 142.1, 140.9, 132.7, 127.1, 126.0, 124.0, 122.2, 118.3, 117.4, 116.0, 66.8, 66.5, 56.6, 56.3, 45.6, 44.2, 41.5, 40.7, 36.4, 36.1, 35.4, 28.6, 27.5, 23.3, 22.5, 22.1, 18.1, 11.2; IR (KBr) ν 3351 (br), 2943, 2872, 1653, 1503, 1454, 1376, 1306, 1047, 908, 732 cm^{-1}; HRMS (ESI): *m/z* calcd for [(M+H)$^+$] = 481.3426, found = 481.3430.

4.1.1.22. (*R*)-*N*-(2-Aminophenyl)-6-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)heptanamide (23b). This was prepared from acid **18** by following the same procedure described for **23a**. The reagents used were as follows: acid **18** (19 mg, 0.030 mmol, 1 equiv), 1,2-phenylenediamine (3.2 mg, 0.030 mmol, 1 equiv), HBTU (12.5 mg, 0.033 mmol, 1.1 equiv), HOBt (20.2 mg, 0.150 mmol, 5 equiv) and DIPEA (15.7 μL, 0.090 mmol, 3 equiv). oxalylchloride (1.2 μL, 0.014 mmol, 1.5 equiv), DIPEA (1.6 μL, 0.027, 3 equiv) and *O*-*tert*-butyldimethylsilyl-*N*-methyl hydroxylamine (2.26 μL, 0.018 mmol, 2 equiv); TBAF (108 μL of a 1 M solution in THF, 0.108 mmol, 4 equiv relative to the protected intermediate) and Et_3N (15 μL, 0.108 mmol, 4 equiv relative to the protected intermediate). Compound **23b** was purified by silica gel column chromatography (50/45/5 EtOAc/hexanes/Et_3N) to afford 19.3 mg (0.027 mmol) of **23b** in 84% yield. R_f = 0.33 (90/10 EtOAc/Et_3N); ^{1}H NMR (400 MHz, CD_3OD) δ 7.06 (1H, d, J = 8.0 Hz), 7.02 (1H, t, J = 7.6 Hz), 6.84 (1H, d, J = 8.0 Hz), 6.71 (1H, t, J = 7.5 Hz), 6.22 (1H, d, J = 11.0 Hz), 5.89 (1H, d, J = 11.0 Hz), 4.10–4.01 (1H, m), 4.00–3.93 (1H, m), 2.83 (1H, dd, J = 12.8, 4.0 Hz), 2.59 (1H, dd, J = 12.8, 3.6 Hz), 2.51–2.35 (3H, m), 2.30–2.10 (3H, m), 2.09–1.23 (17H, m), 1.22–1.08 (1H, m), 1.02 (1H, t, J = 7.2 Hz), 0.97 (3H, d, J = 6.4 Hz), 0.58 (3H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 174.0, 140.9, 132.7, 127.1, 125.9, 124.0, 122.3, 118.3, 117.4, 116.0, 66.8, 66.5, 56.7, 56.3, 45.6, 44.2, 41.4, 40.7, 36.5, 36.2, 36.1, 35.6, 28.6, 27.6, 26.4, 25.7, 23.6, 23.3, 22.1, 18.2, 11.2; IR (KBr) ν 3350 (br), 2943, 2872, 1652, 1503, 1456, 1376, 1306, 1046, 909, 732 cm^{-1}; HRMS (ESI): *m/z* calcd for [(M+H)$^+$] = 495.3587, found = 495.3577.

4.1.1.23. *N*-((*R*)-4-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)pentyl)-2-mercaptoacetamide (24a). EDC·HCl (9.2 mg, 0.048 mmol, 1.2 equiv) was added to a stirred solution of the amine **14** (23 mg, 0.040 mmol, 1 equiv) and 2-(acetylthio)acetic acid (5.9 mg, 0.044 mmol, 1.1 equiv) in CH_2Cl_2 (1 mL) at 0 °C. The solution was stirred at room temperature for 4 h then diluted with CH_2Cl_2 (15 mL), washed with 0.5 M HCl (2 × 5 mL), with satd $NaHCO_3$ (2 × 5 mL), with brine (5 mL) and dried (Na_2SO_4). The solution was concentrated in vacuo to give the protected product as an oil (27 mg, 0.046 mmol) and the product was carried forward without further purification. In a flame-dried round bottom flask under argon atmosphere, a deoxygenated solution of MeONa (2 mg 0.036 mmol, 1 equiv) in MeOH was added by cannula to the acetylated mercaptoacetamide (25 mg, 0.036 mmol, 1 equiv). The solution was stirred at room temperature for 4 h then quenched by the addition of AcOH (1 mL). The solution was concentrated in vacuo then diluted in EtOAc (15 mL). The solution was washed with water (5 mL), brine (5 mL), dried (Na_2SO_4) and concentrated to give the deacetylated compound witch was dissolved in CH_2Cl_2 (1 mL) and CH_3CN (1 mL). HF (50 μL) was added to the solution and the mixture was stirred at room temperature overnight. The reaction mixture was quenched with satd $NaHCO_3$ (2 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic fractions were washed with brine (5 mL) and dried (Na_2SO_4). The solution was concentrated in vacuo then purified by silica gel column chromatography (5% MeOH in CH_2Cl_2) to give **24a** in 69% yield. R_f = 0.5 (10% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (1H, br s), 6.31 (1H, d, J = 11.6 Hz), 5.85 (1H, d, J = 10.8 Hz), 4.19–4.08 (1H, m), 4.08–4.01 (1H, m), 3.35–3.17 (3H, m), 3.80 (1H, d, J = 16.0 Hz), 2.74 (1H, d, J = 17.2 Hz), 2.48 (1H, d, J = 12.8 Hz), 2.26–2.15 (2H, m), 2.04–1.74 (6H, m), 1.73–1.00 (13H, m), 0.93 (3H, d, J = 6.4 Hz), 0.54 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4, 143.3, 131.3, 124.1, 115.4, 67.6, 67.5, 56.60, 56.58, 46.3, 44.9, 42.4, 40.7, 40.3, 37.2, 36.0, 35.7, 29.9, 29.1, 28.6, 23.7, 2.5, 22.5, 19.0, 12.3; IR (KBr) ν 3311 (br), 2925, 2869, 1651, 1558, 1540, 1457, 1261, 1045, 802, 733 cm^{-1}; HRMS (ESI): *m/z* calcd for [(M+Na)$^+$] = 458.2705, found = 458.2698.

4.1.1.24. *N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyloctahydro-1*H*-inden-1-yl)hexyl)-2-mercaptoacetamide (24b). This was prepared from amine **16** by following the same procedure described for **24a**. The reagents used were as follows: amine **16** (29 mg, 0.048 mmol, 1 equiv), 2-(acetylthio)acetic acid (7.1 mg, 0.053 mmol, 1.1 equiv equiv), EDC·HCl (11 mg, 0.058 mmol, 1.2 equiv). Deprotection: acetylated mercaptoacetamide (30 mg, 0.041 mmol, 1 equiv), MeONa (2.2 mg 0.041 mmol, 1 equiv). Compound **32** was purified by silica gel column chromatography (5% MeOH in CH_2Cl_2) to give 13 mg (0.031 mmol) of **32** in 67% yield. R_f = 0.5 (10% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (1H, br s), 6.31 (1H, d, J = 11.4 Hz), 5.85 (1H, d, J = 11.4 Hz), 4.12 (1H, br s), 4.05 (1H, br s), 3.28 (2H, q, J = 6.4 Hz), 3.24 (2H, d, J = 8.8 Hz), 2.80 (1H, d, J = 12.4 Hz), 2.73 (1H, d, J = 13.2 Hz), 2.48 (1H, d, J = 11.2 Hz), 2.27–2.15 (2H, m), 2.05–1.74 (6H, m), 1.73–1.16 (14H, m), 1.13–1.00 (1H, m), 0.92 (3H, d, J = 6.4 Hz), 0.54 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.2, 143.3, 131.4, 124.1, 115.5, 67.6, 67.4, 56.7, 56.5, 46.0, 44.9, 42.4, 40.7, 40.2, 37.4, 36.2, 35.7, 30.1, 29.2, 28.6, 27.9, 23.71, 23.66, 22.5, 19.0, 12.3; IR (KBr) ν 3306 (br), 2935, 2869, 1650, 1550, 1439, 1048, 909, 732 cm^{-1}; HRMS (ESI): *m/z* calcd for [(M+H)$^+$] = 450.3042, found = 450.3043.

4.1.1.25. 2-Amino-*N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-dihydroxycyclohexylidene)ethylidene)-7a-methyloctahydro-1*H*-inden-1-yl)hexyl)acetamide (25a). DCC (2.2 mg, 0.0107 mmol, 1.05 equiv) then DMAP (catalytic amount) were added to a stirred solution of amine **16** (6.2 mg, 0.0102 mmol, 1 equiv) and *N*-Bocglycine (1.9 mg, 0.0107 mmol, 1.05 equiv) in CH_2Cl_2 (150 μL)

at 0 °C. The solution was then stirred at room temperature for 2 h then diluted with CH_2Cl_2 (10 mL), washed with satd $NaHCO_3$ (2 × 5 mL), with brine (5 mL) and dried (Na_2SO_4). The solution was concentrated in vacuo then purified by silica gel column chromatography (50% EtOAc in hexanes) to obtain the protected intermediate as a clear oil (7.3 mg, 0.0096 mmol). The product was then dissolved in CH_3CN (100 μL) and CH_2Cl_2 (100 μL), then HF (one drop of a 48% solution) was added to the solution and the mixture was stirred at room temperature overnight. The reaction mixture was quenched with satd $NaHCO_3$ (2 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic fractions were washed with brine (5 mL) and dried (Na_2SO_4). The solution was concentrated in vacuo then purified by silica gel column chromatography (10/20/17 Et_3N/MeOH/EtOAc) to obtain **25a** in 66% yield (3.3 mg, 0.0070 mmol). R_f = 0.15 (10/20/17 Et_3N/MeOH/EtOAc); ^1H NMR (400 MHz, CD_3OD) δ 6.21 (1H, d, J = 11.2 Hz), 5.80 (1H, d, J = 11.2 Hz), 4.09–4.02 (1H, m), 4.01–3.95 (1H, m), 3.25–3.15 (2H, m), 2.83 (1H, d, J = 11.2 Hz), 2.59 (1H, d, J = 10.0 Hz), 2.41 (1H, d, J = 10.8 Hz), 2.26–2.11 (2H, m), 2.09–1.80 (5H, m), 1.79–1.71 (1H, m), 1.70–1.02 (14H, m), 0.95 (3H, d, J = 6.0 Hz), 0.92–0.84 (2H, m), 0.57 (3H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 169.3, 140.8, 132.7, 122.2, 116.0, 66.8, 66.5, 56.7, 56.3, 45.6, 44.2, 43.0, 41.5, 40.7, 39.2, 36.4, 36.2, 35.6, 29.7, 29.6, 28.6, 27.6, 23.3, 22.1, 18.1, 11.2; IR (KBr) ν 3301 (br), 2927, 2872, 1659, 1642, 1443, 1050, 978, 668 cm^{-1}; HRMS (ESI): m/z calcd for $[(M+H)^+]$ = 433.3425, found = 433.3423.

4.1.1.26. *N*-((*R*)–5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexyl)-2-(dimethylamino)acetamide (25b). EDC·HCl (1.6 mg, 0.011 mmol, 1.1 equiv) was added to a stirred solution of amine **16** (6.3 mg, 0.010 mmol, 1 equiv) and dimethylglycine (1.2 mg, 0.011 mmol, 1.1 equiv) in CH_2Cl_2 (0.2 mL) at 0 °C. The solution was stirred at room temperature for 4 h then diluted with CH_2Cl_2 (10 mL), washed with satd $NaHCO_3$ (2 × 5 mL), with brine (5 mL) and dried (Na_2SO_4). The solution was concentrated in vacuo to give the crude acetamide as a yellow oil. TBAF (4 μL of a 1 M solution in THF, 0.040 mmol, 4 equiv) and Et_3N (4 μL, 0.033 mmol, 3 equiv) were added to a stirred solution of the crude product in THF (0.5 mL) under argon and the mixture was stirred overnight. The solution was concentrated in vacuo and loaded directly onto silica gel column chromatography (80/10/10 EtOAc/MeOH/Et_3N) to obtain **25b** as a clear oil in 58% yield (2.9 mg, 0.006 mmol). R_f = 0.5 (80/10/10 EtOAc/MeOH/Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 11.2 Hz), 5.85 (1H, d, J = 11.2 Hz), 3.32–3.20 (2H, m), 2.93 (1H, s), 2.79 (1H, dd, J = 13.2, 4.0 Hz), 2.74 (1H, dd, J = 14.0, 4.0 Hz), 2.48 (1H, d, J = 14.0 Hz), 2.28 (6H, s), 2.28–2.16 (2H, m), 2.06–1.02 (18H, m), 0.91 (3H, d, J = 6.0 Hz), 0.88–0.80 (2H, m), 0.53 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 143.3, 131.3, 124.1, 115.5, 67.7, 67.5, 63.4, 56.7, 56.5, 46.3, 46.0, 44.9, 42.4, 40.7, 39.2, 37.4, 36.2, 35.7, 30.4, 29.1, 27.9, 23.7, 22.5, 19.0, 12.3; IR (KBr) ν 3332 (br), 2931, 2869, 1657, 1528, 1454, 1047, 732 cm^{-1}; HRMS (ESI): m/z calcd for $[(M+H)^+]$ = 461.3738, found = 431.3735.

4.1.1.27. 2-Bromo-*N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-dihydroxycyclohexylidene)ethylidene)-7a-methyloctahydro-1*H*-inden-1-yl)hexyl)acetamide (25c). DCC (3.5 mg, 0.017 mmol, 1.05 equiv) then DMAP (catalytic amount) were added to a stirred solution of amine **16** (9.7 mg, 0.016 mmol, 1 equiv) and bromoacetic acid (2.33 mg, 0.017 mmol, 1.05 equiv) in CH_2Cl_2 (150 μL) at 0 °C. The solution was then stirred at room temperature for 2 h then diluted with CH_2Cl_2 (10 mL), washed with satd $NaHCO_3$ (2 × 5 mL), with brine (5 mL) and dried (Na_2SO_4). The solution was concentrated in vacuo then purified by silica gel column chromatography (50% EtOAc in hexanes) to afford the TBS-*O*-protected **25c** (10.1 mg, 0.014 mmol); R_f = 0.8 (50% EtOAc in hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 6.47 (1H, br s), 6.15 (1H, d, J = 10.8 Hz), 5.80 (1H, d, J = 10.8 Hz), 4.15–4.00 (2H, m), 3.88 (2H, s), 3.32–3.27 (2H, m), 3.18 (2H, br s), 2.79 (1H, d, J = 12.0 Hz), 2.42–2.30 (2H, m), 2.25 (1H, d, J = 13.6 Hz), 2.09 (1H, dd, J = 12.8, 7.6 Hz), 2.03–1.01 (17H, m), 1.45 (9H, s), 0.90 (3H, d, J = 6.0 Hz), 0.86 (9H, s), 0.85 (9H, s), 0.52 (3H, s), 0.04 (12H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 141.0, 133.9, 121.9, 116.4, 68.3, 68.2, 56.7, 56.5, 46.2, 45.9, 43.9, 40.8, 40.5, 37.0, 36.3, 35.7, 35.1, 30.0, 29.7, 28.9, 28.5, 28.0, 26.09, 26.08, 25.8, 24.9, 23.6, 22.4, 19.0, 18.38, 18.34, 12.3, −4.4, −4.5, −4.6, −4.7. IR (KBr) ν 2930, 2854, 2119, 1655, 1642, 1448, 1252, 1086, 835, 775 cm^{-1}; HRMS (ESI): m/z calcd for $[(M+Na)^+]$ = 746.3970, found = 746.3970.

4.8 mg (0.0066 mmol) of TBS-*O*-protected **25c** were dissolved in CH_3CN (100 μL) and CH_2Cl_2 (100 μL), then HF (one drop of a 48% solution) was added to the mixture was stirred at room temperature overnight. The reaction mixture was quenched with satd $NaHCO_3$ (2 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic fractions were washed with brine (5 mL) and dried (Na_2SO_4). The solution was concentrated in vacuo then purified by silica gel column chromatography (40% acetone in hexanes) to afford 2.5 mg (0.0050 mmol) of **25c** in 65% yield. R_f = 0.15 (40% acetone in hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 6.48 (1H, br s), 6.31 (1H, d, J = 11.4 Hz), 5.85 (1H, d, J = 11.4 Hz), 4.12 (1H, br s), 4.04 (1H, br s), 3.89 (2H, s), 3.29 (2H, q, J = 6.7 Hz), 2.79 (1H, dd, J = 12.2, 3.8 Hz), 2.74 (1H, dd, J = 13.6, 3.6 Hz), 2.26–2.14 (5H, m), 2.04–1.02 (19H, m), 0.92 (3H, d, J = 6.8 Hz), 0.54 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 143.3, 131.3, 124.1, 115.5, 67.6, 67.5, 56.6, 56.5, 46.0, 44.9, 42.4, 40.5, 37.4, 36.2, 35.6, 30.0, 29.7, 29.5, 29.1, 27.9, 23.7, 22.5, 19.0, 12.3; IR (KBr) ν 3305 (br), 2926, 2868, 1659, 1552, 1442, 1216, 1046, 977, 734 cm^{-1}; HRMS (ESI): m/z calcd for $[(M+Na)^+]$ = 518.2240, found = 518.2242.

4.1.1.28. 2-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexylamino)-2-oxoethyl ethyl carbonotrithioate (25d). NaHMDS (42 μL of a 1 M solution in THF, 0.042 mmol, 3 equiv) was added to a stirred solution of ethanethiol (3.1 μL, 0.042 mmol, 1 equiv) in dry THF (200 μL) cooled to 0 °C under argon. The solution was stirred at 0 °C for 10 min then CS_2 (3.3 μL, 0.056 mmol, 4 equiv) was added dropwise and the reaction mixture was stirred at room temperature for 1 h before a solution of TBS-*O*-protected **25c** (7 mg, 0.014 mmol, 1 equiv) in dry THF (150 μL) was added. After stirring for an additional 1 h at room temperature, the reaction mixture was quenched by addition of satd NH_4Cl (3 mL) and extracted with EtOAc (3 × 5 mL). The combined organic fraction were dried (Na_2SO_4) and concentrated in vacuo, the oil was then purified by silica gel column chromatography (50% acetone in hexanes) to afford the trithiocarbonate **25d** in 82% yield (6.4 mg, 0.011 mmol). R_f = 0.2 (50% acetone in hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 11.4 Hz), 6.29 (1H, br s), 5.85 (1H, d, J = 11.4 Hz), 4.15–4.09 (1H, m), 4.07–4.01 (1H, m), 4.06 (2H, s), 3.39 (2H, q, J = 7.6 Hz), 3.23 (2H, d, J = 7.6 Hz), 2.79 (1H, dd, J = 12.4, 3.6 Hz), 2.74 (1H, d, J = 13.2, 3.6 Hz), 2.47 (1H, dd, J = 13.2, 2.8 Hz), 2.25–2.16 (2H, m), 2.04–1.10 (19H, m), 1.37 (3H, t, J = 7.6 Hz), 1.09–0.99 (1H, m), 0.90 (3H, d, J = 6.4 Hz), 0.53 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 223.5, 166.8, 143.0, 131.3, 124.1, 115.5, 67.6, 67.5, 56.6, 56.5, 46.0, 44.9, 42.4, 40.7, 40.2, 39.4, 37.4, 36.2, 35.7, 32.3, 30.0, 29.1, 27.9, 23.7, 23.5, 23.5, 19.0, 13.5, 12.3; (KBr) ν 3294 (br), 2929, 2869, 1653, 1551, 1446, 1375, 1079, 1046, 811 cm^{-1}; HRMS (ESI): m/z calcd for $[(M+H)^+]$ = 554.2796, found = 554.2782.

4.1.1.29. *N*-((*R*)-4-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)pentyl)methanesulfonamide (27a). Methylsulfonyl chloride (2.3 μL, 0.031 mmol, 1.1 equiv) was added to a stirred solution of the amine **14** (16 mg, 0.028 mmol, 1 equiv) and Et_3N (11 μL, 0.084 mmol, 3 equiv) in CH_2Cl_2 (1.5 mL) at 0 °C. The solution was stirred at 0 °C for 1 h then at room temperature for 1 h. The reaction mixture was then diluted with CH_2Cl_2 (5 mL), washed with satd $NaHCO_3$ (2 × 5 mL), water (5 mL), brine (5 mL) then dried (Na_2SO_4). The solution was filtered, concentrated and the residual oil was loaded on silica gel (35% EtOAc in hexanes) to give the sulfonamide as an oil. The oil was then dissolved into CH_2Cl_2 (100 μL) and CH_3CN (100 μL) and then a 48% solution of HF was added (one drop). The solution was stirred at room temperature overnight then quenched cautionly by the addition of a satd $NaHCO_3$ until no effervescence was observed. The solution was extracted with CH_2Cl_2 (3 × 5 mL) then the combined organic layers were washed with H_2O (5 mL), brine (5 mL), dried ($MgSO_4$) and then concentrated in vacuo. The oil was then purified by silica gel column chromatography (5% MeOH in EtOAc) to give 3.7 mg (0.0008 mmol) of the sulfonamide **27a** in 30% yield. R_f = 0.7 (5% MeOH in EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 10.8 Hz), 5.85 (1H, d, J = 10.8 Hz), 4.19–4.00 (2H, m), 3.11 (2H, t, J = 6.8 Hz), 2.96 (3H, s), 2.80 (1H, d, J = 12.4 Hz), 2.74 (1H, d, J = 13.2 Hz), 2.48 (1H, d, J = 12.8 Hz), 2.30–2.15 (3H, m), 2.08–1.02 (17H, m), 0.93 (3H, d, J = 6.4 Hz), 0.54 (3H, s) ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.1, 131.5, 124.0, 115.6, 67.6, 67.5, 56.53, 56.48, 46.0, 44.9, 44.1, 42.4, 40.7, 40.6, 37.4, 36.0, 33.0, 29.1, 27.9, 27.1, 23.7, 22.5, 19.0, 12.3; IR (KBr) ν 3303 (br), 2929, 2871, 1445, 1316, 1261, 1149, 1045, 974, 801, 735 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 462.2624, found = 462.2650.

4.1.1.30. *N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)–4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyloctahydro-1*H*-inden-1-yl)hexyl)methanesulfonamide (27b). This was prepared from amine **16** by following the same procedure described for **27a**. The reagents used were as follows: amine **16** (5.5 mg, 0.009 mmol, 1 equiv), methylsulfonyl chloride (1 μL, 0.01 mmol, 1.1 equiv) and Et_3N (3 μL, 0.003 mmol, 3 equiv). Compound **27b** was purified by silica gel column chromatography (5% MeOH in EtOAc) to give 2 mg (0.004 mmol) the sulfonamide **27b** in 48% yield. R_f = 0.7 (5% MeOH in EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 11.2 Hz), 5.85 (1H, d, J = 11.2 Hz), 4.20–4.10 (1H, m), 4.09–4.00 (1H, m), 3.34 (2H, q, J = 6.4 Hz), 2.96 (3H, s), 2.80 (1H, d, J = 12.0 Hz), 2.74 (1H, d, J = 13.6 Hz), 2.60 (1H, d, J = 13.6 Hz), 2.25–2.15 (2H, m), 2.08–0.85 (20H, m), 0.92 (3H, d, J = 6.4 Hz), 0.54 (3H, s) ^{13}C NMR (75 MHz, $CDCl_3$) δ 131.3, 124.1, 115.5, 100.0, 67.6, 67.5, 56.6, 56.5, 46.0, 44.9, 43.6, 42.4, 40.6, 37.4, 36.2, 35.6, 30.9, 29.9, 29.1, 27.9, 23.7, 23.4, 22.5, 19.0, 12.3; IR (KBr) ν 3310 (br), 2939, 2857, 1750, 1347, 1169, 1030, 698 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 476.2816, found = 476.2804.

4.1.1.31. *N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexyl)trifluoromethanesulfonamide (27c). This was prepared from amine **16** by following the same procedure described for **27a**. The reagents used were as follows: amine **16** (20.9 mg, 0.035 mmol, 1 equiv), trifluoromethanesulfonyl chloride (4 μL, 0.038 mmol, 1.1 equiv) and Et_3N (14 μL, 0.105 mmol, 3 equiv). Compound **27c** was purified by silica gel column chromatography (5% MeOH in EtOAc) to give 6.2 mg (0.012 mmol) the sulfonamide **27c** in 35% yield. R_f = 0.4 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 11.4 Hz), 5.85 (1H, d, J = 11.4 Hz), 4.17–4.08 (1H, m), 4.08–4.00 (1H, m), 3.30 (2H, q, J = 7.2 Hz), 2.79 (1H, dd, J = 12.0, 4.0 Hz), 2.74 (1H, dd, J = 13.2, 4.0 Hz), 2.48 (1H, dd, J = 13.6, 3.2 Hz), 2.26–2.16 (2H, m), 2.04–1.75 (5H, m), 1.74–1.18 (14H, m), 1.13–1.02 (1H, m), 0.92 (3H, t, J = 6.4 Hz), 0.54 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.2, 131.4, 124.1, 115.5, 67.7, 67.5, 56.6, 56.5, 46.0, 44.9, 44.8, 42.4, 40.7, 37.4, 36.2, 35.5, 31.0, 29.1, 27.9, 23.7, 23.1, 22.5, 18.9, 12.3; (KBr) ν 3325 (br), 2940, 2871, 1435, 1371, 1221, 1188, 1148, 1045, 606 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 530.2528, found = 530.2518.

4.1.1.32. *N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexyl)ethanesulfonamide (27d). This was prepared from amine **16** by following the same procedure described for **27a**. The reagents used were as follows: amine **16** (14 mg, 0.024 mmol, 1 equiv), ethanesulfonyl chloride (4.5 μL, 0.048 mmol, 2 equiv) and Et_3N (10 μL, 0.072 mmol, 3 equiv). Compound **27d** was purified by silica gel column chromatography (EtOAc) to give 6.0 mg (0.013 mmol) the sulfonamide **27d** in 35% yield. R_f = 0.2 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 11.4 Hz), 5.85 (1H, d, J = 11.4 Hz), 4.16–4.09 (1H, m), 4.08–4.01 (1H, m), 3.15–3.08 (2H, m), 3.04 (2H, q, J = 7.2 Hz), 2.79 (1H, dd, J = 11.6, 4.0 Hz), 2.74 (1H, d, J = 13.2, 4.0 Hz), 2.48 (1H, dd, J = 13.6, 3.2 Hz), 2.27–2.16 (2H, m), 2.03–1.75 (5H, m), 1.73–1.16 (14H, m), 1.37 (3H, t, J = 7.2 Hz), 1.12–1.08 (1H, m), 0.92 (3H, d, J = 6.4 Hz), 0.53 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.0, 131.1, 123.8, 115.3, 67.4, 67.2, 56.4, 56.3, 46.8, 45.8, 44.6, 43.4, 42.1, 40.4, 37.2, 36.0, 35.4, 30.9, 28.9, 27.7, 23.5, 23.1, 22.2, 18.8, 12.1, 8.4; (KBr) ν 3291 (br), 2939, 2876, 1451, 1371, 1141, 1046, 910, 732 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 490.2967, found = 490.2966.

4.1.1.33. *N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexyl)-2-hydroxyethanesulfonamide (27e). This was prepared from amine **16** by following the same procedure described for **27a**. The reagents used were as follows: amine **16** (14 mg, 0.023 mmol, 1 equiv), 2-hydroxyethanesulfonyl chloride (3.4 mg, 0.046 mmol, 2 equiv) and Et_3N (10 μL, 0.069 mmol, 3 equiv). Compound **27e** was purified by silica gel column chromatography (5% MeOH in EtOAc) to give 4 mg (0.008 mmol) the sulfonamide **27e** in 35% yield. R_f = 0.2 (5% MeOH in EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 11.8 Hz), 5.85 (1H, d, J = 11.8 Hz), 4.19–4.09 (1H, m), 4.09–4.00 (1H, m), 4.07 (2H, t, J = 6.8 Hz), 3.17 (2H, t, J = 6.8 Hz), 3.03 (2H, t, J = 6.8 Hz), 2.79 (1H, d, J = 13.6 Hz), 2.75 (1H, d, J = 12.8 Hz), 2.48 (1H, d, J = 12.0 Hz), 2.17–2.25 (2H, m), 2.03–1.15 (19H, m), 1.14–1.01 (1H, m), 0.92 (3H, d, J = 6.4 Hz), 0.54 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.3, 131.3, 124.1, 115.5, 67.7, 67.5, 57.4, 56.7, 56.5, 53.6, 47.3, 46.0, 44.9, 42.4, 40.7, 37.4, 36.2, 36.0, 29.1, 28.3, 27.9, 23.9, 23.7, 22.5, 19.0, 12.3; (KBr) ν 3354 (br), 2934, 2867, 1724, 1316, 1201, 1170, 1143, 1046, 732 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na$^+$] = 506.2916, found = 506.2919.

4.1.1.34. *N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexyl)butane-1-sulfonamide (27f). This was prepared from amine **16** by following the same procedure described for **27a**. The reagents used were as follows: amine **16** (22.1 mg, 0.036 mmol, 1 equiv), butylsulfonyl chloride (5.2 μL, 0.040 mmol, 1.1 equiv) and Et_3N (15 μL, 0.108 mmol, 3 equiv). Compound **27f** was purified by silica gel column chromatography (EtOAc) to give 10.1 mg (0.020 mmol) the sulfonamide **27f** in 57% yield. R_f = 0.3 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.30 (1H, d, J = 11.4 Hz), 5.84 (1H, d, J = 11.4 Hz), 4.15–4.08 (1H, m), 4.07–3.98 (1H, m), 3.10 (2H, q, J = 6.8 Hz), 3.05–2.97 (2H, m), 2.79 (1H, dd, J = 12.0, 3.6 Hz), 2.74 (1H, dd, J = 13.6, 3.6 Hz), 2.47

(1H, dd, J = 13.6, 3.2 Hz), 2.27–2.16 (2H, m), 2.04–1.16 (23H, m), 1.13–1.01 (1H, m), 0.95 (3H, t, J = 7.6 Hz), 0.91 (3H, d, J = 6.8 Hz), 0.53 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.2, 131.4, 124.0, 115.5, 67.6, 67.4, 56.6, 56.5, 52.6, 46.0, 44.9, 43.6, 42.4, 40.7, 37.4, 36.2, 35.6, 31.1, 29.1, 27.9, 25.9, 23.7, 23.4, 22.5, 21.8, 19.0, 13.8, 12.3; (KBr) ν 3290 (br), 2937, 2871, 1449, 1320, 1141, 1047, 734 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 518.3280, found = 518.3267.

4.1.1.35. *N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexyl)benzenesulfonamide (27g). This was prepared from amine **16** by following the same procedure described for **27a**. The reagents used were as follows: amine **16** (16.2 mg, 0.027 mmol, 1 equiv), benzenesulfonyl chloride (6.8 μL, 0.052 mmol, 2 equiv) and Et_3N (11 μL, 0.078 mmol, 3 equiv). Compound **27g** was purified by silica gel column chromatography (EtOAc) to give 5.1 mg (0.010 mmol) the sulfonamide **27g** in 37% yield. R_f = 0.4 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (2H, d, J = 7.2 Hz), 7.58 (1H, t, J = 7.2 Hz), 7.52 (2H, d, J = 7.2 Hz), 6.31 (1H, d, J = 11.6 Hz), 5.84 (1H, d, J = 11.6 Hz), 4.35 (1H, t, J = 6.0 Hz), 4.15–4.08 (1H, m), 4.08–3.99 (1H, m), 2.95 (2H, q, J = 6.4 Hz), 2.79 (1H, dd, J = 12.4, 4.0 Hz), 2.74 (1H, dd, J = 13.2, 3.6 Hz), 2.48 (1H, dd, J = 13.6, 2.8 Hz), 2.28–2.16 (2H, m), 2.04–1.91 (3H, m), 1.90–1.76 (2H, m), 1.65–1.59 (2H, m), 1.58–1.06 (11H, m), 1.05–0.92 (1H, m), 0.86 (3H, d, J = 6.0 Hz), 0.51 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.2, 140.2, 132.8, 131.4, 127.3, 124.1, 115.5, 67.6, 67.5, 56.5, 46.0, 44.9, 43.5, 42.4, 40.6, 37.4, 36.1, 35.5, 30.3, 29.1, 27.9, 23.2, 22.5, 18.9, 12.3; (KBr) ν 3290 (br), 2940, 2870, 1446, 1323, 1159, 1046, 905, 732, 689, 586 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 538.2967, found = 538.2969.

4.1.1.36. 4-Cyano-*N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexyl)benzenesulfonamide (27h). This was prepared from amine **16** by following the same procedure described for **27a**. The reagents used were as follows: amine **16** (14 mg, 0.024 mmol, 1 equiv), 4-cyanobenzenesulfonyl chloride (9.6 mg, 0.048 mmol, 2 equiv) and Et_3N (10 μL, 0.072 mmol, 3 equiv). Compound **27h** was purified by silica gel column chromatography (EtOAc) to give 8.0 mg (0.014 mmol) the sulfonamide **27h** in 61% yield. R_f = 0.5 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (2H, d, J = 8.0 Hz), 7.83 (2H, d, J = 8.0 Hz), 6.30 (1H, d, J = 11.6 Hz), 5.85 (1H, d, J = 11.6 Hz), 4.56 (1H, t, J = 6.0 Hz), 4.17–4.10 (1H, m), 4.10–4.01 (1H, m), 2.99 (2H, q, J = 6.7 Hz), 2.79 (1H, dd, J = 12.4, 4.0 Hz), 2.73 (1H, dd, J = 13.2, 4.0 Hz), 2.48 (1H, dd, J = 13.2, 3.2 Hz), 2.17–2.29 (2H, m), 2.03–1.90 (3H, m), 1.76–1.88 (2H, m), 1.72–1.61 (2H, m), 1.61–1.08 (11H, m), 1.06–0.92 (1H, m), 0.87 (3H, d, J = 6.0 Hz), 0.51 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.7, 143.1, 133.2, 131.5, 127.9, 124.0, 117.5, 116.6, 115.6, 67.6, 67.5, 56.53, 56.48, 45.9, 44.9, 43.6, 42.4, 40.6, 37.4, 36.1, 35.4, 30.3, 29.1, 27.9, 23.7, 23.2, 22.5, 18.9, 12.3; (KBr) ν 3293 (br), 2939, 2869, 1434, 1332, 1160, 1091, 1045, 837, 735, 634, 574, 517 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 563.2919, found = 563.2918.

4.1.1.37. *N*-((*R*)-6-((1R,3aS,7aR,E)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)heptyl)methanesulfonamide (27i). This was prepared from amine **19** by following the same procedure described for **27a**. The reagents used were as follows: amine **19** (10 mg, 0.016 mmol, 1 equiv), methylsulfonyl chloride (1.4 μL, 0.018 mmol, 1.1 equiv) and Et_3N (7 μL, 0.054 mmol, 3 equiv). **27i** was purified by silica gel column chromatography (5% MeOH in EtOAc) to give 5.5 mg (0.012 mmol) the sulfonamide **27i** in 73% yield. R_f = 0.7 (5% MeOH in EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 11.2 Hz), 5.85 (1H, d, J = 11.2 Hz), 4.18–4.08 (1H, m), 4.07–4.00 (1H, m), 3.13 (2H, q, J = 6.8 Hz), 2.96 (3H, s), 2.80 (1H, d, J = 11.6 Hz), 2.74 (1H, d, J = 12.8 Hz), 2.62 (2H, br s), 2.48 (1H, d, J = 12.8 Hz), 2.26–2.16 (3H, m), 2.10–1.00 (19H, m), 0.91 (3H, d, J = 6.0 Hz), 0.54 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.4, 131.3, 124.1, 115.5, 67.6, 67.5, 56.7, 56.5, 46.0, 44.9, 43.6, 42.4, 40.6, 37.4, 36.2, 35.9, 30.5, 29.2, 27.9, 27.3, 25.9, 23.7, 22.5, 19.0, 12.3; IR (KBr) ν 3294 (br), 2933, 2869, 1453, 1318, 1149, 1048, 975, 732, 523 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 490.2967, found = 490.2967.

4.1.1.38. *N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*1*R*,3a*S*,7a*R*,*E*)–4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H* -inden-1-yl)hexyl)methanesulfamide (28a). *N*-(*tert*-Butoxycarbonyl)-*N*-[4-(dimethylazaniumylidene)-1,4-dihydropyridin-1-ylsulfonyl]azanide[33] **26** (8.6 mg, 0.028 mmol, 1.1 equiv) was added to a stirred solution of amine **14** (15 mg, 0.026 mmol, 1 equiv) in CH_2Cl_2 (100 μL). The mixture was stirred at room temperature for 16 h, then concentrated and directly loaded on silica gel (20% EtOAc in hexanes) to give the protected *tert*-butylsulfamoylcarbamate. A 48% solution of HF (one drop) was added to a stirred solution of the product in CH_2Cl_2 (100 μL) and CH_3CN (100 μL) and the solution was stirred at room temperature overnight. The mixture was quenched cautionly by the addition of satd $NaHCO_3$ until no effervescence was observed then acidified with a 1 M aq solution of citric acid (5 mL). The solution was extracted with CH_2Cl_2 (3 × 5 mL) then the combined organic layers were washed with H_2O (5 mL), brine (5 mL), dried ($MgSO_4$) and then concentrated in vacuo. The oil was purified by silica gel column chromatography (EtOAc) to afford 3.2 mg (0.007 mmol) of the sulfamide **28a** in 27% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 11.4 Hz), 5.86 (1H, d, J = 11.4 Hz), 4.46 (2H, s), 4.24–4.16 (1H, m), 4.10–4.02 (1H, m), 3.20–3.02 (2H, m), 2.80 (1H, dd, J = 12.0, 4.0 Hz), 2.74 (1H, dd, J = 13.6, 4.0 Hz), 2.49 (1H, dd, J = 13.2, 3.2 Hz), 2.28–2.17 (2H, m), 2.04–1.70 (5H, m), 1.74–1.24 (12H, m), 1.18–1.05 (1H, m), 0.94 (3H, d, J = 6.4 Hz), 0.55 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.1, 131.5, 124.1, 115.6, 67.7, 67.5, 56.51, 56.48, 46.0, 44.9, 44.4, 42.4, 40.7, 37.4, 36.0, 33.0, 29.1, 27.9, 26.4, 23.7, 22.5, 19.0, 12.3; IR (KBr) ν 3290 (br), 2940, 2872, 1706, 1434, 1329, 1156, 1045, 735, 668 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 463.2596, found = 463.2606.

4.1.1.39. *N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)–4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)hexyl)methanesulfamide (28b). This was prepared from amine **16** by following the same procedure described for **28a**. The reagents used were as follows: **16** (7 mg, 0.011 mmol, 1 equiv) and **26** (3.8 mg, 0.012 mmol, 1.1 equiv). Compound **28b** was obtained in 79% yield (3.6 mg, 0.008 mmol). R_f = 0.1 (EtOAc); ^{1}H NMR (400 MHz, CD_3OD) δ 6.22 (1H, d, J = 11.2 Hz), 5.88 (1H, d, J = 11.2 Hz), 4.10–3.94 (2H, m), 3.01 (2H, t, J = 6.6 Hz), 2.84 (1H, d; J = 11.6 Hz), 2.59 (1H, d, J = 10.8 Hz), 2.41 (1H, d, J = 12.0 Hz), 2.28–2.12 (3H, m), 2.08–1.99 (2H, m), 1.98–1.90 (1H, m), 1.89–1.80 (1H, m), 1.80–1.72 (1H, m), 1.71–1.20 (12H, m), 1.15–1.05 (1H, m), 0.96 (3H, d, J = 6.4 Hz), 0.94–0.85 (1H, m), 0.57 (53H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 140.9, 132.7, 122.3, 116.0, 66.8, 66.5, 56.7, 56.3, 53.6, 45.6, 44.2, 43.1, 41.5, 40.7, 36.4, 36.2, 35.6, 29.9, 28.6, 27.6, 23.3, 22.1, 18.1, 11.2; IR (KBr) ν 3302 (br), 2935, 2860, 1592, 1328, 1156, 1045, 669, 547 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 477.2757, found = 477.2760. 2606.

4.1.1.40. *N*-((*R*)-5-((1*R*,3a*S*,7a*R*,*E*)–4-(2-((3*R*,5*R*)-3,5-Dihydroxycycloheptylidene)ethylidene)-7a-methyloctahydro-1*H*-inden-1-yl)hexyl)methanesulfamide (28c). This was prepared from amine **19** by following the same procedure described for **28a**.

The reagents used were as follows: **19** (10.3 mg, 0.016 mmol, 1 equiv) and **26** (5.5 mg, 0.018 mmol, 1.1 equiv). Compound **28c** was obtained in 69% yield (5.2 mg, 0.011 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 6.22 (1H, d, J = 11.2 Hz), 5.88 (1H, d, J = 11.2 Hz), 4.07–4.01 (1H, m), 4.00–3.95 (1H, m), 3.00 (2H, t, J = 7.2 Hz), 2.83 (2H, d, J = 15.6 Hz), 2.59 (1H, dd, J = 13.2, 4.0 Hz), 2.44–2.35 (2H, m), 2.30–2.11 (3H, m), 2.05–1.00 (19H, m), 0.95 (3H, d, J = 6.4 Hz), 0.57 (3H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 140.2, 134.4, 121.9, 116.5, 67.6, 66.4, 56.7, 56.3, 45.2, 43.4, 40.7, 37.4, 36.2, 36.0, 28.5, 27.7, 27.4, 25.8, 23.5, 22.3, 18.6, 11.7; IR (KBr) ν 3298 (br), 2945, 2872, 1699, 1440, 1333, 1145, 1044, 732, 667 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 491.2919, found = 491.2914.

4.1.1.41. (*R*,*Z*)-Methyl-7-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-bis-(*tert*-butyldimethylsilyloxy)cyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)-2-(*tert*-butyldimethylsilyloxy)oct-2-enoate (29). In a flame-dried round bottom flask under argon atmosphere at −78 °C, NaHMDS (94 μL of a 1 M solution in THF, 0.095 mmol, 1.3 equiv) was added to a solution of methyl-2-(dimethoxyphosphoryl)-2-methoxyacetate (34 mg, 0.110 mmol, 1.5 equiv) in dry THF (1 mL). The solution was stirred for 15 min at −78 °C, then a solution of aldehyde **15** (44 mg, 0.073 mmol, 1 equiv) in dry THF (0.5 mL) was slowly added by cannula. The solution was then slowly warmed to room temperature and stirred overnight. The solution was quenched by the addition of aq satd NH_4Cl (2 mL) and extracted with Et_2O (3 × 5 mL), the combined organic layers were washed with water (5 mL) and brine (5 mL) then dried (5 mL). The solution was concentrated in vacuo then purified by silica gel column chromatography (5% EtOAc in hexanes) to obtain **29** in 71% yield (41 mg, 0.052 mmol). R_f = 0.7 (10% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.17 (1H, d, J = 10.0 Hz), 5.81 (1H, d, J = 10.0 Hz), 5.51 (1H, t, J = 8.0 Hz), 4.13–4.02 (2H, m), 3.75 (3H, s), 2.81 (1H, d, J = 12.0), 2.49–2.33 (4H, m), 2.25 (1H, d, J = 14.0 Hz), 2.10 (1H, dd, J = 12.2, 8.2 Hz), 2.04–1.94 (2H, m), 1.93–1.83 (1H, m), 1.83–1.73 (1H, m), 1.72–1.22 (14H, m), 1.14–1.01 (1H, m), 1.00–0.91 (12H, m), 0.87 (9H, m), 0.86 (9H, m), 0.53 (3H, s), 0.12 (6H, s), 0.05 (12H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5, 141.0, 140.1, 133.9, 126.6, 122.0, 116.3, 68.3, 68.2, 56.7, 56.5, 51.6, 46.2, 45.8, 43.9, 40.8, 37.0, 36.2, 35.7, 29.9, 28.9, 27.9, 27.4, 26.7, 26.10, 26.07, 25.8, 23.6, 22.4, 19.0, 18.44, 18.38, 18.34, 12.3, −4.4, −4.5, −4.6, −4.7; IR (KBr) ν 2951, 2857, 1727, 1636, 1468, 1360, 1252, 1087, 837, 777 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 811.5524, found = 811.5509.

4.1.1.42. (*R*)-Methyl-7-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-dihydroxycyclohexylidene)ethylidene)-7a-methyloctahydro-1*H*-inden-1-yl)-2-oxooctanoate (30a). A 48% solution of HF (one drop) was added to a solution of **29** (7 mg, 0.009 mmol) in CH_2Cl_2 (0.3 mL) and CH_3CN (0.3 mL) and the solution was stirred at room temperature for 2 h. The mixture was concentrated in vacuo and the residue was loaded directly onto silica gel. Compound **30a** was isolated via FCC (50% EtOAc in hexanes) as a white solid in 85% yield (3.9 mg, 0.007 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (1H, d, J = 11.4 Hz), 5.85 (1H, d, J = 11.4 Hz), 4.18–4.08 (1H, m), 4.08–4.01 (1H, m), 3.87 (3H, s), 2.84 (2H, t, J = 7.2 Hz), 2.79 (1H, dd, J = 12.0, 3.6 Hz), 2.74 (1H, dd, J = 13.2, 3.6 Hz), 2.48 (1H, d, J = 13.6 Hz), 2.28–2.16 (2H, m), 2.06–1.74 (5H, m), 1.72–1.16 (14H, m), 1.14–1.02 (1H, m), 0.91 (3H, m, J = 6.0 Hz), 0.54 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.6, 161.8, 143.3, 131.3, 124.1, 115.5, 67.6, 67.5, 56.7, 53.1, 46.0, 44.9, 42.4, 40.7, 39.6, 37.4, 36.1, 35.7, 29.1, 27.9, 25.7, 23.7, 23.6, 22.5, 19.0, 12.3; IR (KBr) ν 3380 (br), 2941, 2869, 1730, 1437, 1262, 1048, 733, 668 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 469.2930, found = 469.2920.

4.1.1.43. (*R*)-7-((1*R*,3a*S*,7a*R*,*E*)-4-(2-((3*R*,5*R*)-3,5-Dihydroxycyclohexylidene)ethylidene)-7a-methyl-octahydro-1*H*-inden-1-yl)-*N*-methyl-2-oxooctanamide (30b). To a solution of enol ether **29** (29 mg, 0.037 mmol, 1 equiv) and AcOH (11 μL, 0.185 mmol, 1 equiv) in 0.5 mL of MeCN and 0.5 mL of CH_2Cl_2 at 0 °C was added CsF (14 mg, 0.092 mmol, 2.5 equiv) in one portion. The mixture was allowed to warm to room temperature and then stirred for an additional 75 min. The reaction mixture then was diluted with 10 mL of EtOAc and 5 mL of satd $NaHCO_3$. The layers were separated and the organic layer was washed with satd $NaHCO_3$ (5 mL) and brine (5 mL), then dried (Na_2SO_4) and concentrated. Purification of the residue on silica gel (5% EtOAc in hexanes, R_f = 0.4) gave the protected-α-ketoester. $MeNH_3Cl$ (3.4 mg, 0.051 mmol, 1.5 equiv) was added to a stirred solution of the product in NEt_3 (1 mL) at 0 °C. The solution was warmed to room temperature and stirred for 2 days. The solution was concentrated and the residue dissolved in EtOAc (10 mL). The organic layer was washed with satd $NaHCO_3$ (2 × 5 mL), H_2O (5 mL), brine (5 mL) and dried (Na_2SO_4). Purification by silica gel column chromatography (5–10% EtOAc in hexanes) afforded the ketoamide [R_f = 0.2 (10% EtOAc in hexanes)] which was directly submitted to the alcohols deprotection using the same procedure as for **30a**. Purification by silica gel column chromatography (50% EtOAc in hexanes) gave **30b** in 49% yield (8.0 mg, 0.018 mmol). R_f = 0.4 (50% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (1H, br s), 6.31 (1H, d, J = 10.8 Hz), 5.85 (1H, d, J = 10.8 Hz), 4.19–4.00 (2H, m), 2.97–2.85 (5H, m), 2.77 (2H, t, J = 14.1 Hz), 2.48 (1H, d, J = 12.0 Hz), 2.28–2.16 (2H, m), 2.07–1.15 (19H, m), 1.14–1.00 (1H, m), 0.91 (3H, d, J = 6.0 Hz), 0.53 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.5, 161.1, 143.4, 131.3, 124.1, 115.5, 67.7, 67.5, 56.7, 56.5, 46.0, 44.9, 42.4, 40.7, 37.4, 37.0, 36.2, 35.8, 29.2, 27.9, 26.1, 25.8, 23.9, 23.7, 22.5, 19.0, 12.3; IR (KBr) ν 3376 (br), 2928, 1717, 1673, 1538, 1453, 1047, 668 cm^{-1}; HRMS (ESI): m/z calcd for [(M+Na)$^+$] = 468.3096, found = 468.3082.

4.2. Biochemical analysis of hybrid molecules

4.2.1. Cell culture

Cells were purchased from American Type Culture Collection (Manassas, VA) and cultured under recommended conditions. Cells were split at 60–70% confluence, as follows: cells were washed with filtered PBS, split with a 5–10 min incubation with 1 mL Trypsin–EDTA (Invitrogen, Carlsbad, CA) in PBS and collected with medium. Following centrifugation, the supernatant was removed. Cell pellets were resuspended with DMEM-F12, and distributed on fresh media-containing dishes. For cell treatments, AT84 cells were split and after 24 h the medium was changed to DMEM + 10% charcoal-stripped FBS. After an additional 24 h, the media was changed and cells were incubated in DMEM-F12 + 10% charcoal-stripped FBS and treated with 1,25D (Sigma) or hybrid compounds, as indicated in the figures.

4.2.2. RT-PCR analysis

PCR primers were designed using Primer3 software found at http://frodo.wi.mit.edu/ and have already been described.[10] After treatment, cells were washed with PBS, homogenized with 1 mL TRIzol Reagent (Invitrogen, Carlsbad, CA), kept at room temperature for 2–5 min, collected and then kept at −80 °C for at least 1 h. After thawing, 0.2 mL of chloroform was added. After vigorous mixing and storing for 10 min, mixtures were centrifuged (10,000 rpm, 15 min, 4 °C) and the upper transparent layer was transferred to a new tube. Isopropanol (0.5 mL) was added and

the new solution was mixed. After centrifuging at 4 °C, the supernatant was discarded and 1 mL of 75% ethanol was added. After centrifuging and discarding the supernatant, the pellet was air-dried for 5–10 min, and then dissolved in ddH_2O. RNA concentrations were measured using a spectrophotometer, and 1–3 μg of RNA were loaded for reverse transcriptase reactions (total volume: 20 μL). ddH_2O (80 μL) was added to the RT-product, and 1.5 μL of the resulting solution was used as template for the PCR reaction. Reverse transcriptase (Super Script II) was purchased from Invitrogen (Carlsbad, CA). DNA polymerase and dNTP's were ordered from Fermentas (Glen Burnie, MD).

4.2.3. Fluorescence polarization (FP) competition assay

The assay was performed using a vitamin D receptor competitor assay kit (Polarscreen, Invitrogen, Carlsbad, CA) set up using 0.5 nM fluorescent tracer. The assay measures the decrease in FP accompanying loss of binding to the relatively high molecular weight VDR ligand binding domain of the fluorescent tracer due to the presence of a competitor. FP was measured using an Analyst HT fluorimeter (Molecular Devices) configured with absorption and emissions filters as recommended by the kit manufacturers. Dose–response curves and IC_{50} determination were determined using XLfit (IDBS) Sigmoidal Dose–Response Model [fit = (A + ((B − A)/(1 + ((C/x)^D)))); inv = (C/((((B − A)/(y − A)) − 1)^(1/D))); res = (y-fit)]. Note that use of concentrations of some analogs at 1 M or greater was precluded in the assay likely due to their insolubility under assay conditions.

4.2.4. Western blotting

The following primary (1st) antibodies (Ab) were used: α-tubulin (Santa Cruz, sc-8035, Santa Cruz, CA), acetylated α-tubulin (Santa Cruz, sc-23950, Santa Cruz, CA), Ac-histone H4 (Santa Cruz, sc-8660-R), β-actin (Santa Cruz, sc-47778, Santa Cruz, CA). After treatments, cells were washed with PBS, scraped with 1 mL PBS, and pelleted by centrifugation (10,000 rpm, 10 min, 4 °C), and frozen at −80 °C after removing the supernatant. At least 1 h later, cells were suspended for 20 min in lysis buffer (10 mM Tris–Cl pH 8, 60 mM KCl, 1 mM EDTA, 1 mM DTT, and 0.5% NP-40 all in H_2O). Cells were then centrifuged at 4 °C, and supernatant was transferred to a new tube. Protein concentration was measured using Bio-Rad protein assay, and samples were mixed with equal volumes of 2× loading dye [100 mM Tris–Cl pH 6.8, 4% SDS, 0.2% bromophenol blue, 20% glycerol, 4% 2-mercaptoethanol, and 200 mM DTT (latter two added before use) in water]. Samples were loaded in 8–15% acrylamide/bis-acrylamide gels immersed in running buffer (3 g Tris–Cl, 14.4 g glycine, 1 g SDS in 1 L H_2O), and electrophoresed (100 V) until the bottom of samples had run all the way. Protein was transferred to methanol activated PVDF membranes (Bio-Rad, Mississauga, ON) using a power source (100 V, 1 h) and transfer buffer (3.03 g Tris–Cl, 14.4 g glycine, 0.037 g SDS in 1 L H_2O. Membranes where then blocked for 1 h with 7% skim milk in TTBS (TBS: 6.05 g Tris–Cl, 8.76 g NaCl, in 1 L H_2O, TTBS = TBS + 0.1% Tween), and incubated overnight with the 1st Ab solution (1:6000–1:1000 diluted Ab in TBS). Membranes were washed three times with TTBS, once with TBS, and incubated for 1 h with secondary Ab solution (1:3000 diluted Ab in TBS). Membranes were washed again and developed using Western Blot Luminol Reagent (Santa Cruz Biotechnology, Santa Cruz, CA) and Hyperfilm (Amersham Biosciences, Piscataway, NJ).

4.2.5. Fluorogenic HDAC assay

Boc-Lys(Ac)-7-amino-4-methylcoumarin (Boc-Lys(Ac)-AMC) was used as substrate for the HDAC assays. Substrate solution was prepared as follows: Boc-Lys(Ac)-AMC was dissolved in DMSO and diluted with HDAC buffer (15 mM Tris–HCl [pH 8.1], 250 μM EDTA, 250 mM NaCl, 10% glycerol) to give 1 mM solutions containing 1.7% DMSO. HDAC enzyme solutions were prepared by diluting HDAC2, -3 and -6 (Cayman Chemical) to concentrations of 0.04 ng/μL, 0.02 ng/μL and 0.05 ng/μL in HDAC buffer (above). A trypsin solution 10 mg/mL in HDAC buffer was used for development. Release of AMC was monitored by measuring the fluorescence at 460 nm (l_{ex} = 390 nm) with a microplate reader (SpectraMax Gemini) at 37 °C. The AMC signals were recorded against a blank with buffer, substrate and trypsin but without the enzyme. All experiments were carried out at least in triplicate.

For HDAC assays, inhibitor (or none for control) diluted in 50 μL of HDAC buffer was mixed with 10 μL of diluted HDAC2, -3 or -6 solution in HDAC buffer at room temperature. The assay was begun by adding 40 μL of substrate solution in HDAC buffer followed by incubation with stirring at 37 °C. After 30 min, 100 μL of trypsin solution was added. After a further 10 min incubation period with stirring at 37 °C, the release of AMC was monitored by measuring the fluorescence.

4.2.6. EdU Cell growth assay

Click-iT EdU Alexa Fluor high-throughput imaging (HCS) assay was used for assessing cell proliferation. HCS assays were performed on AT84 mouse squamous carcinoma cell line (ATCC) following manufacturer's instruction (Molecular Probes, Invitrogen). Images were analyzed for Hoechst 33342 (350/460 nm) and Alexa Fluro 647 (620/700 nm) using Image Xpress Micro (Molecular Devices, CA, USA). All samples were in triplicate.

4.2.7. Hypercalcemia assay

Eight-week old virgin female FVB mice (Charles River, Quebec, Canada) were housed in a limited access room. Animal husbandry adhered to Canadian Council on Animal Care Standards, and all protocols were approved by the McGill University Health Center Animal Care Utilization Committee. Mice were implanted with Alzet osmotic minipumps (Alzet, Cupertino, CA) containing 1,25D or hybrids. In previous studies we determined that constant infusion of 1,25D above 18 pmol/24 h results in hypercalcemia in the majority of animals.[34] We used a similar protocol here and treated mice with a constant infusion of 12, 24 and 48 pmol/24 h of 1,25D (Leo Pharmaceuticals, Ballerup, Denmark), as a positive control, and increasing concentrations of the analogs (240 and 1200 pmol/24 h).[35] Animals were treated for a total of two weeks. Blood calcium and albumin concentrations (to correct calcium) were monitored pre-treatment and at timed intervals (using 25 μL of plasma) by microchemistry (Kodak Ektachrome, Mississauga, ON).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.03.078.

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