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Generation of α-Acetoxyglycine Residues within Peptide Chains: A New Strategy for the Modification of Oligopeptides

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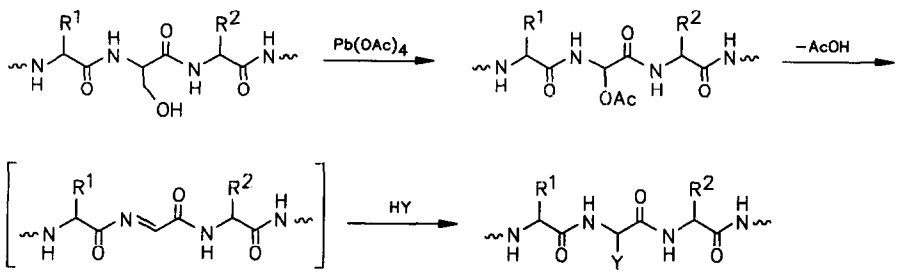
Dedicated to Professor Sir Derek H. R. Barton on the occasion of his 75th birthday

Abstract: Seryl and threonyl peptides are converted into α-acetoxyglycyl peptides by treatment with lead tetraacetate. Reaction of these acetoxy derivatives or the more reactive α-chloroglycyl peptides with thiols, dithiols and carbohydrates allows the attachment of such units to peptide chains. The reaction of α-chloroglycyl peptides with amino acid esters and enamines proceeds with high stereoselectivity and yields peptides with *N,N*-acetal and (2-oxocyclohexyl)glycine moieties, respectively.

Introduction

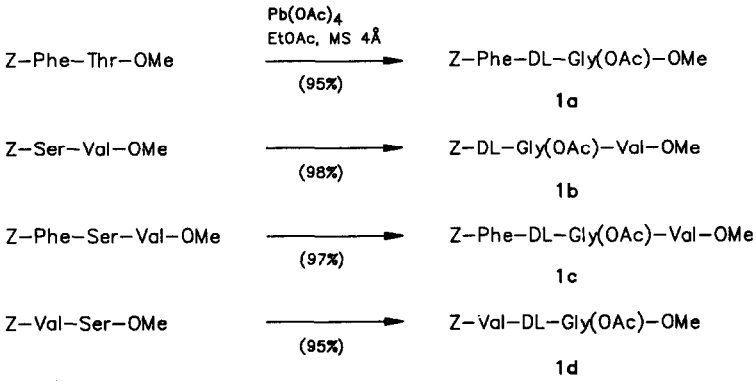
Peptides containing unnatural amino acids are usually prepared by stepwise procedures.[1] In contrast, methods based on the chemical modification of intact oligopeptides have only recently been investigated.[2] We have developed a general method for the introduction of electrophilic glycine equivalents into peptides based on the conversion of serine and threonine residues into α-acetoxyglycine derivatives.[3] The synthetic potential of this approach is indicated in Scheme 1. Elimination of acetic acid by treatment with tertiary amines leads to the *in situ* formation of dehydroglycine residues which readily react with nucleophiles[4] to yield peptides containing modified amino acids.



Scheme 1

Conversion of serine and threonine residues into electrophilic glycine equivalents

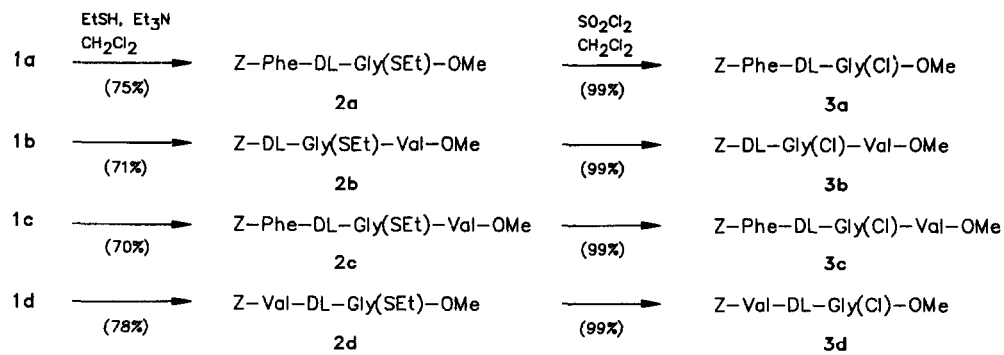
The broad range of our technique is illustrated by the smooth conversion of different types of seryl or threonyl peptides into the corresponding α-acetoxyglycine derivatives **1** by treatment with lead tetraacetate in the presence of molecular sieve 4 Å (Scheme 2). The products are obtained as 1:1 mixtures of diastereomers in yields greater than 90%. The mild reaction conditions permit these transformations to be carried out even in the presence of several oxidation sensitive amino acids, e.g. cystine, methionine and tryptophan. Only histidine and tyrosine require side chain protection.[5]



Scheme 2

In contrast, the generation of electrophilic glycine equivalents by direct bromination of oligopeptides is less regioselective and apt to side reactions due to the drastic reaction conditions.[6]

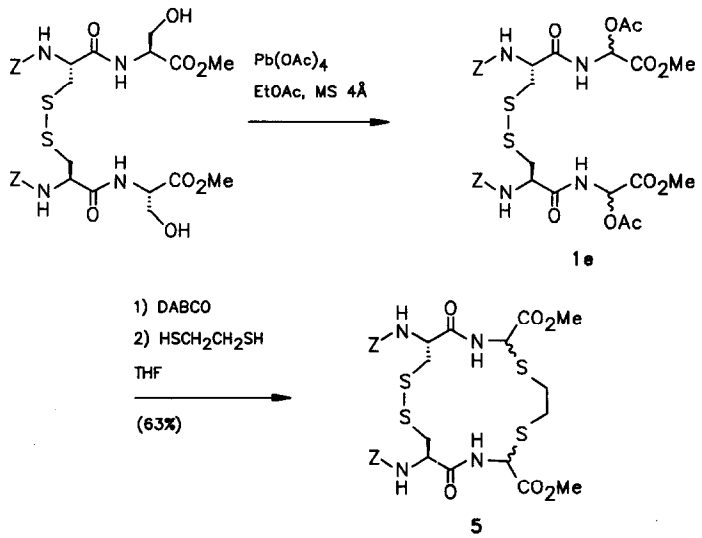
The α-acetoxyglycine derivatives **1** are suitable for reaction with powerful nucleophiles, e.g. thioles and organometallics. In the case of weaker nucleophiles it is advantageous to convert them into the more reactive chlorides **3**. This can easily be achieved by a two-step procedure. Treatment of the acetoxy compounds **1** with thiols provides the α-(alkylthio)glycine derivatives **2** which react with sulfuryl chloride at room temperature[7] to afford the corresponding chlorides **3** as diastereomeric mixtures in high yields (Scheme 3).



Scheme 3

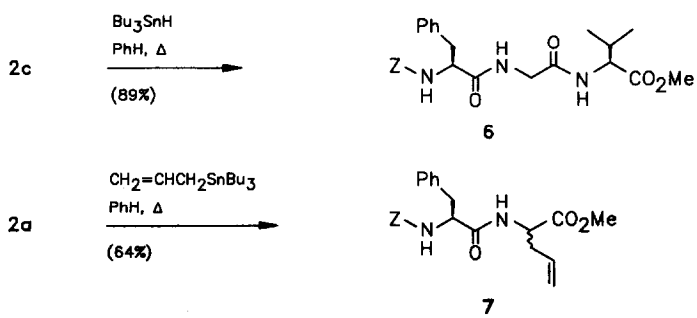
Reaction of α-acetoxy- and α-chloroglycyl peptides with nucleophiles

The reaction of α-acetoxypeptides with thiols allows an easy access to peptides containing α-(alkyl/arylthio)glycyl residues.[3] The potential of this method is demonstrated by the synthesis of a sulfur containing macrocycle as depicted in Scheme 4. Oxidation of the protected cystinylserine peptide **4** with $Pb(OAc)_4$ yields the bis-acetoxy compound **1e** which on treatment with ethane-1,2-dithiol in the presence of diazabicyclo[2.2.2]octane (DABCO) affords the macrocycle **5** in 63% yield as a diastereomeric mixture. The compound is characterized by its spectroscopic data and the FAB-MS spectrum. Structure **5** incorporates an 'inversed' cystine bridge $-SCH_2CH_2S-$ which is inert towards reduction. The synthesis of glutathione analogues with an inverted cystine bridge is being actively pursued.[5]



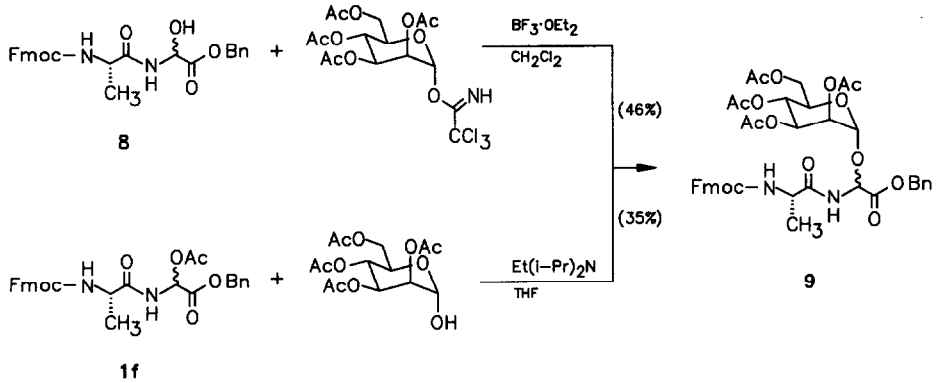
Scheme 4

On treatment of the α-(alkylthio)glycyl peptide **2c** with tri-*n*-butyltin hydride[8] the glycyl derivative **6** is formed in high yield (Scheme 5). This procedure allows the smooth transformation of seryl or threonyl residues in peptides into their glycine analogues. Alternatively, treatment of the α-(ethylthio)glycyl peptide **2a** with allyltri-*n*-butyltin yields the α-allylglycyl derivative **7** as a 1:1 mixture of diastereomers.[2d,9]



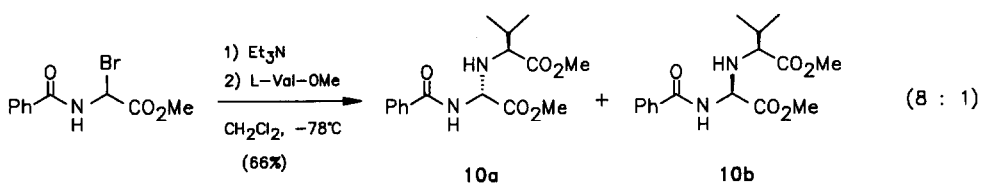
Scheme 5

The reaction of α-acetoxyglycyl peptides **1** with carbohydrates offers perspectives for the design of peptides possessing unique polarities. This is illustrated by the synthesis of lower homologues of *O*-glycosylserines[10] which can be achieved either by reaction of FMOC-Ala-DL-Gly(OH)-OBn (**8**) with the trichloroimidate of tetraacetyl-α-D-mannopyranoside[11] or treatment of FMOC-Ala-DL-Gly(OAc)-OBn (**1f**) with tetraacetyl-α-D-mannopyranose in the presence of Hünig's base (Scheme 6). In both cases a diastereomeric mixture of the α-mannosides **9** is obtained which can be separated by flash chromatography.



Scheme 6

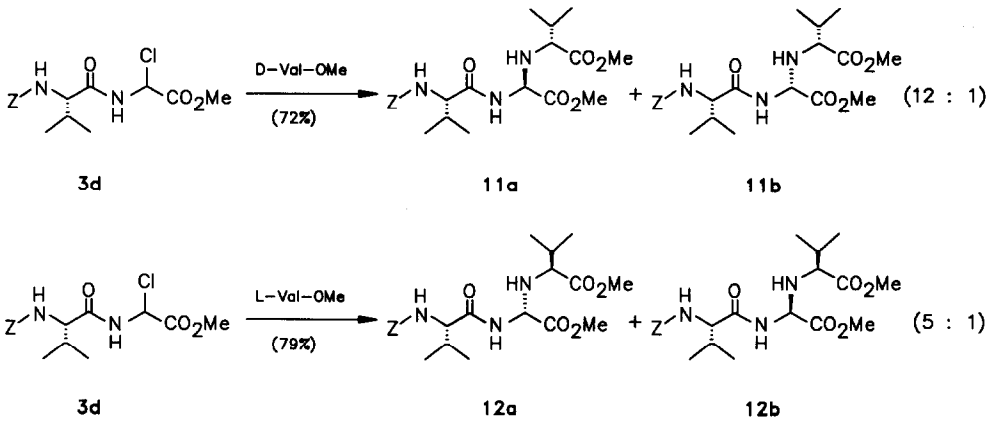
Whereas the examples described above proceed without significant stereoselectivity, the reactions of α-chloroglycyl peptides with amino acid esters exhibit remarkable stereochemical effects. This is disclosed even by simple 2-bromohippuric acid methyl ester which reacts with L-valine methyl ester and triethylamine to yield the adducts **10a** and **10b** with 78% d.e. (Scheme 7). The (*S,S*)-configuration of the main diastereomer **10a** was assigned by X-ray crystallography.[12]



Scheme 7

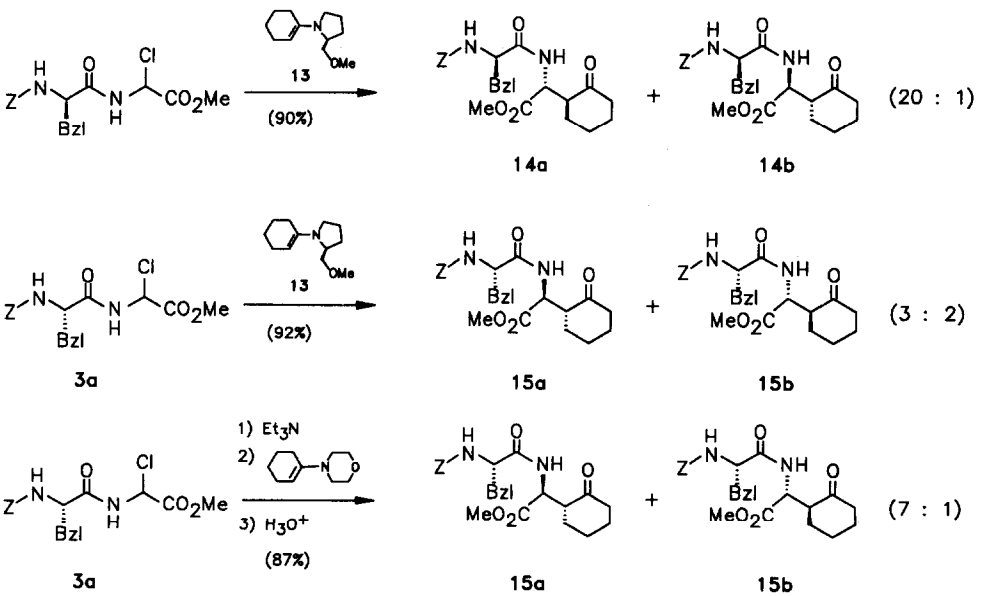
In the case of α-chloroglycyl peptides the stereochemical outcome of this reaction depends strongly on the configuration of the amino acid ester employed. Thus, the reaction of Z-Val-DL-Gly(Cl)-OMe (**3d**) with D-Val-OMe yields preferably diastereomer **11a** with 86% d.e. whereas with L-Val-OMe diastereomer **12a** is obtained with only 66% d.e. (Scheme 8). These observation can be rationalized as 'matched' and 'mismatched' cases using Masamune's terminology for double stereoselection.[13] The main diastereomers are easily purified

by flash chromatography and subsequent recrystallization. The compounds **11** and **12** represent a novel type of pseudopeptide in which a *N,N*-acetal unit functions as a connection between two peptide chains.



Scheme 8

Double stereoselection is also observed in the reaction of α-chloroglycyl peptides with chiral enamines (Scheme 9). Treatment of enamine **13** derived from (*S*)-prolinol methyl ether with Z-D-Phe-DL-Gly(Cl)-OMe yields the 1'*S*,2*R*,5*R*-derivative **14a** with 90% d.e. ('matched case'), whereas Z-L-Phe-DL-Gly(Cl)-OMe (**3a**) affords the 1'*R*,2*S*,5*S*-derivative **15a** with only 20% d.e. ('mismatched case'). In the reaction of **3a** with pro-



Scheme 9

chiral *N*-morpholinocyclohexene, diastereomer **15a** is obtained with 75% d.e. The exclusive formation of the *anti*-diastereomers is in accord with a Diels-Alder-like transition state as was proposed for the analogous reactions of enamines with α-bromohippuric acid esters.[14] The stereochemistry of **15a** was assigned by a X-ray structural analysis.[12]

Acknowledgement

We are indebted to the Deutsche Forschungsgemeinschaft for financial support of this research. S. J. thanks the Fonds der Chemischen Industrie and M. J. the Studienstiftung des Deutschen Volkes for fellowships. M. K. is grateful to the Fonds zur Förderung des wissenschaftlichen Forschung for an Erwin-Schrödinger-Stipendium.

Experimental

General

Melting points were determined with a Büchi melting-point apparatus and are uncorrected. Infrared spectra were obtained using a Bruker IFS 45 spectrometer. NMR spectra were recorded with Bruker AW 80 and Varian VXR 400 S instruments (solutions in $CDCl_3$ unless otherwise stated, Me_4Si as internal reference). Optical rotations were measured with a Perkin Elmer 241 polarimeter. TLC separations were carried out on silica gel TLC plates Merck 60 F_{254}. Flash chromatography was performed according to lit.[15] on Merck silica gel (article no. 9385). Organic solutions were dried over anhydrous $MgSO_4$ and solvent evaporation was carried out at reduced pressure using a rotatory evaporator. Mass spectra were obtained at 70 eV using a Finigan MAT 90 spectrometer equipped with a data system. Elemental analyses were performed at the Institut für Organische Chemie, Universität München.

General procedure for the synthesis of α-acetoxyglycyl peptides **1**

To a stirred solution of the seryl(threonyl) peptide (10 mmol) in dry EtOAc (50 ml) were added molecular sieve 4Å (5 g) and $Pb(OAc)_4$ (0.66 g, 15 mmol) under an argon atmosphere. The reaction mixture was heated under reflux for 2 h and cooled to rt. After filtration through Celite, the organic layer was stirred with aqueous 20% citric acid (100ml) until the solution was nearly colourless. The organic layer was separated, washed with brine, dried and evaporated. The crude products can be directly used for the next steps.

N-Benzyloxycarbonyl-L-phenylalanyl-DL-α-acetoxyglycine methyl ester: (**1a**)*:* From Z-Phe-Thr-OMe, yield 95%; ^{1}H NMR (400 MHz): δ= 2.07 (s, 3H), 3.07-3.12 (m, 2H), 3.74 (s, 3H), 4.50-4.60 (br.m, 1H), 5.07 (s, 2H), 5.41 (br.m, NH), 6.36, 6.38 (2d, *J*=8.9Hz, 1H), 7.15-7.36 (m, 10H), 7.45 (br.m, 1H); $C_{22}H_{24}N_2O_7$ (428.44).

N-Benzyloxycarbonyl-DL-α-acetoxyglycyl-L-valine methyl ester (**1b**)*:* From Z-Ser-Val-OMe, yield 96%; ^{1}H NMR (400 MHz): δ= 0.89-0.97 (m, 6H), 2.09 (s, 3H), 2.15-2.41 (m, 1H), 3.74, 3.75 (2s, 3H), 4.48-4.56 (m, 1H), 5.11 (s, 2H), 5.54 (d, *J*=8.7Hz, NH), 7.29-7.37 (m, 6H); $C_{18}H_{24}N_2O_7$ (380.40).

N-Benzyloxycarbonyl-L-phenylalanyl-DL-α-acetoxyglycyl-L-valine methyl ester (**1c**)*:* From Z-Phe-Ser-Val-OMe, yield 91%; ^{1}H NMR (400 MHz): δ= 0.92-1.00 (m, 6H), 2.08 (s, 3H), 2.14-2.21 (m, 1H), 3.08-3.13 (AB-system, 2H), 3.72 (s, 3H), 4.43-4.58 (m, 2H), 5.07 (s, 2H), 5.36 (br.d, *J*=7.3Hz, NH), 6.39, 6.41 (2d, *J*=8.5Hz, 1H), 6.83 (d, *J*=9.3Hz, NH), 7.13-7.38 (m, 11H); $C_{27}H_{33}N_3O_8$ (527.57).

N-Benzyloxycarbonyl-L-valyl-DL-α-acetoxyglycine methyl ester (**1d**)*:* From Z-Val-Ser-OMe, yield 95%; ^{1}H NMR (400 MHz): δ= 0.91-1.00 (m, 6H), 2.07-2.17 (m, 1H), 2.09 (s, 3H), 3.78 (s, 3H), 4.20 (br.t, *J*=7.4Hz, 1H), 5.08-5.12 (AB-system, 2H), 5.48 (t, *J*=8.4Hz, NH), 6.40, 6.42 (2d, *J*=7.8Hz, 1H), 7.26-7.38 (m, 5H), 7.59 (br.t, *J*=9.4Hz, NH); $C_{18}H_{24}N_2O_7$ (380.40).

N,N'-Bisbenzyloxylcarbonyl-L-cystinyl-DL-α,α'-bisacetoxydiglycine dimethyl ester (**1e**)*:* From $(Z)_2$=$(Cys)_2$=$(Ser\text{-}OMe)_2$, yield 65%, ^{1}H-NMR (80 MHz): δ= 2.08, 2.11 (each s, 3H), 2.75-3.20 (br.m, 4H), 3.70, 3.75 (each s, 3H), 5.00-5.20 (m, 2H), 5.10, 5.20 (each s, 2H), 5.85 (br.d, 2NH), 6.40, 6.42 (2d, *J*=8.5Hz, 2H), 7.35, 7.37 (each s, 5H), 8.90

(br.m, 2NH); ^{13}C-NMR (20.12 MHz) δ= 19.7, 24.3, 52.3, 66.6, 71.5, 127.4, 127.8, 135.8, 156.2, 166.2, 169.1; $C_{32}H_{38}N_4O_{14}S_2$ (766.79).

General procedure for the synthesis of α-(alkylthio)glycyl peptides **2**

To a stirred mixture of α-acetoxyglycyl peptide **1** (10 mmol) and thiol (10 mmol) in dry CH_2Cl_2 at 0°C was added NEt_3 or DABCO (20 mmol) via syringe under an argon atmosphere. After stirring at rt for 15 h, the solution was diluted with *tert*-butyl methyl ether and subsequently washed with 1 N HCl, satd. aqueous $NaHCO_3$ solution and brine. Drying and evaporation of the solvent afforded the α-(alkylthio)glycyl peptides **2** as colourless solids that were recrystallized from EtOAc/petroleum ether (40/60).

N-Benzyloxycarbonyl-L-phenylalanyl-DL-α-(ethylthio)glycine methyl ester (**2a**): Yield 75%; mp 136-138°C; ^{1}H NMR (80 MHz): δ= 1.2 (t, *J*=7.2Hz, 3H), 2.6 (q, *J*=7.2Hz, 2H), 3.7 (s, 3H), 4.4-4.7 (br.m, 1H), 5.0 (s, 2H), 5.4-5.7 (m, 2H), 7.0-7.3 (m, 11H); $C_{22}H_{26}O_5N_2S$ (430.52).

N-Benzyloxycarbonyl-DL-α-(ethylthio)glycyl-L-valine methyl ester (**2b**): Yield 78%, mp 138-141°C; ^{1}H NMR (400 MHz): δ= 0.90-0.97 (m, 6H), 1.23-1.28 (m, 3H), 2.16-2.22 (m, 1H), 2.60-2.73 (m, 2H), 3.74,3.75 (2s, 3H), 4.49-4.55 (m, 1H), 5.11-5.17 (AB-systeme, 2H), 5.45 (dd, *J*=13.7 and 6.9Hz, 1H), 6.04 (2d, *J*=7.4Hz, NH), 7.30-7.36 (s, 5H); FAB MS: 383 (16) [MH$^+$]; $C_{18}H_{26}O_5N_2S$ (382.48): calcd. C 56.53, H 6.85, N 7.32, S 8.38; found C 56.63, H 6.94, N 7.41, S 8.39.

N-Benzyloxycarbonyl-L-phenylalanyl-DL-α-(ethylthio)glycyl-L-valine methyl ester (**2c**): Yield 73%; mp 140-142°C; ^{1}H NMR (400 MHz): δ= 0.90, 0.96 (each d, *J*=6.9Hz, 3H), 1.19 (t, *J*= 7.5Hz, 3H), 2.17-2.21 (m, 1H), 2.50-2.57 (m, 2H), 3.08-3.12 (AB-system, 2H), 3.74 (s, 3H), 4.47, 4.49 (2d, *J*=4.8Hz, 1H), 4.52-4.56 (m, 1H), 5.08 (s, 2H), 5.36 (br.d, *J*=7.0Hz, NH), 5.53, 5.56 (2d, *J*=7.5Hz, 1H), 6.84 (d, *J*=9.5Hz, NH), 7.15-7.37 (m, 10H); FAB MS 530 (11) [MH$^+$]; $C_{27}H_{35}O_6N_3S$ (529.65): calcd. C 61.23, H 6.66, N 7.93, S 6.05; found C 61.41, H 6.70, N 7.93, S 6.04.

N-Benzyloxycarbonyl-L-valyl-DL-α-(ethylthio)glycine methyl ester (**2d**): Yield 78%; mp 138-141°C; IR (KBr): 3292, 2961, 1744, 1691, 1651, 1537, 1454, 1249 cm^{-1}; ^{1}H NMR (80 MHz): δ= 0.85-1.1 (m, 6H), 1.25 (t, *J*=7Hz, 3H), 1.9-2.3 (m, 1H), 2.65 (q, *J*=7Hz, 2H), 3.75 (s, 3H), 3.95-4.2 (m, 1H), 5.1 (s, 2H), 5.3 (br.d, *J*=8Hz, 1H), 5.5 (d, *J*=8Hz, 1H), 6.8 (br.d, *J*=8Hz, 1H), 7.35 (s, 5H); FAB MS: 383 (7) [MH$^+$]; $C_{18}H_{26}O_5N_2S$ (382.48): calcd. C 56.25, H 6.85, N 7.32, S 8.38; found C 56.62, H 6.85, N 7.34, S 8.40.

(4R,15R)-4,15-Bisbenzyloxycarbonylamino-7,12-bismethoxycarbonyl-1,2,8,11-tetrathia-6,13-diaza-5,14-cyclohexadecadione (**5**): From $(Z)_2$=$(Cys)_2$=$(Gly(OAc)\text{-}OMe)_2$ (770 mg, 1 mmol), DABCO (225 mg, 2 mmol), 1,2-ethanethiol (0.08 ml, 0.96 mmol) in THF: Yield 450 mg (63%) colourless oil; ^{1}H NMR (90 MHz) δ= 2.80-3.20 (br.m, 8H), 3.70, 3.72 (each s, 3H), 4.50 (br.m, 2H), 5.06 (br.s, 4H), 5.55 (br.m, 2H), 7.30 (s, 10H); FAB MS 741 (9) [MH$^+$]; $C_{30}H_{36}O_{10}N_4S_4$ (740.88).

General procedure for the synthesis of α-chloroglycyl peptides **3**

To a solution of α-(ethylthio)glycyl peptide **2** (1 mmol) in CH_2Cl_2 (20 ml) was added SO_2Cl_2 in CH_2Cl_2 (1 M, 1.1 ml). After stirring for 15 min the volatile compounds were removed in vacuo. The chloro compounds **3** remained as colourless solids or foams which were used for the next transformations without further purification.

N-Benzyloxycarbonyl-L-phenylalanyl-DL-α-chloroglycine methyl ester (**3a**): Yield 99%; ^{1}H NMR (80 MHz): δ= 3.0 (d, *J*=7Hz, 2H), 3.75 (s, 3H), 4.4-4.65 (br.m, 1H), 5.0 (s, 2H), 5.4-5.65 (m, NH), 6.15, 6.2 (d, *J*=9Hz, 1H), 7.1, 7.2 (each s, 10H), 7.45-7.65 (m, NH); $C_{20}H_{21}N_2O_5Cl$ (404.85).

N-Benzyloxycarbonyl-DL-α-chloroglycyl-L-valine methyl ester (**3b**): Yield ~100%; ^{1}H NMR (80 MHz): δ= 0.95-1.1 (m, 6H), 2.0-2.2 (m, 1H), 3.84 (s, 3H), 4.15-4.3 (m, 1H), 5.1 (s, 2H), 5.4, 5.5 (2d, *J*=9Hz, NH), 6.3, 6.35 (2d, *J*=9Hz, 1H), 7.3-7.4 (m, 5H), 7.65-7.7 (br.m, NH); $C_{16}H_{21}N_2O_5Cl$ (356.806).

N-Benzyloxycarbonyl-L-phenylalanyl-DL-α-chloroglycyl-L-valine methyl ester (**3c**): Yield 97%; ^{1}H NMR (80 MHz): δ= 0.9-1.0 (m, 6H), 2.0-2.15 (m, 1H), 3.1 (d, *J*=7Hz, 2H), 3.7 (s, 3H), 4.14-4.37 (m, 2H), 5.1 (s, 2H), 5.4-5.6 (m, NH), 6.2, 6.3 (2d, *J*=8Hz, 1H), 6.7-6.95 (m, NH), 7.1-7.4 (m, 11H); $C_{25}H_{30}N_3O_6Cl$ (503.98).

N-Benzyloxycarbonyl-L-valyl-DL-α-chloroglycine methyl ester (**3d**): Yield ~100%; ^{1}H NMR (400 MHz): δ= 0.91-1.01 (m, 6H), 2.08-2.20 (m, 1H), 3.84 and 3.85 (s, 3H), 4.22 (m, 1H), 5.07-5.16 (m, 2H), 5.44 and 5.49 (2 br.d, *J*=9Hz, 1H), 6.28 and 6.30 (2 d, *J*=9Hz, 1H), 7.32-7.35 (m, 5H), 7.60-7.62 (m, br., 1H); ^{13}C NMR (100.6 MHz): δ= 17.5, 17.8; 19.0, 19.1; 30.8, 31.3; 53.7, 53.7, 59.6; 59.9, 60.0; 67.3, 67.4; 128.1, 128.2, 128.2, 128.5, 128.5; 136.0; 156.4; 166.6, 166.7; 171.1; $C_{16}H_{21}N_2O_5Cl$ (356.806).

General procedure for the reaction of α-(ethylthio)glycyl peptides **2** *with tri-n-butyltin derivatives*

A mixture of α-(ethylthio)glycyl peptide **2** (1 mmol) with tri-*n*-butyltin hydride or allyltri-*n*-butyltin (1.1 mmol) and AIBN (0.1 mmol) was refluxed in benzene (25ml) for 1 h. After removal of the solvent the crude products were purified by flash chromatography on silica gel [eluant: EtOAc/petroleum ether (40/60) = 1:1 (**6**) or 1:2 (**7**)].

N-Benzyloxycarbonyl-L-phenylalanyl-glycyl-L-valine methyl ester (**6**): From **2c** and tri-*n*-butyltin hydride, yield 85%; mp 131-132°C; IR (KBr): 3317, 1740, 1707, 1653, 1526, 1259, 1215 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ= 0.90, 0.93 (each d, *J*=6.9Hz, 6H), 2.10-2.19 (m, 1H), 2.98-3.15 (AB-system, 2H), 3.68 (s, 3H), 3.80-4.09 (AB-system, 2H), 4.44-4.52 (m, 2H), 5.00-5.08 (AB-system, 2H), 5.63 (d, *J*=7.8Hz, NH) 6.99-7.34 (m, 12H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ= 17.9, 18.9, 31.1, 38.3, 43.2, 52.1, 56.4, 57.4, 67.1, 127.1, 128.0, 128.2, 128.5, 128.7, 129.2, 136.1, 136.4, 156.1, 168.6, 171.8, 172.3; FAB MS 470 (51) [MH^+]; $C_{25}H_{31}N_3O_6$ (370.41): calcd. C 63.95, H 6.65, N 8.95; found C 64.22, H 6.65, N 8.73.

N-Benzyloxycarbonyl-L-phenylalanyl-DL-allylglycine methyl ester (**7**): From **2a** and allyltri-*n*-butyltin, yield 62%; mp 119-120°C; IR (KBr): 3428, 3309, 1734, 1687, 1655, 1536, 1288, 1257 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ= 2.36-2.53 (m, 2H), 3.70,3.71 (each s, 3H), 4.45 (br.q, *J*=6.8Hz,1H), 4.57, 4.59 (t, *J*=5.8Hz, 1H),4.92-5.04 (m, 2H), 5.08, 5.09 (s, 2H), 5.33 (br.s, NH), 5.39-5.58 (m, 1H), 6.28 (br.t, *J*=6.8Hz, NH), 7.18-7.37 (m, 10H); FAB MS 411 (25) [MH^+]; $C_{23}H_{26}N_2O_5$ (410.47).

N-9-Fluorenylmethyloxycarbonyl-L-alanyl-L-serine benzyl ester: Diisopropylethylamine (0.90 ml, 5 mmol) was added dropwise to a solution of Fmoc-Ala-OTDO[16] (2.37 g, 4 mmol) and L-serine benzyl ester *p*-toluenesulfonate (1.47 g, 4 mmol) in dry CH_2Cl_2 (40 ml). After stirring for 60 min the solution was diluted to 200 ml with CH_2Cl_2 and extracted three times with 20% aqueous citric acid, five times with aqueous $NaHCO_3$ and again with citric acid. The organic layer was washed with water and dried. After removal of the solvent the product was recrystallized from EtOAc/petroleum ether. Yield 85%, mp 193-195°C; ^1H NMR. (400 MHz, [D_6]DMSO): δ= 1.23 (d, *J*=7.0Hz, 3H), 3.64 (ddd, *J*=10.9, 5.4 and 5Hz, 1H), 3.74 (ddd, *J*=10.9, 5.4 and 5Hz, 1H), 4.15-4.30 (m, 4H), 4.41 (dt, *J*=7.8 and 5 Hz, 1H), 5.11 (t, *J*=5.4 Hz, 1H), 5.13 (s, 2H), 7.28-7.47 (m, 9H), 5.56 (d, *J*=7.8Hz, 1H), 7.73 (t, *J*=7Hz, 2H), 7.89 (d, *J*=7.6Hz, 2H), 8.15 (d, *J*=8.1Hz, 1H); $C_{28}H_{27}N_2O_6$ (487.53).

N-9-Fluorenylmethyloxycarbonyl-L-alanyl-DL-hydroxyglycine benzyl ester (**8**): To a stirred solution of *N*-9-fluorenylmethyloxycarbonyl-L-alanyl-L-serine benzyl ester (0.98 g, 2 mmol) in dry EtOAc (50 ml) was added 4g of molecular sieve 4 Å and $Pb(OAc)_4$ (1.33 g, 3 mmol) and the mixture was heated under reflux for 2 h under an argon atmosphere. After cooling and filtration through Celite, the organic layer was stirred with 20% aqueous citric acid (15 ml) for 15 min. The organic layer was separated, washed with brine, dried and evaporated. The residue was dissolved in acetone (200ml) and stirred with dilute aqueous $NaHCO_3$ (100 ml) for 30 min. The solution was extracted with EtOAc and the organic layer was dried. Removal of the solvent and recrystallization from CH_2Cl_2/petroleum ether lead to a 1:1 mixture of the D- and L-isomers. Yield 51%, mp 183°C; IR (KBr): 3312, 3066, 2950, 1742, 1689, 1664, 1532, 1478, 1451, 1410, 1377, 1313, 1259, 1103, 1089, 758, 741, 698, 621 cm^{-1}; ^1H-NMR (400 MHz, [D_6]DMSO): δ= 1.20, 1.23 (2d, *J*=7Hz, 3H), 4.13 (dt, *J*=7.3 and 7.3Hz, 1H), 4.19-4.31 (m, 3H), 5.11-5.18 (m, 2H), 5.55 (dd, *J*=8.4 and 6.7Hz, 1H), 6.67, 6.74 (2d, *J*=6.6Hz, 1H), 7.32-7.44 (m, 9H), 7.53, 7.54 (2d, *J*=7.9Hz, 1H), 7.75 -7.89(m, 4H), 8.78, 8.80 (2d, *J*=8.4Hz, 1H); ^{13}C-NMR (100.6 MHz, [D_6]DMSO): δ= 18.5, 47.1, 50.2, 50.3, 66.1, 66.5, 71.8, 71.9, 113.3, 120.5, 125.7, 127.5, 128.1, 128.3, 128.5, 128.8, 136.2, 141.2, 144.2, 144.4, 156.1, 170.0, 170.1, 173.0; $C_{27}H_{26}N_2O_6$ (474.52): calc. C 68.34, H 5.52, N 5.90; found C 68.61, H 5.76, N 5.81.

N-9-Fluorenylmethoxycarbonyl-L-alanyl-DL-α-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyloxy)glycine methyl ester (**9**):

Method A: A solution of **8** (0.95 g, 2 mmol) and *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-trichloracetimidate (0.99 g, 2 mmol) in dry CH_2Cl_2 (30 ml) was stirred with a catalytic quantity of BF_3 x Et_2O for 5 h under an argon atmosphere. The reaction mixture was diluted with CH_2Cl_2 to 100 ml and extracted with aqueous $NaHCO_3$. The organic layer was dried and evaporated. Flash chromatography ($CHCl_3$/acetone 10 :1) afforded one diastereomer of **9** as a colourless foam.

Method B: To a stirred, cooled (-78°C) solution of *N*-9-fluorenylmethoxycarbonyl-L-alanyl-DL-acetoxyglycine benzyl ester (**1f**) (1.03 g, 2 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (0.70 g, 2 mmol) in dry THF (40 ml) was added diisopropylethylamine (0.70 ml, 4 mmol) in THF via syringe under an argon atmosphere. After 2 h of stirring at -78°C the mixture was allowed to warm to rt and after 48 h it was acidified with 20 % aqueous citric acid. Extractive workup with EtOAc, drying of the organic layer and removal of the solvent gave a crude product which was chromatographed as in method A. Yield 46% (method A), 35% (method B), colourless foam; IR (KBr): 3421, 1753, 1699, 1519, 1451, 1369, 1230, 1079, 1055, 760, 742, 698 cm^{-1}; ^1H-NMR (400 MHz): δ= 1.39 (d, *J*=6.6Hz, 3H), 1.96 (s, 3H), 2.03 (s,3H), 2.07 (s,6H), 3.63 (m, 1H), 4.15 (dd, *J*=12.4 and 2.5Hz, 1H), 4.21 (t, *J*=6.6Hz, 1H), 4.26 (m,1H),

4.32 (dd, *J*=12.4 and 5.6Hz, 1H), 4.43 (d, *J*=6.6Hz, 2H), 4.92 (d, *J*=1.3Hz, 1H), 5.05-5.34 (m, 5H), 5.42 (dd, *J*=2.5 and 1.3Hz, 1H), 6.03 (d, *J*=9.5Hz, 1H), 7.26-7.76 (m,14H) ppm; ^{13}C-NMR (100.6 MHz): δ= 18.1, 20.5, 20.68, 20.7, 20.8, 47.1, 51.0, 62.3, 66.1, 67.2, 67.8, 68.7, 71.0, 72.9, 74.9, 95.5, 120.0 (2C), 125.02, 125.04, 127.08, 127.13, 127.7 (2C), 128.2 (2C), 128.58, 128.61 (2C), 134.8, 141.3, 141.4, 143.7, 143.8, 156.1, 166.6, 169.7, 169.8, 170.3, 170.9, 173.4; FAB MS 805.2 (15) [MH$^+$]; $C_{41}H_{44}N_2O_{15}$ x 0.5 H_2O (813.81): calc. C 60.51, H 5.46; found C 60.33, H 5.57.

N-Benzoyl-α-[(1S)-1-(methoxycarbonyl)-2-methylpropyl-amino]glycine methyl ester (**10a/10b**): To methyl 2-benzoylamino-2-bromoacetate (272 mg, 1 mmol) in dry CH_2Cl_2 (10 ml) was added NEt_3 (0.15 ml, 1.1 mmol) at -78°C. After 0.5 h a solution of L-Val-OMe x HCl (168 mg, 1 mmol) and NEt_3 (0.15 ml) in CH_2Cl_2 (10 ml) was added. During 15 h of stirring the solution reached rt and was diluted with *tert*-butyl methyl ether. It was washed twice with water and brine and dried. The diastereomeric ratio (8:1) was determined by 400 MHz ^{1}H NMR spectroscopy. The residue was purified by flash column chromatography on silica gel using petroleum ether/EtOAc (2:1). (*S*)-**10a**: Yield 60 mg (66%), mp 127-128°C; $[\alpha]_D^{25}$ = +19.1 (*c* = 0.8 in $CHCl_3$); IR (KBr): 3355, 2963, 1736, 1648, 1581, 1543; ^{1}H NMR (400 MHz): δ= 0.90 (d, *J*=7Hz, 3H), 0.96 (d, *J*=7Hz, 3H), 1.98 (m, 1H), 2.60 (br.t., *J*=7Hz, 1H), 3.29 (dd, *J*=7, *J*=5Hz, 1H), 3.50 (s, 3H), 3.80 (s, 3H), 5.51 (t, *J*=9Hz, 1H), 6.77 (d, *J*=9Hz, 1H), 7.40-7.55 (m, 3H), 7.74-7.79 (m, 2H), - FAB MS: 323 (18) [MH$^+$]; $C_{16}H_{22}N_2O_5$ (322.361): calcd. C 59.62 H 6.88 N 8.69; found C 59.85 H 7.01 N 8.60.

N-Benzyloxycarbonyl-(S)-valyl-α-[(1R)-1-(methoxycarbonyl)-2-methylpropyl-amino]glycine methyl ester (**11a/11b**): α-Chloropeptide **3d** (356 mg, 1 mmol) was treated with D-Val-OMe (168 mg, 1 mmol) as described for compounds **10**. Diastereomeric ratio: 12:1. (*R*)-**11a**: Yield 323 mg (72%), colourless solid, mp 108-109°C; IR (KBr): 3325, 2962, 1740, 1691, 1656, 1534, 1388, 1370 cm^{-1}; ^{1}H NMR (400 MHz):δ= 0.86, 0.89, 0.92, 0.93 (d, *J*=7Hz, 12H), 1.94 (m, 1H), 2.06 (m, 1H), 2.58 (br., 1H), 3.20 (d, *J*=5Hz, 1H), 3.61 (s, 3H), 3.75 (s, 3H), 4.07 (dd, *J*=8Hz, *J*=6Hz, 1H), 5.10 (s, 2H), 5.30 (d, J=8Hz, 1H), 5.54 (d, *J*=8Hz, 1H), 6.92 (br.d, *J*=8Hz, 1H), 7.28-7.35 (m, 5H); FAB MS: 452 (5) [MH$^+$]; $C_{22}H_{33}N_3O_7$ (451.520): calcd. C 58.52 H 7.37 N 9.31; found C 58.83 H 7.40 N 9.36.

N-Benzyloxycarbonyl-(S)-valyl-α-[(1S)-1-(methoxycarbonyl)-2-methylpropyl-amino]glycine methyl ester (**12a/12b**): α-Chloropeptide **3d** (356 mg, 1 mmol) was treated with L-Val-OMe (168 mg, 1 mmol) as described for compounds **10**. Diastereomeric ratio: 5:1. Yield 358 mg (79%) mixture of diastereomers, 140 mg (31%) pure diastereomer (*S*)-**12a** as a colourless solid, mp 143-144°C; IR (KBr): 3318, 2961, 1745, 1737, 1693, 1649, 1535, 1380, 1370 cm^{-1}; ^{1}H NMR (400 MHz): δ= 0.83, 0.85 (d, *J*=7Hz, 6H), 0.95 (d, *J*=7Hz, 6H), 2.02 (m, 1H), 2.16 (m, 1H), 2.48 (br.s, 1H), 3.22 (s, br., 1H), 3.68 (s, 3H), 3.75 (s, 3H), 4.07 (dd, *J*=9Hz, *J*=5Hz, 1H), 5.07-5.14 (AB, 2H), 5.15-5.26 (m, 2H), 6.62 (br.d, *J*=8Hz, 1H), 7.28-7.37 (m, 5H); $C_{22}H_{33}N_3O_7$ (451.520): calcd. C 58.52 H 7.37 N 9.31; found C 58.63 H 7.33 N 9.22.

General procedure for the reaction of α-chloroglycyl peptides **3** *with cyclic enamines*

To a solution of α-chloroglycyl derivative **3** (1 mmol) in dry THF (15ml) was added NEt_3 (0.07ml, 1 mmol) with stirring at -78°C under an argon atmosphere. The stirring was continued for 30 min and after cooling the mixture to -100°C a precooled (-78°C) solution of enamine (1.1 mmol) in dry THF (5ml) was slowly added. The temperature was maintained 3h at -100°C and 3 h at -78°C. After warming up to room temperature the mixture was hydrolyzed by addition of a few ml of dilute citric acid. The stirring was continued and after 4 h the solution was extracted with EtOAc. The organic layer was washed with water and dried. After removal of the solvent the diastereomeric ratio of the crude products was determined by 400 MHz ^{1}H NMR spectroscopy. The pure products were obtained by flash column chromatography on silica gel using EtOAc/petroleum ether(40/60) 1:1.

N-Benzyloxycarbonyl-D-phenylalanyl-(1'S,2R)/(1'R,2S)-(2'-oxocyclohexyl)glycine methyl ester (**14a/14b**): From Z-D-Phe-D,L-Gly(Cl)-OMe and (2*S*)-*N*-(1'-cyclohexenyl)-2-methoxymethyl-pyrrolidine[17] **13**, diastereomeric ratio: 20:1, yield 90%, colourless oil; ^{1}H NMR (400 MHz): δ= 1.35.2.37 (m, 8H), 2.97-3.33 (m, 3H), 3.66 (s, 3H), 4.48 (q, *J*=6.8Hz, 1H), 4.66, 4.70 (2 dd, *J*=9.6Hz, *J*=3.1Hz, 1H), 5.06 (s, 2H), 5.35 (br.d, *J*=7.9Hz, NH), 6.70 (d, *J*=9.7Hz, NH), 7.15-7.38 (m, 10H); $C_{26}H_{30}N_2O_6$ (466.53).

N-Benzyloxycarbonyl-L-phenylalanyl-(1'R,2S)/(1'S,2R)-(2'-oxocyclohexyl)glycine methyl ester (**15a/15b**): From **3a** and **13** (diastereomeric ratio: 3:2) or *N*-morpholinocyclohexene (diastereomeric ratio: 7:1), yield 87%, mp 89-90°C [(1'*R*,2*S*)-**15a**]; IR (KBr): 3386, 1709, 1671, 1519, 1499, 1275, 1244, 1215 cm^{-1}; ^{1}H NMR (400 MHz): δ= 1.44-1.59 (m, 2H), 1.64-1.75 (m, 2H), 1.87 (br.m, 1H), 2.03-2.11 (m, 2H), 2.27-2.39 (AB-system, 2H), 3.25-3.30 (m, 1H), 3.66 (s, 3H), 4.46 (q, *J*=7.0Hz, 1H), 4.66, 4.70 (2 dd, *J*=9.7Hz, *J*=3.1Hz, 1H), 5.07 (s, 2H), 5.27 (br.d, *J*=7.6Hz, NH), 6.65 (d, *J*=9.4Hz, NH), 7.19-7.37 (m, 10H); ^{13}C NMR (100.6 MHz): δ= 24.7, 27.2, 30.7, 38.2, 41.9,51.8, 52.6, 52.9, 56.0, 66.9, 127.0, 127.9, 128.1, 128.2, 128.5, 128.6, 129.5, 136.1, 136.4, 155.8, 171.1, 171.2, 211.9; FAB MS 467 (38) [MH$^+$]; $C_{26}H_{30}N_2O_6$ (466.53): calcd. C 66.94, H 6.48, N 6.00; found C 66.68, H 6.32, N 6.12.

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