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Development of Amidine-Based Sphingosine Kinase 1 Nanomolar Inhibitors and Reduction of Sphingosine 1-Phosphate in Human Leukemia Cells†

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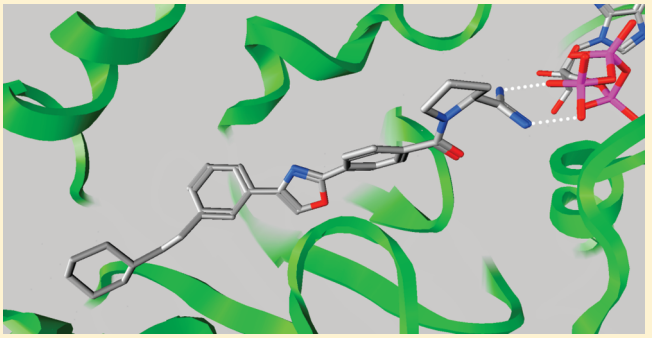
Supporting Information

ABSTRACT: Sphingosine 1-phosphate (S1P) is a bioactive lipid that has been identified as an accelerant of cancer progression. The sphingosine kinases (SphKs) are the sole producers of S1P, and thus, SphK inhibitors may prove effective in cancer mitigation and chemosensitization. Of the two SphKs, SphK1 overexpression has been observed in a myriad of cancer cell lines and tissues and has been recognized as the presumptive target over that of the poorly characterized SphK2. Herein, we present the design and synthesis of amidine-based nanomolar SphK1 subtype-selective inhibitors. A homology model of SphK1, trained with this library of amidine inhibitors, was then used to predict the activity of additional, more potent, inhibitors. Lastly, select amidine inhibitors were validated in human leukemia U937 cells, where they significantly reduced endogenous S1P levels at nanomolar concentrations.



INTRODUCTION

The scientific community has identified the sphingosine kinases (SphKs) as potential therapeutic targets for broad cancer mitigation and chemotherapeutic sensitization.[1,2] The SphKs are the sole producers of sphingosine 1-phosphate (S1P), which regulates cell survival, proliferation, neovascularization, and migration through five G-protein-coupled receptors ($S1PR_{1-5}$) and through other intracellular mechanisms.[3−7] Up-regulation of the SphK1, the first of two SphK isoforms, is found in many cancers (brain,[8,9] bladder,[10] breast,[11,12] colon,[13,14] gastric,[15] head and neck,[16,17] leukemia,[18] non-Hodgkin lymphoma,[19] prostate,[20,21] skin,[22] and squamous cell carcinoma,[23] among others), and the overproduction of S1P has been shown to aid angiogenesis, tumorigenesis, and metastasis.

Because of its deregulation in cancer, SphK1 has been implicated as a potential oncogene;[2,24] however, no genetic mutations have yet been identified, indicating that malignancies may become dependent on SphK1 through a non-oncogene addiction.[25] This theory is appealing because of the central role that S1P plays in the signal amplification of other known oncogenes. SphK1 expression and activation increase with mitogenic signaling from growth factors for a range of receptor tyrosine kinases[26] (epidermal (EGF), vascular endothelial (VEGF), platelet derived (PDGF), among others), estrogen signaling,[27] prolactin expression,[28] and lysophosphatidic acid (LPA) signaling,[29] which indicates SphK1 inhibitors may be capable of counteracting a range of oncogene-accelerated cancers. SphK1 expression has also been shown to protect rapidly dividing cells from hypoxia,[30] autophagy,[31] and chemotherapy.[32] SphK1 siRNA has been shown to slow the rate of growth of cancer cells that have SphK1 overexpression.[20,21,32,33] Breast cancer,[12] gastric cancer,[15] and glioblastoma[8,9] patients with high levels of SphK1 have shorter life expectancies. The relationship between SphK1 and cell survival can be described as linear, with increased S1P facilitating more aggressive and chemotherapeutic resistant cells and decreased S1P leading to a buildup of ceramide, its biosynthetic precursor, and ceramide dependent apoptosis.[34] Indeed, the sphingosine rheostat (Scheme 1) that governs cell fate by controlling the ratio of S1P to ceramide could be manipulated by applying the correct resistance at SphK1 with small molecule inhibitors that "dial down" S1P concentrations.

To state that the less inducible SphK2 is simply the housekeeping isoenzyme of SphK1 would be misleading. Unlike SphK1, which is cytosolic and when phosphorylated translocates to the inner leaflet of the cell membrane,[35] SphK2 is

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Scheme 1. Sphingosine Rheostat

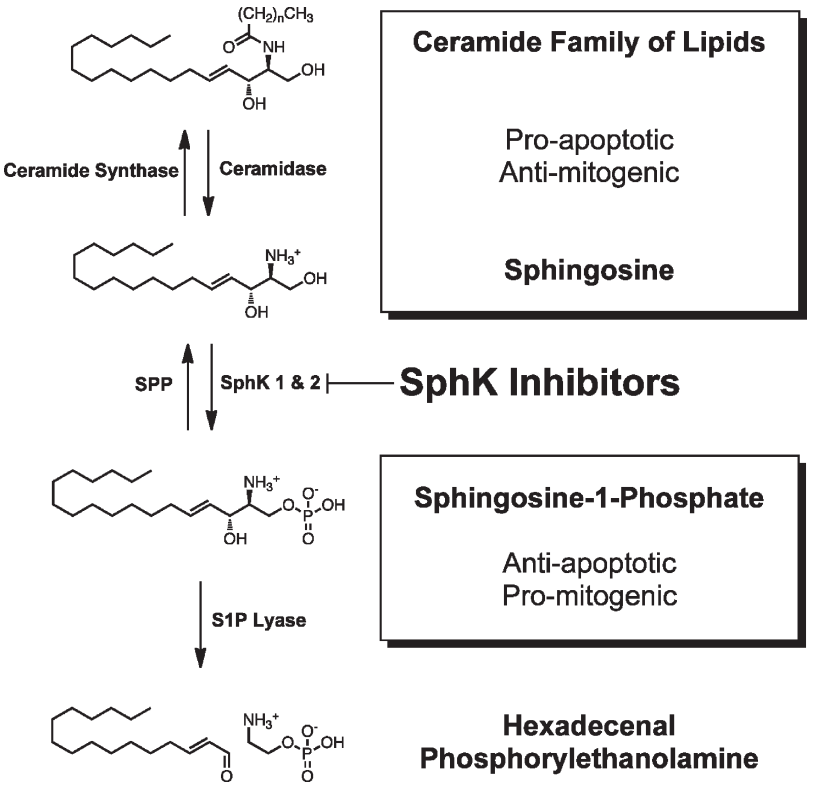
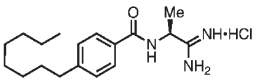
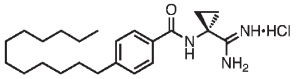
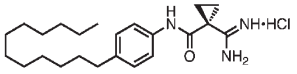


Table 1. Previously Described Amidine-Based Sphingosine Kinase Inhibitors

Compound	Strucure	K_i (μM)[a] SphK1	K_i (μM)[a] SphK2	SphK1 Selectivity[b]
VPC94075		55	20	0.7
1		0.2	0.5	5
2		0.3	6	40

[a] $K_I = [I]/(K'_M/K_M - 1)$. K_M of sphingosine at SphK1 is 10 μM. K_M of sphingosine at SphK2 is 5 μM. [b] Selectivity = $(K_I/K_M)^{SphK2}/(K_I/K_M)^{SphK1}$.

predominately located on or in the organelles, such as the ER or the nucleus.[36] Because of this location, S1P produced by SphK2 in the interior of the cell is not effectively positioned to enter into the inside-out S1P receptor signaling pathway occurring at the cell membrane and therefore does not have the same proliferative effects.[37] Instead, S1P synthesized in the nucleus by SphK2 causes histone deacetylase 1 and 2 (HDAC 1/2) inhibition, p21 gene expression, and cytostasis.[7] SphK2 overexpression causes apoptosis, which is most likely due to its degradation by the proteasome and release of a short proapoptotic BH3-domain present in SphK2 that is absent in SphK1.[38] The relationship between SphK2 and cell survival appears to be parabolic, where up-regulation leads to its degradation and caspase-mediated apoptosis, moderate activity leads to p21 expression and cell cycle arrest, and down-regulation leads to reduced p21 expression and apoptosis or proliferation depending on cell environment.[1]

If SphK inhibitors are to be used to mitigate the presentation of cancer or to retard chemotherapeutic resistance, the question must be raised: Is it necessary to selectively inhibit one of the SphKs or inhibit both enzymes together? The inducibility of SphK1 by mitogenic factors is an indication of disease causing deregulation; however, siRNA experiments demonstrate that "knocking down" SphK2 is more efficacious at retarding cell growth in two glioblastoma cell lines.[9] It is possible that the inhibitor subtype selectivity necessary for effective treatment may be cancer dependent, and our research aim is to synthesize a spectrum of dual and selective SphK inhibitors.

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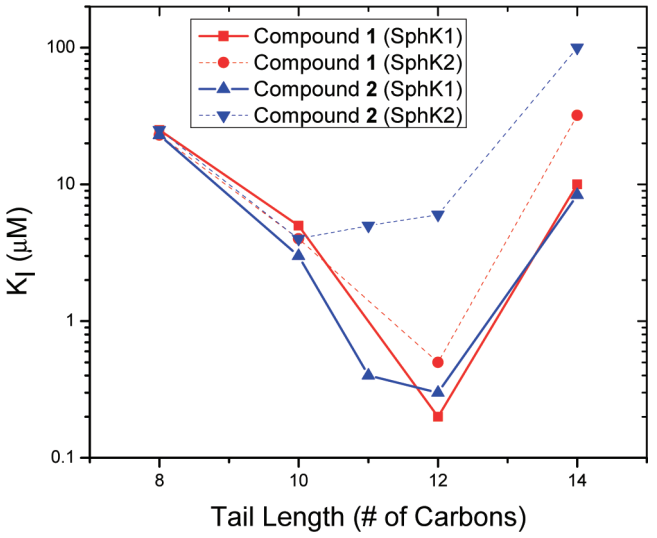

Figure 1. Comparison between tail length and potency for the amide orientations in compounds **1** and **2**. Compound **1** derivatives are shown in red. Compound **2** derivatives are shown in blue. SphK1 inhibition is shown as solid lines. SphK2 inhibition is shown as dotted lines.

Over the past few years several SphK inhibitors have appeared in the literature.[1] A large portion of these are amino alcohol sphingosine analogues that compete for the substrate binding pocket;[39−44] however, the ATP competitive SKI-II is one notable exception.[45] Indeed, sphingosine kinase inhibitors with micromolar K_I values have been effective in vivo in suppressing tumor growth in xenograft models[39,41,46] and inhibited inflammation response in Crohn's,[47] inflammatory bowl,[48] and sepsis[49] disease models. However, there is still a need for a library of potent SphK inhibitors with a range of subtype selectivities that could elucidate the currently enigmatic differences between the SphKs in cancer disease states.

Previous work has led to the generation of submicromolar dual and selective SphK inhibitors **1** and **2**, which were derivatives of the initial hit compound (*S*)-*N*-(1-amino-1-iminopropan-2-yl)-4-octylbenzamide hydrochloride (VPC94075) (Table 1).[50] These amidine-based lipids were selective for the SphKs; they did not inhibit other lipid kinases, such as the diacylglycerol kinases (DGKs), or protein kinases, such as protein kinase C (PKC). They were excellent starting points for drug optimization. The most interesting feature of the preliminary SAR was the selectivity for SphK1 induced by the direction of the amide functional group present in compounds **1** and **2**. The amide-controlled selectivity was dependent on tail length, with a maximum effect only observed in the longer tailed derivatives. Potency and selectivity are affected by tail length and amide configuration as described in Figure 1. Shorter tails (C8 and C10) inhibit both SphK1 and SphK2 equally, but the maximum potency tail length of C12 differentiates dual inhibition and SphK1 selectivity based on amide direction before potencies drop off at longer tail lengths.

These differences can be explained by the tail-binding region of the substrate pocket of SphK1 being larger than that of SphK2, which forces an altered binding position for the inhibitors and causes a repulsive electrostatic interaction for the amide configuration in compound **2**. To exploit this tail length and amide derived selectivity, inhibitors with increased terminal steric bulk and amide rigid analogues derived from proline were synthesized and tested. Scheme 2 shows the individual head and tail optimizations and subsequent partnership to generate compound **38**, which has K_I = 75 nM at SphK1 and is 80-fold selective over SphK2. The library of inhibitors synthesized was then used as a test set in the generation of a SphK1 homology model derived from the solved structure of diacylglycerol kinase β (DGKB).[51] Lastly, a virtual library of possible linkers was docked into the SphK1 model and a class of heteroaromatic compounds with six fewer rotatable bonds was generated and synthesized. Biochemical evaluation led to the identification of the most potent inhibitors of SphK1 reported in the literature to date.[52] Oxazole **56**, which has K_I = 47 nM at SphK1 and 180-fold selectivity, and other amidine-based inhibitors described are shown to significantly reduce S1P concentrations in human leukemia U937 cells at nanomolar concentrations.

RESULTS AND DISCUSSION

Tail Modifications. The tail region was defined to be everything distal to the amidine beyond the amide bond (Scheme 2). Three major modifications were made to the scaffold of compound **2**: aryl deletion, the substitution of terminal ethers, and the substitution of terminal aromatics. The aryl deletion series was synthesized in two steps from the commercially available starting aliphatic amines and 1-cyanocyclopropanecarboxylic acid. In the example shown in Scheme 3, tetradecylamine was coupled using PyBOP to form the nitrile **3a** and then transformed under base catalyzed Pinner conditions[53] to yield the corresponding amidine **4a**.

The ether tail derivatives were then examined, and terminal steric bulk was built into the ether from the corresponding alcohol. In the example synthesis shown in Scheme 4, benzyl alcohol was coupled to 7-bromo-1-heptene using sodium hydride in DMF to form ether **5a**. The terminal olefin was reduced to an alkylborane in situ using 9-BBN and then introduced to Suzuki conditions to be coupled with 1-bromo-4-nitrobenzene to form the arylnitro **6a**. On reduction to the aniline **7a** with zinc dust and amide coupling facilitated by PyBOP to form nitrile **8a**, our standard amidine formation led to the final product **9a**.

The non-ether aromatic tails were synthesized to compare the solubility effects of introducing an ether linkage in the middle of the tail region. In the example synthesis shown in Scheme 5, benzylmagnesium bromide was catalytically converted to its organocuprate with cuprous chloride and coupled to 8-bromo-1-octene to form alkene **10a**. This olefin was identical to that of compound **5a**, with the exception of the ether linkage being substituted with a methylene, and was converted to its corresponding final product under similar chemical transformations.

The K_I values of these tail derivatives were determined by a [γ-^{32}P]ATP in vitro assay[52] of SphK enzymatic activity and are shown in Table 2. The most striking observation about the aryl deletion series **4a**−**c** was the lack of a potency response to changes in tail length. Unlike the aryl-containing analogues described in Figure 1, these saturated tails had a flat SAR in the low micromolar range but did maintain SphK1 selectivity in the longer tailed **4b** and **4c**. It was hypothesized that these more hydrophobic compounds had strong affinities for the active site but were so water insoluble that their active concentrations were small because of aggregation. The more soluble ether tails performed with a more consistent SAR, with the smaller terminal phenyl-containing **9a** being less active than the cyclohexyl **9c** by more than a log order (Table 2). The terminal cyclohexyl

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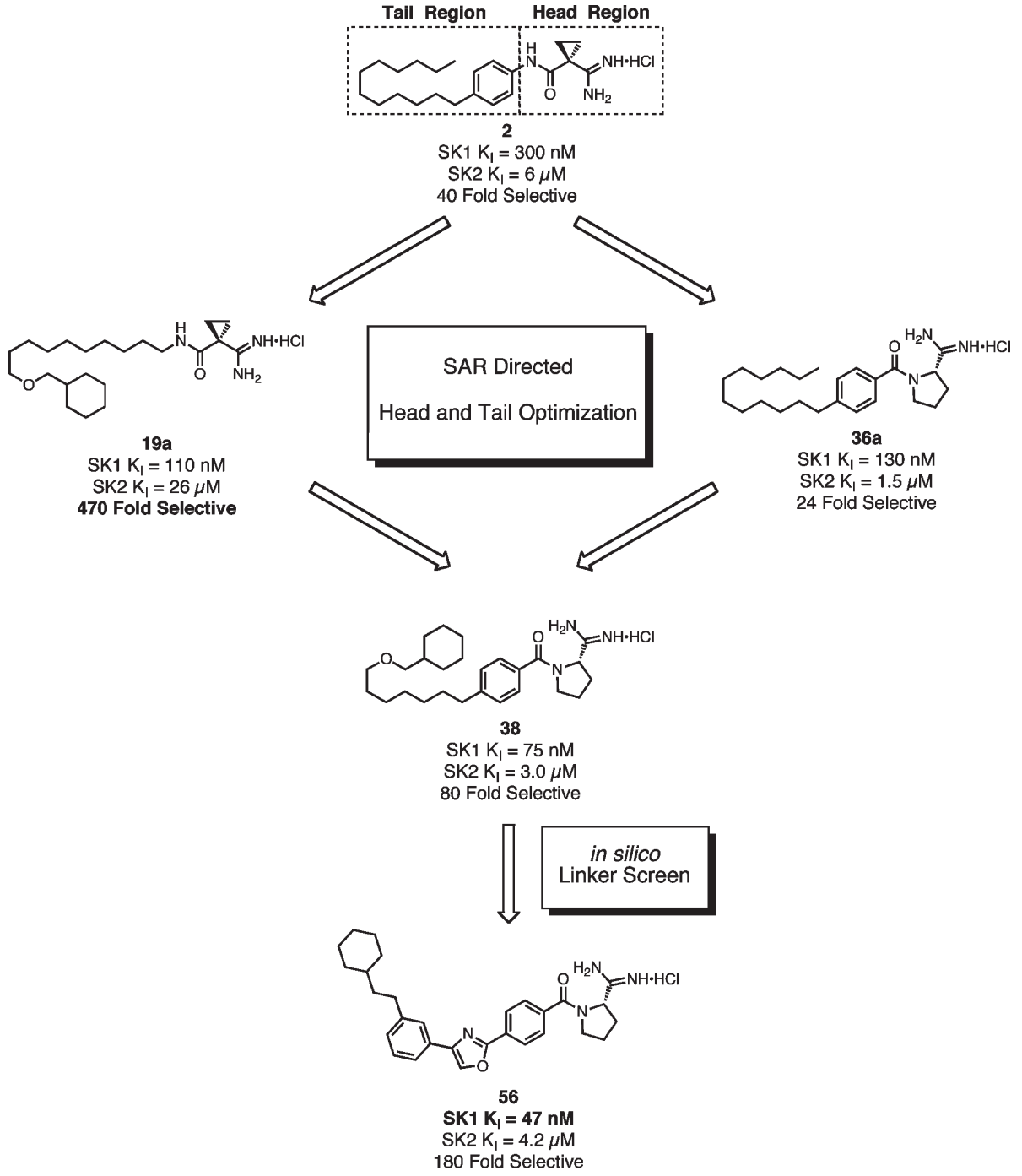
Scheme 2. Outline of Inhibitor Optimization



derivative **9c** was synthesized to evaluate saturation compared to the aromaticity of **9a**, and the positive performance of **9c** suggests a preference for the larger and more hydrophobic terminal cyclohexane. Adding further steric bulk in the adamantyl derivative **9e** caused a loss of activity and selectivity, suggesting an alternative binding conformation for such a large substituent. Short and longer cyclohexyl-containing tails, **9b** and **9d**, respectively, both performed more poorly than **9c**, indicating that is was the optimum length.

Unfortunately, compound **9c** did not yield the substantial gains in potency or selectivity that were expected but did increase water solubility to CLogP = 3.61 versus CLogP = 4.00 for compound **2**.[54] This added polar character allowed us to reconsider the aryl deletion series, and compounds **19a** and **19b** were then synthesized. Shown in Scheme 6 is the example synthesis of **19a**. Cyclohexylmethanol was coupled to 10-bromo-1-decene using sodium hydride in DMF to form ether **15a**. The terminal olefin was converted to the primary alcohol **16a** under hydroboration/oxidation conditions and then displaced to the primary azide **17a** through its mesylate. The azide **17a** was reduced and ligated using Staudinger conditions[55] to form nitrile **18a**, before being converted to amidine **19a**. Compound **19a** proved to be both

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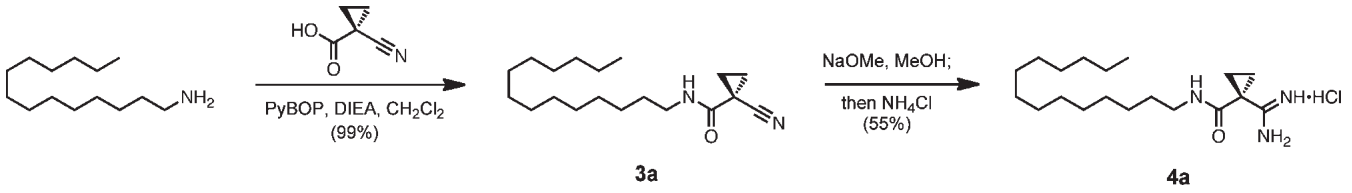
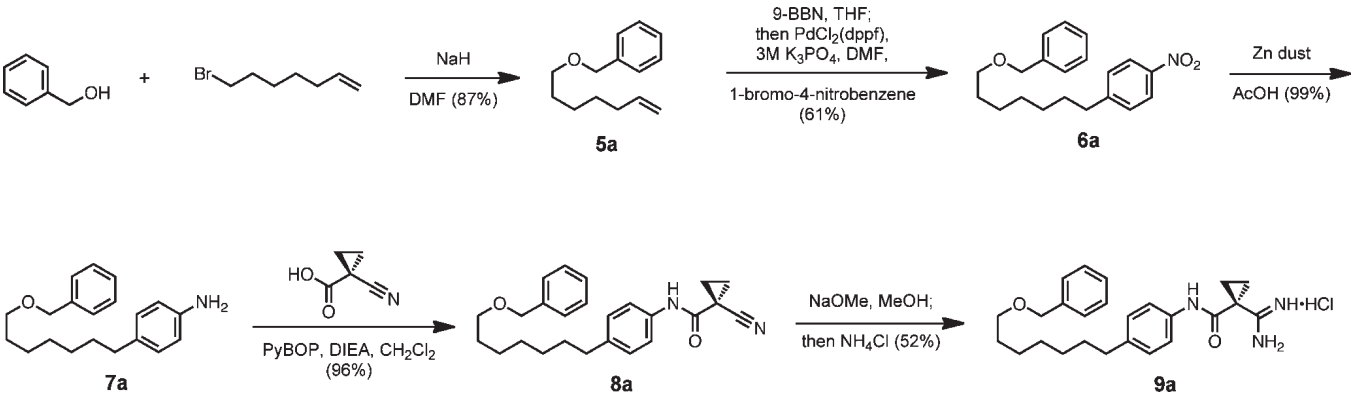
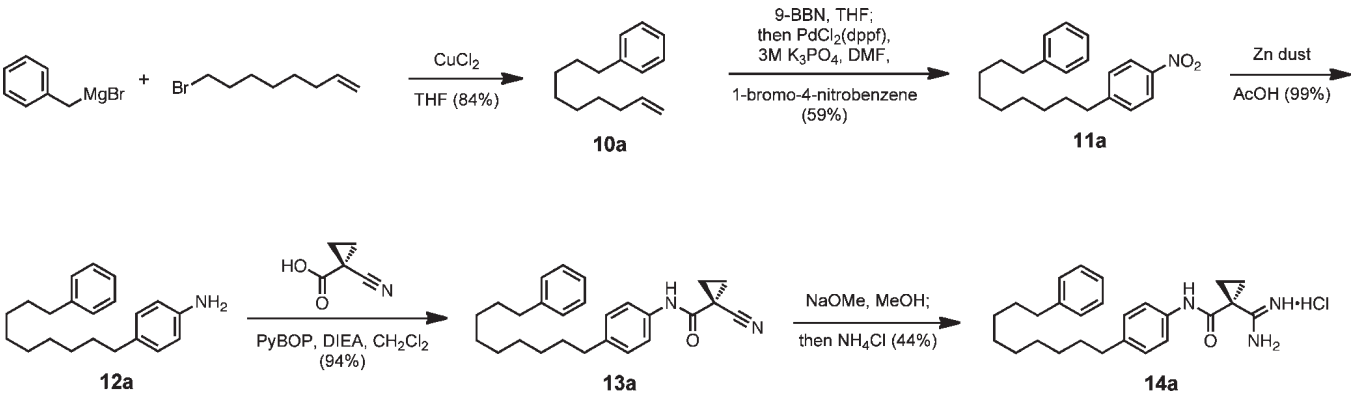
Scheme 3. Example Synthesis of an Aryl Deleted Tail Modification



Scheme 4. Example Synthesis of an Ether Tail Modification



Scheme 5. Example Synthesis of a Non-Ether Tail Modification



more potent, with K_I = 110 nM, and 470-fold selective for SphK1 over SphK2. The reduction in terminal ring size to the cyclopentyl **19b** demonstrated that the steric bulk of the six-membered saturated ring of **19a** was optimal for both potency and selectivity (Table 2).

Having achieved the design of a compound that is $2\,^1/_2$ log order selective for SphK1, our attention shifted to whether the bulkier tail design had aided selectivity in an amide-dependent manner. To test this relationship, the inverted amide derivatives of compounds **9c** and **19a** were synthesized. The synthesis of the aryl containing inverted amide is shown in Scheme 7. Starting from the same terminal alkene used in the synthesis of **9c**, the reduction of **5c** to its alkylborane and coupling under Suzuki conditions to 4-bromobenzaldehyde gave the arylaldehyde **20a**. The aldehyde was then oxidized to benzoic acid **21a** using Pinnick oxidation conditions.[56] The carboxylic acid was coupled to 1-amino-1-cyclopropanecarbonitrile through its acid chloride. Nitrile **22a** was then converted to its amidine to form the desired **23a**. The synthesis of the non-aryl inverted amide analogue **26** was relatively simple, starting with the Williamson ether coupling of cyclohexylmethanol and 11-bromoundecenoic acid (Scheme 8). The acid **24** was then coupled to 1-amino-1-cyclopropanecarbonitrile with PyBOP to form nitrile **25** and converted to the corresponding amidine **26**.

The results from the amide inversion experiments demonstrated that a cyclohexane at the tail terminus does itself increase selectivity for SphK1, as shown in the differences in activity between compounds **1** and **23a** (Table 3). Again, substitution to the smaller cyclopentane reduced activity and selectivity. It was expected that a direct ether substitution in the tail of compound **1** would lead to reduced activity against both kinases equally because of its increased solubility in water; however, compound

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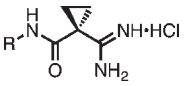
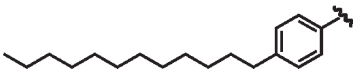
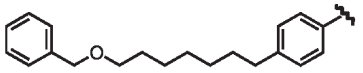
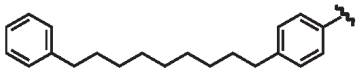
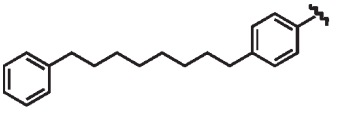
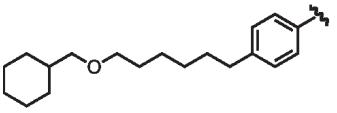
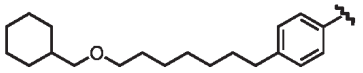
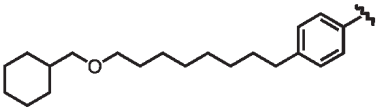
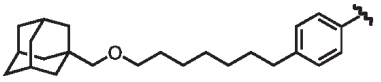
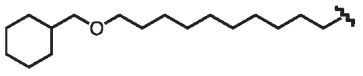
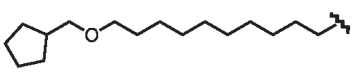
Table 2. K_I for the Tail Modified Compounds



Compound	Tail Group (R)	K_I (μM)[a] SphK1	K_I (μM)[a] SphK2	SphK1 Selectivity[b]
2		0.3	6	40
4a		6	7.5	2.5
4b		5.4	>100	>37
4c		9	>100	>22
9a		5	38	15
14a		0.50	>10	>40
14b		1.3	>10	15
9b		0.39	12	61
9c		0.24	7	58
9d		0.80	>20	>50
9e		1.5	4.6	6.1
19a		0.11	26	470
19b		0.45	25	110

[a] $K_I = [I]/(K'_M/K_M - 1)$. K_M of sphingosine at SphK1 is 10 μM. K_M of sphingosine at SphK2 is 5 μM. [b] Selectivity = $(K_I/K_M)^{SphK2}/(K_I/K_M)^{SphK1}$.

23c lost potency disproportionately leading to a slight degree of SphK1 selectivity. The selectivity was due to the position of the ether linkage along the tail, and compound **30** was synthesized (Scheme SI-1 in Supporting Information) and evaluated to show

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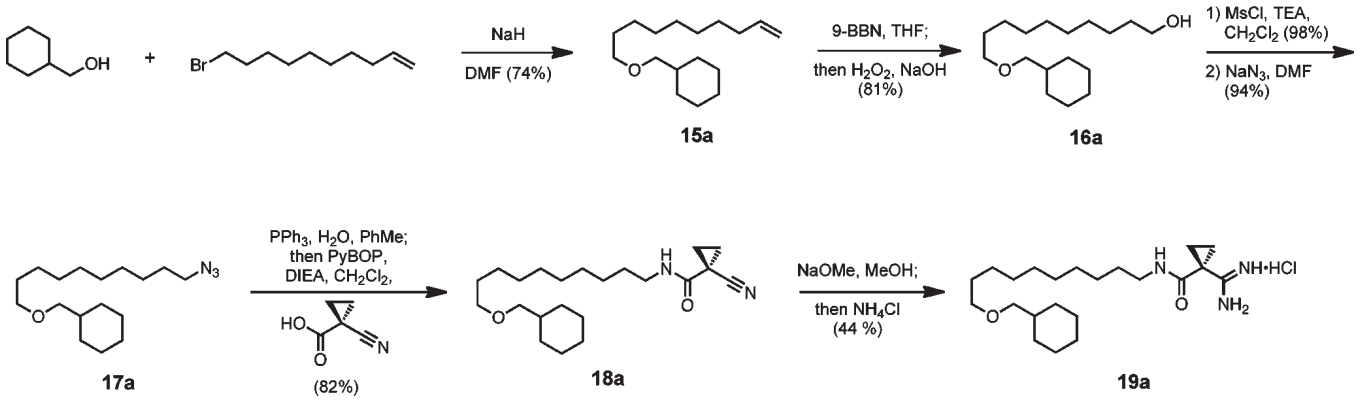
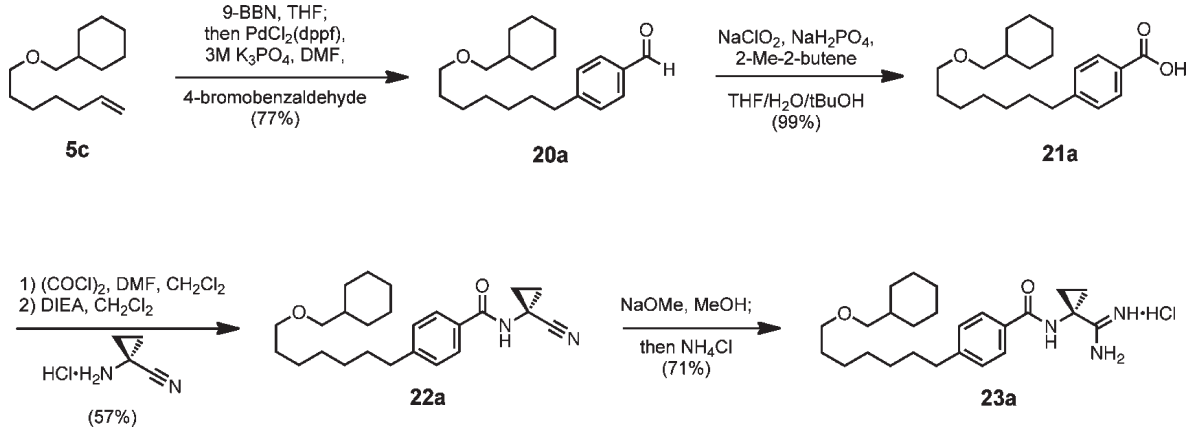
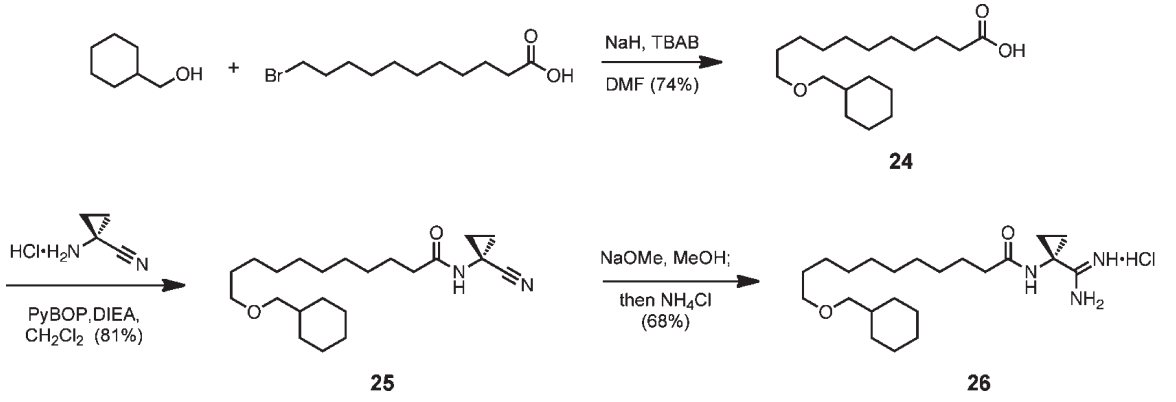
Scheme 6. Example Synthesis of Ether Containing/Aryl Deletion Tail Modified Compounds



Scheme 7. Synthesis of 23a



Scheme 8. Synthesis of 26



no such change in selectivity compared to the saturated parent compound **1**.

An important subtlety of the tail modification data is that the deletion of the aromatic ring present in **9c** and replacement with a three carbon saturated spacer as in **19a** increased both potency and selectivity (Table 2). However, the same conversion from **23a** to **26** increased potency without such an obvious effect on selectivity. One explanation is that a saturated amide increases potency and accentuates the effect that an amide already has on selectivity. On the other hand, a bulky substitution at the tail terminus, such as a cyclohexane, increases potency and selectivity regardless of amide orientation.

Head Group Modifications. An early examination of substitution α to the amidine showed that small substituents, such as

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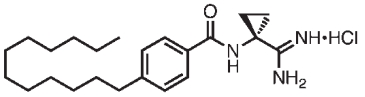
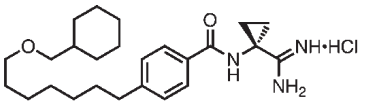
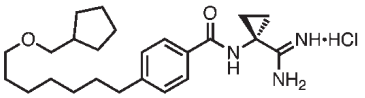
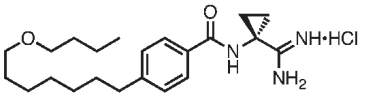
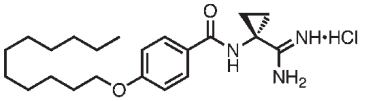
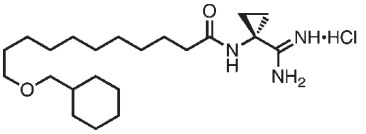
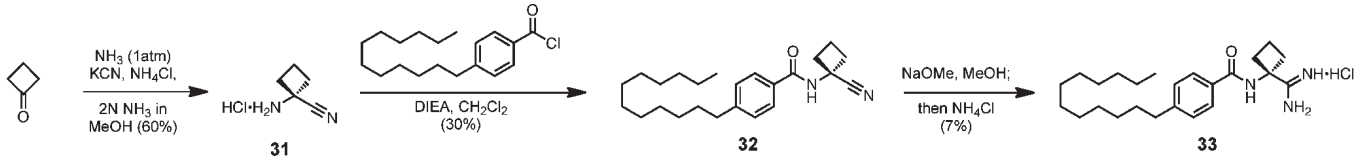
Table 3. K_I for Alternative Amide Configurations

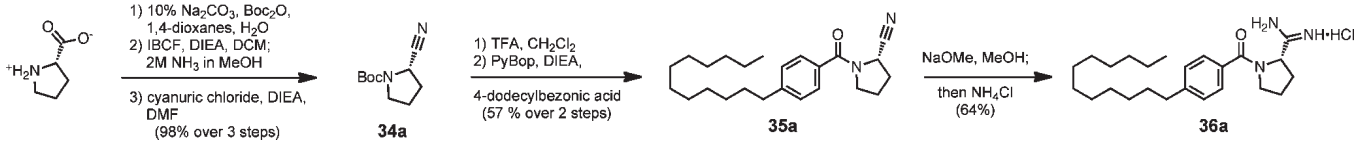
Compound	Structure	K_I (μM)[a] SphK1	K_I (μM)[a] SphK2	SphK1 Selective[b]
1		0.2	0.5	5
23a		0.13	15	240
23b		0.17	18	210
23c		1.3	40	62
30		4.0	10	5
26		0.095	12	250

[a] $K_I = [I]/(K'_M/K_M - 1)$. K_M of sphingosine at SphK1 is 10 μM. K_M of sphingosine at SphK2 is 5 μM. [b] Selectivity = $(K_I/K_M)^{SphK2}/(K_I/K_M)^{SphK1}$.

Scheme 9. Synthesis of the α,α-Cyclobutyl Head Group Analogue 33



Scheme 10. Synthesis of the Proline-Based Rigid Analogue 36a



methyl and cyclopropyl, were tolerated well by the enzyme.[50] It was therefore desirable to test a bulkier cyclobutyl derivative; however, a ring expansion to the cyclobutyl would affect the angle of presentation of the amidine, possibly hindering its function. More promising was a rigid analogue design that restricted the dihedral angle between the position of the amide and that of the amidine. Restricting a bond between such functionally important groups should have an effect on selectivity and potency. Derivatives of both enantiomers of proline provided a synthetically useful avenue to rigidity and would allow freedom of rotation about the amidine while restricting rotation of the amide.

The synthesis of the α,α-cyclobutyl analogue **33** began with the conversion of cyclobutanone under Strecker conditions to 1-amino-1-cyclobutanecarbonitrile **31** (Scheme 9). Immediate acylation with 4-dodecylbenzoyl chloride to form nitrile **32** and conversion to its amidine gave compound **33**. Next, the proline-based rigid analogue syntheses began from the corresponding asymmetric amino acid (Scheme 10). L-Proline was first *N*-Boc protected, before conversion of its carboxylic acid to the primary amide and lastly dehydration of that amide to the nitrile in compound **34a**. The Boc group was then deprotected and the free amine coupled using PyBOP to 4-dodecylbenzoic acid to form compound **35a**. The nitrile was then converted to its

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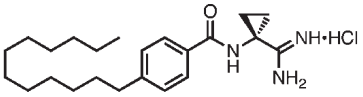
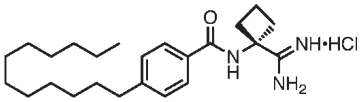
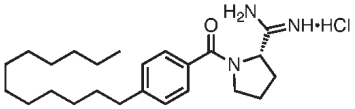
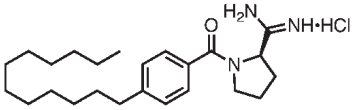
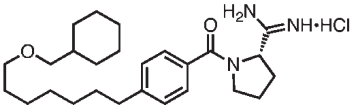
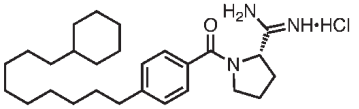
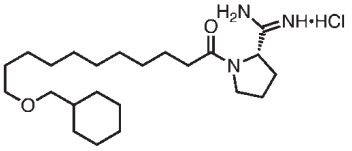
Table 4. K_I for Head Group Modifications

Compound	Structure	K_I (μM)[a] SphK1	K_I (μM)[a] SphK2	SphK1 Selectivity[b]
1		0.2	0.5	5
33		1.6	5	6.3
36a		0.13	1.5	24
36b		16	5	0.6
38		0.075	3	80
47		0.099	5.3	110
40		0.13	8	130

[a] $K_I = [I]/(K'_M/K_M - 1)$. K_M of sphingosine at SphK1 is 10 μM. K_M of sphingosine at SphK2 is 5 μM. [b] Selectivity = $(K_I/K_M)^{SphK2}/(K_I/K_M)^{SphK1}$.

amidine, and the synthesis was repeated for D-proline to produce both enantiomers.

Table 4 shows the biological evaluation of the head group analogues. As suspected, the ring expansion from cyclopropane to the cyclobutane present in **33** worsened activity equally against both SphKs. The proline analogues **36a,b** yielded selectivity as expected, with the (*S*)-configuration derived from L-proline being 24-fold more selective for SphK1, while the (*R*)-enantiomer was slightly SphK2 selective with less potency.

With compound **36a** being more potent and selective for SphK1 than compound **1**, a synthesis combining our best tail derivatives with an (*S*)-proline head group was undertaken (Scheme 11). The aryl **38** and non-aryl **40** were synthesized and evaluated to have K_I of 75 and 130 nM, respectively (Table 4). In previous series there was an increase in activity for the non-aryl over the arylamide substitution (Tables 2 and 3). However, that relationship was for mononitrogen substitution on the amide bonds, while the proline derivatives are dinitrogen substituted. For the proline arylamides, $A^{1,3}$ strain prohibits bond rotation about the carbonyl carbon−aryl bond, effectively rigidifying two bonds compared with compound **23a**. The saturated **40**, which is monosubstituted α to the carbonyl, has the ability to freely rotate and has only one rigidified bond compared with compound **26**. The potency of the proline analogues is therefore dependent on a substitution α to the amide carbonyl that inhibits bond rotation, which prepays the cost of freezing that bond prior to reaching the enzyme active site.

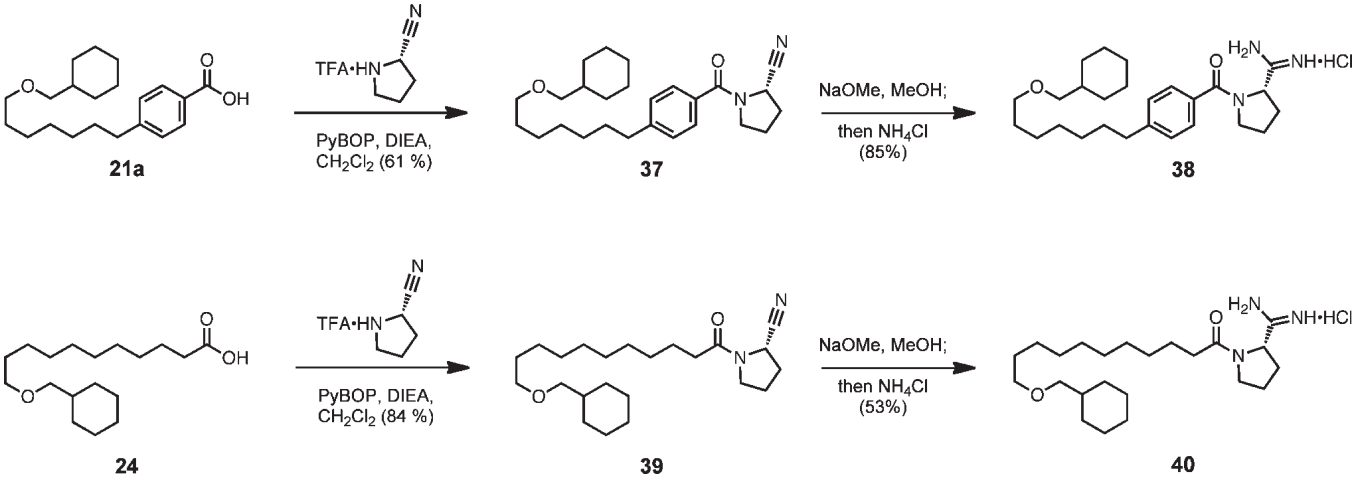
The ether present in the tail increases its calculated water solubility and in the case of **23c** reduces activity versus its non-ether counterpart **1**. A synthesis was then undertaken to eliminate the ether from compound **38** to investigate the limit of such solubility dependence. The synthesis of the non-ether **47** was completed (Scheme SI-2), and it was determined that its lower water solubility caused a decrease in activity (Table 4). The loss of activity for **47** and other compounds with high ClogP suggests an ideal ClogP of around 4.2.

In Silico Linker Screening. Crystal structures of kinases that bear close sequence homology to the ATP binding domain of the SphKs have been solved for YegS,[57,58] a bacterial lipid kinase, phosphofructokinase (PFK),[59,60] and DGKB.[51] Of these structures, DGKB has the greatest overall sequence identity of 20% to SphK1. Cases of such low sequence identity are often referred to as "twilight zone" cases,[61] and a 28 amino acid sequence that defines the substrate binding pocket of SphK1 has no meaningful sequence homology. Modelers tread lightly in such situations, and any conclusions drawn should be supported by experimental data. However, the sequence homology between the two kinases suggests that SphK1 shares the basic quaternary structure of a β-sandwich in DGKB, connected to the ATP binding domain through a hinge.

A homology model of SphK1 was generated (see Supporting Information for details pertaining to model generation and inhibitor docking) from the solved crystal structure of DGKB[51] (Figure 2A). The current library of amidine inhibitors was

dx.doi.org/10.1021/jm2001053 |J. Med. Chem. 2011, 54, 3524–3548

Scheme 11. Combination of Tail and Head Group Modifications



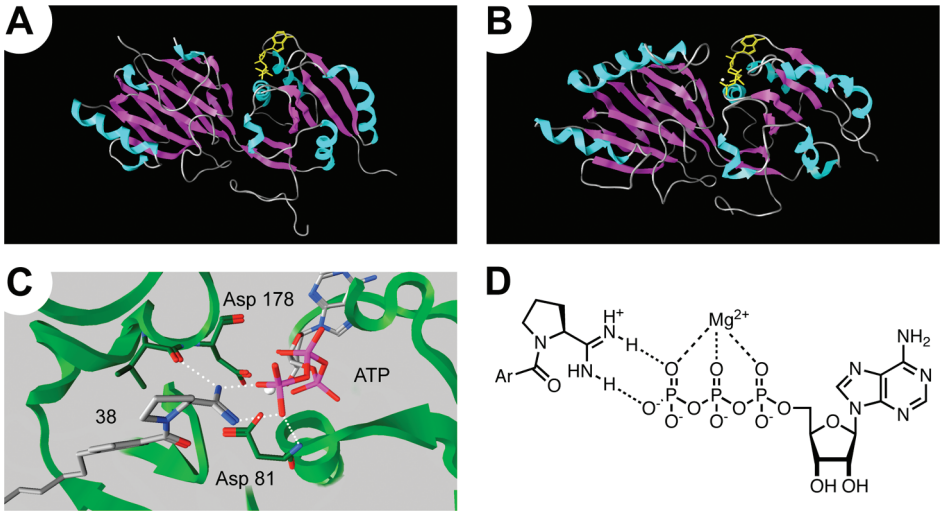


Figure 2. SphK homology model: (A) crystal structure DGKB (α-helices = teal, β-sheets = lavender, ATP = yellow stick model, and Mg^{2+} = white sphere); (B) SphK1 homologue; (C) theoretical binding orientation of compound **38** with ATP and protein backbone; (D) 2D representation of the amidine in compound **38** chelating ATP.

docked into the SphK1 model (Figure 2B) and illuminated an interesting hypothesis of how the amidine may interact with the enzyme. The model suggests that the amidine interacts directly with ATP through a bidentate chelation of its γ phosphate (Figure 2C and Figure 2D). This supports a mechanism of inhibition where SphK first binds ATP and the inhibitor, and the amidine acts to stabilize the [SphK·ATP·I] complex. Using the test set of known amidine-based inhibitors enabled the virtual screening of theoretical amidine inhibitors and a prediction of their enzymatic activity.

Long unrestricted alkyl chains have a large number of rotatable bonds, which add a large entropic cost when forced to lock into a single binding conformation. Our most potent compounds have between 11 and 15 rotatable bonds; thus, it was desirable to reduce these large degrees a freedom by incorporating linker regions that comprise as many ring structures as possible. The SphK1 model suggests a tail binding region that mostly comprises hydrophobic surface area, indicating that this region of the pocket acts as a hydrocarbon ruler designed for sphingosine recognition. Therefore, without much possibility of polar interaction the ideal tail would be one that maximizes the energy associated with ligand and pocket desolvation. Assuming the binding positions of the amidine head group and the cyclohexyl tail fragments were accurate, several hundred possible linkers were created in silico, docked into the SphK1 homology model, and scored (see Supporting Information). These potential linker regions consisted of substituted benzenes, heteroaromatics, saturated rings, fused rings, and alkyl spacers in varying order, and scaffolds were chosen for both their predicted potencies and ease of synthesis. Figure 3 shows the general scaffold picked as a proof of principle for the linker region generation. It is a proline-based rigid analogue series that includes a five-membered heterocycle with an aryl−aryl bond to another benzene that is meta-substituted by a two-carbon spacer to the terminal cyclohexane. The presence of a centralized heterocycle was ideal for solubility manipulation, and the synthesis of the X/Z imidazole, oxazole, and thiazole was undertaken to demonstrate a solubility/activity relationship. Figure 4 illustrates the linker generation process

dx.doi.org/10.1021/jm2001053 |J. Med. Chem. 2011, 54, 3524–3548

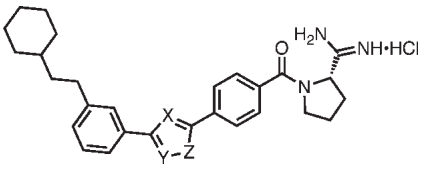


Figure 3. Generic scaffold for the linker design proof of principle.

where the docking conformation of compound **38** (Figure 4A) was fragmented into an arylamide head group and a cyclohexyl tail terminus (Figure 4B) and the in silico linker screening procedure led to a theoretical aromatic tail derivative (Figure 4C).

The synthesis of imidazole **53** began with the hydroboration of vinylcyclohexane and subsequent Suzuki coupling with 3-bromoacetophenone to form ketone **48** (Scheme 12). The ketone was then α-brominated with molecular bromine and displaced by the cesium salt of mono-*tert*-butyl protected terephthalic acid to yield ester **50**. Compound **50** was then cyclized in refluxing xylenes with ammonium acetate to produce imidazole **51**, which was deprotected and coupled to form nitrile **52**. Standard Pinner conditions then yielded the desired imidazole containing amidine **53**. The synthesis of oxazole **56** diverges from that of the imidazole at compound **50**, which is cyclized in AcOH with ammonium acetate to yield the acid deprotected oxazole **54** in one step (Scheme 13). Amide followed by amidine formation then produced the oxazole containing amidine **56**. Synthesis of the thiazole required the conversion of the mono-*tert*-butyl protected terephthalic acid to its terminal amide using isobutyl chloroformate and ammonia in methanol (Scheme 14). This terminal amide could then be transformed into the thioamide **57** using Lawesson's reagent. Thioamide **57** was smoothly coupled then cyclized with the α-bromoketone **49** to yield the thioazole **58**. *tert*-Butyl deprotection, amide formation, and then amidine synthesis produced the desired thioazole containing amidine **60**.

The SphK1 model-predicted and in vitro-determined K_I values for the heterocycle series are listed in Table 5. All three heterocycles were predicted to geometrically fit in the substrate pocket, but the SphK1 model predicted a "Goldilocks" effect based on solubility, where the oxazole **56** with a ClogP of 4.24 should have the lowest K_I of 30 nM. The imidazole **53** and the thiazole **60** were predicted to have lesser potencies due to being too polar and hydrophobic, respectively. On biological evaluation the model performed quite well, yielding the correct order of potency and predicting the actual K_I (47 nM) of the oxazole **56** within the 95% confidence limits. Indeed, the imidazole was the only compound of the three that had an experimentally determined K_I outside the 95% confidence limit, and this is probably due to the ratio of protonated versus neutral states. The pK_a of the protonated imidazole ring is predicted to be around 7 in water, and if one assumes that the charged species has K_I > 10 μM, then that ratio would proportionally reduce the activity of compound **53**. Comparing ClogP to reverse-phase HLPC retention time, which is a standard measure for comparing relative water solubilities, validates this reasoning (Figure SI-3). The retention times of the presented library of amidine containing inhibitors correlates well with ClogP (R^2 = 0.71), and compound **53** is an outlier of this trend (see Supporting Information).

In Vitro Evaluation of Inhibitors in U937 Cells. To evaluate how well these amidine-based inhibitors penetrate and reduce endogenous S1P levels in living cells, U937 cells were pretreated with compounds **1**, **19a**, **38**, and **56** for 2 h (Table 6 and Figure 5). U937 cells are a human monoblastic leukemia cell line, whose S1P levels have been reduced by micromolar concentrations of the known sphingosine kinase inhibitor dimethyl sphingosine (DMS).[40,42] The amidine-based inhibitors indeed showed inhibition at concentrations near the K_I; all showed significant S1P reduction at 100 nM. At 10 nM, lower than the K_I of all the inhibitors, S1P reduction was still observed for compounds **19a** and **38**. In other experiments (not shown), it was determined that the decreased accumulation of S1P in U937 cells was the result of blockade of synthesis rather than increased decay or export of S1P.

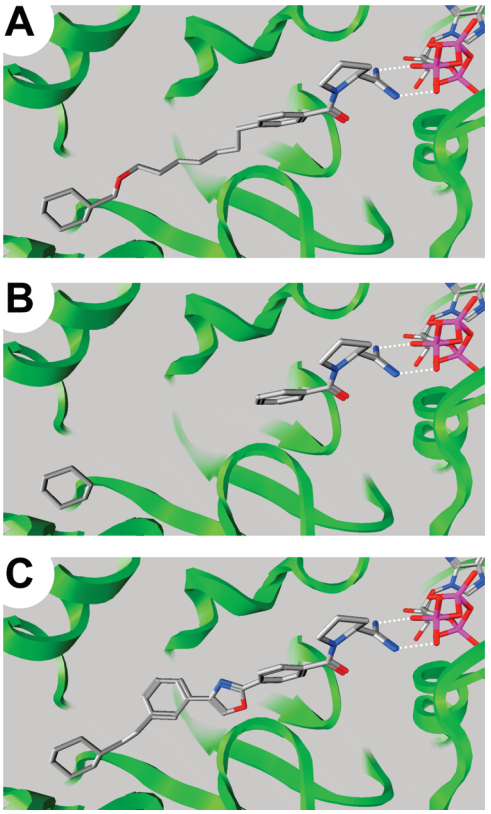
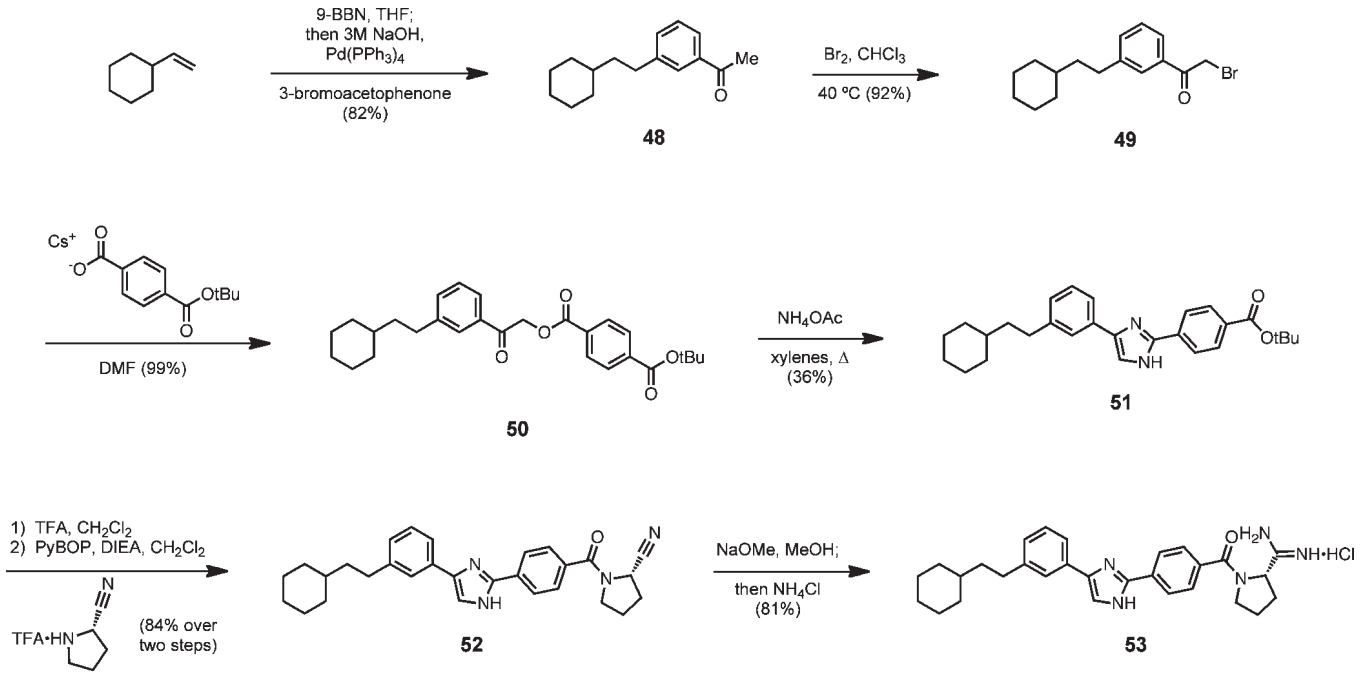


Figure 4. Progression of linker design: (A) theoretical binding orientation of compound **38** chelating ATP (both drawn as stick models); (B) deletion of aliphatic linker between the aryl amide and cyclohexyl groups in **38**; (C) generation of a new linker (**56**) with a reduced number of rotatable bonds.

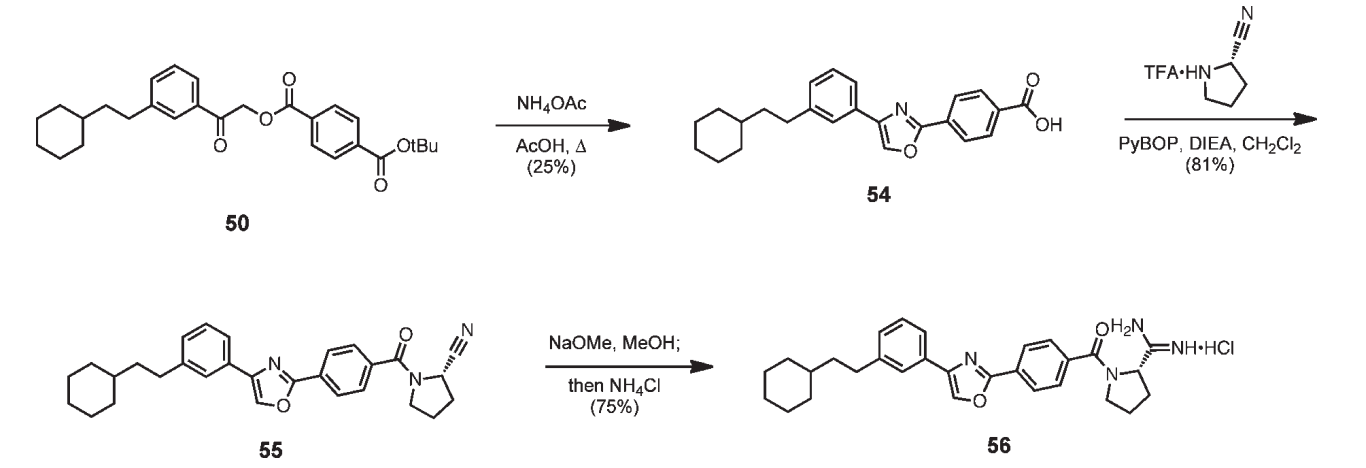
To compare these amidine based inhibitors to other known sphingosine kinase inhibitors, compounds **9ab**[44] and SKI-II[45] were also examined in living U937 cells (Table 6 and Figure 5). Compound **9ab** did not cause S1P reduction at 100 nM, which was expected given its K_I being 1.4 μM for SphK1 and 31 μM for SphK2.[52] However, at 1 μM, nearer to the K_I of compound **9ab** at SphK1, a 40% reduction of S1P is observed. Comparing the K_I values for **9ab** versus those of the SphK1 selective compound **19a**, 110 nM for SphK1 and 26 μM for SphK2 (Table 6), suggests that the observed reduction in S1P levels for **19a** is accomplished through the inhibition of SphK1. SKI-II also fits this trend with a higher SphK1 K_I of 12 μM,[52] and no significant S1P reduction was observed until 10 μM concentration was applied.

A notable outlier in the series is the performance of oxazole **56** on whole cells. With the lowest K_I in the series (47 nM for SphK1), **56** should inhibit S1P production most successfully.

Scheme 12. Synthesis of Imidazole 53



Scheme 13. Synthesis of Oxazole 56



Compound **56** does reduce S1P levels significantly, along with the other amidine inhibitors, at 100 nM, but fails to outperform compounds **19a** and **38** at 10 nM despite having the lowest K_I. This recombinant enzyme versus living cell deviation in activity is subtle and suggests differences in uptake or efflux. Interestingly, S1P reduction in U937 cells by these amidine-based inhibitors did not cause caspase-mediated apoptosis as previous reports have demonstrated with other SphK inhibitors (data not shown).[40,42] However, a more thorough investigation beyond the characterization of these inhibitors is needed to better understand these differences in cytotoxicity.

CONCLUSION

The role of the SphKs as the sole producers of S1P, a lipid promoter for tumorigenesis and angiogenesis, in the sphingosine rheostat illuminates the practicality of an anticancer strategy that targets their activity.[1] Described herein is the optimization and SAR of amidine-based SphK1 subtype-selective inhibitors. The library of inhibitors evaluated were used as a test set in the generation of a SphK1 homology model from the crystal structure of DGKB and used for the in silico design and synthesis of nanomolar SphK1 inhibitors. These inhibitors were found to significantly lower endogenous S1P levels in human leukemia U937 cells at 10 and 100 nM.

EXPERIMENTAL SECTION

Sphingosine Kinase Assay. Human SphK1 and mouse SphK2 cDNAs were used to generate mutant baculoviruses that encoded these proteins. Infection of Sf9 insect cells with the viruses for 72 h resulted in >1000-fold increases in SphK activity in 10000*g* supernatant fluid from

dx.doi.org/10.1021/jm2001053 |*J. Med. Chem.* 2011, 54, 3524–3548

Scheme 14. Synthesis of Thiazole 60

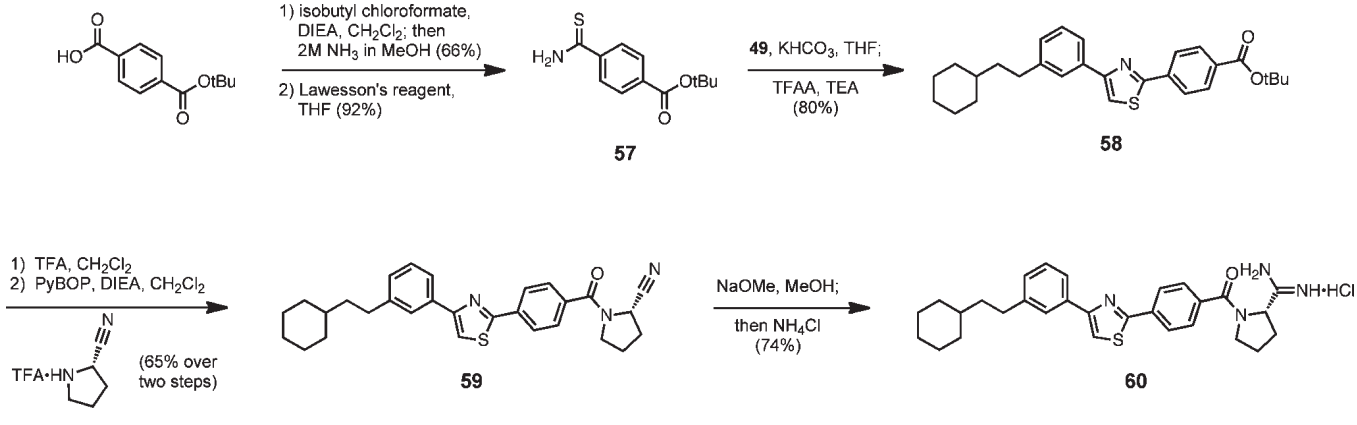
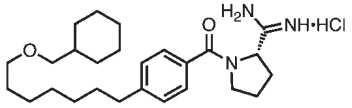
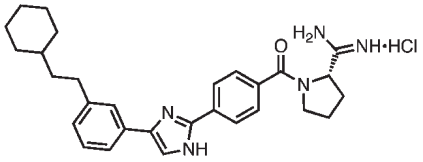
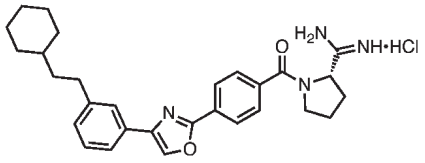
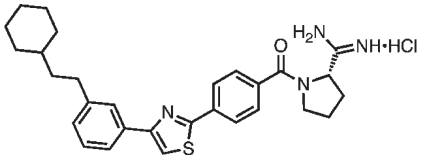


Table 5. K_I for the Heterocycle Linker Series

Compound	Structure	K_I (μM)[a] Predicted SphK1	95% Confidence Lower Limit	95% Confidence Upper Limit	SphK1	SphK2	SphK1 Selectivity[b]
38		-	-	-	0.075	3.0	80
53		0.065	0.01	0.45	2.0	20	20
56		0.031	0.005	0.21	0.047	4.2	180
60		0.035	0.006	0.24	0.11	6.0	110

[a] $K_I = [I]/(K'_M/K_M - 1)$. K_M of sphingosine at SphK1 is 10 μM. K_M of sphingosine at SphK2 is 5 μM. [b] Selectivity = $(K_I/K_M)^{SphK2}/(K_I/K_M)^{SphK1}$.

homogenized cell pellets. The enzyme assay conditions were exactly as described,[52] except infected Sf9 cell extract containing 2−3 μg of protein was used as a source of enzyme.

U937 Cell Culture Assay. U937 cells were grown according to a previously described literature procedure.[40] In general, cells were grown in RPMI 1640 medium enriched with L-glutamine, 10% penicillin and streptomycin, and 10% fetal bovine serum (FBS). Twenty-four hours before dosing with SphK inhibitors, the medium was replaced with medium containing 2% FBS. All cell cultures were grown at a stable temperature of 37 °C, and the SphK inhibitors were dosed for 2 h.

S1P Extraction and LCMS Quantification. Extraction protocols and LCMS procedures were adapted from a previously reported study.[62] Samples of pelleted cells (approximately 4 million) were taken up in 2 mL of 3:1 methanol/chloroform mixture and transferred to a capped glass vial. To this suspension was added 10 μL of internal standard solution containing 1 μM C17 S1P (purchased from Avanti Polar Lipids). The mixture was homogenized via sonication for 10 min and immediately incubated at 48 °C for 16 h. After this time, the mixture was cooled to ambient temperature and 200 μL of 1 M KOH in methanol was added to the suspension. The samples were again sonicated and incubated at 37 °C for an additional 2 h. After this time, the samples were neutralized through the addition of 30 μL of glacial acetic acid and transferred to 2 mL microcentrifuge tubes. Samples were then centrifuge at 10000g for 10 min at 4 °C. The supernatant fluid was collected in a separate glass vial, and the pellets were discarded. The resulting solution was evaporated (to a solid) with a stream of nitrogen.

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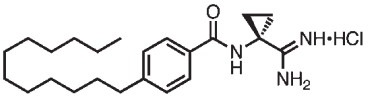
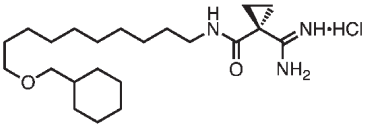
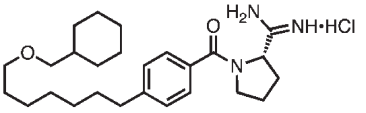
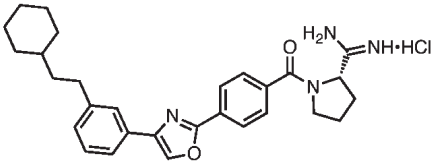
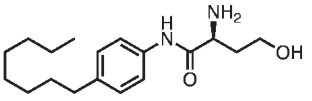
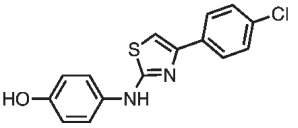
Table 6. K_I for Inhibitors Used for S1P Inhibition in Living U937 Cells

Compound	Structure	K_I (μM)[a] SphK1	K_I (μM)[a] SphK2	SphK1 Selectivity[b]
1		0.2	0.5	5
19a		0.111	26	470
38		0.075	3.0	80
56		0.047	4.2	180
9ab		1.4	31	44
SKI-II		12	33	6

[a] $K_I = [I]/(K'_M/K_M - 1)$. K_M of sphingosine at SphK1 is 10 μM. K_M of sphingosine at SphK2 is 5 μM. [b] Selectivity = $(K_I/K_M)^{SphK2}/(K_I/K_M)^{SphK1}$.

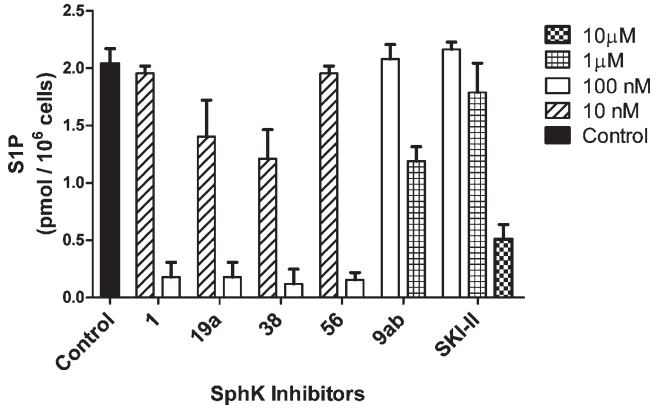


Figure 5. S1P concentrations in human leukemia U937 cells dosed with SphK inhibitors. Error bars indicate 95% confidence limits. Concentrations are indicated by shading and patterns.

Immediately prior to LCMS analysis, the solid material was taken up in 300 μL of methanol and centrifuged at 12000*g* for 12 min at 4 °C. An autosampler vial was loaded with 150 μL of the resulting supernatant for LCMS analysis.

S1P analysis from cellular extracts was performed on an Applied Biosystems 4000 QTrap LC/MS/MS instrument. Chromatographic resolution of analytes was achieved with a Shimadzu LC-20AD system. A binary solvent gradient with a flow rate of 1 mL/min was used to separate sphingolipid analytes by reverse phase chromatography (Supelco Discovery C18 column; 50 mm, 2.1 mm (length, i.d.); 5 μm bead size). Mobile phase A consisted of water/methanol/formic acid (79:20:1), and mobile phase B consisted of methanol/formic acid (99:1). The run started with 100% A for 0.5 min. Solvent B was then increased linearly for 5.1 min to 100% of the total solvent composition and held at 100% for an additional 4.3 min. The column was finally re-equilibrated to 100% for 0.1 min and held for an additional 1 min. The following analytes (and fragmentation patterns) were monitored simultaneously for identification. C17S1P (366.4, 250.4); S1P (380.4, 264.4).

General Synthetic Materials and Methods. All nonaqueous reactions were carried out in oven-dried or flame-dried glassware under an argon or nitrogen atmosphere with dry solvents and magnetic stirring, unless otherwise stated. The argon and nitrogen were dried by passing through a tube of Drierite. Anhydrous diethyl ether (Et_2O), chloroform ($CHCl_3$), dimethylsulfoxide (DMSO), toluene (PhMe), dichloromethane (CH_2Cl_2), methanol (MeOH), ethanol (EtOH), tetrahydrofuran (THF), and *N*,*N*-dimethylformamide (DMF) were purchased from Aldrich or VMR Chemicals and used as received. THF was dried over activated molecular sieves (4 Å) prior to use. All other reagents were purchased from Acros Chemicals and Aldrich Chemicals. Except as indicated otherwise, reactions were monitored by thin layer chromatography (TLC) using 0.25 mm Whatman precoated silica gel plates. Flash chromatography was performed with the indicated solvents and Dynamic Adsorbents silica gel (particle size 0.023−0.040 mm). Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a Varian UnityInova 500/51 or Varian UnityInova 300/54 at 300 K unless otherwise noted. Chemical shifts are reported in ppm (δ) values relative to the solvent as follows: $CDCl_3$ (δ 7.24 for proton and δ 77.0 for carbon NMR), DMSO-d_6 (δ 2.50 for proton and δ 39.5 for carbon NMR),

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CD_3OD (δ 3.31 for proton and δ 47.6 for carbon NMR). All high-resolution mass spectrometry was carried out by the Mass Spectrometry Laboratory in the School of Chemical Sciences at the University of Illinois Urbana-Champagne (Urbana, IL).

TLC stains were as follows. For $KMnO_4$ stain, the mixture was 3 g of $KMnO_4$ and 20 g of K_2CO_3 in 300 mL water and 5 mL of 5% NaOH. For Seebach's dip, to a solution of 25 g of phosphomolybdic acid and 7.5 g cerium(IV) sulfate in 479 mL water was added 25 mL of concentrated sulfuric acid dropwise. For the ninhydrin stain, the mixture was 1.5 g of ninhydrin in 5 mL of AcOH and 500 mL of 95% EtOH. All stains required TLC development on a hot plate set to 80 °C.

Liquid Chromatography and Mass Spectrometry for Evaluation of Chemical Purity. All compounds submitted for biological evaluation were determined to be >95% pure by LCMS evaluation performed by the Mass Spectrometry Laboratory in the School of Chemical Sciences at the University of Illinois Urbana-Champagne (Urbana, IL). High performance liquid chromatography−mass spectrometry (LCMS) was carried out using an Agilent 2.1 mm × 50 mm C-18 column and a Micromass Q-tof Ultima mass spectrometer. Mobile phase A consisted of HPLC grade H_2O and 0.01% TFA. Mobile phase B consisted of MeCN and 0.01% TFA. LCMS identification and purity utilized a binary gradient starting with 90% A and 10% B and linearly increasing to 100% B over the course of 6 min, followed by an isocratic flow of 100% B for an additional 3 min. A flow rate of 0.5 mL/min was maintained throughout the HPLC method. The purity of all products was determined by integration of the total ion count (TIC) spectra and integration of the ultraviolet (UV) spectra at 214 nm. Retention times are abbreviated as t_R; mass to charge ratios are abbreviated as m/z.

General Procedure A: Conversion of Nitriles to Amidines. To a solution of a nitrile (1.0 equiv) in MeOH (0.10 M) was added a 0.5 M solution of sodium methoxide in MeOH (0.50 equiv) at room temperature. The mixture was then heated to 50 °C for 24 h. The intermediate imidate was detectable by TLC; however, since it is in equilibrium with the nitrile, full conversion does not occur. Ammonium chloride (2.0 equiv) was then added in one portion at that temperature and allowed to react until the imidate was completely consumed by TLC analysis. The mixture was then cooled to room temperature and evacuated to dryness to yield a crude solid. The solid was reconstituted with $CHCl_3$ and filtered through a fine glass fritted funnel in order to remove excess ammonium chloride, and the filtrate was again evacuated to dryness. The material was then recrystallized in Et_2O to yield the pure amidine hydrochloride salt. The yields varied greatly depending upon substrate because amidine formation is dependent upon the equilibrium ratio between nitrile and imidate established under the sodium methoxide conditions.

General Procedure B: PyBOP Mediated Couplings of Amines and Anilines to Carboxylic Acids. To a suspension of an amine or aniline (1.0 equiv), carboxylic acid (1.0 equiv), and PyBOP (1.1 equiv) in CH_2Cl_2 at room temperature was added DIEA (4.0 equiv). The mixture was allowed to stir for 4 h unless otherwise stated. The mixture was then evaporated to dryness and immediately purified by flash chromatography.

General Procedure C: Williamson Ether Synthesis. To a solution of an alcohol (2 equiv) in DMF (0.3 M) at 0 °C was added 60% sodium hydride dispersed in mineral oil (2.0 equiv) at 0 °C. Then the mixture was allowed to warm to room temperature and then allowed to react for 45 min. The alkyl bromide was then added in one portion, and the mixture was stirred for 12 h. The reaction was quenched with saturated $NaHCO_3$ (100 × the volume of DMF) and extracted into EtOAc (100 × the volume of DMF). The organic layer was washed 3× with neat water (100 × the volume of DMF), dried with Na_2SO_4, evaporated to a yellow oil, and immediately purified by flash chromatography.

General Procedure D: Suzuki Coupling. To a solution of alkene (1.0 equiv) in THF (0.2 M) at room temperature was added a 0.5 M solution of 9-BBN in THF (2.0 equiv). The mixture was allowed to stir until consumption of the alkene was evident by TLC analysis (4 h unless otherwise stated). The mixture was then treated with 3 M $K_3PO_{4(aq)}$, and diluted with DMF (0.2 M relative to the starting alkene). The aryl bromide and $PdCl_2$(dppf) were then sequentially added, and the mixture was allowed to stir for 16 h. The mixture was diluted with EtOAc (200 × the volume of DMF) and washed 3× with neat water (100 × the volume of DMF). The organic layer was then dried with Na_2SO_4, evaporated to a dark red oil, and immediately purified by flash chromatography.

General Procedure E: Arylnitro Reduction with Zinc Dust. To a solution of an arylnitro (1.0 equiv) in AcOH (0.1 M) at room temperature was added zinc dust (10 equiv) in one portion, and the mixture was allowed to stir for 16 h. The mixture was then diluted with EtOAc and filtered through Celite. The filtrate was evaporated to dryness, coevaporated with PhMe to remove residual AcOH, and immediately purified by flash chromatography.

General Procedure F: Copper Mediated Alkane Synthesis. To a suspension of cuprous chloride (0.05 equiv) in Et_2O (1.0 M relative to the alkyl halide) at −78 °C was added a 2.0 M solution of a Gignard reagent (2 equiv) in THF followed by the addition of an alkyl halide (Br or I) (1.0 equiv). The mixture was allowed to warm to room temperature and held at that temperature until the starting alkene was judged consumed by TLC analysis (4 h unless otherwise stated). The mixture was then cooled to 0 °C and quenched with 1 N HCl (50 × the volume of Et_2O) and extracted into EtOAc (50 × the volume of Et_2O). The organic layer was then dried with Na_2SO_4, evaporated to a dark green oil, and immediately purified by flash chromatography.

General Procedure G: Hydroboration/Oxidation. To a solution of alkene (1.0 equiv) in THF (1.0 M) at room temperature was added a 0.5 M solution of 9-BBN in THF (2.0 equiv), and the mixture was allowed to stir until consumption of the alkene was evident by TLC analysis (4 h unless otherwise stated). The mixture was then cooled to 0 °C and then diluted with EtOH (10 equiv), 3 M $NaOH_{(aq)}$ (1.0 equiv) and slowly treated with 30% H_2O_2 in water (1.4 equiv) sequentially. The mixture was allowed to warm to room temperature and then was stirred for 30 min before being quenched with 1 N HCl (equal to the total volume of THF) and extracted into EtOAc (10 × the total volume of THF). The organic layer was then dried with Na_2SO_4, evaporated to a colorless oil, and immediately purified by flash chromatography.

General Procedure H: Alkyl Mesylate Formation. To a solution of an alcohol (1.0 equiv) in CH_2Cl_2 (0.3 M) at 0 °C was added TEA (2.0 equiv) and then methanesulfonyl chloride (1.1 equiv). The mixture was allowed to warm to room temperature and then was heated to reflux for 30 min. The mixture was then cooled to room temperature, quenched with saturated $NaHCO_3$ (10 × the volume of CH_2Cl_2), and extracted into $CHCl_3$ (200 × the volume of CH_2Cl_2). The organic layer was then dried with Na_2SO_4, evaporated to a yellow oil, and immediately purified by flash chromatography.

General Procedure I: Alkylazide Formation. To a solution of an alkyl mesylate (1.0 equiv) in DMF (0.3 M) at room temperature was added sodium azide (1.1 equiv), and the mixture was heated to reflux for 12 h. The mixture was then cooled to room temperature, diluted with EtOAc (100 × the volume of DMF), and washed 3× with saturated $NaHCO_3$ (100 × the volume of DMF). The organic layer was then dried with Na_2SO_4, evaporated to a yellow oil, and immediately purified by flash chromatography.

General Procedure J: Staudinger Reduction and Ligation. To a solution of an alkylazide (1.0 equiv) and triphenylphosphine (1.1 equiv) in PhMe (0.3 M) was added neat water (2.0 equiv), and the mixture was heated to reflux for 30 min. The mixture was then cooled to room temperature and treated with a solution of 1-cyano-1-cyclopanecarboxylic acid (1.5 equiv), PyBOP (1.5 equiv), and TEA (2.0 equiv) in

CH_2Cl_2 (1.5 M relative to the carboxylic acid). The mixture was allowed to stir for 4 h, then evacuated to a dark red oil, and purified by flash chromatography.

General Procedure K: Pinnick Oxidation. To a solution of an aldehyde (1.0 equiv), NaH_2PO_4 (8.0 equiv), and 2-methyl-2-butene (10 equiv) in THF, water, and ^{t}BuOH (4:4:1) (0.04 M) at room temperature was added sodium chlorite (4 equiv), and the mixture was allowed to stir for 1 h. The mixture was diluted with EtOAc (10 × the volume of the reaction's mixture of solvents) and washed 3× with 1 N HCl (5 × the volume of the reaction's mixture of solvents). The organic layer was then dried with Na_2SO_4 and evaportated to a white solid. No further purification was necessary.

General Procedure L: Acid Chloride Formation. To a solution of a carboxylic acid (1.0 equiv) and DMF (0.05 equiv) in CH_2Cl_2 (0.1 M) at 0 °C was added oxalyl chloride (2.0 equiv) dropwise, and the mixture was allowed to warm to room temperature. The mixture progresses to a yellow green color, and after 3 h the mixture was evaporated to dryness and then immediately purified by flash chromatography.

General Procedure M: Acid Chloride and Amine Coupling. To a solution of an acid chloride (1.0 equiv) in CH_2Cl_2 (0.3 M) at room temperature was added DIEA (4.0 equiv) followed by an amine HCl salt (1.5 equiv), and the mixture was stirred for 12 h. The mixture was then evaporated to dryness and immediately purified by flash chromatography.

General Procedure N: Deprotection of *N*-Boc and *O*-^{t}Bu Ester Protecting Groups. To a solution of either a *N*-Boc or *O*-^{t}Bu protecting group (1.0 equiv) in CH_2Cl_2 (0.2 M) at room temperature was added TFA (0.2 M), and the mixture was reacted until judged complete by TLC analysis (30 min unless otherwise stated). The mixture was then evaporated to dryness and taken on crude.

1-Cyano-*N*-tetradecylcyclopropanecarboxamide (3a). General procedure B was used to couple tetradecan-1-amine (213 mg, 1.00 mmol) and 1-cyano-1-cyclopanecarboxylic acid to yield the title compound. Yield, 99%. White solid. R_f = 0.50 (20% EtOAc in hexanes, $KMnO_4$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.41 (s, 1H), 3.27 (dt, J = 6.5, 5.3 Hz, 2H), 1.65 (dd, J = 8.1, 4.4 Hz, 2H), 1.52 (t, J = 6.9 Hz, 2H), 1.45 (dd, J = 8.0, 4.4 Hz, 2H), 1.36−1.11 (m, 22H), 0.86 (t, J = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.22, 120.54, 40.89, 32.14, 29.87, 29.79, 29.71, 29.58, 29.45, 27.01, 22.91, 17.65, 14.34, 13.67.

1-Cyano-*N*-hexadecylcyclopropanecarboxamide (3b). General procedure B was used to couple hexadecan-1-amine (241 mg, 1.00 mmol) and 1-cyano-1-cyclopanecarboxylic acid to yield the title compound. Yield, 99%. White solid. R_f = 0.52 (20% EtOAc in hexanes, $KMnO_4$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.37 (s, 1H), 3.28 (dt, J = 6.6, 5.3 Hz, 2H), 1.66 (dd, J = 8.1, 4.3 Hz, 2H), 1.58−1.49 (m, 2H), 1.47 (dd, J = 8.1, 4.3 Hz, 2H), 1.39−0.99 (m, 24H), 0.87 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.99, 120.33, 40.68, 31.91, 29.67, 29.56, 29.48, 29.38, 29.22, 26.78, 22.68, 17.43, 14.12, 13.55.

1-Cyano-*N*-octadecylcyclopropanecarboxamide (3c). General procedure B was used to couple octadecan-1-amine (270 mg, 1.00 mmol) and 1-cyano-1-cyclopanecarboxylic acid to yield the title compound. Yield, 99%. White solid. R_f = 0.55 (20% EtOAc in hexanes, $KMnO_4$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.44 (s, 1H), 3.26 (dt, J = 6.6, 5.3 Hz, 2H), 1.65 (dd, J = 8.1, 4.3 Hz, 2H), 1.52 (dt, J = 13.7, 6.9 Hz, 2H), 1.45 (dd, J = 8.1, 4.3 Hz, 2H), 1.26 (m, 26H), 0.86 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.96, 120.28, 40.64, 31.91, 29.68, 29.56, 29.48, 29.36, 29.22, 26.78, 22.67, 17.40, 14.11, 13.42.

1-Carbamimidoyl-*N*-tetradecylcyclopropanecarboxamide Hydrochloride (4a). General procedure A was used to convert **3a** (306 mg, 1.00 mmol) to the title compound. Yield, 55%. White solid. ^{1}H NMR (300 MHz, DMSO) δ 9.11 (s, 2H), 8.97 (s, 2H), 7.80 (t, J = 5.3 Hz, 1H), 3.01 (dd, J = 13.1, 6.5 Hz, 2H), 1.65−0.96 (m, 28H), 0.83 (t, J = 6.5 Hz, 3H). ^{13}C NMR (75 MHz, DMSO) δ 168.54, 167.92, 40.13, 31.98, 29.74, 29.56, 29.48, 29.40, 29.09, 27.05, 22.78, 14.86, 14.65. LCMS: t_R = 4.72; m/z = 324.3. HRMS m/z calcd for $C_{19}H_{38}N_3O$ (M + H), 324.3015; found, 324.3010.

1-Carbamimidoyl-*N*-hexadecylcyclopropanecarboxamide Hydrochloride (4b). General procedure A was used to convert **3b** (320 mg, 1.00 mmol) to the title compound. Yield, 46%. White solid. ^{1}H NMR (300 MHz, DMSO) δ 9.16 (s, 2H), 8.96 (s, 2H), 7.82 (s, 1H), 3.01 (d, J = 5.1 Hz, 2H), 1.51−0.97 (m, 30H), 0.83 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 168.54, 167.92, 40.04, 31.98, 29.74, 29.56, 29.49, 29.40, 27.05, 22.79, 14.86, 14.65. LCMS: t_R = 5.22; m/z = 352.3. HRMS m/z calcd for $C_{21}H_{42}N_3O$ (M + H), 352.3328; found, 352.3329.

1-Carbamimidoyl-*N*-octadecylcyclopropanecarboxamide Hydrochloride (4c). General procedure A was used to convert **3c** (362 mg, 1.00 mmol) to the title compound. Yield, 32%. White solid. ^{1}H NMR (300 MHz, DMSO) δ 9.07 (s, 2H), 8.95 (s, 2H), 7.89 (s, 1H), 3.05 (d, J = 5.1 Hz, 2H), 1.58−0.95 (m, 32H), 0.83 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 168.64, 167.95, 40.09, 32.01, 29.78, 29.59, 29.54, 29.43, 27.12, 22.80, 14.92, 14.70. LCMS: t_R = 6.22; m/z = 380.4. HRMS m/z calcd for $C_{23}H_{46}N_3O$ (M + H), 380.3641; found, 380.3633.

((Hept-6-en-1-yloxy)methyl)benzene (5a). General procedure C was used to couple benzyl alcohol and 7-bromohept-1-ene (1.00 mL, 6.52 mmol) to yield the title compound. Yield, 87%. Yellow oil. R_f = 0.88 (10% EtOAc in hexanes, $KMnO^4$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57−7.19 (m, 5H), 5.89 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H), 5.22−4.89 (m, 2H), 4.57 (s, 2H), 3.54 (t, J = 6.6 Hz, 2H), 2.14 (m, 2H), 1.82−1.61 (m, 2H), 1.59−1.40 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.16, 139.01, 128.62, 127.86, 127.73, 114.65, 73.16, 70.66, 34.08, 29.97, 29.08, 26.04.

((Hex-5-en-1-yloxy)methyl)cyclohexane (5b). General procedure C was used to couple cyclohexylmethanol and 6-bromohex-1-ene (1.00 mL, 7.48 mmol) to yield the title compound. Yield, 70%. Clear and colorless oil. R_f = 0.39 (3% EtOAc in hexanes, $KMnO^4$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.76 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H), 5.10−4.58 (m, 2H), 3.35 (t, J = 6.4, 2H), 3.15 (d, J = 6.5 Hz, 2H), 2.23−0.80 (m, 2H), 1.87−1.05 (m, 13H), 0.87 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.87, 114.59, 77.03, 71.03, 38.28, 33.80, 30.36, 29.42, 26.87, 26.10, 25.73.

((Hept-6-en-1-yloxy)methyl)cyclohexane (5c). General procedure C was used to couple cyclohexylmethanol and 7-bromohept-1-ene (1.00 mL, 6.52 mmol) to yield the title compound. Yield, 70%. Clear and colorless oil. R_f = 0.73 (5% EtOAc in hexanes, $KMnO^4$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.75 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H), 5.07−4.70 (m, 2H), 3.32 (t, J = 6.6 Hz, 2H), 3.14 (d, J = 6.6 Hz, 2H), 2.15−1.90 (m, 2H), 1.84−0.98 (m, 15H), 0.98−0.65 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.04, 114.42, 77.03, 71.18, 38.27, 33.96, 30.37, 29.80, 28.98, 26.88, 26.10, 25.91.

((Oct-7-en-1-yloxy)methyl)cyclohexane (5d). General procedure C was used to couple cyclohexylmethanol and 8-bromooct-1-ene (1.00 mL, 5.98 mmol) to yield the title compound. Yield, 77%. Clear and colorless oil. R_f = 0.43 (3% EtOAc in hexanes, $KMnO^4$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.77 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H), 5.13−4.71 (m, 2H), 3.35 (t, J = 6.6 Hz, 5H), 3.17 (d, J = 6.5 Hz, 2H), 2.12−1.91 (m, 2H), 1.86−1.01 (m, 17H), 0.98−0.74 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.18, 114.36, 77.03, 71.26, 38.29, 33.96, 30.39, 29.92, 29.19, 29.09, 26.89, 26.27, 26.11.

1-((Hept-6-en-1-yloxy)methyl)adamantane (5e). General procedure C was used to couple 1-adamantane-methanol and 7-bromohept-1-ene (1.00 mL, 6.52 mmol) to yield the title compound. Yield, 85%. Clear and colorless oil. R_f = 0.54 (5% EtOAc in hexanes, $KMnO^4$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.79 (ddt, J = 17.0, 10.2, 6.7 Hz, 1H), 5.09−4.77 (m, 2H), 3.36 (t, J = 6.5 Hz, 2H), 2.94 (s, 2H), 2.12−1.86 (m, 5H), 1.79−0.99 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.14, 114.44, 82.10, 71.78, 39.97, 37.49, 34.00, 29.68, 29.00, 28.54, 25.90.

((Hept-6-en-1-yloxy)methyl)cyclopentane (5f). General procedure C was used to couple cyclopentylmethanol and 7-bromohept-1-ene (1.00 mL, 6.56 mmol) to yield the title compound. Yield, 79%. Clear

and colorless oil. R_f = 0.69 (5% EtOAc in hexanes, $KMnO^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.98−5.57 (m, 1H), 5.10−4.79 (m, 2H), 3.38 (t, J = 6.6 Hz, 2H), 3.25 (d, J = 7.1, 2H), 2.24−1.95 (m, 3H), 1.88−1.01 (m, 14H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.13, 114.45, 75.76, 71.17, 39.68, 33.97, 29.94, 29.81, 28.99, 25.92, 25.61.

7-Butoxyhept-1-ene (5g). General procedure C was used to couple butanol and 7-bromohept-1-ene (1.50 mL, 9.84 mmol) to yield the title compound. Yield, 75%. Clear and colorless oil. R_f = 0.74 (10% EtOAc in hexanes, $KMnO_4$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.73 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H), 5.07−4.65 (m, 2H), 3.33 (m, 4H), 2.17−1.79 (m, 2H), 1.62−1.42 (m, 4H), 1.33 (m, 6H), 0.86 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.95, 114.38, 70.96, 70.75, 33.92, 32.05, 29.81, 28.95, 25.89, 19.53, 14.03.

1-(7-(Benzyloxy)heptyl)-4-nitrobenzene (6a). General procedure D was used to couple **5a** (564 mg, 2.76 mmol) and 1-bromo-4-nitrobenzene to yield the title compound. Yield, 61%. Yellow solid. R_f = 0.16 (5% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, J = 7.9 Hz, 2H), 7.45−7.19 (m, 7H), 4.51 (s, 2H), 3.48 (t, J = 6.4 Hz, 2H), 2.70 (t, J = 7.7 Hz, 2H), 1.61 (m, 4H), 1.49−1.17 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.79, 146.20, 138.67, 129.16, 128.36, 127.62, 127.50, 123.55, 72.88, 70.39, 35.83, 30.92, 29.74, 29.23, 29.11, 26.11.

1-(6-(Cyclohexylmethoxy)hexyl)-4-nitrobenzene (6b). General procedure D was used to couple **5b** (1.02 g, 4.56 mmol) and 1-bromo-4-nitrobenzene to yield the title compound. Yield, 52%. Tan oil. R_f = 0.56 (5% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, J = 8.7 Hz, 2H), 7.27 (d, J = 7.8 Hz, 2H), 3.34 (t, J = 6.5 Hz, 2H), 3.15 (d, J = 6.6 Hz, 2H), 2.80−2.58 (m, 2H), 1.86−1.00 (m, 17H), 1.00−0.77 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.97, 146.39, 129.36, 123.74, 77.05, 71.10, 38.25, 35.99, 31.15, 30.38, 29.81, 29.20, 26.88, 26.21, 26.11.

1-(7-(Cyclohexylmethoxy)heptyl)-4-nitrobenzene (6c). General procedure D was used to couple **5c** (951 mg, 4.52 mmol) and 1-bromo-4-nitrobenzene to yield the title compound. Yield, 63%. Tan oil. R_f = 0.61 (5% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, J = 8.7 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H), 3.33 (t, J = 6.5 Hz, 2H), 3.14 (d, J = 6.6 Hz, 2H), 2.75−2.53 (m, 2H), 1.84−0.96 (m, 19H), 0.95−0.70 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.99, 146.36, 129.33, 123.70, 77.03, 71.18, 38.25, 36.02, 31.13, 30.37, 29.89, 29.43, 29.32, 26.88, 26.28, 26.10.

1-(8-(Cyclohexylmethoxy)octyl)-4-nitrobenzene (6d). General procedure D was used to couple **5d** (1.02 g, 4.56 mmol) and 1-bromo-4-nitrobenzene to yield the title compound. Yield, 60%. Tan oil. R_f = 0.64 (5% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, J = 8.6 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 3.36 (t, J = 6.6 Hz, 2H), 3.17 (d, J = 6.6 Hz, 2H), 2.79−2.57 (m, 2H), 1.85−1.00 (m, 21H), 0.86 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.03, 146.41, 129.35, 123.76, 77.06, 71.26, 38.26, 36.07, 31.19, 30.39, 29.93, 29.56, 29.33, 26.89, 26.37, 26.11.

1-(((7-(4-Nitrophenyl)heptyl)oxy)methyl)adamantane (6e). General procedure D was used to couple **5e** (671 mg, 2.77 mmol) and 1-bromo-4-nitrobenzene to yield the title compound. Yield, 59%. Tan oil. R_f = 0.74 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, J = 6.6 Hz, 2H), 7.32 (m, J = 6.6 Hz, 2H), 3.36 (t, J = 6.5 Hz, 2H), 2.93 (s, 2H), 2.81−2.58 (m, 2H), 2.06−1.87 (m, 3H), 1.87−1.09 (m, 22H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.01, 142.80, 132.25, 124.11, 82.13, 71.82, 39.98, 37.48, 36.09, 29.78, 29.46, 29.37, 28.54, 26.27.

4-(7-(Benzyloxy)heptyl)aniline (7a). General procedure E was used to convert **6a** (215 mg, 0.657 mmol) to the title compound. Yield, 99%. Tan oil. R_f = 0.27 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51−7.26 (m, 5H), 7.02 (d, J = 5.3 Hz, 2H), 6.66 (m, J = 5.3 Hz, 2H), 4.57 (s, 2H), 3.52 (m, 4H), 2.70−2.42 (m, 2H), 1.80−1.53 (m, 4H), 1.42 (t, J = 8.6 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.41, 139.03, 133.17, 129.42, 128.65, 127.94, 127.78, 115.48, 73.15, 70.80, 35.37, 32.09, 30.09, 29.69, 29.52, 26.48.

4-(6-(Cyclohexylmethoxy)hexyl)aniline (7b). General procedure E was used to convert **6b** (252 mg, 0.789 mmol) to the title compound. Yield, 99%. Clear and colorless oil. R_f = 0.25 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (d, J = 8.1 Hz, 2H), 6.62 (d, J = 8.1, 2H), 3.56 (s, 2H), 3.40 (t, J = 6.2, 2H), 3.21 (d, J = 6.5, 2H), 2.51 (t, J = 7.6 Hz, 2H), 1.93−1.04 (m, 17H), 1.04−0.73 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.33, 133.10, 129.36, 115.42, 77.07, 71.33, 38.30, 35.27, 32.04, 30.44, 29.95, 29.36, 26.96, 26.34, 26.17.

4-(7-(Cyclohexylmethoxy)heptyl)aniline (7c). General procedure E was used to convert **6c** (663 g, 2.00 mmol) to the title compound. Yield, 99%. Clear and colorless oil. R_f = 0.34 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (d, J = 8.1 Hz, 2H), 6.62 (d, J = 8.2 Hz, 2H), 3.58 (s, 2H), 3.40 (t, J = 6.6 Hz, 2H), 3.23 (d, J = 6.6 Hz, 2H), 2.62−2.41 (m, 2H), 1.97−1.04 (m, 19H), 1.06−0.83 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.36, 133.13, 129.37, 115.43, 77.10, 71.38, 38.32, 35.34, 32.07, 30.47, 30.02, 29.67, 29.50, 26.98, 26.44, 26.20.

4-(8-(Cyclohexylmethoxy)octyl)aniline (7d). General procedure E was used to convert **6d** (572 mg, 1.65 mmol) to the title compound. Yield, 99%. Clear and colorless oil. R_f = 0.38 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (d, J = 8.2 Hz, 2H), 6.62 (d, J = 8.3 Hz, 2H), 3.66−3.43 (m, 2H), 3.39 (t, J = 6.7 Hz, 2H), 3.21 (d, J = 6.6 Hz, 2H), 2.59−2.39 (m, 2H), 1.93−1.02 (m, 21H), 1.06−0.80 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.26, 133.25, 129.35, 115.43, 77.08, 71.37, 38.29, 35.32, 32.07, 30.43, 29.99, 29.70, 29.48, 26.94, 26.43, 26.16.

4-Adamantan-1-ylmethoxy)heptyl)aniline (7e). General procedure E was used to convert **6e** (263 mg, 0.683 mmol) to the title compound. Yield, 99%. Clear and colorless oil. R_f = 0.31 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96 (d, J = 7.8 Hz, 2H), 6.62 (d, J = 7.1 Hz, 2H), 3.54 (s, 2H), 3.37 (t, J = 6.6 Hz, 2H), 2.95 (d, J = 5.1 Hz, 2H), 2.49 (t, J = 6.3 Hz, 2H), 1.96 (s, 3H), 1.85 −1.19 (m, 22H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.22, 133.30, 129.36, 115.44, 82.11, 71.96, 39.98, 37.49, 35.30, 32.02, 29.82, 29.62, 29.47, 28.54, 26.33.

***N*-(4-(7-(Benzyloxy)heptyl)phenyl)-1-cyanocyclopropanecarboxamide (8a).** General procedure B was used to couple **7a** (183 mg, 0.616 mmol) and 1-cyano-1-cyclopanecarboxylic acid to yield the title compound. Yield, 96%. Clear and colorless oil. R_f = 0.47 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.41 (d, J = 8.3 Hz, 2H), 7.38−7.24 (m, 5H), 7.16 (d, J = 8.3 Hz, 2H), 4.51 (s, 2H), 3.48 (t, J = 6.6 Hz, 2H), 2.68−2.42 (m, 2H), 1.78 (dd, J = 8.2, 4.3 Hz, 2H), 1.70−1.52 (m, 6H), 1.46−1.26 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.54, 140.25, 138.93, 134.78, 129.19, 128.59, 127.88, 127.72, 120.86, 120.31, 73.09, 70.69, 35.59, 31.61, 29.99, 29.56, 29.39, 26.39, 18.49, 14.34.

1-Cyano-*N*-(4-(6-(cyclohexylmethoxy)hexyl)phenyl)cyclopropanecarboxamide (8b). General procedure B was used to couple **7b** (205 mg, 0.708 mmol) and 1-cyano-1-cyclopanecarboxylic acid to yield the title compound. Yield, 92%. Clear and colorless oil. R_f = 0.48 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.32 (d, J = 8.2 Hz, 2H), 7.13 (d, J = 8.2 Hz, 2H), 3.36 (t, J = 6.5 Hz, 2H), 3.17 (d, J = 6.5 Hz, 2H), 2.67−2.25 (m, 2H), 1.90−1.02 (m, 21H), 1.02−0.52 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.50, 140.17, 134.75, 129.15, 120.83, 120.27, 77.04, 71.22, 38.27, 35.52, 31.60, 30.40, 29.88, 29.26, 26.91, 26.27, 26.13, 18.47, 14.31.

1-Cyano-*N*-(4-(7-(cyclohexylmethoxy)heptyl)phenyl)cyclopropanecarboxamide (8c). General procedure B was used to couple **7c** (288 mg, 0.950 mmol) and 1-cyano-1-cyclopanecarboxylic acid to yield the title compound. Yield, 93%. Clear and colorless oil. R_f = 0.49 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.38 (d, J = 8.2 Hz, 2H), 7.11 (d, J = 7.8 Hz, 2H), 3.35 (t, J = 6.6 Hz, 2H), 3.19 (t, J = 6.5 Hz, 2H), 2.55 (t, J = 7.6 Hz, 2H),

1.89−0.98 (m, 23H), 0.89 (dd, J = 21.8, 10.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.52, 140.17, 134.79, 129.11, 120.86, 120.25, 77.04, 71.27, 38.26, 35.57, 31.60, 30.40, 29.94, 29.55, 29.38, 26.91, 26.35, 26.13, 18.45, 14.29.

1-Cyano-*N*-(4-(8-(cyclohexylmethoxy)octyl)phenyl)cyclopropanecarboxamide (8d). General procedure B was used to couple **7d** (160 mg, 0.504 mmol) and 1-cyano-1-cyclopanecarboxylic acid to yield the title compound. Yield, 86%. Clear and colorless oil. R_f = 0.62 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.39 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H), 3.36 (t, J = 6.6 Hz, 2H), 3.18 (d, J = 6.6 Hz, 2H), 2.73−2.31 (m, 2H), 1.89−1.02 (m, 25H), 1.02−0.68 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.47, 140.31, 134.69, 129.17, 120.78, 120.28, 77.05, 71.31, 38.26, 35.58, 31.64, 30.40, 29.95, 29.64, 29.38, 26.91, 26.39, 26.13, 18.44, 14.31.

***N*-(4-(7-Adamantan-1-ylmethoxy)heptyl)phenyl)-1-cyanocyclopropanecarboxamide (8e).** General procedure B was used to couple **7e** (83 mg, 0.233 mmol) and 1-cyano-1-cyclopanecarboxylic acid to yield the title compound. Yield, 85%. Clear and colorless oil. R_f = 0.52 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.39 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.3 Hz, 2H), 3.36 (t, J = 6.6 Hz, 2H), 2.94 (d, J = 6.7 Hz, 2H), 2.70−2.43 (m, 2H), 1.95 (s, 3H), 1.85−1.28 (m, 26H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.45, 140.38, 134.60, 129.23, 120.71, 120.32, 82.10, 71.90, 39.96, 37.47, 35.58, 34.30, 31.62, 29.79, 29.54, 29.39, 28.52, 26.30, 18.48, 14.34.

***N*-(4-(7-(Benzyloxy)heptyl)phenyl)-1-carbamimidoylcyclopropanecarboxamide Hydrochloride (9a).** General procedure A was used to convert **8a** (230 mg, 0.590 mmol) to the title compound. Yield, 52%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.54 (s, 1H), 9.15 (s, 2H), 8.88 (s, 2H), 7.44 (d, J = 8.3 Hz, 2H), 7.37−7.20 (m, 5H), 7.10 (d, J = 8.3 Hz, 2H), 4.40 (s, 2H), 3.38 (t, J = 6.4 Hz, 2H), 2.49 (t, J = 7.0 Hz, 2H), 1.66−1.35 (m, 8H), 1.26 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.78, 171.30, 143.91, 143.23, 141.18, 133.40, 132.57, 132.49, 126.14, 76.96, 74.74, 39.69, 36.13, 34.34, 33.81, 33.71, 30.84, 19.96. LCMS: t_R = 4.23; m/z = 408.3. HRMS m/z calcd for $C_{25}H_{34}N_3O_2$ (M + H), 408.2651; found, 408.2646.

1-Carbamimidoyl-*N*-(4-(6-(cyclohexylmethoxy)hexyl)phenyl)cyclopropanecarboxamide Hydrochloride (9b). General procedure A was used to convert **8b** (248 mg, 0.648 mmol) to the title compound. Yield, 41%. White solid. ^{1}H NMR (300 MHz, DMSO) δ 9.67 (s, 1H), 9.03 (s, 4H), 7.47 (d, J = 8.4 Hz, 2H), 7.10 (d, J = 8.4 Hz, 2H), 3.27 (t, J = 6.3 Hz, 2H), 3.10 (d, J = 6.4 Hz, 2H), 2.49 (t, J = 5.0 Hz, 2H), 1.40 (m, 21H), 0.85 (dd, J = 21.4, 11.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.21, 171.45, 143.36, 141.52, 133.61, 126.28, 81.17, 75.51, 42.96, 39.92, 36.41, 35.01, 34.58, 33.81, 31.61, 30.96, 30.79, 20.13. LCMS: t_R = 4.44; m/z = 400.3. HRMS m/z calcd for $C_{24}H_{38}N_3O_2$ (M + H), 400.2964; found, 400.2958.

1-Carbamimidoyl-*N*-(4-(7-(cyclohexylmethoxy)heptyl)phenyl)cyclopropanecarboxamide Hydrochloride (9c). General procedure A was used to convert **8c** (352 mg, 0.888 mmol) to the title compound. Yield, 43%. White solid. ^{1}H NMR (300 MHz, DMSO) δ 9.71 (s, 1H), 9.12 (s, 4H), 7.47 (d, J = 8.4 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 3.27 (t, J = 6.4 Hz, 2H), 3.12 (d, J = 5.9 Hz, 2H), 2.49 (t, J = 7.4 Hz, 2H), 1.78−0.97 (m, 23H), 0.85 (dd, J = 21.6, 10.9 Hz, 2H). ^{13}C NMR (75 MHz, DMSO) δ 168.53, 166.67, 138.61, 136.79, 128.84, 121.51, 76.43, 70.77, 38.21, 35.19, 31.64, 30.27, 29.84, 29.32, 29.23, 26.86, 26.34, 26.05, 15.36. LCMS: t_R = 4.65; m/z = 414.3. HRMS m/z calcd for $C_{25}H_{40}N_3O_2$ (M + H), 414.3121; found, 414.3111.

1-Carbamimidoyl-*N*-(4-(8-(cyclohexylmethoxy)octyl)phenyl)cyclopropanecarboxamide Hydrochloride (9d). General procedure A was used to convert **8d** (178 mg, 0.434 mmol) to the title compound. Yield, 50%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.65 (s, 1H), 9.22 (s, 2H), 8.99 (s, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.11 (d, J = 8.3 Hz, 2H), 3.29 (t, J = 6.3 Hz, 2H), 3.11 (d, J = 6.4 Hz, 2H), 2.50 (t, J = 7.4 Hz, 2H), 1.78−0.99 (m, 25H), 0.99−0.74 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 168.19, 166.47, 138.44, 136.51, 128.62, 121.33, 76.20, 70.55, 37.98, 35.00, 31.39, 30.03, 29.62, 29.24, 28.98, 26.63, 26.14, 25.82, 15.16. LCMS: t_R = 4.86; m/z = 428.3. HRMS m/z calcd for $C_{26}H_{42}N_3O_2$ (M + H), 428.3277; found, 428.3277.

***N*-(4-(7-((Adamantan-1-ylmethoxy)heptyl)phenyl)-1-carbamimidoylcyclopropanecarboxamide Hydrochloride (9e).** General procedure A was used to convert **8e** (79 mg, 0.176 mmol) to the title compound. Yield, 22%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.56 (s, 1H), 9.17 (s, 2H), 8.88 (s, 2H), 7.44 (d, J = 8.5 Hz, 2H), 7.10 (d, J = 8.5 Hz, 2H), 3.28 (t, J = 6.4 Hz, 2H), 2.88 (s, 2H), 2.50 (m, 2H), 1.89 (m, 2H), 1.65 (d, J = 12.1 Hz, 3H), 1.60−1.25 (s, 26H). ^{13}C NMR (126 MHz, DMSO) δ 176.39, 168.07, 167.14, 166.50, 163.30, 138.48, 136.45, 134.79, 128.61, 121.35, 81.37, 71.06, 37.16, 34.94, 34.08, 31.37, 29.93, 29.44, 28.97, 28.04, 26.04, 15.18. LCMS: t_R = 5.65; m/z = 466.4. HRMS m/z calcd for $C_{29}H_{45}N_3O_2$ (M + H), 466.3434; found, 466.3424.

Non-8-en-1-ylbenzene (10a). General procedure F was used to couple benzylmagnesium bromide and 8-bromooct-1-ene (1.00 mL, 5.98 mmol) to yield the title compound. Yield, 84%. Clear and colorless oil. R_f = 0.75 (hexanes, $KMnO^4$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50−6.98 (m, 5H), 5.84 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H), 5.20−4.82 (m, 2H), 2.78−2.47 (m, 2H), 1.89 (dt, J = 7.0, 6.8 Hz, 2H), 1.64 (p, J = 7.0 Hz, 2H), 1.55−1.21 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.09, 139.38, 128.63, 128.45, 125.81, 114.67, 114.42, 36.26, 34.09, 31.80, 29.56, 29.35, 29.20.

Oct-7-en-1-ylbenzene (10b). General procedure F was used to couple benzylmagnesium bromide and 7-bromohept-1-ene (1.00 mL, 6.52 mmol) to yield the title compound. Yield, 84%. Clear and colorless oil. R_f = 0.75 (hexanes, $KMnO^4$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65−7.15 (m, 5H), 5.97 (ddt, J = 16.9, 10.0, 6.7 Hz, 1H), 5.13 (m, 2H), 2.98−2.56 (m, 2H), 2.21 (dt, J = 6.9, 6.5 Hz, 2H), 1.97−1.67 (m, 2H), 1.67−1.28 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.17, 139.42, 128.76, 128.59, 125.95, 114.60, 36.37, 34.20, 31.88, 29.56, 29.41, 29.26.

1-Nitro-4-(9-phenylnonyl)benzene (11a). General procedure D was used to couple **10a** (971 mg, 4.80 mmol) and 1-bromo-4-nitrobenzene to yield the title compound. Yield, 59%. Tan oil. R_f = 0.67 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (d, J = 8.6 Hz, 2H), 7.42−7.30 (m, 4H), 7.30−7.18 (m, 3H), 2.72 (m, 4H), 1.66 (m, 24.4 Hz, 4H), 1.39 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.12, 146.49, 143.12, 129.45, 128.70, 128.55, 125.90, 123.81, 36.33, 36.15, 31.87, 31.31, 29.82, 29.75, 29.66, 29.53.

1-Nitro-4-(8-phenyloctyl)benzene (11b). General procedure D was used to couple **10b** (1.00 g, 5.31 mmol) and 1-bromo-4-nitrobenzene to yield the title compound. Yield, 71%. Tan oil. R_f = 0.49 (5% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, J = 8.8 Hz, 2H), 7.39−7.27 (m, 4H), 7.27−7.15 (m, 3H), 2.72−2.60 (m, 4H), 1.78−1.48 (m, 4H), 1.40 (d, J = 19.9 Hz, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.09, 146.46, 143.08, 129.42, 128.67, 128.52, 125.87, 123.81, 36.25, 36.12, 31.78, 31.26, 29.67, 29.63, 29.55, 29.45.

4-(9-Phenylnonyl)aniline (12a). General procedure E was used to convert **11a** (426 mg, 1.42 mmol) to the title compound. Yield, 99%. Tan oil. R_f = 0.33 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48−7.20 (m, 5H), 7.08 (d, J = 8.3 Hz, 2H), 6.74 (d, J = 8.3 Hz, 2H), 3.58 (s, 2H), 2.87−2.66 (m, 2H), 2.66−2.53 (m, 2H), 1.86−1.57 (m, 4H), 1.42 (s, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.38, 143.27, 133.31, 129.46, 128.76, 128.58, 125.91, 115.54, 36.36, 35.45, 32.21, 31.90, 29.91, 29.71, 29.65.

4-(8-Phenyloctyl)aniline (12b). General procedure E was used to convert **11b** (572 mg, 1.65 mmol) to the title compound. Yield, 99%. Tan oil. R_f = 0.31 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52−7.20 (m, 5H), 7.11 (d, J = 8.3 Hz, 2H), 6.73 (d, J = 8.3 Hz, 2H), 3.81−3.37 (m, 2H), 2.77 (m, 2H), 2.63 (m, 2H), 1.72 (m, 4H), 1.43 (d, J = 19.9 Hz, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ

144.45, 143.29, 133.31, 129.50, 128.81, 128.63, 125.96, 115.58, 36.40, 35.49, 32.25, 31.95, 29.90, 29.75, 29.69.

1-Cyano-*N*-(4-(9-phenylnonyl)phenyl)cyclopropanecarboxamide (13a). General procedure B was used to couple **12a** (251 mg, 0.850 mmol) and 1-cyano-1-cyclopanecarboxylic acid to yield the title compound. Yield, 94%. Clear and colorless oil. R_f = 0.56 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.44 (d, J = 8.4 Hz, 2H), 7.37−7.09 (m, 7H), 2.62 (m, 4H), 1.80 (dd, J = 8.2, 4.4 Hz, 2H), 1.74−1.47 (m, 6H), 1.34 (s, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.53, 143.15, 140.34, 134.78, 129.22, 128.66, 128.49, 125.83, 120.84, 120.33, 36.26, 35.64, 31.79, 31.70, 29.76, 29.59, 29.48, 18.50, 14.37.

1-Cyano-*N*-(4-(8-phenyloctyl)phenyl)cyclopropanecarboxamide (13b). General procedure B was used to couple **12b** (485 mg, 1.72 mmol) and 1-cyano-1-cyclopanecarboxylic acid to yield the title compound. Yield, 91%. Clear and colorless oil. R_f = 0.54 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.45 (d, J = 8.4 Hz, 2H), 7.39−7.28 (m, 2H), 7.28−7.12 (m, 5H), 2.64 (m, 4H), 1.81 (dd, J = 8.2, 4.4 Hz, 2H), 1.75−1.50 (m, 6H), 1.50−1.21 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.59, 143.14, 140.29, 134.85, 129.22, 128.68, 128.52, 125.86, 120.91, 120.35, 36.27, 35.65, 31.81, 31.72, 29.72, 29.60, 29.49, 18.52, 14.38.

1-Carbamimidoyl-*N*-(4-(9-phenylnonyl)phenyl)cyclopropanecarboxamide Hydrochloride (14a). General procedure A was used to convert **13a** (312 mg, 0.803 mmol) to the title compound. Yield, 44%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.61 (s, 1H), 9.21 (s, 2H), 8.97 (s, 2H), 7.46 (d, J = 8.3 Hz, 2H), 7.29−7.19 (m, 2H), 7.18−7.05 (m, 5H), 2.58−2.48 (m, 4H), 1.69−1.31 (m, 8H), 1.23 (s, 10H). ^{13}C NMR (126 MHz, DMSO) δ 168.18, 166.48, 142.74, 138.45, 136.48, 128.68, 128.64, 126.01, 121.35, 35.59, 34.97, 31.44, 29.39, 29.26, 29.06, 29.00, 15.16. LCMS: t_R = 4.79; m/z = 406.3. HRMS m/z calcd for $C_{26}H_{36}N_3O$ (M + H), 406.2858; found, 406.2852.

1-Carbamimidoyl-*N*-(4-(9-phenylnonyl)phenyl)cyclopropanecarboxamide Hydrochloride (14b). General procedure A was used to convert **13b** (560 mg, 1.50 mmol) to the title compound. Yield, 39%. White solid. ^{1}H NMR (300 MHz, DMSO) δ 10.50 (s, 2H), 9.62 (s, 1H), 8.89 (s, 2H), 7.45 (d, J = 7.9 Hz, 2H), 7.17 (m, 7H), 2.71−2.24 (m, 4H), 1.92−0.92 (m, 14H). ^{13}C NMR (75 MHz, DMSO) δ 170.61, 168.67, 166.78, 142.98, 138.63, 136.80, 128.91, 128.87, 126.25, 123.40, 121.59, 35.82, 35.20, 31.67, 30.30, 29.49, 29.30, 29.24, 16.07, 15.23. LCMS: t_R = 4.51; m/z = 392.3. HRMS m/z calcd for $C_{25}H_{34}N_3O$ (M + H), 392.2702; found, 392.2695.

((Dec-9-en-1-yloxy)methyl)cyclohexane (15a). General procedure C was used to couple cyclohexylmethanol and 10-bromodec-1-ene (4.35 mL, 21.7 mmol) to yield the title compound. Yield, 74%. Clear and colorless oil. R_f = 0.86 (10% EtOAc in hexanes, KMnO^a). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.74 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H), 5.13−4.74 (m, 2H), 3.36 (t, J = 6.6 Hz, 2H), 3.17 (d, J = 6.6 Hz, 2H), 2.02 (dt, J = 9.9, 4.0 Hz, 2H), 1.90−0.99 (m, 21H), 0.98−0.70 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.30, 114.31, 77.05, 71.32, 38.29, 34.03, 30.40, 29.97, 29.66, 29.30, 29.14, 26.90, 26.40, 26.12.

((Dec-9-en-1-yloxy)methyl)cyclopentane (15b). General procedure C was used to couple cyclopentylmethanol and 10-bromodec-1-ene (1.00 mL, 4.98 mmol) to yield the title compound. Yield, 76%. Clear and colorless oil. R_f = 0.85 (10% EtOAc in hexanes, KMnO^a). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.80 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H), 5.17−4.75 (m, 2H), 3.39 (t, J = 6.7 Hz, 2H), 3.26 (d, J = 7.1 Hz, 2H), 2.15 (dp, J = 15.0, 7.4 Hz, 1H), 2.03 (m, 2H), 1.82−0.70 (m, 21H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.39, 114.32, 75.77, 71.29, 39.67, 34.04, 29.94, 29.83, 29.67, 29.31, 29.14, 26.40, 25.62.

10-(Cyclohexylmethoxy)decan-1-ol (16a). General procedure G was used to convert **15a** (4.00 g, 15.8 mmol) to the title compound. Yield, 81%. Clear and colorless oil. R_f = 0.68 (30% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.64 (s, 1H), 3.42 (t, J = 6.7 Hz, 2H), 3.23 (t, J = 6.7 Hz, 2H), 3.04 (d, J = 6.6 Hz, 2H), 1.67−1.48 (m, 5H), 1.48−1.32 (m, 5H), 1.24−0.93 (m, 13H), 0.85−0.66 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 76.66, 70.97, 62.19, 37.84, 32.53, 30.00, 29.54, 29.49, 29.46, 29.38, 26.54, 26.03, 25.75.

10-(Cyclopentylmethoxy)decan-1-ol (16b). General procedure G was used to convert **15b** (902 mg, 3.78 mmol) to the title compound. Yield, 85%. Clear and colorless oil. R_f = 0.65 (30% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.69 (s, 1H), 3.38 (t, J = 6.7 Hz, 2H), 3.24 (t, J = 5.9 Hz, 2H), 3.05 (d, J = 6.3 Hz, 2H), 2.14 (sept, J = 7.4 Hz, 1H), 1.96−0.49 (m, 24H).

(((10-Azidodecyl)oxy)methyl)cyclohexane (17a). General procedure H was used to convert **16a** (2.35 g, 8.69 mmol) to 10-(cyclohexylmethoxy)decyl methanesulfonate. 98%. Clear and colorless oil. R_f = 0.26 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.07 (t, J = 6.6 Hz, 2H), 3.23 (t, J = 6.5 Hz, 2H), 3.05 (d, J = 6.6, 2H), 2.86 (s, 3H), 1.69−1.48 (m, 7H), 1.48−1.35 (m, 3H), 1.35−0.94 (m, 15H), 0.86−0.68 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 76.61, 70.88, 70.15, 37.94, 36.99, 30.02, 29.62, 29.33, 29.30, 29.23, 28.98, 28.89, 26.56, 26.04, 25.78, 25.28. General procedure I was then used to convert the alkyl mesylate (2.05 g, 5.88 mmol) to the title compound. Yield, 94%. Clear and colorless oil. R_f = 0.93 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.30 (t, J = 6.6 Hz, 2H), 3.17 (t, J = 7.0 Hz, 2H), 3.12 (d, J = 6.6 Hz, 2H), 1.74−1.56 (m, 5H), 1.56−1.40 (m, 5H), 1.35−1.00 (m, 15H), 0.94−0.76 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 76.71, 70.98, 51.33, 38.03, 30.10, 29.69, 29.42, 29.37, 29.07, 28.78, 26.63, 26.12, 25.85.

(((10-Azidodecyl)oxy)methyl)cyclopentane (17b). General procedure H was used to convert **16b** (904 mg, 3.21 mmol) to 10-(cyclohexylmethoxy)decyl methanesulfonate. R_f = 0.22 (10% EtOAc in hexanes, Seebach's dip). The alkyl mesylate was not purified by flash chromatography in the example and was taken onto the next step crude. This method was later shown to be inferior to that of the synthesis of **17a**. General procedure I was then used to convert the alkyl mesylate to the title compound. Yield, 63% over two steps. Clear and colorless oil. R_f = 0.93 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.38 (t, J = 6.6 Hz, 2H), 3.29−3.14 (m, 4H), 2.24−1.94 (m, 1H), 1.93−0.55 (m, 24H).

1-Cyano-*N*-(10-(cyclohexylmethoxy)decyl)cyclopropanecarboxamide (18a). General procedure J was used to couple **17a** (1.55 g, 5.25 mmol) and 1-cyano-1-cyclopanecarboxylic acid to yield the title compound. Yield, 82%. Clear and colorless oil. R_f = 0.56 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.59 (t, J = 5.4 Hz, 1H), 3.25 (t, J = 6.6 Hz, 2H), 3.14 (dt, J = 6.6, 5.4 Hz, 2H), 3.06 (d, J = 6.6 Hz, 2H), 1.67−1.35 (m, 12H), 1.33 (m, 2H), 1.26−0.95 (m, 15H), 0.79 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.86, 120.06, 76.64, 70.92, 40.48, 37.95, 30.04, 29.63, 29.38, 29.33, 29.26, 29.11, 26.68, 26.57, 26.06, 25.79, 17.22, 13.28.

1-Cyano-*N*-(10-(cyclopentylmethoxy)decyl)cyclopropanecarboxamide (18b). General procedure J was used to couple **17b** (569 mg, 2.02 mmol) and 1-cyano-1-cyclopanecarboxylic acid to yield the title compound. Yield, 84%. Clear and colorless oil. R_f = 0.54 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.52 (s, 1H), 3.32 (t, J = 6.6 Hz, 2H), 3.19 (d, J = 7.1 Hz, 2H), 2.07 (sept, J = 7.4 Hz, 1H), 1.77−0.99 (m, 28H).

1-Carbamimidoyl-*N*-(10-(cyclohexylmethoxy)decyl)cyclopropanecarboxamide Hydrochloride (19a). General procedure A was used to convert **18a** (1.56 g, 4.30 mmol) to the title compound. Yield, 44%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.04 (s, 4H), 7.83 (t, J = 5.6 Hz, 1H), 3.28 (t, J = 6.5 Hz, 2H), 3.11 (d, J = 6.4 Hz, 2H), 3.02 (dt, J = 6.7, 5.6 Hz, 2H), 1.71−1.54 (m, 5H), 1.53−1.00 (m, 24H), 0.94−0.76 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 168.34, 167.68, 76.20, 70.56, 37.97, 30.03, 29.64, 29.47, 29.42, 29.32, 29.23, 26.82, 26.62, 26.17, 25.81, 14.62. LCMS: t_R = 4.29; m/z = 380.3. HRMS m/z calcd for $C_{22}H_{42}N_3O_2$ (M + H), 380.3277; found, 380.3268.

1-Carbamimidoyl-*N*-(10-(cyclohexylmethoxy)decyl)cyclopropanecarboxamide Hydrochloride (19b). General procedure A was used to convert **18b** (591 mg, 1.70 mmol) to the title compound. Yield, 29%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.08 (s, 4H), 7.78 (t, J = 5.4 Hz, 1H), 3.34−3.28 (m, 2H), 3.18 (d, J = 7.0 Hz, 2H), 3.03 (td, J = 6.8, 5.4 Hz, 2H), 2.12−1.95 (m, 1H), 1.68−1.57 (m, 2H), 1.57−1.31 (m, 10H), 1.31−1.10 (m, 16H). ^{13}C NMR (126 MHz, DMSO) δ 168.15, 167.73, 74.92, 70.52, 39.37, 29.63, 29.54, 29.46, 29.41, 29.34, 29.22, 28.91, 26.81, 26.17, 25.42, 16.85, 14.62. LCMS: t_R = 4.08; m/z = 366.3. HRMS m/z calcd for $C_{21}H_{40}N_3O_2$ (M + H), 366.3121; found, 366.3111.

4-(7-(Cyclohexylmethoxy)heptyl)benzaldehyde (20a). General procedure D was used to couple **5c** (1.08 g, 5.12 mmol) and 4-bromobenzaldehyde to yield the title compound. Yield, 77%. Clear and colorless oil. R_f = 0.60 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.94 (t, J = 2.0 Hz, 1H), 7.76 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 3.34 (t, J = 6.6 Hz, 2H), 3.16 (d, J = 6.6 Hz, 2H), 2.74−2.52 (m, 2H), 1.84−1.03 (m, 19H), 0.87 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.83, 150.40, 134.57, 129.96, 129.17, 76.94, 71.13, 38.22, 36.29, 31.16, 30.32, 29.88, 29.43, 29.35, 26.84, 26.27, 26.07.

4-(7-(Cyclopentylmethoxy)heptyl)benzaldehyde (20b). General procedure D was used to couple **5f** (500 mg, 2.55 mmol) and 4-bromobenzaldehyde to yield the title compound. Yield, 77%. Clear and colorless oil. R_f = 0.72 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.89 (s, 1H), 7.72 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 8.1 Hz, 2H), 3.33 (t, J = 6.6 Hz, 2H), 3.19 (d, J = 7.1 Hz, 2H), 2.68−2.49 (m, 2H), 2.08 (sept, J = 7.4 Hz, 1H), 1.77−1.37 (m, 10H), 1.28−1.06 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.97, 150.49, 134.59, 130.01, 129.22, 75.69, 71.13, 39.65, 36.34, 31.18, 29.88, 29.78, 29.45, 29.37, 26.29, 25.60.

4-(7-Butoxyheptyl)benzaldehyde (20c). General procedure D was used to couple **5g** (1.26 g, 7.39 mmol) and 4-bromobenzaldehyde to yield the title compound. Yield, 89%. Clear and colorless oil. R_f = 0.48 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.83 (s, 1H), 7.66 (d, J = 8.2 Hz, 2H), 7.19 (d, J = 8.2 Hz, 2H), 3.26 (m, 4H), 2.70−2.35 (m, 2H), 1.60−1.06 (m, 14H), 0.77 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.53, 150.12, 134.33, 129.69, 128.90, 70.68, 70.46, 36.03, 31.78, 30.90, 29.65, 29.18, 29.08, 26.02, 19.29, 13.84.

***N*-(1-Cyanocyclopropyl)-4-(7-(cyclohexylmethoxy)heptyl) benzamide (22a).** General procedure K was used to convert **20a** (1.25 g, 3.94 mmol) to the corresponding carboxylic acid **21a**, which was taken onto the next reaction crude. General procedure I was then used to convert the crude carboxylic acid to its acid chloride and was used immediately after purification. General procedure M was used to couple the acid chloride and 1-amino-1-cyclopropanecarbonitrile to yield the title compound. Yield, 57% over three steps. While solid. R_f = 0.52 (40% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (dd, J = 8.3, 2.7 Hz, 2H), 7.34−7.12 (m, 2H), 6.97 (d, J = 20.0 Hz, 1H), 3.36 (td, J = 6.6, 2.9 Hz, 2H), 3.18 (dd, J = 6.6, 2.8 Hz, 2H), 2.63 (dd, J = 10.1, 5.2 Hz, 2H), 1.87−0.99 (m, 25H), 0.99−0.73 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.63, 148.03, 130.24, 128.87, 127.66, 120.65, 77.06, 71.26, 38.22, 36.03, 31.30, 30.37, 29.90, 29.49, 29.37, 26.87, 26.31, 26.09, 21.11, 16.99.

***N*-(1-Cyanocyclopropyl)-4-(7-(cyclopentylmethoxy)heptyl)benzamide (22b).** General procedure K was used to convert **20b** (536 mg, 1.77 mmol) to is corresponding carboxylic acid **21b**, which was taken onto the next reaction crude. General procedure I was then used to convert the crude carboxylic acid to its acid chloride and was used immediately after purification. General procedure M was used to couple the acid chloride and 1-amino-1-cyclopropanecarbonitrile to yield the title compound. Yield, 61% over three steps. White solid. R_f = 0.48 (40% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.71 (d, J = 8.2 Hz, 2H), 7.15 (d, J = 8.2 Hz, 2H), 3.36 (t, J = 9.6 Hz, 2H), 3.22 (d, J = 7.2 Hz, 2H), 2.58 (t, J = 6.6 Hz, 2H), 2.18−2.00 (m, 1H), 1.80−0.98 (m, 22H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.68, 147.90, 130.29, 128.80, 127.71, 120.70, 75.76, 71.20, 39.63, 36.02, 31.29, 29.87, 29.81, 29.49, 29.36, 26.31, 25.59, 21.11, 16.92.

4-(7-Butoxyheptyl)-*N*-(1-cyanocyclopropyl)benzamide (22c). General procedure K was used to convert **20c** (1.40 g, 5.07 mmol) to the corresponding carboxylic acid **21c**, which was taken onto the next reaction crude. General procedure I was then used to convert the crude carboxylic acid to its acid chloride and was used immediately after purification. General procedure M was used to couple the acid chloride and 1-amino-1-cyclopropanecarbonitrile to yield the title compound. Yield, 78% over three steps. White solid. R_f = 0.32 (40% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.73 (d, J = 8.1 Hz, 2H), 7.13 (d, J = 8.1 Hz, 2H), 3.57−3.13 (m, 4H), 2.53 (m, 2H), 1.64−1.42 (m, 8H), 1.42−1.13 (m, 10H), 0.86 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.04, 147.95, 130.24, 128.83, 127.77, 120.80, 71.05, 70.83, 36.01, 32.02, 31.29, 29.92, 29.48, 29.36, 26.31, 21.15, 19.56, 16.87, 14.16.

***N*-(1-Carbamimidoylcyclopropyl)-4-(7-(cyclohexylmethoxy) heptyl)benzamide Hydrochloride (23a).** General procedure A was used to convert **22a** (890 mg, 2.25 mmol) to the title compound. Yield, 71%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.12 (s, 1H), 8.86 (s, 2H), 8.57 (s, 2H), 7.82 (d, J = 8.2 Hz, 2H), 7.26 (d, J = 8.2 Hz, 2H), 3.28 (t, J = 6.4 Hz, 2H), 3.11 (d, J = 6.4 Hz, 2H), 2.60 (t, J = 7.5 Hz, 2H), 1.78−0.52 (m, 25H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.08, 172.73, 151.72, 135.97, 133.16, 133.08, 80.94, 75.27, 42.72, 40.10, 37.82, 35.92, 34.78, 34.34, 33.80, 33.69, 31.37, 30.85, 30.55, 23.17. LCMS: t_R = 4.36; m/z = 414.3. HRMS m/z calcd for $C_{25}H_{40}N_3O_2$ (M + H), 414.3121; found, 414.3111.

***N*-(1-Carbamimidoylcyclopropyl)-4-(7-(cyclopentylmethoxy) heptyl)benzamide Hydrochloride (23b).** General procedure A was used to convert **22b** (413 mg, 1.08 mmol) to the title compound. Yield, 73%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.15 (s, 1H), 8.94 (s, 2H), 8.60 (s, 2H), 7.81 (d, J = 7.6 Hz, 2H), 7.26 (d, J = 7.6 Hz, 2H), 3.30 (t, J = 5.6 Hz, 2H), 3.17 (d, J = 7.0 Hz, 2H), 2.60 (t, J = 7.4 Hz, 2H), 2.13−1.94 (m, 1H), 1.75−1.01 (m, 22H). ^{13}C NMR (126 MHz, DMSO) δ 172.42, 167.98, 146.94, 131.23, 128.41, 128.35, 74.92, 70.49, 35.35, 33.06, 31.16, 29.59, 29.54, 29.05, 28.95, 26.10, 25.42, 18.41. LCMS: t_R = 4.29; m/z = 400.3. HRMS m/z calcd for $C_{24}H_{38}N_3O_2$ (M + H), 400.2964; found, 400.2957.

4-(7-Butoxyheptyl)-*N*-(1-carbamimidoylcyclopropyl)benzamide Hydrochloride (23c). General procedure A was used to convert **22c** (791 mg, 2.22 mmol) to the title compound. Yield, 60%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.14 (s, 1H), 8.75 (s, 4H), 7.80 (d, J = 7.7 Hz, 2H), 7.26 (d, J = 7.3 Hz, 2H), 3.29 (m, 4H), 2.60 (t, J = 7.4 Hz, 2H), 1.75−0.93 (m, 18H), 0.93−0.48 (m, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.38, 167.98, 146.95, 131.22, 128.41, 128.34, 70.33, 70.04, 35.35, 33.07, 31.79, 31.17, 29.64, 29.07, 28.94, 26.11, 19.35, 18.40, 14.23. LCMS: t_R = 4.08; m/z = 374.3. HRMS m/z calcd for $C_{22}H_{36}N_3O_2$ (M + H), 374.2808; found, 374.2802.

11-(Cyclohexylmethoxy)undecanoic Acid (24). General procedure C was used to couple cyclohexylmethanol and 11-bromoundecenoic acid (1.00 g, 3.77 mmol), with TBAB (121 mg, 0.38 mmol, 0.1 equiv) being added along with the 11-bromoundecenoic acid in one portion, to yield the title compound. Yield, 74%. Clear and colorless oil. R_f = 0.80 (60% EtOAc in hexanes, $KMnO^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.46 (s, 1H), 3.30 (d, J = 6.6 Hz, 2H), 2.21 (t, J = 7.5 Hz, 2H), 1.72−0.95 (m, 27H), 0.93−0.65 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.87, 76.69, 71.00, 40.15, 34.00, 30.03, 29.52, 29.45, 29.36, 29.32, 29.18, 29.02, 26.52, 25.78, 24.73.

***N*-(1-Cyanocyclopropyl)-11-(cyclohexylmethoxy)undecanamide (25).** General procedure B was used to couple **24** (358 mg, 3.02 mmol) and 1-amino-1-cyclopropanecarbonitirle to yield the title compound. Yield, 81%. White solid. R_f = 0.51 (20% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23−7.13 (m, 1H),

3.34 (t, J = 6.7 Hz, 2H), 3.16 (d, J = 6.6 Hz, 2H), 2.16 (t, J = 7.6 Hz, 2H), 1.78−0.99 (m, 31H), 0.86 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.62, 120.27, 76.79, 71.07, 37.95, 35.86, 30.12, 29.68, 29.49, 29.42, 29.37, 29.28, 29.14, 26.62, 26.11, 25.83, 25.28, 16.58.

***N*-(1-Carbamimidoylcyclopropyl)-11-(cyclohexylmethoxy) undecanamide Hydrochloride (26).** General procedure A was used to convert **25** (791 mg, 2.22 mmol) to the title compound. Yield, 68%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.26−7.91 (m, 5H), 3.28 (t, J = 6.4 Hz, 2H), 3.10 (d, J = 6.4 Hz, 2H), 2.09 (t, J = 7.5 Hz, 2H), 1.72−0.64 (m, 33H). ^{13}C NMR (126 MHz, DMSO) δ 174.53, 172.36, 76.19, 70.55, 37.96, 35.41, 32.38, 30.02, 29.63, 29.49, 29.44, 29.26, 29.12, 26.61, 26.15, 25.81, 24.92, 18.36. LCMS: t_R = 4.36; m/z = 380.3. HRMS m/z calcd for $C_{22}H_{42}N_3O_2$ (M + H), 380.3277; found, 380.3271.

1-Aminocyclobutanecarbonitrile Hydrochloride (31). To a solution of cyclobutanone (930 mg, 1.0 equiv) in 2 N NH_3/MeOH (0.2 M) were added potassium cyanide (2.0 equiv) and ammonium chloride (2.0 equiv). The reaction was run under ammonia gas (1 atm) at room temperature for 17 h. The mixture was then evaporated and the inorganic salts precipitated in $CHCl_3$ and removed via filtration through a fine frit. The solution was diluted in MeOH (100 mL), cooled to 0 °C, and treated with 12.1 N HCl (2.0 equiv). The mixture was allowed to stir for 5 min and then evaporated to dryness to yield the title compound. Yield, 60%. White solid. ^{1}H NMR (300 MHz, CD_3OD) δ 2.73−2.47 (m, 2H), 2.29 (dt, J = 12.7, 8.8 Hz, 2H), 2.19−1.93 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 123.66, 37.44, 35.80, 15.47.

***N*-(1-Cyanocyclobutyl)-4-dodecylbenzamide (32).** General procedure L was used to couple **31** (87 mg, 0.90 mmol) and 4-dodecylbenzoyl chloride to yield the title compound. Yield, 30%. Clear and colorless oil. R_f = 0.48 (25% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (d, J = 8.2 Hz, 2H), 7.99 (d, J = 8.7 Hz, 2H), 7.26 (s, 1H), 3.71−3.52 (m, 2H), 3.44−3.33 (m, 2H), 3.21 (dd, J = 21.0, 9.6 Hz, 2H), 3.11−2.78 (m, 2H), 2.35 (m, 2H), 2.16−1.91 (m, 18H), 1.62 (t, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.71, 147.92, 130.05, 128.73, 127.21, 120.88, 48.00, 35.86, 34.14, 31.90, 31.16, 29.63, 29.56, 29.45, 29.34, 29.22, 22.68, 16.17, 14.13.

***N*-(1-Carbamimidoylcyclobutyl)-4-dodecylbenzamide (33).** General procedure A was used to convert **32** (100 mg, 0.27 mmol) to the title compound. Yield, 7%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.15 (s, 1H), 8.82 (s, 4H), 7.88 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.2 Hz, 2H), 2.68−2.56 (m, 4H), 2.42 (dd, J = 17.6, 11.1 Hz, 2H), 2.10−1.96 (m, 1H), 1.96−1.82 (m, 1H), 1.55 (pent, J = 6.8 Hz, 2H), 1.25 (m, 18H), 0.85 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.70, 166.46, 146.63, 130.64, 128.08, 127.95, 57.31, 34.98, 31.32, 31.01, 30.80, 29.03, 28.85, 28.73, 28.58, 22.12, 15.05, 13.99. LCMS: t_R = 5.29; m/z = 386.3. HRMS m/z calcd for $C_{24}H_{40}N_3O$ (M + H), 386.3171; found, 386.3178.

(*S*)-*tert*-Butyl 2-Cyanopyrrolidine-1-carboxylate (34a). To a solution of L-proline (600 mg, 5.21 mmol) in dioxane (7 mL) and 10% $Na_2CO_{3(aq)}$ (14 mL) was added Boc_2O (2.0 equiv) at room temperature, and the mixture was allowed to stir for 16 h. The mixture was washed 2× with hexanes (100 mL), then acidified with 1 N HCl (100 mL) and extracted 3× with EtOAc (200 mL). The EtOAc layer was dried with Na_2SO_4 and evaporated to a white solid. The crude white solid was then dissolved in CH_2Cl_2 (0.3 M) at 0 °C and treated with TEA (3.0 equiv) and then isobutyl chloroformate (1.1 equiv). The mixture turned turbid after the addition and was allowed to warm to room temperature before slowly clearing over time. After 1 h at room temperature, the mixture was treated with 2 M NH_3 in MeOH (2.0 equiv) and allowed to stir for 6 h. The mixture was evaporated to a white solid and taken on crude. The crude solid was dissolved in DMF (0.1 M) and 2,4,6-collidine (8 equiv) at 0 °C and then treated with cyanuric chloride (3.15 equiv) and allowed to warm to room temperature. The mixture was allowed to stir for 12 h. The mixture was then extracted with EtOAc (20 × the volume of DMF) and washed 3× with saturated $NaHCO_3$ (10 × the volume of DMF), 3× with 1 N HCl (10 × the volume of DMF), and once with brine (10 × the volume of DMF). The organic layer was then dried with Na_2SO_4, evaporated to a yellow oil, and immediately purified by flash chromatography to yield the title compound. Yield, 98%. Clear and colorless oil. R_f = 0.37 (20% EtOAc in hexanes, ninhydrin). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.46−4.10 (m, 1H), 3.30 (dd, J = 13.9, 7.8 Hz, 1H), 3.16 (dd, J = 17.5, 8.1 Hz, 1H), 2.15−1.95 (m, 2H), 1.95−1.76 (m, 2H), 1.39−1.21 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.01, 119.28, 81.08, 47.23, 45.79, 31.68, 30.84, 28.29, 24.73, 23.87.

(*S*)-1-(4-Dodecylbenzoyl)pyrrolidine-2-carbonitrile (35a). General procedure N was use to deprotect **34** (400 mg, 2.05 mmol). General procedure B was used to couple the deprotected **34** and 4-dodecylbenzoic acid to yield the title compound. Yield, 57%. White solid. R_f = 0.75 (40% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, J = 8.0 Hz, 2H), 7.22 (d, J = 7.6 Hz, 2H), 4.96−4.84 (m, 1H), 3.73−3.63 (m, 1H), 3.62−3.52 (m, 1H), 2.68−2.58 (m, 2H), 2.39−2.26 (m, 1H), 2.24−2.10 (m, 1H), 2.06−1.96 (m, 1H), 1.67−1.55 (m, 1H), 1.39−1.18 (m, 20H), 0.87 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.18, 146.28, 128.40, 127.61, 127.17, 118.73, 49.51, 46.87, 35.84, 31.91, 31.20, 30.30, 29.65, 29.56, 29.46, 29.34, 29.23, 25.58, 22.68, 14.12.

(*S*)-1-(4-Dodecylbenzoyl)pyrrolidine-2-carboximidamide Hydrochloride (36a). General procedure A was used to convert **35a** (213 mg, 0.519 mmol) to the title compound. Yield, 64%. White solid. ^{1}H NMR (500 MHz, CD_3OD) δ 7.63 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 4.73 (t, J = 7.5 Hz, 1H), 3.87 (dd, J = 15.4, 9.0 Hz, 1H), 3.73−3.60 (m, 1H), 2.67 (t, J = 7.6 Hz, 2H), 2.54 (dd, J = 13.3, 6.6 Hz, 1H), 2.12−1.93 (m, 1H), 1.71−1.56 (m, 1H), 1.34−1.28 (m, 18H), 0.90 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 171.79, 146.52, 131.91, 128.06, 127.63, 58.94, 50.59, 35.36, 31.66, 31.14, 31.03, 29.33, 29.28, 29.15, 29.06, 28.88, 25.27, 22.32, 13.03. LCMS: t_R = 5.04; m/z = 386.3. HRMS m/z calcd for $C_{24}H_{40}N_3O$ (M + H), 386.3171; found, 386.3175.

(*R*)-1-(4-Dodecylbenzoyl)pyrrolidine-2-carbonitrile (35b). General procedure N was use to deprotect **ent-34a** (275 mg, 1.40 mmol). General procedure B was used to couple the deprotected **ent-34a** and 4-dodecylbenzoic acid to yield the title compound. Yield, 53%. White solid. R_f = 0.75 (40% EtOAc in hexanes, Seebach's dip). ^{1}H and ^{13}C NMR data were identical to the data of **35a**.

(*R*)-1-(4-Dodecylbenzoyl)pyrrolidine-2-carboximidamide Hydrochloride (36b). General procedure A was used to convert **35b** (213 mg, 0.519 mmol) to the title compound. Yield, 73%. White solid. ^{1}H and ^{13}C NMR data were identical to the data of **36a**.

(*S*)-1-(4-(7-(Cyclohexylmethoxy)heptyl)benzoyl)pyrrolidine-2-carbonitrile (37). General procedure N was use to deprotect **34** (179 mg, 0.911 mmol). General procedure B was used to couple the deprotected **34** and **21a** to yield the title compound. Yield, 61%. Clear and colorless oil. R_f = 0.38 (40% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 8.0 Hz, 2H), 4.99−4.68 (m, 1H), 3.72−3.44 (m, 2H), 3.35 (t, J = 6.6 Hz, 2H), 3.16 (d, J = 6.6 Hz, 2H), 2.59 (t, J = 9.7 Hz, 2H), 2.42−1.90 (m, 4H), 1.57 (m, 10H), 1.40−1.01 (m, 9H), 1.01−0.72 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.19, 146.40, 133.46, 128.66, 127.79, 118.72, 77.05, 71.25, 58.68, 49.97, 38.24, 36.02, 31.33, 31.12, 30.37, 29.92, 29.50, 29.36, 26.88, 26.31, 26.10.

(*S*)-1-(4-(7-(Cyclohexylmethoxy)heptyl)benzoyl)pyrrolidine-2-carboximidamide Hydrochloride (38). General procedure A was used to convert **37** (213 mg, 0.519 mmol) to the title compound. Yield, 85%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.11 (s, 2H), 8.85 (s, 2H), 7.61 (d, J = 7.8 Hz, 2H), 7.25 (d, J = 7.7 Hz, 2H), 4.61 (t, J = 6.7 Hz, 1H), 3.81 (dd, J = 14.4, 7.4 Hz, 1H), 3.39 (m, 1H), 3.28 (t, J = 8.5, 2H), 3.09 (d, J = 6.5 Hz, 2H), 2.57 (m, 2H), 2.35−1.87 (m, 4H), 1.71−1.35 (m, 9H), 1.35−0.96 (m, 10H), 0.86 (m, 2H). ^{13}C NMR (126

MHz, DMSO) δ 171.55, 169.93, 145.60, 133.21, 128.38, 128.30, 76.19, 70.53, 58.49, 50.66, 37.97, 35.39, 31.40, 31.13, 30.03, 29.59, 29.04, 26.62, 26.07, 25.81, 25.67. LCMS: t_R = 4.65; m/z = 428.3. HRMS m/z calcd for $C_{26}H_{42}N_3O_2$ (M + H), 428.3277; found, 428.3274.

(*S*)-1-(11-(Cyclohexylmethoxy)undecanoyl)pyrrolidine-2-carbonitrile (39). General procedure N was use to deprotect **34** (371 mg, 1.89 mmol). General procedure B was used to couple the deprotected **34** and **24** to yield the title compound. Yield, 84%. Clear and colorless oil. R_f = 0.29 (40% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.64 (d, J = 6.0 Hz, 1H), 3.53 (dd, J = 8.3, 7.1 Hz, 1H), 3.42−3.22 (m, 3H), 3.09 (d, J = 6.5 Hz, 2H), 2.44−1.84 (m, 6H), 1.78−1.35 (m, 6H), 1.35−0.96 (m, 19H), 0.81 (dd, J = 21.9, 10.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.23, 114.29, 77.45, 71.23, 46.51, 46.38, 38.19, 34.59, 30.31, 30.22, 29.89, 29.68, 29.55, 29.44, 26.83, 26.31, 26.05, 25.30, 24.56.

(*S*)-1-(11-(Cyclohexylmethoxy)undecanoyl)pyrrolidine-2-carboximidamide Hydrochloride (40). General procedure A was used to convert **39** (433 mg, 1.15 mmol) to the title compound. Yield, 53%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 8.89 (s, 4H), 4.45 (dd, J = 9.0, 4.0 Hz, 1H), 3.68 (ddd, J = 9.3, 7.2, 4.8 Hz, 1H), 3.41 (dd, J = 16.6, 7.3 Hz, 1H), 3.34 (s, 1H), 3.27 (t, J = 8.6 Hz, 2H), 3.09 (d, J = 8.5 Hz, 2H), 2.40−2.07 (m, 4H), 2.04−0.98 (m, 27H), 0.93−0.68 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 172.43, 171.78, 76.20, 70.56, 57.22, 47.31, 37.97, 33.86, 31.25, 30.03, 29.47, 29.39, 29.30, 29.23, 26.62, 25.81, 24.72, 24.22. LCMS: t_R = 4.51; m/z = 394.3. HRMS m/z calcd for $C_{23}H_{44}N_3O_2$ (M + H), 394.3434; found, 394.3425.

1-(3-(2-Cyclohexylethyl)phenyl)ethanone (48). To a 0.5 M solution of 9-BBN (1.5 equiv) was added vinylcyclohexane (1.5 mL, 11.3 mmol, 1.5 equiv). The mixture was allowed to stir for 12 h. The mixture was then treated with 3 N $NaOH_{(aq)}$ (1.7 equiv), followed by the solid additions of 3-bromoacetophenone (1.00 mL, 7.56 mmol, 1.0 equiv) and $Pd(PPh_3)_4$ sequentially. The mixture was then heated to reflux for 1 h, then cooled to room temperature and extracted 2× into EtOAc (400 mL). The organic layer was then dried with Na_2SO_4, evaporated to a black oil, and immediately purified by flash chromatography to yield the title compound. Yield, 82%. Clear and colorless oil. R_f = 0.58 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85−7.58 (m, 2H), 7.42−7.07 (m, 2H), 2.59 (t, J = 7.3 Hz, 2H), 2.51 (s, 3H), 1.88−1.53 (m, 5H), 1.53−1.35 (m, 2H), 1.32−1.00 (m, 4H), 0.88 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.29, 143.87, 137.36, 133.36, 128.63, 128.19, 126.02, 39.53, 37.54, 33.46, 33.33, 26.85, 26.79, 26.52.

2-Bromo-1-(3-(2-cyclohexylethyl)phenyl)ethanone (49). To a solution of **48** (2.14 g, 9.28 mmol) in $CHCl_3$ (40 mL, 0.23 M) at 40 °C was added Br_2 (1.0 equiv) dropwise. The reaction was complete immediately after the addition of the bromine, and the mixture was cooled to room temperature and quenched with saturated $NaHCO_3$ (100 mL). The mixture was then extracted 3× with $CHCl_3$ (100 mL). The organic layer was then dried with Na_2SO_4, evaporated to a black oil, and immediately purified by flash chromatography to yield the title compound. Yield, 92%. Yellow oil. R_f = 0.71 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90−7.66 (m, 2H), 7.45−7.28 (m, 2H), 4.42 (s, 2H), 2.74−2.56 (m, 2H), 1.80−1.54 (m, 5H), 1.47 (dt, J = 10.4, 7.1 Hz, 2H), 1.32−1.01 (m, 4H), 0.89 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.54, 144.30, 134.30, 134.21, 129.04, 128.95, 126.56, 39.45, 37.51, 33.49, 33.32, 31.67, 26.89, 26.56.

4-(*tert*-Butoxycarbonyl)benzoic Acid. To a mixture of terephthalic acid (6.64 g, 40.0 mmol), Boc_2O (1.0 equiv), and DMAP (0.25 equiv) were added ^{t}BuOH (60 mL) and THF (20 mL). The mixture was heated to reflux for 24 h. The mixture was then evaporated to dryness and immediately purified by flash chromatography to yield the title compound. Yield, 36%. White solid. R_f = 0.50 (5% MeOH in $CHCl_3$, $KMnO_4$). Rotomer A: ^{1}H NMR (300 MHz, DMSO) δ 8.20−7.74 (m, 4H), 1.54 (s, 9H), 1.41−1.17 (m, 2H). Rotomer B: ^{1}H NMR (300 MHz, DMSO) δ 8.20−7.74 (m, 4H), 1.41−1.17 (m, 9H). Rotomer ratio A/B = 1.95.

***tert*-Butyl (2-(3-(2-Cyclohexylethyl)phenyl)-2-oxoethyl) terephthalate (50).** A solution of 4-(*tert*-butoxycarbonyl)benzoic acid (1.10 g, 4.95 mmol) and Cs_2CO_3 (0.51 equiv) in EtOH (80 mL, 0.06 M) was sonicated for 6 min at room temperature. The solvent was then evaporated and dried under vacuum for 20 min. The cesium carboxylate salt was then dissolved in DMF (30 mL), and **49** (1.2 equiv) was transferred by cannula in DMF (10 mL) at room temperature. The mixture was allowed to stir for 12 h before being diluted with EtOAc (400 mL) and washed 3× with neat water (100 mL). The organic layer was then dried with Na_2SO_4, evaporated to a yellow oil, and immediately purified by flash chromatography to yield the title compound. Yield, 99%. Tan oil. R_f = 0.43 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, J = 6.8 Hz, 2H), 8.01 (d, J = 6.8 Hz, 2H), 7.73 (m, 2H), 7.47−7.04 (m, 2H), 5.55 (s, 2H), 2.62 (t, J = 6.9 Hz, 2H), 1.84−0.52 (m, 22H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.11, 165.50, 164.95, 144.34, 136.30, 134.28, 133.00, 129.95, 129.60, 128.98, 127.82, 125.37, 81.86, 67.01, 39.41, 37.49, 33.44, 33.30, 28.31, 26.83, 26.50.

***tert*-Butyl 4-(4-(3-(2-Cyclohexylethyl)phenyl)-1*H*-imidazol-2-yl)benzoate (51).** To a round-bottom flask, fitted with a deans-stark trap containing 4 Å molecular sieves, were added **50** (1.00 g, 2.22 mmol) and ammonium acetate (5.0 equiv). The mixture was dissolved in xylenes (30 mL, 0.07 M). The mixture was then heated to reflux for 7 h. The mixture was then cooled to room temperature, evaporated to a black oil, and immediately purified by flash chromatography to yield the title compound. Yield, 36%. Yellow solid. R_f = 0.05 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (d, J = 8.4 Hz, 2H), 87.92 (d, J = 8.4 Hz, 2H), 7.62 (s, 1H), 7.52 (d, J = 7.7 Hz, 1H), 7.42 (s, 1H), 7.32−7.15 (m, 1H), 7.05 (d, J = 7.6 Hz, 1H), 2.70−2.39 (m, 2H), 1.85−1.01 (m, 20H), 1.01−0.69 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.90, 146.77, 144.07, 134.13, 131.60, 130.22, 128.88, 127.64, 125.44, 122.74, 81.65, 39.61, 37.65, 33.56, 33.48, 28.42, 26.91, 26.56.

(*S*)-1-(4-(4-(3-(2-Cyclohexylethyl)phenyl)-1*H*-imidazol-2-yl) benzoyl)pyrrolidine-2-carbonitrile (52). General procedure N was used to deprotect the ^{t}Bu ester on compound **51** (193 mg, 0.448 mmol) to yield the corresponding carboxylic acid. In a separate flask, general procedure N was used to deprotect **34** (88 mg, 0.448 mmol). General procedure B was used to couple the deprotected **51** and **34** to yield the title compound. Yield, 84%. Yellow solid. R_f = 0.57 (EtOAc, Seebach's dip). ^{1}H NMR (300 MHz, DMSO) δ 12.81 (s, 1H), 8.22−7.90 (m, 2H), 7.90−7.34 (m, 5H), 7.26 (t, J = 6.8 Hz, 1H), 7.03 (d, J = 6.5 Hz, 1H), 4.88 (dd, J = 7.5, 5.2 Hz, 1H), 4.09 (ddd, J = 12.3, 7.6, 3.3 Hz, 1H), 3.98−3.40 (m, 3H), 3.08−2.66 (m, 2H), 2.66−2.51 (m, 2H), 2.39−0.56 (m, 13H). ^{13}C NMR (75 MHz, DMSO) δ 168.98, 145.55, 143.45, 142.40, 134.95, 133.13, 129.09, 128.77, 128.44, 125.29, 124.94, 122.55, 120.15, 115.64, 49.93, 47.44, 39.89, 37.49, 33.45, 31.92, 30.28, 26.89, 26.52, 25.84.

(*S*)-1-(4-(4-(3-(2-Cyclohexylethyl)phenyl)-1*H*-imidazol-2-yl)benzoyl)pyrrolidine-2-carboximidamide Hydrochloride (53). General procedure A was used to convert **52** (140 mg, 0.310 mmol) to the title compound. Yield, 81%. Yellow solid. ^{1}H NMR (500 MHz, DMSO) δ 12.85 (s, 1H), 9.07 (s, 2H), 8.74 (s, 2H), 8.07 (d, J = 8.2 Hz, 2H), 7.80 (d, J = 8.2 Hz, 2H), 7.65 (m, 2H), 7.25 (t, J = 7.6 Hz, 1H), 7.03 (d, J = 7.2 Hz, 1H), 4.62 (t, J = 7.5 Hz, 1H), 3.93−3.70 (m, 1H), 3.47 (dd, J = 19.6, 12.3 Hz, 1H), 2.67−2.53 (m, 2H), 2.37 (dd, J = 14.8, 8.7 Hz, 1H), 2.02−0.92 (m, 14H). ^{13}C NMR (126 MHz, DMSO) δ 171.36, 169.60, 145.32, 143.20, 142.05, 134.89, 134.67, 132.81, 129.15, 128.91, 126.78, 124.71, 122.30, 120.07, 58.61, 50.66, 39.71, 37.25, 33.22, 31.42, 26.64, 26.28, 25.93. LCMS: t_R = 3.79; m/z = 470.3. HRMS m/z calcd for $C_{29}H_{36}N_5O$ (M + H), 470.2920; found, 470.2916.

4-(4-(3-(2-Cyclohexylethyl)phenyl)oxazol-2-yl)benzoic Acid (54). To a round-bottom flask containing **50** (1.10 g, 2.44 mmol) and

ammonium acetate (5.0 equiv) was added AcOH (20 mL, 0.12 M). The mixture was then heated to reflux for 16 h. The mixture was then cooled to room temperature, evaporated to dryness, and immediately purified by flash chromatography to yield the title compound. Yield, 25%. Yellow solid. R_f = 0.58 (EtOAc, Seebach's dip). Compound was crude by NMR and taken onto the next step without further purification.

(*S*)-1-(4-(4-(3-(2-Cyclohexylethyl)phenyl)oxazol-2-yl)benzoyl)pyrrolidine-2-carbonitrile (55). General procedure N was used to deprotect **34** (88 mg, 0.448 mmol). General procedure B was used to couple the deprotected **34** and crude **54** to yield the title compound. Yield, 81%. Yellow solid. R_f = 0.51 (50% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, J = 8.0 Hz, 2H), 7.96 (s, 1H), 7.81−7.52 (m, 4H), 7.30 (dt, J = 6.6, 4.9 Hz, 1H), 7.14 (d, J = 7.6 Hz, 1H), 5.03−4.69 (m, 1H), 3.91−3.26 (m, 2H), 2.77−2.43 (m, 2H), 2.43−0.62 (m, 17H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.25, 160.94, 144.13, 142.74, 136.60, 134.30, 130.90, 129.79, 128.93, 128.65, 128.28, 126.69, 125.81, 123.13, 118.81, 49.69, 47.18, 39.68, 37.67, 33.54, 30.48, 26.92, 26.57, 25.82.

(*S*)-1-(4-(4-(3-(2-Cyclohexylethyl)phenyl)oxazol-2-yl)benzoyl)pyrrolidine-2-carboximidamide Hydrochloride (56). General procedure A was used to convert **55** (169 mg, 0.373 mmol) to the title compound. Yield, 75%. Yellow solid. ^{1}H NMR (500 MHz, DMSO) δ 9.23 (s, 2H), 9.03 (s, 2H), 8.78 (s, 1H), 8.12 (d, J = 8.4 Hz, 2H), 7.90 (d, J = 8.4 Hz, 2H), 7.73−7.60 (m, 2H), 7.34 (t, J = 7.6 Hz, 1H), 7.16 (d, J = 7.6 Hz, 1H), 4.67 (t, J = 7.5 Hz, 1H), 3.99−3.69 (m, 1H), 3.50−3.37 (m, 1H), 2.61 (t, J = 9.1 Hz, 2H), 2.44−2.30 (m, 1H), 2.01−1.79 (m, 3H), 1.74 (d, J = 13.0 Hz, 2H), 1.69−1.54 (m, 3H), 1.54−1.41 (m, 2H), 1.30−1.00 (m, 4H), 1.00−0.75 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 171.39, 169.19, 160.68, 143.73, 141.87, 137.70, 136.51, 130.93, 129.22, 129.15, 128.68, 126.16, 125.49, 123.13, 58.50, 50.57, 37.24, 33.18, 33.02, 31.46, 26.62, 26.25, 25.61. LCMS: t_R = 4.79; m/z = 471.3. HRMS m/z calcd for $C_{29}H_{35}N_4O_2$ (M + H), 471.2760; found, 471.2759.

***tert*-Butyl 4-Carbamothioylbenzoate (57).** 4-(*tert*-Butoxycarbonyl)benzoic acid (3.24 g, 14.6 mmol) was then dissolved in CH_2Cl_2 (0.3 M) at 0 °C and treated with TEA (3.0 equiv) and then isobutyl chloroformate (1.1 equiv). The mixture turned turbid after the addition and was allowed to warm to room temperature before slowly clearing over time. After 1 h at room temperature, the mixture was treated with 2 N NH_3 in MeOH (2.0 equiv) and allowed to stir for 6 h. The mixture was evaporated to a white solid and purified by flash chromatography to yield the corresponding amide. Yield, 66%. White solid. R_f = 0.39 (60% EtOAc in hexanes, UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, J = 8.1 Hz, 2H), 7.84 (d, J = 8.1 Hz, 2H), 6.48 (s, 2H), 1.59 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.25, 165.10, 137.02, 135.22, 129.87, 127.47, 81.99, 28.35. The amide was then dissolved in THF (0.5 M) and treated with Lawesson's reagent (0.6 equiv) in one portion. The mixture was allowed to stir for 3 h before being evaporated to dryness and purified by flash chromatography to yield the title compound. Yield, 92%. Yellow/green solid. R_f = 0.93 (50% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (500 MHz, DMSO) δ 10.06 (s, 1H), 9.65 (s, 1H), 8.12−7.55 (m, 4H), 1.53 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 199.69, 164.80, 143.60, 133.51, 129.12, 127.88, 81.62, 28.18.

***tert*-Butyl 4-(4-(3-(2-Cyclohexylethyl)phenyl)thiazol-2-yl)benzoate (58).** To a solution of **57** (1.95 g, 8.22 mmol) and $KHCO_3$ (1.1 equiv) in THF (45 mL) at −5 °C was added **49** (2.05 g, 6.63 mmol) in THF (5 mL). The mixture was allowed to warm to room temperature. The mixture was allowed to stir for 2 h and then cooled to −5 °C again and treated with TEA (2.2 equiv) and TFAA (1.1 equiv). The mixture was allowed to warm to room temperature and was stirred for 16 h. The mixture was diluted with $CHCl_3$ (400 mL) and quenched with water (100 mL). The organic layer was then dried with Na_2SO_4, evaporated to dryness, and immediately purified by flash chromatography to yield the title compound. Yield, 80%. Yellow solid. R_f = 0.88 (10% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18−7.95 (m, 4H), 7.84 (s, 1H), 7.81−7.64 (m, 1H), 7.45 (s, 1H), 7.33 (t, J = 7.6 Hz, 1H), 7.17 (d, J = 7.6 Hz, 1H), 2.67 (t, J = 8.6 Hz, 2H), 1.94−1.44 (m, 15H), 1.44−1.06 (m, 5H), 0.96 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.61, 165.35, 157.22, 143.99, 137.37, 135.15, 134.43, 133.24, 130.52, 128.94, 126.92, 126.47, 124.04, 113.70, 81.48, 77.68, 39.74, 37.70, 33.60, 33.48, 28.43, 26.99, 26.65.

(*S*)-1-(4-(4-(3-(2-Cyclohexylethyl)phenyl)thiazol-2-yl)benzoyl)pyrrolidine-2-carbonitrile (59). General procedure N was used to deprotect the ^{t}Bu ester on compound **58** (2.10 mg, 4.69 mmol) to yield the corresponding carboxylic acid. In a separate flask, general procedure N was used to deprotect **34** (919 mg, 4.69 mmol). General procedure B was used to couple the deprotected **58** and **34** to yield the title compound. Yield, 65%. Yellow solid. R_f = 0.36 (50% EtOAc in hexanes, Seebach's dip). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, J = 8.1 Hz, 2H), 7.79 (s, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 7.5 Hz, 2H), 7.48 (s, 1H), 7.30 (t, J = 7.6 Hz, 1H), 7.14 (d, J = 7.7 Hz, 1H), 4.82 (s, 1H), 3.85−3.35 (m, 2H), 2.64 (t, J = 5.3 Hz, 2H), 2.40−1.81 (m, 4H), 1.81−1.39 (m, 7H), 1.39−1.02 (m, 4H), 1.02−0.74 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.21, 168.71, 166.42, 157.05, 144.05, 136.02, 134.33, 128.95, 128.71, 128.51, 126.69, 123.97, 118.92, 113.77, 49.74, 47.23, 39.72, 37.64, 33.55, 33.43, 30.42, 26.93, 26.59, 25.83.

(*S*)-1-(4-(4-(3-(2-Cyclohexylethyl)phenyl)thiazol-2-yl)benzoyl)pyrrolidine-2-carboximidamide Hydrochloride (60). General procedure A was used to convert **59** (682 mg, 1.50 mmol) to the title compound. Yield, 74%. White solid. ^{1}H NMR (500 MHz, DMSO) δ 9.23 (s, 2H), 9.02 (s, 2H), 8.23 (s, 1H), 8.15−8.00 (m, 2H), 7.94−7.60 (m, 4H), 7.37 (t, J = 6.5 Hz, 1H), 7.17 (d, J = 6.4 Hz, 1H), 4.66 (s, 1H), 3.84 (dd, J = 34.3, 15.3 Hz, 1H), 3.53−3.20 (m, 2H), 2.62 (t, J = 5.6 Hz, 2H), 2.53−2.09 (m, 2H), 2.08−1.31 (m, 9H), 1.31−0.96 (m, 4H), 0.89 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.16, 173.99, 171.05, 160.83, 148.44, 142.06, 139.74, 138.98, 133.98, 133.47, 131.24, 131.00, 128.82, 124.56, 120.56, 63.26, 55.32, 41.95, 37.93, 37.79, 36.20, 31.37, 31.00, 30.38. LCMS: t_R = 5.08; m/z = 487.3. HRMS m/z calcd for $C_{29}H_{35}N_4OS$ (M + H), 487.2532; found, 487.2530.

■ ASSOCIATED CONTENT

Supporting Information. Synthetic schemes for compounds **30** and **47**, tabulated results from the inhibitor docking studies, the SphK1/DGKB sequence alignment, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

†PDB code for DGKB: 2QV7.

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■ ABBREVIATIONS USED

9-BBN, 9-borabicyclo[3.3.1]nonane; AcOH, acetic acid; Boc_2O, di-*tert*-butyl dicarbonate; DGK, diacylglycerol kinase; DIEA, *N*,*N*-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; DMS, dimethyl sphingosine; dppf, 1,1′-bis(diphenylphosphino)ferrocene; EGF, epidermal growth factor; EtOAc, ethyl acetate; HDAC, histone deacetylase; LPA, lysophosphatidic acid; MOE, molecular operating environment; PDGF, platelet derived growth factor; PFK, phosphofructokinase; PKC, protein kinase C; PyBOP, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate; S1P, sphingosine 1-phosphate; S1PR, sphingosine 1-phosphate receptor; SAR, structure−activity relationship; SphK, sphingosine kinase; VEGF, vascular endothelial growth factor; TBAB, tetra-*n*-butylammonium bromide; ^{t}BuOH, *tert*-butanol; TEA, triethylamine; TFA, trifluoroacetic acid; TFAA, trifluoroacetic anhydride

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