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Synthesis of the four stereoisomers of cyclobutane analogues of phenylalanine in enantiomerically pure form

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Abstract—All stereoisomers of 1-amino-2-phenylcyclobutanecarboxylic acid—c_4Phe—have been synthesized and the series c_nPhe has thus been completed. The use of two different strategies based on a cyclization reaction, starting from ethyl isocyanoacetate and dialkyl malonate, respectively, gave both *cis*-c_4Phe and *trans*-c_4Phe in racemic form. HPLC resolution of one of the intermediates using a cellulose-derived chiral stationary phase allowed the isolation of the corresponding enantiomerically pure N-protected amino acids, prepared for incorporation into peptides. The relative stereochemistry of enantiopure compounds has been unambiguously assigned.

1. Introduction

Secondary and tertiary structures of peptides turn out to be as crucial as the primary structures for their biological activity. Therefore, to establish the active conformation of peptides we must study their three-dimensional structure, not only for the main chain but also for the side chain moieties, the latter being directly involved in peptide-receptor recognition phenomena that determine the biological specificity. Aromatic amino acids (Phe, Tyr, Trp, His) warrant special attention since aromatic groups on a peptidic ligand often play a central role in the interaction with the receptor. Thus, the introduction of constraints in these amino acids provides a variety of analogues in which the orientation of the aromatic group can be more controlled or even fixed, becoming a valuable tool to study the structure–activity relationships in bioactive peptides.[1]

One of the most attractive series of restricted analogues of phenylalanine is that resulting from tethering Cα to Cβ (Fig. 1), that is, 1-amino-2-phenylcycloalkanecarboxylic acids (c_nPhe).[2] In recent years, we have focussed our interest on the synthesis of these amino acids both in racemic and enantiopure forms. The incorporation of these carbocyclic analogues of phenylalanine into model dipeptides RCO-L-Pro-c_nPhe-NHR′ and the conformational analysis of these peptides has provided evidence of the role of these amino acids in modulating the β-folding mode. A conformational study of RCO-L-Pro-c_3Phe-NHR′ and RCO-L-Pro-*cis*-c_6Phe-NHR′ has already been completed,[3,4] whereas the corresponding analysis of the peptides RCO-L-Pro-*trans*-c_6Phe-NHR′ and RCO-L-Pro-c_5Phe-NHR′ are in progress in our laboratory.[5]

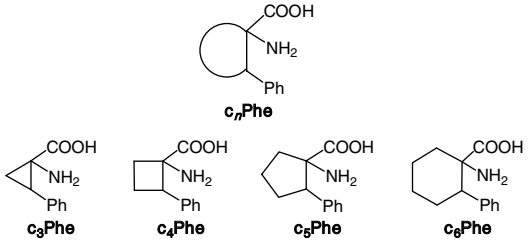

Figure 1. Structures of the series of 1-amino-2-phenylcycloalkanecarboxylic acids (c_nPhe) as conformationally restricted analogues of Phe.

These studies require the use of enantiomerically pure amino acids as starting materials. The synthesis of most of these compounds has already been reported (c_3Phe,[6] c_5Phe,[7] c_6Phe[8]). In order to complete the series of compounds to be studied and to characterize a general behaviour concerning the influence of ring size with the conformational restriction imposed by the

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constrained phenylalanine, conveniently protected c_4Phe was needed (Fig. 1).

Numerous asymmetric syntheses of 1-aminocyclopropane-, 1-aminocyclopentane- and 1-aminocyclohexanecarboxylic acids have been developed. Nevertheless, the corresponding cyclobutane derivatives have received very little attention in recent years.[6a] Only a few racemic syntheses of substituted 1-aminocyclobutanecarboxylic acids have been reported. The small number of references to these cyclobutane amino acids may indicate their unfavourable situation with respect both to small-angle strain and to entropy.

To the best of our knowledge, the only references found in the literature concerning the total synthesis of 2-phenyl-1-aminocyclobutanecarboxylic acid (c_4Phe) describes a cyclization reaction to obtain the carbocycle.[9] Curtius rearrangement of alkyl 2-phenylcyclobutane-1,1-dicarboxylate led to the *trans* isomer of c_4Phe in its racemic form. The synthesis of *cis*-c_4Phe was reported by the same author in a subsequent patent.[9c] In this method the starting material was the previous monoacid and the corresponding rearrangement of the *cis*-substituent was performed. In this patent only very vague mention is made of how the corresponding enantiomers of the amino acids were obtained by fractional crystallization of different diastereomeric salts.

We were encouraged to improve the synthesis of *trans*-c_4Phe and develop a new method to prepare *cis*-c_4Phe. The second objective of this paper is to report the synthesis, for the first time, of enantiomerically pure (1*R*,2*R*)-, (1*S*,2*S*)-, (1*R*,2*S*)- and (1*S*,2*R*)-c_4Phe from the corresponding racemate by HPLC using a polysaccharide-derived chiral stationary phase. Furthermore, X-ray diffraction analysis of some dipeptides derived from the new amino acids was used to determine the absolute configuration of each stereoisomer.

2. Results and discussion

2.1. Synthesis of racemic *cis*- and *trans*-c_4Phe[†]

As mentioned above, although the synthesis of cyclic α-amino acids has attracted a great deal of attention recently, cyclobutane amino acids have not been developed as much as the corresponding compounds with three-, five- or six-membered ring.[6a] Not only are there very few references to enantiopure cyclobutane amino acids, the same applies also to racemic compounds.

One of the first descriptions of the preparation of cyclobutane amino acids included c_4Phe. As stated in the introduction, in 1964 Burger reported the synthesis of different phenylcyclobutane amino acids. In the case of phenylalanine analogues, a cyclization reaction starting from dialkyl malonate and the corresponding nucleophile is reported. This method exploits the different reactivities of the two alkoxycarbonyl esters of the corresponding alkyl 2-phenylcyclobutane-1,1-dicarboxylate, owing to the steric hindrance of the aromatic moiety. Thus, selective hydrolysis of the *trans*-carboxylate ester and different degradation reactions provided the precursors of *cis*- and *trans*-c_4Phe in good yields.[9]

Until 1980 only a few more examples of the synthesis of amino acids containing a cyclobutane ring were reported in the literature.[10] However, since that time several different compounds of this type have been isolated from natural sources and their activity as potential neurotransmitters has been tested.[11]

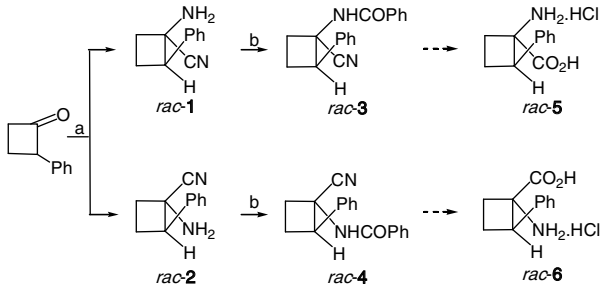
Surprisingly, in recent years cyclobutane amino acids have received increasing attention and several publications have reported new approaches to these compounds. Some of them take into account the use of cyclobutane compounds as starting materials and introduce the amino and acid functions on this skeleton by Bücherer–Bergs or Strecker reactions on the cyclobutanones,[11a,12] or by reaction of sodium azide with the corresponding acid[11b] or sulfinyloxirane.[13] Some strategies based on cyclizations have also been described.[14] More recently, Avenoza reported the synthesis of different 2-substituted cyclobutane amino acids by means of a formal [2+2] cycloaddition (Michael–Dieckmann-type reaction).[15]

Asymmetric versions of different routes have also been developed. Asymmetric Strecker reactions have been applied to 2-substituted cyclobutanones by Frahm et al.[16] and later by Fadel et al.[17] Wanner and co-workers described the use of a chiral glycine equivalent and a strategy based on a cyclization reaction to obtain the four stereoisomers of 1-amino-2-(hydroxymethyl)-cyclobutane carboxylic acid.[18]

Our experience on different approaches to analogues of phenylalanine c_nPhe highlighted the classic Strecker reaction as a useful method. Indeed, we reported an efficient synthesis of *trans*-c_6Phe through a completely diastereoselective Strecker reaction.[8c] The absence of selectivity in this reaction on 2-phenylcyclopentanone allowed us to develop a divergent synthetic route to obtain both *cis*-c_5Phe and *trans*-c_5Phe.[7]

Taking into account all these synthetic precedents, we decided to undertake the preparation of c_4Phe by means of the classic Strecker reaction on 2-phenylcyclobutanone. The cyclic ketone was synthesized as described in the literature.[19] Treatment of 2-phenylcyclobutanone with NaCN and NH_4Cl under the optimal conditions found for the synthesis of *trans*-c_6Phe and c_5Phe in all cases afforded mixtures of *cis*- and *trans*-1-cyano-2-phenylcyclobutylamine (*rac*-**1** and *rac*-**2**, respectively, Scheme 1) in a similar ratio. Conversion of the ketone was not as good as in previous cases (92% for 2-phenylcyclohexanone, 69% for 2-phenylcyclopentanone and 35% for 2-phenylcyclobutanone), probably due to decomposition over the long reaction time. Both isomers were separated and the corresponding amides *rac*-**3** and

[†] *cis*- or *trans*-c_4Phe refers to the relative position between the amino and the phenyl groups in the cyclic analogue of phenylalanine.



Scheme 1. Synthesis of racemic *cis*-c_4Phe and *trans*-c_4Phe precursors by Strecker reaction. Reaction conditions: (a) (i) NaCN, NH_4Cl, 2-propanol, NH_4OH; (ii) column chromatography; (b) BzCl, NEt_3, CH_2Cl_2.

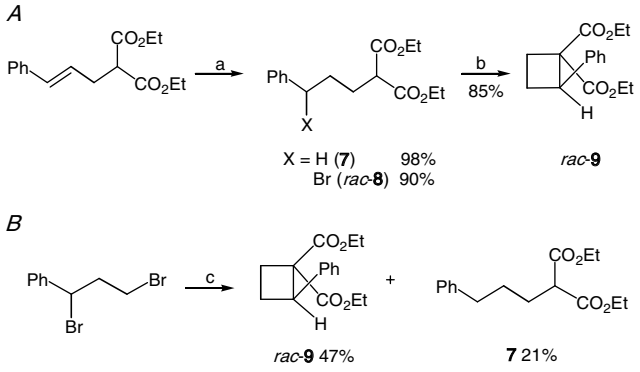
rac-**4** of each amino nitrile were synthesized as precursors of the amino acids (according to our previous experience and taking into account that hydrolysis of the amide nitrile was much more efficient than hydrolysis of the starting amino nitrile). However, in the case of the cyclobutane derivatives, the strong conditions required to hydrolyze the cyano group caused ring opening.

Different attempts to synthesize c_4Phe precursors by means of [2+2] cycloadditions were attempted following reported strategies,[15] but in our case this proved unsuccessful.

At this point a methodology based on a cyclization reaction seemed to be the most appropriate way to synthesize c_4Phe. Our first efforts were directed towards improving the synthesis of *trans*-c_4Phe described in the literature.[9]

We reproduced the route already developed and introduced some changes. The first part of this strategy consists of preparing diethyl 2-phenylcyclobutane-1,1-dicarboxylate (*rac*-**9**). The proposed route started with the alkylation of diethyl malonate with cinnamyl chloride to obtain diethyl cinnamylmalonate.[20] Addition of hydrogen bromide to this compound provided the halogenated compound *rac*-**8**, which was cyclized to the target compound *rac*-**9**. In our case the halogenation reaction could not be reproduced in only one step and this reaction was replaced by a two step process: initial hydrogenation to produce compound **7** and the subsequent introduction of the bromo-substituent in the benzylic position with *N*-bromosuccinimide to give *rac*-**8**. Both reactions gave excellent yields. In the presence of sodium hydride the cyclization reaction on compound *rac*-**8** gave a good yield (Scheme 2A).

Furthermore, we considered the possibility of a double alkylation of diethyl malonate with 1,3-dibromo-1-phenylpropane. This reaction was carried out by heating an equimolecular mixture of the reactants under reflux in tetrahydrofuran in the presence of sodium hydride. Under these conditions a mixture of different compounds was obtained and some of them were isolated by column chromatography; compounds **7** and *rac*-**9** were isolated in 21% and 47% yield, respectively. Thus,

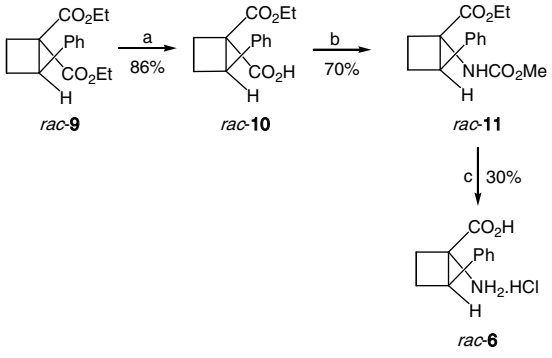


Scheme 2. Synthesis of *rac*-**9**. Reaction conditions: (a) (i) H_2, Pd/C, EtOH; (ii) NBS, BzOOBz, CH_2Cl_2; (b) NaH, THF; (c) $CH_2(CO_2Et)_2$, NaH, THF, reflux.

in only one step diethyl 2-phenylcyclobutane-1,1-dicarboxylate *rac*-**9** can be synthesized in a competitive yield in comparison to the longer route described previously. Moreover, by-product **7** from this reaction can be recycled to obtain the cyclic compound by following the first route (Scheme 2B).

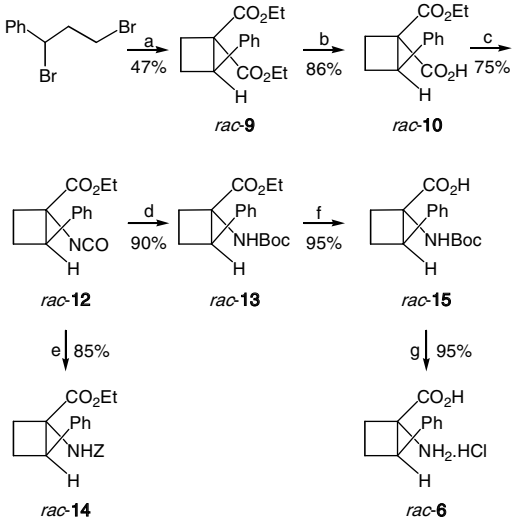
Once the cyclobutane precursor *rac*-**9** had been synthesized, transformation of the ethoxycarbonyl groups into the target amino and carboxylic acid groups constituted the second part of this strategy. The synthetic route started with hydrolysis of the ester group in the *trans* position, followed by Curtius rearrangement in order to obtain the methylcarbamate *rac*-**11**. Although the synthesis of this compound was competitive, we obtained only a low yield in the final hydrolysis to give the amino acid, *trans*-c_4Phe. This reaction must be developed in several steps: first saponification of the ester and then hydrolysis of the carbamate. This last step required strong conditions (reflux in hydrochloric acid) and the final chlorohydrate of the amino acid *rac*-**6** was obtained in low yield, probably due to ring opening (Scheme 3).

This problem was solved by applying the Curtius rearrangement to obtain the corresponding isocyanate compound *rac*-**12** and transforming this into a



Scheme 3. Reaction conditions: (a) (i) NaOH, H_2O, reflux; (ii) HCl; (b) (i) PCl_5, Et_2O; (ii) NaN_3, H_2O, acetone; (iii) toluene, reflux; (iv) MeOH, reflux; (c) (i) NaOH, H_2O, reflux; (ii) HCl, reflux.

compound from which nitrogen deprotection would occur under milder conditions, for example, *tert*-butyl carbamate or benzyl carbamate. Isocyanate *rac*-**12** was converted into the corresponding *N*-Boc *rac*-**13** and *N*-Z *rac*-**14** ester compounds in good yields. Compound *rac*-**13** was chosen to continue the synthetic route because better resolutions could be achieved using chiral HPLC (see Section 2.2). Standard saponification conditions were used to transform *rac*-**13** into *N*-Boc-*trans*-c_4Phe *rac*-**15**. Treatment with ethyl acetate saturated in HCl led to the hydrochloride of *trans*-c_4Phe *rac*-**6** in excellent yield (Scheme 4).

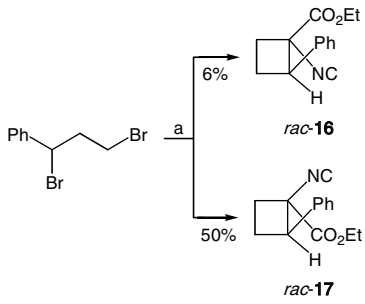


Scheme 4. Reaction conditions: (a) $CH_2(CO_2Et)_2$, NaH, THF, reflux; (b) (i) NaOH, H_2O, reflux; (ii) HCl; (c) (i) PCl_5, Et_2O; (ii) NaN_3, H_2O, acetone; (iii) toluene, reflux; (d) (i) HCl, THF, reflux; (ii) Boc_2O, NEt_3, CH_2Cl_2; (e) (i) HCl, THF, reflux; (ii) ZCl, DIEA, CH_2Cl_2; (f) (i) NaOH, H_2O, reflux; (ii) HCl; (g) 3 N HCl, EtOAc.

On this basis we can report this approach to racemic *trans*-c_4Phe as a facile synthesis that, in six steps, provides the target amino acid in 25% overall yield.

Although cyclic compound *rac*-**9** could also be a precursor of *cis*-c_4Phe, we decided to design a new strategy that was more facile and competitive. Several amino acids,[21] among them 1-aminocyclobutanecarboxylic acid,[14] have been synthesized starting from ethyl isocyanoacetate. The main advantage of this starting material is that the nitrogen atom is already attached to Cα, meaning that a rearrangement reaction would not be necessary.

The double alkylation of ethyl isocyanoacetate with 1,3-dibromo-1-phenylpropane, under conditions reported in the literature,[21] gave a mixture of at least four compounds from which *trans*-isocyanide *rac*-**16** and *cis*-isocyanide *rac*-**17** could be isolated by column chromatography in 6% and 50% yield, respectively (Scheme 5). The relative stereochemistries of the isocyanate esters *rac*-**16** and *rac*-**17** were assigned on the basis of ^{1}H NMR spectroscopy. For compound *rac*-**16**, the *cis*-ethyl protons appear more shielded and thus upfield with respect to *trans*-ethyl protons; this is due to the influence

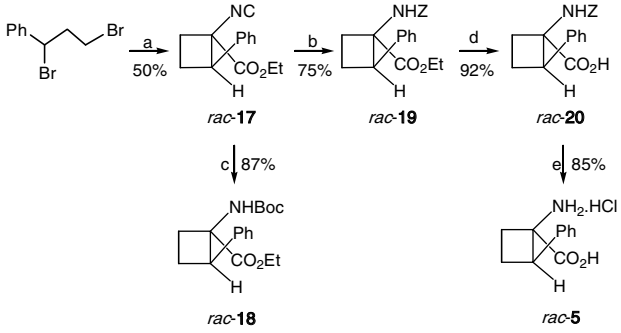


Scheme 5. Reaction conditions: (a) (i) $CNCH_2CO_2Et$, K_2CO_3, Bu_4NHSO_4, MeCN, reflux; (ii) column chromatography.

of the phenyl moiety. In *rac*-**17** the ethyl group appears less shielded and corresponds to a *trans* relative disposition of the ester and phenyl groups.

The corresponding hydrochlorides of the amine esters were obtained by treatment with a few drops of HCl in ethanol. The same conditions as in the case of *trans*-c_4Phe were used to prepare *N*-Boc *rac*-**18** and *N*-Z *rac*-**19** ester compounds of *cis*-c_4Phe. In the case of *cis*-c_4Phe, *rac*-**19** was used to obtain the respective enantiomers by HPLC resolution and we also used this as a precursor for the racemic amino acid (see Section 2.2). In this way *rac*-**19** was used to prepare the N-protected amino acid *N*-Z-*cis*-c_4Phe *rac*-**20**. Deprotection of *rac*-**20** to give *cis*-c_4Phe *rac*-**5** must be controlled carefully to avoid the formation of by-products.

The synthesis of racemic *cis*-c_4Phe can therefore be described as a new route that, in only four steps from 1,3-dibromo-1-phenylpropane and ethyl isocyanoacetate, gives the final amino acid in 30% overall yield (Scheme 6).



Scheme 6. Reaction conditions: (a) (i) $CNCH_2CO_2Et$, K_2CO_3, Bu_4NHSO_4, MeCN, reflux; (ii) column chromatography; (b) (i) HCl, EtOH; (ii) ZCl, DIEA, CH_2Cl_2; (c) (i) HCl, EtOH; (ii) Boc_2O, NEt_3, CH_2Cl_2; (d) (i) NaOH, H_2O, reflux; (ii) HCl; (e) (i) H_2, Pd/C, EtOH; (ii) 3 N HCl, EtOAc.

The *N*-Boc and *N*-Z derivatives of *rac*-**16** were also prepared and were identified as the corresponding compounds *rac*-**13** and *rac*-**14** prepared from *rac*-**9**. In this way we confirmed the relative stereochemistry previously proposed.

2.2. HPLC resolution of *rac*-13 and *rac*-19

In the previous section we described two efficient routes to obtain the target compounds *cis*-c_4Phe and *trans*-c_4Phe in racemic form. In this section we report the isolation of both enantiomers of the amino acids **15** and **20** in enantiomerically pure form. The direct separation of enantiomers by preparative chromatography on chiral stationary phases (CSPs) is nowadays recognized as a powerful tool to obtain enantiopure compounds.[22] In enantioselective liquid chromatography, polysaccharide-derived CSPs from cellulose and amylose are very popular because of their wide applicability and usefulness.[23] Our research group collaborated in the development of new polysaccharide-derived CSPs in order to overcome one of the drawbacks of the former systems: their incompatibility with mobile phases other than hydrocarbons or alcohols, which resulted in swelling or dissolution of the polysaccharide derivative. The main characteristic of these new CSPs is that the mixed polysaccharide derivatives are covalently bonded to an allylsilica gel matrix.[24] This covalent immobilization provides an extremely high stability for these phases in the presence of a wide range of solvents. This synthetic methodology has been extensively studied and reported.[25] Due to the synthetic simplicity of these phases, to their high chemical stability and high selectivity exhibited towards a variety of compounds, these phases are especially suitable for resolutions on a preparative scale—as we have already shown in the preparative enantioseparations of different phenylalanine[7,8b,c,26] and other amino acid[27] surrogates. The recent commercialization of CSPs derived from polysaccharide covalently bonded to the matrix will surely make HPLC resolution even more popular.

Although different non-commercial polysaccharide-derived supports were tested for the resolution of the precursors of *trans*-c_4Phe, *rac*-**13** and *rac*-**14**, and *cis*-c_4Phe, and *rac*-**18** and *rac*-**19**, the only CSP that offered the possibility of separation was the one consisting of mixed 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose covalently attached to allylsilica.[24] A mixture of *n*-hexane/2-propanol was taken as a starting point and different amounts of chloroform and acetone were added to improve the values of the separation and resolution factors. The most representative results are gathered in Table 1.

The precursors of *trans*-c_4Phe, *rac*-**13** and *rac*-**14**, showed satisfactory separation values (Table 1, entries 1 and 5), but these were significantly better for *rac*-**13**—the conditions of this resolution were therefore studied further. Both mixtures of *n*-hexane/2-propanol/acetone and *n*-hexane/2-propanol/chloroform gave similar chromatographic parameters (Table 1, entries 2, 3 and 4). We decided to choose ternary systems with chloroform because reproducibility with this eluent is better than with acetone. The conditions chosen to develop a semi-preparative resolution of *rac*-**13** consisted of a mixture of *n*-hexane/2-propanol/chloroform 95/3/2 (Table 1, entry 3). In order to assess the enantiopurity of the fractions collected we selected other conditions, in which the lower amount of chloroform allowed better UV monitoring (Table 1, entry 2 and Fig. 2).

Separation of *rac*-**18** could not be achieved on the stationary phase derived from cellulose under any chromatographic conditions explored. Resolution of *rac*-**19** was tested with the elution systems previously described. The presence of a certain amount of acetone improved the results (Table 1, entries 8 and 9) and, although chromatographic parameters were comparable to those obtained with chloroform (Table 1, entry 7), retention times and peak widths were more favourable in the former case. Furthermore, the higher ratio of more polar solvents in the mixture (2-propanol and acetone) made these conditions better for a semi-preparative separation due to improved compound solubility. Hence, the best chromatographic system obtained on the CSP derived from the 3,5-dimethylphenylcarbamate of cellulose used a ternary mixture *n*-hexane/2-propanol/acetone 94/4/2 as the eluent (Table 1, entry 9).

Finally, under the conditions described above, the separations of *rac*-**13** and *rac*-**19** were performed at the semi-preparative level. The analytical resolution was scaled-up to the preparative column (150 × 20 mm ID) as described below.

The isolation of *trans*-c_4Phe enantiomers was carried out by HPLC resolution of *rac*-**13** on a 150 × 20 mm ID column filled with the 10-undecenoate/3,5-dimethylphenyl-carbamate of cellulose bonded on allylsilica gel, using a 95/3/2 mixture of *n*-hexane/2-propanol/chloroform as the eluent (flow rate 14 mL/min). Compound

Table 1. Selected chromatographic data for the HPLC resolution of *trans*-c_4Phe and *cis*-c_4Phe precursors on several stationary phases and chromatographic modes

Entry[a]	Compound	Eluent[b] A/B/C/D	Flow rate (mL/min)	λ (nm)	k'_1[c]	α[c]	R_S[c]
1	*rac*-**13**	99/1/0/0	0.7	210	1.13	1.97	6.00
2	*rac*-**13**	97/2/1/0	1	220	1.07	1.85	6.42
3	*rac*-**13**	95/3/2/0	0.7	220	1.35	1.97	4.19
4	*rac*-**13**	97/1/0/2	1	220	1.02	2.02	7.71
5	*rac*-**14**	99/1/0/0	1	210	3.27	1.29	2.08
6	*rac*-**19**	99/1/0/0	1	210	5.64	1.81	4.89
7	*rac*-**19**	97/2/1/0	1	230	2.69	1.97	5.25
8	*rac*-**19**	95/3/0/2	1	230	1.45	1.75	4.86
9	*rac*-**19**	94/4/0/2	0.7	220	1.11	1.86	5.87

[a] Analytical column dimensions: 150 × 4.6 mm ID, injection volume: 5 μL, $c = 5$ mg/mL, samples dissolved in chloroform.
[b] A: *n*-hexane, B: 2-propanol, C: chloroform, D: acetone.
[c] For definition of k' (capacity factor), α (separation factor) and R_S (resolution factor), see Section 4.

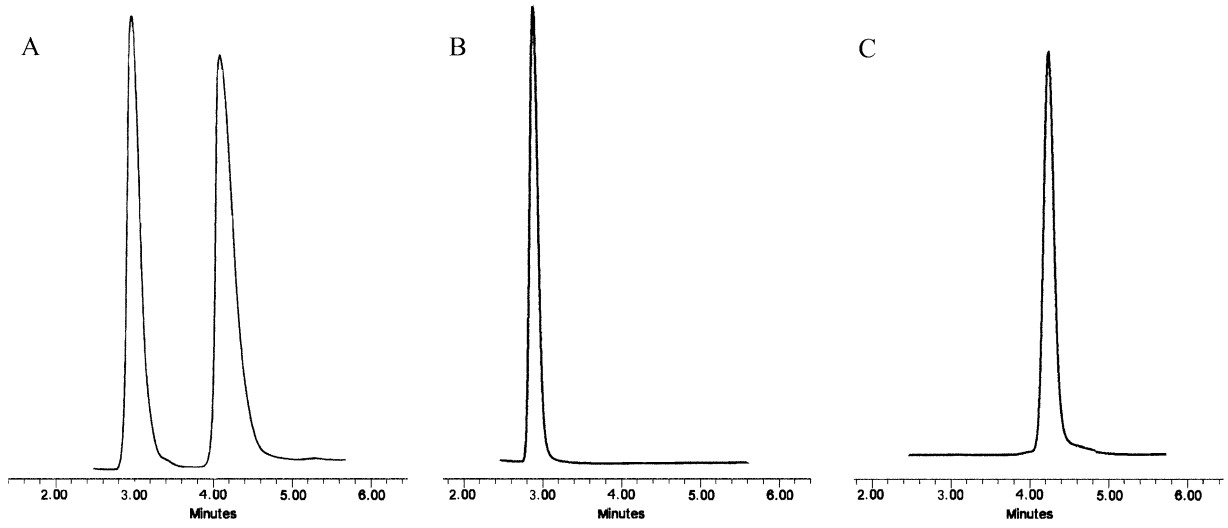


Figure 2. HPLC analytical resolution of *trans*-c$_4$Phe precursor *rac*-**13** (A) and resolved enantiomers (1*S*,2*R*)-**13** (B) and (1*R*,2*S*)-**13** (C). Column: 150 × 4.6 mm ID containing 3,5-dimethylphenylcarbamate of cellulose (CSP-**1**). Eluent: *n*-hexane/2-propanol/chloroform 97/2/1. Flow rate: 1 mL/min. UV detection: 220 nm. Chromatographic parameters: $k'_1 = 1.07$; $\alpha = 1.85$; $R_S = 6.42$.

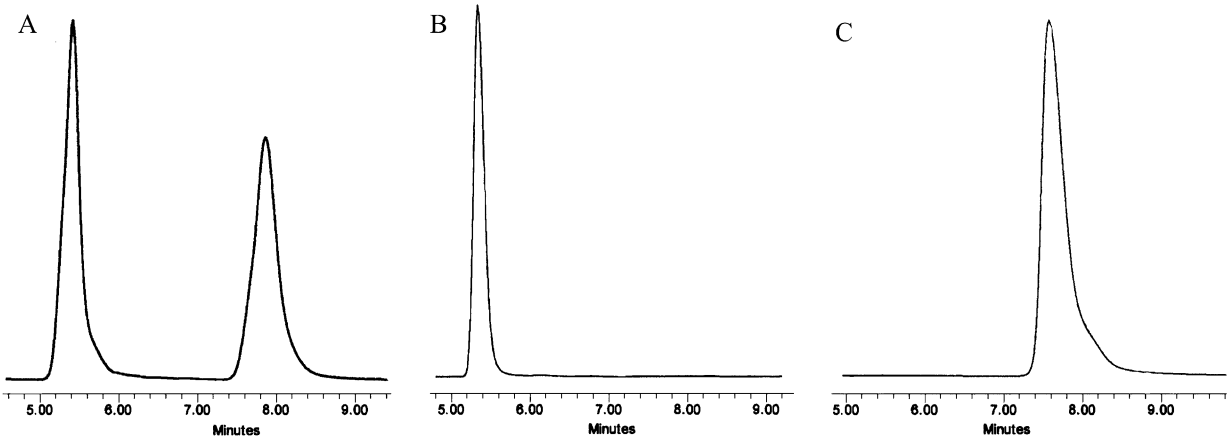


Figure 3. HPLC analytical resolution of *cis*-c$_4$Phe precursor *rac*-**19** (A) and resolved enantiomers (1*R*,2*R*)-**19** (B) and (1*S*,2*S*)-**19** (C). Column: 150 × 4.6 mm ID containing 3,5-dimethylphenylcarbamate of cellulose (CSP-**1**). Eluent: *n*-hexane/2-propanol/acetone 94/4/2. Flow rate: 0.7 mL/min. UV detection: 220 nm. Chromatographic parameters: $k'_1 = 1.11$; $\alpha = 1.86$; $R_S = 5.87$.

rac-**13** (750 mg) was dissolved in chloroform (2.45 mL) and injections of 100 μL were performed approximately every 5 min. The three collected fractions had the following compositions: the first fraction provided 345 mg of the first eluted enantiomer in optically pure form; the second fraction contained 155 mg of a 15/85 mixture of the first and the second eluted enantiomers; the third fraction provided 235 mg of the enantiomerically pure second eluted enantiomer.

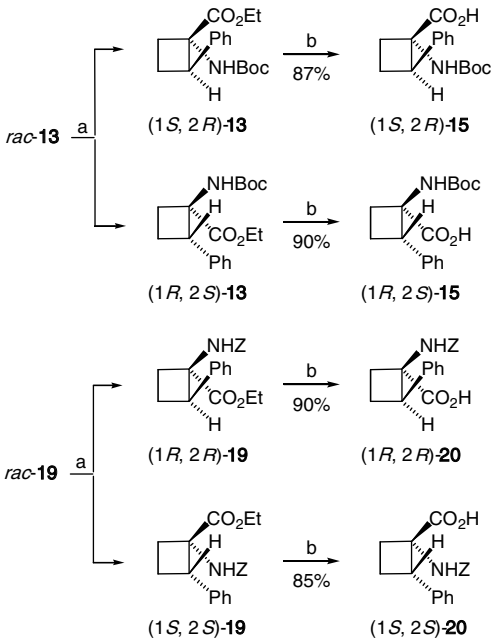
HPLC resolution of rac-**19** was carried out on the same column, but in this case different conditions were employed: a mixture of *n*-hexane/2-propanol/acetone 94/4/2 as eluent at a flow rate of 14 mL/min. Compound rac-**19** (710 mg) was injected as a solution of 300 mg/mL in chloroform (repetitive injections of 200 μL) onto the semi-preparative column and four separate fractions were collected. The first fraction contained 320 mg of the first eluted enantiomer in its enantiomerically pure form. The second fraction (35 mg) contained a 65/35 mixture of the first and the second eluted enantiomers. The third fraction (100 mg) and the last one (235 mg) provided the second eluted enantiomer with high enantiopurities (0.5/99.5 and over 0.1/99.9, respectively).

The enantiomeric purities of the resolved enantiomers of compounds **13** and **19** were quantified at an analytical level (Figs. 2 and 3, respectively), and their absolute configurations were assigned by means of the incorporating the separated enantiomers into dipeptide derivatives.[28]

2.3. Synthesis of *N*-protected amino acids c$_4$Phe in enantiomerically pure form and assignment of absolute configurations

After HPLC resolution of *rac*-**13** and *rac*-**19**, the isolated enantiomers were submitted to basic saponification under the conditions developed for the racemic material. This reaction gave high yields of the enantiopure *N*-protected amino acids, as the *tert*-butylcarbamate for

trans-c$_4$Phe (1*S*,2*R*)-**15** and (1*R*,2*S*)-**15**, and the benzylcarbamate in the case of *cis*-c$_4$Phe (1*R*,2*R*)-**20** and (1*S*,2*S*)-**20** (Scheme 7).



Scheme 7. Synthesis of enantiomerically pure c$_4$Phe derivatives: (a) chiral HPLC resolution; (b) (i) NaOH, H_2O, reflux; (ii) HCl.

At this stage, we had prepared suitable compounds in enantiopure form for further applications of the cyclobutane analogues of phenylalanine, such as their incorporation into a peptide chain.[28]

3. Conclusion

Conformationally restricted phenylalanine analogues *cis*-c$_4$Phe and *trans*-c$_4$Phe have been prepared in their racemic form by means of two different facile and competitive routes based on a cyclization strategy. In the case of *cis*-c$_4$Phe this is a new and rapid synthesis and in the case of the *trans* analogue the reported synthesis[9b] has been improved. For the first time we have developed a strategy for the synthesis of all stereoisomers in enantiomerically pure form of c$_4$Phe: racemic precursors of *cis*-c$_4$Phe and *trans*-c$_4$Phe are resolved by HPLC separation on an easily produced cellulose-derived chiral stationary phase. Moreover, we also prepared the enantiomerically pure restricted amino acids that were appropriately protected for incorporation into peptides, the conformational analysis of which could provide more information about their influence in modulating the β-folding mode.

4. Experimental

4.1. General

All reagents, with the exceptions of diethyl cinnamylmalonate[20] and 1,3-dibromo-1-phenylpropane,[29] were purchased from the Aldrich Chemical Co. (Milwaukee, WI) and used without further purification. Solvents were dried, when necessary, by standard methods. The progress of the reactions was assessed by thin layer chromatography (TLC) on Merck 60 F240 precoated silica gel polyester plates and products were visualized under UV light (254 nm), iodine vapour or ninhydrin reaction as appropriate. Column chromatography was performed using Merck silica gel (40–60 μm). 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 and AV-400 instrument in $CDCl_3$ or D_2O, using the residual solvent signal as the internal standard ([D$_6$]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. HPLC was carried out using a Waters HPLC system equipped with a Waters 600-E pump and a Waters 991 photodiode array detector. The solvents used as HPLC mobile phases were of chromoscan grade. Mixed 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose was prepared and linked to allylsilica gel (Nucleosil 100-5, Machery-Nagel) according to our previously described procedure[24] to give the corresponding CSP. This stationary phase was packed into stainless-steel tubes by the slurry method. The HPLC analytical assays were carried out on 150×4.6 mm ID columns containing this CSP. All analytical assays were performed using mixtures of *n*-hexane/2-propanol, *n*-hexane/2-propanol/chloroform and *n*-hexane/2-propanol/acetone as eluents (flow rate 0.7 and 1 mL/min), with UV monitoring performed at 210, 220 or 230 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 enantiomers, and w_1 and w_2 denote their half-height peak widths; t_0 is the dead time. The semi-preparative HPLC resolution of compounds *rac*-**13** and *rac*-**19** were carried out on a 150×20 mm ID column filled with the previous CSP.

4.2. Synthesis of diethyl 2-(3-phenylpropyl)malonate 7

A solution of diethyl cinnamylmalonate (1.38 g, 5 mmol) in EtOH (7 mL) was hydrogenated at room temperature for 15 h with 10% palladium–carbon (140 mg). Removal of the catalyst and the solvent quantitatively gave the required compound **7** (1.35 g, 4.9 mmol, 98% yield). R_f (hexane/EtOAc 8/2) = 0.8. IR (neat) 1729.8 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.18 (t, 6H, $J = 7.1$ Hz), 1.53–1.64 (m, 2H), 1.83–1.91 (m, 2H), 2.57 (t, 2H, $J = 7.7$ Hz), 3.27 (t, 1H, $J = 7.5$ Hz), 4.11 (q, 4H, $J = 7.1$ Hz), 7.08–7.27 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.00, 28.28, 29.02, 35.41, 51.83, 61.22, 125.79, 128.27, 141.62, 169.31.

4.3. Synthesis of diethyl 2-(3-bromo-3-phenylpropyl)-malonate *rac*-8

Compound **7** (1.39 g, 5 mmol) was dissolved in anhydrous dichloromethane (40 mL). *N*-bromosuccinimide (987 mg, 5.5 mmol) and benzoyl peroxide (18 mg, 0.05 mmol) were added at room temperature. The mixture was heated under reflux for 15 h. After cooling the solids were filtered off and washed with dichloromethane. The washings were combined, dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by flash column chromatography (eluent: hexane/EtOAc 98/2) to give *rac*-**8** as a slightly yellowish oil (1.61 g, 4.5 mmol, 90% yield). R_f (hexane/EtOAc 8/2) = 0.6. IR (neat) 1728.9, 697.1 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.19 (t, 6H, J = 7.1 Hz), 1.74–1.90 (m, 1H), 1.97–2.28 (m, 3H), 3.28 (t, 1H, J = 7.3 Hz), 4.10–4.27 (m, 4H), 4.87 (dd, 1H, J = 6.6, J = 8.1 Hz), 7.19–7.32 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.95, 27.27, 37.26, 51.05, 54.19, 61.38, 127.11, 128.39, 128.67, 141.43, 168.83.

4.4. Synthesis of diethyl 2-phenylcyclobutane-1,1-dicarboxylate *rac*-9

Method A: To a solution of *rac*-**8** (1.43 g, 4.0 mmol) in anhydrous tetrahydrofuran (30 mL) under an inert atmosphere was added NaH (60% dispersion in mineral oil) (160 mg, 4.0 mmol) at 0 °C. The mixture was strirred at room temperature overnight. The solvent was evaporated and the residue was taken up in ether and treated with ice. The organic layer was washed with water, dried over $MgSO_4$ and the solvent was evaporated. Compound *rac*-**9** was purified by flash column chromatography (eluent: hexane/EtOAc 95/5) and obtained as an oil (938 mg, 3.4 mmol, 85% yield).

Method B: To a solution of diethyl malonate (0.606 mL, 4.0 mmol) in anhydrous tetrahydrofuran (30 mL) under an inert atmosphere was added NaH (60% dispersion in mineral oil) (340 mg, 8.5 mmol) at 0 °C. A solution of 1,3-dibromo-1-phenylpropane (1.11 g, 4.0 mmol) in anhydrous tetrahydrofuran (10 mL) was added to the reaction. The mixture was heated under reflux overnight. The solvent was evaporated and the residue was taken up in ether and treated with ice. The organic layer was washed with water, dried over $MgSO_4$ and the solvent was evaporated. Compound *rac*-**9** was purified by flash column chromatography (eluent: hexane/EtOAc 95/5) to give the product as an oil (467 mg, 1.9 mmol, 47% yield).

R_f (hexane/EtOAc 8/2) = 0.4. IR (neat) 1726.0 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.67 (t, 3H, J = 7.1 Hz), 1.19 (t, 3H, J = 7.1 Hz), 2.03–2.21 (m, 2H), 2.48–2.64 (m, 2H), 3.57 (qd, 1H, J = 7.1, J = 10.7 Hz), 3.69 (qd, 1H, J = 7.1, J = 10.7 Hz), 4.08–4.23 (m, 2H), 4.29 (t, 1H, J = 9.4 Hz), 7.08–7.24 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.51, 14.10, 20.57, 25.61, 44.85, 59.59, 60.89, 61.20, 126.84, 127.74, 127.95, 139.21, 169.38, 171.70.

4.5. Synthesis of *trans*-1-(ethoxycarbonyl)-2-phenylcyclobutanecarboxylic acid *rac*-10

A solution of dicarboxylate *rac*-**9** (1.11 g, 4.0 mmol) in ethanol (3 mL) was stirred and heated under reflux with a solution of NaOH (176 mg, 4.4 mmol) in the minimum amount of water at reflux temperature for 1 d. The excess solvent was evaporated and the residue was taken up in water and washed with ether. The aqueous layer was acidified and extracted with ether. The organic portions were collected, dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by flash column chromatography (eluent: hexane/EtOAc 7/3) to give *rac*-**10** as a white solid (854 mg, 3.4 mmol, 86% yield). Mp 116 °C (EtOAc/hexane). R_f (hexane/EtOAc 8/2) = 0.2. IR (nujol) 3100–2900, 1737.6, 1701.9 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.71 (t, 3H, J = 7.1 Hz), 2.08–2.16 (m, 1H), 2.31–2.39 (m, 1H), 2.54–2.69 (m, 2H), 3.72 (m, 2H), 4.30 (t, 1H, J = 9.4 Hz), 7.11–7.24 (m, 5H), 7.21 (br s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.43, 20.49, 25.62, 45.27, 59.32, 61.38, 127.00, 127.59, 128.02, 138.70, 169.24, 177.46.

4.6. Synthesis of methyl *trans*-1-(ethoxycarbonyl)-2-phenylcyclobutylcarbamate *rac*-11

PCl_5 (208 mg, 1.0 mmol) was added to a solution of *rac*-**10** (248 mg, 1.0 mmol) in anhydrous ether (10 mL) and the reaction mixture was stirred at room temperature for 90 min. The solvent was removed under reduced pressure. The oily residue was dissolved in toluene (10 mL) and the solvent and the residual PCl_5 distilled off in vacuo. The acid chloride was dissolved in acetone (5 mL) and a solution of NaN_3 (117 mg, 1.8 mmol) in water (1 mL) was added. The reaction was stirred for 90 min and evaporation of the solvent gave a residue, which was extracted with toluene. The organic solution was dried and, after filtration, MeOH (4 mL) was added. The solution was stirred at 80 °C for 150 min and the solvent was removed under reduced pressure. Purification of the residue by flash column chromatography (eluent: hexane/EtOAc 9/1) afforded *rac*-**11** as a colourless oil (194 mg, 0.7 mmol, 70% yield). R_f (hexane/EtOAc 8/2) = 0.4. IR (nujol) 3348.8, 1727.9, 1518.7 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz, 60 °C) δ 0.78 (t, 3H, J = 7.0 Hz), 2.13–2.22 (m, 1H), 2.39–2.52 (m, 2H), 2.69–2.91 (m, 1H), 3.64 (s, 3H), 3.74 (q, 2H, J = 7.0 Hz), 4.37 (br s, 1H), 5.88 (br s, 1H), 7.03–7.21 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz, 60 °C) δ 13.59, 18.77, 27.38, 47.42, 51.94, 61.25, 64.78, 126.58, 126.67, 128.06, 139.51, 155.59, 171.73.

4.7. Synthesis of *trans*-1-(ethoxycarbonyl)-2-phenylcyclobutylisocyanate *rac*-12

PCl_5 (208 mg, 1.0 mmol) was added to a solution of *rac*-**10** (248 mg, 1.0 mmol) in anhydrous ether (10 mL) and the reaction mixture was stirred at room temperature for 90 min. The solvent was removed under reduced pressure. The oily residue was dissolved in toluene (10 mL) and the solvent and the residual PCl_5 distilled off in vacuo. The acid chloride was dissolved in acetone (5 mL) and a solution of NaN_3 (117 mg, 1.8 mmol) in

water (1 mL) was added. The reaction was stirred for 90 min and evaporation of the solvent gave a residue, which was extracted with toluene. The organic solution was dried and, after filtration, the solution was stirred at 80 °C for 150 min and the solvent was removed under reduced pressure. Purification of the residue by flash column chromatography (eluent: hexane/EtOAc 15/1) afforded *rac*-**12** as an oil (184 mg, 0.8 mmol, 75% yield). R_f (hexane/EtOAc 8/2) = 0.7. IR (nujol) 2253.4, 1731.8. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.80 (t, 3H, $J = 7.2$ Hz), 2.11 (ddd, 1H, $J = 1.5$, $J = 9.2$, $J = 11.8$ Hz), 2.25 (ddd, 1H, $J = 1.2$, $J = 9.6$, $J = 19.2$ Hz), 2.41–2.57 (m, 2H), 3.73–3.85 (m, 2H), 3.91 (t, 1H, $J = 9.9$ Hz), 7.07–7.24 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.53, 18.87, 30.34, 52.28, 62.05, 66.55, 126.10, 126.43, 127.10, 128.23, 138.27, 170.62.

4.8. Synthesis of *trans*-Boc-c$_4$Phe-OEt *rac*-13

A solution of isocyanate *rac*-**12** (736 mg, 3.0 mmol) in tetrahydrofuran (7 mL) and a solution of 2 N hydrochloric acid (2 mL) were mixed and stirred until complete consumption of the starting material was achieved (TLC monitoring, eluent: hexane/EtOAc 8/2). The solvent was evaporated under reduced pressure. The residue was redissolved in water and then lyophilized. The resulting solid was suspended in dichloromethane (10 mL) and NEt_3 (0.431 mL, 3.1 mmol) was added. After 5 min Boc_2O (990 mg, 4.5 mmol) was added and stirring was continued at room temperature. Further portions of Boc_2O (220 mg, 1.0 mmol) were added every 8 h (three extra portions added). When the reaction was complete, dichloromethane (15 mL) was added and the solution was successively washed with 5% aqueous $KHSO_4$ and saturated brine. The organic layer was dried over $MgSO_4$ and the solvent evaporated to yield a residue, which was purified by flash column chromatography (eluent: hexane/EtOAc, 15:1) to give *rac*-**13** as a white solid (862 mg, 2.7 mmol, 90% yield). Mp 186 °C (EtOAc/hexane). R_f (CH_2Cl_2/EtOAc 8/2) = 0.4. IR (nujol) 3248.5, 3123.2, 1700.0 (b), 1602.6 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, 60 °C) δ 0.80 (t, 3H, $J = 7.1$ Hz), 1.39 (s, 9H), 2.11–2.19 (m, 1H), 2.39–2.49 (m, 1H), 2.53–2.62 (m, 2H), 3.72 (m, 2H), 4.14 (br s, 1H), 5.46 (br s, 1H), 7.03–7.20 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz, 60 °C) δ 13.67, 19.23, 28.43, 30.69, 48.21, 60.98, 65.11, 79.91, 126.78, 126.92, 128.10, 139.51, 154.71, 171.75.

4.9. Resolution of *rac*-13: (1*S*,2*R*)-13 and (1*R*,2*S*)-13

HPLC resolution of a solution of *trans*-racemate *rac*-**13** (750 mg) in $CHCl_3$ (2.45 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/2-propanol/chloroform 95/3/2 as the eluent (flow rate: 14 mL/min). A total of 26 injections was required, with one injection performed every 5 min. Three separate fractions were collected. The first, second and third fractions contained, respectively, 100/0 (345 mg), 15/85 (155 mg) and 0/100 (235 mg) mixtures of (1*S*,2*R*)-**13** and (1*R*,2*S*)-**13**. Spectroscopic data for both enantiomers are the same as those described for *rac*-**13**:

(1*S*,2*R*)-**13**: $[\alpha]_D = +80.9$ (*c* 0.75, $CHCl_3$),

(1*R*,2*S*)-**13**: $[\alpha]_D = -79.6$ (*c* 0.79, $CHCl_3$).

4.10. Synthesis of *trans*-Z-c$_4$Phe-OEt *rac*-14

A solution of isocyanate *rac*-**12** (245 mg, 1.0 mmol) in tetrahydrofuran (5 mL) and a solution of 2 N hydrochloric acid (1 mL) were mixed and stirred until complete consumption of the starting material was achieved (TLC monitoring, eluent: hexane/EtOAc 8/2). The solvent was evaporated under reduced pressure. The residue was redissolved in water and then lyophilized. The resulting solid was suspended in dichloromethane (10 mL) and, after cooling to 0 °C, *N*,*N*-diisopropylethylamine (0.701 mL, 4 mmol) and benzyl chloroformate (0.225 mL, 1.5 mmol) were added and the reaction was stirred at room temperature for 1 d. Dichloromethane (15 mL) was added and the solution was successively washed with 5% aqueous $KHSO_4$, 5% aqueous $NaHCO_3$ and saturated brine. The organic layer was dried over $MgSO_4$ and the solvent evaporated to yield a residue, which was purified by flash column chromatography (eluent: hexane/EtOAc 10/1) to give *rac*-**14** as an oil (300 mg, 0.9 mmol, 85% yield). R_f (hexane/EtOAc 8/2) = 0.6. IR (neat) 3353.6, 3340.1, 1726.9, 1710.6, 1514.8, 1498.4 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, 55 °C) δ 0.77 (t, 3H, $J = 7.1$ Hz), 2.12–2.20 (m, 1H), 2.44 (dt, 1H, $J = 9.6$, $J = 19.5$ Hz), 2.53 (t, 1H, $J = 19.1$ Hz), 2.72 (br s, 1H), 5.09 (s, 2H), 5.83 (br s, 1H), 7.01–7.30 (m, 10H). ^{13}C NMR ($CDCl_3$, 100 MHz, 55 °C) δ 13.59, 19.01, 27.90, 47.98, 61.20, 65.12, 66.65, 126.76, 128.05, 128.09, 128.52, 136.60, 139.45, 155.06, 171.54.

4.11. Synthesis of *trans*-Boc-c$_4$Phe-OH *rac*-15

A solution of NaOH (100 mg, 2.5 mmol) in water was added to a suspension of *rac*-**13** (1.0 mmol, 319 mg) in water (5 mL). The reaction mixture was heated under reflux until complete consumption of the starting material was achieved (TLC monitoring, eluent: hexane/EtOAc 8/2). The mixture was cooled to room temperature and water (10 mL) was added. The aqueous layer was washed with dichloromethane (15 mL), acidified with hydrochloric acid to pH 2–3 and then extracted with dichloromethane (3 × 15 mL). Concentration of the organic layer resulted in the precipitation of *rac*-**15** as a white solid (276 mg, 0.9 mmol, 95%). Mp 186 °C (hexane). R_f (CH_2Cl_2/EtOAc 8/2) = 0.4. IR (nujol) 3400–2500, 3257.2, 1702.8, 1456.9 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz, 55 °C) δ 1.37 (s, 9H), 2.13–2.20 (m, 1H), 2.37–2.51 (m, 2H), 2.60–2.66 (m, 1H), 4.03 (br s, 1H), 5.63 (br s, 1H), 7.07–7.30 (m, 5H), 9.53 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz, 55 °C) δ 19.55, 28.36, 28.75, 48.79, 65.07, 80.78, 127.13, 128.29, 138.80, 155.36, 174.82.

4.11.1. (1*S*,2*R*)-*N*-Boc-c$_4$Phe-OH (1*S*,2*R*)-15. An identical procedure to that described above was applied to transform (1*S*,2*R*)-**13** (192 mg, 0.6 mmol) into (1*S*,2*R*)-

15, which was obtained as an oil (157 mg, 0.5 mmol, 90% yield). $[\alpha]_D = +80.5$ (*c* 0.59, $CHCl_3$). Spectroscopic data are the same as those described for *rac*-**15**.

4.11.2. (1*R*,2*S*)-*N*-Boc-c$_4$Phe-OH (1*R*,2*S*)-15. In a similar way to that described above, starting from (1*R*,2*S*)-**13** (192 mg, 0.6 mmol), (1*R*,2*S*)-**15** was obtained as an oil (152 mg, 0.5 mmol, 87% yield). $[\alpha]_D = -77.7$ (*c* 0.66, $CHCl_3$). Spectroscopic data are the same as those described for *rac*-**15**.

4.12. Synthesis of *trans*-c$_4$Phe hydrochloride rac-6

A solution of *trans*-Boc-c$_4$Phe-OH *rac*-**15** (291 mg, 1 mmol) was treated for 30 min with a solution of ethyl acetate saturated with HCl. The solvent was evaporated and the solid was redissolved in water and lyophilized to give *rac*-**6** as a white solid (216 mg, 0.9 mmol, 95% yield). Mp 186–189 °C (dec). IR (nujol) 3300–2200, 1721.2, 1589.1 cm^{-1}. ^1H NMR (D_2O, 300 MHz) δ 2.34–2.46 (m, 2H), 2.58–2.68 (m, 2H), 4.11 (t, 1H, $J = 8.9$ Hz), 7.29–7.41 (m, 5H). ^{13}C NMR (D_2O, 75 MHz) δ 19.26, 25.94, 47.37, 63.19, 127.08, 127.72, 128.61, 137.17, 171.87.

4.13. Synthesis of *trans*-1-(ethoxycarbonyl)-2-phenylcyclobutylisocyanide *rac*-16 and *cis*-1-(ethoxycarbonyl)-2-phenylcyclobutylisocyanide *rac*-17

To a solution of ethyl isocyanoacetate (1.70 g, 1.64 mL, 15 mmol) in acetonitrile (200 mL) were added potassium carbonate (12.50 g, 90 mmol), tetrabutylammonium hydrogensulfate (1.06 g, 3 mmol) and 1,3-dibromo-1-phenylpropane (4.17 g, 15 mmol). The resulting heterogeneous mixture was heated at 70–80 °C until all of the starting electrophile had been consumed (TLC monitoring, eluent: hexane/EtOAc 8/2, approximately 7 d). The reaction mixture was cooled and filtered through Celite® to remove the salts. The solid material was washed with acetonitrile and the filtrate was evaporated. The residue was taken into ether and washed with water and brine and then dried over $MgSO_4$. The residue was purified by flash column chromatography (eluent: hexane/EtOAc 20/1) to give the *trans*-isomer *rac*-**16** (206 mg, 0.9 mmol, 6% yield) and the *cis*-isomer *rac*-**17** (1.72 g, 7.5 mmol, 50% yield) as oily products.

CAUTION: Ethyl isocyanoacetate is a lachrymator and irritant and must be handled with due care.

4.13.1. *trans*-1-(Ethoxycarbonyl)-2-phenylcyclobutylisocyanide *rac*-16. R_f (hexane/EtOAc 8/2) = 0.6. IR (neat) 2133.9, 1739.5 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 0.77 (t, 3H, $J = 7.1$ Hz), 2.16–2.24 (m, 1H), 2.45–2.60 (m, 2H), 2.69–2.76 (m, 1H), 3.78 (m, 2H), 4.15 (t, 1H, $J = 9.8$ Hz), 7.14–7.30 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.73, 19.14, 30.45, 52.64, 62.03, 63.79, 126.83, 127.65, 128.22, 136.66, 160.04, 166.33.

4.13.2. *cis*-1-(Ethoxycarbonyl)-2-phenylcyclobutylisocyanide *rac*-17. R_f (hexane/EtOAc 8/2) = 0.5. IR (neat) 2133.9, 1741.4 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.28 (t, 3H, $J = 7.1$ Hz), 2.22–2.32 (m, 2H), 2.59–2.69 (m, 1H), 2.71–2.79 (m, 1H), 4.14 (t, 1H, $J = 9.3$ Hz), 4.24 (m, 2H), 7.14–7.33 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.03, 20.96, 30.99, 47.77, 62.78, 66.33, 127.68, 127.80, 128.47, 136.27, 161.25, 167.93.

4.14. Synthesis of *cis*-Boc-c$_4$Phe-OEt *rac*-18

A solution of isonitrile *rac*-**17** (229 mg, 1.0 mmol) in ethanol (7 mL) and a few drops of hydrochloric acid was stirred until complete consumption of the starting material was achieved (TLC monitoring, eluent: hexane/EtOAc 8/2). The solvent was evaporated under reduced pressure. The residue was redissolved in water and then lyophilized.

The resulting solid was suspended in dichloromethane (10 mL) and NEt_3 (0.153 mL, 1.1 mmol) was added. After 5 min, Boc_2O (330 mg, 1.5 mmol) was added and stirring was continued at room temperature. Further portions of Boc_2O (88 mg, 0.4 mmol) were added every 8 h (three additional portions added). When the reaction was complete, dichloromethane (15 mL) was added and the solution was successively washed with 5% aqueous $KHSO_4$ and saturated brine. The organic layer was dried over $MgSO_4$ and the solvent evaporated to yield a residue, which was purified by flash column chromatography (eluent: hexane/EtOAc 15:1) to give *rac*-**18** as a white solid (277 mg, 0.9 mmol, 87% yield). Mp 74 °C (EtOAc/hexane). R_f (hexane/EtOAc 8/2) = 0.4. IR (nujol) 3369.1, 3247.6, 3209.0, 1729.8, 1700.0, 1603.5 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz, 60 °C) δ 1.23 (t, 3H, $J = 7.1$ Hz), 1.26 (s, 9H), 2.11–2.20 (m, 2H), 2.40 (qd, 1H, $J = 9.8$, $J = 11.4$ Hz), 2.80 (td, 1H, $J = 9.0$, $J = 11.8$ Hz), 3.90 (t, 1H, $J = 9.1$ Hz), 4.17 (br q, 2H, $J = 7.1$ Hz), 4.40 (br s, 1H), 7.11–7.29 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz, 60 °C) δ 13.77, 20.50, 28.03, 29.48, 46.25, 61.01, 61.84, 79.70, 127.26, 128.01, 128.56, 136.79, 154.84, 172.99.

4.15. Synthesis of *cis*-Z-c$_4$Phe-OEt *rac*-19

A solution of isonitrile *rac*-**17** (687 mg, 3.0 mmol) in ethanol (7 mL) and a few drops of hydrochloric acid was stirred until complete consumption of the starting material was achieved (TLC monitoring, eluent: hexane/EtOAc 8/2). The solvent was evaporated under reduced pressure. The residue was redissolved in water and then lyophilized.

The resulting solid was suspended in dichloromethane (10 mL) and, after cooling to 0 °C, *N*,*N*-diisopropylethylamine (2.103 mL, 12 mmol) and benzyl chloroformate (0.675 mL, 4.5 mmol) were added and the reaction was stirred at room temperature for 1 d. Dichloromethane (15 mL) was added and the solution was successively washed with 5% aqueous $KHSO_4$, 5% aqueous $NaHCO_3$ and saturated brine. The organic layer was dried over $MgSO_4$ and the solvent evaporated to yield a residue, which was purified by flash column chromatography (eluent: hexane/EtOAc 10/1) to give *rac*-**19** as an oil (795 mg, 2.3 mmol, 75% yield). R_f (hexane/EtOAc 6/

4) = 0.7. IR (neat) 3416.3, 3355.6, 1728.9 (b), 1489.8 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz, 55 °C) δ 1.16 (t, 3H, $J = 6.7$ Hz), 2.12–2.20 (m, 1H), 2.36–2.46 (m, 1H), 2.84 (td, 1H, $J = 9.3$, $J = 12.6$ Hz), 3.92 (t, 1H, $J = 9.0$ Hz), 4.14 (br s, 1H), 4.64 (m, 1H), 4.94 (d, 1H, $J = 12.3$ Hz), 4.89 (d, 1H, $J = 12.3$ Hz), 7.08–7.27 (m, 10H). ^{13}C NMR ($CDCl_3$, 100 MHz, 55 °C) δ 14.10, 20.60, 27.38, 46.51, 61.34, 62.08, 66.69, 127.60, 127.99, 128.05, 128.37, 128.83, 136.40, 136.50, 155.57, 172.71.

4.16. Resolution of *rac*-19: (1*R*,2*R*)-19 and (1*S*,2*S*)-19

HPLC resolution of a solution of *cis*-racemate *rac*-**19** (710 mg) in $CHCl_3$ (2.4 mL) was carried out by successive injections of 0.2 mL on a 150 × 20 mm ID column filled with mixed 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose bonded on allylsilica gel and using a mixture of *n*-hexane/2-propanol/acetone 94/4/2 as the eluent (flow rate: 14 mL/min). A total of 13 injections was required, with one injection performed every 10 min. Four separate fractions were collected. The first, second, third and fourth fractions contained, respectively, 100/0 (320 mg), 65/35 (35 mg), 0.5/99.5 (100 mg) and 0.1/99.9 (235 mg) mixtures of the first and the last eluted enantiomers. Spectroscopic data for both enantiomers are the same as those described for *rac*-**19**:

(1*R*,2*R*)-**19**: $[\alpha]_D = -20.9$ (*c* 0.97, $CHCl_3$),

(1*S*,2*S*)-**19**: $[\alpha]_D = +20.1$ (*c* 1.00, $CHCl_3$).

4.17. Synthesis of *cis*-Z-c_4Phe-OH *rac*-20

A solution of NaOH (100 mg, 2.5 mmol) in water was added to a suspension of *rac*-**19** (1.0 mmol, 353 mg) in water (5 mL). The reaction mixture was heated under reflux until complete consumption of the starting material was achieved (TLC monitoring, eluent: hexane/EtOAc 8/2). The mixture was cooled to room temperature and water (10 mL) was added. The aqueous layer was washed with dichloromethane (15 mL), acidified with hydrochloric acid to pH 2–3 and then extracted with dichloromethane (3 × 15 mL). Concentration of the organic layer resulted in the precipitation of *rac*-**20** as a white solid (299 mg, 0.9 mmol, 92%). Mp 128 °C (hexane). R_f (CH_2Cl_2/EtOAc 8/2) = 0.5. IR (nujol) 3334.3, 1721.2, 1668.1, 1529.3 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz, 55 °C) δ 2.19–2.27 (m, 2H), 2.35–2.48 (m, 1H), 2.82 (td, 1H, $J = 9.3$, $J = 12.6$ Hz), 4.07 (t, 1H, $J = 8.9$ Hz), 4.80 (br s, 1H), 4.92 (d, 1H, $J = 12.6$ Hz), 4.97 (d, 1H, $J = 12.2$ Hz), 7.13–7.28 (m, 10H), 9.53 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz, 55 °C) δ 20.60, 27.59, 46.10, 61.89, 67.20, 127.79, 128.13, 128.48, 128.94, 136.07, 136.26, 156.16, 177.24.

4.17.1. (1*R*,2*R*)-*N*-Z-c_4Phe-OH (1*R*,2*R*)-20. An identical procedure to that described above was applied to transform (1*R*,2*R*)-**19** (283 mg, 0.8 mmol) into (1*R*,2*R*)-**20**, which was obtained as an oil (234 mg, 0.7 mmol, 90% yield). $[\alpha]_D = -39.8$ (*c* 0.73, $CHCl_3$). Spectroscopic data are the same as those described for *rac*-**20**.

4.17.2. (1*S*,2*S*)-*N*-Z-c_4Phe-OH (1*S*,2*S*)-20. In a similar way to that described above, starting from (1*S*,2*S*)-**19** (247 mg, 0.7 mmol), (1*S*,2*S*)-**20** was obtained as an oil (194 mg, 0.6 mmol, 85% yield). $[\alpha]_D = +40.1$ (*c* 0.86, $CHCl_3$). Spectroscopic data are the same as those described for *rac*-**20**.

4.18. Synthesis of *cis*-c_4Phe hydrochloride *rac*-5

A solution of *cis*-Z-c_4Phe-OH *rac*-**20** (228 mg, 0.7 mmol) in ethanol (7 mL) was treated at room temperature with 10% palladium–carbon (50 mg) in a hydrogen atmosphere. The reaction was carefully controlled by TLC monitoring (eluent: CH_2Cl_2/EtOAc 8/2) to avoid product decomposition. The catalyst was filtered off and the solvent was evaporated. The resulting residue was treated with a solution of ethyl acetate saturated with HCl for 30 min. The solvent was evaporated and the solid was redissolved in water and washed with several additional portions of CH_2Cl_2 and then lyophilized to give *rac*-**5** (135 mg, 0.6 mmol, 85% yield). Mp 158–161 °C (dec). IR (nujol) 3300–2400, 1736.6, 1569.8 cm^{-1}. ^1H NMR (D_2O, 300 MHz) δ 2.12–2.18 (m, 1H), 2.38–2.46 (m, 1H), 2.68–2.78 (m, 1H), 2.83 (td, 1H, $J = 9.2$, 12.6 Hz), 4.36 (t, 1H, $J = 9.5$ Hz), 7.29–7.50 (m, 5H). ^{13}C NMR (D_2O, 75 MHz) δ 18.94, 26.16, 44.77, 62.96, 127.76, 128.26, 129.19, 135.04, 173.25.

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