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Tetrahedron: *Asymmetry* 17 (2006) 1521–1528
Tetrahedron: *Asymmetry*

Stereoselective synthesis of a new chiral synthon: a cyclic pseudodipeptide containing an aspartic acid derivative and L-valine

Daniele Balducci, Alessandro Grandi, Gianni Porzi* and Sergio Sandri*

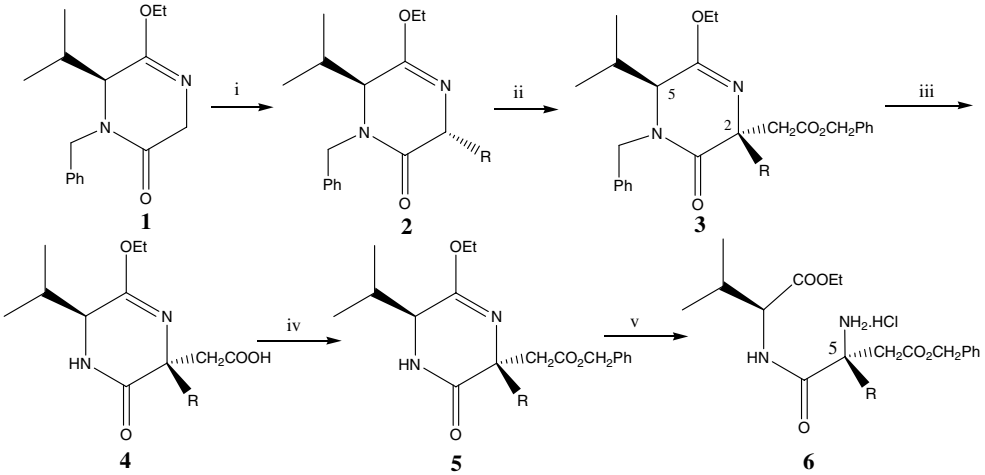
Dipartimento di Chimica 'G.Ciamician', Università di Bologna, Via Selmi 2, 40126 Bologna, Italy

Received 29 March 2006; accepted 22 May 2006

Abstract—A simple stereoselective synthesis of cyclic synthons **4a**,**b** and **10a**,**b**, derived from an L-valine unit and an unnatural derivative of the aspartic acid, has been accomplished starting from the chiral synthon **1** and following the procedure already reported by us for the synthesis of similar compounds. These cyclic synthons are interesting substrates because they can behave as both electrophiles and nucleophiles and could be useful starting materials for the preparation of higher peptides such as, for instance, the unnatural tetrapeptides **15** and **18**, through the condensation of **4a** with **6a** or **13** with **17**, respectively.

1. Introduction

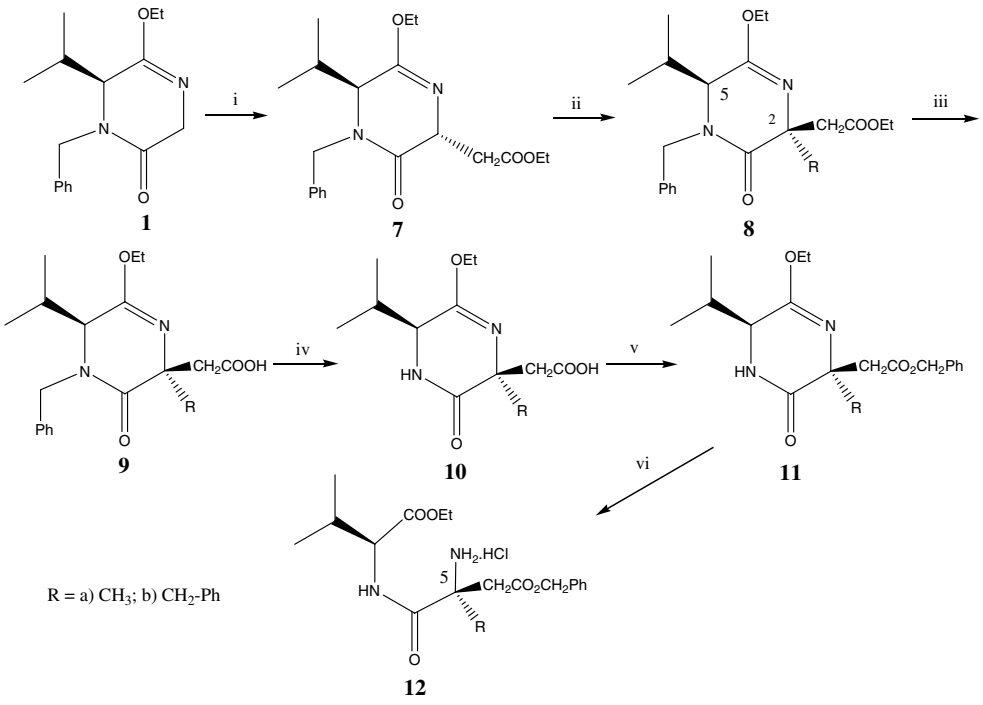
Previously, we reported the stereocontrolled synthesis of unnatural di- and tripeptides containing an L-valine unit and a cyclic nonproteinogenic α-aminoacid.[1,2] As a continuation of our studies in this area, we herein report a simple stereoselective synthesis of cyclic synthons **4** (Scheme 1) and **10** (Scheme 2) derived from an L-valine unit and unnatural α-aminoacids derivatives of aspartic acid. In our opinion, these diastereomeric synthons, which can be



Scheme 1. Reagents and conditions: (i) LHMDS/THF, R–X; (ii) LHMDS/THF, $BrCH_2CO_2CH_2Ph$; (iii) Li/NH_3; (iv) $Br–CH_2Ph$, Et_3N in acetone; (v) 0.5 M HCl.

* Corresponding authors. Fax: +39 051 2099512; e-mail: gianni.porzi@unibo.it

0957-4166/$ - see front matter
doi:10.1016/j.tetasy.2006.05.021



Scheme 2. Reagents and conditions: (i) LHMDS/THF, $BrCH_2CO_2Et$; (ii) LHMDS/THF, R–X; (iii) NaOH/EtOH; (iv) Li/NH_3; (v) $Br–CH_2Ph$, Et_3N in acetone; (vi) 0.5 M HCl.

considered as masked unnatural dipeptides, are interesting because they can act as both electrophiles and nucleophiles and thus be useful as starting materials for preparing higher peptides. When synthon **4** or **10** is converted into the corresponding activated ester (for instance, a pentafluorophenyl-ester), it can be used as an electrophile to accomplish a coupling reaction with a C-protected peptide. As shown, we synthesized pseudotetrapeptide **15** through the condensation of synthon **4a** with pseudodipeptide **6a** (Scheme 3). Analogously, we synthesized the masked pseudotetrapeptide **18** by using the unnatural dipeptide **17**, easily obtained from the intermediate **2a**, as a nucleophile (Scheme 4). Alternatively, by converting the diastereomeric synthon **4** or **10** into **6** or **12**, respectively, we obtained unnatural dipeptides, which can be used as nucleophiles by reaction with an N-protected α-aminoacid or peptide.

Thus, we believe that such new synthons, **4** and **10**, can be considered as masked unnatural dipeptides, useful starting materials for the synthesis of more complex structures.

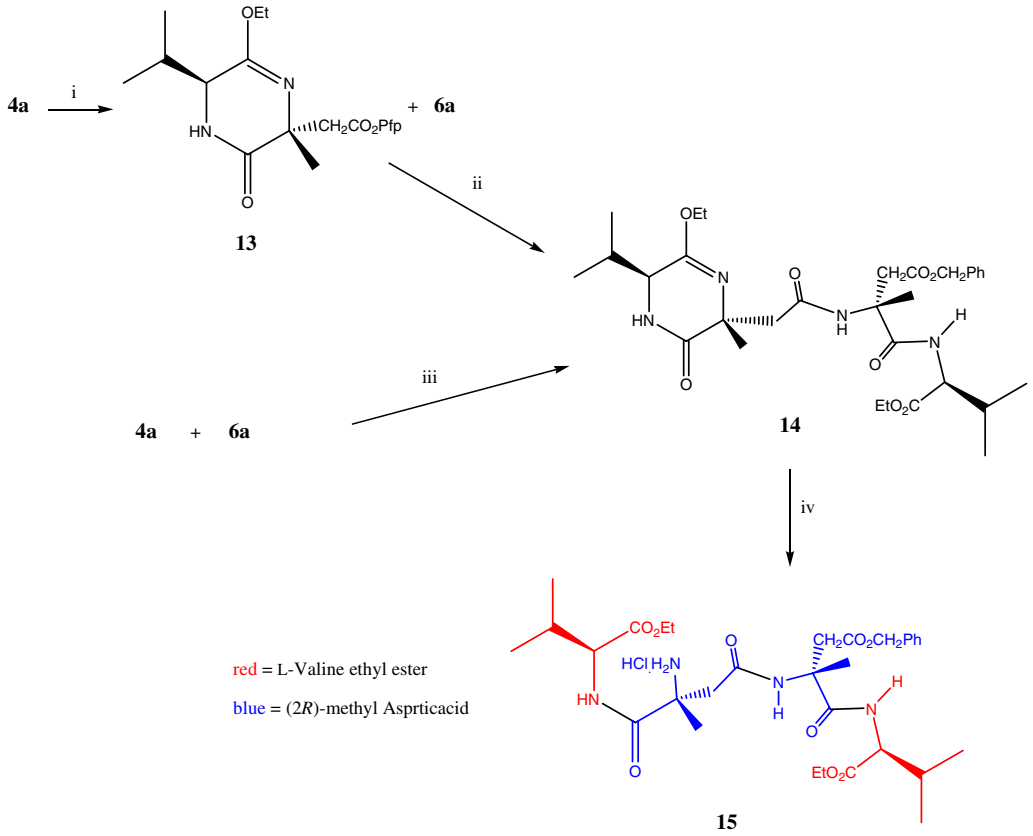
The strategy followed to synthesize the chiral synthons **4** and **10** is based on the experience previously acquired in analogous stereoselective approaches,[3–6] that is starting from the chiral mono-lactim ether **1** easily obtained from L-valine. Herein we report a simple stereoselctive route occurring in good chemical yield and stereochemical control.

2. Results and discussion

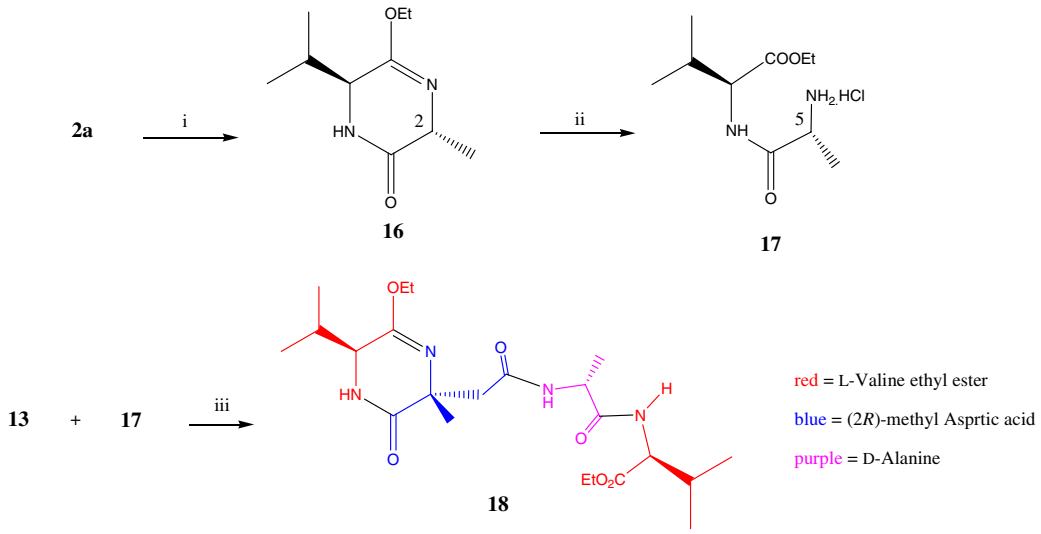
To synthesize chiral synthons **4** and **10**, we followed the strategy based on the use of the mono-lactim ether **1**, which can be easily alkylated twice at the C-2 position with suitable electrophiles. Deprotonation of **1** with LHMDS at −78 °C, followed by alkylation with the appropriate haloderivative (iodomethane or benzylbromide), afforded diastereomer **2a**,**b** in good chemical yield and with a largely prevalent (de 40% for **2a** and 80% for **2b**) 1,4-*trans* stereoselection (ascertained by NOE measurements) with respect to the isopropyl group, as already observed[1–6] in analogous cases (Scheme 1).

Monoalkyl intermediate **2a**,**b**, was submitted to a second alkylation by using benzylbromoacetate as electrophile, to give derivative **3a**,**b**. The reaction occurred in good yield and with practically total regio- and 1,4-*trans*-selection (de ⩾96%), which was ascertained by NOE experiments. After removal of the benzyl groups by the Birch reaction, intermediate **3a**,**b** was converted into the chiral synthon **4a**,**b**, which afforded the corresponding benzyl ester **5a**,**b** after esterification with benzylbromide. Subsequent acid hydrolysis under mild conditions allowed us to obtain the pseudodipeptide **6a**,**b** as the hydrochloride salt.

Diastereomeric pseudodipeptides **12a**,**b**, with an opposite configuration at the C-5 stereocentre with respect to **6a**,**b**, were obtained by inverting the alkylation sequence described above. In the strategy reported in Scheme 2, we employed ethylbromoacetate because in this case the alkylation of **1** with benzylbromoacetate occurred in an inexplicably poor yield. Then, ester **7**, which was obtained in good yield and 70% de, was alkylated with either iodomethane or benzylbromide affording **8a**,**b** with a de >96%. Intermediate ester **8a**,**b** was hydrolyzed in an alkaline medium to **9a**,**b** and the benzyl group then removed by a Birch reaction. Acid derivative **10a**,**b** was converted into the



Scheme 3. Reagents and conditions: (i) pentafluorophenyl trifluoroacetate (CF_3CO_2Pfp) in CH_2Cl_2, pyridine; (ii) **6a** in refluxing THF; (iii) HBTU in CH_3CN, Et_3N at rt; (iv) 0.5 M HCl in EtOH at rt.



Scheme 4. Reagents and conditions: (i) Li/NH_3; (ii) 0.5 M HCl, EtOH at rt; (iii) dry THF, Et_3N at rt.

benzylester **11a**,**b** and then the pseudodipeptide **12a**,**b** was obtained as a hydrochloride salt after acid hydrolysis of the lactim ether function under mild conditions.

In order to show that synthon **4** (a masked unnatural dipeptide) can be considered a useful starting material to synthesize more complex structures, we performed a coupling reaction between **4a** and the C protected pseudodipeptide **6a**. For this purpose, we converted **4a** into the corresponding activated ester **13** by treatment with pentafluorophenyl trifluoroacetate in the presence of pyridine. However, the subsequent coupling reaction occurred very slowly also in refluxing THF, and the product was recovered in an unsatisfactory yield, in addition to other

by-products being generated. Conversely, intermediate **14** was obtained in a satisfactory yield by carrying out the reaction directly on **4a** and by employing the activating reagent HBTU in CH_3CN at room temperature.[7] After acid hydrolysis of lactim derivative **14** under mild conditions, pseudotetrapeptide **15**, containing two L-valine and two (2*R*)-methyl-aspartic acid units, was then recovered (Scheme 3).

It is interesting to note that using reaction of the activated ester **13** with the nucleophile **17** (obtained from **2a** through the Birch reaction and successive acid hydrolysis), the masked pseudotetrapeptide **18** [containing two L-valine units, one (2*R*)-methyl-aspartic acid and one D-alanine] was obtained in good yield (Scheme 4).

In our opinion, the different reactivity observed for the nucleophiles **6a** and **17** towards the activated ester **13** can be ascribed to steric factors: in fact, the two nucleophiles show a considerable difference in the steric hindrance near the amine group.

The pseudotetrapeptide **15** thus obtained is unusual because it is C-terminal at both the ends of the chain, while the two (2*R*)-methyl-aspartic acid (an unnatural α-aminoacid) units are linked to each other.

Finally, we believe that our simple protocol, utilized to synthesize small peptidomimetic structures, provides interesting access to compounds, which could exhibit therapeutic effects similar to natural peptides with the advantage of metabolic stability.[8]

3. Experimental

3.1. General information

^1H and ^{13}C NMR spectra were recorded on a Gemini spectrometer at 300 MHz using $CDCl_3$ as the solvent. Chemical shifts are reported in parts per million relative to $CDCl_3$ and the coupling constants (*J*) are in hertz. IR spectra were recorded on a Nicolet 210 spectrometer. Optical rotation values were measured at 25 °C on a Perkin–Elmer 343 polarimeter. Dry THF was distilled from sodium benzophenone ketyl. Chromatographic separations were performed with silica gel 60 (230–400 mesh). Mass spectra were obtained with HPLC Agilent technologies HP1100 series equipped with diode array detector. MS analysis was obtained by using a Gemini 3U C18 110A column, H_2O/CH_3CN as solvent at 25 °C in a linear gradient from 70/30 to 20/80, respectively, and a flow rate of 0.4 mL/min. The mass revelator was a Hewlett Packard 1100 MSD series equipped with API-ES interface and a single quadruple detector.

3.1.1. (2*R*,5*S*)-4-Benzyl-6-ethoxy-5-isopropyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-pyrazine 2a. Synthesis and spectroscopic data are reported in Ref. 6.

3.1.2. (2*R*,5*S*)-2,4-Dibenzyl-6-ethoxy-5-isopropyl-3-oxo-2,3,4,5-tetrahydro-pyrazine 2b. Compound **2b** was synthesized by alkylating **1** with benzylbromide and following the procedure used for **2a**. After purification by silica gel chromatography, eluting with hexane/ethyl acetate, the product was recovered as an oil in 85% yield. ^1H NMR δ: 0.92 (d, 3H, $J = 7$); 1.02 (d, 3H, $J = 7.9$); 1.26 (t, 3H, $J = 7$); 2.20 (m, 1H); 3.37 (d, 1H, $J = 5.1$); 3.51 (dd, 1H, $J = 1.8, 3.3$); 3.82 (d, 1H, $J = 15$); 4.16 (m, 2H); 4.40 (m, 1H); 5.51 (d, 1H, $J = 15$); 6.82 (m, 2H); 7.30 (m, 8ArH). ^{13}C NMR δ: 13.9, 17.0, 19.6, 31.1, 39.3, 46.4, 59.0, 60.7, 61.0, 125.7, 127.0, 127.3, 127.4, 128.3, 130.4, 135.6, 138.1, 158.3, 169.1. HPLC–MS: 365.2 $[M+H]^+$, 387.2 $[M+Na]^+$, 751.4 $[2M+Na]^+$. IR ($CHCl_3$): 1648.3 cm^{-1} ($\nu_{C=O}$), 1693 cm^{-1} ($\nu_{C=N}$). The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

3.1.3. (2*R*,5*S*)-(4-Benzyl-6-ethoxy-5-isopropyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid benzylester 3a. To a solution of **2a** (14.35 g, 49.8 mmol) in dry THF (150 mL) and cooled at −78 °C, a solution of 1 M LHMDS in THF (50 mL, 50 mmol) was added dropwise under stirring. After about 1 h, benzylbromoacetate (9 mL, 56.8 mmol) was added and the reaction mixture kept under stirring at −78 °C until the reaction was practically complete (about 3 h). Then, water and ethyl acetate were added and after separation the organic solution was evaporated in vacuo. The residue was submitted to purification by silica gel chromatography, eluting with hexane/ethyl acetate and the pure product was recovered as an oil in 88% yield. ^1H NMR δ: 0.89 (d, 3H, $J = 7$); 1.05 (d, 3H, $J = 7$); 1.18 (t, 3H, $J = 7$); 1.53 (s, 3H); 2.2 (m, 1H); 2.71 (d, 1H, $J = 15.8$); 3.4 (d, 1H, $J = 15.8$); 3.73 (d, 1H, $J = 2.6$); 3.79–4.07 (m, 2H); 4.18 (d, 1H, $J = 15$); 5.05 (q_{AB}, 2H, $J = 12.4$); 5.25 (d, 1H, $J = 15$); 7.33 (m, 10ArH). ^{13}C NMR δ: 13.9, 17.0, 20.5, 29.0, 29.3, 46.7, 47.0, 58.6, 60.7, 61.7, 65.7, 127.3, 127.9, 128.2, 128.3, 128.4, 128.5, 135.9, 136.0, 156.9, 170.4, 171.6. $[\alpha]_D = -6.8$ (*c* 1.4, $CHCl_3$). HPLC–MS: 437.5 $[M+H]^+$, 459.5 $[M+Na]^+$, 475.5 $[M+K]^+$, 896 $[2M+Na]^+$. IR ($CHCl_3$): 1647.5 cm^{-1} ($\nu_{C=O}$), 1694 cm^{-1} ($\nu_{C=N}$), 1738 cm^{-1} ($\nu_{C=O}$). Anal. Calcd for $C_{26}H_{32}N_2O_4$: C, 71.53; H, 7.39; N, 6.42. Found: C, 71.85; H, 7.37; N, 6.4.

3.1.4. (2*R*,5*S*)-(2,4-Dibenzyl-6-ethoxy-5-isopropyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid benzylester 3b. Compound **3b** was obtained starting from **2b**, following the procedure used for **3a**. The product was recovered pure as a wax in 90% yield with elution by silica gel chromatography with hexane/ethyl acetate. ^1H NMR δ: 0.33 (d, 3H, $J = 7$); 0.95 (d, 3H, $J = 7.0$); 1.22 (t, 3H, $J = 7$); 1.97 (m, 1H); 2.68 (d, 1H, $J = 16.2$); 3.06 (d, 1H, $J = 12.8$); 3.28 (d, 1H, $J = 12.8$); 3.35 (d, 1H, $J = 16.2$); 3.68 (d, 1H, $J = 2.2$); 4.0 (m, 2H); 4.37 (d, 1H, $J = 15$); 4.86 (d, 1H, $J = 15$); 5.01 (q_{AB} 2H, $J = 12.6$); 7.30 (m, 15 ArH). ^{13}C NMR δ: 13.8, 15.0, 20.5, 29.0, 45.3, 46.8, 48.0, 60.4, 62.2, 62.4, 65.6, 126.4, 126.8, 127.5, 127.8, 127.9, 128.0, 128.1, 131.0, 135.6, 135.9, 136.3, 157.6, 170.1, 170.5. $[\alpha]_D = -26.3$ (*c* 1, $CHCl_3$). HPLC–MS: 513.5 $[M+H]^+$, 535.5 $[M+Na]^+$, 1048 $[2M+Na]^+$. IR ($CHCl_3$): 1647.5 cm^{-1} ($\nu_{C=O}$), 1694 cm^{-1} ($\nu_{C=N}$), 1738 cm^{-1} ($\nu_{C=O}$). Anal. Calcd for $C_{32}H_{36}N_2O_4$: C, 74.97; H, 7.08; N, 5.46. Found: C, 75.21; H, 7.06; N, 5.45.

3.1.5. (2*R*,5*S*)-(6-Ethoxy-5-isopropyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid 4a. A solution of **3a** (5.28 g, 12.11 mmol) in 30 mL of dry THF/*tert*-butanol 9:1 was added to about 100 mL of liquid ammonia cooled at −50 °C. Then, Li (0.34 g, 48.44 mmol) was added in small pieces and the reaction monitored by TLC. The addition of Li was stopped as soon as the reaction mixture became blue, the starting material having disappeared. The reaction was then quenched with NH_4Cl and the cooling bath removed to allow the complete evaporation of NH_3. After the addition of water and CH_2Cl_2, the aqueous solution was acidified to pH = 4 with dilute HCl and the organic solution evaporated to dryness under vacuum. The pure product was recovered as a wax in 84% yield. ^{1}H NMR δ: 0.89 (d, 3H, $J = 6.9$); 1.02 (d, 3H, $J = 6.9$); 1.29 (t, 3H, $J = 7.2$); 1.47 (s, 3H); 2.30 (m, 1H); 2.30 (m, 1H); 2.73 (d, 1H, $J = 16$); 3.05 (d, 1H, $J = 16.5$); 4.0 (m, 1H); 4.12 (m, 2H); 7.37 (s, 1H). ^{13}C NMR δ: 14.0, 16.1, 18.5, 28.6, 30.5, 30.9, 45.3, 57.8, 58.7, 61.5, 157.7, 174.3, 174.9. $[\alpha]_D = -9.2$ (*c* 0.6, $CHCl_3$). HPLC–MS: 257.5 $[M+H]^+$, 536 $[2M+Na]^+$. IR ($CHCl_3$): 1650–1750 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=N}$), 2400–3300 cm^{-1} (broad, ν_{OH}), 3403 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{12}H_{20}N_2O_4$: C, 56.23; H, 7.87; N, 10.93. Found: C, 55.98; H, 7.89; N, 10.95.

3.1.6. (2*R*,5*S*)-(2-Benzyl-6-ethoxy-5-isopropyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid 4b. Compound **4b** was obtained starting from **3b** and following the procedure used for **4a**. The product was recovered pure as a wax in 85% yield. ^{1}H NMR δ: 0.01 (d, 3H, $J = 7$); 0.70 (d, 3H, $J = 7$); 1.25 (t, 3H, $J = 7$); 1.95 (m, 1H); 2.72 (d, 1H, $J = 16.8$); 2.86 (d, 1H, $J = 12.4$); 3.19 (d, 1H, $J = 16.8$); 3.31 (d, 1H, $J = 12.4$); 3.80 (br s, 1H); 4.13 (q, 2H, $J = 7$); 7.18 (m, 5ArH); 7.53 (br s, 1H). ^{13}C NMR δ: 14.2, 14.5, 18.4, 29.3, 44.8, 46.2, 58.2, 61.1, 62.4, 126.6, 127.8, 131.0, 135.9, 158.3, 174.2, 175.0. $[\alpha]_D = +3.2$ (*c* 1.4, $CHCl_3$). HPLC–MS: 333.3 $[M+H]^+$, 455.3 $[M+Na]^+$, 687.6 $[2M+Na]^+$. IR ($CHCl_3$): 1650–1750 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=N}$), 2400–3300 cm^{-1} (broad, ν_{OH}), 3400 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{18}H_{24}N_2O_4$: C, 65.04; H, 7.28; N, 8.43. Found: C, 65.25; H, 7.26; N, 8.41.

3.1.7. (2*R*,5*S*)-(6-Ethoxy-5-isopropyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid benzyl ester 5a. To a solution of **4a** (2.83 g, 11.05 mmol) in acetone (30 mL) and Et_3N (1.32 mL, 11.05 mmol), benzylbromide (1.44 mL, 12.16 mmol) was added and the reaction mixture stirred at room temperature for about 30 h. The reaction mixture was evaporated to dryness under vacuum and to the residue was added water and ethylacetate. The organic solution was evaporated in vacuo and the residue was submitted to silica gel chromatography, eluting with hexane/ethyl acetate. The pure product was recovered as an oil in 70% yield. ^{1}H NMR δ: 0.85 (d, 3H, $J = 7$); 0.94 (d, 3H, $J = 7$); 1.23 (t, 3H, $J = 7$); 1.45 (s, 3H); 2.25 (m, 1H); 2.67 (d, 1H, $J = 16.2$); 3.28 (d, 1H, $J = 16.2$); 3.74 (m, 1H); 4.03 (m, 2H); 5.08 (q_{AB}, 2H, $J = 12.2$); 5.58 (br s, 1H); 7.32 (m, 5ArH). ^{13}C NMR δ: 13.5, 15.8, 17.8, 28.6, 30.1, 45.3, 57.7, 58.1, 60.3, 65.3, 127.5, 127.7, 127.8, 135.5, 157.2, 169.8, 173.5. $[\alpha]_D = -12.8$ (c 0.6, $CHCl_3$). HPLC–MS: 347.5 $[M+H]^+$, 369.5 $[M+Na]^+$, 716 $[2M+Na]^+$. IR ($CHCl_3$): 1645.5 cm^{-1} ($\nu_{C=O}$), 1694.7 cm^{-1} ($\nu_{C=N}$), 1738.5 cm^{-1} ($\nu_{C=O}$), 3405 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{19}H_{26}N_2O_4$: C, 65.87; H, 7.56; N, 8.09. Found: C, 65.74; H, 7.59; N, 8.11.

3.1.8. (2*R*,5*S*)-(2-Benzyl-6-ethoxy-5-isopropyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid benzyl ester 5b. Compound **5b** was obtained starting from **4c**, and following the procedure used for **5a**. The product was recovered pure as an oil in 70% yield. ^{1}H NMR δ: 0.05 (d, 3H, $J = 7$); 0.68 (d, 3H, $J = 7$); 1.24 (t, 3H, $J = 7$); 1.95 (m, 1H); 2.74 (d, 1H, $J = 16.2$); 2.87 (d, 1H, $J = 12.8$); 3.33 (d, 1H, $J = 12.8$); 3.34 (d, 1H, $J = 16.2$); 3.68 (m, 1H); 4,08 (m, 2H); 5.07 (s, 2H); 5.69 (br s, 1H); 7.25 (m, 10ArH). ^{13}C NMR δ: 14.2, 14.6, 18.1, 29.5, 45.5, 46.6, 58.3, 61.1, 62.9, 66.2, 126.6, 127.3, 128.3, 128.5, 131.0, 135.7, 136.0, 158.2, 170.6, 171.9. $[\alpha]_D = +2.7$ (*c* 0.7, $CHCl_3$). HPLC–MS: 423.5 $[M+H]^+$, 445.5 $[M+Na]^+$, 868 $[2M+Na]^+$. IR ($CHCl_3$): 1645 cm^{-1} ($\nu_{C=O}$), 1694.4 cm^{-1} ($\nu_{C=N}$), 1738 cm^{-1} ($\nu_{C=O}$), 3404 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{25}H_{30}N_2O_4$: C, 71.07; H, 7.16; N, 6.63. Found: C, 71.35; H, 7.13; N, 6.61.

3.1.9. Dipeptide [(EtO)Val-(2*R*)-methyl-Asp(OBn)]·HCl 6a. To a solution of **5a** (0.84 g, 2.42 mmol) in ethanol (10 mL), 0.5 M HCl (10 mL, 5 mmol) was added and the reaction mixture stirred at room temperature for 20 h. The acid solution was evaporated in vacuo and the intermediate hydrochloride was isolated as a wax in quantitative yield. ^{1}H NMR δ: 0.92 (m, 6H); 1.24 (t, 3H, $J = 7$); 1.88 (s, 3H); 2.22 (m, 1H); 3.35 (br s, 2H); 4.18 (m, 2H); 4.48 (dd, 1H, $J = 5.1, 7.8$); 5.14 (s, 2H); 7.37 (m, 5ArH); 8.13 (d, 1H, $J = 7.8$); 8.99 (br s, 3H). ^{13}C NMR δ: 14.0, 18.0, 18.8, 22.8, 30.7, 39.8, 58.2, 58.9, 61.3, 67.2, 128.2, 128.3, 128.4, 134.8, 169.5, 171.2, 171.3. $[\alpha]_D = -9.6$ (*c* 0.7, $CHCl_3$). HPLC–MS: 365.4 $[M-HCl+H]^+$, 387.4 $[M-HCl+Na]^+$, 729.8 $[2M-HCl+H]^+$. IR ($CHCl_3$): 1682.5 cm^{-1} ($\nu_{C=O}$), 1734 cm^{-1} ($\nu_{C=O}$), 3000 cm^{-1} ($\nu_{NH_3^+}$), 3420 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{19}H_{29}ClN_2O_5$: C, 56.92; H, 7.29; N, 6.99. Found: C, 57.11; H, 7.28; N, 6.97.

3.1.10. Dipeptide [(EtO)Val-(2*R*)-benzyl-Asp(OBn)]·HCl 6b. Compound **6b** was obtained starting from **5b**, following the procedure used for **6a**. The product was recovered pure as a wax in quantitative yield. ^{1}H NMR δ: 0.92 (m, 6H); 1.34 (t, 3H, $J = 7$); 2.17 (m, 1H); 3.04 (d, 1H, $J = 18.2$); 3.30 (s, 2H); 3.64 (d, 1H, $J = 18.2$); 4.30 (m, 3H); 5.22 (s, 2H); 7.40 (m, 10ArH). ^{13}C NMR δ: 14.9, 19.2, 19.8, 31.9, 39.7, 43.0, 60.5, 62.7, 62.9, 68.9, 129.7, 130.0, 130.1, 130.4, 132.0, 133.6, 136.9, 170.8, 171.3, 173.0. HPLC–MS: 441.5 $[M-HCl+H]^+$, 463.5 $[M-HCl+Na]^+$, 882 $[2M-HCl+H]^+$. IR ($CHCl_3$): 1680.2 cm^{-1} ($\nu_{C=O}$), 1738 cm^{-1} ($\nu_{C=O}$), 2980 cm^{-1} ($\nu_{NH_3^+}$), 3425 cm^{-1} (ν_{NH}). The product was not isolated in sufficiently pure form for the elemental analysis or to measure the specific rotation.

3.1.11. (2*R*,5*S*)-(4-Benzyl-6-ethoxy-5-isopropyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid ethyl ester 7. To a solution of **1** (7.4 g, 27 mmol) in dry THF (100 mL) and cooled at −78 °C, a solution of 1 M LHMDS in THF (27 mL, 27 mmol) was added dropwise under stirring. After about 1 h, ethylbromoacetate (3 mL, 27 mmol) was

added, then the reaction mixture was allowed to warm up to room temperature and kept under stirring, until the reaction was practically complete (overnight). After the addition of water and ethyl acetate, the organic solution was separated and then evaporated in vacuo. The residue was submitted to purification by silica gel chromatography, eluting with hexane/ethyl acetate. The pure product was recovered as an oil in 80% yield. ^{1}H NMR δ: 0.98 (d, 3H, $J = 7$); 1.08 (d, 3H, $J = 7$); 1.24 (t, 3H, $J = 7$); 1.28 (t, 3H, $J = 7$); 2.26 (m, 1H); 2.95 (dd, 1H, $J = 6.3$, 15.9); 3.08 (dd, 1H, $J = 5.7$, 15.9); 3.72 (dd, 1H, $J = 1.8$, 4.5); 3.97 (d, 1H, $J = 15$); 4.02–4.30 (m, 4H); 4.48 (m, 1H); 5.51 (d, 1H, $J = 15$); 7.31 (m, 5ArH). ^{13}C NMR δ: 13.7, 13.8, 17.3, 19.6, 31.2, 38.7, 47.0, 55.1, 59.7, 60.9, 61.9, 127.2, 127.5, 128.3, 135.8, 159.5, 169.1, 171.1. $[\alpha]_D = +31.2$ (c 0.6, $CHCl_3$). HPLC–MS: 361.5 $[M+H]^+$, 383.5 $[M+Na]^+$, 744 $[2M+Na]^+$. IR ($CHCl_3$): 1646.5 cm^{-1} ($\nu_{C=O}$), 1695.5 cm^{-1} ($\nu_{C=N}$), 1740 cm^{-1} ($\nu_{C=O}$). Anal. Calcd for $C_{20}H_{28}N_2O_4$: C, 66.64; H, 7.83; N, 7.77. Found: C, 66.45; H, 7.86; N, 7.79.

3.1.12. (2*S*,5*S*)-(4-Benzyl-6-ethoxy-5-isopropyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid ethyl ester 8a. Compound **8a** was obtained by the alkylation of **7** with CH_3I and following the procedure used to obtain **7**. The product was recovered pure as an oil in 85% yield, after elution by silica gel chromatography with hexane/ethyl acetate. ^{1}H NMR δ: 0.95 (d, 3H, $J = 7$); 1.08 (d, 3H, $J = 7$); 1.25 (m, 6H); 1.58 (s, 3H); 2.23 (m, 1H); 2.82 (br s, 2H); 3.75 (d, 1H, $J = 2.2$); 4.01 (d, 1H, $J = 15$); 4.16 (m, 4H); 5.46 (d, 1H, $J = 15$); 7.30 (m, 5ArH). ^{13}C NMR δ: 13.5, 13.7, 16.2, 20.1, 29.3, 29.4, 45.1, 46.3, 58.6, 59.5, 60.3, 61.0, 127.0, 127.4, 128.2, 135.9, 155.7, 169.7, 171.0. $[\alpha]_D = -3.6$ (c 0.5, $CHCl_3$). HPLC–MS: 375.5 $[M+H]^+$, 772 $[2M+Na]^+$. IR ($CHCl_3$): 1647 cm^{-1} ($\nu_{C=O}$), 1693.8 cm^{-1} ($\nu_{C=N}$), 1739.2 cm^{-1} ($\nu_{C=O}$). Anal. Calcd for $C_{21}H_{30}N_2O_4$: C, 67.35; H, 8.07; N, 7.48. Found: C, 67.56; H, 8.06; N, 7.45.

3.1.13. (2*S*,5*S*)-(2,4-Dibenzyl-6-ethoxy-5-isopropyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid ethyl ester 8b. Compound **8b** was obtained by the alkylation of **7** with benzylbromide and following the procedure used to obtain **7**. The product was recovered as an oil in 80% yield after elution by silica gel chromatography with hexane/ethyl acetate. ^{1}H NMR δ: 0.83 (d, 3H, $J = 7$); 0.90 (d, 3H, $J = 7$); 1.28 (t, 3H, $J = 7$); 1.30 (t, 3H, $J = 7$); 1.98 (m, 1H); 3.03 (m, 3H); 3.23 (d, 1H, $J = 12.4$); 3.44 (d, 1H, $J = 12.4$); 4.20 (m, 5H), 4.83 (d, 1H, $J = 15.4$); 6.75 (m, 2ArH); 7.20 (m, 8ArH). ^{13}C NMR δ: 13.9, 14.0, 16.0, 20.3, 29.0, 45.9, 46.6, 47.2, 60.0, 60.6, 61.0, 63.3, 126.3, 126.8, 127.5, 127.6, 128.1, 130.6, 135.4, 136.5, 157.3, 169.3, 170.0. HPLC–MS: 451.5 $[M+H]^+$, 473.5 $[M+Na]^+$, 489.5 $[M+K]^+$. IR ($CHCl_3$): 1650 cm^{-1} ($\nu_{C=O}$), 1694.8 cm^{-1} ($\nu_{C=N}$), 1738.5 cm^{-1} ($\nu_{C=O}$). The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

3.1.14. (2*S*,5*S*)-(4-Benzyl-6-ethoxy-5-isopropyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid 9a. To **8a** (2.61 g, 6.98 mmol) dissolved in ethanol (10 mL) was added 2 M NaOH (10.5 mL, 21 mmol) and the solution stirred for 24 h at room temperature. The reaction mixture was concentrated under vacuum and the residue was acidified to pH = 4 with diluted HCl in the presence of CH_2Cl_2. The organic solution was separated and then evaporated in vacuo. The product was recovered as a wax in quantitative yield. ^{1}H NMR δ: 0.89 (d, 3H, $J = 7$); 1.10 (d, 3H, $J = 7$); 1.31 (t, 3H, $J = 7$); 1.60 (s, 3H); 2.29 (m, 1H); 2.76 (d, 1H, $J = 15.6$); 3.14 (d, 1H, $J = 15.6$); 3.84 (m, 1H); 3.94 (d, 1H, $J = 15$); 4.13 (m, 2H); 5.52 (d, 1H, $J = 15$); 7.30 (m, 5ArH). ^{13}C NMR δ: 13.6, 16.3, 20.1, 29.3, 29.5, 45.1, 46.6, 58.1, 61.0, 61.3, 127.6, 127.8, 128.6, 135.3, 156.9, 171.3, 173.2. $[\alpha]_D = -1.0$ (c 0.8, $CHCl_3$). HPLC–MS: 347.4 $[M+H]^+$, 369.4 $[M+Na]^+$, 385.4 $[M+K]^+$, 715.8 $[2M+Na]^+$. IR ($CHCl_3$): 1650–1750 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=N}$), 2400–3300 cm^{-1} (broad, ν_{OH}). Anal. Calcd for $C_{19}H_{26}N_2O_4$: C, 65.87; H, 7.56; N, 8.09. Found: C, 65.63; H, 7.59; N, 8.11.

3.1.15. (2*S*,5*S*)-(2,4-Dibenzyl-6-ethoxy-5-isopropyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid 9b. Compound **9b** was obtained starting from **8b**, following the procedure used for **9a**. The product was recovered pure as a wax in quantitative yield. ^{1}H NMR δ: 0.82 (d, 3H, $J = 7$); 0.85 (d, 3H, $J = 7$ Hz); 1.27 (t, 3H, $J = 7$); 2.01 (m, 1H); 2.97 (d, 1H, $J = 15.4$); 3.12 (m, 2H); 3.48 (d, 1H, $J = 15.4$); 3.97 (d, 1H, $J = 15$); 4.23 (m, 2H); 5.03 (d, 1H, $J = 15$); 6.70 (m, 2ArH); 7.25 (m, 8ArH) 12.18 (br s, 1H). ^{13}C NMR δ: 14.0, 16.1, 20.1, 29.0, 46.1, 46.4, 46.9, 60.4, 61.6, 63.0, 126.7, 127.4, 128.0, 128.4, 130.6, 134.4, 135.8, 158.4, 169.4, 172.9. $[\alpha]_D = -52.6$ (c 0.4, $CHCl_3$). HPLC–MS: 423.5 $[M+H]^+$, 445.5 $[M+Na]^+$, 461.5 $[M+K]^+$, 868 $[2M+Na]^+$. IR ($CHCl_3$): 1650–1750 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=N}$), 2400–3300 cm^{-1} (broad, ν_{OH}). Anal. Calcd for $C_{25}H_{30}N_2O_4$: C, 71.07; H, 7.16; N, 6.63. Found: C, 71.4; H, 7.13; N, 6.61.

3.1.16. (2*S*,5*S*)-(6-Ethoxy-5-isopropyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid 10a. Compound **10a** was obtained starting from **9a**, following the procedure used for **4a**. The product was recovered as a wax in 90% yield. ^{1}H NMR δ: 0.87 (d, 3H, $J = 7$); 1.05 (d, 3H, $J = 7$); 1.32 (t, 3H, $J = 7$); 1.48 (s, 3H); 2.38 (m, 1H); 2.87 (q_{AB}, 2H, $J = 15.9$); 4.05 (m, 1H); 4.16 (q, 2H, $J = 7$); 7.15 (br s, 1H). ^{13}C NMR δ: 13.8, 15.9, 18.1, 27.9, 30.3, 44.6, 57.9, 61.7, 157.7, 174.1, 174.7. $[\alpha]_D = -4.3$ (c 0.3, $CHCl_3$). HPLC–MS: 257.3 $[M+H]^+$, 535.6 $[2M+Na]^+$. IR ($CHCl_3$): 1650–1750 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=N}$), 2400–3300 cm^{-1} (broad, ν_{OH}), 3401.7 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{12}H_{20}N_2O_4$: C, 56.23; H, 7.87; N, 10.93. Found: C, 56.43; H, 7.84; N, 10.91.

3.1.17. (2*S*,5*S*)-(2-Benzyl-6-ethoxy-5-isopropyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid 10b. Compound **10b** was obtained starting from **9b**, following the procedure used for **4a**. The product was recovered pure as a wax in 90% yield. ^{1}H NMR δ: 0.69 (d, 3H, $J = 7$); 0.79 (d, 3H, $J = 7$); 1.29 (t, 3H, $J = 7$); 2.02 (m, 1H); 2.52 (d, 1H, $J = 2.2$); 2.84 (d, 1H, $J = 15.8$); 2.85 (d, 1H, $J = 12.6$); 3.20 (d, 1H, $J = 12.6$); 3.24 (d, 1H, $J = 15.8$); 4.20 (m, 2H); 7.20 (m, 5ArH); 7.60 (br s, 1H); 11.92 (br s, 1H). ^{13}C NMR δ: 14.0, 15.9, 17.9, 29.7, 44.5, 46.4, 57.4, 61.6, 62.7, 126.9, 127.8, 130.5, 135.0, 159.0, 173.1, 174.6. $[\alpha]_D = -69$ (c 0.2, $CHCl_3$). HPLC–MS: 333.5 $[M+H]^+$, 688 $[2M+Na]^+$. IR ($CHCl_3$): 1650–1750 cm^{-1}

($\nu_{C=O}$ and $\nu_{C=N}$), 2400–3300 cm^{-1} (broad, ν_{OH}), 3400.8 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{18}H_{24}N_2O_4$: C, 65.04; H, 7.28; N, 8.43. Found: C, 65.23; H, 7.26; N, 8.41.

3.1.18. (2*S*,5*S*)-(6-Ethoxy-5-isopropyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid benzyl ester 11a. Compound **11a** was obtained starting from **10a**, following the procedure used for **5a**. The product was recovered pure as a wax in 70% yield, after elution by silica gel chromatography with hexane/ethyl acetate. ^1H NMR δ: 0.90 (d, 3H, $J = 7$); 1.02 (d, 3H, $J = 7$); 1.22 (t, 3H, $J = 7$); 1.62 (s, 3H); 2.34 (m, 1H); 2.73 (d, 1H, $J = 15$); 3.16 (d, 1H, $J = 15$); 4.04 (m, 3H); 5.09 (q_{AB}, 2H, $J = 12.4$); 5.74 (br s, 1H); 7.30 (m, 5ArH). ^{13}C NMR δ: 13.9, 16.0, 18.2, 28.3, 30.3, 44.6, 58.1, 58.5, 61.1, 65.6, 65.9, 127.8, 127.9, 128.2, 136.1, 157.2, 170.1, 173.7. $[\alpha]_D = -5.2$ (*c* 0.6, $CHCl_3$). HPLC–MS: 347.4 $[M+H]^+$, 348.4 $[M+2H]^+$, 385.4 $[M+K]^+$, 715.8 $[2M+Na]^+$. IR ($CHCl_3$): 1644.7 cm^{-1} ($\nu_{C=O}$), 1695.2 cm^{-1} ($\nu_{C=N}$), 1737.3 cm^{-1} ($\nu_{C=O}$), 3404 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{19}H_{26}N_2O_4$: C, 65.87; H, 7.56; N, 8.09. Found: C, 66.1; H, 7.54; N, 8.07.

3.1.19. (2*S*,5*S*)-(2-Benzyl-6-ethoxy-5-isopropyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid benzyl ester 11b. Compound **11b** was obtained starting from **10c**, following the procedure used for **5a**. The product was recovered as a wax in 70% yield, after elution by silica gel chromatography with hexane/ethyl acetate. ^1H NMR δ: 0.77 (d, 3H, $J = 7$); 0.78 (d, 3H, $J = 7$); 1.29 (t, 3H, $J = 7$); 2.05 (m, 1H); 2.54 (d, 1H, $J = 2.7$); 2.90 (d, 1H, $J = 15.6$); 2.91 (d, 1H, $J = 12.9$); 3.24 (d, 1H, $J = 12.9$); 3.42 (d, 1H, $J = 15.6$); 4.17 (m, 2H); 5.12 (q_{AB}, 2H, $J = 12.3$); 5.43 (br s, 1H); 7.27 (m, 10ArH). ^{13}C NMR δ: 14.0, 15.8, 17.9, 29.6, 44.7, 46.7, 57.6, 61.2, 63.2, 65.8, 126.8, 127.7, 127.8, 128.0, 128.3, 130.6, 135.5, 136.1, 158.5, 170.0, 171.6. HPLC–MS: 423.5 $[M+H]^+$, 868 $[2M+Na]^+$. IR ($CHCl_3$): 1645.3 cm^{-1} ($\nu_{C=O}$), 1694.7 cm^{-1} ($\nu_{C=N}$), 1737.8 cm^{-1} ($\nu_{C=O}$), 3402.5 cm^{-1} (ν_{NH}). The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

3.1.20. Dipeptide [(OEt)Val-(2*S*)-methyl-Asp(OBn)]·HCl 12a. Compound **12a** was obtained starting from **11a**, and following the procedure used for **6a**. The product was recovered pure as a wax in quantitative yield. ^1H NMR δ: 1.00 (d, 3H, $J = 7$); 1.01 (d, 3H, $J = 7$); 1.29 (t, 3H, $J = 7$); 168 (s, 3H); 2.21 (m, 1H); 3.10 (d, 1H, $J = 18.2$); 3.48 (d, 1H, $J = 18.2$); 4.18 (q, 2H; $J = 7.2$); 4.39 (d, 1H, $J = 6.8$); 5.23 (br s, 2H); 7.40 (br s, 5ArH). ^{13}C NMR δ: 14.1, 18.2, 18.9, 22.6, 30.3, 40.0, 58.6, 58.8, 61.2, 67.3, 128.2, 128.3, 128.5, 134.8, 170.0, 171.1, 171.2. $[\alpha]_D = -4.5$ (*c* 0.4, $CHCl_3$). HPLC–MS: 365.4 $[M-HCl+H]^+$, 387.4 $[M-HCl+Na]^+$, 729.8 $[2M-HCl+H]^+$. IR ($CHCl_3$): 1683 cm^{-1} ($\nu_{C=O}$), 1734.8 cm^{-1} ($\nu_{C=O}$), 3000 cm^{-1} ($\nu_{NH_3^+}$), 3425 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{19}H_{29}ClN_2O_5$: C, 56.92; H, 7.29; N, 6.99. Found: C, 56.73; H, 7.32; N, 7.01.

3.1.21. Dipeptide [(OEt)Val-(2*S*)-benzyl-Asp(OBn)]·HCl 12b. Compound **12b** was obtained starting from **11c**, following the procedure used for **6a**. The product was recovered as a wax in quantitative yield. ^1H NMR δ: 1.05 (d, 3H, $J = 7$); 1.08 (d, 3H, $J = 7$); 1.32 (t, 3H, $J = 7$); 2.28 (m, 1H); 3.06 (d, 1H, $J = 18$); 3.65 (d, 1H, $J = 18$); 4.23 (m, 2H), 4.39 (m, 1H); 5.23 (s, 2H); 7.38 (m, 10ArH). ^{13}C NMR δ: 14.9, 19.5, 19.9, 24.5, 31.9, 39.3, 43.0, 60.7, 62.6, 68.8, 129.9, 130.5, 131.9, 133.7, 137.0, 171.3, 172.8, 172.9. HPLC–MS: 441.5 $[M-HCl+H]^+$, 463.5 $[M-HCl+Na]^+$, 904 $[2M-HCl+Na]^+$. IR ($CHCl_3$): 1685 cm^{-1} ($\nu_{C=O}$), 1735.5 cm^{-1} ($\nu_{C=O}$), 2990 cm^{-1} ($\nu_{NH_3^+}$), 3430 cm^{-1} (ν_{NH}). The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

3.1.22. (2*R*,5*S*)-(6-Ethoxy-5-isopropyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-acetic acid pentafluorophenyl ester 13. To a stirred solution of **4a** (1.28 g, 5 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C, pyridine (0.4 mL, 5 mmol) was added followed by pentafluorophenyl trifluoroacetate (1.3 mL, 7.5 mmol). The cooling bath was then removed, the reaction stirred at rt for about 6 h and monitored by TLC. When the reaction was complete, CH_2Cl_2 (10 mL) was added, the organic solution washed with water, dried over $CaCl_2$ and then evaporated to dryness in vacuo. The crude reaction product was purified by silica gel chromatography, eluting with hexane/ethyl acetate and the pure product was obtained as a wax in 90% yield. ^1H NMR δ: 0.91 (d, 3H, $J = 7$); 1.02 (d, 3H, $J = 7$); 1.28 (t, 3H, $J = 7.4$); 1.55 (s, 3H); 2.32 (m, 1H); 2.97 (d, 1H, $J = 16.2$); 3.56 (d, 1H, $J = 16.2$); 4.03 (m, 1H); 4.13 (m, 2H); 5.93 (s, 1H). ^{13}C NMR δ: 13.9, 16.3, 18.4, 29.1, 30.8, 44.8, 55.4, 59.0, 61.4, 133.0–148.0, 134–148 (m, C–F), 158.3, 166.9, 173.2. $[\alpha]_D = -0.2$ (*c* 0.6, $CHCl_3$). HPLC–MS: 423.1 $[M+H]^+$, 424.1 $[M+2H]^+$, 445.1 $[M+Na]^+$, 446.2 $[M+H+Na]^+$, 462.1 $[M+H+K]^+$, 867.2 $[2M+Na]^+$, 868.2 $[2M+H+Na]^+$. IR ($CHCl_3$): 1668.7 cm^{-1} ($\nu_{C=O}$), 1793.6 cm^{-1} ($\nu_{C=N}$), 1783 cm^{-1} ($\nu_{C=O}$), 3400 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{18}H_{19}F_5N_2O_4$: C, 51.59; H, 4.53; N, 6.63. Found: C, 51.73; H, 4.51; N, 6.61.

3.1.23. (2*R*,5*S*,2′*S*,5′*R*)-(6-Ethoxy-5-isopropyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-8′-[3′,6′-diaza-4′,7′-dioxo-2-isopropyl-5′-methyl-5′-(acetic acid benzylester)]-ethyloctanoate 14. Under an inert atmosphere, a solution of **6a** (1.72 g, 4.8 mmol) and triethylamine (1.2 mL, 14.4 mmol) was added dropwise into a stirred solution of **4a** (1.24 g, 4.8 mmol) and HBTU (2.06 g, 5.4 mmol) in CH_3CN (100 mL). The reaction mixture was stirred at rt and after about 24 h, CH_2Cl_2 and then 0.1 M HCl (50 mL) were added. The organic extract was dried over $CaCl_2$ and evaporated in vacuo. The crude reaction product was then purified by silica gel chromatography, eluting with hexane/ethyl acetate. The pure product was obtained as an oil in 80% yield. ^1H NMR δ: 0.90 (m, 12H); 1.25 (m, 6H); 1.44 (s, 3H); 1.61 (s, 3H); 2.20 (m, 2H); 2.51 (d, 1H, $J = 14.6$); 2.86 (d, 1H, $J = 14.6$); 2.98 (d, 1H, $J = 16.6$); 3.25 (d, 1H, $J = 16.6$); 4.15 (m, 5H); 4.43 (dd, 1H, $J = 5.1, 7.8$); 5.10 (s, 2H); 6.34 (br s, 1H); 7.16 (br s, 1H); 7.35 (d, 1H, $J = 7.8$); 7.37 (m, 5ArH). ^{13}C NMR δ: 14.1, 14.2, 16.2, 17.8, 18.4, 18.9, 21.0, 23.4, 28.7, 30.7, 31.0, 40.0, 48.1, 57.7, 58.3, 58.7, 60.3, 61.0, 66.4, 128.1, 128.2, 128.5, 135.5, 151.0, 170.4, 170.9, 171.9, 172.9, 173.5.

$[\alpha]_D = -4.3$ (c 0.8, $CHCl_3$). HPLC–MS: 603.3 $[M+H]^+$, 625.3 $[M+Na]^+$, 641.3 $[M+K]^+$, 1227.6 $[2M+Na]^+$. IR ($CHCl_3$): 1650 cm^{-1} ($\nu_{C=O}$), 1695 cm^{-1} ($\nu_{C=N}$), 1734 cm^{-1} ($\nu_{C=O}$), 3405 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{31}H_{46}N_4O_8$: C, 61.78; H, 7.69; N, 9.3. Found: C, 61.89; H, 7.66; N, 9.27.

3.1.24. Tetrapeptide [(OEt)Val-(2*R*)-methyl-Asp-(2*R*)-methyl-Asp(OBn)-Val(OEt)]·HCl 15. A solution of **14** (0.468 g, 0.8 mmol) in 6.2 mL of ethanol and 3.1 mL of 0.5 M HCl was stirred at rt for about 5 h. The acid solution was evaporated in vacuo and the product as hydrochloride was isolated as a wax in quantitative yield. ^1H NMR δ: 0.93 (d, 3H, $J = 7$); 0.94 (d, 3H, $J = 7$); 0.98 (d, 3H, $J = 7$); 0.99 (d, 3H, $J = 7$); 1.28 (t, 6H, $J = 7$); 1.62 (s, 3H); 1.65 (s, 3H); 2.20 (m, 2H); 2.98 (q_{AB}, 2H, $J = 16.4$); 3.22 (q_{AB}, 2H, $J = 17.8$); 4.22 (m, 6H); 5.16 (s, 2H); 7.37 (m, 5ArH). ^{13}C NMR δ: 14.8, 19.1, 19.4, 19.8, 22.6, 24.4, 31.7, 32.2, 40.4, 41.9, 59.8, 59.9, 60.0, 60.5, 62.6, 62.7, 67.9, 129.6, 129.7, 129.9, 137.6, 170.7, 172.1, 172.2, 173.0, 173.6, 175.1. HPLC–MS: 621.7 $[M-HCl+H]^+$, 622.7 $[M-HCl+2H]^+$, 643.7 $[M-HCl+Na]^+$, 1242.4 $[2M-HCl+H]^+$. IR ($CHCl_3$): 1682.5 cm^{-1} ($\nu_{C=O}$), 1734 cm^{-1} ($\nu_{C=O}$), 2980 cm^{-1} ($\nu_{NH_3^+}$), 3200–3400 cm^{-1} (ν_{NH}). The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

3.1.25. (2*R*,5*S*)-(6-ethoxy-5-isopropyl-2-methyl-3-oxo)-2,3,4,5-tetrahydropyrazine 16. Compound **16** was obtained starting from **2a**, following the procedure used for **4a**. After quenching with NH_4Cl, and evaporation of NH_3, water and CH_2Cl_2 were added and the organic extract evaporated to dryness under vacuum. The pure product was recovered as an oil in quantitative yield. ^1H NMR δ: 0.89 (d, 3H, $J = 7$); 1.01 (d, 3H, $J = 7$); 1.3 (t, 3H, $J = 7$); 1.49 (d, 3H, $J = 7$); 2.25 (m, 1H); 3.5 (q, 1H, $J = 7$); 3.9 (m, 1H); 4.15 (m, 2H); 5.8–6.1 (br s, 1H). ^{13}C NMR δ: 13.8, 16.0, 17.9, 20.4, 32.0, 53.2, 58.2, 60.7, 158.2, 173.4. $[\alpha]_D = +101.2$ (c 0.8, $CHCl_3$). HPLC–MS: 199.3 $[M+H]^+$, 221.3 $[M+Na]^+$, 419.6 $[2M+Na]^+$. IR ($CHCl_3$): 1648.5 cm^{-1} ($\nu_{C=O}$), 1694 cm^{-1} ($\nu_{C=N}$), 3402 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{10}H_{18}N_2O_2$: C, 60.58; H, 9.15; N, 14.13. Found: C, 60.71; H, 9.12; N, 14.09.

3.1.26. Dipeptide [(OEt)Val-(2*R*)-Ala]·HCl 17. Compound **17** was obtained starting from **16**, following the procedure used for **15**. The product was recovered pure as a wax in quantitative yield. ^1H NMR δ: 0.98 (m, 6H); 1.30 (t, 3H, $J = 7$); 1.56 (d, 3H, $J = 7$); 2.23 (m, 1H); 4.09 (q, 1H, $J = 7$); 4.22 (q, 2H, $J = 7$); 4.40 (dd, 1H, $J = 5.1$, 8.4); 8.62 (d, 1H, $J = 8.4$). ^{13}C NMR δ: 14.8, 18.5, 18.7, 19.8, 32.1, 50.5, 59.5, 62.6, 171.8, 173.0. $[\alpha]_D = -9.4$ (c 1.5, $CHCl_3$). HPLC–MS: 217.3 $[M-HCl+H]^+$, 239.3 $[M-HCl+Na]^+$, 455.6 $[2M-HCl+Na]^+$. IR ($CHCl_3$): 1680 cm^{-1} ($\nu_{C=O}$), 1739 cm^{-1} ($\nu_{C=O}$), 2980 cm^{-1} ($\nu_{NH_3^+}$), 3400 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{10}H_{21}ClN_2O_3$: C, 47.52; H, 8.37; N, 11.08. Found: C, 47.33; H, 8.39; N, 11.13.

3.1.27. (2*R*,5*R*,2′*S*,5′*R*)-(6-Ethoxy-5-isopropyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-pyrazin-2-yl)-8′-(3′,6′-diaza-4′,7′-dioxo-2-isopropyl-5′-methyl)-ethyloctanoate 18. Under an inert atmosphere, activated ester **13** (0.312 g, 0.74 mmol) in dry THF (10 mL) was added to a stirred solution of **17** (0.16 g, 0.74 mmol), dissolved in THF (5 mL) and triethylamine (0.1 mL, 0.74 mmol) and the reaction monitored by TLC. After about 12 h at rt, CH_2Cl_2 (15 mL) was added and the organic solution washed with diluted HCl and then with water. The organic extract was dried over $CaCl_2$ and evaporated to dryness in vacuo. The crude reaction product was purified by the silica gel chromatography eluting, with hexane/ethyl acetate. The pure product was recovered as a white waxy solid in 85% yield. ^1H NMR δ: 0.90 (d, 3H, $J = 7$); 0.92 (d, 3H, $J = 7$) 0.96 (d, 3H, $J = 7$); 1.03 (d, 3H, $J = 7$); 1.28 (t, 3H, $J = 7$); 1.30 (t, 3H, $J = 7$); 1.39 (d, 3H, $J = 7.2$); 1.49 (s, 3H); 2.18 (m, 1H); 2.30 (m, 1H); 2.77 (d, 1H, $J = 14.7$); 2.93 (d, 1H, $J = 14.7$); 4.17 (m, 5H); 4.56 (m, 2H); 6.20 (br s, 1H); 6.80 (d, $J = 8.7$); 7.01 (d, 1H, $J = 7.2$). ^{13}C NMR δ: 13.9, 14.0, 16.3, 17.6, 18.2, 18.3, 18.8, 28.0, 30.8, 30.9, 46.8, 48.4, 57.0, 58.3, 58.5, 60.9, 61.4, 157.4, 170.1, 171.8, 172.4, 173.4. $[\alpha]_D = +32.2$ (c 0.9, $CHCl_3$). HPLC–MS: 455.4 $[M+H]^+$, 477.4 $[M+Na]^+$, 931.8 $[2M+Na]^+$. IR ($CHCl_3$): 16450.2 cm^{-1} ($\nu_{C=O}$), 1694 cm^{-1} ($\nu_{C=N}$), 1735.5 ($\nu_{C=O}$), 3402 cm^{-1} (ν_{NH}). Anal. Calcd for $C_{22}H_{38}N_4O_6$: C, 58.13; H, 8.43; N, 12.33. Found: C, 58.4; H, 8.41; N, 12.29.

Acknowledgement

Thanks are due to the University of Bologna for the financial support ('Ricerca Fondamentale Orientata', ex 60%).

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