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Synthesis of cyclopropanoid 2-*epi*-muramyldipeptide analogues as potential immunostimulants

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Abstract—The preparation of cyclopropanoid 2-*epi*-muramyldipeptide analogues from suitable substituted cyclopropylamines is described.

1. Introduction

Over the past two decades intensive investigation of muramyldipeptide (*N*-acetylmuramyl-L-alanyl-D-isoglutamine, MDP) derivatives revealed their adjuvant activity and stimulation of non-specific resistance against bacterial, viral and parasite infections as well as anticancer properties.[1,2] They can also stimulate tumoricidal activity of monocytes[3,4] and of macrophages.[5,6]

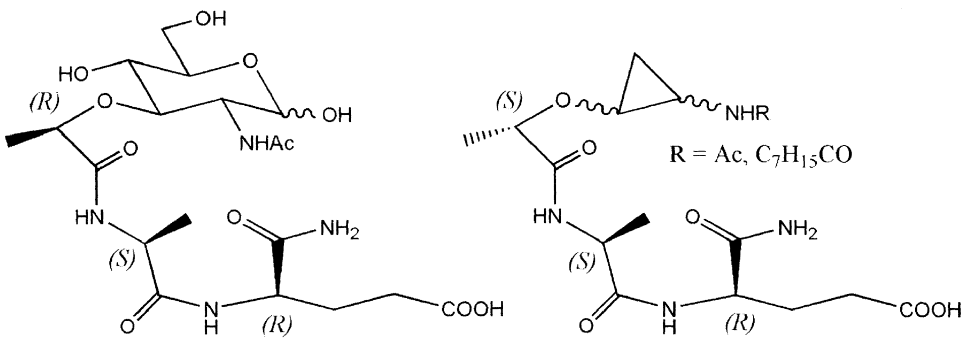
Many MDP derivatives and analogues have been synthesized and evaluated biologically in order to obtain new molecules with improved pharmacological properties.[1,2,7–19] (Fig. 1)

2. Results and discussion

During ongoing QSAR studies of these compounds we became interested in derivatives possessing a (*S*) configuration at the lactic acid residue (Scheme 1).

Although in MDP this stereogenic center is of (*R*) configuration it is of interest to note that the chirality at this center seems to be of minor importance as, e.g., *nor*-MDP derivatives where there is no methyl group present at all at this center, are known to exhibit equal biological activities and even reduced toxicity.[7,8]

Our approach to these (2*S*) configurated cyclopropanoid analogues started from commercially available isopropyl



MDP **cyclopropanoid 2-*epi*-MDP analogues**

Figure 1. Structure of MDP and its 2-*epi*-cyclopropanoid analogues.

Keywords: Cyclopropanoid 2-*epi*-muramyldepeptide; Immunostimulant; Pharmacological.
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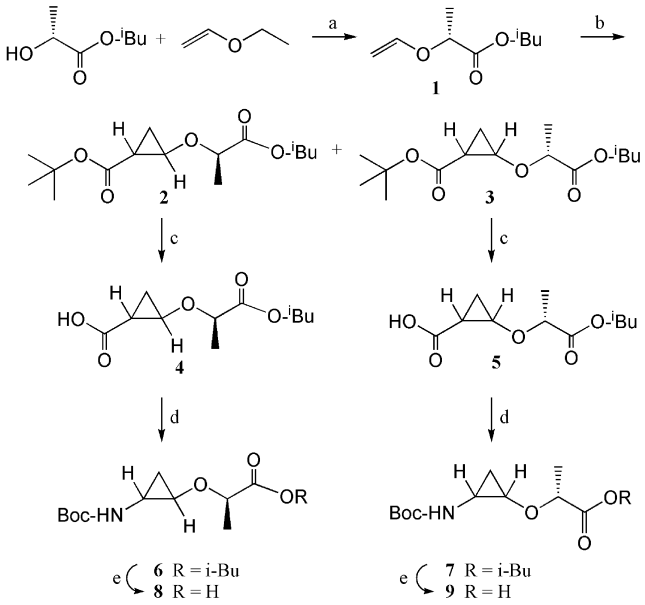


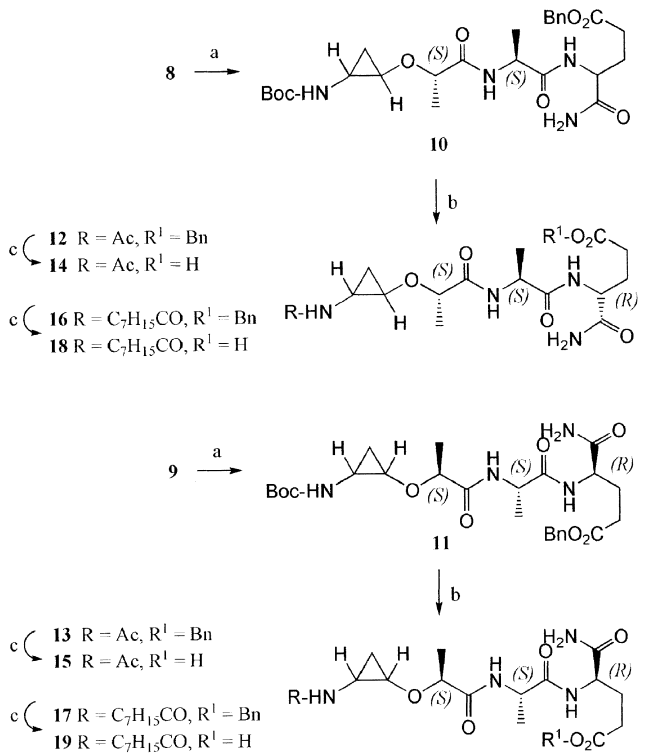
Scheme 1. Reagents: (a) $Hg(OAc)_2$; (b) N_2CHCO_2tBu, $[(Rh(OAc)_2)_2]$; (c) CF_3COOH; (d) DPPA, *t*BuOH, Et_3N; (e) KOH.

(*S*)-2-hydroxypropanoate whose $Hg(OAc)_2$ mediated reaction[20,21] with ethylvinylether resulted in the formation of isopropyl (2*S*)-2-(vinyloxy)propanoate (**1**) whose $Rh[(OAc)_2]_2$ catalyzed reaction with *tert*-butyldiazoacetate[22] yielded a mixture of the cyclopropanes *trans*-**2** and *cis*-**3** that were easily separated by chromatography. Hydrolysis of the ester by treatment of *trans*-**2** with trifluoroacetic acid[23,24] gave the acid *trans*-**4**; similarly from the ester *cis*-**3** the *cis*-configurated acid *cis*-**5** was obtained.

A modified *Curtius* degradation[25] of the acid **4** using diphenylphosphoryl azide (DPPA)/*tert*-butanol/triethylamine gave the *N*-BOC-protected cyclopropylamine *trans*-**6**. In an analogous manner from the *cis*-configurated acid **5** the *N*-BOC-protected amine *cis*-**7** was obtained together with ca 14% of *trans*-**6** invariably formed by epimerization during the reaction.

Alkaline saponification of the ester moiety gave the acids *trans*-**8** and *cis*-**9**, respectively. These acids were subjected to a peptide synthesis using the mixed anhydride method [isobutyl chloroformate/*N*-methyl-morpholine (NMM)][26] and H_2N-L-Ala-D-iGln-γ-OBn as dipeptide. Thus the products *trans*-**10** and *cis*-**11** were obtained in reasonably high yields. Treatment of *trans*-**10** with hydrochloric acid followed by acetylation with acetylchloride/triethylamine gave a mixture of the corresponding *N*-acetyl-derivatives *trans*-**12a**/*trans*-**12b** that could be separated by chromatography. In contrast, treatment of *cis*-**11** with hydrochloric acid followed by acetylation gave a mixture of diastereomeric acetates *cis*-**13** that could not be separated under a variety of different chromatographic conditions.

Debenzylation of *trans*-**12a** and *trans*-**12b** was accomplished by hydrogenolysis in the presence of Pd/C to afford *trans*-**14a** and *trans*-**14b**, respectively. Under the same conditions from *cis*-**13** the final target compound *cis*-**15** was obtained in 95% yield.



Scheme 2. Reactions and conditions: (a) $ClCO_2iBu$, NMM, L-Ala-D-iGln-γ-OBn hydrochloride; (b) HCl in EtOAc then AcCl/Et_3N (for **12** and **13**) or $C_7H_{15}COCl/Et_3N$ (for **16** and **17**); (c) Pd/C, H_2.

Since it has been assumed[27] that lipophilic MDP derivatives induce cellular-specific response and increase non-specific resistance more strongly, the synthesis of more lipophilic derivatives was accomplished by deprotection of *trans*-**10** followed by acylation with octanoyl chloride/triethylamine to afford the corresponding *N*-octanoyl-derivative *trans*-**16**. Similarly from *cis*-**11** lipophilic *cis*-**17** was obtained in 63% yield. Hydrogenolysis of *trans*-**16** or *cis*-**17** gave the final products *trans*-**18** or *trans*-**19** in good yields (Scheme 2).

The determination of the different biological activities of these carbocyclic 2-*epi*-MDP-derivatives as well as the determination of the absolute configuration of the stereogenic centers of the cyclopropane ring are presently performed in our labs.

3. Experimental

3.1. General

Melting points are uncorrected (*Leica*hot stage microscope), optical rotations were obtained using a Perkin–Elmer 341 polarimeter (1 cm micro cell), NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000 or Unity 500 (δ given in ppm, J in Hz, internal Me_4Si, Cp correspond to the atoms of the cyclopropane), IR spectra (film or KBr pellet) on a Perkin–Elmer FT-IR spectrometer Spectrum 1000, MS spectra were taken on a Intectra GmbH AMD 402 (electron impact, 70 eV) or on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument; for elemental analysis a Foss–Heraeus Vario EL instrument was used; TLC was

performed on silica gel (Merck 5554, detection by UV absorption or by treatment with either a solution of 10% sulfuric acid, ammonium molybdate and cerium(IV)) sulfate or a solution of ninhydrine in pyridine followed by gentle heating. The solvents were dried according to usual procedures.

3.1.1. Isopropyl (2*S*)-2-(vinyloxy)propanoate (1). A solution of isopropyl (*S*)-2-hydroxypropanoate (19.8 g, 0.15 mol) and $Hg(OAc)_2$ (47.8 g, 0.15 mol) in ethylvinylether (450 ml, 4.70 mol) was stirred for 7 days at 25 °C under argon. The reaction mixture was diluted with hexane (470 ml) and washed with N aq. KOH solution and brine, dried (Na_2SO_4) and the solvents were removed under diminished pressure. The residue was subjected to chromatography (silica gel, hexane/ethyl acetate 10:1) to afford **1** (11.7 g, 49%) as an oil; R_f (hexane/ethyl acetate 3:2) 0.61; $[\alpha]_D=-68.3$ (*c* 1.01, $CHCl_3$); IR (film): ν=3120*w*, 2985*s*, 2940*m*, 2880*w*, 1755*s*, 1730*s*, 1640*s*, 1620*s*, 1510*m*, 1455*m*, 1375*s*, 1320*s*, 1280*s*, 1190*s*, 1135*s*, 1110*s*, 1045*s*; ^{1}H NMR (200 MHz, $CDCl_3$): δ=6.38 (*dd*, *J*=14.3, 6.8 Hz, 1H, HC=C), 5.07 (*qq*, *J*=6.3 Hz, 1H, CH(*i*Pr)), 4.33 (*q*, *J*=6.8 Hz, 1H, H–C(2)), 4.19 (*dd*, *J*=14.3, −2.5 Hz, 1H, H_2C=C(*trans*)), 4.06 (*dd*, *J*=6.8, −2.5 Hz, 1H, H_2C=C(*cis*)), 1.45 (*d*, *J*=6.8 Hz, 3H, Me), 1.25 (*d*, *J*=6.3 Hz, 3H, Me_A(*i*Pr)), 1.23 (*d*, *J*=6.3 Hz, 3H, Me_B(*i*Pr)); ^{13}C NMR (100 MHz, $CDCl_3$): δ=171.5 (*s*, C=O), 150.3 (*d*, =CH), 88.4 (*t*, $=CH_2$), 72.7 (*d*, C(2)), 68.6 (*d*, CH(*i*Pr)), 21.5 (*q*, Me_A(*i*Pr)), 21.4 (*q*, Me_B(*i*Pr)), 17.7 (*q*, Me); MS (GC–MS, e.i., 70 eV): *m*/*z* (%)=158 (15), 144 (1), 130 (1), 116 (20), 99 (1), 89 (1), 71 (80), 43 (100); Anal. calcd for $C_8H_{14}O_3$ (158.19): C, 60.74; H, 8.92; found: C, 60.88; H, 9.03.

3.1.2. Isopropyl *trans*-(2*S*)-2-[2-(*tert*-butoxycarbonyl) cyclopropyl]-oxypropanoate (*trans*-2) and isopropyl *cis*-(2*S*)-2-[2-(*tert*-butoxycarbonyl)cyclopropyl]oxy-propanoate (*cis*-3). To solution of **1** (8.7 g, 55.0 mmol) in dry CH_2Cl_2 (15 ml) containing $[Rh(OAc)_2)_2]$ (100 mg) a solution of *tert*-butyl diazoacetate (9.4 g, 66.1 mmol) in abs. CH_2Cl_2 (30 ml) was slowly added within 8 h keeping the temperature at 25 °C. Then the solvents were removed under diminished pressure and the residue was subjected to chromatography (silica gel, hexane/ethyl acetate 16:1) to afford *trans*-**2** (7.4 g, 49%) and *cis*-**3** (4.2 g, 28%).

Data for **2**. Oil; R_f (hexane/ethyl acetate 3:2) 0.64; IR (film): ν=2980*s*, 2935*m*, 1750*s*, 1720*s*, 1450*s*, 1395*s*, 1375*s*, 1345*s*, 1275*s*, 1205*s*, 1155*s*,1105*s*, 1045*s*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.07 (*qq*, *J*=6.3 Hz, 1H, CH(*i*Pr)), 4.03 (*q*, *J*=6.8 Hz, 1H, H–C(2)), 3.66 (*ddd*, *J*=6.4, 4.2, 2.3 Hz, 1H, H–C(1) Cp, A), 3.64 (*ddd*, *J*=6.4, 4.7, 2.0 Hz, 1H, H–C(1), Cp, B), 1.83 (*ddd*, *J*=9.0, 6.8, 2.1 Hz, 1H, H–C(2), Cp, A), 1.69 (*ddd*, *J*=9.7, 6.2, 2.1 Hz, 1H, H–C(2), Cp, B), 1.401 (*s*, 9H, *t*Bu, A), 1.396 (*s*, 9H, *t*Bu, B), 1.35 (*d*, *J*=6.8 Hz, 3H, Me, A), 1.34 (*d*, *J*=6.8 Hz, 3H, Me, B), 1.27 (*d*, *J*=6.3 Hz, 3H, Me (*i*Pr)), 1.26 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.25 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.24 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.22–1.16 (*m*, 1H, H_A–C(3) Cp), 1.15–1.10 (*m*, 1H, H_B–C(3) Cp)); ^{13}C NMR (50 MHz, $CDCl_3$): δ=172.1 (*s*, C=O), 171.4 (*s*, C=O), 80.43 (*s*, *t*Bu, A), 80.39 (*s*, *t*Bu, B), 75.6 (*d*, C(2), A), 75.4 (*d*, C(2), B), 68.5 (*d*, CH(*i*Pr)), 59.6 (*d*, C(1) Cp)), 28.0 (*q*, *t*Bu), 22.3 (*d*, C(2), Cp, A), 22.0 (*d*, C(2), Cp, B),21.7 (*q*, Me(*i*Pr)), 21.6 (*q*, Me(*i*Pr)), 18.3 (*q*, Me), 15.5 (*dd*, C(3), Cp, A), 14.6 (*dd*, C(3), Cp, B); MS (GC–MS, e.i., 70 eV): *m*/*z* (%)=216 (5), 199 (10), 185 (20), 174 (35), 157 (16), 145 (16), 133 (29), 129 (27), 101 (27), 91 (73), 84 (100), 73 (25), 57 (33); Anal. calcd for $C_{14}H_{24}O_5$ (272.16): C, 61.74; H, 8.88; found: C, 61.63; H, 8.71.

Data for **3**. Oil; R_f (hexane/ethyl acetate 3:2) 0.58; IR (film): ν=2980*s*, 2935*m*, 1730*s*, 1455*m*, 1380*s*, 1370*s*, 1330*m*, 1280*m*, 1255*m*, 1205*s*, 1145*s*, 1105*s*, 1055*m*, 1020*w*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.08 (*qq*, *J*=6.3 Hz, 1H, CH(*i*Pr), A), 5.06 (*qq*, ^3J=6.3 Hz, 1H, CH(*i*Pr), B), 3.99 (*q*, *J*=6.9 Hz, 1H, H–C(2), A), 3.82 (*q*, *J*=6.9 Hz, 1H, H–C(2), B), 3.76 (*ddd*, *J*=6.7, 6.7, 3.9 Hz, 1H, H–C(1), Cp, A), 3.70 (*ddd*, *J*=6.5, 6.5, 4.7 Hz, 1H, H–C(1), Cp, B), 1.69 (*ddd*, *J*=8.4, 6.8, 6.8 Hz, 1H, H–C(2), Cp, A), 1.58–1.54 (*m*, 2H, H–C(2), Cp, B and H_A–C(3), Cp, A), 1.46 (*s*, 9H, *t*Bu, A), 1.43 (*s*, 9H, *t*Bu, B), 1.42–1.39 (*m*, 1H, HA–C(3), Cp, B), 1.34 (*d*, *J*=6.9 Hz, 3H, Me, A), 1.31 (*d*, *J*=6.9 Hz, 3H, Me, B), 1.27 (*d*, *J*=7.2 Hz, 3H, Me(*i*Pr)), 1.25 (*d*, *J*=6.6 Hz, 3H, Me(*i*Pr)), 1.245 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.24 (*d*, *J*=6.5 Hz, 3H, Me(*i*Pr)), 1.08–1.01 (*m*, 1H, H_B–C(3), Cp, A), 0.87 (*ddd*, *J*=8.6, 6.2, 6.2 Hz, 1H, HB–C(3), Cp, B); ^{13}C NMR (50 MHz, CDCl3): δ=172.2 (*s*, C=O), 168.9 (*s*, C=O, A), 168.4 (*s*, C=O), B), 80.3 (*s*, *t*Bu), 75.6 (*d*, C(2), A), 74.9 (*d*, C(2), B), 68.4 (*d*, CH(*i*Pr), A), 68.2 (*d*, CH(*i*Pr), B), 58.3 (*d*, C(1), Cp, A), 56.8 (*d*, C(1), Cp, B), 28.1 (*q*, *t*Bu), 22.9 (*d*, C(2), Cp), 21.7 (*q*, Me(*i*Pr)), 18.5 (*q*, Me, A), 17.8 (*q*, Me, B), 13.5 (*dd*, C(3), Cp, A), 11.7 (*dd*, C(3), Cp, B); MS (GC–MS, e.i., 70 eV): *m*/*z* (%)=216 (4), 199 (10), 185 (2), 174 (33), 157 (19), 145 (15), 133 (29), 129 (46), 117 (5), 101 (28), 91 (66), 84 (100), 73 (28), 57 (41); Anal. calcd for $C_{14}H_{24}O_5$ (272.16): C, 61.74; H, 8.88; found: C, 61.66; H, 8.74.

3.1.3. *trans*-2-[(1*S*)-2-Isopropoxy-1-methyl-2-oxoethyl] oxy-1-cyclopropanecarboxylic acid (*trans*-4). To a solution of **2** (3.70 g, 13.6 mmol) in dry CH_2Cl_2 (30 ml) a solution of CF_3COOH (9.30 g, 81.6 mmol) in dry CH_2Cl_2 (6 ml) was added at 0 °C and stirring was continued for another 18 h. The solvents were removed under reduced pressure and the residue was dissolved in toluene, the solvent removed and the crude acid *trans*-**4** (2.90 g, 99%) was obtained as a slightly brown oil that was used for the next step without any further purification; IR (film): ν=2985*s*, 2940*m*, 2645*m*, 1730*s*, 1695*s*, 1455*s*, 1375*s*, 1285*s*, 1205*s*, 1180*s*, 1140*s*, 1105*s*, 1045*m*, 1015*m*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.08 (*qq*, *J*=6.3 Hz, 1H, CH(*i*Pr), A), 5.07 (*qq*, *J*=6.3 Hz, 1H, CH(*i*Pr), B), 4.05 (*q*, *J*=6.8 Hz, 1H, H–C(2), A), 4.04 (*q*, *J*=6.8 Hz, 1H, H–C(2), B), 3.80 (*ddd*, *J*=6.5, 4.4, 2.1 Hz, 1H, H–C(1), Cp, A), 3.74 (*ddd*, *J*=6.5, 4.4, 2.1 Hz, 1H, H–C(1), Cp, B), 1.92 (*dd*, *J*=9.6, 6.2, 2.0 Hz, 1H, H–C(2), Cp, A), 1.76 (*ddd*, *J*=9.6, 6.1, 2.0 Hz, 1H, H–C(2), Cp, B), 1.43–1.23 (*m*, 2H, $H_{A,B}$–C(3), Cp), 1.37 (*d*, *J*=6.8 Hz, 3H, Me, A), 1.35 (*d*, *J*=7.0 Hz, 3H, Me, B), 1.259 (*d*, *J*=6.3 Hz, 6H, 2×Me(*i*Pr)), 1.256 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.250 (*d*, *J*=6.1 Hz, 3H, Me(*i*Pr)); ^{13}C NMR (50 MHz, $CDCl_3$): δ=178.5 (*s*, COOH), 172.0 (*s*, C=O, A), 171.8 (*s*, C=O, B), 75.8 (*d*, C(2), A), 75.5 (*d*, C(2), B), 68.8 (*d*, CH(*i*Pr)), 60.5 (*d*, C(1), Cp), 21.6 (*q*, Me(*i*Pr)), 21.2 (*d*, C(2), Cp, A), 20.9 (*d*, C(2), Cp, B), 18.4 (*q*, Me, A), 18.2 (*q*, Me, B), 16.6 (*dd*, C(3), Cp, A), 15.7

(*dd*, C(3), Cp, B); MS (e.i., 70 eV): *m*/*z* (%)=217 (1), 199 (1), 174 (2), 156 (9), 145 (6), 129 (43), 117 (5), 102 (22), 91 (42), 84 (100), 73 (87), 55 (44); HRMS calcd for $C_{10}H_{16}O_5$: 216.0998; found: 216.0998.

3.1.4. *cis*-2-[(1*S*)-2-Isopropoxy-1-methyl-2-oxoethyl]oxy-1-cyclopropanecarboxylic acid (*cis*-5). Following the procedure given for the synthesis of **4** a solution of **3** (2.55 g, 9.4 mmol) in CH_2Cl_2 (30 ml) was treated with CF_3COOH (6.4 g, 56.1 mmol) in CH_2Cl_2 (5 ml) to afford *cis*-**5** (2.00 g, 99%) as a slightly brown oil that was used in the next step without any further purification; IR (film): ν=2985*s*, 2940*s*, 2670*m*, 1740*s*, 1700*s*, 1450*s*, 1375*s*, 1330*m*, 1280*m*, 1215*s*, 1140*s*, 1105*s*, 1050*s*, 1015*m*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.08 (*qq*, *J*=6.3 Hz, 1H, CH(*i*Pr), A), 5.07 (*qq*, *J*=6.3 Hz, 1H, CH(*i*Pr), B), 4.08 (*q*, *J*=6.9 Hz, 1H, H–C(2), A), 3.97 (*q*, *J*=6.9 Hz, 1H, H–C(2), B), 3.862 (*ddd*, *J*=6.6, 6.6, 4.6 Hz, 1H, H–C(1), Cp, A), 3.857 (*ddd*, *J*=6.5, 6.5, 4.6 Hz, 1H, H–C(1), Cp, B), 1.80 (*ddd*, *J*=9.0, 6.7, 6.7 Hz, 1H, H–C(2), Cp, A), 1.74 (*ddd*, *J*=9.1, 6.5, 6.5 Hz, 1H, H–C(2), Cp, B), 1.59 (*ddd*, *J*=6.5, 6.5, 4.7 Hz, 1H, H_A–C(3), Cp, A), 1.46 (*ddd*, *J*=6.4, 6.4, 4.9 Hz, 1H, H_A–C(3), Cp, B), 1.39 (*d*, *J*=6.9, 3H, Me, A), 1.37 (*d*, *J*=6.9, 3H, Me, B), 1.269 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.260 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.253 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.248 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.21 (*ddd*, *J*=9.1, 6.5, 6.5 Hz, 1H, H_B–C(3), Cp, A), 1.11 (*ddd*, *J*=9.1, 6.3, 6.3 Hz, 1H, H_B–C(3), Cp, B); ^{13}C NMR (100 MHz, $CDCl_3$): δ=178.3 (*s*, COOH), 172.3 (*s*, C=O), 172.1 (*s*, C=O), 75.7 (*d*, C(2), A), 75.4 (*d*, C(2), B), 68.8 (*d*, CH(*i*Pr), A), 68.6 (*d*, CH(*i*Pr), B), 59.2 (*d*, C(1), Cp, A), 57.9 (*d*, C(1), Cp, B), 21.61 (*q*, Me(*i*Pr)), 21.57 (*q*, Me(*i*Pr)), 21.3 (*d*, C(2), Cp, A), 20.1 (*d*, C(2), Cp, B), 18.4 (*q*, Me, A), 17.8 (*q*, Me, B), 14.7 (*dd*, C(3), Cp, A), 13.1 (*dd*, C(3), Cp, B); MS (e.i., 70 eV): *m*/*z* (%)=217 (1), 199 (1), 174 (2), 156 (6), 145 (7), 129 (19), 119 (2), 101 (16), 91 (19), 85 (100), 73 (25), 55 (21); HRMS calcd for $C_{10}H_{16}O_5$: 216.0998; found: 216.0997.

3.1.5. Isopropyl *trans*-(2*S*)-2-(2-[(*tert*-butoxycarbonyl) amino]cyclo-propyloxy)propanoate (*trans*-6). To a solution containing **4** (3.80 g, 17.6 mmol), triethylamine (2.67 g, 26.4 mmol) and *tert*-butanol (6.5 g, 87.7 mmol) under argon DPPA (5.81 g, 21.1 mmol) was carefully added. The mixture was heated at 80 °C for 3 h, then the solvents were removed under reduced pressure, and the residue was subjected to chromatography (silica gel, hexane/ethyl acetate 5:1) to afford oily **6** (1.9 g, 55%); R_f (hexane/ethyl acetate 3:2) 0.49; IR (film): ν=3365*m*, 2980*s*, 2935*m*, 1715*s*, 1505*s*, 1455*s*, 1390*s*, 1365*s*, 1255 s, 1165*s*, 1110*s*, 1055*s*, 1020*m*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.08 (*qq*, *J*=6.3 Hz, 1H, CH(*i*Pr), A), 5.06 (*qq*, *J*=6.3 Hz, 1H, CH(*i*Pr), B), 4.52 (*br*, 1H, NH), 4.36 (*q*, *J*=7.0 Hz, 1H, H–C(2), A), 4.16 (*q*, *J*=7.0 Hz, 1H, H–C(2), B), 3.44–3.40 (*m*, 1H, H–C(1), Cp, A), 3.38 (*ddd*, *J*=7.1, 3.9, 1.4 Hz, 1H, H–C(1), Cp, B), 2.71–2.67 (*m*, 1H, H–C(2), Cp, A), 2.56–252 (*m*, 1H, H–C(2), Cp, B), 1.41 (*s*, 9H, *t*Bu), 1.37 (*d*, *J*=7.0 Hz, 3H, Me, A), 1.34 (*d*, *J*=7.0 Hz, 3H, Me, B), 1.27 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.254 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.245 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.238 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 1.12 (*ddd*, *J*=8.8, 6.9, 3.9 Hz, 1H, H_A–C(3), Cp, A), 1.09–1.05 (*m*, 1H, H_A–C(3), Cp, B), 0.89–0.75 (*m*, 1H, H_B–C(3), Cp); ^{13}C NMR (50 MHz, $CDCl_3$): δ=172.5 (*s*, C=O, A), 172.4 (*s*, C=O, B), 156.0 (*s*, C=O, BOC, A), 155.9 (*s*, C=O, BOC, B), 79.4 (*s*, *t*Bu), 74.6 (*d*, C(2)), 68.2 (*d*, CH(*i*Pr), A), 68.1 (*d*, CH(*i*Pr), B), 58.7 (*d*, C(1), Cp), 29.6 (*d*, C(2), Cp, A), 29.1 (*d*, C(2), Cp, B), 28.3 (*q*, *t*Bu, A), 28.2 (*q*, *t*Bu, B), 21.7 (*q*, Me(*i*Pr)), 21.3 (*q*, Me(*i*Pr)), 18.6 (*q*, Me, A), 18.1 (*q*, Me, B), 15.2 (*dd*, C(3), Cp); MS (GC–MS, e.i., 70 eV): *m*/*z* (%)=231 (1), 214 (1), 200 (1), 186 (1), 172 (1), 144 (3), 126 (1), 116 (22), 100 (10), 72 (51), 57 (100); Anal. calcd for $C_{15}H_{29}NO_5$ (303.39): C, 59.38; H, 9.63; N, 4.62; found: 59.27; H, 9.91; N, 4.59.

3.1.6. Isopropyl *cis*-(2*S*)-2-(2-[(*tert*-butoxycarbonyl) amino] cyclo-propyloxy)propanoate (*cis*-7). Following the procedure for the synthesis of **6** from **5** (3.3 g, 15.3 mmol), triethylamine (2.3 g, 23.0 mmol), *tert*-butanol (4.5 g, 61.2 mmol) and DPPA (5.1 g, 18.4 mmol) followed by chromatography (silica gel, hexane/ethyl acetate 6:1) *cis*-**7** (1.0 g, 23%) was obtained as an oil; in addition, *trans*-**6** (0.6 g, 14%) was isolated. R_f (hexane/ethyl acetate 3:2) 0.41; IR (film): ν=3370*m*, 2980*s*, 2935*m*, 1715*s*, 1505*s*, 1455*m*, 1365*s*, 1275*s*, 1210*s*, 1175*s*, 1105*s*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.34 (*br*, 1H, NH, A), 5.07 (*qq*, *J*=6.3 Hz, 1H, CH(*i*Pr), A), 5.05 (*qq*, *J*=6.3 Hz, 1H, CH(*i*Pr), B), 4.80 (*br*, 1H, NH, B), 4.12 (*q*, *J*=6.8 Hz, 1H, H–C(2), A), 4.04 (*q*, *J*=6.8 Hz, 1H, H–C(2), B), 3.45–3.36 (*m*, 1H, H–C(1), Cp), 2.70–2.61 (*m*, 1H, H–C(2), Cp), 1.43 (*s*, 9H, *t*Bu), 1.36 (*d*, *J*=6.8 Hz, 3H, Me), 1.26 (*d*, *J*=6.3 Hz. 3H, Me(*i*Pr)), 1.25 (*d*, *J*=6.3 Hz, 6H, Me(*i*Pr)), 1.23 (*d*, *J*=6.3 Hz, 3H, Me(*i*Pr)), 0.95–0.89 (*m*, 1H, H_A–C(3), Cp), 0.63–0.55 (*m*, 1H, H_B–C(3), Cp); ^{13}C NMR (100 MHz, $CDCl_3$): δ=172.4 (*s*, C=O), 156.8 (*s*, C=O, BOC, A), 156.6 (*s*, C=O, BOC, B), 79.4 (*s*, *t*Bu, A), 79.2 (*s*, *t*Bu, B), 75.8 (*d*, C(2), A), 75.4 (*d*, C(2), B), 68.7 (*d*, CH(*i*Pr), A), 68.4 (*d*, CH(*i*Pr), B), 54.7 (*d*, C(1), Cp), 28.4 (*q*, *t*Bu, A), 28.3 (*q*, *t*Bu, B), 27.4 (*d*, C(2), Cp), 21.73 (*q*, Me(*i*Pr)), 21.71 (*q*, Me(*i*Pr)), 21.68 (*q*, Me(*i*Pr)), 21.65 (*q*, Me(*i*Pr)), 18.4 (*q*, Me), 13.6 (*dd*, C(3), Cp, A), 12.6 (*dd*, C(3), Cp, B); MS (e.i. 70 eV): *m*/*z* (%)=231 (1), 214 (1), 188 (1), 172 (5), 144 (17), 116 (21), 100 (9), 72 (100), 57 (91); Anal. calcd for: $C_{15}H_{29}NO_5$ (303.39): C, 59.38; H, 9.63; N, 4.62; found: C, 59.21; H, 9.79; N, 4.65.

3.1.7. *trans*-(2*S*)-2-(2-[(*tert*-Butoxycarbonyl)amino] cyclo-propyloxy)propanoic acid (*trans*-8). To an ice-cold solution of **6** (0.58 g, 1.93 mmol) in ethanol (6 ml) a solution of KOH (0.34 g, 6.0 mmol) in ethanol (10 ml) was slowly added; the mixture is allowed to warm to 25 °C and stirred at this temperature for 3 h, then the solvents were removed under reduced pressure, and water (15 ml) was added and the pH adjusted to 3. The aqueous phase was extracted with ethyl acetate (4×40 ml), the combined organic phases were dried (Na_2SO_4), and the solvents were evaporated to afford *trans*-**8** (0.45 g, 95%) that was used in the next step without any further purification; IR (film): ν=3340*m*, 2980*s*, 2935*m*, 2625*w*, 1715*s*, 1515*s*, 1455*s*, 1395*s*, 1370*s*, 1255*s*, 1220*s*, 1165*s*, 1135*s*, 1055*m*, 1021*m*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=4.64 (*br*, 1H, NH), 4.49–4.41 (*m*, 1H, H–C(2), A), 4.35–4.27 (*m*, 1H, H–C(2), B), 3.47–3.41 (*m*, 1H, H–C(1), Cp), 2.72–2.68 (*m*, 1H, H–C(2), Cp, A), 2.60–2.57 (*m*, 1H, H–C(2), Cp, B), 1.432 (*s*, 9H, *t*Bu, A), 1.429 (*d*, *J*=7.2 Hz, 3H, Me, A), 1.425 (*s*, 9H, *t*Bu, B), 1.417 (*d*, *J*=7.2 Hz, 3H, Me, B), 1.17–1.09 (*m*, 1H, H_A–C(3), Cp), 0.84–0.78 (*m*, 1H, H_B–C(3), Cp);

^{13}C NMR (50 MHz, $CDCl_3$): δ=176.9 (*s*, C=O, A), 176.5 (*s*, C=O, B), 156.0 (*s*, C=O, BOC), 80.0 (*s*, *t*Bu), 74.4 (*d*, C(2)), 59.1 (*d*, C(1), Cp, A), 58.9 (*d*, C(1), Cp, B), 29.4 (*d*, C(2), Cp), 28.3 (*q*, *t*Bu), 18.5 (*q*, Me, A), 18.0 (*q*, Me, B), 15.1 (*dd*, C(3), Cp); MS (e.i., 70 eV): *m*/*z* (%)=261 (1), 245 (1), 230 (1), 189 (1), 144 (8), 128 (1), 116 (26), 100 (4), 72 (45), 57 (100); HRMS calcd for $C_{12}H_{23}NO_5$: 261.1576; found: 261.1577.

3.1.8. *cis*-(2*S*)-2-(2-[(*tert*-Butoxycarbonyl)amino]cyclopropyloxy)propanoic acid (*cis*-9). Following the procedure given for the synthesis of *trans*-**8** from **7** (0.8 g, 2.79 mmol), KOH (0.45 g, 8.0 mmol) and ethanol (20 ml) the acid *cis*-**9** (600 mg, 90%) was obtained as an oil that was used without any further purification in the next step; IR (film): ν=3335*m*, 2980*s*, 2935*s*, 1715*s*, 1520*s*, 1455*s*, 1395*s*, 1370*s*, 1165*s*, 1055*s*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.60–4.90 (*br*, 2H, COOH, NH), 4.25–4.19 (*m*, 1H, H–C(2), A), 4.15–4.08 (*m*, 1H, H–C(2), B), 3.47–3.40 (*m*, 1H, H–C(1), Cp), 2.71–2.61 (*m*, 1H, H–C(2), Cp), 1.46–1.41 (*m*, 12H, *t*Bu, Me), 1.00–0.95 (*m*, 1H, H_A–C(3), Cp), 0.68–0.59 (*m*, 1H, H_B–C(3), Cp); ^{13}C NMR (100 MHz, $CDCl_3$): δ=176.5 (*s*, C=O, A), 176.1 (*s*, C=O, B), 155.8 (*s*, CO, BOC), 79.9 (*s*, *t*Bu), 75.3 (*d*, C(2), A), 75.0 (*d*, C(2), B), 55.0 (*d*, C(1), Cp), 28.4 (*q*, t Bu), 27.6 (*d*, C(2), Cp), 18.4 (*q*, Me, A), 18.1 (*q*, Me, B), 13.6 (*dd*, C(3), Cp, A), 12.7 (*dd*, C(3), Cp, B); MS (e.i. 70 eV): *m*/*z* (%)=261 (1), 189 (1), 171 (1), 144 (6), 126 (2), 116 (36), 100 (7), 72 (79), 57 (100); HRMS calcd for $C_{12}H_{23}NO_5$: 261.1576; found: 261.1577.

3.1.9. Benzyl *N*-{*trans*-(2*S*)-2-[2-(*tert*-butoxycarbonylamino)cyclopropyloxy]propionyl-L-alanyl-D-isoglutaminate (*trans*-10). BOC-L-alanyl-D-isoglutamine-γ-benzylester (696 mg, 1.71 mmol) was deprotected in ethyl acetate (4 ml) by the addition of a solution of dry hydrochloric acid in ethyl acetate (3.6 N by titration, 2.9 ml, 10 mmol) for 2 h, then the volatiles were removed and the residue was used as obtained. To a solution of **8** (380 mg, 1.55 mmol) in dry ethyl acetate (6 ml) and dry DMF (6 ml) under argon at 0 °C NMM (173 mg, 1.71 mmol) was added. The mixture was cooled to −15 °C and isobutyl chloroformate (233 mg, 1.71) was added dropwise through a syringe. After stirring for 5 min at this temperature, a solution of the deprotected dipeptide (587 mg, 1.71 mmol, vide supra) in a mixture of ethyl acetate (4 ml), DMF (2 ml) and NMM (345 mg, 3.42 mmol) was added and the reaction mixture stirred at ambient temperature for 18 h. The solvents were removed under diminished pressure, the residue suspended in water (20 ml) and extracted with ethyl acetate (4×40 ml), the combined organic phases were dried (Na_2SO_4), and the solvents were removed. The residue was purified by chromatography (silica gel, ethyl acetate/methanol 12:1) to afford **10** (740 mg, 90%) as a white amorphous solid. R_f (ethyl acetate/methanol 10:1) 0.42; IR (KBr): ν=3410*s*, 3285*s*, 3070*w*, 2980*m*, 2935*w*, 1730*s*, 1690*s*, 1640*s*, 1520*s*, 1455*m*, 1390*m*, 1365*m*, 1255*m*, 1170*s*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.36–7.28 (*m*, 5H, Ph), 7.26–7.22 (*br*, 1H, NH), 7.10 (*br*, 1H, NH), 6.74 (*br*, 1H, NH), 5.63 (*br*, 1H, NH), 5.11 (*AB system*, *J*=12.3 Hz, 2H, CH_2–Ph), 4.93 (*br*, 1H, NH, BOC, A), 4.83 (*br*, 1H, NH, BOC, B), 4.46–4.40 (*m*, 1H, CH(iGln)), 4.33–4.26 (*m*, 1H, CH(Lac)), 4.21–4.18 (*m*, 1H, CH(Ala)), 3.40–3.37 (*m*, 1H, H–C(1), Cp, A), 3.35–3.32 (*m*, 1H, H–C(1), Cp, B), 2.69–2.62 (*m*, 1H, H–C(2), Cp, A), 2.61–2.53 (*m*, 2H, H_A–C(4) of iGln and H–C(2), Cp, B), 2.49–2.40 (*m*, 1H, H_B–C(4) iGln), 2.24–2.16 (*m*, 1H, H_A–C(3), iGln), 2.04–1.97 (*m*, 1H, H_B–C(3) iGln), 1.40 (*s*, 9H, *t*Bu), 1.37 (*d*, *J*=7.0 Hz, 3H, Me), 1.35 (*d*, *J*=7.2 Hz, 3H, Me), 1.12–1.05 (*m*, 1H, H_A–C(3), Cp), 0.82–0.77 (*m*, 1H, H_B–C(3), Cp); ^{13}C NMR (100 MHz, CD_3OD): δ=176.3 (*s*, C=O), 175.8 (*s*, C=O), 175.2 (*s*, C=O), 174.4 (*s*, C=O), 159.0 (*s*, C=O, BOC), 137.7 (*s*, Ph), 129.7 (*d*, Ph), 129.4 (*d*, Ph), 129.3 (*d*, Ph), 80.0 (*s*, *t*Bu), 77.3 (*d*, C(2), Lac), 67.5 (*t*, CH_2–Ph), 59.8 (*d*, C(1), Cp), 53.6 (*d*, C(2) iGln), 50.4 (*d*, C(2), Ala), 31.4 (*t*, C(4) iGln), 30.3 (*d*, C(2), Cp), 28.7 (*q*, *t*Bu), 27.9 (*t*, C(3) iGln), 18.5 (*q*, Me), 18.0 (*q*, Me), 17.9 (*q*, Me), 15.0 (*dd*, C(3), Cp); MS (e.i., 70 eV): *m*/*z* (%)=478 (11), 461 (30), 444 (6), 435 (6), 390 (3), 378 (4), 370 (6), 363 (9), 346 (13), 299 (4), 255 (34), 243 (4), 237 (13), 215 (11), 192 (21), 127 (100); Anal. calcd for $C_{29}H_{43}N_3O_8$ (549.66): C, 62.01; H, 7.72; N, 7.48; found: 61.97; H, 7.89; N, 7.49.

3.1.10. Benzyl *N*-{*cis*(2*S*)-2-[2-(*tert*-butoxycarbonyl amino) cyclo-propyloxy]propionyl-L-alanyl-D-iso-glutaminate (*cis*-11). Following the procedure given for the synthesis of **10** from **9** (550 mg, 2.24 mmol), isobutyl chloroformate (336 mg, 2.46 mmol) and NMM (248 mg, 2.46 mmol) in ethyl acetate (5 ml) and DMF (5 ml) followed by the addition of the deprotected dipeptide (845 mg, 2.46 mmol; obtained by deprotection of the Boc-protected dipeptide (1.0 g, 2.46 mmol in abs. ethyl acetate (8 ml) with hydrochloric acid in ethyl acetate (3.6 N by titration, 4.1 ml, 14.8 mmol)) and NMM (497 mg, 4.92 mmol) in ethyl acetate (6 ml) and DMF (4 ml) followed by chromatography (silica gel, ethyl acetate/methanol 20:1) gave *cis*-**11** (536 mg, 45%) as a white amorphous solid; R_f (ethyl acetate/methanol 10:1) 0.34; IR (KBr): ν=3395*s*, 3285*s*, 2980*m*, 2935*w*, 1710*s*, 1685*s*, 1650*s*, 1525*s*, 1455*m*, 1365*m*, 1320*m*, 1260*m*, 1170*s*, 1125*m*, 1085*m*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.37–7.28 (*m*, 5H, Ph), 7.13 (*d*, *J*=8.0 Hz, 1H, NH), 6.98 (*d*, *J*=7.4 Hz, 1H, NH), 6.72 (*br*, 1H, NH), 5.52 (*br*, 1H, NH), 5.24 (*br*, 1H, NH), 5.11 (*AB system*, *J*=12.3 Hz, 2H, CH_2–Ph), 4.48–4.38 (*m*, 2H, CH(iGln), CH(Ala), A), 4.29 (*qd*, *J*=6.9 Hz, 1H, CH(Ala), B), 4.02 (*q*, *J*=6.9 Hz, 1H, CH(Lac)), 3.46–3.42 (*m*, 1H, H–C(1), Cp), 2.71–2.65 (*m*, 1H, H–C(2), Cp), 2.64–2.56 (*m*, 1H, H_A–C(4) iGln), 2.49– 2.41 (*m*, 1H, H_B–C(4) iGln), 2.23–2.15 (*m*, 1H, H_A–C(3) iGln), 2.06–1.97 (*m*, 1H, H_B–C(3) iGln), 1.44 (*s*, 9H, *t*Bu), 1.39 (*d*, *J*=6.8 Hz, 3H, Me), 1.36 (*d*, *J*=7.2 Hz, 3H, Me), 0.92–0.89 (*m*, 1H, H_A–C(3) Cp), 0.69–0.65 (*m*, 1H, H_B–C(3) Cp); ^{13}C NMR (125 MHz, $CDCl_3$): δ=173.7 (*s*, C=O), 173.6 (*s*, C=O), 172.9 (*s*, C=O), 172.8 s, C=O), 172.7 (*s*, C=O), 157.2 (*s*, C=O, Boc, A), 156.8 (*s*, C=O, BOC, B), 135.6 (*s*, Ph), 128.4 (*d*, Ph), 128.1 (*d*, Ph), 128.05 (*d*, Ph), 79.5 (*s*, *t*Bu), 76.9 (*d*, C(2) Lac), 66.4 (*t*, CH_2–Ph), 55.2 (*d*, C(1) Cp, A), 54.9 (*d*, C(1) Cp, B), 52.3 (*d*, C(2) iGln, A), 52.2 (*d*, C(2) iGln, B), 48.6 (*d*, C(2) Ala), 30.45 (*t*, C(4) iGln, A), 30.41 (*t*, C(4) iGln, B), 28.2 (*q*, *t*Bu), 28.7 (*d*, C(2) Cp), 26.8 (*t*, C(3) iGln), 18.4 (*q*, Me, A), 18.0 (*q*, Me, B), 17.8 (*q*, Me), 12.8 (*dd*, C(3) Cp, A), 11.2 (*dd*, C(3) Cp, B); MS (e.i., 70 eV): *m*/*z* (%)=534 (1), 461 (2%), 416 (1), 378 (1), 370 (4), 363 (6), 346 (9), 299 (1), 270 (6), 255 (41), 237 (9), 192 (12), 127 (100); Anal. calcd for $C_{29}H_{43}N_3O_8$ (549.66): C, 62.01; H, 7.72; N, 7.48; found: 61.88; H, 7.82; N, 7.51.

3.1.11. Benzyl *N*-{*trans*-(2*S*)-2-(acetylamino)cyclopropyloxy]-propionyl}-L-alanyl-D-isoglutaminate (*trans*-12). To a solution of **10** (850 mg, 1.59 mmol) in ethyl acetate (10 ml) a solution of hydrochloric acid in ethyl acetate (3.6 N by titration, 6.5 ml, 24 mmol) was added and stirring was continued for another 3 h, then all the volatiles were removed under diminished pressure. The residue was dissolved in CH_2Cl_2 (15 ml) and triethylamine (1.62 mg, 16 mmol) was added. After cooling to 0 °C a solution of acetylchloride (188 mg, 2.4 mmol) in CH_2Cl_2 (5 ml) was added and stirring was continued at 25 °C for 14 h. The solvents were removed under reduced pressure, water (40 ml) was added and extracted with ethyl acetate (4×50 ml); the combined organic phases were dried (Na_2SO_4), the solvent was removed and the residue subjected to chromatography (silica gel, ethyl acetate/methanol 16:1→10:1) to afford **12a** (210 mg, 27%), **12b** (90 mg, 12%) and a mixture of **12a/12b** (310 mg, 41%).

*Data for***12a**. Amorphous white solid; R_f (ethyl acetate/methanol 3:1) 0.36; $[\alpha]_D$ −23.1 (*c*, 0.51 MeOH); IR (KBr): ν=3410*s*, 3280*s*, 3070*w*, 2980*w*, 2930*w*, 1735*s*, 1640*s*, 1545*s*, 1450*m*, 1370*m*, 1295*m*, 1235*m*, 1165*m*, 1095*m*, 1040*w*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.40 (*d*, *J*=7.7 Hz, 1H, NH), 7.36–7.28 (*m*, 5H, Ph), 7.15 (*d*, *J*=7.3 Hz, 1H, NH), 6.88 (*br*, 1H, NH), 5.81 (*br*, 1H, NH), 5.10 (*AB system*, *J*=12.3 Hz, 2H, CH2–Ph), 4.44–4.36 (*m*, 2H, CH(iGln), CH(Ala)), 4.19 (*q*, *J*=6.8 Hz, 1H, CH(Lac)), 3.38–3.36 (*m*, 1H, H–C(1) Cp), 2.79–2.75 (*m*, 1H, H–C(2) Cp), 2.60–2.52 (*m*, 1H, H_A–C(4) iGln), 2.49–2.41 (*m*, 1H, H_B–C(4) iGln), 2.24–2.18 (*m*, 1H, H_A–C(3) iGln), 2.04–1.95 (*m*, 1H, H_B–C(3) iGln), 1.89 (*s*, 3H, Ac), 1.365 (*d*, *J*=6.9 Hz, 3H, Me), 1.359 (*d*, *J*=6.9 Hz, 3H, Me), 1.15–1.10 (*m*, 1H, H_A–C(3) Cp), 0.84–0.79 (*m*, 1H, H_B–C(3) Cp); ^{13}C NMR (100 MHz, $CDCl_3$): δ=176.0 (*s*, C=O), 175.5 (*s*, C=O), 174.9 (*s*, C=O), 174.5 (*s*, C=O), 174.1 (*s*, C=O), 137.4 (*s*, Ph), 129.4 (*d*, Ph), 129.12 (*d*, Ph), 129.10 (*d*, Ph), 77.3 (*d*, C(2) Lac), 67.4 (*t*, CH_2–Ph), 59.4 (*d*, C(1) Cp), 53.7 (*d*, C(2) iGln), 50.5 (*d*, C(2) Ala), 31.5 (*t*, C(4) iGln), 29.8 (*d*, C(2) Cp), 28.0 (*t*, C(3) iGln), 22.4 (*q*, Ac), 18.7 (*q*, Me), 17.9 (*q*, Me), 14.9 (*dd*, C(3) Cp); MS (e.i., 70 eV): *m*/*z* (%)=476 (1), 459 (1), 433 (1), 363 (3), 346 (4), 255 (16), 241 (11), 237 (8), 213 (24), 192 (22), 127 (100); Anal. calcd for $C_{23}H_{32}N_4O_7$ (476.53): C, 57.97; H, 6.77; N, 11.76; found: 57.63; H, 6.63; N, 11.70.

*Data for***12b**: Amorphous white solid. R_f (ethyl acetate/methanol 3:1) 0.36; $[\alpha]_D$ −41.2 (*c*, 0.51 MeOH); IR (KBr): ν=3410*s*, 3280*s*, 3070*w*, 2980*w*, 2930*w*, 1735*s*, 1640*s*, 1545*s*, 1450*m*, 1370*m*, 1295*m*, 1235*m*, 1165*m*, 1095*m*, 1040*w*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.37–7.28 (*m*, 6H, Ph, NH), 7.09 (*d*, *J*=6.2 Hz, 1H, NH), 5.92 (*br*, 1H, NH), 5.59 (*br*, 1H, NH), 5.11 (*AB system*, *J*=12.2 Hz, 2H, CH_2–Ph), 4.45–4.40 (*m*, 1H, CH(iGln)), 4.33 (*qd*, *J*=7.0 Hz, 1H, CH(Ala)), 4.26 (*q*, *J*=6.8 Hz, 1H, CH(Lac)), 3.37–3.34 (*m*, 1H, H–C(1) Cp), 2.72–2.69 (*m*, 1H, H–C(2) Cp), 2.62–2.54 (*m*, 1H, H_A–C(4) iGln), 2.50–2.43 (*m*, 1H, H_B–C(4) iGln), 2.23–2.18 (*m*, 1H, H_A–C(3) iGln), 2.06–1.97 (*m*, 1H, H_B–C(3) iGln), 1.90 (*s*, 3H, Ac), 1.36 (*d*, *J*=6.8 Hz, 3H, Me), 1.35 (*d*, *J*=6.6 Hz, 3H, Me), 1.20–1.16 (*m*, 1H, H_A–C(3) Cp), 0.84–0.79 (*m*, 1H, H_B–C(3) Cp); ^{13}C NMR (100 MHz, CD_3OD): δ=175.3 (*s*, C=O), 174.8 (*s*, C=O), 174.0 (*s*, C=O), 137.4 (*s*, Ph), 129.4 (*d*, Ph), 129.1 (*d*, Ph), 77.3 (*d*, C(2) Lac), 67.4 (*t*, CH_2–Ph), 59.4 (*d*, C(1) Cp), 53.6 (*d*, C(2) iGln), 50.5 (*d*, C(2) Ala), 31.5 (*t*, C(4) iGln), 30.0 (*d*, C(2) Cp), 28.0 (*t*, C(3) iGln), 22.5 (*q*, Ac), 19.0 (*q*, Me), 18.0 (*q*, Me), 14.7 (*dd*, C(3) Cp); MS (e.i., 70 eV): *m*/*z* (%)=476 (1), 459 (1), 433 (1), 363 (3), 346 (4), 255 (16), 241 (11), 237 (8), 213 (24), 192 (22), 127 (100); Anal. calcd for $C_{23}H_{32}N_4O_7$ (476.53): C, 57.97; H, 6.77; N, 11.76; found: 57.70; H, 6.61; N, 11.68.

3.1.12. Benzyl *N*-{*cis*-(2*S*)-2-(acetylamino)cyclopropyloxy]-propionyl}-L-alanyl-D-isoglutaminate (*cis*-13). Following the procedure given for the synthesis of **12** a solution of **11** (190 mg, 0.36 mmol) in ethyl acetate (3 ml) was treated with hydrochloric acid in ethyl acetate (3.6 N, 0.7 ml, 2.5 mmol) followed by acetylation (acetylchloride (71 mg, 0.9 mmol), triethylamine (364 mg, 3.6 mmol in CH_2Cl_2 (5 ml)) to afford after chromatography (silica gel, ethyl acetate/methanol 16:1→10:1) **13** (94 mg, 55%) as an amorphous white solid; R_f (ethyl acetate/methanol 10:1) 0.12; IR (KBr): ν=3405*s*, 3065*w*, 2985*w*, 2935*w*, 1735*m*, 1655*s*, 1535*s*, 1455*m*, 1375*m*, 1320*m*, 1255*m*, 1170*m*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.68 (*br*, 1H, NH), 7.36 (*d*, *J*=8.2 Hz, 1H, NH, A), 7.33–7.28 (*m*, 5H, Ph), 7.24 (*d*, *J*=7.8 Hz, 1H, NH, B), 6.95 (*br*, 1H, NH, A), 6.89 (*br*, 1H, NH, B), 6.80 (*d*, *J*=4.9 Hz, 1H, NH, A), 6.58 (*d*, *J*=4.1 Hz, 1H, NH, B), 6.33 (*br*, 1H, NH, A), 6.28 (*br*, 1H, NH, B), 5.08 (*AB system*, *J*=12.5 Hz, 2H, CH_2–Ph), 4.52 (*qd*, *J*=7.3 Hz, 1H, CH(Ala), A), 4.48 (*qd*, *J*=7.3 Hz, 1H, CH(Ala), B), 4.43–4.38 (*m*, 1H, CH (iGln)), 4.02 (*q*, *J*=6.7 Hz, 1H, CH(Lac), A), 3.95 (*q*, *J*=6.8 Hz, 1H, CH(Lac), B), 3.47–3.42 (*m*, 1H, H–C(1) Cp), 2.92–2.88 (*m*, 1H, H–C(2) Cp, A), 2.77–2.71 (*m*, 1H, H–C(2) Cp, B), 2.53–2.39 (*m*, 2H, H–C(4) iGln), 2.21–2.14 (*m*, 1H, H_A–C(3) iGln), 2.01–1.92 (*m*, 1H, H_B–C(3) iGln), 1.98 (*S*, 3H, Ac, A), 1.88 (*s*, 3H, Ac, B), 1.35 (*d*, *J*=6.8 Hz, 3H, Me), 1.34 (*d*, *J*=6.8 Hz, 3H, Me), 1.32 (*d*, *J*=7.0 Hz, 3H, Me), 1.05–0.99 (*m*, 1H, H_A–C(3) Cp, A), 0.90–0.84 (*m*, 1H, H_A–C(3) Cp, B), 0.70–0.68 (*m*, 1H, H_B–C(3) Cp, A), 0.65–0.61 (*m*, 1H, H_B–C(3) Cp, B); ^{13}C NMR (125 MHz, $CDCl_3$): δ=173.7 (*s*, C=O), 173.6 (*s*, C=O), 173.4 (*s*, C=O), 173.33 (*s*, C=O), 173.30 (*s*, C=O), 173.05 (*s*, C=O), 173.00 (*s*, C=O), 172.5 (*s*, C=O), 172.1 (*s*, C=O), 135.6 (*s*, Ph), 128.6 (*d*, Ph), 128.3 (*d*, Ph), 128.2 (*d*, Ph), 76.9 (*d*, C(2) Lac), 66.7 (*t*, CH_2–Ph), 54.7 (*d*, C(1) Cp, A), 54.6 (*d*, C(1) Cp, B), 52.6 (*d*, C(2) iGln, A), 52.5 (*d*, C(2) iGln, B), 48.7 (*d*, C(2) Ala, A), 48.4 (*d*, C(2) Ala, B), 30.7 (*t*, C(4) iGln, A), 30.6 (*t*, C(4) iGln, B), 28.0 (*d*, C(2) Cp, A), 27.1 (*d*, C(2) Cp, B), 27.08 (*t*, C(3) iGln, A), 26.9 (*t*, C(3) iGln, B), 22.83 (*q*, Ac, A), 22.80 (*q*, Ac, B), 18.5 (*q*, Me), 18.3 (*q*, Me), 18.2 (*q*, Me), 18.1 (*q*, Me), 12.8 (*dd*, C(3) Cp, A), 11.3 (*dd*, C(3) Cp, B); MS (e.i. 70 eV): *m*/*z* (%)=476 (1), 432 (1), 369 (2), 346 (2), 255 (16), 241 (8), 213 (18), 200 (6), 192 (12), 127 (100); Anal. calcd for $C_{23}H_{32}N_4O_7$ (476.23): C, 57.97; H, 6.77; N, 11.76; found: C, 57.79; H, 6.86; N, 11.52.

3.1.13. *trans*-(2*S*)-2-[2-(Acetylamino)cyclopropyloxy]-propionyl-L-alanyl-D-isoglutamine (*trans*-14a). A solution of **12a** (155 mg, 0.33 mmol) in ethanol (35 ml) was hydrogenated for 8 h at 3 bar in the presence of Pd/C (10%, 30 mg). The catalyst was filtered off, the solvent removed, and the residue was subjected to chromatography (silica gel, chloroform/methanol/acetic acid 70:25:5) to

yield **14a** (110 mg, 87%) as an amorphous white solid. R_f ($CHCl_3$/MeOH/AcOH 70:25:5) 0.30; $[\alpha]_D$ −27.2 (*c* 0.53, MeOH); IR (KBr): ν=3415*s*, 2935*w*, 1655*s*, 1560*s*, 1450*m*, 1290*w*, 1160*w*, 1125*w*, 1040*w*; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.61 (*br*, 1H, NH), 8.18 (*br*, 1H, NH), 7.87 (*d*, *J*=7.0 Hz, 1H, NH), 7.32 (*br*, 1H, NH), 7.02 (*br*, 1H, NH), 4.28 (*qd*, *J*=7.0 Hz, 1H, CH(Ala)), 4.14–4.07 (*m*, 1H, CH(iGln)), 4.07 (*q*, *J*=6.6 Hz, 1H, CH(Lac)), 3.48–3.44 (*m*, 1H, H–C(1) Cp), 2.68–2.66 (*m*, 1H, H–C(2) Cp), 2.08 (*t*, *J*=7.3 Hz, 2H, H–C(4) iGln), 1.94–1.84 (*m*, 1H, H_A–C(3) iGln), 1.73 (*s*, 3H, Ac), 1.73–1.69 (*m*, 1H, H_B–C(3) iGln), 1.22 (*d*, *J*=6.6 Hz, 3H, Me), 1.21 (*d*, *J*=6.8, 3H, Me), 0.99–0.94 (*m*, 1H, H_A–C(3) Cp), 0.69–065 (*m*, 1H, H_B–C(3) Cp); ^{13}C NMR (100 MHz, CD_3OD): δ=176.9 (*s*, C=O), 176.0 (*s*, C=O), 174.8 (*s*, C=O), 174.7 (*s*, C=O), 77.4 (*d*, C(2) Lac), 59.5 (*d*, C(1) Cp), 54.3 (*d*, C(2) iGln), 50.4 (*d*, C(2) Ala), 31.6 (*t*, C(4) iGln), 29.8 (*d*, C(2) Cp), 28.8 (*t*, C(3) iGln), 22.5 (*q*, Ac), 18.7 (*q*, Me), 17.9 (*q*, Me), 14.8 (*dd*, C(3)); HPLC–MS (ESI, 4.1 kV, 8 μl/min, N_2, methanol): *m*/*z*=849.3 $[(M_2-H)K_2]^+$ (14%), 833.3 $[(M_2-H)NaK]^+$ (37%), 811.3 $[M_2K]^+$ (54%), 425.3 $[MK]^+$ (96%), 409.7 $[MNa]^+$ (100%); HRMS calcd for $C_{16}H_{26}N_4O_7$: 386.1801; found: 386.1800; Anal. calcd for $C_{16}H_{26}N_4O_7$ (386.41): C, 49.73; H, 6.78; N, 14.50; found: C, 49.52; H, 6.85; N, 14.47.

3.1.14. *trans*-(2*S*)-2-[2-(Acetylamino)cyclopropyloxy]-propionyl-L-alanyl-D-isoglutamine (*trans*-14b). Hydrogenolysis of **12b** (57 mg, 0.12 mmol) in ethanol (20 ml) with Pd/C (10%, 20 mg) as described above followed by chromatography (silica gel, chloroform/methanol/acetic acid 70:25:5) gave **14b** (40 mg, 86%) as an amorphous white solid; R_f ($CHCl_3$/MeOH/AcOH 70.25:5) 0.30; $[\alpha]_D$=−42.0 (*c*, 0.36 MeOH); IR (KBr): ν=3410*s*, 2930*m*, 1660*s*, 1555*s*, 1415*s*, 1300*m*, 1160*w*, 1100*m*, 1050*m*; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.96 (*br*, 1H, NH), 8.42 (*br*, 1H, NH), 7.94 (*d*, *J*=7.3 Hz, 1H, NH), 7.34 (*br*, 1H, NH), 6.98 (*br*, 1H, NH), 4.28 (*qd*, *J*=7.0 Hz, 1H, CH(Ala)), 4.14 (*q*, *J*=6.7 Hz, 1H, CH(Lac)), 4.08–4.01 (*m*, 1H, CH(iGln)), 3.42–3.40 (*m*, 1H, H–C(1) Cp determined in CD_3OD), 2.65–2.63 (*m*, 1H, H–C(2) Cp), 2.01 (*t*, *J*=6.9 Hz, 2H, H–C(4) iGln), 1.90–1.81 (*m*, 1H, H_A–C(3) iGln), 1.78–1.72 (*m*, 1H, H_B–C(3) iGln), 1.74 (*s*, 3H, Ac), 1.22 (*d*, *J*=6.8 Hz, 3H, Me), 1.21 (*d*, *J*=6.8 Hz, 3H, Me), 1.01–0.96 (*m*, 1H, H_A–C(3) Cp), 0.69–0.65 (*m*, 1H, H_B–C(3) Cp); ^{13}C NMR (100 MHz, CD_3OD): δ=176.8 (*s*, C=O), 175.9 (*s*, C=O), 174.7 (*s*, C=O), 77.4 (*d*, C(2) Lac), 59.4 (*d*, C(1) Cp), 54.6 (*d*, C(2) iGln), 50.4 (*d*, C(2) Ala), 31.7 (*t*, C(4) iGln), 29.9 (*d*, C(2) Cp), 29.0 (*t*, C(3) iGln), 22.5 (*q*, Ac), 19.1 (*q*, Me), 17.7 (*q*, Me), 14.9 (*dd*, C(3) Cp); HPLC–MS (ESI, 4.1 kV, 8 μl/min, N_2, methanol): *m*/*z*=849.4 $[(M_2-H)K_2]^+$ (38%), 833.5 $[(M_2-H)NaK]^+$ (27%), 425.5 $[MK]^+$ (100%); HRMS calcd For $C_{16}H_{26}N_4O_7$: 386.1801; found: 386.1802; Anal. calcd for $C_{16}H_{26}N_4O_7$ (386.41): C, 49.73; H, 6.78; N, 14.50; found: C, 49.61; H, 6.95; N, 14.45.

3.1.15. *cis*-(2*S*)-2-[2-(Acetylamino)cyclopropyloxy]propionyl-L-alanyl-D-isoglutamine (*cis*-15). Hydrogenolysis of **13** (33 mg, 0.07 mmol) in ethanol (20 ml) with Pd/C (10%, 10 mg) as described above followed by chromatography (silica gel, chloroform/methanol/acetic acid 70:25:5) yielded **15** (25 mg, 95%) as a white amorphous solid. R_f ($CHCl_3$/MeOH/AcOH 70:25:5) 0.30; IR (KBr): ν=3415*s*, 2930*m*, 1655*s*, 1555*s*, 1445*s*, 1315*m*, 1170*m*, 1125*m*, 1045*w*; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.99 (*br*, 1H, NH), 8.43 (*br*, 1H, NH, A), 8.20 (*br*, 1H, NH, B), 7.83 (*d*, *J*=7.7 Hz, 1H, NH, A), 7.79 (*d*, *J*=7.3 Hz, 1H, NH, B), 7.33 (*br*, 1H, NH, A), 7.29 (*br*, 1H, NH, B), 6.95 (*br*, 1H, NH), 4.27 (*qd*, *J*=7.3 Hz, 1H, CH(Ala), A), 4.24 (*qd*, *J*=6.8 Hz, 1H, CH(Ala), B), 4.05–3.96 (*m*, 1H, CH(iGln), 3.94 (*q*, *J*=6.5 Hz, 1H, CH(Lac), A), 3.87 (*q*, *J*=7.1 Hz, CH(Lac), B), 3.52–3.48 (*m*, 1H, H–C(1), Cp determined in CD_3OD), 2.70–2.64 (*m*, 1H, H–C(2), Cp, A), 2.63–2.58 (*m*, 1H, H–C(2), Cp, B), 2.03–1.97 (*m*, 2H, H–C(4) iGln), 1.87–1.69 (*m*, 2H, H–C(3) iGln), 1.84 (*s*, 3H, Ac, A), 1.77 (*s*, 3H, Ac, B), 1.26 (*d*, *J*=6.9 Hz, 3H, Me), 1.23 (*d*, *J*=6.6 Hz, 3H, Me), 1.21 (*d*, *J*=6.0 Hz, 3H, Me), 1.20 (*d*, *J*=6.4, 3H, Me), 0.90–0.75 (*m*, 2H, H–C(3), Cp); ^{13}C NMR (100 MHz, CD_3OD): δ=176.8 (*s*, C=O), 175.6 (*s*, C=O), 175.4 (*s*, C=O), 175.2 (*s*, C=O), 174.8 (*s*, C=O), 174.7 (*s*, C=O), 77.9 (*d*, C(2) Lac), 55.8 (*d*, C(1) Cp, A), 55.6 (*d*, C(1) Cp, B), 54.7 (*d*, C(2) iGln), 50.4 (*d*, C(2) Ala, A), 50.3 (*d*, C(2) Ala, B), 30.7 (*t*, C(4) iGln), 29.3 (*t*, C(3) iGln), 28.7 (*d*, C(2) Cp, A), 28.2 (*d*, C(2) Cp, B), 22.5 (*q*, Ac, A), 22.4 (*q*, Ac, B), 18.9 (*q*, Me), 18.7 (*q*, Me), 18.1 (*q*, Me), 18.0 (*q*, Me), 12.5 (*dd*, C(3), A), 11.9 (*dd*, C(3), B); HPLC–MS (ESI, 4.1 kV, 8 μl/min, N_2, methanol): *m*/*z*=1253.3 $[(M_3-H)K_2]^+$ (46%), 849.4 $[(M_2-H)K_2]^+$ (61%), 833.5 $[(M_2-H)NaK]^+$ (30%), 425 $[MK]^+$ (100%), 409.7 $[MNa]^+$ (11%); HRMS calcd for $C_{16}H_{26}N_4O_7$: 386.1801; found: 386.1802; Anal. calcd for $C_{16}H_{26}N_4O_7$ (386.41): C, 49.73; H, 6.78; N, 14.50.

3.1.16. Benzyl {*trans*-(2*S*)-2-[2-(octanoylamino)cyclopropyloxy]-propionyl}-L-alanyl-D-isoglutaminate (*trans*-16). According to the procedure given for the synthesis of **12** from **10** (600 mg, 1.12 mmol), hydrochloric acid in ethyl acetate (3.6 N, 2.0 ml, 7.2 mmol) in ethyl acetate (7 ml) followed by acylation with octanoyl chloride (237 mg, 1.46 mmol) and triethylamine (1.13 g, 11.2 mmol) in dichloromethane (9 ml) and chromatographic work-up (silica gel, ethyl acetate/methanol 20:1→15:1) gave **16** (500 mg, 79%) as a white amorphous solid. R_f (ethyl acetate/methanol 10:1) 0.29; IR (KBr): ν=3410*m*, 3280*s*, 3070*w*, 2955*w*, 2930*m*, 2855*w*, 1735*m*, 1680*s*, 1635*s*, 1550*s*, 1455*m*, 1385*w*, 1275*m*, 1235*m*, 1170*m*, 1100*w*, 1040*w*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.47 (*d*, *J*=6.6 Hz, 1H, NH, A), 7.45 (*d*, *J*=7.8 Hz, 1H, NH, B), 7.35–7.27 (*m*, 5H, Ph), 7.24 (*d*, *J*=6.4 Hz, 1H, NH, A), 7.20 (*d*, *J*=7.0 Hz, 1H, NH, B), 6.86 (*br*, 1H, NH, A), 6.82 (*br*, 1H, NH, B), 6.04 (*br*, 1H, NH, A), 5.95 (*br*, 1H, NH, B), 5.89 (*br*, 1H, NH, A), 5.87 (*br*, 1H, NH, B), 5.10 (*AB system*, *J*=12.4 Hz, 2H, CH_2–Ph), 4.46–4.34 (*m*, 2H, CH (Ala), CH(iGln)), 4.25 (*q*, *J*=6.7 Hz, 1H, CH(Lac), A), 4.20 (*q*, *J*=6.8 Hz, 1H, CH(Lac), B), 3.36–3.31 (*m*, 1H, C(1) Cp), 2.75–2.70 (*m*, 1H, H–C(2) Cp), 2.58–2.40 (*m*, 2H, H–C(4) iGln), 2.25–2.14 (*m*, 1H, H_A–C(3) iGln), 2.07 (*t*, *J*=7.5 Hz, 2H, H–C(2) Oct), 2.03–1.96 (*m*, 1H, H_B–C(3) iGln), 1.57–1.51 (*m*, 2H, H–C(3) Oct), 1.35 (*d*, *J*=6.5 Hz, 3H, Me), 1.34 (*d*, *J*=7.0 Hz, 3H, Me), 1.33 (*d*, *J*=6.6 Hz, 3H, Me), 1.29–1.23 (*m*, 8H, Oct), 1.17–1.10 (*m*, 1H, H_A–C(3) Cp), 0.84 (*t*, *J*=6.6 Hz, 3H, Me (Oct)), 0.79–0.75 (*m*, 1H, H_B–C(3) Cp); ^{13}C NMR (100 MHz, CD_3OD): δ=178.0 (*s*, C=O), 177.9 (*s*, C=O), 176.4 (*s*, C=O), 175.9 (*s*, C=O), 175.8 (*s*, C=O), 175.4 (*s*, C=O),

174.4 (*s*, C═O), 137.7 (*s*, Ph), 129.7 (*d*, Ph), 129.4 (*d*, Ph), 129.3 (*d*, Ph), 77.4 (*d*, C(2) Lac), 67.5 (*t*, CH_2–Ph), 59.5 (*d*, C(1) Cp), 53.8 (*d*, C(2) iGln, A), 53.7 (*d*, C(2) iGln, B), 50.60 (*d*, C(2) Ala, A), 50.57 (*d*, C(2) Ala, B), 36.84 (*t*, C(2) Oct, A), 36.79 (*t*, C(2) Oct, B), 32.8 (*t*, Oct), 31.4 (*t*, C(4) iGln), 30.2 (*t*, Oct), 30.0 (*t*, Oct), 29.8 (*d*, C(2) Cp, A), 29.7 (*d*, C(2) Cp, B), 27.9 (*t*, C(3) iGln), 26.8 (*t*, Oct), 23.6 (*t*, Oct), 18.9 (*q*, Me), 18.5 (*q*, Me), 17.9 (*q*, Me), 14.8 (*dd*, C(3) Cp, A), 14.6 (*dd*, C(3) Cp, B), 14.3 (*q*, Me, Oct); MS (e.i. 70 eV): *m*/*z* (%)=560 (4), 543 (3), 517 (2), 453 (2), 363 (2), 346 (3), 325 (4), 297 (12), 282 (2), 255 (26), 237 (4), 192 (7), 183 (10), 127 (100); Anal. calcd for $C_{29}H_{44}N_4O_7$ (560.70): C, 62.12; H, 7.91; N, 9.99; found: C, 61.96; H, 7.95; N, 9.83.

3.1.17. Benzyl {*cis*-(2*S*)-2-[2-(octanoylamino)cyclopropyloxy]propionyl}-L-alanyl-D-isoglutaminate (*cis*-17). According to the procedure given for the synthesis of **12** from **11** (170 mg, 0.32 mmol), hydrochloric acid in ethyl acetate (3.6 N, 0.7 ml, 2.5 mmol) in ethyl acetate (3 ml) followed by acylation with octanoyl chloride (78 mg, 0.48 mmol) and triethylamine (323 mg, 3.2 mmol) in dichloromethane (3 ml) and chromatographic work-up (silica gel, ethyl acetate/methanol 20:1→10:1) gave **17** (112 mg, 63%) as a white amorphous solid. R_f (ethyl acetate/methanol 10:1) 0.27; IR (KBr): ν=3395*m*, 3280*m*, 3065*w*, 2930*m*, 2855*w*, 1710*s*, 1680*m*, 1650*s*, 1540*m*, 1460*m*, 1420*w*, 1380*w*, 1325*m*, 1260*m*, 1175*w*; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.43 (*d*, *J*=7.6 Hz, 1H, NH, A), 7.41 (*d*, *J*=6.8 Hz, 1H, NH, B), 7.38 (*d*, *J*=8.0 Hz, 1H, NH, A), 7.34–7.29 (*m*, 5H, Ph), 7.07 (*d*, *J*=7.6 Hz, 1H, NH, B), 6.91 (*br*, 1H, NH, B), 6.70 (*br*, 1H, NH, B), 6.38 (*d*, *J*=4.9 Hz, 1H, NH, A), 6.07 (*d*, *J*=3.5 Hz, 1H, NH, B), 5.87 (*br*, 1H, NH, A), 5.71 (*br*, 1H, NH, B), 5.10 (*AB system*, *J*=12.3 Hz, 2H, CH_2–Ph), 4.50–4.39 (*m*, 2H, CH(iGln), CH(Ala)), 4.07 (*q*, *J*=6.8 Hz, 1H, CH(Lac) A), 3.98 (*q*, *J*=6.8 Hz, CH(Lac), B), 3.50–3.45 (*m*, 1H, H–C(1) Cp), 2.95–2.91 (*m*, 1H, H–C(2) Cp, A), 2.77–2.72 (*m*, 1H, H–C(2) Cp, B), 2.60–2.52 (*m*, 1H, H_A–C(4) iGln), 2.48–2.39 (*m*, 1H, H_B–C(4) iGln), 2.23–2.12 (*m*, 3H, H_A–C(3) iGln, H–C(2) Oct), 2.02–1.93 (*m*, 1H, H_B–C(3) iGln), 1.63–1.55 (*m*, 2H, H–C(3) Oct), 1.39 (*d*, *J*=7.2 Hz, 3H, Me), 1.37 (*d*, *J*=7.4 Hz, 3H, Me), 1.34 (*d*, *J*=6.8 Hz, 3H, Me), 1.33 (*d*, *J*=7.0 Hz, 3H, Me), 1.30–1.23 (*m*, 8H, Oct), 1.04–0.98 (*m*, 1H, H_A–C(3) Cp), 0.84 (*t*, *J*=6.6 Hz, 3H, Me (Oct)), 0.66–0.60 (*m*, 1H, H_B–C(3) Cp); ^{13}C NMR (100 MHz, $CDCl_3$): δ=175.3 (C═O), 173.9 (*s*, C═O), 173.7 (*s*, C═O), 173.3 (*s*, C═O), 173.2 (*s*, C═O), 135.7 (*s*, Ph), 128.7 (*d*, Ph), 128.4 (*d*, Ph), 128.3 (*d*, Ph), 77.0 (*d*, C(2) Lac), 66.6 (*t*, CH_2–Ph), 54.8 (*d*, C(1) Cp), 52.4 (*d*, C(2) iGln), 48.4 (*d*, C(2) Ala), 36.2 (*t*, C(2) Oct), 31.6 (*t*, Oct), 30.5 (*t*, C(4) iGln), 29.2 (*t*, Oct), 28.9 (*t*, Oct), 28.8 (*t*, C(3) iGln), 27.7 (*d*, C(2) Cp), 25.7 (*t*, Oct), 22.4 (*t*, Oct), 18.3 (*q*, Me), 13.9 (*q*, Me (Oct)), 11.1 (*dd*, C(3) Cp); MS (e.i., 70 eV): *m*/*z* (%)=560 (1), 453 (1), 346 (1), 325 (1), 297 (6), 282 (1), 255 (14), 237 (2), 226 (4), 198 (8), 192 (3), 183 (6), 127 (100); Anal. calcd for $C_{29}H_{44}N_4O_7$ (560.70): C, 62.12; H, 7.91; N, 9.99; found: C, 61.96; H, 7.98; N, 9.87.

3.1.18. *trans*-(2*S*)-2-[2-(Octanoylamino)cyclopropyloxy]-propionyl-L-alanyl-D-isoglutamine (*trans*-18). Hydrogenolysis of **16** (150 mg, 0.27 mmol) in ethanol (30 ml) with Pd/C (10%, 30 mg) followed by chromatography (silica gel, chloroform/methanol/acetic acid 85:10:5) gave **18** (120 mg, 95%) as an amorphous white solid. R_f (chloroform/methanol/acetic acid 85:10:5) 0.35; IR (KBr): ν=3410*s*, 2930*m*, 2860*m*, 1650*s*, 1550*s*, 1450*s*, 1305*m*, 1210*m*, 1165*m*, 1100*m*; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.69 (*br*, 1H, NH), 8.24 (*br*, 1H, NH, A), 8.10 (*br*, 1H, NH, B), 7.90 (*d*, *J*=7.7 Hz, 1H, NH), 7.32 (*br*, 1H, NH), 6.98 (*br*, 1H, NH), 4.27 (*qd*, *J*=7.1 Hz, 1H, CH(Ala)), 4.14 (*q*, *J*=6.9 Hz, 1H, CH(Lac) A), 4.09–4.02 (*m*, 1H, CH(iGln)), 4.07 (*q*, *J*=6.9 Hz, 1H, CH(Lac) B), 3.42–3.39 (*m*, 1H, H–C(1) Cp, A determined in CD_3OD), 3.39–3.35 (*m*, 1H, H–C(1) Cp, B determined in CD_3OD), 2.70–2.61 (*m*, 1H, H–C(2), Cp), 2.02–1.96 (*m*, 4H, H–C(4) iGln, H–C(2) Oct), 1.91–1.82 (*m*, 1H, H_A–C(3) iGln), 1.76–1.70 (*m*, 1H, H_B–C(3) iGln), 1.47–1.42 (*m*, 2H, H–C(3) Oct), 1.28–1.14 (*m*, 14H, 2×Me, Oct), 0.99–0.92 (*m*, 1H, H_A–C(3) Cp), 0.84 (*t*, *J*=6.7 Hz, 3H, Me(Oct)), 0.68–0.64 (*m*, 1H, H_B–C(3) Cp); ^{13}C NMR (100 MHz, CD_3OD): δ=177.6 (*s*, C═O), 177.5 (*s*, C═O), 176.8 (*s*, C═O), 175.6 (*s*, C═O), 174.8 (*s*, C═O), 174.7 (*s*, C═O), 77.3 (*d*, C(2) Lac, A), 77.2 (*d*, C(2) Lac, B), 59.5 (*d*, C(1) Cp, A), 59.4 (*d*, C(1) Cp, B), 54.6 (*d*, C(2) iGln), 50.5 (*d*, C(2) Ala, A), 50.4 (*d*, C(2) Ala, B), 36.9 (*t*, C(2) Oct), 32.8 (*t*, Oct), 30.3 (*t*, C(4) iGln), 30.2 (*t*, Oct), 30.1 (*t*, Oct), 30.0 (*d*, C(2) Cp, A), 29.7 (*d*, C(2) Cp, B), 29.1 (*t*, C(3) iGln), 26.9 (*t*, Oct), 23.6 (*t*, Oct), 19.0 (*q*, Me, A), 18.6 (*q*, Me, B), 17.9 (*q*, Me), 15.0 (*dd*, C(3) Cp, A), 14.8 (*dd*, C(3) Cp, B), 14.3 (*q*, Me (Oct)); HPLC–MS (ESI, 4.1 kV, 8 μl/min, N_2, methanol): *m*/*z*=1487.5 $[(M_3-H)K_2]^+$ (45%), 1017.5 $[(M_2-H)K_2]^+$ (10%), 1001.5 $[(M_2-H)NaK]^+$ (38%), 979.5 $[M_2K]^+$ (79%), 963.5 $[(M_2-H)NaK]^+$ (65%), 509.6 $[MK]^+$ (82%), 493.7 $[MNa]^+$ (100%); HRMS calcd for $C_{22}H_{38}N_4O_7$: 470.2740; found: 470.2741; Anal. calcd for $C_{16}H_{26}N_4O_7$ (470.57): C, 56.15; H, 8.14; N, 11.91; found: C, 56.02; H, 8.29; N, 11.82.

3.1.19. *cis*-(2*S*)-2-[2-(Octanoylamino)cyclopropyloxy]-propionyl-L-alanyl-D-isoglutamine (*cis*-19). Hydrogenolysis of **17** (95 mg, 0.17 mmol) in ethanol (20 ml) with Pd/C (10%, 20 mg) followed by chromatography (silica gel, chloroform/methanol/acetic acid 85:10:5) afforded **19** (65 mg, 81%) as a white amorphous solid. R_f (chloroform/methanol/ acetic acid 85:10:5) 0.20; IR (KBr): ν=3405*s*, 2930*m*, 2855*w*, 1655*s*, 1550*s*, 1450*m*, 1320*m*, 1170*m*, 1105*m*; ^{1}H NMR (400 MHz, DMSO-d_6): δ=9.02 (*br*, 1H, NH), 8.10 (*br*, 1H, NH), 7.82 (*d*, *J*=6.8 Hz, 1H, NH), 7.31 (*br*, 1H, NH, A), 7.27 (*br*, 1H, NH, B), 6.96 (*br*, 1H, NH), 4.24 (*qd*, *J*=7.2 Hz, 1H, CH(Ala)), 4.03–3.99 (*m*, 1H, CH(iGln)), 3.93 (*q*, *J*=6.6 Hz, 1H, CH(Lac), A) 3.83 (*q*, *J*=6.7 Hz, 1H, CH(Lac), B), 3.56–3.52 (*m*, 1H, H–C(1) Cp, A determined in CD_3OD), 3.48–3.44 (*m*, 1H, H–C(1) Cp, B determined in CD_3OD), 2.70–2.61 (*m*, 1H, H–C(2) Cp), 2.10 (*t*, *J*=7.4 Hz, 2H, H–C(2) Oct), 2.05–1.98 (*m*, 2H, H–C(4) iGln), 1.88–1.80 (*m*, 1H, H_A–C(3) iGln), 1.78–1.70 (*m*, 1H, H_B–C(3) iGln), 1.50–1.45 (*m*, 2H, H–C(3) Oct), 1.28–1.20 (*m*, 11H, Me, Oct), 1.18 (*d*, *J*=6.6 Hz, 3H, Me), o.88–0.80 (*m*, 2H, H–C(3) Cp), 0.84 (*t*, *J*=7.0 Hz, 3H, Me(Oct)); ^{13}C NMR (100 MHz, CD_3OD): δ=178.1 (*s*, C═O), 177.7 (*s*, C═O), 176.7 (*s*, C═O), 175.5 (*s*, C═O), 174.7 (*s*, C═O), 77.9 (*d*, C(2) Lac, A), 77.8 (*d*, C(2) Lac, B), 55.9 (*d*, C(1) Cp, A), 55.7 (*d*, C(1) Cp, B), 54.7 (*d*, C(2) iGln), 50.3 (*d*, C(2) Ala), 36.9 (*t*, C(2) Oct), 32.9 (*t*, Oct), 30.4 (*t*, Oct), 30.2 (*t*, C(4) iGln), 30.1 (*t*, Oct), 29.2 (*t*, C(3) iGln), 28.6 (*d*, C(2) Cp, A), 28.0 (*d*, C(2) Cp,

B), 27.1 (*t*, Oct), 23.7 (*t*, Oct), 19.1 (*q*, Me, A), 19.0 (*q*, Me, B), 18.0 (*q*, Me), 14.4 (*q*, Me (Oct)), 12.7 (*dd*, C(3) Cp, A), 11.8 (*dd*, C(3) Cp, B); HPLC–MS (ESI, 4.1 kV, 8 μl/min, N_2, methanol): m/z=1487.5 $[(M_3-H)K_2]^+$ (95%), 1017.3 $[(M_2-H)K_2]^+$ (16%), 979.3 $[(M_2K]^+$ (100%), 509.5 $[MK]^+$ (82%), 493.7 $[MNa]^+$ (37%); Anal. calcd for $C_{22}H_{38}N_4O_7$ (470.57): C, 56.15; H, 8.14; N, 11.91; found: C, 55.96; H, 8.28; N, 11.82.

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