Synthesis of Free and N^{α}-Fmoc-/N^{γ}-Boc-Protected (2*S*,4*S*)- and (2*S*,4*R*)-4-Aminopipecolic Acids

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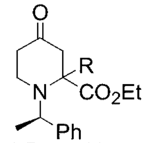
The preparation of (2*S*,4*S*)- and (2*S*,4*R*)-4-aminopipecolic acid, a conformationally constrained basic amino acid bearing orthogonal N^{α}/N^{γ}-protection suitable for solid-phase peptide synthesis, is reported. These amino acids were synthesised from enantiopure ethyl (2*S*)-4-oxo-1-(1-phenylethyl)-piperidine-2-carboxylate (**1**) with introduction of the side-chain amino group by reductive amination, followed by protection/deprotection of the functional groups. A mixture of Fmoc-Sly(Boc)-OH **6** and **7** was used to establish their compatibility in solid-phase peptide synthesis.

Introduction

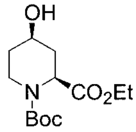
Over recent years considerable efforts have been made in the synthesis of non-natural amino acids employable in the assembly of peptides, with two main goals: i) the synthesis of biologically active peptides capable of surviving proteolysis, and ii) the generation of conformationally constrained peptides able to reproduce the biological activity of larger proteins.[1−3]

As part of a program aimed towards the synthesis of new peptides as pharmacological tools, we required rigid mimics of basic amino acid residues (lysine and ornithine) in both enantiomeric forms, and we focused on a target containing the pipecolic (piperidine-2-carboxylic) acid nucleus, with an additional amino functionality at the C-4.

For both of the natural amino acids cited, an important design consideration may include the restriction of the side chain χ1, χ2 and χ3 (only for lysine) torsion angles,[4] and also of the φ torsion angle between the C^{α} and N^{α} positions. Different conformationally constrained lysine and ornithine analogues have been reported. These include pyrrolidine- and piperidine-based endocyclic-N^{α}/exocyclic-N^{ε} [5−7] or N^{δ} [8−10] analogues, pyrrolidine-containing side chain exocyclic-N^{α}/endocyclic-N^{ε} analogues,[11] azabicyclohexane constrained side chain exocyclic-N^{α}/endocyclic-N^{δ} analogues[12] and anthracene-based exocyclic-N^{α}/exocyclic-N^{ε} analogues.[13]



1 R = α-H
2 R = β-H



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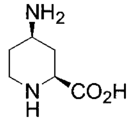

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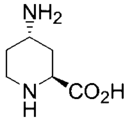

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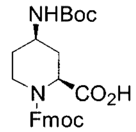

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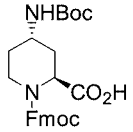
Fmoc-^{cis}Sly(Boc)-OH



7

Fmoc-^{trans}Sly(Boc)-OH

Pipecolic acid substituted with an amino group at C-4, with its constrained φ, χ^1 and χ^2 angles, thus represents an endocyclic-N^{α}/exocyclic-N^{γ} basic constrained amino acid. Our Sly[14] imino acids can also be seen as lysine or ornithine residues bearing shortened side chains. They also mimic the hydrophobicity of lysine, as the two methylene groups formally absent with respect to the lysine side chain are still contained in the pipecolic ring. Furthermore, pipecolic acid derivatives bearing amino functionalities at their C-4 positions are included among the natural occurring non-proteinogenic α-amino acids found in some plants. 4-Aminopipecolic acid has been found in the leaves of *Strophantus scandens* (Apocinaceae),[15−17] its stereochemistry not being assigned with certainty, and (2*S*,4*S*)-4-acetylamino pipecolic acid has been isolated from the leaves of *Calliandra hæmatocephala* (Leguminosæ).[18]

This amino acid moiety has been incorporated into different synthetic biologically active compounds such as cyclic peptides,[19−21] oligomers[22] and small molecules.[23−25] In addition, some biologically active 4-aminopipecolic acid derivatives have also been reported.[26−28] The synthesis of

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racemic 4-aminopipecolic acid through the reduction of 4-aminopicolinic acid with sodium-alcohol has been reported.[15,16,29] Some syntheses of 4-aminopipecolic acids derivatives are known,[28,30,31] but none seems suitable for the synthesis of orthogonally protected 4-aminopipecolic acids **6** and **7**.

In this paper we describe the use of esters **1** and **3** of 4-oxo- and 4-hydroxypipecolic acids to synthesise enantiomerically pure *cis*- and *trans*-(2*S*)-4-aminopipecolic acids, either unprotected (**4** and **5**) or suitably protected for solid-phase peptide synthesis (SPPS) by the Fmoc/*t*Bu strategy (Fmoc-^{cis}Sly-(Boc)-OH **6** and Fmoc-^{trans}Sly-(Boc)-OH **7**).

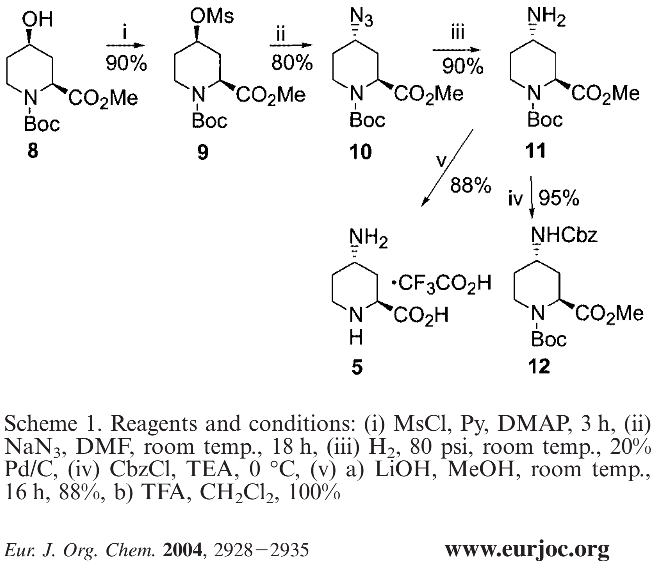
Results and Discussion

We have already described a large-scale method for the asymmetric synthesis of both enantiomers of the protected 4-oxopipecolic acids **1** and **2**, with the 1,3-dipolar cycloaddition between *C*-ethoxycarbonyl-*N*-phenylethylnitrone and but-3-en-1-ol as the key step, followed by the elaboration of the adducts.[32] We have also previously prepared the protected *cis*-4-hydroxypipecolic acid **3** by stereoselective L-Selectride® reduction of the corresponding 4-oxo compound.[33]

The functionality present in these protected 4-oxo- and 4-hydroxypipecolic acids should make them appealing starting material for the synthesis either of 4-aminopipecolic acids **4** and **5**, or of the orthogonally protected amino acids **6** and **7**.

We first investigated which starting material, out of the racemic hydroxy derivative **8** and the oxo derivative **13**, would be preferable for the introduction of an amino functionality at C-4.

In order to synthesise the *trans*-4-aminopipecolic acid isomer we started with an S_N2 reaction on the *cis* alcohol **8**. This compound was treated with methanesulfonyl chloride in pyridine to afford compound **9** in 90% yield and, without further purification, this mesylate was treated with sodium azide in DMF to give the azido compound **10** in 80% yield with inversion of configuration at C-4.



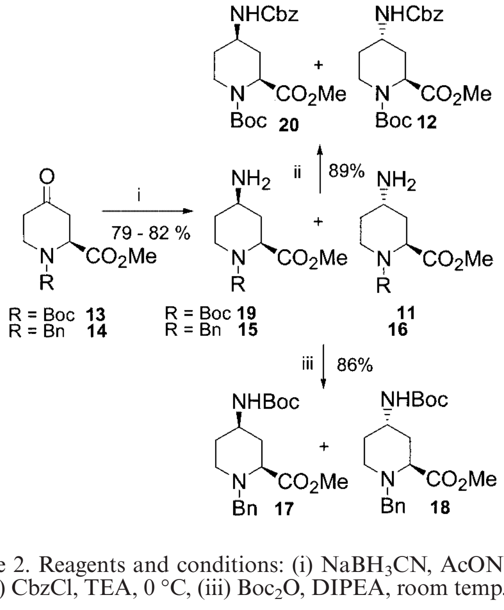
Scheme 1. Reagents and conditions: (i) MsCl, Py, DMAP, 3 h, (ii) NaN_3, DMF, room temp., 18 h, (iii) H_2, 80 psi, room temp., 20% Pd/C, (iv) CbzCl, TEA, 0 °C, (v) a) LiOH, MeOH, room temp., 16 h, 88%, b) TFA, CH_2Cl_2, 100%

Compound **8** could also be transformable into the 4-amino derivative through an aza-Mitsunobu reaction.[34] Indeed, treatment of **8** with PPh_3, diethyl azodicarboxylate (DEAD) and diphenylphosphoryl azide (DPPA)[35] did give the *N*-Boc-4-*trans*-azidopipecolic acid methyl ester (**10**) in low yield.[36] A possible nitrogen donor such as phthalimide was not suitable for that substrate, as the Mitsunobu reaction[37] gave a poor yield (22%) of the corresponding phthalimido ester together with larger amounts (36%) of elimination products.[36]

Hydrogenation of the azide **10** in EtOH in the presence of 20% of palladium/C gave the amino compound **11**, which was directly protected by treatment with benzyl chloroformate to give the final compound **12** in good yield.

Hydrolysis of the methyl ester **11** with lithium hydroxide and subsequent deprotection of the corresponding *N*-Boc acid with trifluoroacetic acid gave the *trans* free amino acid **5** in 88% combined yield.

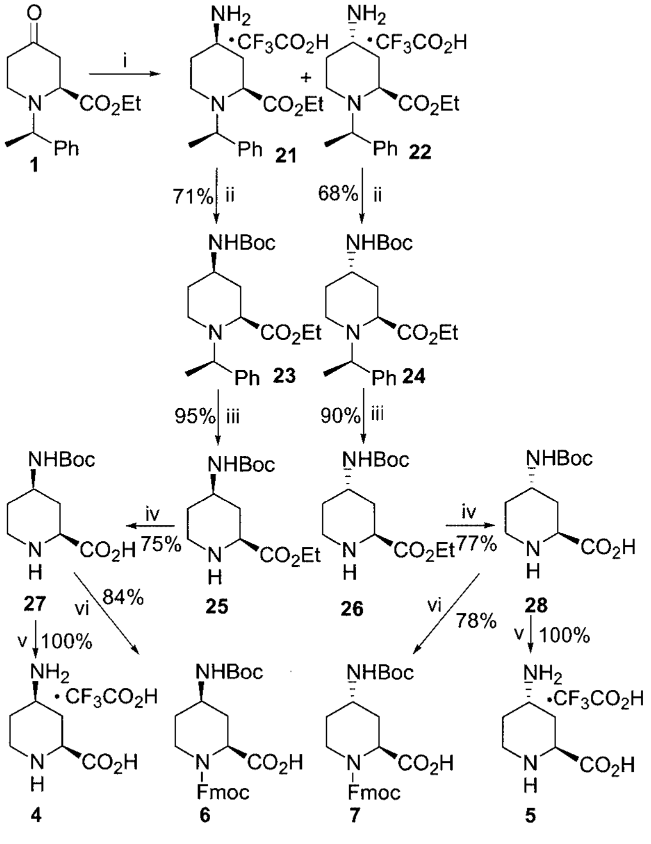
The racemic *N*-Boc- and *N*-Bn-oxopipecolic esters **13** and **14** were then both considered as starting materials. Direct reductive amination[38] with $NaBH_3CN$/ammonium acetate in methanol afforded good yields of equimolecular mixtures of the diastereomeric amines **19** and **11** from the ketone **13** and of the amines **15** and **16** from the ketone **14**. The crude mixture of the amines **15** and **16** was treated with BOC_2O and DIPEA to provide the final compounds **17** and **18**. Similarly, the products **20** and **12** were obtained by treatment of the mixture of **19** and **11** with CbzCl and TEA. The reductive amination of both 4-oxo substrates **13** and **14** was greatly improved by the use of molecular sieves in the reaction mixture (Scheme 2). The synthesis of the above differently protected 4-aminopipecolic acids was accomplished in order to find which pair of diastereoisomers would be most amenable to separation. Attempts to separate the diastereomers by fractional crystallisation failed. Of the candidates, only the couple **15**−**16** proved separable by RP-HPLC. Taking advantage of the experimental conditions found for this pair of compounds, we considered the



Scheme 2. Reagents and conditions: (i) $NaBH_3CN$, $AcONH_4$, 3-Å MS, (ii) CbzCl, TEA, 0 °C, (iii) Boc_2O, DIPEA, room temp., 3 days

use of the reductive amination method for the synthesis of the enantiopure *cis*- and *trans*-4-aminopipecolic acids. Thus, a chromatographic separation followed by suitable deprotection steps might give direct access to both the final compounds **28** and **29**.

Our synthetic route carried out from the enantiopure (1′*R*,2*S*) ketone **1** is shown in Scheme 3. The reductive amination of ketone **1** with ammonium acetate and $NaBH_3CN$ provided an almost equimolecular mixture of the epimeric amines **21** and **22**, in 76% combined yield. These were separated by semipreparative reversed-phase high pressure liquid chromatography (RP-HPLC), and were then in turn protected as N^{γ}-Boc urethanes by standard procedures in good yields.



Scheme 3. Reagents and conditions: (i) a) $NaBH_3CN$, $AcONH_4$, 76%, b) RP-HPLC, (ii) Boc_2O, DIPEA, (iii) H_2, Pd/C(20%), EtOH, (iv) LiOH, MeOH, vi) Fmoc-OSu, 10% Na_2CO_3/H_2O, acetone, room temp., (v) TFA/H_2O

The catalytic hydrogenation of the epimers **23** and **24** was carried out in ethanol in the presence of Pearlman's catalyst. The reaction had gone to completion after one day, to give deprotected N^{α}-amino esters **25** and **26** in high yields. Hydrolysis of ethyl esters **25** and **26** with LiOH gave the acids **27** and **28**, respectively, and these were in turn protected by a (fluorenylmethoxy)carbonyl (Fmoc) group to afford the final orthogonally protected compounds **6** and **7** in good yields.

Stereochemical assignment of compound **6** as the *cis*-2,4-disubstituted piperidine and of **7** as the *trans*-2,4-disubstituted piperidine was achieved by a complete study of the NMR spectroscopic data of the fully deprotected compounds **4** and **5**. The proton assignments were accomplished with the aid of a COSY spectrum, starting from the H-2 protons. The proton H-2 (δ = 3.99 ppm in **4**) displays coupling constants of 12.8 and 3.2 Hz with H-3ax and H-3eq, respectively, requiring H-2 to be axial. The orientation of proton H-4 could not be assigned in the same way, because the coupling constants with protons H-3 and H-5 could not be determined.

The stereochemistry of proton H-4 was determined from ^{1}H-NOESY cross-peaks observed between H-4/H-2 and H-4/H-6ax that indicated their axial orientations (Figure 1). If a chairlike conformation is assumed for the piperidine ring, these NMR considerations are consistent with the *cis* relative configuration for **4** and consequently for **6**. The assignment of the *trans* configuration to the diastereomeric compound **5** is consistent with the observation that its ^{1}H NMR spectrum is identical to that obtained, by deprotection by standard procedures, of the *trans* racemic compound **11** and it is also consistent with NMR spectroscopic data. The resonance frequencies of protons H-4 and H-6 are too close for the different signals to be distinguished. Addition of trifluoroacetic acid to the NMR tube (pH = 1) shifted the H-4 signal (δ = 3.48 ppm) downfield, after which it then appeared as a triplet of triplets with a large coupling constant (14.0 Hz), indicating that this proton was in an axial orientation. The methyne proton H-2 (δ = 4.43 ppm, triplet) shows a low value of the two $J_{H\text{-}2,H\text{-}3}$ coupling constants (< 5 Hz), indicating an axial orientation of the carboxylic group (Figure 1). If a chairlike conformation is assumed for the piperidine ring, these NMR considerations are consistent with a *trans* relative configuration for **5** and consequently for **7**.

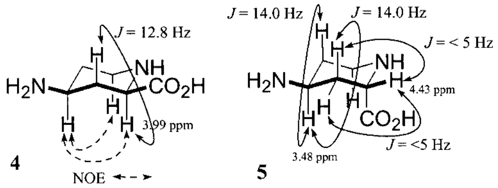


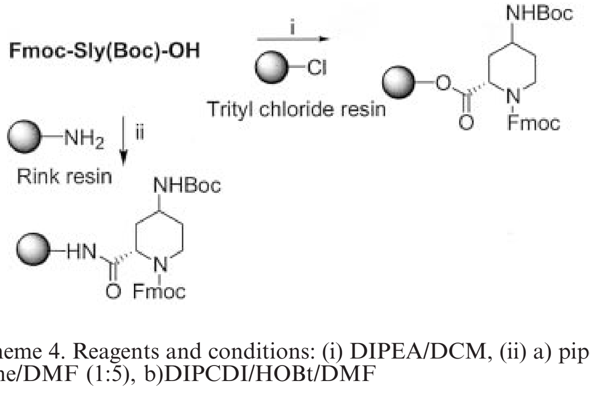
Figure 1. Determination of the relative configuration of compounds **4** and **5** by means of coupling constants and NOE

The assignment of the structure of **5** allowed the relative configurations of 4-*N*-substituted natural pipecolic acids to be elucidated. Marlier et al. reported the isolation of a 4-acetylaminopipecolic acid from the leaves of *Calliandra haematocephala*.[18] They obtained the parent 4-aminopipecolic acid by acid hydrolysis of the corresponding natural amide and ascribed the 2*S* configuration and the *trans* stereochemistry to these amino acids. The *trans* relative configuration was assigned by ^{1}H NMR considerations but the authors wrongly reported the stereoisomer as (2*S*,4*R*) instead of (2*S*,4*S*). Consequently this compound is reported in *Chemical Abstract* as (2*S*) *cis*.[39] Comparison of our ^{1}H

NMR spectra with that reported by Marlier now allows the correct configuration of the natural compound to be unambiguously established as (2*S*,4*S*).

In the earlier literature relating to the natural 4-aminopipecolic acid the stereochemistry was not indicated.

Finally we evaluated the coupling of the constrained Sly amino acids to solid supports, anchoring them to two different resins commonly used for Fmoc/*t*Bu SPPS: Rink resin and trityl chloride resin (Scheme 4). While the Rink resin was chosen for demonstrating the possible preparation of peptide amides containing the Sly amino acids at the *C*-terminal position, the trityl resin was selected to further potential peptide coupling. Coupling of the Sly amino acid to the Rink resin by amide bond formation was performed by use of DIPCDI (1,3-diisopropylcarbodiimide) and HOBt (1-hydroxybenzotriazole) for 16 h at room temperature. The loading determined by the Fmoc release, monitored by UV absorption at 301 nm, showed quantitative anchoring of the amino acid (98% yield). The trityl resin proved preferable because it allows the modulation of the final loading with low amounts of the first amino acid (stoichiometric) for its functionalization, in comparison to the Wang resin, which required 10 equivalents of the first amino acid. Moreover, the steric hindrance of the trityl resin reduces or totally blocks the formation of diketopiperazines at the dipeptide level during the deprotection step, when low resin loadings are used.[35] The use of a such resin is therefore particularly important when the peptide sequence includes elements favouring diketopiperazine formation, such as imino acids stabilizing a *cis* amide bond (as in our Sly amino acid) or a D-/L-amino acid combination. In order to obtain a low resin loading, coupling to the trityl resin (0.080 mmol) was performed with 0.080 mmol of the Sly amino acid, and DIPEA for 2 h at room temperature. The loading (0.27 mmol/g) was determined from the Fmoc release, monitored by UV absorption at 301 nm (34% yield).



Scheme 4. Reagents and conditions: (i) DIPEA/DCM, (ii) a) piperidine/DMF (1:5), b)DIPCDI/HOBt/DMF

Conclusions

In conclusion, two novel basic constrained amino acids **6** and **7**, each incorporating a rigid pipecolic skeleton, were synthesised. These amino acids were synthesised from enantiopure ethyl (2*S*)-4-oxo-1-(1-phenylethyl)piperidine-2-carboxylate (**1**), the side-chain amino group being introduced by reductive amination. Amino acids **6** and **7** are suitably protected for solid-phase organic synthesis. The incorporation of these constrained amino acids into peptides is under investigation.

Experimental Section

General Remarks: Melting points are uncorrected and were measured with a RCH Kofler microscope apparatus. Chromatographic separations were performed under pressure by FCC (flash column chromatography; silica gel unless otherwise stated); R_f values refer to TLC, carried out on 0.25 mm silica gel plates (Merck F_{254}) with the same eluent as indicated for the column chromatography. IR spectra ($CDCl_3$ solution unless otherwise stated) were recorded on a Perkin−Elmer 881 spectrophotometer, and NMR spectra ($CDCl_3$ solution unless otherwise stated) on Varian Gemini 200 and Varian Mercury 400 (^{1}H, 200, 400 MHz; ^{13}C 50, 100 MHz respectively) spectrometers: the chemical shifts are given in ppm from TMS. Coupling constants *J* (in Hz) refer to $^3J_{H,H}$. EI mass spectra were carried out at 70 eV ionizing voltage by direct introduction into a QMD 1000 Carlo−Erba instrument. The electrospray mass spectra were performed on a ThermoFinnigan LCQ Advantage Ion Trap apparatus. Microanalyses were carried out with a Perkin−Elmer 240C elemental analyser. Optical rotation measurements were carried out with a JASCO DIP-360 digital polarimeter. All reactions requiring anhydrous conditions were performed in oven-dried glassware. Analytical reversed-phase HPLC was carried out on a Beckman System Gold instrument fitted with a diode array detector, on a 5 μm (250 × 4.6 mm) C18 Vydac column. Semipreparative purifications were performed with a 10 × 250 mm C18 Vydac column. The flow rates were 1 mL/min for analytical HPLC and 4 mL/min for semipreparative HPLC.

1-*tert*-Butyl 2-Methyl *cis*-4-Hydroxypiperidine-1,2-dicarboxylate (8): A solution of L-Selectride in THF (1.0 M, 1.14 mL) was added dropwise at −78 °C to a previously prepared solution of methyl 1-*tert*-butoxycarbonyl-4-oxopiperidine-2-carboxylate (208 mg, 0.80 mmol) in dry THF (16 mL). The reaction mixture was stirred at −78 °C for 2 h, and the temperature was then slowly increased to 0 °C. After the mixture had been stirred for 30 min, satd. aq. NH_4Cl was added (16 mL). The aqueous layer was extracted with EtOAc (3 × 16 mL). The combined organic layer was washed with water (3 × 20 mL) and brine (3 × 20 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue oil was purified by FCC (CH_2Cl_2/MeOH, 15:1, R_f = 0.34) to afford a colourless oil corresponding to compound **8** (120 mg, 58%). ^{1}H NMR: δ = 1.41 [s, 9 H, C(CH_3)$_3$], 1.50−1.75 (m, 2 H, H-3ax and H-5ax), 1.89 (ddd, *J* = 2.9, 6.8, 15.5 Hz, 1 H, H-5eq), 2.40 (dm, *J* = 2.7 Hz, 1 H, H-3eq), 3.20−3.42 (m, 1 H, H-6ax), 3.71 (s, 3 H, OCH_3), 3.60−3.90 (m, 1 H, H-6eq), 4.08−4.18 (m, 1 H, H-4), 4.65 and 4.80 (rotamers, br. s, 1 H, H-2) ppm. ^{13}C NMR: δ = 27.8 [q, 3 C, C(CH_3)$_3$], 30.6 (t, C-5), 32.9 (t, C-3), 34.6 and 35.7 (rotamers, t, C-6), 49.9 and 51.0 (rotamers d, C-2), 51.5 (q, OCH_3), 62.0 (d, C-4), 79.6 (d, *C*Me$_3$), 155.3 (s, *C*O$_2$*t*Bu), 172.6 (s, *C*O$_2$Me) ppm. MS (EI): *m*/*z* (%) = 200 (6) $[M - CO_2Me]^+$, 144 (60), 100 (50), 82 (21), 57 (100). IR (cm^{-1}): $\tilde{\nu}$ = 2979, 2955, 2904, 1725, 1686. $C_{12}H_{21}NO_5$ (259.30): calcd. C 55.58, H 8.16, N 5.40; found C 55.30, H 8.35, N 5.10.

1-*tert*-Butyl 2-Methyl *cis*-4-[(Methylsulfonyl)oxy]piperidine-1,2-dicarboxylate (9): MsCl (0.036 mL, 0.46 mmol) was added dropwise at 0 °C to a solution of the alcohol **8** (116 mg, 0.45 mmol) and

DMAP (4 mg) in pyridine (1 mL). The mixture was stirred at room temperature for 3 h and was then quenched with satd. aq. NaCl (1 mL) and extracted with CH_2Cl_2 (3 × 2 mL). The organic phases were dried with Na_2SO_4, filtered and concentrated under reduced pressure to give **9** (135 mg, 90%) as a syrup, which was employed in the next step without further purification. An analytical sample was obtained by FCC (CH_2Cl_2/MeOH, 20:1, R_f = 0.34). ^1H NMR: δ = 1.44 [s, 9 H, $C(CH_3)_3$], 1.60−1.82 (m, 2 H, H-3ax and H-5ax), 1.98 (ddd, *J* = 15.0, 7.5, 2.6 Hz, 1 H, H-5eq), 2.60−2.75 (dm, *J* = 16 Hz, 1 H, H-3eq), 2.95 (s, 3 H, SO_2CH_3), 3.20−3.42 (m, 1 H, H-6ax), 3.71 (s, 3 H, OCH_3), 3.80−4.12 (br. m, 1 H, H-6eq), 4.82 and 4.92 (rotamers, br. s, 1 H, H-2), 5.01−5.08 (m, 1 H, H-4) ppm. ^{13}C NMR: δ = 28.3 [q, 3 C, $C(CH_3)_3$], 29.8 (t, C-5), 31.5 (t, C-3), 36.2 (q, SO_2CH_3), 38.5 (t, C-6), 51.2 and 50.1 (rotamers, d, C-2), 51.4 (q, OCH_3), 74.6 (d, C-4), 80.6 (s, *C*Me₃), 151.3 (s, CO_2tBu), 171.2 (s, CO_2Me) ppm. IR (cm^{-1}): $\tilde{\nu}$ = 1720, 1670, 1354. MS (EI): *m*/*z* (%) = 278 (6) [M − CO_2Me], 222 (16), 182 (15), 154 (15), 126 (71), 82 (100), 57 (74). $C_{13}H_{23}NO_7S$ (337.39): calcd. C 46.28, H 6.87, N 4.15; found C 46.46, H 6.97, N 4.08.

1-*tert*-Butyl 2-Methyl *trans*-4-Azidopiperidine-1,2-dicarboxylate (10): NaN_3 (44 mg, 0.68 mmol) was added to a solution of compound **9** (60 mg, 0.18 mmol) in dry DMF (0.5 mL) under nitrogen. The mixture was heated at 60 °C for 18 h, H_2O (0.1 mL) was then added, and the solvent was evaporated under reduced pressure. The crude residue was purified by FCC (CH_2Cl_2; R_f = 0.30) to afford a clear oil corresponding to compound **10** (41 mg, 80%). ^1H NMR: δ = (rotamers) 1.44 [s, 9 H, $C(CH_3)_3$], 1.40−1.75 (m, 2 H, H-3ax and H-5ax), 1.80−2.04 (m, 1 H, H-5eq), 2.38−2.58 (m, 1 H, H-3eq), 2.81−3.12 (m, 1 H, H-4), 3.20−3.42 (m, 1 H, H-6ax), 3.72 (s, 3 H, OCH_3), 4.00−4.20 (m, 1 H, H-6eq), 4.82 and 5.02 (br. s, 1 H, H-2) ppm. ^{13}C NMR (rotamers): δ = 28.3 [q, 3 C, $C(CH_3)_3$], 30.4 (t, C-5), 31.9 (t, C-3), 39.7 and 40.5 (t, C-6), 53.3 and 54.3 (d, C-2), 55.4 (d, C-4), 80.7 (s, *C*Me₃), 151.2 (s, CO_2tBu), 173.5 (s, CO_2Me) ppm. MS (EI): *m*/*z* (%) = 225 (8) [M − CO_2Me]$^+$, 197 (8), 169 (41), 141 (11), 125 (31), 97 (11), 57 (100). IR (cm^{-1}): $\tilde{\nu}$ = 2095, 1724, 1672. $C_{12}H_{20}N_4O_4$ (284.14): calcd. C 50.69, H 7.09, N 19.71; found C 50.44, H 7.19, N 19.91.

1-*tert*-Butyl 2-Methyl *trans*-4-Aminopiperidine-1,2-dicarboxylate (11): The azide **10** (80 mg, 0.28 mmol) was dissolved in MeOH (0.56 mL), Pd/C (20%, 80 mg) was added, and the apparatus was flushed three times with H_2. The reaction mixture was hydrogenated at a pressure of 80 psi at room temperature for 16 h, filtered through a short pad of Celite (3 × 1 mL rinse) and then concentrated under reduced pressure to give the amine **11** (64 mg, 90%), sufficiently pure to be used in the next step without further purification. An analytical sample was obtained by chromatography on basic alumina (CH_2Cl_2, R_f = 0.43). Clear oil. ^1H NMR (mixture of rotamers): δ = 1.42 and 1.45 [s, 9 H, $C(CH_3)_3$], 1.70−1.90 (m, 3 H, H-5eq, H-5ax, H-3ax), 2.20−2.38 (m, 1 H, H-3eq), 2.58−2.80 (m, 1 H, H-4), 2.80−3.10 (m, 1 H, H-6ax), 3.70 (s, 3 H, OCH_3), 3.88−4.13 (m, 1 H, H-6eq), 4.80 and 4.96 (br. s, 1 H, H-2) ppm. ^{13}C NMR (mixture of rotamers): δ = 28.2 [q, 3 C, $(CH_3)_3C$], 34.6 (t, C-5), 36.1 (t, C-3), 40.2 and 41.0 (t, C-6), 45.7 and 45.8 (d, C-4), 52.1 (q, OCH_3), 53.5 and 54.6 (d, C-2), 80.2 (s, *C*Me₃), 155.2 and 155.6 (s, CO_2tBu), 171.9 (s, CO_2Me) ppm. MS (EI): *m*/*z* (%) = 258 (3) [M$^+$], 201 (7), 199 (8), 157 (25), 143 (28), 141 (30), 99 (87), 86 (52), 84 (80), 57 (100). IR (cm^{-1}): $\tilde{\nu}$ = 3356, 3300 (br), 2926, 2851, 1739, 1692. $C_{12}H_{22}N_2O_4$ (258.32): calcd. C 55.80, H 8.58, N 10.84; found C 55.56, H 8.54, N 10.59.

***1-tert*-Butyl 2-Methyl *trans*-4-{[(Benzyloxy)carbonyl]amino}-piperidine-1,2-dicarboxylate (12):** Benzyl chloroformate (0.030 mL, 0.20 mmol) was added dropwise at 0 °C to a solution of the amine **11** (40 mg, 0.15 mmol) and TEA (0.030 mL, 0.20 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred at room temperature for 12 h. The solvent was evaporated, and the residue was purified by flash chromatography (petroleum ether/ethyl acetate, 3:1; R_f = 0.20) to afford a clear oil corresponding to compound **12** (58 mg, 95%). ^1H NMR (mixture of rotamers): δ = 1.41 and 1.44 [s, 9 H, $C(CH_3)_3$], 1.60−1.70 (m, 1 H, H-5ax), 1.95−2.10 (m, 1 H, H-3ax), 2.50 (dm, *J* = 14 Hz, 1 H, H-5eq), 2.95−3.20 (m, 1 H, H-3eq), 3.40−3.65 (m, 1 H, H-6ax), 3.73 (s, 3 H, OCH_3), 3.90−4.05 (m, 2 H, H-6eq, and H-4), 4.54−4.68 (m, 1 H, NHCO), 4.80 and 5.00 (br. s, 1 H, H-2), 5.07 (s, 2 H, OCH_2Ph), 7.32 (s, 5 H, Ar-H) ppm. ^{13}C NMR (mixture of rotamers): δ = 28.3 [q, 3 C, $(CH_3)_3C$], 31.9 (t, C-5), 33.0 (t, C-3), 40.1 and 40.8 (t, C-6), 45.9 (d, C-4), 52.4 (q, OCH_3), 53.5 and 54.6 (d, C-2), 66.8 (t, OCH_2Ph), 80.5 (s, *C*Me₃), 128.1 (d, Ar-H), 128.2 (d, Ar-H), 128.6 (d, Ar-H), 136.3 (s, Ar-H), 155.4 and 155.5 (s, CO_2tBu), 171.4 (s, CO_2Me) ppm. IR (cm^{-1}): $\tilde{\nu}$ = 3439, 2977, 2953, 1760−1660. MS (EI): *m*/*z* (%) = 336 (4) [M − Me_2CH_2]$^+$, 291 (4), 277 (9) [M − CO_2Me], 213 (11), 169 (20), 140 (49), 125 (31), 91 (92), 82 (73), 79 (12), 57 (100). $C_{20}H_{28}N_2O_6$ (392.44): calcd. C 61.21, H 7.19, N 7.14; found C 61.56, H 7.52, N 6.89.

Methyl *cis*- and *trans*-4-Amino-1-benzylpiperidine-2-carboxylate (15 and 16): NH_4AcO (2.58 g, 33.5 mmol) and $NaBH_3CN$ (190 mg, 3.03 mmol) were added to a solution of ketone **16** (0.790 g, 3.03 mmol) in dry MeOH (9.5 mL) in the presence of molecular sieves (3Å). The resulting mixture was stirred at room temperature for 48 h. The mixture was filtered and concentrated under reduced pressure. The resulting residue was purified by chromatography on basic alumina (CH_2Cl_2, R_f = 0.63) to give an equimolecular mixture of the amines **15** and **16** (0.620 g, 82% combined yield). Yellow oil. ^1H NMR: δ = 1.60−1.95 (m, 2 H, H-3 and H-5), 2.18−2.40 (m, 1 H, H-5), 2.35−2.64 (m, 1 H, H-3), 2.74−3.22 (m, 2 H, CH_2Ph), 3.38−3.62 (m, 1 H, H-4), 3.66−3.86 (m, Ar-H) ppm. MS (EI): *m*/*z* (%) = 248 (1) [M$^+$], 189 (4) [M$^+$ − CO_2Me], 174 (39), 172 (22), 91 (100), 86 (22), 84 (35). IR (cm^{-1}): $\tilde{\nu}$ = 3360−3280 (br), 3179, 3085, 3063, 3029, 2980, 2963, 2929, 2855, 1724. $C_{14}H_{20}N_2O_2$ (248.32): calcd. C 67.71, H 8.12, N 11.28; found C 67.56, H 8.02, N 11.48.

Methyl *cis*- and *trans*-1-Benzyl-4-[(*tert*-butoxycarbonyl)amino]piperidine-2-carboxylate (17 and 19): Boc_2O (416 mg, 1.91 mmol) was added portionwise to a solution of the amines **15** and **16** (500 mg, 1.91 mmol) and DIPEA (0.35 mL, 1.91 mmol) in CH_2Cl_2/EtOH (4:1, 4 mL). The mixture was stirred at room temperature for 3 days and was then concentrated under reduced pressure. The residue was partitioned between Et_2O/CH_2Cl_2 (2:1) and $NaHSO_4$ (10%). The aqueous layer was extracted with Et_2O/CH_2Cl_2 (2:1). The solvent was removed from the organic combined solutions and the residue was purified by FCC (CH_2Cl_2/MeOH, 9:1; R_f = 0.51) to give an equimolecular mixture of **17** and **19** (594 mg, 1.64 mmol, 86% yield). Clear oil. ^1H NMR (mixture of rotamers): δ = 1.50−2.09 (m, 2 H, H-3 and H-5), 1.41 and 1.44 [s, 9 H, $C(CH_3)_3$], 2.20−2.40 (m, 1 H, H-3 and H-5), 2.45−2.68 (m, 1 H, H-6), 2.80−3.20 (m, 2 H, H-6 and H-4), 3.32−3.72 (m, 2 H, CH_2Ph), 3.77 and 3.78 (s, 3 H, OCH_3), 4.05 −4.40 (m, 2 H, H-2 and BocN*H*), 4.70−4.85 (m, 1 H), 7.12−7.40 (m, 5 H, Ar-H) ppm. ^{13}C NMR: δ = 31.7 (t), 31.9 (t), 33.7 (t), 35.9 (t), 37.1 (t), 45.9 (t), 47.2 (t), 49.5 (d), 59.9 (d), 60.3 (t), 60.8 (t), 63.0 (d), 64.8 (d), 66.9 (d), 81.1 (s), 127.0 (d), 127.2 (d), 128.1 (d), 128.7 (d), 129.1 (d), 129.3 (d), 137.4 (s), 138.6 (s), 155.0 (s), 172.8 (s), 173.1 (s) ppm. MS (EI): *m*/*z* (%) = 363 (2) [M$^+$], 289 (10), 190 (11), 172 (87), 91 (100), 65 (12), 57 (40), 55 (10). $C_{19}H_{28}N_2O_4$ (348.44): calcd. C 65.49, H 8.10, N 8.04; found C 65.56, H 8.02, N 7.88.

1-*tert*-Butyl 2-Methyl *cis*- and *trans*-4-Aminopiperidine-1,2-dicarboxylate (19 and 11): The procedure described above for the synthesis of the amines **15** and **16** was applied to ketone **13** (790 mg, 3.03 mmol), to give a 3:2 mixture of the amines **19** and **11** (618 mg, 79% combined yield). Purification: FCC on neutral alumina (CH_2Cl_2/MeOH, 25:1; $R_f = 0.41$). Yellow oil. ^1H NMR (amide rotamers): $\delta = 1.42$ and 1.44 [s, 9 H, $C(CH_3)_3$], 1.70−1.90 (m, 3 H), 2.10−2.40 (m, 1 H), 2.50−2.75 (m, 1 H), 2.80−3.10 (m, 1 H), 3.20−3.40 (m, 1 H, **11**), 3.70 (s, 3 H), 3.80−4.18 (m, 1 H, **19**), 4.42−4.65 (m, 1 H, **11**: H-2), 4.96 and 4.80 (br. s, 1 H, **19**: H-2) ppm. ^{13}C NMR (signal of *cis* isomer **19**, amide rotamers): $\delta = 29.5$, 29.6, 31.5, 33.4, 37.3, 38.0, 44.2, 44.9, 45.8, 47.6, 50.4, 51.3, 51.9, 79.8 155.2, 155.6, 173.2 ppm. MS (EI): *m/z* (%) = 258 (11) [M^+], 203 (18), 159 (20), 99 (56), 57 (100). $C_{12}H_{22}N_2O_4$ (258.31): calcd. C 55.80, H 8.58, N 10.84; found C 55.72, H 8.40, N 10.76.

1-*tert*-Butyl 2-Methyl *cis- and trans*-4-{[(Benzyloxy)carbonyl]amino}piperidine-1,2-dicarboxylate (20 and 12): The procedure described above for the synthesis of **12** was applied to a mixture of amines **21** and **13** (80 mg, 0.32 mmol), to give a mixture of protected amines **20** and **12** (141 mg) in 89% yield. Purification: FCC (petroleum ether/ethyl acetate, 3:1; $R_f = 0.20$). Clear oil. ^1H NMR (mixture of rotamers): $\delta = 1.41$ and 1.44 (s, 9 H), 1.70−2.10 (m, 2 H), 1.95−2.10 (m, 1 H), 2.40−2.50 (m, 1 H), 2.95−3.20 (m, 1 H), 3.40−3.68 (m, 1 H), 3.73 and 3.71 (s, 3 H), 3.90−4.10 (m, 2 H), 4.50−4.60 (m, 1 H), 4.80−5.05 (br. m, 1 H), 4.99 and 5.07 (s, 2 H), 7.32 and 7.38 (s, 5 H) ppm. MS (EI): *m/z* (%) = 277 (9), 213 (11), 169 (19), 140 (49), 125 (31), 108 (11), 107 (10), 91 (92), 82 (73), 79 (12), 57 (100). $C_{20}H_{28}N_2O_6$ (392.44): calcd. C 61.21, H 7.19, N 7.14; found C 61.26, H 7.42, N 6.94.

(2*S*,4*R*)-2-(Ethoxycarbonyl)-1-[(1*R*)-1-phenylethyl]piperidin-4-aminium Trifluoroacetate (21) and (2*S*,4*S*)-2-(Ethoxycarbonyl)-1-[(1*R*)-1-phenylethyl]piperidin-4-aminium Trifluoroacetate (22): NH_4OAc (7.26 g, 94.2 mmol) and $NaBH_3CN$ (0.418 g, 6.80 mmol) were added to a solution of ketone **1** (1.73 g, 6.28 mmol) in dry EtOH (8 mL) in the presence of molecular sieves (3 Å). The resulting mixture was stirred at room temperature for 48 h. The mixture was filtered and concentrated under reduced pressure. The resulting residue was purified by chromatography on basic alumina (CH_2Cl_2/MeOH, 20:1; $R_f = 0.26$) to give the amines **21** and **22** (1.32 g, 76% combined yield). These amines were separated by preparative RP-HPLC with isocratic 15% H_2O/CH_3CN containing 0.1% TFA as eluent: 0.67 g of **21** (R_t = 13.1 min) and 0.53 g of **22** (R_t = 16.3 min) were obtained.

Compound 21: Colourless oil. $[\alpha]_D^{22} = -5.2$ (c = 0.2, H_2O). ^1H NMR: $\delta = 1.26$ (t, J = 6.8 Hz, 3 H, OCH_2CH_3), 1.58 (d, J = 6 Hz, 3 H, $NCHCH_3$), 1.95−2.60 (m, 4 H, 2 × H-5 and 2 × H-3), 2.94−3.10 (m, 1 H, H-6ax), 3.10−3.32 (m, 1 H), 3.32−4.00 (m, 2 H), 4.00−4.23 (q, J = 6.8 Hz, 2 H, OCH_2CH_3), 4.52−4.71 (m, 1 H, H-2), 7.25−7.38 (m, 5 H, Ar-H) ppm. MS (EI): *m/z* (%) = 276 (< 1) [M^+], 203 (26) [M − CO_2Et], 186 (4), 171 (2), 160 (7), 105 (100). $C_{18}H_{25}F_3N_2O_4$ (390.40): calcd. C 55.38, H 6.45, N 7.18; found C 55.68, H 6.65, N 7.18.

Compound 22: Colourless oil. $[\alpha]_D^{22} = +7.8$ (c = 0.125, H_2O). ^1H NMR: $\delta = 1.28$ (t, J = 7.1 Hz, 3 H, OCH_2CH_3), 1.46 (d, J = 7.0 Hz, 3 H, $NCHCH_3$), 1.85−2.21 (m, 2 H, H-3 and H-5), 2.38−2.65 (m, 2 H, H-3 and H-5), 2.88−3.10 (m, 1 H, H-6ax), 3.25−4.08 (m, 3 H, H-4, H-6eq and $NCHCH_3$), 4.16−4.34 (m, 2 H, OCH_2CH_3), 4.38−4.52 (m, 1 H, H-2), 7.36−7.45 (m, 5 H, Ar-H) ppm. MS (EI): *m/z* (%) = 276 (< 1) [M^+], 203 (45) [M − CO_2Et]$^+$, 186 (4), 171 (6), 160 (12), 105 (100). $C_{18}H_{25}F_3N_2O_4$ (390.40): calcd. C 55.38, H 6.45, N 7.18; found C 55.49, H 6.62, N 6.88.

Ethyl (2*S*,4*R*)-4-[(*tert*-Butoxycarbonyl)amino]-1-[(1*R*)-1-phenylethyl]piperidine-2-carboxylate (23): The trifluoroacetate salt **21** (340 mg, 0.87 mmol) was dissolved in CH_2Cl_2 (5 mL), anhydrous EtOH (1 mL) and DIPEA (0.32 mL, 1.83 mmol), and Boc_2O (209 mg, 0.96 mmol) was then added portionwise. The resulting mixture was stirred for 5 h at room temperature and was then concentrated under reduced pressure. The residue was purified by FCC with hexane/EtOAc (6:1) as eluent to give **23** ($R_f = 0.57$, 232 mg, 71%). Clear oil. $[\alpha]_D^{23} = +26.2$ (c = 1.04, $CHCl_3$). ^1H NMR: $\delta = 1.25$ (d, J = 6.8 Hz, 3 H, $NCHCH_3$), 1.26 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 1.39 [s, 9 H, $(CH_3)_3C$], 1.63−1.85 (m, 2 H, H-3ax and H-5ax), 2.05−2.32 (m, 2 H, H-3eq and H-5eq), 2.62 (ddd, J = 12.0, J = 5.8, J = 3.6 Hz, 1 H, H-6ax), 3.40−3.68 (m, 3 H, H-2, H-6 and H-4), 3.93 (q, J = 6.8 Hz, 1 H, $NCHCH_3$), 4.17 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 4.62 (d, J = 9.0 Hz, 1 H, BocN*H*), 7.18−7.43 (m, 5 H, Ar-H) ppm. ^{13}C NMR: $\delta = 12.7$ (q, OCH_2CH_3), 14.2 (q, $NCHCH_3$), 28.3 [q, 3C, $(CH_3)_3C$], 31.6 (t), 35.6 (t), 41.9 (t, C-6), 46.3 (d), 58.5 (d), 60.3 (d, C-2), 60.6 (t, OCH_2CH_3), 79.2 (s, *C*Me₃), 126.6 (d, Ar-H), 127.5 (d, 2 C, Ar-H), 127.9 (d, 2 C, Ar-H), 143.9 (s, Ar-H), 154.9 (s, NCO_2tBu), 173.7 (s, CO_2Et) ppm. MS (EI): *m/z* (%) = 303 (19) [M − CO_2Et]$^+$, 262 (27), 203 (26), 186 (89), 105 (100), 91 (68), 82 (25), 56 (31). IR (cm^{-1}): $\tilde{\nu}$ = 3440, 2981, 1710, 1506. $C_{21}H_{32}N_2O_4$ (376.49): calcd. C 66.99, H 8.57, N 7.44; found C 70.04, H 8.44, N 7.78.

Ethyl(2*S*,4*S*)-4-[(*tert*-Butoxycarbonyl)amino]-1-[(1*R*)-1-phenylethyl]piperidine-2-carboxylate (24): The procedure described above for the synthesis of *N*-Boc amine **23** was applied to salt **22** (228 mg, 0.58 mmol), to give (14 h room temp.) the *N*-Boc amine **24** (148 mg, 68%) as a white solid (FCC, hexane/EtOAc, 6:1); $R_f = 0.57$. $[\alpha]_D^{23} = +34.2$ (c = 0.5; $CHCl_3$). M. p. 155−156 °C. ^1H NMR: $\delta = 1.25$ (t, J = 6.8 Hz, 3 H, $NCHCH_3$), 1.31 (d, J = 7.2 Hz, 3 H, OCH_2CH_3), 1.41 [s, 9 H, $(CH_3)_3C$], 1.42−1.82 (m, H-3 and H-5), 2.32 (dm, 12.0 Hz, 1 H, H-5), 2.47 (dm, 12.0 Hz, 1 H, H-3), 2.86 (td, J = 12.0, 3.0 Hz, 1 H, H-6), 3.58 (m, 1 H, H-4), 3.91 (q, J = 6.8 Hz, 1 H, $NCHCH_3$), 3.80−3.98 (m, 1 H, H-6), 4.04−4.18 (m, 3 H, H-2 and OCH_2CH_3), 7.15−7.35 (m, 5 H, Ar-H) ppm. ^{13}C NMR: $\delta = 14.5$ (q, OCH_2CH_3), 21.4 (q, $NCHCH_3$), 28.4 [q, 3C, $(CH_3)_3C$], 32.6 (t), 35.1 (t), 36.5 (t, C-6), 44.5 (d), 56.9 (d), 60.3 (t, OCH_2CH_3), 61.3 (d, C-2), 79.6 (s, *C*Me₃), 126.7 (d, Ar-*C*), 126.9 (d, 2 C, Ar-*C*), 128.3 (d, 2 C, Ar-*C*), 140.9 (s, Ar-*C*), 146.3 (s, NCO_2tBu), 173.0 (s, CO_2Et) ppm. MS (EI): *m/z* (%) = 303 (17) [M − CO_2Et]$^+$, 271 (5), 247 (8), 203 (7), 186 (42), 154 (9), 105 (100), 82 (47). IR (cm^{-1}): $\tilde{\nu}$ = 3445, 2978, 1706, 1492. $C_{21}H_{32}N_2O_4$ (376.49): calcd. C 66.99, H 8.57, N 7.44; found C 66.64, H 8.72, N 7.24.

Ethyl (2*S*,4*R*)-4-[(*tert*-Butoxycarbonyl)amino]piperidine-2-carboxylate (25): Compound **23** (105 mg, 0.28 mmol) was dissolved in EtOH (3.5 mL), $Pd(OH)_2/C$ (20%, 115 mg) was added, and the apparatus was flushed three times with H_2. The reaction mixture was hydrogenated under atmospheric pressure at room temperature for 24 h, filtered through a pad of Celite® (3 × 2 mL of EtOH rinse) and then concentrated under reduced pressure to afford the amine **25** as an oil (73 mg, 95%) sufficiently pure to be used in the next step without purification. Yellow oil. $[\alpha]_D^{23} = +38.2$ (c = 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$): $\delta = 1.27$ (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 1.41 [s, 9 H, $(CH_3)_3C$], 1.60−2.12 (m, 3 H, 2 × H-5 and H-3ax), 2.49 (dm, J = 12.4 Hz, 1 H, H-3eq), 2.94 (td, J = 12.0, J = 3.2 Hz, 1 H, H-6ax), 3.60−3.74 (m, 3 H, H-4, H-6ax and H-2), 4.23 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 4.74 (d, J = 8.4 Hz, 1 H, BocN*H*) ppm. ^{13}C NMR ($CDCl_3$): $\delta = 14.1$ (q, OCH_2CH_3), 28.4 [q, 3 C, $C(CH_3)_3$], 29.1 (t), 33.0 (t), 43.2 (t, C-6), 46.4 (d, C-4), 56.7 (d, C-2), 62.5 (t, OCH_2CH_3), 79.9 (s, *C*Me₃), 154.9 (s, N*C*O_2*t*Bu), 168.6 (s, *C*O_2Et) ppm. MS (EI): *m*/*z* (%) = 227 (2) [M − OEt]$^+$, 199 (78) [M − CO_2Et]$^+$, 143 (86), 81 (100), 56 (40). IR (cm^{-1}): ṽ = 3440, 2981, 1710, 1506. $C_{13}H_{24}N_2O_4$ (272.34): calcd. C 57.33, H 8.88, N 10.29; found C 57.64, H 9.06, N 10.04.

Ethyl (2*S*,4*S*)-4-[(*tert*-Butoxycarbonyl)amino]piperidine-2-carboxylate (26): The procedure described above for the synthesis of amino ester **25** was applied to compound **24** (55 mg, 0.15 mmol), to give amino ester **26** (36 mg, 90%) as a yellow oil. $[\alpha]_D^{23}$ = +13.2 (*c* = 0.5, $CHCl_3$). ^{1}H NMR: δ = 1.25 (t, *J* = 7 Hz, 3 H, OCH_2CH_3), 1.39 [s, 9 H, $(CH_3)_3C$], 1.58 −1.78 (m, 1 H, H-5), 1.85−2.29 (m, 3 H, 2 × H-3 and H-5), 3.00−3.40 (m, 2 H, 2 × H-6), 3.63−3.80 (m, 1 H, H-4), 4.00−4.06 (m, 1 H, H-2), 4.20 (q, *J* = 7.0 Hz, 2 H, OCH_2CH_3), 5.05 (br., 1 H, BocN*H*) ppm. ^{13}C NMR: δ = 14.1 (q, OCH_2CH_3), 28.4 [q, 3 C, $C(CH_3)_3$], 29.3 (t), 31.8 (t), 40.6 (t, C-6), 47.4 (d, C-4), 53.9, C-3), 62.0 (t, OCH_2CH_3), 79.6 (s, *C*Me$_3$), 155.0 (s, N*C*O_2*t*Bu), 170.2 (s, *C*O_2Et) ppm. MS (EI): *m*/*z* (%) = 215 (5), 199 (7) [M − CO_2Et]$^+$, 143 (18), 126 (12), 82 (100), 57 (28). $C_{13}H_{24}N_2O_4$ (272.34): calcd. C 57.33, H 8.88, N 10.29; found C 57.59, H 8.60, N 10.48.

(2*S*,4*R*)-4-[(*tert*-Butoxycarbonyl)amino]piperidine-2-carboxylic Acid (27): A mixture of the ester **25** (64 mg; 0.235 mmol) and LiOH (10.7 mg; 0.45 mmol) in MeOH (1.5 mL) was stirred at room temperature for 24 h. After evaporation of the solvent, the residue was purified by FCC (CH_2Cl_2/MeOH, 4:1; R_f = 0.35) to give pure **27** (43 mg, 75%) as a white solid. M.p. 240 °C (dec.). $[\alpha]_D^{22}$ = +17.6 (*c* = 0.2, H_2O). ^{1}H NMR (D_2O): δ = 1.43 [s, 9 H, $(CH_3)_3C$], 1.46−1.69 (m, 2 H, H-3ax and H-5ax), 2.13 (dm, *J* = 12.8 Hz, 1 H, H-5eq), 2.47 (dm, *J* = 12.4 Hz, 1 H, H-3eq), 3.07 (td, *J* = 13.2, 3.0 Hz, 1 H, H-6ax), 3.49 (ddd, *J* = 13.2, 4.4, 3.0 Hz, 1 H, H-6eq), 3.69 (dd, *J* = 12.8, 3.4 Hz, 1 H, H-2), 3.62−3.80 (m, 1 H, H-4) ppm. ^{13}C NMR (D_2O): δ = 27.2 [q, 3 C, $C(CH_3)_3$], 27.6 (t), 32.2 (t), 41.7 (t, C-6), 45.6 (d, C-4), 57.9 (d, C-2), 80.9 [s, $C(CH_3)_3$], 157.7 (s, N*C*O_2*t*Bu), 172.9 (s, *C*O_2H) ppm. MS (EI): *m*/*z* (%) = 199 (< 1) [M − CO_2H]$^+$, 142 (16), 126 (29), 82 (100), 57 (70), 56 (60). IR (cm^{-1}): ṽ = 1620. $C_{11}H_{20}N_2O_4$ (244.29): calcd. C 54.08, H 8.39, N 19.43; found C 53.84, H 8.16, N 19.70.

(2*S*,4*S*)-4-[(*tert*-Butoxycarbonyl)amino]piperidine-2-carboxylic Acid (28): The procedure described above for the synthesis of acid **27** was applied to ester **26** (33 mg; 0.12 mmol), to give acid **28** (23 mg, 77%) as a white solid (FCC, CH_2Cl_2/MeOH, 4:1; R_f = 0.35). M.p. > 345 °C. $[\alpha]_D^{25}$ = +1.7 (*c* = 1. 02, H_2O). ^{1}H NMR (D_2O): δ = 1.41 [s, 9 H, $(CH_3)_3C$], 1.70−2.01 (m, 2 H, H-3ax and H-5ax), 2.01−2.18 (m, 2 H, H-3eq and H-5eq), 3.10−3.52 (m, 2 H, H-6ax and H-6eq), 3.72−3.84 (m, 2 H, H-2 and H-4) ppm. ^{13}C NMR (D_2O): δ = 27.2 (t), 28.3 [q, 3 C, $C(CH_3)_3$], 31.3 (t), 40.1 (t, C-4), 43.8 (d, C-4), 55.4 (d, C-2), 82.0 [s, $C(CH_3)_3$], 157.9 (s, N*C*O_2*t*Bu), 173.9 (s, *C*O_2H) ppm. MS (EI): *m*/*z* (%) = 199 (< 1) [M − CO_2H]$^+$, 187 (4), 142 (12), 127 (7), 126 (19), 82 (100), 57 (68), 56 (80). IR (cm^{-1}): ṽ = 1620. $C_{11}H_{20}N_2O_4$ (244.29): calcd. C 54.08, H 8.39, N 19.43; found C 54.04, H 8.49, N 19.33.

(2*S*,4*R*)-4-[(*tert*-Butoxycarbonyl)amino]-1-[(9*H*-fluoren-9-yloxy)carbonyl]piperidine-2-carboxylic Acid (6): Fmoc-OSu (80 mg, 0.24 mmol) was added at 0 °C to a suspension of **27** (20 mg 0.082 mmol) in Na_2CO_3 (10%, 0.3 mL) and acetone (2.5 mL). After stirring at room temperature for 6 h, the mixture was concentrated under reduced pressure and the residue was purified by FCC (CH_2Cl_2/MeOH, 20:1, R_f = 0.25) to give **6** (32 mg, 84%) as a white solid. $[\alpha]_D^{25}$ = −20.0 (*c* = 0.34, $CHCl_3$). M. p. 104 °C. ^{1}H NMR (amide rotamers): δ = 1.47 [s, 9 H, $(CH_3)_3C$], 1.60−1.85 (m, 3 H, 2 × H-5 and H-3), 2.63−2.85 (m, 1 H, H-3), 3.48−3.80 (m, 2 H, H-6 and H-4), 3.92−4.12 (m, 1 H, H-6), 4.18−4.30 (m, 1 H, NCO_2CH_2CH), 4.30 −4.47 (m, 2 H, NCO_2CH_2), 4.70 and 4.90 (d, *J* = 4.4 Hz and *J* = 5.2 Hz, 1 H, H-2), 7.26−7.38 (m, 4 H, Ar-H), 7.42−7.62 (m, 2 H, Ar-H), 7.69−7.76 (m, 2 H, Ar-H) ppm. ^{13}C NMR (D_2O): δ = 28.2 (t), 28.3 [q, 3 C, $C(CH_3)_3$], 30.9 (t), 36.1 (t, C-6), 44.1 (d, NCO_2CH_2CH), 47.3 (d, H-4), 51.4 (d, H-2), 67.7 (t, NCO_2CH_2), 81.5 [s, $C(CH_3)_3$], 119.9 (d, Ar-*C*), 125.1 (d, Ar-*C*), 127.1 (d, Ar-*C*), 127.6 (d, Ar-*C*), 141.3 (s, Ar-*C*), 143.9 (s, Ar-*C*), 156.2 (s), 157.8 (s), 175.2 (s, *C*O_2H) ppm. MS (ESI): *m*/*z* (%) = 467 [M + H]$^+$. IR (cm^{-1}): ṽ = 3306, 2981, 2934, 1720, 1691, 1649. $C_{26}H_{30}N_2O_6$ (466.53): calcd. C 66.94, H 6.48, N 6.00; found C 66.64, H 6.58, N 5.80.

(2*S*,4*S*)-4-[(*tert*-Butoxycarbonyl)amino]-1-[(9*H*-fluoren-9-yloxy)carbonyl]piperidine-2-carboxylic Acid (7): Fmoc-OSu (25 mg, 0.074 mmol) was added at 0 °C to a suspension of **28** (10 mg 0.041 mmol) in Na_2CO_3 (10%, 0.07 mL) and acetone (0.7 mL). After stirring at room temperature for 6 h, the mixture was concentrated under reduced pressure and the residue was purified by FCC (CH_2Cl_2/MeOH, 15:1; R_f = 0.25) to give **7** (15 mg, 78%) as a clear oil. $[\alpha]_D^{25}$ = −1.8 (*c* = 0.41, $CHCl_3$). ^{1}H NMR (amide rotamers): δ = 1.47 [s, 9 H, $(CH_3)_3C$], 1.60−2.10 (m, 2 H), 2.43−2.63 (m, 2 H), 3.05−3.80 (m, 3 H), 4.02 −4.30 (m, 2 H), 4.30−4.55 (m, 2 H), 4.40 (dd, *J* = 14.4, *J* = 7.0 Hz, 1 H), 4.85−5.10 (m, 1 H), 7.24−7.41 (m, 4 H, Ar-H), 7.49−7.54 (m, 2, Ar-H), 7.76 (d, *J* = 7 Hz, 2 H, Ar-H) ppm. ^{13}C NMR (D_2O, amide rotamers): δ = 28.4, 29.7, 32.0, 33.1, 40.8, 47.2, 53.9, 68.0, 80.5, 120.0, 125.1, 127.1, 127.7, 141.3, 144.0, 156.3, 160.3, 167.8 ppm. MS (ESI): *m*/*z* (%) = 467 [M + H]$^+$. IR (cm^{-1}): ṽ = 3399, 2977, 1673, 1592. $C_{26}H_{30}N_2O_6$ (466.53): calcd. C 66.94, H 6.48, N 6.00; found C 66.74, H 6.78, N 5.70.

(2*S*,4*R*)-2-Carboxypiperidin-4-aminium Trifluoroacetate (4): Boc-amine **27** (20 mg, 0.082 mmol) was dissolved in a cooled (0 °C) mixture of TFA/H_2O 95:5 (1 mL) and the system was stirred at room temp. After 2 h the solution was concentrated under reduced pressure and the resulting residue was washed with Et_2O to give compound **4** (21 mg, 100%) as a colourless solid. M.p. 314 °C (dec.) $[\alpha]_D^{25}$ = −5.1 (*c* = 0.75, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 1.70−1.98 (m, 2 H, H-3ax and H-5ax), 2.30 (dm, *J* = 12.0 Hz, 1 H, H-5eq), 2.68 (dm, *J* = 13.0 Hz, 1 H, H-3eq), 3.14 (td, *J* = 13.4, 3.2 Hz, 1 H, H-6ax), 3.52−3.71 (m, 2 H, H-4 and H-6eq), 3.99 (dd, *J* = 12.8, 3.2 Hz, 1 H, H-2) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 25.7 (t, C-5), 29.7 (t, C-3), 41.6 (t, C-6), 45.9 (d, C-4), 56.0 (d, C-2), 116.8 (q, $J_{C,F}$ = 293 Hz, *C*F_3CO_2H), 163.2 (q, $J_{C,F}$ = 35.2 Hz, CF_3*C*O_2H), 170.3 (s, *C*O_2H) ppm. MS (ESI): *m*/*z* (%) = 145 [M + H]$^+$. IR (cm^{-1}): ṽ = 1610. $C_8H_{13}F_3N_2O_4$ (258.20): calcd. C 37.21, H 5.07, N 10.85; found C 37.12, H 5.14, N 11.04.

(2*S*,4*S*)-2-Carboxypiperidin-4-aminium Trifluoroacetate (5): The procedure described above for the synthesis of salt **4** was applied to Boc-amine **28** (10 mg, 0.041 mmol), to give salt **5** (11 mg, 100%) as a colourless solid. M.p. 290 °C (dec.). $[\alpha]_D^{25}$ = −10.4 (*c* = 0.64, H_2O). ^{1}H NMR (400 MHz, D_2O + TFA): δ = 1.78−1.85 (m, 1 H, H-5ax), 2.04−2.10 (m, 1 H, H-3 ax), 2.17 (dm, *J* = 13.6 Hz, 1 H, H-5eq), 2.57 (dm, *J* = 14.0 Hz, 1 H, H-3eq), 3.28−3.44 (m, 2 H, H-6eq and H-6ax), 3.48 (tt, *J* = 14.0, 3.6 Hz, 1 H, H-4), 4.43 (t, *J* = 4.8 Hz, 1 H, H-2) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 25.3 (t), 28.1 (t), 39.1 (t, C-6), 43.4 (d, C-4), 54.0 (d, C-2), 115.9 (q, $J_{C,F}$ = 293 Hz, *C*F_3CO_2H), 162.5 (q, $J_{C,F}$ = 35.0 Hz, CF_3*C*O_2H), 170.5 (s, *C*O_2H) ppm. MS (ESI): *m*/*z* (%) = 145 [M + H]$^+$. IR (cm^{-1}): ṽ = 1610. $C_8H_{13}F_3N_2O_4$ (258.20): calcd. C 37.21, H 5.07, N 10.85; found C 37.44, H 4.80, N 11.14.

Anchoring of Fmoc-Sly(Boc)-OH on Trityl Chloride Resin and on Rink Resin

Trityl Chloride Resin: The trityl chloride resin (0.80 mmol/g, 100 mg), previously dried under vacuum, was swollen for 20 min with dry DCM (2 mL). A solution of Fmoc-Sly(Boc)-OH (40 mg, 0.080 mmol) and DIPEA (56 μL, 0.32 mmol) in dry DCM (1 mL) was added to the resin under N_2 and the reaction was allowed to proceed at 25 °C for 2 h with continuous mechanical shaking. The resin was washed with DCM/MeOH/DIPEA (17:2:1, v/v, 3 × 2 mL × 5 min), DCM (3 × 2 mL), DMF (2 × 2 mL) and again with DCM (2 × 2 mL). The resin, dried under vacuum, gave a degree of substitution of 0.27 mmol/g as determined from the Fmoc release monitored by UV absorption at 301 nm (34% yield).

Fmoc-Rink Resin: The Fmoc-Rink resin (0.44 mmol/g, 100 mg) was swollen for 1 h in DMF. The resin was deprotected with 20% piperidine in DMF (2 mL, 2 × 15 min) and was then washed with DMF (5 × 2 mL). DIPCDI (20 μL, 0.22 mmol) was added to a solution of Fmoc-Sly(Boc)-OH (107 mg, 0.22 mmol) and HOBt (30 mg, 0.22 mmol) in DMF (1 mL). After 10 min this solution was added to the resin under N_2 and the system was mechanically stirred for 16 h at 25 °C. The resin was washed with DMF (3 2 mL) and DCM (2 × 2 mL), and was then dried under vacuum. The level of substitution (0.43 mmol/g, 98% yield) was determined from the Fmoc release as monitored by UV absorption at 301 nm.

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