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Regio- and Enantioselectivity of the *Candida antarctica* Lipase Catalyzed Amidations of Cbz-L- and Cbz-D-Glutamic Acid Diesters

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Abstract: *Candida antarctica* lipase (CAL) catalyzed amidation of Cbz-glutamic acid diesters takes place in a regioselective way to give the corresponding monoamide derivatives. The regioselectivity was found to be dependent on the reacting Glu enantiomer. Thus, amidations of Cbz-L-Glu diesters regiospecifically afforded α-amide while the γ-ester is selectively substituted in the D-enantiomer. This enzymatic reaction also shows enantioselectivity when a chiral amine is used as nucleophile.

INTRODUCTION

The synthesis of an oligopeptide analogue undertaken by our group required differentiation between the α– and γ-carbethoxy groups of diethyl N-Cbz-L-glutamate. Modification of one out of several nearly identical groups in a molecule (regioselectivity) is usually very difficult when performed by classical chemical methods because it requires several blocking-deblocking steps which are often not too selective. Nowadays, the application of enzymes to chemical reactions is an increasingly important alternative approach in organic synthesis because, when suitable conditions are found, it may reduce complicated multisteps reactions to a single step. Lipases add an outstanding stability and easy manipulation to the general advantages of using enzymes (mild experimental conditions and high substrate or product specificity) which made them the preferred ones to work in anhydrous organic solvents. Amminolysis of esters is an unnatural reaction for lipases (triacylglycerol hydrolases, EC 3.1.1.3) but there are precedents of lipase-catalyzed amidations of esters by amines,[1] hydrazines[2] and even ammonia.[3] Although some of the first Klibanov´s[4] and Wong´s[5] papers on enzymes in organic solvents dealt with peptide synthesis catalyzed by PPL, regioselective reactions on dicarboxylic aminoacid derivatives as acyl donors are scarcely described: a japanese patent[6] claims the regioselective α-hydrolysis (90% yield) of L-Glu(OBu)-OBu catalyzed by Lipase OF (a Meito Sangyo's lipase from *Candida rugosa*). On the other hand, regioselective γ-hydrolysis catalyzed by the same lipase has been described[7] when the substrates are N-unblocked D-Glu diesters. In this case, the hydrolysis ratio γ:α depends greatly on the alcohol moiety: dicyclopentyl ester displayed a high ratio (20:1) while that of dicyclohexyl, dibenzyl and dibutyl esters were poorer (5.1, 1.6 and 0.8:1 respectively). According to our knowledge, there is not any example of amidations of these bifunctionalyzed aminoacids in organic solvents. In this paper we report the initial results of our studies on the *Candida antarctica* lipase (CAL) mediated regioselective amidation of N-Cbz-L- and N-Cbz-D-glutamic acid diesters and the subsequent formation of the corresponding monoamide derivatives.

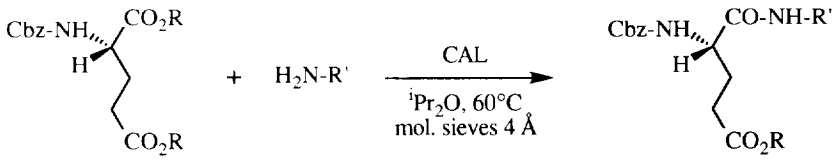
RESULTS AND DISCUSSION

SYNTHESIS

In order to find enzymes capable of catalyzing the reaction, we performed an initial screening of several commercial lipases[8]. In a standard experiment: lipase (50 mg/mL) and molecular sieves 4Å powder (50 mg/mL) were added to a solution of diethyl Cbz-L-glutamate (**1**) (20 mM), n-pentylamine (**3a**) (50 mM) and p-nitrobenzonitrile (20 mM) as internal standard in diisopropylether in screw-cap 2 mL vials. The resulting suspensions were incubated for one day in an orbital shaker at 45°C and analysed by HPLC. The reaction catalyzed by CAL showed a complete conversion into a unique compound while no changes were detected in the other cases, including the blank reaction without enzyme.

According to this promising result, the CAL-reaction was repeated at a preparative scale in order to isolate the product. It was identified (see below, *Structural Assignments*) as the α-monoamide Cbz-L-Glu(OEt)-NH-^{n}Pn (**4a**), displaying a parallel selectivity to that claimed by the above-mentioned patent.[6]

The amidation of **1** was also checked with other amines **3a-e** (Scheme 1) to confirm its regioselectivity and investigate how general the reaction is. These reactions were incubated at 60°C which is nearer to the maximum activity temperature (70-80°C) described by Novo Nordisk for the CAL preparation employed. We found that α-amidation took place in all cases (except with aniline which did not react) while γ-isomer was not detected.



1: R= Et
2: R= Bzl

3a: R'=^{n}Pn
b: R'=Bzl
c: R'=^{i}Pr
d: R'=cHx
e: R'=Ph

4a-d : R= Et
5a :R= Bzl

Scheme 1

The reaction afforded very high conversions (Table 1) with aliphatic amines, both when the amino group was located on a primary or a secondary carbon atom (**3a,b** and **3c,d** respectively), although reaction times were longer in the second case. By comparison, one reaction was carried out at 60 and 45°C (Table 1, entries 4 and 5): it displayed similar results although at different times. No changes were detected in a reaction involving an aromatic amine (aniline **3e**). Moreover, dibenzyl ester (**2**) was also checked as acyl donor. It was also converted into the α-monoamide and comparable results (Table 1) were obtained in a reaction with **3a** in same experimental conditions described for diethyl Cbz-L-glutamate (**1**).

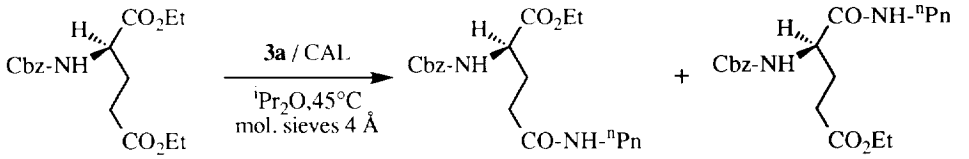
These results suggest that the reaction is strongly enantioselective and the bulky substituent Cbz-NH even as far as in a γ-position inhibits the substitution of the side-chain ester when it is situated in the unfavoured L-configuration and determines the regioselective amidation of the α-position. For the same reasons, amidations on the D-enantiomer should be directed to the γ-ester group.

TABLE 1.—General results of amidation reactions of Cbz-Glu diesters

Entry	Acyl donor	Amine	Temp.(°C)	Product		Time (h)	Conversion(%)[a]		Yield(%)[b]	
				α	γ		α	γ	α	γ
1	**1**	**3a**	60	**4a**		1	100		94	
2	**1**	**3b**	60	**4b**		1	97		66	
3	**1**	**3c**	60	**4c**		6	88		40	
4	**1**	**3d**	60	**4d**		8	91		73	
5	**1**	**3d**	45	**4d**		22	95		--[c]	
6	**1**	**3e**	60	**4e**		24	n.d.		--[c]	
7	**2**	**3a**	60	**5a**		1.5	100		86	
8	**6**	**3a**	60	**8a**	**7a**	24	16	62	--[c]	
9	**6**	**3a**	45	**8a**	**7a**	96	14	69	9	64
10	**1**	**3f**	45	**4f**		48	90		75	
11	**1**	**3g**	45	**4g**		48	n.d.		--[c]	
12	**1**	**3f,g**	45	**4f**		96	91	n.d.	75	
13	**1**	**3f,g**	60	**4f**		24	91	n.d.	--[c]	

[a]. Determined by HPLC; [b]. Isolated product, see Experimental Part. Referred to the acyl donor, yields not optimized;
[c]. Analytical scale only; α, γ. Product substituted at the α or γ position; n.d. Not detected

This hypothesis was verified when diethyl Cbz-D-glutamate (**6**) was incubated with **3a** at 60°C, following the general method described above (Scheme 2). Surprisingly, the reaction was slower and the regioselectivity, although significant, was lower than with the L-isomer **1** (see Table 1). We obtained better results when the reactions were carried out at 45°C and were stopped after 4 days, when they were stabilised. Conversions detected (HPLC) in that conditions were 69 and 14% for **7a** and **8a** respectively and 16% of unchanged **6**.

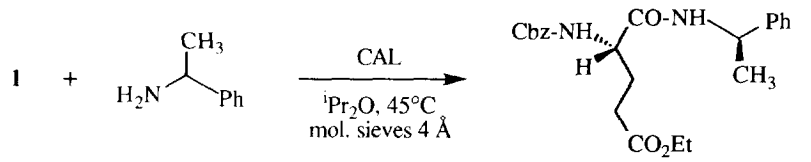


6 **7a** **8a**

Scheme 2

It has been reported that CAL displays also enantioselectivity toward chiral amines when the acyl donors are esters of propiolic and acrylic acids[9], β-arylpropionic acids[10] and β-ketoacids[11]. We checked this feature with our substrate in one reaction: pure enantiomer **1** (20 mM) was incubated with (R)-, (S)- and (R,S)-α-methylbenzylamine (**3f**, **3g** and **3f,g** respectively) (50 mM), CAL (50 mg/mL), molecular sieves 4Å (50 mg/mL) and p-nitrobenzonitrile (20 mM) in anhydrous diisopropylether at 45°C, at a 100 mg scale reaction (Scheme 3). The reaction exhibited an excellent enantioselectivity (Table 1, entries 10 to 13), displaying very good conversion and yield with the (R)-enantiomer **3f** while no reaction was detected with **3g**. As expected, the enzyme selectively distinguished between the enantiomers when the racemic amine **3f,g** was used and 75%

(**3f,g** was initially present at a 2.5:1 excess, that is, 1.25:1 excess of **3f**) of the corresponding (R)-amide was isolated (see Experimental Part). We did not observe formation of the (S)-amide. Same result, although at a shorter time, was obtained when the reaction was carried out at 60°C.



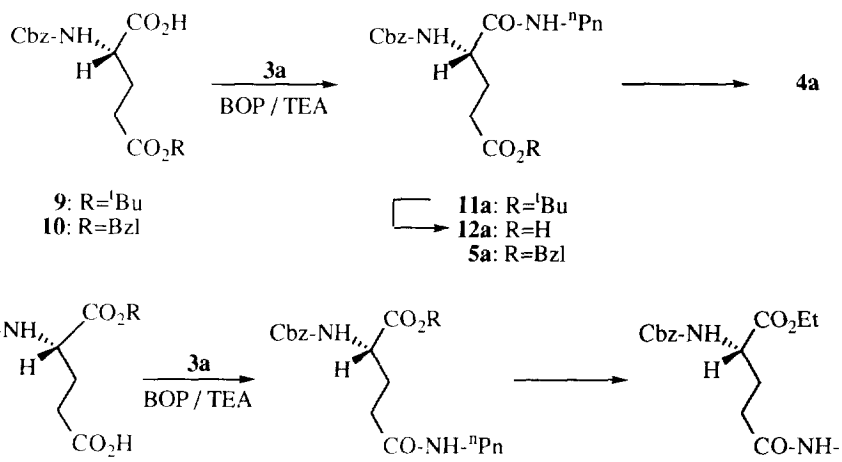
3f : (R)
3g : (S)
3f,g : (R,S)

4f
—
4f

Scheme 3

STRUCTURAL ASSIGNMENTS

As the CAL-catalyzed amidations proceed in a highly regiospecific way, we undertaken the chemical synthesis of some reference compounds to unequivocally determine the amidation position . In particular, the two regioisomeric n-pentyl monoamide Glu derivatives were prepared in both ethyl and benzyl ester form. The benzyl ester derivatives **5a** and **17a** were directly obtained by coupling the commercially available compounds Cbz-L-Glu(OBzl)-OH (**10**) and Cbz-L-Glu-OBzl (**14**), respectively, with n-pentylamine in the presence of BOP (Scheme 4).[12] A similar reaction of compounds **9** and **13** afforded the monoamide derivatives **11a** and **15a**, in which the remaining carboxylic group is protected as the *tert*-butyl ester. Removal of the ^{t}Bu group from these compounds by treatment with TFA, followed by reaction of the resulting carboxylic acids **12a** and **16a** with EtOH and 2-chloro-1-methylpyridinium iodide afforded the expected regioisomeric ethyl derivatives **4a** and **18a**.[13]

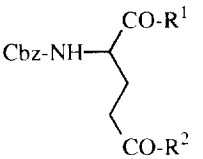


Scheme 4

Compounds **4a** and **5a** were identical (HPLC, NMR) to those Glu derivatives obtained in the CAL-catalyzed reactions of Cbz-L-Glu diesters with n-pentylamine indicating that these reactions exclusively gives the α-amide derivatives. On the other hand, the chemically synthesized compounds **4a** and **18a** showed the same analytical data, with the exception of the sign of the specific rotation, than their corresponding enantiomers **8a** and **7a** respectively, obtained in a regioselective way by the enzymatic amidation of Cbz-D-Glu(OEt)-OEt.

When the ^{1}H NMR spectra of compounds **4a** and **5a** were compared to those of the corresponding diesters **1** and **2**, it was observed that amide substitution at position α produces an upfield shift of the α-CH proton of about 0.2 ppm, while no appreciable change was observed for the γ-CH_2 resonances (Table 2). On the contrary, in the γ-monoamide derivatives **17a** and **18a** the γ-CH_2 protons are more shielded than in the corresponding diester (Δδ ~0.2 ppm). Differences in the ^{13}C NMR spectra of the regioisomeric monoamide derivatives were also found. Thus, α-amide substitution induces downfield shifts on α (~0.8 ppm), β (~0.6 ppm) and γ (~0.3 ppm) carbons.

TABLE 2.—Measured differences in the chemical shifts between Glu-monoamide derivatives and the corresponding diester



			^{1}H NMR (Δδ, ppm)[a]		^{13}C NMR (Δδ, ppm)[a]		
Compd.	R^1	R^2	α-CH	γ-CH_2	α-C	β-C	γ-C
4a(8a)	NH-^{n}Pn	OEt	0.20	-0.02	-0.87	-0.59	-0.31
5a	NH-^{n}Pn	OBzl	0.24	-0.07	-0.78	-0.64	-0.42
4b	NH-Bzl	OEt	0.12	-0.02	-0.94	-0.46	-0.28
4c	NH-iPr	OEt	0.24	-0.01	-0.86	-0.67	-0.28
4d	NH-cHx	OEt	0.26	-0.03	-0.73	-0.56	-0.14
4f	NH-MBzl[b]	OEt	0.17	0.00	-0.82	-0.50	-0.32
17a	OBzl	NH-^{n}Pn	0.11	0.28	-0.21	-0.88	-2.44
18a(7a)	OEt	NH-^{n}Pn	0.07	0.17	-0.33	-1.43	-1.45

[a] $\Delta\delta = \delta_{diester} - \delta_{amide}$; [b]. α-Methylbenzylamine

However, the differences in the ^{13}C chemical shifts of the γ-CONH-^{n}Pn derivatives **17a** and **18a**, when compared to the corresponding diesters, followed the order γ>β>α (Table 2). These differences in the ^{1}H and ^{13}C NMR chemical shifts between the regioisomeric amide derivatives and their symmetric diesters were used to establish the amidation position of compounds **4b-f**, for which no model compounds were synthesized. The calculated Δδ ($\delta_{diester}$- δ_{amide}) values for these compounds were similar to those of α-amidated compounds **4a** and **5a**, and clearly differ from those obtained for their respective regioisomers, **18a** and **17a**.

CONCLUSIONS

We have reported CAL-catalysed amidations of Cbz-Glu diesters. This reaction takes place in a enantioselective manner that determines the regioselectivity of the substitution. Thus, L-Glu derivatives are regiospecifically monosubstituted in the α-ester while γ-ester is preferentially changed in its D-Glu. The reaction is also enantiospecifc for the nucleophile and (R)-α-methylbenzylamine displays good results while (S)-enantiomer remains unchanged.

EXPERIMENTAL

Starting amino acid derivatives were obtained from BACHEM and used without further purification. Diisopropylether was refluxed on sodium wire, distilled and stored on molecular sieves 4Å powder before using. Analytical TLC was performed on aluminium sheets coated with 0.2 mm layer of silica gel 60 F_{254} (Merck). Silica gel 60 (230-400 mesh, Merck) was used for column chromatography. Compounds were detected with UV light (λ= 254 nm) or ninhydrin. ^1H NMR spectra were recorded with a Varian Gemini 200 operating at 200 MHz using Me_4Si as internal standard. ^{13}C NMR spectra were recorded with a Varian Gemini 200 (50 MHz). Carbon assignments were performed by heteronuclear (C-H) correlations (HETCOR). Elemental analyses were obtained on a CHN-O-RAPID apparatus. Analytical HPLC was performed on a Beckman chromatograph using a Spherisorb C_{18} column (4.6 × 250 mm, 5 μm) column with MeOH (A)/H_2O (B) system, A/B = 75:25, as eluent (Flow rate, 1 mL/min) with UV detection λ: 215 nm.

CHEMICAL SYNTHESIS OF MODEL COMPOUNDS:
Cbz-L-Glu(OR)-NH-^{n}Pn (**4a**, **5a**) and Cbz-L-Glu(NH-^{n}Pn)-OR (**17a**, **18a**).

Chemical Amidations

General procedure.– A solution of the corresponding Glu derivative (3 mmol) in dry THF (60 mL) was treated with BOP (1.46 g, 3.3 mmol), TEA (0.46 mL, 3.3 mmol) and n-penthylamine (348 μL, 3 mmol). After stirring at room temperature overnight, the solvent was evaporated to dryness. The resulting residue was dissolved in ethyl acetate and washed twice with H_2O. The organic extract was dried (Na_2SO_4) and then evaporated. The final product was purified on a silica gel column using hexane: ethyl acetate, 3:1.

Cbz-L-Glu(O^{t}Bu)-NH-^{n}Pn (**11a**).- Obtained as a white solid in 68% (0.83 g) from compound **9** (1.012 g, 3 mmol). Elemental analysis: Calcd. for $C_{22}H_{34}N_2O_5$: C 65.00, H 8.43, N 6.89. Found: C 65.29, H 8.28, N 6.71.

Cbz-L-Glu(NH-^{n}Pn)-O^{t}Bu (**15a**).- Obtained as a white solid in 72% (0.85 g) from compound **13** DCHA salt (1.56 g, 3 mmol). Elemental analysis: Calcd. for $C_{22}H_{34}N_2O_5$: C 65.00, H 8.43, N 6.89. Found: C 64.99, H 8.05, N 7.05.

Cbz-L-Glu(OBzl)-NH-^{n}Pn (**5a**).- Obtained as a white solid in 90% (1.19 g) from compound **10** (1.1 g, 3 mmol). $[\alpha]_D$ = -7.8 (c1, MeOH). HPLC: t_R = 12.8 min. Elemental analysis: Calcd. for $C_{25}H_{32}N_2O_5$: C 68.16, H 7.32, N 6.36. Found: C 67.89, H 7.18, N 6.40.

Cbz-L-Glu(NH-^{n}Pn)-OBzl (**17a**).- Obtained as a white solid in 80% (1.05 g) from compound **14** (1.1 g, 3 mmol). $[\alpha]_D$ = -14.4 (c1, MeOH). HPLC: t_R = 11.6 min. Elemental analysis: Calcd. for $C_{25}H_{32}N_2O_5$: C 68.16, H 7.32, N 6.36. Found: C 67.98, H 7.30, N 6.45.

Cleavage of ^{t}Bu Esters

General procedure.– A solution of compound **11a** or **15a** (0.244 g, 0.6 mmol) in CH_2Cl_2 (2 mL) was treated with TFA (2 mL) and stirred at room temperature for 4h. Then, the solvent was evaporated to dryness.

Cbz-L-Glu-NH-^{n}Pn (**12a**).- Obtained as a white solid in 94% (0.197 g) from compound **11a.** Elemental analysis: Calcd. for $C_{18}H_{26}N_2O_5$: C 61.70, H 7.48, N 7.99. Found: C 62.01, H 7.34, N 8.21.

Cbz-L-Glu(NH-^{n}Pn)-OH (**16a**).- Obtained as syrup in 90% (0.190 g) from compound **15a**. Elemental analysis: Calcd. for $C_{18}H_{26}N_2O_5$: C 61.70, H 7.48, N 7.99. Found: C 61.92, H 7.30, N 8.16.

Esterification with EtOH

General procedure.– A solution of compound **12a** or **16a** (67 mg, 0.2 mmol) in toluene (1 mL) was treated with EtOH (100 µL) and tributylamine (114 µL, 0.48 mmol). Then, a solution of 2-chloro-1-methylpyridinium iodide (61 mg, 0.24 mmol) in toluene (1 mL) was added. After stirring at 60°C for 3h, another portion of EtOH (100 µL) was added and the reaction continued at room temperature overnight. Evaporation of the solvents and purification on a silica gel column, using hexane: ethyl acetate (2:1) as eluent, afforded the final pure compound.

Cbz-L-Glu(OEt)-NH-^{n}Pn (**4a**).- Obtained as a white solid in 53% (40 mg) from compound **12a**. $[\alpha]_D$ = -13.5 (c1, MeOH). HPLC: t_R = 7.00 min. Elemental analysis: Calcd. for $C_{20}H_{30}N_2O_5$: C 63.47, H 7.99, N 7.40. Found: C 63.65, H 8.05, N 7.54.

Cbz-L-Glu(NH-^{n}Pn)-OEt (**16a**).- Obtained as syrup in 44% yield (50 mg) from compound **16a**. $[\alpha]_D$ = 18.5 (c1, chloroform). HPLC: t_R = 6.50 min. Elemental analysis: Calcd. for $C_{20}H_{30}N_2O_5$: C 63.47, H 7.99, N 7.40. Found: C 63.38, H 7.99, N 7.58.

CANDIDA ANTARCTICA LIPASE CATALYZED AMIDATIONS

General procedure.– CAL (950 mg) and molecular sieves 4Å powder (950 mg) were added to a solution of the corresponding Glu derivative (0.38 mmoles), amine (0.95 mmoles) and an internal reference (0.38 mM) in diisopropylether dry (19 mL). The reaction vessel was sealed and stirred in an orbital shaker at 45°C (amidation of **6** and those with **3f** and **3f,g**) or 60°C (rest of the reactions). The reactions were periodically controlled and analyzed by HPLC. When the reaction was stabilized, the enzyme and molecular sieves were filtered off and washed with chloroform. The combined organic extract were evaporated to dryness and the resulting residue was purified on a silica gel column. Eluents, initial amounts and reactions times are specified in each case.

Amidations of N-Cbz-L-Glu(OEt)-OEt (1)

Cbz-L-Glu(OEt)-NH-^{n}Pn (**4a**).-Amidation of **1** (128 mg, 0.38 mmol), ^{n}Pn-NH_2 (110 µl, 0.95 mmol), p-nitrobenzonitrile (56 mg, 0.38 mmol), for 1h afforded a white residue which was purified by flash cromatography (hexane: EtOAc, 4:1) to give **4a** as a white solid (135 mg, 94 %). Elemental analysis: Calcd. for $C_{20}H_{30}N_2O_5$: C 63.47, H 7.99, N 7.40. Found: C 63.68, H 8.10, N 7.15.

Cbz-L-Glu(OEt)-NH-Bzl (**4b**).-Amidation of **1** (88 mg, 0.26 mmol), Bzl-NH_2 (71 µl, 0.65 mmol), p-nitrobenzonitrile (38 mg, 0.26 mmol), for 1h afforded a white residue which was purified by flash cromatography (hexane: EtOAc, 2:1) to give first unreacted **1** (2 mg, 2 %) and then **4b** as a white solid (68 mg, 66 %). $[\alpha]_D$ = 1.6 (c1, Chloroform). HPLC: t_R = 5.7 min. Elemental analysis: Calcd. for $C_{22}H_{26}N_2O_5$: C 63.32, H 6.58, N 7.08. Found: C 66.10, H 6.80, N 6.92.

Cbz-L-Glu(OEt)-NH-^{i}Pr (**4c**).-Amidation of **1** (88 mg, 0.26 mmol), ^{i}Pr-NH_2 (55 µl, 0.65 mmol), p-nitrobenzonitrile (38 mg, 0.26 mmol), for 6h afforded a white residue which was purified by flash cromatography (hexane: EtOAc, 2:1) to give first unreacted **1** (5 mg, 6%) and then **4c** as a white solid (36 mg, 40 %). $[\alpha]_D$ = -4.6 (c1, MeOH). HPLC: t_R = 4.5 min. Elemental analysis: Calcd. for $C_{18}H_{26}N_2O_5$: C 61.71, H 7.43, N 8.00. Found: C 62.00, H 7.53, N 7.81.

Cbz-L-Glu(OEt)-NH-cHx (**4d**).-Amidation of **1** (88 mg, 0.26 mmol), cHx-NH_2 (74 µl, 0.65 mmol), p-nitrobenzonitrile (38 mg, 0.26 mmol), for 8h afforded a white residue which was purified by flash cromatography (hexane: EtOAc, 3:1) to give first unreacted **1** (3 mg, 3 %) and then **4d** as a white solid (74 mg, 75 %). $[\alpha]_D$ = -5.7 (c1, MeOH). HPLC: t_R = 7.1 min. Elemental analysis: Calcd. for $C_{21}H_{29}N_2O_5$: C 64.76, H 7.51, N 7.19. Found: C 64.78, H 7.74, N 6.98.

Amidation of Cbz-L-Glu(OBzl)-OBzl (2) with ^{n}Pn-NH_2 (3a)

Cbz-L-Glu(OBzl)-NH-^{n}Pn (**5a**).-Amidation of **2** (120 mg, 0,26 mmol), ^{n}Pn-NH_2 (75 µl, 0,65 mmol), 2-chlorobenzonitrile (36 mg, 0,26 mmol), for 90 min afforded a white residue which was purified by flash cromatography (hexane: EtOAc, 3:1) to give **5a** as a white solid (98mg, 86 %). Elemental analysis: Calcd. for $C_{25}H_{32}N_2O_5$: C 68.16, H 7.32, N 6.36. Found: C 68.31, H 7.51, N 6.29.

Amidation of Cbz-D-Glu(OEt)-OEt (6) with ^{n}Pn-NH_2 (3a)

Cbz-D-Glu(NH-^{n}Pn)-OEt (**7a**) and *Cbz-D-Glu(OEt)-NH-^{n}Pn* (**8a**).-Amidation of **6** (101 mg, 0.30 mmol), ^{n}Pn-NH_2 (87 µl, 0.75 mmol), p-nitrobenzonitrile (44 mg, 0.30 mmol) for 2 days afforded a white residue which was purified by flash cromatography (hexane: EtOAc, 3:1) to give first unreacted **6** (10 mg, 10 %) and the following compounds: **8a** as a white solid (10.2 mg, 9 %). $[\alpha]_D$ = 14.0 (c1, MeOH). HPLC: t_R = 7.0 min. Elemental analysis: Calcd. for $C_{20}H_{30}N_2O_5$: C 63.47, H 7.99, N 7.40 Found: C 63.60, H 8.14, N 7.25. And **7a** as a sirup (73 mg, 64 %). $[\alpha]_D$ = - 19.3° (c1, MeOH). HPLC: t_R = 6.5 min. Elemental analysis: Calcd. for $C_{20}H_{30}N_2O_5$: C 63.47, H 7.99, N 7.40 Found: C 63.41, H 7.81, N 7.56

Amidations of Cbz-L-Glu(OEt)-OEt (1) with (R)- or (R,S)-methylbenzylamine (3f or 3f,g)

Cbz-L-Glu(OEt)-NH-CH(CH_3)-Ph (**4f**).-Amidation of **1** (94 mg, 0.28 mmol), R- or R,S-methylbenzylamine (90 µl, 0.70 mmol), p-nitrobenzonitrile (41 mg, 0.28 mmol), for 2 days afforded a white residue which was purified by flash cromatography (hexane: EtOAc, 3:1) to give first unreacted **1** (6 mg, 6 %) and then **4f** as a white solid (87 mg, 75 %). $[\alpha]_D$ = 20.0 (c1, Chloroform). HPLC: t_R = 6.5 min. Elemental analysis: Calcd. for $C_{23}H_{28}N_2O_5$: C 66.97, H 6.84, N 6.79 Found: C 66.23, H 6.62, N 7.03.

TABLE 3.—Significant ^1H NMR data of Cbz-Glu derivatives (200 MHz, $CDCl_3$, δ ppm)

Compd.	R^1	R^2	α-CH	β-CH_2	γ-CH_2	α-NH	R^1	R^2
1(6)	OEt	OEt	4.39 2.00	2.21	2.40	5.46	4.12 (CH_2, Et)[a] 1.25 (CH_3, Et)[a]	4.20 (CH_2, Et)[a] 1.28 (CH_3, Et)[a]
2	OBzl	OBzl	4.46 2.03	2.37	2.43	5.35	5.09 (CH_2, Bzl)[a]	5.10 (CH_2, Bzl)[a]
4a(8a)	NH-^{n}Pn	OEt	4.19	2.11 1.95	2.42	5.73	6.33 (NH, ^{n}Pn) 0.88 (CH_3, ^{n}Pn)	4.12 (CH_2, Et) 1.24 (CH_3, Et)
4b	NH-Bzl	OEt	4.27	2.15 1.95	2.43	5.73	6.69 (NH, Bzl) 4.42 (CH_2, Bzl)	4.10 (CH_2, Et) 1.23 (CH_3, Et)
4c	NH-iPr	OEt	4.15	2.10 1.95	2.41	5.77	6.20 (NH, ^{i}Pr) 4.02 (CH, ^{i}Pr)	4.11 (CH_2, Et) 1.23 (CH_3, Et)
4d	NH-cHx	OEt	4.13	2.09 1.95	2.43	5.66	6.11 (NH, cHx) 3.73 (CH, cHx)	4.10 (CH_2, Et) 1.25 (CH_3, Et)
4f	NH-MBzl	OEt	4.22	2.01	2.40	5.72	6.72 (NH, MBzl) 1.43 (CH, MBzl)	4.09 (CH_2, Et) 1.21 (CH_3, Et)
5a	NH-^{n}Pn	OBzl	4.22	2.13 1.97	2.50	5.78	6.34 (NH, ^{n}Pn) 0.88 (CH_3, ^{n}Pn)	5.08 (CH_2, Bzl)
11a	NH-^{n}Pn	O^{t}Bu	4.16	2.07	2.42 1.92	5.68 2.30	6.34 (NH, ^{n}Pn)	1.44 (CH_3, ^{t}Bu) 0.89 (CH_3, ^{n}Pn)
12a[b]	NH-^{n}Pn	OH	3.93	1.80	2.17	7.38	7.87 (NH, ^{n}Pn) 0.88 (CH_3, ^{n}Pn)	–
15a	O^{t}Bu	NH-^{n}Pn	4.19	1.85	2.20	5.58	1.43 (CH_3, ^{t}Bu)	5.98 (NH, ^{n}Pn) 0.87 (CH_3, ^{n}Pn)
16a[b]	OH	NH-^{n}Pn	3.91	2.00	2.17	7.59	–	7.78 (NH, ^{n}Pn) 0.88 (CH_3, ^{n}Pn)
17a	OBzl	NH-^{n}Pn	4.35	1.95	2.15	5.65	5.07 (CH_2, Bzl)	5.65 (NH, ^{n}Pn) 0.85 (CH_3, ^{n}Pn)
18a (7a)	OEt	NH-^{n}Pn	4.32	1.97	2.23	5.67	4.19 (CH_2, Et) 1.26 (CH_3, Et)	5.88 (NH, ^{n}Pn) 0.88 (CH_3, ^{n}Pn)

[a] May be interchanged. [b] Registered in DMSO-d_6

TABLE 4.—Significant ^{13}C NMR data of Cbz-Glu derivatives (50 MHz, $CDCl_3$, δ ppm)

Compd.	R^1	R^2	α-CH	β-CH_2	γ-CH_2	α-CO[a]	γ-CO[a]	R^1	R2
1(6)	OEt	OEt	53.31	27.58	30.10	171.45	172.50	13.95 (CH_3)[b]	13.98 (CH_3)[b]
2	OBzl	OBzl	53.39	27.54	29.97	171.58	172.33	66.44 (CH_2)[b]	67.28 (CH_2)[b]
4a (8a)	NH-^{n}Pn	OEt	54.18	28.17	30.41	170.85	173.46	13.91 (CH_3)[b]	14.10 (CH_3)[b]
4b	NH-Bzl	OEt	54.25	28.04	30.38	171.09	173.35	43.47 (CH_2)	14.08 (CH_3)
4c	NH-iPr	OEt	54.17	28.25	30.38	170.07	173.35	41.51 (CH)	14.10 (CH_3)
4d	NH-cHx	OEt	54.04	28.14	30.24	169.74	173.22	48.09 (CH)	13.92(CH_3)
4f	NH-MBzl	OEt	54.13	28.08	30.42	170.08	173.44	48.94 (CH)	14.51 (CH_3)
5a	NH-^{n}Pn	OBzl	54.17	28.18	30.39	170.69	173.09	13.70 (CH_3)	66.53 (CH_2)
17a	OBzl	NH-^{n}Pn	53.60	28.42	32.41	171.60	171.77	67.25 (CH_2)	13.93 (CH_3)
18a (7a)	OEt	NH-^{n}Pn	53.64	29.03	31.55	171.63	171.91	14.07 (CH_3)[b]	14.14 (CH_3)[b]

[a] Assignment of carbonyl carbons was performed by capled ^{13}C NMR spectra of selected compounds. [b] May be interchanged

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