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Structure–Activity Relationship Studies and in Vivo Activity of Guanidine-Based Sphingosine Kinase Inhibitors: Discovery of SphK1- and SphK2-Selective Inhibitors

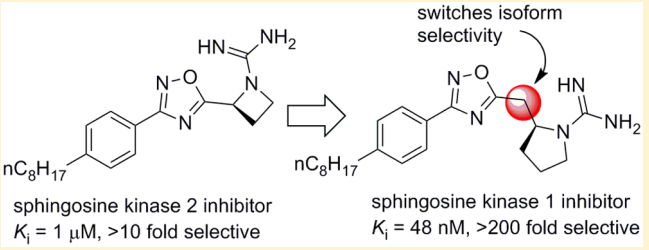
Neeraj N. Patwardhan,[†,#] Emily A. Morris,[†,#] Yugesh Kharel,[§] Mithun R. Raje,[†] Ming Gao,[†] Jose L. Tomsig,[§] Kevin R. Lynch,[§] and Webster L. Santos*[,†]

[†]Department of Chemistry and Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, Virginia 24061, United States

[§]Department of Pharmacology, University of Virginia, Charlottesville, Virginia 22908, United States

Supporting Information

ABSTRACT: Sphingosine 1-phosphate (S1P) is a pleiotropic signaling molecule that acts as a ligand for five G-protein coupled receptors ($S1P_{1-5}$) whose downstream effects are implicated in a variety of important pathologies including sickle cell disease, cancer, inflammation, and fibrosis. The synthesis of S1P is catalyzed by sphingosine kinase (SphK) isoforms 1 and 2, and hence, inhibitors of this phosphorylation step are pivotal in understanding the physiological functions of SphKs. To date, SphK1 and 2 inhibitors with the potency, selectivity, and in vivo stability necessary to determine the potential of these kinases as therapeutic targets are lacking. Herein, we report the design, synthesis, and structure–activity relationship studies of guanidine-based SphK inhibitors bearing an oxadiazole ring in the scaffold. Our studies demonstrate that SLP120701, a SphK2-selective inhibitor (K_i = 1 μM), decreases S1P levels in histiocytic lymphoma (U937) cells. Surprisingly, homologation with a single methylene unit between the oxadiazole and heterocyclic ring afforded a SphK1-selective inhibitor in SLP7111228 (K_i = 48 nM), which also decreased S1P levels in cultured U937 cells. In vivo application of both compounds, however, resulted in contrasting effect in circulating levels of S1P. Administration of SLP7111228 depressed blood S1P levels while SLP120701 increased levels of S1P. Taken together, these compounds provide an in vivo chemical toolkit to interrogate the effect of increasing or decreasing S1P levels and whether such a maneuver can have implications in disease states.



INTRODUCTION

The lysophospholipid sphingosine 1-phosphate (S1P) is a pleiotropic signaling molecule that regulates growth, survival, and migration of many cell types. S1P acts as an extracellular mediator by binding to five G-protein coupled receptors ($S1P_{1-5}$), leading to diverse physiological and pathophysiological processes.[1] Biosynthesis of S1P is realized only by phosphorylation of sphingosine, which is generated by the catabolism of more complex sphingolipids such as sphingomyelin and ceramide or taken up by cells from their environment. The enzyme responsible for this phosphoryl transfer exists as two isoforms encoded by unlinked genes: sphingosine kinase 1 (SphK1) and 2 (SphK2). SphKs have been implicated in a variety of disease states including sickle cell disease,[2] cancer,[1,3] atherosclerosis,[4] and asthma,[5] among others. SphK1's role in cancer is widely studied, where correlations between expression and severity of disease, drug resistance, and/or reduced patient survival have been reported.[3,6] However, pharmacological intervention to lessen SphK1 activity and control the "SphK rheostat", i.e., changing the equilibrium of S1P/Sph ratio, failed to demonstrate statistically significant effects on cell viability, suggesting that S1P's role in oncology or other diseases is complex.[7] Interestingly, the S1P generated by each SphK isoenzyme has been reported to result in opposing biological effects, implying conflicting roles by these enzymes. For example, SphK1 overexpression increases cell survival and proliferation[8] whereas SphK2 overexpression induces cell cycle arrest and apoptosis.[9,10] The latter effect can be explained through the interaction of $Bcl\text{-}_{XL}$ with the BH_3 domain within SphK2.[11] In addition, there is also a difference in their subcellular localization: SphK1 is mostly in the cytoplasm and migrates to the plasma membrane upon phosphorylation[12,13] while SphK2 can localize in the nucleus to inhibit DNA synthesis and regulate HDAC1/2 activity.[14,15] Gene deletion studies in mice indicate that there is some functional redundancy between the two enzymes, as $Sphk1^{-/-}$ and $Sphk2^{-/-}$ mice are viable, fertile, and phenotypically unremarkable.[16,17] However, germ line inactivation of all four SphK alleles is embryonically lethal (day E12.5–13.5) as a result of impaired neurological and vascular development.[18] The circulating level of S1P in SphK1-null mice is reduced by about 2-fold while, curiously, SphK2-null mice have more than double wild type S1P levels in blood and plasma.[18−21]

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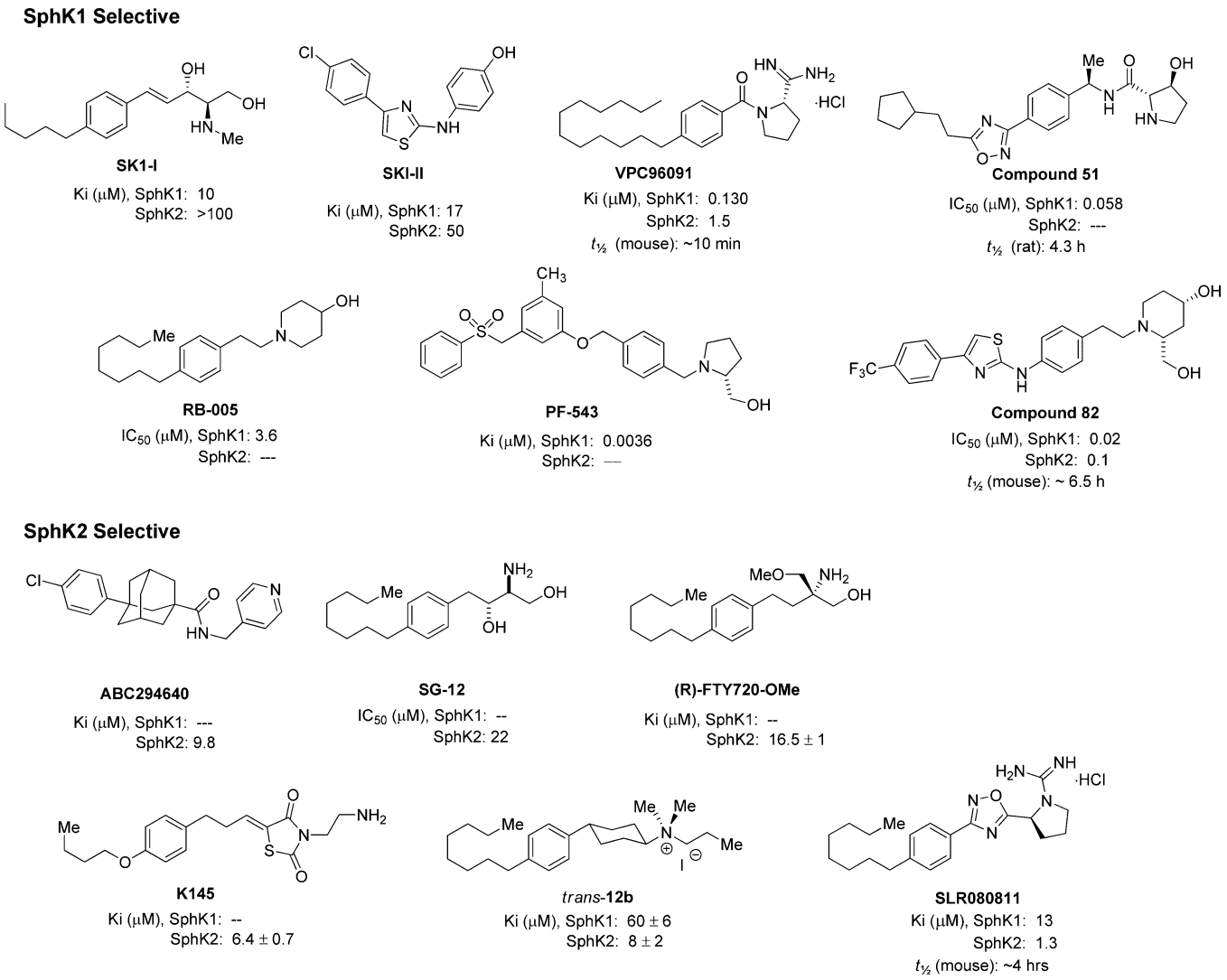

Figure 1. Structures, inhibitory activity, and half-lives of sphingosine kinase inhibitors.

Because of the potential role of S1P in a variety of diseases, pharmacological inhibition of SphKs with small molecules has been a subject of interest both in academia and pharmaceutical industry. Figure 1 illustrates the structures of reported SphK inhibitors.[22] SphK1, for which selective inhibitors have been developed, has been the focus of most studies. Because early inhibitors used to interrogate the function of SphK1 had K_i's in the mid micromolar range (e.g., SK1-I, SKI-II, dimethylsphingosine), some of the biological effects attributed to SphK1 activity may be due to off-target interactions. Indeed, these compounds are best described as molecules that include in their properties "inhibition of SphK1" rather than the commonly used "SphK1 inhibitors". The discovery of selective, nanomolar inhibitors such as PF-543, VPC96091, and compound **51** will be useful chemical tools to better understand SphK1 function, although some of these have limited in vivo stability[23,24] or their effect on S1P levels in vivo have not been documented.[25] The potent, dual SphK1/2 inhibitor, **82**, was recently described to decrease S1P concentration when administered in mice.[7,26] In contrast to SphK1, there is a paucity of SphK2-selective inhibitors;[27] indeed with SphK2, we have found it challenging to duplicate the selectivity and potency readily achieved with SphK1 inhibitors. ABC294640[28] (K_i 10 μM) was the first compound with SphK2 inhibitor properties reported, and it has been deployed in a variety of disease models where it has been reported to have remarkable efficacy. These models include ulcerative colitis,[29] Crohns disease,[30] ischemia/reperfusion injury,[31] osteoarthritis,[32] and colon cancer.[33] However, ABC294640 has at least one reported additional mode of action, that is binding to the estrogen receptor and acting as a partial agonist similar to tamoxifen.[34] Hence, attributing ABC294640 effects to SphK2 is difficult. Other inhibitors such as SG-12,[35] (R)-FTY720-OMe,[36] K145,[37] and *trans*-**12b**[38] are reported as SphK2 inhibitors, albeit with moderate potency and selectivity. Hence, new scaffolds with the potency and selectivity are needed to investigate SphK1 and 2 functions in vivo.[39]

Recently, we reported a novel guanidine-based compound, SLR080811, as a selective SphK2 inhibitor.[40] It was the most potent and selective inhibitor of SphK2 at that time with in vitro activity on U937 cells and in vivo activity in mice. In continuation of our investigations in understanding SphKs, we herein describe the design, synthesis, and structure−activity relationship studies of guanidine-based SphK inhibitors. Our studies reveal SphK1 and SphK2 inhibitors based on the same scaffold but with the distinction of a key methylene unit that induces a switch in isoform selectivity. These inhibitors are the most potent and selective inhibitors reported with in vitro and in vivo effects of altering cellular and blood S1P levels.

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RESULTS AND DISCUSSION

Design of Inhibitors. Previous reports detailed our efforts to develop SphK2-selective inhibitors that led to the identification of quaternary ammonium salts that were fairly potent and moderately selective.[38,41] Compound *trans*-**12b** was shown to be ~8-fold selective toward SphK2 with a K_i of 8 μM (Figure 1). The inhibition data validated our hypothesis that a positive charge is essential for electrostatic interaction, perhaps with the catalytic Asp residues on SphKs, which has been shown to be important for recognition of Sph by the enzyme.[42] Furthermore, recent high resolution structure studies on SphK1 indicated involvement of the catalytic Asp81 residue on the enzyme in the activation of the primary hydroxyl of sphingosine.[43] We thus concluded that a polar, and preferably charged, species was most likely to exhibit the desired inhibition activity toward these enzymes.

To define the structure–activity relationships of SphK inhibitors, we divided the structure of *trans*-**12b** into three regions: the quaternary ammonium group as the head, the cyclohexyl ring as the linker, and the 4-octylphenyl group as the tail (Figure 2). In this paper, we highlight our studies toward the

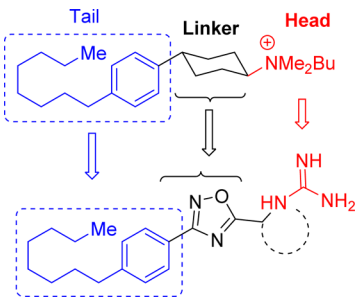

Figure 2. Scaffold modifications toward second generation SphK2 inhibitors.

second generation of SphK inhibitors with a focus on the linker and headgroup region, which features a guanidine functional group. The guanidine group as the warhead is attractive because it is charged under physiological conditions and possesses the ability for electrostatic interaction with the active site Asp residue, an interaction akin to that of quaternary amine group in *trans*-**12b**. We were further encouraged with this strategy because compounds with carboximidamide functionalities (amidines) are present in SphK1 inhibitors.[24,44] As further improvement, we replaced the flexible cyclohexyl ring with a rigid heteroaromatic 1,2,4-oxadiazole linker to minimize the entropic cost of binding and introduce potential hydrogen bond acceptor moieties. The 4-octylphenyl tail was kept constant as previously defined by a prior chain length study.[41]

Chemical Synthesis. The synthesis of guanidine derivatives is shown in Scheme 1. 4-Octyl benzonitrile **2** was synthesized via hydroboration of 1-octene (**1**) with 9-borabicyclo[3.3.1]nonane (9-BBN) followed by a Suzuki–Miyaura cross-coupling reaction of 4-iodobenzonitrile. Treatment of benzonitrile **2** with hydroxylamine hydrochloride and triethylamine in refluxing ethanol affords the common intermediate amidoxime **3** in excellent yield. Subsequent coupling with the desired amino acid using HCTU followed by dehydration at 80 °C generates 1,2,4-oxadiazole **4**. Removal of the Boc group was accomplished with trifluoroacetic acid or by bubbling HCl gas, which produced the desired amine **5** that is converted to bis-Boc protected guanidine **6** by reacting with *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine and Hunig's base over a period of 3 days. Standard deprotection conditions afforded compounds **7a–k**.

To mimic the hydroxyl moieties in sphingosine, hydroxyl groups were installed on the pyrrolidine ring as presented in Scheme 2. Following the standard synthetic sequence of coupling–guanidylation–deprotection, (*S*)-3-hydroxy- and (*R*)-4-hydroxy derivatives **11** and **15**, respectively, were synthesized. Further, we reversed the stereochemistry of the hydroxyl group on the 4-position of the pyrrolidine ring (Scheme 3). Inversion was accomplished under Mitsunobu conditions using benzoic acid as the nucleophile to provide compound **16**. Subsequent saponification afforded the corresponding alcohol, which was converted to product **17**. Derivatives possessing nitrogen in different positions of four (**20a**) or five (**20b**) membered ring heterocycles were synthesized as shown in Scheme 4. Finally, constitutional isomers of the oxadiazole moiety were synthesized. As illustrated in Scheme 5, 1,2,4-oxadiazole **23**, wherein the position of the 4-octylphenyl and pyrrolidine rings are reversed, was generated by coupling of 4-octylbenzoic acid with the amidoxime of Boc-proline to afford compound **22**, which was an intermediate that led to product **23**. The alternative structure, 1,3,4-oxadiazole **26**, was synthesized as depicted in Scheme 6. Key intermediate tetrazole **24** was produced by reacting benzonitrile **3** with NaN_3 at reflux temperature.[45] Under DCC coupling conditions at elevated temperature, compound **25** was isolated and converted to guanidine **26** using standard procedures.

To determine whether amides are a sufficient surrogate for the oxadiazole linker, we synthesized compounds **29–34**. Activation of Boc-proline with ethyl chloroformate followed by addition of 4-decylaniline afforded **27a**,**b** as single enantiomers (Scheme 7). Deprotection, guanidylation, and deprotection yielded guanidines **29a**,**b**. A homologated derivative was achieved by the reduction of benzonitrile **2**, which after subsequent reactions afforded compound **31** (Scheme 8). Further, a reversed amide analogue was synthesized using amide coupling chemistry with 4-ocylbenzoic acid **32**, and following standard protocols yielded guanidine **34** (Scheme 9).

Finally, we inserted carbon spacers between the oxadiazole and pyrrolidine rings. Employing Boc-homoproline and a further homologated derivative afforded compounds **36a**,**b** as shown in Scheme 10.

Structure–Activity Relationships of Analogues and in Vivo Activity. Analysis of reported SphK inhibitors suggests an optimal length, which is a positive charge 18–21 atoms from the omega carbon of the lipid tail. Hence, compounds bearing the guanidine headgroup with a 1,2,4-oxadiazole linked to a 4-octylphenyl chain were synthesized and screened for their inhibitory activity against recombinant Sphk1 and SphK2 at a concentration of 1 μM. Most of these compounds contained a diversifying moiety on the α carbon or on the internal nitrogen because the coupling partners were derived from amino acids.

As shown in Table 1, glycine-derived guanidine **7a** and others bearing methyl (**7c**), isopropyl (**7d**), and hydroxymethyl (**7e**) on the α carbon demonstrated modest inhibition (less than 20% when present at 1 μM). The analogue methylated on the internal nitrogen (**7b**, derived from sarcosine) was inactive. Surprisingly, compound **7f**, a cyclopropyl-containing derivative, showed little activity although cyclopropylamidines were previously found to be nanomolar inhibitors of SphK1.[24,44] Compounds **7a–f** appear to be slightly SphK1 selective. However, when a pyrrolidine ring was introduced (**7g**, SLR080811), a sharp increase in inhibitory activity was observed along with a reversal in selectivity toward

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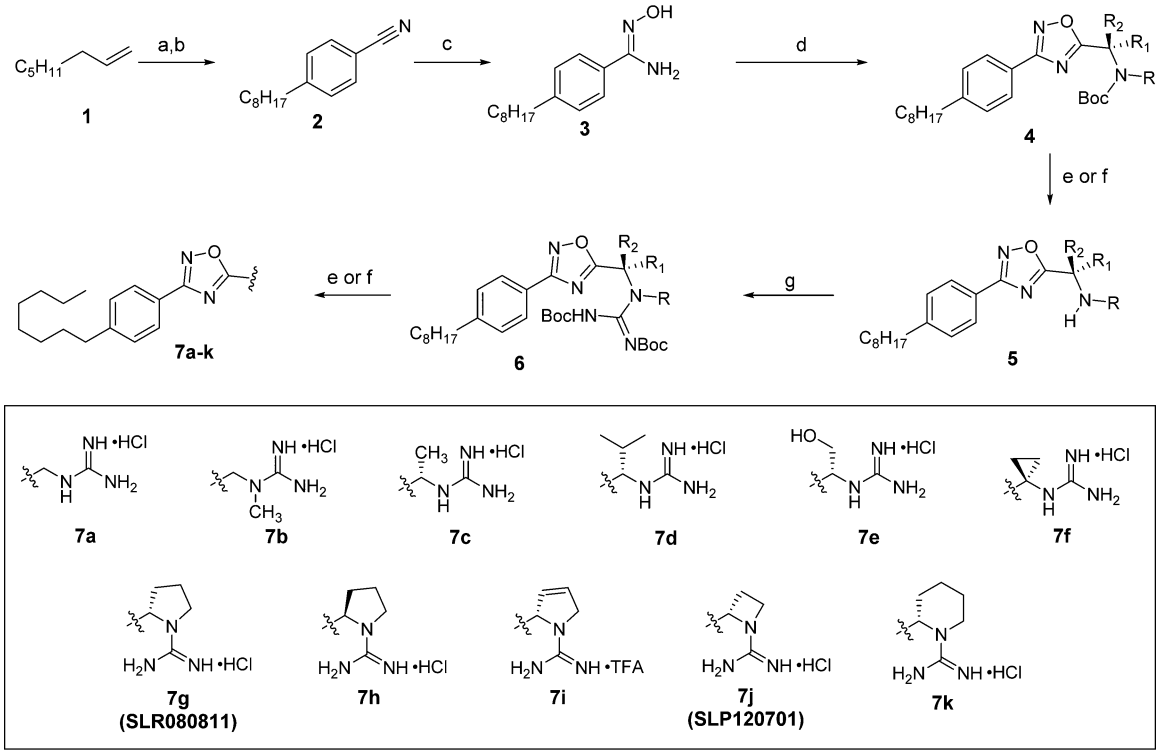
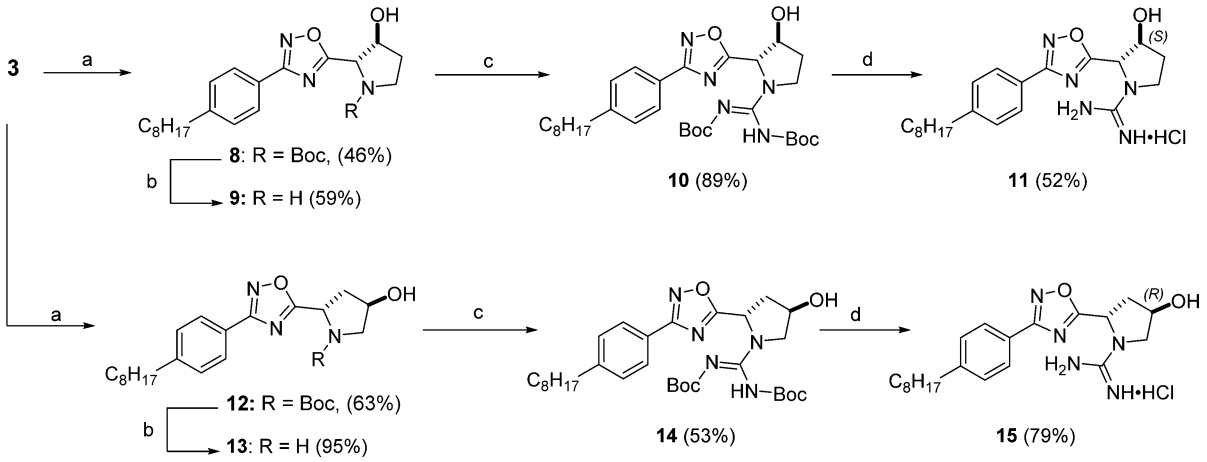
Scheme 1. Synthesis of Analogues 7a–k[a]



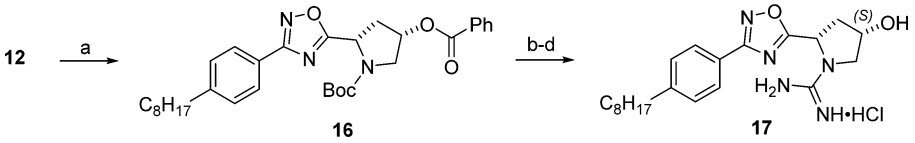
[a]Reagents and conditions; (a) 9-BBN, THF, rt, 18 h; (b) 4-iodobenzonitrile, Cs_2CO_3, Pd(dppf)Cl_2, DMF, 80 °C, 18 h, 62%; (c) NH_2OH·HCl, Et_3N, EtOH, reflux, 2 h, 92%; (d) Boc-protected amino acid, DIEA, HCTU·PF_6, DMF, 80 °C, 18 h, 20–82%; (e) HCl(g), MeOH, 5 min, 59–100%; (f) CF_3COOH, CH_2Cl_2, 2–12 h, 59–100%; (g) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, rt, 3–6 days, 18–81%.

Scheme 2. Synthesis of Derivatives 11 and 15[a]



[a]Reagents and conditions: (a) Boc-amino acid, DIEA, HCTU·PF_6, DMF, 80 °C, 18 h; (b) TFA, CH_2Cl_2, 3–12 h; (c) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, rt, 3 days; (d) HCl(g), MeOH.

Scheme 3. Synthesis of 17[a]



[a]Reagents and conditions: (a) PPh_3, DIAD, PhCOOH, THF, 0 °C, rt, 18 h, 75%; (b) 2 N NaOH, H_2O: THF (1:1), rt, 18 h, 95%; (c) TFA, CH_2Cl_2, 45%; (d) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, rt, 3 days, 79%; (e) HCl(g), MeOH, 22%.

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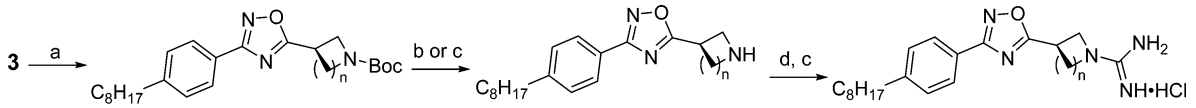
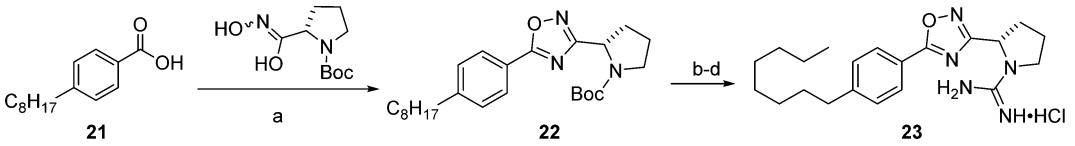
Scheme 4. Synthesis of 18a,b[a]



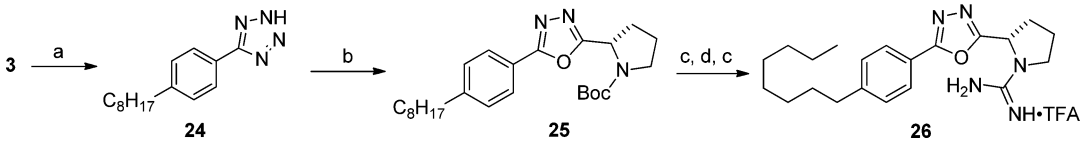
[a]Reagents and conditions: (a) Amino acid, DIEA, HCTU·PF_6, DMF, 80 °C, 18 h; (b) TFA, CH_2Cl_2 3−12 h; (c) HCl(g), MeOH; (d) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, rt, 3 days.

Scheme 5. Synthesis of Reverse 1,2,4-Oxadiazole 23[a]



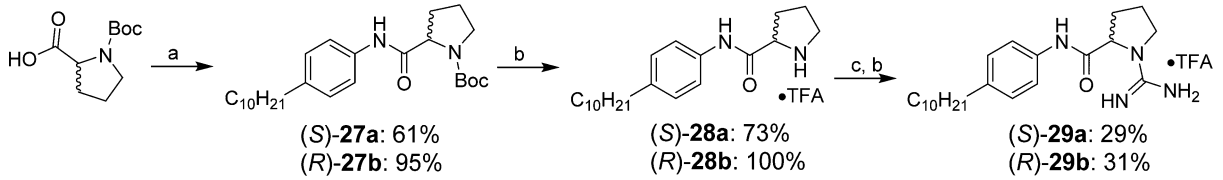
[a]Reagents and conditions: (a) DIEA, HCTU·PF_6, DMF, 80 °C, 18 h, 19%; (b) TFA, CH_2Cl_2, 89%; (c) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, rt, 3 days, 19%; (d) HCl(g), MeOH, 28%.

Scheme 6. Synthesis of 1,3,4-oxadiazole 26[a]



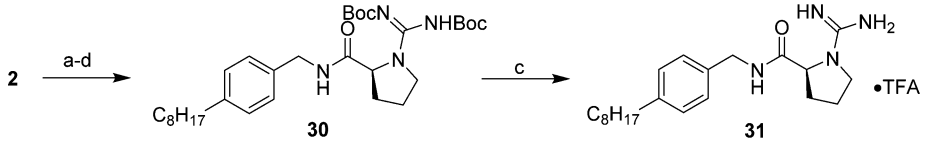
[a]Reagents and conditions: (a) NaN_3, NH_4Cl, DMF, reflux, 4 h, quant; (b) Boc-proline, DCC, toluene, 110 °C, 4 h, 67%; (c) TFA, CH_2Cl_2, quant; (d) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, rt, 3 days.

Scheme 7. Synthesis of Amide Analogues 29a,b[a]



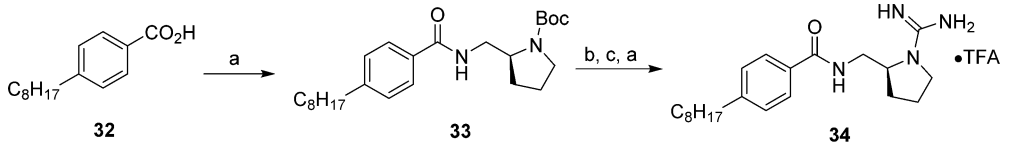
[a]Reagents and conditions: (a) Ethyl chloroformate, TEA, 0 °C, 30 min, then 4-decylaniline, 0 °C; (b) TFA, CH_2Cl_2; (c) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, rt.

Scheme 8. Synthesis of Amide Analogue 31[a]



[a]Reagents and conditions: (a) Lithium aluminum hydride, 0 °C to rt, 1 h; (b) ethyl chloroformate, TEA, Boc-proline, 0 °C to reflux; (c) TFA, CH_2Cl_2; (d) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, rt.

Scheme 9. Synthesis of Amide Analogue 34[a]



[a]Reagents and conditions: (a) EDC, TEA, NHS, CH_2Cl_2, rt then *tert*-butyl (*S*)-2-(aminomethyl)pyrrolidine-1-carboxylate, 85%; (b) TFA, CH_2Cl_2, quant; (c) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, rt, 47%.

SphK2. The calculated K_i for SLR080811 was 13 μM and 1.3 μM for SphK1 and 2, respectively, and it had a 10-fold selectivity toward SphK2 (Table 2). The inhibitory effect of SLR080811 was highly dependent on the (*S*)-stereochemistry at α carbon

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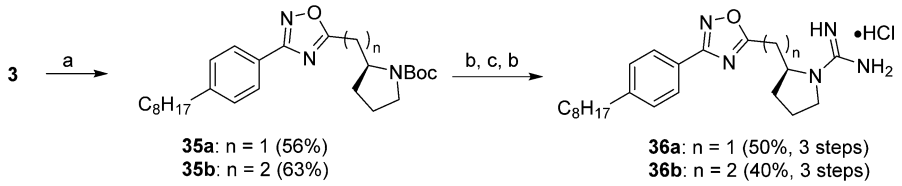
Scheme 10. Synthesis of Homologated Compounds 36a,b[a]



[a]Reagents and conditions: (a) DIEA, HCTU·PF_6, DMF, 80 °C, 18 h; (b) HCl(g), MeOH; (c) *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, rt, 3 days.

center of the pyrrolidine ring as the (*R*)-enantiomer **7h** was markedly less potent as a SphK inhibitor.

In an effort to improve the activity of SLR080811 further, a more conformationally restricted analogue, **7i**, wherein a double bond was introduced on the aliphatic ring, was synthesized. However, this compound was significantly less potent (Table 1). Contraction of the pyrrolidine to a four-membered azetidine ring, **7j** (SLP120701), afforded a compound that was equipotent with SLR080811. In contrast, ring expansion of the pyrrolidine to generate the piperidine analogue, **7k**, resulted in a compound with severely diminished activity, presumably as a result of the change in dihedral angle that orients the guanidine group in a suboptimal position. We next explored the effect of hydroxyl groups on the pyrrolidine ring in an attempt to mimic the hydroxyl groups on Sph. Such a strategy has recently been used with SphK1 inhibitors.[23,26] Compound **11**, which has a (3*S*)-hydroxyl group, was equipotent at both the SphK1 and Sphk2 enzymes, albeit with less potency than the parent compound (Table 1). (4*R*)-**15** retained SphK2 selectivity but was less potent than SLP120701 while its stereoisomer (4*S*)-**17** was significantly less active (Tables 1 and 2). A compound with (3*R*)-hydroxyl group was not tested, as our attempts to synthesize it failed.

To determine the optimal position of the guanidine group around the heterocyclic ring, we installed the nitrogen atom away from the α carbon to generate azetidine (**20a**) and pyrrolidine (**20b**). Both of these compounds were essentially inactive in our assay. We also investigated effect of heteroatoms of the oxadiazole ring, as positional isomers of oxadiazoles are known to possess varying pharmacokinetic properties.[46] Hence, reversed 1,2,4-oxadiazole **23** and 1,3,4-oxadiazole **26** were synthesized. While (5-phenyl)-1,2,4-oxadiazole **23** had inhibitory activity indistinguishable from SLP120701, **26** did not inhibit at 1 μM (Tables 1 and 2). These studies suggest that the isolated nitrogen atom interacts with the target protein, possibly via hydrogen bonding.

In an effort to discover a surrogate for the oxadiazole ring, we installed an amide bond between the phenyl and pyrrolidine rings. Anilide **29a** and **29b** possessing opposite stereochemistry at the α carbon were both inactive at SphK1 and -2. As the amide bonds in these compounds are approximately one bond shorter than the corresponding oxadiazole and can be "off register" in the enzyme binding pocket, we synthesized the homologated benzylamide **31** and found that it possessed no significant inhibitory effect. To perform a thorough analysis, we also synthesized the reversed homologated analogue **34** and found a similar activity. Hence, we conclude that an amide bond is not a sufficient replacement for the oxadiazole ring.

We next focused our attention on the spacing between the oxadiazole and pyrrolidine rings; compound **36a** (SLP7111228) and **36b** contain one or two methylene units, respectively. To our surprise, we observed a complete switch in isoform selectivity as **36a** showed 88% inhibition at SphK1 while exhibiting 20% inhibition at SphK2. The calculated K_i of SLP7111228 for SphK1 is 48 nM and >10 μM for SphK2 (Table 2), affording >200-fold selectivity. This compound is the most potent SphK1-selective compound reported to date. Further extension of the linker in **36b** resulted in substantial decrease in both SphK1 and SphK2 inhibitory activity.

As azetidine derivative SLP120701 had a similar binding constant (K_i = 1.2 μM) but was more selective toward SphK2 than the pyrrolidine analogue SLR080811, we performed biochemical characterization to determine its in vitro and in vivo properties (Table 2 and Figure 3). First, U937 cells (a myeloid cell line isolated from a patient with histiocytic lymphoma), which express both SphK1 and SphK2, were incubated with SLP120701. After cell lysis and sample preparation, the inhibitor, sphingosine, and S1P were quantified using LC-MS-MS. As shown in Figure 3, a dose-dependent accumulation of SLP120701 was observed with a concomitant decrease of S1P and increase in Sph levels. These results suggest that SphKs were inhibited in whole cells and that SLP120701 penetrates these cells effectively.

To determine whether SLP120701 was inhibiting SphK2 in these cells, the cultures were treated with the SphK2-selective substrate, FTY720, and the resulting phosphorylated FTY720 (FTY720-P) was monitored (Figure 4).[47,48] Addition of FTY720 to U937 cells resulted in accumulation of FTY720-P, which should be dampened in the presence of an inhibitor. As expected, increasing SLP120701 from 0.1 to 3 μM was accompanied by decreased amounts of FTY720-P, a trend that reached a minimum at 3 μM. These in vitro studies indicate that the decrease in S1P and FTY720-P as a function of SLP120701 concentration may be attributed to inhibition of SphK2. Note that the IC_{50} of SLP120701 estimated from this data appears to be significantly less than the K_i value, which in our experience results from the avid accumulation of compound in cells and consistent with other studies.[7,40]

To ascertain whether inhibition of SphK2, via enzymatic blockade using SLP120701, has an effect on the levels of sphingoid bases in vivo, we treated C57BL/6 mice with a single intraperitoneal dose of the compound (10 mg/kg) and monitored S1P, Sph, and inhibitor concentration in blood using LC-MS-MS. In contrast to our in vitro studies, S1P levels increased to approximately 2-fold following injection of the SphK2-selective inhibitor (Figure 5). This phenomenon is consistent with S1P levels observed in $Sphk2^{-/-}$ mice where the blood S1P level is 3−4 times higher than wild type control mice.[18−21] We also observed a modest increase in Sph levels (Figure 5B). Further monitoring of compound disappearance from blood suggests a half-life of approximately 8 h, which is significantly longer than that of SLR080811.[40]

To compare the effects of SphK1 inhibition, we similarly subjected U937 cells with SphK1-selective inhibitor SLP7111228. As expected, a dose-dependent accumulation of

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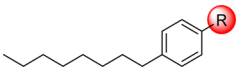
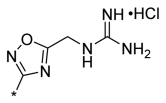
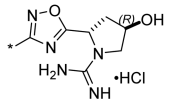
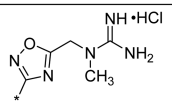
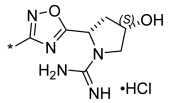
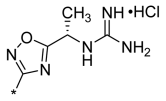
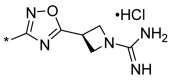
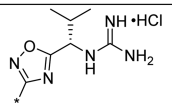
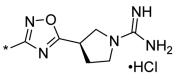
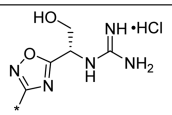
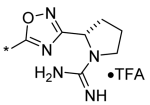
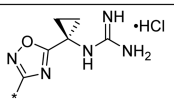
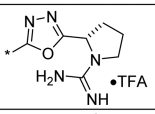
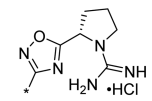
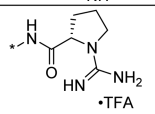
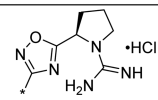
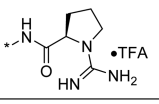
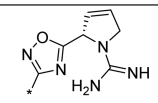
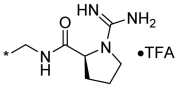
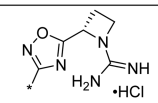
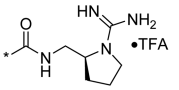
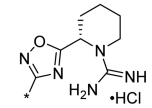
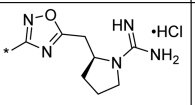
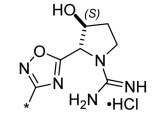
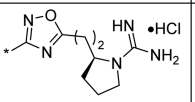
Table 1. SphK1 and SphK2 Inhibitory Activity[a]



Compd	R	SphK1	SphK2	Compd	R	SphK1	SphK2
7a		75	90	**15**		98	70
7b		98	90	**17**		96	90
7c		78	94	**20a**		84	96
7d		70	85	**20b**		88	95
7e		94	99	**23**		91	42
7f		88	98	**26**		96	91
7g		88	44	**29a**		100	83
7h		93	90	**29b**		100	100
7i		94	96	**31**		95	95
7j		92	56	**34**		90	75
7k		98	92	**36a**		12	80
11		69	65	**36b**		77	100

[a]SphK activity is represented as % of control (without inhibitor). Human SphK expression was forced in insect cells infected with recombinant baculovirus, and activity in a cleared lysate was measured using 5 μM (for SphK1) or 10 μM (for SphK2) sphingosine and 250 μM [^{32}P]-ATP. Each compound was assayed at a concentration of 1 μM in triplicate, except for **36a** (assayed at 3 μM with SphK2). Control = activity without inhibitor.

SLP7111228 in these cells is observed (Figure 6A). This property is accompanied by a marked decrease in S1P level in a dose-dependent fashion (Figure 6B). Interestingly, addition of 100 nM of this compound resulted in approximately 90% depression of cellular S1P, which is significantly more compared to SphK2-selective inhibitor SLP120701. When Sph levels were measured as a function of inhibitor concentration, no statistical change was observed (Figure 6C), which is again in contrast with Sphk2 inhibitor. We further determined the effect of SLP120701 on the viability of U937 cells, as recent reports suggested that SphK1-selective inhibitors such as PF-543[23] and compound A/B[7] had no effect on the viability of cancer cells (Figure 6D). Our studies similarly demonstrate that inhibition of SphK1 via SLP120701 up to 3 μM over 24 h has no cytotoxicity effects on cancer cell growth.

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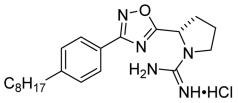
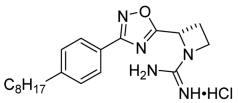
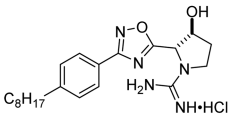
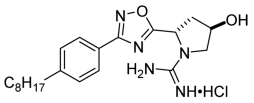
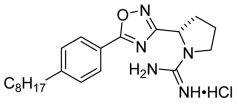
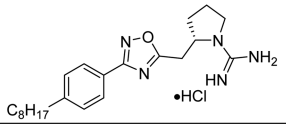
Table 2. K_i of Selected Inhibitors at SphK1 and SphK2

Entry	Compound	Structure	K_i (μM) SphK1	K_i (μM) SphK2
1	**SLR080811 (7g)**		13 ± 1	1.3 ± 0.4
2	**SLP120701 (7j)**		>10	1.2 ± 0.2
3	**SLM120401 (11)**		>10	>10
4	**BD22 (15)**		>10	5.2 ± 1
5	**SLM6111202 (23)**		>10	1.1 ± 0.2
6	**SLP7111228 (36a)**		0.048 ± 0.01	>10

To determine the effect of SLP7111228 in vivo, we injected increasing concentrations of this compound in rats intraperitoneally. As shown in Figure 7A, plasma S1P levels decreased slightly (up to 25% of control) as the inhibitor concentration increases. S1P levels in plasma are known to be significantly lower than in blood. In contrast, blood S1P levels shows a marked dose-dependent depression, which reaches a minimum at about 80% when compared to vehicle (Figure 7B). Finally, our studies suggest that SLP7111228 has an in vivo half-life of over 4 h and that its effect of lowering S1P level is sustained over a period of at least 6 h (Figure 7C,D).

CONCLUSIONS

The second generation inhibitors reported herein feature a guanidine moiety as a warhead and an oxadiazole heterocycle as the linker. Structure−activity studies with this scaffold suggest that conformational restriction of the guanidine group is essential for potent kinase inhibitory activity. Indeed, acyclic analogues along with six membered piperidine or a dehydropyrrolidine derivatives are weak inhibitors of SphK2. Further, decoration of the pyrrolidine ring with a hydroxyl group to mimic the hydroxyl moiety in sphingosine does not increase potency of the compounds, although the compound bearing a (3*R*)-hydroxyl group may have beneficial effect. Our studies also demonstrate that amides are not a good replacement for oxadiazole rings in our scaffold and that the position of the isolated nitrogen of the oxadiazole ring is important; we suspect that this nitrogen atom forms a hydrogen bond with a key amino acid in SphK. A surprising finding in these investigations is that the insertion of a single methylene unit as a spacer between the oxadiazole and pyrrolidine rings resulted in a SphK inhibitor with reversed selectivity, favoring SphK1.

Profiling of SphK1 (SLP7111228) and SphK2 (SLP120701) both in vitro and in vivo reveal interesting observations. First, both inhibitors have an effect of depressing S1P levels in U937 cells. Curiously, SphK1 inhibitors decrease S1P levels further compared to SphK2 inhibitors in vitro. Whether this an activity associated with the potency of these compounds is a possibility, but we note that these compounds are avidly taken up by these cells. Second, in vivo administration of these inhibitors in mice and rats resulted in opposite effects on blood S1P levels. That is, a decrease in blood S1P level is observed with SphK1 inhibitor while there is an increase with SphK2 inhibitors. While these observations are consistent with mouse gene "knock out" studies, our results are notable in that (1) SLP120701 is the second SphK2 inhibitor profiled to result in increased circulating levels of S1P;[40] ABC294640 (a low potency compound that has SphK2 inhibitor properties) had the opposite effect of decreased S1P levels.[49] Our result suggest that increased blood S1P levels are a bona fide property of SphK2-selective inhibitors. (2) SLP120701 has an improved in vivo half-life as compared to the pyrrolidine analogue, SLR080811. (3) SLP7111228 is the most potent and selective (>200 fold) SphK1 inhibitor reported to date with favorable in vivo stability. Interestingly, a single dose of 10 mg/kg in rats decreases blood S1P levels by about 80%. With these two chemical tools in hand, we have the capacity to increase or decrease blood S1P levels, and such studies will aid in determining the pathophysiological effects of such maneuvers in live animals.

EXPERIMENTAL SECTION

Sphingosine Kinase Assays. Recombinant baculovirus encoding either SphK1 or SphK2 was expressed in Sf9 insect cells, cleared lysates were prepared after 48 h, and 1−2 μL (0.02−0.03 mg protein) was used in each assay. Alternately, plasmids encoding SphK1 or SphK2 were used to transfect HEK293T cells, and cleared lysates were prepared after 48 h. SphK activity was measured in kinase assay buffer that consisted of 20 mM Tris-Cl (pH 7.4), 1 mM 2-mercaptoethanol, 1 mM EDTA, 5 mM sodium orthovanadate, 40 mM β-glycerophosphate, 15 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 10 mM $MgCl_2$, 0.5 mM 4-deoxypyridoxine, 10% glycerol, and 0.01 mg/mL each of leupeptin,

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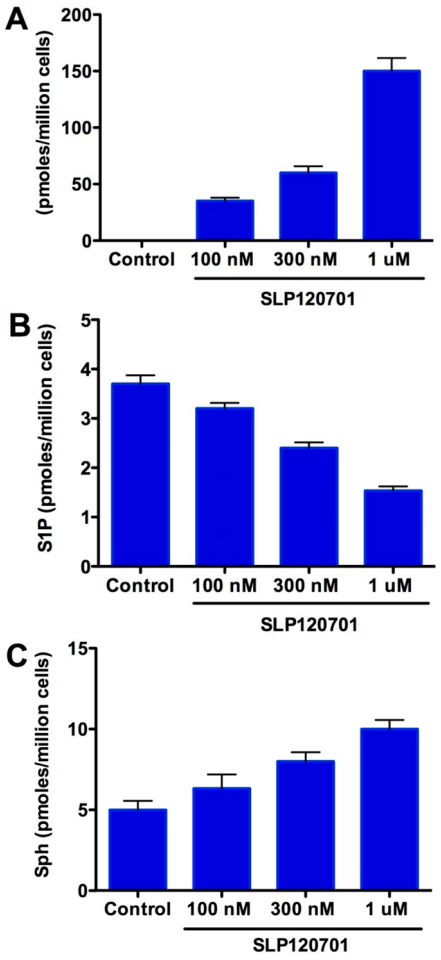

Figure 3. Effect of SLP120701 on sphingolipids in U937 cells. After 2 h of incubation, cells were harvested by centrifugation, lysed, and levels of (A) SLP120701, (B) S1P, and (C) sphingosine were measured using LC-MS-MS. Amounts associated with cells are expressed as the number of pmoles per 10^6 cells. Each value was determined in triplicate.

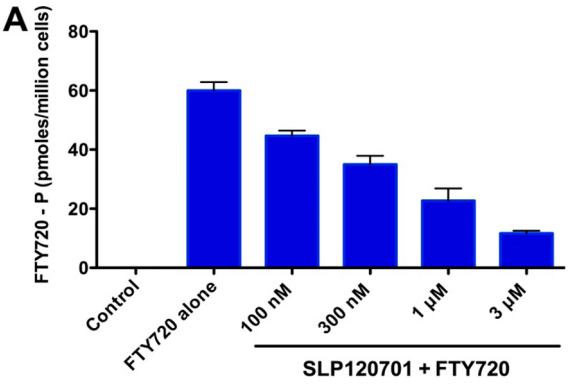

Figure 4. Sphingosine kinase 2 is inhibited by SLP120701. Cultured U937 cells were incubated with 1 μM of FTY720 and increasing concentrations of SLP120701. After 2 h of exposure, cells were harvested by centrifugation, lysed, and levels of FTY720 and FTY720-phosphate (FTY720-P) were quantified using LC-MS-MS. Amounts associated with cells are expressed as the number of pmoles per 10^6 cells. Each value was measured in triplicate.

aprotinin, and soybean trypsin inhibitor. To achieve optimal activity of SphK1 or SphK2, the buffer was supplemented with either 0.5% Triton X-100 or 1 M KCl, respectively. To ascertain any inhibitory effect of

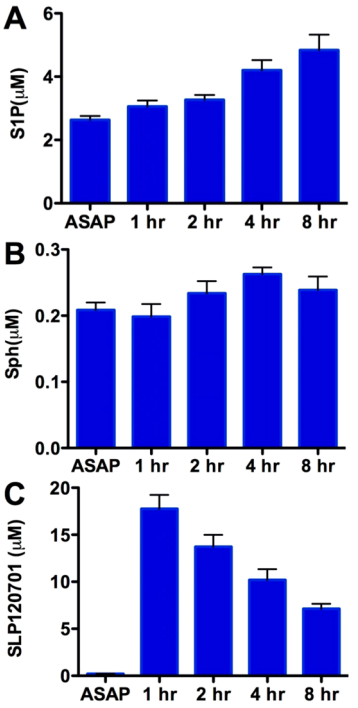

Figure 5. S1P and SLP120701 levels in the blood of mice injected with SLP120701. Wild-type mice were injected (ip) with a single dose (10 mg/kg) and blood was drawn at indicated time points. Levels of S1P (a), sphingosine (b), and SLP120701 (c) from blood samples of WT mice were measured by LC-MS-MS. The standard deviations are values from a group of three to five mice.

compounds, the assay was supplemented with substrate (D-*erythro*-sphingosine, (10 μM for SphK1 and 5 μM for SphK2), an appropriate amount of compounds (to achieve 10−10 000 nM); γ-[^{32}P]ATP (10 μM, specific activity = 8.3 Ci/mmol), and recombinant enzyme (0.02−0.03 mg of total protein). After 20 min at 37 °C, the reaction mixture was extracted with 2 volumes of chloroform/methanol/1 N HCl (100:200:1), and the components in the organic phase were separated by thin layer chromatography using a 1-butanol/glacial acetic acid/water (3:1:1) solvent system. Radiolabeled enzyme products were detected by autoradiography and identified by migration relative to authentic standards. For quantification, the silica gel containing radiolabeled lipid was scraped into a scintillation vial and measured by liquid scintillation counting.

Sample Preparation and LC-MS-MS Analysis. To analyze the lipids and compounds by LC/MS, sample preparation protocols were adapted from a literature procedure,[50] with minor modifications. Cell pellets ((2−4) × 10^6 cells), whole blood (20 μL), or plasma (50 μL) was mixed with 2 mL of a methanol:chloroform solution (3:1) and transferred to a capped glass vial. Suspensions were supplemented with 10 μL of internal standard solution containing 10 pmol each of deuterated (D7) S1P and deuterated (D7) sphingosine. The mixture was placed in a bath sonicator for 10 min and incubated at 48 °C for 16 h. The mixture was then cooled to ambient temperature and mixed with 200 μL of 1 M KOH in methanol. The samples were again sonicated and incubated a further 2 h at 37 °C. Samples were then neutralized by the addition of 20 μL of glacial acetic acid and transferred to 2 mL microcentrifuge tubes. Samples were then centrifuged at 12 000*g* for 12 min at 4 °C. The supernatant fluid was collected in a separate glass vial and evaporated under a stream of nitrogen gas. Immediately prior to LC-MS analysis, the dried material was dissolved in 0.3 mL of methanol and centrifuged at 12 000*g* for 12 min at 4 °C. Fifty microliters of the resulting supernatant fluid was analyzed.

Analyses were performed using liquid chromatography−ESI mass spectrometry (LC-MS) using a triple quadrupole mass spectrometer (AB-Sciex 4000 Q-Trap) coupled to a Shimadzu LC-20AD LC system.

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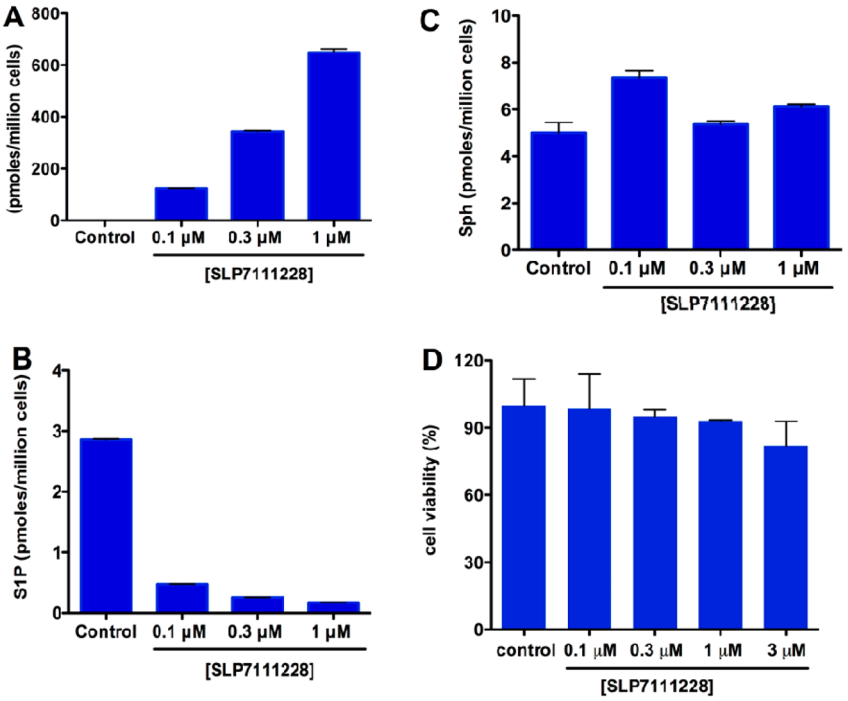

Figure 6. Effect of SLP7111228 on sphingolipids and viability of U937 cells. After 2 h of incubation, cells were harvested by centrifugation and lysed, and levels of (A) SLP7111228, (B) S1P, and (C) sphingosine were measured using LC-MS-MS. Amounts associated with cells are expressed as the number of pmoles per 10^6 cells. Each value was determined in triplicate. (D) Cell viability. Increasing concentration of inhibitor was added to U937 cells and cell growth was measured after 24 h. Each value was determined in duplicate.

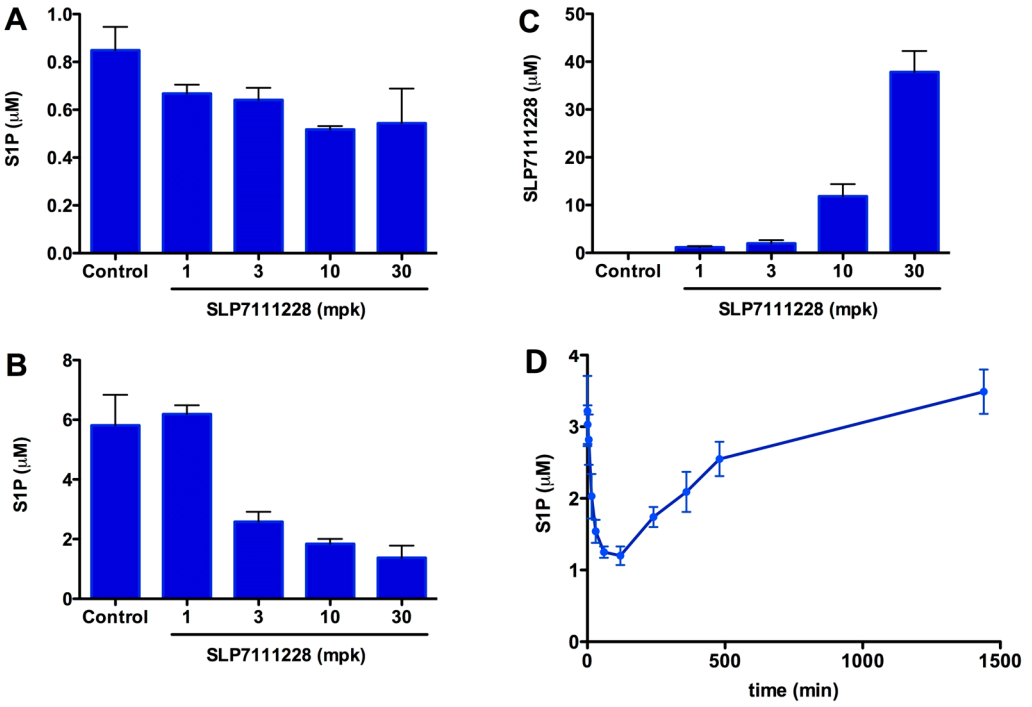

Figure 7. Effect of SLP7111228 upon IP administration in rats. Rats were injected with indicated dose and blood was drawn 2 h postinjection. Levels of S1P in (a) plasma, (b) blood, and (c) SLP7111228 are shown at indicated inhibitor concentration. (d) Time-course experiment with 10 mg/kg SLP7111228 showing blood S1P levels. Samples were analyzed by LC-MS-MS. The standard deviations are values from a group of three to five rats.

A binary solvent gradient with a flow rate of 1 mL/min was used to separate sphingolipids and drugs by reverse phase chromatography using a Supelco Discovery C18 column (50 mm × 2.1 mm, 5 μm bead size). Mobile phase A consisted of water:methanol:formic acid (79:20:1) while mobile phase B was methanol:formic acid (99:1). The run started with 100% A for 0.5 min. Solvent B was then increased linearly to 100% B in 5.1 min and held at 100% for 4.3 min. The column was finally re-equilibrated to 100% A for 1 min. Natural sphingolipids were detected using multiple reaction monitoring (MRM) protocols previously described as follows: S1P (380.4 → 264.4); deuterated (D7)C_{18}S1P (387.4 → 271.3); sphingosine (300.5 → 264.4); deuterated (D7) sphingosine (307.5 → 271.3).[50] Fragmentation of

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compound SLP120701 was analyzed by direct infusion of a 1 μM solution in methanol:formic acid (99:1), and it was found that the transition (356.3→ 126.1) in positive mode provided the most intense signal at the following voltages, DP: 106; EP: 10; CE: 29; CXP: 8. All analytes were analyzed simultaneously using the aforementioned MRMs. Retention times for all analytes under our experimental conditions were between 5.1 and 5.6 min. Quantification was carried out by measuring peak areas using commercially available software (Analyst 1.5.1).

U937 Cell Culture/Viability Assay. U937 cells were grown in RPMI 1640 media supplemented with L-glutamate, 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin at 37 °C in an atmosphere containing 5% CO_2.[51] Twenty-four hours before adding inhibitors, the growth media was replaced with media containing 0.5% FBS. For viability studies, inhibitors were added at the indicated concentrations (0.1, 0.3, 1.0, and 3.0 μM) and after 24 h, trypan blue dye was added to count cells according to manufacturer instructions (Bio-RAD TC10 Automated Cell Counter).

Pharmacokinetic Analysis. Groups of 8−20 week old mice (strain: C57BL6/j) were injected (intraperitoneal route) with either SLP120701 (dose: 10 mg/kg) or an equal volume of vehicle (2% solution of hydroxypropyl-β-cyclodextrin (Cargill Cavitron 82004)). For SLP7111228, three to four Sprague−Dawley strain rats (200−300 g) were injected with 10 mg/kg drug or an equal volume of vehicle for indicated time periods. After injection, animals were bled at the specified time points (ASAP time points were 1−2 min after dosing). Whole blood was processed immediately for LC-MS analysis as described above. Animal protocols were approved prior to experimentation by the University of Virginia's School of Medicine Animal Care and Use Committee.

General Material and Synthetic Procedures. All reactions were conducted in an oven-dried glassware under an inert atmosphere of nitrogen or argon using magnetic stirring. All solvents were dried using the PureSolv solvent purification system prior to use. All other chemical reagents were purchased from commercial sources and were used without further purification. Thin layer chromatography (TLC) was performed either on aluminum-backed silica gel or aluminum oxide (neutral) plates. Column chromatography was performed either on flash grade silica gel (SiO_2, 32−63 μm) or neutral, activated aluminum oxide (Al_2O_3, ∼150 mesh, 58 Å) as solid phase. ^{1}H NMR spectra were recorded at 500 or 400 MHz; the corresponding ^{13}C NMR resonant frequencies were 126 and 101 MHz, respectively. Chemical shifts are reported in ppm from tetramethylsilane (TMS) with the solvent resonance as an internal standard (ex: $CDCl_3$: 7.26 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet, quin = quintet), coupling constants (Hz), and integration. In case of ^{13}C NMR, chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CDCl_3$: 77.16 ppm). Low-resolution mass spectrometry (ESI-MS) was performed on a TSQ triple quadrupole mass spectrometer, equipped with an ESI source, which was used in the positive ion mode. High-resolution mass spectroscopy (HRMS) was performed on an LC/MS time-of-flight mass spectrometer using either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). All melting points are reported without correction. HPLC analyses were performed on an Agilent XDB-C8 reverse phase column using water (with 0.1% v/v of TFA) and acetonitrile as eluents. Optical rotations of final compounds are measured on a Jasco P-2000 polarimeter at room temperature (25 °C). All compounds tested in biological assays are >95% pure by ^{1}H NMR and HPLC analyses unless noted otherwise.

*4-Octylbenzonitrile (**2**).* Oct-1-ene (3 mL, 19.2 mmol) was added to a round-bottom flask containing THF (8 mL). 9-BBN (42 mL, 21.0 mmol) was added as a 0.5 M solution in THF, and the solution was stirred overnight at rt. To the above borane solution was added a solution of 4-iodobenzonitrile (4 g, 17.5 mmol) in DMF (50 mL). The reaction mixture was degassed for 10 min by bubbling N_2 through the solution. Cs_2CO_3 (11.4 g, 34.9 mmol) and $PdCl_2$(dppf) (383 mg, 0.52 mmol) were added together. The resulting reaction mixture was then stirred at 80 °C for 18 h, after which it was poured into a saturated solution of LiBr and extracted three times with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting brown residue was purified by flash chromatography over silica gel (95/5 hexanes/EtOAc) to give the title compound (2.3 g, 62%) as a colorless oil. Analytical data matches with the literature.[52]

*(Z)-N′-Hydroxy-4-octylbenzimidamide (**3**).* Triethylamine (3.9 mL, 27.8 mmol) and hydroxylamine hydrochloride (1.7 g, 24.5 mmol) were added to a solution of **2** (2.4 g, 11.1 mmol) in 95% ethanol (30 mL). The colorless reaction mixture was then refluxed for 4 h. The organic solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (65/35 hexanes/EtOAc) to give the title compound (2.5 g, 92%) as a white solid. Analytical data matches with the literature.[53]

General Procedure A: Coupling of Amidoxime 3 with Amino Acids. DIEA (1.8 equiv) was added to a solution of **3** (1 equiv), and the appropriate Boc-protected amino acid (1.2 equiv) in DMF (0.2 M solution). HCTU (1.5 equiv) was then added to the resulting mixture at rt and stirred at 80 °C for 18 h. At this time, TLC showed complete conversion of starting material. The solution was partitioned between ethyl acetate and water. The organic layer was collected and washed twice with a satd LiBr. The aqueous solution was then back extracted with ethyl acetate. The organic layers were then combined and washed with satd $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel to yield the desired product.

General Procedure B: Deprotection of *t*-Boc Protecting Groups Using HCl(g). Hydrochloric acid gas was bubbled through a solution of the N-Boc protected compound in methanol for 2−5 min or until complete consumption of starting material was observed by TLC. The reaction mixture was concentrated under reduced pressure and triturated with diethyl ether to yield the corresponding free amine hydrochloride salt, which was further purified by trituration with diethyl ether until satisfactory analytical data were obtained.

General Procedure C: Guanidylation of Amines. DIEA (3 equiv) was added to a solution of the corresponding amine hydrochloric acid salt and the reagent (Z)-*tert*-butyl (((*tert*-butoxycarbonyl)imino)-(1*H*-pyrazol-1-yl)methyl)carbamate (1.05 equiv) in acetonitrile (20% vol/wt). The resulting reaction mixture was then stirred at RT until acceptable conversion of the starting amine was observed using TLC. The solvent was then removed under reduced pressure, and the resulting colorless residue was purified by flash column chromatography over silica gel to yield the pure product.

General Procedure D: Deprotection of *t*-Boc Protecting Groups Using TFA. To a solution of Boc-protected intermediate in CH_2Cl_2 was added a 1 N TFA solution in CH_2Cl_2. The resulting solution was stirred at room temperature until complete consumption of the starting material was observed using TLC. The reaction mixture was concentrated under reduced pressure and triturated with diethyl ether to yield the corresponding free amine TFA salt, which was purified by trituration with diethyl ether/hexanes (1/1) until satisfactory analytical data were obtained.

General Procedure E: Amide Coupling of Amino Acids and Amines. The appropriate Boc-protected amino acid (1 equiv) and TEA (1 equiv) were dissolved in tetrahydrofuran (0.2 M solution) and then cooled to 0° C. Ethyl chloroformate (1 equiv) was added dropwise, and the solution was stirred for 30 min at 0 °C. The appropriate amine (1 equiv) was added dropwise. The reaction was stirred another 1 h at 0 °C, 16 h at rt, and 3 h at reflux. After completion, the solution was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to yield the desired product.

***tert*-Butyl ((3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)methyl)-carbamate (4a).** Synthesized by general procedure A. 64% yield, yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 5.38 (br s, 1H), 4.61 (d, J = 5.2 Hz, 2H), 2.64 (t, J = 8.4 Hz, 2H), 1.66−1.56 (m, 2H), 1.46 (s, 9H), 1.36−1.19 (m, 10H), 0.86 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.4, 168.5, 155.6, 146.8, 129.0, 127.5, 123.9, 80.7, 37.3, 36.0, 32.0, 31.3, 29.5, 29.4,

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29.3, 28.4, 22.7, 14.2; HRMS (ESI+): Calcd for $C_{22}H_{33}N_3NaO_3$ [M + Na]$^+$: 410.2420, Found: 410.2451.

***tert*-Butyl Methyl((3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)-methyl)carbamate (4b).** Synthesized by general procedure A. 53% yield, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.2 Hz, 2H), 7.26 (d, J = 7.9 Hz, 2H), 4.66 (d, J = 41.5 Hz, 2H), 3.03 (d, J = 13.2 Hz, 3H), 2.63 (t, J = 7.4 Hz, 2H), 1.62 (quin, J = 7.4 Hz, 2H), 1.46 (d, J = 29.3 Hz, 9H), 1.32−1.21 (m, 10H), 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.2, 168.6, 146.8, 129.0, 127.5, 124.0, 81.0, 45.5, 36.1, 35.3, 32.0, 31.3, 29.6, 29.4, 29.3, 28.4, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{23}H_{36}N_3O_3$ [M + H]$^+$: 402.2756, Found: 402.2742.

(*S*)-*tert*-Butyl (1-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)-ethyl)carbamate (4c). Synthesized by general procedure A. 82% yield, yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 5.29 (br s, 1H), 5.22−5.03 (m, 1H), 2.64 (t, J = 8.0 Hz, 2H), 1.68−1.53 (m, 5H), 1.45 (s, 9H), 1.36−1.17 (m, 10H), 0.86 (t, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.0, 168.4, 154.9, 146.7, 129.0, 127.5, 124.0, 80.5, 44.3, 36.0, 31.9, 31.3, 29.5, 29.3, 29.3, 28.4, 22.7, 20.2, 14.2; HRMS: Calcd for $C_{23}H_{35}N_3NaO_3$ [M + Na]$^+$: 424.2576, Found: 424.2571.

(*S*)-*tert*-Butyl (2-Methyl-1-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)propyl)carbamate (4d). Synthesized by general procedure A. 77% yield, yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, J = 8.1 Hz, 2H), 7.27 (d, J = 8.1 Hz, 2H), 5.28−5.17 (m, 1H), 5.01−4.92 (m, 1H), 2.68−2.60 (m, 2H), 2.33−2.17 (m, 1H), 1.67−1.53 (m, 2H), 1.45 (s, 9H), 1.34−1.16 (m, 10H), 0.98 (d, J = 6.8 Hz, 6H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.9, 168.3, 155.4, 146.7, 129.0, 127.5, 124.1, 80.4, 53.7, 36.0, 32.9, 31.9, 31.3, 29.5, 29.3, 28.4, 22.7, 18.7, 18.0, 14.2; HRMS: Calcd for $C_{25}H_{39}N_3NaO_3$ [M + Na]$^+$: 452.2889, Found: 452.2884.

(*S*)-*tert*-Butyl (2-Hydroxy-1-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate (4e). Synthesized by general procedure A. 28% yield, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.3 Hz, 2H), 7.28 (d, J = 8.3 Hz, 2H), 5.56 (s, 1H), 5.18 (s, 1H), 4.17 (d, J = 8.9 Hz, 1H), 4.04 (d, J = 9.5 Hz, 1H), 2.65 (t, J = 7.6 Hz, 2H), 2.51 (s, 1H), 1.63 (quin, J = 7.6 Hz, 2H), 1.48 (s, 9H), 1.35−1.22 (m, 10H), 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.4, 168.4, 147.0, 129.1, 127.6, 123.8, 64.0, 50.2, 36.1, 32.0, 31.3, 29.6, 29.4, 29.3, 28.4, 22.8, 14.2; HRMS (Mixed ESI): Calcd for $C_{23}H_{36}N_3O_4$ [M + H]$^+$: 418.5505, Found: 418.2692.

***tert*-Butyl (1-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)-cyclopropyl)carbamate (4f).** Synthesized by general procedure A. 62% yield, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 7.5 Hz, 2H), 7.24 (d, J = 8.0 Hz, 2H), 5.53 (s, 1H), 2.70−2.57 (m, 2H), 1.77−1.73 (m, 2H), 1.67−1.56 (m, 2H), 1.49−1.45 (m, 11H), 1.35−1.18 (m, 10H), 0.92−0.82 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.8, 168.5, 155.6, 146.3, 128.8, 127.3, 124.2, 80.5, 35.9, 31.8, 31.2, 30.9, 29.4, 29.3, 29.2, 28.2, 22.6, 19.6, 14.1; HRMS (ESI +): Calcd for $C_{24}H_{35}N_3NaO_3$ [M + Na]$^+$: 436.2576, Found: 436.2530.

(*S*)-*tert*-Butyl 2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)-pyrrolidine-1-carboxylate (4g). Synthesized by general procedure A. 48% yield, yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (d, J = 7.8 Hz, 2H), 7.33−7.18 (m, 2H), 5.21−5.14 (m, 1H, minor rotamer), 5.07−5.01 (m, 1H, major rotamer), 3.75−3.67 (m, 1H, major rotamer), 3.67−3.61 (m, 1H, minor rotamer), 3.59−3.50 (m, 1H, major rotamer), 3.50−3.41 (m, 1H, minor rotamer), 2.66−2.58 (m, 2H), 2.44−2.25 (m, 1H), 2.20−2.05 (m, 2H), 2.05−1.89 (m, 1H), 1.68−1.54 (m, 2H), 1.44 (s, 3H), 1.37−1.16 (m, 16H), 0.85 (t, J = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$, rotamers) δ 180.5 (major), 180.1 (minor), 168.5 (major), 154.3 (minor), 153.6 (major), 146.7 (major), 146.4 (minor), 129.0 (major), 128.9 (minor), 127.5 (minor), 127.5 (major), 124.4 (minor), 124.2 (major), 80.5 (major), 80.4 (minor), 53.9 (major), 46.7 (minor), 46.4 (major), 36.0 (major), 32.5 (major), 32.0 (major), 31.6 (minor), 31.3 (major), 29.5 (major), 29.3 (major), 29.2 (major), 28.5 (minor), 28.2 (major), 24.4 (minor), 23.8 (major), 22.7 (major), 14.2 (major). HRMS (ESI+): Calcd for $C_{25}H_{37}N_3NaO_3$ [M + Na]$^+$: 450.2733, Found: 450.2727.

(*R*)-*tert*-Butyl 2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)-pyrrolidine-1-carboxylate ((*R*)-4h). Synthesized by general procedure A. 73% yield, yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 7.9 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 5.10−4.94 (m, 1H), 3.68 (m, 1H), 3.52 (m, 1H), 2.63 (t, J = 7.5 Hz, 2H), 2.36 (m, 1H), 2.12 (m, 2H), 2.02−1.87 (m, 1H), 1.61 (m, 2H), 1.44 (s, 3H), 1.34−1.15 (m, 16H), 0.85 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.4, 168.3, 153.5, 146.5, 128.9, 128.7, 127.4, 127.3, 124.0, 80.4, 60.3, 53.8, 46.3, 35.9, 32.4, 31.8, 31.4, 31.2, 29.4, 29.3, 29.2, 28.4, 28.1, 23.7, 22.6, 21.0, 14.1, 14.0; HRMS (ESI+): Calcd for $C_{25}H_{38}N_3O_3$ [M + H]$^+$: 428.2913, Found: 428.2908.

***(S)-tert*-Butyl2- (3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)-2,5-dihydro-1*H*-pyrrole-1-carboxylate (4i).** Synthesized by general procedure A. 20% yield, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.2 Hz, 2H), 7.29 (d, J = 8.2 Hz, 2H), 6.09 (dd, J = 24.9, 4.3 Hz, 1H), 5.87, (d, J = 21.6 Hz, 1H), 5.87−5.77 (m, 1H), 4.49−4.30 (m, 2H), 2.65 (t, J = 8 Hz, 2H), 1.63 (quin, J = 7.6 Hz, 2H), 1.48 (s, 3H), 1.28 (d, J = 22.2 Hz, 16H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.28, 177.80, 168.69, 153.26, 146.79, 129.89, 129.08, 127.54, 124.99, 124.14, 81.00, 60.85, 53.46, 36.12, 32.01, 31.37, 29.85, 29.58, 29.38, 28.28, 22.81, 14.24; HRMS (ESI+): Calcd for $C_{25}H_{35}N_3O_3Na$ [M + Na]$^+$: 448.2576, Found: 448.2595.

(*S*)-*tert*-Butyl,2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)-azetidine-1-carboxylate (4j). Synthesized by general procedure A. 73% yield, yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.2 Hz, 2H), 7.38−7.15 (m, 2H), 4.60 (s, 1H), 4.19 (td, J = 9.4, 5.6 Hz, 1H), 2.8 (quin, J = 9.4 Hz, 2H), 2.72−2.40 (m, 2H), 1.83−1.61 (m, 3H), 1.42 (s, 9H), 1.33−1.19 (m, 10H), 1.18 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.0, 168.2, 155.9, 132.0, 127.3, 127.2, 125.2, 93.3, 80.2, 80.1, 31.3, 28.6, 28.5, 28.3, 25.7, 22.5, 19.5, 14.1; HRMS (ESI+): Calcd for $C_{24}H_{35}N_3NaO_3$ [M + Na]$^+$: 436.2576, Found: 436.2571.

(*S*)-*tert*-Butyl 2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)-piperidine-1-carboxylate (4k). Synthesized by general procedure A. 77% yield, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.1 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 5.55 (s, 1H), 4.07 (s, 1H), 3.02 (s, 1H), 2.63 (t, J = 8.1, 6.7 Hz, 2H), 1.98−1.85 (m, 2H), 1.80−1.57 (m, 4H), 1.55−1.38 (m, 9H), 1.35−1.19 (m, 10H), 0.86 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.7, 168.3, 150.8, 146.4, 128.8, 127.4, 124.1, 80.5, 35.9, 31.8, 31.2, 29.4, 29.2, 29.2, 28.3, 27.9, 24.7, 22.6, 20.0, 14.0. HRMS (ESI+): Calcd for $C_{26}H_{39}N_3NaO_3$ [M + Na]$^+$: 464.2889, Found: 436.2874

(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)methanamine (5a). Synthesized by general procedure D. 99% yield, off-white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.08−7.92 (m, 2H), 7.34 (d, J = 8.4 Hz, 2H), 4.86 (br s, 2H), 4.59 (s, 2H), 2.70−2.62 (m, 2H), 1.70−1.57 (m, 2H), 1.37−1.20 (m, 10H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 173.1, 168.5, 147.2, 128.9, 127.2, 123.4, 35.6, 34.9, 31.7, 31.1, 29.2, 29.1, 29.0, 22.4, 13.1; HRMS (ESI+): Calcd for $C_{17}H_{26}N_3O^+$ [M$^+$]: 288.2070, Found: 288.2067.

***N*-Methyl-1-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)-methanamine (5b).** Synthesized by general procedure B. 94% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.13 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 8.3 Hz, 2H), 5.01 (s, 2H), 3.09 (s, 3H), 2.81 (t, J = 7.8, 2H), 1.78 (quin, J = 7.3 Hz, 2H), 1.51−1.38 (m, 10H), 1.01 (t, J = 6.7, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.2, 169.7, 148.5, 130.2, 128.5, 124.6, 44.4, 36.8, 34.2, 33.0, 32.4, 30.5, 30.3, 30.2, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{18}H_{28}N_3O$ [M + H]$^+$: 302.2232, Found: 302.2224.

(*S*)-1-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)ethanamine (5c). Synthesized by general procedure D. 89% yield, white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06−7.89 (m, 2H), 7.35−7.19 (m, 2H), 4.33 (q, J = 6.9 Hz, 1H), 2.70−2.55 (m, 2H), 1.81 (br s, 2H), 1.66−1.52 (m, 5H), 1.36−1.17 (m, 10H), 0.86 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 183.0, 168.3, 146.6, 129.0, 127.5, 124.2, 45.0, 36.0, 32.0, 31.3, 29.5, 29.4, 29.3, 22.7, 21.8, 14.2. HRMS (ESI+): Calcd for $C_{18}H_{28}N_3O^+$ [M$^+$]: 302.2227, Found: 302.2219.

(*S*)-2-Methyl-1-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)-propan-1-amine (5d). Synthesized by general procedure D. 59% yield, white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01−7.92 (m, 2H), 7.30−7.19 (m, 2H), 4.00 (d, J = 5.8 Hz, 1H), 2.61 (t, J = 8.0 Hz, 2H), 2.23−2.11 (m, 1H), 1.73 (br s, 2H), 1.66−1.53 (m, 2H), 1.38−1.16 (m, 10H), 1.03−0.91 (m, 6H), 0.85 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.2, 168.1, 146.6, 129.0, 127.5, 124.2, 55.1, 36.0, 33.6, 31.9,

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31.3, 29.5, 29.3, 22.7, 19.0, 17.9, 14.2. HRMS (ESI+): Calcd for $C_{20}H_{32}N_3O^+$ [M^+]: 330.2540, Found: 330.2532.

(*S*)-2-Hydroxy-1-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)-ethanaminium chloride (5e). Synthesized by general procedure B. 74% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.05 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 4.99 (t, J = 4.5 Hz, 1H), 4.20 (d, J = 4.5 Hz, 2H), 2.73 (t, J = 6.5 Hz, 2H), 1.70 (quin, J = 7.3 Hz, 2H), 1.43−1.28 (m, 10H), 0.93 (t, J = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 175.3, 169.8, 148.6, 130.2, 128.5, 124.7, 61.4, 51.4, 36.9, 33.0, 32.4, 30.5, 30.4, 30.3, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{18}H_{28}N_3O_2$+ [M^+]: 318.2181, Found: 318.2174.

1-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)cyclopropanamine (5f). Synthesized by general procedure D. 100% yield, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 8.2 Hz, 2H), 7.27 (d, J = 8.0 Hz, 2H), 2.70−2.59 (m, 2H), 2.38 (s, 2H), 1.69−1.55 (m, 2H), 1.54−1.46 (m, 2H), 1.36−1.19 (m, 12H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 183.8, 168.4, 146.4, 128.8, 127.3, 124.2, 35.9, 31.9, 31.8, 31.2, 29.4, 29.2, 29.2, 22.6, 19.8, 14.1; HRMS (ESI+): Calcd for $C_{19}H_{28}N_3O$ [M + H]$^+$: 314.2232, Found: 314.2208.

(*S*)-3-(4-Octylphenyl)-5-(pyrrolidin-2-yl)-1,2,4-oxadiazole (5g). Synthesized by general procedure D. 82% yield, off white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01−7.87 (m, 2H), 7.25−7.18 (m, 2H), 4.46 (dd, J = 8.3, 5.6 Hz, 1H), 3.20−3.09 (m, 1H), 3.09−2.94 (m, 1H), 2.60 (t, J = 8.0 Hz, 2H), 2.34 (br s, 1H), 2.29−2.17 (m, 1H), 2.14−2.00 (m, 1H), 1.96−1.76 (m, 2H), 1.65−1.51 (m, 2H), 1.34−1.13 (m, 10H), 0.83 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 181.9, 168.2, 146.5, 128.9, 127.5, 124.2, 54.4, 46.9, 36.0, 31.9, 31.3, 31.2, 29.5, 29.3, 29.3, 25.4, 22.7, 14.2; HRMS (ESI+) calcd for $C_{20}H_{30}N_3O$ [M^+]: 328.2389, Found 328.2354.

(*R*)-3-(4-Octylphenyl)-5-(pyrrolidin-2-yl)-1,2,4-oxadiazole (5h). Synthesized by general procedure D. 100% yield, off white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 7.97 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 7.6 Hz, 2H), 4.65−4.44 (m, 1H), 3.34 (s, 1H), 3.12−3.18 (m, 2H), 2.67 (t, J = 7.6 Hz, 2H), 2.35−2.31 (m, 1H), 2.18−2.14 (m, 1H), 2.05−1.91 (m, 2H), 1.68−1.62 (m, 2H), 1.38−1.25 (m, 10H), 0.90 (t, J = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 182.8, 169.4, 148.0, 130.1, 128.4, 125.4, 55.3, 47.6, 36.9, 33.0, 32.4, 31.9, 30.6, 30.4, 30.3, 26.4, 23.7, 14.5; HRMS (ESI+): Calcd for $C_{20}H_{30}N_3O$ [M + H]$^+$: 328.2389, Found: 328.2383.

(*S*)-2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)-2,5-dihydro-1*H*-pyrrol-1-ium Chloride (5i). Synthesized by general procedure B. 72% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 11.95 (d, J = 8.3 Hz, 2H), 11.31 (d, J = 8.3 Hz, 2H), 10.30 (d, J = 4.5 Hz, 1H), 10.20 (d, J = 5.3 Hz, 1H), 9.97 (s, 1H), 8.37−8.20 (m, 2H), 6.64 (t, J = 7.0 Hz, 2H), 5.61 (quin, J = 7.4 Hz, 2H), 5.33−5.19 (m, 10H), 4.84 (t, J = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 174.7, 170.1, 148.7, 130.3, 130.2, 128.5, 125.2, 124.5, 61.6, 54.0, 36.9, 33.0, 32.4, 30.5, 30.4, 30.3, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{20}H_{28}N_3O^+$ [M^+]: 326.2232, Found: 326.2240.

(*S*)-2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-ium (5j). Synthesized by general procedure B. 94% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 8.5 Hz, 2H), 5.93 (t, J = 8.5 Hz, 1H), 4.39−4.26 (m, 1H), 4.17 (td, J = 10.0, 6.3 Hz, 1H), 3.21−2.96 (m, 3H), 2.67 (t, J = 7.9, 7.4 Hz, 2H), 1.64 (quin, J = 7.3 Hz, 2H), 1.43−1.16 (m, 10H), 0.87 (t, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.8, 168.7, 147.2, 128.8, 127.1, 123.2, 52.8, 44.6, 35.4, 31.6, 31.0, 29.1, 28.9, 28.9, 23.9, 22.3, 13.0; HRMS (ESI+): Calcd for $C_{19}H_{29}ClN_3O$ [M + H]$^+$: 350.1999, Found: 350.2017.

(*S*)-2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-ium Chloride (5k). Synthesized by general procedure B. 95% yield, white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, J = 8.3 Hz, 2H), 7.27 (d, J = 8.8 Hz, 2H), 4.12 (dd, J = 9.8, 3.3 Hz, 1H), 3.20 (dt, J = 12.2, 3.6 Hz, 1H), 2.88−2.77 (m, 1H), 2.65 (t, J = 7.6 Hz, 2H), 2.29−2.10 (m, 2H), 1.94−1.76 (m, 2H), 1.72−1.54 (m, 5H), 1.30 (ddt, J = 24.7, 9.8, 3.9 Hz, 10H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.3, 168.2, 146.5, 128.9, 127.4, 124.1, 53.5, 46.0, 36.0, 31.9, 31.6, 31.2, 30.3, 29.4, 29.3, 29.2, 25.7, 23.6, 22.7, 14.1; HRMS (ESI+): Calcd for $C_{21}H_{32}N_3O^+$ [M^+]: 342.2540, Found: 342.2554.

***tert*-Butyl *N*-[[(*tert*-Butoxy)carbonyl]amino([3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl]methyl amino)methylidene]carbamate (6a).** Synthesized by general procedure C. 78% yield, yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.47 (br s, 1H), 8.99 (t, J = 5.3 Hz, 1H), 7.99−7.95 (m, 2H), 7.28 (d, J = 8.0 Hz, 2H), 4.95 (d, J = 5.3 Hz, 2H), 2.64 (t, J = 7.7 Hz, 2H), 1.67−1.58 (m, 2H), 1.52 (s, 9H), 1.49 (s, 9H), 1.35−1.19 (m, 10H), 0.86 (t, J = 6.3 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.4, 168.5, 163.2, 156.3, 153.1, 146.8, 129.0, 127.6, 123.8, 83.8, 79.9, 37.3, 36.1, 31.9, 31.3, 29.5, 29.3, 29.3, 28.3, 28.1, 22.7, 14.2. HRMS (ESI+): Calcd for $C_{28}H_{44}N_5O_5$ [M + H]$^+$: 530.3342, Found: 530.3302.

***tert*-Butyl (((*tert*-Butoxycarbonyl)amino)(methyl((3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)methyl)amino)carbamate (6b).** Synthesized by general procedure C. 34% yield, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 8.7 Hz, 2H), 4.96 (s, 2H), 3.18 (s, 3H), 2.65 (t, J = 7.3 Hz, 2H), 1.67−1.59 (m, 2H), 1.49 (s, 18H), 1.36−1.21 (m, 10H), 0.87 (t, J = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.0, 168.6, 156.1, 146.8, 129.1, 127.6, 124.0, 77.4, 46.5, 38.0, 36.1, 32.0, 31.4, 29.9, 29.6, 29.4, 29.3, 28.2, 28.1, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{29}H_{46}N_5O_5$ [M + H]$^+$: 544.3499, Found: 544.3453.

***tert*-Butyl *N*-[[(*tert*-Butoxy)carbonyl]amino([(1*S*)-1-[3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl]ethyl]amino)methylidene]-carbamate (6c).** Synthesized by general procedure C. 81% yield, yellow solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.50 (br s, 1H), 8.97 (d, J = 8.1 Hz, 1H), 8.00−7.92 (m, 2H), 7.30−7.23 (m, 2H), 5.82−5.72 (m, 1H), 2.70−2.55 (m, 2H), 1.71−1.36 (m, 5H), 1.51 (s, 9H), 1.47 (s, 9H), 1.36−1.10 (m, 10H), 0.86 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.2, 168.5, 163.4, 155.7, 153.1, 146.7, 129.0, 127.6, 124.0, 83.7, 79.7, 43.8, 36.0, 32.0, 31.3, 29.5, 29.3, 28.3, 28.2, 22.7, 20.1, 14.2. HRMS (ESI+): Calcd for $C_{29}H_{46}N_5O_5$ [M + H]$^+$: 544.3499, Found: 544.3477.

***tert*-Butyl *N*-[[(*tert*-Butoxy)carbonyl]amino({[(1*S*)-2-methyl-1-[3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl]propyl]amino})-methylidene]carbamate (6d).** Synthesized by general procedure C. 43% yield, yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.51 (br s, 1H), 9.05 (d, J = 8.5 Hz, 1H), 7.98 (d, J = 7.3 Hz, 2H), 7.26 (d, J = 7.6 Hz, 2H), 5.59−5.55 (m, 1H), 2.64 (t, J = 7.6 Hz, 2H), 2.43−2.35 (m, 1H), 1.66−1.57 (m, 2H), 1.52 (s, 9H), 1.44 (s, 9H), 1.34−1.19 (m, 10H), 1.05−0.99 (m, 6H), 0.86 (t, J = 6.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.2, 168.3, 163.4, 156.3, 153.2, 146.6, 129.0, 127.6, 124.2, 83.6, 79.6, 53.1, 36.0, 32.4, 31.9, 31.3, 29.5, 29.3, 29.3, 28.3, 28.2, 22.7, 18.6, 18.1, 14.2. HRMS (ESI+): Calcd for $C_{31}H_{50}N_5O_5$ [M + H]$^+$: 572.3812, Found: 572.3802.

(*S*,*E*)-*tert*-Butyl (((*tert*-Butoxycarbonyl)amino)(2-hydroxy-1-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate (6e). Synthesized by general procedure C. 32% yield, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.49 (s, 1H), 9.36 (d, J = 7.6 Hz, 1H), 7.98 (d, J = 8.2 Hz, 2H), 7.29 (d, J = 8.2 Hz, 2H), 5.77 (dt, J = 7.9, 4.1 Hz, 1H), 4.14 (qd, J=, 2H), 2.65 (t, J = 7.9 Hz, 2H), 1.68−1.59 (m, 2H), 1.51 (d, J = 14.0 Hz, 18H), 1.29 (d, J = 17.3 Hz, 10H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.5, 168.5, 163.0, 156.4, 153.0, 147.0, 129.1, 127.7, 123.7, 84.0, 80.0, 64.6, 50.6, 36.1, 32.0, 31.3, 29.6, 29.4, 29.4, 28.4, 28.2, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{29}H_{46}N_5O_6$ [M + H]$^+$: 560.3448, Found: 560.3454.

1,2-Di-Boc-3-(1-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)-cyclopropyl)guanidine (6f). Synthesized by general procedure C. 35% yield, colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.50 (s, 1H), 8.92 (s, 1H), 7.86 (d, J = 8.3 Hz, 2H), 7.18 (d, J = 8.2 Hz, 2H), 2.57 (t, J = 7.5 Hz, 2H), 1.79 (dd, J = 5.5, 8.5 Hz, 2H), 1.60−1.53 (m, 2H), 1.51 (dd, J = 5.5, 8.5 Hz, 2H), 1.45 (s, 9H), 1.32 (s, 9H), 1.26−1.12 (m, 10H), 0.80 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.0, 168.5, 163.3, 156.9, 153.2, 146.3, 128.8, 127.4, 124.3, 83.5, 79.6, 35.9, 31.9, 31.2, 30.7, 29.7, 29.4, 29.2, 28.2, 28.1, 22.7, 20.3, 14.1; HRMS (ESI+): Calcd for $C_{30}H_{46}N_5O_5$ [M + H]$^+$: 556.3499.

(*S*)-*tert*-Butyl (((*tert*-Butoxycarbonyl)imino)(2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (6g). Synthesized by general procedure C. 66% yield, yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (d, J = 8.2 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 5.62−5.54 (m, 1H), 3.93−3.83 (m, 1H), 3.83−3.71 (m, 1H), 2.64 (t, J = 7.9 Hz, 2H), 2.48−2.36 (m, 1H), 2.30−2.09 (m, 2H), 2.08−1.98 (m, 1H), 1.95−1.86 (m, 1H), 1.67−1.57 (m, 2H), 1.54−1.18 (m, 28H), 0.86 (t, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.7, 168.3, 161.9, 153.5, 150.3, 146.5, 128.8, 127.4, 123.9, 82.2, 79.5, 55.3, 49.4,

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35.9, 31.8, 31.2, 29.4, 29.2, 28.1, 23.9, 22.6, 14.1; HRMS (ESI+): Calcd for $C_{31}H_{48}N_5O_5$ $[M + H]^+$: 570.3655, found 570.3605.

(*R*)-*tert*-Butyl (((*tert*-Butoxycarbonyl)amino)(2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methylene)carbamate (6h). Synthesized by general procedure C. 55% yield, colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.10 (s, 1H), 7.99 (d, J = 8.2 Hz, 2H), 7.29 (d, J = 8.2 Hz, 2H), 5.71−5.53 (m, 1H), 3.98−3.87 (m, 1H), 3.84−3.80 (m, 1H), 2.75−2.63 (m, 2H), 2.48−2.44 (m, 1H), 2.35−2.13 (m, 2H), 2.08−2.04 (m, 1H), 1.74−1.61 (m, 2H), 1.48 (s, 18H), 1.38−1.21 (m, 10H), 0.89 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.7, 168.4, 146.5, 129.6, 128.9, 127.5, 124.0, 82.2, 79.6, 55.3, 49.4, 35.9, 31.8, 31.4, 31.2, 29.7, 29.4, 29.2, 28.1, 23.9, 22.6, 14.1; HRMS (ESI+): Calcd for $C_{31}H_{48}N_5O_5$ $[M + H]^+$: 570.3655, Found: 570.3662.

(*S,E*)-*tert*-Butyl (((*tert*-Butoxycarbonyl)amino)(2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)-2,5-dihydro-1*H*-pyrrol-1-yl)methylene)carbamate (6i). Synthesized by general procedure C. 22% yield, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.2 Hz, 2H), 7.27 (d, J = 8.0 Hz, 2H), 6.42 (s, 1H), 6.13 (d, J = 4.6 Hz, 1H), 5.90 (dd, J = 6.4, 2.2 Hz, 1H), 2.65 (t, J = 7.5 Hz, 2H), 1.67−1.59 (m, 2H), 1.50 (d, J = 10.8 Hz, 18H), 1.35−1.22 (m, 10H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.7, 168.6, 129.0, 128.9, 127.6, 124.3, 115.6, 110.2, 53.6, 36.1, 32.0, 31.4, 29.9, 29.6, 29.4, 29.3, 28.3, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{31}H_{46}N_5O_5$ $[M + H]^+$: 568.7286, Found: 568.3478.

(*S*)-*tert*-Butyl (((*tert*-Butoxycarbonyl)amino)(2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-yl)methylene)carbamate (6j). Synthesized by general procedure C. 58% yield, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 8.2 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 4.63 (s, 1H), 4.20 (td, J = 9.4, 5.6 Hz, 1H), 2.85 (quin, J = 9.4 Hz, 1H), 2.66 (t, J = 7.5 Hz, 2H), 2.56 (dq, J = 10.4, 5.0 Hz, 1H), 1.74 (s, 1H), 1.64 (quin, J = 7.4 Hz, 2H), 1.45 (s, 18H), 1.34−1.23 (m, 10H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.1, 171.1, 168.5, 146.7, 128.9, 127.5, 123.8, 60.4, 36.0, 31.8, 31.2, 29.4, 29.2, 29.2, 28.0, 22.6, 22.4, 21.0, 14.2, 14.1; HRMS (ESI+): Calcd for $C_{30}H_{46}N_5O_5$ $[M + H]^+$: 556.3499, Found: 556.3488

(*S*)-*tert*-Butyl (((*tert*-Butoxycarbonyl)amino)(2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)methylene)carbamate (6k). To a microwave safe glass tube were added (*S*)-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-ium **5k** (0.04 g, 0.105 mmol), triethylamine (0.045 mL, 0.32 mmol), *tert*-butyl (((*tert*-butoxycarbonyl)amino)(1*H*-pyrazol-1-yl)methylene)carbamate **9** (0.032 g, 0.105 mmol), and anhydrous acetonitrile (2 mL). The resulting reaction mixture was sealed and heated in a microwave reactor for 3 h at 60 W. After 3 h, the reaction flask was cooled, and the resulting colorless solution was concentrated in vacuo. The resulting pale yellow oil was then purified by flash chromatography to yield product **6k** as colorless oil in 18% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, J = 8.3 Hz, 2H), 7.27 (d, J = 8.3 Hz, 2H), 5.98 (bs, 1H), 3.99 (bs, 1H), 3.67 (s, 1H), 3.35 (t, J = 12.0 Hz, 1H), 3.10 (s, 1H), 2.66 (t, J = 7.9, 7.5 Hz, 2H), 2.41 (d, J = 13.4 Hz, 1H), 2.11 (s, 1H), 1.77 (m, 2H), 1.72−1.60 (m, 4H), 1.56 (d, J = 11.9 Hz, 4H), 1.49 (s, 18H), 1.37−1.21 (m, 10H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.6, 167.3, 154.5, 145.5, 127.8, 126.5, 123.0, 52.8, 34.9, 30.8, 30.2, 28.4, 28.2, 27.1, 27.0, 23.7, 21.6, 19.1, 17.9, 16.6, 13.1; HRMS (ESI+): Calcd for $C_{32}H_{50}N_5O_5$ $[M + H]^+$: 584.3734, Found: 584.3822

1-((3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)methyl)guanidine Hydrochloride (7a). Synthesized by general procedure B. 84% yield, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 4.84 (s, 2H), 2.67 (t, J = 7.6 Hz, 2H), 1.71−1.54 (m, 2H), 1.38−1.19 (m, 10H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 175.5, 168.4, 158.1, 147.0, 128.8, 127.1, 123.7, 37.4, 35.5, 31.7, 31.1, 29.2, 29.1, 29.0, 22.4, 13.1; HRMS (ESI+) m/z calcd for $C_{18}H_{28}N_5O$ $[M + H]^+$: 330.2294, Found: 330.2269.

1-Methyl-1-((3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)methyl)-guanidine Hydrochloride (7b). Synthesized by general procedure B. 15% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (d, J = 8.4 Hz, 2H), 7.39 (d, J = 8.5 Hz, 2H), 5.04 (s, 2H), 3.27 (s, 3H), 2.72 (t, J = 7.4 Hz, 2H), 1.69 (quin, J = 7.3 Hz, 2H), 1.43−1.26 (m, 10H), 0.93 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 175.9, 169.8, 159.6, 148.4, 130.2, 128.4, 125.0, 47.4, 37.8, 36.8, 33.0, 32.4, 30.5, 30.4, 30.3, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{19}H_{30}N_5O$ $[M + H]^+$: 344.2450, Found: 344.2416.

(*S*)-1-(1-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)ethyl)-guanidine Hydrochloride (7c). Synthesized by general procedure B. 82% yield, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 (d, J = 8.1 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 5.28−5.14 (m, 1H), 2.75−2.56 (m, 2H), 1.75 (d, J = 6.9 Hz, 3H), 1.68−1.56 (m, 2H), 1.39−1.17 (m, 10H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 178.5, 168.4, 157.3, 147.0, 128.8, 127.1, 123.8, 45.2, 35.6, 31.7, 31.1, 29.2, 29.1, 29.0, 22.4, 18.0, 13.1; HRMS (ESI+) calcd for $C_{19}H_{30}N_5O$ $[M + H]^+$: 344.2450, Found: 344.2407. $[\alpha]_D$ = −148° (c = 0.0005, methanol).

(*S*)-1-(2-Methyl-1-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)-propyl)guanidine Hydrochloride (7d). Synthesized by general procedure B. 80% yield, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 (d, J = 8.1 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 5.08−4.99 (m, 1H), 2.65 (t, J = 7.9 Hz, 2H), 2.51−2.40 (m, 1H), 1.68−1.56 (m, 2H), 1.38−1.19 (m, 10H), 1.07 (dd, J = 15.1, 6.8 Hz, 6H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 177.4, 168.4, 157.9, 147.0, 128.9, 127.1, 123.7, 54.7, 35.6, 32.4, 31.7, 31.1, 29.2, 29.1, 29.0, 22.4, 17.6, 16.9, 13.1; HRMS (ESI+) calcd for $C_{21}H_{34}N_5O$ $[M + H]^+$: 372.2763, Found: 372.2773. $[\alpha]_D$ = −156° (c = 0.0005, methanol)

(*S*)-Amino((2-hydroxy-1-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)ethyl)amino)methaniminium Chloride (7e). 12% yield, white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 8.00 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 4.90 (d, J = 4.4 Hz, 1H), 4.15−4.10 (m, 2H), 2.68 (t, J = 7.6 Hz, 2H), 1.71−1.55 (m, 2H), 1.39−1.22 (m, 10H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 177.8, 169.8, 159.2, 148.3, 130.2, 128.4, 125.1, 63.4, 53.1, 36.9, 33.0, 32.4, 30.5, 30.4, 30.3, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{19}H_{30}N_5O_2$+ $[M^+]$: 360.2399, Found: 360.2386. $[\alpha]_D$ = +118° (c = 0.00066, methanol).

1-(1-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)cyclopropyl)-guanidine Hydrochloride (7f). Synthesized by general procedure B. 99% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.63 (s, 1H), 7.92 (d, J = 8.2 Hz, 2H), 7.32 (d, J = 8.2 Hz, 2H), 2.67 (t, J = 7.6 Hz, 2H), 1.92 (dd, J = 5.2, 8.4 Hz, 2H), 1.72−1.58 (m, 4H), 1.38−1.24 (m, 10H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 180.6, 169.9, 159.8, 148.2, 130.1, 128.3, 125.2, 36.8, 33.0, 32.4, 31.6, 30.5, 30.4, 30.3, 23.7, 21.1, 14.4; HRMS (ESI+): Calcd for $C_{20}H_{30}N_5O$ $[M + H]^+$: 356.2445, Found 356.2439.

(*S*)-2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboximidamide Hydrochloride (7g). Synthesized by general procedure B. 80% yield, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 7.94 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 5.50−5.38 (m, 1H), 3.80−3.70 (m, 1H), 3.67−3.54 (m, 1H), 2.70−2.39 (m, 4H), 2.28−2.16 (m, 1H), 2.16−1.98 (m, 1H), 1.70−1.55 (m, 2H), 1.39−1.15 (m, 10H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 177.5, 168.4, 155.8, 147.0, 128.8, 127.1, 123.6, 55.1, 35.5, 31.7, 31.4, 31.1, 29.2, 29.0, 28.9, 23.0, 22.4, 13.1; HRMS (ESI+) Calcd for $C_{21}H_{32}N_5O$ $[M + H]^+$: 370.2601, Found: 370.2607. $[\alpha]_D$ = −74.6° (c = 0.005, methanol).

(*R*)-2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboximidamide Hydrochloride (7h). Synthesized by general procedure B. 99%, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.94 (d, J = 7.8 Hz, 2H), 7.32 (d, J = 7.9 Hz, 2H), 5.46−5.42 (m, 1H), 3.79−3.75 (m, 1H), 3.65−3.61 (m, 1H), 2.66 (t, J = 7.5 Hz, 2H), 2.53−2.49 (m, 2H), 2.23−2.19 (m, 1H), 2.10−2.06 (m, 1H), 1.65−1.61 (m, 2H), 1.38−1.26 (m, 10H), 0.88 (t, J = 6.7 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 178.9, 169.7, 157.1, 148.3, 130.2, 128.5, 125.0, 56.6, 36.9, 33.0, 32.8, 32.4, 30.6, 30.4, 30.3, 24.4, 23.7, 14.5; HRMS (ESI+): Calcd for $C_{21}H_{32}N_5O$ $[M^+]$: 370.2601, Found: 370.2596. $[\alpha]_D$ = +78.8° (c = 0.00085, methanol).

(*S*)-Amino(2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)-2,5-dihydro-1*H*-pyrrol-1-yl)methaniminium 2,2,2-Trifluoroacetate (7i). Synthesized general procedure D. 98% yield, white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 7.95 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 6.31−6.28 (m, 1H), 6.19−6.13 (m, 2H), 4.57−4.43 (m, 2H), 2.68 (t, J = 5.9 Hz, 2H), 1.69−1.60 (m, 2H), 1.38−1.24 (m, 10H), 0.89 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 169.9, 157.0, 148.5, 130.2, 129.8, 129.7, 128.4, 125.6, 125.5, 124.8, 56.1, 36.9, 33.0, 32.4, 30.5, 30.4, 30.3, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{21}H_{30}N_5O$+ $[M^+]$: 368.2450, Found: 368.2454. HPLC analysis shows that **7i** is 70% pure.

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(*S*)-2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)azetidine-1-carboximidamide Hydrochloride (7j). Synthesized by general procedure B. 95% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 5.83 (dd, J = 9.3, 5.2 Hz, 1H), 4.37 (q, J = 8.6 Hz, 1H), 4.27 (q, J = 8.7 Hz, 1H), 3.18−2.93 (m, 1H), 2.76−2.55 (m, 3H), 1.64 (q, J = 7.3 Hz, 2H), 1.40−1.19 (m, 12H), 0.88 (t, J = 7.2, 6.5 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 178.2, 169.8, 158.6, 148.4, 130.2, 128.4, 124.9, 58.4, 50.9, 36.9, 33.0, 32.4, 30.4, 23.7, 23.0, 14.4; HRMS (ESI+): Calcd for $C_{20}H_{31}N_5O$ [M^+]: 356.2450, Found: 356.2478. $[\alpha]_D$ = −27.3° (c = 0.00055, methanol).

(*S*)-Amino(2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)-piperidin-1-yl)methaniminium Chloride (7k). Synthesized bu general procedure B. 99% yield, white solid. ^{1}H NMR (500 MHz, MeOD) δ 7.87 (d, J = 8.1 Hz, 2H), 7.25 (d, J = 8.1 Hz, 2H), 5.44 (d, J = 4.0 Hz, 1H), 3.90−4.01 (m, 1H), 3.41−3.50 (m, 1H), 3.13 (d, J = 7.4 Hz, 1H), 2.59 (t, J = 7.6 Hz, 2H), 2.46 (d, J = 14.0 Hz, 1H), 2.10−1.98 (m, 1H), 1.75 (m, 2H), 1.63−1.51 (m, 4H), 1.37 (m, 1H), 1.23 (m, 11H), 0.79 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, MeOD) δ 176.5, 168.5, 158.5, 147.0, 128.8, 127.0, 127.0, 123.6, 54.5, 51.7, 43.4, 42.5, 37.3, 35.4, 31.6, 31.0, 29.1, 29.0, 28.9, 27.3, 23.9, 22.3, 19.1, 17.9, 17.4, 15.9, 13.0, 11.8. HRMS (ESI+): Calcd for $C_{22}H_{34}N_5O^+$ [M^+]: 384.2758, Found: 384.2759. HPLC analysis indicates that compound **7k** is 85% pure.

(2*S*,3*S*)-*tert*-Butyl 3-Hydroxy-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (8). Synthesized by general procedure A. 46% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, J = 8.0 Hz, 2H), 7.28 (d, J = 8.0 Hz, 2H), 4.96 (s, 1H), 4.56 (s, 1H), 3.82−3.68 (m, 2H), 2.69−2.59 (m, 2H), 2.35−2.27 (m, 1H), 2.07−2.00 (m, 1H), 1.66−1.58 (m, 2H), 1.46 (s, 3H), 1.36−1.21 (m, 16H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.3, 168.6, 154.0, 146.9, 129.1, 129.0, 127.5, 123.9, 81.0, 76.1, 62.4, 44.5, 36.1, 32.2, 32.0, 31.3, 29.5, 29.4, 29.3, 28.5, 28.3, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{25}H_{37}N_3O_4$ [M + H]$^+$: 444.2862, Found: 444.2883.

(2*S*,3*S*)-2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-3-ol (9). Synthesized by general procedure D. 59% yield, clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 7.4 Hz, 2H), 4.70 (s, 1H), 3.35 (s, 1H), 2.74−2.52 (m, 2H), 2.43−2.17 (m, 3H), 1.97 (s, 1H), 1.72−1.49 (m, 2H), 1.43−1.13 (m, 10H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.4, 168.4, 146.8, 129.0, 127.6, 124.0, 76.5, 63.3, 45.1, 36.1, 34.4, 32.0, 31.3, 29.6, 29.4, 29.3, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{20}H_{30}N_3O_2$ [M + H]$^+$: 344.2338, Found: 344.2338.

(*E*)-*tert*-Butyl (((*tert*-Butoxycarbonyl)amino)((2*S*,3*S*)-3-hydroxy-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methylene)carbamate (10). Synthesized by general procedure C. 89% yield, colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 7.9 Hz, 2H), 5.52 (s, 1H), 4.64 (s, 1H), 4.06−3.86 (m, 2H), 3.18 (bs, 1H), 2.63 (t, J = 7.1 Hz, 2H), 2.38−2.30 (m, 1H), 2.17−2.05 (m, 1H), 1.68−1.57 (m, 2H), 1.81 (bs, 1H), 1.49−1.40 (m, 18H), 1.33−1.23 (m, 10H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.1, 168.8, 147.2, 129.3, 127.9, 124.2, 75.1, 63.8, 47.4, 36.4, 32.3, 31.7, 30.2, 29.9, 29.7, 29.7, 28.5, 23.1, 14.6; HRMS (ESI+): Calcd for $C_{31}H_{48}N_5O_6$ [M + H]$^+$: 586.3604, Found: 586.3569.

Amino((1*S*,3*S*)-3-hydroxy-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium Chloride (11). Synthesized by general procedure B. 52% yield, white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 7.97 (d, J = 8.2 Hz, 2H), 7.36 (d, J = 8.2 Hz, 2H), 5.24 (s, 1H), 4.80 (d, J = 3.2 Hz, 1H), 3.85−3.82 (m, 2H), 2.70 (t, J = 7.2 Hz, 2H), 2.30−2.14 (m, 2H), 1.72−1.60 (m, 2H), 1.44−1.22 (m, 10H), 0.91 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.65, 169.7, 157.5, 148.4, 130.1, 128.4, 124.7, 76.0, 64.1, 47.3, 36.8, 33.0, 32.5, 32.4, 30.5, 30.4, 30.3, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{21}H_{32}N_5O_2$ [M + H]$^+$: 386.2556, Found: 386.2576.

(2*S*,4*R*)-*tert*-Butyl 4-Hydroxy-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (12). Synthesized by general procedure A. 63% yield, yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, J = 7.9 Hz, 2H), 7.31 (d, J = 7.9 Hz, 2H), 5.42−5.30 (m, 1H, minor rotamer), 5.20 (m, 1H, major rotamer), 4.74−4.56 (m, 1H), 3.89−3.79 (m, 1H), 3.77−3.69 (m, 1H, major rotamer), 3.64−3.53 (m, 1H, minor rotamer), 2.74−2.61 (m, 2H), 2.59−2.40 (m, 1H), 2.38−2.28 (m, 1H), 1.74−1.58 (m, 2H), 1.56−1.16 (m, 19H), 0.90 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$, rotamers) δ 180.3 (major), 168.5 (major), 153.8 (major), 146.7 (major), 146.5 (minor), 129.0 (major), 128.8 (minor), 127.4 (major), 125.8 (minor), 123.9 (major), 81.0 (major), 80.8 (minor), 69.9 (minor), 69.3 (major), 54.8 (major), 52.6 (major), 40.9 (major), 40.2 (minor), 36.0 (major), 31.9 (major), 31.2 (major), 29.4 (major), 29.3 (major), 29.2 (major), 28.3 (minor), 28.1 (major), 22.7 (major), 14.1 (major). HRMS (ESI+) Calcd for $C_{25}H_{37}N_3NaO_4$ [M + H]$^+$: 466.2682, Found: 466.2720.

(3*R*,5*S*)-5-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-3-ol (13). Synthesized by general procedure D. 95% yield, yellow solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (d, J = 8.2 Hz, 2H), 7.19 (d, J = 8.2 Hz, 2H), 4.79−4.63 (m, 1H), 4.57−4.43 (m, 1H), 3.22−3.10 (m, 1H), 3.08−2.95 (m, 1H), 2.77 (br s, 2H), 2.56 (t, J = 7.7 Hz, 2H), 2.35−2.18 (m, 2H), 1.63−1.48 (m, 2H), 1.33−1.10 (m, 10H), 0.80 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 181.3, 168.3, 146.6, 128.9, 127.4, 124.0, 72.2, 55.4, 53.0, 40.7, 36.0, 31.9, 31.2, 29.4, 29.3, 29.2, 22.7, 14.1. HRMS (ESI+) m/z calcd for $C_{20}H_{30}N_3O_2^+$ [M^+]: 344.2333, Found: 344.2336.

***tert*-Butyl (((*tert*-Butoxycarbonyl)imino)((2*S*,4*R*)-4-hydroxy-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)-methyl)carbamate (14).** Synthesized by general procedure C. 53% yield, yellow solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, J = 8.3 Hz, 2H), 7.29 (d, J = 8.3 Hz, 2H), 5.83 (t, J = 8.2 Hz, 1H), 4.72−4.59 (m, 1H), 4.05 (dd, J = 12.5, 3.5 Hz, 1H), 3.85−3.69 (m, 1H), 2.67 (t, J = 7.6 Hz, 2H), 2.63−2.53 (m, 1H), 2.45−2.32 (m, 1H), 1.74−1.60 (m, 2H), 1.47 (s, 18H), 1.39−1.24 (m, 10H), 0.90 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz,) δ 178.5, 168.5, 154.1, 146.6, 128.9, 127.5, 124.0, 69.3, 58.0, 53.7, 40.0, 36.0, 31.9, 31.2, 29.7, 29.4, 29.2, 28.1, 22.7, 14.1. HRMS (ESI +) Calcd for $C_{31}H_{48}N_5O_6$ [M + H]$^+$: 586.3605, Found: 586.3557.

Amino((2*S*,4*R*)-4-hydroxy-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium Chloride (15). Synthesized by general procedure B. 79% yield, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.01−7.93 (m, 2H), 7.41−7.31 (m, 2H), 5.66−5.54 (m, 1H), 4.67−4.58 (m, 1H), 3.96−3.85 (m, 1H), 3.65−3.58 (m, 1H), 2.74−2.61 (m, 3H), 2.58−2.49 (m, 1H), 1.73−1.61 (m, 2H), 1.42−1.24 (m, 10H), 0.91 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 178.9, 169.8, 157.7, 148.4, 130.2, 128.4, 124.9, 69.8, 57.1, 54.9, 41.1, 36.9, 33.0, 32.4, 30.5, 30.4, 30.3, 23.7, 14.5; HRMS (ESI+) m/z calcd for $C_{21}H_{32}N_5O_2$ [M + H]$^+$: 386.2556, Found: 386.2596. $[\alpha]_D$ = −45.5° (c = 0.00055, methanol).

(2*S*,4*S*)-*tert*-Butyl 4-(Benzoyloxy)-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (16). PPh_3 (112 mg, 0.43 mmol), benzoic acid (52 mg, 0.43 mmol), and (2*S*,4*R*)-*tert*-butyl 4-hydroxy-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate **15** were added to THF (1.0 mL) at room temperature. The solution was cooled to 0 °C, and diisopropyl azodicarboxylate (0.08 mL, 0.43 mmol) was added. The solution was warmed to room temperature and stirred overnight. At this time, TLC showed complete conversion of the starting material. The organic solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (15%, ethyl acetate/hexanes) to yield product **16** as a colorless oil. 75% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (d, J = 7.8 Hz, 2H), 7.77 (d, J = 7.6 Hz, 1H), 7.71 (d, J = 7.5 Hz, 1H), 7.36 (t, J = 7.9 Hz, 1H), 7.24 (t, J = 7.8 Hz, 2H), 7.13 (t, J = 7.0 Hz, 2H), 5.65 (s, 1H), 5.28 (d, J = 8.3 Hz, 1H), 4.00−3.80 (m, 2H), 2.80−2.63 (m, 4H), 1.67−1.60 (m, 2H), 1.51 (s, 3H), 1.39 (s, 6H), 1.37−1.23 (m, 10H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.7, 168.5, 165.8, 153.6, 146.6, 133.2, 129.7, 129.4, 129.0, 128.3, 127.5, 124.1, 81.1, 72.4, 53.5, 52.6, 38.0, 36.0, 32.0, 31.3, 29.5, 29.3, 28.5, 28.3, 22.7, 14.2; HRMS (mixed+): Calcd for $C_{32}H_{41}N_3O_5Na$ [M + Na]$^+$: 570.2944, Found: 570.2903.

(2*S*,4*S*)-*tert*-Butyl 4-Hydroxy-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate. Hydrolysis of 4-benzoyl ester protecting group in **16**. (2*S*,4*S*)-*tert*-Butyl 4-(benzoyloxy)-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate **16** (85 mg, 0.16 mmol) was dissolved in equal parts of methanol (0.38 mL) and THF (0.38 mL) and 2 M NaOH (0.15 mL, 2 M) and stirred for 30 min at room temperature under nitrogen. The solution was partitioned between ethyl acetate and water. The aqueous solution was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated via vacuum to yield the

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product as colorless oil. 95% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 8.3 Hz, 2H), 5.11 (d, J = 9.1 Hz, 1H), 4.51 (s, 1H), 3.87−3.63 (m, 2H), 2.64 (t, J = 7.5 Hz, 2H), 2.59−2.50 (m, 1H), 2.35−2.25 (m, 1H), 1.66−1.57 (m, 2H), 1.43 (s, 3H), 1.37−1.19 (m, 16H), 0.86 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 181.5, 168.1, 153.5, 147.1, 129.1, 129.0, 127.5, 123.4, 81.2, 70.6, 56.0, 52.4, 39.6, 36.1, 31.9, 31.3, 29.5, 29.3, 29.3, 28.2, 22.7, 14.2; HRMS (ESI+): Calcd for $C_{25}H_{37}N_3O_4Na$ $[M + Na]^+$: 466.2681, Found: 466.2637.

(2*S*,4*S*)-2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-4-ol. Deprotection of Boc group in (2*S*,4*S*)-*tert*-butyl 4-hydroxy-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate. Synthesized by general procedure D. 48% yield, colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, J = 8.1 Hz, 2H), 7.25 (d, J = 7.6 Hz, 2H), 4.66 (d, J = 6.6 Hz, 1H), 4.53 (s, 1H), 3.91 (s, 2H), 3.27 (s, 2H), 2.67−2.60 (m, 2H), 2.56−2.50 (m, 1H), 2.29 (d, J = 13.8 Hz, 1H), 1.62 (quin, J = 7.5 Hz, 2H), 1.38−1.19 (m, 10H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 181.1, 168.2, 147.0, 129.0, 127.6, 123.7, 72.0, 56.0, 53.1, 40.2, 36.1, 32.0, 31.3, 29.6, 29.4, 29.4, 22.8, 14.2; HRMS (mixed+): Calcd for $C_{20}H_{30}N_3O_2$ $[M + H]^+$: 344.2338, Found: 344.2315.

***tert*-Butyl (((*tert*-Butoxycarbonyl)amino)((2*S*,4*S*)-4-hydroxy-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)-methylene)carbamate.** Guanylation of (2*S*,4*S*)-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-4-ol. Synthesized by general procedure C. 79% yield, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.2 Hz, 2H),7.26 (d, J = 8.2 Hz, 2H), 5.81 (t, J = 8.2 Hz, 1H), 4.63 (s, 1H), 4.02 (dd, J = 12.5 Hz, 1H), 3.79−3.70 (m, 1H), 2.65 (t, J = 7.3 Hz, 2H), 2.57 (dd, J = 13.3, 7.7 Hz, 1H), 2.41−2.29 (m, 1H), 1.62 (quin, J = 7.5 Hz, 2H), 1.45 (s, 18H), 1.35−1.20 (m, 10H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.6, 168.6, 154.2, 146.7, 129.0, 127.6, 124.1, 69.6, 58.1, 53.8, 40.2, 36.1, 32.0, 31.4, 29.6, 29.4, 28.2, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{31}H_{48}N_5O_6$ $[M + H]^+$: 586.3604, Found: 586.3603.

Amino((1*S*,4*S*)-4-hydroxy-2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium Chloride (17). Synthesized by general procedure B. 22% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.97 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 5.59 (dd, J = 7.8, 6.7 Hz, 1H), 4.64 (quin, J = 4.4 Hz, 1H), 3.91 (dd, J = 10.7, 4.7 Hz, 1H), 3.62 (ddd, J = 10.7, 2.9, 1.3 Hz, 1H), 2.70 (t, J = 7.4 Hz, 2H), 2.65 (dtd, J = 8.0, 4.8, 4.1, 1.3 Hz, 1H), 2.54 (ddd, J = 13.3, 6.4, 4.9 Hz, 1H), 1.68 (quin, J = 7.3 Hz, 2H), 1.42−1.21 (m, 10H), 0.91 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 178.9, 169.8, 157.6, 148.4, 130.2, 128.4, 125.0, 69.7, 57.1, 54.9, 41.0, 36.8, 33.0, 32.4, 30.5, 30.4, 30.3, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{21}H_{32}N_5O_2$ $[M^+]$: 386.2556, Found: 386.2548. $[\alpha]_D$ = −40.6° (c = 0.0015, methanol).

***tert*-Butyl 3-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)-azetidine-1-carboxylate (18a).** Synthesized by general procedure A. 77% yield, yellow oil. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.96 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H), 4.48−4.21 (m, 4H), 4.15−3.92 (m, 1H), 2.64 (t, J = 7.5 Hz, 2H), 1.63 (td, J = 15.9, 15.1, 8.5 Hz, 2H), 1.45 (s, 9H), 1.36−1.19 (m, 10H), 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 178.7, 168.6, 155.9, 146.7, 128.9, 127.4, 123.8, 80.1, 35.9, 31.8, 31.2, 29.4, 29.2, 29.2, 28.3, 25.7, 22.6, 14.0; HRMS (ESI+): Calcd for $C_{24}H_{35}N_3NaO_3$ $[M + Na]^+$: 436.2576, Found: 436.2558.

(*R*)-*tert*-Butyl 3-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)-pyrrolidine-1-carboxylate (18b). Synthesized by general procedure A. 77% yield, colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 7.7 Hz, 2H), 3.93−3.37 (m, 5H), 2.68−2.57 (m, 2H), 2.36 (dq, J = 12.0. 6.9 Hz, 2H), 1.61 (quin, J = 7.4 Hz, 2H), 1.46 (s, 9H), 1.34−1.18 (m, 10H), 0.86 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ179.3, 168.5, 154.3, 146.7, 129.0, 127.4, 124.0, 79.8, 49.4, 45.1, 36.7, 36.0, 31.9, 31.3, 29.7, 29.5, 29.3, 29.3, 28.6, 22.7, 14.2; HRMS (ESI+): Calcd for $C_{25}H_{37}N_3O_3Na$ $[M + Na]^+$: 450.2732, Found: 450.2695.

3-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-ium Chloride (19a). Synthesized by general procedure B. 94% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.97 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.4 Hz, 2H), 4.64−4.31 (m, 5H), 2.67 (t, J = 7.5 Hz, 2H), 1.64 (quin, J = 7.4 Hz, 2H), 1.42−1.17 (m, 10H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.9, 169.9, 148.3, 130.2, 128.4, 125.1, 51.0, 36.8, 33.0, 32.4, 30.5, 30.4, 30.3, 30.0, 23.7, 14.4. HRMS (ESI+): Calcd for $C_{19}H_{28}N_3O^+$ $[M^+]$: 314.2227, Found: 314.2221.

(*R*)-3-(4-Octylphenyl)-5-(pyrrolidin-3-yl)-1,2,4-oxadiazole (19b). Synthesized by general procedure B. 79%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 3.57 (ddd, J = 12.7, 9.1, 6.3 Hz, 1H), 3.29 (d, J = 5.9 Hz, 2H), 3.25−3.16 (m, 1H), 3.06−2.96 (m, 1H), 2.67−2.57 (m, 2H), 2.34−2.08 (m, 3H), 1.68−1.55 (m, 2H), 1.27 (d, J = 19.6 Hz, 10H), 0.86 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.1, 168.4, 146.5, 129.0, 127.4, 124.3, 52.6, 47.5, 37.3, 36.0, 32.0, 32.0, 31.3, 29.8, 29.5, 29.3, 29.3, 22.7, 14.2; HRMS (ESI+): Calcd for $C_{23}H_{34}N_2O_2$ $[M + H]^+$: 328.2383, Found: 328.2386.

***tert*-Butyl (((*tert*-Butoxycarbonyl)amino)(3-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-yl)methylene)carbamate.** Guanylation of 3-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-ium chloride **19a**. Synthesized by general procedure C. 57%, yellow oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.01 (s, 1H), 7.95 (d, J = 8.2 Hz, 2H), 7.27 (d, J = 8.1 Hz, 2H), 4.82−4.51 (m, 3H), 4.10 (ddd, J = 15.5, 8.9, 6.6 Hz, 1H), 2.64 (t, J = 8.0, 7.4 Hz, 2H), 1.61 (dt, J = 11.9, 7.1 Hz, 2H), 1.48 (s, 18H), 1.36−1.20 (m, 10H), 0.85 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.2, 168.6, 156.2, 146.7, 128.9, 127.4, 123.7, 35.9, 31.8, 31.1, 29.4, 29.2, 29.2, 28.1, 26.5, 22.6, 14.0; HRMS (ESI+): Calcd for $C_{30}H_{46}N_5O_5$ $[M + H]^+$: 556.3499, Found: 556.3455.

(*R*,*Z*)-*tert*-Butyl (((*tert*-Butoxycarbonyl)imino)(3-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)-carbamate. Guanylation of (*R*)-3-(4-octylphenyl)-5-(pyrrolidin-3-yl)-1,2,4-oxadiazole **19b**. Synthesized by general procedure C. 8% yield, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.3 Hz, 2H), 7.27 (d, J = 8.2 Hz, 2H), 4.05 (s, 2H), 3.87−3.68 (m, 3H), 2.69−2.62 (m, 2H), 2.45 (dt, J = 13.4, 6.5 Hz, 2H), 1.68−1.61 (m, 2H), 1.50 (s, 18H), 1.28 (d, J = 22.8 Hz, 10H), 0.92−0.83 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.6, 171.3, 168.6, 154.4, 146.8, 129.1, 127.6, 124.0, 77.4, 66.0, 60.5, 36.1, 32.0, 31.7, 31.3, 29.8, 29.6, 29.4, 29.4, 28.3, 25.4, 22.8, 21.2, 15.4, 14.3, 14.3, 14.2, 11.6, 1.2; HRMS (ESI+): Calcd for $C_{31}H_{48}N_5O_5$ $[M + H]^+$: 570.3655, Found: 570.3690.

Amino(3-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-yl)methaniminium Chloride (20a). Synthesized by general procedure B. 82% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.96 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 8.3 Hz, 2H), 4.63 (t, J = 8.9 Hz, 2H), 4.50−4.33 (m, 3H), 2.66 (t, J = 7.5 Hz, 2H), 1.62 (quin, J = 7.3 Hz, 2H), 1.42−1.18 (m, 10H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 180.0, 169.8, 158.3, 148.2, 130.1, 128.4, 125.2, 55.8, 36.8, 33.0, 32.4, 30.5, 30.4, 30.3, 27.2, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{20}H_{30}N_5O^+$ $[M^+]$: 356.2445, Found: 356.2418.

(*R*)-Amino(3-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)-pyrrolidin-1-yl)methaniminium Chloride (20b). Synthesized by general procedure B. 72% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 4.13−3.87 (m, 3H), 3.74−3.65 (m, 2H), 2.75−2.62 (m, 3H), 2.53 (s, 1H), 1.72−1.64 (m, 2H), 1.45−1.24 (m, 10H), 0.92 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 180.3, 169.6, 156.4, 148.2, 130.1, 128.3, 125.2, 51.4, 47.7, 37.3, 36.8, 33.0, 32.4, 30.6, 30.5, 30.3, 30.3, 23.7, 14.4; MS: Calcd for $C_{21}H_{32}N_5O$ $[M^+]$: 370.2606, Found: 370.2586.

(*S*)-*tert*-Butyl 2-(5-(4-Octylphenyl)-1,2,4-oxadiazol-3-yl)-pyrrolidine-1-carboxylate (22). Synthesized by general procedure A. 19% yield, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 7.7 Hz, 2H), 7.32 (d, J = 7.8 Hz, 2H), 4.99 (d, J = 4.3 Hz, 1H), 3.73−3.52 (m, 2H), 2.68 (t, J = 7.6 Hz, 2H), 2.32 (s, 1H), 2.19−2.04 (m, 2H), 1.96 (d, J = 5.8 Hz, 1H), 1.69−1.59 (m, 2H), 1.46 (s, 3H), 1.37−1.22 (m, 16H), 0.91−0.85 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ172.9, 159.8, 156.0, 148.6, 129.3, 128.2, 107.7, 80.0, 77.4, 53.5, 46.6, 36.2, 32.0, 31.3, 29.6, 29.4, 29.4, 28.4, 23.5, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{25}H_{38}N_3O_3$ $[M + H]^+$: 428.2913, Found: 428.2918.

(*S*)-2-(5-(4-Octylphenyl)-1,2,4-oxadiazol-3-yl)pyrrolidine-1-carboximidamide Hydrochloride (23). Synthesized by general procedure B. 89% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (d, J = 8.4 Hz, 2H), 7.47 (d, J = 8.5 Hz, 2H), 5.02 (t, J = 7.7 Hz, 1H), 3.63−3.46 (m, 2H), 2.78−2.72 (m, 2H), 2.62 (dtd, J = 12.8, 7.8, 5.2 Hz, 2H), 2.47−2.18 (m, 3H), 1.75−1.63 (m, 2H), 1.41−1.26 (m, 10H), 0.94−0.87 (m, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 178.7, 168.6,

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151.0, 130.6, 129.3, 122.1, 55.8, 47.2, 37.0, 33.0, 32.3, 30.5, 30.3, 30.3, 30.1, 24.5, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{20}H_{30}N_3O$ $[M + H]^+$: 328.2388, Found: 328.2386.

(*S*)-Amino(2-(5-(4-octylphenyl)-1,2,4-oxadiazol-3-yl)-pyrrolidin-1-yl)methaniminium Chloride (23). Synthesized by general procedure B. 28% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.05 (d, J = 6.9 Hz, 2H), 7.43 (d, J = 7.2 Hz, 2H), 5.29 (d, J = 7.3 Hz, 1H), 3.74−3.69 (m, 1H), 3.65−3.53 (m, 1H), 2.73 (t, J = 7.8 Hz, 2H), 2.52−2.39 (m, 2H), 2.19 (s, 2H), 1.70−1.63 (m, 2H), 1.32 (d, J = 20 Hz, 10H), 0.93−0.86 (m, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 178.2, 171.1, 156.9, 150.6, 130.5, 129.2, 122.4, 56.3, 37.0, 33.0, 32.4, 32.3, 30.5, 30.4, 30.3, 24.1, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{21}H_{32}N_5O$ $[M + H]^+$: 370.2606, Found: 370.2617.

5-(4-Octylphenyl)-2*H*-tetrazole (24). To a solution of 4-octylbenzonitrile **3** (120 mg, 0.557 mmol) in DMF (3 mL) were added ammonium chloride (54 mg, 1.003 mmol) and sodium azide (65 mg, 1.003 mmol). The reaction was refluxed for ∼4 h. After the reaction was completed, the solution was cooled to room temperature and filtered, and the residue was washed with acetone. The solvent was then evaporated in vacuo, and the residue was partitioned between equal volumes of hexanes and water. The organic layer was then separated, washed with satd LiBr and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vaccuo to afford **34** in a quantitative yield as colorless oil. The isolated product was sufficiently pure to use in the next reaction. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, J = 8.3 Hz, 2H), 7.26 (d, J = 7.3 Hz, 2H), 2.65 (t, J = 8.1 Hz, 2H), 1.61 (quin, J = 7.4 Hz, 2H), 1.34−1.21 (m, 10H), 0.87 (t, J = 6.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.8, 132.2, 129.3, 119.3, 109.6, 36.3, 32.0, 31.1, 29.5, 29.3, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{15}H_{23}N_4$ $[M + H]^+$: 259.1923, Found: 259.1931.

(*S*)-*tert*-Butyl 2-(5-(4-Octylphenyl)-1,3,4-oxadiazol-2-yl)-pyrrolidine-1-carboxylate (25). Synthesized by general procedure A. 67% yield, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 8.3 Hz, 2H), 7.29 (d, J = 7.5 Hz, 2H), 5.21−5.05 (m, 1H), 3.71−3.34 (m, 2H), 2.65 (t, J = 7.7 Hz, 2H), 2.44−1.68 (m, 4H), 1.62 (q, J = 7.3 Hz, 2H), 1.44 (d, J = 8.6 Hz, 3H), 1.37−1.20 (m, 16H), 0.86 (t, J = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3, 164.8, 154.3, 147.4, 129.2, 126.9, 121.3, 80.3, 58.6, 53.0, 46.5, 36.1, 32.4, 32.0, 31.2, 29.5, 29.4, 29.3, 28.5, 28.4, 23.8, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{25}H_{38}N_3O_3$ $[M + H]^+$: 428.2913, Found: 428.2930.

(*S*)-2-(5-(4-Octylphenyl)-1,3,4-oxadiazol-2-yl)pyrrolidin-1-ium 2,2,2-Trifluoroacetate (26). Deprotection of (*S*)-*tert*-butyl 2-(5-(4-octylphenyl)-1,3,4-oxadiazol-2-yl)pyrrolidine-1-carboxylate **25**. Synthesized by general procedure D. 100% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.4 Hz, 2H), 5.17 (t, J = 7.7 Hz, 1H), 3.61−3.48 (m, 2H), 2.72 (t, J = 7.4 Hz, 2H), 2.68−2.59 (m, 1H), 2.58−2.46 (m, 2H), 2.36−2.22 (m, 2H), 1.68 (quin, J = 7.3 Hz, 2H), 1.40−1.26 (m, 10H), 0.90 (t, J = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 167.8, 163.3, 149.7, 130.5, 128.2, 121.7, 54.7, 47.3, 36.9, 33.0, 32.3, 30.5, 30.4, 30.3, 29.8, 24.6, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{20}H_{30}N_3O$+ $[M + H]^+$: 328.2388, Found: 328.2389.

Guanylation of (*S*)-2-(5-(4-octylphenyl)-1,3,4-oxadiazol-2-yl)-pyrrolidin-1-ium 2,2,2-trifluoroacetate. Synthesized by general procedure C. 30% yield, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.2 Hz, 2H), 5.64 (dd, J = 7.6, 5.3 Hz, 1H), 3.95−3.70 (m, 2H), 2.65 (t, J = 7.4 Hz, 2H), 2.48−2.39 (m, 1H), 2.34−2.16 (m, 2H), 2.07−1.98 (m, 1H), 1.68−1.57 (m, 2H), 1.48 (d, J = 19.1 Hz, 18H), 1.31−1.24 (m, 10H), 0.87 (t, J = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 170.4, 165.9, 165.1, 147.3, 129.2, 127.1, 121.4, 54.3, 49.6, 36.1, 32.0, 31.3, 29.6, 29.4, 29.4, 28.3, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{31}H_{48}N_5O_5$ $[M + H]^+$: 570.3655, Found: 570.3656.

Deprotection by general procedure D. 90% yield, white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 5.43 (dd, J = 7.3, 2.1 Hz, 1H), 3.80 (td, J = 9.2, 2.3 Hz, 1H), 3.63 (td, J = 9.8, 7.2 Hz, 1H), 2.75 (t, J = 7.2 Hz, 2H), 2.63−2.46 (m, 2H), 2.33−2.08 (m, 2H), 1.70 (quin, J = 7.3 Hz, 2H), 1.43−1.27 (m, 10H), 0.92 (t, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 167.5, 165.8, 157.0, 149.4, 130.5, 128.0, 121.9, 55.7, 36.9, 33.0, 32.3, 32.1, 30.5, 30.4, 30.3, 24.1, 23.7, 14.4; HRMS (ESI+): Calcd for $C_{21}H_{32}N_5O$+ $[M + H]^+$: 370.2606, Found: 370.2618.

(*S*)-*tert*-Butyl 2-((4-Decylphenyl)carbamoyl)pyrrolidine-1-carboxylate (27a). Synthesized using general procedure E. 61% yield, colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.37 (s, 1H), 7.41 (d, J = 8.4 Hz, 2H), 7.10 (s, 2H), 4.39 (d, J = 75.3 Hz, 1H), 3.68−3.21 (m, 2H), 2.54 (t, J = 7.5 Hz, 2H), 2.03−1.81 (m, 2H), 1.60−1.53 (m, 2H), 1.49 (s, 9H), 1.34−1.16 (m, 14H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.8, 156.5, 138.8, 136.1, 128.9, 119.8, 81.0, 60.6, 47.3, 35.5, 32.0, 31.7, 29.7, 29.6, 29.4, 29.4, 28.5, 27.2, 24.7, 22.8, 14.2. HRMS (ESI+): Calcd for $C_{26}H_{43}N_2O_3$ $[M + H]^+$: 431.3273, Found: 431.3240.

***(R)*-*tert*-Butyl 2-((4-Decylphenyl)carbamoyl)pyrrolidine-1-carboxylate (27b).** Synthesized using general procedure E. 95% yield, colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.39 (s, 1H), 7.40 (d, J = 8.5 Hz, 2H), 7.07 (s, 2H), 4.38 (d, J = 85.0 Hz, 1H), 3.43 (t, J = 45.4 Hz, 2H), 2.59−2.48 (m, 2H), 2.21 (s, 1H), 2.05−1.84 (m, 3H), 1.52 (d, J = 35.8 Hz, 11H), 1.27 (d, J = 19.0 Hz, 14H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.8, 156.5, 138.5, 136.1, 128.8, 119.7, 80.8, 60.5, 47.3, 35.4, 32.0, 31.6, 29.7, 29.7, 29.6, 29.4, 29.3, 27.4, 24.7, 28.5, 22.8, 14.2. HRMS (ESI+): Calcd for $C_{26}H_{42}N_2O_3Na$ $[M + Na]^+$: 453.3093, Found: 453.3126.

(*S*)-2-((4-Decylphenyl)carbamoyl)pyrrolidin-1-ium 2,2,2-Trifluoroacetate (28a). Synthesized by general procedure D. 73% yield, yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.22 (s, 1H), 7.38 (s, 2H), 7.06 (d, J = 6.3 Hz, 2H), 4.6 (s, 1H), 3.39 (s, 2H), 2.53 (t, J = 8.0 Hz, 2H), 2.20−1.91 (m, 4H), 1.55 (s, 2H), 1.37−1.18 (m, 14H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6, 139.9, 135.0, 129.0, 120.3, 60.4, 46.7, 35.5, 32.1, 31.6, 30.3, 29.8, 29.8, 29.7, 29.5, 29.4, 24.9, 22.8, 14.3; HRMS (ESI+): Calcd for $C_{21}H_{35}N_2O^+$ $[M + H]^+$: 331.2749, Found: 331.2716.

***(R)*-2-((4-Decylphenyl)carbamoyl)pyrrolidin-1-ium 2,2,2-Trifluoroacetate 28b.** Synthesized by general procedure D. Quantitative yield, yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.26 (s, 1H), 7.37 (d, J = 7.9 Hz, 2H), 7.04 (d, J = 7.9 Hz, 2H), 4.80 (s, 1H), 3.34 (d, J = 26.2 Hz, 2H), 2.51 (t, J = 7.9 Hz, 2H), 2.44 (s, 1H), 2.08 (s, 1H), 1.95 (s, 2H), 1.54 (s, 2H), 1.32−1.22 (m, 14H), 0.87 (t, J = 6.6 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.7, 140.0, 135.1, 128.9, 120.5, 60.5, 46.6, 35.6, 32.1, 31.6, 30.2, 29.8, 29.8, 29.7, 29.5, 24.6, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{21}H_{35}N_2O^+$ $[M + H]^+$: 331.2749, Found: 331.2752.

(*S,E*)-*tert*-Butyl (((*tert*-Butoxycarbonyl)imino)(2-((4-decylphenyl)carbamoyl)pyrrolidin-1-yl)methyl)carbamate. Guanylation of (*S*)-2-((4-decylphenyl)carbamoyl)pyrrolidin-1-ium 2,2,2-trifluoroacetate. Synthesized by general procedure C. 55% yield, colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.75 (s, 1H), 7.57 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 5.10 (s, 1H), 3.71 (dt, J = 11.0, 7.3 Hz, 1H), 3.55−3.50 (m, 1H), 2.54 (t, J = 7.9 Hz, 2H), 2.41−2.34 (m, 1H), 2.22−2.15 (m, 1H), 2.06−1.99 (m, 1H), 1.91−1.83 (m, 1H), 1.65−1.53 (m, 2H), 1.51 (s, 18H), 1.31−1.21 (m, 14H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 168.7, 161.9, 155.5, 138.7, 136.3, 128.8, 119.6, 61.8, 50.2, 50.2, 35.5, 32.0, 31.8, 29.8, 29.7, 29.7, 29.5, 29.4, 28.4, 28.3, 28.1, 24.7, 22.8; HRMS (ESI+): Calcd for $C_{32}H_{52}N_4O_5$ $[M + H]^+$: 573.4016, Found: 573.4011.

(*R,E*)-*tert*-Butyl (((*tert*-Butoxycarbonyl)imino)(2-((4-decylphenyl)carbamoyl)pyrrolidin-1-yl)methyl)carbamate. Guanylation of (*R*)-2-((4-decylphenyl)carbamoyl)pyrrolidin-1-ium 2,2,2-trifluoroacetate. Synthesized by general procedure C. 85% yield, colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.75 (s, 1H), 7.57 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 5.11 (s, 1H), 3.72 (dt, J = 10.9, 7.3 Hz, 1H), 3.60−3.49 (m, 1H), 2.54 (t, J = 7.1 Hz, 2H), 2.41−2.35 (m, 1H), 2.24−2.15 (m, 1H), 2.06−2.00 (m, 1H), 1.92−1.84 (m, 1H), 1.60−1.54 (m, 2H), 1.51 (s, 18H), 1.25 (s, 14H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.8, 155.4, 138.7, 136.2, 128.8, 119.5, 61.8, 50.3, 35.5, 32.0, 31.7, 29.8, 29.7, 29.7, 29.5, 29.4, 28.3, 28.1, 24.7, 22.8, 14.3; HRMS (ESI+): Calcd for $C_{32}H_{53}N_4O_5$ $[M + H]^+$: 573.4016, Found: 573.4011.

(*S*)-Amino(2-((4-decylphenyl)carbamoyl)pyrrolidin-1-yl)-methaniminium 2,2,2-Trifluoroacetate (29a). Synthesized by general procedure D. 29% yield, white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (s, 1H), 7.42 (d, J = 7.9 Hz, 2H), 7.09 (d, J = 8.0 Hz, 2H), 4.83 (s, 1H), 3.66 (s, 1H), 3.47 (s, 1H), 2.53 (t, J = 7.6 Hz, 2H), 2.31 (s, 2H), 2.19 (s, 1H), 2.00 (s, 1H), 1.55 (s, 2H), 1.25 (s, 14H), 0.88 (t, J =

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6.7 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.4, 155.4, 139.9, 135.2, 129.0, 120.3, 60.9, 48.4, 35.5, 32.0, 31.7, 31.4, 29.8, 29.8, 29.7, 29.5, 29.4, 24.1, 22.8, 14.3; HRMS (ESI+): Calcd for $C_{22}H_{37}N_4O+$ $[M + H]^+$: 373.2967, Found: 373.2963.

(*R*)-Amino(2-((4-decylphenyl)carbamoyl)pyrrolidin-1-yl)-methaniminium 2,2,2-Trifluoroacetate (29b). Synthesized by general procedure D. 31% yield, white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (s, 1H), 7.45 (d, J = 8.4 Hz, 2H), 7.10 (d, J = 8.4 Hz, 2H), 4.86 (s, 1H), 3.70−3.64 (m, 1H), 3.50−3.42 (m, 1H), 2.53 (t, J = 7.8 Hz, 2H), 2.40−2.16 (m, 3H), 2.06−1.98 (m, 1H), 1.61−1.50 (m, 2H), 1.32−1.19 (m, 14H), 0.87 (t, J = 6.2 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.2, 155.3, 139.8, 135.3, 129.0, 120.3, 60.9, 48.3, 35.5, 32.1, 31.7, 31.4, 29.8, 29.8, 29.7, 29.5, 29.4, 22.8, 14.3; HRMS (ESI+): Calcd for $C_{22}H_{37}N_4O+$ $[M + H]^+$: 373.2967, Found: 373.2949.

(*S*,*Z*)-*tert*-Butyl (((*tert*-Butoxycarbonyl)amino)(2-((4-octylbenzyl)carbamoyl)pyrrolidin-1-yl)methylene)carbamate (30). 4-Octylbenzonitrile **2** (200 mg, 0.93 mmol) was dissolved in tetrahydrofuran and added dropwise to a solution of lithium aluminum hydride (106 mg, 2.79 mmol) in tetrahydrofuran (9 mL) at 0 °C. After the addition, the mixture was warmed to room temperature and stirred for 0.5−1 h. After completion, the reaction was cooled to 0 °C and diluted with diethyl ether. Next, 60 μL of water, 60 μL of 15% NaOH solution, and 180 μL of water were added dropwise in that order. The reaction was heated back up to room temperature and stirred for 0.5 h. Next, the reaction was filtered through a Celite plug and the aqueous layer was washed with diethyl ether 3× and separated. The organic layer was then washed with brine, dried with sodium sulfate, and concentrated in vacuo to give (4-octylphenyl)methanamine without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 8.1 Hz, 2H), 3.83 (s, 1H), 2.59 (t, J = 7.6 Hz, 2H), 1.66−1.54 (m, 2H), 1.37−1.24 (m, 10H), 0.89 (t, J = 6.8 Hz, 3H).

(*S*)-*tert*-Butyl 2-((4-Octylbenzyl)carbamoyl)pyrrolidine-1-carboxylate. Synthesized using general procedure E. Taken to the next step without further purification, colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (d, J = 7.9 Hz, 2H), 7.11 (d, J = 7.8 Hz, 2H), 4.62−16 (m, 3H), 3.50−3.26 (m, 2H), 2.55 (t, J = 7.4 Hz, 2H), 2.40−2.10 (m, 1H), 1.96−1.80 (m, 3H), 1.56 (quin, J = 7.4 Hz, 2H), 1.49−1.19 (m, 19H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.4, 155.8, 142.5, 135.5, 128.8, 128.0, 80.7, 61.4, 60.2, 47.2, 43.3, 35.7, 32.0, 31.6, 29.6, 29.4, 29.3, 28.4, 22.8, 14.2. HRMS (ESI+): Calcd for $C_{25}H_{40}N_2O_3Na$ $[M + Na]^+$: 439.2936, Found: 439.2937.

(*S*)-2-((4-Octylbenzyl)carbamoyl)pyrrolidin-1-ium 2,2,2-Trifluoroacetate. Deprotection of the Boc group in (*S*)-*tert*-butyl 2-((4-octylbenzyl)carbamoyl)pyrrolidine-1-carboxylate. Synthesized using general procedure D. Taken to the next step without further purification, yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (s, 1H), 7.69 (s, 1H), 7.09 (dd, J = 8.5, 2.9 Hz, 4H), 4.58 (s, 1H), 4.33 (d, J = 5.2 Hz, 2H), 3.35−3.18 (m, 2H), 2.53 (t, J = 7.7 Hz, 2H), 2.37−2.25 (m, 1H), 2.00−1.84 (m, 3H), 1.55 (quin, J = 7.4 Hz, 2H), 1.33−1.20 (m, 10H), 0.86 (t, J = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.62, 142.38, 134.87, 128.76, 127.52, 59.67, 46.33, 43.69, 35.73, 32.01, 31.68, 30.20, 29.60, 29.50, 29.40, 24.52, 22.80, 14.24; HRMS (ESI+): Calcd for $C_{20}H_{33}N_2O^+$ $[M + H]^+$: 317.2592, Found: 317.2591.

Guanylation of (*S*)-2-((4-octylbenzyl)carbamoyl)pyrrolidin-1-ium 2,2,2-trifluoroacetate. Synthesized by general procedure C. 46% yield, colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.02 (s, 1H), 7.61 (s, 1H), 7.15 (d, J = 8.2 Hz, 2H), 7.08 (d, J = 8.2 Hz, 2H), 4.98 (t, J = 6.8 Hz, 1H), 4.45 (dd, J = 14.8, 6.0 Hz, 1H), 4.32 (dd, J = 14.8, 5.1 Hz, 1H), 3.63 (dt, J = 11.1, 7.3 Hz, 1H), 3.51−3.44 (m, 1H), 3.54 (t, J = 7.4 Hz, 2H), 2.21 (q, J = 7.0 Hz, 1H), 2.03−1.79 (m, 3H), 1.56 (quin, J = 6.9 Hz, 2H), 1.43 (s, 18H), 1.31−1.19 (m, 10H), 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 155.1, 141.9, 135.4, 128.6, 127.7, 61.5, 49.8, 43.4, 35.7, 32.0, 31.6, 29.6, 29.5, 29.4, 29.0, 28.2, 24.6, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{31}H_{51}N_4O_5$ $[M + H]^+$: 559.3859, Found: 559.3832.

(*S*)-Amino(2-((4-octylbenzyl)carbamoyl)pyrrolidin-1-yl)-methaniminium 2,2,2-Trifluoroacetate (31). Synthesized by general procedure D. 39% yield, yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.09 (s, 4H), 4.64 (s, 1H), 4.40 (dd, J = 14.5, 5.9 Hz, 1H), 4.20 (dd, J = 9.6 4.8 Hz, 1H), 3.64 (s, 1H), 3.44 (s, 1H), 2.54 (t, J = 7.8 Hz, 2H), 2.32−2.05 (m, 3H), 2.01−1.93 (m, 1H), 1.56 (quin, J = 7.8 Hz, 2H), 1.33−1.20 (m, 10H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.2, 155.6, 142.3, 135.0, 128.8, 127.5, 60.5, 48.3, 43.5, 35.7, 32.0, 31.7, 31.3, 29.6, 29.5, 29.4, 24.1, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{21}H_{35}N_4O+$ $[M + H]^+$: 359.2810, Found: 359.2794.

(*S*)-*tert*-Butyl 2-((4-Octylbenzamido)methyl)pyrrolidine-1-carboxylate (33). Dissolve 4-octylbenzoic acid **32** (50 mg, 0.21 mmol) in dichloromethane (1 mL). Add half the amount of TEA (0.04 mL, 0.32 mmol) and 1-hydroxypyrrolidine-2,5-dione (37 mg, 0.32 mmol) with stirring at room temperature. In a separate flask, dissolve EDC (82 mg, 0.43 mmol) in dichloromethane (1 mL) with the other half of TEA (0.04 mL, 0.32 mmol). After the EDC is dissolved, add that into the reaction mixture dropwise over 15−20 min. Stir the reaction for 6 h at rt. After 6 h, add (*S*)-*tert*-butyl 2-(aminomethyl) pyrrolidine-1-carboxylate (299 mg, 1.5 mmol) and stir 16−60 h. After completion, the solution was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to yield product **33** in an 85% yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.77 (d, J = 7.8 Hz, 2H), 7.18 (d, J = 8.2 Hz, 2H), 4.17 (s, 1H), 3.51 (d, J = 13.3 Hz, 1H), 3.41−3.31 (m, 3H), 2.60 (t, J = 7.5 Hz, 2H), 2.08−1.79 (m, 3H), 1.72 (s, 1H), 1.59 (quin, J = 7.2 Hz, 2H), 1.45 (s, 9H), 1.32−1.18 (m, 10H), 0.85 (t, J = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 157.1, 146.4, 131.7, 128.4, 127.2, 80.2, 56.2, 47.2, 47.1, 35.9, 31.9, 31.3, 29.6, 29.5, 29.3, 28.5, 24.0, 22.7, 14.2. HRMS (ESI+): Calcd for $C_{25}H_{41}N_2O_3$ $[M + H]^+$: 417.3117, Found: 417.3088.

(*S*)-Amino(2-((4-octylbenzamido)methyl)pyrrolidin-1-yl)-methaniminium 2,2,2-Trifluoroacetate (34). (*S*)-2-((4-Octylbenzamido)methyl)pyrrolidin-1-ium 2,2,2-Trifluoroacetate. Deprotection of Boc group in (*S*)-*tert*-butyl 2-((4-octylbenzamido)methyl)pyrrolidine-1-carboxylate. Synthesized by general procedure D. 63% yield, yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.43 (s, 1H), 7.72 (d, J = 8.1 Hz, 2H), 7.18 (d, J = 8.0 Hz, 2H), 3.80 (s, 1H), 3.72−3.62 (m, 1H), 3.56 (d, J = 14.0 Hz, 1H), 3.17 (s, 2H), 2.59 (t, J = 7.4 Hz, 2H), 2.05−1.93 (m, 2H), 1.92−1.82 (m, 1H), 1.73−1.64 (m, 1H), 1.57 (quin, J = 6.7 Hz, 2H), 1.33−1.20 (m, 10H), 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.8, 147.9, 130.4, 128.8, 127.5, 60.7, 45.2, 41.4, 36.0, 32.0, 31.3, 29.5, 29.4, 29.4, 27.8, 24.1, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{20}H_{33}N_2O^+$ $[M+]^+$: 317.2592, Found: 317.2564.

(*S*,*Z*)-*tert*-Butyl (((*tert*-Butoxycarbonyl)amino)(2-((4-octylbenzamido)methyl)pyrrolidin-1-yl)methylene)carbamate. Guanylation of (*S*)-2-((4-octylbenzamido)methyl)pyrrolidin-1-ium 2,2,2-trifluoroacetate. Synthesized by general procedure C. 47% yield, colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.29 (s, 1H), 7.86 (d, J = 8.3 Hz, 2H), 7.71 (s, 1H), 7.20 (d, J = 8.2 Hz, 2H), 4.61−4.53 (m, 1H), 3.84 (dt, J = 14.0, 3.7 Hz, 1H), 3.76−3.68 (m, 1H), 3.62−3.41 (m, 2H), 2.62 (t, J = 7.6 Hz, 2H), 2.20−2.12 (m, 1H), 1.94−1.86 (m, 1H), 1.82−1.67 (m, 3H), 1.59 (quin, J = 6.3 Hz, 2H), 1.50 (s, 18H), 1.36−1.18 (m, 10H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.6, 155.1, 146.6, 131.8, 128.4, 127.5, 58.6, 50.8, 42.6, 36.0, 32.0, 31.4, 29.6, 29.4, 29.4, 28.9, 28.3, 24.9, 22.8, 14.2; HRMS (ESI+): Calcd for $C_{31}H_{51}N_4O_5$ $[M + H]^+$: 559.3859, Found: 559.3813.

Deprotection by general procedure D. 47% yield, colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.72 (d, J = 8.1 Hz, 2H), 7.21 (d, J = 8.1 Hz, 2H), 4.05 (s, 1H), 3.54 (s, 1H), 3.35−3.09 (m, 1H), 2.60 (t, J = 7.4 Hz, 2H), 2.13−1.92 (m, 3H), 1.58 (quin, J = 6.9 Hz, 2H), 1.50−1.36 (m, 1H), 1.32−1.22 (m, 10H), 0.87 (t, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.2, 155.5, 147.7, 130.5, 128.8, 127.3, 57.9, 47.0, 42.7, 36.0, 32.0, 31.3, 29.8, 29.6, 29.6, 29.4, 29.4, 27.8, 22.8, 22.7, 14.2; HRMS (ESI+): Calcd for $C_{21}H_{35}N_4O+$ $[M+]^+$: 359.2810, Found: 359.2817.

***tert*-Butyl (*S*)-2-((3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)-methyl)pyrrolidine-1-carboxylate (35a).** Synthesized by general procedure A. 56% yield, colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.3 Hz, 2H), 7.25 (d, J = 7.9 Hz, 2H), 4.39−4.16 (m, 1H), 3.48−3.22 (m, 3H), 3.15−2.94 (m, 1H), 2.63 (t, J = 7.9, 6.8 Hz, 2H), 2.14−1.95 (m, 1H), 1.85 (m, 3H), 1.60 (dq, J = 12.9, 6.4, 5.4 Hz, 2H), 1.45 (s, 9H), 1.36−1.17 (m, 18H), 0.85 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.0, 168.3, 154.1, 146.4, 128.8, 127.3, 124.1, 79.9, 55.1, 46.7, 46.3, 35.9, 31.8, 31.2, 29.4, 29.2, 29.2, 28.4, 22.6, 14.1.

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HRMS (ESI+): Calcd for $C_{26}H_{39}N_3NaO_3$ $[M + Na]^+$: 464.2889, Found: 464.2905.

***tert*-Butyl (*S*)-2-(2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)ethyl)pyrrolidine-1-carboxylate (35b).** Synthesized by general procedure A. 63% yield, colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.0 Hz, 2H), 3.94 (d, J = 51.5 Hz, 1H), 3.45 (s, 0.5 H), 3.34 (s, 1H), 3.12 (q, J = 6.3 Hz, 0.5 H), 2.95 (dq, J = 19.8, 8.5, 7.6 Hz, 2H), 2.65 (t, J = 7.6 Hz, 2H), 2.37−2.15 (m, 1H), 2.09−1.79 (m, 4H), 1.64 (tq, J = 20.6, 13.1, 9.4 Hz, 4H), 1.45 (d, J = 6.8 Hz, 9H), 1.36−1.20 (m, 10H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.6, 168.2, 154.6, 146.4, 128.9, 127.3, 124.3, 79.6, 56.4, 46.2, 40.4, 35.9, 31.8, 31.2, 29.9, 29.4, 29.2, 29.2, 28.7, 28.5, 28.4, 26.6, 26.5, 26.3, 22.6, 14.1. HRMS (ESI+): Calcd for $C_{27}H_{41}N_3NaO_3$ $[M + Na]^+$: 478.3046, Found: 478.3042.

(*S*)-2-((3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)methyl)-pyrrolidin-1-ium Chloride. Deprotection of Boc group in *tert*-butyl (*S*)-2-((3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate. Synthesized by general procedure B. 85% yield, white solid; ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (d, J = 7.9 Hz, 2H), 7.32 (d, J = 7.9 Hz, 2H), 4.18 (s, 1H), 3.61−3.47 (m, 2H), 3.42 (t, J = 6.7 Hz, 2H), 2.67 (t, J = 7.8 Hz, 2H), 2.47−2.36 (m, 1H), 2.12 (dd, J = 20.8, 13.5 Hz, 2H), 1.93 (d, J = 8.4 Hz, 1H), 1.71−1.57 (m, 2H), 1.39−1.22 (m, 10H), 0.88 (t, J = 7.2, 6.7 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 177.4, 169.5, 148.1, 130.1, 128.4, 125.1, 58.4, 47.1, 36.8, 33.0, 32.4, 31.5, 30.5, 30.3, 30.3, 29.8, 24.7, 23.7, 14.4. HRMS (ESI+): Calcd for $C_{21}H_{32}N_3O$ $[M + H]^+$: 342.2540, Found: 342.2537.

(*S*)-2-(2-(3-(4-Octylphenyl)-1,2,4-oxadiazol-5-yl)ethyl)-pyrrolidin-1-ium Chloride. Deprotection of Boc group in *tert*-butyl (*S*)-2-(2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)ethyl)pyrrolidine-1-carboxylate. Synthesized by general procedure B. 90% yield, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.1 Hz, 2H), 3.73 (q, J = 7.6, 6.9 Hz, 1H), 3.44−3.30 (m, 2H), 3.18 (t, J = 7.5 Hz, 2H), 2.98 (dt, J = 7.5 Hz, 1H), 2.68 (t, J = 7.8, 6.7 Hz, 2H), 2.36 (ddd, J = 14.1, 7.1 Hz, 2H), 2.10 (ddt, J = 37.7, 13.2, 8.2 Hz, 1.5H), 1.91 (q, J = 7.1 Hz, 0.5H), 1.84−1.57(m, 3.5H), 1.55−1.45 (m, 2H), 1.40−1.23 (m, 10H), 0.90 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 180.2 (rotamer 1), 178.6 (rotamer 2), 168.1(rotamer 1), 168.0 (rotamer 2), 146.6 (rotamer 1), 146.5 (rotamer 2), 128.7 (rotamer 1), 128.7 (rotamer 2), 126.9 (rotamer 1), 126.9 (rotamer 2), 124.1 (rotamer 1), 124.0 (rotamer 2), 59.7, 45.0, 39.3, 35.5, 31.6, 31.1, 29.6, 29.1(rotamer 1), 29.0 (rotamer 2), 28.9, 28.2 (rotamer 1), 28.1 (rotamer 2), 26.9, 25.9, 25.7, 25.6, 23.2, 23.0, 22.3, 13.1. HRMS (ESI+): Calcd for $C_{22}H_{34}N_3O$ $[M + H]^+$: 356.2696, Found: 356.2703.

***tert*-Butyl (*S,E*)-(((*tert*-Butoxycarbonyl)amino)(2-((3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)-methylene)carbamate.** Guanylation of (*S*)-2-((3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-ium chloride. Synthesized by general procedure C. 71% yield, colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.28 (s, 2H), 7.97 (d, J = 8.3 Hz, 2H), 7.25 (d, J = 6.1 Hz, 2H), 4.76 (dt, J = 11.3, 5.7 Hz, 1H), 3.72−3.58 (m, 2H), 3.48 (s, 1H), 3.12 (dd, J = 15.1, 8.4 Hz, 1H), 2.63 (t, J = 8.2, 6.5 Hz, 2H), 2.29−2.17 (m, 1H), 1.89 (ddd, J = 10.1, 7.4, 4.6 Hz, 1H), 1.85−1.74 (m, 2H), 1.61 (quin, J = 7.3 Hz, 2H), 1.45 (s, 18H), 1.35−1.18 (m, 10H), 0.86 (t, J = 5.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.9, 168.2, 154.0, 146.3, 128.8, 127.4, 124.2, 56.4, 50.0, 35.9, 31.8, 31.6, 31.2, 30.5, 30.3, 29.4, 29.2, 28.1, 25.2, 22.6, 14.1, 14.1. HRMS (ESI+): Calcd for $C_{32}H_{49}N_5NaO_5$ $[M + Na]^+$: 606.3631, Found: 606.3700.

***tert*-Butyl (*S,Z*)-(((*tert*-Butoxycarbonyl)imino)(2-(2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)ethyl)pyrrolidin-1-yl)methyl)-carbamate.** Guanylation of (*S*)-2-(2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)ethyl)pyrrolidin-1-ium chloride. Synthesized by general procedure C. 47% yield, colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, J = 8.3 Hz, 2H), 7.28 (d, J = 8.1 Hz, 2H), 4.58 (s, 1H), 3.62 (ddd, J = 11.4, 9.2, 7.0 Hz, 2H), 3.44 (m, 1H), 3.01 (m, 2H), 2.65 (t, J = 7.7 Hz, 2H), 2.35 (m, 2H), 2.14 (m, 2H), 2.02−1.92 (m, 2H), 1.84−1.68 (m, 3H), 1.63 (m, 3H), 1.48 (s, 18H), 1.37−1.20 (m, 10H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.4, 168.2, 154.4, 146.3, 128.9, 127.3, 124.3, 57.7, 49.9, 35.9, 31.9, 31.2, 29.9, 29.4, 29.3, 29.2, 28.2, 22.9, 22.7, 14.1. HRMS (ESI+): Calcd for $C_{33}H_{51}N_5NaO_5$ $[M + Na]^+$: 620.3788, Found: 620.3770.

(*S*)-Amino(2-((3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)-methyl)pyrrolidin-1-yl)methaniminium Chloride (36a). Synthesized by general procedure B. 83% yield, white solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.94 (d, J = 8.3 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 4.52 (m, 1H), 3.53 (m, 1H), 3.48−3.40 (m, 1H), 2.66 (t, J = 7.9, 7.4 Hz, 1H), 2.27 (m, 1H), 2.07 (m, 3H), 1.63 (m, 2H), 1.29 (m, 10H), 0.87 (t, J = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 176.5, 168.2, 155.0, 146.7, 128.7, 126.9, 123.8, 55.8, 35.4, 31.6, 31.0, 30.2, 29.1, 28.9, 28.9, 28.7, 22.3, 22.2, 13.0. HRMS (ESI+): Calcd for $C_{22}H_{34}N_5O$ $[M + H]^+$: 384.2758, Found: 384.2743. $[\alpha]_D$ = −89.6° (c = 0.005, methanol). HPLC analysis indicates that compound **36a** is 93% pure.

(*S*)-Amino(2-(2-(3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl)-ethyl)pyrrolidin-1-yl)methaniminium Chloride (36b). Synthesized by general procedure B. 95% yield, white solid; ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (d, J = 8.3 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 4.03 (m, 1H), 3.46 (m, 1H), 3.35−3.27 (m, 1H), 3.07−2.89 (m, 2H), 2.59 (t, J = 7.5 Hz, 2H), 2.19 (m, 1H), 2.13−1.97 (m, 3H), 1.91 (m, 2H), 1.55 (m, 2H), 1.33−1.12 (m, 10H), 0.79 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 179.8, 167.8, 154.8, 146.7, 128.8, 126.8, 123.8, 57.6, 35.4, 31.6, 31.0, 29.3, 29.1, 29.0, 28.9, 28.1, 22.4, 22.3, 22.2, 13.0. HRMS (ESI+): Calcd for $C_{23}H_{36}N_5O$ $[M + H]^+$: 398.2914, Found: 398.2905. HPLC analysis indicates that compound **36b** is 89% pure.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra for compounds **2**−**36b**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 540-231-5742. E-mail: santosw@vt.edu.

Author Contributions

#These authors (N.N.P. and E.A.M.) contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): W.L.S. and K.R.L. are among the co-founders of SphynKx Therapeutics LLC, which was created to commercialize S1P-related discoveries, including SphK inhibitors, discovered and characterized in their laboratories. The compounds described in this manuscript are included in a patent application licensed to SphynKx..

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■ ABBREVIATIONS USED

Sph, sphingosine; S1P, sphingosine-1-phosphate; SphK, sphingosine kinase

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