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# Radiosynthesis of a Ligand for Studying the Glycine Transporter: [$^{11}$C]ALX-5407

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## SUMMARY

[$^{11}$C]ALX-5407, R-*N*[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl] sarcosine, a chiral glycine transporter 1 antagonist, was labeled with [$^{11}$C]iodomethane by N-alkylation of methyl ester protected N-normethyl precursor, ALX-5536, and subsequent saponification of the methyl ester protecting group. The time for synthesis, purification, and formulation was 33 minutes with an average specific radioactivity of 3909 mCi/μmol (EOS) and average decay corrected radiochemical yield of 8%.

**Key Words**: Glycine, GlyT1, glycine transporter, ALX-5407, carbon-11, positron emission tomography

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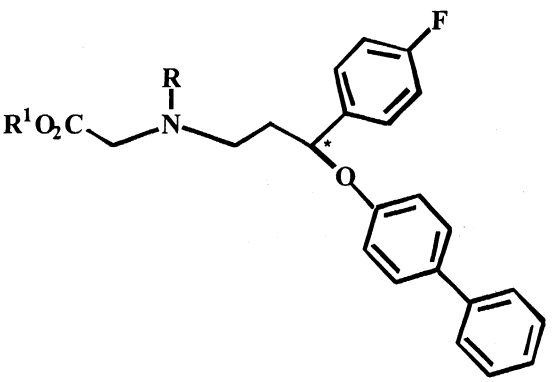
# INTRODUCTION

Glycine, a co-modulator of NMDA, and the NMDA glutamate system have been implicated in the pathophysiology of a number of neuropsychiatric disorders (1,2,3). Glycine has also been proposed as a potential treatment in schizophrenia (4) and is well tolerated in high doses in humans (5). Glycine antagonists have been also been proposed as treatments in ischemia (6,7). Since glycine concentration may be regulated by the glycine transporter (8,9), the glycine transporter may be important in pathophysiology and treatment. For example, a hyperglycinergic model exists for schizophrenia (2) and the reversal of PCP induced hyperactivity has been shown by glycine and glycine uptake inhibitors (10). Thus, it is not surprising that glycine transport inhibitors have been proposed as a potential new class of antipsychotics in the treatment of schizophrenia (11).

Of the various subtypes of the glycine transporter, the glycine transporter type 1 (GlyT1) is expressed in glia and neuronal neocortex in the same areas as high NMDA expression (12). Specifically, we have examined the radiolabeling of a chiral GlyT1 specific antagonist, ALX-5407 (Figure 1). ALX-5407 may be involved in controlling an NMDA function at the modulatory glycine site. Bergeron found that glycine and GlyT1 antagonists, specifically ALX-5407, selectively enhance the amplitude of NMDA glutamatergic components of excitatory




| R | $R^1$ | Compound |
|---|---|---|
| $CH_3$ | H | ALX-5407 |
| $CH_3$ | $CH_3$ | ALX-5406 |
| H | $CH_3$ | ALX-5536 |
| H | H | Saponified ALX-5536 |

Figure 1: Structure of ALX-5407 and related compounds.
The * denotes the chiral center.

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postsynaptic currents (12). The enhancement of these NMDA receptors by such antagonists could provide an appropriate site for therapy where NMDA is hypofunctioning as in schizophrenia (12). We have taken the initial steps of radiolabeling this prototypical chiral glycine transporter antagonist with carbon-11 in the hope that it will be not only useful for imaging pathophysiological changes in schizophrenia cerebral ischemia and other diseases, but also as a potential surrogate marker for therapeutic trials in these disorders. The synthesis, purification and formulation of [$^{11}$C]ALX-5407 from chiral precursors are discussed in this paper.

## RESULTS

[$^{11}$C]ALX-5407 was synthesized using a three step procedure. First, the methyl ester protected N-normethyl precursor, ALX-5536, was reacted with [$^{11}$C]methyl iodide, produced using a General Electric PETtrace MeI Microlab, to prepare the N-[$^{11}$C]methylated methyl ester, [$^{11}$C]ALX-5406. Analytical HPLC of the methylation reaction indicates 60% of the radioactive mixture is the product, [$^{11}$C]ALX-5406, with less than 8% unreacted [$^{11}$C]methyl iodide present and other uncharacterized hydrophilic peaks. Next, the reaction solution containing [$^{11}$C]ALX-5406 and unlabeled ALX-5536 was reacted with decanoyl chloride in the presence of triethylamine to acylate the remaining, unreacted ALX-5536. Third, the methyl ester protecting group was removed using aqueous base to form the product, [$^{11}$C]ALX-5407. This hydrolysis reaction mixture shows less than 5% non-hydrolyzed [$^{11}$C]ALX-5406 present by analytical HPLC.

Acylation of the unreacted ALX-5536 by decanoyl chloride effectively eliminated the problem of separating by chromatography a large quantity of tailing saponified normethyl precursor from the final product, [$^{11}$C]ALX-5407, by derivatizing the unreacted precursor to a compound with a much longer retention time. The decanoic acid produced when the excess decanoyl chloride reacts during the base hydrolysis is also known to have a long retention time. Figure 2 is a typical preparative chromatogram of the base hydrolyzed reaction solution. The uv trace shows a small peak for some saponified normethyl precursor that was not acylated

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by decanoyl chloride with other unknown more hydrophilic peaks and the product peak of no carrier added [$^{11}$C]ALX-5407. The radioactive trace shows [$^{11}$C]ALX-5407 (17%) plus other undetermined hydrophilic peaks.

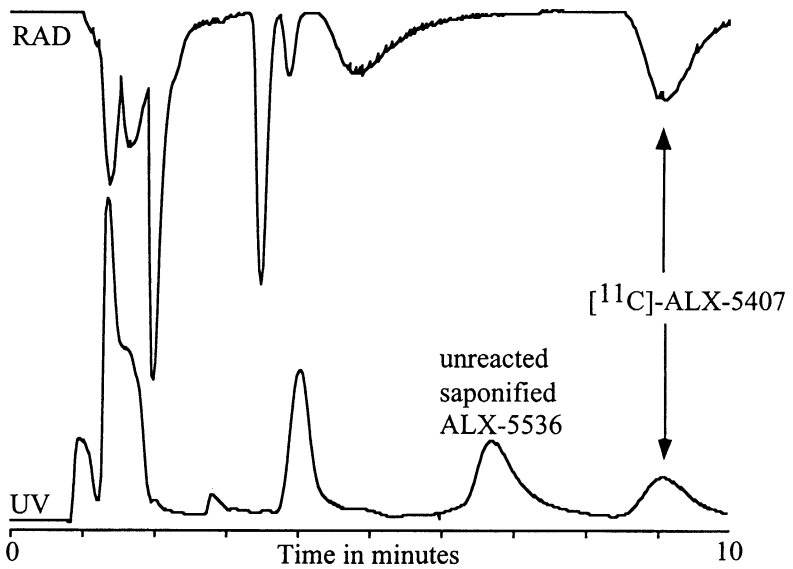


Figure 2: Preparative ultraviolet and radioactive chromatograms of the [$^{11}$C]ALX-5407 reaction mixture.

The synthesis, semipreparative high pressure liquid chromatography (HPLC), and formulation was completed in an average time of 33 minutes (n=3) with an average decay corrected radiochemical yield of 8% based on [$^{11}$C]methyl iodide. An average of 21 mCi of final product had average specific radioactivity was 3909 mCi/μmole at end of synthesis. The final formulated solution was chemically and radiochemically pure (>99%) as determined by analytical HPLC.

## EXPERIMENTAL

ALX-5407, ALX-5406, ALX-5536 were provided enantiomerically pure by Allelix Neuroscience, Cranbury, New Jersey. Dimethylformamide (DMF) was stirred over BaO overnight and vacuum distilled prior to use. All other reagents

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were ACS or HPLC purity. HPLC analysis and purification were performed with two Waters 590EF HPLC pumps, an in-line fixed wavelength (254 nm) detector, and a single two inch NaI crystal radioactive detector. HPLC chromatograms were recorded by a Rainin Dynamax dual channel control/interface module connected to a Macintosh computer with appropriate program software (Dynamax - version 1.4). HPLC semipreparative purification were performed on an Alltech 10 μm C-18 Econosil column (10 x 250 mm) using a mobile phase of 40% acetonitrile / 60% water (0.1 M ammonium formate) at a flow rate of 10 mL/min. Chemical and radiochemical purity were determined using a Novapak 4μm C-18 HPLC column (3.9 x 150 mm) with a mobile phase of 40% acetonitrile / 60% water (0.1 M ammonium formate) at a flow rate of 3 mL/min. A dose calibrator (Capintec 12R) was used for all radioactivity measurements.

### *Radiosynthesis and purification of [$^{11}$C]ALX-5407.*

ALX-5536 (0.5 mg, 1.2 μmoles) was dissolved in 200 μL of DMF and the sealed vial cooled to -78°C. [$^{11}$C]Methyl iodide was synthesized from cyclotron produced [$^{11}$C]carbon dioxide using a General Electric PETtrace MeI Microlab. The [$^{11}$C]methyl iodide was bubbled into the sealed vial containing the precursor, ALX-5536, until the radioactivity reached a plateau. The vial was heated to 80°C for 5 minutes. Triethylamine (10 μL) and decanoyl chloride (10 μL) were added to the vial with subsequent heating for 1 minute at 80°C. Next, 50 μL of 6N NaOH was added and heating at 80°C was continued for 2 minutes. HPLC solvent (200 μL) was added prior to applying the solution to the semipreparative HPLC column. The k' for [ $^{11}$C]ALX-5407 was 8.3 and the k' for the saponified ALX-5563 was 5.9. After collection and vacuum evaporation to dryness, the product was redissolved in 7 mL of sterile normal saline followed by sterile filtration (Millex GV) into a sterile evacuated vial. Sterile 8.4% sodium bicarbonate (3 mL) was added aseptically. The chemical and radiochemical purity of the final solution were determined as described previously.

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## CONCLUSION

[$^{11}$C]ALX-5407 was synthesized in three steps from the methyl ester protected normethyl precursor, ALX-5536 and [$^{11}$C]methyl iodide. The yield and specific radioactivity are sufficient for *in vivo* animal studies; and, possible use (with optimization of the synthetic procedure) in human positron emission tomographic studies.

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