Synthesis, Structure, and Pharmacological Evaluation of the Stereoisomers of Furnidipine

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Received August 16, 1994[⊗]

The synthesis and pharmacological activities of the four stereoisomers of methyl tetrahydrofuran-2-ylmethyl 2,6-dimethyl-4-(2′-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate(furnidipine) are reported. The four isomers were synthesized by a modified Hantzsch synthesis by reaction of (−)- or (+)-tetrahydrofuran-2-ylmethyl 3-aminocrotonate and methyl 2-[(2′-nitrophenyl)methylene]acetoacetate or, alternatively, by reaction of (−)- or (+)-tetrahydrofuran-2-ylmethyl 2-[(2′-nitrophenyl)methylene]acetoacetate and methyl 3-aminocrotonate. The 1:1 diastereomeric mixtures thus obtained were separated by chromatography, using poly(D-phenylglycine) as the chiral stationary phase. The enantiomeric purity of the stereoisomers was determined by a high-performance liquid chromatography–chiral stationary phase technique (HPLC–CSP). Attempts to obtain crystals of a single stereoisomer failed in different solvents, while methanol crystallization of the product obtained from (±)-tetrahydrofuran-2-ylmethyl 2-[(2′-nitrophenyl)methylene]acetoacetate and methyl 3-aminocrotonate yielded good-quality crystals of the most insoluble racemate which proved to be a mixture of the (*SS*)/(*RR*) enantiomers by X-ray crystallography. Conformational analysis of the stereoisomers, assuming rotation of the aryl substituent and ester groups, shows small energy differences (about 4 kcal·mol^{-1}) between the most and the least favorable conformations. Binding studies were performed using [^{3}H]isradipine as a reference ligand. The results showed stereospecificity of the furnidipine isomers in brain, ileum, and cardiac tissues, the (*SS*)- and (*SR*)-isomers clearly being more potent than their (*RR*)- and (*RS*)-enantiomers. The (*SS*)- and (*SR*)-isomers were also more selective on cerebral tissue when compared with ileal and cardiac preparations.

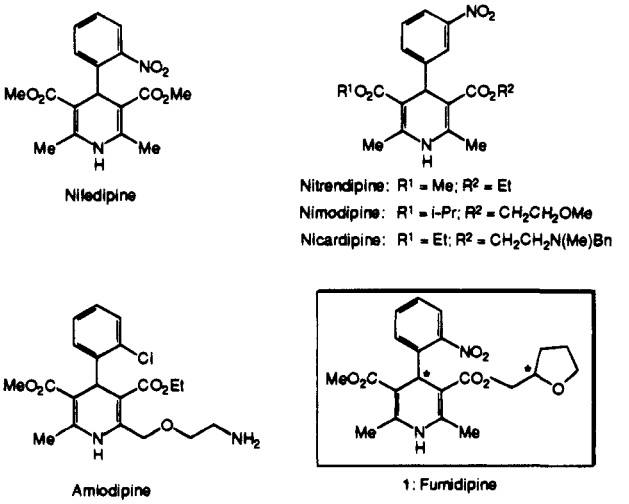
Introduction

The calcium channel antagonists are a group of structurally diverse drugs which inhibit the influx of Ca^{2+} through plasma membrane channels, thus dilating vascular smooth muscle and alleviating the force of cardiac muscle contraction.[1] Some of them, such as diltiazem, verapamil, and nifedipine have been used as antihypertensive agents.[2] To this day the 4-aryl-1,4-dihydropyridine-3,5-dicarboxylic esters of the nifedipine type are the most used among calcium channel blockers,[3] although, as the plasma half-lives of many derivatives are relatively short, these drugs have to be administered repeatedly to achieve clinical efficacy. Symmetrically substituted dihydropyridine drugs are achiral molecules, but when the ester groups are differently substituted, a chiral center appears in the 4-position of the dihydropyridine ring (*e.g.*, nitrendipine, nimodipine, nicardipine, and amlodipine; Chart 1). Some of those chiral calcium antagonists are among the more powerful arterial vasodilators today in use, with relatively small effects on the heart.[1a,4]

The chiral 4-aryl-1,4-dihydropyridines offer an exciting field for the investigation of calcium channels, particularly since the discovery that enantiomers could have exactly the opposite action profile, one of them being a calcium antagonist and the other an agonist.[5,6] The study of these chiral compounds should facilitate information about structural requirements for improved biological activity but also implies a chemical challenge since resolution of enantiomers or development of stereoselective syntheses to obtain the desired stereoisomer is necessary to obtain novel dihydropyridines with improved therapeutical applications.[7]

After screening numerous 4-aryl-1,4-dihydropyridine-3,5-dicarboxylates, we selected one, methyl tetrahy-

Chart 1



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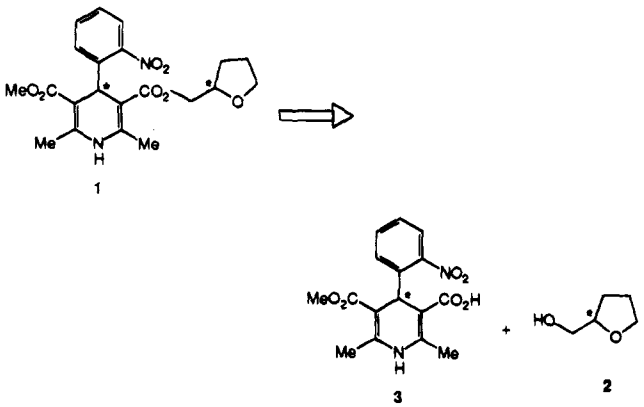
[⊗] Abstract published in *Advance ACS Abstracts*, July 1, 1995.

0022-2623/95/1838-2830$09.00/0

Scheme 1



drofuran-2-ylmethyl 2,6-dimethyl-4-(2′-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**1**, furnidipine),[8] because of its interesting pharmacological profile. It belongs to a new generation of promising Ca^{2+} antagonists, active in the prevention and treatment of different ischemic cardiac tissues (stunned and hibernating myocardium) due to its highly selective effect on the slow vascular calcium channels. Its favorable coronary vasodilator activity could be beneficial for all forms of cardiomyopathy often associated with hypertension and especially that of cardiac ischemia.

Unlike other Ca^{2+} antagonists, furnidipine is highly selective in relaxing both capacitance venous and resistance arterial vessels. As a result, it reduces the preload and the left-ventricular filling pressure. Since, in addition, it reduces the after load by relaxing the resistance vessels, the net result is an increase in cardiac output.

In contrast with other Ca^{2+} antagonists, furnidipine is without influence on the heart conduction system. After intravenous and oral routes, furnidipine was effective in protecting animals against ischemic and reperfusion-induced arrhythmias and in preventing drastic reperfusion-induced hypotension. In myocardial damage induced by isoproterenol, furnidipine decreased mortality and substantially reduced the infarct size.

One of the major effects of furnidipine is the inhibition of calcium influx into smooth vascular muscle tissue. This action, which is highly specific, plays an important role in the prevention of unwanted calcium accumulation in tissues and could be beneficial in the maintenance of the tissular integrity (heart, vessels, brain, etc).

Very small doses (0.2 mg/kg) of furnidipine protected neurones of the hypocampus from destruction. This effect is similar to that of nimodipine (a specific cerebral vasodilator). In mice, subcutaneous doses of 0.3 mg/kg provided significant cerebral protection against ischemia.

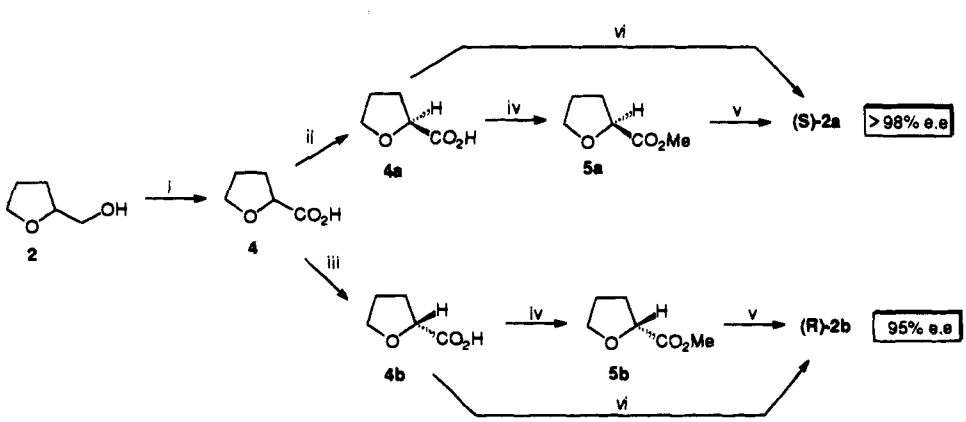
Furnidipine is currently in phase II clinical trials as an agent able to prevent post-reperfusion-induced arrhythmias and stunned myocardium with the potential of therapeutic usefulness in prevention of mortality due to secondary myocardial infarction. We describe the synthesis of diastereomeric and racemic mixtures of **1**, the separation of the isomers, the X-ray analysis of the most insoluble racemate, a conformational study using molecular mechanics and semiempirical molecular orbital calculations, and the pharmacological activity of the four stereoisomers.

Chemistry

At the outset it was envisaged that the four stereoisomers of **1** could be obtained in a simple way by esterification of the (*R*)- and (*S*)-5(methoxycarbonyl)-2,6-dimethyl-4-(2′-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid (**3**) with enantiomerically pure (*R*)- and (*S*)-tetrahydrofurfuryl alcohols **2** (Scheme 1).

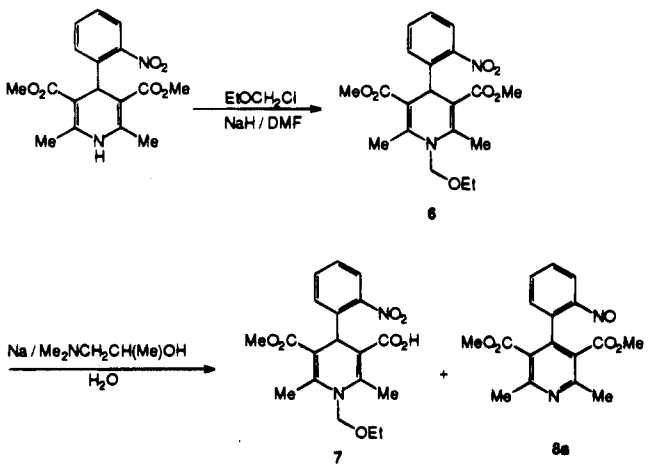
Although the 2-tetrahydrofurfurylmethanol has been resolved by formation of the phthalate acid and recrystallization of the salt formed using brucine as the chiral base,[9a] the data reported refer mainly to the (−)-isomer. The dextrorotatory isomer was also obtained from (*S*)-(−)-1,2,5-pentanetriol,[9b] but no enantiomeric excess was given. Initially we tried the resolution by separation of diastereomeric esters and carbamates obtained with (*R*)-mandelic acid and (*S*)-1-ethylphenylisocyanate, respectively. However, in both cases the oily diastereomeric mixtures could not be separated by chromatography. Therefore, we turned our attention to the resolution of the tetrahydrofuran-2-carboxylic acid. Starting from the cheap racemic alcohol **2** (Scheme 2), we obtained by oxidation with Jones' reagent the corresponding racemic acid **4** which was transformed into diastereomeric salts with brucine and (+)-ephedrine, according to the method described by Belanger et al.[10] Recrystallization of the salts afforded (*R*)- and (*S*)-tetrahydrofuran-2-carboxylic acids with 95% and 98% ee, respectively (85% and 98% ee is described in ref 10). Both enantiomers were converted into the corresponding methyl esters **5** with diazomethane, the

Scheme 2[a]

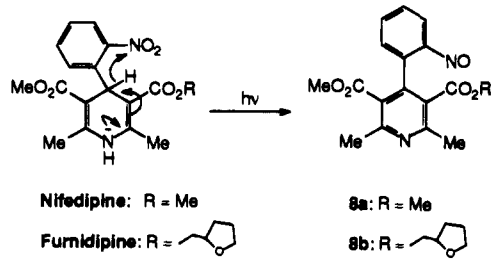


[a] (i) Jones' reagent; (ii) (+)-efedrine; (iii) brucine; (iv) CH_2N_2/Et_2O; (v) $NaBH_4$/EtOH; (vi) $BH_3{\cdot}SMe_2$/THF.

Scheme 3



Scheme 4



esters being finally reduced to the alcohols by the use of $NaBH_4$ without loss of optical purity. Interestingly, the acids can also be directly reduced to the alcohols with a borane–methyl sulfide complex. This result was convenient since it eliminated the need of using diazomethane, and both alcohols were obtained with the same enantiomeric excess as those obtained from the esters.

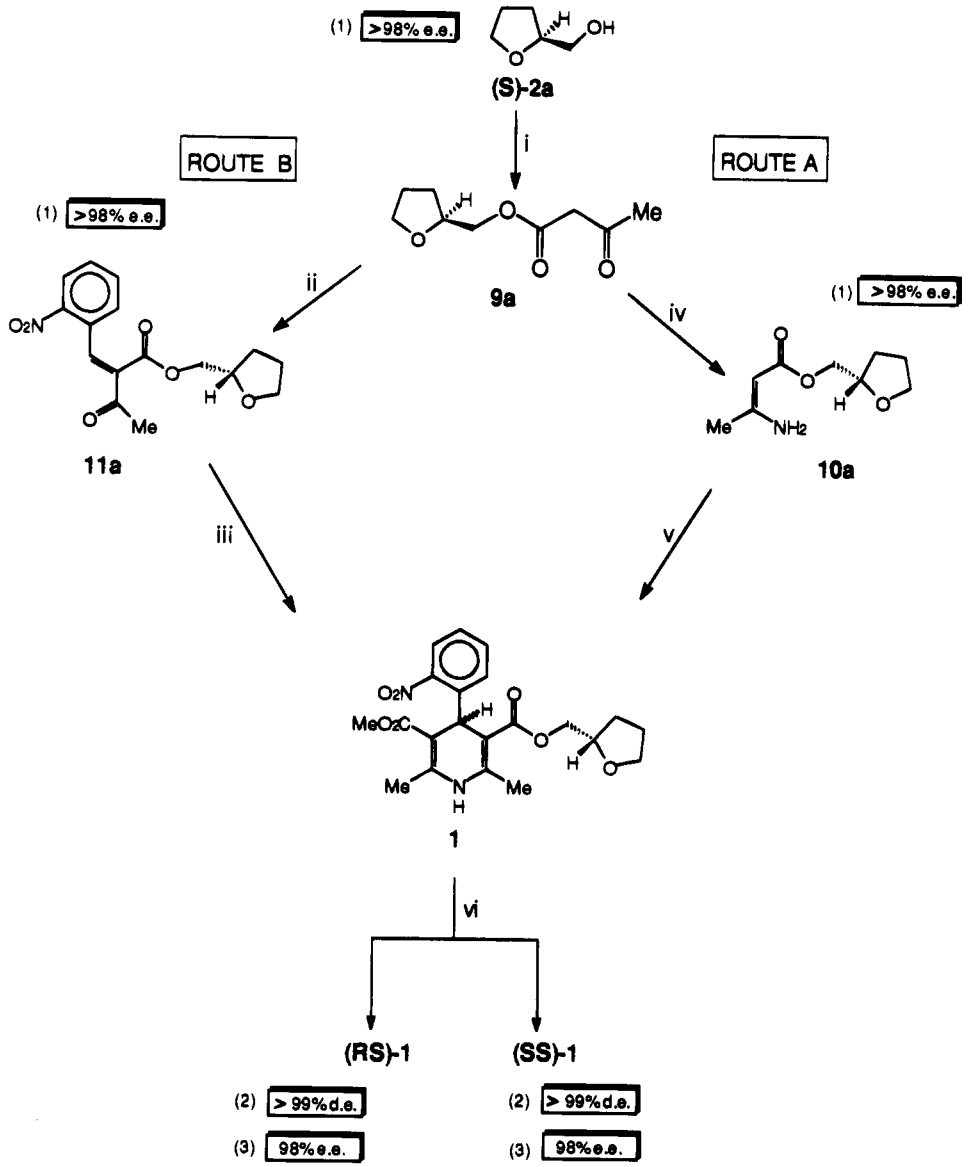
The preparation of pure enantiomers of **3** was first attempted by adapting the method reported by Shibanuma et al.[11] for the resolution of the 5-(methoxycarbonyl)-2,6-dimethyl-4-(3′-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid. In our case the process included protection of the nifedipine nitrogen with chloromethyl ethyl ether, selective hydrolysis of one of the ester groups with sodium in an amino alcohol, deprotection with hydrochloric acid, and resolution of the acid thus obtained. Although we obtained the protected derivative **6** in a 76% yield, the selective hydrolysis was the limiting factor in the conversion of nifedipine to the acid derivative **3** because hydrolysis of **6** gave **7** in a disappointing 12% yield (Scheme 3). Several attempts to improve the yield of **7** by varying the reaction conditions were fruitless, the nitroso derivative **8a** always being isolated as the major product. This result can be explained in terms of the activation of the dihydropyridine ring by the *ortho*-nitro group, toward intramolecular oxido–reduction to the corresponding nitroso pyridine derivative **8a** (Scheme 4) under the hydrolysis conditions. Further experiments (see the Experimental Section) showed that sunlight also promotes the redox process on furnidipine under neutral conditions. A similar process has been described for nifedipine itself and several 2-nitrophenyl derivatives.[12]

The possibility of preparing the enantiomerically pure acid from unsymmetrically substituted 1,4-dihydropyridines, obtained by asymmetric synthesis, was also considered but then discarded since nifedipine analogues bearing 2-nitrophenyl substituents on C-4 could not be prepared in enantiomeric pure form according to two previously reported asymmetric syntheses of chiral dihydropyridines.[13,14] Therefore, we decided to develop a method based on the separation of covalent diastereomers.

The synthetic route starts with enantiomerically pure (*S*)- and (*R*)-tetrahydrofurfuryl alcohols as is shown in Scheme 5. The synthesis (route A) is exemplified by the (*S*)-enantiomer which was transformed into the β-oxoester **9** by reaction with 2,2,6-trimethyl-1,3-dioxo-5-en-4-one in xylene. Treatment of **9** with ammonium acetate in ethanol gave the aminocrotonate **10**, which was condensed with methyl 2-[(2′-nitrophenyl)methylene]acetoacetate, yielding the desired (*RS*)-**1** and (*SS*)-**1** diastereomers via a modified Hantzsch synthesis.[15] Since no asymmetric induction was observed in this route, we also tested an alternative procedure (route B), transforming the β-oxoester **9** into the (phenylmethylene)acetoacetate **11** by reaction with 2-nitrobenzaldehyde. Condensation between **11** and methyl 3-aminocrotonate also gave furnidipine as a diastereomeric mixture. Again, no diastereomeric excess was observed in this route, which in addition gave a lower overall yield due to the formation of nifedipine as a side product (8%) in the last step. The probably mechanism for the formation of the symmetrical 1,4-dihydropyridine is shown in Scheme 6. This involves a retro-Michael process[16] followed by condensation of the methyl (phenylmethylene)acetoacetate **12** with methyl 3-aminocrotonate. The extent of the retro-Michael reaction observed in route B is associated with the bulky ester group in **11a** which favors the process when the less hindered compound is formed. Analogous procedures starting from the (*R*)-tetrahydrofurfuryl alcohol (95% ee) afforded a 1:1 diastereomeric mixture of (*SR*)-**1** and (*RR*)-**1** stereoisomers.

Attempted separations of diastereomers by fractional crystallization using different solvents and solvent mixtures were all unsuccessful. Separation by HPLC using different columns and solvents gave, in the best case, enriched fractions of the stereoisomers, with a diastereomeric excess lower than 25%. Analytical HPLC experiments indicated that the (*RR*)/(*SS*) racemate could be resolved by using an α_1-acid glycoprotein column. Based on this result, which was then used to determine the enantiomeric excess of the separated stereoisomers, we were also able to separate the diastereoisomers by HPLC using a chiral Pirkle column packed with D-phenylglycine. On the basis of the latter analytical result, a semipreparative separation was attempted using a Pirkle column (250 × 10 mm). The (*RS*)/(*SS*) diastereomeric mixture gave three enriched fractions, and repeated chromatography was necessary to obtain pure fractions of the corresponding stereoisomers. Finally we designed a large-scale separation using two tandem Pirkle columns (250 × 30 mm). In a typical separation a dichloromethane solution of diastereomeric furnidipine (80 mg) afforded three fractions (F_1–F_3 exemplified for (*RS*)/(*SS*) in Figure 1) with 99%, 54%, and 80% de. Fraction F_3 rechromatographed under the same conditions gave the (*SS*)-diastereomer in a 96% de (F'_3 fraction in Figure 1). The analytical chromatograms of the F_1 and the rechromatographed

Scheme 5[a]



[a] (i) 2,2,6-Trimethyl-1,3-dioxo-5-en-4-one, 160 °C; (ii) 2-O_2N-C_6H_5-CHO, i-PrOH, piperidine, AcOH, r.t.; (iii) methyl 3-aminocrotonate, i-PrOH, Ar, protected from sunlight; (iv) $H_4N^+AcO^-$, EtOH, reflux; (v) 2-NO_2-$C_6H_4CH{=}C(CO_2CH_3)COCH_3$, i-PrOH, Ar, protected from sunlight; (vi) chiral chromatographic separation. (1) Determined by ^{1}H-NMR analysis with Eu(hfc)$_3$. (2) Determined by HPLC–CSP (Pirkle column). (3) Determined by HPLC–CSP (α-glycoprotein column).

F′$_3$ fractions analyzed on a α_1-acid glycoprotein column are also shown in Figure 2.

Stereochemistry and Conformational Analysis

Initial attempts to obtain suitable crystals for X-ray diffraction by crystallization of one of the separated stereoisomers failed in different solvents. This was unexpected since both racemates of furnidipine, obtained from racemic tetrahydrofurfuryl alcohol and separated by fractional crystallization from ethanol, afforded crystals of each racemate. Thus we decided to submit to X-ray analysis the yellow crystals of the most insoluble racemate. Despite of the low quality of these crystals, X-ray diffraction showed the racemate to be a mixture of (*RR*)- and (*SS*)-isomers. Crystallization from methanol gave more appropriate crystals, and Figure 3 is an ORTEP[17] drawing depicting the (*SS*)-enantiomer as it appears in the crystal while Figure 4 shows the packing[18] in the unit cell and hydrogen bonding involving the oxygen atoms of the tetrahydrofuranyl and nitro groups as H-bond acceptors (for distances and angles, see Table 2). This result showed that both enantiomers crystallize strongly associated and confirmed experimental observations (*e.g.*, the difficulty of separating diastereoisomers by crystallization, easy separation of racemates, problem of obtaining good crystals of single stereoisomers, and even some HPLC results). In the solid-state the dihydropyridine ring is in a boat form[19] flattened at N-1 (stern) and puckered at the C-4 (bow) atoms according to the X-ray numbering.[20] The 4-aryl substituent occupies a pseudoaxial position, almost orthogonal to the plane of the dihydropyridine ring (86°). This pseudoaxial position of the 4-aryl ring, which is reported to be essential for pharmacological activity,[21] is also accompanied by the rotation of the aryl ring with respect to the bisectrize of the intraannular angle C-3–C-4–C-5, the 2-nitrophenyl substituent being rotated 52° and with the nitro group adopting a *synperiplanar* (sp) arrangement lying on the same side as the H-4 hydrogen (the hydrogen at C-4). The nitro group is also

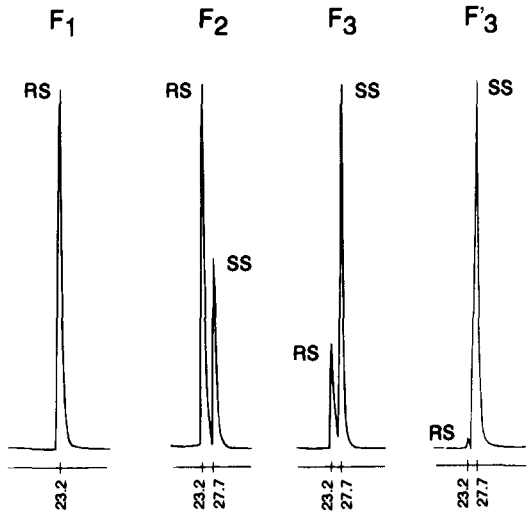


Figure 1. Analytical chromatograms from the HPLC separation of a mixture of (*RS*)-1 and (*SS*)-1 on a Pirkle column (250 × 4.6 mm i.d.). The F'_3 fraction corresponds to rechromatography of F_3 as indicated in the text and the Experimental Section. Samples (20 μL, c = 0.1 mg/mL) were injected in dichloromethane at a flow rate of 1.5 mL/min, eluted with this solvent, and detected by absorbance at 335 nm. Retention times (min) are indicated at each peak.

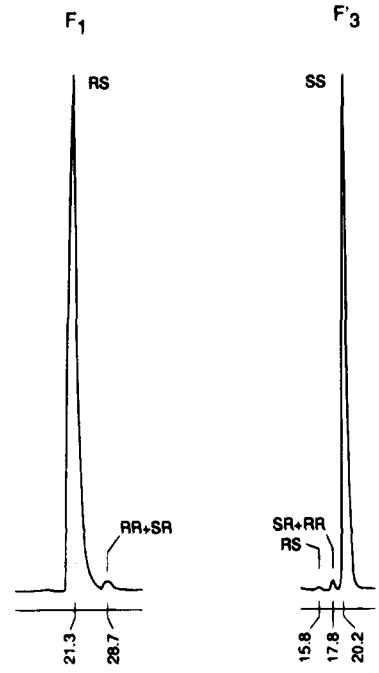


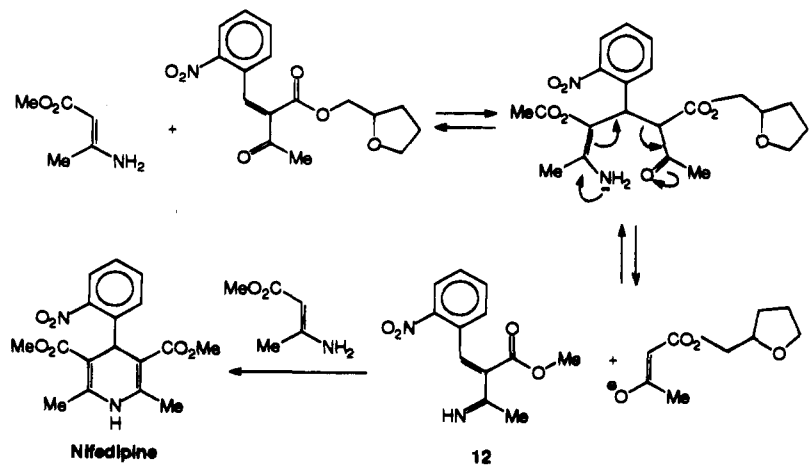
Figure 2. Analytical chromatograms for F_1 and F'_3 fractions on a α_1-acid glycoprotein (100 × 4.6 mm i.d.). Samples (20 μL, c = 0.1 mg/mL) were run with H_2O/i-PrOH (12:88), 0.01 M phosphate buffer, pH = 6.6, as eluent, at a flow rate of 0.2 mL/min and were detected by absorbance at 335 nm. Retention times (min) are indicated at each peak.

rotated 79° with respect to the plane of the aromatic ring.

As expected, both ester groups adopt an "equatorial" arrangement because of the sp^2 hybridization of the dihydropyridine C-3 and C-5 atoms. While the ester group of the tetrahydrofuran-2-ylmethyl substituent is twisted (11°) with respect to the plane of the dihydropyridine ring (C2–C3–C5–C6), the ester group of the methoxycarbonyl substituent is forced about 28° out of coplanarity. Moreover, the ester groups show a preference for the *cis/trans* arrangement[3b,22] with respect to the double bond of the dihydropyridine ring. Although a preference for the *cis/cis* arrangement is usually found for *o*-phenyl-substituted 1,4-dihydropyridines while the *cis/trans* conformations seem to be clearly more favored for derivatives without *ortho* substitution, the *cis* arrangement of at least one of the ester groups seems to be enough for a satisfactory calcium antagonist effect and hydrogen bonding to the receptor.[23] The tetrahydrofuranyl ring adopts a conformation between envelope and half-chair. It is likely that this conformation is slightly forced by the nature of the H-bond acceptor of the O-14 atom. In Tables 1 and 2 and the supporting information, the most significant crystal data are shown.

The conformational behavior of the different stereoisomers was studied using molecular mechanics and semiempirical molecular orbital calculations. Bearing in mind that enantiomers have the same preferred conformations and torsional energy profiles, only the (*SS*)- and (*SR*)-stereoisomers were studied. Geometric optimization was performed using molecular mechanics (cvff force field, Discover 2.9 program[24]). When available, X-ray data were used for the starting point geometries. The resulting structures were further refined using semiempirical methods implemented on the MOPAC 6.0 program.[25] The procedure was as follows: first, previously obtained molecular geometries were optimized using the AM1 Hamiltonian[26] and the Bryoden–Fletcher–Goldfarb–Shanno (BFGS)[27] method. The resulting geometries were then refined with the

Scheme 6



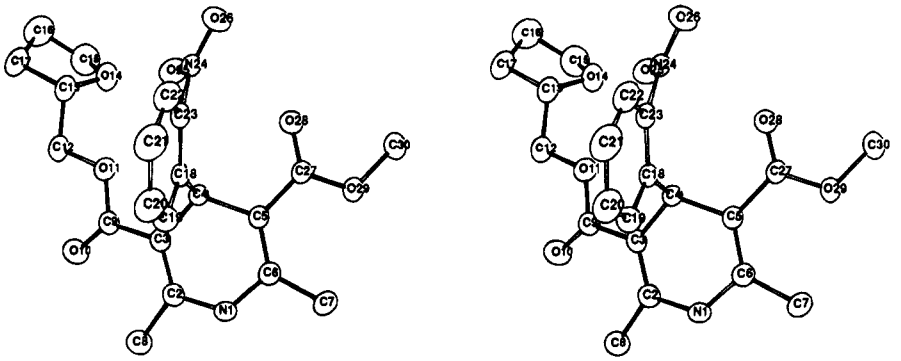


Figure 3. Stereopicture (ORTEP[17]) of the (*SS*)-methyl tetrahydrofuran-2-ylmethyl 2,6-dimethyl-4-(2′-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1). The asymmetric unit is labeled with the atomic numbering[20] used in the crystal structure analysis. H-atom labels are omitted for clarity.

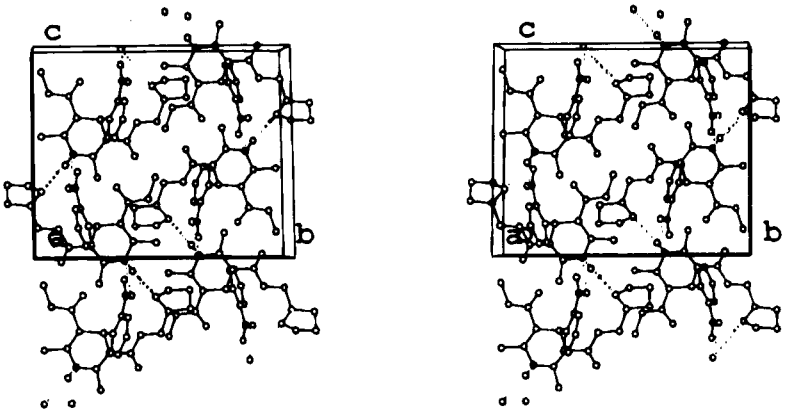


Figure 4. PLUTO[18] stereoview of the molecular packing of 1 ((*SS*)- and (*RR*)-enantiomers) along the *a*-axis showing the hydrogen bonding (nitrogens in black) drawn as dashed lines.

Table 1. Crystal Data of (*SS*)/(*RR*)-1

compound	$C_{21}H_{24}N_2O_7$
M_w	416.430
space group	$P2_1/c$
a, Å	9.988 (1)
b, Å	16.602 (2)
c, Å	13.758 (1)
β, deg	101.75
V, Å^3	2233.6 (4)
Z	4
d_{calcd}, g·cm^{-3}	1.2384
μ(Cu Kα), cm^{-1}	7.461
radiation	Cu Kα
scan mode	$\omega/2\theta$
no. of reflections measd	3636
no. of unique reflections	3270
no. of reflections with $I > 3\sigma(I)$	2430
F(000)	880
R factor	0.044
R_w	0.052
final Δ map (e Å^{-3})	0.27

Table 2. Hydrogen Bonds (Distances in Å and Angles in deg)[a]

donor (D)	H	acceptor (A)	A...H	D...A	D−H−A angle
N-1	H-1	O-14[a]	2.38 (3)	3.219 (3)	165 (3)
N-1	H-1	O-25[a]	2.78 (4)	3.190 (3)	111 (3)

[a] Symmetry operation: X, $^3/_2 - Y$, $^1/_2 + Z$.

help of the eigenvector following (EF) method[28] until a gradient norm of 0.01 kcal/mol was satisfied. The minimum heat of formation obtained was quite similar for both enantiomers: −162.98 kcal/mol for the (*RR*) and −163.16 kcal/mol for the (*RS*). Figure 5 shows the optimized geometries.

The conformational analysis focused on two major aspects: the dihedral angle between the substituted 4-aryl ring and the dihydropyridine ring and the *cis* and *trans* arrangement of the ester groups. In the optimized structures, as in the X-ray structure, the 4-aryl ring appears almost orthogonal to the dihydropyridine ring, with the substituent occupying a pseudoaxial position. In the preferred conformations shown in Figure 5, the dihedral angle (α) defined by H-4−C-4−C-18−C-19 (see Figures 3 and 6) displayed a value of −169.0° for the (*SS*)- and −167.2° for the (*SR*)-enantiomer. In order to evaluate the torsional energy profiles, a grid scan around the dihedral angle α was carried out with a grid resolution of 30°. At each angle a full geometric optimization, except for the angle under study, was performed as described above. The energy dependence on rotation around α is represented in Figure 6. The dihedral angle has two energy minima at 0° and 180°, the energy barrier to rotation being about 6 kcal/mol. These conformations are very close in energy, but, for both stereoisomers, the pseudoaxial conformation (α = 180°) is slightly more stable.

With respect to the conformation of the ester groups, heats of formation have been calculated for the different arrangements by fixing the dihedral angles involved to 0° or 180° (respectively, *cis* or *trans* with respect to the dihydropyridine double bond) and then optimizing the geometry using the aforementioned procedure. The results are represented in Figure 7. Calculations show that the *cis/cis* arrangement is the more stable conformation while the *cis/trans* arrangement, which is adopted in the solid state, is energetically less stable. However, the rotation is relatively unhindered, and it could be expected that all conformations, including the most energetically unfavorable *trans/trans*,[23b] coexist in solu-

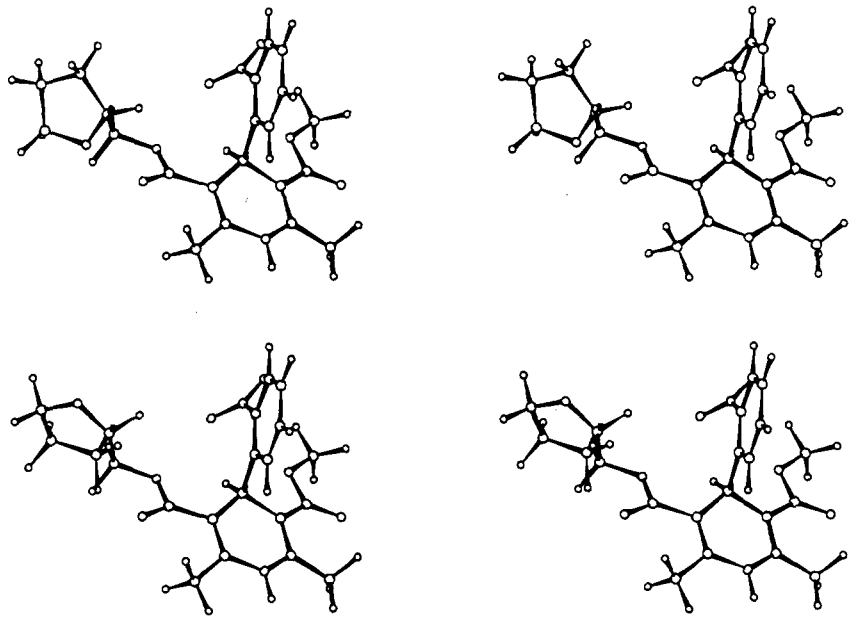


Figure 5. Stereoviews of the optimized geometries for the (*SS*) (top) and (*SR*) (bottom) diastereomers, obtained as described in the text.

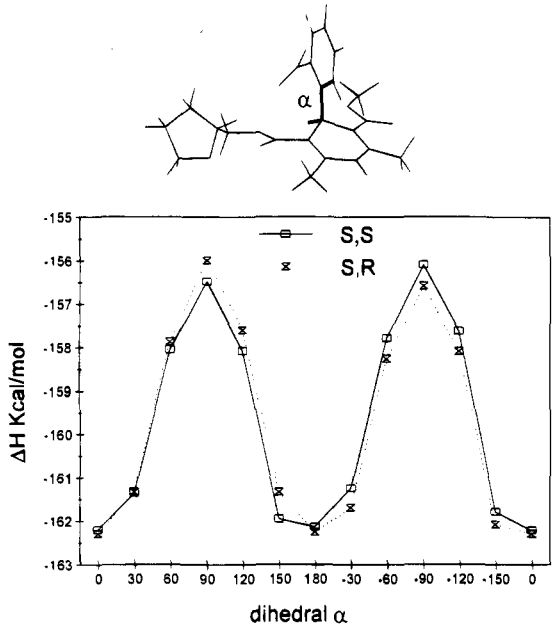


Figure 6. Energy dependence on rotation around the α dihedral for the (*SS*)- and (*SR*)-diastereomers.

tion at room temperature. The differences in the calculated heats of formation for the different conformations between the (*SS*)- and (*SR*)-stereoisomers do not reveal any significant difference in their conformational behavior.

Pharmacology

The effects of four stereoisomers of **1** on [^{3}H]isradipine binding were studied either in guinea pig ileum or rat heart and brain. Figures 8–10 show the inhibition of [^{3}H]isradipine binding. The IC_{50}'s and corresponding K_i's are shown in Table 3.

Whereas ileal longitudinal muscle (vascular smooth muscle) and heart ventricular muscle showed a similar order of potency, namely, *SS* > *SR* > *RR* > *RS*, the cerebral tissue achieved a somehow different stereospecificity profile, since the (*R*)-isomers (C-4 configuration)

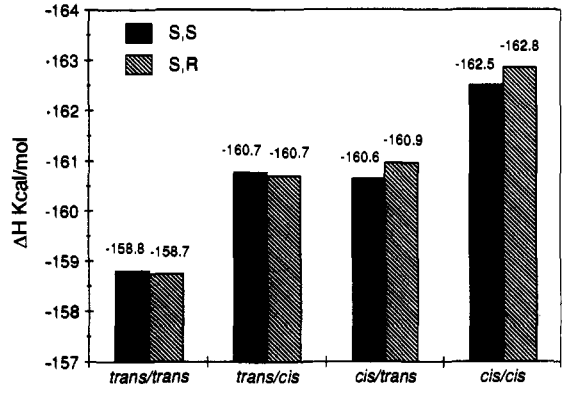


Figure 7. Representation of calculated heats of formation for different ester conformations.

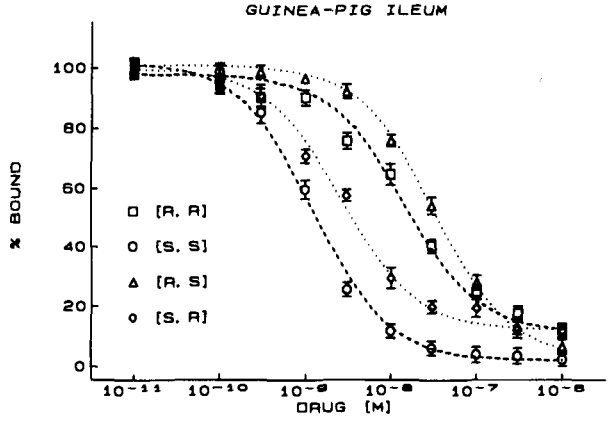


Figure 8. Displacing effect of four stereoisomers of furnidipine on [^{3}H]isradipine binding in guinea pig ileum.

were much less potent than the (*S*)-isomers. In all cases, the affinity of isomers with the *S* configuration on the asymmetric center of the dihydropyridine ring to [^{3}H]isradipine binding sites was greater than that of the isomers having the *R* configuration. The (*SS*)-isomers showed K_i values of 0.58–0.96 nM, the most active isomer, and 12.96–24.76 nM in the case of (*RS*) which was the least active. The brain manifested a more accurate discrimination of the (*SS*)- and (*RR*)-

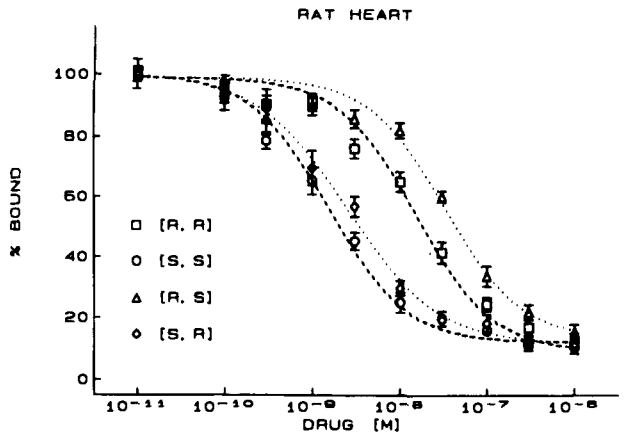


Figure 9. Displacing effect of four stereoisomers of furnidipine on [^{3}H]isradipine binding in rat heart.

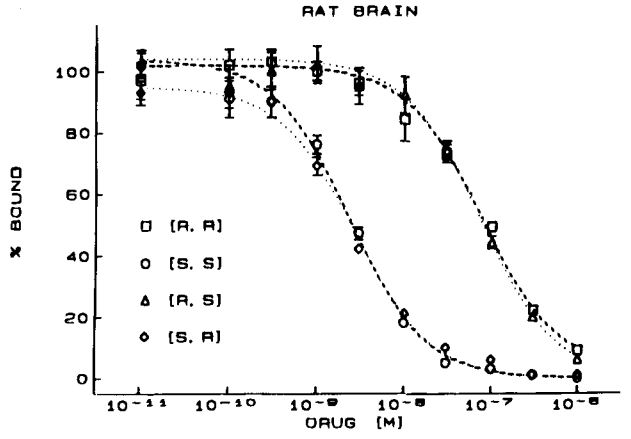


Figure 10. Displacing effect of four stereoisomers of furnidipine on [^{3}H]isradipine binding in rat brain.

Table 3. Effect on the [^{3}H]Isradipine Binding of the Stereoisomers of Fumidipine in Different Tissues

stereoisomer	tissue	IC_{50} (mean ± s.e.m.) (*n*)	K_i (nM)	B_{max} (f mol/mg of protein)	C_H[a]
RS	ileum	33.73 ± 0.86 (8)	24.76	260.7	0.98
RR		23.4 ± 2.54 (8)	11.09	249.8	0.60
SR		2.08 ± 0.54 (8)	2.04	269.0	0.99
SS		1.04 ± 0.24 (8)	0.96	299.2	0.99
RS	heart	45.1 ± 7.70 (9)	12.96	65.3	0.50
RR		15.3 ± 2.12 (9)	5.95	81.4	0.73
SR		2.4 ± 0.96 (9)	1.00	84.7	0.97
SS		1.5 ± 0.30 (9)	0.60	74.9	0.88
RS	brain	86.0 ± 6.80 (6)	18.10	77.2	0.94
RR		81.3 ± 4.11 (6)	17.70	79.1	0.42
SR		2.4 ± 0.58 (6)	0.67	79.7	0.77
SS		2.2 ± 0.89 (6)	0.58	74.9	0.88

[a] C_H: Hill coefficient.

isomers than other tissues, and the difference between IC_{50}'s values reached a factor of about 40 for any (*R*)-isomer (C-4 configuration) with respect to the (*S*)-isomers, while the factor in heart and ileum ranged approximately between 5 and 20. The meaning of this stereospecificity difference in cerebral tissue with respect to cardiac and smooth muscle tissues is unclear. Ishii et al.[29] had interpreted changes in the number of binding sites in the rat brain in the context of the role the central nervous system plays in the development and maintenance of elevated blood pressure.

Considering these results, it clearly emerges that the *S* configuration at C-4 of the dihydropyridine ring is preferred by Ca^{2+} channel binding sites, and hence furnidipine activity would also be favored by this configuration. Although the configuration at C-2 of the tetrahydrofuran ring also exerts some influence on the affinity, the effect that it provokes is clearly less pronounced. Similar results have been previously reported for dihydropyridines with known absolute configuration[30] which have been explained assuming that the two ester groups in calcium antagonists have different functions at the site of action, the smaller one being important for hydrogen bonding with the binding site and the larger one with its lipophilic group playing a significant role at the lipophilic domain of the receptor.

Experimental Section

Chemistry. All melting points were determined on an Electrothermal IA6304 apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 1310 spectrophotometer. The electron-impact (EI-MS) and chemical ionization (CI-MS) mass spectra were measured on a Hewlett-Packard 5988A. Unless otherwise stated, NMR spectra were measured on a Varian Unity 300 (300 MHz) or Varian FT-80A (80 MHz) spectrometer. Microanlyses were determined on a Heraeus CHN Rapid. Preparative high-performance liquid chromatography (HPLC) was carried out on a Shimadzu chromatograph using a Pirkle column (250 × 30 mm i.d.) packed with covalently bonded D-phenylglycine purchased from Hichrom Ltd. The enantiomeric purity of the stereoisomers of 1 was determined by analytical HPLC on a Hewlett-Packard 1050 apparatus using a Chiral-AGP column packet with α_1-acid glycoprotein (100 × 4.6 mm i.d.) purchased from ChromTech. The enantiomeric excess of other compounds was determined by ^{1}H NMR using 0.1 mol of Eu(hfc)^3 per mmol of compound in $CDCl_3$ solutions (7 mg/mL). The diastereomeric excess was determined by analytical HPLC using a Pirkle column (250 × 4.6 mm i.d.) packed with covalently bonded D-phenylglycine purchased from Hichrom Ltd. The optical rotation was measured on a Perkin-Elmer 241 MC polarimeter. Chromatographic separations were performed on a silica gel column by flash chromatography (Kiesselgel 40, 0.040–0.063 mm, Merck). Starting materials were purchased from Aldrich and Janssen and purified before using when necessary. All yields are given after purification.

(±)-Tetrahydrofuran-2-carboxylic Acid (4). A stirred solution of (±)-tetrahydrofurfuryl alcohol (40 g, 0.39 mol) in acetone (700 mL) was cooled to 0 °C, and then Jones's reagent (225 mL) recently prepared was slowly added, keeping the temperature below 20 °C. The addition was continued until the solution developed an orange color which persisted for 5 min (2.5 h was necessary). The solvent was then decanted off, and the green salts were washed with acetone (4 × 50 mL). The excess of oxidant was destroyed with isopropyl alcohol (10 mL), and the solution was adjusted to pH = 7 with $NaHCO_3$. The mixture was filtered and the filtrate concentrated under reduced pressure. The residue was saturated with NaCl and extracted with Et_2O/EtOAc (1:1, 5 × 60 mL). The extracts were dried over $NaSO_4$, and the solvent was eliminated under reduced pressure. The residue was fractionally distilled under vacuum to give 20.32 g (45%) of the racemic acid: bp 66–68 °C/0.05 mmHg (lit.[31] bp 80 °C/13 mmHg).

(*R*)-(+)-Tetrahydrofuran-2-carboxylic Acid (4b). To a hot solution of the racemic acid (47.14 g, 0.40 mol) in ethyl acetate (460 mL) was slowly added brucine dihydrate (86.56 g, 0.20 mol) with stirring. The precipitate formed was allowed to cool to room temperature and filtered. The salt (65.13 g, mp 188–192 °C) was recrystallized twice in acetonitrile (807 mL), with 340 mL of solvent being evaporated each time before filtration (44.36 g, mp 196–197 °C). This salt was recrystallized twice (680 mL of acetonitrile with evaporation of 280 mL), and after filtration 30.95 g was obtained [mp 196.5–197 °C; $[\alpha]_D = -5.6$, $c = 1.02$, MeOH (lit.[10] mp 200–200.5 °C; $[\alpha]_D = -5.8$, $c = 1.00$, MeOH)]. This fraction was treated with a mixture of 1 N hydrochloric acid (62 mL), ethyl acetate (122 mL), and diethyl ether (122 mL) and stirred for 30 min. NaCl

(20 g) was then added, and the brucine hydrochloride thus obtained was filtered and washed with a 1:1 mixture of EtOAc/Et_2O (2 × 20 mL). The aqueous phase was extracted with the same mixture of solvents (5 × 125), and the organic phases were combined, washed with a saturated NaCl solution (244 mL) containing 2.5 mL of 6 N hydrocloric acid, and dried over Na_2SO_4. The solvent was eliminated under reduced pressure and the residue distilled in vacuo to give the (*R*)-(+)-tetrahydrofuran-2-carboxylic acid as a colorless oil [5.42 g, 12%, bp 66–68 °C/0.05 mmHg; $[\alpha]_D$ = +31.1, *c* = 1.02, $CHCl_3$ (lit.[10] $[\alpha]_D$ = +30.4, *c* = 1.01, $CHCl_3$)].

(*S*)-(−)-Tetrahydrofuran-2-carboxylic Acid (4a). The acetonitrile phases obtained from the crystallization of the brucine salt were combined and acidified, the solvent was evaporated, and the residue was distilled as indicated for the (*R*)-enantiomer to give 22.45 g (bp 66–68 °C/0.05 mmHg) of a fraction enriched in the (*S*)-(−)-tetrahydrofuran-2-carboxylic acid. This fraction was dissolved in hot ethyl acetate (127 mL), and (+)-ephedrine 33.76 g, 0.194 mol) was added. The solution was seeded with a few crystals of the salt of (+)-ephedrine obtained with pure (*S*)-acid in the way indicated below and allowed to cool to room temperature. The precipitate was filtered (35.66 g, mp 105–109 °C) and recrystallized three times using 518, 272, and 125 mL of solvent (EtOAc). The salt resulting from the last crystallization [19.58 g, mp 118–118.5 °C; $[\alpha]_D$ = + 13.1, *c* = 1.00, MeOH (lit.[10] mp 116–116.5 °C; $[\alpha]_D$ = +13.8, *c* = 1.00, MeOH)] was treated as indicated for the (*R*)-enantiomer to give the (*S*)-(−)-tetrahydrofuran-2-carboxylic acid as a colorless oil [5.60 g, 12% yield, bp 66–68 °C/0.05 mmHg; $[\alpha]_D$ = −36.0, *c* = 1.21, $CHCl_3$ (lit.[10] $[\alpha]_D$ = −30.14, *c* = 1.21, $CHCl_3$)].

(*S*)-(+)-Methyl and (*R*)-(−)-Methyl Tetrahydrofuran-2-carboxylate (5a and 5b). Into a solution of the corresponding (*R*)- or (*S*)-acids **4a** or **4b** (4.08 g, 35.2 mmol) in Et_2O (5 mL) placed in a flask collector was distilled diazomethane (2.74–2.84 g) prepared from diazald (20 g). The mixture was stirred for 1 h at 0 °C, and then the excess reagent was eliminated by distillation into a solution of AcOH/Et_2O until a colorless distillate was obtained. After elimination of the solvent, the residue obtained was distilled under reduced pressure to give the corresponding ester (4.07 g, 89% yield) as a colorless liquid. (*S*)-(+)-Methyl tetrahydrofuran-2-carboxylate (**5a**): bp 70 °C/0.5 mmHg; $[\alpha]_D$ = +8.5, *c* = 1.04, MeOH (lit.[10] bp 50 °C/0.1 mmHg; $[\alpha]_D$ = +8.1, *c* = 1.05, MeOH). (*R*)-(−)-Methyl tetrahydrofuran-2-carboxylate (**5b**): bp 70 °C/0.5 mmHg; $[\alpha]_D$ = −8.4, *c* = 0.92, MeOH (lit.[10] bp 50 °C/0.1 mmHg; $[\alpha]_D$ = −8.0, *c* = 0.92, MeOH).

(*S*)-(+)-Tetrahydrofuranylmethanol and (*R*)-(−)-2-Tetrahydrofuranylmethanol (2a and 2b). Method A. A solution of the (*R*)- or (*S*)-ester **5a** or **5b** (3.52 g, 27.2 mmol) in ethanol (30 mL) was cooled in an ice–water bath, and $NaBH_4$ (1.54 g, 40.8 mmol) was slowly added. The mixture was heated at reflux for 3 h and then cooled to 0 °C and ajusted to pH = 7 with 15% HCl. The solvent was eliminated under reduced pressure and the aqueous residue saturated with NaCl, extracted with Et_2O, and dried over $MgSO_4$. After elimination of the solvent, the residue was fractionally distilled to give the corresponding (*S*)- or (*R*)-alcohols (2.05 g, 74%). **(*S*)-2a**: bp 185 °C/760 mmHg; $[\alpha]_D$ = +16.6, *c* = 5.35, $CHCl_3$ (lit.[9a] bp 79 °C/20 mmHg; lit.[9b] $[\alpha]_D$ = +14.9, *c* = 5.0, CH_3NO_2); IR ($CHBr_3$) ν_{max} 3406, 2974, 2871, 2258, 1454, 1401, 1041, 923 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.5–1.7 (m, 1H), 1.8–2.0 (m, 1H), 2.45 (s, 1H), 3.48 (dd, 1H, *J* = 11.5 and 6.4 Hz), 3.65 (dd, *J* = 11.5 and 3.2 Hz), 3.7–3.9 (m, 2H), 3.9–4.1 (m, 1H). **(*R*)-2b**: bp 178 °C/760 mmHg; $[\alpha]_D$ = −15.8, *c* = 5.35, $CHCl_3$ (lit.[9a] bp 74.5 °C/14 mmHg; $[\alpha]_D$ = −17.1, *c* = 5.34, $CHCl_3$, *l* = 2).

Method B. A solution of the (*R*)- or (*S*)-acids **4a** or **4b** (1.0 g, 8.60 mmol) in dry THF (12 mL) was cooled to 0 °C, and 2 M BH_3·SMe_2 in THF (1.4 g, 18.50 mmol) was added under Ar. The reaction mixture was stirred for 2 h at room temperature, then cooled to 0 °C, and adjusted to pH = 7 with 17% HCl. The reaction mixture was worked up as indicated in method A to afford 0.50 g (57% yield) of the corresponding alcohol. **(*S*)-2a**: $[\alpha]_D$ = +17.7, *c* = 5.02, $CHCl_3$. **(*R*)-2b**: $[\alpha]_D$ = −17.0, *c* = 4.94, $CHCl_3$.

Methyl 1-(Ethoxymethyl)-2,6-dimethyl-4-(2′-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (6). To a solution of nifedipine (2.0 g, 5.78 mmol) in DMF (8 mL) was slowly added under Ar at −4 °C sodium hydride (430 mg, 14.15 mmol as an 80% dispersion in mineral oil). The mixture was stirred for 80 min at room temperature and then cooled to −4 °C. Chloromethyl ethyl ether (1.34 mL, 14.45 mmol) was added, and stirring was continued at room temperature for 70 min. After that time the reaction mixture was quenched with water (0.8 mL) at 4 °C and extracted with dichloromethane (6 × 20 mL). The organic phase was washed with a saturated NaCl solution (20 mL) and dried over $MgSo_4$ and the solvent eliminated under vacuum. The solid residue was crystallized from methanol to give the dihydropyridine derivative **6** (1.78 g, 76%) as yellow–greenish crystals: mp 134–135 °C; IR (KBr) ν_{max} 1703, 1585, 1530, 1359, 1386, 1281, 1218, 1192, 1073, 999 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60 (d, 1H, *J* = 8.0 Hz), 7.5–7.3 (m, 2H), 7.25 (d, 1H, *J* = 5.6 Hz), 5.61 (s, 1H), 4.90 (s, 1H), 3.63 (s, 6H), 3.55 (*c*, 2H, *J* = 7.1 Hz), 2.47 (s, 6H), 1.29 (t, 3H, *J* = 7.1 Hz); MS (CI) (rel intens) *m/z* 326 (5), 328 (5), 342 (5), 358 (9.5), 372 (100), 373 (22), 403 (8), 404 (32), 405 (7), 432 (10). Anal. ($C_{20}H_{24}N_2O_7$) C, H, N.

1-(Ethoxycarbonyl)-2,6-dimethyl-5-(methoxycarbonyl)-4-(2′-nitrophenyl)-1,4-dihydropyridine-3-carboxylic Acid (7). To (dimethylamino)-2-propanol (9.5 mL) was slowly added sodium (840 mg, 36.70 g), and the mixture was stirred at room temperature for 90 min. Then a solution of water (3 mL) and (dimethylamino)-2-propanol (10 mL) was added dropwise until the sodium was completely dissolved (stirring for 5 h at 40 °C was necessary). The resulting solution was cooled to −4 °C, and a solution of **6** (1.38 g, 4.52 mmol) in benzene (13 mL) was slowly added. The reaction mixture was stirred at room temperature for 2.5 h, the solvent eliminated under reduced pressure, and the residue adjusted in an ice–water bath to ph = 5.3 with 350 mL of a 1.4 M phsophate buffer of pH = 5.1 in an ice–water bath. The aqueous phase was saturated with NaCl and extracted continuously with ethyl ether for 7 h. The extracts were dried over $MgSO_4$, and the solvent was eliminated at reduced pressure to give a residue that was chromatographed on silica gel. Elution with toluene–ethyl acetate (6:4) afforded **7**. Crystallization from methanol (380 mg, 22%) gave yellow crystals: mp 143–145 °C (MeOH); IR (KBr) ν_{max} 1730, 1680, 1660, 1635, 1590, 1570, 1535, 1395, 1370, 1285, 1220, 1200, 1075 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.9–6.9 (m, 5H), 5.61 (s, 1H), 4.85 (s, 1H), 3.62 (s, 3H), 3.52 (q, 2H, *J* = 7.2 Hz), 2.50 (s, 3H), 1.27 (t, 3H, *J* = 7.2 Hz). Anal. ($C_{19}H_{22}N_2O_7$) C, H, N.

Methyl 2,6-Dimethyl-4-(2′-nitrosophenyl)pyridine-3,5-dicarboxylate (8a). Method A. A solution of nifedipine (2.0 g, 5.8 mmol) in dichloromethane (60 mL) was exposed to sunlight at room temperature for 3 days. The solvent was eliminated under vacuum, and the oily residue obtained was chromatographed on silica gel. Elution with acetone–dichoromethane (9:1) gave the title compound that after crystallization from hexane–ethyl acetate (1.34 g, 72%) afforded green crystals: mp 80–82 °C; IR (KBr) ν_{max} 1728, 1558, 1492, 1437, 1293, 1243, 1160, 1110, 1042, 774 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.71 (t, 1H, *J* = 8.8 Hz), 7.51 (d, 1H, *J* = 7.6 Hz), 7.43 (t, 1H, *J* = 8.3 Hz), 6.55 (d, 1H, *J* = 8.8 Hz), 3.38 (s, 6H), 2.66 (s, 6H); MS (CI) (rel intens) *m/z* 59 (42), 152 (24), 193 (23), 237 (19), 252 (28), 253 (41), 267 (31), 269 (100), 284 (22), 328 (24). Anal. ($C_{17}H_{16}N_2O_5$) C, H, N.

Method B. Following the procedure above indicated for the synthesis of the acid **7** and after workup and chromatography of the reaction mixture, 230 mg of **8a** were isolated.

Methyl Tetrahydrofuran-2-ylmethyl 2,6-Dimethyl-4-(2′-nitrosophenyl)pyridine-3,5-dicarboxylate (8b). A solution of furnidipine (1.0 g, 2.40 mmol) in dichloromethane (25 mL) was exposed to sunlight at room temperature for 7 days. The solvent was eliminated under vacuum, and the oily residue obtained was chromatographed on silica gel. Elution with acetone–dichloromethane (9:1) gave the title compound as a blue oil (0.71 g, 71%): IR ($HCBr_3$) ν_{max} 1726, 1558, 1500, 1437, 1291, 1235, 1084, 1041, 691 cm^{-1}; ^1H NMR δ 7.8–7.7 (m, 1H), 7.6–7.5 (m, 1H), 7.5–7.4 (m, 1H), 6.6–6.5 (m, 1H), 3.9–3.8 (m, 1H), 3.8–3.5 (m, 4H), 3.37 (s, 3H), 2.69 (s, 3H), 2.68 (s, 3H), 1.8–1.6 (m, 3H), 1.3–1.1 (m, 1H); MS (CI) (rel intens)

m/z 85 (44), 152 (52), 153 (41), 221 (53), 236 (46), 237 (61), 238 (58), 253 (65), 270 (100), 398 (13). Anal. ($C_{21}H_{22}N_2O_6$) C, H, N.

(*S*)-(+)-Tetrahydrofuran-2-yl and (*R*)-(−)-Tetrahydrofuran-2-yl Acetoacetates (9a and 9b). A mixture of 2,2,6-trimethyl-1,3-diox-5-en-4-one (1.8 g, 12.8 mmol) and the corresponding (*S*)- or (*R*)-2-tetrahydrofuranylmethanol **2a** or **2b** (1.31 g, 12.8 mmol) was heated in xylene (3 mL) at 160 °C for 1 h. The solvent was eliminated and the residue distilled at reduced pressure. **(*S*)-9a**: From 1.50 g (14.7 mmol) of the (*S*)-alcohol **2a**, 2.80 g (91%) were obtained: bp 150 °C/3.5 mmHg; $[\alpha]_D = +21.8$, $c = 1.59$, $CHCl_3$; IR ($CHBr_3$) ν_{max} 3495, 2875, 1743, 1715, 1360, 1315, 1271, 1152, 1084, 1032 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.3−4.0 (m, 3H), 3.9−3.7 (m, 2H), 3.50 (s, 2H), 2.27 (s, 3H), 2.1−1.8 (m, 3H), 1.7−1.5 (m, 1H). **(*R*)-9b:** From 1.31 g (12.8 mmol) of the (*R*)-alcohol **2b**, 2.20 g (94%) were obtained: bp 150 °C/3.5 mmHg; $[\alpha]_D = -20.4$, $c = 1.62$, $CHCl_3$. Anal. ($C_9H_{14}N_4$) C, H, N.

(*S*)-(+)-Tetrahydrofuran-2-yl and (*R*)-(−)-Tetrahydrofuran-2-yl 3-Aminocrotonates (10a and 10b). To a solution of the corresponding (*S*)- or (*R*)-tetrahydrofuran-2-yl acetoacetate **9a** or **9b** (2.0 g, 10.8 mmol) in EtOH (10 mL) was added ammonium acetate (4.16 g, 54.0 mmol), and the mixture was heated at reflux for 6 h. After elimination of the solvent, the residue was purified by crystallization from MeOH. **(*S*)-10a**: From 2.66 g (14.3 mmol) of **9a**, 1.98 g (75%) were obtained: mp 80.5−81.5 °C (white crystals); $[\alpha]_D = +31.7$, $c = 1.11$, $CHCl_3$; IR (KBr) ν_{max} 3412, 3308, 1663, 1623, 1568, 1293, 1160, 1082, 1034, 990 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.58 (s, 1H), 4.2−3.7 (m, 5H), 2.1−1.8 (m, 6H), 1.7−1.5 (m, 1H). **(*R*)-10b**: From 2.0 g (10.8 mmol) of **9b**, 1.47 g (74%) were obtained: mp 79.5−80.5 °C; $[\alpha]_D = -27.3$, $c = 1.22$, $CHCl_3$. Anal. ($C_9H_{15}NO_3$) C, H, N.

(*S*)-(+)-Tetrahydrofuran-2-yl and (*R*)-(−)-Tetrahydrofuran-2-yl 2-[(2′-Nitrophenyl)methylene]acetoacetates (11a and 11b). A mixture of 2-nitrobenzaldehyde (2.52 g, 1.67 mmol), the corresponding (*S*)- or (*R*)-tetrahydrofuran-2-yl acetoacetate **10a** or **10b** (310 mg, 1.67 mmol), piperidine (15 μL), and AcOH (40 μL) was stirred at 40 °C for 30 min, then at room temperature for 15 h, and finally at 0 °C for 1 h. The reaction mixture was cooled to 0 °C and allowed to stand at this temperature overnight. The solid thus obtained was filtered and crystallized from EtOH. **(*S*)-11a**: From 420 mg (2.26 mmol) of **10a**, 490 mg (68%) were obtained: mp 60−62 °C (yellow crystals); $[\alpha]_D = +15.9$, $c = 0.95$, $CHCl_3$; IR (KBr) ν_{max} 1720, 1670, 1567, 1527, 1402, 1379, 1338, 1244, 1079, 988 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.23 (d, 1H, $J = 8.1$ Hz), 8.08 (s, 1H), 7.7−7.4 (m, 3H), 4.2−3.5 (m, 5H), 2.49 (s, 3H), 1.9−1.7 (m, 3H), 1.5−1.2 (m, 1H). **(*R*)-11b**: From 310 mg (1.67 mmol) of **10b**, 340 mg (65%) were obtained: mp 60−62 °C (yellow crystals); $[\alpha]_D = -17.0$, $c = 0.85$, $CHCl_3$. Anal. ($C_{16}H_{17}NO_6$) C, H, N.

(*SS*)-Methyl and (*RS*)-Methyl Tetrahydrofuran-2-ylmethyl 2,6-Dimethyl-4-(2′-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1, Furnidipine). Route A. A mixture of methyl 2-[(2′-nitrophenyl)methylene]acetoacetate (1.76 g, 5.91 mmol) and (*S*)-tetrahydrofuran-2-yl 3-aminocrotonate (**10a**; 680 mg, 5.91 mmol) in i-PrOH (5 mL) under an argon atmosphere and protected from sunlight was heated at reflux temperature for 10 h. The solvent was eliminated at reduced pressure and the residue crystallized from CCl_4 to give 2.39 g (97%) of 1 as a yellow powder, being a 50:50 mixture of the (*SS*)- and (*RS*)-diastereoisomers: mp 102−105 °C.

Route B. A mixture of methyl 3-aminocrotonate (190 mg, 1.63 mmol) and (*S*)-tetrahydrofuran-2-yl 2-[(2′-nitrophenyl)-methylene]acetoacetate (**11a**; 520 mg, 1.63 mmol) in i-PrOH (2 mL) under argon and protected from sunlight was heated at reflux for 10 h. The solvent was eliminated at reduced pressure and the residue crystallized from CCl_4 to give 580 mg (85%) of 1 as a yellow powder, which was identified as a 50:50 mixture of the (*SS*)- and (*RS*)-diastereoisomers.

(*RR*)-Methyl and (*SR*)-Methyl Tetrahydrofuran-2-ylmethyl 2,6-Dimethyl-4-(2′-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1, Furnidipine). Route A. Following the same procedure above indicated in route A, from 500 mg (4.35 mmol) of (*R*)-tetrahydrofuran-2-yl 3-aminocrotonate (**10b**) were obtained 1.77 g (98%) of the (*RR*)/(*SR*) mixture: mp 106−109 °C.

Route B. Following the procedure above indicated in route B, from 480 mg (1.50 mmol) of (*R*)-tetrahydrofuran-2-yl 2-[(2′-nitrophenyl)methylene]acetoacetate (**11b**) were obtained 550 mg (88%) of the (*RR*)/(*SR*) mixture: mp 106−109 °C.

Chromatographic Separation of Diastereomers of Furnidipine. General Procedure. Samples of the mixture of diastereomers were separated on two Pirkle columns (250 × 30 mm) connected in series. Samples (0.5 mL, $c = 80$ mg/mL) were injected as CH_2Cl_2 solutions, at a flow rate of 22 mL/min of CH_2Cl_2 as eluent, and detected by absorbance at 254 nm. In a typical separation a solution of diastereomeric furnidipine (1) afforded three fractions (F_1−F_3 in Figure 1). The fraction F_3 was rechromatographed under the same conditions. From 1.22 g of a 50:50 mixture of (*RS*)/(*SS*)-diastereomers, 0.46 g (38%), of the (*RS*) and 0.22 g (18%) of the (*SS*) were obtained. From 1.0 g of the (*RR*)/(*RS*) mixture, 0.40 g (40%) of the (*SR*) and 0.16 g (16%) of the (*RR*) were obtained.

The diastereomeric excess of the enriched fractions was determined by analytical HPLC using a Pirkle column (250 × 4.6 mm i.d.). Samples (20 μL, $c = 0.1$ mg/mL) were injected in CH_2Cl_2 at a flow rate of 1.5 mL/min and detected by absorbance at 335 nm. The enantiomeric purity of the four stereoisomers of 1 was determined by analytical HPLC using H_2O/i-PrOH (12:88), 0.01 M phoshpate buffer, pH = 6.6, as eluent, and a flow rate of 0.2 mL/min. Injected samples (20 μL, $c = 0.1$ mg/mL) detected by absorbance at 335 nm gave the chromatograms shown in Figure 2.

(*SS*)-1: mp 82−84 °C (CCl_4); $[\alpha]_D = +120.0$, $c = 0.52$, $CHCl_3$; IR (KBr) ν_{max} 3301, 2925, 1704, 1647, 1527, 1488, 1352, 1304, 1271, 1205, 1093 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.69 (dd, 1H, $J = 8$ and 1 Hz), 7.6−7.4 (m, 2H), 7.3−7.2 (m, 1H), 5.99 (bs, 1H), 5.77 (s, 1H), 4.2−3.6 (m, 5H), 3.56 (s, 3H), 2.33 (s, 3H), 2.30 (s, 3H), 2.0−1.7 (m, 3H), 1.6−1.4 (m, 1H). **(*RR*)-1**: mp 81−84 °C (CCl_4); $[\alpha]_D = -121.5$, $c = 0.54$, $CHCl_3$). **(*RS*)-1**: mp 53−55 °C (CCl_4); $[\alpha]_D = -154.3$, $c = 0.49$, $CHCl_3$; IR (KBr) ν_{max} 3297, 2946, 1697, 1528, 1496, 1354, 1275, 1208, 1115 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.8−7.1 (m, 4H), 6.03 (bs, 1H), 5.75 (s, 1H), 4.2−3.6 (m, 5H), 3.54 (s, 3H), 2.31 (s, 3H), 2.99 (s, 3H), 2.0−1.7 (m, 3H), 1.6−1.4 (m, 1H). **(*SR*)-1**: mp 53−55 °C (CCl_4); $[\alpha]_D = +154.7$, $c = 0.51$, $CHCl_3$. Anal. ($C_{21}H_{23}N_2O_7$) C, H, N.

Separation of Racemates of Furnidipine. Furnidipine (7.0 g) obtained from (±)-tetrahydrofuran-2-yl 2-[(2′-nitrophenyl)methylene]acetoacetate was fractionally crystallized three times from ethanol to give two enriched fractions. The most insoluble one (1.57 g, >99% de) was used for the X-ray structure determination ((*SS*)/(*RR*)): mp 169−170 °C (EtOH); IR (KBr) ν_{max} 3301, 2925, 1704, 1647, 1527, 1488, 1352, 1304, 1271, 1205, 1093 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.69 (dd, 1H, $J =$ 8.1 and 1.0 Hz), 7.6−7.4 (m, 2H), 7.3−7.2 (m, 1H), 5.99 (s, 1H), 5.75 (s, 1H), 4.2−3.9 (m, 3H), 3.6−3.9 (m, 2H), 3.56 (s, 3H), 2.33 (s, 3H), 2.30 (s, 3H), 2.0−1.7 (m, 3H, bs), 1.6−1.4 (m, 1H). The most soluble fraction (35 g) enriched in the (*SR*)/(*RS*) racemate (16.5:83.5) was fractionally recrystallized six times from ethanol to give 1.7 g of racemate (>98.2% de): mp 79−81 °C (CCl_4); IR (KBr) ν_{max} 3297, 2946, 2873, 1697, 1528, 1496, 1354, 1275, 1208, 1115, 1096 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.8−7.1 (m, 4H), 6.03 (bs, 1H), 5.78 (s, 1H), 4.2−3.6 (m, 5H), 3.54 (s, 3H), 2.31 (s, 3H), 2.29 (s, 3H), 2.0−1.7 (m, 3H), 1.6−1.4 (m, 1H).

Crystallographic Work. Crystals of the most insoluble racemate obtained after fractional crystallization from absolute ethanol were grown from methanol. Measurements of diffraction were carried out on a Seifert automatic four-circle diffractometer, using graphite-monochromated Cu Kα radiation. The unit cell dimensions were obtained by least-squares refinement from 25 reflections with accurate θ angle. The data were corrected for Lorentz polarization effects. Only reflections with $I > 3\sigma(I)$ were considered. The structure was solved by direct methods (SIR 92),[32] H atoms being located from difference maps. Refinement was carried out using anisotropic temperature factors for non-hydrogen atoms and isotropic factors for H atoms. Atomic scattering factors were taken from

International Tables for X-ray Crystallography[33] and calculations were performed using X-RAY80[34] and PARST.[35] The crystal data and experimental details are summarized in Tables 1 and 2, and the final atomic parameters are given in the supporting information.

Conformational Analysis. All the work was performed on a Silicon Graphics INDIGO workstation. Biosym Software was employed for visualization (Insight-II 2.3) and molecular mechanics calculations (DISCOVER 2.9). For the molecular orbital semiempirical calculations the AM1 method implemented in the standard version of MOPAC 6.0 was used, as described above.

Radioligand Binding Assays. Preparation of Microsomes from Ileum, Heart, and Brain. Membrane preparations and binding assays were essentially as described previously by Bolger et al.[36] Albino guinea pig ileal longitudinal muscle was removed and placed in a Tyrode solution. The longitudinal muscle was removed from the underlying circular muscle. The tissue was then minced with scissors on a cold Tyrode solution and homogenized in 15 volumes·g^{-1} wet weight in an ice-fold 50 mM Tris buffer (pH 7.0, 25 °C) containing (phenylmethyl)sulfonyl fluoride (17.4 mg mL^{-1}). Eight passes of a motor-driven glass–Teflon pestle homogenizer (Potter Elvehjem) were used to prepare the homogenate. This homogenate was then centrifuged at 1000*g* for 20 min at 4 °C (Minifuge T, Heraeus); the supernatant was centrifuged at 45 000*g* for 45 min at 4 °C (Beckman, 18–60M). The resulting pellet was resuspended in a cold 50 mM Tris buffer (66 mg mL^{-1}) and recentrifuged at 45 000*g* for 45 min at 4 °C. The pellet was again resuspended in a 50 mM Tris buffer at a mean protein concentration of 5–10 mg of protein mL^{-1}.

Sprague Dawley rat hearts were quickly excised in full and put in a cold 50 mM Tris buffer (pH 7.4) plus (phenylmethyl)-sulfonyl fluoride. The ventricle muscle was separated, cleaned of fat, minced with scissors, and homogenized in a Sorvall homogenizer three times for 20 s at $^3/_4$ maximum speed at 30-s intervals in an ice bath (3.5 g of tissue in 50 mL of buffer). After suction through two layers of cotton wool, the homogenate was centrifuged at 3000*g* for 10 min at 4 °C. The supernatant was centrifuged at 10 000*g* for 10 min and finally at 100 000*g* in an ultracentrifuge. The pellet was resuspended in a Tris buffer and homogenized in a Teflon–glass Potter Elvehjem homogenizer.

Sprague Dawley rat brains were quickly excised in full, minced with scissors, and washed twice in cold 25 mM HEPES/NaOH and 0.32 M sucrose buffer (pH 7.4), plus 0.1 mM (phenylmethyl)sulfonyl fluoride. The homogenization was carried out in a Teflon Potter homogenizer, at setting 5 seven to ten times for 30 s in an ice bath (1 g of tissue in 10 mL of buffer). The homogenate was centrifuged at 3000 rpm for 5 min at 4 °C, the supernatant was removed, and the pellet was washed and recentrifuged. The combined supernatants were centrifuged four times at 12 500 rpm for 30 min at 4 °C (Biofuge 17RS, Heraeus), and finally the resultant pellets were resuspended together in 25 mM HEPES/NaOH buffer.

Binding Assay. Binding was carried out at room temperature in the presence of 0.5 mM $CaCl_2$ by using 0.2 nM [^{3}H]-(+)-methylisradipine (Amersham; specific activity 85 Ci/mmol). The compounds were added at concentrations ranging from 10^{-6} to 10^{-11} M from stock solutions of 2.5×10^{-4} M in ethanol. After preincubation (5 min) of the microsomes (0.3 mg of protein/mL) with the compounds, the incubation was started by adding the isotope. After 60 min, 1-mL samples were filtered and washed (3 mL each) four times in a M-24R Cell Harvester (Brandel). The filters (Whatman GF/B) were counted in Ready-Protein scintillation fluid (Beckman). Unlabeled or labeled successive concentrations were prepared by diluting the stock solutions with 50 mM Tris buffer. The protein content was determined by the method of Lowry. The microsomes were frozen in liquid nitrogen and kept at −70 °C until used. All experiments were carried out under a sodium lamp. Unspecific binding was calculated from 1 μM cold isradipine.

In radioligand binding studies, all experiments were repeated two to three times in triplicate, with nonspecific binding determined each time, and the analysis was carried out by means of the EBDA-LIGAND program developed by Elsevier–Biosoft.

Acknowledgment. We gratefully acknowledge ALTER S.A. for a studentship (to R.A.) and financial support. We also thank Dr. F. Gago for his helpful contribution in the conformational analysis study.

Supporting Information Available: Crystallographic data (5 pages). Ordering information is given on any current masthead page.

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JM940538D