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Asymmetric Synthesis of a Novel Conformationally Constrained D-Lysine Analogue with a Piperidine Skeleton

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A practical asymmetric synthesis of enantiomerically pure (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-4-[2-(methoxycarbonylamino)ethyl]pipecolic acid starting from easily accessible (*R*)-2-[(*S*)-1,2-bis(benzyloxy)ethyl]-1-[(*S*)-1-phenylethyl]-4-piperidone in around 33 % overall yield has been performed. The efficiency of the synthetic strategy developed for the synthesis of this novel conformationally constrained D-lysine analogue relies on the high-yielding Wadsworth–Emmons reaction of a 2-substituted 4-piperidone and the diastereoselective reduction of the exocyclic C=C double bond at the 4-position of the piperidine ring.

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Introduction

The introduction of non-natural amino acids into biologically active peptides has become one of the most powerful tools to obtain detailed information about their structure–activity relationships.[1] Specific structural, stereoelectronic, steric and conformational properties can be examined by the proper design of such peptides and in many cases the substitution of natural amino acids by conformationally restricted analogues in biologically active peptides has led to derivatives much more selective and efficient than the native peptides. These modified peptides usually show less side effects, an increased selectivity among different receptors, and greater oral bioavailability due to a lower enzymatic degradation that allows a longer biological activity.[2] Therefore, the design and synthesis of conformationally constrained amino acids and their incorporation into peptides have become an essential task for modern drug discovery research. Among the various approaches described to attain conformational rigidity, the incorporation of the amino acid moiety into a ring has been widely used.[3]

Lysine is an important proteinogenic amino acid that strongly stabilises the α-helical conformation of peptides.[4] In recent years the syntheses of several mimetics and analogues of this amino acid have been reported,[5,6] and most of the conformationally constrained lysine analogues described are proline–lysine chimeras.[6] Cyclic amino acids such as proline and pipecolic acid share the ability to exert a significant influence on the local secondary structure of peptides that contain them,[7] and for this reason we considered that a pipecolic acid–lysine chimera (Figure 1) would represent a novel and complementary conformationally constrained lysine analogue.

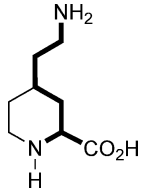

Figure 1. Target pipecolic acid–lysine chimera.

In this paper we report the synthesis of an orthogonally protected *trans*-(2*R*,4*R*)-4-(2-aminoethyl)pipecolic acid derivative in an enantiomerically pure form as a novel constrained analogue of D-lysine in which three torsion angles (χ^1, χ^2 and ϕ) are simultaneously restricted to well-defined values. Our strategy towards the synthesis of this compound relies on diastereoselective functionalisation of 4-piperidone **1** via the corresponding 4-alkylidenepiperidine. The proposed synthesis involves the following steps: (i) Wadsworth–Emmons reaction (WE) of the 4-piperidone with the appropriate phosphonate, (ii) regioselective and stereocontrolled reduction of the exocyclic α,β-unsaturated cyanide moiety present in the compound obtained and (iii) transformation of the piperidine ring substituents to obtain orthogonally protected amine functionalities at the 1- and 4-positions, and the carboxylic acid functionality at the 2-position.

Results and Discussion

The synthesis started from valuable and versatile synthetic intermediate **1**,[8] obtained from (*S*)-*N*-[(*S*)-1-phenylethyl]-2,3-di-*O*-benzylglyceraldimine as previously re-

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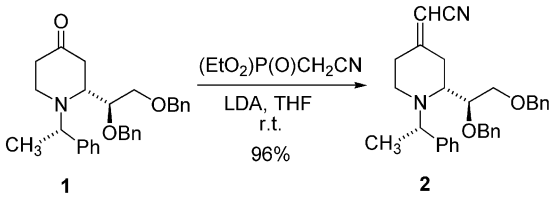
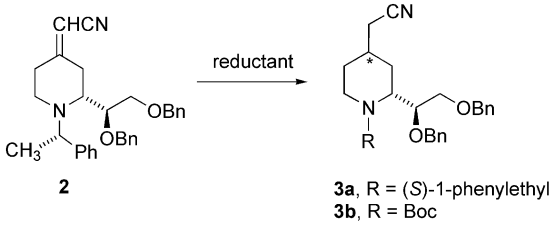
ported.[9] It is worth mentioning that the starting imine is readily available on a multigram scale from inexpensive D-mannitol, which comes from renewable sources.

Wadsworth–Emmons olefination of compound **1** with an excess of diethyl cyanomethylphosphonate using DBU as base in the presence of LiCl in CH_3CN was not satisfactory. After total conversion the resulting *E*/*Z*[10] mixture of compound **2** was contaminated with variable amounts of other unidentified olefinic derivatives, the extent of which depended on the amount of DBU used. Fortunately, the use of LDA (3.5 equiv.) as base led to a 59:41 *E*/*Z* mixture of α,β-unsaturated nitrile **2** in 96% isolated yield when the reaction was carried out with an excess of diethyl cyanomethylphosphonate (3.0 equiv.) at room temperature for 60 min using anhydrous THF as solvent (Scheme 1).



Scheme 1. Synthesis of key intermediate **2**.

Next, diastereoselective reduction of the exocyclic C=C double bond of compound **2** to afford the corresponding saturated 2,4-disubstituted piperidine was attempted under different reaction conditions (Scheme 2) and the best results are collected in Table 1.



Scheme 2. Reduction of key intermediate **2**.

Table 1. Diastereoselective reduction of the C=C double bond of compound **2**.

Reagents	Time [h]	Product	Yield[a] [%]	*cis*/*trans*[b]
H_2, Pt/C	72	**3a**	43	58:42
H_2, PtO_2[c]	15	**3a**	60	60:40
H_2, Pd/C, Boc_2O	72	**3b**	87	65:35
L-Selectride®	20	**3a**	94	13:87

[a] Total yield of the isolated *cis* and *trans* diastereoisomers. [b] Determined by ^{1}H NMR analysis of the crude reaction mixture. [c] 0.1 equiv. of the catalyst was used to avoid concomitant reduction of the cyano group.

Catalytic hydrogenation of compound **2** took place with low diastereoselectivity. By using Pt/C or PtO_2 as the catalyst a *cis*/*trans* mixture (ca. 60:40) of compound **3a** was obtained. Hydrogenation of the C=C double bond with Pd/C was carried out in the presence of Boc_2O in order to protect the free amine that results from the concomitant *N*-debenzylation reaction and, thus, facilitates final product isolation. Under these conditions a *cis*/*trans* mixture (ca. 65:35) of compound **3b** was obtained.

Conjugate reduction of α,β-unsaturated nitrile **2** using an excess of L-Selectride® (4.0 equiv.) in anhydrous THF at –78 °C gave 2,4-disubstituted piperidine **3a** in an excellent yield (94%) and with high *trans* diastereoselectivity (87:13). Compound *trans*-**3a** was easily isolated by column chromatography in a yield of 82% on the gram scale.

The *cis* and *trans* relative configurations of the isolated diastereoisomers of compound **3a** and **3b** were determined on the basis of their NMR spectroscopic data and 2D NOESY experiments (Figure 2) after the complete unequivocal assignment of all the signals in the ^{1}H NMR spectra with the aid of 2D NMR experiments (COSY, HSQC, HMBC).

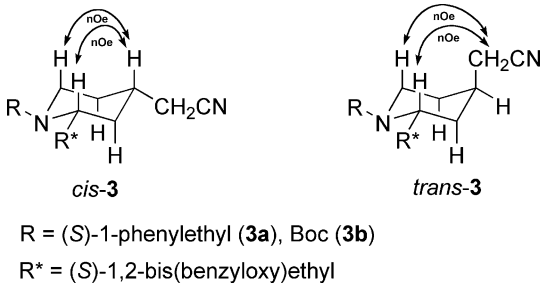


Figure 2. Determination of the relative configurations of *trans*- and *cis*-**3** by means of NOE correlations.

The stereochemical course of the reaction could be rationalised by taking into account the fact that conjugate addition of a hydride to exocyclic α,β-unsaturated nitrile using a sterically demanding reducing agent, such as L-Selectride®, occurs preferentially from the equatorial direction to minimise steric repulsions, yielding *trans*-2,4-disubstituted piperidine as the major product.

The transformation of compound *trans*-**3a** into the orthogonally protected pipecolic acid–lysine chimera was successfully performed as depicted in Scheme 3. Reduction of nitrile using $LiAlH_4$ in THF and reaction of the resulting crude primary amine with methyl chloroformate and potassium carbonate in THF led to carbamate **4** in 83% yield. From this compound the *N*-Boc derivative **5** was obtained in 70% yield by selective hydrogenolytic *N*-debenzylation in the presence di-*tert*-butyl dicarbonate using Pd/C as the catalyst. Next, extensive *O*-debenzylation of **5** to give diol **6** was performed in a nearly quantitative yield by hydrogenolytic debenzylation using molecular hydrogen and catalytic amounts of $Pd(OH)_2$/C. Finally oxidative cleavage of the 1,2-diol moiety by treatment with excess sodium periodate in the presence of a catalytic amount of anhydrous ruthenium trichloride gave the desired compound (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-4-[2-(methoxycarbonylamino)ethyl]-pipecolic acid (**7**) in 74% yield.

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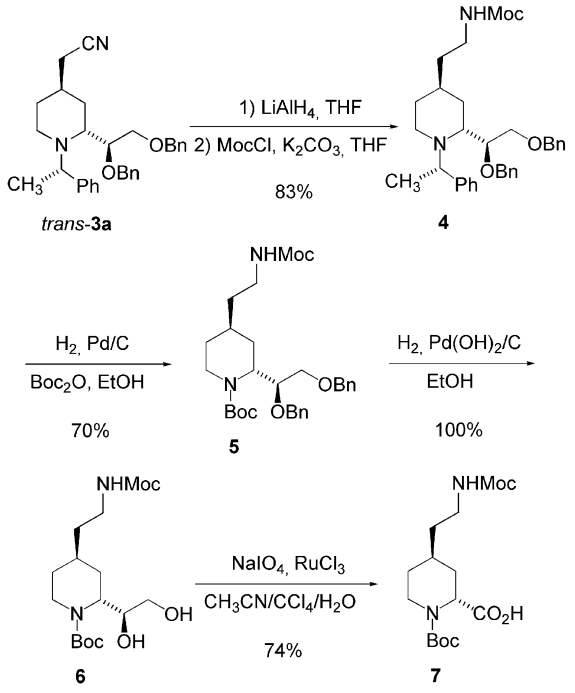

Scheme 3. Synthesis of (2*R*,4*R*)-1-(*tert*-butoxycarbonyl)-4-[2-(methoxycarbonylamino)ethyl]pipecolic acid **8** from *trans*-**3a**.

Conclusions

In summary, enantiomerically pure (2*R,4R*)-1-(*tert*-butoxycarbonyl)-4-[2-(methoxycarbonylamino)ethyl]pipecolic acid is easily accessible from (*R*)-2-[(*S*)-1,2-bis(benzyloxy)-ethyl]-1-[(*S*)-1-phenylethyl]-4-piperidone. The compound obtained, a conformationally constrained D-lysine, is an interesting scaffold to test the role of the lysine side-chain in the conformational preferences of biologically active peptides.

Experimental Section

General: All reagents were of analytical grade and were used as obtained from commercial sources. Reactions were carried out using anhydrous solvents. Whenever possible the reactions were monitored by thin-layer chromatography (TLC). TLC was performed on precoated silica gel polyester plates and products were visualised by using UV light (254 nm) and ethanolic phosphomolybdic acid solution followed by heating. Column chromatography was performed using silica gel (Kiesegel 60, 230–400 mesh). Melting points were determined in open capillaries using a Gallenkamp capillary melting point apparatus and are uncorrected. The FTIR spectra of oils were recorded as thin films on NaCl plates and the FTIR spectra of solids were recorded as KBr pellets using a Thermo Nicolet Avatar 360 FT-IR spectrometer; $\tilde{\nu}_{max}$ values are given for the main absorption bands. NMR spectra were acquired with a Bruker AV-400 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR at room temperature in $CDCl_3$ using a 5 mm probe. The chemical shifts (δ) are reported in parts per million and are referenced to the residual solvent peak. Coupling constants (J) are quoted in hertz. The following abbreviations are used: s, singlet; d, doublet; q, quartet; dd, doublet of doublets; m, multiplet; br. s, broad singlet; br. d, broad doublet; br. dd, broad doublet of doublets. Selective ge-1D NOESY experiments were performed with gradient pulses in the mixing time. Spectra were acquired at 300 K with optimised mixing times and 128 transients per spectrum using the Bruker standard selnogp pulse program. Special precautions such as degassing of the sample were not taken. NOESY spectra were acquired in the phase-sensitive mode with gradient pulses in the mixing time as 2048 × 256 hypercomplex files with eight transients for 256 time increments. A mixing time of 750 ms was used and processing was carried out using a sine-bell-squared function shifted by $\pi/2$ and a states-TPPI method. Special precautions such as degassing of the sample were not taken. Optical rotations were measured with a Jasco 1020 polarimeter at λ = 589 nm and 25 °C in a cell with a 10 cm path length; $[a]_D$ values are given in 10^{-1} deg cm g^{-1} and concentrations are given in g per 100 mL. High-resolution mass spectra were recorded using a Bruker Daltonics MicroToF-Q electrospray instrument from methanolic solutions using the positive electrospray ionisation mode (ESI+). Microanalyses were performed using a Perkin–Elmer 2400 CHNS elemental analyser.

(*E*/*Z*)-(*R*)-4-Cyanomethylene-2-[(*S*)-1,2-bis(benzyloxy)ethyl]-1-[(*S*)-1-phenylethyl]piperidine (2): A 2.0 M solution of LDA in heptane/THF/ethylbenzene (1.75 mL, 3.5 mmol) was added to a solution of diethyl cyanomethylphosphonate (531 mg, 3.0 mmol) in anhydrous THF (10 mL) at room temp. under argon and the mixture was stirred for 10 min. A solution of compound **1** (444 mg, 1.0 mmol) in anhydrous THF (20 mL) was added and the resulting mixture was stirred at room temp. until conversion was complete (ca. 60 min, as monitored by TLC). The reaction was quenched with water (30 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried with anhydrous $MgSO_4$, filtered, evaporated under reduced pressure and subsequently purified by chromatography on silica gel (Et_2O/hexanes, 1:1) to yield compound **2** (447 mg, 96%) as a 59:41 mixture of *E*/*Z* diastereoisomers. Samples of the pure *E* and *Z* diastereoisomers were isolated for analytical purposes by silica gel column chromatography (Et_2O/hexanes, 1:4 → Et_2O/hexanes, 1:1).

(*E*)-2: Oil, $[a]_D^{25}$ = +1.0 (c = 0.87, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 2214, 1685 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.20 (d, J = 6.6 Hz, 3 H), 2.09 (dd, J = 13.7, 7.1 Hz, 1 H), 2.17–2.27 (m, 1 H), 2.39–2.47 (m, 2 H), 2.58 (dd, J = 13.7, 4.0 Hz, 1 H), 2.64–2.72 (m, 1 H), 2.96–3.02 (m, 1 H), 3.57 (dd, J = 10.5, 6.2 Hz, 1 H), 3.81 (br. d, J = 10.5 Hz, 1 H), 3.82–3.88 (m, 1 H), 4.02 (q, J = 6.6 Hz, 1 H), 4.47 (d, J = 12.1 Hz, 1 H), 4.51 (d, J = 12.1 Hz, 1 H), 4.56 (d, J = 11.8 Hz, 1 H), 4.74 (d, J = 11.8 Hz, 1 H), 4.93 (br. s, 1 H), 7.15–7.35 (m, 15 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.3, 31.6, 35.2, 44.3, 56.9, 58.6, 70.8, 72.9, 73.5, 78.3, 92.8, 116.7, 126.8, 127.2, 127.6, 127.7, 127.7, 128.2, 128.3, 128.4, 138.0, 138.5, 144.4, 165.5 ppm. HRMS (ESI$^+$): calcd. for $C_{31}H_{35}N_2O_2$ [M + H]$^+$ 467.2693; found 467.2697.

(*Z*)-2: Oil, $[a]_D^{25}$ = –41.6 (c = 1.02, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 2215, 1686, 1674 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.23 (d, J = 6.6 Hz, 3 H), 1.99–2.07 (m, 1 H), 2.13–2.22 (m, 1 H), 2.40 (dd, J = 14.1, 5.9 Hz, 1 H), 2.46–2.54 (m, 1 H), 2.74 (ddd, J = 12.1, 7.7, 4.1 Hz, 1 H), 2.84 (dd, J = 14.1, 4.6 Hz, 1 H), 3.11–3.18 (m, 1 H), 3.63 (dd, J = 11.0, 6.5 Hz, 1 H), 3.78–3.84 (m, 2 H), 4.04 (q, J = 6.6 Hz, 1 H), 4.47 (d, J = 12.1 Hz, 1 H), 4.54 (d, J = 12.1 Hz, 1 H), 4.57 (d, J = 11.7 Hz, 1 H), 4.74 (d, J = 11.7 Hz, 1 H), 4.97 (br. s, 1 H), 7.14–7.37 (m, 15 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.3, 33.0, 33.8, 44.6, 57.4, 57.8, 70.9, 72.9, 73.5, 79.1, 92.7, 116.8, 126.8, 127.2, 127.4, 127.5, 127.6, 127.7, 128.2, 128.3, 138.3, 138.7, 145.1, 165.5 ppm. HRMS (ESI$^+$): calcd. for $C_{31}H_{35}N_2O_2$ [M + H]$^+$ 467.2693; found 467.2688.

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(2*R*,4*R*)-4-Cyanomethyl-2-[(*S*)-1,2-bis(benzyloxy)ethyl]-1-[(*S*)-1-phenylethyl]piperidine (*trans*-3a): A 1.0 M solution of L-Selectride® in THF (4 mL, 4.0 mmol) was added dropwise to a 59:41 *E*/*Z* mixture of compound **2** (466 mg, 1.0 mmol) dissolved in anhydrous THF (20 mL) at –78 °C under argon and the mixture was stirred for 20 h at –78 °C. The reaction mixture was warmed up to 0 °C and then sat. aq. NH_4Cl (30 mL) was added carefully with stirring at 0 °C. After extraction with Et_2O (3 × 50 mL), the combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents evaporated in vacuo to afford compound **3a** as an 87:13 mixture of *trans*/*cis* diastereoisomers. Purification of the residue by silica gel column chromatography (Et_2O/hexanes, 1:2 → Et_2O) gave *cis*-**3a** (56 mg, 12%) and *trans*-**3a** (384 mg, 82%) as oils.

***trans*-3a:** $[a]_D^{25}$ = –30.2 (*c* = 0.82, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 2245 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.23 (d, *J* = 6.6 Hz, 3 H), 1.20–1.27 (m, 1 H), 1.42 (ddd, *J* = 12.8, 12.8, 3.2 Hz, 1 H), 1.50–1.60 (m, 2 H), 1.97–2.09 (m, 1 H), 2.09 (dd, *J* = 7.4, 1.6 Hz, 2 H), 2.62–2.67 (m, 2 H), 3.08–3.13 (m, 1 H), 3.54 (dd, *J* = 10.4, 4.9 Hz, 1 H), 3.58 (dd, *J* = 10.4, 3.4 Hz, 1 H), 3.83–3.89 (m, 1 H), 3.94 (q, *J* = 6.6 Hz, 1 H), 4.43 (d, *J* = 12.4 Hz, 1 H), 4.46 (d, *J* = 12.4 Hz, 1 H), 4.54 (d, *J* = 11.7 Hz, 1 H), 4.74 (d, *J* = 11.7 Hz, 1 H), 7.09–7.34 (m, 15 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.3, 24.0, 28.8, 29.3, 30.6, 42.4, 54.6, 59.6, 71.9, 72.9, 73.4, 78.8, 118.6, 126.6, 127.2, 127.5, 127.6, 127.6, 127.8, 128.1, 128.3, 128.4, 138.2, 138.9, 146.7 ppm. HRMS (ESI^+): calcd. for $C_{31}H_{37}N_2O_2$ $[M + H]^+$ 469.2850; found 469.2845.

***cis*-3a:** $[a]_D^{25}$ = +9.8 (*c* = 0.66, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 2245 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.99 (ddd, *J* = 12.0, 12.0, 12.0 Hz, 1 H), 1.06 (dddd, *J* = 12.6, 12.6, 12.6, 3.9 Hz, 1 H), 1.16 (d, *J* = 6.5 Hz, 3 H), 1.50–1.63 (m, 2 H), 2.03 (ddd, *J* = 11.8, 11.8, 1.9 Hz, 1 H), 2.14 (dd, *J* = 12.0, 2.5 Hz, 1 H), 2.19 (dd, *J* = 16.7, 6.7 Hz, 1 H), 2.28 (dd, *J* = 16.7, 5.6 Hz, 1 H), 2.42 (ddd, *J* = 11.8, 3.2, 3.2 Hz, 1 H), 2.68–2.74 (m, 1 H), 3.67 (dd, *J* = 10.5, 7.8 Hz, 1 H), 4.04 (d, *J* = 10.5 Hz, 1 H), 4.08–4.15 (m, 2 H), 4.50 (d, *J* = 12.1 Hz, 1 H), 4.60 (d, *J* = 12.1 Hz, 1 H), 4.70 (d, *J* = 11.9 Hz, 1 H), 4.84 (d, *J* = 11.9 Hz, 1 H), 7.17–7.43 (m, 15 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 7.9, 24.3, 31.6, 32.3, 33.8, 44.6, 53.7, 59.1, 71.4, 72.9, 73.6, 77.5, 118.5, 126.5, 127.5, 127.6, 127.6, 127.8, 128.0, 128.3, 128.4, 128.4, 138.3, 138.8, 143.4 ppm. HRMS (ESI^+): calcd. for $C_{31}H_{37}N_2O_2$ $[M + H]^+$ 469.2850; found 469.2865.

(2*R*,4*R*)-2-[(*S*)-1,2-Bis(benzyloxy)ethyl]-4-[2-(methoxycarbonylamino)ethyl]-1-[(*S*)-1-phenylethyl]piperidine (4): A 1.0 M solution of $LiAlH_4$ in THF (2 mL, 2.0 mmol) was added dropwise to a solution of *trans*-**3a** (468 mg, 1.0 mmol) in anhydrous THF (20 mL) at room temp. under argon and the mixture was stirred for 3.5 h. The reaction mixture was cooled to 0 °C and then sat. aq. NH_4Cl (30 mL) was added carefully with stirring. After filtration through a Celite® 545 pad and extraction with Et_2O (3 × 30 mL), the combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents evaporated in vacuo. Methyl chloroformate (189 mg, 2.0 mmol) and anhydrous K_2CO_3 (828 mg, 6.0 mmol) were added successively to a solution of the obtained crude in anhydrous THF (20 mL) at room temp. and the mixture was stirred for 2 h. After filtration through a Celite® 545 pad the solvent was evaporated in vacuo and the residue purified by silica gel column chromatography (EtOAc/EtOH, 4:1) to afford compound **4** (440 mg, 83%) as an oil. $[a]_D^{25}$ = –17.8 (*c* = 1.23, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3347, 1717 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.97–1.08 (m, 1 H), 1.17 (d, *J* = 6.5 Hz, 3 H), 1.18–1.29 (m, 2 H), 1.25–1.32 (m, 1 H), 1.36–1.43 (m, 2 H), 1.47–1.59 (m, 1 H), 2.41–2.57 (m, 2 H), 2.93–3.05 (m, 2 H), 3.03–3.14 (m, 1 H), 3.46 (dd, *J* = 10.7, 5.2 Hz, 1 H), 3.51 (s, 3 H), 3.56 (dd, *J* = 10.7, 2.9 Hz, 1 H), 3.87–4.00 (m, 2 H), 4.40 (s, 2 H), 4.52 (d, *J* = 11.7 Hz, 1 H), 4.66 (d, *J* = 11.7 Hz, 1 H), 4.71–4.79 (m, 1 H), 7.01–7.36 (m, 15 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.8, 28.8, 29.9, 31.1, 36.1, 38.3, 42.5, 51.7, 54.5, 59.3, 71.6, 72.5, 73.2, 78.0, 126.2, 127.0, 127.1, 127.4, 127.5, 127.5, 127.9, 128.0, 128.1, 138.1, 138.9, 147.0, 156.9 ppm. HRMS (ESI^+): calcd. for $C_{33}H_{43}N_2O_4$ $[M + H]^+$ 531.3217; found 531.3223.

(2*R*,4*R*)-1-(*tert*-Butoxycarbonyl)-2-[(*S*)-1,2-bis(benzyloxy)ethyl]-4-[2-(methoxycarbonylamino)ethyl]piperidine (5): Boc_2O (492 mg, 2.25 mmol) and 10% Pd/C (139 mg) were added successively to a solution of **4** (398 mg, 0.75 mmol) in absolute ethanol (12 mL) and the mixture was stirred at room temp. under H_2 for 48 h. After completion of the reaction, the mixture was filtered through Celite® 545 and concentrated in vacuo. The residue was purified by silica gel flash chromatography (Et_2O/hexanes, 1:1 → Et_2O/hexanes, 4:1) to afford compound **5** (276 mg, 70%) as an oil. $[a]_D^{25}$ = –8.1 (*c* = 0.63, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3340, 1718, 1684, 1252 cm^{-1}. ^1H NMR (400 MHz, 328 K, $CDCl_3$): δ = 0.96 (dddd, *J* = 12.6, 12.6, 12.6, 4.4 Hz, 1 H), 1.10–1.31 (m, 3 H), 1.33 (s, 9 H), 1.49 (br. d, *J* = 12.6 Hz, 1 H), 1.53–1.61 (m, 1 H), 1.65 (br. dd, *J* = 13.5, 1.6 Hz, 1 H), 2.63 (br. dd, *J* = 12.8, 12.8 Hz, 1 H), 2.96–3.11 (m, 2 H), 3.53 (dd, *J* = 10.6, 4.9 Hz, 1 H), 3.56 (s, 3 H), 3.58 (dd, *J* = 10.6, 4.2 Hz, 1 H), 3.67–3.73 (m, 1 H), 3.85–4.00 (m, 1 H), 4.26–4.36 (m, 1 H), 4.40–4.47 (m, 1 H), 4.41 (d, *J* = 11.7 Hz, 1 H), 4.44 (d, *J* = 11.9 Hz, 1 H), 4.49 (d, *J* = 11.9 Hz, 1 H), 4.63 (d, *J* = 11.7 Hz, 1 H), 7.13–7.28 (m, 10 H) ppm. ^{13}C NMR (100 MHz, 333 K, $CDCl_3$): δ = 28.5, 29.1, 32.2, 33.1, 37.4, 38.6, 51.9, 55.3, 71.8, 72.6, 73.6, 77.7, 79.2, 127.4, 127.7, 127.8, 128.0, 128.2, 128.4, 138.4, 138.9, 155.3, 157.0 ppm. HRMS (ESI^+): calcd. for $C_{30}H_{42}N_2O_6Na$ $[M + Na]^+$ 549.2935; found 549.2940.

(2*R*,4*R*)-1-(*tert*-Butoxycarbonyl)-2-[(*S*)-1,2-dihydroxyethyl]-4-[2-(methoxycarbonylamino)ethyl]piperidine (6): 20% $Pd(OH)_2/C$ (133 mg) was added to a solution of **5** (265 mg, 0.50 mmol) in absolute ethanol (8 mL) and the mixture was stirred at room temp. under H_2 for 24 h. After completion of the reaction, the mixture was filtered through Celite® 545 and concentrated in vacuo and used in the next step without additional purification. A sample of pure **6** was isolated (as an oil) for analytical purposes by silica gel column chromatography (Et_2O/EtOH, 4:1). $[a]_D^{25}$ = +23.0 (*c* = 0.76, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3345, 1722, 1691, 1256 cm^{-1}. ^1H NMR (400 MHz, 328 K, $CDCl_3$): δ = 1.11 (dddd, *J* = 12.8, 12.8, 12.8, 4.6 Hz, 1 H), 1.26–1.46 (m, 3 H), 1.47 (s, 9 H), 1.67 (br. d, *J* = 12.8 Hz, 1 H), 1.70–1.80 (m, 1 H), 1.81 (br. d, *J* = 13.9 Hz, 1 H), 2.56 (br. s, 2 H), 2.97 (br. dd, *J* = 12.2, 12.2 Hz, 1 H), 3.12–3.31 (m, 2 H), 3.54 (dd, *J* = 11.5, 5.5 Hz, 1 H), 3.67 (s, 3 H), 3.69 (dd, *J* = 11.5, 4.5 Hz, 1 H), 3.88–3.94 (m, 1 H), 4.01–4.13 (m, 1 H), 4.25–4.33 (m, 1 H), 4.58–4.70 (m, 1 H) ppm. ^{13}C NMR (100 MHz, 298 K, $CDCl_3$): δ = 28.4, 28.6, 31.9, 32.8, 37.4, 38.1, 41.1, 51.3, 52.1, 64.3, 72.9, 80.3, 157.2, 157.4 ppm. HRMS (ESI^+): calcd. for $C_{16}H_{30}N_2O_6Na$ $[M + Na]^+$ 369.1996; found 369.2012.

(2*R*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[2-(methoxycarbonylamino)-ethyl]pipecolic Acid (7): $NaIO_4$ (428 mg, 2.0 mmol) was added to a stirred solution of crude **6** (173 mg, 0.50 mmol) obtained as above in $CH_3CN/CCl_4/H_2O$ (2:2:3, 14 mL). After stirring vigorously for 5 min, $RuCl_3$ (6 mg, 0.030 mmol) was added to the mixture and stirring was continued for 2 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and water (10 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL), the combined organic layers were dried with anhydrous $MgSO_4$ and the solvent evaporated in vacuo. The residue was purified by silica gel chromatography (eluent: AcOEt) to afford compound **7** (123 mg, 74%) as an oil. $[a]_D^{25}$ = +25.5 (*c* = 0.64, $CHCl_3$). IR (neat): $\tilde{\nu}$ =

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3700–2250, 3338, 1721, 1671, 1259 cm^{-1}. ^1H NMR (400 MHz, 368 K, $C_6D_5CD_3$): δ = 0.86 (dddd, J = 12.5, 12.5, 12.5, 4.8 Hz, 1 H), 0.99–1.08 (m, 1 H), 1.05–1.16 (m, 2 H), 1.28–1.38 (m, 1 H), 1.36–1.47 (m, 1 H), 1.45 (s, 9 H), 2.10–2.17 (m, 1 H), 2.88–3.03 (m, 2 H), 3.08 (ddd, J = 12.9, 12.9, 2.2 Hz, 1 H), 3.49 (s, 3 H), 4.00–4.11 (m, 1 H), 4.60–5.00 (m, 1 H), 4.89–5.08 (m, 1 H), 8.58 (br. s, 1 H) ppm. ^{13}C NMR (100 MHz, 368 K, $C_6D_5CD_3$): δ = 28.7, 30.3, 31.9, 33.5, 37.0, 38.9, 42.2, 52.0, 54.9, 80.2, 154.7, 156.0, 174.5 ppm. HRMS (ESI^+): calcd. for $C_{15}H_{26}N_2O_6Na$ $[M + Na]^+$ 353.1683; found 353.1678.

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[10] Following complete assignment of the ^1H resonances by 2D NMR studies (COSY, HSQC, HMBC), the *E*/*Z* configuration of the exocyclic double bond was unequivocally determined by a series of selective 1D gradient-enhanced nuclear Overhauser enhancement (ge-1D NOESY) experiments. Diastereomeric ratios were determined by ^1H NMR analyses of the crude reaction mixtures.

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