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Toward stereocontrolled, chemoenzymatic synthesis of unnatural peptides

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Abstract

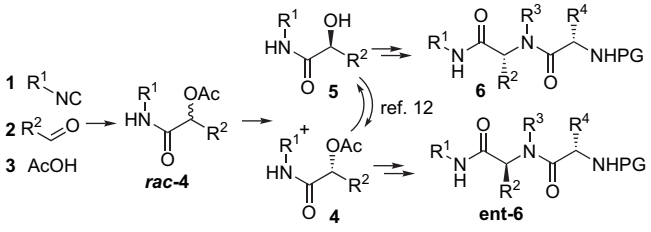
An efficient, chemoenzymatic method for the multicomponent synthesis of unnatural tripeptides is presented. Development of a previously described procedure combines the diversity offered by multicomponent reactions with the selectivity of biocatalysts and allows the convenient introduction of varied amino acid moieties into the tripeptide scaffold, with control of the stereochemistry. Additionally, it allows the introduction of a methyl group to the amide nitrogen, leading to derivatives of *N*-methylated amino acids.

Keywords: Tripeptides; Passerini reaction; Enzymatic hydrolysis; Non-coded aminoacids; *N*-Methylated peptides

1. Introduction

Small peptides and their simple analogues begin to form a group of ubiquitous compounds in medicinal chemistry. Among them, compounds with various activities can be found, such as anti-parasitic,[1] anti-tumor[2] and anti-viral[3] agents, HIV-protease inhibitors,[4] calpain,[5] thrombin[6] and proteasome[7] inhibitors. Modifications of N- and C-terminae were shown to significantly increase the activity,[8] while the introduction of non-coded amino acids[9] and N-alkylation of amide groups[10] have been shown to improve the pharmacokinetic factors of these compounds.

In our previous paper[11] we have presented a method for the synthesis of small peptides, in which hydrolytic enzymes were employed for the enantioselective hydrolysis of compounds obtained in the Passerini multicomponent reaction. Further transformations allowed the synthesis of title compounds (Scheme 1). This method is advantageous as it combines the diversity offered by multicomponent reactions with the selectivity of biocatalysts. The synthesis of tripeptides (R^1=ROOCCH(R)) was, however, not fully accomplished in the cited paper, as the title compounds, bearing an ethyl ester moiety (R^1=$EtOOCCH_2$), were obtained in small yields and with unsatisfactory enantiomeric excesses. The main reasons were that both the enzymatic step and the introduction of azide moiety (precursor of the amino group) proceeded with poor chemoselectivity.



Scheme 1. Basic synthetic concept.

Here we would like to describe our efforts to overcome these drawbacks, by introducing different protection for the C-terminal carboxylic group.

2. Results and discussion

2.1. Synthesis of non-racemic α-hydroxyamides 5

In the first step of our studies, racemic esters of acetic acid were prepared (Scheme 2, **4a** and **4c**), using the Passerini

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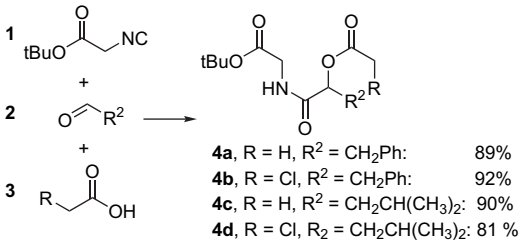
multicomponent reaction, in good yields from *tert*-butyl isocyanoacetate, acetic acid, and respective aldehyde. A group of lipases was tested as catalysts for the stereoselective hydrolysis of these compounds. The group consisted of: *Wheat Germ* Lipase (WGL), Novozym 435, Porcine Pancrease Lipase (PPL), *Candida rugosa* Lipase (CRL), Amano AK Lipase, *Rhizopus niveus* Lipase, *Candida lipolytica* Lipase, *Pseudomonas cepacia* Lipase, and Pig Liver esterase.



Scheme 2. Synthesis of racemic α-acetoxyamides **4**.

Out of the examined enzymes, only WGL catalyzed the hydrolysis, and the stereochemical results were moderate in the case of **4a** and unsatisfactory in the case of **4c** (Table 1, entries 1 and 7, respectively). Therefore a new set of racemic esters (Scheme 2, **4b** and **4d**) was synthesized, using chloroacetic acid in the Passerini reaction, to introduce a better leaving group to the ester. The same group of enzymes as above was tested and this time two additional enzymes PPL and CRL were found to catalyze the hydrolysis (Table 1, entries 4–6 and 10–12).

In the synthesis of enantiomerically enriched alcohol **5a** (Table 1, entries 1–6), the broadening of the enzyme spectrum did not result in the improvement of the enantioselectivity. On the contrary, in the stereocontrolled synthesis of alcohol **5b** (Table 1, entries 7–12), the change of the substrate proved to be successful, as the best result was obtained in the hydrolysis of chloroacetic ester **4d**, catalyzed by PPL. An interesting result was also obtained in terms of the enzyme's enantiomeric preference. In the reaction catalyzed by WGL, different enantiomers of **5b** were obtained when the chloroacetic acid ester was used instead of the acetic one (Table 1, entries 7 and 10, respectively).

Table 1
Chemoenzymatic synthesis of non-racemic compounds **5**

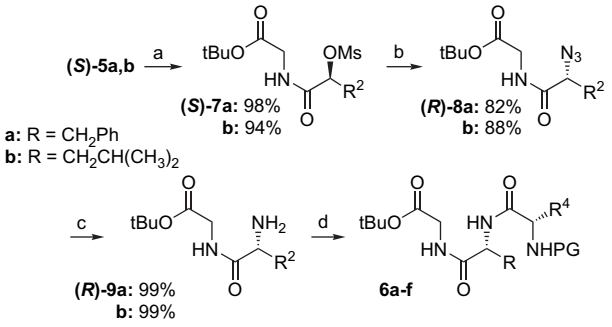
No	Substrate	Enzyme	Time (h)	Product	Yield (%)	ee[a] (%)	E[b]
1	**4a**	WGL	30	(*S*)-**5a**	45	87	28
				(*R*)-**4a**	54	64	
2	**4a**	PPL	48	Conversion<5%			—
3	**4a**	CRL	48	Conversion<5%			—
4	**4b**	WGL	26	(*S*)-**5a**	60	<5	1
				(*R*)-**4b**	16	40	
5	**4b**	PPL	120	(*S*)-**5a**	44	10	1
				(*R*)-**4b**	50	16	
6	**4b**	CRL	26	(*S*)-**5a**	36	47	4
				(*R*)-**4b**	50	35	
7	**4c**	WGL	41	(*S*)-**5b**	58	37	7
				(*R*)-**4c**	38	64	
8	**4c**	PPL	48	Conversion<5%			—
9	**4c**	CRL	48	Conversion<5%			—
10	**4d**	WGL	20	(*R*)-**5b**	81	<5	2
				(*S*)-**4d**	16	63	
11	**4d**	PPL	119	(*S*)-**5b**	64	40	18
				(*R*)-**4d**	32	85	
12	**4d**	CRL	45	(*S*)-**5b**	40	50	9
				(*R*)-**4d**	32	69	

[a] Determined by chiral HPLC.
[b] Calculated from the conversion-independent equation.

In order to assign the configuration of the obtained products, compounds **(*S*)-4** and **(*S*)-5** were synthesized from commercially available, optically pure α-amino acids, by conversion into α-hydroxy acids ($NaNO_2/H_2SO_4$)[13] and EDC mediated coupling[14] of thus obtained compounds with glycine *tert*-butyl ester.

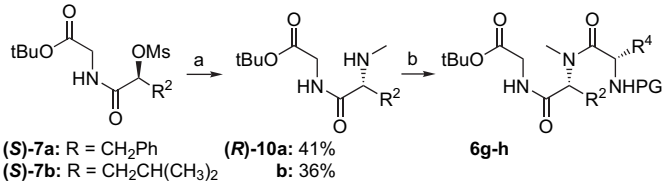
2.2. Synthesis of primary and secondary amines *9*

The next step was to functionalize the hydroxyl group toward amine derivatives. The transformation into NH_2 group was by S_N2 type substitution of methanesulfonic acid esters derived from alcohols **5** (Scheme 3). Enantiopure compounds obtained from the correlation study were used in these syntheses. Compounds **(*R*)-9** were obtained from alcohols **(*S*)-5** in good overall yields (80% both for **(*R*)-9a** and **b**).



Scheme 3. Synthesis of tripeptides **6**. (a) MsCl, Et_3N, DMAP, CH_2Cl_2, rt, 30 min; (b) NaN_3, DABCO, DMAP, benzo-15-crown-5, CH_2Cl_2, 40 °C, 24 h; (c) H_2, Pd/C, methanol, 4 h; (d) Protected amino acid, EDC, HOBt, CH_2Cl_2, rt, 4 h (see Table 3).

The synthesis (Scheme 4) of methylamine derivatives **10a** and **b**, precursors of *N*-methylated peptides, proved to be more problematic. The method used in our previous study, which consisted in the heating of the mesylate in the aqueous methylamine/DMF proved unsuccessful (Table 2, entry 1), probably due to the possible hydrolysis or aminolysis of ester moiety.



Scheme 4. Synthesis of *N*-methylated tripeptides **6**. (a) 5% $MeNH_2$ in methanol, 50 °C, 24 h; (b) EDC, HOBt, CH_2Cl_2, rt, 4 h.

Table 2
Synthesis of **10a**—an optimization study

No	Reaction conditions	Yield (%)
1	40% aq $MeNH_2$, DMF, 50 °C, 24 h	22
2	40% aq $MeNH_2$, DMF, cat. DMAP, 50 °C, 44 h	7
3	$MeNH_2$ hydrochloride, 2 equiv DMAP, 50 °C, 96 h	29
4	33% $MeNH_2$ in ethanol, 50 °C, 24 h	20
5	5% $MeNH_2$ in methanol, 50 °C, 24 h	41
6	5% $MeNH_2$ in methanol, 50 °C, 96 h	16

The addition of catalytic amount of DMAP and the elongation of reaction time (Table 2, entry 2) did not result in the improvement of reaction outcome. To eliminate the water from the reaction media, methylamine hydrochloride was used with DMAP as base and DMF as solvent (Table 2, entry 3). Despite the longer reaction time, the yield improved only slightly. We turned therefore to alcoholic solutions of methylamine. Ethanolic solution with high concentration was used and the yield was only 20% (Table 2, entry 4). The 5% methanolic solution on the other hand gave the best result (Table 2, entry 5), the yield was 41% and the formation of side products was minimized, which simplified the purification. The elongation of reaction time (Table 2, entry 6) did not result in the improvement of yield, probably causing a severe decomposition of the ester moiety. Using the optimized reaction conditions, the methylamine derivative **(*R*)-10b** was synthesized with 36% yield (Scheme 4).

2.3. Synthesis of title peptides *6*

Target tripeptide analogues were prepared by EDC mediated coupling of amines **9** with model amino acids (glycine for **6a**, **b**, **d**, **e**, **g**, **h**, and (*S*)-alanine for **6c** and **f**), bearing a benzyloxycarbonyl (CBz) or *tert*-butoxycarbonyl (Boc) protecting groups on the nitrogen atom (Schemes 3 and 4). Yields of compounds **6** are shown in Table 3.

All of the target compounds were obtained in analytically pure form with very high yields (>95%) in case of the coupling of primary amines (**6a**–**f**). The *N*-methylated peptides (**6g** and **h**) were obtained in lower yields, probably due to steric hindrance.

Table 3
Synthesis of compounds **6**

6	R^2	R^3	R^4	PG	Yield (%)
a	CH_2Ph	H	H	Boc	90
b	CH_2Ph	H	H	CBz	97
c	CH_2Ph	H	CH_3	Boc	99
d	$CH_2CH(CH_3)_2$	H	H	Boc	95
e	$CH_2CH(CH_3)_2$	H	H	CBz	99
f	$CH_2CH(CH_3)_2$	H	CH_3	Boc	99
g	CH_2Ph	CH_3	H	CBz	58
h	$CH_2CH(CH_3)_2$	CH_3	H	CBz	47

3. Summary

In this paper we present the development of our chemoenzymatic, multicomponent methodology for the synthesis of small peptides. Previously reported difficulties were solved by the use of *tert*-butyl ester as the protection of the C-terminus. The enzymatic, enantioselective hydrolysis of racemic intermediate was optimized, where needed, by the introduction of more easily hydrolyzed chloroacetoxy group. Best results were obtained with *Wheat Germ* Lipase and Porcine Pancrease Lipase.

For the ease of configuration determination, a set of model peptides composed of natural amino acids was prepared to demonstrate the synthetic potential of the method. Considering the enormous number of commercially available aldehydes (precursors of amino acid side chain in this method) and the very extensive set of synthetic methods for their preparation, we find our method to be widely applicable.

Small peptides with orthogonal protections on the ends, obtained in this method, are ready for the incorporation into larger biopolymers, using conventional methods. Compounds with compatible protections can be easily deprotected in one step, to yield non-natural tripeptides, compounds of great biological relevance.

4. Experimental

4.1. General

Optical rotations were measured with a JASCO DIP-360 polarimeter. NMR spectra were measured with a Varian 200 GEMINI and Varian 400 GEMINI spectrometer, with TMS as internal standard. TLCs were performed with silica gel 60 (230–400 mesh, Merck) and silica gel 60 PF_{254} (Merck). HPLC experiments were carried in three variants: A: DAICEL CHIRACEL OD-H column with a pre-column, eluent: hexane/*i*-PrOH 95:5 (v/v), flow: 1 mL/min; B: DAICEL CHIRACEL OD-H column with a pre-column, eluent: hexane/*i*-PrOH 9:1 (v/v), flow: 1 mL/min; C; CHIRACEL OJ-H column with a pre-column, eluent: hexane/*i*-PrOH 9:1 (v/v), flow: 1 mL/min; CHN analyses were performed on Perkin–Elmer 240 Elemental Analyzer. MS spectra were recorded on an API 365 (SCIEX) apparatus.

4.2. General procedure for the synthesis of Passerini reaction products ***rac-4***

To a 1 M solution of acetic acid (1 equiv) in CH_2Cl_2 was added aldehyde (1.1 equiv) and then isocyanide (1.1 equiv) at room temperature. After 24 h, the solvent was evaporated and the product was purified by flash chromatography (silica gel, hexane/ethyl acetate, 4:1 v/v).

4.2.1. tert-Butyl rac-(2-acetoxy-3-phenyl-propionylamino)-acetate (***rac-4a***)

Yield: 89% of white crystals; R_f=0.40 (ethyl acetate/hexane 7:3, v/v); mp 99–101 °C (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H, $(CH_3)_3$), 2.06 (s, 3H, CH_3CO), 3.09 (dd, 1H, J=14.4, 7.6 Hz, $PhCH_2$), 3.23 (dd, 1H, J=14.4, 4.4 Hz, $PhCH_2$), 3.81–3.95 (m, 2H, CH_2NH), 5.40 (dd, 1H, J=7.6, 4.4 Hz, CHOAc), 6.47 (br s, 1H, NH), 7.15–7.28 (m, 5H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$):

δ 20.8, 28.0, 37.7, 41.6, 74.1, 82.5, 126.9, 128.3, 129.4, 135.9, 168.4, 169.1, 169.3. HRMS (ESI, $[M+Na]^+$) 344.1453 ($C_{17}H_{23}NO_5Na$: 344.1468). Retention time of enantiomers: t_S=7.93, t_R=9.93 (variant B).

4.2.2. tert-Butyl rac-(2-(chloroacetoxy)-3-phenyl-propionylamino)acetate (**rac-4b**)

Yield: 92% of yellow oil; R_f=0.20 (ethyl acetate/hexane 8:2, v/v). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.46 (s, 9H, (CH_3)$_3$), 3.14 (dd, 1H, J=14.4, 7.8 Hz, PhCH_2), 3.30 (dd, 1H, J=14.4, 4.6 Hz, PhCH_2), 3.81–3.95 (m, 2H, CH_2NH), 4.01–4.07 (m, 2H, CH_2Cl), 5.48 (dd, 1H, J=7.8, 4.6 Hz, C*H*OC(O)), 6.51 (br s, 1H, N*H*), 7.16–7.30 (m, 5H, Ar*H*). ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.4, 38.1, 40.8, 42.2, 76.0, 83.1, 127.5, 128.9, 129.8, 135.6, 166.1, 168.6. HRMS (ESI, $[M+Na]^+$) 378.1088 ($C_{17}H_{22}NO_5NaCl$: 378.1079). Retention time of enantiomers: t_S=8.18, t_R=10.90 (variant B).

4.2.3. tert-Butyl rac-{[2-(acetoxy)-4-methylopentanoyl]-amino}acetate (**rac-4c**)

Yield: 90% of white crystals; R_f=0.44 (ethyl acetate/hexane 7:3, v/v); mp 78–80 °C (ethyl acetate/hexane). Anal. $C_{14}H_{25}NO_5$ requires: C, 58.52%; H, 8.77%; N, 4.87%. Found: C, 58.57%; H, 8.79%; N, 4.76%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (d, J=6.0 Hz, 3H, CH_3CH), 0.93 (d, J=6.0 Hz, 3H, CH_3CH), 1.47 (s, 9H, (CH_3)$_3$C), 1.67–1.76 (m, 3H, C*H*CH_2), 2.16 (s, 3H, CH_3CO), 3.91–3.93 (m, 2H, CH_2NH), 5.21–5.25 (m, 1H, C*H*OAc), 6.50 (br s, 1H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9, 21.7, 23.0, 24.5, 28.0, 40.8, 41.7, 72.6, 82.5, 168.7, 169.8, 170.3. Retention time of enantiomers: t_R=6.91, t_S=7.45 (variant A).

4.2.4. tert-Butyl rac-{[2-(chloroacetoxy)-4-methylo-pentanoyl]-amino}acetate (**rac-4d**)

Yield: 81% of white crystals; R_f=0.50 (ethyl acetate/hexane 7:3, v/v); mp 79–80 °C (ethyl acetate/hexane). Anal. $C_{14}H_{24}ClNO_5$ requires: C, 52.25%; H, 7.52%; N, 4.35%. Found: C, 52.09%; H, 7.66%; N, 4.26%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.92 (d, J=6.0 Hz, 6H, (CH_3)$_2$CH), 1.47 (s, 9H, (CH_3)$_3$C), 1.60–1.85 (m, 3H, C*H*CH_2), 3.92–3.94 (m, 2H, CH_2NH), 4.15–4.16 (m, 2H, ClCH_2), 5.28–5.34 (m, 1H, C*H*OAc), 6.52 (br s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.1, 23.4, 24.9, 28.4, 41.0, 41.1, 42.2, 74.6, 83.0, 169.7. Retention time of enantiomers: t_R=8.16, t_S=9.14 (variant B).

4.3. General procedure for the enzymatic hydrolysis of Passerini reaction products

Ester ***rac*-4** (55 mg) was dissolved in 10 mL of solvent (water/Et_2O 8:2, v/v). Enzyme (9 mg) was added in one portion to the suspension and stirred on a shaker at 300 rpm at room temperature for the amount of time given in Table 1. Extraction with ethyl acetate, concentration in vacuo, and purification of the resulting residue by flash chromatography (silica gel, hexane/ethyl acetate) afforded enaniomerically enriched ester **4** and alcohol **5**. The yields and enantiomeric excesses are given in Table 1.

4.3.1. tert-Butyl (R)-(2-acetoxy-3-phenyl-propionylamino)-acetate (**(R)-4a**)

$[\alpha]_D^{20}$ +32.0 (*c* 0.76, $CHCl_3$, for the enantiopure compound obtained in the correlation study). Other analyses consistent with ***rac*-4a**.

4.3.2. tert-Butyl (R)-(2-(chloroacetoxy)-3-phenyl-propionylamino)acetate (**(R)-4b**)

$[\alpha]_D^{20}$ +7.4 (*c* 1.0, $CHCl_3$, for the compound obtained in the enzymatic step, 35% ee). Other analyses consistent with ***rac*-4b**.

4.3.3. tert-Butyl (R)-{[2-(acetoxy)-4-methylopentanoyl]-amino}acetate (**(R)-4c**)

$[\alpha]_D^{20}$ +22.2 (*c* 1.0, $CHCl_3$, for the enantiopure compound obtained in the correlation study). Other analyses consistent with ***rac*-4c**.

4.3.4. tert-Butyl rac-{[2-(chloroacetoxy)-4-methylo-pentanoyl]amino}acetate (**(R)-4d**)

$[\alpha]_D^{20}$ +8.6 (*c* 0.43, $CHCl_3$, for the compound obtained in the enzymatic step, 85% ee). Other analyses consistent with ***rac*-4d**.

4.3.5. tert-Butyl (S)-(2-(hydroxy)-3-phenyl-propionylamino)-acetate (**(S)-5a**)

White crystals; R_f=0.61 (ethyl acetate/hexane 4:6, v/v); mp 95–96 °C (ethyl acetate/hexane); $[\alpha]_D^{20}$ −69.4 (*c* 1.0, $CHCl_3$, for the enantiopure compound obtained in the correlation study). Anal. $C_{15}H_{21}NO_4$ requires: C, 64.50%; H, 7.58%; N, 5.01%. Found: C, 64.44%; H, 7.59%; N, 4.95. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.47 (s, 9H, (CH_3)$_3$), 2.62 (br s, 1H, O*H*), 2.85 (dd, 1H, J=14.1, 9.2 Hz, PhCH_2), 3.25 (dd, 1H, J=14.1, 3.6 Hz, PhCH_2), 3.81–4.01 (m, 2H, CH_2NH), 4.32 (dd, 1H, J=9.2, 3.6 Hz, C*H*OH), 7.03 (br s, 1H, N*H*), 7.24–7.34 (m, 5H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.4, 28.0, 40.8, 41.5, 72.9, 82.4, 126.9, 128.7, 129.5, 136.9, 168.8, 172.8. Retention time of enantiomers: t_R=14.05, t_S=15.25 (variant A).

4.3.6. tert-Butyl (S)-{[2-(hydroxy)-4-methylopentanoyl]-amino}acetate (**(S)-5b**)

Yellow oil; R_f=0.45 (ethyl acetate/hexane 5:5, v/v); $[\alpha]_D^{20}$ −37.5 (*c* 1.0, $CHCl_3$, for the enantiopure compound obtained in the correlation study). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.94 (d, J=6.6 Hz, 6H, CH_3CH), 1.47 (s, 9H, (CH_3)$_3$C), 1.52–1.86 (m, 3H, C*H*CH_2), 3.12 (br s, 1H, O*H*), 3.80–4.06 (m, 2H, CH_2NH), 4.17 (dd, 1H, J=9.2, 4.0 Hz, C*H*OH), 7.06 (br s, 1H, N*H*). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.7, 23.8, 24.8, 28.4, 41.9, 44.0, 71.0, 82.8, 169.5, 175.2. HRMS (ESI, $[M+Na]^+$) 268.1508 ($C_{12}H_{23}NO_4Na$: 258.1519). Retention time of enantiomers: t_S=5.22, t_R=6.19 (variant C).

4.4. Synthesis of compounds **6a–f**

The transformation of alcohols **(*S*)-5a** and **(*S*)-5b** into amides **6a–f** is further exemplified by the synthesis of **6a**.

*4.4.1. Methanesulfonic acid (S)-1-benzyl-2-[(4-tert-butoxycarbonyl)methylamino]-2-oxoethyl ester ((**S**)-7a)*

Solution of alcohol **(*S*)-5a** (186 mg, 0.67 mmol, from correlation study[12]) in CH_2Cl_2 (6 mL) was cooled to −50 °C. Triethylamine (280 μL, 2.00 mmol) was added in one portion and then methanesulfonyl chloride (103 μL, 1.33 mmol, solution in 1.0 mL of CH_2Cl_2) was added dropwise. The mixture was stirred for 60 min at room temperature, the solvent was evaporated, and the product was purified by flash chromatography (silica gel, hexane/ethyl acetate 85:15, v/v). Yield: 99%, 233 mg of white crystals; R_f=0.81 (hexane/ethyl acetate 4:6, v/v); mp 94−96 °C (ethyl acetate/hexane); $[\alpha]_D^{20}$ −72.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.47 (s, 9H, (CH_3)$_3$), 2.47 (s, CH_3SO_2), 3.04 (dd, 1H, *J*=14.4, 9.6 Hz, PhCH_2), 3.45 (dd, 1H, *J*=14.4, 3.6 Hz, PhCH_2), 3.88−4.02 (m, 2H, CH_2NH), 5.14 (dd, 1H, *J*=10.0, 3.6 Hz, C*H*OMs), 6.82 (br s, 1H, N*H*), 7.27−7.33 (m, 5H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.0, 37.8, 38.6, 41.9, 81.4, 82.6, 127.5, 128.8, 129.7, 135.6, 167.8, 168.0. HRMS (ESI, $[M+Na]^+$) 380.1156 ($C_{16}H_{23}NO_6NaS$: 380.1138).

*4.4.2. tert-Butyl (R)-(2-azido-3-phenyl-propionylamino)-acetate ((**R**)-8a)*

To a solution of methanesulfonic acid ester **(*S*)-7a** (207 mg, 0.58 mmol) in CH_2Cl_2 (10.0 mL) were added 1,4-diaza-bicyclo[2.2.2]octane (104 mg, 0.93 mmol), 4-dimethylaminopyridine (10 mg, 0.08 mmol), sodium azide (76 mg, 1.16 mmol), and benzo-15-crown-5 (10 mg, 0.04 mmol). The mixture was stirred and refluxed. After 24 h the product was purified by flash chromatography (silica gel, hexane/ethyl acetate 3:1, v/v). Yield: 82%, 158 mg of colorless oil; R_f=0.56 (hexane/ethyl acetate 6:4, v/v); $[\alpha]_D^{20}$ −34.1 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.47 (s, 9H, (CH_3)$_3$), 3.00 (dd, 1H, *J*=14.0, 9.0 Hz, PhCH_2), 3.39 (dd, 1H, *J*=14.0, 4.0 Hz, PhCH_2), 3.84−4.01 (m, 2H, CH_2NH), 4.22 (dd, 1H, *J*=9.0, 4.0 Hz, NHCH_2), 6.77 (br s, 1H, N*H*), 7.24−7.37 (m, 5H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.0, 38.8, 41.9, 65.6, 82.6, 127.2, 128.7, 128.8, 129.4, 129.7, 136.2, 168.4, 168.7. HRMS (ESI, $[M+Na]^+$) 327.1435 ($C_{15}H_{20}N_4O_3Na$: 327.1428). IR ($CHCl_3$) ν: 3317, 2980, 2115, 1742, 1667, 1532, 1369, 1250, 1226, 1157, 701 cm^{-1}.

*4.4.3. tert-Butyl (R)-(2-amino-3-phenyl-propionylamino)-acetate ((**R**)-9a)*

Azide **(*R*)-8a** (92 mg, 0.30 mmol) was dissolved in methanol (8 mL). To this mixture 10% Pd/C (10 mg) was added and hydrogen was fluxed through the solution (from a rubber balloon through a needle) for the period of 4 h. Then the reaction mixture was filtered through a bed of Celite and the solvent was evaporated. Yield 99%, 83 mg of colorless oil. $[\alpha]_D^{20}$ +28.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.48 (s, 9H, (CH_3)$_3$), 1.82 (br s, 2H, NH_2), 2.69 (dd, 1H, *J*=13.7, 9.9 Hz, PhCH_2), 3.32 (dd, 1H, *J*=13.7, 3.8 Hz, PhCH_2), 3.66 (dd, 1H, *J*=9.8, 3.8 Hz, NHC*H*), 3.92−4.01 (m, 2H, CH_2NH), 7.22−7.33 (m, 5H, Ar*H*), 7.78 (br s, 1H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.0, 40.8, 41.7, 56.4, 82.2, 126.8, 128.7, 129.3, 129.7, 137.9, 169.1, 174.4. HRMS (positive ESI, $[M-C(CH_3)_3+H]^+$) 223.1080 ($C_{11}H_{15}N_2O_3Na$: 223.1077). IR ($CHCl_3$) ν: 3363, 3316, 2979, 2933, 1741, 1665, 1526, 1368, 1227, 1158, 702 cm^{-1}.

*4.4.4. BocGly-(R)-Phe-GlyO^{t}Bu (**6a**)*

To a solution of amine **(*R*)-9a** (50 mg, 0.18 mmol) in CH_2Cl_2 (8.0 mL) were added Boc-protected glycine (32 mg, 0.18 mmol) and 1-hydroxybenzotriazole (37 mg, 0.27 mmol). The solution was cooled to 0 °C and EDC (39 mg, 0.20 mmol) was added in one portion. After 4 h the solvent was evaporated and the residue was taken up in ethyl acetate (15 mL). This solution was washed with 5% aqueous citric acid solution (2×5 mL), saturated $NaHCO_3$ solution (2×5 mL), and brine (5 mL). The organic phase was dried ($MgSO_4$) and evaporated to yield the analytically pure compound. Yield: 90% of colorless oil; R_f=0.35 (hexane/ethyl acetate 4:6, v/v); $[\alpha]_D^{20}$ +0.9 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H, (CH_3)$_3$), 1.44 (s, 9H, (CH_3)$_3$), 3.05−3.15 (m, 2H, PhCH_2), 3.72−3.80 (m, 2H, Gly-CH_2), 3.78−3.96 (m, 2H, Gly-CH_2), 4.72−4.78 (m, 1H, Phe-C*H*), 5.24 (br s, 1H, N*H*Boc), 6.67 (br s, 1H, N*H*), 6.82 (d, 1H, *J*=8.1 Hz, N*H*), 7.16−7.30 (m, 5H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.0, 28.2, 38.0, 41.9, 54.1, 82.2, 127.0, 128.6, 129.2, 129.7, 136.6, 168.4, 169.6, 170.7. HRMS (ESI, $[M+Na]^+$) 458.2262 ($C_{22}H_{33}N_3O_6Na$: 458.2262). IR ($CHCl_3$) ν: 3312, 2979, 2934, 1741, 1715, 1660, 1527, 1499, 1368, 1249, 1228, 1161, 753, 701 cm^{-1}.

In an analogous manner, compounds **6b−h** were synthesized.

*4.4.5. CBzGly-(R)-Phe-GlyO^{t}Bu (**6b**)*

Yield: 97% of colorless oil; R_f=0.36 (hexane/ethyl acetate 4:6, v/v); $[\alpha]_D^{20}$ +0.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.43 (s, 9H, (CH_3)$_3$), 3.05−3.15 (m, 2H, PhCH_2), 3.72−3.80 (m, 2H, Gly-CH_2), 3.78−3.96 (m, 2H, Gly-CH_2), 4.72−4.84 (m, 1H, BnC*H*), 5.09 (s, 2H, PhCH_2O), 5.70 (br s, 1H, N*H*CBz), 6.78 (br s, 1H, N*H*), 7.04 (d, 1H, *J*=8.2 Hz, N*H*), 7.14−7.43 (m, 5H, Ar*H*). ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.3, 38.5, 42.3, 44.8, 54.6, 67.5, 82.7, 127.3, 128.4, 128.5, 128.8, 128.9, 129.6, 130.0, 136.6, 167.8, 168.8, 169.7, 171.3. HRMS (ESI, $[M+Na]^+$) 492.2084 ($C_{25}H_{31}N_3O_6Na$: 492.2105). IR ($CHCl_3$) ν: 3302, 1729, 1656, 1531, 1368, 1229, 1157, 753, 699 cm^{-1}.

*4.4.6. Boc-(S)-Ala-(R)-Phe-GlyO^{t}Bu (**6c**)*

Yield: 99% of colorless oil; R_f=0.44 (hexane/ethyl acetate 4:6, v/v); $[\alpha]_D^{20}$ −2.2 (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.27 (d, 3H, *J*=7.2 Hz, CH_3CH), 1.43 (s, 9H, (CH_3)$_3$), 1.44 (s, 9H, (CH_3)$_3$), 3.05−3.15 (m, 2H, PhCH_2), 3.76−4.20 (m, 4H, 2×Gly-CH_2), 4.76−4.86 (m, 1H, Phe-C*H*), 5.24 (br s, 1H, N*H*Boc), 6.90 (br s, 1H, N*H*), 6.95 (s, 1H, N*H*), 7.27−7.37 (m, 5H, Ar*H*). ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.4, 29.6, 31.3, 38.0, 42.3, 54.4, 82.5, 127.3, 128.9, 129.6, 136.8, 168.8, 171.3, 173.4. HRMS (ESI, $[M+Na]^+$) 472.2426 ($C_{23}H_{35}N_3O_6Na$: 472.2418). IR (Nujol) ν: 3299, 1748, 1676, 1657, 1651, 1531, 1454, 1367, 1225, 1162 cm^{-1}.

*4.4.7. BocGly-(R)-Leu-GlyO^{t}Bu (**6d**)*

Yield: 95% of colorless oil; R_f=0.30 (hexane/ethyl acetate 5:5, v/v); $[\alpha]_D^{20}$ +25.8 (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89–0.93 (m, 6H, C*H*$_3$CH), 1.42 (s, 9H, (C*H*$_3$)$_3$C), 1.44 (s, 9H, (C*H*$_3$)$_3$C), 1.52–1.78 (m, 3H, C*H*CH$_2$), 3.78–3.92 (m, 4H, 2×Gly-2*H*), 4.48–4.60 (m, 1H, Leu-*H*), 5.37 (br s, 1H, N*H*Boc), 6.82–6.95 (m, 2H, 2N*H*). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.2, 23.3, 25.0, 28.4, 28.6, 31.3, 41.3, 42.3, 51.8, 82.5, 156.5, 169.0, 170.1, 172.4. HRMS (ESI, $[M+Na]^+$) 424.2423 ($C_{19}H_{35}N_3O_6Na$: 424.2418). IR (Nujol) ν: 3251, 1750, 1661, 1528, 1226, 1168 cm^{-1}.

*4.4.8. CBzGly-(R)-Leu-GlyO^{t}Bu (**6e**)*

Yield: 99% of colorless oil; R_f=0.32 (hexane/ethyl acetate 5:5, v/v); $[\alpha]_D^{20}$ +21.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89–0.93 (m, 6H, C*H*$_3$CH), 1.44 (s, 9H, (C*H*$_3$)$_3$C), 1.52–1.78 (m, 3H, C*H*CH$_2$), 3.78–4.00 (m, 4H, 2×Gly-2*H*), 4.48–4.61 (m, 1H, Leu-*H*), 5.10 (s, 2H, PhC*H*$_2$), 5.73 (br s, 1H, N*H*Boc), 6.82–6.88 (m, 2H, 2N*H*), 7.30–7.40 (m, 5H, Ar*H*). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.3, 23.2, 25.1, 28.4, 41.4, 42.3, 51.9, 67.5, 82.7, 128.4, 128.5, 128.9, 136.4, 168.0, 169.6, 172.3. HRMS (ESI, $[M+Na]^+$) 458.2259 ($C_{22}H_{33}N_3O_6Na$: 458.2262). IR ($CHCl_3$) ν: 3300, 2958, 1729, 1652, 1537, 1368, 1236, 1156, 754 cm^{-1}.

*4.4.9. Boc-(S)-Ala-(R)-Leu-GlyO^{t}Bu (**6f**)*

Yield: 99% of white crystals; R_f=0.43 (hexane/ethyl acetate 5:5, v/v); mp 185–187 °C (ethyl acetate/hexane); $[\alpha]_D^{20}$ +18.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89–0.93 (m, 6H, C*H*$_3$CH), 1.34 (d, *J*=7.0 Hz, 3H, Ala-C*H*$_3$), 1.42 (s, 9H, (C*H*$_3$)$_3$C), 1.44 (s, 9H, (C*H*$_3$)$_3$C), 1.52–1.78 (m, 3H, C*H*CH$_2$), 3.78–4.00 (m, 4H, 2×Gly-2*H*), 4.05–4.22 (m, 1H, 2×Ala-*H*), 4.42–4.58 (m, 1H, Leu-*H*), 5.24 (d, *J*=7.0 Hz, 1H, N*H*Boc), 6.83 (d, *J*=9.2 Hz, 1H, Leu-N*H*), 6.97 (br s, H, Gly-N*H*). ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.5, 22.1, 23.4, 25.1, 28.4, 28.6, 41.0, 42.3, 51.8, 80.6, 82.4, 155.9, 169.0, 172.4, 173.4. HRMS (ESI, $[M+Na]^+$) 438.2562 ($C_{20}H_{37}N_3O_6Na$: 438.2575). IR (Nujol) ν: 3290, 3240, 1749, 1677, 1652, 1568, 1532, 1455, 1366, 1226, 1168 cm^{-1}.

*4.5. Synthesis of compounds **6a–h***

The optimized transformation of alcohols **(*S*)-5a** and **(*S*)-5b** into *N*-methylated amides **6g** and **h**, respectively is further exemplified by the synthesis of **6g**.

*4.5.1. CBzGly-(N-Me)-(R)-Phe-GlyO^{t}Bu (**6g**)*

A solution of methanesulfonic acid ester **(*S*)-7a** (60 mg, 0.17 mmol) in methanol (containing 5 wt% of methylamine) was stirred for 24 h in 50 °C. The volatiles were then evaporated and the crude product was purified by flash chromatography (silica gel, ethyl acetate/methanol 9:1, v/v). HRMS (ESI, $[M+H]^+$) 293.1857 ($C_{16}H_{25}N_2O_3$: 293.1860). Obtained compound **(*R*)-10a** was used in further synthesis. To a solution of amine **(*R*)-10a** (28 mg, 0.09 mmol) in CH_2Cl_2 (4.0 mL) were added CBz-protected glycine (21 mg, 0.10 mmol) and 1-hydroxybenzotriazole (22 mg, 0.15 mmol). The solution was cooled to 0 °C and EDC (21 mg, 0.11 mmol) was added in one portion. After 4 h the solvent was evaporated and the residue was taken up in ethyl acetate (15 mL). This solution was washed with 5% aqueous citric acid solution (2×5 mL), saturated $NaHCO_3$ solution (2×5 mL), and brine (5 mL). The organic phase was dried ($MgSO_4$) and evaporated to yield the analytically pure compound. Yield: 58% of colorless oil; R_f=0.35 (hexane/ethyl acetate 4:6, v/v); $[\alpha]_D^{19}$ +52.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.43 (s, 9H, (C*H*$_3$)$_3$), 2.89 (s, 3H, CH_3N), 3.00 (dd, 1H, *J*=14.4, 9.2 Hz, PhC*H*$_2$CH), 3.34 (dd, 1H, *J*=14.6, 6.8 Hz, PhC*H*$_2$CH), 3.67–3.92 (m, 2H, Gly-C*H*$_2$), 3.91–4.07 (m, 2H, Gly-C*H*$_2$), 5.10 (s, 2H, PhC*H*$_2$O), 5.36 (dd, 1H, *J*=9.2, 6.8 Hz, PhC*H*$_2$CH), 5.70 (br s, 1H, N*H*CBz), 6.57 (br s, 1H, N*H*), 7.18–7.44 (m, 5H, Ar*H*). ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.4, 34.0, 42.3, 58.3, 67.3, 82.6, 112.2, 116.4, 126.9, 127.1, 127.7, 128.4, 128.9, 129.0, 159.9, 168.8, 170.0. HRMS (ESI, $[M+Na]^+$) 472.2437 ($C_{23}H_{35}N_3O_6Na$: 472.2418). IR ($CHCl_3$) ν: 3329, 2930, 1727, 1651, 1531, 1455, 1368, 1250, 1157 cm^{-1}.

*4.5.2. CBzGly-(N-Me)-(R)-Leu-GlyO^{t}Bu (**6h**)*

Yield: 47% of yellow oil; R_f=0.38 (hexane/ethyl acetate 5:5, v/v); $[\alpha]_D^{19}$ +83.9 (*c* 0.57, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (d, 3H, *J*=9.6 Hz, C*H*$_3$CH), 0.93 (d, 3H, *J*=9.6 Hz, C*H*$_3$CH), 1.44 (s, 9H, (C*H*$_3$)$_3$C), 1.60–1.78 (m, 3H, C*H*CH$_2$), 2.90 (s, 3H, C*H*$_3$N), 3.78–4.05 (m, 4H, 2×Gly-2*H*), 5.05–5.20 (m, 1H, Leu-*H*), 5.10 (s, 2H, PhC*H*$_2$), 5.80 (br s, 1H, N*H*Boc), 6.47 (br s, 1H, N*H*), 7.32–7.47 (m, 5H, Ar*H*). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.3, 23.4, 25.2, 28.4, 30.0, 36.4, 42.2, 43.3, 55.1, 64.1, 67.3, 113.2, 119.4, 128.4, 128.8, 160.4, 167.5, 167.7, 169.5. HRMS (ESI, $[M+Na]^+$) 506.2254 ($C_{25}H_{33}N_3O_6Na$: 506.2262). IR ($CHCl_3$) ν: 3330, 2957, 2932, 1727, 1651, 1530, 1368, 1251, 1158 cm^{-1}.

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