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Chiral Synthesis and Pharmacological Evaluation of NPS 1407: A Potent, Stereoselective NMDA Receptor Antagonist

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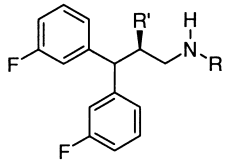
Abstract—The stereoselective synthesis and biological activity of NPS 1407 (**4a**), (*S*)-(−)-3-amino-1,1-bis(3-fluorophenyl)butane, a potent, stereoselective antagonist of the NMDA receptor, are described. The racemate (**4**) was found to be active at the NMDA receptor in an in vitro assay, prompting the synthesis of the individual stereoisomers. The *S* isomer (**4a**) was found to be 12 times more potent than the *R* isomer (**4b**). Compound **4a** demonstrated in vivo pharmacological activity in neuroprotection and anticonvulsant assays. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

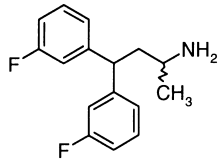
Glutamate is the major excitatory neurotransmitter in the mammalian CNS. Ionotropic glutamate receptors have been classified pharmacologically as *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainate receptors, according to their preferred agonists. Glutamate receptors have been implicated in the physiology and pathophysiology of various neurological and psychiatric diseases and disorders such as ischemic stroke, epilepsy, pain, depression, and various neurodegenerative disorders such as Parkinson's disease. It has been postulated that the NMDA receptor plays a key role in mediating neuronal damage by increasing the cell's permeability to calcium, a known mediator of cellular damage. In animal models of focal ischemia, NMDA receptor antagonists provide profound and consistent cerebroprotection.[1,2]

Many studies support the hypothesis that NMDA receptors may contribute to the development and expression of epilepsy.[1] Noncompetitive NMDA receptor antagonists, including dizocilpine (MK-801) and phencyclidine (PCP), are effective anticonvulsants in animal models of epilepsy. These two specific compounds, however, are not used for the treatment of epilepsy in man due to their undesirable psychotomimetic side effects.

Our initial efforts to develop neuroprotective compounds focused on the Araxin™ compounds (a class of small, highly polar, polycationic arylalkylpolyamine spider toxins and their synthetic analogues). Their ability



1, R = R' = H
2, R = -CH_3, R' = H
3, R = H, R' = -CH_3



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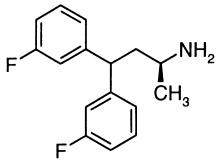
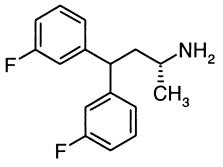


4a



4b

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to selectively block glutamatergic synaptic transmission in the mammalian central nervous system (CNS) is well known.[3,4] Developing arylalkylpolyamines as therapeutic agents was prohibited by unresolvable pharmacokinetic and toxicological problems, directly attributable to their highly polar polyamine chains.[5]

Our attention then turned to the use of pharmaceutically acceptable templates. The diphenylpropylamine skeleton occurs in a wide variety of pharmaceuticals, including antihistamines, analgesics, antitussives, antidiarrheals, antiarrhythmics, antihypertensives, antidepressants, antispasmodics, and anticholinergics. Recently, we reported the discovery of a nonpsychotomimetic class of NMDA receptor open-channel blockers, the diphenylpropylamines; compound **1** is representative of this class.[3,4,6–9] A regiomeric isomer of **4a**, NPS 1506·HCl (**2**·HCl), is currently in human clinical trials for the treatment of stroke.[10,11]

Research from our laboratories concerning the absolute stereochemistry and pharmacological stereoselectivity of the β-methyl analogue, NPS 1392 (**3**), has been reported.[12] The α-methylamino functionality is present in many CNS-active substances including methadone, methadol, promethazine, ephedrine, bupropion, and amphetamine.[13] The α-methyl stereoisomeric pair (**4a** and **4b**) was prepared during our investigation of the structure–activity relationships in this series of diphenylpropylamine NMDA receptor antagonists. Herein we describe the synthesis and pharmacological evaluation of these α-methyl analogues.

Chemistry

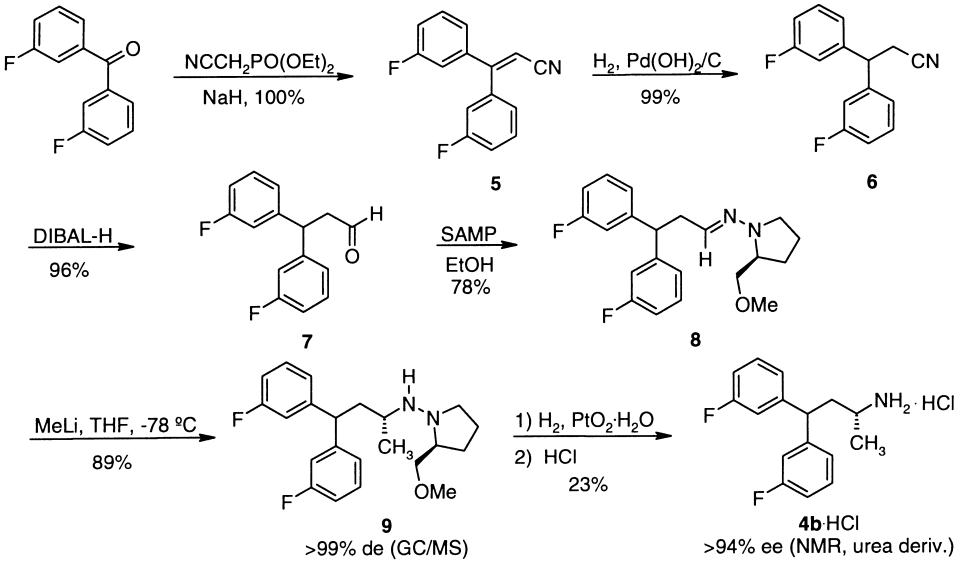
Racemic **4** could not be readily separated using commercially available chiral stationary-phase HPLC techniques. Development of two independent chiral routes served to verify the stereochemical assignment.

The *R* enantiomer (**4b**) was synthesized in a six-step reaction sequence starting from 3,3′-difluorobenzophenone (Method A). The chiral center was introduced by the diastereospecific alkylation of hydrazone **8**, formed from the chiral auxiliary (*S*)-(−)-amino-2-(methoxymethyl)pyrrolidine (SAMP). The resulting diastereomeric hydrazine was then reduced to provide **4b** in high enantiomeric excess (≥94%), as determined by ^{1}H NMR analysis of its diastereomeric urea derivatives.[14] A second method was needed to confirm the chirality assignment suggested by the chemistry in Method A. Both methods were used to prepare both enantiomers.[3,4,6] In Method B, the *S* enantiomer (**4a**) was synthesized in a four-step reaction sequence. The chiral center was formed by the stereospecific Michael addition of chiral amine **11** to benzyl crotonate with ≥99% de/NMR. Reaction of the resultant ester (**12**) with 2 equiv of the Grignard reagent and subsequent dehydration followed by catalytic hydrogenation gave **4a**.

Method A

3,3-Bis(3-fluorophenyl)propionitrile (**5**) was formed quantitatively by the coupling (Horner–Emmons) of commercially available 3,3′-difluorobenzophenone with the sodium anion of diethyl (cyanomethyl)phosphonate in DMF (Scheme 1). Catalytic hydrogenation of the olefinic double bond over Pearlman's catalyst in EtOH provided compound **6**. 3,3-Bis(3-fluorophenyl)propionaldehyde (**7**) was obtained by the diisobutylaluminum hydride (DIBAL-H) reduction of nitrile **6**.

The chiral auxiliary SAMP was introduced by modifying a procedure published by Enders' group.[15] Aldehyde **7** was reacted with the auxiliary hydrazine, (*S*)-(−)-1-amino-2-(methoxymethyl)pyrrolidine (SAMP, ≥97% ee/GLC (Aldrich)), to form **8**. The hydrazone functionality in **8** was then stereospecifically alkylated to form the intermediate diastereomeric hydrazine (**9**). The diastereomeric excess of the methylation reaction to form **9**



Scheme 1.

by reacting **8** with methyllithium was determined to be ≥99%, as measured by GC/MS. Methylcerium[16] and methylytterbium[17] gave diastereomeric excesses of 48% (82% isolated yield) and 99% (84% yield), respectively. The hydrazine's (**9**) N–N bond was then cleaved by catalytic hydrogenolysis over platinum(IV) oxide hydrate. The hydrochloride salt was formed in the usual manner to provide (*R*)-3-amino-1,1-bis(3-fluorophenyl) butane hydrochloride (NPS 1408·HCl, **4b**·HCl).[18]

Method B

The synthesis shown in Scheme 2 began with the mono-*N*-benzylation of (*S*)-(−)-α-methylbenzylamine (ChiraSelect, ≥99.0% ee) with benzyl bromide in *N*,*N*′-dimethylpropyleneurea (DMPU) to provide **11** following a literature procedure.[19] (Compound **11** is now commercially available from Fluka Chemical Corp., Milwaukee, WI). The introduction of chirality was accomplished by Michael addition of the lithium amide of (*S*)-(−)-*N*-benzyl-1-phenylethylamine (**11**) to benzyl crotonate (**10**) (prepared from benzyl bromide and crotonic acid), using a modification of a literature method reporting the preparation of (*R*)-β-aminobutanoic acid.[20] A high degree of diastereospecificity was found for the Michael addition reaction on the basis of ^{1}H NMR studies of the product (**12**), which was measured to be ≥99% de/NMR.

The chiral ester (**12**) was then reacted with the Grignard reagent formed from 3-fluorobromobenzene. Addition of 2 equiv of the Grignard reagent to the ester (**12**) resulted in formation of the tertiary alcohol (**13**). This compound was subsequently dehydrated at 90 °C in a mixture of concentrated sulfuric and glacial acetic acids (1:4) to provide the alkene (**14**). The hydrochloride salt of **14** was formed prior to catalytic hydrogenation. Double-bond reduction and *N*-debenzylation of **14** was achieved with hydrogen gas over Pearlman's catalyst to provide compound **4a**. The final product (**4a**) was found to be of high enantiomeric excess by ^{1}H NMR studies of its diastereomeric urea derivatives.[18] Diastereoisomers were formed from **4a** and **4b** by derivatization of an aliquot in $CDCl_3$ at 25 °C using 1 equiv of (*R*)- and (*S*)-1-(1-naphthyl)ethyl isocyanates (Fluka, ChiraSelect, ≥99%) each.[14]

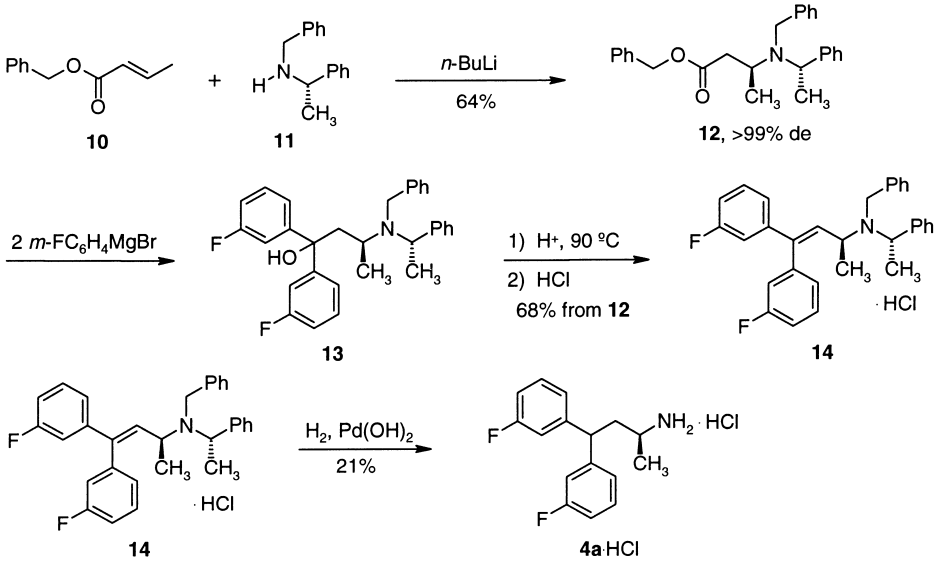
Biological Activity

Functional NMDA receptor antagonism, that is, inhibition of NMDA/glycine-induced increases in cytosolic calcium in cultured rat cerebellar granule cells (RCGCs),[21,22] and displacement of (+)-[^{3}H]-MK-801 from synaptic plasma membranes obtained from rat cortex[23–25] were used to determine potency at the NMDA receptor. Biological data for compounds described in the text and for the well-characterized NMDA receptor antagonist MK-801 (dizocilpine), for comparison, are shown in Table 1.

Compound **4a** (IC_{50} 89 nM) was at least 10-times more potent than its enantiomer (**4b**, IC_{50} 1.11 μM) at inhibiting the NMDA/glycine-induced increase in cytosolic calcium in RCGCs. In this functional assay, the achiral, desmethyl ligand (**1**) had an in vitro potency (IC_{50} 63 nM) similar to that of **4a** for blocking NMDA/glycine-induced channel function. The *S* isomer (**4a**) was somewhat more potent as an NMDA receptor antagonist than the racemate (**4**).

The binding potencies of the compounds were determined at NMDA receptors obtained from rat cortex. The *S* isomer (**4a**, IC_{50} 762 nM) was clearly more potent (fivefold) at displacing [^{3}H]-MK-801 than the *R* isomer (**4b**, IC_{50} 4.50 μM) (Table 1). Interestingly, their unbranched counterpart (compound **1**) was more potent (IC_{50} 230 nM) than either stereoisomer (**4a** or **4b**) in displacing the radioligand.

In comparison to the (*R*)-β-methyl analogue (**3**), the (*S*)-α-methyl analogue (**4a**) was nearly equipotent (IC_{50} 75



Scheme 2.

Table 1. Relative in vitro potencies of selected diphenylpropylamines at NMDA receptors

Compound	Stereochemical configuration	Position of methyl group	NMDA-receptor antagonism (RCGCs) IC_{50} (μM)	Displacement of (+)-[^{3}H]-MK-801 IC_{50} (μM)
MK-801	—	—	0.0034 (6)[a]	0.0056 (11)
1	—	—	0.063 (4)	0.230 (4)
3	*R*	β	0.075 (5)	0.141 (5)
4	(*R*,*S*)	α	0.145 (6)	1.04 (2)
4a	*S*	α	0.089 (4)	0.762 (4)
4b	*R*	α	1.11 (4)	4.50 (2)

[a]Numbers in parentheses indicate the number of experimental determinations.

and 89 nM, respectively) in the RCGC assay. However, compounds **3** and **4a** had a greater than fivefold difference in potency at displacing radioligand from [^{3}H]-MK-801-labeled NMDA receptors (IC_{50} 0.141 and 0.762 μM, respectively).

Compound **4a** was tested in an in vivo model of temporary focal ischemia (rat suture model) by 2-h occlusion of the middle cerebral artery followed by 48 h of reperfusion.[26] Compound **4a** showed a significant neuroprotective effect at doses of 2 mg/kg intraperitoneally (ip) administered 30 min prior to and 3 h after occlusion (data not shown). In comparison to controls, compound **4a** reduced brain damage by 37% [measured reduction in infarct volume ($P < 0.05$, Student's *t*-test)].

The compounds were also tested for anticonvulsant activity in the Frings audiogenic mouse model. These mice are genetically susceptible to sound-induced seizures and display prominent seizure activity in response to a high-intensity sound stimulus.[27] Their seizures respond to a wide range of CNS-active drugs, including previously reported NMDA receptor antagonists, hence, Frings mice are a highly useful screening model for the early identification of potential anticonvulsant drugs.[27]

Male and female Frings audiogenic seizure-susceptible mice (18–25 g) were obtained from an in-house colony at the University of Utah.[27] Groups of eight mice each were treated with varying doses ip of **1**, **4**, **4a**, and **4b**. At the time of peak effect (determined previously in separate experiments), individual mice were placed into round Plexiglas jars (diameter, 15 cm; height, 18 cm) and exposed to a sound stimulus of 110 decibels (11 kHz) delivered for 20 s. Mice were observed for 25 s for the presence or absence of hindlimb tonic extension. Mice not displaying hindlimb tonic extension were considered protected. Toxicity was measured by appearance of tremor. Probit analysis was used to calculate ED_{50} and TD_{50} values.

As shown in Table 2, both enantiomers (**4a** and **4b**) were effective in this model following ip administration. The *S* enantiomer (**4a**) was approximately three-times more potent than the *R* enantiomer (**4b**) and twofold more potent than the racemic mixture (**4**) (ED_{50}s: 2.7, 9.1, and 4.9 mg/kg, respectively). Compound **4a** possessed a long duration of action following ip administration at 10 mg/kg (anticonvulsant activity peaked within 1 h and was maintained for >4 h). Following ip administration to Frings audiogenic mice, all four compounds produced behavioral impairment that was characterized by the presence of tremor. Of the compounds tested, compound **4b** was more toxic than the racemic mixture (**4**) and displayed the lowest protective index (ratio of TD_{50} to ED_{50}) of the compounds tested (PIs: 5.5, 6.4, 1.4, and for compounds **4**, **4a**, and **4b**, respectively). As can be seen, the *S* enantiomer was not only the most potent, but also displayed the greatest protective index. All compounds tested were effective anticonvulsants when given orally.

Summary

In this communication, we have described the synthesis, biological activity, and absolute stereochemical assignment of NPS 1407·HCl (**4a**), a potent NMDA receptor antagonist. Addition of a methyl group alpha to the amine nitrogen of **1** provided a pair of enantiomers (**4a** and **4b**) with significantly different pharmacological potencies. The absolute stereochemical assignment of **4a** was determined using two corroborating and independent synthetic methods of known stereochemical outcome.

Table 2. Relative anticonvulsant potencies of selected diphenylpropylamines in Frings mice

	Mice (ip)		Mice (po)	
Compound	ED_{50} (mg/kg)	TD_{50} (mg/kg)	ED_{50} (mg/kg)	TD_{50} (mg/kg)
1	2.1 (1.5–3.1)[a]	19.9 (15.8–26.5)	9.7 (7.3–13.9)	21.8 (17.2–28.9)
4	4.9 (2.9–6.6)	26.8 (20.0–38.1)[b]	5.1 (2.9–8.2)	18.3 (15.2–28.9)[c]
4a	2.7 (1.5–4.6)	17.4 (11.9–22.7)	7.3 (1.8–11.3)	18.6 (12.9–28.9)
4b	9.1 (6.9–10.9)	13.6 (10.9–16.8)	—	—

[a]Numbers shown in parentheses are 95% confidence intervals.
[b]Marked lethality was observed after 30 (4/7) and 60 (8/8) mg/kg (ip).
[c]LD_{50} (mg/kg, po).

Compound **4a** (*S*-methyl) showed potent, functional in vitro NMDA receptor antagonist activity (IC_{50} 89 nM) with an IC_{50} value similar to that of its unbranched counterpart (**1**, IC_{50} 63 nM). The more-active *S* enantiomer (**4a**, IC_{50} 89 nM) was one order of magnitude more potent than the *R* stereoisomer (**4b**) in the RCGC NMDA receptor assay and approximately six-times more potent than **4b** at displacing radioligand from MK-801-labeled binding sites. Similarly, the *S* enantiomer (**4a**) was found to be three times more potent in vivo against audiogenic seizures than the *R* enantiomer (**4b**) and twofold more potent that the racemate (**4**). Full details of the structure–activity relationships, chemical syntheses, molecular modeling, and pharmacological studies of the diphenylpropylamines as NMDA antagonists will be the subject of future publications.

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18. Compound **4a**: ≥90% ee/NMR (urea derivative); mp 143–145 °C; $[\alpha]_D^{23}$ −4.0° (*c* 0.5, MeOH), $[\alpha]_{365}^{23}$ −16° (*c* 0.5, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4 [3:1]) δ 1.35 (d, 3H, CH_3), 2.23 (m, 1H, CH_2), 2.58 (m, 1H, CH_2), 3.14 (bs, 1H, $CHNH_3^+$), 4.25 (t, 1H, Ar_2CH), 7.25–6.83 (m, 8H, Ar*H*), 8.31 (bs, 3H, NH_3^+); GC/MS t_R 7.02 min, *m*/*z* 261 (M^+). Compound **4b**: ≥94% ee/NMR (urea derivative); mp 119–135 °C; $[\alpha]_D^{23}$ +6.4° (*c* 0.5, MeOH), $[\alpha]_{365}^{23}$ +19° (*c* 0.5, MeOH); ^{1}H NMR data were consistent with those obtained from NMR experiments with the *S* enantiomer.
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