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Preparation of Diastereomerically Pure Immunologically Active Carbocyclic nor-Muramyldipeptide Analogues

Danijel Kikelj*[a], Jurka Kidrič[b], Primož Pristovšek[b], Slavko Pečar[a], Uroš Urleb[a], Aleš Krbavčič[a] and Helmut Hönig[c]

[a]University of Ljubljana, Faculty of Natural Sciences and Technology, Department of Pharmacy, Aškerčeva 9, 61000 Ljubljana, Slovenia

[b]Boris Kidrič Institute of Chemistry, Hajdrihova 19, 61000 Ljubljana, Slovenia

[c]Technische Universität Graz, Institut für Organische Chemie, Stremayrgasse 16, A-8010 Graz, Austria

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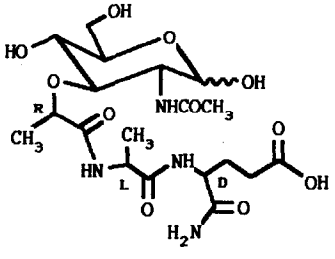
Abstract: The preparation of diastereomerically pure immunologically active carbocyclic *nor*-muramyldipeptide analogues (1'R,2'R)-/(1'S,2'S)-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-isoglutamine and (1'R,2'R)-/(1'S,2'S)-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-glutamic acid is described. The title compounds were synthesized using two independent synthetic routes from racemic *trans*-2-azidocyclohexanol and (1R,2R)-/(1S,2S)-2-aminocyclohexanol, respectively.

The design and synthesis of immune regulatory agents has become a challenging area in modern drug research.[1] In last years immunomodulators derived from bacterial cell wall polymeric constituents have been systematically investigated. The polymeric constitution is not an essential feature and most monomers of bacterial cell wall polymeric components e.g. proteoglycans, lipopolysaccharides and lipoproteins also exhibit strong immunostimulating activities. N-Acetylmuramyl-L-alanyl-D-isoglutamine (muramyldipeptide, MDP) was

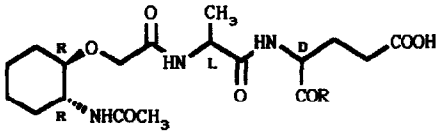


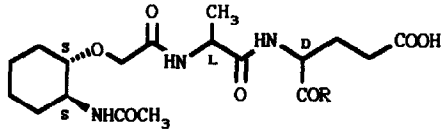
Muramyldipeptide (MDP)

identified in 1974 by Lederer, Kotani and coworkers[2,3] as the minimal immunologically active component of the peptidoglycan monomers. Although in the mean time a number of molecules of both natural and synthetic origin which influence the immune system have been found, MDP still remains one of the most potent immunostimulants identified so far.

In the last decade a number of MDP analogues and derivatives have been synthesized with the aim of obtaining molecules with improved and more defined pharmacological properties.[4,5] Structure-activity relationships for this class of compounds have been established[5,6] and especially in the last few years it became obvious that the intact N-acetyl-D-glucosamine moiety is not essential for the immunostimulant activity of MDP analogues. It can not only be derivatized but also omitted and replaced by acyclic or cyclic moieties as exemplified by biologically active lipophilic 6-O-acyl MPD derivatives,[7-10] FK-156,[11-14] pimelautide,[15,16] N-[2-(2-aminoalkoxy)propanoyl]-L-alanyl-D-isoglutamine derivatives[17] and carbocyclic MDP analogues.[18-21]

We have recently been engaged in the synthesis of novel carbocyclic *nor*-muramyldipeptide analogues in which the N-acetylmuramic acid part of MDP molecule is replaced by *trans*-2-(2'-acetylaminocyclohexyloxy)acetic acid. The recent work of Barton et *al.*[20] on the synthesis of similar MDP analogues containing pseudo-D-glucosamine stimulated us to disclose our own results on the synthesis of optically pure carbocyclic MDP analogues represented by general structures **1a-b** and **2a-b**.



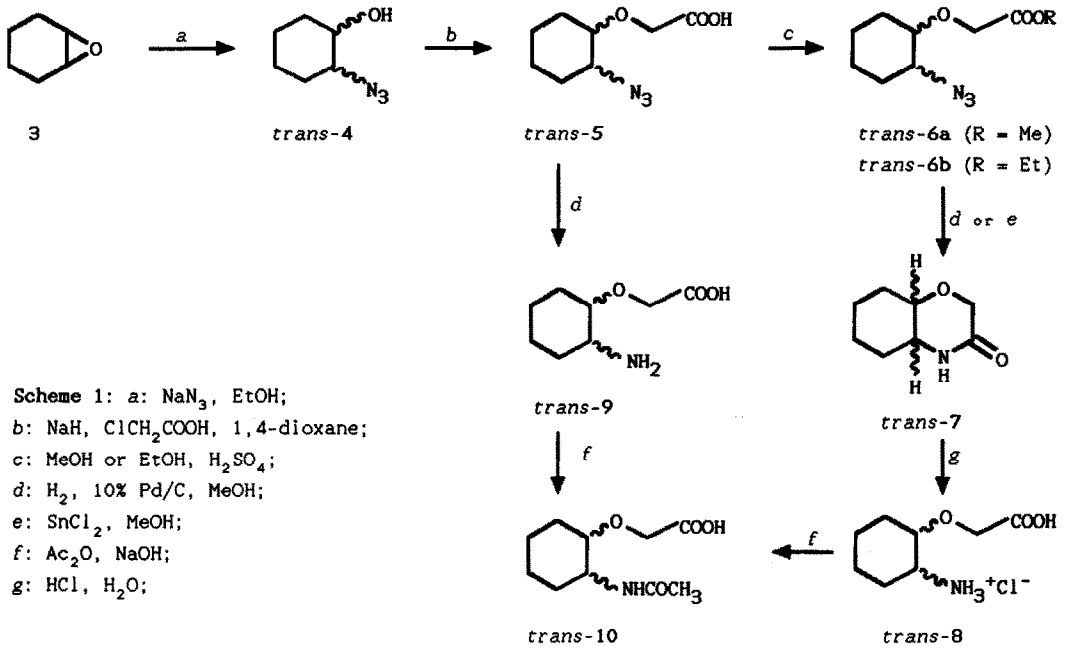


1a: R = NH_2 **1b**: R = NH_2

2a: R = OH **2b**: R = OH

We envisaged the synthesis of **1a-b** and **2a-b** starting from racemic *trans*-2-(2'-acetylaminocyclohexyloxy)acetic acid **10**, which itself was still unknown at the beginning of this work, by coupling it with appropriate benzyl protected dipeptides and consequent chromatographic separation of resulting diastereomeric mixtures followed by hydrogenolytic deprotection. For the preparation of **10** (Scheme 1) racemic *trans*-2-azidocyclohexanol **4**, readily available by ring opening of 1,2-epoxycyclohexane **3** with sodium azide,[22] was transformed into racemic *trans*-2-(2'-azidocyclohexyloxy)acetic acid **5** with chloroacetic acid and sodium hydride in dry 1,4-dioxane as previously reported.[23,24] Catalytic reduction of the azide **5** in methanol in the presence of 10% palladium on charcoal as catalyst produced **9** in excellent yield. Acetylation of the amino acid **9** with acetic anhydride according to a standard procedure in peptide chemistry[25] afforded racemic *trans*-2-(2'-acetylaminocyclohexyloxy)acetic acid **10**. Alternatively, the key intermediate **10** was prepared *via* the morpholinone **7** as described by us previously.[24] The conversion of ester **6** into the morpholinone **7** was initially effected[24] with tin(II)-chloride in methanol followed by aqueous workup (Method A). However, this transformation was achieved more efficiently when catalytic hydrogenation in methanol over 10% palladium on charcoal was used in the reduction step (Method B). Morpholinone **7** could alternatively

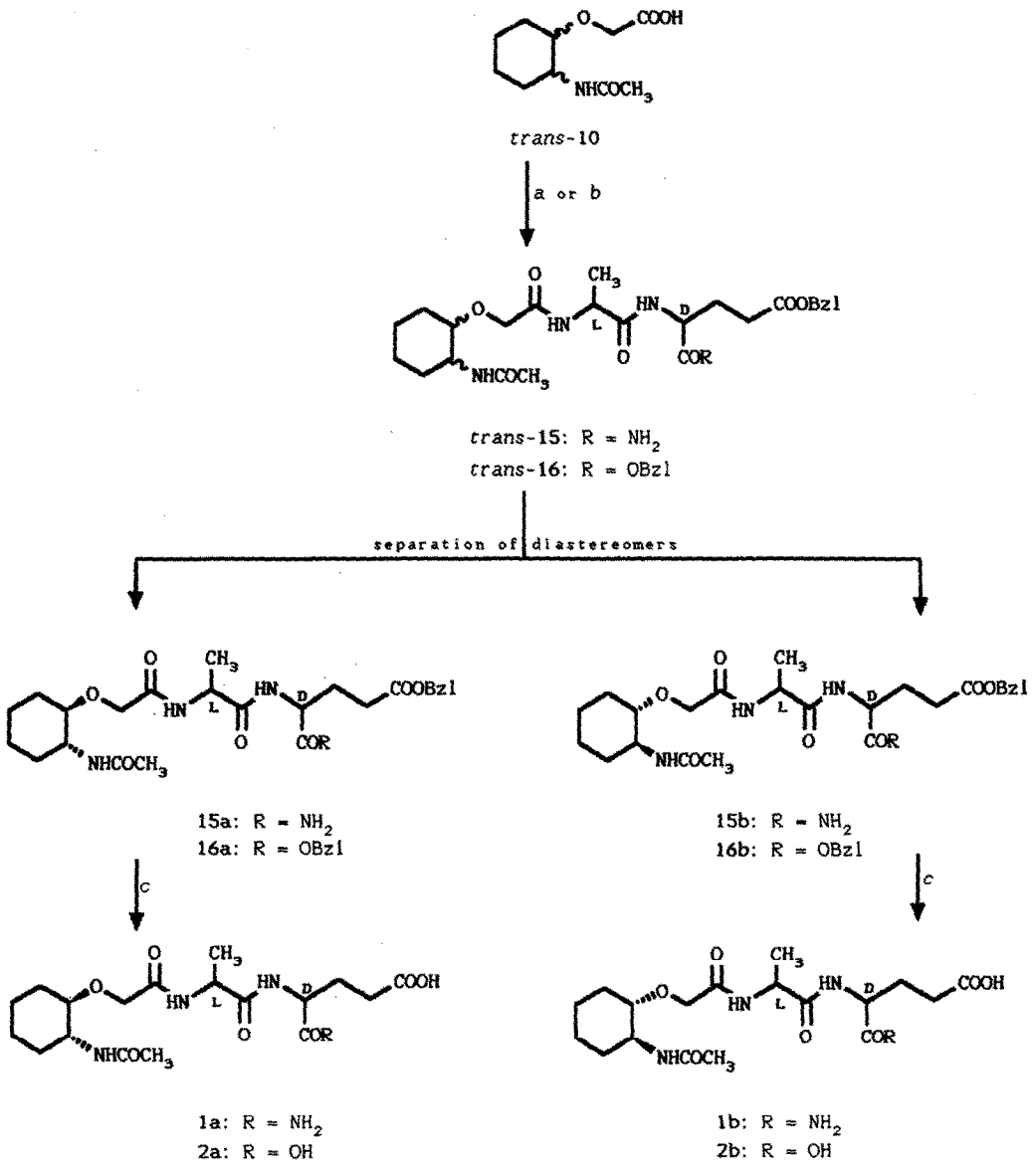
be prepared also from racemic *trans*-2-aminocyclohexanol **11**[26] using the same sequence of reactions as depicted in Scheme 3 for the synthesis of (4aR,8aR)-octahydro-2H-1,4-benzoxazine-3-one **19a** (Method C).



Scheme 1: *a*: NaN_3, EtOH;
b: NaH, $ClCH_2COOH$, 1,4-dioxane;
c: MeOH or EtOH, H_2SO_4;
d: H_2, 10% Pd/C, MeOH;
e: $SnCl_2$, MeOH;
f: Ac_2O, NaOH;
g: HCl, H_2O;

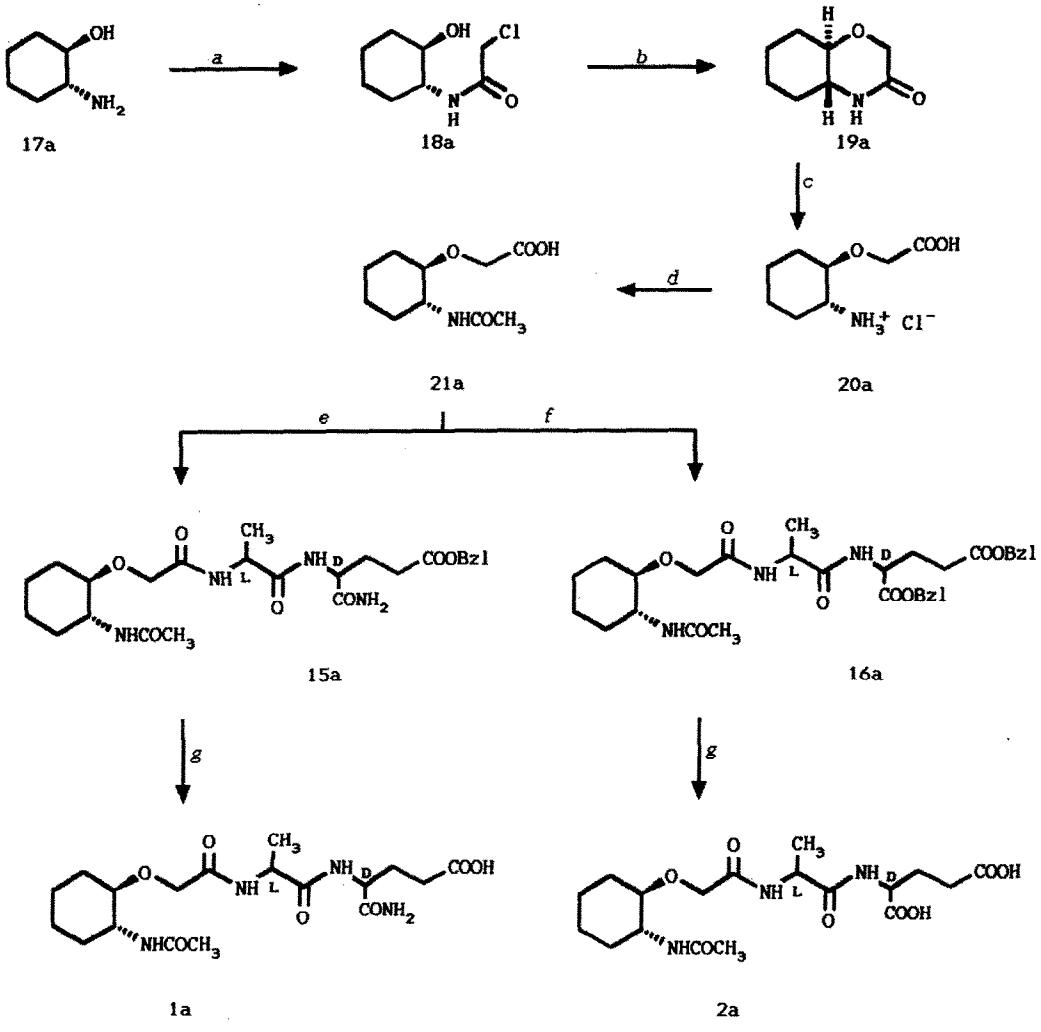
The acid **10** was condensed in the presence of diphenylphosphoryl azide (DPPA)[27-30] and triethylamine with the dipeptide L-alanyl-D-isoglutamine benzyl ester **13**[31,32] to afford benzyl-N-[*trans*-2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-isoglutaminate **15** as a mixture of diastereomers **15a** (R_f = 0.31) and **15b** (R_f = 0.22) which were separated by column chromatography on silica gel using chloroform/methanol (9/1) as eluent. Similarly, condensation of **10** with L-alanyl-D-glutamic acid dibenzyl ester **14**[33] using DPPA/Et_3N as a condensing agent produced dibenzyl-N-[*trans*-2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-glutamate **16** as a mixture of diastereomers **16a** (Rf = 0.59) and **16b** (Rf = 0.37) which could be separated by silica gel column chromatography using as eluent chloroform/ methanol (20/1). Finally, the benzyl protecting group(s) of **15a-b** and **16a-b** were smoothly removed by hydrogenolytic cleavage in methanol in the presence of 10% palladium on charcoal as catalyst to give diastereomerically pure carbocyclic *nor*-MDP analogues **1a-b** and **2a-b** (Scheme 2).

With the pure diastereomers **1a-b** and **2a-b** in hand no statement could be made about the absolute configuration at carbon atoms C-1' and C-2' of the cyclohexane ring. To ascertain the stereochemistry of pure diastereomers **1a-b** and **2a-b** they were prepared *via* an independent synthetic pathway from (1R,2R)- and (1S,2S)-2-aminocyclohexanol respectively which meanwhile became easily available by enzymatic resolution of racemic alkyl



Scheme 2: *a*: L-Ala-D-iGln(OBzl)·HCl, DPPA, Et_3N, DMF; *b*: L-Ala-D-Glu(OBzl)$_2$·HCl, DPPA, Et_3N, DMF; *c*: H_2, 10% Pd/C, MeOH.

esters of *trans*-2-azidocyclohexanol and subsequent hydrogenation thereof.[34] As depicted in Scheme 3, (1R,2R)-2-aminocyclohexanol **17a** was converted with chloroacetyl chloride into (1'R,2'R)-N-(2'-hydroxycyclohexyl)-2-chloroacetamide **18a** which was cyclized with sodium hydride in 1,4-dioxane into the condensed morpholinone **19a**. Hydrolytic cleavage of



Scheme 3: *a*: $ClCH_2COCl$, NaOAc, acetone/H_2O; *b*: NaH, THF; *c*: HCl, H_2O; *d*: Ac_2O, NaOH; *e*: L-Ala-D-iGln(OBzl)·HCl, DPPA, Et_3N, DMF; *f*: L-Ala-D-Glu(OBzl)$_2$·HCl, DPPA, Et_3N, DMF; *g*: H_2, Pd/C, MeOH.

morpholinone **19a** with 18% aqueous hydrochloric acid afforded (1R,2R)-2-carboxymethoxycyclohexylammonium chloride **20a** which was acetylated with acetic anhydride to give (1'R,2'R)-2-(2'-acetylaminocyclohexyloxy)acetic acid **21a**. Coupling of acid **21a** with the dipeptide **13** in the presence of DPPA and Et_3N afforded (1'R,2'R)-benzyl-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-isoglutaminate **15a** whereas with the dipeptide **14** (1'R,2'R)-dibenzyl-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-glutamate **16a** was obtained. Hydrogenolytic deprotection of **15a** and **16a** in methanol with 10% palladium on charcoal as catalyst afforded diastereomerically pure carbocyclic *nor*-MDP analogues **1a**

and **2a**. Using the same synthetic sequence as depicted in Scheme 3 starting from (1S,2S)-2-aminocyclohexanol **17b**, (1'S,2'S)-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-isoglutamine **1b** and (1'S,2'S)-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-glutamic acid **2b** were obtained.

Comparison of specific rotation values of benzyl protected precursors **15a-b** and **16a-b** obtained from optically pure *trans*-2-aminocyclohexanols **17a** and **17b** with the specific rotation values of pure diastereomers obtained by chromatographic separation as well as thin layer chromatography analysis of the products enabled determination of the absolute configuration of the chromatographically separated diastereomers.

The immunological activity of the prepared carbocyclic *nor*-MDP analogues will be reported in the forthcoming paper.

As a basis of our future work on conformational analysis the assignment of ^{1}H and ^{13}C resonances of **1a-b** and **2a-b** in DMSO-d_6 was accomplished using phase sensitive correlation spectroscopy (COSY),[35,36] heteronuclear correlation (HETCOR),[37,38] correlation *via* long range coupling (COLOC)[39] and 2D NOE spectroscopy (NOESY).[40,41] The proton coupling network (1'-, 2'-, all NH- and α-protons) was revealed by homonuclear decoupling and the COSY spectrum. All five carbonyl ^{13}C resonances and the Cβ-iGln signal were assigned on the basis of long range proton-carbon correlation in the COLOC spectra. The proton resonances 3'-, 4'-, 5'- and 6'-H_{eq}, H_{ax} of the cyclohexyl ring and both isoglutamine/-glutamic acid β-protons appear between 1.1 and 2.1 ppm and can only be distinguished by direct proton-carbon coupling in the HETCOR spectrum. All four ring axial proton resonances are located between 1.1 and 1.2 ppm. Their equatorial counterparts are found at lower field[42] (between 1,5 and 2.0 ppm) indicating a relatively rigid cyclohexyl ring structure with both substituents presumably in the equatorial position. The proton 3'-H_{eq} was identified by cross-peaks with 2'-H and acetylamino NH in the NOESY spectra and the proton 6'-H_{eq} by cross-peaks with 1'-H and OCH_2. Only protons and carbons 4' and 5' were left unassigned. The -OCH_2 resonance at approx. 3.9 ppm shows a typical AB pattern with J_{AB} = 15 Hz and (δ_A-δ_B) = 0.16 ppm for **1a** and 0.11 ppm for **1b**. The Hα-Ala quintet is really an overlapping quartet of doublets with J(αH-CH_3) and J(αH-NH) values both being 6.9 Hz. The Hα-iGln multiplet is a part of a complex $A_2BB'MX$ spectrum that includes isoglutamine α-, β-, γ- and NH-protons.

Additionally, the assignment of ^{1}H and ^{13}C resonances of (4aS,8aS)-, (4aR,8aR)-octahydro-2H-1,4-benzoxazin-3-one and *rac-trans*-methyl-2-(2'-azidocyclohexyloxy)acetate[23] based on the chemical shifts of *trans*-2-methyl-octahydro-2H-1,4-benzoxazin-3-one[23,43] and similar substituted morpholin-3-ones[44] was confirmed by DEPT[45], COSY and HETCOR experiments. Protons 1-H and 2-H in compound **20a** as well as 1'-H and 2'-H in compounds **15a**, **16a**, **18a** and **21a** were identified by homonuclear decoupling of the adjacent NH protons.

Experimental

Melting points were determined on a Reichert hot stage microscope and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR spectrometer. Optical rotations were measured on a Perkin-Elmer 1241 MC polarimeter. The reported specific rotation values are average values of ten successive measurements using an integration time of 10 seconds. Elemental analyses were performed at the department of Chemistry, University of Ljubljana on a Perkin-Elmer C,H,N-Analyzer 240 C. Mass spectra were measured on a Varian MAT 311 A mass spectrometer (ionization energy 100 eV). The UV spectra were recorded on a Perkin Elmer 554 UV spectrometer.

NMR spectra were obtained on a Varian VXR-300 spectrometer that operates at 299.94 MHz for ^1H and 75.43 MHz for ^{13}C nuclei. Chemical shifts are reported in ppm relative to tetramethylsilane as internal standard. All experiments were performed at 25 °C with concentrations between 0.3 and 0.1 M in DMSO-d_6 or $CDCl_3$. For the ^1H and ^{13}C spectra a spectral window of 4000 Hz and 13738 Hz respectively were used. The COSY spectrum was recorded with 128 increments and 8 scans each FID, the HETCOR spectrum with 256 increments, 48 scans, both COLOC spectra with 160 increments, 80 scans and all three NOESY spectra with 512 increments, 16 scans. The delays in the HETCOR experiment were optimized for J_{CH} = 140 Hz. In the COLOC experiments delays Δ_1 = 25 or 30 ms and Δ_2 = 30 ms were used. NOESY spectra were recorded with mixing times 0.3, 0.5 and 0.8 s. Gaussian window functions were employed for filtering in 2D spectra. Some routine ^1H- and ^{13}C-NMR spectra were measured on a JEOL-FX-90-Q and on a Bruker WM-250 spectrometers.

rac-trans-Ethyl-2-(2'-Azidocyclohexyloxy)acetate (**6b**): A solution of crude *trans*-2-(2'-azidocyclohexyloxy)acetic acid **5**[23] (11.16 g, 56 mmol) and conc. H_2SO_4 (0.8 ml) in dry ethanol (30 ml) was refluxed for 3 h, poured into ice water (150 ml) and extracted with *n*-hexane (4 x 60 ml). The combined extracts were dried over $MgSO_4$, filtered and evaporated *in vacuo*. The residual oil was distilled *in vacuo* to give pure **6b**; yield: 8.6 g (68 %); b.p. 129 °C/0.5 Torr; IR (Film): 2940, 2860, 2100, 1755, 1730, 1450, 1385, 1265, 1200, 1150, 1125, 1030, 975, 915, 850 cm^{-1}; ^1H-NMR (300 MHz, $CDCl_3$): δ = 1.29 (t, 3H, J = 7.1 Hz, CH_3), 1.19-1.41 (m, 4H, $4H_{ax}$), 1.66-1.78 (m, 2H, $2H_{eq}$), 1.96-2.19 (m, 2H, $2H_{eq}$), 3.16-3.26 (m, 1H, 2'-H), 3.33-3.42 (m, 1H, 1'-H), 4.23 (q, 2H, J = 7.1 Hz, CH_2), 4.24 (s, 2H, OCH_2) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.7 (CH_3), 23.1 (C-4'/5'), 23.4 (C-4'/5'), 29.9 (C-3'), 29.9 (C-6'), 60.4 (OCH_2), 64.3 (2'-H), 66.8 (OCH_2), 82.0 (1'-H), 170.1 (CO) ppm; Anal. calcd. for $C_{10}H_{17}N_3O_3$: C 52.85, H 7.54, N 18.49, found C 52.52, H 7.52, N 18.67.

rac-trans-Octahydro-2H-1,4-benzoxazin-3-one (**7**). *Method B:* *rac-trans*-Methyl-2-(2'-azidocyclohexyloxy)acetate **6a**[23] (1.00 g, 4.69 mmol) was dissolved in methanol (10 ml) and hydrogenated over 10% palladium on charcoal (150 mg) for 1 h at room temperature and

normal pressure. The catalyst was removed by filtration and methanol was evaporated *in vacuo* to yield **7** (0.71 g, 97 %); m.p. 179-181 °C which was in all respects (IR, ^{1}H-NMR) identical with the product obtained by Method A.[24]

rac-trans-2-(2'-Aminocyclohexyloxy)acetic Acid (**9**): A solution of *rac-trans*-2-(2'-azidocyclohexyloxy)acetic acid **5** (360 mg, 1.81 mmol) in methanol (10 ml) was hydrogenated in the presence of 10% palladium on charcoal for 1 h under normal pressure at room temperature. The catalyst was filtered off and methanol was removed *in vacuo* to give 307 mg (98 %) of **9** which was recrystallized from methanol/ether; m.p. 191-193 °C; IR (KBr): 3680-3235 br, 3235-2300 br, 2226, 1646, 1558, 1452, 1406, 1316, 1119, 1055, 1020, 991, 908, 855, 706, 588, 545, 466 cm^{-1}; UV (CH_3OH): λ_{max} (logϵ) = 204 (3.17); ^{1}H-NMR (300 MHz, DMSO-d_6/D_2O): δ = 0.95-1.35 (m, 4H, $4H_{ax}$), 1.45-1.70 (m, 2H, 4'-H_{eq}, 5'-H_{eq}), 1.85-1.95 (m, 1H, H_{eq}), 1.95-2.08 (m, 1H, H_{eq}), 2.75-2.90 (m, 1H, 2'-H), 3.00-3.16 (m, 1H, 1'-H), 3.75 (AB-system, 2H, J = 16.5 Hz, OCH_2) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 24.3 (C-4'/5'), 24.5 (C-4'/5'), 30.3 (C-3'/6'), 31.0 (C-3'/6'), 55.2 (C-2'), 68.9 (OCH_2), 81.1 (C-1'), 178.0 (CO) ppm; Anal. calcd. for $C_8H_{15}NO_3$: C 55.47, H 8.73, N 8.09, found: C 55.23, H 8.97, N 8.14.

rac-trans-2-(2'-Acetylaminocyclohexyloxy)acetic Acid (**10**): To a stirred solution of *trans*-2-(2'-aminocyclohexyloxy)acetic acid **9** (1.105 g, 6.39 mmol) in 1N NaOH (6.39 ml) was added at 5 °C first 1N NaOH (1.28 ml) and then acetic anhydride (0.130 g, 1.28 mmol). When the anhydride disappeared and a homogeneous solution formed, the addition of the same amounts of 1N NaOH and acetic anhydride was repeated four more times. After the addition of the last portion, stirring was continued for 30 min, the solution was acidified to pH 2 and extracted with ethyl acetate (5 x 30 ml). The organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The solid residue was recrystallized from chloroform/ethyl acetate/*n*-hexane to give 0.96 g (70 %) of **10**; m.p. 130-132 °C, which was in all respects (IR, ^{1}H-NMR) identical with compounds **21a** and **21b** described below; Anal. calcd. for $C_{10}H_{17}NO_4$: C 55.80, H 7.96, N 6.51, found: C 55.92, H 8.09, N 6.32.

rac-trans-N-(2'-Hydroxycyclohexyl)-2-chloroacetamide (**12**). **12** was prepared from *rac-trans*-2-aminocyclohexanol (1.15 g, 10 mmol) following the same procedure as described below for the synthesis of **18b**; yield: 1.50 g (78%); m.p. 108-109 °C; IR (KBr): 3320, 2950, 2875, 1650, 1560, 1465, 1420, 1345, 1275, 1250, 1145, 1075, 1060, 990, 955, 875, 780 cm^{-1}; UV (CH_3CN): λ_{max}(log ϵ) = 219 (3.01) nm; ^{1}H-NMR (100 MHz, $CDCl_3$): δ = 1.26-2.16 (m, 8H, $4CH_2$), 3.36-3.71 (m, 3H, OH, 1'-H, 2'-H), 4.09 (s, 2H, CH_2Cl), 6.67 (s br, 1H, NH) ppm; Anal. calcd. for $C_8H_{14}ClNO_2$: C 50.13, H 7.36, N 7.31, found: C 50.29, H 7.42, N 7.05.

rac-trans-Octahydro-2H-1,4-benzoxazin-3-one (7). *Method C*: 7 was prepared from *rac-trans*-N-(2'-hydroxycyclohexyl)-2-chloroacetamide 12 (1.00 g, 5.2 mmol) using the procedure described below for the synthesis of **19a** ; yield: 0.69 g (85 %); m.p. 180-181 °C; The spectroscopic data (^{1}H-NMR, ^{13}C-NMR, IR, UV) were identical with the spectroscopic data of **19a** below; Anal. calcd. for $C_8H_{13}NO_2$: C 61.91, H 8.44, N 9.03, found: C 61.86, H 8.49, N 9.02.

(1'S,2'S)-N-(2'-Hydroxycyclohexyl)-2-chloroacetamide (**18b**): To a stirred suspension of (1S,2S)-2-aminocyclohexanol[47] (1.15 g, 10 mmol) and sodium acetate (1.64 g, 20 mmol) in a mixture of acetone (20 ml) and water (7 ml) chloroacetyl chloride (1.13 g, 10 mmol) was added dropwise over 5 min at 0-5 °C. After the addition was complete the mixture was stirred for 1 h at room temperature and subsequently evaporated to dryness. After addition of chloroform (50 ml), the resulting solution was washed subsequently with water (20 ml) and saturated NaCl solution (20 ml), dried ($MgSO_4$), filtered and evaporated *in vacuo*. The resulting crude **18b** (1.54 g, 81%) was recrystallized from ethyl acetate; m.p. 137-139 °C; $[\alpha]_D^{20}$= +46.7 (c = 0.1, methanol); IR (KBr): 3308, 2944, 2856, 1645, 1548, 1456, 1409, 1264, 1060, 1046, 974, 772 cm^{-1}; UV (CH_3OH): λ_{max} (log ϵ) = 202 (3.74) nm; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.00-1.30 (m, 4H, $4H_{ax}$), 1.42-1.70 (m, 2H, 4'-H_{eq}, 5'-H_{eq}), 1.70-1.90 (m, 2H, 3'-H_{eq}, 6'-H_{eq}), 3.18-3.30 (m, 1H, 2'-H), 3.30-3.42 (m, 1H, 1'-H), 4.01 (s, 2H, CH_2Cl) 4.58 (d, 1H, J = 5.04 Hz, OH), 7.96 (d, 1H, J = 7.86 Hz, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 23.2 (C-4'/5'), 23.5 (C-4'/5'), 30.2 (C-3'/6'), 33.5 (C-3'/6'), 42.4 (C-2), 54.2 (C-1'), 70.3 (C-2'), 164.8 (C-1) ppm; Anal. calcd. for $C_8H_{14}ClNO_2$: C 50.13, H 7.36, N 7.31, found: C 50.24, H 7.52, N 7.58.

(1'R,2'R)-N-(2'-Hydroxycyclohexyl)-2-chloroacetamide (**18a**): **18a** was prepared from (1R,2R)-2-aminocyclohexanol hydrochloride[48] (1.52 g, 10 mmol) according to the procedure described above for the synthesis of **18b**; yield: 1.34 g (70 %); m.p. 138-140 °C; $[\alpha]_D^{20}$= -39.6 (c = 0.1, methanol); The spectroscopic data for **18a** (IR, ^{1}H-NMR, ^{13}C-NMR) were identical to the spectroscopic data of **18b**; Anal. calcd. for $C_8H_{14}ClNO_2$: C 50.13, H 7.36, N 7.31, found: C 50.49 H 7.62, N 7.42.

(4aR,8aR)-Octahydro-2H-1,4-benzoxazin-3-one (**19a**): To a stirred solution of (1'R,2'R)-N-(2'-hydroxycyclohexyl)-2-chloroacetamide **18a** (1.00 g 5.2 mmol) in dry tetrahydrofurane (20 ml) precooled to 0 °C was added sodium hydride (0.18 g, 7.5 mmol). The mixture was allowed to reach room temperature and stirred for 14 h, whereupon water (3 ml) was added and the mixture was stirred for additional 10 min. Tetrahydrofurane and water were removed *in vacuo* and the residue was dissolved in chloroform (50 ml). The organic solution was washed successively with water (20 ml) and satd. NaCl solution (20 ml), dried over Na_2SO_4, filtered and evaporated *in vacuo*. The solid residue was recrystallized from ethyl acetate to give pure **19a**; yield: 0.685 g (85 %); m.p. 165-167 °C; $[\alpha]_D^{20}$= -46.1 (c = 0.1, methanol); IR (KBr): 3182, 3079, 2938, 2863, 1700, 1646, 1415, 1370, 1360,

1306, 1109, 793, 490 cm^{-1}; UV (CH_3OH): λ_{max}(log ϵ) = 202 (3.81) nm; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.18-1.45 (m, 4H, $4H_{ax}$), 1.65-2.05 (m, 4H, $4H_{eq}$), 3.10-3.26 (m, 2H, 4a-H, 8a-H), 4.24 (AB-system, 2H, J = 16.85 Hz, OCH_2), 7.80 (s br, 1H, NH) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 23.3 (C-6/7), 24.1 (C-6/7), 29.7 (C-5/8), 30.6 (C-5/8), 55.8 (C-4a), 67.8 (C-2), 77.8 (C-8a), 169.8 (C-3) ppm; Anal. calcd. for $C_8H_{13}NO_2$: C 61.91, H 8.44, N 9.03, found: C 62.13, H 8.41, N 9.15.

(4aS,8aS)-Octahydro-2H-1,4-benzoxazin-3-one (19b): Obtained in the same way as **19a** from (1'S'2'S)-N-(2'-hydroxycyclohexyl)-2-chloroacetamide **18b** (1.92 g 10 mmol); yield: 1.41 g (91%); m.p. 165-167 °C; $[\alpha]_D^{20}$ = +45.2 (c = 0.1, methanol); The spectroscopic data (IR, ^{1}H-NMR, ^{13}C-NMR) were identical to the spectroscopic data of **19a**; Anal. calcd. for $C_8H_{13}NO_2$: C 61.91, H 8.44, N 9.03, found: C 62.14, H 8.77, N 8.84.

(1R,2R)-2-Carboxymethoxycyclohexylammonium Chloride (20a): A solution of (4aR,8aR)-octahydro-2H-1,4-benzoxazin-3-one **19a** (1.20 g, 7.73 mmol) in 18 % aqueous hydrochloric acid (42 ml) was refluxed for 4 h and evaporated *in vacuo* to a white solid which was recrystallized from ethanol/ether; yield: 1.47 g, (91%); m.p. 218-220 °C; $[\alpha]_D^{20}$= -94.5 (c = 0.1, methanol); IR (KBr): 3650-2400, 1735, 1600, 1582, 1484, 1412, 1219, 1129, 1036, 1008, 823, 685 cm^{-1}; UV (CH_3OH): λ_{max}(log ϵ) = 201 (2.32) nm; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.05-1.32 (m, 3H, $3H_{ax}$), 1.35-1.55 (m, 1H, $1H_{ax}$), 1.58-1.75 (m, 2H, 4-H_{eq}, 5-H_{eq}), 1.95-2.05 (m, 1H, 3/6-H_{eq}), 2.05-2.20 (m, 1H, 3/6-H_{eq}), 2.80-2.95 (m, 1H, 1-H), 3.30-3.42 (m, 1H, 2-H), 4.15 (s, 2H, OCH_2), 8.25 (s br, 3H, NH_3^+), 12.85 (s br, 1H, COOH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 23.2 (C-4/5), 23.3 (C-4/5), 28.6 (C-3/6), 29.5 (C-3/6), 53.7 (C-1), 65.6 (OCH_2), 79.2 (C-2), 172.6 (CO) ppm; Anal. calcd. for $C_8H_{16}ClNO_3$: C 45.83, H 7.69, N 6.68, found: C 46.15, H 7.88, N 7.05.

(1S,2S)-2-Carboxymethoxycyclohexylammonium Chloride (20b): Obtained in the same way as **20a** from (4aS,8aS)-octahydro-2H-1,4-benzoxazin-3-one **19b** (0.83 g, 5.35 mmol); yield: 0.86 g (77%); m.p. 218-220 °C; $[\alpha]_D^{20}$= +92.4 (c = 0.1, methanol); The spectroscopic data (IR, ^{1}H-NMR, ^{13}C-NMR) were identical with the spectroscopic data of compound **20a**; Anal. calcd. for $C_8H_{16}ClNO_3$: C 45.83, H 7.69, N 6.68, found: C 45.86, H 7.79, N 7.04.

(1'R,2'R)-2-(2'-Acetylaminocyclohexyloxy)acetic Acid (21a): To a stirred solution of (1R,2R)-2-carboxymethoxycyclohexylammonium chloride **20a** (1.34 g, 6.39 mmol) in 2N NaOH (6.39 ml) was added at 5 °C first 1N NaOH (1.28 ml) and then acetic anhydride (0.130 g, 1.28 mmol). When the anhydride disappeared and a homogeneous solution was formed, the addition of the same amounts of 1N NaOH and acetic anhydride was repeated four more times. After the addition of the last portion, stirring was continued for 30 min, the solution was acidified to pH 2 and extracted with ethyl acetate (5 x 30 ml). The organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The solid residue was recrystallized from chloroform/ethyl acetate/*n*-hexane to give 1.14 g (83 %)

of **21a**; m.p.130-132 °C; $[\alpha]_D^{20}$ = -44.2 (c = 0.1, methanol); IR (KBr): 3331, 2940, 2867, 2800-2500, 1725, 1613, 1555, 1436, 1372, 1212, 1125, 949, 894, 682, 660 cm^{-1}; UV (CH_3OH): λ_{max}(log ϵ) = 201 (3.77) nm; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.95-1.10 (m, 1H, $1H_{ax}$), 1.10-1.40 (m, 3H, $3H_{ax}$), 1.60-1.70 (m, 1H, 4'/5'-H_{eq}), 1.70-1.80 (m, 1H, 4'/5'-H_{eq}), 2.00-2.20 (m, 1H, 3'/6'-H_{eq}), 2.09 (s, 3H, CH_3), 2.45-2.60 (m, 1H, 3'/6'-H_{eq}), 3.10-3.20 (m, 1H, 1'-H) 3.40-3.55 (m, 1H, 2'-H), 4.14 (AB-system, 2H, J = 17.6 Hz, OCH_2), 8.55 (d, 1H, J = 3.96 Hz, NH), 12.20 (s br, 1H, COOH) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.3 (CH_3), 23.4 (C-4'/5'), 23.8 (C-4'/5'), 29.5 (C-3'/6'), 30.4 (C-3'/6'), 54.4 (C-2'), 64.4 (OCH_2), 79.9 (C-1'), 172.3 (CO), 174.2 (CO) ppm; Anal. calcd. for $C_{10}H_{17}NO_4$: C 55.80, H 7.96, N 6.51, found: C 55.93, H 7.91, N 6.86.

(1'S,2'S)-2-(2'-Acetylaminocyclohexyloxy)acetic Acid (21b): **21b** was prepared in the same way as **21a** from (1S,2S)-2-carboxymethoxycyclohexylammonium chloride **20b** (0.67 g, 3.20 mmol); yield: 0.50 g (72 %); m.p. 130-132 °C; $[\alpha]_D^{20}$ = +43.5 (c = 0.1, methanol); The spectroscopic data (IR, ^{1}H-NMR, ^{13}C-NMR) were identical with the spectroscopic data of **21a**. Anal. calcd. for $C_{10}H_{17}NO_4$: C 55.80, H 7.96, N 6.51, found: C 55.77, H 7.91, N 6.79.

(1'R,2'R)-Dibenzyl-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-glutamate (16a): To a stirred solution of dibenzyl-L-alanyl-D-glutamate hydrochloride (869 mg, 2 mmol) and (1'R,2'R)-2-(2'-acetylaminocyclohexyloxy)acetic acid **21a** (430 mg, 2 mmol) in dry N,N-dimethylformamide (10 ml) were added at 0-2 °C DPPA (550 mg, 2 mmol) and triethylamine (0.56 ml, 4 mmol). The mixture was stirred for 1h at 0-2 °C and then for 60 h at room temperature. Ethyl acetate (40 ml) was added and the organic solution was extracted subsequently with 10% citric acid (3x5 ml), water (3x5 ml), saturated NaCl solution (3x5 ml), saturated $NaHCO_3$ solution (3x5 ml), water (3x5 ml) and saturated NaCl solution (3x5 ml). The organic phase was dried over $MgSO_4$, filtered and evaporated *in vacuo* to give crude **16a** which was purified by column chromatography on silica gel (100 g) with chloroform/methanol (9/1) as eluent; yield: 1.00 g (84 %), colourless viscous oil; $[\alpha]_D^{20}$ = -10.0 (c = 0.1, methanol); IR (KBr): 3287, 3066, 2934, 2858, 1744, 1649, 1548, 1446, 1374, 1271, 1169, 966, 753, 696, 578 cm^{-1}; UV (CH_3OH): λ_{max}(log ϵ) = 202 (4.39) nm; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.00-1.30 (m, 4H, $4H_{ax}$), 1.23 (d, 3H, J = 7.02 Hz, CH_3-Ala), 1.50-1.70 (m, 2H, 4'-H_{eq}, 5'-H_{eq}), 1.80 (s, 3H, $COCH_3$), 1.80-2.12 (m, 4H, 3'-H_{eq}, 6'-H_{eq}, CH_2-βGlu), 2.46 (t, 2H, J = 7.60 Hz, CH_2-γGlu), 3.06-3.18 (m, 1H, 1'-H), 3.50-3.62 (m, 1H, 2'-H), 3.91 (AB-system, 2H, J = 15.14 Hz, OCH_2), 4.30-4.42 (m, 2H, CH-Glu, CH-Ala), 5.08 (s, 2H, CH_2-benzyl), 5.11 (s, 2H, CH_2-benzyl), 7.30-7.40 (m, 10H, 10H-arom.), 7.56 (d, 1H, J = 7.78 Hz, NH), 7.89 (d, 1H, J = 7.63 Hz, NH-acetyl-amino), 8.53 (d, 1H, J = 7.78 Hz, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 18.9 (CH_3-Ala), 22.8 ($CO\underline{C}H_3$), 23.4 (C-4'/5'), 23.7 (C-4'/5'), 25.9 (C-βGlu), 29.7 (C-γGlu), 29.9 (C-3'/6'), 31.0 (C-3'/6'), 47.3 (C-αAla), 51.2 (C-αGlu), 51.8 (C-2'), 65.5 (CH_2-benzyl), 66.0 (CH_2-benzyl), 67.3 (C-2), 80.5 (C-1'), 127.7, 127.8, 127.9, 128.0, 128.4 (10 CH-arom.), 135.8 (C-arom.), 136.0 (C-arom.), 168.9, 169.2, 171.2, 171.9,

172.3 (5 CO) ppm; Anal. calcd. for $C_{32}H_{41}N_3O_8$: C 64.52, H 6.94, N 7.05, found: C 64.04, H 6.85, N 7.29.

(1'S,2'S)-Dibenzyl-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-glutamate (16b): **16b** was prepared from (1'S,2'S)-2-(2'-acetylaminocyclohexyloxy)acetic acid **21b** (430 mg, 2 mmol) according to the procedure described above for the synthesis of **16a**; yield: 1.03 g (86 %); colourless viscous oil; $[\alpha]_D^{20}$ = +19.6 (c = 0.1, methanol); IR (KBr): 3391, 3296, 3066, 2935, 2859, 1740, 1654, 1542, 1453, 1375, 1262, 1166, 1113, 967, 739, 698, 579 cm^{-1}; UV (CH_3OH): λ_{max}(log ϵ) = 203 (4.45) nm; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.00-1.30 (m, 4H, $4H_{ax}$), 1.23 (d, 3H, J = 7.02 Hz, CH_3-Ala), 1.50-1.70 (m, 2H, 4'-H_{eq}, 5'-H_{eq}), 1.80 (s, 3H, $COCH_3$), 1.80-2.12 (m, 4H, 3'-H_{eq}, 6'-H_{eq}, CH_2-βGlu), 2.43 (t, 2H, J = 7.63 Hz, CH_2-γGlu), 3.06-3.18 (m, 1H, 1'-H), 3.50-3.62 (m, 1H, 2'-H), 3.93 (AB-system, 2H, J = 15.29 Hz, OCH_2), 4.30-4.42 (m, 2H, CH-Glu, CH-Ala), 5.08 (s, 2H, CH_2-benzyl), 5.12 (s, 2H, CH_2-benzyl), 7.30-7.40 (m, 10H, 10H-arom.), 7.53 (d, 1H, J = 7.63 Hz, NH), 7.91 (d, 1H, J = 7.75 Hz, NH-acetylamino), 8.49 (d, 1H, J = 7.75 Hz, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 18.8 (CH_3-Ala), 22.8 ($CO\underline{C}H_3$), 23.4 (C-4'/5'), 23.7 (C-4'/5'), 25.9 (C-βGlu), 29.6 (C-γGlu), 29.9 (C-3'/6'), 30.9 (C-3'/6'), 47.4 (C-αAla), 51.1 (C-αGlu), 51.8 (C-2'), 65.4 (CH_2-benzyl), 65.9 (CH_2-benzyl), 67.5 (C-2), 80.7 (C-1'), 127.7, 127.8, 127.9, 127.9, 128.3 (10 CH-arom.), 135.7 (C-arom.), 135.9 (C-arom.), 168.8, 169.2, 171.2, 171.8, 172.1 (5 CO) ppm;. Anal. calcd. for $C_{32}H_{41}N_3O_8$: C 64.52, H 6.94, N 7.05, found: C 64.37, H 6.92, N 7.00.

(1'R,2'R)-Benzyl-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-isoglutaminate 15a: To a stirred solution of benzyl-L-alanyl-D-isoglutaminate hydrochloride (687 mg, 2 mmol) and (1'R,2'R)-2-(2'-acetylaminocyclohexyloxy)acetic acid **21a** (430 mg, 2 mmol) in dry N,N-dimethylformamide (9 ml) were added at 0-2 °C DPPA (550 mg, 2 mmol) and triethylamine (0.56 ml, 4 mmol). The mixture was stirred for 1h at 0-2 °C and subsequently for 60 h at room temperature. Ethyl acetate (40 ml) was added and the resulting organic solution was extracted with 10% citric acid (3 x 5 ml). The citric acid phase was extracted with ethyl acetate (5 x 25 ml)[46] and combined six ethyl acetate extracts were washed with water (3 x 20 ml), saturated NaCl solution (3 x 20 ml), saturated $NaHCO_3$ solution (3 x 20 ml), water (3 x 20 ml) and saturated NaCl solution (3 x 20 ml). The organic phase was dried over $MgSO_4$, filtered and evaporated *in vacuo* to give 0.59 g (58 %) of crude (1'R,2'R)-benzyl-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-isoglutaminate which was recrystallized from ethyl acetate/*n*-hexane; m.p. 173-175 °C; $[\alpha]_D^{20}$ = -20.0(c = 0.1 methanol; IR (KBr): 3290, 3089, 2938, 2859, 1735, 1648, 1553, 1450, 1376, 1318, 1238, 1171, 1127, 1101, 965, 745, 695, 608 cm^{-1}; UV (CH_3OH) λ_{max}(log ϵ) = 202 (4.33) nm; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.05-1.35 (m, 4H, $4H_{ax}$), 1.23 (d, 1H, J = 7.08 Hz, CH_3-Ala), 1.55-1.67 (m, 2H, 4'-H_{eq}, 5'-H_{eq}), 1.70-1.85 (m, 2H, 3'/6'-H_{eq}, H-βiGln), 1.83 (s, 3H, CH_3CO), 1.95-2.10 (m, 2H, 3'/6'-H_{eq}, H-βiGln), 2.38 (t, 2H, J = 7.82 Hz, CH_2-γiGln), 3.05-3.18 (m, 1H, 1'-H), 3.50-3.65 (m, 1H, 2'-H), 3.91 (AB-system, 2H, J = 15.20 Hz, OCH_2),

4.15-4.25 (m, 1H, CH-iGln), 4.25-4.40 (m, 1H, CH-Ala), 5.08 (s, 2H, CH_2-benzyl), 7.13 (s, 1H, NH), 7.22-7.45 (m, 6H, 5H-arom., NH), 7.60 (d, 1H, J = 7.03 Hz, NH), 7.89 (d, 1H, J = 7.82 Hz, NH-acetylamino), 8.26 (d, 1H, J = 8.11 Hz, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 18.3 (CH_3-Ala), 22.8 ($CO\underline{C}H_3$), 23.4 (C-4'/5'), 23.7 (C-4'/5'), 26.8 (C-βiGln), 29.9 (C-3'/6'), 30.0 (C-γiGln), 30.9 (C-3'/6'), 47.8 (C-αAla), 51.5 (C-αiGln), 51.7 (C-2'), 65.4 (CH_2-benzyl), 67.5 (C-2), 80.7 (C-1'), 127.8, 127.9, 128.3, (5CH-arom.), 136.1 (C-arom.), 169.0, 169.5, 172.0, 172.1, 172.8 (5 CO) ppm; Anal. calcd. for $C_{25}H_{36}N_4O_7$: C 59.51, H 7.19, N 11.10, found: C 59.42, H 7.13, N 11.04.

(1'S,2'S)-Benzyl-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-isoglutaminate 15b was prepared from (1'S,2'S)-2-(2'-acetylaminocyclohexyloxy)acetic acid **21b** (430 mg, 2 mmol) according to the procedure described above for the synthesis of **15a**; yield: 0.43 g (43 %); m.p. 199-202 °C; $[\alpha]_D^{20}$= +13.9 (c = 0.1, methanol); IR (KBr): 3410, 3353, 3277, 3180, 3075, 2946, 2859, 1729, 1655, 1555, 1452, 1369, 1271, 1174, 1101, 964, 918, 854, 750, 694, 612, 537 cm^{-1}; UV (CH_3OH): λ_{max}(log ε) = 202 (4.26) nm; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.05-1.35 (m, 4H, $4H_{ax}$), 1.24 (d, 1H, J = 6.89 Hz, CH_3-Ala), 1.55-1.65 (m , 2H, 4'-H_{eq}, 5'-H_{eq}) 1.70-1.85 (m, 2H, 3'/6'-H_{eq}, H-βiGln), 1.81 (s, 3H, CH_3CO), 1.95-2.10 (m, 2H, 3'/6'-H_{eq}, H-βiGln), 2.37 (t, 2H, J = 7.67 Hz, CH_2-γiGln), 3.05-3.18 (m, 1H, 1'-H), 3.50-3.65 (m, 1H, 2'-H), 3.93 (AB-system, 2H, J = 15.24 Hz, OCH_2), 4.15-4.25 (m, 1H, CH-iGln), 4.25-4.40 (m, 1H, CH-Ala), 5.08 (s, 2H, CH_2-benzyl), 7.13 (s, 1H, NH), 7.22-7.45 (m, 6H, 5H-arom, NH), 7.64 (d, 1H, J= 6.97 Hz, NH), 8.03 (d, 1H, J = 7.56 Hz, NH-acetylamino), 8.34 (d, 1H, J = 8.31 Hz, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 18.4 (CH_3-Ala), 22.8 ($CO\underline{C}H_3$), 23.3 (C-4'/5'), 23.7 (C-4'/5'), 26.8 (C-βiGln), 30.0 (C-3'/6'), 30.0 (C-γiGln), 30.8 (C-3'/6'), 47.9 (C-αAla), 51.6 (C-αiGln), 51.8 (C-2'), 65.4 (CH_2-benzyl), 67.7 (C-2), 80.7 (C-1'), 127.8, 127.9, 128.3 (5CH-arom.), 136.1 (C-arom.), 168.9, 169.4, 171.9, 172.1, 172.9 (5CO) ppm; Anal. calcd. for $C_{25}H_{36}N_4O_7$: C 59.51, H 7.19, N 11.10, found: C 59.22, H 7.25, N 10.67.

(1'R,2'R)-Dibenzyl-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-glutamate 16a and **(1'S,2'S)-Dibenzyl-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-glutamate 16b** *via* chromatographic separation of diastereomers: Dibenzyl-N-[*trans*-2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-glutamate as a mixture of diastereomers (**16**) was prepared from dibenzyl-L-alanyl-D-glutamate hydrochloride (869 mg, 2 mmol) and *rac-trans*-2-(2'-acetylaminocyclohexyloxy)acetic acid **10** (430 mg, 2 mmol) as described above for the synthesis of **16a**; yield: 1.036 g (87 %). Separation of the diastereomeric mixture into pure diastereomers **16a** (Rf = 0.59) and **16b** (Rf = 0.37) was accomplished by column chromatography on silica gel (100 g) with chloroform/methanol (20/1) as eluent.

16a: yield: 0.35 g; m.p. 104-107 °C; $[\alpha]_D^{20}$= -12.2 (c = 0.1, methanol); The spectroscopic data (IR, ^{1}H-NMR, ^{13}C-NMR, UV) were identical to the spectroscopic data of **16a** prepared from **21a** (see above). Anal. calcd. for $C_{32}H_{41}N_3O_8$: C 64.52, H 6.94, N 7.05, found: C 64.85, H 6.76, N 7.08.

16b: yield: 0.32 g, colourless viscous oil; $[\alpha]_D^{20}$= +17.1 (c = 0.1, methanol); The spectroscopic data (IR, ^{1}H-NMR, ^{13}C-NMR, UV) were identical to the spectroscopic data of **16b** prepared from **21b** (see above); MS calcd. 595.2894, found 595.2877; Anal. calcd. for $C_{32}H_{41}N_3O_8 \times H_2O$: C 62.62, H 7.06, N 6.85, found: C 62.97, H 7.13, N 7.17.

(1'R,2'R)-Benzyl-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-isoglutaminate 15a and **(1'S,2'S)-Benzyl-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-isoglutaminate 15b** *via* chromatographic separation of diastereomers: Benzyl-N-[*trans*-2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-isoglutaminate as a mixture of diastereomers (**15**) was prepared from benzyl-L-alanyl-D-isoglutaminate hydrochloride (687 mg, 2 mmol) and *rac-trans*-2-(2'-acetylaminocyclohexyloxy)acetic acid **10** (430 mg, 2 mmol) as described above for the synthesis of **15a**; yield: 0.454 g (45 %). Separation of the diastereomeric mixture into pure diastereomers **15a** (Rf = 0.31) and **15b** (Rf = 0.22) was performed by column chromatography on silica gel (120 g) using chloroform/methanol (9/1) as eluent.

15a: yield: 0.215 g; m.p. 169-172 °C; $[\alpha]_D^{20}$= -20.2 (c = 0.1, methanol); The spectroscopic data (IR, ^{1}H-NMR, ^{13}C-NMR, UV) were identical to the spectroscopic data of **15a** prepared from **21a** (see above); Anal. calcd. for $C_{25}H_{36}N_4O_7$: C 59.51, H 7.19, N 11.10, found: C 59.46, H 7.19, N 10.83.

15b: yield: 0.125 g; m.p. 196-198 °C; $[\alpha]_D^{20}$= +13.2 (c = 0.1, methanol); The spectroscopic data (IR, ^{1}H-NMR, ^{13}C-NMR, UV) were identical to the spectroscopic data of **15b** prepared from **21b** (see above); MS calcd. 504.2584, found 504.2582.

(1'R,2'R)-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-glutamic acid (2a): **16a**[49] (596 mg, 1 mmol) was dissolved in methanol (20 ml) and hydrogenated over 10% palladium on charcoal (90 mg) for 1h at room temperature and normal pressure. After filtration and evaporation of methanol *in vacuo* pure **2a** was obtained as a white amorphous solid foam; yield: 411 mg (99 %); $[\alpha]_D^{20}$ = -27.6 (c = 0.1, methanol); IR (KBr): 3340, 3096, 2939, 2900-2500 br, 1734, 1654, 1542, 1449, 1378, 1213, 1116, 969, 856, 668, 586 cm^{-1}; UV (CH_3OH): λ_{max}(log ϵ) = 202 (4.03) nm; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.05-1.25 (m, 4H, 4H_{ax}), 1.24 (d, 3H, J = 7.02 Hz, CH_3-Ala), 1.55-1.70 (m, 2H, 4'-H_{eq}, 5'-H_{eq}), 1.70-1.90 (m, 2H, 3'-H_{eq}, H-βGlu), 1.82 (s, 3H, $COCH_3$), 1.95-2.10 (m, 2H, 6'-H_{eq}, H-βGlu), 2.26 (t, 2H, J = 7.3 Hz, CH_2-γGlu), 3.10-3.20 (m, 1H, 1'-H), 3.50-3.65 (m, 1H, 2'-H), 3.91 (AB-system, 2H, J = 15.26 Hz, OCH_2), 4.18-4.30 (m, 1H, CH-Glu), 4.36-4.44 (m, 1H, CH-Ala), 7.54 (d, 1H, J = 7.78 Hz, NH-Ala), 7.91 (d, 1H, J = 7.75 Hz, NH-acetylamino), 8.35 (d, 1H, J = 7.93 Hz, NH-Glu), 12.4 (s br, 2H, 2COOH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 19.1 (CH_3-Ala), 22.9 ($CO\underline{C}H_3$), 23.5 (C-4'/5'), 23.8 (C-4'/5'), 26.4 (C-βGlu), 29.9 (C-γGlu), 29.9 (C-6'), 31.1 (C-3'), 47.3 (C-αAla), 51.1 (C-αGlu), 51.8 (C-2'), 67.4 (C-2), 80.5 (C-1'), 169.1 (CO-acetylamino), 169.2 (CO-acetyl), 172.1 (CO-Ala), 173.0 (COOH), 173.6 (δ-COOH) ppm; Anal. calcd. for $C_{18}H_{29}N_3O_8$: C 52.04, H 7.04, N 10.11; found: C 52.00, H 7.18, N 10.03.

(1'S,2'S)-N-[2-(2'-acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-glutamic acid (2b): 2b was prepared in analogy to 2a from (1'S,2'S)-dibenzyl-N-[2-(2'-acetylaminocyclohexyloxy)-acetyl]-L-alanyl-D-glutamate 16b[50] (596 mg, 1 mmol); yield: 407 mg (98%), white amorphous solid foam; $[\alpha]_D^{20}$ = +14.3 (c = 0.1, methanol); UV (CH_3OH): λ_{max}(log ϵ) = 201 (4.05) nm; IR (KBr): 3338, 3083, 2938, 2900-2300 broad, 1734, 1654, 1543, 1449, 1377, 1211, 1115, 969, 857, 668, 590 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.05-1.30 (m, 4H, $4H_{ax}$), 1.25 (d, 3H, J = 6.90 Hz, CH_3-Ala), 1.52-1.70 (m, 2H, 4'-H_{eq}, 5'-H_{eq}), 1.70-1.90 (m, 2H, 3'-H_{eq}, H-βGlu), 1.80 (s, 3H, $COCH_3$), 1.90-2.05 (m, 2H, 6'-H_{eq}, H-βGlu), 2.25 (t, 2H, J = 7.57 Hz, CH_2-γGlu), 3.08-3.18 (m, 1H, 1'-H), 3.48-3.60 (m, 1H, 2'-H), 3.93 (AB-system, 2H, J = 15.38 Hz, OCH_2), 4.18-4.27 (m, 1H, CH-Glu), 4.32-4.42 (m, 1H, CH-Ala), 7.53 (d, 1H, J = 7.76 Hz, NH-Ala), 7.92 (d, 1H, J = 7.57 Hz, NH-acetylamino), 8.33 (d, 1H, J = 7.87 Hz, NH-Glu), 12.4 (s br, 2H, 2COOH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 19.2 (CH_3-Ala), 22.9 ($CO\underline{C}H_3$), 23.5 (C-4'/5'), 23.8 (C-4'/5'), 26.4 (C-βGlu), 29.9 (C-γGlu), 30.1 (C-6'), 31.0 (C-3'), 47.5 (C-αAla), 51.0 (C-αGlu), 51.9 (C-2'), 67.7 (C-2), 80.7 (C-1'), 168.9 (CO-acetylamino), 169.2 (CO-acetyl), 171.9 (CO-Ala), 173.0 (COOH), 173.6 (δ-COOH) ppm; MS calcd. 415.1955, found 415.1953; Anal. calcd. for $C_{18}H_{29}N_3O_8$: C 52.04, H 7.04, N 10.11; found: C 52.19, H 7.23, N 9.79.

(1'R,2'R)-N-[2-(2'-Acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-isoglutamine (1a): 1a was prepared by hydrogenolysis of 15a[49] (505 mg, 1 mmol) as described above for the preparation of 2a; yield: 385 mg (93 %); m.p. 216-218 °C; $[\alpha]_D^{20}$ = -25.4 (c = 0.1, methanol); IR (KBr): 3432, 3390, 3287, 3084, 2938, 2861, 1731, 1647, 1557, 1441, 1369, 1271, 1176, 1125, 1036, 971, 935, 845, 578 cm^{-1}; UV (CH_3OH): λ_{max}(log ϵ) = 202 (4.11) nm; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.04-1.30 (m, 4H, $4H_{ax}$), 1.24 (d, 3H, J = 6.84 Hz, CH_3-Ala), 1.50-1.70 (m, 2H, 4'-H_{eq}, 5'-H_{eq}), 1.67-1.82 (m, 2H, 3'-H_{eq}, H-βiGln), 1.84 (s, 3H, CH_3CO), 1.90-2.08 (m, 2H, 6'-H_{eq}, H-βiGln), 2.23 (t, 2H, J = 7.57 Hz, CH_2-γiGln), 3.05-3.20 (m, 1H, 1'-H), 3.50-3.64 (m, 1H, 2'-H), 3.91 (AB-system, 2H, J = 15.16 Hz, OCH_2), 4.12-4.24 (m, 1H, CH-iGln), 4.25-4.40 (m, 1H, CH-Ala), 7.13 (s, 1H, NH_2), 7.35 (s, 1H, NH_2), 7.58 (d, 1H, J = 6.84 Hz, NH-Ala), 7.92 (d, 1H, J = 7.87 Hz, NH-acetylamino), 8.27 (d, 1H, J = 8.06 Hz, NH-iGln), 12.1 (s br, 1H, COOH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 18.3 (CH_3-Ala), 22.8 ($CO\underline{C}H_3$), 23.4 (C-4'/5'), 23.8 (C-4'/5'), 26.9 (C-βiGln), 29.9 (C-6'), 30.1 (C-γiGln), 31.0 (C-3'), 47.9 (C-αAla), 51.8 (C-2'), 67.4 (C-2), 80.7 (C-1'), 169.6 (CO-acetyl), 169.6 (CO-acetylamino), 172.1 (CO-Ala), 173.1 ($CONH_2$), 173.8 (COOH) ppm; MS calcd. 414.2115, found 414.2113; Anal. calcd. for $C_{18}H_{30}N_4O_7$: C 52.16, H 7.30, N 13.52, found: C 51.91, H 7.21, N 13.50.

(1'S,2'S)-N-[2-(2'-Acetylaminocyclohexyloxy)acetyl]-L-alanyl-D-isoglutamine (1b): 1b was prepared by hydrogenolysis of 15b[50] (505 mg, 1 mmol) as described above for the synthesis of 2a; yield: 385 mg (93 %) of white amorphous solid foam; $[\alpha]_D^{20}$ = +18.6 (c = 0.1, methanol); IR (KBr): 3393, 3095, 2938, 2862, 1731, 1654, 1546, 1449, 1377, 1247, 1117, 1037, 973, 938, 861, 577 cm^{-1}; UV (CH_3OH): λ_{max}(log ϵ) = 201 (4.06) nm; ^{1}H-NMR (300 MHz,

DMSO-d_6): δ = 1.04-1.30 (m, 4H, $4H_{ax}$), 1.24 (d, 3H, J = 6.84 Hz, CH_3-Ala), 1.50-1.70 (m, 2H, 4'-H_{eq}, 5'-H_{eq}), 1.67-1.82 (m, 2H, 3'-H_{eq}, H-βiGln), 1.81 (s, 3H, CH_3CO), 1.90-2.08 (m, 2H, 6'-H_{eq}, H-βiGln), 2.21 (t, 2H, J = 7.55 Hz, CH_2-γiGln), 3.05-3.20 (m, 1H, 1'-H), 3.50-3.62 (m, 1H, 2'-H), 3.92 (AB-system, 2H, J = 15.14 Hz, OCH_2), 4.12-4.24 (m, 1H, CH-iGln), 4.25-4.40 (m, 1H, CH-Ala), 7.11 (s, 1H, NH_2), 7.35 (s, 1H, NH_2), 7.58 (d, 1H, J = 6.89 Hz, NH-Ala), 7.89 (d, 1H, J = 7.81 Hz, NH-acetylamino), 8.20 (d, 1H, J = 8.30 Hz, NH-iGln), 12.1 (s br, 1H, COOH) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 18.6 (CH_3-Ala), 22.9 ($CO\underline{C}H_3$), 23.4 (C-4'/5'), 23.7 (C-4'/5'), 27.0 (C-βiGln), 30.0 (C-6'), 30.1 (C-γiGln), 30.9 (C-3'), 47.9 (C-αAla), 51.7 (C-2'), 67.7 (C-2), 80.7 (C-1'), 168.9 (CO-acetylamino), 169.4 (CO-acetyl), 171.9 (CO-Ala), 173.0 ($CONH_2$), 173.8 (COOH) ppm; MS calcd. 414.2115, found 414.2113; Anal. calcd. for $C_{18}H_{30}N_4O_7$ x $1.5H_2O$: C 48.97, H 7.53, N 12.69, found: C 48.83, H 7.38, N 12.78.

References and Notes

1. Devlin, J.P.; Hargrave, K.D. *Tetrahedron* **1989**, *45*, 4327-4369.
2. Ellouz, F.; Adam, A., Ciobaru, R., Lederer, E. *Biochem. Biophys. Res. Commun.* **1974**, *59*, 1317-1325.
3. Kotani, S.; Watanabe, Y.; Kinoshita, F.; Shimono, T.; Morisaki, I.; Shiba, T.; Kusumoto, S.; Tarumi, Y.; Ikenaka, K. *Biken J.* **1975**, *18*, 105-111.
4. Baschang, G. *Tetrahedron*, **1989**, *45*, 6331-6660.
5. Adam, A.; Lederer, E. *Med. Res. Rev.* **1984**, *4*, 111-152.
6. Lefrancier, P.; Lederer, E. *Pure Appl. Chem.* **1987**, *59*, 449-454.
7. Azuma, I.; Sugimura, K.; Yamawaki, M.; Uemiya, M.; Kusumoto, S.; Okada, S.; Shiba, T.; Yamamura, Y. *Infect. Immun.* **1978**, *20*, 600-607.
8. Shiba, T.; Okada, S.; Kusumoto, S.; Azuma, I.; Yamamura, Y. *Bull. Chem. Soc. Japan* **1978**, *51*, 3307-3311.
9. Uemiya, M.; Sugimura, K.; Kusama, T.; Saiki, I.; Yamawaki, M.; Azuma, I.; Yamamura, Y. *Infect. Immun.* **1979**, *24*, 83-89.
10. Inage, M.; Imoto, M.; Kambayashi, Y.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1980**, *21*, 3767-3770.
11. Okada, S.; Takeno, H.; Hemmi, K.; Kitaura, Y.; Hashimoto, M. *Chem. Pharm. Bull.* **1985**, *33*, 889-892.
12. Hemmi, K.; Takeno, H.; Okada, S.; Nakaguchi, O.; Kitaura, Y.; Hashimoto, M. *J. Am. Chem. Soc.* **1981**, *103*, 7026-7028.
13. Hemmi, K.; Takeno, H.; Okada, S.; Nakaguchi, O.; Kitaura, Y.; Hashimoto, M. *Tetrahedron Lett.* **1982**, *23*, 693-696.
14. Gotoh, T.; Nakahara, K.; Iwami, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1982**, *35* 1280-1285.

15. Migliore-Samour, D.; Bouchaudon, J.; Floc'h, F.; Zerial, A.; Ninet, L.; Werner, G.; Jolles, P. *Life Sci.* **1980**, *26*, 883-888.
16. Werner, G.H.; Floc'h, F.; Migliore-Samour, D.; Jolles, P. *Experientia* **1986**, *42*, 521-531.
17. Danklmaier, J.; Hönig, H. *Liebigs Ann. Chem.* **1990**, 145-150.
18. Hasegawa, A.; Okumura, H.; Kiso, M. *Gifu Daigaku Nogakubu Kenkyu Hokoku* **1979**, *42*, 169-175.
19. Azuma, I.; Okumura, H.; Saiki, I.; Kiso, M.; Hasegawa, A.; Tanio, Y.; Yamamura, Y. *Infect. Immun.* **1981**, *33*, 834-839.
20. Barton, D.H.R.; Camara, J.; Dalko, P.; Gero, S.D.; Quiclet-Sire, B.; Stütz, P. *J. Org. Chem.* **1989**, *54*, 3764-3766.
21. Gero, S.; Sire, B,; Dalko, P.; Philippe, M. *German Pat. Appl. DE 3634013* (6.10.1986).
22. Swift, G.; Swern, D. *J. Org. Chem.* **1967**, *32*, 511-517.
23. Gruevski, M.; Kidrič, J.; Kikelj, D.; Krbavčič, A.; Pečar, S.; Urleb, U. *Synth. Commun.* **1989**, *19*, 2665-2675.
24. Kikelj, D.; Krbavčič, A.; Pečar, S.; Tomažič, A. *Monatsh. Chem.* **1991**, *122*, 275-281.
25. Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis* Springer-Verlag, Berlin, Heidelberg, New York, Tokyo **1986**, pp. 231.
26. Winstein, S.; Boschan, R. *J. Am. Chem. Soc.* **1950**, *72*, 4669-4677.
27. Shiori, T.; Yamada, S.I. *Chem. Pharm. Bull.* **1974**, *22*, 849-854.
28. Shiori, T.; Yamada, S.I. *Chem. Pharm. Bull.* **1974**, *22*, 855-858.
29. Shiori, T.; Yamada, S.I. *Chem. Pharm. Bull.* **1974**, *22*, 859-863.
30. Shiori, T.; Ninomiya, K.; Yamada, S.I. *J. Am. Chem. Soc.* **1972**, *94*, 6203-6205.
31. Lefrancier, P.; Bricas, E. *Bull. Soc. Chem. Biol.* **1967**, *49*, 1257-1271.
32. Kusumoto, S.; Tarumi, Y.; Ikenaka, K.; Shiba, T. *Bull. Chem. Soc. Jap.* **1976**, *49*, 533-539.
33. Lefrancier, P.; Choay, J.; Derrien, M.; Lederman, I. *Int. J. Pept. Protein Res.* **1977**, *9*, 249-257.
34. Faber, K.; Hönig, H.; Seufer-Wasserthal, P. *Tetrahedron Lett.* **1988**, *29*, 1903-1905.
35. Bodenhausen, G.; Freeman, R.; Niedermeyer, R.; Turner, D.L. *J. Magn. Reson.* **1977**, *26*, 133-164.
36. Bachmann, P.; Aue, W.P.; Mueller, L.; Ernst, R.R. *J. Magn. Reson.* **1977**, *28*, 29-39.
37. Bax, A.; Morris, G.A.; *J. Magn. Reson.* **1981**, *42*, 501-505.
38. Bax, A. *J. Magn. Reson.* **1983**, *53*, 512-520.
39. Kessler, H.; Griesinger, C.; Zarbock, J.; Loosli, H.R. *J. Magn. Reson.* **1984**, *57*, 331-336.
40. Macura, S.; Huang, Y.; Suter, D.; Ernst, R.R. *J. Magn. Reson.* **1981**, *43*, 259-281.
41. States, J.D.; Haberkorn, R.A.; Ruben, D.J. *J. Magn. Reson.* **1982**, *48*, 286-292.
42. Pretsch, E.; Seibl, J.; Simon, W. *Tabellen zur Strukturaufklärung organischer Verbindungen mit spektroskopischen Methoden*, Springer-Verlag, Berlin, Heidelberg **1981**.

43. Since the resonances of 4a-H and 8a-H in **7a** and **7b** overlap, the C-H correlation was established in similar *rac-trans*-2-methyl-octahydro-2H-1,4-benzoxazine-3-one[23].
44. Danklmaier, J.; Hönig, H.; *Liebigs Ann. Chem.* **1988**, 1149-1153.
45. Bendall, M.R.; Pegg, D.T. *J. Magn. Reson.* **1983**, *53*, 272-296.
46. Due to a good solubility of **15a** and **15b** in the aqueous citric acid solution a reextraction of the aqueous phase was necessary.
47. $[\alpha]_D^{20}$ = +37.7 (c = 2, H_2O).
48. $[\alpha]_D^{20}$ = -34.5 (c = 2, H_2O).
49. Obtained from (1R,2R)-2-aminocyclohexanol.
50. Obtained from (1S,2S)-2-aminocyclohexanol.

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