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Peptides and peptidoaldehydes as substrates for the Pictet–Spengler reaction

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The Pictet–Spengler (PS) reaction was performed with various types of substrates: H-Trp-OMe and dipeptides with N-terminal Trp as arylethylamine components and Z-protected amino aldehydes and peptidoaldehydes as carbonyl components. We found that the C-terminal part of Trp derivatives did not have any influence on the stereoselectivity of the reaction and the results are the same for simple esters of Trp and dipeptides.

On the contrary, the selectivity of the PS reaction with peptidoaldehydes with L configuration of the C-terminus residue is totally different from that obtained with simple L-amino aldehydes. It allows us to obtain *cis* stereoisomers, which cannot be isolated from the reaction with amino aldehydes. But the utility of the peptidoaldehydes as substrates for the PS reaction is reduced by the side formation of enamides which decrease the yield of cyclization. Copyright © 2013 European Peptide Society and John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: Pictet–Spengler reaction; amino aldehydes; peptidoaldehydes; chemical ligation; beta-turn

Introduction

The Pictet–Spengler (PS) reaction is a condensation between arylethylamine and carbonyl components [1,2]. During the reaction, the new, six-membered ring is formed, and a stereogenic center is generated [3–5]. The reaction is useful in the field of heterocycles, but also constrained analogs of aromatic amino acids may be prepared [6–12]. Such compounds exhibit biological activity [13] or, after incorporation into the peptide chains, may change the pharmacological properties of the parent peptides and provide insight into their bioactive conformation [14–16].

Additionally, the PS reaction may be used as a kind of specific, irreversible chemical ligation between peptides with N-terminal aromatic amino acid residues and peptidoaldehydes [17–19]. The conformation of the new, six-membered ring is responsible for the arrangement of peptides chains.

In our earlier studies, we performed the PS cyclization between H-Trp-OCH_3 and α-amino aldehydes (derived from L or D amino acids) with Z protection of the amino group [20,21]. We obtained 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines as products. It was found, that the cyclization was diastereoselective, and 'match' situation was observed for D-amino aldehydes and only one (*cis*) isomer was obtained, whereas 'mismatch' situation was characteristic for L-amino aldehydes and two isomers (*cis* and *trans*) were isolated with the significance dominance of isomer *trans* [20].

In our further studies, we have investigated the diastereoselectivity of the PS reaction, performed between model dipeptides with N-terminal Trp residue and amino aldehydes or between H-Trp-OCH_3 and peptidoaldehydes. For analogs obtained from dipeptides, we also determined the conformation of the six-membered ring and speculated on the possibility of the β-turn induction.

Obtained results show the limitations of the application of the PS cyclization as a chemical ligation method.

Results and Discussion

PS Reaction between Dipeptides and Z-protected Amino Aldehydes

Dipeptides H-Trp-Ala-OCH_3 and H-Trp-Leu-OCH_3 were prepared by the standard Boc-procedure in solution. Z-protected α-amino aldehydes were obtained via the Ferentz–Castro method [22] and used without further purification to avoid the racemization [23–25].

The PS reaction was performed with 5 eq of TFA (Scheme 1). The temperature was controlled – for the first 5 h, the reaction was stirred under −30 °C, and then left overnight at room temperature (RT). The ratio of stereoisomers was determined by ^{1}H-NMR. The results are given in Table 1. The comparison with our earlier observations for H-Trp-OCH_3 shows that the ratio of *cis* and *trans* diastereomers is roughly the same (Entry 1–9) [20]. For D-amino aldehydes, the match situation is observed and only

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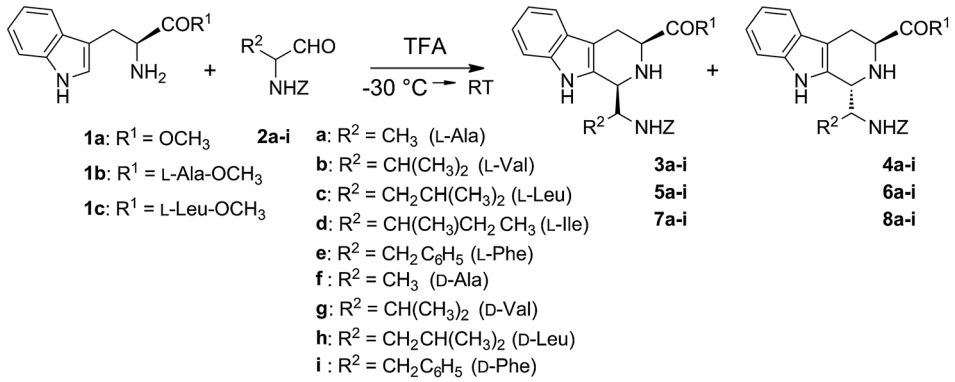

Scheme 1. Pictet–Spengler reaction between dipeptides and α-amino aldehydes.

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

Aldehyde **2**		***cis/trans*** **[%]** H-Trp-OCH_3 **3/4**	***cis/trans*** **[%]** H-Trp-Ala-OCH_3 **5/6**	***cis/trans*** **[%]** H-Trp-Leu-OCH_3 **7/8**
a	Z-L-Ala-H	35/65[a]	28/72 (42%)[b]	30/70 (57%)[b]
b	Z-L-Val-H	0/100	0/100 (61%)	0/100 (50%)
c	Z-L-Leu-H	27/73	30/70 (55%)	20/80 (85%)
d	Z-L-Ile-H	0/100	0/100 (61%)	0/100 (91%)
e	Z-L-Phe-H	25/75	30/70 (60%)	26/74 (56%)
f	Z-D-Ala-H	100/0	100/0 (44%)	100/0 (53%)
g	Z-D-Val-H	100/0	100/0 (50%)	100/0 (59%)
h	Z-D-Leu-H	100/0	100/0 (66%)	100/0 (70%)
i	Z-D-Phe-H	100/0	100/0 (57%)	100/0 (77%)

[a]The overall yields of both diastereomers are given in the reference [20].
[b]The overall yield of both diastereomers after the silica gel purification.

cis isomer is formed, whereas L-amino aldehydes give the mixture of products with the dominance of *trans* isomer.

It means that the C-terminus of Trp derivatives does not have any influence on the structure of the transition state, which determines the selectivity of the reaction.

The conformations of the newly created six-membered ring were studied by 2D NMR ROESY spectra. The NOE effect between H-1 and H-3 protons was crucial for the distinction of *cis* and *trans* diastereomers [26], and the exchange of magnetization among H-3 and H-4 protons was diagnostic for predictions of the ring's conformation. The six-membered ring in all obtained derivatives has the twisted chair conformation. In all *cis* isomers, both substituents are pseudoequatorially located. There are not any differences in relation to analogs derived from H-Trp-OCH_3. The more interesting situation is for *trans* analogs. In our earlier studies, we found that the ester group in compounds **4a–e** was pseudoaxially located (NOE effect between H-3 and both H-4 protons), whereas the substituent on C-1 was pseudoequatorially located [20]. It was confirmed by the crystal structure of one of those analogs (unpublished results).

For *trans* analogs **6a** and **8a**, we observed the strong NOE effect between H-3 and only one H-4a proton. The very weak interactions were observed for the second H-4b proton. It means that the peptide chain at C-3 is pseudoequatorially rather than pseudoaxially located. For analogs **6b–e** and **8b–e** (Figure 1), the NOE effect with H-4b was stronger. It is probably due to the equilibrium between possible conformations of the six-membered ring.

In the literature, it was postulated that disubstituted six-membered ring in tetrahydro-β-carboline can induce β-turn and it is related to *trans* rather than *cis* isomers [27]. We also found that *trans* analogs **6a** and **8a** may be able to induce β-turn. The results for the other *trans* analogs are not so clear, that is why for further studies, we have chosen the compound **6a**. A 10-membered ring with the hydrogen bond between the nitrogen of the peptide chain and the oxygen of the carbonyl group of Z could be formed. For such stabilization of the structure, the NOE effect should be observed between H-3 (for a pseudoaxial position of this proton) and H-1′. And, indeed such interaction through space was observed. The presence of the NOE effect between H-1 and NH(Z) and between H-3 and NHpep also supports the assumption about the β-turn. The chemical shift of NHpep is 7.16 ppm. ^{1}H and ROESY spectra were also recorded at 245 K to check the shift of the signal of that proton [28]. The signal was downfield shifted. All NOE effects were the same as observed at RT. For *trans* diastereomers with sterically hindered side chains, the interaction through space was found between NH(Z) and H-1′ as well as H-3, but any effect was observed with H-1. It confirms that the equilibrium between nonhydrogen-bonded and hydrogen-bonded state is shifted to nonhydrogen-bonded. The chemical shift of NHpep is 6.60 to 6.93.

To confirm the hydrogen bond stabilization, we synthesized compound **9** (Figure 2). There are two possibilities of hydrogen bond formation between two arms: with 10-membered and/or 14-membered rings [27]. Additionally, it is also possible to link NHAla(ald) with CO(Z). According to ROESY spectra (some

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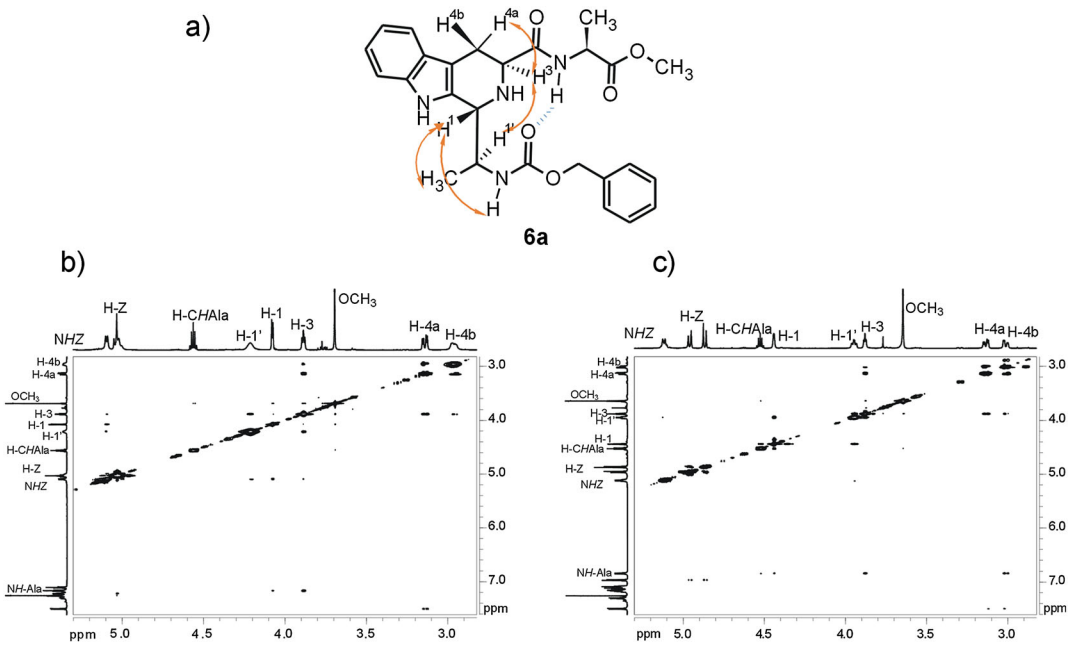


Figure 1. (a) Schematic representation of the NOE effect in molecule stabilized by hydrogen bond (dash line); (b) ROESY spectrum of compound 6a; (c) ROESY spectrum of compound 6d.

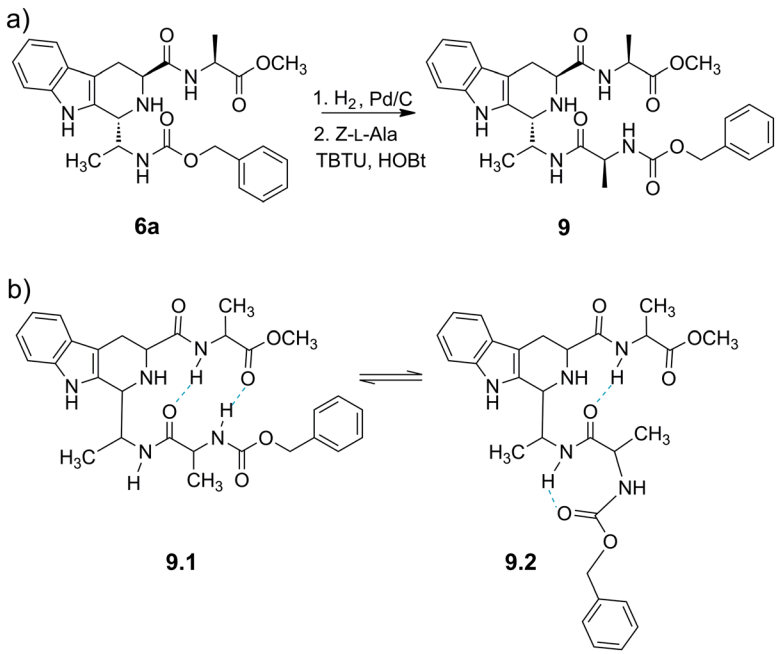


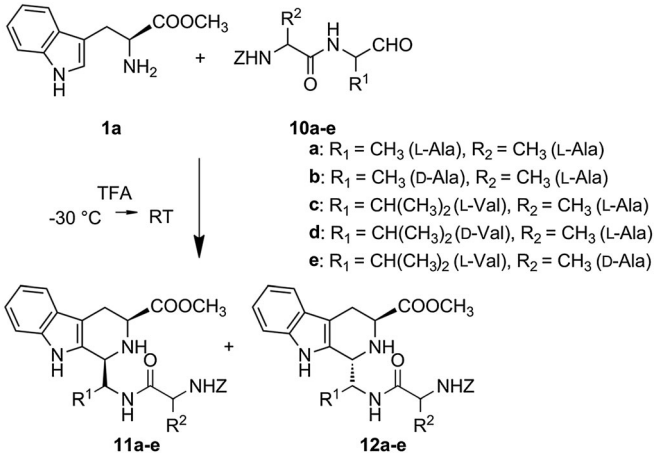
Figure 2. (a) Synthesis of compound 9; (b) Schematic presentation of the possible hydrogen bonds' stabilization of the structure of compound 9.

signals were overlaid) at 245 K, we supposed that in compound **9**, a 10-membered ring rather than a 14-member ring is formed by hydrogen bond stabilization and equilibrium is shifted into **9.2**.

PS Reaction between H-Trp-OCH$_3$ and Peptidoaldehydes

To obtain a long peptide with the central unit of 1,2,3,4-tetrahydro-β-carboline, the constrained derivative of H-Trp-OH can be prepared firstly by the PS reaction with amino aldehydes, and then coupled with appropriate amino acids residues. We wanted to check the synthetic possibility of the PS reaction with peptidoaldehydes. According to our knowledge, there are relatively few studies in that topic [17,18].

We performed that cyclization between H-Trp-OCH$_3$ and 5 dipeptidoaldehydes (**10a–e**) in the presence of 5 eq of TFA (Scheme 2). Peptidoaldehydes were obtained by the LiAlH$_4$ reduction of Weinreb amides of appropriate dipeptides [29,30]. The yield of the reduction step was about 60%. The lower yield



Scheme 2. The Pictet–Spengler reaction with peptidoaldehydes.

of crude peptidoaldehydes in comparison to simple amino aldehydes may be caused by the complexation of the lithium cation by the amide groups (peptide bonds) and better solubility of such complex in aqueous media [29,30].

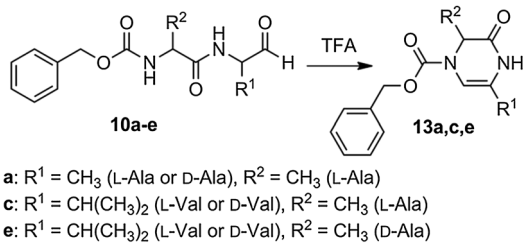
The N-terminal amino acid residue in dipeptidoaldehydes was the same in four cases (L-Ala), and one peptidoaldehyde was synthesized with opposite configuration of N-terminus. C-terminal residue contained different side chains (Ala or Val) and stereochemistries (**10a–e**). The ratios of obtained diastereoisomers are given in Table 2. We compared the obtained results with the selectivity of the reaction with amino aldehydes [20]. The reactions with peptidoaldehydes with D-residues at C-terminus were completely selective and only *cis* isomers **11** were formed. This observation is the same as for Z-D-Xaa-H. The ratio of *cis/trans* isomers obtained from peptidoaldehydes with L-residues strongly depended on the structure of that moiety. Small side chain, as in Ala, favored *cis* isomer, and the ratio was inversed in comparison to Z-L-Ala-H (Table 1, Entry 1). Totally surprising results were obtained for Z-L-Val-H. Almost equimolar mixture of *cis/trans* diastereomers was obtained. The stereochemistry of the N-terminus residue of peptidoaldehydes did not affect the stereoselectivity of the cyclization; the same results were obtained for aldehydes **10c** and **10e**. Products of the PS cyclization with **10c** and **10e** are interesting, especially as there was not possible to obtain *cis* 1,3-disubstituted tetrahydro-β-carboline unit in the reaction with Z-L-Val-H [20].

The total yield of the PS reaction with peptidoaldehydes was lower (50–80%, determined from NMR spectra of the mixture) than for Z-protected amino aldehydes. The reason for it was that peptidoaldehydes reacted in two ways: as components for the PS

Table 2. The ratio of *cis/trans* isomers obtained from peptidoaldehydes (determined by ^{1}H-NMR)

Peptidoaldehyde 10		*cis/trans* [%] 11/12
a	Z-L-Ala-L-Ala-H	68/32 (54%)[a]
b	Z-L-Ala-D-Ala-H	100/0 (60%)
c	Z-L-Ala-L-Val-H	42/58 (76%)
d	Z-L-Ala-D-Val-H	100/0 (74%)
e	Z-D-Ala-L-Val-H	42/58 (81%)

[a]The overall yield of both diastereomers based on ^{1}H-NMR, with reference to the integration of OCH_3 signals of products and H-Trp-OCH_3.



Scheme 3. Peptidoaldehyde cyclization in the presence of a strong acid.

cyclization, but also as substrates for side intramolecular cyclization. Peptidoaldehydes are very sensitive to the presence of acid (Scheme 3) and undergo intramolecular cyclization and elimination [31–35]. The side products were isolated (~20%) and determined as enamides **13**. This side reaction may limit the application of peptidoaldehydes for chemical transformations, demanding the use of a strong acid.

Conclusion

The PS reaction was performed with various types of substrates. We showed that both peptides with N-terminal Trp and peptidoaldehydes may react as substrates for the PS cyclization. The C-terminal part of Trp residue did not have any influence on the stereoselectivity, and the results obtained for peptides are the same as for H-Trp-OMe. The tetrahydro-β-carboline moiety incorporated into the peptide chain may induce β-turn and impose the definite position of the peptide arms. The hydrogen bond stabilization depends on the size of the substituent at C-1.

The selectivity of the PS reaction with peptidoaldehydes with L configuration of the C-terminal residue is totally different from that obtained with Z-L-Xaa-H. It allows us to obtain *cis* stereoisomers, which cannot be isolated from the reaction with amino aldehydes. Such stereoselectivity may result from the different stabilization of the intermediate iminium cation [20,21]. The additional amide bond of peptidoaldehyde may form the hydrogen bond, which stabilizes the transition state leading to the *cis* isomer.

The utility of the peptidoaldehydes as substrates for the PS reaction is reduced by the side formation of enamides which decrease the yield of cyclization. It may limit the application of the PS cyclization as a chemical ligation method for peptides with N-terminal aromatic residue and peptides with aldehyde residue at C-terminal.

Materials and Methods

Reagents and solvents were purchased from commercial suppliers and used as received.

Reversed phase HPLC was performed using a reversed phase C-12 column (Jupiter 4u Proteo 90A, ID = 0.46 cm, L = 25 cm). Gradient 1: $t = 0$ min, 97% A, 3% B, $t = 20$ min, 97% A, 3% B was used, flow rate: 1 mL min^{-1}, $\lambda = 210$ nm. Gradient 2: $t = 0$ min, 97% A, 3% B, $t = 20$ min, 97% A, 3% B was used, flow rate: 1 mL min^{-1}, $\lambda = 210$ nm. The mobile phases (water A, acetonitrile B) contained 0.05% TFA (Gradient 1) or 0.05% HCOOH (Gradient 2). TLC analysis was performed on precoated plates of silica gel 60 F_{254} (Merck). Silica gel 60 (0.063–0.2 mm) from Merck was used for flash chromatography. All 1D and 2D NMR spectra were recorded on 200, 500, or 700 MHz spectrometers. ^{13}C-NMR spectra were acquired as 2D Heteronuclear Single Quantum

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Coherence (HSQC) (quaternary and carbonyl carbons were not observed). For ROESY measurements, the standard pulse sequence was applied with mixing time of 200 ms. Mass spectra were recorded on a LCT time of flight mass spectrometer using electrospray ionization (positive ion mode) or liquid chromatography–mass spectrometry.

Dipeptides H-Trp-Ala-OCH_3 and H-Trp-Leu-OCH_3 were prepared by standard Boc-procedure in solution; TBTU was used as coupling reagent.

α-amino aldehydes **2a–i** derived from D or L amino acids with Z protection of amino group were prepared according to the procedure previously described [22] and used in the next step without purification. Peptidoaldehydes **10a-e** were obtained via Weinreb amides using the procedure given by Fehrentz [29,30], the exception was the amount of $LiAlH_4$. In all cases, 5 eq of reducing agent was used. After the classical work up, crude peptidoaldehydes were obtained and used in the next step without purification.

Z-L-Ala-L-Ala-H 10a

Yield: 74% (crude), ^{1}H-NMR (200 MHz, $CDCl_3$), δ 1.32 to 1.42 (m, 6H, 2xCH_{3Ala}), 4.23 to 4.33 (m, 1H, α-CH_{L-Ala}), 4.47 (m, 1H, J = 7.2 Hz, α-CH_{L-Ala}), 5.12 (bs, 2H, CH_{2Cbz}), 5.34 (d, 1H, J = 7.8 Hz, NH_{Cbz}), 6.78 (bs, 1H, NH_{L-Ala}), 7.29 to 7.38 (m, 5 h, Ar), 9.51 (s, 1H, CHO).

Z-L-Ala-D-Ala-H 10b

Yield: 65% (crude), ^{1}H-NMR (200 MHz, $CDCl_3$), δ 1.32 to 1.42 (m, 6H, 2xCH_{3Ala}), 4.23 to 4.33 (m, 1H, α-CH_{L-Ala}), 4.47 (m, 1H, J = 7.2 Hz, α-CH_{D-Ala}), 5.12 (bs, 2H, CH_{2Cbz}), 5.34 (d, 1H, J = 7.8 Hz, NH_{Cbz}), 6.78 (bs, 1H, NH_{D-Ala}), 7.29 to 7.38 (m, 5 h, Ar), 9.51 (s, 1H, CHO).

Z-L-Ala-L-Val-H 10c

Yield: 67% (crude), ^{1}H-NMR (200 MHz, $CDCl_3$), δ 0.93 (d, 3H, J = 7 Hz, CH_{3Val}), 0.97 (d, 3H, J = 6.9 Hz, CH_{3Val}), 1.41 (d, 1H, J = 7.1 Hz, CH_{3Ala}), 2.20 to 2.31 (m, 1H, CH_{Val}), 4.18 to 4.43 (m, 1H, α-CH_{Ala}), 4.53 (dd, 1H, J = 8 Hz, J = 4.5 Hz, α-CH_{Val}), 5.12 (bs, 2H, CH_{2Cbz}), 5.35 (d, 1H, J = 6.8 Hz, NH_{Cbz}), 6.65 (bd, 1H, J = 5.8 Hz, NH_{Val}), 7.30 to 7.37 (m, 5 h, Ar), 9.63 (s, 1H, CHO).

Z-L-Ala-D-Val-H 10d

Yield: 68% (crude), ^{1}H-NMR (200 MHz, $CDCl_3$), δ 0.92 (d, 3H, J = 7 Hz, CH_{3Val}), 0.99 (d, 3H, J = 6.8 Hz, CH_{3Val}), 1.41 (d, 1H, J = 7.1 Hz, CH_{3Ala}), 2.23 to 2.39 (m, 1H, CH_{Val}), 4.26 to 4.41 (m, 1H, α-CH_{Ala}), 4.55 (dd, 1H, J = 8 Hz, J = 4.4 Hz, α-CH_{Val}), 5.13 (bs, 2H, CH_{2Cbz}), 5.34 (d, 1H, J = 6.8 Hz, NH_{Cbz}), 6.69 (bs, 1H, NH_{Val}), 7.30 to 7.38 (m, 5 h, Ar), 9.61 (s, 1H, CHO).

Z-D-Ala-L-Val-H 10c

Yield: 62% (crude), ^{1}H-NMR (200 MHz, $CDCl_3$), δ 0.92 (d, 3H, J = 7 Hz, CH_{3Val}), 0.99 (d, 3H, J = 6.8 Hz, CH_{3Val}), 1.42 (d, 1H, J = 7.1 Hz, CH_{3Ala}), 2.24 to 2.38 (m, 1H, CH_{Val}), 4.27 to 4.39 (m, 1H, α-CH_{Ala}), 4.55 (dd, 1H, J = 8 Hz, J = 4.4 Hz, α-CH_{Val}), 5.13 (bs, 2H, CH_{2Cbz}), 5.34 (d, 1H, J = 6.2 Hz, NH_{Cbz}), 6.69 (bs, 1H, NH_{Val}), 7.30 to 7.38 (m, 5 h, Ar), 9.63 (s, 1H, CHO).

General Procedure for the PS Reaction

Hydrochlorides of H-Trp-OMe and dipeptides, before the PS reaction, were converted into the free bases by the stirring in the mixture of $CHCl_3$ (H-Trp-OCH_3*HCl) or AcOEt (H-Trp-Ala-OCH_3*HCl and H-Trp-Leu-OCH_3*HCl) and saturated solution of $NaHCO_3$ for 30 min. Layers were separated, and the organic one was washed with brine and dried over $MgSO_4$. After evaporation, yellowish solids were obtained. All the PS cyclizations were performed as previously reported. 1.38 mmol of aldehyde dissolved in 8 mL CH_2Cl_2 was added to 1.25 mmol of H-Trp-OCH_3 or H-Trp-Ala-OCH_3 or H-Trp-Leu-OCH_3 in 8 mL CH_2Cl_2. 5 eq (0.481 mL) of TFA in 1.5 mL CH_2Cl_2 was added in three portions. The reaction mixtures were stirred for 5 h (in the case of amino aldehydes) or 3 h (in the case of peptidoaldehydes) at −30 °C and then at RT overnight. Then, the mixtures were diluted with CH_2Cl_2, and saturated solution of $NaHCO_3$ was used to neutralize TFA. Organic phases were extracted with sat. $NaHCO_3$, washed with brine, and dried over $MgSO_4$. The ratios of the *cis/trans* isomers in the crude mixtures were determined by ^{1}H-NMR (based on the integration of separated peaks of methyl ester groups). Derivatives of dipeptides and amino aldehydes (**5,6,7,8a–i**) were separated by flash chromatography ($CHCl_3$-Acetone). Derivatives of H-Trp-OCH_3 and peptidoaldehydes were prepurified on silica gel to separate byproducts **13a,c,e**; afterwards, *cis/trans* isomers **11,12a,c,e** were separated by semi-preparative HPLC (t = 0 min, 75% A, 25% B, t = 20 min, 45% A, 55% B for **11,12a**; t = 0 min, 65% A, 35% B, t = 20 min, 35% A, 65% B for **11,12c,e**; the mobile phases (water, acetonitrile) contained 0.05% HCOOH to obtain 20 to 30 mg of pure isomers *cis* and *trans*.

Compound **5a (*cis* L-Ala/Ala(pep)**: Yield: 11% (after purification), HPLC (Gradient 1) t_R = 15.37 min. (Gradient 1), R_f ($CHCl_3$-Acetone 6:4) = 0.57, ^{1}H-NMR (700 MHz, $CDCl_3$), δ 1.12 (d, 3H, J = 6.3 Hz, H-2′), 1.48 (d, 3H, J = 7 Hz, $CH_{3Alapep}$), 2.72 (ddd, 1H, J = 15.3 Hz, J = 11.3 Hz, J = 2.4 Hz, H-4), 3.26 (dd, 1H, J = 15.4 Hz, J = 2.9 Hz, H-4), 3.58 (dd, 1H, J = 11.2 Hz, J = 4.3 Hz, H-3), 3.78 (s, 3H, OCH_3), 4.38 (bs, 2H, H-1 + H-1′), 4.66 (p, 1H, J = 7.0 Hz, CH_{Alapep}), 5.14 (q, 2H, J = 12.3 Hz, CH_{2Z}), 5.34 (d, 1H, J = 8.0 Hz, NH_Z), 7.10 to 7.50 (m, 10H, Ar + NH_{Alapep}), 8.29 (s, 1H, NH_{ind}), ^{13}C-NMR (175 MHz, as HSQC, $CDCl_3$), δ 18.3 ($CH_{3Alapep}$), 18.4 (C-2′), 23.8 (C-4), 47.6 (CH_{Alapep}), 49.2 (C-1′), 52.4 (OCH_3), 54.0 (C-3), 56.9 (C-1), 66.9 (CH_{2Z}), 111.0 to 128.0 (Ar). ROESY (700 MHz) H-1 + H-1′ (H-2′, H-3, NH_Z), H-3 (H-1, H-4, NH_{Alapep}), H-4 (H-3, Ar), H-2′ (H1 + H1, NH_Z, NH_{ind}), CH_{Alapep}($CH_{3Alapep}$, NH_{Alapep}). ESI-MS, *m/z*: 479 $[M+H]^+$, 501 $[M+Na]^+$. Exact mass calculated for $C_{26}H_{30}N_4O_5Na$ $[M+Na]^+$: 501.2114, found: 501.2109.

Compound **6a (*trans* L-Ala/Ala(pep)**: Yield: 31% (after purification), HPLC t_R = 15.18 min. (Gradient 1), R_f ($CHCl_3$-Acetone 6:4) = 0.43, ^{1}H-NMR (700 MHz, $CDCl_3$), δ 1.36 (d, 3H, J = 6.4 Hz, H-2′), 1.39 (d, 3H, J = 7.2 Hz, $CH_{3Alapep}$), 2.97 (bdd, 1H, J = 13.9 Hz, J = 5.2 Hz, H-4), 3.14 (dd, 1H, J = 15.8 Hz, J = 5.4 Hz, H-4), 3.69 (s, 3H, OCH_3), 3.88 (t, 1H, J = 6.3 Hz, H-3), 4.08 (d, 1H, J = 4.9 Hz H-1), 4.22 (bs, 1H, H-1′), 4.56 (p, 1H, J = 7.0 Hz, CH_{Alapep}), 5.03 (m, 2H, CH_{2Z}), 5.10 (d, 1H, J = 8.4 Hz, NH_Z), 7.10 to 7.51 (m, 10H, Ar + NH_{Alapep}), 8.19 (s, 1H, NH_{ind}). ^{13}C-NMR (175 MHz, as HSQC, $CDCl_3$), δ 18.3 ($CH_{3Alapep}$), 18.4 (C-2′), 23.8 (C-4), 47.9 (CH_{Alapep}), 49.3 (C-1′), 52.4 (OCH_3), 54.0 (C-3), 55.2 (C-1), 66.9 (CH_{2Z}), 111.0 to 128.0 (Ar). ROESY (700 MHz) H-1 (H-2′, NH_Z, NH_{Alapep}, NH_{ind}), H-3 (H-2′, H-4, H-1′, NH_{Alapep}), H-4 (H-3, Ar), H-1′(H-3, H-2′, NH_Z), H-2′ (H1, H-3, H1″, NH_Z), CH_{Alapep}($CH_{3Alapep}$, NH_{Alapep}). ESI-MS, *m/z*: 479 $[M+H]^+$, 501 $[M+Na]^+$. Exact mass calculated for $C_{26}H_{30}N_4O_5Na$ $[M+Na]^+$: 501.2114, found: 501.2103.

5f (cis D-Ala/Ala(pep): Yield: 44% (after purification), HPLC $t_R = 15.52$ min. (Gradient 1), R_f ($CHCl_3$-Acetone 6:4) = 0.50, ^{1}H-NMR (700 MHz, $CDCl_3$), δ 1.39 (d, 3H, $J = 5.6$ Hz, H-2′), 1.47 (d, 3H, $J = 7$ Hz, $CH_{3Alapep}$), 2.77 (ddd, 1H, $J = 15.4$ Hz, $J = 11.9$ Hz, $J = 2.1$ Hz, H-4), 3.19 (ddd, 1H, $J = 14.9$ Hz, $J = 4.4$ Hz, $J = 1.6$ Hz, H-4), 3.69 (dd, 1H, $J = 11.2$ Hz, $J = 4.4$ Hz, H-3), 3.78 (s, 3H, OCH_3), 4.29 (bs, 1H, H-1), 4.46 (bs, 1H, H-1′), 4.67 (p, 1H, $J = 7.0$ Hz, CH_{Alapep}), 4.95 (bs, 2H, CH_{2Z}), 5.19 (bs, 1H, NH_Z), 7.07 to 7.47 (m, 10H, Ar + NH_{Alapep}), 8.47 (s, 1H, NH_{ind}). ^{13}C-NMR (175 MHz, as HSQC, $CDCl_3$), δ 17.7 (C-2′), 18.3 ($CH_{3Alapep}$), 25.7 (C-4), 47.7 (CH_{Alapep}), 47.8 (C-1′), 52.5 (OCH_3), 57.5 (C-1), 57.8 (C-3), 66.8 (CH_{2Z}), 111.3 to 128.4 (Ar). ROESY (700 MHz) H-1 (H-H-3, H-1′), H-3 (H-1, H-4, NH_{Alapep}), H-4 (H-3, Ar), H-2′ (H-1, H-1′, NH_Z), CH_{Alapep} ($CH_{3Alapep}$). ESI-MS, *m/z*: 479 $[M+H]^+$, 501 $[M+Na]^+$. Exact mass calculated for $C_{26}H_{30}N_4O_5Na$ $[M+Na]^+$: 501.2114, found: 501.2119

Compound **7a (*cis* L-Ala/Leu(pep)**: Yield: 16% (after purification), HPLC $t_R = 16.98$ min. (Gradient 1), R_f ($CHCl_3$-Acetone 6:4) = 0.71, ^{1}H-NMR (700 MHz, $CDCl_3$), δ 0.98 (*pseudo*t, 6H, $J = 6.0$ Hz, $CH_{3Leupep}$), 1.12 (d, 3H, $J = 6.3$ Hz, H-2′), 1.61 to 1.75 (m, 3H, $CH_{2Leupep} + CH_{Leupep}$), 2.71 (ddd, 1H, $J = 15.3$ Hz, $J = 11.2$ Hz, $J = 2.5$ Hz, H-4), 3.26 (ddd, 1H, $J = 15.2$ Hz, $J = 4.0$ Hz, $J = 1.4$ Hz, H-4), 3.60 (dd, 1H, $J = 11.2$ Hz, $J = 4.3$ Hz, H-3), 3.76 (s, 3H, OCH_3), 4.35 (bs, 1H, H-1′), 4.38 (bs, 1H, H-1), 4.70 (td, 1H, $J = 8.4$ Hz, $J = 5.6$ Hz, α-CH_{Leupep}), 5.12 to 5.17 (m, 2H, CH_{2Z}), 5.33 (d, 1H, $J = 7.4$ Hz, NH_Z), 7.10 to 7.50 (m, 10H, Ar + NH_{Leupep}), 8.28 (s, 1H, NH_{ind}). ^{13}C-NMR (175 MHz, as HSQC, $CDCl_3$), δ 15.3 (C-2′), 21.8 ($CH_{3Leupep}$), 22.6 ($CH_{3Leupep}$), 25.0 (CH_{Leupep}), 25.8 (C-4), 41.4 ($CH_{2Leupep}$), 49.3 (C-1′), 50.4 (α-CH_{Leupep}), 52.3 (OCH_3), 56.8 (C-1), 57.3 (C-3), 66.9 (CH_{2Z}), 111.0-128.5 (Ar). ROESY (700 MHz, $CDCl_3$) H-1 (H-3, H-2′, NH_Z, NH_{ind}), H-3 (H-1, H-4, NH_{Leupep}), H-4 (H-3, Ar), H-1′ (H-2′, NH_{ind}), H-2′ (H-1, H-1′), α-CH_{Leupep} ($CH_{2Leupep}$ + CH_{Leupep}, $CH_{3Leupep}$, NH_{Leupep}). ESI-MS, *m/z*: 521 $[M+H]^+$, 543 $[M+Na]^+$. Exact mass calculated for $C_{29}H_{36}N_4O_5Na$ $[M+Na]^+$: 543.2583, found: 543.2591.

Compound **8a (*trans* L-Ala/Leu(pep)**: Yield: 41% (after purification), HPLC $t_R = 16.91$ min. (Gradient 1), R_f ($CHCl_3$-Acetone 6:4) = 0.63, ^{1}H-NMR (700 MHz, $CDCl_3$), δ 0.89 (d, 6H, $J = 6.5$ Hz, $CH_{3Leupep}$), 1.35 (d, 3H, $J = 6.5$ Hz, H-2′), 1.49 to 1.53 (m, 1H, $CH_{2Leupep}$), 1.58 (sept, 1H, $J = 6.3$ Hz, CH_{Leupep}), 1.62 to 1.66 (m, 1H, $CH_{2Leupep}$), 2.96 (bdd, 1H, $J = 15.4$ Hz, $J = 6.3$ Hz, H-4), 3.16 (dd, 1H, $J = 15.8$ Hz, $J = 5.3$ Hz, H-4), 3.67 (s, 3H, OCH_3), 3.90 (t, 1H, $J = 6.2$ Hz, H-3), 4.12 (d, 1H, $J = 5.0$ Hz H-1), 4.22 (bs, 1H, H-1′), 4.60 (td, 1H, $J = 8.6$ Hz, $J = 5.4$ Hz, α-CH_{Leupep}), 5.00 to 5.06 (m, 2H, CH_{2Z}), 5.10 (d, 1H, $J = 7.4$ Hz, NH_Z), 6.97 (bd, 1H, $J = 5.9$ Hz, NH_{Leupep}), 7.10 to 7.51 (m, 9H, Ar), 8.19 (s, 1H, NH_{ind}). ^{13}C-NMR (175 MHz, as HSQC, $CDCl_3$), δ 18.2 (C-2′), 21.8 ($CH_{3Leupep}$), 22.7 ($CH_{3Leupep}$), 23.8 (C-4), 24.8 (CH_{Leupep}), 41.4 ($CH_{2Leupep}$), 49.3 (C-1′), 50.5 (α-CH_{Leupep}), 52.1 (OCH_3), 54.0 (C-3), 55.1 (C-1), 66.8 (CH_{2Z}), 111.0-128.5 (Ar). ROESY (700 MHz, $CDCl_3$) H-1 (H-2′, NH_Z), H-3 (H-4, H-1′, H-2′, NH_{Leupep}), H-4 (H-3, Ar), H-1′ (H-3, H-2′), H-2′ (H-1, H-3, H-1′, NH_Z), α-CH_{Leupep} ($CH_{2Leupep}$, CH_{Leupep}, $CH_{3Leupep}$, NH_{Leupep}). ESI-MS, *m/z*: 521 $[M+H]^+$, 543 $[M+Na]^+$. Exact mass calculated for $C_{29}H_{36}N_4O_5Na$ $[M+Na]^+$: 543.2583, found: 543.2575.

Compound **7f (*cis* D-Ala/Leu(pep)**: Yield: 53% (after purification), HPLC $t_R = 17.14$ min. (Gradient 1), R_f ($CHCl_3$-Acetone 6:4) = 0.54, ^{1}H-NMR (700 MHz, $CDCl_3$), δ 0.96 (d, 3H, $J = 6.4$ Hz, $CH_{3Leupep}$), 0.98 (d, 3H, $J = 6.3$ Hz, $CH_{3Leupep}$), 1.39 (d, 3H, $J = 6.0$ Hz, H-2′), 1.60 to 1.73 (m, 3H, $CH_{2Leupep} + CH_{Leupep}$), 2.77 (ddd, 1H, $J = 15.3$ Hz, $J = 11.2$ Hz, $J = 2.1$ Hz, H-4), 3.20 (ddd, 1H, $J = 15.4$ Hz, $J = 4.3$ Hz, $J = 1.4$ Hz, H-4), 3.71 (dd, 1H, $J = 11.1$ Hz, $J = 4.3$ Hz, H-3), 3.76 (s, 3H, OCH_3), 4.30 (bs, 1H, H-1), 4.45 (bs, 1H, H-1′), 4.73 (td, 1H, $J = 8.4$ Hz, $J = 5.6$ Hz, α-CH_{Leupep}), 4.96 (bs, 2H, CH_{2Z}), 517 (d, 1H, $J = 7.0$ Hz, NH_Z), 6.92 (d, 1H, $J = 8.5$ Hz, NH_{Leupep}), 7.10 to 7.48 (m, 9H, Ar), 8.44 (s, 1H, NH_{ind}). ^{13}C-NMR (175 MHz, as HSQC, $CDCl_3$), δ 17.7 (C-2′), 21.9 ($CH_{3Leupep}$), 22.7 ($CH_{3Leupep}$), 24.9 (CH_{Leupep}), 25.8 (C-4), 41.5 ($CH_{2Leupep}$), 47.9 (C-1′), 50.3 (α-CH_{Leupep}), 52.3 (OCH_3), 57.5 (C-1), 57.9 (C-3), 66.9 (CH_{2Z}), 111.2 to 128.6 (Ar). ROESY (700 MHz, $CDCl_3$) H-1 (H-3, H-2′, NH_{ind}), H-3 (H-1, H-4, NH_{Leupep}), H-4 (H-3, NH_{Leupep}, Ar), H-1′ (H-1, H-2′, NH_{ind}), H-2′ (H-1, H-1′, NH_Z), α-CH_{Leupep} ($CH_{2Leupep}$ + CH_{Leupep}, $CH_{3Leupep}$ NH_{Leupep}). ESI-MS, *m/z*: 521 $[M+H]^+$, 543 $[M+Na]^+$ ESI-MS, *m/z*: 521 $[M+H]^+$, 543 $[M+Na]^+$. Exact mass calculated for $C_{29}H_{36}N_4O_5Na$ $[M+Na]^+$: 543.2583, found: 543.2586.

Peptide 9

0.42 mmol (0.2 g) of compound **6a** was dissolved in 15 mL CH_3OH and 10% Pd/C was added. The suspension was vigorously stirred and saturated with hydrogen under atmospheric pressure, until the starting material was not detected (5 h). The reaction mixture was filtrated through a Celite pad and the filtrate evaporated to dryness. 0.38 mmol (0.13 g) of crude product was redissolved in 10 mL CH_2Cl_2 and cooled in the ice bath. 0.42 mmol (0.093 g) Z-Ala-OH, followed by 0.42 mmol (56 mg) HOBt and 0.42 mmol (0.134 g) TBTU, and 0.76 mmol (0.133 mL) DIPEA were added. The reaction mixture was stirred for 4 h and then the solvent was evaporated. The residue was redissolved in 15 mL AcOEt and extracted with 1 M HCl, sat. $NaHCO_3$ and washed with brine, dried over Na_2SO_4. The crude product was purified by flash chromatography ($CHCl_3$-Acetone).

Yield: 62% (after purification), R_f ($CHCl_3$-Acetone 6:4) = 0.38, ^{1}H-NMR (700 MHz, $CDCl_3$), δ 1.16 (d, 3H, $J = 6.7$ Hz, $CH_{3AlaCbz}$), 1.28 (d, 3H, $J = 6.8$ Hz, H-2′), 1.40 (d, 3H, $J = 7.2$ Hz, $CH_{3Alapep}$), 2.91 (ddd, 1H, $J = 15.9$ Hz, $J = 7.5$ Hz, $J = 1.4$ Hz, H-4), 3.16 (dd, 1H, $J = 15.9$ Hz, $J = 5.2$ Hz, H-4), 3.68 (s, 3H, OCH_3), 3.87 (bt, $J = 6.1$ Hz, H-3), 4.13 (bs, 1H, CH_{AlaZ}), 4.24 (bd, 1H, $J = 3.4$ Hz H-1), 4.35 (m, 1H, H-1′), 4.57 (p, 1H, $J = 7.0$ Hz, CH_{Alapep}) 5.06 to 5.16 (bs, 2H, CH_{2Cbz}), 5.33 (bs, 1H, NH_Z), 6.49 (d, 1H, $J = 8.1$ Hz, $NH_{H-1'}$), 7.06 to 7.50 (m, 9H, Ar), 7.21 (bs, 1H, NH_{Alapep}), 8.48 (s, 1H, NH_{ind}). ^{13}C-NMR (175 MHz, as HSQC, $CDCl_3$), δ 17.1 (C-2′), 17.8 ($CH_{3Alapep}$), 17.9 (CH_{3AlaZ}), 23.9 (C-4), 47.8 (CH_{Alapep}), 47.9 (C-1′), 51.2 (CH_{AlaZ}), 52.1 (OCH_3), 53.8 (C-3), 53.9 (C-1), 66.9 (CH_{2Z}), 94.3-128.6 (Ar). ROESY (700 MHz, $CDCl_3$, t = 245.2 K) H-1 + CH_{AlaZ} (H-2′ + CH_{3AlaZ}, NH_Z, $NH_{H-1'}$), H-3 (H-2′ + CH_{3AlaZ}, H-4, H-1′, NH_{Alapep}), H-4 (H-3), H-1′ (H-2′ + CH_{3AlaZ}, H-3, NH_{ind}), H-2′ + CH_{3AlaZ} (H-1 + CH_{AlaZ}, H-3, H-1′, NH_Z,), CH_{Alapep} ($CH_{3Alapep}$, NH_{Alapep}).

Compound **11a (*cis* L-Ala-L-Ala)**: Yield: 37% (on the basis of ^{1}H-NMR, with reference to the integration of OCH_3 signals of products and H-Trp-OCH_3), HPLC $t_R = 13.5$ min. (Gradient 2), ^{1}H-NMR (500 MHz, $CDCl_3$), δ 1.07 (d, 3H, $J = 5.8$ Hz, H-2′), 1.39 (d, 3H, $J = 7.1$ Hz, CH_{3AlaZ}), 2.81 (bt, 1H, $J = 13.0$ Hz, H-4), 3.13 (dd, 1H, $J = 14.8$ Hz, $J = 3.0$ Hz, H-4), 3.73 (bd, 1H, $J = 9.5$ Hz, H-3), 3.81 (s, 3H, OCH_3), 4.29 (m, 1H, α-CH_{AlaZ}), 4.43 (bs, 1H, H-1), 4.63 (m, 1H, H-1′), 5.12 (q, 2H, $J = 12.2$ Hz, CH_{2Z}), 5.64 (bd, 1H, $J = 5.5$ Hz, NH_Z), 7.17 (d, 1H, $J = 7.3$ Hz, NH_{Ala}), 7.07 to 7.46 (m, 9H, Ar), 9.05 (s, 1H, NH_{ind}). ^{13}C-NMR (125 MHz, as HSQC, $CDCl_3$), δ 14.6 (C-2′), 18.8 (CH_{3AlaZ}), 25.8 (C-4), 47.8 (C-1′), 51.1 (α-CH_{AlaZ}), 52.3 (OCH_3), 55.8 (C-1), 56.0 (C-3), 67.5 (CH_{2Z}), 111.5 to 128.4 (Ar). ROESY (500 MHz, $CDCl_3$) H-1 (H-3, H-1′, H-2′), H-3 (H-1, H-4), H-4 (H-3, Ar), H-1′ (H-1, H-2′, NH_{ind}), H-2′ (H-1, H-1′, Ar), α-CH_{AlaZ} (CH_{3AlaZ}, NH_Z, NH_{Ala}). ESI-MS, *m/z*: 479 $[M+H]^+$, 501 $[M+Na]^+$. Exact mass calculated for $C_{26}H_{30}N_4O_5Na$ $[M+Na]^+$: 501.2114, found: 501.2111.

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Compound **12a (*trans* L-Ala-L-Ala)**: Yield: 17% (on the basis of ^{1}H-NMR, with reference to the integration of OCH_3 signals of products and H-Trp-OCH_3), HPLC t_R = 13.7 min. (Gradient 2), ^{1}H-NMR (500 MHz, $CDCl_3$), δ 0.7 (d, 3H, *J* = 6.0 Hz, H-2′), 1.28 (d, 3H, *J* = 6.8 Hz, CH_{3AlaZ}), 3.07 (ddd, 1H, *J* = 15.3 Hz, *J* = 6.1 Hz, *J* = 2.0 Hz, H-4), 3.28 (d, 1H, *J* = 15.2 Hz, H-4), 3.62 (s, 3H, OCH_3), 3.99 (m, 1H, H-1′), 4.06 (dd, 1H, *J* = 6.1 Hz, *J* = 2.5 Hz H-3), 4.51 (m, 1H, α-CH_{AlaZ}), 4.56 (bs, 1H, H-1), 4.98 to 5.06 (m, 2H, CH_{2Z}), 5.46 (bd, 1H, *J* = 6.5 Hz, NH_Z), 6.57 (d, 1H, *J* = 7.5 Hz, NH_{Ala}), 7.04 to 7.45 (m, 9H, Ar), 8.55 (s, 1H, NH_{ind}). ^{13}C-NMR (125 MHz, as HSQC, $CDCl_3$), δ 17.6 (CH_{3AlaZ}), 18.8 (C-2′), 23.3 (C-4), 47.4 (α-CH_{AlaZ}), 50.6 (C-1′), 52.1 (OCH_3), 53.3 (C-1), 53.8 (C-3), 67.0 (CH_{2Z}), 111.4-128.5 (Ar). ROESY (500 MHz, $CDCl_3$) H-1 (NH_{ind}), H-3 (H-4, CH_{3AlaZ}), H-4 (H-3, Ar), H-1′ (H-2′, NH_{Ala}), H-2′ (H-1′, NH_Z, Ar), α-CH_{AlaZ} (CH_{3AlaZ}, NH_{ind}). ESI-MS, *m/z*: 479 $[M+H]^+$, 501 $[M+Na]^+$. Exact mass calculated for $C_{26}H_{30}N_4O_5Na$ $[M+Na]^+$: 501.2114, found: 501.2119.

Compound **11b (*cis* L-Ala-D-Ala)**: Yield: 60% (on the basis of ^{1}H-NMR, with reference to the integration of OCH_3 signals of products and H-Trp-OCH_3), HPLC t_R = 13.8 min. (Gradient 2), ^{1}H-NMR (500 MHz, $CDCl_3$), δ 1.1 (d, 3H, *J* = 5.7 Hz, H-2′), 1.24 (d, 3H, *J* = 6.7 Hz, CH_{3AlaZ}), 2.82 (ddd, 1H, *J* = 14.7 Hz, *J* = 11.1 Hz, *J* = 2.3 Hz, H-4), 3.14 (ddd, 1H, *J* = 14.9 Hz, *J* = 3.7 Hz, *J* = 1.2 Hz, H-4), 3.75 (dd, 1H, *J* = 10.8 Hz, *J* = 3.2 Hz H-3), 3.82 (s, 3H, OCH_3), 4.04 (m, 1H, H-1′), 4.40 (bs, 1H, H-1), 4.52 (m, 1H, α-CH_{AlaZ}), 4.82 (d, 1H, *J* = 11.9 Hz, CH_{2Z}), 5.02 (d, 1H, *J* = 12.1 Hz, CH_{2Z}), 5.14 (bd, 1H, *J* = 5.8 Hz, NH_Z), 6.75 (d, 1H, *J* = 8.0 Hz, NH_{D-Ala}), 7.04 to 7.45 (m, 9H, Ar), 8.99 (s, 1H, NH_{ind}). ^{13}C-NMR (125 MHz, as HSQC, $CDCl_3$), δ 16.3 (CH_{3AlaZ}), 18.1 (C-2′), 25.5 (C-4), 47.1 (α-CH_{AlaZ}), 50.8 (C-1′), 52.6 (OCH_3), 56.0 (C-3), 56.3 (C-1), 67.2 (CH_{2Z}), 111.4 to 128.4 (Ar). ROESY (500 MHz, $CDCl_3$) H-1 (H-3, CH_{3AlaZ}), H-3 (H-1, H-4, CH_{3AlaZ}), H-4 (H-3, Ar), H-1′ (H-2′, NH_{D-Ala}), H-2′ (H-1′, NH_Z), α-CH_{AlaZ} (CH_{3AlaZ}, NH_{ind}). ESI-MS, *m/z*: 479 $[M+H]^+$, 501 $[M+Na]^+$. Exact mass calculated for $C_{26}H_{30}N_4O_5Na$ $[M+Na]^+$: 501.2114, found: 501.2119.

Compound **11c (*cis* L-Ala-L-Val)**: Yield: 30% (on the basis of ^{1}H-NMR, with reference to the integration of OCH_3 signals of products and H-Trp-OCH_3), HPLC t_R = 14.1 min. (Gradient 2), ^{1}H-NMR (500 MHz, $CDCl_3$), δ 0.83 (d, 3H, *J* = 6.6 Hz, H-3′), 0.91 (d, 3H, *J* = 6.6 Hz, H-3′), 1.34 (d, 3H, *J* = 6.8 Hz, CH_{3AlaZ}), 2.15 (m, 1H, H-2′), 2.79 (bt, 1H, *J* = 13.0 Hz, H-4), 3.11 (dd, 1H, *J* = 14.8 Hz, *J* = 2.8 Hz, H-4), 3.70 (bd, 1H, *J* = 9.5 Hz, H-3), 3.80 (s, 3H, OCH_3), 4.20 to 4.25 (m, 2H, H-1′ + α-CH_{AlaZ}), 4.28 (bs, 1H, H-1), 5.06 (q, 2H, *J* = 11.9 Hz, CH_{2Z}), 5.56 (bd, 1H, *J* = 4.6 Hz, NH_Z), 6.74 (d, 1H, *J* = 7.5 Hz, NH_{Val}), 7.06 to 7.45 (m, 9H, Ar), 9.03 (s, 1H, NH_{ind}). ^{13}C-NMR (125 MHz, as HSQC, $CDCl_3$), δ 18.3 (C-3′ + CH_{3AlaZ}), 20.3 (C-3′), 25.8 (C-4), 28.9 (C-2′), 51.3 (α-CH_{AlaZ}), 52.3 (OCH_3), 55.3 (C-1), 56.3 (C-3), 57.5 (C-1′), 67.5 (CH_{2Z}), 111.4 to 128.4 (Ar). ROESY (500 MHz, $CDCl_3$) H-1 (H-3, H-3′), H-3 (H-1, H-4), H-4 (H-3, Ar), H-1′ + α-CH_{AlaZ} (H-2′, H-3′, CH_{3AlaZ}, NH_Z, NH_{Val}, NH_{ind}), H-2′ (, H-3′, H-1′ + α-CH_{AlaZ}), H-3′ (H-1′ + α-CH_{AlaZ}, H-2′, NH_{Val}). ESI-MS, *m/z*: 507 $[M+H]^+$, 529 $[M+Na]^+$. Exact mass calculated for $C_{28}H_{34}N_4O_5Na$ $[M+Na]^+$: 529.2427, found: 529.2416

Compound **12c (*trans* L-Ala-L-Val)**: Yield: 46% (on the basis of ^{1}H-NMR, with reference to the integration of OCH_3 signals of products and H-Trp-OCH_3), HPLC t_R = 15.6 min. (Gradient 2), ^{1}H-NMR (500 MHz, $CDCl_3$), δ 0.4 (d, 3H, *J* = 6.6 Hz, CH_{3AlaZ}), 0.98 (d, 3H, *J* = 6.5 Hz, H-3′), 1.14 (d, 3H, *J* = 6.7 Hz, H-3′), 1.81 (m, 1H, H-2′), 3.04 (ddd, 1H, *J* = 15.4 Hz, *J* = 6.3 Hz, *J* = 1.6 Hz, H-4), 3.30 (d, 1H, *J* = 15.2 Hz, H-4), 3.61 (s, 3H, OCH_3), 3.99 (m, 1H, α-CH_{AlaZ}), 4.02 to 4.06 (m, 2H, H-3 + H-1′), 4.79 (bs, 1H, H-1), 4.97 to 5.03 (m, 2H, CH_{2Z}), 5.70 (bd, 1H, *J* = 7.0 Hz, NH_Z), 6.65 (d, 1H, *J* = 9.3 Hz, NH_{Val}), 7.01 to 7.42 (m, 9H, Ar), 8.51 (s, 1H, NH_{ind}). ^{13}C-NMR (125 MHz, as HSQC, $CDCl_3$), δ 18.8 (CH_{3AlaZ}), 19.5 (C-3′), 20.3 (C-3′), 23.0 (C-4), 29.7 (C-2′), 49.8 (C-1), 50.6 (α-CH_{AlaZ}), 52.1 (OCH_3), 54.1 (C-3), 56.5 (C-1′), 67.0 (CH_{2Z}), 111.1 to 128.3 (Ar). ROESY (500 MHz, $CDCl_3$) H-1 (H-3 + H-1′, H-2′, H-3′, NH_{ind}), H-3 + H-1′ (H-1, H-4, H-2′, H-3′, NH_{ind}), H-4 (H-3 + H-1′, Ar), H-2′ (H-1, H-3 + H-1′, H-3′, NH_{Val}), H-3′ (H-1, H-3 + H-1′, H-2′), α-CH_{AlaZ} (CH_{3AlaZ}, NH_{Val}). ESI-MS, *m/z*: 507 $[M+H]^+$, 529 $[M+Na]^+$. Exact mass calculated for $C_{28}H_{34}N_4O_5Na$ $[M+Na]^+$: 529.2427, found: 529.2443.

Enamide **13a**: HPLC t_R = 17.3 min. (Gradient 1), ^{1}H-NMR (200 MHz, $CDCl_3$), duplication of some signals resulted from *cis/trans* isomerization of amide (urethane – Z) bond δ 1.29 (duplicated d, 3H, *J* = 6.9 Hz, CH_{3AlaZ}), 1.86 (d, 3H, *J* = 5.1 Hz, CH_{3Ala}), 4.70 to 4.90 (duplicated m, 1H, CH_{Ala}), 5.20 (bs, 2H, CH_{2Z}), 5.96 and 6.08 (2xs, 1H, NH_{CO}), 7.37 (m, 5H, Ar), 7.62 and 7.68 (duplicated s, 1H, HCC). ESI-MS, *m/z*: 283 $[M+Na]^+$, 543 $[2M+Na]^+$, 803 $[3M+Na]^+$, 1063 $[4M+Na]^+$

Enamide **13c**: HPLC t_R = 19.3 min. (Gradient 1), ^{1}H-NMR (200 MHz, $CDCl_3$), duplication of some signals resulted from *cis/trans* isomerization of amide (urethane – Z) bond δ 1.14 (d, 6H, *J* = 6.8 Hz, CH_{3Val}), 1.29 (duplicated d, 3H, *J* = 6.9 Hz, CH_{3Ala}), 2.39 (s, 1H, *J* = 6 Hz, CH_{Val}), 4.70 to 4.90 (duplicated m, 1H, CH_{Ala}), 5.21 (s, 2H, CH_{2Z}), 5.97 and 6.09 (2xs, 1H, NH_{CO}), 7.37 (m, 5H, Ar), 7.94 (bs, 1H, HC = C). ESI-MS, *m/z*: 311 $[M+Na]^+$, 599 $[2M+Na]^+$, 887 $[3M+Na]^+$, 1175 $[4M+Na]^+$

Enamide **13e**: HPLC t_R = 19.4 min. (Gradient 1), ^{1}H-NMR (200 MHz, $CDCl_3$), duplication of some signals resulted from *cis/trans* isomerization of amide (urethane – Z) bond δ 1.14 (d, 6H, *J* = 6.8 Hz, CH_{3Val}), 1.29 (duplicated d, 3H, *J* = 6.9 Hz, CH_{3Ala}), 2.39 (s, 1H, *J* = 6 Hz, CH_{Val}), 4.71 to 4.91 (duplicated m, 1H, CH_{Ala}), 5.21 (s, 2H, CH_{2Z}), 5.98 and 6.10 (2xs, 1H, NH_{CO}), 7.37 (m, 5H, Ar), 7.65 (bs, 1H, HC = C). ESI-MS, *m/z*: 289 $[M+H]^+$

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