Synthesis and Biological Evaluation of Chiral α-Aminoanilides with Central Antinociceptive Activity

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Tocainide and related optically active chiral α-aminoanilides were synthesized and tested *in vivo* via the hot plate test to evaluate their central analgesic action. The aims of the study were to verify if a**)** the increase in lipophilicity, obtained by the introduction of an alkyl group on the steric center (**3f**−**i**), and the replacement of the C═O group with the C═S (**10**) group as well as the introduction of a methyl or ethyl group on the amidic nitrogen atom (**8a**−**c**) would produce an increase in central analgesic efficacy with respect to Tocainide; b**)** the 2,6-xylidide moiety is crucial for high analgesic activity (**3b**−**e**); c**)** the hydrogen atom bonded to the amidic nitrogen moiety is an essential pharmacophoric element for analgesic activity. Among all the synthesized compounds, **3f** showed antinociceptive properties with a good enantioselective index.

Introduction

Traditional pain treatments (i.e., those based on the use of NSAIDs and opioid analgesics) have been known to exhibit several side effects, and this is why there has been a lot interest in the area of the so-called 'adjuvant analgesics', "drugs that have primary indications other than pain but may be analgesics in selected circumstances", in the past 20 years.[1] In this heterogeneous group of alternative drugs for palliative therapy, local anaesthetics (LAs) have been specifically targeted for the treatment of neuropathic pain, and the mechanism of their antinociceptive effect may be due to the blocking of afferent stimuli. However, the ability of these agents to stabilize membranes is not limited to the peripheral nerves;[2] in fact once they cross the blood−brain barrier (BBB), LAs exert marked effects on the central nervous system (CNS). Indeed several LA-like compounds have exerted various systemic effects both in laboratory models and humans.[3] For example, procaine is one of foremost LAs investigated for both its systemic effects and for its neuropsychopharmacological properties. However, it presents some disadvantages due its rapid metabolism and relatively high incidence of allergic phenomena. It because of these reasons that lidocaine (the amide-type substitute of procaine[4]) is currently the LA under greatest investigation as a systemic adjunct in the treatment of pain. Surprisingly, analgesic plasma lidocaine EC_{50}[5,6] values are about 100 times lower than those required to obtain a peripheral blockade of sodium channels (Na^+).[7] These findings suggest that the systemic antinociceptive effects of lidocaine may be ascribed to synergic mechanisms, implicating targets other than sodium channels.[8,9] In agreement with the above hypothesis, we have previously reported that lidocaine, when given systemically, produces significant antinociception in mice and rats through the activation of the cholinergic system via antagonism of the presynaptic muscarinic receptors.[10] It has recently been proposed that antagonism of the presynaptic muscarinic receptors contributes to the effects of procaine on the brain's superior functions.[11] Due to their poor oral bioavailability, lidocaine and procaine may not be considered as the best tools for the investigation of alternative systemic mechanisms of antinociception. Therefore, orally active analogues of lidocaine such as mexiletine (Mex) and tocainide (Toc) were used. In fact in our previous work[12] we demonstrated that (−)-*R*-Toc is the eutomer responsible for exerting analgesia. In particular, biological tests have shown that the antinociceptive effect of the (−)-*R* enantiomer, such as the analgesia induced by lidocaine, procaine, and Mex, is due to a highly stereoselective central presynaptic cholinergic mechanism of action.[13] Thus, in light of these results, the interpretation of Lindstrom and Lindblom[14,15] should be reconsidered. They hypothesized that analgesia induced by Toc might depend on the blocking of Na^+ channels of hyperexcited nerve membranes in the pain-producing foci. This hypothesis prompted us to synthesize further anilide derivatives, structurally related to Toc, with the aim of identifying the chemical features specifically related to the antinociceptive activity and to gain a better insight to the mechanism involved in their analgesic action. In particular, the goals of this study were to verify the following:

a**)** if the increase in lipophilicity, obtained by replacing the hydrogen atom, both on the steric center and on the amidic nitrogen atom, with alkyl groups, and by substituting the C═O group with a more lipophilic moiety, such as C═S, leads to an increase in central analgesic efficacy;

b**)** if the 2,6-xylididic moiety is essential to preserve analgesic activity;

c**)** if the hydrogen atom bonded to the amidic nitrogen is a pharmacophoric element for analgesic action.

Chemistry. The synthesis of compounds **3a**−**i** (Scheme 1) was carried out using, as starting materials, the commercially available amino acids alanine, valine, and isoleucine, in their optically active forms. These α-amino acids were converted into their *N*-Boc derivatives **1a**−**c** by a reaction of 2-*tert*-butoxycarbonylimino-2-phenylacetonitrile (Boc-ON)[16] and triethylamine (Et_3N) in a mixture of dioxane and water. The *N*-*tert*-butoxycarbonyl-α-amino acids were then condensed with suitably substituted aromatic anilines in the presence of EEDQ,

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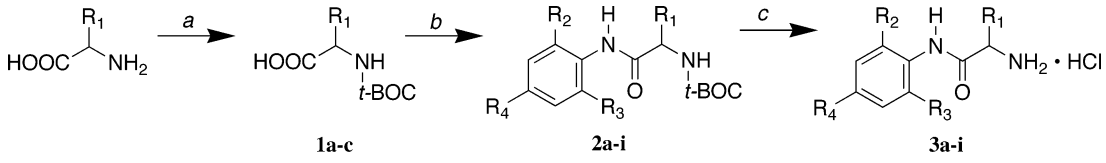
Scheme 1[a]



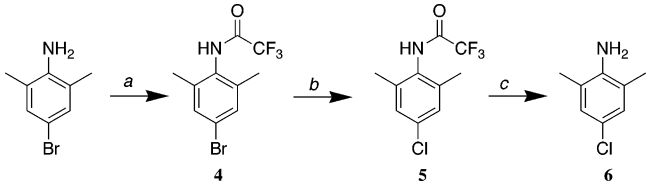
1a-c **2a-i** **3a-i**

1a: $R_1 = CH_3$
1b: $R_1 = CH(CH_3)_2$
1c: $R_1 = CH_2\text{-}CH(CH_3)_2$

	R_1	R_2	R_3	R_4
2a, 3a	CH_3	CH_3	CH_3	H
2b, 3b	CH_3	H	H	Cl
2c, 3c	CH_3	CH_3	CH_3	Cl
2d, 3d	CH_3	CH_3	H	H
2e, 3e	CH_3	CH_3	H	Cl
2f, 3f	$CH(CH_3)_2$	CH_3	CH_3	H
2g, 3g	$CH(CH_3)_2$	CH_3	H	Cl
2h, 3h	$CH_2\text{-}CH(CH_3)_2$	CH_3	CH_3	H
2i, 3i	$CH(CH_3)_2$	CH_3	CH_3	Cl

[a] Reagents and conditions: (a) Boc-ON, Et_3N, dioxane/H_2O, rt: (b) EEDQ or IIDQ, Et_3N, $CHCl_3$, anilines, Δ, 6 h; (c) HCl_g, anhyd Et_2O.

Scheme 2[a]



4 **5** **6**

[a] Reagents and conditions: (a) $(CF_3CO)_2O$, anhyd THF, N_2; (b) CuCl, anhyd DMSO, N_2; (c) HCl 17%/THF (1:1).

a peptide coupling reagent that avoids racemization,[17,18] producing the desired 2-(*tert*-butoxycarbonylamino)-*N*-arylalkanamides **2a**−**h**. Removal of the N-protecting group by gaseous HCl gave 2-amino-N^1-arylalkanamide hydrochlorides **3a**−**h**. The compound **2i**, N^2-(*tert*-butoxycarbonyl)-N^1-(4-chloro-2,6-dimethylphenyl)valinamide, was synthesized using IIDQ instead of EEDQ, as previous experimental situations proved that EEDQ did not give the desired product.

In spite of the higher reactivity[19] of IIDQ when compared to EEDQ, the yield of this reaction was lower than that recorded in the synthesis of previous compounds. The aromatic amine (4-chloro-2,6-dimethylaniline) used to prepare the compound **2i**, was synthesized *ex novo*. The synthesis of this substrate was carried out as reported in Scheme 2: commercially available 4-bromo-2,6-dimethylaniline was converted to *N*-(4-bromo-2,6-dimethylphenyl)-2,2,2-trifluoracetamide (**4**) by reacting it with trifluoroacetic anhydride in anhydrous THF under a nitrogen athmosphere. Then, **4** was converted to *N*-(4-chloro-2,6-dimethylphenyl)-2,2,2-trifluoroacetammide (**5**) with CuCl as source of chloride ions, in anhydrous DMSO.[20] Finally, removal of the trifluoroacetyl group to give **6** was carried out using an equivolumetric mixture of THF and HCl 17%, under reflux, for 17 h. *N*-Alkyl-N^1-aryl-2-(*tert*-butoxycarbonylamino)alkanamides (**7a**−**c**) (Scheme 3) were prepared by reacting **2a** and **2f** with CH_3I or C_2H_5I, as alkylant reagents, in DMF/H_2O in the presence of $Ba(OH)_2$. Even under basic conditions, no racemization was detected (as previously reported for the chiral compounds obtained under the same experimental conditions).[21]

The corresponding hydrochlorides **8a**,**b** were prepared by treatment of **7a**,**b** with gaseous HCl, by using anhydrous Et_2O as the solvent. **8c** instead was obtained as the tartrate salt by using L-tartaric acid. 2-*tert*-Butoxycarbonylamino-*N*-(2,6-dimethylphenyl)propanethioamide (**9**) was prepared, as reported in Scheme 4, by the reaction of **2a** with 2,4-bis-(*p*-pentyloxyphenyl)-1,2,3,4-dithiadiphosphethane 2,4-disulfide,[22] a sulfuration reagent used instead of LR (Lawesson's reagent) in the presence of less polar solvents. This reaction was carried out in benzene at 60 °C, under reflux, for 2 days. The corresponding hydrochloride (**10**) was prepared as described in the Experimental Section.

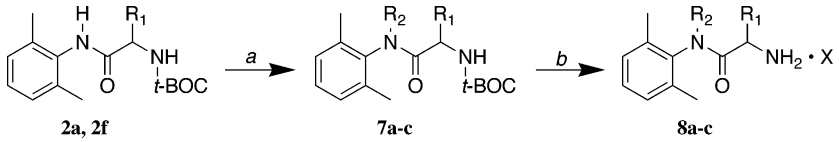
Pharmacological Results. The antinociceptive properties of Toc and its analogues were investigated using the mouse hot plate test. As shown in Table 1, the compounds **3a** (Toc), **3c**, **3e**, **3f**, **3g**, and **3h** showed antinociceptive properties. In particular, an increase in the licking latency values was selectively obtained with the *R* isomer of **3a**, **3f**, and **3g**. Conversely, no stereoselective antinociception was detected for compounds **3c**, **3e**, and **3h**. The increase in the pain threshold produced by (−)-*R*-Toc and (−)-*R*-**3f** was prevented by pretreatment with the muscarinic antagonist atropine, and the acetylcholine depletor Hemicolinium-3 (HC-3) administered 15 min and 1 h respectively, before the test (Table 2). By contrast, the antinociception induced by (*R*)-**3c**, (*S*)-**3c**, (*R*)-**3e**, (*S*)-**3e**, (*R*)-**3g**, (*R*)-**3h**, and (*S*)-**3h** was not visibly modified by atropine pretreatment. None of the other investigated compounds showed antinociceptive effect. At the highest effective doses, all antinociceptive compounds did not modify the animals' behavior. Furthermore, the motor coordination of animals treated with the above-mentioned compounds was evaluated using the rota-rod test. As reported in Table 3, **3a** (Toc), **3c**, **3e**, **3f**, **3g**, and **3h** did not produce any impairment of motor coordination in comparison to the saline-treated group. The number of falls by control animals progressively decreased at every measurement since the mice learned how to balance on the rotating rod.

Discussion

In the following discussion one must remember that in vivo tests, such as the hot plate and rota-rod tests, while obviously speeding up the selection of the compounds with the most promising clinical properties, do not always allow sound structure−activity relationships to be established. In fact, the resulting activity is the consequence of both pharmacokinetic and pharmacodynamic properties that may be differently affected by structural modifications.

All the synthesized compounds can be considered as chiral analogues of Toc, an antiarrhythmic drug of the IB[23] class once used in treatment of symptomatic life-threatening ventricular arrhythmias.[24] Furthermore, Toc is effective as an analgesic in the treatment of trigeminal neuralgia in humans, as well as the nociceptive effect in rats.[25] It is noteworthy that many chiral

Scheme 3[a]

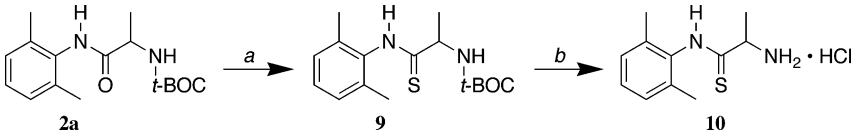


2a: $R_1 = CH_3$
2f: $R_1 = CH(CH_3)_2$

	R_1	R_2	X
7a, 8a	CH_3	CH_3	HCl
7b, 8b	CH_3	CH_2-CH_3	HCl
7c, 8c	$CH(CH_3)_2$	CH_3	$C_4H_6O_6$

[a] Reagents and conditions: (a) DMF/H_2O, $BaOH_2$, CH_3I or CH_3CH_2I, rt; (b) HCl_g, anhyd Et_2O, or L-tartaric acid, EtOH.

Scheme 4[a]



[a] Reagents and conditions: (a) 2,4-Bis(*p*-pentyloxyphenyl)-1,2,3,4-dithiadiphosphethane-2,4-disulfide, benzene, 70 °C; (b) HCl_g, anhyd Et_2O.

Table 1. Effect of Toc and Toc Analogues in the Mouse Hot-Plate Test (52.5 °C)

		licking latency (s)	
compd	treatment (mg/kg s.c.)	before treatment	after treatment
saline		14.3 ± 0.4	15.1 ± 1.2
(*R*)-**3a** Toc	(50)	13.8 ± 1.1	26.5 ± 1.7**
(*S*)-**3a** Toc	(50)	15.4 ± 1.0	14.3 ± 1.5
(*R*)-**3b**	(5)	14.3 ± 0.6	17.3 ± 2.1
(*S*)-**3b**	(5)	13.9 ± 0.8	15.8 ± 1.9
(*R*)-**3c**	(30)	14.1 ± 1.0	23.2 ± 1.8**
(*S*)-**3c**	(30)	13.8 ± 0.9	25.1 ± 2.0**
(*R*)-**3d**	(10)	14.0 ± 1.3	17.1 ± 2.1
(*S*)-**3d**	(15)	13.2 ± 1.2	16.2 ± 2.0
(*R*)-**3e**	(50)	14.9 ± 0.7	25.5 ± 2.0**
(*S*)-**3e**	(50)	14.5 ± 1.0	21.1 ± 1.3**
(*R*)-**3f**	(10)	13.8 ± 0.5	30.3 ± 1.7**
(*S*)-**3f**	(10)	15.6 ± 1.3	15.8 ± 0.6
(*R*)-**3g**	(30)	15.6 ± 1.4	27.9 ± 2.1**
(*S*)-**3g**	(40)	13.8 ± 0.6	19.9 ± 2.0*
(*R*)-**3h**	(10)	16.2 ± 1.0	20.7 ± 1.3*
(*S*)-**3h**	(10)	15.3 ± 1.4	21.3 ± 1.7*
(*R*)-**3i**	(50)	14.4 ± 1.2	17.0 ± 1.4
(*S*)-**3i**	(50)	14.0 ± 0.8	17.2 ± 1.7
(*R*)-**8a**	(10)	14.7 ± 1.0	18.2 ± 2.5
(*S*)-**8a**	(10)	13.9 ± 1.1	16.7 ± 1.9
(*R*)-**8b**	(5)	15.8 ± 0.9	16.0 ± 1.8
(*S*)-**8b**	(5)	13.2 ± 0.8	15.1 ± 1.3
(*R*)-**8c**	(20)	13.5 ± 1.3	13.6 ± 2.1
(*S*)-**8c**	(10)	13.5 ± 0.9	13.6 ± 1.7
(*R*)-**10**	(50)	14.0 ± 0.8	18.8 ± 2.4
(*S*)-**10**	(50)	14.3 ± 0.8	18.6 ± 1.9

[a] The licking latency values were recorded in correspondence with the maximum antinociceptive effect of each compound. *P < 0.05, **P < 0.01 in comparison with saline−saline treated mice.

local anaesthetic drugs are used in clinical therapy in their racemic form. In the last 20 years it was proved, that in all cases, the enantiomers of these drugs had a different pharmacological and pharmacokinetic profile;[26] in particular, the introduction of Mex and Toc in clinical therapy led chemists to review previous studies on each of the single enantiomers of local anaesthetic drugs. It was then demonstrated that the enantiomers of Mex, for example, showed both a different pharmacokinetic profile[27] as well as a diverse inhibitory effect for the clorgiline resistant monoamino oxidase (CRAO).[28] The *R* isomer of Toc showed a higher antiarrhythmic activity than that of the *S* isomer.[29,30] In light of these results, we recently reported the synthesis of a new series of rigid[21,31] or sterically hindered compounds, in order to evaluate potential central analgesic action.[32] Some compounds showed high stereoselective central analgesic activity,[12] probably due to the activation of the central cholinergic system. In this experimental protocol (−)-*R*-Toc was found to be more active than the (+)-*S*-enantiomer. These findings prompted us to plan a new series of chiral-α-aminoanilides, structurally related to Toc with the aim of evaluating the effect of some modified structural moieties (aromatic group, alkylic chain, amidic moiety) on analgesic activity.

Therefore, we synthesized a first series of compounds, (**3a**−**e**) in which we introduced substituents with different electronic properties on the aromatic ring. As reported in Table 1, among all the alanino-anilides, only compounds **3a**, **3c**, and **3e** showed analgesic activity; in the same series only compound **3a** gave rise to a stereoselective analgesic effect, probably due to the xylididic moiety. By these preliminary results we deduced that the 2,6-xylididic moiety was important in central analgesic action. To verify the influence of steric hindrance on the stereogenic center, the compounds **3f**−**i** were also synthesized and tested. First we substituted the methyl group with an isopropyl moiety on the steric center (**3f**, **3g**, **3i**). As reported in Table 1, all compounds, with the exception of **3i**, exhibited antinociceptive properties. In particular **3f** is the most active of the series and shows both potency and stereoselectivity higher than tocainide itself.[33,34] To confirm the influence of the alkylic chain on the chiral carbon atom, we synthesized compound **3h**, that possesses the isobutyl chain. Biological results disclose that the increment of the steric hindrance on this position, such as in **3h**, causes analgesic activity even if it is lower than that caused by **3f**.

To complete our investigation, we synthesized and tested compounds **8a** and **8b**, that possess an alkylic group (methyl and ethyl) on the amidic moiety. Furthermore, we obtained **8c** by performing the same substitution on **3f** which, as mentioned above, is the most active among all the tested compounds. The molecules so obtained (**8a**, **8b** and **8c**) did not show any central analgesic action. To clarify if this loss of activity is dependent on the increment of lipophilicity, due to an insertion of an alkylic moiety on the nitrogen amidic atom, or due to the loss of a hydrogen bond on the receptor site, we synthesized compound

Table 2. Effect of Toc and Active Toc Analogues in the Mouse Hot-Plate Test (52.5 °C)[a]

		licking latency (s)			
			after treatment		
compd	treatment (mg/kg s.c.)	before treatment	without pretreatment	pretreatment with atropine	pretreatment with HC-3
saline		14.3 ± 0.4	15.1 ± 1.2	14.8 ± 1.6	17.5 ± 0.9
(*R*)-**3a** Toc	(50)	13.8 ± 1.1	26.5 ± 1.7**	17.5 ± 1.5	18.6 ± 1.3
(*R*)-**3c**	(30)	14.1 ± 1.0	23.2 ± 1.8**	23.7 ± 1.8**	
(*S*)-**3c**	(30)	13.8 ± 0.9	25.1 ± 2.0**	26.6 ± 2.4**	
(*R*)-**3e**	(50)	14.9 ± 0.7	25.5 ± 2.0**	25.0 ± 2.7**	
(*S*)-**3e**	(50)	14.5 ± 1.0	21.1 ± 1.3**	20.5 ± 2.1**	
(*R*)-**3f**	(10)	13.8 ± 0.5	30.3 ± 1.7**	16.9 ± 2.0**	17.5 ± 2.2
(*R*)-**3g**	(30)	15.6 ± 1.4	27.9 ± 2.1**	26.4 ± 2.0**	
(*R*)-**3h**	(10)	16.2 ± 1.0	20.7 ± 1.3*	23.9 ± 1.8**	
(*S*)-**3h**	(10)	15.3 ± 1.4	21.3 ± 1.7*	20.7 ± 1.7**	

[a] The licking latency values were recorded in correspondence with the maximum antinociceptive effect of each compound. Atropine and hemicolinium-3 (HC-3) at the Dose of 5 mg/kg i.p. and 1 μg per mouse icv, respectively, were administered *$P < 0.05$, **$P < 0.01$ in comparison with saline−saline treated mice.

Table 3. Lack of Effect of Some Compounds on Motor Coordination Expressed as Falls from the Rota-rod[a]

		falls from the rota-rod			
			after treatment		
treatment	dose, mg kg^{-1}	before treatment	15 min	30 min	45 min
saline		2.4 ± 0.4	1.6 ± 0.3	1.1 ± 0.3	0.9 ± 0.2
(*R*)-**3a**	(50)	2.9 ± 0.5	1.7 ± 0.4	1.4 ± 0.2	0.9 ± 0.3
(*S*)-**3c**	(30)	2.7 ± 0.4	1.4 ± 0.3	1.2 ± 0.4	1.0 ± 0.2
(*R*)-**3e**	(50)	2.8 ± 0.4	1.5 ± 0.3	1.1 ± 0.3	0.8 ± 0.3
(*R*)-**3f**	(10)	3.1 ± 0.5	2.3 ± 0.4	1.2 ± 0.2	0.9 ± 0.3
(*R*)-**3g**	(30)	2.6 ± 0.4	1.5 ± 0.5	1.3 ± 0.4	0.8 ± 0.3
(*R*)-**3h**	(10)	2.5 ± 0.6	2.2 ± 0.5	1.5 ± 0.5	0.8 ± 0.3

[a] Each value represents the mean of 6−8 mice. *$P < 0.01$ in comparison with CMC-treated controls.

10. In this compound the increase in lipophilicity was realized by replacing the C═O with the C═S group. Here, also we recorded no antinociceptive action. These results suggest that the amidic moiety (NH−CO) is crucial in preserving analgesic activity. In conclusion among all tested compounds only **3a**, **3c**, **3e**, **3f**, **3g**, and **3h** showed analgesic behavior, with ED_{50} values between 5.7−39.2 mg kg/s.c. (Figure 1). So as to clarify the mechanism of action of these compounds, we administered the muscarinic antagonist atropine to antagonize their analgesic action. The administration of atropine was able to oppose this effect only in the case of compounds (−)-*R*-**3a** and (−)-*R*-**3f**. We can therefore suggest that the antinociception, induced by these two compounds, is of the cholinergic type. Furthermore, these compounds produced analgesia without altering their performance in the rota-rod test. We can therefore suppose that the observed increase in the pain threshold corresponds to an actual analgesic effect. This is because the dose of atropine employed in the present study was able to selectively prevent muscarinic antinociception without modifying the analgesia induced by the modulation of other neurotransmitters, such as opioid and GABA.[10,35] The most likely site of analgesic action is in the central nervous system, since the increase in the pain threshold induced by (−)-*R*-**3a** and (−)-*R*-**3f**, is antagonized by the acethylcholine depletor HC-3, that was administered directly into the cerebral ventricles. Furthermore, the prevention of (−)-*R*-**3a** and (−)-*R*-**3f** antinociception, exerted by HC-3, suggests that this effect is due to a presynaptic mechanism of action. This is since a depletion of cerebral acethylcholine is obtained after HC-3 administration. Concerning the antinociceptive effect produced by **3c**, **3e**, **3g**, and **3h**, no mechanism of action has so far been explained. Nevertheless, a cholinergic type of analgesia may be ruled out, since the increase in pain threshold induced by these compounds was not prevented by atropine.

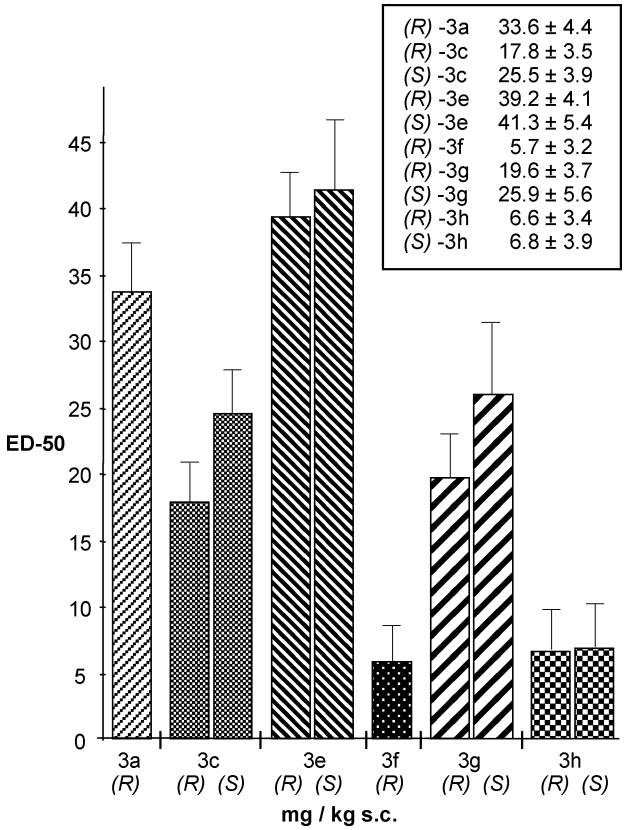


Figure 1. ED_{50} values of molecules possessing an antinociceptive activity.

As reported in this paper, preliminary exploration of the mechanism of action of the compounds shows that they present affinity for the muscarinic receptor. Among the studied com-

Table 4. Dose−Response Curve of (*R*)-**3f** on Mouse Hot-Plate Test[a]

		licking latency in mice (s)				
			after treatment			
treatment	dose, mg kg^{-1}	before treatment	15 min	30 min	45 min	60 min
saline		14.4 ± 1.1	15.6 ± 1.3	14.9 ± 1.3	15.9 ± 1.8	16.0 ± 1.7
(*R*)-**3f**	(1)	13.8 ± 1.2	16.5 ± 1.6	17.2 ± 2.0	15.3 ± 1.7	14.5 ± 1.6
(*R*)-**3f**	(5)	15.1 ± 1.4	23.7 ± 1.5*	20.6 ± 2.3*	17.1 ± 2.0	14.9 ± 1.5
(*R*)-**3f**	(10)	14.7 ± 0.9	30.3 ± 1.7*	23.6 ± 1.7*	18.5 ± 1.2	15.1 ± 1.3

[a] $P < 0.01$ in comparison with saline controls. Each value represents the mean of at least 10 mice.

pounds, **3f** shows outstanding potency, being active at a dose of 10 mg/kg via subcutaneous (s.c.) (Table 4). In addition, analgesia induced by this compound, prevented by HC-3 treatment administered at the dose of 1 μg via intracerebroventricular (i.c.v.), seems to depend on the blocking of the central presynaptic muscarinic autoreceptors that are physiologically involved, via a feedback mechanism, in the inhibition of acethylcholine release. Starting with **3f** as the lead compound, design and synthesis of new analogues are in progress so as to collect more data on the molecular mechanism of action as well as on the effect other chemical structural modifications have on the antinociceptive action.

Conclusions

In conclusion, the results obtained by screening the compounds studied *via* the hot plate and rota-rod tests suggests the following:

(1) The increase in lipophilicity obtained via the methylation of the nitrogens and the substitution of the C═O group with its isologous C═S did not result in an augmentation of the analgesic effect. In fact compounds **8**−**10** did not exhibit any central antinociceptive action in the hot-plate test.

(2) The 2,6-xylididic moiety is essential for analgesic activity; in fact derivative **3b** that does not possess a xylididic ring did not present any analgesic effect, as present in compound **3c** but not in compound **3i**. It is important to underline that the coexistence of the Cl atom in the para position and the methyl group in the ortho position, as in **3e**, restores the analgesic effect, although it results in the loss of stereoselectivity.

(3) An increase in steric hindrance at the chiral center results in the formation of the most active compound of the series (**3f**), but further lengthening of the alkyl chain, as in **3h**, causes a drastic reduction in activity.

(4) Furthermore, one may observe that compounds **8a**−**c** do not exhibit any analgesic activity although steric hindrance at the chiral center is absent and they possess the 2,6-xylididic moiety. In light of this evidence, one may conclude that the presence of an intramolecular hydrogen bond between the amidic and aminic functions is crucial in allowing the molecule to assume the ideal conformation for receptor binding.

(5) Finally, the biological results suggest that an increase in the pain threshold is related to the stimulation of the central cholinergic system without any side effects. In particular, compound **3f** could be a starting point for the discovery of new compounds potentially useful in analgesia and in the treatment of age-related degenerative syndromes of the CNS.

Experimental Section

Pharmacology. Pharmacological Methods. Male Swiss albino mice (23−30 g) from Morini (San Polo d' Enza, Italy) breeding farm were used. Fifteen mice were housed per cage. The cages were placed in the experimental room 24 h before the acclimatization test. The animals were kept at 23 ± 1 °C with a 12 h light/dark cycle, light at 7 a.m., with food and water *ad libitum*. All experiments were carried out according to the guidelines of the European Community Council.

Hot Plate Test. The method adopted was described by O'Callaghan and Holzman.[36] Mice were placed inside a stainless steel container, thermostatically set at 52.5 ± 0.1 °C in a precision water-bath from KW Mechanical Workshop, Siena, Italy. Reaction times (s) were measured with a stop-watch before and at regular intervals up to a maximum of 60 min after treatment. The endpoint used was the licking of the fore or hind paws. Those mice scoring below 12 and over 18 s in the pretest were rejected (30%). An arbitrary cutoff time of 45 s was adopted.

The computer program ALLFIT[37] was used for the analysis of the sigmoidal dose−response table (Table 4) obtained in functional studies; the program uses the constrained four-parameter logistic model to obtain estimates of half-maximal effective concentration (EC_{50}) values (Figure 1). All the quoted values are mean ± SEM.

Rota-rod Test. The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with a nonslippery surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into five equal sections by six disks. Thus, up to five mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 rpm. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s according to Vaught.[38] The performance time was measured before and 15, 30, and 45 min after treatment.

Drugs. The following drugs were used: atropine sulfate (Sigma), hemicholinium-3 hydrobromide (HC-3) (R.B.I.). Other chemicals were of the highest quality commercially available. All drugs were dissolved in isotonic (0.9% NaCl) saline solution. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 mL kg $^{-1}$ by s.c. route or 5 μL by i.c.v. route. I.c.v. administration was performed under ether anaesthesia using isotonic saline as the solvent, according to the method described by Haley and McCormick.[39] Briefly, during anaesthesia, mice were grasped firmly by the loose skin behind the head. A 0.4 mm external diameter, hypodermic needle attached to a 10 μL syringe was inserted perpendicularly through the skull at a depth of no more than 2 mm into the brain of the mouse where 5 μL were then administered. The injection site was 1.5 mm from either side of the midline of a line drawn through to the anterior base of the ears. To ascertain that the drugs were administered exactly into the cerebral ventricle, some mice were i.c.v. injected with 5 μL of diluted 1:10 India ink and their brains examined macroscopically after sectioning.

Chemistry. General. Melting points were determined on a Gallenkamp melting point apparatus in open glass capillary tubes. The infrared spectra were recorded on a Perkin-Elmer Spectrum One FT spectrophotometer, and band positions were given in reciprocal centimeters (cm^{-1}). ^{1}H NMR routine spectra were recorded on a Varian XL 390 spectrometer (90 MHz) using $CDCl_3$ as the solvent, unless otherwise indicated, and TMS as the internal reference. ^{1}H 300-MHz and ^{13}C 75-MHz spectra were recorded on a Varian Mercury 300 spectrometer. Chemical shifts were reported in ppm relative to solvent resonance: $CDCl_3$, δ 7.26 (^{1}H NMR) and δ 77.3 (^{13}C NMR); $(CD_3)_2SO$, δ 2.50 (^{1}H NMR); CD_3OD, δ 3.31 (^{1}H NMR) and δ 47.8 (^{13}C NMR), unless otherwise indicated. Amino proton assignments were confirmed by D_2O exchange. *J* values are given in hertz. EIMS spectra were recorded with a Hewlett-Packard 6890−5973 MSD gas chromatograph/mass spec-

trometer at low resolution; where amine salts are concerned, MS analyses were performed on the corresponding free base forms obtained by extraction. Elemental analyses were performed on a Eurovector Euro EA 3000 elemental analyzer; the data for C, H, and N were within ±0.4 of the theoretical values for all final compounds except (+)-(*S*)-**3d**, (−)-(*R*)-**3h**, (+)-(*S*)-**3h**, (+)-(*S*)-**8b**. Optical rotations were measured on a Perkin-Elmer Mod 341 spectropolarimeter; concentrations were expressed in g/100 mL and the cell length was 1 dm. Thus, $[\alpha]^{20}_D$ values were given in units of 10^{-1} deg cm^2 g^{-1}. Silica gel chromatographic separations were performed by flash chromatography with silica gel (Kieselgel 60, 0.040−0.063 mm, Merk) packed in glass columns, using the technique described by Still et al.[40] The weight of the silica gel was approximately 100 times that of the substance, unless noted otherwise. The eluting solvent indicated in parentheses for each purification was determined by TLC, that was performed on precoated silica gel on aluminum sheets (kieselgel 60, F_{254}, Merck). TLC plates were visualized with UV light and/or in an iodine chamber.

Chemicals and Reagents. Boc-ON reagent [2-(*tert*-butoxycarbonylimino)-2-phenylacetonitrile], the enantiomers of alanine, isoleucine, and valine, 2,6-dimethylaniline, 4-bromo-2,6-dimethylaniline, 2-methylaniline, 4-chloroaniline, 4-chloro-2-methylaniline, EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline), and IIDQ (2-isobuthoxy-1-isobuthoxycarbonyl-1,2-dihydroquinoline) were purchased from Aldrich Chemical Co.; 4-bromo-2,6-dimethylaniline was purchased from Lancaster Synthesis, Inc. in the highest commercially available quality. Yields refer to purified products. The structures of the compounds were confirmed by routine spectrometric analyses. Only spectra for compounds that, to our knowledge, never have been previously described, are given. Even though *N*-*tert*-butoxycarbonylamino acids are commercialy available, compounds **1a**−**c** were obtained from the corresponding amino acids via the N-protection reaction using Boc-ON reagent as reported above.[12,21] (+)-(*R*) and (−)-(*S*)-**2a**, (+)-(*R*) and (−)-(*S*)-**2e**, (−)-(*R*) and (+)-(*S*)-**3a**, and (−)-(*R*) and (+)-(*S*)-**3e** were prepared as previously described.[12] Solvents were RP grade, unless otherwise indicated.

The procedures used to prepare the compounds shown in Schemes **1**−**4** are as follows.

General Procedure for the Synthesis of 2-(*tert*-Butoxycarbonylamino)-*N*-arylalkanamides (2a−i). The preparation of (+)-(*R*)-N^2-(*tert*-butoxycarbonyl)-N^1-(2,6-dimethylphenyl)valinamide [(+)-(*R*)-**2f**] can be taken as the reference procedure for the synthesis of 2-(*tert*-butoxycarbonylamino)-*N*-arylalkanamides (**2a**−**i**).

A solution of (+)-(*R*)-*N*-(*tert*-butoxycarbonyl)valine [(+)-(*R*)-**1f**, 0.22 g, 10 mmol], 2,6-dimethylaniline (0.13 g, 1.1 mmol), freshly recrystallized, EEDQ (0.30 g, 1.2 mmol), and triethylamine (0.15 g, 1.5 mmol) in 50 mL of $CHCl_3$ was refluxed for 6 h. The solvent was evaporated, and the residue, dissolved in EtOAc, was extracted with a 10% HCl solution. The organic layer was then washed with 0.1 M NaOH and H_2O, dried over anhydrous Na_2SO_4, and then evaporated under reduced pressure to give a crude product, that was purified by recrystallization, producing (+)-(*R*)-**2f** as a white solid (64 mg, 20% yield): mp 203−204 °C; $[\alpha]^{20}_D$ = +56 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$): 3678, 3415 (NH), 1692 (CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (d, *J* = 6.9 Hz, 3H, C*H*$_3$CHCH$_3$), 1.07 (d, *J* = 6.9 Hz, 3H, CH$_3$CHC*H*$_3$), 1.45 (s, 9H, CH$_3$C), 2.19 (s, 6H, CH$_3$Ar), 2.24−2.36 (m, 1H, CH$_3$C*H*CH$_3$), 4.06 (apparent t, *J* = 7.8 Hz, 1H, C*H*NH), 5.15 (br s, 1H; CHN*H*), 7.02−7.11 (m, 3H, ArH), 7.50 (br s, 1H; N*H*Ar); MS (70 eV): *m*/*z* (%) 320 (M$^+$, 6), 57 (100).

(+)-(*R*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(4-chlorophenyl)alaninamide [(+)-(*R*)-2b]: 72% yield, mp hygroscopic (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = +44 (*c* 1.3, MeOH); IR (KBr): 3300 (NH); 1664 (CO) cm^{-1}; ^{1}H NMR is reported in the literature[41] (MS (70 eV): *m*/*z* (%) 298 (M$^+$, 17), 44 (100).

(−)-(*S*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(4-chlorophenyl)alaninamide [(−)-(*S*)-2b]: 77% yield, mp 139−140 °C (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = −44 (*c* 1.3, MeOH).

(+)-(*R*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(4-chloro-2,6-dimethylphenyl)alaninamide [(+)-(*R*)-2c]: 20% yield, mp 188−189 °C (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = +60 (*c* 1, $CHCl_3$); IR ($CHCl_3$): 3430 (NH); 1720 (NHCOO), 1694 (NHCO) cm^{-1}; ^{1}H NMR (90 MHz) δ 1.40 (d, overlapping s at 1.43, *J* = 9 Hz, 3H, C*H*$_3$CH), 1.43 (s overlapping d at 1.40, 9H, C*H*$_3$C), 2.03 (s, 3H, CH$_3$Ar), 2.1 (s, 3H, CH$_3$Ar), 4.4 (apparent quintet, *J* = 8 Hz, 1H, C*H*NH), 5.5 (br d, *J* = 8 Hz, 1H; CHN*H*), 6.8−7.2 (m, 2H, Ar), 8.2 (br s, 1H; N*H*); MS (70 eV): *m*/*z* (%) 326 (M$^+$, 6), 44 (100).

(−)-(*S*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(4-chloro-2,6-dimethylphenyl)alaninamide [(−)-(*S*)-2c]: 30% yield, mp 187−188 °C (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = −57 (*c* 1, $CHCl_3$).

(+)-(*R*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(2-methylphenyl)alaninamide [(+)-(*R*)-2d]: 50% yield, mp 97−99 °C (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = +53 (*c* 1.2, MeOH); IR ($CHCl_3$): 3439 (NH), 1700 (CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (d, overlapping s at 1.46, *J* = 6.4 Hz, 3H, C*H*$_3$CH), 1.46 (s, 9H, C*H*$_3$C), 2.26 (s, 3H, CH$_3$Ar), 2.1 (s, 3H, CH$_3$Ar), 4.33 (br, apparent quintet, *J* = 7.1 Hz, 1H, C*H*CH$_3$), 4.9 (br s, 1H, CHN*H*), 7.02−7.10 (m, 1H, Ar HC-4), 7.14−7.24 (m, 2H, Ar HC-3,5), 7.90 (d, *J* = 7.7 Hz, 1H, Ar HC-6), 8.13 (bs, 1H, ArNH); MS (70 eV): *m*/*z* (%): 278 (M$^+$, 16), 44 (100).

(−)-(*S*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(2-methylphenyl)alaninamide [(−)-(*S*)-2d]: 82% yield, mp 97−99 °C (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = −54 (*c* 1.2, MeOH).

(+)-(*R*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(4-chloro-2-methylphenyl)alaninamide [(+)-(*R*)-2e]: 20% yield, mp 132−134 °C (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = +89 (*c* 0.6, CH_2Cl_2); IR ($CHCl_3$): 3440 (NH), 1700 (CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (d, *J* = 8.9 Hz, 3H, C*H*$_3$CH), 1.46 (s, 9H, C*H*$_3$C), 2.22 (s, 3H, CH$_3$Ar), 4.31 (br, apparent quintet, *J* = 7.0 Hz, 1H, C*H*CH$_3$), 4.97 (br d, 1H, CHN*H*), 7.14 (br s overlapping m at, 7.12−7.18, 1H, Ar HC-3), 7.12−7.18 (m overlapping s at 7.14, *J* = 6.7 Hz, 1H, Ar HC-5), 7.86 (d, *J* = 9.2 Hz, 1H, Ar HC-6), 8.24 (bs, 1H, ArNH).

(−)-(*S*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(4-chloro-2-methylphenyl)alaninamide [(−)-(*S*)-2e]: 30% yield, mp 133−135 °C (benzene/hexane); $[\alpha]^{20}_D$ = −86 (*c* 2.0, CH_2Cl_2).

(−)-(*S*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(4-chloro-2-methylphenyl)alaninamide [(−)-(*S*)-2f]: 40% yield, mp 200−202 °C (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = −63 (*c* 1.0, $CHCl_3$).

(+)-(*R*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(4-chloro-2-methylphenyl)valinamide [(+)-(*R*)-2g]: 35% yield, mp 142−144 °C (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = +55 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$): 3436 (NH), 1694 (CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (d, *J* = 6.9 Hz, 3H, C*H*$_3$CHCH$_3$), 1.04 (d, *J* = 6.9 Hz, 3H, CH$_3$CHC*H*$_3$), 1.45 (s, 9H, CH$_3$C), 2.22 (s overlapping apparent octet at 2.31, 3H, CH$_3$Ar), 2.31 (apparent octet overlapping s at 2.22, *J* = 6.8 Hz, 1H, CH$_3$C*H*CH$_3$), 4.00 (dd, *J* = 8.2, 6.6 Hz, 1H, CHN*H*), 5.05 (br s, 1H, CHN*H*), 7.1−7.2 (m, 2H, Ar HC-3,5), 7.76 (d overlapping br s at 7.81, *J* = 9.2 Hz, 1H, Ar HC-6), 7.8 (br s, 1H, ArNH); MS (70 eV): *m*/*z* (%): 340 (M$^+$, 13), 57 (100).

(−)-(*S*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(4-chloro-2-methylphenyl)valinamide [(−)-(*S*)-2g]: 50% yield, mp 142−144 °C (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = −57 (*c* 3.0, MeOH).

(+)-(*R*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(2,6-dimethylphenyl)isoleucinamide [(+)-(*R*)-2h]: 40% yield, mp 193−195 °C (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = +69 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (d overlapping d at 1.00, *J* = 6.66 Hz, 3H, C*H*$_3$CHCH$_3$), 1.00 (d overlapping d at 0.97, *J* = 6.9 Hz, 3H, CH$_3$CHC*H*$_3$), 1.46 (s, 9H, CH$_3$C), 1.52−1.62 (m, 1H, CH$_3$C*H*CH$_3$), 1.68−1.88 (m, 2H, C*H*$_2$CH), 4.25−4.35 (m, 1H, C*H*NH), 6.00 (br d, *J* = 6.2 Hz, N*H*CH), 7.00−7.15 (m, 3H, Ar), 7.60 (br s, 1H, NHAr), 7.8 (br s, 1H, ArNH); MS (70 eV): *m*/*z* (%) 334 (M$^+$, 1); 86 (100).

(−)-(*S*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(2,6-dimethylphenyl)isoleucinamide [(−)-(*S*)-2h]: 50% yield, mp 193−195 °C (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = −45 (*c* 1.0, $CHCl_3$).

(+)-(*R*)-N^2-(*tert*-Butoxycarbonyl)-N^1-(4-chloro-2,6-dimethylphenyl)valinamide [(+)-(*R*)-2i]: IIDQ was used as the condensing agent; 20% yield, mp 171−173 °C (EtOAc/petroleum ether);

$[\alpha]^{20}_D$ = +68 (*c* 1, $CHCl_3$); IR ($CHCl_3$): 3436 (NH), 1723 (NHCOO), 1694 (NHCO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.0 (d overlapping d at 1.05, *J* = 6 Hz, 3H, C*H*$_3$CHCH$_3$), 1.05 (d overlapping d at 1.0, *J* = 6 Hz, 3H, CH$_3$CHC*H*$_3$), 1.4 (s, 9H, CH$_3$C), 2.1 (s overlapping m at 2.0−2.3, 6H, CH$_3$Ar), 2.0−2.3 (m, 1H, CH$_3$C*H*CH$_3$), 4.1 (apparent t, *J* = 9 Hz, 1H, CHN*H*), 5.4 (br d, *J* = 9 Hz, 1H, CHN*H*), 7.05 (s, 2H, Ar), 7.7 (br s, 1H, ArNH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.4 (1C), 18.8 (1C), 19.9 (2C), 28.5 (3C), 30.0 (1C), 60.8 (1C), 80.3 (1C), 128.1 (2C), 132.5 (1C), 132.6 (1C), 137.4 (2C), 156.4 (1C), 171.0 (1C); MS (70 eV): *m*/*z* (%): 354 (M^+, 3), 72 (100).

(−)-(*S*)-*N*^{2}-(*tert*-Butoxycarbonyl)-*N*^{1}-(4-chloro-2,6-dimethylphenyl)valinamide [(−)-(*S*)-2i]: 25% yield, mp 170−171 °C (EtOAc/petroleum ether); $[\alpha]^{20}_D$ = −71 (*c* 1, $CHCl_3$).

General Procedure for the Synthesis of 2-Amino-*N*^{1}-arylalkanamides Hydrochlorides (3a−i). The preparation of (−)-(*R*)-*N*^{1}-(2,6-dimethylphenyl)valinamide hydrochloride [(−)-(*R*)-**3f**] will be described. (+)-(*R*)-*N*^{2}-(*tert*-butoxycarbonyl)-*N*^{1}-(2,6-dimethylphenyl)valinamide [(+)-(*R*)-**2f**] (0.32 g, 1.0 mmol) in 10 mL of anhydrous diethyl ether was saturated with gaseous HCl and stirred at room temperature for 15 min. After removal of the solvent under reduced pressure, the residue was purified by recrystallization from MeOH/Et_2O, to give white needle like crystals [(−)-(*R*)-**3f**] (80 mg, 40%), mp 260−261 °C; $[\alpha]^{20}_D$ = −49.8 (*c* 1.6, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 1.15 (d, *J* = 6.9 Hz, 3H, C*H*$_3$CHCH$_3$), 1.23 (d, *J* = 7.1 Hz, 3H, CH$_3$CHC*H*$_3$), 2.25 (s, 6H, CH$_3$Ar), 2.40−2.54 (m, 1H, CH$_3$C*H*CH$_3$), 4.07 (d, *J* = 4.2 Hz, 1H, C*H*CO), 7.08−7.12 (m, 3H, Ar); ^{13}C NMR (75 MHz, CD_3OD) δ 16.1 (1C), 17.6 (2C), 18.2 (1C), 30.6 (1C), 58.6 (1C), 127.6 (2C), 133.4 (1C), 135.6 (2C), 167.3 (1C).

(−)-(*R*)-*N*^{1}-(4-Chlorophenyl)alaninamide hydrochloride [(−)-(*R*)-3b]: 82% yield, mp 151−152 °C (MeOH/Et_2O); $[\alpha]^{20}_D$ = −8.1 (*c* 1.0, MeOH); the ^1H NMR spectrum recorded on the free base obtained via extraction from an analytical sample of the salt, was in agreement with that reported in the literature for the (+)-(*S*)-**3b**.[42] Anal. ($C_9H_{12}Cl_2N_2O$) C, H, N.

(+)-(*S*)-*N*^{1}-(4-Chlorophenyl)alaninamide hydrochloride [(+)-(*S*)-3b]: 15% yield, mp hygroscopic (EtOH/Et_2O); $[\alpha]^{20}_D$ = +8.3 (*c* 1.2, MeOH). Anal. ($C_9H_{12}Cl_2N_2O{\cdot}0.5H_2O$) C, H, N.

(−)-(*R*)-*N*^{1}-(4-Chloro-2,6-dimethylphenyl)alaninamide hydrochloride [(−)-(*R*)-3c]: 41% yield, mp 251−252 °C (EtOH/Et_2O); $[\alpha]^{20}_D$ = −37 (*c* 2, MeOH). Because of the formation of an aggregated form in CD_3OD solution, the NMR spectra were more complex than expected. Both xylididic methyls and Ar HC-3,5 were anisochronous. ^1H NMR (300 MHz, CD_3OD) δ 1.71 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH), 2.21 (s, 3H, CH$_3$Ar C-2), 2.27 (s, 3H, CH$_3$Ar C-6), 4.26 (q, *J* = 7.1 Hz, 1H, CH$_3$C*H*), 7.10 (d, *J* = 8.2 Hz, 1H, Ar HC-3), 7.26 (d, *J* = 8.2 Hz, 1H, Ar HC-5); ^{13}C NMR (75 MHz, CD_3OD) δ 16.8 (1C), 17.1 (1C), 23.0 (1C), 49.2 (1C), 128.2 (1C), 128.5 (1C), 132.1 (1C), 133.8 (1C), 134.8 (1C), 168.7 (1C). Anal. ($C_{11}H_{16}Cl_2N_2O{\cdot}0.5H_2O$) C, H, N.

(+)-(*S*)-*N*^{1}-(4-Chloro-2,6-dimethylphenyl)alaninamide hydrochloride [(+)-(*S*)-3c]: 78% yield, mp 255−256 °C (EtOH/Et_2O); $[\alpha]^{20}_D$ = +35 (*c* 2, MeOH). Anal. ($C_{11}H_{16}Cl_2N_2O$) C, H, N.

(−)-(*R*)-*N*^{1}-(2-Methylphenyl)alaninamide hydrochloride [(−)-(*R*)-3d]: 47% yield, mp 221−222 °C (EtOH/Et_2O); $[\alpha]^{20}_D$ = −27 (*c* 1, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 1.66 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH), 2.27 (s, 3H, CH$_3$Ar C-2), 4.21 (q, *J* = 7.1 Hz, 1H, CH$_3$C*H*), 7.13−7.21 (m, 1H, Ar HC-3,4), 7.21−7.24 (m, 1H, Ar HC-5), 7.34 (dd, *J* = 7.1 Hz, 2.2 Hz 1H, Ar HC-6); ^{13}C NMR (75 MHz, CD_3OD) δ 16.8 (1C), 16.9 (1C), 49.4 (1C), 125.8 (1C), 126.3 (1C), 126.8 (1C), 130.6 (1C), 133.2 (1C), 134.7 (1C), 168.8 (1C). Anal. ($C_{10}H_{15}ClN_2O$) C, H, N.

(+)-(*S*)-*N*^{1}-(2-Methylphenyl)alaninamide hydrochloride [(+)-(*S*)-3d]: 50% yield, mp 224−225 °C (EtOH/Et_2O); $[\alpha]^{20}_D$ = +23 (*c* 1.2, MeOH). Anal. ($C_{10}H_{15}ClN_2O{\cdot}0.5H_2O$) C, H, N.

(+)-(*S*)-*N*^{1}-(2,6-Dimethylphenyl)valinamide hydrochloride [(+)-(*S*)-3f]: 66% yield, mp 256−258 °C (MeOH/Et_2O); $[\alpha]^{20}_D$ = +43 (*c* 1, MeOH). Anal. ($C_{13}H_{21}ClN_2O$) C, H, N.

(−)-(*R*)-*N*^{1}-(4-Chloro-2-methylphenyl)valinamide hydrochloride [(−)-(*R*)-3g]: 40% yield, mp 230−231 °C (MeOH/Et_2O); $[\alpha]^{20}_D$ = −46.0 (*c* 1.6, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 1.14 (d, *J* = 6.9 Hz, 3H, C*H*$_3$CHCH$_3$), 1.17 (d, *J* = 7.1 Hz, 3H, CH$_3$CHC*H*$_3$), 2.29 (s overlapping apparent octet at 2.35, 3H, CH$_3$-Ar), 2.35 (octet, *J* = 6.9 Hz, 1H, CH$_3$C*H*CH$_3$), 3.99 (d, *J* = 5.7 Hz, 1H, CHCO), 7.20 (dd, *J* = 8.5, 2.4 Hz, 1H, Ar HC-5), 7.28 (d, *J* = 2.2 Hz 1H, Ar HC-3), 7.40 (d, *J* = 8.6, 1H, Ar HC-6); ^{13}C NMR (75 MHz, CD_3OD, δ 46.3) δ 15.3 (1C), 15.6 (1C), 16.4 (1C), 29.0 (1C), 57.3 (1C), 124.7 (1C), 125.5 (1C), 128.8 (1C), 130.2 (1C), 132.1 (1C), 133.5 (1C), 166.0 (1C). Anal. ($C_{12}H_{18}Cl_2N_2O$) C, H, N.

(+)-(*S*)-*N*^{1}-(4-Chloro-2-methylphenyl)valinamide hydrochloride [(+)-(*S*)-3g]: 98% yield, mp 219−220 °C (MeOH/Et_2O); $[\alpha]^{20}_D$ = +40 (*c* 1.5, MeOH). Anal. ($C_{12}H_{18}Cl_2N_2O$) C, H, N.

(−)-(*R*)-*N*^{1}-(2,6-Dimethylphenyl)leucinamide hydrochloride [(−)-(*R*)-3h]: 40% yield, mp 170−171 °C (abs EtOH/Et_2O); $[\alpha]^{20}_D$ = −47 (*c* 1.6, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 1.09 (d, *J* = 6.0 Hz, 6H, C*H*$_3$CH), 1.85−1.95 (br m, 3H, C*H*$_2$C*H*CH$_3$), 2.23 (s 6H, CH$_3$Ar), 4.05−4.15 (m, 1H, CHCO), 7.88−7.90 (m, 3H, Ar). Anal. ($C_{14}H_{23}ClN_2O$) C, H, N.

(+)-(*S*)-*N*^{1}-(2,6-Dimethylphenyl)leucinamide hydrochloride [(+)-(*S*)-3h]: 77% yield, mp 168−170 °C (MeOH/Et_2O); $[\alpha]^{20}_D$ = +48 (*c* 1.7, MeOH). Anal. ($C_{14}H_{23}ClN_2O$) C, H, N.

(−)-(*R*)-*N*^{1}-(4-Chloro-2,6-dimethylphenyl)valinamide hydrochloride [(−)-(*R*)-3i]: 25% yield, mp 248−250 °C (EtOH/Et_2O); $[\alpha]^{20}_D$ = −39 (*c* 1, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 1.14 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH CH$_3$), 1.21 (d, *J* = 7.1 Hz, 3H, CH$_3$-CHC*H*$_3$), 2.23 (s 6H, CH$_3$Ar), 2.40−2.52 (m, 1H, CH$_3$C*H*CH$_3$), 4.05 (d, *J* = 4.1 Hz, 1H, C*H*CH), 7.14 (s, 2H, Ar). ^{13}C NMR (75 MHz, CD_3OD) δ 16.0 (1C), 17.5 (2C), 18.2 (1C), 30.6 (1C), 58.6 (1C), 127.8 (2C), 132.3 (1C), 132.8 (1C), 137.8 (2C), 167.4 (1C); MS (70 eV): *m*/*z* (%): 254 (M^+, 2), 72 (100). Anal. ($C_{13}H_{20}Cl_2N_2O{\cdot}3/4H_2O$) C, H, N.

(+)-(*S*)-*N*^{1}-(4-Chloro-2,6-dimethylphenyl)valinamide hydrochloride [(+)-(*S*)-3i]: 30% yield, mp 246−248 °C (EtOH/Et_2O); $[\alpha]^{20}_D$ = +46 (*c* 1, MeOH). Anal. ($C_{13}H_{20}Cl_2N_2O{\cdot}H_2O$) C, H, N.

***N*-(4-Bromo-2,6-dimethylphenyl)-2,2,2-trifluoracetamide (4).** A solution of 4-bromo-2,6-dimethylaniline (0.50 g, 1.69 mmol) in 10 mL of anhydrous THF was added dropwise to a solution of trifluoroacetic anhydride (0.79 g, 3.75 mmol) in anhydrous THF (20 mL) under vigorous stirring, at 0 °C. The reaction mixture was stirred at room temperature for 14 h and, after removing the solvent under reduced pressure, taken up with EtOAc. The organic layer was washed twice with 2 N HCl. The aqueous layers were extracted twice with EtOAc, and the three organic phases so obtained were pooled together and dried over Na_2SO_4. The solvent was then removed under reduced pressure giving **4** which was then purified by crystallization (EtOAc/petroleum ether) giving a pale brown solid (0.35 g, 47%): mp 142−143 °C; IR ($CHCl_3$) 3410, 3239 (NH), 1745 (CO) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 2.18 (s, 6H, CH$_3$), 7.31 (s, 2H, ArH); ^{13}C (75 MHz, CD_3OD) δ 16.6 (2C), 116.5 (q, *J* = 287.4 Hz, 1C), 121.4 (1C), 130.9 (2C), 131.4 (1C), 137.9 (2C), 156.4 (q, *J* = 37.2 Hz, 1C). MS (70 eV) *m*/*z* (%): 295 (M^+, 62), 147 (100).

***N*-(4-Chloro-2,6-dimethylphenyl)trifluoroacetamide (5).**[20] A mixture of **4** (3.0 g, 10.1 mmol) and CuCl (6.0 g, 61 mmol) in 30 mL of anhydrous DMSO was heated at 188 °C for 6 h; the reaction mixture was then cooled at room temperature and poured into water. The resultant crude solid was collected and redissolved with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **5** (0.63 g, 84%) as a brown solid: mp 135−137 °C; IR (KBr) 3457, 3240 (NH), 1714 (CO) cm^{-1}; ^1H NMR (300 MHz, CD_3Cl_3) δ 2.15 (s, 6H, CH$_3$), 7.08 (s, 2H, ArH), 7.71 (br s, 1H, NH); MS (70 eV) *m*/*z* (%) 251 (M^+, 85), 182 (100).

4-Chloro-2,6-dimethylaniline (6).[43] A solution of **5** (1.00 g, 4 mmol) in a mixture of HCl 17% (25 mL) and THF (25 mL) was refluxed for 7 h; water was then added in order to quench the reaction, and the aqueous phase was extracted three times with Et_2O. The aqueous phase was treated with 4 N NaOH until it reached pH 14 and was then extracted with Et_2O (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and

concentrated under reduced pressure to give **6** (0.6 g, 98%) as a yellow oil: ^{1}H NMR (300 MHz, CD_3Cl_3) δ 2.15 (s, 6H, CH_3), 3.54 (br s, 2H, NH_2), 6.93 (s, 2H, ArH); MS (70 eV) *m*/*z* (%)155 (M^+, 100). The corresponding hydrochloride was obtained by dissolving 0.5 g of **6** in Et_2O and then adding few drops of 2 N HCl. On standing, a gray solid formed: mp 238−239 (lit.: 239.0−239.5 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.31 (s, 6H, CH_3), 7.17 (s, 2H, ArH), 9.53 (br s, 3H, NH_3).

General Procedure for the Synthesis of *N*-Alkyl-*N*^{1}-Aryl-2-(*tert*-butoxycarbonylamino)alkanamides (7a−c). The preparation of (−)-(*R*)-*N*^{2}-*tert*-butoxycarbonyl-*N*^{1}-(2,6-dimethylphenyl)-*N*-methylalaninamide (**7a**) can be taken as the reference procedure for the synthesis of *N*-alkyl-*N*^{1}-aryl-2-(*tert*-butoxycarbonylamino)-alkanamides (**7a**−**c**). *N*^{2}-(*tert*-Butoxycarbonyl)-*N*^{1}-(2,6-dimethylphenyl)alaninamide (**2a**) (0.298 g, 1 mmol) was dissolved in DMF/H_2O (30 mL)/(10 mL). $Ba(OH)_2 \cdot 8H_2O$ (1.9 g, 6 mmol) and CH_3I (0.85 g, 6 mmol) were added. This suspension was stirred at room temperature for 12 h. The yellow reaction mixture was then filtered to remove the excess $Ba(OH)_2$, followed by three extractions with petroleum ether. The organic layer was then dried over anhydrous Na_2SO_4, and the solvent was then removed under reduced pressure to give a crude yellowish oil (0.28 g, 90%): $[\alpha]^{20}_D$ = −47 (*c* 1, $CHCl_3$); MS (70 eV): *m*/*z* (%) 306 (M^+, 1), 44 (100).

(+)-(*S*)-*N*^{2}-*tert*-Butoxycarbonyl-*N*^{1}-(2,6-dimethylphenyl)-*N*^{1}-methylalaninamide [(+)-(*S*)-7a]: 80% yield, oil; $[\alpha]^{20}_D$ = +49 (*c* 1, $CHCl_3$).

(−)-(*R*)-*N*^{2}-*tert*-Butoxycarbonyl-*N*^{1}-(2,6-dimethylphenyl)-*N*^{1}-ethylalaninamide [(−)-(*R*)-7b]: 50% yield, oil; $[\alpha]^{20}_D$ = −28 (*c* 1, $CHCl_3$); IR ($CHCl_3$): 3433 (NH), 1705 (NHCOO), 1650 (NHCO), cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.04 (d, *J* = 6.72 Hz, 3H, C*H*$_3$CH), 1.12 (t, *J* = 7.3 Hz, 3H, C*H*$_3$ CH_2), 1.39 (s, 9H, CH_3 C), 2.21 (s, 3H, CH_3Ar), 2.22 (s, 3H, CH_3Ar), 3.27 (dt, *J* = 13.4, 7.2 Hz, 1H, C*H*HCH$_3$), 3.9−4.1 (m, 2H; CH*H*CH_3 + C*H*CH_3), 5.42 (br d, *J* = 8.4, 1H, NHCO), 7.0−7.2 (m, 3H, ArH); MS (70 eV): *m*/*z* (%) 320 (M^+, 1), 44 (100).

(+)-(*S*)-*N*^{2}-*tert*-Butoxycarbonyl-*N*^{1}-(2,6-dimethylphenyl)-*N*^{1}-ethylalaninamide [(+)-(*S*)-7b]: 65% yield, oil; $[\alpha]^{20}_D$ = +30 (*c* 1, $CHCl_3$).

(−)-(*R*)-*N*^{2}-*tert*-Butoxycarbonyl-*N*^{1}-(2,6-dimethylphenyl)-*N*^{1}-methylvalinamide [(−)-(*R*)-7c]: 60% yield, oil; $[\alpha]^{20}_D$ = −21 (*c* 1, $CHCl_3$); IR ($CHCl_3$): 1707 (CO, carbamate), 1649 (CO, amide) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.67 (d, *J* = 6.9 Hz, 3H, C*H*$_3$CHCH$_3$), 0.80 (d, *J* = 6.9 Hz, 3H, CH_3CHC*H*$_3$), 1.39 (s, 9H, CH_3C), 1.62−1.76 (m, 1H, CH_3C*H*CH_3), 2.17 (s, 3H, CH_3Ar), 2.23 (s, 3H, CH_3Ar), 3.14 (s, 3H, CH_3N), 3.94 (dd, *J* = 6.8, 4.4 Hz, 1H, C*H*NH), 5.25 (br d, *J* = 9.6 Hz, 1H, NHCO), 7.05−7.22 (m, 3H, ArH); MS (70 eV); *m*/*z* (%) 334 (M^+, 1), 57 (100).

(+)-(*S*)-*N*^{2}-*tert*-Butoxycarbonyl-*N*^{1}-(2,6-dimethylphenyl)-*N*^{1}-methylvalinamide [(+)-(*S*)-7c]: 65% yield, oil; $[\alpha]^{20}_D$ = +31 (*c* 1, $CHCl_3$).

(−)-(*R*)-*N*^{1}-(2,6-Dimethylphenyl)-*N*^{1}-methylalaninamide hydrochloride [(−)-(*R*)-8a]:[44] 50% yield, mp 190−205 °C (abs EtOH/Et_2O); $[\alpha]^{20}_D$ = −25 (*c* 1.75, MeOH); IR (KBr): 1651 (CO) cm^{-1}; two forms were present in CD_3OD solution; for the sake of simplicity, only the predominating one will be described; ^{1}H NMR (300 MHz) δ 1.18 (d, *J* = 6.9 Hz, 3H, C*H*$_3$CH), 2.26 (s, 3H, CH_3 Ar), 2.28 (s, 3H, CH_3 Ar), 3,19 (s, 3H, CH_3N), 3.63 (q, *J* = 7.0, 1H, CH), 7.22−7.31 (m, 3H, ArH); ^{13}C NMR (75 MHz, CD_3OD) δ 15.1 (1C), 16.6 (1C), 16.9 (1C), 34.8 (1C), 35.9 (1C), 129.3 (1C), 129.5 (1C), 129.6 (1C), 135.7 (1C), 136.0 (1C), 138.8 (1C), 169.6 (1C); MS (70 eV) *m*/*z* (%) 206 (M^+, 1), 44 (100). Anal. ($C_{12}H_{19}ClN_2O$) C, H, N.

(+)-(*S*)-*N*^{1}-(2,6-Dimethylphenyl)-*N*^{1}-methylalaninamide hydrochloride [(+)-(*S*)-8a]: 70% yield, mp 191−193 °C (MeOH/Et_2O); $[\alpha]^{20}_D$ = +40 (*c* 1.75, MeOH). Anal. ($C_{12}H_{19}ClN_2O$) C, H, N.

(−)-(*R*)-*N*^{1}-(2,6-Dimethylphenyl)-*N*^{1}-ethylalaninamide hydrochloride [(−)-(*R*)-8b]: 40% yield, mp 162−163 °C (MeOH/Et_2O); $[\alpha]^{20}_D$ = −90 (*c* 1.5, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.99 (d, *J* = 6.9 Hz, 3H, C*H*$_3$-CH), 1.04 (t, *J* = 7.3 Hz, 3H, CH_3 CH_2), 2.18 (s, 3H, CH_3Ar), 2.22 (s, 3H, CH_3Ar), 3.19 (dt, *J* = 14.0, 7.0 Hz, 1H, C*H*HCH$_3$), 3.65−3.70 (m, 1H, C*H* CH_3), 3.96 (dt, *J* = 14.2, 7.2 Hz, 1H, CH*H*CH$_3$), 7.08−7.20 (m, 3H, Ar), 8.24 (br s, 3H, NH_3). Anal. ($C_{13}H_{21}ClN_2O$), C, H, N.

(+)-(*S*)-*N*^{1}-(2,6-Dimethylphenyl)-*N*^{1}-ethylalaninamide hydrochloride [(+)-(*S*)-8b]: 50% yield, mp 163−164 °C (MeOH/Et_2O); $[\alpha]^{20}_D$ = +60 (*c* 1.0, MeOH). Anal. ($C_{13}H_{21}ClN_2O$), C, H, N % was found to be −0.49 of the theoretical value.

(−)-(*R*)-*N*^{1}-(2,6-Dimethylphenyl)-*N*^{1}-methylvalinamide L-Tartrate [(−)-(*R*)-8c·$C_4H_6O_6$]. A solution of (−)-(*R*)-1-*N*^{1}-(*tert*-butoxycarbonyl)-*N*^{1}-(2,6-dimethylphenyl)-*N*^{1}-methylvalinamide [(−)-(*R*)-**7c**, 1 mmol] in EtOAc was treated with 48% HBr under vigorous stirring at room temperature for 1 h. After this time, the organic layer was removed under reduced pressure and the aqueous phase was alkalinized with 2 N NaOH, extracted twice with EtOAc, and dried over Na_2SO_4 to give a yellow oil that was converted into the corresponding tartrate as follows. A solution of **8c** (1 mmol) in EtOH was mixed with a solution of L-tartaric acid (1 mmol) in EtOH and stirred at room temperature for 10 min; the solvent was then removed under reduced pressure giving **8c** as a white solid that was recrystallized from acetone to give **8c·tartrate** in the form of white crystals (0.27 g, 70%). mp 146−148 °C; $[\alpha]^{20}_D$ = −28 (*c* 1.4, MeOH). In CD_3OD solution two forms were present; for the sake of simplicity, only the predominating NMR spectrum will be described: ^{1}H NMR (300 MHz) δ 0.76 (d, *J* = 7.1 Hz, 3H, C*H*$_3$-CHCH$_3$), 0.95 (d, *J* = 7.2 Hz, 3H, CH_3CHC*H*$_3$), 1.79−1.86 (m, 1H, CH_3C*H*CH_3), 2.25 (s, 3H, CH_3Ar), 2.26 (s, 3H, CH_3Ar), 3.19 (s, 3H, CH_3N), 3.57 (d, *J* = 2.7 Hz, 1H, CHCO), 4.40 (s, 2H, CHOH), 7.20−7.30 (m, 3H, ArH); ^{13}C NMR (75 MHz, CD_3OD, δ 46.2) δ 13.5 (1C), 15.1 (1C), 15.3 (1C), 16.8 (1C), 26.0 (1C), 33.4 (1C), 55.3 (1C), 71.4 (2C), 127.5 (1C), 127.9 (2C), 134.0 (1C), 134.5 (1C), 137.3 (1C), 167.3 (1C), 174.1 (2C). Anal. ($C_{18}H_{28}N_2O_7 \cdot H_2O$) C, H, N.

(+)-(*S*)-*N*^{1}-(2,6-Dimethylphenyl)-*N*^{1}-1-methylvalinamide L-tartrate [(+)-(*S*)-8c·$C_4H_6O_6$]: 80% yield, mp 160−163 °C; $[\alpha]^{20}_D$ = +41 (*c* 1.8, MeOH). Anal. ($C_{18}H_{28}N_2O_7 \cdot H_2O$) C, H, N.

(+)-(*R*)-2-*tert*-Butoxycarbonylamino-*N*-(2,6-dimethylphenyl)-propanethioamide [(+)-(*R*)-9]. A solution of **2a** (0.29 g, 1 mmol) and 2,4-bis-(*p*-pentyloxyphenyl)-1,2,3,4-dithiadiphosphethane 2,4-disulfide (0.52 g, 1mmol) in benzene was refluxed for 2 days. After this time, the reaction mixture was washed with Na_2CO_3 5% (pH 12), and the alkaline layer was extracted three times with Et_2O. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure, giving a yellowish oil that was purified by silica gel chromatography (petroleum ether/EtOAc 2:1) to produce **9** (0.15 g, 50%) as a white solid: mp 150−152 °C; $[\alpha]^{20}_D$ = +34.2 (*c* 2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (s, 9H, CH_3C), 1.61 (d, *J* = 6.8 Hz, 3H, C*H*$_3$CH), 2.21 (s, 6H, CH_3Ar), 4.80 (apparent quintet, *J* = 6.8 Hz, 1H, CH), 5.60 (br d, *J* = 6.8 Hz, 1H, NHCOO), 7.08−7.11 (m, 2H, Ar HC-3,5), 7.15−7.20 (m, 1H, Ar HC-4), 9.82 (br s, 1H, NHAr); MS (70 eV) *m*/*z* (%) 308 (M^+, 22), 293 (100).

(−)-(*S*)-2-*tert*-Butoxycarbonylamino-*N*-(2,6-dimethylphenyl)-propanethioamide [(−)-(*S*)-9]: 60% yield, mp 148−150 °C; $[\alpha]^{20}_D$ = −16.9 (*c* 2, $CHCl_3$).

(−)-(*R*)-2-Amino-*N*-(2,6-dimethylphenyl)propanethioamide Hydrochloride [(−)-(*R*)-10]. Obtained in a 30% yield via the method reported above for its oxygenated isologous (**3a**): mp 230 °C dec (EtOH/Et_2O); $[\alpha]^{20}_D$ = −59 (*c* 2, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.65 (br d, *J* = 6.2, 3H, C*H*$_3$CH), 2.04 (s, 3H, CH_3Ar), 2.12 (s, 3H, CH_3Ar), 2.72 (br s, 3H, NH_3), 4.80−4.82 (m, 1H, CH), 6.94−7.00 (m, 3H, ArH), 8.39 (br s, 1H, NHCS); Anal. ($C_{11}H_{17}ClN_2S$) C, H, N.

(+)-(*S*)-2-Amino-*N*-(2,6-dimethylphenyl)propanethioamide hydrochloride [(+)-(*S*)-10]: 60% yield, mp 230 °C dec (EtOH/Et_2O); $[\alpha]^{20}_D$ = +58 (*c* 2, MeOH). Anal. ($C_{11}H_{17}ClN_2S \cdot 1/3 \cdot H_2O$) C, H, N.

Supporting Information Available: Elemental analysis of the compounds submitted for pharmacological evaluation. This material is available free of charge via the Internet at http://pubs.acs.org.

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