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Syntheses of *R* and *S* Isomers of AF-DX 384, a Selective Antagonist of Muscarinic M_2 Receptors

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Abstract—Enantiomers of 5,11-dihydro-11-[2-[2-[(*N*,*N*-dipropylaminomethyl)piperidin-1-yl]ethylamino]-carbonyl]-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (AF-DX 384) **1**, have been synthesized from (*S*)-(+) and (*R*)-(−)-2-[*N*,*N*-dipropylaminomethyl]piperidine **4**. The enantiomeric excess of **1** has been determined by capillary electrophoresis by using the α-highly sulphated cyclodextrin (α-HSCD) as chiral selector within the running electrolyte. (*S*)-(+)-(**4**) was prepared from (*S*)-(−)-pipecolic acid in a 4-step procedure (overall yield: 30%, ee: 99%) and (*R*)-(−)-AF-DX 384 from (*R*)-(+)-pipecolic acid. The (*R*)-(−) isomer exhibited in vitro a 23-fold higher affinity than its enantiomer (*S*)-(+) towards muscarinic receptors of subtype 2.

Introduction

Acetylcholine mediates a number of central and peripheral functions such as smooth muscle contraction and dilatation, cardiac inotropic effects, salivation as well as sleep mechanisms and cognition.[1] These effects are the result of an interaction of acetylcholine to five muscarinic receptor subtypes (M_1–M_5) (mAChR) distributed in different organs.[2] Among these receptor subtypes, M_2 receptors, were originally described as binding sites with high affinity for AF-DX 384 **1**,[3–7] AF-DX 116 **2**[8–11] (Scheme 1) or methoctramine, low affinity for pirenzepine, hexahydrosiladifenidol.[12] These receptors were found mainly in the heart of mammals, and thought to be an homogeneous population in this organ. Although the latter point has recently been readdressed[13] and heart muscarinic receptors proven to correspond to a heterogeneous population of M_1, M_2 and M_3 receptors, M_2 receptors have been demonstrated to be involved in the cardiac pathophysiology such as cardiomyopathies, cardiac ischemia and heart failure.

Cholinergic muscarinic receptor ligands have been radiolabelled and studied in vivo using positron emission tomography (PET) or single photon emission computed tomography (SPECT).[14] However, most of the currently used radiotracers (or their analogues), except bromocaramiphen[15] and (*R*,*S*)-fluoromethyl QNB,[16] were found to be nonselective with regard to the muscarinic receptor subtypes.[14,16] AF-DX 384 **1** and AF-DX 116 **2** have been described as selective antagonists of M_2 receptors endowed with a nanomolar affinity. Their tritiated analogues were tested for their in vivo binding and metabolisation characteristics in rats.[17] Based on these results, racemic AF-DX 384 has been synthesized [3,18] and labelled with carbon-11 (β^+ emitter, $t_{1/2}$: 20 min).[19] However, knowing that the spatial arrangement of the moieties interacting with mAChRs plays a decisive role for affinity to the receptor, the possibility exists that enantiomers of **1** might demonstrate a high eudismic ratio. For example, a high stereoselectivity ratio has been reported for hyoscyamine enantiomers,[20] and many other chiral antagonists.[1,21] Consequently, both

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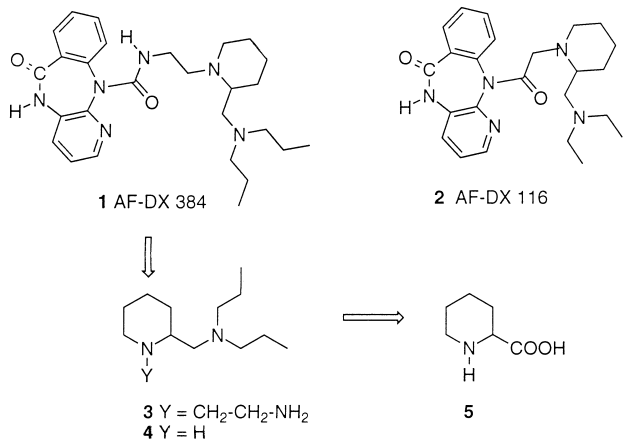


Scheme 1. Structures of AF-DX 116 and AF-DX 384.

enantiomers of a muscarinic ligand are generally synthesized for a comparison of their relative affinities towards mAChRs (for chiral muscarinic antagonists, see refs. 22–25). In order to examine pharmacokinetics and pharmacological profiles of individual enantiomers of AF-DX 384 **1** we describe here their syntheses and the evaluation of their in vitro affinity towards muscarinic M_2 receptors. The labelling of both enantiomers of AF-DX 384 with carbon-11 would allow the in vivo determination of the specific and nonspecific binding of the more active enantiomer as it was shown for dexetimide, levetimide and analogues.[26–28]

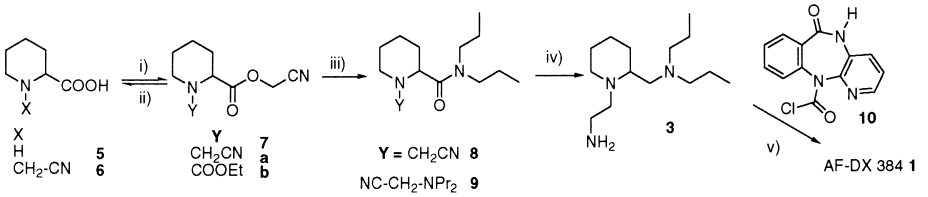
Results and Discussion

Enantiomers of (dipropylaminomethyl)piperidine **4** are the key chiral compounds in the synthesis of (+)- and (−)-AF-DX 384 (+)- and (−)-**1**. 2-(Dialkylaminomethyl)piperidines are generally prepared by catalytic hydrogenation of the corresponding pyridines,[3,29,30] or by hydride reduction of a piperidine carboxamide.[31–33] Reductive amination of a *N*-protected piperidine carboxaldehyde[29] and functional transformations of piperidine methanol have also been reported.[18,31] The preparation of optically active 2-(dialkylaminomethyl)piperidines required either the resolution of the racemic compound[10] or a synthesis starting from enantiomerically pure piperidine carboxylic acid (pipecolic acid) **5**.[32–34] An elegant approach using (−)-2-cyano-6-phenyloxazolopiperidine has recently been described.[35]

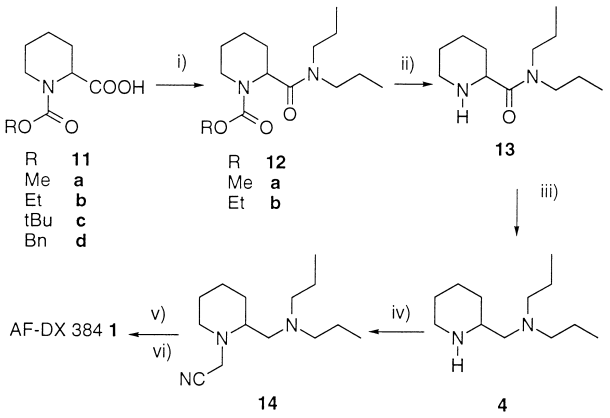
Synthesis of (−)-4 by resolution. The resolution of piperidine (±)-(**4**), according to a procedure described for an analogue,[10] was first attempted to access to (+)- and (−)-(**4**). Enantiomer (−)-(**4**) was obtained in 35% yield by treatment of (±)-(**4**) with one equivalent of L-(+) tartaric acid and by crystallization of one diastereomeric salt from isopropanol. However, the enantiomeric excess (ee) did not exceed 60%. Attempts to use other solvents (or mixture of solvents) or to modify the molar ratio of tartaric acid to amine (±)-(**4**) did not improve the ee. This prompted us to develop a new route to the optical isomers of **4** starting from the commercially available (*R*)-(+) and (*S*)-(−)-pipecolic acid.

Synthesis of (±)-AF-DX 384 1 from (±)-pipecolic acid 5. Due to the flexible nature of the piperidine ring and the strong neighbouring group participation of the ring nitrogen, the functional transformations of pipecolic acid (+)- or (−)-**5**, without racemization, are not necessarily straightforward.[18,32] Thus, the synthesis of AF-DX 384 **1** was first studied using the racemic acid (±)-**5**. Attempts to transform pipecolic acid (±)-**5** or its methyl ester into amide (±)-**13** without protecting the heterocyclic nitrogen failed. A three-step procedure was first envisaged for the synthesis of polyamine (±)-**3** (Scheme 1). It was based on the use of a cyanomethyl group which can act both as a protective group[36,37] and as a latent aminoethyl moiety. Moreover, it has been shown that a cyanomethyl ester is more reactive than a carboxylic acid in amidation reactions[38–42] and that this reaction proceeds without racemization when no carbonyl group is present β to the stereogenic centre.[43–45] Reaction of acid (±)-**5** with chloroacetonitrile and Et_3N allowed both *N*-alkylation and esterification of the acid. Ester (±)-**7a** was obtained in 99% yield. Its reaction with an excess of dipropylamine in acetonitrile did not give any amide (±)-**8**. Deprotection of the ester was partially observed with the formation of *N,N*-dipropylaminoacetonitrile **9** (12%).[46] Using lithium dipropylamide in THF–hexane, the nitrile **9** was formed with a 60% yield. The reaction of dipropylamine with ester (±)-**7a** was also attempted in the presence of sodium cyanide, an efficient catalyst of aminolysis.[47] When the reaction was carried out in acetonitrile, amine nitrile **9** was the major product (71% isolated) together with the *N*-cyanomethyl acid (±)-**6**. Meanwhile, in methanol, amide (±)-**8** was obtained in more than 60% yield. An in situ transesterification of the cyanomethyl ester could explain this result.[47] Amine (±)-**3** was obtained in 42% yield by reduction of amide (±)-**8** with lithium aluminium hydride.[18] Reaction of (±)-**3** with chlorocarbamate **10**[18] in acetonitrile led to (±)-AF-DX 384 **1** in 65% yield (Scheme 2).

Piperidine carboxamides are usually prepared from acid **5** after protection of the amine function with a methoxy,[48] ethoxy,[29] *tert*-butyloxy,[31,49] benzyloxy[29,32–34] group. The carbamates (±)-**11a**, (±)-**11b**, (±)-**11c** and (±)-**11d**, respectively, were prepared in more than 75% yield. All attempts (mixed anhydride using isobutyl chloroformate,[34] acid activation,[31,50] or use of a tin derivative[51]) to effect amidation of (±)-**11c** or benzyloxycarbonyl (±)-**11d** derivatives with *N,N*-dipropylamine failed. Amide (±)-**12b** was obtained in 81% by reaction of *N,N*-dipropylamine with cyanomethyl ester (±)-**7b** or in 95% yield by activation of the acid (±)-**11b** with isobutyl chloroformate in the presence of Et_3N, followed by reaction with *N,N*-dipropylamine. Amidation of *N*-methoxycarbonyl acid (±)-**11a** was attempted under different conditions (mixed anhydride method, thionyl chloride in the presence of DMAP).[62] The highest yield was obtained when the acid (±)-**11a** was first transformed into its dicyclohexylammonium



Scheme 2. Reagents and conditions: (i) $ClCH_2CN$, MeCN: **7a** (80 °C, 22 h) 99%; **7b** (24 h, rt) 81%; (ii) $HNPr_2$, NaCN, MeCN, 80 °C, 24 h; (iii) $HNPr_2$, NaCN, MeOH, 65 °C, 10 h, **8**: 67%; (iv) $LiAlH_4$, 0 °C, 2 h, 72%; (v) 80 °C, 4 h, 65%.



Scheme 3. Synthesis of amine (±)-**4**. Reagents and conditions: (i) $(C_6H_{11})_2NH$, $HNPr_2$, DBU, rt, 48 h, **12a**: 55%; (ii) Me_3SiI, 70 °C, 38 h, 88%; (iii) $LiAlH_4$, 70 °C, 24/h, 68%; (iv) $BrCH_2CN$, 70 °C, 5 h, 75%; (v) $LiAlH_4$, 0 °C, 2 h, 72%; (v) 80 °C, 4 h, 65%.

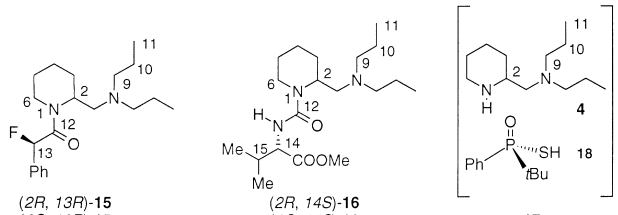
salt[53] then treated sequentially with thionyl chloride and *N,N*-dipropylamine in the presence of diazabicycloundecene (DBU) which gave amide (±)-**12a** in 55% yield. Attempts to prepare (±)-**13** from (±)-**11a** using conditions previously described ($SOCl_2$ then amine) for similar compounds,[30] gave only tars (Scheme 3).

Deprotection (hydrochloric acid[54] or trifluoroacetic acid [55]) of the ethoxycarbonyl group of (±)-**12b** led to the crude amide (±)-**13** in a low yield (<40%). On the other hand, deprotection of the *N*-methoxycarbonyl group with iodotrimethylsilane,[56] yielded to piperidine carboxamide (±)-**13** in 88% yield. Reduction of the amide function with $LiAlH_4$ then gave the amine (±)-**4** which was transformed into (±)-AF-DX 384 **1** according to a described procedure.[18]

Synthesis of (*R*)-(−)-AF-DX 384 and (*S*)-(+)-AF-DX 384: determination of the optical purities. The same method was followed for preparing both enantiomers of AF-DX 384 **1**. Starting from (*S*)-(−)-pipecolic acid, (+)-amine **4** and (+)-AF-DX 384 **1** were obtained. Similarly, (*R*)-(+)-pipecolic acid gave amine (−)-**4** which was transformed into (−)-**1**. The formation of amine (+)-(**4**) and (+)-AF-DX 384 **1** from (*S*)-(−)-pipecolic acid allowed the assignment of the absolute configuration *S* to the (+) enantiomer of **1** and thus *R* to (−)-**4** and (−)-**1**. The optical rotations of piperidines **3**, **4** and AF-DX 384 being unknown, different methods were investigated for the determination of the enantiomeric purity of these compounds. Chiral lanthanide shift reagent ($(Eu(hfc)_3$)[57,58] did not show any separation of the diastereoisomeric signals in the ^1H NMR spectra of amides (±)-**8** and (±)-**13**. Chiral HPLC was used for the separation of enantiomers of amide **12a** but all tested columns (see experimental) failed in the cases of polyamines **3**, **4** and AF-DX 384 **1**. Among the large variety of reagents and methods developed for the determination of the enantiomeric composition of amines,[57–59] we first chose to derivatize optically enriched amines **4** and **3** with α-methoxy-α-trifluoromethylphenylacetyl chloride (Mosher reagent).[60] However, no clear separation of the fluorine resonances were observed in ^{19}F NMR spectra of the corresponding amides. On the other hand, the ^{19}F NMR spectrum of the adducts **15**, formed from (*R*)-(−)-α-fluorophenylacetyl chloride[61,62] and amine (±)-**4,** exhibited four well separated doublets (each diastereoisomer with their rotamers). The difficulties encountered to prepare enantiomerically pure (*R*)-(−)-α-fluorophenylacetyl chloride and the kinetic resolution observed when trying to determine the ee of amide **13**, limited the use of this fluorinated reagent. Amine **4** was thus derivatized with (*S*)-(−)-methyl-2-isocyanato-3-methylbutanoate. In the ^1H NMR spectra of the diastereoisomeric mixture **16**, the diastereoisomeric excess (de) could be easily measured by comparison of integration curves of the proton to the carboxylate group (Scheme 4).

We also analyzed the ^{31}P NMR spectrum of the mixed complex **17** formed between amine **4** and (*S*)-(−)-*tert*-butylphenylphosphinothioic acid **18**.[63] Unfortunately, no distinct phosphorus signals were observed and no attempts were made to search for another phosphorous derivative.[64] Surprisingly, in the ^1H NMR spectrum of **17**, the two methyl triplets were clearly separated and their integration gave a good and rapid measurement of the de. To our knowledge, it is the first use of the phosphorus derivative **18** for the determination of ee of a chiral secondary amine using ^1H NMR spectroscopy. Table 1 summarizes the chemical shifts differences measured for the separated signals with the different reagents.

Attempts to use the chiral solvating agent **18** with the primary amine **3** failed. Finally, due to its high efficiency in the separation of enantiomers,[67,68] we attempted high performance capillary electrophoresis (HPCE) to determine the ee of AF-DX 384. We first studied the introduction of highly sulphated cyclodextrins (HSCD) (α-HSCD, β-HSCD or γ-HSCD) in the running electrolyte. In these context, cyclodextrins were used both as carrier giving a negative charge to the analysed enantiomers via inclusion complexes, and as chiral selector, if the complex formation constants of the enantiomers with the



Scheme 4. Structures of the compounds used for determination of enantiomeric purity of amine **4**.

chiral cavity of the cyclodextrins are different. In order to increase the differences of electrophoretic behaviour of the diastereoisomeric complexes ((+) AF-DX 384/HSCD and (−)AF-DX 384/HSCD), we also analysed the racemic (±) AF-DX 384 in acidic medium (phosphate buffer, pH 2.5). Under these conditions, the enantiomers had a positive charge and their electrophoretic mobility was opposite to the electrophoretic mobility of the cyclodextrin complexes. With such a strategy, the selectivity obtained in HPCE for the three HSCD studied was maximum after optimisation of the phosphate buffer concentration and of the HSCD concentration. Co-injection of each enantiomer in the racemic mixture leads to peak identification. Whatever the HSCD used (α, β or γ), AF-DX 384 racemic mixture was resolved, and detected at the anodic end of the capillary. The resolution (*R*s) obtained, when HSCDs were introduced at a 5% (w/w) concentration in the running electrolyte, increased when the cyclodextrin internal diameter decreased: *R*s (α-HSCD) > *R*s (β-HSCD) > *R*s (γ-HSCD). Consequently the ee of the optically enriched AF-DX 384 was determined using α-HSCD as chiral selector, assuming that both enantiomers have the same UV molecular absorption coefficient whatever their state (i.e. as complexes or not).[69] The apparent migration mobility of the two diastereoisomeric complexes being different, the ee determined in HPCE were calculated with corrected areas which are defined as area/migration time ratio. Each enantiomer of AF-DX 384 was obtained in 99% ee from (*S*) or (*R*)-pipecolic acid, via amine (*S*)-(+)- or (*R*)-(−)-**4** (ee >99% determined from ^{1}H NMR of **17**). This confirmed that no racemization was observed when using this synthetic route.

Biological Evaluation

Racemic and enantiomers of AF-DX 384, in comparison with the nonselective, high-affinity muscarinic antagonist atropine were assayed for their affinity to rat heart M_2 muscarinic receptors against [^{3}H]-*N*-methyl scopolamine. As shown in Table 2, analysis of competition curves confirmed that (±)-**1** has a high affinity towards muscarinic receptors albeit lower than atropine.

The (*R*)-(−)-(**1**) isomer was found to exhibit a 23-fold higher affinity than its (*S*)-(+)-(**1**) enantiomer. Compared to AF-DX 116 (selectivity (+):(−): 8)[10] the eudismic ratio is slightly higher. Its value was closer to the one reported for analogues of QNB[65] but remains low in comparison to hyoscyamine, procyclidine or benzetimide (affinities ratio (*S*):(*R*), respectively: 330, 0.003 and >4000).[1]

Conclusion

In summary, enantiomers of AF-DX 384 **1** have been synthesized from (*R*)-(+) and (*S*)-(−)-pipecolic acids with a 99% ee. This method allowed the assignment of their absolute configurations, the (+) enantiomer being obtained from (*S*)-(−)-pipecolic acid, and the (−) one from the (*R*)-(+)-acid **5**. For the first time, the phosphorus reagent **18** has been used in conjunction with ^{1}H NMR to measure the ee of a secondary amine (**4**). The validity of the method was confirmed by comparison of the results with those obtained after derivatization of **4**

Table 1. ^{1}H NMR and ^{19}F NMR nonequivalences

Compounds	Nuclei	Position	Solvent	Multiplicity, δ (ppm) (configurations)		Δδ ppm
15	F[a]	13	$CDCl_3$	d, −168.8;	d, −171.5;	2.7
				d, −172.4[b] (2*R*, 13*S*)	d, −175.5[c] (2*S*,13*S*)	3.1
16	H	H-14	$CDCl_3$	dd, 4.28 (*2S,14S*)	dd, 4.41 (*2R,14S*)	0.13
17	H	Me-11	$CDCl_3$	t, 0.78 (*2S,S*p)	t, 0.66 (*2R,S*p)	0.12

[a] ^{19}F NMR.
[b] Rotamers: ratio 52:48.
[c] Rotamers: ratio 65:35.

Table 2. Affinities of tested compounds for M_2 muscarinic receptors (rat heart tissue)

Compounds	pIC_{50}[a]	Compounds	pIC_{50}[b]
Atropine	8.70 ± 0.33		
(±)-AF-DX 384 **1**	7.36 ± 0.30	(±)-AF-DX 116 **2**	6.85
(*R*)-(−)AF-DX 384 (*R*)-(−)-(**1**)	8.02 ± 0.51	(+)-AF-DX 116 (+)-(**2**)[c]	7.30
(*S*)-(+)AF-DX 384 (*S*)-(+)-(**1**)	6.66 ± 0.38	(−)-AF-DX 116 (−)-(**2**)[c]	6.39

[a] pIC_{50} values represent the negative logarithm of the concentration that produces 50% inhibition of the antagonist maximal response and are expressed as means ± SEM, $n = 3$ using 5×10^{-10} M [^{3}H]methyl scopolamine.
[b] Data from refs 3–7 and obtained from competition studies against 3.10^{-10} M [^{3}H]methyl scopolamine.
[c] The absolute configuration was not determined.[3–7]

and measurements of the diastereoisomeric excesses either by ^{1}H NMR or HPLC. The ee of enantiomers of AF-DX 384 has been determined by capillary electrophoresis using 5% (w/w) α-highly sulphated cyclodextrin in acidic medium (phosphate buffer, pH 2.5). Finally, the affinities of the enantiomers of AF-DX 384 toward M_2 receptors were evaluated and the (*R*)-(−)-enantiomer was shown to be the eutomer.

Experimental

General techniques

All reactions were carried out under a nitrogen atmosphere. Tetrahydrofuran (THF) was distilled from sodium benzophenone. (±)-2-(*N,N*-Dipropylaminomethyl)piperidine **4**,[18] (*R*)-(−)-α-fluoro phenyl acetic acid, (*R*)-(−)-α-fluoro phenyl acetyl chloride,[61] (±)-[(*N*-ethoxycarbonyl) piperidine]-2-carboxylic acid **11b**[29] (±)-[*N*-(*tert*-butoxycarbonyl)piperidine]-2-carboxylic acid **11c**[31,49] and (±)-[(*N*-benzyloxycarbonyl)piperidine]-2-carboxylic acid **11d**,[29] were synthesized according to described methods. (*S*)-(−)-*tert*-Butyl phenyl phosphinothioic acid was kindly provided by M. Mikolajczyk (Poland). All other reagents were used as obtained from commercial sources (purity >98% Janssen Chimica, Aldrich or Sigma). Organic solutions were dried over anhydrous $MgSO_4$ and evaporated at <50 °C under reduced pressure.

Thin-layer chromatography was performed on silica gel 60 F-254 plates (0.1 mm, Merck) with iodine and/or UV detection. Chromatographic separations were made on silica gel columns (Kieselgel 60, 70–230 mesh, Merck). Analytical high performance liquid chromatography (HPLC) was carried out with a Waters instrument (detector 486 and pump 510 or detector M996 (200–300 nm) and pump 600). The following columns (0.46×25 cm): Daicel Chiralcel OD, OJ, DNBPGJ. T. Baker, Nucleodex β-OH, Nucleosil chiral-2 were tested for the chiral separations.

Enantiomeric resolution by capillary electrophoresis

All solutions were prepared by using the 18 MΩ.cm water (Millipore, Bedford, MA, USA). The highly sulphated cyclodextrins were purchased from Beckman (Beckman, Fullerton, CA, USA). Triethylammonium phosphate of analytical purity was purchased from Aldrich. All these reagents were used without further purification.

All analyses were carried out on a P/ACE 2100 system (Beckman, Fullerton, CA, USA) fitted with an UV detector. The acquisition and the processing of data were performed with a Dell computer by means of P/ACE Station software (Beckman). The samples were systematically injected in hydrodynamic mode (injection pressure: 0.5 psi or 3.4 kPa) and their analysis were achieved on a fused silica capillary of 50 μm id presenting a total length of 57 cm (50 cm effective length to detection window). Injections were performed at the cathodic end of the capillary. The temperature was fixed at 22 °C. The pH of the solutions was measured at the temperature of the experiment, with a model Φ pH meter (Beckman) and before each analysis the capillary was flushed with water (1 min) then with the electrolyte (10 min). The rinsing pressure is equal to 20 psi. Finally the electrolytes were systematically degassed by sonication (Brandson).

Optical rotations were measured on a Perkin–Elmer 241 LC polarimeter at λ = 589 nm. $[\alpha]_D$ Values are given in units of 10^{-1} deg cm^2 g^{-1}. Microanalyses were carried out at CNRS centre (Vernaison, France). Melting points (MP) were determined on a Gallenkamp apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance DPX 250 (^{1}H: 250 MHz; ^{13}C: 62 MHz; ^{31}P: 101 MHz; ^{19}F: 235 MHz) or a Bruker Avance DRX 400 (400 and 100 MHz, ^{1}H and ^{13}C, respectively) spectrometers. Chemical shifts in $CDCl_3$ are reported in δ units (parts per million) from tetramethylsilane (^{1}H and ^{13}C), fluorotrichloromethane (^{19}F) as internal standards or phosphoric acid (^{31}P) as external standard. Multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). Coupling constants (*J*) are given in Hertz. All the attempts to determine the optical purities using a chiral lanthanide shift reagent were made by recording the ^{1}H NMR spectra of compounds (racemic or optically enriched, 0.05 mmol in 0.5 mL in $CDCl_3$) in the presence of incremental additions (from 0.1 to 0.5 equiv) of Europium(III) tris[3-heptafluorobutanoyl-(1*R*)-camphorate] [Eu(hfc)$_3$]. IR spectra were recorded on a Perkin–Elmer 16 PC-FT-IR spectrometer. Electron impact (EI, 70 ev) mass spectra were recorded with a Nermag R10 instrument and high resolution mass spectra (HRMS) on a Jeol JMSD 300 or a Jeol AX 500.

Resolution of (±)-2-[*N,N*-dipropylaminomethyl]piperidine 4. Diamine (±) **4** (1 g, 0.5 mmol) and (L)-(+) tartaric acid (0.758 g, 0.5 mmol, $[\alpha]_D^{22}$ +12° (*c* 20, H_2O)) were dissolved in hot isopropanol (400 mL). The mixture was allowed to crystallize at room temperature. After 96 h, the white and round crystals were collected (mp 132–133 °C after two crystallizations) then dissolved in dichloromethane. Aqueous sodium hydroxide (15%) was added and the mixture vigorously stirred for 1 h. The organic layer was separated, washed with brine, and dried. The solvent was removed under vacuum to yield (*R*)-(−)-**4** as a pale-yellow oil (0.350 g, 35%). Bp 0.002 mmHg 87.5 °C; $[\alpha]_D^{22}$ −49 ee: 58%.

(*S,R*) and (*R,R*) 1-[2-(*N,N*-Dipropylaminomethyl)piperidin-1-yl]-2-fluoro-2-phenyl-ethanone 15. (*R*)-(−)-α-Fluoro phenyl acetyl chloride (0.022 mL, 0.15 mmol) was added dropwise to the amine (±)-**4** (0.015 g, 0.09 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was stirred at room temperature for 2 h. Aqueous sodium hydroxide (2 mL, 15%) was added and the mixture was vigorously stirred for 10 min. After extraction with CH_2Cl_2 (2×5 mL), the organic layer was successively washed with $NaHCO_3$ (2×5 mL, saturated solution) and brine then dried. The solution was concentrated to give the title compounds (*S,R*)-**15** and (*R,R*)-**15** as a pale-yellow oil (0.015 g, 52%). IR ν_{max} (cm^{-1}, NaCl) 1652; ^{19}F NMR (235 MHz, $CDCl_3$) (*S,R*)-**15**: δ 169.1 (d, *J* = 51.7 Hz, 0.52 F), 172.6 (d, *J* = 51.7 Hz, 0.48 F);

(*R*,*R*)-**15**: δ 171.4 (d, $J=51.7$ Hz, 0.65 F), 175.7 (d, $J=51.7$ Hz, 0.45 F); HRMS *m*/*z* calcd for $C_{20}H_{31}N_2OF$: 334.24203, found 334.23559; MS (EI^+) 334 (M^+, 100), 319 (61), 305 (29), 286 (23), 267 (80).

(*S*,*R*)- and (*S*,*S*)-[2-[2-(*N*,*N*-dipropylaminomethyl)piperidin-1-yl-carbonyl]amino]-3-methyl butanoic acid methyl ester 16. The isocyanate (0.19 mmol) was added dropwise to a solution of amine (±)-**4** (0.038 g, 0.19 mmol) in $CDCl_3$ (1.5 mL) at room temperature The mixture was stirred for 3 h and an aliquot was removed for ^1H NMR analysis. The ureas were isolated as followed. CH_2Cl_2 was added to the reaction mixture and the organic layer was washed successively with HCl (3 M), NaOH (15%), H_2O then dried. After evaporation of the solvent, the diastereoisomeric ureas **16** were isolated and characterized without further purification. IR ν_{max} (cm^{-1}, NaCl) 3350, 1742, 1644 and 1520; ^1H NMR (250 MHz, $CDCl_3$) δ 6.78 (bd, 1H, $J=5.9$ Hz, CON*H*CH*i*PrCOOCH$_3$), 6.18 (bd, 1H, $J=5.9$ Hz, CON*H*CH*i*PrCOOCH$_3$), 4.41 (dd, 1H, $J=8.0$ and 4.9 Hz, CONHC*Hi*PrCOOCH$_3$ (*R*,*S*)), 4.28 (dd, 1H, $J=8.7$ and 5.7 Hz, CONHC*Hi*PrCOOCH$_3$ (*S*,*S*)), 3.97–4.20 (m, 5H), 3.72 (s, 3H, CONHCH*i*PrCOOC*H*$_3$), 3.70 (s, 3H, CONHCH*i*PrCOOC*H*$_3$), 2.56–2.87 (m, 3H), 2.31–2.52 (m, 8H), 1.92–2.30 (m, 5H), 1.35–1.80 (m, 19H), 0.81–0.98 (m, 24H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.3, 12.3, 18.7, 18.8, 19.4, 19.6, 19.8, 20.1, 20.1, 20.4, 25.4, 25.5, 31.7, 31.8, 39.2, 52.0, 52.1, 53.4, 57.9, 58.5; 59.2, 59.4, 63.6, 159.0, 174.2, 174.4; HRMS, *m*/*z* calcd for $C_{19}H_{37}O_3N_3$: 355.28347, found 355.29143; MS (EI^+) 355 (100), 343 (14), 331 (24), 312 (11), 281 (50), 269 (24).

(*S*)-(−)-*tert*-Butyl phenyl phosphinothioic acid–amine (±)-(4) complex 17. (*S*)-(−)-*tert*-Butyl phenyl phosphinothioic acid **18** (0.008 g, 0.037 mmol) and amine (±)-**4** (0.006 g, 0.030 mmol) in $CDCl_3$ (0.6 mL) were mixed in an NMR tube and the spectrum recorded after a few min. IR ν_{max} (cm^{-1}, KBr) 3424 and 1018; ^1H NMR (250 MHz, $CDCl_3$) δ 7.20–7.30 (m, 10H, Ph), 4.28 (m, 1H), 4.13 (m, 1H), 3.45 (m, 2H), 3.25 (m, 1H), 3.05 (td, 1H, $J=12$ and 2.7 Hz), 2.51–2.95 (m, 10H), 2.45 (td, 1H, $J=12.0$ and 2.7 Hz), 1.20–2.05 (m, 23H), 1.10 (s, 9H, C(CH$_3$)$_3$), 1.00 (s, 9H, C(CH$_3$)$_3$), 0.78 (t, 6H, $J=7.2$ Hz, CH$_2$CH$_3$, (*S*,*S*)), 0.66 (t, 6H, $J=7.3$ Hz, CH$_2$CH$_3$, (*S*,*R*)); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 11.1, 11.2, 17.4, 17.7, 21.7, 22.4, 25.2, 25.2, 27.0, 35.6, 36.8, 43.9, 44.2, 53.1, 55.5, 55.6, 56.7, 56.8, 127.0, 127.2, 129.6, 129.7, 132.8, 132.9, 138.1, 139.5; ^{31}P NMR (101.3 MHz, $CDCl_3$) 83.0.

Cyanomethyl *N*-(cyanomethyl)-2-piperidine carboxylate (±)-7a. A mixture of pipecolic acid (±)-**5** (0.500 g, 3.88 mmol), triethylamine (2.7 mL, 19.3 mmol) and chloroacetonitrile (2.2 mL, 19 mmol) in acetonitrile (30 mL) was heated at 80 °C for 22 h. After cooling to room temperature, the volatile compounds were evaporated under vacuum and the residue was treated with dichloromethane (25 mL). The organic layer was washed with brine (50 mL), dried and evaporated. Chromatography of the residue on silica gel (eluent CH_2Cl_2:MeOH, 97:3) gave the title compound (±)-**7a** (0.830 g, 99%); $R=0.75$ (CH_2Cl_2:MeOH, 85:15); mp 48 °C; IR (KBr, cm^{-1}) 2234 (CN), 1754 (COO); ^1H NMR δ 4.86 and 4.75 (AB, $J_{AB}=15.7$ Hz, 2H, OC*H*$_2$CN), 3.78 and 3.56 (AB, $J_{AB}=17.6$ Hz, 2H, NC*H*$_2$CN), 3.36 (dd, $J_{2ax,3eq}=3.6$ Hz, $J_{2ax,3ax}=9.1$ Hz, 1H, H-2ax), 2.92 (m, 1H, H-6eq), 2.59 (m, 1H, H-6ax), 2.13–2.00 (m, 1H, H-3), 1.82–1.60 (m, 4H, H-5, 1 H-4, 1 H-3), 1.49–1.41 (m, 1H, H-4); ^{13}C NMR δ 171.0 (CO), 114.8 (NCH$_2$*C*N), 114.1 (OCH$_2$*C*N), 62.1 (C_2), 51.5 (C_6), 48.8 (O*C*H$_2$CN), 44.0 (N*C*H$_2$CN), 29.5 (C_3), 24.9 (C_5), 22.2 (C_4); MS (EI) *m*/*z* 208 ($M+1^+$, 0.3), 207 (M^+, 0.5), 123 (100), 84 (18), 55 (22), 49 (30), 42 (18), 41 (34); HRMS calcd for $C_{10}H_{13}N_3O_2$: 207.1008; found: 207.1012.

(±)-*N*-(Cyanomethyl)piperidine-2-carboxylic acid (±)-6. A mixture of ester (±)-**7a** (0.730 g, 3.48 mmol), dipropylamine (12 mL, 87 mmol) and sodium cyanide (0.427 g, 8.7 mmol) in dry acetonitrile (30 mL) was heated at 80 °C for 20 h. After cooling to room temperature and concentration, the residue was treated with CH_2Cl_2 (50 mL). The organic layer was washed with brine (2×30 mL) and then acidified at pH 3.5 by slow addition of $KHSO_4$ (saturated solution). The organic layer was dried then concentrated to give (*N*,*N*-dipropylamino)acetonitrile **9** (0.345 g, 71%).[46] The aqueous layer was continuously extracted with chloroform for 24 h. The residue of the organic layer was dried then concentrated to give the title compound (±)-**6** (0.146 g, 25%). R_f 0.65 (CH_2Cl_2:MeOH, 85:15); mp 133 °C; IR (NaCl, cm^{-1}) 3428 (COOH), 2514 (CN), 1718 (CO); ^1H NMR δ 8.70 (s, 1H, COO*H*); 3.94 and 3.60 (AB, $J_{AB}=17.6$ Hz, 2H, NC*H*$_2$CN), 3.25 (dd, $J=3.3$ and 10.0 Hz, 1H, NC*H*CH$_2$), 2.94 (m, 1H, H-6eq), 2.62 (m, 1H, H-6ax), 2.12–2.06 (m, 1H, NCHC*H*$_2$), 1.82–1.60 (m, 4H), 1.49–1.39 (m, 1H); ^{13}C NMR δ 176.9 (*C*OOH), 114.8 (*C*N), 63.4 (N*C*H); 52.0 (N*C*H$_2$CH$_2$), 43.9 (N*C*H$_2$CN), 29.8 (NCH*C*H$_2$), 24.8 (NCH$_2$*C*H$_2$), 22.7 (NCH$_2$CH$_2$*C*H$_2$); MS (EI) *m*/*z*: 168 (M^+, 0.02); 123 (100), 55 (24), 54 (16), 42 (18), 41 (41); HRMS calcd for $C_8H_{12}N_2O_2$: 168.0899; found: 168.0900.

(±)-[(*N*′-Cyanomethyl)piperidine-2-(*N*,*N*-dipropyl)carboxamide (±)-8. To a solution of the ester (±)-**7a** (0.536 g, 2.59 mmol) in methanol (25 mL), dipropylamine (1.77 mL, 12.93 mmol) and sodium cyanide (0.317 g, 6.46 mmol) were added under nitrogen atmosphere. The reaction mixture was heated at 80 °C for 10 h. After cooling and evaporation of the volatile compounds, the resulting oil was treated with $CHCl_3$ (15 mL) and washed with water (15 mL). The organic layer was dried and evaporated. The residue was purified by chromatography on silical gel (eluent $CHCl_3$:MeOH, 97:3) to give the title compound (±)-**8** (0.260 g, 67%) as a yellow oil. R_f 0.9 (CH_2Cl_2:MeOH, 85:15); IR (KBr, cm^{-1}): 2224 (CN), 1670 (CO); ^1H NMR δ 3.58 and 2.82 (AB, $J_{AB}=15.4$ Hz, 2H, NC*H*$_2$CN), 3.63–3.42 (m, 4H, NC*H*$_2$CH$_2$), 3.20–3.13 (m, 1H, H-2), 2.88–2.84 (m, 1H), 2.48–2.44 (m, 1H), 2.30–2.24 (m, 1H), 2.08 (m, 1H), 1.83–1.78 (m, 1H), 1.62–1.18 (m, 7H); 0.90 (t, $J=7.4$ Hz, 3H, C*H*$_3$), 0.84 (t, $J=7.4$ Hz, 3H, C*H*$_3$); ^{13}C NMR δ 169.0 (*C*O), 118.6 (*C*N), 55.7 (C-6), 52.0 (N*C*H$_2$CN), 50.5 (C-2), 50.0 (N*C*H$_2$CH$_2$), 27.4 (C-4), 25.0 (C-3), 24.4 (C-5), 20.5 (*C*H$_2$CH$_3$), 11.4 (*C*H$_3$); MS (EI) *m*/*z*: 251 (M^+, 0.8), 97

(64), 84 (65), 49 (87), 43 (70), 42 (54), 41 (100); HRMS calcd for $C_{14}H_{25}N_3O$: 251.1998; found: 251.2007.

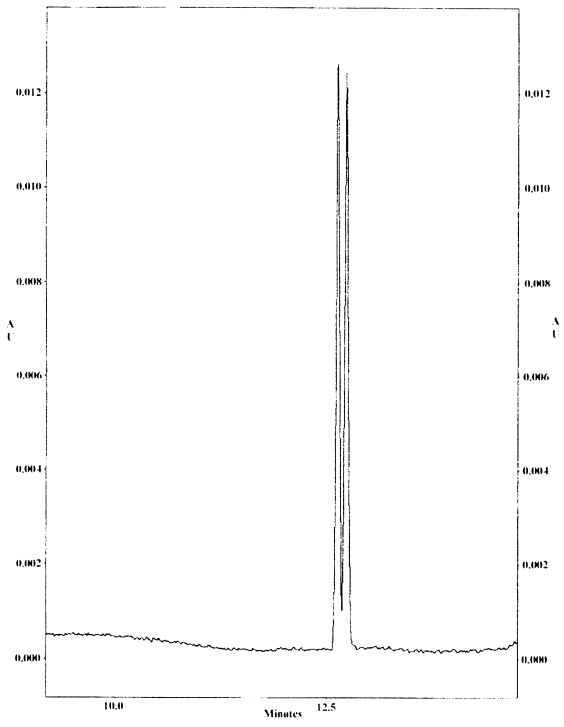
(±)-Cyanomethyl [(*N*-ethoxycarbonyl)piperidine]-2-carboxylate (±)-7b. A mixture of acid (±)-**11b** (0.050 g, 0.25 mmol), triethylamine (0.069 mL, 0.5 mmol) and chloroacetonitrile (0.031 mL, 0.25 mmol) in CH_2Cl_2 (1 mL) was stirred for 24 h at room temperature. After evaporation of the volatile compounds, CH_2Cl_2 (20 mL) was added. The organic layer was washed with brine (25 mL) then dried. After concentration, the residue was purified by column chromatography (eluent CH_2Cl_2 then MeOH) to give the title compound (±)-**7b** (0.048 g, 81%) as a yellow oil. R_f 0.85 ($CHCl_3$:MeOH, 90:10); IR (NaCl, cm^{-1}): 2862 (CN), 1758 (COO), 1696 (NCO); ^1H NMR δ 5.00 (s, 0.62H, H-2), 4.91 (s, 0.38H, H-2), 4.79 (AB, J_{AB} = 15.9 Hz, 2H, OCH_2CN), 4.16 (q, J = 7.0 Hz, 2H, CH_3CH_2O), 4.04–3.99 (m, 1H, H-6), 3.07–2.93 (m, 1H, H-6), 2.25–2.20 (m, 1H, H-3), 1.93–1.67 (m, 3H, H-5, H-4, H-3), 1.53–1.42 (m, 2H, H-5, H-4), 1.28–1.25 (m, 3H, CH_3); ^{13}C NMR δ 170.6 ($COOCH_2CN$), 156.6 ($COOCH_2CH_3$), 114.3 (*C*N), 61.9 (CH_2CH_3), 54.6 (C-2), 48.8 (CH_2CN), 41.8 (C-6), 26.7 (C-3), 24.5 (C-5), 20.7 (C-4), 14.6 (CH_3); MS (EI) *m/z*: 241 ($M+1^+$, 6), 240 (M^+, 6), 128 (100); HRMS calcd for $C_{11}H_{16}N_3O_4$: 240.1110; found: 240.1123.

(*S*)-(−)-[(*N*-Methoxycarbonyl)piperidine]-2-carboxylic acid (*S*)-(−)-(11a). To a solution of pipecolic acid (*S*)-(−)-**5** (0.183 g, 1.42 mmol) in water (3 mL, *c* 0.51 M, pH 7.3) at 4 °C were added dropwise and simultaneously the methyl chloroformate (1.67 mmol) and aqueous NaOH (2 N, 1.45 mL, 2.84 mmol) in order to maintain the reaction mixture weakly basic (pH 8–9). The mixture was stirred at room temperature overnight. The aqueous layer was washed with Et_2O (10 mL) then acidified (pH 3) with 6 N HCl. The acidic aqueous layer was extracted with Et_2O (3×10 mL) and the combined extracts were dried and evaporated to give the protected piperidine (*S*)-(−)-**11a**. White solid (0.257 g, 97%). R_f 0.4 (CH_2Cl_2:MeOH, 97:3); mp 108 °C; $[\alpha]_D^{22}$ −55° (*c* 6.9, $CHCl_3$); IR (NaCl, cm^{-1}): 3060 (COOH), 1744 (OCON), 1666 (CO); ^1H NMR δ 9.02 (s, 1H, COO*H*), 4.90 (m, 1H, H-2), 4.10 (m, 1H, H-6), 3.73 (s, 3H, CH_3), 3.04 (m, 1H, H-6), 2.32 (m, 1H, H-3), 1.8–1.5 (m, 3H, H-3, H-5, H-4), 1.5–1.2 (m, 2H, H-4, H-5); ^{13}C NMR δ 176.6 (C-7), 157.5 (C-9), 53.1 (C-10), 48.1 (C-2), 41.9 (C-6), 26.6 (C-3), 24.5 (C-5), 20.7 (C-4); MS (EI) *m/z:* 188 ($M+1^+$, 3), 187 (M^+, 5), 156 (100). Anal. calcd for $C_8H_{13}NO_4$: C, 51.34; H, 7.00; N, 7.48; O, 34.17. Found: C, 51.11; H, 6.83; N, 7.42 O 34.12

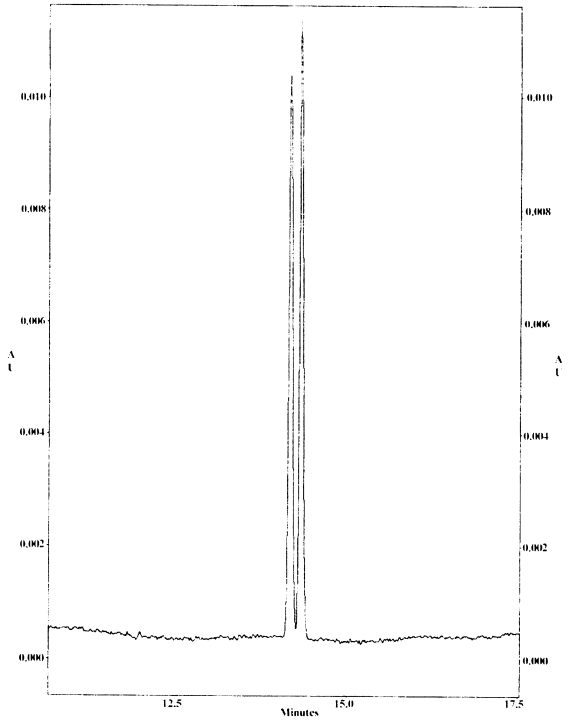
(*S*)-(−)-*N,N*-Dipropyl [(*N′*-methoxycarbonyl)piperidine]-2-carboxamide (*S*)-(−)-12a. To a solution of (*S*)-(−)-**11a** (0.257 g, 1.37 mmol) in AcOEt (2 mL), was added dicyclohexylamine (0.296 mL, 1.49 mmol) at room temperature. After stirring for 1 h, the precipitate was collected and recrystallised from Et_2O to give a yellow solid of [(*N*-methoxycarbonyl)piperidine]-2-dicyclohexylammonium carboxylate (0.506 g, 100%). IR (KBr, cm^{-1}): 1550 (NH_2^+), 1450 (COO^-). To a stirred solution of this salt (0.506 g, 1.37 mmol) in CH_2Cl_2 (8 mL), pyridine (0.14 mL, 1.78 mmol) and thionyl chloride (0.12 mL, 1.64 mmol) were added dropwise at room temperature under nitrogen atmosphere. The mixture was stirred at room temperature for 30 min before addition, at this temperature, of dipropylamine (0.18 mL, 1.05 mmol) and DBU (0.30 mL, 2.05 mmol) in CH_2Cl_2 (2 mL). After 48 h of stirring, brine (5 mL) was poured into the mixture, and the product was extracted with AcOEt (2×10 mL). The organic layers were washed successively with HCl 3 N (3×10 mL), NaOH 1 N (2×10 mL), and brine (10 mL) then dried. Evaporation of the volatile compounds gave a crude product, which was purified by chromatography on silica gel (CH_2Cl_2:MeOH, 97:3) to give (*S*)-(−)-**12a** (yellow oil, 0.1484 g, 55%). R_f 0.6 (CH_2Cl_2:MeOH, 97:3); $[\alpha]_D^{22}$ −28° (*c* 4, $CHCl_3$) ee >99% (HPLC, Chiralcel OD, 220 nm, *n*-heptane:isopropanol, 95:5; 0.6 mL min^{-1}: 12 min). The spectral data of the two rotamers (70:30) were identical with those previously described.[66] HRMS calcd for $C_{14}H_{26}N_2O_3$ 270.1943; found: 270.1932; anal. calcd C, 62.19; H, 9.69; N, 10.36; O, 17.75. Found: C, 62.09; H, 9.57; N, 10.18; O, 17.75.

(±)-*N,N*-Dipropyl [(*N′*-ethoxycarbonyl)piperidine]-2-carboxamide (±)-12b. A solution of acid (±)-**11b** (1.014 g, 5.04 mmol) chloroform (12 mL) and triethylamine (728 mL, 5.24 mmol) was cooled to −15 °C. Isobutyl chloroformate was added dropwise (654 mL, 5.04 mmol) and the reaction mixture stirred at −15 °C for 1 h. Dipropylamine (691 mL, 5.04 mmol) was then added dropwise and allowed to react with stirring at −15 °C for 3 h. After addition of $CHCl_3$ (20 mL), the organic layer was successively washed with brine (3×20 mL), ice-cooled citric acid (2×15 mL, 10% solution), $NaHCO_3$ (10 mL, saturated solution) and finally with brine (3×20 mL) and dried. Evaporation of the volatile compounds gave the title compound (±)-**12b** (1.34 g, 95%). R_f 0.5 ($CHCl_3$:MeOH, 97:3); IR (NaCl, cm^{-1}): 1690 (NCOO), 1644 (CON); ^1H NMR δ 5.01 (s, 0.90H, H-2), 4.90 (s, 0.10H, H-2), 4.13 (q, J = 7.0 Hz, 2H, CH_2CH_3), 3.96–3.90 (m, 1H, H-6), 3.54–3.42 (m, 2H), 3.25–2.98 (m, 3H), 1.89–1.43 (m, 10H), 1.25 (t, 3 H, J = 7.0 Hz, CH_2CH_3), 0.92 (t, J = 7.4 Hz, 3H, ($NCH_2CH_2CH_3$)), 0.88 (t, J = 7.4 Hz, 3H, ($NCH_2CH_2CH_3$)); ^3C NMR δ 172.3 (*C*ON), 156.7 (*C*OO), 61.5 (CH_2CH_3), 50.9 (C-2), 49.6 and 47.7 (NCH_2CH_2), 42.0 (C-6), 27.3 (C-3), 25.0 (C-5), 21.0 (C-4), 19.5 ($CH_2CH_2CH_3$), 14.7 (COO CH_2CH_3), 11.4 (CH_2CH_2 CH_3); MS (EI) *m/z*: 285 ($M+1^+$, 5), 284 (M^+, 7), 157 (62), 156 (100), 55 (32), 43 (49), 42 (21), 41 (52); HRMS calcd for $C_{15}H_{28}N_2O_3$ 284.20999; found: 284.19996.

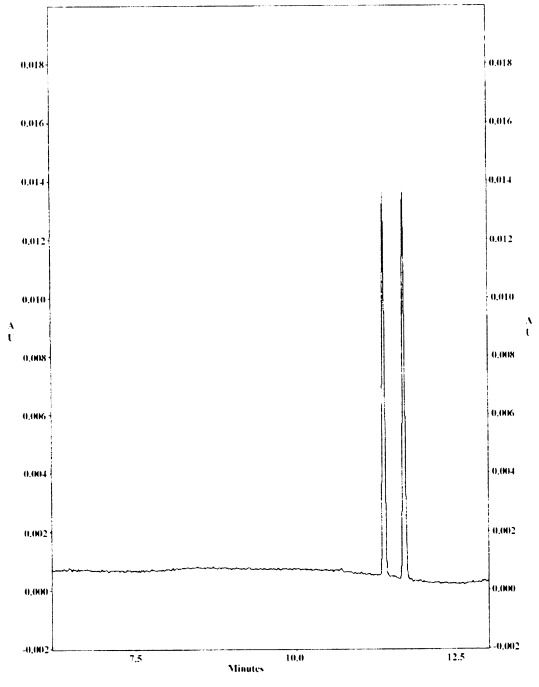
(*S*)-(−)-(*N,N*-Dipropyl)piperidine-2-carboxamide (*S*)-(−)-13. Iodotrimethylsilane (0.2 mL, 4.2 mmol) was added dropwise to a solution of the carbamate (*S*)-(−)-**12a** (0.115 g, 0.42 mmol) in $CHCl_3$ (15 mL) at room temperature. The solution was then heated to reflux for 16 h. Methanol (15 mL) was added and the reflux continued overnight. The solution was concentrated. The residue was diluted in CH_2Cl_2 (10 mL) and washed with HCl 3 N (3×10 mL). The aqueous layer was made basic with NaOH 2 N and extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried and concentrated. The residue was purified by column chromatography (eluent CH_2Cl_2:MeOH:C_6H_{12}:NH_4OH



γ-HSCD



β-HSCD



α-HSCD

Figure 1. Separation of AF-DX 384 enantiomers as a function of the HSCD introduced into the electrolyte. Conditions: applied voltage: −15 kV; anodic detection λ = 200 nm; electrolyte: triethylammonium phosphate 25×10^{-3} M/HSCD 5% (w/w), pH = 2.5; hydrodynamic injection 1 μL of an ethanolic solution of (±)AF-DX 384 (1.2 mg/mL).

(28%), 68:15: 15:2) to give the title compound (*S*)-(−)-**13** (0.0784 g, 88%). R_f 0.7 (CH_2Cl_2:MeOH:C_6H_{12}:NH_4OH, 68:15: 15:2); $[\alpha]_D^{22}$ −11° (*c* 4, $CHCl_3$), ee > 99% determined by HPLC of the amide (*S*)-(−)-**12a** formed by reaction of (*S*)-(−)-**13** with methyl chloroformate under the conditions described previously. IR (NaCl, cm^{-1}): 3502 (NH); 1642 (CON); ^{1}H NMR δ 3.47–3.43 (m, 1H, H-2), 3.33–3.25 (m, 1H), 3.16–3.06 (m, 4H, NCH_2CH_2), 2.62 (dt, *J* = 11.5 and 4.2 Hz, 1H, H-6), 2.39 (s, 1H, N*H*), 1.82–1.64 (m, 1H, H-4), 1.60–1.25 (m, 9H, $CH_2CH_2CH_3$, H-3, H-4, H-5), 0.89 (t, *J* = 7.3 Hz, 3H, CH_3), 0.77 (t, *J* = 7.3 Hz, 3H, CH_3); ^{13}C NMR δ 173.3 (*C*ON), 56.5 (C-2), 49.3 ($CONCH_2$), 47.6 ($CONCH_2$), 45.7 (C-6), 30.4 (C-3), 26.8 (C-5), 24.5 (C-4), 22.7 ($CONCH_2CH_2$), 20.9 ($CONCH_2CH_2$), 11.4 (CH_3); *m*/*z*: 213 ($M+1^+$, 0.38); 212 (M^+, 1.0); 84 (100); HRMS calcd for $C_{12}H_{24}N_2O$ 212.1883; found: 212.1889.

(*S*)-(+)-2-(*N*,*N*-Dipropylaminomethyl)piperidine (*S*)-(+)-(4). A mixture of $LiAlH_4$ (0.120 g, 3.10 mmol) in THF (1 mL) and amide (*S*)-(−)-**13** (0.326 g, 1.5 mmol) in THF (4 mL) was stirred at 70 °C for 2 h. After cooling to 0 °C, water (0.112 mL, 6.20 mmol), NaOH (15%, 0.112 mL) and water (0.340 mL) were successively added and the mixture stirred for 2 h. After filtration, the solution was concentrated. Bulb-to-bulb distillation of the residue gave the title compound (*S*)-(+)-(**4**) (0.238 g, 80%) $[\alpha]_D^{22}$ +74° (*c* 2.5, $CHCl_3$) (ee > 99% see theoretical part).

(*S*)-(−)-[2-(*N*′,*N*′-Dipropylaminomethyl)piperidin-1-yl]-acetonitrile (*S*)-(−)-(14). Alkylation of amine (*S*)-(+)-**4**

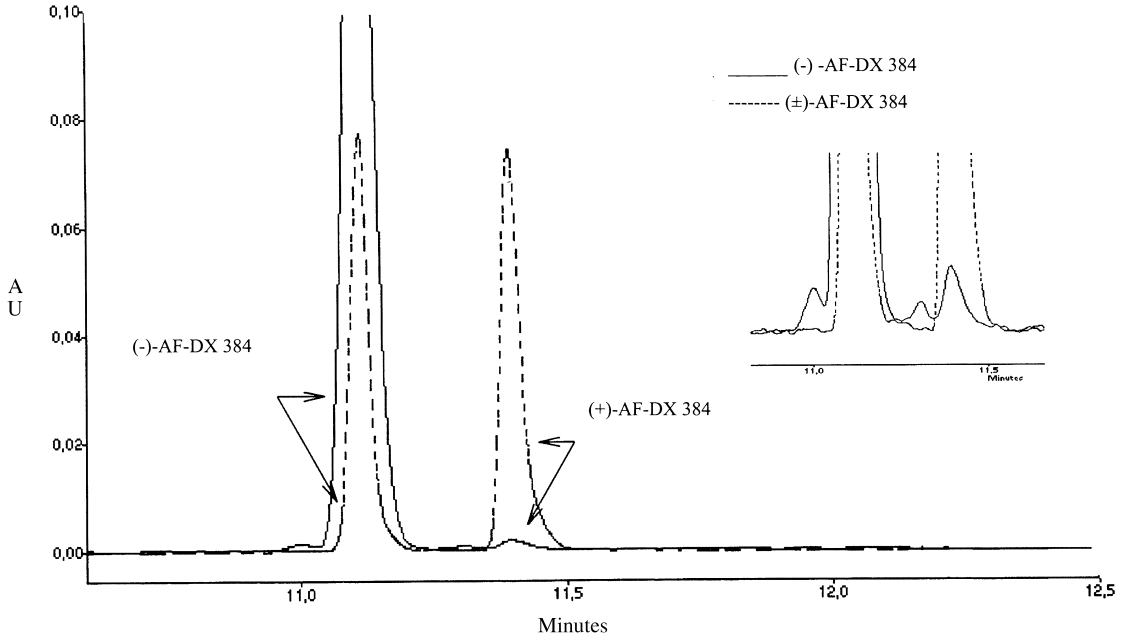


Figure 2. Example of (−) AF-DX 384 enantimoer identification. Conditions: identical to those reported in Figure 1.

with bromoacetonitrile was carried out according to a previously described method,[18] and led to (*S*)-(−)-**14**. $[\alpha]_D^{22}$ −19° (*c* 1.7, $CHCl_3$).

(*S*)-(−)-2-[-2-(*N′*,*N′*-Dipropylaminomethyl)piperidin-1-yl]-ethan-1-amine (*S*)-(−)-3. (*S*)-(−)-**14** (0.051 g, 0.22 mmol) in THF (0.6 mL) was added dropwise to $LiAH_4$ (0.017 g, 0.44 mmol) in THF (0.12 mL). The mixture was stirred at 70 °C for 2 h. After cooling, water (0.88 mmol, 0.016 mL), NaOH (15%, 0.016 mL) and water (0.048 mL) were successively added and the mixture stirred for 2 h. The solids were filtered and the solution concentrated. Bulb-to-bulb distillation of the residue gave the title compound (*S*)-(−)-**3** (0.038 g, 72%). $[\alpha]_D^{22}$ −61° (*c* 1, $CHCl_3$).

In Figure 1, we have shown the electropherograms in optimised conditions, for the (±) AF-DX 384 with each HSCD and in Figure 2, an example of (*S*)-(−)-AF-DX 384 enantiomer identification (retention times: (*S*)-(−)-AF-DX 384: 11.1 min; (*R*)-(+)-AF-DX 384: 11.4 min)

(*S*)-(+)-5,11-Dihydro-11-[2-[2-[(*N*,*N*-dipropylaminomethyl)piperidin-1-yl]-ethylamino]-carbonyl]-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (*S*)-(+)-1. (*S*)-(+)-**1** AF-DX 384 $[\alpha]_D^{22}$ +12° (*c* 1, $CHCl_3$) was obtained from (*S*)-(−)-**3** according to the procedure previously described.[18] Data from capillary electrophoresis: RT 11.4 min, ee 99%. The same reactions were used to prepare (*R*)-(−)-AF-DX 384 from (*R*)-(−)-**4**. Data from capillary electrophoresis: RT 11.1 min, ee 99%.

Biological evaluation. Muscarinic M_2 binding affinities (IC_{50}) of the synthesized compounds (*S*)-(+)-**1**, (R)-(−)-**1** and atropine (as reference) were determined on rat heart tissue under competition conditions. Membranes preparation was conducted on freshly dissected heart from male Sprague–Dawley rats,[4] through homogenisation in 10 volumes of 50 mM Tris–HCl buffer (pH 7.4 at +4 °C) with a Polytron (2×30 s, maximal setting) followed by ultracentrifugation (23,000 × *g*, 60 min, +4 °C). Aliquot fractions of proteins (30 mg) were incubated for 90 min at room temperature in the presence of 0.5 nM [^{3}H]N-methyl-scopolamine (NEN-DuPont, France; specific activity: 84 Ci/mmol) and 6–8 increasing concentrations of competitors in a final volume of 200 mL of 50 mM Tris–HCl (pH 7.4) buffer. Incubation was stopped by rapid filtration over Whatman GF/B glass fiber filters presoaked with 0.3% polyethylenimine using a Brandell Cell Harvester. Nonspecific binding was determined in the presence of 10^{-6} M atropine. Affinity values were calculated through the use of the EBDA/Ligand software and expressed as $pIC_{50} \pm SEM$.

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