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Diastereoselective Pictet–Spengler condensation of tryptophan with α-amino aldehydes as chiral carbonyl components

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Abstract

The Pictet–Spengler reaction of Trp with α-amino aldehydes derived from L and D-amino acids was studied in terms of double stereodifferentiation. The results observed for D-amino aldehydes represent 'matched' situation (one diastereoisomer was formed) whereas with L-amino aldehydes 'mismatched' (two diastereoisomers were formed). The conformation of newly formed six-membered ring was analyzed. It was found that stable conformers were different for cis and trans isomers.

1. Introduction

Since its discovery, the Pictet–Spengler reaction[1] has been extensively studied in the areas of syntheses of different heterocycle systems. One of the possible uses of this reaction is in preparation of restricted analogs of aromatic amino acids occurring in proteins (Phe, Tyr, Trp, His). These aromatic amino acids very often play a crucial role in the interaction of ligand with the corresponding receptor-protein and therefore many efforts have been done to obtain their cyclic or constrained analogs. Those analogs have been introduced into the bioactive peptides to obtain local constraints and therefore provide insight into the structures required for bioactivity. Conformational restrictions of such residues have been a successful strategy to influence the biological activity, potency, selectivity, metabolic stability, and many other pharmacological properties.[2–6]

Tryptophan is often a significant part of peptide ligands, which determines their affinity for receptors therefore different constrained analogs of this amino acid have recently gained considerable importance in the structure–activity studies of bioactive peptides. One of the most successfully applied analogs of constrained tryptophan is the one in which the α-nitrogen has been linked to the aromatic ring through a methylene unit, resulting in the six-membered ring derivative 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (Tcc). It is prepared via a Pictet–Spengler cyclization from chiral tryptophan ester and formaldehyde.[7,8] Other aldehydes generate a new stereogenic center. Moreover, the products of the Pictet–Spengler reaction can be treated as 1,3-disubstituted tetrahydro-β-carbolines and cis/trans isomers can be obtained. Therefore, the main challenge of this cyclization is stereoselectivity and the ratio of isomers. Different conditions, such as temperature, solvents, and achiral carbonyl substrates, were studied to improve the selectivity of the Pictet–Spengler reaction.[9–12] It was found that the major compound was the cis isomer when reactions were carried at low temperature. It is also well known that the N_b-benzyl or N_b-diphenylmethyl substituted tryptophan esters favored trans isomers.[13–17] Recently it has been shown that the Pictet–Spengler reaction of tryptophan allyl ester is cis-specific.[18] Other synthetic strategies used to influence the results of the Pictet–Spengler condensation include the use of chiral catalysts,[19] chiral auxiliaries,[20,21] or optically active carbonyl compounds.[22]

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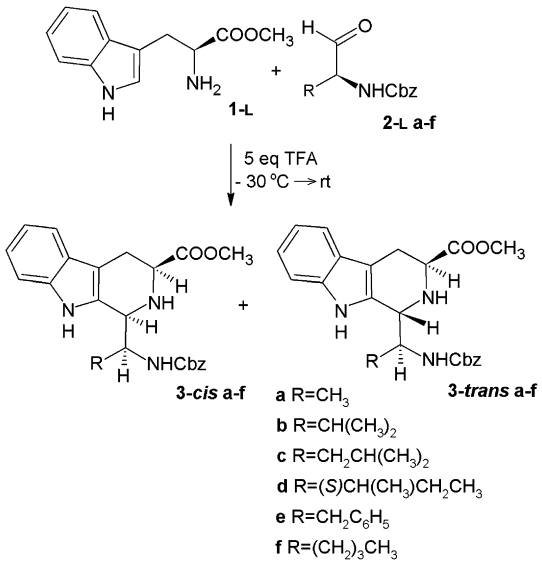
In this paper we present the diastereoselectivity study of the Pictet–Spengler condensation of tryptophan and α-amino aldehydes derived from L or D-amino acids as chiral carbonyl components. In the literature there are some examples of using α-amino aldehydes in the Pictet–Spengler reaction.[23–29] We have been studied the dependence of isomeric products from the configuration of the aldehyde and hypothesized on the intermediate iminium cation structure as the factor responsible for the stereoselectivity.

The cis and trans products of such condensations can serve as constrained tryptophan dipeptide mimetics. Such mimetics can be incorporated into the peptide sequence to reduce the flexibility of the peptide chain and probe the bioactive conformation. The trans steroisomer of tetrahydro-β-carboline derivatives could serve as β-turn dipeptide mimetic as it was shown for similar tetrahydroisoquinoline compounds by Grieco[28] and Lesma.[30] Such mimetics incorporated into peptide chain could introduce β-turn motif in the particular part of peptide chain.

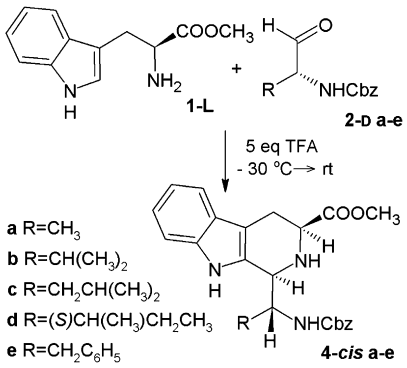
2. Results and discussion

The Pictet–Spengler reaction is an intramolecular cyclization of the intermediate iminium ion of tryptophan. We utilized a conventional Pictet–Spengler synthetic protocol. Methyl esters of L and D-tryptophan were prepared by a standard procedure, α-amino aldehydes **2a–f** were prepared in good or excellent yields via the Fehrentz–Castro procedure[31] and used immediately without purification to avoid racemization.[32] For the protection of the α-amino function of the amino aldehydes, benzyloxycarbonyl group (Cbz) was chosen. The reaction was performed in CH_2Cl_2 in the presence of 5 equiv of TFA. The temperature conditions were controlled, for 5 h the reaction was stirred at −30 °C and then stirring was continued at room temperature overnight.

Two series of tetrahydro-β-carboline analogs were prepared, with the moiety of L or D α-amino aldehydes (Schemes 1 and 2).



Scheme 1. Pictet–Spengler cyclization of L-Trp–OMe with α-amino aldehydes derived from L-amino acids.



Scheme 2. Pictet–Spengler cyclization of L-Trp–OMe with α-amino aldehydes derived from D-amino acids.

The resulting diastereomeric products (cis/trans) were easily separable. The ratio before purification and after silica gel column was comparable. The well separated signals of the methyl ester protons allowed us to define the ratio of diastereomers by NMR measurements before purification was undertaken (Fig. 1).

The configuration at C-1 (the C-3 configuration was known, because L-Trp–OMe or D-Trp–OMe **1** was used as the starting material) was determined by 2D NMR (ROESY spectra) analysis[33] and compared with Cook's method of assignment based on ^{13}C NMR.[34]

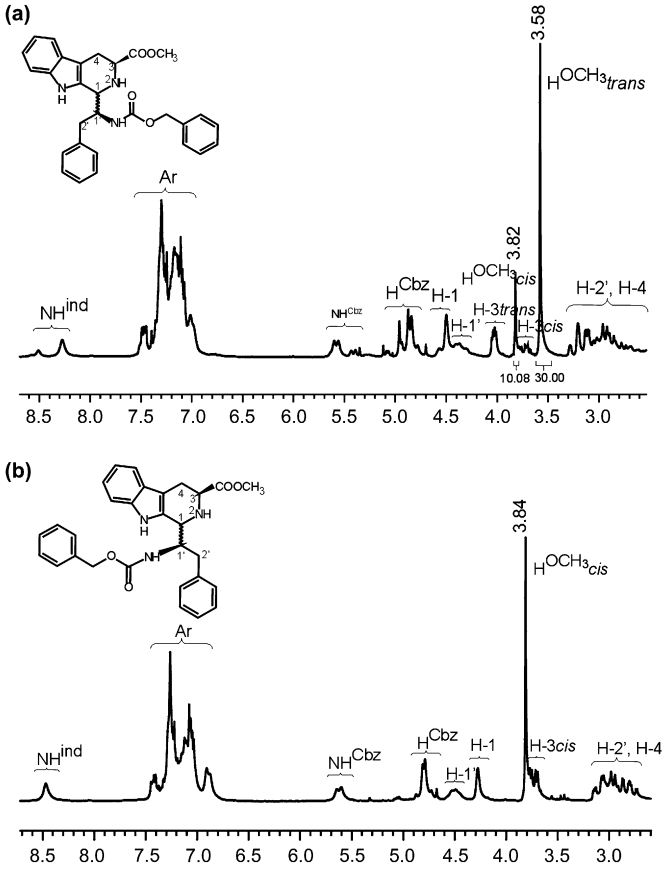


Figure 1. 200 MHz ^1H NMR spectra before purification for analogs of L (a) and D (b) amino aldehydes.

The NOE effect observed between the H-1 and H-3 protons was diagnostic for identification of the cis configuration. For the trans diastereomers, the exchange of magnetization among the H-1 and H-3 protons was not observed (Fig. 2).

Our results (Table 1) showed that the Pictet–Spengler reaction of L-Trp–OMe with L-amino aldehydes preferentially led to trans isomers (**3a–f**). In the case of D-amino aldehydes we expected that the ratio of products would be switched. But reactions selectively led to the cis isomers (**4a–e**). No trans products were observed. Our results from the Pictet–Spengler condensation were opposite to the ones obtained by Grieco and co-workers.[29] They showed that the reaction of Fmoc–L-Ala–H with L-DOPA carried at room temperature leads to the cis isomer as the main product. To prove our results we performed the epimerization studies and refluxed small samples of **3-*cis***, **3-*trans***, and **4-*cis*** in MeOH in the presence of TFA. The ratio of cis/trans isomers was not changed after 3 h of reflux.

The Pictet–Spengler cyclization was also performed with the D-Trp–OMe and both Cbz–L-Leu–H and Cbz–D-Leu–H (**2c**). From the reaction with L-amino aldehyde only one isomer was obtained and this was designated as the cis isomer. In the case of D-amino aldehydes, the trans isomer was instead favored (the cis compound was the minor product).

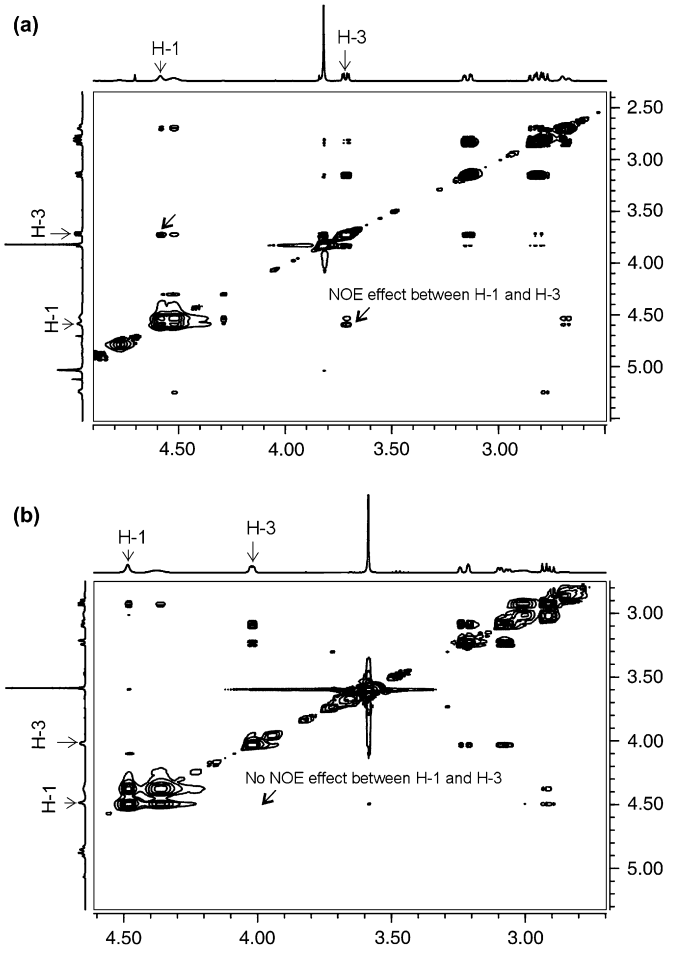


Figure 2. 500 MHz ROESY spectra for **3-*cis* e** (a) and **3-*trans* e** (b).

Table 1
The ratio of cis/trans stereoisomers determined by NMR measurements

Tryptophan	α-Amino aldehyde	cis/trans ratio determined by NMR [%]	
L-Trp	Cbz–L-Ala–H	**3-*cis* a/3-*trans* a**	35/65
L-Trp	Cbz–L-Val–H	**3-*cis* b/3-*trans* b**	0/100
L-Trp	Cbz–L-Leu–H	**3-*cis* c/3-*trans* c**	27/73
L-Trp	Cbz–L-Ile–H	**3-*cis* d/3-*trans* d**	0/100
L-Trp	Cbz–L-Phe–H	**3-*cis* e/3-*trans* e**	29/71
L-Trp	Cbz–L-Nle–H	**3-*cis* f/3-*trans* f**	25/75
L-Trp	Cbz–D-Ala–H	**4-*cis* a**	100/0
L-Trp	Cbz–D-Val–H	**4-*cis* b**	100/0
L-Trp	Cbz–D-Leu–H	**4-*cis* c**	100/0
L-Trp	Cbz–D-allo-Ile–H	**4-*cis* d**	100/0
L-Trp	Cbz–D-Phe–H	**4-*cis* e**	100/0
D-Trp	Cbz–L-Leu–H	**5-*cis* c**	100/0
D-Trp	Cbz–D-Leu–H	**6-*cis* c/6-*trans* c**	15/85

In general, when both substrates for Pictet–Spengler cyclization have the same configuration (L,L or D,D) the diastereomeric mixture of *cis* and *trans* 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines is formed ('mismatched' situation) and the major compound is the trans diastereomer (65–100%). In the case when the configuration of the stereogenic centers in the substrates is in opposition to each other (L,D or D,L) the P–S cyclization gave only cis diastereomer ('matched' situation).

We hypothesized that the Felkin–Ahn model and hydrogen bonded intermediate can explain the observed results (Fig. 3a and b). This is in agreement with earlier model of Thal presented for tryptamine.[25,27]

In the reaction with L-amino aldehydes nucleophilic attack occurs preferentially from the face opposite to the ester group. A hydrogen bond is formed between iminium and benzyloxycarbonyl groups and stabilizes such pseudocyclic structure, explaining the predomination of the trans diastereomer. On the

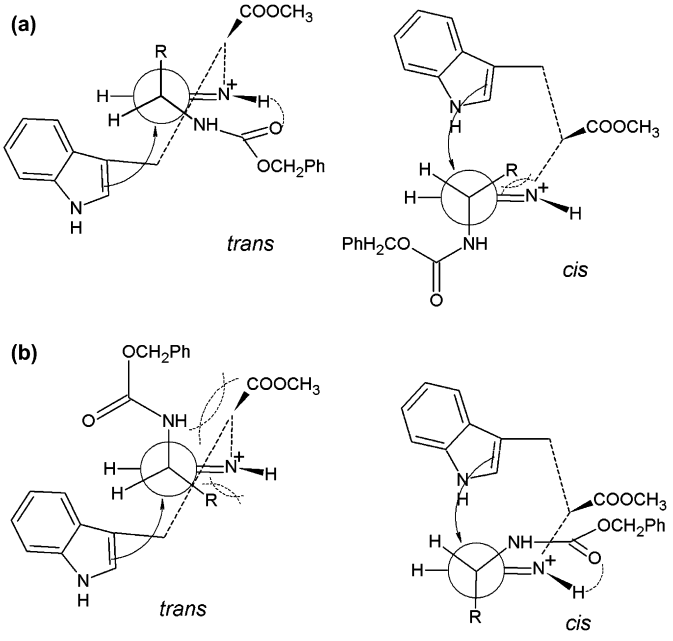


Figure 3. (a) For L-amino aldehydes and (b) for D-amino aldehydes.

other hand for the cis isomer the nucleophilic attack occurs from the same face as the ester group and there is a slight unfavorable steric interaction between R and the iminium nitrogen. The smaller the R, the higher the amount of cis isomer that is observed, this explains the highest amount of cis compound with Cbz–L-Ala–H. For β-branched amino aldehydes the steric hindrance results in unfavorable interactions and in those cases the cis isomer is not formed.

For D-amino aldehydes, attack from the ester group face is preferred. Because the hydrogen bond stabilizes the intermediate structure, only the cis compound is formed. This is because, in the intermediate leading to the trans diastereomer there would be unfavorable interactions, and the transition state would be less stable and in effect trans isomer is not formed.

Cook and co-workers[16,34] found that of the two possible half chair conformations for cis isomer, conformer **B** should represent the more stable form (Fig. 4). Both substituents are located in the equatorial position and are devoid of the unfavorable 1,3-interactions. This is in agreement with our interpretation. For the trans isomer they established that conformer **D** is more stable, this is opposite to our observations.

We suppose that for our compounds, the more stable conformer is **C**. The values of the coupling constant (^3J) between H-3 and H-4 protons were compared with typical values of vicinal H, H coupling constant in cyclohexane (H^a–H^a ^3J 8–13 Hz, H^a–H^e ^3J 2–6 Hz, H^e–H^e ^3J 1–4 Hz) and with values calculated for **3-*cis* c** and **3-*trans* c** by PCModel[35] (Table 2). This proves that the chemical environment for H-3 proton is different in cis and trans diastereomers.

These observations were confirmed by ROESY spectra (Fig. 5). For cis compounds the NOE effect was observed between the H-3 proton and only one H-4a proton. The $COOCH_3$ group was located in the equatorial position. Such an observation is possible only in the **B** conformer. On the other hand the NOE effect for the trans isomer was observed between H-3 and both H-4a and H-4b protons. This means that $COOCH_3$ group is located in the axial position. This suggests that the conformational equilibrium is shifted to the **C** form for the trans analog.

Table 2
Experimental and calculated values of ^3J for cis and trans isomers

^3J (H-3, H-4) for cis diastereomer [Hz]			^3J (H-3, H-4) for trans diastereomer [Hz]		
Experimental values	Calculated values		Experimental values	Calculated values	
	A	**B**		**C**	**D**
11.50	4.71	11.81	6.0	5.49	11.74
4.00	1.65	3.88	2.0	1.34	4.28

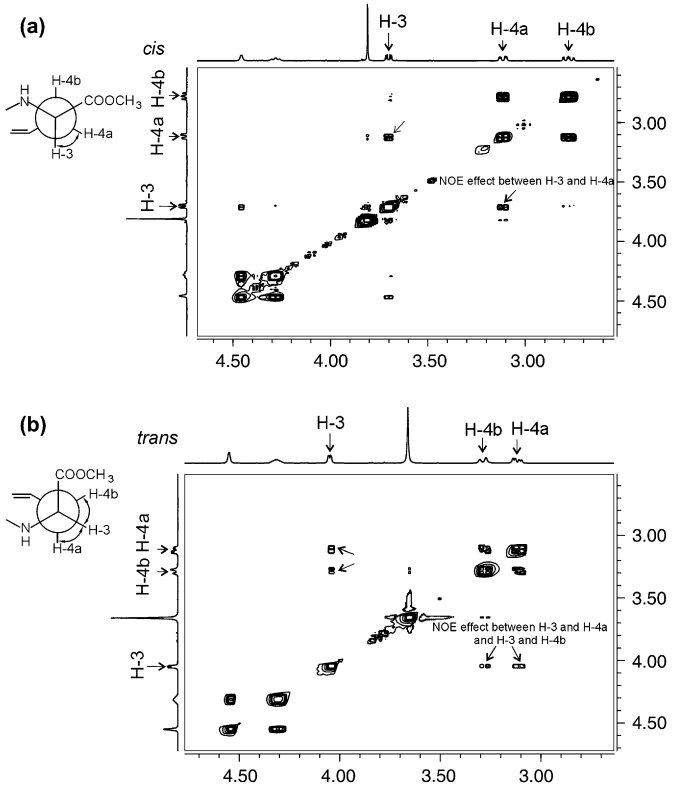


Figure 5. 500 MHz ROESY spectra illustrating mutual interactions of the H-3 and H-4 protons.

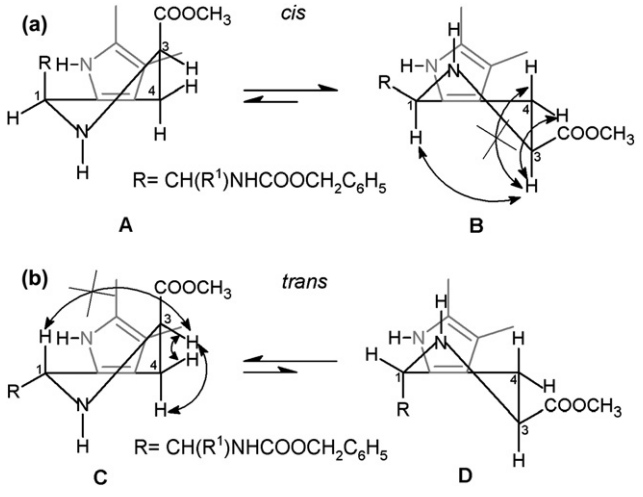


Figure 4. Conformational analysis of *cis* (a) and *trans* (b) 1,3-disubstituted tetrahydro-β-carbolines derived from α-amino aldehydes.

3. Conclusion

We studied the dependence of diastereomeric products of the Pictet–Spengler cyclization from the structure and chirality of substrates. We have found that there is chirality transfer from C-2 of α-amino aldehyde to the newly created stereogenic center in the tetrahydro-β-carboline derivatives. When the reaction is performed with L-Trp–OMe, L-amino aldehydes lead to the mixture of products with the dominance of the trans isomer (more than 65%), however, the reaction with D-amino aldehydes is completely selective and leads to only one diastereomer established as the *cis* tetrahydro-β-carboline derivative. Condensation of D-Trp–OMe and L-aldehydes gives only the cis isomer while the reaction with

D-aldehydes provides a mixture of cis/trans isomers with dominance of the trans compound.

Futhermore, we have demonstrated that the ester group in the trans isomer is located in the axial position and more stable is the conformer when R group (at C-1) is equatorially located. For the cis isomer the more stable conformer is the one in which both groups are equatorially located.

Our results are potentially useful for the synthesis of a wide range of building blocks, peptidomimetic or alkaloid motifs, which needs defined configuration of substituents. The trans steroisomer of tetrahydro-β-carboline derivatives incorporated into peptide chain could introduce β-turn motif in the particular part of peptide chain. It is also possible to use the obtained tetrahydro-β-carbolines for further cyclization with the formation of additional five- or six-membered rings.

4. Experimental

4.1. General

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

RP-HPLC was performed using a RP C-18 column (Discovery BIO Wide Pore C18, ID=0.46 cm, L=25 cm, PS=5 μm). Gradient t=0 min, 97% A, 3% B, t=20 min, 30% A, 70% B was used, flow rate: 1 mL min^{-1}, λ=230 nm. The mobile phases (water, acetonitrile) contained 0.05% TFA. 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 ^{1}H NMR spectra were recorded on a Varian Unity Plus 200 or 500 MHz spectrometers at 300 K with TMS as the internal standard. ^{13}C NMR spectra were acquired as 2D HSQC (quaternary and carbonyl carbons were not observed). The 2D COSY and ROESY spectra were acquired using 500 or 700 MHz spectrometers. For ROESY measurements the standard pulse sequence was applied with mixing time of ms. Mass spectra were recorded on a LCT TOF spectrometer using electrospray ionization (positive ion mode). Optical rotations were measured on a Perkin–Elmer 241 polarimeter. Melting points were measured on a Stuart Scientific melting point apparatus and are uncorrected.

α-Amino aldehydes **2a–f** derived from D or L-amino acids with Cbz for protection of the amino group were prepared according to the procedure previously described and used in the next step without purification.[31]

L and D-Trp–OMe·HCl was converted into the free base by the stirring in the mixture of $CHCl_3$ 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 a yellowish solid was obtained.

4.2. Standard procedure for the Pictet–Spengler reaction

Cbz-L-amino aldehydes or Cbz-D-amino aldehydes (3.3 mmol) were dissolved in CH_2Cl_2 (15 mL) and a solution of L-Trp–OMe or D-Trp–OMe (0.65 g, 2.98 mmol) in CH_2Cl_2 (15 mL) was added. The reaction mixture was cooled to −30 to −40 °C and TFA (1.148 mL, 14.9 mmol) in CH_2Cl_2 (2 mL) was added dropwise. The reaction mixture was cooled (−30 °C) for 5 h and left overnight at room temperature. Then the mixture was diluted with 20 mL CH_2Cl_2 and saturated solution of $NaHCO_3$ (25 mL) was used to neutralize the residual TFA. It was stirred for 30 min and layers were separated. The organic phase was extracted with saturated $NaHCO_3$ (3×20 mL), washed with brine (2×20 mL), and dried over $MgSO_4$. After evaporation yellow foams were obtained. The ratio of the cis/trans isomers in the crude mixture after the reaction was determined by ^{1}H NMR (based on the integration of separated peaks of methyl ester group). Isomers were isolated by flash chromatography ($CHCl_3$–Acetone).

4.2.1. Methyl ester of (1S,3S,1′S) and (1R,3S,1′S)-1-[1′-[(N-benzyloxycarbonyl)amino]ethyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**3-cis a** and **3-trans a**)

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

cis **3a**: Yield: 21%; yellowish foam, mp 76–79 °C; $[\alpha]_D^{20}$ −62.6 (c 1, $CHCl_3$); t_R=16.26 min; R_f ($CHCl_3$–Acetone 8:2) 0.50; IR (KBr): cm^{-1} 3335, 3059, 3033, 2951, 1697, 1511, 1453, 1337, 1267, 1238, 1059, 741, 697; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05 (d, J=6.5 Hz, 3H, CH_3), 2.79 (m_{ABx}, J=13 Hz, J=3 Hz, 1H, H-4a), 3.12 (ddd_{ABx}, J=14.5 Hz, J=2.75 Hz, J=1.5 Hz, 1H, H-4b), 3.73 (dd, J=11.5 Hz, J=4 Hz, 1H, H-3), 3.81 (s, 3H, OCH_3), 4.35 (br s, 1H, H-1′), 4.44 (br s, 1H, H-1), 5.11 and 5.15 (q_{AB}, J=12 Hz, 2H, CH_2Ph), 5.41 (d, J=7 Hz, 1H, NHCbz), 7.09–7.48 (m, 9H, Ar), 8.42 (s, 1H, NH_{in}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.5 (CH_3), 25.8 (C-4), 49.5 (C-1′), 52.2 (OCH_3), 55.9 (C-1), 56.0 (C-3), 67.0 (CH_2Ph), 111.0–129.0 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-3, H-2′), H-3 (H-1, H-4a, H-1′), H-4a (H-3, H-4b), H-4b (H-4a), H-1′ (H-3, H-2′), H-2′ (H-1, H-1′); ESI m/z: 408.2 $[M+H]^+$, 430.2 $[M+Na]^+$; exact mass calcd for $C_{23}H_{25}N_3O_4Na$ $[M+Na]^+$: 430.1743. Found: 430.1740.

trans **3a**: Yield: 45%; white foam, mp 65–67 °C; $[\alpha]_D^{20}$ +86.9 (c 1, $CHCl_3$); t_R=16.31 min; R_f ($CHCl_3$–Acetone 8:2) 0.34; IR (KBr): cm^{-1} 3348, 3059, 3032, 2951, 1705, 1502, 1453, 1341, 1330, 1220, 1057, 742, 698; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.33 (d, J=6 Hz, 3H, CH_3), 3.09 (ddd_{ABx}, J=15 Hz, J=6.25 Hz, J=2 Hz, 1H, H-4a), 3.24 (d_{ABx}, J=14.5 Hz, 1H, H-4b), 3.63 (s, 3H, OCH_3), 4.04 (dd, J=5.75 Hz, J=2.75 Hz, 1H, H-3), 4.36 (br s, J=1.5 Hz, 1H, H-1′), 4.53 (br s, 1H, H-1), 4.96 (s, 2H, CH_2Ph), 5.39 (d, J=9 Hz, 1H, NHCbz), 7.07–7.49 (m, 9H, Ar), 8.40 (s, 1H, NH_{in}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 18.3 (CH_3), 23.6 (C-4), 49.3 (C-1′), 52.3 (OCH_3), 53.5 (C-1), 54.2 (C-3), 66.7 (CH_2Ph), 111.4–128.6 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-2′), H-3 (H-4a, H-4b), H-4a (H-3, H-4b), H-4b (H-3, H-4a), H-1′ (H-2′), H-2′ (H-1, H-1′); ESI m/z: 408.2 $[M+H]^+$, 430.2 $[M+Na]^+$; exact mass calcd for $C_{23}H_{25}N_3O_4Na$ $[M+Na]^+$: 430.1743. Found: 430.1742.

*4.2.2. Methyl ester of (1S,3S,1′S) and (1R,3S,1′S)-1-[1′-[(N-benzyloxycarbonyl)amino]-2′-methyl-propyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**3-cis b** and **3-trans b**)*

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

trans **3b**: Yield: 57%; white foam, mp 69–72 °C; $[\alpha]_D^{20}$ +91.8 (*c* 1, $CHCl_3$); t_R=17.54 min; R_f ($CHCl_3$–Acetone 8:2) 0.58; IR (KBr): cm^{-1} 3351, 3059, 3032, 2960, 1706, 1505, 1454, 1330, 1301, 1220, 741, 698; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.08 (d, *J*=7 Hz, 3H, C*H*$_3$), 1.18 (d, *J*=6.5 Hz, 3H, C*H*$_3$), 1.82–1.89 (m, 1H, H-2′), 3.09 (dd$_{ABx}$, *J*=14.75 Hz, *J*=6 Hz, 1H, H-4a), 3.28 (d$_{ABx}$, *J*=15.5 Hz, 1H, H-4b), 3.63 (s, 3H, OC*H*$_3$), 3.84 (td, *J*=10 Hz, *J*=1.5 Hz, 1H, H-1′), 4.00 (d, *J*=5 Hz, 1H, H-3), 4.74 (s, 1H, H-1), 4.79 and 4.91 (q$_{AB}$, *J*=13 Hz, 2H, C*H*$_2$Ph), 5.37 (d, *J*=9 Hz, 1H, N*H*Cbz), 6.84–7.49 (m, 9H, Ar), 8.32 (s, 1H, N*H*$_{in}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.5, 20.2 (*C*H$_3$), 23.0 (C-4), 30.2 (*C*H), 49.9 (C-1), 52.1 (OC*H*$_3$), 54.1 (C-3), 58.7 (C-1′), 66.3 (*C*H$_2$Ph), 111.4–128.3 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-1′, H-3′), H-3 (H-4a, H-4b), H-4a (H-3, H-4b), H-4b (H-3, H-4a), H-1′ (H-1′, H-3′), H-2′ (H-3′), H-3′ (H-1′, H-2′); ESI *m/z*: 436.2 $[M+H]^+$, 458.2 $[M+Na]^+$; exact mass calcd for $C_{25}H_{29}N_3O_4Na$ $[M+Na]^+$: 458.2056. Found: 458.2056.

*4.2.3. Methyl ester of (1S,3S,1′S) and (1R,3S,1′S)-1-[1′-[(N-benzyloxycarbonyl)amino]-3′-methyl-butyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**3-cis c** and **3-trans c**)*

The ratio of cis/trans isomers determined before purification by ^{1}H NMR (200 MHz) was 27/73.

cis **3c**: Yield: 13%; white foam, mp 67–69 °C; $[\alpha]_D^{20}$ −61.5 (*c* 1, $CHCl_3$); t_R=18.78 min; R_f ($CHCl_3$–Acetone 8:2) 0.62; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.83 (d, *J*=6.5 Hz, 3H, C*H*$_3$), 0.87 (d, *J*=7 Hz, 3H, C*H*$_3$), 0.99 (pseudo-t, *J*=12.25 Hz, 1H, H-2′a), 1.49 (pseudo-td, *J*=12.5 Hz, *J*=3.25 Hz, 1H, H-3′), 1.64 (m, 1H, H-2b′), 2.78 (m$_{ABx}$, *J*=13 Hz, *J*=2.63 Hz, 1H, H-4a), 3.12 (ddd$_{ABx}$, *J*=14.5 Hz, *J*=4 Hz, *J*=2 Hz, 1H, H-4b), 3.70 (dd, *J*=10.5 Hz, *J*=4 Hz, 1H, H-3), 3.81 (s, 3H, OC*H*$_3$), 4.28 (br t, *J*=10 Hz, 1H, H-1′), 4.46 (br s, 1H, H-1), 5.14 (s, 2H, C*H*$_2$Ph), 5.17 (d, *J*=8.5 Hz, 1H, N*H*Cbz), 7.10–7.49 (m, 9H, Ar), 8.36 (s, 1H, N*H*$_{in}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.5, 23.7 (*C*H$_3$), 24.7 (*C*H), 25.7 (C-4), 37.7 (*C*H$_2$), 52.2 (C-1′), 52.3 (OC*H*$_3$), 56.2 (C-3), 56.8 (C-1), 67.2 (*C*H$_2$Ph), 111.2–128.6 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-3, H-2′, H-4′), H-3 (H-1, H-4a, H-1′), H-4a (H-3, H-4b), H-4b (H-4a), H-1′ (H-3, H-2′, H-4′), H-2′ (H-1, H-1′, H-3′, H-4′), H-3′ (H-1′, H-2′, H-4′), H-4′ (H-1, H-1′, H-2′, H-3′); ESI *m/z*: 472.2 $[M+Na]^+$; exact mass calcd for $C_{26}H_{31}N_3O_4Na$ $[M+Na]^+$: 472.2212. Found: 472.2219.

trans **3c**: Yield: 41%; white solid, mp 152–153 °C; $[\alpha]_D^{20}$ +81.4 (*c* 1, $CHCl_3$); t_R=19.14 min; R_f ($CHCl_3$–Acetone 8:2) 0.47; IR (KBr): cm^{-1} 3412, 3060, 3034, 2957, 2865, 1723, 1495, 1454, 1224, 1213, 1051, 738, 694; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.03 (d, *J*=6 Hz, 3H, C*H*$_3$), 1.07 (d, *J*=6 Hz, 3H, C*H*$_3$), 1.47 (m, 1H, H-2′a), 1.61 (m, 1H, H-3′), 1.83 (m, 1H, H-2b′), 3.12 (dd$_{ABx}$, *J*=15 Hz, *J*=5.25 Hz, 1H, H-4a), 3.29 (d$_{ABx}$, *J*=15 Hz, 1H, H-4b), 3.66 (s, 3H, OC*H*$_3$), 4.05 (d, *J*=4 Hz, 1H, H-3), 4.32 (m, 1H, H-1′), 4.55 (s, 1H, H-1), 4.84 and 4.94 (q$_{AB}$, *J*=12.25 Hz, 2H, C*H*$_2$Ph), 5.29 (d, *J*=9 Hz, 1H, N*H*Cbz), 6.94–7.52 (m, 9H, Ar), 8.30 (s, 1H, N*H*$_{in}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.4, 23.2 (*C*H$_3$), 23.2 (C-4), 24.9 (*C*H), 41.4 (*C*H$_2$), 51.1 (C-1′), 52.1 (OC*H*$_3$), 52.6 (C-1), 54.1 (C-3), 66.4 (*C*H$_2$Ph), 111.3–128.3 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-2′), H-3 (H-4a, H-4b), H-4a (H-3, H-4b), H-4b (H-3, H-4a), H-1′ (H-2′, H-3′, H-4′), H-2′ (H-1, H-1′, H-4′), H-3′ (H-1′, H-4′), H-4′ (H-1′, H-2′, H-3′); ESI *m/z*: 450.3 $[M+H]^+$, 472.2 $[M+Na]^+$; exact mass calcd for $C_{26}H_{31}N_3O_4Na$ $[M+Na]^+$: 472.2212. Found: 472.2226.

*4.2.4. Methyl ester of (1R,3S,1′S,2′S)-1-[1′-[(N-benzyloxycarbonyl)amino]-2′-methyl-butyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**3-trans d**)*

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

trans **3d**: Yield: 68%; yellowish solid, mp 138–141 °C; $[\alpha]_D^{20}$ +112.8 (*c* 1, $CHCl_3$); t_R=18.49 min; R_f ($CHCl_3$–Acetone 8:2) 0.68; IR (KBr): cm^{-1} 3401, 3355, 3060, 3034, 2967, 2948, 1730, 1716, 1497, 1456, 1295, 1224, 1204, 1040, 734, 695; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.96 (t, *J*=7.25 Hz, 3H, C*H*$_3$ a), 1.15 (d, *J*=7 Hz, 3H, C*H*$_3$ b), 1.59–1.73 (m, 3H, H-2′ and H-3′), 3.09 (ddd$_{ABx}$, *J*=15.5 Hz, *J*=7 Hz, *J*=2 Hz, 1H, H-4a), 3.29 (d$_{ABx}$, *J*=15.5 Hz, 1H, H-4b), 3.63 (s, 3H, OC*H*$_3$), 3.90 (td, *J*=10 Hz, *J*=2 Hz, 1H, H-1′), 4.00 (d, *J*=4.5 Hz, 1H, H-3), 4.73 (s, 1H, H-1), 4.76 and 4.91 (q$_{AB}$, *J*=12.75 Hz, 2H, C*H*$_2$Ph), 5.41 (d, *J*=10 Hz, 1H, N*H*Cbz), 6.81–7.48 (m, 9H, Ar), 8.47 (s, 1H, N*H*$_{in}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.2, 15.5 (*C*H$_3$), 22.9 (C-4), 26.1 (*C*H$_2$), 36.5 (*C*H), 50.0 (C-1), 52.1 (OC*H*$_3$), 54.3 (C-3), 57.1 (C-1′), 66.2 (*C*H$_2$Ph), 111.4–128.2 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-1′, H-2′, H-5′), H-3 (H-4a, H-4b), H-4a (H-3, H-4b), H-4b (H-3, H-4a), H-1′ (H-1, H-2′, H-3′, H-5′), H-2′ (H-1, H-1′, H-4′, H-5′), H-3′ (H-1′, H-2′, H-4′), H-4′ (H-2′, H-5′), H-5′ (H-1, H-1′, H-2′); ESI *m/z*: 472.2 $[M+Na]^+$; exact mass calcd for $C_{26}H_{31}N_3O_4Na$ $[M+Na]^+$: 472.2212. Found: 472.2228.

*4.2.5. Methyl ester of (1S,3S,1′S) and (1R,3S,1′S)-1-[1′-[(N-benzyloxycarbonyl)amino]phenylethyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**3-cis e** and **3-trans e**)*

The ratio of cis/trans isomers determined before purification by ^{1}H NMR (200 MHz) was 25/75.

cis **3e**: yield: 12%; white foam, mp 83–85 °C; $[\alpha]_D^{20}$ −9.4 (*c* 0.52, $CHCl_3$); t_R=19.00 min; R_f ($CHCl_3$–Acetone 8:2) 0.61; IR (KBr): cm^{-1} 3335, 3060, 3030, 2951, 2847, 1703, 1511, 1453, 1437, 1343, 1267, 1218, 1041, 742, 698; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.67–2.85 (m$_{ABx}$ and m$_{AB}$, 3H, H-4a and H-2′), 3.14 (ddd$_{ABx}$, *J*=15 Hz, *J*=3.75 Hz, *J*=1.75 Hz, 1H, H-4b), 3.72 (dd, *J*=11 Hz, *J*=4 Hz, 1H, H-3), 3.82 (s, 3H, OC*H*$_3$), 4.52 (br s, 1H, H-1′), 4.59 (s, 1H, H-1), 5.03 (s, 2H, C*H*$_2$Ph), 5.24 (d, *J*=7 Hz, 1H, N*H*Cbz),

7.08–7.5 (m, 14H, Ar), 8.54 (s, 1H, NH_{in}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.7 (C-4), 34.8 (C-2′), 52.2 (OCH_3), 55.7 (C-1′), 55.8 (C-1), 56.1 (C-3), 67.0 (CH_2Ph), 111.2–129.0 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-3, H-2′), H-3 (H-1, H-4a, H-1′), H-4a (H-3, H-4b), H-4b (H-4a), H-1′ (H-3, H-2′), H-2′ (H-1, H-1′); ESI *m/z*: 506.2 $[M+Na]^+$; exact mass calcd for $C_{29}H_{29}N_3O_4Na$ $[M+Na]^+$: 506.2056. Found: 506.2052.

trans **3e**: Yield: 40%; white foam, mp 71–72 °C; $[\alpha]_D^{20}$ +16.7 (*c* 1, $CHCl_3$); t_R=19.20 min; R_f ($CHCl_3$–Acetone 8:2) 0.45; IR (KBr): cm^{-1} 3350, 3061, 3029, 2950, 2854, 1704, 1497, 1454, 1221, 1048, 1027, 742, 699; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.89–3.00 (m_{AB}, 2H, H-2′), 3.08 (ddd_{ABx}, *J*=15 Hz, *J*=5.75 Hz, *J*=1.13 Hz, 1H, H-4a), 3.23 (br d_{ABx}, *J*=15.5 Hz, 1H, H-4b), 3.59 (s, 3H, OCH_3), 4.02 (br, 1H, H-3), 4.38 (br s, 1H, H-1′), 4.49 (s, 1H, H-1), 4.84 and 4.89 (q_{AB}, *J*=12 Hz, 2H, CH_2Ph), 5.54 (d, *J*=9 Hz, 1H, N*H*Cbz), 7.00–7.47 (m, 9H, Ar), 8.21 (s, 1H, NH_{in}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 23.3 (C-4), 38.6 (C-2′), 50.4 (C-1), 52.0 (OCH_3), 54.2 (C-3), 55.1 (C-1′), 66.5 (CH_2Ph), 111.2–129.2 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-2′), H-3 (H-4a, H-4b), H-4a (H-3, H-4b), H-4b (H-3, H-4a), H-1′ (H-2′), H-2′ (H-1, H-1′); ESI *m/z*: 484.2, 506.2 $[M+Na]^+$; exact mass calcd for $C_{29}H_{29}N_3O_4Na$ $[M+Na]^+$: 506.2056. Found: 506.2055.

4.2.6. Methyl ester of (1S,3S,1′S) and (1R,3S,1′S)-1-[1′-[(N-benzyloxycarbonyl)amino]pentyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**3-cis f** and **3-trans f**)

The ratio of cis/trans isomers determined before purification by ^{1}H NMR (200 MHz) was 29/71.

cis **3f**: yield: 16%; white foam, mp 62–64 °C; $[\alpha]_D^{20}$ −45.0 (*c* 1, $CHCl_3$); t_R=18.73 min; R_f ($CHCl_3$–Acetone 8:2) 0.62; IR (KBr): cm^{-1} 3329, 3054, 3033, 2956, 2856, 1745, 1700, 1539, 1454, 1254, 742, 696; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.80 (t, *J*=7.25 Hz, 3H, H-5′), 1.14–1.49 (m, 6H, H-2′, H-3′, and H-4′), 2.78 (m_{ABx}, *J*=13.125 Hz, *J*=3.25 Hz, *J*=2.5 Hz, 1H, H-4a), 3.11 (ddd_{ABx}, *J*=14.75 Hz, *J*=4.25 Hz, *J*=1.75 Hz, 1H, H-4b), 3.71 (dd, *J*=11 Hz, *J*=4 Hz, 1H, H-3), 3.81 (s, 3H, OCH_3), 4.2 (m, 1H, H-1′), 4.45 (s, 1H, H-1), 5.14 (s, 2H, CH_2Ph), 5.25 (d, *J*=8.5 Hz, 1H, N*H*Cbz), 7.09–7.49 (m, 9H, Ar), 8.43 (s, 1H, NH_{in}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0 (CH_3), 22.5, 28.7 (CH_2), 25.8 (C-4), 52.1 (OCH_3), 54.3 (C-1′), 56.1 (C-3), 56.5 (C-1), 67.0 (CH_2Ph), 111.2–128.5 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-3, H-CH_2), H-3 (H-1, H-4a, H-1′), H-4a (H-3, H-4b), H-4b (H-4a), H-1′ (H-3, H-CH_2), H-CH_2 (H-1, H-1′), H-4′ (H-1, H-1′, H-2′, H-3′); ESI *m/z*: 450.2 $[M+H]^+$, 472.2 $[M+Na]^+$; exact mass calcd for $C_{26}H_{31}N_3O_4Na$ $[M+Na]^+$: 472.2212. Found: 472.2220.

trans **3f**: Yield: 44%; white foam; $[\alpha]_D^{20}$ +81.9 (*c* 1, $CHCl_3$); t_R=18.98 min; R_f ($CHCl_3$–Acetone 8:2) 0.48; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.93 (t, *J*=6.75 Hz, 3H, H-5′), 1.32–1.64 (m, 6H, H-2′, H-3′, and H-4′), 3.09 (ddd_{ABx}, *J*=15 Hz, *J*=6.25 Hz, *J*=1.75 Hz, 1H, H-4a), 3.26 (d_{ABx}, *J*=15.5 Hz, 1H, H-4b), 3.63 (s, 3H, OCH_3), 4.02 (dd, *J*=6 Hz, *J*=2.25 Hz, 1H, H-3), 4.17 (m, 1H, H-1′), 4.57 (s, 1H, H-1), 4.85 and 4.91 (q_{AB}, *J*=12.25 Hz, 2H, CH_2Ph), 5.32 (d, *J*=10 Hz, 1H, N*H*Cbz), 6.94–7.49 (m, 9H, Ar), 8.35 (s, 1H, NH_{in}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1 (CH_3), 22.7, 28.5, 32.2 (CH_2), 23.2 (C-4), 52.0 (C-1), 52.1 (OCH_3), 53.1 (C-1), 54.2 (C-3), 66.4 (CH_2Ph), 111.3–128.3 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-CH_2), H-3 (H-4a, H-4b), H-4a (H-3, H-4b), H-4b (H-3, H-4a), H-1′ (H-CH_2); ESI *m/z*: 450.3 $[M+H]^+$, 472.2 $[M+Na]^+$; exact mass calcd for $C_{26}H_{31}N_3O_4Na$ $[M+Na]^+$: 472.2212. Found: 472.2228.

4.2.7. Methyl ester of (1S,3S,1′R)-1-[[(N-benzyloxycarbonyl)amino]ethyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**4-cis a**)

^{1}H NMR (200 MHz) before purification indicated only one product (determined as cis isomer).

Yield: 81%; yellowish foam, mp 68–71 °C; $[\alpha]_D^{20}$ −92.5 (*c* 1, $CHCl_3$); t_R=16.59 min; R_f ($CHCl_3$–Acetone 8:2) 0.5; IR (KBr): cm^{-1} 3344, 3059, 3033, 2951, 1732, 1704, 1504, 1453, 1268, 1220, 1057, 741, 697; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.38 (d, *J*=6.5 Hz, 3H, CH_3), 2.81 (m_{ABx}, *J*=13.25 Hz, *J*=2.5 Hz, 1H, H-4a), 3.15 (ddd_{ABx}, *J*=14.5 Hz, *J*=4.13 Hz, *J*=1.5 Hz, 1H, H-4b), 3.80 (dd, *J*=11 Hz, *J*=4 Hz, 1H, H-3), 3.83 (s, 3H, OCH_3), 4.32 (s, 1H, H-1), 4.46 (br s, 1H, H-1′), 4.91 (s, 2H, CH_2Ph), 5.49 (d, *J*=8.5 Hz, 1H, N*H*Cbz), 7.02–7.49 (m, 9H, Ar), 8.59 (s, 1H, NH_{in}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 18.3 (CH_3), 25.3 (C-4), 48.4 (C-1′), 52.5 (OCH_3), 57.4 (C-1), 56.3 (C-3), 66.7 (CH_2Ph), 111.6–128.6 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-3, H-2′), H-3 (H-1, H-4a), H-4a (H-3, H-4b), H-4b (H-4a), H-1′ (H-2′), H-2′ (H-1, H-1′); ESI *m/z*: 408.2 $[M+H]^+$, 430.2 $[M+Na]^+$, 837.2 $[2M+Na]^+$; exact mass calcd for $C_{23}H_{25}N_3O_4Na$ $[M+Na]^+$: 430.1743. Found: 430.1743.

4.2.8. Methyl ester of (1S,3S,1′R)-1-[1′-[(N-benzyloxycarbonyl)amino]-2′-methyl-propyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**4-cis b**)

^{1}H NMR (200 MHz) before purification indicated only one product (determined as cis isomer).

Yield: 89%; yellowish solid, mp 156–159 °C; $[\alpha]_D^{20}$ −86.4 (*c* 1, $CHCl_3$); t_R=17.67 min; R_f ($CHCl_3$–Acetone 8:2) 0.69; IR (KBr): cm^{-1} 3420, 3365, 3343, 3029, 2957, 2895, 2848, 1721, 1497, 1454, 1446, 1222, 1215, 1043, 1035, 748, 699; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.09 (d, *J*=6 Hz, 3H, CH_3), 1.13 (d, *J*=6 Hz, 3H, CH_3), 1.91–1.97 (m, 1H, H-2′), 2.83 (m_{ABx}, *J*=13.13 Hz, *J*=2.5 Hz, 1H, H-4a), 3.14 (ddd_{ABx}, *J*=15 Hz, *J*=3.88 Hz, *J*=1.5 Hz, 1H, H-4b), 3.80 (dd, *J*=11 Hz, *J*=4.5 Hz, 1H, H-3), 3.82 (s, 3H, OCH_3), 3.89 (td, *J*=10.13 Hz, *J*=2.5 Hz, 1H, H-1′), 4.55 (d, *J*=1.5 Hz, 1H, H-1), 4.80 and 4.91 (q_{AB}, *J*=12.75 Hz, 2H, CH_2Ph), 5.44 (d, *J*=10 Hz, 1H, N*H*Cbz), 6.84–7.48 (m, 9H, Ar), 8.47 (s, 1H, NH_{in}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.8, 20.2 (CH_3), 25.1 (C-4), 29.9 (*C*H), 52.2 (OCH_3), 53.9 (C-1), 56.2 (C-3), 58.1 (C-1′), 66.1 (CH_2Ph), 111.7–128.3 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-3, H-1′, H-3′), H-3 (H-1, H-4a), H-4a (H-3, H-4b), H-4b (H-4a), H-1′ (H-1, H-2′, H-3′), H-2′ (H-3′), H-3′ (H-1, H-1′, H-2′); ESI *m/z*: 458.2 $[M+Na]^+$; exact mass calcd for $C_{25}H_{29}N_3O_4Na$ $[M+Na]^+$: 458.2056. Found: 458.2058.

*4.2.9. Methyl ester of (1S,3S,1′R)-1-[1′-[(N-benzyloxycarbonyl)amino]-3′-methyl-butyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**4-cis c**)*

^{1}H NMR (200 MHz) before purification indicated only one product (determined as cis isomer).

Yield: 76%; yellowish solid, mp 63–65 °C; $[\alpha]_D^{20}$ −67.1 (*c* 1, $CHCl_3$); t_R=18.82 min; R_f ($CHCl_3$–Acetone 8:2) 0.71; IR (KBr): cm^{-1} 3328, 3060, 3033, 2954, 2868, 1734, 1694, 1538, 1509, 1454, 1437, 1267, 1218, 1051, 740, 697; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.99 (d, *J*=6 Hz, 3H, C*H*$_3$), 1.04 (d, *J*=6.5 Hz, 3H, C*H*$_3$), 1.41 (m, 1H, H-2′a), 1.68 (m, 1H, H-2′b), 1.75 (m, 1H, H-3′), 2.82 (m_{ABx}, *J*=13.125 Hz, *J*=2.63 Hz, 1H, H-4a), 3.14 (dd_{ABx}, *J*=13 Hz, *J*=3.25 Hz, 1H, H-4b), 3.80 (dd, *J*=11 Hz, *J*=4.5 Hz, 1H, H-3), 3.82 (s, 3H, OC*H*$_3$), 4.30 (s, 1H, H-1), 4.37 (br m, 1H, H-1′), 4.80 and 4.89 (q_{AB}, *J*=12.5 Hz, 2H, C*H*$_2$Ph), 5.34 (d, *J*=10 Hz, 1H, N*H*Cbz), 6.87–7.47 (m, 9H, Ar), 8.49 (s, 1H, N*H*$_{in}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.1, 23.4 (*C*H$_3$), 25.0 (C-4), 25.1 (*C*H), 41.3 (*C*H$_2$), 50.3 (C-1′), 52.3 (O*C*H$_3$), 56.2 (C-3), 57.2 (C-1), 66.4 (*C*H$_2$Ph), 111.5–128.3 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-3, H-2′, H-3′, H-4′), H-3 (H-1, H-4a, H-1′), H-4a (H-3, H-4b), H-4b (H-4a), H-1′ (H-3, H-2′, H-3′, H-4′), H-2′ (H-1, H-1′, H-3′, H-4′), H-3′ (H-1, H-1′, H-2′, H-4′), H-4′ (H-1, H-1′, H-2′, H-3′); ESI *m/z*: 450.3 $[M+H]^+$, 472.2 $[M+Na]^+$; exact mass calcd for $C_{26}H_{31}N_3O_4Na$ $[M+Na]^+$: 472.2212. Found: 472.2219.

*4.2.10. Methyl ester of (1S,3S,1′R,2′S)-1-[1′-[(N-benzyloxycarbonyl)amino]-2′-methyl-butyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**4-cis d**)*

^{1}H NMR (200 MHz) before purification indicated only one product (determined as cis isomer).

Yield: 60%; yellowish solid, mp 109–112 °C; $[\alpha]_D^{20}$ −81.9 (*c* 1, $CHCl_3$); t_R=18.66 min; R_f ($CHCl_3$–Acetone 8:2) 0.78; IR (KBr): cm^{-1} 3398, 3312, 3060, 3033, 2965, 2933, 2876, 1741, 1700, 1538, 1501, 1454, 1269, 1219, 1046, 741, 697; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.02 (t, *J*=7.5 Hz, 3H, C*H*$_3$ a), 1.06 (d, *J*=6 Hz, 3H, C*H*$_3$ b), 1.34 (m, 1H, H-2′), 1.69 (m, 1H, H-3′a), 1.79 (m, 1H, H-3′b), 2.83 (m_{ABx}, *J*=13 Hz, *J*=2.75 Hz, *J*=2.25 Hz, 1H, H-4a), 3.13 (dd_{ABx}, *J*=14.5 Hz, *J*=3.25 Hz, 1H, H-4b), 3.80 (dd, *J*=11.5 Hz, *J*=4.25 Hz, 1H, H-3), 3.82 (s, 3H, OC*H*$_3$), 3.90 (td, *J*=9.625 Hz, *J*=1.83 Hz, 1H, H-1′), 4.54 (s, 1H, H-1), 4.80 and 4.91 (q_{AB}, *J*=12.75 Hz, 2H, CH_2Ph), 5.38 (d, *J*=10.5 Hz, 1H, NHCbz), 6.84–7.48 (m, 9H, Ar), 8.31 (s, 1H, N*H*$_{in}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 10.8, 15.8 (*C*H$_3$), 24.9 (C-4), 26.0 (*C*H$_2$), 35.9 (*C*H), 52.2 (O*C*H$_3$), 54.0 (C-1), 56.1 (C-3), 56.3 (C-1′), 66.2 (*C*H$_2$Ph), 111.5–128.3 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-3, H-2′, H-3′, H-4′, H-5′), H-3 (H-1, H-4a), H-4a (H-3, H-4b), H-4b (H-4a), H-1′ (H-2′, H-3′, H-4′), H-2′ (H-1, H-1′, H-3′, H-4′), H-3′ (H-1, H-1′, H-2′, H-4′), H-4′ (H-1, H-1′, H-2′, H-3′), H-5′ (H-1, H-2′, H-3′); ESI *m/z*: 450.2 $[M+H]^+$, 472.2 $[M+Na]^+$; exact mass calcd for $C_{26}H_{31}N_3O_4Na$ $[M+Na]^+$: 472.2212. Found: 472.2217.

*4.2.11. Methyl ester of (1S,3S,1′R)-1-[1′-[(N-benzyloxycarbonyl)amino]phenylethyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**4-cis e**)*

^{1}H NMR (200 MHz) before purification indicated only one product (determined as cis isomer).

Yield: 92%; white solid, mp 98–100 °C; $[\alpha]_D^{20}$ −30.8 (*c* 1, $CHCl_3$); t_R=19.24 min; R_f ($CHCl_3$–Acetone 8:2) 0.69; IR (KBr): cm^{-1} 3327, 3060, 3030, 2951, 2848, 1730, 1697, 1539, 1497, 1454, 1267, 1224, 1059, 741, 697; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.83 (m_{ABx}, *J*=13.25 Hz, *J*=3 Hz, *J*=2.5 Hz, 1H, H-4a), 2.94–3.06 (m_{AB}, 2H, H-2′), 3.12 (ddd_{ABx}, *J*=15 Hz, *J*=3.5 Hz, *J*=1 Hz, 1H, H-4b), 3.75 (dd, *J*=11.5 Hz, *J*=3.75 Hz, 1H, H-3), 3.84 (s, 3H, OC*H*$_3$), 4.29 (s, 1H, H-1), 4.53 (br s, 1H, H-1′), 4.81 and 4.86 (q_{AB}, *J*=12.25 Hz, 2H, C*H*$_2$Ph), 5.59 (d, *J*=7 Hz, 1H, N*H*Cbz), 6.93–7.45 (m, 14H, Ar), 8.39 (s, 1H, N*H*$_{in}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.0 (C-4), 38.2 (C-2′), 52.3 (O*C*H$_3$), 54.0 (C-1′), 54.7 (C-1), 56.1 (C-3), 66.5 (*C*H$_2$Ph), 111.4–128.9 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-3, H-2′), H-3 (H-1, H-4a, H-1′), H-4a (H-3, H-4b), H-4b (H-4a), H-1′ (H-3, H-2′), H-2′ (H-1′); ESI *m/z*: 506.2 $[M+Na]^+$; exact mass calcd for $C_{29}H_{29}N_3O_4Na$ $[M+Na]^+$: 506.2056. Found: 506.2055.

*4.2.12. Methyl ester of (1S,3R,1′S)-1-[1′-[(N-benzyloxycarbonyl)amino]-3′-methyl-butyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**5-cis c**)*

^{1}H NMR (200 MHz) before purification indicated only one product (determined as cis isomer).

Yield: 90%; yellowish solid, mp 63–65 °C; $[\alpha]_D^{20}$ +66.6 (*c* 1, $CHCl_3$); t_R=18.51 min; R_f ($CHCl_3$–Acetone 8:2) 0.71; IR (KBr): cm^{-1} 3324, 3061, 3033, 2955, 2869, 1734, 1695, 1538, 1508, 1454, 1437, 1266, 1218, 1051, 740, 697; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.10 (d, *J*=6.5 Hz, 3H, C*H*$_3$), 1.04 (d, *J*=6.5 Hz, 3H, C*H*$_3$), 1.40 (m, 1H, H-2′a), 1.68 (m, 1H, H-2′b), 1.75 (m, 1H, H-3′), 2.83 (m_{ABx}, *J*=12.88 Hz, *J*=2.38 Hz, 1H, H-4a), 3.14 (ddd_{ABx}, *J*=15 Hz, *J*=3.75 Hz, *J*=1.25 Hz, 1H, H-4b), 3.80 (dd, *J*=11.25 Hz, *J*=4.25 Hz, 1H, H-3), 3.83 (s, 3H, OC*H*$_3$), 4.31 (s, 1H, H-1), 4.38 (br m, 1H, H-1′), 4.81 and 4.91 (q_{AB}, *J*=12.5 Hz, 2H, C*H*$_2$Ph), 5.37 (d, *J*=10 Hz, 1H, N*H*Cbz), 6.87–7.47 (m, 9H, Ar), 8.55 (s, 1H, N*H*$_{in}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.2, 23.4 (*C*H$_3$), 25.0 (C-4), 25.0 (*C*H), 41.2 (*C*H$_2$), 50.3 (C-1′), 52.3 (O*C*H$_3$), 56.2 (C-3), 57.3 (C-1), 66.4 (*C*H$_2$Ph), 111.4–128.2 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-3, H-2′), H-3 (H-1, H-4a), H-4a (H-3, H-4b), H-4b (H-4a), H-1′ (H-2′, H-3′, H-4′), H-2′ (H-3′, H-4′), H-3′ (H-2′, H-4′), H-4′ (H-1′, H-2′); ESI *m/z*: 450.3 $[M+H]^+$, 472.3 $[M+Na]^+$; exact mass calcd for $C_{26}H_{31}N_3O_4Na$ $[M+Na]^+$: 472.2212. Found: 472.2219.

*4.2.13. Methyl ester of (1S,3R,1′R) and (1R,3R,1′R)-1-[1′-[(N-benzyloxycarbonyl)amino]-3′-methyl-butyl]-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**6-cis c** and **6-trans c**)*

The ratio of cis/trans isomers determined before purification by ^{1}H NMR (200 MHz) was 15/85.

cis **6c**: Yield: 6%; white foam, mp 67–69 °C; $[\alpha]_D^{20}$ +61.8 (*c* 1, $CHCl_3$); t_R=18.56 min; R_f ($CHCl_3$–Acetone 8:2) 0.67; IR (KBr): cm^{-1} 3388, 3061, 3033, 2954, 2869, 1703, 1513, 1454, 1437, 1268, 1220, 1028, 740, 698; ^1H NMR (500 MHz, $CDCl_3$): δ 0.83 (d, *J*=6.5 Hz, 3H, CH_3), 0.87 (d, *J*=7 Hz, 3H, CH_3), 0.97 (pseudo-t, *J*=11.5 Hz, 1H, H-2′a), 1.49 (pseudo-td, *J*=12.875 Hz, *J*=3.75 Hz, 1H, H-3′), 1.64 (m, 1H, H-2b′), 2.78 (m_{ABx}, *J*=12.75 Hz, *J*=2.875 Hz, 1H, H-4a), 3.12 (ddd_{ABx}, *J*=14.5 Hz, *J*=4 Hz, *J*=2 Hz, 1H, H-4b), 3.71 (dd, *J*=11 Hz, *J*=4 Hz, 1H, H-3), 3.81 (s, 3H, OCH_3), 4.29 (br t, *J*=10.25 Hz, 1H, H-1′), 4.46 (br s, 1H, H-1), 5.14 (s, 2H, CH_2Ph), 5.19 (d, *J*=9 Hz, 1H, N*H*Cbz), 7.10–7.50 (m, 9H, Ar), 8.42 (s, 1H, NH_{in}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.4, 23.6 (*C*H_3), 24.8 (*C*H), 25.8 (C-4), 37.6 (*C*H_2), 52.1 (C-1′), 52.2 (O*C*H_3), 56.1 (C-3), 56.7 (C-1), 67.0 (*C*H_2Ph), 111.2–128.5 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-3), H-3 (H-1, H-4a, H-1′), H-4a (H-3, H-4b), H-4b (H-4a), H-1′ (H-3, H-2′, H-4′), H-2′ (H-1′, H-3′, H-4′), H-3′ (H-1′, H-2′, H-4′), H-4′ (H-1′, H-2′, H-3′); ESI *m*/*z*: 472.2 $[M+Na]^+$; exact mass calcd for $C_{26}H_{31}N_3O_4Na$ $[M+Na]^+$: 472.2212. Found: 472.2232.

trans **6c**: Yield: 49%; white solid, mp 149–152 °C; $[\alpha]_D^{20}$ −80.4 (*c* 1, $CHCl_3$); t_R=18.96 min; R_f ($CHCl_3$–Acetone 8:2) 0.44; IR (KBr): cm^{-1} 3412, 3388, 3369, 3060, 3034, 2958, 2865, 1724, 1495, 1454, 1224, 1213, 1051, 738, 694; ^1H NMR (500 MHz, $CDCl_3$): δ 0.99 (d, *J*=7 Hz, 3H, CH_3), 1.04 (d, *J*=6.5 Hz, 3H, CH_3), 1.43 (m, 1H, H-2′a), 1.58 (m, 1H, H-3′), 1.79 (m, 1H, H-2b′), 3.09 (ddd_{ABx}, *J*=15 Hz, *J*=6.25 Hz, *J*=2 Hz, 1H, H-4a), 3.26 (d_{ABx}, *J*=15.5 Hz, 1H, H-4b), 3.63 (s, 3H, OCH_3), 4.02 (dd, *J*=6 Hz, *J*=2.25 Hz, 1H, H-3), 4.28 (br s, 1H, H-1′), 4.52 (s, 1H, H-1), 4.81 and 4.91 (q_{AB}, *J*=12.25 Hz, 2H, CH_2Ph), 5.27 (d, *J*=9.5 Hz, 1H, N*H*Cbz), 6.91–7.50 (m, 9H, Ar), 8.31 (s, 1H, NH_{in}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.3, 23.1 (*C*H_3), 23.1 (C-4), 24.8 (*C*H), 41.3 (*C*H_2), 51.0 (C-1′), 52.0 (O*C*H_3), 52.5 (C-1), 54.1 (C-3), 66.3 (*C*H_2Ph), 111.3–128.3 (Ar); ROESY (500 MHz, $CDCl_3$) H-1 (H-2′, H-3′), H-3 (H-4a, H-4b), H-4a (H-3, H-4b), H-4b (H-3, H-4a), H-1′ (H-2′, H-4′), H-2′ (H-1′, H-4′), H-3′ (H-4′), H-4′ (H-2′, H-3′); ESI *m*/*z*: 450.3 $[M+H]^+$, 472.3 $[M+Na]^+$; exact mass calcd for $C_{26}H_{31}N_3O_4Na$ $[M+Na]^+$: 472.2212. Found: 472.2216.

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References and notes

1. Pictet, A.; Spengler, T. *Chem. Ber.* **1911**, *44*, 2030.
2. Tourwé, D.; Verschueren, K.; Frycia, A.; Davis, P.; Porreca, F.; Hruby, V. J.; Toth, G.; Jaspers, H.; Verheyden, P.; Van Binst, G. *Biopolymers* **1996**, *38*, 1.
3. Hruby, V. J.; Li, G.; Haskell-Luevano, C.; Shenderovich, M. *Biopolymers* **1997**, *43*, 219.
4. Gibson, S. E.; Guillo, N.; Tozer, M. J. *Tetrahedron* **1999**, *55*, 585.
5. Hruby, V. J.; Balse, P. M. *Curr. Med. Chem.* **2000**, *7*, 945.
6. Cowell, S. M.; Lee, Y. S.; Cain, J. P.; Hruby, V. J. *Curr. Med. Chem.* **2004**, *11*, 2785.
7. Tatsui, G. *J. Pharm. Soc. Jpn.* **1928**, *48*, 92.
8. Snyder, H. R.; Walker, H. G.; Werber, F. X. *J. Am. Chem. Soc.* **1949**, *71*, 527.
9. Bailey, P. D.; Hollinshead, S. P.; Malcy, N. R. *Tetrahedron Lett.* **1987**, *28*, 5177.
10. Sandrin, J.; Hollinshead, S. P.; Cook, J. M. *J. Org. Chem.* **1989**, *54*, 5636.
11. Bailey, P. D.; Hollinshead, S. P.; Malcy, N. R.; Morgan, K.; Palmer, S. J.; Prince, S. N.; Reynolds, C. D.; Wood, S. D. *J. Chem. Soc., Perkin Trans. 1* **1993**, 431.
12. Bailey, P. D.; Moore, M. H.; Morgan, K. M.; Smith, D. I.; Vernon, J. M. *Tetrahedron Lett.* **1994**, *35*, 3587.
13. Soerens, D.; Sandrin, J.; Ungemach, F.; Mokry, P.; Wu, G. S.; Yamanaka, E.; Hutchins, L.; DiPierro, M.; Cook, J. M. *J. Org. Chem.* **1979**, *44*, 535.
14. Ungemach, F.; DiPierro, M.; Weber, R.; Cook, J. M. *J. Org. Chem.* **1981**, *46*, 164.
15. Deng, L.; Czerwinski, K.; Cook, J. M. *Tetrahedron Lett.* **1991**, *32*, 175.
16. Cox, E. D.; Cook, J. M. *Chem. Rev.* **1995**, *95*, 1797.
17. Cox, E. D.; Hamaker, L. K.; Li, J.; Yu, P.; Czerwinski, K.; Deng, L.; Bennet, D. W.; Cool, J. M.; Watson, W. H.; Krawiec, M. *J. Org. Chem.* **1997**, *62*, 44.
18. Alberch, L.; Bailey, P. D.; Clingan, P. D.; Mills, T. J.; Price, R. A.; Pritchard, R. G. *Eur. J. Org. Chem.* **2004**, 1887.
19. Taylor, M. S.; Jacobson, E. N. *J. Am. Chem. Soc.* **2004**, *126*, 10558.
20. Gremmen, C.; Willemse, B.; Wanner, M. J.; Koomen, G.-J. *Org. Lett.* **2000**, *2*, 1955.
21. Tsuji, R.; Nakagawa, M.; Nishida, A. *Tetrahedron: Asymmetry* **2003**, *14*, 177.
22. Larghi, E. L.; Amongero, M.; Bracca, A. B. J.; Kaufman, T. S. *ARKIVOC* **2005**, *xii*, 98.
23. Plate, R.; van Hout, R. H. M.; Bahm, H.; Ottenheijm, H. C. J. *J. Org. Chem.* **1987**, *52*, 555.
24. Nakagawa, M.; Liu, J.-J.; Hino, T. *J. Am. Chem. Soc.* **1989**, *111*, 2721.
25. Melnyk, P.; Ducrot, P.; Thal, C. *Tetrahedron* **1993**, *49*, 8589.
26. De la Figura, N.; Alkorta, I.; García-López, M. T.; Herranz, R.; González-Muñiz, R. *Tetrahedron* **1995**, *51*, 7841.
27. Ducrot, P.; Rabhi, C.; Thal, C. *Tetrahedron* **2000**, *56*, 2683.
28. Grieco, P.; Campiglia, P.; Gomez-Monterrey, I.; Novellino, E. *Tetrahedron Lett.* **2002**, *43*, 6297.
29. Gomez-Monterrey, I. M.; Campiglia, P.; Bertamino, A.; Mazzoni, O.; Diurno, M. V.; Novellino, E.; Grieco, P. *Tetrahedron* **2006**, *62*, 8083.
30. Lesma, G.; Meschini, E.; Recca, T.; Sacchetti, A.; Silvani, A. *Tetrahedron* **2007**, *63*, 5567.
31. Fehrentz, J.-A.; Castro, B. *Synthesis* **1983**, 676.
32. Jurczak, J.; Golebiowski, A. *Chem. Rev.* **1989**, *89*, 149.
33. Wang, H.; Usui, T.; Osada, H.; Ganesan, A. *J. Med. Chem.* **2000**, *43*, 1577.
34. Ungemach, F.; Soerens, D.; Weber, R.; DiPierro, M.; Campos, O.; Mokry, P.; Cook, J. M.; Silverton, J. V. *J. Am. Chem. Soc.* **1980**, *102*, 6976.
35. PCMODEL for Windows, Version 9.00.0; Serene Software: Bloomington, IN, August 2004.