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Aspartame analogues containing 1-amino-2-phenylcyclohexanecarboxylic acids (c_6Phe)

Alberto Avenoza,[a] Miguel París,[a] Jesús M. Peregrina,[a] Myriam Alías,[b] María P. López,[b] José I. García[b] and Carlos Cativiela[b,*]

[a]*Departamento de Química, Universidad de La Rioja, Grupo de Síntesis Química de La Rioja, U.A.-C.S.I.C., 26006 Logroño, Spain*
[b]*Departamento de Química Orgánica, Facultad de Ciencias, Instituto de Ciencia de Materiales de Aragón, Universidad de Zaragoza-C.S.I.C., 50009 Zaragoza, Spain*

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Abstract—This report describes the synthesis and the conformational analysis of the optically pure dipeptides analogues of aspartame: H-(*S*)-Asp-(1*R*,2*R*)-c_6Phe-OMe and H-(*S*)-Asp-(1*S*,2*S*)-c_6Phe-OMe, in which the Phe residue of aspartame has been replaced by a restricted Phe with a cyclohexane skeleton: 1-amino-2-phenylcyclohexanecarboxylic acid (c_6Phe). Of these, only the dipeptide that incorporates (1*R*,2*R*)-c_6Phe is sweet, whereas that incorporates (1*S*,2*S*)-c_6Phe is bitter. This relationship between the absolute configuration of the dipeptides and the properties is explained through the different conformational behaviour displayed by each molecule, based on molecular mechanics and molecular dynamics calculations, including solvent effects.

1. Introduction

Aspartame (H-(*S*)-Asp-(*S*)-Phe-OMe), a dipeptide approximately 150–200 times sweeter than sucrose,[1] was discovered by accident in 1965, when James Schlatter, a chemist of G. D. Searle Company was testing an anti-ulcer drug. Due to the explosion of no calorie or low calorie foods in the marketplace and taking into account that aspartame is made up primarily of two amino acids, it was initially regarded as the perfect artificial sweetener. However, since its initial approval by Food and Drug Administration (FDA) in 1981 for use in United States as food additive, some questions have arisen about its safety. Because of this, aspartame may be the most systematically studied food additive.

In recent years, several efforts have been made in order to elucidate the stereochemical basis of sweet taste and two models have emerged as the most appropriates: Temussi[2] and Goodman.[3] The core of the difference between both models is the conformational flexibility of peptide tastants like aspartame or its dipeptide analogues and consequently the difficulty to elucidate which is the bioactive conformation of those aspartame-like sweeteners. Thus, in order to develop a three-dimensional receptor model, several conformationally restricted analogues of aspartame have been synthesised and studied. Since it has been shown that many changes are well tolerated in the phenylalanine (Phe) of the dipeptide[4–6] and taking into consideration that one approach involves the use of constrained amino acids,[7,8] the Phe of aspartame have been replaced by different conformationally restricted amino acids and, in particular, different α-methyl α-amino acids have been used.[9–11] More restriction was observed when the Phe was substituted by cyclopropane amino acids: 1-aminocyclopropanecarboxylic acid (Acc)[12,13] and 1-amino-2-phenylcyclopropanecarboxylic acids (c_3Phe)[14] but, of these, only H-Asp-Acc-OR are sweet whereas all four isomers of H-Asp-c_3Phe-OMe are tasteless.

However, to the best of our knowledge, Phe of aspartame has never been replaced by cyclohexane-α-amino acids, in which the torsional angle χ^1 is restricted by tethering C_α to C_β.[15] In this field, we have recently reported the synthesis of 1-amino-2-phenylcyclohexanecarboxylic acids (c_6Phe) using a Diels–Alder reaction[16] as conformationally constrained Phe analogues and their incorporation into some dipeptides to modulate the β-folding mode.[17] Moreover, the influence of side chain restriction and NH…π interaction on the β-turn folding modes of dipeptides incorporating c_6Phe derivatives have been studied.[18]

Therefore, we have considered the replacement of Phe by c_6Phe in the aspartame dipeptide. Thus, in this paper we report the synthesis and the conformational behaviour of

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* Corresponding author. Tel./fax: +34-976-761-210; e-mail: cativiela@posta.unizar.es.
Abbreviations: Cbz, carbobenzyloxycarbonyl; Et_2O, diethyl ether; EtOAc, ethyl acetate; EtOH, ethanol; MeOH, methanol; NMM, *N*-methylmorpholine; O^{t}Bu, *tert*-butoxy; OMe, methoxy; OR, alkoxy; TFA, trifluoroacetic acid; Bz, benzoyl.

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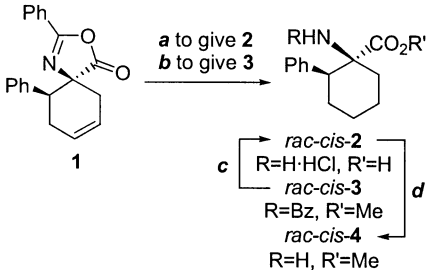


Scheme 1. *Reagents and conditions*: (a) Ref. 16. (b) and (c) Ref. 20. (d) $SOCl_2$, MeOH.

two stereoisomers of H-(*S*)-Asp-c_6Phe-OMe as conformationally resctricted aspartame analogues.

2. Results and discussion

2.1. Peptide synthesis

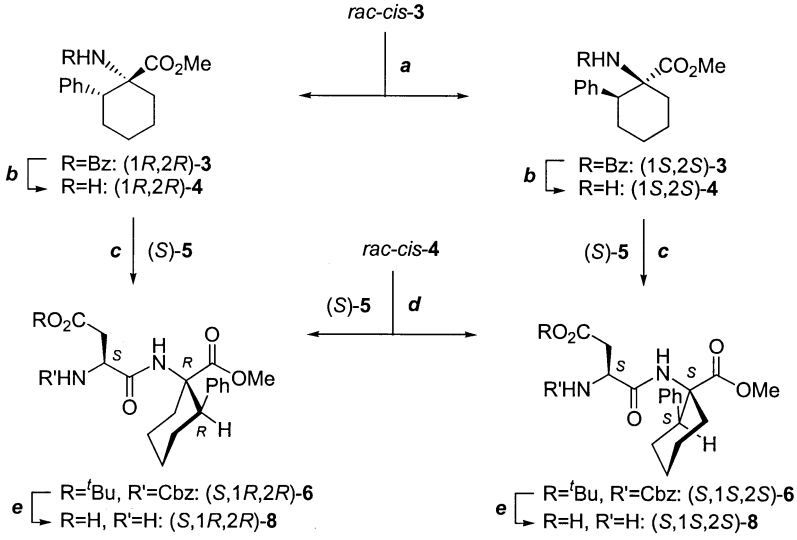
We have developed two synthesis of the aspartame analogues. Firstly, our starting material was Bz-*rac-cis*-c_6Phe-OMe, *rac-cis*-**3**, which was previously obtained[16] from **1**, a precursor of *rac-cis*-c_6Phe·HCl, *rac-cis*-**2** (Scheme 1). Thus, in order to obtain the optically pure amino esters *cis*-**4**, the separation of the enantiomerically pure Bz-(1*R*,2*R*)-c_6Phe-OMe and Bz-(1*S*,2*S*)-c_6Phe-OMe, (1*R*,2*R*)-**3** and (1*S*,2*S*)-**3**, from Bz-*rac-cis*-c_6Phe-OMe (*rac-cis*-**3**) was achieved by chiral HPLC using a covalently bonded amylose-derived CSP on a semipreparative column.[19,20] Once enantiomerically pure precursors were available, two easy steps, hydrolysis followed by formation of the methyl ester with thionyl chloride/methanol, led respectively to pure (1*R*,2*R*)-**4** and (1*S*,2*S*)-**4** in high yields (Scheme 2).

Starting from (1*R*,2*R*)-**4** and (1*S*,2*S*)-**4**, we assayed different methods to couple these amino esters with the conveniently protected aspartic acid Cbz-(*S*)-Asp(O^{t}Bu)-OH, (*S*)-**5**. Of all coupling conditions tried the best results were obtained when we used ^{i}BuOCOCl in the presence of NMM in CH_2Cl_2 at -15°C.[21,22] In this way, we synthesised the diastereoisomers (*S*,1*R*,2*R*)-**6** and (*S*,1*S*,2*S*)-**6** with an overall yield of 93% from amino esters (1*R*,2*R*)-**4** and (1*S*,2*S*)-**4**, respectively (Scheme 2). Both optically pure compounds were characterised by their spectral data.

An alternative way to obtain the protected dipeptides (*S*,1*R*,2*R*)-**6** and (*S*,1*S*,2*S*)-**6** as optically pure compounds involves the same synthetic procedure but now starting from H-*rac-cis*-c_6Phe-OMe (*rac-cis*-**4**) and further chromatographic separation of the corresponding diastereoisomeric mixture[19] (Scheme 2). The synthesis of amino ester *rac-cis*-**4** was achieved from *rac-cis*-**2**, in good yield, by formation of the methyl ester with thionyl chloride/methanol (Scheme 1). Coupling of *rac-cis*-**4** with (*S*)-**5** provided the mixture of diastereoisomers in excellent yield. Expected separation of two stereoisomers could not be achieved by flash chromatography under any conditions tested. This difficulty prompted us to explore the use of semipreparative HPLC for the resolution of (*S*,1*R*,2*R*)-**6** and (*S*,1*S*,2*S*)-**6**.

Resolution was firstly examined at the analytical scale in normal phase using silica as stationary phase. Elution and detection conditions of the best-performed separations are given in Table 1. Thus, the same amylose-based chiral column previously used in the separation of *rac-cis*-**3**, proved also to be very efficient in other semipreparative resolutions of phenylalanine surrogates,[23,24] was examined. The mixture of *n*-hexane/2-propanol/chloroform indicated in Table 1 was therefore selected as an eluent in an attempt to optimise the resolution in relation with the column loadability.

The extension of the analytical conditions to the semipreparative scale proved to be very efficient (α and R_s values are given in Table 1). The sample was dissolved in chloroform and the separation was achieved by successive injections using the peak shaving technique. Thus, 2.1 g of diastereomeric mixture afforded 1.6 g of optically pure material (ca. 0.8 g of each diastereoisomer) in a single passage through a 150 mm×20 mm ID column and with a



Scheme 2. *Reagents and conditions*: (a) Chiral HPLC. (b) (i) 6N HCl; (ii) $SOCl_2$, MeOH. (c) ^{i}BuOCOCl, NMM, CH_2Cl_2. (d) (i) ^{i}BuOCOCl, NMM, CH_2Cl_2; (ii) HPLC. (e) (i) TFA/CH_2Cl_2; (ii) H_2, Pd/C.

Table 1. Selected chromatographic data for the HPLC resolution of (*S*,1*R*,2*R*)-**6** and (*S*,1*S*,2*S*)-**6** on several stationary phases and chromatographic modes

Chromatographic mode	Column	Eluent[a] A/B/C	Flow (mL/min)	λ (nm)	k_1'	α	R_s
Normal phase	Nova-Pak[b]	98/2/0	0.7	210	1.15	1.19	1.96
Normal phase	Spherisorb[c]	98/2/0	1	210	2.19	1.18	2.12
Chiral phase[d]	Analytical[e]	98/2/0	1	210	2.35	1.71	3.09
Chiral phase[d]	Analytical[e]	96/2/2	1	225	2.81	1.68	1.73
Chiral phase[d]	Semipreparative[f]	96/2/2	18	265	1.79	1.55	0.94

For the definition of k', α and R_s see Section 4.
[a] A: *n*-hexane, B: 2-propanol, C: chloroform.
[b] Radial-pak cartridge, 4 μm Nova-Pak®, 100 mm×8 mm ID (analytical scale).
[c] Steel column, 5 μm Spherisorb®, 250 mm×4.6 mm ID (analytical scale).
[d] Mixed 10-undecenoate/3,5-dimethylphenylcarbamate of amylose bonded to 5 μm allylsilica.
[e] Steel column, 150 mm×4.6 mm ID.
[f] Steel column, 150 mm×20 mm ID.

total time of 4 h to complete the process. The optical purity of the compounds was checked on the analytical column.

Direct assignment of their absolute configuration could not be achieved because none of the diastereoisomers **6** gave single crystals suitable for X-ray diffraction analysis. Thus, in order to assign the stereochemistry of both dipeptides, we compared their spectral data with those corresponding to the dipeptides previously synthesised from each enantiomer (1*R*,2*R*)-**4** and (1*S*,2*S*)-**4**.

Deprotection of each diastereoisomer was carried out in two steps: firstly TFA in CH_2Cl_2 afforded (*S*,1*R*,2*R*)-**7** and (*S*,1*S*,2*S*)-**7**, further hydrogenolysis using palladium on carbon as a catalyst led to the optically pure analogues of aspartame H-(*S*)-Asp-(1*R*,2*R*)-c_6Phe-OMe and H-(*S*)-Asp-(1*S*,2*S*)-c_6Phe-OMe, (*S*,1*R*,2*R*)-**8** and (*S*,1*S*,2*S*)-**8**.

2.2. Peptide taste determination

Taste tests were carried out by a 'sip and spit' qualitative assessment of solutions of the compounds using a three-volunteer taste panel. The analogues were tasted in water at room temperature without any pH adjustment at 0.5% (w/v) concentration. These taste determinations show that compound (*S*,1*R*,2*R*)-**8** is sweet, although less potent than aspartame, with a metallic aftertaste; whereas compound (*S*,1*S*,2*S*)-**8** is bitter.

2.3. Molecular modelling studies

Molecular modelling studies carried out for aspartame and some of its analogues have led to a widely accepted model which relates the topochemical arrangement of the different groups of the dipeptide with the taste.[3] Following this

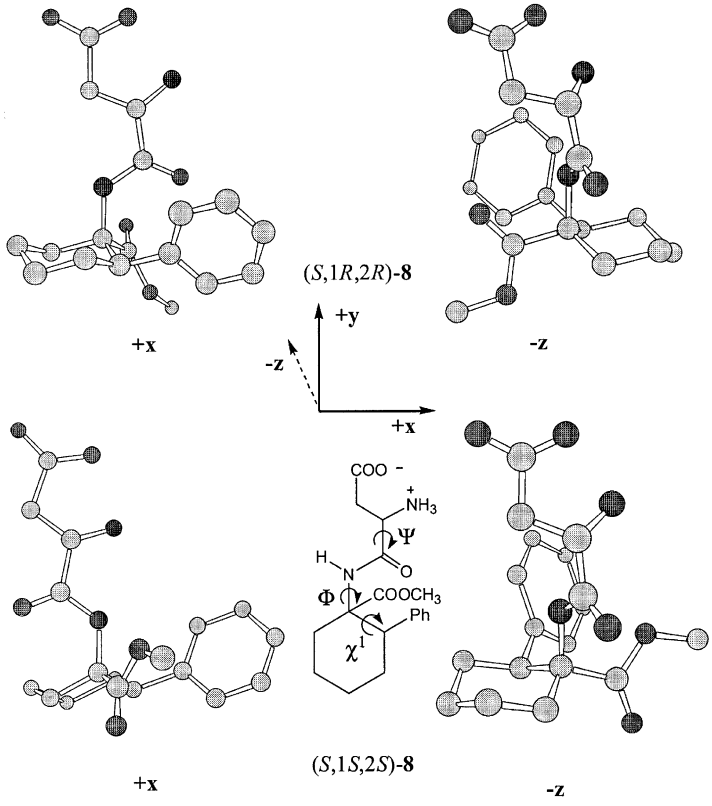


Figure 1. Calculated minimum energy conformations of (*S*,1*R*,2*R*)-**8** and (*S*,1*S*,2*S*)-**8** (hydrogen atoms have been omitted for clarity).

Table 2. Calculated energies (kcal/mol) and some selected dihedral angles for the most stable conformations of (*S*,1*R*,2*R*)-**8** and (*S*,1*S*,2*S*)-**8**

Compound	Conformer	Dihedral angles[a]			E_{MM2}	ΔG_{solv}	E_{Tot}	ΔE_{Tot}
		Ψ	Φ	χ^1				
(*S*,1*R*,2*R*)-**8**	+x	−156.3	−45.8	−35.7	−56.4	−79.8	−136.2	0.0
	−z	−40.9	+94.4	−54.7	−58.9	−73.3	−132.2	4.0
(*S*,1*S*,2*S*)-**8**	+x	+19.2	−93.5	+59.4	−58.5	−81.1	−139.6	0.8
	−z	−72.6	−75.2	+59.7	−63.1	−77.3	−140.4	0.0

[a] See Fig. 1 for the definition of the dihedral angles.

model, the sweet taste is associated to a L-shaped conformation, in which the hydrophobic group is pointing to the arbitrarily chosen +x coordinate axis (Fig. 1). On the other hand, the bitter taste is associated to the conformation in which the same group points in the −z direction, other possible arrangements leading to tasteless compounds.

In general, most of molecular modelling studies on this kinds of systems obviate solvation effects, that however, can be important for relatively high polar molecules as the dipeptides considered. In order to account for solvation effects, at least in an approximate manner, we envisaged a mixed scheme, combining molecular mechanics and quantum mechanics calculations. Thus, single point energy semiempirical AM1 calculations on the MM2 optimised structures (see Experimental for details) were carried out, using the COSMO continuum model, with the dielectric constant of water. The solvation energy was obtained as the energy difference between the solvated and the isolated structures, and then this energy was added to the MM2 steric energy to obtain the relative energies of the different conformers. Although this procedure does not allow to take into account solvent effects during the geometry optimisation, it has the advantage of conjugating the strength of MM2 force field for conformational analysis with that of a successful quantum mechanical method for calculating the solvation energy.

Table 2 gathers the minimum energy values obtained for conformations +x and −z of compounds (*S*,1*R*,2*R*)-**8** and (*S*,1*S*,2*S*)-**8**. The corresponding structures are shown in Fig. 1.

As can be seen, based on total energies, compound (*S*,1*R*,2*R*)-**8** should be sweet (since conformer +x is more stable than conformer −z), whereas compound (*S*,1*S*,2*S*)-**8** should be bitter (since the reverse relative stability is obtained). It can be then concluded that molecular mechanics calculations are in good agreement with the taste experimentally found for both analogues, which supports the validity of the model assumed for the structure–taste relationship.

From the methodological point of view, it is worth noting that inclusion of solvation energy is fundamental to arriving to this result. Thus, in both cases, the gas phase MM2 energies indicate the −z conformation as the most stable; however, the +x conformation is differentially more solvated than the −z conformation, giving rise to a reversal of stability in solution in the case of (*S*,1*R*,2*R*)-**8**.

3. Conclusion

Two new aspartame derivatives incorporating the constrained phenylalanine, c_6Phe, have been prepared showing different properties depending on the stereochemistry of the modified phenylalanine residue. The relations between the absolute configuration of the dipeptides and the properties are explained through the different conformational behaviour displayed by each molecule. The use of new constrained phenylalanines is in progress and the obtained results will be published in due course.

4. Experimental

4.1. General

4.1.1. Instrumentation. Solvents were purified according to standard procedures. Analytical TLC was performed using Merck 60 SI F_{254} precoated silica gel polyester plates using the following solvent systems: 1 (Hexane–EtOAc, 5:2); 2 (CH_2Cl_2–MeOH, 8:2). The products were examined by UV fluorescence or developed by iodine or by ninhydrin chromatic reaction as appropriate. Column chromatography was performed using silica gel 60 (230–400 mesh). Melting points were determined on a Büchi SMP-20 apparatus and were not corrected. IR spectra were registered on a Mattson Genesis FTIR spectrophotometer; ν_{max} is given for the main absorption bands. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity-300 or a Bruker ARX-300 instruments. Spectra were recorded in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard (chemical shifts are reported in ppm on the δ scale, coupling constants in Hz). Optical rotations were measured on a Perkin–Elmer 241 polarimeter-C in a 1 dm cell of 1 mL capacity. Microanalyses were carried out on a Perkin–Elmer 200 C, H, N, S analyser and were in good agreement with the calculated values. Mass spectra were obtained in the +FAB mode on a high resolution VG-autospectrometer.

4.1.2. HPLC. HPLC was carried out using a Waters HPLC system equipped with a Waters 600-E pump and a Waters 991 photodiode array detector. Radial-Pak cartridge 4 μm Nova-Pak® 100 mm×8 mm ID, steel column 5 μm Spherisorb® Silica 250 mm×4.6 mm ID and steel column 5 μm Xterra™ RP_{18} 4.6 mm×150 mm ID were purchased from Waters Chromatografia S.A. The chiral stationary phase consisting of a mixed 10-undecenoate/3,5-dimethylphenylcarbamate of amylose bonded to allylsilica was prepared according to a previously described procedure[25,26] by means of a radical reaction between the alkenyl groups in the polysaccharide and the modified silica (100 Å, 5 μm).

The chiral stationary phase obtained in this way was packed into stainless tubes by the slurry method. The analytical assays were carried out on a 150 mm×4.6 mm ID column and the semipreparative resolution was achieved on a 150 mm×20 mm ID column. All analytical assays and semipreparative chromatography were performed under the conditions given in Table 1. The solvents used as mobile phases were chromoscan grade from LabScan.

The capacity (k'), selectivity (α) and resolution (R_s) values were calculated according to the equations $k'_R=(t_R-t_0)/t_0$, $\alpha=k'_2/k_1'$, $R_s=1.18(t_2-t_1)/(w_2+w_1)$. Subscripts 1 and 2 refer to the first and second eluted diastereoisomer, respectively; t_R (R=1,2) are their retention times, and w_2 and w_1 denote their bandwidths at half-heigth; t_0 is the dead time.

4.1.3. Molecular modelling methodology. All computer simulations were carried out using the Chem3D program.[27] Molecular Mechanics and Molecular Dynamics were achieved by means of the MM2 force field,[28] as implemented in Chem3D. Molecular Dynamics trajectories were collected in periods of 1 ns, using a step interval of 1 fs, and a target temperature of 373 K. It is well-known that the dihedral angles of the Asp residue remains essentially unchanged for all minimum energy conformations of aspartame, and that this disposition is very similar to the X-ray crystal structure.[3] Consequently, the geometry of this residue was kept fixed in the disposition of the X-ray crystal structure of aspartame throughout all the simulations.

Solvation effects were taken into account by means of single point energy semiempirical AM1[29] calculations on the MM2 optimised structures, using the COSMO continuum model,[30] with the dielectric constant of water.

4.2. Synthesis of *rac-cis*-c$_6$Phe-OMe (*rac-cis*-4)

The preparation of 1-amino-2-phenylcyclohexane-1-carboxylic acid hydrochloride, *rac-cis*-**2**, from the cycloadduct obtained in the Diels–Alder reaction of 1,3-butadiene and (*Z*)-2-phenyl-4-benzylidene-5(4*H*)-oxazolone has already been reported.[16,20] In order to obtain the corresponding methyl ester, *rac-cis*-**4**, the following procedure was carried out.

$SOCl_2$ (0.9 mL, 11.8 mmol) was added dropwise to a stirred solution of MeOH (35 mL) cooled in an ice-bath. After 15 min of stirring at that temperature, *rac-cis*-**2** (2.13 g, 7.9 mmol) was added to the reaction mixture and reflux for 8 h. After the addition of 3 further portions of $SOCl_2$/MeOH, prepared as above mentioned, the solvents were eliminated in vacuo. The residue was partitioned between 5% $NaHCO_3$/EtOAc. The organic layer was washed with an additional portion of 5% $NaHCO_3$, dried and concentrated in vacuo to afford the racemic α-amino ester (yield 88%).

4.3. General procedure for the synthesis of protected dipeptides (*S*,1*R*,2*R*)-6, (*S*,1*S*,2*S*)-6

4.3.1. Procedure A. Cbz-(*S*)-Asp(O^{t}Bu)-OH, (*S*)-**5**, (2.46 g, 7.6 mmol) and NMM (768 mg, 7.6 mmol) were dissolved in dry CH_2Cl_2 (100 mL) under an inert atmosphere. The mixture was cooled at −15°C and a precooled solution of ^{i}BuOCOCl (1.03 g, 7.6 mmol) in dry CH_2Cl_2 (25mL) was added. After stirring for 20 min, a precooled solution in CH_2Cl_2 (25 mL) of H-*rac-cis*-c$_6$Phe-OMe, *rac-cis*-**4** (1.73 g, 7.4 mmol), which was used as obtained in Section 4.2 without any further purification, was added dropwise to the above solution. The reaction was stirred at −15°C for 1 h, then the solution was allowed to warm to room temperature. The solution was diluted with CH_2Cl_2 and washed with 5% $KHSO_4$, 5% $NaHCO_3$, brine, dried and evaporated to dryness. The product was purified by silica gel column chromatography using hexane–ethyl acetate (5:2) as eluent yielding the protected dipeptides in high yield (>90%).

Diastereomeric mixture of protected dipeptides (*S*,1*R*,2*R*)-**6** and (*S*,1*S*,2*S*)-**6** purified by silica gel column chromatography was resolved by semipreparative HPLC as described below.

4.3.2. Procedure B. The reported general synthetic way was applied separately to the optically pure enantiomers H-(1*R*,2*R*)-c$_6$Phe-OMe, (1*R*,2*R*)-**4**, and H-(1*S*,2*S*)-c$_6$Phe-OMe, (1*S*,2*S*)-**4**. Each protected dipeptide **6** was purified by silica gel column chromatography using hexane–ethyl acetate (5:2) as eluent. The optically pure diastereoisomers (*S*,1*R*,2*R*)-**6** and (*S*,1*S*,2*S*)-**6** are then obtained separately in high yield (>90%).

4.3.3. Cbz-(*S*)-Asp(O^{t}Bu)-(1*R*,2*R*)-c$_6$Phe-OMe, (*S*,1*R*,2*R*)-6. Mp 50°C. Rf_1=0.35. $[\alpha]_D^{20}=-14.9$ (*c* 0.4, $CHCl_3$). IR (nujol): 3354, 1724, 1708, 1689, 1679 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.10–1.54 (m, 2H), 1.41 (s, 9H), 1.55–2.08 (m, 5H), 2.59 (dd, 1H, *J*=17.7, 7.8 Hz), 2.72–2.96 (m, 2H), 3.06 (dd, 1H, *J*=13.2, 3.4 Hz), 3.40 (s, 3H), 4.45–4.60 (m, 1H), 5.08 (d, 1H, *J*=12.0 Hz), 5.15 (d, 1H, *J*=12.0 Hz), 5.81 (d, 1H, *J*=6.6 Hz), 6.79 (s, 1H), 7.00–7.10 (m, 2H), 7.20–7.40 (m, 8H). ^{13}C NMR ($CDCl_3$): δ 20.7, 25.7, 26.4, 28.0, 30.6, 37.7, 49.7, 51.1, 51.8, 64.1, 67.1, 81.8, 127.5, 127.6, 128.2, 128.3, 128.6, 128.6, 136.1, 139.9, 155.7, 170.0, 171.3, 172.9. MS (*m*/*z*, %): 539 [(M+1)$^+$, 81]. Anal. Calcd for $C_{30}H_{38}N_2O_7$: C, 66.89; H, 7.11; N, 5.20. Found: C, 66.97; H, 7.06; N, 5.14.

4.3.4. Cbz-(*S*)-Asp(O^{t}Bu)-(1*S*,2*S*)-c$_6$Phe-OMe, (*S*,1*S*,2*S*)-6. Mp 59°C. Rf_1=0.35. $[\alpha]_D^{20}=+27.8$ (*c* 0.5, $CHCl_3$). IR (nujol): 3372, 1721, 1703, 1688, 1676 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.25–1.50 (m, 2H), 1.37 (s, 9H), 1.60–2.05 (m, 5H), 2.50 (dd, 1H, *J*=17.1, 5.4 Hz), 2.84 (dd, 1H, *J*=17.1, 5.4 Hz), 2.94 (m, 1H), 3.08 (m, 1H), 3.42 (s, 3H), 4.42 (m, 1H), 5.13 (d, 1H, *J*=11.7 Hz), 5.20 (d, 1H, *J*=11.7 Hz), 6.14 (d, 1H, *J*=8.7 Hz), 6.68 (s, 1H), 7.05–7.15 (m, 2H), 7.15–7.25 (m, 3H), 7.25–7.45 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 20.5, 25.7, 26.6, 27.9, 30.9, 36.4, 49.7, 51.7, 51.8, 64.2, 67.2, 81.6, 127.5, 127.7, 127.9, 128.1, 128.3, 128.6, 136.1, 139.9, 156.1, 170.4, 171.4, 173.1. MS (*m*/*z*, %): 1099 [(2M+Na)$^+$, 11], 561 [(M+Na)$^+$, 100], 539 [(M+1)$^+$, 32]. Anal. Calcd for $C_{30}H_{38}N_2O_7$: C, 66.89; H, 7.11; N, 5.20. Found: C, 67.08; H, 7.03; N, 5.24.

4.4. Resolution of two stereoisomers of Cbz-(*S*)-Asp(O^{t}Bu)-*cis*-c$_6$Phe-OMe: isolation of (*S*,1*R*,2*R*)-6, (*S*,1*S*,2*S*)-6

HPLC resolution of a mixture of (*S*,1*R*,2*R*)-**6** and (*S*,1*S*,2*S*)-

6 (2.1 g) dissolved in chloroform (4.2 mL) was carried out by successive injections of 0.2 mL on a 150 mm×20 mm column filled with mixed 10-undecenoate/3,5-dimethylphenylcarbamate of amylose bonded to allylsilica using a mixture of *n*-hexane/2-propanol/chloroform 96/2/2 as the eluent (flow rate 18 mL/min). Each run was collected into three separated fractions. The combined first fractions, collected between 4.3 and 5.7 min, contained 804 mg of optically pure first eluted diastereoisomer (*S*,1*R*,2*R*)-**6**. The combined last fractions (collected between 7.1 and 10.5 min) contained 797 mg of optically pure more strongly retained diastereoisomer (*S*,1*S*,2*S*)-**6**.

4.5. General procedure for the synthesis of (*S*,1*R*,2*R*)-7, (*S*,1*S*,2*S*)-7

The corresponding protected dipeptide **6** (1 mmol) was dissolved in CH_2Cl_2 (15 mL). Then, TFA (7.5 mL) was added. The solution was stirred at room temperature for 1.5 h. TFA and CH_2Cl_2 were evaporated under reduced pressure to afford the final product.

4.5.1. Cbz-(*S*)-Asp-(1*R*,2*R*)-c$_6$Phe-OMe, (*S*,1*R*,2*R*)-7. This compound was prepared according to the procedure described above: Cbz-(*S*)-Asp(O^{t}Bu)-(1*R*,2*R*)-c$_6$Phe-OMe, (*S*,1*R*,2*R*)-**6** (646 mg, 1.2 mmol); CH_2Cl_2 (20 mL); TFA (10 mL). Yield: quantitative, mp 61°C. Rf_1=0.07, Rf_2=0.77. $[\alpha]_D^{20}$=−31.6 (*c* 0.38, $CHCl_3$). IR (nujol): 2300–3600, 1723, 1708, 1688, 1675 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.10–1.58 (m, 2H), 1.58–1.74 (m, 2H), 1.74–1.90 (m, 2H), 1.98 (ddd, 1H, *J*=13.5, 13.2, 2.7 Hz), 2.69 (dd, 1H, *J*=17.1, 6.3 Hz), 2.82–3.02 (m, 2H), 3.08 (dd, 1H, *J*=12.0, 3.0 Hz), 3.42 (s, 3H), 4.10 (br s, 1H), 4.59 (m, 1H), 5.09 (d, 1H, *J*=11.7 Hz), 5.17 (d, 1H, *J*=11.7 Hz), 5.71 (d, 1H, *J*=8.7 Hz), 6.56 (s, 1H), 6.95–7.05 (m, 2H), 7.15–7.25 (m, 3H), 7.25–7.40 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 20.6, 25.6, 26.4, 30.7, 35.4, 49.4, 51.3, 52.0, 64.6, 67.6, 127.5, 127.7, 128.4, 128.5, 128.6, 128.7, 135.7, 139.5, 156.2, 170.8, 172.7, 175.4. MS (*m*/*z*, %): 506 [(M+H+Na)$^+$, 77], 483 [(M+H)$^+$, 52], 423 [(M−COOCH$_3$)$^+$, 20]. Anal. Calcd for $C_{26}H_{30}N_2O_7$: C, 64.72. H, 6.27; N, 5.81; Found: C, 64.81; H, 6.18; N, 5.86.

4.5.2. Cbz-(*S*)-Asp-(1*S*,2*S*)-c$_6$Phe-OMe, (*S*,1*S*,2*S*)-7. This compound was prepared according to the procedure described above: Cbz-(*S*)-Asp(O^{t}Bu)-(1*S*,2*S*)-c$_6$Phe-OMe, (*S*,1*S*,2*S*)-**6** (646 mg, 1.2 mmol); CH_2Cl_2 (20 mL); TFA (10 mL). Yield: quantitative, mp 183°C. Rf_1=0.07, Rf_2=0.77. $[\alpha]_D^{20}$=+22.8 (*c* 0.5, $CHCl_3$). IR (nujol): 3283, 1749, 1699, 1684, 1676 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.18–1.55 (m, 2H), 1.55–1.90 (m, 4H), 1.97 (ddd, 1H, *J*=14.7, 13.2, 2.0 Hz), 2.64 (dd, 1H, *J*=17.1, 5.4 Hz), 3.43 (s, 3H), 4.49 (m, 1H), 5.13 (d, 1H, *J*=12.0 Hz), 5.22 (d, 1H, *J*=12.0 Hz), 6.01 (d, 1H, *J*=8.7 Hz), 6.63 (s, 1H), 7.00–7.20 (m, 2H), 7.21–7.42 (m, 8H). ^{13}C NMR ($CDCl_3$): δ 20.6, 25.6, 26.5, 29.7, 30.8, 35.0, 49.6, 51.3, 52.0, 64.4, 67.5, 127.6, 128.2, 128.5, 128.7, 139.7, 156.3, 170.5, 172.8, 175.2. MS (*m*/*z*, %): 505 [(M+Na)$^+$, 100], 483 [(M+H)$^+$, 93], 451 [(M+H–MeOH)$^+$, 30], 437 [(M−COOH)$^+$, 46], 423 [(M−COOCH$_3$)$^+$, 46]. Anal. Calcd for $C_{26}H_{30}N_2O_7$: C, 64.72; H, 6.27; N, 5.81. Found: C, 64.83; H, 6.31; N, 5.79.

4.6. General procedure for the synthesis of (*S*,1*R*,2*R*)-8, (*S*,1*S*,2*S*)-8

The corresponding semi-protected dipeptide **7** (1 mmol) was dissolved in 30 mL EtOAc/MeOH (1:1) and hydrogenated at room temperature in the presence of 10% palladium/carbon (45 mg) for 12 h. The solution was filtered, evaporated under reduced pressure and further lyophilised to afford a white solid. Both deprotected dipeptides were shown to be optically pure by reversed-phase HPLC (column: 5 μm Xterra™ RP_{18}, 4.6 mm× 150 mm ID). The elutions were performed isocratically with 20% CH_3CN/80% 0.0125 M NaH_2PO_4 (pH 2.5) (v/v) at a flow rate of 1 mL/min, with UV detection at 210 nm.

4.6.1. H-(*S*)-Asp-(1*R*,2*R*)-c$_6$Phe-OMe, (*S*,1*R*,2*R*)-8. This compound was prepared according to the procedure described above: Cbz-(*S*)-Asp-(1*R*,2*R*)-c$_6$Phe-OMe, (*S*,1*R*,2*R*)-**7** (433 mg, 0.9 mmol); 30 mL EtOAc/MeOH; 10% Pd/C (45 mg). Yield: 95.5%, mp 140–145°C. $[\alpha]_D^{20}$=−4.9 (*c* 0.5, MeOH). IR (nujol): 3600–2400, 1729, 1689, 1679 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.09–1.80 (m, 5H), 1.81–2.00 (m, 1H), 2.10–2.30 (m, 1H), 2.50 (m, 1H), 2.62 (dd, 1H, *J*=17.0, 6.6 Hz), 2.71 (dd, 1H, *J*=17.0, 6.6 Hz), 2.99 (dd, 1H, *J*=12.9, 2.7 Hz), 3.29 (s, 3H), 4.17 (t, 1H, *J*=6.6 Hz), 7.10–7.40 (m, 5H), 8.09 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 20.3, 25.3, 26.5, 30.5, 37.8, 49.6, 49.9, 51.2, 63.3, 126.9, 128.0, 128.1, 140.7, 168.8, 171.8, 172.1. MS (*m*/*z*, %): 393 [(M−H+2Na)$^+$, 100], 371 [(M+Na)$^+$, 89], 349 [(M+H), 23]. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 62.21; H, 6.97; N, 7.99.

4.6.2. H-(*S*)-Asp-(1*S*,2*S*)-c$_6$Phe-OMe, (*S*,1*S*,2*S*)-8. This compound was prepared according to the procedure described above: Cbz-(*S*)-Asp-(1*S*,2*S*)-c$_6$Phe-OMe, (*S*,1*S*,2*S*)-**7** (433 mg, 0.9 mmol); 30 mL EtOAc/MeOH; 10% Pd/C (45 mg). Yield: 98%, mp 145–150°C. $[\alpha]_D^{20}$=+65.9 (*c* 0.5, MeOH). IR (nujol): 3600–2400, 1728, 1687 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.25–1.80 (m, 5H), 1.81–2.00 (m, 1H), 2.17 (m, 1H), 2.22 (dd, 1H, *J*=16.2, 8.7 Hz), 2.34 (dd, 1H, *J*=16.2, 4.8 Hz), 2.63 ("d", 1H, *J*=12.9 Hz), 2.94 (dd, 1H, *J*=12.9, 2.4 Hz), 3.26 (s, 3H), 3.70 (m, 1H), 7.10–7,30 (m, 5H), 8.30 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 20.1, 25.3, 26.5, 30.6, 32.9, 48.7, 49.9, 51.2, 62.9, 126.9, 127.9, 128.0, 129.2, 140.7, 171.5, 172.4, 172.5. MS (*m*/*z*, %): 697 [(2M+H)$^+$, 8], 371 [(M+Na)$^+$, 42], 349 [(M+H)$^+$, 100]. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04; Found: C, 62.17; H, 6.98; N, 8.09.

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