Bioorganic & Medicinal Chemistry xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of 2-(5-methyl-4-phenyl-2-oxopyrrolidin-1-yl)-acetamide stereoisomers as novel positive allosteric modulators of sigma-1 receptor

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ARTICLE INFO

Article history:
Received 24 November 2012
Revised 21 February 2013
Accepted 1 March 2013
Available online xxxx

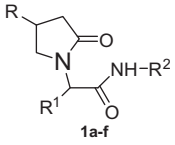
Keywords:
2-(5-Methyl-4-phenyl-2-oxopyrrolidin-1-yl)-acetamide
Enantiomers
Sigma-1 receptor
Agonist
Modulation

ABSTRACT

Novel positive allosteric modulators of sigma-1 receptor represented by 2-(5-methyl-4-phenyl-2-oxopyrrolidin-1-yl)-acetamide enantiomers were synthesised using an asymmetric Michael addition of 2-nitroprop-1-enylbenzene to diethyl malonate. Following the chromatographic separation of the methyl *erythro*- and *threo*-4-nitro-3*R*- and 3*S*-phenylpentanoate diastereoisomers, target compounds were obtained by their reductive cyclisation into 5-methyl-4-phenylpyrrolidin-2-one enantiomers and the attachment of the acetamide group to the heterocyclic nitrogen. Experiments with electrically stimulated rat vas deference contractions induced by the PRE-084, an agonist of sigma-1 receptor, showed that (4*R*,5*S*)- and (4*R*,5*R*)-2-(5-methyl-4-phenyl-2-oxopyrrolidin-1-yl)-acetamides with an *R*-configuration at the C-4 chiral centre in the 2-pyrrolidone ring were more effective positive allosteric modulators of sigma-1 receptor than were their optical antipodes.

1. Introduction

During the past decade, there has been significant interest in the investigation of Structure–Activity Relationship (SAR) aimed at searching for new nootropic pharmaceuticals for the treatment of cognition/memory disorders. Currently, drugs **1a**–**f** containing the pyrrolidin-2-one pharmacophore and representing the so-called racetam family play a key role in the treatment of these disorders. For example, piracetam (**1a**), pramiracetam (**1b**), etiracetam (**1c**), nefiracetam (**1d**), oxiracetam (**1e**) and phenylpiracetam (**1f**) are drugs of choice for the specific therapy of cognition/memory disorders.[1] However, the discovery of new effective pharmaceuticals that improve neurotransmission in the human brain is critical because it affects mental and cognitive abilities.



1a-f

a) piracetam (R=R^1=R^2=H);
b) pramiracetam (R=R^1=H, R^2=(CH$_2$)$_2$N(Pr-*i*)$_2$);
c) etiracetam (R=R^2=H, R^1=Et);
d) nefiracetam (R=R^2=H, R^1=2,6-Me$_2$C$_6$H$_3$);
e) oxiracetam (R=OH, R^1=R^2=H);
f) phenylpiracetam (R=Ph, R^1=R^2=H)

Our previous investigations in this field of medicinal chemistry were aimed at the resolution of racemic phenylpiracetam **1f** into individual stereoisomers and their subsequent pharmaceutical analysis. The *R*-phenylpiracetam 4*R*-**1f** was found to be a more effective antidepressant, analgesic, muscle relaxant and psycho-stimulating compound than was the *S*-antipode 4*S*-**1f**.[2] These results were in good agreement with pharmacological data demonstrating the effectiveness of employing the conformational variation of chiral centre(s) of racemic molecules, wherein the enantiomeric resolution can be used to afford the optimal biological effect.

The objective of this investigation is the resolution of a known racemic 4,5-disubstituted piracetam analogue, 2-(5-methyl-4-phenyl-2-oxopyrrolidin-1-yl)-acetamide (**2**),[3] whose nootropic properties could be improved by regulating the stereochemistry of the C4 and C5 positions of the pyrrolidin-2-one ring.

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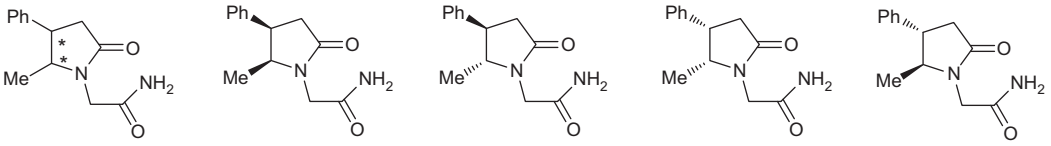
http://dx.doi.org/10.1016/j.bmc.2013.03.016



They attracted our attention due to the unexpected activity discovered for compound (4*R*,5*S*)-**2a** during in vitro CNS pharmacological target profiling, which was performed in a commercially available panel of 77 radioligand binding assays (CEREP, France).[4] The only target site for its activity (inhibition or enhancement of radioligand binding for more than 20%) was sigma receptor, where this compound increased the specific binding of radioligand, thus suggesting a positive modulatory activity of (4*R*,5*S*)-**2a** on sigma receptors.

Recent studies consider sigma-1 receptor (sig-1R) as an emerging new CNS drug target. This receptor plays an important role in neuronal plasticity, a process implicated in the pathophysiology of neuropsychiatric diseases, such as Alzheimer's disease, major depressive disorders, and schizophrenia.[5,6] Sig-1R has been thoroughly studied to elucidate possible neuropharmacological applications, mainly in learning and memory processes, including depression, anxiety, schizophrenia, analgesia and some effects of drug abuse.[7] As a chaperone protein, sig-1R modulates the intracellular calcium signalling of the endoplasmatic reticulum. Several studies suggest that sig-1R receptor is involved in memory processes and their agonists have demonstrated effectiveness in the treatment of cognitive impairments in experimental animal models.[8]

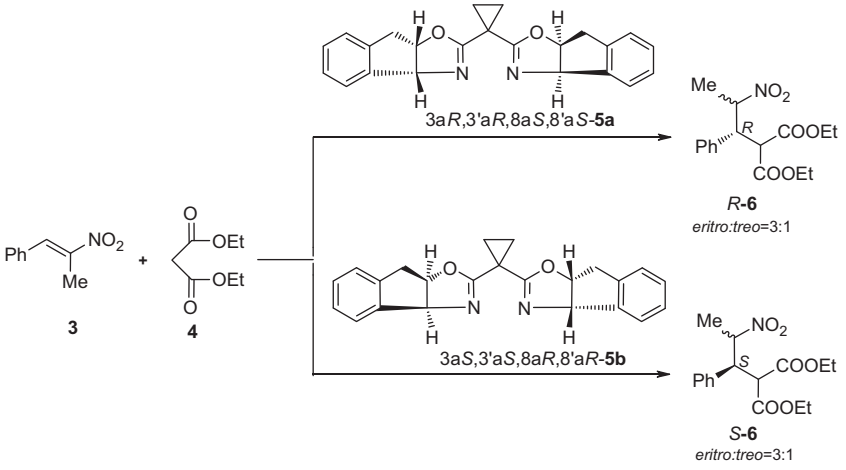
To date, we have not found any data about the effect of piracetam and its structural analogues on sig-1R. Therefore, our research is aimed at the synthesis of 2-(5-methyl-4-phenyl-2-oxopyrrolidin-1-yl)-acetamide (**2**) four individual enantiomers 4*R*,5*S*-**2a**, 4*R*,5*R*-**2b**, 4*S*,5*R*-**2c**, and 4*S*,5*S*-**2d** for the purpose to compare the modulatory activity of these compounds with respect to sig-1R using the isolated vas deferens experimental model.

2. Chemistry

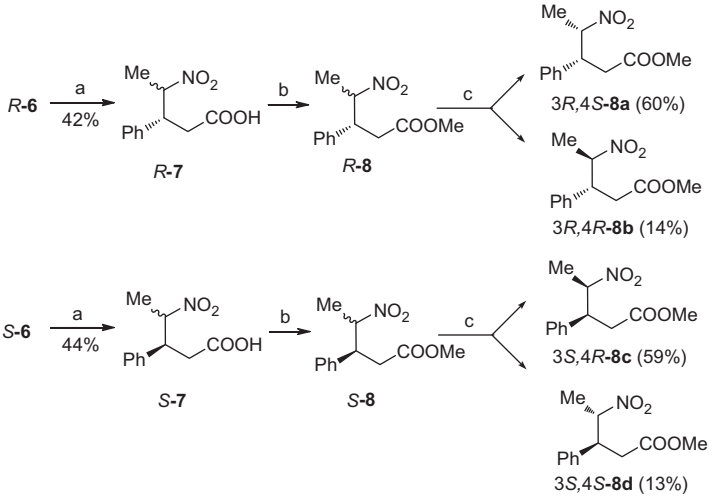
No evidence was found in the literature related to the chiral resolution of racemic 2-(5-methyl-4-phenyl-2-oxopyrrolidin-1-yl)-acetamide (**2**) or the asymmetric synthesis of its separate enantiomers from chiral or nonchiral reagents. Accordingly, we describe the special methodology for the preparation of these compounds. The crucial part of the synthesis consists of the asymmetric Michael addition of 2-nitroprop-1-enylbenzene (**3**) to diethyl malonate (**4**) catalysed by the chiral 2,2′-cyclopropylidene-bis-oxazoline compound **5**, magnesium triflate and an organic base according to the methodology developed by Barnes et al.[9] As a result, diethyl (1*R*)-2-(2-nitro-1-phenylpropyl)-malonate (*R*-**6**) was obtained as a mixture of *erythro*- and *threo*-diastereoisomers in 87% yield and with optical purity 94% in the case of (3a*R*,3′a*R*,8a*S*,8′a*S*)-2,2′-cyclopropylidenebis-[3a,8a]-dihydro-8*H*-indeno-[1,2-*d*]-oxazole ((3a*R*,3′a*R*,8a*S*,8′a*S*)-**5a**). The substitution of the catalyst by its optical antipode (3a*S*,3′a*S*,8a*R*,8′a*R*)-**5b** resulted in the preparation of a second pair of diastereoisomers *S*-**6** in 83% yield and with optical purity 95%. The introduction of the methyl group in nitrostyrene at least 10 times hindered the rate of the condensation compared with that of 2-nitrovinylbenzene, as discussed in the study.[9] The usage of twofold amount of the chiral catalyst **5** allowed to reduce the reaction time up to 72 h (Scheme 1).

Subsequent chemical manipulations with diastereoisomeric mixtures *R*-**6** and *S*-**6** were aimed at obtaining suitable derivatives for resolution by column chromatography. Methyl (3*R*)- and (3*S*)-4-nitro-3-phenylpentanoates *R*-**8** and *S*-**8** were found to be the most suitable candidates for this purpose. According to this precondition, compounds *R*-**6** and *S*-**6** were subjected to acidic hydrolysis and decarboxylation. Intermediate (3*R*)- and (3*S*)-4-nitro-3-phenylpentanoic acids *R*-**7** and *S*-**7** were esterified by methanol, and the obtained diastereoisomeric mixtures of esters *R*-**8** and *S*-**8** were separated by column chromatography on silica gel, affording the *erythro*- and *threo*-isomers of 4-nitro-3-phenylpentanoate: 3*R*,4*S*-**8a**, 3*R*,4*R*-**8b**, 3*S*,4*R*-**8c** and 3*S*,4*S*-**8d** (Scheme 2).

The resulting methyl 4-nitro-3-phenylpentanoates **8a**–**d** were converted into target compounds 4*R*,5*S*-**2a**, 4*R*,5*R*-**2b**, 4*S*,5*R*-**2c**, and 4*S*,5*S*-**2d** using typical reactions for the preparation of 2-(2-oxopyrrolidin-1-yl)-acetamide analogues:



Scheme 1. The preparation of diethyl (1*R*)- and (1*S*)-2-(2-nitro-1-phenylpropyl)-malonates *R*-**6** and *S*-**6**.

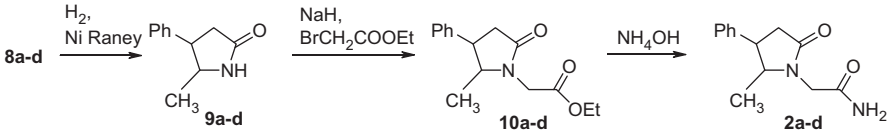


Scheme 2. The preparation of methyl (3*R*)- and (3*S*)-4-nitro-3-phenylpentanoates **8a–d**. Reagents and conditions: (a) 36% HCl and CH_3COOH mixture (1:3), reflux, 18 h; (b) MeOH, $SOCl_2$ (cat) 20 h, reflux; (c) chromatographic separation on silica gel.

(a) Hydrogenation of each stereoisomer **8a–d** in the presence of Ni Raney catalyst accompanied by the cyclisation of intermediate ethyl 4-amino-3-phenylpentanoates into the appropriate 5-methyl-4-phenylpyrrolidin-2-ones: 4*R*,5*S*-**9a**, 4*R*,5*R*-**9b**, 4*S*,5*R*-**9c**, and 4*S*,5*S*-**9d**.

(b) The treatment of the individual 5-methyl-4-phenylpyrrolidin-2-ones **9a–d** with sodium hydride and ethyl bromoacetate.

(c) Carbamoylation of the ethyl 2-(5-methyl-2-oxo-4-phenylpyrrolidin-1-yl)-acetates 4*R*,5*S*-**10a**, 4*R*,5*R*-**10b**, 4*S*,5*R*-**10c**, and 4*S*,5*S*-**10d** with ammonium hydroxide.[3,10]



2, 8, 9, 10 a = 4*R*,5*S*; **b=** 4*R*,5*R*; **c=** 4*S*,5*R*;**d =** 4*S*,5*S*

The target enantiomers 4*R*,5*S*-**2a**, 4*R*,5*R*-**2b**, 4*S*,5*R*-**2c**, and 4*S*,5*S*-**2d** were recrystallised from water and according to chiral chromatography data their optical purity was in the range 90–99%. The angles of optical rotation for these compounds were in agreement with the stereochemistry of phenyl and methyl groups (Table 1) and consistent with the X-ray analysis of the two enantiomers 4*R*,5*S*-**2a** and 4*R*,5*R*-**2b** (Figs. 1 and 2). The ORTEP diagram of these compounds demonstrates that the crystal structure of 4*R*,5*R*-**2b** features two independent molecules in the asymmetric unit. Both molecules are connected by a centre of pseudoinversion. The main bond lengths and angles of 4*R*,5*S*-**2a** and 4*R*,5*R*-**2b** are provided in Table 2.

Table 1
Optical rotation angles for 2-(5-methyl-2-oxo-4-phenyl-pyrrolidin-1-yl)-acetamide (**2**) stereoisomers

Compound	$[\alpha]_D^{20}$	Solvent, concentration
4*R*,5*S*-**2a**	−96.7°	*c* 0.05, MeOH
4*R*,5*R*-**2b**	+22.9°	*c* 0.05, MeOH
4*S*,5*R*-**2c**	+94.1°	*c* 0.05, MeOH
4*S*,5*S*-**2d**	−26.0°	*c* 0.05, MeOH

3. Results and discussion

The positive allosteric modulatory activity of the 2-(5-methyl-4-phenyl-2-oxopyrrolidin-1-yl)-acetamide enantiomers **2a–d** with respect to sig-1R was evaluated using the electrically stimulated rat vas deferens model.[11] The action was determined by comparing the vas deferens contraction heights induced by selective sig-1R agonist PRE-084 (2-morpholin-4-ylethyl-1-phenylcyclohexane-1-carboxylate) in the absence of the tested compound (control) and after preincubation with a 10 μM solution of each enantiomer.

The intensity of the electrically stimulated contractions of rat vas deferens in the presence of 100 μM of PRE-084 was 122 ± 11%. The tested compounds alone did not alter the height of contractions of electrically stimulated vas deferens. In contrast, the preincubation of vas deferens in 10 μM solution of tested enantiomers 10 min before the addition of the PRE-084 increased the

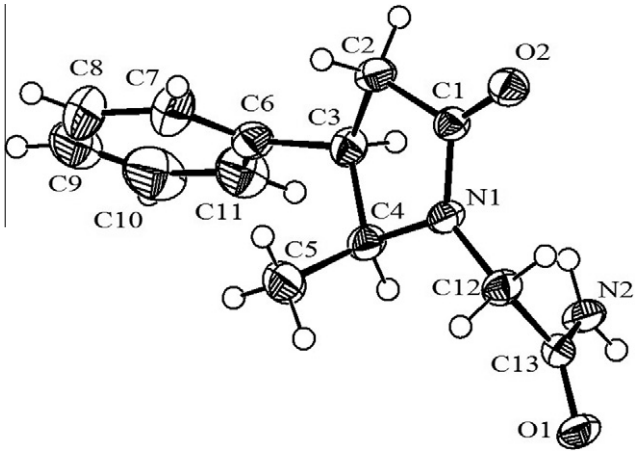


Figure 1. ORTEP diagram of the crystal structure of 4*R*,5*S*-**2a**.

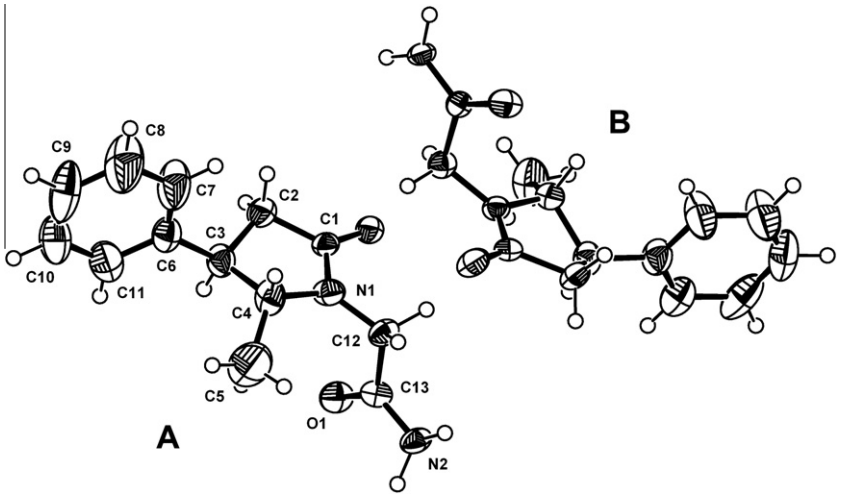


Figure 2. ORTEP diagram of the crystal structure of 4*R*,5*R*-**2b**.

Table 2
Principal bond lengths (l) and valence angles (ω) for 4*R*,5*S*-**2a** and 4*R*,5*R*-**2b** compounds

Bond	l (Å)		
	4*R*,5*S*-**2a**	4*R*,5*R*-**2b**	
		Molecule A	Molecule B
N1–C1	1.345 (2)	1.354 (6)	1.325 (6)
N1–C12	1.449 (2)	1.437 (6)	1.457 (6)
C1–C2	1.512 (2)	1.499 (7)	1.521 (7)
C2–C3	1.524 (2)	1.523 (7)	1.531 (7)
C3–C4	1.551 (2)	1.528 (7)	1.536 (7)
C3–C6	1.513 (2)	1.508 (7)	1.525 (7)
C4–N1	1.476 (2)	1.480 (6)	1.472 (6)
C4–C5	1.518 (2)	1.502 (8)	1.490 (7)
C12–C13	1.520 (2)	1.530 (7)	1.514 (7)
C13–O1	1.235 (2)	1.231 (5)	1.244 (5)
C13–N2	1.319 (2)	1.318 (7)	1.332 (7)
Angle	ω (°)		
	4*R*,5*S*-**2a**	4*R*,5*R*-**2b**	
		Molecule A	Molecule B
C1–N1–C4	113.9 (1)	112.2 (4)	115.0 (4)
C1–N1–C12	123.4 (1)	122.8 (4)	121.3 (4)
C4–N1–C12	122.1 (1)	125.0 (5)	121.1 (4)
N1–C1–C2	107.7 (1)	108.4 (5)	107.1 (5)
C1–C2–C3	103.6 (1)	104.5 (5)	102.9 (5)
C2–C3–C4	103.0 (1)	102.9 (5)	103.0 (5)
C3–C4–N1	100.7 (1)	103.0 (5)	100.0 (5)
N1–C12–C13	113.9 (1)	111.2 (5)	112.0 (5)
C12–C13–O1	119.0 (1)	120.8 (5)	120.5 (5)
C12–C13–N2	118.0 (1)	115.9 (5)	116.3 (5)

Table 3
Positive allosteric modulating effect of enantiomers **2a–d** in rat vas deferens contraction experiments

Enantiomers	% Of increase*
4*R*,5*S*-**2a**	222 ± 37**
4*R*,5*R*-**2b**	191 ± 23**
4*S*,5*R*-**2c**	141 ± 40
4*S*,5*S*-**2d**	147 ± 31

* The response of isolated vas deferens to selective sig-1R agonist PRE-084 was compared without (control) or in the presence of enantiomers **2a–d** at the concentration of 10 μM. All results are expressed as a mean ± SEM of six vas deferens.
** P <0.01 paired student t-test.

intensity of observed contractions. As shown in Table 3, this increase in activity was inherent to all enantiomers. However, the contractions in the case of 4*R*,5*S*-**2a** and 4*R*,5*R*-**2b** were observed to be at least two times higher than those of the control (Table 3).

4. Conclusions

In summary, four individual enantiomers of 2-(5-methyl-4-phenyl-2-oxopyrrolidin-1-yl)-acetamide 4*R*,5*S*-**2a**, 4*R*,5*R*-**2b**, 4*S*,5*R*-**2c**, and 4*S*,5*S*-**2d** were obtained using an asymmetric Michael addition of 2-nitroprop-1-enylbenzene to diethyl malonate catalysed by the chiral 2,2′-cyclopropylidene-bis-oxazoline. This reaction was followed by the chromatographic separation of the diastereoisomeric mixtures of methyl *erythro*- and *threo*-4-nitro-3*R*-phenylpentanoate (*R*-**8**) and methyl *erythro*- and *threo*-4-nitro-3*S*-phenylpentanoate (*S*-**8**), the reductive cyclisation of each enantiomer into the appropriate pyrrolidin-2-one and the final conversion of these compounds into target N-acetamide derivatives. The evaluation of the in vitro biological effect of these compounds using electrically stimulated rat vas deferens contractions induced by selective sigma-1 receptor agonist PRE-084 demonstrated that enantiomers 4*R*,5*S*-**2a** and 4*R*,5*R*-**2b** with the *R*-configuration at the C-4 chiral centre in the 2-pyrrolidone ring are more effective positive allosteric modulators of sigma-1 receptor than are their optical antipodes.

5. Experimental

5.1. Chemistry

All chemicals were supplied by Acros and Aldrich. The melting points were determined using a Boetius PHMK melting point apparatus and are reported uncorrected. ^{1}H and ^{13}C NMR spectra were obtained using a Varian 400 MHz Mercury-400 spectrometer and $CDCl_3$ as the solvent. The chemical shifts are reported in δ values (ppm) relative to an internal TMS standard. The abbreviations are s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad. High-resolution mass spectra were obtained using a Micromass Quatro Micro™ API with MeCN as the solvent. Optical rotation values at 405 nm were measured on a Perkin–Elmer 141 Polarimeter. Elemental analyses (C, H, N) were performed on a Carlo Erba 1108 analyser and were found to be within ±0.4% of the theoretical values. The purity of the compounds was determined by TLC using Merck 60F254 silica gel, whereas Merck Kieselgel (silica gel 0.063–0.230 mm) was used for column chromatography.

5.1.1. Diethyl (1*R*)-2-(2-nitro-1-phenylpropyl)-malonate (*R*-6)

To a solution of (3a*R*,3′a*R*,8a*S*,8′a*S*)-2,2′-cyclopropylidenebis-[3a,8a]-dihydro-8*H*-indeno-[1,2-*d*]-oxazole (**5a**) (420 mg, 1.18 mM) in chloroform (amylene stabilised) (5 ml) in a 250 ml reaction flask, magnesium triflate (378 mg, 1.18 mM) and water (25 μl) were added at room temperature, and the mixture was allowed to stir under argon for 1 h. Molecular sieves (1.0 g) were added to the mixture, and the mixture was stirred for an additional 30 min. The obtained suspension was diluted with 45 ml of a chloroform solution containing diethylmalonate (1.67 g, 10.2 mM), 2-nitroprop-1-enylbenzene (1.63 g, 10.0 mM) and a mixture of morpholine (46 μl) and tetra-methylguanidine (46 μl). The reaction mixture was stirred at 20–25 °C for 72 h. The degree of conversion and the selectivity were determined by a chiral HPLC analysis [Chiralpak IC, 4.6 × 250 mm, 1.0 ml/min, eluent *i*-PrOH–hexane (1:9)] every 12 h. After the completion of the reaction, the reaction mixture was diluted with hexane (50 ml) and stirred for 20 min. Subsequently, the solid was filtered off and the filtrate was washed with 5% aqueous HCl (2 × 50 ml), brine (2 × 50 ml), dried over anhydrous Na_2SO_4. The drying reagent was removed by filtration and the solution was concentrated under reduced pressure. The residue was purified by silica chromatography using ethylacetate/hexane (1:10) and fractions with R_f 0.28 were collected. Yield: 87% (2.8 g). According to the chiral HPLC, the obtained low-melting yellow solid was a mixture of *erythro*- and *threo*-isomers of diethyl (1*R*)-2-(2-nitro-1-phenylpropyl)-malonate in a ratio 3:1. Optical purity: 94%. *erythro*-Isomer ^{1}H NMR ($CDCl_3$); δ = 0.85 (t, 3H, J = 7.0 Hz, CH_2CH_3), 1.15–1.27 (m, 3H, CH_2CH_3), 1.37 (d, 3H, J = 6.8 Hz, CH_3CHNO_2), 3.63–3.93 (m, 3H, CH_2CH_3, COC*H*CO), 4.07–4.29 (m, 3H, CH_2CH_3), 5.07–5.16 (m, 1H, C*H*NO_2), 6.99–7.28 (m, 5H, C_6H_5); *threo*-isomer ^{1}H NMR ($CDCl_3$); δ = 0.93 (t, 3H, J = 7.0 Hz, $3CH_2CH_3$), 1.15–1.27 (m, 3H, CH_2CH_3), 1.29 (d, 1H, J = 6.8 Hz, CH_3CHNO_2), 3.63–3.93 (m, 3H, CH_2CH_3, COC*H*CO), 4.07–4.29 (m, 3H, CH_2CH_3, PhC*H*), 4.29–5.06 (m, 1H, C*H*NO_2), 6.99–7.28 (m, 5H, C_6H_5); HRMS: calculated for $[C_{16}H_{21}NO_6+H]^+$: 324.1444; found: 324.1442. Anal. Calcd for $C_{16}H_{21}NO_6$ (323.35): C, 59.43; H, 6.55; N, 4.33. Found: C, 59.51; H, 6.46; N, 4.27.

5.1.2. Diethyl (1*S*)-2-(2-nitro-1-phenylpropyl)-malonate (*S*-6)

The substitution of chiral catalyst **5a** with its optical antipode **5b** in Section 5.1.1 afforded the diastereoisomeric mixture ***S*-6**. Yield: 83% (2.67 g). According to the chiral HPLC, the obtained low-melting yellow solid was a mixture of *erythro*- and *threo*-isomers of diethyl 2-(2-nitro-1(*S*)-phenylpropyl)-malonate in a ratio of 3:1. Optical purity: 95%. *erythro*-Isomer ^{1}H NMR ($CDCl_3$); δ = 0.85 (t, 3H, J = 7.0 Hz, CH_2CH_3), 1.15–1.27 (m, 3H, CH_2CH_3), 1.37 (d, 3H, J = 6.8 Hz, CH_3CHNO_2), 3.63–3.93 (m, 3H, CH_2CH_3, COC*H*CO), 4.07–4.29 (m, 3H, CH_2CH_3, PhC*H*), 5.07–5.16 (m, 1H, C*H*NO_2), 6.99–7.28 (m, 5H, C_6H_5); *threo*-Isomer ^{1}H NMR ($CDCl_3$); δ = 0.93 (t, 1H, J = 7.0 Hz, CH_2CH_3), 1.15–1.27 (m, 3H, CH_2CH_3), 1.29 (d, 1H, J = 6.8 Hz, CH_3CHNO_2), 3.63–3.93 (m, 3H, CH_2CH_3, COC*H*CO), 4.07–4.29 (m, 3H, CH_2CH_3, PhC*H*), 4.29–5.06 (m, 1H, C*H*NO_2), 6.99–7.28 (m, 5H, C_6H_5); HRMS: calculated for $[C_{16}H_{21}NO_6+H]^+$: 324.1444; found: 324.1443. Anal. Calcd for $C_{16}H_{21}NO_6$ (323.35): C, 59.43; H, 6.55; N, 4.33. Found: C, 59.49; H, 6.54; N, 4.27.

5.1.3. (3*R*)-4-Nitro-3-phenylpentanoic acid (*R*-7)

Diethyl (1*R*)-2-(2-nitro-1-phenylpropyl)-malonate (2.00 g, 6.18 mM) was refluxed in a mixture of acetic and 36% hydrochloric acids at a ratio of 1:3 (30 ml) for 18 h. After the completion of the reaction, the reaction mixture was cooled and concentrated under reduced pressure. The residue was purified by silica column chromatography using ethylacetate/hexane (1:5), and fractions with R_f 0.22 were collected. The obtained yellow solid was a mixture of *erythro*- and *threo*-isomers of (3*R*)-4-nitro-3-phenylpentanoic acid in a ratio of 4:1. Yield: 42% (579 mg). *erythro*-Isomer ^{1}H NMR ($CDCl_3$); δ = 1.48 (d, 3H, J = 6.6 Hz, 5-CH_3), 2.61–2.91 (m, 2H, 2-CH_2), 3.54–3.70 (m, 1H, 3-H), 4.77–4.87 (m, 1H, 4-H), 7.05–7.48 (m, 5H, C_6H_5); *threo*-isomer ^{1}H NMR ($CDCl_3$); δ = 1.27 (d, 3H, J = 6.6 Hz, 5-CH_3), 2.61–2.91 (m, 2H, 2-CH_2), 3.54–3.70 (m, 1H, 3-H), 4.66–4.70 (m, 1H, 4-H), 7.05–7.48 (m, 5H, C_6H_5); HRMS: calculated for $[C_{11}H_{13}NO_4+H]^+$: 224.0921; found: 224.0917.

5.1.4. (3*S*)-4-Nitro-3-phenylpentanoic acid (*S*-7)

The substitution of ***R*-6** with its optical antipode ***S*-6** in Section 5.1.3 afforded a diastereoisomeric mixture of *erythro*- and *threo*-isomers of (3*S*)-4-nitro-3-phenylpentanoic acid in a ratio of 4:1. Yield: 44% (606 mg). *erythro*-Isomer ^{1}H NMR ($CDCl_3$); δ = 1.48 (d, 3H, J = 6.6 Hz, 5-CH_3), 2.61–2.91 (m, 2H, 2-CH_2), 3.54–3.70 (m, 1H, 3-H), 4.77–4.87 (m, 1H, 4-H), 7.05–7.48 (m, 5H, C_6H_5); *threo*-isomer ^{1}H NMR ($CDCl_3$); δ = 1.27 (d, 3H, J = 6.6 Hz, 5-CH_3), 2.61–2.91 (m, 2H, 2-CH_2), 3.54–3.70 (m, 1H, 3-H), 4.66–4.70 (m, 1H, 4-H), 7.05–7.48 (m, 5H, C_6H_5); HRMS: calculated for $[C_{11}H_{13}NO_4+H]^+$: 224.0921; found: 224.0919.

5.1.5. Methyl *erythro*-(3*R*,4*S*)-4-nitro-3-phenylpentanoate (3*R*,4*S*-8a)

A mixture of *erythro*- and *threo*-isomers of (3*R*)-4-nitro-3-phenylpentanoic acid (*R*-**7**) (500 mg, 2.42 mM) and thionyl chloride (61 μl, 1.0 mM) in methanol (20 ml) was refluxed for 6 h. The reaction mixture was cooled and concentrated under reduced pressure. The residue was purified by silica column chromatography using ethylacetate/hexane (1:15). The fractions with R_f 0.18 containing methyl *erythro*-(3*R*,4*S*)-4-nitro-3-phenylpentanoate were collected and evaporated under reduced pressure. Yield: 345 mg (60%) of low melting yellow solid. Optical purity 93% according to chiral HPLC. ^{1}H NMR ($CDCl_3$); δ = 1.48 (d, 3H, J = 6.6 Hz, 5-CH_3), 2.58–2.85 (m, 2H, CH_2), 3.53 (s, 3H, OCH_3), 3.56–3.71 (m, 1H, 3-H), 4.79–4.88 (m, 1H, 4-H), 7.07–7.31 (m, 5H, C_6H_5); HRMS: calculated for $[C_{12}H_{15}NO_4+H]^+$: 238.1077; found: 238.1074. Anal. Calcd for $C_{12}H_{15}NO_4$ (237.26): C, 60.75; H, 6.37; N, 5.90. Found: C, 60.53; H, 6.24; N, 5.82.

5.1.6. Methyl *threo*-(3*R*,4*R*)-4-nitro-3-phenylpentanoate (3*R*,4*R*-8b)

The fractions with R_f 0.26 containing methyl *threo*-(3*R*,4*R*)-4-nitro-3-phenylpentanoate obtained during the chromatographic separation in Section 5.1.5 were collected and evaporated under reduced pressure. Yield: 80 mg (14%) of low melting yellow solid *threo*-(3*R*,4*R*)-4-nitro-3-phenylpentanoate. Optical purity 82% according to chiral HPLC. ^{1}H NMR ($CDCl_3$); δ = 1.27 (d, 3H, J = 6.6 Hz, 5-CH_3), 2.58–2.85 (m, 2H, 2-CH_2), 3.46 (s, 3H, OCH_3), 3.56–3.71 (m, 1H, 3-H), 4.68–4.77 (m, 1H, 4-H), 7.07–7.31 (m, 5H, C_6H_5); HRMS: calculated for $[C_{12}H_{15}NO_4+H]^+$: 238.1077; found: 238.1075. Anal. Calcd for $C_{12}H_{15}NO_4$ (237.26): C, 60.75; H, 6.37; N, 5.90. Found: C, 60.39; H, 6.28; N, 5.75.

5.1.7. Methyl *erythro*-(3*S*,4*R*)-4-nitro-3-phenylpentanoate (3*S*,4*R*-8c)

A mixture of *erythro*- and *threo*-isomers of (3*S*)-4-nitro-3-phenylpentanoic acid (*S*-**7**) (500 mg, 2.42 mM) and thionyl chloride (61 μl, 1.0 mM) in methanol (20 ml) was refluxed for 6 h. The reaction mixture was cooled and concentrated under reduced pressure. The residue was purified by silica column chromatography using ethylacetate/hexane (1:15). The fractions with R_f 0.18 containing methyl *erythro*-(3*S*,4*R*)-4-nitro-3-phenylpentanoate were collected and evaporated under reduced pressure. Yield: 340 mg (59%) of low melting yellow solid. Optical purity 93% according to chiral HPLC. ^{1}H NMR ($CDCl_3$); δ = 1.48 (d, 3H, J = 6.6 Hz, 5-CH_3), 2.58–2.85 (m, 2H, CH_2), 3.53 (s, 3H, OCH_3), 3.56–3.71 (m, 1H, 3-H), 4.79–4.88 (m, 1H, 4-H), 7.07–7.31 (m, 5H, C_6H_5); HRMS: calculated

for $[C_{12}H_{15}NO_4+H]^+$: 238.1077; found: 238.1074. Anal. Calcd for $C_{12}H_{15}NO_4$ (237.26): C, 60.75; H, 6.37; N, 5.90. Found: C, 60.63; H, 6.29; N 5.78.

5.1.8. Methyl *threo*-(3*S*,4*S*)-4-nitro-3-phenylpentanoate (3*S*,4*S*-8d)

The factions with R_f 0.26 containing methyl *threo*-(3*S*,4*S*)-4-nitro-3-phenylpentanoate obtained during the chromatographic separation in Section 5.1.7 were collected and evaporated under reduced pressure. Yield: 78 mg (13%) of low melting yellow solid. Optical purity 94% according to chiral HPLC. ^{1}H NMR ($CDCl_3$); δ = 1.27 (d, 3H, J = 6.6 Hz, 5-CH_3), 2.58–2.85 (m, 2H, 2-CH_2), 3.46 (s, 3 H, OCH_3), 3.56–3.71 (m, 1H, 3-H), 4.68–4.77 (m, 1H, 4-H); 7.07–7.31 (m, 5H, C_6H_5); HRMS: calculated for $[C_{12}H_{15}NO_4+H]^+$: 238.1077; found: 238.1073. Anal. Calcd for $C_{12}H_{15}NO_4$ (237.26): C, 60.75; H, 6.37; N, 5.90. Found: C, 60.60; H, 6.31; N, 5.77.

5.1.9. *erythro*-(4*R*,5*S*)-5-Methyl-4-phenylpyrrolidin-2-one (4*R*,5*S*-9a)

The hydrogenation was performed using a stirring suspension of methyl *erythro*-(3*R*,4*S*)-4-nitro-3-phenylpentanoate (3*R*,4*S*-**8a**) (600 mg, 2.52 mM) in ethanol (40 ml) and 1 ml of 50% Ni Raney slurry in water at 50 °C and 50 atm for 18 h. After the completion of the reaction, the reaction mixture was cooled, and the catalyst was filtered off and washed with 30 ml of ethanol. The filtrate was concentrated under reduced pressure. The purification of the residue by column chromatography on silica gel using CH_2Cl_2/EtOH (20:1) and collecting fractions with R_f 0.40 afforded *erythro*-(4*R*,5*S*)-5-methyl-4-phenylpyrrolidin-2-one as a white solid. Yield: 80% (353 mg). ^{1}H NMR ($CDCl_3$); δ = 0.75 (d, 3H, J = 6.5 Hz, 5-CH_3), 2.55–2.69 (m, 2H, 3-CH_2), 3.64–3.72 (m, 1H, 4-H), 3.96–4.04 (m, 1H, 5-H), 6.78 (br s, 1H, NH), 7.07–7.33 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$); δ = 17.50, 35.22, 44.08, 53.72, 126.51, 127.12, 127.92, 128.50, 128.80, 138.81, 177.84; HRMS: calculated for $[C_{11}H_{13}NO+Na]^+$: 198.0895; found: 198.0900.

5.1.10. *threo*-(4*R*,5*R*)-5-Methyl-4-phenylpyrrolidin-2-one (4*R*,5*R*-9b)

The substitution of 3*R*,4*S*-**8a** with 3*R*,4*R*-**8b** in Section 5.1.9 afforded (4*R*,5*R*)-5-methyl-4-phenylpyrrolidin-2-one. Yield: 85% (375 mg). ^{1}H NMR ($CDCl_3$); δ = 1.20 (d, 3H, J = 6.5 Hz, 5-CH_3), 2.48–2.57 (m, 1H, 3-CH_2), 2.65–2.74 (m, 1H, 3-CH_2), 2.98–3.07 (m, 1H, 4-H) 3.65–3.75 (m, 1H, 5-H), 6.76 (br s, 1H, NH), 7.07–7.33 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$); δ = 20.39, 39.33, 49.66, 57.68, 127.23 (C-2, C-6 aromatic), 127.37, 128.82 (C-3, C-5 aromatic), 140.84, 176.48;. HRMS: calculated for $[C_{11}H_{13}NO+Na]^+$: 198.0895; found: 198.0888.

5.1.11. *erythro*-(4*S*,5*R*)-5-Methyl-4-phenylpyrrolidin-2-one (4*S*,5*R*-9c)

The substitution of 3*R*,4*S*-**8a** with 3*S*,4*R*-**8c** in Section 5.1.9 afforded (4*S*,5*R*)-5-methyl-4-phenylpyrrolidin-2-one. Yield: 84% (371 mg). ^{1}H NMR ($CDCl_3$); δ = 0.75 (d, 3H, J = 6.5 Hz, 5-CH_3), 2.55–2.69 (m, 2H, 3-CH_2), 3.64–3.72 (m, 1H, 4-H), 3.96–4.04 (m, 1H, 5-H), 6.78 (br s, 1H, NH), 7.07–7.33 (m, 5H, C_6H_5). ^{13}C NMR ($CDCl_3$); δ = 17.53, 35.21, 44.07, 53.72, 126.50, 127.13, 127.91, 128.51, 128.80, 138.83, 177.74; HRMS: calculated for $[C_{11}H_{13}NO+Na]^+$: 198.0895; found: 198.0901.

5.1.12. *threo*-(4*S*,5*S*)-5-Methyl-4-phenylpyrrolidin-2-one (4*S*,5*S*-9d)

The substitution of 3*R*,4*S*-**8a** with 3*S*,4*S*-**8d** in Section 5.1.9 afforded (4*S*,5*S*)-5-methyl-4-phenylpyrrolidin-2-one. Yield: 84% (371 mg). ^{1}H NMR ($CDCl_3$); δ = 1.20 (d, 3H, J = 6.5 Hz, 5-CH_3), 2.48–2.57 (m, 1H, 3-CH_2), 2.65–2.74 (m, 1H, 3-CH_2), 2.98–3.07 (m, 1H, 4-H) 3.65–3.75 (m, 1H, 5-H), 6.76 (br s, 1H, NH), 7.07–7.33 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$); δ = 20.38, 39.36, 49.64, 57.71, 127.22 (C-2, C-6 aromatic), 127.37, 128.82 (C-3, C-5 aromatic), 140.85, 176.59; HRMS: calculated for $[C_{11}H_{13}NO+Na]^+$: 198.0895; found: 198.0890.

5.1.13. Ethyl *erythro*-(4*R*,5*S*)-2-(5-methyl-4-phenylpyrrolidin-1-yl)-acetate (4*R*,5*S*-10a)

A solution of *erythro*-(4*R*,5*S*)-5-methyl-4-phenylpyrrolidin-2-one (4*R*,5*S*-**9a**) (351 mg, 2.00 mM) in toluene (30 ml) was added to a suspension of sodium hydride (56 mg, 2.35 mM) in toluene (30 ml). The stirred mixture was heated at 80–90 °C for 30 min and then cooled to room temperature. Ethyl bromoacetate (368 mg, 2.20 mM) was added to the reaction mixture, which was heated to 110–120 °C for 6 h and concentrated under reduced pressure. The residue was dissolved in toluene (30 ml). The obtained solution was washed with 5% aqueous HCl (2 × 50 ml), brine (2 × 50 ml), dried over anhydrous Na_2SO_4. The drying reagent was removed by filtration, and the solution was concentrated under reduced pressure. The residue was purified by silica column chromatography using CH_2Cl_2/MeOH (20:1). The fractions with R_f 0.48 were collected and evaporated under reduced pressure, affording ethyl (4*R*,5*S*)-2-(5-methyl-4-phenylpyrrolidin-1-yl)-acetate (367 mg, 70%) as a colourless oil. ^{1}H NMR ($CDCl_3$); δ = 0.72 (d, 3H, J = 6.6 Hz, 5-CH_3), 1.23 (t, 3H, J = 7.0 Hz, CH_2CH_3), 2.60–2.91 (d, 2H, J = 8.5 Hz, 3-CH_2), 3.65–3.74 (m, 1H, 4-H), 3.66 (d, 2H, J = 17.7 Hz, NCH_2COO), 4.01–4.10 (m, 1H, 5-H), 4.10–4.20 (m, 2H, CH_2CH_3), 4.38 (d, 1H, J = 17.7 Hz, NCH_2COO), 7.09–7.31 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$); δ = 14.14, 14.62, 35.21, 42.07, 42.29, 57.62, 61.34, 127.14 (C-2, C-6 aromatic), 128.03, 128.51 (C-3, C-5 aromatic), 138.88, 168.89, 174.69; HRMS: calculated for $[C_{15}H_{29}NO_3+H]^+$: 262.1443; found: 262.1433.

5.1.14. Ethyl *threo*-(4*R*,5*R*)-2-(5-methyl-4-phenylpyrrolidin-1-yl)-acetate (4*R*,5*R*-10b)

The substitution of 4*R*,5*S*-**9a** with 4*R*,5*R*-**9b** in Section 5.1.13 afforded (4*R*,5*R*)-2-(5-methyl-4-phenylpyrrolidin-1-yl)-acetate. Yield: 72% (377 mg). ^{1}H NMR ($CDCl_3$ δ = 1.16 (d, 3H, J = 6.3 Hz, 5-CH_3), 1.23 (t, 3H, J = 7.0 Hz, CH_2CH_3), 2.53–2.63 (m, 1H, CH_2), 2.76–2.86 (m, 1H, CH_2), 2.92–3.01 (m, 1H, 4-H), 3.71 (d, 1H, J = 17.7 Hz, NCH_2COO), 3.74–3.83 (m, 1H, 5-H), 4.10–4.20 (m, 3H, CH_2CH_3), 4.38 (d, 1H, J = 17.8 Hz, NCH_2COO), 7.18–7.33 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$), δ = 14.14, 17.97, 38.89, 41.88, 47.22, 61.22, 61.35, 127.25 (C-2, C-6 aromatic), 127.54, 128.33 (C-3, C-5 aromatic), 141.07, 168.75, 174.23; HRMS: calculated for $[C_{15}H_{29}NO_3+H]^+$: 262.1443; found: 262.1423.

5.1.15. Ethyl *erythro*-(4*S*,5*R*)-2-(5-methyl-4-phenylpyrrolidin-1-yl)-acetate (4*S*,5*R*-10c)

The substitution of 4*R*,5*S*-**9a** with 4*S*,5*R*-**9c** in Section 5.1.13 afforded (4*S*,5*R*)-2-(5-methyl-4-phenylpyrrolidin-1-yl)-acetate. Yield: 70% (367 mg). ^{1}H NMR ($CDCl_3$); δ = 0.72 (d, 3H, J = 6.6 Hz, 5-CH_3), 1.23 (t, 3H, J = 7.0 Hz, CH_2CH_3), 2.60–2.91 (d, 2H, J = 8.5 Hz, 3-CH_2), 3.65–3.74 (m, 1H, 4-H), 3.66 (d, 2H, J = 17.7 Hz, NCH_2COO), 4.01–4.10 (m, 1H, 5-H), 4.10–4.20 (m, 2H, CH_2CH_3), 4.38 (d, 1H, J = 17.7 Hz, NCH_2COO), 7.09–7.31 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$), δ = 14.13, 14.65, 35.21, 42.07, 42.29, 57.62, 61.34, 127.14 (C-2, C-6 aromatic), 128.03, 128.51 (C-3, C-5 aromatic), 138.88, 168.89, 174.68; HRMS: calculated for $[C_{15}H_{29}NO_3+H]^+$: 262.1443; found: 262.1448.

5.1.16. Ethyl *threo*-(4*S*,5*S*)-2-(5-methyl-4-phenylpyrrolidin-1-yl)-acetate (4*S*,5*S*-10d)

The substitution of 4*R*,5*S*-**9a** with 4*S*,5*S*-**9d** in Section 5.1.13 afforded (4*S*,5*S*)-2-(5-methyl-4-phenylpyrrolidin-1-yl)-acetate. Yield: 72% (377 mg). ^{1}H NMR ($CDCl_3$); δ = 1.16 (d, 3H, J = 6.3 Hz, 5-CH_3), 1.23 (t, 3H, J = 7.0 Hz, CH_2CH_3), 2.53–2.63 (m, 1H, CH_2),

2.76–2.86 (m, 1H, CH_2), 2.92–3.01 (m, 1H, 4-H), 3.71 (d, 1H, J = 17.7 Hz, NCH_2COO), 3.74–3.83 (m, 1H, 5-H), 4.10–4.20 (m, 3H, CH_2CH_3), 4.38 (d, 1H, J = 17.8 Hz, NCH_2COO), 7.18–7.33 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$), δ = 14.14, 17.97, 38.89, 41.87, 47.21, 61.22, 61.35, 127.25 (C-2, C-6 aromatic), 127.53, 128.33 (C-3, C-5 aromatic), 141.06, 168.75, 174.23; HRMS: calculated for $[C_{15}H_{29}NO_3+H]^+$: 262.1443; found: 262.1456.

5.1.17. *erythro*-(4*R*,5*S*)-2-(5-Methyl-2-oxo-4-phenyl-pyrrolidin-1-yl)-acetamide (4*R*,5*S*-2a)

A solution of ethyl (4*R*,5*S*)-2-(5-methyl-4-phenylpyrrolidin-1-yl)-acetate (4*R*,5*S*-**10a**) (350 mg, 1.34 mM) in methanol (30 ml) was treated with 25% aqueous ammonium (10 ml) for 12 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by column chromatography using CH_2Cl_2/EtOH (20:1). The fractions with R_f 0.32 were collected and evaporated under reduced pressure, affording *erythro*-(4*R*,5*S*)-2-(5-methyl-2-oxo-4-phenyl-pyrrolidin-1-yl)-acetamide (249 mg, 80%) as a white solid recrystallised from water. Mp 169–171 °C. $[\alpha]_D^{20}$ −96.7° (*c* 0.05, MeOH). Optical purity 99.3% according to chiral HPLC. ^1H NMR ($CDCl_3$); δ = 0.77 (d, 3H, J = 6.6 Hz, 5-CH_3), 2.62–2.81 (m, 2H, 3-CH_2), 3.66–3.75 (m, 1H, 4-H), 3.75 (d, 1H, J = 16 Hz, NCH_2COO), 3.98–4.08 (m, 1H, 5-H), 4.04 (d, 1H, J = 16 Hz, NCH_2COO), 5.48 and 6.29 (br s, br s, 2H, NH_2), 7.07–7.32 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$); δ = 14.72, 34.69, 42.40, 45.47, 59.03, 127.33 (C-2, C-6 aromatic), 127.87, 128.65 (C-3, C-5 aromatic), 138.19, 170.92, 175.10; HRMS: calculated for $[C_{13}H_{16}N_2O_2+Na]^+$: 255.1109; found: 255.1113. Anal. Calcd for $C_{13}H_{16}N_2O_2$ (232.28): C, 67.22; H, 6.94; N, 12.06. Found: C, 67.31; H, 6.99; N, 12.10.

5.1.18. *threo*-(4*R*,5*R*)-2-(5-Methyl-2-oxo-4-phenyl-pyrrolidin-1-yl)-acetamide (4*R*,5*R*-2b)

The substitution of 4*R*,5*S*-**10a** with 4*R*,5*R*-**10b** in Section 5.1.17 afforded (4*R*,5*R*)-2-(5-methyl-4-phenylpyrrolidin-1-yl)-acetamide. Yield: 72% (224 mg). Mp 117–118 °C. $[\alpha]_D^{20}$ +22.9 (*c* 0.05, MeOH). Optical purity 90.6% according to chiral HPLC. ^1H NMR ($CDCl_3$); δ = 1.23 (d, 3H, J = 6.2 Hz, 5-CH_3), 2.52–2.62 (m, 1H, 3-CH_2), 2.77–2.86 (m, 1H, 3-CH_2), 3.95–3.05 (m, 1H, 4-H), 3.67–3.85 (m, 1H, 5-H), 3.85 (d, 1H, J = 16 Hz, NCH_2COO), 3.97 (d, 1H, J = 16 Hz, NCH_2COO), 5.54 and 6.25 (br s, br s, 2H, NH_2), 7.16–7.33 (m, 5H, C_6H_5). ^{13}C NMR ($CDCl_3$); δ = 18.32, 38.54, 45.10, 47.15, 62.51, 127.33 (C-2, C-6 aromatic), 127.43, 128.95 (C-3, C-5 aromatic), 140.65, 170.80, 174.72; HRMS: calculated for $[C_{13}H_{16}N_2O_2+Na]^+$: 255.1109; found: 255.1115. Anal. Calcd for $C_{13}H_{16}N_2O_2$ (232.28): C, 67.22; H, 6.94; N, 12.06. Found: C, 67.25; H, 6.98; N, 12.08.

5.1.19. *erythro*-(4*S*,5*R*)-2-(5-methyl-2-oxo-4-phenyl-pyrrolidin-1-yl)-acetamide (4*S*,5*R*-2c)

The substitution of 4*R*,5*S*-**10a** with 4*S*,5*R*-**10c** in Section 5.1.17 afforded (4*S*,5*R*)-2-(5-methyl-2-oxo-4-phenyl-pyrrolidin-1-yl)-acetamide. Yield: 72% (224 mg). Mp 169–171 °C. $[\alpha]_D^{20}$ +96.7° (*c* 0.05, MeOH). Optical purity 99.0% according to chiral HPLC.

^1H NMR ($CDCl_3$); δ = 0.77 (d, 3H, J = 6.6 Hz, 5-CH_3), 2.62–2.81 (m, 2H, 3-CH_2), 3.66–3.75 (m, 1H, 4-H), 3.75 (d, 1H, J = 16 Hz, NCH_2COO), 3.98–4.08 (m, 1H, 5-H), 4.04 (d, 1H, J = 16 Hz, NCH_2COO), 5.48 and 6.29 (br s, br s, 2H, NH_2), 7.07–7.32 (m, 5H, C_6H_5). ^{13}C NMR ($CDCl_3$); δ = 14.72, 34.69, 42.40, 45.47, 59.03, 127.33 (C-2, C-6 aromatic), 127.87, 128.65 (C-3, C-5 aromatic), 138.19, 170.91, 175.10; HRMS: calculated for $[C_{13}H_{16}N_2O_2+Na]^+$: 255.1109; found: 255.1116. Anal. Calcd for $C_{13}H_{16}N_2O_2$ (232.28): C, 67.22; H, 6.94; N, 12.06. Found: C, 67.30; H, 6.95; N, 12.11.

5.1.20. *threo*-(4*S*,5*S*)-2-(5-Methyl-2-oxo-4-phenyl-pyrrolidin-1-yl)-acetamide (4*S*,5*S*-2d)

The substitution of 4*R*,5*S*-**10a** with 4*S*,5*S*-**10d** in Section 5.1.17 afforded (4*S*,5*S*)-2-(5-methyl-4-phenylpyrrolidin-1-yl)-acetamide. Yield: 72% (224 mg). Mp 117–118 °C. $[\alpha]_D^{20}$ −26.0 (*c* 0.05, MeOH). Optical purity 93.8% according to chiral HPLC.

^1H NMR ($CDCl_3$); δ = 1.23 (d,3H, J = 6.2 Hz, 5-CH_3), 2.52–2.62 (m, 1H, 3-CH_2), 2.77–2.86 (m, 1H, 3-CH_2), 3.95–3.05 (m, 1H, 4-H), 3.67–3.85 (m, 1H, 5-H), 3.85 (d, 1H, J = 16 Hz, NCH_2COO), 3.97 (d, 1H, J = 16 Hz, NCH_2COO), 5.54 and 6.25 (br s, br s, 2H, NH_2), 7.16–7.33 (m, 5H, C_6H_5). ^{13}C NMR ($CDCl_3$); δ = 18.33, 38.55, 45.10, 47.15, 62.51, 127.33 (C-2, C-6 aromatic), 127.43, 128.95 (C-3, C-5 aromatic), 140.65, 170.80, 174.71; HRMS: calculated for $[C_{13}H_{16}N_2O_2+Na]^+$: 255.1109; found: 255.1107. Anal. Calcd for $C_{13}H_{16}N_2O_2$ (232.28): C, 67.22; H, 6.94; N, 12.06. Found: C, 67.24; H, 6.96; N, 12.09.

5.2. Determination of optical purity by chiral HPLC

Chiral HPLC measurements were performed on a Waters Alliance (Waters Corporation, Milford, USA) LC system equipped with a 2695 separation module, quaternary pump, degasser, autosampler and column thermostat. Waters 2489 double-absorbed UV detector at 210 nm was used for the analysis. The output signal was monitored and processed using Waters Empower 2 software. The separation of *R*-**6** and *S*-**6** was performed on polysaccharide-based immobilised columns Chiralpak IA (250 × 4.6 mm I.D., with a particle size 5 μm) using the *i*-PrOH/*n*-hexane (1:9) mobile phase. The separation of 3*R*,4*S*-**8**, 3*R*,4*R*-**8**, 3*S*,4*S*-**8**, and 3*S*,4*R*-**8** was performed on coated Lux Cellulose-1 (150 × 4.6 mm I.D., with a particle size 5 μm) columns using EtOH/*n*-hexane (1:99) as the mobile phase. The separation of 4*R*,5*S*-**2**, 4*R*,5*R*-**2**, 4*S*,5*S*-**2**, and 4*S*,5*R*-**2** was performed on coated Lux Amylose-2 (150 × 4.6 mm I.D., with a particle size 5 μm) columns using EtOH/*n*-hexane (15:85) as the mobile phase. The chromatographic runs were performed at a flow rate of 1.0 ml/min and a column temperature of 25 °C. The injection volume was 10 μl and the analytical sample concentration was 0.5 mg/ml. The enantiomeric elution order was established by analysing racemic mixture and individual enantiomer samples.

5.3. X-ray crystallographic analysis

Diffraction data for the *erythro*-(4*R*,5*S*)-2-(5-methyl-2-oxo-4-phenyl-pyrrolidin-1-yl)-acetamide (4*R*,5*S*-**2a**) and *threo*-(4*R*,5*R*)-2-(5-methyl-2-oxo-4-phenyl-pyrrolidin-1-yl)-acetamide (4*R*,5*R*-**2b**) were collected on a Bruker-Nonius KappaCCD diffractometer using monochromic graphite Mo Kα radiation (λ = 0.71073 Å). The crystal structures were solved by a direct method and refined by a full-matrix least squares method.[12,13] Non-hydrogen atoms were refined using an anisotropic approximation, whereas H-atoms were refined by the riding model. Crystal data for 4*R*,5*S*-**2**: orthorhombic, a = 6.3800(2), b = 9.9220(3), c = 20.1510(6) Å, V = 1275.61(7) Å^3, Z = 4, and the space group is $P2_12_12_1$. A total of 2905 reflection intensities were collected up to $2\theta_{max}$ = 55°; for the structural refinement, 2367 reflections with $I > 2\sigma(I)$ were used. The final *R*-factor was 0.041. Crystal data for 4*R*,5*R*-**2**: monoclinic; a = 11.2168(5), b = 9.0714(4), c = 13.3621(7) Å, β = 109.849(2)°; V = 1278.9(1) Å^3, Z = 4, and the space group is $P2_1$. A total of 3571 reflection intensities were collected up to $2\theta_{max}$ = 58°; for the structure refinement, 1930 independent reflections with $I > 3\sigma(I)$ were used. The final *R*-factor was 0.054. Crystallographic data for the compounds were deposited into the Cambridge Crystallographic Data Centre as a Supplementary Publication Number CCDC 905537 for 4*R*,5*S*-**2a** and CCDC 904950 for 4*R*,5*R*-**2b**, respectively.

Copies of the data could be obtained, free of charge, by application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.

5.4. The experimental model of vas deferens isolation and stimulation in vitro

Wistar rats were sacrificed by decapitation. Both vas deferens were excised, immersed in ice-cold Krebs–Henseleit (K–H) buffer solution (content in mmol/l: NaCl 118.0, KCl 4.75, $CaCl_2$ 2.52, $MgCl_2$ 1.64, $NaHCO_3$ 24.88, K_2HPO_4 1.18, glucose 10.0, EDTA 0.05) and cleaned from the surrounding tissues. The proximal portions of the ductus deferens (~15 mm) were mounted in 50 ml organ baths and incubated in K–H buffer solution bubbled with 95% CO_2 and 5% O_2 at 32 °C. The passive tension was fixed at 1.0 g and every 15 min, the buffer solution in the organ bath was changed. After a 60 min adaptation period, the isolated vas deferens were stimulated with an electrical current at the frequency of 0.1 Hz, pulse duration of 1 ms and at a voltage of 50 V. When the electrical current induced stable contraction amplitude, cumulative doses (from 1 to 100 μM) of selective sig-1R agonist PRE-084 were added. After reaching the plateau contraction amplitude at the highest studied agonist concentration (100 μM), the electrical stimulation was turned off and the isolated vas deferens was washed several times with the K–H buffer solution. After 30 min, electrical stimulation was resumed using the same parameters. When the electrical current induced stable contraction amplitude, the tested compounds were added to the isolated vas deferens at a concentration of 10 μM. After 10 min of electrical stimulation, a cumulative dose of PRE-084 was added. The response to the sig-1R agonist before and after the addition of tested compound was calculated as a percentage increase relative to the baseline contraction amplitude. All results are expressed as a mean ± SEM. The data were evaluated using an analysis of variance (ANOVA). Whenever ANOVA was significant, additional multiple comparisons were performed using a Newman–Keuls post-hoc test. *P*-values of less than 0.05 were considered to be significant.

Acknowledgment

This work was supported by European Regional Development Fund Project: *ERAF 2DP/2.1.1.1.0/10/APIA/VIAA/059.*

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.03.016. These data include MOL files and InChiKeys of the most important compounds described in this article.

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