

## Synthesis and Biological Activity of Novel Statine Derivatives Containing Ferrocenyl Moiety

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Na busca por novos inibidores de protease aspartica, conjugados de ferroceno com estatina foram planejados e sintetizados através de reação de acoplamento usando o protocolo padrão *N,N'*-diciclohexilcarboimida (DCC) e 1-hidroxibenzotriazol (HOBt). Os novos compostos foram caracterizados por espectroscopia de IR, <sup>1</sup>H NMR, MS e análise elemental. Os resultados do bioensaio mostraram que alguns dos novos compostos podem servir como ponto de partida.

In a search for new aspartic protease inhibitors, conjugates of ferrocene with statine were designed and synthesized by coupling reaction using the standard *N,N'*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) protocol. The title compounds were characterized by IR, <sup>1</sup>H NMR spectroscopy, MS and elemental analysis. The results of bioassay showed that some title compounds could serve as a starting point.

**Keywords:** statine, ferrocene, aspartic protease, synthesis, biological activity

### Introduction

Aspartic proteases are involved in many biological pathways in fungi, plants, humans, parasites, retroviruses.<sup>1-2</sup> *Aspergillus oryzae* aspartic proteinase is found to have activity not only for the activation of trypsinogen but also for the activation of chymotrypsinogen with the cleavage of the Arg15-Ile16 bond.<sup>3</sup> Statine, the (3*S*, 4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid configurational isomer is a key component of pepstatin and of other synthetic peptide inhibitors of aspartic protease.<sup>4</sup> It is well known that long-chain peptides can exhibit poor penetration and are generally unsuitable for a metabolically stable drug, because of enzymatic degradation.<sup>5</sup> In many case, this inconvenience can be circumvented by shortening the length of the inhibitors.

Ferrocene is a member of a special organometallic group known as metallocenes or "sandwich" molecules. Among metallocenes there are compounds with antiproliferative effect, antibiotics, and inhibitors of enzymes.<sup>6-9</sup> Conjugates of ferrocene with well-known drugs were reported.<sup>10-11</sup> Ferrocene-containing compounds often possess unexpected biological activity.<sup>12</sup> We report here the search for shorter aspartic protease inhibitors resulting from introducing

ferrocenyl unit into statine and synthesized the novel statine derivatives which might provide interesting biological properties. The structure of target compounds is listed in Table 1.

### Experimental

#### Materials and equipment

All the reactions were carried out under a nitrogen atmosphere with the exclusion of moisture. All the amino acids used were L-amino acids. N-Boc-statine and N-Boc-AHPPA were synthesized according to the method described in the literature.<sup>13</sup> Ferrocenylmethylamine was synthesized by the literature method of Kelly *et al.*<sup>14</sup> For syntheses of compounds **4** and **10** see reference.<sup>15</sup> Solvents were purified and dried by standard methods. The melting points were determined on an XT-4 micro melting point apparatus and uncorrected. IR spectra were recorded on an EQUINOX-55 spectrometer on a KBr matrix. <sup>1</sup>H NMR spectra were recorded on an INOVA-400 NMR spectrometer using TMS as an internal standard. Chemical shift values ( $\delta$ ) are given in ppm. Elemental analyses were performed on a Vario EL III CHNS analyzer. Electrospray mass spectra were obtained with an MALDI-TOF Mass spectrometer. 200-300 mesh silica gel was used for column chromatography.

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**Table 1** Structure of target compounds and their biological activities

Compound	R <sup>1</sup>	*1	R <sup>2</sup>	Inh @ 5 × 10 <sup>-4</sup> mol L <sup>-1</sup>
1	Boc	<i>S</i>		24%
2	Boc	<i>S</i>		41%
3	Boc	<i>R</i>		no active
4	Boc	<i>S</i>		56.6%
5	Boc	<i>R</i>		no active
6	Boc	<i>S</i>		21.3%
7	Boc	<i>S</i>		59.1%
8		<i>S</i>		61.2%
9	Boc	<i>S</i>	CH <sub>2</sub> Fc	13.6%
10	Boc-AHPPA	<i>S</i>	Val-CH <sub>2</sub> Fc	68.3%
11		<i>S</i>		37.4%

### General procedure A: Coupling reactions using DCC / HOBt

The amino compound (1 mmol) was dissolved in DCM (1 mL) and DMF (1 mL). Boc-amino acid (1.25 mmol) and HOBt (1.4 mmol) were added followed by a solution of DCC (1.4 mmol) in DCM (3 mL). The reaction mixture was allowed to stir at 0 °C for 5 h and at room temperature overnight. DCU was filtrated, and the filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate, washed successively with cold 1 mol L<sup>-1</sup> HCl, saturated NaHCO<sub>3</sub>, and saturated NaCl, and dried (MgSO<sub>4</sub>). The peptide was purified by silica gel chromatography (3% methanol in chloroform (v/v)).

### General procedure B: Removal of the tert-butoxycarbonyl group

The Boc-peptide (1 equiv.) in a solution of 4 mol L<sup>-1</sup> HCl in dioxane (10 equiv. of HCl) was stirred at room temperature and the reaction monitored by TLC. Complete reaction was generally achieved in about 30 min. Excess reagent was removed under reduced pressure to give a solid residue. The residue was washed three times with ethyl ether and dried in vacuum over KOH and P<sub>2</sub>O<sub>5</sub> for several hours. The resulting hydrochloride was used without further purification. The hydrochloride was neutralized with NMM in DCM to give free amine.

### Bioassay method

The inhibition of the tested bacteria (*Aspergillus oryzae*) was determined with the method of dull colony notation.<sup>16</sup> All

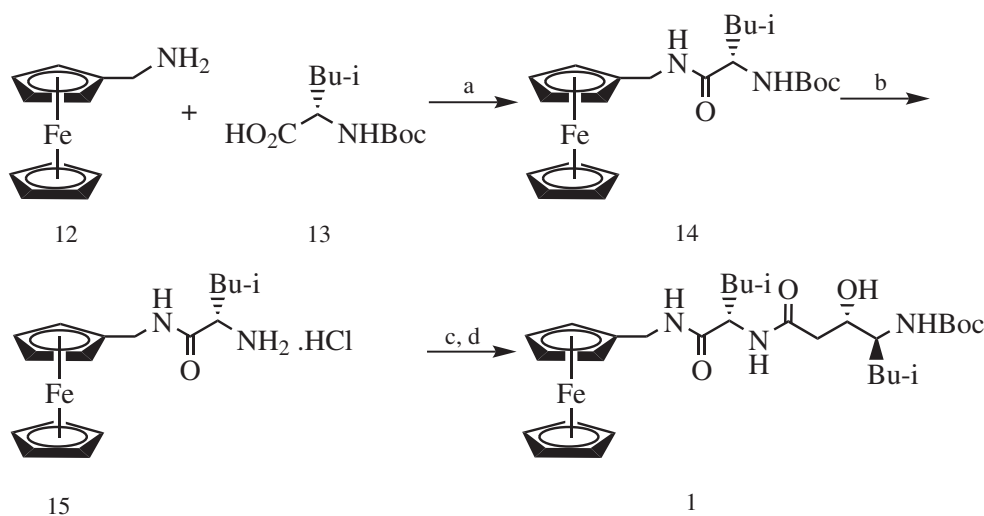
compounds tested were dissolved in DMSO (500 μmol) and subsequently diluted in the culture medium before treatment of the suspension of the tested bacteria. The suspension at three concentrations was inoculated on the culture medium treated with compound and incubated at room temperature for 60 h. In parallel, the tested bacterial was treated with DMSO as control. For each compound, three repetitions were conducted to ensure the reliability of the results. The percent inhibition was determined using the following relationship: inhibition rate (%) = [av colony numbers of control (not treated with compound) - av colony numbers smeared with drugs] / av colony numbers without drugs.

## Results and Discussion

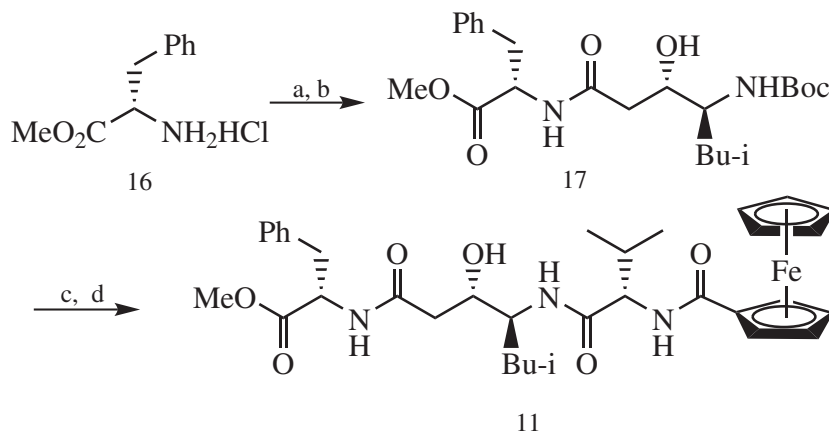
The representative synthesis of various inhibitors containing ferrocenyl moiety is outlined in Scheme 1 and 2. Boc-protected leucine **13** was coupled to aminomethylferrocene **12** using DCC and HOBt as the coupling agent. The Boc-group was removed by treatment with 4 mol L<sup>-1</sup> HCl in dioxane, neutralized with *N*-methylmorpholine (NMM), and the resulting free amine was allowed to couple subsequently with *N*-Boc-statine using the same coupling sequence to give the compound **1**. Other compounds (such as **5**, **7**, etc.) in Table 1 were prepared by analogous sequence as described above.

Phenylalanine methyl ester hydrochloride **16** was neutralized with NMM. Using the method described above, compound **11** was obtained in 27.6% yield for the coupling/deprotection steps.

The target compounds are characterized by IR and <sup>1</sup>H NMR spectroscopy, MALDI-TOF MS and elemental analyses. The results are in accordance with the expected



**Scheme 1.** Synthesis of compound **1**. Reagents and conditions: (a) DCC/HOBt; (b) 4 mol L<sup>-1</sup> HCl/dioxane; (c) NMM/DCM, (d) *N*-Boc-statine, DCC/HOBt, 50.7% for steps (a)-(c).



**Scheme 2.** Synthesis of compound **11**. Reagents and conditions: (a) NMM/DCM, (b) N-Boc-statine, DCC/HOBt; (b) 4 mol L<sup>-1</sup> HCl/dioxane; (c) NMM/DCM; (d) FcCO-Val-OH, DCC/HOBt, 27.6% for steps (a)-(d).

structures. In the mass spectra they give peaks corresponding to the molecular ion. In the case of compounds **1-10**, an intense fragment ion was observed at  $m/z$  199 and is due the  $[\text{FcCH}_2]^+$  cation. Fragment ions corresponding to  $[\text{M}-65]^+$  were also noted. This corresponds to loss of the unsubstituted ( $\eta^5\text{-C}_5\text{H}_5$ ) ring.

For compounds **1-10**, their <sup>1</sup>H NMR spectra are characteristic: the ferrocenyl substituent gives rise to a five-proton singlet for the unsubstituted cyclopentadienyl ring and a multiplet for the monosubstituted ring. The protons of the methylene unit adjacent to the ferrocene moiety appear as a doublet often overlapping with the singlet of the ( $\eta^5\text{-C}_5\text{H}_5$ ) ring.

In compound **11**, the ( $\eta^5\text{-C}_5\text{H}_5$ ) ring appears as a singlet in <sup>1</sup>H NMR spectrum at  $\delta$  4.21 whereas the *meta* and *ortho* protons on the ( $\eta^5\text{-C}_5\text{H}_4$ ) ring are present at  $\delta$  4.27 and 4.71, respectively. The characteristic bands of the ferrocenyl group in the IR spectra of the compound **2** appear at 3091, 1440, 1277, 1175, 1041 and 744 cm<sup>-1</sup>.

All the target compounds were screened against the tested bacteria (*Aspergillus oryzae*) and the results of their biological activity are shown in Table 1. Replacing valine with leucine, phenylalanine or 4-nitro-phenylalanine residue renders lower activity (**4** vs **1**, **2**, **6**, Table 1). This might suggest that the valine residue is necessary. Truncation of the C-terminus by removal of the C-terminal amino acid residue resulted in some loss of potency (**1**, **2**, **4**, **7** vs **9**, Table 1). Compound **8** was found to have an activity of 61.2% inhibition and higher than compound **4**. This might indicate that amino acid residue of N-terminus is very important. Compound **3** and **5** having the *R*-configuration, and as expected they have no activity. Replacing Boc group with FcCO-Val residue leads to inhibitor **11** with 37.4% inhibition. Interestingly, *bis*-statine based compound **10** has an activity of 68.3% inhibition and higher than others.

## Conclusions

In conclusion, a novel series of statine derivatives with short peptides containing ferrocenyl moiety have been prepared. All the compounds were characterized by elemental analyses, MS, <sup>1</sup>H NMR and IR spectra. The results of bioassay showed that compounds **4**, **8** and **11** might serve as a starting point.

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## Supplementary Information

Supplementary data are available free of charge at <http://jbsc.sbj.org.br>, as PDF file.

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17. Abbreviations: N-Boc-AHPPA, (3S, 4S)- (tert-Butoxycarbonylamino)-3-hydroxy-5-phenyl-pentanoic acid; AHPPA, (3S, 4S)-4-amino-3-hydroxy-5-phenyl-pentanoic acid; Nph, 4-nitro-phenylalanine.

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### Boc-Leu-NH-CH<sub>2</sub>Fc **14**

Yellow sticky oil, yield 73.5%,  $[\alpha]_D^{20} = -1.8^\circ$  (c 0.11, MeOH); IR (KBr): 3313, 3093, 2967, 2872, 1655, 1520, 1293 and 1168 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  0.93-0.98 {m, 6H, (CH<sub>3</sub>)<sub>2</sub>CH}, 1.05-1.09 (m, 1H, CHMe<sub>2</sub>), 1.44 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 1.65-1.76 (m, 2H, CH<sub>2</sub>CHMe<sub>2</sub>), 3.72 (d,  $J=0.8$  Hz, 1H, CHCO), 4.11-4.16 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 4.87 (br, 1H, NH), 6.38 (br, 1H, NH). Anal. Calcd for C<sub>25</sub>H<sub>29</sub>FeN<sub>3</sub>O<sub>3</sub>: C 59.18, H 5.76, N 8.28; found C 59.20, H 5.83, N 8.24.

### Boc-Phe-NH-CH<sub>2</sub>Fc **17**

Yellow sticky oil, yield 71.4%,  $[\alpha]_D^{20} = +3.1^\circ$  (c 0.16, MeOH); IR (KBr): 3415, 3004, 2926, 1711, 1516, 1419, 1362, 1222, 1031, 903 and 531 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.39 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 3.05 (d,  $J=8$  Hz, 2H, CH<sub>2</sub>Ph), 4.03-4.19 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 4.32 (br, 1H, CHCO), 5.10 (s, 1H, NH), 6.10 (s, 1H, NH), 7.18-7.30 (m, 5H, ArH). Anal. Calcd for C<sub>25</sub>H<sub>30</sub>FeN<sub>3</sub>O<sub>3</sub>: C 64.94, H 6.54, N 6.06; found C 65.17, H 6.83, N 6.21.

### Boc-Val-NH-CH<sub>2</sub>Fc **18**

Yellow sticky oil, yield 77.2%,  $[\alpha]_D^{20} = -0.6^\circ$  (c 0.51, MeOH); IR (KBr): 3311, 3092, 2967, 2874, 1656, 1520, 1244 and 1169 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  0.96 (q,  $J=0.8$  Hz, 6H, (CH<sub>3</sub>)<sub>2</sub>CH), 1.17-1.25 (m, 1H, CHMe<sub>2</sub>), 1.44 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 3.87 (t,  $J=0.4$  Hz, 1H, CHCO), 4.13-4.17 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 5.00 (br, 1H, NH), 6.15 (br, 1H, NH). Anal. Calcd for C<sub>21</sub>H<sub>30</sub>FeN<sub>3</sub>O<sub>3</sub>: C 60.88, H 7.30, N 6.76; found C 60.75, H 7.18, N 6.55.

### Boc-Nph-NH-CH<sub>2</sub>Fc **19**

Yellow sticky oil, yield 65.5%,  $[\alpha]_D^{20} = +0.97^\circ$  (c 0.23, MeOH); IR (KBr): 3305, 3086, 2931, 2868, 1656, 1602, 1521, 1452, 1345, 1248, 1165, 1021, 854 and 486 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.39 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 3.11

(q,  $J=0.8$  Hz, 2H, CH<sub>2</sub>Ph), 3.21-3.26 (m, 1H, CHCO), 4.05-4.19 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 5.08 (d,  $J=5.6$  Hz, 1H, NH), 6.07 (d,  $J=6.6$  Hz, 1H, NH), 7.33 (q,  $J=8.8$  Hz, 2H, ArH), 8.15 (q,  $J=6.4$  Hz, 2H, ArH). Anal. Calcd for C<sub>25</sub>H<sub>29</sub>FeN<sub>3</sub>O<sub>3</sub>: C 59.18, H 5.76, N 8.28; found C 59.20, H 5.83, N 8.24.

### Boc-Val-Phe-NH-CH<sub>2</sub>Fc **20**

Yellow sticky oil, yield 71.4%,  $[\alpha]_D^{20} = -9.4^\circ$  (c 0.16, MeOH); IR (KBr): 3422, 2932, 1663, 1497, 1439, 1390, 1253, 1101 and 700 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  0.79-0.91 {m, 6H, (CH<sub>3</sub>)<sub>2</sub>CH}, 1.38 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 1.43-1.45 (m, 1H, CHMe<sub>2</sub>), 2.99 (m, 2H, CH<sub>2</sub>Ph), 3.04 (t,  $J=1.6$  Hz, 1H, CHCO), 3.87 (t,  $J=6$  Hz, 1H, CHCO), 4.04-4.17 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 4.62 (d,  $J=6.4$  Hz, 1H, NH), 4.81 (br, 1H, NH); 6.17 (br, 1H, NH); 7.28-7.16 (m, 5H, ArH). Anal. Calcd for C<sub>30</sub>H<sub>39</sub>FeN<sub>3</sub>O<sub>4</sub>: C 64.17, H 7.00, N 7.48; found C 64.31, H 7.14, N 7.36.

### Boc-Statine-Leu-NH-CH<sub>2</sub>Fc **1**

Yellow solid, yield 49.2%, m.p. 71-72 °C,  $[\alpha]_D^{20} = -41^\circ$  (c 0.10, MeOH); IR (KBr): 3325, 3092, 2957, 2930, 2870, 1689, 1645, 1535, 1465, 1366, 1248, 1169, 1045, 881 and 644 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  0.75-0.98 {m, 12H, (CH<sub>3</sub>)<sub>2</sub>CH, (CH<sub>3</sub>)<sub>2</sub>CHCH<sub>2</sub>}, 1.23-1.49 (m, 6H, CH<sub>2</sub>CHMe<sub>2</sub>, CH<sub>2</sub>CHMe<sub>2</sub>), 1.53 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 2.23-2.40 (m, 2H, CH<sub>2</sub>CO), 3.64-3.74 (m, 1H, NCH-Bu-i), 3.87-3.95 (m, 2H, NCHCO, CH-O), 3.97-4.09 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 4.30 (q, 1H,  $J=8$  Hz, NH), 4.65 (d,  $J=8$  Hz, 1H, NH), 6.32 (d,  $J=8$  Hz, 1H, NH). MALDI-TOF MS:  $m/z = 586$  [M+1]<sup>+</sup>. Anal. Calcd for C<sub>30</sub>H<sub>47</sub>FeN<sub>3</sub>O<sub>5</sub>: C 61.53, H 8.09, N 7.18; found C 61.64, H

### Boc-Statine-Phe-NH-CH<sub>2</sub>Fc **2**

Yellow solid, yield 53.6%, m.p. 79-81 °C,  $[\alpha]_D^{20} = -44^\circ$  (c 0.21, MeOH); IR (KBr): 3310, 3088, 3031, 2956, 2929, 2869, 1688, 1643, 1532, 1452, 1365, 1248, 1168, 1044, 817 and 744 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  0.75-0.86

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{m, 6H, (CH<sub>3</sub>)<sub>2</sub>CHCH<sub>2</sub>}, 1.33-1.43 (m, 3H, CH<sub>2</sub>CHMe<sub>2</sub>), 1.56 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 2.17-2.36 (m, 2H, CH<sub>2</sub>CO), 2.90-3.07 (m, 2H, CH<sub>2</sub>Ph), 3.45-3.57 (m, 1H, NCH-Bu-i), 3.84-3.93 (m, 2H, NCHCO, CH-O), 3.95-4.04 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 4.62 (d, *J*=9.6 Hz, 1H, NH), 5.98 (br, 1H, NH), 6.46 (d, *J*=8 Hz, 1H, NH), 7.10-7.20 (m, 5H, ArH). MALDI-TOF MS: *m/z* =619 (M<sup>+</sup>). Anal. Calcd for C<sub>33</sub>H<sub>45</sub>FeN<sub>3</sub>O<sub>5</sub>: C 63.97, H 7.32, N 6.78; found C 63.74, H 7.45, N 6.62.

#### Boc-(3*R*, 4*S*)-Statine-Phe-NH-CH<sub>2</sub>Fc 3

Yellow solid, yield 53.6%, m.p. 85-87 °C, [α]<sub>D</sub><sup>20</sup> = -30.7° (*c* 0.17, MeOH); IR (KBr): 3333, 3088, 2953, 2930, 2853, 1681, 1633, 1532, 1449, 1368, 1248, 1169, 698 and 670 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.85-0.93 (m, 6H, (CH<sub>3</sub>)<sub>2</sub>CHCH<sub>2</sub>), 1.31-1.33 (m, 3H, CH<sub>2</sub>CHMe<sub>2</sub>), 1.57 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 2.25-2.43 (m, 2H, CH<sub>2</sub>CO), 3.00-3.14 (m, 2H, CH<sub>2</sub>Ph), 3.52-3.59 (m, 1H, NCH-Bu-i), 3.73-3.91 (m, 2H, NCHCO, CH-O), 4.03-4.10 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 5.99 (br, 1H, NH), 6.38 (d, *J*=7.6 Hz, 1H, NH), 6.47 (d, *J*=8.0 Hz, 1H, NH), 7.17-7.24 (m, 5H, ArH). MALDI-TOF MS: *m/z* =619 (M<sup>+</sup>). Anal. Calcd for C<sub>33</sub>H<sub>45</sub>FeN<sub>3</sub>O<sub>5</sub>: C 63.97, H 7.32, N 6.78; found C 63.81, H 7.19, N 6.90.

#### Boc-Statine-Val-NH-CH<sub>2</sub>Fc 4

Yellow solid, yield 50.7%, m.p. 86-88 °C [lit. <sup>[15]</sup> 86-88 °C], [α]<sub>D</sub><sup>20</sup> = -41.4° (*c* 0.23, MeOH); IR (KBr): 3326, 3095, 2929, 2851, 1688, 1631, 1533, 1444, 1367, 1246, 1167, 1046, 821, 641 and 482 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.82-0.95 {m, 12H, (CH<sub>3</sub>)<sub>2</sub>CH, (CH<sub>3</sub>)<sub>2</sub>CHCH<sub>2</sub>}, 1.20-1.48 (m, 4H, CHMe<sub>2</sub>, CH<sub>2</sub>CHMe<sub>2</sub>), 1.59 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 2.32-2.43 (m, 2H, CH<sub>2</sub>CO), 3.48 (q, 1H, *J*=0.4 Hz, NCH-Bu-i), 3.83-3.95 (m, 2H, NCHCO, CH-O), 4.12-4.21 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 4.73 (d, *J*=12 Hz, 1H, NH), 6.24 (br, 1H, NH), 6.51 (d, *J*=8 Hz, 1H, NH). MALDI-TOF MS: *m/z* =571 (M<sup>+</sup>). Anal. Calcd for C<sub>29</sub>H<sub>45</sub>FeN<sub>3</sub>O<sub>5</sub>: C 60.94, H 7.94, N 7.35; found C 60.85, H 7.88, N 7.29.

#### Boc-(3*R*, 4*S*)-Statine-Val-NH-CH<sub>2</sub>Fc 5

Yellow solid, yield 54.3%, m.p. 89-91 °C, [α]<sub>D</sub><sup>20</sup> = -27° (*c* 0.21, MeOH); IR (KBr): 3326, 3091, 2954, 2932, 2871, 1685, 1626, 1538, 1440, 1367, 1277, 1175, 1041, 744 and 483 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.80-0.95 {m, 12H, (CH<sub>3</sub>)<sub>2</sub>CH, (CH<sub>3</sub>)<sub>2</sub>CHCH<sub>2</sub>}, 1.44 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 1.50-1.68 {m, 4H, CHMe<sub>2</sub>, CH<sub>2</sub>CHMe<sub>2</sub>}, 2.33-2.40 (m, 2H, CH<sub>2</sub>CO), 3.46-3.56 (m, 1H, NCH-Bu-i), 3.71-3.95 (m, 2H, NCHCO, CH-O), 4.02-4.18 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 4.73 (d, *J*=9.6 Hz, 1H, NH), 6.22 (br, 1H, NH), 6.51 (d, *J*=8.4 Hz, 1H, NH). MALDI-TOF MS: *m/z* =572 [M+H]<sup>+</sup>. Anal. Calcd for C<sub>29</sub>H<sub>45</sub>FeN<sub>3</sub>O<sub>5</sub>: C 60.94, H 7.94, N 7.35; found C 60.73, H 8.01, N 7.17.

#### Boc-Statine-Nph-NH-CH<sub>2</sub>Fc 6

Yellow solid, yield 40.5%, m.p. 96-98 °C, [α]<sub>D</sub><sup>20</sup> = -39° (*c* 0.15, MeOH); IR (KBr): 3319, 3077, 2956, 1689, 1647, 1522, 1445, 1345, 1167, 1046, 845 and 486 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.92 {q, *J*=1.2 Hz, 6H, (CH<sub>3</sub>)<sub>2</sub>CH}, 1.07-1.11 (m, 1H, CHMe<sub>2</sub>), 1.44 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 1.56-1.71 (m, 2H, CH<sub>2</sub>CHMe<sub>2</sub>), 2.31-2.40 (m, 2H, CH<sub>2</sub>CO), 3.14-3.22 (m, 2H, CH<sub>2</sub>-Ar), 3.43-3.51 (m, 1H, NCH-Bu-i), 3.71-3.95 (m, 2H, NCHCO, CH-O), 4.07-4.16 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 4.71 (d, *J*=4.4 Hz, 1H, NH), 6.42 (d, *J*=4.4 Hz, 1H, NH), 6.69 (d, *J*=8.0 Hz, 1H, NH), 7.33 (q, *J*=5.2 Hz, 2H, ArH), 8.08 (q, *J*=8.4 Hz, 2H, ArH). MALDI-TOF MS: *m/z* =664 (M<sup>+</sup>). Anal. Calcd for C<sub>33</sub>H<sub>44</sub>FeN<sub>4</sub>O<sub>7</sub>: C 59.64, H 6.67, N 8.43; found C 59.67, H 6.71, N 8.53.

#### Boc-Statine-Val-Phe-NH-CH<sub>2</sub>Fc 7

Yellow solid, yield 30.2%, m.p. 166-168 °C, [α]<sub>D</sub><sup>20</sup> = -53° (*c* 0.10, MeOH); IR (KBr): 3422, 2932, 1663, 1497, 1439, 1390, 1253, 1101 and 700 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.86-0.99 {m, 12H, (CH<sub>3</sub>)<sub>2</sub>CH, (CH<sub>3</sub>)<sub>2</sub>CHCH<sub>2</sub>}, 1.28-1.38 (m, 2H, CHMe<sub>2</sub>, CHMe<sub>2</sub>), 1.44 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 1.50-1.69 {m, 2H, CH<sub>2</sub>-Pr-i}, 2.49-2.62 (m, 2H, CH<sub>2</sub>CO), 3.05-3.17 (m, 2H, CH<sub>2</sub>Ph, NCH-Bu-i), 3.50 (t, *J*=3.6 Hz, 1H, NCHCO), 3.89 (br, 1H, CH-O), 4.00-4.17 (m, 12H, NCHCO, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 4.86 (d, *J*=7.6 Hz, 1H, NH), 5.04 (d, *J*=8.4 Hz, 1H, NH), 5.51 (br, 1H, NH), 6.38 (br, 1H, NH), 7.30-7.10 (m, 5H, ArH). MALDI-TOF MS: *m/z* =718 (M<sup>+</sup>). Anal. Calcd for C<sub>38</sub>H<sub>54</sub>FeN<sub>4</sub>O<sub>6</sub>: C 63.50, H 7.57, N 7.80; found C 63.62, H 7.66, N 7.65.

#### Boc-Val-Statine-Val-NH-CH<sub>2</sub>Fc 8

Yellow solid, yield 32.4%, m.p. 142-144 °C, [α]<sub>D</sub><sup>20</sup> = -49° (*c* 0.10, MeOH); IR (KBr): 3326, 3086, 2929, 2850, 1687, 1627, 1573, 1534, 1367, 1245, 1167, 1087 and 642 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.81-0.96 {m, 18H, (CH<sub>3</sub>)<sub>2</sub>CH, (CH<sub>3</sub>)<sub>2</sub>CH, (CH<sub>3</sub>)<sub>2</sub>CHCH<sub>2</sub>}, 1.04-1.43 (m, 3H, CHMe<sub>2</sub>, CHMe<sub>2</sub>, CHMe<sub>2</sub>), 1.57 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 1.61-1.70 (CH<sub>2</sub>-Pr-i), 2.33-2.38 (2H, CH<sub>2</sub>CO), 3.45-3.56 (m, 2H, NCH-Bu-i, NCHCO), 3.71-3.99 (m, 2H, NCHCO, CH-O), 4.02-4.17 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 4.76 (d, *J*=9.2 Hz, 1H, NH), 6.21 (br, 1H, NH), 6.51 (d, *J*=8.8 Hz, 1H, NH), 6.65 (br, 1H, NH). MALDI-TOF MS: *m/z* =670 (M<sup>+</sup>). Anal. Calcd for C<sub>34</sub>H<sub>54</sub>FeN<sub>4</sub>O<sub>6</sub>: C 60.89, H 8.12, N 8.35; found C 61.01, H 8.20, N 8.29.

#### Boc-Statine-NH-CH<sub>2</sub>Fc 9

Yellow solid, yield 57.7%, m.p. 109-111 °C, [α]<sub>D</sub><sup>20</sup> = -21° (*c* 0.17, MeOH); IR (KBr): 3327, 3095, 2929, 2850, 1688, 1627, 1534, 1366, 1246, 1168, 816, 643 and 482 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.88 {q, *J*=0.4 Hz, 6H, (CH<sub>3</sub>)<sub>2</sub>CH}, 1.26-1.39 (m, 3H, CH<sub>2</sub>CHMe<sub>2</sub>), 1.58 {s,

9H, (CH<sub>3</sub>)<sub>3</sub>}, 2.28-2.39 (m, 2H, CH<sub>2</sub>CO), 3.39-3.56 (m, 1H, NCH-Bu-i), 3.95-3.98 (m, 1H, CH-O), 4.01-4.20 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 4.72 (d, *J*=9.6 Hz, 1H, NH), 6.09 (br, 1H). MALDI-TOF MS: *m/z* =472 (M<sup>+</sup>). Anal. Calcd for C<sub>24</sub>H<sub>36</sub>FeN<sub>2</sub>O<sub>4</sub>: C 61.02, H 7.68, N 5.93; found C 61.44, H 7.65, N 6.01.

**Boc-AHPPA-Statine-Val-NH-CH<sub>2</sub>Fc 10**

Yellow solid, yield 20.3%, m.p. 125-127 °C [lit. <sup>[15]</sup> 125-127 °C], [α]<sub>D</sub><sup>20</sup> = -62.6° (*c* 0.10, MeOH); IR (KBr): 3323, 3088, 2960, 2928, 1648, 1534, 1451, 1366, 1247, 1167, 1047, 817, 699 and 483 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.82-0.98 {m, 12H, (CH<sub>3</sub>)<sub>2</sub>CH, (CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>CH}, 1.33-1.38 (m, 2H, CHMe<sub>2</sub>, CHCH<sub>2</sub>Me<sub>2</sub>), 1.42 {s, 9H, (CH<sub>3</sub>)<sub>3</sub>}, 1.54-1.69 (m, 2H, CHCH<sub>2</sub>Me<sub>2</sub>), 2.24-2.53 (m, 4H, CH<sub>2</sub>CO, CH<sub>2</sub>CO), 2.91 (t, 2H, *J*=2.8 Hz, CH<sub>2</sub>Ph), 3.49-3.52 (m, 1H, NCHCO), 3.74-3.78 (m, 2H, CH-O, CH-O), 4.01-4.09 (m, 2H, NCHBn, NCHBu-i), 4.12-4.26 (m, 11H, C<sub>5</sub>H<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>, CH<sub>2</sub>N), 4.97 (d, 1H, *J*=8.8 Hz, NH), 6.24 (br, 1H, NH), 6.38 (br, 1H, NH), 6.76 (d, 1H, *J*=8.8 Hz, NH), 7.18-7.27

(m, 5H, ArH). MALDI-TOF MS: *m/z* =762 (M<sup>+</sup>). Anal. Calcd for C<sub>40</sub>H<sub>58</sub>FeN<sub>4</sub>O<sub>7</sub> · H<sub>2</sub>O: C 61.53, H 7.75, N 7.18; found C 61.55, H 7.78, N 7.21.

**FcCO-Val-Statine-Phe-OMe 11**

Yellow solid, yield 27.6%, m.p. 105-107 °C, [α]<sub>D</sub><sup>20</sup> = -50° (*c* 0.13, MeOH); IR (KBr): 3447, 3097, 2925, 1774, 1678, 1488, 1367, 1260, 1055, 881, 746, 523 and 493 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.91-0.99 {m, 12H, (CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>, (CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>}, 1.39-1.42 (m, 2H, CHMe<sub>2</sub>, CHMe<sub>2</sub>), 1.59-1.75 (m, 2H, CH<sub>2</sub>-Pr-i), 2.37-2.38 (d, *J*=6.4 Hz, 2H, CH<sub>2</sub>CO), 3.01 (q, *J*=6.4 Hz, 2H, CH<sub>2</sub>Ph), 3.10 (q, *J*=5.6 Hz, 2H, NCH-Bu-i), 3.73 (s, 3H, CH<sub>3</sub>O), 4.02-4.12 (br, 1H, NCHCO), 4.21 (s, 5H, Fc), 4.27 (d, *J*=2 Hz, 2H, FcCO), 4.71 (d, *J*=1.2 Hz, 2H, FcCO), 4.79 (q, *J*=6.4 Hz, 1H, NH), 5.98 (d, *J*=7.6 Hz, 1H, NH), 6.41 (d, *J*=9.2 Hz, 1H), 7.07-7.24 (m, 5H, ArH). MALDI-TOF MS: *m/z* =647 (M<sup>+</sup>). Anal. Calcd for C<sub>34</sub>H<sub>45</sub>FeN<sub>3</sub>O<sub>6</sub>: C 63.06, H 7.00, N 6.49; found C 63.23, H 7.11, N 6.38.