Synthesis of Spacered Cyclopropanoid Muramyldipeptide Analogues as Potential Immunostimulants

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A novel class of cyclopropanoic muramyldipeptide analogues containing an additional methylene spacer between the cyclopropane ring and the lactyl residue has been prepared by a straightforward synthesis starting from isopropyl (*R*)-lactate.

Key words: Muramyldipeptide Analogues, Cyclopropanes, Immunostimulants

Introduction

Interest in immunostimulation [1] has been fuelled by the advances in bacterial cell wall chemistry and by the rapidly expanding knowledge of endogenous mediators of cell-differentiation and cooperation. Thus, over the past two decades muramyldipeptide (N-acetyl-muramyl-L-alanyl-D-isoglutamine, MDP) and its derivatives have been investigated in detail and many of these compounds revealed adjuvant activity and showed stimulation of nonspecific resistance against bacterial, viral and parasite infections [2 – 4] as well as anticancer activities. Albeit this progress, many of the compounds exhibited undesired side effects, among them pyrogenicity, induction of arthritis, transient leucopenia as well as sensitivization to endotoxin [5 – 7].

Structure activity relationships for these MDP analogues have been established [8, 9] and as a result it has been assumed that an intact carbohydrate part is not essential for establishing immunostimulant activity. Several carbocyclic [10 – 12] as well as acyclic analogues [13] have been synthesized and tested. Even more recently, the synthesis of lipophilic phosphonate [14], phosphonamidate [14], adamantyl [15] or acridine substituted analogues [16, 17] has been reported.

Results and Discussion

Cyclopropanes and cyclopropanoid modified biomolecules have been in the focus of synthetic interest for several years and the synthesis of highly functionalized cyclopropanes has become a viable discipline in its own right [18]. In our own approach to novel and unprecedented MDP analogues we decided to prepare target compounds possessing a spacered cyclopropane moiety instead of the carbohydrate portion.

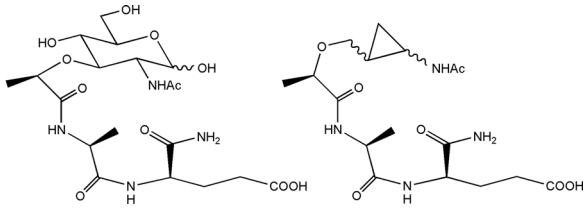

Fig. 1. MDP and its spacered cyclopropanoid analogues.

Thus, allylation of commercially available isobutyl (*R*)-lactate with allyl bromide in the presence of Ag_2O [19] gave 78% of isobutyl (2*R*)-(allyloxy)propanoate (**1**) (Scheme 1) whose rhodium acetate catalyzed reaction [20] with *tert*-butyl diazoacetate furnished a 1:1:2:2 mixture of the corresponding *cis*- and *trans*-configurated cyclopropanes **2** that could not be separated even by exhaustive chromatography under a variety of different conditions.

Selective cleavage of the *tert*-butyl ester leaving the isopropyl ester intact was performed by treatment of **2** with trifluoroacetic acid [21, 22]. The acid **3** was then subjected to a modified *Curtius*-degradation using diphenylphosphoryl azide (DPPA) [23] in the presence of *tert*-butanol and triethylamine. Thus, the N-Boc-protected cyclopropylamine **4** could be obtained

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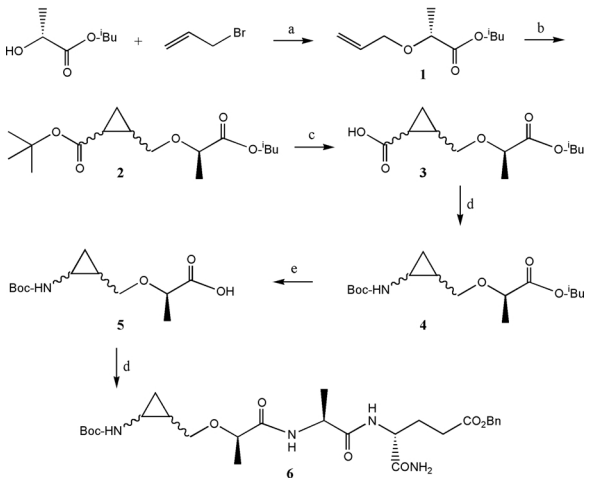
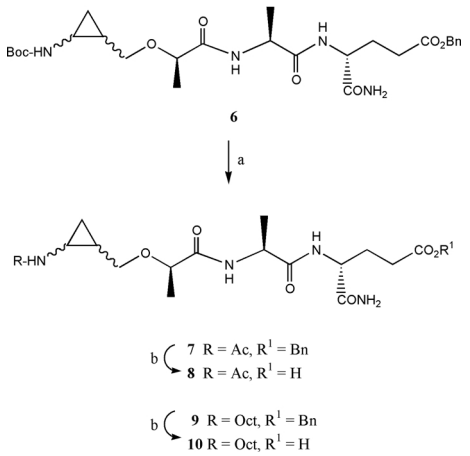


Scheme 1. Reagents: a) $MgSO_4/Ag_2O$; b) N_2CHCO_2 *t*Bu/[$(Rh(OAc)_2)_2$]; c) CF_3COOH; d) DPPA, *t*BuOH, NEt_3; e) KOH in EtOH; f) $ClCO_2$*i*Bu/NMM, then H_2N-L-Ala-D-*i*Gln-OBn.



Scheme 2. Reagents: a) HCl/EtOAc then AcCl/NEt_3 (for **7**) or $C_7H_{15}COCl/Et_3N$ (for **9**); b) Pd/C, H_2, 3 bar.

in 51% yield. Hydrolysis of the isopropyl ester was accomplished with potassium hydroxide in ethanol to afford the free acid **5** which was subjected to a peptide synthesis using the mixed-anhydride protocol [24]. Thus, treatment of **5** with isobutyl chloroformate/N-methyl-morpholine (NMM) followed by the addition of L-alanyl-D-*i*-glutamine-γ-benzyl ester (that was freshly prepared from commercially available Boc-L-Ala-D-*i*Gln-OBn by acidic cleavage of the Boc group using hydrochloric acid in ethyl acetate) gave 86% of the protected derivative **6**.

The Boc group was cleaved by treatment of **6** with dry hydrochloric acid in ethyl acetate and the resulting amine was acetylated by reaction with acetyl chloride/triethylamine. Hydrogenolysis of the N-acetyl-derivative **7** using Pd/C as the catalyst finally gave the target molecule **8**. Similarly from **6** the octanoyl derivative **9** was obtained by deprotection of **6** followed by acylation using octanoyl chloride/triethylamine. Tedious chromatography of the resulting mixture of products allowed the separation of *cis*-configurated **9a** as well as of *trans*-**9b**. Hydrogenolysis of **9a**/**9b** finally gave **10a** and **10b**, respectively.

The determination of the different biological activities of these novel carbocyclic MDP analogues is still in progress.

Experimental Section

General

See ref. [23].

*Isobutyl (2R)-2-(allyloxy)propanoate (**1**)*

To a solution of isobutyl (*R*)-lactate (7.3 g, 0.05 mol) in hexane (200 ml) a mixture of Ag_2O (30.0 g, 0.13 mol) and $MgSO_4$ (1.2 g, 0.01 mol) was added. In the dark, allyl bromide (9.1 g, 0.075 mol) was slowly added and stirring at r.t. was continued for another 24 h. The suspension was filtered, the residue washed with hexane and the combined organic phases were evaporated. The remaining oil (5.9 g, 78%) was used without further purification in the next step. An analytical sample could be obtained by chromatography (silica gel, hexane/ethyl acetate 3:2). – R_F (hexane/ethyl acetate 3:2) 0.73. – $[\alpha]_D$ 70.6 (*c*, 1.0 $CHCl_3$). – IR (film): ν = 3080w, 2965s, 2875m, 1750s, 1650w, 1470m, 1370m, 1275m, 1200s, 1140s, 1065m, 1025m cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.90 (dddd, J = 17.2, 10.4, 6.1, 5.5 Hz, 1 H, HC=C), 5.27 (dd, J = 17.2, −1.7 Hz, 1 H, $H_2C=C_{trans}$), 5.18 (dd, J = 10.4, −1.7 Hz, 1 H, $H_2C=C_{cis}$), 4.14 (dd, J = 12.5, 5.5 Hz, 1 H, 1-$H_{A,allyl}$), 4.01 (q, J = 6.9 Hz, 1 H, 2-H), 3.96 – 3.87 (m, 3 H, 1-$H_{B,allyl}$ and CH_2 of *i*Bu), 1.95 (qqt, J = 6.7 Hz, CH of *i*Bu), 1.41 (d, J = 6.9 Hz, 3 H, Me), 0.93 (d, J = 6.7 Hz, 6 H, 2 x Me of *i*Bu). – ^{13}C NMR (50 MHz, $CDCl_3$): δ = 173.3 (s, C=O), 134.1 (d, =CH), 117.6 (t, $=CH_2$), 74.0 (d, 2-C), 71.0 (CH_2O), 70.8 (t, CH_2O), 27.7 (d, CH), 19.0 (q, Me), 18.7 (q, Me). – MS (GC-MS, EI, 70 eV): *m*/*z* (%) = 186 (1), 171 (1), 130 (7), 115 (1), 103 (1), 85 (75), 74 (14), 57 (35), 41 (100). – Analysis for $C_{10}H_{18}O_3$ (186.25): calcd. C 64.49, H 9.74; found C 64.13, H 9.83.

*Isobutyl (2R)-2-{[2-(tert-butoxycarbonyl)cyclopropyl]methoxy}propanoate (**2**)*

To a solution of **1** (4.70 g, 25.3 mmol) in abs. dichloromethane containing [$(Rh(OAc)_2)_2$] (100 mg) was added a solution of *tert*-butyl diazoacetate (5.2 g, 36.6 mmol) in abs. dichloromethane (20 ml) very slowly (approx. 20 h). The solvents were removed under diminished pressure and the residue was subjected to chromatography (silica gel, hexane/ethyl acetate 16:1) to afford oily **2** (5.90 g, 78%) as a mixture of isomers (cis_A : cis_B : $trans_A$: $trans_B$ = 1:1:2:2 (by ^{1}H NMR)). – R_F (hexane/ethyl acetate 3:2) 0.69. – IR (film) ν = 2970s, 2875s, 1725s, 1470s, 1395s, 1370s, 1325s, 1260s, 1210s, 1150s, 1070s, 1030s, 990m cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.05 – 3.85 (m, 4 H, 2-H, H_A-$CH_2O_{cis,A}$, CH_2 of *i*Bu), 3.70 (dd, J = 10.3, 7.2 Hz, 1 H, H_A-$CH_2O_{cis,B}$), 3.61 (dd, J = 10.3, 7.0 Hz, 1 H, H_B-$CH_2O_{cis,B}$), 3.53 (dd, J = 10.2, 6.2 Hz, H_A-$CH_2O_{trans,A}$), 3.45 - 3.34 (m, 3 H, H_B-$CH_2O_{cis,A}$, $CH_2O_{trans,B}$), 3.28 (dd, J = 10.2, 6.4 Hz, 1 H, H_B-$CH_2O_{trans,A}$), 1.94 (qqt, J = 6.7 Hz, 1 H, CH of *i*Bu), 1.74 – 1.54 (m, 1 H, 2-H of cp), 1.48 – 1.40 (m, 1 H, 1-H of cp), 1.46 (s, 9 H, *t*Bu), 1.44 (s, 9 H, *t*Bu), 1.43 (s, 9 H, *t*Bu), 1.42 (s, 9 H, *t*Bu), 1.394 (d, J = 6.8 Hz, 3 H, Me), 1.390 (d, J = 6.8 Hz, 3 H, Me), 1.36 (d, J = 6.8 Hz, 3 H, Me), 1.14 – 1.09 (m, 1 H, 3-$H_{A,trans}$ of cp), 1.02 – 0.94 (m, 2 H, 3-H_{cis} of cp), 0.923 (d, J = 6.6 Hz, 6 H, 2 x Me), 0.915 (d, J = 6.6 Hz, 6 H, 2 x Me), 0.83 – 0.77 (m, 1 H, 3-$H_{B,trans}$ of cp). – ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.1 (s, C=O), 172.9 (s, C=O), 172.88 (s, C=O), 172.85 (s, C=O), 172.55 (s, C=O), 172.49 (s, C=O), 171.4 (s, C=O), 171.2 (s, C=O), 80.4 (s, O*C*$(CH_3)_3$), 80.2 (s, O*C*$(CH_3)_3$), 74.9 (d, 2-$C_{cis,A}$), 74.7 (d, 2-$C_{trans,A}$), 74.6 (d, 2-$C_{trans,B}$), 74.4 (d, 2-$C_{cis,B}$), 71.6 (t, $CH_2O_{trans,A}$), 71.4 (t, $CH_2O_{trans,B}$), 70.8 (t, CH_2 of *i*Bu), 70.7 (t, CH_2 of *i*Bu), 70.66 (t, CH_2 of *i*Bu), 68.1 (t, $CH_2O_{cis,A}$), 67.6 (t, $CH_2O_{cis,B}$), 28.23 (q, OC(*C*$H_3)_3$), 28.20 (q, OC(*C*$H_3)_3$), 27.8 (d, CH of *i*Bu), 21.0 (d, 2-$C_{trans,A}$ of cp), 20.9 (d, 2-$C_{trans,B}$ of cp), 20.8 (d, 2-$C_{cis,A}$ of cp), 20.2 (d, 2-$C_{cis,B}$ of cp), 19.6 (q, Me), 19.3 (d, 1-C of cp), 19.1 (q, Me), 18.9, 18.8, 18.74, 18.69, 18.65, 18.57 (Me or 1-C of cp), 13.0 (dd, 3-$C_{trans,A}$ of cp), 12.7 (dd, 3-$C_{trans,B}$ of cp), 11.9 3-$C_{cis,A}$ of cp), 11.4 (dd, 3-$C_{cis,B}$ of cp). – MS (GC-MS, EI, 70 eV): *m*/*z* (%) = 301 (1), 245 (9), 227 (23), 216 (3), 198 (7), 188 (15), 170 (4), 155 (7), 143 (59), 117 (14), 99 (100). – Analysis for $C_{16}H_{28}O_5$ (300.40): calcd. C 63.97, H 9.40; found C 63.81, H 9.56.

*2-({[(1R) 2-Isobutoxy-1-methyl-2-oxoethyl]oxy}methyl)-1-cyclopropanecarboxylic acid (**3**)*

To a solution of **2** (5.0 g, 16.7 mmol) in dry dichloromethane (100 ml) at 0 °C under argon a solution of trifluoroacetic acid (11.4 g, 100 ml) in dry dichloromethane (20 ml) was slowly added and stirring was continued for 18 h. The solvents were removed under diminished pressure, toluene (twice 40 ml) added and evaporated and oily **3** (4.1 g, 100%) was obtained. It was used in the next step without any purification. – IR (film): ν = 2965s, 2875m, 1735s, 1700s, 1470m, 1370m, 1275m, 1200s, 1170s, 1140s, 1070m, 1025m, 985m cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.02 – 3.85 (m, 4 H, H_A-$CH_2O_{cis,A}$, 2-H, CH_2 of *i*Bu), 3.72 – 3.65 (m, 2 H, $CH_2O_{cis,B}$), 3.50 (dd, J = 10.2, 6.0 Hz, 1 H, H_A-$CH_2O_{trans,A}$), 3.47 – 3.39 (m, 3 H, H_B-$CH_2O_{cis,A}$, $CH_2O_{trans,B}$), 3.28 (dd, J = 10.2, 6.2 Hz, 1 H, H_B-$CH_2O_{trans,A}$), 1.94 (qqt, J = 6.7 Hz, CH of *i*Bu), 1.81 – 1.73 (m, 1 H, 2-H of cp), 1.72 – 1.65 (m, 1 H, 1-H_{cis} of cp), 1.62 – 1.55 (m, 1 H, 1-H_{trans} of cp), 1.391 (d, J = 6.8 Hz, 3 H, Me), 1.386 (d, J = 6.8 Hz, 3 H, Me), 1.35 (d, J = 6.8 Hz, 3 H, Me), 1.27 – 1.23 (m,1 H, 3-$H_{A,trans}$ of cp), 1.19 – 1.12 (m, 1 H, 3-$H_{A,cis}$ of cp), 1.10 – 1.06 (m, 1 H, 3-$H_{B,cis}$ of cp), 0.98 – 0.93 (m, 3-$H_{B,trans}$ of cp), 0.92 (d, J = 6.9 Hz, 6 H, 2 x Me), 0.91 (d, J = 6.6 Hz, 6 H, 2 x Me). – ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.72 (s, COOH), 179.65 (s, COOH), 178.7 (s, COOH), 178.3 (s, COOH), 173.3 (s, C=O), 173.1 (s, C=O), 173.01 (s, C=O), 172.98 (s, C=O), 75.0 (d, 2-$C_{cis,A}$), 74.8 (d, 2-$C_{trans,A}$), 74.7 (d, 2-$C_{trans,B}$), 74.6 (d, 2-$C_{cis,B}$), 71.1 (t, CH_2), 70.93 (t, CH_2), 70.92 (t, CH_2), 70.90 (t, CH_2), 70.87 (t, CH_2), 70.85 (t. CH_2), 68.1 (t, $CH_2O_{cis,A}$), 67.6 (t, $CH_2O_{cis,B}$), 27.78 (d, CH of *i*Bu), 27.75 (d, CH of *i*Bu), 22.37 (d, 2-$C_{trans,A}$ of cp), 22.35 (d, 2-$C_{trans,B}$ of cp), 21.9 (d, 2-$C_{cis,A}$ of cp), 21.5 (d, 2-$C_{cis,B}$ of cp), 19.05 (q, Me of *i*Bu), 19.04 (q, Me of *i*Bu), 18.7, 18.6, 18.4, 18.1, 17.40, 17.36 (Me and 1-C of cp), 13.7 (dd, 3-$C_{trans,A}$ of cp), 13.6 (dd, 3-$C_{trans,B}$ of cp), 13.1 (dd, 3-$C_{cis,A}$ of cp), 13.0 (dd, 3-$C_{cis,B}$ of cp). – MS (EI, 70 eV): *m*/*z* (%) = 245 (5), 227 (37), 198 (5), 188 (5), 171 (3), 143 (39), 125 (4), 116 (14), 113 (41), 99 (100). – HRMS for $C_{12}H_{20}O_5$: calcd. 244.1310; found 244.1311.

*Isobutyl (2R) 2-({2-[(tert-butoxycarbonyl)amino]cyclopropyl}methoxy)propanoate (**4**)*

To a solution of **3** (7.0 g, 28.7 mmol) containing triethylamine (4.3 g, 42.6 mmol) and *tert*-butanol (8.5 g, 115 mmol) under argon DPPA (9.5 g, 34.5 mmol) was carefully added and the mixture heated for 40 h at 50 °C. The volatiles were removed under reduced pressure and the residue was subjected to chromatography (silica gel, hexane/ethyl acetate 5:1) to afford oily **4** (4.6 g, 51%). – R_F (hexane/ethyl acetate 3:2) 0.62. – IR (film): ν = 3355m, 2970s, 2875m, 1720s, 1510s, 1470m, 1390m, 1365s, 1255s, 1170s, 1140s, 1065m, 1025m cm^{-1}. – ^{1}H NMR (400 MHz, CD_3OD): δ = 5.85 (br, 1 H, NH, determined in $CDCl_3$, *cis*), 4.70 (br, 1 H, NH, determined in $CDCl_3$, *trans*), 4.12 – 4.00 (m, 1 H, 2-H), 3.96 – 3.88 (m, 2 H, CH_2 of *i*Bu), 3.60 – 3.50 (m, 1 H, H_A-CH_2O_{cis}), 3.43 (dd, J = 10.3, 6.5 Hz, 1 H, H_A-CH_2O_{trans}), 3.34 (dd, J = 10.3, 6.9 Hz, 1 H, H_B-CH_2O_{trans}), 3.29 – 3.22 (m, 1 H, H_B-CH_2O_{cis}), 2.66 – 2.56 (m, 1 H, 2-H_{cis} of cp), 2.38 – 2.32

(*m*, 1 H, 2-H$_{trans}$ of cp), 1.94 (qqt, $J = 6.6$ Hz, 1 H, CH of *i*Bu), 1.43 (s, 9 H, tBu), 1.364 (d, $J = 6.9$ Hz, 3 H, Me), 1.359 (d, $J = 6.8$ Hz, 3 H, Me), 1.355 (d, $J = 6.9$ Hz, 3 H, Me), 1.29 – 1.19 (m, 1 H, 1-H$_{cis}$ of cp), 1.18 – 1.10 (m, 1 H, 1-H$_{trans}$ of cp), 0.95 (d, $J = 6.6$ Hz, 6 H, 2 x Me of *i*Bu), 0.95 – 0.88 (m, 1 H, 3-H$_{A,cis}$ of cp), 0.70 – 0.63 (m, 2 H, 3-H$_{trans}$ of cp), 0.48 – 0.37 (m, 1 H, 3-H$_{B,cis}$ of cp). – ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 174.3$ (s, C=O), 173.0 (s, C=O), 172.9 (s, C=O), 157.8 (s, C=O of Boc), 157.0 (s, C=O of Boc), 156.6 (s, C=O, C=O of Boc), 156.1 (s, C=O of Boc), 79.1 (s, O*C*(CH$_3$)$_3$), 78.8 (s, O*C*(CH$_3$)$_3$), 74.43 (d, 2-C$_{trans,A}$), 74.37 (d, 2-C$_{trans,B}$), 74.3 (d, 2-C$_{cis,A}$), 74.1 (d, 2-C$_{cis,B}$), 71.7 (t, CH$_2$O$_{trans,A}$), 71.4 (t, CH$_2$O$_{trans,B}$), 70.7 (t, CH$_2$ of *i*Bu), 70.63 (t, CH$_2$ of *i*Bu), 70.57 (t, CH$_2$ of *i*Bu), 69.8 (t, CH$_2$O$_{cis,A}$), 68.9 (t, CH$_2$O$_{cis,B}$), 28.30 (q, OC(*C*H$_3$)$_3$), 28.26 (q, OC(*C*H$_3$)$_3$), 28.1 (d, 2-C of cp), 27.6 (d, CH of *i*Bu), 27.2 (d, 2-C of cp), 19.9 (d, 1-C of cp), 18.91 (q, Me), 18.86 (q, Me), 18.86 (q, Me), 18.81 (q, Me), 18.64 (q, Me), 18.57 (q, Me), 18.5 (q, Me), 16.1 (d, 1-C of cp), 12.6 (dd, 3-C of cp), 12.04 (dd, 3-C of cp), 11.98 (dd, 3-C of cp), 11.3 (dd, 3-C of cp). – MS (EI, 70 eV): *m*/*z* (%) = 316 (1), 260 (12), 242 (1), 216 (6), 199 (1), 170 (10), 147 (6), 114 (51), 113 (50), 70 (99), 69 (100), 57 (100). – Analysis for $C_{16}H_{29}NO_5$ (315.41): calcd. C 60.93, H 9.27, N 4.44; found C 60.81, H 9.42, N 4.51.

*(2R) 2-({2-[tert-Butoxycarbonyl)amino]cyclopropyl}methoxy)propanoic acid (***5***)*

To a solution of **4** (4.6 g, 14.6 mmol) in ethanol (40 ml) at 0 °C a solution of potassium hydroxide (2.5 g, 44.6 mmol) in ethanol (50 ml) was added. The reaction was stirred at r.t. for 2 h, then the solvents were removed under diminished pressure and the residue was suspended in water (50 ml), the pH was adjusted to 3 by the addition of hydrochloric acid (10%) and the aq. phase was extracted with ethyl acetate (4 × 100 ml). The combined organic phases were dried (Na_2SO_4) and the solvents removed to afford oily **5** (3.8 g, 100%) that was used for the next step without any further purification. IR (film): $\nu = 3335$m, 2980s, 2935s, 1715s, 1515s, 1455m, 1395s, 1370s, 1255s, 1170s, 1065s, 1025m cm^{-1}. – ^{1}H NMR (400 MHz, CD_3OD): $\delta = 5.42$ (br, 1 H, determined in $CDCl_3$, *cis*), 4.82 (br, 1 H, NH, determined in $CDCl_3$, *trans*), 4.05 – 3.96 (m, 1H, 2-H), 3.62 – 3.32 (m, 2 H, CH$_2$O), 2.66 – 2.58 (m, 1 H, 2-H$_{cis}$ of cp), 2.38 – 2.32 (m, 1 H, 2-H$_{trans}$ of cp), 1.43 (s, 9 H, *t*Bu), 1.363 (d, $J = 6.8$ Hz, 3 H, Me), 1.360 (d, $J = 6.9$ Hz, 3 H, Me), 1.31 – 1.20 (m, 1 H, 1-H$_{cis}$ of cp), 1.19 – 1.10 (m, 1 H, 1-H$_{trans}$ of cp), 0.96 – 0.89 (m, 1 H, 3-H$_{A,cis}$ of cp), 0.72 – 0.64 (m, 2 H, 3-H$_{trans}$ of cp), 0.46 – 0.41 (m, 1 H, H$_B$-3$_{cis,A}$ of cp), 0.40 – 0.35 (m, 1 H, H$_B$-3$_{cis,B}$ of cp). – ^{13}C NMR (125 MHz, CD_3OD): $\delta = 177.06$ (s, C=O), 176.99 (s, C=O), 176.89 (s, C=O), 176.85 (s, C=O), 159.4 (s, C=O of Boc), 159.03 (s, C=O of Boc), 159.02 (s, C=O of Boc), 80.2 (s, O*C*(CH$_3$)$_3$), 75.5 (d, 2-C), 75.3 (d, 2-C), 75.1 (d, 2-C), 72.8 (t, CH$_2$O$_{trans,A}$), 72.7 (t, CH$_2$O$_{trans,B}$), 70.4 (t, CH$_2$O$_{cis,A}$), 69.9 (t, CH$_2$O$_{cis,B}$), 28.74 (q, OC(*C*H$_3$)$_3$), 28.73 (q, OC(*C*H$_3$)$_3$), 28.1 (d, 2-C of cp), 20.4 (d, 1-C of cp), 19.1 (q, Me), 19.0 (q, Me), 18.9 (q, Me), 17.3 (d, 1-C of cp), 12.3 (dd, 3-C of cp), 12.1 (dd, 3-C of cp), 11.8 (dd, 3-C of cp). – MS (EI, 70 eV): *m*/*z* (%) = 260 (4), 244 (8), 232 (3), 203 (3), 186 (2), 170 (3), 143 (3), 128 (3), 113 (58), 100 (10), 70 (26), 57 (100). – HRMS for $C_{12}H_{21}NO_5$: calcd. 259.1420; found 259.1419.

*Benzyl N-[(2 R) 2-{[((tert-butoxycarbonyl)amino)cyclopropyl]methoxy}propio nyl]-L-alanyl-D-isoglutaminate (***6***)*

To a solution of Boc-L-alanyl-D-isoglutamine-γ-benzyl ester (5.7 g, 14.0 mmol, Bachem) in abs. ethyl acetate (45 ml) a solution of dry hydrochloric acid in abs. ethyl acetate (3.6 N by titration, 23.6 ml, 85 mmol) was added and the mixture was stirred for 3 h at r.t. The volatiles were removed and the crude hydrochloride was used without any further purification. To a solution of **5** (3.2 g, 12.3 mmol) in dry ethyl acetate (30 ml) and abs. dimethylformamide (30 ml) under argon NMM (1.42 g, 14.1 mmol) was added at 0 °C, then the mixture was cooled to −15 °C and isobutyl chloroformate (1.92 g, 14.1 mmol) was slowly added. After stirring for 5 min at −15 °C a solution of the deprotected dipeptide (*vide supra*, 4.8 g, 14.0 mmol) dissolved in a mixture containing NMM (2.84 g, 28.2 mmol), ethyl acetate (40 ml) and dimethylformamide (40 ml) was added. Stirring at r.t. was continued for 18 h, then the solvents were removed under reduced pressure, the residue suspended in water (100 ml) and extracted with ethyl acetate (4 × 150 ml). The combined organic phases were dried (Na_2SO_4), the solvents removed, and the residue was subjected to chromatography (silica gel, ethyl acetate/methanol 16:1 → 10:1) to afford **6** (5.8 g, 86%) as a white amorphous solid. – R_F (ethyl acetate/methanol 10:1) 0.43. – IR (KBr): $\nu = 3400$m, 3335m, 3290m, 2980w, 2935w, 1730s, 1670s, 1650s, 1530m, 1450w, 1390m, 1365m, 1310w, 1250m, 1170m, 1100m, 1065w, 1025w cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.77$ (br, 1 H, NH), 7.36 – 7.25 (m, 6 H, NH and Ph), 6.88 (br, 1 H, NH), 6.83 (br, 1 H, NH), 6.79 (br, 1 H, NH), 5.09 (*AB* system, $J = 12.4$ Hz, 2 H, CH$_2$-Ph), 4.88 (br, 1 H, NH of Boc), 4.48 – 4.41 (m, 1 H, CH of *i*Gln), 4.35 – 4.29 (m, 1 H, CH of Ala), 4.10 – 4.03 (m, 1 H, CH of Lac), 3.86 – 3.80 (m, 1 H, CH of Lac), 3.70 – 2.95 (m, 2 H, CH$_2$O), 2.94 – 2.64 (m, 1 H, 2-H$_{cis}$ of cp), 2.59 – 2.51 (m, 1 H, H$_A$-4 of *i*Gln), 2.47 – 2.40 (m, 1 H, H$_B$-4 of *i*Gln), 2.39 – 2.33 (m,1 H, 2-H$_{trans}$ of cp), 2.24 – 2.17 (m, 1 H, H$_A$-3 of *i*Gln), 2.04 – 1.95 (m, 1 H, H$_B$-3 of *i*Gln), 1.411 (s, 9 H, tBu), 1.405 (s, 9 H, tBu), 1.400 (s, 9 H, tBu), 1.39 – 1.31 (m, 6 H, Me of Ala and Me of Lac), 1.28 – 1.15 (m, 1 H, 1-H of cp), 1.00 – 0.94 (m, 1 H, H$_A$-3$_{cis}$ of cp), 0.77 – 0.69 (m, 1 H, H$_A$-3$_{trans}$ of cp), 0.67 – 0.61 (m, 1 H, H$_B$-3$_{trans}$ of cp),

0.41 – 0.37 (m, 1 H, H_B-3$_{cis}$ of cp). – ^{13}C NMR (100 MHz, CD_3OD): δ = 176.2 (s, C=O), 175.94 (s, C=O), 175.87 (s, C=O), 175.80 (s, C=O), 175.71 (s, C=O), 175.70 (s, C=O), 174.73 (s, C=O), 174.70 (s, C=O), 159.2 (s, C=O of Boc), 159.1 (s, C=O of Boc), 158.9 (s, C=O of Boc), 137.3 (s, Ph), 129.4 (d, Ph), 129.0 (d, Ph), 80.1 (s, O*C*(CH_3)$_3$), 77.5 (d, 2-$C_{cis,A}$ of Lac), 77.0 (d, 2-$C_{trans,A}$ of Lac), 76.8 (d, 2-$C_{trans,B}$ of Lac), 76.4 (d, 2-$C_{cis,B}$ of Lac), 72.8 (t, CH_2O), 72.3 (t, CH_2O), 70.3 (t, CH_2O), 69.7 (t, CH_2O), 67.4 (t, CH_2-Ph), 53.5 (d, 2-C of *i*Gln), 50.5 (d, 2-C of Ala), 31.43 (t, 4-C of *i*Gln), 31.41 (t, 4-C of *i*Gln), 29.4 (d, 2-C of cp), 29.3 (2-C of cp), 28.82 (q, OC(CH_3)$_3$), 28.80 (q, OC(*C*H_3)$_3$), 28.12 (t, 3-C of *i*Gln), 28.07 (t, 3-C of *i*Gln), 21.0 (d, 1-C of cp), 20.7 (d, 1-C of cp), 19.2 (q, Me), 18.92 (q, Me), 18.88 (q, Me), 18.0 (q, Me), 17.9 (q, Me), 11.9 (dd, 3-C of cp). – MS (EI, 70 eV): *m*/*z* (%) = 548 (1), 505 (1), 474 (3), 457 (2), 448 (2), 430 (5), 404 (5), 380 (11), 363 (8), 346 (3), 313 (12), 285 (21), 257 (21), 237 (13), 229 (56), 192 (100), 127 (24), 113 (74), 91 (91). – Analysis for $C_{27}H_{40}N_4O_8$ (548.64): calcd. C 59.11, H 7.35, N 10.21; found C 59.01, H 7.49, N 10.30.

*Benzyl N-[(2R) 2-{[(2-acetylamino)cyclopropyl]methoxy}propionyl]-L-alanyl-D-isoglutaminate (***7***)*

To a solution of **6** (1.0 g, 1.8 mmol) in ethyl acetate (8 ml) a solution of dry hydrochloric acid in ethyl acetate (3.6 N by titration, 3.3 ml, 11.8 mmol) was added and the reaction was stirred for 3 h at r.t., then the volatiles were removed and the residue was dissolved in dichloromethane (30 ml) containing triethylamine (1.8 g, 18 mmol). After cooling to 0 °C a solution of acetyl chloride (200 mg, 2.5 mmol) in dichloromethane (5 ml) was added dropwise and stirring at r.t. was continued for another 18 h. The solvents were removed under reduced pressure, water (50 ml) was added and the solution was extracted with ethyl acetate (4 × 50 ml). The combined organic phases were dried (Na_2SO_4), the solvent was evaporated and the residue subjected to chromatography (silica gel, ethyl acetate/methanol 13:1 → 10:1) to afford **7** (594 mg, 67%) as a white amorphous solid. R_F (ethyl acetate/methanol 10:1) 0.12. – IR (KBr): ν = 3280s, 3070m, 2930m, 1730s, 1650s, 1540s, 1455m, 1370m, 1245m, 1170m, 1110m cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.32 (d, J = 8.3 Hz, 1 H, NH), 8.12 (d, J = 7.1 Hz, 1 H, NH), 7.56 – 7.47 (m, 2 H, NH), 7.39 (br, 1 H, NH), 7.34 – 7.26 (m, 5 H, Ph), 7.14 (br, 1 H, NH), 6.97 (br, 1 H, NH), 6.47 (br, 1 H, NH), 6.43 (br, 1 H, NH), 6.32 (br, 1 H, NH), 6.10 (br, 1 H, NH), 6.06 (br, 1 H, NH), 6.01 (br, 1 H, NH), 5.07 (AB system, J = 12.5 Hz, 2 H, CH_2-Ph), 4.59 – 4.53 (m, 1 H, CH of Ala), 4.43 – 4.35 (m, 2 H, CH of *i*Gln and CH of Ala), 4.14 (q, J = 6.7 Hz, 1 H, CH of Lac), 3.89 (dd, J = 9.8, 4.4 Hz, 1 H, H_A-$CH_2O_{trans,A}$), 3.82 (q, J = 6.9 Hz, 1 H, CH of Lac), 3.74 (dd, J = 10.0, 5.2 Hz, H_A-$CH_2O_{cis,A}$), 3.69 (dd, J = 11.8, 4.6 Hz, 1 H, H_A-$CH_2O_{cis,B}$), 3.59 (dd, J = 11.2, 4.8 Hz, 1 H, H_A-$CH_2O_{trans,B}$), 3.33 (dd, J = 11.0, 11.0 Hz, 1 H, H_B-$CH_2O_{cis,B}$), 3.11 (dd, J = 10.0, 10.0 Hz, 1 H, H_B-$CH_2O_{cis,A}$), 3.06 (dd, J = 11.3, 9.0 Hz, 1 H, H_B-CH_2O,$_{trans,B}$), 2.79 – 2.70 (m, 1 H, 2-H of cp), 2.69 (dd, J = 9.8, 9.8 Hz, 1 H, H_B-$CH_2O_{trans,A}$), 2.54 – 2.38 (m, 2 H, 4-H of *i*Gln), 2.25 – 2.14 (m, 1 H, 3-H_A of *i*Gln), 2.05 – 1.95 (m, 1 H, 3-H_B of *i*Gln), 1.94 (s, 3 H, Ac), 1.92 (s, 3 H, Ac), 1.90 (s, 3 H, Ac), 1.88 (s, 3 H, Ac), 1.40 – 1.31 (m, 6 H, Me of Ala, Me of Lac), 1.28 – 1.10 (m, 1 H, 1-H of cp), 1.03 – 0.95 (m, 1 H, 3-$H_A$cis of cp), 0.85 – 0.79 (m, 1 H, 3-$H_{Btrans,A}$ of cp), 0.77 – 0.70 (m, 1 H, 3-$H_{Atrans,B}$ of cp), 0.69 – 0.61 (m, 1 H, 3-$H_{B,trans}$ of cp), 0.41 – 0.35 (m, 1 H, 3-$H_{B,cis}$ of cp). – ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.01 (s, C=O), 173.99 (s, C=O), 173.99 (s, C=O), 173.90 (s, C=O), 173.8 (s, C=O), 173.7 (s, C=O), 173.03 (s, C=O), 172.99 (s, C=O), 172.97 (s, C=O), 172.90 (s, C=O), 172.86 (s, C=O), 172.84 (s, C=O), 172.79 (s, C=O), 172.7 (s, C=O), 172.4 (s, C=O), 172.1 (s, C=O), 135.55 (s, Ph), 135.52 (s, Ph), 128.37 (d, Ph), 128.36 (d, Ph), 128.08 (d, Ph), 128.06 (d, Ph), 128.0 (d, Ph), 76.8 (d, 2-$C_{cis,A}$ of Lac), 76.3 (d, 2-$C_{trans,A}$ of Lac), 75.4 (d, 2-$C_{cis,B}$ of Lac), 75.0 (d, 2-$C_{trans,B}$ of Lac), 72.2 (t, $CH_2O_{trans,A}$), 71.0 (t, $CH_2O_{trans,B}$), 69.4 (t, $CH_2O_{cis,A}$), 68.9 (t, $CH_2O_{cis,B}$), 66.54 (t, CH_2-Ph), 66.51 (t, CH_2-Ph), 66.47 (t, CH_2-Ph), 53.0 (d, 2-$C_{cis,A}$ of *i*Gln), 52.9 (d, 2-$C_{trans,A}$ of *i*Gln), 52.6 (d, 2-$C_{trans,B}$ of *i*Gln), 52.5 (d, 2-$C_{cis,B}$ of *i*Gln), 49.3 (d, 2-C of Ala), 49.2 (d, 2-C of Ala), 48.9 (d, 2-C of Ala), 30.7 (t, 4-C of *i*Gln), 30.6 (t, 4-C of *i*Gln), 29.1 (d, 2-$C_{trans,A}$ of cp), 28.6 (d, 2-$C_{trans,B}$ of cp), 27.4 (d, 2-$C_{cis,A}$ of cp), 27.03 (t, 3-C of *i*Gln), 26.98 (d, 2-$C_{cis,B}$ of cp), 26.7 (t, 3-C of *i*Gln), 26.5 (t, 3-C of *i*Gln), 22.94 (q, Ac), 22.86 (q, Ac), 22.75 (q, Ac), 20.9, 20.0, 18.7, 18.4, 18.2, 17.9, 17.83, 17.77, 17.70, 17.0, 16.4 (Me or 1-C), 10.9 (dd, 3-$C_{cis,A}$ of cp), 10.2 (dd, 3-$C_{trans,A}$ of cp), 10.0 (dd, 3-$C_{trans,B}$ of cp), 9.3 (3-$C_{cis,B}$ of cp). – MS (EI, 70 eV): *m*/*z* (%) = 490 (1), 472 (1), 446 (3), 380 (3), 363 (9), 297 (1), 272 (10), 255 (34), 237 (5), 227 (100), 192 (9), 144 (20), 112 (66), 91 (48). – Analysis for $C_{24}H_{24}N_4O_7$ (490.56): calcd. C 58.76, H 6.99, N 11.42; found C 58.60, H 7.11, N 11.52.

*(2R) 2-{[2-(Acetylamino)cyclopropyl]methoxy}propionyl-L-alanyl-D-isoglutamine (***8***)*

Hydrogenolysis of **7** (350 mg, 0.7 mmol) in ethanol (50 ml) in the presence of Pd/C (10%, 50 mg) for 8 h at 3 bar followed by filtration and chromatography (chloroform/ethanol/acetic acid 70:25:5) gave **8** (280 mg, 100%) as a white amorphous solid. R_F (chloroform/ethanol/acetic acid 70:25:5) 0.38. – IR (KBr): ν = 3415s, 1655s, 1540s, 1450m, 1375m, 1300w, 1255w, 1175w, 1110w, 1060w cm^{-1}. – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.20 – 8.14 (m, 1 H, NH), 7.98 – 7.88 (m, 2 H, NH), 7.34 (br, 1 H, NH), 7.06 (br, 1 H, NH), 4.27 – 4.22 (m, 1 H, CH of *i*Gln), 4.18 – 4.11 (m, 1 H, CH of Ala), 3.97 (q, J = 6.8 Hz, 1 H, CH of Lac), 3.85 (q, J = 6.7 Hz, 1 H, CH of Lac), 3.79 (q, J = 6.9 Hz, 1 H, CH

of Lac), 3.48 – 3.20 (m, 2 H, CH_2O), 2.75 – 2.65 (m, 1 H, 2-H_{trans} of cp), 2.62 – 2.56 (m, 1 H, 2-H_{cis} of cp), 2.17 – 2.13 (m, 2 H, 4-H of *i*Gln), 1.99 – 1.88 (m, 1 H, 3-H_A of *i*Gln), 1.79 (s, 3 H, Ac), 1.78 (s, 3 H, Ac), 1.74 (s, 3 H, Ac), 1.73 – 1.67 (m, 1 H, 3-H_B of *i*Gln), 1.26 – 1.18 (m, 6 H, Me of Ala and Me of Lac), 1.16 – 1.02 (m, 1 H, 1-H of cp), 0.87 – 0.81 (m, 1 H, 3-$H_{A,cis}$ of cp), 0.62 – 0.52 (m, 2 H, 3-H_{trans} of cp), 0.42 – 0.38 (m, 1 H, 3-$H_{B,cis,A}$ of cp), 0.37 – 0.32 (m, 1 H, 3-$H_{B,cis,B}$ of cp). – ^{13}C NMR (125 MHz, CD_3OD): $\delta = 176.24$ (s, C=O), 176.15 (s, C=O), 175.1 (s, C=O), 175.0 (s, C=O), 174.7 (s, C=O), 77.4 (d, 2-$C_{cis,A}$ of Lac), 77.0 (d, 2-$C_{trans,A}$ of Lac), 76.32 (d, 2-$C_{cis,B}$ of Lac), 76.26 (d, 2-$C_{trans,B}$ of Lac), 72.9 (t, $CH_2O_{trans,A}$), 72.2 (t, $CH_2O_{trans,B}$), 70.2 (t, $CH_2O_{cis,A}$), 69.9 (t, $CH_2O_{cis,B}$), 53.93 (d, 2-C of *i*Gln), 53.90 (d, 2-C of *i*Gln), 53.8 (d, 2-C of *i*Gln), 50.62 (d, 2-C of Ala), 50.59 (d, 2-C of Ala), 50.6 (d, 2-C of Ala), 31.8 (t, 4-C of *i*Gln), 29.1 (d, 2-C_{trans} of cp), 28.2 (t, 3-C of *i*Gln), 28.0 (d, 2-$C_{cis,A}$ of cp), 27.7 (d, 2-$C_{cis,B}$ of cp), 22.5 (q, Ac), 22.4 (q, Ac), 21.1, 20.6, 20.3, 19.1, 18.9, 17.9, 17.8, 17.5 (Me or 1-C of cp), 11.1 (dd, 3-$C_{trans,A}$ of cp), 10.8 (dd, 3-$C_{trans,B}$ of cp), 10.7 (dd, 3-$C_{cis,A}$ of cp), 9.5 (dd, 3-$C_{cis,B}$ of cp). – MS (EI, 70 eV): *m/z* (%) = 400 (1), 382 (2), 356 (1), 323 (1), 290 (1), 272 (12), 255 (15), 227 (62), 210 (2), 195 (2), 184 (6), 156 (7), 144 (29), 112 (100). – Analysis for $C_{17}H_{28}N_4O_7$ (400.44): calcd. C 50.99, H 7.04, N 14.06; found C 50.78, H 7.23, N 13.88.

*Benzyl N-[(2R) 2-{[cis-(2-octanoylamino)cyclopropyl]methoxy}propionyl-L-alanyl-D-isoglutaminate (cis-***9a***) and benzyl N-[(2R) 2-{[trans-(2-octanoylamino)cyclopropyl]methoxy}propionyl]-L-alanyl-D-isoglutaminate (trans-***9b***)*

Following the procedure given for the synthesis of **7** from **6** (1.40 g, 2.55 mmol) in abs. ethyl acetate (25 ml) deprotection was performed with hydrochloric acid in ethyl acetate (3.6 N, 4.7 ml, 16.9 mol) followed by acylation with octanoyl chloride (0.62 g, 3.83 mmol) / triethylamine (2.58 g, 25.5 mmol) in abs. dichloromethane (40 ml). Chromatography (silica gel, ethyl acetate/methanol 19:1 → 10:1) gave diasteromer *trans*-**9a** (80 mg, 5%), diastereomer *cis*-**9b** (60 mg, 4%) and a mixture of stereoisomers (960 mg, 66%).

Data for *cis*-**9a**: white amorphous solid. – R_F (ethyl acetate/methanol 10:1) 0.16. – IR (KBr): ν = 3405s, 3285s, 3065w, 2930m, 2855m, 1730m, 1675s, 1645s, 1545s, 1455m, 1420m, 1390m, 1310m, 1245m, 1170m, 1105m, 1070w cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.19$ (d, $J = 6.8$ Hz, 1 H, NH), 7.36 – 7.27 (m, 6 H, NH and Ph), 7.06 (br, 1 H, NH), 5.89 (br, 1 H, NH), 5.88 (br, 1 H, NH), 5.09 (AB system, $J = 12.3$ Hz, 2 H, CH_2-Ph), 4.50 – 4.40 (m, 1 H, CH of *i*Gln), 4.32 (qd, $J = 6.9$ Hz, 1 H, CH of Ala), 4.19 (q, $J = 6.6$ Hz, 1 H, CH of Lac), 3.59 (dd, $J = 11.3$, 4.9 Hz, 1 H, H_A-CH_2O), 3.07 (dd, $J = 11.3$, 8.8 Hz, 1 H, H_B-CH_2O), 2.76 – 2.72 (m, 1 H, 2-H of cp), 2.56 – 2.47 (m, 1 H, 4-H_A of *i*Gln), 2.46 – 2.39 (m, 1 H, 4-H_B of *i*Gln), 2.25 – 2.19 (m, 1 H, 3-H_A of *i*Gln), 2.10 (t, $J = 7.6$ Hz, 2 H, 2-H of oct), 2.07 – 1.97 (m, 1 H, 3-H_B of *i*Gln), 1.61 – 1.53 (m, 2 H, 3-H of oct), 1.36 (d, $J = 7.0$ Hz, 3 H, Me), 1.33 (d, $J = 6.8$ Hz, 3 H, Me), 1.30 – 1.20 (m, 8 H, oct), 1.15 – 1.08 (m, 1 H, 1-H of cp), 0.85 (t, $J = 6.9$ Hz, 3 H, Me of oct), 0.76 – 0.69 (m, 1 H, 3-H_A of cp), 0.68 – 0.61 (m, 1 H, 3-H_B of cp). – ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 175.2$ (s, C=O), 174.1 (s, C=O), 173.8 (s, C=O), 173.1 (s, C=O), 172.9 (s, C=O), 135.6 (s, Ph), 128.5 (d, Ph), 128.2 (d, Ph), 128.1 (d, Ph), 74.6 (d, 2-C of Lac), 70.6 (t, CH_2O), 66.6 (t, CH_2-Ph), 52.6 (d, 2-C of *i*Gln), 49.5 (d, 2-C of Ala), 36.5 (t, 2-C of oct), 31.7 (t, oct), 30.7 (t, 4-C of *i*Gln), 29.2 (t, oct), 29.0 (t, oct), 28.6 (t, 2-C of cp), 26.6 (t, 3-C of *i*Gln), 25.7 (t, oct), 22.6 (t, oct), 20.4 (d, 1-C of cp), 18.1 (q, Me), 17.3 (q, Me), 14.1 (q, Me of oct), 10.3 (dd, 3-C of cp). – MS (EI, 70 eV): *m/z* (%) = 505 (1), 475 (1), 458 (1), 448 (1), 430 (3), 404 (4), 380 (9), 363 (7), 346 (3), 335 (3), 313 (16), 285 (14), 257 (11), 237 (11), 229 (45), 211 (11), 192 (99), 185 (8), 144 (21), 127 (17), 113 (56), 91 (73), 69 (100). – Analysis for $C_{30}H_{46}N_4O_7$ (574.72): calcd. C 62.69, H 8.07, N 9.75; found C 62.47, H 8.19, N 9.84.

Data for *trans*-**9b**: white amorphous solid. – R_F (ethyl acetate/methanol 10:1) 0.16. – IR (KBr): ν = 3405s, 3285s, 3065w, 2930m, 2855m, 1730m, 1675s, 1645s, 1545s, 1455m, 1420m, 1390m, 1310m, 1245m, 1170m, 1105m, 1070w cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.19$ (d, $J = 7.5$ Hz, 1 H, NH), 7.36 – 7.29 (m, 5 H, Ph), 7.23 (d, $J = 8.1$ Hz, 1 H, NH), 7.15 (br, 1 H, NH), 5.78 (br, 1 H, NH), 5.38 (br, 1 H, NH), 5.09 (AB system, $J = 12.3$ Hz, 2 H, CH_2-Ph), 4.46 (qd, $J = 7.5$ Hz, 1 H, CH, Ala), 4.45 – 4.41 (m, 1 H, CH of *i*Gln), 3.86 (q, $J = 6.7$ Hz, 1 H, CH of Lac), 3.71 (dd, $J = 11.7$, 4.6 Hz, 1 H, H_A-CH_2O), 3.32 (dd, $J = 11.7$, 10.4 Hz, 1 H, H_B-CH_2O), 2.79 – 2.76 (m, 1 H, 2-H of cp), 2.61 – 2.53 (m, 1 H, 4-H_A of *i*Gln), 2.48 – 2.40 (m, 1 H, 4-H_B of *i*Gln), 2.28 – 2.20 (m, 1 H, 3-H_A of *i*Gln), 2.10 (t, $J = 7.7$ Hz, 2 H, oct), 2.07 – 1.98 (m, 1 H, 3-H_B of *i*Gln), 1.61 – 1.55 (m, 2 H, 3-H oct), 1.44 (d, $J = 7.1$ Hz, 3 H, Me), 1.38 (d, $J = 6.6$ Hz, 3 H, Me), 1.31 – 1.20 (m, 9 H, 1-H of cp and oct), 1.07 – 1.01 (m, 1 H, 3-H_A of cp), 0.85 (t, $J = 6.7$ Hz, 3 H, Me, oct), 0.39 – 0.35 (m, 1 H, 3-H_B of cp). – ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 175.8$ (s, C=O), 174.2 (s, C=O), 173.5 (s, C=O), 173.3 (s, C=O), 172.6 (s, C=O), 135.6 (s, Ph), 128.5 (d, Ph), 128.2 (d, Ph), 128.1 (d, Ph), 77.4 (d, 2-C of Lac), 69.9 (t, CH_2O), 66.7 (t, CH_2-Ph), 53.0 (d, 2-C of *i*Gln), 49.2 (d, 2C of Ala), 36.7 (t, 2-C of oct), 31.7 (t, oct), 30.5 (t, 4-C of *i*Gln), 29.2 (t, oct), 29.0 (t, oct), 27.1 (d, 2-C of cp), 26.7 (t, 3-C of *i*Gln), 26.9 (t, oct), 18.9 (d, 1-C of cp), 17.5 (q, Me), 17.4 (q, Me), 14.1 (q, Me of oct), 10.0 (dd, 3- of cp). – MS (EI, 70 eV): *m/z* (%) = 505 (1), 475 (1), 458 (1), 448 (1), 430 (4), 404 (6), 380 (12), 363 (3), 346 (4), 335 (1), 313 (10), 285 (12), 257 (15), 237 (11), 229 (52), 211 (9), 192 (82), 185 (4), 144 (17), 127 (21), 113 (43), 91 (63), 69 (100). – Analysis for $C_{30}H_{46}N_4O_7$ (574.72): calcd. C 62.69, H 8.07, N 9.75; found C 62.52, H 8.23, N 9.86.

*(2R) 2-{[cis-2-(octanoylamino)cyclopropyl]methoxy}propionyl-L-alanyl-D-isoglutamine (cis-***10a***)*

Hydrogenolysis of **9a** (75 mg, 0.13 mmol) in ethanol (30 ml) in the presence of Pd/C as described above followed by chromatography (silica gel, chloroform/ethanol/acetic acid 70:25:5) gave **10** (60 mg, 95%) as a white amorphous solid. R_F (chloroform, ethanol/acetic acid 70:20:5) 0.42. – IR (KBr): ν = 3290s, 2930m, 2860m, 1650s, 1555s, 1455m, 1260m, 1210w, 1170w, 1110m cm^{-1}. – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.60 (br, 1H, NH), 8.01 (d, J = 7.3 Hz, 1 H, NH), 7.95 (d, J = 4.6 Hz, 1 H, NH), 7.33 (br, 1 H, NH), 7.00 (br, 1 H, NH), 4.23 (qd, J = 7.0 Hz, 1 H, CH of Ala), 4.10 – 4.03 (m, 1 H, CH of *i*Gln), 3.97 (q, J = 6.4 Hz, 1 H, CH of Lac), 3.47 (dd, J = 10.9, 5.9 Hz, 1 H, H_A-CH_2O determined in CD_3OD), 3.31 (m, 1 H, H_B-CH_2O, determined in CD_3OD), 2.62 – 2.58 (m, 2-H of cp), 2.05 – 1.95 (m, 4 H, H4-H of *i*Gln, 2-H of oct), 1.94 – 1.84 (m, 1 H, H_A-3 of *i*Gln), 1.75 – 1.68 (m, 1 H, H_B-3 of *i*Gln), 1.49 – 1.42 (m, 2 H, 3-H of oct), 1.29 – 1.18 (m, 14 H, Me, Me, Oct), 1.10 – 1.02 (m, 1H, 1-H of cp), 0.84 (t, J = 6.7 Hz, 3 H, Me of oct), 0.61 – 0.51 (m, 2 H, 3-H of cp). – ^{13}C NMR (100 MHz, CD_3OD): δ = 177.5 (*s*, C=O), 186.7 (*s*, C=O), 176.2 (*s*, C=O), 176.0 (*s*, C=O), 174.8 (s, C=O), 76.3 (2-C of Lac), 72.2 (t, CH_2O), 54.5 (d, 2-C of *i*Gln), 50.6 (d, 2-C of Ala), 36.9 (t, 2-C of oct), 32.9 (t, oct), 30.7 (t, 4-C of *i*Gln), 30.3 (t, oct), 30.1 (t, oct), 29.2 (t, 3-C of oct), 29.1 (d, 2-C of cp), 27.0 (t, oct), 23.7 (t, oct), 20.5 (d, 1-C of cp), 19.0 (q, Me), 17.9 (q, Me of oct), 11.0 (dd, 3-C of cp). – HPLC-MS (ESI, 4.1 kV, 8 μl/min N_2, methanol): *m/z* (%) = 1007.4 $[M_2K]^+$ (100), 991.5 $[M_2Na]^+$ (86), 969.5 $[M_2H]^+$ (7), 523.5 $[MK]^+$ (49), 507.9 $[MNa]^+$ (55), 485.5 $[MH]^+$ (22). – HRMS for $C_{23}H_{40}N_4O_7$: calcd. 484.2898; found 484.2897. – Analysis for $C_{23}H_{40}N_4O_7$ (484.60): calcd. C 57.01, H 8.32, N 5.11; found C 57.13, H 8.54, N 11.41.

*(2R) 2-{[trans-2-(Octanoylamino)cyclopropyl]methoxy}-propionyl-L-alanyl-D-isoglutamine (trans-***10b***)*

Hydrogenolysis of **9b** (45 mg, 0.08 mmol) in ethanol (25 ml) in the presence of Pd/C (10%, 20 mg) as described above followed by chromatography (silica gel, chloroform/ethanol/acetic acid 70:25.5) gave **10b** (31 mg, 82%) as a white amorphous solid. – R_F (chloroform/ethanol/acetic acid 70:25:5) 0.43. – IR (KBr): ν = 3405s, 2955s, 2925s, 2855s, 1730m, 1655s, 1555s, 1455m, 1260m, 1110m, 1070w cm^{-1}. – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.80 (br, 1 H, NH), 7.99 (br, 1 H, NH), 7.92 (d, J = 7.1 Hz, 1 H, NH), 7.30 (br, 1 H, NH), 6.93 (br, 1 H, NH), 4.19 (qd, J = 7.1 Hz, 1 H, CH of Ala), 4.02 – 3.98 (m, 1 H, CH of *i*Gln), 3.74 (q, J = 6.8 Hz, 1 H, CH of Lac), 3.56 (dd, J = 11.0, 5.7 Hz, 1 H, H_A-CH_2O, determined in CD_3OD), 3.38 (dd, J = 11.0, 8.9 Hz, 1 H, H_B-CH_2O, determined in CD_3OD), 2.75 – 2.69 (m, 1 H, 2-H of cp), 2.02 – 1.96 (m, 4 H, 4-H of *i*Gln and 2-H of oct), 1.88 – 1.80 (m, 1 H, 3-H_A of *i*Gln), 1.75 – 1.65 (m, 1 H, 3-H_B of *i*Gln), 1.47 – 1.38 (m,2 H, 3-H of oct), 1.30 – 1.11 (m, 14 H, 1-H of cp, 2 x Me and oct), 1.10 – 1.00 (m, 1 H, 3-H_A of cp), 0.81 (t, J = 6.9 Hz, Me of oct), 0.46 – 0.42 (m, 1 H, 3-H_B of cp). – ^{13}C NMR (100 MHz, CD_3OD): δ = 178.2 (s, C=O), 176.7 (s, C=O), 176.4 (s, C=O), 174.8 (s, C=O), 77.6 (d, 2-C of Lac), 70.3 (t, CH_2O), 54.6 (d, 2-C of *i*Gln), 50.7 (d, 2-C of Ala), 36.9 (t, 2-C of oct), 32.9 (t, oct), 30.7 (t, 4-C of *i*Gln), 30.3 (t, oct), 30.2 (t, oct), 29.3 (t, 3-C of *i*Gln), 28.0 (d, 2-C of cp), 27.1 (t, oct), 23.7 (t, oct), 19.2 (d, 1-C of cp), 18.1 (q, Me), 17.8 (q, Me), 14.4 (q, Me of oct), 9.6 (dd, 3-C of cp). – HPLC-MS (ESI, 4.1 kV, 8 μl/min N_2, methanol): *m/z* (%) = 1007.4 $[M_2K]^+$ (100), 991.4 $[M_2Na]^+$ (83), 969.5 $[M_2H]^+$ (6), 523.7 $[MK]^+$ (71), 507.8 $[MNa]^+$ (100), 485.5 $[MH]^+$ (29). – HRMS for $C_{23}H_{40}N_4O_7$: calcd. 484.2898; found 484.2898. – Analysis for $C_{23}H_{40}N_4O_7$ (484.60): calcd. C 57.01, H 8.32, N 5.11; found C 56.82, H 8.47, N 11.46.

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