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ENANTIOSELECTIVE SYNTHESIS OF A PYRROLO-BENZOTHIADIAZINE DERIVATIVE
S 18986, A NEW AMPA RECEPTOR POSITIVE MODULATOR

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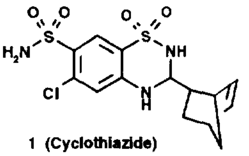
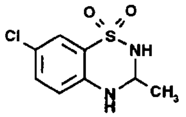
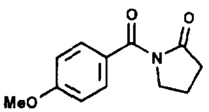
Abstract: Enantioselective reduction of pyrrolo-benzothiadiazine **6** using various chiral reducing agents has been studied and led to efficient synthesis of **8** (S 18986) which was established to be of (*S*) configuration by single crystal X-ray diffraction analysis. S 18986 is a potent and selective AMPA positive modulator which displays total stereoselectivity, the (*R*) enantiomer being completely inactive.

The excitatory amino acid glutamate plays a major role in learning and memory processes[1]. Recent studies indicate that molecules which reduce desensitization of AMPA-selective glutamate receptors through a positive allosteric modulation may improve cognition[2] and could be useful in the treatment of memory and learning disorders resulting from aging, central nervous system trauma, stroke, and neurodegenerative disorders such as Alzheimer's disease. Among these potential drugs are the benzothiadiazines, cyclothiazide[3] **1** and IDRA-21[4] **2**, and the benzamides, aniracetam[5] **3**, BA-14[6] **4** and BA-74[7] **5**.


1 (Cyclothiazide)


2 (± IDRA 21)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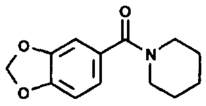
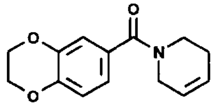

4 (BA-14)


5 (BA-74)

There is evidence that this putative allosteric site on the AMPA receptor displays stereoselectivity in its interaction with the ligand. In a studies on cyclothiazide[8] **1**, Cordi et al. have shown, after isolation and testing of the separate enantiomers, that potentiation of AMPA transmission was sensitive to the absolute stereochemistry of the ligand. In the same benzothiadiazine series, Guidoti[9,10] has shown that the cognition-enhancing IDRA-21 **2** also displays pronounced stereoselectivity. Only the (+) enantiomer was identified as

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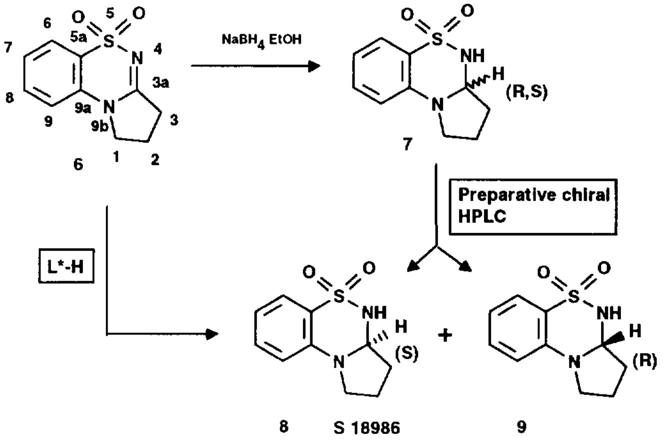
being pharmacologically active although a complete *in vivo* comparison between racemic and (+) IDRA-21 could not be carried out due to insufficient available quatities of (+) IDRA-21 in the absence of a stereoselective synthesis.

Recently we discovered that the known racemic pyrrolo-benzothiadiazine 7[11] potentiates response of AMPA-activated current in *Xenopus* oocytes expressing AMPA receptor[12] (Table 1). At 60 μM it produces a two fold increase of the signal elicited by an application of 30 μM AMPA. As compound 7 bears a chiral center at C_{3a}, we suspected that the activity could be related to the intrinsic properties of a single enantiomer. We report here the identification, characterization and asymmetric synthesis of the active enantiomer of 7.

Racemic 7 was initially synthesized by reduction of **6** (Scheme 1) with sodium borohydride.[11] Chiral resolution was achieved by preparative chiral HPLC[13] using chiralpak AS eluting with heptane/ethanol (1:2). The two enantiomers were tested in *Xenopus* oocytes (Table 1). All activity resided in the second enantiomer eluted from the column which was established to be of S configuration (compound **8**, S 18986) by single crystal X-ray diffraction experiments.[14] Moreover, S 18986 showed selective activity for AMPA receptor since no effect was observed when the agonists were kainate or NMDA (Table 2).



Scheme 1.

In order to prepare larger quantities of the (*S*) enantiomer S 18986 **8** for biological evaluation, we required a synthetic alternative and therefore decided to investigate asymmetric reduction of the C_{3a}=N double bond of precursor **6**. Although many examples of the asymmetric reduction of prochiral ketones to chiral alcohols have been described, only a few successful, enantioselective reductions of prochiral imines to amines have been achieved.[15] [16]

Table 1. AMPA-activated current potentiation

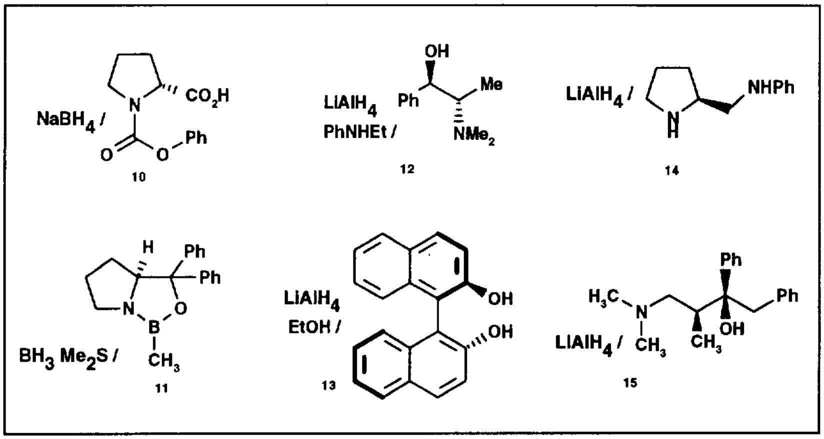
compound	EC_{2X} (μM)	EC_{5X} (μM)
Cyclothiazide	2	10
BA-14	300	n.t
BA-74	45	110
racemic	60	160
(*R*)-enantiomer	300	n.t.
(*S*)-enantiomer	35	70

Table 2. Selectivity of S 18986 for AMPA versus Kainate and NMDA

	AMPA	KA	NMDA
(*S*)-enantiomer	35	>300	>>300

For example chiral sodium acyloxyborohydride, **10** (Table 3), which is easily prepared by the reaction of $NaBH_4$ with 3 (*S*)-*N*-benzyloxycarbonylproline has proven useful for reduction of certain cyclic imines as shown by Iwakuma.[17] Applied to our substrate, **10** was completely ineffective (Table 3, entry 1). This is probably due to the poor reactivity of this complex toward carbon-nitrogen double bonds. The same lack of reactivity was found with oxazaborolidine hydride **11**[18] (entry 2). Hence we looked at methodologies used to enantioselective prochiral ketone reductions. The more efficient methods are based on chirally modified aluminium hydride[19] and therefore we investigated the use of four such complexes **12-15** as represented in table 3. Aluminium hydride was complexed with (-)-*N*-methyl ephedrine[20] , (*R*)-binaphtol[21] and (*S*)-2-(anilomethyl)pyrrolidine[10] according to literature procedures. Unfortunately, in all these cases (entries 3,4,5), none or very little induction was observed. In contrast, reduction of **6** with Mosher's reagent[11], prepared by mixing 1 molar equivalent of lithium aluminium hydride with 2.3 molar equivalents of amino carbinol (+)-(2*S*,3*R*)-4-dimethylamino-3-methyl-1,2-diphenyl-2-butanol (chirald®) gave moderate to good enantioselectivity (entry 6-12). Solvent effects were studied at 0 °C in tetrahydrofuran, diethyl ether, diglyme, dimethoxyethane, (entries 7, 10, 11, 12) and showed ether to be the better solvent providing excellent enantioselectivity (76% ee) with complete conversion[12]. This is in contrast to the moderate ee values observed, for the same conditions but conducted in tetrahydrofuran (24% ee), diglyme (22% ee) and dimethoxyethane (12% ee). Lowering the reaction temperature in tetrahydrofuran (0 → - 70°C) resulted in increased enantioselectivity (24 → 40% ee) but reduced dramatically the conversion yield (98 → 23%). At the same temperature (- 70 °C) no reaction took place in ether (entry 9) due to the very low solubility of **6** under such conditions, while at room temperature a lower enantioselectivity was observed (64% ee compare to 76% ee at 0 °C).

Table 3. Asymmetric reduction of 6 using various chiral reducing agents 10-15



Entry	Chiral reducing agent	Temp (°C)	Solvent	configuration at C3a	ee[a] (%)	Conversion[b] (%)
1	**10**	0→rt	THF	--	--	no reaction
2	**11**	0→rt	THF	--	0	< 10
3	**12**	-70→0	THF	*S*	8	99
4	**13**	-70→0	THF	*S*	6	20
5	**14**	-70→0	THF	*S*	16	83
6	**15**	-70	THF	*S*	40	23
7	**15**	0	THF	*S*	24	98
8	**15**	rt	THF	*S*	18	98
9	**15**	-70	Et_2O	*S*	--	no reaction
10	**15**	**0**	**Et_2O**	***S***	**76**	**98**
11	**15**	rt	Et_2O	*S*	64	98
12	**15**	0	Diglyme	*S*	22	77
13	**15**	0	DME	*S*	12	57

a) Determined by HPLC-analysis using a Chiralcel OD column as described in ref 13
b) Determined by HPLC

In his study on the asymmetric reduction of acetophenone derivatives, Mosher[23] had showed that the absolute stereochemistry of the reduction product was often a function of the age of this chiral complex: when the reagent was refluxed for 10 min and then allowed to stand overnight, the opposite enantiomer was produced with good enantioselectivity. In the reduction of **6**, we did not observe this stereochemical time-dependent reversal but a dramatic decrease of enantioselectivity (76 → 14% ee). Another important aspect of this asymmetric reduction is the ease with which the chiral auxiliary **15** can be regenerated in high yield (95%) by simple dilute acid extraction.[25] Optical purification of compound **8** was realized by recrystallization: Typically, the enantioselective reduction delivers reaction mixtures in ee of around 76% contaminated with 1-

2% of starting material. It is crucial to eliminate this impurity to acquire enantiomeric purity >99%. Hence, the crude reaction mixture is stirred overnight at 20 °C, in a defined volume of acetonitrile (0.7 × ee % mL/g where ee is determined by chiral HPLC), the white powder is filtered and discarded (racemic mixture). The filtrate has been enriched in the (*S*) enantiomer up to the eutectic ratio of 0.095/0.905. After evaporation of the solvent, the solid is dissolved at reflux temperature in the minimum amount of acetonitrile (usually around 15 ml/g) and let crystallized very slowly at 50-55 °C without stirring. The large crystals are quickly filtered and recrystallized a second time as described above to give enantiomerically pure S 18986. At lower crystallization temperature, a second crop of smaller crystals appeared which are of much lower optical purity and in some conditions may be racemic. The mother liquors of these crystallizations which have typically ee of 60%, can be recycled by specific dissolution of the first eutectic as described above. This surprising behavior could be explained by the existence of two different crystalline forms (polymorphes), each endowed with a different eutectic composition in acetonitrile. The first crystalline form is favored at low ee and appears to be catalyzed by the presence of a trace of unreduced material, has an eutectic composition around 81% ee at 20 °C. The second form being favored at higher ee, has an eutectic composition slightly lower than 81% ee at 20 °C, but much lower at 50-55 °C.

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[12] In short, *Xenopus* oocytes were injected with 50 ng of poly(A)$^+$ mRNA prepared from rat cerebral cortex and isolated using oligo-dT cellulose chromatography. The oocytes were incubated at 18 °C for 2-3 days to allow

expression. Electrical recording were performed in a plexiglass bath at room temperature. Recording were carried out in «OR2» medium using a 2-electrode voltage clamp with a third electrode in the bath as voltage reference. The holding potential was usually adjusted to -60mV. All agents were bath applied and the responses were measured as the peak of inward current. Dose-response curves were constructed with increasing concentration of the tested compound in the presence of 30 μM AMPA and expressed as EC_{2X} and EC_{5X} (concentration of compound required to double or quintuple respectively, AMPA inward current). Each experiment was reproduced (3 <n <6) and s.e.m. for each concentration were maximum 25 % of the increase.

[13] Chirapak AS (50x2 cm); Flow rate = 1.5 mL/min;.UV absorbance was monitored at 275 nm. A complete separation was obtained by single injection of 80 mg of racemate dissolved in 2.5 mL of acetone. The retention times of the 2 enantiomers were 4.98 min and 5.98 min respectively. The enantiomeric purity of each isomer was monitored on chiralcel OD column eluted with *n*-heptane/isopropanol/diethylamine (300/700/0.5). Each enantiomer was then recrystallized from ethyl acetate.

[14] X-ray studies were realized by C. Pascard and J. Guilhem at the Institut de Chimie des Substances Naturelles, CNRS, Laboratoire de Cristallochimie, Gif sur Yvette (France).

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[24] Experimental procedure: An ether solution (80 mL), containing the chiral amino alcohol **15** (24.12 g, 85.09 mmol) was added at 0 °C to a magnetically stirred solution of $LiAlH_4$ 1M in ether (37.82 ml, 37.87 mmol) diluted in 200 mL of ether. A white precipitate began to form when about two third of **15** was added. The reaction mixture was vigorously stirred at 0 °C during 15 min. The cyclothiadiazine **6** was added in several portions and the mixture was stirred 4 h at 0 °C. The reaction was hydrolyzed by adding dropwise 100 mL of 1N HCl (vigorous evolution of gas) and then extracted with ethyl acetate. The organic extracts were washed (1N HCl, brine), dried ($MgSO_4$), and evaporated under vacuum to give reduced products as a white solid (4.18 g, 69%). The white solid (ee: 76%, contaminated by 2% of starting material) was stirred at room temperature for 20 h in 218 mL acetonitrile. The white powder was filtered, the filtrate concentrated (3.86 g, ee: 81%) and then recrystallized in 58 mL acetonitrile by dissolving at reflux temperature, and then cooling without stirring on a heating plate at 55 °C. The large transparent crystals are collected by quick filtration (2.28 g; ee: 91%) and crystallized in the same way in 28 ml acetonitrile affording the desired compound (1.17 g, ee: >99%).$[\alpha]_D$ = +260° (0.5 mg/ml 95% ethanol). 1H NMR (DMSO-d6) δ 7.55 (dd,1H), 7.5 (broad s, 1H), 7.4 (td, 1H), 6.75 (td, 1H), 6.7 (dd, 1H), 5.0 (m, 1H), 3.5 (m, 1H), 3.2 (m, 1H), 2.3 (m, 1H), 1.7-2.2 (m, 3H).

Extraction of chiral ligand **15**: Neutralization of the acid extract of the reaction gave precipitation of a first part of chiral amino alcohol **15** (15.77 g, 65%) and extraction of the filtrate with ethyl acetate gave a second part (7.33 g, 30%).

[25] Enantiomeric purity of amino alcohol **15** was monitored as described in ref 13

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