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Synthesis and biological activity of the calcium modulator (*R*) and (*S*)-3-methyl 5-pentyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate

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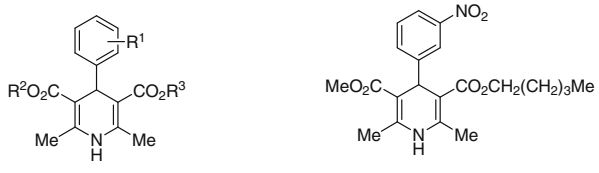
ABSTRACT

An efficient total synthesis of (*R*) and (*S*)-3-methyl 5-pentyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate in high optical purities is reported. The useful step is the resolution of racemic 2, 6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid by using commercially available *Cinchona* alkaloids cinchonidine and quinidine as the resolving agents. Under the optimum conditions, the optical purities for *R*- and *S*-enantiomers are extremely high (ee >99.5%). The further dihydropyridine receptor binding activity assay shows that the *S*-enantiomer is more potent than *R*-enantiomer both in rat cardiac (approximately 19 times) and cerebral cortex membrane (12 times).

1,4-Dihydropyridines (1,4-DHPDs), a class of calcium modulators, are widely used in clinic because of their biological actions as drugs in vasodilatation, hepatoprotection, neuromodulatory, cognition, memory enhancing, neuroprotection, anti-atherosclerosis, anti-diabetes, antioxidant, anti-mutagenic, and anti-tumor.[1] The different activity of 1,4-dihydropyridines derivatives often derives from the functional groups substituted on the 1,4-DHPDs moiety. When substituents on the left side differ from those on the right side of 1,4-DHPDs, the molecule is chiral, with the carbon at position 4 as the stereogenic centre and exists as two enantiomers. Some years ago, a great deal of effort was devoted to investigating the role of the chirality in dihydropyridine ring. The enantiomers of an unsymmetrical 1,4-DHPDs usually differ in their biological activities and even have an exactly opposite activity profile.[2] For example, the *S*-enantiomer of manidipine is about 30–80 times more potent than the *R*-isomer in its antihypertensive action.[3,4] In some case, the activity of each enantiomer was often totally different, one of them being a calcium antagonist of the channel and the other an agonist.[5] Chiral 4-aryl-1,4-DHPDs have been extensively investigated as calcium modulator for the last two decades and some chiral 1,4-DHPDs have been synthesized by several research groups through an asymmetric induction[6] or through the resolution of racemic mixture.[3,7]

3-Methyl 5-pentyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (MN9202, **1,** Fig. 1) is a promising 1,4-DHPDs analogue screened by our group, which have potent pharmacological activities[8] in the treatment of myocardial hypertrophy, membrane lipid peroxidation, platelet aggregation, antiproliferation, anticonvulsant, anti-hypercholesterolemia, and anti-atherosclerosis. All of these earlier studies were, however, only carried out with racemic MN9202 on calcium channels. The title compounds have never been prepared in enantiomerically pure form, and the role of stereoselectivity in MN9202 were not addressed.

In light of the chiral and chemical peculiarities of dihydropyridines, the investigation whether the chirality at position 4 of MN9202 influence the pattern of calcium channel modulators became very important for improving the therapeutical application. For this purpose, the single enantiomers were synthesized and the dihydropyridine receptor binding activities were also detected in rat cardiac and cerebral cortex membrane.



1,4-Dihydropyridines (1,4-DHPDs) MN9202 (1)

Figure 1. Chemical structures of 1,4-dihydropyridine derivatives.

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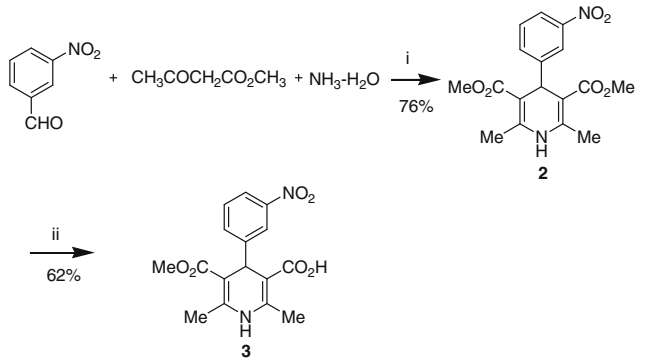


Scheme 1. Reagents and conditions: (i) EtOH, rt, 1 h; refluxing, 7 h; (ii) 10 M NaOH, MeOH, refluxing, 9 h; 2 M HCl, pH = 3.

The synthesis of racemic 2,6-dimethyl-5-methoxy carbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid **3** was illustrated in Scheme 1. Using commercially available 3-nitrobenzaldehyde, methyl 3-oxobutanoate, and $NH_3{\cdot}H_2O$ as starting materials, the 3,5-dimethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate **2** was prepared in 76% yield via the Hantzsch three component condensation in the usual manner according to similar method described in previous Letter.[9] When treated **2** with 10 M NaOH in MeOH–H_2O, the desired product **3** was obtained in 62% yield after recrystallization with MeOH–H_2O.

With the acid **3** in hand, the resolution protocol using chiral organic bases was tried. Shibanuma et al.[7b] have reported the resolution method using cinchonine and cinchonidine to obtain the single enantiomers of **3**, but the resolution process need the protected and deprotected approach for NH in 1,4-DHPDs ring. The optically active 1,4-dihydropyridine derivatives have been synthesized from the enantiomers of the 1-ethoxymethylated carboxylic acid followed by removal of the prior NH-protecting group. We attempted to use unprotected racemic acid **3** for direct resolution and the resolution was investigated in detail. The results were shown in Table 1. When using cinchonidine and quinidine as chiral resolving agents, the unprotected racemic acid can be resolved by the formation of 3-cinchonidine salt and 3-quinidine salt. In the resolution process, the solvent was very important and has been chosen carefully because of its effect on the efficiency of resolution and the ease of crystallization.[10] When performed the racemic acid with resolving agent cinchonidine in EtOH and acetone, the resolution was totally inefficient and the ee value was very poor (Table 1, entries 1 and 2). DMF allowed an improvement for ee value to 99%, but the crystallization yield was very low (26%, entry 3). Addition of water to the DMF has shown the favorable effect in crystallization. But while the water was increased, the ee value was decreased (entries 4–7). When DMF/H_2O (8:5, v/v) was used, the enantiomeric acid **3** was obtained successfully in high ee and yield (entry 5). Solvent of DMF/H_2O (8:5) was also efficient when performed the racemic acid with resolving agent quinidine (entry 8). The free enantiomeric acid **3** was obtained by treating the salt with 2 M HCl after work-up.[11] The cinchonidine and quinidine can be recovered[12] from the mother liquor of resolution due to their alkaloid feature.[13] When the recovered bases were reused in the next resolution, no significant loss in activity was observed (at least 5, data were not shown). The absolute configurations at the C-4 carbon have been established that (+)-**3a** and (−)-**3b** have *S*- and *R*-configurations at C-4, respectively, which were based on the optical value of **3a** and **3b** with the compounds reported in literature,[7b,c] whose absolute configurations have been determined by X-ray crystallography.[7f]

After obtained the key intermediates, enantiomeric dihydropyridinecarboxylic acids **3a** and **3b**, the esterification was carried out easily (Scheme 2). The resulting enantiomeric dihydropyridinecarboxylic acid **3a** or **3b** was converted to corresponding acid chloride by treatment with $SOCl_2$ in CH_2Cl_2–DMF (4:1) at −20 °C. The acid chloride was not isolated and directly treated with *n*-pentanol to give the final enantiomeric MN9202 **1a** or **1b** in the yield 78% and 76%, respectively.[14] The absolute configuration of (−)-**1a** was proven to be *R* which determined by the precursor of (*S*)-**3a** because of no inversion in the esterification reaction. The racemic MN9202 was synthesized by the method mentioned above when using the racemic dihydropyridinecarboxylic acid **3** instead of enantiomeric acid, the structure of MN9202 was identical to that reported by us using another synthetic method. In order to further investigate the therapeutical application, the optical purities of MN9202 enantiomers were also determined by chiral high-pressure-liquid-chromatography columns AD-H. Under the optimum conditions,[15] the racemic MN9202 as a reference standard can be extremely separated and the enantiomeric excess of **1a** or **1b** were both determined.

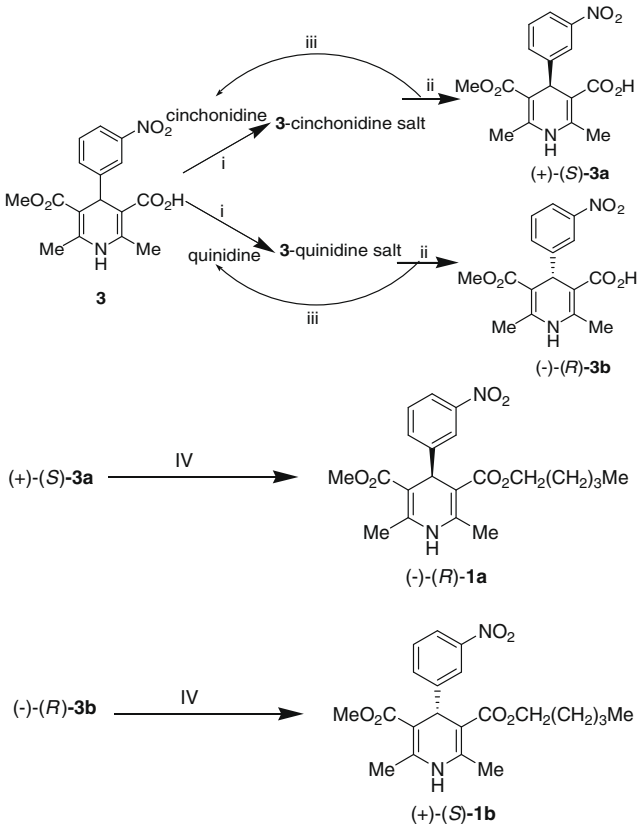
In order to evaluate pharmacological profiles of the MN9202 enantiomers, we performed competition binding experiments with [^{3}H]-nitrendipine in rat cardiac and cerebral cortex membrane homogenate by the general method.[7f] The concentrations of 50% inhibition of nitrendipine binding (IC_{50}) are shown in Table 2. The (*S*)-MN9202 has higher affinity than the (*R*)-MN9202 both in the cardiac and cerebral cortex membrane, approximately 19 times more potent in cardiac and 12 times in cerebral cortex membrane. The binding results also indicate that both (*R*)- and (*S*)-MN9202 exhibit differences in tissue selectivity and have slightly high affinity in the cardiac membrane.

In summary, we have identified an efficient process to the synthesis of (*R*) and (*S*)-methyl pentyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate (MN9202) in high optical purities. The cinchonidine and quinidine-derived resolution protocol is completely enantioselective for the dihydropyridinecarboxy-

Table 1
The resolution of racemic 1,4-dihydropyridine acid **3**

Entry	Resolving agent	Solvent	Yield[a] (%)	ee[b] (%)	Absolute configuration[c]
1	Cinchonidine	EtOH	52	63.4	*S*
2	Cinchonidine	Acetone	49	72.1	*S*
3	Cinchonidine	DMF	26	99.3	*S*
4	Cinchonidine	DMF–H_2O (8:2)	32	99.5	*S*
5	Cinchonidine	DMF–H_2O (8:5)	41	>99.5	*S*
6	Cinchonidine	DMF–H_2O (8:8)	44	97.8	*S*
7	Cinchonidine	DMF–H_2O (8:11)	46	95.0	*S*
8	Quinidine	DMF–H_2O (8:5)	43	>99.5	*R*
9	Quinidine	DMF–H_2O (8:8)	46	96.6	*R*

[a] Based on the total racemic **3**, recrystallized twice.
[b] Determined after esterification by chiral HPLC.
[c] Established by comparing the optical value of **3a** or **3b** with the compounds reported in the literature.



Scheme 2. Reagents and conditions: (i) solvent, refluxing, 15 min; rt, overnight; (ii) 0.5 M HCl, 0 °C; (iii) recovering of cinchonidine or quinidine: 40%NaOH, rt; (iv) $SOCl_2$, CH_2Cl_2–DMF (4:1, v/v), −20 °C.

Table 2
Inhibition of [^{3}H]-nitrendipine binding to rat cardiac and cerebral cortex membrane homogenate

Entry	Drug	IC_{50}[a] (nM)	
		Cardiac membrane	Cerebral cortex membrane
1	(−)-(*R*)-**1a**	8.14 ± 1.10[c]	17.62 ± 1.86
2	(+)-(*S*)-**1b**	0.42 ± 0.06[b,c]	1.48 ± 0.12[b]
3	Racemic **1**	0.76 ± 0.09	3.62 ± 0.32

[a] Concentrations of 50% inhibition of [^{3}H]-nitrendipine binding, each value represents the mean ± SD (n = 3).
[b] P <0.01, compared with (−)-(*R*)-**1a**.
[c] P <0.01, compared with cerebral cortex membrane.

lic acids **3**, and cinchonidine and quinidine can be reused in the next steps, which is amenable to scale-up for the production of MN9202. This approach can be also used to synthesis of others chiral dihydropyridines and open up a new field to investigate the chirality influence in MN9202. The dihydropyridine receptor binding assay demonstrated that the stereochemistry at C-4 is crucial and *S*-enantiomer was defined as active isomer which is more potent than *R*-enantiomer both in rat cardiac and cerebral cortex membrane.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.104.

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11. *Preparation of the optical isomers of 2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid*: A mixture of **3** (4 g, 12 mmol) and cinchonidine (3.55 g, 12 mmol) in DMF (8 mL) was refluxed for 15 min and then added heated water (5 mL). The above mixture was cooled and allowed to stand at ambient temperature for 18 h. The resulting precipitates were collected by filtration and air-dried to give the impure **3**-cinchonidine salt. After recrystallized twice from DMF–H_2O (8:5), the pure salt was obtained (3.1 g) in 41% yield (82% based on the isomer). Mp 193–195 °C; $[\alpha]_D^{25}$ −58.4(*c* 0.5, acetone). A suspension of the pure salt (2.0 g, 3 mmol) in EtOAc (100 mL) was treated with 0.5 M HCl (40 mL) under stirring and cooling in an ice-bath and then the aqueous layer was removed and extracted with EtOAc. The combined organic layer were washed with brine, dried over Na_2SO_4 and evaporated in vacuo to give (+)-**3a** (0.88 g). (+)-(*S*)-**3a**: 84% yield. Mp 193–195 °C; $[\alpha]_D^{25}$ +24.2 (*c* 0.5, acetone) [lit.[7b] mp 194–195 °C; $[\alpha]_D^{22}$ +19.1 (*c* 0.556, acetone)]; ^{1}H NMR (400 MHz, DMSO-d_6/$CDCl_3$(1:1)) δ 8.62 (s, br, 1H), 8.07 (s, 1H), 7.96 (d, *J* = 7 Hz, 1H), 7.64 (d, *J* = 8 Hz, 1H), 7.44–7.43 (m, 1H), 5.06 (s, 1H), 3.60 (s, 3H), 2.34 (s, 3H), 2.33 (s, 3H); ESI-MS *m*/*z*: 333 (M+H$^+$); HREI-MS calcd for $C_{16}H_{16}N_2O_6$ 332.1008; Found 332.1021; IR (KBr): 3354, 1675, 1662, 1261, 1161, 766, 578 cm^{-1}. The **3**-quinidine salt and optical isomer **3b** was obtained as a similar procedure mentioned above when using quinidine as resolution agent. **3**-quinidine salt: 43% yield. Mp 199–201 °C, $[\alpha]_D^{25}$ +119.8(*c* 0.5, acetone). (−)-(*R*)-**3b**: 93%yield. Mp 190–192 °C; $[\alpha]_D^{25}$ −24.0 (*c* 0.5, acetone) [lit.[7b] mp 196–197 °C; $[\alpha]_D^{22}$ −19.6 (*c* 0.542, acetone)]; ESI-MS *m*/*z*: 333 (M+H$^+$); HREI-MS calcd for $C_{16}H_{16}N_2O_6$ 332.1008; found 332.1016; the ^{1}H NMR and IR spectra of (−)-(*R*)-**3b** were identical to those of another enantiomer (+)-(*S*)-**3a**.
12. *Recovering of cinchonidine and quinidine*: To the corresponding aqueous layer (obtained after treated with HCl) was added 40% NaOH. The resulted aqueous layer (pH 10) was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4 and evaporated in vacuo to give cinchonidine (93% yield) and quinidine (95% yield).
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14. *Synthesis of (R) and (S)-3-methyl-5-pentyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate*: A suspension of (+)-**3a** (0.52 g, 1.5 mmol) in CH_2Cl_2–DMF (4:1, 5 mL) was cooled at −20 °C in an ice-bath under N_2, and $SOCl_2$ (0.2 g, 1.6 mmol) was added dropwise under this temperature. After adding, the mixture was stirred for another 30 min. Then a solution of *n*-pentanol in CH_2Cl_2 (2 mL) was added dropwise to the reaction mixture under the same condition over a period of 10 min. The reaction mixture was stirred for 9 h (TLC) and diluted with CH_2Cl_2 (50 mL) and wished with water (50 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (30 mL × 3). The combined organic layer were washed with brine, dried

over Na_2SO_4 and evaporated in vacuo. The residue was purified by column chromatography on silica gel (elute: petroleum ether/ethyl acetate = 3:1, v/v). The fractions containing the desired compound were combined and evaporated in vacuo to give (*R*)-MN9202 (0.46 g). (*S*)-MN9202 was obtained from (*R*)-**3b** by using a similar manner. (−)-(*R*)-**1a**: 76%yield. Mp 112–113 °C; $[\alpha]_D^{25}$ −9.7 (*c* 0.5, acetone); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 8.01 (d, *J* = 8 Hz, 1H), 7.64 (d, *J* = 8 Hz, 1H), 7.37 (dd, *J* = 8 Hz, *J* = 8 Hz, 1H), 5.70 (s, 1H), 5.10 (s, 1H), 4.10–3.96 (m, 2H), 3.65 (s, 3H), 2.38 (s, 3H), 2.37 (s, 3H), 1.62–1.53 (m, 2H), 1.32–1.19 (m, 4H), 0.86 (t, *J* = 7 Hz, 3H); ESI-MS *m*/*z*: 403 ($M+H^+$); HREI-MS calcd for $C_{21}H_{26}N_2O_6$ 402.1791; found 402.1802; IR (KBr): 3440, 3364, 2957, 2928, 2360, 2341, 1649, cm^{-1}. (+)-(*S*)-**1b**: 83%yield. Mp 112–113 °C; $[\alpha]_D^{25}$ +9.8 (*c* 1.0, acetone); ESI-MS *m*/*z*: 403 ($M+H^+$); HREI-MS calcd for $C_{21}H_{26}N_2O_6$ 402.1791; found 402.1785; the ^{1}H NMR and IR spectra of (+)-(*S*)-**1b** were identical to those of another enantiomer (−)-(*R*)-**1a**.

15. *Enantiomeric purity analysis:* The optical purity was checked by chiral HPLC under the following conditions: Chiral AD-H (4.6 mm × 250 mm); column temperature, 30 °C; eluent, *n*-hexane/*i*-propanol (95:5, v/v); flow rate, 0.7 mL/min; detection, UV 254 nm (Dionex instrument). Retention times (Shown in Figure 2, see Supplementary data): $t_{(-)\text{-}(R)\text{-}\mathbf{1a}}$ = 29 min; $t_{(+)\text{-}(S)\text{-}\mathbf{1b}}$ = 33 min.