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Facile synthesis and highly efficient resolution of a constrained cyclopropane analogue of phenylalanine

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Abstract—Enantiomerically pure (2*R*,3*R*)- and (2*S*,3*S*)-1-(*N*-*tert*-butoxycarbonyl)amino-2,3-diphenyl-1-cyclopropanecarboxylic acids have been prepared by HPLC resolution of a racemic precursor. The complete stereoselectivity of the cyclopropanation process, together with the high efficiency of the subsequent transformations and the chromatographic resolution, allowed the preparation of optically pure compounds on a multigram scale. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

The incorporation of rigid amino acid surrogates into bioactive peptides constitutes a very useful approach for the construction of molecules of pharmaceutical interest.[1–3] Reduction of flexibility not only results in retarded enzymatic degradation, but also can lead to enhanced selectivity by preclusion of those conformers that are responsible for undesired bioactivity, i.e. for side effects.

In addition, constrained peptide analogues are of great value in structure–function relationship studies aimed at elucidating the biologically active conformation.[3,4] In these studies, special attention must be paid to the three-dimensional arrangement of the side chain moieties since they are directly involved in peptide-receptor recognition and some of them are strictly required for bioactivity. The presence of constrained amino acids with specifically oriented side chains[4–6] provides very valuable information about the spatial requirements of the side substituents for optimal interaction with the receptor.

Cyclopropane α-amino acids constitute a special class of side chain conformationally constrained residues. The rigid three-membered ring forces the substituents to adopt a well-defined orientation with respect to the peptide backbone, an orientation that is fixed by the stereochemistry at the α and β carbons. Cyclopropane analogues of several proteinogenic amino acids, mainly the phenylalanine derivative (denoted as c_3Phe in Fig. 1), have been inserted into a number of peptides in order to carry out structural[7–14] and biological[12–19] studies.

Very recently, the behaviour of a cyclopropane residue bearing two phenyl groups in a *trans* relative disposition, which can be viewed as a highly restricted phenylalanine surrogate ($c_3$diPhe, Fig. 1), has been explored[20–22] Interestingly, the replacement of phenylalanine with each of the $c_3$diPhe enantiomers in a midsize peptide has led either to the stabilization or to the disruption of the helical conformation adopted by the parent peptide.[20] Moreover, we have shown[22] by X-ray diffraction experiments that a dipeptide incorporating (2*R*,3*R*)$c_3$diPhe adopts a γ-turn,[23] a conformation that is very rarely found in crystalline short linear peptides.

The striking conformational features exhibited by $c_3$diPhe evidences the high potential of this residue for the induction of specific secondary structures. However, the exploitation of this constrained phenylalanine analogue relies firstly in ready accessibility to the enantiomerically pure material. With the aim of obtaining significant amounts of the two enantiomers we envisaged their preparation by combining a racemic synthesis with a resolution procedure. We describe here an efficient and convenient route for the multigram-scale preparation of both enantiomers of $c_3$diPhe in optically

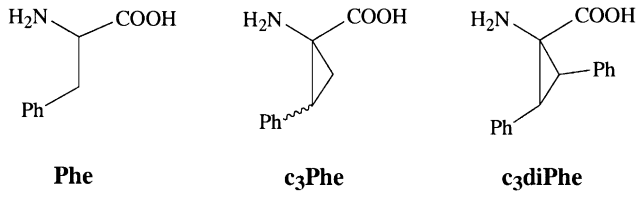

Figure 1. Structure of cyclopropane analogues of phenylalanine.

Keywords: cyclopropaneamino acids; constrained phenylalanines; 1,3-dipolar cycloaddition; unsaturated 5(4*H*)-oxazolone; chiral stationary phase; HPLC resolution.

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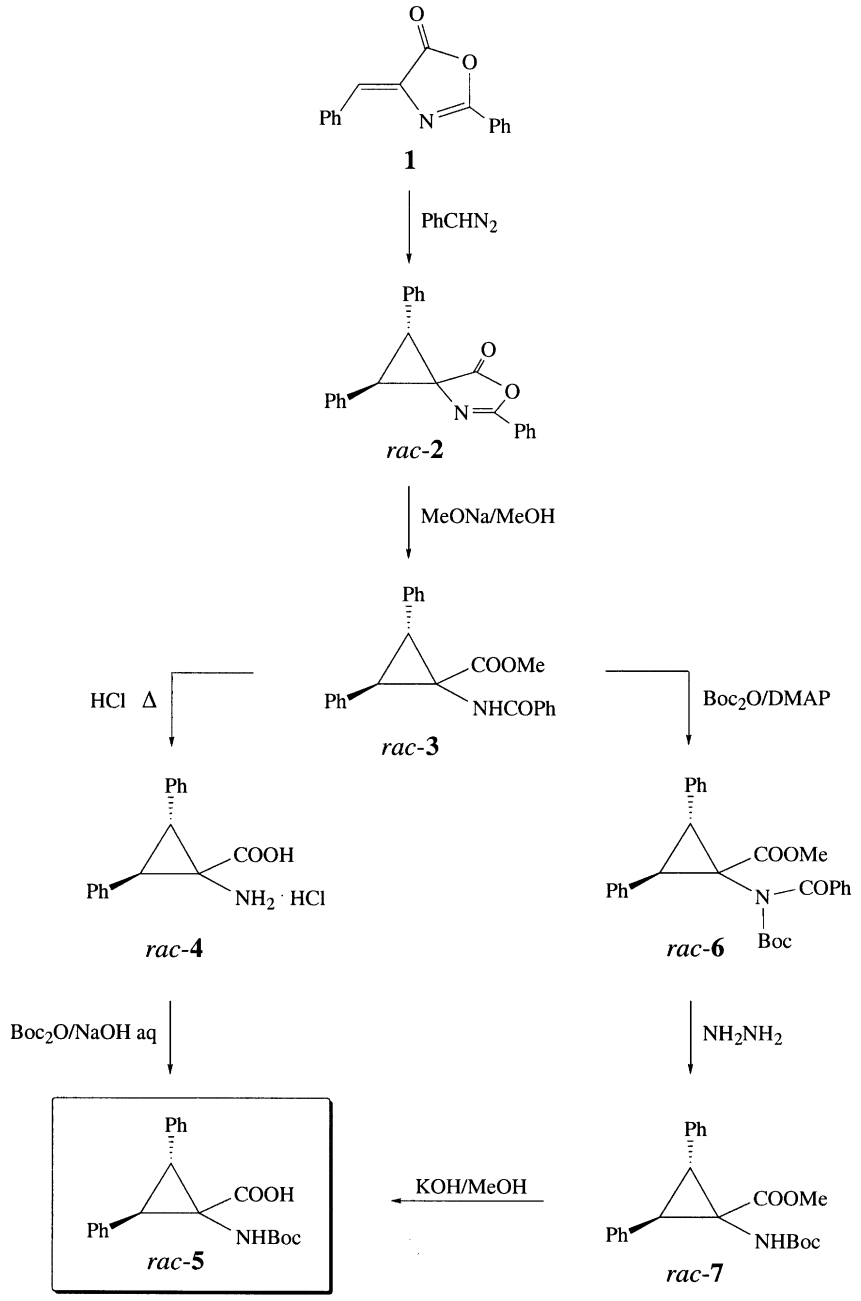
pure form by HPLC resolution of a racemic precursor using a non-commercial polysaccharide-derived chiral stationary phase. Given that the main application for the desired products is their incorporation into peptides, we selected as the target compounds the *N*-Boc amino acids as they are suitably protected for this purpose. To the best of our knowledge, no route to racemic $c_3$diPhe derivatives is available to date, and only one asymmetric synthesis has been described.[20]

2. Results and discussion

2.1. Racemic synthesis

Among the various strategies for the synthesis of cyclopropane α-amino acids, the 1,3-dipolar cycloaddition of diazoalkanes with α,β-dehydroamino acid derivatives has been widely applied.[24–27] In this context, we have described[28] a synthetic route to c_3Phe based on the reaction of phenyldiazomethane with α,β-dehydroalanine protected as an imino ester. Unfortunately, this methodology cannot be extended to the preparation of $c_3$diPhe, since the analogous α,β-dehydrophenylalanine derivative exhibits such a low reactivity that it remains inert even to the action of diazomethane.[29]

The synthesis of $c_3$diPhe following this strategy therefore required a more activated alkene moiety. Unsaturated 5(4*H*)-oxazolones can be viewed as cyclic α,β-dehydroamino acid derivatives and, in fact, their exocyclic double bond has, on numerous occasions, shown excellent



Scheme 1. Synthetic route to racemic $c_3$diPhe derivatives.

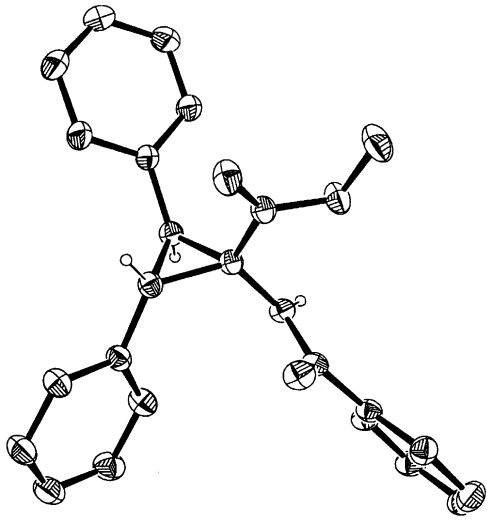


Figure 2. X-Ray molecular structure of *rac*-**3**.

behaviour as a dipolarophile towards diazoalkanes.[24,25,27,30] Thus, several 4-arylmethylene[31–33] and heteromethylene[34–36] derivatives have been reacted with diazomethane to give 2-substituted cyclopropaneamino acids. In particular, 2-phenyl-4-benzylidene-5(4*H*)-oxazolone has proven a convenient substrate for the preparation of c_3Phe,[31,32] thus demonstrating a higher reactivity in comparison with the open-chain dehydrophenylalanine derivative mentioned above.

These considerations, together with our previous experience[27,37–40] in the synthesis and reactivity of unsaturated 5(4*H*)-oxazolones, led us to select (*Z*)-2-phenyl-4-benzylidene-5(4*H*)-oxazolone (**1**) as the starting material for the preparation of $c_3$diPhe. Compound **1** is readily obtained as the major isomer by condensation of hippuric acid and benzaldehyde following the classical Erlenmeyer–Plöchl synthesis.[41] When oxazolone **1** was treated with a solution of phenyldiazomethane (very easily generated from benzaldehyde tosylhydrazone under phase-transfer catalysis conditions[42]), a slow evolution of gas was observed. The process was completed in 24 h, after which time ^{1}H NMR analysis of the crude reaction mixture showed the presence of only one compound. This product was identified as the spirocyclopropane *rac*-**2**, with the two phenyl substituents in a *trans* relative disposition (Scheme 1). This stereochemical assignment was based on the absence of any one effect between the two cyclopropane protons and in spite of the high value of the coupling constant between them (9.6 Hz), which seemed to be indicative of a *cis* disposition (for vicinal cyclopropane protons $J_{cis}>J_{trans}$,[43] and the J_{trans} magnitude rarely exceeds 9 Hz). The *trans* stereochemistry was later confirmed by X-ray diffraction analysis of a derivative (vide infra). Since the intermediate Δ^1-pyrazoline could not be isolated or even detected, it was not possible to discern whether the complete stereoselectivity of the process was attained in the first step (Δ^1-pyrazoline formation) or if a mixture of *cis* and *trans* pyrazolines was formed and, on extrusion of nitrogen, they gave rise to the same cyclopropane derivative.

The next step in our synthetic route involved nucleophilic opening of the oxazolone ring. This process was carried out by treatment of *rac*-**2** with methanol in the presence of a catalytic amount of sodium methoxide and provided the corresponding benzamido ester (*rac*-**3**) in high yield. This compound afforded single-crystals suitable for X-ray diffraction analysis, allowing us to confirm the *trans* relative configuration of the two aromatic substituents (Fig. 2). Removal of the protecting groups in *rac*-**3** was performed by refluxing in HCl. The harsh experimental conditions required to complete hydrolysis proved compatible with the stability of the cyclopropane moiety and so the amino acid hydrochloride (*rac*-**4**) was isolated in almost quantitative yield.

Subsequent introduction of Boc protection on the amino function turned out to be troublesome, a situation we previously observed when working with other hindered quaternary amino acids. When *rac*-**4** was treated with Boc_2O (di-*tert*-butyl dicarbonate) under the usual conditions,[44] i.e. in a mixture of aqueous NaOH and dioxane as the solvent, the (*N*-*tert*-butoxycarbonyl)amino acid (*rac*-**5**) was formed but reproducible results could not be obtained, with yields varying from 40 to 70%. Strict control of the pH of the reaction mixture appeared to be crucial to achieve high conversions and the efficiency of the process also varied depending on the scale of the reaction.

These difficulties prompted us to explore an alternative route for the synthesis of *rac*-**5**, based on the methodology developed by Burk and Allen[45] for direct amide/urethane exchange. In the first step, the benzamido group in *rac*-**3** was acylated by reaction with Boc_2O in the presence of a catalytic amount of 4-(dimethylamino)pyridine (Scheme 1). The imide obtained (*rac*-**6**) was then subjected to debenzoylation by treatment with hydrazine monohydrate to afford compound *rac*-**7**. Both steps proceeded under very mild conditions and by-products arising from hydrazinolysis of the ester moiety or from urethane cleavage in *rac*-**6** were not detected. Finally, saponification of the ester substituent in *rac*-**7** with a methanolic solution of KOH led to the desired *N*-Boc amino acid (*rac*-**5**), which was isolated in 83% yield from *rac*-**3**.

This route led to the conversion of the starting oxazolone into the final *N*-Boc amino acid with an overall yield of 76% for a total of five steps. Moreover, as already reported,[45] it is not necessary to isolate the intermediate imide due to the fact that the amide function in *rac*-**3** can be transformed into the urethane moiety in *rac*-**7** through a one-pot process. Chromatographic purification can thus be reduced to a minimum as the crude reaction products can be used in the subsequent step and only a silica gel column in necessary prior to the final saponification. This fact, together with the high overall yield and the easy transformations involved, make this methodology particularly appropriate for large-scale preparations.

2.2. HPLC Resolution

Once an efficient racemic route to the target compound had been developed, we undertook the preparation of the product in enantiomerically pure form by HPLC resolution of one of the synthetic intermediates using a chiral

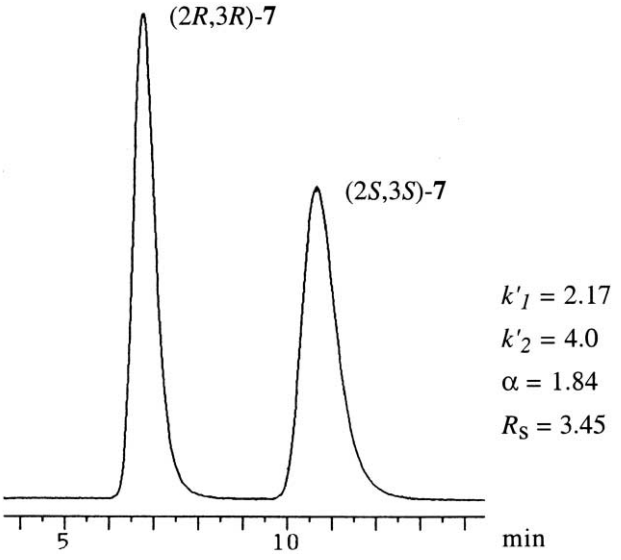


Figure 3. HPLC Analytical resolution of *rac*-**7**. Column: 150×4.6 mm ID, Eluent: *n*-hexane/2-propanol 94/6. Flow rate: 0.7 mL min^{-1}. UV detection at 220 nm. See Section 4 for definition of the chromatographic parameters.

stationary phase. Specifically, a non-commercial polysaccharide-derived support consisting of mixed 10-undecenoate/3,5-dimethylphenylcarbamate of amylose covalently attached to allylsilica gel[46,47] was used. The excellent chiral discrimination ability exhibited by this stationary phase towards a variety of compounds,[46] together with its high chemical stability, make it especially suitable for resolutions on a preparative scale. The efficiency of this system has actually been demonstrated in the preparative enantioseparations of different phenylalanine surrogates.[48,49]

Firstly, the resolution of derivatives *rac*-**2**, *rac*-**6**, *rac*-**3** and *rac*-**7** was investigated on an analytical scale. The first two compounds were quickly eluted and could not be separated under any of the chromatographic conditions tested, while *rac*-**3** and *rac*-**7** were completely resolved using mixtures of *n*-hexane/2-propanol as the mobile phase. The optimal separation was obtained for *rac*-**7** (Fig. 3).

From these results, *rac*-**7** was selected for preparative chromatography. This compound had the added advantage of being the immediate precursor to the desired *N*-Boc amino acid. The extension of the analytical conditions (see Fig. 3) to the preparative scale proved extremely efficient. Thus, 7.0 g of racemate afforded as much as 6.7 g of optically pure material (ca. 3.5 g and 3.2 g of the first and second eluted enantiomer, respectively) in a single passage through a 150×20 mm column and with a total time of 10 h to complete the process. The optical purity of the resolved enantiomers was assessed at analytical level, and assignment of their absolute configurations was carried out after conversion into the respective *N*-Boc amino acids (see Section 2.4). It should be mentioned that the high performance of this enantioseparation was due in part to the high stability of the silica-bound stationary phase in the presence of chlorinated solvents, a distinct advantage over the polysaccharide-derived phases commercially available.[50,51] The sample could thus be injected in a very high concentration (500 mg of racemate per mL of dichloromethane), a concentration unattainable in the *n*-hexane/2-propanol mixture used as the eluent.

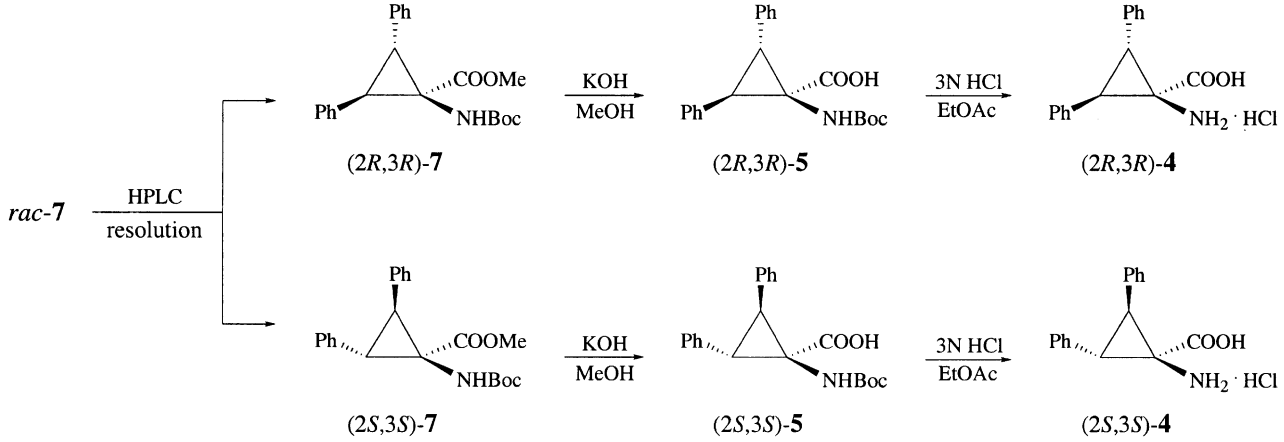
2.3. Preparation of enantiomerically pure compounds

After HPLC resolution of *rac*-**7**, the isolated enantiomers were submitted to saponification under the conditions previously developed for the racemic material. This process afforded the desired *N*-Boc amino acids in enantiomerically pure form (Scheme 2). A small quantity of these products was treated with a saturated solution of HCl in anhydrous ethyl acetate to provide the optically pure amino acids, which were prepared for characterization purposes.

2.4. Assignment of absolute configurations

The absolute configurations of all the enantiomerically pure derivatives prepared were determined by comparison of the optical rotations of the *N*-Boc amino acids with the values reported in the literature.[20] This allowed us to assign a (2*R*,3*R*) stereochemistry to the compounds obtained from the first eluted enantiomer of **7**, and a (2*S*,3*S*) configuration to the more strongly retained enantiomer of **7** and its derivatives (Scheme 2).

This assignment was confirmed when both (2*R*,3*R*)- and (2*S*,3*S*)-**5** were coupled to L-proline and the physical and



Scheme 2. Synthetic route to enantiomerically pure $c_3$diPhe derivatives.

spectroscopic properties of the resulting dipeptides were compared to those of samples characterised by X-ray diffraction analysis.[22]

3. Conclusion

We have developed a very efficient methodology for the synthesis of both enantiomers of $c_3$diPhe, a highly constrained phenylalanine surrogate, in optically pure form and appropriately protected for incorporation into peptides. Starting from readily available substrates and through high-yield transformations, a racemic precursor has been prepared and subjected to HPLC resolution on a non-commercial amylose-derived chiral stationary phase to afford large quantities of enantiomerically pure compounds.

4. Experimental

4.1. General

Melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. 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 apparatus at room temperature, unless otherwise indicated, using the residual solvent signal as the internal standard; chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hertz. Optical rotations were measured in a cell with a 10 cm pathlength at 22°C using a Perkin–Elmer 241-C polarimeter. Elemental analyses were carried out on a Perkin–Elmer 200 C, H, N, S analyzer. TLC was performed on Merck 60 F_{240} precoated silica gel polyester plates and products were visualised under UV light (254 nm) and iodine vapour. Column chromatography was performed using silica gel (Kieselgel 60). (*Z*)-2-Phenyl-4-benzylidene-5(4*H*)-oxazolone (**1**) was prepared[41] by condensation of hippuric acid (benzoylglycine) and benzaldehyde in the presence of anhydrous sodium acetate and acetic anhydride. Phenyldiazomethane (caution! this compound must be handled in a well-ventilated hood) was generated[42] as a toluene solution through the basic decomposition of benzaldehyde tosylhydrazone under phase-transfer catalysis conditions. This hydrazone was prepared[52] by refluxing benzaldehyde and tosylhydrazide in acetic acid, and has recently become commercially available.

4.2. X-Ray diffraction

Colourless single crystals of *rac*-**3** were obtained by slow evaporation from a methanolic solution. The X-ray diffraction data were collected at room temperature on a Siemens P4 four-circle diffractometer, using graphite-monochromated Mo-Kα radiation (λ=0.71073 Å). Reflections were measured in the $\omega/2\theta$-scan mode in the θ range 2–26°. The structure was solved by direct methods using SHELXS 97[53] and refinement was performed using SHELXL 97[54] by the full-matrix least-squares technique with anisotropic thermal factors for heavy atoms. Hydrogen atoms were located by calculation (with the exception of the cyclopropane and NH 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: monoclinic, $P2_1/c$; a=9.934(4) Å, b=22.433(10) Å, c=10.071(4) Å, β=111.84(2)°; Z=4; d (calcd)=1.184 g cm^{-3}; reflections collected/independent: 8946/3883 [R(int)=0.0325]; data/parameters: 3883/254; final R indices [$I>2\sigma(I)$]: R_1=0.046, wR_2=0.117; final R indices (all data): R_1=0.081, wR_2=0.153.

4.3. High performance liquid chromatography

HPLC was carried out on a system equipped with a Waters 600-E pump and a Waters 991 photo-diode array detector. The preparation of the chiral stationary phase, consisting of mixed 10-undecenoate/3,5-dimethylphenylcarbamate of amylose covalently bonded to allylsilica gel, has already been described.[46,47] The solvents used as mobile phases were of spectral grade. The HPLC analytical assays were carried out on a 150×4.6 mm ID column with an eluent flow rate of 0.7 mL min^{-1} and UV monitoring at 220 nm. The capacity (k'), selectivity (α) and resolution (R_s) factors are defined as follows: $k'=(t_r-t_0)/t_0$, $\alpha=k'_2/k'_1$, $R_s = 2(t_2 - t_1)/(w_2 + w_1)$, where subscripts 1 and 2 refer to the first and second eluted enantiomer, respectively, t_r (r=1,2) are their retention times, and w_1 and w_2 denote their baseline peak widths; t_0 is the dead time. The preparative resolution of *rac*-**7** was carried out on a 150×20 mm ID column. A mixture of *n*-hexane/2-propanol 94/6 was used as the eluent, at a flow rate of 14 mL min^{-1}. UV detection was performed at 265 nm.

4.4. *c*-1,*t*-2,5-Triphenyl-4-aza-6-oxaspiro[2,4]hept-4-en-*r*-7-one, *rac*-2

A freshly prepared[42] solution of phenyldiazomethane (150 mmol) in toluene (800 mL) was added to (*Z*)-2-phenyl-4-benzylidene-5-(4*H*)-oxazolone[41] (**1**) (18.68 g, 75 mmol) and the reaction mixture was stirred at room temperature with protection from light for 24 h. Excess diazoalkane was then destroyed by the addition of a small quantity of silica gel. After filtration, the solvent was evaporated under reduced pressure to yield crude *rac*-**2**, which was used in the next step without further purification. An analytically pure sample was obtained by recrystallization from diethyl ether. Mp 134°C. R_f (hexanes/ethyl acetate 9/1)=0.26. IR (nujol) 1802, 1636 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.78 (d, 1H, J=9.6 Hz); 3.97 (d, 1H, J=9.6 Hz); 7.27–7.54 (m, 13H); 7.94 (d, 2H, J=7.5 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 41.31; 42.99; 59.28; 126.12; 127.42; 127.63; 127.93, 128.45; 128.67; 128.69; 128.90; 132.32; 132.65; 134.58; 162.0; 174.01. Anal. Calcd for $C_{23}H_{17}NO_2$: C, 81.40; H, 5.05; N, 4.13. Found: C, 81.29; H, 5.09; N, 4.11.

4.5. Methyl 1-benzamido-*c*-2,*t*-3-diphenyl-*r*-1-cyclopropanecarboxylate, *rac*-3

A 0.1% solution of sodium methoxide in absolute methanol (40 mL) was added to crude *rac*-**2** obtained in the previous step, and the reaction mixture was vigorously stirred at room temperature until TLC analysis indicated that the starting material had disappeared (ca. 30 min). The product was collected by vacuum filtration and washed with small portions of cold methanol; white solid (25.53 g,

68.81 mmol, 92% yield for two steps). Mp 216°C. R_f (hexanes/ethyl acetate 6/4)=0.36. IR (nujol) 3240, 1731, 1644 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 3.49 (s, 3H); 3.52 (d, 1H, J=9.0 Hz); 3.78 (d, 1H, J=9.0 Hz); 6.17 (bs, 1H); 7.26–7.68 (m, 15H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 36.32; 39.37; 44.98; 52.39; 126.92; 127.28; 127.79; 128.15; 128.61; 128.79; 128.82; 129.40; 131.83; 134.04; 134.33; 135.11; 168.43; 169.37. Anal. Calcd for $C_{24}H_{21}NO_3$: C, 77.61; H, 5.70; N, 3.77. Found: C, 77.79; H, 5.62; N, 3.81.

4.6. Methyl 1-(*N*-*tert*-butoxycarbonyl)benzamido-*c*-2,*t*-3-diphenyl-*r*-1-cyclopropanecarboxylate, *rac*-6

A mixture of *rac*-**3** (20.50 g, 55.26 mmol), 4-(dimethylamino)pyridine (1.35 g, 11.05 mmol) and di-*tert*-butyl dicarbonate (18.07 g, 82.89 mmol) in tetrahydrofuran (55 mL) was stirred at room temperature. After 48 h, another portion of di-*tert*-butyl dicarbonate (6.02 g, 27.63 mmol) was added and stirring was continued for an additional 24 h. The solvent was then removed and the residue chromatographed (eluent: hexanes/ethyl acetate 8/2) to give *rac*-**6** as a colourless oil, which crystallised upon standing (23.65 g, 50.21 mmol, 91% yield). Mp 140°C. R_f (hexanes/ethyl acetate 8:2)=0.31. IR (nujol) 1736, 1684 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz, 60°C) δ 0.90 and 1.07 (s, 9H); 3.35 and 3.38 (s, 3H); 3.65 and 3.73 (d, 1H, J=9.2 Hz); 3.81 and 4.06 (d, 1H, J=9.2 Hz); 6.96–7.53 (m, 15H). Note: due to the dynamic equilibrium between rotamers, two sets of signals were observed for some protons; for the same reason, the ^{13}C NMR spectrum was inconclusive and data are therefore not included. Anal. Calcd for $C_{29}H_{29}NO_5$: C, 73.87; H, 6.20; N, 2.97. Found: C, 74.02; H, 6.12; N, 3.05.

4.7. Synthesis and HPLC resolution of 7

4.7.1. Methyl 1-(*N*-*tert*-butoxycarbonyl)amino-*c*-2,*t*-3-diphenyl-*r*-1-cyclopropanecarboxylate, *rac*-7. A solution of *rac*-**6** (22.75 g, 48.30 mmol) in tetrahydrofuran/methanol 1/1 (180 mL) was treated with hydrazine monohydrate (3.38 g, 67.62 mmol) and the reaction was stirred at room temperature for 48 h. After evaporation of the solvent, a mixture of hexanes/diethyl ether 2/1 (150 mL) was added. The insoluble material was filtered off under reduced pressure and washed thoroughly with hexanes/diethyl ether 2/1. The filtrate was concentrated to a thick oil, which was purified by chromatography (eluent: hexanes/ethyl acetate 8/2) to provide *rac*-**7** as a white solid (16.72 g, 45.56 mmol, 94% yield). Mp 109–110°C. R_f (hexanes/ethyl acetate 8/2)=0.22. IR (nujol) 3362, 1722, 1708 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz, 60°C) δ 1.36 (s, 9H); 3.28 (d, 1H, J=9.0 Hz); 3.46 (s, 3H); 3.63 (d, 1H, J=9.0 Hz); 4.71 (bs, 1H); 7.19–7.47 (m, 10H). ^{13}C NMR ($CDCl_3$ 75 MHz, 60°C) δ 28.25; 36.11; 39.12; 46.58; 51.94; 80.10; 127.17; 127.32; 128.13; 128.42; 128.93; 129.24; 134.83; 135.45; 155.72; 169.84. Anal. Calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81. Found: C, 71.67; H, 6.91; N, 3.77.

4.7.2. Resolution of *rac*-7: methyl (2*R*,3*R*)- and (2*S*,3*S*)-1-(*N*-*tert*-butoxycarbonyl)amino-2,3-diphenyl-1-cyclopropanecarboxylate, (2*R*,3*R*)-7 and (2*S*,3*S*)-7. HPLC resolution of *rac*-**7** (7.0 g) dissolved in dichloromethane (14 mL) was carried out by successive injections of 0.27 mL on a 150×20 mm ID column filled with 10-undecenoate/3,5-dimethylphenylcarbamate of amylose bonded on allylsilica gel and using a mixture of *n*-hexane/2-propanol 94/6 as the eluent (flow rate 14 mL min^{-1}). A total of 66 injections were required with one injection being performed every 9 min. Four separate fractions were collected. Evaporation of the first fraction provided 3.47 g of enantiomerically pure (2*R*,3*R*)-**7**. The fourth fraction gave 3.22 g of enantiomerically pure (2*S*,3*S*)-**7**. The second (46 mg) and third (270 mg) fractions, respectively, contained 66/34 and 1/99 mixtures of (2*R*,3*R*)-**7**/(2*S*,3*S*)-**7** (2*R*,3*R*)-**7**: mp 79–80°C. $[\alpha]_D$=+62.9 (c=0.50, $CHCl_3$). (2*S*,3*S*)-**7**: mp 78–80°C. $[\alpha]_D$=−63.8 (c=0.54, $CHCl_3$). Spectroscopic data for both (2*R*,3*R*)-**7** and (2*S*,3*S*)-**7** were the same as those described above for *rac*-**7**.

4.8. Synthesis of racemic and enantiomerically pure 5

4.8.1. 1-(*N*-*tert*-butoxycarbonyl)amino-*c*-2,*t*-3-diphenyl-*r*-1-cyclopropanecarboxylic acid, *rac*-5. A 2N solution of KOH in methanol (100 mL) was added to *rac*-**7** (8.50 g, 23.16 mmol) and the reaction mixture was stirred at room temperature for 2 days. After evaporation of the solvent, the remaining residue was redissolved in water (100 mL). Neutralization with 5% aqueous $KHSO_4$ resulted in the precipitation of the desired product, which was collected by filtration under reduced pressure and washed several times with water; white solid (7.92 g, 22.44 mmol, 97% yield). Mp 216°C (dec.). IR (nujol) 3400–2400, 3226, 1697, 1650 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz, 60°C) δ 1.39 (s, 9H); 3.22 (d, 1H, J=8.8 Hz); 3.69 (d, 1H, J=8.8 Hz); 4.75 (bs, 1H); 7.20–7.46 (m, 10H). ^{13}C NMR (DMSO-d_6, 75 MHz, 75°C) δ 27.53; 33.77; 35.91; 47.21; 77.54; 125.83; 126.17; 127.17; 127.38; 128.32; 128.48; 135.54; 135.63; 154.75; 169.56. Anal. Calcd for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96. Found: C, 71.51; H, 6.50; N, 4.01.

4.8.2. (2*R*,3*R*)-1-(*N*-*tert*-butoxycarbonyl)amino-2,3-diphenyl-1-cyclopropanecarboxylic acid, (2*R*,3*R*)-5. In a similar way to that described above, starting from (2*R*,3*R*)-**7** (3.35 g, 9.13 mmol), (2*R*,3*R*)-**5** was obtained as a white solid (3.11 g, 8.81 mmol, 97% yield). Mp 114°C. $[\alpha]_D$=+77.6 (c=0.46, $CHCl_3$) [lit.[20] +70.1 (c=1.03, $CHCl_3$)]; $[\alpha]_D$=+111.0 (c=0.50, MeOH). Spectroscopic data were identical to those reported for *rac*-**5**.

4.8.3. (2*S*,3*S*)-1-(*N*-*tert*-butoxycarbonyl)amino-2,3-diphenyl-1-cyclopropanecarboxylic acid, (2*S*,3*S*)-5. An identical procedure to that described above was applied to the transformation of (2*S*,3*S*)-**7** (3.11 g, 8.47 mmol) to (2*S*,3*S*)-**5** (2.86 g, 8.10 mmol, 96% yield). Mp 115–116°C. $[\alpha]_D$=−79.8 (c=0.49, $CHCl_3$) [lit.[20] −66.8 (c=1.28, $CHCl_3$)]; $[\alpha]_D$=−109.5 (c=0.50, MeOH). Spectroscopic data were the same as those described for *rac*-**5**.

4.9. Synthesis of racemic and enantiomerically pure 4

4.9.1. 1-Amino-*c*-2,*t*-3-diphenyl-*r*-1-cyclopropanecarboxylic acid hydrochloride, *rac*-4. A mixture of 6N HCl (60 mL) and acetic acid (30 mL) was added to *rac*-**3** (3.0 g, 8.09 mmol) and the reaction mixture was heated under reflux for 24 h. The solvent was evaporated to dryness and

the resulting solid was washed with diethyl ether in order to remove benzoic acid. Final lyophilization afforded pure *rac*-**4** as a white solid (2.25 g, 7.77 mmol, 96% yield). Mp 204°C (dec.). IR (nujol) 3600–2400, 1751, 1729 cm^{-1}. ^1H NMR (D_2O, 300 MHz) δ 3.41 (d, 1H, J=8.7 Hz); 3.72 (d, 1H, J=8.7 Hz); 7.20–7.44 (m, 10H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 32.71; 34.80; 44.31; 127.15; 127.81; 128.04; 128.51; 128.82; 129.95; 132.39; 134.24; 167.93. Anal. Calcd for $C_{16}H_{16}ClNO_2$: C, 66.32; H, 5.57; N, 4.83. Found: C, 65.97; H, 5.65; N, 4.91.

4.9.2. (2*R*,3*R*)-1-Amino-2,3-diphenyl-1-cyclopropanecarboxylic acid hydrochloride, (2*R*,3*R*)-4. A 3N solution of HCl in anhydrous ethyl acetate (6 mL) was added to (2*R*,3*R*)-**5** (180 mg, 0.51 mmol) and the reaction mixture was stirred at room temperature until completion (ca. 30 min, TLC monitoring). After evaporation of the solvent, the residue was taken up in water and lyophilised to afford pure (2*R*,3*R*)-**4** as a white solid (147 mg, 0.51 mmol, 100% yield). Mp 174–175°C (dec.). $[\alpha]_D$=+17.3 (c=0.24, MeOH). Spectroscopic data were the same as those described for *rac*-**4**.

4.9.3. (2*S*,3*S*)-1-Amino-2,3-diphenyl-1-cyclopropanecarboxylic acid hydrochloride, (2*S*,3*S*)-4. An identical procedure to that described above was used for the conversion of (2*S*,3*S*)-**5** (138 mg, 0.39 mmol) into (2*S*,3*S*)-**4** (112 mg, 0.39 mmol, 100% yield). Mp 175°C (dec.). $[\alpha]_D$=−18.8 (c=0.21, MeOH). Spectroscopic data were the same as those reported for *rac*-**4**.

5. Supporting information

A complete list of crystallographic data, atom coordinates, thermal parameters, bond lengths, bond angles and torsion angles is available from the Cambridge Crystallographic Data Centre.

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