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α- and β-Substituted phosphonate analogs of LPA as autotaxin inhibitors

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Abstract—Autotaxin (ATX) is an attractive pharmacological target due to its lysophospholipase D activity which leads to the production of lysophosphatidic acid (LPA). Blockage of ATX produced LPA by small molecules could be a potential anticancer chemotherapy. In our previous study, we have identified the two β-hydroxy phosphonate analogs of LPA (compounds **f17** and **f18**) as ATX inhibitors. With this work, we investigated α- and β-substituted phosphonate analogs of LPA and evaluated them for ATX inhibitory activity. The stereochemistry of β-hydroxy phosphonates was also studied.
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1. Introduction

Autotaxin (ATX, NPP2) is a member of the nucleotide pyrophosphatase and phosphodiesterase (NPP) family of enzymes which are able to hydrolyze phosphoester bonds in nucleotides.[1,2] It can be biosynthesized by a variety of cells and tissues and highly expressed in various malignancies.[3–8] ATX is a multifunctional enzyme which potently stimulates cancer cell proliferation and tumor cell motility, augments the tumorigenicity, and induces angiogenic response.[3,9–14] In 2002, two independent laboratories discovered that ATX is identical to plasma lysophospholipase D (lysoPLD) and acts by hydrolyzing lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA).[15,16] LPA is a lipid mediator that signals cell proliferation, migration, and survival by activating its specific extracellular and intracellular receptors.[17–27] The generation of LPA by ATX can fully account for the biological effects of ATX in cell culture. Considering the fact that ATX is the major enzyme in biosynthesis of LPA in plasma, the development of ATX inhibitors could lead to novel anticancer chemotherapies.

Keywords: Autotaxin; ATX; Phosphonates; LPA.
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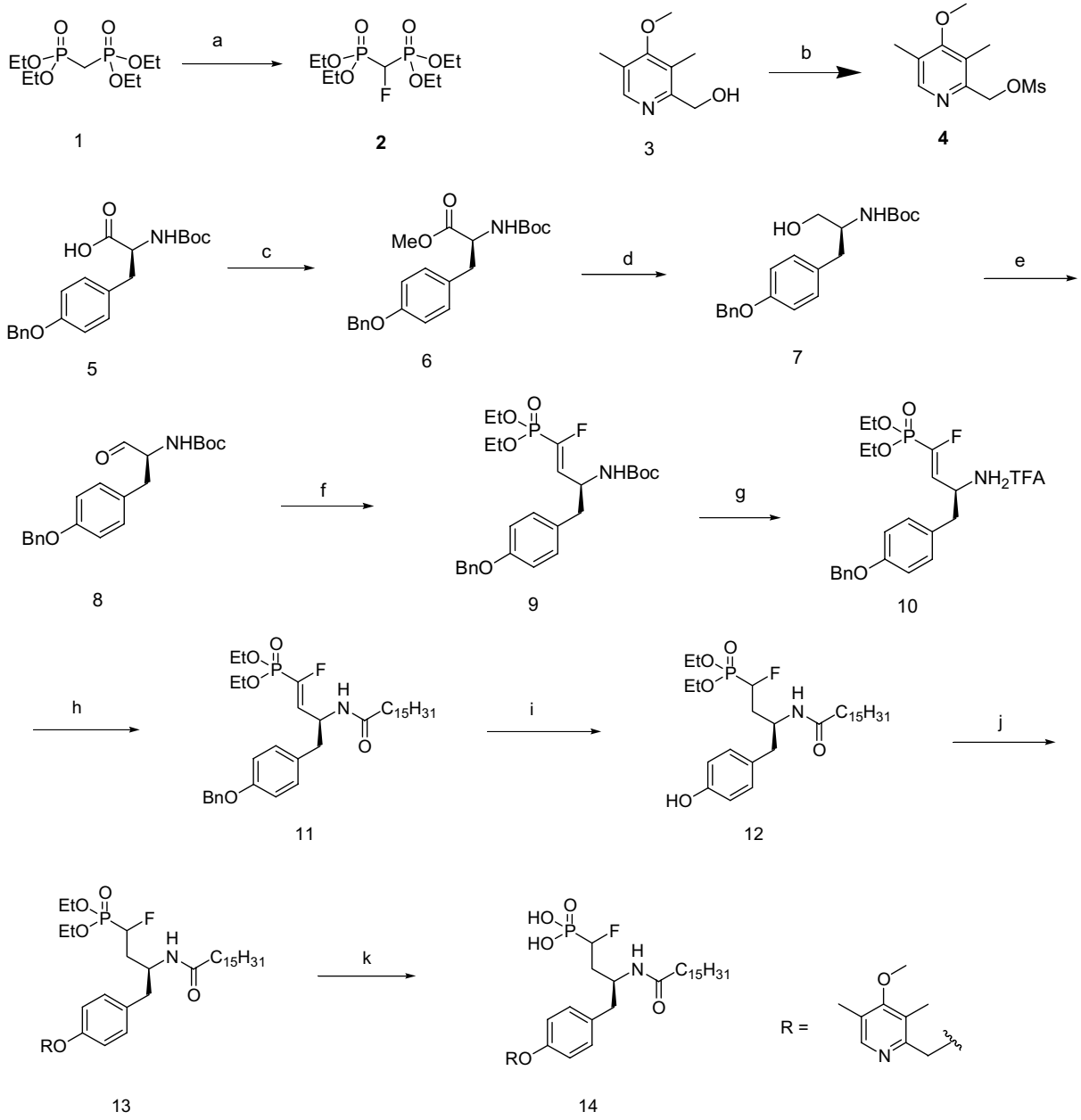
There are a limited number of ATX inhibitors which have been reported.[28–32] In our laboratory, we have developed a series of β-keto and β-hydroxy phosphonate derivatives and tested the ATX inhibitory activity in a choline detection assay.[33] Among them, two β-hydroxy phosphonates originated from L-tyrosine were identified as the lead compounds and they inhibited approximately 70% of ATX activity at 1 μM. The pK_a of the second phosphonate proton (~7.6) can be influenced by the substitutions on the α- and β-positions of the phosphonate.[34] This electronic property is believed to affect enzyme–substrate interaction.[35] In this report, we synthesized both α- and β-substituted phosphonate analogs based on the lead compounds and investigated their activities on ATX inhibition.

2. Results and discussion

2.1. Chemistry

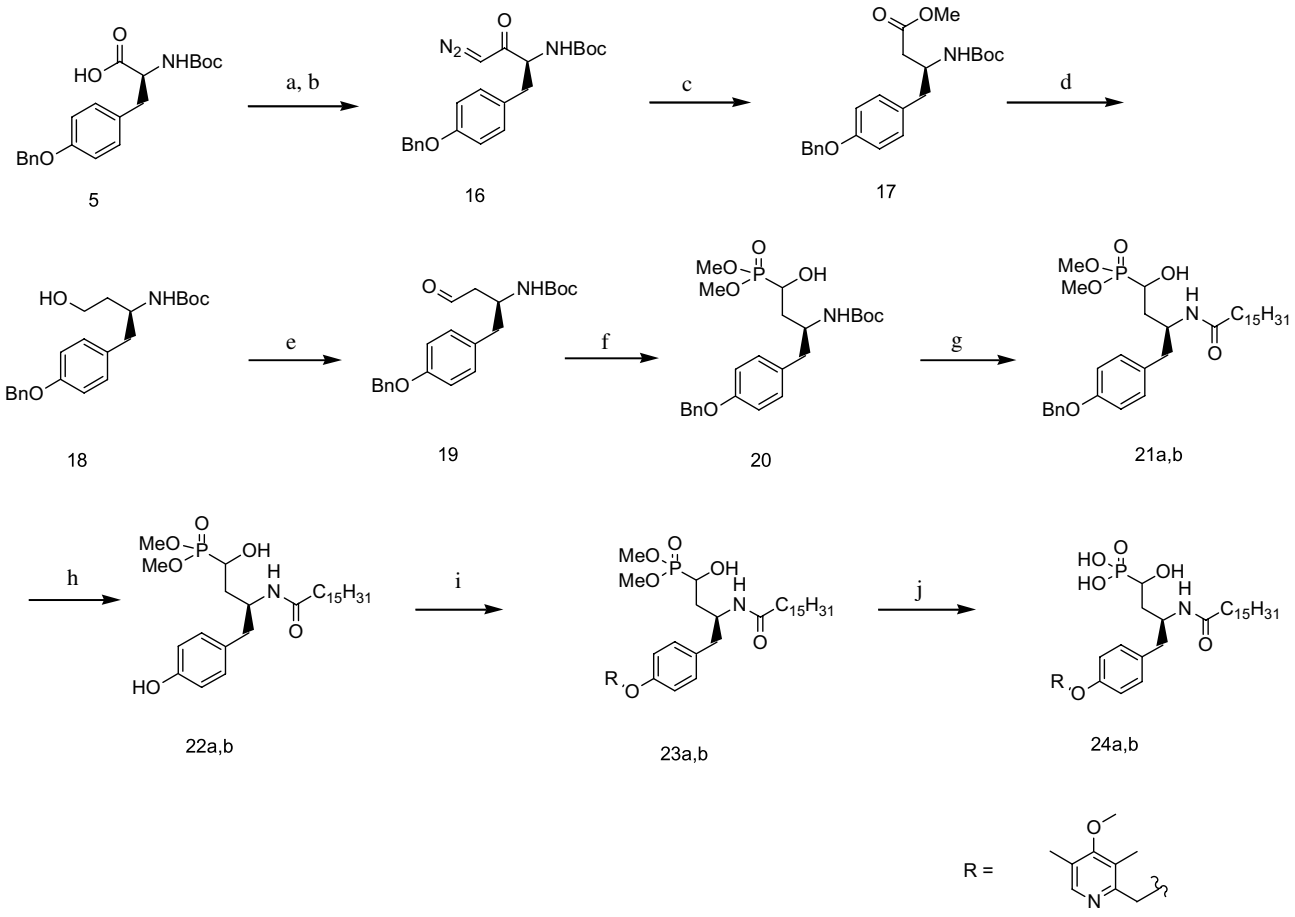
Synthesis of α-fluoro phosphonates (Scheme 1) began with esterification of the free acid of *N*-*tert*-butyl carbamate, *O*-benzyl protected L-tyrosine **5**, with trimethylsilyl diazomethane. The methyl ester **6** was reduced to the corresponding alcohol with sodium borohydride in the presence of calcium chloride and then oxidized to the desired aldehyde **8** under the Swern oxidative condition. The tetraethyl monofluoromethylene diphosphonate **2** was formed by the fluorination of the tetraethyl



Scheme 1. Synthesis of **14**. Reagents and conditions: (a) NaH, THF, 0 °C; Selectfluor, rt, overnight, 56%; (b) NEt_3, CH_2Cl_2, 0 °C, then MsCl, 5 h, 90%; (c) TMS–CHN_2, 0 °C, 10 min, anhydrous MeOH, 100%; (d) $NaBH_4$, $CaCl_2$, THF, EtOH, 3 h, 0 °C—rt, 94%; (e) oxalyl chloride, DMSO, CH_2Cl_2, −78 °C, NEt_3, 2 h, 83%; (f) **2**, *n*-BuLi, THF, −78 °C, 2 h, 70%; (g) TFA, CH_2Cl_2, rt, 5 h; (h) palmitoyl chloride, TEA, 0 °C, 3 h; (i) $Pd(OH)_2$, H_2, EtOH, rt, overnight, 85%, three steps; (j) **4**, K_2CO_3, acetone, 18-crown-6, reflux, overnight, 90%; (k) TMS–Br, then MeOH and H_2O, rt, overnight, 82%.

diphosphonate anion with Selectfluor.[36] Next, treatment of the monofluorodiphosphonate **2** with *n*-butyl lithium at −78 °C generated the lithiated carbanion, which condensed with the aldehyde to afford α-fluorovinylphosphonate **9**. The protecting N-Boc group was removed by treatment of trifluoroacetic acid and the remaining ammonium trifluoroacetic acid salt was acylated with palmitoyl chloride to generate amide **11**. After dual hydrogenation/hydrogenolysis of the olefin and benzyl group, respectively, the resulting phenol **12**, which was a mixture of two diastereomers, was coupled with the mesylate **4** under basic conditions to give the ether mixture **13**. The α-fluoro phosphonate **14** was obtained by trimethylsilyl bromide mediated deprotection of diethyl esters.[37]

Synthesis of α-hydroxy phosphonates (Scheme 2) commenced with the Arndt–Eistert reaction. Treatment of *N-tert*-butyl carbamate, *O*-benzyl ether protected L-tyrosine **5** with isobutyl chloroformate gave the mixed anhydride. The diazoketone **16** was prepared by adding the pre-cooled freshly distilled diazomethane to the mixed anhydride. Next, a carbene mediated Wolff rearrangement furnished the transformation from a diazoketone to a ketene, which underwent a nucleophilic addition by methanol to afford the desired homologated



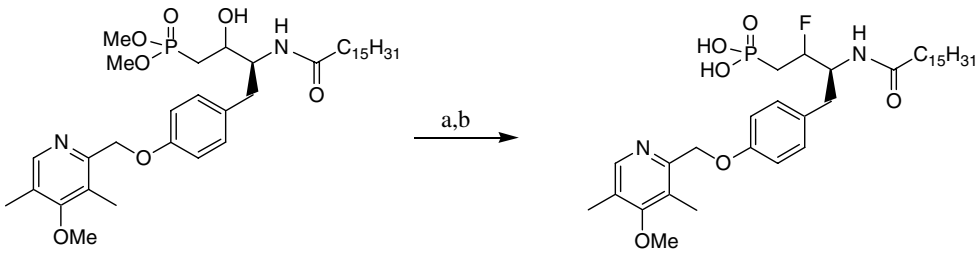
Scheme 2. Synthesis of **24a** and **b**. Reagents and conditions: (a) KOH, 65 °C, isobutyl chloroformate, THF, NEt_3, 0 °C; (b) CH_2N_2, overnight, 51%; (c) silver benzoate, NEt_3, MeOH, THF, −78–0 °C, overnight, 73%; (d) $NaBH_4$, $CaCl_2$, THF, EtOH, 0 °C—rt, overnight, 97%; (e) oxalyl chloride, DMSO, CH_2Cl_2, −78 °C, NEt_3, 2 h, 94%; (f) dimethyl phosphite NaH, THF, 0 °C, 1 h, 60%; (g) TFA, CH_2Cl_2, rt, 5 h, then palmitoyl chloride, NEt_3, CH_2Cl_2, 0 °C, 3 h, 82%; (h) $Pd(OH)_2$, H_2, EtOH, rt, overnight, 58–73%; (i) 4-methoxy-3,5-dimethyl-2-pyridine-methanol, DIAD, PPh_3, NEt_3, CH_2Cl_2, 0 °C—rt, overnight, 80%; (j) TMS–Br, then MeOH and H_2O rt, overnight, 90%.

methyl ester **17**. The aldehyde **19** was obtained by a two-step reduction/oxidation process. Then the sodium anion of dimethyl phosphite was generated and reacted with the aldehyde to give α-hydroxy phosphonates **20**. Next, the N-Boc protecting group was removed and the resulting ammonium trifluoroacetate salt was acylated with palmitoyl chloride to generate amides **21a** and **b**. The two diastereomers in terms of α-hydroxy group were separated by column chromatography at this step. The exact stereochemistry for each diastereomer was determined (unpublished results). The benzyl ether was cleaved by treatment with palladium hydroxide on carbon and the resulting phenols **22a** and **b** were reacted with 4-methoxy-3,5-dimethyl-2-pyridine-methanol under Mitsunobu condition to give **23a** and **b**. Finally, the fully functionalized α-hydroxy phosphonates were obtained by the same method mentioned above.

The transformation of the β-hydroxy phosphonates (**49a** and **b**)[33] to β-fluoro phosphonates (**25a** and **b**) was accomplished by using diethylaminosulfur trifluoride (DAST) (Scheme 3). The product was then formed with the above-mentioned deprotection of the phosphonate group using TMS–Br.

2.2. Biological evaluation

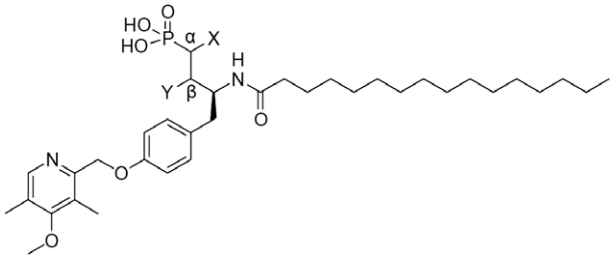
The ATX activity was measured in the presence of the compounds under different concentrations (100 μM, 10 μM, and 1 μM). The ATX activity without compounds was used as the standard (100% activity). The data are shown in Table 1. α-Hydroxy (**24a** and **b**) and α-fluoro (**14a** and **b**) phosphonates did not show any substantial ATX inhibition. The β-fluoro phosphonate demonstrated the same levels of activity as the lead compounds (**f17** and **f18**)[33] at higher concentrations (10 μM and 100 μM); however it lost some potency at the lowest concentration (1 μM). Further optimization was attempted on β-hydroxy and β-keto phosphonates. We investigated a variety of natural amino acid backbones and the compounds and data are shown in Table 2. Most of the compounds could inhibit 50–80% ATX activity at the highest concentration and lost the potency when lower concentrations were applied. The β-keto phosphonate derived from L-proline **38** has proved to be the most potent β-keto phosphonate which could inhibit 80% of ATX activity at medium concentration (10 μM). Tryptophan was chosen to compare with the linker region in tyrosine. The compounds were not as



49a,b 25a,b

Scheme 3. Synthesis of **25a** and **b**. Reagents and conditions: (a) DAST, CH_2Cl_2, −78 °C, 1 h, 50%; (b) TMS–Br, CH_2Cl_2, rt, 4 h, then H_2O and MeOH, overnight, 62%.

Table 1. ATX inhibitory evaluation of compounds **14**, **24**, and **25**



Compounds	X	α	Y	β	ATX activity (%)		
					1 μM	10 μM	100 μM
24a	OH	*S*	H		71	70	53
24b	OH	*R*	H		73	68	87
14	F[a]	*RS*	H		74	68	25
f18	H		OH	*b*[b]	63	21	8
f17	H		OH	*a*	27	13	6
25	H		F[a]	*RS*	59	22	11

[a] Racemic products were tested.
[b] *a* refers to the diastereomer that elutes first, *b* refers to the diastereomer that elutes second from normal phase silica gel based column chromatography.

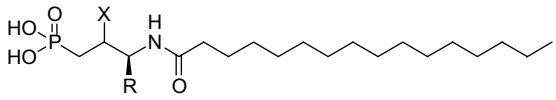
potent as the lead compounds and the β-hydroxy phosphonate with 4-methoxy-3,5-dimethyl-pyridyl moiety **44** did show higher potency than the one with benzyl group **45**.

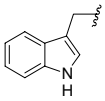
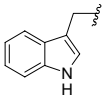
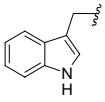
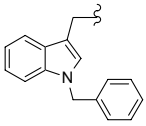
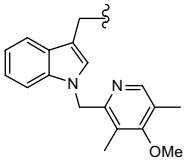
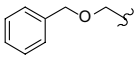
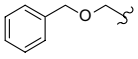
2.3. Structure determination of lead compounds

The lead compounds **f17** and **f18** are diastereomers originating from reduction of β-keto phosphonate. To further study this reaction and determine the absolute stereochemistry of β-hydroxy phosphonates, a variety of reductive agents and reaction conditions were applied (Table 3). Sodium borohydride gave diastereoselectivity in 1:2.5 ratio favoring the more polar isomer. Lewis acid mediated reduction gave higher reaction yields but lost the diastereoselectivity. Application of bulky hydride reducing reagents such as lithium triethylborohydride (Super-Hydride) and lithium tris[(3-ethyl-3-pentyl)oxy]aluminohydride resulted in lower reaction yields but significantly improved the selectivity.

The relationship between the dihedral angle and the vicinal coupling constant ^3J was given theoretically by the Karplus relationship.[38] Due to the single bond rotation the coupling constants are revealed as an average value contributed from relatively stable rotational isomers. It is expected that the ^3J difference between *syn* and *anti* isomers could be enlarged if the hydroxyl group and amide in the β-hydroxy phosphonate substrate are fixed in a ring form which prevents a free rotation of carbon bond. Oxazolidines **47** and **48** were prepared (Scheme 4) from β-hydroxy phosphonates **49a** and **49b** (**49a** was the less polar isomer and **49b** was the more polar isomer). The results of the decoupling study show that the *J* values between geminal benzylic protons H_3 and H_4 are approximately 14 Hz in both oxazolidines (Fig. 1). These two protons couple with H_2 to give *J* values corresponding to 6 Hz and 9 Hz, respectively. The ^3J values between H_1 and H_2 are close to 0 Hz in **47** and 5 Hz in **48**. According to the Karplus relationship, **47** has the *anti* configuration and the less polar isomer **49a** corresponds to the *R* alcohol; **48** has the *syn* configuration and the more polar isomer **49b** corresponds to the *S* alcohol. This result is consistent with the reported ^3J values of oxazolidone derivatives of α-amino-β-hydroxy acids.[39,40] Taken into account the outcome of diastere-

Table 2. ATX inhibitory evaluation of compounds **26**–**46**



Compounds	X	R	ATX activity (%)		
			1 μM	10 μM	100 μM
26	=O	–H	79	95	76
27	–OH[a]	–H	88	88	56
28	=O	$-CH_3$	60	67	72
29	–OH[a]	$-CH_3$	50	14	25
30	=O	$-CH(CH_3)_2$	109	77	24
31	–OH (*a*)[b]	$-CH(CH_3)_2$	115	57	13
32	–OH (*b*)[b]	$-CH(CH_3)_2$	72	78	23
33	=O	$-CH_2CH(CH_3)_2$	89	48	25
34	–OH (*a*)	$-CH_2CH(CH_3)_2$	68	53	23
35	–OH (*b*)	$-CH_2CH(CH_3)_2$	93	64	52
36	–OH (*a*)	$-CH_2Ph$	88	70	70
37	–OH (*b*)	$-CH_2Ph$	92	99	67
38	=O	$-CH_2CH_2CH_2-$[c]	49	18	9
39	–OH[a]	$-CH_2CH_2CH_2-$[c]	83	43	16
40	=O		91	70	28
41	–OH (*a*)		88	51	25
42	–OH (*b*)		93	75	26
43	–OH[a]		80	35	28
44	–OH[a]		64	28	13
45	–OH (*a*)		83	41	19
46	–OH (*b*)		78	46	61

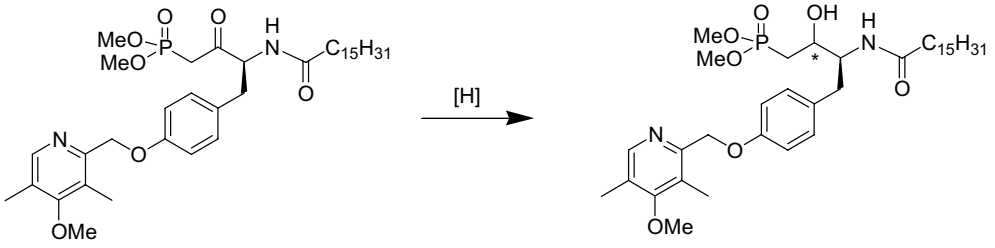
[a] Racemic products were tested.
[b] *a* refers to the diastereomer that elutes first, *b* refers to the diastereomer that elutes second from normal phase silica gel based column chromatography.
[c] The compounds were synthesized from protected L-proline.

oselectivity, the reaction is likely governed by Felkin–Ahn model (Fig. 2).

3. Conclusion

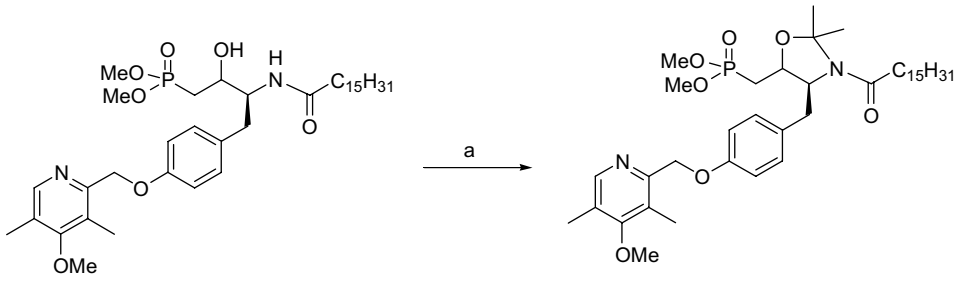
We have synthesized a series of α-/β-substituted phosphonate analogs of LPA and evaluated them for ATX inhibitory activity. The β-substituted analogs showed higher potency than the α-substituted analogs. Further structural optimization was attempted on β-keto and β-hydroxy phosphonates. We investigated a variety of amino acid backbones. Some analogs showed comparable potency with the lead compounds (**f17** and **f18**) at high concentrations (10 μM and 100 μM). However, at the lowest concentration (1 μM), these newer analogs

Table 3. Reduction of β-keto phosphonate



Entry	Hydride	Conditions	Yield (%)	Ratio (*a*:*b*)[a]
1	$NaBH_4$	EtOH, 0 °C	75	1:2.5
2	$NaBH_4$, $CeCl_3$	EtOH, −78 °C	82	1:1
3	$NaBH_4$, $CeCl_3$	EtOH, 0 °C	85	1:1
4	$NaBH_4$, $MgCl_2$	EtOH, 0 °C	60	1:1
5	$Li(C_2H_5)_3BH$	THF, 0 °C	33	1:9
6	$LiAl[OC(C_2H_5)_3]_3H$	THF, 0 °C	40	1:11
7	Catecholborane	THF, 0 °C	NR[b]	NR

[a] *a* refers to the diastereomer that elutes first, *b* refers to the diastereomer that elutes second.
[b] NR, no reaction.



Scheme 4. Synthesis of **47** and **48**. Reagents and conditions: (a) 2-methoxypropene, CSA, CH_2Cl_2, 0 °C, 30–35%.

showed reduced potency compared to the lead compounds. The stereochemistry of the β-hydroxy phosphonates was also determined by ^1H homonuclear decoupling study. The most potent compound (**f17**) was proven to be a β-hydroxy phosphonate with *R*-hydroxy moiety. Additional synthetic efforts are needed to fully elucidate the SAR of the phosphonate analogs and toward the goal of developing potent ATX inhibitors as the potential anticancer chemotherapy.

4. Experimental

4.1. Materials

Chemicals for syntheses were purchased from Aldrich Chemical Company (Milwaukee, WI), Acros Chemical Company (Morris Plains, NJ), Novabiochem Chemical Company (Laufelfingen, Switzerland), and Fluka Chemical Company (Milwaukee, WI), and were used without further purification. All reactions were carried out under an inert atmosphere of nitrogen unless otherwise stated. All solvents were filtered through alumina (activity 1) immediately prior to use in reactions. In certain case, THF was filtered through alumina and subsequently distilled over a mixture of sodium and benzophenone immediately prior to use. Merck silica gel F-254 precoated, aluminum backed plates and Merck Silica Gel 60 (230–400 mesh) or Silicycle Ultra pure silica gel (230–400 mesh) were used in thin layer chromatography and flash column chromatography, respectively. Analtech (Newark, NJ) alumina GF Preparative TLC plates (500 μm, 1000 μm, and 2000 μm 20 × 20 cm) were used for preparative TLC separation. All nuclear magnetic resonance (NMR) spectra were collected using a Varian UnityInova 300 NMR spectrometer at 300 MHz and chemical shifts are reported in ppm.

4.2. General procedures

4.2.1. General procedure A: acylation of amines. To a solution of the amine (1 equiv) to be acylated and TEA (3 equiv) in CH_2Cl_2 at 0 °C was added the appropriate acyl chloride dropwise by syringe. The reaction mixture was then allowed to stir for several hours. After completion of the reaction as determined by TLC, it was quenched with 10% aqueous HCl and further extracted with 10% aqueous HCl. The aqueous layer was then ex-

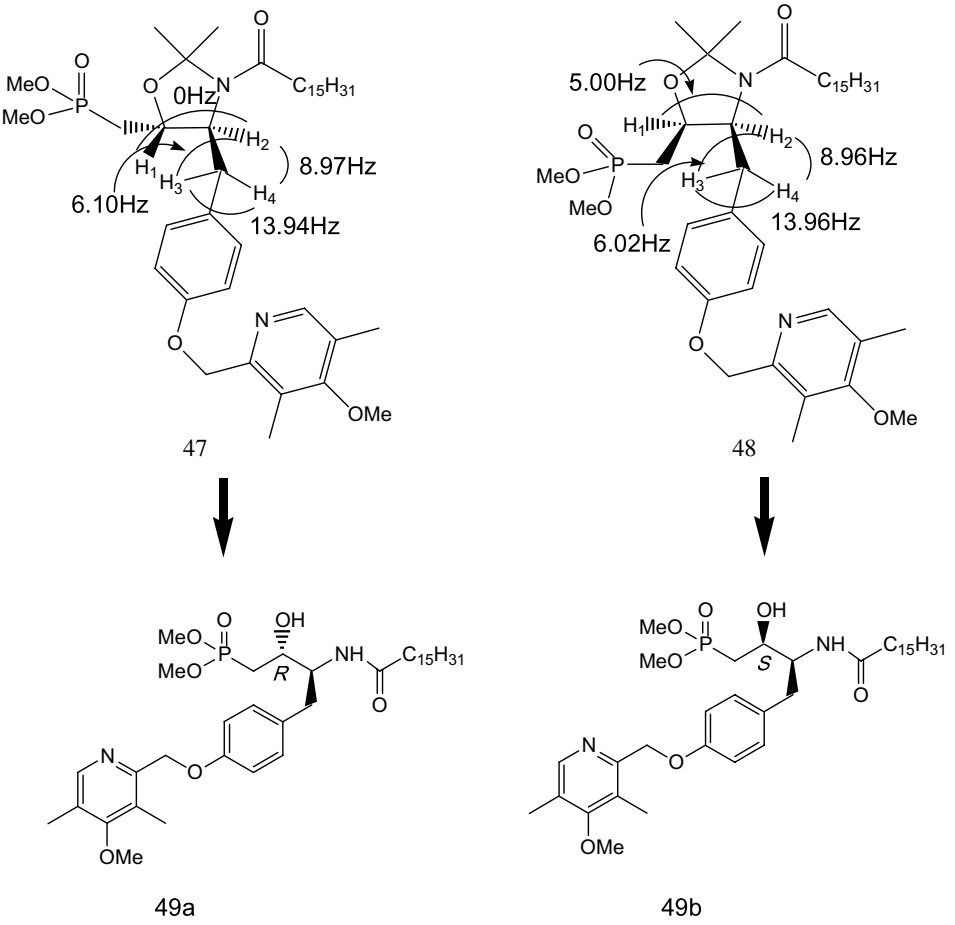


Figure 1. ^{1}H homonuclear decoupling study.

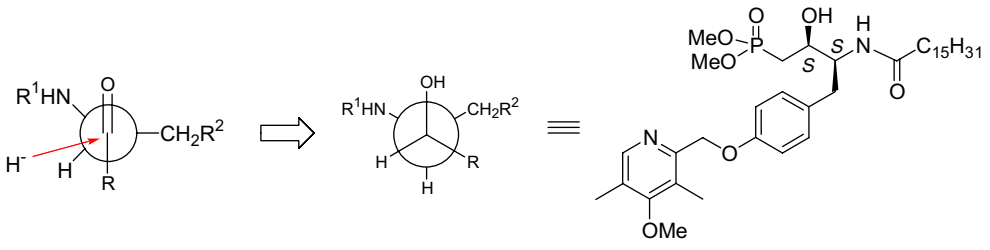


Figure 2. Modified Felkin–Ahn model of reductive reaction.

tracted once with CH_2Cl_2 followed by a brine extraction of the organic layer. The organic layer was then dried using magnesium sulfate, filtered, and concentrated under reduced pressure. Then the product was purified using the appropriate purification techniques.

4.2.2. General procedure B: palladium hydroxide on carbon mediated hydrogenolysis of benzyl ethers. To a solution of the benzyl ether to be cleaved in ethanol was added a catalytic amount of 20% palladium hydroxide on carbon; the reaction was then subjected to a hydrogen atmosphere (typically 1ATM) and was allowed to stir overnight. The following day the reaction mixture was filtered through a pad of Celite to remove all palladium using methanol as the eluent followed by removal of all solvent under reduced pressure. Then any purification that was deemed necessary was undertaken.

4.2.3. General procedure C: sodium borohydride reduction of esters. $NaBH_4$ (2 equiv) and $CaCl_2$ (2 equiv) are dissolved in ethanol so that a solution of milky appearance is formed. This solution is cooled to 0 °C and the ester to be reduced is dissolved in THF, so that a 2:1 ethanol/THF mixture will be the resulting solution ratio. Then the ester in THF is added dropwise to the reaction mixture via canula. The reaction mixture is allowed to slowly warm to rt and then it is stirred overnight. The following day the reaction is quenched with a saturated solution of aqueous ammonium chloride, then extracted with EtOAc, brine, dried with sodium sulfate, and concentrated under reduced pressure. The product is then

either purified via flash column chromatography or used unpurified.

4.2.4. General procedure D: Swern oxidation of alcohols. To dry dichloromethane in a flame-dried RBF was added oxalyl chloride (1.5 equiv, 2 M in CH_2Cl_2), the mixture was then cooled to −78 °C before a solution of dimethylsulfoxide (3 equiv) in CH_2Cl_2 was added dropwise via syringe. The resulting mixture was stirred at this temperature for 30 min before a solution of the alcohol (1 equiv) to be oxidized in CH_2Cl_2 was added dropwise via canula. The mixture was stirred for 30 min before triethyl amine was added via syringe; the resulting mixture was stirred for an additional 15 min before it was warmed to rt. Then a 10% HCl solution in water was added to the reaction mixture until it turned acidic as indicated by pH paper. Then the organic layer was extracted with another small portion of 10% HCl, then the aqueous layer was twice back extracted with CH_2Cl_2. The organic layer was further extracted with brine, dried using magnesium sulfate, filtered, and then concentrated under reduced pressure. The resulting aldehyde was either purified using flash column chromatography or used unpurified.

4.2.5. General procedure E: mesylate coupling. To a stirring solution of phenol (1 equiv), potassium carbonate (4 equiv), and appropriate mesylate (or alkyl halide) (1.2 equiv) in acetone was added a catalytic amount of 18-crown-6 and the reaction mixture was heated to reflux. The reaction was maintained at this temperature overnight and the next morning allowed too cool to room temperature. After sufficient cooling the reaction mixture was concentrated under reduced pressure and then the resulting mixture was solvated with ethyl acetate and extracted with ammonium chloride, then the aqueous layer was further extracted with ethyl acetate, the organic layer was washed with brine, dried with sodium sulfate, filtered, and then concentrated under reduced pressure. The product was then purified using flash column chromatography.

4.2.6. General procedure F: synthesis of β-keto phosphonates. To a solution of diethylmethylphosphonate (1 equiv) in THF at −78 °C was added *n*-BuLi (1.1 equiv, 2.5 M) dropwise via syringe. The reaction mixture was allowed to stir at this temperature for 1 h before a solution of the ester (1.05 equiv) in THF was added. The reaction mixture was stirred for an additional hour before a saturated aqueous solution of ammonium chloride was added and the reaction mixture was allowed to slowly warm to room temperature. Then the reaction mixture was extracted with ethyl acetate, followed by brine, dried over sodium sulfate and concentrated under reduced pressure. The product was then purified using flash column chromatography.

4.2.7. General procedure G: sodium borohydride reduction of ketones. $NaBH_4$ (2 equiv) is dissolved in ethanol so that a solution of milky appearance is formed. This solution is cooled to 0 °C and the ketone to be reduced is dissolved in THF, so that a 2:1 ethanol/THF mixture will be the resulting solution ratio. Then the ester in THF is added dropwise to the reaction mixture via canula. The reaction mixture is allowed to slowly warm to rt and then it is stirred overnight. The following day the reaction is quenched with a saturated solution of aqueous ammonium chloride, then extracted with EtOAc, brine, dried with sodium sulfate, and concentrated under reduced pressure. The product was then purified using flash column chromatography.

4.2.8. General procedure H: bromotrimethylsilane mediated deprotection of phosphate and phosphonate esters. To a stirring solution of the ester to be deprotected is added bromotrimethylsilane (10 equiv). The reaction mixture is then allowed to stir for about 4 h and progress is monitored by TLC; after completion of the reaction the solvent is removed under reduced pressure and then the resulting oil is solvated with 95% methanol 5% water and the reaction mixture is allowed to stir overnight. The following day the solvent is removed under reduced pressure and the appropriate purification technique is employed.

4.2.9. General procedure I: mitsunobu coupling. To a solution of the alcohol (2 equiv), the coupling phenol or acid (1 equiv), diisopropylethyl amine (2 equiv), and polymer bound triphenylphine (2 equiv) in CH_2Cl_2 at 0 °C was added diisopropylazodicarboxylate (2 equiv). The reaction mixture was then allowed to slowly warm to room temperature and to stir overnight; the following day the reaction mixture is diluted with methanol and filtered through a pad of Celite using methanol as the eluent. The organic layer is then concentrated under reduced pressure and the product is purified using flash column chromatography.

4.3. Synthesis of compounds 2–25, 47, and 48

4.3.1. [(Diethoxy-phosphoryl)-fluoro-methyl]-phosphonic acid diethyl ester (2). To a solution of sodium hydride (700 mg, 60%, 17.5 mmol) in THF at 0 °C was added a solution of (diethoxy-phosphorylmethyl)-phosphonic acid diethyl ester (5 g, 17.3 mmol) in THF. The reaction mixture was allowed to stir at this temperature for 1 h before solid Selectfluor (6.146 g, 17.3 mmol) was added to the mixture. The reaction mixture was stirred at room temperature overnight and then the solid was filtered off through a pad of Celite using methanol as the eluent, concentrated under reduced pressure, and purified using flash column chromatography (4% MeOH:EtOAc, stained with Henessian stain) to yield the monofluorinated product in 3.0 g (56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (t, 12 H, J = 7.31 Hz), 4.28 (p, 8H, J = 8.05 Hz), 5.50 (m, 1H).

4.3.2. Methanesulfonic acid 4-methoxy-3,5-dimethyl-pyridin-2-ylmethyl ester (4). To a stirring solution of (4-methoxy-3,5-dimethyl-pyridin-2-yl)-methanol (500 mg, 3.0 mmol) and triethylamine (0.63 ml, 4.52 mmol) in CH_2Cl_2 at 0 °C was slowly added methane sulfonylchloride (0.28 ml, 3.62 mmol) via syringe. The reaction mixture was slowly warmed to room temperature and stirred for an additional 4 h at which time the reaction was stopped. It was stopped prematurely and some

starting material was retained. The solvent was removed under reduced pressure and then the resulting deep red oil was placed directly onto a flash column and purified via flash column chromatography (1:1 EtOAc/hexanes) to give 660 mg product (90%). ^{1}H NMR (300 MHz, $CDCl_3$) 2.15 (s, 3H), 2.24 (s, 3H), 3.67 (s, 3H), 4.57 (s, 2H), 8.10 (s, 1H); ^{13}C NMR δ 11.11, 13.54, 46.02, 60.12, 126.04, 126.79, 149.60, 154.73, 164.53.

4.3.3. 3-(4-Benzyloxy-phenyl)-2-*tert*-butoxycarbonylamino-propionic acid methyl ester (6). To a solution of N-Boc *O*-Bnz L-tyrosine (2.5 g, 6.74 mmol) in anhydrous methanol was added trimethylsilyl diazomethane (2 M in ether, 5.1 ml, 10.1 mmol) dropwise to the stirring mixture at 0 °C until no more nitrogen gas was evolved and a bright yellow color persisted (about 20 min). The mixture was then concentrated under reduced pressure and purified by flash column chromatography (20% EtOAc:hexanes) to yield the crude product 2.8 g. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 9H), 3.03 (m, 2H), 3.69 (s, 3H), 4.57 (m, 1H), 5.02 (s, 2H), 5.08 (m, 1H), 6.91 (d, 2H, J = 8.42 Hz), 7.05 (d, 2H, J = 8.42 Hz), 7.31–7.44 (m, 5H). ^{13}C NMR δ 28.58, 37.72, 52.41, 54.86, 70.22, 115.20, 127.75, 128.23, 128.63, 128.85, 130.61, 137.31, 158.17, 172.70.

4.3.4. [1-(4-Benzyloxy-benzyl)-2-hydroxy-ethyl]-carbamic acid *tert*-butyl ester (7). This compound was synthesized according to general procedure C from **6** (2.8 g, 7.27 mmol) to yield the resulting alcohol in 2.26 g (94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 9H), 2.78 (d, 2H, J = 7.32 Hz), 3.60 (m, 2H), 3.82 (b, 1H), 5.04 (s, 2H), 6.92 (d, 2H, J = 8.78 Hz), 7.13 (d, 2H, J = 8.42 Hz), 7.31–7.45 (m, 5H).

4.3.5. [1-(4-Benzyloxy-benzyl)-2-oxo-ethyl]-carbamic acid *tert*-butyl ester (8). This compound was synthesized according to general procedure D from **7** (2.26 g, 8.79 mmol) to yield the resulting aldehyde in 1.87 g (83%).^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 3.06 (d, 2H, J = 6.58 Hz), 4.38 (d, 1H, J = 6.59 Hz), 5.04 (s, 2H), 5.11 (m, 1H), 6.92 (d, 2H, J = 8.42 Hz), 7.09 (d, 2H, J = 8.42 Hz), 7.31–7.44 (m, 5H), 9.62 (s, 1H). ^{13}C NMR δ 28.54, 34.78, 61.14, 70.26, 80.37, 115.34, 127.72, 128.25, 128.85, 128.91, 130.63, 137.18, 158.14, 199.91.

4.3.6. [4-(4-Benzyloxy-phenyl)-3-*tert*-butoxycarbonylamino-1-fluoro-but-1-enyl]-phosphonic acid diethyl ester (9). To a solution of **2** (500 mg, 1.63 mol) in THF at −78 °C was added a solution of *n*-butyl lithium (0.67 ml, 2.5 M in THF). The reaction mixture was allowed to stir for 1 h and then a solution of **8** (775 mg, 2.18 mmol) in THF was added and the reaction mixture was allowed to slowly warm to room temperature. The reaction mixture was allowed to stir overnight and the next day the reaction was quenched with ammonium chloride and then the aqueous layer was extracted with EtOAc, then the organic layer was extracted with brine, the organic was then dried with sodium sulfate, filtered, and then concentrated under reduced pressure. The product was purified via flash column chromatography (1:1 EtOAc/hexanes) to afford the product in 600 mg (70%) yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (m, 6H); 1.46 (m, 9H); 2.04 (s, 1H), 2.78 (m, 2H), 4.07 (m, 4H), 4.88 (m, 1H), 5.02 (s, 2H), 5.15 (m, 1H), 5.95 (m, 1H), 6.92 (d, 2H, J = 8.45 Hz), 7.14 (d, 2H, J = 8.46 Hz), 7.44 (m, 5H).

4.3.7. [4-(4-Benzyloxy-phenyl)-1-fluoro-3-hexadecanoylamino-but-1-enyl]-phosphonic acid diethyl ester (11). A solution of **9** (600 mg, 1.18 mmol) in dichloromethane and trifluoroacetic acid (2:1) was stirred for several hours and progress was monitored by TLC. After the reaction had proceeded to completion the solvent was removed under reduced pressure and the remaining TFA was removed after repeated azeotropic distillations with both diethyl ether and methanol. The product was prepared from palmitoyl chloride according to the general procedure A. The crude products were collected without purification in a yield of 860 mg.

4.3.8. [1-Fluoro-3-hexadecanoylamino-4-(4-hydroxy-phenyl)-butyl]-phosphonic acid diethyl ester (12). This compound was synthesized according to the general procedure B from **11** (860 mg) in a yield of 570 mg (85%, three steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, 3H, J = 5.12 Hz), 1.21 (s, 24H), 1.30 (m, 6H), 1.52 (m, 2H), 2.09 (t, 3H, J = 7.31 Hz), 2.72 (m, 2H), 3.61(s, 1H), 4.14 (m, 4H), 4.39 (b, 1H), 4.82 (m, 1H), 6.11 (t, 1H, J = 8.79 Hz), 6.72 (d, 2H, J = 8.05 Hz), 6.95 (d, 2H, J = 7.32 Hz). ^{13}C NMR δ 14.32, 16.52, 16.60, 16.65, 22.89, 25.90, 25.99, 29.47, 29.49, 29.57, 29.73, 29.86, 29.89, 29.91, 32.12, 36.99, 37.01, 63.54, 64.02, 70.71, 115.73, 127.25, 128.00, 128.09, 130.48, 130.50, 156.02, 173.87, 174.13.

4.3.9. {1-Fluoro-3-hexadecanoylamino-4-[4-(4-methoxy-3,5-dimethyl-pyridin-2-ylmethoxy)-phenyl]-butyl}-phosphonic acid dimethyl ester (13). This compound was synthesized according to the general procedure E from **12** (1.098 g, 1.97 mmol) and **4** (531 mg, 2.17 mmol) in a yield of 1.151 g (83%). The product was then purified by using flash column chromatography (5% MeOH:CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, 3H, J = 6.5 Hz), 1.21 (m, 26H), 1.29 (m, 8H), 1.52 (p, 2H, J = 6.5 Hz), 2.08 (m, 3H), 2.23 (s, 3H), 2.29 (s, 3H), 2.77 (m, 2H), 3.76 (s, 3H), 4.135 (q, 4H, J = 8.1 Hz), 4.36 (m, 1H), 5.09 (s, 2H), 5.69–5.76 (m, 1H), 6.92 (d, 2H, J = 8,8 Hz), 7.06 (d, 2H, J = 8.5 Hz), 8.20 (s, 1H).

4.3.10. {1-Fluoro-3-hexadecanoylamino-4-[4-(4-methoxy-3,5-dimethyl-pyridin-2-ylmethoxy)-phenyl]-butyl}-phosphonic acid (14). This product was formed by using general procedure H from **13** (70 mg, 0.099 mmol) to afford 41 mg yield (63%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, J = 6.8 Hz, 3H); 1.27 (m, 26H); 1.52 (m, 2H); 1.53 (m, 2H); 2.15 (m, 4H); 2.39 (s, 3H); 2.46 (s, 3H); 2.76 (m, 2H); 4.10 (s, 3H); 4.30 (m, 1H); 5.35 (s, 2h); 7.05 (d, J = 8.4 Hz, 2H); 7.23 (d, J = 7.9 Hz, 2H); 8.42 (s, 1H); elemental analysis CHN calcd % C = 64.59%, H = 8.67%, N = 4.30%; found C = 62.51%, H = 8.61%, N = 4.22%.

4.3.11. [1-(4-Benzyloxy-benzyl)-3-diazo-2-oxo-propyl]-carbamic acid *tert*-butyl ester (16). To N-Boc, *O*-benzyl L-tyrosine acid (4 g, 10.8 mmol) in THF at 0 °C in a erlenmeyer flask free of internal scratches is added isobutyl chloroformate (2.206 g, 16.2 mmol). The reaction mixture is allowed to stir for 30 min before a solution of ethereal diazomethane (approximately 2.3 g, 54 mmol) is added via canula. Reaction mixture is allowed to slowly warm to room temperature and then allowed to stir overnight. The following day the reaction mixture was then stirred open to air in order to allow for unreacted diazomethane to dissipate. After a few hours the reaction mixture is extracted (sodium bicarbonate, then brine), dried (sodium sulfate), and then concentrated under reduced pressure. The reaction mixture is purified via flash chromatography (50% EtOAc in hexanes) to yield the product (2.16 g, 51%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 9H), 2.96 (d, 2H, J = 6.77 Hz), 4.36 (b, 1H), 5.05 (s, 2H), 5.16 (b, 1H), 5.23 (b, 1H), 6.91 (d, 2H, J = 8.71 Hz), 7.10 (d, 2H, J = 8.71 Hz), 7.30–7.44 (m, 5H); ^{13}C NMR δ 28.58, 37.98, 54.73, 58.90, 70.27, 115.28, 127.78, 128.82, 128.87, 130.69, 137.23, 158.09.

4.3.12. 4-(4-Benzyloxy-phenyl)-3-*tert*-butoxycarbonylamino-butyric acid methyl ester (17). To a solution of the α-diazoketone **16** (2.16 g, 5.47 mmol), TEA (2.3 ml, 16.4 mmol) in 25 ml anhydrous methanol, and a small amount of THF (added to help solubilize the diazoketone) in a flask wrapped with tin foil, to protect reaction from light, and cooled to −78 °C was added a catalytic amount of silver benzoate. Silver benzoate was dissolved in a small amount of TEA before it is added to reaction mixture. Reaction mixture was allowed to stir at −78 °C for several hours before a second portion of silver benzoate (catalytic amount, dissolved in TEA) was added. Then reaction mixture was allowed to slowly warm to room temperature and then allowed to stir overnight. The next day the reaction mixture is filtered through silica gel by using EtOAc as eluent to remove any silver salts that are left over from the reaction. The mixture is then concentrated under reduced pressure and purified via flash column chromatography (20% EtOAc in hexanes) to yield the product (1.63 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 9H), 2.47 (m, 2H), 2.75 (m, 2H), 3.66 (s, 3H), 4.12 (b, 1H), 5.02 (s, 2H), 5.11 (b, 1H), 6.90 (d, 2H, J = 8.61 Hz), 7.10 (d, 2H, J = 8.42 Hz), 7.30–7.44 (m, 5H); ^{13}C NMR δ 28.66, 37.88, 39.80, 49.25, 51.92, 70.22, 79.59, 115.14, 127.75, 128.20, 128.85, 130.34, 130.66, 137.39, 155.45, 157.85, 172.41.

4.3.13. [1-(4-Benzyloxy-benzyl)-3-hydroxy-propyl]-carbamic acid *tert*-butyl ester (18). This product was synthesized according to general procedure C using **17** as the starting material (1.63 g, 3.95 mmol). The product was purified by flash column chromatography (EtOAc) and the yield was 1.41 g (97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.81 (b, 2H), 2.75 (d, 2H, J = 4.95 Hz), 3.65 (b, 2H), 4.02 (b, 1H), 4.78 (d, 1H, J = 8.98 Hz), 5.03 (s, 2H), 5.06 (b, 1H), 6.92 (d, 2H, J = 8.72 Hz), 7.11 (d, 2H, J = 8.73 Hz), 7.30–7.46 (m, 5H); ^{13}C NMR δ 28.69, 37.85, 40.81, 48.69, 59.19, 70.30, 79.94, 115.14, 127.78, 128.26, 128.90, 130.53, 130.63, 137.39, 157.10, 157.77.

4.3.14. [1-(4-Benzyloxy-benzyl)-3-oxo-propyl]-carbamic acid *tert*-butyl ester (19). This compound was prepared from **18** (1.41 g, 3.81 mmol) by using general procedure D. The product was purified by flash column chromatography (20% EtOAc in hexanes) in a yield of 1.32 g (94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 9H), 2.55 (m, 2H), 2.77 (m, 2H), 4.22 (b, 1H), 4.85 (d, 1H, J = 8.06 Hz), 5.03 (s, 2H), 5.06 (b, 1H), 6.94 (d, 2H, J = 8.60 Hz), 7.11 (d, 2H, J = 8.43 Hz), 7.32–7.46 (m, 5H), 9.68 (s, 1H); ^{13}C NMR δ 28.63, 40.15, 47.78, 48.05, 70.27, 79.86, 115.28, 127.78, 128.26, 128.87, 130.02, 130.63, 137.31, 157.98, 201.44.

4.3.15. [4-(4-Benzyloxy-phenyl)-3-*tert*-butoxycarbonylamino-1-hydroxy-butyl]-phosphonic acid dimethyl ester (20). To a solution of 60% NaH in mineral oil (215 mg, 5.38 mmol) in THF at 0 °C was added dimethyl phosphite (0.5 ml, 5.38 mmol) dropwise via syringe. The resulting mixture was allowed to stir at this temperature for 1 h before a solution of aldehyde **19** (1.32 g, 3.58 mmol) was added. The reaction mixture is then allowed to stir for 15 min before it was quenched with saturated ammonium chloride in water. The organic layer is then extracted with EtOAc, followed by brine, dried with sodium sulfate, and then concentrated under reduced pressure. The product was purified by flash column chromatography (EtOAc) in a yield of 1.02 g (60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (s, 9H), 1.66 (m, 1H), 1.89 (m, 1H), 2.75 (d, 2H, J = 6.41 Hz), 3.75 (m, 6H), 4.05 (m, 2H), 4.80 (d, 1H, J = 8.97 Hz), 4.99 (s, 2H), 6.88 (d, 2H, J = 7.33 Hz), 7.07 (d, 2H, J = 7.33 Hz), 7.25–7.40 (m, 5H); ^{13}C NMR δ 28.55, 37.02, 40.47, 48.08, 48.29, 53.61, 63.28, 65.52, 70.22, 80.26, 115.01, 115.20, 127.70, 128.18, 128.82, 130.07, 130.50, 130.80, 157.40, 157.82.

4.3.16. [4-(4-Benzyloxy-phenyl)-3-hexadecanoylamino-1-hydroxy-butyl]-phosphonic acid dimethyl ester (21a and b). This compound was prepared from **20**. The N-Boc protecting group was removed with the same method in preparation of **11** and then the TFA salt was reacted with palmitoyl chloride according to the general procedure A. The products were purified by column chromatography (2% MeOH in CH_2Cl_2) in a yield of 1.1 g (82% two steps). Two diastereomers were separated in the ratio of 1:4.

Compound **21a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 5.85 Hz), 1.25 (s, 24H), 1.48 (p, 2H, J = 6.96 Hz), 1.69 (m, 1H), 2.00 (m, 1H), 2.12 (dt, 2H, J = 2.56, 7.30 Hz), 2.79 (dq, 2H, J = 5.49, 15.37 Hz), 3.81 (dd, 6H, J = 2.92, 10.61 Hz), 3.92 (m, 1H), 4.33 (m, 1H), 5.01 (s, 2H), 6.18 (d, 1H, J = 8.79 Hz), 6.89 (d, 2H, J = 8.05 Hz), 7.08 (d, 2H, J = 8.42 Hz), 7.25–7.41 (m, 5H); ^{13}C NMR δ 14.40, 22.95, 26.07, 29.41, 29.62, 29.76, 29.97, 32.19, 36.75, 37.50, 40.23, 47.09, 47.28, 53.71, 53.79, 63.20, 65.47, 70.25, 115.25, 127.67, 128.20, 128.82, 129.81, 129.94, 130.31, 137.23, 157.93, 165.76, 175.72.

Compound **21b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 6.22 Hz), 1.23 (s, 24H), 1.51 (p, 2H, J = 6.95 Hz), 1.85 (m, 1H), 1.99 (m, 1H), 2.12 (t, 2H, J = 8.42 Hz), 2.80 (d, 2H, J = 6.59 Hz), 3.74 (t, 6H, J = 9.88 Hz), 4.04 (m, 1H), 4.25 (m, 1H), 5.00 (s, 2H), 6.18 (d, 1H, J = 7.69 Hz), 6.88 (d, 2H, J = 8.42 Hz), 7.09 (d, 2H, J = 8.79 Hz), 7.25–7.42 (m, 5H); ^{13}C NMR δ 14.40, 22.95, 25.94, 29.57, 29.62, 29.68, 29.81, 29.92, 29.97, 32.19, 35.90, 37.13, 40.17, 49.49, 49.71, 53.45, 53.53, 53.82, 65.47, 67.66, 70.25, 115.06, 127.70, 128.20, 128.82, 130.25, 130.66, 157.82, 173.96.

4.3.17. [3-Hexadecanoylamino-1-hydroxy-4-(4-hydroxy-phenyl)-butyl]-phosphonic acid dimethyl ester (22a and b). The two diastereomers were synthesized according to general procedure B from **21a** (290 mg, 0.46 mmol) and **21 b** (190 mg, 3.01 mmol) in the yields of 95 mg (58%) and 180 mg (73%).

Compound **22a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 6.59 Hz), 1.23 (s, 24H), 1.50 (p, 2H, J = 5.85 Hz), 1.68 (m, 1H), 1.95 (m, 1H), 2.13 (t, 2H, J = 8.05 Hz), 2.74 (m, 2H), 3.79 (dd, 6H, J = 4.76, 10.24 Hz), 3.89 (m, 1H), 4.34 (m, 1H), 6.14 (d, 1H, J = 8.78 Hz), 6.73 (d, 2H, J = 8.42 Hz), 6.96 (d, 2H, J = 8.42 Hz); ^{13}C NMR δ 14.34, 22.90, 26.02, 29.44, 29.54, 29.58, 29.72, 29.89, 29.94, 32.14, 36.74, 36.87, 40.14, 53.67, 53.74, 53.96, 54.06, 115.88, 128.08, 130.31, 155.94, 175.84.

Compound **22b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 6.95 Hz), 1.23 (s, 24H), 1.51 (p, 2H, J = 6.90 Hz), 1.82 (m, 1H), 1.99 (m, 1H), 2.12 (t, 2H, J = 7.68 Hz), 2.72 (d, 2H, J = 5.49 Hz), 3.73 (t, 6H, J = 9.88 Hz), 4.00 (t, 1H, J = 7.69 Hz), 4.24 (m, 1H), 6.38 (m, 1H), 6.74 (d, 2H, J = 8.41 Hz), 6.95 (d, 2H, J = 8.42 Hz); ^{13}C NMR δ 14.34, 22.92, 25.92, 29.54, 29.59, 29.79, 29.95, 32.14, 37.04, 53.49, 53.58, 53.76, 54.06, 115.78, 128.43, 130.58, 155.80, 174.36.

4.3.18. {3-Hexadecanoylamino-1-hydroxy-4-[4-(4-methoxy-3,5-dimethyl-pyridin-2-ylmethoxy)-phenyl]-butyl}-phosphonic acid dimethyl ester (23a and b). The two diastereomers were synthesized according to general procedure I from **22a** (250 mg, 0.474 mmol) and **22b** (106 mg, 0.201 mmol) in the yields of 216 mg (67%) and 86 mg (63%).

Compound **23a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 7.0 Hz), 1.24 (m, 26H); 1.49 (p, 2H, J = 7.0 Hz), 1.68 (m, 1H), 2.02 (m, 1H), 2.12 (m, 2H), 2.27 (s, 3H), 2.32 (s, 3H), 2.79 (dq, 2H, J = 7.9, 14.9 Hz), 3.78 (s, 3H), 3.81 (dd, 6H, J = 4.9, 10.3 Hz), 3.87 (m, 1H), 4.36 (m, 1H), 5.12 (s, 2H), 5.72 (d, 1H, J = 8.6 Hz), 6.95 (d, 2H, J = 8.8 Hz), 7.07 (d, 2H, J = 8.8 Hz), 8.23 (s, 1H).

Compound **23b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 6.9 Hz), 1.23 (m, 26H), 1.53 (m, 2H), 1.83 (m, 1H), 2.03 (m, 1H), 2.09 (t, 2H, J = 8.5 Hz), 2.26 (s, 3H), 2.31 (s, 3H), 2.79 (d, 2H, J = 6.2 Hz), 3.75 (m, 9H), 4.01 (m, 1H), 4.12 (q, 1H, J = 6.5 Hz), 4.21 (m, 1H), 5.11 (s, 2H), 5.99 (d, 1H, J = 7.7 Hz), 6.93 (d, 2H, J = 8.8 Hz), 7.07 (d, 2H, J = 8.8 Hz), 8.23 (s, 1H).

4.3.19. {3-Hexadecanoylamino-1-hydroxy-4-[4-(4-methoxy-3,5-dimethyl-pyridin-2-ylmethoxy)-phenyl]-butyl}-phosphonic acid (24a and b). The two diastereomers were synthesized according to general procedure H from **23a** (56 mg, 0.0825 mmol) and **23b** (86 mg, 0.127 mmol) in the yields of 43 mg (80%) and 70 mg (86%).

Compound **24a**: ^{1}H NMR (300 MHz, CD_3OD) δ 0.89 (t, 3H, J = 6.8 Hz, 3H), 1.26 (m, 26H), 1.51 (m, 2H), 1.83 (m, 2H), 2.13 (t, 2H, J = 6.6 Hz), 2.35 (s, 3H), 2.39 (s, 3H), 2.75 (d, 2H, J = 6.6 Hz), 3.38 (m, 1H), 3.99 (s, 2H), 4.32 (m, 1H), 4.94 (s, 2H), 5.25 (m, 1H), 6.99 (d, 2H, J = 9.2 Hz), 7.19 (d, 2H, J = 8.2 Hz), 8.35 (s, 1H); ^{13}C NMR (300 MHz, CD_3OD) δ 14.03, 14.46, 23.75, 27.20, 30.32, 30.48, 30.61, 30.83, 33.10, 37.29, 41.67, 93.43, 98.51, 115.92, 131.68, 133.47, 157.96, 170.14; elemental analysis CHN calcd % C = 64.79%, H = 8.86%, N = 4.32%; found C = 64.06%, H = 9.14%, N = 4.56%.

Compound **24b**: ^{1}H NMR (300 MHz, CD_3OD) δ 0.89 (t, 3H, J = 6.5 Hz), 1.27 (m, 26H), 1.46 (p, 2H, J = 7.3 Hz), 1.84 (m, 1H), 1.99 (m, 1H), 2.06 (t, 2H, J = 6.9 Hz), 2.36 (s, 3H), 2.41 (s, 3H), 2.60 (m, 1H), 2.90 (m, 1H), 3.82 (m, 1H), 4.01 (s, 2H), 4.30 (m, 1H), 5.27 (s, 2H), 6.68 (d, 1H, J = 8.1 Hz), 7.01 (m, 2H), 7.20 (d, 2H, J = 7.0 Hz), 8.37 (s, 1H); ^{13}C NMR (300 MHz, CD_3OD) δ 9.95, 12.88, 13.27, 22.57, 25.89, 29.04, 29.07, 29.31, 29.34, 29.41, 29.45, 29.64, 31.90, 36.15, 36.67, 38.41, 60.38, 66.73, 95.54, 114.65, 114.79, 130.22, 130.54, 132.42, 174.37; elemental analysis CHN calcd % C = 64.79%, H = 8.86%, N = 4.32%; found C = 62.91%, H = 8.94%, N = 4.03%.

4.3.20. {2-Fluoro-3-hexadecanoylamino-4-[4-(4-methoxy-3,5-dimethyl-pyridin-2-ylmethoxy)-phenyl]-butyl}-phosphonic acid (25a). To a solution of **49a** (30 mg, 0.04 mmol) in CH_2Cl_2 at −78 °C was added via cannula a pre-cooled solution of DAST (0.015 ml, 0.11 mmol) CH_2Cl_2. The reaction mixture was allowed to warm to room temperature and after 1 h, a saturated solution of sodium bicarbonate was added and the mixture was diluted with EtOAc. The mixture was extracted with EtOAc and combined organic layers were washed with brine and dried over magnesium sulfate and concentrated under vacuum. The residue was purified by column chromatography (4% MeOH:CH_2Cl_2) to give the dimethyl ester product in 14 mg (47%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 7.57 Hz), 1.25 (s, 24H), 1.61 (p, 2H, J = 7.10 Hz), 2.05 (m, 2H), 2.26 (s, 3H), 2.28 (t, 2H, J = 7.48 Hz), 2.32 (s, 3H), 2.75 (dq, 2H, J = 8.32, 15.05 Hz), 3.75 (m, 6H), 3.77 (s, 3H), 4.38 (q, 1H, J = 7.48 Hz), 4.87 (dq, 1H, J = 3.49, 10.48 Hz), 5.12 (s, 2H), 6.95 (d, 2H, J = 8.73 Hz), 7.12 (d, 2H, J = 8.73 Hz), 8.23 (s, 1H). MS (ESI) *m/z* 679.6 $[M+H]^+$. Compound **25a** was prepared according to the general procedure H to give 8 mg (62%). ^{1}H NMR (300 MHz, CD $_3$OD) δ 0.89 (t, 3H, J = 6.35 Hz), 1.28 (s, 24H), 1.64 (p, 2H, J = 6.80 Hz), 2.19 (dd, 2H, J = 7.26, 19.05 Hz), 2.40 (s, 3H), 2.45 (t, 2H, J = 7.71 Hz), 2.50 (s, 3H), 2.63 (dd, 1H, J = 9.53,

14.06 Hz), 3.19 (dd, 1H, J = 5.44, 14.97 Hz), 3.68 (m, 6H), 3.91 (m, 1H), 4.16 (s, 3H), 5.45 (s, 2H), 7.23 (d, 2H, J = 8.62 Hz), 7.38 (d, 2H, J = 8.62 Hz), 8.50 (s, 1H). MS (ESI) m/z 651.2 $[M+H]^+$.

4.3.21. {2-Fluoro-3-hexadecanoylamino-4-[4-(4-methoxy-3,5-dimethyl-pyridin-2-ylmethoxy)-phenyl]-butyl}-phosphonic acid (25b). It was prepared from **49b** with the same method as in the preparation of **25a** with same reaction yield. ^{1}H NMR (300 MHz, CD_3OD) δ 0.89 (t, 3H, J = 6.54 Hz), 1.28 (s, 24H), 1.67 (p, 2H, J = 6.94 Hz), 2.15 (m, 2H), 2.40 (s, 3H), 2.47 (t, 2H, J = 6.54 Hz), 2.50 (s, 3H), 2.95 (m, 1H), 3.94 (m, 1H), 4.16 (s, 3H), 5.39 (m, 1H), 5.45 (s, 2H), 7.10 (m, 1H), 7.29 (m, 4H), 8.51 (s, 1H). MS (ESI) m/z 651.8 $[M+H]^+$.

4.3.22. {3-Hexadecanoyl-4-[4-(4-methoxy-3,5-dimethyl-pyridin-2-ylmethoxy)-benzyl]-2,2-dimethyl-oxazolidin-5-ylmethyl}-phosphonic acid dimethyl ester (47). 2-Methoxypropene (0.06 ml, 0.63 mmol) was added to a solution of **49a** (80 mg, 0.12 mmol) in CH_2Cl_2 at 0 °C, then CSA (5.5 mg, 0.02 mmol) was added and the mixture was allowed to warm to room temperature overnight. The next day the reaction mixture was diluted with CH_2Cl_2 and washed with saturated sodium bicarbonate solution then dried over magnesium sulfate and concentrated under vacuum. The residue was purified by preparative TLC (4% MeOH:CH_2Cl_2) to give 27 mg product (32%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 6.06 Hz), 1.24 (bs, 24H), 1.60 (m, 2H), 1.63 (s, 3H), 1.66 (s, 3H), 2.00 (m, 2H), 2.25 (s, 3H), 2.30 (s, 3H), 2.85 (m, 2H), 3.50 (m, 6H), 3.77 (s, 3H), 4.13 (dd, 1H, J = 5.30, 9.60 Hz), 4.33 (m, 1H), 5.12 (s, 2H), 6.98 (d, 2H, J = 8.61 Hz), 7.11 (d, 2H, J = 8.61 Hz), 8.24 (s, 1H). ^{13}C NMR δ 11.14, 13.62, 14.37, 22.95, 25.43, 28.13, 28.58, 29.62, 29.70, 29.81, 29.94, 31.25, 32.16, 33.07, 35.85, 40.68, 52.46, 52.54, 60.15, 64.61, 64.66, 70.99, 74.89, 115.41, 126.76, 129.57, 130.58, 149.33, 154.33, 154.38, 158.01, 170.59.

4.3.23. {3-Hexadecanoyl-4-[4-(4-methoxy-3,5-dimethyl-pyridin-2-ylmethoxy)-benzyl]-2,2-dimethyl-oxazolidin-5-ylmethyl}-phosphonic acid dimethyl ester (48). 2-Methoxypropene (0.06 ml, 0.63 mmol) was added to a solution of **49b** (80 mg, 0.12 mmol) in CH_2Cl_2 at 0 °C, then CSA (5.5 mg, 0.02 mmol) was added and the mixture was allowed to warm to room temperature overnight. The next day the reaction mixture was diluted with CH_2Cl_2 and washed with saturated sodium bicarbonate solution then dried over magnesium sulfate and concentrated under vacuum. The residue was purified by preparative TLC (4% MeOH:CH_2Cl_2) to give 25 mg product (30%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 6.72 Hz), 1.24 (br s, 24H), 1.60 (s, 3H), 1.65 (m, 2H), 1.77 (s, 3H), 2.10 (m, 2H), 2.26 (s, 3H), 2.30 (s, 3H), 2.65 (m, 1H), 2.95 (dd, 1H, J = 5.60, 4.18 Hz), 3.77 (s, 3H), 3.78 (m, 6H), 4.06 (m, 1H), 4.60 (m, 1H), 5.11 (s, 2H), 6.95 (d, 2H, J = 8.21 Hz), 7.07 (d, 2H, J = 8.21 Hz), 8.25 (s, 1H). ^{13}C NMR δ 11.17, 13.65, 14.40, 22.95, 24.25, 25.16, 27.22, 27.65, 29.49, 29.62, 29.73, 29.78, 29.97, 32.19, 35.34, 35.63, 52.19, 52.75, 53.21, 61.38, 61.49, 70.97, 72.17, 101.76, 115.54, 129.89, 130.74, 149.38, 154.35, 158.04, 171.31.

4.4. Synthesis of compounds 26–46

These compounds were synthesized from appropriate amino acids by using General procedures A, E, F, G, and H.[24]

4.4.1. (3-Hexadecanoylamino-2-oxo-propyl)-phosphonic acid (26). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, 3H, J = 7.03 Hz), 1.28 (s, 24H), 1.62 (q, 2H, J = 7.03 Hz), 2.25 (t, 2H, J = 7.03 Hz), 3.15 (m, 2H), 4.20 (s, 2H). MS (ESI) m/z 392.5 $[M+H]^+$.

4.4.2. (3-Hexadecanoylamino-2-hydroxy-propyl)-phosphonic acid (27). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, 3H, J = 6.93 Hz), 1.28 (s, 24H), 1.62 (q, 2H, J = 6.93 Hz), 1.90 (m, 2H), 2.22 (t, 2H, J = 6.94 Hz), 3.22 (dd, 1H, J = 6.63, 14.47 Hz), 3.38 (dd, 1H, J = 3.38, 14.32 Hz), 4.03 (m, 1H). MS (ESI) m/z 394.3 $[M+H]^+$.

4.4.3. (3-Hexadecanoylamino-2-oxo-butyl)-phosphonic acid (28). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, 3H, J = 6.93 Hz), 1.28 (s, 24H), 1.33(d, 3H, J = 7.42 Hz), 1.61 (q, 2H, J = 7.28 Hz), 2.23 (t, 2H, J = 7.43 Hz), 3.20 (m,2H), 4.50 (m, 1H). MS (ESI) m/z 406.5 $[M+H]^+$.

4.4.4. (3-Hexadecanoylamino-2-hydroxy-butyl)-phosphonic acid (29). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, 3H, J = 6.96 Hz), 1.14 (d, 3H, J = 6.50 Hz), 1.29 (s, 24H), 1.61 (q, 2H, J = 6.86 Hz), 1.91 (m, 2H), 2.19 (t, 2H, J = 6.97 Hz), 3.70 (m, 1H), 3.91(m, 2H). MS (ESI) m/z 408.4 $[M+H]^+$.

4.4.5. (3-Hexadecanoylamino-4-methyl-2-oxo-pentyl)-phosphonic acid (30). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (m, 9H), 1.28 (s, 24H), 1.61 (m, 2H), 2.13 (m, 1H), 2.26 (t, 2H, J = 7.52 Hz), 4.31 (m, 1H), 8.21 (b, 1H). MS (ESI) m/z 434.6 $[M+H]^+$.

4.4.6. (3-Hexadecanoylamino-2-hydroxy-4-methyl-pentyl)-phosphonic acid (31). ^{1}H NMR (300 MHz, CD_3OD) δ 0.93 (m, 9H), 1.29 (s, 24H), 1.63 (p, 2H, J = 7.09 Hz), 1.92 (m, 2H), 2.28 (m, 1H), 2.40 (t, 2H, J = 7.10 Hz), 3.58 (m, 2H). MS (ESI) m/z 436.1 $[M+H]^+$.

4.4.7. (3-Hexadecanoylamino-2-hydroxy-4-methyl-pentyl)-phosphonic acid (32). ^{1}H NMR (300 MHz, CD_3OD) δ 0.89 (m, 9H), 1.28 (s, 24H), 1.62 (p, 2H, J = 6.62 Hz), 1.95 (m, 2H), 2.28 (m, 1H), 2.25 (t, 2H, J = 7.66 Hz), 3.67 (m, 2H), 3.78 (m, 1H). MS (ESI) m/z 436.1 $[M+H]^+$.

4.4.8. (3-Hexadecanoylamino-5-methyl-2-oxo-hexyl)-phosphonic acid (33). ^{1}H NMR (300 MHz, CD_3OD) δ 0.94 (m, 9H), 1.28 (s, 24H), 1.64 (m, 5H), 2.25 (t, 2H, J = 6.73 Hz), 3.16 (m, 2H), 4.59 (m, 1H). MS (ESI) m/z 448.4 $[M+H]^+$.

4.4.9. (3-Hexadecanoylamino-2-hydroxy-5-methyl-hexyl)-phosphonic acid (34). ^{1}H NMR (300 MHz, CD_3OD) δ 0.92 (m, 9H), 1.28 (s, 24H), 1.62 (m, 5H), 1.90 (m, 2H), 2.25 (t, 2H, J = 7.27 Hz), 3.72 (m, 1H), 3.99 (m, 1H). MS (ESI) m/z 450.4 $[M+H]^+$.

4.4.10. (3-Hexadecanoylamino-2-hydroxy-5-methyl-hexyl)-phosphonic acid (35). ^{1}H NMR (300 MHz, CD_3OD) δ 0.92 (m, 9H), 1.28 (s, 24H), 1.43 (m, 2H), 1.61 (m, 3H), 1.91 (m, 2H), 2.22 (t, 2H, J = 7.41 Hz), 3.72 (m, 1H), 3.84 (m, 1H), 3.95 (b, 1H). MS (ESI) m/z 450.2 $[M+H]^+$.

4.4.11. (3-Hexadecanoylamino-2-hydroxy-4-phenyl-butyl)-phosphonic acid (36). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, 3H, J = 7.05 Hz), 1.28 (s, 24H), 1.46 (p, 2H, J = 7.05 Hz), 1.92 (m, 2H), 2.13 (t, 2H, J = 7.40 Hz), 2.80 (m, 1H), 2.93 (m, 1H), 4.07(m, 1H), 4.16 (m, 1H), 7.24 (m, 5H). MS (ESI) m/z 484.4 $[M+H]^+$.

4.4.12. (3-Hexadecanoylamino-2-hydroxy-4-phenyl-butyl)-phosphonic acid (37). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, 3H, J = 6.74 Hz), 1.29 (s, 24H), 1.37 (p, 2H, J = 6.37 Hz), 1.90 (m, 2H), 2.04 (t, 2H, J = 7.11 Hz), 2.60 (m, 1H), 3.15 (m, 1H), 3.95 (m, 1H), 4.12 (m, 1H), 7.22 (m, 5H). MS (ESI) m/z 484.5 $[M+H]^+$.

4.4.13. [2-(1-Hexadecanoyl-pyrrolidin-2-yl)-2-oxo-ethyl]-phosphonic acid (38). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, 3H, J = 7.16 Hz), 1.29 (s, 24H), 1.59 (q, 2H, J = 7.16 Hz), 2.00 (m, 2H), 2.21 (m, 2H), 2.36 (t, 2H, J = 7.47 Hz), 3.12 (m, 1H), 3.21 (m, 1H), 3.62 (m, 2H), 4.66 (m, 1H). MS (ESI) m/z 432.4 $[M+H]^+$.

4.4.14. [2-(1-Hexadecanoyl-pyrrolidin-2-yl)-2-hydroxy-ethyl]-phosphonic acid (39). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, 3H, J = 7.51 Hz), 1.24 (s, 24H), 1.61 (q, 2H, J = 7.00 Hz), 1.92 (m, 2H), 2.05 (m, 4H), 2.38 (t, 2H, J = 7.51 Hz), 3.59 (m, 2H), 4.10 (m, 1H), 4.25 (m,1H). MS (ESI) m/z 434.7 $[M+H]^+$.

4.4.15. [3-Hexadecanoylamino-4-(1H-indol-3-yl)-2-oxo-butyl]-phosphonic acid (40). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, 3H, J = 6.98 Hz), 1.28 (s, 24H), 1.45 (q, 2H, J = 6.98 Hz), 2.13 (t, 2H, J = 7.29 Hz), 3.10 (m, 2H), 3.17 (m, 2H), 7.05 (m, 2H), 7.08 (s, 1H), 7.31 (d, 1H, J = 8.25 Hz), 7.58 (d, 1H, J = 8.25 Hz). MS (ESI) m/z 521.6 $[M+H]^+$.

4.4.16. [3-Hexadecanoylamino-2-hydroxy-4-(1H-indol-3-yl)-butyl]-phosphonic acid (41). ^{1}H NMR (300 MHz, CD_3OD) δ 0.89 (t, 3H, J = 7.09 Hz), 1.27 (s, 24H), 1.46 (m, 2H), 1.93 (m, 2H), 2.16 (t, 2H, J = 7.00 Hz), 3.06 (m, 2H), 4.15 (m, 1H), 4.25 (m, 1H), 7.03 (m, 2H), 7.09 (s, 1H), 7.30 (d, 1H, J = 8.42 Hz), 7.62 (d, 1H, J = 8.42 Hz), 8.25 (s, 1H). MS (ESI) m/z 523.9 $[M+H]^+$.

4.4.17. [3-Hexadecanoylamino-2-hydroxy-4-(1H-indol-3-yl)-butyl]-phosphonic acid (42). ^{1}H NMR (300 MHz, CD_3OD) δ 0.89 (t, 3H, J = 7.38 Hz), 1.28 (br s, 26H), 2.11 (m, 2H), 2.14 (t, 2H, J = 7.98 Hz), 2.93 (m, 1H), 3.21 (m, 1H), 4.09 (m, 1H), 4.37 (m, 1H), 7.01 (m, 2H), 7.08 (s, 1H), 7.30 (d, 1H, J = 8.60 Hz), 7.61 (d, 1H, J = 8.60 Hz). MS (ESI) m/z 523.9 $[M+H]^+$.

4.4.18. [4-(1-Benzyl-1H-indol-3-yl)-3-hexadecanoylamino-2-hydroxy-butyl]-phosphonic acid (43). ^{1}H NMR (300 MHz, CD_3OD) δ 0.89 (t, 3H, J = 6.51 Hz), 1.27 (br s, 26H), 2.03 (t, 2H, J = 6.76 Hz), 3.73 (d, 2H, J = 9.85 Hz), 4.02 (m, 1H), 4.27 (m, 1H), 5.30 (s, 2H), 7.01–7.07 (m, 3H), 7.09 (s, 1H), 7.20–7.33 (m, 5H), 7.62 (d, 1H, J = 7.88 Hz). MS (ESI) m/z 613.3 $[M+H]^+$.

4.4.19. {3-Hexadecanoylamino-2-hydroxy-4-[1-(4-methoxy-3,5-dimethyl-pyridin-2-ylmethyl)-1H-indol-3-yl]-butyl}-phosphonic acid (44). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, 3H, J = 7.40 Hz), 1.28 (s, 26H), 1.65 (m, 2H), 2.01 (t, 2H, J = 7.93 Hz), 2.40 (s, 3H), 2.46 (s, 3H), 3.24 (m, 2H), 3.34 (s, 3H), 3.37 (m, 2H), 4.15 (s, 2H), 5.73 (d, 1H, J = 7.17 Hz), 7.10 (s, 1H), 7.08–7.18 (m, 2H), 7.24 (d, 1H, J = 6.86 Hz), 7.70 (d, 1H, J = 7.17 Hz), 8.36 (s, 1H). MS (ESI) m/z 672.3 $[M+H]^+$.

4.4.20. (4-Benzyloxy-3-hexadecanoylamino-2-hydroxy-butyl)-phosphonic acid (45). ^{1}H NMR (300 MHz, CD_3OD) δ 0.89 (t, 3H, J = 7.22 Hz), 1.27 (s, 24H), 1.61 (q, 2H, J = 7.23 Hz), 1.88–1.96 (m, 2H), 2.26 (t, 2H, J = 7.22 Hz), 3.60 (m, 2H), 4.16 (m, 1H), 4.25 (m, 1H), 4.53 (s, 2H), 7.25–7.35 (m, 5H). MS (ESI) m/z 514.3 $[M+H]^+$.

4.4.21. (4-Benzyloxy-3-hexadecanoylamino-2-hydroxy-butyl)-phosphonic acid (46). ^{1}H NMR (300 MHz, CD_3OD) δ 0.89 (t, 3H, J = 6.86 Hz), 1.27 (s, 24H), 1.59 (q, 2H, J = 6.86 Hz), 1.89–2.00 (m, 2H), 2.22 (t, 2H, J = 6.86 Hz), 3.86 (m, 2H), 4.07 (m, 1H), 4.52 (s, 2H), 7.27–7.35 (m, 5H). MS (ESI) m/z 514.2 $[M+H]^+$.

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