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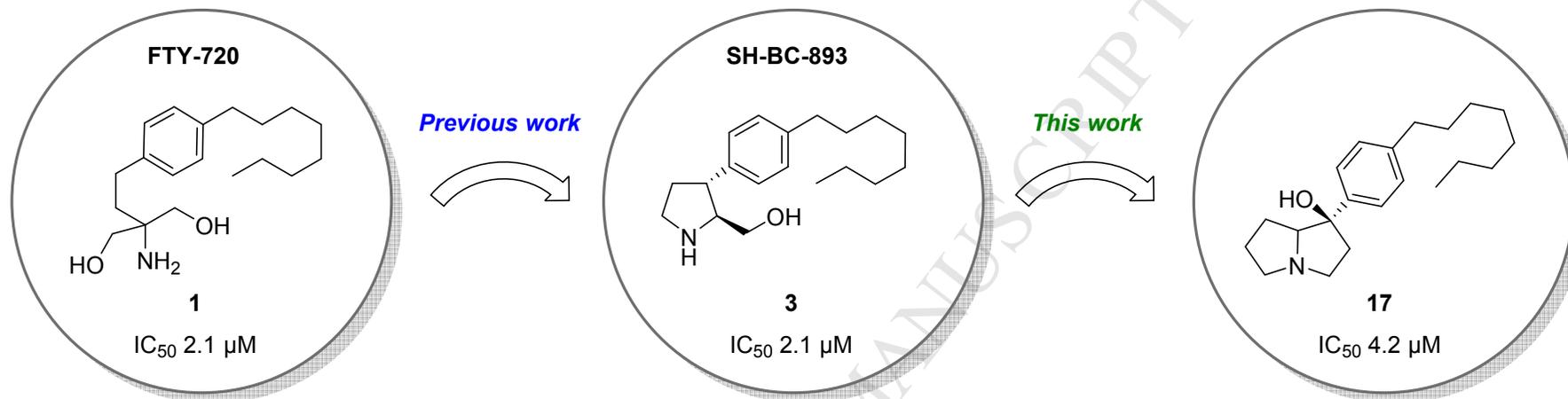
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GRAPHICAL ABSTRACT





Research Paper

In search of constrained FTY720 and phytosphingosine analogs as dual acting anticancer agents targeting metabolic and epigenetic pathways

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Abstract

A series of compounds containing pyrrolidine and pyrrolizidine cores with appended hydrophobic substituents were prepared as constrained analogs of FTY720 and phytosphingosine. The effect of these compounds on the viability of cancer cells, on downregulation of the nutrient transport systems, and on their ability to cause vacuolation was studied. An attempt to inhibit HDACs with some phosphate esters of our analogs was thwarted by our failure to reproduce the reported inhibitory action of FTY720-phosphate.

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1. Introduction

Cancer chemotherapy remains an enigmatic and challenging endeavor. In spite of heroic efforts and impressive advances on many fronts, major obstacles such as resistance and toxicity plague the search for effective drugs [1-4]. Compounds that exploit the metabolic differences between cancer and normal cells provide an alternative to toxic systemic chemotherapies or therapies targeting oncogenic signal transduction cascades [5-7]. FTY720 (Gilenya), an approved drug for the treatment of multiple sclerosis [8] is also known to have a cytotoxic effect against cancer cells [9]. However, when phosphorylated it exerts profound cardiovascular effects at higher doses [10]. We have previously shown that pyrrolidines related to ring-constrained analogs of synthetic sphingolipids such as FTY720 (Gilenya, fingolimod) **1** (Figure 1) and natural phytosphingosine **2** kill cancer cells by interfering with one or more nutrient transport systems required for sustenance [11-13]. This strategy of “starving cancer cells to death” has been effectively demonstrated *in vitro* and *in vivo* with the analog **3** [14], which unlike FTY720, is not phosphorylated *in vivo* and avoids the cardiovascular effects induced by FTY720-phosphate through its interaction with sphingosine-1-phosphate receptors [15-20].

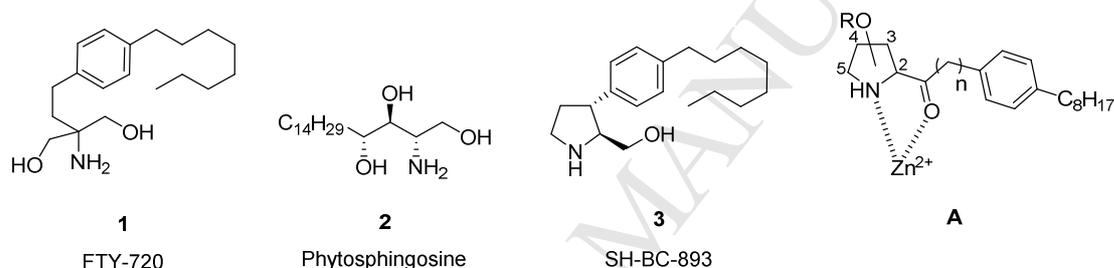


Figure 1. Reported anticancer compounds and a new proposed congener A

We have shown that four of the nutrient acquisition mechanisms used by mammalian cells, namely transporter-mediated import of amino acids and glucose, receptor-mediated LDL uptake and processing, autophagy, and macropinocytosis, are inhibited by analog **3** [14]. Remarkably, cytotoxicity is limited to cancer cells, most likely because non-transformed cells can adapt to the stress caused by nutrient deprivation by altering their metabolic program. The ability of analog **3** to kill cancer cells at ca.2 μM doses is attributed in part to PP2A activation which restricts access to nutrients by down-regulating amino acid and glucose transporters from the cell surface and blocks lysosomal fusion (visualized as extensive vacuolation, Supplementary Figure 1) [14]. Variations of chain length, stereochemistry, and functional group manipulations were also performed to establish thresholds of activity for each of three phenotypes: viability, transporter loss, and vacuolation [13]. The favorable activity profile and promising pharmacological properties of analog **3**, including water solubility, encouraged us to study related analogs with a view to better understanding the role of the structural and functional features that contribute to cancer cell killing. As a first objective, we were particularly interested to find new structural variants of the original analog **3** that were

differentiated with regard to down-regulation and/or vacuolation, the latter property being an indication of a compound's ability to block lysosomal fusion. We initially prepared analogs in which the extremity of the C-3-aryloctyl chain was modified. As a second objective, we considered transposing the C-3-aryloctyl chain in analog **3** to the C-2 position of the pyrrolidine ring and including polar substituents within geometric proximity to the nitrogen atom. A third objective was to determine if these new analogs now carrying the aryloctyl chain containing polar groups but branched at the C-2-position of the pyrrolidine ring, could inhibit histone deacetylases (HDACs) [21-23]. Considering the position of the nitrogen atom in the pyrrolidine ring, we speculated that placing a proximal keto group could possibly result in bidentate metal coordination in a cellular environment to target zinc-dependent metalloenzymes, thereby leading to a dual mode of antiproliferative activity on cancer cells in addition to activation of PP2A. Following this notion, we were encouraged that in 2014 Spiegel et al. had reported that FTY720-phosphate was an inhibitor of HDACs by virtue of an increase in acetylation of lysine residues [24-25]. However, Gardner et al. were unable to confirm this in a different cell type [26]. Nevertheless, we considered a generic compound represented by structure A as a constrained C-2-substituted pyrrolidine variant of FTY720 that would combine the attributes of the lead compound **3** while also having the potential to function as an inhibitor of HDACs (Figure 1).

Results and discussion

2.1 New C-3 arylchain-modified variants of analog **3**

As previously reported, constrained analog **3** was similarly active to FTY720 (Table 1) while lacking the dose-limiting cardiotoxicity associated with FTY720 [11-14]. In an effort to further probe the structural characteristics of analog **3** vis-a-vis cytotoxicity, nutrient transporter down-regulation, and vacuolation, we turned our attention to the hydrophobic aryloctyl appendage.

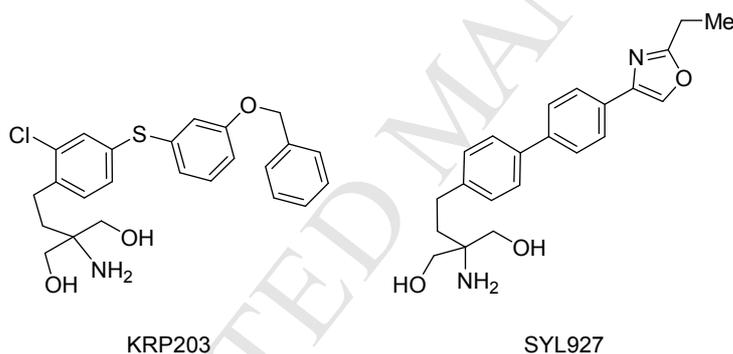


Figure 2. Immunosuppressant KRP-203 and S1P1 agonist SYL927

Inspired by the structure of the immunosuppressant KRP-203 (Figure 2) [27] we wanted to investigate the effect of inserting a benzyl ether moiety at the extremity of the aryloctyl chain as shown in compound **4** (Table 1) (see Experimental Section).

Next, we introduced a 2-methyl oxazoline moiety at the extremity of the octyl chain, as found in the selective S1P1 agonist SYL927 [28] to obtain analogs **5** and **6**. Cytotoxicity assays measured as IC_{50} showed a 4-7 fold reduction in potency for compounds **4**, **5**, **6** and **7** compared to analog **3** (Table 1). The loss of activity of the 2-octylketone **7** indicated that polar groups at the extremity of the octyl chain are detrimental to activity. These analogs down-regulated the nutrient transporter-associated protein CD98 at high concentrations, but

failed to vacuolate cells even at 40 μM , a dose where CD98 down-regulation and cell killing were robust, suggesting

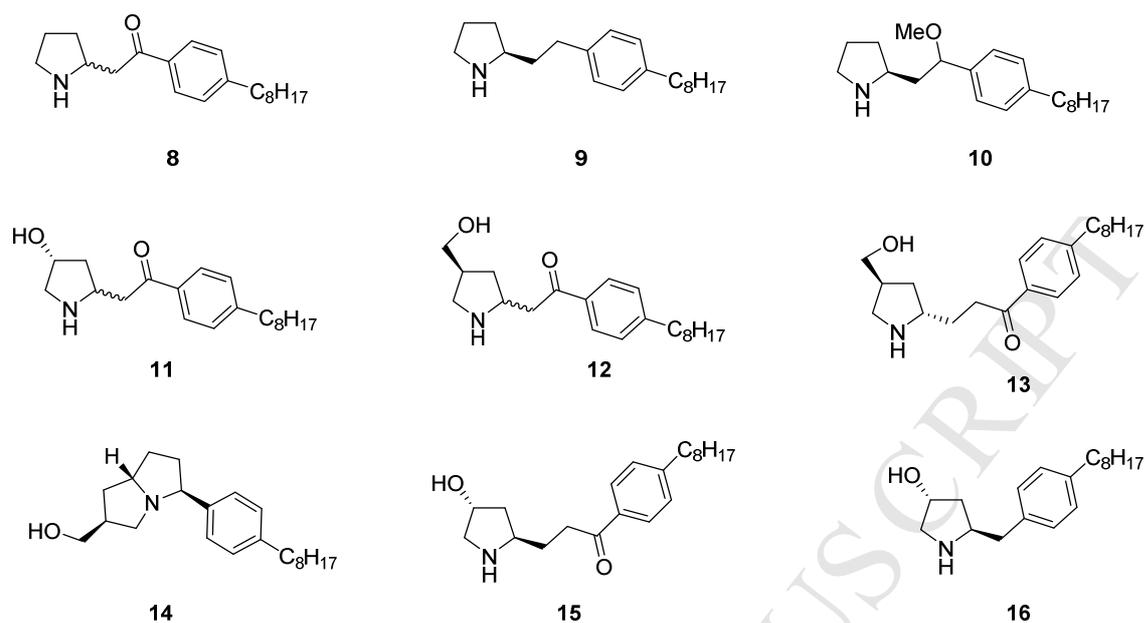
Table 1. Cytotoxicity, CD98 down-regulation and vacuolation profiles of the new C-3-arylalkyl chain variants of analog 3

Comp.	IC ₅₀ (μM) [95% CI]		%CD98 down-regulation			Vacuolation score		
			2.5 μM	10 μM	40 μM	2.5 μM	10 μM	40 μM
1	2.1	[1.9, 2.4]	30	63	n.d.	+++	+++	n.d.
3	2.1	[2.0, 2.2]	41	66	n.d.	++	+++	n.d.
4	10.9	[8.7, 13.7]	11	14	51	n.d.	0	0
5	10.2	[8.7, 12.0]	5	18	61	n.d.	0	0
6	8.4	[7.0, 10.1]	5	33	61	n.d.	0	0
7	15.1	[11.2, 20.3]	-15	-8	23	n.d.	0	0

that they lacked the ability to block lysosomal fusion. In view of these results, we decided not to pursue further modifications within the 3-aryloctyl appendage of the lead compound **3**.

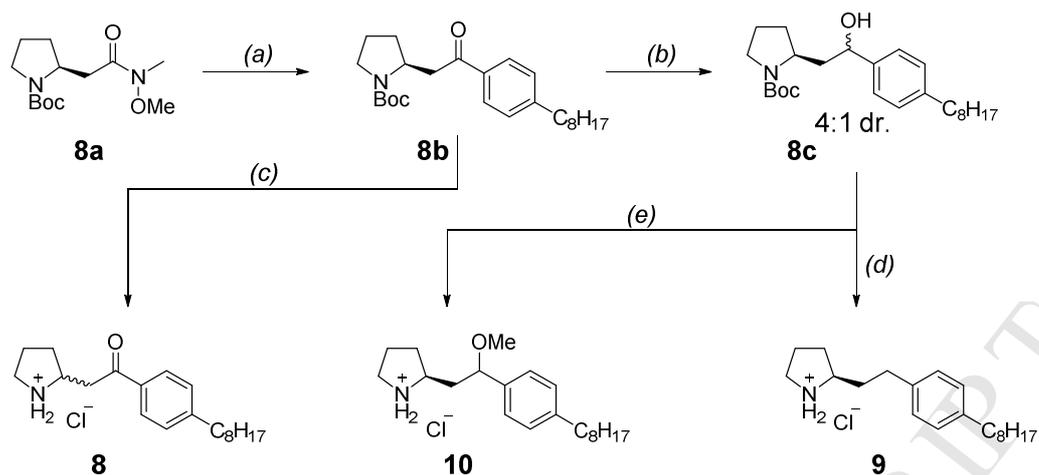
2.2 Extended C-2 modified variants of analog 3

Being cognizant that the pyrrolidine core had to maintain its basic character for cytotoxicity [11-14], we next sought to probe the positioning of the aryloctyl side chain within the pyrrolidine ring. To this end, we considered branching at C-2 to generate a series of 2-substituted pyrrolidines with extended chains. Anticipating the need for a polar group within the chain for possible metal chelation and HDAC inhibition, we initially considered placing a ketone at the beta and gamma positions next to the pyrrolidine ring (Table 2). We were pleased that the modified variants in this new series exhibited cytotoxicity similar to analog **3**. They also down-regulated nutrient transporters and vacuolated at concentrations near their IC₅₀, suggesting that they most likely share the same mechanism of action as analog **3** (Table 2).

Table 2. Cytotoxicity, CD98 down-regulation and vacuolation profiles of the C-2 modified variants of analog 3 (as HCl salts)

Comp.	IC50 (μM) [95% CI]		%CD98 down-regulation			Vacuolation score		
			2.5 μM	10 μM	40 μM	2.5 μM	10 μM	40 μM
3	2.1	[2.0, 2.2]	42	66	n.d.	++	+++	n.d.
8	2.5	[2.3, 2.8]	28	57	n.d.	+	+++	n.d.
9	2.8	[2.6, 3.0]	41	63	n.d.	++	+++	n.d.
10	3.0	[2.7, 3.3]	16	58	n.d.	0	+++	n.d.
11	2.1	[1.5, 2.8]	22	46	n.d.	+++	+++	n.d.
12	2.0	[1.6, 2.6]	44	60	n.d.	++	+++	n.d.
13	4.0	[3.5, 4.6]	8	46	n.d.	0	+++	n.d.
14	12.2	[11.4, 13.0]	0	32	68	0	0	+
15	2.1	[1.9, 2.3]	15	52	n.d.	++	+++	n.d.
16	2.2	[2.1, 2.3]	40	63	n.d.	++	+++	n.d.

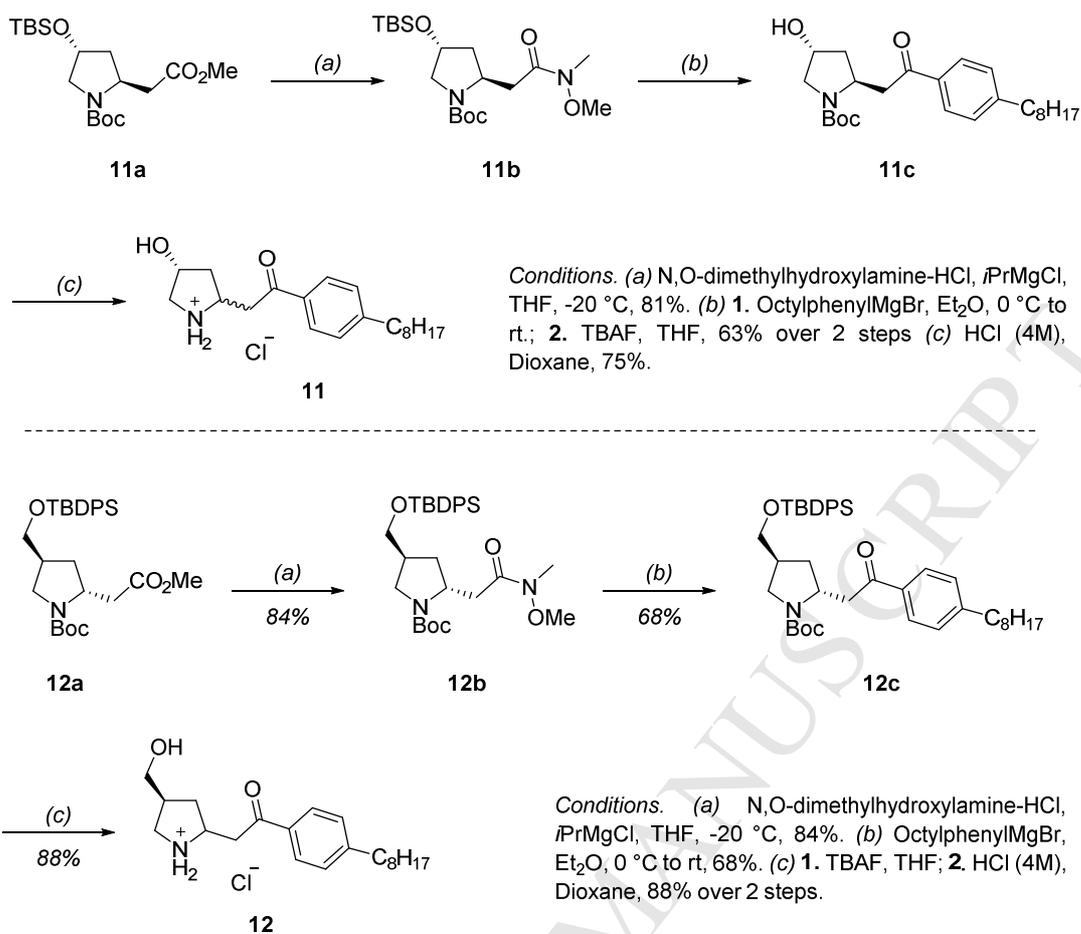
Compounds **8**, **9**, and **10** were prepared from the Weinreb amide derivative of L-homoproline **8a** previously reported by Georg *et al.* [29] (Scheme 1). Treatment of **8a** with 3 equivalents of octylphenylmagnesium bromide in Et_2O at 0°C led to benzylic ketone **8b** as a versatile common intermediate. The presence of by-products from the reagent necessitated careful chromatography of the crude reaction product affording 62% yield of pure **8b**. Removal of the N-Boc group with 4 N HCl in dioxane afforded **8** as a mixture of enantiomers due to rapid racemization in methanol or water (*vide infra*). Ketone **8b** was reduced with NaBH_4 to give the corresponding benzylic alcohol **8c** as a 4:1 diastereomeric mixture that could be easily separated by column chromatography.



Conditions. (a) OctylPhenylMgBr, Et₂O, 0 °C to r.t., 62% (b) NaBH₄, MeOH, 0 °C to r.t., 99%. (c) HCl (4M), Dioxane, 99%. (d) **1.** H₂, Pd/C, EtOH; **2.** HCl (4M), Dioxane, 73% over 2 steps. (e) **1.** Diastereomeric FCC Separation; **2.** NaH, MeI, THF; **3.** HCl (4M), Dioxane, 38% over 2 steps.

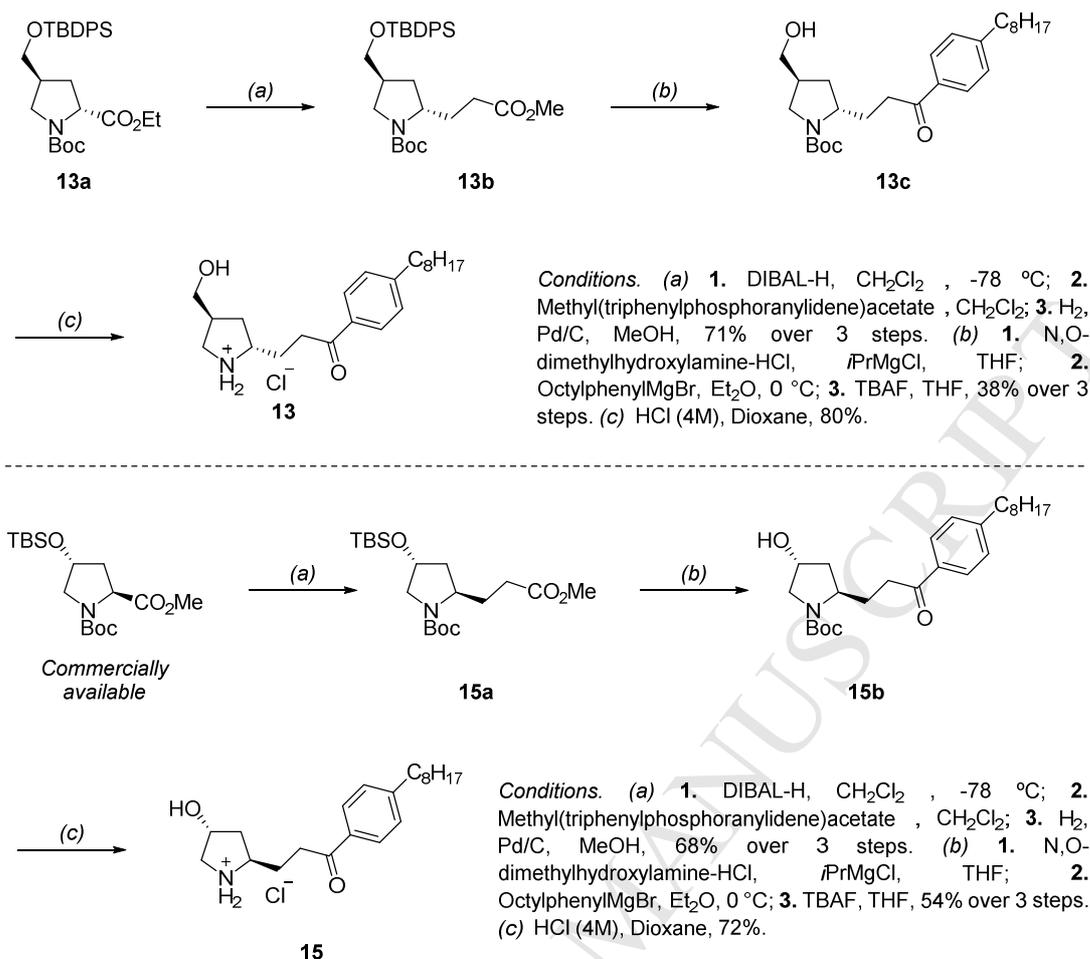
Scheme 1. Synthesis of 2-substituted pyrrolidines

Although the stereochemistry of each diastereomer was not determined, the major diastereomer was converted to the methyl ether, then deprotected to afford product **10**. The crude diastereomeric mixture of **8c** was also catalytically hydrogenated at atmospheric pressure in ethanol, affording product **9** after final removal of the N-Boc protective group.



Scheme 2. Synthesis of 2- and 4-substituted pyrrolidines

Products **11** and **12** were obtained starting from the 4-substituted homoprolines **11a** and **12a** respectively (Scheme 2) (see Supporting Information for the synthesis of **11a** and **12a**). The corresponding Weinreb amides **11b** and **12b** were treated with octylphenylmagnesium bromide to give ketones **11c** and **12c** with acceptable yields. Careful chromatographic purification to separate by-products resulting from the Grignard reagent followed by treatment with TBAF and acid led to products **11** and **12**. Products **13** and **15** were prepared starting from **13a** (Scheme 3) (see Supporting Information for the synthesis of **13a**) and commercially available 1-(tert-butyl) 2-methyl (2*S*,4*R*)-4-((tert-butyldimethylsilyl)oxy)pyrrolidine-1,2-dicarboxylate. Reduction of the ester group to the corresponding aldehyde with DIBAL-H, followed by a Wittig reaction with methyl(triphenylphosphoranylidene)acetate, and catalytic hydrogenation afforded the ester intermediates **13b** and **15a**. The corresponding Weinreb amides were subsequently reacted with octylphenylmagnesium bromide to give ketones **13c** and **15b**, in 37% and 54% yield respectively. Removal of the OTBDPS and N-Boc groups afforded products **13** and **15**.

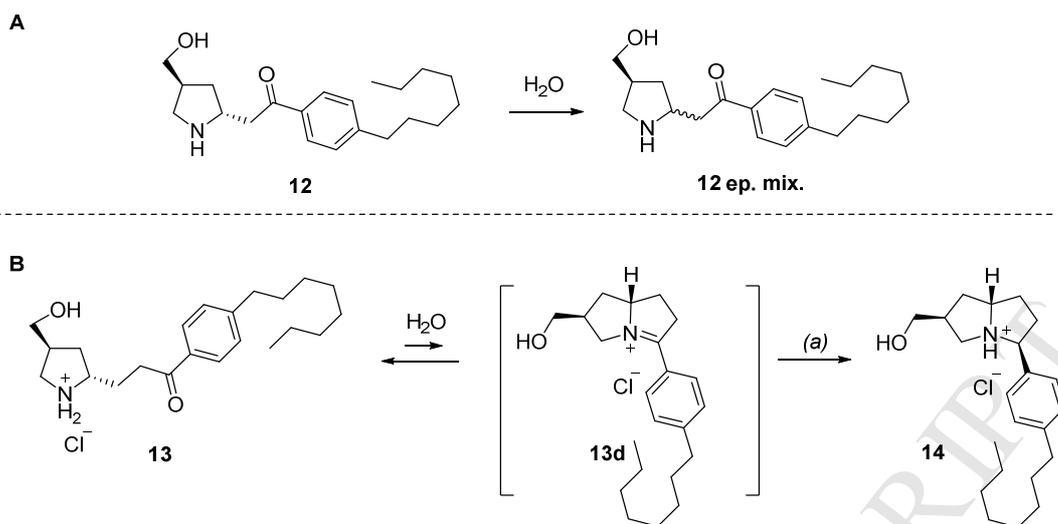


Scheme 3. Synthesis of C-2 substituted extended pyrrolidines

We observed that **11** and **12** epimerized at the C-2 position when dissolved in protic solvents. In the presence of D_2O at room temperature, deuterium was incorporated at C-2 confirming a fast enolization followed by β -elimination and ring closure. Keto pyrrolidine **12** was stable only in very acidic conditions ($\text{pH} \leq 3$ in H_2O), while epimerization was extremely fast in basic conditions. The incorporation of deuterium is complete in 30 min at $\text{pH} = 10$, and in 3 hours at $\text{pH} = 7$. A similar behavior had already been observed for the chlorpromazine-like central nervous system depressant Su17595A, [30], the muscle relaxant tolperisone [31], and the antiarrhythmic drug moricizine [32], albeit at a slower rate.

When the keto group was further removed from the pyrrolidine ring by extending the chain length as in **13**, the desired ketone was found to be in equilibrium with an azabicyclic salt resulting from intramolecular iminium ion formation (2:1 mixture in H_2O) (**13d**), which upon reduction with NaBH_4 led to **14** (Scheme 4, B). The same behavior was observed in the case of compound **15**.

Surprisingly the new bicyclic derivative **14** maintained a reasonable cytotoxic activity (IC_{50} 12.2 μM). We therefore decided to further investigate this new structural analog and we prepared the bicyclic enantiopure



Conditions: (a) NaBH₄, MeOH, 0 °C to rt, 99%

Scheme 4. Epimerization and cyclization processes observed

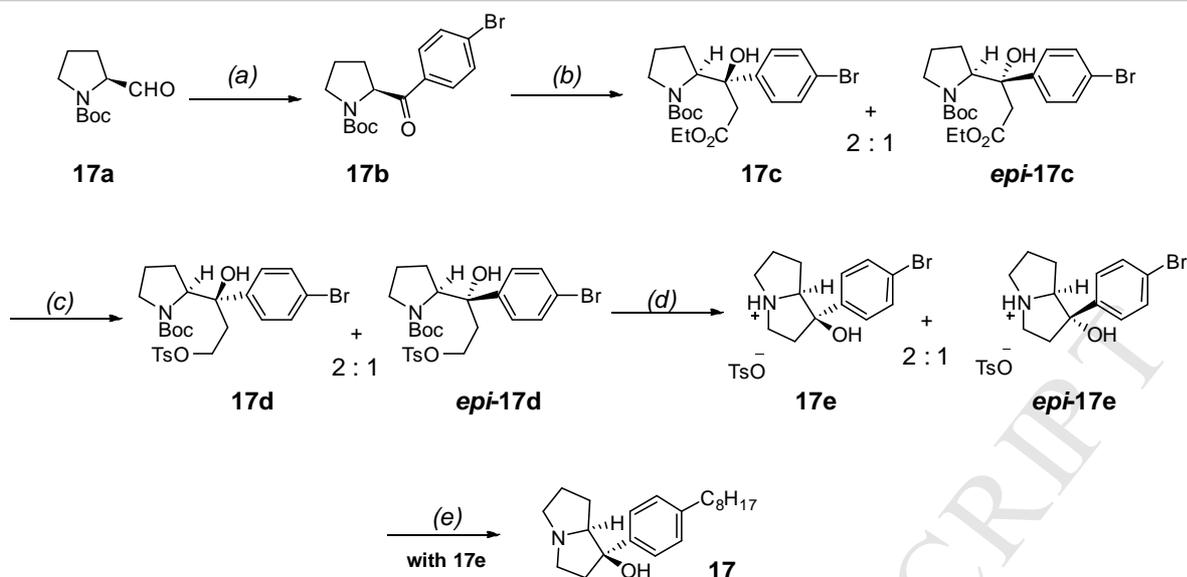
pyrrolidine **17** bearing the aryloctyl appendage on C-3, in analogy with our lead compound **3**, as well as the monocyclic variant **18**, to check the effect of the substituent position and the nature of the azacycles (Scheme 5).

We started the synthesis from the readily available (*S*)-prolinal which was arylated and reoxidized to the corresponding ketone since the addition of the corresponding aryl Grignard on the Weinreb amide resulted in decomposition of the reagent without conversion of the substrate (Scheme 5). Subsequent addition of the lithium enolate of ethyl acetate delivered **17c** as a mixture of diastereomers that could not be separated at this stage. The ester was further reduced to the alcohol and tosylated, which when warmed up to 105 °C in toluene, underwent a spontaneous deprotection/cyclisation, leading to the formation of the bicyclic structure **17e** and its epimer (*epi*-**17e**) as tosylate salts in a ratio of 2:1.

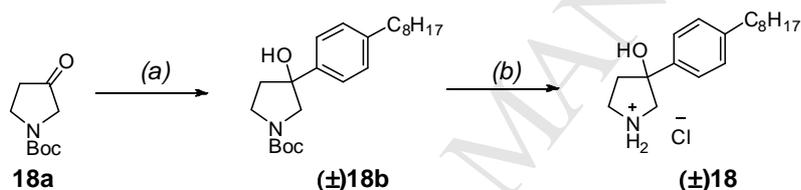
The diastereomers were separated at this stage, delivering the major product **17e** in 38% yield. Suzuki coupling of **17e** followed by hydrogenation of the resulting alkene delivered **17**. The moderate yield over the two last steps was attributed to the highly sensitive benzylic alcohol decomposing easily under acidic conditions.

The racemic compound **18** was easily accessed from N-Boc 3-pyrrolidinone which was arylated with the octylphenylmagnesium bromide and deprotected under acidic conditions.

Interestingly, compound **17** proved to be more active than **14** with an IC₅₀ in the same range as the monocyclic C-2 substituted variants while **18** was less active than **17** (Figure 3), indicating that the increased steric hindrance generated by the pyrrolidine was tolerated.



Conditions: (a) 1. 1,4-dibromobenzene, *n*-BuLi, THF, -78 °C; 2. DMP, CH₂Cl₂, 41% over 2 steps. (b) LDA, EtOAc, THF, -78 °C, 76%. (c) 1. LiBH₄, THF, 0°C to rt; 2. Ts₂O, Pyridine, CH₂Cl₂, 52% over 2 steps. (d) PhMe, 105 °C, 55%. (e) 1. Octyne, catecholborane, NaHCO₃, Pd(PPh₃)₄, DME; 2. H₂, Pd/C, EtOAc, then HCl, 29% over 2 steps.



Conditions: (a) OctylphenylMgBr, Et₂O, 61%. (b) HCl (4M), dioxane, 45%

Scheme 5. Pyrrolizidine and pyrrolidine syntheses

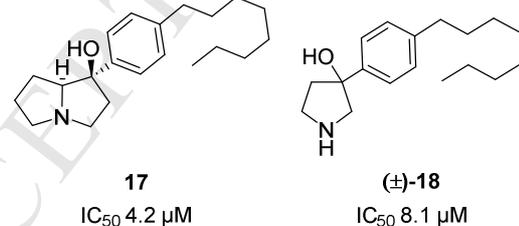
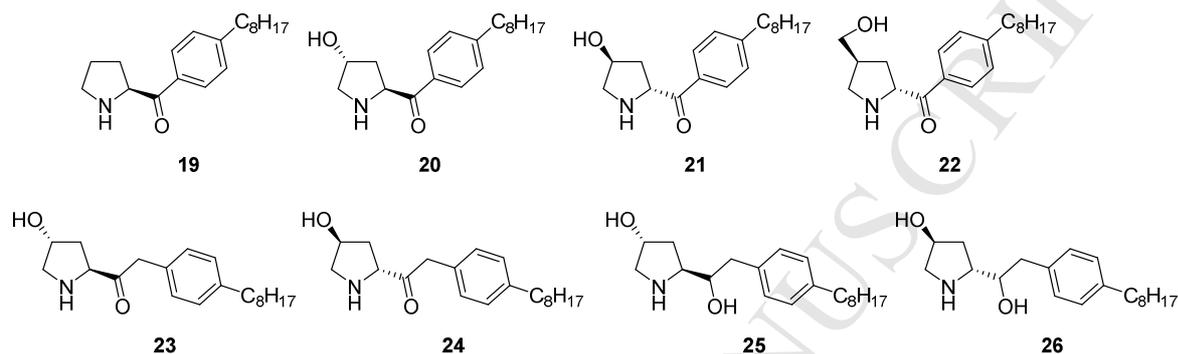


Figure 3. Pyrrolizidine and pyrrolidine analogs

2.3 Repositioning the keto group in extended C-2 modified variants of analog 3

In considering an alternative position of the keto group in the chain, we placed it on the α -position of C-2 branched aryloctyl pyrrolidine analogs (Table 3). This would avoid the issues of partial epimerization issues encountered due to beta elimination and ring closure as described above, although the basicity of the pyrrolidine nitrogen could be diminished due to the inductive effect of the carbonyl group [33]. In general, the cytotoxic activity of the C-2 ketoaryl compounds was significantly reduced compared to **3** and to the corresponding extended keto variants (compare Tables 2 and 3). This indicated that a carbonyl group adjacent to the pyrrolidine nitrogen atom on a chain at C-2 was not well tolerated, whereas alcohols **25** and **26** retained activity similar to the lead compound **3**.

Table 3. Cytotoxicity, CD98 down-regulation and vacuolation profiles of the C-2 substituted α -keto and α -hydroxy pyrrolidines

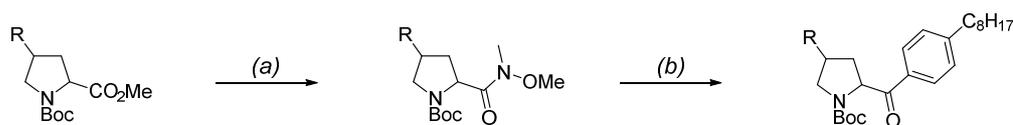


Comp.	IC50 (μ M) [95% CI]		%CD98 down-regulation			Vacuolation score		
			2.5 μ M	10 μ M	40 μ M	2.5 μ M	10 μ M	40 μ M
3	2.1	[2.0, 2.2]	42	66	n.d.	++	+++	n.d.
19	< 40		-21	-4	33	0	0	+++
20	28.1	[26.1, 30.2]	2	9	48	0	0/+	+++
21	15.3	[14.6, 15.9]	12	51	41	0	+++	+++
22	19.6	[18.7, 20.5]	0	19	61	0	0	+++
23	18.6	[15.5, 22.3]	13	23	59	0	0	+++
24	17.1	[14.5, 20.2]	11	16	54	0	0	+++
25	3.0	[2.6, 3.5]	19	62	n.d.	+	+++	n.d.
26	1.8	[1.7, 2.0]	9	62	n.d.	0	++	n.d.

Nevertheless, all of the C-2 keto compounds were able to down-regulate nutrient transporters at elevated concentrations. The same trend held for vacuolation, as **21** reached maximum vacuolation at 10 μ M (Table 3). The negative influence of the α -keto group in the chain was further seen by the 5-10 fold decrease in cytotoxicity when comparing compounds **20** and **21** to **16**. It is also notable that, at the concentration where compound **26** kills 50% of cells, no down-regulation of nutrient transporter proteins or vacuolation was observed, suggesting an alternative mode of action.

Compounds **20**, **22** and **23**, as well as the corresponding reduction products **16** and **25** were synthesized as single enantiomers from the intermediates **20b** and **22b** (Scheme 6). The Weinreb amides **20b** and **22b** were reacted with octylphenylmagnesium bromide to give the corresponding ketones, which were deprotected to

give **20c** and **22c**. Treatment with acid afforded the pyrrolidines **20** and **22** respectively.



19a R=H (2*S*, 4*R*)

20a R=OTBS (2*S*, 4*R*)

13a R=CH₂OTBDPS (2*R*, 4*S*)

19b R=H (2*S*, 4*R*) 89%

20b R=OTBS (2*S*, 4*R*) 95%

22b R=CH₂OTBDPS (2*R*, 4*S*) 99%

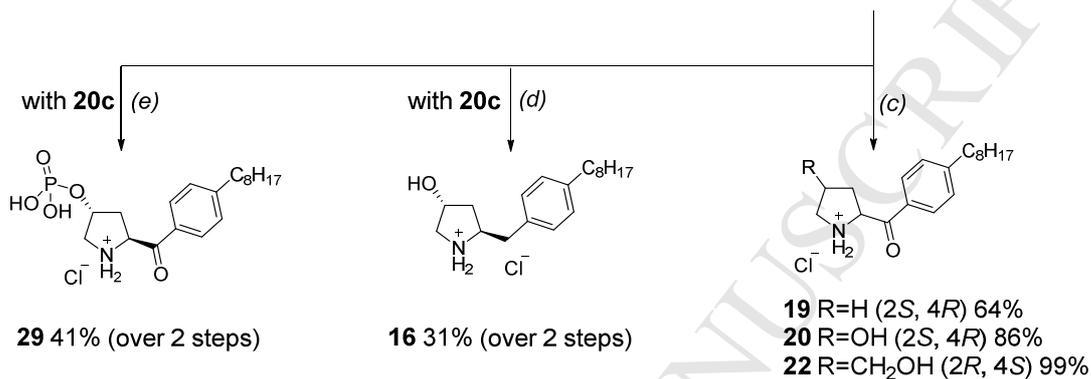
19c R=H (2*S*, 4*R*) 65%

20c R=OH (2*S*, 4*R*) 65%

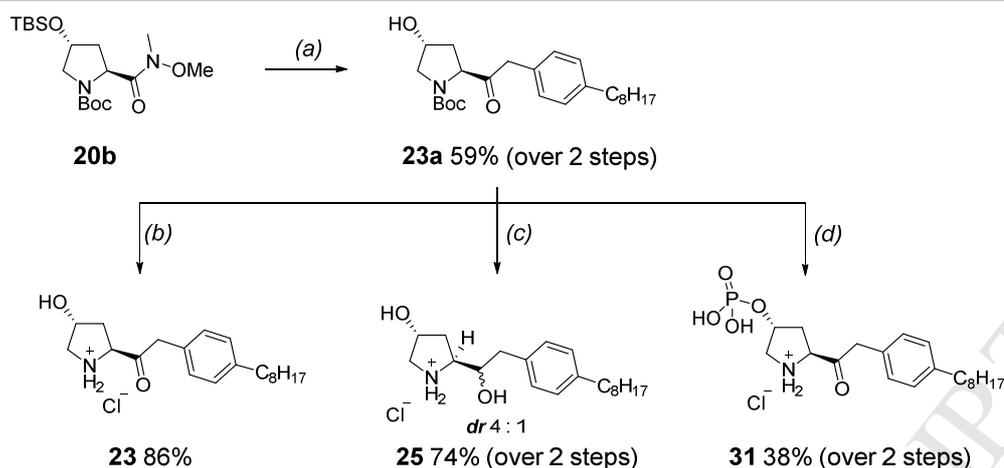
(over 2 steps)

22c R=CH₂OH (2*R*, 4*S*) 48%

(over 2 steps)



Conditions. (a) N,O-dimethylhydroxylamine-HCl, *i*PrMgCl, THF, -20 °C. (b) **1.** OctylphenylMgBr, Et₂O, 0 °C; **2.** TBAF, THF (c) HCl (4M), Dioxane. (d) **1.** H₂, Pd/C, EtOH; **2.** HCl (4M), Dioxane. (e) **1.** P(O*t*Bu)₂NEt₂, tetrazole, THF, 0 °C then *t*BuOOH; **2.** HCl (4M), Dioxane



Conditions. (a) **1.** OctylphenylMgBr, Et₂O, 0 °C; **2.** TBAF, THF (b) HCl (4M), Dioxane. (c) **1.** NaBH₄, MeOH; **2.** HCl (4M), Dioxane. (d) **1.** P(O*t*Bu)₂NEt₂, tetrazole, THF, 0 °C then *t*BuOOH; **2.** HCl (4M), Dioxane

Scheme 6. Syntheses of the α -keto and α -hydroxy C2-substituted pyrrolidines and their phosphate esters

Pd/C catalyzed hydrogenation of the keto group in **22c** followed by N-Boc deprotection led to **16** in modest yield. Following a similar protocol, we prepared the extended ketone intermediate **23a** that was transformed to **23** and **25**, the latter consisting of a 4:1 mixture of diastereomers (Scheme 6).

As previously stated, we proposed to prepare constrained C-2 substituted α -hydroxy pyrrolidine analogs of FTY720 bearing a phosphate group to test them as HDAC inhibitors. To this end we prepared phosphate esters **29** and **31** as well as their enantiomers **30** and **32** (not shown) by standard methods (Scheme 6).

2.4 Anticipated HDAC activity presents a conundrum

The nuclear zinc-dependent metalloprotease enzymes known as histone deacetylases have been the subject of extensive studies over the past decades because they play a major role in regulating gene expression [34]. Chromatin remodeling affects the accessibility of DNA to transcription factors and thus plays a central role in controlling gene expression and determining cellular phenotypes. The acetylation and deacetylation of N-acetyl lysine residues catalyzed by histone acetylases (HATs) and histone deacetylases (HDACs) respectively, is a tightly controlled process that, if perturbed, can lead to cancer. [34] Consequently, the search for HDAC inhibitors as drugs to combat cancer has been of considerable interest. [21-23] Inspired by the structure of the natural product trichostatin A (TSA) (**27**) [35], Breslow and coworkers [36] developed the simple suberoylanilide hydroxamic acid (SAHA) (**28**), which is marketed under the trade name Zolinza (Vorinostat) (Figure 4).

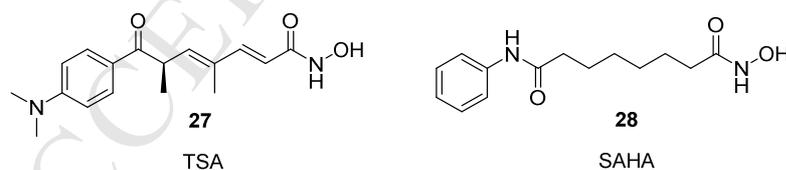


Figure 4. Classical HDAC inhibitors

Previous studies from our laboratory have focused on exploring the importance of the chain length and incorporation of an α -alkoxy chain as well as stereochemical features on a SAHA motif on HDAC inhibitory activity [37-38]. Relying on molecular modeling and the crystal structure of HDAC 8 [39] we reported on a series of macrocyclic inhibitors with a nanomolar activity profile against a number of class I and class II HDACs [40].

As previously mentioned, we were intrigued by the reports by Spiegel and coworkers [24-25] that FTY720-phosphate and sphingosine-1-phosphate [41] are inhibitory toward HDACs. In these studies, nuclear SphK2 was required to observe FTY720- and sphingosine-1-phosphate-dependent increases in several histone acetylation marks. The (*S*)-phosphate formed *in vivo* from FTY720 would mimic the natural sphingosine phosphate intracellularly and act as a ‘synthetic’ inhibitor. Their results showed that FTY720-P inhibited recombinant HDACs 1, 2, 3 and 8, approaching the activity of SAHA. Using the reported crystal structure of HDAC 2, the Spiegel group conducted molecular docking studies of FTY720-P on the active site and concluded that the binding mode was very similar to SAHA and sphingosine-1-phosphate. They hypothesized that the juxtaposition of the primary amino group and the hydroxymethyl group bearing the (*S*)-configured phosphate in FTY720 might act similarly to the hydroxamic acid group in SAHA and the phosphate in S1P. They further invoked a favorable π - π interaction of the phenyl group in FTY720-P with Phe206 and Phe151 (Figure 5).

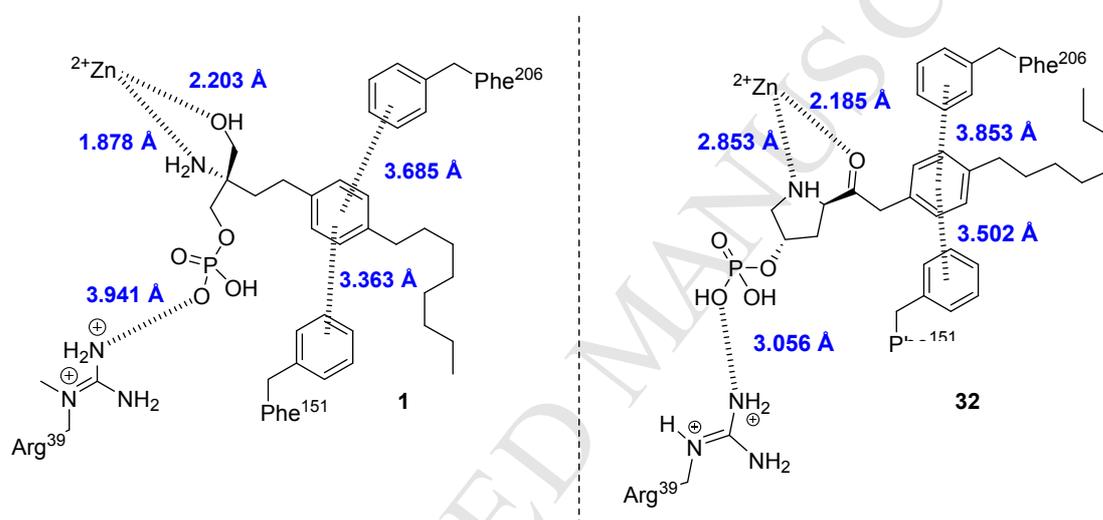
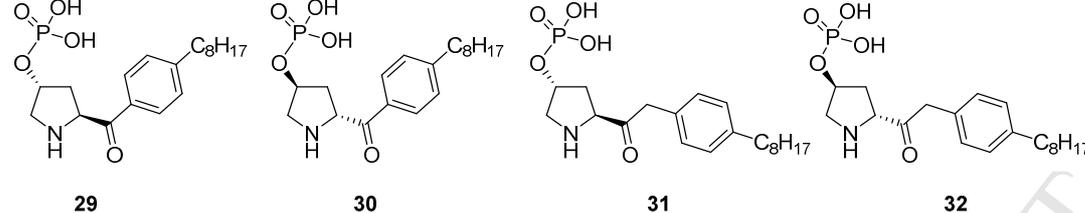


Figure 5. Proposed binding mode of FTY720-phosphate (Spiegel) and a hypothetical pose for a synthetic C-2-keto arylalkyl pyrrolidine 3-phosphate (32)

Encouraged by these highly intriguing results from the Spiegel group, we were poised to test phosphate analogs **29-32** sharing similar interactions as suggested by a docking study using FITTED against HDACs. [42-43]. However, prior to undertaking this initiative, we wanted to see if these phosphates exhibited any cytotoxic activity. While it was not surprising that the phosphate esters **29**, **30**, **31**, and **32** were totally inactive in down-regulation and vacuolation tests compared to their hydroxy pyrrolidine ketone progenitors **20**, **21**, **23** and **24**, as the charged phosphate should not be able to enter the cell, we were surprised to find that these analogs were cytotoxic at IC_{50} 12.9 μM , 12.8 μM , 25.0 μM and 25.3 μM respectively (Table 4). The cytotoxicity of these phosphate esters over the unphosphorylated progenitors despite the absence of transporter loss or vacuolation suggests that they could act through a distinct mechanism, possibly targeting a

receptor on the cell surface.

Table 4. Cytotoxicity, CD98 down-regulation and vacuolation profiles of phosphate ester analogs



Comp.	IC50 (μM) [95% CI]		%CD98 down-regulation			Vacuolation score		
			2.5 μM	10 μM	40 μM	2.5 μM	10 μM	40 μM
3	2.1	[2.0, 2.2]	42	66	n.d.	++	+++	n.d.
29	12.9	[11.9, 14.0]	n.d.	3	-8	0	0	0
30	12.8	[11.7, 14.0]	n.d.	-9	0	0	0	0
31	25.0	[18.1, 34.4]	n.d.	3	0	n.d.	0	0
32	25.3	[19.5, 32.9]	n.d.	5	8	n.d.	0	0

We then focused on establishing the parameters to test HDAC activity. Pleasingly, the reported activity of SAHA was confirmed in both our *in vitro* and cell-based test systems (85% inhibition at 2 μM concentration). However, FTY720-P, reported to exhibit 60-75% inhibition at 5 μM by Spiegel, [24-25] was inactive up to 20 μM in our hands despite the fact that its integrity and phosphorylation were confirmed by UPLC-ESI-MS/MS (Supplemental Figures 2A, B and 3 and data not shown). Assays performed using **12**, **25**, **26**, **31**, and **32** that employed SAHA as a positive control failed to detect HDAC inhibition (Supplemental Figure 2B, C). Examination of histone acetylation in intact cells also failed to confirm a role for FTY720-P as an HDAC inhibitor (Supplemental Figure 3). Since other groups have reported difficulty detecting increased acetylation of specific lysine residues [26], we utilized antibodies against multiple acetylation sites on both H3 and H4; however, no changes in histone acetylation were detected in cells treated with FTY720-P despite consistent and robust increases in acetylation in cells treated with SAHA. Clearly, this disappointing outcome presented an unexplained conundrum that led us to refrain from conducting further HDAC inhibition tests with additional compounds in this series. Given that the parent compound FTY720-P did not inhibit HDAC activity, our intended objective of achieving dual-action inhibitors was not realized.

2. Conclusions

In conclusion, we have reported that placing the aryloctyl group found in the anticancer analog **3** in the C-2 position of the pyrrolidine maintains cytotoxic activity. However, introduction of a carbonyl group in between the aryloctyl appendage and the pyrrolidine ring resulted in variable levels of activity depending on its position along the chain. Placing the carbonyl group two or three carbon atoms removed from the pyrrolidine ring was generally better tolerated with an activity profile only slightly diminished compared to the lead

analog **3**, whereas attempts to bring it closer were detrimental to the activity, likely due to a decrease of the nitrogen basicity. Our attempts to mimic the HDAC inhibiting activity of FTY720-phosphate as reported by Spiegel and coworkers with a series of constrained C-2 keto pyrrolidine analogs and their phosphate esters were thwarted by the fact that in our hands FTY720-phosphate did not act as an inhibitor. Thus, our search for a dual-action anticancer agent derived from a synthetic pyrrolidine-based sphingolipid was only partially validated with a new series of C-2 alkyl and C-2 ketoalkyl arylloctyl pyrrolidines. Gratifyingly, these maintained cytotoxicity, nutrient transporter down-regulation, and vacuolation activity profiles equal to that of the lead analog **3** with the notable exception of **4**, **5**, **6**, and **7**, which do not vacuolate even at high concentrations, and **26**, which triggers changes in endolysosomal trafficking at elevated doses but appears to kill cells by binding to an additional target. Our efforts also uncovered pyrrolizidines with appended arylloctyl chains as new cytotoxic agents. Studies are in progress to better understand the anti-neoplastic actions of synthetic sphingolipids and to identify their direct protein targets in cells.

3. Experimental section

4.1 Chemistry: General information

All reactions involving moisture sensitive compounds were performed in flame-dried glassware under a positive pressure of dry, oxygen free, argon and in dry solvents. Anhydrous solvents were distilled under a positive pressure of argon before use and dried by standard methods. THF, ether, CH₂Cl₂ and toluene were dried by the SDS (Solvent Delivery System). Commercial grade reagents were used without further purification. Silica column chromatography was performed on 230-400 mesh silica gel. Thin layer chromatography (TLC) was carried out on glass-backed silica gel plates. Visualisation was effected by UV light (254 nm) or by staining with potassium permanganate solution, cerium ammonium molybdate or *p*-anisaldehyde followed by heating. ¹H and ¹³C NMR spectra were recorded on Bruker AV-400 and AV-500 MHz spectrometers at room temperature (298 K). Chemical shifts are reported in parts per million (ppm) referenced from CDCl₃ (δ_{H} : 7.26 ppm and δ_{C} : 77.0 ppm). Coupling constants (*J*) are reported in Hertz (Hz). Multiplicities are given as multiplet (m), singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin.) and broad (br.). Infrared spectra were recorded on a FT-IR spectrometer and are reported in reciprocal centimetres (cm⁻¹). Optical rotations were determined on an Anton Paar MCP 300 polarimeter at 589 nm. Specific rotations are given in units of 10⁻¹ deg cm² g⁻¹. High resolution mass spectra (HRMS) were performed by the “Centre régional de spectroscopie de masse de l’Université de Montréal” with electrospray ionisation (ESI) coupled to a quantitative time-of-flight (TOF) detector.

General procedure A for *N*-Boc deprotection

HCl (500 μ L, 4M in dioxane, excess) was added to substrate in dry dioxane. The reaction was stirred at rt until disappearance of the starting material by TLC analysis. The solution was then concentrated *in vacuo* in several cycles co-distilling with dry dioxane.

General procedure B for removal of silyl ethers

TBAF (1.1 eq., 1.0 M in THF) was added to a solution of substrate in dry THF (C = 0.06 M). The reaction

was then stirred at rt until disappearance of the starting material by TLC analysis. The solution was diluted with saturated aq. NaHCO₃ solution and EtOAc. The aqueous layer was extracted x2 with EtOAc. The organic layers were combined, washed x1 with brine, dried over Na₂SO₄, filtered, concentrated.

General procedure C for addition of Grignard reagents

A 3-neck round-bottomed flask equipped with a thermometer and a condenser was flame-dried and flushed with Ar. The flask was then charged with Mg⁰ (1.1 eq.), a single I₂ crystal and another vacuum/Ar cycle was performed. Et₂O (C = 0.65 M) was added resulting in a bright orange suspension of Mg⁰ pellets. Phenyl octyl bromide (1.0 eq.) was then added in one portion and the suspension was heated *via* a heatgun until the internal temperature reached 32 °C and stabilized for 5-10 seconds, indicating that the Grignard formation had started. The reaction was stirred at rt until disappearance of the starting material by ¹H NMR analysis (e.g. ≈ 1h). The Grignard solution (3.0 eq., C = 0.65 M) was then syringed to another flask containing substrate (1.0, eq.) in dry Et₂O (C = 0.05 M). The solution was stirred at rt until disappearance of the starting material by TLC analysis. Saturated aqueous NH₄Cl solution was added and the aqueous layer was extracted x2 with EtOAc. The organic layers were collected, washed x1 brine, dried over Na₂SO₄, filtered, concentrated *in vacuo*.

tert-Butyl (2*S*,3*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(4-(3-hydroxyprop-1-yn-1-yl)phenyl)-5-oxopyrrolidine-1-carboxylate (**4b**) (See Supp. Info for a Scheme)

tert-Butyl (2*S*,3*R*)-3-(4-bromophenyl)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-5-oxopyrrolidine-1-carboxylate **4a** (182 mg, 0.30 mmol, 1.0 equiv.) was dissolved in dry DMF (1mL) and Et₃N (3 mL), then propargyl alcohol (0.14 mL, 2.40 mmol, 8.0 equiv.) was added. Inside the solution was bubbling a flow of Ar for 20 min before to add sequentially CuI (17 mg, 0.09 mmol, 0.3 equiv.) and Pd(PPh₃)₄ (52 mg, 0.05 mmol, 1.5 equiv.). The bubbling of Ar was continued for further 15 min, then the mixture was warmed to 70 °C and stirred for 18 h in a sealed flask. The mixture was extracted with an aqueous NH₄OH 1 M solution (100 mL) mixed with brine (100 mL) and EtOAc (100 mL x 4 times). The combined organic layers were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified by silica gel column chromatography with hexane/EtOAc (gradient from 8:2 to 6:2) to afford the title compound **4b** as a brown oil (68 mg, 39%). R_f: 0.54 (Hexane/EtOAc 6:4). [α]_D²⁰ +12.9° (c 0.5, CHCl₃). IR (neat), ν_{max}: 3435, 3165, 3071, 2927, 2856, 2167, 2079, 1778, 1748, 1713, 1366, 1149, 1107, 1029, 882, 700. ¹H NMR (CDCl₃, 500 MHz), δ: 7.70-7.60 (m, 4H), 7.48-7.36 (m, 8H), 7.09 (d, *J* = 8.2 MHz, 2H), 4.53-4.47 (m, 2H), 4.08-4.07 (m, 1H), 3.97 (dd, *J* = 10.6 MHz, *J* = 4.4 MHz, 1H), 3.80 (dd, *J* = 12.9 MHz, *J* = 2.6 MHz, 1H), 3.48 (broad d, *J* = 9.4 MHz, 1H), 3.19 (dd, *J* = 17.9 MHz, *J* = 9.4 MHz, 1H), 2.55 (dd, *J* = 17.9 MHz, *J* = 2.6 MHz, 1H), 1.85-1.75 (m, 1H), 1.42 (s, 9H), 1.08 (s, 9H) ppm. ¹³C NMR (CDCl₃, 125 MHz), δ: 173.9, 149.7, 144.5, 135.7, 135.6, 133.0, 132.6, 130.1, 128.1, 128.0, 126.6, 121.7, 87.8, 85.2, 83.4, 66.6, 64.3, 51.8, 39.8, 38.7, 28.1, 27.0, 19.4, 1.2 ppm. HRMS (ESI) calcd. for C₃₅H₄₂NO₅Si⁺ (M+H)⁺: 584.28268, found: 584.28328.

tert-Butyl (2*S*,3*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(4-(3-hydroxypropyl)phenyl)pyrrolidine-1-carboxylate (**4c**) (See Supp. Info for a Scheme)

tert-Butyl (2*S*,3*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(4-(3-hydroxyprop-1-yn-1-yl)phenyl)-5-oxopyrrolidine-1-carboxylate **4b** (52 mg, 0.09 mmol) was dissolved in EtOH (0.80 mL) and Pd/C (10%, 48 mg, 0.05 mmol, 0.5 equiv.) was added. The air was pumped out of the flask and replaced by H₂. Upon completion (24 h), the reaction mixture was filtered through celite. The solvent was removed under reduced pressure to afford a colorless oil. The crude was dissolved in dry THF (2 mL) and the solution was cooled to 0 °C. Borane dimethyl sulfide complex (0.05 mL, 0.53 mmol, 6.0 equiv.) was then added and the reaction was warmed up to room temperature and stirred for 18 h. The mixture was quenched with H₂O (1 mL) and the solvent was removed under reduced pressure. The residue was extracted with an aqueous NaHCO₃ saturated solution (50 mL) and CH₂Cl₂ (50 mL x 4 times). The combined organic layers were dried over Na₂SO₄ and filtered. The solvent was removed under reduced pressure to afford **4c** as a pale yellow oil (51 mg, 98% over

two steps). R_f : 0.58 (Hexane/EtOAc 6:4). $[\alpha]_D^{20}$ -6.0° (c 0.5, CHCl₃). IR (neat), ν_{\max} : 3461, 3070, 3047, 2958, 2929, 2858, 2166, 2115, 2010, 1692, 1589, 1514, 1472, 1454, 1426, 1391, 1364, 1254, 1166, 1106, 1070, 1033, 1006, 987, 923, 856, 821, 772, 737, 700, 643, 608. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.70-7.62 (m, 4H), 7.46-7.34 (m, 6H), 7.15-7.06 (m, 4H), 4.20-3.35 (m, 7H), 2.74-2.64 (m, 1H), 2.59-2.52 (m, 1H), 2.31-2.17 (m, 1H), 1.95-1.83 (m, 2H), 1.68-1.55 (m, 1H), 1.50 (s, 3H), 1.33 (s, 6H), 1.06 (s, 9H), 0.98-0.92 (m, 1H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 154.4, 154.3, 141.1, 140.2, 135.8, 133.7, 133.5, 129.8, 129.7, 128.8, 127.9, 127.8, 127.7, 127.6, 127.4, 127.2, 79.5, 65.7, 65.5, 63.7, 62.5, 47.3, 46.6, 46.4, 45.5, 37.8, 34.3, 33.1, 32.0, 31.8, 28.7, 28.6, 27.0, 24.7, 19.5, 19.4, 14.0 ppm. HRMS (ESI) calcd. for C₃₅H₄₈NO₄Si⁺ (M+H)⁺: 574.33471, found: 574.33298.

(2*S*,3*R*)-3-(4-(3-(Benzyloxy)propyl)phenyl)-2-(hydroxymethyl)pyrrolidin-1-ium chloride (**4**) (See Supp. Info for a Scheme)

tert-Butyl (2*S*,3*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(4-(3-hydroxypropyl)phenyl)pyrrolidine-1-carboxylate **4c** (34 mg, 0.06 mmol, 1.0 equiv.) was dissolved in dry THF (0.50 mL) and the solution was cooled to 0 °C. To this solution NaH (60% in mineral oil, 12 mg, 0.30 mmol, 5.0 equiv.) was slowly added in one portion, then benzyl bromide (0.03 mL, 0.24 mmol, 4.0 equiv.) was added dropwise. The reaction mixture was stirred for 2 h, then quenched with the addition of an aqueous NH₄Cl saturated solution (1 mL) and extracted with EtOAc (4 x 30 mL). The combined organic layers were washed x1 with brine (30 mL), dried over Na₂SO₄ and filtered. The solvent was removed under reduced pressure to afford a pale yellow oil. This oil was dissolved in dry THF (1 mL) and the solution was cooled to 0 °C. Tetrabutylammonium fluoride solution (1M in THF, 0.30 mL, 0.30 mmol, 5.0 equiv.) was added and the reaction mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was quenched with an aqueous NaHCO₃ saturated solution (20 mL) and extracted three times with CH₂Cl₂ (30 mL). The combined organic layers were washed with brine (30 mL), dried over Na₂SO₄ and filtered. The solvent was removed under reduced pressure to afford a pale yellow oil. To this crude a HCl 1M solution in dioxane (0.50 mL, 0.50 mmol, 8.0 equiv.) was added. The reaction mixture was stirred at room temperature for 1 h, then the solvent was removed under reduced pressure. The crude mixture was purified by silica gel column chromatography with CH₂Cl₂/MeOH (gradient from 9:1 to 6:4) to give a pale yellow oil. This oil was dissolved in water, filtered through a plastic syringe filter (pore size: 0.45 μ m) and lyophilized to afford the title compound **4** as a white solid (20 mg, 93% over three steps). R_f : 0.72 (CH₂Cl₂/MeOH 8:2). $[\alpha]_D^{20}$ -19.2° (c 0.1, MeOH). IR (neat), ν_{\max} : 3254, 2922, 2848, 2496, 1719, 1595, 1516, 1494, 1476, 1452, 1427, 1391, 1353, 1307, 1265, 1206, 1174, 1117, 1067, 1046, 998, 920, 858, 841, 812, 791, 745, 699. ¹H NMR (MeOD, 500 MHz), δ : 7.37-7.18 (m, 9H), 4.50 (s, 2H), 3.75 (t, J = 5.6 MHz, 2H), 3.71-3.53 (m, 2H), 3.49 (J = 6.0 MHz, 2H), 3.45-3.35 (m, 1H), 3.35-3.25 (m, 1H), 2.70 (t, J = 7.2 MHz, 2H), 2.53-2.40 (m, 1H), 2.30-2.18 (m, 1H), 1.94-1.85 (m, 2H) ppm. ¹³C NMR (CDCl₃, 125 MHz), δ : 141.5, 138.4, 135.7, 128.9, 128.0, 127.6, 127.3, 127.2, 72.6, 72.2, 71.1, 69.0, 67.1, 60.8, 58.3, 44.7, 44.6, 42.4, 32.5, 31.5, 31.2 ppm. HRMS (ESI) calcd. for C₂₁H₂₈NO₂⁺ (M+H)⁺: 326.21146, found: 326.21200.

tert-Butyl (2*S*,3*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(4-(7-hydroxyhept-1-yn-1-yl)phenyl)-5-oxopyrrolidine-1-carboxylate (**5b**) (See Supp. Info for a Scheme)

tert-Butyl (2*S*,3*R*)-3-(4-bromophenyl)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-5-oxopyrrolidine-1-carboxylate **5a** (436 mg, 0.72 mmol, 1.0 equiv.) was dissolved in dry DMF (3 mL) and Et₃N (1 mL), then 1-heptynol (0.18 mL, 1.44 mmol, 2.0 equiv.) was added. Inside the solution was bubbling a flow of Ar for 20 min before adding CuI (41 mg, 0.22 mmol, 0.3 equiv.) and Pd(PPh₃)₄ (125 mg, 0.11 mmol, 0.15 equiv.). The bubbling of Ar was continued for further 15 min, then the mixture was warmed to 70 °C and stirred for 18 h in a sealed flask. An aqueous NH₄OH 1 M solution (100 mL) mixed with brine (100 mL) was added and the mixture was extracted with EtOAc (4 x 100 mL). The combined organic layers were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified by silica gel column

chromatography with hexane/EtOAc (gradient from 8:2 to 3:7) to afford the title compound **5b** as a brown oil. (345 mg, 75%). R_f : 0.51 (Hexane/EtOAc 5:5). $[\alpha]_D^{20} +12.3^\circ$ (c 0.5, CHCl_3). IR (neat), ν_{max} : 3489, 3166, 2932, 2858, 2154, 1781, 1748, 1713, 1366, 1303, 1255, 1149, 1072, 822, 700. $^1\text{H NMR}$ (CDCl_3 , 500 MHz), δ : 7.70-7.64 (m, 4H), 7.48-7.40 (m, 8H), 7.37 (d, $J = 7.4$ MHz, 2H), 7.10 (d, $J = 7.4$ MHz, 2H), 4.09 (broad s, 1H), 4.00 (dd, $J = 10.6$ MHz, $J = 4.0$ MHz, 1H), 3.83 (d, $J = 10.6$ MHz, 1H), 3.67 (t, $J = 6.4$ MHz, 2H), 3.51 (d, $J = 8.9$ MHz, 1H), 3.22 (dd, $J = 17.8$ MHz, $J = 9.6$ MHz, 1H), 2.58 (d, $J = 17.8$ MHz, 1H), 2.43 (t, $J = 6.4$ MHz, 2H), 1.86 (broad s, 1H), 1.68-1.50 (m, 4H), 1.44 (s, 9H), 1.11 (s, 9H) ppm. $^{13}\text{C NMR}$ (CDCl_3 , 125 MHz), δ : 174.0, 149.6, 143.3, 135.6, 135.5, 132.9, 132.6, 132.2, 130.0, 128.0, 127.9, 126.3, 123.0, 90.6, 83.2, 80.2, 66.6, 64.2, 62.7, 39.7, 38.6, 32.3, 28.5, 28.0, 26.9, 25.1, 19.4, 19.2 ppm. HRMS (ESI) calcd. for $\text{C}_{39}\text{H}_{50}\text{NO}_5\text{Si}^+$ ($\text{M}+\text{H}$) $^+$: 640.34528, found: 640.34597.

tert-Butyl (2*S*,3*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(4-(6-oxohexyl)phenyl)pyrrolidine-1-carboxylate (**5c**) (See Supp. Info for a Scheme)

tert-Butyl (2*S*,3*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(4-(7-hydroxyhept-1-yn-1-yl)phenyl)-5-oxopyrrolidine-1-carboxylate **5b** (312 mg, 0.49 mmol, 1.0 equiv.) was dissolved in EtOH (2.5 mL) and Pd/C (10%, 164 mg, 0.15 mmol, 0.3 equiv.) was added. The air was pumped out of the flask and replaced by H_2 . Upon completion (18 h), the reaction mixture was filtered through Celite. The solvent was removed under reduced pressure to afford a colorless oil. The crude was dissolved in dry THF (5 mL) and the solution was cooled to 0 °C, then borane dimethyl sulfide complex (0.18 mL, 1.94 mmol, 4.0 equiv.) was added and the reaction was allowed to warm to room temperature and stirred for 24 h. The mixture was quenched with H_2O (1 mL) and the solvent was removed under reduced pressure. An aqueous NaHCO_3 saturated solution (80 mL) was added and the solution was extracted with CH_2Cl_2 (4x 80 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to afford a slight yellow oil. This crude was used directly for the next step without further purification. A solution of dimethyl sulfoxide (0.08 mL, 1.18 mmol, 2.4 equiv.) in CH_2Cl_2 (2 mL) was added dropwise, via cannula, over 10 min to a stirred solution of oxalyl chloride (0.05 mL, 0.59 mmol, 1.2 equiv.) in CH_2Cl_2 (2 mL) at -78 °C. The mixture was stirred at -78 °C for 15 min and then a solution of the previous alcohol in CH_2Cl_2 (3 mL) was added dropwise, via cannula, over 10 min. The mixture was stirred at -78 °C for 1 h and then Et_3N (0.25 mL, 1.76 mmol, 3.6 equiv.) was added dropwise over 5 min. The mixture was warmed to 0 °C over a period of 3.5 h, then quenched with an aqueous NaHCO_3 saturated solution (50 mL) and warmed to room temperature. The separated aqueous layer was extracted with CH_2Cl_2 (50 mL x 3 times), and the combined organic layers were dried over Na_2SO_4 and concentrated in vacuum to leave a yellow oil which was purified by silica gel column chromatography with hexane/EtOAc (gradient from 9:1 to 8:2) to afford the title compound **5c** as a colourless oil (220 mg, 72% over three steps). R_f : 0.59 (Hexane/EtOAc 8:2). $[\alpha]_D^{20} -9.7^\circ$ (c 0.3, CHCl_3). IR (neat), ν_{max} : 3072, 3050, 2951, 2831, 2740, 1732, 1692, 1426, 1390, 1164, 1105, 1070, 1034, 822, 701. $^1\text{H NMR}$ (CDCl_3 , 500 MHz, mixture of rotamers), δ : 9.76 (s, 1H), 7.71-7.63 (m, 4H), 7.46-7.33 (m, 6H), 7.16-7.06 (m, 4H), 4.20-3.35 (m, 6H), 2.62-2.55 (m, 2H), 2.42 (t, $J = 7.2$ MHz, 2H), 2.32-2.18 (m, 1H), 1.99-1.85 (m, 1H), 1.70-1.57 (m, 4H), 1.55-1.42 (m, 4H), 1.41-1.30 (m, 9H), 1.07 (s, 9H) ppm. $^{13}\text{C NMR}$ (CDCl_3 , 125 MHz, mixture of rotamers), δ : 202.8, 154.3, 154.2, 141.1, 140.8, 135.6, 133.6, 133.4, 129.7, 129.6, 128.6, 127.8, 127.8, 127.7, 127.3, 127.2, 79.4, 79.1, 65.6, 65.4, 63.6, 62.1, 60.4, 53.4, 47.2, 46.5, 46.3, 45.4, 43.9, 35.4, 32.9, 31.9, 31.3, 29.1, 29.0, 28.6, 28.4, 26.9, 22.0, 19.4, 19.3 ppm. HRMS (ESI) calcd. for $\text{C}_{39}\text{H}_{54}\text{NO}_4\text{Si}^+$ ($\text{M}+\text{H}$) $^+$: 628.38166, found: 628.38194.

tert-Butyl (2*S*,3*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(4-((*E*)-7-(2-methyloxazol-4-yl)hept-6-en-1-yl)phenyl)pyrrolidine-1-carboxylate (**5d**) (See Supp. Info for a Scheme)

A solution of Lithium bis(trimethylsilyl)amide (1 M in THF, 0.49 mL, 0.49 mmol, 2 equiv.) was added over 5 min via syringe to a stirred solution of dimethyl((2-methyloxazol-4-yl)methyl)phosphonate. The oxazole phosphonate derivative was prepared by using standard procedures from the known 4-hydroxymethyl-2-

methyloxazole [44] (101 mg, 0.49 mmol, 2.0 equiv.) in dry THF (2 mL) at $-78\text{ }^{\circ}\text{C}$. The resulting orange solution was stirred at $-78\text{ }^{\circ}\text{C}$ for 30 min and then a solution of the aldehyde derivative **5c** (154 mg, 0.25 mmol, 1.0 equiv.) in dry THF (2 mL) was added via cannula. The mixture was allowed to warm slowly to room temperature over 5 h, stirred overnight, and then quenched with an aqueous NH_4Cl saturated solution (20 mL) and diluted with EtOAc (50 mL). The organic extract was washed with brine (50 mL), then dried over Na_2SO_4 , filtered and evaporated to dryness. The residue was purified by silica gel column chromatography with hexane/EtOAc (gradient from 9:1 to 8:2) to afford the title compound **5d** as a colourless oil (88 mg, 51%). R_f : 0.36 (Hexane/EtOAc 85:15). $[\alpha]_{\text{D}}^{20}$ -15.2° (c 0.5, CHCl_3). IR (neat), ν_{max} : 3072, 3045, 2956, 2855, 2032, 2022, 1692, 1654, 1516, 1488, 1447, 1410, 1391, 1270, 1070, 982, 742, 700. ^1H NMR (CDCl_3 , 400 MHz, mixture of rotamers), δ : 7.79-7.66 (m, 4H), 7.46-7.25 (m, 7H), 7.15-7.05 (m, 4H), 6.40 (dt, $J = 15.6\text{ MHz}$, $J = 7.0\text{ MHz}$, 1H), 6.17 (d, $J = 15.6\text{ MHz}$, 1H), 4.25-3.35 (m, 5H), 2.63-2.52 (m, 2H), 2.44 (s, 3H), 2.35-2.15 (m, 3H), 2.00-1.85 (m, 1H), 1.70-1.25 (m, 16H), 1.08 (s, 9H) ppm. ^{13}C NMR (CDCl_3 , 100 MHz, mixture of rotamers), δ : 161.6, 154.4, 141.2, 139.2, 135.7, 133.6, 132.9, 129.8, 128.7, 127.8, 127.2, 118.2, 79.4, 72.7, 65.7, 64.7, 63.6, 48.1, 46.6, 35.6, 32.9, 32.0, 31.5, 29.3, 29.1, 28.7, 28.5, 27.0, 19.4, 14.0 ppm. HRMS (ESI) calcd. for $\text{C}_{44}\text{H}_{59}\text{N}_2\text{O}_4\text{Si}^+$ ($\text{M}+\text{H}$) $^+$: 707.42386, found: 707.42492.

(2*S*,3*R*)-2-(Hydroxymethyl)-3-(4-((*E*)-7-(2-methyloxazol-4-yl)hept-6-en-1-yl)phenyl)pyrrolidin-1-ium chloride (**5**) (See Supp. Info for a Scheme)

tert-Butyl (2*S*,3*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(4-((*E*)-7-(2-methyloxazol-4-yl)hept-6-en-1-yl)phenyl)pyrrolidine-1-carboxylate **5d** (27 mg, 0.04 mmol, 1.0 equiv.), was dissolved in dry THF (1 mL) and the solution was cooled to $0\text{ }^{\circ}\text{C}$. Tetrabutylammonium fluoride solution (1M in THF, 0.08 mL, 0.08 mmol, 2.0 equiv.) was added and the reaction mixture was warmed to room temperature and stirred for 24 h. The reaction mixture was quenched with an aqueous NaHCO_3 saturated solution (20 mL) and extracted with CH_2Cl_2 (30 mL x 3 times). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to afford a pale yellow oil. To this crude a HCl 1M solution in dioxane (0.50 mL, 0.50 mmol, 13 equiv.) was added. The reaction mixture was stirred at room temperature for 1 h, then the solvent was removed under reduced pressure. The crude mixture was purified by silica gel column chromatography with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (gradient from 9:1 to 6:4) to give a yellow oil. This oil was dissolved in water, filtered through a plastic syringe filter (pore size: 0.45 μm) and lyophilized to afford the title compound **5** as a white solid (15 mg, 96% over two steps). R_f : 0.59 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 8:2). $[\alpha]_{\text{D}}^{20}$ -16.5° (c 0.2, MeOH). IR (neat), ν_{max} : 3331, 3254, 2929, 2857, 2029, 2020, 1654, 1515, 1487, 1449, 1407, 1270, 1079, 982, 742, 705, 576. ^1H NMR (MeOD, 500 MHz), δ : 7.63 (s, 1H), 7.27 (d, $J = 11.0\text{ MHz}$, 2H), 7.22 (d, $J = 11.0\text{ MHz}$, 2H), 6.38-6.29 (m, 1H), 6.21 (d, $J = 15.8\text{ MHz}$, 1H), 3.88-3.75 (m, 1H), 3.70-3.53 (m, 3H), 2.63 (t, $J = 7.7\text{ MHz}$, 2H), 2.53-2.42 (m, 1H), 2.45 (s, 3H), 2.30-2.15 (m, 3H), 1.70-1.57 (m, 2H), 1.55-1.30 (m, 6H) ppm. ^{13}C NMR (MeOD, 125 MHz), δ : 162.3, 142.3, 138.7, 135.5, 134.3, 132.4, 128.8, 127.1, 117.7, 67.1, 58.2, 44.7, 44.5, 35.0, 32.4, 32.3, 31.2, 28.8, 28.7, 28.6, 12.1 ppm. HRMS (ESI) calcd. for $\text{C}_{23}\text{H}_{33}\text{N}_2\text{O}_2^+$ ($\text{M}+\text{H}$) $^+$: 369.25365, found: 369.25509.

(2*S*,3*R*)-2-(Hydroxymethyl)-3-(4-(7-(2-methyloxazol-4-yl)heptyl)phenyl)pyrrolidin-1-ium chloride (**6**) (See Supp. Info for a Scheme)

tert-Butyl (2*S*,3*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(4-((*E*)-7-(2-methyloxazol-4-yl)hept-6-en-1-yl)phenyl)pyrrolidine-1-carboxylate **5d** (34 mg, 0.05 mmol, 1.0 equiv.) was dissolved in freshly distilled Et_3N (1 mL) and Pd/C (10%, 20 mg, 0.02 mmol, 0.4 equiv.) was added. The air was pumped out of the flask and replaced by H_2 . Upon completion (6 h), the reaction mixture was filtered through celite. The solvent was removed under reduced pressure to afford a colorless oil. This oil was dissolved in dry THF (1 mL) and the solution was cooled to $0\text{ }^{\circ}\text{C}$. Tetrabutylammonium fluoride solution (1M in THF, 0.10 mL, 0.10 mmol, 2.0 equiv.) was added and the reaction mixture was warmed to room temperature and stirred for 24 h. The reaction mixture was quenched with an aqueous NaHCO_3 saturated solution (30 mL) and extracted with

CH₂Cl₂ (30 mL x 4 times). The combined organic layers were washed with brine (60 mL), dried over Na₂SO₄ and filtered. The solvent was removed under reduced pressure to afford a pale yellow oil. To this crude a HCl 1M solution in dioxane (0.50 mL, 0.50 mmol, 10 equiv.) was added. The reaction mixture was stirred at room temperature for 1 h, then the solvent was removed under reduced pressure. The crude mixture was purified by silica gel column chromatography with CH₂Cl₂/MeOH (gradient from 9:1 to 6:4) to give a pale yellow oil. This oil was dissolved in water, filtered through a plastic syringe filter (pore size: 0.45 μm) and lyophilized to afford the title compound **6** as a white solid (17 mg, 88% over three steps). R_f: 0.59 (CH₂Cl₂/MeOH 8:2). [α]_D²⁰ -22.9° (c 0.2, MeOH). IR (neat), ν_{max}: 3390, 3192, 2924, 2855, 2344, 2244, 2166, 2038, 1709, 1654, 1607, 1515, 1381, 1227, 1181, 1065, 1032, 1019, 819, 723, 599, 543. ¹H NMR (DMSO, 500 MHz), δ: 9.86 (broad s, 1H), 9.02 (broad s, 1H), 7.67 (s, 1H), 7.25 (d, J = 8.1 MHz, 2H), 7.16 (d, J = 8.1 MHz, 2H), 3.62-3.43 (m, 3H), 3.42-3.35 (m, 1H), 3.25-3.12 (m, 3H), 2.56-2.52 (m, 2H) 2.39-2.35 (m, 1H) 2.36 (s, 3H), 2.34-2.25 (m, 1H), 2.07-1.98 (m, 1H), 1.68 (m, 4H), 1.32-1.20 (m, 9H) ppm. ¹³C NMR (DMSO, 125 MHz), δ: 160.7, 141.3, 139.7, 136.7 134.3, 128.6, 127.5, 66.5, 62.8, 58.5, 48.6, 44.3, 44.2, 34.7, 32.5, 31.0, 28.8, 28.7, 28.5, 27.7, 25.3, 13.5 ppm. HRMS (ESI) calcd. for C₂₃H₃₅N₂O₂⁺ (M+H)⁺: 371.26930, found: 371.26964.

tert-Butyl (2*S*,3*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(4-(7-oxooct-1-yn-1-yl)phenyl)pyrrolidine-1-carboxylate (**7b**) (See Supp. Info for a Scheme)

A solution of commercially available oct-7-yn-2-one **A** (176 mg, 1.42 mmol, 2 eq.) in dry DMF (500 μL) was added to a solution of **7a** (422 mg, 0.71 mmol) in dry DMF (1.2 mL) and Et₃N (727 μL). The resulting mixture was degassed by bubbling argon for 20 min, before adding CuI (41 mg, 0.231 mmol, 0.3 eq.) and Pd(PPh₃)₄ (82 mg, 0.071 mmol, 0.1 eq.). After having been degassed again for additional 15 min, the reaction was stirred for 48 hours at 70 °C. Afterwards, the mixture was diluted with a combination of NH₄OH 1 M (25 mL) and brine (50 mL) and the product was extracted with EtOAc (4 x 30 mL). The combined organic layers were dried over Na₂SO₄, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane 1:6, R_f: 0.09) to give **7b** as a yellow oil (236 mg, 52%). [α]_D²⁵ -0.8 (c 1.55, CHCl₃). IR (neat), ν_{max}: 2931, 2858, 1690, 1510, 1472, 1427, 1391, 1364, 1240, 1166, 1107, 1070, 987, 823, 773, 740, 702, 605, 541, 504 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ: 7.67-7.62 (m, 4 H), 7.42-7.35 (m, 6 H), 7.31 (t, J = 9.1 Hz, 2H), 7.07 (d, J = 7.5 Hz, 2 H), 4.13 (dd, J = 10.1, 3.4 Hz, 0.4 H), 3.89 (br. s, 0.4 H), 3.79-3.75 (m, 1.8 H), 3.72-3.69 (m, 1 H), 3.64-3.59 (m, 1 H), 3.56-3.52 (m, 0.4 H), 3.44-3.38 (m, 1 H), 2.49 (t, J = 7.3 Hz, 2 H), 2.42 (t, J = 6.9 Hz, 2 H), 2.30-2.22 (m, 1 H), 2.15 (s, 3 H), 1.93-1.86 (m, 1 H), 1.77-1.72 (m, 2 H), 1.63-1.58 (m, 2 H), 1.50 (s, 3.6 H), 1.33 (s, 5.4 H), 1.06 (s, 9 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ: 208.7, 154.2, 143.3, 142.7, 135.6, 133.7, 133.5, 133.4, 133.3, 131.8, 129.7, 129.6, 127.7, 127.3, 127.1, 122.1, 89.5, 80.7, 79.5, 79.2, 65.4, 65.1, 63.5, 62.1, 47.0, 46.5, 46.4, 45.6, 43.2, 32.7, 31.7, 29.9, 28.6, 28.4, 28.1, 26.8, 23.0, 19.2 ppm. HRMS (ESI) calcd. for C₄₀H₅₂NO₄Si (M+H)⁺ 638.36601, found 638.36681.

tert-Butyl (2*S*,3*R*)-2-(hydroxymethyl)-3-(4-(7-oxooctyl)phenyl)pyrrolidine-1-carboxylate (**7c**) (See Supp. Info for a Scheme)

7b (200 mg, 0.31 mmol) was dissolved in EtOAc (5 mL) and Pd/C (10%, 37 mg) was added to the resulting solution. The air was removed from the flask under vacuum and replaced with hydrogen (balloon). The reaction was vigorously stirred overnight at room temperature. Afterwards, the mixture was filtered through a celite pad, washing with EtOAc. The collected solution was concentrated *in vacuo*, affording the intermediate (2*S*,3*R*)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-(4-(7-oxooctyl)phenyl)pyrrolidine-1-carboxylate a colorless oil (201 mg, 99%). [α]_D²⁵ +8.5 (c 0.87, CHCl₃). IR (neat), ν_{max}: 2929, 2856, 1692, 1514, 1472, 1427, 1392, 1365, 1254, 1166, 1108, 987, 856, 822, 772, 740, 701, 607, 504 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ: 7.73-7.63 (m, 4 H), 7.42-7.35 (m, 6 H), 7.15-7.08 (m, 4 H), 4.16-4.13 (m, 0.4 H), 3.90 (br. s, 0.4 H), 3.84-3.76 (m, 1.8 H), 3.72-3.69 (m, 1 H), 3.66-3.60 (m, 1 H), 3.58-3.54 (m, 0.4 H), 3.44-3.39 (m, 1 H), 2.59-2.55 (m, 2 H), 2.41 (t, J = 7.4 Hz, 2 H), 2.30-2.21 (m, 1 H), 2.13 (s, 3 H), 1.95-1.88 (m, 1 H), 1.62-1.55 (m, 4 H), 1.50 (s, 3.6 H), 1.33 (br. s, 9.4 H), 1.06 (s, 9 H) ppm. ¹³C NMR (CDCl₃, 125 MHz,

mixture of rotamers), δ : 209.2, 154.3, 154.2, 141.0, 140.9, 135.6, 134.8, 133.8, 133.6, 133.5, 133.4, 129.7, 129.6, 128.6, 127.7, 127.3, 127.1, 79.4, 79.0, 65.5, 65.3, 63.5, 62.1, 47.1, 46.4, 46.2, 45.3, 43.7, 35.4, 32.9, 31.9, 31.3, 29.8, 29.1, 29.0, 28.6, 28.5, 28.4, 28.0, 26.9, 26.5, 23.7, 19.4. 19.3 ppm. HRMS (ESI) calcd. for $C_{40}H_{56}NO_4Si$ (M+H)⁺ 642.3973, found 642.40020.

(2*S*,3*R*)-2-(((tert-butyl)phenylsilyloxy)methyl)-3-(4-(7-oxooctyl)phenyl)pyrrolidine-1-carboxylate (50 mg, 0.078 mmol) was submitted to general procedure B. The crude was purified by flash column chromatography (EtOAc/hexane 1:1, Rf: 0.29) to give **7c** as a colorless oil (31 mg, 99%). IR (neat), ν_{\max} : 3402, 2928, 2855, 1690, 1666, 1514, 1454, 1394, 1365, 1251, 1165, 1115, 1084, 1054, 968, 854, 772, 535 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz), δ : 7.15-7.11 (m, 4 H), 3.91-3.88 (m, 1 H), 3.76-3.73 (m, 2 H), 3.61 (dd, J = 11.5, 6.9 Hz, 1 H), 3.34 (td, J = 10.5, 6.4 Hz, 1 H), 2.90 (br. s, 1 H), 2.58-2.55 (m, 2 H), 2.41 (t, J = 7.4 Hz, 2 H), 2.13 (s, 3 H), 1.99-1.91 (m, 1 H), 1.62-1.54 (m, 4 H), 1.50 (s, 9 H), 1.34-1.30 (m, 4 H) ppm. ¹³C NMR (CDCl₃, 125 MHz), δ : 209.3, 156.5, 141.6, 138.0, 128.7, 127.5, 80.5, 67.0, 65.7, 47.5, 47.1, 43.7, 35.4, 32.8, 31.2, 29.9, 29.0, 28.5, 23.7 ppm. HRMS (ESI) calcd. for $C_{24}H_{37}NO_4Na$ (M+Na)⁺ 426.26150, found 426.26007.

8-(4-((2*S*,3*R*)-2-(hydroxymethyl)pyrrolidin-3-yl)phenyl)octan-2-one hydrochloride (**7**) (See Supp. Info for a Scheme)

Prepared according to general procedure A, starting from **7c** (20 mg, 0.050 mmol). The crude was recrystallized from EtOAc to give product **7** as a white solid (16 mg, 94%). For biological testing a portion of the product was dissolved in the minimum amount of HPLC grade water, filtered (pore size = 0.45 μ m) and lyophilized. $[\alpha]_D^{25}$ +16.4 (c 0.55, CHCl₃). IR (neat), ν_{\max} : 3277, 2922, 2848, 1707, 1589, 1517, 1464, 1401, 1367, 1332, 1216, 1161, 1110, 1064, 1020, 965, 928, 819, 775, 716, 654, 634, 591, 542, 503 cm⁻¹. ¹H NMR (CD₃OD, 500 MHz), δ : 7.27 (d, J = 8.2 Hz, 2 H), 7.22 (d, J = 8.2 Hz, 2 H), 3.77 (q, J = 6.2 Hz, 1 H), 3.66-3.60 (m, 2 H), 3.58 (ddd, J = 11.8, 8.8, 3.3 Hz, 1 H), 3.41 (ddd, J = 11.8, 9.9, 7.3 Hz, 1 H), 3.31-3.27 (m, 1 H), 2.64-2.61 (m, 2 H), 2.50-2.45 (m, 3 H), 2.28-2.20 (m, 1 H), 2.14 (s, 3 H), 1.66-1.60 (m, 2 H), 1.59-1.53 (m, 2 H), 1.37-1.32 (m, 4 H) ppm. ¹³C NMR (CD₃OD, 125 MHz), δ : 210.7, 142.2, 135.5, 128.8, 127.1, 67.1, 58.2, 44.7, 44.5, 42.8, 35.0, 32.4, 31.1, 28.6, 28.4, 23.4 ppm.

tert-Butyl (*S*)-2-(2-(4-octylphenyl)-2-oxoethyl)pyrrolidine-1-carboxylate (**8b**)

Prepared according to general procedure C, starting from **8a** [25] (300 mg, 1.10 mmol). The crude was purified by flash column chromatography (EtOAc/hexane 1:6, Rf: 0.17) to give **8b** as a colorless oil (274 mg, 62%). $[\alpha]_D^{25}$ -22.9 (c 1.3, CHCl₃). IR (neat), ν_{\max} : 2924, 2854, 1680, 1606, 1455, 1391, 1365, 1277, 1169, 1116, 1012, 989, 772, 545 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.96-7.87 (m, 2 H), 7.25 (d, J = 7.5 Hz, 2 H), 4.34-4.29 (m, 1 H), 3.74 (br. d, J = 14.8 Hz, 0.5 H), 3.47 (br. d, J = 15.2 Hz, 0.5 H), 3.40 (br. s, 1 H), 3.32 (br. s, 1 H), 2.85-2.73 (m, 1 H), 2.64 (br. s, 2 H), 2.03 (br. s, 1 H), 1.90-1.79 (m, 2 H), 1.75 (br. s, 1 H), 1.64-1.57 (m, 2 H), 1.45 (s, 9 H), 1.30-1.22 (m, 10 H), 0.86 (t, J = 7.0 Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 198.8, 198.3, 154.4, 154.3, 149.1, 148.7, 134.6, 128.7, 128.5, 128.4, 79.7, 79.2, 54.5, 54.3, 46.7, 46.2, 43.7, 43.0, 36.0, 31.9, 31.3, 31.1, 30.3, 29.4, 29.3, 29.2, 28.6, 23.6, 22.8, 22.6, 14.1 ppm. HRMS (ESI) calcd. for $C_{25}H_{40}NO_3$ (M+H)⁺ 402.30027, found 402.29965.

(*S*)-1-(4-Octylphenyl)-2-(pyrrolidin-2-yl)ethan-1-one hydrochloride (**8**)

Prepared according to general procedure A, starting from **8b** (100 mg, 0.25 mmol). The crude was purified by flash column chromatography (EtOH/CH₂Cl₂ 1:4, Rf: 0.45) to give product **8** as a white solid (84 mg, 99%). Note: the product racemized spontaneously when dissolved in MeOH or H₂O. For biological testing a portion of this solid was dissolved in the minimum amount of HPLC grade water, filtered (pore size = 0.45 μ m) and lyophilized. $[\alpha]_D^{25}$ -39.1 (c 0.23, CHCl₃). IR (neat), ν_{\max} : 2921, 2852, 1678, 1605, 1589, 1466, 1377, 1222, 1188, 1032, 976, 914, 822, 770, 569 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz), δ : 9.52 (br. s, 2 H), 7.86 (d, J = 8.1 Hz, 2 H), 7.18 (d, J = 7.8 Hz, 2 H), 4.17-4.09 (m, 1 H), 3.89 (dd, J = 18.4, 5.9 Hz, 1 H), 3.50 (dd, J = 18.4, 6.8

Hz, 1 H), 3.40 (t, $J = 7.2$ Hz, 2 H), 2.61–2.57 (m, 2 H), 2.37–2.29 (m, 1 H), 2.10–1.95 (m, 2 H), 1.81–1.70 (m, 1 H), 1.59–1.54 (m, 2 H), 1.30–1.24 (m, 10 H), 0.88 (t, $J = 6.8$ Hz, 3 H) ppm. ^{13}C NMR (CDCl_3 , 125 MHz), δ : 196.8, 149.6, 133.6, 128.7, 128.4, 56.0, 45.0, 40.5, 36.0, 31.9, 31.0, 30.6, 29.7, 29.4, 29.3, 29.2, 23.6, 22.6, 14.1 ppm. HRMS (ESI) calcd. for $\text{C}_{20}\text{H}_{32}\text{NO}$ (M^+) 302.24784, found 302.24782.

tert-Butyl (2*S*)-2-(2-hydroxy-2-(4-octylphenyl)ethyl)pyrrolidine-1-carboxylate (**8c**)

NaBH_4 (4.9 mg, 0.13 mmol, 1.5 eq.) was added to a solution of **8b** (35 mg, 0.087 mmol) in MeOH (3 mL) at 0 °C. The resulting mixture was stirred for 2 hours at the same temperature. Afterwards, the reaction was quenched with brine (1 mL), the MeOH was removed *in vacuo* and the product was extracted with EtOAc (4 x 4 mL). The organic layers were dried over Na_2SO_4 , filtered and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane 1:6, then EtOAc/hexane 1:4 Rf: 0.38 and 0.19) to give **8c diast1** (7 mg, 20%) and **8c diast2** (28 mg, 80%) as colorless oils. **8c diast1**: $[\alpha]_{\text{D}}^{20}$ -8.0 (*c* 0.2, MeOH). IR (neat), ν_{max} : 3406, 2923, 2851, 1723, 1671, 1397, 1245, 1168, 1104, cm^{-1} . ^1H NMR (CDCl_3 , 300 MHz), δ : 7.28 (d, $J = 7.9$ Hz, 2 H), 7.13 (d, $J = 8.0$ Hz, 2 H), 5.33 (br. s, 1 H), 4.64–4.57 (m, 1 H), 4.33–4.25 (m, 1 H), 3.37 (t, $J = 6.6$ Hz, 2 H), 2.60–2.54 (m, 2 H), 2.03–1.93 (m, 2 H), 1.91–1.87 (m, 2 H), 1.72–1.67 (m, 2 H), 1.62–1.54 (m, 2 H), 1.49 (s, 9 H), 1.25 (br. s, 10 H), 0.87 (t, $J = 6.8$ Hz, 3 H) ppm. ^{13}C NMR (CDCl_3 , 75 MHz), δ : 156.7, 141.6, 141.5, 128.2, 125.6, 80.0, 69.8, 54.0, 46.6, 46.3, 35.6, 31.9, 31.5, 31.2, 29.7, 29.5, 29.3, 28.5, 23.6, 22.7, 14.1 ppm. HRMS (ESI) calcd. for $\text{C}_{25}\text{H}_{41}\text{NO}_3\text{Na}$ ($\text{M}+\text{Na}^+$) 426.29787, found 426.29919. **8c diast2** $[\alpha]_{\text{D}}^{20}$ -52.5 (*c* 0.8, MeOH). IR (neat), ν_{max} : 3413, 2924, 2854, 1668, 1393, 1365, 1247, 1168, 1103, 849, 772, 557 cm^{-1} . ^1H NMR (CDCl_3 , 300 MHz), δ : 7.26 (d, $J = 7.8$ Hz, 2 H), 7.13 (d, $J = 7.7$ Hz, 2 H), 4.74 (br. s, 1 H), 4.10 (br. s, 1 H), 3.31 (br. s, 2 H), 2.60–2.55 (m, 2 H), 2.14 (br. s, 1 H), 2.05–1.93 (m, 1 H), 1.89–1.79 (m, 2 H), 1.69 (br. s, 2 H), 1.61–1.54 (m, 2 H), 1.46 (s, 9 H), 1.30–1.26 (m, 10 H), 0.87 (t, $J = 6.8$ Hz, 3 H) ppm. ^{13}C NMR (CDCl_3 , 75 MHz), δ : 155.4, 142.3, 141.6, 128.3, 125.5, 79.7, 72.5, 55.7, 46.4, 46.3, 35.6, 32.4, 31.9, 31.5, 29.7, 29.5, 29.3, 29.2, 28.5, 23.8, 22.6, 14.1 ppm. HRMS (ESI) calcd. for $\text{C}_{25}\text{H}_{41}\text{NO}_3\text{Na}$ ($\text{M}+\text{Na}^+$) 426.29787, found 426.29907.

(*R*)-2-(4-Octylphenethyl)pyrrolidine hydrochloride (**9**)

8c (12 mg, 0.0297 mmol) was dissolved in EtOH (3 mL) and Pd/C (10%, 7 mg) was added to the resulting solution. The air was removed from the flask under vacuum and replaced with hydrogen (balloon). The reaction was vigorously stirred overnight at room temperature. Afterwards, the mixture was filtered through a celite pad, washing with EtOH. The collected solution was concentrated *in vacuo*, affording the intermediate *tert*-Butyl (*R*)-2-(4-octylphenethyl)pyrrolidine-1-carboxylate as a colorless oil (9 mg, 78%). $[\alpha]_{\text{D}}^{20}$ -36.0 (*c* 0.45, CHCl_3). IR (neat), ν_{max} : 2924, 2853, 1694, 1514, 1455, 1391, 1364, 1254, 1169, 1100, 771 cm^{-1} . ^1H NMR (CDCl_3 , 400 MHz, mixture of rotamers), δ : 7.09 (s, 4 H), 3.85 (br. s, 0.4 H), 3.75 (br. s, 0.6 H), 3.41 (br. s, 0.8 H), 3.32 (br. s, 1.2 H), 2.58–2.54 (m, 4 H), 2.14 (br. s, 0.4 H), 2.04–1.87 (m, 1.6 H), 1.85–1.80 (m, 2 H), 1.72 (br. s, 1 H), 1.63–1.55 (m, 3 H), 1.45 (s, 9 H), 1.32–1.24 (m, 10 H), 0.88 (t, $J = 6.8$ Hz, 3 H) ppm. ^{13}C NMR (CDCl_3 , 75 MHz, major rotamer), δ : 154.6, 140.3, 139.2, 128.3, 128.1, 79.0, 56.9, 46.1, 36.4, 35.5, 32.4, 31.9, 31.6, 30.6, 29.7, 29.5, 29.4, 29.2, 28.6, 23.2, 22.7, 14.1 ppm. HRMS (ESI) calcd. for $\text{C}_{25}\text{H}_{41}\text{NO}_2\text{K}$ ($\text{M}+\text{K}^+$) 426.27744, found 426.27543.

tert-Butyl (*R*)-2-(4-octylphenethyl)pyrrolidine-1-carboxylate (9 mg, 0.023 mmol) was submitted to general procedure A. The crude was purified by flash column chromatography (EtOH/ CH_2Cl_2 1:8, Rf: 0.16) to give product **9** as a white solid (7 mg, 93%). For biological testing a portion of this solid was dissolved in the minimum amount of HPLC grade water, filtered (pore size = 0.45 μm) and lyophilized. $[\alpha]_{\text{D}}^{25}$ -4.0 (*c* 0.35, CHCl_3). IR (neat), ν_{max} : 2921, 2852, 2751, 1591, 1514, 1455, 1418, 1042, 815, 722, 554 cm^{-1} . ^1H NMR (CDCl_3 , 500 MHz), δ : 9.69 (br. s, 1 H), 9.19 (br. s, 1 H), 7.12 (d, $J = 8.0$ Hz, 2 H), 7.05 (d, $J = 8.0$ Hz, 2 H), 3.56–3.48 (m, 1 H), 3.44–3.37 (m, 1 H), 3.35–3.30 (m, 1 H), 2.80–2.74 (m, 1 H), 2.71–2.65 (m, 1 H), 2.55–2.52 (m, 2 H), 2.37–2.30 (m, 1 H), 2.15–2.08 (m, 1 H), 2.06–1.97 (m, 2 H), 1.96–1.88 (m, 1 H), 1.72–1.64 (m, 1 H), 1.59–1.53 (m, 2 H), 1.29–1.25 (m, 10 H), 0.87 (t, $J = 7.0$ Hz, 3 H) ppm. ^{13}C NMR (CDCl_3 , 125 MHz), δ :

140.9, 137.2, 128.6, 128.3, 60.0, 44.8, 35.5, 34.0, 32.5, 31.9, 31.6, 30.4, 29.7, 29.5, 29.4, 29.3, 23.5, 22.7, 14.1 ppm. HRMS (ESI) calcd. for $C_{20}H_{34}N$ (M)⁺ 288.26858, found 288.26992.

(2S)-2-(2-Methoxy-2-(4-octylphenyl)ethyl)pyrrolidine hydrochloride (10)

NaH (1.3 mg, 60% dispersion in mineral oil, 0.033 mmol, 1.2 eq.) was added to a solution of **8c diast2** (11 mg, 0.027 mmol) in dry THF (1 mL) at 0 °C. The resulting mixture was stirred at the same temperature for 1 h, before adding methyl iodide (5 μ L, 0.081 mmol, 3 eq.). Then, the reaction was stirred at room temperature for 3 h, before being quenched with water (1 mL). The product was extracted with EtOAc (3 x 2 mL) and the combined organic layers were dried over MgSO₄, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane 1:6, Rf: 0.16) to give the intermediate tert-Butyl (2S)-2-(2-methoxy-2-(4-octylphenyl)ethyl)pyrrolidine-1-carboxylate as a colorless oil (7 mg, 64%). $[\alpha]_D^{25}$ -77.1 (c 0.35, CHCl₃). IR (neat), ν_{max} : 2924, 2854, 1692, 1454, 1391, 1364, 1251, 1170, 1102, 771 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.23-7.13 (m, 4 H), 4.11 (br. s, 1 H), 4.02 (br. s, 0.5 H), 3.93 (br. s, 0.5 H), 3.39 (br. s, 0.5 H), 3.29 (br. s, 1.5 H), 3.16 (s, 3 H), 2.60-2.57 (m, 2 H), 2.27 (br. s, 1 H), 1.82-1.73 (m, 2 H), 1.62-1.56 (m, 3 H), 1.46 (s, 9 H), 1.30-1.26 (m, 10 H), 0.88 (t, *J* = 6.9 Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 154.5, 142.2, 139.5, 128.4, 126.5, 81.6, 79.0, 78.7, 56.3, 54.7, 46.3, 46.1, 42.6, 35.7, 31.9, 31.5, 30.9, 30.3, 29.7, 29.5, 29.3, 29.2, 28.6, 23.7, 23.2, 22.6, 14.1 ppm. HRMS (ESI) calcd. for $C_{26}H_{44}NO_3$ (M+H)⁺ 418.33210, found 418.33141.

tert-Butyl (2S)-2-(2-methoxy-2-(4-octylphenyl)ethyl)pyrrolidine-1-carboxylate (6 mg, 0.014 mmol) was submitted to general procedure A. The crude was purified by flash column chromatography (EtOH/CH₂Cl₂ 1:10, Rf: 0.20) to give product **10** as a white solid (3 mg, 60%). For biological testing a portion of this solid was dissolved in the minimum amount of HPLC grade water, filtered (pore size = 0.45 μ m) and lyophilized. $[\alpha]_D^{25}$ -55.0 (c 0.20, CHCl₃). IR (neat), ν_{max} : 2921, 2851, 2766, 1459, 1107, 1033, 827, 722, 564 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz), δ : 10.53 (br. s, 1 H), 8.54 (br. s, 1 H), 7.19 (d, *J* = 8.1 Hz, 2 H), 7.14 (d, *J* = 8.1 Hz, 2 H), 4.35 (dd, *J* = 10.2, 2.9 Hz, 1 H), 3.91 (br. s, 1 H), 3.51-3.46 (m, 1 H), 3.38-3.33 (m, 1 H), 3.20 (s, 3 H), 2.59-2.56 (m, 2 H), 2.28-2.21 (m, 2 H), 2.07-2.03 (m, 2 H), 1.95-1.92 (m, 1 H), 1.73-1.67 (m, 1 H), 1.61-1.55 (m, 2 H), 1.30-1.24 (m, 10 H), 0.87 (t, *J* = 7.0 Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz), δ : 143.2, 137.3, 128.7, 126.4, 82.4, 58.9, 56.5, 44.5, 40.1, 35.7, 31.9, 31.4, 30.7, 29.7, 29.5, 29.3, 29.2, 23.1, 22.6, 14.1 ppm. HRMS (ESI) calcd. for $C_{21}H_{36}NO$ (M)⁺ 318.27914, found 318.28009.

tert-Butyl (2S,4R)-4-((tert-butyldimethylsilyloxy)-2-(2-methoxy-2-oxoethyl)pyrrolidine-1-carboxylate (11a)

Commercially available 1-(tert-butyl) 2-methyl (2S,4R)-4-((tert-butyldimethylsilyloxy)pyrrolidine-1,2-dicarboxylate (200 mg, 0.56 mmol, 1.0 eq.) was dissolved in MeOH (1 mL) and an aqueous LiOH (330 μ L, 1M, 1.5 eq.) was added. The solution was stirred at 45 °C for 3h whereby TLC analysis indicated that the reaction had gone to completion. A 5% (w/w) aqueous HCl solution was added dropwise until pH = 2, whereby a white precipitate was formed. The mixture was extracted with Et₂O (2 x 5 mL). The resulting organic layer was collected, dried over Na₂SO₄, filtered and concentrated to afford an incolor oil which was brought to the next step without further purification.

The incolor oil (120 mg, 0.35 mmol) was dissolved back in THF (2 mL) then Et₃N (97 μ L, 0.70 mmol, 2 eq.) and isobutylchloroformate (58 μ L, 0.310 mmol, 1.6 eq.) were added. The resulting mixture was stirred at room temperature for 1 h. Afterwards, the reaction was cooled down to 0 °C and a freshly prepared solution of CH₂N₂ in Et₂O was added dropwise until the resulting mixture remained bright yellow. Then, the reaction was stirred for 1h at 0 °C and for 30 min at room temperature, adding further CH₂N₂ any time the mixture had turned back to colorless. After a persistant yellow color remained, the flask was cooled down again to 0 °C and a 0.5 M solution of acetic acid in water was slowly added until the mixture turned colorless. Then, the layers were separated and the aqueous one was extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with water (5 mL) and brine (5 mL), dried over Na₂SO₄, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane 1:3, Rf: 0.21) to give the diazo

intermediate as a pale yellow oil. This intermediate was redissolved in dry MeOH (2 mL) and a solution of silver benzoate (16 mg, 0.07 mmol, 0.2 eq.) in Et₃N (97 μ L, 0.70 mmol, 2 eq.) was added to this mixture under an argon atmosphere. Then, the flask was wrapped in aluminum foil and the reaction was refluxed for 2 h. Afterwards, the mixture was left to reach room temperature, filtered through a celite pad washing with abundant EtOAc and concentrated. The residue was purified by flash column chromatography (hexane/EtOAc 8:2 Rf: 0.28) to give **11a** as a colorless oil (77 mg, 59% over 2 steps). $[\alpha]_D^{25}$ -74.1 (*c* 0.78, CHCl₃). IR (neat), ν_{\max} : 2929, 1739, 1693, 1472, 1152, 1108, 853, 774 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 4.32-4.28 (p, *J* = 4.3 Hz, 1 H), 4.24-4.16 (m, 1 H), 3.65 (s, 3 H), 3.43-3.33 (m, 2 H), 2.99-2.86 (dd, *J* = 11.1, 4.6 Hz, 1 H), 2.37 (m, 1 H), 2.09 (m, 1 H), 1.84 (m, 1 H), 1.44 (s, 9 H), 0.85 (s, 9 H), 0.04 (s, 6 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 172.0, 171.9, 155.0, 154.9, 79.9, 79.5, 70.2, 69.6, 55.1, 54.7, 53.0, 51.6, 41.2, 40.5, 39.6, 38.9, 28.6, 25.8, 18.1 ppm. HRMS (ESI) calcd. for C₂₅H₄₀NO₃ (M+H)⁺ 374.23680, found 374.23637.

tert-Butyl (2*S*,4*R*)-4-((*tert*-butyldimethylsilyl)oxy)-2-(2-(methoxy(methyl)amino)-2-oxoethyl)pyrrolidine-1-carboxylate (**11b**)

Isopropyl magnesium chloride (240 μ L, 2 M in THF, 0.48 mmol, 6 eq.) was added dropwise to a solution of **11a** (30.0 mg, 0.08 mmol) and *N*,*O*-dimethylhydroxylamine (23 mg, 0.24 mmol, 3 eq.) in dry THF (1 mL) at -20 °C. The resulting mixture was allowed to reach 0 °C over 3 h, then, it was stirred at the same temperature overnight. Afterwards, the reaction was quenched by adding a few drops of water. Then, the mixture was filtered on a celite pad washing with abundant EtOAc and concentrated. The residue was purified by flash column chromatography (hexane/EtOAc 7:3 Rf: 0.35) to give **11b** as a colorless oil (26 mg, 81%). $[\alpha]_D^{25}$ -56.6 (*c* 0.58, CHCl₃). IR (neat), ν_{\max} : 2954, 1692, 1390, 1252, 1156, 835 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 4.34-4.26 (m, 2 H), 3.68 (s, 3 H), 3.43-3.32 (m, 2 H), 3.16-3.02 (m, 4 H), 2.46 (bs, 1 H), 2.12 (bs, 1 H), 1.87 (bs, 1 H), 1.45 (s, 9 H), 0.85 (s, 9 H), 0.04 (s, 6 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 172.7, 172.4, 155.0, 79.7, 79.3, 70.3, 69.7, 61.4, 55.1, 54.5, 53.1, 41.4, 40.6, 37.7, 36.7, 32.1, 32.1, 28.6, 28.5, 25.9, 25.9, 18.1 ppm. HRMS (ESI) calcd. for C₂₅H₄₀NO₃ (M+H)⁺ 403.26230, found 403.26277.

tert-Butyl (2*S*,4*R*)-4-hydroxy-2-(2-(4-octylphenyl)-2-oxoethyl)pyrrolidine-1-carboxylate (**11c**)

11b was submitted to general procedure C (11 mg, 0.02 mmol). The crude yellow oil was submitted to general procedure B without further purification. The resulting residue was purified by flash column chromatography (hexane/EtOAc 8:2 Rf: 0.33) to give **11c** as a yellow oil (5 mg, 63% over 2 steps). $[\alpha]_D^{25}$ +50.4 (*c* 0.25, CHCl₃). IR (neat), ν_{\max} : 2953, 1856, 1783, 1251, 932, 704 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.89 (bs, 2 H), 7.26-7.24 (m, 2 H), 4.44-4.40 (m, 2 H), 3.90-3.87 (m, 0.57 H), 3.67-3.60 (m, 1 H), 3.46 (m, 0.64 H), 2.89-2.84 (dd, *J* = 15.5, 9.6 Hz, 1 H), 2.65-2.62 (m, 2 H), 2.22 (m, 2 H), 1.90 (bs, 1 H), 1.62-1.59 (m, 2 H), 1.45 (s, 9 H), 1.32-1.23 (m, 10 H), 0.88-0.85 (t, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 198.9, 198.2, 171.3, 154.9, 149.3, 149.1, 134.6, 128.8, 128.5, 80.3, 79.7, 69.9, 69.4, 60.5, 54.9, 54.6, 53.3, 43.3, 40.3, 36.1, 32.0, 31.2, 29.5, 29.4, 29.3, 28.6, 22.8, 21.2, 14.3, 14.2 ppm. HRMS (ESI) calcd. for C₂₅H₄₀NO₃ (M+H)⁺ 418.29519, found 418.29710.

(2*S*,4*R*)-4-Hydroxy-2-(2-(4-octylphenyl)-2-oxoethyl)pyrrolidin-1-ium chloride (**11**)

11 was synthesized in accordance with the general procedure A, starting from **11c** (21 mg, 0.05 mmol). **11** was obtained as a white solid (13 mg, 75%). IR (neat), ν_{\max} : 3310, 2921, 1669, 1605, 1277 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of diastereomers), δ : 7.71-7.70 (d, *J* = 8.2 Hz, 2 H), 7.00-6.99 (d, *J* = 8.0 Hz, 2 H), 4.51 (s, 1 H), 4.07-4.04 (dd, *J* = 11.3, 6.4 Hz, 1 H major isomer), 3.99-3.96 (dd, *J* = 9.0, 6.2 Hz, 1 H minor isomer), 3.41-3.24 (m, 2 H), 2.37-2.05 (m, 3 H), 1.77-1.63 (m, 1 H), 1.36 (m, 2 H), 1.18-1.14 (m, 10 H), 0.78-0.76 (t, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of diastereomers), δ : 198.5, 149.1, 133.5, 128.5, 128.5, 69.1, 69.1, 54.3, 54.0, 52.8, 38.5, 37.7, 35.6, 31.8, 30.8, 29.4, 29.4, 29.3, 22.6, 13.8 ppm. HRMS (ESI)

calcd. for $C_{25}H_{40}NO_3$ (M+H)⁺ 318.24276, found 318.24284.

1-(tert-butyl) 2-ethyl (2R,4R)-4-(((tert-butyl)diphenylsilyl)oxy)methyl)-5-oxopyrrolidine-1,2-dicarboxylate (12a3) and 1-(tert-butyl) 2-ethyl (2R,4S)-4-(((tert-butyl)diphenylsilyl)oxy)methyl)-5-oxopyrrolidine-1,2-dicarboxylate (epi-12a3) (See Supp. Info for a Scheme)

HCl (84 mL, 0.2 N in H₂O, 16.8 mmol, 1 eq.) was added dropwise to a solution of **12a1** (5.25 g, 16.8 mmol) in MeOH (125 mL) and the resulting solution was stirred at room temperature for 1 h. Afterwards, a small amount of bromocresol green was added to monitor the pH of the solution and NaBH₃CN (2.11 g, 33.6 mmol, 2 eq.) was added portion wise (4 portions over 2 h). Meanwhile, the pH of the solution had been corrected by adding a few drops of HCl (0.2 N in H₂O), anytime the pH indicator had turned blue. HCl has always been added in the minimal amount necessary to make the indicator turn back to yellow. The reaction was stirred at room temperature for 48 hours, continuing monitoring and correcting the pH when needed. Eventually, a few drops of NaHCO₃ satd. solution were added until the indicator turned blue and the mixture was concentrated *in vacuo* to remove the organic solvent. Brine (130 mL) was added to the mixture and the product was extracted in EtOAc (3 x 130 mL). The organic layers were dried over Na₂SO₄, filtered and concentrated. The resulting residue was purified by flash column chromatography (MeOH/CH₂Cl₂ 1:20, R_f: 0.24) to give the intermediate alcohol **12a2** (3.38 g, 70%) as a 2:1 mixture of diastereoisomers. This intermediate was redissolved in dry DMF (60 mL) and imidazole (2.41 g, 35.4 mmol, 3 eq.) was added to the resulting solution. Then, this mixture was cooled down to 0 °C and TBDPSCI (4.60 mL, 17.7 mmol, 1.5 eq.) was added dropwise. The resulting solution was stirred at room temperature for 3 h, before adding EtOH (1 mL) and stirring for additional 30 min. Eventually, the mixture was poured into water (400 mL) and the product was extracted into Et₂O (3 x 300 mL). The organic layers were dried over Na₂SO₄, filtered and concentrated. The resulting residue was purified by flash column chromatography (Et₂O/hexane 1:2 R_f: 0.13 and 0.06, then Et₂O/hexane 1:1) to give **12a3** (2.98 g, 48%) and **epi-12a3** (1.12 g, 18%) as colorless oils. The stereochemistry was assigned by performing NOESY experiments (through space coupling observed between 2-H and 4-H in **12a3**, **epi-12a3**) **12a3**: [α]_D²⁵ +28.3 (c 2.7, CHCl₃). IR (neat), ν_{max} : 2931, 1792, 1746, 1718, 1472, 1428, 1369, 1313, 1278, 1188, 1151, 1111, 1007, 966, 913, 848, 822, 734, 702, 613, 504 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz), δ : 7.66-7.62 (m, 4 H), 7.43-7.37 (m, 6 H), 4.62 (dd, *J* = 9.7, 3.2 Hz, 1 H), 4.24 (q, *J* = 7.1 Hz, 2 H), 4.03 (dd, *J* = 10.2, 4.8 Hz, 1 H), 3.80 (dd, *J* = 10.2, 3.4 Hz, 1 H), 2.82-2.77 (m, 1 H), 2.46-2.40 (m, 1H), 2.17-2.12 (m, 1 H), 1.50 (s, 9 H), 1.30 (t, *J* = 7.1 Hz, 3 H), 1.03 (s, 9 H) ppm. ¹³C NMR (CDCl₃, 125 MHz), δ : 173.3, 171.6, 149.3, 135.7, 135.5, 133.2, 132.6, 129.8, 127.7, 83.4, 62.9, 61.6, 57.6, 44.5, 27.8, 26.8, 25.2, 19.2, 14.1 ppm. HRMS (ESI) calcd. for C₂₉H₃₉NO₆SiNa (M+Na)⁺ 548.24389, found 548.24492. **epi-12a3**: [α]_D²⁵ +7.7 (c 3.6, CHCl₃). IR (neat), ν_{max} : 2931, 1791, 1746, 1718, 1473, 1428, 1369, 1318, 1151, 1109, 1032, 970, 909, 822, 781, 734, 702, 613, 504 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz), δ : 7.66-7.61 (m, 4 H), 7.44-7.35 (m, 6 H), 4.50 (dd, *J* = 8.9, 7.5 Hz, 1 H), 4.22-4.13 (m, 2 H), 3.93 (dd, *J* = 10.3, 6.7 Hz, 1 H), 3.87 (dd, *J* = 10.3, 4.1 Hz, 1 H), 2.79 (dddd, *J* = 9.5, 8.7, 6.7, 4.1 Hz, 1 H), 2.52-2.45 (m, 1H), 2.16 (ddd, *J* = 13.2, 8.7, 7.5 Hz, 1 H), 1.49 (s, 9 H), 1.25 (t, *J* = 7.1 Hz, 3 H), 1.04 (s, 9 H) ppm. ¹³C NMR (CDCl₃, 125 MHz), δ : 172.7, 171.3, 149.2, 135.6, 135.5, 133.2, 132.9, 129.7, 127.7, 83.5, 62.3, 61.5, 57.6, 45.4, 27.8, 26.7, 24.3, 19.2, 14.0 ppm. HRMS (ESI) calcd. for C₂₉H₃₉NO₆SiNa (M+Na)⁺ 548.24389, found 548.24498.

1-(tert-Butyl) 2-ethyl (2R,4S)-4-(((tert-butyl)diphenylsilyl)oxy)methyl)pyrrolidine-1,2-dicarboxylate (13a) (See Supp. Info for a Scheme)

LiEt₃BH (4.68 mL, 1 M in THF, 4.68 mmol, 1.2 eq.) was added dropwise to a solution of **12a3** (2.05 g, 3.90 mmol) in anhydrous THF (70 mL) at -78 °C under an argon atmosphere and the resulting solution was stirred at the same temperature for 30 min. Afterwards, the reaction was quenched with NaHCO₃ satd. sol. (20 mL) and allowed to reach 0 °C, then a few drops of H₂O₂ 30% were added and the mixture was stirred at 0 °C for 20 min. Eventually, the organic solvent was removed under *vacuo* and the remaining aqueous layer was extracted with CH₂Cl₂ (3 x 120 mL). The combined organic layers were dried over Na₂SO₄, filtered and

concentrated, affording a colorless oil. This intermediate hemiaminal was redissolved in anhydrous CH_2Cl_2 (70 mL) and Et_3SiH (1.25 mL, 7.8 mmol, 2 eq.) was added to the solution under an argon atmosphere. The resulting mixture was cooled down to $-78\text{ }^\circ\text{C}$ and BF_3OEt_2 (481 μL , 3.90 mmol, 1 eq.) was added dropwise. The reaction was stirred at $-78\text{ }^\circ\text{C}$ for 30 min, before adding NaHCO_3 satd. sol. (20 mL) and allowing the mixture to reach room temperature. The product was extracted with CH_2Cl_2 (3 x 120 mL) and the organic extracts were dried over Na_2SO_4 , filtered and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane 1:6, Rf: 0.24) to give **13a** as a colorless oil (1.52 g, 76% over two steps). $[\alpha]_{\text{D}}^{25} +24.7$ (c 1.5, CHCl_3). IR (neat), ν_{max} : 2931, 2858, 1744, 1699, 1473, 1427, 1389, 1365, 1257, 1188, 1110, 1030, 939, 870, 823, 741, 702, 611, 505 cm^{-1} . ^1H NMR (CDCl_3 , 500 MHz, mixture of rotamers), δ : 7.65–7.63 (m, 4 H), 7.44–7.37 (m, 6 H), 4.34 (dd, $J = 7.9, 4.1$ Hz, 0.4 H), 4.24–4.14 (m, 2.6 H), 3.73 (dd, $J = 10.6, 7.7$ Hz, 0.6 H), 3.64–3.59 (m, 2.4 H), 3.29 (dd, $J = 10.6, 7.4$ Hz, 0.6 H), 3.23, (dd, $J = 10.5, 7.4$ Hz, 0.4 H), 2.60–2.51 (m, 1 H), 2.13–2.04 (m, 1H), 2.03–1.98 (m, 1 H), 1.47 (s, 3.6 H), 1.42 (s, 5.4 H), 1.30–1.25 (m, 3 H), 1.06 (s, 3.6 H), 1.05 (s, 5.4 H) ppm. ^{13}C NMR (CDCl_3 , 125 MHz, mixture of rotamers), δ : 173.2, 172.9, 154.4, 153.7, 135.5, 133.4, 133.3, 129.7, 127.7, 79.8, 79.7, 64.8, 60.9, 60.8, 59.0, 58.7, 48.9, 48.8, 39.8, 38.9, 33.0, 32.2, 28.4, 28.3, 26.8, 19.2, 14.3, 14.1 ppm. HRMS (ESI) calcd. for $\text{C}_{29}\text{H}_{42}\text{NO}_5\text{Si}$ ($\text{M}+\text{H}$) $^+$ 512.28268, found 512.28059.

(2*R*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(((*tert*-butyldiphenylsilyl)oxy)methyl)pyrrolidine-2-carboxylic acid (**12a4**) (See Supp. Info for a Scheme)

NaOH (290 μL , 1 N in H_2O , 0.29 mmol, 1.5 eq.) was added to a solution of **13a** (99 mg, 0.19 mmol) in MeOH (1.2 mL) and the resulting mixture was vigorously stirred for 24 h. Afterwards, the organic solvent was concentrated *in vacuo* and the residue was suspended in brine (20 mL). Afterwards, while gradually acidifying to pH=2 by adding HCl 0.2 N, the product was extracted with CH_2Cl_2 (6 x 20 mL). The organic layers were dried over Na_2SO_4 , filtered and concentrated, affording **12a4** (93 mg, 99%) as a colorless solid. $[\alpha]_{\text{D}}^{25} +19.3$ (c 0.9, MeOH). IR (neat), ν_{max} : 2929, 1699, 1390, 1366, 1162, 1108, 998, 906, 823, 739, 700, 608, 503 cm^{-1} . ^1H NMR (CD_3OD , 500 MHz, mixture of rotamers), δ : 7.74–7.66 (m, 4 H), 7.47–7.37 (m, 6 H), 4.30–4.21 (m, 1 H), 3.66–3.57 (m, 3 H), 3.37–3.33 (m, 1 H), 2.57–2.54 (m, 1 H), 2.17–2.11 (m, 1H), 2.06–2.03 (m, 1 H), 1.48 (s, 3.6 H), 1.44 (s, 5.4 H), 1.06 (s, 9 H) ppm. ^{13}C NMR (CD_3OD , 125 MHz, mixture of rotamers), δ : 175.9, 154.9, 154.6, 135.9, 135.3, 134.6, 133.1, 129.6, 129.5, 129.0, 127.5, 127.2, 80.0, 79.8, 64.8, 64.6, 59.5, 48.9, 48.6, 39.7, 38.9, 32.8, 32.1, 27.4, 27.2, 26.0, 25.8, 18.7, 18.5 ppm. HRMS (ESI) calcd. for $\text{C}_{27}\text{H}_{38}\text{NO}_5\text{SiNa}$ ($\text{M}+\text{Na}$) $^+$ 506.23332, found 506.23394.

tert-Butyl (2*R*,4*S*)-4-(((*tert*-butyldiphenylsilyl)oxy)methyl)-2-(2-methoxy-2-oxoethyl)pyrrolidine-1-carboxylate (**12a**) (See Supp. Info for a Scheme)

Et_3N (54 μL , 0.388 mmol, 2 eq.) and isobutylchloroformate (40 μL , 0.310 mmol, 1.6 eq.) were added to a solution of **12a4** (94 mg, 0.194 mmol) in anhydrous THF (2 mL) at $0\text{ }^\circ\text{C}$ and the resulting mixture was stirred at room temperature for 1 h. Afterwards, the reaction was cooled down to $0\text{ }^\circ\text{C}$ and a freshly prepared solution of CH_2N_2 in Et₂O was added dropwise until the resulting mixture remained bright yellow. Then, the reaction was stirred for 1h at $0\text{ }^\circ\text{C}$ and for 30 min at room temperature, adding further CH_2N_2 any time the mixture had turned back to colorless. After a persistent yellow color remained, the flask was cooled down again to $0\text{ }^\circ\text{C}$ and a 0.5 M solution of acetic acid in water was slowly added until the mixture turned colorless. Then, the layers were separated and the aqueous one was extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with water (5 mL) and brine (5 mL), dried over Na_2SO_4 , filtered and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane 1:3, Rf: 0.21) to give the diazo intermediate as a pale yellow oil. This intermediate was redissolved in dry MeOH (2 mL) and a solution of silver benzoate (9 mg, 0.0388 mmol, 0.2 eq.) in Et₃N (54 μL , 0.388 mmol, 2 eq.) was added to this mixture under an argon atmosphere. Then, the flask was wrapped in aluminum foil and the reaction was refluxed for 2 h. Afterwards, the mixture was left to reach room temperature, filtered through a celite pad washing with

abundant EtOAc and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane 1:4, Rf: 0.27) to give **12a** as a colorless oil (54 mg, 55% over two steps). $[\alpha]_D^{25} +21.2$ (c 1.0, CHCl₃). IR (neat), ν_{\max} : 2931, 2858, 1737, 1692, 1428, 1388, 1365, 1253, 1161, 1109, 823, 740, 702, 611, 504 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.64-7.62 (m, 4 H), 7.44-7.37 (m, 6 H), 4.21 (br. s, 0.5 H), 4.13 (br. s, 0.5 H), 3.67 (s, 3 H), 3.63-3.60 (m, 1 H), 3.58-3.51 (m, 1.5 H), 3.43 (br. s, 0.5 H), 3.25-3.16 (m, 1 H), 2.91, (d, $J = 14.2$ Hz, 0.5 H), 2.80 (d, $J = 14.6$ Hz, 0.5 H), 2.50-2.44 (m, 1 H), 2.33 (dd, $J = 14.6, 9.7$ Hz, 1H), 1.86 (br. s, 1 H), 1.80-1.77 (m, 1 H), 1.46 (s, 9 H), 1.05 (s, 9 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 171.9, 154.3, 154.1, 135.5, 133.5, 129.7, 127.9, 127.7, 79.6, 79.3, 65.3, 54.0, 51.6, 49.1, 39.3, 39.2, 38.7, 38.4, 33.7, 33.1, 28.5, 26.8, 19.2 ppm. HRMS (ESI) calcd. for C₂₉H₄₂NO₅Si (M+H)⁺ 512.28268, found 512.28235.

tert-Butyl (2*R*,4*S*)-4-(((*tert*-butyldiphenylsilyl)oxy)methyl)-2-(2-(methoxy(methyl)amino)-2-oxoethyl)pyrrolidine-1-carboxylate (**12b**)

12a (53 mg, 0.104 mmol) was submitted to the procedure from **11b**. The residue was purified by flash column chromatography (EtOAc/hexane 1:1, Rf: 0.30) to give **12b** as a colorless oil (47 mg, 84%). $[\alpha]_D^{25} +23.3$ (c 0.6, CHCl₃). IR (neat), ν_{\max} : 2931, 2857, 1690, 1385, 1364, 1256, 1162, 1109, 1000, 906, 870, 823, 739, 702, 611, 504 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.65-7.62 (m, 4 H), 7.44-7.36 (m, 6 H), 4.24 (br. s, 1 H), 3.68 (s, 3 H), 3.65-3.62 (m, 1 H), 3.56 (br. s, 1.5 H), 3.45 (br. s, 0.5 H), 3.24 (br. s, 1 H), 3.17 (s, 3 H), 3.00, (d, $J = 14.7$ Hz, 0.5 H), 2.88 (d, $J = 13.6$ Hz, 0.5 H), 2.52-2.45 (m, 2 H), 1.89-1.81 (m, 2 H), 1.46 (s, 9 H), 1.04 (s, 9 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 172.3, 154.2, 135.6, 135.5, 133.6, 129.7, 127.7, 79.5, 79.1, 65.4, 61.2, 54.0, 49.1, 39.2, 38.4, 36.8, 36.4, 33.8, 32.0, 28.5, 26.8, 19.2 ppm. HRMS (ESI) calcd. for C₃₀H₄₅N₂O₅Si (M+H)⁺ 541.30923, found 541.30687.

tert-Butyl (2*R*,4*S*)-4-(((*tert*-butyldiphenylsilyl)oxy)methyl)-2-(2-(4-octylphenyl)-2-oxoethyl)pyrrolidine-1-carboxylate (**12c**)

Prepared according to general procedure C, starting from **12b** (30 mg, 0.055 mmol). The crude was purified by flash column chromatography (EtOAc/hexane 1:10, Rf: 0.17) to give **12c** as a colorless oil (25 mg, 68%). $[\alpha]_D^{25} +5.3$ (c 0.3, CHCl₃). IR (neat), ν_{\max} : 2924, 2853, 1671, 1606, 1515, 1458, 1390, 1366, 1175, 1111, 823, 739, 701, 611, 504 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.95 (d, $J = 7.3$ Hz, 1 H), 7.90 (d, $J = 6.8$ Hz, 1 H), 7.64-7.61 (m, 4 H), 7.43-7.35 (m, 6 H), 7.27-7.26 (m, 2 H), 4.38-4.34 (m, 1 H), 3.72 (d, $J = 14.8$ Hz, 0.5 H), 3.61 (br. s, 1 H), 3.58-3.53 (m, 1.5 H), 3.50-3.43 (m, 1 H), 3.29-3.25 (m, 0.5 H), 3.22-3.19 (m, 0.5 H), 2.88-2.78 (m, 1 H), 2.65 (br. s, 2 H), 2.54-2.48 (m, 1 H), 1.87-1.75 (m, 2 H), 1.65-1.59 (m, 2 H), 1.47 (s, 4.5 H), 1.45 (s, 4.5 H), 1.31-1.26 (m, 10 H), 1.03 (s, 9 H), 0.88 (t, $J = 6.9$ Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 198.7, 198.3, 154.4, 154.2, 134.6, 133.5, 129.7, 128.7, 128.5, 128.4, 127.7, 79.7, 79.3, 65.4, 65.2, 54.4, 49.2, 43.9, 43.2, 39.2, 38.4, 36.0, 33.7, 32.7, 31.8, 31.1, 29.7, 29.4, 29.3, 29.2, 28.5, 26.8, 22.6, 19.2, 14.1 ppm. HRMS (ESI) calcd. for C₄₂H₆₀NO₄Si (M+H)⁺ 670.42861, found 670.42981.

2-(2*R*,4*S*)-4-(Hydroxymethyl)pyrrolidin-2-yl)-1-(4-octylphenyl)ethan-1-one hydrochloride (**12**)

Prepared according to general procedure B, starting from **12c** (14 mg, 0.021 mmol). The crude was purified by flash column chromatography (EtOAc/hexane 1:1, Rf: 0.28) to give the intermediate *tert*-Butyl (2*R*,4*S*)-4-(hydroxymethyl)-2-(2-(4-octylphenyl)-2-oxoethyl)pyrrolidine-1-carboxylate as a colorless oil (9 mg, 99%). $[\alpha]_D^{25} +7.5$ (c 0.4, CHCl₃). IR (neat), ν_{\max} : 3439, 2924, 2854, 1673, 1606, 1394, 1366, 1255, 1173, 1123, 772, 558 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.94 (d, $J = 7.7$ Hz, 1 H), 7.89 (d, $J = 7.9$ Hz, 1 H), 7.27-7.24 (m, 2 H), 4.42-4.37 (m, 1 H), 3.72 (d, $J = 15.5$ Hz, 0.5 H), 3.63 (br. s, 1.5 H), 3.58-3.49 (m, 1.5 H), 3.47 (br. s, 0.5 H), 3.26-3.23 (m, 0.5 H), 3.16-3.13 (m, 0.5 H), 2.91-2.80 (m, 1 H), 2.65 (br. s, 2 H), 2.55-2.46 (m, 1 H), 1.89-1.80 (m, 2 H), 1.64-1.59 (m, 2 H), 1.46-1.40 (m, 9 H), 1.31-1.25 (m, 10 H), 0.88 (t, $J = 7.0$ Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 198.5, 198.0, 154.2, 148.8, 149.0,

134.5, 128.5, 128.4, 127.7, 79.7, 79.5, 65.2, 54.4, 49.2, 49.0, 43.9, 43.2, 39.2, 38.4, 36.0, 33.7, 32.7, 31.8, 31.1, 29.7, 29.4, 29.3, 29.2, 28.5, 26.8, 22.6, 14.1 ppm. HRMS (ESI) calcd. for $C_{26}H_{42}NO_4$ (M+H)⁺ 432.31084, found 432.30903.

tert-Butyl (2*R*,4*S*)-4-(hydroxymethyl)-2-(2-(4-octylphenyl)-2-oxoethyl)pyrrolidine-1-carboxylate (8 mg, 0.019 mmol) was submitted to general procedure A. The crude was purified by flash column chromatography (EtOH/CH₂Cl₂ 1:4, R_f: 0.15) to give product **12** as a white solid (6 mg, 88%). Note: the product epimerized spontaneously on C-2 when dissolved in MeOH or H₂O, giving a 1:1 mixture of diastereoisomers. For biological testing a portion of the product was dissolved in the minimum amount of HPLC grade water, filtered (pore size = 0.45 μm) and lyophilized. IR (neat), ν_{\max} : 3376, 2922, 2852, 1675, 1605, 1570, 1465, 1378, 1282, 1184, 1039, 906, 815, 722, 549 cm⁻¹. ¹H NMR (CD₃OD, 500 MHz, 1:1 mixture of diastereoisomers), δ : 7.98 (d, *J* = 8.3 Hz, 4 H), 7.38 (d, *J* = 8.3 Hz, 4 H), 4.18–4.12 (m, 1 H), 4.09–4.04 (m, 1 H), 3.76–3.65 (m, 4 H, partially deuterated), 3.63–3.58 (m, 2 H), 3.47–3.39 (m, 4 H, partially deuterated), 3.20–3.13 (m, 2 H), 2.74–2.71 (m, 4 H), 2.67–2.58 (m, 2 H), 2.44–2.38 (m, 1 H), 2.22–2.17 (m, 1 H), 1.99 (dt, *J* = 13.5, 8.4 Hz, 1 H), 1.70–1.62 (m, 5 H), 1.36–1.30 (m, 20 H), 0.91 (t, *J* = 7.0 Hz, 6 H) ppm. ¹³C NMR (CD₃OD, 125 MHz, 1:1 mixture of diastereoisomers), δ : 197.1, 149.7, 133.6, 128.6, 128.0, 62.4, 61.9, 56.2, 55.3, 39.6, 39.0, 35.5, 32.9, 32.6, 31.6, 30.9, 29.1, 29.0, 28.9, 22.3, 13.0 ppm. HRMS (ESI) calcd. for $C_{21}H_{34}NO_2$ (M)⁺ 332.25841, found 332.25898.

tert-Butyl (2*S*,4*S*)-4-(((tert-butyl)diphenylsilyl)oxy)methyl)-2-(3-methoxy-3-oxopropyl)pyrrolidine-1-carboxylate (**13b**)

DIBAL-H (760 μL, 1 M in CH₂Cl₂, 0.76 mmol, 2 eq.) was added dropwise to a solution of **13a** (194 mg, 0.38 mmol) in dry CH₂Cl₂ (3.5 mL) at -78 °C. The resulting mixture was stirred at the same temperature for 2 hours, before quenching the reaction with MeOH (100 μL). Then, the solution was allowed to reach room temperature and a 2 M solution of potassium sodium tartrate in water (3.5 mL) was added. The resulting mixture was vigorously stirred at room temperature for 30 min, before separating the layers. The aqueous one was extracted with CH₂Cl₂ (3 x 7 mL) and the combined organic layers were dried over MgSO₄, filtered and concentrated, affording a colorless oil. This intermediate aldehyde was redissolved in dry CH₂Cl₂ (3.5 mL) and methyl(triphenylphosphoranylidene)acetate (191 mg, 0.57 mmol, 1.5 eq.) was added at 0 °C. The resulting solution was stirred for 1 h at 0 °C and for 1 h at room temperature, before being cooled down again to 0 °C and quenched with NH₄Cl satd. sol. (3.5 mL). The layers were separated and the aqueous one was extracted with CH₂Cl₂ (3 x 7 mL). The combined organic layers were washed with water (7 mL) and brine (7 mL), dried over Na₂SO₄, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane 1:4, R_f: 0.30) to give the intermediate tert-Butyl (2*R*,4*S*)-4-(((tert-butyl)diphenylsilyl)oxy)methyl)-2-((E)-3-methoxy-3-oxoprop-1-en-1-yl)pyrrolidine-1-carboxylate a colorless oil (143 mg, 72%). $[\alpha]_D^{25} +36.4$ (c 1.1, CHCl₃). IR (neat), ν_{\max} : 2931, 2858, 1725, 1693, 1428, 1387, 1364, 1265, 1162, 1107, 978, 861, 823, 740, 701, 611, 503 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.65–7.62 (m, 4 H), 7.43–7.37 (m, 6 H), 6.87–6.97 (m, 1 H), 5.83 (t, *J* = 14.5 Hz, 1 H), 4.53 (br. s, 0.4 H), 4.37 (br. s, 0.6 H), 3.75 (s, 1.8 H), 3.73 (s, 1.2 H), 3.61 (d, *J* = 6.3 Hz, 2 H), 3.60–3.57 (m, 0.6 H), 3.50–3.46 (m, 0.4 H), 3.27 (t, *J* = 9.4 Hz, 0.6 H), 3.21–3.18 (m, 0.4 H), 2.50–2.41 (m, 1 H), 1.97–1.86 (m, 1 H), 1.80 (dd, *J* = 6.4, 2.5 Hz, 0.6 H), 1.78 (dd, *J* = 6.4, 2.5 Hz, 0.4 H), 1.46 (s, 5.4 H), 1.41 (s, 3.6 H), 1.05 (s, 9 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 166.8, 154.2, 154.1, 148.7, 148.4, 135.5, 133.3, 129.7, 127.9, 127.7, 120.0, 79.7, 65.0, 64.9, 57.7, 57.4, 51.6, 49.0, 48.9, 39.3, 38.4, 34.1, 33.4, 28.4, 28.2, 26.8, 19.2 ppm. HRMS (ESI) calcd. for $C_{30}H_{42}NO_5Si$ (M+H)⁺ 524.28270, found 524.28136.

tert-Butyl (2*R*,4*S*)-4-(((tert-butyl)diphenylsilyl)oxy)methyl)-2-((E)-3-methoxy-3-oxoprop-1-en-1-yl)pyrrolidine-1-carboxylate (122 mg, 0.23 mmol) was dissolved in MeOH (9 mL) and Pd/C (10%, 29 mg) was added to the resulting solution. The air was removed from the flask under vacuum and replaced with hydrogen (balloon). The reaction was vigorously stirred overnight at room temperature. Afterwards, the

mixture was filtered through a celite pad, washing with MeOH. The collected solution was concentrated *in vacuo*, affording **13b** as a colorless oil (122 mg, 99%). $[\alpha]_{\text{D}}^{20} +20.9$ (c 0.9, CHCl₃). IR (neat), ν_{max} : 2931, 2857, 1737, 1690, 1427, 1388, 1364, 1254, 1169, 1109, 823, 739, 701, 610, 504 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.70-7.63 (m, 4 H), 7.44-7.37 (m, 6 H), 3.88 (br. s, 0.5 H), 3.80 (br. s, 0.5 H), 3.67 (s, 3 H), 3.58 (d, $J = 6.2$ Hz, 2 H), 3.49-3.43 (m, 0.5 H), 3.40-3.35 (m, 0.5 H), 3.29-3.24 (m, 0.5 H), 3.22-3.17 (m, 0.5 H), 2.54-2.48 (m, 1 H), 2.32 (br. s, 2 H), 2.04-1.92 (m, 1 H), 1.75-1.66 (m, 3 H), 1.46 (s, 9 H), 1.05 (s, 9 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 173.9, 173.7, 154.7, 154.5, 135.5, 133.5, 129.7, 127.7, 79.4, 79.1, 65.6, 65.4, 56.5, 51.5, 48.9, 39.5, 38.7, 33.4, 32.8, 31.1, 30.2, 30.0, 28.5, 26.8, 19.2 ppm. HRMS (ESI) calcd. for C₃₀H₄₄NO₅Si (M+H)⁺ 526.2983, found 526.2993.

tert-Butyl (2S,4S)-4-(hydroxymethyl)-2-(3-(4-octylphenyl)-3-oxopropyl)pyrrolidine-1-carboxylate (13c)

13b (122 mg, 0.23 mmol) was submitted to the procedure reported for **12b**. The crude was purified by flash column chromatography (EtOAc/hexane 1:1, R_f: 0.28) to give the intermediate *tert-Butyl (2S,4S)-4-(((tert-butyl)diphenylsilyloxy)methyl)-2-(3-(methoxy(methyl)amino)-3-oxopropyl)pyrrolidine-1-carboxylate* as a colorless oil (111 mg, 87%). $[\alpha]_{\text{D}}^{25} +20.2$ (c 1.1, CHCl₃). IR (neat), ν_{max} : 2930, 2857, 1688, 1386, 1364, 1254, 1175, 1109, 997, 823, 740, 702, 611, 504 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.65-7.63 (m, 4 H), 7.44-7.36 (m, 6 H), 3.92 (br. s, 0.5 H), 3.83 (br. s, 0.5 H), 3.68 (s, 3 H), 3.59 (d, $J = 6.1$ Hz, 2 H), 3.51-3.45 (m, 0.5 H), 3.43-3.37 (m, 0.5 H), 3.30-3.25 (m, 0.5 H), 3.17 (br. s, 3.5 H), 2.57-2.51 (m, 1 H), 2.43 (br. s, 2 H), 1.94 (br. s, 1 H), 1.71 (br. s, 3 H), 1.45 (s, 9 H), 1.04 (s, 9 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 174.4, 174.2, 154.7, 135.5, 133.5, 129.6, 127.7, 79.2, 78.9, 65.7, 65.5, 61.1, 56.8, 48.8, 39.5, 38.7, 33.6, 33.0, 32.2, 30.0, 29.8, 29.7, 29.2, 28.5, 26.8, 19.2 ppm. HRMS (ESI) calcd. for C₃₁H₄₇N₂O₅Si (M+H)⁺ 555.3249, found 555.3268.

tert-Butyl (2S,4S)-4-(((tert-butyl)diphenylsilyloxy)methyl)-2-(3-(methoxy(methyl)amino)-3-oxopropyl)pyrrolidine-1-carboxylate (34 mg, 0.061 mmol) was submitted in sequence to general procedures C and B. The crude was purified by flash column chromatography (EtOAc/hexane 1:1, R_f: 0.20) to give **13c** as a colorless oil (12 mg, 44% over two steps). $[\alpha]_{\text{D}}^{25} +6.3$ (c 0.6, CHCl₃). IR (neat), ν_{max} : 3437, 2923, 2854, 1678, 1605, 1391, 1364, 1253, 1174, 1122, 770, 567 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.87 (d, $J = 8.3$ Hz, 2 H), 7.26-7.24 (m, 2 H), 4.01 (br. s, 0.5 H), 3.95 (br. s, 0.5 H), 3.64-3.60 (m, 2 H), 3.47-3.44 (m, 1 H), 3.31-3.26 (m, 0.5 H), 3.18-3.12 (m, 0.5 H), 3.09-3.02 (m, 0.5 H), 2.96 (br. s, 1.5 H), 2.64 (t, $J = 7.6$ Hz, 2 H), 2.54 (br. s, 1 H), 2.04 (br. s, 1 H), 1.88-1.74 (m, 3 H), 1.64-1.54 (m, 2 H), 1.41 (s, 9 H), 1.30-1.25 (m, 10 H), 0.87 (t, $J = 7.0$ Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 199.8, 199.2, 154.8, 148.8, 148.6, 134.6, 128.6, 128.2, 79.5, 79.2, 64.8, 56.8, 49.0, 48.4, 39.6, 38.8, 36.0, 35.7, 35.3, 33.8, 33.2, 31.8, 31.1, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 28.5, 27.5, 22.6, 14.1 ppm. HRMS (ESI) calcd. for C₂₇H₄₄NO₄ (M+H)⁺ 446.32650, found 446.32667.

3-((2S,4S)-4-(Hydroxymethyl)pyrrolidin-2-yl)-1-(4-octylphenyl)propan-1-one hydrochloride (13) and (2S)-2-(hydroxymethyl)-5-(4-octylphenyl)-1,2,3,6,7,7a-hexahydropyrrolizin-4-ium chloride (13d)

Prepared according to general procedure A, starting from **13c** (6 mg, 0.013 mmol). The crude was triturated and washed with Et₂O, affording **13** as a white solid (4 mg, 80%). Note: in CD₃OD the product was slowly but completely converted into the bicyclic salt **13d**. On the other hand, in D₂O the two species resulted in equilibrium, giving a mixture with a 2:1 constant ratio in favor of the open compound **13**. For biological testing a portion of the product was dissolved in the minimum amount of HPLC grade water, filtered (pore size = 0.45 μm) and lyophilized. **13**: ¹H NMR (CD₃OD, 500 MHz), δ : 7.94 (d, $J = 8.3$ Hz, 2 H), 7.33 (d, $J = 8.3$ Hz, 2 H), 3.75-3.67 (m, 1 H), 3.63-3.53 (m, 2 H), 3.50 (dd, $J = 11.8, 8.2$ Hz, 1 H), 3.23 (dt, $J = 13.9, 6.9$ Hz, 2 H, partially deuterated), 3.12 (dd, $J = 11.8, 6.8$ Hz, 1 H), 2.71-2.67 (m, 2 H), 2.66-2.60 (m, 1 H), 2.21-2.01 (m, 3 H), 1.91 (dt, $J = 13.5, 9.1$ Hz, 1 H), 1.68-1.61 (m, 2 H), 1.33-1.29 (m, 10 H), 0.89 (t, $J = 6.9$ Hz, 3 H) ppm. **13d**: IR (neat), ν_{max} : 3410, 2923, 2853, 1645, 1605, 1456, 1417, 1373, 1296, 1190, 1045, 811, 566

cm⁻¹. ¹H NMR (CD₃OD, 500 MHz), δ: 7.93 (d, *J* = 8.4 Hz, 2 H), 7.56 (d, *J* = 8.4 Hz, 2 H), 5.09–5.00 (m, 1 H), 4.23–4.17 (m, 1 H), 4.14–4.06 (m, 2 H, partially deuterated), 3.71 (dd, *J* = 6.0, 1.3 Hz, 2 H), 3.68–3.61 (m, 1 H, partially deuterated), 3.02–2.95 (m, 1 H), 2.80–2.76 (m, 2 H), 2.60 (dt, *J* = 12.7, 7.6 Hz, 1 H), 2.28 (ddd, *J* = 12.8, 7.4, 2.0 Hz, 1 H), 2.13–2.02 (m, 1 H), 1.98–1.90 (m, 1 H), 1.72–1.65 (m, 2 H), 1.35–1.29 (m, 10 H), 0.89 (t, *J* = 6.9 Hz, 3 H) ppm. ¹³C NMR (CD₃OD, 125 MHz), δ: 178.8, 152.6, 131.2, 129.5, 123.6, 75.7, 63.2, 51.4, 43.4, 41.1, 35.6, 31.6, 31.1, 30.7, 29.1, 29.0, 28.9, 26.6, 22.3, 13.0 ppm.

((2S,5S)-5-(4-Octylphenyl)hexahydro-1H-pyrrolizin-2-yl)methanol hydrochloride (14)

NaBH₄ (0.5 mg, 0.012 mmol, 1.5 eq.) was added to a solution of **13d** (3 mg, 0.008 mmol) in MeOH (300 μL) at 0 °C. The resulting mixture was stirred for 1 hour at the same temperature. Afterwards, the reaction was quenched with HCl 1 N (20 μL) and concentrated. The crude was purified by flash column chromatography (MeOH/CH₂Cl₂ 1:4, R_f: 0.58) to give product **14** as a white solid (3 mg, 99%, dr. 10:1). The stereochemistry was assigned by performing NOESY experiments (through space coupling observed between 2-H and 5-H in the major diastereoisomer). For biological testing the product was dissolved in the minimum amount of HPLC grade water, filtered (pore size = 0.45 μm) and lyophilized. [α]_D²⁵ -58.7 (*c* 0.15, MeOH). IR (neat), ν_{max}: 3417, 2923, 2853, 1518, 1456, 1089, 1037, 833, 535 cm⁻¹. ¹H NMR (CD₃OD, 500 MHz), δ: 7.49 (d, *J* = 8.1 Hz, 2 H), 7.32 (d, *J* = 8.1 Hz, 2 H), 4.52–4.46 (m, 1 H), 4.42 (dd, *J* = 10.9, 6.7 Hz, 1 H), 3.69 (dd, *J* = 11.1, 5.2 Hz, 1 H), 3.62 (dd, *J* = 11.1, 5.9 Hz, 1 H), 3.45 (dd, *J* = 11.8, 6.6 Hz, 1 H), 3.09 (dd, *J* = 11.8, 10.5 Hz, 1 H), 2.92–2.83 (m, 1 H), 2.67–2.64 (m, 2 H), 2.54–2.51 (m, 1 H), 2.41–2.35 (m, 2 H), 2.09–2.05 (m, 2 H), 2.00–1.92 (m, 1 H), 1.65–1.59 (m, 2 H), 1.32–1.28 (m, 10 H), 0.89 (t, *J* = 6.9 Hz, 3 H) ppm. ¹³C NMR (CD₃OD, 125 MHz), δ: 145.1, 130.2, 129.2, 127.9, 72.7, 68.6, 60.7, 54.1, 39.5, 35.2, 32.8, 32.7, 31.6, 31.2, 31.1, 29.1, 29.0, 28.9, 22.3, 13.0 ppm. HRMS (ESI) calcd. for C₂₂H₃₆NO (M)⁺ 330.2797, found 330.2793.

tert-Butyl (2R,4R)-4-((tert-butyldimethylsilyl)oxy)-2-(3-methoxy-3-oxopropyl)pyrrolidine-1-carboxylate (15a)

15a was submitted to the Horner-Wadsworth-Emmons extension procedure from **13b**, starting from commercially available 1-(tert-butyl) 2-methyl (2S,4R)-4-((tert-butyldimethylsilyl)oxy)pyrrolidine-1,2-dicarboxylate (200 mg, 0.56 mmol). The residue was purified by flash column chromatography (hexane/EtOAc 8:2 R_f: 0.38) to give the intermediate tert-Butyl (2S,4R)-4-((tert-butyldimethylsilyl)oxy)-2-((E)-3-methoxy-3-oxoprop-1-en-1-yl)pyrrolidine-1-carboxylate as a colorless oil (150 mg, 69% over 2 steps). [α]_D²⁵ -3.0 (*c* 3.15, CHCl₃). IR (neat), ν_{max}: 2977, 2926, 2855, 1701, 1396, 1260, 987, 753 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ: 6.85–6.84 (m, 1 H), 5.90–5.75 (d, *J* = 15.0 Hz, 1 H), 4.57–4.47 (m, 1 H), 4.35–4.33 (t, *J* = 7.8 Hz, 1 H), 3.75 (s, 3 H), 3.47–3.36 (m, 2 H), 2.10–2.06 (m, 1 H), 1.85–1.80 (m, 1 H), 1.45 (s, 9 H), 0.89 (s, 9 H), 0.07 (s, 6 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ: 200.3, 200.0, 166.9, 154.7, 149.3, 148.9, 128.5, 127.6, 127.0, 120.0, 79.9, 70.0, 69.7, 69.5, 56.9, 55.3, 54.8, 51.6, 41.5, 40.6, 36.9, 28.4, 28.2, 25.7, 17.9 ppm. HRMS (ESI) calcd. for C₂₅H₄₀NO₃ (M+H)⁺ 618.35301, found 618.35381.

tert-Butyl (2S,4R)-4-((tert-butyldimethylsilyl)oxy)-2-((E)-3-methoxy-3-oxoprop-1-en-1-yl)pyrrolidine-1-carboxylate (150 mg, 0.39 mmol) was dissolved in MeOH (15 mL) and Pd/C (10%, 47 mg) was added to the resulting solution. The air was removed from the flask under vacuum and replaced with hydrogen (balloon). The reaction was vigorously stirred overnight at room temperature. Afterwards, the mixture was filtered through a celite pad, washing with MeOH. The collected solution was concentrated *in vacuo*, affording **15a** as a colorless oil (149 mg, 99%). [α]_D²⁰ -29.0 (*c* 0.44, CHCl₃). IR (neat), ν_{max}: 2929; 1739; 1693; 1390; 1154; 833; 773 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ: 4.33–4.32 (q, 1 H), 4.10–3.95 (m, 1 H), 3.66 (s, 3 H), 3.48–3.32 (m, 2 H), 2.30 (bs, 2 H), 2.07–1.96 (m, 2 H), 1.78–1.71 (m, 2 H), 1.45 (s, 9 H), 0.86 (s, 9 H), 0.05 (s, 6 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ: 174.1, 155.3, 79.6, 79.4, 70.5, 70.0, 55.6, 55.0, 54.7, 51.7, 40.8, 40.1, 31.0, 30.7, 30.5, 30.2, 28.6, 25.9, 18.1 ppm. HRMS (ESI) calcd. for C₂₅H₄₀NO₃ (M+H)⁺ 388.25140, found 388.25200.

tert-Butyl (2*R*,4*R*)-4-hydroxy-2-(3-(4-octylphenyl)-3-oxopropyl)pyrrolidine-1-carboxylate (**15b**)

15a (334.0 mg, 0.86 mmol) was submitted to the procedure from **12b**. The residue was purified by flash column chromatography (hexane/EtOAc 6:4 Rf: 0.25) to give the intermediate *tert*-Butyl (2*R*,4*R*)-4-((*tert*-butyldimethylsilyloxy)-2-(3-(methoxy(methyl)amino)-3-oxopropyl)pyrrolidine-1-carboxylate as a colorless oil (328 mg, 92%).

tert-Butyl (2*R*,4*R*)-4-((*tert*-butyldimethylsilyloxy)-2-(3-(methoxy(methyl)amino)-3-oxopropyl)pyrrolidine-1-carboxylate (100 mg, 0.24 mmol) was submitted in sequence to general procedures C and B. The resulting residue was purified by flash column chromatography (hexane/EtOAc 4:6 Rf: 0.33) to give **15b** as a yellow oil (61 mg, 59% over 2 steps). $[\alpha]_D^{25}$ -19.0 (c 0.40, CHCl₃). IR (neat), ν_{\max} : 2922, 1672, 1411 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.90-7.88 (d, *J* = 8.2 Hz, 2 H), 7.29-7.27 (d, *J* = 9.3 Hz, 2 H), 4.48-4.47 (m, 1 H), 4.12-4.09 (m, 1 H), 3.60-3.58 (d, *J* = 11.7 Hz, 1 H), 3.47-3.43 (dd, *J* = 12.0, 4.6 Hz, 1 H), 2.99-2.95 (m, 2 H), 2.69-2.66 (t, *J* = 6.4 Hz, 2 H), 2.21-2.11 (m, 2 H), 1.91-1.87 (m, 3 H), 1.66-1.63 (m, 2 H), 1.46 (s, 9 H), 1.33-1.28 (m, 10 H), 0.92 (t, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 199.4, 155.2, 148.8, 134.5, 129.5, 128.6, 128.2, 79.6, 70.0, 55.6, 54.7, 40.3, 36.0, 31.9, 31.1, 29.7, 29.6, 29.4, 29.3, 29.2, 28.5, 22.7, 14.1, 14.1 ppm. HRMS (ESI) calcd. for C₂₅H₄₀NO₃ (M+H)⁺ 454.29280, found 454.29428.

(2R,4R)-4-Hydroxy-2-(3-(4-octylphenyl)-3-oxopropyl)pyrrolidin-1-ium chloride (**15**)

15 was synthesized in accordance with the general procedure A, starting from **15b** (43 mg, 0.10 mmol). **15** was obtained as a white solid (26 mg, 72%). As for **13**, **15** revealed prone to cyclize spontaneously to **15d** in protic solvent such as MeOH. IR (neat), ν_{\max} : 2977, 2926, 2855, 1701, 1396, 1260, 987, 753 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, opened form **15**), δ : 7.96-7.94 (d, *J* = 8.3 Hz, 2 H), 7.35-7.33 (d, *J* = 8.3 Hz, 2 H), 4.54 (m, 1 H), 3.95-3.90 (m, 1 H), 3.48-3.43 (dd, *J* = 12.5, 4.1 Hz, 1 H), 3.28-3.19 (m, 3 H), 2.71-2.67 (m, 2 H), 2.26-2.10 (m, 3 H), 1.87-1.80 (m, 1 H), 1.65 (m, 2 H), 1.34-1.29 (m, 10 H), 0.91-0.88 (t, *J* = 6.85 Hz, 3 H) ppm. ¹H NMR (CDCl₃, 500 MHz, cyclized form **15d**), δ : 8.00-7.98 (d, *J* = 8.5 Hz, 1 H), 7.59-7.57 (d, *J* = 8.4 Hz, 2 H), 5.24 (m, 1 H), 4.93-4.90 (m, 1 H), 4.33-4.29 (m, 1 H), 4.17-4.13 (m, 2 H), 3.74 (m, 1 H), 2.83-2.79 (m, 2 H), 2.64 (m, 1 H), 2.36-2.32 (dd, *J* = 13.1, 6.1 Hz, 1 H), 2.12-2.09 (m, 1 H), 1.97-1.89 (m, 1 H), 1.71 (m, 2 H), 1.36-1.31 (m, 10 H), 0.93-0.90 (t, *J* = 6.9 Hz, 3 H) ppm. HRMS (ESI) calcd. for C₂₅H₄₀NO₃ (M+H)⁺ 618.35301, found 618.35381.

(S)-*tert*-Butyl 2-(4-bromobenzoyl)pyrrolidine-1-carboxylate (**17b**)

*n*BuLi (2.5 M in hexane, 11.4 mL, 24 mmol, 2.0 eq.) was added dropwise via syringe to a stirred and cooled to -78 °C solution of 1,4-dibromobenzene (5.88 g, 24.9 mmol, 2.06 eq.) in dry THF (42 mL) under Ar. Stirring was continued for 30 min before *N*-Boc-D-prolinal **17a** (2.2038 g, 1.0 mmol) in THF (18 mL) was added via syringe over ca 5 min. Stirring was continued for a further 15 min and the mixture was quenched by addition of sat. NH₄Cl (20 mL) and H₂O (10 mL). The bulk of the THF was then removed *in vacuo* and the mixture was extracted into Et₂O (30 mL), washed once with brine (15 mL) and dried (MgSO₄). Evaporation of the solvent and chromatography over SiO₂ (2.5 x 40 cm) using 13% EtOAc-hexanes afforded (*S*)-*tert*-butyl 2-((4-bromophenyl) (hydroxy)methyl) pyrrolidine-1-carboxylate as a mixture of diastereomers (2.3 g, 59%).

Dess-Martin periodinane (3.5 g, 8.2 mmol, 1.3 eq.) was added as a solid over ca 2 min to a stirred and cooled (0 °C) solution of (*S*)-*tert*-butyl 2-((4-bromophenyl) (hydroxy)methyl)pyrrolidine-1-carboxylate (mixture of diastereomers, 2.3 g, 6.5 mmol, 1.0 eq.) in CH₂Cl₂ (24 mL). The flask was capped with a glass stopper and stirring was continued overnight. The mixture was then quenched by the addition of sat. NaHCO₃ (10 mL) and H₂O (5 mL) and stirring was continued for 30 min. The mixture was then filtered through Celite (2 x 3cm), washing the filter cake with CH₂Cl₂. The aqueous layer was extracted once with CH₂Cl₂ (10 mL) and the combined organic was dried (MgSO₄), evaporated, and chromatographed over SiO₂ (2.5 x 30 cm) using 13% EtOAc-hexanes to afford (*S*)-*tert*-butyl 2-(4-bromobenzoyl)pyrrolidine-1-carboxylate **17b** (1.6011 g,

70%). HRMS (ESI) calcd. for $C_{16}H_{20}BrNO_3$ (M+Na)⁺ 376.05188, found 376.05202.

tert-Butyl (*S*)-2-((*R*)-1-(4-bromophenyl)-3-ethoxy-1-hydroxy-3-oxopropyl)pyrrolidine-1-carboxylate (**17c**) and *tert*-butyl (*S*)-2-((*S*)-1-(4-bromophenyl)-3-ethoxy-1-hydroxy-3-oxopropyl)pyrrolidine-1-carboxylate (**epi-17c**) BuLi (2.5 M in hexane, 5.5 mL, 13.7 mmol, 3.0 eq.) was added via syringe to a stirred and cooled to -78 °C solution of *i*-Pr₂NH (1.9 mL, 13.6 mmol, 3.0 eq.) in THF (12 mL). The cooling bath was removed for 10 min and then replaced and stirring was continued for a further 10 min before EtOAc (1.5 mL, 15.4 mmol, 3.4 eq.) was added dropwise via syringe. Stirring was then continued for 45 min before (*S*)-*tert*-butyl-2-(4-bromobenzoyl)pyrrolidine-1-carboxylate **17b** (1.6011 g, 4.55 mmol, 1.0 eq.) in THF (5 mL + 1 mL rinse) was added at a slow dropwise rate via syringe (ca 15 min). Stirring was then continued for 20 min and then the mixture was quenched by the addition of sat. NH₄Cl (5 mL) and H₂O (5 mL). The mixture was diluted with Et₂O (30 mL) and washed once with H₂O (20 mL), once with brine (20 mL) and dried (Na₂SO₄). Evaporation of the solvent provided (*S*)-*tert*-butyl 2-((*S*)-1-(4-bromophenyl)-3-ethoxy-1-hydroxy-3-oxopropyl)pyrrolidine-1-carboxylate **17c** and (*S*)-*tert*-butyl 2-((*R*)-1-(4-bromophenyl)-3-ethoxy-1-hydroxy-3-oxopropyl)pyrrolidine-1-carboxylate **epi-17c** as a mixture (1.54 g, 76%), which was used directly in the next step without further purification. HRMS (ESI) calcd. for $C_{25}H_{40}NO_3$ (M+H)⁺ 464.10431, found 464.10432.

tert-Butyl (*S*)-2-((*R*)-1-(4-bromophenyl)-1-hydroxy-3-(tosyloxy)propyl)pyrrolidine-1-carboxylate (**17d**) and (*S*)-*tert*-butyl 2-((*S*)-1-(4-bromophenyl)-1-hydroxy-3-(tosyloxy)propyl)pyrrolidine-1-carboxylate (**epi-17d**)

LiBH₄ solution (2 M in THF, 1.1 mL, 2.2 mmol, 0.6 eq.) was added via syringe to a stirred and cooled to 0 °C solution of esters (*S*)-*tert*-butyl 2-((*S*)-1-(4-bromophenyl)-3-ethoxy-1-hydroxy-3-oxopropyl)pyrrolidine-1-carboxylate **17c** and (*S*)-*tert*-butyl 2-((*R*)-1-(4-bromophenyl)-3-ethoxy-1-hydroxy-3-oxopropyl)pyrrolidine-1-carboxylate **epi-17c** (mixture from previous step, 1.54 g, 3.48 mmol, 1.0 eq.) in THF (10 mL) under Ar. The ice-bath was left in place but not recharged and stirring was continued for 7 h. The mixture was then quenched by the careful addition of H₂O (3 mL) and then NaHCO_{3aq}(sat., 5 mL). EtOAc (10 mL) was then added and the biphasic mixture was stirred for 1 h. The aqueous phase was extracted once with EtOAc (10 mL) and the combined organic was washed once with brine (10 mL) and dried (Na₂SO₄). Evaporation of the solvent and filtration of the residue through a plug of SiO₂ (2 x 4 cm) using 40% EtOAc-hexanes (ca 100 mL, TLC control) afforded the alcohols (*S*)-*tert*-butyl 2-((*R*)-1-(4-bromophenyl)-1,3-dihydroxypropyl)pyrrolidine-1-carboxylate and (*S*)-*tert*-butyl 2-((*S*)-1-(4-bromophenyl)-1,3-dihydroxypropyl)pyrrolidine-1-carboxylate as a mixture (1.38 g, 72%).

Et₃N (0.93 mL, 6.7 mmol, 1.2 eq.) followed by TsCl (1.16 g, 6.1 mmol, 1.1 eq.) were added to a stirred solution of alcohols (*S*)-*tert*-butyl-2-((*R*)-1-(4-bromophenyl)-1,3-dihydroxypropyl) pyrrolidine -1-carboxylate and (*S*)-*tert*-butyl-2-((*S*)-1-(4-bromophenyl)-1,3-dihydroxypropyl) pyrrolidine-1-carboxylate mixture from previous step (2.22 g, 5.55 mmol, 1.0 eq.) in CH₂Cl₂ (10 mL). The flask was capped with a glass stopper and stirred for 8 h. The mixture was diluted with CH₂Cl₂ (10 mL), washed once with H₂O (25 mL), and dried (MgSO₄). Removal of the solvent *in vacuo* and chromatography over SiO₂ (2.5 x 35 cm) using 10% EtOAc-hexanes afforded the tosylates (*S*)-*tert*-butyl 2-((*R*)-1-(4-bromophenyl)-1-hydroxy-3-(tosyloxy)propyl)pyrrolidine-1-carboxylate **17d** and (*S*)-*tert*-butyl 2-((*S*)-1-(4-bromophenyl)-1-hydroxy-3-(tosyloxy)propyl)pyrrolidine-1-carboxylate **epi-17d** as a mixture (2.23 g, 72%).

(*1R*)-1-(4-Bromophenyl)-1-hydroxyoctahydro-pyrrolizin-4-ium 4-methylbenzenesulfonate (**17e**) and (*1S*)-1-(4-bromophenyl)-1-hydroxyoctahydro-pyrrolizin-4-ium 4-methylbenzenesulfonate (**epi-17e**)

A solution of tosylates (*S*)-*tert*-butyl 2-((*R*)-1-(4-bromophenyl)-1-hydroxy-3-(tosyloxy)propyl)pyrrolidine-1-carboxylate **17d** and (*S*)-*tert*-butyl 2-((*S*)-1-(4-bromophenyl)-1-hydroxy-3-(tosyloxy)propyl)pyrrolidine-1-carboxylate **epi-17d** (2.2 g, 4.0 mmol, 1.0 eq.) was stirred for 10h at 110 °C in PhMe (18 mL) under Ar. The solvent was then removed *in vacuo* and the residue was chromatographed over SiO₂ (2.5 x 35 cm) using 10-20% MeOH-CHCl₃ to give a faster eluting fraction ((*1R*,7a*S*)-1-(4-bromophenyl)-1-

hydroxyoctahydropyrrolizin-4-ium 4-methylbenzenesulfonate) **17e** and a slower eluting fraction ((1*S*,7*aS*)-1-(4-bromophenyl)-1-hydroxyoctahydropyrrolizin-4-ium 4-methylbenzenesulfonate) **epi-17e**. Mixed fractions were discarded. After removal of the solvent, the faster eluting diastereomer was crystallized from CH₂Cl₂-*t*-BuOMe (note 1) to provide ((1*R*,7*aS*)-1-(4-bromophenyl)-1-hydroxyoctahydropyrrolizin-4-ium 4-methylbenzenesulfonate **17e** (0.7 g, 38%). The slower eluting fraction was also evaporated to dryness and the solid residue was suspended in CH₂Cl₂ (3 mL) and filtered through a syringe filter (25 mm, PTFE 0.45 μm) to remove silica washing with three portions of CH₂Cl₂ (3 ml each) (note 2). The solvent was then removed *in vacuo* and the solid residue was crystallized from CH₂Cl₂-*t*-BuOMe (note 3) to provide (1*S*,7*aS*)-1-(4-bromophenyl)-1-hydroxyoctahydropyrrolizin-4-ium 4-methylbenzenesulfonate **epi-17e** (0.3 g, 17%). **17e**: mp 143 °C. $[\alpha]_D^{25} +21.0$ (*c* 0.65, MeOH). IR (neat), ν_{\max} : 3371, 3673, 1657, 1394, 561 cm⁻¹. ¹H NMR (400 MHz, CDCl₃), δ : 1.74-1.83 (m, 1 H), 2.05-2.13 (m, 1 H), 2.24-2.33 (m, 2 H), 2.39 (s, 3 H), 2.45 (dd, *J* = 5.4, 13.4 Hz, 1 H), 2.81 (ddd, *J* = 7.2, 13.0, 13.0 Hz, 1 H), 3.03-3.09 (m, 1 H), 3.32 (ddd, *J* = 5.9, 12.8, 12.8 Hz, 1 H), 3.55 (ddd, *J* = 6.0, 6.0, 11.7 Hz, 1 H), 3.80-3.87 (br s, 1 H), 3.94-3.99 (m, 1 H), 4.38 (app dd, *J* = 4.5, 8.0 Hz, 1 H), 7.18 (d, *J* = 8.1 Hz, 2 H), 7.36 (d, *J* = 8.7 Hz, 2 H), 7.45 (d, *J* = 8.7 Hz, 2 H), 7.71 (d, *J* = 8.1 Hz, 2 H), 11.26 (br s, 1 H) ppm. ¹³C NMR (100 MHz, CDCl₃), δ : 21.8, 22.8, 27.5, 42.6, 53.7, 55.7, 79.4, 122.3, 126.1, 127.7, 129.4, 132.0, 140.3, 141.0, 142.0 ppm. LRMS found *m/z* 282.0. **epi-17e**: mp = 177.5-178.5 °C. IR (neat), ν_{\max} : 1234, 1185, 1009, 815, 696, 568, 478 cm⁻¹. ¹H NMR (400 MHz, CDCl₃), δ : 1.18-1.26 (m, 1 H), 1.77-1.84 (m, 1 H), 1.93-2.01 (m, 2 H), 2.39 (s, 3 H), 2.53-2.66 (overlapping m, 2 H), 2.97 (ddd, *J* = 11.4, 9.6, 6.8 Hz, 1 H), 3.26 (dd, *J* = 11.6, 6.4 Hz, 1 H), 3.98 (ddd, *J* = 11.3, 6.1, 6.1 Hz, 1 H), 4.31 (ddd, *J* = 11.9, 11.9, 7.1 Hz, 1 H), 4.61 (dd, *J* = 10.0, 8.2 Hz, 1 H), 5.80 (br s, 1 H), 7.18 (d, *J* = 7.9 Hz, 2 H), 7.33 (d, *J* = 8.6 Hz, 2 H), 7.49 (d, *J* = 8.6 Hz, 2 H), 7.73 (d, *J* = 7.9 Hz, 2 H) ppm. ¹³C NMR (100 MHz, CDCl₃), δ : 4.3, 21.8, 26.0, 29.8, 33.5, 54.2, 57.2, 81.8, 123.1, 126.3, 128.6, 129.3, 132.2, 139.4, 140.9, 141.8 ppm. LRMS found *m/z* 282.0. Note 1: Diastereomer (1*R*,7*aS*)-1-(4-bromophenyl)-1-hydroxyoctahydropyrrolizin-4-ium 4-methylbenzenesulfonate was dissolved in ca. 10 mL of CH₂Cl₂ and was then brought to boiling with a heat gun. *t*-BuOMe (ca 5 mL) was then added and the solution was allowed to crystallize overnight, the flask being left completely open. The next day the flask was capped with a glass stopper and cooled at ca -15 °C (freezer section of fridge) for a further 10 h. Filtration afforded flocculent white needles of ((1*R*,7*aS*)-1-(4-bromophenyl)-1-hydroxyoctahydropyrrolizin-4-ium 4-methylbenzenesulfonate. Note 2: The syringe filter was attached to a syringe and the plunger was removed. The suspension was then transferred to this syringe via Pasteur pipette and forced through the filter by re-inserting the plunger. Note 3: Diastereomer (1*S*,7*aS*)-1-(4-bromophenyl)-1-hydroxyoctahydropyrrolizin-4-ium 4-methylbenzenesulfonate was dissolved in ca 10 mL of CH₂Cl₂ with stirring in an oil bath set at 45 °C. *t*Bu-OMe was added (ca 3mL) and stirring was discontinued. The oil bath was shut off but left in place and the mixture was allowed to crystallize overnight, the flask being left completely open. The next day the flask was capped with a glass stopper and cooled at ca -15 °C (freezer section of fridge) for a further 10 h. Filtration afforded small needles.

(1R)-1-(4-Octylphenyl)hexahydro-1H-pyrrolizin-1-ol hydrochloride (17)

To a solution of catecholborane (135 μL, 1.0 M in THF, 0.135 mmol, 1.5 eq.) was added octyne dropwise (19.9 μL, 0.135 mmol, 1.5 eq.). Gas formation was observed during the addition. The colorless solution was refluxed for 2h then cooled back to rt. In another flask, **17e** (41 mg, 0.09 mmol, 1.0 eq.) was dissolved in a biphasic mixture of DME (1.1 mL) and aqueous NaHCO₃ (1 M) solution, then the octyne/catecholborane solution was syringed into the flask. Pd(PPh₃)₄ (3.1 mg, 0.0027 mmol, 0.03 eq.) was added and the overall white suspension was refluxed overnight. Et₂O and brine were added and the aqueous layer was extracted x2 with Et₂O. The organic layers were collected, dried over Na₂SO₄, filtered through Celite, concentrated. The crude oil was chromatographed over SiO₂ (9:1 DCM/MeOH) to deliver an orange oil (18 mg). The product was engaged in the next step without further purification. The oil (12 mg, 0.038 mmol, 1.0 eq.) was dissolved in MeOH (1.2 mL) and Pd/C was added in one portion (0.4 mg, 0.0038 mmol, 0.1 eq.). The flask was purged x3 with H₂ and the black suspension was stirred for 1h30. Then HCl (9.5 μL, 4 M, 0.038 mmol, 1.0 eq.) was

added and the solution was stirred for 2h, then filtered through a pad of Celite and concentrated. The resulting crude was chromatographed on reversed C18 column (0 to 20% MeCN in H₂O) to deliver **17** as a colorless oil (9 mg, 29% over two steps). $[\alpha]_{\text{D}}^{25}$ -16.0 (*c* 0.275, MeOH) IR (neat), ν_{max} : 3357, 2923, 1593, 1349 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.14-7.08 (m, 4 H), 4.78 (bs, 1 H), 4.60-4.57 (t, *J* =, 0.47 H), 4.54-4.50 (t, *J* = 14.8, 0.53 H), 3.91-3.67 (m, 3 H), 3.54-3.47 (m, 1 H), 2.58-2.54 (m, 2 H), 2.30-2.25 (m, 0.53 H), 2.13-2.09 (m, 0.47 H), 1.84-1.81 (m, 1 H), 1.58-1.56 (m, 2 H), 1.58-1.40 (m, 25 H), 1.29-1.25 (m, 13 H), 0.89-0.86 (t, *J* =, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 207.1, 207.1, 154.6, 153.8, 142.2, 141.9, 130.6, 130.3, 129.7, 129.6, 129.0, 128.9, 83.1, 83.1, 83.1, 83.0, 82.9, 80.9, 80.5, 75.7, 75.6, 74.8, 74.8, 63.4, 62.5, 53.8, 53.7, 53.5, 53.5, 47.6, 46.3, 32.0, 30.0, 30.0, 29.6, 29.4, 28.5, 28.4, 22.8, 14.2 ppm. HRMS (ESI) calcd. for C₂₃H₃₇NO₃Na (M+H)⁺ 316.2635, found 316.2644.

tert-Butyl 3-hydroxy-3-(4-octylphenyl)pyrrolidine-1-carboxylate ((±)**18b**)

(±)**18b** was synthesized in accordance with the general procedure C, starting from **18a** (100 mg, 0.54 mmol). The resulting residue was purified by flash column chromatography (hexane/EtOAc 8:2, R_f: 0.28) to give (±)**18b** as a pale yellow oil (123 mg, 61%). IR (neat), ν_{max} : 3392, 2923, 1670, 1412, 1134 cm⁻¹. ¹H NMR (500 MHz, CDCl₃, mixture of rotamers) δ 7.37 (t, *J* = 6.6 Hz, 2H), 7.18 (d, *J* = 8.0 Hz, 2H), 3.80 – 3.49 (m, 4H), 2.65 – 2.50 (m, 2H), 2.36 – 2.09 (m, 2H), 1.67 – 1.54 (m, 2H), 1.47 (d, *J* = 11.1 Hz, 9H), 1.38 – 1.11 (m, 10H), 0.88 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (125 MHz, CDCl₃, mixture of rotamers) δ 154.9, 154.8, 142.8, 140.1, 128.7, 125.2, 125.2, 80.7, 79.9, 79.6, 59.7, 58.8, 45.2, 44.7, 39.6, 38.9, 35.7, 32.0, 31.6, 29.9, 29.6, 29.5, 29.4, 28.7, 22.8, 14.3. HRMS (ESI) calcd. for C₂₃H₃₇NO₃Na (M+Na)⁺ 398.2666, found 398.2681.

3-Hydroxy-3-(4-octylphenyl)pyrrolidin-1-ium chloride ((±)**18**)

18 was synthesized in accordance with the general procedure A, starting from **18b** (25 mg, 0.066 mmol). The resulting residue was triturated with a mixture of CH₂Cl₂/Et₂O (9:1) to give **18** as a pale yellow oil (9.6 mg, 45 %). R_f: 0.18 CH₂Cl₂/MeOH 9:1, 1% Et₃N). IR (neat), ν_{max} : 3385, 2922, 1617, 1379, 1179, 1086 cm⁻¹. ¹H NMR (500 MHz, MeOD) δ 7.44 (d, *J* = 8.1 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 3.61 (d, *J* = 9.8 Hz, 2H), 3.43 (dd, *J* = 30.3, 11.5 Hz, 2H), 2.68 – 2.55 (m, 2H), 2.43 (dd, *J* = 23.4, 10.3 Hz, 1H), 2.33 (d, *J* = 11.0 Hz, 1H), 1.61 (d, *J* = 7.3 Hz, 2H), 1.40 – 1.22 (m, 10H), 0.89 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (125 MHz, MeOD) δ 142.7, 137.9, 128.3, 125.1, 79.6, 56.6, 48.1, 48.0, 47.8, 47.6, 47.4, 47.3, 47.1, 44.4, 38.1, 35.0, 31.6, 31.3, 29.2, 29.0, 28.9, 22.3, 13.0. HRMS (ESI) calcd. for C₁₈H₂₉NO (M+H)⁺ 276.2322, found 276.2326.

tert-Butyl (S)-2-(methoxy(methyl)carbamoyl)pyrrolidine-1-carboxylate (**19b**)

To a -20 °C cooled solution containing **19a** (321 mg, 1.4 mmol, 1.0 eq.) and N,O-dimethylhydroxylamine-HCl (205 mg, 2.10 mmol, 1.5 eq.) in dry THF (1 mL) was added *i*PrMgCl (1.4 mL, 2.8 mmol, 2.0 M in THF, 2.0 eq.) dropwise. The light brown solution was stirred at -10 °C for 20 min whereby additional N,O-dimethylhydroxylamine-HCl (205 mg, 2.10 mmol, 1.5 eq.) was added in one portion followed by *i*PrMgCl (1.4 mL, 2.8 mmol, 2.0 M in THF, 2.0 eq.) dropwise. The reaction was stirred for 20 additional minutes at -10 °C whereby TLC analysis indicated that the reaction had gone to completion. Saturated aqueous NH₄Cl solution (5 mL) was added and the resulting aqueous layer was extracted with EtOAc (2 x 5 mL). The organic layers were collected, dried over Na₂SO₄, filtered, concentrated to give **19b** as a pure incolor oil (321 mg, 89%) which was brought to the next step without further purification (R_f: 0.34 hexanes/EtOAc 5:5). $[\alpha]_{\text{D}}^{20}$ -13.9 (*c* 1.25, CHCl₃). The data matched those reported in the literature. [45]

tert-Butyl (S)-2-(4-octylbenzoyl)pyrrolidine-1-carboxylate (**19c**)

19c was synthesized in accordance with the general procedure C, starting from **19b** (100 mg, 0.39 mmol). The resulting residue was purified by flash column chromatography (hexane/EtOAc 8:2 R_f: 0.32) to give **19c** as a yellow oil (98 mg, 65%). $[\alpha]_{\text{D}}^{20}$ -78.0 (*c* 0.21, CHCl₃). IR (neat), ν_{max} : 2925, 2584, 1687, 1391, 1160 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ 7.88 (dd, *J* = 19.0, 8.2 Hz, 2H), 7.25 (dd, *J* = 15.6, 7.8 Hz, 2H), 5.37 – 5.16 (m,

1H), 3.76 – 3.41 (m, 2H), 2.64 (dt, $J = 11.2, 7.8$ Hz, 2H), 2.37 – 2.22 (m, 1H), 1.99 – 1.85 (m, 3H), 1.67 – 1.55 (m, 2H), 1.46 (s, 4H), 1.36 – 1.20 (m, 16H), 0.87 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (CDCl_3 , 125 MHz, mixture of rotamers), δ : 198.7, 198.2, 154.6, 154.0, 149.1, 149.1, 133.0, 132.9, 128.8, 128.8, 128.8, 128.4, 79.8, 79.7, 61.4, 61.1, 46.9, 46.8, 36.1, 32.0, 31.2, 29.5, 29.4, 29.3, 28.6, 28.3, 23.7, 22.8, 14.2 ppm. HRMS (ESI) calcd. for $\text{C}_{24}\text{H}_{37}\text{NO}_3$ ($\text{M}+\text{Na}$) $^+$ 410.26660, found 410.26710.

(S)-2-(4-Octylbenzoyl)pyrrolidin-1-ium chloride (19)

19 was synthesized in accordance with the general procedure A, starting from **19c** (27 mg, 0.07 mmol). The resulting residue was triturated with EtOAc to give **19** as a white powder (14 mg, 64%). $[\alpha]_{\text{D}}^{20}$ -38.7 (c 0.16, CHCl_3). IR (neat), ν_{max} : 2923, 2853, 1686, 1397, 1250, 997 cm^{-1} . ^1H NMR (500 MHz, MeOD) δ 8.00 (d, $J = 8.3$ Hz, 2H), 7.43 (d, $J = 8.3$ Hz, 2H), 5.34 (dd, $J = 9.3, 7.1$ Hz, 1H), 3.45 (dt, $J = 15.3, 6.1$ Hz, 2H), 2.79 – 2.61 (m, 1H), 2.24 – 1.90 (m, 2H), 1.73 – 1.58 (m, 2H), 1.42 – 1.23 (m, 10H), 0.90 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (CDCl_3 , 125 MHz, mixture of rotamers), δ : 194.9, 152.9, 132.1, 130.8, 130.7, 64.8, 47.8, 37.3, 33.3, 32.6, 31.4, 30.9, 30.7, 30.6, 25.5, 24.0, 14.7 ppm. HRMS (ESI) calcd. for $\text{C}_{19}\text{H}_{30}\text{NO}$ ($\text{M}+\text{H}$) $^+$ 288.23219, found 288.23192.

tert-Butyl (2S,4R)-4-((tert-butyldimethylsilyloxy)-2-(methoxy(methyl)carbamoyl)pyrrolidine-1-carboxylate (20b)

20b was synthesized in accordance with the procedure from **19b**, starting from **20a** (50.0 mg, 0.14 mmol). The crude incolor oil of **20b** (54 mg, 95%) was brought to the next step without further purification (Rf: 0.25 hexanes/EtOAc 7:3). $[\alpha]_{\text{D}}^{25}$ -14.0 (c 0.50, CHCl_3). The spectral datas matched those reported in the litterature.⁷

tert-Butyl (2R,4S)-4-((tert-butyldimethylsilyloxy)-2-(methoxy(methyl)carbamoyl)pyrrolidine-1-carboxylate (21b)

21b was synthesized in accordance with the procedure of its enantiomer **20b**, starting from **21a** (300 mg, 0.84 mmol). **21b** was obtained as a colorless oil (298 mg, 91 %) which was brought to the next step without further purification (Rf: 0.25 hexanes/EtOAc 7:3). $[\alpha]_{\text{D}}^{25}$ +13.0 (c 1.00, CHCl_3). The spectral datas matched those reported for its enantiomer.

tert-Butyl (2S,4R)-4-hydroxy-2-(4-octylbenzoyl)pyrrolidine-1-carboxylate (20c)

20b (200 mg, 0.52 mmol) was submitted in sequence to general procedures C and B. The resulting residue was purified by flash column chromatography (hexane/EtOAc 4:6 Rf: 0.35) to give **20c** as a yellow oil (135 mg, 65% over 2 steps). $[\alpha]_{\text{D}}^{25}$ -6.6 (c 0.27, CHCl_3). IR (neat), ν_{max} : 2924, 1686, 1605, 1399, 1158 cm^{-1} . ^1H NMR (CDCl_3 , 500 MHz, mixture of rotamers), δ : 7.95-7.90 (m, 2 H), 7.31-7.27 (m, 2 H), 5.51-5.48 (t, $J = 7.6$ Hz, 0.45 H), 5.42-5.39 (t, $J = 8.1$ Hz, 0.55 H), 4.55 (s, 1 H), 3.80-3.56 (m, 2 H), 2.71-2.68 (m, 2 H), 2.41-2.37 (m, 1 H), 2.09-2.04 (m, 1 H), 1.74-1.62 (m, 3 H), 1.49 (s, 3 H), 1.32-1.26 (m, 15 H), 0.92-0.89 (t, $J = 7.0$ Hz, 3 H) ppm. ^{13}C NMR (CDCl_3 , 125 MHz, mixture of rotamers), δ : 199.0, 198.5, 154.9, 154.4, 149.7, 149.6, 133.4, 133.3, 129.2, 129.1, 129.1, 128.7, 80.7, 80.4, 71.0, 70.3, 60.0, 59.7, 55.6, 40.1, 39.3, 36.4, 32.2, 31.5, 31.4, 29.8, 29.6, 29.6, 28.8, 28.5, 23.0, 14.5 ppm. HRMS (ESI) calcd. for $\text{C}_{25}\text{H}_{40}\text{NO}_3$ ($\text{M}+\text{H}$) $^+$ 426.26150, found 426.26245.

tert-Butyl (2R,4S)-4-hydroxy-2-(4-octylbenzoyl)pyrrolidine-1-carboxylate (21c)

21b (298 mg, 0.77 mmol) was submitted in sequence to general procedures C and B. The resulting residue was purified by flash column chromatography (hexane/ EtOAc 4:6 Rf: 0.35) to give **21c** as a yellow oil (205 mg, 62% over 2 steps). $[\alpha]_{\text{D}}^{25}$ +38.1 (c 1.65, CHCl_3). The spectral datas matched those reported for its enantiomer.

(2S,4R)-4-Hydroxy-2-(4-octylbenzoyl)pyrrolidin-1-ium chloride (20)

20 was synthesized in accordance with the general procedure A, starting from **20c** (18 mg, 0.07 mmol). **20** was obtained as a white solid (18 mg, 86%). $[\alpha]_D^{25}$ -30.6 (*c* 0.80, CHCl₃). IR (neat), ν_{\max} : 2923, 2470, 2070, 1596, 1463, 1119, 973 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz), δ : 7.99-7.98 (d, *J* = 8.2 Hz, 1 H), 7.43-7.42 (d, *J* = 7.9 Hz, 2 H), 5.51-5.47 (dd, *J* = 10.3, 8.2 Hz, 1 H), 4.61-4.60 (m, 1 H), 3.39 (s, 2 H), 2.73-2.71 (m, 2 H), 2.66-2.62 (dd, *J* = 12.9, 8.2 Hz, 1 H), 2.07-2.02 (ddd, *J* = 13.8, 10.4, 4.2 Hz, 1 H), 1.68-1.65 (m, 2 H), 1.34-1.28 (m, 10 H), 0.91-0.88 (t, *J* = 7.0 Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz), δ : 194.6, 162.8, 152.6, 131.8, 130.4, 130.4, 71.3, 63.3, 55.0, 40.5, 37.0, 33.0, 32.2, 30.5, 30.4, 30.3, 23.7, 14.4 ppm. HRMS (ESI) calcd. for C₂₅H₄₀NO₃ (M+H)⁺ 304.22770, found 304.22860.

(2R,4S)-4-Hydroxy-2-(4-octylbenzoyl)pyrrolidin-1-ium chloride (21)

21 was synthesized in accordance with the general procedure A, starting from **21c** (30 mg, 0.07 mmol). **21** was obtained as a white solid (16 mg, 64%). $[\alpha]_D^{25}$ +25.6 (*c* 0.34, CHCl₃). The spectral datas matched those reported for its enantiomer.

(3R,5R)-5-(4-Octylbenzyl)pyrrolidin-3-ol (16)

20c (20 mg, 0.050 mmol) was dissolved in EtOH (5 mL) and Pd/C (10%, 24 mg) was added to the resulting solution. The air was removed from the flask under vacuum and replaced with hydrogen (balloon). The reaction was vigorously stirred for 24 hours at room temperature. Afterwards, the mixture was filtered through a celite pad, washing with abundant EtOH, and the collected solution was concentrated *in vacuo*. The crude was purified by flash column chromatography (EtOAc/hexane 1:1, R_f: 0.35) to give the intermediate tert-Butyl (2*R*,4*R*)-4-hydroxy-2-(4-octylbenzyl)pyrrolidine-1-carboxylate as a colorless oil (6 mg, 32%). $[\alpha]_D^{25}$ -32.7 (*c* 0.30, CHCl₃). IR (neat), ν_{\max} : 3408, 2923, 2853, 1694, 1668, 1513, 1455, 1393, 1365, 1253, 1153, 1116, 981, 858, 770, 553 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.11-7.04 (m, 4 H), 4.22-4.14 (m, 2 H), 3.50 (br. s, 0.6 H), 3.35 (br. s, 0.4 H), 3.30 (br. s, 1 H), 3.09 (br. s, 1 H), 2.68 (br. s, 0.4 H), 2.63 (br. s, 0.6 H), 2.57-2.54 (m, 2 H), 1.87 (br. s, 2 H), 1.60-1.55 (m, 2 H), 1.52 (s, 9 H), 1.31-1.26 (m, 10 H), 0.87 (t, *J* = 7.0 Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 154.9, 141.0, 135.3, 129.3, 128.4, 79.7, 69.7, 69.4, 57.3, 54.5, 40.3, 39.4, 35.6, 31.9, 31.6, 29.5, 29.3, 29.2, 28.6, 22.6, 14.1 ppm. HRMS (ESI) calcd. for C₂₄H₃₉NO₃Na (M+Na)⁺ 412.28222, found 412.28110.

tert-Butyl (2*R*,4*R*)-4-hydroxy-2-(4-octylbenzyl)pyrrolidine-1-carboxylate (6 mg, 0.015 mmol) was submitted to general procedure A. The crude was triturated in Et₂O to give product **16** as a white solid (5 mg, 100%). For biological testing a portion of the product was dissolved in the minimum amount of HPLC grade water, filtered (pore size = 0.45 μm) and lyophilized. $[\alpha]_D^{25}$ +4.0 (*c* 0.25, MeOH). IR (neat), ν_{\max} : 3318, 2920, 2851, 1515, 1437, 1394, 1314, 1266, 1159, 1080, 1063, 1031, 961, 772, 720, 615, 531, 450 cm⁻¹. ¹H NMR (CD₃OD, 500 MHz), δ : 7.25 (d, *J* = 8.1 Hz, 2 H), 7.21 (d, *J* = 8.1 Hz, 2 H), 4.56 (t, *J* = 4.2 Hz, 1 H), 4.12-4.05 (m, 1 H), 3.50 (dd, *J* = 12.4, 4.2 Hz, 1 H), 3.19 (d, *J* = 12.4 Hz, 1 H), 3.05 (d, *J* = 7.6 Hz, 2 H), 2.63-2.60 (m, 2 H), 2.13 (dd, *J* = 13.7, 5.8 Hz, 1 H), 1.90 (ddd, *J* = 13.7, 11.6, 4.2 Hz, 1 H), 1.65-1.59 (m, 2 H), 1.34-1.31 (m, 10 H), 0.92 (t, *J* = 7.0 Hz, 3 H) ppm. ¹³C NMR (CD₃OD, 125 MHz), δ : 141.9, 133.6, 128.7, 128.4, 69.0, 60.3, 53.0, 39.5, 37.1, 35.1, 31.6, 31.3, 29.2, 29.0, 28.9, 22.3, 13.0 ppm.

tert-Butyl (2R,4S)-4-(((tert-butyl)diphenylsilyloxy)methyl)-2-(methoxy(methyl)carbamoyl)pyrrolidine-1-carboxylate (22b)

Isopropyl magnesium chloride (585 μL, 2 M in THF, 1.17 mmol, 6 eq.) was added dropwise to a solution of **13a** (100 mg, 0.195 mmol) and N,O-dimethylhydroxylamine (87 mg, 0.89 mmol, 4.5 eq.) in dry THF (1 mL) at -20 °C. The resulting mixture was kept at the same temperature and stirred for 1 h. Afterwards, the reaction was quenched at -20 °C by canulating the solution to an aqueous NH₄Cl satd. sol. (5 mL) and the product was extracted with EtOAc (3 x 5 mL). The organic layers were washed with brine (5 mL), dried over Na₂SO₄, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane 1:2, R_f:

0.09) to give **22b** as a colorless oil (102 mg, 99%). $[\alpha]_D^{25} +13.6$ (*c* 1.7, CHCl₃). IR (neat), ν_{\max} : 2931, 2858, 1696, 1472, 1427, 1388, 1365, 1319, 1256, 1164, 1109, 999, 940, 909, 879, 823, 741, 702, 613, 504, 489 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.65-7.63 (m, 4 H), 7.43-7.37 (m, 6 H), 4.75 (d, *J* = 6.5, 0.5 H), 4.65 (d, *J* = 6.2, 0.5 H), 3.80-3.78 (m, 0.5 H), 3.77 (s, 1.5 H), 3.70 (s, 1.5 H), 3.69-3.65 (m, 0.5 H), 3.59 (dd, *J* = 6.1, 1.7 Hz, 2 H), 3.35 (dd, *J* = 10.5, 7.0 Hz, 0.5 H), 3.29 (dd, *J* = 10.5, 7.1 Hz, 0.5 H), 3.20 (s, 3 H), 2.69-2.55 (m, 1 H), 2.11 (dt, *J* = 12.9, 8.8 Hz, 0.5 H), 2.03 (dt, *J* = 12.9, 9.5 Hz, 0.5 H), 1.95-1.90 (m, 1 H), 1.46 (s, 4.5 H), 1.42 (s, 4.5 H), 1.05 (s, 4.5 H), 1.04 (s, 4.5 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 173.1, 154.5, 153.8, 135.5, 133.4, 133.3, 129.6, 127.7, 79.5, 79.4, 65.0, 61.3, 61.2, 56.7, 56.4, 49.3, 49.1, 39.5, 38.6, 32.7, 32.0, 28.5, 28.4, 26.8, 26.7, 19.2 ppm. HRMS (ESI) calcd. for C₂₉H₄₃N₂O₅Si (M+H)⁺ 527.29358, found 527.29360.

tert-Butyl (2*R*,4*S*)-4-(((*tert*-butyldiphenylsilyl)oxy)methyl)-2-(4-octylbenzoyl)pyrrolidine-1-carboxylate (**22c**)

Prepared by applying in sequence general procedures C and B, starting from **22b** (85 mg, 0.16 mmol). The crude was purified by flash column chromatography (EtOAc/hexane 1:1, R_f: 0.16) to give **22c** as a colorless oil (32 mg, 48% over two steps). $[\alpha]_D^{25} +16.6$ (*c* 0.65, CHCl₃). IR (neat), ν_{\max} : 3434, 2924, 2854, 1687, 1605, 1394, 1365, 1223, 1161, 1129, 998, 882, 769 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.89 (d, *J* = 8.1 Hz, 0.8 H), 7.85 (d, *J* = 8.1 Hz, 1.2 H), 7.27 (d, *J* = 8.1 Hz, 1.2 H), 7.24 (d, *J* = 8.1 Hz, 0.8 H), 5.37 (dd, *J* = 9.4, 2.4 Hz, 0.4 H), 5.24 (dd, *J* = 9.3, 3.6 Hz, 0.6 H), 3.82-3.75 (m, 1 H), 3.66-3.57 (m, 2 H), 3.33 (dd, *J* = 10.7, 6.9 Hz, 0.6 H), 3.27 (dd, *J* = 10.6, 7.8 Hz, 0.4 H), 2.67-2.62 (m, 2 H), 2.57-2.46 (m, 1 H), 2.20 (dt, *J* = 12.9, 9.0 Hz, 0.6 H), 2.12 (dt, *J* = 12.2, 9.6 Hz, 0.4 H), 2.03-1.99 (m, 1 H), 1.93 (br. s, 0.4 H), 1.76 (br. s, 0.6 H), 1.66-1.58 (m, 2 H), 1.45 (s, 3.6 H), 1.30-1.27 (m, 10 H), 1.26 (s, 5.4 H), 0.87 (t, *J* = 6.9 Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 198.3, 197.8, 154.5, 153.9, 149.1, 132.6, 132.4, 128.7, 128.6, 128.3, 79.9, 79.8, 64.1, 64.0, 61.0, 60.9, 49.3, 48.9, 39.7, 38.8, 36.0, 33.1, 32.2, 31.8, 31.1, 31.0, 29.4, 29.2, 28.5, 28.2, 22.6, 14.1 ppm. HRMS (ESI) calcd. for C₂₅H₃₉NO₄Na (M+Na)⁺ 440.27713, found 440.27711.

((2*R*,4*S*)-4-(Hydroxymethyl)pyrrolidin-2-yl)(4-octylphenyl)methanone hydrochloride (**22**)

Prepared according to general procedure A, starting from **22c** (6 mg, 0.014 mmol). The crude was triturated in Et₂O to give product **22** as a white solid (5 mg, 100%). For biological testing a portion of the product was dissolved in the minimum amount of HPLC grade water, filtered (pore size = 0.45 μm) and lyophilized. $[\alpha]_D^{25} +46.4$ (*c* 0.25, CHCl₃). IR (neat), ν_{\max} : 3370, 2922, 2852, 1683, 1605, 1570, 1464, 1416, 1400, 1373, 1350, 1310, 1263, 1182, 1164, 1092, 1060, 1013, 989, 967, 901, 722, 528 cm⁻¹. ¹H NMR (CD₃OD, 500 MHz), δ : 8.01 (d, *J* = 8.3 Hz, 2 H), 7.45 (d, *J* = 8.3 Hz, 2 H), 5.43 (t, *J* = 7.9 Hz, 1 H), 3.70 (dd, *J* = 10.9, 4.7 Hz, 1 H), 3.65 (dd, *J* = 10.9, 5.0 Hz, 1 H), 3.62 (dd, *J* = 11.2, 6.8 Hz, 1 H), 3.34-3.30 (m, 1 H), 2.77-2.73 (m, 2 H), 2.60-2.52 (m, 2 H), 2.19-2.13 (m, 1 H), 1.71-1.65 (m, 2 H), 1.37-1.31 (m, 10 H), 0.91 (t, *J* = 7.0 Hz, 3 H) ppm. ¹³C NMR (CD₃OD, 125 MHz), δ : 193.1, 151.1, 130.2, 129.0, 63.2, 61.7, 48.0, 39.5, 35.6, 32.3, 31.6, 30.8, 29.1, 29.0, 28.9, 22.3, 13.0 ppm. HRMS (ESI) calcd. for C₂₀H₃₂NO₂ (M)⁺ 318.24276, found 318.24265.

tert-Butyl (2*S*,4*R*)-4-hydroxy-2-(2-(4-octylphenyl)acetyl)pyrrolidine-1-carboxylate (**23a**)

Prepared by applying in sequence general procedures C using octyl benzyl bromide [46] then B starting from **20b** (500 mg, 1.24 mmol). The resulting residue was purified by flash column chromatography (hexane/EtOAc 4:6, R_f: 0.40) to give **23a** as a yellow oil (303 mg, 59% over 2 steps). $[\alpha]_D^{25} -66.5$ (*c* 1.55, CHCl₃). IR (neat), ν_{\max} : 3433, 2923, 1676, 1394, 1159 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.14-7.09 (m, 4 H), 4.61-4.54 (m, 1 H), 4.35 (bs, 1 H), 3.81 (s, 0.75 H), 3.74-3.67 (m, 1.25 H), 3.63-3.61 (m, 0.63 H), 3.53-3.44 (m, 1.37 H), 2.58-2.55 (t, *J* = 7.7 Hz, 2 H), 2.08-1.79 (m, 2 H), 1.59-1.56 (m, 2 H), 1.46 (s, 3.51 H), 1.39 (s, 5.19 H), 1.30-1.26 (m, 10 H), 0.89-0.86 (t, *J* = 7.0 Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 207.9, 207.2, 154.8, 154.2, 142.0, 141.7, 130.6, 130.3, 129.6, 129.5, 128.8, 128.7, 80.7, 80.3, 70.4, 69.5, 63.4, 62.7, 55.2, 55.2, 47.1, 46.1, 35.6, 31.9, 31.5, 29.5, 29.3, 29.3,

28.3, 22.7, 14.1 ppm. HRMS (ESI) calcd. for $C_{25}H_{40}NO_3$ (M+H)⁺ 318.24276, found 318.24270.

tert-Butyl (2R,4S)-4-hydroxy-2-(2-(4-octylphenyl)acetyl)pyrrolidine-1-carboxylate (24a)

Prepared by applying in sequence general procedures C using octyl benzyl bromide [46] then B starting from **21b** (484 mg, 1.20 mmol). The resulting residue was purified by flash column chromatography (hexane/EtOAc 4:6, Rf: 0.40) to give **24a** as a yellow oil (340 mg, 68% over 2 steps). $[\alpha]_D^{25} +65.3$ (c 0.19, $CHCl_3$). The spectral datas matched those reported for its enantiomer.

(2S,4R)-4-Hydroxy-2-(2-(4-octylphenyl)acetyl)pyrrolidin-1-ium chloride (23)

23 was synthesized in accordance with the general procedure A, starting from **23a** (25 mg, 0.06 mmol). **23** was obtained as a white powder (18 mg, 86%). $[\alpha]_D^{25} +91.9$ (c 0.09, $CHCl_3$). IR (neat), ν_{max} : 3364, 3191, 2953, 2703, 1716, 1332, 1071, 763, 682 cm^{-1} . ¹H NMR ($CDCl_3$, 500 MHz), δ : 7.19-7.16 (s, 4 H), 4.79-4.75 (dd, $J = 7.5$ Hz, 1 H), 4.56 (m, 1 H), 3.92 (s, 2 H), 3.32-3.26 (m, 2H), 2.61 (dd, $J = 10.8, 7.8$ Hz, 2 H), 2.49-2.45 (dd, $J = 13.4, 7.7$ Hz, 1 H), 2.06-2.00 (ddd, $J = 13.5, 11.1, 4.0$ Hz, 1 H), 1.61-1.59 (m, 2 H), 1.32-1.28 (m, 10 H), 0.93-0.88 (t, $J = 6.98$ Hz, 3 H) ppm. ¹³C NMR ($CDCl_3$, 125 MHz), δ : 202.3, 201.9, 145.0, 142.0, 129.6, 129.4, 128.5, 128.4, 128.2, 69.6, 68.7, 64.6, 64.2, 53.4, 63.1, 35.1, 31.6, 31.3, 29.2, 29.0, 28.9, 22.3, 13.0 ppm. HRMS (ESI) calcd. for $C_{25}H_{40}NO_3$ (M+H)⁺ 318.24276, found 318.24750.

(2R,4S)-4-Hydroxy-2-(2-(4-octylphenyl)acetyl)pyrrolidin-1-ium chloride (24)

24 was synthesized in accordance with the general procedure A, starting from **24a** (17 mg, 0.04 mmol). **24** was obtained as a white powder (15 mg, 98%). $[\alpha]_D^{25} -80.0$ (c 0.04, $CHCl_3$). The spectral datas matched those reported for its enantiomer.

(2S,4R)-4-Hydroxy-2-(1-hydroxy-2-(4-octylphenyl)ethyl)pyrrolidin-1-ium chloride (25)

$NaBH_4$ (2.2 mg, 0.058 mmol, 1.2 eq.) was added in one portion to a solution of **23a** (20.0 mg, 0.050 mmol, 1.0 eq.) in dry MeOH (0.8 mL). The solution was stirred at rt for 2h. Saturated aqueous NH_4Cl solution was added and the resulting aqueous layer was extracted with EtOAc (1 x 2 mL). The organic layers were combined, washed with brine (1 x 2 mL), dried over Na_2SO_4 , filtered, concentrated. The resulting residue was purified by flash column chromatography (hexane/EtOAc 4:6, Rf: 0.38) to give the intermediate *tert-Butyl (2S,4R)-4-hydroxy-2-(1-hydroxy-2-(4-octylphenyl)ethyl)pyrrolidine-1-carboxylate* as a colorless oil (15 mg, 75%). $[\alpha]_D^{20} -11.7$ (c 1.02, $CHCl_3$). IR (neat), ν_{max} : 3409, 2923, 1664, 1403, 1160, 990, 771 cm^{-1} . ¹H NMR ($CDCl_3$, 500 MHz, mixture of rotamers), δ : 7.17-7.15 (d, $J = 7.8$ Hz, 2 H), 7.11-7.09 (d, $J = 7.9$ Hz, 2 H), 4.40 (bs, 1 H), 4.11-4.09 (m, 1 H), 3.83 (bs, 1 H), 3.65 (bs, 1 H), 3.39-3.36 (dd, $J = 12.1, 4.2$ Hz, 1 H), 2.82-2.78 (m, 1 H), 2.57-2.54 (m, 3 H), 2.09-2.06 (m, 1 H), 1.89-1.77 (m, 2 H), 1.65 (bs, 1 H), 1.60-1.55 (m, 2 H), 1.46 (s, 9 H), 1.31-1.25 (m, 10 H), 0.89-0.86 (t, $J = 7.0$ Hz, 3 H), ppm. ¹³C NMR ($CDCl_3$, 125 MHz, mixture of rotamers), δ : 158.1, 157.9, 141.1, 135.6, 129.5, 128.6, 80.9, 80.3, 73.0, 70.0, 55.6, 35.7, 32.0, 31.7, 29.6, 29.5, 29.4, 28.6, 28.5, 22.8, 14.2 ppm. HRMS (ESI) calcd. for $C_{25}H_{40}NO_3$ (M+H)⁺ 442.29278, found 442.29415.

tert-Butyl (2S,4R)-4-hydroxy-2-(1-hydroxy-2-(4-octylphenyl)ethyl)pyrrolidine-1-carboxylate (6 mg, 0.014 mmol) was submitted to the general procedure A. **25** was obtained as a white powder (5 mg, 98%). IR (neat), ν_{max} : 3363, 2955, 1315, 968, 557 cm^{-1} . ¹H NMR ($CDCl_3$, 500 MHz, mixture of diastereomers), δ : 7.20-7.19 (d, $J = 8.1$ Hz, 2 H), 7.15-7.13 (d, $J = 8.1$ Hz, 2 H), 4.55-4.53 (m, 1 H), 3.92-3.88 (m, 1 H), 3.79-3.74 (m, 1 H), 3.30-3.29 (m, 2 H), 3.22-3.19 (m, 1 H), 2.83-2.80 (m, 2 H), 2.60-2.57 (t, $J = , 2$ H), 2.08-2.04 (m, 1 H), 1.97-1.91 (m, 1 H), 1.61-1.58 (m, 2 H), 1.33-1.29 (m, 10 H), 0.91-0.89 (t, $J = 7.0$ Hz, 3 H) ppm. ¹³C NMR ($CDCl_3$, 125 MHz, mixture of diastereomers), δ : 142.7, 135.8, 130.7, 129.8, 72.8, 71.2, 63.9, 54.4, 42.2, 38.1, 36.7, 33.2, 33.0, 30.8, 30.6, 30.5, 23.9, 14.6 ppm. HRMS (ESI) calcd. for $C_{25}H_{40}NO_3$ (M+H)⁺ 320.25835, found 320.25841.

(2R,4S)-4-Hydroxy-2-(1-hydroxy-2-(4-octylphenyl)ethyl)pyrrolidin-1-ium chloride (26)

24a (14 mg, 0.03 mmol) was submitted to the procedure of its enantiomer **23a**. The resulting residue was purified by flash column chromatography (hexane/EtOAc 4:6, Rf: 0.38) to give the intermediate tert-Butyl (2*R,4S*)-4-hydroxy-2-(1-hydroxy-2-(4-octylphenyl)ethyl)pyrrolidine-1-carboxylate as a colorless oil (10 mg, 71 %). The spectral datas matched those reported for its enantiomer.

tert-Butyl (2*R,4S*)-4-hydroxy-2-(1-hydroxy-2-(4-octylphenyl)ethyl)pyrrolidine-1-carboxylate (10 mg, 0.024 mmol) was submitted to the general procedure A. **26** was obtained as a white powder (8 mg, 95 %). The spectral datas matched those reported for its enantiomer.

(2S,4R)-2-(4-Octylbenzoyl)-4-(phosphonooxy)pyrrolidin-1-ium chloride (29)

Di-tert-butyl diethylphosphoramidite (93%, 44 μ L, 0.15 mmol, 2.0 eq.) and tetrazole (0.45 M in ACN, 0.22 mmol, 3.0 eq.) were added dropwise to a solution of **20c** (30.0 mg, 0.074 mmol, 1.0 eq.) in dry THF (1 mL) at 0°. The resulting mixture was allowed to warm up to rt and stirred for 1.5 hours. The reaction was cooled back to -30 °C whereby tBuOOH (5.0 M, 0.30 mmol, 4.0 eq.) was added dropwise. The resulting mixture was stirred at -30 °C for 15 minutes and at rt for 15 additional minutes. Afterwards, the reaction was cooled back to 0 °C whereby an aqueous NaHSO₃ solution (10% w/w, 2 mL) was added dropwise. The aqueous layer was extracted with EtOAc (3 x 2 mL). The resulting organic layer was washed with brine (2 mL), dried over Na₂SO₄, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane 5:5 + 0.5% Pyridine, Rf: 0.32) to give the intermediate tert-Butyl (2*S,4R*)-4-((di-tert-butoxyphosphoryl)oxy)-2-(4-octylbenzoyl)pyrrolidine-1-carboxylate as a colorless oil (26 mg, 62%). $[\alpha]_D^{25}$ -11.7 (*c* 1.02, CHCl₃). IR (neat), ν_{\max} : 2977, 2926, 2855, 1701, 1396, 1260, 987, 753 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.91-7.90 (d, *J* = 8.2 Hz, 0.85 H), 7.87-7.86 (d, *J* = 8.2 Hz, 1.15 H), 7.28-7.26 (d, *J* = 8.7 Hz, 1.15 H), 7.25-7.24 (d, *J* = 8.7 Hz, 0.85 H), 5.47-5.43 (t, *J* = 8.0 Hz, 0.4 H), 5.37-5.33 (t, *J* = 8.2 Hz, 0.6 H), 4.91 (m, 1 H), 3.93-3.90 (dd, *J* = 12.2 Hz, 0.6 H), 3.87-3.85 (m, 0.4 H), 3.75-3.69 (m, 1 H), 2.67-2.62 (m, 2 H), 2.62-2.54 (m, 1 H), 2.09-2.01 (m, 1 H), 1.62 (m, 2 H), 1.50-1.48 (m, 18 H), 1.44 (s, 4 H), 1.30-1.22 (m, 15 H), 0.87 (t, *J* = 7.4 Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 198.5, 198.0, 154.3, 153.7, 149.5, 149.4, 133.1, 133.0, 128.9, 128.9, 128.8, 128.5, 83.1, 83.0, 83.0 83.0, 80.4, 80.2, 75.8, 75.8, 75.2, 75.2, 59.5, 59.2, 53.7, 53.7, 53.4, 53.3, 36.2, 32.0, 31.2, 30.1, 30.0, 29.5, 59.3, 28.5, 28.2, 22.8, 14.2 ppm. HRMS (ESI) calcd. for C₂₅H₄₀NO₃ (M+H)⁺ 618.35301, found 618.35381.

tert-Butyl (2*S,4R*)-4-((di-tert-butoxyphosphoryl)oxy)-2-(4-octylbenzoyl)pyrrolidine-1-carboxylate (25 mg, 0.04 mmol) was submitted to general procedure A. **29** was obtained as a white solid (12 mg, 66%). $[\alpha]_D^{25}$ -29.3 (*c* 0.37, CHCl₃). IR (neat), ν_{\max} : 2923, 1685, 1165, 1032, 922, 513 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 8.01-7.99 (d, *J* = 7.4 Hz, 2 H), 7.42-7.40 (d, *J* = 7.8 Hz, 2 H), 5.53 (bs, 1 H), 4.98 (bs, 1 H), 3.70 (bs, 1 H), 3.44 (bs, 1 H), 2.99 (bs, 1 H), 2.73-2.70 (t, *J* = 7.6, 2 H), 2.08 (m, 1 H), 1.67-1.64 (m, 2 H), 1.34-1.25 (m, 10 H), 0.90-0.87 (t, *J* = 7.0 Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 193.0, 151.2, 130.3, 129.1, 129.0, 74.2, 61.9, 52.7, 38.0, 35.6, 31.6, 30.8, 29.1, 29.0, 28.9, 22.3, 13.0 ppm. HRMS (ESI) calcd. for C₂₅H₄₀NO₃ (M+H)⁺ 384.19344, found 384.19257.

(2S,4R)-2-(4-Octylbenzoyl)-4-(phosphonooxy)pyrrolidin-1-ium chloride (30)

21c (50 mg, 0.12 mmol) was submitted to the procedure of its enantiomer. The residue was purified by flash column chromatography (EtOAc/hexane 5:5 + 0.5% Pyridine, Rf: 0.32) to give the intermediate tert-Butyl (2*R,4S*)-4-((di-tert-butoxyphosphoryl)oxy)-2-(4-octylbenzoyl)pyrrolidine-1-carboxylate as a colorless oil (71 mg, 63%). $[\alpha]_D^{25}$ +11.0 (*c* 0.53, CHCl₃). The spectral datas matched those reported for its enantiomer.

tert-Butyl (2*R,4S*)-4-((di-tert-butoxyphosphoryl)oxy)-2-(4-octylbenzoyl)pyrrolidine-1-carboxylate (27 mg, 0.05 mmol) was submitted to general procedure A. **30** was obtained as a white solid (14 mg, 78%). $[\alpha]_D^{25}$ +27.2 (*c* 0.55, CHCl₃). The spectral datas matched those reported for its enantiomer.

(2S,4R)-2-(2-(4-Octylphenyl)acetyl)-4-(phosphonoxy)pyrrolidin-1-ium chloride (31)

23a (68 mg, 0.16 mmol) was submitted to the procedure from **31**. The resulting residue was purified by flash column chromatography (hexane/EtOAc 6:4, Rf: 0.37) to give the intermediate tert-Butyl (2*S*,4*R*)-4-((di-tert-butoxyphosphoryl)oxy)-2-(2-(4-octylphenyl)acetyl)pyrrolidine-1-carboxylate as a colorless oil (65 mg, 67 %). $[\alpha]_{\text{D}}^{25}$ -45.2 (*c* 0.65, CHCl₃). IR (neat), ν_{max} : 2925, 1696, 1393, 1262, 1160, 989 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz, mixture of rotamers), δ : 7.14-7.08 (m, 4 H), 4.78 (bs, 1 H), 4.60-4.57 (t, *J* = 8.9, 7.4 Hz, 0.47 H), 4.54-4.50 (t, *J* = 9.1, 7.7 Hz, 0.53 H), 3.91-3.67 (m, 3 H), 3.54-3.47 (ddd, *J* = 16.6, 13.4, 3.3 Hz, 1 H), 2.58-2.54 (m, 2 H), 2.30-2.25 (m, 0.53 H), 2.13-2.09 (m, 0.47 H), 1.84-1.81 (m, 1 H), 1.58-1.56 (m, 2 H), 1.58-1.40 (m, 25 H), 1.29-1.25 (m, 13 H), 0.89-0.86 (t, *J* = 7.0 Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz, mixture of rotamers), δ : 207.1, 207.1, 154.6, 153.8, 142.2, 141.9, 130.6, 130.3, 129.7, 129.6, 129.0, 128.9, 83.1, 83.1, 83.1, 83.0, 82.9, 80.9, 80.5, 75.7, 75.6, 74.8, 74.8, 63.4, 62.5, 53.8, 53.7, 53.5, 53.5, 47.6, 46.3, 32.0, 30.0, 30.0, 29.6, 29.4, 28.5, 28.4, 22.8, 14.2 ppm. HRMS (ESI) calcd. for C₂₅H₄₀NO₃ (M+H)⁺ 610.38672, found 610.38470.

tert-Butyl (2*S*,4*R*)-4-((di-tert-butoxyphosphoryl)oxy)-2-(2-(4-octylphenyl)acetyl)pyrrolidine-1-carboxylate (30 mg, 0.05 mmol) was submitted to general procedure A. **31** was obtained as a purple paste (12 mg, 57%). $[\alpha]_{\text{D}}^{25}$ +4.7 (*c* 1.35, CHCl₃). IR (neat), ν_{max} : 2922, 1724, 1514, 1173, 1009 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz), δ : 7.17 (m, 4 H), 5.00 (bs, 1 H), 4.82-4.77 (m, 1 H), 3.98-3.92 (s, 2 H), 3.60-3.57 (d, *J* = 12.7 Hz, 1 H), 3.42-3.39 (d, *J* = 9.4 Hz, 1 H), 2.83-2.78 (m, 1 H), 2.62-2.58 (m, 2 H), 2.18-2.13 (m, 1 H), 1.60 (m, 2 H), 1.33-1.29 (m, 10 H), 0.92-0.88 (t, *J* = 10.7 Hz, 3 H) ppm. ¹³C NMR (CDCl₃, 125 MHz), δ : 201.7, 142.0, 141.6, 129.5, 129.5, 129.4, 129.4, 128.5, 128.4, 128.2, 75.0, 74.4, 74.4, 64.5, 64.1, 63.8, 63.3, 52.4, 52.3, 45.1, 35.1, 31.6, 31.3, 29.2, 29.0, 28.9, 22.3, 13.0 ppm. HRMS (ESI) calcd. for C₂₅H₄₀NO₃ (M+H)⁺ 398.20909, found 398.20750.

(2R,4S)-2-(2-(4-Octylphenyl)acetyl)-4-(phosphonoxy)pyrrolidin-1-ium chloride (32)

24a (68 mg, 0.16 mmol) was submitted to the procedure of its enantiomer. The resulting residue was purified by flash column chromatography (hexane/EtOAc 6:4, Rf: 0.37) to give the intermediate tert-Butyl (2*R*,4*S*)-4-((di-tert-butoxyphosphoryl)oxy)-2-(2-(4-octylphenyl)acetyl)pyrrolidine-1-carboxylate as a colorless oil (66 mg, 68 %). $[\alpha]_{\text{D}}^{25}$ +53.1 (*c* 0.85, CHCl₃). The spectral datas matched those reported for its enantiomer.

tert-Butyl (2*R*,4*S*)-4-((di-tert-butoxyphosphoryl)oxy)-2-(2-(4-octylphenyl)acetyl)pyrrolidine-1-carboxylate (35 mg, 0.06 mmol) was submitted to general procedure A. **32** was obtained as a purple paste (15 mg, 60%). $[\alpha]_{\text{D}}^{25}$ -5.0 (*c* 1.20, CHCl₃). The spectral datas matched those reported for its enantiomer.

4.2 Biology methods

4.2.1 Compounds.

Compound stocks (2.5-50 mM) were prepared in water with the exception of **5**, **6**, **15**, **20**, **21**, **23**, **24**, **27**, **28**, **29**, and **30**, which were prepared in DMSO. FTY720 (S)-Phosphate was purchased from Cayman Chemical Company (cat# 10006408) and prepared in DMSO immediately before use. Vorinostat (suberoylanilide hydroxamic acid, SAHA) was purchased from LC Labs (cat# V-8477) and prepared in DMSO.

4.2.2 Cell Culture Studies.

FL5.12 cells (murine hematopoietic stem cells originally obtained from Craig Thompson, Memorial Sloan Kettering Cancer Center) were maintained at 5-750,000 cells/mL, with a density of 400-600,000 cells/mL the day of treatment. FL5.12 cells were cultured in RPMI 1640 (Mediatech) supplemented with 500 pg/mL recombinant murine IL-3 (cat# 575502, Biolegend), 10% Fetal Bovine Serum (Sigma-Aldrich), 1% HEPES buffer (Mediatech), 2 mM L-Glutamine (Mediatech), 55 μM β-mercaptoethanol (Sigma-Aldrich), and antibiotics. MEFs (murine embryonic fibroblasts generated in-house or obtained from Ashley Snider, Stony Brook School of Medicine) were maintained in DMEM with 4.5 g/L glucose and L-glutamine (Mediatech)

supplemented with 10% Fetal Bovine Serum (Sigma-Aldrich) and antibiotics. Cells were screened for Mycoplasma every 3 months using the Look-Out Mycoplasma PCR Detection kit (cat# MP0035, Sigma-Aldrich).

4.2.3 Viability Assays.

FL5.12 cells were treated at 25,000/mL. Viability was determined at 48 h by vital dye exclusion (4'-6-diamidino-2-phenylindole (DAPI) or propidium iodide) using flow cytometry. IC₅₀ values were calculated using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA)

4.2.4 Nutrient Transporter Expression.

Surface CD98 (4F2hc, SLC3A2) expression in FL5.12 cells plated at 225-250,000/mL was measured after a 3 h treatment using phycoerythrin (PE)-conjugated anti-mouse CD98 antibody (Biolegend). PE-conjugated rat IgG2a κ antibody (Biolegend) was used as an isotype control. 150-200,000 cells were stained in triplicate in 100 μ L FACS block (PBS with 10% FBS and 0.05% NaN₃) containing 0.25 μ L antibody on ice for 30 min, washed twice with 1 mL FACS wash (PBS containing 2% FCS and 0.05% NaN₃), and analyzed on a BD LSR II or FACSCalibur flow cytometer. Data was processed using FlowJo (Treestar), where analysis was limited to viable cells as determined by vital dye exclusion (DAPI) or forward/side scatter.

4.2.5 Vacuolation Assay.

FL5.12 cells were treated at a density of 200,000/mL for 3 hours before being imaged at 100X using brightfield microscopy on a Nikon TE2000-S fluorescence microscope equipped with DIC filters. Vacuoles in at least 5 images were qualitatively evaluated for at least 2 biological replicates in each experimental condition. Scores were assigned as follows: 0 = no vacuoles, + = some cells vacuolated, ++ = most cells have at least 1 vacuole, +++ = all cells heavily vacuolated. Two researchers scored each replicate independently to mitigate any potential biases.

4.2.6 HDAC activity assays.

HDAC activity was measured for recombinant HDAC1 (Cayman, cat# 10011617) or HeLa extract (Enzo, cat# BML-KI137) using the Enzo COLOR DE LYS HDAC colorimetric activity assay kit (cat# BML-AK501-0001) or the Cayman HDAC Fluorometric Activity Assay Kit (cat# 10011563). Reaction mixtures containing acetylated lysine substrate were incubated at 37°C for 15-30 min. After developer was added, plates were incubated for 10-15 min at 37°C and absorbance at 405 nm measured with an Epoch microplate spectrophotometer (BioTek) or 15 min at RT and fluorescence measured with a SpectraMax Gemini XS plate reader (Molecular Devices) with excitation at 355 nm and emission at 460 nm. For fluorescence measurements, each reaction was conducted \pm trichostatin A to generate corrected fluorescence values per the Cayman protocol.

4.2.7 Western blot.

Cell lysates were prepared in RIPA lysis buffer (10 mM Tris-Cl (pH 8.0), 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 140 mM NaCl) containing 1X complete protease inhibitor (Pierce cat# 88265). Where indicated, histones were acid extracted in 0.2 N HCl overnight at 4°C. Protein concentration was quantified using Pierce BCA protein assay (Thermo Scientific cat# 23223) and samples prepared in 1X NuPage sample buffer (Invitrogen cat#NP0007) with 5 mM DTT. Samples were run on 4-12% NuPAGE gels (cat# NP0336BOX), transferred to BioTrace NT nitrocellulose membranes (Pall cat# 66485), incubated 1 h in blocking solution (5% bovine serum albumin, 7.7 μ M NaN₃ in 1X TBST), incubated with primary antibodies (H3K9, cat#9649; H3K18, cat#13998; H3, cat#4499; H4K5, cat#8647; H4K8, cat#2594; H4, cat#2935, all) overnight at 4°C in blocking solution, and with secondary antibodies (IRDye 800 or 680 conjugates, cat# 926-3211 or 926-6802, LI-COR) for 1 h at RT. Membranes were analyzed on a LI-COR Odyssey SA Imager and

analyzed using Image Studio software (LI-COR).

4.2.8 Statistics.

IC₅₀ values are shown with the 95% confidence interval as calculated in GraphPad Prism. CD98 loss is given as the mean of at least 2 biological replicates.

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Biology figures; Spectroscopic data, X-ray data (See Supporting Informations)

In search of constrained FTY720 and phytosphingosine analogs as dual acting anticancer agents targeting metabolic and epigenetic pathways.

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Highlights of the submitted manuscript:

1. Pyrrolizidines inhibit transporters of cell surface expression in cancer cells
2. Substituents on pyrrolidines affect vacuolation/nutrient transporter inhibition
3. Probing functional group tolerance of the hydrophobic substituents
4. Rationale and synthesis of constrained pyrrolidine phosphate esters as dual agents