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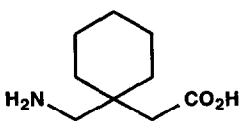
ENANTIOSELECTIVE SYNTHESIS OF PD144723: A POTENT STEREOSPECIFIC ANTICONVULSANT.

Po-wai Yuen,* Gerald D. Kanter, Charles P. Taylor and Mark G. Vartanian
Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105.

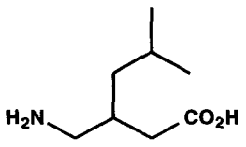
Abstract: PD144723, S-(+)-3-isobutyl GABA, is structurally related to both the inhibitory neurotransmitter γ-aminobutyric acid and the novel anticonvulsant gabapentin. That the S-enantiomer is more potent than the R-(-)-enantiomer, PD144550, for *in vitro* displacement of [^{3}H]gabapentin at the gabapentin binding site illustrates the stereoselectivity of this site. The two enantiomers were prepared by Evans' chiral oxazolidinone alkylation chemistry.

γ-Aminobutyric acid (GABA) and L-glutamic acid are the two major neurotransmitters that regulate neuronal activity in the brain. L-glutamic acid is an excitatory neurotransmitter whereas GABA is the major inhibitory transmitter.[1] An imbalance in the concentration of these neurotransmitters can lead to convulsive states. It has been shown by Karlsson *et al.*[2] that convulsions result when the concentration of GABA in the brain diminishes below a threshold level. However, seizures appear to terminate when the GABA levels in the brain rise during convulsion.[3] Therefore, it is clinically relevant to be able to control convulsive states by controlling the metabolism of GABA.

The most straight forward approach for treatment of seizures is to administer GABA into a convulsing subject. Unfortunately, this approach works only if GABA is injected directly into the brain of a convulsing animal[3a] presumably because of its inability to cross the blood-brain barrier. An alternate approach is to develop a lipophilic GABA-mimetic to circumvent this problem. A number of 3-alkyl GABA analogues were synthesized and tested for anticonvulsant activity by the Silverman group.[4] Among the compounds that they have studied, (±)-3-isobutyl GABA is by far the most *in vivo* active anticonvulsant in this class. Although previous studies by Silverman show that (±)-3-isobutyl GABA is an L-glutamic acid decarboxylase (GAD) activator, this effect is only significant at concentrations above 1.0 mM.[4c] Thus, the GAD activating property of 3-isobutyl GABA cannot account for its *in vivo* anticonvulsant activity. Recently, Hill and coworkers[5a,b] described a novel high affinity gabapentin binding site and suggested that the anticonvulsant activity of gabapentin may be due to its interaction at this site. 3-Isobutyl GABA **2**, being structurally related to gabapentin **1**, may be active through its interaction with this binding site. Not suprisingly, (±)-3-isobutyl GABA was found to displace tritiated gabapentin *in vitro* with an IC_{50} of 83 nM.[5b]

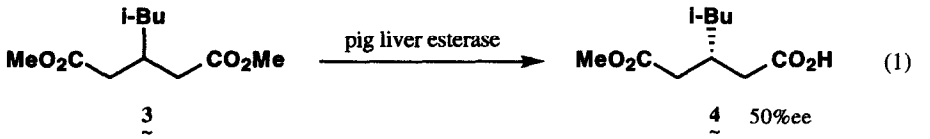


Gabapentin, 1



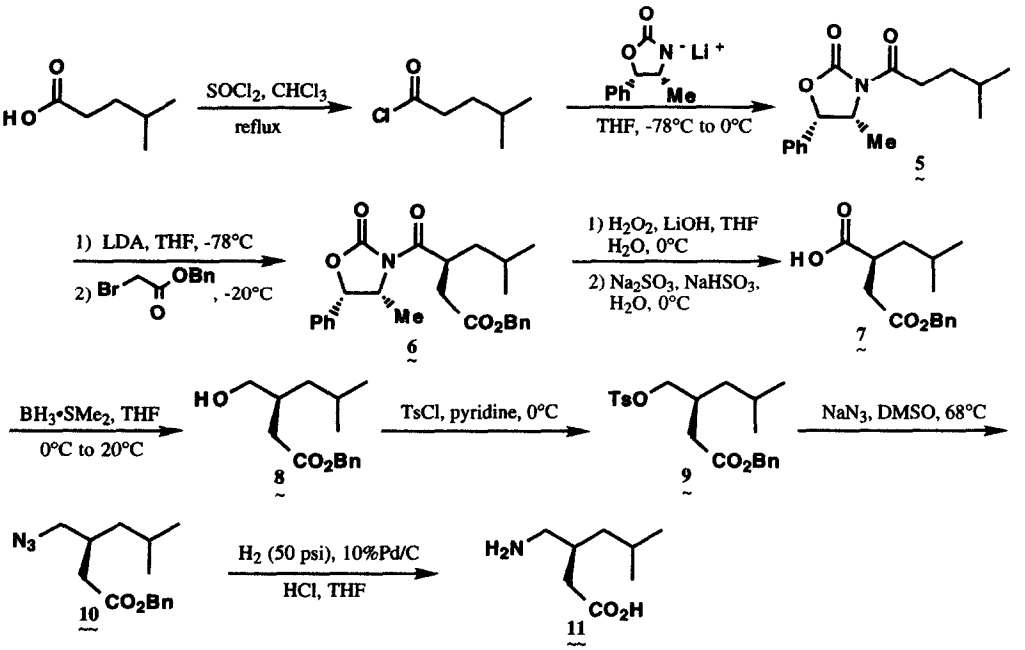
3-Isobutyl GABA, 2

In order to study the stereospecificity of this binding site, an enantioselective synthesis of both enantiomers of 3-isobutyl GABA is required. The method described by Silverman[6] for the preparation of (R)- and (S)-4-amino-3-methylbutanoic acids was investigated. However, the hydrolysis of 3-isobutylglutarate diester **3** using pig liver esterase (PLE, E.C. 3.1.1.1) gave the desired acid ester **4** in only 50% ee (Equation 1).



Another approach based on Evans' chiral oxazolidinone alkylation reaction[7] was studied (Scheme 1). Thus, 4-methylpentanoic acid was converted into the corresponding acid chloride by thionyl chloride treatment, followed by acylation of the anion of (4R,5S)-(+)-4-methyl-5-phenyl-2-oxazolidinone to give the acyloxazolidinone **5** in 64% yield. This acyloxazolidinone was then alkylated with benzyl bromoacetate to give the alkylated product **6** in 53% yield and >95%ee.[8] The chiral auxiliary on the acyloxazolidinone **6** was removed by lithium hydroxide/hydrogen peroxide treatment followed by a reductive work-up with a solution of sodium bisulphite which was pre-adjusted to pH = 7 with sodium sulphite. The resulting acid **7** was reduced to the corresponding alcohol **8** using borane dimethylsulfide complex. The alcohol **8** was converted to the azide **10** by first treatment with tosyl chloride in pyridine and then with sodium azide in dimethyl sulphoxide at 65°C. The azide **10** was then converted to (S)-3-isobutyl GABA, PD144723,[9] by hydrogenation using palladium catalyst. The (R)-enantiomer, PD144550, was prepared by the same method using (4S,5R)-(-)-4-methyl-5-phenyl-2-oxazolidinone as the chiral auxiliary.

Scheme 1: Synthesis of (S)-(+)-3-isobutyl GABA, PD144723.



Partially purified rat neocortex synaptic plasma membranes[5c] were used in binding studies. The membrane preparation (0.1-0.3 mg protein) was incubated with 20 nM [^{3}H]gabapentin in 10 mM HEPES buffer (sodium free, pH 7.4 at 20°C) in the presence of varying concentrations of test compound for 30 min at room temperature before filtering onto GFB filters under vacuum. Filters were washed three times with 5 mL of ice-cold 100 mM NaCl solution and dpm bound to filters was determined using liquid scintillation counting. Non-specific binding

was defined by dpm bound in the presence of 10 μM of (±)-3-isobutyl GABA. The (S)-enantiomer, PD144723, was found to be the most potent compound yet studied for displacement of [^{3}H]gabapentin from the gabapentin binding site *in vitro*, having an IC_{50} of 37 nM. The (R)-enantiomer, PD144550, displaced [^{3}H]gabapentin at a much higher concentration with an IC_{50} of 620 nM. The comparatively poor binding of PD144550 at the gabapentin binding site compared with PD144723 illustrates the stereoselectivity of this site (Fig. 1).

Male CF-1 strain mice (20-25 g) were used in anticonvulsant testing. Maximal electroshock was delivered with corneal electrodes as previously described by Krall and coworkers.[10] It is consistent with the binding data, that PD144723 (ED_{50} = 20 mg/Kg) is much more active than both gabapentin (ED_{50} = 87 mg/Kg) and PD144550 (ED_{50} > 300 mg/Kg) in blocking maximal electroshock seizures in mice (Fig. 2). It is also worth noting that PD144550 is inactive in this maximal electroshock seizures model even with an i.v. dose of 300 mg/kg.

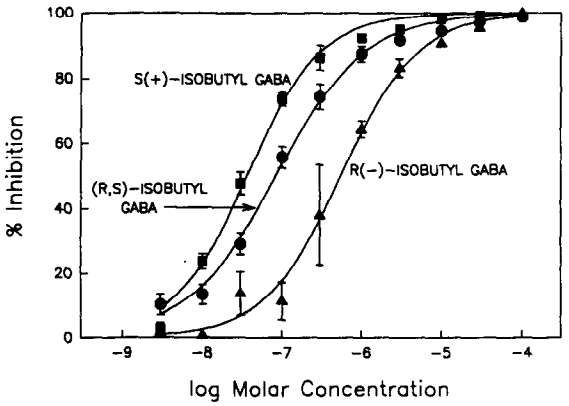


Fig. 1: Inhibition of [^{3}H]gabapentin binding by 3-isobutyl GABA racemate and enantiomers. IC_{50} values are: gabapentin 0.08 μM, (±)-isobutyl GABA 0.083 μM, R-(-)-enantiome 0.62 μM, S-(+)-enantiomer 0.037 μM.

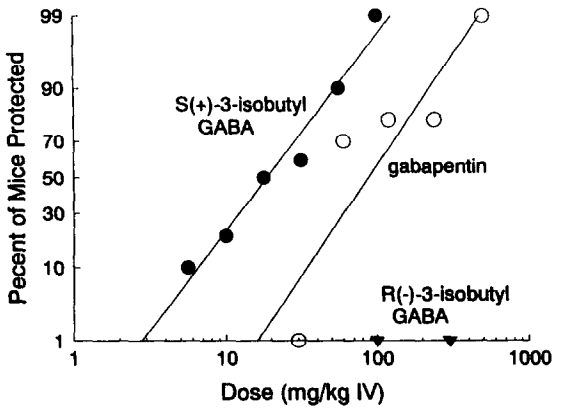


Fig. 2: Dose-response experiments with the enantiomers of 3-isobutyl GABA compared with those for gabapentin with intravenous administration to mice. Straight lines represent best fit probit analyses.

Although a defined physiological role of the gabapentin binding site remains to be elucidated, the present results further support that action of gabapentin and 3-isobutyl GABA at the high-affinity gabapentin binding site is related to anticonvulsant action *in vivo*.

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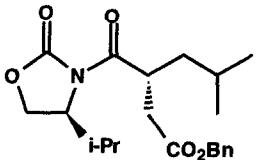
8. The enantiomeric excess was determined by 300 MHz ^{1}H NMR comparison of the (R)- and (S)-MTPA esters of alcohol **8**. The absolute stereochemistry of acyloxazolidinone **6** was determined based on an X-ray crystallographic analysis of the acyloxazolidinone **6a** and its subsequence conversion into the (R)-enantiomer, PD144550.



6a

9. All new compounds gave satisfactory spectral and analytical data. For PD144723: ^{1}H NMR (300 MHz, D_2O) δ 3.00 (ABX, 2H, J_{AB}=12.95 Hz, J_{AX}=13.16 Hz, J_{BX}=5.36 Hz, υ_{AB}=22.87 Hz), 2.39 (m, 2H), 2.19 (m, 1H), 1.62 (m, 1H), 1.20 (m, 2H), 0.88 (d, 6H, J=6.44 Hz). MS (EI): m/z 160(MH^+), 142. IR (KBr): 3431, 2899, 1553, 1369 cm^{-1}. Anal. Calcd for $C_8H_{17}NO_2$: C, 60.35; H, 10.76; N, 8.80. Found: C, 60.21; H, 10.69; N, 8.92. $[\alpha]_D^{23}$ = +10.52° (c 1.06, H_2O). mp = 186-188°C.

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