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The Enantiomer of Strychnine is a Weak Antagonist of the Inhibitory Glycine Receptor

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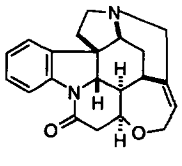
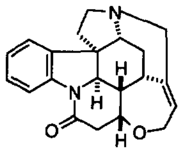
Abstract. *ent*-Strychnine (**2**) was synthesized for the first time from (1*R*,4*S*)-(+)-4-hydroxy-2-cyclopentenyl acetate (**3**) by a slight modification of the sequence recently employed to prepare natural (–)-strychnine. *ent*-Strychnine is about a 1000 fold weaker antagonist of the inhibitory glycine receptor expressed in oocytes than natural (–)-strychnine.

Glycine and γ-aminobutyric acid (GABA) are the main inhibitory neurotransmitters in the central nervous system of both vertebrates and invertebrates.[3] Glycine receptors are localized mainly in the spinal cord and lower brainstem, where glycine is the predominant inhibitory neurotransmitter. Biochemical and molecular cloning investigations have demonstrated that the inhibitory glycine receptor is a member of the ligand-gated ion channel family of receptors.[4-6] In a manner similar to the $GABA_A$ receptor, ligand binding to the glycine receptor inhibits neuronal firing by increasing chloride permeability of the neuronal membrane and thus antagonizing membrane depolarization. The plant alkaloid strychnine (**1**) is the best characterized high-affinity antagonist of the inhibitory glycine receptor (often called the strychnine-sensitive glycine receptor). Strychnine binding abolishes glycinergic inhibition and results in overexcitation of the motor system and muscular convulsions. Upon UV irradiation $[^3H]$strychnine behaves like a photoaffinity agent and is covalently attached to the 48-kD transmembrane α_I subunit of the glycine receptor. This incorporation of strychnine is blocked by glycine indicating that the α subunit also contains the glycine binding site.[5]



(–)-strychnine (**1**)



ent-strychnine (**2**)

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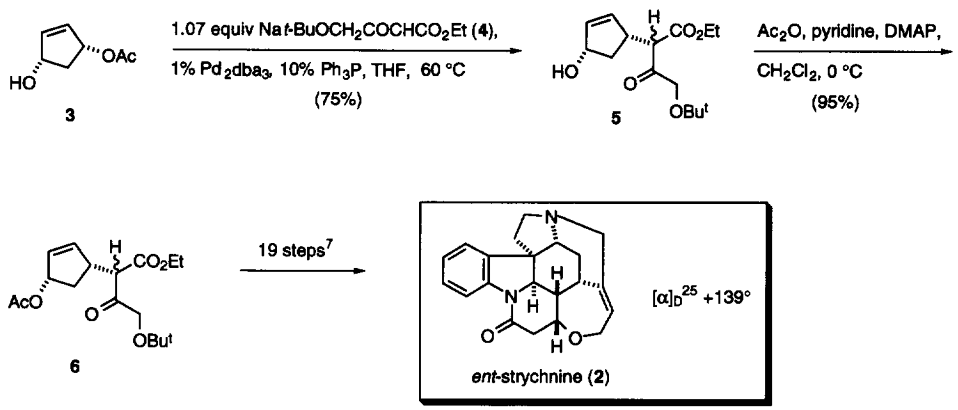
In this Letter we report that the enantiomer of strychnine (*ent*-strychnine, **2**) is a significantly weaker antagonist of the glycine receptor than the natural plant alkaloid. This result establishes that strychnine binding to the glycine receptor involves specific three-dimensional interactions.

The first asymmetric total synthesis of (–)-strychnine (**1**) was recently accomplished[7] in ~3% overall yield from readily available enantiopure (1*R*,4*S*)-(+)-4-hydroxy-2-cyclopentenyl acetate (**3**).[8] By design, a slight modification of this synthesis strategy allowed *ent*-strychnine to be prepared for the first time (Scheme I). Direct palladium-catalyzed coupling[9] of **3** with the sodium salt **4** of ethyl 4-*t*-butoxy-3-oxobutanoate[10] cleanly gave the *cis*-adduct **5** (a 1:1 mixture of epimers) in 75% yield.[11] Acetylation then provided **6** (95% yield). The cyclopentenyl β-keto ester **6**, which is enantiomeric with an intermediate employed in the synthesis of (–)-strychnine, was then converted to *ent*-strychnine (**2**) by the established sequence.[7] *Ent*-strychnine showed $[\alpha]_D^{25}$ +139° (c = 0.4, $CHCl_3$), which is identical in magnitude but opposite in sign to the optical rotation of natural (–)-strychnine.[7,12]

Scheme I. Total Synthesis of *ent*-Strychnine (**2**).



The efficacy of *ent*-strychnine was assessed in voltage-clamped *Xenopus* oocytes expressing glycine receptors following the injection of rat spinal cord mRNA. We have shown previously that, upon application of glycine, the oocytes elicit large chloride currents that are abolished by a micromolar concentration of strychnine.[13] As seen in Figure 1, at a membrane potential of –60 mV, 10^{-6} M strychnine fully inhibits the current induced by 5×10^{-4} M glycine. In contrast, the same concentration of *ent*-strychnine leaves the glycine response unchanged; although at much higher concentrations *ent*-strychnine is able to block glycine receptors. As shown in Figure 2, 10^{-4} M *ent*-strychnine is needed to fully block glycine receptors. Our data shows that the concentration of *ent*-strychnine that antagonizes half of the glycine response (IC_{50}) is about 1000 times larger than that of strychnine.

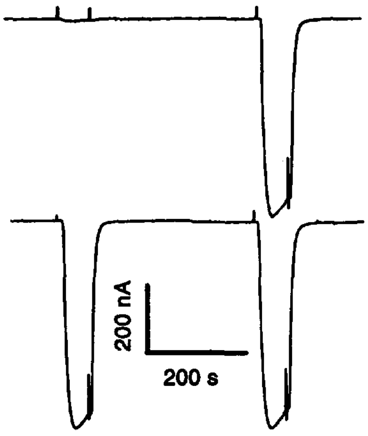


Figure 1. Effect of strychnine (top trace) and *ent*-strychnine (bottom trace) on glycine receptors expressed in *Xenopus laevis* oocytes. For both traces, 5 x 10^{-4}M glycine and 10^{-6}M antagonist were coapplied between the two leftmost tick marks. Control responses, on the right side of the traces, were subsequently elicited by applying glycine alone.

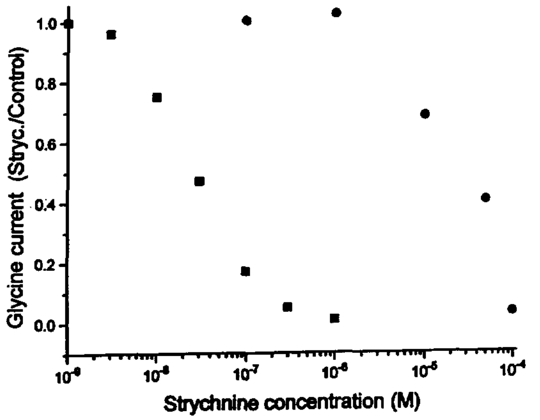


Figure 2. Dose-dependent inhibition of glycine-produced currents by strychnine (filled squares) and *ent*-strychnine (filled circles). Points are plotted as the ratio between the response in presence of the antagonist and the control response, versus the strychnine or *ent*-strychnine concentration. Each point represents data from 2-6 oocytes.

In summary, we have synthesized the enantiomer of strychnine and shown that it blocks glycine receptors with a much lower affinity than strychnine. The recent discovery that the strychnine-binding subunit of the glycine receptor exists in several different molecular forms[5,13] raises the question of the action of *ent*-strychnine on each of these variants. So far, the interaction between strychnine and the glycine receptor has been studied by modifying the strychnine-binding subunit. It has been determined, for instance, that natural strychnine binds to a site located within amino-acids 171-220 of the α subunit of the glycine receptor and that four amino-acid residues on the extracellular loop of the glycine receptor play a crucial role in strychnine binding.[14,15] The availability of *ent*-strychnine may prove especially useful for gaining more information about the spatial arrangement of these and other amino acids involved in strychnine action. Since the glycine receptor belongs to a family of proteins sharing much structural homology, knowledge of the three-dimensional conformation of the glycine receptor could provide insight about the geometry of the extracellular portion of other ligand-gated ion channels, like the GABA and the nicotinic receptors.

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