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[^{3}H]-(*R*)-NPTS, a Radioligand for the Type 1 Glycine Transporter

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Abstract—The synthesis of NPTS, **6**, a potent inhibitor of the type 1 glycine transporter (GlyT1) is described, as well as preparation of **6** in optically active and tritiated form for use as a radioligand for affinity displacement assay of GlyT1.

GlyT1, the type 1 glycine transporter, and its type 2 counterpart GlyT2 mediate the specific reuptake of glycine during synaptic transmission.[1] GlyT1 and GlyT2 belong to the Na+/Cl-dependent family of transporters, which includes transporters for the neurotransmitters dopamine, serotonin, adrenaline, and GABA.[2] Glycine is an obligatory co-agonist at the NMDA class of glutamate receptors, and since a deficiency in NMDA neurotransmission is thought to play a role in the etiology of schizophrenia, an increase of glycine levels has been proposed as a novel approach to treating psychosis.[3] Clinical studies with high dose glycine therapy, which improves outcome of schizophrenic patients with negative symptoms, supports the idea that augmenting NMDA transmission by increasing glycine levels can benefit psychotic patients.[4] Blockade of GlyT1-mediated reuptake of glycine may provide the same benefit, as GlyT1 is co-localized with NMDA receptors.[5] Recently, the first potent and selective GlyT1 inhibitor was reported, (*R*)-N[3-(4′fluorophenyl)-3-(4′phenylphenoxy)propyl]-sarcosine, ALX 5407.[6,7] NFPS, the racemic form of ALX 5407, has been shown to augment downstream effects of NMDA transmission, supporting the proposed role of GlyT1 in regulating NMDA transmission.[8] We began a program to explore the role of GlyT1 inhibitors in treating schizophrenia by developing an analogue of ALX 5407, (*R*)-*N*[3-phenyl-3-(4′-(4-toluoyl)phenoxy)-propyl]sarcosine ((*R*)-NPTS) as a suitable radioligand for a GlyT1 binding affinity assay (Fig. 1). We report herein the SAR and synthetic studies leading to NPTS, as well as the synthesis of [^{3}H]-(*R*)-NPTS.

The key step in the synthesis of compound **6** is the palladium-mediated boronic acid coupling with the acid chloride **4** to produce the requisite benzophenone derivative **5** (Fig. 2). This step follows a recent literature report, and afforded comparable yields and similar versatility in our hands.[9] As shown in Figure 1, **6** was somewhat weaker than **1** in a standard rat synaptosomal uptake assay using tritiated glycine. We decided to use **6** as the basis for a radioligand binding assay with improved sensitivity for detecting new GlyT1 inhibitors.

Synthesis of a suitable precursor in optically active form for radiolabeled (*R*)-NPTS is outlined in Figure 3. The challenge of preparing the water soluble amino alcohol **8** was solved with the Staudinger reaction.[10] The commercially available *R*-(+)-3-chloro-1-phenyl-1-propanol, **7**, was converted to the iodide and then to the azide, followed by reduction with triphenylphosphine and water in tetrahydrofuran. Without isolation, the amino alcohol **8** was reacted with sodium hydride in NMP and

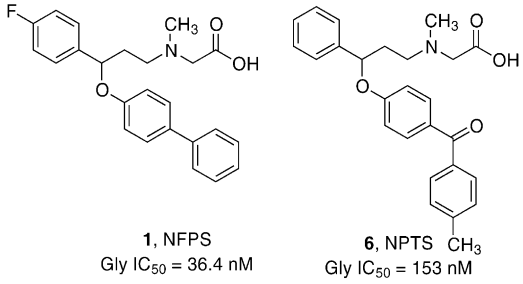

Figure 1. Structures of NFPS, **1**, and NPTS, **6**, and their activity in an assay measuring inhibition of [^{3}H]-glycine uptake into rat synaptosomes.

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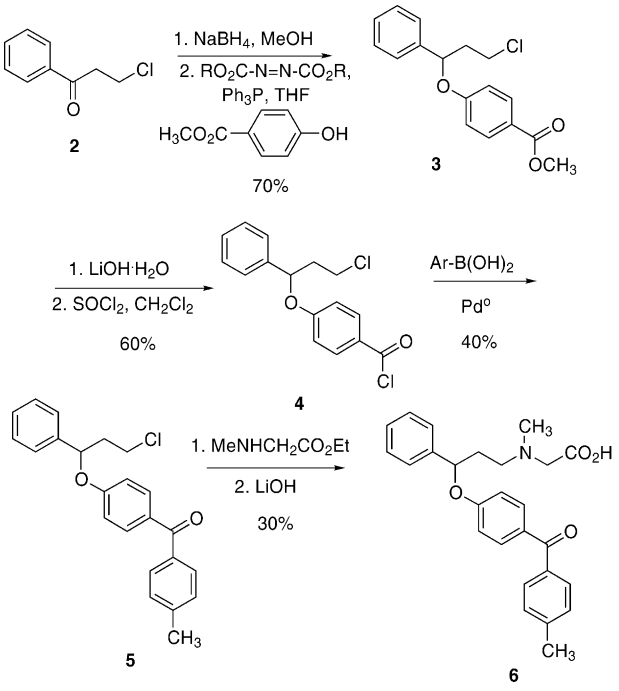

Figure 2. Preparation of NPTS in racemic form.

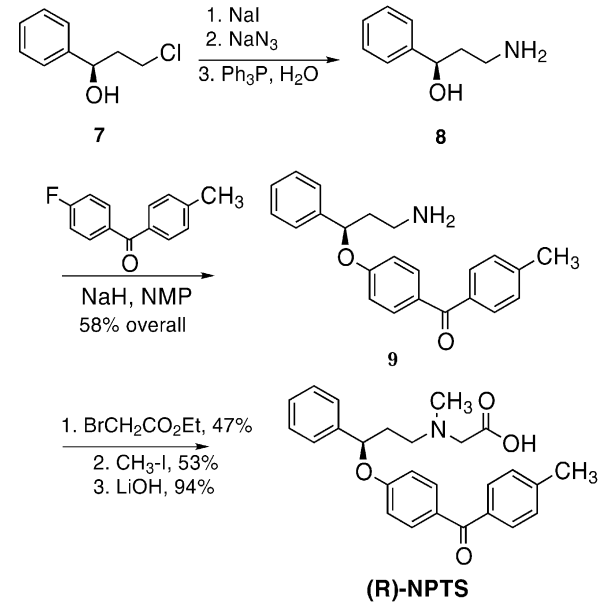

Figure 3. Synthesis of (*R*)-NPTS.

p-fluoro(4′-methyl)benzophenone, in overall 58% yield to this point, in analogy with earlier work on the synthesis of fluoxetine.[11] The final steps are carried out as before, and the opposite enantiomer was prepared from the corresponding *S*-(−)-alcohol. [^{3}H]-(*R*)-NPTS was prepared by substituting tritiated methyl iodide in the penultimate step, affording after purification material of >98% purity and 71 Ci/mmol.[12]

In studies of binding to the cloned human GlyT1c subtype of the GlyT1 transporter expressed in HEK 293 cells, [^{3}H]-(*R*)-NPTS showed a K_d value of 1 nM with

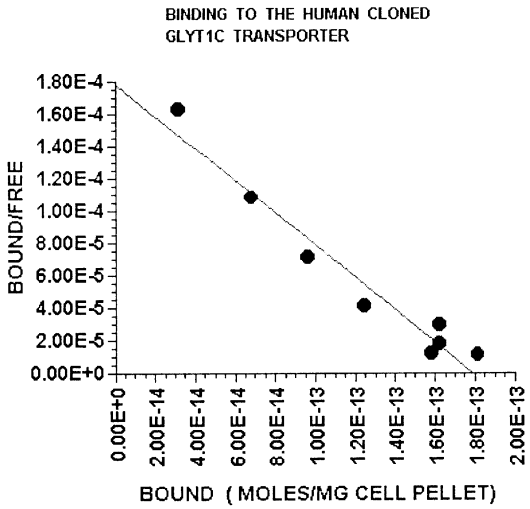

Figure 4. Scatchard plot of [^{3}H]-(*R*)-NPTS binding to HEK 293 cells expressing the GlyT1c transporter.

saturable binding by Scatchard analysis (Fig. 4). Using tissue from rat hippocampus, [^{3}H]-(*R*)-NPTS gave a K_d value of 0.8 nM, while compound **1** in the same tissue gave a K_i value of 1.6 nM.

The use of [^{3}H]-NPTS in a radioligand binding assay provides a higher level of sensitivity than the standard tritiated glycine uptake assay, which should facilitate SAR efforts in this new area.

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11. Robertson, D. W.; Krushinski, J. H.; Fuller, R. W.; Leander, J. D. *J. Med. Chem.* **1988**, *31*, 1412.
12. (*R*)-NPTS: $[\alpha]_D = -1.72°$. ^{13}C NMR (δ, $CDCl_3$): 21.80, 33.32, 41.65, 54.18, 56.73, 77.52, 115.68, 126.04, 128.54, 129.11, 129.23, 130.19, 131.07, 132.35, 135.27, 139.70, 143.06, 160.92, 167.88, 195.46. [^{3}H]-(*R*)-NPTS was purified by HPLC using an Inertsil ODS 10×250 mm column and a 20 mM aq potassium phosphate buffer, pH = 3 gradient with acetonitrile.
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