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

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H-(*S*)-Asp-(1*R*,2*S*)-c_6Phe-OMe and H-(*S*)-Asp-(1*S*,2*R*)-c_6Phe-OMe, in which the Phe residue of aspartame has been replaced by a restricted Phe with a cyclohexane skeleton: 1-amino-2-phenylcyclohexanecarboxylic acid (c_6Phe).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

1. Introduction

The discovery of the dipeptide sweetener Aspartame[1] [H-(*S*)-Asp-(*S*)-Phe-OMe] has led to the synthesis of a large number of dipeptide analogues in an attempt to find a better taste profile and to elucidate the mechanism for the sweet response.

Analysis of the preferred conformations of aspartame using a combination of X-ray crystallography, ^{1}H NMR spectroscopy and molecular mechanics calculations has led several authors to propose different models for the tastant–receptor interaction. Two such models have emerged as the most appropriate: Temussi[2] and Goodman.[3] The difference between the two models concerns the conformational flexibility of aspartame dipeptides. As a result, the availability of conformationally restricted analogues is very important to establish the molecular arrays required for sweet and bitter tastes. The incorporation of side-chain constrained amino acids constitutes a powerful tool to explore this aspect. Restrictions on the χ^1 torsion angles will affect the conformational preferences of the side substituents and have an impact on the rotameric distribution with respect to unmodified residues. Thus, the incorporation of amino acids with well-defined χ^1 tendencies into bioactive peptides may provide fundamental insights into the precise conformational requirements of the side-chain groups to fit the receptor binding site.

Several conformationally restricted analogues of aspartame in which the Phe residue has been replaced by different conformationally restricted amino acids have been synthesised and studied. Particular examples have incorporated different 1-aminocycloalkanecarboxylic acids[4,5] and 1-amino 2-phenylcyclopropanecarboxylic acids (c_3Phe).[6] Some of the resulting compounds were found to be sweet whereas others are bitter or tasteless.

The presence in the phenylalanine analogue of a cyclohexane structure, which tethers C_α to C_β, (c_6Phe) appears to be very efficient for the restriction of the side-chain flexibility and is far superior to other restrictions such as α- and/or β-methylation. For each enantiomer only two χ^1 arrangements are accessible, with one of these being strongly discriminated by the propensity of the bulky phenyl group to occupy equatorial positions in a chair conformation. As a result, (1*S*,2*R*)-c_6Phe and (1*S*,2*S*)-c_6Phe can be viewed as frozen *gauche* (+) side-chain analogues of (*S*)-Phe and, conversely, (1*R*,2*S*)-c_6Phe as *gauche* (−) analogues of (*R*)-Phe.[7]

Due to this interesting restriction, and as an extension of our studies on constrained analogues of aspartame, we previously described the replacement of the Phe residue with

Keywords: Strecker; Cyclohexanes; Constrained phenylalanines; Aspartame analogues; Computer-assisted methods; Chiral stationary phase; HPLC resolution.
Abbreviations: TEA, triethylamine; Cbz, benzyloxycarbonyl; EtOAc, ethyl acetate; MeOH, methanol; NMM, *N*-Methylmorpholine; O^{t}Bu, *tert*-butoxy; OMe, methoxy; TFA, trifluoroacetic acid; Bz, benzoyl.
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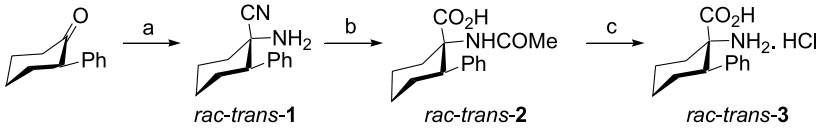


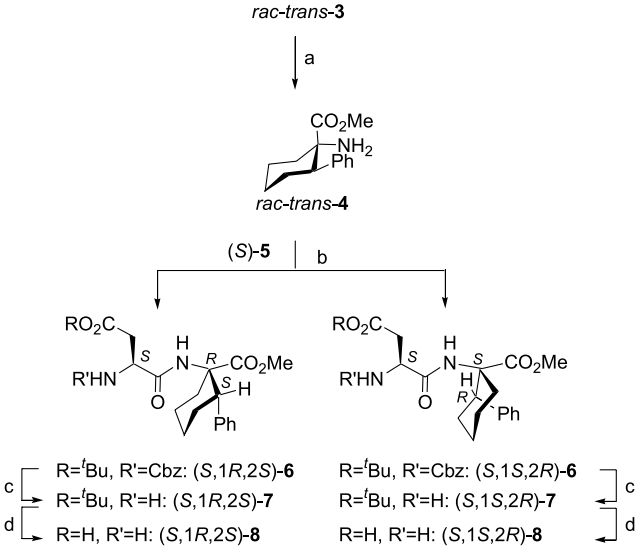
Scheme 1. Synthetic route to racemic *trans*-c_6Phe. Reagents and conditions: (a) NaCN, NH_4Cl, ^{i}PrOH, NH_4OH. (b) CH_3COCl, TEA, CH_2Cl_2. (c) 12 N HCl, reflux.

two enantiomers of *cis*-c_6Phe, namely (1*R*,2*R*)-c_6Phe and (1*S*,2*S*)-c_6Phe, which resulted in a sweet and a bitter compound, respectively.[8] The different organoleptical properties of the two molecules are related to the different conformational behaviour of each dipeptide, as exemplified by molecular calculations. These conformational preferences depend on the absolute configuration of each enantiomer of the Phe analogue.

cis-c_6Phe *trans*-c_6Phe

In the course of our work on the synthesis of conformationally restricted phenylalanines, we recently developed[9] a convenient route for the preparation of *trans*-c_6Phe in racemic form by a Strecker reaction on 2-phenylcyclohexanone. The importance of the absolute configuration of the Phe analogue in the sweet properties of the aspartame dipeptide makes the incorporation of the two enantiomers of *trans*-c_6Phe, i.e. (1*S*,2*R*)-c_6Phe and (1*R*,2*S*)-c_6Phe, indispensable to complete the synthesis and conformational studies of aspartame analogues incorporating all four isomers of restricted phenylalanine c_6Phe.

We report here the synthesis and conformational behaviour of H-(*S*)-Asp-(1*R*,2*S*)-c_6Phe-OMe and H-(*S*)-Asp-(1*S*,2*R*)-c_6Phe-OMe as conformationally restricted aspartame analogues.



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

2. Results and discussion

2.1. Peptide synthesis

The first step in the synthesis of the aspartame analogues involved the preparation of *rac-trans*-**3**. As previously described by our group,[9] a convenient route for the preparation of *trans*-c_6Phe in its racemic form involves a Strecker reaction of 2-phenylcyclohexanone and subsequent hydrolysis of the product (Scheme 1). The synthesis of amino ester *rac-trans*-**4** was achieved in moderate yield from *rac-trans*-**3** by formation of the methyl ester with thionyl chloride/methanol (Scheme 2). Once this starting material had been obtained, we attempted to couple these amino esters, *rac-trans*-**4**, with conveniently protected aspartic acid Cbz-(*S*)-Asp(O^{t}Bu)-OH, (*S*)-**5**. Coupling by the mixed-anhydride procedure,[10,11] using ^{i}BuOCOCl in the presence of NMM in CH_2Cl_2 at −15 °C, afforded a mixture of the desired diastereoisomers (*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6** in very good yield.

However, all attempts to separate the diastereoisomers by flash chromatography were unsuccessful. We therefore decided to explore the use of semi-preparative HPLC for the resolution of (*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6**. Analytical separation was initially examined in normal phase using silica as the stationary phase, but separation was not observed under any of the conditions tested. Better results were obtained on an amylose-derived chiral stationary phase, which has proved to be very efficient in other semi-preparative resolutions of phenylalanine surrogates.[8,12–16] Elution and detection conditions for the best separations are given in Table 1 and the analytical resolution is shown in Figure 1.

Table 1. Selected chromatographic data for the HPLC resolution of (*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6** on the amylose-derived chiral stationary phase

Column	Eluent[a] A/B/C	Flow (mL/min)	λ (nm)	k_1'	α	R_s
Analytical[b]	97/3/0	0.8	210	1.97	1.25	1.67
Semi-preparative[c]	96/3/1	15	254	1.79	1.22	0.90

For the definition of k', α and R_s see Section 4.
[a] A: *n*-hexane, B: 2-propanol, C: chloroform.
[b] Steel column, 150 mm×4.6 mm ID, t_o=2.64 min. c=5 mg/mL. Temperature: 25 °C.
[c] Steel column, 150 mm×20 mm ID, t_o=2.52 min. c=200 mg/mL. Temperature: 25 °C.

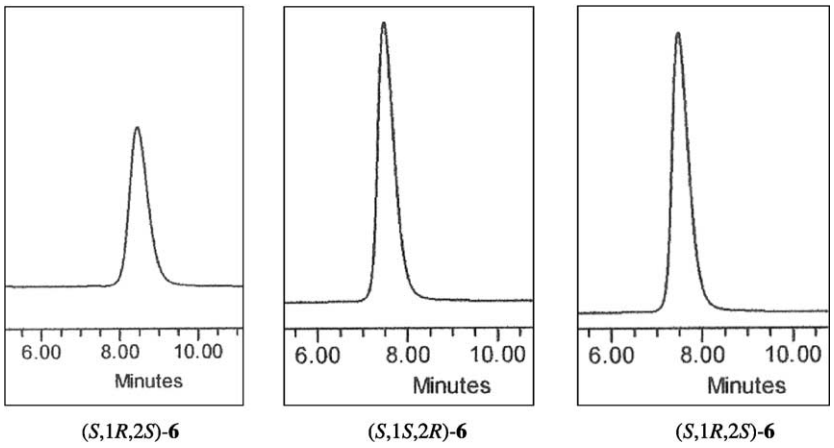


Figure 1. HPLC analytical resolution of Cbz-Asp(O^{t}Bu)-*rac*-*trans*-c_6Phe-OMe and resolved diastereoisomers (*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6**.

For the extension of the analytical conditions to the semi-preparative scale we selected the mixture *n*-hexane/2-propanol/chloroform indicated in Table 1. This solvent system was found to be the most appropriate eluent to optimise the resolution in relation to the column loadability.

Direct assignment of the stereochemistry of the dipeptides (*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6** was made by comparing their spectral data with those corresponding to the dipeptides synthesised by coupling the two optically pure enantiomers (1*R*,2*S*)-**4** and (1*S*,2*R*)-**4**, prepared from optically pure amino acids (1*R*,2*S*)-**3** and (1*S*,2*R*)-**3**,[9] with protected aspartic acid (*S*)-**5**.

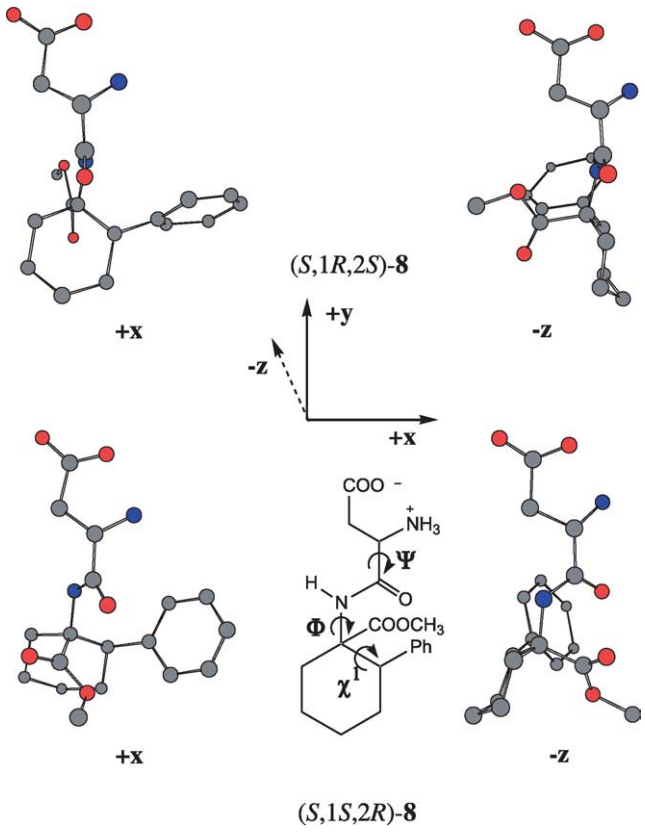


Figure 2. Calculated minimum energy conformations of (*S*,1*R*,2*S*)-**8** and (*S*,1*S*,2*R*)-**8** (hydrogen atoms have been omitted for clarity).

The final steps leading to deprotected dipeptides were carried out with each diastereoisomer as follows. Firstly, treatment with TFA in CH_2Cl_2 afforded (*S*,1*R*,2*S*)-**7** and (*S*,1*S*,2*R*)-**7**. Secondly, hydrogenolysis using palladium on carbon as a catalyst and further purification by RP-HPLC gave the optically pure analogues of aspartame: H-(*S*)-Asp-(1*R*,2*S*)-c_6Phe-OMe and H-(*S*)-Asp-(1*S*,2*R*)-c_6Phe-OMe, (*S*,1*R*,2*S*)-**8** and (*S*, 1*S*,2*R*)-**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*S*)-**8** is sweet, albeit less potent than aspartame, whereas compound (*S*,1*S*,2*R*)-**8** is bitter.

2.3. Molecular modelling studies

The research carried out on the elucidation of detailed structure-taste relationships of aspartame and its analogues has led to a widely accepted model that has considerable predictive power for related molecules.[3] According to this model, the sweet taste is associated with an L-shaped conformation, in which the hydrophobic group projects along the +x axis (Fig. 2). A reverse L-shaped structure, in which the hydrophobic group points to the −z axis, is associated with a bitter taste. Other possible topochemical arrays would lead to tasteless compounds.

In order to account for solvation effects, we decided to carry out the same mixed scheme as reported previously,[8] combining molecular mechanics and quantum mechanics calculations. Thus, single point energy semi-empirical AM1 calculations on the MM2 optimised structures (see Section 4 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 this energy was then added to the MM2 steric energy to obtain the relative energies of the different conformers. Although this procedure does not take into account solvent effects during the geometry optimisation, it has the advantage of combining

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

Compound	Conformer	Dihedral angles[a]			E_{MM2}	ΔG_{solv}	E_{Tot}	ΔE_{Tot}
		Ψ	Φ	χ^1				
(*S*,1*R*,2*S*)-**8**	+x	−56.3	175.5	−58.2	−58.9	−77.7	−136.6	0
	−z	−55.7	90.4	−60.3	−54.3	−81.9	−136.2	0.4
(*S*,1*S*,2*R*)-**8**	+x	−89.5	39.2	57.2	−51.2	−80.0	−131.2	3.2
	−z	−166.6	45.7	60.8	−55.4	−79.0	−134.4	0

[a] See Figure 2 for the definition of the dihedral angles.

the strength of the MM2 force field for conformational analysis with that of a successful quantum mechanical method for calculating the solvation energy.

The minimum energy values obtained for conformations +x and −z for compounds (*S*,1*R*,2*S*)-**8** are gathered in Table 2 and the corresponding structures are shown in Figure 2. As can be seen, based on total energies, compound (*S*,1*R*,2*S*)-**8** should be sweet (since conformer +x is more stable than conformer −z), whereas compound (*S*,1*S*,2*R*)-**8** should be bitter (since the reverse relative stability is obtained).

Analysis of the results obtained for the four new analogues of aspartame reveals that the conformational preferences of the c_6Phe residues have been shifted with respect to their (*S*)-Phe and (*R*)-Phe counterparts.[17] Thus, in (1*S*,2*R*)-c_6Phe and (1*S*,2*S*)-c_6Phe, analogues of (*S*)-Phe, a *gauche* (+) side-chain conformation is shown, while this rotamer proves to be the least favoured for the (*S*)-Phe residue. As for (1*R*,2*R*)-c_6Phe and (1*R*,2*S*)-c_6Phe, analogues of (*R*)-Phe, a *gauche* (−) disposition is exhibited, whereas this rotamer is the least favoured for the (*R*)-Phe residue.

It has been reported[3,18] that in the minimum energy conformations calculated for sweet H-(*S*)-Asp-(*S*)-Phe-OMe, the side chain in (*S*)-Phe can assume any of the three possible staggered C_α-C_β rotamers: *gauche* (+), *gauche* (−) and *anti*; but the conformation responsible for sweet taste demands a value of $\chi^1 = -60°$ in the C-terminal residue.

In addition, it should be noted that the incorporation into aspartame of c_6Phe residues with a *gauche* (−) preference, i.e. (1*R*,2*R*)-c_6Phe and (1*R*,2*S*)-c_6Phe, leads to sweet derivatives whereas replacement by c_6Phe residues where a gauche (+) preference is exhibited, i.e. (1*S*,2*S*)-c_6Phe and (1*S*,2*R*)-c_6Phe, leads to bitter analogues. This fact suggests that, for a sweet response, the stereochemistry at C_α in the Phe residue is not as crucial as the value of the dihedral angle χ^1, with the latter being necessary but not sufficient on its own.

It can therefore be concluded that the results of molecular mechanics calculations are in good agreement with the taste experimentally found. Once again, the procedure followed to describe the solvation effects has proved to be both convenient and very useful, as the results obtained in vacuo would have led to incorrect taste predictions for some of the new analogues; e.g. the case of (*S*,1*R*,2*R*)-**8**.[8]

These results support the model reported by Goodman et al.[3] which has been assumed to explain the structure-taste relationship in all four aspartame analogues that incorporate c_6Phe.

3. Conclusion

Two new aspartame derivatives that incorporate the constrained phenylalanines *trans*-c_6Phe have been synthesised. The isomer H-(*S*)-Asp-(1*R*,2*S*)-c_6Phe-OMe is sweet whereas isomer H-(*S*)-Asp-(1*S*,2*R*)-c_6Phe-OMe is bitter. The relationship between the absolute configurations of the dipeptides and the taste response can be explained in terms of the different conformational behaviour displayed by each molecule.

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 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 Varian Unity-300 or Bruker ARX-300 instruments, using 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 are in good agreement with the calculated values.

4.1.2. High performance liquid chromatography. HPLC was carried out using a Waters HPLC system equipped with a Waters 600-E pump and a Waters 991 photodiode array detector. The chiral stationary phase, which consisted of mixed 10-undecenoate/3,5-dimethylphenylcarbamate of amylose bonded to allylsilica, was prepared according to a previously described procedure.[19,20] The analytical assays were carried out on a 150 mm×4.6 mm ID column and the semi-preparative resolution was achieved on a 150 mm× 20 mm ID column. All analytical assays and semi-preparative chromatography were performed under the conditions given in Table 1. Final products were purified

on a Xterra™ MS C_8 250 mm×4.6 mm ID column. The solvents used as mobile phases were of spectral grade.

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 diastereoisomers, respectively; t_R ($R = 1, 2$) are their retention times, and w_2 and w_1 denote their bandwidths at half height; t_0 is the dead time.

4.1.3. Molecular modelling methodology. All computer simulations were carried out using the Chem3D program.[21] Molecular Dynamics were obtained by means of the MM2 force field,[22] 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 473 K. It is well known that the dihedral angles of the Asp residue remain 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 position of the X-ray crystal structure of aspartame throughout all the simulations.

Solvation effects were taken into account by means of single point energy semi-empirical AM1[23] calculations on the MM2 optimised structures, using the COSMO continuum model[24,25] with the dielectric constant of water.

4.2. Synthesis of *rac-trans*-c_6Phe-OMe, *rac-trans*-4

The synthesis of 1-amino-2-phenylcyclohexane-1-carboxylic acid hydrochloride, *rac-trans*-**3**, was performed as described previously.[9] The corresponding methyl ester, *rac-trans*-**4**, was prepared using the following procedure.

$SOCl_2$ (1.8 mL, 24.67 mmol) was added dropwise to a stirred solution of MeOH (20 mL) cooled in an ice-bath. After 15 min stirring at that temperature, *rac-trans*-**3** (316 mg, 1.24 mmol) was added to the reaction mixture and this was heated under reflux for 8 h. A further 3 portions of $SOCl_2$/MeOH, prepared as described above, were added and the solvents were removed 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 42%).

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

Cbz-(*S*)-Asp(O^{t}Bu)-OH, (*S*)-**5**, (198 mg, 0.61 mmol) and NMM (62 mg, 0.61 mmol) were dissolved in dry CH_2Cl_2 (15 mL) under an inert atmosphere. The mixture was cooled to −15 °C and a precooled solution of ^{t}BuOCOCl (84 mg, 0.61 mmol) in dry CH_2Cl_2 (3 mL) was added. The mixture was stirred for 20 min and a precooled solution of H-*rac-trans*-c_6Phe-OMe, *rac-trans*-**4** (137 g, 0.51 mmol)—obtained as described in Section 4.2 and used without further purification—in CH_2Cl_2 (3 mL) was added dropwise. The reaction mixture was stirred at −15 °C for 1 h and the solution was allowed to warm up 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 to give the protected dipeptides in high yield (>99%). The diastereomeric mixture of protected dipeptides was purified by silica gel column chromatography and then resolved by semi-preparative HPLC as described below.

4.3.1. Resolution of two stereoisomers of Cbz-(*S*)-Asp(O^{t}Bu)-*rac-trans*-c_6Phe-OMe: isolation of (*S*,1*R*,2*S*)-6 and (*S*,1*S*,2*R*)-6. HPLC resolution of a mixture of (*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6** (235 mg) dissolved in chloroform (1.2 mL) was carried out by successive injections of 0.10 mL onto a 150 mm×20 mm column filled with mixed 10-undecenoate-3,5-dimethyl phenylcarbamate of amylose bonded to allylsilica. A mixture of *n*-hexane/2-propanol/chloroform (96/3/1) was used as the eluent (flow rate 15 mL/min). Each run was collected into three separate fractions: 6.5–7.2, 7.2–8.2 and 8.2–9.6 min. In this way 85 mg and 53 mg of the less and more strongly retained diastereoisomers were obtained, respectively. The combined second fractions, containing 95 mg of diastereomeric mixture, were reinjected to afford 23 mg and 32 mg of the less and more strongly retained diastereoisomers, respectively. As a result, 108 mg of optically pure first eluted diastereoisomer and 85 mg of optically pure last eluted diastereoisomer were obtained.

4.3.2. CBz-(*S*)-Asp(O^{t}Bu)-(1*R*,2*S*)-c_6Phe-OMe, (*S*,1*R*,2*S*)-6. Mp: oil. $Rf_1 = 0.40$. $[\alpha]_D^{20} = -5.45$ ($c = 1$, $CHCl_3$). IR: 3336.94; 1727.73; 1711.31; 1693.51; 1678.15 cm^{-1}. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 1.2–2.0 (m, 5H); 1.38 (m, 9H); 2.1–2.5 (m, 3H); 2.54 (dd, 1H, $J = 6$ Hz, $J = 16.5$ Hz); 2.9 (m, 1H); 3.56 (m, 1H); 3.62 (s, 3H); 4.49 (m, 1H); 5.09 (m, 2H); 5.84 (d, 1H, $J = 7.8$ Hz); 7.0–7.4 (m, 11H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 172.34; 170.96 169.38; 155.79; 140.97; 136.30; 128.54; 128.33; 128.23; 128.18; 128.06; 127.20; 81.67; 67.01; 64.26; 51.95; 51.59; 47.07; 37.54; 32.35; 28.39; 27.97; 25.02; 22.54. Anal. Calcd for $C_{30}H_{38}N_2O_7$: C, 66.89; H, 7.11; N, 5.20. Found: C, 66.99; H, 7.11; N, 5.17.

4.3.3. CBz-(*S*)-Asp(O^{t}Bu)-(1*S*,2*R*)-c_6Phe-OMe, (*S*,1*S*,2*R*)-6. Mp: oil. $Rf_1 = 0.40$. $[\alpha]_D^{20} = +30.34$ ($c = 0.92$, $CHCl_3$). IR: 3336.58; 1729.23; 1678.47 cm^{-1}. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 1.2–2.5 (m, 8H); 1.45 (s, 9H); 2.58 (dd, 1H, $J = 6$ Hz, $J = 18.3$ Hz); 2.87 (dd, 1H, $J = 17.1$ Hz, $J = 3$ Hz); 3.57 (m, 1H); 3.63 (s, 3H); 4.45 (m, 1H); 5.07 (s, 2H); 5.93 (d, 1H, $J = 8.4$ Hz); 7.1–7.4 (m, 11H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 172.93; 171.49; 169.85; 156.51; 141.35; 136.68; 129.01; 128.91; 128.88; 128.60; 128.46; 127.59; 82.09; 67.56; 64.88; 52.47; 47.28; 37.33; 32.39; 29.82; 28.77; 28.50; 25.32; 22.97. Anal. Calcd for $C_{30}H_{38}N_2O_7$: C, 66.89; H, 7.11; N, 5.20. Found: C, 66.75; H, 7.15; N, 5.27.

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

The corresponding protected dipeptide (1 mmol) was dissolved in CH_2Cl_2 (15 mL) and TFA (7.5 mL) was added. The solution was stirred at room temperature for

1.5 h. The TFA and CH_2Cl_2 were evaporated under reduced pressure to afford the final product.

4.4.1. CBz-(*S*)-Asp-(1*R*,2*S*)-c$_6$Phe-OMe, (*S*,1*R*,2*S*)-7. This compound was prepared according to the procedure described above. Cbz-(*S*)-Asp(O^{t}Bu)-(1*R*,2*S*)-c$_6$Phe-OMe, (*S*,1*R*,2*S*)-**6**, (100 mg, 0.19 mmol); CH_2Cl_2 (3 mL); TFA (1.55 mL). Yield: quantitative. Mp: oil. $Rf_1=0.04$, $Rf_2=0.79$. $[\alpha]_D^{20}=-19.76$ ($c=0.47$, $CHCl_3$). IR (nujol): 2500–3600; 1725.02 cm^{-1}. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 1.2–2.0 (m, 5H); 2.0–2.4 (m, 3H); 2.72 (dd, 1H, $J=6.1$ Hz $J=17.3$ Hz); 2.92 (m, 1H); 3.55 (m, 1H); 3.64 (s, 3H); 4.54 (m, 1H); 5.09 (s, 2H); 5.92 (d, 1H, $J=8$ Hz); 6.8–7.4 (m, 11H); 8.50 (s, 1H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 175.58; 172.49; 169.68; 159.76; 156.13; 140.60; 135.78; 128.58; 128.32; 128.15; 127.29; 67.50; 64.61; 52.24; 51.41; 47.26; 35.87; 31.93; 29.37; 24.93; 22.68. Anal. Calcd for $C_{26}H_{30}N_2O_7$: C, 64.72; H, 6.27; N, 5.81. Found: C, 64.77; H, 6.20; N, 5.74.

4.4.2. CBz-(*S*)-Asp-(1*S*,2*R*)-c$_6$Phe-OMe, (*S*,1*S*,2*R*)-7. This compound was prepared according to the procedure described above. Cbz-(*S*)-Asp(O^{t}Bu)-(1*S*,2*R*)-c$_6$Phe-OMe, (*S*,1*S*,2*R*)-**6**, (65 mg, 0.12 mmol); CH_2Cl_2 (2 mL); TFA (1 mL). Yield: quantitative. Mp: oil. $Rf_1=0.04$, $Rf_2=0.79$. $[\alpha]_D^{20}=+16.63$ ($c=0.19$, $CHCl_3$). IR (nujol): 2500–3600; 1723.26; 1686.86; 1671.37; 1660.74 cm^{-1}. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 1.1–2.0 (m, 5H); 2.05 (m, 1H); 2.15 (m, 1H); 2.30 (m, 1H); 2.63 (dd, 1H, $J=4.2$ Hz, $J=12.9$ Hz); 2.84 (dd, 1H, $J=3$ Hz, $J=12.6$ Hz); 3.47 (m, 1H); 3.56 (s, 3H); 4.46 (m, 1H); 4.98 (m, 2H); 5.93 (d, 1H, $J=6.6$ Hz); 6.87 (s, 1H); 6.9–7.4 (m, 11H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 175.74; 172.47; 169.42; 156.17; 140.72; 135.98; 128.54; 128.30; 128.24; 128.15; 128.02; 127.19; 67.32; 64.48; 52.11; 51.54; 46.95; 35.44; 31.92; 28.30; 24.87; 22.45. Anal. Calcd for $C_{26}H_{30}N_2O_7$: C, 64.72; H, 6.27; N, 5.81. Found: C, 64.79; H, 6.30; N, 5.77.

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

The corresponding semi-protected dipeptide (1 mmol) was dissolved in EtOAc/MeOH (1:1) (30 mL) 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 purified by reversed phase HPLC (column: 5 μm Xterra™ MS C_8, 150×4.6 mm ID). The elutions were performed isocratically with 20% CH_3CN/80% H_2O (v/v) at a flow rate of 2 mL(min, with UV detection at 220 nm.

4.5.1. H-(*S*)-Asp-(1*R*,2*S*)-c$_6$Phe-OMe, (*S*,1*R*,2*S*)-8. This compound was prepared according to the procedure described above. Cbz-(*S*)-Asp-(*S*,1*R*,2*S*)-c$_6$Phe-OMe, (*S*,1*R*,2*S*)-**7**, (78 mg, 0.16 mmol); EtOAc/MeOH (6 mL); 10% Pd/C (15 mg). Yield: 97%. Mp: 144 °C. $Rf_2=0.51$. $[\alpha]_D^{20}=-14.76$ ($c=0.49$, MeOH). IR (nujol): 2400–3600; 1720.20; 1690.31 cm^{-1}. ^{1}H NMR (D_2O 300 MHz) δ 1.5–1.7 (m, 2H); 1.8–2.2 (m, 5H); 2.37 (m, 1H); 2.90 (m, 2H); 3.19 (dd, 1H, $J=3$ Hz, $J=6$ Hz); 3.58 (s, 3H); 4.31 (t, 1H, $J=6$ Hz); 7.3–7.5 (m, 5H). ^{13}C NMR (D_2O/MeOH-d_4 75 MHz) δ 174.13; 173.74; 167.47; 140.69; 128.91; 128.26; 127.35; 63.55; 52.13; 49.78; 36.30; 30.59; 27.63; 22.95; 21.25. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 62.12; H, 6.95; N, 8.07.

4.5.2. H-(*S*)-Asp-(1*S*,2*R*)-c$_6$Phe-OMe, (*S*,1*S*,2*R*)-8. This compound was prepared according to the procedure described above. Cbz-(*S*)-Asp-(*S*,1*S*,2*R*)-c$_6$Phe-OMe, (*S*,1*S*,2*R*)-**7**, (47 mg, 0.1 mmol); EtOAc/MeOH (4 mL); 10% Pd/C (5 mg). Yield: 84%. Mp: 165 °C. $Rf_2=0.51$. $[\alpha]_D^{20}=+36.94$ ($c=0.71$, MeOH). IR (nujol): 2400–3600; 1730.81; 1684.52 cm^{-1}. ^{1}H NMR (D_2O 300 MHz) δ 1.4–1.6 (m, 1H); 1.6–1.8 (m, 2H); 1.8–2.0 (m, 3H); 2.15 (m, 1H); 2.45 (m, 1H); 2.53 (dd, 1H, $J=6$ Hz, $J=13.2$ Hz); 2.71 (dd, 1H, $J=4.2$ Hz, $J=12.9$ Hz); 3.03 (dd, 1H, $J=3$ Hz, $J=6.9$ Hz); 3.53 (s, 3H); 4.22 (t, 1H, $J=4.2$ Hz); 7.2–7.4 (m, 5H). ^{13}C NMR (D_2O/MeOH-d_4 75 MHz) δ 175.79; 173.61; 167.86; 140.98; 129.13; 128.18; 127.25; 63.28; 52.06; 50.44; 49.52; 37.14; 32.35; 27.78; 23.38; 21.35. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 62.25; H, 6.91; N, 8.09.

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