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Synthesis and stereochemical analysis of β-nitromethane substituted γ-amino acids and peptides†

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The high diastereoselectivity in the Michael addition of nitromethane to α,β-unsaturated γ-amino esters, crystal conformations of β-nitromethane substituted γ-amino acids and peptides are studied. Results suggest that the *N*-Boc protected amide NH, conformations of α,β-unsaturated γ-amino esters and alkyl side chains play a crucial role in dictating the high diastereoselectivity of nitromethane addition to *E*-vinylogous amino esters. Investigation of the crystal conformations of both α,β-unsaturated γ-amino esters and the Michael addition products suggests that an H–C^{γ}–C^{β}=C^{α} eclipsed conformer of the unsaturated amino ester leads to the major (*anti*) product compared to that of an N–C^{γ}–C^{β}=C^{α} eclipsed conformer. The major diastereomers were separated and subjected to the peptide synthesis. The single crystal analysis of the dipeptide containing β-nitromethane substituted γ-amino acids reveals a helical type of folded conformation with an isolated H-bond involving a nine-atom pseudocycle.

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Introduction

Due to their structural diversity and functional versatility, backbone homologated α-amino acids and their oligomers, such as β-and γ-peptides, emerged as a very important class of molecules in medicinal chemistry.[1] The β- and γ-peptides have been shown to adopt stable secondary structures, such as helices, β-sheets and reverse turns, similar to the natural protein secondary structures. Additionally, the backbone functionalized γ^4-amino acids, such as β-keto γ^4-amino acids, β-hydroxy-γ^4-amino acids and α,β-unsaturated γ^4-amino acids, are highly versatile non-natural amino acids present in many biologically active peptide natural products.[2] Synthetic and naturally occurring peptides containing these backbone functionalized γ-amino acids have been used as inhibitors of cysteine, serine and aspartic acid proteases.[3] In addition, simple unprotected β-alkyl or dialkyl substituted γ-amino acids (pregabalin and gabapentin) have also been marketed as anticonvulsant drugs for the neuropathic pain as well as for the treatment of epilepsy.[4]

Nitroalkane chemistry is unique with respect to the diverse functional group transformations. Nitroalkanes have been used as effective precursors for the synthesis of amines, carboxylic acids, aldehydes, ketones, substituted alkanes, alkenes, complex heterocyclic structures and more.[5]

With these divergent functional group transformations of nitroalkanes in mind, we asked the question whether these functional properties of nitroalkanes can be transformed to the γ-amino acids and to the peptides, since they are very attractive entities from the perspective of medicinal chemistry. Hence, we anticipate that α,β-unsaturated γ-amino esters may serve as ideal precursors for the synthesis of β-nitromethane functionalized γ-amino acids using Michael addition. A variety of nucleophiles and catalysts have been demonstrated for the C–C and C–X (X = N, S, O, *etc.*) bond formation using the Michael addition reaction.[6] In addition, nitromethane Michael addition to the *N*-carbamate protected vinylogous amino esters has also been reported, however, these reports have offered no insight into the diastereoselectivity and the mechanism of the conjugate addition.[7] Recently, we established the synthesis of *E*-vinylogous amino acids and their utilization in the construction of hybrid peptides.[8] We sought to utilize these amino acids as substrates in the Michael addition reaction with nitromethane to understand the diastereoselectivity as well as to obtain stereochemically pure β-nitromethane substituted γ-amino acids to synthesize hybrid peptides. Herein, we are reporting the highly diastereoselective Michael addition of nitromethane to various *N*-Boc-(*S*,*E*)-α,β-unsaturated γ-amino esters and the crystal conformations of β-nitromethane substituted γ-amino acids and peptides. Results suggest that the high diastereoselectivity is mainly depending on the conformations of the

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†Electronic supplementary information (ESI) available: Crystallographic information, copies of ^{1}H and ^{13}C NMR spectra as well as HPLC chromatograms are available. CCDC 802255, 825102, 907818–907820. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob27070f

E-vinylogous amino esters, amino acid side chains and the amide NH (Boc-NH). In addition, the X-ray analysis demonstrates that the β-nitromethane substituted γ-dipeptide adopted a novel C_9-helical type of conformation in single crystals.

Results and discussion

Synthesis of (*E*)-vinylogous amino esters from α-amino acids

The *N*-protected (*S*,*E*)-vinylogous amino esters (**1**) were synthesized starting from the *N*-protected amino aldehydes using the Wittig reaction[8a] as shown in Scheme 1. The *N*-Boc amino aldehydes were synthesized from the oxidation of corresponding amino alcohols.[9] A variety of *E*-vinylogous amino esters were synthesized using this protocol (Scheme 1, **1a–l**). Instructively, many of these *N*-protected *E*-vinylogous amino esters were prone to crystallize immediately after the column purification. The crystal conformations of the compounds **1b** and **1c** are shown in Fig. 1.[8a]

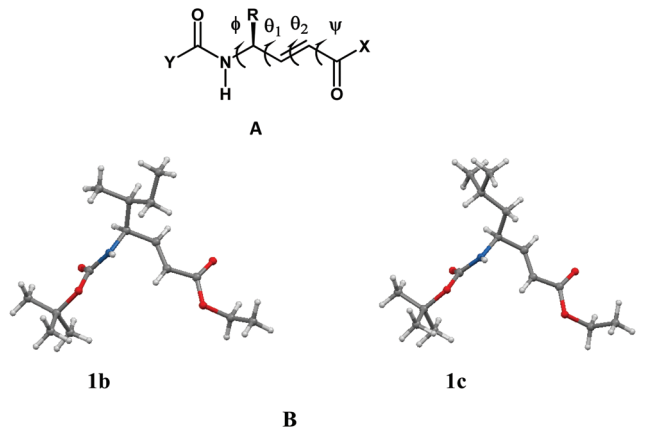
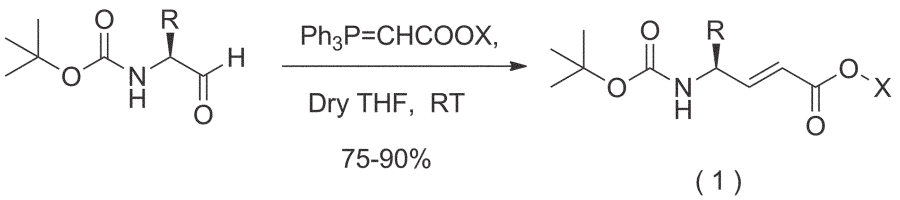


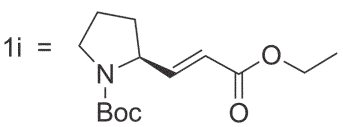
Fig. 1 A. The local torsional variables of vinylogous residues. B. X-ray structures of *N*-Boc-(*S*,*E*)-vinylogous amino esters **1b** and **1c** showing the N–C^{γ}–C^{β}=C^{α} eclipsed conformations.[8a]

Conjugate addition of nitromethane to (*E*)-vinylogous amino esters

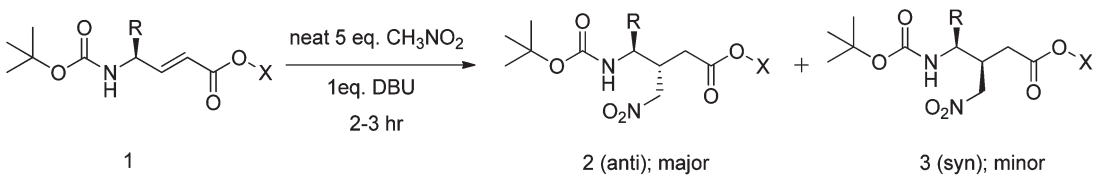
We anticipate that Michael addition of nitromethane to all *E*-vinylogous esters (**1a–l**) can be performed in the presence of a base. The schematic representation of the conjugate addition in the presence of a base is shown in Scheme 2. Conditions for the Michael addition were optimized using the base DBU.[7a] To begin with, ethyl ester of *N*-Boc-(*S*,*E*)-α,β-unsaturated γ-valine (Boc-(*S*,*E*)-dgV-OEt, **1a**) was subjected to the Michael addition



1a: R = -CH(CH$_3$)$_2$, X = -CH$_2$-CH$_3$; 1b: R = -CH(CH$_3$)-CH$_2$-CH$_3$, X = -CH$_2$-CH$_3$;
1c: R = -CH$_2$-CH(CH$_3$)$_2$, X = -CH$_2$-CH$_3$; 1d: R = -CH$_2$-NHBoc, X = -CH$_2$-CH$_3$;
1e: R = -CH$_2$-C$_6$H$_5$, X = -CH$_2$-CH$_3$; 1f: R = -CH$_2$-O^{t}Bu, X = -CH$_2$-CH$_3$;
1g: R = -CH$_2$-COO^{t}Bu, X = -CH$_2$-CH$_3$; 1h: R = -CH$_3$, X = -CH$_2$-CH$_3$;
1i: R = -Proline, X = -CH$_2$-CH$_3$; 1j: R = -CH(CH$_3$)$_2$, X = -CH$_2$-C$_6$H$_5$;
1k: R = -CH$_2$-CH(CH$_3$)$_2$, X =-CH$_2$-C$_6$H$_5$; 1l: R = -CH$_2$-C$_6$H$_5$, X = -CH$_2$-C$_6$H$_5$;



Scheme 1 Synthesis of α,β-unsaturated γ-amino esters using the Wittig reaction.



Scheme 2 Synthesis of β-nitromethane substituted γ-amino esters.

Table 1 List of β-nitromethane substituted γ-amino acids and their diastereomeric ratios

Entry **1**	R	X	Product (% yield) **2** + **3**	dr (*anti* : *syn*) **2** : **3**
A	$-CH(CH_3)_2$	$-C_2H_5$	90	89 : 11[a,c]
B	$-CH(CH_3)-CH_2-CH_3$	$-C_2H_5$	89	83 : 17[a,c]
C	$-CH_2-CH(CH_3)_2$	$-C_2H_5$	86	82 : 18[c]
D	$-CH_2-NHBoc$	$-C_2H_5$	87	90 : 10[a]
E	$-CH_2-C_6H_5$	$-C_2H_5$	88	80 : 20[c]
F	$-CH_2-O^tBu$	$-C_2H_5$	84	100 : 0[a,c]
G	$-CH_2-COO^tBu$	$-C_2H_5$	82	81 : 19[a]
H	$-CH_3$	$-C_2H_5$	92	80 : 20[b]
I	Proline side chain	$-C_2H_5$	91	61 : 39[a]
J	$-CH(CH_3)_2$	$-CH_2C_6H_5$	80	86 : 14[a]
K	$-CH_2-CH(CH_3)_2$	$-CH_2C_6H_5$	79	81 : 19[b]
L	$-CH_2-C_6H_5$	$-CH_2C_6H_5$	72	89 : 11[a]

[a] Determined by reverse phase HPLC. [b] Determined by NMR analysis. [c] Determined by isolated products.

reaction using nitromethane in the presence of DBU. The Michael addition product was isolated with 90% yield in 2.5 h at room temperature. Interestingly, the HPLC analysis of the crude product reveals the 89.5 : 9.5 diastereomeric ratio of *anti* (**2**) : *syn* (**3**) products, respectively. Both major (**2**) and minor (**3**) products were separated using column chromatography and analyzed. Encouraged by the high diastereoselectivity, we further subjected other vinylogous amino acids (**1b–l**) to the Michael addition reaction and the diastereomeric products were isolated in moderate to good yields (72–92%). The analyses of all nitromethane conjugate addition products reveal that *anti* (**2b–l**) is a major product in all the cases. The diastereomeric major (**2a**, **2b**, **2c** and **2e**) and minor (**3a**, **3b**, **3c** and **3e**) products of **1a**, **1b**, **1c** and **1e** were separated using column chromatography and the diastereomeric ratio was measured using the isolated yields. Surprisingly, compound **1f** gave a single *anti* isomer **2f**. As the reactions were performed in small scale, only major *anti* Michael addition products (**2g**, **2j**, **2k** and **2l**) were isolated using column chromatography for the compounds **1g**, **1j**, **1k** and **1l**. In addition, we found difficult to separate the diastereomers for the compounds **1d**, **1h** and **1i** from the column chromatography. However, the diastereomeric ratio (**2** : **3**) was measured for all the crude products either using HPLC or by the ^{1}H NMR (Table 1). Instructively, high diastereoselectivity was observed for all *E*-vinylogous esters except the proline side-chain containing **1i**. In addition, both benzyl and ethyl esters gave similar yields and diastereoselectivity of the nitromethane Michael addition. Out of all Michael addition products (**2a–l** and **3a–l**) in Table 1, we were able to obtain the single crystals for **2c** and its X-ray structure is shown in Fig. 2.

In contrast to the nucleophilic addition at the carbonyl compounds containing α-chiral center, the stereochemical outcome of 1,4 nucleophilic addition to α,β-unsaturated esters containing γ-stereogenic center is sometimes bewildering to rationalize. However, the intriguing results of conjugate addition of nitromethane to *N*-Boc-protected vinylogous amino esters **1a–e**, **1h** and **1j–k**, a single *anti* isomer of **1f** and the

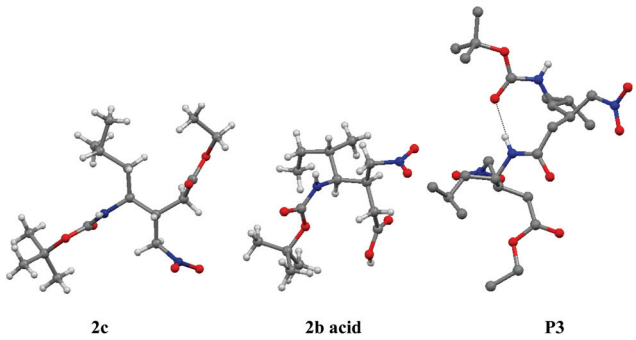
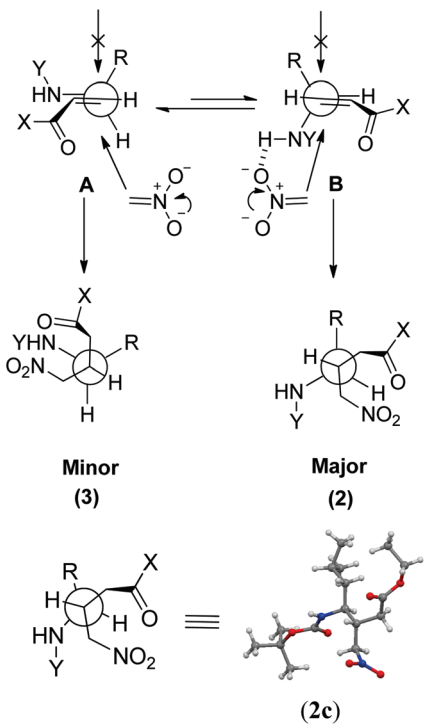


Fig. 2 X-ray crystal structures of Boc-NH-γL(β-CH_2NO_2)-COOEt (**2c**), Boc-γI-(β-CH_2NO_2)-COOH (**2b acid**) and dipeptide Boc-NH-γV(β-CH_2NO_2)-γL-(β-CH_2NO_2)-COOEt (**P3**). The backbone and side-chain H-atoms of dipeptide are not shown for clarity.

almost equal diastereomeric ratio from **1i**, enable us to rationalize a plausible mechanism of the Michael addition starting from the crystal structures of *E*-vinylogous amino esters. The local conformations of vinylogous residues were determined by introducing additional torsional variables θ_1 ($N-C^{\gamma}-C^{\beta}=C^{\alpha}$) and θ_2 ($C^{\gamma}-C^{\beta}=C^{\alpha}-C'$) along with the ϕ and ψ as shown in Fig. 1. The crystal conformations of vinylogous amino esters from the literature[8,10] and from the present work suggest that they mainly adopt either $N-C^{\gamma}-C^{\beta}=C^{\alpha}$ (θ_1 = ~0°) or $H-C^{\gamma}-C^{\beta}=C^{\alpha}$ (θ_1 = ~120°) eclipsed conformations (Fig. 1). This is not surprising because in their seminal work Houk and colleagues[11] and Wiberg *et al.*[12] proposed that the eclipsed conformations of olefins and the carbonyls are stabilized by



Scheme 3 Schematic representation of the highly diastereoselective Michael addition of nitromethane to the *E*-vinylogous amino esters. The crystal structure of **2c** representing the stereochemical outcome from the conformer **B**.

$\sigma \rightarrow \pi^*$ donations. As the C–C bond is a better σ donor than the C–N bond, from the analysis of twenty one units of vinylogous residues in both monomers as well as in peptide crystals (see ESI†), we observed that the amino acid side-chains are nearly perpendicular to the double bonds. In addition, the analysis also reveals the preference of an N–C^{γ}–C^{β}=C^{α} eclipsed conformation in monomer esters and an H–C^{γ}–C^{β}=C^{α}eclipsed conformation in amides/peptides. Based on these analyses, we speculate that both eclipsed conformers **A** and **B** are in equilibrium in solution (Scheme 3).

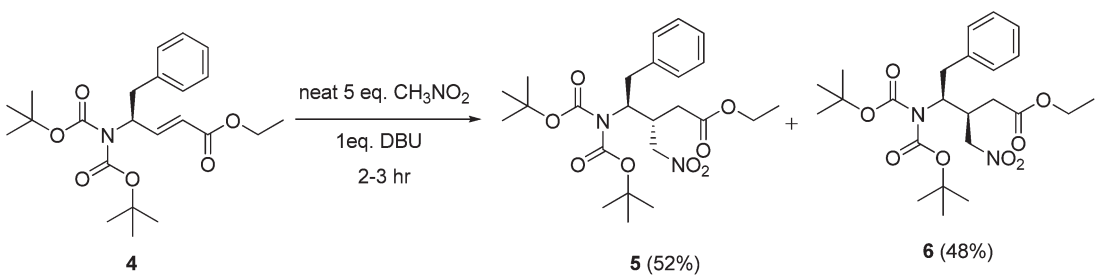
The preference for **A** over **B** is may be due to the reduced **A**[1,3] strain. In the conformer **B**, both C^{γ}–H and C^{α}–H are in the same plane separated by a distance of 2.3 Å, whereas in the conformer **A**, N–H is twisted upwards in all N–C–C=C eclipsed conformations (in single crystals) probably to reduce the steric strain (N–H···H–C^{α} dist., 2.75 Å and N–H···H–C^{α} angle 67°) with C^{α}–H. The crystal structure analysis also suggests that the approach of the nucleophile from top is restricted due to the steric clash with an R group (Scheme 3) as the R-group projected perpendicular to the double bonds. Based on these analyses, we propose that a nucleophile must approach the conjugated ester from the opposite face of the R group to avoid the steric clash. In addition, the excellent diastereoselectivity observed in all *E*-vinylogous amino esters containing amide NH except proline (**1i**), suggesting the significant role of the amide NHs in dictating the high diastereoselectivity. In order to understand the role of NH in the conjugate addition, we performed the conjugate addition reaction on ethyl ester of *N*,*N*-di-Boc-protected (*S*,*E*)-α,β-unsaturated γ-phenylalanine (**4**, Scheme 4). Instructively, the conjugate addition of nitromethane yields the diastereomeric ratio (**5** + **6**) 52 : 48 similar to the compound **1i** suggesting the pivotal role of amide NH. We speculate that amide NH may be involved in the H-bond formation with the incoming nucleophile and stabilizing the nucleophilic approach. Based on these overall results we propose the possible reaction mechanism for the conjugate addition of the nitromethane as shown in Scheme 3. The conformer **B** leads to the major (*anti*) product due to the H-bond mediated stabilization of the nucleophile in the conjugate addition, whereas the conformer **A** disfavored the formation of major (*syn*) as there is no favorable H-bond stabilization for the nucleophilic approach. Further, the analysis of the crystal structure **2c** reveals that it adopted a similar structure as anticipated from the conformer **B**.

β-Nitromethane substituted γ-peptides and crystal conformations

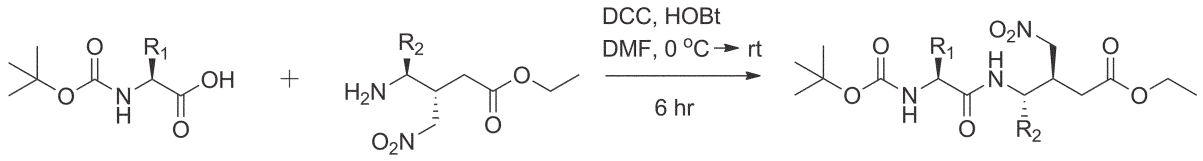
In order to synthesize peptides containing β-nitromethane substituted γ-amino acids, we subjected ethyl esters of *N*-protected nitroamino acids for the saponification and *N*-Boc deprotection using 1 N NaOH and 50%TFA in DCM, respectively. The free amines and carboxylic acids were directly used for the peptide synthesis without further characterization. However, we were able to obtain the single crystals for **2b** after the ester hydrolysis and its structure is shown in Fig. 2. The peptide couplings were carried out using standard DCC/HOBt coupling conditions (Scheme 5). The list of β-nitromethane substituted γ-hybrid peptides (**P1–7**) synthesized using this procedure is shown in Scheme 5. All peptides were isolated in moderate yields (55–78%) after column chromatography. Out of all the peptides in Scheme 5, **P3** gave single crystals after the slow evaporation from the solution of EtOAc and its X-ray structure is shown in Fig. 2. Similar to the vinylogous amino acids (Fig. 1), the backbone torsional angles of β-nitromethane substituted γ-amino acids were analyzed by introducing additional torsional variables θ_1 and θ_2.[8] The torsional variables of β-nitromethane substituted γ-amino residues are tabulated in Table 2. Inspection of the crystal structure reveals that the independent amino acids in the dipeptide adopted g^+, g^+ backbone conformations similar to the amino acid crystal structure **2b**. In contrast to the *anti* conformation of **2c** (along the C3–C4 bond), the amino acid **2b** and amino acid residues in the dipeptide **P3** follow the general trend of tetraalkyl substituted ethane by adopting the *gauche* conformations.[13] Instructively, the analysis of the crystal structure of **P3** reveals that it adopts a C_9-helical type of structure in single crystals.[14] The right handed helical twist is stabilized by a 9-atom involved intramolecular H-bond between the urethane carbonyl and NH of the C-terminal residue with C=O···H–N distance 1.995 Å [O···N dist. 2.845 Å] and O···H–N bond angle 170°.

Conclusions

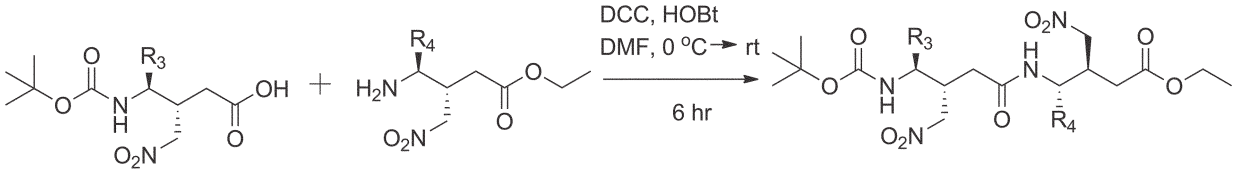
Overall, the β-nitromethane substituted γ-amino acids were synthesized with high diastereoselectivity in the absence of any metal or chiral catalysts. The high diastereoselectivity of the conjugate addition of nitromethane to the α,β-unsaturated



Scheme 4 Conjugate addition of nitromethane to *N*,*N*-di-Boc-protected *E*-vinylogous amino ester.



P1 : R_1 = -CH_3 ; R_2 = -$CH_2C_6H_5$; 78%
P2 : R_1 = -$CH_2C_6H_5$; R_2= -$CH(CH_3)_2$; 70%



P3 : R_3 = -$CH(CH_3)_2$;R_4 = -CH_2-$CH(CH_3)_2$; 60%
P4 : R_3= -CH_2-$CH(CH_3)_2$;R_4 = -$CH(CH_3)$-CH_2-CH_3 ; 64%
P5 : R_3= -CH_2O^tBu ;R_4 = -$CH(CH_3)_2$; 55%
P6 : R_3= -$CH(CH_3)_2$;R_4 = -$CH(CH_3)_2$; 58%
P7 : R_3= -$CH(CH_3)_2$;R_4 = -$CH_2C_6H_5$; 65%

Scheme 5 Synthesis of peptides containing β-nitromethane substituted γ-amino acids.

Table 2 Torsional values of nitro amino acids and nitropeptide

Compound	Residue	ϕ	θ_1	θ_2	ψ	Back bone conformation
2c	γL(β-CH_2NO_2)	−110	−179	89	−146	t, g^+
2b acid	γI(β-CH_2NO_2)	−110	67	72	−145/36	g^+, g^+
P3	γV(β-CH_2NO_2)	−100	68	72	−87	g^+, g^+
	γL(β-CH_2NO_2)	−99	69	66	36	g^+, g^+

γ-amino esters depends on the two relatively stable stereo conformers of the unsaturated esters as well as the steric effects of the amino acid side-chains. In addition amide NH plays a crucial role in dictating the high diastereoselectivity in an $H–C^{\gamma}–C^{\beta}=C^{\alpha}$ eclipsed conformer by stabilizing the nucleophilic approach through intermolecular H-bonding. The C_9 helical signature of the dipeptide and the ability of nitroalkane's diverse functional group transformations offer the glimpse of the potential to generate a new class of peptide foldamers with diverse functional group transformations from β-nitromethane substituted γ-amino acids.

Experimental section

General experimental details

Solvent THF was distilled from sodium/benzophenone. Column chromatography was performed on silica gel (120–200 mesh). *N*-Protected amino aldehydes and Wittig ylides were synthesized using the reported procedures.[8a] IR spectra were recorded on an FT-IR spectrophotometer by using KBr pellets and NaCl plates. Wavelengths of maximum absorbance (ν_{max}) are quoted in wavenumbers (cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded on 400 MHz/500 MHz and on 100 MHz/125 MHz respectively, using residual solvents as internal standards ($CDCl_3$ and DMSO). Chemical shifts (δ) reported in parts per million (ppm) and coupling constants (J) reported in Hz. Mass spectra were recorded by MALDI TOF/TOF and Electron Spray Ionization (ESI). Diastereomeric ratios were determined by Reverse Phase HPLC using an analytical C_{18} column (5 μm, 4.6 × 250 mm), MeOH–H_2O = 75 : 25 (except for 2i + 3i mixture, MeOH–H_2O = 50 : 50) as a mobile phase with flow rate 0.75 mL min^{-1}.

General procedure for synthesis of vinylogous amino esters (1)

The *N*-protected amino aldehyde (10 mmol) was dissolved in dry THF (40 mL) under the N_2 atmosphere. To this solution Wittig ylide (11.5 mmol) was added. The progress of the reaction was monitored by TLC. After the completion of the reaction (8 h), THF was evaporated and the product was purified by column chromatography using a 5 : 95 ethyl acetate–pet ether solvent system.

(*S*,*E*)-Ethyl-4-(*tert*-butoxycarbonylamino)-5-methylhex-2-enoate (1a).[8a] White solid (yield 2.43 g, 90%); mp 59 °C; UV (λ_{max})

216 nm; $[\alpha]_D^{25}$ −3.40 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85–6.81 (dd, 1H, *J* = 15.8 Hz, *J* = 5.2 Hz, –C*H*=CH–COOEt), 5.92–5.88 (d, 1H, *J* = 15.6 Hz, –CH=C*H*–COOEt), 4.55 (d, 1H, *J* = 6.8 Hz, NH), 4.18–4.16 (q, *J* = 6.88 Hz, 2H, –OCH_2), 1.86–1.84 (m, 1H, –C*H*–CH=CH), 1.42 (s, 9H, Boc), 1.29–1.25 (t, *J* = 7.32 Hz, 3H, –OCH_2–CH_3), 0.93–0.88 (q, 6H, *J* = 6.4 Hz, $C(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$); 166.3, 155.4, 147.4, 121.5, 79.7, 60.5, 56.7, 32.3, 28.4, 18.9, 17.0, 14.3; MALDI TOF/TOF *m*/*z* calcd for $C_{14}H_{25}NO_4$ [M + Na^+] 294.1681, observed 294.1686.

(4*S*,5*R*,*E*)-Ethyl-4-(*tert*-butoxycarbonylamino)-5-methylhept-2-enoate (1b).[8a] White solid (yield 2.62 g, 92%); mp 62 °C; UV (λ_{max}) 216 nm; $[\alpha]_D^{25}$ −11.20 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87 (d, 1H, *J* = 15.6 Hz, –C*H*=CH–COOEt), 5.92 (d, 1H, *J* = 15.6 Hz, –CH=C*H*–COOEt), 4.58 (dd, 1H, *J* = 17.9 Hz, *J* = 9.2 Hz, –C*H*–CH=CH–), 4.32 (br, 1H, NH), 4.2 (q, *J* = 7.3 Hz, 2H, –OCH_2), 1.66 (m, 2H, –CH–CH_2–CH_3), 1.45 (s, 9H, Boc), 1.29 (t, *J* = 7.1 Hz, 3H, –OCH_2–CH_3), 1.14 (m, 1H, –C*H*–CH_2–CH_3), 0.91–0.86 (m, 6H, 2 × –CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 155.3, 147.1, 121.6, 79.6, 60.4, 55.7, 39.0, 28.4, 25.3, 15.3, 14.2, 11.6; MALDI TOF/TOF *m*/*z* value for $C_{15}H_{27}NO_4$ [M + Na^+] 308.1838 (calcd), 308.1837 (found).

(*S*,*E*)-Ethyl-4-(*tert*-butoxycarbonylamino)-6-methylhept-2-enoate (1c).[8a] White solid (2.70 g, 95%); mp 55 °C; UV (λ_{max}) 218 nm; $[\alpha]_D^{25}$ −25.50 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.85–6.81 (dd, *J* = 16 Hz, *J* = 5.5 Hz, 1H, C*H*=CHCO_2Et), 5.93–5.90 (d, 1H, *J* = 16 Hz, CH=C*H*CO_2Et), 4.45 (br, 1H, NH), 4.33 (br, 1H, –C*H*–CH=CH), 4.21–4.17 (q, 2H, *J* = 7 Hz, –OCH_2), 1.72–1.67 (m, 1H, C*H*–$(CH_3)_2$), 1.44 (s, 9H, Boc), 1.40–1.37 (t, 2H, *J* = 7 Hz, CH_2CH–$(CH_3)_2$), 1.30–1.27 (t, *J* = 7 Hz, 3H, –OCH_2CH_3), 0.94–0.93 (d, *J* = 6.5 Hz, 6H, CH–$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 155.1, 148.0, 120.4, 79.7, 60.5, 49.8, 43.9, 28.4, 24.7, 22.7, 22.2, 14.3; MALDI TOF/TOF *m*/*z* value for $C_{15}H_{27}NO_4$ [M + Na^+] 308.1838 (calcd), 308.1840 (found).

(*R*,*E*)-Ethyl-4,5-bis((*tert*-butoxycarbonyl)amino)pent-2-enoate (1d). White solid (2.66 g, 70%); mp 120 °C; UV (λ_{max}) 218 nm; $[\alpha]_D^{25}$ −3.0 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.82 (dd, 1H, *J* = 15.8 Hz, *J* = 5.3 Hz, –C*H*=CH–COOEt), 5.95 (dd, 1H, *J* = 15.8 Hz, *J* = 1.8 Hz, –CH=C*H*–COOEt), 5.28 (bs, 1H, NH), 4.86 (bs, 1H, NH), 4.36 (m, 1H, –C*H*–CH=CH–), 4.16 (q, 2H, *J* = 7.3 Hz, –OC$H_2$$CH_3$), 3.28 (m, 2H, –CH–C$H_2$–NHBoc), 1.4 (bs, 18H, 2 Boc), 1.25 (t, 3H, *J* = 7.1 Hz, –OCH_2CH_3); ^{13}C NMR δ 166.1, 156.7, 155.4, 145.4, 122.3, 79.9, 60.6, 52.8, 43.6, 28.4, 28.3, 28.0, 14.2; MALDI TOF/TOF *m*/*z* value for [M + Na^+] $C_{17}H_{30}N_2O_6$ 381.1996 (calcd), 381.1979 (found).

(*S*,*E*)-Ethyl-4-((*tert*-butoxycarbonyl)amino)-5-phenylpent-2-enoate (1e). White powder (4.01 g, 84%); mp 70 °C; UV (λ_{max}) 206 nm, 255 nm; $[\alpha]_D^{25}$ −2.0 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.14 (m, 5H, aromatic), 6.91–6.86 (dd, *J* = 5.04 Hz, *J* = 11 Hz, 1H, –C*H*=CHCO_2Et), 5.85–5.81 (d, *J* = 17.4, 1H, –CH=C*H*CO_2Et), 4.59 (br, 1H, NH), 4.52 (m, 1H, –C*H*–CH=CH), 4.18–4.13 (q, *J* = 6.8 Hz, 2H, –OCH_2), 2.92–2.85 (m, 2H, CH_2–Ph), 1.37 (s, 9H, Boc), 1.27–1.23 (t, *J* = 7.3 Hz, 3H, –OCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 155.0, 147.6, 136.4, 129.4, 128.6, 126.9, 121.1, 79.9, 60.5, 52.3, 40.9, 28.3, 14.3; MALDI TOF/TOF *m*/*z* value for $C_{18}H_{25}NO_4$ [M + Na^+] 342.1681 (calcd), 342.1657 (found).

(*R*,*E*)-Ethyl-5-*tert*-butoxy-4-(*tert*-butoxycarbonylamino)pent-2-enoate (1f).[8a] Colorless oil (2.55 g, 81%); UV (λ_{max}) 211 nm; $[\alpha]_D^{25}$ +4.4 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$), δ 6.87 (dd, *J* = 15.6 Hz, *J* = 5.5 Hz, 1H, –CH–C*H*=CH–), 5.90 (dd, *J* = 15.8 Hz, *J* = 1.6 Hz, 1H, –CH–CH=C*H*–), 4.99 (br, 1H, NH), 4.33 (br, 1H, –C*H*–CH=CH–), 4.14 (q, *J* = 7.3 Hz, 2H, –OCH_2–), 3.42 (d, *J* = 4.1 Hz, 2H, CH_2–O^{t}Bu), 1.40 (s, 9H, Boc), 1.23 (t, 3H, *J* = 7.1 Hz, –OCH_2–CH_3), 1.11 (s, 9H, O^{t}Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 155.3, 146.9, 121.7, 79.7, 73.4, 63.2, 60.4, 51.7, 28.4, 27.4, 14.2; MALDI TOF/TOF *m*/*z* value for $C_{16}H_{29}NO_5$ [M + Na^+] 338.1943 (calcd), 338.1904 (found).

(*S*,*E*)-6-*tert*-Butyl-1-ethyl 4-((*tert*-butoxycarbonyl)amino)hex-2-enedioate (1g). White solid (2.9 g, 80%); mp 68 °C; UV (λ_{max}) 206 nm; $[\alpha]_D^{25}$ −10.0 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.86 (dd, 1H, *J* = 15.6 Hz, *J* = 5 Hz, C*H*=CH–COOEt), 5.92 (dd, 1H, *J* = 15.6 Hz, *J* = 1.8 Hz, CH=C*H*–COOEt), 5.3 (d, 1H, *J* = 8.2 Hz, NH), 4.63 (m, 1H, –C*H*–CH=CH–), 4.17 (q, 2H, *J* = 7.2 Hz, –O$CH_2$$CH_3$), 2.53 (m, 2H, –C$H_2$COO^{t}Bu), 1.42 (two s, 18H, Boc and ^{t}Bu), 1.26 (t, 3H, *J* = 7.1 Hz, –OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.0, 166.1, 154.9, 146.8, 121.5, 81.8, 79.9, 60.5, 48.3, 39.8, 28.4, 28.4, 14.3; MALDI TOF/TOF *m*/*z* value for $C_{17}H_{29}NO_6$ [M + Na^+] 366.1887 (calcd), 366.1813 (found).

(*S*,*E*)-Ethyl-4-(*tert*-butoxycarbonylamino)pent-2-enoate (1h).[8a] Colorless oil (yield 2.25 g, 93%); UV (λ_{max}) 216 nm; $[\alpha]_D^{25}$ −20.8 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87–6.82 (dd, 1H, *J* = 15.6 Hz, *J* = 4.1 Hz, –C*H*=CH–COOEt), 5.89–5.85 (d, 1H, *J* = 15.6 Hz, –CH=C*H*–COOEt), 4.5 (br, 1H, NH), 4.38 (m, 1H, –C*H*–CH=CH–), 4.16 (q, 2H, *J* = 7.3 Hz, –OCH_2), 1.432 (s, 9H, Boc), 1.265–1.247 (m, 6H, –CH(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 154.9, 154.9, 120.2, 79.8, 60.5, 47.0, 28.4, 20.4, 14.3; MALDI TOF/TOF *m*/*z* value for $C_{12}H_{21}NO_4$ [M + Na^+] 266.1368 (calcd), 266.1365 (found).

(*S*,*E*)-*tert*-Butyl-2-(3-ethoxy-3-oxoprop-1-enyl)pyrrolidine-1-carboxylate (1i).[8a] Colorless oil (2.23 g, 83%); UV (λ_{max}) 214 nm; $[\alpha]_D^{25}$ −72.0 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83–6.78 (d, *J* = 15.6 Hz, 1H, –CH–C*H*=CH–), 5.83–5.80 (d, *J* = 15.2 Hz, 1H, –CH–CH=C*H*–), 4.51–4.37 (br, 1H, –C*H*–CH=CH–), 4.21–4.17 (q, *J* = 6.4 Hz, 2H, –OCH_2), 3.44–3.43 (t, *J* = 6 Hz, 2H, Boc-N–CH_2), 2.14–2.05 and 1.89–1.82 (m, 4H, –CH_2–CH_2–), 1.421 (s, 9H, Boc), 1.31–1.27 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 154.3, 148.5, 120.4, 79.6, 60.3, 57.8, 46.2, 31.7, 28.3, 22.9, 14.2; MALDI TOF/TOF *m*/*z* value for $C_{14}H_{23}NO_4$ [M + Na^+] 292.1525 (calcd), 292.1520 (found).

(*S*,*E*)-Benzyl-4-((*tert*-butoxycarbonyl)amino)-5-methylhex-2-enoate (1j). White solid (2.84 g, 82%); mp 83 °C; UV (λ_{max}) 217 nm, 314 nm; $[\alpha]_D^{25}$ −19.0 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34 (m, 5H, aromatic), 6.91 (d, 1H, *J* = 15.3 Hz, –C*H*=CH–COOBz), 5.97 (d, 1H, *J* = 15.9 Hz, –CH=C*H*–COOBz), 5.17 (bs, 2H, –CH_2–C_6H_5), 4.55 (d, 1H, *J* = 7.2 Hz, NH), 4.19 (m, 1H, –C*H*–CH=CH–), 1.86 (m, 1H, –C*H*$(CH_3)_2$), 1.43 (s, 9H, Boc), 0.91 (dd, *J* = 16.2 Hz, *J* = 5.8 Hz, 6H, –CH(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.1, 155.4, 148.2,

135.9, 128.6, 128.3, 121.1, 79.8, 66.4, 56.7, 32.3, 28.4, 18.9, 18.0; MALDI TOF/TOF *m*/*z* value for [M + Na^+] $C_{19}H_{27}NO_4$ 356.1832 (calcd), 356.1856 (found).

(*S*,*E*)-Benzyl-4-((*tert*-butoxycarbonyl)amino)-6-methylhept-2-enoate (1k). White solid (2.59 g, 70%); mp 60 °C; UV (λ_{max}) 217 nm, 314 nm; $[\alpha]_D^{25}$ −33.0 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.3 (m, 5H, aromatic), 6.89 (dd, 1H, *J* = 15.6 Hz, *J* = 5 Hz, –C*H*═CH–COOBz), 5.97 (dd, 1H, *J* = 15.8 Hz, *J* = 1.6 Hz, –C*H*═CH–COOBz), 5.16 (m, 2H, $-CH_2C_6H_5$), 4.69 (d, 1H, *J* = 8.2 Hz, NH), 4.35 (m, 1H, –C*H*–CH═CH–), 1.67 (m, 1H, –C*H*$(CH_3)_2$), 1.43 (s, 9H, Boc), 1.36 (t, *J* = 7.3 Hz, 2H, –CH_2–CH$(CH_3)_2$), 0.92 (d, 6H, *J* = 6.9 Hz, –CH(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.2, 155.2, 149.8, 135.9, 128.6, 128.3, 128.3, 120.0, 79.6, 66.3, 49.8, 43.7, 28.4, 24.4, 22.8, 22.2; MALDI TOF/TOF value for [M + Na^+] $C_{20}H_{29}NO_4$ 370.1989 (calcd), 370.1960 (found).

(*S*,*E*)-Benzyl-4-((*tert*-butoxycarbonyl)amino)-5-phenylpent-2-enoate (1l). White solid (3.15 g, 78%); mp 80 °C; UV (λ_{max}) 207 nm, 314 nm; $[\alpha]_D^{25}$ −1.0 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.25 (m, 10H, aromatic), 6.96 (d, *J* = 15.6 Hz, 1H, –C*H*═CH–COOBz), 5.91 (d, *J* = 15.9 Hz, 1H, –CH═C*H*OOBz), 5.16 (s, 2H, –CH_2–C_6H_5), 4.62 (br, 1H, NH), 4.54 (br, 1H, –C*H*–CH_2–Phe), 2.88 (m, 2H, –CH–CH_2–Phe), 1.38 (s, 9H, Boc); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.0, 155.0, 148.3, 136.4, 135.9, 129.4, 128.6, 128.2, 126.9, 120.7, 79.9, 66.3, 52.3, 40.9, 28.3, 28.0; MALDI TOF/TOF *m*/*z* value for [M + Na^+] $C_{23}H_{27}NO_4$ 404.1832 (calcd), 404.1885 (found).

Synthesis of *N*-protected β-nitromethane substituted γ-amino esters through the Michael addition of nitromethane to *N*-protected α,β-unsaturated γ-amino esters (2 + 3). The *N*-Boc α,β-unsaturated γ-amino ester **1** (2.0 mmol) was dissolved in neat nitromethane (10.0 mmol). To this solution DBU (2.0 mmol) was added under a N_2 atmosphere. The reaction mixture was stirred at room temperature for about 2.5–3 h and the progress of the reaction was monitored by TLC. After completion of the reaction, EtOAc (100 mL) was added to the reaction mixture, and washed with H_2O (3 × 50 mL), 1 N HCl (2 × 50 mL) and brine (2 × 50 mL) solution. Then the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography to get the pure diastereomers (**2** and **3**). The ratio of diastereoselectivities was calculated using Reverse Phase HPLC chromatograms, ^{1}H NMR integrals or from the isolated yields.

(3*R*,4*S*)-Ethyl-4-(*tert*-butoxycarbonylamino)-5-methyl-3-(nitromethyl)hexanoate (2a)^{anti}. Light yellow oil (0.57 g, 89%, dr 89 : 11); $[\alpha]_D^{25}$ +35.0 (*c* 1.0, MeOH); the dr determined by a Reverse Phase HPLC analytical C_{18} column (5 μm, 4.6 × 250 mm), flow rate 0.75 mL min^{-1}, R_t (major, **2a**^{anti}) = 33.3 min, R_t (minor, **3a**^{syn}) = 32.3 min; IR ν (cm^{-1}) 3360, 2970, 2930, 1710, 1550, 1510, 1380, 1240, 1170, 1100, 1030; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.4 (s, 9H, Boc), 4.35 (d, *J* = 10.1, 1H, NH), 3.60–3.54 (m, 1H, γ CH), 1.87–1.81 (m, 1H, –C*H*$(CH_3)_2$), 0.98 (d, *J* = 6.9 Hz, 6H, CH_3), 0.88 (d, *J* = 6.9 Hz, 6H, CH_3), 2.92–2.84 (m, 1H, β CH), 4.55–4.44 (m, 2H, CH_2–NO_2), 2.53–2.38 (m, 2H, α CH_2), 4.16 (q, *J* = 8 Hz, 2H, –OCH_2–), 1.23 (t, *J* = 8 Hz, 3H, –OCH_2–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 156.3, 79.9, 76.3, 61.0, 56.9, 36.6, 34.4, 29.3, 28.3, 20.1, 16.8, 14.2; MALDI TOF/TOF *m*/*z* value for $C_{15}H_{27}N_2O_6$ [M + Na^+] 355.1840 (calcd), 355.1829 (found).

(3*S*,4*S*)-Ethyl-4-((*tert*-butoxycarbonyl)amino)-5-methyl-3-(nitromethyl)hexanoate (3a)^{syn}. Colorless oil; yield (0.08 g, 11%); $[\alpha]_D^{25}$ −9.0 (*c* 1.0, MeOH); IR ν cm^{-1} 3370, 2988, 2870, 1720, 1555, 1399, 1234, 1190, 1113, 1020; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.53–4.39 (m, 3H, $-CH_2NO_2$ and NH), 4.13 (q, *J* = 7.3 Hz, 2H, –OCH_2CH_3), 3.51–3.45 (m, 1H, γ CH), 2.98–2.9 (m, 1H, β CH), 2.47–2.30 (m, 2H, α CH_2), 1.77–1.69 (m, 1H, –C*H*$(CH_3)_3$), 1.4 (s, 9H, Boc), 1.25 (t, *J* = 7.1 Hz, 3H, –OCH_2CH_3), 0.98–0.91 (m, 6H, –C$(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.1, 156.1, 79.7, 76.8, 61.1, 56.9, 36.1, 32.3, 30.1, 28.3, 28.1, 19.9, 18.1, 14.1; MALDI TOF/TOF *m*/*z* value for $C_{19}H_{28}N_2O_6$ [M + Na^+] 355.1840 (calcd), 355.1829 (observed).

(3*R*,4*S*,5*S*)-Ethyl-4-(*tert*-butoxycarbonylamino)-5-methyl-3-(nitromethyl)heptanoate (2b)^{anti}. Yellow oil (0.56 g, 83%, dr 83 : 17); $[\alpha]_D^{25}$ +13.0 (*c* 1.0, MeOH); the dr determined by a Reverse Phase HPLC analytical C_{18} column (5 μm, 4.6 × 250 mm), flow rate 0.75 mL min^{-1}, R_t (major, **2b**^{anti}) = 24.9 min, R_t (minor, **3b**^{syn}) = 23.2 min; IR ν (cm^{-1}) 3370, 2970, 2930, 1700, 1550, 1520, 1380, 1250, 1170, 1030; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.54–4.41 (m, 2H, –CH_2–NO_2), 4.28 (d, *J* = 10.5 Hz, 1H, NH), 4.12 (q, *J* = 8 Hz, 2H, –OCH_2–CH_3), 3.58 (m, 1H, γ CH), 2.91 (m, 1H, β CH), 2.45 (m, 2H, α CH), 1.51 (m, 2H, –CH–CH_2–CH_3), 1.4 (s, 9H, Boc), 1.24 (m, 4H, –OCH_2–CH_3 and –C*H*–), 0.92 (m, 6H, –CH_2–CH_3 and CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 155.9, 79.7, 76.1, 61.0, 50.2, 42.0, 39.4, 34.2, 28.3, 25.0, 23.4, 21.5, 14.1; MALDI TOF/TOF *m*/*z* value for $C_{16}H_{30}N_2O_6$ [M + Na^+] 369.2002 (calcd), 369.2072 (found).

(3*S*,4*S*,5*R*)-Ethyl-4-((*tert*-butoxycarbonyl)amino)-5-methyl-3-(nitromethyl)heptanoate (3b)^{syn}. Light yellow oil; yield (0.12 g, 17%); $[\alpha]_D^{25}$ −17.0 (*c* 1.0, MeOH); IR ν cm^{-1} 3380, 2970, 2944, 1717, 1563, 1400, 1277, 1190, 1020; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 4.56–4.41 (m, 3H, NH and $-CH_2NO_2$), 4.16 (q, *J* = 7.3 Hz, 2H, –OCH_2CH_3), 3.93 (m, 1H, γ CH), 3.05–2.93 (m, 1H, β CH), 2.43–2.31 (m, 2H, $-CH_2NO_2$), 1.60–1.52 (m, 1H, –C*H*$(CH_3)CH_2CH_3$), 1.43 (s, 9H, Boc), 1.27 (t, *J* = 7.1 Hz, –OCH_2CH_3), 0.93–0.89 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 172.0, 156.0, 79.7, 76.7, 61.1, 55.8, 36.6, 35.9, 32.1, 28.2, 28.0, 24.8, 16.0, 14.1, 11.1; MALDI TOF/TOF *m*/*z* value for $C_{19}H_{28}N_2O_6$ [M + Na^+] 369.2002 (calcd), 369.2072 (observed).

(3*R*,4*S*)-Ethyl-4-(*tert*-butoxycarbonylamino)-6-methyl-3-(nitromethyl)heptanoate (2c)^{anti}. White solid (0.55 g, 82%, dr 82 : 18); mp 67 °C; $[\alpha]_D^{25}$ −148.0 (*c* 1.0, MeOH); the dr determined by a Reverse Phase HPLC analytical C_{18} column (5 μm, 4.6 × 250 mm), flow rate 0.75 mL min^{-1}, R_t (major, **2c**^{anti}) = 33.3 min, R_t (minor, **3c**^{syn}) = 32.3 min; IR ν (cm^{-1}) 3370, 2980, 2940, 1710, 1550, 1520, 1380, 1250, 1160, 1010, 904, 852, 764, 629; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.47 (m, 2H, –CH_2–NO_2), 4.27 (d, *J* = 9.2 Hz, 1H, NH), 4.14 (q, *J* = 8 Hz, 2H, –OCH_2–CH_3), 3.79 (m, 1H, γ CH), 2.75 (m, 1H, β CH), 2.45 (m, 2H, α CH_2), 1.63 (m, 2H, CH_2), 1.4 (s, 9H, Boc), 1.33 (m, 1H, CH), 1.24

(t, J = 7.3 Hz, 3H, –OCH$_2$–*CH*$_3$), 0.91 (d, J = 6.9 Hz, 3H, CH$_3$), 0.87 (d, J = 6.9 Hz, 3H, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.2, 156.3, 79.3, 76.2, 60.9, 56.6, 36.4, 36.1, 34.6, 28.3, 23.7, 16.3, 14.1, 11.4; MALDI TOF/TOF m/z value for $C_{16}H_{30}N_2O_6$ [M + Na$^+$] 369.2002 (calcd), 369.2062 (found).

(3*S*,4*S*)-Ethyl-4-((*tert*-butoxycarbonyl)amino)-6-methyl-3-(nitromethyl)heptanoate (3c)^{syn}. Colorless oil; yield (0.12 g, 18%); $[\alpha]_D^{25}$ −30.0 (c 1.0, MeOH); IR ν cm^{-1} 3380, 2990, 1730, 1560, 1523, 1387, 1277, 1182, 1030; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 4.59–4.42 (m, 3H, NH and –CH$_2$NO$_2$), 4.16 (q, J = 7.3 Hz, 2H, –OC*H*$_2$CH$_3$), 3.81–3.84 (m, 1H, γ CH), 2.86–2.79 (m, 1H, β CH), 2.51–2.33 (m, 2H, α CH$_2$), 1.69–1.62 (m, 1H, –C*H*(CH$_3$)$_3$), 1.40 (s, 9H, Boc), 1.29–1.26 (t, J = 7.1 Hz, 3H, –OCH$_2$C*H*$_3$), 0.96–0.91 (m, 6H, –CH(C*H*$_3$)$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz) 171.9, 155.7, 79.7, 76.6, 61.0, 49.7, 42.1, 38.8, 32.3, 28.2, 28.1, 24.9, 23.1, 21.7, 14.1; MALDI TOF/TOF m/z value for $C_{19}H_{28}N_2O_6$ [M + Na$^+$] 369.2002 (calcd), 369.2062 (observed).

Ethyl-4,5-bis((*tert*-butoxycarbonyl)amino)-3-(nitromethyl)-pentanoate (crude mixture, 2d + 3d, inseparable diastereomers). White solid (0.69 g, 87%, dr 90 : 10); the dr determined by a Reverse Phase HPLC analytical C$_{18}$ column (5 μm, 4.6 × 250 mm), flow rate 0.75 mL min^{-1}, R_t (major, **2d**^{anti}) = 25.96 min, R_t (minor, **3d**^{syn}) = 25.26 min; ^{1}H NMR (500 MHz, CDCl$_3$) 5.19–5.06 (br, 2NH), 4.61–4.48 (m, 2H, –CH$_2$NO$_2$), 4.17–4.13 (m, 2H, –OCH$_2$CH$_3$), 3.83–3.78 (m, 1H, γ CH), 3.29 (m, 2H, –CH$_2$NHBoc), 2.85–2.83 (m, 1H, β CH), 2.62–2.44 (br m, 2H, α CH$_2$), 1.41 (br m, 18H, 2Boc), 1.27–1.24 (br m, 3H, –OCH$_2$CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.9, 171.4, 156.9, 156.4, 80.0, 76.1, 61.1, 60.5, 53.1, 52.8, 42.3, 42.1, 36.4, 34.1, 31.9, 31.0, 29.7, 29.4, 28.3, 28.3, 22.7, 14.7. MALDI TOF/TOF m/z value for $C_{13}H_{24}N_2O_6$ [M + Na$^+$] 419.2160 (calcd), 419.2174 (found).

(3*R*,4*S*)-Ethyl-4-(*tert*-butoxycarbonylamino)-3-(nitromethyl)-5-phenyl pentanoate (2e)^{anti}. White solid (0.6 g, 80%, dr 80 : 20); mp 117 °C; $[\alpha]_D^{25}$ −114.2 (c 1.0, MeOH); the dr determined by isolated products; IR ν (cm^{-1}) 3370, 2970, 2930, 1700, 1550, 1510, 1380, 1250, 1170, 1030, 860, 766; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.23 (m, 5H, aromatic), 4.54 (m, 2H, –*CH*$_2$–NO$_2$), 4.34 (d, J = 8.24 Hz, 1H, NH), 4.15 (q, J = 7.2 Hz, 2H, –O*CH*$_2$–CH$_3$), 3.99 (m, 1H, γ CH), 2.91 (m, 2H, CH$_2$–Phe), 2.74 (m, 1H, β CH), 2.55 (m, 2H, α CH$_2$), 1.29 (s, 9H, Boc), 1.26 (t, J = 7.32 Hz, 3H, –OCH$_2$–*CH*$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.2, 155.6, 137.0, 129.1, 128.7, 126.9, 79.9, 76.0, 61.1, 53.1, 38.8, 38.3, 34.3, 28.2, 14.2; MALDI TOF/TOF m/z value for $C_{19}H_{28}N_2O_6$ [M + Na$^+$] 403.1840 (calcd), 403.1816 (found).

(3*S*,4*S*)-Ethyl-4-((*tert*-butoxycarbonyl)amino)-3-(nitromethyl)-5-phenyl pentanoate (3e)^{syn}. White solid (0.15 g, 20%); mp 120 °C; $[\alpha]_D^{25}$ −15.0 (c 1.0, MeOH); the dr determined by isolated products; IR ν (cm^{-1}) 3374, 2980, 2930, 1718, 1691, 1553, 1520, 1378, 1249, 1175, 1022, 701, 605; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.33–7.18 (m, 5H, aromatic), 4.59–4.51 (m, 3H, NH and –CH$_2$NO$_2$), 4.18 (q, J = 7.2 Hz, 2H, –O*CH*$_2$CH$_3$), 4.09 (m, 1H, γ CH), 2.94–2.88 (m, 1H, β CH), 2.58–2.53 (m, 2H, α CH$_2$), 1.33–1.27 (m, 12H, Boc and –OCH$_2$*CH*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz) 171.9, 155.6, 136.9, 129.0, 128.7, 126.9, 79.9, 74.7, 61.2, 52.7, 39.2, 37.7, 32.4, 29.7, 28.2, 14.2; MALDI TOF/TOF m/z value for $C_{19}H_{28}N_2O_6$ [M + Na$^+$] 403.1840 (calcd), 403.1816 (observed).

(3*R*,4*R*)-Ethyl-5-*tert*-butoxy-4-(*tert*-butoxycarbonylamino)-3-(nitromethyl)pentanoate (2f)^{anti}. Yellow oil (0.63 g, 84%, dr 100 : 0); $[\alpha]_D^{25}$ +131.0 (c 1.0, MeOH); the dr determined by a Reverse Phase HPLC analytical C$_{18}$ column (5 μm, 4.6 × 250 mm), flow rate 0.75 mL min^{-1}, R_t (single isomer, **2f**^{anti}) = 25.3 min; IR ν (cm^{-1}) 3380, 2970, 2930, 2800, 1720, 1550, 1500, 1370, 1240, 1170, 1090, 1020, 874, 816; ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.95 (d, J = 9.2 Hz, 1H, NH), 4.57 (m, 2H, –*CH*$_2$–NO$_2$), 4.12 (q, J = 7.3 Hz, 2H, –O*CH*$_2$–CH$_3$), 3.88 (m, 1H, γ CH), 3.45 (m, 2H, *CH*$_2$–O^{t}Bu), 2.91 (m, 1H, β CH), 2.49 (m, 2H, α CH$_2$), 1.41 (s, 9H, Boc), 1.24 (t, J = 7.2 Hz, 3H, –OCH$_2$–*CH*$_3$), 1.14 (s, 9H, ^{t}Bu); ^{13}C NMR δ 171.4, 155.8, 79.9, 73.5, 62.1, 60.9, 50.9, 37.0, 33.9, 28.3, 27.3, 14.2; MALDI TOF/TOF m/z value for $C_{17}H_{32}N_2O_7$ [M + Na$^+$] 399.2102 (calcd), 399.2140 (found).

(3*R*,4*S*)-1-*tert*-Butyl-6-ethyl 3-(*tert*-butyloxycarbonylamino)-4-(nitromethyl)hexanedioate (2g)^{anti}. White solid (0.66 g, 82%, dr 81 : 19); mp 86 °C; $[\alpha]_D^{25}$ −3.0 (c 1.0, MeOH); the dr determined by a Reverse Phase HPLC analytical C$_{18}$ column (5 μm, 4.6 × 250 mm), flow rate 0.75 mL min^{-1}, R_t (major, **2g**^{anti}) = 30.0 min, R_t (minor, **3g**^{syn}) = 26.7 min; IR ν (cm^{-1}) 3350, 2980, 2930, 1700, 1550, 1520, 1380, 1240, 1170, 1030, 860, 775; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.19 (d, J = 9.2 Hz, 1H, NH), 4.55 (m, 2H, –*CH*$_2$–NO$_2$), 4.14 (m, 2H, –O*CH*$_2$–), 4.04 (m, 1H, γ CH), 2.9 (m, 1H, β CH), 2.48 (m, 4H, α and δ CH$_2$'s), 1.45 (s, 9H, Boc), 1.4 (s, 9H, ^{t}Bu),1.26 (t, J = 5.6 Hz, 3H, –OCH$_2$–*CH*$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.1, 170.2, 155.5, 81.9, 80.1, 78.1, 61.1, 49.0, 38.2, 34.1, 28.3, 28.0, 14.1; HRMS m/z value for $C_{18}H_{32}N_2O_8$ [M + Na$^+$] 427.2051 (calcd), 427.2063 (found).

Ethyl-4-((*tert*-butoxycarbonyl)amino)-3-(nitromethyl)pentanoate (crude mixture, 2h + 3h, inseparable diastereomers). Light yellow oil (0.560 g, 92%, dr 80 : 20); the dr determined by NMR analysis; ^{1}H NMR (500 MHz, CDCl$_3$) 4.58–4.44 (m, 2H, –C*H*$_2$NO$_2$), 4.18–4.14 (m, 2H, –OC*H*$_2$–CH$_3$), 3.92–3.82 (m, 1H, γ CH), 2.84–2.77 (m, 1H, β CH), 2.56–2.38 (m, 2H, α CH$_2$), 1.44 (br, 9H, Boc), 1.28–1.26 (m, 3H, –OCH$_2$C*H*$_3$), 1.22–1.21 (br, 3H, side chain –CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.9, 171.4, 155.4, 79.8, 79.5, 76.3, 76.2, 60.9, 60.3, 47.5, 47.3, 39.8, 39.4, 33.8, 32.7, 28.6, 18.5, 17.7, 14.0; MALDI TOF/TOF m/z value for $C_{13}H_{24}N_2O_6$ [M + Na$^+$] 327.1527 (calcd), 327.1554 (found).

***tert*-Butyl-2-(4-ethoxy-1-nitro-4-oxobutan-2-yl)pyrrolidine-1-carboxylate (crude mixture, 2i + 3i, inseparable diastereomers).** Yellow oil (0.6 g, 91%, dr 61 : 49); the dr determined by a Reverse Phase HPLC analytical C$_{18}$ column (5 μm, 4.6 × 250 mm), flow rate 0.75 mL min^{-1}, R_t (major, **2i**^{anti}) = 52.3 min, R_t (minor, **3i**^{syn}) = 47.9 min; ^{1}H NMR (400 MHz, DMSO) δ 4.53 (br, 2H, C*H*$_2$NO$_2$), 4.07–3.93 (m, 2H, –OC*H*$_2$CH$_3$), 3.73 (br, 1H, γ CH), 3.32–3.19 (m, 2H, –C*H*$_2$NBoc), 3.02–2.95 (m, 1H, β CH), 2.31–2.19 (m, 2H, α CH$_2$), 1.69 (br, 4H, –CH$_2$–CH$_2$–), 1.36 (br, 9H, Boc), 1.15–1.52 (m, 3H, –OCH$_2$C*H*$_3$); ^{13}C NMR (100 MHz, DMSO) δ 170.8, 154.9, 154.8, 154.1, 79.4, 79.2, 77.6, 77.39, 60.7, 58.6, 58.2, 47.2, 37.4, 33.2, 32.2, 28.5, 27.2, 24.0, 23.5, 14.5. MALDI TOF/TOF m/z value for $C_{15}H_{26}N_2O_6$ [M + Na$^+$] 353.1683 (calcd), 353.1686 (found).

(3*R*,4*S*)-Benzyl-4-((*tert*-butoxycarbonyl)amino)-5-methyl-3-(nitromethyl)hexanoate (2j)^{anti}. Colorless oil (dr 86 : 14); $[\alpha]_D^{25}$ −42.0 (*c* 1.0, MeOH); the dr determined by a Reverse Phase HPLC analytical C_{18} column (5 μm, 4.6 × 250 mm), flow rate 0.75 mL min^{-1}, R_t (major, **2j**^{anti}) = 28.9 min, R_t (minor, **3j**^{syn}) = 26.4 min; IR ν (cm^{-1}) 3380, 2970, 2390, 1710, 1550, 1510, 1380, 1240, 1170, 1150, 966, 753, 689; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.32 (m, 5H, aromatic), 5.13 (s, 2H, –CH_2–Phe), 4.53–4.41 (m, 2H, α CH_2), 4.34 (d, *J* = 10.1 Hz, NH), 3.60–3.54 (m, 1H, γ CH), 2.93–2.85 (m, 1H, β CH), 2.62–2.43 (m, 2H, –CH_2–NO_2), 1.84–1.77 (m, 1H, δ CH), 1.42 (s, 9H, Boc), 0.95 (d, *J* = 6.9 Hz, 3H, CH_3), 0.88 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 156.5, 135.7, 128.8, 128.6, 127.2, 80.1, 76.0, 67.0, 57.1, 36.8, 34.6, 29.5, 28.5, 20.2, 17.1; MALDI TOF/TOF *m*/*z* value for $C_{20}H_{30}N_2O_6$ [M + Na$^+$] 417.1996 (calcd), 417.1915 (observed).

(3*R*,4*S*)-Benzyl-4-((*tert*-butoxycarbonyl)amino)-6-methyl-3-(nitromethyl)heptanoate (2k)^{anti}. White solid (dr 81 : 19); mp 85 °C; $[\alpha]_D^{25}$ −7.0 (*c* 1.0, MeOH); the dr determined by ^{1}H NMR; IR ν (cm^{-1}) 3382, 2969, 2361, 1727, 1685, 1545, 1510, 1451, 1381, 1255, 1156, 962, 746, 696; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.31 (m, 5H, aromatic), 5.13 (s, 2H, –OCH_2–Phe), 4.53–4.41 (m, 2H, –CH_2NO_2), 4.33 (br, 1H, NH), 3.82 (m, 1H, γ CH), 2.81–2.78 (m, 1H, β CH), 2.62–2.57 (m, 2H, α CH_2), 1.63 (m, 1H, ω CH), 1.42 (s, 9H, Boc), 1.37–1.29 (m, 2H, δ CH_2), 0.91 (d, *J* = 6.4 Hz, 3H, CH_3), 0.87 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 155.9, 135.5, 128.6, 128.4, 127.0, 79.8, 76.0, 66.8, 65.2, 60.4, 50.2, 42.1, 39.4, 28.3, 25.0, 23.4, 21.5, 21.1, 14.2; MALDI TOF/TOF *m*/*z* value for $C_{21}H_{32}N_2O_6$ [M + Na$^+$] 431.2153 (calcd), 431.2160 (observed).

(3*R*,4*S*)-Benzyl-4-((*tert*-butoxycarbonyl)amino)-3-(nitromethyl)-5-phenyl pentanoate (2l)^{anti}. White solid (dr 89 : 11); mp 106 °C; $[\alpha]_D^{25}$ −112.0 (*c* 1.0, MeOH); the dr determined by a Reverse Phase HPLC analytical C_{18} column (5 μm, 4.6 × 250 mm), flow rate 0.75 mL min^{-1}, R_t (major, **2l**^{anti}) = 37.6 min, R_t (minor, **3l**^{syn}) = 35.7 min; IR ν (cm^{-1}) 3370, 2978, 2929, 2363, 2342, 1726, 1693, 1542, 1509, 1364, 1248, 1147, 1108, 982, 845, 754, 734, 702, 638; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.12 (m, 10H, aromatic), 5.15 (s, 2H, –OCH_2–Phe), 4.60–4.48 (m, 2H, –CH_2–NO_2), 4.36 (d, *J* = 9.2 Hz, 1H, NH), 4.05–3.98 (m, 1H, γ CH), 2.95–2.86 (m, 2H, –CH–CH_2–Phe), 2.76–2.53 (m, 3H, β CH and α CH_2), 1.30 (s, 9H, Boc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 155.6, 136.9, 135.4, 129.0, 128.7, 128.5, 126.9, 80.0, 75.8, 66.9, 61.8, 38.9, 38.3, 34.3, 28.2; HR-MS *m*/*z* value for $C_{24}H_{30}N_2O_6$ [M + Na$^+$] 465.1996 (calcd), 465.1989 (observed).

General procedure for the synthesis of di-Boc protected vinylogous amino ester (4)

Di-*tert*-butyl dicarbonate (0.436 mL, 2 mmol), DMAP (0.122 g, 1 mmol) and compound **1e** were dissolved in dry acetonitrile (2 mL). The resulting solution was stirred for about 6 h. After that the reaction mixture was dissolved in 50 mL of EtOAc and washed with 1 M HCl (3 × 50 mL) and brine solution (3 × 50 mL). Then the organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The product was purified by column chromatography using 2 : 98 ethyl acetate–pet ether solvent systems.

Boc_2N-dgF-OEt (4). Colorless liquid; yield 20%; $[\alpha]_D^{25}$ −29.0 (*c* 1.0, MeOH); UV (λ_{max}) 218 nm, 256 nm; ^{1}H NMR δ (ppm) 7.29–7.18 (m, 5H, aromatic), 7.10 (dd, *J* = 16 Hz, *J* = 5 Hz, 1H, α CH), 5.91 (dd, *J* = 15.8 Hz, *J* = 2.1 Hz, 1H, β CH), 5.21–5.15 (m, 1H, γ CH), 4.19 (q, *J* = 7.3 Hz, 2H, –OCH_2CH_3), 3.19 (m, 2H, –$CH_2C_6H_5$), 1.41 (s, 18H, $(Boc)_2$), 1.28 (t, *J* = 7.1 Hz, 3H, –OCH_2CH_3); ^{13}C NMR δ (ppm) 166.0, 152.1, 147.0, 137.3, 129.3, 128.3, 126.5, 121.5, 82.5, 60.3, 57.9, 38.4, 27.8, 14.1; HR-MS *m*/*z* value for $C_{23}H_{33}NO_6$ [M + Na$^+$] 442.2200 (calcd), 442.2212 (observed).

$(Boc)_2$N-γF(β-CH_2NO_2)-OEt (5 + 6, diastereomeric mixture). Light yellow liquid; yield 88%; the dr determined by ^{1}H NMR analysis; ^{1}H NMR δ (ppm) 7.31–7.26 (m, 5H, Ar), 7.24–7.16 (m, 5H, Ar′), 5.41–5.24 (m, 2H, –CH_2NO_2) and 4.75–4.39 (m, 2H, –CH_2NO_2), 4.19–4.09 (m, 6H, 2 –OCH_2CH_3, 2 γ CH), 3.08–2.47 (m, 10H, 2 β CH's, 2 –$CH_2C_6H_5$ and 2 –CH_2COOEt), 1.51–1.49 (m, 18H, 2Boc), 1.30–1.25 (m, 28H, 2 Boc and 2 –OCH_2CH_3); HR-MS *m*/*z* value for $C_{24}H_{36}N_2O_8$ [M + Na$^+$] 503.2364 (calcd), 503.2346 (observed).

General procedure for peptide synthesis

Saponification of the ethyl ester of *N*-Boc-protected β-nitromethane substituted γ-amino acids. The Boc-γX(β-CH_2NO_2)-COOEt (3 mmol) was dissolved in 4 mL methanol followed by 8 mL 1 N NaOH was added slowly to the solution. The reaction mixture was stirred for about 24 h. The progress of the reaction was monitored by TLC. After completion of the reaction, methanol was evaporated. The aqueous layer was diluted with water (50 mL), acidified (pH ~ 4) with 5% HCl and extracted with EtOAc (3 × 30 mL). The combined organic layer was washed with brine solution (2 × 10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to get free carboxylic acid. The carboxylic acids were directly used for the peptide synthesis without further purification.

Deprotection of an *N*-Boc-group from ethyl ester of β-nitromethane substituted γ-amino acids. The solution of Boc-NH-γX(β-CH_2NO_2)-COOEt (3.6 mmol) in 3 mL of DCM was cooled at 0 °C followed by addition of neat TFA. After completion of the reaction (1 h), the solvent was evaporated under reduced pressure. The residue was then treated with saturated Na_2CO_3 solution in cold condition. The aqueous layer was extracted with EtOAc (3 × 40 mL). The combined organic layer was washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to 3 mL.

Coupling strategy. The solution of NH_2-γX(β-CH_2NO_2)-COOEt in EtOAc (3.6 mmol) was added to the solution of Boc-γX(β-CH_2NO_2)-COOH in DMF (3.0 mmol) under ice cold conditions. The mixture was treated with DCC (3.0 mmol) and HOBt (3.0 mmol). The reaction mixture was stirred for about 12 h at room temperature and the completion of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with EtOAc (100 mL) and DCU was filtered through the sintered funnel. The EtOAc layer was washed with brine (3 × 50 mL) followed by 5% aq. HCl

(3 × 50 mL), 10% aq. Na_2CO_3 (1 × 50 mL), brine (3 × 50 mL) and dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography using EtOAc/pet ether to get the pure product.

Boc-Ala-γPhe(β-CH_2NO_2)-COOEt ***(P1)***. White solid (0.94 g, 78%); mp 108 °C; $[\alpha]_D^{20}$ −29.0 (*c* 1.0, MeOH); IR ν (cm^{-1}) 3360, 3318, 2980, 1727, 1680, 1544, 1449, 1378, 1318, 1255, 1164, 1068, 1027, 704, 602; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.15 (m, 5H, aromatic), 6.39 (d, *J* = 9.2 Hz, 1H, NH), 4.74 (d, *J* = 6 Hz, 1H, NH), 4.60–4.48 (m, 2H, –CH_2NO_2), 4.41–4.35 (m, 1H, α Ala CH), 4.17 (q, *J* = 7 Hz, 2H, –OCH_2–CH_3), 3.99–3.93 (m, 1H, γ CH), 3.02–2.95 (m, 1H, β CH), 2.93–2.74 (m, 2H, α CH_2), 2.65–2.46 (m, 2H, –CH_2–Phe), 1.43 (s, 9H, Boc), 1.28–1.25 (t, *J* = 7.1 Hz, 3H, –OCH_2CH_3), 1.1 (d, *J* = 6 Hz, 3H, –CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 171.2, 155.6, 136.9, 129.0, 128.7, 126.9, 79.2, 75.9, 61.1, 51.3, 50.7, 38.6, 38.3, 34.4, 28.3, 17.6, 14.2; MALDI TOF/TOF *m*/*z* value for $C_{22}H_{33}N_3O_7$ [M + Na^+] 474.2211 (calcd); 474.2294 (observed).

Boc-Phe-γVal(β-CH_2NO_2)-COOEt, ***(P2)***. White solid (0.67 g, 70%); mp 110 °C; $[\alpha]_D^{20}$ −14.0 (*c* 1.0, MeOH); IR ν (cm^{-1}) 3281, 2971, 2925, 1713, 1684, 1651, 1553, 1461, 1380, 1250, 1172, 1097, 960, 857, 754, 706; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.21 (m, 5H, aromatic), 6.02 (d, *J* = 10.1 Hz, 1H, NH), 5.05 (d, *J* = 7.8 Hz, 1H, NH), 4.28 (q, *J* = 7.8 Hz, 1H, α Phe), 4.15 (q, *J* = 7.3 Hz, 2H, –OCH_2CH_3), 4.02 (m, 2H, –CH_2NO_2), 3.85 (m, 1H, γ CH), 3.06 (m, 2H, –CH_2–Phe), 2.75 (m, 1H, β CH), 2.26 (m, 2H, α CH_2), 1.79 (m, 1H, –C*H*$(CH_3)_2$), 1.41 (s, 9H, Boc), 1.27 (t, *J* = 7.1 Hz, 3H, –OCH_2–CH_3), 0.88 (m, 6H, –$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 171.0, 136.5, 129.3, 128.9, 127.2, 80.6, 75.7, 61.0, 56.5, 55.0, 37.4, 36.2, 34.2, 29.2, 28.3, 20.0, 17.1, 14.2; HR-MS *m*/*z* value for $C_{24}H_{37}N_3O_7$ [M + Na^+] 502.2524 (calcd), 502.2513 (found).

Boc-γVal(β-CH_2NO_2)-γLeu(β-CH_2NO_2)-COOEt ***(P3)***. Light yellow solid (0.9 g, 70%); mp 93 °C; $[\alpha]_D^{20}$ −12.0 (*c* 1.0, MeOH); IR ν (cm^{-1}) 3258, 3067, 2967, 1744, 1651, 1555, 1460, 1379, 1293, 1253, 1167, 1107, 865, 742; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.25 (d, *J* = 9.2 Hz, 1H, NH), 4.62–4.49 (m, 2H, –CH_2NO_2), 4.43–4.30 (m, 3H,–CH_2NO_2 and NH), 4.26–4.19 (m, 1H, γ CH), 4.14 (q, *J* = 7.3 Hz, 2H, –OCH_2–), 3.40–3.34 (m, 1H, γ CH), 3.02–2.95 (m, 1H, β CH), 2.89–2.82 (m, 1H, β CH), 2.60–2.37 (m, 2H, α CH_2), 2.27–2.25 (m, 2H, α CH_2), 1.70–1.65 (m, 2H, Leu δ CH_2), 1.60–1.55 (m, 1H, Val δ CH), 1.41 (s, 9H, Boc), 1.38–1.34 (m, 1H, Leu ω CH), 1.24 (t, *J* = 7.1 Hz, 3H, –OCH_2–CH_3), 1.00–0.86 (m, 12H, Leu and Val side chain $(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 170.5, 157.2, 80.4, 75.9, 61.0, 60.5, 57.9, 48.5, 41.7, 39.0, 37.1, 37.0, 34.4, 30.2, 28.3, 25.0, 23.3, 21.6, 21.1, 19.9, 19.4, 14.2; MALDI TOF/TOF *m*/*z* value for $C_{24}H_{44}N_4O_9$ [M + Na^+] 555.3001 (calcd), 555.3064 (observed).

Boc-γLeu(β-CH_2NO_2)-γIle(β-CH_2NO_2)-COOEt ***(P4)***. White solid (0.655 g, 64%); mp 103 °C; $[\alpha]_D^{20}$ −6.0 (*c* 1.0, MeOH); IR ν (cm^{-1}) 3394, 2967, 2362, 1700, 1654, 1553, 1457, 1378, 1254, 1169, 1035; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.08 (d, *J* = 10.1 Hz, 1H, NH), 4.62–4.51 (m, 2H, –CH_2NO_2), 4.46–4.32 (m, 3H, –CH_2NO_2 and NH), 4.12 (q, *J* = 6.9 Hz, 2H, –OCH_2–CH_3), 4.03–3.9 (m, 1H, γ CH), 3.8 (m, 1H, γ CH), 3.03 (m, 1H), 2.79 (m, 1H), 2.59–2.38 (m, 2H), 2.3 (d, *J* = 6.4 Hz, 2H), 1.66–1.43 (m, 4H), 1.40 (s, 9H), 1.23 (t, *J* = 7.1 Hz, 3H), 0.97–0.84 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 156.8, 80.4, 76.0, 75.7, 60.9, 54.6, 49.9, 42.3, 40.1, 37.0, 36.2, 36.1, 34.8, 28.3, 25.3, 24.5, 23.0, 21.7, 16.2, 14.2, 11.2; MALDI TOF/TOF *m*/*z* value for $C_{25}H_{46}N_4O_9$ [M + $Na]^+$ 569.3157 (calcd), 569.3146 (found).

Boc-γSer(OBu^t) (β-CH_2NO_2)-γVal(β-CH_2NO_2)-COOEt ***(P5)***. Yellow oil (0.56 g, 55%); $[\alpha]_D^{20}$ −8.0 (*c* 1.0, MeOH); IR ν (cm^{-1}) 3323, 2973, 1721, 1552, 1376, 1242, 1173, 1085, 1028, 873, 701; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.32 (d, *J* = 10.1 Hz, 1H), 5.04 (d, *J* = 9.2 Hz, 1H), 4.88 (dd, *J* = 13.1 Hz, *J* = 4.4 Hz, 1H), 4.58 (m, 2H), 4.29 (m, 1H), 4.16–4.06 (m, 3H), 3.96 (m, 1H), 3.87 (m, 1H), 3.53 (m, 2H), 2.91 (m, 2H), 2.58–2.24 (m, 4H), 1.41 (s, 9H), 1.24 (m, 6H), 1.15 (s, 9H), 0.92 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 156.7, 80.5, 76.2, 73.9, 63.8, 61.0, 60.5, 55.5, 54.9, 50.3, 49.3, 39.5, 37.2, 36.8, 33.9, 29.3, 28.3, 27.3, 24.9, 21.1, 20.2, 17.0, 14.2; HR-MS *m*/*z* value for $C_{25}H_{46}N_4O_{10}$ [M + Na^+] 585.3106 (calcd), 585.3110 (found).

Boc-γVal(β-CH_2NO_2)-γVal(β-CH_2NO_2)-COOEt ***(P6)***. Yellow solid (0.55 g, 58%); mp 120 °C; $[\alpha]_D^{20}$ −5.0 (*c* 1.0, MeOH); IR ν (cm^{-1}) 3427, 3254, 3062, 2964, 2950, 2564, 1742, 1642, 1554, 1461, 1295, 1253, 1166, 1033, 885, 735; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (d, *J* = 10.1 Hz, 1H, NH), 4.57 (m, 2H, –CH_2NO_2), 4.38 (m, 3H, NH and –CH_2NO_2), 4.14 (q, *J* = 7.3 Hz, 2H, –CH_2CH_3), 3.96 (dt, *J* = 10.1 Hz, *J* = 6.6 Hz, 1H), 3.38 (td, *J* = 9.4 Hz, *J* = 3.7 Hz, 1H), 2.97 (m, 2H), 2.49 (m, 2H), 2.3 (d, *J* = 6.9 Hz, 2H), 1.42 (m, 11H, Boc and 2 $CH(CH_3)_2$), 1.24 (t, *J* = 7.1 Hz, 3H, –OCH_2–CH_3), 0.95 (m, 12H, 2 –CH(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 170.8, 157.2, 80.5, 76.0, 61.0, 58.0, 55.1, 37.2, 37.0, 36.5, 34.6, 34.0, 30.3, 29.4, 28.3, 25.6, 25.0, 20.1, 19.9, 19.5, 17.5, 14.2; MALDI TOF/TOF *m*/*z* value for $C_{23}H_{42}N_4O_9$ [M + Na^+] 541.2844 (calcd), 541.2827 (observed).

Boc-γVal(β-CH_2NO_2)-γPhe(β-CH_2NO_2)-COOEt ***(P7)***. White solid (0.85 g, 65%); mp 147 °C; $[\alpha]_D^{20}$ −15.0 (*c* 1.0, MeOH); IR ν (cm^{-1}) 3360, 2973, 2929, 1729, 1687, 1552, 1437, 1377, 1294, 1246, 1168, 1086, 1021, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.14 (m, 5H, aromatic), 6.36 (d, *J* = 8.7 Hz, 1H), 4.67–4.54 (m, 2H), 4.51–4.42 (m, 1H), 4.3 (d, *J* = 9.6 Hz, 1H), 4.17–4.01 (m, 4H), 3.36–3.30 (m, 1H), 2.99–2.91 (m, 2H), 2.87–2.80 (m, 1H), 2.72–2.47 (m, 4H), 2.21–2.02 (m, 2H), 1.66–1.58 (m, 1H), 1.42 (m, 9H), 1.24 (t, *J* = 7.1 Hz, 3H), 0.92 (dd, *J* = 16.3 Hz, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 170.4, 157.1, 136.9, 128.9, 128.7, 127.0, 80.4, 76.0, 75.6, 61.1, 57.8, 51.5, 38.6, 38.3, 37.0, 34.4, 30.0, 28.3, 20.0, 18.8, 14.2; MALDI TOF/TOF *m*/*z* value for $C_{31}H_{42}N_4O_9$ [M + Na^+] 589.2844 (calcd), 589.2828 (found).

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References

1 (*a*) A. Abel, *Advances in Amino Acid Mimetics and Peptidomimetics*, JAI, Stanford, 1999, vol. 2; (*b*) W. M. Kazmierski, *Methods in Molecular Medicine: Peptidomimetic Protocols*, Humana, Totowa, NJ, 1999; (*c*) D. Seebach, A. K. Beck and D. J. Bierbaum, *Chem. Biodiversity*, 2004, **1**, 1111–1239; (*d*) W. S. Horne and S. H. Gellman, *Acc. Chem. Res.*, 2008, **41**, 1399–1408; (*e*) R. P. Cheng, S. H. Gellman and W. F. DeGrado, *Chem. Rev.*, 2001, **101**, 3219–3232; (*f*) P. G. Vasudev, S. Chatterjee, N. Shamala and P. Balaram, *Chem. Rev.*, 2011, **111**, 657–687; (*g*) J. L. Price, W. S. Horne and S. H. Gellman, *J. Am. Chem. Soc.*, 2010, **132**, 12378–12387; (*h*) P. Schramm, G. V. M. Sharma and H.-J. Hofmann, *Biopolymers*, 2010, **94**, 279–291.

2 (*a*) P. G. Williams, R. E. Moore and V. J. Paul, *J. Nat. Prod.*, 2003, **66**, 1356–1363; (*b*) T. L. Simmons, L. M. Nogle, J. Media, F. A. Valeriote, S. L. Mooberry and W. H. Gerwick, *J. Nat. Prod.*, 2009, **72**, 1011–1016; (*c*) T. Golakoti, W. Y. Yoshida, S. Chaganty and R. E. Moore, *Tetrahedron*, 2000, **56**, 9093–9102; (*d*) J. Maibaum and D. H. Rich, *J. Org. Chem.*, 1988, **53**, 869–873; (*e*) R. G. Linington, B. R. Clark, E. E. Trimble, A. Almanza, L.-D. Uren, D. E. Kyle and W. H. Gerwick, *J. Nat. Prod.*, 2009, **72**, 14–17; (*f*) J. A. Nieman, J. E. Coleman, D. J. Wallace, E. Piers, l. Y. Lim, M. Roberge and R. J. Andersen, *J. Nat. Prod.*, 2003, **66**, 183–199.

3 (*a*) C. L. Moore, D. D. Leatherwood, T. S. Diehl, D. J. Selkoe and M. S. Wolfe, *J. Med. Chem.*, 2000, **43**, 3434–3442; (*b*) C. Palomo, J. I. Miranda and A. Linden, *J. Org. Chem.*, 1996, **61**, 9196–9201; (*c*) R. P. Hanzlik and S. A. Thompson, *J. Med. Chem.*, 1984, **27**, 711–712; (*d*) A. Breuning, B. Degel, F. Schulz, C. Buchold, M. Stempka, U. Machon, S. Heppner, C. Gelhaus, M. Leippe, M. Leyh, C. Kisker, J. Rath, A. Stich, J. Gut, P. J. Rosenthal, C. Schmuck and T. Schirmeister, *J. Med. Chem.*, 2010, **53**, 1951–1963; (*e*) N. Schaschke and C. P. Sommerhoff, *ChemMedChem*, 2010, **5**, 367–370.

4 (*a*) G. J. Sill, *Curr. Opin. Pharmacol.*, 2006, **6**, 108–113; (*b*) R. B. Silverman, *Angew. Chem., Int. Ed.*, 2008, **47**, 3500–3504.

5 (*a*) N. Ono, *The Nitro Group in Organic Synthesis*, Wiley-VCH, New York, 2001; (*b*) F. A. Luzzio, *Tetrahedron*, 2001, **57**, 915–945; (*c*) R. Ballini, G. Bosica, D. Fiorini, A. Palmieri and M. Petrini, *Chem. Rev.*, 2005, **105**, 933–971; (*d*) A. G. M. Barrett and G. G. Graboski, *Chem. Rev.*, 1986, **86**, 751–762; (*e*) E. Breuer, H. G. Aurich and A. Nielsen, *Nitrones, Nitronates & Nitroxides, The Chemistry of Functional Groups*, ed. S. Patai, J. Wiley & Sons, New York, 1992.

6 (*a*) P. Perlmutter, *Conjugate Addition Reactions in Organic Synthesis*, Pergamon, Oxford, 1992, p. 339; (*b*) J. Christoffers and A. Baro, *Angew. Chem., Int. Ed.*, 2003, **42**, 1688–1690; (*c*) A. Alexakis and C. Benhaim, *Eur. J. Org. Chem.*, 2002, 3221–3236; (*d*) S. B. Tsogoeva, *Eur. J. Org. Chem.*, 2007, 1701–1716; (*e*) A. S. Kireev, M. Manpadi and A. Kornienko, *J. Org. Chem.*, 2006, **71**, 2630–2640; (*f*) S. Hanessian, J. Ma and W. Wang, *J. Am. Chem. Soc.*, 2001, **123**, 10200–10206; (*g*) Y. Yamamoto, Y. Chounan, S. Nishii, T. Ibuka and H. Kitahara, *J. Am. Chem. Soc.*, 1992, **114**, 7652–7660; (*h*) N. Asao, T. Shimada, T. Sudo, N. Tsukada, K. Yazawa, Y. S. Gyoung, T. Uyehara and Y. Yamamoto, *J. Org. Chem.*, 1997, **62**, 6274–6282; (*i*) N. Sewald, K. D. Hiller, M. Körner and M. Findeisen, *J. Org. Chem.*, 1998, **63**, 7263–7274; (*j*) T. Cailleau, J. W. B. Cooke, S. G. Davies, K. B. Ling, A. Naylor, R. L. Nicholson, P. D. Price, P. M. Roberts, A. J. Russell, A. D. Smith and J. E. Thomson, *Org. Biomol. Chem.*, 2007, **5**, 3922–3931.

7 (*a*) J. S. Plummer, L. A. Emery, M. A. Stier and M. J. Suto, *Tetrahedron Lett.*, 1993, **34**, 7529–7532; (*b*) Y. Kimura, S. Aarashi, M. Takahashi and I. Hayakawa, *Chem. Pharm. Bull.*, 1994, **42**, 1442–1454; (*c*) N. Ohtake, K. Yamada, E. Mano, O. Okamoto, R. Ushijima and S. Nakagawa, *J. Antibiot.*, 1997, **50**, 567–585; (*d*) S. Hanessian, R. Buckle and M. Bayrakdarian, *J. Org. Chem.*, 2002, **67**, 3387–3397; (*e*) V. Beylin, D. C. Boyles, T. T. Curran, D. Macikenas, R. V. Parlett IV and D. Vrieze, *Org. Process Res. Dev.*, 2007, **11**, 441–449.

8 (*a*) S. M. Mali, A. Bandyopadhyay, S. V. Jadhav, M. Ganesh Kumar and H. N. Gopi, *Org. Biomol. Chem.*, 2011, **9**, 6566–6574; (*b*) A. Bandyopadhyay, S. M. Mali, P. Lunawat, K. M. P. Raja and H. N. Gopi, *Org. Lett.*, 2011, **13**, 4482–4485; (*c*) A. Bandyopadhyay and H. N. Gopi, *Org. Lett.*, 2012, **14**, 2770–2773.

9 (*a*) M. Ocejo, J. L. Vicario, D. Badia, L. Carrillo and E. Reyes, *Synlett*, 2005, 2110–2112; (*b*) S. V. Jadhav, A. Bandyopadhyay, S. N. Benke, S. M. Mali and H. N. Gopi, *Org. Biomol. Chem.*, 2011, **9**, 4182–4187.

10 M. Hagihara, N. J. Anthony, T. J. Stout, J. Clardy and S. L. Schreiber, *J. Am. Chem. Soc.*, 1992, **114**, 6568–6571.

11 A. E. Dorigo, D. W. Pratt and K. N. Houk, *J. Am. Chem. Soc.*, 1987, **109**, 6591–6600.

12 K. B. Wiberg and E. Martin, *J. Am. Chem. Soc.*, 1985, **107**, 5035–5041.

13 S. Brownstein, J. Dunogues, D. Lindsay and K. U. Ingold, *J. Am. Chem. Soc.*, 1977, **99**, 2073–2078.

14 P. G. Vasudev, N. Shamala, K. Ananda and P. Balaram, *Angew. Chem., Int. Ed.*, 2005, **44**, 4972–4975.