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Synthesis and biological evaluation of sphingosine kinase substrates as sphingosine-1-phosphate receptor prodrugs

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ABSTRACT

In the search for bioactive sphingosine 1-phosphate (S1P) receptor ligands, a series of 2-amino-2-heterocyclic-propanols were synthesized. These molecules were discovered to be substrates of human-sphingosine kinases 1 and 2 (SPHK1 and SPHK2). When phosphorylated, the resultant phosphates showed varied activities at the five sphingosine-1-phosphate (S1P) receptors ($S1P_{1-5}$). Agonism at $S1P_1$ was displayed in vivo by induction of lymphopenia. A stereochemical preference of the quaternary carbon was crucial for phosphorylation by the kinases and alters binding affinities at the S1P receptors. Oxazole and oxadiazole compounds are superior kinase substrates to FTY720, the prototypical prodrug immunomodulator, fingolimod (FTY720). The oxazole-derived structure was the most active for human SPHK2. Imidazole analogues were less active substrates for SPHKs, but more potent and selective agonists of the $S1P_1$ receptor; additionally, the imidazole class of compounds rendered mice lymphopenic.

1. Introduction

Five membrane-bound sphingosine 1-phosphate (S1P, Fig. 1) receptors control physiological processes, including heart rate, tissue permeability,[1] wound healing,[2] immune cell trafficking[3] and oligodendrocyte function.[4] Receptor expression and metabolism of sphingolipid signaling molecules enable endogenous S1P to control these diverse functions with specificity while being present at concentrations of 200–450 nM in plasma.[5,6] Our laboratories have attempted to describe these signaling pathways by investigating the structure–activity-relationship of individual S1P receptors through the synthesis and biological characterization of non-natural S1P receptor ligands. Previously, we reported diverse classes of S1P analogues with various receptor affinities; including $S1P_4$ and $S1P_{1,5}$ selective agonists,[7,8] as well as some of the first $S1P_{1,3}$ antagonists.[9,10]

Important in the successful development of S1P ligands is their incorporation into sphingosine metabolism. In view of natural S1P biosynthesis and degradation, one pathway for ligand inactivation involves lysophospholipid phosphatases.[11,12] These enzymes dephosphorylate S1P and related molecules to primary alcohols that are physiologically inactive at the five receptors. We and others illustrated the synthesis of phosphonate mimetics that are more chemically resistant to phosphatase activity (bioactive **VPC44152**, Fig. 1).[10,13] This report describes the synthesis and biological characterization of S1P ligands that are prone to phosphorylation

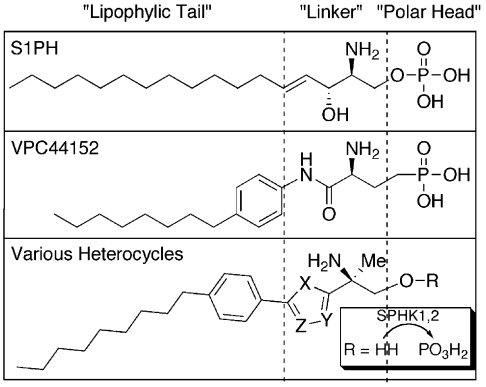

Figure 1. S1P is active at five S1P receptors ($S1P_{1-5}$). **VPC44152** is a non-hydrolysable phosphonate agonist at $S1P_{1,4,5}$. Various 2-amino-2-heterocyclic propanols were designed as prodrugs (converted by sphingosine kinases to active phosphates) for targeting S1P receptors.

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by one or both of the known sphingosine kinases (SPHKs).[14] Substrates for SPHKs may obtain therapeutically useful equilibriums between their alcohol and phosphate states in vivo, as investigated by $S1P_1$ induced lymphopenia.

A series of 2-amino-2-heterocyclic-propanols were investigated, based on our previous discovery of $S1P_1$ selective agonists that contained N-aryl amide moieties within their linker region (Fig. 1). This series was tested for activity at the known mouse (mSPHK) and human (hSPHK) sphingosine kinases. These potential kinase substrates were tested in vitro; and, following chemical phosphorylation, the compounds were evaluated at the five individual S1P receptors; and finally, the substrates were tested in vivo, for the induction of $S1P_1$ mediated lymphopenia.

Imidazole, oxazole, and oxadiazole containing compounds are phosphorylated by SPHKs, with hSPHK2 being the more active species. This activity was dependant on the chirality of the C-2 carbon. One of two oxadiazoles was a better kinase substrate for than FTY720, the prototypical S1P prodrug. The corresponding oxazole showed the highest activity at SPHK2. Imidazole based compounds were comparatively less active substrates at the SPHKs but their phosphorylated congeners were more potent and selective agonists at the $S1P_1$ receptor. meta-Substituted compounds in these series (found to be antagonists of $S1P_{1,3}$ receptors) were not substrates for SPHKs. This is consistent with our previous model, in which the stereochemical preference for antagonism is opposite to that favored by enzymatic phosphorylation.

2. Chemistry

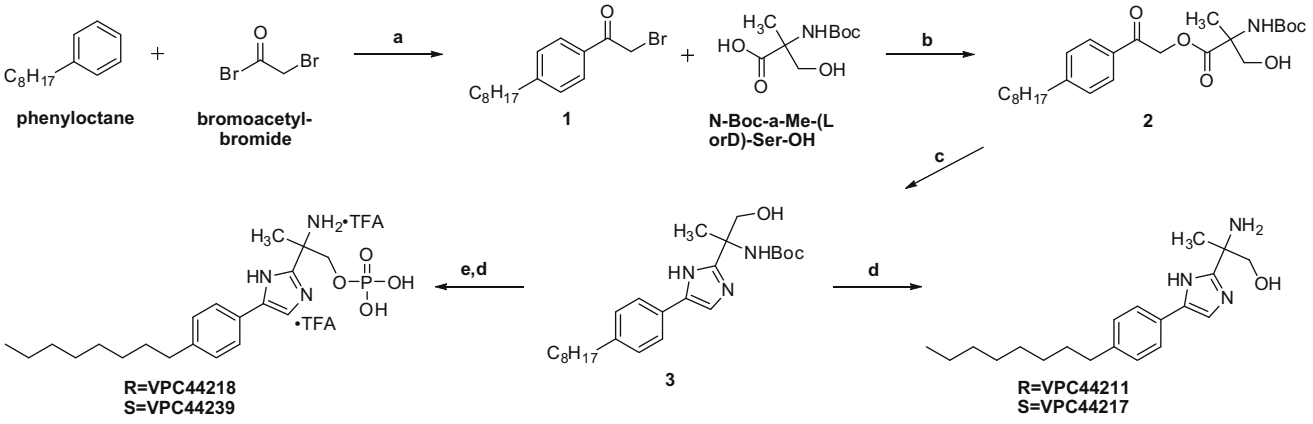
The synthesis of 4(5)-phenylimidazoles (Scheme 1) was envisioned through a Davidson-like cyclodehydration.[15–19] Compound **1** was attained from the Friedel–Crafts acylation of commercially available 1-phenyloctane and 2-bromoacetyl bromide as previously described.[10] *N*-Boc-α-methylserines were converted to their cesium salts under sonication,[20] and alkylated with α-bromoketone **1** to form the desired α-acyloxyketones, ***R*-** and ***S*-2**, in robust yields. α-Acyloxyketones were cyclized to optically active phenylimidazoles ***R***- and ***S*-3** by careful heating with NH_4OAc in xylenes. The 2-amino-1-propanols, **3**, were deprotected under acidic conditions and neutralized to yield the optically active final compounds **VPC44211** and **VPC44217**. N-Boc protected compounds were also converted to the corresponding phosphates by standard phosphoramidite methodology. Subsequent deprotection provided the bis-ammonium trifluoroacetate salts **VPC44218** and **VPC44239**.

A procedure to create the 4-phenyloxazole ring system (Scheme 2) was readily available; however, the acidic conditions necessary for the cyclization meant that new protection schemes for the α-methyl-serine were necessary.[21] A *tert*-butyldiphenylsilyl (TBDPS) ether was used successfully[22] with a benzyloxycarbonyl (Cbz) protection for the amine. Literature procedures for the selective hydrolysis of the methyl ester protected acid (**6**) in the presence of the TBDPS ether further increased the utility of this protection scheme.[23] Formation of the corresponding α-acyloxyketone proceeded smoothly, but the cyclization step to form the desired intermediate **9** proved low yielding. The 4-phenyloxazole was converted by standard methods to amino alcohol **VPC92153** and amino phosphate **VPC92249**.

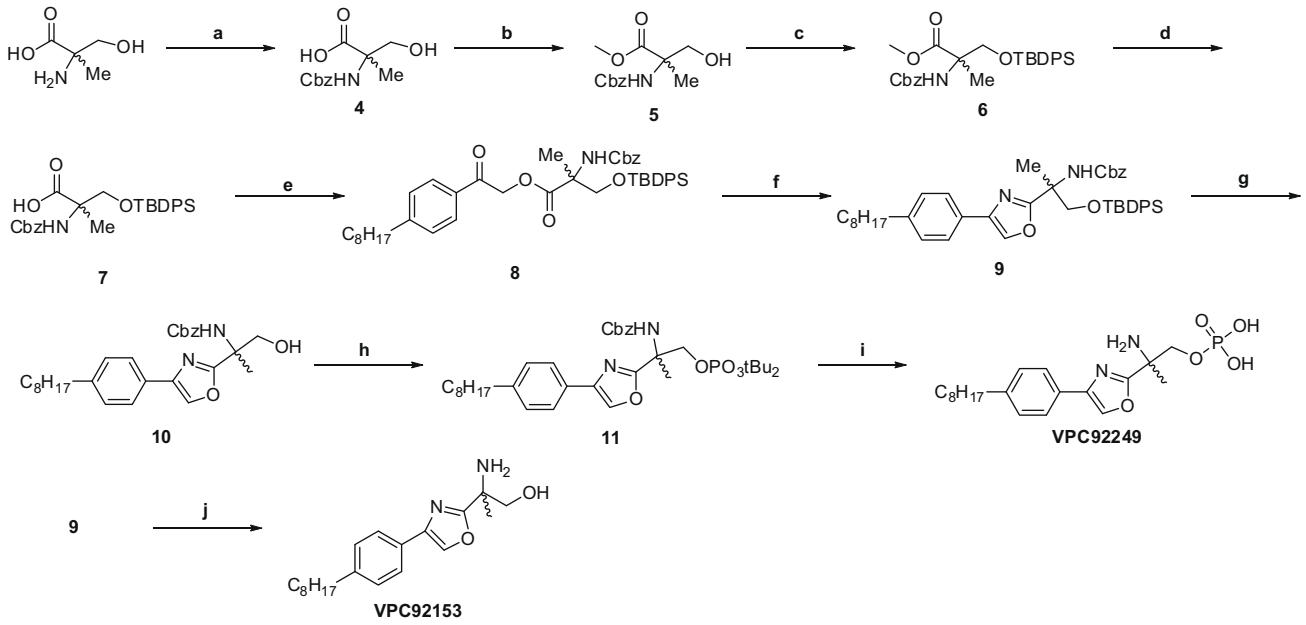
1,2,4-Oxadiazoles are established peptide bond mimetics and comparable to the 4(5)-phenylimidazoles.[24] They are smaller in diameter, considerably less basic than their imidazole counterparts, and allow for hydrogen-bond acceptance, but not donation. Two isomers exist in which the nitrogen atom occupies a similar location compared with the imidazole ring. To approach the two isomers, a common pathway for construction of the oxadiazole ring was desired. Previously, 1,2,4-oxadiazoles were constructed by the condensation of activated carboxylic acids with amidoximes in the presence of strong base.[25–30] Several common condensation methods suggested by the literature for coupling carboxylic acids and amidoximes afforded little or no success (DCC,[31] EDC,[32] and DIC/HOBT[33]). Following the literature through extensions to mild condensation strategies, a general method for the conjoining of various carboxylic acids and amidoximes remained elusive.[34] We found PyBOP, the common and mild condensation reagent, worked well for the coupling of both oxadiazole isomers.

With this strategy in hand, the synthesis of the 1,2,4-oxadiazole isomer commenced with the conversion of commercially available 4-iodobenzonitrile to the *para*-alkynylaniline **12** through a Verkade-modified Sonogashira reaction (Scheme 3).[35] Selective reduction of the arylalkyne was accomplished by hydrogenation over Lindlar's catalyst to afford para-octylbenzonitrile **13**. Using methods pioneered by Tiemann and Kruger,[36] and optimized by Eitner and Weitz,[37] hydroxylamine heated in ethanol gave reliable yields of the amidoxime **14**.[38,39]

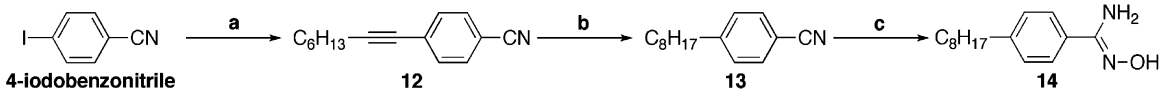
Commercially available 2-methyl-(D,L)-serine was converted to acid **15** in two convenient steps (Scheme 4). Carboxylic acid **15** was coupled with amidoxime **14** to form acylamidoxime **16** following our newly established PyBOP coupling strategy. The resulting intermediate was cyclized, providing near quantitative yields of **17**. Global deprotection was successful upon the addition of



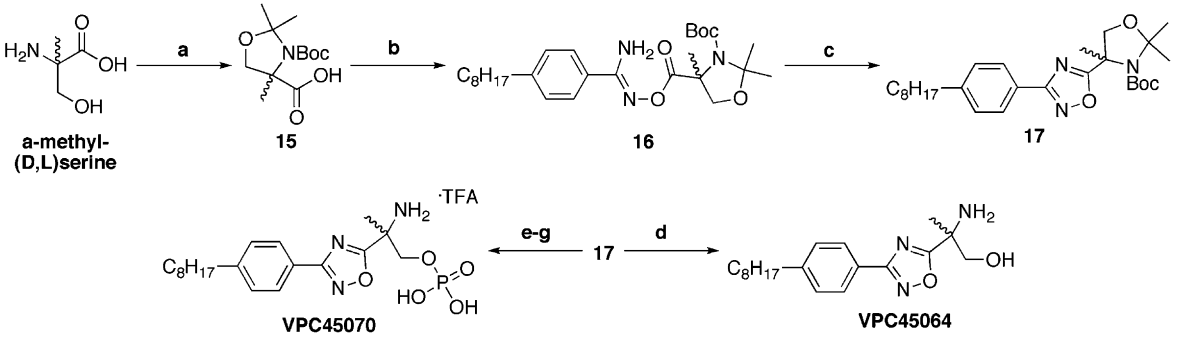
Scheme 1. Synthesis of chiral 4(5)-phenylimidazoles. Reagents and conditions: (a) $AlCl_3$, neat, 0 °C to rt, 4 h. (84%); (b) Cs_2CO_3, EtOH, sonication, 5–10 min.; then α-bromoketone in DMF, rt, overnight (86–94%); (c) NH_4OAc, xylenes, Dean-Stark, 110–120 °C, 1–3 h, 50–60%; (d) TFA, CH_2Cl_2, 0 °C to rt, 4 h, 80–84%; (e) *N,N*-di-*iso*-propyl-di-*tert*-butyl-phosphoramidite, 3% tetrazole in acetonitrile, CH_2Cl_2, 4–8 h; then 30% $H_2O_{2(aq)}$ rt, 4 h, 33–37%.



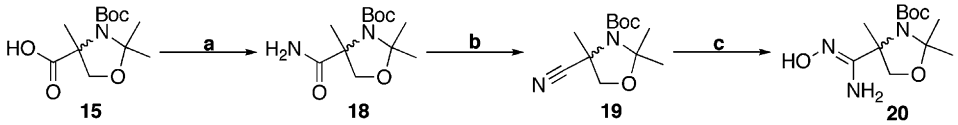
Scheme 2. Synthesis of 4-phenyloxazoles. Reagents and conditions: (a) 10% satd aq Na_2CO_3, dioxanes, *N*-(benzyloxycarbonyloxy)succinimide; (b) $TMSCHN_2$, 6:1 benzene/MeOH (79% over two steps); (c) TBDPSCl, imidazole, DMAP, CH_2Cl_2 (82%); (d) NaOH, H_2O/*i*-PrOH (96%); (e) Cs_2CO_3, EtOH, sonication, 5–10 min; then α-bromoketone in DMF, rt, overnight (94%); (f) NH_4OAc, AcOH, 90 °C, 10 h (33%); (g) *t*-Bu_4NF, THF (77%); (h) *N,N*-di-*iso*-propyl-di-*tert*-butyl-phosphoramidite, 3% tetrazole in acetonitrile, CH_2Cl_2, overnight; then 30% $H_2O_{2(aq)}$ rt, 4 h, 59%; (i) Pd/C, H_2, EtOH, rt, overnight (97%); then TFA, CH_2Cl_2, 4 h (quantitative); (j) *t*-Bu_4NF, THF (77%); then Pd/C, H_2, EtOH (quantitative).



Scheme 3. Synthesis of benzylamidoxime **14**. Reagents and conditions: (a) $Pd(OAc)_2$, Bu_4NOAc, 1-octyne, DMF, rt, overnight (85–99%); (b) H_2, Pd on $BaSO_4$, EtOH, 45 psi, rt, 1 h (>95%); (c) $NH_2OH{\cdot}HCl$, Et_3N, 95% $EtOH_{(aq)}$, 75 °C, 3 h (74%).



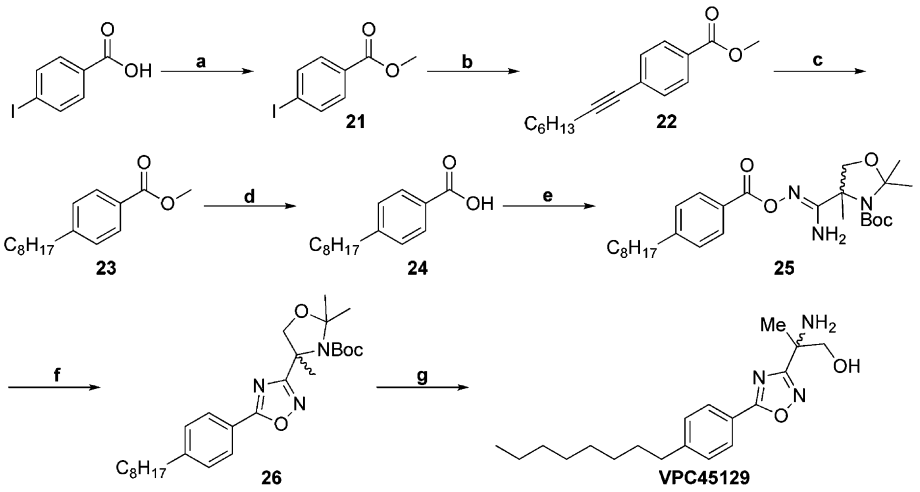
Scheme 4. Reagents and conditions: (a) (i) 10% Na_2CO_3 in H_2O, rt, 5–10 min; then Boc_2O in dioxanes, rt, 0.5–2 days; (ii) 2,2-dimethoxypropane, BF_3 OEt_2, acetone, rt, 1–3 h (>95%, two steps); (b) PyBOP, *i*-Pr_2NEt, CH_2Cl_2, rt, 4 h, 88%; (c) DMF, 110 °C, 3–4 h (71%); (d) TFA, CH_2Cl_2, rt, 3 h; then $NaHCO_3$, rt, 15 min, 90%; (e) (i) TFA, CH_2Cl_2, rt, 3 h; then $NaHCO_3$, rt, 15 min, 90%; (ii) 10% Na_2CO_3 in H_2O, rt, 5–10 min; then Boc_2O in dioxanes, rt, 4–6 h, (67%, two steps); (f) *N,N*-di-*iso*-propyl-di-*tert*-butyl-phosphoramidite, 3% tetrazole in acetonitrile, CH_2Cl_2, overnight; then 30% $H_2O_{2(aq)}$ rt, 4 h, 57%; (g) TFA, CH_2Cl_2, rt, 3 h, >80%.



Scheme 5. Reagents and conditions: (a) (i) *iso*-butyl chloroformate, Et_3N, THF, −10 °C, 30 min; (ii) NH_4OH, 0 °C to rt, 1 h, 60–80%; (b) Et_3N, trifluoroacetic anhydride (TFAA), 0.1 M, THF, 0 °C to rt, 30 min, 85–95%; (c) $NH_2OH{\cdot}HCl$, Et_3N, 95% $EtOH_{(aq)}$, 75 °C, 3–5 h, 85%.

TFA to attain 3-phenyl-1,2,4-oxadiazole **VPC45064** following basic workup. Protected amino alcohol **17** was also converted to the ammonium phosphate **VPC45070** under standard conditions.

Inversion of the oxadiazole substitution pattern relied on the proper conversion of α-methyl serine to the amidoxime derivative **20** (Scheme 5). The desired 5-phenyl-1,2,4-oxadiazole more closely



Scheme 6. Reagents and conditions: (a) MeOH, $SOCl_2$, 0 °C to rt, 24 h, 81%; (b) 1-octyne, $Pd(OAc)_2$, Bu_4NOAc, DMF, rt, overnight, 83%; (c) H_2, Pd/C, EtOH, rt, 4–6 h, 99%; (d) 20% $NaOH_{(aq)}$, 95% EtOH(aq), rt, 2 h, then 1 N H_2SO_4 rt, 15 min, 99%; (e) **20**, PyBOP, *i*-Pr_2NEt, CH_2Cl_2, rt, 4 h (43%); (f) DMF, 110 °C, 16 h, (60%); (g) TFA, CH_2Cl_2, rt, 3 h; then $NaHCO_3$, rt, 15 min, >90%.

approximates the position of the nitrogens in the 4(5)-phenylimidazole compounds. Carboxylic acid **15** was converted to the primary amide **18** through formation of the mixed anhydride followed by addition of either $NH_{3(g)}$ or $NH_4OH_{(aq)}$. Convenient and effective dehydration conditions[40] were used to convert the amide to the nitrile-serinoid **19**. Treatment of this nitrile with hydroxylamine yielded the desired amidoxime analogue of serine **20**.

The methyl benzoyl ester **21**, previously synthesized by esterification (Scheme 6) provided the methyl *para*-octynylbenzoyl ester **22** by a modified Sonogashira coupling. Hydrogenation over Pd/C was achieved to yield the alkylbenzoyl ester **23**. Saponification of **23** provided the *para*-octylbenzoic acid **24** efficiently, which was condensed with the sterically congested amidoxime **20** to yield **25** under PyBOP coupling conditions. The purified intermediate was cyclized to the 5-phenyl-1,2,4-oxadiazole, **26**, as previously described. The *N*-Boc and *N*,*O*-isopropylidene were deprotected with TFA and treated with basic conditions, providing the desired 2-amino-1-propanol **VPC45129.**

Once obtained, **VPC45129** was subjected to anhydrous *N*-Boc protection and subsequent phosphorylation and deprotection to yield the corresponding phosphate (**VPC46023**) as a white solid (Scheme 7).

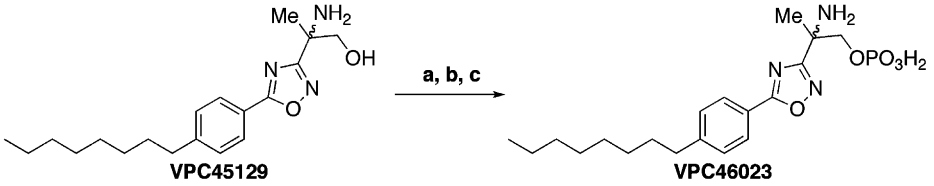
Synthesis of a 4-phenylthiazole derivative began with the serine-derived amide **18**, which was next converted to the thioamide **27** with the use of Lawesson's reagent (Scheme 8). The α-iminothioketone formed by the base-initiated *S*-alkylation of compound **27** was dehydrated in situ to give a separable mixture of the desired thiazole **28** and the incomplete dihydrothiazole **29**.[41,42] This one pot reaction was not optimized, but on re-treatment of the dihydrothiazole intermediate **29** with dry lutidine and TFAA, the dehydration was completed in excellent yields. Thiazole **28** was deprotected with TFA and neutralized to provide the desired aminoalcohol **VPC45214**.

3. Biology

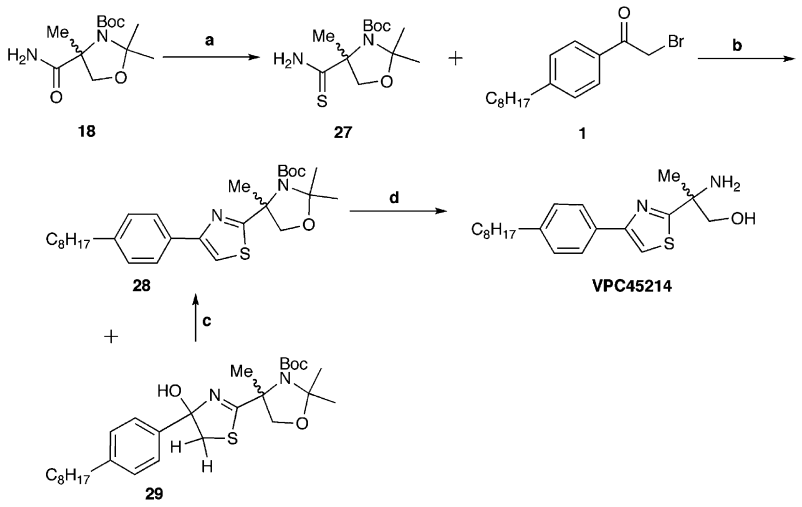
The final 2-heterocyclic-2-amino-1-propanols were analyzed as substrates of four SPHKs (h-SPHK1,2 and m-SPHK1,2, as previously described[43]). Phosphorylation was compared to that of the natural substrate of the kinases, D-erythro-sphingosine.

Most of our compounds (Fig. 2) exhibited activity at SPHK2 with the exception of the 4-phenylthiazole (**VPC45124**). The (*S*)-stereoisomer of the imidazole (**VPC44217**) was virtually inactive at the kinases while the *R*-stereoisomer (**VPC44211**), having the natural configuration about the quaternary carbon, had approximately 20% the activity of sphingosine at hSPHK2. This stereoselective preference was upheld when comparing the racemic mixture of the 3-phenyl-1,2,4-oxadiazole (**VPC45064**) and its *R*- stereoisomer (**VPC45080**), and recapitulates the observed stereoselectivity of SPHK2 for the methylated FTY720 analogs, AAL149 and AAL151.[44] The 5-phenyl-1,2,4-oxadiazole (**VPC45129**) and the 4-phenyloxazole (**VPC92153**) performed exceptionally well in the phosphorylation assay. **VPC92153** displayed the best activity at SPHK2. While **VPC45129** displayed moderate activity at SPHK2, it was the only alcohol in the series to have significant activity at SPHK1. It should be noted that very few synthetic analogs display activity at SPHK1, making this particular oxadiazole-containing compound unusual.

Due to extensive work by our laboratories and others, it is now well understood that lymphopenia induced by S1P receptor agonists, such as FTY720, is the direct result of potency at the $S1P_1$ receptor after in vivo phosphorylation by SPHK2.[43] It has also been demonstrated that the bradycardia evoked by FTY720 is linked to



Scheme 7. Synthesis of 5-phenyl-1,2,4-oxadiazole phosphate **VPC46032**. Reagents and conditions: (a) Boc_2O, TEA, CH_2Cl_2; (b) tetrazole, MeCN, *N*,*N*-diisopropylditertbutylphosphoramidite; 30% H_2O_2; (c) TMSBr, DCM (32% over three steps).



Scheme 8. Synthesis of thiazole **VPC45214**. (a) Lawesson's reagent, THF, rt, 4 h, 46%; (b) (i) $KHCO_3$, DME, −15 °C, 15 min; (ii) α-bromoketone, −15 °C, 30 min, then rt, 30 min; (iii) TFAA, lutidine, DME, −15 °C to rt, 12 h, 39%; (c) TFAA, lutidine, DME, −15 °C to rt, overnight, >95%; (d) TFA, CH_2Cl_2, rt, 6 h; then $NaHCO_3$, 15 min, 62%.

agonism at the $S1P_3$ receptor, at least in rodents.[45] Thus it is of interest to determine receptor activity in assessing aminoalcohols as S1P receptor prodrug agonists. Each phosphate was subjected to our standard GTP-[γ-^{35}S] assay as previously described.[6–10]

On initial examination of the data (Table 1), the selectivity between the $S1P_1$ and $S1P_3$ receptor has been greatly improved relative to FTY720. In most cases a difference of two log orders of selectivity was observed. The only exception was the 3-phenyl-1,2,4-oxadiazole phosphate (**VPC45070**) which was considerably less potent at $S1P_1$ and was equipotent at $S1P_3$. These phosphates also appear to be good agonists for the $S1P_4$ receptor, providing some insight into $S1P_4$ agonist SAR. However, while these analogs are approximately equipotent to the natural ligand, S1P itself is a surprisingly poor agonist. Experimental potencies of S1P at $S1P_4$ are in the high nanomolar range according to our assays.

The 4-phenylthiazole phosphate was not included in the receptor screening because it was such a poor substrate for the SPHKs. The ligand was, therefore an unlikely candidate as a $S1P_1$ receptor prodrug. Because of the detrimental side effects of FTY720's potency at the $S1P_3$ receptor, and the ability of this class of heterocycles to discriminate between $S1P_1$ and $S1P_3$, the therapeutic potential of these compounds becomes immediately apparent.

While receptor data provides some insight as to how these compounds should work as a potential therapy, the ultimate test of these heterocyclic sphingosine analogs lies in their ability to induce lymphopenia in vivo. Disappointingly, most of these aminoalcohols were not effective at lowering lymphocyte counts despite their unprecedented activity at the SPHKs and receptor potencies (data not shown). However, the 4-phenylimidazole analogs performed exceptionally well; lowering lymphocyte counts approximately 75% in most cases (Fig. 3).

The imidazole containing compounds that exhibited low nanomolar binding constants effectively induced lymphopenia in mice.

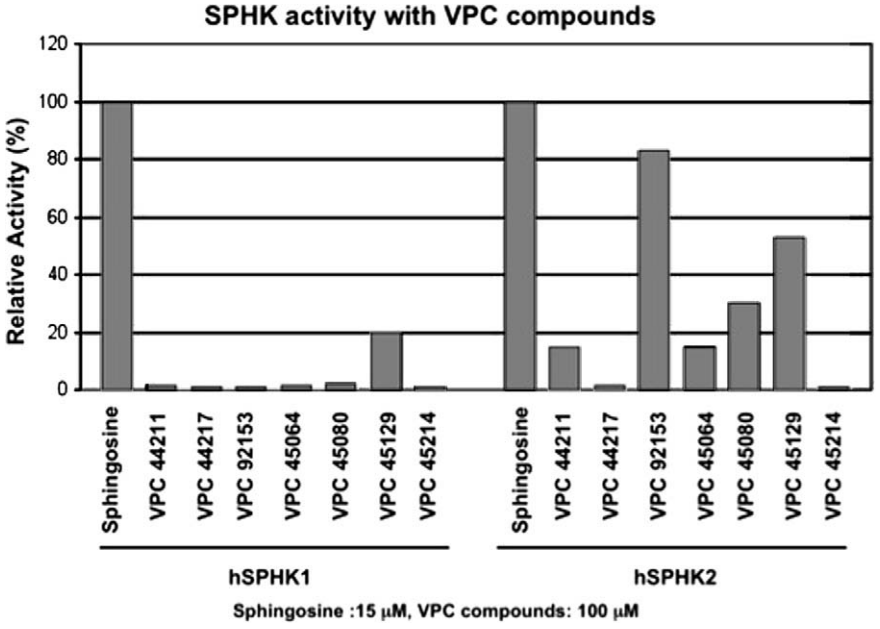


Figure 2. Comparison of imidazole, thiazole, oxadiazole and oxazole 2-amino-alcohols. Compounds were analyzed as possible substrates for the kinases hSPHK1 (hSK1) and hSPHK2 (hSK2).

Table 1
GTP-[γ-^{35}S] ASSAYS

	S1P Receptors									
	S1P$_1$		**S1P$_2$**		**S1P$_3$**		**S1P$_4$**		**S1P$_5$**	
	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}
S1P	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
FTY720P	0.28	1.00	NAA	0.00	0.23	0.50	0.15	0.78	4.32	0.56
VPC44218	1.09	0.87	NAA	0.00	50.77	0.65	0.59	0.93	3.62	0.83
VPC44239	8.09	0.92	NAA	0.00	1,000	0.61	4.13	1.19	16.15	0.83
VPC45070	24.40	0.89	NAA	0.00	22.99	0.51	0.52	0.81	4.35	0.73
VPC46023	2.50	1.00	NA	0.00	NA	NA	NA	NA	21.60	1.00
VPC92249	4.28	0.80	NA	0.00	52.52	1.00	NA	NA	24.07	0.60

NAA = no agonist activity detected. NA = not assessed. [a] EC_{50} and [b] E_{MAX} values were normalized to those of S1P. In a typical assay, the EC_{50} value of S1P was 10 nM (100 nM at S1P$_4$) and the membrane bound GTP[γ-^{35}S] varied from 1000 to 3000 cpm in response to increasing S1P concentrations.

S1P analogues containing the natural configuration (**VPC44211**) induced lymphopenia for more than 20 h while the unnatural aminoalcohol (**VPC44217**) did not cause this effect. This finding is consistent with our initial kinase studies, where the analog with the unnatural stereochemistry was a poor substrate for either sphingosine kinase. Although this study produced only a single heterocyclic analog of desirable activity in vivo, we have demonstrated there is a clear structure–activity-relationship for this class of SPHK substrates. Elucidating the elements that make these amino alcohols substrates for SPHK1 and SPHK2 while dialing out S1P$_3$ potency, it becomes possible to deliver immunosuppressants with significantly less detrimental S1P$_3$ related side effects.

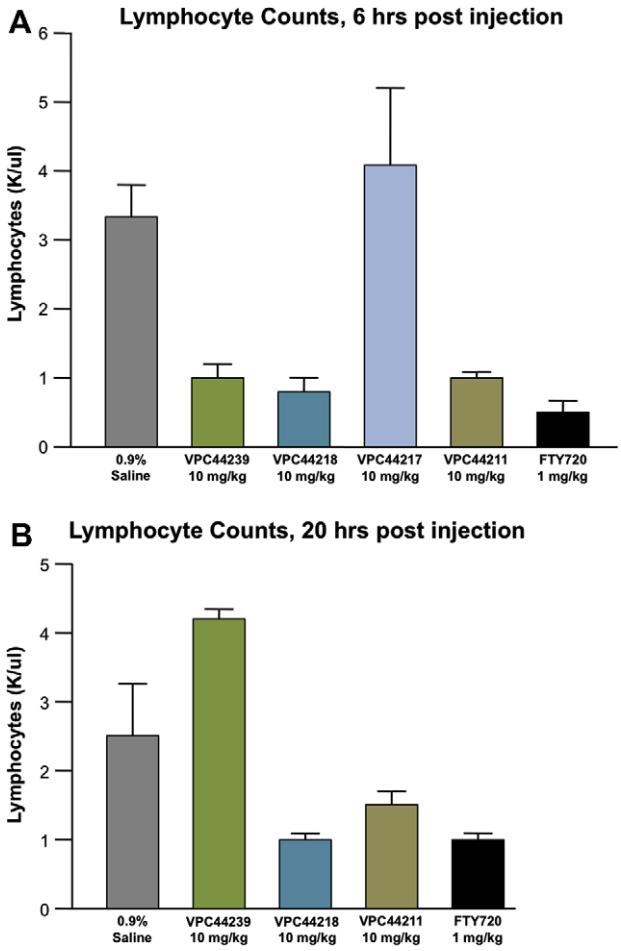


Figure 3. (A) Four chiral phenylimidazole compounds **VPC44239**, **VPC44218**, **VPC44217** and **VPC44211** were compared for their stimulation of lymphopenia using our standard in vivo assay.[6] (B) Twenty hours post ip injection, the alcohol **VPC44211** and phosphate **VPC44218** treated mice experienced nearly equivalent levels of lymphocyte depletion from the periphery.[46]

4. Conclusion

At the outset of this study, we sought to not only further our understanding of the elements of SPHK1 and SPHK2 substrate SAR, but also design more metabolically stable S1P$_1$ receptor agonist prodrugs. Additionally, we hoped to improve S1P$_1$/S1P$_3$ receptor selectivity, which would make these compounds more attractive as potential therapeutic agents. While in vitro data was initially quite promising, our newly synthesized series of heterocyclic S1P receptor prodrugs did not prove viable therapeutic candidates after in vivo analysis of lymphocyte levels. A likely cause for this result is the rate of dephosphorylation of these analogs by any number of lysophospholipid phosphatases, which would be revealed by a low agonist (phosphate): parent (alcohol) drug ratio in plasma. Another possibility is rapid clearance of these compounds in mice. We are currently evaluating these possibilities.

Due to their implication in a number of disease states, such as cancer and tumor growth, inhibitors of the sphingosine kinases are quite desirable. Here, we have presented a body of research that displays some of the most remarkable substrates of SPHK1 and SPHK2 yet reported in the literature; compounds whose rates of phosphorylation are beginning to approach that of the natural ligand. We hope to take this data forward in an effort to design a novel class of SPHK inhibitors that could be used as tools to answer questions about the role S1P in various disease states. Such tools have the potential to validate sphingosine kinases as drug targets.

5. Experimental

5.1. General procedure for O-alkylation of α-bromoketones

A mixture of Cs_2CO_3 (0.51 mmol) and carboxylic acid (1.00 mmol) in absolute EtOH (30 mL) was sonicated for five to ten minutes until nearly homogenous. The solvent was removed by rotary evaporation then dried in vacuo for 30 min. The cesium carboxylate was reconstituted in DMF and the primary halide (1.00 mmol) was added in one portion. The mixture was stirred for 8–16 h at ambient temperature then diluted with EtOAc. The precipitate was filtered and washed with EtOAc. The filtrate was concentrated to dryness and the crude material was purified by column chromatography.

5.2. General procedure for the cyclization of imidazoles

A solution of an α-acyloxyketone (1 mmol) and NH_4OAc (5 mmol) in xylenes (20 mL) was stirred in a round-bottomed flask. The apparatus was affixed with a Dean-Stark trap, filled with

xylenes, and a reflux condenser. The solution was heated between 110 °C and 120 °C for 1–5 h. The reaction darkened over this time and was monitored by TLC until forward progression appeared to subside or a byproduct began to form. When finished, the reaction mixture was concentrated to dark oil that was purified by column chromatography.

5.3. General procedure for the deprotection of N-Boc amines and di-*tert*-butyl-phosphate esters

To a 1:1 solution of TFA/CH_2Cl_2 (10 mL) was added the protected substrate (1 mmol). The solution was stirred at room temperature for 1–4 h until no starting material remained by TLC. The solution was concentrated under reduced pressure and repeatedly co-evaporated with diethyl ether (5 × 5 mL) to remove residual TFA. Amino alcohols were reconstituted in a mixture of 1:1 $NaHCO_3$/EtOAc and stirred for 10–15 min. The layers were separated and the aqueous layer was extracted with EtOAc (3×). The organic layers were combined, dried (Na_2SO_4), and concentrated to dryness by rotary evaporation. The crude mixture was separated by column chromatography and analyzed by one or more of the following methods including ^{1}H NMR, ^{13}C NMR, and MS. For the deprotection of phosphates, the desired compound was triturated with water (or methanol/ether). The liquid was decanted and the solid was then stirred in Et_2O until it became a free flowing solid under the organic solvent. The precipitate was filtered and washed with a small amount of Et_2O to yield the final compound as a solid that was analyzed by ^{1}H NMR, ^{13}C NMR, and MS.

5.4. General procedure for amidoxime formation from nitriles

A nitrile (1.0 mmol) was dissolved in 95% EtOH (1.5 mL). Triethylamine (2.3 mmol) and hydroxylamine hydrochloride (2.2 mmol) were added and the reaction mixture was heated to more than 70 °C for 3 h. At some time over the course of heating, the reaction mixture became a homogenous solution and the progress was monitored by TLC. Generally, by the end of 3 h, no starting material remained and the solution was concentrated to a slurry that could be precipitated from H_2O, as well as various organic solvents. The solid was filtered through a medium fritted funnel and washed with first H_2O and then cold hexanes. The remaining precipitate was dried to a solid and characterized by NMR and MS techniques.

5.4.1. 2-Bromo-1-(4-octyl-phenyl)-ethanone (1)

To a solution of aluminum chloride (0.46 g, 3.45 mmol) in 1,2-dichloroethane (1.8 mL) stirring at 0 °C was added 2-bromoacetylbromide (0.73 g, 3.6 mmol) dropwise. Once added, phenyl octane (0.571 g, 3.0 mmol) was added dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and was stirred for an additional 4 h. After this time, the reaction mixture was cooled to 0 °C and water was added to quench the excess aluminum chloride. The organic layer was isolated and evaporated to dryness. The crude material was purified via flash chromatography and 0.573 g (1.8 mmol, 60%) of the title product was recovered. R_f (10% EtOAc/hexanes) = 0.53. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.91 (d, J = 8.07 Hz, 2H), 7.30 (d, J = 8.07 Hz, 2H), 4.44 (s, 2H), 2.67 (t, J = 8.06, 2H), 1.63 (quintet, J = 7.69 Hz, 2H), 1.30–1.27 (m, 10H), 0.88 (t, J = 6.91 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 191.16, 150.5, 139.9, 132.1, 129.56, 129.39, 36.73, 32.49, 31.68, 31.61, 30.06, 29.92, 29.86, 23.32, 14.78.

5.4.2. 2-*tert*-Butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid 2-(4-octyl-phenyl)-2-oxo-ethyl ester ((*2S*)-2)

The ester was formed by the general procedure above with N-Boc-α-Me-(L)-Ser-OH (2.5 G, 11.405 mmol), Cs_2CO_3 (1.891 G, 5.817 mmol), EtOH (33 mL), DMF (65 mL) and **1** (3.550 g, 11.405 mmol). The crude material was concentrated to dryness and purified by column chromatography (350 mL SiO_2, 1:3 EtOAc/Hexanes) to give 4.419 g (86%) of clear oil as the desired ester. R_f = 0.50. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.82 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 5.61 (d, J = 6.3 Hz, 1H), 5.52 (s, 1H), 5.35 (d, J = 6.3 Hz, 1H), 4.19 (m, 1H), 3.94 (dd, J = 12.1, 4.6 Hz, 1H), 3.73 (t, J = 11.2 Hz, 1H), 2.67 (t, J = 7.7 Hz, 2H), 1.67–1.56 (m, 5H), 1.45 (s, 9H), 1.35–1.21 (m, 10H), 0.87, (t, J = 6.8 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 193.18, 173.04, 155.76, 150.66, 131.21, 129.10, 128.19, 79.88, 68.26, 66.68, 61.34, 36.18, 31.91, 31.08, 29.45, 29.29, 28.38, 22.72, 14.18 ppm.

5.4.3. 2-tert-Butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid 2-(4-octyl-phenyl)-2-oxo-ethyl ester ((*2R*)-2)

The ester was formed by the general procedure above with the optically active amino acid *N*-Boc-α-Me-(D)-Ser-OH (176 mg, 0.803 mmol), Cs_2CO_3 (133 mg, 410 mmol), EtOH (2.3 mL), **1** (250 mg, 0.803 mmol), DMF (4.5 mL). Following purification by column chromatography (150 mL SiO_2, 1:3 EtOAc/Hexanes) 320 mg (89%) of a clear oil was obtained as the desired ester. R_f = 0.50. ^{1}H and ^{13}C NMR data were consistent with that of (*2S*)-**2**.

5.4.4. (*2R*)-{2-Hydroxy-1-methyl-1-[5-(4-octyl-phenyl)-1H-imidazol-2-yl]-ethyl}-carbamic acid tert-butyl ester ((*2R*)-3)

The general imidazole cyclization was employed with (*2S*)-**2** (4.419 G, 9.829 mmol) and NH_4OAc (3.8 g, 49.145 mmol) in xylenes (200 mL). The crude dark oil was purified by column chromatography (250 mL SiO_2, 1:1 EtOAc/hexanes) to yield 2.436 g of an off-white solid. R_f = 0.31. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.55 (s, 2H), 7.16 (d, J = 7.9 Hz, 2H), 7.11 (s, 1H), 5.85 (s, 1H), 4.26 (d, J = 11.0 Hz, 1H), 3.66 (d, J = 11.4 Hz, 1H), 2.59 (t, J = 7.7 Hz, 2H), 1.67 (s, 3H), 1.68–1.58 (m, 2H), 1.42 (s, 9H), 1.38–1.22 (m, 10H), 0.88 (t, J = 6.6. Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 156.95, 151.34, 141.91, 128.90, 127.59, 124.74, 110.13, 80.37, 69.58, 54.71, 35.85, 32.06, 31.66, 29.68, 29.47, 28.50, 22.84, 22.12, 14.30 ppm.

5.4.5. (*2S*)-{2-Hydroxy-1-methyl-1-[5-(4-octyl-phenyl)-1*H*-imidazol-2-yl]-ethyl}-carbamic acid tert-butyl ester ((*2S*)-3)

The general imidazole cyclization was employed with (*2R*)-**2** (320 mg, 0.712 mmol), NH_4OAc (274 mg, 3.56 mmol), and xylenes (15 mL) to yield 183 mg of an off-white solid after purification by column chromatography (100 mL SiO_2, 1:1 EtOAc/hexanes). R_f = 0.31. ^{1}H and ^{13}C NMR data were consistent with that of (*2S*)-**3**.

5.4.6. (*2R*)-2-Amino-2-[5-(4-octyl-phenyl)-1H-imidazol-2-yl]-propan-1-ol (VPC44211)

To N-Boc amino alcohol (*2R*)-**3** (1.266 G, 2.947 mmol) stirring in CH_2Cl_2 (95 mL) was added Et_3SiH (1.18 mL, 7.368 mmol) followed by TFA (35 mL). The reaction was stirred under positive pressure (N_2) with a venting needle. Progress was measured by TLC revealing the completed reaction within 4–5 h. The solution was concentrated to a sticky solid, which was further dried by co-evaporation with Et_2O (3x20 mL). The solid was reconstituted in EtOAc (25 mL), washed with 2 N NaOH (3 × 15 mL), dried (Na_2SO_4), concentrated to $\leqslant$1 mL of solvent and precipitated by the addition of Et_2O to yield 784 mg (81%) an off-white solid. R_f (1:1 acetone/chloroform) = 0.12. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.56 (d, J = 7.3 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 7.17 (s, 1H), 3.97 (d, J = 10.8 Hz, 1H), 3.60 (dd, J = 10.8, 2.2 Hz, 1H), 2.60 (t, J = 7.4 Hz, 2H), 2.35 (br s, 1H), 1.61 (m, 2H), 1.47 (s, 3H), 1.38–1.18 (m, 10H), 0.88 (t, J = 6.1 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 173.08, 153.63, 142.07, 129.03, 124.89, 111.16, 92.28, 72.30, 54.14, 35.95, 32.16, 31.71, 29.76, 29.54, 26.12, 22.95, 14.37 ppm. MS (ESI+) m/z 330 $[M+H]^+$.

5.4.7. 2-Amino-2-[5-(4-octyl-phenyl)-1H-imidazol-2-yl]-propan-1-ol (VPC44217)

The general acidic deprotection was utilized with TFA (1.3 mL) and (*2S*)-**3** (70 mg, 0.132 mmol) in 1.3 mL of CH_2Cl_2. The final compound was triturated with Et_2O to yield 35 mg (80%) a white solid. R_f (1:1 acetone/chloroform) = 0.12. MS (ESI+) *m/z* 330 $[M+H]^+$. ^1H and ^{13}C NMR data was consistent with that of **VPC44211**.

5.4.8. (*2R*)-Phosphoric acid mono-{2-amino-2-[5-(4-octyl-phenyl)-1H-imidazol-2-yl]-propyl} ester (VPC44218)

To (*2R*)-**3** (111 mg, 0.258 mmol) was added a 3% tetrazole in MeCN (1.53 mL, 0.517 mmol). The mixture was diluted with the smallest amount of CH_2Cl_2 necessary to achieve homogeneity. The reaction was stirred for 15–60 min at ambient temperatures, then N,N-di-*iso*-propyl-di-*tert*-butyl-phosphoramidite (0.16 mL, 0.517 mmol) was added. As this solution stirred overnight, di-*iso*-propylamine precipitated out as a white solid. When a sufficient amount of starting material was consumed (monitored by TLC), a 30% solution of hydrogen peroxide (1.17 mL, 1.034 mmol) was added. The mixture was stirred for 4 h, during which time it quickly became homogenous. The organic solution was washed with saturated $NaHCO_3$, dried with Na_2SO_4, filtered and concentrated in vacuo. The crude material was purified by column chromatography (150 mL SiO_2, 30% acetone/$CHCl_3$) to yield 35 mg (21%) of a purified white solid. (Impure product was collected from column containing di-iso-propylamine.) R_f = 0.50 ^1H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.54 (br s, 2H), 7.16–7.09 (m, 3H), 6.17 (br s, 1H), 4.60–4.39 (m, 2H), 2.55 (t, J = 7.0 Hz, 2H), 1.77 (s, 3H), 1.64–1.54 (m, 2H), 1.49–1.35 (m, 27H), 1.30–1.17 (m, 10H), 0.85 (m, 3H) ppm.

The protected phosphate (35 mg, 0.056 mmol) was converted to the title compound the general acid mediated deprotection to yield the final compound as 28 mg (80%) of a white solid. ^1H NMR (300 MHz, DMSO-d_6, 23 °C, δ): 9.46 (s, 1H), 7.68 (d, *J* = 8.1 Hz, 2H), 7.55 (s, 1H), 7.18 (d, *J* = 7.7 Hz, 2H), 4.26–4.15 (m, 2H), 4.13–4.04 (m, 2H), 2.60–2.53 (m, 2H), 2.51 (s, 9H), 1.63 (s, 3H), 1.62–1.50 (m, 2H), 1.35–1.20 (m, 10H), 0.85, (t, *J* = 7.0 Hz, 3H) ppm. ^{13}C NMR (300 MHz, DMSO-d_6, 23 °C, δ): 166.91, 145.44, 140.58, 131.52, 128.61, 128.37, 124.31, 116.75, 67.34, 55.46, 46.22, 38.02, 31.23, 30.86, 29.74, 28.96, 28.80, 28.30, 23.17, 22.34, 13.82. MS (ESI+) *m/z* 410 $[M+H]^+$.

5.4.9. (*2S*)-Phosphoric acid mono-{2-amino-2-[5-(4-octyl-phenyl)-1H-imidazol-2-yl]-propyl} ester (VPC44239)

The above procedure was performed to phosphorylate (*2S*)-**3** (183 mg, 0.426 mmol) with phosphoramidite (0.27 mL, 0.852 mmol), 3% tetrazole in MeCN (2.5 mL, 0.852 mmol), and an incorrect amount of 30% H_2O_2 (0.07 mL, 0.054 mmol). Following the standard workup the crude material was purified by column chromatography (150 mL SiO_2, 1:1 EtOAc/hexanes) to yield 97 mg (37%) of a purified white solid. The protected phosphate (97 mg, 0.156 mmol) was converted to the title compound by the general deprotection. Following standard workup, 79 mg (82%) of the desired material was attained as a white solid. Characterization was consistent with the spectral data for **VPC44218**. MS (ESI+) m/z 410 $[M+H]^+$.

5.4.10. Methyl 2-(benzyloxycarbonylamino)-3-hydroxy-2-methylpropanoate (5)

α-Methyl D,L-serine (8.4 mmol) was dissolved in 22.7 mL of a 10% Na_2CO_3 solution. To this solution was added of N-(benzyloxycarbonyloxy) succinamide (16.8 mmol) followed by 16.8 mL of 1,4-dioxanes. The reaction mixture was then stirred for 24 h at which time it was extracted with diethyl ether (3 × 15 mL). The aqueous layer was then acidified to a pH of 3 and extracted with ethyl acetate (4 × 20 mL). The organic layers were combined and washed once with brine and dried with sodium sulfate. The organic layer was evaporated and **4** was isolated as a thick oil.

The acid **4** was next dissolved in 168 mL of a mixture of methanol and benzene (1:6). To this solution was added 2 M TMS–diazomethane in diethyl ether (9.24 mmol), which turned the solution yellow. The reaction mixture was stirred for approximately 1 h at ambient temperature. At this time, glacial acetic acid was dripped into the solution until the yellow color dissipated. The solution was next concentrated to an oil and reconstituted in ethyl acetate. The organic layer was washed with ammonium chloride (3 × 15 mL) and brine (1 × 15 mL) then dried with sodium sulfate. The title product, **5**, was concentrated to an oil and purified by flash chromatography yielding 2.0 g (7.5 mmol, 89%) of the title compound after two steps. R_f (50% EtOAc/hexanes) = 0.3 ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.47–7.35 (m, 5H), 5.72 (br s, 1H), 5.08 (s, 2H), 4.01–3.83 (m, 1H), 3.83–3.75 (m, 1H) 3.75 (s, 3H), 3.10 (br s, 1H), 1.49 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 173.90, 155.90, 128.85, 128.52, 128.39, 67.17, 66.77, 53.18, 20.68.

5.4.11. 2-Benzyloxycarbonylamino-3-(*tert*-butyl-diphenyl-silanyloxy)-2-methyl-propionic acid methyl ester (6)

To a solution of alcohol **5** (0.748 g, 2.8 mmol) stirring in 5.6 mL of N,N-dimethylformamide was added imidazole (0.42 g, 6.16 mmol) followed by *tert*-butyldiphenylchlorosilane (TBDPSCl, 0.846 g, 3.08 mmol). The solution was allowed to stir at room temperature for 4 h. After this time, the reaction mixture was diluted with 30 mL of ethyl acetate and washed thoroughly with water (3 × 5 mL) and brine (6 × 7 mL). The organic layer was then dried over sodium sulfate and evaporated to dryness. The crude mixture was purified with flash chromatography and 1.011 g (1.99 mmol, 71%) of the title product was recovered. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.63–7.60 (m, 5H), 7.46–7.34 (m, 10H), 5.93 (br s, 1H), 5.11 (s, 2H), 4.09 (d, *J* = 9.01 Hz, 1H), 3.91 (d, *J* = 9.67 Hz, 1H), 3.73 (s, 3H), 1.54 (s, 3H), 1.04 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 173.69, 155.23, 138.14, 135.82, 133.15, 130.15, 128.82, 128.10, 67.17, 66.69, 60.71, 52.94, 26.98, 19.61, 14.83.

5.4.12. 2-Benzyloxycarbonylamino-3-(tert-butyl-diphenyl-silanyloxy)-2-methyl-propionic acid (7)

To a stirring solution of the methyl ester **6** (1.011 g, 1.99 mmol) in 39.8 mL of *iso*-propanol was added a solution of sodium hydroxide (0.16 g, 4.0 mmol) in water (13.3 mL). The reaction mixture was heated to 60 °C and allowed to stir for 1 h while being monitored by TLC. At the end of this time, the reaction mixture was poured into a 1:1 mixture of diethyl ether and water (100 mL total) and the aqueous layer was acidified to a pH of approximately 3 using 1 M HCl (aq). The aqueous layer was extracted with diethyl ether (3 × 20 mL). The organic layers were combined and washed once with brine (15 mL) and dried over sodium sulfate. No further purification was necessary and 0.978 g (1.99 mmol, quant.) of the acid was recovered. R_f (25% EtOAc/hexanes) = 0.0. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.73–7.70 (m, 1H), 7.62–7.59 (m, 4H), 7.43–7.33 (m, 10H), 5.84 (br s, 1H), 5.09 (s, 2H), 4.03 (d, *J* = 7.29 Hz, 1H), 3.94 (d, *J* = 10 Hz, 1H), 1.55 (s, 3H), 1.01 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 178.05, 156.48, 135.76, 135.05, 132.85, 130.16, 128.78, 128.36, 128.25, 128.05, 67.05, 66.91, 61.61, 26.94, 19.55.

5.4.13. 2-Benzyloxycarbonylamino-3-(tert-butyl-diphenyl-silanyloxy)-2-methyl-propionic acid 2-(4-octyl-phenyl)-2-oxo-ethyl ester (8)

The acid **7** (1.8 mmol) was dissolved in 5.7 mL of ethanol; to this solution was added cesium carbonate (0.97 mmol). The mixture was sonicated for 10 min after which time the reaction mixture was evaporated to a solid and dried in vacuo for approximately 30 min. After this time the solid was reconstituted in 11.8 mL of

dimethylformamide followed by the addition of the primary bromide **1** (1.8 mmol). After stirring approximately 12–15 h at ambient temperature, the reaction mixture was diluted with approximately 20–30 mL of ethyl acetate and filtered through a fritted funnel. The filtrate was washed several times with water and brine. The organic layer was dried with sodium sulfate and evaporated to dryness. The crude material was purified by flash chromatography to yield 1.237 g (1.7 mmol, 94%) of the title product. R_f (10% EtOAc/hexanes) = 0.35. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.85–7.72 (m, 3H), 7.65–7.62 (m, 4H), 7.44–7.26 (m, 12H), 5.89 (br s, 1H), 5.1 (s, 2H), 4.19–4.17 (m, 1H), 4.09 (d, J = 9.62 Hz, 1H), 2.67 (t, J = 7.37 Hz, 2H), 1.69 (s, 3H), 1.63 (quintet, J = 7.06 Hz, 2H), 1.31–1.26 (m, 10H), 1.05 (s, 9H), 0.89 (t, J = 7.05 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm): 190.92, 163.77, 149.87, 135.59, 134.80, 132.95, 131.76, 129.82, 128.92, 128.51, 128.01, 127.88, 127.73, 66.51, 66.38, 36.09, 34.41, 31.86, 31.06, 29.41, 29.22, 26.77, 26.57, 22.67, 21.07, 19.36, 14.22, 14.13.

5.4.14. {2-(*tert*-Butyl-diphenyl-silanyloxy)-1-methyl-1-[4-(4-octyl-phenyl)-oxazol-2-yl]-ethyl}-carbamic acid benzyl ester; compound with methane (9)

To a solution of the keto-ester (**8**, 1.237 g, 1.7 mmol) in acetic acid (6.07 mL) was added ammonium acetate (0.331 g, 4.25 mmol). The reaction mixture was heated to 100 °C for 10 h. At the end of this time, the reaction mixture was evaporated to dryness and reconstituted in ethyl acetate (50 mL). This solution was washed with sodium bicarbonate (3 × 10 mL) and brine (1 × 10 mL). The organic layer was finally dried over sodium sulfate and purified via flash chromatography to recover 0.4 g (0.56 mmol, 33%) of the desired oxazole. R_f (10% EtOAc/hexanes) = 0.61. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.78–7.57 (m, 8H), 7.49–7.21 (m, 12H), 6.17 (br s, 1H), 5.12 (s, 2H), 4.14 (d, J = 7.29 Hz, 1H), 4.01 (d, J = 9.6 Hz, 1H), 2.64 (t, J = 8.06 Hz, 2H), 1.79 (s, 3H), 1.62 (quintet, J = 7.3 Hz, 2H), 1.32–1.23 (m, 10H), 0.99 (s, 9H), 0.90 (t, J = 6.92 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 165.59, 155.34, 142.90, 135.97, 135.74, 135.33, 133.37, 130.04, 128.94, 128.73, 128.47, 128.25, 128.00, 127.95, 126.76, 125.85, 36.03, 32.14, 29.54, 26.88, 22.64, 19.42, 14.38.

5.4.15. {2-Hydroxy-1-methyl-1-[4-(4-octyl-phenyl)-oxazol-2-yl]-ethyl}-carbamic acid benzyl ester (10)

The silyl ether (**9**, 0.4 g, 0.56 mmol) was dissolved in a 1 M solution of tetrabutylammonium fluoride in THF (1.12 mL). This solution was allowed to stir at room temperature for 17 h. At the end of this time, the reaction mixture was evaporated to dryness and reconstituted in 30 mL of ethyl acetate. This solution was then washed with ammonium chloride (3 × 7 mL) and brine (1 × 7 mL). The organic layers were finally dried over sodium sulfate and evaporated to dryness. The product was isolated using flash chromatography yielding 0.2 g (0.43 mmol, 77%) of the title product. R_f (10% EtOAc/hexanes) = 0.32. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.83 (s, 1H), 7.73–7.71 (m, 1H), 7.61 (d, J = 8.07 Hz, 2H), 7.42–7.34 (m, 4H), 7.21 (d, J = 8.07 Hz, 2H), 5.95 (br s, 1H), 5.08 (s, 2H), 4.09 (dd, J = 3.45 Hz, 1H), 3.90 (dd, J = 5.76, 1H), 2.62 (t, J = 8.07 Hz, 2H), 1.74 (s, 3H), 1.62 (quintet, J = 7.68 Hz, 2H), 1.30–1.27 (m, 10H), 0.88 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 165.41, 153.66, 143.47, 135.04, 133.49, 129.01, 128.78, 128.39, 127.95, 125.73, 68.33, 67.13, 36.00, 32.12, 31.67, 29.72, 29.50, 26.79, 22.92, 14.37.

5.4.16. 2-Amino-2-[4-(4-octyl-phenyl)-oxazol-2-yl]-propan-1-ol (VPC92153)

To a solution of the protected amine **10** (0.23 mmol) in 5 mL of ethanol was added a catalytic amount of 20% activated palladium on charcoal (10% w/w). This mixture was then degassed with hydrogen and stirred vigorously under a hydrogen atmosphere for 12–15 h at room temperature. At the end of this time, the reaction mixture was filtered through Celite and the crude mixture was concentrated to dryness and purified via preparatory thin-layer chromatography to provide 4.9 mg of the final amine. R_f (80% EtOAc/hexanes) = 0.16. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.82 (s, 1H), 7.61 (d, J = 8.02, 2H), 7.21 (d, J = 7.70, 2H), 3.95 (d, J = 10.89 Hz, 1H), 3.67 (d, J = 10.89, 1H), 2.62 (t, J = 7.69 Hz, 2H), 1.92 (br s, 2H), 1.61 (quintet, J = 7.37 Hz, 2H), 1.5 (s, 3H), 1.30–1.25 (m, 10H), 0.88 (t, J = 7.06 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 168.25, 143.20, 132.86, 128.82, 127.96, 125.43, 69.94, 54.34, 35.75, 31.87, 31.42, 29.71, 29.47, 29.26, 23.65, 22.67, 14.12. HRMS (electrospray) [M+H] calculated = 331.2386, found 331.2383.

5.4.17. Benzyl 1-(di-*tert*-butoxyphosphoryloxy)-2-(4-(4-octylphenyl)oxazol-2-yl)propan-2-ylcarbamate (11)

To a solution of the alcohol (**10**, 0.81 mmol) in THF (4 mL) was added 1H-tetrazole (2.43 mmol) and *N,N*-diisopropyldi-*tert*-butylphosphoramidite (1.62 mmol). The solution was allowed to stir for 24 h at which time 30% hydrogen peroxide in H_2O (3.24 mmol) was added. The reaction mixture was allowed to stir for an additional 15 h at which time the reaction mixture was evaporated to dryness and diluted in EtOAc. The organic layer was then washed with sodium metabisulfate, sodium bicarbonate, and brine. The organic layer was dried, evaporated and the crude material was purified by flash chromatography to recover 31 mg (0.047 mmol, 59%) of the title product. R_f = 0.32; ^{1}H NMR (300 MHz, CDCl3) δ (ppm) 7.81 (s, 1H), 7.60 (d, J = 8.1, 2H), 7.34 (m, 5H), 7.19 (d, J = 8.1, 2H), 5.09 (s, 2H), 4.39 (m, 2H), 2.61 (m, 2H), 1.69–1.55 (m, 2H), 1.44 (s, 9H), 1.40 (s, 9H), 1.26 (m, 10H), 0.88 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 164.07, 155.00, 143.21, 141.11, 133.64, 128.91, 128.67, 128.27, 125.77, 83.16, 69.69, 66.71, 56.68, 56.58, 35.98, 32.10, 31.65, 29.92, 29.70, 29.50, 22.90, 21.97, 21.38, 14.35.

5.4.18. 2-amino-2-(4-(4-octylphenyl)oxazol-2-yl)propyl dihydrogen phosphate (VPC92249)

The protected amine (0.475 mmol) was dissolved in 10 mL of 95% ethanol. To this solution was added a catalytic amount of 10% palladium on activated carbon. The ambient atmosphere was purged and replaced with hydrogen; the reaction mixture was then stirred vigorously for 15 hours. At this time the mixture was filtered through Celite and evaporated to dryness. 0.46 mmol (97%) of the deprotected amine was recovered. A small amount of the free amine (0.046 mmol) was then dissolved in DCM (0.46 mL) and TFA (0.46 mL). The reaction mixture was stirred for 2 h, at which time it was evaporated to dryness and recrystallized with hexanes, ether, and methanol to give 19 mg (.046 mmol, 100%)of the title product. ^{1}H NMR (500 MHz, DMSO) δ (ppm) 8.67 (s, 1H), 7.69 (d, J = 7.8, 0H), 7.25 (d, J = 7.7, 0H), 4.21 (s, 2H), 3.60 (br s, 4H), 2.56 (m, 2H), 1.57 (m, 2H), 1.26 (m, 10H), 0.83 (m, 3H). ^{13}C NMR (126 MHz, DMSO) δ (ppm): 161.63, 143.31, 140.71, 136.81, 128.80, 128.20, 125.91, 68.03, 56.06, 35.37, 31.72, 31.30, 22.54, 20.54, 14.41. HRMS (electrospray) [M+H] calculated = 411.2049, found 411.2057.

5.4.19. 4-Oct-1-ynyl-benzonitrile (12)

A mixture of 4-iodobenzonitrile (229 mg, 1 mmol), $Pd(OAc)_2$ (5 mg, 0.02 mmol) and Bu_4NOAc (452 mg, 1.5 mmol) were dried under reduced pressure for 1 h. To the mixture, under an inert atmosphere, was added dry DMF (3 mL), followed by 1-octyne (0.10 mL, 1 mmol). The solution faded from orange to dark brown over the following 5–15 min and, after 1–4 h, was complete by TLC analysis. The reaction mixture was extracted with Et_2O (4×) and the organic layers were combined, washed with water and brine to remove DMF and concentrated to a brown liquid. The material was purified by flash chromatography (0–10% EtOAc in

hexanes) to yield 209 mg (99%) of a yellow liquid. R_f (5% EtOAc in hexanes) = 0.63. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.54 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 8.6 Hz, 2H), 2.40 (t, J = 7.7 Hz, 2H), 1.58 (quintet, J = 7.3 Hz, 2H), 1.44–1.37 (m, 2H), 1.35–1.23 (m 4H), 0.88 (t, J = 6.9 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 132.15, 131.98, 129.25, 118.71, 110.83, 95.81, 95.41, 79.52, 31.40, 28.68, 28.50, 22.62, 19.60, 14.15 ppm.

5.4.20. 4-Octyl-benzonitrile (13)

Alkynyl benzonitrile **12** (191 mg, 0.904 mmol) was dissolved in 45 mL of absolute ethanol and placed in a Parr reactor. To this solution was added 90 mg (0.1 G/mmol) of 10% Pd on $BaSO_4$. The reactor was filled with H_2 and allowed to stir for 5 min before a vacuum was pulled and sustained for an additional 5 min. This cycle was repeated two more times before a pressure of 36 psi of H_2 was maintained for 50 min. At this time the vessel was disassembled and the mixture was filtered over Celite and washed with Et_2O. The organic layer was condensed to 195 mg (100%) of a light yellow oil. The oil was determined to be >97% pure by NMR and used in the following step without further purification. R_f (5% EtOAc in hexanes) = 0.69. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.54 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.1 Hz, 2H), 2.64 (t, J = 7.7 Hz, 2H), 1.60 (quintet, J = 7.3 Hz, 2H), 1.39–1.12 (m 10H), 0.86 (t, J = 6.5 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 148.67, 132.12, 129.26, 119.24, 109.50, 36.17, 31.92, 31.07, 29.46, 29.28, 22.73, 14.18 ppm.

5.4.21. *N*-Hydroxy-4-octyl-benzamidine (14)

The general procedure for amidoxime formation was used to convert benzonitrile **13** (195 mg, 0.904 mmol) in 95% EtOH (1.4 mL) to the desired product with triethylamine (0.29 mL, 2.079 mmol) and hydroxylamine hydrochloride (138 mg, 1.989 mmol) The remaining precipitate was dried to 187 mg (74%) of an off-white solid. R_f (5% MeOH in $CHCl_3$) = 0.59. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 8.29 (br s, 1H), 7.52 (d, J = 7.7 Hz, 2H), 7.18 (d, J = 7.7 Hz, 2H), 5.22 (br s, 1H), 2.59 (t, J = 7.5 Hz, 2H), 1.68–1.53 (m, 2H), 1.32–1.21 (m, 10H), 0.87 (t, J = 6.4 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 175.87, 145.77, 128.91, 126.15, 95.49, 46.00, 35.96, 32.06, 31.47, 29.63, 29.44, 22.5, 14.31 ppm. MS (ESI+) m/z 349 $[M+H]^+$.

5.4.22. (*R,S*)-2,2,4-Trimethyl-oxazolidine-3,4-dicarboxylic acid 3-tert-butyl ester (15)

D,L-α-Me-Ser-OH (0.5 G, 4.2 mmol) was dissolved in a 10% solution of Na_2CO_3 (11.5 mL). To this solution was added Boc_2O (2.748 G, 12.6 mmol) in dioxanes (8.4 mL) and the mixture was allowed to stir for 36 h. When completed the reaction mixture was washed with Et_2O (3 × 25 mL) and was acidified with 10% HCl before being extracted with EtOAc (5 × 25 mL). The EtOAc layers were combined, dried (Na_2SO_4) and concentrated to an amorphous white solid.

The white solid, following 3 h of drying, but without further purification, was dissolved in dry acetone (10.5 mL)and 2,2-dimethoxypropane (6.7 mL). To this solution was added $BF_3{\cdot}OEt_2$ (0.03 mL, 0.21 mmol). The reaction was stirred for 3 h at room temperature and concentrated to a brown solid. The solid was dissolved in EtOAc (25 mL), washed with H_2O (3 × 15 mL), dried (Na_2SO_4), and concentrated to 1.140 G (>95%, two steps) of off-white solid. R_f (10% MeOH/$CHCl_3$) = 0.65. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 11.46 (br s, 1H), 4.14–4.04 (m, 1H), 3.78–3.70 (m, 1H), 1.57–1.43 (9H), 1.40–1.31 (m, 9H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): major rotamer = 177.87, 150.92, 96.46, 80.89, 73.78, 65.26, 28.30, 26.24, 23.31, 21.68; and minor rotamer = 177.31, 151.80, 95.66, 81.08, 73.30, 65.72, 28.38, 26.86, 24.77, 20.55 ppm.

5.4.23. (S)-2,2,4-Trimethyl-oxazolidine-3,4-dicarboxylic acid 3-*tert*-butyl ester (S1)

The procedures for **15** were carried out with N-Boc-α-Me-L-Ser-OH (150 mg, 0.684 mmol) in dry acetone (1.71 mL) and 2,2-dimethoxypropane (1.44 mL, 8.895 mmol) with $BF_3{\cdot}OEt_2$ (0.01 mL, 0.023 mmol). The title compound was isolated as previously described to give 161 mg (91%) of a glassy yellow solid. R_f, ^{1}H NMR, and ^{13}C NMR were consistent with racemic **15**.

5.4.24. Compound 16

To a solution of amino acid **15** (174 mg, 0.672 mmol) stirring in dry CH_2Cl_2 (17 mL) was added PyBOP (350 mg, 0.672 mmol) and i-Pr_2NEt (0.12 mL, 0.672 mm), followed by amidoxime **14** (167 mg, 0.672 mmol). The reaction was stirred at room temperature for 12–16 h. The mixture was diluted with Et_2O (15 mL) and washed with saturated aqueous NH_4Cl (2 × 5 mL) and brine (2 × 5 mL), then concentrated and purified by column chromatography (100 mL SiO_2, 1:3 EtOAc/hexanes) to give 289 mg (88%) of an off-white solid. R_f (1:3 EtOAc/hexanes) = 0.44. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): major rotamer = 7.56 (d, J = 7.7 Hz, 2H), 7.14 (d, J = 7.7 Hz, 2H), 5.68 (br s, 1H), 5.23 (s, 1H), 4.26 (d, J = 8.8 Hz, 1H), 3.79 (d, J = 8.0 Hz, 1H), 2.56 (t, J = 7.7 Hz, 2H), 1.66 (s, 3H), 1.60 (d, J = 11.5 Hz, 6H), 1.45 (s, 9H), 1.28–1.18 (m, 10H), 0.83 (t, J = 6.4 Hz, 3H); and minor rotamer = 7.56 (d, J = 7.7 Hz, 2H), 7.14 (d, J = 7.7 Hz, 2H), 5.68 (br s, 1H), 5.23 (s, 1H), 4.16 (d, J = 8.8 Hz, 1H), 3.82 (d, J = 8.0 Hz, 1H), 2.56 (t, J = 7.7 Hz, 2H), 1.64 (s, 3H), 1.57 (d, J = 11.5 Hz, 6H), 1.37 (s, 9H), 1.28–1.18 (m, 10H), 0.83 (t, J = 6.4 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): (two rotamers) 170.15, 168.97, 160.28, 157.18, 152.49, 151.08, 146.26, 128.66, 126.78, 96.03, 95.73, 81.15, 73.82, 73.36, 65.80, 65.31, 35.82, 31.89, 31.28, 29.47, 29.23, 28.50, 28.17, 27.04, 26.20, 24.88, 24.79, 23.66, 22.70, 22.13, 21.12, 14.46 ppm.

5.4.25. Compound S2

The above procedure was performed to condense amidoxime **14** (154 mg, 0.621 mmol) and protected amino acid **S1** (161 mg, 0.621 mmol) with PyBOP (323 mg, 0.621 mmol) and *i*-Pr_2NEt (0.11 mL, 0.621 mmol) in dry CH_2Cl_2 (16 mL). The reaction was purified by flash chromatography (100 mL SiO_2, 1:3 EtOAc/hexanes) to give 248 mg (82%) of an off-white solid. R_f, ^{1}H NMR, and ^{13}C NMR were consistent with racemic **16**.

5.4.26. (*R,S*)-2,2,4-Trimethyl-4-[3-(4-octyl-phenyl)-[1,2,4]oxadiazol-5-yl]-oxazolidine-3-carboxylic acid tert-butyl ester (17)

Condensation product **16** (289 mg, 0.590 mmol) was dissolved in dry DMF (12 mL) and heated to 110 °C for 6 h. After this time, the reaction was concentrated and purified by flash chromatography (100 mL SiO_2, 1:3 EtOAc/hexanes) to yield 198 mg (71%) of the title product as clear oil. Crude material (~10%) was isolated and saved for further purification if needed. R_f (1:3 EtOAc/hexanes) = 0.83. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): major rotamer = 7.97 (d, J = 8.5 Hz, 2H), 7.28 (d, J = 8.5 Hz, 2H), 4.24 (d, J = 8.8 Hz, 1H), 3.99 (d, J = 8.8 Hz, 1H), 2.64 (t, J = 7.7 Hz, 2H), 1.91 (s, 3H), 1.75 (s, 3H), 1.70 (s, 3H), 1.49–1.43 (m, 2H), 1.36–1.23 (m, 10H), 1.21 (s, 9H), 0.86 (t, J = 6.7 Hz, 3H); and minor rotamer = 7.95 (d, J = 8.5 Hz, 2H), 7.25 (d, J = 8.5 Hz, 2H), 4.19 (d, J = 8.8 Hz, 1H), 3.97 (d, J = 8.8 Hz, 1H), 2.62 (t, J = 7.7 Hz, 2H), 1.96 (s, 3H), 1.74 (s, 3H), 1.63 (s, 3H), 1.49–1.43 (m, 2H), 1.36–1.23 (m, 10H), 1.25 (s, 9H), 0.86 (t, J = 6.7 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): (two rotamers) 180.79, 168.73, 150.85, 14683, 146.48, 129.17, 128.99, 127.66, 127.59, 124.22, 96.87, 96.07, 95.54, 81.28, 81.02, 74.88, 74.88, 74.52, 61.03, 36.21, 32.09, 31.48, 29.67, 29.47, 28.56, 28.26, 27.17, 26.59, 25.38, 23.90, 23.02, 22.89, 21.99, 14.34 ppm.

5.4.27. (*S*)-2,2,4-Trimethyl-4-[3-(4-octyl-phenyl)-[1,2,4]oxadiazol-5-yl]-oxazolidine-3-carboxylic acid *tert*-butyl ester S3)

Condensation product **S2** (248 mg, 0.506 mmol) was dissolved in dry DMF (10 mL) and heated to 110 °C for 24 h. After this time, the reaction was concentrated to a crude yellow oil and purified by flash chromatography (100 mL SiO_2, 1:3 EtOAc/hexanes) to yield 198 mg (71%) of the title product as clear oil. R_f, ^1H NMR, and ^{13}C NMR were consistent with racemic **17**.

5.4.28. (*2R,S*)-2-Amino-2-[3-(4-octyl-phenyl)-[1,2,4]oxadiazol-5-yl]-propan-1-ol (VPC45064)

General acid deprotection was performed on oxadiazole **17** (76 mg, 0.161 mmol) in a 1:1 solution of TFA/CH_2Cl_2 (4 mL). Standard basic workup yielded the crude product, which was purified by flash chromatography (10% MeOH in $CHCl_3$) to 48 mg (90%) of clear liquid. R_f (10% MeOH in $CHCl_3$) = 0.50. ^1H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.98 (d, J = 8.1 Hz, 2H), 7.27 (d, J = 8.1 Hz, 2H), 3.98 (d, J = 11.2 Hz, 1H), 3.71 (d, J = 11.2 Hz, 1H), 2.70–2.58 (m, 4H), 1.63 (quintet, J = 6.9 Hz, 2H), 1.54 (s, 3H), 1.38–1.23 (m, 10H), 0.88 (t, J = 6.5 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 183.42, 173.11, 168.25, 146.89, 129.13, 127.60, 92.19, 69.54, 55.1, 36.15, 32.04, 31.40, 29.61, 29.45, 23.81, 22.85, 14.28 ppm. MS (ESI+) m/z 332 $[M+H]^+$.

5.4.29. (*2S*)-2-Amino-2-[3-(4-octyl-phenyl)-[1,2,4]oxadiazol-5-yl]-propan-1-ol (VPC45080)

General acid deprotection was performed on oxadiazole **S3** (192 mg, 0.407 mmol) in a 1:1 solution of TFA/CH_2Cl_2 (8 mL). Standard basic workup yielded the crude product, which was purified by flash chromatography (10% MeOH in $CHCl_3$) to 115 mg (85%) of clear liquid. R_f, ^1H NMR, ^{13}C NMR, and MS were consistent with racemic **VPC45064**.

5.4.30. (*2R,S*)-Phosphoric acid mono-{2-amino-2-[3-(4-octyl-phenyl)-[1,2,4]oxadiazol-5-yl]-propyl} ester (VPC45070)

Oxadiazole **17** (67 mg, 0.142 mmol) was stirred in a solution containing TFA (2 mL) and CH_2Cl_2 (2 mL). After 3 h the reaction mixture was evaporated to dryness and re-dissolved in 10% Na_2CO_3 (aq) (0.28 mL). To the reaction was added Boc_2O (62 mg, 0.284 mmol) in dioxanes (0.28 mL). The reaction mixture was concentrated, dissolved in EtOAc, washed with brine and purified by flash chromatography (50 mL SiO_2, 1:3 EtOAc/hexanes) to yield 41 mg (67%) of clear oil. R_f (1:3 EtOAc/hexanes) = 0.21. ^1H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.98 (d, J = 8.1 Hz, 2H), 7.28 (d, J = 8.1 Hz, 2H), 5.55 (s, 1H), 4.12 (d, J = 11.5 Hz, 1H), 3.90 (d, J = 11.5 Hz, 1H), 3.40 (br s, 1H), 2.65 (t, J = 7.7 Hz, 2H), 1.74 (s, 3H), 1.63 (quintet, J = 6.8 Hz, 2H), 1.40 (s, 9H), 1.34–1.22 (10H), 0.87 (t, J = 6.7 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 146.89, 129.09, 127.64, 123.94, 95.50, 68.04, 52.17, 36.17, 32.05, 31.43, 29.63, 29.44, 28.38, 22.87, 22.30, 14.32 ppm.

To the resulting alcohol (41 mg, 0.095 mmol) was added a 3% solution of 1H-tetrazole (0.56 mL, 0.190 mmol) in acetonitrile. The mixture was diluted with the smallest amount of CH_2Cl_2 necessary to achieve homogeneity. The reaction was stirred for 15–60 min at ambient temperatures, then *N,N*-di-*iso*-propyl-di-*tert*-butyl-phosphoramidite (0.06 mL, 0.190 mmol) was added. As this solution stirred overnight, di-*iso*-propylamine precipitated out as a white solid. When a sufficient amount of starting material was consumed (monitored by TLC), a 30% solution of hydrogen peroxide (0.05 mL, 0.380 mmol) was added. The mixture was stirred for 4 h, during which time it quickly became homogenous. The organic solution was washed with saturated $NaHCO_3$, dried with Na_2SO_4, filtered and concentrated in vacuo. The mixture was separated by column chromatography (50 mL SiO_2, 1:3 EtOAc/hexanes) to yield 34 mg (57%) of the desired compound as clear oil. R_f (1:3 EtOAc/hexanes) = 0.30. ^1H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.96 (d, J = 6.7 Hz, 2H), 7.26 (d, J = 6.7 Hz, 2H), 5.97 (br s, 1H), 4.32 (m, 2H), 2.64 (t, J = 7.5 Hz, 2H), 1.84 (s, 3H), 1.69–1.57 (m, 2H), 1.48 (s, 9H), 1.45 (s, 9H), 1.40 (s, 9H), 1.33–1.24 (m, 10H), 0.87 (t, J = 5.9 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 168.53, 158.86, 146.66, 129.03, 127.65, 124.25, 121.65, 83.42, 70.01, 56.05, 36.17, 32.07, 31.42, 30.03, 29.95, 29.65, 29.47, 29.44, 28.45, 22.87, 14.32 ppm.

The phosphotriester (34 mg, 0.055 mmol) was finally deprotected by general acid deprotection, involving a 1:1 solution of TFA/CH_2Cl_2 (1 mL). Standard purification procedures yielded 15 mg (51%) of the desired TFA salt as a white solid. The final phosphate did not have was insoluble in common in NMR solvents therefore no spectra was recorded. MS (ESI+) m/z 412 $[M+H]^+$.

5.4.31. 4-Carbamoyl-2,2,4-trimethyl-oxazolidine-3-carboxylic acid tert-butyl ester (18)

5.4.31.1. Method A ($NH_{3(g)}$). To a solution of **15** (150 mg, 0.578 mmol) in anhydrous THF (1.2 mL) at −20 °C was added Et_3N (0.08 mL, 0.578 mmol) then iso-butyl chloroformate (IBCF) (0.08 mL, 0.578 mmol) in a dropwise manner. The reaction mixture was allowed to stir for 25–35 min at this low temperature. Following this time, the bath was replaced with a MeCN/dry ice bath, the temperature was allowed to equilibrate and $NH_{3(g)}$ was bubbled through the reaction mixture for 10 min. This mixture was then allowed to warm to room temperature and stir overnight. The reaction mixture was transferred to a larger reaction vessel with Et_2O and concentrated to a yellow solid. The solid was dissolved in H_20 (10 mL) and EtOAc (10 mL) and separated. The aqueous layer was extracted with EtOAc (3 × 10 mL). The organic layers were combined and washed with H_2O (2 × 10 mL) then brine (1 × 10 mL) before being dried (Na_2SO_4) and concentrated to 109 mg (73%) of yellow oil which could be crystallized from Et_2O/hexanes to a white solid. R_f (1:1 EtOAc/hexanes) = 0.28. ^1H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 5.16 (br s, 2H), 3.77 (d, J = 6.9 Hz, 2H), 1.64–1.46 (m, 9H), 1.42 (s, 9H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 176.18, 157.83, 81.32, 71.26, 69.36, 28.45, 28.01, 19.10 ppm.

5.4.31.2. Method B (NH_4OH). To a solution of **15** (100 mg, 0.386 mmol) in anhydrous THF (0.75 mL) at −10 °C was added iPr_2-NEt (0.07 mL, 0. 386 mmol) then IBCF (0.06 mL, 0.386 mmol) in a dropwise manner. The reaction mixture was allowed to stir for 10–15 min at this low temperature before the addition of (aq). NH_4OH (1.65 mL, 12.57 mmol). The reaction mixture was stirred vigorously at 0 °C for 3 h, then reduced to the aqueous layer and extracted with EtOAc (3 × 10 mL). The organic layers were combined and washed with H_2O (2 × 10 mL) followed by brine (1 × 10 mL), then dried (Na_2SO_4) and concentrated to 112 mg (>95%)of yellow oil. The oil became an off white solid upon extensive drying and could be crystallized (60–80%) from Et_2O/hexanes to a white solid. See Method **A** for physical data.

5.5. 4-Cyano-2,2,4-trimethyl-oxazolidine-3-carboxylic acid tert-butyl ester (19)

Primary amide **18** (95 mg, 0.368 mmol) was dissolved in dry THF (3.7 mL) and stirred at 0 °C. Et_3N (0.10 mL, 0.747 mmol) then TFAA (0.05 mL, 0.368 mmol) were added dropwise, in that order, at the reduced temperature. The mixture was allowed to warm to room temperature. TLC analysis revealed no starting material at 10 minutes and the reaction was concentrated to dryness following a total of 30 min. Thorough drying under vacuum yielded 84 mg (95%) of the desired compound. No further purification was performed as the compound was >95% pure as determined by ^1H NMR. R_f (1:1 EtOAc/hexanes) = 0.88. ^1H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 4.31 (d, J = 9.0 Hz, 1H), 3.89 (d, J = 9.0 Hz, 1H),

1.69 (s, 3H), 1.58–1.53 (m, 6H), 1.51 (s, 9H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 143.73, 119.33, 73.72, 36.89, 28.52, 26.17, 24.34 ppm. MS (ESI+) *m/z* 241 $[M+H]^+$.

5.5.1.4-(*N*-Hydroxycarbamimidoyl)-2,2,4-trimethyl-oxazolidine-3-carboxylic acid tert-butyl ester (20)

The above procedure for amidoxime formation was performed to convert nitrile **19** to the desired compound with $NH_2OH{\cdot}HCl$ (54 mg, 0.770 mmol), Et_3N (0.11 mL, 0.805 mmol) and 95% EtOH (0.55 mL). Following standard workup the compound was further purified by column chromatography (50 mL SiO_2, 5% MeOH in $CHCl_3$) to yield 81 mg (85%) of the title compound. R_f (5% MeOH in $CHCl_3$) = 0.60. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 6.45 (br s, 1H), 5.14 (br s, 1H), 4.64 (br s, 1H), 4.39–4.00 (m, 1H), 3.79–3.58 (m, 1H), 1.69–1.48 (m, 9H), 1.47–1.32 (m, 9H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 176.36, 154.73, 152.72, 81.46, 74.68, 46.05, 28.61, 25.72, 21.21 ppm.

5.5.2. 4-Iodo-benzoic acid methyl ester (21)

To a heterogeneous solution of 4-iodobenzoic acid (496 mg, 2.00 mmol) dissolved in anhydrous methanol (5 mL), at 0 °C, was added drop-wise thionyl chloride (0.51 mL, 7.00 mmol). Following addition, the solution was allowed to warm to room temperature and stir overnight (16 h). The reaction mixture was concentrated to dryness by rotary evaporation and co-evaporated with Et_2O (3 × 5 mL) to remove excess ($SOCl_2$). The crude material was recrystallized from MeOH, filtered, and the precipitate washed with cold MeOH to yield 425 mg (81%) of an off white solid. R_f (10% MeOH in CH_2Cl_2) = 0.92. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.81, (d, *J* = 8.5 Hz, 2H), 7.74 (d, *J* = 8.5 Hz, 2H), 3.91 (s, 3H) ppm.

5.5.3. 4-Oct-1-ynyl-benzoic acid methyl ester (22)

Aryl iodide **21** (425 mg, 1.622 mmol) and 1-octyne (0.16 mL, 1.622 mmol) were coupled using the above Sonogashira general procedure. Pd(II)$(OAc)_2$ (7 mg, 0.032 mmol), Bu_4NOAc (734 mg, 2.433 mmol) and dry DMF (5 mL) were used to gain the desired compound as a crude brown liquid. The material was purified by flash chromatography (150 mL SiO_2, 5% EtOAc in hexanes) to yield 328 mg (83%) of yellow oil. R_f (5% EtOAc in hexanes) = 0.55. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.92 (d, *J* = 8.5 Hz, 2H), 7.40 (d, *J* = 8.5 Hz, 2H), 3.86 (s, 3H), 2.38 (t, *J* = 7.1 Hz, 2H), 1.64–1.51 (quintet, *J* = 7.1 Hz, 2H), 1.47–1.36 (m, 2H), 1.33–1.23 (m, 4H), 0.87 (t, *J* = 6.9 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 166.69, 131.54, 129.48, 129.08, 128.89, 94.08, 80.23, 52.19, 31.47, 28.74, 28.67, 22.69, 19.61, 14.17 ppm.

5.5.4. 4-Octyl-benzoic acid methyl ester (23)

To a solution of alkyne **22** (328 mg, 1.342 mmol) dissolved in anhydrous EtOH (10 mL) was added 10% Pd/C (20% by weight). The reaction flask was repeatedly filled with H_2 (balloon) and evacuated under vacuum. Finally, after three cycles of the previous step, the mixture was allowed to stir under an H_2 atmosphere until determined complete by TLC (purging procedure was repeated following TLC analysis). The reaction mixture was filtered through Celite and washed with methanol. The filtrate was concentrated to 329 mg (99%) of the title compound, requiring no further purification. R_f (5% EtOAc in hexanes) = 0.59. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.94 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 3.87 (s, 3H), 2.63 (t, *J* = 7.7 Hz, 2H), 1.60 (quintet, *J* = 7.5 Hz, 2H), 1.35–1.20 (m, 10), 0.87 (t, *J* = 6.8 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 167.19, 148.52, 129.72, 128.46, 127.71, 51.91, 36.10, 31.96, 31.24, 29.53, 29.37, 22.78, 14.18 ppm.

5.5.5. 4-Octyl-benzoic acid (24)

NaOH (1.5 G) was stirred in H_2O until homogenous. To this solution was added a solution of **23** (329 mg, 1.325 mmol) in 95% EtOH (7.5 mL). The reaction was allowed to stir at room temperature for 2 h, at which time no starting material was observed by TLC analysis. The reaction mixture was acidified with 1 N H_2SO_4 and extracted with Et_2O (5 × 20 mL). The organic layers were combined, washed with H_2O (2 × 20 mL), and dried (Na_2SO_4). The solvent was concentrated to 308 mg (99%) of an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 11.29 (br s, 1H), 8.06 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 2H), 2.70 (t, *J* = 7.7 Hz, 2H), 1.66 (quintet, *J* = 7.5 Hz, 2H), 1.42–1.21 (m, 10), 0.91 (t, *J* = 6.8 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 172.78, 149.75, 130.49, 128.73, 127.04, 36.31, 32.07, 31.31, 29.65, 29.50, 29.45, 22.68, 14.29 ppm.

5.5.6. Compound 25

To a solution of carboxylic acid **24** (69 mg, 0.296 mmol) stirring in dry CH_2Cl_2 (7.5 mL) was added PyBOP (154 mg, 0.296 mmol) and i-Pr_2NEt (0.05 mL, 0.296 mmol), followed by amidoxime **20** (81 mg, 0.296 mmol). The reaction was stirred at room temperature for 12–16 h. The mixture was diluted with Et_2O (15 mL) and washed with saturated aqueous NH_4Cl (2 × 5 mL) and brine (2 × 5 mL), then concentrated and purified by column chromatography (75 mL SiO_2, 1:9 EtOAc/hexanes) to give 62 mg (43%) of the desired compound. R_f (1:4 EtOAc/hexanes) = 0.72. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.93 (d, *J* = 6.0 Hz, 2H), 7.24 (d, *J* = 6.0 Hz, 2H), 5.66 (br s, 1H), 5.02–4.90 (m, 1H), 3.91–3.67 (m, 1H), 2.65 (t, *J* = 7.5 Hz, 2H), 1.75 (s, 3H), 1.70–1.55 (m, 9H), 1.47 (s, 9H), 1.36–1.16 (m, 10H), 0.87 (t, *J* = 6.6 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 163.96 m 148.71, 129.67, 128.68, 127.21, 95.60, 95.44, 81.48, 36.17, 31.98, 31.30, 29.56, 29.40, 29.35, 28.53, 22.79, 14.24 ppm.

5.5.7. 2,2,4-Trimethyl-4-[5-(4-octyl-phenyl)-[1,2,4]oxadiazol-3-yl]-oxazolidine-3-carboxylic acid tert-butyl ester (26)

Condensation product **25** (62 mg, 0.127 mmol) was dissolved in dry DMF (2.5 mL) and heated to 110 °C for 16 h. After this time, the reaction was concentrated to crude yellow oil and purified by flash chromatography (50 mL SiO_2, 1:4 EtOAc/hexanes) to yield 36 mg (60%) of the title product as clear oil. R_f (1:4 EtOAc/hexanes) = 0.52. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 8.02 (d, *J* = 8.3 Hz, 2H), 7.33 (d, *J* = 8.3 Hz, 2H), 4.18 (d, *J* = 8.7 Hz, 1H), 3.94 (d, *J* = 8.7 Hz, 1H), 2.68 (t, *J* = 7.6 Hz, 2H), 1.86 (s, 3H), 1.78 (s, 2H), 1.72 (s, 3H), 1.69–1.58 (m, 2H), 1.37–1.14 (m, 10H), 1.20 (s, 9H), 0.87 (t, *J* = 6.7 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 171.66, 135.50, 129.46, 129.27, 128.34, 96.58, 92.28, 80.29, 75.00, 74.87, 60.79, 46.66, 36.35, 32.11, 31.39, 29.68, 29.52, 28.69, 28.42, 26.79, 23.85, 22.92, 22.73, 21.68, 14.35 ppm.

5.5.8. 2-Amino-2-[5-(4-octyl-phenyl)-[1,2,4]oxadiazol-3-yl]-propan-1-ol (VPC45129)

The oxazolidine, **26**, was deprotected by general acid deprotection, involving a 1:1 solution of TFA/CH_2Cl_2 (1.5 mL). Standard basic workup yielded the free amino alcohol as 30 mg (98%) of a white solid. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 9.07 (br s, 2H), 7.86 (d, *J* = 7.8 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 4.23 (dd, *J* = 35.4, 12.0 Hz, 2H), 2.60 (t, *J* = 7.7 Hz, 2H), 1.93 (s, 3H), 1.65–1.52 (m, 2H), 1.35–1.22 (m, 10H), 0.88 (t, *J* = 6.6 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 177.09, 169.99, 149.09, 129.25, 128.58, 121.06, 65.83, 58.38, 36.32, 32.09, 31.31, 29.66, 29.54, 29.48, 22.89, 20.59, 14.34 ppm. MS (ESI+) *m/z* 332 $[M+H]^+$.

5.5.9. 2-Amino-2-(5-(4-octylphenyl)-1,2,4-oxadiazol-3-yl)propyl dihydrogen phosphate (VPC46023)

The amino alcohol **VPC45129** (0.0211 mmol) was dissolved in dichloromethane (0.2 mL). To this solution was added triethylamine (0.0633 mmol) and ditertbutyldicarbonate (0.0232 mmol) at 0 °C. This solution was allowed to stir for 4 h to completion at

this time the reaction mixture was diluted with diethyl ether and washed with 10% HCl, $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ filtered through a fritted funnel and concentrated to an oil. The crude reaction mixture was then immediately dissolved in a solution of 3% tetrazole in acetonitrile (0.12 mL) and stirred vigorously. To this solution was added 0.0417 mmol of N,N-diisopropyldi*tert*butylphosphoramidite and the mixture was allowed to react for 15 h. At this time 0.0839 mmol of hydrogen peroxide were added as a 30% solution in water. The oxidation was allowed to proceed for four more hours at which time the crude organic reaction mixture was washed with $NaHCO_3$, and dried with sodium sulfate and concentrated to an oil. This mixture was then dissolved in DCM (0.2 mL) and bromotrimethylsilane (0.211 mmol) was added at ambient temperature via syringe. The solution was stirred for 4 h after which time the crude mixture was evaporated to dryness and reconstituted in 95% methanol in water (1 mL) and stirred overnight. This mixture was then evaporated to dryness followed by co-evaporation with diethylether and methanol until a paste was obtained. Final crystallization was achieved with the addition of water to yield the title phosphate (2.8 mg, 0.0068 mmol) as a fine white solid. The final product was analyzed and submitted for biological evaluation. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 8.09 (d, J = 8.2, 1H), 7.45 (d, J = 8.2, 1H), 4.48 (d, J = 4.4, 1H), 4.35 (d, J = 4.1, 1H), 2.83–2.65 (m, 1H), 1.81 (s, 1H), 1.73–1.58 (m, 1H), 1.45–1.19 (m, 2H), 0.89 (t, J = 4.9, 1H). ^{13}C NMR (75 MHz, CD3OD) δ (ppm) 130.82, 129.85, 118.61, 114.82, 69.75, 37.14, 33.04, 32.46, 30.67, 24.82, 23.78, 20.82. MS (electrospray) [M+H] = 412.

5.5.10. 2,2,4-Trimethyl-4-thiocarbamoyl-oxazolidine-3-carboxylic acid tert-butyl ester (27)

To a stirred solution of **18** (297 mg, 1.150 mmol) in THF (1.5 mL) at room temperature was added Lawesson's reagent (233 mg, 0.575 mmol) portion-wise. The reaction mixture was allowed to stir for 4 h and monitored by TLC. The reaction mixture was concentrated and purified by column chromatography (100 mL SiO_2, 1:9 EtOAc/hexanes) to yield 145 mg (46%) of the title compound. R_f (1:9 acetone/$CHCl_3$) = 0.46. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.98 (br s, 1H), 4.98–4.22 (m, 1H), 3.84 (d, J = 10.0 Hz, 1H), 1.79 (s, 3H), 1.61 (s, 3H), 1.55 (s, 3H), 1.45 (s, 9H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 155.03, 81.82, 71.22, 51.76, 28.51, 26.72, 26.54, 24.43 ppm.

5.5.11. 2,2,4-Trimethyl-4-[4-(4-octyl-phenyl)-thiazol-2-yl]-oxazolidine-3-carboxylic acid tert-butyl ester (28)

To a solution of **27** (145 mg, 0.528 mmol) in DME (5 mL) stirring at −15 °C (ethylene glycol/dry ice bath) was added $KHCO_{3(s)}$. The mixture was stirred vigorously for 15 min before the addition of α-bromoketone **1** (164 mg, 0.528 mmol). The reaction was held at −15 °C for an additional 30 min, and then warmed to rt for 30 min. The reaction was cooled to −15 °C again and a solution of Et_3N (0.59 mL, 4.228 mmol), TFAA (0.30 mL, 2.114 mmol), and DME (2.5 mL) was cannulated into the reaction vessel. The reaction was stirred and warmed overnight (16 h) to a yellow solution with white precipitate. The mixture was concentrated and reconstituted in H_2O (15 mL) and $CHCl_3$ (15 mL). The layers were separated and the aqueous layer was extracted with $CHCl_3$ (3 × 15 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated before being further purified by flash chromatography (150 mL SiO_2, 1:9 EtOAc/hexanes) to yield 100 mg (39%) of clear oil. R_f (1:9 EtOAc/hexanes) = 0.38. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.80 (d, J = 7.6 Hz, 2H), 7.35 (s, 1H), 7.22 (d, J = 7.6 Hz, 2H), 4.22 (d, J = 9.0 Hz, 1H), 4.02 (d, J = 9.0 Hz, 1H), 2.62 (t, J = 7.7 Hz, 2H), 1.95 (s, 3H), 1.81 (s, 3H), 1.72–1.56 (m, 5H), 1.36–1.16 (m, 19H), 0.88 (t, J = 6.4 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 184.57, 155.04, 151.55, 143.18, 132.30, 128.98, 126.42, 111.91, 96.50, 95.53, 80.49, 65.32, 35.95, 32.11, 31.67, 29.71, 29.52, 29.49, 28.65, 28.44, 25.71, 25.17, 24.21, 22.89 ppm.

5.5.12. 2-Amino-2-[4-(4-octyl-phenyl)-thiazol-2-yl]-propan-1-ol (VPC45214)

The thiazole **28** was deprotected under General Acidic Conditions, involving a 1:1 solution of TFA/CH_2Cl_2 (3.4 mL). The standard workup, under basic conditions, yielded the free amino alcohol as 37 mg (62%) of a white solid. R_f (1:1 acetone/$CHCl_3$) = 0.36. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.76 (d, J = 8.1 Hz, 2H), 7.34 (s, 1H), 7.22 (d, J = 8.1 Hz, 2H), 3.94 (d, J = 10.8 Hz, 1H), 3.72 (d, J = 10.8 Hz, 1H), 2.74 (s, 3H), 2.62 (t, J = 7.7 Hz, 2H), 1.67–1.58 (m, 2H), 1.56 (s, 3H), 1.37–1.22 (m, 10H), 0.88 (t, J = 6.7 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 155.36, 143.42, 135.12, 131.90, 129.05, 126.40, 112.11, 71.88, 57.89, 35.06, 32.13, 31.68, 29.72, 29.52, 26.83, 22.92, 14.37 ppm. MS (ESI+) m/z 347 $[M+H]^+$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.04.015.

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