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A new stereocontrolled synthesis of uncommon tripeptides derived from 2,6-diaminopimelic acid (2,6-DAP)

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Abstract—The stereocontrolled synthesis of uncommon tripeptides **8** and **11a–c**, structural variants of 2,6-diaminopimelic acid, was carried out starting from the mono-lactim ether **1** easily obtained from L-valine. The configurations of the introduced stereogenic centers were assigned on the basis of ^{1}H NMR spectroscopic data.

1. Introduction

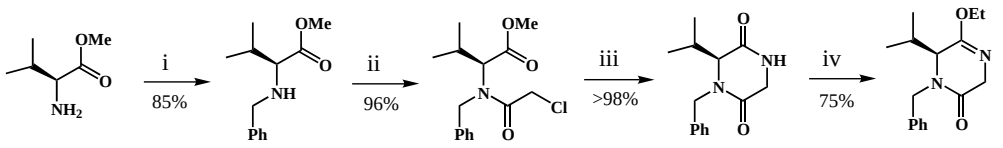
Owing to our interest in new stereoselective approaches for the synthesis of α-amino acids[1] and peptides,[2,3] we have recently focused our attention towards new structural variants of 2,6-diaminopimelic acid (2,6-DAP) for their potential antibacterial activity.[4] In continuation of our program we have directed our efforts towards producing new tripeptides, such as **8** and **11a–c**, containing the 2,6-DAP acid and valine units at the ends of the chain, i.e. Val-(2,6-DAP)-Val, the latter being monoalkyl derivatives of **8**. Such tripeptides are unusual, because they are C-terminal at both the ends of the chain. The strategy employed is based on experience previously acquired on the asymmetric synthesis of (+)- and (−)-2,6-DAP,[5] (+)- and (−)-2,7-diaminosuberic acid,[6] (+)-*o*-phenylene-bis-alanine[6] and on the stereoselective synthesis of sterically constrained dipeptides.[3]

2. Results and discussion

In the present study we have followed a strategy which makes use of the mono-lactim ether **4**, a chiral synthon easily synthesized starting from L-valine (Scheme 1).

When the lithium enolate of synthon **4** was treated with 0.5 equiv. of 1,3-diiodopropane in THF at −78°C a practically total conversion into the diastereomers **5** and **6** is obtained in (Scheme 2).

The prevalence of the diastereomer **5** is controlled by the stereochemistry of the *iso*-propyl group at C(6): in fact, as already observed for similar mono-[2] and bis-lactim ethers,[7] the *trans*-addition of electrophile is generally preferred because the *si* face of the lithium enolate of **4** is shielded by the sterically demanding *iso*-propyl group. Nevertheless, the formation of the minor diastereomer **6** is, most probably, because the stereo-



1 **2** **3** **4**

Scheme 1. (i) $PhCH_2Br$/pyridine in CH_2Cl_2; (ii) $ClCH_2COCl$/TEA in CH_2Cl_2; (iii) 10 M NH_3 in EtOH; (iv) Et_3OBF_4 in CH_2Cl_2.

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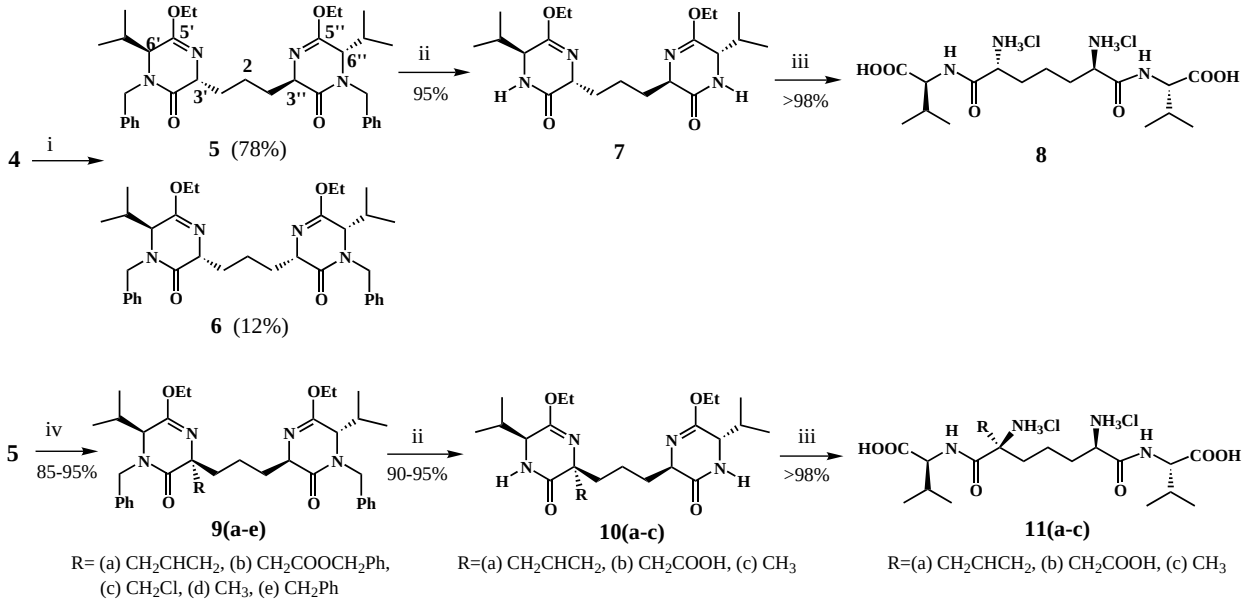

Scheme 2. (i) 1 M LHMDS/THF, $I(CH_2)_3I$; (ii) Na/NH_3; (iii) 1 M HCl at 60°C; (iv) 1 M LHMDS/THF, RX (see Table 1).

selectivity in the first alkylation of **4** is generally variable, giving low to moderate *cis*-induction.[2,7]

As reported in Scheme 2, the monoalkylated intermediates **9a–e** were obtained by adding one equivalent of alkylating reagent (allyl bromide, benzyl bromoacetate, chloroiodomethane, iodomethane or benzyl bromide) to the lithium enolate of the pure isomer **5** in THF at −78°C. The conversion of **5** into **9a–e** occurs in good to very good yields and with high *trans* stereocontrol as can be seen from the data collected in Table 1. The alkylation reactions with allyl bromide and chloro- or iodomethane furnished a small quantity of dialkylated product, which is easily separable by silica gel chromatography.

The stereochemistry of the intermediates **5** and **6** was established on the basis of the ^1H NMR spectra considering that the former stereoisomer has a C_2 axis. In fact, in the product **5** the signals for both *iso*-propyl groups (at 0.94 and 1.06 ppm) overlap as do the signals for the benzylic protons (at 3.88 and 5.57 ppm), indicating their magnetic equivalence. It is also worth pointing out the overlapping signals for the C(3′) and C(3″) protons at 3.69 ppm and those for the C(5′)- and C(5″)-OC_2H_5 methyl protons at 1.17 ppm.

Conversely, in the stereoisomer **6**, the above-mentioned protons being magnetically inequivalent, have different chemical shifts, i.e. the overlap observed for **5** does not occur (see Section 4). Thus, it can be inferred that the stereoisomer **5** possesses *trans–trans* relative configuration, while **6** has *trans–cis* configuration. Nevertheless, the absolute configuration was unequivocally assigned by converting **5** into the corresponding (2*R*,6*R*)-DAP through acid hydrolysis by refluxing with 57% HI.[5,6] Analogously, the stereoisomer **6** was converted into the optically inactive (2*R*,6*S*)-*meso*-DAP.

The second alkylation, i.e. the conversion of **5** into **9a–e**, again occurs with *trans* stereoselectivity with respect to the *iso*-propyl group, as already observed for similar substrates.[3,7] Nevertheless, the configuration of the introduced stereogenic centre of monoalkylated **9a–e** was ascertained on the basis of the nOe measurements, as already observed for similar substrates.[2] On this basis, the nOe observed for C(6′)H (at 3.74 ppm) of derivative **9d** upon irradiation of the C(3′) methyl protons (at 1.42 ppm) is consistent with a *trans* relationship between the CH_3 and *iso*-propyl groups. Confirmation of the *trans* stereochemistry has been obtained from the derivative **9e** that shows a higher field resonance of the C(6′)H (3.21 ppm) with respect to C(6″)H (3.72 ppm). In fact, the upfield shift is induced by the shielding effect of the phenyl ring of the C(3′) benzyl group, which lies in the axial-like position adopting the preferential 'aryl inside' arrangement. As previously observed in analogous substrates,[3] this shielding (0.5 ppm) is clear evidence that the benzyl group lies *trans* with respect to the *iso*-propyl group.

The intermediates **9a–d** were then converted into the corresponding tripeptides **11a–c** through a Birch reaction (Na/NH_3), which furnishes derivatives **10a–c**, followed by heterocyclic ring cleavage by hydrolysis with 1N aqueous HCl (Scheme 2).

Table 1. Diastereoselective alkylation of **5**

Alkylating reagent		Yield (%)[a] of **9a–e**
R	X	
a- CH_2CHCH_2	Br	90
b- $CH_2CO_2CH_2Ph$	Br	95
c- CH_2Cl	I	90
d- CH_3	I	85
e- CH_2Ph	Br	90

[a] Determined by ^1H NMR.

3. Conclusion

The reported strategy provides a new approach to the asymmetric synthesis of unusual tripeptides (C-terminal at both ends of the chain) starting from a chiral synthon easily synthesized from L-valine. This synthetic strategy is a versatile approach because it allows to produce a wide variety of tripeptides, as well those with potential biological activity.[8,9] In fact, tripeptides containing various bis(α-amino acid) units and different terminal amino acids could be prepared by reacting a chiral synthon obtained from a suitable α-amino acid and an appropriate dihalo derivative.

4. Experimental

4.1. General information

^{1}H and ^{13}C NMR spectra were recorded on a Gemini spectrometer at 300 MHz using $CDCl_3$ as solvent, unless otherwise stated. Chemical shifts are reported in ppm relative to $CDCl_3$ or to 1,4-dioxane if D_2O is used. The coupling constants (J) are in Hz. Optical rotation values were measured 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).

4.2. (*S*)-*N*-Benzylvaline methyl ester 1

To a solution of (*S*)-valine methyl ester (20.1 g, 153.4 mmol) in CH_2Cl_2 (100 mL) and pyridine (13.5 mL, 168.8 mmol) was added benzyl bromide (20.1 mL, 168.77 mmol) at 0°C and the reaction stirred overnight at room temperature. After addition of water (100 mL) the organic solution was separated and the solvent removed under vacuum. The crude product was purified by silica gel chromatography with hexane/ethyl acetate to afford pure product in 85% yield. ^{1}H NMR δ: 0.96 (d, 3H, $J=7$); 0.98 (d, 3H, $J=7$); 1.86–2.03 (m, 1H); 3.05 (d, 1H, $J=6.2$); 3.61 (d, 1H, $J=13.2$); 3.74 (s, 3H); 3.86 (d, 1H, $J=13.2$); 7.3 (m, 5ArH). ^{13}C NMR δ: 18.5, 19.2, 31.6, 51.1, 52.4, 66.3, 126.7, 128, 139.8, 175.4. $[\alpha]_D$ −53.9 (c 1.03, $CHCl_3$).

4.3. (*S*)-*N*-Benzyl-*N*-chloroacetylvaline methyl ester 2

A solution of **1** (17.6 g, 79.6 mmol) in CH_2Cl_2 (100 mL) was treated with TEA (13.3 mL, 95.4 mmol) and the solution was cooled to 0°C. Chloroacetyl chloride (7.6 mL, 95.4 mmol) was added dropwise and the reaction monitored by TLC. Water (100 mL) was added, the organic layer was collected and concentrated in vacuo. After purification by silica gel chromatography with hexane/ethyl acetate, the pure product was obtained in 96% yield. ^{1}H NMR δ: 0.97 (d, 3H, $J=6.8$); 1.01 (d, 3H, $J=6.8$); 2.28–2.45 (m, 1H); 3.44 (s, 3H); 3.97 (d, 1H, $J=2.2$); 4.38 (d, 1H, $J=10.4$); 4.75 (s, 2H); 4.90 (d, 1H, $J=10.4$); 7.1–7.4 (m, 5ArH). ^{13}C NMR δ: 18.2, 19.7, 27.9, 41.4, 48.2, 51.5, 62.2, 125.4, 127.3, 127.5, 127.9, 128.6, 136, 167.7, 170. $[\alpha]_D$ −78.8 (c 1.06, $CHCl_3$).

4.4. (2*S*)-1-*N*-Benzyl-2-isopropyl-pyperazine-3,6-dione 3

The intermediate **2** (23 g, 77.3 mmol) was dissolved in ca. 10 M ethanolic ammonia solution (100 mL). The flask was sealed and the reaction stirred overnight at rt. After evaporation of NH_3, the solution was concentrated in vacuo and a mixture of water/ethyl acetate was added to the residue. The organic extract was concentrated under vacuum and the residue submitted to silica gel chromatography eluting with hexane/ethyl acetate. The pure product was obtained as a solid in practically quantitative yield (mp 107–109°C). ^{1}H NMR δ: 1.06 (d, 3H, $J=7$); 1.13 (d, 3H, $J=7$); 2.20–2.36 (m, 1H); 3.68 (d, 1H, $J=4.8$); 3.93 (d, 1H, $J=15$); 3.98 (dd, 1H, $J=3$, 17.6); 4.16 (d, 1H, $J=17.6$); 5.45 (d, 1H, $J=15$); 6.42–6.62 (bs, 1H); 7.31 (m, 5ArH). ^{13}C NMR δ: 17.6, 19.7, 31.8, 45.1, 48.2, 64.5, 127.8, 128.7, 135.3, 164.6, 168. $[\alpha]_D$ +10.8 (c 1.012, $CHCl_3$).

4.5. (6*S*)-1-Benzyl-5-ethoxy-3,6-dihydro-6-isopropyl-pyrazine-2-one 4

A solution of **3** (19 g, 77.2 mmol) in dry CH_2Cl_2 (100 mL) was added to triethyloxonium tetrafluoroborate prepared from BF_3/etherate (23.5 mL) and epichlorohydrin (11 mL) as reported in the literature.[10] The reaction mixture was stirred overnight at rt under inert atmosphere, then it was added to phosphate buffer solution at pH 7. The organic layer was separated, evaporated in vacuo and the residue submitted to chromatographic separation by silica gel eluting with hexane/ethyl acetate. The reaction product was recovered pure as an oil in 75% yield. ^{1}H NMR δ: 0.95 (d, 3H, $J=7.2$); 1.05 (d, 3H, $J=7.2$); 1.26 (t, 3H, $J=7.4$); 2.15–2.31 (m, 1H); 3.65 (m, 1H); 3.93 (d, 1H, $J=15$); 3.98–4.23 (m, 4H); 5.48 (d, 1H, $J=15$); 7.3 (m, 5ArH): ^{13}C NMR δ: 14.1, 17.4, 19.9, 31.8, 47.1, 50.8, 61.3, 127.5, 127.9, 128.6, 135.8, 160.2, 168.0. $[\alpha]_D$ +68.0 (c 1.1, $CHCl_3$). Anal. calcd for $C_{16}H_{22}N_2O_2$: C, 70.04; H, 8.08; N, 10.21. Found: C, 70.3; H, 8.1; N, 10.18%.

4.6. Conversion of 4 into 5 and 6

To a solution of **4** (5.4 g, 19.6 mmol), in dry THF (100 mL) and cooled at −78°C, a solution of LHMDS in THF (1 M, 21.6 mL) was added dropwise under stirring. After about 1 h, 1,3-diiodopropane (1.13 mL, 9.8 mmol) was added and the reaction was monitored by TLC. When the reaction was complete, the mixture was allowed to warm up to room temperature under stirring. Water and ethyl acetate are added and after separation the organic solution was evaporated in vacuo. The residue was submitted to silica gel chromatography eluting with hexane/ethyl acetate and the diastereomers **5** and **6** are separated.

4.6.1. 1-[(3′*R*,6′*S*)-1′-Benzyl-5′-ethoxy-3′,6′-dihydro-6′-isopropylpyrazin-3′-yl-2′-one]-3-[(3″*R*,6″*S*)-1″-benzyl-5″-ethoxy-3″,6″-dihydro-6″-isopropylpyrazin-3″-yl-2″-one] propane 5. Compound **5** was isolated pure as a solid (mp 149–51°C) in 78% yield. ^{1}H NMR δ: 0.94 (d, 6H, J=7); 1.06 (d, 6H, J=7); 1.17 (t, 6H, J=7.2); 1.4–1.6 (m, 2H); 1.62–1.75 (m, 2H); 1.9–2.32 (m, 4H); 3.69 (dd, 2H, J=1.7, 2.2); 3.88 (d, 2H, J=15); 3.95–4.2 (m, 6H); 5.57 (d, 2H, J=15); 7.15 (m, 10ArH); ^{13}C NMR δ: 14.1, 17.6, 19.9, 20.6, 31.5, 33.7, 47, 57.8, 61, 61.5, 127.3, 127.6, 128.5, 136.2, 158.6, 170.5. $[\alpha]_D$ +41.6 (c 1.0, $CHCl_3$). Anal. calcd for $C_{35}H_{48}N_4O_4$: C, 71.4; H, 8.22; N, 9.52. Found: C, 71.65; H, 8.25; N, 9.48%.

4.6.2. 1-[(3′*R*,6′*S*)-1′-Benzyl-5′-ethoxy-3′,6′-dihydro-6′-isopropylpyrazin-3′-yl-2′-one]-3-[(3″*S*,6″*S*)-1″-benzyl-5″-ethoxy-3″,6″-dihydro-6″-isopropylpyrazin-3″-yl-2″-one] propane 6. Compound **6** was isolated pure, as an oil, in 12% yield. ^{1}H NMR δ: 0.89 (d, 3H, J=6.9); 0.9 (d, 3H, J=6.9); 1.02 (d, 3H, J=6.9); 1.06 (d, 3H, J=6.9); 1.19 (t, 3H, J=7.4); 1.22 (t, 3H, J=7.4); 1.5–1.8 (m, 2H); 1.9 (m, 2H); 2.07–2.27 (m, 4H); 3.65 (dd, 1H, J=1.5, 3.9); 3.68 (dd, 1H, J=2.1, 3.3); 3.90 (d, 1H, J=15); 3.92 (d, 1H, J=15.3); 4.1 (m, 6H); 5.45 (d, 1H, J=15.3); 5.47 (d, 1H, J=15); 7.25 (m, 10ArH). ^{13}C NMR δ: 14.2, 14.3, 17.6, 18, 20.1, 20.6, 23, 30.5, 31.6, 33.8, 36.5, 47, 47.2, 57.7, 60, 60.9, 61, 61.1, 61.8, 127.4, 127.5, 127.8, 128.1, 128.6, 136.1, 136.3, 157.1, 158.8, 170.5. $[\alpha]_D$ −20.0 (c 1.014, $CHCl_3$).

4.7. Alkylation of 5

To a solution of **5** (0.588 g, 1 mmol) in dry THF (30 mL) and cooled at −78°C was added a solution of LHMDS in THF (1 M, 1.1 mL) under stirring. After about 1 h, the appropriate alkylating reagent (1 mmol) was added and the reaction was then monitored by TLC. When the reaction was practically complete, the mixture was allowed to warm to room temperature under stirring. Water and ethyl acetate are added and after separation the organic solution was evaporated in vacuo. The residue was submitted to silica gel chromatography eluting with hexane/ethyl acetate.

4.7.1. 1-[(3′*R*,6′*S*)-3′-Allyl-1′-benzyl-5′-ethoxy-6′-hydro-6′-isopropylpyrazin-3′-yl-2′-one]-3-[(3″*R*,6″*S*)-1″-benzyl-5″-ethoxy-3″,6″-dihydro-6″-isopropylpyrazin-3″-yl-2″-one] propane 9a. Allyl bromide was used as alkylating reagent. ^{1}H NMR δ: 0.82 (d, 3H, J=6.8); 0.89 (d, 3H, J=6.8); 1.0–1.3 (m, 6H); 1.2 (m, 6H); 1.5–1.72 (m, 2H); 1.75–2.1 (m, 4H); 2.15–2.3 (m, 2H); 2.42 (dd, 1H, J=7.2, 13.2); 2.7 (dd, 1H, J=7.5, 13.2); 3.65 (m, 2H); 3.87 (d, 1H, J=15); 3.89 (d, 1H, J=15); 3.9–4.2 (m, 5H); 4.93–5.1 (m, 2H); 5.47 (d, 2H, J=15); 5.42–5.61 (m, 1H); 7.25 (m, 10ArH). ^{13}C NMR δ: 14.2, 17.1, 17.6, 19.8, 20, 20.7, 29.6, 31.5, 34.3, 41, 44.9, 46.6, 47.2, 57.7, 60.6, 60.8, 61, 61.7, 63.7, 118, 127.4, 127.9, 128.4, 128.5, 128.6, 134.2, 136, 136.4, 155.7, 158.7, 170.6, 171.5. $[\alpha]_D$ −10.7 (c 1, $CHCl_3$). Anal. calcd for $C_{38}H_{52}N_4O_4$: C, 72.58; H, 8.34; N, 8.91. Found: C, 72.7; H, 8.36; N, 8.88%.

4.7.2. 1-[(3′*R*,6′*S*)-1′-Benzyl-3′-benzyloxyacetil-5′-ethoxy-6′-hydro-6′-isopropylpyrazin-3′-yl-2′-one]-3-[(3″*R*,6″*S*)-1″-benzyl-5″-ethoxy-3″,6″-dihydro-6″-isopropylpyrazin-3″-yl-2″-one] propane 9b. Benzyl bromoacetate was used as alkylating reagent. ^{1}H NMR δ: 0.85 (d, 3H, J=6.8); 0.92 (d, 3H, J=6.8); 1.04 (d, 6H, J=7); 1.12–1.23 (m, 6H); 1.5–2.3 (m, 8H); 2.81 (d, 1H, J=16); 3.32 (d, 1H, J=16); 3.7 (m, 2H); 3.8–4.2 (m, 6H); 4.28 (d, 1H, J=15); 5.04 (q_{AB}, 2H, J=12); 5.1 (d, 1H, J=15); 5.47 (d, 1H, J=15); 7.3 (m, 15ArH). ^{13}C NMR δ: 14, 17, 17.5, 19.4, 19.9, 20.8, 26.8, 29.5, 31.5, 33.9, 41.7, 44, 47.1, 47.5, 57.4, 60.6, 60.9, 61.3, 61.7, 62, 65.7, 127.1, 127.3, 127.6, 127.9, 128.2, 128.5, 135.8, 136.1, 136.3, 156.8, 158.7, 170.2, 170.7, 171.6. $[\alpha]_D$ −3.1 (c 1.014, $CHCl_3$). Anal. calcd for $C_{44}H_{56}N_4O_6$: C, 74.97; H, 8.01; N, 7.95. Found: C, 75.2; H, 8.03; N, 7.93%.

4.7.3. 1-[(3′*R*,6′*S*)-1′-Benzyl-3′-chloromethyl-5′-ethoxy-6′-hydro-6′-isopropylpyrazin-3′-yl-2′-one]-3-[(3″*R*,6″*S*)-1″-benzyl-5″-ethoxy-3″,6″-dihydro-6′-isopropylpyrazin-3″-yl-2″-one] propane 9c. Chloroiodomethane was used as alkylating reagent. ^{1}H NMR δ: 0.87 (d, 3H, J=7); 0.93 (d, 3H, J=7); 1.06 (d, 6H, J=7.4); 1.24 (t, 3H, J=7.4); 1.26 (t, 3H, J=7.4); 1.5–1.78 (m, 2H); 1.8–2.15 (m, 4H); 2.18–2.32 (m, 2H); 3.65 (d, 1H, J=9.6); 3.69 (m, 1H); 3.77 (d, 1H, J=2.4); 3.91 (d, 1H, J=15); 3.97 (d, 1H, J=15); 4.05–4.18 (m, 5H); 4.18 (d, 1H, J=9.6); 5.49 (d, 1H, J=15); 5.62 (d, 1H, J=15); 7.29 (m, 10ArH). ^{13}C NMR δ: 14.2, 17, 17.6, 19.9, 20.1, 20.6, 29.5, 31.6, 34.1, 39.4, 46.6, 47.3, 53.6, 57.6, 60.7, 61.1, 61.8, 64.7, 127.5, 128.5, 128.6, 135.5, 136.3, 157.8, 158.9, 169.5, 170.4. $[\alpha]_D$ −5.3 (c 1, $CHCl_3$). Anal. calcd for $C_{36}H_{49}ClN_4O_4$: C, 67.85; H, 8.79; Cl, 5.56. Found: C, 68.1; H, 8.81; Cl, 5.55%.

From the reaction mixture the following sub-products were isolated.

***cis–trans* derivative (3*S*′,6*S*′,3″*R*,6″*S*)**: ^{1}H NMR δ: 0.92 (d, 6H, J=7); 1.05–1.11 (m, 6H); 1.23–1.31 (m, 6H); 1.6–2.28 (m, 8H); 3.68 (m, 2H); 3.78 (d, 1H, J=2.2); 3.9–4.2 (m, 7H); 5.47 (d, 1H, J=15); 5.63 (d, 1H, J=15); 7.28 (m, 10ArH). ^{13}C NMR δ: 14.3, 17, 17.9, 20.6, 20.7, 21.7, 29.4, 30.4, 36.7, 39.5, 46.6, 47, 53.7, 59.6, 60.6, 60.9, 61.1, 64.7, 127.5, 127.9, 128.1, 128.6, 128.7, 135.4, 136, 157.2, 158, 169.5, 170.1.

Dialkylated product (3′*R*,6′*S*,3″*R*,6″*S*)-3′,3″dichloromethyl derivative: ^{1}H NMR δ: 0.91 (d, 6H, J=7); 1.07 (d, 6H, J=7); 1.28 (t, 6H, J=7.2); 1.5–1.7 (m, 2H); 1.8–2.0 (m, 4H); 2.18–2.35 (m, 2H); 3.61 (d, 2H, J=10); 3.79 (d, 2H, J=2.4); 4.01 (d, 2H, J=15); 4.15 (m, 6H); 5.60 (d, 2H, J=15); 7.34 (m, 10ArH). ^{13}C NMR δ: 14.2, 16.9, 18.5, 20.6, 29.3, 39.8, 46.6, 53.6, 60.7, 61, 64.5, 127.6, 128.5, 135.3, 158, 169.3. $[\alpha]_D$ −59.7 (c 0.63, $CHCl_3$).

4.7.4. 1-[(3′*S*,6′*S*)-1′-Benzyl-5′-ethoxy-6′-hydro-6′-isopropyl-3′-methyl-pyrazin-3′-yl-2′-one]-3-[(3″*R*,6″*S*)-1″-benzyl-5″-ethoxy-3″,6″-dihydro-6″-isopropylpyrazin-3″-yl-2″-one] propane 9d. Iodomethane was used as alkylating reagent. ^{1}H NMR δ: 0.88 (d, 3H, J=7); 0.93 (d, 3H, J=7); 1.05 (d, 3H, J=7); 1.07 (d, 3H, J=7); 1.21 (t, 3H, J=7.4); 1.25 (t, 3H, J=7.4); 1.44 (s, 3H); 1.5–2.1 (m, 6H); 2.15–2.3 (m, 2H); 3.67 (dd, 1H, J=1.4, 3.6); 3.73 (d, 1H, J=2.6); 3.85–4.2 (m, 7H); 5.51 (d, 2H, J=15); 7.3 (m, 10ArH). ^{13}C NMR δ: 14.3, 17.4, 17.5, 17.6, 19.8, 20.1, 20.8, 28.8, 30.1, 31.6, 34.3, 41.2, 46.7, 47.2, 57.7, 60.6, 61, 61.2, 61.7, 127.4, 127.8, 127.9, 128.6, 136.4, 136.5, 154.9, 158.7, 170.6, 173.3. $[\alpha]_D$ +9.1 (c 0.316, $CHCl_3$). Anal. calcd for $C_{36}H_{50}N_4O_4$: C, 71.73; H, 8.36; N, 9.29. Found: C, 71.95; H, 8.38; N, 9.25%.

4.7.5. 1-[(3′*R*,6′*S*)-1′,3′-Dibenzyl-5′-ethoxy-6′-hydro-6′-isopropylpyrazin-3′-yl-2′-one]-3-[(3″*R*,6″*S*)-1″-benzyl-5″-ethoxy-3″,6″-dihydro-6″-isopropylpyrazin-3″-yl-2″-one] propane 9e. Benzyl bromide was used as the alkylating reagent. ^{1}H NMR δ: 0.8 (d, 3H, J=6.9); 0.91 (d, 3H, J=6.9); 0.96 (d, 3H, J=6.9); 1.08 (d, 3H, J=6.9); 1.28 (t, 6H, J=6.9); 1.65–2.3 (m, 8H); 3.03 (d, 1H, J=12.5); 3.21 (d, 1H, J=2.4); 3.34 (d, 1H, J=12.5); 3.72 (dd, 1H, J=1.8, 3.9); 3.88 (d, 1H, J=15.6); 3.95 (d, 1H, J=15); 4.25 (m, 5H); 5.25 (d, 1H, J=15); 5.53 (d, 1H, J=15.6); 7 (m, 15ArH). ^{13}C NMR δ: 14.2, 14.3, 16.8, 17.6, 19.9, 20, 20.5, 29.2, 31.6, 34.3, 41.9, 46.1, 46.4, 47.3, 57.7, 60.2, 60.5, 61, 61.8, 65.2, 126.1, 127.1, 127.5, 127.8, 127.9, 128, 128.4, 128.7, 130.8, 135.5, 136.5, 137.9, 156, 158.9, 170.7, 171.

4.8. General procedure for the Birch reaction on intermediates 5 and 9a–d

To a stirred solution of Na (0.435 g, 18.9 mmol) dissolved in liquid ammonia (60 mL), cooled at –50°C under an inert atmosphere, was added a solution of intermediate **5** or **9a–d** (1.35 mmol) in THF/t-butanol 9:1 (10 mL). After 5 minutes the reaction was quenched with NH_4Cl (1.07 g) and the cooling bath was removed allowing the complete removal of NH_3. After addition of water, the aqueous solution was extracted with ethyl acetate and the organic solution evaporated to dryness under vacuum. The product was recovered in very high yield.

4.8.1. 1-[(3′*R*,6′*S*)-5′-Ethoxy-3′,6′-dihydro 6′-isopropyl-1′*H*-pyrazin-3′-yl-2′-one]-3-[(3″*R*,6″*S*)-5″-ethoxy-3″,6″-dihydro-6″-isopropyl-1″*H*-pyrazin-3″-yl-2″-one] propane 7. Starting from **5**, the pure product was isolated in 95% yield. ^{1}H NMR δ: 0.87 (d, 6H, J=7); 1 (d, 6H, J=7); 1.29 (t, 6H, J=7); 1.4–1.65 (m, 2H); 1.9 (m, 4H); 2.15–2.35 (m, 2H); 3.9 (dd, 2H, J=2.2, 5.2); 4.03 (m, 2H); 4.09–4.21 (m, 4H), 6.07 (bs, 2H). ^{13}C NMR δ: 14.2, 16.2, 18.2, 21, 32, 34.1, 57.6, 58.4, 61.1, 158, 172.4. $[\alpha]_D$ +108 (c 1, $CHCl_3$).

4.8.2. 1-[(3′*R*,6′*S*)-3′-Allyl-5′-ethoxy-6′-hydro-6′-isopropyl-1′*H*-pyrazin-3′-yl-2′-one]-3-[(3″*R*,6″*S*)-5″-ethoxy-3″,6″-dihydro-6″-isopropyl-1″*H*-pyrazin-3″-yl-2″-one] propane 10a. Starting from **9a**, the pure product was isolated in 90% yield. ^{1}H NMR δ: 0.81 (d, 3H, J=6.9); 0.82 (d, 3H, J=6.9); 0.96 (d, 3H, J=7.2); 0.98 (d, 3H, J=7.2); 1.27 (m, 6H); 1.5–1.7 (m, 1H); 1.75–1.9 (m, 2H); 1.98 (m, 2H); 2.15–2.35 (m, 4H); 2.62 (dd, 1H, J=7.2, 12.9); 3.82 (dd, 1H, J=2.1, 4.8); 3.91 (dd, 1H, J=1.2, 2.7); 4.0–4.21 (m, 5H); 5.05 (m, 2H); 5.58–5.72 (m, 1H); 6.4–6.8 (bs, 2H). ^{13}C NMR δ: 14.3, 15.9, 16.7, 18.3, 19.7, 30.7, 32.2, 34.2, 39.8, 46, 57.5, 58.1, 60.8, 60.9, 63.8, 118.3, 133.3, 157.1, 158.2, 172.4, 173.3. $[\alpha]_D$ +94.3 (c 1, $CHCl_3$).

4.8.3. 1-[(3′*R*,6′*S*)-3′-Carboxymethylen-5′-ethoxy-6′-hydro-6′-isopropyl-1′*H*-pyrazin-3′-yl-2′-one]-3-[(3″*R*,6″*S*)-5″-ethoxy-3″,6″-dihydro-6″-isopropyl-1″*H*-pyrazin-3″-yl-2″-one] propane 10b. Starting from **9b**, the pure product was isolated in 90% yield. ^{1}H NMR δ: 0.66 (d, 3H, J=6.6); 0.68 (d, 3H, J=6.6); 0.83 (t, 6H, J=7.2); 0.9–1.05 (m, 2H); 1.15 (m, 6H); 1.25–1.45 (m, 2H); 1.5–1.75 (m, 2H); 1.8–2.1 (m, 2H); 2.27 (d, 1H, J=16); 2.73 (d, 1H, J=15.8); 3.76 (t, 1H, J=2.6); 3.87–3.99 (m, 4H); 4.0–4.15 (m, 2H). ^{13}C NMR δ: 14.7, 17.3, 17.8, 18.4, 18.7, 20.6, 24.4, 31.4, 34, 34.4, 41.6, 49.7, 49.8, 58.3, 59.4, 62.1, 62.3, 63.7, 159.7, 160.3, 173.6, 177.1, 178.6.

4.8.4. 1-[(3′*S*,6′*S*)-5′-Ethoxy-6′-hydro-6′-isopropyl-3′-methyl-1′*H*-pyrazin-3′-yl-2′-one]-3-[(3″*R*,6″*S*)-5″-ethoxy-3″,6″-dihydro-6″-isopropyl-1″*H*-pyrazin-3″-yl-2″-one] propane 10c. Starting from **9c**, the pure product was isolated in 95% yield. ^{1}H NMR δ: 0.79 (d, 3H, J=7); 0.83 (d, 3H, J=7); 0.95 (d, 3H, J=7.2); 1.03 (d, 3H, J=7.2); 1.25 (t, 3H, J=7); 1.26 (t, 3H, J=7); 1.33 (s, 3H); 1.4–2.37 (m, 8H); 3.78 (dd, 1H, J=2.2, 4.8); 3.94 (dd, 1H, J=1, 2.8); 3.4–4.16 (m, 5H); 7.77 (bs, 1H); 7.93 (bs, 1H). ^{13}C NMR δ: 14.2, 15.9, 16.1, 16.6, 18.2, 18.4, 20.1, 29, 30.8, 32, 34.2, 40.6, 57.4, 58.1, 58.3, 60.7, 60.9, 61, 156, 158.1, 172.5, 174.7. $[\alpha]_D$ +58.3 (c 1.01, CH_3OH).

4.9. General procedure for the hydrolysis of 7 and 10a–c to tripeptides

The hydrolysis was performed by heating **7** or **10a–c** (0.7 mmol) at 60°C in aqueous HCl (1 M, 5 mL). The reaction was monitored by TLC and by ^{1}H NMR. After about 40 h the acid solution was evaporated in vacuo and the residue submitted to NMR analysis. The pure product was isolated in practically quantitative yield.

4.9.1. Tripeptide [(HO)Val-(2*R*,6*R*)-DAP-Val(OH)]·2HCl 8. Compound **8** was obtained as a solid (decomposes at 148°C) starting from **7**. ^{1}H NMR δ (D_2O): 0.80 (d, 12H, J=6.9); 1.2–1.35 (m, 2H); 1.7–1.9 (m, 4H); 2.0–2.12 (m, 2H); 3.97 (t, 2H, J=6.3); 4.1 (d, 2H, J=5.4). ^{13}C NMR δ (D_2O): 18.1, 19.3, 20.3, 30.3, 31.2, 53.4, 59.3, 170.1, 175.4. $[\alpha]_D$ –56.7 (c 1.014, H_2O). Anal. calcd for $C_{17}H_{34}Cl_2N_4O_6$: C, 44.26; H, 7.43; Cl, 15.37. Found: C, 44.42; H, 7.45; Cl, 15.32%.

4.9.2. Tripeptide [(HO)Val-(2*R*,6*R*)-2-allyl-2,6-DAP-Val(OH)]·2HCl 11a. **11a** was obtained as a solid (decomposes at 138°C) starting from **10a**. ^{1}H NMR δ (D_2O): 0.8 (m, 12H); 1.2–1.5 (m, 2H); 1.7–2.2 (m, 6H); 2.58 (dd, 1H, $J=7.4$, 14.4); 2.74 (dd, 1H, $J=6.6$, 14.4); 4 (m, 1H); 4.15 (m, 2H), 5.18–5.25 (m, 2H); 5.50–5.67 (m, 1H). ^{13}C NMR δ (D_2O): 18, 18.6, 18.9, 19.2, 30.4, 31.5, 35.6, 40.8, 53.4, 59.2, 59.9, 64, 66.2, 66.6, 123.7, 129, 170.2, 171.1, 175.3, 175.4. $[\alpha]_D$ –26.1 (*c* 0.6, 1N HCl). Anal. calcd for $C_{20}H_{38}Cl_2N_4O_6$: C, 47.9; H, 7.64; Cl, 14.14. Found: C, 48.1; H, 7.66; Cl, 14.1%.

4.9.3. Tripeptide [(HO)Val-(2*R*,6*R*)-2-carboxymethylene-2,6-DAP-Val(OH)]·2HCl 11b. **11b** was obtained as an oil starting from **10b**. ^{1}H NMR δ (D_2O): 0.78–0.84 (m, 12H); 1.1–1.5 (m, 2H); 1.72 (m, 2H); 1.95 (m, 2H); 2.13 (m, 2H); 3.04 (d, 1H, $J=18.6$); 3.33 (d, 1H, $J=18.6$); 4.02 (t, 1H, $J=6.3$); 4.2 (d, 1H, $J=5.7$); 4.22 (d, 1H, $J=5.1$). ^{13}C NMR δ (D_2O): 17.9, 18.1, 18.4, 19.1, 30.3, 31.2, 36, 39.5, 53.4, 59.2, 59.7, 61.5, 170.4, 170.8, 173.2, 175.3, 175.5. $[\alpha]_D$ –41 (*c* 1.16, 1N HCl). Anal. calcd for $C_{19}H_{36}Cl_2N_4O_8$: C, 43.94; H, 6.99; Cl, 13.65. Found: C, 43.1; H, 7.12; Cl, 13.6%.

4.9.4. Tripeptide [(HO)Val-(2*S*,6*R*)-2-methyl-2,6-DAP-Val(OH)]·2HCl 11c. **11c** was obtained as a solid (decomposes at 131°C) starting from **10c**. ^{1}H NMR δ (D_2O): 0.84 (m, 12H); 1.1–1.4 (m, 12H); 1.56 (s, 3H); 1.75–1.94 (m, 4H); 2.05–2.15 (m, 2H); 4.0 (t, 1H, $J=6.2$); 4.18 (m, 2H). ^{13}C NMR δ (D_2O): 18, 18.1, 18.5, 19, 19.2, 19.3, 22.6, 30.4, 31.4, 37.1, 53.4, 59.1, 59.6, 61, 170.2, 172.2, 175.2, 175.3. $[\alpha]_D$ –29.5 (*c* 0.51, 1N HCl). Anal. calcd for $C_{18}H_{36}Cl_2N_4O_6$: C, 45.48; H, 7.63; Cl, 14.91. Found: C, 45.58; H, 7.65; Cl, 14.85%.

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References

1. (a) Porzi, G.; Sandri, S. *Tetrahedron: Asymmetry* **1994**, *5*, 453; (b) D′Arrigo, M. C.; Porzi, G.; Sandri, S. *J. Chem. Res. (S)* **1995**, 430; (c) Porzi, G.; Sandri, S. *Tetrahedron: Asymmetry* **1996**, *7*, 189; (d) Porzi, G.; Sandri, S. *Tetrahedron: Asymmetry* **1998**, *9*, 3411; (e) Di Felice, P.; Maestri, M.; Paradisi, F.; Porzi, G.; Sandri, S. *Tetrahedron: Asymmetry* **1999**, *10*, 4709.
2. (a) Favero, V.; Porzi, G.; Sandri, S. *Tetrahedron: Asymmetry* **1997**, *8*, 599; (b) Di Felice, P.; Porzi, G.; Sandri, S. *Tetrahedron: Asymmetry* **1999**, *10*, 2191.
3. Porzi, G.; Sandri, S.; Verrocchio, P. *Tetrahedron: Asymmetry* **1998**, *9*, 119 and references cited therein.
4. Cox, R. J. *Nat. Prod. Rep*. **1996**, *13*, 29.
5. Paradisi, F.; Porzi, G.; Rinaldi, S.; Sandri, S. *Tetrahedron: Asymmetry* **2000**, *11*, 1259–1262.
6. Paradisi, F.; Porzi, G.; Rinaldi, S.; Sandri, S. *Tetrahedron: Asymmetry* **2000**, *11*, 4617–4622.
7. Lange, M.; Undheim, K. *Tetrahedron* **1998**, *54*, 5337 and references cited therein.
8. Bouchaudon, J.; Dutruc-Rosset, G.; Farge, D.; James, C. *J. Chem. Soc., Perkin Trans. 1* **1989**, 695.
9. Fischer, P. M.; Solbakken, M.; Undheim, K. *Tetrahedron* **1994**, *50*, 2277.
10. Meerwein, H. *Org. Synth*. **1966**, *46*, 113.