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Syntheses of [^{11}C] and [^{3}H] LY274601, a serotonin$_{1A}$ receptor agonist

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Summary

LY274601, R-(+)-8-thiomethyl-2-(di-n-propylamino)tetralin, a serotonin (5-HT)$_{1A}$ receptor full agonist with high affinity (Ki: 0.6nM) and selectivity, was labeled with ^{11}C for imaging 5-HT$_{1A}$ receptor sites *in vivo* by positron emission tomography (PET). [^{11}C]LY274601 was synthesized by *S*-methylation of the normethyl precursor unmasked *via* hydrolysis of the butyrate thioester of LY274601. The methylation reaction with [^{11}C]iodomethane proceeded quickly and efficiently in DMF at 40°C, yielding the radiotracer in an average overall radiochemical yield of 35.7±9.8%. The synthesis time including HPLC purification and formulation for injection was approximately 30 min. The specific activity was 630±78mCi/μmol at the end of synthesis (E.O.S.). This labeling procedure was also employed in the preparation of [^{3}H]LY274601, R-(+)-8-[^{3}H]methylthio-2-(di-n-propyl-amino)tetralin, from [^{3}H]iodomethane.

Key Words: radiotracer, synthesis, serotonin$_{1A}$ receptor, carbon-11, tritium, positron emission tomography

Introduction

A PET radiotracer which selectively labels the serotonin (5-HT)$_{1A}$ receptor is of interest to study various affective disorders such as anxiety, depression, motion sickness or eating disorders. In the past, several agonists as well as antagonists of the receptor have been labeled with ^{11}C or ^{18}F and evaluated as radiotracers for imaging 5-HT$_{1A}$ receptor sites by PET (1-6). These studies revealed large differences in the *in vivo* biodistribution between 5-HT$_{1A}$ receptor agonist radiotracers and antagonist tracers.

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Comparing *in vivo* behavior of antagonist PET radiotracers with that of agonists would be informative, since agonist radiotracers label only the high affinity state of receptors (*i.e.* coupled with G protein) while antagonist PET tracers such as [^{11}C]WAY-100635 (4, 6) label not only G protein coupled receptors but also the low affinity uncoupled form. Therefore, agonist PET radiotracers might yield different information on the 5-HT$_{1A}$ receptor functional status than that obtained with antagonist tracers. Here we report radiolabeling of R-(+)-8-methylthio-2-(di-n-propylamino)tetralin, LY274601, a 5-HT$_{1A}$ full agonist with a Ki value of 0.6 nM (7), with ^{11}C. The agonist binds to the 5-HT$_{1A}$ receptor with a greater than 2000 and 500 fold affinity over D_1 and D_2 receptors, respectively. Its binding selectivity over other 5-HT receptor groups and subtypes such as 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_{1B}$ or 5-HT$_{1C}$ is 400 - 2000 fold. LY274601 has a similar chemical structure to 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin, with the only difference being the methylthio group at the 8 position. It is suggested in the literature that the structural difference provides a longer duration in treatment of psychiatric disorders (7).

In addition, we report labeling of LY274601 with ^{3}H. [^{3}H]LY274601 would be a useful ligand for *in vitro* binding studies of 5-HT$_{1A}$ receptors and *in vivo* biodistribution studies in small animals. It would be particularly interesting to compare the biodistribution of [^{3}H]LY274601 with that of a selective 5-HT$_{1A}$ antagonist radiotracer using *ex vivo* autoradiography to reveal more detailed structural differences in target tissues than is possible by PET.

Results and Discussion

[^{11}C]LY274601 was synthesized by *S*-methylation of the normethyl precursor unmasked via hydrolysis of the butyrate thioester of LY274601 (**b**) (Figure 1).

As previously reported (8), the butyryl thioester functionality was easily removed by hydrolysis with tetrabutylammonium hydroxide yielding normethyl LY274601, a thiol (**b**). The *S*-methylation reaction between the unmasked thiol and [^{11}C]iodomethane proceeded quickly at 40-45°C yielding [^{11}C]LY274601 (**c**) in an average radiochemical yield of 35% (decay corrected) in 1 min. Synthesis time including HPLC purification and formulation for injection was approximately 30 min. Average specific activity was 636±79 mCi/μmol at the end of synthesis. Radiochemical purity was greater than 98 %. Compared with the previously-reported radiosynthesis of [^{11}C]8-OH-DPAT or its analog (1, 9), which was carried out by *N*-propylation with [^{11}C]iodopropane, [^{11}C]LY274601 can be synthesized with [^{11}C]iodomethane by a simpler and less time consuming procedure with higher efficiency.

The butyrate thioester precursor (**a**) was also used in the preparation of [^{3}H]LY274601 with high specific activity [^{3}H]iodomethane. Since the [^{3}H]iodomethane was supplied in toluene, the *S*-methylation reaction in toluene between the unmasked thiol (**b**) and iodomethane was studied first using unlabeled iodomethane. HPLC analyses confirmed that the *S*-methylation reaction in toluene proceeded as efficiently as in DMF.

Therefore, [^{3}H]iodomethane in toluene was used without distillation. The tritiation procedure yielded [^{3}H]LY274601 in an isolated radiochemical yield of 8 %. The specific activity was 31.8 mCi/μmol. The radiochemical purity was greater than 98%. The HCl salt of [^{3}H]LY274601 was found stable for up to 3 months while the free base suffered decomposition. Compared with the previously-reported 6-step procedure for the tritiation of 8-OH-DPAT (10, 11) which could be applied to the preparation of [^{3}H]LY274601, the method described here is simpler, less time consuming and more efficient.

The utility of thioester precursors for *S*-methylations has been validated in the preparation of [^{11}C] and [^{3}H]McN5652 using [^{11}C] or [^{3}H]iodomethane (8). Similar procedures were successfully employed here for the preparation of [^{11}C] and [^{3}H]LY274601. Thioester precursors offer greater stability than normethyl thiol precursors. Thioesters are resistant to oxidation, but easily hydrolyzed by base such as tetrabutylammonium hydroxide (12,13). The unmasked thiols readily methylate with reagents such as iodomethane. Thus, with thioester precursors and radiolabeled iodomethane *S*-methylations occur with high efficiency at 40-45°C. According to HPLC analyses, 70-80 % of ^{11}C from [^{11}C]iodomethane was incorporated into the LY molecules (**c**) in 1 min.

These [^{11}C] and [^{3}H] labeled radioligands are currently being used to study their *in vivo* binding to 5-HT$_{1A}$ receptor sites.

Experimental

Authentic LY274601 was provided as a gift from Dr. John M. Schauss of Lilly Research Laboratories, Indianapolis, IN, USA. ^{1}H-N.M.R. spectroscopic analyses were performed by Spectral Data Services, (Champaign, IL, USA) on a 360 MHz instrument using $(CH_3)_4Si$ as an internal standard. High resolution mass spectrometric analyses were performed by Mass Spectrometry Service Laboratory, University of Minnesota. [^{3}H]Iodomethane was purchased from Amersham Life Science (Elk Grove, IL, USA).

Synthesis of [^{11}C]LY274601 (**c**)

[^{11}C]LY274601 was synthesized by *S*-methylation of the normethyl precursor unmasked via hydrolysis of the butyrate thioester of LY274601(**b**) (Figure 1). The hydrolysis of the thioester was performed according to the literature procedure (8). Ten microliters of tetrabutylammonium hydroxide (1 M in methanol) were added to 0.5 mg of the butyrate thioester (**a**) in a 1 mL glass reaction vial and stirred on a vortex mixer. After 10 min, 200 μL DMF was added, and the reaction vial was sealed and cooled in an ethanol-dry ice bath. [^{11}C]Iodomethane, synthesized by a procedure previously described (14) was introduced by a stream of helium into the cooled reaction vial. When the level of radioactivity reached a maximum, the carrier gas flow was stopped and the reaction vessel submerged in a 40-45°C water bath for 1 min. The reaction mixture was diluted with 200 μL HPLC solvent (acetonitrile:water, 60/40, containing 0.1 N ammonium formate) and applied to a semipreparative C-18 column (Alltech Econosil, 25 cm x 10 mm i.d.).

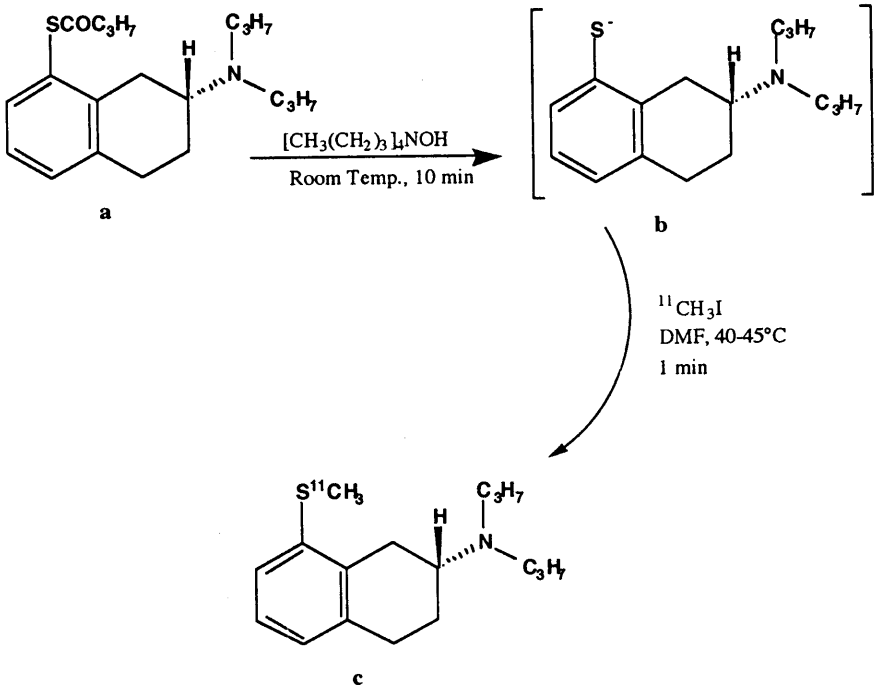

Figure 1. Radiosynthesis of [^{11}C]LY274601

HPLC separation of the product was carried out using the above-mentioned mobile phase at a flow of 9 mL/min. The effluent was monitored with a u.v. detector at 254 nm (Dynamax UV-1) and a radioactivity detector with a photodiode and a $CdWO_4$ crystal (Biomedical Instrumentation Group, University of Pennsylvania). The eluant with a radioactivity peak corresponding to the retention time of the authentic LY274601 (t_R = 7.7 min, k' = 7.6) was collected in a flask, diluted with 20 mL sterile water and passed through a C-18 Sep-Pak Light (Waters-Millipore). The Sep-Pak was washed with 5 mL of sterile water and flushed with air. [^{11}C]LY274601 was eluted from the Sep-Pak with 1 mL of ethanol (200 proof, USP; Spectrum). The ethanol solution was diluted with 20 mL sterile water and filtered through a sterile 0.22 μm filter (Millex-GV) into a sterile evacuated vial (Mediphysics).

After formulation of the [^{11}C]LY274601 solution, the specific activity was determined as previously described (14). An aliquot of the final solution of known volume was applied to an analytical reversed phase HPLC column (Alltech C-18, 25 cm x 4.6 mm i.d.) eluted with a mobile phase of actonitrile and water (60:40) containing 0.1 N ammonium formate. The mass of the [^{11}C]LY274601 was determined by comparing the u.v. absorption of the [^{11}C]LY274601 solution to that of a standard LY274601 solution of a known concentration. The specific activity at the end of synthesis (E.O.S.) was calculated by dividing the radioactivity of the final solution by the mass of [^{11}C]LY274601.

Synthesis of [^{3}H]LY274601

Under an argon atmosphere, tetrabutylammonium hydroxide in methanol (10 μL, 1.0 M) was added to the butyrate thioester (**a**) (1.4 mg) contained in a 1 mL septum-sealed glass vial. After 10 min, [^{3}H]iodomethane (10 mCi, 85 mCi/μmol, Amersham Life Science) in 500 μL toluene was added and the solution heated to 80°C for 5 min. Unreacted [^{3}H]iodomethane was subsequently removed by bubbling argon through the solution and trapped in a charcoal column filter. The reaction mixture was purified via semi-preparative HPLC as described above for [^{11}C]LY274601. Fractions were collected manually (30 s) and an aliquot from each fraction was dissolved in 10 mL of scintillation cocktail (Ultima Gold, Packard) and counted using a scintillation counter (Tri-Carb 2300TR). Fractions with a radioactivity peak corresponding to the retention time of the authentic LY274601 were combined, diluted with 30 mL of sterile water, and passed through a C-18 Sep-Pak Light. The Sep-Pak was washed with 5 mL sterile water and flushed with argon. [^{3}H]LY274601 was eluted with 1 mL ethanol containing 0.5 % HCl. Specific activity and radiochemical purity were determined by injecting an aliquot of known activity onto an analytical column (Alltech Econosil C-18, 25 cm x 4 mm i.d.) and comparing the uv absorption peak area to that of a standard of a known concentration.

Synthesis of butyrate thioester precursor (**a**)

Fifty mg of LY274601 was demethylated with a 5-fold excess of sodium thiomethoxide as previously described (15). After heating at 160°C for 1h, the reaction mixture was cooled to room temperature, and 10-fold excess of butyryl chloride was added. The reaction mixture was stirred for 2-3 min and quenched by addition of 5 mL water. The product was extracted into dichloromethane, dried and the solvent evaporated under vacuum. Purification of the butyrate thioester was performed by semipreparative HPLC (Alltech C-18 Econosil, 25 cm x 10 mm i.d.) with acetonitrile-water (60/40) containing 0.1 N ammonium formate at a flow of 9 mL/min. The eluant containing butyrate thioester of LY274601 was collected in a flask, diluted with 30 mL water and passed through a C-18 Sep-Pak Light. The Sep-Pak was washed with 10 mL of water and flushed with air. The thioester was eluted from the Sep-Pak with 1 mL of methanol. Then the solvent was removed by rotary evaporation. The identification of the thioester was confirmed by NMR spectroscopy and mass spectrometry. In addition, the thioester was converted back to LY274601 with unlabeled iodomethane using the procedure described above for the radiosyntheses. 1H NMR ($CDCl_3$) δ 2.7-3.7 (m, 7H, aliph), 1.0 (t, 9H, J = 7.2 Hz, $CH_3CH_2CH_2$), 1.9-2.0 (m, 6H, $CH_3CH_2CH_2$), 2.8-3.2 (m, 6H, $CH_3CH_2CH_2$), 6.94 (d, 1H, arom), 7.06 (d, 1H, arom), 7.18 (t, 1H, arom). HRMS-EI ($C_{20}H_{31}ONS$): m/z calcd 333.213; found 333.214 [M+].

Conclusion

The highly selective and potent 5-HT_{1A} agonist, LY274601, can be labeled efficiently and quickly with ^{11}C by *S*-methylation of the normethyl precursor unmasked via *in situ* hydrolysis of its butyrate thioester. Using a similar procedure [^{3}H]LY274601 can be also synthesized with high efficiency.

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References

1. Thorell JO, Stone-Elander S, and Ingvar M - J Labelled Compds Radiopharm 35 : 496 (1994)
2. Thorell JO, Hedberg MH, Johansson AM, and Hacksell U - J Labelled Compds Radiopharm 37: 314 (1996)
3. Visser GM, van Waarde A, Medema J, and Vaalburg W - J Labelled Compds Radiopharm 37: 280 (1996)
4. Mathis CA, Simpson NR, Mahmood K, Kinahan PE, and Mintun MA - Life Sci 55: PL 403-7 (1994)
5. Wilson AA, DaSilva JN, and Houle S - J Labelled Compds R adiopharm 37: 295 (1996)
6. Pike VW, McCarron JA, Lammerstma AA, Hume SP, Poole K, Grasby PM, Malizia A, Cliffe IA, Fletcher A, and Bench CJ - Eur J Pharm 283 : R 1-3 (1995)
7. Foreman MM, Fuller RW, Leander JD, Nelson DL, Calligaro DO, Lucaites VL, Wong DT, Zhang L, Barrett JE, and Schaus JM - Drug Dev Res 34 : 66-85 (1995)
8. Suehiro M, Musachio JL, Dannals RF, Mathews WB, Ravert HT, Scheffel U, and Wagner HN Jr.- Nucl Med Biol 22 : 543-5 (1995)
9. Hallidin C, Swahn C-G, Suhara T, Farde L, Karlsson P, Sokoloff P, and Sedvall G - J Labelled Compds Radiopharm 35 : 471 (1994)
10. Gozlan H, El Mestikawy, Pìchat L, Glowinski J and Hamon M. - Nature 305: 140-2 (1983)
11. Hacksell U, Svensson U, Nilsson JLG, Hjorth S, Carlsson, Wikström H, Lindberg P, Sanchez D - J Med Chem 22: 1469-75 (1979)
12. Harnish DP and Tarbell DS - Anal Chem 21 : 968-70 (1949)
13. Zervas L and Photaki I - J Am Chem Soc 84 : 3889-94 (1962)
14. Dannals RF, Ravert HT, Wilson AA, and Wagner HN Jr. - Int J Appl Radiat Isot 37 : 433-4 (1986)
15. Suehiro M, Ravert HT, Dannals RF, Scheffel U, ad Wagner HN Jr - J Labelled Compds Radiopharm 31 : 841-9 (1992)

