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# Synthesis of enantiomerically pure β,β-diphenylalanine (Dip) and fluorenylglycine (Flg)

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**Abstract**—A new strategy for the preparation of both enantiomers of two phenylalanine analogues, β,β-diphenylalanine and fluorenylglycine, has been developed. The combination of a high yielding racemic synthesis and a very efficient resolution procedure has provided significant amounts of each amino acid in enantiomerically pure form and suitably protected for use in peptide synthesis. This methodology can be easily applied to the preparation of larger quantities of enantiopure compounds.


## 1. Introduction

The incorporation of conformationally constrained α-amino acids into bioactive peptides is considered a very useful strategy in the search for pharmaceutical applications of these systems.[1–4] The restriction of conformational freedom in peptides results in increased metabolic stability and can also lead to enhanced selectivity. Additionally, structure–activity relationship studies on constrained peptide analogues are crucial in the elucidation of the biologically active conformation.[5,6] In this context, aromatic amino acids (Phe, His, Tyr, Trp) are of special importance since aromatic side chains often play a key role in peptide-receptor recognition processes. Controlling the orientation of these aromatic groups is therefore very important and the introduction of additional substituents is regarded as a powerful tool for achieving this aim.[2,6,7]

Among phenylalanine analogues, β,β-diphenylalanine (Dip, Fig. 1) bears an additional phenyl group at the β-position. This non-proteinogenic amino acid has frequently been incorporated into biologically active peptides as a replacement for Phe and has provided some selective and potent ligands.[8–15] Indeed, over a dozen patents related to the incorporation of Dip into bioactive peptides show the high potential of this amino acid in the design of peptide analogues. Another attractive phenylalanine surrogate, closely related to Dip, is fluorenylglycine (Flg, Fig. 1) where the two phenyl rings are covalently linked. This amino acid has been studied to a much lesser extent than Dip and has replaced Phe in only a few active peptides.[13–15] Despite the structural similarity between Dip and Flg, the relative orientation of the two aromatic rings differs substantially, being almost perpendicular in the former amino acid and coplanar in the latter. This can lead to different conformational and biological behaviours.

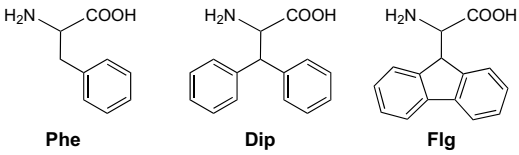


**Figure 1.** Structure of the phenylalanine (Phe) analogues synthesized in this work: β,β-diphenylalanine (Dip) and fluorenylglycine (Flg).

For incorporation into peptide sequences, the availability of large quantities of these amino acids in their enantiomerically pure form becomes a relevant issue. Several synthetic procedures for the preparation of Dip,[14–29] either in racemic[14,16–21] or enantiomerically pure[15,22–29] form, have been described. In terms of the enantiopure compound, the reported routes starting from more readily available substrates are those using L-serine derivatives,[26,29] which lead to the isolation of (*R*)-Dip. The (*S*)-enantiomer is, however, less accessible through these synthetic paths. These particular strategies cannot be applied to the synthesis of Flg and the same holds true for most of the other procedures described for Dip. There are

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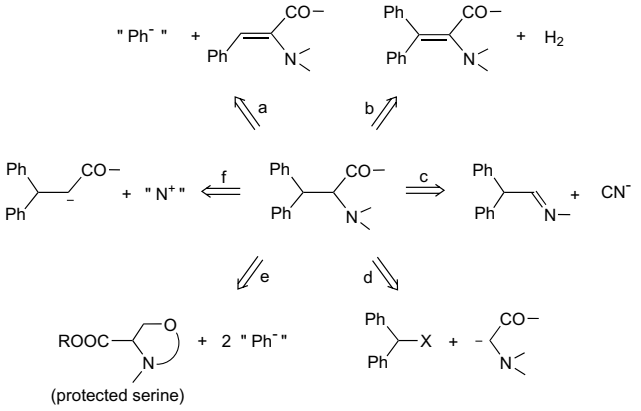
indeed only two methods for the preparation of Dip that have been extended to the synthesis of enantiomerically pure Flg,[15,22,23] one of which proceeds with very poor selectivity. This situation prompted us to undertake the development of a new synthetic pathway starting from readily available substrates that would allow the isolation of both enantiomers of Flg. Given its structural similarity to Dip, we were interested in expanding this methodology to the preparation of this amino acid as well.

## 2. Results and discussion

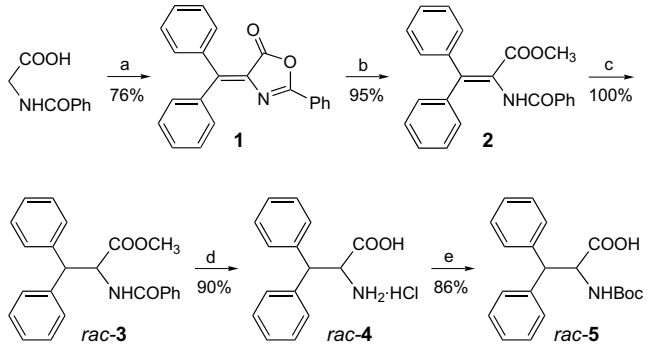
A retrosynthetic analysis of Dip (Scheme 1) shows that the existing procedures for its preparation, either in racemic or enantiomerically pure form, cover the majority of the general strategies applied for the synthesis of α-amino acids. However, one particularly widely used approach is absent, namely the hydrogenation of an α,β-dehydroamino acid derivative (Scheme 1, route b).




**Scheme 1.** Retrosynthetic analysis for Dip.

Such an α,β-dehydroamino acid precursor of Dip could be obtained from the corresponding unsaturated 5(4*H*)-oxazolone. These heterocyclic compounds have frequently been used for the preparation of a wide variety of amino acids.[30,31] Our experience in the synthesis and reactivity of unsaturated 5(4*H*)-oxazolones[30,32] prompted us to explore the isolation of Dip and Flg starting from the corresponding substrate of this type.

2-Phenyl-4-(diphenylmethylene)-5(4*H*)-oxazolone **1** was selected as the starting material for the preparation of Dip. The synthesis of this compound cannot be achieved[33] through the classical Erlenmeyer–Plöchl method,[34] that is, by condensation of hippuric acid (*N*-benzoylglycine) and benzophenone in the presence of sodium acetate and acetic anhydride. Indeed, it was only in 1992 that the synthesis of this oxazolone was first described.[35] The procedure involves cyclization of hippuric acid prior to its reaction with benzophenone *N*-methylimine. Following this strategy, we were able to obtain oxazolone **1** (Scheme 2) using commercially available benzophenone imine instead of the *N*-methylimine derivative, in a yield comparable to that of the original method.[35]



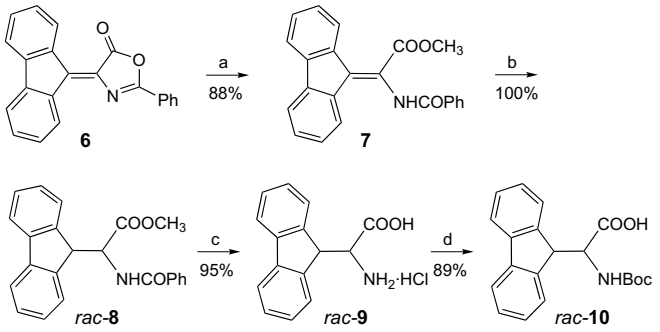

**Scheme 2.** Synthesis of racemic *N*-Boc protected Dip. Reagents and conditions: (a) (i) ClCOOEt, $NEt_3$, benzene; (ii) $Ph_2C{=}NH$, benzene, 50–55 °C; (b) MeONa, MeOH; (c) $H_2$ Pd/C, MeOH/$CH_2Cl_2$ 40 °C; (d) 6 M HCl/AcOH, reflux; (e) $Boc_2O$, aq 1 M NaOH/dioxane.

The next step in our synthetic route involved nucleophilic opening of the oxazolone ring. Treatment of compound **1** with methanol in the presence of a catalytic amount of sodium methoxide afforded the corresponding benzamido ester **2** in high yield. Subsequently, hydrogenation of the tetrasubstituted double bond was attempted under the usual conditions, that is, using palladium–carbon as the catalyst and methanol as the solvent. Due to the low solubility of compound **2** in this medium, the reaction only took place in high dilution and could not be scaled up. The introduction of a small percentage of dichloromethane as a co-solvent, along with mild heating of the reaction mixture, enabled the successful hydrogenation of **2**, affording *rac*-**3** in quantitative yield.

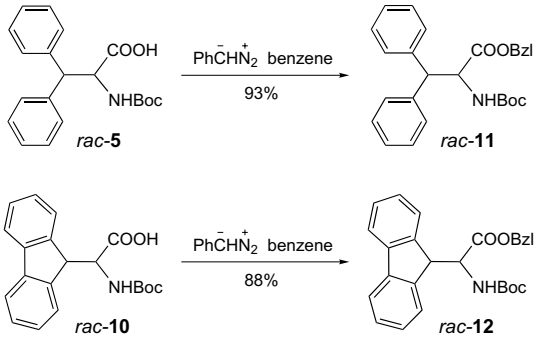
This compound was heated under reflux in a mixture of HCl and acetic acid to afford the amino acid hydrochloride *rac*-**4**. The presence of acetic acid proved critical in this step, ensuring the solubility of the starting material. The subsequent introduction of Boc protection on the amino function was achieved by treatment with di-*tert*-butyl dicarbonate under standard conditions,[36] leading to *rac*-**5** in good yield. Mild heating was needed to guarantee the solubility of the amino acid.

This synthetic route (Scheme 2) enabled the conversion of the starting oxazolone **1** into the final *N*-Boc amino acid *rac*-**5** with an overall yield of 74% in four steps. This compound is suitably protected for use in standard peptide synthesis.

Following a parallel synthetic procedure, the *N*-Boc derivative of fluorenylglycine was obtained from the corresponding oxazolone. In this case, 2-phenyl-4-(9-fluorenylidene)-5(4*H*)-oxazolone **6** was synthesized as previously described[21,37] by the condensation of hippuric acid and 9-fluorenone under classical Erlenmeyer–Plöchl conditions.[34] Oxazolone **6** was then transformed as shown in Scheme 3. The main difference in comparison to the synthetic route leading to Dip was observed in the hydrogenation of the double bond in **7**, which in this case was straightforward. The different reactivities of **7** and **2** are due to the planarity of the aromatic system in the Flg deriv-




**Scheme 3.** Synthesis of racemic *N*-Boc protected Flg. Reagents and conditions: (a) MeONa, MeOH; (b) $H_2$ Pd/C, MeOH/$CH_2Cl_2$; (c) 6 M HCl/AcOH, reflux; (d) $Boc_2O$, aq 1 M NaOH/dioxane.




**Scheme 4.** Synthesis of substrates for HPLC resolution (Bzl: benzyl).

ative. Following this route, the *N*-Boc protected amino acid *rac*-**10** was obtained in 74% overall yield from oxazolone **6**.

In this way, several grams of racemic Dip and Flg could be prepared through synthetic paths involving easy transformations that, with their high overall yields, are suitable for scaling-up to larger quantities.

Once an efficient route to the target compounds in racemic form had been developed, we attempted to isolate them in enantiomerically pure form. With this aim in mind, we addressed the resolution of synthetic precursors by HPLC using a chiral column. The direct separation of enantiomers by preparative chromatography on chiral stationary phases is currently recognized as a powerful tool to obtain enantiopure compounds.[38,39] In particular, polysaccharide-derived phases are very popular because of their wide applicability and usefulness.[40–45] Our research group has successfully employed amylose- and cellulose-derived phases in the preparative enantioseparations of different protected non-natural amino acids.[46–55] In these particular stationary phases, the chiral support is covalently bonded to the silica gel matrix, which results in an extremely high stability in the presence of all organic solvents. This feature, together with the high enantiodiscrimination exhibited towards a variety of compounds, makes these stationary phases especially suitable for resolutions on a preparative scale. Very recently, such phases have become commercially available. Specifically, a Chiralpak® IA column containing 3,5-dimethylphenylcarbamate of amylose as the chiral selector was used in this work (our previous resolutions were carried out on similar columns made in the laboratory).

Among the precursors of the target amino acids, only the benzamido esters *rac*-**3** and *rac*-**8** are suitable substrates for chromatography. However, the harsh conditions needed for the removal of the ester and amide groups in the subsequent hydrolysis step would certainly lead to partial epimerization, thus hampering the isolation of optically pure compounds. To avoid this problem, the acid moiety in the *N*-Boc amino acids *rac*-**5** and *rac*-**10** was temporarily protected as a benzyl ester. This could be readily achieved by the reaction with phenyldiazomethane (Scheme 4).

Firstly, the resolution of *rac*-**11** and *rac*-**12** was tested on an analytical scale using mixtures of *n*-hexane/2-propanol as eluents. An excellent separation of the enantiomers of *rac*-**11** was obtained by using an 80/20 mixture of *n*-hexane/2-propanol as the elution system (Fig. 2). Addition of *tert*-butyl methyl ether was required for the resolution of *rac*-**12** and optimal results were finally attained on elution with *n*-hexane/*tert*-butyl methyl ether/2-propanol 80/19/1 (Fig. 3).

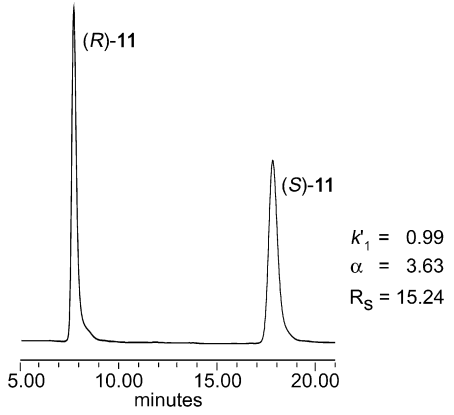



**Figure 2.** HPLC resolution of the Dip precursor *rac*-**11** at the analytical level. Column: Chiralpak® IA (250 × 4.6 mm ID). Eluent: *n*-hexane/2-propanol 80/20. Flow rate: 0.9 mL/min. UV detection: 210 nm. See Section 4.2 for definition of the chromatographic parameters.

The preparative enantioseparations were then performed by extension of the analytical conditions to the preparative scale. Thus, resolution of *rac*-**11** (2.15 g) afforded as much as 2.11 g of enantiomerically pure material (1.06 g and 1.05 g of the first and second eluted enantiomer, respectively). In a similar way, the resolution of *rac*-**12** (2.94 g) provided 1.36 g of the first eluted enantiomer and 1.33 g of the more strongly retained enantiomer, both in enantiomerically pure form. These separations were achieved in a single passage of the racemate through the column. Optimization of the resolution procedure by reinjection of the remaining enantioenriched material was not attempted. The enantiomeric purity of the resolved enantiomers was assessed at the analytical level.

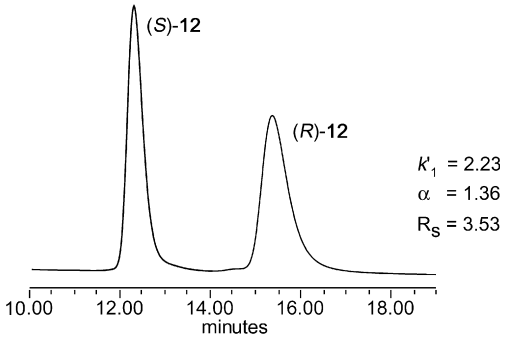
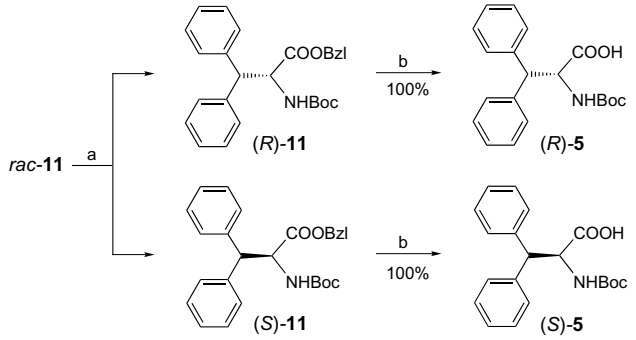
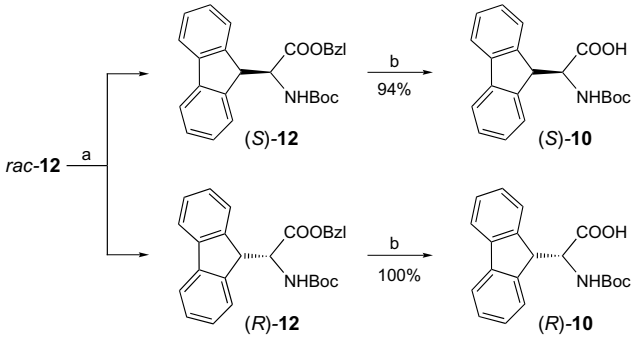



**Figure 3.** HPLC resolution of the Flg precursor *rac*-**12** at the analytical level. Column: Chiralpak® IA (250 × 4.6 mm ID). Eluent: *n*-hexane/*tert*-butyl methyl ether/2-propanol 80/19/1. Flow rate: 0.9 mL/min. UV detection: 220 nm. See Section 4.2 for definition of the chromatographic parameters.

Finally, the isolated enantiopure material was subjected to catalytic hydrogenation to provide the desired enantiopure *N*-Boc amino acids in quantitative yield (Schemes 5 and 6).




**Scheme 5.** Synthesis of enantiomerically pure *N*-Boc protected Dip. Reagents and conditions: (a) chiral HPLC; (b) $H_2$ Pd/C, AcOEt.




**Scheme 6.** Synthesis of enantiomerically pure *N*-Boc protected Flg. Reagents and conditions: (a) chiral HPLC; (b) $H_2$ Pd/C, AcOEt.

The absolute configurations of all the enantiomerically pure derivatives were determined by comparison of the specific rotations measured for the final *N*-Boc amino acids with the values reported in the literature.[15,25–27] This allowed us to assign an (*R*)-configuration to the first eluted enantiomer of **11** and its derivative, and an (*S*)-configuration to the more strongly retained enantiomer of **11** and the *N*-Boc amino acid obtained from it (Scheme 5). As far as **12** is concerned, the less strongly retained enantiomer was found to be (*S*), while the last enantiomer was assigned an (*R*)-configuration (Scheme 6).

## 3. Conclusion

We have developed an efficient and practical strategy for the synthesis of each enantiomer of two phenylalanine analogues, β,β-diphenylalanine (Dip) and fluorenylglycine (Flg). Starting from readily available substrates and using high-yielding transformations, a racemic precursor has been prepared and subjected to HPLC resolution on an amylose-derived chiral stationary phase to afford significant amounts (over 1.0 g) of enantiomerically pure compounds. The enantiopure amino acids obtained in this way are suitably protected for incorporation into peptides. The methodology is amenable to the synthesis of larger quantities.

## 4. Experimental

### 4.1. General

All reagents were purchased from the Aldrich Chemical Co. (Milwaukee, WI) and used without further purification. The progress of the reactions was monitored by thin layer chromatography (TLC) on Macherey-Nagel Polygram syl G/UV precoated silica gel polyester plates. The products were visualized under UV light (254 nm) and iodine vapour. Column chromatography was performed using SDS 60 ACC silica gel (50–70 μm). Melting points were determined on a Gallenkamp apparatus and are uncorrected. IR spectra were registered on a Mattson Genesis FTIR spectrophotometer; $\nu_{max}$ is given for the main absorption bands. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker ARX-300 instrument at room temperature, unless otherwise indicated, in $CDCl_3$ or DMSO-$d_6$, using the residual solvent signal as the internal standard; chemical shifts ($\delta$) are expressed in ppm and coupling constants ($J$) in Hertz. Optical rotations were measured at room temperature using a JASCO P-1020 polarimeter. High-resolution mass spectra were obtained on a Bruker Microtof-Q spectrometer. Phenyldiazomethane was generated[56] as a benzene solution through the basic decomposition of benzaldehyde tosylhydrazone under phase-transfer catalysis conditions. 2-Phenyl-4-(9-fluorenylidene)-5(4*H*)-oxazolone **6** was prepared[21,37] by condensation of hippuric acid and 9-fluorenone in the presence of anhydrous sodium acetate and acetic anhydride.

### 4.2. High performance liquid chromatography (HPLC)

HPLC was carried out using a Waters HPLC system equipped with a Waters 600-E pump and a Waters 996 photodiode-array detector. The solvents used as mobile phases were of chromoscan grade. Chiralpak® IA columns (Daicel, Tokyo, Japan) of 250 × 4.6 mm ID and 250 × 20 mm ID were used for the analytical and preparative scale separations, respectively. Analytical assays were

performed using mixtures of *n*-hexane/2-propanol and *n*-hexane/2-propanol/*tert*-butyl methyl ether as eluents (flow rate 0.9 mL/min). The preparative HPLC resolution of compounds *rac*-**11** and *rac*-**12** was carried out using, respectively, mixtures of *n*-hexane/2-propanol 80/20 and *n*-hexane/*tert*-butyl methyl ether/2-propanol 80/19/1 as eluents working at a flow rate of 18 mL/min. The capacity ($k'$), selectivity ($\alpha$) and resolution ($R_S$) factors are defined as follows: $k' = (t_r - t_0)/t_0$, $\alpha = k'_2/k'_1$, $R_S = 1.18\ (t_2 - t_1)/(w_2 + w_1)$, where subscripts 1 and 2 refer to the first and second eluted enantiomers, $t_r$ ($r = 1, 2$) are their retention times, and $w_1$ and $w_2$ denote their half-height peak widths; $t_0$ is the dead time.

### 4.3. Synthesis of 2-phenyl-4-(diphenylmethylene)-5(4*H*)-oxazolone, 1

A solution of ethyl chloroformate (3.52 g, 32.40 mmol) in benzene (16 mL) was added dropwise to a slurry of hippuric acid (5.80 g, 32.40 mmol) and triethylamine (3.27 g, 32.40 mmol) in benzene (40 mL). The resulting mixture was stirred at room temperature for 4 h. The residue was filtered off and the filtrates were added dropwise to a solution of benzophenone imine (2.93 g, 16.20 mmol) in benzene (10 mL). The reaction mixture was stirred at 50–55 °C for 1 h, after which the solvent was removed under reduced pressure. The solid residue was suspended in EtOH (45 mL) and collected by filtration; yellow solid (4.01 g, 12.34 mmol, 76% yield). Mp 187 °C (EtOH). IR (Nujol) $\nu$ 1796, 1636 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 7.30–7.65 (m, 13H), 8.07–8.12 (m, 2H). $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 125.81, 127.91, 128.09, 128.21, 128.84, 129.69, 130.30, 130.52, 130.89, 132.60, 132.90, 136.86, 138.59, 150.39, 162.21, 165.48. HRMS (ESI) $C_{22}H_{15}NO_2Na$ $[M+Na]^+$: calcd 348.09950, found 348.09854.

### 4.4. Synthesis of methyl 2-benzamido-3,3-diphenylpropenoate, 2

A 2% solution of sodium methoxide in absolute methanol (7 mL) was added to **1** (3.82 g, 11.75 mmol) and the reaction mixture was vigorously stirred at room temperature for 30 min. The product was collected by vacuum filtration and washed with small portions of cold methanol; white solid (3.99 g, 11.18 mmol, 95% yield). Mp 202 °C (MeOH). IR (Nujol) $\nu$ 3290, 1731, 1640 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 3.57 (s, 3H), 7.15–7.51 (m, 14H), 7.61–7.67 (m, 2H). $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 52.01, 126.01, 127.32, 128.12, 128.18, 128.70, 128.74, 128.96, 129.32, 129.85, 132.09, 133.21, 136.10, 138.67, 139.49, 165.09, 166.04. HRMS (ESI) $C_{23}H_{19}NO_3Na$ $[M+Na]^+$: calcd 380.12571, found 380.12702.

### 4.5. Synthesis of methyl 2-benzamido-3,3-diphenylpropanoate, *rac*-3

A solution of **2** (3.72 g, 10.42 mmol) in a mixture of methanol/dichloromethane 9/1 (80 mL) was hydrogenated at 40 °C in the presence of 10% palladium–carbon (125 mg). After 48 h, filtration of the catalyst on Celite® and evaporation of the solvent afforded pure *rac*-**3** as a white solid (3.73 g, 10.39 mmol, 100% yield). Mp 162 °C (MeOH). IR (Nujol) $\nu$ 3391, 1733, 1643 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 3.54 (s, 3H), 4.58 (d, 1H, $J = 8.3$ Hz), 5.58 (dd, 1H, $J = 8.3$, 8.3 Hz), 6.32 (d, 1H, $J = 8.3$ Hz), 7.18–7.60 (m, 15H). $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 52.26, 53.55, 55.58, 126.98, 127.20, 127.45, 128.22, 128.52, 128.57, 128.63, 128.92, 131.79, 133.75, 139.48, 140.00, 167.20, 172.33. HRMS (ESI) $C_{23}H_{21}NO_3Na$ $[M+Na]^+$: calcd 382.14136, found 382.14034.

### 4.6. Synthesis of 2-amino-3,3-diphenylpropanoic acid hydrochloride, *rac*-4

Acetic acid (40 mL) and 6 M HCl (40 mL) were added to *rac*-**3** (3.63 g, 10.11 mmol) and the reaction mixture was heated under reflux for 24 h. The solvent was evaporated and the resulting solid was taken up in 0.5 M HCl (150 mL) and washed with chloroform (3 × 50 mL). The aqueous phase was concentrated and lyophilized to afford pure *rac*-**4** as a white solid (2.52 g, 9.09 mmol, 90% yield). Mp 180 °C (dec). IR (Nujol) $\nu$ 3500–2800, 1750 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$, 300 MHz) $\delta$ 4.29 (d, 1H, $J = 10$ Hz), 4.75 (d, 1H, $J = 10$ Hz), 7.17–7.56 (m, 10H). $^{13}C$ NMR (DMSO-$d_6$, 75 MHz) $\delta$ 53.03, 55.38, 127.16, 127.34, 128.34, 128.45, 128.49, 128.85, 139.01, 139.62, 169.79. HRMS (ESI) $C_{15}H_{16}NO_2$ $[M-Cl]^+$: calcd 242.11810, found 242.11610.

### 4.7. Synthesis of 2-(*N*-*tert*-butoxycarbonylamino)-3,3-diphenylpropanoic acid, *rac*-5

A suspension of *rac*-**4** (2.39 g, 8.60 mmol) in a mixture of dioxane (17.2 mL) and aqueous 1 M NaOH (17.2 mL) was cooled in an ice-water bath. Di-*tert*-butyl dicarbonate (2.06 g, 9.46 mmol) was added and the reaction mixture kept at 35 °C for 15 h. A further 4.30 mmol of di-*tert*-butyl dicarbonate was then added and stirring continued for an additional 24 h. The solvent was evaporated and the residue taken up in water (200 mL) and washed with hexanes (2 × 60 mL). The aqueous phase was acidified with solid citric acid to pH 2–3. The product was collected by filtration under reduced pressure and washed with water; white solid (2.51 g, 7.37 mmol, 86% yield). Mp 133 °C (hexanes). IR (Nujol) $\nu$ 3378, 3200–2700, 1692, 1602, 1582 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 1.32 (s, 9H), 4.44 (d, 1H, $J = 7.3$ Hz), 4.76 (d, 1H, $J = 8.8$ Hz), 5.04 (dd, 1H, $J = 8.8$, 7.3 Hz), 7.14–7.29 (m, 10H). $^{13}C$ NMR ($CDCl_3$, 75 MHz, 60 °C) $\delta$ 28.28, 53.22, 57.20, 80.73, 127.17, 127.30, 128.41, 128.64, 128.80, 128.82, 139.62, 140.31, 155.50, 175.29. HRMS (ESI) $C_{20}H_{23}NO_4Na$ $[M+Na]^+$: calcd 364.15193, found 364.15106.

### 4.8. Synthesis of benzyl 2-(*N*-*tert*-butoxycarbonylamino)-3,3-diphenylpropanoate, *rac*-11

A freshly prepared solution of phenyldiazomethane (10.74 mmol) in benzene (80 mL) was added slowly to a suspension of *rac*-**5** (2.44 g, 7.16 mmol) in benzene (25 mL) at room temperature until a permanently coloured solution was obtained. Excess diazoalkane was then destroyed by the addition of a small quantity of silica gel. The crude product obtained after evaporation of the solvent was purified by column chromatography (eluent:

hexanes/ethyl acetate 8/2) to afford pure *rac*-**11** as a white solid (2.86 g, 6.64 mmol, 93% yield). Mp 91 °C (hexanes/ethyl acetate). IR (Nujol) $\nu$ 3348, 1731, 1695 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz, 60 °C) $\delta$ 1.34 (s, 9H), 4.36 (d, 1H, $J = 8.4$ Hz), 4.76 (d, 1H, $J = 8.8$ Hz), 4.94 (s, 2H), 5.08 (dd, 1H, $J = 8.8$, 8.4 Hz), 6.99–7.33 (m, 15H). $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 28.25, 53.70, 56.85, 67.04, 80.14, 127.10, 127.15, 128.21, 128.37, 128.42, 128.59, 128.64, 128.72, 135.12, 139.66, 140.23, 155.21, 172.03. HRMS (ESI) $C_{27}H_{29}NO_4Na$ $[M+Na]^+$: calcd 454.19888, found 454.19725.

*Caution*: Phenyldiazomethane must be handled with care in a well-ventilated hood.

### 4.9. Resolution of *rac*-11: isolation of benzyl (*R*)- and (*S*)-2-(*N*-*tert*-butoxycarbonylamino)-3,3-diphenylpropanoate, (*R*)-11 and (*S*)-11

HPLC resolution of *rac*-**11** (2.15 g) dissolved in chloroform (4.30 mL) was carried out by successive injections of 0.9 mL on a 250 × 20 mm ID Chiralpak® IA column using a mixture of *n*-hexane/2-propanol 80/20 as the eluent (flow rate: 18 mL/min; UV detection: 265 nm). Three separate fractions were collected. Enantiomerically pure (*R*)-**11** (1.062 g) was isolated by evaporation of the first fraction. In a similar way, (*S*)-**11** (1.048 g) was obtained in optically pure form from the third fraction. (*R*)-**11**: white solid; mp 120 °C; $[\alpha]_D = -33.1$ (*c* 0.64, $CHCl_3$). (*S*)-**11**: white solid; mp 120 °C; $[\alpha]_D = +33.4$ (*c* 0.62, $CHCl_3$). Spectroscopic data for both (*R*)- and (*S*)-**11** are the same as those described above for *rac*-**11**.

### 4.10. Synthesis of (*R*)-2-(*N*-*tert*-butoxycarbonylamino)-3,3-diphenylpropanoic acid, (*R*)-5

A solution of (*R*)-**11** (974 mg, 2.26 mmol) in ethyl acetate (50 mL) was hydrogenated at room temperature in the presence of 10% palladium–carbon (97 mg). After 24 h, filtration of the catalyst and evaporation of the solvent afforded pure (*R*)-**5** as a white solid (770 mg, 2.26 mmol, 100% yield). Mp 152 °C. $[\alpha]_D = -36.8$ (*c* 0.60, MeOH) [lit.[25] −35.7 (*c* 1.0, MeOH); lit.[26] −36.2 (*c* 1.0, MeOH); lit.[27] −35.8 (*c* 1.0, MeOH)]. Spectroscopic data are identical to those reported for *rac*-**5**.

### 4.11. Synthesis of (*S*)-2-(*N*-*tert*-butoxycarbonylamino)-3,3-diphenylpropanoic acid, (*S*)-5

An identical procedure to that described above was applied to transform (*S*)-**11** (989 mg, 2.29 mmol) into (*S*)-**5** (780 mg, 2.29 mmol, 100% yield). Mp 152 °C. $[\alpha]_D = +36.7$ (*c* 0.59, MeOH) [lit.[15] +33.6 (*c* 0.50, MeOH); lit.[25] +32.2 (*c* 1.0, MeOH)]. Spectroscopic data are the same as those described for *rac*-**5**.

### 4.12. Synthesis of methyl 2-benzamido-2-(9-fluorenylidene)acetate, 7

A 2% solution of sodium methoxide in absolute methanol (20 mL) was added to **6** (6.55 g, 20.27 mmol) and the reaction mixture stirred at room temperature until TLC analysis indicated that the starting material had been consumed (ca. 6 h). The product was collected by vacuum filtration and washed with small portions of cold methanol; yellow solid (6.33 g, 17.83 mmol, 88% yield). Mp 214–215 °C (MeOH). IR (Nujol) $\nu$ 3248, 1727, 1644 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 3.96 (s, 3H), 7.08–7.96 (m, 13H), 8.44 (sa, 1H). $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 53.01, 119.75, 120.43, 123.57, 124.25, 126.40, 127.19, 127.54, 128.65, 129.09, 132.26, 132.79, 135.52, 136.27, 139.87, 140.71, 164.93, 165.80. HRMS (ESI) $C_{23}H_{17}NO_3Na$ $[M+Na]^+$: calcd 378.10859, found 378.11006.

### 4.13. Synthesis of methyl 2-benzamido-2-(9-fluorenyl)-acetate, *rac*-8

A solution of **7** (6.09 g, 17.15 mmol) in methanol/dichloromethane 85/15 (60 mL) was hydrogenated at room temperature in the presence of 10% palladium–carbon (300 mg). After 2 h, filtration of the catalyst on Celite® and evaporation of the solvent afforded pure *rac*-**8** (6.11 g, 17.11 mmol, 100% yield) as a white solid. Mp 173 °C. IR (Nujol) $\nu$ 3274, 1734, 1643 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 3.78 (s, 3H), 4.70 (d, 1H, $J = 3.0$ Hz), 5.68 (dd, 1H, $J = 3.0$, 8.7 Hz), 5.97 (d, 1H, $J = 8.7$ Hz), 7.22–7.81 (m, 13H). $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 49.20, 52.59, 54.04, 119.78, 120.18, 124.71, 124.86, 126.81, 127.40, 127.59, 128.04, 128.42, 131.57, 133.80, 141.00, 141.16, 142.20, 142.84, 167.28, 171.47. HRMS (ESI) $C_{23}H_{19}NO_3Na$ $[M+Na]^+$: calcd 380.12626, found 380.12507.

### 4.14. Synthesis of 2-amino-2-(9-fluorenyl)acetic acid hydrochloride, *rac*-9

Acetic acid (50 mL) and 6 M HCl (50 mL) were added to *rac*-**8** (5.16 g, 14.45 mmol) and the reaction mixture was heated under reflux for 48 h. The solvent was evaporated and the resulting solid was washed with small portions of diethyl ether. The residue was dissolved in water and lyophilized; white solid (3.77 g, 13.69 mmol, 95% yield). Mp 234 °C (dec). IR (Nujol) $\nu$ 3350–2800, 1722 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$, 300 MHz) $\delta$ 4.65 (m, 1H), 4.85 (m, 1H), 7.32–7.59 (m, 5H), 7.84–7.91 (m, 3H), 8.49 (bs, 3H). $^{13}C$ NMR (DMSO-$d_6$, 75 MHz) $\delta$ 47.06, 53.85, 120.27, 124.83, 125.54, 127.23, 127.41, 128.10, 128.23, 140.92, 141.07, 141.16, 141.58, 168.97. HRMS (ESI) $C_{15}H_{14}NO_2$ $[M-Cl]^+$: calcd 240.10245, found 240.10188.

### 4.15. Synthesis of 2-(*N*-*tert*-butoxycarbonylamino)-2-(9-fluorenyl)acetic acid, *rac*-10

A suspension of *rac*-**9** (3.06 g, 11.10 mmol) in a mixture of dioxane (22.2 mL) and aqueous 1 M NaOH (22.2 mL) was cooled in an ice-water bath. Di-*tert*-butyl dicarbonate (2.66 g, 12.21 mmol) was added and the reaction mixture kept at room temperature for 15 h. Another portion of di-*tert*-butyl carbonate (1.21 g, 5.55 mmol) was then added and stirring continued for an additional 24 h. After evaporation of the solvent, the residue was taken up in water (200 mL) and washed with hexanes (2 × 60 mL). The aqueous phase was then acidified with solid citric acid to pH 2–3. The product was collected by vacuum filtration and washed with water; white solid (3.35 g, 9.89 mmol, 89%

yield). Mp 235 °C (diisopropyl ether). IR (Nujol) $\nu$ 3409, 3200–2850, 1725, 1705 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 1.15 (s, 9H), 4.28 (m, 1H), 4.56 (m, 1H), 5.16 (m, 1H), 7.12–7.78 (m, 8H). $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 28.05, 48.77, 55.13, 80.47, 119.85, 120.30, 124.65, 124.98, 127.42, 127.95, 128.40, 128.92, 140.78, 141.20, 142.38, 142.80, 155.80, 175.36. HRMS (ESI) $C_{20}H_{21}NO_4Na$ $[M+Na]^+$: calcd 362.13628, found 362.13558.

### 4.16. Synthesis of benzyl 2-(*N*-*tert*-butoxycarbonylamino)-2-(9-fluorenyl)acetate, *rac*-12

A freshly prepared solution of phenyldiazomethane (12.83 mmol) in benzene (90 mL) was added slowly to a suspension of *rac*-**10** (2.90 g, 8.55 mmol) in benzene (25 mL) at room temperature until a permanently coloured solution was obtained. Excess diazoalkane was then destroyed by the addition of a small quantity of silica gel. The crude product obtained after evaporation of the solvent was purified by column chromatography (eluent: hexanes/ethyl acetate 8/2) to afford pure *rac*-**12** as a white solid (3.23 g, 7.53 mmol, 88% yield). Mp 104 °C (hexanes/ethyl acetate). IR (Nujol) $\nu$ 3441, 1736, 1712 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz, 60 °C) $\delta$ 1.28 (s, 9H), 4.54 (m, 2H), 5.13 (s, 2H), 5.18 (m, 1H), 7.12–7.38 (m, 10H), 7.55–7.70 (m, 3H). $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 28.07, 49.24, 55.29, 67.36, 79.88, 119.71, 120.07, 124.71, 124.74, 127.12, 127.21, 127.74, 127.98, 128.43, 128.53, 134.96, 141.14, 141.26, 142.10, 142.83, 155.34, 171.16. HRMS (ESI) $C_{27}H_{27}NO_4Na$ $[M+Na]^+$: calcd 452.18323, found 452.18326.

*Caution*: Phenyldiazomethane must be handled with care in a well-ventilated hood.

### 4.17. Resolution of *rac*-12: isolation of benzyl (*R*)- and (*S*)-2-(*N*-*tert*-butoxycarbonylamino)-2-(9-fluorenyl)acetate, (*R*)-12 and (*S*)-12

HPLC resolution of *rac*-**12** (2.94 g) dissolved in chloroform (7.35 mL) was carried out by successive injections of 0.25 mL on a 250 × 20 mm ID Chiralpak® IA column using a mixture of *n*-hexane/*tert*-butyl methyl ether/2-propanol 80/19/1 as the eluent (flow rate: 18 mL/min; UV detection: 235 nm). Three separate fractions were collected. The first, second and third fractions contained, respectively, 100/0 (1.357 g), 45/55 (210 mg) and 0/100 (1.331 g) mixtures of (*S*)-**12**/(*R*)-**12**. (*S*)-**12**: white solid; mp 95 °C; $[\alpha]_D = +3.4$ (*c* 0.61, $CHCl_3$). (*R*)-**12**: white solid; mp 95 °C; $[\alpha]_D = -3.6$ (*c* 0.61, $CHCl_3$). Spectroscopic data for both (*R*)- and (*S*)-**12** are the same as those described above for *rac*-**12**.

### 4.18. Synthesis of (*S*)-2-(*N*-*tert*-butoxycarbonylamino)-2-(9-fluorenyl)acetic acid, (*S*)-10

A solution of (*S*)-**12** (1.086 g, 2.53 mmol) in ethyl acetate (50 mL) was hydrogenated at room temperature in the presence of 10% palladium–carbon (110 mg). After 8 h, filtration of the catalyst and evaporation of the solvent afforded pure (*S*)-**10** as a white solid (803 mg, 2.37 mmol, 94% yield). Mp 167 °C. $[\alpha]_D = +53.9$ (*c* 0.60, MeOH) [lit.[15] +49.7 (*c* 0.50, MeOH)]. Spectroscopic data are identical to those reported for *rac*-**10**.

### 4.19. Synthesis of (*R*)-2-(*N*-*tert*-butoxycarbonylamino)-2-(9-fluorenyl)acetic acid, (*R*)-10

An identical procedure to that described above was applied to transform (*R*)-**12** (1.270 g, 2.96 mmol) into (*R*)-**10** (999 mg, 2.95 mmol, 100% yield). Mp 167 °C. $[\alpha]_D = -53.8$ (*c* 0.62, MeOH). Spectroscopic data are the same as those described for *rac*-**10**.

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