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Synthesis of N^{α}-Z, N^{β}-Fmoc or Boc protected α-hydrazinoacids and study of the coupling reaction in solution of N^{α}-Z-α-hydrazinoesters

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Abstract—The preparation of chiral orthogonally protected N^{α}-Z, N^{β}-Fmoc- or Boc-α-hydrazinoacids derivatives, directly suitable for SPPS, is described in six steps with good yields starting from the corresponding α-aminoacids. The coupling reaction assays performed in liquid phase between N^{α}-Z-hydrazinoesters and *N*-Fmoc-α-aminoacids demonstrated the low reactivity of the hydrazinoester derivatives. However, we found that the acid fluoride method allowed the formation of hydrazinodipeptides almost quantitatively.

1. Introduction

Hydrazinopeptides are a class of peptide analogues for which one (or more) peptidic bond(s) has (have) been replaced by one (or more) hydrazidic bond(s).[1] As recently demonstrated by Seebach and Lelais,[2] the hydrazidic bond is very resistant to protease and hydrazinopeptides could be interesting peptidomimetic candidates in drug design. Moreover, the few investigations concerning the structural analysis of foldamers constructed starting from α-hydrazinoacids seem to demonstrate that the presence of supplementary nitrogen atoms in the backbone leads to new forms of intramolecular structuration.[3] Compared to other pseudopeptides, hydrazinopeptides have not received much attention from the scientific community, the main reason is that among the numerous described methods,[4] few can be applied generally to provide enantiomerically pure α-hydrazinoacids directly suitable for oligomerization. Furthermore, the presence of two reactive nitrogens (N^{α} and N^{β}) on the α-hydrazinoacids leads to a problem of regioselectivity when performing acylation.

A few years ago,[5,6] we demonstrated that enantiomerically pure N^{α},N^{β}-bis and triprotected α-hydrazinoesters can be synthesized by using the Mitsunobu protocol as the key reaction. One important feature of this strategy is the possibility to introduce different protecting groups on the N^{α} and N^{β} positions, which can lead to the formation of N^{α},N^{β}-orthogonally bisprotected α-hydrazinoesters. In this paper, we demonstrate that the results previously obtained[5,6] can be extended to the preparation of N^{β}-*tert*-butyloxycarbonyl (Boc) and N^{β}-fluorenomethyloxycarbonyl (Fmoc) protected α-hydrazinoacid derivatives, compounds, which are directly suitable for peptide synthesis on solid support (SPPS). We also report the first results obtained, involving N^{α}-Z-α-hydrazinoesters in a coupling process in solution, which led to the preparation of hydrazinodipeptides.

2. Results and discussion

N,*N*-Triprotected α-hydrazinoesters **4** were prepared via the Mitsunobu reaction between the acidic partner *N*-benzyloxycarbonylaminophthalimide **3** and the corresponding α-hydroxyesters **2** prepared from **1** via a procedure previously described in the literature.[7] As we demonstrated before, this reaction proceeds via a total inversion of configuration of the stereogenic carbon and leads to the formation of enantiomerically pure compounds **4**.[6] As R is not too hindered, this procedure allowed the formation of the corresponding compounds **4** with good yields (Scheme 1); however, lower yield (30%) was obtained when R=$CH(CH_3)CH_2CH_3$.

As we described in a previous paper,[8] the presence of the phthalimide group is one of the key factors for the success of the Mitsunobu reaction. Unfortunately, as other authors reported, we were confronted with the difficulty of finding

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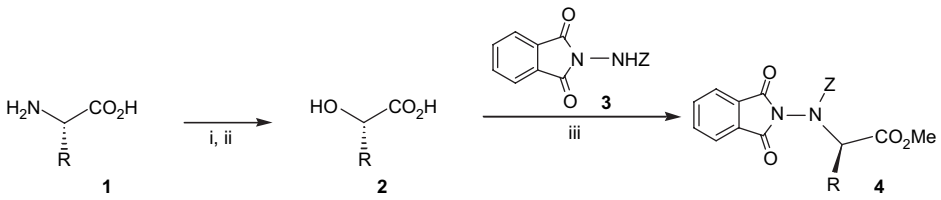
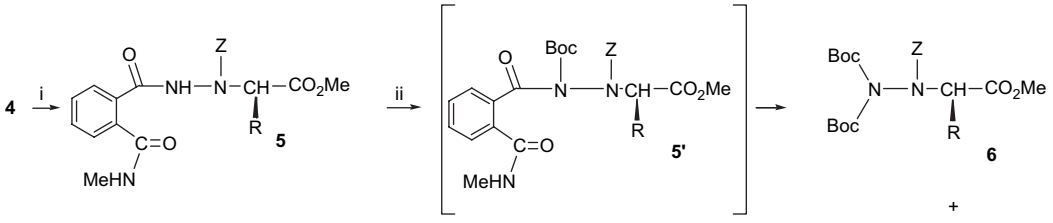


R	4 yields % (from 2)
H	93
CH_3	96
$CH_2CH(CH_3)_2$	97
$CH_2C_6H_5$	64
$CH(CH_3)_2$	64
$CH(CH_3)$-CH_2CH_3	30

Scheme 1. *Reagents, conditions and yields*: (i) $NaNO_2$, CH_3COOH; (ii) $SOCl_2$, MeOH and (iii) DBAD or DEAD, PPh_3, THF.

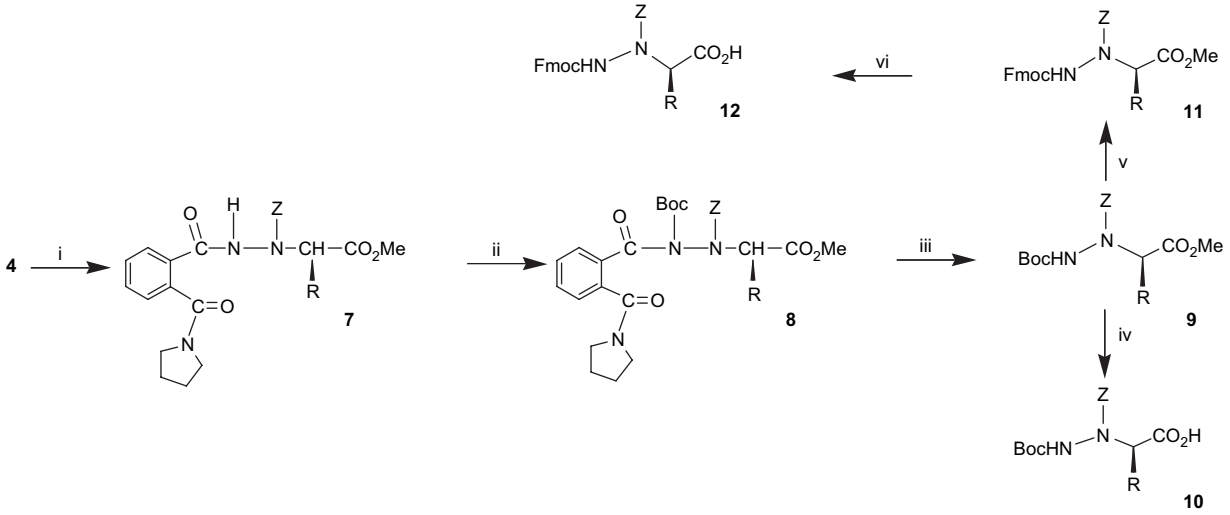
general and mild conditions to remove the phthaloyl group.[9] For this reason, we tried to find conditions that allowed the conversion of the phthalimide group of compounds **4** into more convenient protecting groups. The results are given in (Schemes 2 and 3) Table 1. We first demonstrated that the phthalimide group could be converted into triprotected compounds **6** by using a two-step, one-pot protocol[10] (Scheme 2).

If we consider the recent work published by Bonnet et al.,[11] the preparation of this kind of compounds is of interest, since the fully protected α-hydrazinoacid derivatives are required



R	6 yields % (from 4)
H	97
CH_3	70
$CH_2CH(CH_3)_2$	38
$CH(CH_3)_2$	66

Scheme 2. *Reagents and conditions*: (i) $MeNH_2$/MeOH/THF and (ii) Boc_2O/DMAP/THF.



Scheme 3. *Reagents and conditions*: (i) pyrrolidine, THF; (ii) Boc_2O, DMAP, THF; (iii) $MeNH_2$, MeOH, THF; (iv) (a) LiOH, H_2O, THF; (b) HCl 10%; (v) (a) HCl, AcOEt; (b) Fmoc-Cl, Na_2CO_3, dioxane and (vi) NaOH, $CaCl_2$, *i*-PrOH–H_2O.

Table 1. Preparation of N^{α},N^{β}-protected hydrazinoacids **10** and **12**

Entry	R	**7**[a]	**8**[b]	**9**[c]	**10**[d]	**11**[e]	**12**[f]
1	$-CH_3$	97	93	85	83	98	98
2	–H	99	96	96	98	75	96
3	$-CH(CH_3)CH_2CH_3$	63	81	74	70	[g]	nd
4	$-CH_2CH(CH_3)_2$	100	83	83	80	[g]	nd
5	$-CH_2Ph$	79	76	72	81	[g]	nd
6	$-CH(CH_3)_2$	65	70	93	75	[g]	nd

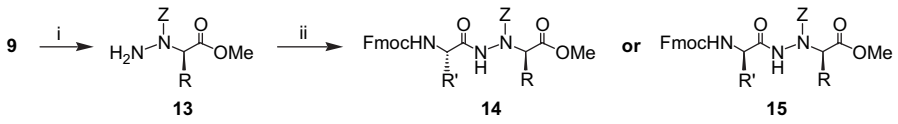
[a] Yields in pure compounds **7** calculated from **4**.
[b] Yields in pure compounds **8** calculated from **7**.
[c] Yields in pure compounds **9** calculated from **8**.
[d] Yields in pure compounds **10** calculated from **9**.
[e] Yields in pure compounds **11** calculated from **9**.
[f] Yields in pure compounds **12** calculated from **11**.
[g] Problem of purification of **11**.

to avoid polymerization side reactions in SPPS. Bisprotected compounds **9** can also be obtained by using a multi-step protocol (Scheme 3). In fact, the difference between these two procedures depends on the nature, primary or secondary, of the nucleophilic amine used to open the phthalimide ring. In both cases, the fixation of the electron withdrawing Boc group onto the hydrazide function followed by a nucleophilic displacement allowed the dephthaloylation. The action of $MeNH_2$ on *N*,*N*-triprotected α-hydrazinoesters **4** gives rise to the opening of phthaloyl ring leading to compounds **5**, which can be isolated if necessary.[12] The use of Boc_2O/DMAP allowed the selective protection of the nitrogen of the hydrazide group of compound **5**, without affecting the methyl amide. The latter can react with the carbonyl function of the benzoyl hydrazide leading to the splitting of the C–N bond with formation of methylphthalimide. An attack of a second equivalent of Boc_2O results in the formation of the triprotected hydrazinoesters **6**. Thus, when methylamine is used, the C–N bond splitting occurred via an intramolecular reaction involving the non-isolable intermediate **5′**.[5] On the contrary this last step is no more possible when using a secondary amine like pyrrolidine. In this case, the Boc protected compound **8** was stable and can be isolated. The splitting of the C–N bond only occurred in a supplementary step by the action of methylamine. The method using the pyrrolidine presents two main advantages: (1) it avoids the use of $Mg(ClO_4)_2$, which was previously described[13] as the only way to obtain compound **9** from compound **6** and (2) it allows the isolation of intermediated compounds **7** and **8** with good yields. Unfortunately, in spite of considerable efforts, the protocol described to obtain compounds **9** cannot be transposed for the preparation of Fmoc protected analogues **11**. No reaction occurred when Fmoc–Cl or Fmoc–OSu was used as an electrophile, even in the presence of DMAP as catalyst. However, we demonstrated that compounds **11** can be obtained from **9** by a two-step procedure (see v, Scheme 3).

To reach our goal, which was the obtention of N^{α},N^{β}-orthogonally bisprotected α-hydrazinoacids directly suitable for peptidic synthesis on solid phase, the last step to perform was the conversion of the ester group of compounds **9** and **11** into the corresponding acids **10** and **12**. Depending on the nature of the protection of the hydrazine, two strategies were used. LiOH was convenient to convert in good yields compound **9** into the corresponding compounds **10**. However, these conditions cannot be used to obtain compounds **12** starting from **11** because of the lability of the Fmoc group under alkaline conditions. We showed that this problem can be avoided by using NaOH in the presence of $CaCl_2$[14] in a mixture of isopropanol and water, which lead to the formation of corresponding compounds **12**. For two examples (R=H and R=Me) the α-hydrazinoacids **12** were isolated in pure form. Unfortunately, in other cases, the degradation of compounds **11** during purification impeded the obtention of **12** in pure form. However, it is interesting to note that these crude products **12** can be involved in peptidic synthesis on solid phase (to be published). To conclude this first part, we have shown that N^{α},N^{β}-orthogonally bisprotected α-hydrazinoacids **10** and **12** can be obtained starting from the corresponding α-aminoacids **1** in six or seven steps in acceptable overall yields.

Very few reactions are reported in the literature describing the coupling of α-hydrazinoacid derivatives. Collet et al.[15] and more recently, Lelais and Seebach[2] demonstrated that N^{α}-benzyl α-hydrazinoacid derivatives, obtained by electrophilic amination method[16] can be linked to α-aminoacids or oligomerized in solution with fair yields using classical coupling reagents. Collet et al.[17] pointed out the problem of the regioselectivity between the N^{α} and the N^{β} positions when unhindered lateral chains were present on the α-hydrazinoacid, which can be circumvented by the use of N^{α}-benzyl protected derivatives. As it has been shown above, our method allowed the preparation of N^{α}-Z protected α-hydrazinoacids. This protecting group is very widely employed as permanent protecting group in SPPS because its removal can occur simultaneously with the cleavage of the peptide from the solid support. So, we decided to perform coupling reactions between α-hydrazinoesters **13** (obtained from **9** using classical Boc deprotection conditions) and Fmoc protected α-aminoacids in solution, but keeping in mind the possibility to use them later in SPPS (Scheme 4 and Table 2).

The first reactions were performed between compound **13** (R=Me) and *N*-Fmoc-L-Ala-OH in order to find the best conditions of coupling (entries 1–7). Surprisingly, using activated esters and Leuch's anhydride (not reported results) no coupling reaction occurred. Furthermore, the classical method TBTU/HOBt/DIEA[18] leads, in our case, to the formation of hydrazinodipeptides with a fair yield of 50% (entry 1). In a same manner, poor results were obtained when *O*-(7-azabenzotriazol-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)[19] was used as coupling reagent (entries 2 and 3), even in the presence of *N*-methylmorpholine (NMM), the conditions described by Lelais and Seebach.[2] This result suggests that N^{α}-Z protected α-hydrazinoacids are less reactive than N^{α}-benzyl protected one and can be explained by the more electron withdrawing ability of Z group. Fortunately, we demonstrated that the coupling reaction can lead to the desired product with 73% yield when using HOBt–DCC[20] (entry 4). Better results were obtained with $SOCl_2$[21] or isobutyl chloroformate[22] (entries 5 and 6), however, the use of these reactants necessitate low temperature conditions (−20 °C), which are difficultly compatible with the solid phase synthesis.[21] For this reason, we will prefer the use of the acid fluoride method[23] (entry 7), which can be performed at room temperature. In fact, the protocol described in the litterature[23] suggested the addition of the acid fluoride to a cold solution (−20 °C) of



Scheme 4. *Reagents and conditions*: (i) HCl 3 N/AcOEt and (ii) FmocNH–CH(R′)–COOH, for coupling conditions, see Table 2.

Table 2. Coupling reactions of α-hydrazinoesters **13** with FmocNH–CH(R′)–COOH

Entry	R	R′	Coupling conditions	*T* (°C)	Yields[a] in **14** or **15** (%)
1	$-CH_3$	$-CH_3$	HOBt/TBTU/DIEA[18]	20	50
2	$-CH_3$	$-CH_3$	HATU/HOBt/DIEA[19]	20	19
3	$-CH_3$	$-CH_3$	HATU/NMM[2]	20	30
4	$-CH_3$	$-CH_3$	HOBt/DCC[20]	20	73
5	$-CH_3$	$-CH_3$	$SOCl_2/CH_2Cl_2$[21]	−20	90
6	$-CH_3$	$-CH_3$	Isobutyl chloroformate/NMM[22]	−20	86
7	$-CH_3$	$-CH_3$	*N*-Fmoc-L-Ala-F[23,b]	20	98
8	$-CH_3$	$-CH_3$	*N*-Fmoc-D-Ala-F[23,b]	20	98
9	$-CH_3$	$-CH(CH_3)_2$	*N*-Fmoc-L-Val-F[23,b]	20	78
10	$-CH(CH_3)CH_2CH_3$	$-CH(CH_3)_2$	*N*-Fmoc-L-Val-F[23,b]	20	68

[a] Yields in pure **14** or **15** calculated from **13**.
[b] Acid fluoride of Fmoc-Ala-OH was previously synthesized and isolated before the coupling reaction. A cold solution (−10 °C) of Fmoc-Ala-F in DCM is added.

α-aminoester and to perform the reaction at room temperature. Keeping in mind the possibility to use this protocol in solid phase synthesis, we decided to add a cold solution of the acid fluoride (−10 °C) to the corresponding hydrazinoester and to perform the reaction at room temperature. These conditions led to the formation of the desired products with 98% yield. The optical purity of compound **14** (R=R′=Me) was evaluated up to 95% by comparing its ^{13}C NMR data with those of the corresponding diastereoisomer **15** obtained by coupling *N*-Fmoc-D-Ala-OH with **13** R=Me (entry 8). A difference can be observed if we compare the chemical shift of the corresponding C_α (δC_α(**14**)=49.8 ppm and δC_α(**15**)=49.5 ppm). As it is possible to check from the Figure 1, both spectra of **14** and **15** exhibit a single signal for the C_α.

To assess the acid fluoride procedure, we coupled an hindered aminoacid (*N*-Fmoc-L-Val-F) with an unhindered hydrazinoester **13** R=Me or with an hindered hydrazinoester **13** R=CH(Me)CH_2CH_3 (entries 9 and 10). In both cases, the corresponding dipeptides **14** were obtained in 78% and 68% yields, respectively. These results suggest that this procedure could be easily extended to the formation of a large number of hydrazinodipeptides.

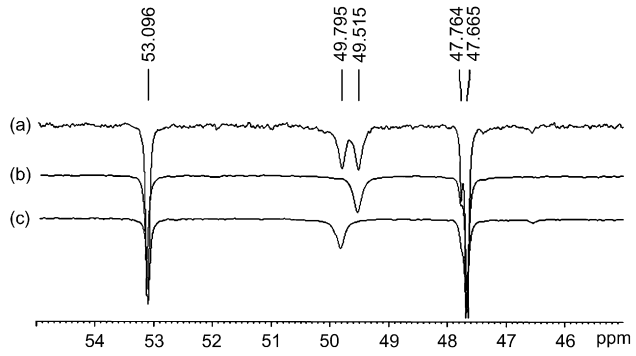


Figure 1. Part of ^{13}C NMR spectra of: (a) mixture of **14** (R=R′=Me) and **15** (R=R′=Me); (b) compound **15** (R=R′=Me) and (c) compound **14** (R=R′=Me).

3. Conclusion

We have shown that chiral orthogonally protected N^{α}-(Z)-N^{β}-(Fmoc or Boc), protected α-hydrazinoacid derivatives, directly suitable for SPPS, can be prepared in six steps and in reasonable yields starting from the corresponding α-aminoacids. The coupling reaction assays performed between N^{α}-Z-α-hydrazinoesters and *N*-Fmoc-α-aminoacids demonstrated the low reactivity of α-hydrazinoester when the N^{α} is protected by a benzyloxycarbonyl group. However, we find that the use of the acid fluoride method allowed the formation of hydrazinodipeptides **14** and **15** in good yields. The use of these conditions for the synthesis of hydrazinopeptides in SPPS is under active investigation.

4. Experimental

4.1. General

Tetrahydrofuran was dried by distillation over sodium benzophenone ketyl. Unless otherwise stated, reagents were purchased from chemical companies and used without prior purification. Reactions were monitored by thin layer chromatography (TLC) using aluminium-backed silica gel plates (Macherey–Nagel ALUGRAM® SIL G/UV_{254}). TLC spots were viewed under ultraviolet light and by heating the plate after treatment with a staining solution of phosphomolybdic acid. Product purifications were performed using Geduran 60 H Silica Gel (63–200 mesh). Reagent grade solvents were used as received. ^1H NMR and ^{13}C (300 MHz) spectra were recorded on a Bruker Advancer 300. Multiplicities are reported as follow: s=singlet, t=triplet, q=quadruplet, m=multiplet. IR spectra were recorded on a Bruker Tensor

27. Electron impact mass spectra were performed on a Pro-MALDI/FTMS apparatus in the 'Laboratoire de Spectrométrie de Masse et de Chimie Laser—Université de Metz—France'.

4.2. General procedure for the preparation of compounds 4

To a solution of *N*-benzyloxycarbonylaminophthalimide **3** (5 mmol), PPh_3 (2 g, 7.5 mmol) and α-hydroxyester (RCH(OH)COOMe, 5 mmol) **2** in dry THF (50 mL) and under nitrogen was added in one portion diethylazodicarboxylate (DEAD) or di-*tert*-butylazodicarboxylate (DBAD) (7.5 mmol) with stirring at 0–5 °C. The resulting solution was stirred at room temperature for 0.5 h (monitored by TLC until completion) or 3 h in the special case of R=$CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$ and concentrated in vacuo. The residue was triturated in AcOEt, and the most of triphenylphosphine oxide and diethyl (or di-*tert*-butyl) hydrazinedicarboxylate was removed by filtration. The filtrate was evaporated and the residue was chromatographied on silica gel.

The spectroscopic data of compounds **4**, R=H, CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH_2C_6H_5$ were described elsewhere.[6]

4.2.1. N^{α}-Benzyloxycarbonyl-N^{β}-phthalimidohydrazino-isoleucine methyl ester (4, R=$CH(CH_3)CH_2CH_3$).

Oil; IR (NaCl) ν_{max}/cm^{-1}: 1800, 1739; ^1H NMR (300 MHz, $CDCl_3$): δ 7.89–7.85 (m, 2H, arom Pht), 7.81–7.72 (m, 2H, arom Pht), 7.39–7.33 (m, 2H, arom Ph), 7.20–7.12 (m, 3H, arom Ph), 5.32–5.00 (m, 2H, CH_2 Z), 4.87 (d, 0.6H, J=11.2 Hz, αCH), 4.65 (d, 0.4H, J=12 Hz, αCH), 3.71 (s, 3H, O–CH_3), 2.04–1.72 (m, 2H, γCH_2), 1.42–1.22 (m, 4H, βCH and γCH_3), 1.00–0.74 (m, 3H, δCH_3); ^{13}C NMR ($CDCl_3$) δ 169.9, 169.7 (*C*OOCH$_3$), 166.7, 116.3, 166.2, 165.8 (O=*C*–Pht), 154.9, 154.7 (N–*C*OOCH$_2$C$_6$H$_5$), 136.0 (C arom), 135.5, 135.4 (CH arom Pht), 130.7, 130.3 (CH arom Pht), 129.3, 129.1, 129.0, 128.8, 128.7, 127.7 (CH arom), 124.6 (CH arom Pht), 70.1, 69.4 (CH_2 Z), 66.9, 65.6 (αCH), 53.5 (O–*C*H$_3$), 35.4 (βCH), 26.3 (γCH_2), 15.3 (βCH), 12.0 (δCH_3); HRMS calcd for $C_{23}H_{24}N_2O_6$ [M+Na$^+$] *m*/*z* 447.15266, found 447.1527.

4.3. General procedure for the preparation of compounds 6

To a solution of compounds **3** (3 mmol) in THF (20 mL) was added at room temperature 3 equiv of methylamine (9 mmol, 2 M in MeOH). The mixture was stirred at room temperature until completion (about 5 h, monitored by TLC). The solvent and the excess of amine were removed in vacuo. The residue was dissolved in THF (20 mL), Boc_2O (3 equiv, 9 mmol) and a catalytic amount of DMAP were added. The mixture was stirred at room temperature until completion (about 6 h, monitored by TLC). Then, the solvent and the excess of amine were removed in vacuo and compounds **6** were purified by column chromatography.

4.3.1. N^{α}-(Benzyloxycarbonyl)-N^{β},N^{β}-[bis(*tert*-butyloxycarbonyl)]-hydrazinoglycine methyl ester (6, R=H).

White solid; IR (NaCl) ν_{max}/cm^{-1}: 3325, 1770, 1733, 1717; ^1H NMR (300 MHz, $CDCl_3$) δ 7.46–7.13 (m, 5H, arom), 5.29–5.07 (m, 2H, CH_2 Z), 4.37–4.15 (m, 2H, αCH_2), 3.81–3.55 (m, 3H, O–CH_3), 1.64–1.33 (m, 18H, Boc); ^{13}C NMR ($CDCl_3$) δ 171.5, 168.5, 168.4 (*C*OOCH$_3$), 155.5, 154.9 (*C*OOCH$_2$Ph), 150.7, 150.5 (*C*OO*t*-Bu), 136.2, 136.1 (C arom), 128.9, 128.7, 128.6, 128.4 (CH arom), 84.6, 84.5 (*C*Boc), 69.0, 68.8 (CH_2 Z), 53.9 (O–CH_3), 52.4 (αCH_2), 28.2, 27.9 (CH_3 Boc); HRMS calcd for $C_{21}H_{30}N_2O_8$ [M+Na$^+$] *m*/*z* 461.18944, found 461.1894.

4.3.2. N^{α}-(Benzyloxycarbonyl)-N^{β},N^{β}-[bis(*tert*-butyloxycarbonyl)]-hydrazinoalanine methyl ester (6, R=CH_3).

Oil; IR (NaCl) ν_{max}/cm^{-1}: 1800, 1764, 1734; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38–7.26 (m, 5H, arom), 5.29–5.17 (m, 2H, CH_2 Z), 4.58 (q, J=7 Hz, 1H, αCH), 3.68 (s, 3H, O–CH_3), 1.48, 1.47, 1.40 (3s, 18H, Boc), 1.26 (d, J=7 Hz, 3H, βCH_3); ^{13}C NMR ($CDCl_3$) δ 173.1 (*C*OOCH$_3$), 155.5 (*C*OOCH$_2$Ph), 150.3 (*C*OO*t*-Bu), 136.3, 135.4, 130.6, 130.2 (CH arom), 128.8, 128.7, 128.6, 128.4 (CH arom), 84.0 (*C*Boc), 68.7 (CH_2 Z), 54.9 (αCH), 53.9 (O–CH_3), 28.1 (CH_3 Boc), 14.7 (βCH_3); HRMS calcd for $C_{22}H_{32}N_2O_8$ [M+Na$^+$] *m*/*z* 475.20509, found 475.2052.

4.3.3. N^{α}-(Benzyloxycarbonyl)-N^{β},N^{β}-[bis (*tert*-butyloxycarbonyl)]-hydrazinovaline methyl ester (6, R=$CH(CH_3)_2$).

Oil; IR (NaCl) ν_{max}/cm^{-1}: 3332, 1748, 1733, 1717; ^1H NMR (300 MHz, $CDCl_3$) δ 7.49–7.14 (m, 5H, H arom), 5.25–5.05 (m, 2H, O–CH_2), 4.56–4.23 (m, 1H, N–CH), 3.75–3.48 (m, 3H, O–CH_3), 1.51–1.34 (m, 19H, 2$(CH_3)_3$, CH), 1.13–0.97 (m, 3H, CH_3), 0.94–0.77 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 171.7, 169.9 (*C*OOCH$_3$), 156.0 (*C*OOCH$_2$Ph), 151.8, 151.7 (*C*OO*t*-Bu), 136.3 (C arom), 129.0, 128.9, 128.8, 128.7, 128.5 (CH arom), 84.6, 84.5, 84.2 (*C*$(CH_3)_3$), 69.3 (O–*C*H$_2$), 67.0 (N–*C*H), 52.3 (O–CH_3), 29.1 (CH), 28.4, 28.3, 28.2 (C(*C*H$_3$)$_3$), 21.0, 19.3 (2CH_3); HRMS calcd for $C_{24}H_{36}N_2O_8$ [M+Na$^+$] *m*/*z* 503.23639, found 503.2364.

4.3.4. N^{α}-(Benzyloxycarbonyl)-N^{β},N^{β}-[bis(*tert*-butyloxycarbonyl)]-hydrazinoleucine methyl ester (6, R=$CH_2CH(CH_3)_2$).

Oil; IR (NaCl) ν_{max}/cm^{-1}: 3361, 1762, 1745, 1733; ^1H NMR (300 MHz, $CDCl_3$) δ 7.43–7.17 (m, 5H, H arom), 5.25–5.07 (m, 3H, O–CH_2), 3.84–3.52 (m, 1H, N–CH), 3.84–3.52 (m, 3H, O–CH_3), 1.66–1.27 (m, 21H, CH, CH_2 and 2$(CH_3)_3$), 1.06–0.80 (m, 6H, 2CH_3); ^{13}C NMR ($CDCl_3$) δ 171.2 (*C*OOCH$_3$), 155.6 (*C*OOCH$_2$Ph), 151.5 (*C*OO*t*-Bu), 136.4 (C arom), 129.1, 128.8, 128.6 (CH arom), 84.6 (*C*$(CH_3)_3$), 69.2 (O–*C*H$_2$), 60.4, 59.6 (N–*C*H), 52.7 (O–CH_3), 38.4 (CH_2), 28.7 (*C*H$(CH_3)_2$), 28.4 (C(*C*H$_3$)$_3$), 23.7, 22.5, 22.4 (2CH_3); HRMS calcd for $C_{25}H_{38}N_2O_8$ [M+Na$^+$] *m*/*z* 517.25204, found 517.2523.

4.4. General procedure for the preparation of compounds 7

To a solution of compound **4** (3 mmol) in THF (20 mL) was added at room temperature 3 equiv of pyrrolidine (9 mmol). The mixture was stirred at room temperature during 3 h for R=H and CH_3 (monitored by TLC until completion) or 5 h for the other compounds. The solvent and the excess of amine were removed in vacuo and the residue was chromatographied on silica gel.

4.4.1. N^{α}-(Benzyloxycarbonyl)-N^{β}, [2-(pyrrolidine-1-carbonyl)-benzoyl]-hydrazinoglycine methyl ester (7, R=H). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3277, 1795, 1771, 1746; ^1H NMR (300 MHz, $CDCl_3$) δ 9.03–8.93 (m, 1H, βNH), 7.95–7.70 (m, 1H, arom), 7.61–7.20 (m, 8H, arom), 5.30–5.14 (m, 2H, CH_2 Z), 4.44–4.31 (m, 2H, αCH_2), 3.83–3.46 (m, 5H, O–CH_3, pyrro), 3.19–3.07 (m, 2H, pyrro), 2.04–1.82 (m, 4H, pyrro); ^{13}C NMR ($CDCl_3$) δ 170.3, 170.1 (*C*OOCH$_3$), 167.3 (O*C*-pyrro), 166.8 (N–*C*OC_6H_4), 156.4, 155.6 (*C*OOCH$_2$Ph), 137.7, 137.5, 136.8, 136.3 (C arom), 135.5 (CH arom), 132.4, 132.3 (C arom), 130.8, 130.5, 130.3, 129.9, 129.2, 129.0, 128.8, 128.2, 127.3, 124.7 (CH arom), 69.6, 69.2, 69.0 (CH_2 Z), 53.1 (O–*C*H$_3$), 54.1, 53.1 (αCH_2), 49.7, 46.7, 46.4, 45.8, 26.5, 25.1 (CH_2 pyrro); HRMS calcd for $C_{23}H_{25}N_3O_6$ [M+Na$^+$] *m*/*z* 462.16356, found 462.1635.

4.4.2. N^{α}-(Benzyloxycarbonyl)-N^{β}, [2-(pyrrolidine-1-carbonyl)-benzoyl]-hydrazinoalanine methyl ester (7, R=CH$_3$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3247, 1786, 1742, 1716; ^1H NMR (300 MHz, $CDCl_3$) δ 9.19–8.43 (m, 1H, βNH), 7.90–7.06 (m, 9H, arom Phe), 5.29–4.74 (m, 3H, CH_2 Z, αCH), 3.81–3.34 (m, 5H, O–CH_3, CH_2 pyrro), 3.17–2.92 (m, 2H, CH_2 pyrro), 2.02–1.54 (m, 4H, 2CH_2 pyrro), 1.43 (d, *J*=7 Hz, 3H, βCH_3); ^{13}C NMR ($CDCl_3$) δ 172.6 (*C*OOCH$_3$), 169.8, 169.5 (O*C*-pyrro), 168.6, 168.1 (N–*C*OC_6H_4), 160.0, 155.7 (*C*OOCH$_2$Ph), 137.5 (C arom), 136.8, 136.3 (C arom), 130.8, 129.6, 128.8, 128.0 (CH arom), 68.6, 68.5 (CH_2 Z), 56.5 (αCH), 52.9 (O–*C*H$_3$), 49.3, 46.1, 26.1, 24.8 (N–(*C*H$_2$)$_4$), 14.6 (βCH_3); HRMS calcd for $C_{24}H_{27}N_3O_6$ [M+Na$^+$] *m*/*z* 476.17921, found 476.1793.

4.4.3. N^{α}-(Benzyloxycarbonyl)-N^{β}, [2-(pyrrolidine-1-carbonyl)-benzoyl]-hydrazinovaline methyl ester (7, R=CH(CH$_3$)$_2$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3275, 1741, 1734, 1717; ^1H NMR (300 MHz, $CDCl_3$) δ 9.20–8.90 (m, 1H, βNH), 7.89–7.70 (m, 1H, arom), 7.62–7.26 (m, 8H, arom), 5.30–5.10 (m, 2H, CH_2 Z), 4.93–4.36 (m, 1H, αCH), 3.86–3.51 (m, 5H, O–CH_3, pyrro), 3.35–2.93 (m, 2H, pyrro), 2.47–2.14 (m, 1H, βCH), 2.04–1.60 (m, 4H, pyrro), 1.11–0.89 (m, 6H, γCH_3); ^{13}C NMR ($CDCl_3$) δ 171.4 (*C*OOCH$_3$), 170.1 (O*C*-pyrro), 167.2 (N–*C*OC_6H_4), 160.0, 155.7 (*C*OOCH$_2$Ph), 137.5 (C arom), 136.8, 136.3 (C arom), 130.9, 130.6, 129.8, 129.0, 128.7, 128.2, 126.9 (CH arom), 69.0 (CH_2 Z), 65.6 (αCH), 52.8 (O–*C*H$_3$), 49.7, 46.5 (CH_2 pyrro), 28.6 (βCH), 26.3, 25.0 (2CH_2 pyrro), 20.1, 19.7 (γCH_3); HRMS calcd for $C_{26}H_{31}N_3O_6$ [M+Na$^+$] *m*/*z* 504.21051, found 504.2109.

4.4.4. N^{α}-(Benzyloxycarbonyl)-N^{β}, [2-(pyrrolidine-1-carbonyl)-benzoyl]-hydrazinoleucine methyl ester (7, R=CH$_2$CH(CH$_3$)$_2$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3171, 1772, 1743, 1718; ^1H NMR (300 MHz, $CDCl_3$) δ 8.68 (s, 1H, βNH), 7.76–7.48 (m, 1H, arom), 7.44–7.07 (m, 8H, arom), 5.25–4.82 (m, 3H, CH_2 Z, αCH), 3.65 (s, 3H, O–CH_3), 3.54–3.46 (m, 2H, pyrro), 3.05–2.90 (m, 2H, pyrro), 1.98–1.49 (m, 7H, pyrro, βCH_2 and γCH), 0.91–0.75 (m, 6H, δCH_3); ^{13}C NMR ($CDCl_3$) δ 172.3 (*C*OOCH$_3$), 169.5 (O*C*-pyrro), 167.2 (N–*C*OC_6H_4), 156.3 (*C*OOCH$_2$Ph), 136.8, 136.5 (C arom), 132.1 (CH arom), 130.9 (C arom), 130.0, 129.7, 129.5, 128.8, 128.4, 127.8, 126.6 (CH arom), 68.7 (CH_2 Z), 60.0, 59.0 (αCH), 52.9 (O–*C*H$_3$), 49.3, 46.1 (2*C*H$_2$ pyrro), 38.1, 37.8 (*C*H$_2$), 26.1, 24.8 (2*C*H$_2$ pyrro), 24.7 (γCH), 23.6, 21.7 (δCH_3); HRMS calcd for $C_{27}H_{33}N_3O_6$ [M+Na$^+$] *m*/*z* 518.22616, found 518.2265.

4.4.5. N^{α}-(Benzyloxycarbonyl)-N^{β}, [2-(pyrrolidine-1-carbonyl)-benzoyl]-hydrazinoisoleucine methyl ester (7, R=CH(CH$_3$)CH$_2$CH$_3$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3245, 1772, 1741, 1717, 1696; ^1H NMR (300 MHz, $CDCl_3$) δ 9.22–8.91 (m, 1H, βNH), 7.97–7.65 (m, 1H, arom), 7.56–7.22 (m, 8H, arom), 5.30–5.05 (m, 2H, CH_2 Z), 4.98–4.48 (m, 1H, αCH), 3.88–3.41 (m, 5H, O–CH_3, pyrro), 3.29–2.89 (m, 2H, pyrro), 2.05–1.70 (m, 4H, pyrro), 1.60–1.40 (m, 1H, βCH), 1.33–1.16 (m, 2H, γCH_2), 1.05–0.72 (m, 2H, δCH_3); ^{13}C NMR ($CDCl_3$) δ 171.0 (*C*OOCH$_3$), 169.7 (O*C*-pyrro), 166.9 (N–*C*OC_6H_4), 156.2 (*C*OOCH$_2$Ph), 136.5 (C arom), 131.7 (CH arom), 130.5, 130.2 (C arom), 129.3, 128.7, 128.3, 127.8, 126.6 (CH arom), 68.6 (CH_2 Z), 63.8 (αCH), 60.5 (γCH_2), 52.4 (O–*C*H$_3$), 49.3, 46.1 (2CH_2 pyrro), 34.7 (βCH), 26.1, 24.8 (2CH_2 pyrro), 15.7 (βCH_3), 11.6 (δCH_3); HRMS calcd for $C_{27}H_{33}N_3O_6$ [M+Na$^+$] *m*/*z* 518.22616, found 518.2263.

4.4.6. N^{α}-(Benzyloxycarbonyl)-N^{β}, [2-(pyrrolidine-1-carbonyl)-benzoyl]-hydrazinophenylalanine methyl ester (7, R=CH$_2$C$_6$H$_5$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3289, 1792, 1743, 1718; ^1H NMR (300 MHz, $CDCl_3$) δ 8.83–8.92 (m, 1H, βNH), 8.00–7.08 (m, 14H, arom), 5.37–4.90 (m, 3H, CH_2 Z and αCH), 3.83–3.47 (m, 5H, O–CH_3 et pyrro), 3.40–2.93 (m, 4H, pyrro et βCH_2), 2.04–1.63 (m, 4H, pyrro); ^{13}C NMR ($CDCl_3$) δ 171.7 (*C*OOCH$_3$), 170.1 (O*C*-pyrro), 167.9 (N–*C*OC_6H_4), 156.6 (*C*OOCH$_2$Ph), 137.8 (C arom), 137.0, 136.4 (C arom), 135.3, 134.4, 132.7, 132.6, 132.2, 131.2 (CH arom), 131.0, 130.4, 128.7, 128.2, 126.8, 124.5, 124.1, 122.2 (CH arom), 69.0 (CH_2 Z), 53.1 (O–*C*H$_3$), 49.7, 46.6 (2*C*H$_2$ pyrro), 35.5 (βCH_2), 26.1, 24.8 (2CH_2 pyrro); HRMS calcd for $C_{30}H_{31}N_3O_6$ [M+Na$^+$] *m*/*z* 552.21051, found 552.2103.

4.5. General procedure for the synthesis of compound 8

Compound **7** (3 mmol) was dissolved in THF (20 mL) and 1.5 equiv of Boc_2O and a catalytic amount of DMAP were added. The mixture was stirred at room temperature for 6 h until completion (monitored by TLC). The solvent was removed in vacuo, and the residue was chromatographied on silica gel.

4.5.1. N^{α}-(Benzyloxycarbonyl)-N^{β}, [2-(pyrrolidine-1-carbonyl)-benzoyl]-N^{β}, *tert*-butyloxycarbonyl-hydrazinoglycine methyl ester (8, R=H). Oil; IR (NaCl) ν_{max}/cm^{-1}: 1810, 1735, 1680; ^1H NMR (300 MHz, $CDCl_3$) δ 7.23–7.09 (m, 9H, arom), 5.28–5.15 (m, 2H, CH_2 Z), 4.27–4.01 (m, 2H, αCH_2), 3.80–3.33 (m, 7H, O–CH_3 et pyrro), 1.88–1.75 (m, 4H, pyrro), 1.31–1.85 (m, 9H, Boc); ^{13}C NMR ($CDCl_3$) δ 169.9, 169.5 (*C*OOCH$_3$), 169.0, 168.6 (O*C*-pyrro), 168.4 (N–*C*OC_6H_4), 155.8, 155.5 (*C*OOCH$_2$Ph), 151.0 (*C*O*O*t-Bu), 136.7 (C arom Z), 136.3, 135.8 (C arom C_4H_6), 129.4, 129.2, 128.9, 128.6, 128.1, 127.8, 127.2, 127.0 (CH arom), 85.5 (*C*Boc), 69.4 (CH_2 Z), 54.1, 53.1 (αCH_2), 52.6, 52.5 (O–*C*H$_3$), 49.7, 46.5 (2*C*H$_2$ pyrro), 28.2, 28.1 (CH_3 Boc) et 26.7, 25.1 (2*C*H$_2$ pyrro); HRMS calcd for $C_{28}H_{33}N_3O_8$ [M+Na$^+$] *m*/*z* 562.21599, found 562.2163.

4.5.2. N^{α}-(Benzyloxycarbonyl)-N^{β}, [2-(pyrrolidine-1-carbonyl)-benzoyl]-N^{β}, *tert*-butyloxycarbonyl-hydrazino-alanine methyl ester (8, R=CH_3). Oil; IR (NaCl) ν_{max}/cm^{-1}: 1825, 1733, 1684; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69–7.12 (m, 9H, arom), 5.22–5.01 (m, 2H, CH_2 Z), 4.92 (q, J=7 Hz, 0.26H, αCH), 4.84 (q, J=7 Hz, 0.20H, αCH), 4.71 (q, J=7 Hz, 0.33H, αCH), 4.62 (q, J=7 Hz, 0.24H, αCH), 3.66–3.52 (m, 5H, O–CH_3 et pyrro), 3.39–3.19 (m, 2H, pyrro), 2.01–1.73 (m, 4H, pyrro) et 1.55–1.35 (m, 12H, βCH_3 et Boc); ^{13}C NMR ($CDCl_3$) δ 171.5, 171.3, 171.2 (*C*OOCH$_3$), 171.1, 170.0, 169.7, 169.5 (O*C*-pyrro), 168.2, 168.1, 168.0 (N–*C*OC_6H_4), 154.9, 154.8, 154.7 (*C*OOCH$_2$Ph), 151.4, 151.2, 151.1 (*C*O*O*t-Bu), 137.3, 137.1, 136.9, 136.0, 135.8, 134.8 (C arom), 128.7, 128.5, 128.3, 127.6, 127.3, 126.9 (CH arom), 85.0, 84.9, 84.8 (*C*Boc), 68.9, 68.8 (CH_2 Z), 58.8, 58.1, 57.7, 56.7 (αCH), 52.6, 52.5 (O–*C*H$_3$), 49.2, 45.9, 26.3, 24.8 (N–(*C*H$_2$)$_4$), 27.7, 27.6, 27.5 (CBoc), 14.5, 14.4 (CH_3); HRMS calcd for $C_{29}H_{35}N_3O_8$ [M+Na$^+$] *m*/*z* 576.23164, found 576.2316.

4.5.3. N^{α}-(Benzyloxycarbonyl)-N^{β}, [2-(pyrrolidine-1-carbonyl)-benzoyl]-N^{β}, *tert*-butyloxycarbonyl-hydrazino-valine methyl ester (8, R=$CH(CH_3)_2$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 1786, 1740, 1723; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03–7.18 (m, 9H, arom), 5.41–4.87 (m, 2.5H, 2CH_2 Z, 0.5H, αCH), 4.84–4.37 (m, 0.5H, αCH), 3.92–3.55 (m, 5H, O–CH_3 et pyrro), 3.26–2.97 (m, 2H, pyrro), 2.04–1.58 (m, 4H, pyrro), 1.48–0.83 (m, 16H, γCH_3, βCH, Boc); ^{13}C NMR ($CDCl_3$) δ 170.1, 170.0, 169.9 (*C*OOCH$_3$), 169.6, 169.5, 169.4 (O*C*-pyrro), 168.2, 168.1, 168.0 (N–*C*OC_6H_4), 155.7, 155.6, 155.0, 154.9 (*C*OOCH$_2$Ph), 151.9, 151.8, 151.5, 151.4 (*C*O*O*t-Bu), 138.3, 138.0, 137.7 (C arom Z), 136.0, 135.9, 135.8, 134.0, 133.8, 133.7 (C arom C_6H_4), 131.4, 131.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.4, 127.2, 126.9 (CH arom), 85.2, 85.1 (*C*Boc), 69.1, 69.0, 68.8 (CH_2 Z), 66.8 (αCH), 52.2, 52.1 (O–*C*H$_3$), 49.8, 46.0, 45.7 (CH_2 pyrro), 29.1, 29.0 (βCH), 27.7, 27.6, 27.5, 27.4 (CH_3 Boc), 26.1, 24.8, 24.7 (CH_2 pyrro), 20.0, 19.4, 19.3, 19.2 (γCH_3); HRMS calcd for $C_{31}H_{39}N_3O_8$ [M+Na$^+$] *m*/*z* 604.26294, found 604.2630.

4.5.4. N^{α}-(Benzyloxycarbonyl)-N^{β}, [2-(pyrrolidine-1-carbonyl)-benzoyl]-N^{β}, *tert*-butyloxycarbonyl-hydrazino-leucine methyl ester (8, R=$CH_2CH(CH_3)_2$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 1792, 1756, 1733, 1718, 1669; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72–7.13 (m, 9H, arom), 5.33–4.68 (m, 3H, CH_2 Z, αCH), 3.63–2.99 (m, 7H, O–CH_3 et pyrro), 1.84–1.32 (m, 7H, pyrro, γCH and βCH_2), 1.22–0.81 (m, 15H, δCH_3 et Boc); ^{13}C NMR ($CDCl_3$) δ 171.1, 171.0 (*C*OOCH$_3$), 170.1, 169.8 (O*C*-pyrro), 168.4, 168.2 (N–*C*OC_6H_4), 155.4, 155.3, 154.8 (*C*OOCH$_2$Ph), 151.8, 151.5 (*C*O*O*t-Bu), 138.2, 138.1, 137.9, 137.8 (C arom Z), 136.2, 136.1, 134.4, 134.3 (C arom C_6H_4), 131.3, 131.1, 128.9, 128.7, 128.6, 128.5, 128.4, 127.7, 127.5, 127.2 (CH arom), 85.3 (*C*Boc), 69.2, 69.1 (CH_2 Z), 60.6, 60.1, 59.3, 58.4 (αCH), 52.7, 52.6, 52.5 (O–*C*H$_3$), 49.1, 46.2, 46.0, 45.9 (CH_2 pyrro), 38.9, 38.6, 38.5, 38.4 (βCH_2), 27.9, 27.7 (CH_3 Boc), 26.4 (CH_2 pyrro), 25.3, 25.2 (γCH), 25.0 (CH_2 pyrro), 23.5, 23.4, 22.7, 22.6, 22.4 (δCH_3); HRMS calcd for $C_{32}H_{41}N_3O_8$ [M+H$^+$] *m*/*z* 596.2972, found 596.2979.

4.5.5. N^{α}-(Benzyloxycarbonyl)-N^{β}, [2-(pyrrolidine-1-carbonyl)-benzoyl]-N^{β}, *tert*-butyloxycarbonyl-hydrazino-isoleucine methyl ester (8, R=$CH(CH_3)CH_2CH_3$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 1749, 1732, 1717, 1684, 1670; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99–7.15 (m, 9H, arom), 5.36–5.08 (m, 2H, CH_2 Z), 4.89–4.68 (m, 0.77H, αCH), 4.63–4.45 (m, 0.23H, αCH), 3.79–3.05 (m, 7H, O–CH_3 et pyrro), 2.04–1.57 (m, 4H, pyrro) et 1.45–0.71 (m, 18H, βCH, γCH_2, γCH_3, Boc); ^{13}C NMR ($CDCl_3$) δ 170.7, 170.5, 170.3 (*C*OOCH$_3$), 169.8 (O*C*-pyrro), 168.6 (N–*C*OC_6H_4), 156.0 (*C*OOCH$_2$Ph), 151.7 (*C*O*O*t-Bu), 138.6, 138.4 (C arom Z), 136.3, 136.2, 134.4, 134.3 (C arom C_6H_4), 131.7, 131.3, 129.4, 129.2, 129.1, 129.0, 128.9, 128.7, 128.6, 128.5, 128.4, 127.7, 127.2 (CH arom), 85.5, 85.4 (*C*Boc), 69.4, 69.2 (CH_2 Z), 66.3 (αCH), 61.1 (γCH_2), 52.6, 52.5 (O–*C*H$_3$), 49.2, 49.1, 46.1, 46.0 (CH_2 pyrro), 35.6, 35.4 (βCH), 28.1, 27.9, 27.8 (CH_3 Boc), 26.2, 26.1 (CH_2 pyrro), 16.1, 11.5 (β, δCH_3); HRMS calcd for $C_{32}H_{41}N_3O_8$ [M+Na$^+$] *m*/*z* 618.27859, found 618.2785.

4.5.6. N^{α}-(Benzyloxycarbonyl)-N^{β}, [2-(pyrrolidine-1-carbonyl)-benzoyl]-N^{β}, *tert*-butyloxycarbonyl-hydrazino-phenylalanine methyl ester (8, R=$CH_2C_6H_5$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 1806, 1754, 1727, 1638, 7.81–7.07; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81–7.07 (m, 14H, arom), 5.35–4.82 (m, 3H, CH_2 Z, αCH), 3.62–2.98 (m, 9H, O–CH_3, βCH_2 et pyrro), 1.97–1.66 (m, 4H, pyrro), 1.31–0.95 (m, 9H, Boc); ^{13}C NMR ($CDCl_3$) δ 170.3, 170.1, 170.0 (*C*OOCH$_3$), 169.8, 169.7 (O*C*-pyrro), 168.4, 168.3 (N–*C*OC_6H_4), 155.1, 154.6 (*C*OOCH$_2$Ph), 151.8, 151.7, 151.5, 151.3 (*C*O*O*t-Bu), 138.1, 138.0, 137.3, 137.1, 137.0, 136.8, 136.1, 136.0, 134.6, 134.5 (C arom), 131.3, 131.2, 131.1, 130.1, 130.0, 129.9, 129.1, 129.0, 128.9, 128.8, 128.7, 128.5, 128.4, 128.2, 127.5, 127.3 (CH arom), 85.6, 85.5, 85.3 (*C*Boc), 69.8, 69.4, 69.3 (CH_2 Z), 63.9, 63.7, 63.5, 62.5, 61.8 (αCH), 52.6, 52.4 (O–*C*H$_3$), 49.3, 49.2, 46.3, 46.1, 46.0 (CH_2 pyrro), 36.2, 36.1 (βCH_2), 28.3, 27.9, 27.7 (CH_3 Boc) et 26.5, 26.4, 25.0 (CH_2 pyrro); HRMS calcd for $C_{35}H_{39}N_3O_8$ 629.27 [M+Na$^+$] *m*/*z* 652.26294, found 652.2632.

4.6. General procedure for the preparation of compound 9

Compound **8** (3 mmol) was dissolved in THF (20 mL) and a solution of methylamine (4.5 mmol, 2 M in MeOH) was added at room temperature. After 3 h (R=H, CH_3, CH_2Ph) or 5 h (until completion monitored by TLC), the solvent and the excess of amine were removed in vacuo and **9** was purified by column chromatography.

4.6.1. N^{α}-(Benzyloxycarbonyl)-N^{β} (*tert*-butyloxycarbonyl)-hydrazinoglycine methyl ester (9, R=H). White solid; IR (NaCl) ν_{max}/cm^{-1}: 3325, 1733, 1717; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.18 (m, 5H, arom), 6.88–6.37 (m, 1H, βNH), 5.25–5.06 (m, 2H, CH_2 Z), 4.46–4.24 (m, 2H, αCH_2), 3.82–3.61 (m, 3H, O–CH_3) et 1.61–1.31 (m, 9H, Boc); ^{13}C NMR ($CDCl_3$) δ 170.6 (*C*OOCH$_3$), 156.6 (*C*OOCH$_2$Ph), 155.6 (*C*O*O*t-Bu), 136.3 (C arom), 129.1, 129.0, 128.8, 128.7, 128.4 (CH arom), 82.5 (*C*Boc), 69.2, 69.0 (CH_2 Z), 52.9 (O–CH_3), 51.9 (αCH_2) et 28.7 (CH_3 Boc); HRMS calcd for $C_{16}H_{22}N_2O_6$ [M+Na$^+$] *m*/*z* 361.13701, found 361.1372.

4.6.2. N^{α}-(Benzyloxycarbonyl)-N^{β} (*tert*-butyloxycarbonyl)-hydrazinoalanine methyl ester (9, R=CH_3). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3320, 1743, 1717; ^1H NMR (300 MHz, $CDCl_3$) δ 7.58–7.17 (m, 5H, arom), 6.88–6.35 (m, 1H, βNH), 5.47–4.66 (m, 3H, CH_2 Z et αCH), 3.96–3.56 (m, 3H, O–CH_3), 1.60–1.32 (m, 12H, Boc et βCH_3), 173.1 (*C*OOCH$_3$), 156.5 (*C*OOCH$_2$Ph), 155.7 (*COO*t-Bu), 136.2 (C arom), 128.9, 128.6, 128.4, 128.3 (CH arom), 81.6 (*C*Boc), 68.7 (CH_2 Z), 57.6, 56.4 (αCH), 52.8 (O–CH_3), 28.5 (CH_3 Boc) et 14.7 (βCH_3); HRMS calcd for $C_{17}H_{24}N_2O_6$ [M+Na$^+$] *m/z* 375.15266, found 375.1528.

4.6.3. N^{α}-(Benzyloxycarbonyl)-N^{β} (*tert*-butyloxycarbonyl)-hydrazinovaline methyl ester (9, R=$CH(CH_3)_2$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3332, 1747, 1717; ^1H NMR (300 MHz, $CDCl_3$) δ 7.51–7.20 (m, 5H, arom), 6.80–6.34 (m, 1H, βNH), 5.29–5.05 (m, 2H, CH_2 Z), 4.82–4.38 (m, 1H, αCH), 3.81–3.56 (m, 3H, O–CH_3), 2.34–2.01 (m, 1H, βCH), 1.53–1.30 (m, 9H, Boc), 1.16–0.80 (m, 6H, γCH_3); ^{13}C NMR ($CDCl_3$) δ 171.8 (*C*OOCH$_3$), 157.2 (*C*OOCH$_2$Ph), 155.2 (*COO*t-Bu), 136.3 (C arom), 129.1, 128.8, 128.5 (CH arom), 81.9 (*C*Boc), 69.1 (CH_2 Z), 65.9 (αCH), 52.7 (O–CH_3), 29.1 (βCH), 28.7 (CH_3 Boc) et 20.3, 19.6 (2CH_3); HRMS calcd for $C_{19}H_{28}N_2O_6$ [M+Na$^+$] *m/z* 403.18396, found 403.1838.

4.6.4. N^{α}-(Benzyloxycarbonyl)-N^{β} (*tert*-butyloxycarbonyl)-hydrazinoleucine methyl ester (9, R=$CH_2CH(CH_3)_2$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3334, 1740, 1684; ^1H NMR (300 MHz, $CDCl_3$) δ 7.49–7.17 (m, 5H, arom), 6.70–6.23 (m, 1H, βNH), 5.35–4.67 (m, 3H, CH_2 Z, αCH), 3.84–3.54 (m, 3H, O–CH_3), 1.88–1.24 (m, 12H, βCH_2, γCH, Boc) et 1.05–0.79 (m, 6H, δCH_3); ^{13}C NMR ($CDCl_3$) δ 173.5 (*C*OOCH$_3$), 157.3 (*C*OOCH$_2$Ph), 155.4 (*COO*t-Bu), 136.3 (C arom), 129.1, 128.8, 128.5 (CH arom), 81.8 (*C*Boc), 69.1 (CH_2 Z), 60.4, 59.4 (αCH), 53.0 (O–CH_3), 38.2 (βCH_2), 29.1 (γCH), 28.7 (CH_3 Boc), et 23.1, 21.9 (δCH_3); HRMS calcd for $C_{20}H_{30}N_2O_6$ [M+Na$^+$] *m/z* 417.19961, found 417.1995.

4.6.5. N^{α}-(Benzyloxycarbonyl)-N^{β} (*tert*-butyloxycarbonyl)-hydrazinoisoleucine methyl ester (9, R=$CH(CH_3)CH_2CH_3$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3333, 1747, 1716; ^1H NMR (300 MHz, $CDCl_3$) δ 7.43–7.12 (m, 5H, arom), 6.83–6.32 (m, 1H, βNH), 5.27–4.50 (m, 3H, CH_2 Z, αCH), 3.86–3.56 (m, 3H, O–CH_3), 1.70–1.15 (m, 12H, Boc, βCH, γCH_2) et 1.13–0.76 (m, 6H, δCH_3); ^{13}C NMR ($CDCl_3$) δ 172.6 (*C*OOCH$_3$), 157.6 (*C*OOCH$_2$Ph), 136.3 (C arom), 129.1, 128.8, 128.5 (CH arom), 81.8 (*C*Boc), 69.2 (CH_2 Z), 64.4 (αCH), 52.7 (O–CH_3), 35.9 (βCH), 28.7 (CH_3 Boc), 26.5 (γCH_2), 16.3, 12.3 (β, δCH_3); HRMS calcd for $C_{20}H_{30}N_2O_6$ [M+Na$^+$] *m/z* 417.19961, found 417.1993.

4.6.6. N^{α}-(Benzyloxycarbonyl)-N^{β} (*tert*-butyloxycarbonyl)-hydrazinophenylalanine methyl ester (9, R=$CH_2C_6H_5$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3313, 1794, 1744, 1717; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38–7.09 (m, 10H, arom), 6.86–6.35 (m, 1H, βNH), 5.35–4.77 (m, 3H, CH_2 Z, αCH), 3.75–3.41 (m, 3H, O–CH_3), 3.26–3.03 (m, 2H, βCH_2), 1.49–1.27 (m, 9H, Boc); ^{13}C NMR ($CDCl_3$) δ 171.5 (*C*OOCH$_3$), 156.4 (*C*OOCH$_2$Ph), 155.4 (*COO*t-Bu), 137.2, 136.1 (C arom), 129.6, 128.9, 128.6, 128.4, 127.9, 127.8, 127.3, 127.1 (CH arom), 81.9 (*C*Boc), 68.8 (CH_2 Z), 62.2 (αCH), 52.8 (O–CH_3), 35.5 (βCH_2), 28.5 (CH_3 Boc); HRMS calcd for $C_{23}H_{28}N_2O_6$ [M+Na$^+$] *m/z* 451.18396, found 451.1840.

4.7. General procedure for the preparation of compound 10

α-Hydrazinoester **9** (1 mmol) was dissolved in 10 mL of THF and LiOH (10 mL, 2.5 M) was added. The mixture was stirred at room temperature for 1 h until completion (monitored by TLC). The mixture was poured into water and extracted three times with chloroform. The aqueous layer was then acidified to pH 1 and extracted eight times with EtOAc. The combined organic layers were dried over $MgSO_4$ and evaporated to give the desired α-hydrazinoacid **10**.

4.7.1. N^{α}-(Benzyloxycarbonyl)-N^{β} (*tert*-butyloxycarbonyl)-hydrazinoglycine (10, R=H). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3466–3126, 1734, 1718; ^1H NMR (300 MHz, DMSO) δ 9.83–8.91 (m, 1H, COOH), 7.39–7.27 (m, 5H, arom), 5.76–5.73 (m, 1H, βNH), 5.24–4.48 (m, 2H, CH_2 Z), 4.33–3.72 (m, 2H, αCH_2) et 1.61–1.10 (m, 9H, Boc); ^{13}C NMR ($CDCl_3$) δ 156.6 (*C*OOCH$_2$Ph), 155.6 (*COO*t-Bu), 136.3 (C arom), 129.1, 129.0, 128.8, 128.7, 128.4 (CH arom), 82.5 (*C*Boc), 69.2, 69.0 (CH_2 Z), 51.9 (αCH_2), 28.7 (CH_3 Boc).

4.7.2. N^{α}-(Benzyloxycarbonyl)-N^{β} (*tert*-butyloxycarbonyl)-hydrazinoalanine (10, R=CH_3). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3500–3000, 1743, 1717; ^1H NMR (300 MHz, $CDCl_3$) δ 9.34–9.12 (m, 1H, COOH), 7.68–6.98 (m, 6H, arom, βNH), 5.20–4.66 (m, 3H, CH_2 Z, αCH), 1.45–1.28 (m, 12H, Boc, βCH_3); ^{13}C NMR ($CDCl_3$) δ 175.4, 175.0 (*C*OOH), 157.4 (*C*OOCH$_2$Ph), 156.4 (*COO*t-Bu), 136.0 (C arom), 128.9, 128.8, 128.7, 128.5, 128.3, 127.9, 127.5 (CH arom), 82.9, 82.5 (*C*Boc), 69.2, 69.0, 68.6 (CH_2 Z), 58.6 (αCH), 28.6, 28.5, 28.0 (CH_3 Boc), 15.0, 14.6 (βCH_3); HRMS calcd for $C_{16}H_{22}N_2O_6$ [M+Na$^+$] *m/z* 361.13701, found 361.1372.

4.7.3. N^{α}-(Benzyloxycarbonyl)-N^{β} (*tert*-butyloxycarbonyl)-hydrazinovaline (10, R=$CH(CH_3)_2$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3490–3170, 1736, 1715; ^1H NMR (300 MHz, $CDCl_3$) δ 10.92–9.67 (m, 1H, COOH), 7.70–6.59 (m, 6H, 5 arom, βNH), 5.35–4.93 (m, 2H, CH_2 Z), 4.81–3.88 (m, 1H, αCH), 2.56–2.16 (m, 1H, βCH), 1.58–0.90 (m, 15H, Boc, γCH_3); ^{13}C NMR ($CDCl_3$) δ 177.6, 176.9 (*C*OOH), 156.8 (*C*OOCH$_2$Ph), 136.0 (C arom), 129.1, 128.9, 128.6 (CH arom), 83.4 (CBoc), 69.3, 69.1 (CH_2 Z), 67.1 (αCH), 30.3 (βCH), 28.7 (CH_3 Boc) et 20.9, 17.9 (γCH_3); HRMS calcd for $C_{18}H_{26}N_2O_6$ [M+Na$^+$] *m/z* 389.16831, found 389.1682.

4.7.4. N^{α}-(Benzyloxycarbonyl)-N^{β} (*tert*-butyloxycarbonyl)-hydrazinoleucine (10, R=$CH_2CH(CH_3)_2$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3400–3186, 1718; ^1H NMR (300 MHz, $CDCl_3$) δ 10.47–8.94 (m, 1H, COOH), 7.61–7.06 (m, 6H, 5 arom, βNH), 5.47–4.34 (m, 3H, CH_2 Z, αCH), 1.94–1.05 (m, 12H, γCH, βCH_2, Boc), 1.02–0.55 (m, 6H, δCH_3); ^{13}C NMR ($CDCl_3$) δ 156.7 (*C*OOCH$_2$Ph), 136.3 (C arom), 129.2, 128.9, 128.6 (CH arom), 85.8 (*C*Boc), 69.8, 69.3

(CH_2 Z), 60.5 (αCH), 38.5, 38.1 (βCH_2), 29.1 (γCH), 28.6 (CH_3 Boc), et 23.8, 21.9 (δCH_3); HRMS calcd for $C_{19}H_{28}N_2O_6$ [M+Na$^+$] *m/z* 403.18396, found 403.1839.

4.7.5. N^{α}-(Benzyloxycarbonyl)-N^{β} (*tert*-butyloxycarbonyl)-hydrazinoisoleucine (10, R=CH(CH_3)CH_2CH_3). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3500–3124, 1733, 1716; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.13–9.31 (m, 1H, COOH), 7.49–6.55 (m, 6H, 5 arom, βNH), 5.56–4.05 (m, 3H, CH_2 Z, αCH), 2.23–1.16 (m, 12H, Boc, γCH_2, βCH), 1.09–0.72 (m, 6H, βCH_3, δCH_3); ^{13}C NMR ($CDCl_3$) δ 177.7, 177.2 (*C*OOH), 157.1 (*C*OOCH_2Ph), 136.0 (C arom), 129.1, 128.9, 128.5 (CH arom), 82.3 (CBoc), 69.3, 69.0 (CH_2 Z), 68.1 (αCH), 37.1 (βCH), 28.8, 28.6 (CH_3 Boc), 26.8, 26.6, 26.3 (γCH_2), 16.6, 16.1, 12.4, 12.2 (β, δCH_3); HRMS calcd for $C_{19}H_{28}N_2O_6$ [M+Na$^+$] *m/z* 403.18396, found 403.1837.

4.7.6. N^{α}-(Benzyloxycarbonyl)-N^{β} (*tert*-butyloxycarbonyl)-hydrazinophenylalanine (10, R=$CH_2C_6H_5$). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3601–3225, 1734, 1717; ^{1}H NMR (300 MHz, DMSO) δ 9.98–6.72 (m, 13H, arom, βNH, COOH), 5.49–4.60 (m, 3H, CH_2 Z, αCH), 3.07–2.68 (m, 1H, βCH_2), 2.67–2.53 (m, 1H, βCH_2) 1.68–1.22 (m, 9H, Boc); ^{13}C NMR (DMSO) δ 157.1, 156.8 (*C*OOCH_2Ph), 155.6 (*C*OO*t*-Bu), 142.5, 140.8, 137.2, 136.2 (C arom), 129.0, 128.3, 128.1, 127.9, 127.8, 127.3, 127.0 (CH arom), 81.9 (*C*Boc), 66.5 (CH_2 Z), 64.2, 62.8 (αCH_2), 28.0 (CH_3 Boc); HRMS calcd for $C_{22}H_{26}N_2O_6$ [M+Na$^+$] *m/z* 437.16831, found 437.1685.

4.8. General procedure for the preparation of compound 11

A solution of dry hydrochloric acid in EtOAc (3 N, 3 mL) was added to compound **9** (3 mmol). The mixture was stirred at room temperature for 1 h until completion (monitored by TLC). The mixture was poured into saturated solution of $NaHCO_3$ (pH 7–8) and extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was dissolved in a 10% solution of Na_2CO_3 in water (7.95 mL, 7.5 mmol). Dioxane (4.5 mL) is added and the mixture is stirred in an ice-water bath. 9-Fluorenylmethyl chlorocarbonate (1.16 g, 4.5 mmol) is added in small portions and stirring is continued at an ice-water bath temperature for 30 min and then at room temperature for 12 h. The reaction mixture is poured into water (180 mL), the solution is acidified to pH 1, and then extracted with diethyl ether. The combined organic layers were dried over $MgSO_4$ and evaporated in vacuo. The residue was chromatographied on silica gel.

4.8.1. N^{α}-(Benzyloxycarbonyl)-N^{β} (9*H*-fluoren-9-ylmethoxycarbonyl)-hydrazinoalanine methyl ester (11, R=CH_3). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3311, 1741, 1717; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03–7.09 (m, 14H, arom), 7.02–6.24 (m, 1H, βNH), 5.52–4.65 (m, 3H, CH_2 Z, αCH), 4.73–4.09 (m, 3H, CH Fmoc et CH_2 Fmoc), 3.85–3.56 (m, 3H, O–CH_3), 1.70–1.32 (m, 3H, βCH_3); ^{13}C NMR ($CDCl_3$) δ 172.7, 171.1 (*C*OOCH_3), 157.2, 157.0 (*C*OOCH_2Ph), 156.9, 156.7, 156.1 (*C*OOFmoc), 144.0, 143.7, 141.6, 136.0 (C arom), 128.6, 128.4, 128.3, 128.0, 127.3, 125.3, 120.2 (CH arom), 68.5, 68.1, 67.9 (CH_2 Z), 61.9, 61.5 (CH_2 Fmoc), 56.3 (αCH), 52.5, 52.3 (O–CH_3), 47.3 (CH Fmoc) et 14.3 (βCH_3); HRMS calcd for $C_{27}H_{26}N_2O_6$ [M+Na$^+$] *m/z* 497.16831, found 497.1682.

4.8.2. N^{α}-(Benzyloxycarbonyl)-N^{β} (9*H*-fluoren-9-ylmethoxycarbonyl)-hydrazinoglycine methyl ester (11, R=H). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3383, 1771, 1747, 1734, 1716; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01–6.72 (m, 14H, arom, βNH), 5.37–4.95 (m, 2H, CH_2 Z), 4.58–4.02 (m, 5H, αCH_2, CH_2 Fmoc, CH Fmoc), 3.80–3.63 (m, 3H, O–CH_3); ^{13}C NMR ($CDCl_3$) δ 170.5 (*C*OOCH_3), 156.4 (*C*OOCH_2Ph), 155.8 (*C*O Fmoc), 144.0, 141.9, 136.1 (C arom), 129.4, 129.1, 128.9, 128.6, 128.4, 127.9, 127.7 (CH arom), 69.4 (CH_2 Z), 68.4 (CH_2 Fmoc), 53.0 (O–CH_3), 52.8 (αCH_2), 47.6 (CH Fmoc); HRMS calcd for $C_{26}H_{24}N_2O_6$ [M+Na$^+$] *m/z* 483.15266, found 483.1530.

4.9. General procedure for the preparation of compound 12

α-Hydrazinoester **11** (1 mmol) was dissolved in 2.25 mL of $CaCl_2$ (0.8 M) in *i*-PrOH–H_2O, 7:3, at room temperature. NaOH (1.2 equiv) was added and the mixture was stirred for 7 h at room temperature. The mixture was poured into water, acidified and extracted five times with EtOAc. The combined organic layers were dried over $MgSO_4$ and evaporated to give the desired α-hydrazinoacid **12**.

4.9.1. N^{α}-(Benzyloxycarbonyl)-N^{β} (9*H*-fluoren-9-ylmethoxycarbonyl)-hydrazinoglycine (12, R=H). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3682–3085, 1734, 1717, 1653; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.38–9.70 (m, 1H, COOH), 8.29–6.93 (m, 14H, arom, βNH), 5.44–4.67 (m, 2H, CH_2 Z), 4.60–4.12 (m, 5H, αCH_2, CH_2 Fmoc, CH Fmoc); ^{13}C NMR ($CDCl_3$) δ 157.1 (*C*OOCH_2Ph), 143.5, 140.8, 136.3 (C arom), 128.9, 128.4, 128.3, 127.9, 127.8, 127.7, 127.4, 125.3, 121.4, 120.1 (CH arom), 67.4, 67.2 (CH_2 Z), 66.7, 66.6 (CH_2 Fmoc), 59.8 (αCH_2), 51.9 (CH Fmoc); HRMS calcd for $C_{25}H_{22}N_2O_6$ [M+Na$^+$] *m/z* 469.13701, found 469.1370.

4.9.2. N^{α}-(Benzyloxycarbonyl)-N^{β} (9*H*-fluoren-9-ylmethoxycarbonyl)-hydrazinoalanine (12, R=CH_3). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3447–3133, 1743, 1718; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.74–9.67 (m, 1H, COOH), 8.11–6.73 (m, 14H, arom, βNH), 5.29–4.63 (m, 3H, CH_2 Z, αCH), 4.57–3.96 (m, 3H, CH Fmoc, CH_2 Fmoc), 1.77–1.35 (m, 3H, βCH_3); ^{13}C NMR ($CDCl_3$) δ 177.9 (*C*OOH), 156.4 (*C*OOCH_2Ph), 144.2, 143.8, 141.9, 136.0 (C arom), 129.1, 128.9, 128.5, 127.8, 125.6, 120.7 (CH arom), 69.3, 68.6 (CH_2 Z), 61.1 (CH_2 Fmoc), 56.9 (αCH), 47.6 (CH Fmoc), 14.8 (βCH_3); HRMS calcd for $C_{26}H_{24}N_2O_6$ [M+Na$^+$] *m/z* 483.15266, found 483.1527.

4.10. General procedure for the preparation of compound 13

A solution of dry hydrochloric acid in EtOAc (3 N, 3 mL) was added to compound **9** (3 mmol). The mixture was stirred at room temperature for 1 h until completion (monitored by TLC). The mixture was poured into saturated solution of $NaHCO_3$ (pH 7–8) and extracted three times with EtOAc.

The combined organic layers were dried over $MgSO_4$ and evaporated.

4.10.1. N^{α}-(Benzyloxycarbonyl)-hydrazinoalanine methyl ester (13, R=CH_3). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3404, 1744, 1706; ^1H NMR (300 MHz, $CDCl_3$) 7.53–7.17 (m, 5H, H arom), 5.25–5.03 (m, 2H, CH_2 Z), 4.99–4.62 (m, 1H, βNH), 4.12–3.81 (m, 2H, βNH, αCH), 3.80–3.54 (m, 3H, O–CH_3), 1.61–1.30 (m, 3H, βCH_3); ^{13}C NMR ($CDCl_3$) δ 172.7 (*C*OOCH$_3$), 156.5 (*C*OOCH$_2$Ph), 136.2 (C arom), 129.1, 128.8, 128.5 (CH arom), 68.5 (CH_2 Z), 57.1 (αCH), 52.8 (O–CH_3), 15.3 (βCH_3); HRMS calcd for $C_{11}H_{14}N_2O_4$ [M+Na$^+$] *m/z* 253.1183, found 253.1169.

4.10.2. N^{α}-(Benzyloxycarbonyl)-hydrazinoisoleucine methyl ester (13, R=CH(CH_3)–CH_2–CH_3). Oil; IR (NaCl) ν_{max}/cm^{-1}: 3352, 1743, 1705; ^1H NMR (300 MHz, $CDCl_3$) 7.56–7.16 (m, 5H, H arom), 5.25–5.07 (m, 2H, CH_2 Z), 4.82–4.29 (m, 1H, βNH), 4.22–3.81 (m, 2H, βNH, αCH), 3.77–3.51 (m, 3H, O–CH_3), 2.34–2.09 (m, 1H, βCH), 1.63–1.38 (m, 1H, γCH_2), 1.32–1.08 (m, 1H, γCH_2), 1.10–0.73 (m, 6H, βCH_3 et γCH_3); ^{13}C NMR ($CDCl_3$) δ 172.1 (*C*OOCH$_3$), 158.6 (*C*OOCH$_2$Ph), 136.7 (C arom), 129.1, 128.8, 128.5 (CH arom), 68.6 (CH_2 Z), 64.9 (αCH), 52.4 (O–CH_3), 35.3, 34.6 (βCH); 26.8, 26.5 (γCH_2), 16.7, 16.4 (βCH_3), 11.9, 11.4 (γCH_3); HRMS calcd for $C_{14}H_{20}N_2O_4$ [M+Na$^+$] *m/z* 295.1652, found 295.1652.

4.11. Procedures for coupling reactions (see Table 2)

4.11.1. TBTU/HOBt/DIEA (entry 1, Table 2). To a stirred solution of *N*-Fmoc-L-Ala-OH (1.35 mmol) in 3 mL of dry THF was added at 0 °C successively HOBt (1.35 mmol), TBTU (1.35 mmol), a cold solution of **13** (0.45 mmol) in 5 mL of dry THF and DIEA (4.5 mmol). After 15 min at 0 °C, the mixture was allowed to warm to room temperature and stirred overnight. The solvent was evaporated and the residue was chromatographied on silica gel (50%).

4.11.2. DCC/HOBt (entry 4, Table 2). To a stirred solution of **13** (1 equiv), *N*-Fmoc-L-Ala-OH (1 equiv) and HOBt (1.5 equiv) in dry THF (0.5 M) was added at 0 °C DCC (1 equiv). After 1 h at 0 °C, the mixture was allowed to warm to room temperature and stirred overnight. The DCHU was filtered, the solvent was evaporated and the residue was dissolved in EtOAc. The mixture was stirred for 2 h at −20 °C and filtered. The solvent was evaporated and the residue was chromatographied on silica gel (73%).

4.11.3. HATU/HOBt/DIEA (entry 2, Table 2). To a stirred solution **13** (0.7 mmol), *N*-Fmoc-L-Ala-OH (0.7 mmol) and HOBt (2.1 mmol) in 3 mL of dry THF was added at 0 °C successively HATU (0.7 mmol) and DIEA (2.1 mmol). After 15 min at 0 °C, the mixture was allowed to warm to room temperature and stirred overnight. The solvent was evaporated and the residue was chromatographied on silica gel (19%).

4.11.4. HATU/NMM (entry 3, Table 2). Compound **13** (5 mmol) was dissolved in 2.5 mL of CH_2Cl_2 and cooled in an ice bath. To the mixture was successively added, NMM (15 mmol), a solution of *N*-Fmoc-L-Ala-OH (5 mmol) in 1.3 mL of CH_2Cl_2 and HATU (5 mmol). The mixture was allowed to warm to room temperature and stirred overnight. Saturated aqueous $NaHCO_3$ solution was added, and the mixture was stirred for another 10 min. The mixture was diluted with H_2O and extracted with Et_2O (3×). The combined organic layer were dried over $MgSO_4$ and concentrated under reduced pressure. Then the residue was purified by column chromatography (30%).

4.11.5. $SOCl_2$ (entry 5, Table 2). A solution of *N*-Fmoc-L-Ala-OH (3 mmol) in 17 mL of dry CH_2Cl_2 was treated with $SOCl_2$ (30 mmol) and the mixture refluxed for 2 h under nitrogen. Solvent and excess of $SOCl_2$ were removed in vacuo and the residue was dissolved in 3.4 mL of CH_2Cl_2. Addition of 34 mL of hexane allowed pure *N*-Fmoc-L-Ala-Cl to precipitate. To a stirred solution of **13** (1.77 mmol) in a solution of $NaHCO_3$ (3.76 mmol) in 6.3 mL of dry CH_2Cl_2 was added at −10 °C dropwise *N*-Fmoc-L-Ala-Cl in 2.5 mL of CH_2Cl_2. The mixture was allowed to warm to 0 °C for 1 h, then 10 h more at room temperature. NaCl was filtered and the solvent was evaporated. The residue was chromatographied on silica gel (90%).

4.11.6. Mixte anhydride (entry 6, Table 2). To a solution of *N*-Fmoc-L-Ala-OH (5 mmol), NMM (5 mmol) in 5 mL of THF was added at −10 °C successively, isobutyl chloroformate (5 mmol) and a solution of **13** (5 mmol) in 5 mL of THF. After half an hour at −10 °C and 2 h at room temperature, the mixture was filtered and the solvent was evaporated. Then the residue was purified by column chromatography (86%).

4.11.7. Acid fluoride (entries 7–10, Table 2). To a stirred solution of the Fmoc aminoacid (2 mmol) in dry CH_2Cl_2 (10 mL) and pyridine (2 mmol, 2 mL) kept under nitrogen atmosphere was added cyanuric fluoride (4 mmol, 0.33 mL) at −20 °C. After stirring at room temperature for 3 h, crushed ice was added along with 10 mL of additional CH_2Cl_2. The organic layer was separated and the aqueous layer extracted once with 5 mL of CH_2Cl_2. The combined organic layers were extracted with 10 mL of ice-cold water and dried ($MgSO_4$), and the solvent was removed with a rotary evaporator at room temperature to give the pure acid fluoride. A cold solution (−10 °C) of *N*-Fmoc-Xaa-F in 2.5 mL of CH_2Cl_2 is added dropwise to a stirred solution of **13** (1.77 mmol) and $NaHCO_3$ (3.76 mmol) in 6.3 mL of dry CH_2Cl_2. The mixture was allowed to warm to 20 °C (room temperature) and stirred for 10 h. NaF was filtered and the solvent was evaporated. The residue was chromatographied on silica gel (98%).

4.11.8. *N*-Fmoc-(*S*)-Ala-(*R*)-h(Z)Ala-OMe (14, R′=R=Me). Wax solid; IR (NaCl) ν_{max}/cm^{-1}: 3309, 1718; ^1H NMR (300 MHz, $CDCl_3$) δ 8.34–7.14 (m, 15H, arom, βNH), 5.59–4.72 (m, 4H, CH_2 Z, αCH hAla, NH Ala), 4.54–4.05 (m, 4H, CH Fmoc, CH_2 Fmoc, αCH Ala), 3.89–3.48 (m, 3H, O–CH_3), 1.70–1.35 (m, 6H, CH_3 Ala, CH_3 hAla); ^{13}C NMR ($CDCl_3$) δ 173.4 (*C*OOCH$_3$), 156.6 (*C*O Fmoc), 155.9 (*C*O Z), 144.5, 144.4, 142.0, 136.3 (C arom), 129.1, 128.9, 128.4, 127.9, 125.7, 120.7 (CH arom), 69.1 (CH_2 Z), 68.0 (CH_2 Fmoc), 56.6 (αCH hAla), 53.1 (O–CH_3), 49.8 (αCH hAla), 47.8 (CH Fmoc), 18.6, 15.1 (CH_3 Ala, CH_3 hAla); HRMS calcd for $C_{30}H_{31}N_3O_7$ [M+Na$^+$] *m/z* 568.20542, found 568.2052.

4.11.9. *N*-Fmoc-(*S*)-Val-(*R*)-h(Z)Ala-OMe (14, R′=*i*-Pr, R=Me). Wax solid; IR (NaCl) ν_{max}/cm^{-1}: ^1H NMR (300 MHz, $CDCl_3$) δ 8.47–7.97 (m, 1H, βNH), 7.94–6.99 (m, 13H, arom), 5.77–5.28 (m, 1H, αNH), 5.18–4.91 (m, 2H, CH_2 Z), 4.85–4.28 (m, 2H, CH Fmoc, αCH hAla), 4.28–3.99 (m, 3H, CH_2 Fmoc, αCH Val), 3.77–3.46 (m, 3H, O–CH_3), 2.46–1.94 (m, 1H, βCH Val), 1.60–1.32 (m, 3H, CH_3 hAla), 1.11–0.63 (γCH_3 Val); ^{13}C NMR ($CDCl_3$) δ 175.3, 175.4 (CONH), 171.9 (*C*OOCH_3), 157.1 (*C*OOCH_2Ph), 155.9 (*C*OOFmoc), 144.5, 144.3 (C arom Fmoc), 136.2 (C arom Z), 129.1, 128.9, 128.6, 128.4, 127.8, 125.8, 120.6 (CH arom), 68.7, 67.9, 67.7 (CH_2 Z), 61.1 (CH_2 Fmoc), 59.8 (αCH Val), 56.7 (αCH hAla), 53.1 (O–CH_3), 47.9, 47.8 (CH Fmoc), 31.9, 31.2 (βCH Val), 21.7, 19.9, 19.7, 18.3 (γCH_3 Val), 15.3, 14.9 (βCH_3 hAla); HRMS calcd for $C_{32}H_{35}N_3O_7$ [M+Na$^+$] *m*/*z* 596.23672, found 596.2367.

4.11.10. *N*-Fmoc-(*S*)-Val-(*R*)-h(Z)Ile-OMe (14, R′=*i*-Pr, R=$CH(CH_3)CH_2CH_3$). Wax solid; IR (NaCl) ν_{max}/cm^{-1}: 3310, 1772, 1717; ^1H NMR (300 MHz, $CDCl_3$) δ 8.50–8.14 (m, 1H, βNH), 7.83–7.12 (m, 14H, arom), 5.81–5.27 (m, 1H, NH), 5.24–4.97 (m, 2H, CH_2 Z), 4.92–4.56 (m, 1H, αCH hIle), 4.50–3.83 (m, 4H, CH Fmoc, CH_2 Fmoc, αCH Val), 3.81–3.49 (m, 3H, O–CH_3), 2.42–1.76 (m, 2H, βCH hIle, βCH Val), 1.64–0.37 (m, 19H, βCH_3 hIle, 2γCH_3 Val, γCH_2 hIle, γCH_3 hIle); ^{13}C NMR ($CDCl_3$) δ 175.7 (CONH), 172.5 (*C*OOCH_3), 157.2 (*C*OOCH_2Ph), 156.9 (*C*OOFmoc), 144.6, 144.4, 144.3, 141.9 (C arom Fmoc), 136.1 (C arom Z), 129.1, 129.0, 128.8, 128.5, 128.4, 127.7, 125.7, 120.6 (CH arom), 69.3 (CH_2 Z), 68.0 (CH_2 Fmoc), 59.7 (αCH Val), 59.4 (αCH hIle), 52.8 (O–CH_3), 47.7 (CH Fmoc), 35.4 (βCH hIle), 30.9 (βCH Val), 26.3 (γCH_2 hIle), 19.8 (γCH_3 Val), 18.1 (γCH_3 Val), 16.6 (βCH_3 hIle), 11.9 (γCH_3 hIle); HRMS calcd for $C_{34}H_{39}N_3O_7$ [M+Na$^+$] *m*/*z* 616.3017, found 616.3033.

4.11.11. *N*-Fmoc-(*R*)-Ala-(*R*)-h(Z)Ala-OMe (15). Wax solid; IR (NaCl) ν_{max}/cm^{-1}: 3292, 1717; ^1H NMR (300 MHz, $CDCl_3$) δ 8.21 (s, 1H, βNH), 7.82–7.21 (m, 14H, arom), 5.56 (s, 1H, NH Ala), 5.26–4.78 (m, 3H, CH_2 Z, αCH hAla), 4.54–4.05 (m, 4H, CH Fmoc, CH_2 Fmoc, αCH Ala), 3.79–3.48 (m, 3H, O–CH_3), 1.60–1.10 (m, 6H, CH_3 Ala, CH_3 hAla); ^{13}C NMR ($CDCl_3$) δ 173.5 (*C*OOCH_3), 156.6 (*C*O Fmoc), 155.9 (*C*O Z), 144.4, 144.3, 141.9, 136.0 (C arom), 129.1, 128.9, 128.7, 128.4, 127.7, 125.7, 120.6 (CH arom), 69.2 (CH_2 Z), 67.8 (CH_2 Fmoc), 56.5 (αCH hAla), 53.1 (O–CH_3), 49.5 (αCH hAla), 47.7 (CH Fmoc), 18.9, 14.9 (CH_3 Ala, CH_3 hAla); HRMS calcd for $C_{30}H_{31}N_3O_7$ [M+Na$^+$] *m*/*z* 568.20542, found 568.2053.

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