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Research Article

Synthesis Of Tripeptide Derivatives Of α -Aminoalkylphosphonate Esters
For Prostate-Specific Antigen Inhibition

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ABSTRACT

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Recently, we reported the rational ligand design of a new lead compound R/S-diphenyl [N-benzyloxy carbonyl amino(4-carbamoylphenyl) methyl] phosphonate as an inhibitor of prostate-specific antigen (PSA), a serine protease implicated in the advancement of prostate tumor progression.¹ In this work, we present the synthesis of tripeptide derivatives of the aforementioned lead using a convergent synthetic scheme. The solution phase synthesis present itself as a challenge compared to solid phase synthesis but allows scalability without the need of specialized resins or kits while incorporating mild protection/deprotection/coupling reagents and conditions. These compounds may be potential drug candidates for targeting PSA for prostate cancer treatment.

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INTRODUCTION:

Prostate Specific Antigen (PSA) is being used as a biological indicator for the progression of the disease state.²⁻⁴ Some evidence has shown that PSA is responsible for activating growth factors that are responsible for carcinogenesis,⁵⁻⁷ thus, inhibiting PSA could potentially block/reduce proliferation or metastasis of prostate cancer (PCa) cells. Recently, we reported a new lead compound **1**, diphenyl [N-benzyloxycarbonylamino(4-carbamoylphenyl)methyl] phosphonate (**Figure 1**), as a PSA inhibitor by targeting the P1 pocket of the PAS protein through the molecular modeling AutoDock 4 program, where the P1 site displays binding specificity to the R-group of the α -aminoalkylphosphonate.⁸ The α -aminoalkylphosphonates have previously shown inhibition of a variety of serine proteases^{9, 10-12} and can be tuned synthetically to be site-specific for the P1 pocket of the protein. The class of compounds, diphenyl α -aminoalkylphosphonates, are an appealing class of compounds not only due to their documented irreversible reactivity with serine proteases but also the ease of synthesis.^{9,13,14} The compounds also display a significant range of pH stability and are not susceptible to hydrolysis or nucleophilic attack at neutral pH's from 5.0 to 8.0, optimal for therapeutic

use.¹⁵ Moreover, the phenoxy leaving groups can be tailored to improve the lability of the leaving group by substituting with nitro- or chlorophenoxy groups, thereby increasing the electrophilicity of the phosphorous.¹⁶ The compounds can also be coupled to peptides to achieve better binding-site recognition and specificity via amide coupling of smaller peptides.

In our previous study, it was determined by the docking studies that **1** had scored the highest out of chosen compounds in this study, with a free energy score of -8.29/-9.14 kJ·mol⁻¹ for *R/S*, respectively. Both stereoisomers were consistent with respect to their interactions between the P1 pocket residues of THR₁₉₀, SER₂₁₇, & SER₂₂₇ and the carbamoyl moiety of the ligand. The model predicts that both hydroxyls of THR₁₉₀ and SER₂₂₇ form hydrogen bonds with the C=O of the carbamoyl, with distances of 2.2 Å and 2.5 Å, respectively. Concurrently, the amide proton forms a hydrogen bond within the distance of 2.2 Å of the carbonyl of the SER₂₁₇ amide. This push-pull hydrogen bonding between the S1/P1 groups of the inhibitor/protein is most likely responsible in stabilizing the binding conformation. However, the IC₅₀ of **1** and other studied compounds are in the μ M range, which is not sufficient as a drug molecule.

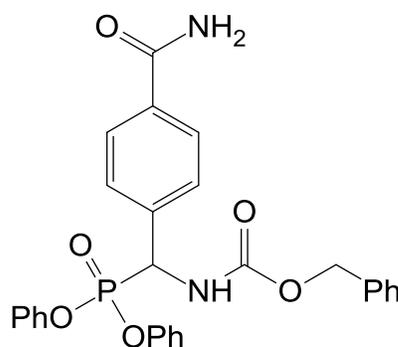


Figure 1. Structure of compound 1

Choosing an appropriate ligand for a biological target is a particularly challenging task in medicinal chemistry. Although a *de novo* approach may be suitable for finding novel binding pairs, it can be a time consuming process. In this work, peptide derivatives of the α -aminoalkylphosphonates were chosen and synthesized according to virtual screening results of 722 tripeptide mimics using an incremental

grow method. The peptide moieties were chosen based on their variety of functional group specificity to the P1 pocket of PSA. The synthesis of several derivatives are discussed in this work.

In the design of tripeptide mimics containing the S1 phenylglycine derivative, the benzyloxycarbonyl (also called Carboxybenzyl, Cbz) group in **1** was removed from the structure and S2 &

S3 amino acids were added to the N-terminus of the S1 anchor (**Figure 2**). Naming of atoms with associated amino acid fragments of the ligand are also shown in **Figure 2**. We modeled a total of 722 ligands

using the Raccoon AutoDock Virtual Screen¹⁷ - combinations of 19 primary D-amino acids were used for S2 and S3 sites of the ligand (**Figure 2**).

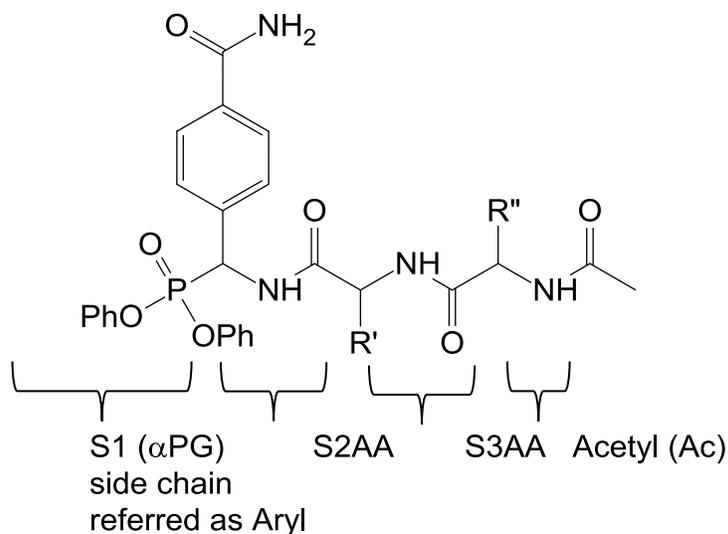


Figure 2– Atom/group assignments of the tripeptide mimics containing the S1 phenylglycine derivative.

Due to the chiral center involved, compounds isolated from reaction steps are racemic mixtures. Since both showed activity in our previous study, both *R*- and *S*- enantiomers of the compounds were designed and synthesized. Most of them have high

binding scores. Several top scoring poses from the molecular docking studies of the tripeptide mimics are listed in **Table 1**, which were synthesized and discussed in the work.

	S3AA	S2AA	Binding Score (kJ/mol)
7a	Val	Leu	R: -8.00 S: -12.25
7b	Ala	Val	R: -8.25 S: -9.66
7c	Gly	Phe	R: -11.17 S: -10.30

Table 1 – Selected AutoDock binding scores of several tripeptide diphenyl phosphonates.

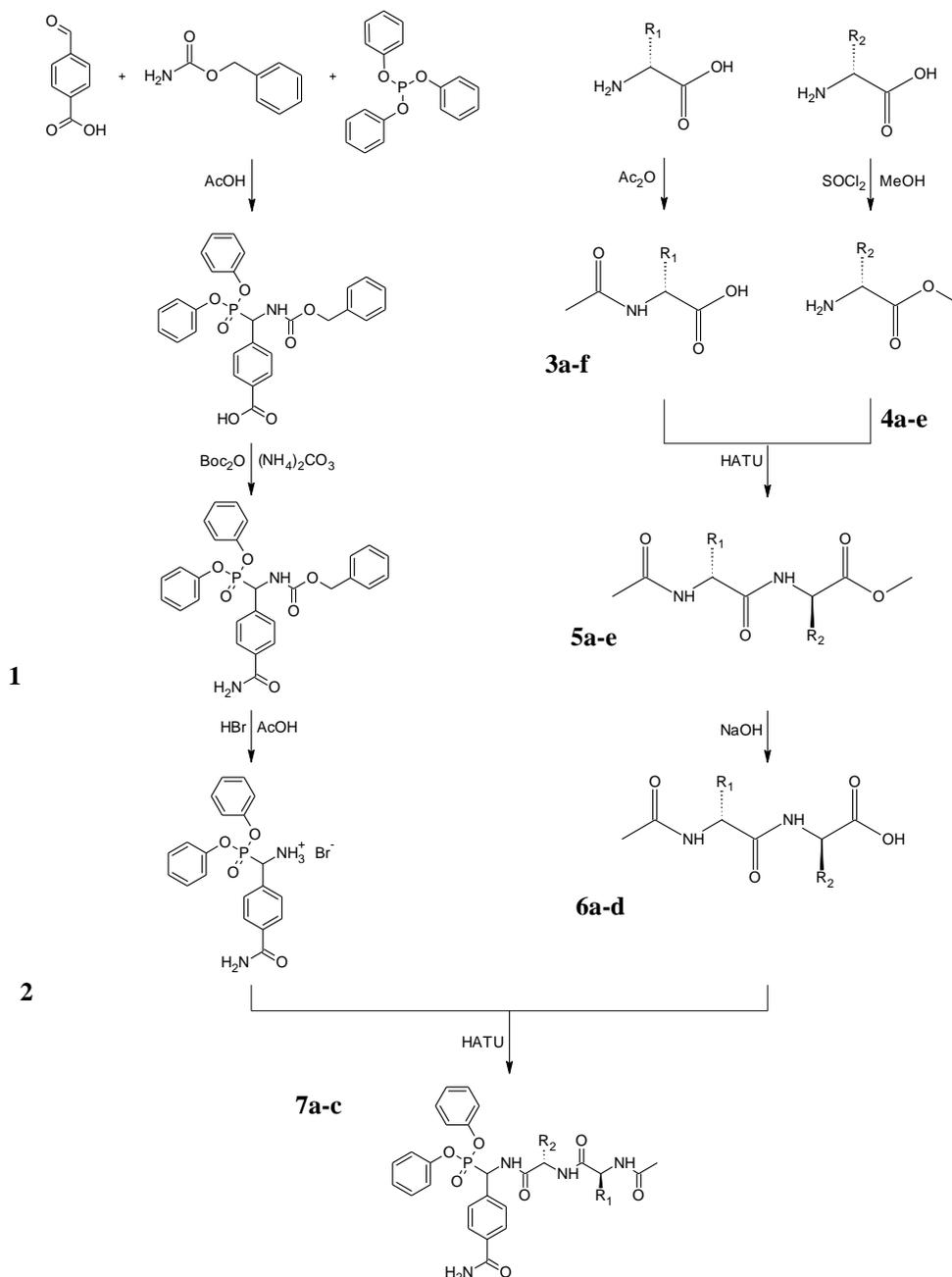


Figure 3 – Synthetic scheme for the synthesis of peptidyl α -amino alkylphosphonates. Compound variants are numbered in bold.

Kabachnik-Fields reaction and the cleavage of the Cbz protecting group.

The synthesis of diphenyl α -aminoalkylphosphonates followed a simple Kabachnik-Fields reaction using a 3-component, 2-step, one-pot synthesis consisting of an aldehyde, an amine, and a trialkylphosphite.¹⁸ The reaction, known as the Kabachnik-Fields reaction,^{19,20} can be completed with modest yields and purification of products *via* crystallizations. Then the carboxylic

acid was then converted to amide. The nature of the activating group in amide synthesis and its effect in changing the compound's stereochemistry must be considered in peptide synthesis.²¹ The leaving group for these activated carboxylic acids is the chloride ion, which is extremely labile and will consequently lead to the formation of the oxalzone enolate intermediate and thus a change in chirality. Carbodiimides are also extremely effective in activating carboxylic acids *via*

formation of an *O*-acylisourea, which is subsequently react with an amine to synthesize the amide.²² The issue with this reaction is the degree of racemization/epimerization and the formation of a urea by-product that is difficult to remove. To reduce this undesired effect, carbodiimides are often used with catalytic amounts of 1-hydroxy-7-azabenzotriazole (HOAt) or 1-hydroxybenzotriazole (HOBt).^{23,24} The synthetic importance of hydroxybenzotriazoles cannot be stressed enough, as it has led to the development of single molecule dual-purpose coupling agents. Compound **1** was synthesized according to this method and the detailed study can be found in our previous report.¹

Cleavage of the Cbz protecting group from **1** was performed with considerable high yields (92%). The crude product of **2** can be easily isolated as a hydrobromide salt upon trituration with anhydrous diethyl ether and subsequently recrystallized from MeOH/ether. Alternatively, Cbz deprotection can be achieved using H₂ and Pd/C, although HBr/AcOH is quite efficacious without the requirement of the pyrophoric catalyst or a highly-combustible gas.

Dipeptide Synthesis

The two divisions in peptide synthesis are solid-phase²⁵ and solution phase peptide synthesis. The solution-phase peptide synthesis approach was chosen (**Figure 4**) due to ease of performing reactions in a scalable fashion. Using D-amino acids as starting materials, both substrates have to be N-protected for S3-positioned substrate and *O*-protected for the S2-positioned substrate before coupling. Protection of the COOH- terminus is a facile procedure typically performed in literature²⁶⁻²⁸ by dissolving the amino

acid into MeOH, followed with excess addition of thionyl chloride to generate the acyl chloride *in situ*. The acyl chloride immediately reacts with the MeOH solvent to generate the methyl ester product as an HCl salt. Thionyl chloride is added to the reaction vessel at 0 °C since the formation of the acyl chloride is an exothermic process.

Protection of the primary amine can be achieved via amide formation using an appropriate *N*-protecting group. For protections that require removal later on in the synthetic scheme, carbamates are very useful protecting groups due to their varying ranges of pH stability. For example, Cbz and tert-butyloxycarbonyl (Boc) protecting groups are acid-labile while Fluorenylmethyloxycarbonyl (Fmoc) is base-labile at room temperature. Acetyl and trifluoroacetyl groups display some robustness and are quite small compared to the aforementioned list but can be removed using Zn/HCl or basic conditions (pH > 12) with heat. Since *N*-deprotection is not being performed, the acetyl group was chosen. This can be achieved by heating or refluxing acetic anhydride with the free amine using methanol or glacial acetic acid as the solvent. Excess solvent can be removed with ease using rotatory evaporation and recrystallization of the residue with an appropriate solvent.

Once the appropriate protections have been performed, the coupling reaction to synthesize the amide product is the following step. This can be achieved using HBTU, an efficient coupling agent that surpasses the efficacy of carbodiimides such as dicyclohexylcarbodiimide or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.²⁹ HBTU is a cheaper alternative than HATU but displays nearly identical results.³⁰

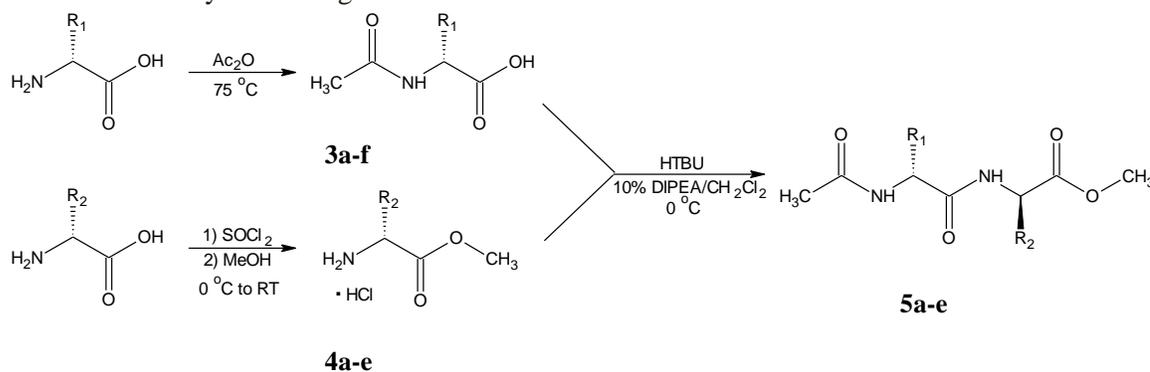
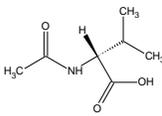
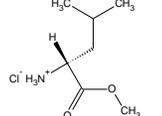
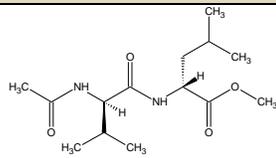
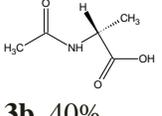
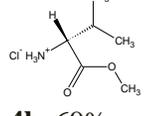
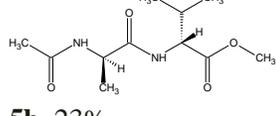
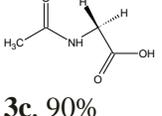
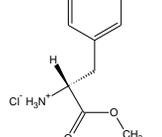
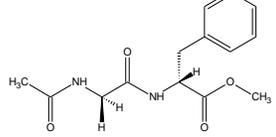
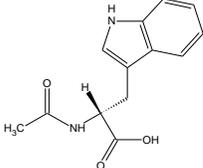
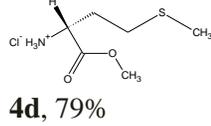
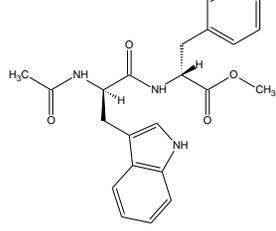
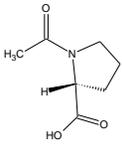
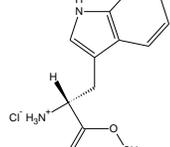
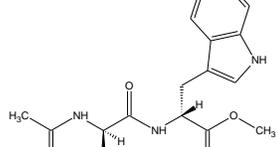


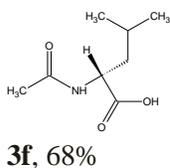
Figure 4 – Dipeptide synthesis scheme using a solution-phase HBTU coupling procedure.

Summary of reaction yields of the dipeptide synthesis are shown in **Table 2**. Both *N*- and *O*-protections were performed with product yields ranging from modest to excellent. Alanine acetylation worked particularly poorly, in part due to difficulty in precipitating out the product out of the mother liquor. A mixed solvent system using ethyl acetate/petroleum ether may help improve yields in future work. Carboxylic acid protections using thionyl chloride in methanol were consistent with yields and purification of product. Crystallization of the products from MeOH/diethyl ether are the optimal mixed solvent system for HCl salts.

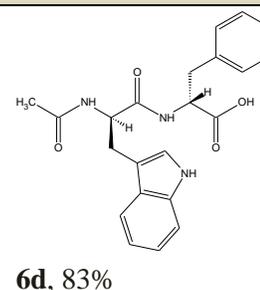
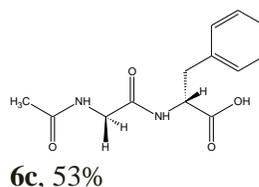
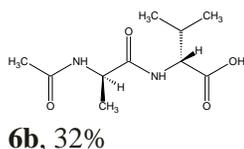
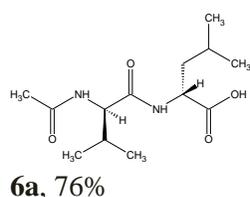
Coupling procedures for di- and tripeptides showed mixed success in the solution phase synthesis. Isolation of the products were somewhat difficult to isolate and most products were obtained as oily residues after the initial work-up. Trituration with a non-polar solvent and subsequent recrystallization was implemented into the procedures to alleviate these issues and achieve spectroscopic purity. This often had to be utilized in the case of amino acids/peptides containing aliphatic side chains. Peptides are notorious for difficulty in crystallization and typically have to be precipitated to afford the synthesized product.³¹

Table 2 – Summary of reaction yields for the synthesis of peptidomimetic substrates from D-amino acid starting materials

Acetylation	Esterification	HBTU Coupling
 <p>3a, 70%</p>	 <p>4a, 82%</p>	 <p>5a, 53%</p>
 <p>3b, 40%</p>	 <p>4b, 69%</p>	 <p>5b, 23%</p>
 <p>3c, 90%</p>	 <p>4c, 86%</p>	 <p>5c, 80%</p>
 <p>3d, 64%</p>	 <p>4d, 79%</p>	 <p>5d, 78%</p>
 <p>3e, 71%</p>	 <p>4e, 91%</p>	 <p>5e, 76%</p>



Ester Deprotection (6a-d)



For NMR spectra of amino acids and coupled peptides, diastereotopic proton splitting is widely seen in compounds containing methylene protons and at least one chiral center. For the dipeptide N-acetyl-D-glycine-D-tryptophan methyl ester (**5e**) in **Figure 5**, H_a and H_b diastereotopic protons of the glycyl fragment display a distinct splitting pattern that looks similar to a doublet of quartets. Reaction progress for

protections, deprotections, and couplings can be tracked via analysis of amide proton splitting and methyl/COOH protons. This facilitates analysis of these compounds, especially in the case of convoluted splitting patterns that arise in the final step in the synthesis. Additionally, the compound N-acetyl-D-proline exhibits cis/trans isomerism of both 1H and ^{13}C signals, analogous to those published in literature.³²

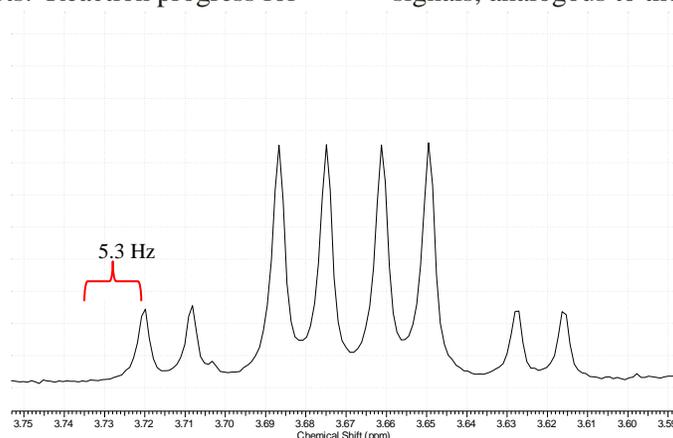


Figure 5 – 1H NMR of N-acetyl-D-glycine-D-tryptophan methyl ester (**5e**), displaying diastereotopic splitting of methylene protons, H_aH_b , of the glycyl fragment.

The final step peptide coupling

The final step in the synthetic route were not without its own difficulties. Final peptide couplings have been previously shown to have lower yields and longer reaction times.³³ Yields were less than half of the expected amount for the given reactions (**Table 3**). 1H NMR analysis revealed the formation of diastereomers, which was confirmed using ^{31}P NMR

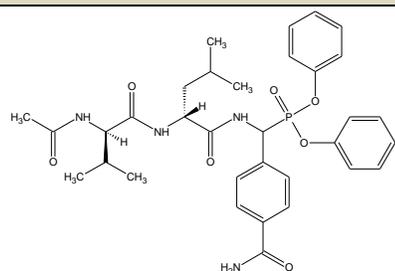
if additional product was available for use. For **7a**, 3 diastereomers are apparent from 1H and ^{31}P studies, suggesting that epimerization of the dipeptide may have occurred. From peak integration, the ratio of the diastereomeric products are 1:0.39:0.74 for the peaks at 13.82, 13.73, and 13.57, respectively. For **7c**, only two diastereotopic peaks were observed with a ratio of

1:0.36. Diastereomer peak assignment was not possible using the NMR techniques described here.

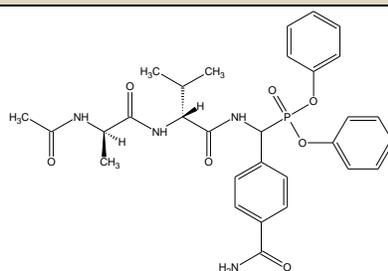
IR spectroscopy confirmed the presence of functional groups characteristic of intermediates and the final products. For acetylated intermediates, C=O ($\sim 1700\text{ cm}^{-1}$) and N-H (2° amide, $\sim 3100\text{ cm}^{-1}$) stretches were observed while for methyl ester synthesis resulted in amine salts with broad N-H

stretches at $\sim 2200\text{--}3100\text{ cm}^{-1}$. Compound **2** also exhibited a similar N-H stretch, as expected. Peptide intermediates had shown expected 2° amide stretches and the final products displayed the presence of the 1° amide mode in addition to the aforementioned IR absorptions. A representative IR spectrum of the compounds can be found in **Figure 6**.

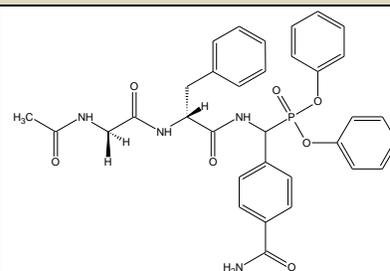
HATU Coupling



7a, 43%



7b, 34%



7c, 39%

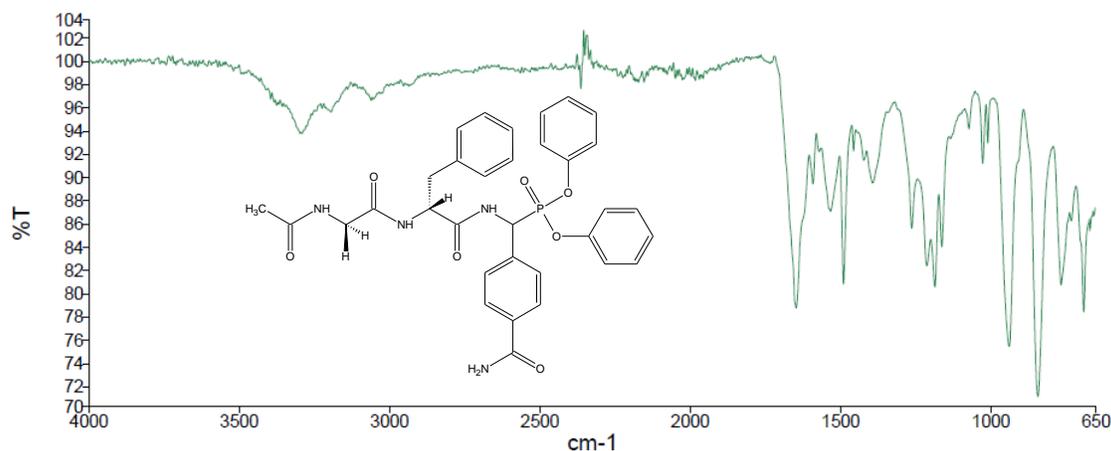


Figure 6. IR of compound 7c.

Experimental:

Solvents and instruments

All chemicals were purchased from Fisher Scientific and were used as received. Deuterated solvents were purchased from Acros Organics. All D-amino acids and coupling reagents were purchased from Alfa Aesar with purity greater than 98+% and were used as received. Solvents were purchased from

Pharmo-AAPER and Alfa Aesar. Anhydrous methanol (MeOH) used in reactions was prepared by refluxing the solvent with I_2 chips and Mg turnings for 30 minutes, followed by distillation of MeOH which was subsequently stored over 3 \AA molecular sieves. NMR spectra for ^1H , ^{13}C , and ^{31}P nuclei were obtained

on Varian INNOVA 300 MHz and 500 MHz NMR spectrometers. Broad-band proton-decoupled ^{31}P spectra were recorded using 85% phosphoric acid in a sealed capillary as an internal standard. Infrared spectra were obtained using Perkin-Elmer Spectrum One Fourier Transform infrared absorbance spectrometers using the universal diamond attenuated total reflectance (ATR) accessory. HRMS experiments were conducted using Thermo-Finnigan tandem Linear Ion Trap / Fourier Transform-Ion Cyclotron Resonance mass spectrometer (LTQ FT-ICR MS). Samples were directly injected into the MS using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). Solvent matrices included mixtures of MeOH, ACN, H_2O , and/or HCO_2H . No effort was made to separate the enantiomeric or diastereomeric final products.

4.2 Synthesis

Diphenyl [amino(4-carbamoylphenyl)methyl] phosphonate hydrobromide (2)

Under inert atmosphere, Diphenyl [N-benzyloxycarbonylamino(4-carbamoylphenyl)methyl] phosphonate (540 mg, 1.04 mmol) was suspended in a round bottom containing 8 mL hydrobromic acid solution, 33% in acetic acid. The reaction was stirred for 3 hours at room temperature, followed by addition of excess diethyl ether and agitated for an additional hour. The reaction mixture was placed in $-10\text{ }^\circ\text{C}$ environment to promote trituration of the crude product. The crude material was filtered, washed with ether, and recrystallized in methanol/ether to afford a white crystalline product (445 mg, 92%); ^1H NMR (DMSO, 500 MHz) δ 5.76 (d, $J = 17.5$ Hz, 1H), 6.95 (d, $J = 8.8$ Hz, 2H), 7.11 (d, $J = 8.8$ Hz, 2H), 7.23 (m, 2H), 7.34 (m, 2H), 7.41 (m, 2H), 7.50/8.06 (s, cis/trans, 2H), 7.76 (dd, $J = 8.3$ Hz & $J = 1.9$ Hz, 2H), 7.98 (d, $J = 8.3$ Hz, 2H), 9.50 (s, 3H); ^{13}C NMR (DMSO, 300 MHz) δ 49.26, 51.32, 120.28, 120.33, 120.42, 120.48, 125.69, 125.99, 128.00, 128.03, 128.82, 128.90, 130.06, 130.18, 133.22, 133.29, 135.28, 149.28, 149.33, 149.40, 149.45, 167.22; ^{31}P NMR (DMSO, 300 MHz) δ 12.26; HRMS (APCI, MeOH) m/z calculated for $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_4\text{P}$ (M+1) 383.11552, found 383.11578.

N-acetyl-D-valine (3a)

D-valine (600 mg, 5.12 mmol) was placed in a round-bottom flask containing 3 mL methanol and stirred at $70\text{ }^\circ\text{C}$ until dissolution. Acetic anhydride (1.5 mL, 15.87 mmol) was slowly added to the stirring solution with the conditions maintained until completion of the reaction (16 hours). The solvent was reduced using rotatory evaporation and the residue was dissolved with hot ethyl acetate and cooled to $-16\text{ }^\circ\text{C}$. The product precipitated out of solution as a white powder (570 mg, 70%); ^1H NMR (DMSO, 500 MHz) δ 0.88 (d, $J = 5.3$ Hz, 6H), 1.87 (s, 3H), 2.01 (m, 1H), 4.12 (m, 1H), 7.97 (d, $J = 6.8$ Hz, 1H), 12.47 (br. s, 1H); ^{13}C NMR (DMSO, 300 MHz) δ 18.12, 19.23, 22.39, 29.84, 57.25, 169.64, 173.30; HRMS (ESI, ACN/ H_2O / HCO_2H 70/30/0.01) m/z calculated for $\text{C}_7\text{H}_{14}\text{NO}_3$ (M+1) 160.09682, found 160.09682.

N-acetyl-glycine (3b)

Glycine (1.80 g, 24 mmol) was partially dissolved in 20 mL methanol. Acetic anhydride (6.8 mL, 72 mmol) was slowly added to the solution and the reaction was refluxed for 6 hours. Volatiles were removed using rotatory evaporation and the residue was dissolved in ethyl acetate and cooled to $0\text{ }^\circ\text{C}$ to afford a white crystalline solid that was filtered and washed with cold ethyl acetate (2.21 g, 90%); ^1H NMR (DMSO, 500 MHz) δ 1.85 (s, 3H), 3.72 (d, $J = 5.8$ Hz, 2H), 8.16 (m, 1H), 12.47 (s, 1H); ^{13}C NMR (DMSO, 300 MHz) δ 22.47, 40.87, 170.05, 171.71; HRMS (ESI, ACN/ H_2O / HCO_2H 70/30/0.01) m/z calculated for $\text{C}_4\text{H}_8\text{NO}_3$ (M+1) 118.04987, found 118.04987.

N-acetyl-D-alanine (3c)

D-alanine (500 mg, 5.61 mmol) was suspended in 2.5 mL glacial acetic acid, stirring at room temperature. Acetic anhydride (1 mL, 10.58 mmol) was added dropwise to the reaction vessel and was stirred overnight, at which dissolution was achieved. The solvent was reduced using rotatory evaporation and the residue was dissolved with hot ethyl acetate and cooled to $-16\text{ }^\circ\text{C}$. The product precipitated from the mother liquor as a white powder (300 mg, 40%); ^1H NMR (DMSO, 300 MHz) δ 1.24 (d, $J = 7.6$ Hz, 3H), 1.83 (s, 3H), 4.17 (quint, $J = 7.3$ Hz (x4), 1H), 8.17 (d, $J = 7.0$ Hz, 1H), 12.50 (br. s, 1H); ^{13}C NMR (DMSO, 300 MHz) δ 17.25, 22.38, 47.50, 169.12, 174.40;

HRMS (ESI, ACN/H₂O/HCO₂H 70/30/0.01) *m/z* calculated for C₅H₁₀NO₃ (M+1) 132.06552, found 132.06551.

N-acetyl-D-proline (3d)

D-proline (173 mg, 1.50 mmol) was stirred in 5 mL solution of 1,4-dioxane/water (1:1 mixture) and heated to 70 °C to achieve dissolution. Acetic anhydride (150 μL, 1.59 mmol) was slowly added to the solution and the sealed round bottom was stirred overnight under maintained heating. Volatiles were removed under reduced pressure and the solution was acidified with 10% HCl until pH 1, then extracted with ethyl acetate (x3). The combined organic phases were concentrated in vacuo and cooled to -16 °C to afford a white crystalline solid (168 mg, 71%); ¹H NMR (DMSO, 500 MHz) δ 1.84/1.90 (m, 2H), 1.86/1.97 (s, 3H), 2.05/2.24 (m, 1H), 2.13 (m, 1H), 3.35/3.51 (m, 2H), 4.19/4.46 (dd, *J* 8.7 Hz & 3.5 Hz, 1H), 12.51 (br. s, 1H); ¹³C NMR (DMSO, 300 MHz) δ 22.41, 24.33, 29.03, 30.80, 45.75, 47.20, 58.09, 59.34, 168.20, 168.46, 173.50, 173.83; HRMS (ESI, ACN/H₂O/HCO₂H 70/30/0.01) *m/z* calculated for C₇H₁₂NO₃ (M+1) 158.08117, found 158.08117.

N-acetyl-D-tryptophan (3e)

D-tryptophan (600 mg, 2.94 mmol) was suspended in 6 mL water containing sodium bicarbonate (540 mg, 6.14 mmol). While stirring, acetic anhydride (1.8 mL, 19.04 mmol) was added dropwise and the reaction mixture was heated to 40 °C to achieve dissolution. The reaction was maintained at the aforementioned conditions for 3 hours. Upon completion, the mixture was cooled to 0 °C overnight to afford a pale yellow solid, which was filtered and washed with cold water and used without further purification (460 mg, 64%); ¹H NMR (DMSO, 500 MHz) δ 1.79 (s, 3H), 2.98 (dd, *J* = 14.6 & 8.8 Hz, 1H), 3.15 (dd, *J* = 14.8 & 5.1 Hz, 1H), 4.45 (m, 1H), 6.98 (t, *J* = 7.5 Hz, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 7.13 (d, *J* = 2.4 Hz, 1H), 7.33 (d, *J* = 7.8 Hz, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 10.82 (s, 1H), 12.57 (br. s, 1H); ¹³C NMR (DMSO, 300 MHz) δ 22.48, 27.21, 53.07, 110.04, 111.45, 118.22, 118.43, 120.98, 123.61, 127.25, 136.15, 169.33, 173.68; HRMS (ESI, ACN/H₂O/

HCO₂H 70/30/0.01) *m/z* calculated for C₁₃H₁₅N₂O₃ (M+1) 247.10772, found 247.10820.

N-acetyl-D-leucine (3f)

D-leucine (500 mg, 5.12 mmol) was suspended in 2 mL acetic acid and stirred at room temperature. Acetic anhydride (1 mL, 10.58 mmol) was slowly added to the solution and was allowed to stir overnight. The solvent was reduced using rotatory evaporation and the residue was dissolved with hot ethyl acetate and cooled to -16 °C. The product precipitated out of solution as a white powder (450 mg, 68%); ¹H NMR (DMSO, 500 MHz) 0.87 (dd, *J* = 14.6 Hz & 6.4 Hz, 6H), 1.49 (m, 2H), 1.62 (m, 1H), 1.84 (s, 3H), 4.20 (m, 1H), 8.08 (d, *J* = 8.2 Hz, 1H), 12.45 (br. s, 1H); ¹³C NMR (DMSO, 300 MHz) δ 21.31, 22.33, 22.85, 24.33, 50.21, 169.30, 174.27; HRMS () *m/z* calculated for C_xH_xNO_x (M+1), found .

D-leucine methyl ester hydrochloride (4a)

D-leucine (600 mg, 4.57 mmol) was dissolved in 9 mL of dry methanol and subsequently cooled to 0 °C using an ice water bath. While stirring, thionyl chloride (3 mL, 41.35 mmol) was added drop-wise to the solution via syringe. After addition, the reaction vessel was removed from the ice water bath and stirred overnight at room temperature. The solvent was reduced in vacuo and the crude solid was dissolved in minimal hot methanol. Upon adding diethyl ether, the product crystallized out at room temperature as white needles (680 mg, 82%); ¹H NMR (DMSO, 300 MHz) δ 0.89 (d, *J* = 6.4 Hz, 6H), 1.72 (m, 3H), 3.73 (s, 3H), 3.90 (t, *J* = 7.0 Hz (x2), 1H), 8.76 (br. s, 3H); ¹³C NMR (DMSO, 300 MHz) δ 22.07, 23.67, 39.11, 50.45, 52.69, 170.28; HRMS (ESI, ACN/H₂O/HCO₂H 70/30/0.01) *m/z* calculated for C₇H₁₆NO₂ (M+1) 146.11756, found 146.11755.

D-valine methyl ester hydrochloride (4b)

D-valine (300 mg, 2.56 mmol) was dissolved in 4 mL of dry methanol and subsequently cooled to 0 °C using an ice water bath. While stirring, thionyl chloride (1.5 mL, 20.68 mmol) was added drop-wise to the solution via syringe. After addition, the reaction vessel was removed from the ice water bath and stirred overnight at room temperature. The solvent was reduced in

vacuo and the crude solid was dissolved in minimal hot methanol. Upon adding diethyl ether, the product crystallized out at $-16\text{ }^{\circ}\text{C}$ as white cubic crystals (297 mg, 69%); ^1H NMR (DMSO, 500 MHz) δ 0.95 (d, $J = 6.8$ Hz, 3H), 0.99 (d, $J = 6.8$ Hz, 3H), 2.20 (m, 1H), 3.76 (s, 3H), 3.85 (d, $J = 4.9$ Hz, 2H), 8.62 (s, 3H); ^{13}C NMR (DMSO, 300 MHz) δ 17.59, 18.41, 29.33, 52.59, 57.24, 169.28; HRMS (ESI, MeOH/H₂O/HCO₂H 50/50/0.01) m/z calculated for C₆H₁₄NO₂ (M+1) 132.10191, found 132.10197.

D-tryptophan methyl ester hydrochloride (4c)

D-tryptophan (600 mg, 2.94 mmol) was dissolved in 9 mL of dry methanol and subsequently cooled to $0\text{ }^{\circ}\text{C}$ using an ice water bath. While stirring, thionyl chloride (3 mL, 41.35 mmol) was added drop-wise to the solution via syringe. After addition, the reaction vessel was removed from the ice water bath and stirred overnight at room temperature. The solvent was reduced in vacuo and the crude solid was dissolved in minimal hot methanol. Upon adding diethyl ether, the product crystallized out at $-16\text{ }^{\circ}\text{C}$ as a pale purple solid (683 mg, 91%); ^1H NMR (DMSO, 500 MHz) δ 3.30 (m, 2H), 3.65 (s, 3H), 4.22 (t, $J = 6.3$ Hz, 1H), 7.01 (m, 1H), 7.09 (m, 1H), 7.24 (d, $J = 2.4$ Hz, 1H), 7.37 (d, $J = 7.8$ Hz, 1H), 7.50 (d, $J = 7.8$ Hz, 1H), 8.55 (s, 3H), 11.09 (s, 1H); ^{13}C NMR (DMSO, 300 MHz) δ 26.15, 52.65, 52.75, 106.40, 111.66, 118.08, 118.65, 121.18, 125.07, 126.96, 136.27, 169.79; HRMS (ESI, ACN/H₂O/HCO₂H 70/30/0.01) m/z calculated for C₁₂H₁₅N₂O₂ (M+1) 219.11280, found 219.11279.

D-methionine methyl ester hydrochloride (4d)

D-methionine (200 mg, 1.34 mmol) was dissolved in 3 mL of dry methanol and subsequently cooled to $0\text{ }^{\circ}\text{C}$ using an ice water bath. While stirring, thionyl chloride (1 mL, 12.38 mmol) was added drop-wise to the solution via syringe. After addition, the reaction vessel was removed from the ice water bath and stirred overnight at room temperature. The solvent was reduced in vacuo and the oil was dissolved in minimal hot methanol. The product crystallized out at $-16\text{ }^{\circ}\text{C}$ as a white crystalline solid (212 mg, 79%); ^1H NMR (DMSO, 500 MHz) δ 2.05 (s, 3H), 2.08 (m, 2H), 2.59 (m, 2H), 3.76 (s, 3H), 4.09 (t, $J = 6.2$ Hz (x2), 1H), 8.73 (br. s, 3H); ^{13}C NMR (DMSO, 300 MHz) δ 14.23,

28.33, 29.33, 50.78, 52.81, 169.62; HRMS (APCI, MeOH) m/z calculated for C₆H₁₄NO₂S (M+1) 164.07398, found 164.07393.

D-phenylalanine methyl ester hydrochloride (4e)

D-phenylalanine (1 g, 6.05 mmol) was suspended in 15 mL of dry methanol and subsequently cooled to $0\text{ }^{\circ}\text{C}$ using an ice water bath. While stirring, thionyl chloride (5 mL, 68.92 mmol) was added drop-wise to the solution via syringe. After addition, dissolution was achieved and the reaction vessel was removed from the ice water bath and stirred overnight at room temperature. The solvent was reduced in vacuo and the solid was dissolved in minimal hot methanol. Upon addition of diethyl ether, the product crystallized out at room temperature as white needles (1.12 g, 86%); ^1H NMR (DMSO, 500 MHz) δ 3.14 (m, 1H), 3.30 (m, 1H), 3.64 (s, 3H), 4.21 (dd, $J = 7.3$ & 5.6 Hz, 1H), 7.30 (m, 5H), 8.99 (br. s, 3H); ^{13}C NMR (DMSO, 300 MHz) δ 35.82, 52.52, 53.38, 127.25, 128.61, 129.43, 134.90, 169.33; HRMS (ESI, ACN/H₂O/HCO₂H 70/30/0.01) m/z calculated for C₁₀H₁₄NO₂ (M+1) 180.10191, found 180.10184.

N-acetyl-D-valine-D-leucine methyl ester (5a)

N-acetyl-D-valine (325 mg, 2.04 mmol) was dissolved in 8 mL of a 10% diisopropylethylamine in dichloromethane solution, then cooled to $0\text{ }^{\circ}\text{C}$ in an ice water bath. While stirring, HBTU (765 mg, 2.01 mmol) was added and the reaction was stirred for 20 minutes. After activation, D-leucine methyl ester (320 mg, 2.20 mmol) was added to the reaction vessel. The reaction was allowed to slowly warm to room temperature over a period of 4 hours. After completion, the solvent was reduced in vacuo and the residue was partially dissolved in ethyl acetate and washed & extracted with a saturated solution of ammonium chloride, sodium bicarbonate, and brine, successively. The organic phase was extracted and dried with magnesium sulfate, filtered, and dried to afford the crude product. The spectroscopically pure product was isolated via crystallization in ethyl acetate/ hexanes at $-16\text{ }^{\circ}\text{C}$ as a white solid (306 mg, 53%); ^1H NMR (CDCl₃, 500 MHz) δ 0.95 (m, 12H), 1.58 (m, 3H), 2.03 (s, 3H), 2.08 (dq, $J = 13.2$ & 6.8 (x3) Hz), 1H), 3.73 (s, 3H), 4.28 (dd, $J = 8.8$ & 6.8 Hz, 1H),

4.58 (td, $J = 8.6$ Hz (x2), 1H) 6.14 (m, 2H); HRMS (ESI, ACN/H₂O/HCO₂H 70/30/0.01) m/z calculated for C₁₄H₂₇N₂O₄ (M+1) 287.19653, found 287.19660.

N-acetyl-D-alanine-D-valine methyl ester (5b)

N-acetyl-D-alanine (140 mg, 1.07 mmol) was dissolved in 4 mL of a 10% diisopropylethylamine in dichloromethane solution, then cooled to 0 °C in an ice water bath. While stirring, HBTU (390 mg, 1.03 mmol) was added and the reaction was stirred for 20 minutes. After activation, D-valine methyl ester (172 mg, 1.03 mmol) was added to the reaction vessel. The reaction was allowed to slowly warm to room temperature over a period of 4 hours. After completion, the solvent was reduced in vacuo and the residue was dissolved in dichloromethane and washed & extracted with a saturated solution of ammonium chloride, sodium bicarbonate, and brine, successively. The organic phase was extracted and dried with magnesium sulfate, filtered, and dried to afford an oil, which was dissolved in a mixture of ethyl acetate and hexanes and cooled to -16 °C. The product was precipitated as fine white needles (60 mg, 23%); ¹H NMR (CDCl₃, 500 MHz) δ 0.86 (m, 6H), 1.17 (t, $J = 6.8$ Hz (x2), 3H), 1.82 (d, $J = 3.4$ Hz, 3H), 2.03 (m, 1H), 3.63 (d, $J = 6.3$ Hz, 3H), 4.17 (m, 1H), 4.40 (m, 1H), 8.00 (t, $J = 7.6$ Hz (x2), 1H), 8.07 & 8.18 (d, $J = 8.3$ Hz, 1H); HRMS (ESI, ACN/H₂O/ HCO₂H 50/50/0.01) m/z calculated for C₁₁H₂₁N₂O₄ (M+1) 245.14958, found 245.14979.

N-acetyl-glycine-D-phenylalanine methyl ester (5c)

N-acetyl-glycine (184 mg, 1.57 mmol) was dissolved in 6 mL of a 10% diisopropylethylamine in dichloromethane solution, then cooled to 0 °C in an ice water bath. While stirring, HBTU (590 mg, 1.56 mmol) was added and the reaction was stirred for 15 minutes. After activation, D-phenylalanine methyl ester hydrochloride (340 mg, 1.58 mmol) was added to the reaction vessel. The reaction was allowed to slowly warm to room temperature over a period of several hours. After completion, the solvent was reduced in vacuo and the residue was dissolved in dichloromethane and washed & extracted with a saturated solution of ammonium chloride, sodium bicarbonate, and brine, successively. The organic

phase was extracted and dried with magnesium sulfate, filtered, and dried to afford an oil, which was consequently dissolved in hot ethyl acetate. At -16 °C, the product precipitated as fine white crystals (348 mg, 80%); ¹H NMR (DMSO, 300 MHz) δ 1.82 (s, 3H), 2.96 (m, 2H), 3.59 (s, 3H), 3.67 (m, 2H), 4.47 (td, $J = 8.2$ Hz (x2) & 5.9 Hz, 1H), 7.23 (m, 5H), 8.03 (t, $J = 5.6$ Hz (x2), 1H), 8.30 (d, $J = 7.6$ Hz, 1H); HRMS (APCI, MeOH) m/z calculated for C₁₄H₁₉N₂O₄ (M+1) 279.13393, found 279.13424.

N-acetyl-D-tryptophan-D-phenylalanine methyl ester (5d)

N-acetyl-D-tryptophan (190 mg, 0.77 mmol) was suspended in 3 mL of a 10% diisopropylethylamine in dichloromethane solution, then cooled to 0 °C in an ice water bath. While stirring, HBTU (292 mg, 0.77 mmol) was added and the reaction was stirred for 20 minutes. After activation, D-phenylalanine methyl ester hydrochloride (170 mg, 0.79 mmol) was added to the reaction vessel. The reaction was allowed to slowly warm to room temperature overnight. After completion, the solvent was reduced in vacuo and the residue was dissolved in ethyl acetate and washed & extracted with a saturated solution of ammonium chloride, sodium bicarbonate, and brine, successively. The organic phase was extracted and dried with magnesium sulfate, filtered, and dried to afford an oil, which was consequently dissolved in ethyl acetate/hexanes. At -16 °C, the product precipitated as a white solid (245 mg, 78%); ¹H NMR (DMSO, 300 MHz) δ 1.75 (s, 3H), 2.86 (m, 4H), 3.59 (d, $J = 14.1$ Hz, 3H), 4.52 (m, 2H), 7.02 (m, 3H), 7.25 (m, 6H), 7.56 (t, $J = 8.8$ Hz (x2), 1H), 7.97 (dd, $J = 10.0$ Hz & 8.2 Hz, 1H), 8.47 (m, 1H), 10.76 (d, $J = 15.2$ Hz, 1H); HRMS (APCI, MeOH) m/z calculated for C₂₃H₂₆N₃O₄ (M+1) 408.19178, found 408.19151.

N-acetyl-D-glycine-D-tryptophan methyl ester (5e)

N-acetyl-glycine (92 mg, 0.78 mmol) was dissolved in 4 mL of a 10% diisopropylethylamine in dichloromethane solution, then cooled to 0 °C in an ice water bath. While stirring, HBTU (292 mg, 0.77 mmol) was added and the reaction was stirred for 20 minutes. After activation, D-tryptophan methyl ester (200 mg, 0.78 mmol) was added to the reaction vessel.

The reaction was allowed to slowly warm to room temperature over a period of 4 hours. After completion, the solvent was reduced in vacuo and the residue was partially dissolved in ethyl acetate and washed & extracted with a saturated solution of ammonium chloride, sodium bicarbonate, and brine, successively. The organic phase was extracted and dried with magnesium sulfate, filtered, and dried to afford an oil, which was dissolved in hot ethyl acetate and cooled to -16 °C. The product was precipitated as a pale yellow solid (185 mg, 76%); ¹H NMR (DMSO, 500 MHz) δ 1.83 (s, 3H), 3.11 (m 2H), 3.58 (s, 3H), 3.69 (m, 2H), 4.52 (q, *J* = 7.5 Hz, 1H), 6.99 (t, *J* = 7.8 Hz, 1H), 7.06 (t, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 2.5 Hz, 1H), 7.33 (d, *J* = 8.3 Hz, 1H), 7.48 (d, *J* = 7.9 Hz, 1H), 8.04 (t, *J* = 5.8 Hz, 1H), 8.23 (d, *J* = 7.8 Hz, 1H), 10.88 (s, 1H); HRMS (ESI, ACN/H₂O/HCO₂H 70/30/0.01) *m/z* calculated for C₁₆H₂₀N₃O₄ (M+1) 318.14483, found 318.14546.

N-acetyl-D-valine-D-leucine (6a)

N-acetyl-D-valine-D-leucine methyl ester (200 mg, 0.70 mmol) was dissolved in 4 mL of absolute ethanol and cooled to 0 °C in an ice water bath. Subsequently, 620 μL of 5% sodium hydroxide solution was slowly added and the reaction was stirred for an additional 1.5 hours. After completion, 10% HCl solution was added dropwise to acidify the solution to pH 1 and ethanol was slowly removed using light vacuum to afford the solid as white crystals (145 mg, 76%); ¹H NMR (DMSO, 500 MHz) δ 0.85 (m, 12H), 1.51 (m, 2H), 1.62 (m, 1H), 1.85 (s, 3H), 1.95 (dq, *J* = 13.2 & 6.8 (x3) Hz, 1H), 4.19 (m, 2H), 7.82 (d, *J* = 9.2 Hz, 1H), 8.11 (d, *J* = 7.8 Hz, 1H), 12.43 (br. s, 1H); HRMS (ESI, ACN/H₂O/HCO₂H 50/50/0.01) *m/z* calculated for C₁₃H₂₅N₂O₄ (M+1) 295.16283, found 295.16318.

N-acetyl-D-alanine-D-valine (6b)

N-acetyl-D-alanine-D-valine methyl ester (100 mg, 0.41 mmol) was dissolved in 2 mL of methanol and cooled to 0 °C in an ice water bath. Subsequently, 1 mL of 5% NaOH solution was slowly added and the reaction was stirred for an additional 3 hours. After completion, solvent was reduced in vacuo and the mixture was acidified with 10% HCl solution. The aqueous phase was extracted three times with ethyl

acetate and the organic phases were combined, dried with MgSO₄, and the solid filtered off. The solvent of the organic phase was removed using rotatory evaporation, resulting in a colorless oil residue. The residue was dissolved in hot ethyl acetate, adding a small portion of hexanes. The product precipitated out of solution at -16 °C as a pale green solid (30 mg, 32%); ¹H NMR (DMSO, 500 MHz) δ 0.86 (m, 6H), 1.18 (m, 3H), 1.83 (s, 3H), 2.03 (m, 1H), 4.13 (m, 1H), 4.38 (m, 1H), 7.89 (d, *J* = 8.2 Hz, 1H), 8.01 (m, 1H); HRMS (APCI, MeOH) *m/z* calculated for C₁₀H₁₉N₂O₄ (M+1) 231.13393, found 231.13417.

N-acetylglycine-D-phenylalanine (6c)

N-acetylglycine-D-phenylalanine methyl ester (340 mg, 1.22 mmol) was dissolved in 4 mL of methanol and cooled to 0 °C in an ice water bath. Subsequently, 4 mL of 5% NaOH solution was slowly added and the reaction was stirred for an additional 3 hours. After completion, solvent was reduced in vacuo and the mixture was acidified with 10% HCl solution. The aqueous phase was extracted three times with ethyl acetate and the organic phases were combined, dried with MgSO₄, and the solid filtered off. The solvent of the organic phase was removed using rotatory evaporation, resulting in a slightly yellow oil residue. The residue was dissolved in hot ethyl acetate, adding a small portion of hexanes to promote crystallization of the product as a white solid (172 mg, 53%); ¹H NMR (DMSO, 300 MHz) δ 1.82 (s, 3H), 2.87 (dd, *J* = 14.1 Hz & 8.8 Hz, 1H), 3.04 (dd, *J* = 13.5 Hz & 5.3 Hz, 1H), 3.65 (m, 2H), 4.42 (td, *J* = 8.5 Hz (x2) & 5.3 Hz, 1H), 7.23 (m, 5H), 8.02 (t, *J* = 5.6 Hz (x2), 1H), 8.11 (d, *J* = 7.6 Hz, 1H), 12.77 (br. s, 1H); HRMS (APCI, MeOH) *m/z* calculated for C₁₃H₁₇N₂O₄ (M+1) 265.11828, found 265.11858.

N-acetyl-D-tryptophan-D-phenylalanine (6d)

N-acetyl-D-tryptophan-D-phenylalanine methyl ester (140 mg, 0.34 mmol) was dissolved in 2 mL of methanol and cooled to 0 °C in an ice water bath. Subsequently, 2 mL of 5% NaOH solution was slowly added and the reaction was stirred for an additional 2 hours. After completion, solvent was reduced in vacuo and the mixture was acidified with 10% HCl solution. The acidified solution was allowed to stir at

room temperature for approximately 1 hour, during which the product precipitated out of solution as a white solid (112 mg, 83%); ^1H NMR (Acetone- d_6 300 MHz) δ 1.83 (s, 3H), 3.11 (m, 4H), 4.86 (m, 2H), 7.03 (m, 3H), 7.19 (m, 5H), 7.39 (m, 2H), 7.59 (m, 1H), 10.03 (m, 1H); HRMS (APCI, MeOH) m/z calculated for $\text{C}_{22}\text{H}_{24}\text{N}_3\text{O}_4$ (M+1) 394.17613, found 394.17599.

Diphenyl [N-(N-acetyl-D-valyl-D-leucyl)amino(4-carbamoylphenyl)methyl] phosphonate (7a)

N-acetyl-D-valine-D-leucine (30 mg, 0.11 mmol) was dissolved in 2 mL of a 10% diisopropylethylamine in dichloromethane solution and cooled to 0 °C in an ice water bath. While stirring, HATU (43 mg, 0.11 mmol) was added to the reaction mixture. After the activation period of 15 minutes, diphenyl [amino(4-carbamoylphenyl)methyl] phosphonate (43 mg, 0.11 mmol) was added and the reaction was allowed to continue stirring overnight. Volatiles were removed using rotatory evaporation and the residue was washed with saturated ammonium chloride, sodium bicarbonate, and brine solutions, successively, extracting into ethyl acetate. The organic phase was dried with magnesium sulfate, filtered, concentrated using vacuum, and cooled to -16 °C to afford a white solid (31 mg, 43%); ^1H NMR (DMSO, 300 MHz) δ 0.80 (m, 12H), 1.46 (m, 3H), 1.86 (m, 3H), 1.88 (m, 1H), 4.18 (m, 1H), 4.59 (m, 1H), 5.95 (m, 1H), 6.99 (m, 3H), 7.09 (m, 1H), 7.20 (m, 2H), 7.34 (m, 5H), 7.69 (m, 2H), 7.88 (m, 3H), 7.98 (m, 1H), 8.07/8.28/8.38 (d, $J = 8.2$ Hz, 1H), 9.20/9.39/9.49 (m, 1H); ^{31}P NMR (DMSO, 300 MHz) δ 13.57, 13.73, 13.82; HRMS (ESI, ACN/ H_2O / HCO_2H 70/30/0.1) m/z calculated for $\text{C}_{33}\text{H}_{42}\text{N}_4\text{O}_7\text{P}$ (M+1) 637.27856, found 637.27998.

Diphenyl [N-(N-acetyl-D-alanyl-D-valyl)amino(4-carbamoylphenyl)methyl] phosphonate (7b)

N-acetyl-D-alanine-D-valine (15 mg, 65 μmol) was dissolved in 1 mL of a 10% diisopropylethylamine in dichloromethane solution. While stirring at room temperature, HATU (25 mg, 65 μmol) was added to the reaction mixture. After the activation period of 15 minutes, diphenyl [amino(4-carbamoylphenyl)methyl] phosphonate (25 mg, 54

μmol) was added and the reaction was allowed to continue stirring overnight. After completion, the mixture was dissolved into ethyl acetate and was subsequently washed with water, sodium bicarbonate (x2), and brine solutions, successively. The organic phase was dried with magnesium sulfate, filtered, concentrated using vacuum. The product precipitated out of ethyl acetate/hexanes at -16 °C to afford a pale yellow solid (11 mg, 34%); ^1H NMR (DMSO, 300 MHz) δ 0.80 (m, 6H), 1.13 (m, 3H), 1.80 (m, 3H), 4.35 (m, 1H), 4.59 (m, 1H), 5.99 (m, 1H), 6.98 (m, 3H), 7.09 (m, 1H), 7.20 (m, 2H), 7.36 (m, 5H), 7.69 (m, 2H), 7.86 (m, 3H), 7.99 (m, 1H), 8.13 (m, 1H) 9.33 (m, 1H), 9.57 (m, 1H); HRMS (APCI, MeOH) m/z calculated for $\text{C}_{30}\text{H}_{36}\text{N}_4\text{O}_7\text{P}$ (M+1) 595.23161, found 595.23151.

Diphenyl [N-(N-acetyl-D-glycyl-D-phenylalanyl)amino(4-carbamoylphenyl)methyl] phosphonate (7c)

N-acetylglycine-D-phenylalanine (150 mg, 0.57 mmol) was dissolved in 3.5 mL of a 10% diisopropylethylamine in dichloromethane solution and cooled to 0 °C in an ice water bath. While stirring, HATU (218 mg, 0.57 mmol) was added to the reaction mixture. After the activation period of 20 minutes, diphenyl [amino(4-carbamoylphenyl)methyl] phosphonate hydrobromide salt (260 mg, 0.56 mmol) was added and the reaction was allowed to continue stirring overnight. The reaction contents were partially dissolved into ethyl acetate and washed with sodium bicarbonate (x3) and brine solutions, successively. The organic phase was dried with magnesium sulfate, filtered, and concentrated using rotatory evaporation. The crude solid was dissolved in a mixture of hot ethyl acetate and diethyl ether and subsequently cooled to -16 °C to afford a white solid (140 mg, 39%); ^1H NMR (DMSO, 500 MHz) δ 1.79/1.84 (s, 3H), 2.86 (m, 2H), 3.65 (m, 2H), 4.82/4.90 (m, 1H), 5.96 (m, 1H), 7.00 (m, 3H), 7.20 (m, 8H), 7.36 (m, 5H), 7.63/7.74 (m, 2H), 7.97 (m, 4H), 8.15/8.22 (d, $J = 8.8$ Hz, 1H), 9.49/9.68 (d, $J = 9.8$ Hz, 1H); ^{31}P NMR (DMSO, 300 MHz) δ 15.46, 15.66; HRMS (APCI, MeOH) m/z calculated for $\text{C}_{33}\text{H}_{34}\text{N}_4\text{O}_7\text{P}$ (M+1) 629.21596, found 629.21584.

RESULTS AND DISCUSSIONS:

For the synthesis of α -aminoalkylphosphonates and its peptide derivatives, a convergent synthetic scheme was devised similar to that published by Oleksyszyn et al.⁹ A convergence synthesis was chosen rather than a linear route to improve the overall efficiency of the route. The synthetic scheme is shown in **Figure 3**. From the left-hand route, the first step is the Kabachnik-Fields reaction to synthesize the N-protected α -aminoalkylphosphonate. The para substituent on the compound can vary depending on the aldehyde substrate, or a derivative can be synthesized after the first step of the route. The N-protecting group, a benzyl carbamate, can be removed using a strong acid such as HBr to afford the ammonium salt of the compound. At this point in the synthetic scheme, this intermediate can be coupled to a dipeptide via amide condensation using a stoichiometric coupling reagent such as 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxid hexafluorophosphate (HATU) or *N,N,N',N'*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU).

In order to synthesize dipeptide substrates for the final reaction step, protected amino acids must first be coupled, as shown on the right-hand side of the scheme. Each amino acid is either N-acetylated using acetic anhydride under reflux conditions or O-protected as the methyl ester. After amide coupling mediated by HATU or HBTU, the methyl ester is cleaved with dilute NaOH to yield the N-protected dipeptides. The two routes converge at the final step to yield the tripeptide mimic containing the phosphonate moiety at the S1 position.

CONCLUSION

The synthetic scheme described in this work is a viable method for the synthesis of the peptide derivatives α -aminoalkylphosphonates. The overall synthetic route is a simple approach for the synthesis of peptidomimetics containing a diphenyl phosphonate moiety at the COOH-terminus. Solution phase synthesis of dipeptides were completed with moderate yields overall, but the scalability of the reactions greatly benefits this approach compared to

solid-phase methods. Additionally, none of the products required chromatographic separation to achieve spectroscopic purity, as determined by NMR. In addition, diastomeric ratio of tripeptide mimics can be determined using either ¹H or ³¹P NMR via integration of nuclei signals.

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