Coumarin Derivatives as Protease-Sensitive Prodrugs☆

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Summary

To overcome the lack of selectivity of anticancer drugs toward malignant cells, the development of prodrugs, which could be activated selectively by tumour-specific proteases is the goal of these studies. In this work tripartate prodrugs have been evaluated consisting of a carrier unit and a spacer group, which allows for intramolecular cyclisation while releasing the third component, the compound attached to the carboxylic acid moiety of the spacer group. As carrier units amino acids or peptides have been used, which are required for recognition by the protease. As the spacer unit the "trimethyl-lock"-spacer has been applied; as a model leaving group *p*-anisidine was attached to the carboxylic acid moiety. It was intended to test the compounds for their releasing rate of *p*-anisidine. Two of the evaluated compounds, **9b** and **9h**, were degraded with half-lives of 46 min at room temperature. However, the poor solubility in aqueous solutions proved the major disadvantage of the TML-based prodrugs.

Introduction

One of the current problems in cancer therapy is the poor selectivity of the present anticancer drugs toward cancer cells. A possible approach to overcoming this lack of selectivity is the design of anticancer prodrugs. Recently tumour-selective properties have been identified that make it possible to evaluate prodrugs that can be activated in the vicinity of the tumour due to tumour-selective activation mechanisms[1]. For example, elevated levels of proteases are described for malignant tumours. Increased levels of proteases can be caused either by the tendency of the tumour to spread through the extracellular matrix (tumour invasion and metastasis) or by liberation from infiltrating cells as lytic enzymes against the tumour. The activation of prodrugs by the serine proteases plasmin[4] and chymotrypsin[5] and the cysteine protease cathepsin B has been described[6]. It has been well established that several proteases, especially cathepsin B are expressed tumour-specifically, which would predestine them for the tumour-selective activation of prodrugs by proteases.

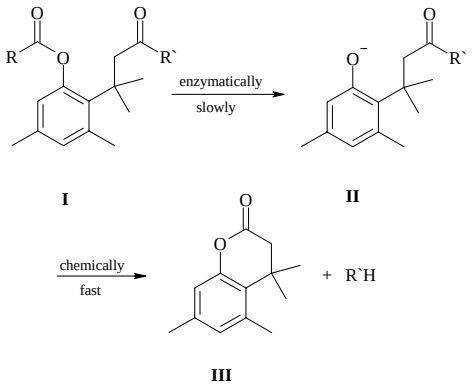
Polymorphonuclear elastase (PMN-E) is a serine protease, which has been found to occur in a number of malignant diseases in significantly increased levels. Examples are lung[7], breast[8], and colon cancer[9], but also inflammatory diseases like rheumatoid arthritis[10]. In case of breast cancer, PMN-E has even been used as a marker for the recurrence or the progress of the disease. Although this increased enzyme occurrence is well known, no PMN-E-sensitive prodrugs have been described until now.

Protease-cleavable prodrugs need to fulfil two main criteria: On the one hand they have to contain the peptide sequence required for the recognition by the protease (carrier unit). On the other hand the release of the attached drug has to be ensured. Sterical hindrance during the release of the attached drug could be prevented by introducing a spacer unit between carrier unit and drug (tripartate prodrug, Figure 1).

Figure 1. Tripartate prodrugs.

The suitability of coumarin acids and its derivatives for the design of esterase-cleavable prodrugs has been shown in several studies[11]. Upon unmasking the hydroxyl group the ring-opened coumarin derivatives usually undergo spontaneous lactonisation, thereby releasing the compounds attached to the carboxylic acid moiety (Scheme 1). In case of **I** the lactonisation is the result of the "trimethyl lock", which was determined to strongly increase the rate of the lactonisation reaction due to a restriction of rotational freedom, termed stereopopulation control[12] . As a result of this intramolecular strain, intermediate **II** shows a half-life of approximately 100 s in aqueous solutions.

In this paper the synthesis of prodrugs based on the "trimethyl lock" system as the spacer unit is described. Since PMN-E has a substrate specificity for amino acids with short aliphatic side chains in P_1, different peptide sequences containing alanine and valine in P_1 will be introduced. For our investigations we chose *p*-anisidine as a model leaving group.



Scheme 1

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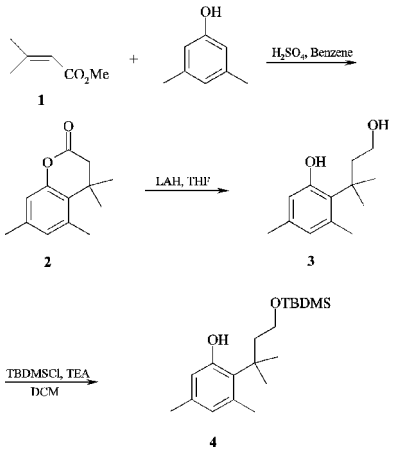
The evaluated compounds were tested for their cleavage by porcine pancreatic elastase, which was used as a model enzyme.

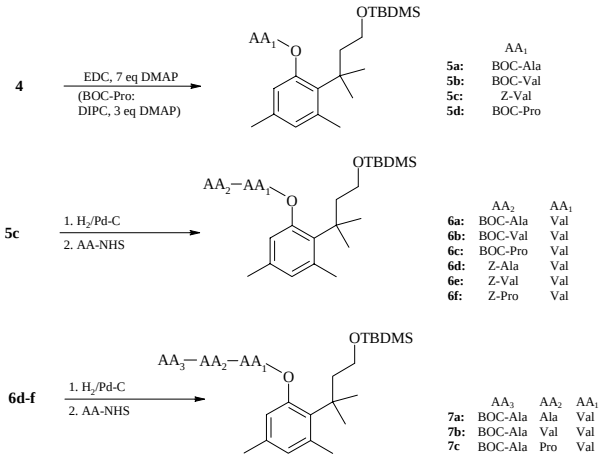
Results and Discussion

To prevent the facile lactonisation of the "trimethyl lock" system, a multistep synthesis is required: the first step is the reduction of the carboxylic acid to the alcohol, followed by the introduction of an O-protective group to selectively acylate the phenolic hydroxyl group. The attached peptide now functions as a "protective group" for the phenolic hydroxyl group and thus allows the re-oxidation of the alcoholic function to the acid.

3-(2-Hydroxy-4,6-dimethylphenyl)-3,3-dimethylpropanol (**3**) was synthesised by reacting commercially available 3,5-dimethylphenol and methyl 3,3-dimethylacrylate (**1**) in refluxing benzene under catalysis of conc. sulfuric acid and subsequent reduction of the coumarin derivative **2** with LAH in THF[11a]. The TBDMS-protective group was introduced following the procedure described by Wang et al. (Scheme 2) (**4**)[11b].



Scheme 2



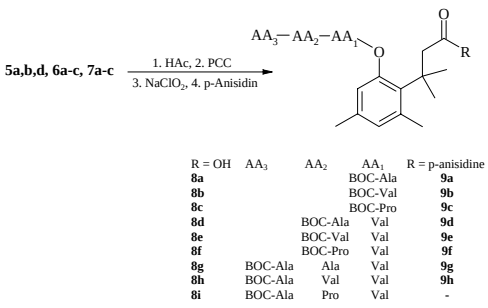
Scheme 3

O-Acylation of **4** with amino acids activated as *p*-nitrophenol esters, as described by Wang et al., did not work in our hands, even under varied conditions (reaction time, temperature, solvent, equivalents of base, etc.). However, the conditions described by Greenwald and co-workers, who used EDC or DIPC as coupling reagents and up to 7 eq DMAP as base yielded the desired compounds. *O*-Acylated compounds **5a**, **b**, and **d** were obtained in acceptable yields (Scheme 3)[11c].

In order to synthesise the oligopeptide derivatives, two strategies can be applied: **4** can be directly acylated with di- or tripeptides or the peptide chain can be build up in a stepwise fashion. The direct acylation of **4** with different tripeptides either activated as *p*-nitrophenyl esters or by using different coupling reagents was unsuccessful, however. For the stepwise synthesis strategy it is necessary to selectively deblock the amino terminus. Therefore, we tried to remove the N-terminal BOC-protective group of compound **5a**. Unfortunately the parallel cleavage of the TBDMS-protective group occurred instead, probably due to its lability under strong acidic and basic conditions. An alternative to generate the free amino terminus is the introduction of Z as N-protective group (**5c**), which can be cleaved during hydrogenolysis by using Pd-C as catalyst. Under these conditions, the N-protective group was cleaved selectively, so that the N-terminal unprotected compounds could be obtained and reacted with BOC- and Z-amino acid-*N*-hydroxysuccinimide esters without further purification. The dipeptide-spacer compounds **6a–f** were obtained (Scheme 3). *N*-Hydroxysuccinimide esters were prepared from the N-protected amino acid by reacting with 2.1 eq *N*-hydroxysuccinimide and 1.1 eq DCC following standard coupling procedures. Repeated hydrogenolysis of compounds **6d–f** and subsequent reaction with BOC-amino acid-NHS gave the tripeptide-spacer derivatives **7a–c** (Scheme 3).

Cleavage of the TBDMS-group in a mixture of glacial acetic acid, THF and water followed by stepwise oxidation of the alcohol to the appropriate aldehyde with pyridinium-chlorochromate and then to the carboxylic acid by using sodium chlorite as mild oxidising reagents led to the compounds **8a–i** (Scheme 4). Attachment of the *p*-anisidine residue was performed with DCC/HOBt as coupling reagents and led to compounds **9a–h** (Scheme 4). The coupling of compound **8i** was not successful.

To determine whether the synthesised prodrugs could be activated by elastase, the respective compounds were dissolved in acetonitrile and then diluted with tris-buffer (0.1 M,



Scheme 4

pH 8.0). All compounds were only poorly soluble in aqueous solvents. Up to 15% of acetonitrile had to be used to prepare solutions with concentrations between 0.05 and 0.1 mM. It has been described that many enzymes are still active in organic solvents, but the enzyme activity decreases with increasing amounts of organic solvent [13]. Our investigations show that PPE losses two third of its activity after an incubation of 2 h in buffered solution. Additionally, the enzyme activity decreases by 50% with every 10% increase in acetonitrile concentration. The use of surfactants (Pluronic F 127, Tween 80, Chremophor EL, Triton X 100; 1% or 10%, respectively) failed to increase the solubility. Despite these solubility problems, the stability of compounds **9a–h** was investigated by incubating them with purified PPE (Tris, 0.1 M, pH 8.0). At various times 20 µl aliquots were withdrawn and immediately analysed by RP-HPLC (detection wavelength: 250 nm, solvent: KH_2PO_4 (20 mM, pH 3.3)/acetonitrile = 30/70). In case of compounds **9a** and **9c–g**, no hydrolysis was observed during the course of the experiment. On the other hand, compounds **9b** and **9h** were degraded with half-lives of 46 min each, as judged by HPLC-peak area (Figure 1). Running a linear gradient (30 min 95/5 to 20/80 KH_2PO_4 (20 mM, pH 3.3)/acetonitrile) BOC-Val(OH) and BOC-Ala-Val-Val(OH) could be detected as the primary cleavage products. Cleavage occurred only in presence of the active enzyme, without enzyme and with enzyme denaturised by heating no hydrolysis was observed. Nevertheless, it was not possible to quantify these compounds and their cleavage products within one HPLC-experiment because all compounds show immense differences in polarity and solubility in aqueous solution. Therefore, further investigations in order to determine the rate of *p*-anisidine release were not performed.

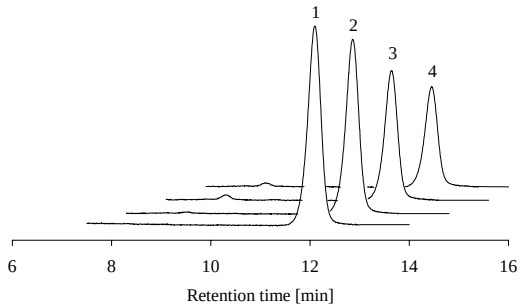


Figure 2. HPLC chromatogram showing the degradation of **9b** catalysed by PPE, at time 0, PPE was added. At various times (1: 0 min; 2: 20 min; 3: 40 min; 4: 60 min) aliquots were withdrawn and immediately loaded onto the HPLC column.

The experiments described above demonstrate that the poor solubility is a major drawback of the TML-based prodrugs. At the time we planned the syntheses we were aware, that hydrophobicity might lead to a poor solubility of these compounds in aqueous solvents. However, synthesis and enzymatic activation of coumarin-based prodrugs have been described in several publications – in no cases were solubility problems reported. As a measure for hydrophobicity of organic compounds the octanol-water partition coefficient *p* is commonly used. In order to obtain comparable values, the log p-values were estimated by computational methods:[14] most of the synthesised compounds gave log *p* values between 4 and 6, which is in good accordance with the poor solubility in aqueous solutions observed before. If in these calculations the N-terminal BOC-group is replaced by a MeOSuc- or an Ac-group, the water solubility increases about a factor of 50. Replacement of the *p*-anisidine residue by the more polar sulfanilic acid did not further increase the water solubility. The *O*-acylation of compound **4** with MeOSuc- or Ac-amino acids, however, did not proceed in acceptable yields, so that the theoretical results could not be confirmed.

The results demonstrate the general feasibility of the concept to activate TML-prodrugs by elastase. Further experiments are planned in order to increase the water solubility of these compounds by a suitable derivatisation strategy.

Acknowledgement

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Experimental

General: Mp: not corrected, Linström apparatus.– IR spectra (cm^{-1}): Perkin-Elmer 1600 series FTIR; in KBr, if not noted otherwise.– NMR spectra: Bruker DPX 200 (200 MHz) and Bruker DPX 200 (300 MHz); δ in ppm; ^1H values from spectra in $CDCl_3$, if not noted otherwise; standard reference for all spectra in $CDCl_3$ and d_4-MeOH was TMS (δ = 0 ppm) or (in cases of silyl groups containing compounds) the signal of the undeuterated solvent in D_2O.– Elemental analysis: Perkin-Elmer elemental analyser 2400 CHN.– Mass spectra: Intectra AMD 402/3.– Chromatography: cc: Merck silica gel 60 (7734); tlc: Merck Alufolien, silica gel 60 F_{254}.– HPLC: Merck Hitachi, LaChrom; Pump: L-7100; Detector: DAD L-7450.

Solvents were dried/purified following standard laboratory procedures (e.g. DCM by refluxing over P_4O_{10} for 2 h and storing the distilled solvent over 4Å molecular sieve) or were purchased commercially. Unless noted otherwise, moisture and air sensitive reactions were conducted under a dry nitrogen atmosphere. All amino acids used in this work are L-amino acids. PPE was purchased from Serva (20929).

Abbreviations: Ac: Acetyl; BOC: *tert*-butyloxycarbonyl; DCC: dicyclohexyl carbodiimide; DCM: dichloromethane; DIPC: diisopropyl carbodiimide; DMAP: dimethylaminopyridine; EDC: *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; LAH: lithiumaluminium hydride; MeOSuc: Methoxysuccinyl; NHS: *N*-hydroxysuccinimide ester; HOBt: 1-hydroxybenzotriazol hydrate; PCC: pyridinium chlorochromate; PE: petrol ether; PPE: porcine pancreatic elastase; TEA: triethyl amine; TBDMSCl: *tert*-butyldimethylsilyl chloride, THF: tetrahydrofuran; TML: trimethyllock; TMSCl: trimethyl chlorosilane; Z: benzyloxycarbonyl.

Methyl-3,3-dimethylacrylate (**1**)[11a]

Yield: 14.4 g (0.13 mol, 63%) of a colourless liquid which was directly used for the next step without further purification.– $C_6H_{10}O_2$ (114.14).

4,4,5,7-Tetramethyl-3,4-dihydrocoumarin (**2**)[11a]

Yield: 14.9 g (0.073 mol, 87%) of a colourless liquid which was directly used for the next step without further purification.– ^1H NMR: δ = 1.44 (s, 6H, CH_3), 2.27 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.59 (s, 2H, CH_2), 6.74 (m, 2H, Ar-H).– $C_{13}H_{16}O_2$ (204.27).

3-(2-Hydroxy-4,6-dimethylphenyl)-3,3-dimethylpropan-1-ol (**3**)[11a]

Yield: 13.2 g (63 mmol, 92%) of a colourless solid, which was directly used for the next step without further purification.– ^1H NMR: δ = 1.52 (s, 6H, CH_3), 2.13 (s, 3H, CH_3), 2.23 (t, *J* = 7.2 Hz, 2H, CH_2), 2.45 (s, 3H, CH_3), 3.58 (t, *J* = 7.2 Hz, 2H, CH_2), 6.31, 6.65 (s, 1H, Ar-H).– $C_{13}H_{20}O_2$ (208.30).

*1-O-(tert-Butyldimethylsilyl)-3-(2-hydroxy-4,6-dimethylphenyl)-3,3-dimethylpropanol (**4**)*[11b]

Yield: 18.0 g (60.0 mmol, 86%) of a colourless solid.– ^{1}H NMR: δ = 0.07 (s, 6H, Si-CH_3), 0.87 (s, 9H, Si(CH_3)$_3$), 1.54 (s, 6H, CH_3), 2.11 (t, *J* = 7.0 Hz, 2H, CH_2), 2.18 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 3.59 (t, *J* = 7.0 Hz, 2H, CH_2), 5.66 (s, 1H, OH), 6.40, 6.48 (s, 1H, Ar-H).– $C_{13}H_{34}O_2Si$ (250.50).

*General Procedure for the O-Acylation of **4** with N-Protected Amino Acids*

N-Protected amino acid (2 eq), **4** (1 eq), and DMAP (7 eq) were dissolved in dry DCM and the solution cooled to 0 °C. To the solution was added EDC (2.9 eq) and the mixture was subsequently stirred at r.t. overnight. It was diluted with DCM, and washed three times with 1% $NaHCO_3$ and 1 N HCl. After drying with Na_2SO_4 the solvent was removed in vacuo. After cc (10% ethyl acetate in cyclohexane) **5** was obtained.

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(BOC-alanyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropanol (**5a**)*[11b]

Starting from BOC-alanine (500 mg, 2.64 mmol), **4** (425 mg, 1.32 mmol), DMAP (1130 mg, 9.24 mmol), and EDC (585 mg, 3.79 mmol), **5a** (470 mg, 0.96 mmol, 36%) was obtained as a colourless liquid.– ^{1}H NMR: δ = –0.03 (s, 6H, $SiMe_2$), 0.87 (s, 9H, t-Bu), 1.46 (s, 6H, CH_3; s, 9H, t-Bu), 1.59 [d, *J* = 7.3 Hz, 3H, CH_3(Ala)], 2.05 (m, 2H, CH_2), 2.25 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 3.52 (m, 2H, CH_2), 4.55 [m, 1H, α-H(Ala)], 5.16 (m, 1H, NH), 6.54, 6.85 (s, 1H, Ar-H).– IR: ν = 3454, 1770, 1758, 1246 cm^{-1}.– $C_{27}H_{47}NO_5Si$ (493.76).

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(BOC-valyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropanol (**5b**)*

Starting from BOC-valine (575 mg, 2.64 mmol), **4** (425 mg, 1.32 mmol), DMAP (1130 mg, 9.24 mmol) and EDC (585 mg, 3.79 mmol), **5b** (570 mg, 1.09 mmol, 41%) was obtained as a colourless liquid.– ^{1}H NMR: δ = –0.03 (s, 6H, $SiMe_2$), 0.87 (s, 9H, t-Bu), 1.06, 1.12 [d, *J* = 6.9 Hz, 3H, CH_3(Val)], 1.50 (s, 6H, CH_3; 9H, t-Bu), 2.03 (m, 2H, CH_2), 2.26 (s, 3H, CH_3), 2.41 [m, 1H, β-H(Val)], 2.56 (s, 3H, CH_3), 3.51 (m, 2H, CH_2), 4.48 [m, 1H, α-H(Val)], 5.14 (m, 1H, NH), 6.51, 6.85 (s, 1H, Ar-H).– IR: ν = 3458, 2976, 1757, 1715, 1246, 1174 cm^{-1}.– $C_{29}H_{51}NO_5Si$ (521.82).

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(Z-valyloxy)-4,6-dimethylphenyl]-3,3- dimethylpropanol (**5c**)*

Starting from Z-valine (727 mg, 2.89 mmol), **4** (465 mg, 1.45 mmol), DMAP (1240 mg, 10.12 mmol), and EDC (804 mg, 4.19 mmol), **5c** (522 mg, 0.94 mmol, 65%) was obtained as a colourless liquid.– ^{1}H NMR: δ = -0.03 (s, 6H, $SiMe_2$), 0.87 (s, 9H, t-Bu), 1.08, 1.15 [d, *J* = 6.9 Hz, 3H, CH_3(Val)], 1.48 (s, 6H, CH_3), 2.05 (m, 2H, CH_2), 2.27 (s, 3H, CH_3), 2.47 [m, 1H, β-H(Val)], 2.57 (s, 3H, CH_3), 3.51 (m, 2H, CH_2), 4.57 [m, 1H, α-H(Val)], 5.18 (s, 2H, CH_2), 5.40 (m, 1H, NH), 6.51, 6.86 (s, 1H, Ar-H), 7.40 (m, 5H, Ar-H).– IR: ν = 3433, 2994, 1769, 1758, 1374, 1246, 1056 cm^{-1}.– $C_{32}H_{49}NO_5Si$ (555.83).

*O-Acylation of **4** with BOC-Proline*

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(BOC-prolyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropanol (**5d**)*[11c]

Yield: 100 mg (0.10 mmol, 84%) of a colourless liquid.– ^{1}H NMR: δ = –0.03 (s, 6H, $SiMe_2$), 0.87 (s, 9H, t-Bu), 1.65 (s, 6H, CH_3; 9H, t-Bu), 2.01 [m, 2H, CH_2; 4H, 3′-H, 4′-H (Pro)], 2.26 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 3.54 [m, 2H, CH_2; 2H, 5'-H (Pro)], 4.56 [m, 1H, α-H(Pro)], 6.59, 6.85 (s, 1H, Ar-H).– IR: ν = 2976, 1762, 1701, 1397, 1256, 1142 cm^{-1}.– $C_{29}H_{49}NO_5Si$ (519.80).

General Procedure for the Lengthening of the Peptide Chain with N-Protected Amino Acid N-Hydroxysuccinimide Esters

The appropriate Z-protected compound was dissolved in dry ethanol (50 ml) and Pd-C (100 mg) was added. The solution was degassed and subsequently stirred in an hydrogen atmosphere until the reaction was complete (tlc-control, ca. 2.5 h). The catalyst was filtered off and the solution concentrated in vacuo. The residue was directly used for the next step without further purification and characterisation.

The desired N-unprotected compound (1 eq) was dissolved in dry DCM and the appropriate N-protected amino acid-NHS (2 eq) was added. The solution was stirred until the reaction was complete (tlc), the solvent was evaporated, and the residue taken up in ethyl acetate. The organic phase was washed with 1% $NaHCO_3$-solution (3×) and 1 M HCl and dried with Na_2SO_4. The solvent was evaporated in vacuo and the residue purified by cc (cyclohexane/ethyl acetate = 7/3).

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(BOC-alanylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropanol (**6a**)*

Starting from **5c** (983 mg, 1.77 mmol), **6a** (228 mg, 0.39 mmol, 22%) was obtained as a colourless liquid.– ^{1}H NMR: δ = –0.03 (s, 6H, $SiMe_2$), 0.87 (s, 9H, t-Bu), 1.05, 1.08 [d, *J* = 6.9 Hz, 3H, CH_3(Val)], 1.37 [d, *J* = 7.3 Hz, 3H, CH_3(Ala)], 1.46 (s, 9H, t-Bu; 6H, CH_3), 2.03 (m, 2H, CH_2), 2.23 (s, 3H, CH_3), 2.46 [m, 1H, β-H(Val)], 2.53 (s, 3H, CH_3), 3.47 [t, *J* = 7.4 Hz, 2H, CH_2), 4.21 [m, 1H, α-H(Ala)], 4.78 [dd, *J* = 4.4 and 9.0 Hz, 1H, α-H(Val)], 5.02 (m, 1H, NH), 6.49 (s, 1H, Ar-H), 6.73 (m, 1H, NH), 6.83 (s, 1H, Ar-H).– IR: ν = 3450, 2994, 1770, 1758, 1635, 1245 cm^{-1}.– $C_{31}H_{56}N_2O_6Si$ (580.39).

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(BOC-valylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropanol (**6b**)*

Starting from **5c** (727 mg, 1.30 mmol), **6b** (290 mg, 0.47 mmol, 36%) was obtained as a colourless liquid.– ^{1}H NMR: δ = –0.03 (s, 6H, $SiMe_2$), 0.84 (s, 9H, t-Bu), 0.94, 0.96 [2d, *J* = 6.6 Hz, 6H, CH_3(Val)], 1.44 (s, 9H, t-Bu), 1.45 (s, 6H, CH_3), 2.05 [m, 2H, CH_2; 1H, β-H(Val)], 2.23 (s, 3H, CH_3), 2.45 [m, 1H, β-H(Val)], 2.53 (s, 3H, CH_3), 3.47 (m, 2H, CH_2), 3.93 [dd, *J* = 6.5 and 8.8 Hz, 1H, α-H(Val)], 4.78 [dd, *J* = 4.4 and 8.8 Hz, 1H, α-H(Val)], 5.11 (m, 1H, NH), 6.40 (m, 1H, NH), 6.48, 6.83 (s, 1H, Ar-H).– IR: ν = 3324, 2961, 1769, 1758, 1246, 1051 cm^{-1}.– $C_{34}H_{60}N_2O_6Si$ (620.95).

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(BOC-prolylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropanol (**6c**)*

Starting from **5c** (654 mg, 1.18 mmol), **6c** (407 mg, 0.66 mmol, 56%) was obtained as a colourless liquid.– ^{1}H NMR: δ = –0.03 (s, 6H, $SiMe_2$), 0.87 (s, 9H, t-Bu), 1.04, 1.05 [d, *J* = 6.9 Hz, 3H, CH_3(Val)], 1.44 (s, 9H, t-Bu; 6H, CH_3), 1.97 (m, 2H, CH_2; 4H, 3′-H, 4′-H (Pro)], 2.23 (s, 3H, CH_3), 2.45 [m, 1H, β-H(Val)], 2.53 (s, 3H, CH_3), 3.45 [m, 2H, CH_2; 2H, 5′-H(Pro)], 4.37 [m, 1H, -H(Pro)], 4.76 [m, 1H, α-H(Val)], 6.48 (m, 1H, Ar-H; 1H, NH), 6.82 (s, 1H, Ar-H).– IR: ν = 3456, 2958, 2856, 1757, 1691, 1394, 1248, 1167, 1090 cm^{-1}.– $C_{34}H_{58}N_2O_6Si$ (618.93).

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(Z-alanylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropanol (**6d**)*

Starting from **5c** (867 mg, 1.56 mmol), **6d** (539 mg, 0.91 mmol, 58%) was obtained as a colourless liquid.– ^{1}H NMR: δ = –0.03 (s, 6H, $SiMe_2$), 0.87 (s, 9H, t-Bu), 1.06 [m, 6H, CH_3(Val)], 1.47 [m, 6H, CH_3; 3H, CH_3(Ala)], 2.04 (m, 2H, CH_2), 2.22 (s, 3H, CH_3), 2.49 [m, 1H, β-H(Val)], 2.56 (s, 3H, CH_3), 3.51 (t, *J* = 7.5 Hz, 2H, CH_2), 4.34 [m, 1H, α-H(Ala)], 4.79 [dd, *J* = 4.6 and 9.0 Hz, 1H, α-H(Val)], 5.15 (s, 2H, CH_2), 5.75 (m, 1H, NH), 6.52 (s, 1H, Ar-H), 6.61 (m, 1H, NH), 6.86 (s, 1H, Ar-H), 7.35 (m, 5H, Ar-H).– IR: ν = 3308, 2958, 1769, 1758, 1246, 1051 cm^{-1}.– $C_{32}H_{54}N_2O_6Si$ (590.88).

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(Z-valylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropanol (**6e**)*

Starting from **5c** (653 mg, 1.18 mmol), **6e** (431 mg, 0.69 mmol, 58%) was obtained as a colourless liquid.– ^{1}H NMR: δ = –0.03 (s, 6H, $SiMe_2$), 0.84 (s, 9H, t-Bu), 0.94, 0.97, 1.05, 1.07 [d, *J* = 6.9 Hz, 3H, CH_3(Val)], 1.44 (s, 6H, CH_3), 2.05 [m, 2H, CH_2; 1H, β-H(Val)], 2.24 (s, 3H, CH_3), 2.43 [m, 1H, β-H(Val)], 2.53 (s, 3H, CH_3), 3.47 (t, *J* = 7.5 Hz, 2H, CH_2), 4.05 [dd, *J* = 6.5 and 8.6 Hz, 1H, α-H(Val)], 4.78 [dd, *J* = 4.4 and 8.9 Hz, 1H, α-H(Val)], 5.12 (s, 2H, CH_2), 5.41 (d, *J* = 8.6 Hz, 1H, NH), 6.35 (d, *J* = 9.0 Hz, 1H, NH), 6.48, 6.83 (s, 1H, Ar-H), 7.35 (m, 5H, Ar-H).– IR: ν = 2959, 1757, 1691, 1395, 1248, 1167 cm^{-1}.– $C_{37}H_{58}N_2O_6Si$ (654.97).

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(Z-prolylvaloxy)-4,6-dimethylphenyl]-3,3-dimethylpropanol (**6f**)*

Starting from **5c** (727 mg, 1.31 mmol), **6f** (446 mg, 0.73 mmol, 56%) was obtained as a colourless liquid.– ^{1}H NMR: δ = –0.03 (s, 6H, $SiMe_2$), 0.87 (s, 9H, t-Bu), 1.01 [m, 6H, CH_3(Val)], 1.46 (s, 6H, CH_3), 2.06 [m, 2H, CH_2; 4H, 3′-H, 4é-H (Pro)], 2.27 (s, 3H, CH_3), 2.42 [m, 1H, β-H(Val)], 2.56 (s, 3H, CH_3), 3.51 [m, 2H, CH_2; 2H, 5′-H (Pro)], 4.44 [m, 1H, α-H(Pro)], 4.73 [dd, *J* = 4.4 and 9.0 Hz, 1H, α-H(Val)], 5.21 (s, 2H, CH_2), 6.51 (m, 1H, Ar-H; 1H, NH), 6.85 (s, 1H, Ar-H), 7.35 (m, 5H, Ar-H).– IR: ν = 2994, 1770, 1758, 1246, 1055 cm^{-1}.– $C_{37}H_{56}N_2O_6Si$ (652.95).

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(BOC-alanylalanylvaloxy)-4,6-dimethylphenyl]-3,3-dimethylpropanol (**7a**)*

Starting from **6d** (533 mg, 0.90 mmol), **7a** (200 mg, 0.30 mmol, 33%) was obtained as a colourless solid.– Mp: 67 °C.– ^{1}H NMR: δ = 0.03 (s, 6H, $SiMe_2$), 0.87 (s, 9H, t-Bu), 1.07, 1.09 [d, *J* = 6.9 Hz, 3H, CH_3(Val)], 1.39, 1.43 [d, *J* = 7.0 Hz, 3H, CH_3(Ala)], 1.43 (s, 9H, t-Bu; 3H, CH_3), 1.60 (s, 3H, CH_3), 2.07 (m, 2H, CH_2), 2.26 (s, 3H, CH_3), 2.49 [m, 1H, β-H(Val)], 2.56 (s, 3H, CH_3), 3.50 [t, *J* = 7.4 Hz, 2H, CH_2), 4.18, 4.53 [m, 1H, α-H(Ala)], 4.77 [dd, *J* = 4.4 and 9.0 Hz, 1H, α-H(Val)], 4.97 (m, 1H, NH), 6.51 (s, 1H, Ar-H), 6.64, 6.68 (m, 1H, NH), 6.85 (s, 1H, Ar-H).– IR: ν = 3302, 2952, 2934, 1758, 1693, 1644, 1517, 1472, 1396, 1247, 1167 cm^{-1}.– $C_{35}H_{61}N_3O_7Si$ (663.98).

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(BOC-alanylvalylvaloxy)-4,6-dimethylphenyl]-3,3-dimethylpropanol (**7b**)*

Starting from **6e** (449 mg, 0.69 mmol), **7b** (201 mg, 0.29 mmol, 42%) was obtained as a colourless solid.– Mp: 85 °C.– ^{1}H NMR: δ = –0.03 (s, 6H, $SiMe_2$), 0.87 (s, 9H, t-Bu), 1.06, 1.12 [2d, *J* = 6.9 Hz, 6H, CH_3(Val)], 1.38 (d, *J* = 7.0 Hz, 3H, CH_3(Ala)], 1.48 (s, 9H, t-Bu; 6H, CH_3), 2.13 [m, 2H, CH_2; 1H, β-H(Val)], 2.26 (s, 3H, CH_3), 2.46 [m, 1H, β-H(Val)], 2.56 (s, 3H, CH_3), 3.50 (t, *J* = 7.4 Hz, 2H, CH_2), 4.25 [m, 1H, α-H(Ala)], 4.39 [m, 1H, α-H(Val)], 4.80 [dd, *J* = 4.6 and 8.9 Hz, 1H, α-H(Val)], 5.23 (d, *J* = 7.5 Hz, 1H, NH), 6.52, 6.86 (s, 1H, Ar-H), 6.78 (d, *J* = 8.8 Hz, 1H, NH), 6.93 (d, *J* = 8.7Hz, 1H, NH).– IR: ν = 3306, 2959, 2928, 1755, 1691, 1648, 1514, 1471, 1390, 1366, 1253, 1169 cm^{-1}.– $C_{37}H_{65}N_3O_7Si$ (692.03).

*1-O-(tert-Butyldimethylsilyl)-3-[2-N-(BOC-alanylprolylvaloxy)-4,6-dimethylphenyl]-3,3-dimethylpropanol (**7c**)*

Starting from **6f** (475 mg, 0.73 mmol), **7c** (150 mg, 0.23 mmol, 32%) was obtained as a colourless liquid.– ^{1}H NMR: δ = –0.03 (s, 6H, $SiMe_2$), 0.87 (s, 9H, t-Bu), 1.12 [m, 6H, CH_3(Val)], 1.38 [d, *J* = 6.8 Hz, 3H, CH_3(Ala)], 1.47 (s, 9H, t-Bu; 6H, CH_3), 2.06 (m, 2H, CH_2; 4H, 3′-H, 4′-H (Pro)], 2.27 (s, 3H, CH_3), 2.47 [m, 1H, β-H(Val)], 2.56 (s, 3H, CH_3), 3.51 [t, *J* = 7.4 Hz, 2H, CH_2), 3.70 (m, 2H, 5′-H(Pro)], 4.55 [m, 1H, α-H(Ala)], 4.76 [m, 1H, α-H(Pro); 1H, α-H(Val)], 5.43 (m, 1H, NH), 6.54, 6.84 (s, 1H, Ar-H), 7.47 (m, 1H, NH).– IR: ν = 2964, 2933, 1751, 1688, 1641, 1512, 1366, 1249, 1172 cm^{-1}.– $C_{37}H_{63}N_3O_7Si$ (690.01).

*General Procedure for the Cleavage of the TBDMS-Protective Group and the Oxidation of **5–7** to the Carboxylic Acids*

Either **5**, **6**, or **7** (1 mmol) was dissolved in THF (2 ml), water (2 ml), and glacial acetic acid (6 ml). The solution was stirred for 1 h at r.t. and the solvent was subsequently removed in vacuo. The residue was used for the next step without further purification.

PCC (2 eq) were dissolved in dry DCM. To this solution was added a solution of the appropriate alcohol (1 eq) in dry DCM. During the addition the colour of the mixture turned to black. The reaction was completed while stirring overnight. The solvent was evaporated, the residue taken up in a minimum of DCM and filtrated over a short silica gel column (4 cm, ether). The eluent was evaporated to dryness and the residue was used for the next step without further purification.

A solution of the appropriate aldehyde (1 eq) and NaH_2PO_4 (0.6 eq) in water (1.4 ml) and ACN (3.9 ml) was cooled to 0 °C by an ice-salt bath. To this solution, was added dropwise a solution of $NaClO_2$ (2.5 eq, 80%) in water (5 ml). The reaction mixture was stirred for 1 h at 0 °C, then it was allowed to warm to r.t. One equivalent of Na_2SO_3 was added to decompose HOCl and H_2O_2. The pH was adjusted to 2 with 1 N HCl and the product extracted with ethyl acetate (3 × 20 ml). The combined organic phases were dried with Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by cc (5% MeOH in DCM).

*3-[2-N-(BOC-alanyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionic Acid (**8a**)*[11b]

Starting from **5a** (496 mg, 1.00 mmol), a colourless liquid (103 mg, 0.26 mmol, 26%) was obtained.– ^{1}H NMR: δ = 1.45 (s, 9H, t-Bu), 1.57 [m, 6H, CH_3; 3H, CH_3(Ala)], 2.22, 2.54 (s, 3H, CH_3), 2.82 (s, 2H, CH_2), 4.51 [m, 1H, α-H(Ala)], 5.12 (m, 1H, NH), 6.52, 6.82 (s, 1H, Ar-H).– IR: = 3354, 2994, 1769, 1758, 1246, 1057 cm^{-1}.– $C_{21}H_{31}NO_6$ (393.48).

*3-[2-N-(BOC-valyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionic Acid (**8b**)*

Starting from **5b** (896 mg, 1.72 mmol), a viscous yellowish liquid (204 mg, 0.49 mmol, 29%) was obtained.– ^{1}H NMR: δ = 1.04, 1.10 [d, *J* = 6.8 Hz, 3H, CH_3(Val)], 1.44 (s, 9H, t-Bu), 1.55 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 2.35 (m, 1H, β-H), 2.54 (s, 3H, CH_3), 2.84 (m, 2H, CH_2), 4.41 [m, 1H, α-H(Val)], 5.11 (m, 1H, NH), 6.52, 6.82 (s, 1H, Ar-H).– IR: = 3358, 2976, 1757, 1715, 1369, 1246, 1174 cm^{-1}.– MS (FAB): *m/z* = 444.1 [M+Na]$^+$.– $C_{23}H_{35}NO_6$ (412.54).

*3-[2-N-(BOC-prolyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionic Acid (**8c**)*[11c]

Starting from **5d** (369 mg, 0.71 mmol), a viscous liquid (166 mg, 0.40 mmol, 56%) was obtained.– ^{1}H NMR: δ = 1.46 (s, 9H, t-Bu), 1.65, 1.68 (s, 3H, CH_3), 2.00, 2.31 [m, 4H, 3′-H, 4′-H (Pro)], 2.23 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.84 (s, 2H, CH_2), 3.54 [m, 2H, 5′-H(Pro)], 4.56 [m, 1H, α-H(Pro)], 6.56, 6.82 (s, 1H, Ar-H).– IR: ν = 3433, 2976, 1762, 1701, 1397, 1142 cm^{-1}.– $C_{23}H_{33}NO_6$ (419.52). C, H, N (*0.5 H_2O).

*3-[2-N-(BOC-alanylvaloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionic Acid (**8d**)*

Starting from **6a** (280 mg, 0.48 mmol), a viscous yellowish liquid (139 mg, 0.28 mmol, 59%) was obtained.– ^{1}H NMR: δ = 1.06 [m, 6H, CH_3(Val)], 1.33 [d, *J* = 7.3 Hz, 3H, CH_3(Ala)], 1.55, 1.60 (s, 3H, CH_3), 1.43 (s, 9H, t-Bu), 2.21 (s, 3H, CH_3), 2.39 [m, 1H, β-H(Val)], 2.54 (s, 3H, CH_3), 2.80 (m, 2H, CH_2), 4.21 [m, 1H, α-H(Ala)], 4.68 [m, 1H, α-H(Val)], 5.09 (m, 1H, NH), 6.50, 6.82 (s, 1H, Ar-H), 7.04 (m, 1H, NH).– IR: ν = 3427, 2973, 1709, 1529, 1392, 1368, 1173 cm^{-1}.– MS (EI): 492 (0.6), 297 (8), 241 (34), 241 (12), 215 (68), 204 (13), 187 (38), 72 (100).– MS (FAB): *m/z* = 515.3 [M+Na]$^+$.– $C_{26}H_{40}N_2O_7$ (492.62).

*3-[2-N-(BOC-valylvaloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionic Acid (**8e**)*

Starting from **6b** (367 mg, 0.59 mmol), a viscous yellowish liquid was obtained (242 mg, 0.46 mmol, 79%).– ^{1}H NMR: δ = 0.96, 1.11 [2d, *J* = 6.8 Hz, 6H, CH_3(Val)], 1.42 (s, 9H, t-Bu), 1.55, 1.62 (s, 3H, CH_3), 2.15 [m, 1H, β-H(Val)], 2.21 (s, 3H, CH_3), 2.40 [m, 1H, β-H(Val)], 2.54 (s, 3H, CH_3), 2.80 (m, 2H, CH_2), 3.95, 4.70 [m, 1H, α-H(Val)], 5.16 (m, 1H, NH), 6.50 (s, 1H, Ar-H), 6.82 (s, 1H, Ar-H; m, 1H, NH), 8.02 (s, 1H, COOH).– IR: ν = 3308, 2966, 1757, 1709, 1664, 1525, 1389, 1367, 1175 cm^{-1}.– MS (EI): 299 (18), 248 (30), 215 (12), 116 (10), 72 (100).– MS (FAB): *m/z* = 543.4 [M+Na]$^+$.– $C_{28}H_{44}N_2O_7$ (520.67).

*3-[2-N-(BOC-prolylvaloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionic Acid (**8f**)*

Starting from **6c** (481 mg, 0.78 mmol), a viscous yellowish liquid was obtained (182 mg, 0.35 mmol, 45%).– ^{1}H NMR: δ = 1.05, 1.09 [d, *J* = 6.8 Hz, 3H, CH_3(Val)], 1.46 (s, 9H, t-Bu), 1.58, 1.62 (s, 3H, CH_3), 1.90 (m, 4H, 3′-H, 4′-H (Pro)], 2.22 (s, 3H, CH_3), 2.41 [m, 1H, β-H(Val)], 2.54 (s, 3H, CH_3), 2.82 (m, 2H, CH_2), 3.43 (m, 2H, 5′-H(Pro)], 4.40 [m, 1H, α-H(Pro)], 4.64 [m, 1H, α-H(Val)], 6.51, 6.82 (s, 1H, Ar-H), 7.69 (m,

1H, NH), 8.02 (s, 1H, COOH).– IR: ν = 3422, 2968, 2930, 1668, 1392, 1166 cm^{-1}.– MS (EI): 297 (45), 241 (66), 213 (50), 183 (12), 114 (43), 70 (100).– MS (FAB): *m/z* = 541.3 $[M+Na]^+$.– $C_{28}H_{42}N_2O_7$ (518.67).

*3-[2-N-(BOC-alanylalanylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionic Acid (**8g**)*

Starting from **7a** (189 mg, 0.28 mmol), a viscous yellowish liquid (90 mg, 0.16 mmol, 57%) was obtained.– ^{1}H NMR: δ = 1.04 [m, 6H, CH_3(Val)], 1.28 [m, 6H, CH_3(Ala)], 1.42 (s, 9H, t-Bu), 1.55, 1.59 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 2.41 [m, 1H, β-H(Val)], 2.54 (s, 3H, CH_3), 2.79 (m, 2H, CH_2), 4.16, 4.53, 4.68 (m, 1H, α-H), 5.14 (m, 1H, NH), 6.49, 6.81 (s, 1H, Ar-H), 7.05 (d, *J* = 7.4 Hz, 1H, NH), 7.21 (d, *J* = 8.8 Hz, 1H, NH).– IR: ν = 3303, 2970, 2932, 1760, 1715, 1650, 1540, 1392, 1248, 1174 cm^{-1}.– MS (FAB): *m/z* = 586.5 $[M+Na]^+$.– $C_{29}H_{45}N_3O_8$ (563.69).

*3-[2-N-(BOC-alanylvalylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionic Acid (**8h**)*

Starting from **7b** (242 mg, 0.35 mmol), a yellowish solid (134 mg, 0.23 mmol, 65%) was obtained.– Mp: 109° C.– ^{1}H NMR: δ = 0.89, 1.05 [m, 6H, CH_3(Val)], 1.27 (m, 3H, CH_3(Ala)], 1.42 (s, 9H, t-Bu), 1.53, 1.61 (s, 3H, CH_3), 2.16 [m, 1H, β-H(Val)], 2.20 (s, 3H, CH_3), 2.34 [m, 1H, β-H(Val)], 2.54 (s, 3H, CH_3), 2.80 (m, 2H, CH_2), 4.18, 4.33, 4.70 (m, 1H, α-H), 5.23 (m, 1H, NH), 6.48, 6.80 (s, 1H, Ar-H), 7.14, 7.18 (m, 1H, NH).– IR: ν = 3292, 2994, 1770, 1758, 1374, 1246, 1053 cm^{-1}.– MS (ESI): *m/z* = 614.5 $[M+Na]^+$.– $C_{31}H_{49}N_3O_8$ (591.75).

*3-[2-N-(BOC-alanylprolylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionic Acid (**8i**)*

Starting from **7c** (138 mg, 0.20 mmol), a viscous yellowish liquid (58 mg, 0.09 mmol, 45%) was obtained.– ^{1}H NMR: δ = 1.05 [m, 6H, CH_3(Val)], 1.31 [m, 3H, CH_3(Ala)], 1.40 (s, 9H, t-Bu), 1.54, 1.57 (s, 3H, CH_3), 1.98 (m, 3H, 3′-H, 4′-Hs (Pro); 1H, β-H(Val)], 2.19 (s, 3H, CH_3), 2.30 [m, 1H, 3′-H(Pro)], 2.52 (s, 3H, CH_3), 2.79 (m, 2H, CH_2), 3.58 (m, 2H, 5′-H(Pro)], 4.56 (m, 3H, α-H), 6.48, 6.79 (s, 1H, Ar-H), 7.44 (d, *J* = 8.4 Hz, 1H, NH), 7.55 (d, *J* = 8.2 Hz, 1H, NH).– IR: ν = 3220, 2962, 2930, 1759, 1691, 1640, 1531, 1453, 1366, 1254, 1169, 1092 cm^{-1}.– MS (ESI): *m/z* = 612.4 $[M+Na]^+$; (FAB): *m/z* = 612.4 $[M+Na]^+$.– $C_{31}H_{47}N_3O_8$ (589.73).

*General Procedure for the Attachment of **8** with p-Anisidine*

To a solution of the appropriate starting compound (1 eq) in dry DCM (3 ml), was added DCC (1.2 eq) at 0 °C and the resulting mixture was stirred for 10 min. Then HOBt (1.1 eq) was added and the mixture was stirred for 10 min, followed by the addition of *p*-anisidine (1.1 eq) and DMAP (0.2 eq). The reaction mixture was stirred for 1 h at 0 °C and stirring was continued at r.t. overnight. The solvent was evaporated, the residue taken up in ethyl acetate, and the precipitated urea was separated by filtration. The organic phase was washed with sat. $NaHCO_3$-solution (3×20 ml), 10% citric acid-solution (2×20 ml), and water (2×20 ml) and dried with Na_2SO_4. The solvent was removed in vacuo and the crude product purified by cc (ethyl acetate/PE).

*N-(4-Methoxyphenyl)-3-[2-N-(BOC-alanyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionamide (**9a**)*

Starting from **8a** (100 mg, 0.25 mmol), DCC (62 mg, 0.30 mmol), HOBt (42 mg, 0.28 mmol), *p*-anisidine (34 mg, 0.28 mmol), and DMAP (6 mg, 0.05 mmol), **9a** (57 mg, 0.11 mmol, 46%) was obtained as a colourless solid.– Mp: 85° C.– ^{1}H NMR: δ = 1.40 (s, 9H, t-Bu), 1.57 [d, *J* = 7.5 Hz, 3H, CH_3(Ala)], 1.68, 1.70 (s, 3H, CH_3), 2.21, 2.46 (s, 3H, CH_3), 2.53, 2.63 (s, 1H, CH_2), 3.73 (s, 3H, OCH_3), 4.51 [dq, *J* = 7.5 Hz, 1H, α-H(Ala)], 5.14 (d, *J* = 7.5 Hz, 1H, NH), 6.54 (s, 1H, Ar-H), 6.73 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.78 (s, 1H, Ar-H), 7.19 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.77 (s, 1H, NH).– IR: ν = 3346, 2976, 2927, 1762, 1663, 1512, 1366, 1245, 1160, 1065 cm^{-1}.– $C_{28}H_{38}N_2O_6$ (498.62). C, H, N.

*N-(4-Methoxyphenyl)-3-[2-N-(BOC-valyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionamide (**9b**)*

Starting from **8b** (191 mg, 0.45 mmol), DCC (112 mg, 0.54 mmol), HOBt (76 mg, 0.50 mmol), *p*-anisidine (61 mg, 0.50 mmol), and DMAP (11 mg, 0.09 mmol), **9b** (137 mg, 0.26 mmol, 58%) was obtained as a colourless solid.– Mp: 141 °C.– ^{1}H NMR: δ = 1.09, 1.15 [d, *J* = 6.9 Hz, 3H, CH_3(Val)], 1.43 (s, 9H, t-Bu), 1.70 (s, 6H, CH_3), 2.23, 2.47 (s, 3H, CH_3), 2.42 [m, 1H, β-H(Val)], 2.55, 2.65 (s, 1H, CH_2), 3.75 (s, 3H, OCH_3), 4.10 [dd, *J* = 4.9 and 8.9 Hz, 1H, α-H(Val)], 4.99 (d, *J* = 8.9 Hz, 1H, NH), 6.54, 6.80 (s, 1H, Ar-H), 6.74, 7.21 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.73 (s, 1H, NH).– IR: ν = 3334, 2966, 1745, 1537, 1512, 1244 cm^{-1}.– $C_{30}H_{42}N_2O_6$ (526.68). C, H, N.

*N-(4-Methoxyphenyl)-3-[2-N-(BOC-prolyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionamide (**9c**)*

Starting from **8c** (146 mg, 0.35 mmol), DCC (86 mg, 0.42 mmol), HOBt (59 mg, 0.38 mmol), *p*-anisidine (47 mg, 0.38 mmol), and DMAP (9 mg, 0.07 mmol), **9c** (125 mg, 0.24 mmol, 68%) was obtained as a colourless solid.– Mp: 84 °C.– ^{1}H NMR: δ = 1.43 (s, 9H, t-Bu), 1.69, 1.75 (s, 3H, CH_3), 2.44 [m, 4H, 3′,4′-H(Pro)], 2.22, 2.57 (s, 3H, CH_3), 2.73, 2.80 (s, 1H, CH_2), 3.54 [m, 2H, 5′-H (Pro)], 3.74 (s, 3H, OCH_3), 4.61 [m, 1H, α-H(Pro)], 6.58, 6.78 (s, 1H, Ar-H), 6.74, 7.22 (d, *J* = 9.0 Hz, 2H, Ar-H), 8.05 (s, 1H, NH).– IR: ν = 3349, 2975, 1763, 1675, 1537, 1512, 1408, 1146 cm^{-1}.– $C_{30}H_{40}N_2O_6$ (524.66). C, H, N.

*N-(4-Methoxyphenyl)-3-[2-N-(BOC-alanylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionamide (**9d**)*

Starting from **8d** (80 mg, 0.16 mmol), DCC (40 mg, 0.19 mmol), HOBt (27 mg, 0.18 mmol), *p*-anisidine (22 mg, 0.18 mmol), and DMAP (4 mg, 0.03 mmol), **9d** (24 mg, 0.04 mmol, 25%) was obtained as a colourless solid.– Mp: 103° C.– ^{1}H NMR: δ = 1.09, 1.11 [d, *J* = 6.8 Hz, 3H, CH_3(Val)], 1.32 [d, *J* = 7.1 Hz, 3H, CH_3(Ala)], 1.43 (s, 9H, t-Bu), 1.68 (s, 6H, CH_3), 2.22 (s, 3H, CH_3), 2.41 [m, 1H, β-H(Val)], 2.59 (s, 3H, CH_3), 2.54, 2.66 (s, 1H, CH_2), 3.74 (s, 3H, OCH_3), 4.22 [dq, *J* = 7.1 Hz, 1H, α-H(Ala)], 4.62 [dd, *J* = 5.0 and 8.4 Hz, 1H, α-H(Val)], 4.93 (d, *J* = 8.4 Hz, 1H, NH), 6.52 (s, 1H, Ar-H), 6.75 (d, *J* = 9.2 Hz, 2H, Ar-H), 6.79 (s, 1H, Ar-H), 7.05 (m, 1H, NH), 7.26 (d, *J* = 9.2 Hz, 2H, Ar-H), 7.99 (s, 1H, NH).– IR: ν = 3314, 2973, 2930, 1759, 1708, 1652, 1512, 1365, 1243, 1173 cm^{-1}.– $C_{33}H_{47}N_3O_7$ (597.76). C, H, N.

*N-(4-Methoxyphenyl)-3-[2-N-(BOC-valylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionamide (**9e**)*

Starting from **8e** (160 mg, 0.31 mmol), DCC (77 mg, 0.37 mmol), HOBt (52 mg, 0.34 mmol), *p*-anisidine (42 mg, 0.34 mmol), and DMAP (8 mg, 0.06 mmol), **9e** (43 mg, 0.07 mmol, 22%) was obtained as a colourless solid.– Mp: 153° C.– ^{1}H NMR: δ = 0.90, 0.91 [d, *J* = 6.6 Hz, 3H, CH_3(Val)], 1.09, 1.12 [d, *J* = 6.6 Hz, 3H, CH_3(Val)], 1.41 (s, 9H, t-Bu), 1.68 (s, 6H, CH_3), 2.03 [m, 1H, β-H(Val)], 2.21 (s, 3H, CH_3), 2.39 [m, 1H, β-H(Val)], 2.48 (s, 3H, CH_3), 2.66, 2.73 (s, 1H, CH_2), 3.73 (s, 3H, OCH_3), 3.94, 4.62 [m, 1H, α-H(Val)], 5.03 (d, *J* = 8.6 Hz, 1H, NH), 6.50 (s, 1H, Ar-H), 6.69 (d, *J* = 8.0 Hz, 1H, NH), 6.75 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.79 (s, 1H, Ar-H), 7.26 (d, *J* = 9.0 Hz, 2H, Ar-H), 8.22 (s, 1H, NH).– IR: ν = 3332, 2960, 2929, 1762, 1687, 1654, 1514, 1245, 1172 cm^{-1}.– $C_{35}H_{51}N_3O_7$ (625.81). C, H, N.

*N-(4-Methoxyphenyl)-3-[2-N-(BOC-prolylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionamide (**9f**)*

Starting from **8f** (120 mg, 0.23 mmol), DCC (57 mg, 0.28 mmol), HOBt (39 mg, 0.25 mmol), *p*-anisidine (31 mg, 0.25 mmol), and DMAP (6 mg, 0.05 mmol), **9f** (30 mg, 0.05 mmol, 21%) a colourless solid was obtained. The purity of the compound was 85% (RP-HPLC).– Mp: 83° C.– ^{1}H NMR: δ = 1.09 [m, 6H, CH_3(Val)], 1.43 (s, 9H, t-Bu), 1.69 (s, 6H, CH_3), 1.85 (m, 4H, 3′-H, 4′-H (Pro)], 2.21 (s, 3H, CH_3), 2.46 [m, 1H, β-H(Val)], 2.53 (s, 3H, CH_3), 2.68, 2.74 (s, 1H, CH_2), 3.37 (m, 2H, 5′-H(Pro)], 3.73 (s, 3H, OCH_3), 4.37 [m, 1H, α-H(Pro)], 4.57 [m, 1H, α-H(Val)], 6.51 (s, 1H, Ar-H), 6.74 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.77 (s, 1H, Ar-H), 7.25 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.92 (m, 1H, NH), 8.17 (s, 1H, NH).– IR: ν = 3320, 2971, 2931, 1761, 1678, 1511, 1396, 1244, 1171 cm^{-1}.– $C_{35}H_{49}N_3O_7$ (623.79).

*N-(4-Methoxyphenyl)-3-[2-N-(BOC-alanylalanylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionamide (***9g)**

Starting from **8g** (78 mg, 0.14 mmol), DCC (35 mg, 0.17 mmol), HOBt (24 mg, 0.15 mmol), *p*-anisidine (19 mg, 0.15 mmol), and DMAP (3 mg, 0.03 mmol), **9g** (33 mg, 0.05 mmol, 35%) was obtained as a colourless solid.– Mp: 99° C.– ^{1}H NMR: δ = 1.04 [m, 6H, CH_3(Val)], 1.28 [m, 6H, CH_3(Ala)], 1.42 (s, 9H, t-Bu), 1.67, 1.70 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 2.41 [m, 1H, β-H(Val)], 2.50 (s, 3H, CH_3), 2.71, 2.78 (s, 1H, CH_2), 3.73 (s, 3H, OCH_3), 4.17 (m, 1H, α-H), 4.58 (m, 2H, α-H), 5.28 (m, 1H, NH), 6.55 (s, 1H, Ar-H), 6.75 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.79 (s, 1H, Ar-H), 7.23 (m, 1H, NH), 7.26 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.53 (d, *J* = 9.2 Hz, 1H, NH), 8.11 (s, 1H, NH).– IR: ν = 3320, 2974, 2932, 1655, 1512, 1245, 1170 cm^{-1}.– $C_{36}H_{52}N_4O_8$ (668.84). C, H, N.

*N-(4-Methoxyphenyl)-3-[2-N-(BOC-alanylvalylvalyloxy)-4,6-dimethylphenyl]-3,3-dimethylpropionamide (***9h)**

Starting from **8h** (71 mg, 0.12 mmol), DCC (30 mg, 0.14 mmol), HOBt (20 mg, 0.13 mmol), *p*-anisidine (16 mg, 0.13 mmol), and DMAP (3 mg, 0.03 mmol), **9h** (36 mg, 0.05 mmol, 43%) was obtained as a colourless solid.– Mp: 165° C.– ^{1}H NMR: δ = 0.91, 0.92, 1.09, 1.13 [d, *J* = 6.8 Hz, 3H, CH_3(Val)], 1.31 (d, *J* = 7.3 Hz, 3H, CH_3(Ala)], 1.43 (s, 9H, t-Bu), 1.68, 1.70 (s, 3H, CH_3), 2.16 [m, 1H, β-H(Val)], 2.22 (s, 3H, CH_3), 2.39 [m, 1H, β-H(Val)], 2.50 (s, 3H, CH_3), 2.59, 2.78 (s, 1H, CH_2), 3.74 (s, 3H, OCH_3), 4.14 [m, 1H, α-H(Val)], 4.37 [dq, *J* = 7.3 Hz, 1H, α-H(Ala)], 4.63 [dd, *J* = 5.4 and 7.7 Hz, 1H, α-H(Val)], 5.15 (d, *J* = 9.6 Hz, 1H, NH), 6.53 (s, 1H, Ar-H), 6.76 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.79 (s, 1H, Ar-H), 6.96 (m, 1H, NH), 7.18 (m, 1H, NH), 7.26 (d, *J* = 9.0 Hz, 2H, Ar-H), 8.11 (m, 1H, NH).– IR: ν = 3321, 2967, 2932, 1760, 1651, 1512, 1367, 1245, 1171 cm^{-1}.– $C_{38}H_{56}N_4O_8$ (696.89). C, H, N.

HPLC Experiments

The respective compound was dissolved in acetonitrile and diluted with tris-buffer [0.1 M, pH 8.0; final concentration acetonitrile: 15%; final concentrations of compounds [mM] (retention times, solvent 1): **9a**: 0.055 (8.35 min); **9b**: 0.040 (12.17 min); **9c**: 0.042 (12.37 min); **9d**: 0.046 (9.11 min); **9e**: 0.043 (12.19 min); **9f**: 0.042 (11.93 min); **9g**: 0.040 (6.91 min); **9h**: 0.038 (8.78 min)]. The assays were performed at room temperature with a PPE concentration of 75 µg/ml (stock solution of PPE was prepared in 1 mM acetic acid). At various times 20 µl aliquots were withdrawn and immediately analysed by HPLC (detection wavelength: 250 nm; column: CC 250/4 Nucleosil 100-5 C_{18} HD; oven temperature: 30° C; flow: 0.7 ml/min; mobile phase: KH_2PO_4 (20 mM, pH 3.3)/acetonitrile [either 30/70 (solvent 1) or a linear gradient: within 30 min from 95/5 to 20/80 (solvent 2)].

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