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Synthesis and biological evaluation of γ-aminophosphonates as potent, sub-type selective sphingosine 1-phosphate receptor agonists and antagonists[§]

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Abstract

The synthesis of *N*-arylamide phosphonates and related arylether and arylamine analogues provided potent, subtype-selective agonists and antagonists of the five known sphingosine 1-phosphate (S1P) receptors ($S1P_{1-5}$). To this end, the syntheses of phosphoserine mimetics - selectively protected and optically active phosphonoserines - are described. *In vitro* binding assays showed that the implementation of phosphonates as phosphate mimetics provided compounds with similar receptor binding affinities as compared to their phosphate precursors. *Meta*-substituted arylamide phosphonates were discovered to be antagonists of the $S1P_1$ and $S1P_3$ receptors. When administered to mice, an antagonist blocked the lymphopenia evoked by a S1P receptor agonist and caused capillary leakage in both lung and kidney.

Keywords

Sphingosine 1-phosphate; VPC23019; VPC44116; FTY720; Immune-modulation

1. Introduction

Sphingosine 1-phosphate (S1P, Figure 1) receptors ($S1P_{1-5}$) are integral membrane G protein-coupled receptors that were initially referred to as endothelial differentiation gene (EDG) receptors, EDG-1, -5, -3, -6 and -8, respectively.[1] These receptors provide control over numerous aspects of cellular physiology when activated by endogenous S1P.[2,3] Particular

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[§] Initial synthesis and binding affinities of compounds **12a**,**b**, and **d** were presented: Foss, F. W. Jr.; Clemens, J. J.; Davis, M. D.; Lynch, K. R.; Macdonald, T. L. Abstracts of Papers, 228th National Meeting of the American Chemical Society, Philadelphia, PA **2004**.

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attention has been accorded to the role of the $S1P_1$ receptor in modulating the immune system since the discovery of FTY720, an agonist at the $S1P_{1,3,4,5}$ receptors (Figure 1). $S1P_1$ receptor agonists have been shown to inhibit the egress of T-lymphocytes from secondary lymphoid tissues, and thus are thought to direct effector T-cells away from sites of inflammation.[4]

FTY720 was developed from synthetic analog studies of myriocin (ISP-1).[5] Eventually discovered to be a pro-drug, FTY720 is activated *in vivo* when phosphorylated by sphingosine kinase type 2 to form FTY720-P.[6] The biological activity of FTY720 indicated that S1P receptors are valid targets for the treatment of autoimmune disorders and allograft rejection.[7] It is hoped that S1P receptor agonists can modulate immune system function without the toxic liabilities attendant to existing immuno-therapeutics such as calcineurin inhibitors and corticosteroids.[7–9]

The success of FTY720 in human investigation has prompted synthetic efforts to provide both pharmacological tools to study S1P signaling and therapeutics.[10–15] We reported previously the synthesis of aryl-amide containing phosphates (Figure 1, **VPC22173** and **VPC23019)** and profiled their binding affinities at S1P receptors *in vitro*.[14,16] Among these compounds were the first $S1P_1$ receptor antagonists, *e.g.* **VPC23019**.[16] In an effort to discover compounds with increased resistance to phosphatase-catalyzed hydrolysis (the deactivation pathway of S1P analogs), the synthesis of the corresponding phosphonates is reported herein. Further, the synthesis of related aryl-amine and aryl-ether containing phosphonates is discussed.

To initiate this work, strategies were pursued for the efficient synthesis of chiral phosphonoserines **4a** and **4b**, which are non-natural amino acids used to study protein phosphorylation.[17] Previous syntheses of note include Barton and Vonder-Embse's synthesis of the fully unprotected phosphonoserine from N-Cbz-glutamic acid in 4 steps and 58% yield, involving the use of white phosphorous (P_4).[18] Perich and Johns published two syntheses, the most recent in 42% yield and seven steps, from properly protected glutamic acid and using a Barton – McCombie deoxygenation.[19] Finally, other methods employed include enzymatic chiral resolutions of racemic materials, [20–22] and the induction of chirality by chiral auxiliaries.[23–25] Methods reported in this paper lead to chiral phosphonoserines with protecting groups amenable to our synthetic approach, as well as peptide synthesis, in good yield from commercially available *L*- or *D*- serine and *R*- or *S*-glycidol.

Derived from protected *R*- and *S*- phosphonoserines **4a** and **4b**, N-aryl-amide phosphonates **12a–f** provided similar binding affinities at S1P receptors as their phosphate precursors. Synthesis of the α-fluorophosphonate **13** showed similarly potent binding compared to its corresponding phosphonate **12a**. Phosphonate analogues **12c**, **12d** (**VPC44116**) and **12f**, of our previously described $S1P_{1,3}$ antagonists,[16] proved to retain their activity as antagonists and provided pharmacological tools for *in vivo* studies. While a new class of arylether phosphonates **18a, 18b** and **19** were relatively weak partial agonists or inactive, the aryl-amine **26** retained similar activity to its amide precursor.

2. Results and Discussion

2.1 Chemistry

2.1.1 Synthesis of aryl-amide-phosphonates 12a–f and 13—The production of phosphonate analogues containing an amide linker region was envisaged through the condensation of chiral phosphonoserines (*L*-or *D*-2-(N-tert-butoxycarbonyl amine)-4-phosphonyl butyric acids) **4a** and **4b** with various substituted anilines. The initial efforts (Scheme 1) towards this protected unnatural amino acid began with the synthesis of Garner's Aldehyde, **1**, from commercially available L-serine over five steps.[26] A Horner-Wadsworth-Emmons olefination performed from one of two bis-phosphonates installed the α-methylene or α-fluorophosphonates in **2a** or **2b**, respectively.[27] The fluorinated bisphosphonate used to synthesize **2b** was derived, as previously described by Prestwich,[28] from the commercially available tetraethyl methylenebisphosphonate used to arrive at **2a.**

The resulting olefins **2a** and **2b** were reduced by hydrogenation over Pd/C to **3a** and **3b**. Selective acetonide deprotection proved to be low yielding in our initial efforts;[29] however, this led to the use of a convenient, simultaneous deprotection and oxidation with the Jones reagent in acetone.[30] This un-optimized method led to the protected amino-acids **(*R*)-4a,b** in 40% and 18% yields, respectively, in 3 steps from Garner's Aldehyde.

A second avenue (Scheme 2) led to a shorter and more efficient synthesis of both **(*S*)-** and **(*R*)-4a**. For example, (*R*)-(+)-glycidol was benzyl protected,[31] and the epoxide was opened by the resultant carbanion of diethyl methylphosphonate and *n*-BuLi, in the presence of $BF_3{\cdot}OEt_2$, to yield alcohol **6**.[32] Installation of an azide at the 3-hydroxyl position proved to be the major impediment to this approach, as has been noted by others for similar substrates.[33] Mesylate formation followed by azide substitution suffered from a tendency for elimination over a range of temperatures. Therefore, a milder method of azide formation was pursued.

Diphenylphosphoryl azide (DPPA) under Mitsunobu conditions was successful in assembling the desired azides, **7**, despite reported difficulties arising from congested secondary alcohols.[34] After extensive isolation efforts, near quantitative yields were found via this procedure. Purification issues led to the investigation of more convenient Mitsunobu reagents to preclude the difficult separation of polar products from the complex reaction mixture (polar phosphonate **7** was nearly inseparable from both triphenyl- and tributylphosphane oxides). The use of commercially available polymer-bound triphenylphosphine yielded equivalent conversions to azide **7**. With the use of three equivalents of phosphine, the desired transformation was completed in fewer than 20 hours. The inclusion of a polymer-bound phosphine reagent allowed for standard purification of the crude reaction mixture, after filtration of the resultant phosphane oxide.

A two-step reduction of **7**, first in the presence of H_2, Boc_2O and Lindlar's catalyst,[35] followed by H_2, Pd/C, yielded the protected amino alcohol **9** in 72% over two steps. Oxidation of the primary alcohol was performed by means of $RuCl_{3(cat.)}/NaIO_4$ conditions[36] (76%) to yield compound **(*R*)-4a** in 40% yield over 6 steps from optically active glycidol. **(*R*)-** and **(*S*)-4a** were further derived to the corresponding diastereomers, **(*R*)-** and **(*S*)-4a-Phe-OMe**, through a PyBOP mediated condensation with *L*-phenylalanine methyl ester.[38] This was done to ensure high enantiomeric excess of the desired carboxylic acids. These condensation reactions were high yielding and arrived at individual stereoisomers as determined by NMR.

Compounds **12a-f** and **13** (Scheme 3) were synthesized by PyBOP initiated condensations[37] of **(*R*)-** or **(*S*)-4a** or **(*R*)-4b** with various aniline compounds **10a–d**. Aqueous soluble carbodiimide (EDC methiodide) was also investigated as a condensation reagent; however, this method generally produced lower yields. The alkane hydrocarbons of unavailable alkylanilines were installed through Sonogashira couplings with 3-iodo-nitrobenzene,[38] followed by concomitant reduction of the nitro group and resulting triple bond. Pd couplings were also successfully performed following the amide formation with *m*- or *p*- iodoaniline to complete the desired phosphonates in a linear fashion. The compounds **11a–f** were deprotected with bromotrimethylsilane followed by hydrolysis of the ensuing phosphonate silyl-oxy-esters. These conditions conveniently deprotected the *N*-Boc group, as well, to yield compounds **12a–f**. α-Fluorophosphonate **13** was synthesized by the same protocol from **4b** and **10a**.

While the synthesis described from glycidol retained the reported efficiency up to half-gram scale, increasing material to greater than one gram proved detrimental to the formation of azide **7**. Due to the demand for greater quantities of compounds **13b** and **13d**, further optimization of our synthesis from serine was undertaken (Scheme 4). Acetonide protected vinyl phosphonate **2** was converted to phosphonoserine **4a** in two convenient steps. Compound **2**, as displayed in Scheme 1, undergoes reduction within four to six hours under an atmosphere of H_2 and in the presence of 10% Pd/C ($20^{w}/_{w}$ %). It was observed that a trace amount of the alcohol **9a** was present at this time. The reaction was allowed to stir for one day at room temperature, and it was estimated by TLC that nearly half of the acetonide was hydrolyzed. Following further investigation, the use of an additional half equivalent of 10% Pd/C ($10^{w}/_{w}$%), added after one day of vigorous stirring, converted the remaining material to the reduced alcohol **9a** in less than 72 hours.[39] This selective acetonide deprotection proved to be effective with greater than five grams of material and yielded more efficient conversions (>95%) than 1.5 equivalents of *p*-toluenesulfonic acid in ethanol (65–75 %). The alcohol was then converted to **4a** with $RuCl_3/NaIO_4$ conditions, as previously described.

2.1.2 Synthesis of aryl ether phosphonates 18a, 18b, and 19—
Using glycidol as an alternative starting material to serine led conveniently to phenolic ether compounds (Scheme 5). The synthesis began with a Mitsunobu condensation between *p*-octylphenol and glycidol. Chiral epoxides **14** were then opened, as previously described, to alcohols **15**. For these substrates, DPPA/Mitsunobu conditions yielded products with sufficiently disparate polarities from tributylphosphane oxide. The azides, **16**, were reduced and deprotected as described above to give enantiomers **18a** and **18b**. Compound **15a** was deprotected to form **19** to confirm the overall effects of an amine at the 3-position of **18b**.

2.1.3 Synthesis of aryl amine phosphonate 26—
Secondary amine **26** was synthesized to view the effects of reducing the amide bond in **12d** (Scheme 6) while retaining a functional group capable of donating a hydrogen bond. After activation of *p*-octylaniline by mono-tosyl protection, the amine was condensed under Mitsunobu conditions to form epoxide **21**. Consecutive nucleophilic ring opening, azide formation with DPPA/Mitsunobu conditions, and reduction yielded amine **24**. Deprotection of the N-tosyl group proved difficult. Sluggish reaction times and low yields were discovered with the planned Mg/MeOH deprotection,[41] and dissolving metal conditions were used. Solid sodium in $NH_{3(l)}$ unmasked the desired secondary amine in an un-optimized 26% yield. Compound **25** was deprotected with TMSBr to yield the ammonium bromide salt **26** .

2.2 Biological Evaluation

2.2.1 [γ^{35}S]-GTP Binding Assay—
γ^{35}S-GTP dependant receptor binding activity (Table 1) was determined *in vitro* for S1P, FTY720-P, and all final compounds, as previously communicated.[16] Briefly, the expression of individual human S1P receptors and individual G protein subunits was forced in HEK293T cells. The membrane bound G protein α subunits yielded data by binding the labeled, non-hydrolyzable [γ^{35}S]-GTP when activated by an extracellular ligand. Following our discovery of the $S1P_{1,3}$ antagonist **VPC23019 (B)**, the meta-substituted analogues were analyzed for their ability to antagonize S1P’s endogenous activity in the [γ^{35}S]-GTP assays. The effects on S1P’s endogenous binding constant were determined as previously discussed.[16]

Para substituted phosphonates **12a, 12b (VPC44152), 12e**, **18a, 18b**, and **19** showed various activities as agonists. Phosphonate **12b (VPC44152)** was twice as potent as corresponding phosphate **A (VPC22173)** at $S1P_1$ and $S1P_3$, while less potent at $S1P_4$ and $S1P_5$. Phosphonate **12a** gained activity across all receptors with comparison to **A** and displayed similar potency to FTY720-P and S1P at $S1P_1$. The replacement of the amide linkage with an ether resulted in the loss of activity, at $S1P_1$ and $S1P_3$, for **18a** compared to **12b**, implicating the importance of available hydrogen-bond donation alpha to the phenyl ring. Interestingly, epimer **18b** was considerably less potent than **18a** at $S1P_1$ but displayed modest activity at all five S1P receptors.

γ-Hydroxyphosphonate **19** was less potent than **18a** at $S1P_1$ and functionally inactive at $S1P_{2–5}$, which is consistent with the two point binding model for S1P receptor interaction.[42] Compared with our previously described phosphate agonist **A**, phosphonates **12a**, **12b (VPC44152)**, and **12e** retained similar potency and efficacy. Meta-substituted compounds **12c**, **12d (VPC44116)**, and **12f** showed no agonist activity at $S1P_1$ and $S1P_3$ receptors; rather, meta substituted compounds displayed antagonists activity against S1P binding to the $S1P_1$ and $S1P_3$ receptors.

To characterize these compounds, Schild regressions were performed as described in earlier work.[16] These experiments revealed arylamides **12d** and **12f** as potent antagonists at the $S1P_1$ and $S1P_3$ receptors. Arylamine **26** displayed antagonist activity at both receptors with a preference for $S1P_3$. The most promising antagonist, **12d** (**VPC44116**), was compared with its phosphate precursor **VPC23019. VPC23019** and **VPC44116** were nearly indistinguishable in their affinity for the $S1P_1$ and $S1P_3$ receptors (K_i values of about 30nM and 300nM, respectively). This was described in more detail by radioligand displacement experiments, described previously,[16] revealing IC_{50}s for the phosphate and phosphonate to be 31 nM and 72 nM, respectively (not shown).

We have demonstrated previously that **VPC44116** opposes the protective effect of FTY720 in a mouse model of acute renal injury.[43] To characterize this compound further, we injected mice with doses up to 45 mg/kg body weight and measured blood lymphocytes. Lymphopenia (a decrease in circulating lymphocytes below the normal range) is a convenient index of $S1P_1$ receptor agonist action. The quintessential S1P agonist, FTY720, has been proposed to operate as a functional antagonist through receptor desensitization mechanisms[44] – a hypothesis that suggests a direct receptor antagonist would behave likewise. Nevertheless, no significant change in circulating lymphocyte numbers was observed at any dose of **VPC44116** tested (not shown). However, **12d** (**VPC44116** (*meta*)) blocked the lymphopenia evoked by its positional isomer, the agonist **12b** (**VPC44152** (*para*)) (Figure 2). A S1P receptor antagonist similar to **VPC44116** but containing a hexyl (vs. octyl in **VPC44116**) group caused vascular leakage in lung when administered to mice.[45] To learn whether **VPC44116** behaved similarly, we injected **VPC44116** into mice followed by Evans blue dye as described previously.[43] After sacrifice, we found extravasation of the dye into lung and kidney, but not into heart, liver, testes or intestine (Figure 3).

3. Conclusion

The synthetic methods described provide entry to multiple oxidation states and/or orthogonal protecting groups of phosphonoserines, including an isoelectric α-fluorophosphonate. This is accomplished from two convenient materials of the current chiral pool, *L*- or *D*- serine (eight steps and 39% yield) and *R*- or *S*- glycidol (six steps and 40% yield). Optically active agonists and antagonists of the S1P receptors are described. Replacing the phosphorous-oxygen bond with a phosphorous-carbon bond provides bioactive agents with putative resistance to degradative phosphatase activity. The phosphonate-containing antagonist, **12d** (**VPC44116**), opposed the lymphopenia evoked by its agonist isomer, **12b** (**VPC44152**), but did not affect numbers of circulating lymphocytes when injected alone. However, injection of **VPC44116** alone caused capillary leakage in lung and kidney.

4. Experimental

4.1. General Experimental

All reactions were performed under an inert atmosphere using flame dried glassware. Reaction solvents methylene chloride, diethyl ether, tetrahydrofuran, and toluene were obtained from OptiDry canisters (<50 ppm H_2O, Fisher Scientific) and passed through an activated alumina (activity I) column, directly into the reaction flask when possible. Dimethylformamide was obtained from an OptiDry canister without further drying prior to use. All other solvents were used as obtained. All commercially available reagents were purchased from either Aldrich (Milwaukee, WI), Sigma (St. Louis, MO), Acros (Pittsburg, PA), or Advanced Chem Tech (Louisville, KY) and used as obtained unless otherwise stated. The reactions were monitored by analytical thin layered chromatography using Merck silica gel F-254 pre-coated aluminum-backed plates. R_f values refer to column chromatography eluent, unless otherwise noted. When not reported, R_f value ≈ 0.00. Silicycle Ultra Pure Silica Gel (230–400 mesh) or Fisher Scientific Silica Gel 60 Sorbent (230–400 mesh) was used for all normal phase chromatography. All yields refer to chromatographically and spectroscopically pure compounds, unless otherwise indicated.

Optical rotations were measured on a Perkin-Elmer model 343 polarimeter with a sodium lamp at 23 ± 2 °C in the stated solvent; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. ^1H and ^{13}C NMR spectra were recorded on UnityInova 300 (75) and 500 (125) MHz spectrometers (Varian). Chemical shifts are reported in δ (ppm) units using ^1H (residual) and ^{13}C signals from $CDCl_3$ as an internal standard (7.26 and 77.23 ppm, respectively) unless otherwise specified. Elemental analysis was performed by Atlantic Microlab, Inc. (Norcross, GA) for C, H, and N. Elemental analyses were run in duplicate after thorough drying, before submission and when obtained by the vendor. Low Resolution Electrospray Ionization (ESI) was performed at the University of Virginia Mass Spectometry Laboratory. High-Resolution Mass Spectrometry (HRMS) was performed at the Mass Spectrometry Laboratory at University of Illinois Urbana-Champaign (Micromass Q-T of Ultima).

4.1.1. 4-formyl-2,2-dimethyloxazolidine-3-carboxylic acid *tert*-butyl ester (1)—*L*-serine (5.00 g, 0.048 mol) was dissolved in 100 mL of methanol and cooled to <0 °C (brine/ice). Thionyl chloride (20.8 mL, 0.286 mol) was added slowly by syringe. The mixture was stirred overnight and then concentrated and co-evaporated with ether multiple times to eliminate excess thionyl chloride and provide the desired methyl ester that was shown to be >95% pure by ^1H NMR. ^1H NMR (300 MHz, CD_3OD, 23 °C, δ): 4.07 (t, J = 4.0 Hz, 1H), 3.98 (d, J = 4.0 Hz, 2H), 3.83 (s, 3H) ppm.

The amino ester was reconstituted in CH_2Cl_2 (100 mL) and triethylamine (16.6 mL, 0.119 mol) was added dropwise at 0 °C. To this stirring solution was added di-*tert*-butyl dicarbonate (11.420 g, 0.052 mol) in one portion. The reaction was stirred until the starting material was consumed, as determined by TLC (1:1 EtOAc/Hex). The reaction mixture was concentrated and dissolved in EtOAc (50 mL) then washed with saturated $NaHCO_{3(aq)}$ (3×25 mL) followed by brine (3×25 mL). The organic layer was dried over $Na_2SO_{4(s)}$, filtered and concentrated. ^1H NMR (300 MHz, $CDCl_3$, 23°, δ): 5.54 (brd, 1H), 4.35 (m, 1H), 3.89 (ddd, J = 13.8, 11.2, and 3.7 Hz, 2H), 3.76 (s, 3H), 2.76 (bs, 1H), 1.43 (s, 9H) ppm.

The crude oil was dissolved in acetone (120 mL) and 2,2-dimethoxypropane (87 mL, 15 eq.). The solution was stirred at room temperature and $BF_3 \cdot OEt_2$ (1.2 mL, 9.52 mmol) was added. The reaction mixture turned a yellow-orange hue and was stirred for 2.5 hours. When the reaction was complete, by TLC analysis, the solution was treated with 99% Et_3N (1.2 mL) and the solvent was removed. The brown oil was then partitioned between diethyl ether and saturated $NaHCO_{3\,(aq)}$. The aqueous layer was extracted with diethyl ether (4×25 mL) and the organic layers were combined, dried (Na_2SO_4), and concentrated to a yellow oil (> 90% pure by ^1H NMR). $R_{f(1:1\ EtOAc/Hexanes)}$ = 0.73. ^1H NMR (300 MHz, CD_3OD, 23 °C, δ): Major rotamer = 4.37 (dd, J = 7.0, 3.1 Hz, 1H), 4.13 (dt, J = 9.23, 7.0Hz, 2H), 3.74 (S, 3H), 1.52 (s, 3H), 1.48 (s, 3H), 1.40 (s, 9H) ppm; Minor rotamer = 4.48 (dd, J = 6.6, 2.6 Hz, 1H), 4.04 (dt, J = 7.3, 2.9 Hz, 2H), 3.74 (s, 3H), 1.66 (s, 3H), 1.62 (s, 3H) 1.48 (s, 9H) ppm. ^{13}C NMR (300 MHz, CD_3OD, 23 °C, δ): Major rotamer = 151.38, 80.51, 66.45, 59.46, 52.49, 28.46, 27.60, 25.15, 24.57 ppm; Minor rotamer = 151.38, 80.51, 66.20, 59.38, 52.61, 28.54, 27.60, 26.21, 25.35 ppm.

A mixture of $NaBH_4$ (2.247 g, 59.08 mmol) and LiCl (2.505 g, 59.08 mmol) was prepared in EtOH (42 mL), at 0 °C. A solution of the purified acetonide (7.659 g, 29.54 mmol) dissolved in THF (30 mL) was then added dropwise to the reaction mixture. The mixture was warmed to room temperature and stirred for four hours. After four hours the precipitate was filtered over celite and washed with EtOH. The filtrate was then concentrated and reconstituted in H_2O (50 mL) and EtOAc (50 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3×25 mL). The organic layers were combined and washed with brine (2×25 mL), dried ($Na_2SO_{4\,(s)}$), concentrated, and the crude oil was purified by column chromatography (25–50% EtOAc/Hexanes) to give 6.101 g (89%) of the alcohol as a white solid. $Rf_{(1:1\ EtOAc/Hexanes)}$ = 0.86. ^1H NMR (300 MHz, $CDCl_3$, 35 °C, δ) = 4.07 (m, 2H), 3.75 (s, 1H), 3.68 (m, 2H), 1.52 (s, 6H), 1.49 (s, 9H) ppm.

To a stirring solution of 2.0 M oxalyl chloride, $(COCl)_2$, (11.78 mL, 23.56 mmol) at −78 °C was added drop-wise a solution of DMSO (2.1 mL, 23.56 mmol) in CH_2Cl_2 (33 mL). This solution was allowed to stir at −78 °C for 15 minutes before a solution of the primary alcohol in CH_2Cl_2 was added drop-wise. The solution was stirred for 35 minutes before Et_3N was added in a drop-wise manner at −78 °C. Following the addition of base, the reaction mixture was allowed to warm to 0 °C and the reaction was quenched with saturated $NH_4Cl_{(aq)}$. The mixture was separated and the organic layer was washed with sat. $NaHCO_{3(aq)}$ (2×25 mL) followed by brine (2×25 mL). The organic layer was dried ($MgSO_4$) and concentrated to a clear oil which was used immediately in the following reaction without further purification. $R_{f(1:3\ EtOAc/Hexanes)}$ = 0.35. ^1H NMR (300 MHz, $CDCl_3$, 23 °C, δ) = Major rotamer: 9.35 (d, 1H, *J* = 2.4 Hz), 4.02 (m, 1H), 3.88 (m, 2H), 1.43 (s, 3H), 1.34 (s, 3H), 1.22 (9H) ppm; and Minor rotamer: 9.38 (m, 1H), 4.13 (m, 1H), 3.88 (m, 2H), 1.39 (s, 3H), 1.33–1.27 (m, 12H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ) = Major rotamer: 198.84, 151.07, 94.72, 80.55, 64.53, 63.58, 27.99, 25.51, 23.54 ppm; and Minor rotamer: 199.02, 152.29, 94.05, 80.92, 64.60, 63.16, 28.03, 26.45, 24.47 ppm.

4.1.2.1 4-[2-(diethoxyphosphoryl)vinyl]-2,2-dimethyloxazolidine-3-carboxylic acid *tert*-butyl ester ((*3R*)-2a)—

To tetraethyl methylenebisphosphonate (4.28 mL, 17.278 mmol) in THF (40 mL) at −78 °C was added 2.5 M *n*-BuLi in Hexanes (6.28 mL, 15.707 mmol). After 15 minutes of stirring at low temperature, aldehyde **1** (3.601 g, 15.707 mmol) was added in THF (40 mL) and the reaction was allowed to warm to room temperature with continued stirring overnight. The reaction mixture was concentrated to 1 to 2 mLs and purified by column chromatography (500 mL SiO_2, 5% MeOH in $CHCl_3$) to yield 5.440 g (95%, two steps) of clear oil. R_f = 0.65. ^1H NMR (300 MHz, $CDCl_3$, 23 °C, δ) = 6.63 (dt, 1H, *J* = 5.9, 17.8 Hz), 5.72 (dt, 1H, *J* = 15.8, 17.8 Hz), 4.40 (dt, 1H), 4.04 (m, 5H), 3.77 (m, 1H), 1.59 (m, 3H), 1.42 (m, 12H), 1.25 (dt, 6H, *J* = 1.5, 7.0 Hz) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 157.4, 150.3, 127.3, 119.7, 117.2, 92.6, 67.5, 62.1, 59.6 (d, *J* = 22.7 Hz), 28.5, 26.7, 25.7, and 16.6 (d, *J* = 8.1 Hz) ppm. $[\alpha]^{23}_D$ = −64.1° (c = 1.00, MeOH). HRMS (ES+) calculated *m/z* = 364.1889, experimental *m/z* = 364.1893.

4.1.2.2 4-[2-(diethoxyphosphoryl)vinyl]-2,2-dimethyloxazolidine-3-carboxylic acid *tert*-butyl ester ((*3S*)-2a)—

The above procedure for the synthesis of **(*3R*)-2a** was employed using the epimer of **1** (1.146 g, 5.00 mmol) in THF (15 mL), 2.5M *n*-BuLi in Hexanes (2.00 mL, 5.00 mmol) and tetraethyl methylenebisphosphonate (1.36 mL, 5.5 mmol) yielded 1.728 g (>95%, two steps) of clear oil. Rf, ^1H and ^{13}C NMR were consistent with data reported for **(*3R*)-2a**. $[\alpha]^{23}_D$ = +65.9° (c = 1.03, MeOH). HRMS (ES+) calculated *m/z* = 364.1889, experimental *m/z* = 364.1893.

4.1.3 4-[2-(diethoxyphosphoryl)-2-fluorovinyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (2b)—The above procedure for synthesis of **2a** was employed using **1** (229 mg, 1.00 mmol) in THF (2.5 mL), tetraethyl 2-fluoromethylene-bisphosphonate (337 mg, 1.10 mmol) in 2.5 mL of THF, and 2.5M *n*-BuLi in Hexanes (0.4 mL, 1.00 mmol). The reaction yielded 119 mg (31%) of **2b** as a clear liquid. $Rf_{(EtOAc)}$ = 0.36. ^1H NMR (300 MHz, $CDCl_3$, 23°C, δ): 5.92 (dt, J = 37.6, 7.9 Hz, 1H), 3.71 (m, 1H), 4.10 (m, 5H), 3.72 (m, 1H), 1.41 (m, 6H), 1.39 (m, 9H), 1.29 (m, 6H) ppm.

4.1.4. General Procedure I: Hydrogenation /or Hydrogenolysis (3a) To a solution of phosphonate **2a** (845 mg, 2.33 mmol) dissolved in 25 mL of anhydrous EtOH was added 10% Pd/C (20% $^w/_w$). The reaction flask was repeatedly filled with H_2 (balloon) and evacuated. Following three to five repetitions, the reaction was allowed to stir under an H_2 atmosphere for 4 h. The mixture was filtered over celite and washed with EtOH. The filtrate, which required no further purification, was concentrated to 844 mg (99%) of clear oil. $Rf_{(EtOAc)}$ = 0.29. ^1H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 4.09 (dq, *J* = 3.3, 7.0 Hz, 4H), 3.94 (m, 1H), 3.68 (m, 2H), 1.80 (m, 4H), 1.59 (s, 3H) 1.54 (s, 3H), and 1.32 (t, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 156.4, 92.5, 89.8, 65.0, 61.9 (d, *J* = 6.6Hz), 31.2, 28.6, 23.4 (d, *J* = 20.1Hz), 21.6 (d, *J* = 20.1 Hz), 16.7 (d, *J* = 6.0Hz) ppm.

4.1.5. 4-[2-(diethoxyphosphoryl)-2-fluoroethyl]-2,2-dimethyloxazolidine-3-carboxylic acid *tert*-butyl ester (3b)—General Procedure I was performed on **2b** (141 mg, 0.37 mmol) to give 125 mg (88%) of **3b** as a clear oil. ^1H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 5.24 (d, *J* = 14.3 Hz, 1H), 4.76 (m, 1H), 4.14 (m, 4H), 4.03 (m, 1H), 3.84 (dd, *J* = 8.1 Hz, 2H), 3.60 (m, 1H), 2.17 (m, 2H), 1.50 (m, 3H), 1.40 (m, 12H), 1.28 (m, 6H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 1.52.96, 68.24, 66.42, 63.34, 55.68, 28.44, 23.20, 16.55 ppm.

4.1.6. 2-*tert*-butoxycarbonylamino-4-(diethoxyphosphoryl)butyric acid ((*2R*)-4a)—In a stirring solution of **3a** (844 mg, 2.31 mmol) and 5 mL of acetone, at 0 °C was added Jones' reagent (1.73 mL, 4.62 mmol). The reaction mixture was allowed to warm to room temperature and the stirring was continued for 12 hours. After this time the mixture was transferred to a larger flask then celite and isopropyl alcohol were added. The mixture was stirred for 15 minutes and the precipitate was filtered, washed with acetone, and made alkaline by the addition of sat. $NaHCO_{3\ (aq)}$. The solution was concentrated to remove organic solvents and washed with EtOAc (3 x 25 mL). The aqueous layer was acidified to a pH ~3 by the addition of solid citric acid and extracted with CH_2Cl_2 (5 x 25 mL). The combined extracts were washed with brine (3 x 15 mL) and dried over solid $MgSO_4$. The solvent was concentrated to 460 mg (59%) of a white solid, which could be recrystallized from Et_2O/Hexanes. ^1H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 10.41 (brs, 1H), 5.38 (d, *J* = 7.5 Hz, 1H), 4.32 (m, 1H), 4.10 (t, J = 7.3 Hz, 4H), 1.95 (m, 4H), 1.43 (s, 9H), 1.31 (dt, J = 7.0, 1.5 Hz, 6H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23°C, δ): 190.58, 157.35, 62.47, 53.57, 28.49, 25.81, 22.44, 16.50 ppm.

4.1.7. 2-tert-butoxycarbonylamino-4-(diethoxyphosphoryl)-4-fluorobutyric acid ((*2R*)-4b)—Similar procedures for the synthesis of **(*2R*)-4a** were followed using **3b** (125 mg, 0.326 mmol), 1 mL of acetone, and Jones' reagent (0.25 mL, 0.625 mmol) to yield 40 mg (48%) of **(*2R*)-4b** as a white solid. ^1H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 5.90 (m, 1H), 5.50 (m, 2H), 5.00 (dt, *J* = 46.4, 8.8 Hz, 1H), 4.31 (m, 1H), 4.17 (m, 4H), 2.38 (m, 2H), 1.41 (s, 9H), 1.32 (dt, *J* = 7.0, 2.2 Hz, 6H) ppm.

4.1.8.1 2-benzyloxymethyloxirane ((*2S*)-5)—To a stirring mixture of BnBr (0.72 mL, 6 mmol), 60% NaH (193 mg, 4.8 mmol) suspended in mineral oil, and 5 mL of DMF was added *R*-glycidol (0.26 mL, 4 mmol) dissolved in 3 mL of DMF, dropwise and at 0 °C. The solution was added via syringe over 30 minutes at which time the reaction was allowed to stir for an additional 3 hours while warming to room temperature. The crude material was diluted with 50 mL of EtOAc and washed with NH_4Cl (3×50 mL), $NaHCO_3$ (3×50 mL), LiBr (3×25 mL) and brine (3×50 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to clear oil. The crude oil was purified by flash chromatography (~200 mL SiO_2, 1:4 EtOAc/Hexanes) to yield 515 mg (78%) of clear oil. $R_{f(1:4\ EtOAc/Hex.)}$ = 0.31. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.31 (m, 5H), 4.56 (dd, J = 18.7, 12.1 Hz, 2H), 3.74 (dd, J = 11.4, 2.9 Hz, 1H), 3.39 (dd, J = 11.4, 5.9 Hz, 1H), 3.14 (dq, J = 2.9, 0.9 Hz, 1H), 2.73 (t, J = 4.6 Hz, 1H), 2.57 (dd, J = 5.1, 2.6 Hz, 1H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 137.83, 128.21, 128.19, 127.49, 72.96, 70.65, 50.58, 43.85 ppm.

4.1.8.2 2-benzyloxymethyloxirane ((*2R*)-5)—The above procedures for the formation of **(*2S*)-5** were used to form 1.232 g (75%) of the title compound from *S*-glycidol (0.66 mL, 10.0 mmol). $Rf_{(1:4\ EtOAc/Hex.)}$ = 0.31. ^{1}H and ^{13}C NMR data were consistent with **(*2S*)-5**.

4.1.9.1 General Procedure II: Nucleophilic Epoxide Opening ((*3S*)-6) To a solution of diethyl methylphosphonate (2.04 mL, 14.1 mmol) dissolved in THF (14 mL), stirring at −78 °C, was added 2.5M *n*-BuLi (5.65 mL, 14.1 mmol) dropwise. The mixture was stirred for 15 minutes at −78 °C and **(*3S*)-5** (773 mg, 4.71 mmol) dissolved in THF (2.4 mL) followed by $BF_3·OEt_2$ (2.32 mL, 18.8 mmol) were added dropwise at −78 °C. Stirring was continued at −78 °C for two additional hours before the reaction was quenched by the dropwise addition of $NH_4Cl_{(aq)}$ (~2 mL). The mixture was allowed to warm to room temperature overnight, concentrated to a yellow oil, and purified by column chromatography (~300 mL of SiO_2, 1:1 acetone/chloroform) to give 1.424 g (96%) of a faintly yellowed liquid. $Rf_{(1:1\ acetone/chloroform)}$ = 0.39. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.34 (m, 5H), 4.55 (s, 2H), 4.10 (m, 4H), 3.85 (m, 1H), 3.49 (dd, *J* = 9.5, 3.3 Hz, 1H), 3.38 (dd, *J* = 9.7, 7.3 Hz, 1H), 1.85 (m, 4H), 1.31 (t, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 137.74, 127.81, 127.12, 127.07, 127.00, 73.63, 72.69, 69.20 (d, J = 16.6 Hz), 60.99 (d, J = 6.6 Hz), 26.12 (d, J = 4.5 Hz), 22.03, 20.15, 15.93 (d, J = 6.0 Hz) ppm. $[\alpha]^{23}_D$ = −11.9° (c = 1.13, MeOH).

4.1.9.2 (4-benzyloxy-3-hydroxybutyl)phosphonic acid diethyl ester ((*3R*)-6)— Compound **(*2R*)-5** (1.232 g, 7.50 mmol) was converted to **(*3R*)-6** as in the above reaction to yield 1.311 g (55%) of the title compound as a faintly yellowed liquid. $Rf_{(1:1\ acetone/chloroform)}$ = 0.39. See **(*3S*)-6** for corresponding ^{1}H and ^{13}C NMR data. $[\alpha]^{23}_D$ = +10.4° (c = 1.06, MeOH).

4.1.10.1 (3-azido-4-benzyloxybutyl)phosphonic acid diethyl ester ((*3R*)-7)—To **(*3S*)-6** (0.992 g, 3.136 mmol) dissolved in CH_2Cl_2 (4 mL) at 0 °C was added polymer-bound-Ph_3P (3.1 g, ~9.3 mmol), followed by DPPA (0.74 mL, 3.450 mmol) then DIAD (0.68 mL, 3.450 mmol) via syringe. The reaction mixture was allowed to warm to room temperature and was stirred overnight. By morning no starting material was present by TLC analysis. The reaction mixture was filtered through celite, which was rinsed with methanol, and the filtrate was concentrated to about 2 g of crude yellow oil. Further purification was acheived by column chromatography (~250 mL of SiO_2, 1:9 acetone/chloroform) to yield 952 mg (96%) of clear oil. $Rf_{(1:9\ acetone/chloroform)}$ = 0.31. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C,δ): 7.34 (m, 5H), 4.56 (s, 2H), 4.09 (m, 4H), 3.56 (m, 3H), 1.80 (m, 4H), 1.32 (dt, *J* = 7.0, 1.1 Hz, 6H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 137.78, 128.62, 128.51, 127.98, 127.76, 73.54, 72.58, 61.87, 24.37, 23.33, 21.44, 16.61 (d, J = 6.1 Hz) ppm.

4.1.10.2 (3-azido-4-benzyloxybutyl)phosphonic acid diethyl ester ((*3S*)-7)— Compound **(*3R*)-6** (741 mg, 2.342 mmol) was converted to **(*3S*)-7** as the above reaction to yield 585 mg (73% isolated yield) of the title compound. Rf$_{(1:9 \text{ acetone/chloroform})}$ = 0.31. See **(*3S*)-7** for corresponding ^{1}H and ^{13}C NMR data.

4.1.11.1 (4-benzyloxy-3-*tert*-butoxycarbonylaminobutyl)phosphonic acid diethyl ester ((*3R*)-8)—

Compound **(*3R*)-7** (1.412 g, 4.137 mmol) was stirred in 32 mL of methanol and Boc$_2$O (0.993 g, 4.551 mmol) followed by Lindlar's catalyst (285 mg, 20% by weight) were added. The reaction mixture was stirred vigorously and a balloon of H$_{2(g)}$ was affixed. The apparatus was purged numerous times by cycling between vacuum (5×5 min) and H$_{2(g)}$. Finally H$_2$ atmosphere was applied and maintained for 24h at room temperature. At this time no starting material was visible by TLC analysis and the reaction mixture was filtered through Celite 545 with the aid of methanol. The solvent was condensed and the resultant clear oil was purified by column chromatography (~150mL of SiO$_2$, 1:9 acetone/chloroform) to yield 1.324 g (77%) of clear oil. Rf$_{(5\% \text{ MeOH in CHCl3})}$ = 0.35. ^{1}H NMR (300 MHz, CDCl$_3$, 23 °C, δ): 7.30 (m, 5H), 4.94 (d, *J* = 8.5 Hz, 1H), 4.48 (d, *J* = 4.2 Hz, 2H), 4.06 (m, 4H), 3.74 (m, 1H), 3.45 (d, *J* = 3.5 Hz, 2H), 1.81 (m, 4H), 1.42 (s, 9H), 1.29 (t, *J* = 6.9 Hz, 6H) ppm. ^{13}C NMR (300 MHz, CDCl$_3$, 23 °C, δ): 155.63, 137.95, 128.38, 128.22, 127.70, 127.59, 79.25, 73.15, 71.73, 61.54 (d, *J* = 6.8 Hz), 50.65 (d, *J* = 19.3 Hz), 28.35, 25.33, 23.34, 21.46, 16.45 (d, *J* = 6.8 Hz) ppm.

4.1.11.2 (4-benzyloxy-3-*tert*-butoxycarbonylaminobutyl)phosphonic acid diethyl ester ((*3S*)-8)—

Compound **(*3S*)-7** was converted to **(*3S*)-8** by the above method to yield 605 mg of a clear oil, which was >95% pure by NMR and was carried on to reaction **4.1.12.2** without further purification. Rf$_{(5\% \text{ MeOH in CHCl3})}$ = 0.35. See **(*3S*)-8** for corresponding ^{1}H and ^{13}C NMR data.

4.1.12.1 (3-*tert*-butoxycarbonylamino-4-hydroxybutyl)phosphonic acid diethyl ester ((*3R*)-9)—

General Procedure I was utilized for the hydrogenolysis of compound **(*3R*)-8** (1.201 g, 2.891 mmol) to give 2.714 g (94%) of **(*3R*)-9** as a clear oil. No further purification was necessary. Rf$_{(5\% \text{ MeOH in CHCl3})}$ = 0.09. ^{1}H NMR (300 MHz, CDCl$_3$, 23 °C, δ): 5.33 (d, J = 6.9 Hz), 4.00 (m, 5H), 3.52 (m, 2H), 1.69 (brm, 5H), 1.34 (s, 9H), 1.23 (t, J = 6.9 Hz, 6H) ppm. ^{13}C NMR (300 MHz, CDCl$_3$, 23 °C, δ): 156.20, 79.25, 64.31, 61.76, 57.30, 52.74 (d, J_P = 19.3 Hz), 33.31 (d, J_P = 15.5 Hz), 28.44, 24.32, 23.17, 16.46 (d, J_P = 5.8 Hz) ppm. $[\alpha]^{23}_D$ = −10.8° (c ≈ 1.00, MeOH).

4.1.12.2 (3-*tert*-butoxycarbonylamino-4-hydroxybutyl)phosphonic acid diethyl ester ((*3S*)-9)—

The crude material from **(*3S*)-8** (605 mg, 1.4 mmol) was converted to **(*3S*)-9** by General Procedure I. The resultant oil was purified by flash chromatography (100 mL SiO$_2$, 5% methanol in chloroform) to yield 347 mg (72% over two steps) as clear oil. Rf$_{(5\% \text{ MeOH in CHCl3})}$ = 0.09. See **(*3R*)-9** for corresponding ^{1}H and ^{13}C NMR data. $[\alpha]^{23}_D$ = +10.4° (c = 1.00, MeOH).

4.1.12.3 (3-tert-butoxycarbonylamino-4-hydroxybutyl)-phosphonic acid diethyl ester ((*3R*)-9) from (*3R*)-2a—

To a solution of compound **2a** (5.211 g, 14.340 mmol) in EtOH (145 mL) was added 10% Pd/C (2.8 g, 20% $^w/_w$) [caution: Pd/C may spark on contact with ground glass joint; funnel and joint were rinsed with EtOH prior to attaching ground glass apparatus.] The round-bottomed flask was affixed with a three-way adapter w/stopcock. The adapter was fitted with a balloon of H$_2$ and a vacuum hose. The system was purged of air and filled with H$_2$ repeatedly in five minute increments. After 3 cycles the vacuum was removed and mixture was opened to H$_2$ atmosphere. After 1.5 days the reaction had preceded ~60% by TLC and a second portion of Pd/C (1.4 g, 10% $^w/_w$) was added as well as more EtOH (50 mL). Following another 1.5 days the reaction had progressed to one spot by TLC analysis. The mixture was filtered over celite and washed with MeOH (4×50 mL). The solvent was evaporated and NMR analysis showed the resultant clear oil (4.72 g, 14.34 mmol) to be >95% pure. ^{1}H and ^{13}C NMR are consistent with data reported for **9a** from **8a**. $[\alpha]^{23}_D = -10.2°$ (c = 1.00, MeOH). HRMS (ES+) calculated *m/z* = 326.1733, experimental *m/z* = 326.1737.

4.1.12.3 (3-*tert*-butoxycarbonylamino-4-hydroxybutyl-phosphonic acid diethyl ester ((*3S*)-9) from (*3S*)-2a—

The above procedure was repeated with **(*3S*)-2a** (1 g, 2.75 mmol), 10% Pd/C (1.5 g then 0.75 g) in EtOH (75 mL then 25 mL) to yield 0.905 g (> 95%) of clear oil. The physical data was consistent with that of compound **(*3S*)-9** derived from **(*3S*)-8**. HRMS (ES+) calculated *m/z* = 326.1733, experimental *m/z* = 326.1729.

4.1.13.1 TEMPO Oxidation: ((*2R*)-4a)

To a mixture of **(*3R*)-9** (181 mg, 0.72 mmol) and iodosobenzenediacetate (430 mg, 1.335 mmol) and $NaHCO_3$ (1.44 mmol) stirring in 22 mL of 1:1 MeCN/H_2O was added 2,2,6,6-tetramethyl-1-piperidinyloxy (20 mg, 0.128 mmol). The mixture was stirred vigorously at room temperature for three hours then diluted with 10 mL of chloroform and extracted with Na_2CO_3. Ethyl acetate was added to the aqueous layer and the solution was acidified with 1N HCl and extracted (5×15 mL) with EtOAc. The organic layers were combined, dried over $Na_2SO_{4(s)}$, filtered and evaporated to an amorphous solid. The desired material was used without further purification in the next series of reactions. $Rf_{(AcOH/MeOH/CH2Cl2) = 0.40.}$ ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 9.91 (brs, 1H), 5.41 (m, 1H), 4.31 (m, 1H), 4.08 (m, 4H), 2.02 (m, 4H), 1.41 (s, 9H), 1.29 (m, 6H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 173.79, 135.57, 80.15, 62.53 (d, J_P = 6.0Hz), 57.58, 53.52, 28.41, 25.66, 22.40, 20.51, 16.46 (d, J_P = 6.0 Hz) ppm. HRMS (ES+) calculated *m/z* = 340.1525, experimental *m/z* = 340.1514.

4.1.13.2 $RuCl_3/NaIO_4$ Oxidation: ((*2S*)-4a)

To **(*3S*)-9** (231 mg, 0.710 mmol) in 1.4 mL of CCl_4, 1.4 mL of MeCN, and 2.2 mL of H_2O was added $NaIO_4$ (456 mg, 2.130 mmol) followed by $RuCl_3$ hydrate (3 mg, 0.016 mmol). By TLC analysis all the alcohol was consumed in 5 minutes (presumably to the aldehyde; $Rf_{(1:9\ MeOH/CHCl3)} \approx 1$). After 1.5 hours, only a baseline spot was visible by TLC and the reaction was diluted with H_2O and extracted with $CHCl_3$ (5×15 mL). The organic layers were combined and dried over $MgSO_4$, then concentrated and purified by flash chromatography (~75 mL of SiO_2, 10 to 25% MeOH in CH_2Cl_2) to yield 184 mg (76%) of an amorphous white solid. $Rf_{(AcOH/MeOH/CH2Cl2)}$ = 0.40. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 11.32 (s, 1H), 5.39 (d, *J* = 7.2 Hz), 4.23 (m, 1H), 4.03 (dq, *J* = 7.2, 2.4 Hz, 4H), 1.93 (m, 4H), 1.35 (s, 9H), 1.23 (t, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 173.81, 155.59, 79.94, 62.30, 53.32 (d, *J* = 19.8 Hz), 28.29, 25.55, 22.30, 20.40, 16.33 (d, J = 6.1 Hz) ppm. HRMS (ES+) calculated *m/z* = 340.1525, experimental *m/z* = 340.1525.

4.1.14.1 General Procedure III: PyBOP Condensation (11a)

To a solution of the protected amino acid **(*2R*)-4a** (50 mg,0.147 mmol) in dry CH_2Cl_2 (4 mL) was added PyBOP (77 mg, 0.147 mmol) and DIEA (0.03 mL, 0.147 mmol), followed by *p*-decylaniline (34 mg, 0.147 mmol). The reaction was stirred at room temperature for 12 hours then concentrated and purified by column chromatography (0 to 20% acetone in chloroform) to 35 mg of clear oil. $Rf_{(1:1\ EtOAc/Hex.)}$=0.15. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 9.16 (s, 1H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.10 (d, *J* = 8.5 Hz, 2H), 5.69 (d, *J* = 6.5 Hz, 1H), 4.44 (m, *J* = 7.3, 1.2 Hz, 1H), 4.12 (m, 4H), 2.54 (t, *J* = 7.7 Hz, 2H), 2.07 (m, *J* = 7.7 Hz, 2H), 1.88 (m, 2H), 1.56 (p, *J* = 6.9 Hz, 2H), 1.44 (s, 9H), 1.32 (dt, *J* = 11.9, 7.3 Hz, 6H), 0.87 (t, J = 6.9 Hz, 3H) ppm.

4.1.14.2 [3-*tert*-butoxycarbonylamino-3-(4-octylphenylcarbamoyl)propyl]-phosphonic acid diethyl ester (11b)—

General Procedure III was used to convert **(*2R*)-4a** (50 mg, 0.147 mmol) and *p*-octylaniline (0.05 mL, 0.147 mmol) to the title compound. The crude material was purified through flash chromatography (~ 50 mL of SiO_2, 1:9 Acetone/

$CHCl_3$) to give 54 mg (70%) of clear oil. Rf$_{\text{(1:9 Acetone/ CHCl3)}}$ = 0.2. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 9.22 (s, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 5.77 (d, *J* = 8.1 Hz, 1H), 4.44 (m, 1H), 4.10 (m, 4H), 2.53 (t, *J* = 7.9 Hz, 2H), 2.09 (m, 2H), 1.87 (m, 2H), 1.55 (p, *J* = 6.8 Hz, 2H), 1.43 (s, 9H), 1.31 (dt, *J* = 10.6, 7.0 Hz, 6H), 0.86 (t, *J* = 7.0, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 169.75, 155.96, 139.21, 135.76, 128.99, 120.12, 80.30, 62.29 (d, J = 22.2 Hz), 35.63, 32.13, 31.78, 29.72, 29.51, 28.59, 26.52 (d, J = 5.0 Hz), 23.08, 22.91, 21.20, 16.67 (d, J = 7.6 Hz), 14.35 ppm.

4.1.14.3 [3-*tert*-butoxycarbonylamino-3-(3-heptylphenylcarbamoyl)propyl]-phosphonic acid diethyl ester (11c)—General Procedure III was used to convert **(2*R*)-4a** (50 mg, 0.147 mmol) and *m*-heptylaniline[39] (0.28 mg, 0.147 mmol) to the title compound. The crude material was purified through flash chromatography (~50 mL of SiO_2, 1:3 Acetone/ $CHCl_3$) to give 35 mg (46%) of clear oil. Rf$_{\text{(1:3 Acetone/ CHCl3)}}$=0.55. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 9.22 (s, 1H), 7.40 (s, 1H), 7.39 (d, *J* = 9.2 Hz, 1H), 7.19 (t, *J* = 7.7 Hz, 1H), 6.91 (d, *J* = 7.3 Hz, 1H), 5.68 (d, *J* = 7.3 Hz, 1H), 4.46 (m, 1H), 4.11 (m, 4H), 2.55 (t, *J* = 7.7 Hz, 2H), 2.07 (m, 2H), 1.89 (m, 2H), 1.58 (p, *J* = 7.3 Hz, 2H), 1.44 (s, 9H), 1.32 (dt, *J* = 11.9, 6.9 Hz, 6H), 0.86 (t, *J* = 6.9 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 169.72, 144.14, 138.00, 128.90, 124.61, 123.12, 119.93, 117.28, 78.50, 36.21, 62.15, 36.21, 31.99, 31.67, 29.53, 29.38, 28.52, 26.44, 22.58, 21.18, 16.64, 14.29 ppm.

4.1.14.4 [3-*tert*-butoxycarbonylamino-3-(4-octylphenylcarbamoyl)propyl]-phosphonic acid diethyl ester (11d)—General Procedure III was used to convert **(2*R*)-4a** (1.122 g, 3.307 mmol) and *m*-octylaniline (0.815 g, 3.968 mmol) to the title compound. The crude material was purified through flash chromatography (~ 300 mL of SiO_2, 1:9 acetone/ $CHCl_3$) to give 1.134 g (65%) of translucent oil. R$_{\text{f (3:7 acetone/CHCl3)}}$ = 0.58. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 9.28 (s, 1H), 7.41 (s, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.18 (t, *J* = 8.1 Hz, 1H), 6.90 (d, *J* = 7.7 Hz, 1H), 5.74 (d, *J* = 8.1 Hz, 1H), 4.46 (m, 1H), 4.12 (ddt, *J* = 24.6, 7.3, 3.5 Hz, 4H), 2.54 (t, *J* = 7.3 Hz, 2H), 2.07 (sep., J = 6.5 Hz, 2H), 1.90 (dq, *J* = 18.4, 13.5, 6.5 Hz, 2H), 1.57 (p, *J* = 7.7 Hz, 2H), 1.43 (m, 9H), 1.31 (dt, *J* = 10.0, 7.3 Hz, 6H), 1.28 (m, 12H), 0.86 (t, *J* = 6.5 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 169.77, 144.07, 138.04, 128.85, 124.53, 120.33, 119.94, 118.11, 117.29, 80.20, 62.36, 62.04, 54.79, 36.20, 32.06, 31.65, 30.07, 29.66, 29.56, 29.43, 29.13, 28.51, 26.46, 23.03, 22.83, 22.78, 21.16, 16.64, 16.55, 14.28 ppm.

4.1.14.5 [3-*tert*-butoxycarbonylamino-3-(4-octylphenylcarbamoyl)propyl]-phosphonic acid diethyl ester (11e)—General Procedure III was used to convert **(2*S*)-4a** (50 mg, 0.147 mmol) and *p*-octylaniline (0.05 mL, 0.147 mmol) to the title compound. The crude material was purified through flash chromatography (~ 50 mL of SiO_2, 1:9 Acetone/ $CHCl_3$) to give 54 mg (70%) of clear oil. Rf$_{\text{(1:9 Acetone/ CHCl3)}}$ = 0.2. See **11b** for corresponding ^{1}H and ^{13}C NMR data.

4.1.14.6 [3-*tert*-butoxycarbonylamino-3-(3-octylphenylcarbamoyl)propyl]-phosphonic acid diethyl ester (11f)—General Procedure III was used to convert **(2*S*)-4a** (34 mg, 0.100 mmol) and *m*-octylaniline[39] (0.02 mL, 0.100 mmol) to the title compound. The crude material was purified through flash chromatography (~50 mL of SiO_2, 1:1 EtOAc/ Hex.) to give 23 mg (45%) of an off-white solid. Rf$_{\text{(1:1 EtOAc/Hex.)}}$=0.10. See **11d** for corresponding ^{1}H and ^{13}C NMR data.

4.1.15.1 General Procedure IV: Phosphonate Diester Deprotection (12a) The phosphonate diester **11a** (35 mg, 0.063 mmol) was dissolved in 0.65 mL of CH_2Cl_2 to which bromotrimethylsilane (0.08 mL, 0.630 mmol) was added via syringe. The solution was allowed to stir for four to six hours. The off-white solution was then concentrated and reconstituted in

> 0.65 mL of 95% methanol in water. The new solution was allowed to stir for four hours to ensure hydrolysis and was concentrated and co-evaporated with methanol and diethyl ether until a pasty solid was attained. The compound was triturated to a fine white solid on addition of water. The solid was filtered through a fine fritted funnel and washed with water (3×) followed by cold pentane (3×) to yield 31 mg of the desired compound as a white solid. ^{1}H NMR (300 MHz, CD_3OD, 23 °C, δ): 7.51 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.4 Hz, 2H), 4.12 (t, *J* = 6.4 Hz, 1H), 2.58 (t, *J* = 7.7 Hz, 2H), 2.24 (m, 2H), 1.86 (m, 2H), 1.60 (p, *J* = 6.7 Hz, 2H), 1.28 (m, 12H), 0.90 (t, *J* = 7.0 Hz, 3H) ppm. ^{13}C NMR (300 MHz, CD_3OD, 23 °C, δ): 141.04, 130.04, 121.40, 114.14, 56.36, 36.47, 33.21, 32.88, 30.89, 30.86, 30.74, 30.60, 30.41, 23.88, 14.59 ppm. MS (ESI+) *m/z* 399 $[M+H]^+$.

4.1.15.2 [3-amino-3-(4-octylphenylcarbamoyl)propyl]-phosphonic acid (12b – VPC44152)—General Procedure IV was used to globally deprotect compound **11b** in formation of the title compound as 47 mg (100%) of an off-white solid. ^{1}H NMR (300 MHz, CD_3OD, 23 °C, δ): 7.51 (d, *J* = 8.3 Hz, 2H), 7.17 (d, *J* = 8.3 Hz, 2H), 4.13 (t, *J* = 6.5 Hz, 1H), 2.59 (t, *J* = 7.9 Hz, 1H), 2.24 (m, 2H), 1.88 (m, 2H), 1.60 (p, *J* = 6.2 Hz, 2H), 1.31 (m, 10H), 0.89 (t, *J* = 7.0 Hz, 3H) ppm. ^{13}C NMR (300 MHz, CD_3OD, 23 °C, δ): 140.90, 136.48, 129.69, 121.25, 66.69, 57.13, 36.32, 33.02, 32.72, 30.56, 30.41, 30.27, 23.71, 22.89, 22.46, 14.43 ppm. MS (APCI) *m/z* = 371.9 $[M+1]^+$, 370.80 $[M, 100\%]^+$. Elemental CHN: calculated C = 47.90%, H = 7.15%, N = 6.21%; found C = 48.21%, H = 7.25%, N = 6.02%.

4.1.15.3 [3-amino-3-(3-heptylphenylcarbamoyl)propyl]-phosphonic acid (12c)— General Procedure IV was used to globally deprotect compound **11c** in formation of the title compound as 25 mg (84%) of a white solid. ^{1}H NMR (300 MHz, $(CD_3)_2SO$, 23 °C, δ): 8.26 (m, 1H), 7.42 (m, 1H), 7.26 (t, J = 6.8 Hz, 1H), 6.97 (d, J = 7.5 Hz, 1H), 3.98 (m, 1H), 2.55 (m, 2H), 2..03 (m, 2H), 1.60 (m, 4H), 1.26 (m, 8H), 0.85 (m, 3H) ppm. ^{13}C NMR (300 MHz, $(CD_3)SO$, 23 °C, δ): 166.79, 143.28, 137.83, 128.94, 124.37, 119.27, 116.95, 53.25, 35.17, 31.27, 30.87, 28.60, 28.54, 25.36, 24.32, 22.51, 22.11, 13.99 ppm. MS (ESI+) *m/z* 357 $[M+H]^+$.

4.1.15.4 [3-amino-3-(3-octylphenylcarbamoyl)propyl]-phosphonic acid (12d – VPC44116)—General Procedure IV was used to globally deprotect compound **12d** in formation of the title compound as 28 mg (90%) of a white solid. ^{1}H NMR (300 MHz, CD_3OD, 23 °C, δ): 7.45 (m, 2H), 7.23 (t, *J* = 7.8 Hz, 1H), 6.97 (d, *J* = 7.6 Hz, 1H), 4.15 (m, 1H), 2.59 (t, *J* = 7.3 Hz, 2H), 2.25 (m, 2H), 1.89 (m, 2H), 1.61 (m, 2H), 1.31 (m, 10H), 0.89 (t, J = 6.8 Hz, 3H) ppm. ^{13}C NMR (300 MHz, CD_3OD, 23 °C, δ): 167.99, 139.32, 130.01, 126.19, 121.28, 119.44, 118.72, 55.39, 55.20, 37.04, 33.17, 32.77, 30.74, 30.47, 29.72, 27.11, 25.42, 23.87, 23.61, 14.58 ppm. MS (APCI) *m/z* = 371.9 $[M+1]^+$, 370.80 $[M, 100\%]^+$. HRMS (ES+) calculated *m/z* = 371.2100, experimental *m/z* = 371.2108. Elemental CHN: calculated C = 47.90%, H = 7.15%, N = 6.21%; found C = 48.11%, H = 7.14%, N = 6.14%.

4.1.15.5 [3-amino-3-(4-octylphenylcarbamoyl)propyl]-phosphonic acid (12e)— General Procedure IV was used to globally deprotect compound **11e** in formation of the title compound as 25 mg (96%) of a white solid. ^{1}H NMR (300 MHz, CD_3OD, 23 °C, δ): 7.47 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.1 Hz, 2H), 4.25 (m, 1H), 3.95 (t, *J* = 6.8 Hz, 1H), 2.57 (t, *J* = 7.5 Hz, 2H), 2.22 (m, 2H), 1.97 (m, 1H), 1.84 (m, 2H), 1.58 (m , 2H), 1.26 (m, 10H), 0.87 (t, *J* = 6.6 Hz, 3H) ppm. ^{13}C NMR (300 MHz, CD_3OD, 23 °C, δ): 140.90, 136.48, 129.69, 121.25, 66.69, 57.13, 36.32, 33.02, 32.72, 30.56, 30.41, 30.27, 23.71, 22.89, 22.46, 14.43 ppm. MS (ESI+) *m/z* 371 $[M+H]^+$.

4.1.15.6 [3-amino-3-(3-octylphenylcarbamoyl)propyl]-phosphonic acid (12f)— General Procedure IV was used to globally deprotect compound **11f** in formation of the title compound as 20 mg (100%) of a white solid. ^{1}H NMR (300 MHz, CD_3OD, 23 °C, δ): 7.44 (s, 1H), 7.42 (d, *J* = 10.0 Hz, 1H), 7.24 (t, *J* = 7.6 Hz, 1H), 6.98 (d, *J* = 7.8 Hz, 1H), 4.08 (t, *J* = 6.6 Hz, 1H), 2.60 (t, *J* = 7.3 Hz, 2H), 2.23 (m, 2H), 1.89 (m, 2H), 1.61 (quin., *J* = 6.8Hz, 2H), 1.32 (m, 10H), 0.89 (t, J = 6.6 Hz, 3H) ppm. ^{13}C NMR (300 MHz, CD_3OD, 23 °C, δ): 167.99, 139.32, 130.01, 126.19, 121.28, 119.44, 118.72, 55.39, 55.20, 37.04, 33.17, 32.77, 30.74, 30.47, 29.72, 27.11, 25.42, 23.87, 23.61, 14.58 ppm. MS (ESI+) *m/z* 371 $[M+H]^+$.

4.1.15.7 [3-amino-3-(4-decylphenylcarbamoyl)-2-fluoropropyl]-phosphonic acid (13)—Compounds **(*3R*)-4b** and *p*-decylaniline were converted to the title compound by application of first; General Procedure III to yield 22 mg (24%) of a clear liquid, $Rf_{(1:1\ EtOAc/Hexanes)}$=0.21. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 8.78 (d, *J* = 38.9, 1H), 7.43 (d, *J* = 7.7 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 5.56 (d, *J* = 22.6 Hz, 1H), 4.99 (m, 1H), 4.56 (m, 1H), 4.22 (m, 4H), 2.55 (t, *J* = 7.9 Hz, 2H), 2.40 (m, 2H), 1.56 (p, *J* = 7.0 Hz, 2H), 1.45 (s, 9H), 1.36 (dt, *J* = 7.2, 7.0 Hz, 6H), 1.25 (m, 12H), 0.87 (t, *J* = 6.6 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 129.01, 120.11, 100.16, 63.98, 63.47, 51.81, 51.50, 25.59, 32.10, 31.74, 29.83, 29.81, 29.71, 29.53, 29.45, 28.50, 28.48, 22.89, 16.63, 14.33 ppm. General Procedure IV was then implemented to give 19 mg (100%) of the title compound as a white solid. ^{1}H NMR (300 MHz, CD_3OD, 23 °C, δ): 7.50 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.4 Hz, 2H), 5.02 (m, 1H), 4.26 (m, 1H), 4.11 (m, 1H), 2.59 (t, J = 7.6 Hz, 2H), 2.39 (m, 1H), 1.60 (p, *J* = 7.0 Hz, 2H), 1.29 (m, 12H), 0.90 (t, J = 6.6 Hz, 3H) ppm. ^{13}C NMR (300 MHz, CD_3OD, 23 °C, δ): 166.71, 140.61, 135.83, 129.57, 120.97, 36.04, 32.64, 32.29, 30.32, 30.21, 30.05, 29.89, 23.36, 16.81, 14.37 ppm. MS (ESI+) *m/z* 417 $[M+H]^+$.

4.1.16.1 General Procedure V: Standard Mitsunobu Conditions (14a) To a solution of *p*-octylphenol (1.000 g, 4.847 mmol) in 5 mL of anhydrous THF was added (*R*)-(+)- glycidol (0.35 mL, 5.331 mmol) and Ph_3P (1.398 g, 5.331 mmol). The reaction mixture was cooled to 0 °C and DIAD (1.05 mL, 5.331 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 24 hours. A white precipitate formed upon addition of Et_2O and was filtered off through a fine fritted funnel. The filtrate was concentrated and purified by column chromatography (~200 mL SiO_2, $CHCl_3$) to give 1.023 g (80%) of **14a** as colorless oil. $Rf_{(CHCl3)}$=0.66. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.09 (d, J = 8.4 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.19 (dd, *J* = 11.2, 3.3 Hz, 1H), 3.95 (dd, *J* = 11.0, 5.5 Hz, 1H), 3.35 (p, *J* = 4.8 Hz, 1H), 2.90 (t, *J* = 4.2 Hz, 1H), 2.75 (dd, *J* = 4.8, 2.6 Hz, 1H), 2.54 (t, *J* = 7.5 Hz, 2H), 1.56 (m, 2H), 1.28 (m, 10H), 0.88 (t, J = 7.0 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 156.70, 135.92, 129.51, 114.63, 69.02, 50.44, 45.01, 35.26, 32.10, 31.93, 29.70, 29.49, 22.89, 14.33 ppm.

4.1.16.2 2-(4-octylphenoxymethyl)-oxirane (14b)—The title compound was formed, through General Procedure V, starting with 1.000 g of (*S*)-(-)-glycidol, as 1.073 g (84%) of a clear oil following column chromatography (~200 mL SiO_2, $CHCl_3$) $Rf_{(CHCl3)}$=0.66. ^{1}H and ^{13}C NMR were consistent with those of epimer **14a**.

4.1.17.1 [3-hydroxy-4-(4-octylphenoxy)-butyl]-phosphonic acid diethyl ester (15a)—General Procedure II was used to transform **14a** (1.023 g, 3.899 mmol) to 1.400 g (87%) of **15a** as a clear oil after column chromatography (~300 mL SiO_2, 1:4 acetone/ chloroform). $Rf_{(1:4\ acetone/chloroform)}$ =0.33. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.09 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 8.5 Hz, 2H), 4.11 (m, 4H), 4.02 (m, 1H), 3.90 (m, 2H), 2.53 (t, *J* = 7.7 Hz, 2H), 1.92 (m, 4H), 1.56 (p, J = 8.1 Hz, 2H), 1.30 (m, 16H), 0.87 (t, J = 6.9 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 156.70, 135.89, 129.54, 114.54, 71.77, 70.08, 62.02, 35.26, 32.11, 31.95, 29.70, 29.49, 26.59, 23.12, 22.89, 21.23, 16.69, 14.33 ppm.

4.1.17.2 [3-hydroxy-4-(4-octylphenoxy)-butyl]-phosphonic acid diethyl ester (15b)—General Procedure II was used to transform **14b** (1.073 g, 4.089 mmol) to 1.215 g (72%) of **15b** as a clear oil after column chromatography (~300 mL SiO_2, 1:4 acetone/chloroform). $Rf_{(1:4\ \text{acetone/chloroform})}$ =0.33.

4.1.18.1 General Procedure VI: Azide Formation with Free Phosphine (16a) To a stirring solution of **15a** (500 mg, 1.206 mmol) in 1.6 mL of CH_2Cl_2 at 0 °C was added Ph_3P (348 mg, 1.327 mmol). The solution was stirred for 15 minutes and then DIAD (0.26 mL, 1.327 mmol) and DPPA (0.29 mL, 1.327 mmol) were added consecutively, dropwise. The reaction was allowed to warm to room temperature and stirred overnight. Mixture was concentrated and purified by chromatography (~150 mL SiO_2, 1:9 Acetone/$CHCl_3$) followed by (~100 mL SiO_2, 50% to 80% Et_2O in petroleum ether) to yield 518 mg (98%) of non-viscous clear liquid. $Rf_{(1:9\ \text{Acetone/CHCl3})}$=0.44. 1H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.04 (d, J = 8.6 Hz, 2H), 6.77 (d, J = 8.8 Hz, 2H), 4.05 (m, 4H), 3.95 (m, 2H), 3.75 (m, 1H), 2.48 (t, J = 7.9 Hz, 2H), 1.80 (m, 4H), 1.51 (p, J = 7.3 Hz, 2H), 1.27 (t, J = 7.0 Hz, 6H), 1.23 (m, 10H), 0.82 (t, J = 6.6 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 156.11, 135.80, 129.29, 114.30, 70.45, 61.67, 60.95, 35.01, 31.84, 31.68, 29.44, 29.22, 24.16, 23.10, 22.63, 21.21, 16.41, 14.06 ppm.

4.1.18.2 [3-azido-4-(4-octylphenoxy)-butyl]-phosphonic acid diethyl ester (16b) —General Procedure VI was used to form the title compound from alcohol **15b**. Purification consisted of two chromatography steps. First a column (~250 mL of SiO_2, 1:9 Acetone/$CHCl_3$) was run to yield the desired product with $OPPh_3$. The crude material was then run through a short plug (~50 mL of SiO_2) with $CHCl_3$ as the eluent. The phosphine oxide did not more over this system and the plug yielded 354 mg (67%) of a clear liquid. Data were consistent with that of **16a.**

4.1.19.1 [3-amino-4-(4-octylphenoxy)-butyl]-phosphonic acid diethyl ester (17a) —General Procedure I was used to reduce the azide **16a** to 488 mg of amine **17a** as a yellow liquid. Further purification was not necessary and the product was carried on to the final deprotection. ^1H NMR (300 MHz, CD_3OD, 23 °C, δ): 6.97 (d, J = 8.4 Hz, 2H), 6.74 (d, J = 8.1 Hz, 2H), 5.43 (m, 2H), 3.99 (m, 4H), 3.90 (m, 2H), 2.43 (t, J = 7.9 Hz, 2H), 1.89 (m, 4H), 1.47 (p, J = 7.0 Hz, 2 H), 1.21 (m, 16H), 0.79 (t, J = 6.7 Hz, 3H) ppm. ^{13}C NMR (300 MHz, CD_3OD, 23 °C, δ): 168.83, 156.23, 135.57, 129.18, 114.35, 69.57, 61.82, 51.05, 49.84, 34.96, 31.81, 31.66, 29.41, 29.20, 24.83, 22.73, 22.58, 20.85, 16.34, 14.03 ppm.

4.1.19.2 [3-amino-4-(4-octylphenoxy)-butyl]-phosphonic acid diethyl ester (17b) —General Procedure I was used to reduce the azide **16b** to 333 mg of amine **17b** as a light yellow liquid. Further purification was not necessary and the product was carried on to the final deprotection. Data were similar to that obtained for **17a.**

4.1.20.1 [3-amino-4-(4-octylphenoxy)-butyl]-phosphonic acid (18a)—General Procedure IV was used to deprotect compound **17a** in formation of the title compound as 500 mg (97%) of an off-white solid. ^1H NMR (300 MHz, CD_3OD, 23 °C, δ): 7.12 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 4.22 (dd, J = 10.4, 3.5 Hz, 1H), 4.07 (dd, J = 10.7, 6.1 Hz, 1H), 3.68 (m, 1H), 2.55 (t, J = 7.3 Hz, 2H), 2.09 (m, 2H), 1.88 (m, 2H), 1.57 (p, J = 7.7 Hz, 2H), 1.30 (m, 10H), 0.89 (t, J = 6.8 Hz, 3H) ppm. ^{13}C NMR (300 MHz, CD_3OD, 23 °C, δ): 13764, 130.64, 115.70, 101.52, 68.14, 36.14, 33.18, 33.07, 30.73, 30.58, 30.39, 25.28, 24.70, 23.87, 14.58 ppm. MS (ESI+) m/z 358 $[M+H]^+$.

4.1.20.2 [3-amino-4-(4-octylphenoxy)-butyl]-phosphonic acid (18b)—General Procedure IV was used to deprotect compound **17b** in formation of the title compound as 278 mg (79%) of an off-white solid. ^1H NMR, ^{13}C NMR, and MS (ESI+) were consistent with those obtained for compound **18a**. Elemental CHN: calculated C = 60.49%, H = 9.02%, N = 3.92%; found C = 60.21%, H = 9.00%, N = 3.68%.

4.1.21. [3-hydroxy-4-(4-octylphenoxy)-butyl]-phosphonic acid (19)—General Procedure IV was used to deprotect compound **15a** (25 mg, 0.060 mmol) in formation of the title compound. Further purification was performed by recrystallization from EtOAc and Hexanes to yield 22 mg (79%) of a white solid. ^{1}H NMR (300 MHz, CD_3OD, 23 °C, δ): 7.07 (m, 2H), 6.85 (m, 2H), 3.90 (m, 3H), 2.53 (m, 2H), 1.95 (m, 2H), 1.78 (m, 2H), 1.29 (m, 10H), 0.89 (m, 3H) ppm. MS (ESI-) m/z 357 $[M-H]^-$.

4.1.22. 4-methyl-*N*-(3-octylphenyl)-benzenesulfonamide (20)—To a stirring solution of *m*-octylaniline[40] (1.000 g, 4.87 mmol) in 5 mL of pyridine at 0 °C was added tosyl chloride (928 mg, 4.87 mmol). The reaction mixture was warmed to room temperature. After one hour, the mixture was diluted with EtOAc and washed with 1N HCl (3×), $NaHCO_3$ (2×), and brine (2×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by column chromatography (~150 mL SiO_2, 1:19 Acetone/Chloroform) to yield 1.700 g (97%) of a white solid. Rf $_{\text{(1:19 Acetone/Chloroform)}}$ = 0.70. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.72 (d, 2H), 7.59 (bs, 1H), 7.19 (d, *J* = 7.8 Hz, 2H), 7.03 (q, 4H), 2.51 (t, *J* = 7.2Hz, 2H), 2.34 (s, 3H), 1.54 (p, *J* = 7.6 Hz, 2H), 1.27 (m, 10H), 0.88 (t, *J* = 6.8 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 144.2, 140.5, 136.7, 134.7, 130.1, 129.7, 127.9, 122.4, 35.8, 32.4, 31.9, 30.0, 29.8, 23.2, 22.0, 14.6. MS (ESI) *m/z* 360 $[M+H]^+$.

4.1.23. 4-methyl-*N*-(3-octylphenyl)-*N*-oxiranylmethylbenzenesulfonamide (21)—General Procedure V was used to couple **20** (0.809 g, 2.25 mmol) with (*R*)-(+)-glycidol (0.23 mL, 3.375 mmol) to form 644 mg (69%) of the title compound **21** after flash chromatography (~150 mL SiO_2, 1:4 EtOAc/Hexanes), as a clear oil. Rf$_{\text{(1:4 EtOAc/Hex.)}}$ = 0.36. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.45 (d, J = 8.1 Hz, 2H), 7.20 (m, 2H), 7.07 (m, 1H), 7.02 (m, 1H), 6.89 (m, 1H), 6.82 (m, 1H), 3.65 (ddd, *J* = 35.2, 14.3, 5.3 Hz, 2H), 3.08 (m, 1H), 2.63 (m, 1H), 2.49 (t, *J* = 7.5 Hz, 2H), 2.37 (s, 3H), 2.16 (m, 1H), 1.48 (p, *J* = 6.8 Hz, 2H), 1.25 (m, 10H), 0.85 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 144.01, 143.51, 139.60, 138.83, 129.39, 127.68, 126.10, 53.65, 50.24, 45.78, 35.56, 31.84, 31.24, 29.41, 29.15, 22.64, 21.47, 14.10 ppm.

4.1.24. {3-hydroxy-4-[(3-octylphenyl)-(toluene-4-sulfonyl)-amino]-butyl}-phosphonic acid diethyl ester (22)—General Procedure II completed the synthesis of 853 mg (97%) of compound **22** from **21** as a clear oil following column chromatography (SiO_2, 5 to 20% Acetone in $CHCl_3$). Rf$_{\text{(1:4 EtOAc/Hex.)}}$ = 0.05. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.41 (dd, *J* = 8.1, 6.5 Hz, 2H), 7.18 (d, *J* = 7.7 Hz, 2H), 7.13 (d, *J* = 8.1, 1H), 7.04 (d, *J* = 7.7 Hz, 1H), 6.85 (m, 1H), 6.73 (m, 1H), 4.00 (m, 4H), 3.61 (m, 2H), 3.47 (m, 2H), 2.45 (t, *J* = 8.1Hz, 2H), 1.76 (m, 4H), 1.45 (m, 2H), 1.24 (m, 16H), 0.83 (t, *J* = 6.9 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 143.63, 139.60, 138.92, 134.77, 129.50, 129.44, 128.93, 128.31, 127.88, 126.04, 68.80 (d, *J* = 13.5 Hz), 61.74 (d, *J* = 5.8 Hz), 56.23 (d, *J* = 7.7 Hz), 35.66, 31.93, 31.35, 29.49, 29.30, 28.98, 27.10 (d, *J* = 4.8 Hz), 22.70, 21.59, 20.48, 16.47 (d, *J* = 5.8 Hz), 14.18 ppm.

4.1.25. {3-azido-4-[(3-octylphenyl)-(toluene-4-sulfonyl)-amino]-butyl}-phosphonic acid diethyl ester (23)—General Procedure VI transformed alcohol **22** (302 mg, 0.532 mmol) into azide **23**. The crude material was purified twice by chromatography (~150 mL of SiO_2, 10%Acetone/$CHCl_3$) followed by (~100 mL of SiO_2, 7:3 EtOAc/Hexanes) to yield 267 mg (85%) as a clear oil. [Another 5% of the desired product was isolated with residual phosphine oxide.] Rf$_{\text{(1:9 Acetone/CHCl3)}}$ = 0.43. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.38 (d, *J* = 8.4 Hz, 2H), 7.19 (d, *J* = 7. Hz, 2H), 7.07 (d, *J* = 7.7 Hz, 1H), 6.86 (d, *J* = 13.2

Hz, 1H), 6.78 (m, 1H), 4.02 (m, 4H), 3.53 (m, 3H), 2.48 (t, *J* = 7.5 Hz, 2H), 2.37 (s, 3H), 1.75 (m, 4H), 1.46 (p, *J* = 7.0 Hz, 2H), 1.25 (m, 16H), 0.84 (t, *J* = 7.0 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 144.36, 143.80, 139.38, 134.55, 132.04, 129.48, 129.05, 128.45, 127.87, 125.73, 61.75 (d, J = 6.6 Hz), 61.32 (d, J = 16.1 Hz), 54.74, 35.68, 31.92, 31.33, 29.49, 29.28, 28.97, 28.56, 25.30 (d, J = 4.5 Hz), 22.70, 21.59, 16.49 (d, J = 6.0 Hz), 14.16 ppm.

4.1.26. {3-amino-4-[(3-octylphenyl)-(toluene-4-sulfonyl)-amino]-butyl}-phosphonic acid diethyl ester (24)—Compound **23** (267 mg, 0.450 mmol) was dissolved in 50 mL of a 20:1 MeOH/HCl solution. To this solution was added ~0.5 g of $Pd(OH)_2$ and the apparatus was assembled and experiment run analogous to that of General Procedure I. When no starting material remained (~four hours by TLC) the mixture was filtered over celite and washed with 2% Et_3N in MeOH. The filtrate was concentrated to yield 268 mg (100%) of the title compound as yellow oil. No further purification was required. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.26 (d, *J* = 7.9 Hz, 2H), 7.11 (d, *J* = 7.9 Hz, 2H), 7.06 (d, *J* = 7.7 Hz, 1H), 6.97 (d, *J* = 7.5 Hz, 1H), 6.72 (d, *J* = 7.7 Hz, 1H), 6.60 (m, 1H), 4.02 (bs, 2H), 3.87 (m, 4H), 3.30 (m, 2H), 2.63 (m, 1H), 2.35 (t, *J* = 7.7 Hz, 2H), 2.27 (s, 3H), 1.59 (m, 4H), 1.33 (m, 2H), 1.12 (m, 16H), 0.72 (t, *J* = 6.6 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 144.36, 143.95, 138.95, 137.75, 129.45, 128.99, 128.49, 128.07, 127.68, 125.79, 61.96 (d, J = 7.1 Hz), 56.05, 48.67 (t, J = 21.7), 35.48, 31.76, 31.22, 29.33, 29.13, 26.26 (d, J = 7.6 Hz), 22.52, 22.22, 21.29, 20.35, 16.08 (d, J = 6.6 Hz), 13.86 ppm.

4.1.27. [3-amino-4-(3-octylphenylamino)-butyl]-phosphonic acid diethyl ester (25)—A solution of $Na_{(s)}$ (70 mg, 3.06 mmol) in 25 mL of $NH_{3(l)}$ was contained in a 2-neck flask fitted with a cold finger cooled to −78 °C and stir bar. Compound **24** (182 mg, 0.306 mmol) diluted in a minimal amount of THF was quickly added to the solution and the reaction was allowed to proceed for no longer than 5 minutes, at which time ethanol was added under vigorous stirring and the reaction was allowed to warm to room temperature. The reaction mixture was concentrated to dryness, dissolved in EtOAc, washed with $NaHCO_3$ (3×) and brine (1×). The organic layer was dried then columned (~75 mL of SiO_2, 5% MeOH in $CHCl_3$). The desired product was found to be a clear oil, in 25 mg (25%) quantities. Starting material (28%) was also recovered. $R_{f(5\% \text{ MeOH in CHCl3})}$ = 0.17. ^{1}H NMR (300 MHz, $CDCl_3$, 23 °C, δ): 7.07 (t, *J* = 7.5 Hz, 1H), 6.53 (t, *J* = 7.0 Hz, 1H), 6.44 (m, 2H), 4.08 (m, 4H), 3.70 (m, 1H), 3.11 (m, 3H), 2.50 (t, *J* = 7.9 Hz, 2H), 1.83 (m, 4H), 1.57 (p, *J* = 7.3 Hz, 2H), 1.31 (m, 16H), 0.87 (t, *J* = 7.0 Hz, 3H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 23 °C, δ): 157.35, 148.46, 129.30, 118.14, 113.44, 110.41, 62.05, 61.72, 58.20, 47.13, 36.41, 34.55, 32.10, 31.72, 29.92, 29.73, 29.64, 29.49, 28.18 (d, J = 8.6 Hz), 22.88, 16.69 (d, J = 7.1 Hz), 14.32 ppm.

4.1.28. [3-amino-4-(3-octylphenylamino)-butyl]-phosphonic acid (26)—The deprotection of **25** (23 mg, 0.056 mmol) was carried out by General Procedure IV to give 20 mg (83%) of the title compound as a colorless solid. ^{1}H NMR (300 MHz, CD_3OD, 23 °C, δ): 7.36 (dd, J = 7.9, 3.7 Hz, 1H), 7.26 (t, J = 1.7 Hz, 1H), 7.21 (d, J = 7.9 Hz, 1H), 7.13 (d, J = 7.7 Hz, 1H), 3.70 (m, 3H), 2.65 (t, J = 7.5 Hz, 2H), 2.14 (m, 2H), 1.95 (m, 2H), 1.64 (p, J = 7.5 Hz, 2H), 1.31 (m, 10H), 0.89 (t, J = 7.0 Hz, 3H) ppm. MS (ESI+) m/z 357 $[M+H]^+$.

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References and Notes

1. Lynch KR. Biochim et Biophys Acta 2002;1582:70–71.
2. Hla T. Pharmacol Rev 2003;47:401–407.

3. Watterson K, Sankala H, Milstien S, Spiegel S. Prog in Lipid Res 2003;42:344–357. [PubMed: 12790117]
4. Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V, Allende ML, Proia RL, Cyster JG. Nature 2004;427:355–360. [PubMed: 14737169]
5. For a convenient review: Im D-S. TRENDS in Pharmacol Sci 2003;24:12–4.
6. Kharel Y, Lee S, Snyder AH, Sheasley-O'Neill SL, Morris MA, Setiady Y, Zhu R, Zigler MA, Burcin TL, Ley K, Tung KSK, Engelhard VH, Macdonald TL, Lynch KR. J Biol Chem 2005;280:36865–36872. [PubMed: 16093248]
7. Rosen J, Sanna G, Alfonso C. Immunol Rev 2003;195:160–177. [PubMed: 12969317]
8. Ferguson RM, Henry ML, Elkhammas EA, Davies EA, Bumgardner GL, Pelletier RP, Rajab A. Am J Surg 2003;186:306–311. [PubMed: 12946838]
9. Lei G, Suzuki AS, Goto T, Kokubo T, Miyamoto M, Kimura H. Transplant Proc 2000;32:1628. [PubMed: 11119867]
10. For a review of initial studies: Kiuchi M, Adachi K, Kohara T, Minoguchi M, Hanano T, Aoki Y, Mishina T, Arita M, Nakao N, Ohtsuki M, Hoshino Y, Teshima K, Chiba K, Sasaki S, Fujita T. J Med Chem 2000;43:2946–2961. [PubMed: 10956203]
11. Kiuchi M, Adachi K, Tomatsu A, Chino M, Tadeda S, Tanaka Y, Maeda Y, Sato N, Mitsutomi N, Sugahara K, Chiba K. Bioorg Med Chem 2005:425–432. [PubMed: 15598563]
12. Hale JJ, Neway W, Mills SG, Hajdu R, Keohane CA, Rosenbach M, Milligan J, Shei GJ, Chrebet G, Bergstrom H, Card D, Koo GC, Koprak SL, Jackson JJ, Rosen H, Mandala S. Bioorg & Med Chem Let 2004;14(12):3351–3355. [PubMed: 15149705]
13. Lim HS, Park JJ, Ko K, Lee MH, Chung SK. Bioorg Med Chem 2004;14:2499–2503.
14. Clemens JJ, Davis MD, Lynch KR, Macdonald TL. Bioorg Med Chem Lett 2003;13:3401–3404. [PubMed: 14505636]
15. Clemens JJ, Davis MD, Lynch KR, Macdonald TL. Bioorg Med Chem Lett 2004;14:4903–4906. [PubMed: 15341948]
16. Davis MD, Clemens JJ, Macdonald TL, Lynch KR. J Biol Chem 2005;280:9833–9841. [PubMed: 15590668]
17. Shapiro S, Buechler D, Enz A, Pombo-Villar E. Tetrahedron Lett 1994;35:1173–1176.
18. Barton DHR, Vonder Embse RA. Tetrahedron 1998;54:12475–12496.
19. Glenn T, Perich JW, Johns RB. Aust J Chem 1992;45:1225–40.
20. Antczak K, Szewczyk J. Phos Sulf 1985;22:247–251.
21. Rozhko LF, Klochkov SG, Ragulin VV, Tsvetkov EN. Russ J Gen Chem 1999;69:1093–1096.
22. Yokomatsu T, Sato M, Shibuya S. Tetrahedron Asymm 1996;7:2743–2745.
23. Jiao XY, Chen WY, Hu BF. Synth Comm 1992;22:1179–1186.
24. Shapiro G, Buechler D, Ojea V, Pombo-Villar E, Ruiz M, Weber HP. Tetrahedron Lett 1993;34:6255–6258.
25. Villanueva JM, Collignon N. Tetrahedron 1983;39:1299–1305.
26. Williams L, Zhang Z, Shao F, Carroll PJ, Joullié MM. Tetrahedron 1996;52:11673–11694.
27. For a review of vinyl phosphonate formations see: Minami T, Motoyoehiya J. Synthesis 1992:333–349.
28. Xu Y, Qian L, Prestwich GD. Org Lett 2003;5:2267–2270. [PubMed: 12816425]
29. Selective cleavage of the acetonide in **3a** or **3b** while retaining N-*tert*-butoxy-carbamate was unsuccessful by: Dowex 50×8 in EtOH; Camphorsulfonic acid in MeOH; and 0.5M HCl/THF; and low yielding by cat. p-TsOH in H_2O/MeOH; and trifluoroacetic acid/CH_2Cl_2 followed by Boc_2O, 10% Na_2CO_3 in Dioxane/H_2O.
30. Yonezawa Y, Shimizu K, Yoon K, Shin C. Synthesis 2000;5:634–636.
31. Tse B. J Am Chem Soc 1996;118:7094–7100.
32. Li Z, Racha S, Dan L, El-Subbagh H, Abushanab E. J Org Chem 1993;58:5779–5783.
33. Sun C, Bittman R. J Org Chem 2004;69:7694–7699. [PubMed: 15497998]
34. Lal B, Pramanik BN, Manhas MS, Bose AK. Tetrahedron Lett 1977;23:1977–1980.
35. Reddy PG, Pratap RV, Kumar GDK, Mohanty SK, Baskaran S. Eur J Org Chem 2002:3740–3743.

36. Carlsen PHJ, Katsuki T, Martin VS, Sharpless KB. J Org Chem 1981;46:3936–3938.
37. Coste J, Le-Nguyen D, Castro B. Tetrahedron Lett 1990;31:205–208.
38. Jones L II, Schumm JS, Tour JM. J Org Chem 1997;62:1388–1410.The authors further suggest Urgaonkar S, Verkade JG. J Org Chem 2004;69:5752–5755. [PubMed: 15307751]
39. Reaction times were longer for runs where Pd/C accumulated on the walls of the reaction flask.
40. Preparations of meta-substituted anilines were performed as described in Ref. 38 (Jones II, L.; Schumm, J. S.; Tour, J. M.).
41. Nyasse B, Grehn L, Ragnarsson U. Chem Commun 1997:1017–1018.
42. Parrill AL, Wang D, Bautista DL, Van Brocklyn JR, Lorincz Z, Fischer DJ, Baker DL, Liliom K, Spiegel S, Tigyi G. J Biol Chem 2000;275:39379–39384. [PubMed: 10982820]
43. Awad AS, Ye H, Huang L, Li L, Foss FW, Macdonald TL, Lynch KR, Okusa MD. Am J Phys 2006;290:1516–1524.
44. Matloubian M, Lo CG, Cinamon G, Lesneski MG, Xu Y, Brinkmann V, Allende ML, Proia RL, Cyster JG. Nature 2004;427:355–360. [PubMed: 14737169]
45. Sanna MG, Wang SK, Gonzalez-Cabrera PJ, Don A, Marsolais D, Matheu MP, Wei SH, Parker I, Jo E, Cheng WC, Cahalan MD, Wong CH, Rosen H. Nature Chem Biol 2006;2:434–441. [PubMed: 16829954]

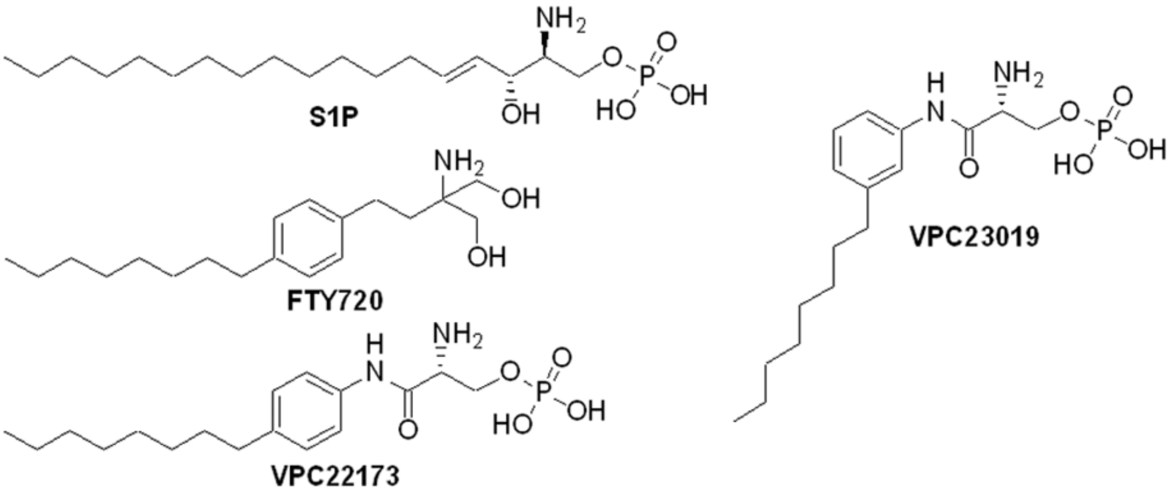


Figure 1.

Structures of endogenous sphingosine 1-phosphate (S1P) and the S1P receptor ligands, FTY720, **VPC22173** and **VPC23019**.

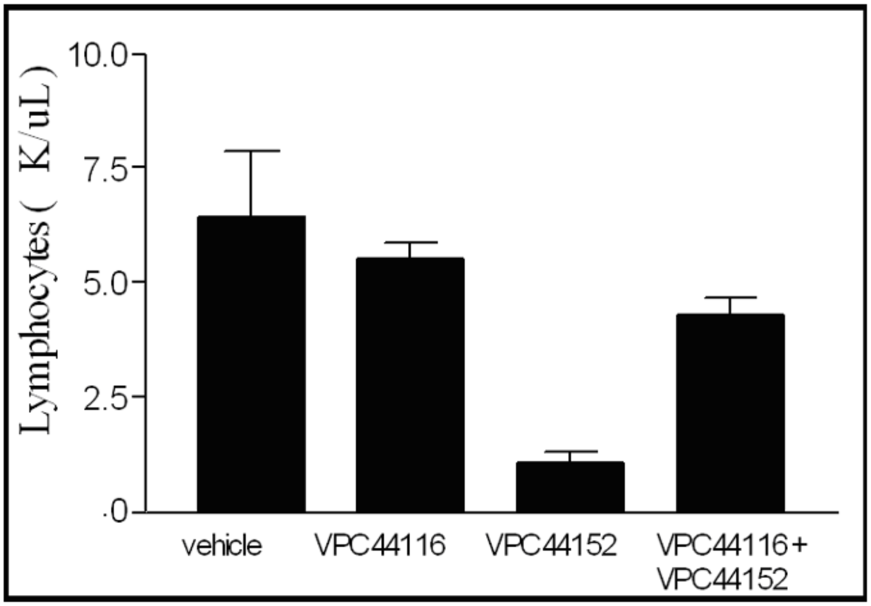


Figure 2.

The lymphopenia evoked by the S1P agonist, **12b** (**VPC44152**) is blocked by co-administration of the S1P receptor antagonist **12d** (**VPC44116**). Groups of 3 C57BL/6 x sv129/J mice injected with vehicle (2% hydroxypropyl β-cyclodextrin), **VPC44116** (22 mg/kg) and/or **VPC44152** (18 mg/kg). After 16 hours, blood was drawn from the orbital sinuses and lymphocytes were measure with a Hemavet blood analyzer. Data are presented as mean ± S.E.

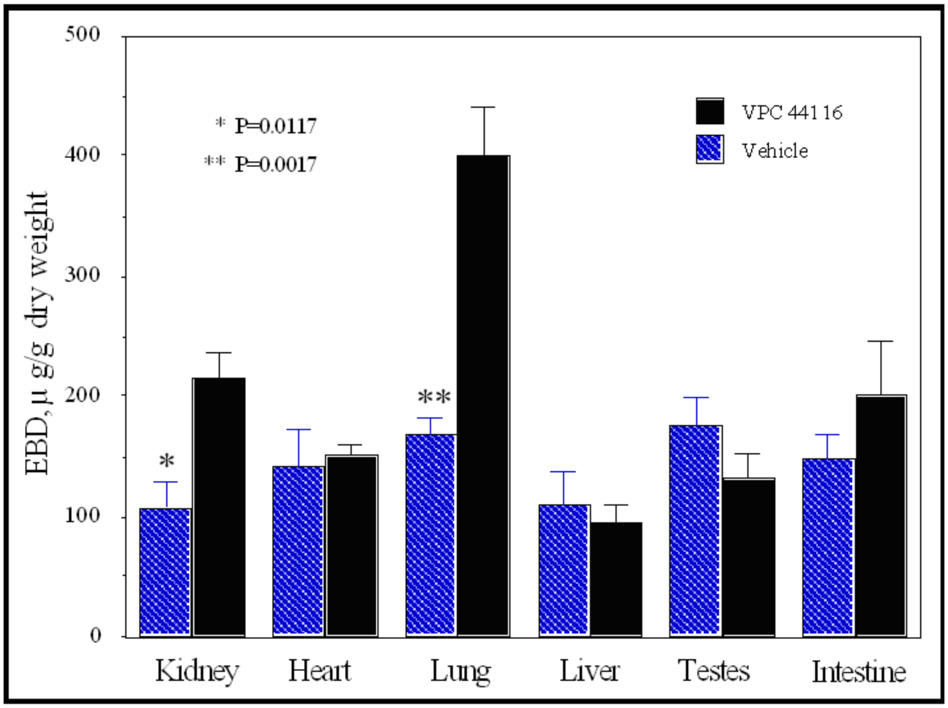
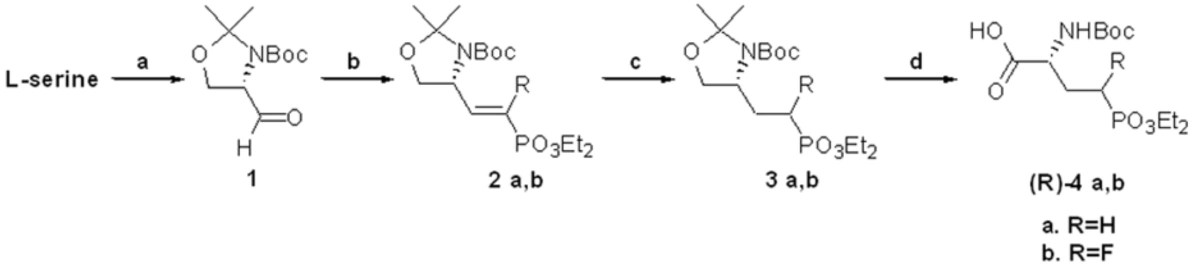


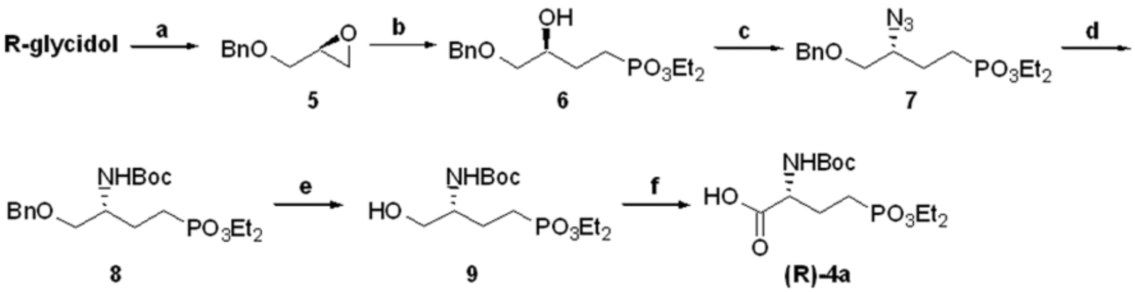
Figure 3.

Effect of **VPC44116** on vascular permeability. C57BL/6 mice were treated with vehicle (2% hydroxypropyl β-cyclodextrin) or **VPC44116** (25 mg/kg) two hours prior to injection 2% Evans Blue dye (EBD) (20 mg/kg) into the jugular vein 30 min. before harvesting tissues. EBD was extracted into formamide, measured in a spectrometer and the amount of extravasated EBD in tissues was calculated from a standard curve. Values are means ± SE; n = 4 for each group. $^{**}P < 0.05$, $^{**}P < 0.01$ compared with vehicle treatment.



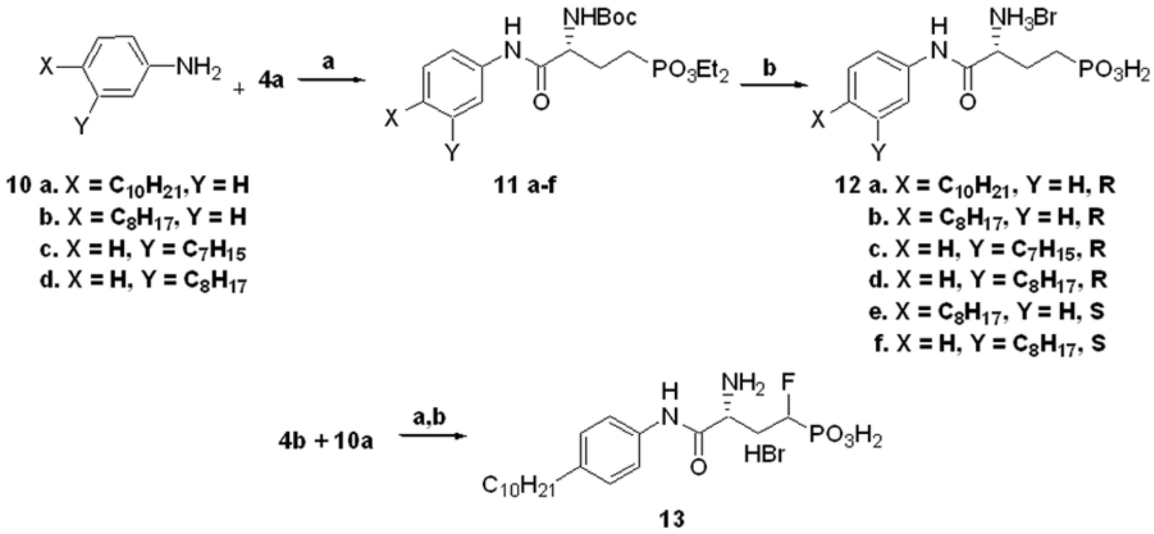
Scheme 1.

Synthesis of **(*3R*)-4a,b** – Method A: a.) i. $SOCl_2$, MeOH, rt, 16h ii. Boc_2O, Et_3N, CH_2Cl_2, rt, 12h iii. 2,2-dimethoxypropane, p-TsOH, CH_2Cl_2, 0 °C to rt, 2h 62% (3 steps) iv. $NaBH_4$, LiCl, 3:2 EtOH/THF, 0 °C to rt, 4h 89% v. DMSO, $(COCl)_2$, CH_2Cl_2, −78 °C, then Et_3N −78 °C to rt, 2–4 h, 97% b.) tetraethyl methylenebisphosphonate, *n*-BuLi, THF, −78 °C, rt, overnight, 75% (**2a**) or tetraethyl 2-fluoromethynebisphosphonate, *n*-BuLi, THF, -78 °C, rt, overnight, 31% (**2b**) c.) H_2, Pd/C, rt., 12h, EtOH, 99% (**3a**) and 88% (**3b**) d.) Jones reagent, acetone, 0 °C to rt., 12h then, isopropyl alcohol, celite, rt, 15min., 59% (**4a**) and 48% (**4b**).



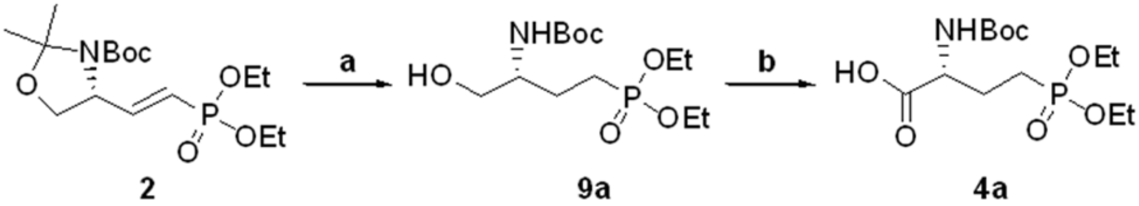
Scheme 2.

Synthesis of ***(3R)***-[or **(*3S*)-**]**4** – Method B: a.) BnBr, DMF, 60% NaH, 0 °C to rt, 3h, 78% b.) $CH_3PO_3Et_2$, *n*-BuLi, BF_3·OEt_2, THF, −78 °C to rt, 3h then, NH_4Cl, 1h, 96% c.) DPPA, DIAD, 3%-polymer-bound $PhPPh_2$, CH_2Cl_2, 0 °C to rt, 20h, 96%, d.) Boc_2O, H_2 (balloon), 20%$^{w/w}$ Lindlar's catalyst, MeOH, rt, 24h 77% e.) H_2 (balloon), Pd/C, EtOH, rt, 24h, 94% f.) TEMPO, bis(acetoxy)iodosobenzene, $NaHCO_3$, 1:1 CH_3CN/H_2O, rt, 3h., 38% or $RuCl_3$·hydrate, $NaIO_4$, 3:2:2 $H_2O/CH_3CN/CCl_4$, rt, 3h, 76%.



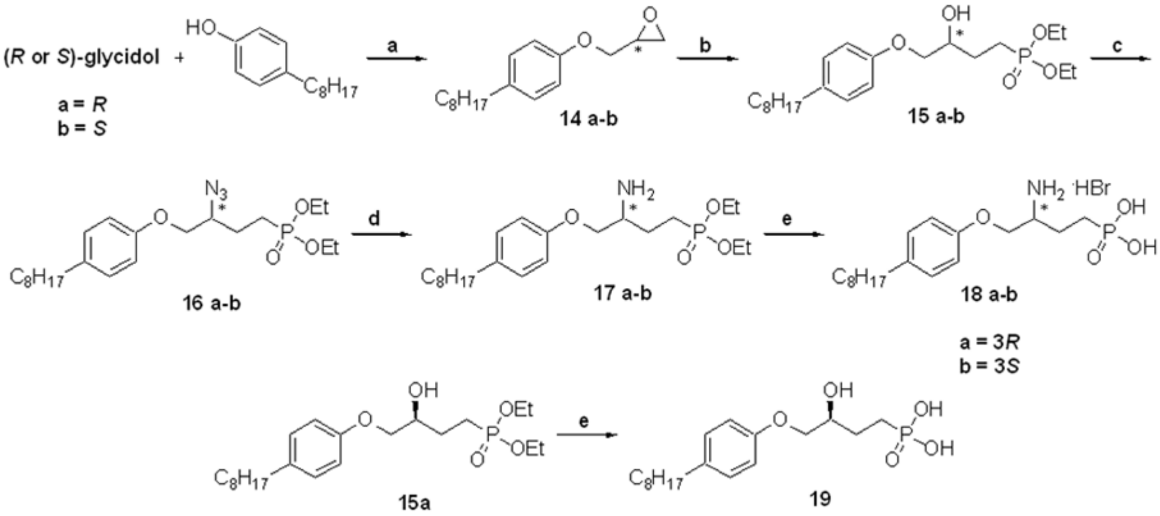
Scheme 3.

Synthesis of arylamide phosphonates **12 a–f & 13**: a) PyBOP, **10 a–d**, di-*iso*- propyl ethylamine (DIEA), CH_2Cl_2, 24–70% b.) TMSBr, CH_2Cl_2, rt, 4-6h then, 95:5 MeOH/H_2O, rt, 1–4h, 45-100%.



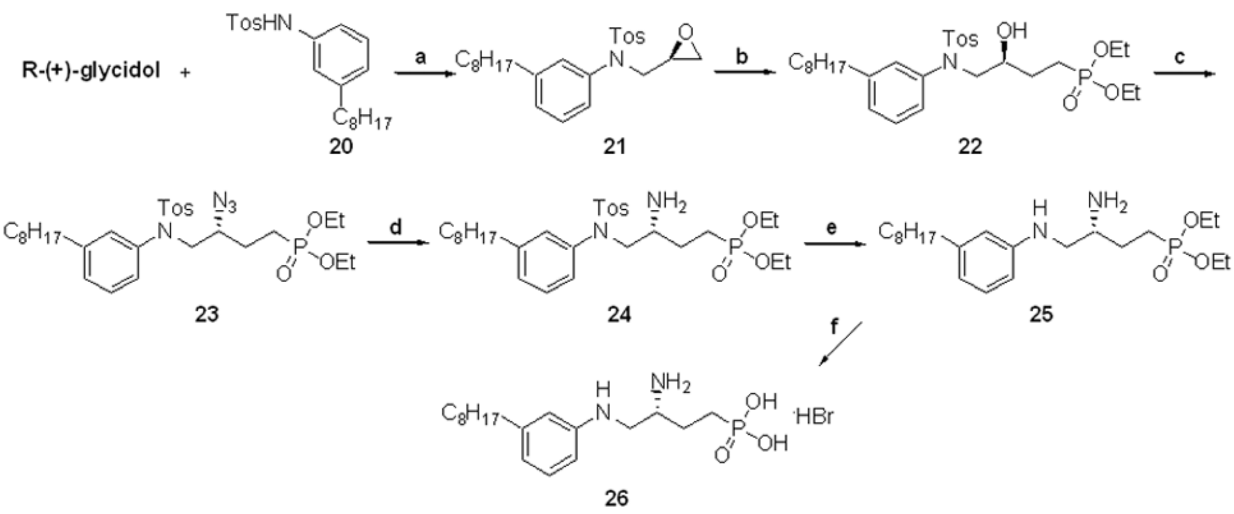
Scheme 4.

Efficient synthesis of **4a** from **2**a: a.) H_2, 10% Pd/C (20 $^{w}/_{w}$% followed by 10 $^{w}/_{w}$%), EtOH, rt, 3d, >95% b.) $RuCl_3{\cdot}H_2O_x$, $NaIO_4$, 2:2:3 $CCl_4/CH_3CN/H_2O$, rt, 1–3h, 79%.



Scheme 5.

Synthesis of arylether phosphonate analogues **18 a–b** and **19**: a.) DIAD, PPh_3, THF, 0 °C to rt, overnight, 80–84% b.) $CH_3PO_3Et_2$, n-BuLi, $BF_3 \cdot OEt_2$, THF, −78 °C to rt, 3h then, NH_4Cl, 1h, 72–87% c.) DPPA, DIAD, PPh_3, CH_2Cl_2, 0 °C to rt, 20h, 67–98% d.) H_2 (balloon), Pd/C, EtOH, formic acid cat., rt, 3.5h, quantitative e.) TMSBr, CH_2Cl_2, rt, 4–6h then, 95:5 MeOH/H_2O, rt, 2–4h, 79–100%.



Scheme 6.

Synthesis of arylamine phosphonate analogue **26**: a.) DIAD, PPh_3, THF, 0 °C to rt, overnight, 69% b.) $CH_3PO_3Et_2$, *n*-BuLi, $BF_3{\cdot}OEt_2$, THF, –78 °C to rt, 2h then, NH_4Cl, 2h, 97% c.) DPPA, DIAD, PPh_3, CH_2Cl_2, 0 °C to rt, 20h, 85% (containing 5% $OPPh_3$) d.) H_2 (balloon), 20%^{w}/^{w} $Pd(OH)_2$, 20:1 MeOH/conc. HCl, 1h, 100% e.)$Na_{(s)}$, $NH_{3(l)}$, –78 °C, 5 min.then EtOH, 25% (recovered 28% starting material) f.) TMSBr, CH_2Cl_2, rt, 4–6h then, 95:5 MeOH/H_2O, 4h, rt, 95%.

Table 1

[γ-^{35}S]-GTP binding assay in HEK293T cells over-expressed with subtype specific S1P receptors.[a]

			Receptors									
			$S1P_1$		$S1P_2$		$S1P_3$		$S1P_4$		$S1P_5$	
Compound	**linker**	**Head Group**	**EC_{50}**[a]	**Emax**	**EC_{50}**	**Emax**	**EC_{50}**	**Emax**	**EC_{50}**	**Emax**	**EC_{50}**	**Emax**
FTY720-P	H_2CCH_2	phosphate	1.3	1.00	NA	0.00	0.1	0.50	4.0	0.90	4.0	0.56
VPC22173	amide	phosphate	58.0	1.10	NA	0.00	450.0	0.35	500.0	1.10	52.0	0.79
VPC2301S	amide	phosphate	NA	0.00	NA	0.00	NA	0.00	120.0	1.13	480.0	0.57
12 a	amide	phosphonate	3.6	0.96	270.0	0.50	43.0	0.88	230.0	0.67	30.0	0.77
b	amide	phosphonate	27.0	0.93	NA	0.00	270.0	0.38	2,300.0	0.80	76.0	0.64
c	amide	phosphonate	NA	0.00	n/a	n/a	NA	0.00	n/a	n/a	n/a	n/a
d	amide	phosphonate	NA	0.00	NA	0.00	NA	0.00	6,100.0	1.42	33.0	0.73
6	amide	phosphonate	1200.00	NA	NA	0.00	15000	0.30	n/a	n/a	n/a	n/a
f	amide	phosphonate	NA	0.00	NA	0.00	NA	0.00	n/a	n/a	n/a	n/a
13	amide	fluorophosphonate	2.1	0.99	490.0	0.47	23.0	0.96	170.0	0.68	19.0	0.77
18 a	ether	phosphonate	68.0	0.70	NA	0.00	NA	0.00	147.0	0.71	31.0	0.41
b	ether	phosphonate	140.0	0.64	530.0	0.50	100.0	0.53	150.0	0.90	47.0	0.50
19	ether	phosphonate	33.0	0.43	NA	0.00	NA	0.00	440,000.0	0.45	NA	0.00
26	amine	phosphonate	30.0	0.56	NA	0.00	NA	0.00	340.0	1.18	41.0	0.61

[a] EC_{50}s are nM and determined by the mean of at least three experiments.

Emax values are normalized to the maximal activation of endogenous S1P, at each receptor.

NA = no activation

n/a = not available