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Synthesis of rigid tryptophan mimetics by the diastereoselective Pictet–Spengler reaction of β^3-*homo*-tryptophan derivatives with chiral α-amino aldehydes

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The Pictet–Spengler (PS) cyclizations of β^3-*h*Trp derivatives as arylethylamine substrates were performed with L-α-amino and D-α-amino aldehydes as carbonyl components. During the PS reaction, a new stereogenic center was created, and the mixture of *cis/trans* 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines was obtained. The ratio of *cis/trans* diastereomers depends on the stereogenic centre of used amino aldehyde and the size of substituents. It was confirmed by 1H and 2D NMR (ROESY) spectra. The conformations of cyclic products were studied by 2D NMR ROESY spectra. Products of the PS condensation after removal of protecting group(s) can be incorporated into a peptide chain as tryptophan mimetics with the possibility of the β-turn induction.

Keywords: tryptophan mimetics; building blocks; beta-turn; P-S cyclisation; diastereoselectivity

Introduction

Tryptophan is often a key pharmacophore, which determines the affinity of peptide ligands for their receptors [1,2]. Various constrained tryptophan analogues or tryptophan containing motifs have been utilized to generate highly potent and selective ligands to biological target receptors [3–5]. Cyclic analogues of tryptophan, which introduce local constraints and reduce the flexibility of the indole moiety, are very valuable tools to probe the bioactive conformation of the peptide ligands [6–9]. Such rigid compounds may be used to replace the natural amino acid residues in the peptide sequence to improve biological activity, potency, metabolic stability and other properties of parent peptides. The usage of the Pictet–Spengler (PS) reaction [10,11] was one of the possibilities used to prepare such analogues by freezing the indole moiety in tryptophan [12]. The PS reaction is a cyclization based on the reaction between arylethylamine and carbonyl components. The main advantage of this reaction is the formation of a product with stable C–C bond in one single step [13]. During the PS reaction of tryptophan with aldehyde, a new ring is formed and stereogenic centre is generated [14–16]. The products of the condensation containing 1,2,3,4-tetrahydro-β-carboline skeleton can be obtained as *cis/trans* isomers. Diastereomeric ratio of the products depends on the conditions of the reaction [17–19] and the structure of the substrates [20–22].

The heterocyclic skeleton of 1,2,3,4-tetrahydro-β-carbolines possess multiple sites for functionalization. Therefore, they are an ideal choice for the design of pharmacophore-based libraries in drug discovery, through the generation of a large number of structurally diverse compounds [23]. There is also a possibility to incorporate such 1,2,3,4-tetrahydro-β-carbolines into a peptide chain to obtain peptidomimetics with rigid analogues of tryptophan.

The PS cyclization of α-Trp derivatives with Z protected α-amino aldehydes (prepared from L-amino and D-amino acids) was performed in our previous study [17,24,25]. It was found that the cyclisation was diastereoselective, and for D-amino aldehydes, only *cis* isomer occurs; for L-amino aldehydes, two isomers were formed with the dominance of isomer *trans* [24,26].

β-Amino acids represent an important class of biologically relevant molecules. Oligomers composed of β-amino acids can form predictable secondary structures stable to metabolic transformations, and they can mimic α-peptides in peptide–protein interactions [25,27,28]. For these reasons, β-amino acids are very useful for peptidomimetics [29] design [30]. In this paper, we present the diastereoselectivity studies of PS condensation of L-α-amino and D-α-amino aldehydes as carbonyl components with methyl ester of L-β^3-homo-tryptophan (β^3-*h*TrpOCH$_3$) and N-terminal L-β^3-homo-tryptophan dipeptides (β^3-*h*TrpAlaOCH$_3$ and β^3-*h*TrpLeuOCH$_3$) as arylethylamine substrates. We describe the conformation of a newly created 6-membered ring. We have studied the dependence of the ratio of the isomeric products on the configuration of aldehyde. These analogues can be used as rigid dipeptide mimetics, which may be incorporated into the peptide sequence to reduce the flexibility of the peptide chain and probe the bioactive conformation.

β-Turns often play a crucial role in bioactive peptides as a molecular recognition element [31] and initiation sites for protein folding

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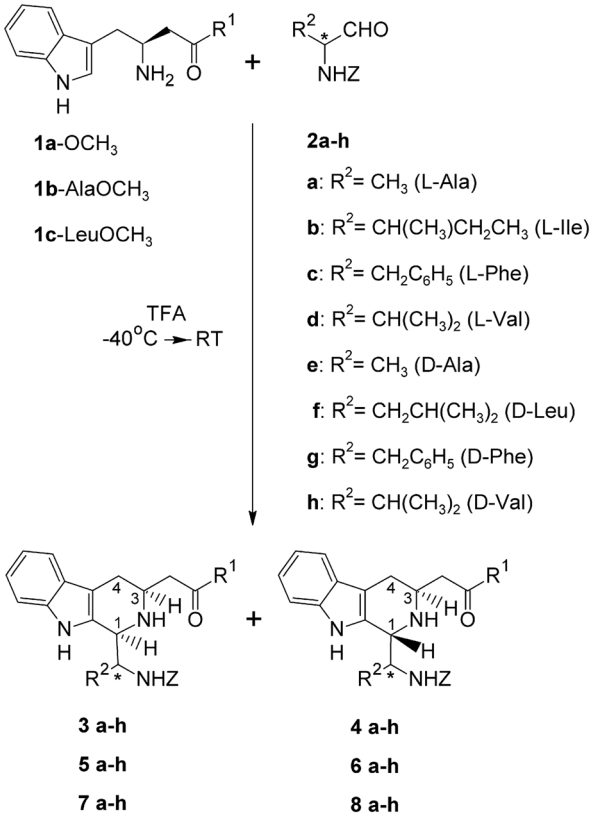
[32,33]. The design and synthesis of analogues that can mimic these secondary structural elements is a valuable tool for the study of bioactive structures of pharmacophore for better understanding the molecular mechanisms of peptide–proteins or protein–protein interactions and to provide potent therapeutic agents [16,34]. We will discuss this possibility of the β-turn induction by products of PS condensation.

Results and discussion

The synthesis of β^3-*h*TrpOCH$_3$was accomplished by Arndt-Eistert homologation of L-α-tryptophan [35]. Dipeptides β^3-*h*TrpAlaOCH$_3$ and β^3-*h*TrpLeuOCH$_3$ were prepared by the standard Boc-procedure in solution. α-Amino aldehydes protected by a benzyloxycarbonyl group (Z), were prepared with good yields via the Ferentz–Castro procedure [36] and used instantly without purification to avoid racemization. The PS cyclizations of different L-β^3-*homo*-tryptophan derivatives with L-α-amino and D-α-amino aldehydes were performed in the presence of 5 equivalents of TFA and at low temperature to avoid racemization of chiral amino aldehydes (Scheme 1) [17,24,37–39]. The reaction was stirred at −40 °C for the first 5 h, and then stirring was continued at room temperature overnight.

The ratio of stereoisomers was determined by ^{1}H NMR spectra of the crude mixture after the reaction. The diastereomeric products (*cis/trans*) were separated in a silica gel column. The conformations of the individual isomers were determined by 2D NMR ROESY spectra. The exchange of magnetization (NOE effect) among H-1 and H-3 protons was diagnostic for identification of



Scheme 1. PS reaction between β^3-*h*Trp derivatives and α-amino aldehydes.

cis/trans diastereoisomers. The results of all performed reactions are summarized in Table 1.

The PS reactions of all used β^3-*h*Trp derivatives (β^3-*h*TrpOCH$_3$, β^3-*h*TrpAlaOCH$_3$ and β^3-*h*TrpLeuOCH$_3$) with L-amino aldehydes preferentially led to *trans* isomers. Only reactions between β^3-*h*TrpOCH$_3$ and Z-L-Ala-CHO led to the product with opposite stereoselectivity, and *cis* isomer occurs as the main product. The reactions with D-amino aldehydes were totally selective, and only *cis* diastereoisomer was formed. The comparison of these results with the study conducted under the same conditions with α-Trp derivatives and L-amino and D-amino aldehydes shows that the ratio of *cis/trans* diastereoisomers is very similar [24,26]. These and our previous results showed that the ratio of *cis/trans* diastereomers depended on the stereogenic centre of amino aldehyde. To explain the difference between the stereoselectivity of the PS reaction of L-Ala-CHO with β^3-*h*TrpOCH$_3$ and dipeptides, we hypothesized the mechanisms of these reactions. The Felkin–Ahn asymmetric induction model and hydrogen bonded intermediate can explain this result (Figure 1).

For β^3-*h*Trp derivatives, there is a possibility to form two different pseudocyclic structures: 6-membered and 7-membered. Six-membered ring is formed when the intramolecular hydrogen bond links ester group and iminium cation. Such a situation is responsible for diastereoselectivity of the PS reaction with Z-L-Ala-CHO. Additional -CH$_2$- group in β^3-*h*Trp main-chain seems to facilitate the hydrogen bond formation in the 6-membered ring; thus, the amount of *cis* isomer is increased (Table 1), comparing with α-Trp [24]. For L-amino aldehydes with bigger side chains (Z-L-Ile-CHO), 7-membered ring with an intramolecular hydrogen bond between iminium cation and Z-group determines the selectivity, and *trans* isomer is predominantly formed. For the *cis* isomer, there is an unfavorable steric interaction between side chain of amino aldehyde (R) and iminium nitrogen. The smaller and less branched R, the higher amount of *cis* isomer was observed. For D-amino aldehydes, only a *cis* isomer was formed because of the unfavorable interactions in the intermediate state leading to the *trans* isomer (Figure 2).

The conformations of the newly created 6-membered ring were studied by 2D NMR ROESY spectra. The NOE effect among H-3 and H-4 protons was crucial for predictions of the ring's conformation. For *cis* isomer, there are two possible twisted chair conformations (Figure 3), but NOE effect between H-3 and both H-4 protons confirmed only the more stable form B with both substituents equatorially located. All *cis* isomers adopt the conformation with both substituents (on C-1 and C-3) equatorially located. For *trans* analogs, the results were more variable. For compounds 4a-h obtained from β^3-*h*TrpOCH$_3$, we observed the NOE effect between

Table 1. The ratio of *cis/trans* isomers determined* by ^{1}H NMR

	Amino aldehyde 2a-h	β^3-*h*TrpOCH$_3$ *cis/trans* [%] 3a-h/4a-h	β^3-*h*TrpAlaOCH$_3$ *cis/trans* [%] 5a-h/6a-h	β^3-*h*TrpLeuOCH$_3$ *cis/trans* [%] 7a-h/8a-h
a	Z-L-Ala-H	60/40	35/65	39/61
b	Z-L-Ile-H	0/100	0/100	0/100
c	Z-L-Phe-H	47/53	26/73	21/79
d	Z-L-Val-H	0/100	0/100	0/100
e	Z-D-Ala-H	100/0	100/0	100/0
f	Z-D-Leu-H	100/0	100/0	100/0
g	Z-D-Phe-H	100/0	100/0	100/0
h	Z-D-Val-H	100/0	100/0	100/0

* before purification

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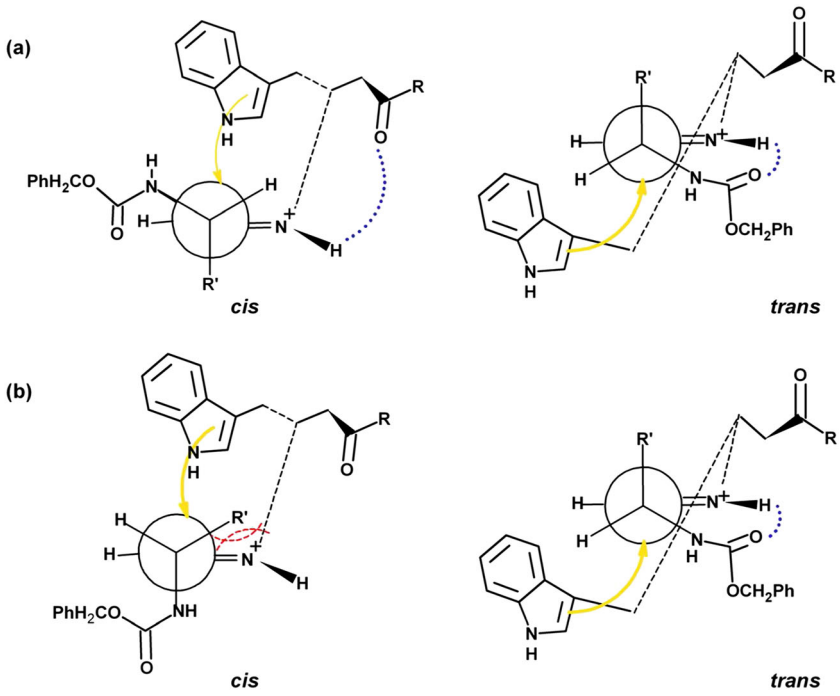

Figure 1. Hypothesis of the mechanism for PS reaction between β^3-*h*Trp derivatives and L-amino aldehydes with (a) smaller side chains, (b) bigger side chains.

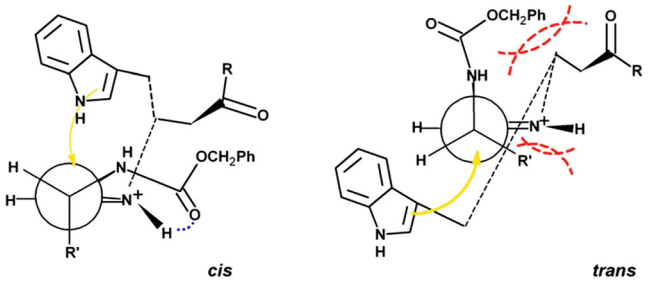

Figure 2. Hypothesis on the mechanism for PS reaction between D-amino aldehydes and β^3-*h*Trp derivatives.

H-3 and both H-4 protons, which means that the conformational equilibrium is shifted to the C form where the -CH_2COOCH_3 group was axially and substituent on carbon C-1 equatorially located. For *trans* analogs 6a-h, 8a-h**,** we observed a strong NOE effect between H-3 and one of H-4 protons; for second H-4 proton, the effect was weak or did not occur. These observations indicate that for the peptide chain at C-3, an equatorial position is more preferable then axial. To sum up, in the case of *trans* isomers with small C-3 substituents (-CH_2COOCH_3), the group is axially located; for larger groups, peptide moieties are equatorially located.

A general definition of a β-turn states that any tetrapeptide chain in which the distance between the C$\alpha(i)$ and the C$\alpha(i+3)$ is below 7 and which occurs in non-helical region constitutes a β-turn [40]. The pseudo 10-membered ring often, but not always, is formed by an intramolecular hydrogen bond between the CO of the first residue (i) and the NH of the fourth residue ($i+3$). According to literature [26,41] and our previous study [24], it is considered that disubstituted 6-membered rings in tetrahydro-β-carboline can induce β-turns. Such structures are more commonly found in *trans* isomers; therefore, we speculate that it is possible that *trans* analogs 6a-h and 7a-4 can have the ability to form β-turns. Hypothetically, there is a possibility to form two different pseudocyclic structures: 9-membered and 11-membered. A 9-membered ring is formed when the intramolecular hydrogen bond links an amino group derived from aldehyde and oxygen of the carbonyl group in peptide chain (Figure 4a). An 11-membered ring with an intramolecular hydrogen bond between oxygen of the Z-group and an amine group in a peptide chain may also arise (Figure 4b). To confirm these conjectures and find out which of these structures are the most energetically favorable, the optimization using molecular mechanics (HyperCube, HyperChem 8.0) was performed. We found that the structure with an 11-membered ring is preferable, which is consistent with literature data for peptidomimetics containing β-amino acids [42]. The calculated distance between hydrogen and oxygen was below 2.5 , and between C$\alpha(i)$ and oxygen, in which our analogues take place C$\alpha(i+3)$, was below 7 [40] (Figure 5).

The results of the molecular modeling were confirmed by 2D NMR ROESY spectra. We observed the NOE effects for analogs 6a and 7a between protons marked by arrows in Figure 5, which is in agreement with the modeled structure.

Conclusion

In conclusion, our studies have shown that L-β^3-*homo*-tryptophan and peptides with N-terminal L-β^3-*homo*-tryptophan residue can be used as substrates for PS cyclization. During the PS reactions between β^3-*h*Trp derivatives and L-amino aldehydes *cis* and *trans* products are formed with the dominance of *trans* isomer with the exception of reaction β^3-*h*TrpOCH_3 with Z-L-Ala-CHO. In the reaction with L-amino aldehydes with side chains branched on carbon β (Z-L-Ile-H, Z-L-Val-H) only *trans* isomer is obtained. The PS reactions with D-amino aldehydes are fully selective and only *cis* diastereomer is formed. The C-terminal part of the β^3-*h*Trp residue does not have any influence on the selectivity of the PS reactions, and the results obtained for methyl ester of β^3-*h*Trp are the same as for peptides with N-terminal β^3-*h*Trp. The conformations of the

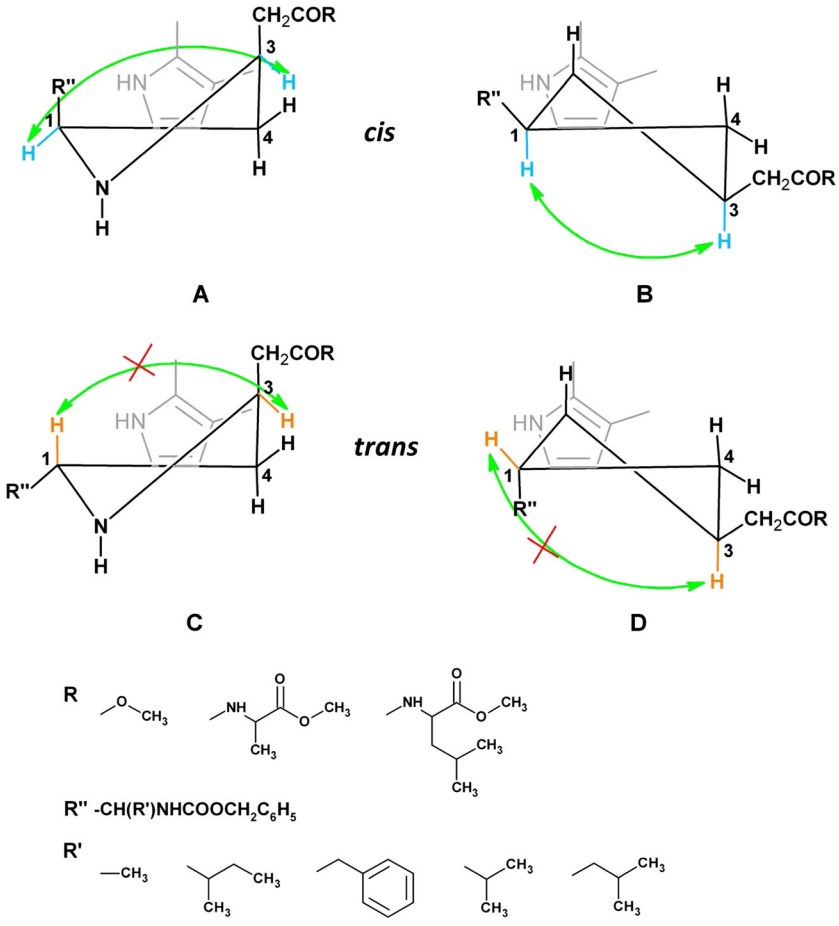

Figure 3. The conformations of the newly created 6-membered rings adopted by the (a) cis and (b) trans products of PS cyclization.

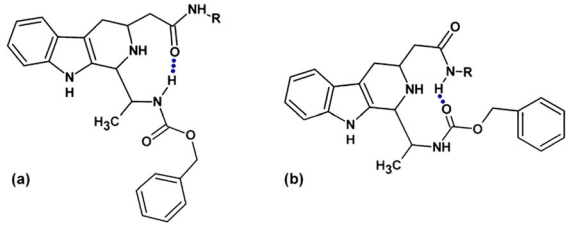

Figure 4. Hypothetical 9-membered (a) and 11-membered (b) pseudocyclic forms of β^3-*homo*-tryptophan dipeptides.

newly created 6-membered ring depends on the size of C-terminal part of β^3-*h*Trp residue. In *trans* isomers small C-terminal substituents (-CH_2COOCH_3) are axially located, big ones (peptide moieties) are equatorially located.

Obtained 1,2,3,4-tetrahydro-β-carbolines after removal of protecting group(s) could be incorporated into a peptide chain to obtain peptidomimetics with rigid analogues of tryptophan. The tetrahydro-β-carbolines (6a and 7a) moiety that are incorporated into the peptide chain may induce β-turn and impose the definite position of the peptide chains.

Experimental section

General procedure

The chemicals and solvents were used as received from commercial suppliers (Merck, Warsaw, Poland; Sigma-Aldrich, Poznan, Poland;). The RP–HPLC analysis and purifications were carried out using C12 analytical (Jupiter 4u Proteo 90A, 250 × 4,6 mm, 4 micron, linear gradient 1: t = 0 min, 97%A, 3%B, t = 20 min, 3%A, 97%B, flow rate 1 ml min^{-1}, λ = 254 nm) and C12 semi-preparative (Jupiter 4u Proteo 90A, 250 × 10 mm, 4 micron, linear gradient 2: t = 0 min, 65%A, 35%B, t = 30 min, 65%A, 35%B, flow rate 3 ml min^{-1}, λ = 254 nm) columns on a Shimadzu instrument (Shimadzu, Duisburg, Germany). The mobile phases (water A, acetonitrile B) contained 0,05% (v/v) TFA. TLC analysis was performed on precoated plates of silica gel 60 F254. Silica gel 60 (0.063–0.2) was used for flash chromatography. Mass analyses were performed on a LCT TOF spectrometer using electrospray ionization (positive ion mode) or LC–MS (Shimadzu, Duisburg, Germany). NMR spectra were recorded on Varian NMR System 200 or 700 MHz spectrometers (Agilent, Boeblingen, Germany). ^{13}C NMR spectra were acquired as 2D HSQC (quaternary and carbonyl carbons were not observed). A methyl ester of β^3-*homo*-tryptophan was prepared by standard procedures. Dipeptides β^3-*h*TrpAlaOCH$_3$ and β^3-*h*TrpLeuOCH$_3$ were prepared by standard Boc-procedure in solution, O-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (TBTU) was used as coupling reagent. The protecting group Boc was removed in the presence of TFA and then methyl ester of β^3-*homo*-tryptophan and dipeptides were converted into free bases by the stirring in the mixture of AcOEt and saturated solution of $NaHCO_3$ for 30 min. An organic layer was separated, washed with brine and dried over $MgSO_4$, filtered out and the solvent was evaporated to obtain a yellowish solid. α-Amino aldehydes derived from D-amino or L-amino

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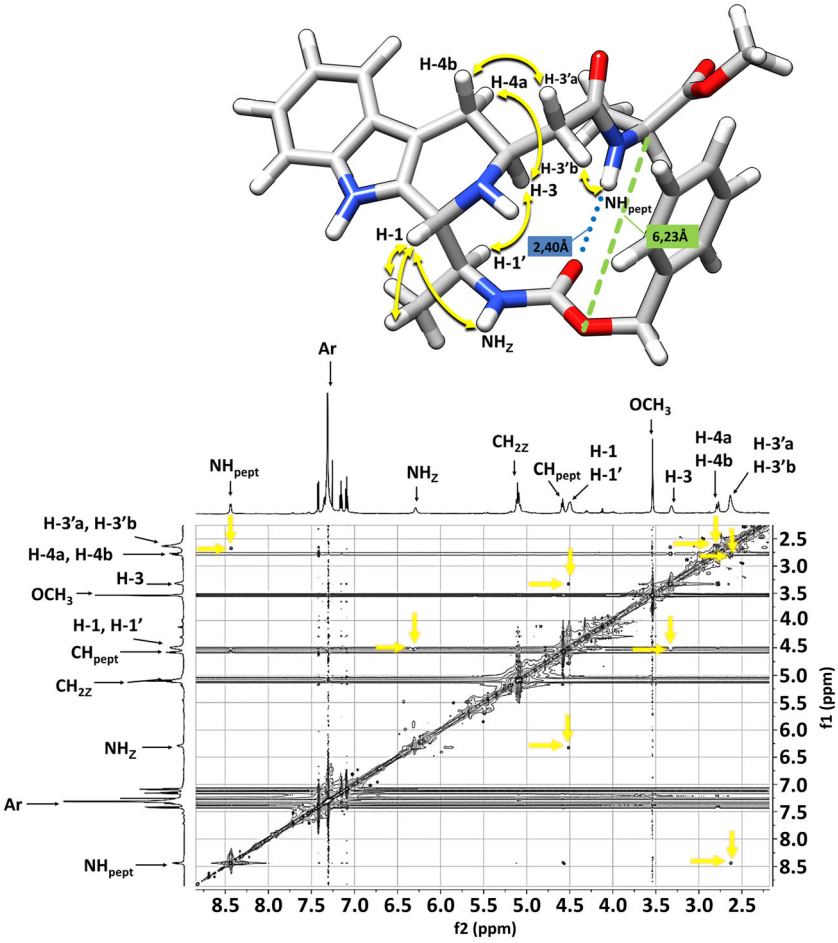

Figure 5. Schematic presentation of the NOE effect (yellow arrows) in the molecule adopting the β-turn structure stabilized by a hydrogen bond (blue dotted line) and a ROESY spectrum of the compound 6a.

acids with Z protection of amino group were prepared in good or excellent yields via the Fehrentz–Castro [36] procedure and used immediately without purification to avoid racemization.

Preparation of boc-β^3-homo-tryptophan

Synthesis of 3-(tert-butyloxycarbonylamino)-1-diazo-4-indolebutan-3-one

Triethylamine (0.830 g, 0.741 ml, 8.21 mmol) and then ethyl chloroformate (0.895 g, 0.792 ml, 8.21 mmol) were added to a solution of Boc-tryptophan (2.50 g, 8.21 mmol) in anhydrous tetrahydrofuran (40 ml) through a rubber septum under argon at 0 °C. After 15 min, a white precipitate of triethylammonium chloride appears, the stirring was stopped, the septum was replaced with a funnel and a freshly prepared diazomethane ethereal solution was added. The mixture was slowly stirred for 3 h (the reaction was monitored by TLC). The excess diazomethane was destroyed by an addition of a few drops of acetic acid and a saturated aqueous solution sodium bicarbonate (10 ml) was added carefully. The aqueous layer was separated and the organic layer was washed with a saturated aqueous sodium chloride (3 × 30 ml). The organic layer was dried over MgSO4, filtered off and the solvent was evaporated. The crude α-aminodiazoketone was used directly in the next step and solvents were used as received from commercial suppliers.

Homologation of 3-(tert-butyloxycarbonylamino)-1-diazo-4-indolebutan-3-one to Boc-β^3-homo-tryptophan

N-protected α-aminodiazoketone was dissolved in ethyl acetate (6 ml per 0,1 g of crude α-aminodiazoketone). Silver benzoate (4% mol) and silica gel (1 g per 0.1 g α-aminodiazoketone) were added, and the mixture (under the exclusion of light) was stirred on the rotary evaporator for 8 h at 45 °C (the reaction was monitored by TLC). The silica gel was filtered off and washed with AcOEt. The ethyl acetate was evaporated to yield Boc-β^3-*homo*-tryptophan yellow solid (yield 76%). $[\alpha]_D^{20} = -14.0$ (c 1, CH_3OH); HPLC (grad. 1) $t_R = 17{,}8$ min; Rf ($CHCl_3$–MeOH 9 : 1) = 0.28; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.40 (s, 9H, CH_3); 2.49–2.62 (m, 2H, CH_2); 2.93–3.05 (m, 2H, CH_2); 5.08 (m, 1H, C*H*); 7.01–7.32 (m, 4H, Ar); 7.64 (d, J = 7.75 Hz, 1H, Ar); 8.02 (bs, 1H, NH_{ind}); ESI *m/z*: 218.90 $[M–Boc]^+$, 340.85 $[M + Na]^+$, 356.85 $[M + K]^+$;

General procedure for Pictet-Spengler reaction

0.50 mmol of β^3-*h*TrpOCH$_3$ or β^3-*h*TrpAlaOCH$_3$ or β^3-*h*TrpAlaOCH$_3$ was dissolved in 10 ml CH_2Cl_2 and added to 0.60 mmol of amino aldehyde in 10 ml CH_2Cl_2 and mixture was cooled to −40 °C. 5 eq (0.192 ml) of TFA in 1.5 mL CH_2Cl_2 was added in three portions. The reaction mixtures were stirred for 5 h at -40 °C and then at RT overnight. The mixtures were diluted with CH_2Cl_2, and a saturated solution of $NaHCO_3$ was added to neutralize TFA. Organic layers were separated, extracted with saturated solution of $NaHCO_3$, after

then washed with brine and dried over $MgSO_4$, filtrated off and the solvent was evaporated to crude yellowish solids or oils. The ratios of the *cis/trans* isomers in the crude mixtures were determined by ^{1}HNMR (based on the integration of separated peaks of methyl ester groups). Crude mixtures were purified and *cis/trans* isomers were separated by flash chromatography ($CHCl_3$/AcOEt) or by semi-preparative HPLC to obtain 10–20 mg of pure isomers *cis* and *trans*.

Tcc(Z-L-Ala)CH_2COOCH_3 3a and 4a

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 60/40.

cis 3a: Yield: 28%; yellowish foam; $[\alpha]_D^{20} = -14.0$ (*c* 1, CH_3OH); t_R (grad. 1) = 15.81 min; t_R (grad. 2) = 11.07 min; R_f ($CHCl_3$ – Acetone 3:2) = 0.62; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.03 (d, J = 6.8 Hz, 3H, CH_3); 2.51 (m, 1H, H-4b); 2.65 (dd, J = 17.6 Hz, J = 6.2 Hz, 2H, H-3′a and H-3′b); 2.81 (m, 1H, H-4a); 3.34 (dq, J = 8.8 Hz, J = 4.7 Hz, 1H, H-3); 3.72 (s, 3H, OCH_3); 4.33 (bs, 1H, H-1′); 4.39 (s, 1H, H-1); 5.13 (q, J = 12.3 Hz, 2H, CH_2Ph); 5.49 (d, J = 8.3 Hz, 1H, NHZ); 7.05-7.52 (m, 10H, Ar, NH); 8.56 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 14.4 (CH_3); 27.9 (C-4); 40.4 (C-3′); 49.6 (C-1′); 50.5 (C-3); 51.6 (OCH_3); 56.4 (C-1); 66.9 (CH_2Ph); 94.9–127.9 (Ar); ROESY (700 MHz, CDCl3) H-1 (H-3, NHZ, CH_3); H-3 (H-1, H-4a, H-3′a and H-3′b), H-4a (H-3, H-4b, H-3′a and H-3′b), H-4b (H-4a, H-3′a and H-3′b), H-3′a and H-3′b (H-3, H-4a, H-3b), H-1′(CH_3), CH_3 (H-1′, NHZ), NHZ (H-1, CH_3); ESI *m/z*: 422 $[M+H]^+$, 444 $[M+Na]^+$; exact mass calculated for $C_{24}H_{27}N_3O_4$ $[M+H]^+$: 422. 2074, found: 422.2090.

trans 4a: Yield: 19%; yellowish foam; $[\alpha]_D^{20} = +79.3$ (*c* 1, CH_3OH); t_R (grad. 1) = 15.89 min; t_R (grad. 2) = 11.15 min; R_f ($CHCl_3$ – Acetone 3:2) = 0.40; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.32 (d, J = 6.5 Hz, 3H, CH_3); 2.51 (m, 1H, H-4b); 2.64 (dd, J = 15.9 Hz, J = 9.8 Hz, 2H, H-3′a and H-3′b); 3.02 (d, J = 4.9 Hz, 1H, H-4a); 3.69 (s, 3H, OCH_3); 3.79 (m, 1H, H-3); 4.10 (bs, 1H, H-1′); 4.33 (s, 1H, H-1); 4.97 (s, 2H, CH_2Ph); 5.60 (d, J = 8.2 Hz, 1H, NHZ); 7.06–7.50 (m, 10H, Ar, NH); 8.60 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 17.8 (CH_3); 26.6 (C-4); 37.3 (C-3′); 47.6 (C-3); 48.6 (C-1′); 51.5 (OCH_3); 53.4 (C-1); 66.2 (CH_2Ph), 95.4-128.2 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (NHZ, CH_3); H-3 (H-4a, H-4b, H-3′a and H-3′b), H-4a (H-3, H-4b, H-3′a and H-3′b), H-4b (H-3, H-4a, H-3′a and H-3′b), H-3′a and H-3′b (H-3, H-4a, H-3b), H-1′(CH_3, NHZ), CH_3 (H-1, H-1′, NHZ), NHZ (H-1, H-1′, CH_3); ESI *m/z*: 422 $[M+H]^+$, 444 $[M+Na]^+$; exact mass calculated for $C_{24}H_{27}N_3O_4$ $[M+H]^+$: 422. 2074, found: 422.2086.

Tcc(Z-L-Ile)CH_2COOCH_3 4b

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 0/100

trans 4b: Yield: 37%; yellowish foam; $[\alpha]_D^{20} = +69.3$ (*c* 1, CH_3OH); t_R (grad. 1) = 17.17 min; R_f ($CHCl_3$ – Acetone 3:2) = 0.72; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.99 (m, 6H, CH_3), 1.22 (m, 2H, $CH(CH_3)CH_2CH_3$), 1.98 (m, 1H, $CH(CH_3)CH_2CH_3$); 2.78 (dd, J = 17.4 Hz, J = 4.6 Hz, 1H, H-3′b); 2.92 (dd, J = 16.6 Hz, J = 6.6 Hz, 1H, H-4b); 3.09 (m, 1H, H-3′a); 3.24 (dt, J = 24.5 Hz, J = 12.3 Hz, 1H, H-4a); 3.69 (s, 3H, OCH_3); 4.21 (dt, J = 19.0 Hz, J = 9.4 Hz, 1H, H-1′); 4.30 (bs, 1H, H-3); 4.91 (d, J = 6.5 Hz, 1H, H-1); 5.02 (s, 2H, CH_2Ph); 6.35 (d, J = 9.4 Hz, 1H, NHZ); 6.99–7.54 (m, 10H, Ar, NH); 8.78 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 11.1 (CH_3); 17.1 (CH_3); 23.6 ($CH(CH_3)CH_2CH_3$); 24.1 (C-4); 34.4 (C-3′); 34.7 ($CH(CH_3)CH_2CH_3$); 48.6 (C-3); 52.7(C-1′); 53.4 (OCH_3); 56.6 (C-1′); 67.9 (CH_2Ph), 111.8–128.9 (Ar); ROESY (700 MHz, CDCl3) H-1 (H-1′, H-3′a, H-3′b, $CH(CH_3)CH_2CH_3$); H-3 (H-4a, H-4b, H-3′a, H-3′b), H-4a (H-3, H-4b, H-3′a and H-3′b), H-4b (H-3, H-4a, H-3′a and H-3′b), H-3′a (H-1, H-3, H-4a, H-3b), H-3′a (H-1, H-3, H-4a, H-3b), H-1′(H-1, H-3, NHZ, $CH(CH_3)CH_2CH_3$, $CH(CH_3)CH_2CH_3$, CH_3), $CH(CH_3)CH_2CH_3$ (H-1, H-1′, $CH(CH_3)CH_2CH_3$, CH_3, NH), NHZ (H-1′); NH ($CH(CH_3)CH_2CH_3$); ESI *m/z*: 464 $[M+H]^+$; exact mass calculated for $C_{27}H_{33}N_3O_4$ $[M+H]^+$: 464.2544, found: 464,2551.

Tcc(Z-L-Phe)CH_2COOCH_3 3c and 4c

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 47/53.

cis 3c: Yield: 23%; yellowish foam; $[\alpha]_D^{20} = -51.1$ (*c* 1, CH_3OH); t_R (grad. 1) = 16.20 min; R_f ($CHCl_3$ – Acetone 3:2) = 0.83; ^{1}H NMR (700 MHz, $CDCl_3$) δ 2.90 (m, 2H, H-4a and H-4b); 3.02 (m, 2H, CH_2Ph); 3.14 (d, J = 13.7 Hz, 2H, H-3′a and H-3′b); 3.58 (m, 1H, H-3); 3.73 (s, 3H, OCH_3); 4.76 (s, 1H, H-1′); 4.88 (q, J = 12.5 Hz, 2H, CH_2Ph_Z); 5.15 (s, 1H, H-1); 6.15 (s, 1H, NHZ); 6.91–7.43 (m, 14H, Ar); 8.78 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 24.5 (C-4), 35.7 (C-3′), 36.1 (CH_2Ph), 52.9 (C-3), 53.3 (OCH_3), 54.5 (C-1′), 59.1 (C-1), 67.2 (CH_2Ph_Z), 111.7–129.0 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-1′, H-3′a, H-3′b, $CH_2CH(CH_3)_2$); H-3 (H-4a, H-4b, H-3′a, H-3′b), H-4a (H-3, H-4b, H-3′a and H-3′b), H-4b (H-3, H-4a, H-3′a and H-3′b), H-3′a (H-1, H-3, H-4a, H-3b), H-3′a (H-1, H-3, H-4a, H-3b), H-1′(H-1, H-3, NHZ, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$, CH_3, NH), $CH_2CH(CH_3)_2$ (H-1, H-1′, $CH_2CH(CH_3)_2$, CH_3, NH), NHZ (H-1′); NH (H-1′, $CH_2CH(CH_3)_2$); ESI *m/z*: 498 $[M+H]^+$, 520 $[M+Na]^+$; exact mass calculated for $C_{30}H_{31}N_3O_4$ $[M+H]^+$: 498.2387, found: 498.2409.

trans 4c: Yield: 37%; yellowish foam; $[\alpha]_D^{20} = +69.3$ (*c* 1, CH_3OH); t_R (grad. 1) = 17.17 min; R_f ($CHCl_3$ – Acetone 3:2) = 0.72; ^{1}H NMR (700 MHz, $CDCl_3$) δ 2.83 (m, 1H, H-3′b); 2.99 (m, 2H, H-4b and CH_2Ph); 3.11 (m, 1H, H-3′a); 3.22 (m, 2H, H-4b and CH_2Ph); 3.63 (s, 3H, OCH_3); 4.32 (s, 1H, H-3); 4.47 (s, 1H, H-1); 4.82 (d, J = 12.4 Hz, 1H, H-1′); 4.94 (dd, J = 31.9 Hz, J = 11.0 Hz, 2H, CH_2Ph_Z); 6.16 (s, 1H, NHZ); 6.85–7.56 (m, 14H, Ar); 9.54 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 24.5 (C-4), 35.2 (C-3′), 37.5 (CH_2Ph), 48.4 (C-3), 53.25 (OCH_3), 54.2 (C-1), 55.4 (C-1′), 66.9 (CH_2Ph_Z), 111.8–128.9 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-4b and CH_2Ph, H-4a and CH_2Ph_{Phe}, NHZ); H-3 (H-4b and CH_2Ph, H-4b and CH_2Ph, H-3′b, H-3′a), H-4a and CH_2Ph (H-1, H-3, H-4b and CH_2Ph), H-4b and CH_2Ph (H-1, H-3, H-4a and CH_2Ph), H-3′a (H-3, H-3′b), H-3′b (H-3, H-3′a), H-1′ (H-4a and CH_2Ph, H-4b and CH_2Ph), NHZ (H-1); ESI-MS m/z: 498 $[M+H]^+$, 520 $[M+Na]^+$ exact mass calculated for $C_{30}H_{31}N_3O_4$ $[M+H]^+$: 498.2387, found: 498.2410.

Tcc(Z-L-Val)CH_2COOCH_3 4d

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 0/100.

trans 4d: Yield: 35%; yellowish foam; $[\alpha]_D^{20} = +22.6$ (*c* 1, CH_3OH); t_R (grad. 1) = 16.52 min; R_f ($CHCl_3$ – Acetone 3:2) = 0.63; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.06 (t, J = 7.2 Hz, 6H, CH_3); 1.85 (ddd, J = 15.8 Hz, J = 10.0 Hz, J = 4.6 Hz, 1H, $CH(CH_3)_2$); 2.42 (dd, J = 16.1 Hz, J = 4.0 Hz, 1H, H-4b); 2.52 (m, 1H, H-3′b); 2.67 (dt, J = 18.7 Hz, J = 9.3 Hz, 1H, H-4a); 3.05 (m, 1H, H-3′a); 3.69 (s, 3H, OCH_3); 3.81 (dd, J = 9.9 Hz, J = 2.4 Hz, 1H, H-3); 3.85 (dt, J = 9.7 Hz, J = 2.4 Hz, 1H, H-1′); 4.39 (s, 1H, H-1); 5.11 (m, 2H, CH_2Ph_Z); 5.43 (d, J = 9.9 Hz, 1H, NHZ); 6.86–7.47 (m, 9H, Ar); 8.30 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 19.4 (CH_3), 26.7 (C-4), 30.1 ($CH(CH_3)_2$), 36.9 (C-3′), 48.1 (C-3), 48.9 (C-1), 52.7 (OCH_3), 58.7 (C-1′), 66.9 (CH_2Ph_Z), 111.8–128.9 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-1′, H-3′a, $CH(CH_3)_2$, CH_3, NHZ); H-3 (H-3′a, H-3′b, H-4a, H-4b), H-4a (H-3, H-4b); H-4b (H-3, H-4b); H-3′a (H-1, H-3, H-3′b); H-3′b (H-3, H-3′a); H-1′ (H-1, $CH(CH_3)_2$, CH_3, NHZ, NH_{ind}); $CH(CH_3)_2$ (H-1, H-1′, CH_3),

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CH_3 (H-1, H-1′, $CH(CH_3)_2$); N*H*Z (H-1′); NH_{ind} (H-1′, Ar); ESI *m/z*: 450 $[M+H]^+$; exact mass calculated for $C_{26}H_{31}N_3O_4$ $[M+H]^+$: 450.2387, found: 450.2392.

Tcc(Z-D-Ala)CH_2COOCH_3 3e

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 100/0.

cis 3e: Yield: 39%; yellowish foam; $[\alpha]_D^{20} = +16.7$ (*c* 1, CH_3OH); t_R (grad. 1) = 15.84 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.65; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.60 (d, J = 6.2 Hz, 3H, CH_3); 3.04 (dd, J = 15.9 Hz, J = 4.0 Hz, 1H, H-4b); 3.11 (m, 1H, H-3′b); 3.14 (m, 1H, H-4a); 3.41 (dd, J = 17.6 Hz, J = 7.9 Hz, 1H, H-3′a); 3.78 (s, 3H, OCH_3); 3.79 (bs, 1H, H-3); 4.76 (bs, 1H, H-1′); 4.89 (s, 1H, H-1); 4.95 (d, J = 12.6 Hz, 2H, CH_2Ph); 5.35 (s, 1H, N*H*Z); 6.84-7.67 (m, 10H, Ar, N*H*); 9.23 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 16.7 (CH_3); 24.3 (C-4); 35.7 (C-3′); 46.6 (C-1′); 52.7 (OCH_3); 54.1 (C-3); 60.1 (C-1); 67.8 (CH_2Ph); 111.9–128.4 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, CH_3); H-3 (H-1, H-4a, H-3′a, H-3′b), H-4a (H-3, H-4b, H-3′a, H-3′b), H-4b (H-4a, H-3′a, H-3′b), H-3′a (H-3, H-4a, H-3b), H-3′b (H-3, H-4a, H-3b), H-1′(CH_3), CH_3 (H-1, H-1′); ESI *m/z*: 422 $[M+H]^+$, 444 $[M+Na]^+$; exact mass calculated for $C_{24}H_{27}N_3O_4$ $[M+H]^+$: 422.2074 found: 422.2079.

Tcc(Z-D-Leu)CH_2COOCH_3 3f

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 100/0.

cis 3f: Yield: 62%; yellowish foam; $[\alpha]_D^{20} = +18.7$ (*c* 1, CH_3OH); t_R (grad. 1) = 17.29 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.78; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.95 (dd, J = 13.6 Hz, J = 6.2 Hz, 6H, CH_3); 1.48 (m, 1H, $CH_2CH(CH_3)_2$); 1.74 (m, 1H, $CH_2CH(CH_3)_2$); 1.92 (m, 1H, $CH_2CH(CH_3)_2$); 2.89 (d, J = 5.3 Hz, 2H, H-4a and H-4b); 2.92 (d, J = 5.0 Hz, 1H, H-3′b); 3.15 (m, 1H, H-3′a); 3.64 (s, 1H, H-3); 3.75 (s, 3H, OCH_3); 4.56 (m, 1H, H-1′); 4.78 (s, 1H, H-1); 4.91 (d, J = 4.2 Hz, 2H, CH_2Ph); 6.72 (d, J = 8.7 Hz, 1H, N*H*Z); 6.94–7.40 (m, 9H, Ar); 9.23 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 21.9 (CH_3), 24.4 (C-4), 24.9 ($CH_2CH(CH_3)_2$), 35.8 (C-3′), 39.3 ($CH_2CH(CH_3)_2$), 49.10 (C-1′), 51.7 (OCH_3), 54.4 (C-3), 60.6 (C-1), 67.6 (CH_2Ph), 112.6–129.0 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, H-1′, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$); H-3 (H-1, H-4a and H-4b, H-3′a, H-3′b), H-4a and H-4b (H-3, H-3′a, H-3′b), H-3′a (H-3, H-4a and H-3b), H-3′b (H-3, H-4a and H-3b), H-1′(H-1, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$, N*H*Z), $CH_2CH(CH_3)_2$ (H-1, H-1′, N*H*Z, $CH_2CH(CH_3)_2$, CH_3), N*H*Z (H-1′, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$, CH_3); ESI *m/z*: 464 $[M+H]^+$, exact mass calculated for $C_{27}H_{33}N_3O_4$ $[M+H]^+$: 464.2544 found: 464.2551.

Tcc(Z-D-Phe)CH_2COOCH_3 3g

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 100/0.

cis 3g: Yield: 47%; yellowish foam; $[\alpha]_D^{20} = +66.2$ (*c* 1, CH_3OH); t_R (grad. 1) = 16.51 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.83; ^{1}H NMR (700 MHz, $CDCl_3$) δ 2.94 (dd, J = 17.9 Hz, J = 3.5 Hz, 1H, H-3′b), 2.99 (dd, J = 16.6 Hz, J = 4.2 Hz, 1H,H-4b), 3.10 (m, 1H, H-4a); 3.14 (dd, J = 14.1 Hz, J = 7.3 Hz, 1H, CH_2Ph); 3.28 (dd, J = 13.9 Hz, J = 8.8 Hz, 1H, CH_2Ph); 3.39 (dd, J = 18.0 Hz, J = 9.7 Hz, 1H, H-3′a); 3.71 (m, 1H, H-3); 3.84 (s, 3H, OCH_3); 4.78 (s, 1H, H-1); 4.85 (m, 1H, H-1′); 4.89 (dt, J = 20.8 Hz, J = 10.4 Hz, 2H, CH_2Ph_Z); 5.09 (s, 1H, N*H*Z); 6.93–7.48 (m, 14H, Ar); 9.11 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 24.7 (C-4), 35.5 (C-3′), 36.7 (CH_2Ph), 52.2 (C-1′), 53.7 (OCH_3), 53.9 (C-3), 58.1 (C-1), 66.9 (CH_2Ph_Z), 111.9–129.0 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, CH_2Phe); H-3 (H-1, H-4b, H-3′a, H-3′b), H-4a (H-4b, H-3′a), H-4b (H-3, H-4a), H-3′a (H-3, H-4a, H-3′b), H-3′b (H-3, H-3′a), H-1′ (CH_2Ph), CH_2Ph (H-1, H-1′); ESI *m/z*: 498 $[M+H]^+$, 520 $[M+Na]^+$ exact mass calculated for $C_{30}H_{31}N_3O_4$ $[M+H]^+$: 498.2387 found: 498.2407.

Tcc(Z-D-Val)CH_2COOCH_3 3h

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 100/0.

cis 3h: Yield: 48%; yellowish foam; $[\alpha]_D^{20} = +25.6$ (*c* 1, CH_3OH); t_R (grad. 1) = 16.15 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.81; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.11 (d, J = 6.3 Hz, 3H, CH_3); 1.22 (d, J = 6.6 Hz, 3H, CH_3); 2.19 (m, 1H, $CH(CH_3)_2$); 2.95 (dd, J = 18.0 Hz, J = 2.3 Hz, 1H, H-3′b); 3.03 (dd, J = 15.8 Hz, J = 3.9Hz, 1H, H-4b); 3.21 (m, 1H, H-4a); 3.49 (dd, J = 18.1 Hz, J = 10.4 Hz, 1H, H-3′a); 3.76 (s, 1H, H-3); 3.81 (s, 3H, OCH_3); 4.21 (dd, J = 10.3 Hz, J = 8.0 Hz, 1H, H-1′); 4.94 (s, 2H, CH_2Ph); 4.96 (s, 1H, H-1); 5.09 (s, 1H, N*H*Z); 6.88–7.51 (m, 9H, Ar); 8.18 (bd, J = 37.3 Hz, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 18.8 (CH_3), 24.5 (C-4), 29.0 ($CH(CH_3)_2$), 35.2 (C-3′), 53.2 (OCH_3), 54.5 (C-3), 56.6 (C-1′), 57.2 (C-1), 67.5 (CH_2Ph), 111.9–128.2 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, H-1′, $CH(CH_3)_2$); H-3 (H-1, H-4b, H-3′a, H-3′b), H-4a (H-4b), H-4b (H-3, H-4a, H-3′b), H-3′a (H-3, H-3′b), H-3′b (H-3, H-4b, H-3′a), H-1′ (H-1, $CH(CH_3)_2$, CH_3), $CH(CH_3)_2$ (H-1, H-1′, CH_3); ESI *m/z*: 450 $[M+H]^+$, exact mass calculated for $C_{26}H_{31}N_3O_4$ $[M+H]^+$: 450.2387, found: 450.2385.

Tcc(Z-L-Ala)CH_2-Ala-$COOCH_3$ 5a and 6a

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 35/65.

cis 5a: Yield: 17%; yellowish foam; $[\alpha]_D^{20} = -9.9$ (*c* 1, CH_3OH); t_R (grad. 1) = 15.64 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.51; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.34 (q, J = 6.1 Hz, 3H, CH_{3pept}); 1.54 (d, J = 6.3 Hz, 3H, CH_3); 2.41 (m, 1H, H-3′b); 2.53 (m, 3H, H-4b and H-3′a); 3.16 (dd, J = 18.0 Hz, J = 5.6 Hz, 1H, H-4a); 3.58 (s, 1H, H-3); 3.67 (s, 3H, OCH_3); 4.42 (m, 1H, N*H*Z); 5.52 (m, 1H, CH_{pept}); 4.66 (s, 1H, H-1′); 4.90 (d, J = 9.5 Hz, 1H, H-1); 5.02 (dd, J = 25.7 Hz, J = 11.9 Hz, 2H, CH_2Ph); 6.72 (s, 1H, NH_{pept}); 7.00–7.51 (m, 9H, Ar); 8.85 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 18.4 (CH_{3pept}), 20.2 (CH_3), 23.9 (C-4), 38.2 (C-3′), 46.3 (C-1′), 52.5 (C-3), 52.6 (OCH_3), 53.7 (C-1′), 54.3 (CH_{pept}), 67.1 (CH_2Ph), 111.3–128.6 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, CH_3); H-3 (H-1, H-3′a and H-4b, H-3′b), H-4a (H-4b and H-3′a, H-3′b), H-4b and H-3′a (H-3, H-4a, H-3′b), H-3′b (H-3, H-3′a and H-4b), H-1′ (H-3, CH_3, N*H*Z), CH_{pept} (CH_{3pept}), CH_3 (H-1′, N*H*Z), CH_{3pept} (NH_{pept}), N*H*Z (H-1′, CH_3), NH_{pept}(CH_{3pept}); ESI *m/z*: 493 $[M+H]^+$; exact mass calculated for $C_{27}H_{32}N_4O_5$ $[M+H]^+$: 493.2445 found: 493.2468.

trans 6a: Yield: 27%; yellowish foam; $[\alpha]_D^{20} = +18.1$ (*c* 1, CH_3OH); t_R (grad. 1) = 15.10 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.71; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.36 (q, J = 6.5 Hz, 3H, CH_{3pept}); 1.42 (d, J = 6.0 Hz, 3H, CH_3); 2.59 (m, 1H, H-4b); 2.63 (d, J = 7.1 Hz, 1H, H-4a); 2.78 (m, 2H, H-3′a and H-3′b); 3.32 (bs, 1H, H-3); 3.56 (s, 3H, OCH_3); 4.52 (dd, J = 7.5 Hz, 2H, CH_{pept} and H-1′); 4.57 (m, 1H, H-1); 5.10 (td, J = 25.6 Hz, J = 7.5 Hz, 2H, CH_2Ph); 6.27 (s, 1H, N*H*Z); 7.07–7.49 (m, 9H, Ar); 8.44 (s, 1H, NH_{pept}); 8.85 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 15.1 (CH_{3pept}), 17.9 (CH_3), 27.9 (C-4), 41.5 (C-3′), 48.4 (C-1), 48.8 (C-1′), 52.2 (C-3), 52.5 (OCH_3), 57.7 (CH_{pept}), 67.2 (CH_2Ph), 111.2–130.9 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (N*H*Z, CH_3); H-3 (H-4a, H-1′, H-3′a and H-3′b), H-4a (H-3, H-3′a and H-3′b), H-4b (H-3, H-3′a and H-3′b), H-3′a and H-3′b (H-3, H-4a, H-4b), H-1′ (H-3), CH_{pept} (CH_{3pept}), CH_3 (H-1), CH_{3pept} (NH_{pept}), N*H*Z (H-1), NH_{pept}

(CH_{3pept}); ESI *m/z*: 493 $[M+H]^+$; exact mass calculated for $C_{27}H_{32}N_4O_5$ $[M+H]^+$: 493.2445 found: 493.2467.

Tcc(Z-L-Ile)CH_2-Ala-$COOCH_3$ 6b

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 0/100.

trans 6b: Yield: 37%; yellowish foam; $[\alpha]_D^{20} = +8.4$ (*c* 1, CH_3OH); t_R (grad. 1) = 17.97 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.36; ^1H NMR (700 MHz, $CDCl_3$) δ 0.96 (m, 6H, CH_3); 1.02 (d, J = 6.7 Hz, 3H, CH_{3pept}); 1.24 (m, 1H, $CH(CH_3)CH_2CH_3$); 1.66 (m, 2H, $CH(CH_3)CH_2CH_3$); 2.37 (m, 1H, H-4b); 2.42 (m, 2H, H-3′a and H-3′b); 2.94 (dd, J = 15.2 Hz, J = 4.2 Hz, 1H, H-4a); 3.66 (dd, J = 9.6 Hz, J = 4.5 Hz, 1H, H-3); 3.70 (s, 3H, OCH_3); 3.90 (d, J = 9.9 Hz, J = 2.2 Hz, 1H, H-1′); 4.36 (s, 1H, CH_{pept}); 4.71 (m, 1H, H-1); 4.85 (dd, J = 66.7 Hz, J = 12.7 Hz, 2H, CH_2Ph); 5.57 (d, J = 5.7 Hz, 1H, NHZ); 6.90 (d, J = 7.5 Hz, 1H, NH_{pept}); 7.02–7.43 (m, 9H, Ar); 8.42 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 16.1 (CH_3), 21.8 (CH_{3pept}), 24.9 ($CH(CH_3)CH_2CH_3$), 25.8 ($CH(CH_3)CH_2CH_3$), 28.2 (C-4), 40.5 (C-3′), 50.2 (CH_{pept}), 50.6 (C-1), 52.6 (OCH_3), 58.1 (C-1′), 69.8 (CH_2Ph), 111.4–128.5 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (CH_{3pept}, NHZ); H-3 (H-4a, H-4b, H-3′a and H-3′b), H-4a (H-3, H-4b, H-3′a and H-3′b), H-4b (H-3, H-4a, H-3′a and H-3′b), H-3′a and H-3′b (H-3, H-4a, H-4b, CH_{pept}), H-1′ (CH_3, $CH(CH_3)CH_2CH_3$, $CH(CH_3)CH_2CH_3$, NHZ), CH_{pept}(CH_{3pept}), $CH(CH_3)CH_2CH_3$ (H-1′, $CH(CH_3)CH_2CH_3$, CH_3, NHZ), NH_{pept} (CH_{pept}), NHZ (H-1, H-1′, $CH(CH_3)CH_2CH_3$); ESI *m/z*: 535 $[M+H]^+$, 557 $[M+Na]^+$; exact mass calculated for $C_{30}H_{38}N_4O_5$ $[M+H]^+$: 535.2915, found: 535.2941.

Tcc(Z-L-Phe)CH_2COOCH_3 5c and 6c

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 26/73.

cis 5c: Yield: 39%; yellowish foam; $[\alpha]_D^{20} = -12.9$ (*c* 1, CH_3OH); t_R (grad. 1) = 17.19 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.55; ^1H NMR (700 MHz, $CDCl_3$) δ 0.98 (m, 3H, CH_3); 1.63 (d, J = 8.7 Hz, 2H, CH_2Ph); 3.13 (d, J = 15.2 Hz, 1H, H-3′b); 3.20 (d, J = 8.1 Hz, 2H, H-4a and H-4b); 3.25 (m, 1H, H-3′a); 3.59 (s, 1H, H-3); 3.75 (s, 3H, OCH_3); 3.90 (s, 1H, H-1′); 4.57 (s, 1H, CH_{pept}); 4.79 (dd, J = 47.7 Hz, 12.7 Hz, 2H, CH_2Ph_Z); 4.89 (bs, 1H, H-1); 5.30 (bs, 1H, NHZ); 6.78–7.64 (m, 15H, Ar and NH_{pept}); 8.86 (s, 1H, NH_{ind}); 8.93 (s, 1H, NH); ^{13}C NMR (700 MHz, $CDCl_3$) δ 21.3 (CH_3), 25.2 (C-4), 37.4 (C-3′), 38.4 (C-3), 39.9 (CH_2Ph), 52.2 (CH_{pept}), 53.1 (C-1), 53.2 (OCH_3), 55.1 (C-1′), 67.2 (CH_2Ph_Z), 111.8–129.1 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, H-4a and H-4b, H-3′a, NH); H-3 (H-1, H-4a and H-4b, H-3′a, H-3′b), H-4a and H-4b (H-1, H-3′b), H-3′a (H-1, H-3′b), H-3′b (H-1, H-4a and H-4b, H-1′, H-3′a), H-1′ (H-1, H-3′b, CH_2Ph, NH), CH_{pept} (CH_3, NH_{pept}), CH_2Ph (H-1′), NH_{pept} (CH_{pept}), NH (H-1, H-1′); ESI *m/z*: 569 $[M+H]^+$, 591 $[M+Na]^+$; exact mass calculated for $C_{33}H_{36}N_4O_5$ $[M+H]^+$: 569.2758 found: 569.2764.

trans 6c: Yield: 23%; yellowish foam; $[\alpha]_D^{20} = +21.5$ (*c* 1, CH_3OH); t_R (grad. 1) = 17.73 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.43; ^1H NMR (700 MHz, $CDCl_3$) δ 0.84 (m, 3H, CH_3); 1.43 (m, 2H, CH_2Ph); 2.87 (m, 1H, H-4b); 3.03 (m, 2H, H-3′a and H-3′b); 3.19 (m, 1H, H-4a); 3.51 (m, 1H, H-3); 3.59 (s, 3H, OCH_3); 4.21 (s, 1H, H-1′); 4.34 (bs, 1H, H-1); 4.49 (m, 1H, CH_{pept}); 4.87 (m, 1H, NHZ); 5.02 (dd, J = 24.0 Hz, 9.2 Hz, 2H, CH_2Ph_Z); 6.87–7.51 (m, 14H, Ar); 7.79 (s, 1H, NH_{pept}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 22.3 (CH_3), 24.6 (C-3), 37.3 (C-4), 39.9 (CH_2Ph), 49.4 (C-1′), 51.7 (CH_{pept}), 51.9 (OCH_3), 53.8 (C-1), 65.8 (C-3), 67.2 (CH_2Ph_Z), 111.7–128.9 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-1′, H-3′a and H-3′b); H-3 (H-4a, H-3′a and H-3′b), H-4a (H-3, H-4b, H-3′a and H-3′b), H-4b (H-4a, H-3′a and H-3′b), H-3′a and H-3′b (H-1, H-3, H-4a, H-4b), H-1′ (H-1, CH_2Ph, NHZ), CH_{pept} (CH_3, NH_{pept}), CH_2Phe (H-1′), NH_{pept} (CH_{pept}); ESI-MS m/z: 569 $[M+H]^+$, 591 $[M+Na]^+$; exact mass calculated for $C_{33}H_{36}N_4O_5$ $[M+H]^+$: 569.2758 found: 569.2767.

Tcc(Z-L-Val)CH_2-Ala-$COOCH_3$ 6d

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 0/100.

trans 6d: Yield: 35%; yellowish foam; $[\alpha]_D^{20} = +3.9$ (*c* 1, CH_3OH); t_R (grad. 1) = 16.43 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.38; ^1H NMR (700 MHz, $CDCl_3$) δ 1.09 (t, J = 6.6 Hz, 3H, CH_3); 1.14 (d, J = 6.6 Hz, 3H, CH_3); 1.38 (d, J = 7.1 Hz, CH_{3pept}); 1.95 (m, 1H, $CH(CH_3)_2$); 2.52 (d, J = 8.1 Hz, 1H, H-4b); 2.60 (m, 2H, H-3′a and H-3′b); 2.75 (m, 1H, H-4a); 3.44 (bs, 1H, H-3); 3.67 (s, 3H, OCH_3); 3.87 (m, 1H, H-1); 4.50 (s, 1H, H-1′); 4.60 (dd, J = 14.3 Hz, J = 7.1 Hz, 1H, CH_{pept}); 4.89 (dd, J = 55.0 Hz, J = 12.5 Hz, 2H, CH_2Ph); 5.11 (s, 1H, NHZ); 6.88–7.45 (m, 9H, Ar); 7.48 (s, 1H, NH_{pept}); 8.35 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 18.30 (CH_{3pept}), 20.0 (CH_3), 27.5 (C-4), 29.7 ($CH(CH_3)_2$), 42.1 (C-3′), 47.9 (CH_{pept}), 51.1 (C-3), 52.4 (OCH_3), 54.2 (C-1′), 57.9 (C-1), 67.7 (CH_2Ph), 111.4-128.4 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-1′, $CH(CH_3)_2$, CH_3), H-3 (H-4a , H-3′a and H-3′b), H-4a (H-3, H-4b, H-3′a and H-3′b), H-4b (H-4a, H-3′a and H-3′b), H-3′a and H-3′b (H-3, H-4a, H-4b), H-1′ (H-1, $CH(CH_3)_2$, CH_3); $CH(CH_3)_2$ (H-1, H-1′, CH_3, NHZ), CH_3 (H-1, H-1′, $CH(CH_3)_2$), CH_{pept}(CH_{3pept}, NH_{pept}), NH_{pept} (CH_{pept}), NHZ (H-1′, $CH(CH_3)_2$); ESI *m/z*: 521 $[M+H]^+$, 543 $[M+Na]^+$; exact mass calculated for $C_{29}H_{36}N_4O_5$ $[M+H]^+$: 521.2772 found: 521.2781.

Tcc(Z-D-Ala)CH_2-Ala-$COOCH_3$ 5e

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 100/0.

cis 5e: Yield: 63%; yellowish foam; $[\alpha]_D^{20} = +15.6$ (*c* 1, CH_3OH); t_R (grad. 1) = 15.63 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.50; ^1H NMR (700 MHz, $CDCl_3$) δ 1.41 (m, 6H, CH_{3pept} and CH_3); 2.65 (d, J = 11.8 Hz, 1H, H-4b); 2.74 (m, 3H, H-3′a and H-3′b); 2.85 (d, J = 12.9 Hz, 1H, H-4a); 3.50 (m, 1H, H-3); 3.73 (s, 3H, OCH_3); 4.43 (bs, 1H, H-1); 4.59 (m, 2H, H-1′ and CH_{pept}); 4.92 (s, 2H, CH_2Ph); 5.13 (m, 1H, NHZ); 7.04–7.45 (m, 9H, Ar); 7.81 (s, 1H, NH_{pept}); 8.58 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 17.7 (CH_{3pept}), 21.8 (CH_3), 26.8 (C-4), 42.1 (C-3′), 47.5 (C-1′), 52.3 (C-3), 52.5 (OCH_3), 58.2 (C-1′), 66.4 (CH_{pept}), 66.7 (CH_2Ph), 111.4–128.2 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, CH_{3pept} and CH_3); H-3 (H-1, H-4a, H-4b), H-4a (H-3, H-4b, H-3′a and H-3′b), H-4b (H-3, H-4a, H-3′a and H-3′b), H-3′a and H-3′b (H-4a, H-4b), H-1′ and CH_{pept} (CH_{3pept} and CH_3, NH_{ind}), NH_{pept} (CH_{3pept} and CH_3); ESI *m/z*: 493 $[M+H]^+$; exact mass calculated for $C_{27}H_{32}N_4O_5$ $[M+H]^+$: 493.2445 found: 493.2458.

Tcc(Z-D-Leu)CH_2-Ala-$COOCH_3$ 5f

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 100/0.

cis 5f: Yield: 65%; yellowish foam; $[\alpha]_D^{20} = +123.2$ (*c* 1, CH_3OH); t_R (grad. 1) = 17.03 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.56; ^1H NMR (700 MHz, $CDCl_3$) δ 0.98 (dt, J = 16.7 Hz, J = 8,3 Hz, 6H, CH_3); 1.51 (d, J = 7.3 Hz, 3H, CH_{3pept}); 1.79 (m, 2H, $CH_2CH(CH_3)_2$); 1.95 (m, 1H, $CH_2CH(CH_3)_2$); 2.93 (d, J = 14.5 Hz, 1H, H-3′b); 3.10 (m, 1H, H-4b); 3.14 (m, 1H, H-4a), 3.49 (m, 1H, H-3′a); 3.76 (s, 3H, OCH_3); 3.87 (bs, 1H, H-3); 4.54 (m, 1H, CH_{pept}); 4.72 (t, J = 10.5 Hz, 1H, H-1′); 4.76 (s, 1H, H-1); 4.92 (dd, J = 33.1 Hz, J = 12.7 Hz, 2H, CH_2Ph); 5.15 (s, 1H, NHZ); 6.92–7.45 (m, 10H, Ar and NH_{ind}); 8.13 (s, 1H, NH_{pept}); 8.93 (s, 1H, NH); ^{13}C NMR (700 MHz, $CDCl_3$) δ 16.9 (CH_{3pept}), 21.4 (CH_3), 24.9 ($CH_2CH(CH_3)_2$ and C-4), 37.7 (C-3′), 39.9 ($CH_2CH(CH_3)_2$), 48.9 (C-1′), 49.0 (CH_{pept}), 53.2 (OCH_3), 55.0 (C-3), 60.0 (C-1), 67.6 (CH_2Ph), 111.8–128.6 (Ar); ROESY (700 MHz,

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$CDCl_3$) H-1 (H-3, H-1′, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$, N*H*); H-3 (H-1, H-4b, H-3′a, N*H*), H-4a (H-4b), H-4b (H-3a, H-3, H-3′a), H-3′a (H-3, H-4b, H-3′b), H-3′b (H-3′a), H-1′ (H-1, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$), $CH_{pept}(CH_{3pept})$, $CH_2CH(CH_3)_2$ (H-1, H-1′, $CH_2CH(CH_3)_2$), $CH_2CH(CH_3)_2$ (H-1, H-1′, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$, CH_3, N*H*), NH_{pept} (CH_{pept}), N*H* (H-1, H-3, $CH_2CH(CH_3)_2$); ESI *m/z*: 535 $[M+H]^+$; exact mass calculated for $C_{30}H_{38}N_4O_5$ $[M+H]^+$: 535.2915, found: 535.2914.

Tcc(Z-D-Phe)CH$_2$COOCH$_3$ 5g

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 100/0.

cis 5g: Yield: 39%; yellowish foam; $[\alpha]_D^{20} = +58.5$ (*c* 1, CH_3OH); t_R (grad. 1) = 16.76 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.54; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.42 (s, 3H, CH_3); 1.54 (d, J = 7.0 Hz, 2H, CH_2Ph); 3.10 (s, 1H, H-3′b); 3.17 (d, J = 15.2 Hz, 2H, H-4a and H-4b); 3.27 (d, J = 9.7 Hz, 1H, H-3′a); 3.54 (m, 1H, H-3); 3.77 (s, 3H, OCH_3); 4.44 (s, 1H, H-1); 4.54 (bs, 1H, H-1′); 4.88 (s, 1H, CH_{pept}); 5.09 (dd, J = 40.2 Hz, J = 13.6 Hz, 2H, CH_2Ph_Z); 5.44 (s, 1H, N*HZ*);7.07–7.49 (m, 14H, Ar); ^{13}C NMR (700 MHz, $CDCl_3$) δ 16.8 (CH_3), 17.1 (CH_2Ph); 24.9 (C-3′), 37.1 (C-4), 48.8 (C-1), 49.3 (C-1′), 53.2 (OCH_3), 58.7 (CH_{pept}), 64.3 (C-3), 67.1 (CH_2Ph_Z), 111.5–129.1 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3); H-3 (H-1, H-4a and H-4b, H-3′a, H-3′b), H-4a and H-4b (H-3, H-3′a, H-3b); H-3′a (H-3, H-4a and H-4b, H-3′b), H-3′b (H-3, H-4a and H-4b, H-3′a), H-1′ (CH_2Ph), CH_{pept} (CH_3), CH_2Ph (H-1′), CH_3 (CH_{pept}); ESI *m/z*: 569 $[M+H]^+$, 591 $[M+Na]^+$; exact mass calculated for $C_{33}H_{36}N_4O_5$ $[M+H]^+$: 569.2758 found: 569.2783.

Tcc(Z-D-Val)CH$_2$-Ala-COOCH$_3$ 5h

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 100/0.

cis 5h: Yield: 39%; yellowish foam; $[\alpha]_D^{20} = +8.4$ (*c* 1, CH_3OH); t_R (grad. 1) = 15.55 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.52; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.08 (d, J = 6.4 Hz, 3H, CH_3); 1.23 (d, J = 6.5 Hz, 3H, CH_3); 1.48 (d, J = 7.4 Hz, 3H, CH_{3pept}); 2.13 (m, 1H, $CH(CH_3)_2$); 2.80 (d, J = 13.8 Hz,1H, H-3′b); 3.11 (dd, J = 13.7 Hz, J = 9.1 Hz, 1H, H-4a and H-4b); 3.42 (dd, J = 15.1 Hz, J = 11.8 Hz, 1H, H-3′a); 3.77 (s, 3H, OCH_3); 3.90 (s, 1H, H-3); 4.22 (m, 1H, H-1′); 4.53 (m, 1H, CH_{pept}); 4.94 (s, 2H, CH_2Ph); 4.99 (s, 1H, H-1); 5.11 (s, 1H, N*HZ*); 6.98–7.47 (m, 10H, Ar and NH_{ind}); 8.30 (bs, 1H, NH_{pept}); 8.85 (s, 1H, N*H*); ^{13}C NMR (700 MHz, $CDCl_3$) δ 16.6 (CH_{3pept}), 20.0 (CH_3), 20.6 (CH_3), 25.2 (C-4), 28.7 ($CH(CH_3)_2$), 38.1 (C-3′), 48.9 (CH_{pept}), 53.0 (OCH_3), 55.7 (C-3), 56.8 (C-1), 57.0 (C-1′), 66.6 (CH_2Ph), 111.8–128.2 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, H-1′, N*H*); H-3 (H-1, H-4a and H-4b, H-3′a, H-3′b), H-4a and H-4b (H-3, H-3′a, H-3′b), H-3′a (H-3, H-4a and H-4b, H-3′b), H-3′b (H-3, H-4a and H-4b, H-3′a), H-1′ (H-1, $CH(CH_3)_2$, CH_3, N*H*), $CH(CH_3)_2$ (H-1′, CH_3), $CH_{pept}(CH_{3pept}$, NH_{pept}), NH_{pept} (CH_{pept}), N*H* (H-1, H-1′); ESI *m/z*: 521 $[M+H]^+$, 543 $[M+Na]^+$; exact mass calculated for $C_{29}H_{36}N_4O_5$ $[M+H]^+$: 521.2772 found: 521.2770.

Tcc(Z-L-Ala)CH$_2$-Leu-COOCH$_3$ 7a and 8a

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 39/61.

cis 7a: Yield: 18%; yellowish foam; $[\alpha]_D^{20} = -29.9$ (*c* 1, CH_3OH); t_R (grad. 1) = 17.15 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.63; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.78–0.99 (m, 6H, CH_{3pept}); 1.43 (bs, 3H, CH_3); 1.55 (bs, 2H, $CH_2CH(CH_3)_2$); 1.76 (bs, 1H, $CH_2CH(CH_3)_2$); 2.41 (d, J = 13.0 Hz, 1H, H-4b); 2.51 (m, 2H, H-3′a and H-3′b); 2.60 (m, 1H, H-4a); 3.12 (bs, 1H, H-3); 3.73 (s, 3H, OCH_3); 4.15 (s, 1H, H-1′); 4.43 (s, 1H, CH_{pept}); 4.91 (d, J = 9.4 Hz, 1H, H-1); 5.01 (dd, J = 20.9 Hz, J = 12.0 Hz, 2H, CH_2Ph); 5.14 (m, 1H, N*HZ*); 7.01–7.60 (m, 9H, Ar); ^{13}C NMR (700 MHz, $CDCl_3$) δ 18.5 (CH_3), 21.9 (CH_{3pept}), 23.8 (C-4), 24.8 ($CH_2CH(CH_3)_2$), 38.2 (C-3′), 39.8 ($CH_2CH(CH_3)_2$), 46.2 (C-1), 48.1 (C-1′), 51.1 (CH_{pept}), 52.4 (C-3), 52.6 (OCH_3), 67.8 (CH_2Ph), 111.4–130.5 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, CH3), H-3 (H-1, H-4a, H-3′a and H-3′b), H-4a (H-3, H-4b, H-3′a and H-3′b), H-4b (H-4a, H-3′a and H-3′b), H-3′a and H-3′b (H-3, H-4a, H-4b), H-1′ (CH_3), CH_3 (H-1′), CH_{pept} ($CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$), $CH_2CH(CH_3)_2$ (CH_{pept}, $CH_2CH(CH_3)_2$, CH_{3pept}), $CH_2CH(CH_3)_2$ (CH_{pept}, $CH_2CH(CH_3)_2$, CH_{3pept}), CH_{3pept} ($CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$); ESI *m/z*: 535 $[M+H]^+$, 557 $[M+Na]^+$; exact mass calculated for $C_{30}H_{38}N_4O_5$ $[M+H]^+$: 535.2915 found: 535.2929.

trans 8a: Yield: 27%; yellowish foam; $[\alpha]_D^{20} = +27.9$(*c* 1, CH_3OH); t_R (grad. 1) = 17.23 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.32; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.89 (m, 6H, CH_{3pept}); 1.13 (m, 3H, CH_3); 1.63 (dt, J = 14.1 Hz, J = 6.4 Hz, 2H, $CH_2CH(CH_3)_2$); 1.70 (dq, J = 20.1 Hz, J = 6.6 Hz, 1H, $CH_2CH(CH_3)_2$); 2.62 (m, 1H, H-4b); 2.71 (m, 1H, H-4a); 2.81 (d, J = 17.3 Hz, 2H, H-3′b and H-3′a); 3.39 (bs, 1H, H-3); 3.55 (s, 3H, OCH_3); 3.72 (m, 1H, H-1); 3.85 (s, 1H, H-1′); 4.50 (s, 1H, N*HZ*); 4.61 (dd, J = 14.3 Hz, J = 7.0 Hz, 1H, CH_{pept}); 5.06 (t, J = 12.3 Hz, 2H, CH_2Ph); 6.24 (s, 1H, N*H*); 7.03–7.49 (m, 9*H*, Ar); 8.23 (s, 1H, NH_{pept}); 8.97 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 14.8 (CH_3), 21.2 (CH_{3pept}), 24.8 ($CH_2CH(CH_3)_2$), 27.6 (C-4), 38.5 (C-3′), 40.7 ($CH_2CH(CH_3)_2$), 48.8 (C-1′), 51.0 (CH_{pept}), 51.8 (C-1), 52.4 (C-3); 53.4 (OCH_3), 66.9 (CH_2Ph), 111.2–129.3 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (CH_3); H-3 (H-4b, H-3′b and H-3′a), H-4a (H-4b, H-3′a and H-3′b, N*H*), H-4b (H-3, H-4a, H-3′a and H-3′b, N*H*), H-3′a and H-3′b (H-3, H-4a, H-4b, N*H*), H-1′ (CH_3), CH_3 (H-1′, N*HZ*), CH_{pept} ($CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$, NH_{pept}), $CH_2CH(CH_3)_2$ (CH_{3pept}, CH_{pept}, NH_{pept}), $CH_2CH(CH_3)_2$ (CH_{3pept}, CH_{pept}), CH_{3pept} ($CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$), NH_{pept} ($CH_2CH(CH_3)_2$, CH_{pept}), NH (H-4a, H-4b, H-3′a and H-3′b), NH_{ind} (Ar); ESI *m/z*: 535 $[M+H]^+$, 557 $[M+Na]^+$; exact mass calculated for $C_{30}H_{38}N_4O_5$ $[M+H]^+$: 535.2915 found: 535.2940.

Tcc(Z-L-Ile)CH$_2$-Leu-COOCH$_3$ 8b

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 0/100.

trans 8b: Yield: 39%; yellowish foam; $[\alpha]_D^{20} = +35.7$ (*c* 1, CH_3OH); t_R (grad. 1) = 18.50 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.32; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.91 (m, 6H, CH_3); 0.96 (m, 6H, CH_{3pept}); 1.19 (m, 1H, $CH(CH_3)CH_2CH_3$); 1.21 (dd, J = 13.8 Hz, J = 7.2 Hz, $CH_2CH(CH_3)_2$); 1.68 (m, 2H, $CH_2CH(CH_3)_2$); 1.73 (m, 2H, $CH(CH_3)CH_2CH_3$); 2.35 (m, 2H, H-3′a and H-3′b); 2.60 (m, 1H, H-4b); 2.73 (m, 1H, H-4a); 3.60 (s, 3H, OCH_3); 3.73 (t, J = 8.3 Hz, 1H, H-3); 4.02 (s, 1H, H-1′); 4.12 (dd, J = 14.3 Hz, J = 7.1 Hz, 1H, H-1); 4.60 (d, J = 5.5 Hz, 1H, CH_{pept}); 5.07 (s, 2H, CH_2Ph); 5.59 (s, 1H, N*HZ*); 6.43–7.43 (m, 9H, Ar); 8.04 (s, 1H, NH_{pept}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 10.7 (CH_3), 17.0 (CH_3), 21.6 (CH_{3pept}), 24.9 ($CH_2CH(CH_3)_2$), 26.0 ($CH(CH_3)CH_2CH_3$), 29.5 ($CH_2CH(CH_3)_2$), 35.6 (C-4), 40.0 (C-3′), 40.9 ($CH(CH_3)CH_2CH_3$), 50.8 (CH_{pept}), 51.8 (C-3), 53.2 (OCH_3), 59.3 (C-1′), 60.3 (C-1), 67.1 (CH_2Ph), 111.3–128.3 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-1′, CH_3); H-3 (H-4b), H-4a (H-4b, H-3′a, H-3′b), H-4b (H-3, H-4a, H-3′a, H-3′b), H-3′a (H-4a, H-4b, H-3′b), H-3′b (H-4a, H-4b, H-3′a), H-1′ (H-1, $CH(CH_3)CH_2CH_3$, N*HZ*), $CH(CH_3)CH_2CH_3$ (H-1′, $CH(CH_3)CH_2CH_3$, CH_3, N*HZ*), $CH(CH_3)CH_2CH_3$ ($CH(CH_3)CH_2CH_3$, CH_3), CH_3 ($CH(CH_3)CH_2CH_3$, $CH(CH_3)CH_2CH_3$), CH_{pept} ($CH_2CH(CH_3)_2$), $CH_2CH(CH_3)_2$ ($CH_2CH(CH_3)_2$, CH_{3pept}), $CH_2CH(CH_3)_2$ ($CH_2CH(CH_3)_2$, CH_{3pept}, CH_{pept}), N*H*pept (CH_{pept}), N*HZ* ($CH(CH_3)CH_2CH_3$); ESI *m/z*: 577 [M

$+H]^+$, 599 $[M+Na]^+$; exact mass calculated for $C_{33}H_{44}N_4O_5$ $[M+H]^+$: 577.3384 found: 577.3394.

Tcc(Z-L-Phe)CH$_2$-Leu-COOCH$_3$ 7c and 8c

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 21/79.

cis 7c: Yield: 18%; yellowish foam; $[\alpha]_D^{20} = -7.9$ (*c* 1, CH_3OH); t_R (grad. 1) = 18.37 min, t_R (grad. 1) = 15.03 min; R_f ($CHCl_3$ – Acetone 3:2) = 0.49; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.94 (m, 6H, CH_3); 1.37 (m, 2H, CH_2Ph); 1.64 (m, 3H, $CH_2CH(CH_3)_2$); 2.61 (m, 1H, H-4b); 2.87 (m, 1H, H-4a); 3.04 (m, 2H, H-3′a and H-3′b); 3.69 (s, 1H, H-3); 3.73 (s, 3H, OCH_3); 4.35 (s, 1H, H-1′); 4.53 (s, 1H, CH_{pept}); 4.69 (s, 1H, H-1); 4.70 (m, 1H, N*HZ*); 5.07 (m, 2H, CH_2Ph_Z); 6.95–7.36 (m, 14H, Ar); 8.45 (s, 1H, NH_{ind}) ^{13}C NMR (700 MHz, $CDCl_3$) δ 22.5 (CH_3), 24.8 ($CH_2CH(CH_3)_2$), 27.9 (C-4), 28.3 (CH_2Ph), 37.3 (C-3′), 41.2 ($CH_2CH(CH_3)_2$), 50.8 (C-1), 52.4 (OCH_3), 54.9 (C-1′), 63.8 (C-3), 66.4 (CH_{pept}), 66.7 (CH_2Ph), 111.4–126.6 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (CH_2Ph, NH_{ind}); H-3 (H-1′, H-4a, H-4b, H-3′a and H-3′b), H-4a (H-4b, H-3), H-4b (H-4a, H-3), H-3′a and H-3′b (H-3, H-1′), H-1′ (H-3, H-3′a and H-3′b), CH_{pept} (CH_3, $CH_2CH(CH_3)_2$), $CH_2CH(CH_3)_2$ (CH_{pept}, CH_3), CH_3 ($CH_2CH(CH_3)_2$), NH_{ind} (H-1, Ar); ESI *m/z*: 611 $[M+H]^+$; exact mass calculated for $C_{36}H_{42}N_4O_5$ $[M+H]^+$: 611.3228 found: 611.3246.

trans 8c: Yield: 42%; yellowish foam; $[\alpha]_D^{20} = +31.9$ (*c* 1, CH_3OH); t_R (grad. 1) = 18.45 min, t_R (grad. 2) = 15.14 min; R_f ($CHCl_3$ – Acetone 3:2) = 0.34; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.93 (m, 6H, CH_3); 1.61 (m, 4H, CH_2Ph and $CH_2CH(CH_3)_2$); 1.97 (bs, 1H, $CH_2CH(CH_3)_2$); 2.48 (m, 1H, H-4b); 2.71 (m, 2H, H-3′a and H-3′b); 2.81(m, 1H, H-4a); 3.33 (s, 1H, H-3); 3.57 (s, 3H, OCH_3); 4.53 (s, 2H, H-1′ and CH_{pept}); 4.68 (s, 1H, H-1); 4.98 (m, 2H, CH_2Ph_Z); 5.81 (bs, 1H, N*HZ*); 7.12–7.31 (m, 14H, Ar); 7.45 (s, 1H, NH_{pept}); 8.45 (s, 1H, NH_{ind}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 22.5 (CH_3), 24.8 ($CH_2CH(CH_3)_2$), 28.1(C-4), 35.5 (C-3′), 41.1 (CH_2Ph), 42.5 ($CH_2CH(CH_3)_2$), 50.6 (C-1), 52.2 (OCH_3), 52.2 (C-3), 55.4 (C-1′), 56.9 (CH_{pept}), 66.7 (CH2Ph$_Z$), 111.9–128.3 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (CH_2Ph and $CH_2CH(CH_3)_2$); H-3 (H-1′ and CH_{pept}, H-4b, H-3′a and H-3′b), H-4a (H-4b, H-3′a and H-3′b, H-1′ and CH_{pept}), H-4b (H-4a, H-3′a and H-3′b), H-3′a and H-3′b (H-4a, H-4b, Ar, NH_{pept}), H-1′ and CH_{pept} (H-3, H-4a, H-3′a and H-3′b, Ar, NH_{pept}, NH_{ind}), CH_2Ph and $CH_2CH(CH_3)_2$ (H-1, $CH_2CH(CH_3)_2$, CH_3Leu), CH_3Leu ($CH_2CH(CH_3)_2$, CH_2Ph and $CH_2CH(CH_3)_2$), NH_{pept} (H-1′ and CH_{pept}), NH_{ind} (H-1′ and CH_{pept}); ESI-MS m/z: 611 $[M+H]^+$; exact mass calculated for $C_{36}H_{42}N_4O_5$ $[M+H]^+$: 611.3228 found: 611.3246.

Tcc(Z-L-Val)CH$_2$-Leu-COOCH$_3$ 8d

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 0/100.

trans 8d: Yield: 51%; yellowish foam; $[\alpha]_D^{20} = +23.3$ (*c* 1, CH_3OH); t_R (grad. 1) = 18.02 min; R_f ($CHCl_3$ – Acetone 3:2) = 0.46; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.92 (q, J = 5.4 Hz, 6H, CH_{3pept}); 0.99 (m, 6H, CH_3); 1.57 (m, 1H, $CH_2CH(CH_3)_2$); 1.67 (m, 2H, $CH_2CH(CH_3)_2$); 2.02 (m, 1H, $CH(CH_3)_2$); 2.37 (m, 1H, H-3′b); 2.41 (d, J = 10.5 Hz, 1H, H-4b); 2.51 (d, J = 14.2 Hz, 1H, H-3′a); 2.72 (d, J = 13.6 Hz, 1H, H-4a); 3.19 (bs, 1H, H-3); 3.57 (s, 3H, OCH_3); 3.98 (m, 1H, H-1); 4.36 (s, 1H, H-1′); 4.64 (m, 1H, CH_{pept}); 5.11 (m, 2H, CH_2Ph); 5.59 (m, 1H, N*HZ*); 6.89 (m, 1H, NH_{ind}); 6.86–7.47 (m, 9H, Ar); 7.91 (s, 1H, NH_{pept}); 8.34 (s, 1H, N*H*); ^{13}C NMR (700 MHz, $CDCl_3$) δ 19.6 (CH_{3pept}), 20.1 (CH_3), 24.95 ($CH_2CH(CH_3)_2$); 29.1 (C-4), 29.7 ($CH(CH_3)_2$), 41.1 ($CH_2CH(CH_3)_2$), 42.7 (C-3′), 50.6 (CH_{pept}), 53.7 (C-3), 52.3 (OCH_3), 57.0 (C-1′), 59.4 (C-1), 67.1 (CH_2Ph_Z), 111.0–128.4 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-1′, $CH(CH_3)_2$, N*H*), H-3 (H-4b, H-3′a, H-3′b, H-1′, NH_{pept}), H-4a (H-4b, H-3′a, H-3′b), H-4b (H-3, H-4a, H-3′a, H-3′b), H-3′a (H-3, H-4a, H-4b, H-3′b, NH_{pept}), H-3′a (H-3, H-4a, H-4b, H-3′b, NH_{pept}), H-1′ (H-1, H-3, N*H*), $CH(CH_3)_2$ (H-1, CH_3, NH, N*HZ*), CH_3 ($CH(CH_3)_2$), CH_{pept} ($CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$, NH_{pept}), $CH_2CH(CH_3)_2$ ($CH_2CH(CH_3)_2$, CH_{3pept}, CH_{pept}), $CH_2CH(CH_3)_2$ ($CH_2CH(CH_3)_2$, CH_{3pept}, $CH_2CH(CH_3)_2$), CH_{3pept} ($CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$), NH_{pept} (H-3, H-3′a, H-3′b, CH_{pept}, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$), N*H* (H-1, H-1′, *CH*$(CH_3)_2$, Ar), N*HZ* (H-1, H-1′, CH_2Ph, $CH(CH_3)_2$); ESI *m/z*: 563 $[M+H]^+$; exact mass calculated for $C_{32}H_{42}N_4O_5$ $[M+H]^+$: 563.3228 found: 563.3231.

Tcc(Z-D-Ala)CH$_2$-Leu-COOCH$_3$ 7e

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 100/0.

cis 7e: Yield: 43%; yellowish foam; $[\alpha]_D^{20} = +58.8$ (*c* 1, CH_3OH); t_R (grad. 1) = 17.19 min; R_f ($CHCl_3$ – Acetone 3:2) = 0.61; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.97 (dd, J = 21.5 Hz, J = 6.6 Hz, 6H, CH_{3pept}); 1.50 (d, J = 7.0 Hz, 3H, CH_3); 1.66 (m, 2H, $CH_2CH(CH_3)_2$); 1.86 (m, 1H, $CH_2CH(CH_3)_2$); 2.78 (d, J = 11.9 Hz, 1H, H-3′b); 3.09 (m, 1H, H-4b); 3.15 (dd, J = 16.0 Hz, J = 4.2 Hz, 1H, H-4a); 3.31 (m, 1H, H-3′a); 3.78 (s, 3H, OCH_3); 3.89 (bs, 1H, H-3); 4.47 (m, 1H, CH_{pept}); 4.82 (bs, 1H, H-1); 4.84 (bs, 1H, H-13); 4.94 (q, J = 12.7 Hz, 2H, CH_2Ph); 6.93 (d, J = 9.9 Hz, 1H, N*HZ*); 6.98–7.49 (m, 9H, Ar); 8.83 (s, 1H, NH_{ind}); 8.95 (s, 1H, NH_{pept}); ^{13}C NMR (700 MHz, $CDCl_3$) δ 17.2 (CH_3), 21,9 (CH_{3pept}), 22.7 (CH_{3pept}), 24.6 (C-4), 24.9 ($CH_2CH(CH_3)_2$), 39.0 ($CH_2CH(CH_3)_2$), 39.7 (C-4), 46.3 (C-1), 52.0 (OCH_3), 52.4 (CH_{pept}), 55.8 (C-3), 60.2 (C-1′), 67.2 (CH_2Ph), 111.8–128.2 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, CH_3, NH_{ind}); H-3 (H-1, H-4b, H-3′a, H-3′b), H-4a (H-3b, H-3′b), H-4b (H-3, H-4a, H-3′a), H-3′a (H-3, H-4b, H-3′b), H-3′b (H-3, H-4a, H-3′a), H-1′ (CH_3, N*HZ*), CH_3 (H-1, H-1′, N*HZ*), CH_{pept} ($CH_2CH(CH_3)_2$, NH_{pept}), $CH_2CH(CH_3)_2$ ($CH_2CH(CH_3)_2$, CH_{3pept}), $CH_2CH(CH_3)_2$ ($CH_2CH(CH_3)_2$, CH_{3pept}, CH_{pept}, NH_{pept}), CH_{3pept} ($CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$), NH_{pept} ($CH_2CH(CH_3)_2$, CH_{pept}), N*H*ind (H-1, Ar), N*HZ* (H-1′, CH_3); ESI *m/z*: 535 $[M+H]^+$, 557 $[M+Na]^+$; exact mass calculated for $C_{30}H_{38}N_4O_5$ $[M+H]^+$: 535.2915 found: 535.2924 Df. 1.68 ppm.

Tcc(Z-D-Leu)CH$_2$-Leu-COOCH$_3$ 7f

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 100/0.

cis 7f: Yield: 67%; yellowish foam; $[\alpha]_D^{20} = +50.9$ (*c* 1, CH_3OH); t_R (grad. 1) = 18.61 min; R_f ($CHCl_3$ – Acetone 3:2) = 0.52; ^{1}H NMR (700 MHz, $CDCl_3$) δ 0.86–1.03 (m, 12H, CH_3); 1.54 (ddd, J = 13.0 Hz, J = 5.3 Hz, J = 3.3 Hz, 1H, $CH_2CH(CH_3)_2$); 1.67 (m, 1H, $CH_2CH(CH_3)_{2pept}$); 1.79 (m, 2H, $CH_2CH(CH_3)_{2pept}$); 1.93 (m, 2H, $CH_2CH(CH_3)_2$); 2.88 (d, J = 14.3 Hz, 1H, H-3′b); 3.11 (m, 1H, H-4b); 3.16 (m, 1H, H-4a); 3.53 (m, 1H, H-3′a); 3.78 (s, 3H, OCH_3); 3.89 (bs, 1H, H-3); 4.51 (m, 1H, CH_{pept}); 4.72 (t, J = 10.5 Hz, 1H, H-1′); 4.78 (s, 1H, H-1); 4.92 (m, 2H, CH_2Ph); 5.15 (m, 1H, N*HZ*); 6.92–7.64 (m, 9H, Ar); 8.29 (s, 1H, NH_{pept}); 8.51 (s, 1H, N*H*); 8.91 (s, 1H, NH_{ind}) ^{13}C NMR (700 MHz, $CDCl_3$) δ 21.3 (CH_3), 23.1 (CH_{3pept}), 25.1 (C-4), 38.4 (C-3′), 39.5 ($CH_2CH(CH_3)_{2pept}$ and $CH_2CH(CH_3)_{2pept}$), 40.1 ($CH_2CH(CH_3)_2$ and $CH_2CH(CH_3)_2$), 49.2 (C-1′), 52.2 (CH_{pept}), 53.1 (OCH_3), 54.9 (C-3), 60.0 (C-1), 67.6 (CH_2Ph), 111.9–128.5 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, $CH_2CH(CH_3)_2$, N*H*, NH_{ind}); H-3 (H-1, H-4b, H-3′a, H-3′b), H-4a (H-4b, H-3′a, H-3′b), H-4b (H-3, H-4a, H-3′a, H-3′b), H-3′a (H-3, H-4a, H-4b, H-3′b), H-3′b (H-3, H-4a, H-4b, H-3′a), H-1′ ($CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$, N*H*), $CH_2CH(CH_3)_2$ (H-1′, $CH_2CH(CH_3)_2$), $CH_2CH(CH_3)_2$ (H-1, H-1′, $CH_2CH(CH_3)_2$, CH_3, N*H*), CH_{pept} ($CH_2CH(CH_3)_{2pept}$, $CH_2CH(CH_3)_{2pept}$, NH_{pept}), $CH_2CH(CH_3)_{2pept}$ ($CH_2CH(CH_3)_{2pept}$, CH_{3pept}),

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$CH_2CH(CH_3)_{2pept}$ ($CH_2CH(CH_3)_{2pept}$, CH_{3pept}, CH_{pept}), NH_{pept} (CH_{pept}), N*H* (H-1, H-1′, $CH_2CH(CH_3)_2$), NH_{ind} (H-1, Ar); ESI *m/z*: 577 $[M+H]^+$; exact mass calculated for $C_{33}H_{44}N_4O_5$ $[M+H]^+$: 577.3384 found: 577.3388.

Tcc(Z-D-Phe)CH$_2$-Leu-COOCH$_3$ 7g

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 100/0.

cis 7g: Yield: 38%; yellowish foam; $[\alpha]_D^{20} = +67.1$ (*c* 1, CH_3OH); t_R (grad. 1) = 18.59 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.46; ^1H NMR (700 MHz, $CDCl_3$) δ 0.96 (dd, J = 16.4 Hz, J = 6.2 Hz, 6H, CH_3); 1.67 (m, 2H, CH_2Ph); 1.76 (m, 1H, $CH_2CH(CH_3)_2$); 1.83 (m, 2H, $CH_2CH(CH_3)_2$); 3.12 (d, J = 15.2 Hz, 1H, H-4b); 3.20 (d, J = 15.2 Hz, 2H, H-3′a and H-3′b); 3.25 (m, 1H, H-4a); 3.59 (m, 1H, H-3); 3.75 (s, 3H, OCH_3); 3.90 (s, 1H, H-1′); 4.57 (s, 1H, CH_{pept}); 4.79 (dd, J = 47.7 Hz, J = 12.7 Hz, 2H, CH_2Ph_Z), 4.89 (s, 1H, H-1); 5.30 (m, 1H, N*H*Z); 6.78–7.64 (m, 15H, Ar and NH_{ind}); 8.86 (s, 1H, NH_{pept}); 8.93 (s, 1H, N*H*); ^{13}C NMR (700 MHz, $CDCl_3$) δ 21.3 (CH_3), 24.9 ($CH_2CH(CH_3)_2$); 25.2 (C-4), 37.4 (C-3′), 38.4 (C-1), 39.9 (CH_2Ph), 41.2 ($CH_2CH(CH_3)_2$) 52.2 (CH_{pept}), 53.1 (C-1), 53.2 (OCH_3), 55.1 (C-1′), 67.2 (CH_2Ph_Z), 111.8–129.1 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, H-3′a and H-3′b, N*H*); H-3 (H-1, H-4a), H-4a (H-3, H-4b, H-3′a and H-3′b), H-4b (H-4a, H-3′a and H-3′b), H-3′a and H-3′b (H-1, H-4a, H-4b), H-1′ (H-1, CH_2Ph, NH), CH_2Ph (H-1′), CH_{pept} ($CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$, NH_{pept}), $CH_2CH(CH_3)_2$ ($CH_2CH(CH_3)_2$, CH_3, CH_{pept}), $CH_2CH(CH_3)_2$ ($CH_2CH(CH_3)_2$, CH_3, CH_{pept}), CH_3 ($CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$), NH_{pept} (CH_{pept}), N*H* (H-1, H-1′); ESI *m/z*: 611 $[M+H]^+$; exact mass calculated for $C_{36}H_{42}N_4O_5$ $[M+H]^+$: 611.3228 found: 611.3255.

Tcc(Z-D-Val)CH$_2$-Ala-COOCH$_3$ 7h

The ratio of *cis/trans* isomers determined before purification by 1H NMR (200 MHz) was 100/0.

cis 7 h: Yield: 67%; yellowish foam; $[\alpha]_D^{20} = +40.5$ (*c* 1, CH_3OH); t_R (grad. 1) = 18.00 min; R_f ($CHCl_3$ – Acetone 3 : 2) = 0.52; ^1H NMR (700 MHz, $CDCl_3$) δ 0.96 (m, 6H, CH_{3pept}); 1.08 (m, 3H, CH_3); 1.19 (m, 3H, CH_3); 1.65 (m, 1H, $CH(CH_3)_2$); 1.77 (m, 2H, $CH_2CH(CH_3)_2$); 2.17 (m, 1H, $CH_2CH(CH_3)_2$); 2.84 (d, J = 14.5 Hz, 1H, H-3′b); 3.12 (m, 2H, H-4a and H-4b); 3.43 (d, J = 14.7 Hz, 1H, H-3′a); 3.75 (s, 3H, OCH_3); 3.87 (s, 1H, H-3); 4.22 (m, 1H, H-1′); 4.52 (dd, J = 14.5 Hz, J = 7.3 Hz, 1H, H-1); 4.93 (m, 2H, CH_2Ph); 4.98 (m, 1H, CH_{pept}); 5.11 (s, 1H, N*H*Z); 6.97–7.43 (m, 9H, Ar); 8.35 (s, 1H, NH_{pept}); 9.02 (s, 1H, N*H*); ^{13}C NMR (700 MHz, $CDCl_3$) δ 19.8 (CH_{3pept}), 20.2 (CH_3), 24.7 ($CH_2CH(CH_3)_2$); 25.1 (C-4), 28.7 ($CH_2CH(CH_3)_2$), 39.7 ($CH(CH_3)_2$), 42.7 (C-3′), 51.7 (C-1), 52.8 (OCH_3), 55.7 (C-3), 55.9 (CH_{pept}), 57.0 (C-1′), 66.6 (CH_2Ph), 111.8–128.1 (Ar); ROESY (700 MHz, $CDCl_3$) H-1 (H-3, $CH(CH_3)_2$, CH_3); H-3 (H-1, H-4a and H-4b, H-3′a, H-3b), H-4a and H-4b (H-3, H-3′b), H-3′a (H-3′b), H-3′b (H-3, H-4a and H-4b, H-3′a, NH_{pept}), H-1′ ($CH(CH_3)_2$, N*H*Z, N*H*), $CH(CH_3)_2$ (H-1′, CH_3, N*H*, N*H*Z), CH_3 ($CH(CH_3)_2$), CH_{pept}($CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$, N*H*pept), $CH_2CH(CH_3)_2$ ($CH_2CH(CH_3)_2$, CH_{3pept}, CH_{pept}), $CH_2CH(CH_3)_2$ ($CH_2CH(CH_3)_2$, CH_{3pept}, CH_{pept}), CH_{3pept} ($CH_2CH(CH_3)$, $CH_2CH(CH_3)_2$), NH_{pept} (H-3′b, CH_{pept}, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$), N*H* (H-1′, $CH(CH_3)_2$, Ar), N*H*Z (H-1′, $CH(CH_3)_2$); ESI *m/z*: 563 $[M+H]^+$; exact mass calculated for $C_{32}H_{42}N_4O_5$ $[M+H]^+$: 563.3228 found: 563.3252.

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