Synthesis and Preparative Resolution of the *trans*-Cyclohexane Analogues of Phenylalanine

Carlos Cativiela,*[a] Pilar López,*[a] and Marta Lasa[a]

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Different approaches to the synthesis of enantiomerically pure (1*R*,2*S*)- and (1*S*,2*R*)-1-amino-2-phenylcyclohexanecarboxylic acids (*trans*-c_6Phe) through a racemic pathway followed by semi-preparative HPLC of a racemic precursor have been studied. The complete diastereoselectivity of the Strecker reaction and the high efficiency of the subsequent transformations into the amino acid favour this method in comparison to the Diels–Alder route. The relative stereochemistry of the amino acid and its precursors has been unambiguously assigned. The preparation of the final enantiomerically pure amino acids and their corresponding *N*-Boc derivatives was carried out by HPLC resolution of one of the intermediates using a cellulose-derived chiral stationary phase.

Introduction

The incorporation of rigid amino acid surrogates with conformational constraints into peptides with biological activity is a very useful tool for the construction of molecules of pharmaceutical interest.[1−5] In this context, phenylalanine (Phe) is a key amino acid because it is located in the pharmacophoric regions of many bioactive peptides and seems to play an important role in recognition processes, often through hydrophobic contacts.[6−8] Several analogues of phenylalanine with limited conformational flexibility have been developed by different structural modifications[9] in order to increase metabolic stability and elucidate the conformation in which the peptide binds to the receptor. The use of synthetic amino acids with rigidly oriented side chains is an invaluable tool in this investigation.

A specific orientation of the side chains of phenylalanine can be attained by the insertion of an alkylidene bridge between the α- and β-carbons of the amino acid, thus providing a series of 1-amino-2-phenylcycloalkanecarboxylic acids (c_nPhe). Incorporation of this kind of amino acid into peptides induces folded secondary structures that are typically observed in cyclic α,α-disubstituted glycines.[10] Such structures allow the influence of the restricted side-chain mobility on the peptide conformations to be studied.

As part of our investigations[11−15] into the conformational preferences of this family of amino acids in model dipeptides RCO-L-Pro-c_nPhe-NHR′, we found that the *cis*-cyclohexane surrogates of phenylalanine, (1*R*,2*R*)-c_6Phe and (1*S*,2*S*)-c_6Phe, have a stabilising effect on the β-turn structure.[13,14] Conformational analysis of the *cis*-c_6Phe-containing dipeptides in the solid state[13] and in solution[14] provides evidence of the importance of these amino acids in modulating the β-folding mode — (1*R*,2*R*)-c_6Phe shows a clear preference for the type II β-turn, whereas the (1*S*,2*S*) enantiomer greatly stabilises the βI turn. In addition, the conformational tendencies of *cis*-c_6Phe were assessed by computational methods.[16] In a similar way, the corresponding counterpart of these *cis* surrogates — the *trans* derivatives (1*R*,2*S*)-c_6Phe and (1*S*,2*R*)-c_6Phe — were also studied theoretically.[16] In this case, the results of the calculations were not conclusive and the two computational methods used showed significant discrepancies. In spite of the interest of this structural study, the lack of experimental data did not allow the theoretical results to be corroborated or contradicted, because the RCO-L-Pro-*trans*-c_6Phe-NHR′ dipeptides needed for the conformational analysis had not been synthesised.

Both (1*R*,2*S*)- and (1*S*,2*R*)-c_6Phe are available through asymmetric Diels−Alder reactions[17] using chiral cyanocinnamates derived from (*R*)-pantolactone and (*S*)-ethyl lactate, respectively, as dienophiles. However, when both enantiomers are needed it may be more convenient to perform a racemic synthesis followed by a resolution procedure. This strategy allows the use of only one starting product for obtaining both enantiomers and, moreover, avoids the duplication of the synthetic steps.

From a racemic point of view, the synthesis of differently substituted 1-aminocyclohexanecarboxylic acids has been performed from the Bücherer hydantoins or, sometimes, by the Strecker reaction.[18] The structural assignments of

[a] Departamento de Química Orgánica, ICMA, Universidad de Zaragoza-CSIC,
50009 Zaragoza, Spain
Fax: + 34-976-761-210
E-mail: pilopez@unizar.es; cativiela@unizar.es

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cyclic amino acids synthesised by these routes have been, to say the least, controversial. In particular, 1-amino-2-phenylcyclohexanecarboxylic acid was described for the first time by a Bücherer–Bergs synthesis.[19] In this case a single spot was observed in the TLC for the obtained hydantoin and, hence, after hydrolysis, only one isomer of c_6Phe with unknown relative *cis*/*trans* stereochemistry was reported by the authors. Later, the Strecker reaction and further hydrolysis of nitrile were applied to the synthesis of c_6Phe.[20] In this case the authors assumed (but didn't prove) the relative configuration of the amino acid as opposite to that found from the Bücherer synthesis, in a similar manner to that described in the synthesis of related 2-alkyl-1-aminocyclohexanecarboxylic acids.[18] The melting point was the only described physical characteristic of c_6Phe obtained by both the Bücherer–Bergs and Strecker routes.[19,20] Different attempts to determine the relative stereochemistry on the basis of NMR studies of several derivatives[21] or precursors[22] led to contradictory assignments, therefore the synthesis of *trans*-c_6Phe by Strecker or related reactions remains so far uncertain.

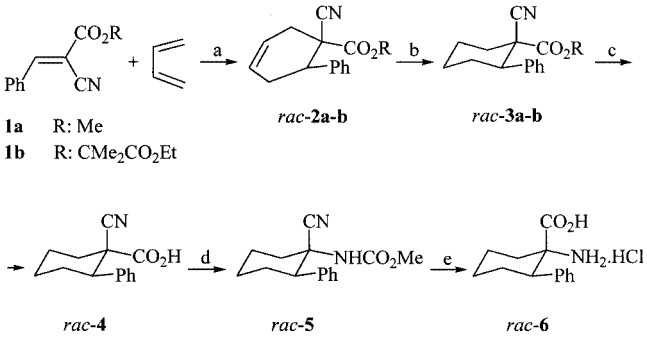
To the best of our knowledge, another route to racemic *trans*-c_6Phe where the relative configuration has been undoubtedly and specifically determined has not been reported in the literature. Therefore we would like to report here an efficient synthesis of unequivocally assigned *trans*-c_6Phe in racemic form through a racemic Diels–Alder or Strecker synthesis. The preparation of enantiomerically pure (1*R*,2*S*)- and (1*S*,2*R*)-c_6Phe was achieved from the racemate by HPLC using a polysaccharide-derived chiral stationary phase. The molecular structure of one of the resolved enantiomers has been obtained by X-ray diffraction analysis. Given that the main application of the desired products is their incorporation into peptides, we also synthesised the enantiopure *N*-Boc amino acids, which are suitably protected for this purpose.

Results and Discussion

Synthesis of Racemic *trans*-c_6Phe

Two alternative synthetic routes to obtain the racemic *trans*-c_6Phe were developed. Firstly, given our wide experience in this area, we considered the Diels–Alder reaction between 1,3-butadiene and the appropriate dienophile as the key step in the synthesis. As we reported previously, the excellent dienophilic nature of unsaturated 5(4*H*)-oxazolones allowed the synthesis of racemic *cis*-c_6Phe using (*Z*)-2-phenyl-4-benzylidene-5(4*H*)-oxazolone as the starting material.[23,24] However, the synthesis of racemic *trans*-c_6Phe employing the corresponding (*E*)-oxazolone as a dienophile could not be achieved due to isomerization between the *Z* and *E* isomers. The less stable *E* compound readily isomerizes to the more stable (*Z*)-oxazolone in the presence of a Lewis acid, the required catalyst for the Diels–Alder reaction, and a mixture of the corresponding adducts of both dienophiles is obtained.[25,26] The use of solid catalysts, such as silica gel treated with $TiCl_4$ or $ZnCl_2$ supported on silica gel, promoted the Diels–Alder reaction of cyclopentadiene with (*E*)-2-phenyl-4-benzylidene-5(4*H*)-oxazolone in very high yields without isomerization.[27] Unfortunately, reaction between the (*E*)-oxazolone and 1,3-butadiene, a much less reactive diene than cyclopentadiene, could not be achieved.

As an alternative to the use of unsaturated 5(4*H*)-oxazolones as dienophiles in Diels–Alder reactions, (*E*)-2-cyanocinnamates have proved to be excellent precursors for amino acids.[28–30,17] In an attempt to obtain *trans*-c_6Phe in its racemic form, we carried out the Diels–Alder reaction between 1,3-butadiene and two achiral (*E*)-2-cyanocinnamic esters — methyl ester **1a** and the ester of ethyl 2-hydroxy-2-methylpropionate **1b** (Scheme 1). In the latter case, it was expected that a seven-membered chelate complex between the dienophile and the catalyst would be formed, a situation in agreement with the model for chelate complexes proposed and confirmed by Helmchen[31] to explain the results obtained in the cycloadditions of acrylates of (*S*)-ethyl lactate. The formation of structurally different dienophile–catalyst complexes is a function of the Lewis acid used in the Diels–Alder reaction and this factor determines the course of the reaction. For this reason the behaviour of $TiCl_4$ and $AlCl_2Et$ as cycloaddition catalysts was investigated.



Scheme 1. Synthesis of racemic *trans*-c_6Phe by Diels–Alder reaction: (a) Lewis acid (see Table 1), CH_2Cl_2, 0 °C; (b) H_2/ Pd/C, EtOH; (c) (i) KOH, EtOH, reflux; (ii) 12 N HCl; (d) (i) PCl_5, toluene; (ii) NaN_3, acetone; (iii) toluene, MeOH, reflux; (e) 12 N HCl, reflux

Compound **1b** was prepared according to the previously reported procedure for methyl (*E*)-2-cyano-3-phenylpropanoate (**1a**),[32] and dienophiles **1a** and **1b** were both reacted with 1,3-butadiene under several sets of conditions (Table 1). Our previous studies on the Diels–Alder reaction between chiral (*E*)-2-cyanocinnamates and 1,3-butadiene have shown that in the absence of a catalyst the reaction does not occur and good conversions could only be obtained using equimolar quantities of Lewis acids.[17] For this reason, all reactions studied were performed in the presence of a Lewis acid with a catalyst/dienophile ratio of one.

In the case of dienophile **1a** (R = Me), $TiCl_4$ was not effective even when the **1a**/diene ratio was increased to 1:10 (entries 1 and 2) and reaction was only observed when $AlCl_2Et$ was employed as the catalyst, in this case leading to

Table 1. Results obtained in the reaction of (*E*)-2-cyanocinnamates **1a,b** with 1,3-butadiene[a]

Entry	Dienophile	**1**:Diene ratio	Catalyst	*t* (h)	Yield[b] [%]
1	**1a**[c]	1:5	$TiCl_4$	72	–
2	**1a**[c]	1:10	$TiCl_4$	30	–
3	**1a**	1:5	$AlCl_2Et$	72	76[d]
4	**1b**	1:5	$TiCl_4$	72	78[d]
5	**1b**	1:5	$AlCl_2Et$	72	33[e]

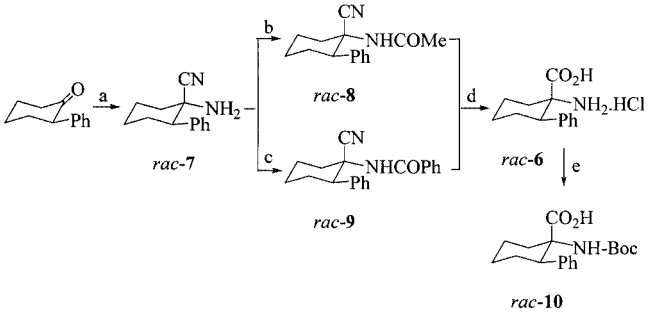
[a] All reactions were carried out in dry CH_2Cl_2 under an inert atmosphere at 0 °C, with one equivalent of Lewis acid. [b] Yield or conversion percentage. [c] Decomposition of the dienophile without formation of cycloadducts was observed. [d] Products isolated by column chromatography. [e] Conversion percentage determined by ^{1}H NMR spectroscopy.

the adduct *rac*-**2a** in very good yield (76% isolated product, entry 3). The impossibility of forming a chelate complex with $TiCl_4$ could account for the unsuccessful behaviour of this catalyst with **1a**. Conversely, the very good result obtained for **1b** [R = $C(CH_3)_2CO_2Et$] with $TiCl_4$ (78% yield of isolated *rac*-**2b**, entry 4) confirms, as expected, the formation of a chelate complex. In the case of this latter dienophile, the use of $AlCl_2Et$ as the catalyst led only to a moderate result (33% conversion, entry 5), probably due to the higher degree of steric hindrance of the **1b**–catalyst complex. Bearing in mind the simple preparation of dienophile **1a** and the good result obtained with this dienophile in conjunction with $AlCl_2Et$, **1a** was selected as the starting material for the synthesis of *trans*-c_6Phe by Diels–Alder reaction.

The cycloadducts *rac*-**2a,b** were hydrogenated over palladium-carbon to give cyclohexane derivatives *rac*-**3a,b** (Scheme 1). These compounds were saponified with 10% KOH/ethanol to afford *trans*-1-cyano-2-phenylcyclohexanecarboxylic acid (*rac*-**4**). Curtius rearrangement of the corresponding acyl azide led to cyano carbamate derivative *rac*-**5**, which was finally hydrolysed to give the hydrochloride of *trans*-c_6Phe (*rac*-**6**). When the precursor *rac*-**5** was subjected to hydrolysis with concentrated hydrochloric acid under reflux, a mixture of two products was formed under all conditions tested (long reaction times, addition of acetic acid or isopropyl alcohol to increase the solubility of the starting material). One of these products was confirmed as the desired *trans*-c_6Phe hydrochloride *rac*-**6** and the second one appeared to be the hydrochloride of *trans*-1-cyano-2-phenylcyclohexylamine, which would result from the partial hydrolysis of *rac*-**5**. The hydrochloride of *trans*-c_6Phe *rac*-**6** was thus obtained in 65% yield, a result that unfortunately could not be improved.

As mentioned in the introduction, an alternative way to obtain both diastereomers of c_6Phe in its racemic form involves a Strecker or similar reaction starting from 2-phenylcyclohexanone. Different attempts carried out many years ago to synthesise the racemic target compound by this route were not conclusive with respect to the *cis*/*trans* stereochemistry of the amino acid formed, probably due to the precarious characterisation techniques employed at that time.[19,20] More recently, synthesis of some enantiomerically pure *cis*- and *trans*-2-substituted 1-aminocyclohexanecarboxylic acids, constrained analogues of isoleucines[33] and threonines,[34] has been described by means of asymmetric Strecker reactions between 2-substituted cyclohexanones and a chiral amine. To the best of our knowledge, the reaction of 2-phenylcyclohexanone has not been reported.

Taking into account that the Strecker reaction seems to be a good route to obtain our target amino acid and that the stereochemistry of this synthetic procedure remains undetermined, we were encouraged to employ it to obtain *trans*-c_6Phe in a diastereomerically pure form. Treatment of 2-phenylcyclohexanone with NaCN and NH_4Cl under the classical conditions reported in the literature[35] afforded mixtures of *cis*- and *trans*-1-cyano-2-phenylcyclohexylamine in which the *trans* isomer *rac*-**7** was the major component (Scheme 2). The diastereomeric *cis*/*trans* ratio in these mixtures was approximately 1:5. The reaction did not go to completion, however, and unchanged 2-phenylcyclohexanone was detected by ^{1}H NMR spectroscopy. Longer reaction times and the use of an excess of both inorganic salts led to an increase in the amount of *trans* isomer, which was the only product when the reaction was carried out with a ratio of 2-phenylcyclohexanone/NaCN/NH_4Cl of 1:2:2 after stirring for seven days. It therefore seems that *trans*-1-cyano-2-phenylcyclohexylamine (*rac*-**7**) is the thermodynamically favoured isomer. This assumption was confirmed when it was observed that solutions of the less stable *cis* isomer and a very small quantity of 2-phenylcyclohexanone in $CHCl_3$ evolved to mixtures of *cis* and *trans* isomers.



Scheme 2. Synthesis of racemic *trans*-c_6Phe and racemic *N*-Boc *trans*-c_6Phe by Strecker reaction: (a) NaCN, NH_4Cl, *i*PrOH, NH_4OH; (b) AcCl, NEt_3, CH_2Cl_2; (c) BzCl, NEt_3, CH_2Cl_2; (d) 12 N HCl, reflux; (e) TMAH, acetonitrile, Boc_2O

The relative stereochemistry of the major isomer, a *trans* disposition between the phenyl and amino group (Scheme 2), was initially assigned on the basis of ^{1}H NMR spectroscopy, taking into account that the deshielding effect of the triple bond of the cyano group causes the benzylic proton of the *cis* derivative to resonate at lower field than the corresponding protons in the *trans* isomer.

All attempts at direct hydrolysis of the amino nitrile *rac*-**7** were unsuccessful: the cyano group remained unchanged

within the limits of detection upon treatment with concentrated hydrochloric acid under reflux and the reaction led only to the starting material *rac*-**7** as the corresponding hydrochloride. Addition of acetic acid or isopropyl alcohol and long reaction times led to mixtures of *rac*-**6** and the hydrochloride of *rac*-**7**. This result is not surprising given the results of the study of the hydrolysis reaction of cyano carbamate *rac*-**5** described above. In fact, the spectroscopic data of the hydrochloride of *rac*-**7** were the same as those obtained for the by-product formed on acid hydrolysis of cyano carbamate *rac*-**5**.

This unusual resistance to hydrolysis could be due to the presence, in the most energetically favourable chair form, of the large phenyl group in the equatorial position of the cyclohexane ring and *gauche* to the axial nitrile, which leaves this group in a highly hindered environment. Other authors have also found this behaviour in the hydrolysis of the Strecker product of some 2-substituted cyclohexanones.[18,22] With the aim of increasing the reactivity of the cyano group by modifying the conformational equilibrium and the solubility of the starting material, two different amides of the amino nitrile *rac*-**7** were synthesised. Acetamide *rac*-**8** and benzamide *rac*-**9** were obtained from compound *rac*-**7** under standard conditions; these reactions gave excellent yields (Scheme 2). The hydrolysis of both compounds by refluxing in concentrated HCl or 2-propanol/12 N HCl mixtures resulted in quantitative transformation into the *trans*-c_6Phe hydrochloride *rac*-**6**.

The relative stereochemistry of the amino nitrile *rac*-**7** was unequivocally assigned by treating the amino acid hydrochloride *rac*-**6** (obtained after hydrolysis) with propylene oxide under reflux in order to obtain the free *trans*-c_6Phe. Comparison of the spectroscopic data for the free amino acid with those reported in the literature[17] definitively showed a *trans* disposition between the phenyl and amino groups. This unequivocal assignment allowed confirmation of the relative stereochemistry of the major isomer of the amino nitrile *rac*-**7**, which had previously been proposed on the basis of ^{1}H NMR chemical shifts. Moreover, the controversy about the assignment of the relative stereochemistry in the Strecker reaction of 2-phenylcylohexanone is solved: this route leads to *trans*-c_6Phe, in agreement with Sacripante's configurational assignments.[22]

An efficient route to the target compound *trans*-c_6Phe in its racemic form has therefore been developed (three synthetic steps from the commercially available 2-phenylcyclohexanone, 77% overall yield), and this racemic compound can now be directly used in the synthesis of diastereomeric peptides, although for this purpose the amino acids must be conveniently protected.

In order to obtain the *N*-Boc-protected derivative *rac*-**10** (Scheme 2) it was necessary to study methods for the subsequent introduction of Boc protection on the amino function. However, the sterically hindered nature of $C^{\alpha,\alpha}$-disubstituted amino acids means that problems are often encountered in protecting their amino group. Indeed, the use of standard conditions [NaOH, dioxane, H_2O and *tert*-butyl dicarbonate (Boc_2O)] requires reaction times that are much longer than those needed for natural amino acids and the yields are lower. This lack of reactivity is probably due to the aqueous conditions and the slow course of the reaction, a situation that leads to hydrolysis of the Boc_2O before it can react with the sterically hindered amino acid. These difficulties can be overcome by working in a polar aprotic solvent with a lipophilic base (tetramethylammonium hydroxide, TMAH), which enhances the amino acid salt's solubility in the organic solvent.[36] This methodology (TMAH, acetonitrile, Boc_2O) was used in several different attempts to protect the amino group of *trans*-c_6Phe. The solubility of the starting material proved to be critical for the reaction yield: when the tetramethylammonium salt was not completely dissolved, the reaction proceeded very slowly and gave a very poor yield. Because of the low solubility of the starting material in acetonitrile (even working at 50 °C), it was not possible to perform the *N*-protection directly starting from the *trans*-c_6Phe hydrochloride *rac*-**6** — prior liberation of the free amino acid was necessary. Even when using these conditions, free *trans*-c_6Phe could only be dissolved with mild heating of the reaction mixture (TMAH, acetonitrile). Once a homogeneous solution had been obtained, reaction with *tert*-butyl dicarbonate could be carried out. *N*-Protection progressed with continuous feeding of Boc_2O and the *N*-Boc derivative *rac*-**10** was obtained in 73% yield after a reaction time of two days.

HPLC Analytical Resolution of *trans*-c_6Phe Derivatives

Once an efficient route to the target compound had been developed, we undertook the preparation of both enantiomers of the amino acid *trans*-c_6Phe in optically 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.[37] CSPs derived from cellulose and amylose are extremely popular and their utility in enantioselective liquid chromatography is well documented.[38−42] Nevertheless, these phases have one major drawback and this is their incompatibility with mobile phases other than hydrocarbons or alcohols: the polysaccharide derivative, which is coated onto a silica matrix, is swollen or dissolved by strong solvents such as chloroform, tetrahydrofuran or ethyl acetate, among others.[41,43] This phenomenon considerably reduces the choice of mobile phase, thus limiting the possibility of increasing selectivity and of improving the solubility of the racemate. This last factor is critical in preparative chromatography since it strongly limits the loading capacity of the column and therefore the amount of sample that can be separated by injection.[43−45]

Different attempts have been described to overcome this problem by covalently fixing the chiral selector to a matrix.[46] Our research group has collaborated in the development of new polysaccharide-derived CSPs where mixed polysaccharide derivatives are covalently bonded to an allylsilica gel matrix.[47,48] This covalent immobilisation results in an extremely high stability for these phases in the presence of a wide range of solvents, including all the solvents men-

tioned above.[49] Although these phases are not yet commercially available, the synthetic methodology has been extensively studied and reported.[50] Due to their synthetic simplicity and to their high stability and selectivity, this kind of phase has been used in different separations at analytical level[51−54] and also on a preparative scale.[55] In particular, the efficiency of this system has been demonstrated in the preparative enantioseparation of different phenylalanine surrogates.[24,56−59]

The HPLC analytical resolution of derivatives *rac*-**7**−**9** was tested on four columns containing silica-bonded phases derived from different mixed polysaccharides: 10-undecenoate/3,5-dimethylphenylcarbamate of cellulose (CSP-**1**), 10-undecenoate/phenylcarbamate of cellulose (CSP-**2**), 10-undecenoate/3,5-dimethylphenylcarbamate of amylose (CSP-**3**), 10-undecenoate/4-methylbenzoate of cellulose (CSP-**4**). Mixtures of *n*-hexane/2-propanol and *n*-hexane/ethanol were tested as eluents (flow rate 0.8−1 mL/min), with UV monitoring performed at 210 nm. Table 2 contains the most representative results. None of the *trans*-c_6Phe precursors could be resolved on CSP-**4**. Compound *rac*-**7** could not be separated under any chromatographic conditions explored, while compound *rac*-**9** was resolved on the three systems CSP-**1**, -**2** and -**3** (see Table 2, entries 4, 6 and 7). CSP-**1** was also able to distinguish between the two enantiomers of *rac*-**8** (entry 1). The best analytical chromatographic separation achieved was that involving benzamide derivative *rac*-**9** on CSP-**1** (derived from the 3,5-dimethylphenylcarbamate of cellulose; entry 4), which gave the highest values for the separation factor α and resolution R_s.

Table 2. Selected chromatographic data for the HPLC resolution of *trans*-c_6Phe precursors on several stationary phases and chromatographic modes[a]

Entry	Compound	CSP	Eluent A/B[b]	λ (nm)	k'_1 [c]	α[c]	R_s[c]
1	*rac*-**8**[d]	CSP-**1**	93:7	210	1.53	1.24	1.95
2	*rac*-**8**[e]	CSP-**1**	93:7	230	1.29	1.17	0.95
3	*rac*-**8**[f]	CSP-**1**	93:7	235	1.24	1.13	0.68
4	*rac*-**9**[d]	CSP-**1**	95:5	210	1.51	1.64	3.56
5	*rac*-**9**[e]	CSP-**1**	95:5	250	1.79	1.48	2.49
6	*rac*-**9**[d]	CSP-**2**	98:2	210	2.36	1.39	0.99
7	*rac*-**9**[d]	CSP-**3**	97:3	210	1.79	1.37	2.02

[a] Column dimensions: 150 × 4.6 mm ID, flow rate: 1 mL/min, injection volume: 5 μL, samples dissolved in chloroform. [b] A: *n*-hexane, B: *i*PrOH. [c] For the definition of k' (capacity factor), α (separation factor) and R_s (resolution factor), see Exp. Sect. [d] c = 5 mg/mL. [e] Overload mode, c = 100 mg/mL. [f] Overload mode, c = 500 mg/mL.

Although the mechanism for chiral discrimination on CSPs derived from arylcarbamates of polysaccharides remains ambiguous, the interaction between the carbamate residues of the chiral selector with the racemates is thought to be the most important factor for effective enantiodiscrimination.[41,42]. In such a case, hydrogen bonding and dipole−dipole interactions between selector and solute involving the N−H and C=O groups of the carbamate would be expected to play a significant role in the chiral resolution, as would additional π-π interactions involving the aryl groups of the carbamate residue and the aromatic groups of the solute. This hypothesis could account for the results obtained in the separation of compounds *rac*-**8** and *rac*-**9**: benzamide derivative *rac*-**9** has an N−H amide bond that is more polar than that in acetamide compound *rac*-**8**, and also has an additional phenyl group near the amide group. Therefore, its interaction with the chiral selector should be stronger and its resolution higher (compare entries 4 and 1 in Table 2).

HPLC Semi-Preparative Resolution of Optically Pure (1*R*,2*S*)-8 and (1*S*,2*R*)-8

The column saturation capacity (W_s) was calculated working in an overload mode with the analytical column (150 × 4.6 mm ID) by injecting increasing amounts of compound. Once we determined W_s we could scale-up the analytical resolution to the preparative column. When a concentrated sample of benzamide *rac*-**9** (c = 100 mg/mL) was injected into the analytical column, values of α and R_s were still very favourable for an efficient separation (Table 2, entry 5). Unfortunately, compound *rac*-**9** is only sparingly soluble, even in chloroform, and more concentrated solutions could not be prepared. Thus, despite the good chromatographic parameters found for the separation, the maximum permitted concentration of derivative *rac*-**9** led to a very low loading capacity in the semi-preparative column (10 mg). The acetamide derivative *rac*-**8** was therefore selected as the *trans*-c_6Phe precursor to be resolved into its enantiomers by chiral HPLC. Although the enantiomers had a poorer separation, their higher solubility allowed more concentrated samples to be injected (up to a concentration of 500 mg/mL, entry 7) and a loading capacity of 50 mg was obtained.

Semi-preparative resolution of acetamide *rac*-**8** was performed working in repetitive injection mode on a 150 × 20 mm ID column. The enantiomeric fractionation was achieved in two steps. In the first purification step, 1.1 g of product was dissolved in 2.2 mL of chloroform and injected at intervals of 12 min (28 injections of 0.1 mL) onto the semi-preparative column. Each run was collected into three separate fractions. The first and last fractions contained, respectively, 400 mg of the less strongly retained enantiomer in its enantiomerically pure form and 330 mg of the more strongly retained enantiomer with 97% *ee*. The need for a second chromatographic step could not be avoided, since all attempts at crystallisation of the partially resolved material failed to increase the enantiopurity. The final purification step involved reinjecting the fraction enriched in the second enantiomer and the new fractions were collected in such a way that optically pure enantiomer was obtained (210 mg). The optical purity of the resolved enantiomers was assessed at the analytical level, as shown in the HPLC chromatograms of Figure 1.

At this stage, the first eluted enantiomer of **8** gave single crystals suitable for X-ray diffraction analysis, which allowed us to confirm the *trans* relative configuration of the phenyl and acetamido groups (Figure 2).

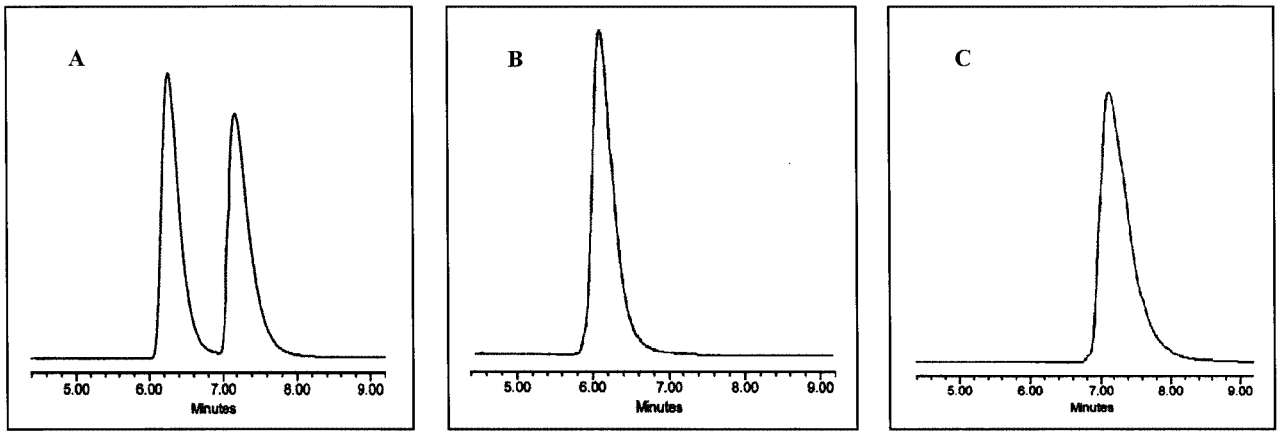


Figure 1. HPLC analytical resolution of acetamide *rac*-**8** (A) and resolved enantiomers (1*R*,2*S*)-**8** (B) and (1*S*,2*R*)-**8** (C): column: 150 × 4.6 mm ID containing 3,5-dimethylphenylcarbamate of cellulose (CSP-**1**); eluent: *n*-hexane/2-propanol (93:7); flow rate: 1 mL/min; UV detection: 210 nm; chromatographic parameters: $k'_1 = 1.53$; $\alpha = 1.24$; $R_S = 1.95$

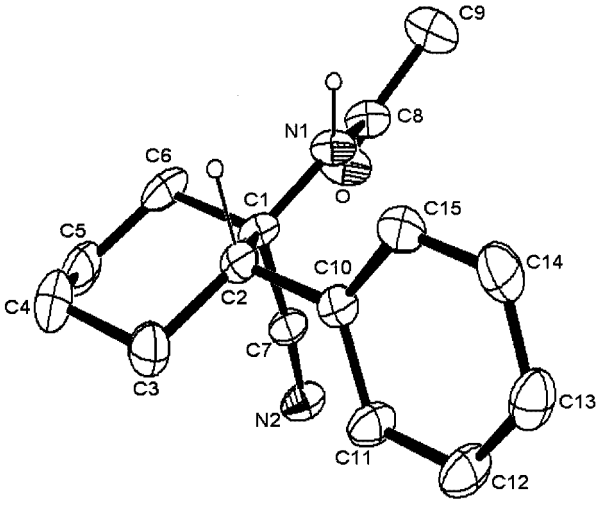
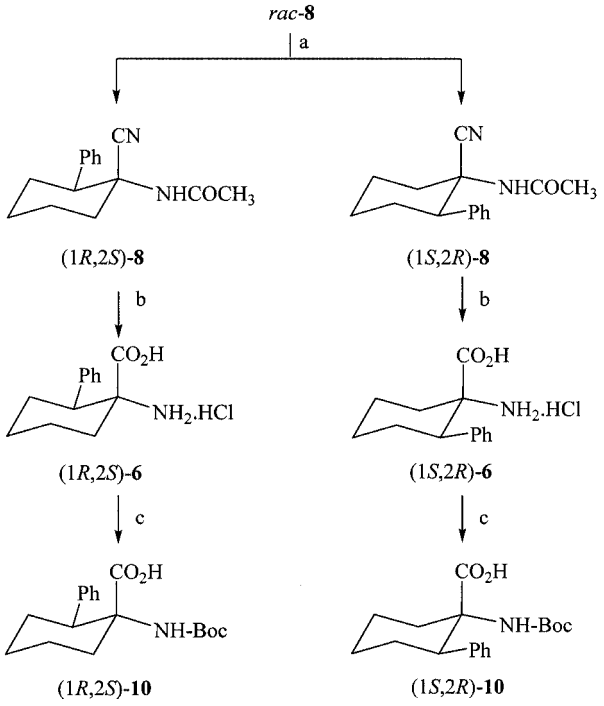


Figure 2. X-ray diffraction structure of (1*R*,2*S*)-**8** with atom numbering; hydrogen atoms, with the exception of the NH and benzylic protons, are omitted for clarity



Scheme 3. Synthesis of enantiomerically pure *trans*-c_6Phe derivatives: (a) chiral HPLC resolution; (b) 12 N HCl, reflux; (c) TMAH, acetonitrile, Boc_2O

Isolation of Enantiomerically Pure (1*R*,2*S*)-6 and (1*S*,2*R*)-6 and Assignment of Absolute Configuration − Preparation of Optically Pure *N*-Boc Derivatives (1*R*,2*S*)-10 and (1*S*,2*R*)-10

After HPLC resolution of *rac*-**8**, 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*S*)-**6** and (1*S*,2*R*)-**6** (Scheme 3).

A small quantity of each of these products was treated with propylene oxide under reflux in order to obtain the free amino acids for identification purposes. Thus, the absolute configuration of both optically pure enantiomers of derivative **8** was determined by comparing the optical rotations of the corresponding free amino acids with the values reported in the literature.[17] This allowed us to assign a (1*R*,2*S*) configuration to the first eluted enantiomer of **8** and a (1*S*,2*R*) stereochemistry to the more strongly retained enantiomer and, likewise, the absolute stereochemistry of their derived amino acids.

This procedure (racemic synthesis followed by chiral HPLC separation) is a valuable tool when both enantiomers are needed in an enantiopure form as, even without optimisation (reinjection of the enantioenriched second fraction was not attempted in the present work, but it can be done it if necessary), chiral resolution led to 36% yield of (1*R*,2*S*)-**8** and 20% of (1*S*,2*R*)-**8** from the racemic mixture. Taking into account the overall yield of the racemic synthesis, this means that, starting from 10 mmol of commercial 2-phenylcyclohexanone, 2.5 mmol of hydrochloride

amino acid (1*R*,2*S*)-**6** and 1.5 mmol of (1*S*,2*R*)-**6** are obtained. The alternative asymmetric procedure described in the introduction[17] led, in five steps, to 2.1 mmol of (1*R*,2*S*)-**6** and 2.3 mmol of (1*S*,2*R*)-**6** starting, respectively, from 5 mmol of noncommercial chiral cyanocinnamates derived from (*R*)-pantolactone or (*S*)-ethyl lactate as dienophiles. Both routes are competitive and their results comparable, and we can choose one of them as a function of our necessities: a specific chiral dienophile if our interest is to obtain only one enantiomer or the racemic synthesis to obtain both enantiomers starting from a commercial nonchiral precursor.

The enantiopure *N*-Boc amino acids (1*R*,2*S*)-**10** and (1*S*,2*R*)-**10** were synthesised from the corresponding amino acid hydrochlorides (1*R*,2*S*)-**6** and (1*S*,2*R*)-**6** under the conditions previously developed for the racemic material (Scheme 3). It is worth noting that the solubilities of the tetramethylammonium salts of the enantiopure amino acids are higher than that of the racemic material and, as a consequence, *N*-protection was easier.

Conclusion

We have developed a strategy for the synthesis of both enantiomers of *trans*-c_6Phe, a conformationally restricted phenylalanine analogue. Starting from readily available substrates and through high-yield transformations, a racemic precursor has been prepared and subjected to HPLC resolution on a noncommercial cellulose-derived chiral stationary phase. The enantiopure amino acids obtained in this way were appropriately protected for incorporation into peptides.

Experimental Section

General Remarks: All reagents 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 checked by thin layer chromatography (TLC) on Merck 60 F240 precoated silica gel polyester plates and 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 SMP-20 apparatus and were not corrected. IR spectra were registered on a Mattson Genesis FTIR spectrophotometer; $\tilde{\nu}_{max}$ 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 $CDCl_3$ 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. 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, 10-undecenoate/phenylcarbamate of cellulose, 10-undecenoate/3,5-dimethylphenylcarbamate of amylose and 10-undecenoate/4-methylbenzoate of cellulose were prepared and linked to allylsilica gel (Nucleosil 100−5, Machery-Nagel) according to our previously described procedure[47,48] to give CSP-**1**, CSP-**2**, CSP-**3** and CSP-**4**, 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/2-propanol or *n*-hexane/ethanol as eluents (flow rate 0.8−1 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 semi-preparative HPLC resolution of compound **8** was carried out on a 150 × 20 mm ID column filled with CSP-**1**. A mixture of *n*-hexane/2-propanol (93:7) was used as the eluent with a flow rate of 18 mL/min. UV detection was performed at 235 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 with the analytical 150 × 4.6 mm ID column by injecting increasing amounts of sample at different concentrations, as indicated in Table 2. In this way, the capacity of the semi-preparative column was established as 50 mg and the optimum concentration of the sample as 500 mg/mL.

(1-Carboxyethyl-1-methyl)ethyl Cyanoacetate: Under an inert atmosphere, PCl_5 (2.08 g, 10 mmol) was added in small portions to a solution of cyanoacetic acid (0.85 g, 10 mmol) in anhydrous diethyl ether (5 mL). After complete dissolution of the PCl_5 (20 min approximately), the solvent was evaporated under vacuum. The resulting acid chloride is very air-sensitive and must be used as soon as possible. The acid chloride was added to a mixture of ethyl 2-hydroxy-2-methylpropionate (0.7 mL, 5 mmol), AgCN (1 g, 7.5 mmol) and anhydrous toluene (20 mL). The reaction mixture was heated under reflux for 8 h and, after cooling, the solvent was evaporated under reduced pressure. CH_2Cl_2 was added and the insoluble residue was filtered off. The solvent was removed and the product purified by column chromatography to give an oil (0.7 g, 3.5 mmol, 70% yield). R_f (CH_2Cl_2/EtOAc, 8:2) = 0.82. IR (neat): $\tilde{\nu}$ = 1745.27, 2263.07 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.23 (t, J = 7.2 Hz, 3 H), 1.57 (s, 6 H), 3.45 (s, 2 H), 4.17 (q, J = 7.2 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 13.78, 24.19, 24.75, 61.55, 80.90, 112.78, 161.88, 171.15 ppm.

(1-Carboxyethyl-1-methyl)ethyl (*E*)-2-Cyanocinnamate (1b): Benzaldehyde (0.61 mL, 6 mmol) was added dropwise to a mixture of (1-carboxyethyl-1-methyl)ethyl cyanoacetate (1.025 g, 5.15 mmol), ammonium acetate (403 mg, 5.15 mmol) and acetic acid (0.44 mL, 7.18 mmol) and the mixture was placed in a flask equipped with an automatic water separator. The reaction was heated under reflux for 15 h and, after cooling, the solvent was evaporated. The product was purified by column chromatography (hexane/EtOAc, 5:1). Cyanocinnamate **1b** was obtained as a colourless oil (1.3 g, 4.53 mmol, 88% yield) that crystallised upon standing as a white solid. M.p. 51 °C. R_f (hexane/EtOAc, 8:2) = 0.33. IR (nujol): $\tilde{\nu}$ = 2222.57, 1739.48, 1717.31, 1597.74 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.25 (t, J = 7.0 Hz, 3 H), 1.67 (s, 6 H), 4.21 (q, J = 7.0 Hz, 2 H), 7.45−7.55 (m, 3 H), 7.95−7.98 (m, 2 H), 8.20 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 14.05, 24.51, 61.62, 80.75, 102.82, 115.27, 129.30, 131.16, 131.39, 133.46, 155.47,

161.26, 171.67 ppm. $C_{16}H_{17}NO_4$: calcd. C 66.89, H 5.96, N 4.88; found C 66.70, H 5.94, N 4.85.

Methyl *trans*-1-Cyano-6-phenyl-3-cyclohexenecarboxylate (*rac*-2a): Under an inert atmosphere, 5 mL of a 1 M solution of $AlEtCl_2$ in CH_2Cl_2 (5 mmol) was added to a solution of methyl (*E*)-2-cyanocinnamate (935 mg, 5 mmol) in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 1 h, the solution was cooled to 0 °C and a solution of 1,3-butadiene (1.35 g, 2.18 mL, 25 mmol) in dry CH_2Cl_2 (2 mL) was added. The reaction mixture was stirred for 72 h at 0 °C and was then quenched by the addition of $Na_2CO_3{\cdot}10H_2O$. The filtrate was evaporated to give a yellow oil, which was purified by column chromatography. The cycloadduct *rac*-**2a** was obtained as a colourless oil (929 mg, 3.85 mmol, 77% yield). R_f (hexane/EtOAc, 8:2) = 0.36. IR (neat): $\tilde{\nu}$ = 3033.49, 2243.78, 1742.38, 1657.52 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ = 2.30−2.38 (m, 1 H), 2.51−2.61 (m, 1 H), 2.63−2.76 (m, 1 H), 2.85−2.92 (m, 1 H), 3.16 (dd, *J* = 5.0, *J* = 11.6 Hz, 1 H), 3.36 (s, 3 H), 5.65−5.70 (m, 1 H), 5.85−5.89 (m, 1 H), 7.17−7.30 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 29.77, 34.98, 45.69, 49.39, 52.99, 118.07, 121.61, 127.23, 127.91, 128.00, 128.55, 138.78, 168.92 ppm.

(1-Carboxyethyl-1-methyl)ethyl *trans*-1-Cyano-6-phenyl-3-cyclohexenecarboxylate (*rac*-2b): Under an inert atmosphere, 1 mL of a 1 M solution of $TiCl_4$ in CH_2Cl_2 (1 mmol) was added to a solution of (1-carboxyethyl-1-methyl)ethyl (*E*)-2-cyanocinnamate (287 mg, 1 mmol) in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 1 h, the solution was cooled to 0 °C and a solution of 1,3-butadiene (540 mg, 0.87 mL, 10 mmol) in dry CH_2Cl_2 (1 mL) was added. The reaction mixture was stirred for 72 h at 0 °C and was then quenched by the addition of $Na_2CO_3{\cdot}10H_2O$. The filtrate was evaporated to give a yellow oil, which was purified by column chromatography. The cycloadduct *rac*-**2b** was obtained as a white solid (264 mg, 0.78 mmol, 78% yield). M.p. 85 °C (EtOAc/hexane). R_f (hexane/EtOAc, 8:2) = 0.58. IR (nujol): $\tilde{\nu}$ = 2240.89, 1736.59, 1655.59 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ = 1.08 (s, 3 H), 1.14 (t, *J* = 7.00 Hz, 3 H), 1.28 (s, 3 H), 2.33−2.42 (m, 1 H), 2.62−2.74 (m, 1 H), 2.81−2.89 (m, 1 H), 3.22 (dd, *J* = 5.5, *J* = 11.8 Hz, 1 H), 3.95−4.15 (m, 2 H), 5.70−5.76 (m, 1 H), 5.89−5.94 (m, 1 H), 7.23−7.30 (m, 3 H), 7.37−7.40 (m, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 13.79, 22.72, 24.46, 30.55, 35.27, 45.21, 48.71, 61.18, 80.21, 117.98, 121.55, 127.33, 127.76, 128.20, 128.35, 129.09, 129.38, 138.89, 167.01, 171.16 ppm. $C_{20}H_{23}NO_4$: calcd. C 70.36, H 6.79, N 4.10; found C 70.34, H 6.58, N 4.20.

Methyl *trans*-1-Cyano-2-phenylcyclohexanecarboxylate (*rac*-3a): A solution of *rac*-**2a** (1.206 g, 5 mmol) in EtOH (40 mL) was hydrogenated at room temperature in the presence of 10% palladium/carbon (300 mg) until completion of the reaction (1 day). The catalyst was filtered off and the solvent evaporated to afford *rac*-**3a** as a white solid in quantitative yield. M.p. 70 °C (CH_2Cl_2/hexane). R_f (hexane/EtOAc, 8:2) = 0.38. IR (nujol): $\tilde{\nu}$ = 2241.85, 1734.66 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ = 1.40−1.56 (m, 1 H), 1.70−2.22 (m, 7 H), 3.05 (dd, *J* = 3.3, *J* = 12.9 Hz, 1 H), 3.51 (s, 3 H), 7.24−7.30 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 21.83, 25.43, 28.67, 34.81, 49.07, 52.98, 53.28, 118.00, 127.86, 127.94, 128.49, 139.77, 169.38 ppm. $C_{15}H_{17}NO_2$: calcd. C 74.05, H 7.04, N 5.76; found C 74.23, H 7.01, N 5.78.

(1-Carboxyethyl-1-methyl)ethyl *trans*-1-Cyano-2-phenylcyclohexanecarboxylate (*rac*-3b): A solution of *rac*-**2b** (341 mg, 1 mmol) in EtOH (10 mL) was hydrogenated at room temperature in the presence of 10% palladium/carbon (85 mg) until completion of the reaction (1 day). The catalyst was filtered off and the solvent evaporated to give *rac*-**3b** as a white solid (306 mg, 0.89 mmol, 89% yield). M.p. 73 °C (CH_2Cl_2/hexane). R_f (hexane/EtOAc, 8:2) = 0.57. IR (nujol): $\tilde{\nu}$ = 2237.03, 1739.48 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ = 1.15 (t, *J* = 7.3 Hz, 3 H), 1.23 (s, 3 H), 1.35 (s, 3 H), 1.72−1.99 (m, 7 H), 2.22−2.29 (m, 1 H), 3.05 (dd, *J* = 3.3, *J* = 12.6 Hz, 1 H), 3.97−4.16 (m, 2 H), 7.20−7.30 (m, 3 H), 7.33−7.36 (m, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 13.96, 21.85, 23.09, 24.92, 25.43, 29.15, 35.21, 48.57, 52.72, 61.37, 80.34, 118.00, 127.73, 128.36, 128.43, 139.76, 167.56, 171.50 ppm. $C_{20}H_{25}NO_4$: calcd. C 69.95, H 7.34, N 4.08; found C 69.72, H 7.30, N 4.09.

***trans*-1-Cyano-2-phenylcyclohexanecarboxylic Acid (*rac*-4):** Methyl ester *rac*-**2a** (973 mg, 4 mmol) was heated under reflux with 10% KOH/EtOH (50 mL) for 15 h. The solvent was evaporated to dryness and the residue was taken up in water (25 mL) and washed with CH_2Cl_2 (3 × 25 mL). The aqueous layer was acidified by addition of 12 N HCl and the product extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were dried and the solvent removed to give pure acid *rac*-**4** as a white solid (770 mg, 3.36 mmol, 84% yield). M.p. 170−171 °C (CH_2Cl_2/hexane). IR (nujol): $\tilde{\nu}$ = 3140.52, 2257.28, 1747.20 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ = 1.41−1.56 (m, 1 H), 1.71−2.15 (m, 6 H), 2.21−2.29 (m, 1 H), 3.05 (dd, *J* = 3.3, *J* = 12.9 Hz, 1 H), 7.25−7.33 (m, 5 H), 9.02 (br. s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 21.85, 25.38, 28.87, 34.94, 48.43, 53.52, 117.55, 127.95, 128.04, 128.59, 139.52, 173.90 ppm. $C_{14}H_{15}NO_2$: calcd. C 73.34, H 6.59, N 6.11; found C 73.15, H 6.14, N 6.07.

Methyl *trans*-1-Cyano-2-phenylcyclohexylcarbamate (*rac*-5): PCl_5 (0.21 g, 1 mmol) was added to a solution of carboxylic acid *rac*-**4** (229 mg, 1 mmol) in dry Et_2O (10 mL). The reaction mixture was stirred at room temperature for 90 min. The solvent and most of the PCl_5 was removed under reduced pressure. In order to complete the removal of the residual PCl_5, toluene was added and then removed under reduced pressure (3 × 5 mL). The acid chloride was dissolved in acetone (3 mL) and a solution of NaN_3 (114 mg, 1.75 mmol) in water (1 mL) was added. The reaction mixture was stirred at room temperature for 90 min. The solvent was removed and the residue was extracted with toluene. The organic solution was dried over $MgSO_4$ and, after filtration, MeOH (4 mL) was added. The reaction mixture was stirred at 80 °C for 2 h and the solvent was removed under reduced pressure to afford a white solid (212 mg, 0.82 mmol, 82% yield). M.p. 145−6 °C (CH_2Cl_2/hexane). R_f (hexane/EtOAc, 8:2) = 0.18. IR (nujol): $\tilde{\nu}$ = 3346.50, 2237.30, 1726.16 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ = 1.36−1.49 (m, 1 H), 1.57−1.69 (m, 1 H), 1.73−1.95 (m, 4 H), 2.06−2.21 (m, 1 H), 2.71 (dd, *J* = 3.1, *J* = 12.7 Hz, 1 H), 3.01−3.10 (m, 1 H), 3.58 (s, 3 H), 4.84 (br. s, 1 H), 7.31−7.44 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 22.72, 25.48, 30.17, 36.77, 51.50, 52.27, 57.14, 118.53, 128.37, 128.48, 129.27, 138.35, 154.76 ppm. $C_{15}H_{18}N_2O_2$: calcd. C 69.74, H 7.02, N 10.84; found C 69.89, H 6.99, N 11.01.

Hydrolysis of Methyl *trans*-1-Cyano-2-phenylcyclohexylcarbamate: A suspension of cyano-carbamate *rac*-**5** (0.5 mmol, 129 mg) in 12N HCl (10 mL) was heated under reflux for 3 d. The solvent was removed under vacuum and the residue partitioned between H_2O and Et_2O. The phases were separated and the aqueous layer was washed with Et_2O (3 × 5 mL) and the solvents evaporated to dryness to afford the amino acid hydrochloride *rac*-**6** and the hydrochloride of *trans*-1-cyano-2-phenylcyclohexylamine in a 65:35 mixture. The ^1H and ^{13}C NMR spectroscopic data of *trans*-c_6Phe hydrochloride were identical to those obtained by the alternative Strecker synthetic pathway. Spectroscopic data for the hydrochloride of *trans*-1-cyano-2-phenylcyclohexylamine: ^1H NMR (D_2O,

300 MHz): δ = 1.34−1.42 (m, 1 H), 1.63−1.72 (m, 1 H), 1.82−2.00 (m, 7 H), 2.33−2.37 (m, 1 H), 2.93−2.97 (m, 1 H), 7.30−7.42 (m, 5 H) ppm. ^{13}C NMR (D_2O, 75 MHz): δ = 22.38, 24.19, 29.39, 35.27, 49.65, 57.48, 116.22, 128.67, 129.13, 129.45, 136.74.

***trans*-1-Cyano-2-phenylcyclohexylamine (*rac*-7):** NH_4Cl (2.675 g, 50 mmol) and NaCN (2.451 g, 50 mmol) were added to a solution of 2-phenylcyclohexanone (4.356 g, 25 mmol) in a mixture of *i*PrOH and 30% NH_4OH (20 mL/25 mL). The mixture was stirred for one week at room temperature. The organic solvent was evaporated under reduced pressure and the liquid residue was extracted with CH_2Cl_2 (3 × 40 mL). The organic layers were combined, dried over $MgSO_4$ and the solvent evaporated to yield a solid, which was purified by column chromatography or recrystallisation to give the amino nitrile *rac*-**7** as a white solid (4.61 g, 23 mmol, 92% yield). M.p. 125 °C (EtOAc/hexane). R_f (CH_2Cl_2/EtOAc, 8:2) = 0.40. IR (nujol): $\tilde{\nu}$ = 3377, 3309, 2217 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.34−1.49 (m, 1 H), 1.60−2.10 (m, 8 H), 2.11−2.18 (m, 1 H), 2.55 (dd, J = 3.3, J = 12.7 Hz, 1 H), 7.28−7.42 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 23.21, 25.70, 29.48, 39.23, 53.05, 56.11, 122.79, 127.86, 128.63, 128.76, 139.37 ppm. $C_{13}H_{16}N_2$: calcd. C 77.96, H 8.05, N 13.99; found C 78.09, H 8.11, N 14.06.

***trans*-*N*-(1-cyano-2-phenylcyclohexyl)acetamide (*rac*-8):** Under an inert atmosphere, NEt_3 (1.515 g, 2.1 mL, 15 mmol) and acetyl chloride (589 mg, 0.53 mL, 7.5 mmol) were added to an ice-cooled solution of amino nitrile *rac*-**7** (1 g, 5 mmol) in dry CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature overnight. The reaction mixture was successively washed with aqueous solutions of 5% $NaHCO_3$, saturated NaCl, 2% H_2SO_4, and saturated NaCl. The organic phase was dried over $MgSO_4$ and filtered. Evaporation of the solvent yielded a solid, which was purified by column chromatography (eluent: CH_2Cl_2/EtOAc/toluene, 10:1:0.1) to afford the acetamide derivative *rac*-**8** as a white solid (1.102 g, 4.55 mmol, 91% yield). M.p. 154 °C (hexane). R_f (CH_2Cl_2/EtOAc, 8:2) = 0.46. IR (nujol): $\tilde{\nu}$ = 3332.41, 1645.95, 1530.24 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.72−1.96 (m, 6 H), 1.80 (s, 3 H), 2.06−2.22 (m, 1 H), 2.90 (dd, J = 2.9, J = 12.5 Hz, 1 H), 2.96−3.04 (m, 1 H), 5.45 (br. s, 1 H), 7.30−7.48 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 22.83, 23.61, 25.43, 30.07, 36.09, 50.48, 57.45, 118.30, 128.30, 129.12, 138.78, 169.48 ppm. $C_{15}H_{18}N_2O$: calcd. C 74.35, H 7.49, N 11.56; found C 74.51, H 6.91, N 11.57.

Resolution of Acetamide *rac*-8: (1*R*,2*S*)- and (1*S*,2*R*)-*N*-(1-Cyano-2-phenylcyclohexyl)acetamide: HPLC resolution of *rac*-**8** (1.1 g) dissolved in $CHCl_3$ (2.2 mL) was carried out by successive injections of 0.1 mL of solution onto 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 and 2-propanol (93:7) as the eluent (flow rate: 18 mL/min). A total of 28 injections was required, with one injection performed every 12 min. Three separate fractions were collected. The first, second and third fractions contained, respectively, 100:0 (400 mg), 27:73 (325 mg) and 1.5:98.5 (330 mg) mixtures of (1*R*,2*S*)-**8** and (1*S*,2*R*)-**8**. Reinjection of the third fraction in a manner similar to that described above led to 220 mg of enantiomerically pure (1*S*,2*R*)-**8**. Optimisation of the resolution procedure by reinjection of the enantioenriched second fraction was not attempted.

(1*R*,2*S*)-(8): M.p. 154 °C (hexane/EtOAc). $[\alpha]_D$ = +109.9 (c = 1.01, $CHCl_3$).

(1*S*,2*R*)-(8): M.p. 155 °C (hexane/EtOAc). $[\alpha]_D$ = −109.1 (c = 1.01, $CHCl_3$).

***trans*-*N*-(1-Cyano-2-phenylcyclohexyl)benzamide (*rac*-9):** Under an inert atmosphere, NEt_3 (303 mg, 0.42 mL, 3 mmol) and benzoyl chloride (211 mg, 0.18 mL, 1.5 mmol) were added to an ice-cooled solution of amino nitrile *rac*-**7** (200 mg, 1 mmol) in dry CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was successively washed with 5% aqueous $NaHCO_3$ and water. The organic phase was dried over $MgSO_4$ and filtered. Evaporation of the solvent yielded a solid, which was purified by column chromatography to afford benzamide *rac*-**9** as a white solid (289 mg, 0.95 mmol, 95% yield). M.p. 183−4 °C (EtOAc/hexane). R_f (hexane/EtOAc, 8:2) = 0.34. IR (nujol): $\tilde{\nu}$ = 3413, 1664, 1525 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.39−1.54 (m, 1 H), 1.72−2.00 (m, 5 H), 2.16−2.31 (m, 1 H), 3.00 (dd, J = 3.2, J = 12.7 Hz, 1 H), 3.15−3.24 (m, 1 H), 6.22 (br. s, 1 H), 7.29−7.50 (m, 10 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 22.81, 25.51, 29.87, 36.25, 51.13, 57.41, 118.23, 126.79, 128.32, 128.58, 128.61, 129.31, 131.97, 133.61, 138.62, 166.50 ppm. $C_{20}H_{20}N_2O$: calcd. C 78.92, H 6.62, N 9.20; found C 78.79, H 6.50, N 9.16.

***trans*-c$_6$Phe Hydrochloride (*rac*-6):** A solution of acetamide *rac*-**8** (242 mg, 1 mmol) or benzamide *rac*-**9** (304 mg, 1 mmol) in 12 N hydrochloric acid (10 mL) was heated under reflux for 1 d. Where necessary, a mixture of *i*PrOH and 12 N hydrochloric acid (3:10) can be used to improve the solubility of the starting material. The solvent was evaporated and the resulting solid was partitioned between CH_2Cl_2 and water. The aqueous phase was washed with three additional portions of CH_2Cl_2 and then lyophilized to give 235 mg of *rac*-**6** (0.92 mmol, 92% yield). M.p. 288−296 °C (dec.). IR (nujol): $\tilde{\nu}$ = 1733.70, 1747.20 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz): δ = 1.28−1.40 (m, 1 H), 1.62−1.72 (m, 3 H), 1.77−1.86 (m, 1 H), 1.88−2.01 (m, 1 H), 2.14−2.23 (m, 1 H), 2.24−2.35 (m, 1 H), 2.81 (dd, J = 3.7, J = 12.9 Hz, 1 H), 7.16−7.22 (m, 2 H), 7.24−7.34 (m, 3 H) ppm. ^{13}C NMR (D_2O, 75 MHz): δ = 21.36, 24.61, 28.51, 33.73, 50.32, 63.55, 128.43, 128.59, 129.25, 138.61, 172.64 ppm.

(1*R*,2*S*)-c$_6$Phe Hydrochloride [(1*R*,2*S*)-6]: In a similar way to that described above, starting from (1*R*,2*S*)-**8** (29 mg, 0.12 mmol), (1*R*,2*S*)-**6** was obtained as a white solid (28 mg, 0.11 mmol, 92% yield). M.p. 304−308 °C (dec.). $[\alpha]_D$ = +23.1 (c = 1.14, 6 M HCl/H_2O). Spectroscopic data are the same as those described for *rac*-**6**.

(1*S*,2*R*)-c$_6$Phe Hydrochloride [(1*S*,2*R*)-6]: An identical procedure to that described above was applied to transform (1*S*,2*R*)-**8** (29 mg, 0.12 mmol) into (1*S*,2*R*)-**6** (30 mg, 0.12 mmol, 98% yield). M.p. 295−302 °C (dec.). $[\alpha]_D$ = −23.6 (c = 2.72, 6 M HCl/H_2O). Spectroscopic data are the same as those described for *rac*-**6**.

***trans*-*N*-Boc-c$_6$Phe (*rac*-10):** A solution of *trans*-c$_6$Phe hydrochloride *rac*-**6** (0.5 mmol, 127 mg) in ethanol (5 mL) and propylene oxide (1.5 mL) was heated under reflux for 1 h. The solvent was then 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 and TMAH (0.5 mmol, 93 mg) were added to CH_3CN (20 mL) and the mixture was stirred at 40−50 °C until a solution had formed. Once complete dissolution was achieved, Boc_2O (0.75 mmol, 164 mg) was added and stirring was continued at room temperature for 2 d. On the third day, a further 0.5 mmol of Boc_2O was added and the same procedure was repeated on the fourth and the fifth days. After 5 days of reaction, the CH_3CN 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 white solid was washed with additional portions of hexane

to give 116 mg (0.36 mmol, 73% yield) of *N*-Boc derivative *rac*-**10**. M.p. 168 °C (EtOAc/hexane). R_f (hexane/EtOAc, 8:2) = 0.35. IR (nujol): $\tilde{\nu}$ = 3399.46, 1722.35, 1676.06 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz, 60 °C): δ = 1.43 (s, 9 H), 1.40−2.00 (m, 5 H), 2.20−2.40 (m, 3 H), 3.43 (dd, J = 3.9, J = 12.3 Hz, 1 H), 5.35 (br. s, 1 H), 7.18−7.28 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 22.60, 25.20, 28.45, 28.69, 33.60, 48.33, 63.49, 79.97, 127.15, 128.25, 128.83, 141.14, 154.74, 176.88 ppm. $C_{18}H_{25}NO_4$: calcd. C 67.69, H 7.89, N 4.39; found C 67.57, H 7.46, N 4.32.

(1*R*,2*S*)-*N*-Boc-c$_6$Phe [(1*R*,2*S*)-10]: In a similar way to that described above, starting from (1*R*,2*S*)-**6** (77 mg, 0.3 mmol), (1*R*,2*S*)-**10** was obtained (48 mg, 0.15 mmol, 50% yield). $[\alpha]_D$ = −51.3 (c = 1.33, $CHCl_3$). Spectroscopic data are the same as those described for *rac*-**10**.

(1*S*,2*R*)-*N*-Boc-c$_6$Phe [(1*S*,2*R*)-10]: An identical procedure to that described above was applied to transform (1*S*,2*R*)-**6** (39 mg, 0.15 mmol) into (1*R*,2*S*)-**10** (22 mg, 0.07 mmol, 46% yield). Spectroscopic data are the same as those described for *rac*-**10**.

X-ray Crystallographic Study: Colourless single crystals of (1*R*,2*S*)-**8** were obtained by slow evaporation of solvent from a hexane/EtOAc solution. Crystal size: 0.25 × 0.55 × 0.50 mm. The X-ray diffraction data were collected at 293(2) K on a Siemens P4 four-circle diffractometer, using graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å). Reflections were measured in the $\omega/2\theta$-scan mode in the θ range 2−25.5°. The structure was solved by direct methods using SHELXS-97 (G. M. Sheldrick, SHELXS-97, University of Göttingen, **1997**) and refinement was performed using SHELXL-97 (G. M. Sheldrick, SHELXL-97, University of Göttingen, **1997**) by the full-matrix least-squares technique with anisotropic thermal factors for heavy atoms. Hydrogen atoms were located by calculations (with the exception of NH and benzylic protons, which were found on the E-map) and affected by an isotropic thermal factor fixed to 1.2-times the U_{eq} of the carrier atom (1.5 for the methyl protons). Crystallographic data for $C_{15}H_{18}N_2O$ (M_r = 242.31): tetragonal $P4_12_12$; a = 9.576(3) Å, b = 9.576(3) Å, c = 31.282(17) Å; Z = 8; d(calcd.) = 1.122 g cm^{-3}; μ = 0.071. Reflections collected/independent: 5984/2655 [R_{int} = 0.1014]; data/parameters: 2655/165; final R_{int} [$I > 2\sigma(I)$]: R_1 = 0.0514, wR_2 = 0.1149; final R_{int} (all data): R_1 = 0.1247, wR_2 = 0.1483.
CCDC-226680 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336-033; E-mail: deposit@ccdc.cam.ac.uk].

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