# Solid-Phase Synthesis of Novel Trimers Containing a Phenylstatine Core and Analysis by High-Resolution Magic Angle Spinning

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*Received January 2, 2007*

Here we describe a multistep solid-phase synthetic approach for the addition of amino acid residues to both the C- and N-termini of a phenylstatine core, yielding a library aimed at the development of structure−activity relationships in the S2 and S2′ regions of the aspartyl proteases. Optimization of the synthetic strategy was performed on the basis of the in situ analysis of the compounds bound to the solid support through high-resolution magic angle spinning NMR Spectroscopy (HR-MAS NMR).

## Introduction

Although peptides display a diverse range of biological properties and their use as drugs is usually compromised by their instability, low bioavailability, and poor pharmacological profiles, they are still useful tools to enhance the knowledge of the recognition site of their biological receptors.

Aspartyl proteases are a well-characterized class of enzymes that play an important role in mammals, plants, fungi, parasites, and retrovirus.[1] This family cleaves proteins through a general acid−base mechanism using a water molecule activated by two aspartate residues as the nucleophile (Figure 1). To take advantage of the enzymatic mechanism, most known aspartyl protease inhibitors are transition-state mimics designed to displace the activated catalytic water. Thus secondary alcohols, diols, and amines often appear as the central "core" of the aspartyl protease inhibitors. The core may also include groups that contribute to specificity through interactions with the S1 or S1′ binding pockets of the protease.[2] Binding affinity and selectivity can then be enhanced by the addition of amino acid residues (or equivalents) to both the C- and N-termini of the core to interact with additional specificity pockets (S2, S2′, etc.).[3]

Most reported aspartyl protease libraries have focused on a single "core" transition state isostere, varying both the number and structure of amino acid residues for the N- and C-termini.[4] This approach is illustrated by our recent lead-generation effort for BACE.[5] In this example, a library of pentamers was generated to establish structure−activity relationships using the known statine isostere flanked by valine residues as the central unit and exhaustively varying the N- and C-termini residues with the natural amino acids to give a library of 20 × 20 compounds. Herein, we report on the synthesis of a library of trimers to explore the S2 and S2′ regions of the aspartyl proteases, as shown in Figure 2. In particular, the choices of phenylstatine isostere as the core structure and the *S*-stereochemistry for the hydroxyl group were based upon the known preferences of the aspartyl protease BACE-1.[6] A minimal number of representative amino acids (6) were attached to both sides of the phenylstatine moiety to reduce the total library size. This set was chosen to represent acidic (Glu), basic (Lys), aromatic (Phe), aliphatic (Ala, Val), and polar (Ser) amino acids. Trimers rather than pentamers were targeted to minimize the peptide length and explore the minimum size of inhibitors which are needed to evaluate the potential of a given core structure (Figure 2).

Furthermore, because of the observation of low yields during the solid-phase synthesis of the trimers, we used NMR spectroscopy for characterization of the organic reactions on solid supports. There are two NMR strategies currently available to obtain such data. The first involves using standard NMR methods on resin samples that are swollen with a solvent and placed into conventional liquid high-resolution probes. While it is possible to obtain high-quality $^{13}C$ spectra from such samples (gel-phase $^{13}C$ NMR), significant line-broadening resulting from residual dipolar coupling and the heterogeneity of the sample still persists, and $^{1}H$ NMR is not accessible.[7−9] The major limitations of this approach are its intrinsic poor sensitivity and the information content being limited to $^{13}C$ spectra. The second strategy involves rotating the solvent swollen resins at an angle of 54.7° to reduce the line-broadening, resulting in line widths that approach those obtained in solution-phase NMR. This technique (high-resolution magic angle spinning, HR-MAS, NMR spectroscopy) has emerged as a powerful tool for in situ characterization of a compound anchored to a solid support[10−12] and can be widely applied to compounds attached to resins provided some degree of mobility is introduced into the sample by an appropriate swelling of the

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10.1021/cc070001o CCC: $37.00 
Published on Web 07/14/2007

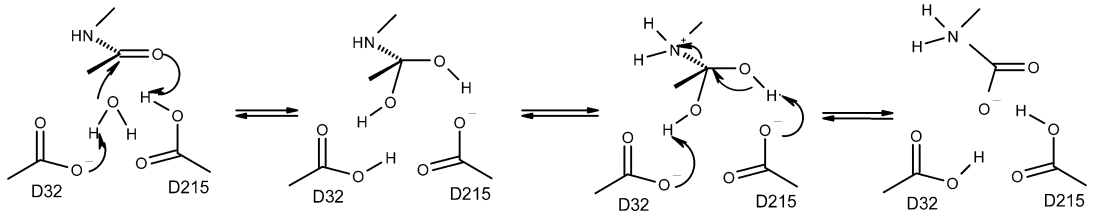



**Figure 1.** Catalytic mechanism for substrate hydrolysis by aspartic proteases.

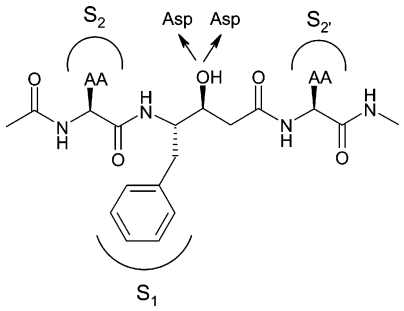



**Figure 2.** Phenylstatine trimer library.

solid support.[10−12] The HR-MAS analysis of the intermediates and final products led to the optimization of the synthetic strategy during the library construction.

## Results and Discussion

In recent years, the use of alkoxybenzaldehyde-based linkers for the production of carboxamides has attracted a significant interest. The first linker described for this purpose was based on trialkoxy systems related to the amide-releasing PAL resin.[13] It was soon realized, however, that such electronic-rich systems were not always required to facilitate cleavage, particularly when the leaving group was a secondary amine. Thus, new linkers based on simply monoalkoxy benzaldehydes have been exploited. The AMEBA resin (acid-sensitive aldehyde resin, **I**) was selected for our initial studies because it is among the most versatile of the formyl linkers.[14] Initial functionalization involved the conversion of the resin-bound formyl group to the corresponding secondary amine **II** using excess of methylamine in the presence of $NaBH(OAc)_3$ in trimethylorthoformate (TMOF) as outlined in Scheme 1. The next synthetic step was the incorporation of the first protected amino acid using bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBrOP) as the coupling agent to afford **III**.

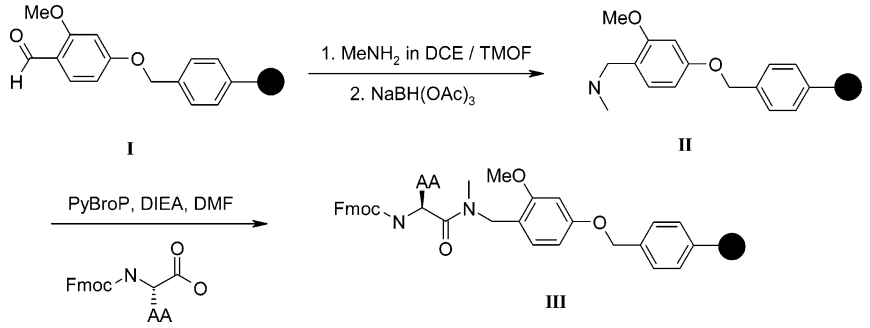
Because the yields obtained after two reaction steps were poor, a more sensitive analytical technique to both monitor the progress of the solid-phase reactions and to obtain detailed structural information was sought. In this context, HR-MAS NMR was chosen to confirm the structure of the intermediates bound to the resin, to investigate the possible formation of side products, and to quantify the loading of the compound on the solid support.

Prior to the characterization of the intermediates, a $^1$H HR-MAS spectrum was acquired of **I** to identify the signals arising from the polymer support itself. The spectrum exhibited broad peaks between 6.3 and 7.3 ppm and between 1.3 and 2.0 ppm corresponding to the aromatic and aliphatic parts of the polystyrene matrix, respectively (Figure 3a). Additional peaks arising from the resonances of the alkoxy-benzaldehyde linker were also identified, for instance, the signal at 10.31 ppm that corresponded to the benzaldehyde proton (H11 in Figure 3a). The signals of the linker were sharper than those of the resin matrix because of its higher degree of rotational freedom.

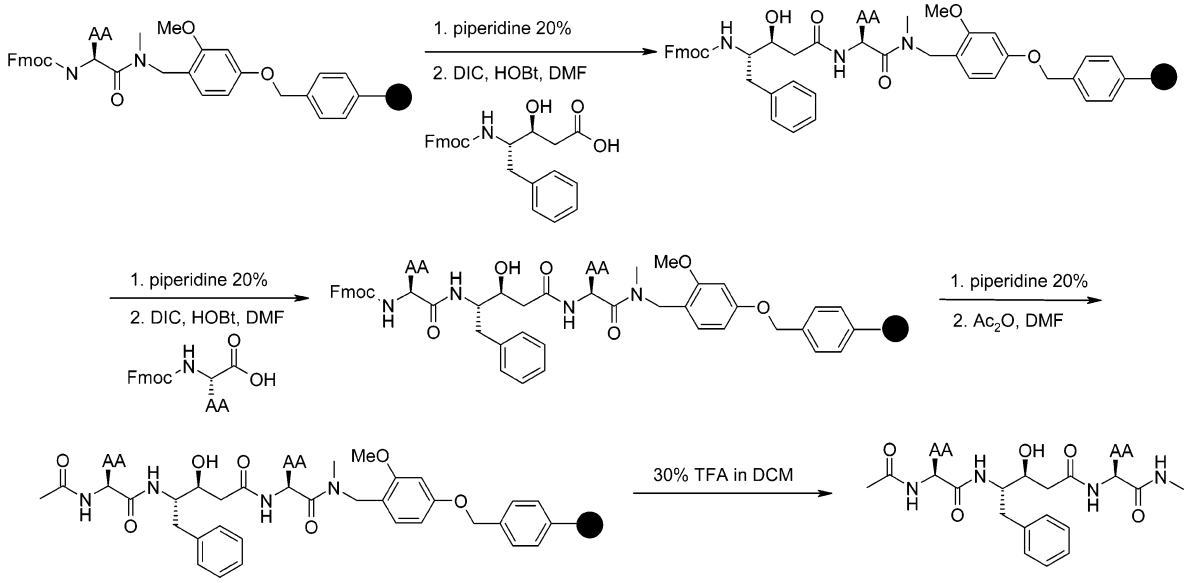
The first step of the synthetic scheme was monitored on the solid support. The $^1$H HRMAS spectrum of the reaction outcome showed the absence of the signal at 10.31 ppm and the presence of a signal at 8.62 ppm attributed to the HC═N proton of the undesired imine intermediate (H11 in Figure 3b), indicating that the reaction was not yet driven to completion. The use of longer reaction times resulted in the disappearance of the diagnostic benzaldehyde and imine signals (Figure 3c), confirming the complete reduction of the aldehyde to the amine under these conditions. The new peak at 2.39 ppm was assigned to the N-methyl protons of amine **II** (13 in Figure 3c).

The second synthetic step involving the coupling of the Fmoc-protected residue to the amine **II** was also monitored by HR-MAS. Inspection of the $^1$H spectrum revealed the appearance of four weak signals between 7.2 and 7.8 ppm that corresponded to the aromatic protons of the Fmoc protecting group, thus confirming the incorporation of the residue into the resin (Figure 4b). The yields of solid-phase reactions were determined by comparison of the intensity of a proton signal of the resin-bound product with that of a

**Scheme 1.** Reductive Amination of **I** and Incorporation of the First Protected Amino Acid into the Solid Support



**Scheme 2.** Solid-Phase Synthesis of the Trimers




proton signal of the linker.[15,16] In this case, the aromatic signal of the Fmoc protecting group at 7.74 ppm, which accounted for two aromatic protons and appeared to be well-separated from the resin background, was compared with that of the methylene protons of the linker (H1 in Figure 4b). The determined yields were quite low (10−20%), in complete agreement with those obtained through the conventional Fmoc quantification procedure upon cleavage of the protecting group from the resin.

Because the $^1$H spectrum also showed the presence of strong signals at 2.10 and 2.15 ppm that could not be attributed to the targeted molecule, the formation of a side product on the solid support was inferred to be the cause of the poor yields. The signals showed correlations to carbon resonances at 21.7 and 22.1 ppm in a $^1$H−$^{13}$C HSQC HR-MAS experiment and were attributed to the two rotamers of the tertiary amide shown in Figure 4b. The side product was generated via acetylation of **II** with the reducing agent

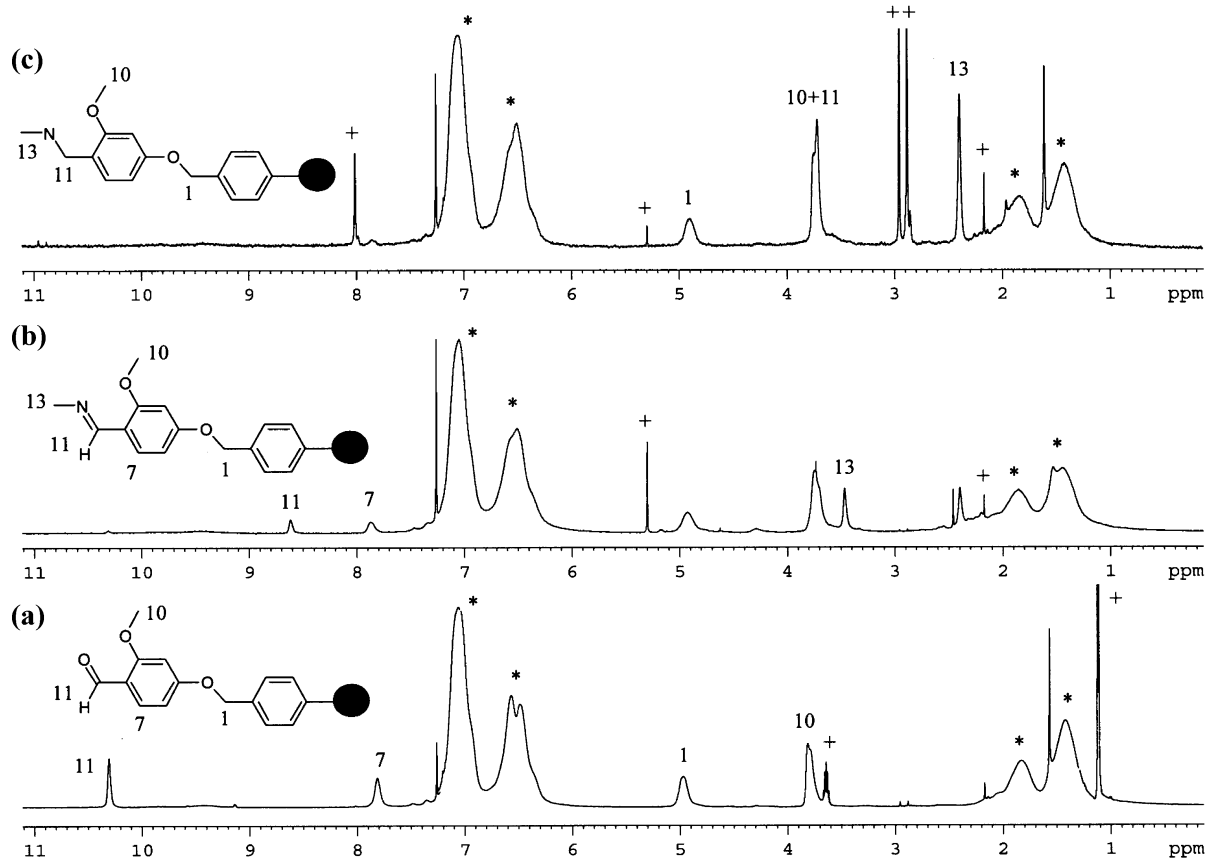



**Figure 3.** $^1$H HRMAS (500 MHz) spectra in $CDCl_3$ of the AMEBA resin **I** (a), the imine intermediate (b), and the amine **II** (c) attached to the solid support. The broad peaks arising from the polystyrene matrix and the sharp signals of additional solvents (acetone, diisopropyl ether, $CH_2Cl_2$, and DMF) are labeled with an asterisk (*) and a plus sign (+), respectively.

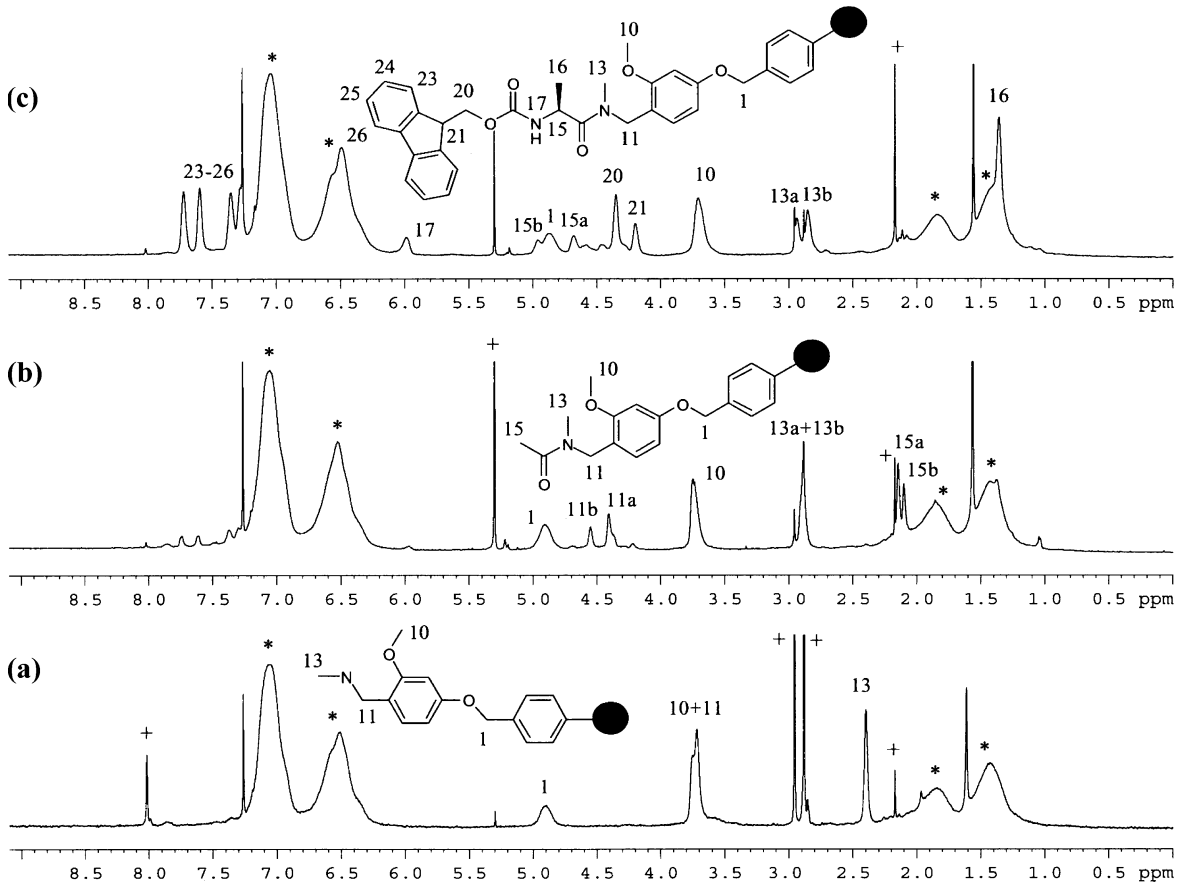



**Figure 4.** $^1$H HRMAS NMR (500 MHz) spectra in $CDCl_3$ of amine **II** (a), the side product originated from the undesired acetylation of **II** (b), and compound **III** (c) attached to the solid support. The broad peaks arising from the polystyrene matrix and the sharp signals of additional solvents (acetone, $CH_2Cl_2$, and DMF) are labeled with an asterisk (*) and a plus sign (+), respectively. The labels a and b are used to distinguish the signals corresponding to each rotamer.

$NaBH(OAc)_3$ that had not been completely removed from the solid support after the first synthetic step. More aggressive washing of the resin upon the reductive amination to eliminate the excess of reducing agent avoided the formation of the undesired product and led to excellent yields, as measured either through HR-MAS or the conventional Fmoc methodology. A detailed HR-MAS analysis was undertaken for **III** (AA = alanine) for an in situ characterization of the growing compound. The analysis of the $^1$H spectrum was complicated by the splitting of signal as a consequence of the slow interconversion of the *syn* and *anti* conformational isomers around the C−N bond of the tertiary amide. Homonuclear (COSY) and heteronuclear (edited-HSQC) 2D HR-MAS NMR experiments were therefore needed to achieve a full assignment of the proton resonances (Figure 5). The alanine $\beta$-methyl protons appeared at 1.36 ppm in the $^1$H spectrum (16 in Figure 4c), and the α-proton of the alanine was identified for both rotamers through the coupling to the $\beta$-methyl protons observed in the COSY spectrum (Figure 5). Furthermore, the edited-HSQC spectrum allowed the discrimination of $CH_2$ versus the CH and $CH_3$ groups (the signals appear with opposite phase in the spectrum), leading to the assignment of the rest of the aliphatic resonances as shown in Figure 5.

Once the resin had been functionalized with the first amino acid, elongation of the peptide was accomplished by deprotection of the N-terminal group with 20% piperidine in DMF and coupling of the Fmoc-protected phenylstatine in DMF with DIC as coupling agent and HOBt as additive.

Completion of the amidation reaction was monitered using a Kaiser test until no amine was detected in the reaction. This test is very useful and straightforward for the detection of primary amines attached to the resin. In some cases, recoupling with stronger agent, HATU, was needed to complete the reaction. After Fmoc deprotection, the six resins were split six ways and subsequently coupled to the to the N-terminus of the chain for each of the designated six amino acids using the same procedure as before to produce the 36 protected peptides. Removal of the Fmoc group and acetylation of the final peptide yielded the corresponding anchored trimer. No acetylation of the free hydroxy group of the phenylstatine core was detected by HPLC or NMR in any case, probably because of the greater reactivity and steric accessibility of the amino terminal group compared to the more hindered hydroxyl group. As in previous steps, structure verification of some of the compounds before cleavage from the resin involved a combination of 1D and 2D HR-MAS NMR experiments.

Several TFA concentrations were tested to determine the best conditions for full cleavage of the compound from the resin. It was found that at least 30% TFA in DCM was needed to ensure full cleavage of the compound from the resin. In addition, triisopropylsilane (2.5%) was added as scavenger when Ser($^t$Bu) was present in the peptidic sequence

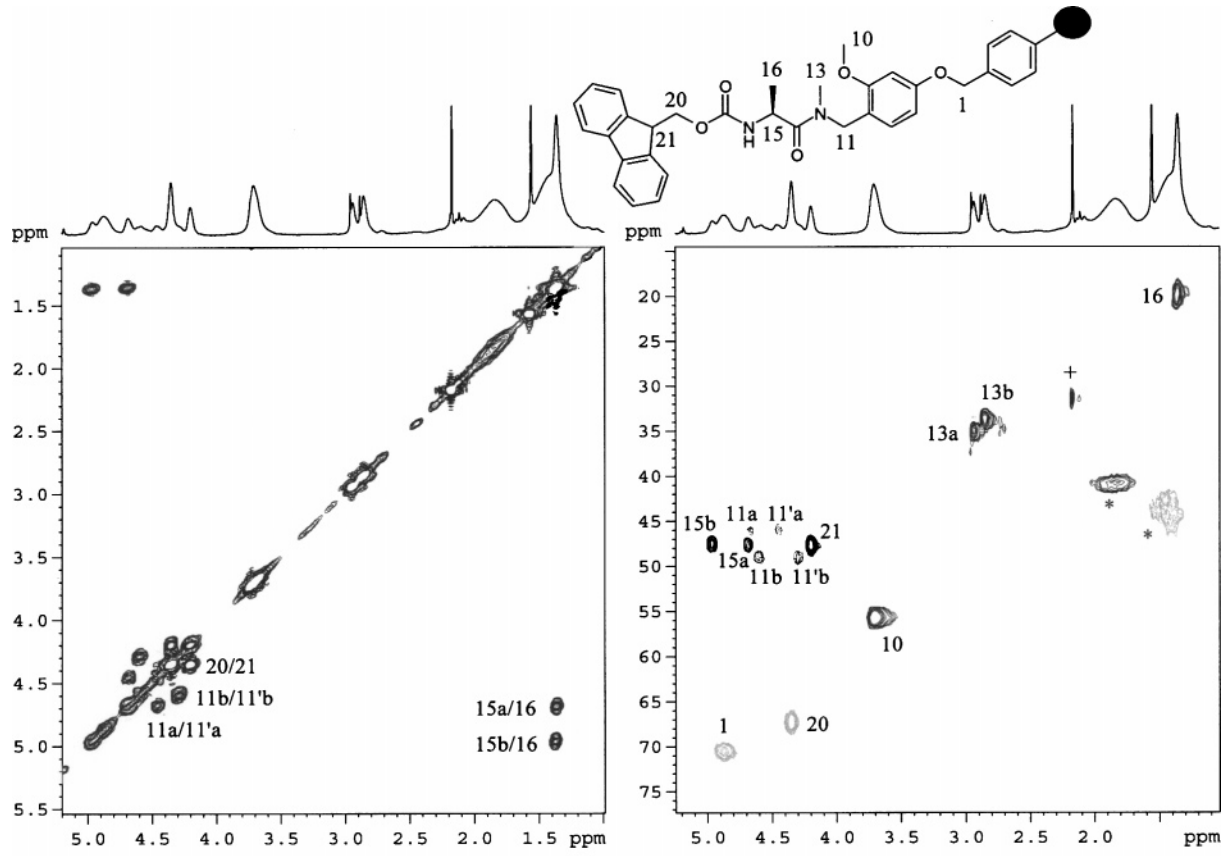



**Figure 5.** COSY (left) and edited-HSQC (right) HRMAS NMR (500 MHz) spectra in $CDCl_3$ of **III** (AA = alanine). The black and gray lines in the HSQC spectrum represent the opposite sign of the $CH_2$ versus the CH and $CH_3$ peaks. The labels a and b are used to distinguish the signals corresponding to each rotamer.

**Table 1.** Purities of the Trimers upon Cleavage from the Resins as Determined by HPLC under Basic Conditions

| entry | trimer | purity (%) | entry | trimer | purity (%) |
|---|---|---|---|---|---|
| 1 | Ac-Ala-PhStatine-Ala-NHMe | 75 | 19 | Ac-Ala-PhStatine-Glu-NHMe | 70 |
| 2 | Ac-Phe-PhStatine-Ala-NHMe | 83 | 20 | Ac-Phe-PhStatine-Glu-NHMe | 73 |
| 3 | Ac-Val-PhStatine-Ala-NHMe | 89 | 21 | Ac-Val-PhStatine-Glu-NHMe | 65[a] |
| 4 | Ac-Glu-PhStatine-Ala-NHMe | 70 | 22 | Ac-Glu-PhStatine-Glu-NHMe | 64[a] |
| 5 | Ac-Lys-PhStatine-Ala-NHMe | 95 | 23 | Ac-Lys-PhStatine-Glu-NHMe | 56 |
| 6 | Ac-Ser-PhStatine-Ala-NHMe | 49 | 24 | Ac-Ser-PhStatine-Glu-NHMe | 50 |
| 7 | Ac-Ala-PhStatine-Phe-NHMe | 74 | 25 | Ac-Ala-PhStatine-Lys-NHMe | 95 |
| 8 | Ac-Phe-PhStatine-Phe-NHMe | 78 | 26 | Ac-Phe-PhStatine-Lys-NHMe | 88 |
| 9 | Ac-Val-PhStatine-Phe-NHMe | 86 | 27 | Ac-Val-PhStatine-Lys-NHMe | 93 |
| 10 | Ac-Glu-PhStatine-PheNHMe | 48a | 28 | Ac-Glu-PhStatine-Lys-NHMe | 89 |
| 11 | Ac-Lys-PhStatine-Phe-NHMe | 80 | 29 | Ac-Lys-PhStatine-Lys-NHMe | *b* |
| 12 | Ac-Ser-PhStatine-Phe-NHMe | 83 | 30 | Ac-Ser-PhStatine-Lys-NHMe | 76 |
| 13 | Ac-Ala-PhStatine-Val-NHMe | 79 | 31 | Ac-Ala-PhStatine-Ser-NHMe | 56 |
| 14 | Ac-Phe-PhStatine-Val-NHMe | 62 | 32 | Ac-Phe-PhStatine-Ser-NHMe | 88 |
| 15 | Ac-Val-PhStatine-Val-NHMe | 78 | 33 | Ac-Val-PhStatine-Ser-NHMe | 86 |
| 16 | Ac-Glu-PhStatine-Val-NHMe | 63 | 34 | Ac-Glu-PhStatine-Ser-NHMe | 68 |
| 17 | Ac-Lys-PhStatine-Val-NHMe | 81 | 35 | Ac-Lys-PhStatine-Ser-NHMe | 82 |
| 18 | Ac-Ser-PhStatine-Val-NHMe | 64 | 36 | Ac-Ser-PhStatine-Ser-NHMe | 59 |

[a] These samples were analyzed under acidic conditions. [b] Quality of the HPLC signal was not good enough to provide an accurate assessment of purity

to achieve complete deprotection of Ser side chain. The purity of the final compounds was assessed by HPLC-UV (Table 1).

## Conclusions

We have reported a synthetic procedure that successfully allows for full diversification about the phenylstatine core. Using this approach, several trimers containing a phenylstatine core were prepared in library format that will allow us to search for structure−activity relationships in the S2−S2′ region on our panel cassette of aspartyl proteases. From an analytical standpoint, the difficulties associated with the monitoring of solid-phase reactions have been overcome with the use of HR-MAS NMR spectroscopy. This technique provided precise structural characterization of the intermediates attached to the solid support that is not attainable through

any other analytical technique. In particular, a side product formed during the coupling of the first amino acid was identified through HR-MAS analysis enabling the optimization of a synthetic procedure that avoided side-product formation. In addition, HRMAS was also used for quantification of the compound loading on the solid support, and the results so obtained were similar to those of the destructive Fmoc quantification procedure.

## Experimental Section

**Materials and Methods.** 4-Formyl-3-methoxy-phenyloxymethyl polystyrene resin (loading 0.92 mmol/g, 200−400 mesh) was purchased from Bachem. Amino acids were purchased from Novabiochem and Bachem, and the coupling agents were obtained from Novabiochem. All coupling reactions were performed in plastic syringes fitted with polystyrene frits. The solid-phase reactions were analyzed by HR-MAS NMR spectroscopy or after product cleavage from the resin sample by UV quantification. Chromatographic analysis was carried out on an Agilent HP1100 liquid chromatography system. Eluents for acidic conditions, A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile), were used in a gradient (0.5 min at 5% B, 5−60% B in 4.5 min). Eluents for basic conditions, A (ammonium bicarbonate, 10 mM in water) and B (acetonitrile), were used in a gradient (0.5 min at 5% B, 5−60% of B in 4.5 min). The flow rate was 1 mL/min, and the analytical HPLC column used was a 4.6 × 50 mm XTerra C-18 column. The 36 final compounds were characterized by mass spectrometry (MSD quadrupole mass spectrometer, Agilent Technologies), and the structures of 8 representative compounds were also confirmed by NMR spectroscopy. Liquid-phase $^1$H NMR spectra were acquired on a Bruker Avance DPX 300 MHz spectrometer equipped with an inverse probe at 25 °C. The proton chemical shifts were referenced to the residual DMSO signal at 2.5 ppm.

**HR-MAS NMR Experiments.** The resin samples (3 mg) were introduced into a 4 mm rotor and swollen in $CDCl_3$. The spectra were acquired on a Bruker Avance DRX 500 MHz spectrometer equipped with a 4 mm HR-MAS probe at 25 °C. The samples were spun at 4 kHz. Proton and carbon chemical shifts were referenced to the residual solvent signals at 7.26 and 77.1 ppm, respectively. $^1$H spectra were acquired using 32 K data points, which were zero-filled to 64 K data points prior to Fourier transformation. Absolute-value COSY and phase-sensitive HSQC spectra were acquired using gradient-selection techniques. Acquisition data matrices were defined by 1 K × 256 points in $t_2$ and $t_1$, respectively, and multiplied by appropriate window functions and zero-filled to 2 K × 512 matrices prior to Fourier transformation. Baseline correction was applied in both dimensions. Spectra were processed using the Bruker XWINNMR program on a Silicon Graphics computer.

**General Procedure for the Anchoring of First Amino Acid to the Resin.** 4-Formyl-3-methoxy-phenyloxymethyl polystyrene resin (7 g) was swollen in DCE for 1 h at room temperature in a 500 mL glass reactor fitted with a frit filter. Methylamine (2 M in THF, 10 equiv) was dissolved in DCE/TMOF (2:1); then it was added to the resin, and the slurry was agitated in an orbital shaker for 1 h at room temperature. Then, $NaBH(OAc)_3$ (10 equiv) in DCE/TMOF (2:1) was added to get a liquid gel. Mixture was shaken overnight at room temperature. After that, the resin was washed thoroughly with MeOH (×5), DCM (×3), DIPEA, 10% in DMF (×3), DMF (×4), and DCM (×3) and dried under vacuum overnight prior to the next coupling reaction.

N-methylated resin (3 g) was swollen in DCM, and DMF and Fmoc-AA-OH (3 equiv) dissolved in the minimum amount of DMF were added. Then DIEA (6 equiv) and PyBrOP (3 eq) were added, and the mixture was agitated overnight at room temperature. The resin was then washed with DMF (×3) and DCM (×3) and dried under vacuum. After this step, aliquots of the resin were taken to calculate the loading by HR-MAS NMR or Fmoc quantification. In the second case, between 2 and 5 mg of resin were taken and treated with 2 mL of 20% piperidine in DMF. This solution was diluted with DCM, and its absorbance at 300 nm was measured in a spectrophotometer. From the molar absortivity of the Fmoc-piperidine adduct at 300 nm, we calculated the concentration and therefore the resin loading.

**General Procedure for the Elongation of the Peptidyl Chain.** After the first amino acid coupling, the resin was acetylated with acetic anhydride (6 mmol) and DIPEA (12 mmol) in DMF. After 20 min, the resin was washed with DMF (×3), and the Fmoc group was removed with 20% piperidine in DMF (2 × 1 min and 2 × 10 min). After the mixture was washed with DMF and DCM, Fmoc-PhStatine-OH (3 equiv), HOBt (3 equiv), and DIC (3 equiv) were added to the resin in the minimum amount of DMF, and mixture was shaken at room temperature at least for 2 h until the Kaiser test became negative. In case coupling was not complete, recoupling was performed with Fmoc-PhStatine-OH (3 equiv), DIPEA (6 equiv), and HATU (3 equiv) in DMF for 90 min. The Fmoc group was removed with piperidine as shown before.

The intermediate peptidyl resins were respectively split into six batches of equal amounts to introduce the next point of diversity. HOBt (4 equiv) and DIC (4 equiv) in DMF were added to approximately 500 mg of resin Fmoc-AA-OH (4 equiv), and the mixture was agitated for 2 h. When the Kaiser test was positive, a new coupling was performed as explained previously. The Fmoc group was removed, and after the resin was washed, acetic anhydride (10 equiv) and DIPEA (20 equiv) in DMF were added. The mixture was agitated for 20 min at room temperature, and after that time, the resin was washed and dried under vacuum.

**General Procedure for Cleavage and Deprotection.** Peptidyl resins were placed in 16 × 100 mm screw-cap glass tubes and treated with 6−7 mL of 30% TFA in DCM. When Ser was present in the peptidic sequence, 2% TIPS was added to the cleavage cocktail. The mixtures were shaken for 3 h at room temperature, and then the solvent was removed in the Genevac evaporator.

**Ac-Ala-PhStatine-Ala-NHMe (1).** ES-MS Calcd ($C_{20}H_{31}N_4O_5$): [$MH^+$] 407.5. Found: [$MH^+$] 407.3.

**Ac-Phe-PhStatine-Ala-NHMe (2).** $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.17 (d, $J$ = 7.1 Hz, 3 H), 1.74 (s, 3 H), 2.12 (m, 2 H), 2.56 (d, $J$ = 4.6 Hz, 3 H), 2.69 (m, 2 H), 2.85 (dd,

$J$ = 13.5, 5.9 Hz, 1 H), 2.95 (dd, $J$ = 14.0, 4.5 Hz, 1 H), 3.90 (m, 2 H) 4.17 (m, 1 H), 4.52 (m, 1 H), 5.06 (d, $J$ = 5.3 Hz, 1 H), 7.23 (m, 10 H), 7.70 (d, $J$ = 9.1 Hz, 1 H), 7.77 (q, $J$ = 4.7 Hz, 1 H), 7.85 (d, $J$ = 7.5 Hz, 1 H), 8.04 (d, $J$ = 8.48 Hz, 1 H). ES-MS Calcd ($C_{26}H_{35}N_4O_5$): [$MH^+$] 483.6. Found: [MH+] 483.3.

**Ac-Val-PhStatine-Ala-NHMe (3).** ES-MS Calcd ($C_{22}H_{35}N_4O_5$): [$MH^+$] 435.5. Found: [$MH^+$] 436.1.

**Ac-Glu-PhStatine-Ala-NHMe (4).** ES-MS Calcd ($C_{22}H_{33}N_4O_7$): [$MH^+$] 465.5. Found: [$MH^+$] 464.8.

**Ac-Lys-PhStatine-Ala-NHMe (5).** ES-MS Calcd ($C_{23}H_{38}N_5O_5$): [$MH^+$] 464.6. Found: [$MH^+$] 464.3.

**Ac-Ser-PhStatine-Ala-NHMe (6).** ES-MS Calcd ($C_{20}H_{31}N_4O_6$): [$MH^+$] 423.5. Found: [$MH^+$] 423.2.

**Ac-Ala-PhStatine-Phe-NHMe (7).** $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.15 (d, $J$ = 7.3 Hz, 3 H), 1.83 (s, 3 H), 2.11 (d, $J$ = 6.7 Hz, 2 H), 2.56 (d, $J$ = 4.6 Hz, 3 H), 2.67 (m, 2 H), 2.81 (dd, $J$ = 13.7, 5.9 Hz, 1 H), 3.01 (dd, $J$ = 13.9, 4.8 Hz, 1 H), 3.87 (m, 2 H), 4.25 (m, 1 H), 4.37 (m, 1 H), 4.99 (d, $J$ = 5.3 Hz, 1 H), 7.21 (m, 10 H), 7.56 (d, $J$ = 9.3 Hz, 1 H), 7.84 (q, $J$ = 4.7 Hz, 1 H), 7.96 (d, $J$ = 7.3 Hz, 1 H), 7.97 (d, $J$ = 8.1 Hz, 1 H). ES-MS Calcd ($C_{26}H_{35}N_4O_5$): [$MH^+$] 483.6. Found: [$MH^+$] 483.3.

**Ac-Phe-PhStatine-Phe-NHMe (8).** ES-MS Calcd ($C_{32}H_{39}N_4O_5$): [$MH^+$] 559.7. Found: [$MH^+$]: 559.5.

**Ac-Val-PhStatine-Phe-NHMe (9).** ES-MS Calcd ($C_{28}H_{39}N_4O_5$): [$MH^+$] 511.6. Found: [$MH^+$] 511.5.

**Ac-Glu-PhStatine-PheNHMe (10).** ES-MS Calcd ($C_{28}H_{37}N_4O_7$): [$MH^+$] 541.6. Found: [$MH^+$] 541.3.

**Ac-Lys-PhStatine-Phe-NHMe (11).** ES-MS Calcd ($C_{29}H_{42}N_5O_5$): [$MH^+$] 540.7. Found: [$MH^+$] 540.4.

**Ac-Ser-PhStatine-Phe-NHMe (12).** $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.87 (s, 3 H), 2.12 (m, 2 H), 2.56 (d, $J$ = 4.4 Hz, 3 H), 2.66 (dd, $J$ = 13.7, 8.0 Hz, 1 H), 2.71 (dd, $J$ = 13.9, 9.5 Hz, 1 H), 2.81 (dd, $J$ = 13.6, 6.6 Hz, 1 H), 3.00 (dd, $J$ = 13.9, 4.7 Hz, 1 H), 3.54 (m, 2 H), 3.81 (m, 1 H), 3.90 (m, 1 H), 4.28 (m, 1 H), 4.34 (m, 1 H), 4.94 (t, $J$ = 5.5 Hz, 1 H), 4.98 (d, $J$ = 5.4 Hz, 1 H), 7.20 (m, 10 H), 7.60 (d, $J$ = 9.1 Hz, 1 H), 7.81 (m, 2 H), 7.86 (d, $J$ = 7.9 Hz, 1 H). ES-MS Calcd ($C_{26}H_{35}N_4O_6$): [$MH^+$] 498.6. Found: [$MH^+$] 498.4.

**Ac-Ala-PhStatine-Val-NHMe (13).** ES-MS Calcd ($C_{22}H_{35}N_4O_5$): [$MH^+$] 435.5. Found: [$MH^+$] 435.3.

**Ac-Phe-PhStatine-Val-NHMe (14).** ES-MS Calcd ($C_{28}H_{39}N_4O_5$): [$MH^+$] 511.6. Found: [$MH^+$] 511.3.

**Ac-Val-PhStatine-Val-NHMe (15).** ES-MS Calcd ($C_{24}H_{39}N_4O_5$): [$MH^+$] 463.6. Found: [$MH^+$] 463.3.

**Ac-Glu-PhStatine-Val-NHMe (16).** ES-MS Calcd ($C_{24}H_{37}N_4O_7$): [$MH^+$] 493.6. Found: [$MH^+$] 493.3.

**Ac-Lys-PhStatine-Val-NHMe (17).** ES-MS Calcd ($C_{26}H_{42}N_5O_5$): [$MH^+$] 492.6. Found: [$MH^+$] 492.4.

**Ac-Ser-PhStatine-Val-NHMe (18).** $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 0.81 (d, $J$ = 6.9 Hz, 6 H), 1.87 (s, 3 H), 1.93 (m, 1 H), 2.24 (m, 2 H), 2.56 (d, $J$ = 4.6 Hz, 3 H), 2.65 (dd, $J$ = 13.5, 7.7 Hz, 1 H), 2.83 (dd, $J$ = 13.5, 6.3 Hz, 1 H), 3.53 (t, $J$ = 5.6 Hz, 2 H), 3.87 (m, 2 H), 3.98 (dd, $J$ = 8.1, 6.5 Hz, 1 H), 4.27 (dd, $J$ = 13.5, 6.1 Hz, 1 H), 5.05 (t, $J$ = 5.5 Hz, 1 H), 5.09 (d, $J$ = 5.5 Hz, 1 H), 7.21 (m, 5 H), 7.58 (d, $J$ = 8.3 Hz, 1 H), 7.68 (d, $J$ = 9.1 Hz, 1 H), 7.85 (q, $J$ = 4.0 Hz, 1 H), 7.94 (d, $J$ = 7.7 Hz, 1 H). ES-MS Calcd ($C_{22}H_{35}N_4O_6$): [$MH^+$] 451.5. Found: [$MH^+$] 451.3

**Ac-Ala-PhStatine-Glu-NHMe (19).** ES-MS Calcd ($C_{22}H_{33}N_4O_7$): [$MH^+$] 465.5. Found: [$MH^+$] 465.3.

**Ac-Phe-PhStatine-Glu-NHMe (20).** ES-MS Calcd ($C_{28}H_{37}N_4O_7$): [$MH^+$] 541.6. Found: [$MH^+$] 541.3.

**Ac-Val-PhStatine-Glu-NHMe (21).** ES-MS Calcd ($C_{24}H_{37}N_4O_7$): [$MH^+$] 493.6. Found: [$MH^+$] 493.3.

**Ac-Glu-PhStatine-Glu-NHMe (22).** $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.68 (m, 2 H), 1.87 (m, 5 H), 2.20 (m, 6 H), 2.56 (d, $J$ = 4.6 Hz, 3 H), 2.65 (dd, $J$ = 14.2, 8.2 Hz, 1 H), 2.82 (dd, $J$ = 13.5, 5.1 Hz, 1 H), 3.90 (m, 2 H), 4.18 (m, 2 H), 5.08 (d, $J$ = 5.3 Hz, 1 H), 7.21 (m, 5 H), 7.57 (d, $J$ = 9.3 Hz, 1 H), 7.81 (q, $J$ = 4.4 Hz, 1 H), 7.88 (d, $J$ = 7.7 Hz, 1 H), 7.98 (d, $J$ = 7.7 Hz, 1 H). ES-MS Calcd ($C_{24}H_{35}N_4O_9$): [$MH^+$] 523.6. Found: [$MH^+$] 523.3.

**Ac-Lys-PhStatine-Glu-NHMe (23).** ES-MS Calcd ($C_{25}H_{40}N_5O_7$): [$MH^+$] 522.6. Found: [$MH^+$] 522.3.

**Ac-Ser-PhStatine-Glu-NHMe (24).** ES-MS Calcd ($C_{22}H_{33}N_4O_8$): [$MH^+$] 481.5. Found: [$MH^+$] 481.3.

**Ac-Ala-PhStatine-Lys-NHMe (25).** ES-MS Calcd ($C_{23}H_{38}N_5O_5$): [$MH^+$] 464.6. Found: [$MH^+$] 464.3.

**Ac-Phe-PhStatine-Lys-NHMe (26).** ES-MS Calcd ($C_{29}H_{42}N_5O_5$): [$MH^+$] 540.7. Found [$MH^+$] 540.4.

**Ac-Val-PhStatine-Lys-NHMe (27).** $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 0.78 (dd, $J$ = 6.6, 2.5 Hz, 6 H), 1.27 (m, 4 H), 1.46 (m, 1 H), 1.61 (m, 1 H), 1.88 (m, 4 H), 2.14 (dd, $J$ = 14.2, 4.4 Hz, 1 H), 2.24 (dd, $J$ = 14.5, 8.5 Hz, 1 H), 2.56 (d, $J$ = 4.7 Hz, 3 H), 2.63 (dd, $J$ = 13.2, 9.1 Hz, 1 H), 2.85 (dd, $J$ = 13.9, 5.7 Hz, 1 H), 3.89 (m, 1 H), 3.94 (m, 1 H), 4.08 (dd, $J$ = 8.2, 7.6 Hz, 1 H), 4.12 (ddd, $J$ = 8.5, 5.4 Hz, 1 H), 7.21 (m, 5 H), 7.54 (d, $J$ = 8.8 Hz, 1 H), 7.78 (m, 3 H). ES-MS Calcd ($C_{26}H_{42}N_5O_5$): [$MH^+$] 491.6. Found: [$MH^+$] 491.9.

**Ac-Glu-PhStatine-Lys-NHMe (28).** ES-MS Calcd ($C_{25}H_{40}N_5O_7$): [$MH^+$] 522.6. Found: [$MH^+$] 522.3

**Ac-Lys-PhStatine-Lys-NHMe (29).** ES-MS Calcd ($C_{26}H_{45}N_6O_5$): [$MH^+$] 521.7. Found: [$MH^+$] 521.4.

**Ac-Ser-PhStatine-Lys-NHMe (30).** ES-MS Calcd ($C_{23}H_{38}N_5O_6$): [$MH^+$] 480.6. Found: [$MH^+$] 480.3.

**Ac-Ala-PhStatine-Ser-NHMe (31).** ES-MS Calcd ($C_{20}H_{31}N_4O_6$): [$MH^+$] 423.5. Found: [$MH^+$] 423.2.

**Ac-Phe-PhStatine-Ser-NHMe (32).** ES-MS Calcd ($C_{26}H_{35}N_4O_6$): [$MH^+$] 499.6. Found: [$MH^+$] 499.3.

**Ac-Val-PhStatine-Ser-NHMe (33).** $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 0.78 (d, $J$ = 6.7 Hz, 6 H), 1.89 (m, 4 H), 2.22 (m, 2 H), 2.57 (d, $J$ = 4.4 Hz, 3 H), 2.63 (dd, $J$ = 13.6, 9.0 Hz, 1 H), 2.84 (dd, $J$ = 13.7, 5.7 Hz, 1 H), 3.55 (m, 2 H), 3.92 (m, 2 H), 4.10 (dd, $J$ = 8.6, 7.1 Hz, 1 H), 4.17 (m, 1 H), 4.84 (t, $J$ = 5.7 Hz, 1 H), 5.09 (d, $J$ = 5.1 Hz, 1 H), 7.22 (m, 5 H), 7.59 (d, $J$ = 8.9 Hz, 1 H), 7.75 (d, $J$ = 7.9 Hz, 1 H), 7.80 (m, 2 H). ES-MS Calcd ($C_{22}H_{35}N_4O_6$): [$MH^+$] 451.5. Found: [$MH^+$] 451.3.

**Ac-Glu-PhStatine-Ser-NHMe (34).** ES-MS Calcd ($C_{22}H_{33}N_4O_8$): [$MH^+$] 481.5. Found: [$MH^+$] 481.3.

**Ac-Lys-PhStatine-Ser-NHMe (35).** ES-MS Calcd ($C_{23}H_{38}N_5O_6$): [$MH^+$] 480.6. Found: [$MH^+$] 480.3.

**Ac-Ser-PhStatine-Ser-NHMe (36).** $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.87 (s, 3 H), 2.25 (d, $J$ = 6.7 Hz, 2 H), 2.57

(d, $J = 4.4$ Hz, 3 H), 2.67 (dd, $J = 13.5$, 8.3 Hz, 1 H), 2.83 (dd, $J = 13.5$, 6.5 Hz, 1 H), 3.53 (m, 4 H), 3.90 (m, 2 H), 4.13 (m, 1 H), 4.30 (m, 1 H), 4.84 (t, $J = 5.6$ Hz, 1 H), 4.99 (t, $J = 5.5$ Hz, 1 H), 5.08 (d, $J = 5.3$ Hz, 1 H), 7.21 (m, 5 H), 7.69 (m, 2 H), 7.77 (q, $J = 4.0$ Hz, 1 H), 7.90 (d, $J = 7.7$ Hz, 1 H). ES-MS Calcd ($C_{20}H_{31}N_4O_7$): [$MH^+$] 439.5. Found: [$MH^+$] 439.0.

**Acknowledgment.** We thank James McCarthy for his helpful advice on the preparation of this paper, Maria Teresa Diez and Alfonso Rivera for their technical support with the HPLC analysis, and Rafael Ferritto for his supervision during this work.

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CC070001O