

Enantiodivergent Synthesis of (*R*)- and (*S*)-Rolipram

Joachim Demnitz, Luigi LaVecchia, Edmond Bacher, Thomas H. Keller, Thomas Müller, Friedrich Schürch, Hans-Peter Weber and Esteban Pombo-Villar*

Preclinical Research, Novartis Pharma Ltd, CH-4002 Basel, Switzerland,
phone +41 61 324 9865, fax +41 61 324 9794, e-mail esteban.pombo@pharma.novartis.com

Received: 2 November 1997 / Accepted: 23 February 1998 / Published: 10 March 1998

Abstract: Both enantiomers of rolipram (**1**) have been prepared in large quantity from a common intermediate *rac*-3-(3'-cyclopentyloxy-4'-methoxy)phenyl-4-nitro butyric acid (**6**), which was resolved by way of the two readily separable diastereoisomeric amides obtained with (*S*)-(-)-phenylethylamine. Reduction of the nitro group and intramolecular transamidation gave (*R*)-(-)-**1** and (*S*)-(+)-**1**, respectively. CD spectra of both enantiomers of rolipram are reported and discussed. Both enantiomers of rolipram presented the same potency of inhibitory activity against recombinant cyclic-AMP-selective phosphodiesterase (PDE4) subtypes.

Keywords: Rolipram, phosphodiesterase, circular dichroism (CD) spectra, enantiodivergent synthesis.

Introduction

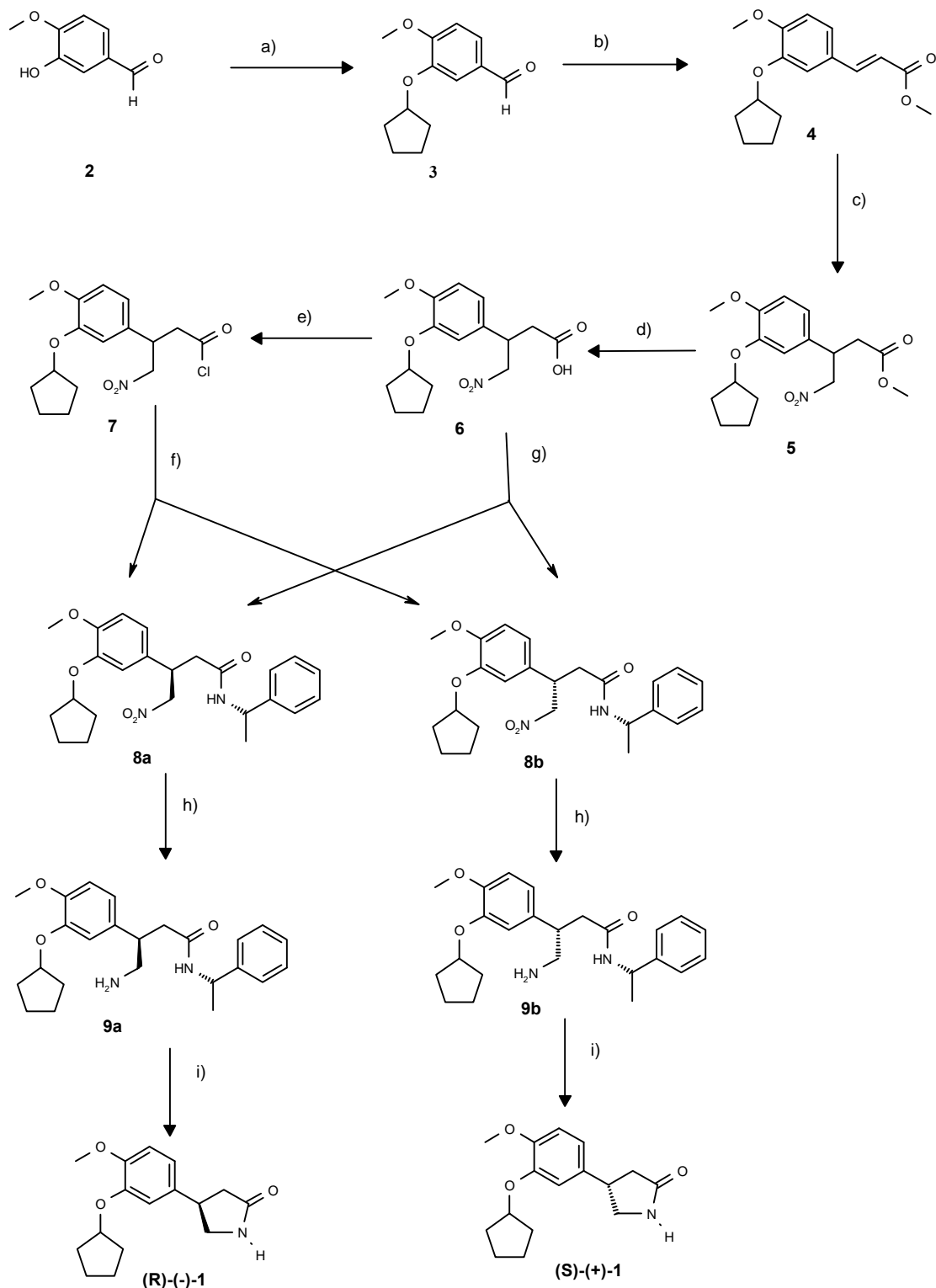
Rolipram (**1**) is a compound with varied biological activity. In particular, attention has been drawn to the emetic [1], antiinflammatory [2], immunosuppressant [3], antidepressive [4,5], putative antiparkinsonian [6], and neuroprotective [7] effects. The best characterised biochemical activity of **1** is the selective inhibition of the cAMP-selective phosphodiesterase family known as Type IV (PDE4). Its selectivity for this subtype of phosphodiesterases is the hallmark of the classification of these enzymes [8].

Although **1** has been often used as the racemate in biological experiments, the biological activity of the enantiomers may be widely divergent [9-12]. Therefore we considered it desirable to use this compound routinely as a single enantiomer for both *in vitro* and *in vivo* studies. This made it necessary to obtain sufficiently large

quantities of both enantiomers. In this paper we report a new synthesis of the enantiomers of **1**, and the characterisation of these compounds as inhibitors of PDE4 enzyme subtypes.

The industrial synthesis of *rac*-(**1**) and resolution of the enantiomers either chromatographically [13-15] or by classical (enzymatic) resolution of an intermediate in the synthesis [16] has been reported. Asymmetric syntheses of **1** have been recently reviewed [17]. Additionally, several recent enantioselective syntheses of **1** [18-19] have been reported. For our purposes, however, we required larger quantities of both enantiomers, and the chiral chromatographic methods, although perfectly suitable for the separation of a few grams of material, were not readily suitable for the quantities required. It seemed to us that an enantiodivergent synthesis, where diastereoisomeric intermediates could be readily separated, would provide an alternative fast and efficient route to both enantiomers.

* To whom correspondence should be addressed.



Scheme 1. Enantiodivergent synthesis of rolipram: a) BnBu_3NBr , $\text{C}_5\text{H}_9\text{Br}$, KOH , H_2O , toluene, 93%. b) $\text{CH}_2(\text{COOH})\text{COOMe}$, aniline, piperidine, py, 85°C , 89%. c) CH_3NO_2 , tetramethylguanidine, 75°C , 24h, 78%. d) NaOH , MeOH , 94%. e) SO_2Cl_2 , CH_2Cl_2 , 93%. f) (*S*)-phenylethylamine, CH_2Cl_2 , reflux, 70%. g) (*S*)-phenylethylamine, HOBT, DMAP, EDCI, DMF , 0°C -RT, 79% h) Pd/C , 0.2 bar H_2 , DMF , 20 h, 100%. i) xylene, reflux 3-4h, 54%.

Additionally, from a previous synthesis of *rac*-**1** [20], the known racemic ester **5** [21] was available to us in large quantities. We thus envisaged a strategy where the racemic acid could be converted to a mixture of readily separable diastereoisomeric amides, which could then be converted to the cyclic pyrrolidinones (+)-**1** and (–)-**1** respectively. In our initial experiments, the acid **6** was converted to the corresponding mixture of diastereoisomeric amides with either the Evans auxiliary, (+)-3-benzyl-oxazolidin-2-one or with (*S*)-phenylethylamine. The latter mixture of diastereoisomers proved to be the easiest to separate, so we decided to use this chiral amine in the enantiodivergent step.

Results and Discussion

Synthesis

We have devised an improved procedure for the synthesis of cyclopentyl isovanillin (**3**) from isovanillin (**2**) utilising phase-transfer catalysis. The clean condensation of **3** with monomethyl malonate with concomitant decarboxylation directly gave the known unsaturated ester **4** [22] in very high yield. Base-catalysed Michael addition of nitromethane to **4** proceeded smoothly to the known ester **5** [21], which was readily hydrolysed to the acid **6**. Conversion of **6** to a mixture of the diastereoisomeric amides **8a** and **8b** was performed either directly with *N*-(3-dimethylaminopropyl)-*N'*-ethyl-carbodiimide hydrochloride (EDCI) and hydroxybenzotriazole (HOBT) activation (ca 70% yield), or via the acid chloride **7** (ca. 75% yield over both steps). Chromatographic separation yielded the crystalline (+)-**8a** and (–)-**8b**. Reduction to the corresponding amines **9a** or **9b** could be performed with Raney nickel, but this needed long reaction times and did not provide consistently reproducible results. Platinum oxide was also adequate as a catalyst, but on a large scale, some over-reduction of the aromatic phenylethylamine moiety was observed. For this purpose, catalytic hydrogenation with palladium on carbon was far more effective and convenient. Heating **9a** in refluxing xylene for 3–4 h effected transamidation to give crystalline (*R*)-(–)-**1** in 54% yield (99.70% e.e.). Analogous treatment of **9b** provided (*S*)-(+)-**1** in 54% yield (98.95% e.e.).

CD-spectroscopy

The compounds prepared here present two different types of chromophores which may give rise to Cotton effects (CE's), namely the phenyl and the amide groups.

The π - π^* transitions of the phenyl group are associated with CE's in the region of 250–260 nm, around 210 nm, and in the region of 190 nm, attributed to the 1L_b , 1L_a , and $^1B_{a,b}$ transitions, respectively [23]. The amide chromophore can present characteristic CE's around 220 nm, attributed to the n- π^* transition, and around 190 nm attributed to the π - π^* transition [24]. The CD spectra of the diastereoisomeric pairs **8a**, **8b**, and **9a**, **9b**, as well as those of the enantiomers (+)-**1** and (–)-**1** are shown in **Figure 1**, and detailed in **Table 1**. In both pairs of diastereoisomers **8** and **9**, the presence of two differentially substituted benzylic chiral moieties may be expected to give rise to additional CE's. In this case, however, all four diastereoisomeric amides of (*S*)-phenylethyl amine show pronounced negative CE's in the region 190–215 nm, analogous to those found for (*S*)-phenylethylacetamide (**10**) [25,26]. The CD spectrum of **10** in methanol, presents minima at λ_{\max} ($\Delta\epsilon$) = 197.5 (–28.5), 211 (–13.3), and 215 (–12.8) nm, which are associated with the corresponding 1L_a absorption band. The absorptions due to this moiety dominate the chiroptical properties of the acyclic amides **8** and **9** in this region. There are, however, clear differences between the diastereoisomers **8a** and **8b** particularly in the region associated with the 1L_b band. In the case of **9a** and **9b**, both compounds show negative CE's in the region 190–220 nm, and a positive CE near 240 nm, and **9a** presents positive CE's in the 240–270 nm region. Although **9b** also presents mainly positive CE's in this latter region, it shows a weak negative CE at 251 nm.

The effect of substituents on the benzene moiety, can have pronounced effects on the observed CE's [27,28]. The CD spectrum of (*S*)-phenylethylamine shows a positive CE at the origin of the 1L_b band, λ_{\max} 268 ($\Delta\epsilon_{\max}$ +0.11). Upon substitution at the 4-position of the benzene ring with a Me, Br or MeO group, an inversion of the sign of the corresponding CE's is observed (see **Table 1**). A similar substituent in the 3-position, on the other hand, enhances the CD effect observed in the unsubstituted case. However, for the corresponding acetamide, **10**, a different substituent effect is observed; in fact, the CE's of the 4-MeO derivative (**11**) and the parent molecule are very similar, but the 3-MeO analogue (**12**) shows a CE with the opposite sign [25]. From these data alone, one cannot confidently predict the effect of two alkoxy substitutions on the CD spectrum of the corresponding acetamides.

In the region of 250–290 nm, (*S*)-**1** shows a broad CE (λ_{\max} = 280.5, $\Delta\epsilon_{\max}$ = +0.27), of the same sign as those of (*S*)-phenylethylamine acetamide (**10**) and p-MeO-phenylethylamine acetamide (**11**).

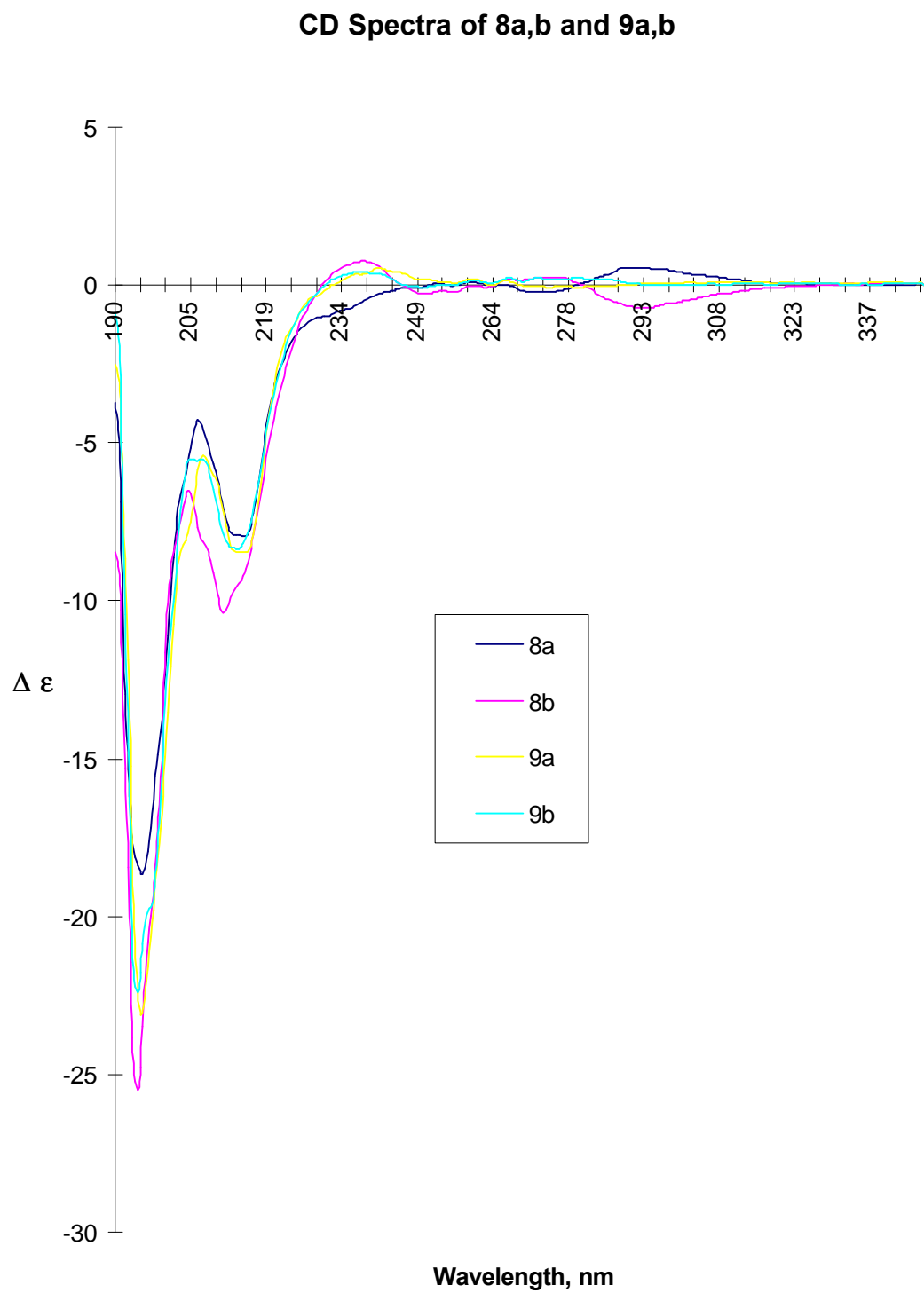


Figure 1. CD Spectra of **8a,b** and **9a,b**. Conditions: $c = 2.00$ mg/ml, MeOH, $l = 0.01$ cm.

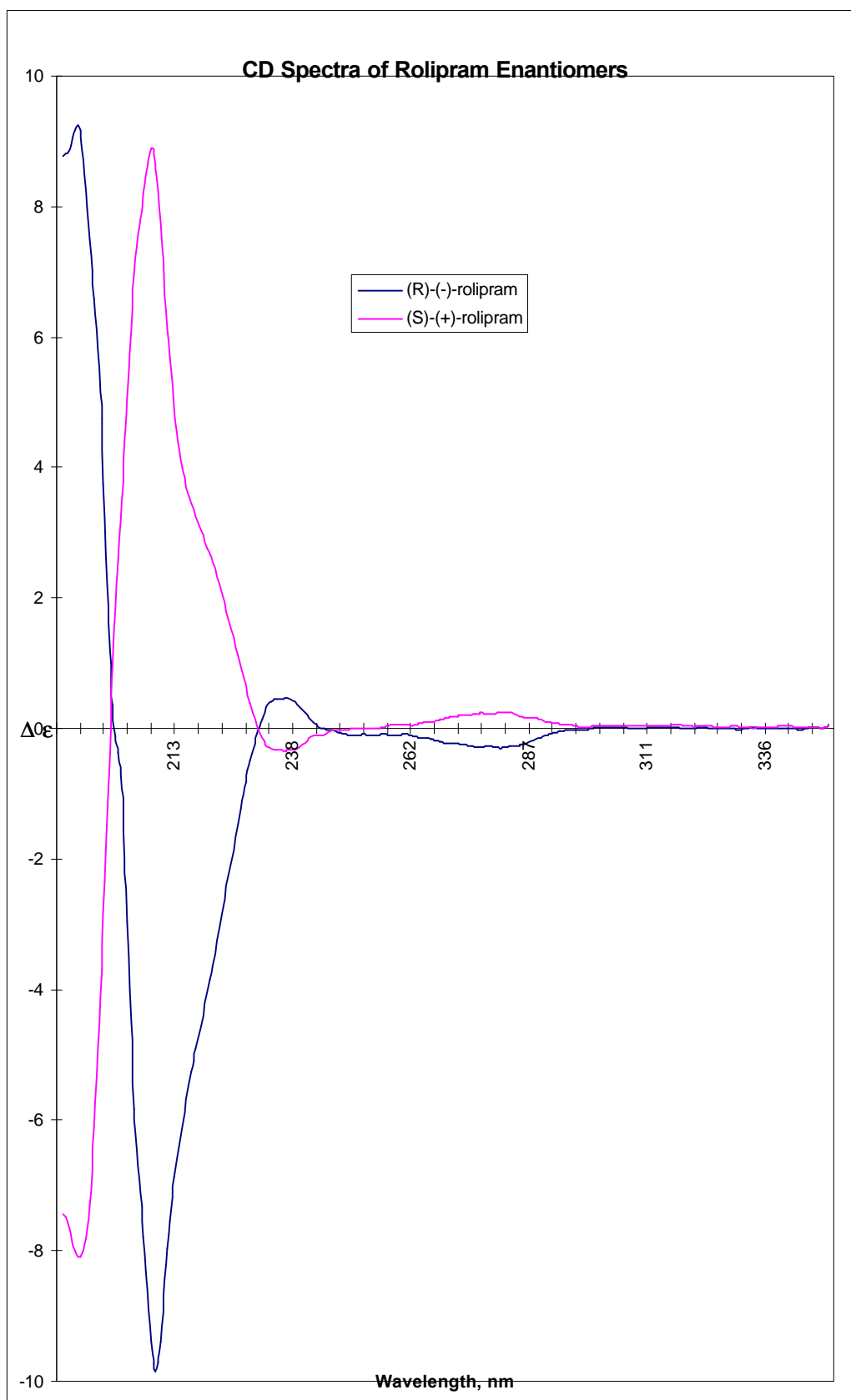


Figure 2. CD Spectra of the rolipram enantiomers. Conditions: $c = 2.00$ mg/ml, MeOH, $l = 0.01$ cm.

Table 1. CD spectral Data in MeOH.

Compound	λ_{\max} (nm)	$\Delta \epsilon$
8a	296.5	0.717 ^a
	277.5	-0.203 ^a
	211 (sh)	<i>ca.</i> 7.9 ^b
	215	-7.952 ^b
	194	-18.387 ^b
8b	302.5	-0.561 ^a
	272.5	0.541 ^a
	215 (sh)	<i>ca.</i> 10 ^b
	211.5	-10.373 ^b
	194	-25.552 ^b
9a	267	0.176 ^c
	261	0.213 ^c
	245	0.330 ^c
	215	-8.435 ^b
	195.5	-23.081 ^b
9b	268	0.242 ^c
	251	-0.123 ^c
	239.5	0.482 ^c
	214	-8.365 ^b
	205.5	-5.540 ^b
	194.5	-22.422 ^b
(R)-(-)-1	280	-0.242 ^c
	236	0.478 ^c
	209.5	-9.857 ^b
	192.5	9.186 ^b
(S)-(+)-1	280.5	0.270 ^c
	237.5	-0.351 ^c
	208.5	8.893 ^b
	193.5	-8.105 ^b

Table 1 continued.

10 (S)-phenylethylamine acetamide	267.5	0.224 ^d
	260.5	0.231 ^d
	254.5	0.126 ^d
	248	0.047 ^d
	234	0.037 ^d
	215	-12.862 ^b
	211	-13.332 ^b
11 (S)-4-MeO -phenylethylamine acetamide ^e	197.5	-28.522 ^b
	282	+0.21
	275	+0.23
	267	+0.14
	225	-12.1
12 (S)-3-MeO- phenylethylamine acetamide ^e	200	-33.0
	279	-0.31
	275	-0.35
	201	-39.5

^{a)} $l = 1.00$ cm, $c = 2.00$ mg/ml.

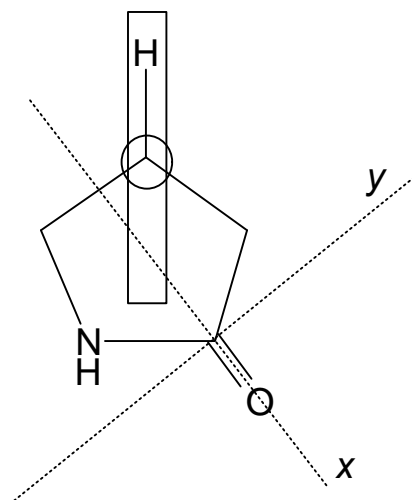
^{b)} $l = 0.01$ cm, $c = 2.00$ mg/ml.

^{c)} $l = 0.01$ cm, $c = 10.0$ mg/ml.

^{d)} $l = 0.01$ cm, $c = 20.0$ mg/ml.

^{e)} data from ref. [25]. in ethanol as solvent..

a)



b)

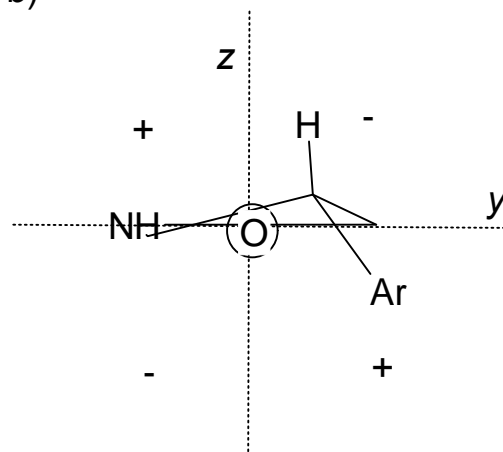


Figure 3. Application of the lactam octant rule. a) the xy -plane is defined by the N(1)-C(2)-O plane, z -axis coming out of the plane. Rotating structure around the y -axis, and viewing the molecule down the x -axis results in the projection shown in b). In this case all the substituents are in the quadrants behind the yz -plane. The aromatic moiety falls on the lower right quadrant, therefore the sign of the CE associated with the $n\text{-}\pi^*$ transition at 220 nm in (*R*)-rolipram is expected to be positive.

Interestingly, the enantiomers of **1** present characteristic bisignate CD spectra in the region of 190–230 nm, as seen in **Figure 2**. In this wavelength range, the corresponding transitions would be the allowed $\pi\text{-}\pi^*$ electric dipole and the forbidden $n\text{-}\pi^*$ magnetic dipole transitions of the amide, and the 1L_a aromatic band, although contributions from the ${}^1B_{a,b}$ aromatic transitions could also be important. The CD spectrum of (*S*)-phenylethylamine shows a positive Cotton effect at 212 nm, assigned to the 1L_a transition, and a negative one at 184 nm associated with the ${}^1B_{a,b}$ transition. Additionally, the amine moiety contributes two further maxima in this region, associated with the $n\text{-}\sigma^*$ transition, a positive one at 193 nm and a negative one at 225 nm [27,29]. This is in contrast to the acetamide **11**, where a strong negative CE at 197.5 nm is observed, and weaker, but still negative CE's at 211 and 215 nm, as discussed above. For (*S*)-(+)-**1** the CE at 208 nm is positive, while that at 193.5 nm is negative, both being of approximately equal intensity. The amide $n\text{-}\pi^*$ transition gives rise to a CE observed as a shoulder at ca. 215 nm, of a sign predicted by application of the lactam octant rule [30,31], as indicated in **Figure 3**, and also similar to that seen in the CD spectrum of (*R*)-(-)-4-hydroxypiperidin-2-one [32]. Although more detailed studies are necessary to fully assign the CE's described to specific electronic transitions or exciton coupling effects [33,34], these observations provide a reference for chiroptical properties in similar systems.

Pharmacological activity

In our hands, partially purified PDE4 enzyme from human neutrophils was inhibited by rolipram with $\text{pIC}_{50}=5.5$ with no observable difference in activity between the two enantiomers (see **Table 2**. Note that potencies are given as pIC_{50} *i.e.* the $-\log \text{IC}_{50}$). This

result is similar to findings by Souness [9] and Torphy [35] on partially purified PDE4 from guinea pig eosinophils and human monocytes respectively, which show little discrimination between (*R*)- and (*S*)-rolipram with IC_{50} 's in the micromolar range [36–38].

Since we have recently started to use the four PDE4 isoenzymes A–D [39] for our routine screening, it was interesting to examine the behaviour of rolipram in these assays. There has been, as far as we are aware, only one report which examines the stereoselective inhibition of the PDE4A–D by rolipram [38]. Torphy found that with the exception of PDE4D, where the inhibitory potencies were roughly equal, (*R*)-rolipram was more potent than its antipode with eudismic ratios of 3 to 9. The pIC_{50} 's for (*R*)-rolipram ranged from of 7.2 (PDE4A) to 6.5 (PDE4D).

Rolipram is generally a less potent inhibitor on the PDE4 isoenzymes prepared in our labs (see **Table 2**) when compared to the results from the SmithKline Beecham group. Furthermore (*R*)-rolipram tends to be more potent than (*S*)-rolipram in all the assays, with eudismic ratios between 3 and 5.

A number of explanations have been proposed to account for the conflicting results obtained in PDE4 inhibition studies with rolipram. For example, several splice variants are known for each of the human PDE4A, PDE4B and PDE4D [40], and altered sensitivity of enzymes toward inhibition due to protein phosphorylation has been proposed [40]. The most popular hypothesis so far, assumes that PDE4 is capable of assuming different conformational states, to which rolipram-like compounds can bind with different potencies [37,38,41].

We are currently investigating the behaviour of several novel classes of rolipram-based PDE4 inhibitors, with the hope of gaining further insight into the fascinating behaviour of the PDE4 enzymes. These results will be communicated in due course.

Table 2. Biological data for (*R*)- and (*S*)-rolipram.^a

	Neutrophil PDE4	PDE4A	PDE4B	PDE4C	PDE4D	Rolipram binding ^b
(<i>R</i>)-rolipram	5.5 (± 0.2)	5.9 (± 0.1)	6.1 (± 0.3)	5.4 (± 0.1)	6.4 (± 0.2)	8.5 (± 0.3)
(<i>S</i>)-rolipram	5.5 (± 0.2)	5.4 (± 0.1)	5.6 (± 0.2)	4.9 (± 0.1)	5.8 (± 0.1)	7.6 (± 0.1)

^a pIC_{50} 's (± SEM); n = 3–5.

^b Inhibition of [³H]-rolipram binding to rat brain membranes.

Experimental Section

General

Melting points were determined on a Buechi 535 and are uncorrected. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. IR spectra (ν, cm^{-1}) were recorded on a Bruker IFS-66 spectrometer. ^1H -, and ^{13}C -NMR spectra were recorded on a Bruker DPX 300 MHz spectrometer. Chemical shifts (δ) are given in ppm values using CDCl_3 as internal standard. Mass spectra were recorded on a VGTS-250 or a VG 70-SE spectrometer. CD spectra (λ_{max} (nm), $[\text{Q}]$ (degrees $\text{cm}^2 \text{dmol}^{-1}$)) were measured on a Jobin Yvon CD6 circular dichrograph. Solvent cutoff was defined for reference solvent absorbance = 2. Molar dichroic absorption ($\Delta\epsilon$) was calculated from the molar ellipticity according to the equation $[\text{Q}] = 3298 \Delta\epsilon$ [42]. Enantiomeric purity was determined by HPLC on a 5 μ Chiralcel OD column, 25 x 0.46 cm (N. Djordjevic, G. Lerch). Microanalyses were performed on a Leco CHN-800 or Leco RO-478 respectively. Silica gel chromatography was performed with columns of various lengths and diameters using Merck's silica gel 60, particle size 0.040-0.063 mm as stationary phase. Hexane fraction boiling at 65-70 °C was used as solvent.

Cyclopentyl isovanillin (3)

Benzyltributyl-ammonium bromide (80.2 g, 0.255 mol) and bromo-cyclopentane (394 ml, 3.6 mol) were added to a rapidly stirred solution of isovanillin (288 g, 1.8 mol) in toluene (1620 ml), and this suspension was heated to 80-83 °C. To the resulting solution aqueous potassium hydroxide (225 g in 570 ml water) was added dropwise during 1 h, maintaining the solution at 80-83 °C. After 3 h the solution was cooled to room temperature, the two phases separated, and the upper toluene phase dried (MgSO_4) and evaporated under vacuum. The residue was distilled to yield **3** (676 g, 93%) as a faintly green oil, b.p. 120 °C /0.1 mm. IR (neat): 1689, 1585, 1265, 810. MS (EI): 220 (M^+), 152 (M-cyclopentyl). ^1H -NMR (CDCl_3): 1.5-2.0 (m, 8H, CH_2), 3.95 (s, 1H, CH_3), 4.81-4.91 (m, 1H, CH), 6.95-7.95 (m, 3H, Ar-H), 9.85 (s, 1H, CH). Anal. Calcd for $\text{C}_{13}\text{H}_{16}\text{O}_3$: C, 70.9; H, 7.3; O, 21.8. Found: C, 70.7; H, 7.3; O, 21.7.

3-cyclopentyloxy-4-methoxy-methyl cinnamate (4)

Aniline (13.6 ml) and piperidine (13.6 ml) were added to a solution of (568.9 g, 2.59 mol) **3** in pyridine (1633 ml), and the resulting solution was heated to 85 °C. Monomethyl malonate (672.7g, 5.69 mol) was then added

to this solution during 1 h, ensuring that the internal temperature did not exceed 90 °C. Stirring was continued for an additional 5 h at the same temperature. The mixture was cooled down to room temperature and slowly added to a mixture of 2N hydrochloric acid (13.6 l) and ice. The aqueous phase was extracted with 5.6 l EtOAc (2 x 2.8 l), and the combined organic extracts washed with brine, and dried (MgSO_4). The solution was evaporated to dryness under reduced pressure, and the residue dissolved in Et_2O (770 ml), and cooled to 5 °C. Hexane (2.3 l) was added to this solution over 30 min during which a suspension formed, which was stirred for an additional 1h, filtered, washed with precooled diethyl ether/hexane (240 ml 1:3) and dried overnight to give **4** (498.2 g, 69.7 %) as colourless crystals, mp. 56.8-58.8 °C. The mother liquor was subjected to chromatography (3.8 kg Silica gel, hexane-EtOAc 3:1) to yield, after crystallisation as described above, additional **4** (136.1 g, 19.1 %), mp. 60-61 °C. IR (KBr): 1717, 1638, 1513, 1270, 1171, MS (EI): 276 (M^+), 207, 198, 192, 176. ^1H -NMR (CDCl_3): 1.55-1.97 (m, 8H, CH_2), 3.75 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 4.74 (m, 1H, CH), 6.24 (d, 1H, CH, $J=15.9$ Hz), 6.92 (dd, 2H, Ar-H, $J=8.2$ Hz) 7.01-7.05 (m, 1H, Ar-H), 7.57 (d, 1H, CH, $J=15.9$ Hz). Anal. Calcd for $\text{C}_{16}\text{H}_{20}\text{O}_4$: C, 69.6; H, 7.3; O, 23.2. Found: C, 69.0; H, 7.1; O, 23.0.

3(3'-cyclopentyloxy-4'-methoxy) phenyl-4-nitro methyl butyrate (5)

A solution of **4** (550.6 g, 1.99 mol) and 1,1,3,3-tetramethyl guanidine (53.9 ml) in nitromethane (1090 ml) was stirred for 24 h at 75 °C. After cooling the mixture to room temperature, EtOAc (1000 ml) was added to it. The biphasic mixture was poured into 2 N HCl (1500 ml), the organic phase separated, and the aqueous phase extracted with EtOAc (1000 ml). The combined organic phases were washed with 2 N HCl (1500 ml), followed by brine, and dried (MgSO_4). Evaporation under vacuum yielded crude **5** (677 g) which was purified by chromatography (12 kg silica gel, hexane-ethyl acetate 3:1) to give **5** (523 g, 78 %) as a white solid, mp. 84.1-85.1 °C. IR (KBr): 1744, 1733, 1551, 1518, 1258, 1237. MS (EI): 337 (M^+), 269, 222, 238, 222, 191, 164, 150, 131. ^1H -NMR (CDCl_3): 1.50-1.92 (m, 8H, CH_2), 2.68 (d, 2H, CH_2 , $J=7.3$ Hz), 3.57 (s, 3H, CH_3), 3.75 (s, 3H, CH_3), 3.84 (m, 1H, CH), 4.59 (ddd, 2H, CH_2 , $J=12.4$ and 7.2 Hz), 4.69 (m, 1H, CH), 6.59-6.80 (m, 3H, Ar-H). Anal. Calcd for $\text{C}_{17}\text{H}_{23}\text{NO}_6$: C, 60.5; H, 6.9; O, 28.5. Found: C, 60.6; H, 6.7; O, 28.0.

3(3'-cyclopentyloxy-4'-methoxy) phenyl-4-nitro butyric acid (6)

Sodium hydroxide solution (2 N, 720 ml) was added dropwise to a cold (0 °C) suspension of **5** (72 g, 0.21 mol) in methanol (1440 ml) while the internal temperature did not exceed 20 °C. After the addition was complete, the ice bath was removed and the mixture stirred for an additional 1.5 h. Methanol was removed from the reaction mixture under vacuum, and to the remaining aqueous solution EtOAc (1400 ml) was added. The mixture was acidified to pH=2 with HCl (2 N, 720 ml). The two layers were separated and the aqueous layer extracted twice with EtOAc (2 x 700 ml). The combined organic extracts were dried (MgSO₄) and evaporated to yield **6** (68.5 g, 99.3%) as beige crystals. The obtained crude material was almost pure and could be used directly for the subsequent step. Recrystallisation from EtOAc-hexane (1:1, 140 ml) proceeded to give **6** (64.5, 93.5 %) mp 119-121 °C. IR (KBr): 1703, 1590, 1553, 1260. MS (FAB): 323 (M⁺), 263, 255. ¹H-NMR (CDCl₃): 1.54-1.98 (m, 8H, CH₂), 2.72 (ddd, 2H, CH₂, J=16.6 and 7.5 Hz), 3.74 (s, 3H, CH₃), 3.81 (m, 1H, CH), 4.56 (ddd, 2H, CH₂, J=12.5 and 7.7 Hz), 4.68 (m, 1H, CH), 6.65-6.76 (m, 1H, Ar-H), 6.71 (dd, 2H, Ar-H, J=8.5 Hz). Anal. Calcd for C₁₆H₂₁NO₆: C, 59.4; H, 6.6; N, 4.3. Found: C, 59.6; H, 6.4; N, 4.2.

3(3'-Cyclopentyloxy-4'-methoxy) phenyl-4-nitro butyryl chloride (7)

Thionyl chloride (24 ml, 0.33 mol) was added over 15 min. to a suspension of **6** (64.7 g, 0.2 mol) in CH₂Cl₂ (470 ml) at room temperature. After complete addition the suspension was heated to reflux for 3 h. The yellow solution was evaporated to give **7** (69.5 g) as a brown oil which solidified upon cooling. This crude material was taken up in Et₂O (100 ml) and CH₂Cl₂ (50 ml), and crystallised by slow addition of the hexane fraction (500 ml) giving rise to **7** (63.7 g, 93.2 %) as beige crystals, mp 75-76 °C. IR (KBr): 1788, 1551, 1260. MS (EI): 341 (M⁺), 305, 273. ¹H-NMR (CDCl₃): 1.56-1.88 (m, 8H, CH₂), 3.28 (ddd, 2H, CH₂, J=17.5 and 7.9 Hz), 3.76 (s, 3H, CH₃), 3.87 (m, 1H, CH), 4.55 (ddd, 2H, CH₂, J=12.7 and 7.2 Hz), 4.69 (m, 1H, CH), 6.64-6.77 (m, 1H, Ar-H), 6.71 (dd, 2H, Ar-H, J=8 Hz). Anal. calcd for C₁₆H₂₀NO₅Cl: C, 56.2; H, 5.9; N, 4.1. Found: C, 56.6; H, 5.9; N, 4.2.

(3R)-(3'-Cyclopentyloxy-4'-methoxy) phenyl-4-nitro butyryl - (S)-(-)- phenyl-ethylamide (8a) and (3S)-(3'-Cyclopentyloxy-4'-methoxy) phenyl-4-nitro butyryl -(S)-(-)- phenyl-ethylamide (8b)

Via carboxylic acid **6**

4-Dimethylaminopyridine (116 g, 0.95 mol), solid hydroxybenzotriazole (HOBT, 73.2 g, 0.54 mol), and (146g, 0.45 mol) solid **6** were added in sequence to a

cooled (0°C), stirred solution of (S)-(-)-1-phenyl ethylamine (54.7 g, 0.45 mol) in DMF (2700 ml). After stirring for 15 min, *N*-(3-dimethylaminopropyl)-*N'*-ethyl-carbodiimide hydrochloride (EDCI, 97.9 g, 0.51 mol) was added in portions over 15 min. Stirring was continued at 0°C for 1 h. The ice bath was removed, and the solution stirred overnight. After cooling the mixture to 5-10 °C, EtOAc (2000 ml) was added and the solution washed with 2N HCl (4500 ml). The aqueous phase was extracted with EtOAc (1800 ml) and the combined organic extracts were dried (MgSO₄). Filtration and evaporation under reduced pressure yielded a mixture of diastereoisomers **8a** and **8b**. Purification by chromatography as described below gave **8a** (51.8 g, 26.8 %), **8b** (57 g, 29.5 %) and a mixed fraction (45 g 23 %) which was not further purified. For analytical data see below.

Via acid chloride **7**

A solution of (S)-(-)-1-phenyl-ethylamine (49 ml, 0.39 mol) in CH₂Cl₂ (60 ml) was added dropwise over 15 min to a refluxing solution of **7** (60 g, 0.175 mol) in CH₂Cl₂ (660 ml). After maintaining reflux for 2 h, the reaction was complete. The solution was cooled to room temperature and washed successively with 2N HCl (240 ml), 2N NaHCO₃ (240 ml), and brine (240 ml). The united aqueous phases were extracted twice with CH₂Cl₂ (240 ml and 120 ml). The combined organic phases were dried (MgSO₄), and evaporated under vacuum to furnish a diastereomeric mixture of **8a** and **8b** (76.2 g). The mixture was dissolved in CH₂Cl₂ (500 ml) and subjected to chromatography (silica gel EtOAc-hexane fraction-CH₂Cl₂ 50:30:20) yielding **8a** (28.9 g, 38.7 %) and **8b** (31 g, 41.5 %).

8a: white beige crystals, mp 143-144 °C, [α]_D²⁰ = +7.15 (c=0.47 acetone). CD (c= 2.00 mg/ml, MeOH, l = 0.01 cm) 194.5 (-18.387), 206.5 (-4.276), 211 (sh, ca. 7.9), 215 (-7.952), 275.5 (-0.225), 294.5 (0.551). CD (c= 2.00 mg/ml, MeOH, l = 1.00 cm) 277.5 (-0.203), 296.5 (0.717). IR (KBr): 3336, 1643, 1550. MS (EI): 427 (MH⁺), 323, 281. ¹³C-NMR (CDCl₃): 21.81, 24.30, 33.04, 40.45, 40.77, 49.17, 56.34, 79.87, 80.56, 112.59, 114.75, 119.55, 126.16, 126.32, 127.75, 128.97, 131.07, 143.00, 148.23, 150.03, 169.03. ¹H-NMR (CDCl₃): 1.27 (d, 3H, CH₃, J=6.9 Hz), 1.53-1.83 (m, 8H, CH₂), 2.50 (ddd, 2H, CH₂, J=14.6 and 7.3 Hz), 3.75 (s, 3H, CH₃), 3.82 (m, 1H, CH), 4.61 (ddd, 2H, CH₂, J=12.3 and 8 Hz), 4.65 (m, 1H, CH), 4.96 (m, 1H, CH), 5.49 (d, 1H, NH, J=8 Hz), 6.65-6.73 (m, 1H, Ar-H), 6.67 (dd, 2H, Ar-H, J=8.9 Hz), 7.10-7.26 (m, 5H, Ar-H). Anal. Calcd for C₂₄H₃₀N₂O₅: C, 67.6; H, 7.1; N, 6.6. Found: C, 67.5; H, 7.2; N, 6.6.

8b: white beige crystals, mp 158-159 °C, [α]_D²⁰ = -38.3 (c=0.52 acetone). CD (c= 2.00 mg/ml, MeOH, l = 0.01 cm) 194.5 (-25.552), 204.5 (-6.524), 211.5 (-10.373),

215 (sh, *ca.* 10), 239.5 (0.750), 295.5 (-0.733).). CD (*c* = 2.00 mg/ml, MeOH, *l* = 1.00 cm) 272.5 (0.541), 302.5 (-0.561). IR (KBr): 3368, 1648, 1637, 1555, 1521. MS (EI): 427 (MH⁺), 323, 282. ¹³C-NMR (CDCl₃): 21.77, 24.24, 32.98, 40.63, 40.78, 48.97, 56.24, 79.93, 80.71, 112.50, 114.76, 119.33, 126.11, 127.55, 128.81, 130.91, 142.79, 148.19, 149.95, 168.90. ¹H-NMR (CDCl₃): 1.34 (d, 3H, CH₃, J=6.9 Hz), 1.50-1.97 (m, 8H, CH₂), 2.50 (ddd, 2H, CH₂, J=14.4 and 7.7 Hz), 3.75 (s, 3H, CH₃), 3.84 (m, 1H, CH), 4.60 (ddd, 2H, CH₂, J=12.4 and 8 Hz), 4.59 (m, 1H, CH), 4.97 (m, 1H, CH), 5.45 (d, 1H, NH, J=8Hz), 6.62-6.73 (m, 1H, Ar-H), 6.67 (dd, 2H, Ar-H, J=8.8 Hz), 6.96-7.19 (m, 5H, Ar-H). Anal. Calcd for C₂₄H₃₀N₂O₅: C, 67.6; H, 7.1; N, 6.6. Found: C, 67.5; H, 7.1; N, 6.6.

(3R)(3'-Cyclopentyloxy-4'-methoxy) phenyl-4-amino butyryl - (S)-(-)- phenyl-ethylamide (9a)

A suspension of **8a** (38.7 g, 0.09 mol) and 10% Pd/C (10 g) in isopropanol (2900 ml) and DMF (580 ml) was hydrogenated at room temperature and 0.2 bar pressure overnight (20 h). The reaction mixture was filtered over celite, washed with isopropanol and concentrated under vacuum to dryness yielding crude **9a** (40 g) as off-white solid, which was used without further purification for the subsequent step. For analytical data, a sample was purified from a different experiment (Silica gel, EtOAc-MeOH-NH₄OH 80:20:2) R_f = 0.4. mp 94-95 °C, [α]_D²⁵ = -59.2° (*c* = 0.54, MeOH), CD (*c* = 2.00 mg/ml, MeOH, *l* = 0.01 cm) 195.5 (-23.081), 208 (-5.479), 215 (-8.435), 242 (0.533). CD (*c* = 10.00 mg/ml, MeOH, *l* = 0.01 cm) 245 (0.330), 261 (0.213), 267 (0.176). IR (KBr): 3326, 2925, 1641, 1538, 1520. MS (EI): 397 (MH⁺). ¹³C-NMR (CDCl₃): 21.94, 24.37, 33.16, 41.84, 45.98, 47.69, 48.97, 56.53, 80.89, 112.77, 115.22, 120.11, 126.46, 127.61, 128.94, 134.86, 143.51, 148.29, 149.46, 170.99. ¹H-NMR (CDCl₃): 1.20 (d, 3H, CH₃, J=6.9 Hz), 1.52-1.93 (m, 8H, CH₂), 2.40 (ddd, 2H, CH₂, J=14.0 and 6.5 Hz), 2.70-2.89 (m, 2H, CH₂), 2.94-3.05 (m, 1H, CH), 3.75 (s, 3H, CH₃), 4.66 (m, 1H, CH), 4.93 (m, 1H, CH), 5.68 (d, 1H, NH, J=7.8Hz), 6.66-6.76 (m, 1H, Ar-H), 6.70 (dd, 2H, Ar-H, J=8.8 Hz), 7.08-7.45 (m, 5H, Ar-H).

(3S)(3'-Cyclopentyloxy-4'-methoxy) phenyl-4-amino butyryl - (S)-(-)- phenyl-ethylamide (9b)

A suspension of **8b** (57.1 g, 0.13 mol) and 10% Pd/C (14.3 g) in isopropanol (4300 ml) and DMF (850 ml) was hydrogenated as described above to give crude **9b** (57.9g, > 100%) as a viscous oil, which was of sufficient purity for the subsequent step. A sample of this oil obtained in a separate experiment was purified by chromatography (Silica gel, EtOAc-MeOH-NH₄OH 80:20:2; R_f = 0.1) for chiroptical measurements. [α]_D²⁵ = -20.3° (*c* = 1.3, MeOH), CD (*c* = 2.00 mg/ml, MeOH, *l* = 0.01 cm) 194.5

(-22.422), 205.5 (-5.540), 214 (-8.365), 239 (0.419). CD (*c* = 10.00 mg/ml, MeOH, *l* = 0.01 cm) 239.5 (0.482), 251 (-0.123), 268 (0.242). IR (KBr): 3291, 2961, 1641, 1515, 1259. MS (EI): 397 (MH⁺), 367, 299, 194. ¹³C-NMR (CDCl₃): 22.05, 24.35, 33.11, 41.74, 45.95, 47.74, 48.68, 56.41, 80.65, 112.70, 115.40, 119.94, 126.24, 127.15, 128.72, 134.75, 143.45, 148.17, 149.33, 171.08. ¹H-NMR (CDCl₃): 1.38 (d, 3H, CH₃, J=6.9 Hz), 1.45-1.84 (m, 8H, CH₂), 2.48 (ddd, 2H, CH₂, J=13.9 and 8.7 Hz), 2.84-2.97 (m, 2H, CH₂), 3.07-3.11 (m, 1H, CH), 3.83 (s, 3H, CH₃), 4.70 (m, 1H, CH), 5.02 (m, 1H, CH), 5.89 (d, 1H, NH, J=7.9Hz), 6.65-6.83 (m, 1H, Ar-H), 6.74 (dd, 2H, Ar-H, J=8.7 Hz), 6.98-7.23 (m, 5H, Ar-H).

(4R)-(3'-Cyclopentyloxy-4'-methoxy)phenyl-2-pyrrolidone ((R)-(-)-1)

A suspension of **9a** (38.3 g, 96.5 mmol) in xylene (2300 ml, isomeric mixture) was heated to reflux for 3-4 h. The clear yellowish solution was evaporated to dryness under vacuum. The resulting solid was suspended in t-butyl methyl ether (500 ml) and stirred overnight at room temperature. Finally the suspension was cooled to -5 °C, filtered, washed with precooled (-5 °C) t-butyl methyl ether (400 ml), and dried overnight at 50 °C, 2 mbar to give **(R)-(-)-1** (14.3 g, 54 %) as off white crystals, mp 127-130 °C, [α]_D²⁵ = -35° (*c* = 0.5, MeOH), Enantiomeric purity (5 μ Chiralcel OD column, 25 x 0.46 cm, mobile phase: CH₃CN 0.6 ml min⁻¹, retention time 10.3 min): e.e. 99.70% CD (*c* = 2.00 mg/ml, MeOH, *l* = 0.01 cm) 192.5 (9.186), 209.5 (-9.857), 237 (0.461), 282.5 (-0.299). CD (*c* = 10.00 mg/ml, MeOH, *l* = 0.01 cm) 236 (0.478), 280 (-0.242). IR (KBr): 3196, 3094, 1709, 1689, 1517. MS (EI): 275 (M⁺), 207, 177, 150, 135, 107. ¹³C-NMR (CDCl₃): 26.02, 34.82, 40.15, 41.99, 51.79, 58.16, 82.62, 114.25, 115.88, 120.81, 136.59, 149.92, 151.21, 179.82. ¹H-NMR (CDCl₃): 1.48-1.93 (m, 8H, CH₂), 2.52 (ddd, 2H, CH₂, J=16.9 and 8.9 Hz), 3.50 (ddd, 2H, CH₂, J=9.2 and 7.3 Hz), 3.55 (m, 1H, CH), 3.76 (s, 3H, CH₃), 4.70 (m, 1H, CH), 6.57 (s, 1H, NH), 6.69-6.77 (m, 3H, Ar-H). Anal. Calcd for C₁₆H₂₁NO₃: C, 69.8; H, 7.7; N, 5.1. Found: C, 69.7; H, 7.5; N, 5.1.

(4S)-(3'-Cyclopentyloxy-4'-methoxy)phenyl-2-pyrrolidone ((S)-(+)-1)

A solution of **9b** (57.8 g, 146 mmol) in xylene (3000 ml) was subjected to the same procedure as described above for **(R)-(-)-1** giving **(S)-(+)-1** (21.5 g, 54 %) as off-white crystals, mp 131-134 °C, [α]_D²⁵ = +31° (*c* = 0.6, MeOH) Enantiomeric purity: (5 μ Chiralcel OD column, 25 x 0.46 cm, mobile phase: CH₃CN 0.6 ml min⁻¹, retention time 9.5 min): 98.95% e.e. CD (*c* = 2.00 mg/ml, MeOH, *l* = 0.01 cm) 193.5 (-8.105), 208.5 (8.893), 237.5 (-0.336), 283.5 (0.248). CD (*c* = 10.00 mg/ml, MeOH, *l* =

0.01 cm) 237.5 (-0.351), 280.5 (0.270). IR (KBr): 3196, 3094, 1709, 1688, 1517. MS (EI): 276 (MH⁺), 208, 150, 107. ¹³C-NMR (CDCl₃): 26.36, 35.16, 40.53, 42.32, 52.16, 58.50, 82.96, 114.60, 116.24, 121.16, 136.95, 150.26, 151.54, 180.24. ¹H-NMR (CDCl₃): 1.52-1.92 (m, 8H, CH₂), 2.52 (ddd, 2H, CH₂, J=16.9 and 8.8 Hz), 3.50 (ddd, 2H, CH₂, J=9.2 and 7.3 Hz), 3.55 (m, 1H, CH), 3.76 (s, 3H, CH₃), 4.70 (m, 1H, CH), 6.43 (s, 1H, NH), 6.69-6.77 (m, 3H, Ar-H). Anal. calcd for C₁₆H₂₁NO₃: C, 69.8; H, 7.7; N, 5.1. Found: C, 69.7; H, 7.8; N, 5.1.

(S)-Phenylethylamine acetamide (10)

Acetic anhydride (10.2 g, 0.1 mmol) was added dropwise to a solution of (S)-(-)-1-phenyl-ethylamine (12.11 g, 0.1 mmol) in toluene (20 ml) at such a rate that slow reflux was maintained. When the addition was complete, the solution was allowed to cool to rt. and hexane (20 ml) added with stirring. After leaving for 8 h at 4 °C, the precipitate was filtered off, washed with hexane (10 ml) and dried (50 °C, 0.4 mbar, 8h) to give crystals (8.66 g, 53.5%) which were of high purity, and were used directly for CD measurements. mp. 103 °C [Lit [Ref. 26] 102-103 °C], [α]_D²⁰ = -152.8° (c = 10.9, EtOH). CD (c = 2.00 mg/ml, MeOH, l = 0.01 cm) (λ_{max} nm, (Δε)): 215(-12.862), 211(-13.332), 197.5(-28.522) CD (c = 20.00 mg/ml, MeOH, l = 0.01 cm) (λ_{max} nm, (Δε)): 267.5 (0.224), 260.5 (0.231), 254.5 (0.126), 248 (0.047), 234 (0.037).

Inhibition of cAMP phosphodiesterase (PDE4) from human neutrophil homogenate

Phosphodiesterase was prepared from human neutrophils by ultrasonic homogenisation. Activity was assayed by the column method of Thompson [43]. Inhibitors were dissolved in DMSO and diluted to the required concentration in buffer T (MgCl₂ 5mM, mercaptoethanol 3.6 mM, bovine serum albumin 1mg/ml, tris-hydroxymethyl-aminomethane 40mM, pH 8.0) containing 10% DMSO.

Inhibition of Human cAMP-specific phosphodiesterase (PDE4) isoenzymes

PDE4 activity was assessed as previously described [40]. With the exception of PDE4B (rat; expressed in *S. cerevisiae*), all isoenzyme preparations were from human sources: PDE4A, PDE4B, PDE4D expressed in *S. cerevisiae*.

Inhibition of rolipram binding to rat brain membranes

Binding of [³H]-rolipram to rat brain membranes was performed according to published procedures [40] adapted for use in 96-well microtitre plates.

Acknowledgement: The authors thank Mr. G. Lerch and Dr. N. Djordjevic for enantiomeric purity determination, and Dr. H.U. Gremlich for measurement of the CD spectra.

References

1. Heaslip, R.J.; Evans, D.Y. *Eur. J. Pharmacol.* **1995**, *286*, 281-290.
2. Sekut, L.; Yarnall, D.; Stimpson, S.A.; Noel, L.S.; Bateman-Fite, R.; Clark, R.L.; Brackeen, M.F.; Menius, J.A.; Conolly, K.M. *Clin. Exp. Immunol.* **1995**, *100*, 126-132.
3. Sommer, N.; Loeschmann, P.A.; Northoff, G.H.; Weller, M.; Steinbrecher, A.; Steinbach, J.P.; Richtenfels, R.; Meyermann, R.; Reithmueller, A.; Fontana, A.; Dichgans, J.; Martin, R.; *Nature Med.* **1995**, *1*, 244-248.
4. Nibuya, M.; Nestler, E.J.; Duman, R.S. *J. Neurosci.* **1996**, *16*, 2365-2372.
5. Schneider, H.H.; Schmiechen, R.; Brenzinski, M.; Seidler, J. *Eur. J. Pharmacol.* **1986**, *127*, 105-115.
6. Casacchia, M.; Meco, G.; Castellana, F.; Bedini, L.; Cusimano, G.; Agnoli, A. *Pharmacol. Res. Commun.*, **1983**, *15*, 329-334.
7. Kato, H.; Araki, T.; Itoyama, Y.; Kogure, K. *Eur. J. Pharmacol.* **1995**, *272*, 107-110.
8. Beavo, J.A.; Reifsnnyder, D.H. *Trends Pharmacol. Sci.* **1990**, *11*, 150-155.
9. Souness, J.E.; Scott, L.C. *Biochem. J.* **1993**, *291*, 389-395.
10. Semmler, J.; Wachtel, H.; Endres, S. *Int. J. Immunopharmacol.* **1993**, *15*, 409-413.
11. Schultz, J.E.; Folkers, G. *Pharmacopsychiat.* **1988**, *21*, 83-86.
12. Schneider, H.H.; Yamaguchi, M.; Andrews, J.S.; Stephens, D.N. *Pharmacol. Biochem. Behav.* **1995**, *50*, 211-217.
13. Bender, P.E. PCT International Application WO 921959 A1, 92.11.12; Blashke, G. *J. Liq. Chromatography* **1986**, *9*, 341-368.
14. Baures, P.W.; Eggleston, D.S.; Erhard, K.F.; Cieslinski, L.B.; Torphy, T.J.; Christensen, S.B. *J. Med. Chem.* **1993**, *36*, 3274-3277.
15. Kuesters, E.; Spöndlin, Ch. *J. Chromatogr., A*, **1996**, *737*, 333-337.
16. Petzoldt, K.; Schmiechen, R.; Hamp, K. Ger. Offen. DE 3921593 A1, 91.01.10.
17. Mulzer, J. *J. Prakt. Chem.* **1994**, *336*, 287.
18. Braun, M.; Opdenbusch, K.; Unger, C. *Synlett* **1995**, *11*, 1174-6.

19. Honda, T.; Ishikawa, F.; Kanai, K.; Sato, S.; Daishiro, K.; Tominaga, H. *Heterocycles* **1996**, *42*, 109-12; Diaz, A.; Siro, J.G.; Garcia-Navio, J. L.; Vaquero, J.J.; Alvarez-Builla, J. *Synthesis* **1997**, 559-562. Langlois, N.; Wang, H.-S. *Synth. Commun.* **1997**, *27*, 3133-3144.
20. Demnitz, J.; LaVecchia, L. unpublished work.
21. Backstrom, R.; Honkanen, E.; Raasmaja, A; Linden, I.B. PCT International Application WO 9116303 A1, 31 October, 1991.
22. Brackeen, M.F.; Stafford, J.A.; Cowan, D.J.; Brown, P.J.; Domanico, P.L.; Feldman, P.L.; Rose, D.; Strickland, A.B.; Veal, J.M.; Verghese, M. *J. Med. Chem.* **1995**, *38*, 4848-4854.
23. Smith, H.E., in: *Circular Dichroism, Principles and Applications*, Nakanishi, K.; Berova, N.; Woody, R.W., eds., VCH Publishers, New York: **1994**, pp413-442.
24. Woody, R.W. in: *Circular Dichroism, Principles and Applications*, Nakanishi, K.; Berova, N.; Woody, R.W., eds., VCH Publishers, New York: **1994**, p474..
25. Horiba, M.; Yamamoto, S.; Oi, N. *Agric. Biol. Chem.* **1982**, *46*,1219-1224.
26. Dem'yanovich, V.M.; Shishkina, I.N. *Khim. Geterosikl. Soedin.* **1996**, *7*, 944-949.
27. Fontana, L.P.; Smith, H.E. *J. Org. Chem.* **1987**, *52*, 3386-3389.
28. Pickard, S.T.; Smith, H.E. *J. Amer. Chem. Soc.* **1990**, *112*, 5741-5747.
29. Johnson, Jr., W.C.; Fontana, L.P.; Smith, H.E. *J. Amer.Chem.Soc.* **1987**, *109*, 3361-3366.
30. Schellman, J.A.; Lifson, S. *Biopolymers* **1973**, *12*, 315-327.
31. Ong, E.C.; Cusachs, L.C.; Weigang, Jr., O.E. *J. Chem. Phys.* **1977**, *67*, 3289-3297.
32. Geiger, R.E.; Wagnière, G.H. *Helv. Chim. Acta* **1975**, *58*, 738-747.
33. Person, R.V.; Monde, K.; Humpf, H.-U.; Berova, N.; Nakanishi, K. *Chirality* **1995**, *7*, 128-135
34. Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy, Exciton Coupling in Organic Stereochemistry*, University Science Books, Oxford University Press: Oxford, **1983**. Nakanishi, K.; Berova, N. in: *Circular Dichroism, Principles and Applications*, Nakanishi, K.; Berova, N.; Woody, R.W., eds., VCH Publishers, New York: **1994**, pp 361-398.
35. Torphy, T.J.; Stadel, J.M.; Burman, M.; Cieslinski, L.B.; McLaughlin, M.M.; White, J.R.; Livi, G.P. *J. Biol. Chem.* **1992**, *267*, 1798-1804.
36. Nemoz, G.; Moueqqit, M.; Prigent, A.; Pacheco, H. *Eur. J. Biochem.* **1989**, *184*, 511-520.
37. Souness, J.E. *Phosphodiesterase Inhibitors*; Schudt, C.; Dent, G.; Rabe, K.F. Ed.; Academic Press: London, 1996; 173-184.
38. Christensen, S.B.; DeWolf, W.E.; Ryan, M.D.; Torphy, T.J. *Phosphodiesterase Inhibitors*; Schudt, C.; Dent, G.; Rabe, K.F. Ed.; Academic Press: London, 1996; 185-207.
39. Engels, P.; Sullivan, M.; Müller, T.; Lübbert, H. *FEBS Lett.* **1995**, *358*, 305-310.
40. Müller, T.; Engels, P.; Fozard, J. *Trends in Pharm. Sci.* **1996**, *17*, 294-298.
41. Rocque, W.J.; Tian, G.; Wiseman, J.S.; Holmes, W.D.; Zajac-Thompson, I.; Willard, D.H.; Patel, I.R.; Wisely, G.B.; Clay, W.C.; Kadwell, S.H.; Hoffman, C.R.; Luther, M.A. *Biochemistry* **1997**, *36*, 14250.
42. Rodger, A.; Nordén, B. *Circular Dichroism and Linear Dichroism*; Oxford University Press, Oxford, **1997**, p144.
43. Thompson, W.J; Terasaki, W.J.; Epstein, P.M.; Strada, S.J. *Adv. Second Messenger Phosphoprot. Res.* **1979**, *10*, 69-92.
44. Schneider, H.H.; Schmiechen, R.; Brezinski, M.J. *Eur. J. Pharmacol.* **1986**, *127*, 105-115.

Sample Availability: Not available.