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Circular dichroism studies on absolute configuration assignment of peptidomimetics with a terminal chiral 3-arylpropionic acid unit

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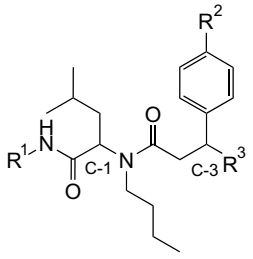
Abstract—The relationship between the molecular structure of peptidomimetics **1** and their chiroptical properties has been studied using circular dichroism spectroscopy. It was demonstrated that the sign of the Cotton effects occurring around 230 and 270 nm enables the direct assignment of stereochemistry at C-1 and C-3 carbon atoms. The TD-DFT calculations of the circular dichroism (CD) spectrum of one of the model compounds confirm the absolute stereochemistry assignment made experimentally. Thus, CD spectroscopy proved to be a simple, reliable and general tool for the determination of the absolute configuration of the stereogenic centers of peptidomimetics with a terminal 3-arylpropionic group.

1. Introduction

Currently, medicinal chemists have dedicated much attention to the novel classes of peptide-like compounds, peptidomimetics, which exhibit therapeutic activity.[1,2] It is well known that there is a close relationship between their biological activity and stereostructure. In this context, there is a need for new synthetic methods leading to chiral peptidomimetics with defined absolute configuration. Therefore, a reliable assignment of configuration at all stereogenic centers present in peptidomimetics is rapidly becoming a key issue.

Peptidomimetics possessing a terminal 3-arylpropionic acid are known to exhibit biological activity.[3–6] According to the literature, the configuration at C-3 of this group has a significant influence on their biological activity.[4–6] Nevertheless, the problem of the absolute configuration assignment often remains unsolved in the literature. For example, Tucker[5] and Brady[6] designed a series of potent thrombin inhibitors possessing a 3-arylpropionic unit. A clear difference in the potency between the diastereoisomers was observed, although, the stereochemistry at the C-3 atom was not established.

Recently, we reported a novel approach to the synthesis of non-racemic peptidomimetics of structure **1a–d** (Fig. 1) with a combination of enzymatic desymmetrization of 3-phenylglutaric anhydrides with the Ugi multi-component reaction.[7,8]



1a, R =R^3= CH_2CO_2Et, R^2 = H
1b, R^1 = Bn, R^2 = H, R^3= CH_2CO_2Et
1c, R^1 = Bn, R^2 = Cl, R^3= CH_2CO_2Et
1d, R^1 = Bn, R^2 = OMe, R^3= CH_2CO_2Et

Figure 1. Peptidomimetics of structure **1**.

Since over the course of the aforementioned reaction the diastereoisomers are formed, we needed to solve the problem of the assignment of their relative and absolute configurations. The initial circular dichroism (CD) studies of compounds **1a–d**, proved that the less polar diastereoisomers of **1a–d** possessed the same relative configurations

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at C-1 and C-3.[8] Similarly, all the more polar diastereoisomers possessed the opposite relative configuration to their less polar counterparts. We initially assumed that the Smith's benzene sector rule[9] could be used to assign absolute configuration at the C-3 carbon atom. A direct assignment of absolute configuration at the C-1 carbon atom was a more complex problem, which could not be resolved with the data we had in our hands. To unambiguously determine the absolute configuration at this stereogenic center we decided to undertake extended chiroptical studies. For this purpose we synthesized all the possible stereoisomers of peptidomimetics **1a** and **1b** (four for the **a** series and four for the **b** series, respectively) as model compounds (Fig. 1) for CD analysis. On the basis of the obtained data we intended to establish a general correlation between Cotton effects (CEs) present in the CD spectra and the stereo-structure of peptidomimetics possessing a terminal 3-arylpropionic acid unit.

2. Results and discussion

2.1. Chemistry

The diastereoisomers with a defined configuration at C-1 and C-3 carbons were obtained by the four-step functionalization of L- and D-leucine **2** and *ent*-**2**, respectively, leading to the respective derivatives **6** followed by their coupling with optically active 3-phenylglutaric acid monoethyl esters **7** and *ent*-**7**, as shown in Scheme 1.

Coupling of the Cbz-protected amino acids **3** with glycine ethyl ester hydrochloride using DCC in the presence of hydroxybenzotriazole led to the formation of amides **4a** and *ent*-**4a** in 61% and 78% yield, respectively. Analogous coupling with benzylamine gave the compounds **4b** in 80% yield. Amine **6b** was obtained in a two-reaction sequence. Catalytic hydrogenation of amide **4b** gave L-leucine benzylamide **5b** in 81% yield. The reductive alkylation with butanal in the presence of $NaBH_3CN$ afforded amine **6b** in 47% yield. The overall yield for the two steps was 38%. To simplify the reaction procedure and to avoid side reactions for the glycine series, the compounds **6a**, *ent*-**6a** and *ent*-**6b** were obtained without isolation of the respective intermediates **5**. After deprotection of the amine group, the resulting crude compounds **5** were used directly in subsequent aminoalkylation reaction to give secondary amines **6** in 29–47% yield. Chiral monoesters **7** (80% ee) and *ent*-**7** (77% ee) were obtained following the enzymatic desymmetrization procedure reported earlier by us.[10]

A key synthetic step was the coupling of amines **6** with monoesters **7**. This was achieved by mixed anhydride methodology. This approach, in comparison to the coupling with DCC/HOBt, gave comparable yields (Table 1), although the purification procedure was simplified. The diastereoisomers of peptidomimetics **1a** and **1b** were synthesized in yields ranging from 16% to 45% (Table 1). No attempt was made to optimize the reaction.

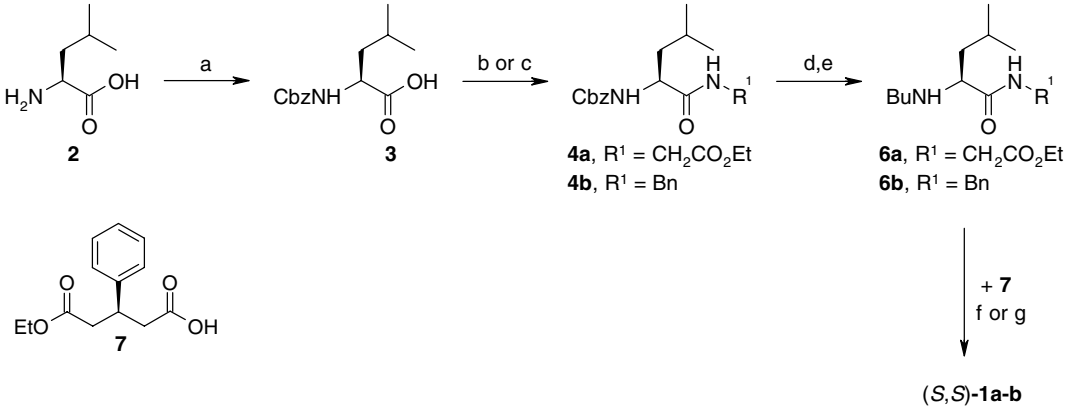
Table 1. Synthesis of model compounds **1a** and **1b** by coupling of amines **6** with monoacids **7**

Entry	Amine **6**	Acid **7**	Reaction time [h]	Yield [%]	Product
1	*ent*-**6a**	**7**	24	45	(1*R*,3*S*)-**1a**
2	**6a**	*ent*-**7**	16	24	(1*S*,3*R*)-**1a**
3	**6a**	**7**	5.5	16	(1*S*,3*S*)-**1a**
4	*ent*-**6a**	*ent*-**7**	24	32	(1*R*,3*R*)-**1a**
5	*ent*-**6b**	**7**	24	28	(1*R*,3*S*)-**1b**
6	**6b**	*ent*-**7**	24	26 (28)[a]	(1*S*,3*R*)-**1b**
7	**6b**	**7**	24	31 (26)[a]	(1*S*,3*S*)-**1b**
8	*ent*-**6b**	*ent*-**7**	24	17	(1*R*,3*R*)-**1b**

[a] By coupling using DCC and HOBt in CH_2Cl_2.

2.2. Configuration assignment by CD

The above synthetic approach gave us an access to the model compounds **1** with unambiguously defined stereochemistry at C-1 and C-3. The respective CD and UV data are presented in Table 2. The CD and UV spectra of the compounds (1*S*,3*S*)-**1a** and (1*R*,3*R*)-**1a** are shown in Figure 2.



Scheme 1. The synthetic strategy leading to model compounds **1a** and **1b** with a defined stereochemistry at C-1 and C-3. Reagents and conditions: (a) CbzCl, NaOH, 0 °C, ~90%; (b) Gly-OEt·HCl, DCC, HOBt, Et_3N, CH_2Cl_2, 68–78%; (c) $BnNH_2$, DCC, HOBt, CH_2Cl_2, 80%; (d) H_2, Pd/C, MeOH; (e) PrCHO, $NaBH_3CN$, Na_2SO_4, THF, 29–47% for two steps d and e; (f) $ClCO_2Et$, Et_3N, acetone, 16–45%; (g) DCC, HOBt, CH_2Cl_2, 26–28%.

Table 2. UV and CD data of the model compounds **1a** and **1b** recorded in MeCN

Entry	Compound	UV ε (λ)		CD $\Delta\varepsilon$ (λ)			
1	(1*R*,3*S*)-**1a**	330 (257)	9000 (209)	+2.79 (191.0)	+1.29 (206.0)	+7.71 (230.0)	−0.03 (269.0)
2	(1*S*,3*R*)-**1a**	350 (257)	14,100 (208^{sh})	−2.20 (190.0)	−1.64 (207.0^{sh})	−7.41 (230.0)	+0.03 (266.0)
3	(1*S*,3*S*)-**1a**	475 (256)	11,700 (206^{sh})	+8.07 (190.0)	−1.28 (208.5^{sh})	−9.73 (229.0)	−0.09 (269.0)
4	(1*R*,3*R*)-**1a**	300 (257)	9600 (208)	−7.98 (191.5)	+1.25 (207.5^{sh})	+10.12 (230.0)	+0.10 (267.5)
5	(1*R*,3*S*)-**1b**	510 (257)	25,000 (208^{sh})	−3.16 (197.5)	+2.18 (208.5)	+10.83 (230.0)	−0.03 (269.5)
6	(1*S*,3*R*)-**1b**	580 (257)	23,600 (207^{sh})	+2.00 (196.5)	−1.75 (210.5)	−8.23 (230.5)	+0.02 (267.5)
7	(1*S*,3*S*)-**1b**	520 (257)	20,300 (208^{sh})	+10.85 (193.0)	−1.36 (210.5^{sh})	−9.22 (229.0)	−0.02 (269.5)
8	(1*R*,3*R*)-**1b**	480 (258)	27,000 (206^{sh})[a]	−13.21 (194.0)	+2.55 (210.0)	+12.73 (230.0)	+0.08 (267.5)

The UV and CD values are given as ε (λ) and $\Delta\varepsilon$ (λ), respectively.
[a] An additional strong absorption band at 192 nm was observed.

As can be seen from Tables 1 and 2, four CD bands are present in the CD spectra of the compounds investigated. However, only two CD bands of lowest energy, occurring around 270 and 230 nm were of interest for the purpose of our studies.

The long-wavelength CD band at approximately 268 nm is associated with the electronic absorption observed at ca. 257 nm (vide Fig. 2). This Cotton effect (CE) can be attributed to the ^1L_b benzene transition. Although its amplitude is low, as expected, the band is well developed and can be undoubtedly used for a correlation between the structure and CE signs. The circular dichroic assignment for this band is based on the benzene sector rule.[9] According to the rule, the absolute configuration of a stereogenic center contiguous to the benzene ring can be established unambiguously on the basis of the sign of this excitation. Therefore, we can use this regularity to assign the configuration at the C-3 stereogenic center of compounds **1**. The presence of an additional phenyl substituent in the **1b** series should not affect the assignment because the sign of the ^1L_b CE depends exclusively on the stereochemistry of the stereogenic center directly connected to the benzene ring.[9] In the case of peptidomimetics **1**, the rule predicts a negative sign of the CE around 268 nm for diastereoisomers with an (*S*)-absolute configuration and a positive one for diastereoisomers with an (*R*)-absolute configuration at the C-3 carbon atom. The spectroscopic data are in excellent agreement with the predicted ones, as evident from Table 2 and Figure 2.

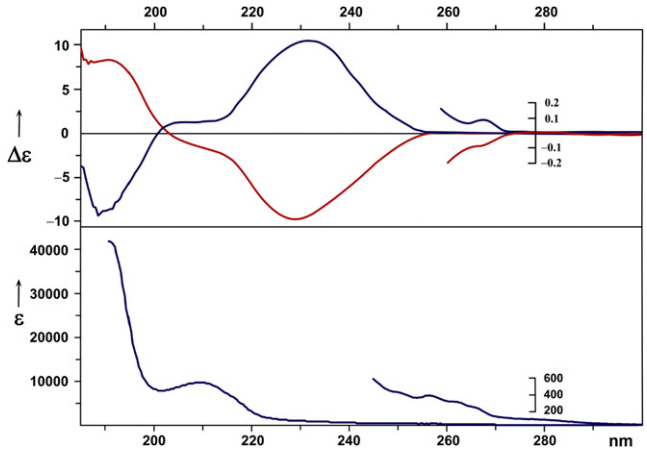


Figure 2. CD spectra of compounds (1*S*,3*S*)-**1a** (——) and (1*R*,3*R*)-**1a** (——) recorded in MeCN.

The next CD band at 230 nm does not possess any corresponding electronic absorption (vide Table 2). This band most probably represents a sum of contributions from the ester, amide, and benzene (^1L_a) chromophores present in the molecules. A relatively strong intensity of this absorption suggests the presence of different electronic transitions of approximately the same energy.

Regarding this CD band, the investigated compounds fall into two different classes. In the first class, consisting of compounds (1*R*,3*S*)-**1a**, (1*R*,3*R*)-**1a**, (1*R*,3*S*)-**1b**, and (1*R*,3*R*)-**1b**, the sign of the 230 nm CD band is positive, whereas in the second class, represented by compounds (1*S*,3*R*)-**1a**, (1*S*,3*S*)-**1a**, (1*S*,3*S*)-**1b**, and (1*S*,3*R*)-**1b**, the sign of this band is negative. Both classes represent the opposite configurations at stereogenic center C-1: the absolute configuration is (1*R*) for the first class and (1*S*) for the second class. Thus, it can be concluded that the stereochemistry at C-1 is responsible for the sign of this CD band, independent of the R^1 substituent (Fig. 1). Consequently, it can be stated that despite other differences in the molecular structure, the negative CE at 230 nm correlates to the (1*S*) and the positive CE corresponds to the (1*R*) absolute configuration in the investigated peptidomimetics.

The regularities above allow the assignment of the absolute configuration at both C-1 and C-3 stereogenic centers of the peptidomimetics obtained by the Ugi reaction **1a**–**d**. Since the negative CE around 230 nm points out unambiguously to the (*S*)-absolute configuration at C-1, the configuration at this carbon atom in compounds **1a**, **1b**, **1c**, and **1d** displaying a negative sign of this band (Table 3) should be (1*S*). Conversely, compounds **1a**, **1b**, **1c**, and **1d** with a positive sign of the 230 nm CD band possess a (1*R*)-absolute configuration (Table 3).

All compounds obtained by the Ugi reaction should possess an absolute (*S*)-configuration at C-3, based on enzyme enantioselectivity.[8,10] Thus, on the basis of the benzene sector rule, the sign of the 268 nm CD band should be negative. As can be seen from Table 3, the majority of compounds studied fulfill this criterion. In the case of products **1a**, **1b**, and **1d**, a negative sign of the band points out to (3*S*) absolute configuration, which very nicely corroborates our expectation. Two stereoisomers of compound **1c**, however, deviate from the regularity, displaying a positive 268 nm CD band for the assumed (3*S*) absolute configuration. Nevertheless, it is well documented that the benzene

Table 3. UV and CD data of the compounds **1a**, **1b**, **1c**, and **1d** obtained by the Ugi reaction, recorded in MeCN

Entry	Compound	UV ε (λ)		CD $\Delta\varepsilon$ (λ)			
1	(1*S*,3*S*)-**1a**	280 (257)	15,900 (205^{sh})	+3.10 (193.0)	−0.46 (210.5)	−4.56 (228.5)	−0.05 (268.0)
2	(1*R*,3*S*)-**1a**	260 (257)	14,700 (205^{sh})	−0.69 (197.0)	+1.17 (207.0^{sh})	+6.92 (229.5)	−0.03 (268.0)
3	(1*S*,3*S*)-**1b**	450 (257)	24,500 (207^{sh})	+12.1 (191.5)	−2.40 (210.5^{sh})	−9.23 (229.5)	−0.03 (268.0)
4	(1*R*,3*S*)-**1b**	480 (257)	24,300 (207^{sh})	−1.68 (197.5)	+1.16 (209.5)	+6.96 (230.5)	−0.02 (268.0)
5	(1*S*,3*S*)-**1c**	490 (266)	11,200 (225^{sh})	+4.61 (197.0)	−1.54 (209.0^{sh})	−5.72 (232.0)	+0.09 (265.5)
6	(1*R*,3*S*)-**1c**	590 (268)	14,200 (226^{sh})	+2.37 (197.0)	+1.91 (210.0^{sh})	+6.38 (230.0)	+0.05 (265.5)
7	(1*S*,3*S*)-**1d**	1630 (275)	14,000 (225^{sh})	+7.70 (193.0)	−1.56 (209.0)	−6.29 (235.0)	−0.09 (278.0)
8	(1*R*,3*S*)-**1d**	1530 (275)	12,800 (225^{sh})	−1.47 (195.5)	+1.57 (207.5)	+4.25 (234.0)	−0.08 (281.5)

The UV and CD values are given as ε (λ) and $\Delta\varepsilon$ (λ), respectively.

ring substituents, which have a positive spectroscopic moment, for example, a chlorine atom, especially at the *para* position, lead to an opposite sign of the ^1L_b CEs in comparison to the unsubstituted counterpart.[11,12]

Thus, the CD data for **1c** validates our assumption as well. It should be noted, however, that for enantiomers or local enantiomers of chiral benzene compounds possessing ring substituents, the benzene sector rule for sign prediction has to be used carefully. The vibronic and induced contributions to the ^1L_b CEs should be considered to fully ascertain a sign prediction.

A lower amplitude of the 230 nm CD band was observed for the majority of Ugi compounds in comparison to the model compounds (Tables 2 and 3). This may be due to the use of enantiomerically pure amino acids (>98% ee) as substrates in the synthesis of the latter.

3. TD-DFT calculations

In order to verify the absolute configuration determined experimentally, we performed a theoretical determination of the absolute configuration by a calculation of CD spectra of the model compound (*S*,*S*)-**1a** using time-dependent density functional theory (TD-DFT). Methods based on density functional theory were found to be attractive in terms of accuracy and computational effort, which hold true not only for ground states but also for electronically excited states.[13–15] Therefore the TD-DFT method, in which excited-state properties are determined from the linear response of the molecules to an external continuous wave field, has became an important tool for the theoretical treatment of molecular electronic excitation spectra.[16–21]

A fundamental prerequisite for the computational calculation of CD spectra is required for all CD-relevant conformational species of the respective molecule. In our case, the conformational analysis of (*S*,*S*)-**1a** was carried out in two steps. From the results of a conformational search using both MM3 molecular mechanics force field and CONFLEX software,[22] we obtained the set of conformers of (*S*,*S*)-**1a** accessible at ambient temperature. From these conformers we selected those having the relative energies in the range from 0.0 to 20 kcal mol^{-1}, and their structures were fully optimized with the use of the AM1 semi-empirical method implemented in the Gaussian package.[23] For each conformer, the single point energy at the b3lyp/6-31g(d) level was calculated. In the case of (*S*,*S*)-**1a** all AM1 optimized structures converged to the two conformers, which were fully optimized at b3lyp/6-31g(d) level of theory. Frequency calculations at the b3lyp/6-31g(d) level proved that those conformations are stable. For both conformers, percentage populations were calculated on the basis of ΔE and ΔG values, using Boltzmann statistics and $T = 298$ K (see Table 4). Due to the significant difference between them, both ΔE and ΔG conformer distribution values were taken under further considerations.[24]

Table 4. Calculated B3LYP/6-31g(d) relative energies (ΔE and ΔG) and populations of conformers of (S,*S*)-**1a**

Conformer	ΔE (population) [kcal mol^{-1}]	ΔG (population) [kcal mol^{-1}]
A	0.00 (58.9%)	1.42 (8.0%)
B	0.21 (41.1%)	0.00 (92.0%)

The DFT calculated structures of (*S*,*S*)-**1a** are shown in Figure 3. Both conformers show structural similarities: (i) the presence of intramolecular hydrogen NH···O=C bonds; (ii) the bent structure (as a consequence of stabilizing hydrogen bond), and (iii) position of all bulky substituents outside the 'center' of the molecule. The lengths of the hydrogen NH···O=C bonds range from 2.013 to 3.066 Å. In the case of the conformer A, the shortest hydrogen bond occurs between the NH hydrogen atom and C=O group of the tertiary amide, the second one—between the NH and C=O ester group is slightly longer, whereas in case of conformer B the shortest one involves a NH hydrogen atom and C=O ester carbonyl group, opposite to NH group. The hydrogen bond between a NH and tertiary amide C=O group is longer by about 1 Å and does not affect the conformation. In both cases, the distance between the NH hydrogen atom and neighboring C=O oxygen atom is over 3 Å and for this reason there are no hydrogen bonds between NH and terminal ester groups.

It is well known that the combination of b3lyp hybrid functional with a large basis set augmented with diffuse functions (e.g., aug-cc-pvtz) provided good or excellent results for TD-DFT calculations of chiroptical properties. Due to the size of (*S*,*S*)-**1a**, we chose even smaller split-valence basis set augmented with the diffuse functions 6-311++g(d,p) and for each conformer we calculated singlet

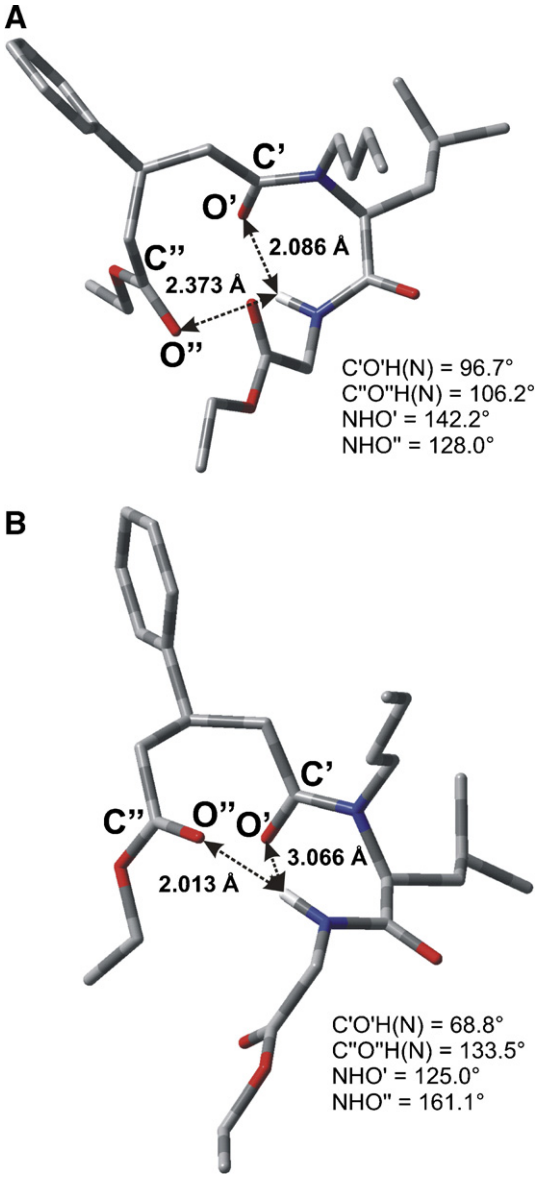


Figure 3. Computed low-energy conformers **A** (upper) and **B** (lower) of (*S*,*S*)-**1a**, with intramolecular hydrogen bonds shown. All CH hydrogen atoms were omitted for clarity.

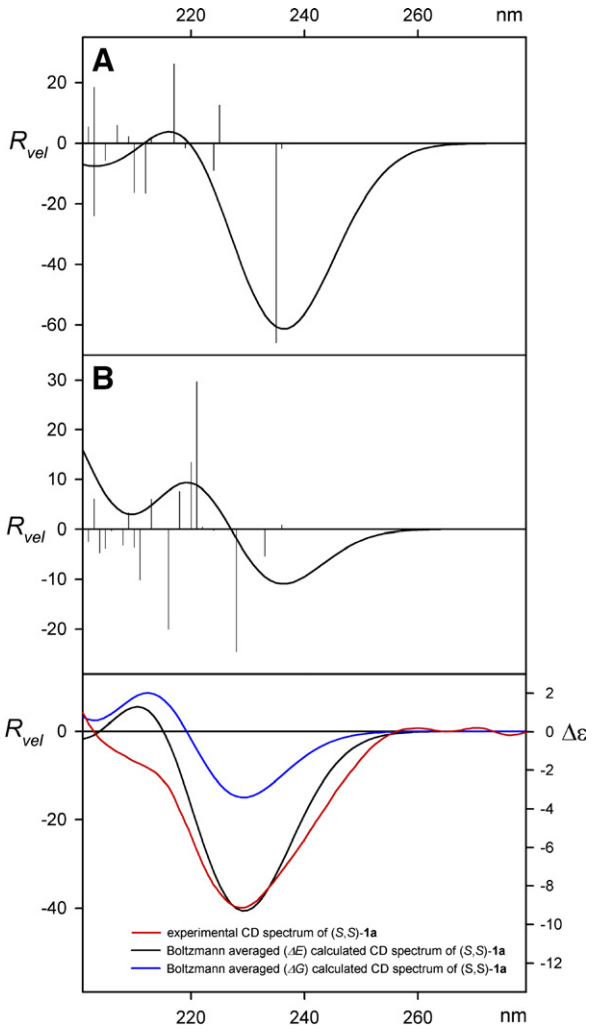


Figure 4. Calculated by TD DFT/b3lyp/6-311++g(d,p) method CD for conformers of (*S*,*S*)-**1a**: **A**—upper panel; **B**—middle panel, and the comparison between measured CD spectrum and Boltzman averaged calculated CD spectra of (*S*,*S*)-**1a**—the lowest panel (note that in this case the calculated spectra were scaled by the factor of 0.97 to match experimental bands). Vertical bars represent the calculated rotatory strengths.

states at b3lyp/6-311++g(d,p) level of theory. The theoretical calculation of the CD and UV spectra of low-energy conformers of (*S*,*S*)-**1a** by TD-DFT/b3lyp/6-311++g(d,p) method afforded the curves illustrated in Figure 4.

The TD-DFT singlet states calculations well reproduce the experimental data in the region between 250 and 200 nm, although the excitation energies were underestimated by about 5 nm. The calculated spectra for both conformers show that the first negative Cotton effect at around 235 nm originated mainly from HOMO(−1) or HOMO(−2)–LUMO(+1) excitations. This band represents a sum of contributions from the ester, amide, and benzene chromophores present in the molecules. Note that in the case of conformer **B**, a small difference in conformation caused a decrease of the amplitude of the first Cotton effect and an increase of the amplitude of the second Cotton effect at higher energy region. However, the Boltzmann averaged spectra (shown in Fig. 4) are in good agreement with the measured ones, independent of the method of population calculations used. A good overall agreement has now been found between the experimental and computed signs of the Cotton effects. This correlation applies not only to the long-wavelength Cotton effects but also to those in the range of 200–215 nm, which has not been invoked previously for the absolute configuration-spectra correlation.

Unfortunately the singlet states calculations do not reproduce the spectral region at lower energies. According to the UV experimental data, the lowest energy transitions are forbidden due to their low intensity and posses the vibronic character. In order to verify this hypothesis, we calculated the triplet states for the investigated molecules. Although the calculated transition energies are in fair agreement with the experimental data, the calculated oscillator and

rotatory strengths have zero values and for this reason, the theoretical confirmation of Smith's benzene sector rule for these compounds was not possible at this level of theory.

It is evident that the basic pattern of the CD and UV spectral curves, that is, the sign, position, intensity, and shape of the bands, is well reproduced by the calculation. It is noteworthy that the calculated structures and the theory data points out the presence of intramolecular hydrogen bonding. This observation is in perfect agreement with the bent structure for peptidomimetics (*S*,*S*)-**1** that we postulated previously on the basis of ^{1}H NMR data.[8] Thus, our absolute configurational assignment is now theoretically proven. Since the absolute configuration of the model compound (*S*,*S*)-**1a** is fixed as (1*S*,3*S*), the comparison of the present calculated and observed CD data leads to the unambiguous determination of the absolute configuration of the other compounds discussed here. Accordingly, the absolute stereochemistry of the other compounds **1** is theoretically confirmed.

4. Conclusions

In conclusion, the chiroptical correlation for peptidomimetics **1a**–**d** obtained via an enzymatic/Ugi reaction combination has been carried out. A direct assignment of the absolute configuration at the C-1 and C-3 atoms was possible on the basis of the signs of the Cotton effects around 268 m and 230 nm only. The CD method proposed here allowed the assignment of the absolute configuration in this class of peptidomimetics independently of the synthetic method used. The CD spectroscopy appeared to be a convenient, reliable and very fast technique for peptidomimetic configurational assignment. It was demonstrated that the CD spectra are highly sensitive to changes in the structure of the investigated compounds as well. These effects cannot be studied easily by any other spectroscopic method. The TD-DFT calculations of the theoretical CD proved the empirical assignment to be correct. Considering the fact that the molecule computed here possesses an enormous flexibility and complexity in relation to the number of chromophores, the agreement between theory and experiment has to be judged as excellent. This agreement again confirms the configuration previously determined and, in addition, demonstrates the efficiency of the method.

We would like to emphasize the fact that the chiroptical correlation between stereostructure and the CD bands signs, proposed here, can be applied to a broad variety of peptidomimetics or naturally occurring products possessing terminal 3-arylpropionic acid unit in their structure. It should be noted that the benzene sector rule for sign prediction has to be used with due care.

5. Experimental

5.1. General

CD spectra were measured using a JASCO J-715 spectropolarimeter in 1 cm and 1 mm cells in acetonitrile at concentrations of approximately 2×10^{-4} M. The UV and CD data for all the compounds **1** are summarized in Tables 2 and 3. NMR spectra were recorded in $CDCl_3$ with TMS as an internal standard using a 200 MHz Varian Gemini 200 or a Bruker DMX 500 Avance (500 MHz) spectrometers. Chemical shifts are reported in parts per million and coupling constants (*J*) are given in hertz (Hz). MS spectra were recorded using an API-365 (SCIEX) apparatus. Optical rotations were measured in a 1 dm cell of 1 mL capacity using a Jasco DIP-360 polarimeter operating at 589 nm. Concentrations *c* are given in 10 mg/mL. Elemental analyses were performed using a CHN Perkin–Elmer 240 apparatus. Melting points are uncorrected. All reactions were monitored by TLC on Merck 60 F_{254} silica gel plates. Preparative TLC was performed on 0.5 mm Kieselgel 60 F_{254} plates. Column chromatography was performed using Merck 60/230–400 mesh silica gel.

5.2. Computational methods

In our computations all the excited-state calculations have been performed based upon the ground state geometries of single molecules with the use of a Gaussian program package.[23] Thus the results correspond to vertical transitions, and the excitation energies can be compared with the band maxima in the experimental spectra. Rotatory strengths were calculated using length and velocity representations. Length and velocity rotatory strengths are equal and origin-independent at the complete basis set limit. Herein the differences between the length and velocity calculated values of rotatory strengths were quite small and for this reason only velocity rotatory strengths were taken into considerations. The CD and UV spectra were simulated by overlapping Gaussian functions for each transition according to the procedure described by Grimme et al.[25] No correlation for the medium dielectric constant was implemented. The conformational analysis has been performed with the use of CaChe program package.[22]

5.3. The general procedure for the synthesis of the model compounds 1a and 1b

To a cooled solution of monoester **7** (0.08 mmol, 18 mg) in acetone (0.5 mL), Et_3N (0.09 mmol, 12 μL) was added. After stirring for 5 min, the solution of ethyl chloroformate (0.09 mmol, 9 μL) in acetone (0.5 mL) was added dropwise over 10 min. After 10 min, a solution of amine **6** (0.07 mmol) in acetone (0.5 mL) was added dropwise. The mixture was stirred at 0 °C for 1 h and at rt overnight. The solvent was evaporated, the residue was dissolved in Et_2O and washed subsequently with citric acid (1 M aqueous solution), $NaHCO_{3satd}$ and brine. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo. The product was purified by flash chromatography (silica gel, hexane/EtOAc as eluents).

5.3.1. Peptidomimetic (1*R*,3*S*)-1a: (3*S*)-4-{butyl-[(1*R*)-1-(ethoxycarbonylmethyl-carbamoyl)-3-methyl-butyl]-carbamoyl}-3-phenyl-butyric acid ethyl ester. Yield 45%: transparent oil; $R_f = 0.35$ (hexane/EtOAc, 6:4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.84–0.96 (m, 9H) 1.15 (t, $J = 7.1$ Hz, 3H), 1.26 (t, $J = 7.1$ Hz, 3H), 1.25–1.32 (m,

2H), 1.35–1.45 (m, 2H), 1.48–1.56 (m, 2H), 1.75–1.81 (m, 1H), 2.66 (dd, $J = 7.7$ Hz, $J = 15.4$ Hz, 1H), 2.71–2.77 (m, 2H), 2.80 (dd, $J = 7.1$ Hz, $J = 15.4$ Hz, 1H), 3.05–3.20 (m, 2H), 3.76 (dd, $J = 5.7$ Hz, $J = 18.0$ Hz, 1H), 3.77–3.82 (m, 1H), 3.85 (dd, $J = 6.0$ Hz, $J = 18.0$ Hz, 1H), 4.04 (dq, $J = 2.6$ Hz, $J = 7.1$ Hz, 2H), 4.17 (q, $J = 7.1$ Hz, 2H), 4.96 (t, $J = 7.0$ Hz, 1H), 6.55 (br s, 1H), 7.17–7.21 (m, 1H), 7.22–7.30 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) 13.6, 14.0, 14.1, 20.2, 22.2, 22.5, 22.8, 24.7, 32.2, 36.7, 38.9, 39.7, 40.5, 41.1, 45.5, 60.4, 61.1, 126.8, 127.4, 128.5, 143.1, 169.4, 171.8, 172.7; MS (ESI): $m/z = 513$ ($[M+Na]^+$, 64%), 491 ($[M+H]^+$, 100%); ESI-MS HR: m/z calcd for $C_{27}H_{43}N_2O_6$: 491.3121; found: m/z: 491.3134.

5.3.2. Peptidomimetic (1*S*,3*R*)-1a: (3*R*)-4-{butyl-[(1*S*)-1-(ethoxycarbonylmethyl-carbamoyl)-3-methyl-butyl]-carbamoyl}-3-phenyl-butyric acid ethyl ester. Yield 24%: transparent oil; $R_f = 0.35$ (hexane/EtOAc, 6:4); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.85–0.95 (m, 9H). 1.08 (dt, $J = 2.2$ Hz, $J = 7.1$ Hz, 3H), 1.19 (t, $J = 7.1$ Hz, 3H), 1.25–1.50 (m, 5H), 1.55–1.80 (m, 2H), 2.55–2.77 (m, 4H), 3.00–3.15 (m, 2H), 3.50–3.60 (m, 1H), 3.65–3.80 (m, 2H), 3.97 (q, $J = 2.6$ Hz, $J = 7.1$ Hz, 2H), 4.10 (q, $J = 7.1$ Hz, 2H), 4.90 (m, 1H), 6.48 (br s, 1H), 7.10–7.30 (m, 5H). Analytical data were almost the same as for the compound (1*R*,3*S*)-**1a**.

5.3.3. Peptidomimetic (1*S*,3*S*)-1a: (3*S*)-4-{butyl-[(1*S*)-1-(ethoxycarbonylmethyl-carbamoyl)-3-methyl-butyl]-carbamoyl}-3-phenyl-butyric acid ethyl ester. Yield 16%: transparent oil; $R_f = 0.31$ (hexane/EtOAc, 6:4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.79 (d, $J = 6.5$ Hz, 3H), 0.85 (d, $J = 6.6$ Hz, 3H), 0.88–0.97 (m, 3H), 1.15 (t, $J = 7.1$ Hz, 3H), 1.26 (t, $J = 7.1$ Hz, 3H), 1.24–1.32 (m, 2H), 1.35–1.55 (m, 4H), 1.68–1.75 (m, 1H), 2.60–2.80 (m, 4H), 3.05–3.20 (m, 2H), 3.73–3.84 (m, 1H), 3.85 (d, $J = 5.3$ Hz, 1H), 3.98 (d, $J = 6.3$ Hz, 1H), 4.00–4.08 (m, 2H), 4.17 (q, $J = 7.1$ Hz, 2H), 4.97 (t, $J = 7.4$ Hz, 1H), 7.00 (t, $J = 5.3$ Hz, 1H), 7.17–7.30 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.6, 14.0, 14.1, 20.3, 22.1, 22.8, 24.5, 31.9, 36.5, 38.8, 39.6, 40.7, 41.1, 60.4, 61.1, 126.8, 127.2, 127.4, 128.5, 143.1, 169.5, 172.0, 172.1, 172.8; MS (ESI): $m/z = 513$ ($[M+Na]^+$, 50%), 491 ($[M+H]^+$, 100%); ESI-MS HR: m/z calcd for $C_{27}H_{43}N_2O_6$: 491.3121; found: m/z: 491.3105.

5.3.4. Peptidomimetic (1*R*,3*R*)-1a: (3*R*)-4-{butyl-[(1*R*)-1-(ethoxycarbonylmethyl-carbamoyl)-3-methyl-butyl]-carbamoyl}-3-phenyl-butyric acid ethyl ester. Yield 32%: transparent oil; $R_f = 0.31$ (hexane/EtOAc, 6:4); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.71–0.97 (m, 9H), 1.08 (t, $J = 7.1$ Hz, 3H), 1.19 (t, $J = 7.1$ Hz, 3H), 1.20–1.23 (m, 3H), 1.24–1.99 (m, 7H), 2.60–2.80 (m, 4H), 3.05–3.20 (m, 2H), 3.73–3.84 (m, 1H), 3.76–3.89 (m, 2H), 4.03 (q, $J = 7.1$ Hz, 2H), 4.17 (q, $J = 7.1$ Hz, 2H), 4.97 (m, 1H), 7.00 (br s, 1H), 7.17–7.30 (m, 5H). Analytical data were almost the same as for the compound (1*S*,3*S*)-**1a**.

5.3.5. Peptidomimetic (1*R*,3*S*)-1b: (3*S*)-4-[((1*R*)-1-benzyl-carbamoyl-3-methyl-butyl)-butyl-carbamoyl]-3-phenyl-butyric acid ethyl ester. Yield 27%: transparent oil; $R_f = 0.60$ (hexane/EtOAc, 6:4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83–0.92 (m, 9H), 1.14 (t, $J = 7.1$ Hz, 3H), 1.17–1.33 (m, 4H) 1.36–1.48 (m, 1H), 1.49–1.61 (m, 1H), 1.69–1.84 (m, 1H), 2.63 (dd, $J = 7.5$ Hz, $J = 15.5$ Hz, 1H), 2.66–2.72 (m, 2H), 2.75 (dd, $J = 7.3$ Hz, $J = 15.5$ Hz, 1H), 3.05–3.15 (m, 2H), 3.73 (quintet, $J = 7.3$ Hz, 1H), 4.01 (dq, $J = 3.6$ Hz, $J = 7.1$ Hz, 2H), 4.17 (dd, $J = 5.6$ Hz, $J = 14.9$ Hz, 1H), 4.33 (dd, $J = 6.4$ Hz, $J = 14.9$ Hz, 1H), 4.94 (br s, 1H), 6.66 (br s, 1H), 7.13–7.31 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.6, 14.0, 20.2, 22.2, 22.9, 24.7, 32.0, 36.7, 38.8, 39.7, 40.5, 43.2, 45.2, 60.4, 126.8, 126.9, 127.1, 127.2, 127.3, 127.5, 127.7, 128.4, 128.5, 128.6, 138.4, 143.0, 171.4, 171.8, 172.5; ESI-MS: $m/z = 517$ ($[M+Na]^+$, 100%); ESI-MS HR: m/z calcd for $[M+Na]^+$, $C_{30}H_{42}N_2O_4Na$: 517.3037; found: 517.3062.

5.3.6. Peptidomimetic (1*S*,3*R*)-1b: (3*R*)-4-[((1*S*)-1-benzyl-carbamoyl-3-methyl-butyl)-butyl-carbamoyl]-3-phenyl-butyric acid ethyl ester. Yield 27%: transparent oil; $R_f = 0.60$ (hexane/EtOAc, 6:4); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.79–1.00 (m, 9H), 1.14–1.47 (m, 7H), 1.53–1.69 (m, 2H), 1.70–1.90 (m, 1H), 2.55–2.82 (m, 4H), 3.05–3.15 (m, 2H), 3.74 (quintet, $J = 7.4$ Hz, 1H), 4.01 (q, $J = 7.1$ Hz, 2H), 4.16 (dd, $J = 6.5$ Hz, $J = 14.8$ Hz, 1H), 4.34 (dd, $J = 5.6$ Hz, $J = 14.8$ Hz, 1H), 4.94 (t, $J = 7.6$ Hz, 1H), 6.63 (br s, 1H), 7.12–7.39 (m, 10H). Analytical data were almost the same as for the compound (1*R*,3*S*)-**1b**.

5.3.7. Peptidomimetic (1*S*,3*S*)-1b: (3*S*)-4-[((1*S*)-1-benzyl-carbamoyl-3-methyl-butyl)-butyl-carbamoyl]-3-phenyl-butyric acid ethyl ester. Yield 31%: transparent oil; $R_f = 0.58$ (hexane/EtOAc, 6:4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.79 (d, $J = 6.5$ Hz, 3H), 0.82–0.97 (m, 6H), 1.12 (t, $J = 7.1$ Hz, 3H), 1.18–1.33 (m, 4H), 1.35–1.45 (m, 1H), 1.47–1.57 (m, 1H), 1.70–1.77 (m, 1H), 2.64 (dd, $J = 5.4$ Hz, $J = 7.3$ Hz, 2H), 2.69 (dd, $J = 7.3$ Hz, $J = 8.5$ Hz, 2H), 3.05–3.15 (m, 2H), 3.75 (quintet, $J = 7.3$ Hz, 1H), 3.98 (dq, $J = 4.3$ Hz, $J = 7.1$ Hz, 2H), 4.35 (d, $J = 6.0$ Hz, 2H), 4.94 (t, $J = 7.4$ Hz, 1H), 6.95 (br s, 1H), 7.14–7.31 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.6, 14.0, 20.2, 22.1, 22.2, 22.9, 24.6, 31.9, 36.6, 38.8, 39.5, 40.8, 43.3, 45.0, 60.4, 126.8, 127.1, 127.2, 127.7, 128.5, 128.6, 138.4, 140.1, 171.5, 172.0, 172.6; ESI-MS: $m/z = 517$ ($[M+Na]^+$, 100%); ESI-MS HR: m/z calcd for $[M+Na]^+$, $C_{30}H_{42}N_2O_4Na$: 517.3037; found: 517.3020.

5.3.8. Peptidomimetic (1*R*,3*R*)-1b: (3*R*)-4-[((1*R*)-1-benzyl-carbamoyl-3-methyl-butyl)-butyl-carbamoyl]-3-phenyl-butyric acid ethyl ester. Yield 28%: transparent oil; $R_f = 0.58$ (hexane/EtOAc, 6:4); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.83–1.00 (m, 9H), 1.14 (t, $J = 7.1$ Hz, 3H), 1.05–1.80 (m, 7H), 2.60–2.77 (m, 4H), 3.03–3.18 (m, 2H), 3.73 (quintet, $J = 7.4$ Hz, 1H), 3.99 (q, $J = 7.1$ Hz, 2H), 4.28 (d, $J = 5.8$ Hz, 2H), 4.76 (dd, $J = 6.5$ Hz, $J = 8.8$ Hz, 1H), 6.96 (br s, 1H), 7.14–7.30 (m, 10H). Analytical data were almost the same as for the compound (1*S*,3*S*)-**1b**.

5.3.9. *N*-Benzyloxycarbonyl-L-leucine 3: (2*S*)-benzyloxycarbonylamino-4-methyl-pentanoic acid.[26] To a solution of L-leucine (**2**, 8.15 mmol, 1.07 g) in 2 M $NaOH_{aq}$ (10 mL), cooled to 0 °C, benzyl chloroformate (9.45 mmol, 1.40 mL) was added dropwise over 30 min. The mixture

was stirred at rt for another 2 h, then acidified with HCl_{concd}. The aqueous phase was extracted with EtOAc (3×15 mL) and the combined organic layers were dried ($MgSO_4$). The solvent was evaporated in vacuo to give crude *N*-benzyloxycarbonyl-L-leucine **3** as a transparent oil in 92% yield: $[\alpha]_D^{24} = -16.8$ (*c* 2.03, EtOH), (lit. −16.7 (*c* 2.0, EtOH)[26]); ^1H NMR (200 MHz, $CDCl_3$) δ 0.80–1.00 (m, 6H), 1.50–1.80 (m, 3H), 4.30–4.50 (m, 1H), 5.11 (s, 2H), 5.26 (d, $J = 8.6$ Hz, 1H), 7.34 (s, 5H).

5.3.10. *N*-Benzyloxycarbonyl-D-leucine *ent*-3: (2*R*)-benzyloxycarbonylamino-4-methyl-pentanoic acid.[27] The compound *ent*-**3** was synthesized as described for the compound **3**. 90% yield: transparent oil; $[\alpha]_D^{22} = +16.9$ (*c* 2.00, EtOH), (lit. +14.7 (*c* 1.0, MeOH)[27]); ^1H NMR δ (200 MHz, $CDCl_3$) 0.80–1.00 (m, 6H), 1.50–1.80 (m, 3H), 4.30–4.50 (m, 1H), 5.11 (s, 2H), 5.26 (d, $J = 8.6$ Hz, 1H), 7.34 (s, 5H).

5.3.11. *N*-(*N*-Benzyloxycarbonyl-D-leucyl)-glycine ethyl ester *ent*-4a: ((2*R*)-2-benzyloxycarbonylamino-4-methyl-pentanoylamino)-acetic acid ethyl ester. Cbz-D-leucine *ent*-**3** (3.95 mmol, 1.048 mg), glycine ethyl ester hydrochloride (4.03 mmol, 553 mg), and HOBt (4.05 mmol, 547 mg) were suspended in dry CH_2Cl_2 (10 mL) and cooled to 0 °C. DCC (4.00 mmol, 540 mg) was added, and the mixture stirred for 15 min at 0 °C. Then Et_3N (2.50 mmol, 350 μL) was added dropwise over 15 min. The mixture was stirred at 0 °C for another 30 min, and then at rt overnight. The precipitated urea was filtered off and the solution concentrated in vacuo. The residue was dissolved in Et_2O (5 mL) and washed successively with citric acid (1 M aqueous solution), $NaHCO_{3satd}$ and brine. The organic layer was dried over $MgSO_4$ and the solvent evaporated in vacuo to give the crude product. Recrystallization from Et_2O/hexane gave compound *ent*-**4a** in 78% yield: white crystals; mp 96 °C; $[\alpha]_D^{25} = +27.6$ (*c* 1.00, MeOH); ^1H NMR (200 MHz, $CDCl_3$) δ 0.93 (d, $J = 5.8$ Hz, 6H), 1.27 (t, $J = 7.1$ Hz, 3H), 1.50–1.80 (m, 3H), 4.00 (d, $J = 5.1$ Hz, 2H), 4.19 (q, $J = 7.1$ Hz, 2H), 4.15–4.30 (m, 1H), 5.10 (d, $J = 2.1$ Hz, 2H), 5.37 (d, $J = 8.4$ Hz, 1H), 6.70 (br s, 1H), 7.34 (s, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 23.1, 24.8, 41.5, 53.6, 61.7, 67.3, 128.2, 128.4, 128.6, 148.5, 156.4, 169.9, 172.5; Anal. Calcd for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 7.99. Found: C, 61.97; H, 7.58; N, 8.04.

5.3.12. *N*-(*N*-Benzyloxycarbonyl-L-leucyl)-glycine ethyl ester 4a: ((2*S*)-2-benzyloxycarbonylamino-4-methyl-pentanoylamino)-acetic acid ethyl ester.[28] The compound **4a** was synthesized as described for the compound *ent*-**4a**. 61% yield: white crystals (Et_2O/hexane); mp 98 °C (lit. 101[15]); $[\alpha]_D^{25} = -27.5$ (*c* 1.05, MeOH) (lit. $[\alpha]_D^{23} = -26.4$ (*c* 1.94, EtOH)[28]); ^1H NMR (200 MHz, $CDCl_3$) δ 0.93 (d, $J = 5.8$ Hz, 6H), 1.27 (t, $J = 7.1$ Hz, 3H), 1.50–1.80 (m, 3H), 4.00 (d, $J = 5.1$ Hz, 2H), 4.19 (q, $J = 7.1$ Hz, 2H), 4.15–4.30 (m, 1H), 5.10 (d, $J = 2.1$ Hz, 2H), 5.37 (d, $J = 8.4$ Hz, 1H), 6.70 (br s, 1H), 7.34 (s, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 23.1, 24.8, 41.5, 53.6, 61.7, 67.3, 128.2, 128.4, 128.6, 148.5, 156.4, 169.9, 172.5; Anal. Calcd for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 7.99. Found: C, 61.74; H, 7.76; N, 7.72.

5.3.13. *N*-Benzyloxycarbonyl-D-leucine benzylamide *ent*-4b: (2*R*)-2-benzyloxycarbonylamino-4-methyl-pentanoic acid benzylamide. Cbz-D-leucine *ent*-**3** (4.00 mmol, 1.06 g), benzylamine (4.02 mmol, 440 μL), and HOBt (4.07 mmol, 550 mg) were dissolved in dry CH_2Cl_2 (10 mL) and cooled to 0 °C. DCC (4.10 mmol, 846 mg) was then added. The mixture was stirred for 15 min at 0 °C and at rt overnight. The precipitated urea was filtered off and the solution was concentrated in vacuo. The residue was dissolved in EtOAc (5 mL) and washed successively with citric acid (1 M aqueous solution), $NaHCO_{3satd}$ and brine. The organic layer was dried over $MgSO_4$ and the solvent evaporated in vacuo to give a crude solid product. This was recrystallized from Et_2O/hexane to give product *ent*-**4b** in 80% yield: white crystals, mp 103–108 °C; $[\alpha]_D^{26} = +18.5$ (*c* 1.26, EtOH); ^1H NMR (200 MHz, $CDCl_3$) δ 0.92 (d, $J = 6.0$ Hz, 6H), 1.20–1.80 (m, 3H), 4.10–4.30 (m, 1H), 4.40–4.49 (m, 2H), 5.08 (s, 2H), 5.23–5.42 (m, 1H), 6.80 (br s, 1H), 7.20–7.40 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.0, 24.8, 41.6, 43.5, 53.7, 67.1, 127.5, 127.7, 128.1, 128.2, 128.6, 128.7, 135.2, 138.1, 156.4, 172.4; Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 70.45; H, 7.56; N, 8.11.

5.3.14. *N*-Benzyloxycarbonyl-L-leucine benzylamide 4b: (2*S*)-2-benzyloxycarbonylamino-4-methyl-pentanoic acid benzylamide.[29] The compound **4b** was synthesized as described for compound *ent*-**4b**. 81% yield: white crystals (Et_2O/hexane); 110 °C (lit. 112–113[29]); $[\alpha]_D^{22} = -14.1$ (*c* 1.20, EtOH) (lit. $[\alpha]_D = -16.6$ (*c* 1.20, EtOH)[29]); ^1H NMR (200 MHz, $CDCl_3$) δ 0.85–0.92 (m, 6H), 1.20–1.90 (m, 3H), 4.10–4.30 (m, 1H), 4.39–4.49 (m, 2H), 5.07 (s, 2H), 5.21–5.38 (m, 1H), 6.80 (br s, 1H), 7.20–7.40 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.0, 24.8, 41.6, 43.5, 53.7, 67.1, 127.5, 127.7, 128.1, 128.2, 128.6, 128.7, 135.2, 138.1, 156.4, 172.4; Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.02; H, 7.41; N, 8.02.

5.3.15. L-Leucine benzylamide 5b: (2*S*)-2-amino-4-methyl-pentanoic acid benzylamide.[30] Hydrogen gas was bubbled through the stirred solution of Cbz-D-leucine benzylamide **4b** (1.84 mmol, 654 mg) and palladium (10% Pd/C, 360 mg) in methanol (20 mL). After 2 h, the catalyst was filtered off and the solution was evaporated to give product **5b** as a transparent oil in 81% yield: ^1H NMR (200 MHz, $CDCl_3$) δ 0.85–0.92 (m, 6H), 1.20–1.90 (m, 3H), 3.30–3.49 (m, 1H), 4.42 (d, $J = 5.9$ Hz, 2H), 7.20–7.40 (m, 5H), 7.70 (br s, 1H); ESI-MS: *m/z* 221 ($[M+H]^+$,100%); ESI-MS HR: *m/z* calcd for $C_{13}H_{21}N_2O$ ($[M+H]^+$): 221.1648; found: 221.1662.

5.3.16. *N*-Butyl-L-leucine benzylamide 6b: (2*S*)-2-(butylamino)-4-methyl-pentanoic acid benzylamide. To a cooled 0 °C suspension of $NaBH_3CN$ (1.84 mmol, 116 mg), Na_2SO_4 (166 mg), and L-leucine benzylamide **5b** (0.38 mmol, 83 mg) in dry THF, butanal (0.66 mmol, 60 μL) was added dropwise over 30 min. After stirring for 2.5 h at 0 °C, 10 mL of water was added and the aqueous slurry extracted with CH_2Cl_2 (3×10 mL). The combined organic phases were dried over $MgSO_4$ and the solvent evaporated in vacuo. The product was purified by

flash chromatography (hexane → hexane/EtOAc, 6:4) to give compound **6b** as a transparent oil that solidified upon standing. Yield 47%: mp 39 °C; $[\alpha]_D^{23} = -7.3$ (*c* 1.01, MeOH); ^{1}H NMR δ (200 MHz, $CDCl_3$) 0.82–0.93 (m, 9H), 1.20–1.49 (m, 4H), 1.50–1.90 (m, 3H), 2.41–2.63 (m, 2H), 3.06 (dd, $J = 9.6$ Hz, $J = 4.8$ Hz, 1H), 4.45 (d, $J = 5.9$ Hz, 2H), 7.20–7.40 (m, 5H), 7.64 (t, $J = 5.0$ Hz, 1H); ESI-MS: *m/z* 277 ($[M+H]^+$, 100%); ESI-MS HR: *m/z* calcd for $[M+H]^+$, $C_{17}H_{29}N_2O$ ($[M+H]^+$): 277.2274; found: 277.2280.

5.4. The general procedure for the synthesis of compound 6 in a one-pot procedure from the respective compound 4

Hydrogen gas was bubbled through a stirred solution of Cbz-protected leucine amide **4** (1.10 mmol) and palladium (10% Pd/C, 166 mg) in absolute EtOH (10 mL). After 2 h, the catalyst was filtered off and the ethanolic solution cooled to 0 °C. Dry Na_2SO_4 (~400 mg) was added, followed by butanal (2.22 mmol, 200 μL), and $NaBH_3CN$ (2.15 mmol, 135 mg) was added portionwise. After stirring for 1.5 h at 0 °C, the solvent was evaporated and the residue suspended in water (10 mL). The aqueous slurry was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated in vacuo. The product **6** was purified by flash chromatography (hexane → hexane/EtOAc, 6:4).

5.4.1. *N*-Butyl-D-leucine benzylamide (*ent*-6b): (2*R*)-2-(butylamino)-4-methyl-pentanoic acid benzylamide. 29% yield: transparent oil; $[\alpha]_D^{23} = +5.1$ (*c* 1.54, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.82–1.00 (m, 9H), 1.10–1.49 (m, 4H), 1.50–1.69 (m, 2H), 1.70–1.90 (m, 1H), 2.41–2.63 (m, 2H), 3.12 (dd, $J = 9.4$ Hz, $J = 4.4$ Hz, 1H), 4.43 (d, $J = 5.9$ Hz, 2H), 7.20–7.40 (m, 5H), 7.70 (br s, 1H); ^{13}C NMR δ 13.7, 20.2, 21.8, 23.2, 25.1, 32.3, 42.8, 48.7, 61.6, 127.2, 127.4, 127.7, 128.5, 128.6, 138.6, 175.1.

5.4.2. *N*-(*N*-Butyl-L-leucyl)-glycine ethyl ester 6a: ((2*S*)-2-butylamino-4-methyl-pentanoylamino)-acetic acid ethyl ester. 46% yield: transparent oil; $[\alpha]_D^{23} = -13.0$ (*c* 1.15, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.80–0.99 (m, 9H), 1.21 (t, $J = 7.1$ Hz, 3H), 1.19–1.60 (m, 4H), 1.61–1.80 (m, 3H), 2.51 (t, $J = 6.6$ Hz, 2H), 3.00 (dd, $J = 4.8$ Hz, $J = 7.5$ Hz, 1H), 3.96 (d, $J = 6.0$ Hz, 1H), 4.00 (d, $J = 6.0$ Hz, 1H), 4.13 (q, $J = 7.1$ Hz, 2H), 7.72 (t, $J = 5.3$ Hz, 1H); ^{13}C NMR δ 13.9, 14.1, 20.3, 21.8, 23.2, 25.1, 32.4, 40.8, 48.6, 61.1, 61.4, 169.8, 175.6; APCI-MS *m/z*: 273 ($[M+H]^+$, 100%); ESI-MS HR: *m/z* calcd for $[M+H]^+$, $C_{14}H_{29}N_2O_3$ ($[M+H]^+$): 273.2178; found: 273.21616.

5.4.3. *N*-(*N*-Butyl-D-leucyl)-glycine ethyl ester *ent*-6a: ((2*R*)-2-butylamino-4-methyl-pentanoylamino)-acetic acid ethyl ester. 44% yield: transparent oil, purified by flash chromatography (hexane → hexane/EtOAc, 6:4 → $CHCl_3$/MeOH/NH_3, 89:19:1); $[\alpha]_D^{23} = +16.8$ (*c* 0.72, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.80–0.99 (m, 9H), 1.24 (t, $J = 7.1$ Hz, 3H); 1.24–1.60 (m, 4H), 1.61–1.80 (m, 3H), 2.54 (t, $J = 6.5$ Hz, 2H), 3.03 (dd, $J = 4.5$ Hz, $J = 9.0$ Hz, 1H), 3.96 (d, $J = 5.6$ Hz, 1H), 4.00 (dd, $J = 5.9$ Hz, 1H), 4.15 (q, $J = 7.1$ Hz, 2H), 7.72 (t, $J = 5.3$ Hz, 1H); ^{13}C NMR δ 13.9, 14.1, 20.3, 21.8, 23.2, 25.1, 32.4, 40.8, 48.6, 61.1, 61.4, 169.8, 175.6; ESI-MS HR: *m/z* calcd for $[M+H]^+$, $C_{14}H_{29}N_2O_3$ ($[M+H]^+$): 273.2178; found: 273.2181.

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References

1. Loughlin, W. A.; Tyndall, J. D. A.; Glenn, M. P.; Fairlie, D. P. *Chem. Rev.* **2004**, *104*, 6085.
2. Tyndall, J. D. A.; Pfeiffer, B.; Abbenante, G.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 793.
3. Leban, J. J.; Landavazo, A.; McDermed, J. D.; Diliberto, E. J., Jr.; Jansen, M.; Stockstill, B.; Kull, F. C., Jr. *J. Med. Chem.* **1994**, *37*, 439.
4. Needham, J.; Kelly, M. T.; Ishige, M.; Andersen, R. J. *J. Org. Chem.* **1994**, *59*, 2058.
5. Tucker, T. J.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Lucas, B. J.; Sisko, J. T.; Lynch, J. J.; Lyle, E. A.; Baskin, E. P.; Woltmann, R. F.; Appleby, S. D.; Chen, I.-W.; Dancheck, K. B.; Naylor-Olsen, A. M.; Krueger, J. A.; Cooper, C. M.; Vacca, J. P. *J. Med. Chem.* **1997**, *40*, 3687.
6. Brady, S. F.; Stauffer, K. J.; Lumma, W. C.; Smith, G. M.; Ramjit, H. G.; Lewis, S. D.; Lucas, B. J.; Gardell, S. J.; Lyle, E. A.; Appleby, S. D.; Cook, J. J.; Holahan, M. A.; Stranieri, M. T.; Lynch, J. J., Jr.; Lin, J. H.; Chen, I.-W.; Vastag, K.; Naylor-Olsen, A. M.; Vacca, J. P. *J. Med. Chem.* **1998**, *41*, 401.
7. Ostaszewski, R.; Portlock, D. E.; Fryszkowska, A.; Jeziorska, K. *Pure Appl. Chem.* **2003**, *75*, 413.
8. Fryszkowska, A.; Frelek, J.; Ostaszewski, R. *Tetrahedron* **2005**, *61*, 6064.
9. Smith, H. E. Circular dichroism of the benzene chromophore. In *Circular Dichroism Principles and Applications*; Nakanishi, K., Berova, N., Woody, R. W., Eds.; VSH: New York, 2000; pp 397–429.
10. Fryszkowska, A.; Komar, M.; Koszelewski, D.; Ostaszewski, R. *Tetrahedron: Asymmetry* **2005**, *16*, 2475.
11. Fontana, L. P.; Smith, H. E. *J. Org. Chem.* **1987**, *52*, 3386.
12. Pickard, S. T.; Smith, H. E. *J. Am. Chem. Soc.* **1990**, *112*, 5741.
13. Koch, W.; Holthausen, M. C. *A Chemist's Guide to Density Functional Theory*; Wiley-VCH: Weinheim, 2000.
14. Adamo, C.; di Matteo, A.; Barone, V. From Classical Density Functionals to Adiabatic Connection Methods. The State of the Art. In *Advances in Quantum Chemistry*; Löwdin, P.-O., Ed.; Academic Press, 1999; Vol. 36.
15. Geerlings, P.; De Proft, F.; Langenaeker, W. *Chem. Rev.* **2003**, *103*, 1793.
16. Furche, F. J. *Chem. Phys.* **2001**, *114*, 5982.
17. Casida, M. E.; Jamorski, C.; Casida, K. C.; Salahub, D. R. *J. Chem. Phys.* **1998**, *108*, 4439.
18. Stratmann, E. R.; Scuseria, G. E.; Frisch, M. J. *J. Chem. Phys.* **1998**, *109*, 8218.
19. Gross, E. K. U.; Kohn, W. *Adv. Quantum Chem.* **1990**, *21*, 255.
20. Gross, E. K. U.; Dobson, J. F.; Petersilka, M. *Top. Curr. Chem.* **1996**, *181*, 81.

21. Bauernschmitt, R.; Ahlrichs, R. *Chem. Phys. Lett.* **1996**, *256*, 454.
22. CaChe Ws Pro 5.0 Fujitsu Ltd.
23. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A. J.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 03*; Gaussian: Pittsburgh, PA, 2001.
24. A recent paper has pointed out the need of using ΔG values rather than ΔE values for calculation of conformer distributions: Lattanzi, A.; Viglione, R. G.; Scettri, A.; Zanasi, R. *J. Phys. Chem. A* **2004**, *108*, 10749.
25. Diedrich, C.; Grimme, S. *J. Phys. Chem. A* **2003**, *107*, 2524.
26. Anbazhagan, M.; Reddy, T. I.; Rajappa, S. *J. Chem. Soc., Perkin Trans. 1* **1997**, *11*, 1623.
27. Moriniere, J.-L.; Danree, B.; Lemoine, J.; Guy, A. *Synth. Commun.* **1988**, *22*, 441.
28. Shiina, I.; Suenaga, Y.; Nakano, M.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **2000**, *73*, 2811.
29. Williams, M. W.; Young, G. T. *J. Chem. Soc.* **1964**, 3701.
30. Giordano, C.; Gallina, C.; Ottaviano, V.; Consalvi, V.; Scandurra, R. *Eur. J. Med. Chem. Chim. Ther.* **1992**, *27*, 865.