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Synthesis of a novel conformationally restricted Val-Phe dipeptidomimetic

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Abstract: A method for the synthesis of (3(*R*,*S*),6*S*,11b(*R*,*S*))-1,3,4,6,7,11*b*-hexahydro-4-oxo-3-phthalimidopyrido[2,1-*a*]isoquinoline-6-carboxylic acid **2** as a new conformationally restricted dipeptidomimetic of Val-Phe is reported. It involved cyclisation via an intramolecular electrophilic addition at the reactive bridgehead carbon. This new scaffold can be used as a building block in the preparation of libraries of peptidomimetics. Copyright © 2005 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Val-Phe peptidomimetic; pyridoisoquinoline; building block

INTRODUCTION

Among the numerous therapeutic agents that have benefited from the peptidomimetic design [1,2], two main classes of potential drugs useful in the treatment or prevention of cancer and neurodegenerative disorders are of particular interest. For example, some anti-metastatic agents have been designed in the cancer field [3,4], and for Alzheimer's disease some promising approaches have been proposed [5,6].

We have recently developed a program dealing with farnesyltransferase (FTase) inhibitors able to interfere with the Ras-MAP kinase cascade or with the PI3 kinase/AKT survival pathway [7,8].

In this paper, we report the synthesis of (3(*R*,*S*),6*S*,11b(*R*,*S*))-1,3,4,6,7,11*b*-hexahydro-4-oxo-3-phthalimidopyrido[2,1-*a*]isoquinoline-6-carboxylic acid **2** as a novel dipeptidomimetic of Val-Phe where the side chain of Val is modified inside a hexahydropyridone ring and the benzyl side chain of Phe is restricted in tetrahydroisoquinoline (Chart 1).

To study the issue of geometrical similarities between the designed dipeptidomimetic **2** and the natural fragment, we performed a molecular modeling study on the two structures.

A representative sample of the conformational space was generated by a 200-cycle simulated annealing experiment. Computation began at 1000 K and the system was maintained at that temperature for 2000 fs. The temperature was then reduced with exponential ramping until 300 K was reached. At this point, the lowest energy conformation was selected and minimised. A superposition of the minimised conformation of Val-Phe and **2** (Figure 1) resulted in an RMS of 0.907, as calculated on the skeleton atoms. The results obtained suggest that the geometry of the skeleton was conserved, but the mimicking Phe side-chain of **2** was constrained into a *cis* amide conformation imposed by lactam geometry.

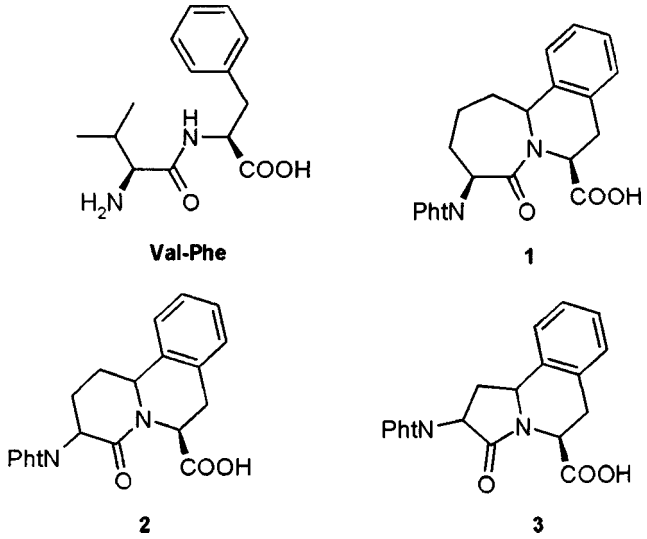

Chart 1 Constrained peptidomimetics of Val-Phe.

This constrained Val-Phe dipeptidomimetic offers several advantages over its peptidic model due to the presence of the ternary nitrogen lactam: (i) improved metabolic stability, (ii) decrease in entropy and (iii) absence of intramolecular or intermolecular hydrogen bonding.

In our recent research program [7,8], it was proved essential to replace the Val-Phe peptidic part in the FTase inhibitor CVFM [9,10] by a non-peptidic linker able to resist peptidases hydrolysis. Moreover, the Val-Phe sequence was found to be a recognition

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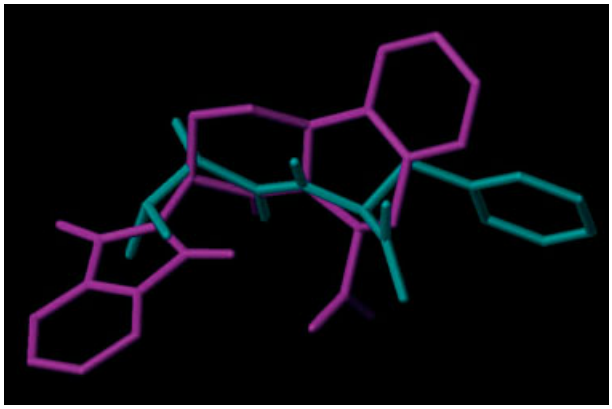


Figure 1 Superposition of the minimised conformation of Val-Phe (cyan) and dipeptidomimetic **2** (magenta).

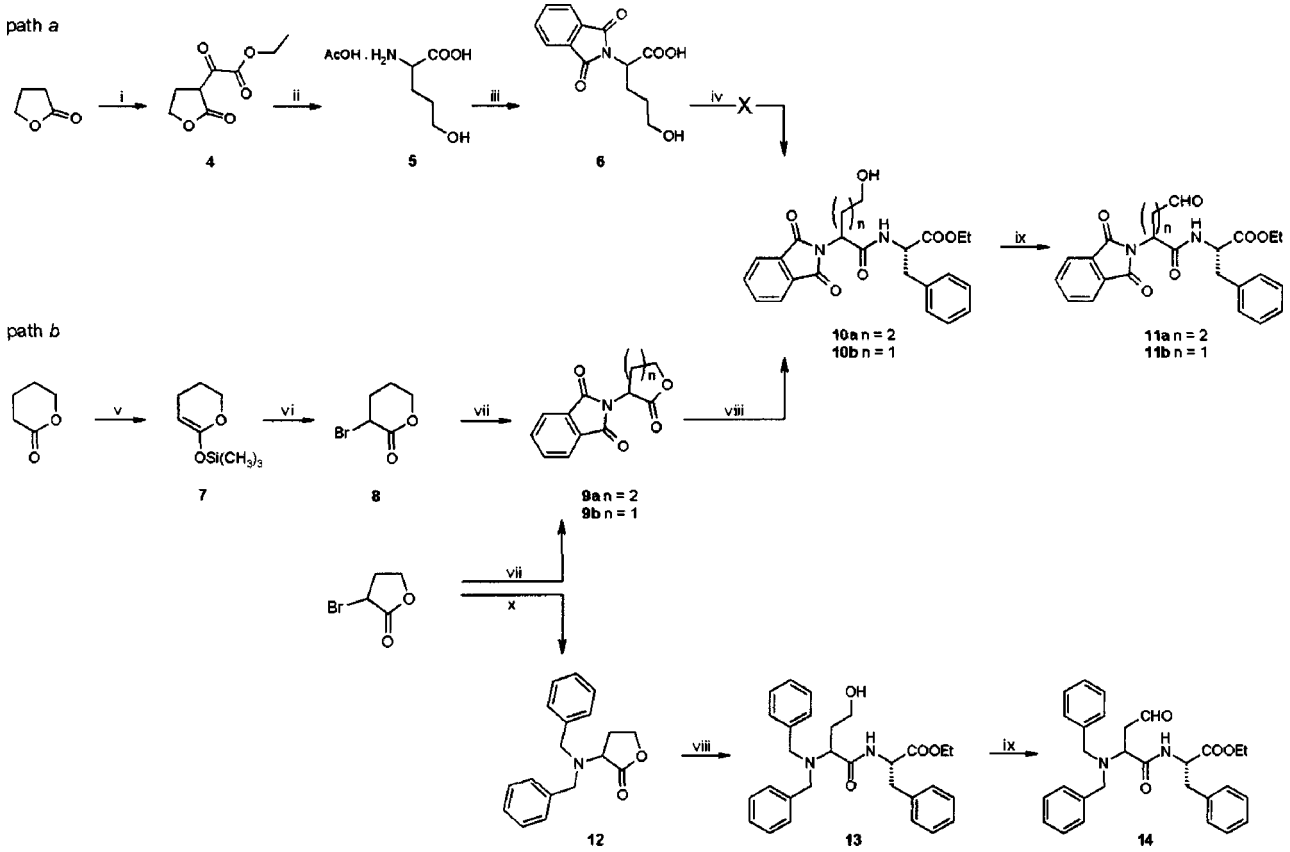
motif inducing a marked increase in FSH/LH activity [11], which participates in the charge-relay system of carboxypeptidase Y [12] and in the inhibition of betaine : homocysteine-*S*-methyltransferase [13].

RESULTS AND DISCUSSION

We have previously used a method [14] described for the synthesis of a 7,6-fused azepinone, i.e. azepinoisoquinoline **1**, and we decided to apply and to adapt a similar method to the preparation of the 6,6- and 5,6-fused analogs, pyrido- and pyrroloisoquinolines **2** and **3** respectively (Chart 1).

Pyridoisoquinoline **2** can be obtained from the key intermediate **10a** (Scheme 1) where the primary amine is masked as a phthalimido group. This choice was guided by two criteria: first, the protective group must occupy two valences of the nitrogen atom in order to allow regioselective cyclisation between the phenylalaninate nitrogen atom and the carbaldehyde of **11a**; second, the protective group must be resistant to the strong acidic conditions of cyclisation.

The synthesis of **10a** was initially envisaged from γ-butyrolactone (Scheme 1, path *a*). 2-Amino-5-hydroxypentanoic acid **5** was obtained in a one pot sequence by acid hydrolysis of 2-ketolactone **4** obtained from Claisen condensation between γ-butyrolactone and diethyl oxalate, spontaneous decarboxylation of



Scheme 1 Reagents and conditions; (i) diethyl oxalate, EtONa, Et_2O, rt, 2 h, 50%; (ii) a. 2 N H_2SO_4, reflux, 4 h; b. NaOH; c. NH_3, H_2/Raney Ni, 50 bars, 90 °C, 4 h; d. AcOH, 45%; (iii) Pht=N-COOEt, 1.5 M Na_2CO_3, rt, 2 h, 25%; (iv) H-Phe-OEt.HCl, NMM, HOBt, EDCI, CH_2Cl_2, rt, 18 h; (v) a. LDA, THF, −78 °C, 10 min; b. $(CH_3)_3SiCl$, rt, 1 h, 60%; (vi) Br_2, TEA, CH_2Cl_2, −15 °C, 30 min, 95%; (vii) potassium phthalimidate, xylene, reflux, 3 h, 21% (**9a**), 35% (**9b**); (viii) H-Phe-OEt.HCl, sodium 2-ethylhexanoate, THF, reflux, 36 h, 50% (**10a**), 60 h, 20% (**10b**), 72 h, 45% (**13**); (ix) oxalyl chloride, DMSO, TEA, CH_2Cl_2, −78 °C, rt, 30 min, 70% (**11a**), 30% (**11b**), 20% (**14**); (x) dibenzylamine, TEA, DMF, 0 °C, 2 h, 50%.

the intermediate α-keto acid and catalytic reduction (Raney nickel) [15] of the imine generated by reaction with ammonia. The 2-amino acid **5** was then protected as phthalimide **6** with *N*-ethoxycarbonylphthalimide. Surprisingly, the standard peptidic coupling procedure of *N*-phthaloyl amino acid **6** with ethyl phenylalaninate failed to give the corresponding pseudopeptide **10a**.

Consequently, another method was used to obtain intermediate **10a**, by reacting valerolactone **9a** with ethyl phenylalaninate (Scheme 1, path *b*). Silyl enolether **7** resulted from the enolisation of δ-valerolactone with LDA (THF, $-78\,^{\circ}C$) and etherification with trimethylsilyl chloride [16]. Reaction temperature was kept below $-70\,^{\circ}C$ to avoid the formation of 2-trimethylsilyl-δ-valerolactone resulting from *C*-silylation. Bromination of **7** gave a good yield of 2-bromovalerolactone **8** [17]. Subsequent Gabriel reaction between bromide **8** and potassium phthalimidate under xylene reflux gave 2-phthalimido-δ-valerolactone **9a**. Dipeptide **10a** was then obtained (50% yield) by aminolysis of lactone **9a** with ethyl phenylalaninate and sodium 2-ethylhexanoate, which was found to serve both as a base and as a catalyst [18].

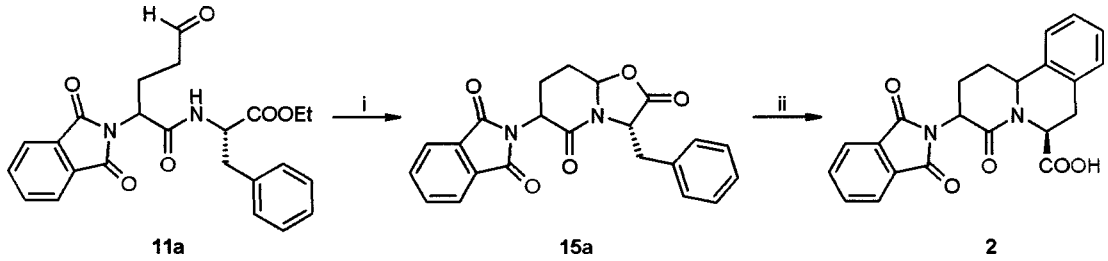
Swern oxidation of primary alcohol **10a** gave a good yield of the corresponding dipeptidyl aldehyde **11a**.

Under reflux in acidic conditions, aldehyde **11a** cyclised into pyrido[2,1-*b*][1,3]oxazole-2,5-dione **15a** (Scheme 2). Two mechanisms may account for this cyclisation [14].

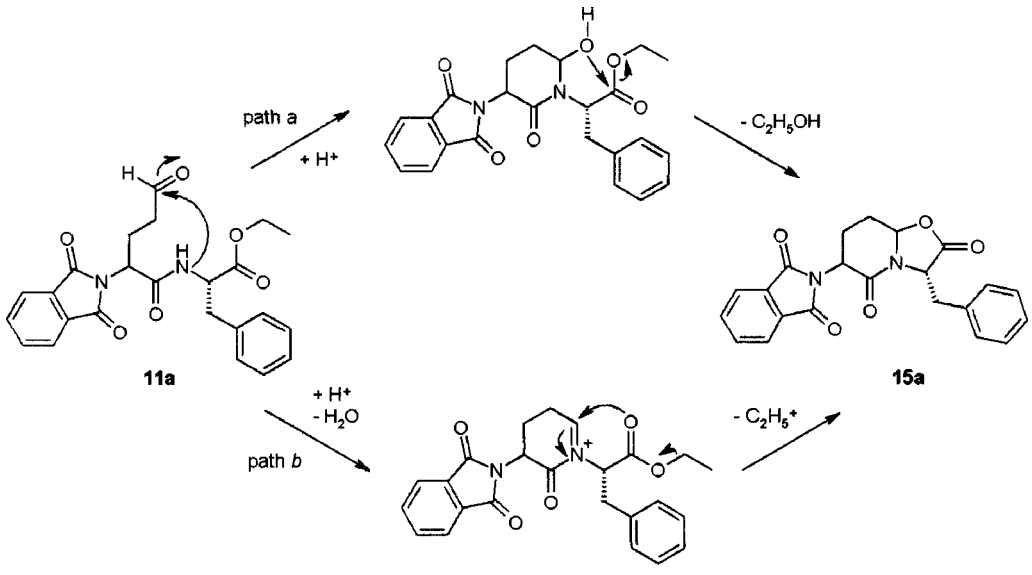
The bicyclic lactam **15a** (Scheme 3) may arise either (path *a*) from direct lactonisation of the cyclic hemi-*N*-acylaminal with the proximal alkyl ester or (path *b*) from a nucleophilic attack of the activated *N*-acyliminium by the carboxylic ester followed by loss of the alkyl group [19,20]. Neither of these intermediates could be detected in the reaction mixture. Further treatment with triflic acid and triflic anhydride induced cyclisation of bicycle **15a** into tricyclic pyrido[2,1-*a*]isoquinoline **2**. The proposed mechanism [14] involved cyclisation *via* an intramolecular electrophilic addition to the reactive bridgehead carbon (Scheme 4).

This explains why compounds **15a** and **2** were obtained as a mixture of inseparable diastereomers.

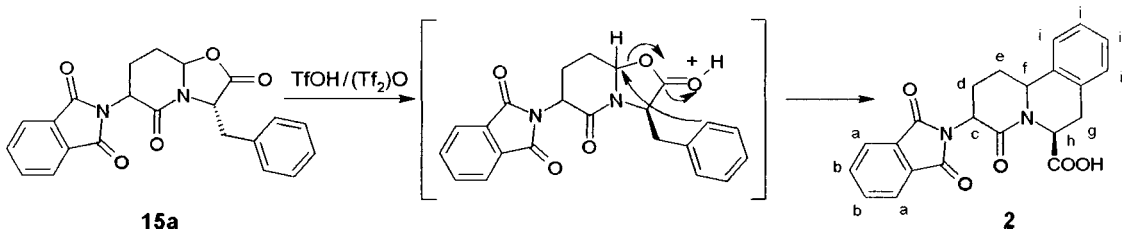
The same synthesis strategy was envisaged to prepare pyrroloisoquinoline **3** (Chart 1). Commercial 2-bromo-γ-butyrolactone was used as starting material and reacted with potassium phthalimidate to give 2-phthalimidobutyrolactone **9b** (Scheme 1). Alcohol **10b** and aldehyde **11b** were obtained according to the reaction conditions leading to compounds **10a**



Scheme 2 Reagents and conditions; (i) TFA, $CHCl_3$, reflux, 6 days, 65%; (ii) TfOH, $(Tf)_2O$, CH_2Cl_2, rt, 21 h, 45%.



Scheme 3 Proposed mechanisms for cyclisation into pyrido[2,1-*b*][1,3]oxazole-2,5-dione **15a**.



Scheme 4 Proposed mechanism for cyclisation into pyrido[2,1-*a*]isoquinoline **2**.

and **11a**. However, cyclisation of aldehyde **11b** in the presence of trifluoroacetic acid failed to give the corresponding pyrrolo[2,1-*b*][1,3]oxazole-2,5-dione, homolog of **15a**, owing to its marked instability in these conditions. The same conclusions (instability and absence of cyclisation) were drawn when the phthalimido protective group was replaced by a dibenzylamino group (Scheme 1): aldehyde **14** did not evolve into the expected condensed bicyclic lactam whose sterically hindered core is obvious as is the benzyl substituent.

CONCLUSION

This work reports an efficient synthesis of (3(*R*,*S*),6*S*, 11b(*R*,*S*))-1,3,4,6,7,11*b*-hexahydro-4-oxo-3-phthalimidopyrido[2,1-*a*]isoquinoline-6-carboxylic acid **2**, a new and versatile conformationally constrained dipeptidomimetic. Conversion of the *N*-phthalimido protecting group into the free amine is readily obtained by treatment with hydrazine in methanol. This new fused tricycle also constitutes an interesting pattern in the building of chemical libraries and in the development of enlarged chemical structures with biological and pharmacological properties. Though the method described herein is capable of generating the azepino- and pyridoisoquinolines **1** and **2**, it, however, failed to give the pyrroloisoquinoline **3**.

EXPERIMENTAL

Structure Calculations

Molecular modeling studies were performed using SYBYL [21] software version 6.91 running on a Silicon Graphics Octane2 workstation. Three-dimensional models of Val-Phe and **2** were built from a library of standard fragments, and their geometry was subsequently optimised using the Amber 7.0 force field [22,23] including the electrostatic term calculated from Gasteiger and Hückel atomic charges. The method of Powell available in the Maximin 2 procedure was used for energy minimisation until the gradient value was smaller than 0.001 kcal mol^{-1} $Å^{-1}$.

Chemistry

Column chromatography was performed on silica gel 60 230–400 Mesh (Merck). Melting points were determined on a Büchi SMP 20 capillary melting point apparatus and remained uncorrected. The IR spectra were recorded with a Bruker Vector 22 spectrophotometer; absorbances are reported in ν (cm^{-1}). The ^1H NMR spectra were recorded at 300 MHz on a Bruker AC 300 spectrometer using tetramethylsilane as an internal standard. Chemical shifts are expressed in ppm (δ), J values are in hertz and the splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; dd, doublet of doublets; m, massif or multiplet. The mass spectra were recorded on a quadripolar Finnigan Mat SSQ 710 instrument in either electronic impact or chemical ionisation mode. Elemental analyses for C, H, N were performed by the 'Service Central d'Analyses' at the CNRS, Vernaison, France.

Ethyl 2-oxo-2-((*3R,S*)-2-oxotetrahydrofuran-3-yl)acetate(4)

Sodium ethanolate (23.0 g, 1.0 mol of sodium in 100 ml of ethanol) was added to 500 ml of anhydrous diethyl ether and cooled in an ice bath. Butyrolactone (76.9 ml, 1.0 mol) and diethyl oxalate (137 ml, 1.0 mol) were added dropwise, and the solution was stirred at room temperature for 2 h. Cooled water, and then 3 N sulfuric acid was added to the reaction mixture. The aqueous layer was extracted twice with diethyl ether, the organic layer was dried over magnesium sulfate and the solvent evaporated. The residue was purified by distillation under reduced pressure to give a colourless oil (50%); b.p. 120–130 °C (0.5 mbar); IR: 1740 (CO), 1705 (CO), 1638 (CO); ^1H NMR ($CDCl_3$): 1.39 (t, 3H, $J = 7.1$), 3.30 (m, 2H), 4.37 (q, 2H, $J = 7.1$), 4.50 (t, 2H, $J = 7.2$), 7.27 (m, 1H); MS (electronic impact): m/z 186 (M^+); Anal. Calcd. for $C_8H_{10}O_5$: C, 51.61; H, 5.41; O, 42.97; Found: C, 51.77; H, 5.52; O, 42.93.

(*2R,S*)-2-Amino-5-hydroxypentanoic Acid Acetate (5)

Lactone **4** (16.7 g, 90.0 mmol) was stirred under reflux in 2 N sulfuric acid (90 ml) until the end of gas release (3–5 h). The reaction mixture was alcalinised with concentrated sodium hydroxide, cooled in an ice bath and saturated with ammonia. The salts were filtered off and Raney nickel was added to the solution. The mixture was heated at 90 °C under 50 bars of hydrogen pressure. After 4 h, heating was stopped and the mixture was stirred at room temperature for 18 h. The solution was filtered, concentrated, and the residue was dissolved in ethanol. The salts were filtered off and the amino acid **5** was isolated after adding glacial acetic acid as a colourless solid (45%) which was used as such in the following reaction. m.p. 217–218 °C; IR: 3420 (OH), 1710 (CO); MS (electronic impact):

m/z 133 (M^+); Anal. Calcd. for $C_7H_{15}NO_5$: C, 43.52; H, 7.83; N, 7.25; Found: C, 43.60 ; H, 7.78; N, 7.41.

(2*R*,*S*)-5-Hydroxy-2-phthalimidopentanoic Acid (6)

Amino acid **5** (2.90 g, 15.0 mmol) was dissolved in aqueous sodium carbonate (1.5 M, 20 ml, 30.0 mmol). *N*-ethoxycarbonylphthalimide (3.30 g, 154 mmol) was then added and the reaction mixture was stirred at room temperature for 2 h. The salts were filtered off, the solution was cooled to 0 °C and acidified with 6 N hydrochloric acid. After extraction with chloroform, the organic layer was washed with water, dried, the solvent was evaporated, and the residue was purified by column chromatography (methylene chloride/methanol 95 : 5) to give a colourless solid (25%); m.p. 164–166 °C; IR: 3464 (OH), 1780 (CO), 1717 (CO); ^{1}H NMR ($CDCl_3$): 1.90–2.35 (m, 4H), 3.36 (s, 1H), 4.40 (m, 2H), 5.11 (m, 1H), 7.85–7.98 (m, 4H); MS (electronic impact): *m/z* 263 (M^+); Anal. Calcd. for $C_{13}H_{13}NO_5$: C, 59.31; H, 4.98; N, 5.32; Found: C, 59.18; H, 5.03; N, 5.39.

(3,4-Dihydro-2*H*-pyran-6-yloxy)trimethylsilane (7)

A solution of lithium diisopropylamide in tetrahydrofuran/heptane (2 M, 53 ml, 106 mmol) was diluted in 100 ml of tetrahydrofuran. The solution was cooled to −78 °C and δ-valerolactone (9.10 ml, 100 mmol) in 10 ml of tetrahydrofuran was added dropwise. The mixture was then stirred for 10 min. Chlorotrimethylsilane (21.6 ml, 170 mmol) was added in one batch and the mixture was stirred for 1 h at room temperature. The solvents were removed under vacuum. The residue was dissolved in anhydrous pentane. The salts were filtered off and the solvent was evaporated. The residue was purified by distillation under reduced pressure to give a colourless liquid, which was stored at 4 °C (60%); b.p. 72 °C (45 mbars); IR: 1688 (O-Si); ^{1}H NMR ($CDCl_3$): 0.18 (s, 9H), 1.50–2.26 (m, 4H), 3.70 (t, 1H, $J = 4.0$), 4.02 (t, 2H, $J = 4.5$); MS (electronic impact): *m/z* 172 (M^+); Anal. Calcd. for $C_8H_{16}O_2Si$: C, 55.77; H, 9.36; O, 18.57; Found: C, 55.61; H, 9.52; O, 18.52.

2-Bromo-δ-valerolactone (8)

Triethylamine (5.94 ml, 42.7 mmol) was added to a solution of enol ether **7** (6.39 g, 37.1 mmol) in 50 ml of methylene chloride and the mixture was cooled to −15 °C. A solution of bromine (1.91 ml, 37.1 mmol) in 10 ml of methylene chloride was added dropwise and the solution was stirred at −15 °C for 30 min. The reaction mixture was washed twice with saturated aqueous ammonium chloride, dried over magnesium sulfate and evaporated to dryness to give a yellow oil (95%); IR: 1740 (CO); ^{1}H NMR ($CDCl_3$): 1.84 (m, 1H), 2.13–2.53 (m, 3H), 4.34 (m, 1H), 5.54 (m, 2H); MS (electronic impact): *m/z* 179 (M^+); Anal. Calcd. for $C_5H_7BrO_2$: C, 33.55; H, 3.94; O, 17.87; Found: C, 33.41; H, 4.04; O, 17.89.

(2*R*,*S*)-2-Phthalimido-δ-valerolactone (9a)

2-Bromo-δ-valerolactone **8** (6.33 g, 35.4 mmol) and potassium phthalimidate (6.55 g, 35.4 mmol) were dissolved in 30 ml of xylene and the mixture was heated at reflux for 3 h. The solvent was removed under vacuum and the residue was dissolved in methylene chloride/water. The salts were filtered off. The organic layer was separated, washed with water, dried over magnesium sulfate and the solvent evaporated. The residue was purified by column chromatography (cyclohexane/ethyl acetate 6 : 4) to give a colourless solid which was re-crystallised from toluene (21%); m.p. 166–167 °C; IR: 1780 (CO), 1717 (CO); ^{1}H NMR ($CDCl_3$): 2.14 (m, 2H), 2.52 (m, 2H), 4.54 (t, 2H, $J = 6.1$), 4.93 (dd, 1H, $J = 7.5$, $J = 12.0$), 7.76 (dd, 2H, $J = 3.0$, $J = 5.4$), 7.98 (dd, 2H, $J = 3.0$, $J = 5.4$); MS (electronic impact): *m/z* 245 (M^+); Anal. Calcd. for $C_{13}H_{11}NO_4$: C, 63.67; H, 4.52; N, 5.71; Found: C, 63.79; H, 4.44; N, 5.58.

Ethyl ((2*R*,*S*)-(5-hydroxy-1-oxo-2-phthalimido)pentyl)phenylalaninate (10a)

Valerolactone **9a** (1.80 g, 7.35 mmol), ethyl phenylalaninate hydrochloride (2.53 g, 11.0 mmol) and sodium 2-ethylhexanoate (3.06 g, 18.4 mmol) were dissolved in 50 ml of anhydrous tetrahydrofuran. The mixture was stirred and heated at reflux in a nitrogen atmosphere for 36 h. The solvent was removed under vacuum. The residue was dissolved in ethyl acetate and the solution was washed successively with 1 N hydrochloric acid, saturated aqueous sodium hydrogen carbonate and brine. The organic layer was dried over magnesium sulfate and the solvent was evaporated. The residue was purified by column chromatography (cyclohexane/ethyl acetate 65 : 35) to give a colourless solid (50%); m.p. 197–199 °C; IR: 3364 (OH), 1776 (CO), 1715 (CO), 1610 (CO); ^{1}H NMR ($CDCl_3$): 1.24 (m, 3H), 1.46 (m, 2H), 2.23 (m, 2H), 3.16 (m, 2H), 3.54 (m, 2H), 4.15 (m, 2H), 4.69–4.97 (m, 2H), 6.78–7.29 (m, 6H), 7.73 (m, 2H), 7.85 (m, 2H); MS (electronic impact): *m/z* 438 (M^+); Anal. Calcd. for $C_{24}H_{26}N_2O_6$: C, 65.74; H, 5.98; N, 6.39; Found: C, 65.87; H, 5.86; N, 6.52.

Ethyl ((2*R*,*S*)-1,5-dioxo-2-phthalimidopentyl) phenylalaninate (11a)

A solution of oxalyl chloride (0.38 ml, 4.45 mmol) in 30 ml of anhydrous methylene chloride was cooled to −78 °C and a mixture of dry dimethyl sulfoxide (0.63 ml, 8.89 mmol) and methylene chloride (1.50 ml) was added dropwise in a nitrogen atmosphere. After 10 min, a solution of dipeptide **10a** (1.50 g, 3.42 mmol) in methylene chloride (15 ml) was added dropwise. After 15 min, triethylamine (1.91 ml, 13.7 mmol) was added and the mixture was stirred at −78 °C for 10 min and then warmed to 0 °C. The mixture was partitioned between ethyl acetate/ethyl ether (1 : 1) and water. The organic layer was washed successively with 1 N hydrochloric acid and brine, then dried over magnesium sulfate, filtered and concentrated. The residue was purified by column chromatography (cyclohexane/ethyl acetate 6 : 4) to give aldehyde **11a** as an oil (70%); IR: 2726 (CH), 1779 (CO), 1718 (CO), 1649 (CO), 1616 (CO); ^{1}H NMR ($CDCl_3$): 1.29 (m, 3H), 1.87 (m, 2H), 2.44 (m, 2H), 3.19 (m, 2H), 4.17 (m, 2H), 4.57–5.07 (m, 2H), 7.04–7.49 (m, 6H), 7.76 (m, 2H), 7.87 (m, 2H), 8.95 (s, 1H); MS (electronic impact): *m/z* 436 (M^+); Anal. Calcd. for $C_{24}H_{24}N_2O_6$: C, 66.05; H, 5.54; N, 6.42; Found: C, 65.92; H, 5.63; N, 6.49.

(3*R*,6(*R*,*S*),8a(*R*,*S*))-3-Benzyl-6-phthalimidoperhydropyrido(2,1-*b*)(1,3)oxazole-2,5-dione (15a)

A solution of aldehyde **11a** (2.62 g, 6.0 mmol) and trifluoroacetic acid (15.3 ml, 198 mmol) in chloroform (50 ml) was

refluxed for 6 days in a nitrogen atmosphere. The cooled solution was neutralised with saturated aqueous sodium bicarbonate and extracted twice with methylene chloride. The organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated. The residue was purified by column chromatography (cyclohexane/ethyl acetate 65 : 35) to give a colourless hygroscopic solid (65%); IR: 1803 (CO), 1719 (CO), 1678 (CO); ^{1}H NMR ($CDCl_3$): 1.65–2.40 (m, 4H), 3.33 (m, 2H), 4.43 (m, 1H), 4.74–4.91 (m, 2H), 7.32–7.51 (m, 5H), 7.78 (m, 2H), 7.93 (m, 2H); MS (electronic impact): m/z 390 (M^+); Anal. Calcd. for $C_{22}H_{18}N_2O_5$: C, 67.69; H, 4.65; N, 7.18; Found: C, 67.48; H, 4.71; N, 7.23.

(3(*R,S*),6*S*,11b(*R,S*))-1,3,4,6,7,11*b*-Hexahydro-4-oxo-3-phthalimidopyrido(2,1-*a*)isoquinoline-6-carboxylic Acid (2)

A solution of azlactone **15a** (1.29 g, 3.30 mmol) in 50 ml of methylene chloride was added to a cooled mixture (10 °C) of triflic acid (11.7 ml, 132 mmol) and triflic anhydride (2.78 ml, 16.5 mmol). The solution was stirred at room temperature for 21 h. The reaction mixture was poured into iced water and extracted with ethyl acetate. The organic extract was washed with water and brine, dried and concentrated. The residue was purified by column chromatography (methylene chloride/methanol 95 : 5) to give a colourless solid (45%); m.p. 152–154 °C; IR: 3566 (OH), 1778 (CO), 1716 (CO), 1653 (CO); ^{1}H NMR ($CDCl_3$): 2.11–2.56 (m, 4H, Hd, He), 3.28 (m, 2H, Hg), 4.87 (m, 1H, Hf), 4.97 (m, 1H, Hh), 5.05 (m, 1H, Hc), 7.02–7.31 (m, 4H, Hi), 7.66 (m, 2H, Hb), 7.84 (m, 2H, Ha); MS (electronic impact): m/z 390 (M^+), 346; Anal. Calcd. for $C_{22}H_{18}N_2O_5$: C, 67.69; H, 4.65; N, 7.18; Found: C, 67.51; H, 4.72; N, 7.27.

(2*R,S*)-2-Phthalimido-γ-butyrolactone (9b)

This lactone was prepared from 2-bromo-γ-butyrolactone (7.0 g, 42.4 mmol), as described for compound **9a**. Compound **9b** was purified by column chromatography (cyclohexane/ethyl acetate 75 : 25) to give a yellow solid which was recrystallised from toluene (35%); m.p. 173–175 °C; IR: 1784 (CO), 1768 (CO), 1714 (CO); ^{1}H NMR ($CDCl_3$): 2.55–2.66 (m, 1H), 2.79 (m, 1H), 4.41 (m, 1H), 4.65 (m, 1H), 5.11 (m, 1H), 7.71–7.79 (m, 2H), 7.85–7.92 (m, 2H); MS (chemical ionisation): m/z 232 (MH^+); Anal. Calcd. for $C_{12}H_9NO_4$: C, 62.34; H, 3.92; N, 6.06; Found: C, 62.26; H, 4.04, N, 5.98.

Ethyl ((2*R,S*)-(4-hydroxy-1-oxo-2-phthalimido) butyl)phenylalaninate (10b)

Butyrolactone **9b** (4.0 g, 17.3 mmol), ethyl phenylalaninate hydrochloride (5.95 g, 25.9 mmol) and sodium 2-ethylhexanoate (7.19 g, 43.2 mmol) were dissolved in 100 ml of anhydrous tetrahydrofuran. The mixture was stirred and heated at reflux in a nitrogen atmosphere for 60 h. The solvent was removed under vacuum. The residue was dissolved in ethyl acetate, the salts were filtered off and the solution was washed successively with 1 N hydrochloric acid, saturated aqueous sodium hydrogen carbonate and brine. The organic layer was dried over magnesium sulfate and the solvent was evaporated. The residue was purified by column chromatography (cyclohexane/ethyl acetate 4 : 6) to give a colourless oil (20%); IR: 3360 (OH), 1777 (CO), 1715 (CO), 1615 (CO); ^{1}H NMR ($CDCl_3$): 1.22 (m, 3H), 2.32 (m, 1H), 2.48 (m, 1H), 3.11 (m, 2H), 3.60 (m, 1H), 3.72 (m, 1H), 4.13 (q, 2H, $J = 7.1$), 4.83 (m, 1H), 5.03 (m, 1H), 6.96 (d, 1H, $J = 12.0$), 7.05–7.26 (m, 5H), 7.75 (m, 2H), 7.84 (m, 2H); MS (electronic impact): m/z 424 (M^+); Anal. Calcd. for $C_{23}H_{24}N_2O_6$: C, 65.08; H, 5.70; N, 6.60; Found: C, 64.70; H, 6.03; N, 6.48.

Ethyl ((2*R,S*)-1,4-dioxo-2-phthalimidobutyl) phenylalaninate (11b)

The aldehyde **11b** was prepared from **10b** (0.50 g, 1.18 mmol), as described for compound **11a**. Compound **11b** was purified by column chromatography (cyclohexane/ethyl acetate 75 : 25) to give an oil (30%); IR: 1774 (CO), 1715 (CO), 1649 (CO); ^{1}H NMR ($CDCl_3$): 1.33 (m, 3H), 1.48–1.59 (m, 2H), 3.31 (m, 2H), 4.23 (m, 2H), 4.69–4.98 (m, 1H), 5.20–5.33 (m, 1H), 7.21–7.39 (m, 6H), 7.73–7.77 (m, 2H), 7.85–7.89 (m, 2H); MS (electronic impact): m/z 422 (M^+); Anal. Calcd. for $C_{23}H_{22}N_2O_6$: C, 65.40; H, 5.25; N, 6.63; Found: C, 65.56; H, 5.17; N, 6.45.

(2*R,S*)-2-dibenzylamino-γ-butyrolactone (12)

Triethylamine (7 ml, 50.0 mmol) was added to a solution of dibenzylamine (4.80 g, 25.0 mmol) in 30 ml of dimethylformamide and the mixture was stirred at 0 °C for 30 min. A solution of 2-bromo-γ-butyrolactone (2.10 ml, 25.0 mmol) in 10 ml of dimethylformamide was added dropwise and the reaction mixture was stirred for 2 h at 0 °C. The solvent was removed under vacuum. The residue was dissolved in methylene chloride and washed with 5% aqueous sodium hydrogen carbonate and water. The organic layer was dried over magnesium sulfate and the solvent was evaporated. The residue was purified by column chromatography (cyclohexane/ethyl acetate 85 : 15) to give a colourless solid (50%); IR: 1763 (CO); ^{1}H NMR (DMSO-d_6): 2.25–2.35 (m, 2H), 3.70 (d, 2H, $J = 13.9$), 3.78–3.82 (m, 1H), 3.95 (d, 2H, $J = 13.9$), 4.06–4.15 (m, 1H), 4.28–4.39 (m, 1H), 7.20–7.56 (m, 10H); MS (chemical ionisation): m/z 282 (MH^+); Anal. Calcd. for $C_{18}H_{19}NO_2$: C, 76.84; H, 6.81; N, 4.98; Found: C, 76.85; H, 6.76; N, 4.92.

Ethyl ((2*R,S*)-(2-dibenzylamino-4-hydroxy-1-oxo)butyl)phenylalaninate (13)

Butyrolactone **12** (4.0 g, 14.2 mmol), ethyl phenylalaninate hydrochloride (4.90 g, 21.3 mmol) and sodium 2-ethylhexanoate (5.90 g, 35.5 mmol) were dissolved in 100 ml of anhydrous tetrahydrofuran. The mixture was stirred and heated at reflux in a nitrogen atmosphere for 72 h. The solvent was removed under vacuum. The residue was dissolved in chloroform, the salts were filtered off and the solution was washed successively with 1 N hydrochloric acid, saturated aqueous sodium hydrogen carbonate and brine. The organic layer was dried over magnesium sulfate and the solvent was evaporated. The residue was purified by column chromatography (cyclohexane/ethyl acetate 6 : 4) to give a yellow oil (45%); IR: 3367 (OH), 1738 (CO), 1668 (CO); ^{1}H NMR (DMSO-d_6): 1.09–1.16 (m, 3H), 1.24–1.32 (m, 2H), 2.17–2.25 (m, 2H), 3.04–3.71 (m, 6H), 3.80–3.89 (m, 2H), 4.20–4.28 (m, 1H), 4.79–4.89 (m, 1H), 7.03–7.41 (m, 15H), 7.95 (d, 0.5H, $J = 7.0$), 8.05 (d, 0.5H, $J = 7.0$); MS (chemical ionisation): m/z 475 (MH^+); Anal. Calcd. for $C_{29}H_{34}N_2O_4$: C, 73.39; H, 7.22; N, 5.90; Found: C, 73.48; H, 7.22; N, 5.91.

Ethyl ((2*R*,*S*)-2-dibenzylamino-1,4-dioxobutyl)-phenylalaninate (14)

The aldehyde **14** was prepared from alcohol **13** (0.50 g, 1.06 mmol), as described for compound **11a**. Aldehyde **14** was purified by column chromatography (cyclohexane/ethyl acetate 7 : 3) to give an oil (20%); IR: 1739 (CO), 1697 (CO), 1685 (CO); ^{1}H NMR ($CDCl_3$): 1.66–1.70 (m, 3H), 2.04–2.07 (m, 2H), 3.21–3.24 (m, 2H), 4.19–4.26 (m, 2H), 4.33–4.39 (m, 1H), 4.92–5.04 (m, 5H), 5.70–5.89 (m, 1H), 7.12–7.53 (m, 15H), 9.73 (d, 1H, $J = 7.2$); MS (chemical ionisation): m/z 473 (MH^+); Anal. Calcd. for $C_{29}H_{32}N_2O_4$: C, 73.71; H, 6.83; N, 5.93; Found: C, 73.67; H, 6.84; N, 5.97.

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