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Synthesis of enantiomerically pure *cis*- and *trans*-cyclopentane analogues of phenylalanine

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Abstract—For the first time, all stereoisomers of 1-amino-2-phenylcyclopentanecarboxylic acid—c_5Phe—have been synthesised. A Strecker reaction on 2-phenylcyclopentanone and further transformations of each amino nitrile into the amino acid provides *cis*-c_5Phe and *trans*-c_5Phe with high efficiency. A divergent synthetic route was then developed to obtain the target compounds *cis*- and *trans*-c_5Phe in their racemic form. The preparation of the final enantiomerically pure amino acids and their corresponding *N*-protected derivatives was also achieved by HPLC resolution of one of the intermediates using a cellulose-derived chiral stationary phase. The relative stereochemistry of each amino acid and its precursors have been unambiguously assigned.
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1. Introduction

Structure–activity relationship studies on bioactive peptides are essential for establishing the active conformation of these systems. In this context, the side-chain moieties warrant special attention since they are directly involved in peptide-receptor recognition phenomena and determine biological specificity. The use of constrained amino acids with rigidly oriented side chains provides very valuable information in such investigations.[1] In particular, restricted amino acid analogues with aromatic rings have been used for this purpose with some of them being met with considerable success.[2,3] Over the course of our studies into the conformational tendencies of carbocyclic analogues of phenylalanine (Phe) in model dipeptides a series of 1-amino-2-phenyl-cycloalkanecarboxylic acids (c_nPhe) was synthesised. In particular, we reported the synthesis of all the stereoisomers of c_3Phe[4] and c_6Phe.[5] Nevertheless, in spite of the potential interest of c_5Phe, its stereoisomers have not yet been reported.

To the best of our knowledge, the only reference in the literature concerning the synthesis of 2-phenyl-1-aminocyclopentanecarboxylic acid (c_5Phe) describes a Bücherer–Bergs reaction starting from 2-phenylcyclo-

pentanone that, after hydrolysis of the corresponding hydantoin, led to only one isomer of c_5Phe in its racemic form,[6] without specifying the stereochemistry.

Herein, we report the development of a highly efficient synthesis for both *cis*- and *trans*-c_5Phe in racemic form and the preparation of enantiomerically pure (1*R*,2*R*)-, (1*S*,2*S*)-, (1*R*,2*S*)- and (1*S*,2*R*)-c_5Phe, suitably protected to be incorporated into a peptide chain (Fig. 1).
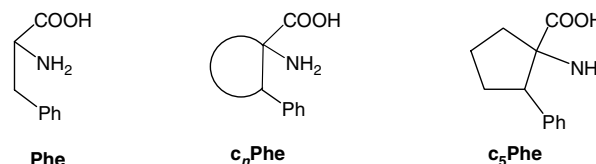

Figure 1. Structure of 1-amino-2-phenylcycloalkanecarboxylic acids (c_nPhe) as conformationally restricted analogues of Phe.

2. Results and discussion

2.1. Synthesis of racemic *cis*- and *trans*-c_5Phe[†]

In recent years, a number of *cis*- and *trans*-1-aminocyclo-pentanecarboxylic acids with different substituents in

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† *cis*- or *trans*-c_5Phe refers to the relative position between the amino and the phenyl groups in the cyclic analogue of phenylalanine.

the ring have been synthesised, most times in enantio-merically pure form. In particular, cyclic analogues of glutamate have been extensively studied, due to their importance as agonists or antagonists of the glutamate receptors. Other cyclopentane amino acid analogues, as serine or threonine, have also been reported. The synthetic routes to obtain 1-aminocyclopentane-carboxylic acids can be divided into two groups.[‡] The first group involves the formation of the cyclopentane ring and includes cyclisation by alkylation of a glycine equivalent with a 1,4-dielectrophile[7] and ring closing metathesis (RCM) on geminal alkenyl or alkynyl bislac-tims[8] or other glycine equivalents.[9] A recent example of this last synthetic pathway was reported by Trost, who described the synthesis of a precursor of *cis*-1-amino-2-phenylcyclopentanecarboxylic acid through a RCM reaction on an α,α-dialkenyl *N*-acylglycinate, although free amino acid was not reported.[10] Other methodologies, such as palladium-mediated cyclisation,[11] enantio-selective alkylidene carbene C–H insertion reactions[12] or synthesis of the cyclopentane ring through sequential aldol reactions[13] and intramolecular aldol condensa-tion[14] have also been used. On the other hand, the intro-duction of the amino and acid functions on the cyclopentane skeleton was carried out in a traditional way by Bücherer–Bergs or Strecker reactions on the corresponding cyclopentanones. The Bücherer–Bergs reaction has been applied to the synthesis of different cyclopentane derived α-amino acids in their racemic form[6,15] and, more recently, to obtain enantiopure cyclopentane Glu analogues.[16] Although less used, the Strecker reaction has also proven to be convenient for the synthesis of amino acids with a cyclopentane skele-ton.[17] Next to these approaches, the construction of a quaternary α-carbon by direct organocatalytic amina-tion of β-dicarbonyl compounds has been reported.[18]

As described in the introduction, the only reported way to obtain the amino acid c₅Phe involves a Bücherer–Bergs reaction starting from 2-phenylcyclopentanone.[6] In this case, a single spot in TLC was observed for the obtained hydantoin and, hence, after hydrolysis under strong conditions (H₂SO₄, 150 °C) only one isomer of c₅Phe with an unknown relative *cis/trans* stereochemis-try was described by the authors. The melting point was the only described physical characteristic of c₅Phe obtained. Taking into account that the epimerisation during the hydrolysis process at elevated temperatures has been described for different spirohydantoins obtained from 2-substituted cyclopentanones,[15a,b] the described synthesis of the amino acid c₅Phe is, at the least, questionable.

Over the course of our work on the synthesis of cyclic analogues of phenylalanine, we recently described an efficient synthesis of *trans*-c₆Phe through a completely diastereoselective Strecker reaction and further hydro-lysis of an amide derived from the amino nitrile.[5c]

Although the asymmetric Strecker reaction on different 2-substituted cyclopentanones was described,[17b,d] to the best of our knowledge, the reaction of 2-phenyl-cyclopentanone has not yet been reported for such a route and we were therefore encouraged to study this approach to obtain the different isomers of c₅Phe.

The 2-phenylcyclopentanone used as the starting mate-rial was synthesised as described in the literature by reaction of phenylmagnesium bromide and 2-chloro-cylopentanone.[19] Treatment of 2-phenylcyclopentanone with NaCN and NH₄Cl under the optimal conditions found for the Strecker reaction on 2-phenylcyclohexa-none[5c] (ketone/NaCN/NH₄Cl in a ratio of 1:2:2, long reaction times) afforded in all cases mixtures of *cis*- and *trans*-1-cyano-2-phenylcyclopentylamine (*rac*-**1** and *rac*-**2**, respectively, Scheme 1) in a 1:1 ratio. It was found that the longer the reaction time, the higher the yield of the products. After 10 days of reaction, a mixture of stereoisomers was obtained with 69% conver-sion, a result that unfortunately could not be improved upon, probably due to the decomposition of the starting ketone at longer reaction times. Both isomers were sep-arated and purified by column chromatography and were obtained as colourless oils that would not crystal-lise on standing at low temperatures or by the addition of solvents.

The relative stereochemistry of the separated isomers was initially assigned on the basis of ¹H NMR spectro-scopy, taking into account the deshielding effect of the triple bond of the cyano group on the hydrogen atom in the relative *cis*-disposition. In this case, the benzylic proton of the *cis*-derivative *rac*-**1** resonates at a lower field than the corresponding proton in the *trans*-isomer *rac*-**2**.

The applied reaction conditions (room temperature, long reaction times) are consistent with a thermodynam-ically controlled reaction. Moreover, after purification by column chromatography, both *rac*-**1** and *rac*-**2** evolve on standing, to mixtures of *cis*- and *trans*-isomers.

It is noteworthy that the result of the Strecker reaction on 2-phenylcyclopentanone (an equimolecular mixture of *cis*- and *trans*-isomers) was so different from the behaviour of 2-phenylcyclohexanone under the same conditions (completely diastereoselective *trans* selec-tion). To understand this result, a molecular modelling study of the amino nitrile precursors of c₅Phe (*cis*-amino nitrile **1** and *trans*-amino nitrile **2**) and the correspond-ing amino nitrile precursors of c₆Phe was performed at molecular mechanics (MM) and DFT levels. The results indicate the greater thermodynamic stability of the *trans*-amino nitrile precursor of c₆Phe, whereas in the case of c₅Phe, the energy difference between *cis*- and *trans*-diastereomers is much lower, which is consistent with the reaction mixtures of both isomers obtained in the experiments.

In order to prevent the establishment of the equilibrium between *cis*- and *trans*-isomers *rac*-**1** and *rac*-**2** after their purification, both isomeric amino nitriles were

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[‡] References concerning the synthesis of cyclopentanic aminoacids incorporated in rigid bi- or tricyclic structures are deliberately not included in this revision.
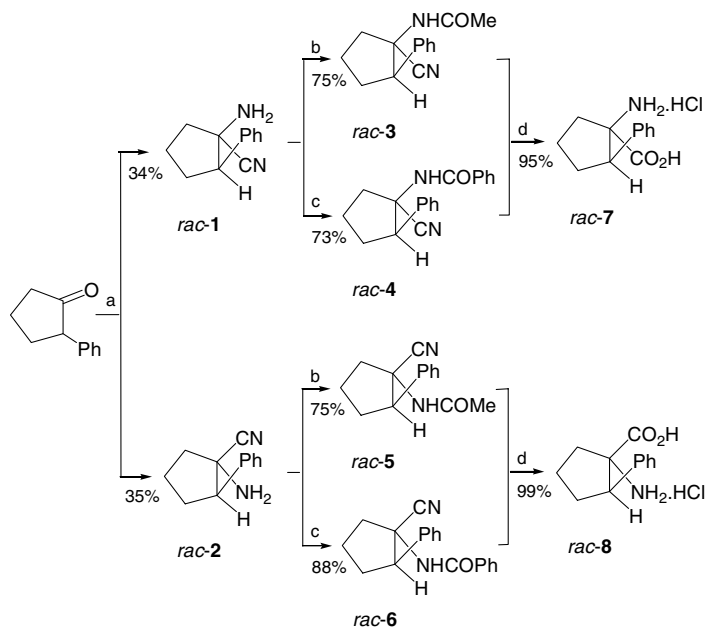
Scheme 1. Synthesis of racemic *cis*-c$_5$Phe and *trans*-c$_5$Phe by Strecker reaction. Reaction conditions: (a) (i) NaCN, NH$_4$Cl, *i*-PrOH, NH$_4$OH; (ii) column chromatography; (b) AcCl, NEt$_3$, CH$_2$Cl$_2$; (c) BzCl, NEt$_3$, CH$_2$Cl$_2$; (d) 12 N HCl, reflux.
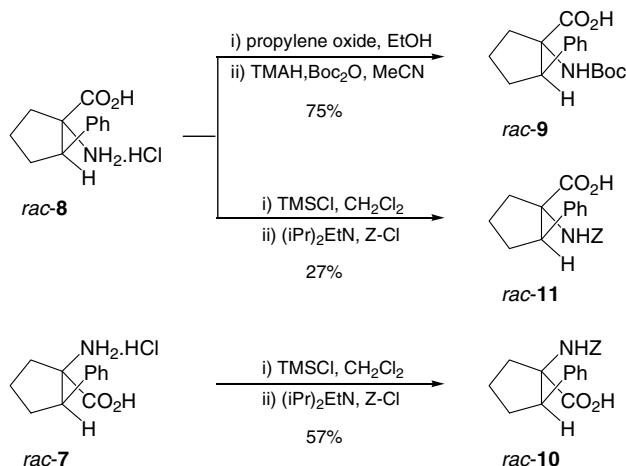
transformed into derivatives immediately after being isolated by column chromatography. Thus, acetamide and benzamide derivatives of each amino nitrile were synthesised, since we have previously reported that the hydrolysis of acetamido- or benzamidonitriles is much more efficient than hydrolysis of the starting amino nitrile.[5c] On the other hand, these amides proved to be very convenient substrates for the enantiomeric resolution through chiral HPLC using polysaccharide carbamates as chiral selectors in the stationary phase. As indicated in Scheme 1, acetamide *rac*-**3** and benzamide *rac*-**4** were obtained from *cis*-compound *rac*-**1** under standard conditions with excellent yields. In a similar way, acetamide *rac*-**5** and benzamide *rac*-**6** were obtained from *trans*-isomer *rac*-**2**.

Moreover, these amido derivatives allowed us to assign the relative stereochemistry of each diastereoisomer by NOE experiments. For example, irradiation of the NH of the acetamido group in *rac*-**3** and *rac*-**5** induces enhancement of the benzylic proton in *rac*-**5** (4.6%) and a significantly smaller effect (0.7%) in *rac*-**3**. In the same way, irradiation of the NH of the benzamido group in *rac*-**4** and *rac*-**6** shows a greater NOE effect on the benzylic proton of *rac*-**6** (6.8%) than on the corresponding proton of *rac*-**4** (0.9%). These NOEs are consistent with a *cis*-configuration between the phenyl and amido groups in *rac*-**3** and *rac*-**4** and, hence, between the phenyl and amine in *rac*-**1**. Correspondingly, *rac*-**2**, *rac*-**5** and *rac*-**6** can be assigned as precursors of *trans*-c$_5$Phe.

Treatment of *rac*-**3** and *rac*-**4** with concentrated HCl under reflux resulted in a quantitative transformation into *cis*-c$_5$Phe hydrochloride *rac*-**7**. Likewise, hydrolysis of both *rac*-**5** and *rac*-**6** led to *trans*-c$_5$Phe hydrochloride *rac*-**8**.

A divergent synthetic route to the target compounds *cis*- and *trans*-c$_5$Phe in their racemic forms was then developed (three synthetic steps from 2-phenylcyclopentanone, with a similar overall yield for each compound of around 25%). These racemic compounds were now ready for direct use in the synthesis of diastereomeric peptides. For this purpose, the amino acids must be suitably protected.

In order to obtain the *N*-Boc-protected derivatives, we employed the same methodology (TMAH, acetonitrile, Boc$_2$O) as used to protect the amino group of sterically hindered *trans*-c$_6$Phe.[5c] Under these conditions, as observed previously, the solubility of the starting material proved to be critical for the success of the reaction: when the amino acid tetramethylammonium salt was not completely in solution, the reaction proceeded very slowly and gave a very poor yield. The low solubility of the starting materials in acetonitrile meant that it was not possible to perform directly the *N*-protection starting from the corresponding c$_5$Phe hydrochlorides *rac*-**7** and *rac*-**8**, and prior to liberation of the free amino acids was necessary through a treatment with propylene oxide. Mild heating of the reaction mixture (TMAH, acetonitrile, 40 °C) enabled free *trans*-c$_5$Phe to be dissolved and the reaction with *tert*-butyl dicarbonate was carried out. *N*-Protection progressed by successive feeding with Boc$_2$O and Boc-*trans*-c$_5$Phe *rac*-**9** was finally obtained in 75% yield after a reaction time of 2 days (Scheme 2). However, free *cis*-c$_5$Phe could not be dissolved under any conditions and, after addition of *tert*-butyl dicarbonate and successive feeding, only the starting material was recovered. This lack of reactivity of *rac*-**7** is probably due to the presence of the large phenyl group in a *cis* relative position with respect to the amine. The different behaviour of *rac*-**7** and *rac*-**8** in this *N*-protection process is consistent with the

i) propylene oxide, EtOH
ii) TMAH, Boc$_2$O, MeCN
75%

rac-**8**

rac-**9**

i) TMSCl, CH$_2$Cl$_2$
ii) (iPr)$_2$EtN, Z-Cl
27%

rac-**11**

rac-**7**

i) TMSCl, CH$_2$Cl$_2$
ii) (iPr)$_2$EtN, Z-Cl
57%

rac-**10**

Scheme 2. Synthesis of racemic N-protected trans-c$_5$Phe and cis-c$_5$Phe.

relative stereochemistry assigned to the two stereoisomers by NMR studies.

The resistance to N-protection in the presence of bulky protecting groups has also been noted in other cycloalkane amino acids, in which the amino and phenyl groups are in a cis disposition, as in the case of cis-c$_6$Phe.[5b] To overcome the low reactivity of the amino group, we carried out the same strategy as used in the synthesis of N-protected derivatives of cis-c$_6$Phe: that is, prior to transformation of the carboxylic acid function into its trimethylsilyl ester. As observed previously, this transitory protection of the carboxylate provides an excellent yield in the subsequent reaction of the amino group with a chloroformate. This methodology (addition of chlorotrimethylsilane and subsequent reaction of the amine with benzyl chloroformate) led to Z-cis-c$_5$Phe rac-**10** from rac-**7** in 57% yield (Scheme 2). The same reaction, starting from trans-amino acid rac-**8**, provided Z-trans-c$_5$Phe rac-**11** with a lower yield (27%, Scheme 2). The low reactivity of rac-**8** under these reaction conditions is in agreement with a cis geometry between the carboxylic acid and phenyl group and is consistent with the relative stereochemical assignment established previously.

The relative stereochemistry of the amino nitriles rac-**1** and rac-**2**, and their corresponding derivatives rac-**3**–rac-**11**, was later unequivocally assigned by X-ray crystal structure analysis of some dipeptides derived, respectively, from amino acids rac-**7** and rac-**8**.

2.2. HPLC resolution of rac-4 and rac-6

Once an efficient route to the target compounds had been developed, we undertook the preparation of both enantiomers of amino acids **7** and **8** in enantiomerically pure form. Although enantiomers can be obtained by the separation of diastereoisomers, the direct separation of enantiomers by preparative chromatography on chiral stationary phases (CSPs) is today recognised as a powerful alternative.[20] Polysaccharide-derived CSPs

from cellulose and amylose are extremely popular because of their wide applicability and usefulness in enantioselective liquid chromatography.[21] Our research group collaborated in the development of new polysaccharide-derived CSPs where mixed polysaccharide derivatives are covalently bonded to an allylsilica gel matrix.[22] The covalent immobilisation results in an extremely high stability for these phases in the presence of a wide range of solvents. Although these phases are not commercially available, the synthetic methodology has been extensively studied and reported.[23] The preparation of these phases has been meticulously described, so that these in-house materials are readily prepared with reproducible chromatographic results. The synthetic simplicity, high chemical stability and the great selectivity exhibited towards a variety of compounds make these phases especially suitable for resolutions on a preparative scale, as shown in the preparative enantioseparations of different phenylalanine[4,24] and other amino acid[25] surrogates.

Herein, two non-commercial polysaccharide-derived supports consisting of mixed 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose[22] and amylose,[26] respectively, covalently attached to allyl silica were used (CSP-1 and CSP-2). The HPLC analytical resolution of the precursors of cis-c$_5$Phe (rac-**3**, rac-**4**) and trans-c$_5$Phe (rac-**5**, rac-**6**) was tested on both columns. Different mixtures of n-hexane/i-PrOH and n-hexane/i-PrOH/acetone were tested as eluents (flow rate 1 mL/min) with UV monitoring performed at 210 nm. The most representative results are shown in Table 1.

Resolution of cis-acetamido derivative rac-**3** on CSP-1 was unsatisfactory, with low values for the separation and resolution factors on using mixtures of hexane/i-PrOH as the eluent. This result could not be improved by adding a certain amount of acetone to the elution system (Table 1, entries 1 and 2). Separation of rac-**3** could not be achieved on CSP-2 under any chromatographic conditions explored. However, cis-benzamido derivative rac-**4** was resolved on both columns (entries 3–5), with the best chromatographic results obtained on CSP-1 (derived from the 3,5-dimethylphenylcarbamate of cellulose) using a ternary mixture (hexane/i-PrOH/acetone 95/3/2) as the elution system (entry 4).

Resolution of trans derivatives rac-**5** and rac-**6** was similar to that achieved for the cis compounds. Separation of trans-acetamido derivative rac-**5** could not be achieved on CSP-1 (low α and R_s, entries 7 and 8) or on CSP-2 (no resolution). As in the case of rac-**4**, trans-benzamido derivative rac-**6** was resolved on both columns with good chromatographic parameters (entries 9 and 11) and these could be improved on CSP-1 by adding a certain amount of acetone to the eluent. The use of a 95/3/2 mixture of hexane/i-PrOH/acetone as the elution system gave the highest values for the separation factor α and resolution R_s (entry 10).

Although the mechanism for chiral discrimination on CSPs derived from arylcarbamates of polysaccharides is not clear due to its complexity, the interaction

Table 1. Selected chromatographic data for the HPLC resolution of *cis*-c$_5$Phe and *trans*-c$_5$Phe precursors on several stationary phases and chromatographic modes

Entry	Compound	CSP	Eluent[c] A/B/C	λ (nm)	k'_1 [d]	α[d]	R_s[d]
1	*rac*-**3**	CSP-1[a]	93/7/0	210	3.04	1.08	—
2	*rac*-**3**	CSP-1[a]	95/2/3	210	4.25	1.06	—
3	*rac*-**4**	CSP-1[a]	98/2/0	210	5.31	1.30	1.50
4	*rac*-**4**	CSP-1[a]	95/3/2	210	2.71	1.23	1.88
5	*rac*-**4**	CSP-2[a]	98/2/0	210	3.50	1.15	0.70
6	*rac*-**4**	CSP-1[b]	95/3/2	270	3.24	1.14	0.83
7	*rac*-**5**	CSP-1[a]	93/7/0	210	3.24	1.09	—
8	*rac*-**5**	CSP-1[a]	95/3/2	210	4.46	1.06	—
9	*rac*-**6**	CSP-1[a]	95/5/0	210	3.41	1.24	1.32
10	*rac*-**6**	CSP-1[a]	95/3/2	210	3.09	1.22	1.97
11	*rac*-**6**	CSP-2[a]	98/2/0	210	5.20	1.24	1.36
12	*rac*-**6**	CSP-1[b]	95/3/2	270	3.49	1.25	1.21

[a] Analytical column dimensions: 150 × 4.6 mm ID, flow rate: 1 mL/min, injection volume: 5 µL, *c* = 5 mg/mL, samples dissolved in chloroform.
[b] Semipreparative column dimensions: 150 × 20 mm ID, flow rate: 18 mL/min, injection volume: 100 µL, *c* = 300 mg/mL, samples dissolved in chloroform.
[c] A: *n*-hexane, B: 2-propanol, C: acetone.
[d] For definition of *k'* (capacity factor), α (separation factor) and R_s (resolution factor), see Experimental section.

between the carbamate residues of the chiral selector with the racemate, mainly through hydrogen bonds involving the carbamate groups, is proposed as the most important factor for an effective enantiodiscrimination.[21b,21c] Additional π–π interactions involving aryl groups of the carbamate residue and aromatic groups of solute are also considered in this recognition mechanism. This hypothesis could account for the results obtained in the separation of compounds *rac*-**3**–**6**: benzamide derivatives *rac*-**4** and *rac*-**6** have an N–H amide bond that is more polar than the one in acetamide compounds *rac*-**3** and *rac*-**5**. Furthermore, the benzamide derivatives also have an additional phenyl group near the amide group. The interactions of these compounds with the chiral selector should therefore be stronger and their resolution higher, a situation consistent with the experimental results.

Finally, under the conditions described above, the separations of *rac*-**4** and *rac*-**6** were performed at the semipreparative level. The column saturation capacity (W_s) was calculated working in overload mode with the analytical column (150 × 4.6 mm ID) by injecting increasing amounts of compound. When concentrated samples of benzamides *rac*-**4** and *rac*-**6** (300 mg/mL CHCl$_3$) were injected into the analytical column, values of α and R_s were still very favourable for an efficient separation. Once W_s had been determined, we scaled-up the analytical resolution to the preparative column (150 × 20 mm ID) and a loading capacity of 30 mg was obtained for each compound. Values of α and R_s for the semipreparative resolution of *rac*-**4** and *rac*-**6** are given in entries 6 and 12, respectively.

HPLC resolution of *rac*-**4** (1 g) dissolved in chloroform (3.3 mL) was carried out by successive injections of 100 µL at intervals of 10 min on a 150 × 20 mm ID column filled with the 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose bonded on allylsilica gel, using a 95/3/2 mixture of hexane/*i*-PrOH/acetone as the eluent (flow-rate 18 mL min^{-1}). Four separate fractions were collected. Evaporation of the first fraction provided

440 mg of the first eluted enantiomer in its enantiomerically pure form. The second fraction contained 45 mg of an 85/15 mixture of the first and the second eluted enantiomers. The third and fourth fractions provided 150 and 350 mg, respectively, of mixtures 15/85 and 4/96 enriched in the last eluted enantiomer. These last fractions were collected and reinjected under the same conditions to give 315 mg of the last eluted enantiomer in an enantiomerically pure form.

In a similar way, HPLC resolution of *rac*-**6** was carried out using the same column and under the same conditions. In this case, a solution of 1 g of *rac*-**6** in 3.3 mL of chloroform was injected (repetitive injections of 100 µL each 12 min) onto the semipreparative column and three separate fractions were collected. The first fraction contained 440 mg of the enantiomerically pure first eluted enantiomer and the third fraction provided 525 mg of the last eluted enantiomer in a 9/91 enantiomeric ratio. The second fraction (25 mg) contained a 90/10 mixture of the first and the second eluted enantiomers. The final purification step involved reinjecting the fraction enriched in the second enantiomer and the new fractions were collected in such a way that the enantiomerically pure enantiomer was obtained (330 mg).

The enantiopurity of the resolved enantiomers of compounds **4** and **6** was assessed at the analytical level (Figs. 2 and 3, respectively). The absolute configurations of the compounds were assigned by X-ray diffraction analysis of some c$_5$Phe-containing dipeptides.[27] In this way we were able to establish that the absolute configuration of the enantiomer of *cis* benzamide **4** eluted first by HPLC was (1*R*,2*R*) and the second one had a (1*S*,2*S*) stereochemistry. Likewise, the less strongly retained isomer of *trans* benzamide **6** could be assigned as (1*R*,2*S*) and the last eluted enantiomer as (1*S*,2*R*).

Moreover, this unequivocal assignment allowed the confirmation of the relative stereochemistry of the amino nitriles *rac*-**1** and *rac*-**2**, which had previously been proposed on the basis of ^{1}H NMR shifts and NOE effects.
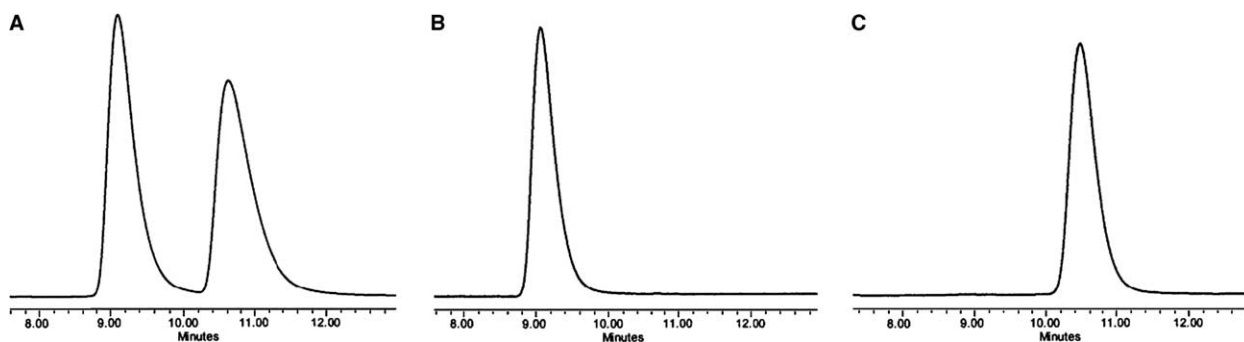
Figure 2. HPLC analytical resolution of *cis* benzamide *rac*-**4** (A) and resolved enantiomers (1*R*,2*R*)-**4** (B) and (1*S*,2*S*)-**4** (C). Column: 150 × 4.6 mm ID containing 3,5-dimethylphenylcarbamate of cellulose (CSP-**1**). Eluent: *n*-hexane/2-propanol/acetone 95/3/2. Flow rate: 1 mL/min. UV detection: 210 nm. Chromatographic parameters: $k'_1 = 2.71$; $\alpha = 1.23$; $R_s = 1.88$.
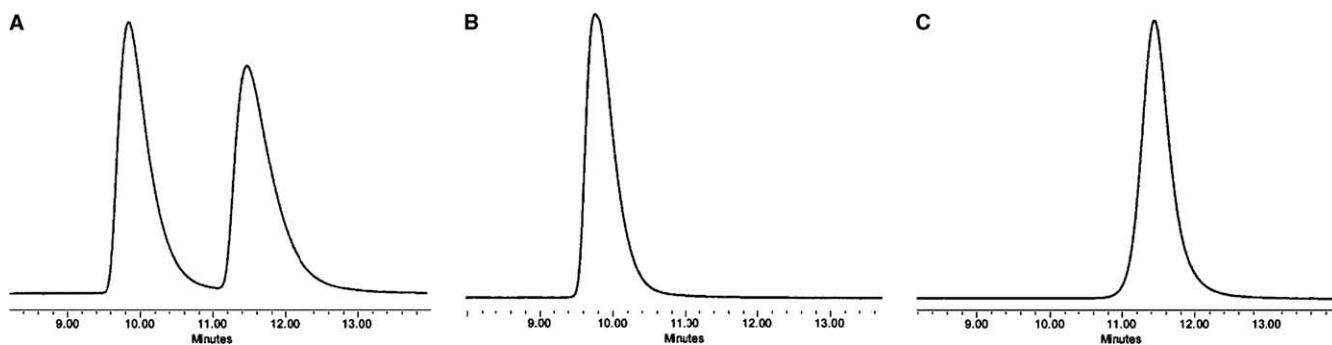

Figure 3. HPLC analytical resolution of *trans* benzamide *rac*-**6** (A) and resolved enantiomers (1*R*,2*S*)-**6** (B) and (1*S*,2*R*)-**6** (C). Column: 150 × 4.6 mm ID containing 3,5-dimethylphenylcarbamate of cellulose (CSP-**1**). Eluent: *n*-hexane/2-propanol/acetone 95/3/2. Flow rate: 1 mL/min. UV detection: 210 nm. Chromatographic parameters: $k'_1 = 3.09$; $\alpha = 1.22$; $R_s = 1.97$.

2.3. Synthesis of c₅Phe amino acids in enantiomerically pure form and their *N*-protected derivatives

After HPLC resolution of *rac*-**4** and *rac*-**6**, the isolated enantiomers were submitted to acid hydrolysis under the conditions previously developed for the racemic material. This process afforded in high yields the desired enantiopure amino acid hydrochlorides (1*R*,2*R*)-**7**, (1*S*,2*S*)-**7**, (1*R*,2*S*)-**8** and (1*S*,2*R*)-**8**, respectively (Scheme 3).

Enantiopure Z-protected amino acids (1*R*,2*R*)-**10** and (1*S*,2*S*)-**10** were synthesised from the corresponding amino acid hydrochlorides (1*R*,2*R*)-**7** and (1*S*,2*S*)-**7** under the conditions previously developed for the racemic material (Scheme 3). Respectively, *N*-Boc amino acids (1*R*,2*S*)-**9** and (1*S*,2*R*)-**9** were obtained from the corresponding enantiopure amino acids under the conditions that we have previously established for the racemate (Scheme 3).

3. Conclusion

Starting from readily available substrates and using high-yield transformations, conformationally restricted phenylalanine analogues *cis*-c₅Phe and *trans*-c₅Phe have been prepared for the first time. Moreover, we have developed a strategy for the synthesis of all stereoisomers of c₅Phe in enantiomeric pure forms: the appropri-

ate racemic precursors of *cis*-c₅Phe and *trans*-c₅Phe were subjected to HPLC resolution on an easily prepared cellulose-derived chiral stationary phase. The enantiopure amino acids obtained in this way were suitably protected for incorporation into peptides whose structural and biological studies could clarify certain aspects about the effects induced by this type of conformational restriction and the influence of the absolute configurations of the stereogenic centres.

4. Experimental

4.1. General

All reagents, with the exception of 2-phenylcyclopentanone, were purchased from the Aldrich Chemical Co. (Milwaukee, WI) and used without further purification. 2-Phenylcyclopentanone was synthesised as described in the literature by the reaction of phenylmagnesium bromide and 2-chlorocyclopentanone.[19] Solvents were dried, when necessary, by standard methods. The progress of the reactions was checked by thin layer chromatography (TLC) on Merck 60 F240 precoated silica gel polyester plates while products were visualised under UV light (254 nm), iodine vapour or ninhydrin chromatic reaction as appropriate. Column chromatography was performed using Merck silica gel (40–60 μm). The solvents used as HPLC mobile phases were of chromoscan grade. Melting points were determined on a Büchi
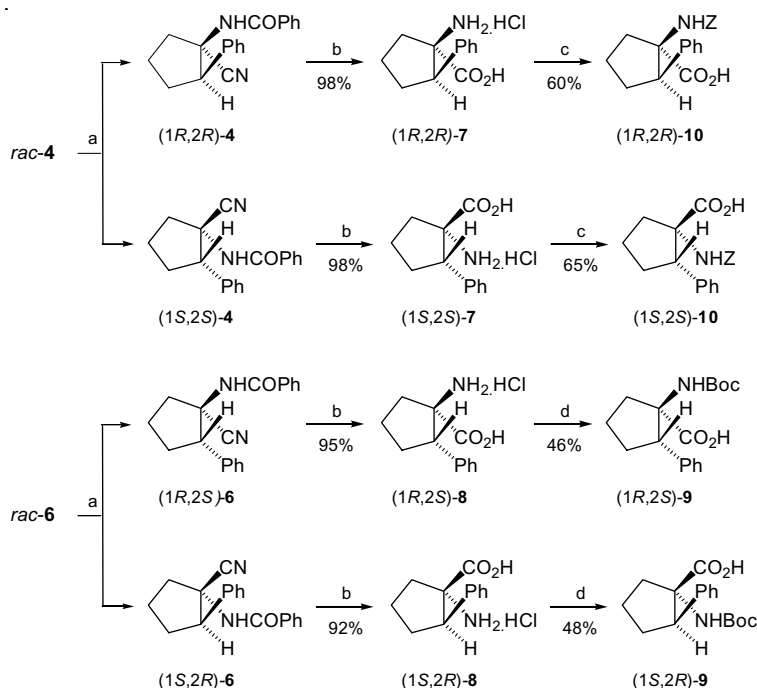
Scheme 3. Synthesis of enantiomerically pure c₅Phe derivatives. Reaction conditions: (a) chiral HPLC resolution; (b) 12 M HCl, reflux; (c) (i) TMSCl, CH₂Cl₂; (ii) (*i*-Pr)₂EtN, Z-Cl; (d) (i) propylene oxide, EtOH; (ii) TMAH, acetonitrile, Boc₂O.

SMP-20 apparatus and were not corrected. IR spectra were registered on a Mattson Genesis FTIR spectrophotometer; v_{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 instrument in CDCl₃ or D₂O, using the residual solvent signal as the internal standard ([D₆]acetone was used as an external reference for the ^{13}C spectra); chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hertz. Optical rotations were measured at room temperature using a Perkin–Elmer 241 Polarimeter-C in a 10 cm cell of 1 mL capacity. Microanalyses were carried out on a Perkin–Elmer 200 C, H, N, S analyser. High-resolution mass spectra were obtained on a high-resolution VG-autospectrometer. HPLC was carried out using a Waters HPLC system equipped with a Waters 600-E pump and a Waters 991 photodiode array detector. Mixed 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose and 10-undecenoate/3,5-dimethylphenylcarbamate of amylose were prepared and linked to allylsilica gel (Nucleosil 100-5, Machery-Nagel) according to a previously described procedure[22,26] to give CSP-**1** and CSP-**2**, respectively. These stationary phases were packed into stainless-steel tubes by the slurry method. The HPLC analytical assays were carried out on 150 × 4.6 mm ID columns containing these CSPs. All analytical assays were performed using mixtures of *n*-hexane/*i*-PrOH and *n*-hexane/*i*-PrOH/acetone as eluents (flow rate 1 mL/min), with UV monitoring performed at 210 nm. The capacity (k'), selectivity (α) and resolution (R_s) factors are defined as follows: $k' = (t_r - t_0)/t_0$, $\alpha = k'_1/k'_2$, $R_s = 1.18(t_2 - t_1)/(w_2 + w_1)$, where subscripts 1 and 2 refer to the first and second eluted enantiomer, and w_1 and w_2 denote their half-height peak widths; t_0 is the dead time. The semipreparative HPLC resolution of

compounds **4** and **6** was carried out on a 150 × 20 mm ID column filled with CSP-**1**. A mixture of *n*-hexane/*i*-PrOH/acetone (95/3/2) was used as the eluent with a flow rate of 18 mL/min. UV detection was performed at 270 nm. Both the column loading capacity, W_s (defined as the maximum sample mass that the column can hold), and the optimum sample concentration were calculated for the analytical 150 × 4.6 mm ID column by injecting increasing amounts of sample at different concentrations. In this way, the capacity of the semiprepative column was established as 30 mg and the optimum concentration of the sample as 300 mg/mL.

4.2. Strecker reaction on 2-phenylcyclopentanone. Synthesis of *cis*-1-cyano-2-phenylcyclopentylamine *rac*-1 and *trans*-1-cyano-2-phenylcyclopentylamine *rac*-2

To a solution of 2-phenylcyclopentanone (800 mg, 5.0 mmol) in a mixture of *i*-PrOH/30% NH₄OH (5 mL/25 mL) were added NH₄Cl (669 mg, 12.5 mmol) and NaCN (623 mg, 12.5 mmol). The mixture was stirred for 10 days at room temperature. The organic solvent was evaporated under reduced pressure and the liquid residue extracted with CH₂Cl₂ (3 × 25 mL). The organic layers were combined, dried over MgSO₄ and the solvent evaporated to yield a residue, which was purified by flash column chromatography (eluent: hexane/EtOAc 8/2) to give the *cis*-isomer *rac*-**1** (316 mg, 1.70 mmol, 34% yield) and the *trans*-isomer *rac*-**2** (325 mg, 1.75 mmol, 35% yield) as oily products.

4.2.1. *cis*-1-Cyano-2-phenylcyclopentylamine *rac*-1. R_f (CH₂Cl₂/EtOAc 8/2) = 0.7. IR (neat) 3373.4, 3314.4, 2223.0 cm⁻¹. ^{1}H NMR (CDCl₃, 300 MHz) δ 1.24 (br s, 2H), 1.84–2.08 (m, 4H), 2.27–2.38 (m, 2H), 3.35 (dd,

1H, $J = 7.4$, $J = 11.8$), 7.27–7.43 (m, 5H). ^{13}C NMR (CDCl$_3$, 75 MHz) δ 21.65, 27.46, 40.28, 55.37, 56.55, 124.33, 127.75, 128.52, 128.76, 136.08.

4.2.2. *trans*-1-Cyano-2-phenylcyclopentylamine *rac*-2.

R_f (CH$_2$Cl$_2$/EtOAc 8/2) = 0.5. IR (neat) 3350–3310, 2220.2 cm^{-1}. ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.89–2.26 (m, 7H), 2.37–2.43 (m, 1H), 2.98 (dd, 1H, $J = 8.0$, $J = 11.5$), 7.30–7.40 (m, 5H). ^{13}C NMR (CDCl$_3$, 75 MHz) δ 20.26, 28.75, 39.83, 56.77, 61.12, 122.55, 127.69, 128.02, 128.41, 137.20.

4.3. *cis*-N-(1-Cyano-2-phenylcyclopentyl)acetamide (*rac*-3)

Under an inert atmosphere, Et$_3$N (304 mg, 0.416 mL, 3.0 mmol) and acetyl chloride (118 mg, 0.106 mL, 1.5 mmol) were added to an ice-cooled solution of *cis*-amino nitrile *rac*-1 (186 mg, 1.0 mmol) in dry CH$_2$Cl$_2$ (10 mL). The reaction mixture was stirred at room temperature overnight. The reaction mixture was successively washed with aqueous solutions of 5% NaHCO$_3$, 2 M H$_2$SO$_4$ and saturated NaCl. The organic phase was dried over MgSO$_4$ and filtered. Evaporation of the solvent yielded a residue, which was purified by flash column chromatography (eluent: hexane/EtOAc 8/2) to afford acetamide derivative *rac*-3 as a white solid (171 mg, 0.75 mmol, 75% yield). Mp 155–156 °C (EtOAc/hexane). R_f (CH$_2$Cl$_2$/EtOAc 8/2) = 0.6. IR (Nujol) 3392.4–3209.2, 2234.7, 1676.3, 1521.1 cm^{-1}. ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.80 (s, 3H), 1.92–2.04 (m, 2H), 2.10–2.30 (m, 2H), 2.50–2.64 (m, 2H), 3.75 (dd, 1H, $J = 7.7$, $J = 9.9$), 5.24 (br s, 1H), 7.34–7.46 (m, 5H). ^{13}C NMR (CDCl$_3$, 75 MHz) δ 21.73, 23.12, 28.27, 38.06, 53.85, 57.06, 120.93, 128.33, 129.17, 135.99, 169.73. Anal. Calcd for C$_{14}$H$_{16}$N$_2$O: C, 73.66; H, 7.06; N, 12.27. Found: C, 73.54; H, 6.95; N, 12.17.

4.4. *cis*-N-(1-Cyano-2-phenylcyclopentyl)benzamide *rac*-4

Under an inert atmosphere, Et$_3$N (1.515 g, 2.1 mL, 15.0 mmol) and benzoyl chloride (1.054 g, 0.808 mL, 7.5 mmol) were added to an ice-cooled solution of *cis*-amino nitrile *rac*-1 (930 mg, 5.0 mmol) in dry CH$_2$Cl$_2$ (30 mL). The reaction mixture was stirred at room temperature overnight. The reaction mixture was successively washed with aqueous solutions of 5% NaHCO$_3$, 2 N H$_2$SO$_4$ and saturated NaCl. The organic phase was dried over MgSO$_4$ and filtered. Evaporation of the solvent yielded a residue, which was purified by flash column chromatography (eluent: hexane/EtOAc 8/2) to afford the benzamide derivative *rac*-4 as a white solid (1.060 g, 3.65 mmol, 73% yield). Mp 142 °C (EtOAc/hexane). R_f (hexane/EtOAc 8/2) = 0.2. IR (Nujol) 3387.8, 2237.3, 1649.5, 1514.3 cm^{-1}. ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.95–2.05 (m, 2H), 2.20–2.32 (m, 2H), 2.64–2.69 (m, 2H), 3.85 (dd, 1H, $J = 8.1$, $J = 9.6$), 5.99 (br s, 1H), 7.24–7.48 (m, 5H). ^{13}C NMR (CDCl$_3$, 75 MHz) δ 22.27, 28.35, 38.47, 54.44, 56.92, 120.94, 126.68, 128.31, 128.54, 128.62, 129.37, 132.01, 133.07, 135.73, 166.56. Anal. Calcd for C$_{19}$H$_{18}$N$_2$O: C, 78.59; H, 6.25; N, 9.65. Found: C, 78.23; H, 6.18; N, 9.20.

4.5. Resolution of benzamide *rac*-4: (1*R*,2*R*) and (1*S*,2*S*)-N-(1-cyano-2-phenylcyclopentyl)benzamide

HPLC resolution of *cis* racemate *rac*-4 (1.0 g) dissolved in CHCl$_3$ (3.3 mL) was carried out by successive injections of 0.1 mL on a 150 × 20 mm ID column filled with mixed 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose bonded on allylsilica gel (CSP-1) and using a mixture of *n*-hexane/*i*-PrOH/acetone 95/3/2 as the eluent (flow rate: 18 mL/min). A total of 35 injections were required, with one injection performing every 10 min. Four separate fractions were collected. The first, second, third and fourth fractions contained, respectively, 100/0 (440 mg), 85/15 (45 mg), 15/85 (150 mg) and 4/96 (350 mg) mixtures of (1*R*,2*R*)-4 and (1*S*,2*S*)-4. The combined third and fourth fractions were reinjected in a way similar to that described above and led to 315 mg of enantiomerically pure (1*S*,2*S*)-4. Spectroscopic data of both enantiomers are the same as those described for *rac*-4.

(1*R*,2*R*)-4: Mp 147 °C (EtOAc/hexane). $[\alpha]_D = -13.9$ (*c* 1.02, CHCl$_3$).

(1*S*,2*S*)-4: Mp 147 °C (EtOAc/hexane). $[\alpha]_D = +13.3$ (*c* 1.03, CHCl$_3$).

4.6. *cis*-c$_5$Phe hydrochloride *rac*-7

A solution of *cis*-acetamide *rac*-3 (228 mg, 1.0 mmol) or *cis* benzamide *rac*-4 (290 mg, 1.0 mmol) in 12 M hydrochloric acid (5 mL) was heated under reflux for 1 day. The solvent was evaporated and the resulting solid was partitioned between diethyl ether and water. The aqueous phase was washed with several additional portions of diethyl ether and then lyophilised to give 230 mg of the hydrochloride *rac*-7 (0.95 mmol, 95% yield). Mp 245–250 °C (dec). IR (Nujol) 3194.3, 1744.8 cm^{-1}. ^{1}H NMR (D$_2$O, 300 MHz) δ 1.84–2.23 (m, 5H), 2.49–2.58 (m, 1H), 3.78 (dd, 1H, $J = 7.5$, $J = 12.3$), 7.20–7.23 (m, 2H), 7.28–7.37 (m, 3H). ^{13}C NMR (D$_2$O, 75 MHz) δ 22.66, 28.41, 36.31, 52.74, 68.87, 128.32, 128.57, 129.38, 134.92, 174.82.

4.6.1. (1*R*,2*R*)-c$_5$Phe hydrochloride (1*R*,2*R*)-7.

An identical procedure to that described above was applied to transform (1*R*,2*R*)-4 (261 mg, 0.9 mmol) into (1*R*,2*R*)-7 as a white solid (213 mg, 0.88 mmol, 98% yield). Mp 242 °C (dec). $[\alpha]_D = -40.2$ (*c* 1.18, 6 M HCl/H$_2$O). Spectroscopic data are the same as those described for *rac*-7.

4.6.2. (1*S*,2*S*)-c$_5$Phe hydrochloride (1*S*,2*S*)-7.

In a similar way to that described above, starting from (1*S*,2*S*)-4 (232 mg, 0.8 mmol), (1*S*,2*S*)-7 was obtained as a white solid (189 mg, 0.78 mmol, 98% yield). Mp 241 °C (dec). $[\alpha]_D = +41.2$ (*c* 1.15, 6 M HCl/H$_2$O). Spectroscopic data are the same as those described for *rac*-7.

4.7. *cis*-Z-c$_5$Phe-OH *rac*-10

Under an inert atmosphere, chlorotrimethylsilane (272 mg, 0.324 mL, 2.5 mmol) was added to an ice-cooled

suspension of hydrochloride *rac*-**7** (242 mg, 1.0 mmol) in anhydrous CH_2Cl_2 (7 mL). The reaction was stirred at 0 °C for 10 min and then heated under reflux for 1 h. After cooling to 0 °C, benzyl chloroformate (216 mg, 0.210 mL, 1.4 mmol) and *N,N*-diisopropylethylamine (323 mg, 0.438 mL, 2.5 mmol) were added and the reaction stirred at room temperature for 1 day. The solvent was evaporated to dryness and the residue taken up in saturated aqueous $NaHCO_3$ (5 mL). Diethyl ether (15 mL) and water (10 mL) were added and after stirring for 10 min, the layers were separated. The aqueous phase was washed with an additional portion of ether, acidified by the addition of a solution of $KHSO_4$, and then extracted several times with CH_2Cl_2. After drying and filtering, removal of the solvent afforded *rac*-**10** as a white solid. Additional purification was carried out by flash column chromatography (eluent: hexane/EtOAc 8/2) to afford the *N*-Z derivative *rac*-**10** (193 mg, 0.57 mmol, 57% yield). Mp 133 °C (EtOAc/hexane). R_f (CH_2Cl_2/EtOAc 8/2) = 0.3. IR (Nujol) 3396.4, 3140.2, 1728.0, 1689.1 cm^{-1}. ^1H NMR (CDCl$_3$, 300 MHz, 60 °C) δ 1.87–2.04 (m, 2H), 2.15–2.24 (m, 2H), 2.49–2.69 (m, 2H), 3.79 (dd, 2H, $J = 7.8$, $J = 10.7$), 4.80 (br s, 1H), 4.98 (d, 1H, $J = 12.5$), 5.05 (d, 1H, $J = 12.1$), 7.25–7.37 (m, 10H), 9.16 (br s, 1H). ^{13}C NMR (CDCl$_3$, 75 MHz, 60 °C) δ 23.11, 30.21, 37.08, 54.25, 66.78, 67.68, 127.64, 127.80, 128.04, 128.25, 128.40, 128.56, 128.88, 136.12, 136.98, 156.21, 179.53. Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.95; H, 6.18; N, 3.92.

4.7.1. (1*R*,2*R*)-*N*-Z-c₅Phe-OH (1*R*,2*R*)-10. An identical procedure to that described above was applied to transform (1*R*,2*R*)-**7** (242 mg, 1.0 mmol) into (1*R*,2*R*)-**10** as an oil (204 mg, 0.60 mmol, 60% yield). $[\alpha]_D = -32.5$ (*c* 0.50, CHCl$_3$). Spectroscopic data are the same as those described for *rac*-**10**.

4.7.2. (1*S*,2*S*)-*N*-Z-c₅Phe-OH (1*S*,2*S*)-10. In a similar way to that described above, starting from (1*S*,2*S*)-**7** (218 mg, 0.9 mmol), (1*S*,2*S*)-**10** was obtained as an oil (199 mg, 0.59 mmol, 65% yield). $[\alpha]_D = +30.0$ (*c* 0.78, CHCl$_3$). Spectroscopic data are the same as those described for *rac*-**10**.

4.8. *trans*-*N*-(1-Cyano-2-phenylcyclopentyl)acetamide (*rac*-5)

Under an inert atmosphere, Et$_3$N (304 mg, 0.416 mL, 3.0 mmol) and acetyl chloride (118 mg, 0.106 mL, 1.5 mmol) were added to an ice-cooled solution of *trans*-amino nitrile *rac*-**2** (186 mg, 1.0 mmol) in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature overnight. The reaction mixture was successively washed with aqueous solutions of 5% Na-HCO$_3$, 2 M H$_2$SO$_4$ and saturated NaCl. The organic phase was dried over MgSO$_4$ and filtered. Evaporation of the solvent yielded a residue, which was purified by flash column chromatography (eluent: hexane/EtOAc 8/2) to afford the *trans* acetamide derivative *rac*-**5** as a white solid (171 mg, 0.75 mmol, 75% yield). Mp 134 °C (EtOAc/hexane). R_f (CH_2Cl_2/EtOAc 8/2) = 0.4. IR (Nujol) 3244.5, 2237.8, 1647.35, 1529.8 cm^{-1}. ^1H NMR (CDCl$_3$, 300 MHz) δ 1.96 (s, 3H), 1.98–2.27 (m,

5H), 2.93–3.00 (m, 1H), 3.29 (dd, 1H, $J = 8.1$, $J = 10.3$), 5.83 (br s, 1H), 7.34–7.42 (m, 5H). ^{13}C NMR (CDCl$_3$, 75 MHz) δ 20.77, 23.32, 28.46, 38.20, 54.38, 61.11, 118.83, 128.39, 128.43, 129.02, 136.79, 169.94. Anal. Calcd for $C_{14}H_{16}N_2O$: C, 73.66; H, 7.06; N, 12.27. Found: C, 73.45; H, 6.93; N, 12.10.

4.9. *trans*-*N*-(1-Cyano-2-phenylcyclopentyl)benzamide (*rac*-6)

Under an inert atmosphere, Et$_3$N (1.515 g, 2.1 mL, 15.0 mmol) and benzoyl chloride (1.054 g, 0.808 mL, 7.5 mmol) were added to an ice-cooled solution of *trans*-amino nitrile *rac*-**2** (930 mg, 5.0 mmol) in dry CH_2Cl_2 (30 mL). The reaction mixture was stirred at room temperature overnight and then successively washed with aqueous solutions of 5% NaHCO$_3$, 2 M H$_2$SO$_4$ and saturated NaCl. The organic phase was dried over MgSO$_4$ and filtered. Evaporation of the solvent yielded a residue, which was purified by flash column chromatography (eluent: hexane/EtOAc 8/2) to afford the *trans* benzamide derivative *rac*-**6** as a white solid (1.278 g, 4.40 mmol, 88% yield). Mp 122 °C (EtOAc/hexane). R_f (CH_2Cl_2/EtOAc 8/2) = 0.2. IR (Nujol) 3271.0–3245.9, 1638.7 cm^{-1}. ^1H NMR (CDCl$_3$, 300 MHz) δ 2.08–2.36 (m, 5H), 2.99–3.08 (m, 1H), 3.45 (dd, 1H, $J = 8.1$, $J = 10.3$), 6.56 (br s, 1H), 7.31–7.49 (m, 8H), 7.64–7.67 (m, 2H). ^{13}C NMR (CDCl$_3$, 75 MHz) δ 20.85, 28.24, 38.17, 54.48, 61.24, 118.74, 126.99, 128.24, 128.37, 128.60, 129.00, 132.10, 133.06, 136.83, 167.11. Anal. Calcd for $C_{19}H_{18}N_2O$: C, 78.59; H, 6.25; N, 9.65. Found: C, 78.45; H, 6.15; N, 9.79.

4.10. Resolution of benzamide *rac*-6: (1*R*,2*S*) and (1*S*,2*R*)-*N*-(1-cyano-2-phenylcyclopentyl)benzamide

HPLC resolution of *trans* racemate *rac*-**6** (1.0 g) dissolved in CHCl$_3$ (3.3 mL) was carried out by successive injections of 0.1 mL on a 150 × 20 mm ID column filled with mixed 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose bonded on allylsilica gel (CSP-**1**) and using a mixture of *n*-hexane/*i*-PrOH/acetone 95/3/2 as the eluent (flow rate: 18 mL/min). A total of 35 injections were required, with one injection performing every 12 min. Three separate fractions were collected. The first, second and third fractions contained, respectively, 100/0 (440 mg), 90/10 (25 mg) and 9/91 (525 mg) mixtures of (1*R*,2*S*)-**6** and (1*S*,2*R*)-**6**. Reinjection of the third fraction in a similar way to that described above led to 330 mg of enantiomerically pure (1*S*,2*R*)-**6**.

(1*R*,2*S*)-**6**: Mp 120 °C (EtOAc/hexane). $[\alpha]_D = -90.1$ (*c* 1.02, CHCl$_3$).

(1*S*,2*R*)-**6**: Mp 118 °C (EtOAc/hexane). $[\alpha]_D = +88.2$ (*c* 1.00, CHCl$_3$).

4.11. *trans*-c₅Phe hydrochloride *rac*-8

A solution of *trans* acetamide *rac*-**5** (228 mg, 1.0 mmol) or *trans* benzamide *rac*-**6** (290 mg, 1.0 mmol) in 12 M hydrochloric acid (5 mL) was heated under reflux for 1 day. The solvent was evaporated and the resulting

solid was partitioned between CH_2Cl_2 and water. The aqueous phase was washed with several additional portions of CH_2Cl_2 and then lyophilised to give 239 mg of *rac*-**8** (0.99 mmol, 99% yield). Mp 254–256 °C (dec). IR (Nujol) 3325–2250, 1749.2, 1734.8 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz) δ 1.94–2.18 (m, 4H), 2.34–2.44 (m, 1H), 2.56–2.64 (m, 1H), 3.43 (dd, 1H, $J = 7.1$, $J = 12.1$), 7.31–7.40 (m, 5H). ^{13}C NMR (D_2O, 75 MHz) δ 22.38, 30.56, 34.39, 55.18, 69.64, 128.30, 128.88, 136.24, 173.07.

4.11.1. (1*R*,2*S*)-c₅Phe hydrochloride (1*R*,2*S*)-8.
In a similar way to that described above, starting from (1*R*,2*S*)-**6** (261 mg, 0.9 mmol), (1*R*,2*S*)-**8** was obtained as a white solid (207 mg, 0.86 mmol, 95% yield). Mp 253–255 °C (dec). $[\alpha]_D = +9.9$ (*c* 1.02, 6 M HCl/H_2O). Spectroscopic data are the same as those described for *rac*-**8**.

4.11.2. (1*S*,2*R*)-c₅Phe hydrochloride (1*S*,2*R*)-8.
An identical procedure to that described above was applied to transform (1*S*,2*R*)-**6** (203 mg, 0.7 mmol) into (1*S*,2*R*)-**8** as a white solid (156 mg, 0.64 mmol, 92% yield). Mp 251–253 °C (dec). $[\alpha]_D = -9.9$ (*c* 1.02, 6 M HCl/H_2O). Spectroscopic data are the same as those described for *rac*-**8**.

4.12. *trans*-Boc-c₅Phe-OH *rac*-9

A solution of *trans*-c₅Phe hydrochloride *rac*-**8** (1.0 mmol, 242 mg) in ethanol (10 mL) and propylene oxide (3 mL) was heated under reflux for 1 h. The solvent was evaporated under reduced pressure. In order to complete the removal of the residual propylene oxide and the by-products of the reaction, Et_2O was added and then removed under vacuum (3 × 5 mL). The resulting solid was suspended in acetonitrile (20 mL) and TMAH (tetramethylammonium hydroxide) was added (1 mmol, 181 mg). The mixture was stirred at 40–50 °C until a solution was formed. Once complete dissolution was achieved, Boc_2O (1.5 mmol, 327 mg) was added at room temperature and the mixture was stirred for 2 days. On the third day, a further 1.0 mmol of Boc_2O (218 mg) was added and stirred for a further 1 day. The acetonitrile was removed under vacuum and the residue was partitioned between H_2O and Et_2O. The aqueous layer was acidified with solid citric acid to pH 3–4 and then extracted with EtOAc. Concentration of the organic layer resulted in the precipitation of a solid, a process that was completed by adding a portion of hexane. The product was recrystallised (EtOAc/hexane) to give 229 mg of a white solid (0.75 mmol, 75% yield). Mp 188 °C (EtOAc/hexane). R_f (CH_2Cl_2/EtOAc 6/4) = 0.7. IR (Nujol) 3296.7–3251.4, 1696.1, 1638.2 cm^{-1}. ^{1}H NMR (CDCl$_3$, 300 MHz, 60 °C) δ 1.74 (s, 9H), 1.97–2.18 (m, 3H), 2.24–2.38 (m, 2H), 2.58–2.69 (m, 1H), 3.53–3.65 (m, 1H), 5.35 (br s, 1H), 7.19–7.31 (m, 5H). ^{13}C NMR (CDCl$_3$, 75 MHz, 60 °C) δ 22.85, 28.39, 30.20, 35.94, 54.18, 70.43, 80.29, 127.29, 128.05, 128.32, 138.48, 155.19, 177.84. Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.75; H, 7.46; N, 4.49.

4.12.1. (1*R*,2*S*)-*N*-Boc-c₅Phe-OH (1*R*,2*S*)-9.
In a similar way to that described above, starting from (1*R*,2*S*)-**8** (193 mg, 0.8 mmol), (1*R*,2*S*)-**9** was obtained as a white solid (112 mg, 0.37 mmol, 46% yield). Mp 164 °C (EtOAc/hexane). $[\alpha]_D = -75.8$ (*c* 0.91, CHCl$_3$). Spectroscopic data are the same as those described for *rac*-**9**.

4.12.2. (1*S*,2*R*)-*N*-Boc-c₅Phe-OH (1*S*,2*R*)-9.
An identical procedure to that described above was applied to transform (1*S*,2*R*)-**8** (145 mg, 0.6 mmol) into (1*S*,2*R*)-**9** as a white solid (88 mg, 0.29 mmol, 48% yield). Mp 164 °C (EtOAc/hexane). $[\alpha]_D = +74.6$ (*c* 0.98, CHCl$_3$). Spectroscopic data are the same as those described for *rac*-**9**.

4.13. *trans*-Z-c₅Phe-OH *rac*-11

Under an inert atmosphere, chlorotrimethylsilane (103 mg, 0.097 mL, 0.8 mmol) was added to an ice-cooled suspension of hydrochloride *rac*-**8** (73 mg, 0.3 mmol) in anhydrous CH_2Cl_2 (5 mL). The reaction was stirred at 0 °C for 10 min and then heated under reflux for 1 h. After cooling to 0 °C, benzyl chloroformate (60 mg, 0.059 mL, 0.4 mmol) and *N,N*-diisopropylethylamine (97 mg, 0.131 mL, 0.8 mmol) were added and the reaction stirred at room temperature for 1 day. The solvent was evaporated to dryness and the residue was taken up in a saturated solution of $NaHCO_3$ (5 mL). Diethyl ether (15 mL) and water (10 mL) were added and after stirring for 10 min, the layers were separated. The aqueous phase was washed with an additional portion of ether, acidified by the addition of a solution of $KHSO_4$, and then extracted several times with CH_2Cl_2. After drying and filtering, removal of the solvent afforded a residue that was purified by flash column chromatography (eluent: hexane/EtOAc 8/2) to afford the *N*-Z derivative *rac*-**11** (27 mg, 0.08 mmol, 27% yield). R_f (CH_2Cl_2/EtOAc 8/2) = 0.5. IR (Nujol) 3241.1, 1694.8, 1641.3 cm^{-1}. ^{1}H NMR (CDCl$_3$, 300 MHz) δ 2.01–2.56 (m, 6H), 3.71 (br s, 1H), 5.07 (d, 1H, $J = 12.1$), 5.14 (d, 1H, $J = 12.1$), 5.74 (br s, 1H), 7.11–7.35 (m, 10H), 8.75 (br s, 1H). ^{13}C NMR (CDCl$_3$, 75 MHz) δ 23.11, 30.21, 35.48, 53.83, 66.69, 70.28, 127.39, 127.85, 128.08, 128.19, 128.35, 128.53, 136.31, 137.95, 154.97, 177.96. Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.89; H, 6.22; N, 3.98.

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