Comparative Lipase-Catalyzed Hydrolysis of Ethylene Glycol Derived Esters. The 2-Methoxyethyl Ester as a Protective Group in Peptide and Glycopeptide Synthesis

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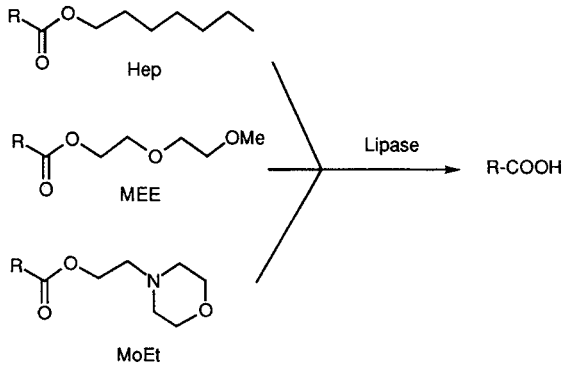
Dedicated to Professor Peter Welzel on the occasion of his 60th birthday.

Comparison of the lipase-catalyzed cleavage of polar esters derived from ethylene glycol proved 2-methoxyethyl (ME) esters most favorable protecting groups for the carboxylic function of peptides and glycopeptides. They combine high substrate acceptance and high yields of hydrolysis with favorable physicochemical properties and advantageous solubility. The application of this polar ester as protecting group was extended to *N*-glycosylated amino acids and *N*-glycopeptides. The selective removal of ME esters by lipases was achieved under mild conditions (pH 7.0 and 37°C), leaving all other linkages including peptide bonds and other ester protecting groups unaffected.

Enzymatic methods are useful in glycopeptide synthesis.[1] To form peptide bonds[2] and intersaccharidic and saccharide-peptide linkages,[3,4] various biocatalysts and methods have been developed and provide efficient ways to construct large polyfunctional molecules. Enzymes may also be used for the protection and deprotection of functional groups. During the past years, efficient combinations of protecting groups have been reported, which permit selective enzymatic deprotections of carboxylic, amino, thiol and hydroxyl groups.[5] The application of enzymatic methods are advantageous in glycopeptide synthesis because of their selectivity. Enzymes often show high regio- and stereoselectivity,[6] and they are able to distinguish even between similar parts of molecules. In addition, strongly acidic and basic conditions are avoided, since enzymes operate under very mild conditions, usually in neutral media at temperatures of 20 to 50°C. Thus, epimerization within the peptide portions or β-elimination of carbohydrate side chains under basic conditions as well as cleavage or anomerization of the glycosidic bonds under acidic conditions can be prevented.[7] In particular, at the end of the synthesis of large glycopeptides, mild deprotection conditions are required during the long reaction times. There are some protecting groups cleavable by enzymes which could be applied to the final deblocking of peptides and glycopeptides.[8] Most of them are esters, which can be hydrolyzed by proteases, esterases and lipases. In contrast to proteases, lipases do not attack peptide bonds. This is why lipases can be more generally applied than proteases in protecting group chemistry.

We here report on a comparative study of esters derived from ethylene glycol as the carboxylic protecting groups for amino acids, peptides and glycopeptides. These investigations aimed at esters with optimum properties in both, synthesis and hydrolysis. As the cleavage of ester protecting groups by lipases has been described only for *O*-glycopeptides so far, extension of this methodology to *N*-glycopeptides was also examined. Among the described protecting groups, heptyl-[9,10], morpholinoethyl-[11] and 2-(2-methoxyethoxy)ethyl (MEE)[12] esters have successfully been applied to glycopeptide synthesis (Scheme 1). For instance, a broad range of peptide heptyl esters, are hydrolyzed by lipase N from *Rhizopus niveus*.[9] Also *O*-glycosylated amino acid and peptide heptyl esters were converted to corresponding acids.[10] However, some hydrophobic peptide heptyl esters were not accepted as substrates by lipases.[9] The substrate acceptance was improved by introducing glycol-based esters which have enhanced solubility in aqueous solutions. Hydrophilic 2-(2-methoxyethoxy)ethyl esters proved to be distinctly better substrates to lipases than heptyl esters. Even peptide esters which contain hydrophobic amino acids in the *C*-terminal region were hydrolyzed in high yields.[12] However, some unsolved problems remained: The reaction of some glycosylated amino acids, e.g. *O*-(*N*-acetylgalactosaminyl) serine and threonine MEE esters, gave only poor yields in lipase-promoted hydrolysis. Moreover, additional purification steps are necessary to obtain pure peptides because of the oily consistence of most MEE esters.



OHep = heptyl ester; OMEE = 2-(2-methoxyethoxy)ethyl ester; OMoEt = 2-(N-morpholino)ethyl ester

Scheme 1. Esters useful in glycopeptide synthesis.

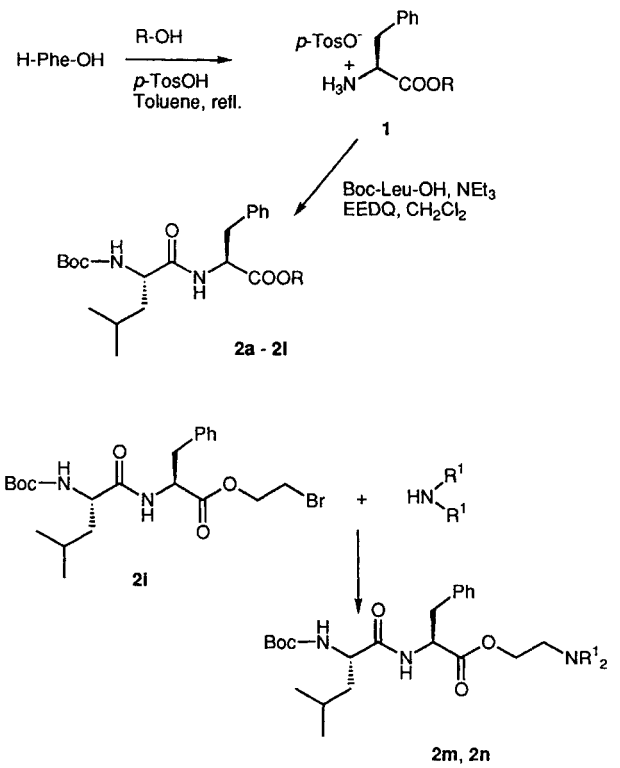
To combine high substrate acceptance, optimum purification properties and high solubility in aqueous solutions, various esters of dipeptides were synthesized and examined in lipase-catalyzed hydrolysis. As model compounds, esters **2** of the hydrophobic dipeptide *N*-*tert*-butyloxycarbonyl-L-leucyl-L-phenylalanine were chosen as difficult substrates. The corresponding heptyl ester is not cleaved by lipases.[9]

Most of the amino acid esters required for the synthesis of the model peptide esters are readily accessible from amino acids and the ethylene glycol derived alcohol by

azeotropic esterification in the presence of *p*-toluenesulfonic acid (Scheme 2 and Table 1). Subsequent coupling with *N*-Boc-leucine using ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate (EEDQ)[13] gave the dipeptide esters **2a–l**. The dipeptide (2-aminoethyl) esters **2m** and **2n** were obtained by reaction of 2-bromoethyl ester[11,14] **2i** with the corresponding amines.

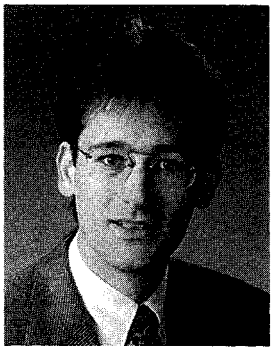
Out of 34 lipases investigated in screening assays with dipeptide esters **2**, the enzymes quoted in Table 2 proved to be most effective in cleaving the esters. In consideration of their stability in the presence of an organic cosolvent and of their proteolytic activity, lipase N (Amano) from *Rhizopus niveus*, lipase M (Amano) from *Mucor javanicus* and lipase A6 (Amano) from *Aspergillus niger* showed the most advantageous properties. All lipases given in Table 2 are commercially available, inexpensive and retain some extent of their activity in the presence of an organic cosolvent over several days. For some lipases, accompanying proteolytic activity has to be inhibited, in particular, if prolonged reaction times are required as for esters of larger glycopeptides.

The results of the comparative investigation (Scheme 3) are shown in Table 1. This data illustrates that dipeptide esters **2a–e** and **2n**, which are soluble in aqueous phosphate buffer after addition of small amounts of acetone, are readily cleaved by lipase N and give yields of more than 85 %. Slow or no hydrolysis was observed in screening assays with various lipases for compounds **2f–m**, including the heptyl ester **2l**.



Scheme 2. Synthesis of phenylalanine esters **1** and Boc-leucyl-phenylalanine esters **2**.

Biological Sketches



Markus Gewehr was born in Kastellaun, Germany, in 1966. He studied chemistry at the Johannes Gutenberg University in Mainz. In 1990/91 he worked as a research fellow with Prof. N. Ise and Prof. H. Kitano at Kyoto University, Japan. Under the supervision of Prof. H. Kunz, he received his Ph.D. in 1996. His research interests were enzymatic protection group techniques for the synthesis of peptides and glycopeptides and the application of enzymatic methods in SPPS. He is now working in the Crop Protection Research Department of BASF AG, Ludwigshafen.

Horst Kunz, born 1940 in Frankenhausen (Saxony), studied chemistry at the Humboldt-Universität Berlin and at the Universität Mainz. He completed his Ph.D. under the supervision of Leopold Horner on syntheses of cyclic organophosphorus compounds in 1969. His "Habilitation", completed in 1977, dealt with ester analogues of acetylcholine and their application in protecting-group chemistry. He was appointed to Associate Professor for Organic Chemistry in 1979 and to Full Professor of Bioorganic Chemistry in 1988 at the Universität Mainz. His research is focussed on the synthesis of glycopeptides, methods of peptide and carbohydrate chemistry and on stereoselective synthesis.

Table 1. Synthesis of Phenylalanine Esters **1** and Boc-leucyl-phenylalanine Esters **2**; EEDQ = Ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate.

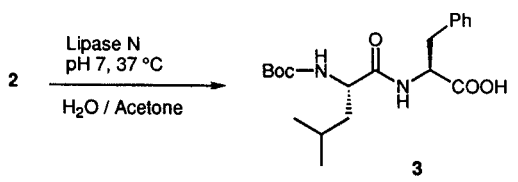
R[a]	Tos-OH H-Phe-OR				Dipeptide Ester	Synthesis Yield [%]	Hydrolysis 1 d[i] (yield [%])[j]
		mp [°C]	$[\alpha]_D^{22}$	R_f			
$-(CH_2)_2OCH_3$ (ME)	**1a**	93	+0.2[e]	0.77[b]	**2a**	79	quantitative (91)
$-(CH_2)_2O(CH_2)_2OCH_3$ (MEE)	**1b**	oil	−0.8[d,f]	0.53[c]	**2b**	81	quantitative (94)
$-[(CH_2)_2O]_3CH_3$ (MEEE)	**1c**	oil	+4.5[e,f]	0.65[b]	**2c**	67	quantitative (86)
$-(CH_2)_2OiPR$	**1d**	105	−3.1[e]	0.60[c]	**2d**	84	quantitative (85)
$-(CH_2)_2O(CH_2)_2OC_2H_5$ (EEE)	**1e**	oil	−0.9[e]	0.71[b]	**2e**	65	quantitative (90)
$-[(CH_2)_3O]_2CH_3$ (MPP)	**1f**	144	+0.55[e]	0.78[b]	**2f**	72	incomplete
$-CH_3$ (Me)	**1g**	amorph.	+11.4[e,g]	0.81[b]	**2g**	66	incomplete
$-(CH_2)_2Br$ (BrEt)	**1h**	119	−9.1[d]	0.70[c]	**2h**	76	incomplete
$-(CH_2)_2O(CH_2)_3CH_3$ (BE)	**1i**	oil	−2.2[e]	0.70[c]	**2i**	61	incomplete
$-[(CH_2)_2O]_3(CH_2)_3CH_3$ (BEEE)	**1k**	oil	−0.4[d]	0.72[c]	**2k**	79	incomplete
$-(CH_2)_6CH_3$ (Hep)	**1l**	amorph.	−2.3[d,h]	0.81[b]	**2l**	85	none
$-(CH_2)_2N(C_2H_5)_2$					**2m**	49	none
2-(N-morpholino)ethyl (MoEt)					**2n**	62	quantitative (90)

[a] Abbreviations: ME = 2-methoxy ethyl ester, MEE = 2-(2-methoxyethoxy)ethyl ester, MEEE = 2-[2-(2-methoxyethoxy)ethoxy]ethyl ester, EEE = 2-(2-ethoxyethoxy)ethyl ester, MPP = 3-(3-methoxypropoxy)propyl ester, Me = methyl ester, BrEt = 2-bromoethyl ester, BE = 2-butoxyethyl ester, BEEE = 2-[2-(2-butoxyethoxy)ethoxy]ethyl ester, Hep = heptyl ester; MoEt = *N*-(morpholino)ethyl ester.
[b] MeOH/EtOAc 3:1.
[c] MeOH/EtOAc 1:3.
[d] (c 1, $CHCl_3$).
[e] (c 1, MeOH).
[f] Compounds **1b**, **1c** and **1k** contain > 10% of the corresponding alcohol.
[g] Lit.[29]: +13.1 (c 7.5, MeOH).
[h] Lit.[9b]: +9.7 (c 2.1, MeOH.
[i] Conversion according to TLC.
[j] Isolated yields.

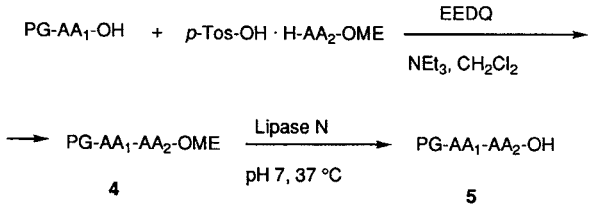
Table 2. Lipases Effective in the Selective Hydrolysis of Amino Acid, Peptide and Glycopeptide Esters.

Name of lipase	Supplier	Origin
lipase N	Amano	*Rhizopus niveus*
lipase M	Amano	*Mucor javanicus*
lipase A6	Amano	*Aspergillus niger*
lipase AP6	Fluka	*Aspergillus niger*
lipase CE	Amano	*Humicola lanuginosa*
lipase D	Amano	*Rhizopus delemar*
lipase F-AP15	Amano	*Rhizopus javanicus*
lipase L-3126 Type VII	Sigma	*Candida cylincracea*
lipase M10	Amano	*Mucor javanicus*
lipase R	Amano	*Penicillium roqueforti*
lipase from *Rhizopus arrhizus*	Boehringer Mannheim	*Rhizopus arrhizus*

Including the criterion of useful physicochemical properties, the 2-methoxyethyl ester **2a** most advantageously fulfills the requirements defined above: High solubility



Scheme 3. Comparison of various esters in lipase-catalyzed hydrolyses of Boc-Leu-Phe-OR.



Scheme 4. Synthesis of dipeptide ME esters **4** from the hydrotosylates of amino acid ME ester and the hydrolysis of **4** catalyzed by lipase N to give dipeptides **5**; PG = protective group; AA = amino acid.

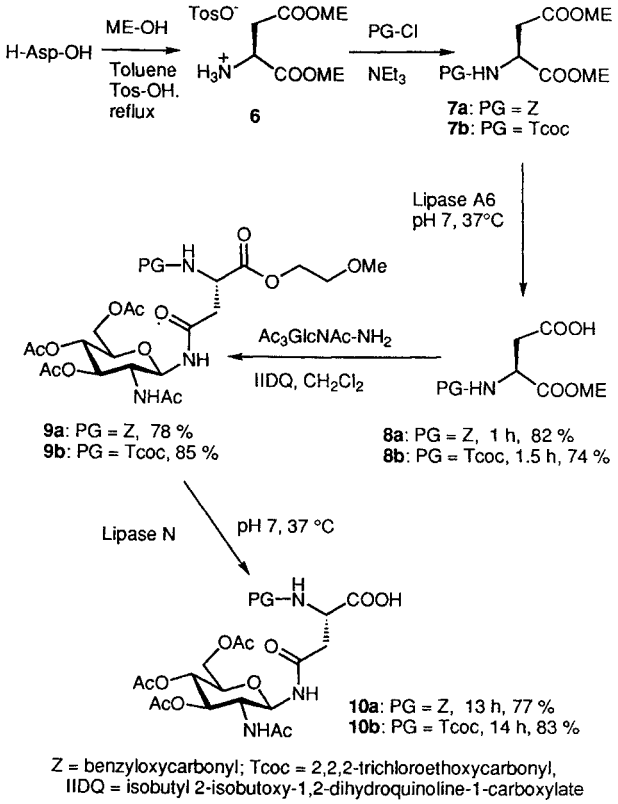
in a mixture of aqueous sodium phosphate buffer with small amounts of acetone as the cosolvent, high yield of enzymatic hydrolysis and simple purification of the amino acid ester salt **1** as well as of the dipeptide ester **2a**.

To investigate the influence of the C-terminal amino acid and the amino protecting group, dipeptide esters **4** were synthesized and hydrolyzed using lipase N (Scheme 4). Most peptide ME esters were obtained in pure crystalline form. The results given in Table 3 show, that neither the type of amino acids nor that of the amino protecting group have an inhibitory influence on the cleavage of the ME ester. Only Fmoc protection, which decreases the solubility of compound **4f**, results in a lower rate of hydrolysis. In all lipase-catalyzed hydrolyses of peptide ME esters, no undesired side reactions were observed. The *N*-terminal *tert*-butyloxycarbonyl (Boc)-, benzyloxycarbonyl (Z)-, 9-fluorenylmethoxycarbonyl[15] (Fmoc)-

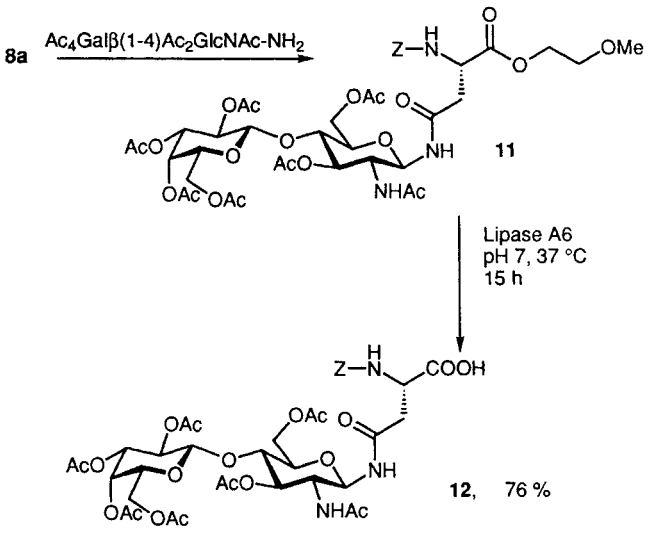
Table 3. Synthesis of Dipeptide ME Esters **4** from the Hydrotosylates of Amino Acid ME Ester and the Hydrolysis of **4** Catalyzed by Lipase N to Give Dipeptides **5**; PG = Protective Group; AA = Amino Acid.

PG	AA_1	AA_2	H-AA_2-OME[a] mp	$[\alpha]_D^{22}$ (c 1, CH_3OH)	Dipeptide ME ester	Yield [%]	Dipeptide	Hydrolysis Yield [%]
Boc	Phe	Ala	208 °C	+ 5.7	**4a**	95	**5a**	82
Boc	Gly	Leu	118 °C	+ 6.8	**4b**	87	**5b**	91
Boc	Ser	Leu			**4c**	85	**5c**	90
Z	Thr	Gly	176 °C		**4d**	89	**5d**	94
Aloc	Val	Ser	160 °C	+ 4.4	**4e**	78	**5e**	92
Fmoc	Gly	Ser			**4f**	67	**5f**	79

[a] Hydrotoluenesulfonate



Scheme 5. Synthesis of aspartic acid di-ME esters **7**, regioselective hydrolysis by lipase A6 and cleavage of ME esters of *N*-glycosylated asparagine derivatives **9**, giving building blocks **10** for the chain elongation in peptide synthesis. PG = protecting group.



Scheme 6. Hydrolysis of asparagine ester **11**, containing a disaccharide of lactosamine type I.

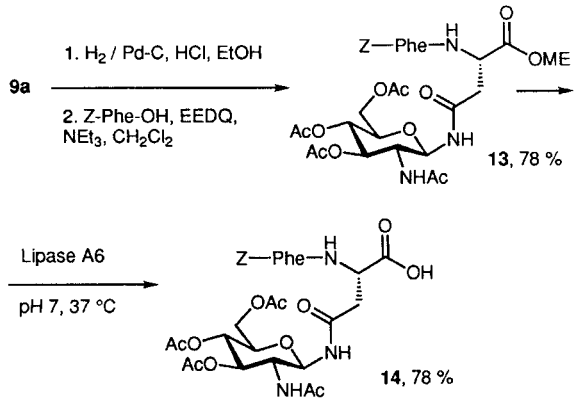
and allyloxycarbonyl[16] (Aloc)-groups remained unaffected in the obtained dipeptides **5**.

In glycopeptide synthesis, the use of 2-methoxyethyl (ME) ester displays further advantages. To explore the capacity of the ME ester in the synthesis of *N*-glycosylated peptides, *N*-glycosyl asparagine building blocks must be generated first. It has been shown that papain can be used to selectively cleave methyl,[17] heptyl and 2-(2-methoxyethoxy)ethyl esters of *O*-glycosylated serine and threonine derivatives[18] as well as methyl esters of *N*-glycosylated asparagine.[19] *N*-Protected aspartic acid α-monoesters are usually prepared by selective hydrolysis of the diesters or via selective formation of β-esters.[20] The alternative nucleophilic opening of aspartic acid anhydrides leads to mixtures of products and results in only moderate yields of the desired products.[21]

Because of the regioselectivity of enzymes, an efficient access to aspartic acid derivatives selectively deblocked at the β-carboxylic function is possible. *N*-Protected aspartic acid di-2-methoxyethyl esters **7** were synthesized in two steps in high yields (Scheme 5). Treatment of compounds **7** with lipase A6 at pH 7 and 37° C for 60–90 minutes yielded aspartic acid derivatives **8** with an unprotected β-carboxylic function. The α-ME esters remained intact. After reaction times of at least 8 hours, both ester functions were hydrolyzed giving the *N*-terminally protected aspartic acid. It is interesting to note that opposite selectivity was observed for the hydrolysis of aspartic acid diallyl and dimethyl esters catalyzed by papain or thermitase.[22] In these cases, the α-ester was selectively cleaved giving side-chain protected aspartic acid derivatives. Compounds **8** can be reacted with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glycopyranosylamine[7b] and isobutyl 2-isobutoxy-1,2-dihydroquinoline-1-carboxylate (IIDQ)[23] to form the *N*-glycosylated asparagine

esters **9a** or **9b**, respectively. Treatment of these *N*-glycosylasparagine esters **9** with lipase N for 12 hours furnished the C-terminally deblocked compounds **10**. Reaction of **8** with 2-acetamido-3,6-di-*O*-acetyl-4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glycopyranosylamine[24] resulted in the formation of the lactosamine asparagine ester conjugate **11** (Scheme 6). In contrast to the hydrolysis of compounds **9a** and **9b**, lipase A6 was used in the hydrolysis of this ME ester giving the lactosamine asparagine building block **12** in higher yield than with lipase N. In all reactions, selective hydrolysis of the ME ester was achieved under mild reaction conditions (pH 7.0, 37° C). Neither the *O*-acetyl groups within the carbohydrate portions nor the amino protecting groups or the glycosidic bonds were affected. Derivatives **10** and **12** can be directly used as building blocks for glycopeptide synthesis in solution[24] as well as on solid phase.[25]

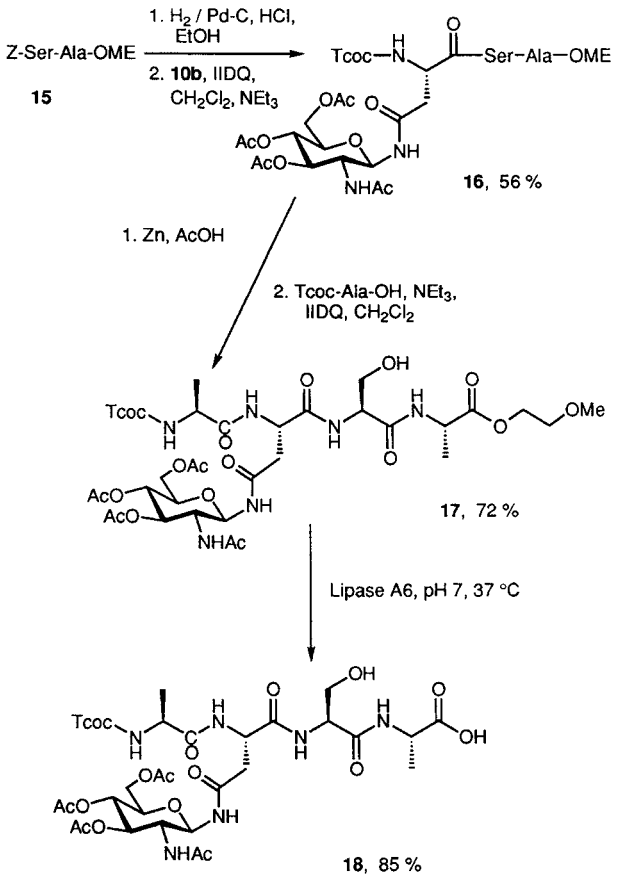
In order to demonstrate the properties of ME esters in the synthesis of *N*-glycopeptides, compounds **9a** and **10b** were used in the synthesis of glycopeptides **14** and **18**. The *N*-glycosyl dipeptide **13** was chosen as a model substrate because of its hydrophobic character. As was shown earlier, the enzymatic cleavage of esters of *O*-glycosyl amino acids in some cases gave unsatisfying results.[8c,10] Therefore, the Z group of **9a** was hydrogenolyzed in the presence of hydrogen chloride to yield the *N*-glycosyl asparagine ester as the hydrochloride. The ME ester remained stable under these conditions (Scheme 7). After drying in high vacuum, deprotonation and condensation with Z-Phe-OH, DCC (dicyclohexyl carbodiimide)[26] and HOBt (1-hydroxybenzotriazole),[27] the Z-protected glyco-dipeptide ME ester **13** was obtained. Cleavage of its ME ester using lipase A6 was carried out in aqueous phosphate buffer at pH = 7.0. The *N*-glycosylated dipeptide **14** was isolated in a yield of 78 %.



Scheme 7. Synthesis of *N*-glycosylated dipeptide ester **13** and removal of the ME ester protecting group using lipase; DCC = dicyclohexyl carbodiimide; HOBt = hydroxybenzotriazole.

The glycotetrapeptide ME ester **17** was prepared according to Scheme 8. Like the MEE ester,[12,18] the ME ester is also stable under the conditions required for the removal of *N*-terminal protecting groups, commonly applied in peptide synthesis. Benzyloxycarbonyl (Z)-seryl-alanine-2-methoxyethyl ester **15** obtained from Z-serine and alanine ME ester according to Scheme 4 was subjected to hydrogenation of the Z group. Condensation of the seryl-alanine ME ester with trichloroethoxycarbonyl (Tcoc)-protected *N*-glycosyl asparagine **10b** using IIDQ gave the protected *N*-glycotripeptide ME ester **16**. Treatment of **16** with zinc in acetic acid resulted in the selective removal of the Tcoc group.[28] Also under these conditions, the ME ester was not affected. The obtained amino-deblocked *N*-glycotripeptide ME ester was condensed with Tcoc-alanine to furnish the desired Tcoc-protected *N*-glycotetrapeptide ME ester **17** in an overall yield of 72 %.

The ME ester of **17** was selectively cleaved by lipase A6 at pH = 7 and 37° C in phosphate buffer containing 30 % of acetone. The glycosidic bond, the peptide linkages and all other protective groups, in particular, the ester protecting groups of the carbohydrate hydroxylic functions, remained unaffected. The selectively carboxy-deblocked compound **18** was obtained in a yield of 85 % after lyophilization and recrystallization from methanol/diethyl ether.



Scheme 8. Synthesis of *N*-glycosylated tetrapeptide ME ester **17** and hydrolysis of the ME ester by lipase.

In conclusion, these results illustrate the advantageous properties of the 2-methoxyethyl (ME) ester in the synthesis of peptides and *N*-glycopeptides. In comparison to other esters, cleavable by enzymes, peptide ME esters of various structure are accepted as substrates to lipases.

In addition, these ME esters show enhanced solubility in water-containing solutions. Therefore, ME esters of hydrophobic peptides and glycopeptides can be subjected to lipase-catalyzed hydrolysis. Moreover, the ME esters compared to long-chain ethylene glycol esters show improved properties during purification procedures. Most peptide ME esters readily crystallize, whereas many MEE esters are oily liquids. The regioselective lipase-catalyzed hydrolysis of the β-ME ester of aspartic acid di-ME esters opens up a short and efficient way to aspartic acid synthons required for the synthesis of *N*-glycosyl asparagine building blocks. Finally, the lipase-catalyzed cleavage can be applied to the synthesis of *N*-glycopeptides. The selective cleavage of the ME ester under mild conditions prevents any undesired attack at other linkages of the molecules, like peptide and glycosidic bonds or other protecting groups including the ester protecting groups within the carbohydrate portions.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz ^{1}H and 50.3 MHz ^{13}C) and on a Bruker AM 400 (400 MHz ^{1}H, 100.6 MHz ^{13}C). Mps are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Flash chromatography was carried out on silica gel purchased from J. T. Baker (0.030–0.060 mm), E. Merck, Darmstadt (0.040–0.063 mm) and Amicon (0.035–0.070 mm). The phosphate buffer solution was prepared from a solution of disodium hydrogenphosphate (36.5 g, 0.2 mol) in H_2O (1 L), adjusted to pH 7 by addition of H_3PO_4. For compounds **2**, NMR data of typical examples of each series are given.

L-Phenylalanine Ester Hydrogentoluenesulfonate (1):
A mixture of phenylalanine (3.3 g, 20 mmol), *p*-TsOH · H_2O (5.7 g, 30 mmol) and the corresponding ethylene glycol derived alcohol (0.1 mol) in benzene (100 mL) was refluxed until H_2O (2.8 mL) has separated. The solvent was removed in vacuo. In case of compounds **1a, 1d, 1h** and **1l**, the remaining crude phenylalanine ester hydrogentosylate was stirred with Et_2O. All other crude phenylalanine ester salts (see Table 1) were dried in vacuo and used directly in the synthesis of dipeptide esters **2**.

***N-tert*-Butyloxycarbonyl-L-leucyl-L-phenylalanine Esters 2a–l:**
A solution of *N-tert*-butyloxycarbonyl-L-leucine (1.4 g, 6 mmol) and ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate[13] (1.7 g, 7 mmol) in anhyd CH_2Cl_2 (50 mL) were stirred for 15 min. Phenylalanine ester salt **1** (7 mmol) and Et_3N (0.7 g, 7 mmol) were added and the stirring was continued, until Boc-leucine was completely consumed (monitoring by TLC). The solution was washed with 0.1 M HCl (2 × 30 mL) and sat. aq $NaHCO_3$ (2 × 30 mL) and dried ($MgSO_4$). The solvent was evaporated in vacuo. The product was purified by flash chromatography. Yields are given in Table 1. Physical and elemental analysis data are given in Table 4.
As examples, NMR data of compounds **2a**, **2b**, **2k** and **2l** are given:

2a:

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.90 (d, $J_{\delta H,\gamma H}$ = 3.7 Hz, 6H, 2 $(CH_3)_{Leu}$); 1.21–1.27 (m, 1H, (γ-CH)$_{Leu}$); 1.42 (s, 9H, C$(CH_3)_3$); 1.56–1.60 (m, 2H, (β-CH_2)$_{Leu}$); 3.12 (s_b, 2H, (β-CH_2)$_{Phe}$); 3.35 (s, 3H, $(OCH_3)_{ME}$); 3.54 (t, J_{vic} = 4.1 Hz, 2H, $(CH_2O)_{ME}$); 4.07–4.12 (m, 1H, (α-CH)$_{Leu}$); 4.23 (t, J_{vic} = 3.8 Hz, 2H, $(COOCH_2)_{ME}$); 4.84–4.88 (m, 2H, (α-CH)$_{Phe}$) and (NH)$_{Phe}$); 6.56 (d, $J_{NH,\alpha H}$ = 7.2 Hz, 1H, (NH)$_{Leu}$); 7.13–7.24 ((ArH)$_{Phe}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 21.81, 22.73 (CH_3 Leu); 24.55 (g-CH Leu); 28.16 (C$(CH_3)_3$); 37.74 (β-CH_2 Phe); 41.21 (β-CH_2 Leu); 53.05 (α-CH Phe, α-CH Leu); 58.69 $((OCH_3)_{ME})$; 64.14 $((COOCH_2)_{ME})$; 69.95 $((CH_2O)_{ME})$; 79.75 (C$(CH_3)_3$); 126.87, 128.33, 129.29 (ArC); 135.76 ((CAr)$_{ipso}$); 155.39 (urethane C=O); 171.10 (amide C=O); 172.12 (ester C=O).

Table 4. Elemental Analysis and Physical Data of Dipeptide Esters Boc-Leu-Phe-OR **2**.

dipeptide ester	mp	$[\alpha]_D^{22}$	R_f EtOAc	elemental analysis calcd./found
2a	64°C	−6.7[d]	0.76	C 63.90 H 8.31 N 6.42
2b	oil	−14.5[d]	0.71	C 62.48 H 8.39 N 5.83
2c	oil[a]	−1.1[d]	0.67	
2d	68°C	−9.0[d]	0.71	C 64.63 H 8.68 N 6.03 C 64.17 H 8.57 N 6.31
2e	oil	−3.9[d]	0.68	C 63.14 H 8.56 N 5.66 C 63.33 H 8.48 N 5.69
2f	88°C	−10.2[d]	0.73	C 63.76 H 8.72 N 5.51 C 63.09 H 8.71 N 5.47
2g[b]	74°C	−19.2[d]	0.73	
2h	119°C	−9.2[d]	0.74	C 54.44 H 6.85 N 5.77 C 54.32 H 6.92 N 5.74
2i	97°C	−15.1[d]	0.79	C 65.25 H 8.84 N 5.85 C 64.64 H 8.75 N 5.87
2k	oil	−31.4[d]	0.72	C 63.58 H 8.89 N 4.94 C 63.19 H 9.34 N 4.93
2l[c]	amorph.	−20.0[e]		
2m	50°C	−3.7[d]	0.27	C 65.38 H 9.07 N 8.80 C 65.10 H 9.46 N 8.38
2n	97°C	−27.8[d]	0.35	C 63.52 H 8.41 N 8.55 C 64.47 H 8.45 N 8.63

[a] Compound contains > 10% of the corresponding alcohol (^{1}H NMR); MS (FAB, $C_{27}H_{44}N_2O_8$, MH^+): calcd 525.3, found 525.5.
[b] Lit.[30a]: mp 78–79°C; Lit.[30b]: $[a]_D^{22}$ = 19.5 (c = 1, $CHCl_3$).
[c] Lit.: $[\alpha]_D^{22}$ = −21.1 (c = 1, CH_3OH.
[d] (c = 1, $CHCl_3$).
[e] (c = 1, CH_3OH).

2b:

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.89 (d, $J_{\delta H,\gamma H}$ = 4.2 Hz, 6H, 2 $(CH_3)_{Leu}$); 1.41 (s, 9H, C$(CH_3)_3$); 1.43–1.52 (m, 1H, (γ-CH)$_{Leu}$); 1.55–1.62 (m, 2H, (β-CH_2)$_{Leu}$); 3.11 (d, $J_{\beta H,\alpha H}$ = 5.6 Hz, 1H, (β-CH_2)$_{a,Phe}$); 3.13 (d, $J_{\beta H,\alpha H}$ = 5.5 Hz, 1H, (β-CH_2)$_{b,Phe}$); 3.36 (s, 3H, $(OCH_3)_{ME}$); 3.51–3.54 (m, 2H, $(CH_2OCH_3)_{ME}$); 3.58–3.66 (m, 4H, $2(CH_2O)_{ME}$); 4.06 (m, 1H, (α-CH)$_{Leu}$); 4.25 (t, J_{vic} = 4.6 Hz, 2H, $(COOCH_2)_{ME}$); 4.79–4.89 (m, 2H, (α-CH)$_{Phe}$) und (NH)$_{Phe}$); 6.56 (d, $J_{NH,\alpha H}$ = 7.6 Hz, 1H, (NH)$_{Leu}$); 6.56 (d, J_{vic} = 7.6 Hz, 2H, (ArH)$_{o,Phe}$); 7.14–7.26 (m, 3H, (ArH)$_{m,p,Phe}$).
^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 21.74, 22.71 (CH_3 Leu); 24.46 (γ-CH Leu); 28.10 (C$(CH_3)_3$); 37.62 (β-CH_2 Phe); 41.10 (β-CH_2 Leu); 53.00 (α-CH Leu); 54.52 (α-CH Phe); 58.81 $((OCH_3)_{ME})$; 64.24 $((COOCH_2)_{ME})$; 68.56 $(COOCH_2CH_2)$; 70.28 (CH_2OCH_3); 71.67 $(CH_2CH_2OCH_3)$; 79.69 (C$(CH_3)_3$); 126.81, 128.27, 129.25 (ArC); 135.71 ((CAr)$_{ipso}$); 155.33 (urethane C=O); 171.03 (amide C=O); 172.11 (ester C=O).

2k:

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.87 (s, 3H, $(CH_3)_{BEEE}$); 0.90 (d, $J_{\delta H,\gamma H}$ = 3.8 Hz, 6H, $2(CH_3)_{Leu}$); 1.23–1.38 (m, 3H, (γ-CH Leu), $(CH_2CH_3)_{BEEE}$); 1.40 (s, 9H, C$(CH_3)_3$); 1.46–1.65 (m, 4H, (β-CH_2)$_{Leu}$ and $(CH_2C_2H_5)_{BE}$); 3.10 (d, $J_{\beta H,\alpha H}$ = 5.8 Hz, 1H, (β-CH_2)$_{a,Phe}$); 3.12 (d, $J_{\beta H,\alpha H}$ = 5.6 Hz, 1H, (β-CH_2)$_{b,Phe}$); 3.41 (t, J_{vic} = 6.7 Hz, 2H, $(OCH_2C_3H_7)_{BE}$); 3.51–3.66 (m, 10H, $5(CH_2O)_{BE}$); 4.05–4.08 (m, 1H, (α-CH)$_{Leu}$); 4.23 (t, J_{vic} = 4.8 Hz, 2H, $(COOCH_2)_{BEEE}$); 4.79–4.88 (m, 2H, (α-CH)$_{Phe}$) and (NH)$_{Phe}$); 6.61 (d, $J_{NH,\alpha H}$ = 7.7 Hz, 1H, (NH)$_{Leu}$); 7.09–7.29 (m, 5H, (ArH)$_{Phe}$).
^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 3.82 $((CH_3)_{BEEE})$; 19.19, 19.20 $((CH_2CH_3)_{BEEE}$, $(CH_2C_2H_5)_{BEEE})$; 21.92, 22.83 (CH_3 Leu); 24.65 (γ-CH Leu); 28.26 (C$(CH_3)_3$); 37.82 (β-CH_2 Phe); 41.32 (β-CH_2 Leu); 53.14 (α-CH Leu, α-CH Phe); 64.42 $((COOCH_2)_{BEEE})$; 68.71 $((COOCH_2CH_2)_{BEEE})$; 70.01, 70.56, 70.59, 70.64 (4 $(CH_2O)_{BEEE}$); 71.13 $((OCH_2C_3H_7)_{BEEE})$; 79.38 (C$(CH_3)_3$); 126.97, 128.43, 129.40

(ArC); 135.86 ((CAr)$_{ipso}$); 155.14 (urethane C=O); 171.15 (amide C=O); 172.16 (ester C=O).

2l:

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.84–0.92 (m, 9 H, 2(CH_3)$_{Leu}$, (CH_3)$_{Hep}$); 1.18–1.34 (m, 10 H, (CH_2)$_{Hep}$); 1.40 (s, 9 H, C(CH_3)$_3$); 1.46–1.63 (m, 3 H, (β-CH_2)$_{Leu}$, (γ-CH)$_{Leu}$); 3.08 (s_b, 2 H, (β-CH_2)$_{Phe}$); 4.01–4.07 (m, 3 H, (α-CH)$_{Leu}$, (COOCH_2)$_{Hep}$); 4.79–4.82 (m, 1 H, (α-CH)$_{Phe}$); 4.87 (d, $J_{NH,\alpha H}$ = 7.9 Hz, 1 H, (NH)$_{Phe}$); 6.53 (d, $J_{NH,\alpha H}$ = 7.9 Hz, 1 H, (NH)$_{Leu}$); 6.97–7.26 (m, 5 H, (ArH)$_{Phe}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 13.72 (CH_3 Hep); 14.20 ((CH_2)$_{Hep}$); 22.27, 22.59 (CH_3 Leu); 24.47 (γ-CH Leu); 25.49 ((CH_2)$_{Hep}$); 28.04 (C(CH_3)$_3$); 28.20, 28.56, 31.38 (3 (CH_2)$_{Hep}$); 37.85 (β-CH_2 Phe); 41.12 (β-CH_2 Leu); 52.98, 53.14 (α-CH Phe and α-CH Leu); 65.38 ((COOCH_2)$_{Hep}$); 79.77 (C(CH3)3); 126.77, 128.22, 128.99, 129.09 (ArC); 135.67 ((CAr)$_{ipso}$); 155.24 (urethane C=O); 171.05 (amide C=O); 171.83 (ester C=O).

N-tert-Butyloxycarbonyl-L-leucyl-L-phenylalanine 2-Aminoethyl Esters 2m and 2n:

To morpholine or diethylamine, respectively (50 mL) stirred at 0°C dipeptide-(2-bromoethyl)ester[14] **2i** (1.9 g, 4 mmol) was added in small portions. The stirring was continued for 2 h at r.t. After evaporation of the amine in vacuo, the residue was purified by flash chromatography. Yields are given in Table 1. Physical and elemental analysis data are quoted in Table 4.

2m:

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.83 (d, $J_{\delta H,\gamma H}$ = 2.5 Hz, 3 H, (CH_3)$_{Leu}$); 0.87 (d, $J_{\delta H,\gamma H}$ = 3.0 Hz, 3 H, (CH_3)$_{Leu}$); 1.08 (d, J_{vic} = 7.1 Hz, 6 H, (NCH$_2$CH_3)); 1.18–1.63 (m, 3 H, (γ-CH)$_{Leu}$, (β-CH_2)$_{Leu}$); 1.44 (s, 9 H, C(CH_3)$_3$); 2.44 (t, J_{vic} = 4.9 Hz, 2 H, COOCH$_2$CH_2); 3.13 (d, J_{vic} ≐ 5.3Hz, 2 H, (β-CH_2)$_{Phe}$); 3.37 (q, J_{vic} = 7.2 Hz, 4 H, (NCH_2CH$_3$)); 3.97–4.09 (m, 1 H, (α-CH)$_{Leu}$); 4.03 (t, J_{vic} = 4.4 Hz, 2 H, COOCH_2); 4.54–4.62 (m, 1 H, (α-CH)$_{Phe}$); 4.96 (d, $J_{NH,\alpha}$ H = 8.3Hz, 1 H, (NH)Phe); 6.63 (d, $J_{NH,\alpha H}$ = 7.3 Hz, 1 H, (NH)$_{Leu}$); 7.07–7.28 ((ArH)$_{Phe}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 15.00 (CH_3 ester); 21.88, 22.87 (CH_3 Leu); 24.66 (γ-CH Leu); 28.27 (C(CH_3)$_3$); 37.84 (β-CH_2 Phe); 41.16, 43.79 (NCH_2CH$_3$, β-CH_2 Leu); 53.09, 53.76 (α-CH Phe, α-CH Leu); 56.38 (COOCH_2CH_2); 62.39 (COOCH_2CH$_2$); 80.16 (C(CH$_3$)$_3$); 127.35, 128.64 (ArC); 136.53 ((CAr)$_{ipso}$); 155.39 (urethane C=O); 170.22, 171.83 (amide C=O, ester C=O).

2n:

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.86 (d, $J_{\delta H,\gamma H}$ = 2.3 Hz, 3 H, (CH_3)$_{Leu}$); 0.89 (d, $J_{\delta H,\gamma H}$ = 2.4 Hz, 3 H, (CH_3)$_{Leu}$); 1.08–1.23 (m, 1 H, (γ-CH)$_{Leu}$); 1.40 (s, 9 H, C(CH_3)$_3$); 1.46–1.71 (m, 2 H, (β-CH_2)$_{Leu}$); 2.41 (t, J_{vic} = 4.6 Hz, 4 H, 2(NCH$_2$)$_{EtMo}$); 2.52 (t, J_{vic} = 5.7Hz, 2 H, (COOCH$_2$CH_2)$_{EtMo}$); 3.09 (d, J_{vic} = 6.0 Hz, 2 H, (β-CH_2)$_{Phe}$); 3.65 (t, J_{vic} = 4.6 Hz, 4 H, 2(CH$_2$O)$_{EtMo}$); 4.05–4.12 (m, 1 H, (α-CH)$_{Leu}$); 4.17 (t, J_{vic} = 5.8 Hz, 2 H, (COOCH$_2$)$_{EtMo}$); 4.75–4.88 (m, 2 H, (α-CH)$_{Phe}$, (NH)$_{Phe}$); 6.67 (d, $J_{NH,\alpha H}$ = 7.7 Hz, 1 H, (NH)$_{Leu}$); 7.09–7.25 ((ArH)$_{Phe}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 22.00 (CH_3 Leu); 24.74 (γ-CH Leu); 28.31 (C(CH_3)$_3$); 38.28 (β-CH_2 Phe); 41.41 (β-CH_2 Leu); 52.12, 53.63, 54.70 (α-CH Phe, α-CH Leu, NCH_2CH$_2$O); 57.04 (COOCH$_2$CH_2); 61.80 (COOCH_2CH$_2$); 66.41 (NCH$_2$CH_2O); 79.78 (C(CH$_3$)$_3$); 126.83, 128.34, 129.18 (ArC); 135.84 ((CAr)$_{ipso}$); 155.90 (urethane C=O); 171.68, 171.99 (amide C=O, ester C=O).

N-tert-Butyloxycarbonyl-L-leucyl-L-phenylalanine (3):

To a solution of Boc-dipeptide ester **2** (2–3 mmol) in acetone (4–8 mL), aq sodium phosphate buffer (0.2 M, 30 mL, pH = 7) was added and the mixture was warmed to 37°C. Lipase N (Amano) (200 mg) was added. The solution was shaken for 15 h. After addition of brine (50 mL), the product was extracted with EtOAc (3 × 40 mL). If necessary, HOAc was added before extraction. After drying ($MgSO_4$), the solvent was evaporated in vacuo and the residue purified by flash chromatography (petroleum ether/EtOAc 2:1 + HOAc 1 %). Yields of Boc-Leu-Phe-OH are given in Table 3. Amorphous; $[\alpha]_D^{22}$ = −10.9 (c = 1, MeOH); (Lit.[31]: $[\alpha]_D^{22}$ = −10.2 (c = 0.8, MeOH)); R_f = 0.78 (EtOAc/HOAc 10:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.88 (d, $J_{\delta H,\gamma H}$ = 3.1 Hz, 3 H, (CH_3)$_{Leu}$); 0.92 (d, $J_{\delta H,\gamma H}$ = 3.2 Hz, 3 H, (CH_3)$_{Leu}$); 1.20–1.29 (m, 1H, (γ-CH)$_{Leu}$); 1.41 (s, 9 H, C(CH_3)$_3$); 1.50–1.63 (m, 2 H, (β-CH_2)$_{Leu}$); 2.85 (dd, J_{gem} = 13.1 Hz, J_{vic} = 6.0 Hz, 1 H, (β-CH_2)$_{a,Phe}$); 3.06 (dd, J_{gem} = 13.2 Hz, J_{vic} = 6.2 Hz, 1 H, (β-CH_2)$_{b,Phe}$); 3.99–4.04 (m, 1 H, (α-CH)$_{Leu}$); 4.80–4.88 (m, 2 H, (α-CH)$_{Phe}$, (NH)$_{Phe}$); 6.50 (d, $J_{NH,\alpha H}$ = 7.6 Hz, 1 H, (NH)$_{Leu}$); 7.12–7.34 ((ArH)$_{Phe}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 21.83, 22.69 (CH_3 Leu); 24.50 (γ-CH Leu); 28.18 (C(CH_3)$_3$); 37.65 (β-CH_2 Phe); 40.09 (β-CH_2 Leu); 52.88, 53.06 (α-CH Phe, α-CH Leu); 79.61 (C(CH$_3$)$_3$); 126.65, 128.37, 129.18 (ArC); 134.82 ((CAr)$_{ipso}$); 155.41 (urethane C=O); 170.99 (amide C=O); 174.22 (acid C=O).

***N*-Protected Dipeptide (2-Methoxyethyl) Esters 4:**

To a stirred mixture of amino acid (50 mmol) and *p*-TsOH · H_2O(11.4 g, 60 mmol) in benzene (200 mL), 2-methoxyethanol (10.9 mL, 0.2 mol) was added. The mixture was refluxed using a water separator at 90°–100°C, until 6.1 mL of H_2O separated. Solvent and excess of alcohol were evaporated in vacuo. The residue was recrystallized from MeOH/Et_2O if possible. The obtained crude hydro-*p*-toluenesulfonate of the amino acid (2-methoxyethyl) ester (10 mmol) was added to a solution of the *N*-protected amino acid (6 mmol) and ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate[13] (1.7 g, 7 mmol) in anhyd CH_2Cl_2 (50 mL). Et_3N (0.7 g, 10 mmol) was added, and the solution was stirred for 4 h at r.t. After washing with 0.1 M HCl (2 × 50 mL), sat. aq $NaHCO_3$ (2 × 50 mL) and sat. aq brine (50 mL) and drying ($MgSO_4$), the solvent was evaporated in vacuo. The residue was purified by recrystallization from MeOH/Et_2O or by flash chromatography (petroleum ether/EtOAc 4:1). Yields of the synthesized *N*-protected dipeptide ME esters **4** are given in Table 3.

N-tert-Butyloxycarbonyl-L-phenylalanyl-L-alanine (2-Methoxyethyl) Ester (4a):

Colorless crystals; mp. 78°C; $[\alpha]_D^{22}$ = +15.3 (c = 1, MeOH); R_f = 0.71 (EtOAc).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.35 (d, $J_{\alpha H,\beta H}$ = 8.8 Hz, 3 H, (CH_3)$_{Ala}$); 1.38 (s, 9 H, C(CH_3)$_3$); 3.05 (d, $J_{\beta H,\alpha H}$ = 6.6 Hz, 2 H, (CH_2)$_{Phe}$); 3.35 (s, 3 H, (OCH$_3$)$_{ME}$); 3.56 (t, J_{vic} = 4.6Hz, 2 H, (CH$_2$O)$_{ME}$); 4.25 (t, J_{vic} = 4.6 Hz, 2 H, (COOCH$_2$)$_{ME}$); 4.33 (t, $J_{\alpha H,\beta H}$ = 6.5 Hz, 1 H, (α-CH)$_{Ala}$); 4.52 (t, $J_{\alpha H,\beta H}$ = 7.2 Hz, 1 H, (α-CH)$_{Phe}$); 5.01 (s, 1 H, (NH)$_{Phe}$); 6.51 (d, $J_{NH,\alpha H}$ = 6.9 Hz, 1 H, (NH)$_{Ala}$); 7.21–7.31 (m, 5 H, (ArH)$_Z$).

^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 18.02 (CH_3 Ala); 28.11 (C(CH_3)$_3$); 38.33 (β-CH_2 Phe); 48.05 (α-CH Ala); 55.49 (α-CH Phe); 58.74 ((OCH$_3$)$_{ME}$); 64.11 ((COOCH$_2$)$_{ME}$); 70.05 ((CH$_2$O)$_{ME}$); 79.90 (C(CH$_3$)$_3$); 126.67, 128.38, 129.25 (ArC); 136.63 ((CAr)$_{ipso}$); 155.28 (urethane C=O); 170.90 (amide C=O); 172.32 (ester C=O).

Anal. Calcd for $C_{20}H_{30}N_2O_6$ (394.5): C, 60.90; H, 7.67; N, 7.10. Found: C, 60.86; H, 7.79; N, 7.17.

N-tert-Butyloxycarbonyl-L-glycyl-L-leucine (2-Methoxyethyl) Ester (4b):

Colorless crystals; mp. 61°C; $[\alpha]_D^{22}$ = −20.0 (c = 1, MeOH); R_f = 0.65 (EtOAc).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.89 (d, $J_{\gamma H,\delta H}$ = 5.4 Hz, 6 H, 2(CH_3)$_{Leu}$); 1.40 (s, 9 H, C(CH_3)$_3$); 1.55–1.62 (m, 3 H, (β-CH_2)$_{Leu}$ and (γ-CH)$_{Leu}$); 3.32 (s, 3 H, (OCH$_3$)$_{ME}$); 3.54 (t, J_{vic} = 4.7 Hz, 2 H, (CH$_2$O)$_{ME}$); 3.78 (d, $J_{\alpha H,NH}$ = 4.8 Hz, 2 H, (CH_2)$_{Gly}$); 4.22 (t, J_{vic} = 4.6 Hz, 2 H, (COOCH$_2$)$_{ME}$); 4.55–4.69 (m, 1 H, (α-CH)$_{Leu}$); 5.35 (t, $J_{NH,\alpha H}$ = 4.9 Hz, 1 H, (NH)$_{Gly}$); 6.68 (d, $J_{NH,\alpha H}$ = 6.9 Hz, 1 H, (NH)$_{Leu}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 21.57, 22.46 (CH_3 Leu); 24.49 (γ-CH Leu); 27.99 (C(CH_3)$_3$); 40.94 (β-CH_2 Leu); 43.92 (α-CH_2 Gly); 50.58 (α-CH Leu); 58.50 ((OCH$_3$)$_{ME}$); 63.81 ((COOCH$_2$)$_{ME}$); 69.93 ((CH$_2$O)$_{ME}$); 79.93 (C(CH$_3$)$_3$); 120.81, 126.29, 127.54, 128.98, 129.19 (ArC); 135.88 ((CAr)$_{ipso}$); 155.97 (urethane C=O); 169.40 (amide C=O); 172.51 (ester C=O).

Anal. Calcd for $C_{16}H_{30}N_2O_6$ (346.4): C, 55.47; H, 8.73; N, 8.09. Found: C, 55.30; H, 8.85; N, 7.91.

N-*tert*-Butyloxycarbonyl-L-seryl-L-leucine (2-Methoxyethyl) Ester (4c):

Colorless crystals; mp. 47°C; $[\alpha]_D^{22} = -20.8$ ($c = 1$, MeOH); $R_f = 0.42$ (EtOAc).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 0.90$ (d, $J_{\gamma H,\delta H} = 6.1$ Hz, 3 H, $(CH_3)_{Leu}$); 0.97 (d, $J_{\gamma H,\delta H} = 5.8$ Hz, 3 H, $(CH_3)_{Leu}$); 1.38–1.41 (m, 1 H, $(\gamma\text{-CH})_{Leu}$); 1.42 (s, 9 H, $C(CH_3)_3$); 1.53–1.60 (m, 2 H, $(\beta\text{-CH}_2)_{Leu}$); 3.36 (s, 3 H, $(OCH_3)_{ME}$); 3.61 (t, $J_{vic} = 5.0$ Hz, 2 H, $(CH_2O)_{ME}$); 3.77–4.09 (m, 2 H $(\beta\text{-CH}_2)$Ser); 4.23 (t, $J_{vic} = 4.8$ Hz, 2 H, $(COOCH_2)_{ME}$); 4.31 (m, 1 H, $(\alpha\text{-CH})_{Ser}$); 4.49–4.52 (m, 1 H, $(\alpha\text{-CH})_{Leu}$); 5.82 (d, $J_{NH,\alpha H} = 7.5$ Hz, 1 H, $(NH)_{Ser}$); 6.87 (d, $J_{NH,\alpha H} = 7.0$ Hz, 1 H, $(NH)_{Leu}$).

^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 21.89$, 22.36 (CH_3 Leu); 25.01 (τ-CH Leu); 27.84 ($C(CH_3)_3$); 40.87 (β-CH_2 Leu); 48.91 (γ-CH Leu); 56.52 (α-CH Ser); 58.51 ($(OCH_3)_{ME}$); 60.99 (β-CH_2 Ser); 64.16 ($(COOCH_2)_{ME}$); 68.91 ($(CH_2O)_{ME}$); 80.05 ($C(CH_3)_3$); 155.47 (urethane C=O); 169.02 (amide C=O); 171.97 (ester C=O).

Anal. Calcd for $C_{17}H_{32}N_2O_7$ (376.5): C, 54.24; H, 8.57 N, 7.44. Found: C, 54.14; H, 8.71; N, 7.07.

N-Benzyloxycarbonyl-L-threonyl-glycine (2-Methoxyethyl) Ester (4d):

Colorless solid; $[\alpha]_D^{22} = -6.8$ ($c = 1$, MeOH); $R_f = 0.31$ (EtOAc).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.19$ (d, $J_{\gamma H,\beta H} = 3.2$ Hz, 3 H, $(CH_3)_{Thr}$); 3.35 (s, 3 H, $(OCH_3)_{ME}$); 3.56 (t, $J_{vic} = 5.0$ Hz, 2H, $(CH_2O)_{ME}$); 4.04 (d, $J_{\alpha H,NH} = 5.5$ Hz, 2 H, $(CH_2)_{Gly}$); 4.18–4.24 (m, 1 H, $(\alpha\text{-CH})_{Thr}$); 4.27 (t, $J_{vic} = 4.2$ Hz, 2 H, $(COOCH_2)_{ME}$); 4.42–4.50 (m, 1 H, $(\beta\text{-CH})_{Thr}$); 5.10 (s, 2 H, $(CH_2)_Z$); 5.92 (d, $J_{NH,\alpha H} = 7.8$ Hz, 1 H, $(NH)_{Thr}$); 7.13 (t, $J_{NH,\alpha H} = 4.9$ Hz, 1 H, $(NH)_{Gly}$); 7.32 (s, 5 H, $(ArH)_Z$).

^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 18.20$ (CH_3 Thr); 41.21 (α-CH_2 Gly); 58.74 ($(OCH_3)_{ME}$); 59.17 (α-CH Thr); 64.23 ($(COOCH_2)_{ME}$); 67.11, 67.73 ($(CH_2)_Z$, (β-$CH)_{Thr}$); 70.03 ($(CH_2O)_{ME}$); 127.75, 127.88, 128.03, 128.11, 128.45 (ArC); 136.12 ($(CAr)_{ipso}$); 156.76 (urethane C=O); 169.80 (amide C=O); 171.32 (ester C=O).

Anal. Calcd for $C_{17}H_{24}N_2O_7$ (368.4): C, 55.43; H, 6.57; N, 7.60. Found: C, 55.48; H, 6.56; N, 7.27.

N-Allyloxycarbonyl-L-valyl-L-serine (2-Methoxyethyl) Ester (4e):

Colorless oil; $[\alpha]_D^{22} = -7.5$ ($c = 1$, MeOH); $R_f = 0.22$ (EtOAc).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 0.93$ (d, $J_{\gamma H,\beta H} = 7.8$ Hz, 3 H, $(CH_3)_{Val}$); 0.97 (d, $J_{\gamma H,\beta H} = 6.9$ Hz, 3 H, $(CH_3)_{Val}$); 2.08 (q, $J_{\beta H,\gamma H} = 6.6$ Hz, 1 H, $(\beta\text{-CH})_{Val}$); 2.92 (s_b, 1 H, $(OH)_{Ser}$); 3.35 (s, 3 H, $(OCH_3)_{ME}$); 3.58 (t, $J_{vic} = 4.7$ Hz, 2 H, $(CH_2O)_{ME}$); 3.83 (dd, $J_{gem} = 11.6$, $J_{\alpha H,\beta H} = 3.2$ Hz, 1 H, $(\beta\text{-CH}_2)_{a,Ser}$); 4.00 (dd, $J_{gem} = 11.6$, $J_{\alpha H,\beta H} = 4.7$ Hz, 1 H, $(\beta\text{-CH}_2)_{b,Ser}$); 4.25 (dt, $J_{gem} = 12.0$ Hz, $J_{vic} = 4.6$ Hz, 1 H, $(COOCH_2)_{a,ME}$); 4.37 (dt, $J_{gem} = 12.0$, $J_{vic} = 4.8$ Hz, 1 H, $(COOCH_2)_{b,ME}$); 4.52 (d, $J_{vic} = 4.3$ Hz, 2 H, $(CH_2)_{Aloc}$); 4.66–4.73 (m, 2 H, $(\alpha\text{-CH})_{Ser}$ and $(\alpha\text{-CH})_{Val}$); 5.17 (dd, $J_{gem} = 10.4$ Hz, $J_{vic} = 1.3$ Hz, 1 H, $(CH_2=)_{Aloc,cis}$); 5.23 (dd, $J_{gem} = 10.8$ Hz, $J_{vic} = 1.4$ Hz, 1 H, $(CH_2=)_{Aloc,trans}$); 5.58 (d, $J_{NH,\alpha H} = 8.7$ Hz, 1 H, $(NH)_{Val}$); 5.78–5.94 (m, 1 H, $(CH)_{Aloc}$); 7.11 (d, $J_{NH,\alpha H} = 7.8$ Hz, 1 H, $(NH)_{Ser}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 17.84$, 19.07 (CH_3 Val); 31.26 (β-CH Val); 54.61 (α-CH Val); 58.76 ($(OCH_3)_{ME}$); 60.25 (α-CH Ser); 62.79 (β-CH_2 Ser); 64.23 ($(COOCH_2)_{ME}$); 65.85 (CH_2=$CHCH_2$); 70.08 ($(CH_2O)_{ME}$); 117.69 (CH_2=$CHCH_2$); 132.61 (CH_2=$CHCH_2$); 156.61 (urethane C=O); 170.26 (amide C=O); 171.76 (ester C=O).

Anal. Calcd for $C_{15}H_{26}N_2O_7$ (346.4): C, 52.01; H, 7.57; N, 8.09. Found: C, 51.89; H, 7.68; N, 8.09.

N-Fluorenyl-9-methoxycarbonyl-L-glycyl-L-serine (2-Methoxyethyl) Ester (4f):

Colorless crystals; mp. 107°C; $[\alpha]_D^{22} = +4.4$ ($c = 1$, MeOH); $R_f = 0.62$ (EtOAc).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 3.35$ (s, 3 H, $(OCH_3)_{ME}$); 3.54 (t, $J_{vic} = 3.8$ Hz, 2 H, $(CH_2O)_{ME}$); 3.86 (d, $J_{gem} = 10.4$ Hz, $J_{\alpha H,\beta H} = 4.1$ Hz, 1H, $(\beta\text{-CH}_2)_{a,Ser}$); 3.99–4.06 (m, 3 H, $(\beta\text{-CH}_2)_{b,Ser}$, $(CH_2)_{Gly}$); 4.22–4.54 (2 m, 4 H, $(\alpha\text{-CH})_{Ser}$, $(H\text{-}9)_{Fmoc}$, $(COOCH_2)_{ME}$); 4.50 (d, $J_{vic} = 5.6$ Hz, 2 H, $(CH_2O)_{Fmoc}$); 5.38 (t, $J_{NH,\alpha H} = 8.9$ Hz, 1 H, $(NH)_{Gly}$); 7.34 (d, $J_{NH,\alpha H} = 8.8$ Hz, 1 H, $(NH)_{Ser}$); 7.28–7.40 (m, 4 H, $(H\text{-}2, H\text{-}3, H\text{-}6, H\text{-}7)_{Fmoc}$); 7.60 (d, $J_{vic} = 8.0$ Hz, 2 H, $(H\text{-}1, H\text{-}8)_{Fmoc}$); 7.72 (d, $J_{vic} = 7.6$ Hz, 2 H, $(H\text{-}4, H\text{-}5)_{Fmoc}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 39.72$ (α-CH_2 Gly); 47.27 ($(C\text{-}9)_{Fmoc}$); 57.45 (α-CH Ser); 58.88 ($(OCH_3)_{ME}$); 63.01 (β-CH_2 Ser); 63.33 ($(COOCH_2)_{ME}$); 67.50 ($(CH_2O)_{Fmoc}$); 69.81 ($(CH_2O)_{ME}$); 124.77, 126.34, 127.05, 127.81 (ArC); 141.72, 142.83, 142.50 ($(ArC)_{ipso}$); 156.10 (urethane C=O); 170.26 (amide C=O); 171.20 (ester C=O).

Anal. Calcd for $C_{23}H_{26}N_2O_7$ (442.5): C, 62.43; H, 5.92; N, 6.33. Found: C, 62.48; H, 5.96; N, 6.35.

Lipase-Catalyzed Hydrolysis of Peptide ME esters. *N*-Protected Dipeptides 5:

N-Protected dipeptide 2-methoxyethyl esters **4** (3 mmol) were dissolved in acetone (5–15 mL). Aqueous sodium phosphate buffer (0.2 M, 50 mL, pH = 7) was added, and the mixture was warmed up to 37°C. After addition of lipase N (Amano) (300 mg), the solution or suspension, respectively, was shaken for 15 h to 2 d at 37°C. It was saturated with NaCl and the dipeptide extracted with EtOAc. If necessary, HOAc was added before extraction. The organic layer was dried ($MgSO_4$) and the solvent removed in vacuo. The residue was purified by flash chromatography (EtOAc/HOAc 100:1). The yields are given in Table 3.

N-tert-Butyloxycarbonyl-L-phenylalanyl-L-alanine (5a):

Colorless crystals; mp. 96°C (Lit.[32]: 88°C); $[\alpha]_D^{22} = +9.3$ ($c = 1$, $CHCl_3$) (Lit.[32]: $[\alpha]_D^{22} = +11.6$ ($c = 2$, $CHCl_3$)); $R_f = 0.75$ (EtOAc/HOAc 10:1).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.29$ (d, $J_{\alpha H,\beta H} = 8.8$ Hz, 3 H, $(CH_3)_{Ala}$); 1.41 (s, 9 H, $(CH_3)_3$); 3.11 (d, $J_{\beta H,\alpha H} = 5.1$ Hz, 2 H, $(CH_2)_{Phe}$); 4.31–4.41 (m, 1 H, $(\alpha\text{-CH})_{Ala}$); 4.49 (t, $J_{\alpha H,\beta H} = 6.8$ Hz, 1 H, $(\alpha\text{-CH})_{Phe}$); 5.41 (d, $J_{NH,\alpha H} = 7.4$ Hz, 1 H, $(NH)_{Phe}$); 6.73 (d, $J_{NH,\alpha H} = 7.1$ Hz, 1 H, $(NH)_{Ala}$); 7.29–7.44 (m, 5 H, $(ArH)_{Phe}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 16.99$ (CH_3 Ala); 27.82 ($C(CH_3)_3$); 38.34 (β-CH_2 Phe); 49.40 (α-CH Ala); 53.71 (α-CH Phe); 79.93 ($C(CH_3)_3$); 126.29, 128.53, 128.98, 129.19 (ArC); 135.89 ($(CAr)_{ipso}$); 155.87 (urethane C=O); 169.51 (amide C=O); 174.46 (acid C=O).

Anal. Calcd for $C_{17}H_{24}N_2O_5$ (336.4): C, 60.70; H, 7.19; N, 8.33. Found: C, 60.98; H, 6.96; N, 7.87.

N-tert-Butyloxycarbonyl-L-glycyl-L-leucine (5b):

Colorless crystals; mp 96°C (Lit.[33a]: 112°C); $[\alpha]_D^{22} = -18.6$ ($c = 1$, MeOH); (Lit.[33b]: $[\alpha]_D^{22} = -21.0$ ($c = 0.2$, CH_3OH); $R_f = 0.46$ (EtOAc/HOAc 50:1).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 0.78$ (d, $J_{\gamma H,\delta H} = 6.1$ Hz, 3H, $2(CH_3)_{Leu}$); 0.83 (d, $J_{\gamma H,\delta H} = 5.9$ Hz, 3 H, $2(CH_3)_{Leu}$); 1.30–1.38 (m, 1 H, (g-CH Leu)); 1.41 (s, 9 H, $C(CH_3)_3$); 1.47–1.65 (m, 2H, $(\beta\text{-CH}_2)_{Leu}$); 3.85 (d, $J_{\alpha H,NH} = 6.0$ Hz, 2 H, $(CH_2)_{Gly}$); 4.34–4.45 (m, 1 H, $(\alpha\text{-CH})_{Leu}$); 5.86 (t, $J_{NH,\alpha H} = 5.2$ Hz, 1 H, $(NH)_{Gly}$); 6.90 (d, $J_{NH,\alpha H} = 7.7$ Hz, 1 H, $(NH)_{Leu}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 21.99$, 22.58 (CH_3 Leu); 23.89 (γ-CH Leu); 27.94 ($C(CH_3)_3$); 40.91 (β-CH_2 Leu); 42.93 (α-CH_2 Gly); 52.69 (α-CH Leu); 80.86 ($C(CH_3)_3$); 120.72, 126.14, 127.44, 128.88, 129.02 (Ar–C); 135.24 ((C–$Ar)_{ipso}$); 155.79 (urethane C=O); 171.60 (amide C=O); 174.71 (acid C=O).

N-tert-Butyloxycarbonyl-L-seryl-L-leucine (5c):

Colorless crystals; mp 138°C; $[\alpha]_D^{22} + 7.1$ ($c = 1$, MeOH); $R_f = 0.31$ (EtOAc/HOAc 10:1).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 0.92$ (d, $J_{\gamma H,\delta H} = 5.3$ Hz, 3 H, $(CH_3)_{Leu}$); 0.95 (d, $J_{\gamma H,\delta H} = 5.7$ Hz, 3 H, $(CH_3)_{Leu}$); 1.38 (s, 9 H, $C(CH_3)_3$); 1.41–1.63 (m, 3 H, $(\beta\text{-CH}_2)_{Leu}$ and $(\gamma\text{-CH})_{Leu}$); 3.56–3.92 (m, 2 H $(\beta\text{-CH}_2)_{Ser}$); 4.18–4.49 (m, 2 H, $(\alpha\text{-CH})_{Ser}$ and $(\alpha\text{-CH})_{Leu}$); 5.81 (d, $J_{NH,\alpha H} = 7.4$ Hz, 1 H, $(NH)_{Ser}$); 6.34 (d, $J_{NH,\alpha H} = 6.6$ Hz, 1 H, $(NH)_{Leu}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 21.95$, 22.62 (CH_3 Leu); 25.04 (γ-CH Leu); 27.82 ($C(CH_3)_3$); 40.91 (β-CH_2 Leu); 48.62 (α-CH Leu); 56.55 (α-CH Ser); 61.57 (β-CH_2 Ser); 79.38 ($C(CH_3)_3$); 155.42 (urethane C=O); 171.72 (amide C=O); 173.78 (acid C=O).

Anal. Calcd for $C_{14}H_{26}N_2O_6$ (318.4): C, 52.82; H, 8.23; N, 8.80. Found: C, 52.93; H, 8.50; N, 8.42.

N-Benzyloxycarbonyl-L-threonyl-glycine (5d):
Colorless oil; $[\alpha]_D^{22} - 36.3$ ($c = 1$, DMF) (Lit.[34]: $[\alpha] = -33.0$ ($c = 0.2$, DMF)); $R_f = 0.43$ (EtOAc/HOAc 10:1).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.07$ (d, $J_{\gamma H,\beta H} = 5.0$ Hz, 3 H, $(CH_3)_{Thr}$); 3.85 (d, $J_{\gamma H,NH} = 7.6$ Hz, 2 H, $(CH_2)_{Gly}$); 4.28–4.44 (m, 2 H, $(\alpha\text{-}CH)_{Thr}$, $(\beta\text{-}CH)_{Thr}$); 5.11 (s, 2 H, $(CH_2)_Z$); 7.44 (s, 5 H, $(ArH)_Z$); 7.62 (d, $J_{NH,\alpha H} = 8.9$ Hz, 1 H, $(NH)_{Thr}$); 7.73 (t, $J_{NH,\alpha H} = 7.9$ Hz, 1 H, $(NH)_{Gly}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 20.00$ (CH_3 Thr); 40.03 (α-CH_2 Gly); 57.17 (α-CH Thr); 66.21, 67.06 ($(CH_2)_Z$, β-CH Thr); 127.12, 127.35, 127.73, 128.49 (ArC); 135.61 ($(CAr)_{ipso}$); 154.90 (urethane C=O); 169.23 (amide C=O); 172.66 (acid C=O).

N-Allyloxycarbonyl-L-valyl-L-serine (5e):

Colorless oil; $[\alpha]_D^{22} = -16.9$ ($c = 1$, MeOH); $R_f = 0.56$ (EtOAc/HOAc 5:1).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 0.89$ (d, $J_{\gamma H,\beta H} = 6.4$ Hz, 3 H, $(CH_3)_{Val}$); 0.94 (d, $J_{\gamma H,\beta H} = 6.3$ Hz, 3 H, $(CH_3)_{Val}$); 2.10–2.26 (m, 1 H, $(\beta\text{-}CH)_{Val}$); 3.63 (dd, $J_{gem} = 9.7$ Hz, $J_{\alpha H,\beta H} = 4.7$ Hz, 1 H, $(\beta\text{-}CH_2)_{Ser,a}$); 3.81 (dd, $J_{gem} = 9.8$ Hz, $J_{\alpha H,\beta H} = 4.7$ Hz, 1 H, $(\beta\text{-}CH_2)_{Ser,b}$); 3.92–4.01 (m, 1 H, $(\alpha\text{-}CH)_{Val}$); 4.49 (d, $J_{vic} = 5.3$ Hz, 2 H, $CH_2{=}CHCH_2)_{Aloc}$); 4.53–4.66 (m, 1 H, $(\alpha\text{-}CH)_{Ser}$); 5.21 (dd, $J_{gem} = 10.3$ Hz, $J_{vic} = 1.5$ Hz, 1 H, $(CH_2{=}CHCH_2)_{Aloc,cis}$); 5.33 (dd, $J_{gem} = 11.2$ Hz, $J_{vic} = 1.4$ Hz, 1 H, $(CH_2{=}CHCH_2)_{Aloc,trans}$); 5.69–5.81 (m, 1 H, $(CH)_{Aloc}$); 7.02 (d, $J_{NH,\alpha H} = 7.7$ Hz, 1 H, $(NH)_{Val}$); 7.91 (d, $J_{NH,\alpha H} = 7.8$ Hz, 1 H, $(NH)_{Ser}$).
^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 18.54$, 18.98 (CH_3 Val); 31.19 (β-CH Val); 53.62 (α-CH Val); 57.43 (α-CH Ser); 61.37 (β-CH_2 Ser); 64.98 ($CH_2{=}CHCH_2$); 117.68 ($CH_2{=}CHCH_2$); 132.65 ($CH_2{=}CHCH_2$); 155.18 (urethane C=O); 169.67 (amide C=O); 171.66 (acid C=O).

Anal. calcd for $C_{12}H_{20}N_2O_6$ (288.3): C, 49.99; H, 6.99; N, 9.72. Found: C, 49.65 ; H, 7.38 ; N, 9.43.

N-Fluorenyl-9-methoxycarbonyl-L-glycyl-L-serine (5f):
Colorless crystals; mp 184°C; $[\alpha]_D^{22} - 3.1$ ($c = 1$, MeOH); $R_f = 0.71$ (EtOAc/HOAc 5:1).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 3.80$ (d, $J_{gem} = 0.9$ Hz, $J_{\alpha H,\beta H} = 3.9$ Hz, 1 H, $(\beta\text{-}CH_2)_{a,Ser}$); 3.86–3.99 (m, 3H, $(\beta\text{-}CH_2)_{b,Ser}$, $(CH_2)_{Gly}$); 4.23 (t, $J_{H9,CH2} = 7.3$ Hz, 1 H, $(H\text{-}9)_{Fmoc}$); 4.38–4.42 (m, 1 H, $(\alpha\text{-}CH)_{Ser}$); 4.40 (d, $J_{vic} = 6.4$ Hz, 2 H, $(CH_2O)_{Fmoc}$); 7.03 (t, $J_{NH,\alpha H} = 8.4$ Hz, 1 H, $(NH)_{Gly}$); 7.26–7.41 (m, 5 H, $(NH)_{Ser}$, $(H\text{-}2, H\text{-}3, H\text{-}6, H\text{-}7)_{Fmoc}$); 7.58 (d, $J_{vic} = 8.3$ Hz, 2 H, $(H\text{-}1, H\text{-}8)_{Fmoc}$); 7.74 (d, $J_{vic} = 8.0$ Hz, 2 H, $(H\text{-}4, H\text{-}5)_{Fmoc}$).
^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 40.04$ (α-CH_2 Gly); 47.14 ($(C\text{-}9)_{Fmoc}$); 56.58 (α-CH Ser); 62.76 (β-CH_2 Ser); 67.29 $((CH_2O)_{Fmoc})$; 120.36, 124.07, 126.10, 127.23 (ArC); 141.35, 142.97 $((ArC)_{ipso})$; 155.15 (urethane C=O); 171.21 (amide C=O); 173.50 (acid C=O).

Anal. Calcd for $C_{20}H_{20}N_2O_6$ (384.4): C, 62.49; H, 5.24; N, 7.29. Found: C, 62.94; H, 5.33; N, 6.86.

L-Aspartic Acid Di-ME-ester Hydrogentoluenesulfonate (6):
Methoxyethanol (50 mL, 0.6 mol) was added to a stirred mixture of aspartic acid (13.3 g, 0.1 mol) and p-TsOH · H_2O (22.8 g, 0.12 mol) in benzene (300 mL). The mixture was refluxed for 12 h. The solvent was evaporated in vacuo. The crude aspartic diester salt was used directly in the synthesis of *N*-protected compounds **7**; yellow oil; $[\alpha]_D^{22} = -2.6$ ($c = 1.5$, MeOH); $R_f = 0.55$ (MeOH/EtOAc 3:1).

N-Benzyloxycarbonyl-L-aspartic Acid Di(2-methoxyethyl) Ester (7a) and N-2,2,2-Trichloroethoxycarbonyl-L-aspartic Acid Di(2-methoxyethyl) Ester (7b):
To a stirred solution of crude **6** (3 g, 5.9 mmol) in CH_2Cl_2 (40 mL) was added dropwise at 0°C benzyl chloroformate (0.9 mL, 6.4 mmol) or 2,2,2-trichloroethyl chloroformate (0.8 mL, 6.2 mmol). Subsequently, Et_3N (1.7 mL, 12.3 mmol) was added dropwise. The solution was stirred at r.t. for 5 h, washed with 0.5 M HCl (2 × 30 mL) and dried ($MgSO_4$). After evaporation of the solvent, the residue was purified by flash chromatography (petroleum ether/EtOAc 2:1).

7a: Yield 1.8 g (80 %); colorless oil; $[\alpha]_D^{22} = 14.5$ ($c = 1$, MeOH); $R_f = 0.61$ (EtOAc), $R_f = 0.20$ (EtOAc/petroleum ether 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.90$ (dd, $J_{gem} = 15.2$ Hz, $J_{\beta H,\alpha H} = 3.8$ Hz, 1 H, $(\beta\text{-}CH_2)_{a,Asp}$); 3.13 (dd, $J_{gem} = 15.9$ Hz, $J_{\beta H,\alpha H} = 4.6$ Hz, 1 H, $(\beta\text{-}CH_2)_{b,Asp}$); 3.31, 3.32 (2 s, 6 H, OCH_3); 3.36–3.47 (m, 4 H, $2(CH_2OCH_3)_{ME}$); 4.09–4.26 (m, 4 H, $(COOCH_2)_{ME}$); 4.49 (d, $J_{\alpha H,NH} = 8.3$ Hz, 1 H, $(\alpha\text{-}CH)_{Asp}$); 5.11 (s, 2 H, $(CH_2O)_Z$); 5.71 (d, $J_{NH,\alpha H} = 8.5$ Hz, 1 H, $(NH)_{Asp}$); 7.35 (s, 5 H, $(ArH)_Z$).
^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 36.75$ (β-C Asp); 50.65 (α-C Asp); 58.83 (OCH_3); 63.74, 64.25 $((COOCH_2)_{ME})$; 67.17 $((CH_2O)_Z)$; 70.09 $(CH_2O)_{ME}$); 128.21, 128.59, 128.74 (ArC); 136.62 $((ArC)_{ipso})$; 154.85 (urethane C=O); 170.18, 170.38 (ester C=O).

7b: Yield 2.0 g (80 %); colorless oil; $[\alpha]_D^{22} = -20.7$ ($c = 1$, MeOH); $R_f = 0.58$ (EtOAc).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.76$ (dd, $J_{gem} = 15.1$ Hz, $J_{\beta H,\alpha H} = 3.7$ Hz, 1 H, $(\beta\text{-}CH_2)_{a,Asp}$); 3.10 (dd, $J_{gem} = 14.9$ Hz, $J_{\beta H,\alpha H} = 4.5$ Hz, 1 H, $(\beta\text{-}CH_2)_{b,Asp}$); 3.34, 3.36 (2 s, 6 H, OCH_3); 3.39–3.49 (m, 4 H, $2(CH_2OCH_3)_{ME}$); 4.21–4.33 (m, 4 H, $(COOCH_2)_{ME}$); 4.51 (d, $J_{\alpha H,NH} = 9.1$ Hz, 1 H, $(a\text{-}CH)_{Asp}$); 4.75 (s, 2 H, $(CH_2)_{Tcoc}$); 6.01 (d, $J_{NH,\alpha H} = 9.0$ Hz, 1 H, NH).
^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 36.77$ (β-C Asp); 50.35 (α-C Asp); 58.36 (OCH_3); 63.82, 64.77 $((COOCH_2)_{ME})$; 70.19 $(CH_2O)_{ME}$); 74.56 $((CH_2)_{Tcoc})$; 95.01 $((Cl_3C)_{Tcoc})$; 154.51 (urethane C=O); 170.13, 170.25 (ester C=O).

N-Benzyloxycarbonyl-L-aspartic Acid α-(2-Methoxyethyl) Ester (8a) and N-2,2,2-Trichloroethoxycarbonyl-L-aspartic Acid α-(2-Methoxyethyl) Ester (8b):
To aqueous sodium phosphate buffer (0.2 M, 20 mL, pH = 7) at 37°C was added *N*-protected aspartic acid di-ME ester **7** (1.5 mmol) and lipase A6 (Amano) (200 mg). After shaking for 90 min, the solution was saturated with brine and the product extracted with EtOAc (5 × 20mL). The organic layer was dried ($MgSO_4$), and the solvent evaporated in vacuo. The remainder was purified by flash chromatography (petroleum ether/EtOAc/HOAc 100:50:1).

8a: Yield 0.4 g (82 %); colorless oil; $[\alpha]_D^{22} = -3.2$ ($c = 1$, MeOH); $R_f = 0.47$ (EtOAc/HOAc 50:1), $R_f = 0.38$ ($CHCl_3$/MeOH/HOAc 120:10:5).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.88$ (dd, $J_{gem} = 16.8$ Hz, $J_{\beta H,\alpha H} = 4.4$ Hz, 1 H, $(\beta\text{-}CH_2)_{a,Asp}$); 3.09 (dd, $J_{gem} = 17.0$ Hz, $J_{\beta H,\alpha H} = 3.9$ Hz, 1 H, $(\beta\text{-}CH_2)_{b,Asp}$); 3.34 (s, 3 H, OCH_3); 3.55 (t, $J_{vic} = 4.4$ Hz, 2 H, $(CH_2O)_{ME}$); 4.24 (t, $J_{vic} = 5.0$ Hz, 2 H, $(COOCH_2)$); 4.67 (ddd, $J_{\alpha H,NH} = 8.3$ Hz, $J_{\alpha H,\beta aH} = 4.3$ Hz, $J_{\alpha H,\beta bH} = 4.0$ Hz, 1 H, $(\alpha\text{-}CH)_{Asp}$); 5.11 (s, 2 H, $(CH_2)_Z$); 5.97 (d, $J_{NH,\alpha H} = 8.6$ Hz, 1 H, NH); 7.25–7.33 (m, 5 H, ArH); 7.66 (s_b, 1 H, COOH).
^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 36.22$ (β-C Asp); 50.16 (α-C Asp); 58.63 $((OCH_3)_{ME})$; 63.73 $((COOCH_2)_{ME})$; 67.07 $((CH_2O)_Z)$; 69.92 $((CH_2O)_{ME})$; 127.98, 128.08, 128.40 (ArC); 135.95 $((ArC)_{ipso})$; 156.02 (urethane C=O); 170.57 (ester C=O); 174.54 (acid C=O).

Anal. Calcd for $C_{15}H_{19}NO_7$ (325.3): C, 55.38; H, 5.89; N, 4.31. Found: C, 55.47; H, 5.88; N, 4.02.

8b: Yield 0.4 g (74 %); colorless solid; $[\alpha]_D^{22} = -20.7$ ($c = 1$, MeOH); $R_f = 0.46$ (EtOAc/HOAc 50:1).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.96$ (dd, $J_{gem} = 17.3$ Hz, $J_{\beta H,\alpha H} = 4.6$ Hz, 1 H, $(\beta\text{-}CH_2)_{a,Asp}$); 3.05 (dd, $J_{gem} = 17.3$ Hz, $J_{\beta H,\alpha H} = 4.7$ Hz, 1H, $(\beta\text{-}CH_2)_{b,Asp}$); 3.34 (s, 3 H, OCH_3); 3.36–3.50 (m, 2 H, $(CH_2OCH_3)_{ME}$); 4.15–4.29 (m, 2 H, $(COOCH_2)_{ME}$); 4.51–4.57 (m, 1 H, $(\alpha\text{-}CH)_{Asp}$); 4.74 (s, 2 H, $(CH_2)_{Tcoc}$); 6.00 (d, $J_{NH,\alpha H} = 8.1$ Hz, 1 H, NH).
^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 36.20$ (β-C Asp); 50.64 (α-C Asp); 58.85 (OCH_3); 63.84 $((COOCH_2)_{ME})$; 69.01 $(COOCH_2CH_2)$; 74.70 $((CH_2)_{Tcoc})$; 95.30 $((Cl_3C)_{Tcoc})$; 154.61 (urethane C=O); 168.70, (ester C=O); 174.13 (acid C=O).

Anal. Calcd for $C_{10}H_{14}NO_7Cl_3$ (366.6): C, 32.76; H, 3.85; N, 3.82. Found: C, 32.48; H, 3.90; N, 3.55.

N^2-Benzyloxycarbonyl-N^4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-asparagine (2-Methoxyethyl) Ester (9a) and

N^2-2,2,2-Trichloroethoxycarbonyl-N^4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-asparagine (2-Methoxyethyl) Ester (9b):

To a solution of **8a** (1.2 g, 4 mmol) or **8b** (1.5 g, 4.1 mmol), respectively, in anhyd CH_2Cl_2 (30 mL), isobutyl 2-isobutoxy-1,2-dihydroquinoline-1-carboxylate[23] (IIDQ 1.4 g, 4.7 mmol) was added. The solution was stirred for 15 min. After addition of a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine (1.4 g, 4 mmol) in anhyd CH_2Cl_2 (20 mL), the stirring was continued for 8 h. The solution was washed twice with sat. aq $NaHCO_3$ (2 × 30 mL), 0.1 M HCl (30 mL), sat. brine (30 mL) and dried ($MgSO_4$). The solvent was evaporated in vacuo and the crude product was purified by flash chromatography (EtOAc).

9a: Yield 2.0 g (78 %); colorless crystals; mp. 201°C; $[\alpha]_D^{22} = +6.6$ ($c = 1$, MeOH); $R_f = 0.43$ ($CHCl_3$/MeOH 10:1); $R_f = 0.52$ (acetone).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.91$ (s, 3H, NH*Ac*); 2.00, 2.02, 2.04 (3s, 9H, OAc); 2.69 (dd, $J_{gem} = 17.1$ Hz, $J_{\beta H,\alpha H} = 4.2$ Hz, 1H, $(\beta\text{-}CH_2)_{a,Asn}$); 2,86 (dd, $J_{gem} = 17.1$ Hz, $J_{\beta H,\alpha H} = 4.0$ Hz, 1H, $(\beta\text{-}CH_2)_{b,Asn}$); 3.34 (s, 3H, OCH_3); 3.55 (t, $J_{vic} = 4.2$ Hz, 2H, $(CH_2O)_{ME}$); 3.71–3.74 (m_c, 1H, H-5); 4.00–4.29 (m, 5H, $(COOCH_2)_{ME}$, H-2, H-6); 4.60 (ddd, $J_{\alpha H,NH} = 8.2$ Hz, $J_{\alpha H,\beta aH} = J_{\alpha H,\beta bH} = 4.2$ Hz, 1H, $(\alpha\text{-}CH)_{Asn}$); 5.03–5.07 (m, 3H, H-1, H-3, H-4); 5.08 (s, 2H, $(CH_2O)_Z$); 5.96 (d, $J_{NH,\alpha H} = 8.3$ Hz, 1H, $(\alpha\text{-}NH)_{Asn}$); 6.23 (d, $J_{NH,H2} = 8.2$ Hz, 1H, NHAc); 7.26 (d, $J_{NH,H1} = 5.2$ Hz, 1H, $(\beta\text{-}NH)_{Asn}$); 7.31 (m, 5H, $(ArH)_Z$).

^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 20.45, 20.56, 20.59$ (CH_3COO); 22.80 (CH_3CONH); 37.89 (β-C Asn); 50.61 (α-C Asn); 52.91 (C-2); 58.74 (-OCH_3); 61.75 (C-6); 64.44 ($(COOCH_2)_{ME}$); 66.88 ($(CH_2O)_Z$); 67.86 (C-5); 70.01 (CH_2OCH_3); 72.96, 73.42 (C-3, C-4); 79.44 (C-1); 127.93, 128.04, 128.38 (ArC); 136.10 $(ArC)_{ipso}$); 155.93 (urethane C=O); 169.21, 170.54, 170.98 (COOME, CH_3COO); 171.30 (amide C=O); 172.00 (CH_3CONH).

Anal. Calcd for $C_{29}H_{39}N_3O_{14}$ (653.6): C, 53.29; H, 6.01; N, 6.42. Found: C, 53.35; H, 6.10; N, 6.40.

9b: Yield 2.2 g (76 %); colorless crystals; mp. 93°C; $[\alpha]_D^{22} = +102.5$°C ($c = 1$, MeOH); $R_f = 0.58$ ($CHCl_3$/MeOH 5:1); $R_f = 0.23$ ($CHCl_3$/MeOH 10:1).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.96$ (s, 3H, NH*Ac*); 2.01, 2.04, 2.06 (3s, 9H, OAc); 2.73 (dd, $J_{gem} = 17.2$ Hz, $J_{\beta H,\alpha H} = 4.5$ Hz, 1H, $(\beta\text{-}CH_2)_{a,Asn}$); 2.91 (dd, $J_{gem} = 17.0$ Hz, $J_{\beta H,\alpha H} = 4.6$ Hz, 1H, $(\beta\text{-}CH_2)_{b,Asn}$); 3.36 (s, 3H, OCH_3); 3.58 (t, $J_{vic} = 4.6$ Hz, 2H, $(CH_2O)_{ME}$); 3.71–3.74 (m_c, 1H, H-5); 4.02–4.31 (m, 5H, $(COOCH_2)_{ME}$, H-2, H-6); 4.60 (ddd, $J_{\alpha H,NH} = 8.7$ Hz, $J_{\alpha H,\beta aH} = J_{\alpha H,\beta bH} = 4.3$ Hz, 1H, $(\alpha\text{-}CH)_{Asn}$); 4.71 (s, 2H, $(CH_2O)_{Tcoc}$); 5.04–5.12 (m, 3H, H-1, H-3, H-4); 6.30 (d, $J_{NH,\alpha H} = 7.7$ Hz, 2H, $(\alpha\text{-}NH)_{Asn}$ and NHAc); 7.34 (d, $J_{NH,H1} = 8.4$ Hz, 1H, $(\beta\text{-}NH)_{Asn}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 20.48, 20.58, 20.61$ (CH_3COO); 22.89 (CH_3CONH); 37.58 (β-C Asn); 50.82 (α-C Asn); 53.12 (C-2); 58.80 (OCH_3); 61.89 (C-6); 64.66 ($(COOCH_2)_{ME}$); 68.05 (C-5); 70.08 (CH_2OCH_3); 72.90, 73.61 (C-3, C-4); 74.69 ($(CH_2O)_{Tcoc}$); 79.63 (C-1); 95.32 ($(Cl_3C)_{Tcoc}$); 154.36 (urethane C=O); 169.23, 170.34, 170.54, 170.57 (COOME, CH_3COO); 171.38 (amide C=O); 172.08 (CH_3CONH).

Anal. Calcd for $C_{24}H_{34}N_3O_{14}Cl_3$ (694.9): C, 41.48; H, 4.93; N, 6.05. Found: C, 41.46; H, 4.99; N, 5.89.

N^2-Benzyloxycarbonyl-N^4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-asparagine (10a) and N^2-2,2,2-Trichloroethoxycarbonyl-N^4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-asparagine (10b):

To a solution of **9a** (1.0 g, 1.5 mmol) in acetone (5 mL) or **9b** (2.0 g, 2.9 mmol) in acetone (12 mL), respectively, aq sodium phosphate buffer (0.2 M, 40 mL, pH = 7) and lipase A6 (Amano) (200 mg) were added. After shaking for 14 h at 37°C, the solution was saturated with NaCl and the product extracted with EtOAc (5 × 30 mL). The organic layer was dried ($MgSO_4$) and the solvent evaporated in vacuo. The remaining residue was purified by flash chromatography (EtOAc/HOAc 100:1).

10a: Yield 690 mg (77 %); colorless crystals; mp. 211°C; $[\alpha]_D^{22} = -66.1$ ($c = 1$, MeOH); $R_f = 0.26$ ($CHCl_3$/MeOH 10:1); $R_f = 0.42$ (EtOAc/HOAc 10:2).

^{1}H NMR (200 MHz, DMSO-d_6): $\delta = 1.90$ (s, 3H, NH*Ac*); 1.91, 1.96, 1.99 (3s, 9H, OAc); 2.41–2.89 (m, 2H, $(\beta\text{-}CH_2)_{Asn}$); 3.47–3.84 ($m_c$, 1H, H-5); 4.02–4.38 (m, 3H, H-2, H-6); 4.76 (m, 1H, $(\alpha\text{-}CH)_{Asn}$); 5.01 (s, 2H, $(CH_2O)_Z$); 5.05–5.21 (m, 3H, H-1, H-3, H-4); 7.35 (m, 5H, $(ArH)_Z$); 7.39 (d, $J_{NH,\alpha H} = 6.4$ Hz, 1H, $(\alpha\text{-}NH)_{Asn}$); 7.90 (d, $J_{NH,H2} = 8.2$ Hz, 1H, NHAc); 8.59 (d, $J_{NH,H1} = 9.1$ Hz, 1H, $(\beta\text{-}NH)_{Asn}$).

^{13}C NMR (50.3 MHz, DMSO-d_6): $\delta = 20.31, 20.45, 20.76$ (CH_3COO); 22.48 (CH_3CONH); 36.79 (β-C Asn); 50.02 (α-C Asn); 52.03 (C-2); 61.75 (C-6); 65.40 ($(CH_2O)_Z$); 67.73 (C-5); 72.20, 73.02 (C-3, C-4); 77.94 (C-1); 126.65, 127.66, 128.24 (ArC); 136.77 $(ArC)_{ipso}$); 155.73 (Urethane C=O); 169.22, 169.40, 169.68 (CH_3COO); 169.95 (amide C=O); 172.85 (CH_3CONH, acid C=O).

Anal. Calcd for $C_{26}H_{33}N_3O_{13}$ (595.6): C, 52.44; H, 5.58; N, 7.06. Found: C, 52.02; H, 5.74; N, 7.40.

10b: Yield 1.5 g (83 %); colorless crystals; mp. 188°C; $[\alpha]_D^{22} = +110.1$ ($c = 1$, MeOH); $R_f = 0.51$ ($CHCl_3$/MeOH 5:1); $R_f = 0.38$ ($CHCl_3$/MeOH 10:1).

^{1}H NMR (200 MHz, DMSO-d_6): $\delta = 1.98$ (s, 3H, NH*Ac*); 2.03, 2.04, 2.07 (3s, 9H, OAc); 2.85 (d, $J_{gem} = 15.1$ Hz, 1H, $(\beta\text{-}CH_2)_{a,Asn}$); 2.96 (d, $J_{gem} = 15.0$ Hz, 1H, $(\beta\text{-}CH_2)_{b,Asn}$); 3.84 (d, $J_{vic} = 8.7$ Hz, 1H, H-5); 4.06–4.23 (m, 2H, H-2, H-6); 4.61 (dd, $J_{\alpha H,NH} = 8.0$ Hz, $J_{\alpha H,\beta H} = 4.3$ Hz, 1H, $(\alpha\text{-}CH)_{Asn}$); 4.75 (s, 2H, $(CH_2O)_{Tcoc}$); 5.09–5.25 (m, 3H, H-1, H-3, H-4); 6.59 (d, $J_{NH,\alpha H} = 8.1$ Hz, 1H, $(\alpha\text{-}NH)_{Asn}$); 7.20 (d, $J_{NH,H2} = 9.6$ Hz, 1H, NHAc); 7.53 (d, $J_{NH,H1} = 7.9$ Hz, 1H, $(\beta\text{-}NH)_{Asn}$).

^{13}C NMR (50.3 MHz, DMSO-d_6): $\delta = 20.75$ (CH_3COO); 22.65 (CH_3CONH); 37.45 (β-C Asn); 50.50 (α-C Asn); 53.37 (C-2); 62.09 (C-6); 68.36 (C-5); 72.87, 73.44 (C-3, C-4); 74.78 ($(CH_2O)_{Tcoc}$); 79.08 (C-1); 95.34 ($(Cl_3C\text{-})_{Tcoc}$); 154.74 (urethane C=O); 169.70, 170.92, 171.13 (CH_3COO); 171.31 (amide C=O); 173.37 (CH_3CONH); 173.57 (acid C=O).

Anal. Calcd for $C_{21}H_{28}N_3O_{13}Cl_3$ (636.8): C, 39.61; H, 4.43; N, 6.60. Found: C, 39.46; H, 4.65; N, 6.45.

N^2-Benzyloxycarbonyl-N^4-(2-acetamido-3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl)-L-asparagine (2-Methoxyethyl) Ester (11):

A solution of **8a** (1.0 g, 3 mmol) in anhyd CH_2Cl_2 (30 mL) and isobutyl 2-isobutoxy-1,2-dihydroquinoline-1-carboxylate[23] (1.2 g, 4 mmol) was stirred at r.t. for 15 min. A solution of 2-acetamido-3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosylamine (1.4 g, 2 mmol) in anhyd CH_2Cl_2 (20 mL) was added. After stirring for 13 h, the solution was washed with sat. aq $NaHCO_3$ (2 × 30 mL), 0.1 M HCl (30 mL) and sat. brine (30 mL) and dried ($MgSO_4$). The solvent was evaporated in vacuo, and the crude product was purified by flash chromatography (petroleum ether/EtOAc 2:1); Yield 1.5 g (81 %); colorless crystals; mp. 126°C; $[\alpha]_D^{22} = +5.3$ ($c = 1$, MeOH); $R_f = 0.40$ ($CHCl_3$/MeOH 10:1).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.88$ (s, 3H, NH*Ac*); 1.94, 2.01, 2.06, 2.09, 2.12, 2.13 (6s, 18H, OAc); 2.56 (dd, $J_{gem} = 13.1$ Hz, $J_{\beta H,\alpha H} = 3.5$ Hz, 1H, $(\beta\text{-}CH_2)_{a,Asn}$); 2.81 (dd, $J_{gem} = 14.0$ Hz, $J_{\beta H,\alpha H} = 3.6$ Hz, 1H, $(\beta\text{-}CH_2)_{b,Asn}$); 3.35 (s, 3H, OCH_3); 3.58 (t, $J_{vic} = 4.5$ Hz, 2H, $(CH_2O)_{ME}$); 3.67–3.91 (m, 4H, $(H\text{-}5)_{Glc}$), $(H\text{-}2)_{Glc}$, $(H\text{-}4)_{Glc}$, $(H\text{-}6)_{a,Glc}$); 4.03 (dd, $J_{gem} = 11.2$ Hz, $J_{6,H5} = 7.0$ Hz, 1H, $(H\text{-}6)_{a,Gal}$); 4.11–4.21 (m, 5H, $(H\text{-}6)_{b,Glc}$, $(H\text{-}6)_{b,Gal}$, $(H\text{-}5)_{Gal}$, $(COOCH_2)_{ME}$); 4.47–4.48 (m, 1H, $(\alpha\text{-}CH)_{Asn}$); 4.70 (d, $J_{H1,H2} = 6.4$ Hz, 1H, $(H\text{-}1)_{Gal}$); 4.99 (s, 2H, $(CH_2O)_Z$); 5.02–5.03 (m, 1H, $(H\text{-}3)_{Gal}$); 5.09 (d, $J_{H1,H2} = 9.1$ Hz, 1H, $(H\text{-}1)_{Glc}$); 5.17–5.25 (m, 3H, $(H\text{-}3)_{Glc}$, $(H\text{-}2)_{Gal}$, $(H\text{-}4)_{Gal}$); 6.13 (d, $J_{NH,\alpha H} = 7.8$ Hz, 1H, $(\alpha\text{-}NH)_{Asn}$); 6.33 (d, $J_{NH,H2} = 8.5$ Hz, 1H, NHAc); 7.53 (d, $J_{NH,H1} = 8.5$ Hz, 1H, $(\beta\text{-}NH)_{Asn}$); 7.56–7.67 ($(ArH)_Z$).

^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 20.14, 20.21, 20.34, 20.94, 20.98$ (CH_3COO); 22.57 (CH_3CONH); 36.89 (β-C Asn); 51.34 (α-C Asn); 53.96 (C-2 Glc); 58.83 (OCH_3); 60.48 (C-6 Glc); 61.74 (C-6 Gal); 64.37 ($(COOCH_2)_{ME}$); 65.03 ($(CH_2O)_Z$); 65.58 (C-4 Gal); 67.76, 68.22

(C-2 Gal, C-3 Gal); 69.81 (C-5 Gal); 70.40 (CH_2OCH_3); 71.41 (C-4 Glc); 73.19 (C-5 Glc); 76.92 (C-3 Glc); 80.33 (C-1 Glc); 100.03 (C-1 Gal); 126.73, 127.46, 128.70 (ArC); 136.67 $(ArC)_{ipso}$); 155.21 (urethane C=O); 165.96, 168.36, 169.22, 169.23, 170.10, 170.16, 170.67, 171.80 (CH_3COO, *C*OOME); 172.20 (amide C=O); 172.44 (CH_3CONH).

Anal. Calcd for $C_{41}H_{55}N_3O_{22}$ (941.9): C, 52.28; H, 5.89; N, 4.46. Found: C, 51.88; H, 5.91; N, 4.90.

N^2-Benzyloxycarbonyl-N^4-(2-acetamido-3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl)-L-asparagine (12):

To a solution of **11** (0.9 g, 1 mmol) in acetone (3 mL), aq sodium phosphate buffer solution (0.2 M, 20 mL, pH = 7) and lipase A6 (Amano) (300 mg) were added. After shaking for 15 h at 37°C, the solution was saturated with NaCl and the product extracted with EtOAc (5 × 30 mL). The organic layer was dried ($MgSO_4$), and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (EtOAc/HOAc 100:1); yield 670 mg (76 %); colorless crystals; mp. 185°C; $[\alpha]_D^{22} = +87.7$ ($c = 0.5$, MeOH); $R_f = 0.59$ ($CHCl_3$/MeOH/HOAc 2:2:1); $R_f = 0.39$ (EtOAc/HOAc 5:1).

^{1}H NMR (200 MHz, DMSO-d_6): $\delta = 1.92$ (s, 3H, NH*Ac*); 2.01, 2.02, 2.03, 2.07, 2.10, 2.12 (6s, 18H, OAc); 2.62 (d, $J = 3.8$ Hz, 1H, $(\beta\text{-}CH_2)_{a,Asn}$); 2.83 (dd, $J_{gem} = 13.4$ Hz, $J_{\beta H,\alpha H} = 3.7$ Hz, 1H, $(\beta\text{-}CH_2)_{b,Asn}$); 3.75–3.89 (m, 4H, $(H\text{-}5)_{Glc}$, $(H\text{-}2)_{Glc}$, $(H\text{-}4)_{Glc}$, $(H\text{-}6)_{a,Glc}$); 4.00 (dd, $J_{gem} = 11.7$ Hz, $J_{H6,H5} = 8.0$ Hz, 1H, $(H\text{-}6)_{a,Gal}$); 4.10–4.28 (m, 3H, $(H\text{-}6)_{b,Glc}$, $(H\text{-}6)_{b,Gal}$, $(H\text{-}5)_{Gal}$); 4.31–4.35 (m, 1H, $(\alpha\text{-}CH)_{Asn}$); 4.61 (d, $J_{H1,H2} = 7.2$ Hz, 1H, $(H\text{-}1)_{Gal}$); 5.07 (s, 2H, $(CH_2O)_Z$); 5.11–5.16 (m, 1H, $(H\text{-}3)_{Gal}$); 5.29 (d, $J_{H1,H2} = 9.0$ Hz, 1H, $(H\text{-}1)_{Glc}$); 5.35–5.56 (m, 3H, $(H\text{-}3)_{Glc}$, $(H\text{-}2)_{Gal}$, $(H\text{-}4)_{Gal}$); 7.33 (d, $J_{NH,\alpha H} = 7.9$ Hz, 1H, $(\alpha\text{-}NH)_{Asn}$); 7.54 (s, 5H, $(ArH)_Z$); 7.85 (d, $J_{NH,H2} = 8.6$ Hz, 1H, NHAc); 8.58 (d, $J_{NH,H1} = 8.6$ Hz, 1H, $(\beta\text{-}NH)_{Asn}$).

^{13}C NMR (50.3 MHz, DMSO-d_6): $\delta = 20.03$, 20.13, 20.16, 20.79, 20.97 (CH_3COO); 22.16 (CH_3CONH); 36.68 (β-C Asn); 49.81 (α-C Asn); 53.79 (C-2 Glc); 60.27 (C-6 Glc); 61.38 (C-6 Gal); 65.29 $((CH_2O)_Z)$; 65.59 (C-4 Gal); 67.70, 68.67 (C-2 Gal, C-3 Gal); 69.98 (C-5 Gal); 70.22 (C-4 Glc); 72.49 (C-5 Glc); 75.69 (C-3 Glc); 80.23 (C-1 Glc); 100.13 (C-1 Gal); 126.08, 127.47, 128.07 (ArC); 136.39 $(ArC)_{ipso}$); 155.43 (urethane C=O); 166.71, 168.21, 169.41, 169.46, 170.05, 170.29, 170.55 (*C*OOME); 171.76 (amide C=O); 172.82 (CH_3CONH, acid C=O).

Anal. Calcd for $C_{38}H_{49}N_3O_{21}$ (883.8): C, 51.66; H, 5.59; N, 4.75. Found: C, 51.66; H, 5.11; N, 4.57.

N-Benzyloxycarbonyl-L-phenylalanyl-N^4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-asparagine (2-Methoxyethyl) Ester (13):

The 2-(*N*-glycosyl)asparagine ester **9a** (1.3 g, 2 mmol) was dissolved in EtOH (50 mL). Conc. HCl (0.2 mL) and palladium (5 %) on charcoal (100 mg) were added. Hydrogenation was carried by stirring the solution for 6 h under H_2 atmosphere. After filtration on Hyflo-Supercell, the solvent was evaporated in vacuo. The residue was dissolved in anhyd CH_2Cl_2 (50 mL) and added to a ice-cooled solution of *N*-benzyloxycarbonyl-L-phenylalanine (0.4 g, 1.4 mmol), dicyclohexyl carbodiimide (0.4 g, 2 mmol) and 1-hydroxybenzotriazole (0.5 g, 3 mmol) in anhyd CH_2Cl_2 (50 mL), which already had been stirred for 15 min. Et_3N (0.3 mL, 2 mmol) was added, and the solution was stirred for 16 h at r.t. After washing with 0.1 M HCl (2 × 50 mL), sat. aq $NaHCO_3$ (2 × 50 mL), sat. brine (50 mL) and drying ($MgSO_4$), the solvent was evaporated in vacuo, the residue was purified by flash chromatography (petroleum ether/EtOAc 4:1); Yield 0.9 g (78 %); colorless crystals; mp. 118°C; $[\alpha]_D^{22} = -7.5$ ($c = 1$, MeOH); $R_f = 0.46$ (EtOAc).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.89$ (s, 3H, NH*Ac*); 2.02, 2.04, 2.08 (3s, 9H, OAc); 2.67 (dd, $J_{gem} = 15.9$ Hz, $J_{\beta H,\alpha H} = 4.1$ Hz, 1H, $(\beta\text{-}CH_2)_{a,Asn}$); 2.92 (dd, $J_{gem} = 16.0$ Hz, $J_{\beta H,\alpha H} = 4.5$ Hz, 1H, $(\beta\text{-}CH_2)_{b,Asn}$); 3.03 (dd, $J_{gem} = 14.3$ Hz, $J_{vic} = 4.9$ Hz, 1H, $(\beta\text{-}CH_2)_{a,Phe}$); 3.21 (dd, $J_{gem} = 13.9$ Hz, $J_{vic} = 5.7$ Hz, 1H, $(\beta\text{-}CH_2)_{b,Phe}$); 3.35 (s, 3H, OCH_3); 3.43 (t, $J_{vic} = 4.5$ Hz, 2H, $(CH_2O)_{ME}$); 3.75–3.77 (m, 1H, H-5); 4.02–4.21 (m, 4H, $(COOCH_2)_{ME}$, H-2, H-6); 4.49–4.57 (m, 1H, $(\alpha\text{-}CH)_{Asn}$); 4.86–5.00 (m, 2H, H-1, $(\alpha\text{-}CH)_{Phe}$); 5.04–5.12 (m, 2H, H-3, H-4); 5.13 (s, 2H, $(CH_2O)_Z$); 6.34 (d, $J_{NH,\alpha H} = 7.1$ Hz, 1H, $(NH)_{Phe}$); 6.80 (d, $J_{NH,H2} = 8.0$ Hz, 1H, NHAc); 7.11–7.35 (m, 11H, $(ArH)_Z$), $(ArH)_{Phe}$, $(\beta\text{-}NH)_{Asn}$); 7.50 (d, $J_{NH,\alpha H} = 7.7$ Hz, 1H, $(\alpha\text{-}NH)_{Asn}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 20.20$, 20.27, 20.79 (CH_3COO); 23.13 (CH_3CONH); 37.24, 37.35 (β-C Asn, β-C Phe); 49.93 (α-C Asn); 52.68 (C-2); 53.73 (α-CH Phe); 58.74 (OCH_3); 62.13 (C-6); 64.26 $((COOCH_2)_{ME})$; 67.74 $((CH_2O)_Z)$; 67.95 (C-5); 69.72 (CH_2OCH_3); 73.43, 73.44 (C-3, C-4); 78.97 (C-1); 125.65, 127.16, 127.40, 128.78, 129.22, 129.70 (ArC); 136.01, 136.77 $(ArC)_{ipso}$); 155.68 (urethane C=O); 169.50, 170.01, 170.16, 172.73 (*C*OOME, CH_3COO, amide C=O); 172.45 (CH_3CONH).

Anal. Calcd for $C_{38}H_{48}N_4O_{15}$ (800.8): C, 56.99; H, 6.04; N, 7.00. Found: C, 56.76; H, 6.11; N, 6.67.

N-Benzyloxycarbonyl-L-phenylalanyl-N^4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-asparagine (14):

To a solution of glycodipeptide ester **13** (0.9 g, 1.1 mmol) in acetone (5 mL), aq sodium phosphate buffer (0.2 M, 40 mL, pH = 7) and lipase A6 (Amano) (200 mg) were added. The mixture was shaken for 18 h at 37°C, the solution saturated with brine and the product extracted with EtOAc (5 × 50mL). The organic layer was dried ($MgSO_4$) and the solvent evaporated in vacuo. The residue was purified by flash chromatography (EtOAc); yield 640 mg (78%); colorless crystals; mp. 84°C; $[\alpha]_D^{22} = +16.6$ ($c = 1$, MeOH); $R_f = 0.54$ (EtOAc/HOAc 5:1).

^{1}H NMR (200 MHz, DMSO-d_6): $\delta = 1.90$ (s, 3H, NH*Ac*); 1.98, 2.03, 2.07 (3s, 9H, OAc); 2.60–2.68 (m, 1H, $(\beta\text{-}CH_2)_{a,Asn}$); 2.88 (dd, $J_{gem} = 16.2$ Hz, $J_{\beta H,\alpha H} = 4.8$ Hz, 1H, $(\beta\text{-}CH_2)_{b,Asn}$); 3.06 (dd, $J_{gem} = 15.1$ Hz, $J_{\beta H,\alpha H} = 4.8$ Hz, 1H, $(\beta\text{-}CH_2)_{a,Phe}$); 3.13 (d, $J_{\beta H,\alpha H} = 5.3$ Hz, 1H, $(b\text{-}CH_2)_{b,Phe}$); 3.80–3.83 (m, 1H, H-5); 4.00–4.12 (m, 2H, H-2, H-6); 4.81 (mc, 1H, $(\alpha\text{-}CH)_{Asn}$); 4.92–5.09 (m, 4H, H-1, $(\alpha\text{-}CH)_{Phe}$, H-3, H-4); 5.10 (s, 2H, $(CH_2O)Z$); 7.33–7.37 (m, 10H, $(ArH)_Z$), $(ArH)_{Phe}$); 7.41 (d, $J_{NH,\alpha H} = 8.7$ Hz, 1H, $(NH)_{Phe}$); 7.74 (d, $J_{NH,H2} = 8.9$ Hz, 1H, NHAc); 7.91 (d, $J_{NH,\alpha H} = 8.7$ Hz, 1H, $(\alpha\text{-}NH)_{Asn}$); 8.13 (d, $J_{NH,H1} = 8.9$ Hz, 1H, $(\beta\text{-}NH)_{Asn}$).

^{13}C NMR (200 MHz, DMSO-d_6): $\delta = 20.15$, 20.80, 21.31 (CH_3COO); 22.40 (CH_3CONH); 36.75, 37.78 (β-C Asn, β-C Phe); 47.10 (α-C Asn); 52.38 (C-2); 53.69 (α-CH Phe); 62.11 (C-6); 67.17 $((CH_2O)_Z)$; 68.50 (C-5); 73.51, 73.56 (C-3, C-4); 79.09 (C-1); 125.66, 126.53, 127.18, 128.86, 129.56 (ArC); 136.57, 136.94 (ArC)ipso); 155.37 (urethane C=O); 169.67, 170.58, 170.86, 171.09 (CH_3COO, amide C=O); 172.16, 172.51 (CH_3CONH, acid C=O).

Anal. Calcd for $C_{44}H_{59}N_5O_{16}$ (914.0): C, 56.60; H, 5.70; N, 7.54. Found: C, 56.23; H, 5.61; N, 7.25.

N-Benzyloxycarbonyl-L-seryl-L-alanine (2-Methoxyethyl) Ester (15):

A solution of *N*-benzyloxycarbonyl-L-serine (9.6 g, 40 mmol) and isobutyl 2-isobutoxy-1,2-dihydroquinoline-1-carboxylate (13.2 g, 44 mmol) in anhyd CH_2Cl_2 (200 mL) was stirred for 15 min. A solution of crude L-alanine-2-methoxyethyl ester hydrotoluenesulfonate (19 g, 45 mmol, see general procedure for compounds **4**) and Et_3N (6.5 mL, 45 mmol) in anhyd CH_2Cl_2 (50 mL) was added. The solution was stirred for 5 h, washed with 1.0 M HCl (2 × 80 mL), sat. aq $NaHCO_3$ (2 × 80 mL), sat. brine (80 mL) and dried ($MgSO_4$). After evaporation of the solvent, the residue was purified by flash chromatography (petroleum ether/EtOAc 4:1); yield 13.0 g (88 %); colorless crystals; mp. 67°C; $[\alpha]_D^{22} = -7.2$ ($c = 1$, MeOH); $R_f = 0.60$ ($CHCl_3$/MeOH/HOAc 60:10:5); $R_f = 0.26$ (EtOAc).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.39$ (d, $J_{\beta H,\alpha H} = 7.2$ Hz, 3H, $(CH_3)_{Ala}$); 3.35 (s, 3H, $(OCH_3)ME$); 3.56 (t, $J_{vic} = 4.4$ Hz, 2H, $(CH_2O)_{ME}$); 3.66–4.08 (m, 3H (OH)Ser, $(\beta\text{-}CH_2)_{Ser}$); 4.12–4.25 (m, 3H, (α-CH)Ala and $(COOCH_2)_{ME}$); 4.55 (t, $J_{vic} = 7.3$ Hz, 1H, $(\alpha\text{-}CH)_{Ser}$); 5.09 (s, 2H, $(CH_2)_Z$); 5.93 (d, $J_{NH,\alpha H} = 7.5$ Hz, 1H, $(NH)_{Ser}$); 7.15 (d, $J_{NH,\alpha H} = 6.8$ Hz, 1H, $(NH)_{Ala}$); 7.32 (s, 5H, $(ArH)_Z$).

^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 17.36$ (CH_3 Ala); 48.27 (α-CH Ala); 55.61 (α-CH Ser); 58.73 $((OCH_3)_{ME})$; 62.85 ($\beta\text{-}CH_2$ Ser); 64.16 $((COOCH_2)_{ME})$; 66.96 $((CH_2)_Z)$; 70.02 $((CH_2O)_{ME})$; 127.84, 128.02, 128.35 (ArC); 135.98 $((CAr)_{ipso})$; 156.26 (urethane C=O); 170.43 (amide C=O); 172.62 (ester C=O).

Anal. Calcd for $C_{30}H_{44}N_5O_{17}Cl_3$ (853.1): C, 55.43; H, 6.57; N, 7.60. Found: C, 55.45; H, 6.48; N, 7.47.

N^2-2,2,2-Trichloroethoxycarbonyl-N^4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-asparaginyl-L-seryl-L-alanine (2-Methoxyethyl) Ester (16):

To a solution of dipeptide ester **15** (2.0 g, 5.5 mmol) in EtOH (50 mL), conc. HCl (0.1 mL) and palladium (5 %) on charcoal (100 mg) were added. The solution was stirred for 5 h under H_2 atmosphere. After filtration through Hyflo-Supercell, the solvent was evaporated in vacuo. The residue was dissolved in anhyd CH_2Cl_2 (30 mL), and this solution was added to a ice-cooled solution of **10b** (2.9 g, 4.6 mmol), dicyclohexyl carbodiimide (1.3 g, 6.5 mmol) and 1-hydroxybenzotriazole (1.0 g, 6.5 mmol) in anhyd CH_2Cl_2 (50 mL), which had been stirred for 15 min. Et_3N (0.7 mL, 4.8 mmol) was added. The solution was stirred for 15 h at r.t., washed with 0.1 M HCl (2 × 50 mL), sat. aq $NaHCO_3$ (2 × 50 mL), sat. aq brine (50 mL) and dried ($MgSO_4$). After evaporation of the solvent, the residue was purified by flash chromatography (petroleum ether/EtOAc 4:1); yield 2.2 g (56 %); colorless crystals; mp. 113°C; $[\alpha]_D^{22} = -5.2$ ($c = 1$, MeOH); $R_f = 0.55$ ($CHCl_3$/MeOH 5:1).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.31$ (d, $J_{\beta H,\alpha H} = 7.4$ Hz, 3 H, $(CH_3)_{Ala}$); 1.83 (s, 3 H, NH*Ac*); 2.02, 2.04, 2.09 (3 s, 9 H, OAc); 2.59 (dd, $J_{gem} = 15.6$ Hz, $J_{\beta H,\alpha H} = 4.8$ Hz, 1 H, $(\beta\text{-}CH_2)_{a,Asn}$); 2.82 (dd, $J_{gem} = 14.9$ Hz, $J_{\beta H,\alpha H} = 4.7$ Hz, 1 H, $(\beta\text{-}CH_2)_{b,Asn}$); 3.33 (s, 3 H, OCH_3); 3.49 (t, $J_{vic} = 4.8$ Hz, 2 H, $(CH_2O)_{ME}$); 3.48–3.61 (m, 2 H, $(\beta\text{-}CH_2)_{Ser}$); 3.79 (d, $J_{H5,H4} = 8.9$ Hz, 1 H, H-5); 3.96–4.06 (m, 1 H, $(\alpha\text{-}CH)_{Ala}$); 4.22–4.37 (m, 6 H, H-2, $(H\text{-}6)_a$, $(H\text{-}6)_b$, $(\alpha\text{-}CH)_{Ser}$, $(COOCH_2)_{ME}$); 4.56 (s, 2 H, $(CH_2O)_{Tcoc}$); 4.78 (d, $J_{\alpha H,NH} = 8.7$ Hz, 1 H, $(\alpha\text{-}CH)_{Asn}$); 5.01–5.12 (m, 1 H, H-1); 5.04–5.12 (m, 2 H, H-3, H-4); 6.16 (d, $J_{NH,\alpha H} = 7.9$ Hz, 1 H, $(\alpha\text{-}NH)_{Asn}$); 6.52 (d, $J_{NH,\alpha H} = 7.4$ Hz, 1 H, $(NH)_{Ser}$); 6.74 (d, $J_{NH,H1} = 7.4$ Hz, 1 H, (NHAc) or $(NH)_{Ala}$); 6.81 (d, $J_{NH,\alpha H} = 8.7$ Hz, 1 H, (NHAc) or $(NH)_{Ala}$); 8.11 (d, $J_{NH,H1} = 8.3$ Hz, 1 H, $(\beta\text{-}NH)_{Asn}$).

^{13}C NMR (50.3 MHz, $CDCl_3$): $\delta = 18.01$ (CH_3 Ala); 19.94, 20.21, 20.47 (CH_3COO); 22.24 (CH_3CONH); 37.60 (β-C Asn); 48.12 (α-C Ala); 51.15 (α-C Asn); 54.92 (C-2); 55.13 (α-C Ser); 58.96 (OCH_3); 61.47 (β-C Ser); 61.87 (C-6); 64.41 ($COOCH_2$); 68.34, 68.54 (C-5, $COOCH_2CH_2$); 69.94, 72.48 (C-3, C-4); 73.35 ($(CH_2O)_{Tcoc}$); 79.85 (C-1); 96.03 ($(Cl_3C)_{Tcoc}$); 155.00 (urethane C=O); 169.38 (CH_3CONH); 169.52, 169.73, 170.29 (CH_3COO); 170.54, 170.90, 171.15, 171.38 (amide C=O); 172.38 (ME ester C=O).

N^2-2,2,2-Trichloroethoxycarbonyl-L-alanyl-N^4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-asparaginyl-L-seryl-L-alanine (2-Methoxyethyl) Ester (17):

Zn (2.5 g, 37 mmol) was added to 1 M HCl (50 mL) and stirred for 10 min. After filtration and washing with HOAc (3 × 20 mL), the Zn was added to a solution of glycotripeptide **16** (2.0 g, 2.3 mmol) in HOAc (30 mL). The mixture was stirred for 6 h, filtered, and the solvent was evaporated in vacuo. The residue was dried in high vacuum and dissolved in DMF/CH_2Cl_2 (1:1, 30 mL). This solution was added to a solution of *N*-2,2,2-trichloroethoxycarbonyl-L-alanine (1.1 g, 4 mmol), dicyclohexyl carbodiimide (0.8 g, 4 mmol) and 1-hydroxybenzotriazole (0.8 g, 5 mmol) in anhyd CH_2Cl_2 (30 mL), which had already been stirred for 15 min. Et_3N (0.3 mL, 2.3 mmol) was added. The solution was stirred for 10 h at r.t., filtered and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (petroleum ether/EtOAc 1:1); Yield 1.5 g (72 %); colorless solid; $[\alpha]_D^{22} = -15.3$ ($c = 1$, MeOH); $R_f = 0.44$ ($CHCl_3$/MeOH 5:1).

^{1}H NMR (200 MHz, DMSO-d_6): $\delta = 1.22$ (d, $J_{\beta H,\alpha H} = 7.0$ Hz, 3 H, $(CH_3)_{Ala,N\text{-}term}$); 1.27 (d, $J_{\beta H,\alpha H} = 7.3$ Hz, 3 H, $(CH_3)_{Ala,C\text{-}term}$); 1.76 (s, 3 H, NH*Ac*); 1.91, 1.96, 2.00 (3 s, 9 H, OAc); 2.50–2.70 (m, 2 H, $(\beta\text{-}CH_2)_{a,Asn}$, $(\beta\text{-}CH_2)_{b,Asn}$); 3.25 (s, 3 H, OCH_3); 3.33 (t, $J_{vic} = 4.8$ Hz, 2 H, $(CH_2O)_{ME}$); 3.46–3.60 (m, 2 H, $(\beta\text{-}CH_2)_{Ser}$); 3.82–3.97 (m, 3 H, H-5, $(\alpha\text{-}CH)_{Ala,C\text{-}term}$, $(\alpha\text{-}CH)_{Ala,N\text{-}term}$); 4.15–4.39 (m, 6 H, H-2, $(H\text{-}6)_a$, $(H\text{-}6)_b$, $(\alpha\text{-}CH)_{Ser}$, $(COOCH_2)_{ME}$); 4.77 (s, 2 H, $(CH_2O)_{Tcoc}$); 4.80–4.87 (m, 2 H, H-1, $(\alpha\text{-}CH)_{Asn}$); 5.06–5.21 (m, 2 H, H-3, H-4); 7.40 (d, $J_{NH,\alpha H} = 7.5$ Hz, 1 H, $(NH)_{Ser}$); 7.88 (m_c, 1 H, $(NH)_{Ala}$); 7.91 (d, $J_{NH,\alpha H} = 4.9$ Hz, 1 H, $(NH)_{Ala}$); 7.96 (d, $J_{NH,\alpha H} = 7.5$ Hz, 1 H, $(\alpha\text{-}NH)_{Asn}$); 8.11 (d, $J_{NH,H2} = 7.4$ Hz, 1H, (NHAc)); 8.66 (d, $J_{NH,H1} = 8.7$ Hz, 1 H, $(\beta\text{-}NH)_{Asn}$).

^{13}C NMR (50.3 MHz, DMSO-d_6): $\delta = 17.23$ (CH_3 Ala); 18.08 (CH_3 Ala); 20.39, 20.52 (CH_3COO); 22.68 (CH_3CONH); 36.85 (β-C Asn); 47.48 (α-C Ala); 48.58 (α-C Ala); 51.13 (α-C Asn); 52.12 (C-2); 55.08 (α-C Ser); 58.02 (OCH_3); 60.01 (β-C Ser); 61.59 (C-6); 64.31 ($COOCH_2$); 68.27 (C-5); 72.27 ($COOCH_2CH_2$); 73.35, 73.54 (C-3, C-4); 73.88 ($(CH_2O)_{Tcoc}$); 77.97 (C-1); 96.00 ($(Cl_3C)_{Tcoc}$); 154.10 (urethane C=O); 169.30 (CH_3CONH); 169.44, 169.55 (CH_3COO); 170.03, 170.55, 171.94 (amide C=O, ester C=O).

MS (FAB, $C_{33}H_{49}N_6O_{18}Cl_3$, MH^+): calcd 923.2, found 923.2.

N^2-2,2,2-Trichloroethoxycarbonyl-L-alanyl-N^4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-asparaginyl-L-seryl-L-alanine (18):

To a solution of glycotetrapeptide ester **17** (1.2 g, 1.3 mmol) in acetone (3 mL), aq sodium phosphate buffer (0.2 M, 10 mL, pH = 7) and lipase A6 (Amano) (500 mg) were added. The mixture was shaken for 17 h at 37°C. After lyophilization, the residue was dissolved in MeOH (7 mL) and crystallized by addition of Et_2O ; yield 1.0 g (85 %); colorless crystals; mp. 137°C; $[\alpha]_D^{22} = -69.7$ ($c = 1$, MeOH); $R_f = 0.41$ ($CHCl_3$/MeOH/HOAc 60:10:5); $R_f = 0.13$ ($CHCl_3$/MeOH 5:1).

^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.20$ (d, $J_{\beta H,\alpha H} = 6.6$ Hz, 3 H, $(CH_3)_{Ala,N\text{-}term}$); 1.26 (d, $J_{\beta H,\alpha H} = 7.0$ Hz, 3 H, $(CH_3)_{Ala,C\text{-}term}$); 1.74 (s, 3 H, NH*Ac*); 1.89, 1.95, 1.98 (3 s, 9 H, OAc); 2.44–2.63 (m, 2 H, $(\beta\text{-}CH_2)_{a,Asn}$, $(\beta\text{-}CH_2)_{b,Asn}$); 3.53–3.60 (m, 2 H, $(\beta\text{-}CH_2)_{Ser}$); 3.75–3.85 (m, 2H, $(\alpha\text{-}CH)_{Ala,C\text{-}term}$, $(\alpha\text{-}CH)_{Ala,N\text{-}term}$); 3.91–3.94 (m, 1 H, H-5); 4.15–4.27 (m, 3 H, H-2, $(H\text{-}6)_a$, $(H\text{-}6)_b$,); 4.36 (d, $J_{\alpha H,NH} = 6.5$ Hz, 1H, $(\alpha\text{-}CH)_{Ser}$); 4.75 (d, $J_{\alpha H,NH} = 10.2$ Hz, 1 H, $(\alpha\text{-}CH)_{Asn}$); 4.80 (s, 2 H, $(CH_2O)_{Tcoc}$); 4.83 (m, 1 H, H-1); 5.07–5.17 (m, 2 H, H-3, H-4); 7.38 (d, $J_{NH,\alpha H} = 7.9$ Hz, 1 H, $(NH)_{Ser}$); 7.87 (d, $J_{NH,\alpha H} = 6.5$ Hz, 1 H, $(NH)_{Ala}$); 7.90 (m_c, 1 H, $(NH)_{Ala}$); 7.93 (d, $J_{NH,\alpha H} = 7.1$ Hz, 1 H, $(\alpha\text{-}NH)_{Asn}$); 8.09 (d, $J_{NH,H2} = 6.7$ Hz, 1 H, (NHAc)); 8.63 (d, $J_{NH,H1} = 8.8$ Hz, 1 H, $(\beta\text{-}NH)_{Asn}$); 10.50 (s_{br}, 1 H, COOH).

^{13}C NMR (100.6 MHz, DMSO-d_6): $\delta = 17.23$ (CH_3 Ala); 18.03 (CH_3 Ala); 20.32, 20.45 (CH_3COO); 22.61 (CH_3CONH); 37.87 (β-C Asn); 47.46 (α-C Ala); 48.55 (α-C Ala); 51.20 (α-C Asn); 51.20 (C-2); 55.03 (α-C Ser); 61.57 (β-C Ser); 61.73 (C-6); 68.34 (C-5); 72.30, 73.35 (C-3, C-4); 73.59 ($(CH_2O)_{Tcoc}$); 78.01 (C-1); 95.99 ($(Cl_3C\text{-})_{Tcoc}$); 154.06 (urethane C=O); 169.22 (CH_3CONH); 169.40, 169.45, 169.54 (CH_3COO); 169.94, 170.02, 170.53, 171.88 (amide C=O); 173.79 (acid C=O).

MS (FAB, $C_{30}H_{43}N_6O_{17}Cl_3$, MH^+): calcd 865.2, found 865.7.

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