Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 15 (2004) 1651–1658

Diastereoselective synthesis of chiral nonracemic naphthylaminoalcohols with analgesic activity

Ornella Azzolina,[a,*] Simona Collina,[a] Gloria Brusotti,[a] Guya Loddo,[a] Laura Linati,[b] Enrica Lanza[c] and Victor Ghislandi[a]

[a]*Dipartimento di Chimica Farmaceutica, Università di Pavia, Viale Taramelli 12, 27100 Pavia, Italy*
[b]*Centro Grandi Strumenti, Università di Pavia, Via Bassi 21, 27100 Pavia, Italy*
[c]*Dipartimento di Farmacologia Sperimentale ed Applicata, Università di Pavia, Viale Taramelli 14, 27100 Pavia, Italy*

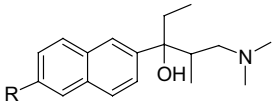
Received 16 March 2004; accepted 23 March 2004
Available online 21 April 2004

Abstract—The diastereoselective synthesis of chiral nonracemic naphthylaminoalcohols has been accomplished. The diastereoisomers, obtained in high enantiomeric excesses, were investigated by ^{1}H NMR and HPLC analyses. The configurational assignment was performed by NOESY ^{1}H NMR spectroscopy. Pharmacological evaluation of the analgesic activity by means of the hot plate test is described.

1. Introduction

Recent research has been focused on the design, synthesis and pharmacological evaluation of numerous compounds for developing new, safe and effective drugs. Several naphthylaminoalcohols, bearing either one or two stereogenic centres (series I[1] and series II[2,3], Fig. 1) showed interesting opioid-like analgesic properties. We previously reported[2] the preparation and the preliminary pharmacological profile of compound **1** (Fig. 2), which gave rise to two racemic pairs (2*RS*,3*SR*) and (2*RS*,3*RS*). Starting from racemic or optically active 1-dimethylamino-2-methyl-pentan-3-one **2** [(*RS*)-**2**, (*R*)-**2** or (*S*)-**2**], the synthetic procedure led overall to the isolation of (2*RS*,3*RS*)-**1**, (2*R*,3*R*)-**1** or (2*S*,3*S*)-**1**, respectively; in each case only traces of the other isomers were present in the mother liquor. Owing to their difficult isolation, only small amounts of racemic (2*RS*,3*SR*)-**1** and the corresponding enantiomers were isolated. Since a pharmacological enantioselectivity was observed[2] in mice by hot plate test (HPT), the availability of (2*R*,3*S*)-**1** and (2*S*,3*R*)-**1** isomers becomes important for gaining a better understanding of the relevance of the stereochemical features in the antinociceptive activity.

Figure 1.



Compound	R
1	OH
3	H
4	OTHP[a]

[a]THP = tetrahydropyranyl

Figure 2.

An efficient diastereoselective synthesis of either the (2*RS*,3*RS*) or (2*RS*,3*SR*) racemates of **3** and **4** (Fig. 2), performed via organolithium addition to (*RS*)-**2**, has recently been developed.[4] The stereoselective behaviour of this reaction, which depended on solvent, temperature, organolithium reagents either with or without chelation control was also studied.

* Corresponding author. Tel.: +00390382507356; fax: +00390382422-975; e-mail: ornella.azzolina@unipv.it

0957-4166/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2004.03.036

Herein, we report the preparation of (2*R*,3*S*)-**1** and (2*S*,3*R*)-**1** and of all stereoisomers of **3** via organolithium addition to ketone **2**. ^{1}H NMR and HPLC analyses were used to investigate the diastereomeric excess of the crude products. Configurational assignment of diastereoisomers of **3** was attributed by NOESY ^{1}H NMR while the evaluation of the enantiomeric excess was determined with chiral HPLC analysis. The in vivo evaluation of the analgesic activity by HPT is also reported.

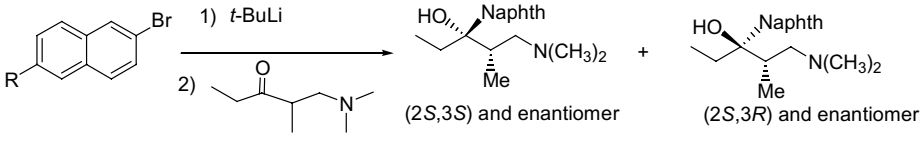
2. Results and discussion

The synthesis of racemic and enantiomeric **1**, **3** and **4** was accomplished via nucleophilic addition of the appropriate naphthalenic anion to ketone **2**. The results of elemental analyses, IR and ^{1}H NMR spectra were in agreement with the assigned structures for all the compounds.

Firstly, we synthesized compound **3**, starting from (*RS*)-**2**, following the procedure already used for **1**,[2] but with suitable modifications. Quenching with water (instead of HCl) and subsequent acid–base work-up generated the pure mixture of the two racemic pairs of **3** (Table 1, entry 1), as clearly evidenced by ^{1}H NMR (Fig. 3) and HPLC analyses (Table 2). Subsequent flash chromatography allowed the separation of the two racemic pairs, as evidenced by the chromatograms reported in Figure 4.

In order to assign the configuration, NOESY experiments were performed on the major racemic pair of **3**·HCl and on (2*RS*,3*RS*)-**1**·HCl, whose configuration had already been established by X-ray analysis of (2*R*,3*R*)-**1**·HCl.[2] Both compounds showed the same significant NOE effects (Fig. 5A) corresponding to the proton interaction between the methylene group linked to the stereogenic carbon C_3 and the methyl group linked to C_2, respectively. This interaction is possible only for the (2*R*,3*R*) and the (2*S*,3*S*) enantiomers, as

Table 1. Diastereoselective arylation of ketone **2**[4]



Entry	Compound	Solvent	*T* (°C)[a]	Diastereoisomeric ratio[b]
1	**3**	Diethylether	−50	59:41
2	**3**	THF	+10	86:14
3	**3**	Toluene	+60	48:52
4	**4**	THF	+10	72:28
5	**4**	THF	−60	40:60
6	**4**	Toluene	+60	71:29

[a] Ketone addition.
[b] (2*RS*,3*RS*)/(2*RS*,3*SR*), determined by HPLC (see Table 2).

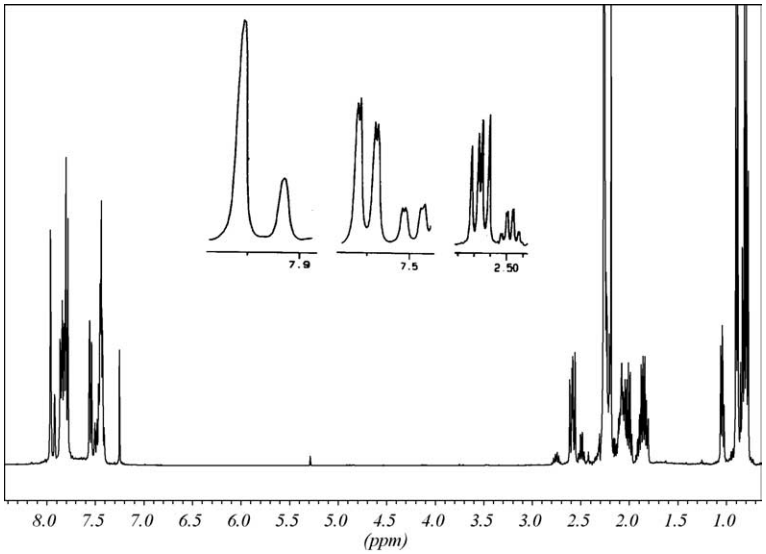


Figure 3. ^{1}H NMR spectrum of the two racemic pairs (2*RS*,3*RS*)-**3** and (2*RS*,3*SR*)-**3**.

Table 2. Analytical diastereoisomeric resolution of racemic pairs of **1**, **3** and **4** on NovaPak Silica column (150×3.9 mm)

Compound	Mobile phase[a]	t_{R1} (min) (2*RS*,3*SR*)	t_{R2} (min) (2*RS*,3*RS*)
1	90/10/0.3	11.6	18.8
3	95/5/0.2	4.4	6.9
4	95/5/0.2	5.7	9.5
	90/10/0.3	4.0	5.6

[a] Solvent mixture: *n*-hexane/IPA/triethylamine (v/v/v); flow rate = 0.5 mL/min; UV detector: $\lambda = 273$ nm.

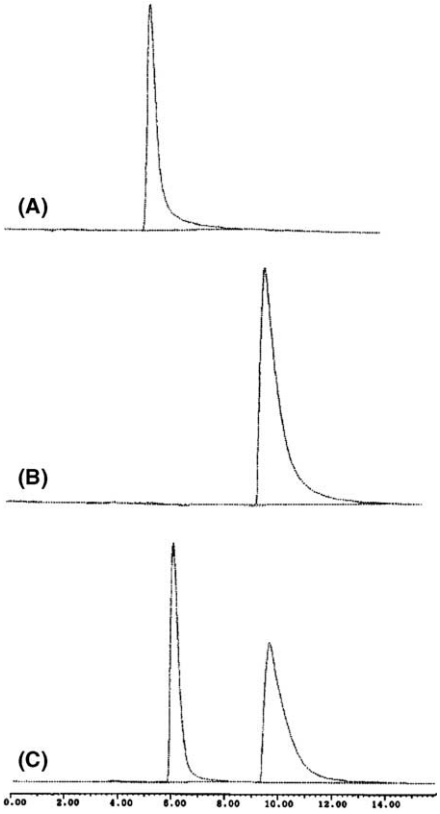


Figure 4. Chromatographic resolution of (2*RS*,3*SR*)-**3** and (2*RS*,3*RS*)-**3** on Hypersil Silica. (2*RS*,3*SR*)-**3** (A); (2*RS*,3*RS*)-**3** (B); mixture of the racemic pairs before flash chromatography (C).

confirmed by the molecular modeling study according to Ewig et al.[5] (a molecular view of (2*R*,3*R*)-**3** is reported in Fig. 5B). Therefore, the (2*RS*,3*RS*) configuration was assigned to the major product **3** of the reaction.

In order to improve the selectivity, the nucleophilic addition of the organolithium reagent from 2-bromonaphthalene was investigated.[4] The synthetic procedure was performed on a small scale, in different solvents and at different temperatures in order to obtain the naphthalenic anion, which would react in situ with (*RS*)-**2**. Quenching with polymer-supported carboxylic acid (IRC50), followed by a catch and release protocol, performed with acidic anion-exchange resin (Amberlyst 15) and NH_3 solution in methanol, gave the pure diastereoisomeric mixture, directly available for HPLC analysis. Data reported in Table 1 show that both solvent and temperature influence the diastereoselection of the synthetic process.

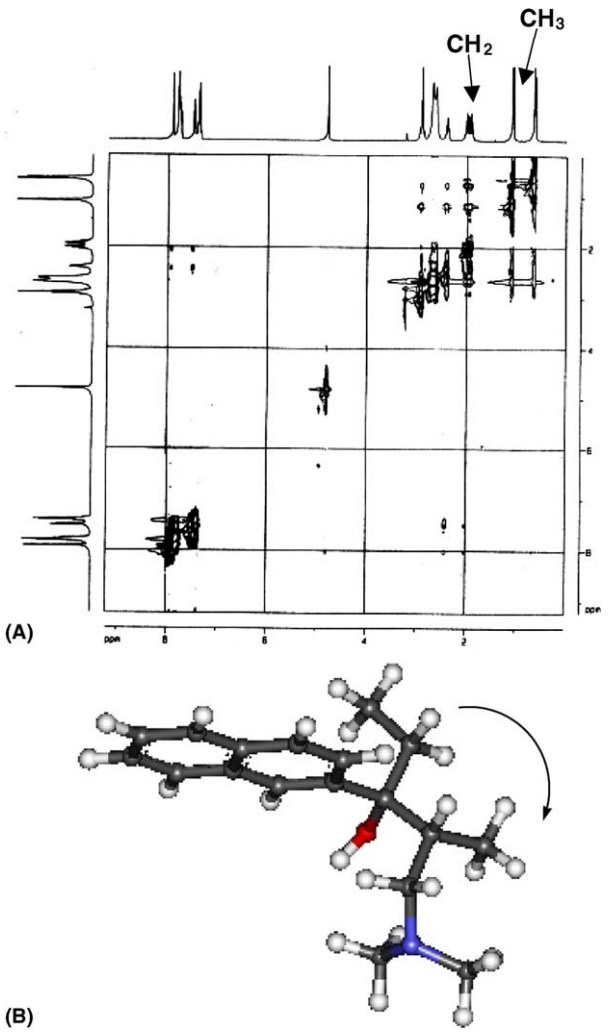
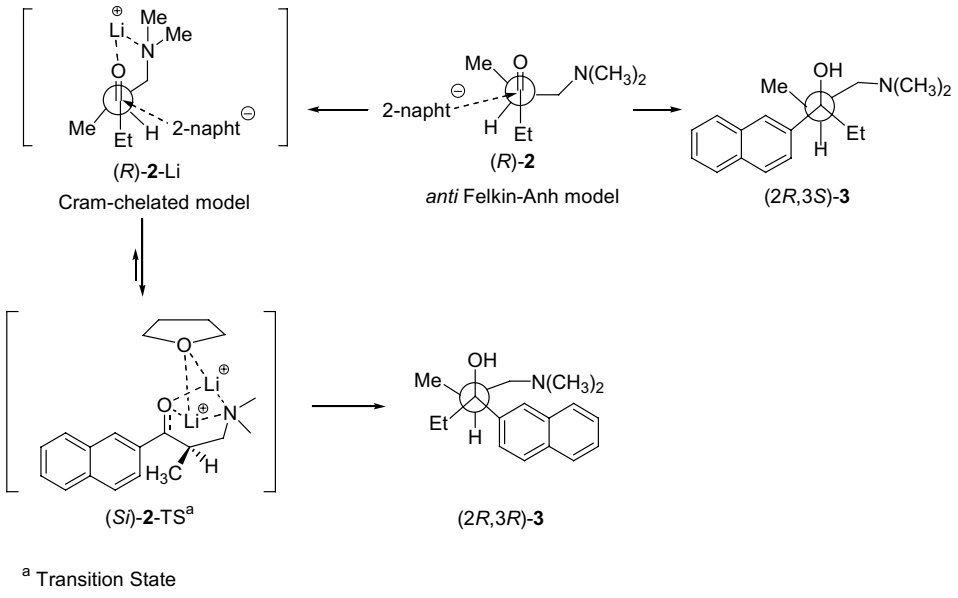


Figure 5. ^{1}H NMR and NOESY spectra of (2*RS*,3*RS*)-**3** (A) and conformational view of (2*R*,3*R*)-**3** (B).

To explain the diastereoselectivity, we have hypothesized[4] an intermediate chelate (Scheme 1). The difference in the diastereoselection is due to the stability of the Cram-chelate intermediate **2**-Li. (*R*)-**2**-Li [or (*S*)-**2**-Li] and the O of THF should also coordinate a second atom of lithium, giving rise to a more stable polycyclic intermediate (*Si*)-**2**-TS [or (*Re*)-**2**-TS]. Conversely, the non-coordinating solvent toluene should promote diastereoselective behaviour via the *anti*-Felkin–Anh model.

The encouraging results in the synthesis of (2*RS*,3*RS*)-**3** prompted us to perform the synthesis of **4**, which gave a mixture of diastereoisomers, both in THF at −60 °C and in toluene at +60 °C (Table 1, entries 5 and 6). The ^{1}H NMR spectrum suggested a DOSY (*D*iffusion *O*rder *S*pectroscop*Y*) experiment (Fig. 6). A pulsed field gradient NMR spectroscopy allowed us to measure the translational diffusion of molecules in solution. Depending on the value of the apparent diffusion coefficients, in 1D spectra the signals of the components of a mixture can be separated.[6,7] Two different series of signals were registered: the first one aligned at $y = -9.204$



Scheme 1.

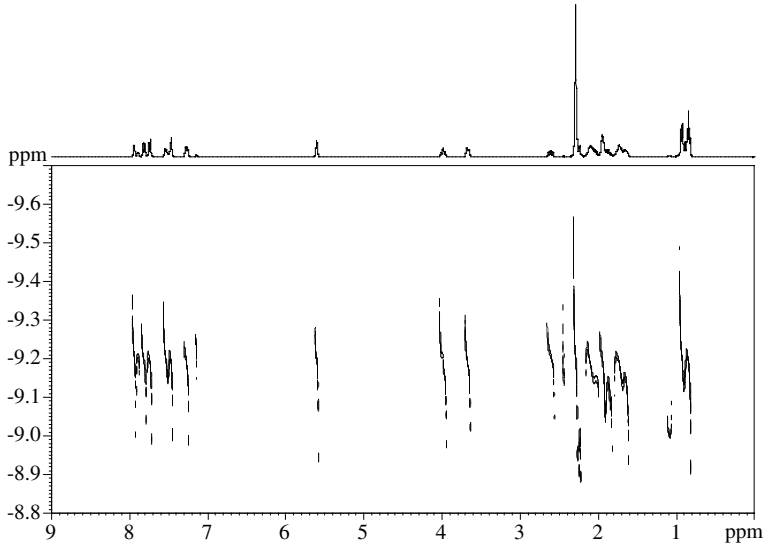


Figure 6. ^{1}H NMR DOSY spectrum of crude **4** (Table 1, entry 5).

and the second one aligned at $y = -9.187$, showing the presence of two racemic pairs. It is reasonable to suppose that the presence of the third stereogenic centre (due to the THP moiety) does not affect the physicochemical features of the isomers significantly.

The synthesis of **4** in THF at −60 °C and in toluene at +60 °C (Table 1, entries 5 and 6) showed an unexpected switchover in the diastereoselectivity with respect to **3**. This difference could be due to the formation of a complex between the tetrahydropyranyl moiety and the lithium atom, thus modifying the diastereoselective behaviour according to the *anti*-Felkin–Anh model; on the other hand the noncoordinating solvent toluene can promote the reaction towards the Cram model.[4]

The configuration of (2*RS*,3*RS*)-**4** was attributed to the major racemic pair of the crude **4** by comparison with (2*RS*,3*RS*)-**1**[2] on the basis of the retention times before and after the cleavage of the THP group (Table 2). The work-up of **4** with CH_2Cl_2 and Amberlyst 15 provided the cleavage of the THP protective group, thus affording pure **1**.

Considering these results, we also prepared the enantiomers of **1** and **3** by employing (*R*)-**2** [or (*S*)-**2**], which had already been synthesized by us.[2] The different reaction conditions (Table 3) mainly promoted compound (2*R*,3*R*) [or (2*S*,3*S*)] and (2*R*,3*S*) [or (2*S*,3*R*)], respectively. The diastereoisomeric mixtures obtained so far can be easily separated by flash chromatography on silica gel, affording enantiomerically pure naph-

Table 3. Experimental conditions for arylation of (*R*)-**2** and (*S*)-**2**

Entry	Ar-Br	Ketone	Solvent	*T* (°C)	Yield (%)[a]	Diastereoisomeric ratio
1	2-Br-naphthalene	(*R*)-**2**	THF	+10	83.2	88/12[b]
2	2-Br-naphthalene	(*R*)-**2**	Toluene	+60	70.1	46/54[b]
3	2-Br-naphthalene	(*S*)-**2**	THF	+10	85.5	83/17[c]
4	2-Br-naphthalene	(*S*)-**2**	Toluene	+60	72.2	49/51[c]
5	2-Br-6-OTHP-naphthalene	(*R*)-**2**	THF	−60	71.9	42/58[b]
6	2-Br-6-OTHP-naphthalene	(*S*)-**2**	THF	−60	87.4	42/58[c]

[a] Isolated yields by flash chromatography.
[b] (2*R*,3*R*)/(2*R*,3*S*).
[c] (2*S*,3*S*)/(2*S*,3*R*).

thylaminoalcohols with an enantiomeric excess ⩾94% (Table 4).

Liquid chromatography procedures were attempted using several chiral stationary phases in order to develop a suitable method for the evaluation of the enantiomeric excess of all enantiomers of **1** and **3**. Baseline separation was successfully achieved on analytical Chiralpak AD column (Table 5).

Finally the in vivo pharmacological profile on the analgesic activity of compounds **1** and **3**, investigated by HPT in mice, is reported.[8,9] Experimental data (Table 6) clearly show that the stereoisomers possess relevant analgesic activity, with the exception of enantiomer (2*S*,3*S*)-**1** ($AD_{50} = 31.86$ μmol/kg). In particular, (2*RS*,3*RS*)-**1** possesses antinociceptive properties comparable to morphine while (2*RS*,3*SR*)-**1** is about 26 times more potent. These preliminary data encouraged the investigation of enantiomers (2*R*,3*S*)-**1** and (2*S*,3*R*)-**1**. The AD_{50} values clearly show the role of stereochemistry in the analgesic activity, being (2*S*,3*R*), the configuration of the eutomer.

3. Conclusion

A practical and convenient diastereoselective procedure has been developed for the synthesis of pure diastereoisomers **1** and **3**, depending on the experimental conditions and has been successively applied to the preparation of all enantiomers by arylation of (*R*)-**2** or (*S*)-**2**. To the best of our knowledge, this result constitutes the first diastereoselective synthesis of chiral non-racemic naphthylaminoalcohols.

Concerning the pharmacological activity, morphine-like analgesic properties were evidenced for all compounds by the hot plate test in mice. In particular, the most active compound (2*S*,3*R*)-**1** will be investigated further to acquire more information about its biological profile.

Table 4. Optical properties of compounds **1** and **3**

Compound	[α] (λ, nm) (*c*, MeOH)	Ee (%)[a]
(+)-(2*S*,3*R*)-**1**	+16.45 (405) (0.46)	94.0
(−)-(2*R*,3*S*)-**1**	−16.9 (405) (0.46)	97.3
(+)-(2*R*,3*R*)-**3**	+47.1 (405) (0.50)	94.3
(−)-(2*S*,3*S*)-**3**	−48.1 (405) (0.54)	97.6
(+)-(2*S*,3*R*)-**3**	+39.2 (589) (0.46)	98.1
(−)-(2*R*,3*S*)-**3**	−40.3 (589) (0.48)	99.0

[a] Determined by chiral HPLC (see Table 5).

Table 6. Analgesic activity in the HPT

Compound	AD_{50}[a] (μmol/kg)	Conf. limits
Morphine	10.67	7.94–14.80
(2*RS*,3*RS*)-**1**[b]	10.31	2.09–50.33
(2*R*,3*R*)-**1**[b]	9.72	6.45–17.34
(2*S*,3*S*)-**1**[b]	31.86	28.21–35.93
(2*RS*,3*SR*)-**1**	0.41	0.04–4.73
(2*S*,3*R*)-**1**	0.32	0.07–1.58
(2*R*,3*S*)-**1**	1.11	0.62–2.03
(2*RS*,3*RS*)-**3**	1.23	0.23–6.70
(2*RS*,2*SR*)-**3**	3.90	3.58–4.34

[a] Determined in mice.
[b] See Ref. 2.

4. Experimental

4.1. General

Commercially available reagents and solvents were used as received from the supplier unless otherwise specified. Technical grade Amberlyst and Amberlite resins were used after washing with suitable solvents. Diethyl ether, toluene and tetrahydrofuran (THF) were distilled over sodium.[9] All moisture sensitive reactions were carried out under a nitrogen atmosphere. Unless otherwise specified, reactions involving polymers were carried out on a IKA KS 130 basic laboratory shaker at 250 rpm. NMR spectra were performed at 9.4 T (TMS as internal standard, $\delta = 0$) with an ADVANCE spectrometer at

Table 5. Chiral resolution of enantiomers of **1** and **3** on a Chiralpak AD column (250×4.6 mm)

Compound	Mobile phase[a]	t_{R1} (min) (config.)	t_{R2} (min) (config.)	[α]	R_s
1	95/5/0.1	21.92 (2*S*,3*S*)	26.14 (2*R*,3*R*)	1.2	1.2
1	95/5/0.1	19.71 (2*S*,3*R*)	28.60 (2*R*,3*S*)	1.45	1.6
3	98/2/0.1	6.12 (2*R*,3*R*)	7.29 (2*S*,3*S*)	1.2	1.4
3	98/2/0.1	5.86 (2*R*,3*S*)	8.50 (2*S*,3*R*)	1.45	1.8

[a] Solvent mixture: *n*-hexane/IPA/diethylamine (v/v/v); flow rate = 0.5 mL/min; UV detector: λ = 273 nm.

400 MHz, mod. Bruker Germany and a BB1 5 mm probe; chemical shifts are given in ppm. Elemental analyses were executed by Carlo Erba 1106 C, H, N analyzer. Analytical TLC were performed using precoated glass-backed plates (Fluka Kieselgel 60 F_{254}) and visualized by ultra-violet radiation, acidic ammonium molybdate (IV) or potassium permanganate. The diastereoisomeric separation of **1** and **3** were performed on a Biotage Flash Chromatography System using Flash 40+M, 4.0×15.0 cm prepacked cartridges.

HPLC analyses were performed on a system consisting of a Waters pump mod. 510, a Reodyne 7125 injector (20 μL sample loop) and a Perkin–Elmer, mod. LC-95, double wavelength UV detector (the wavelength was fixed at 273 nm). Experimental data were analyzed with the Hewlett–Packard 3395 HPLC integrator. Achiral HPLC analyses were performed on NovaPak silica column (150×3.9 mm, 4 μm) and Hypersil silica (250×4.0 mm, 5 μm). Elution was carried out using the isocratic conditions reported in Table 2. Chiral HPLC analyses were performed on Chiralpak AD column (Daicel) (250×3 mm, 5 μm); elution was carried out using *n*-hexane and isopropyl alcohol (IPA). All the solvents were purchased by Carlo Erba and are HPLC grade. Optical rotations were recorded on a Jasco DIP 1000 polarimeter at a concentration of 1% in methanol.

4.2. Preparation of (2*RS*,3*RS*)-1-dimethylamino-2-methyl-3-naphthalen-2-yl-pentan-3-ol, (2*RS*,3*RS*)-3 and (2*RS*,3*SR*)-1-dimethylamino-2-methyl-3-naphthalen-2-yl-pentan-3-ol, (2*RS*,2*SR*)-3

2-Bromonaphthalene (5 g, 24.0 mmol) was dissolved in dry diethyl ether (100 mL) under a nitrogen atmosphere, the solution cooled to −78 °C and *t*-BuLi (48 mmol, 15 mL, 1.7 M in pentane) then added dropwise. After 1 h at the same temperature, the mixture was allowed to warm to −50 °C and then a solution of (*RS*)-**2** (2.75 g, 19.2 mmol) in dry diethyl ether (25 mL) added.

The stirring was continued for 3 h at −50 °C and then the reaction mixture allowed to warm to +10 °C and quenched with water (80 mL). The aqueous phase was extracted with diethyl ether (3×40 mL) and the combined organic phases extracted further with 5% aqueous solution of DL-tartaric acid. The acidic aqueous layer was made alkaline with Na_2CO_3 (pH 9) and, after extraction with CH_2Cl_2 and evaporation of the solvent under vacuum, 3.96 g (yield 76.1%) of (2*RS*,3*RS*)-**3** and (2*RS*,3*SR*)-**3** were obtained as a yellow oil with diastereoisomeric ratio 59/41 (Table 1, entry 1) determined by HPLC (Table 2).

4.3. Preparation of (2*RS*,3*RS*)-1-dimethylamino-2-methyl-3-[6-(tetrahydro-pyran-2-yloxy)-naphthalen-2-yl]-pentan-3-ol and (2*RS*/3*SR*)-1-dimethylamino-2-methyl-3-[6-(tetrahydro-pyran-2-yloxy)-naphthalen-2-yl]-pentan-3-ol, (2*RS*,3*RS*)- and (2*RS*,3*SR*)-4

2-Bromo-6-OTHP-naphthalene (6 g, 19.5 mmol) was dissolved in dry THF (100 mL) under a nitrogen atmosphere, the solution cooled to −78 °C and *t*-BuLi (39 mmol, 23 mL, 1.7 M in pentane) then added dropwise. After 1 h at the same temperature, the mixture was allowed to warm to −60 °C and a solution of (*RS*)-**2** (1.8 g, 15.6 mmol) in dry THF (20 mL) then added.

Stirring was continued for 3 h at −60 °C and then the reaction mixture quenched with water (80 mL). The aqueous phase was extracted with diethyl ether (3×40 mL) and the combined organic phases further extracted with 5% aqueous solution of DL-tartaric acid. The acidic aqueous layer was made alkaline with Na_2CO_3 (pH 9) and, after extraction with CH_2Cl_2 and evaporation of the solvent under vacuum, 5.66 g (yield 78.2%) of (2*RS*,3*RS*)-**4** and (2*RS*,3*SR*)-**4** were obtained as a yellow oil with a diastereoisomeric ratio 40/60 (Table 1, entry 5) determined by HPLC (Table 2).

4.4. General procedure for arylation of ketone 2

To a solution of the appropriate starting material (24 mmol) in the appropriate solvent (100 mL) was added *t*-BuLi (48 mmol, 28 mL 1.7 M in pentane) and the reaction mixture kept at −78 °C for 1 h. A solution of (*RS*)-**2**, (*R*)-**2** $\{[\alpha]_D^{22} = -34.7,\ d_{22}{}^4 = 0.85517\}$ or (*S*)-**2** $\{[\alpha]_D^{22} = +34.6,\ d_{22}{}^4 = 0.85517\}$ (19.2 mmol in 25 mL of the appropriate solvent) was then added, according to the experimental conditions (Tables 1 and 3). After 4 h the reaction was quenched by the addition of polymer-supported carboxylic acid (IRC50) and the mixture stirred for 30 min and then filtered. Evaporation of the organic solvent yielded a mixture of the diastereoisomeric pairs and the unreacted starting material, as evidenced by HPLC analysis (Table 2). Crude products were purified by the addition of CH_2Cl_2 and Amberlyst 15. The mixture was shaken for 1 h and, after filtration, the resin suspended in 10 mL of NH_3 in methanol (3.5 M) and shaken overnight; the resin was filtered off and washed with ammonia solution. Evaporation of the solvent gave a pale yellow oil, corresponding to **1** or **3**, as a mixture of different ratios of diastereoisomers (HPLC, Tables 2 and 5).

Separation of diastereoisomeric mixtures were achieved through flash chromatography by eluting with *n*-hexane 2/EtOAc 8/NH_4OH 0.1 (1000 mL) and EtOAc 10/NH_4OH 0.1 (1000 mL) for compound **1** and *n*-hexane 7/EtOAc 3/NH_4OH 0.1 (1000 mL) and *n*-hexane 5/EtOAc 5/NH_4OH 0.1 (1000 mL) for compound **3**.

4.4.1. (2*RS*,3*SR*)-1. White solid, mp 198–200 °C, $R_f = 0.53$ (CH_2Cl_2 53/MeOH 47/DEA 1). ^1H NMR (400 MHz, $CDCl_3$, TMS): $\delta_H = 7.5$ (m, 6H, aromatic); 2.30 [s, 6H, $N(CH_3)_2$]; 2.16 (m, 2H, CH_2–CH_3, $J = 11$ Hz); 1.98 (m, 1H, CH, $J = 7$ Hz); 1.83 (dd, 2H, CH_2–N); 0.94 (t, 3H, CH_3–CH_2, $J = 7$ Hz); 0.80 (d, 3H, CH_3–CH, $J = 7$ Hz). Elemental analysis: $C_{18}H_{25}NO_2$ requires C, 75.22; H, 8.77; N, 4.87. Found: C, 75.68; H, 8.40; N, 4.78.

4.4.2. (−)-(2*R*,3*S*)-1. Yellow oil. For [α] and ee% values, see Table 4. ^{1}H NMR spectrum is identical to that of the corresponding racemate. Elemental analysis: $C_{18}H_{25}NO_2$ requires C, 75.22; H, 8.77; N, 4.87. Found: C, 75.45; H, 8.90; N, 4.62.

4.4.3. (+)-(2*S*,3*R*)-1. Yellow oil. For [α] and ee% values, see Table 4. ^{1}H NMR spectrum is identical to that of the corresponding racemate. Elemental analysis: $C_{18}H_{25}NO_2$ requires C, 75.22; H, 8.77; N, 4.87. Found: C, 75.09; H, 8.90; N, 4.52.

4.4.4. (2*RS*,3*SR*)-3. White solid, mp 69.8–71.0 °C, $R_f = 0.76$ (EtOAc 90/*n*-hexane 10/NH_4OH 1). ^{1}H NMR (400 MHz, CD_3OD, TMS): $\delta_H = 7.80$ (m, 4H, aromatic); 7.48 (m, 3H, aromatic); 3.04 (dd, 1H, N–*H*CH, $J = 13.1$, 6.9 Hz); 2.83 (s, 3H, N–CH_3); 2.75 (s, 3H, N–CH_3); 2.68 (dd, 1H, N-HC*H*, $J = 13.1$, 6.9 Hz); 2.42 (qdd, 1H, CH_3–C*H*, $J = 7.1$, 6.9, 6.9 Hz); 2.18 (qd, 1H, CH_3–*H*CH, $J = 7.4$ Hz); 1.94 (qd, 1H, CH_3–HC*H*, $J = 7.4$ Hz); 0.83 (d, 3H, CH_3–CH, $J = 7.1$ Hz); 0.71 (t, 3H, CH_3–CH_2, $J = 7.30$ Hz). Elemental analysis: $C_{18}H_{25}NO$ requires C, 79.66; H, 9.28; N, 5.16. Found: C, 79.72; H, 9.32; N, 5.18.

4.4.5. (−)-(2*R*,3*S*)-3. Yellow oil. For [α] and ee% values, see Table 4. ^{1}H NMR spectrum is identical to that of the corresponding racemate. Elemental analysis: $C_{18}H_{25}NO$ requires C, 79.66; H, 9.28; N, 5.16. Found: C, 79.54; H, 9.11; N, 5.30.

4.4.6. (+)-(2*S*,3*R*)-3. Yellow oil. For [α] and ee% values, see Table 4. ^{1}H NMR spectrum is identical to that of the corresponding racemate. Elemental analysis: $C_{18}H_{25}NO$ requires C, 79.66; H, 9.28; N, 5.16. Found: C, 79.70; H, 9.26; N, 5.08.

4.4.7. (2*RS*,3*RS*)-3. White solid, mp 120.0–121.4 °C, R_f 0.52 (EtOAc 90/*n*-hexane 10/NH_4OH 0.1). ^{1}H NMR (400 MHz, CD_3OD, TMS): $\delta_H = 7.97$ (s, 1H, aromatic); 7.86 (m, 3H, aromatic); 7.51 (m, 3H, aromatic); 2.99 (d, 2H, N–CH_2, $J = 6.26$ Hz); 2.77 (s, 3H, N–CH_3); 2.71 (s, 3H, N–CH_3); 2.45 (q, 1H, CH_3–C*H*, $J = 6.70$ Hz); 2.05 (m, 2H, CH_3–CH_2); 1.12 (d, 3H, CH_3–CH, $J = 6.87$ Hz); 0.74 (t, 3H, CH_3–CH_2, $J = 7.36$ Hz). Elemental analysis: $C_{18}H_{25}NO$ requires C, 79.66; H, 9.28; N, 5.16. Found: C, 79.78; H, 9.30; N, 5.15.

4.4.8. (−)-(2*S*,3*S*)-3. Yellow oil. For [α] and ee% values, see Table 4. ^{1}H NMR spectrum is identical to that of the corresponding racemate. Elemental analysis: $C_{18}H_{25}NO$ requires C, 79.66; H, 9.28; N, 5.16. Found: C, 79.33; H, 9.15; N, 5.24.

4.4.9. (+)-(2*R*,3*R*)-3. Yellow oil. For [α] and ee% values, see Table 4. ^{1}H NMR spectrum is identical to that of the corresponding racemate. Elemental analysis: $C_{18}H_{25}NO$ requires C, 79.66; H, 9.28; N, 5.16. Found: C, 79.67; H, 8.98; N, 5.20.

4.5. ^{1}H NMR spectroscopy

^{1}H,^{1}H-COSY and ^{1}H-NOESY NMR spectra of (2*RS*,3*RS*)-**1**·HCl and **3**·HCl were performed at 9.4 T, in CD_3OD at room temperature (TMS as internal standard, $\delta = 0$).

4.6. Molecular modeling study

A systematic conformational search of compound (2*R*,3*R*)-**3**·HCl was performed using the MMFF94 force field as implemented in the MOE 2001.01 software.[5] Only those conformations with energies within 10 kcal/mol of the lowest energy structure were considered. One of the low-energy conformations obtained is represented in Figure 5B.

4.7. Pharmacology

Male adult Swiss mice weighing 30 ± 5 g were used. Antinociception was estimated by means of the hot plate test (HPT).[7] Compounds were dissolved in saline solution and administered within 1 h from dissolution. The response to the thermal stimulus was evaluated using a copper plate heated to 55 °C. The time at which mice displayed a nociceptive response by sitting on its hind legs and licking, was measured in seconds. Once the basal animal reaction time was determined, to establish the dose–response curve, groups of 10 mice were treated (via sc injection) with increasing doses of the compounds. Control animals received the same volume of saline solution. The reaction time to the pain stimulus was measured 20 min following injection. The reaction time of the control animals (cut off time) was 23 ± 2 sec. AD_{50} values and their 95% confidence intervals were determined using a computerized program.[8] Experimental data are reported in Table 6.

References and notes

1. Azzolina, O.; Collina, S.; Brusotti, G.; Rossi, D.; Callegari, A.; Linati, L.; Barbieri, A.; Ghislandi, V. *Tetrahedron: Asymmetry* **2002**, *13*, 1073–1081.
2. Ghislandi, V.; Azzolina, O.; Collina, S.; Paroli, E.; Antonilli, L.; Giuseppetti, G.; Tadini, C. *Chirality* **1994**, *6*, 389–399.
3. Collina, S.; Azzolina, O.; Vercesi, D.; Benevelli, F.; Callegari, A.; Tadini, C.; Lanza, E.; Barbieri, A.; Ghislandi, V. *Bioorg. Med. Chem.* **2000**, *8*, 769–775.
4. Azzolina, O.; Collina, S.; Brusotti, G.; Rossi, D.; Linati, L.; Lanza, E.; Ghislandi, V. *Tetrahedron Lett.* **2004**, *45*, 1355–1357.
5. Ewig, C. S.; Berry, R.; Dinur, U.; Hill, J. R.; Hwang, M. J.; Li, H.; Maple, J.; Peng, Z.; Stockfish, T. P.; Thacher, T. S.;

Yan, L.; Ni, X.; Hagler, A. T. *J. Comput. Chem.* **2001**, *22*, 1782.
6. Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 288.
7. Morris, K. F.; Johnson, C. S., Jr. Diffusion Ordered 2D NMR Spectroscopy. *J. Am. Chem. Soc.* **1992**, *114*, 3139.
8. Woolfe, G.; Mac Donald, A. D. *J. Pharmacol. Exp. Ther.* **1944**, *80*, 300.
9. Tallarida, R. J.; Murray, R. B. *Manual of Pharmacological Calculation with Computer Programs*; Springer: Berlin, 1987; pp 26–31.