Synthesis of Phthalimido-Desmuramylpeptide Analogues as Potential Immunomodulating Agents

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The preparation of immunologically active phthalimido desmuramylpeptide analogues **2e-h**, **4c-d**, and **7b** is described. The *N*-acetylmuramic acid in the muramyl dipeptide has been replaced by a phthaloylated acyclic moiety such as *N*-phthaloylated amino acids **1a-c**, 2-(2-phthalimidoethoxy)acetic acid **3**, or by the carbocyclic rac. *trans*-2-(2'-phthalimidocyclohexyloxy)acetic acid **6**.

Synthese von Phthalimido-desmuramylpeptid-Analogen mit potentiell immunmodulierenden Eigenschaften

Die Synthese der immunologisch wirksamen Phthalimido-desmuramylpeptid-Analoga **2e-h**, **4c-d** und **7b** mit einem *N*-phthaloylierten acyclischen Teil wie z.B. *N*-phthaloylierten Aminosäuren **1a-c**, sowie 2-(2-phthalimidoethoxy)essigsäure **3** oder einer carbocyclischen rac. *trans*-2-(2'-Phthalimidocyclohexyloxy)acetyl Gruppe **6** als Ersatz für den *N*-Acetylmuramylsäure Rest wird beschrieben.

The constituents of bacterial cell wall stimulate nonspecific and specific host defences and exhibit strong immunomodulating activity[1].

For adjuvant activity the minimum structural component of the peptidoglycan monomers has been identified as *N*-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP)[2-5]. Although MDP has interesting properties *in vitro*, its application is limited due to its short duration of action and undesired side effects[6]. Therefore, a lot of lipophilic derivatives have been synthesized, where *N*-acetylglucosamine which is not an essential part of MDP for the immunostimulant activity[7,8], has been replaced by different cyclic[9,10] or acyclic structures[11,13].

Although the phthalimido group is mostly used as a protective group in organic synthesis[15], there are also some phthalimido compounds acting on the immune system. Thalidomide, a derivative of glutamic acid, has immunosuppressive effects, and it is effectively used in the treatment of erythema nodosum leprosum[16,17]. The effect of two soluble thalidomide derivatives on lymphocite stimulation was described[18]. The *N*-phthaloylated derivative of 2-amino-4-chlorophenylthiazole-5-acetic acid has immunosuppressive activity[19], and in contrast phthalimido substituted 1-aziridine carboxylic acid derivatives have immunostimulant activity[20].

All these facts and our previous interest in this field of research prompted us to prepare this type of more lipophilic MDP analogues to provide new data about SAR from biological testing[21].

The acyclo concept in design of MDP analogues where the *N*-acetylmuramic acid part of MDP was replaced with an acyclic moiety such as *N*-phthaloylated amino acids **1a-c** or 2-(2-phthalimidoethoxy)acetic acid **3** has been used by *Hoenig*[11] and by ourselves[12a,12b,13]. The aromatic part of the phthalimido group could be considered as a good π-electronic substitute for a glucosamine part of the parent molecule. Thus, the acyclic phthalimido substituted carboxylic acid derivatives **1a-c** were synthesized according to lit.[22]. The protected dipeptides Boc-L-alanyl-D-isoglutamine benzyl ester[23] and Boc-L-alanyl-D-glutamic acid dibenzylester[24] were prepared by known procedures. After the split off of a protective group, the free amino group of the dipeptide was coupled with *N*-phthaloylated carboxylic acid derivatives **1a-c** in the presence of diphenylphosphoryl azide[25] and triethylamine to give compounds **2a-d** which gave the debenzylated products **2e-h** after catalytic hydrogenation over 10% Pd/C (Scheme 1).

With the synthesis of nor-MDP analogues where the D-lactyl residue is modified, we wanted to omit toxicity and pyrogenicity of MDP[26], and different lengths of acyclic side chains were used to get information about structure-activity relationship.

2-(2-Phthalimidoethoxy)-acetic acid **3**[27] is a bioisosteric analogue of compound **1c**, where a methylene group was replaced by an oxygen atom. Condensation of compound **3** with the dipeptide L-Ala-D-iGln(OBzl) in the presence of DPPA/Et_3N gave benzyl *N*-(2-(2-phthalimidoethoxy)acetyl)-L-alanyl-D-isoglutaminate **4a**. In the same way the reaction between **3** and the dipeptide L-alanyl-D-glutaminic acid dibenzylester proceeded to afford dibenzyl *N*-(2-(2-phthalimidoethoxy)acetyl)-L-alanyl-D-glutamate **4b**. The benzyl protecting groups of compounds **4a** and **4b** were removed by catalytical hydrogenation (10% Pd/C) to give acyclic nor-MDP analogues **4c** and **4d** (Scheme 2). Hydrogenolytic cleavage of benzyl groups proceeded mostly in glacial acetic acid in order to avoid ring opening of the phthalimido moiety.

The third type of MDP-analogues prepared was the *N*-phthaloylated carbocyclic compound **7b**. In this compound the *N*-acetylmuramic acid part of the MDP molecule was replaced by *rac trans*-2-(2'-phthalimidocyclohexyloxy)acetic acid **6**. Acylation of *rac trans*-2-(2-aminocyclohexyloxy)acetic acid (**5**)[28] with *N*-carbethoxyphthalimide gave compound **6** and subsequent condensation with the dipeptide L-Ala-D-iGln benzyl ester in the presence of DPPA/Et_3N afforded benzyl *N*-(*trans*-2-(2-phthalimidocyclohexyloxy)acetyl)-L-alanyl-D-isoglutaminate (**7a**) as a

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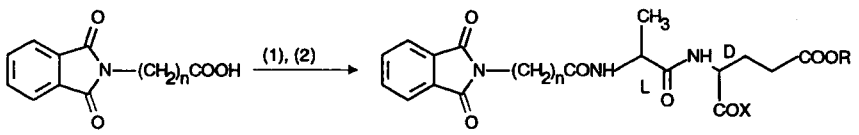


1a-c 2a-h

(1): L-Ala-D-iGln(OBzl) · HCl or L-Ala-D-Glu(OBzl)$_2$ · HCl

(2): H_2/ Pd/C (10%)/ HOAc

	n
1a	2
1b	3
1c	4

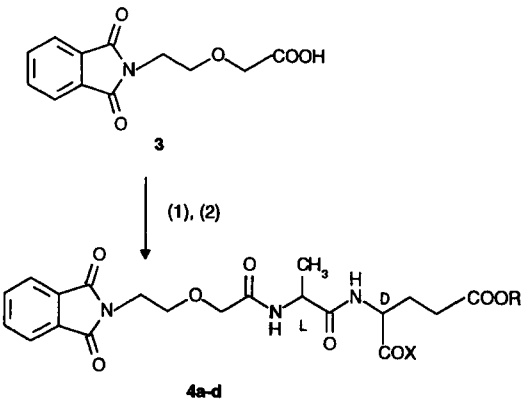
mixture of diastereomers. Finally, the benzyl protecting group of compound **7a** was removed by catalytic hydrogenation to give compound **7b** (Scheme 3).

Some of the synthesized compounds have been tested in *in vivo* preliminary immunorestoration tests[21]. After immunosuppression of mice with cyclophosphamide (25 mg/kg *p.o.*) (days 2, 4, 6), they were infected with *Candida albicans* and treated with the test compounds (days 1, 3, 5, *i.p.* administration) in four different doses (0.1, 1.0, 10 and 100 mg/kg) or with vehicle alone. A survival of the test animals > 30% is significant and a consequence of the immunorestoration activity of the applied compounds. In immunorestoration test (azimexone as a

	n	X	R
2a	2	NH_2	Bzl
2b	3	NH_2	Bzl
2c	4	NH_2	Bzl
2d	4	OBzl	Bzl
2e	2	NH_2	H
2f	3	NH_2	H
2g	4	NH_2	H
2h	4	OH	H

Scheme 1

standard - 40% increased survival), compound **2g** showed the same effect as azimexone, while compounds **4c** and **4d** were less active (20% increased survival), but azimexone has a two times lower molecular mass and the efficacy of the test compounds **2g** and **4d** in immunorestoration test is thus also higher as compared to that of azimexone.



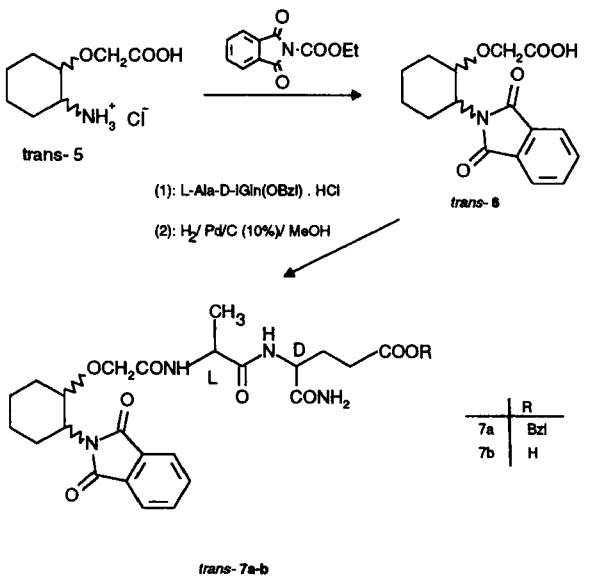
3 4a-d

(1): L-Ala-D-iGln(OBzl) · HCl or L-Ala-D-Glu(OBzl)$_2$ · HCl

(2): H_2/ Pd/C (10%)/ HOAc

	X	R
4a	NH_2	Bzl
4b	OBzl	Bzl
4c	NH_2	H
4d	OH	H

Scheme 2



trans-5 trans-6 trans-7a-b

(1): L-Ala-D-iGln(OBzl) . HCl

(2): H_2/ Pd/C (10%)/ MeOH

	R
7a	Bzl
7b	H

Scheme 3

Experimental Part

Melting points: Reichert hot stage microscope, uncorrected.- IR: Perkin-Elmer FTIR 1600.- ^{1}H-NMR: 300 MHz, Varian VXR-300. Tetramethylsilane as internal standard.- Microanalyses: Perkin-Elmer elemental analyzer 2400 CHN.- Optical rotations: Perkin-Elmer 240 MC polarimeter.

Coupling of ***1a-c, 3, and 6*** *with benzyl L-alanyl-D-isoglutaminate or dibenzyl L-alanyl-D-glutamate: General procedure*

To a stirred solution of corresponding phthalimidocarboxylic acid derivative **1a-c**, **3**, and **6** (1.6 mmol) and benzyl L-alanyl-D-isoglutaminate hydrochloride (548 mg, 1.6 mmol) or dibenzyl L-alanyl-D-glutamate hydrochloride (696 mg, 1.6 mmol) in dry DMF, diphenylphosphorylazide (495 mg, 1.8 mmol) and triethylamine (323 mg, 3.2 mmol) were added at 0°C. After stirring for 1 h at this temp., stirring was continued for 24 h at room temp. Ethyl acetate (40 ml) was added and the solution was extracted subsequently with 10% citric acid (3 x 10 ml), water (3 x 10 ml), saturated NaCl solution (3 x 10 ml), saturated $NaHCO_3$ solution (3 x 10 ml), water (3 x 10 ml), and satd. NaCl solution (3 x 10 ml). The org. phase was dried (anhydrous $MgSO_4$) and the solvent removed under reduced pressure.

Benzyl N-(3-phthalimidopropionyl)-L-alanyl-D-isoglutaminate (**2a**)

Prepared from **1a** and benzyl L-alanyl-D-isoglutaminate hydrochloride and recrystallized from acetone.- Yield 757 mg (93%), m.p. 216-220°C, $[\alpha]^{20}_D$ = -8.9° (c = 0.4, MeOH).- IR (KBr): 3330; 3300; 3080; 2960; 1777; 1750-1720; 1650; 1450; 1380; 1280; 1210 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.14 (d, 3H, J = 6.8 Hz, CH_3-Ala); 1.7-1.82 and 1.98-2.1 (2m, 1H each, CH_2-β-iGln); 2.36 (t, J = 7.8 Hz, 2H, CH_2-γ-iGln or C$\underline{H_2}$CONH); 2.45-2.52 (m, 2H, CH_2-γ-iGln or C$\underline{H_2}$CONH); 3.76 (t, J = 7.3 Hz, 2H, NCH_2); 4.12-4.24 (m, 2H, CH-Ala, CH-iGln); 5.07 (s, 2H, CH_2-benzyl); 7.13 and 7.29 (2s, 1H each, NH_2-iGln); 7.28-7.37 (m, 5H, phenyl); 7.82-7.87 (m, 4H, phthaloyl); 8.14 (d, J = 8.06 Hz, 1H, NH); 8.32 (d, J = 6.7 Hz, 1H, NH).- $C_{26}H_{28}N_4O_7$ (508.5) Calcd. C 61.4 H 5.51 N 11.0 Found C 61.5 H 5.84 N 11.0.

Benzyl N-(4-phthalimidobutanoyl)-L-alanyl-D-isoglutaminate (**2b**)

Prepared from **1b** and benzyl L-alanyl-D-isoglutaminate hydrochloride and recrystallized from acetone.- Yield 677 mg (81%), m.p. 190-191°C, $[\alpha]^{20}_D$ = -9.2° (c = 0.2, MeOH).- IR (KBr): 3330; 3210; 3050; 2980; 1777; 1750-1720; 1650; 1460; 1380; 1280; 1210 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.16 (d, J = 7.08 Hz, 3H, CH_3-Ala); 1.7-1.9 (m, 3H, 1CH_2, CH_2-β-iGln); 2.01-2.15 (m, 1H, CH_2-β-iGln); 2.17 (t, J = 8.0 Hz, 2H, CH_2-γ-iGln or C$\underline{H_2}$CONH); 2.36 (t, J = 7.8 Hz, 2H, C$\underline{H_2}$-γ-iGln or C$\underline{H_2}$CONH); 3.57 (t, J = 6.9 Hz, 2H, NCH_2); 4.1-4.25 (m, 2H, CH-Ala, CH-iGln); 5.06 (s, 2H, CH_2-benzyl); 7.13 (s, 1H, NH_2-iGln); 7.3-7.45 (m, 6H, 1H, NH_2-iGln, phenyl); 7.8-7.95 (m, 4H, phthaloyl); 8.13-8.20 (d, 2H, NH).- $C_{27}H_{30}N_4O_7$ (522.6) Calcd. C 62.1 H 5.75 N 10.7 Found C 62.1 H 5.95 N 10.4.

Benzyl N-(5-phthalimidopentanoyl)-L-alanyl-D-isoglutaminate (**2c**)

Prepared from **1c** and benzyl L-alanyl-D-isoglutaminate hydrochloride and recrystallized from MeOH/diethyl ether.- Yield 558 mg (65%), m.p. 172-174°C, $[\alpha]^{20}_D$ = -10.1° (c = 0.3, MeOH).- IR (KBr): 3300; 3220; 3050; 2980; 1750; 1730-1700, 1680; 1460; 1380; 1280; 1210 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.14 (d, J = 7.1 Hz, 3H, CH_3-Ala), 1.4-1.5 (m, 4H, 2CH_2); 1.70-1.85 and 1.95-2.01 (2m, 1H each, CH_2-β-iGln); 2.17 (deg. t, 2H, CH_2-γ-iGln or C$\underline{H_2}$CONH); 2.34 (t, J = 8.1 Hz, CH_2-γ-iGln or C$\underline{H_2}$CONH); 3.54 (t, J = 6.7 Hz, 2H, NCH_2); 4.1-4.2 (m, 2H, CH-Ala, CH-iGln); 5.05 (s, 2H, CH_2-benzyl); 7.08 (s, 1H, NH_2-iGln); 7.33-7.4 (m, 6H, NH_2-iGln, phenyl); 7.76-7.90 (m, 4H, phthaloyl); 8.06-8.08 (deg. d, 2H, NH).- $C_{28}H_{32}N_4O_7$ (536.6) Calcd. C 62.6 H 5.96 N 10.4 Found C 62.7 H 6.28 N 10.5.

Dibenzyl N-(5-phthalimidopentanoyl)-L-alanyl-D-glutamate (**2d**)

Prepared from **1c** and dibenzyl L-alanyl-D-glutamate hydrochloride and recrystallized from EtOH/diethyl ether.- Yield 703 mg (70%), m.p. 114-117°C, $[\alpha]^{20}_D$ = -23° (c = 0.4, MeOH).- IR (KBr): 3320; 3080; 2980-2940; 1740-1710; 1660-1640; 1560; 1450; 1350; 1280-1260; 1180-1160 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.13 (d; J = 7.1 Hz, 3H, CH_3-Ala), 1.42-1.58 (m; 4H, 2CH_2), 1.81-1.88 and 2.0-2.01 (2m; 1H each, CH_2-β-Glu), 2.13 (t; J = 6.1 Hz, 2H, CH_2-γ-Glu or C$\underline{H_2}$CONH), 2.45 (t; J = 8.1 Hz, 2H, CH_2-γ-Glu or C$\underline{H_2}$CONH), 3.54 (t; J = 6.8 Hz, NCH_2), 4.24-4.40 (m; 2H, CH-Ala, CH-iGln), 5.07 and 5.10 (2s; 2H each, 2CH_2 benzyl), 7.3-7.4 (m; 10 H, 2 x phenyl), 7.80-7.98 (m; 4H, phthaloyl), 8.00 (d; J = 7.5 Hz, 1H, NH), 8.3 (d; J = 7.6 Hz, 1H, NH).- $C_{35}H_{37}N_3O_8$ (627.7) Calcd. C 67.0 H 5.94 N 6.7 Found C 66.6 H 6.13 N 6.3.

Hydrogenolitic cleavage of benzyl protecting groups - General procedure

Compounds **2a-d**, **4a-b** (1 mmol) were dissolved in glacial acetic acid and compound **7a** (1 mmol) in methanol and hydrogenated for 4.6 h over 10% Pd/C (50-100 mg) at room temp. and normal pressure. After filtration, the solvent was removed *in vacuo*.

N-(3-Phthalimidopropionyl)-L-alanyl-D-isoglutamine (**2e**)

Prepared from **2a**, yield 406 mg (97%), m.p. 220-222°C, $[\alpha]^{20}_D$ = -9.5° (c = 0.1, MeOH).- IR (KBr): 3312; 3080; 2960; 1727; 1672; 1613; 1540; 1380; 1161 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.15 (d; J = 7.1 Hz, 3H, CH_3-Ala), 1.62-1.78 and 1.90-2.14 (2m; 1H each, CH_2-β-iGln), 2.20 (t; J = 7.5 Hz, 2H, CH_2-γ-iGln or C$\underline{H_2}$CONH), 2.45-2.56 (m; 2H, CH_2-γ-iGln or C$\underline{H_2}$CONH), 3.77 (t; J = 7.3 Hz, 2H, NCH_2), 4.10-4.24 (m; 2H, CH-Ala, CH-iGln), 7.11 and 7.26 (2s; 1H, each, NH_2-iGln), 7.81-7.88 (m; 4H, phthaloyl), 8.12 (d; J = 8.3 Hz, 1H, NH), 8.30 (d; J = 6.4 Hz, 1H, NH).- $C_{19}H_{22}N_4O_7$ (418.4) Calcd. C 54.5 H 5.30 N 13.4 Found C 54.2 H 5.47 N 13.0.

N-(4-Phthalimidobutanoyl)-L-alanyl-D-isoglutamine (**2f**)

Yield 311 mg (72%), m.p. 202-205°C, $[\alpha]^{20}_D$ = -8.6° (c = 0.4, MeOH).- IR (KBr): 3320; 3210; 3050; 2960; 1730-1710; 1650; 1560-1530; 1410; 1340 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.15 (d; J = 7.2 Hz, 3H, CH_3-Ala), 1.65-1.90 (m; 3H, 1CH_2, 1H, CH_2-β-iGln), 1.90-2.10 (m; 1H, CH_2-β-iGln), 2.17 (m; 4H, CH_2-γ-iGln and CH_2CONH), 3.58 (t; J = 6.8 Hz, 2H, NCH_2), 4.10-4.25 (m; 2H, CH-Ala, CH-iGln), 7.10 and 7.30 (2s; 1H each, NH_2-iGln), 7.80-7.95 (m; 4H, phthaloyl), 8.10 (d; J = 7.3 Hz, 2H, 2 NH).- $C_{20}H_{24}N_4O_7$ (432.4) Calcd. C 55.5 H 5.55 N 12.9 Found C 55.1 H 5.28 N 12.6.

N-(5-Phthalimidopentanoyl)-L-alanyl-D-isoglutamine (**2g**)

Yield 438 mg (98%), m.p. 190-192°C, $[\alpha]^{20}_D$ = -9.5° (c = 0.4, MeOH).- IR (KBr): 3300; 3220; 3050; 2980; 1750; 1730-1700; 1680; 1460; 1380 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.16 (d; J = 7.0 Hz, 3H, CH_3-Ala), 1.43-1.58 (m; 4H, 2CH_2), 1.61-1.76 and 1.88-2.11 (2m; 1H each, CH_2-β-iGln), 2.15 (m; 4H, CH_2-γ-iGln and C$\underline{H_2}$CONH), 3.54 (t; J = 6.8 Hz, 2H, NCH_2), 4.08-4.22 (m; 2H, CH-Ala, CH-iGln), 7.05 and 7.28 (2s; 1H each, NH_2-iGln), 7.78-7.86 (m; 4H, phthaloyl), 8.05 (d; J = 7.2 Hz, 2H, 2 NH).- $C_{21}H_{26}N_4O_7$ (446.5) Calcd. C 56.5 H 5.87 N 12.5 Found C 56.4 H 5.54 N 12.3.

N-(5-Phthalimidopentanoyl)-L-alanyl-D-glutamic acid (**2h**)

Yield 336 mg (75%), m.p. 101-105°C, $[\alpha]^{20}_D$ = -23.1° (c = 0.4, MeOH).- IR (KBr): 3320; 3280; 3080; 2960; 2940; 1730-1710; 1650; 1560-1530; 1410; 1340 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.16 (d; J = 6.0 Hz, 3H, CH_3-Ala), 1.40-1.65 (m; 4H, $2CH_2$), 1.82-1.86 and 2.0-2.10 (2m; 1H each, CH_2-β-Glu), 2.12 (m; 2H, CH_2-γ-Glu or $C\underline{H}_2CONH$), 2.20-2.35 (m; 2H, CH_2-γ-Glu or $C\underline{H}_2CONH$), 3.55 (t; J = 6.5 Hz, 2H, NCH_2), 4.10-4.40 (m; 2H, CH-Ala, CH-Glu), 7.80-7.90 (m; 4H, phthaloyl), 8.00 (s; 2H, 2 NH).- $C_{21}H_{25}N_3O_8$ (447.5) C 56.4 H 5.63 N 9.4 Found C 56.2 H 5.85 N 9.5.

Benzyl N-(2-(2-phthalimidoethoxy)-acetyl)-L-alanyl-D-isoglutaminate (**4a**)

Prepared from **3** (398 mg, 1.6 mmol) and benzyl L-alanyl-D-isoglutaminate hydrochloride (548 mg, 1.6 mmol) similarly as compound **2a**. The precipitated solid was dissolved in DMF, the mixture was diluted with ethyl acetate (40 ml), 10% citric acid was added (80 ml), and the solution was extracted with ethyl acetate (4 x 100 ml). The separated product was filtered off and recrystallized from ethanol; yield 0.76 g (88%), m.p. 205-206°C, $[\alpha]^{20}_D$ = +10.3° (c = 0.3, DMF).- IR (KBr): 3440; 3380; 3275; 3050; 2945; 1765; 1710; 1660; 1515; 1390; 1270; 1195; 1140; 1030; 1020; 760; 725 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.19 (d; J = 6.8 Hz, 3H, CH_3-Ala), 1.68-1.85 and 1.93-2.10 (2m; 1H each, CH_2-β-iGln), 2.35 (t; J = 7.7 Hz, 2H, CH_2-γ-iGln), 3.68 (t; J = 5.0 Hz, 2H, $NC\underline{H}_2CH_2O$), 3.82 (t; J = 5.0 Hz, 2H, $NCH_2C\underline{H}_2O$), 3.91 (s; 2H, $OC\underline{H}_2CO$), 4.10-4.21 (m; 1H, CH-iGln), 4.27 (t; J = 6.1 Hz, 1H, CH-Ala), 5.08 (s; 2H, $COOC\underline{H}_2Ph$), 7.11 and 7.33 (2s; 1H each, $CONH_2$), 7.36 (s; 5H, phenyl), 7.81-7.86 (m; 4H, phthaloyl), 7.69, 8.16 (2d; J = 7.6 Hz, 1H each, NH).- $C_{27}H_{30}N_4O_8$ (538.5) Calcd. C 60.2 H 5.62 N 10.4 Found C 60.1 H 5.78 N 10.4.

Dibenzyl N-(2-(2-phthalimidoethoxy)-acetyl)-L-alanyl-D-glutamate (**4b**)

Prepared from **3** and dibenzyl L-alanyl-D-glutamate hydrochloride and purified by cc (silica gel; chloroform/methanol). Yield 662 mg (66%), m.p. 108-109°C, $[\alpha]^{20}_D$ = +3.17° (c = 0.3, MeOH).- IR (KBr): 3302; 1774; 1745; 1719; 1699; 1649; 1521; 1406; 1270; 1207; 1187, 1115; 1022; 870 cm^{-1}.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.40 (d; J = 7.1 Hz, 3H, CH_3-Ala), 1.68-1.85 and 1.92-2.07 (2m; 1H each, CH_2-β-Glu), 2.39 (t; J = 8.1 Hz, 2H, CH_2-γ-Glu), 3.64-3.77 (m; J = 5.1 Hz, 2H, $NC\underline{H}_2CH_2O$), 3.86-3.97 (m; 4H, $NCH_2C\underline{H}_2O$ and $OC\underline{H}_2CO$-), 4.4-4.52 (m; 1H, CH-Glu), 4.53-4.63 (m; 1H, CH-Ala), 5.08, 5.1 (2s; 2H each, $COOC\underline{H}_2C_6H_5$), 7.07, 7.11 (2s; 1H each, NH), 7.27-7.40 (m; 10 H, phenyl), 7.66-7.89 (m; 4H, phthaloyl).- $C_{34}H_{35}N_3O_9$ (629.7) Calcd. C 64.9 H 5.60 N 6.7 Found C 64.7 H 5.71 N 6.8.

N-(2-(2-Phthalimidoethoxy)-acetyl)-L-alanyl-D-isoglutamine (**4c**)

Yield 388 mg (85%), m.p. 229-232°C, $[\alpha]^{20}_D$ = +12.5° (c = 0.3, DMF).- IR (KBr): 3440; 3360; 3274; 2910; 1755; 1665; 1635; 1540; 1390; 1130 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.20 (d; J = 6.8 Hz, 3H, CH_3-Ala), 1.79-1.89 and 1.94-2.02 (2m; 1H each, CH_2-β-iGln), 2.19 (t; J = 7.8 Hz, 2H, CH_2-γ-iGln), 3.69 (t; J = 5.3 Hz, 2H, $NC\underline{H}_2CH_2O$), 3.81 (t; J = 5.30 Hz, 2H, $NCH_2C\underline{H}_2O$), 3.91 (s; 2H, $OC\underline{H}_2CO$), 4.03-4.09 (m; 1H, CH-iGln), 4.28 (t; J = 7.0 Hz, 1H, CH-Ala), 7.09 and 7.29 (2s; 1H each, $CONH_2$), 7.68 (d; J = 7.0 Hz, 1H, NH), 7.89-7.99 (m; 4H, phthaloyl), 8.14 (d; J = 8.1 Hz, 1H, NH).- $C_{20}H_{24}N_4O_8$ (448.4) Calcd. C 53.6 H 5.39 N 12.5 Found C 53.4 H 5.65 N 12.1.

N-(2-(2-Phthalimidoethoxy)-acetyl)-L-alanyl-D-glutamic acid (**4d**)

Yield 500 mg (91%), m.p. 192-195°C, $[\alpha]^{20}_D$ = -4.9° (c = 0.2, MeOH).- IR (KBr): 3372; 3311; 2952; 1768; 1747; 1708; 1660; 1636; 1559; 1393; 1205; 1135; 1021; 719 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.20 (d; J = 6.9 Hz, 3H, CH_3-Ala), 1.68-1.84 and 1.90-2.06 (2m; 1H each, CH_2-β-Glu), 2.41 (t; J = 7.3 Hz, 2H, CH_2-γ-Glu), 3.69 (t; J = 5.2 Hz, 2H, $NC\underline{H}_2CH_2O$), 3.81 (t; J = 5.2 Hz, 2H, $NCH_2C\underline{H}_2O$), 3.91 (s; 2H, $OC\underline{H}_2CO$), 4.14-4.24 (m; 1H, CH-iGln), 4.28-4.41 (m; 1H, CH-Ala), 7.57 (d; 1H, NH), 7.80-7.93 (m; 4H, phthaloyl), 8.24 (d; 1H, NH), 12.4 (br s; 2H, COOH).- $C_{20}H_{23}N_3O_9$ (449.4) Calcd. C 53.4 H 5.16 N 9.4 Found C 53.4 H 5.22 N 9.4.

rac-trans-2-(2'-Phthalimidocyclohexyloxy)acetic acid (**6**)

To a stirred solution of *rac-trans*-2-carboxymethoxycyclohexylammonium chloride (**5**) (1.00 g, 4.77 mmol) and sodium carbonate (1.06 g, 10.02 mmol) in water (10 ml) *N*-ethoxycarbonylphthalimide (1.10 g, 5.01 mmol) was added and the mixture was stirred for 20 h at room temp., whereon it was extracted with chloroform and acidified to pH 2 with 6 M HCl. Crude **6** separated as a colourless oil which solidified upon standing. It was filtered off and dried to give 0.92 g (64%) of **6** which was recrystallized from ethanol (4 ml); white crystals, m.p. 190-192°C.- IR (KBr): 3461, 3049, 2940, 1770, 1710, 1399, 1386, 1250, 1157, 1132, 1090, 1022, 920, 719 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 1.1-1.45 (m; 3H, $3H_{ax}$), 1.7-1.9 (m; 3H, H_{ax}, $2H_{eq}$), 2.1-2.35 (m; 2H, $2H_{eq}$), 3.9-4.1 (m; 4H, 1'-H, 2'-H, OCH_2), 7.8-7.95 (m; 4H, $4H_{ar}$), 12.4 (s br; 1H, COOH).- $C_{16}H_{17}NO_5$ (303.3) Calcd. C 63.35 H 5.65 N 4.62 Found C 63.57 H 5.77 N 4.86.

Benzyl N-(trans-2-(2'phthalimidocyclohexyloxy)acetyl)-L-alanyl-D-isoglutaminate (**7a**)

Prepared from **6** (465 mg, 1.53 mmol) and benzyl L-alanyl-D-isoglutaminate hydrochloride (527 mg, 1.53 mmol) as described for **2a**.- The crude product was purified by dissolution in ethyl acetate (5 ml) and precipitation with *n*-hexane (5 ml). The resulting colourless oil was dried *in vacuo*; yield 490 mg (54%), white amorphous foam; m.p. 70-74°C.- IR (KBr): 3700-3150 br, 3070, 3050, 2940, 2862, 1770, 1715, 1665, 1525, 1455, 1390, 1160, 1115, 1090, 990, 905, 870, 722, 698, 640, 525 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 0.88 (1.07) (d; J = 7.2 Hz, 3H, CH_3-Ala), 1.15-1.42 (m; 3H, $3H_{ax}$), 1.62-1.85 (m; 4H, H_{ax}, $2H_{eq}$, H-β-iGln), 1.88-2.30 (m; 3H, $2H_{eq}$, H-β-iGln), 2.32 (t; J = 7.5 Hz, 2H, CH_2-γ-iGln), 3.78 (3.84) (AB-system, J = 15.1 Hz, 2H, OCH_2), 3.95-4.20 (m; 4H, CH-iGln, CH-Ala, 1'-H, 2'-H), 5.08 (5.10) (s; 2H, CH_2-benzyl), 7.05 (7.08) (s; br; 1H, NH), 7.22-7.42 (m; 7H, 5H arom., 2 NH), 7.26-7.38 (m; 4H, 4H arom.), 8.05 (8.08) (d; J = 7.2 Hz, NH).- $C_{31}H_{36}N_4O_8$ (592.6) Calcd. C 62.82 H 6.12 N 9.45 Found C 63.12 H 6.13 N 9.50.

N-(trans-2-(2'phthalimidocyclohexyloxy)acetyl)-L-alanyl-D-isoglutamine (**7b**)

Yield 402 mg (80%), m.p. 104-107°C.- IR (KBr): 3700-3100 br, 2940, 2860, 1770, 1710, 1655, 1525, 1450, 1385, 1250, 1160, 1110, 1085, 905, 870, 795, 720, 635, 525 cm^{-1}.- ^{1}H-NMR ([D$_6$]DMSO): δ (ppm) = 0.89 (1.08) (d; J = 7.2 Hz, 3H, CH_3-Ala), 1.16-1.45 (m; 3H, $3H_{ax}$), 1.60-1.85 (m; 4H, H_{ax}, $2H_{eq}$, H-β-iGln), 1.85-2.30 (m; 3H, $2H_{eq}$, H-β-iGln), 2.16 (t; J = 7.2 Hz, 2H, CH_2-γ-iGln), 3.77 (3.83) (AB-system, J = 16.6 Hz, 2H, OCH_2), 3.95-4.25 (m; 4H, CH-iGln, CH-Ala, 1'-H, 2'-H), 7.01 (7.05) (s; br; 1H, NH), 7.24 (7.30) (s br; 1H, NH), 7.22 (7.38) (d; J = 7.2 Hz, 1H, NH), 7.78-7.95 (m; 4H, 4H arom.), 8.02 (8.06) (d; J = 7.2 Hz, 1H, NH), 12.1 (s br; 1H, COOH).- $C_{24}H_{30}N_4O_8$ (502.5) Calcd. C 50.93 H 7.12 N 9.90 Found C 50.93 H 7.47 N 9.82.

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