Synthesis of H-3 Labeled 5-Fluoro-3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-piperazinyl]propyl]-1H-indole, a Serotonergic Agent with Potential Antidepressant Activity

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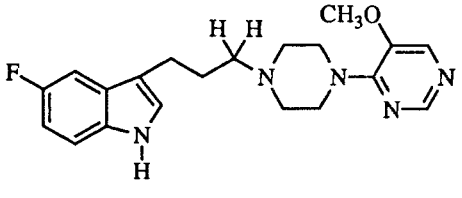
Summary

The title compound was prepared by the $LiAlT_4$ reduction of 5-fluoro-3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-piperazinyl]-3-oxopropyl]-1H-indole. The radiochemical purity of the product was 98.9% and the specific activity was calculated as 53.0 Ci/mmol from HPLC analyses and 55.6 Ci/mmol from ^3H NMR measurements.

Keywords: Lithium aluminum tritide, serotonergic activity, potential antidepressant.

Introduction

BMS 181,101, 5-fluoro-3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-piperazinyl]propyl]-1H-indole, **1a**, is presently in Phase II clinical studies as a potential antidepressant.[1]



1a

In animals, BMS 181,101 has a unique pharmacological profile, appearing to have a dual serotonergic mechanism of action. Its activity combines potent 5-HT uptake inhibition (IC_{50} < 1 nm) with dynamic $5\text{-}HT_{1D}$ activation, thereby enhancing overall 5-HT

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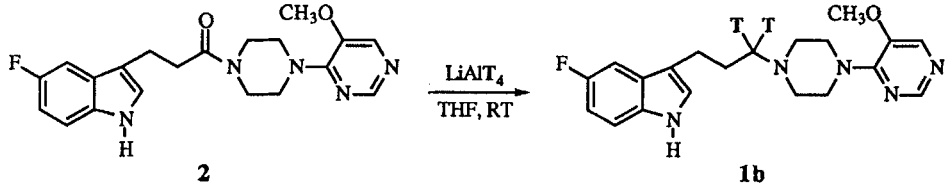
neurotransmission. To further understand the receptor binding characteristics and localization of this compound, we prepared high specific activity H-3 labeled BMS 181,101, **1b**, through the $LiAlT_4$ reduction of the corresponding amide, **2** (Scheme 1).

Scheme 1. Radiochemical synthesis of H-3 labeled BMS 181,101, **1b**.

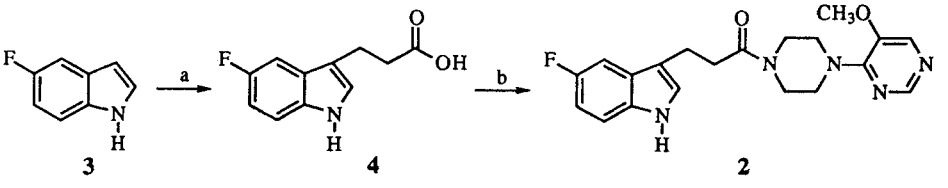


2 **1b**

Results and Discussion

The objective of this work was to prepare H-3 labeled BMS 181,101 with a specific activity of at least 40 Ci/mmol. This requirement, coupled with the structure of **1a** and the availability of high specific activity $LiAlT_4$,[2] prompted us to evaluate the reduction of **2** with $LiAlH_4$. Synthesis of **2** was achieved in an overall yield of 30% from 5-fluoroindole (Scheme 2). In this approach, 5-fluoroindole (**3**) was reacted with acrylic acid to form the corresponding propionic acid derivative (**4**), which was then condensed with 1-(5-methoxy-4-pyrimidinyl)piperazine to form the desired amide, **2**.

Scheme 2. Synthesis of 5-fluoro-3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-piperazinyl]-3-oxo-propyl]-1H-indole, **2**.



3 **4** **2**

Reagents: (a) Acrylic acid, Acetic anhydride, Acetic acid, 90°C, N_2, 57%.
(b) 1-(5-Methoxy-4-pyrimidinyl)piperazine, Diphenylphosphoryl azide, Et_3N, DMF, 23°C, N_2, 52%.

In preliminary non-radioactive experiments, the reduction of **2** with a 2-3 fold molar excess of $LiAlH_4$ gave **1a** in only moderate yields. However, the lack of unwanted side products permitted a simple and efficient HPLC purification of the reaction mixture. In the radiolabeling experiment (Scheme 1) we used a twofold molar excess of $LiAlT_4$ hydride equivalents and obtained an 18% yield of the desired product, **1b**. As described above for the non-radioactive reduction, we observed only the desired labeled product and unreacted starting material.

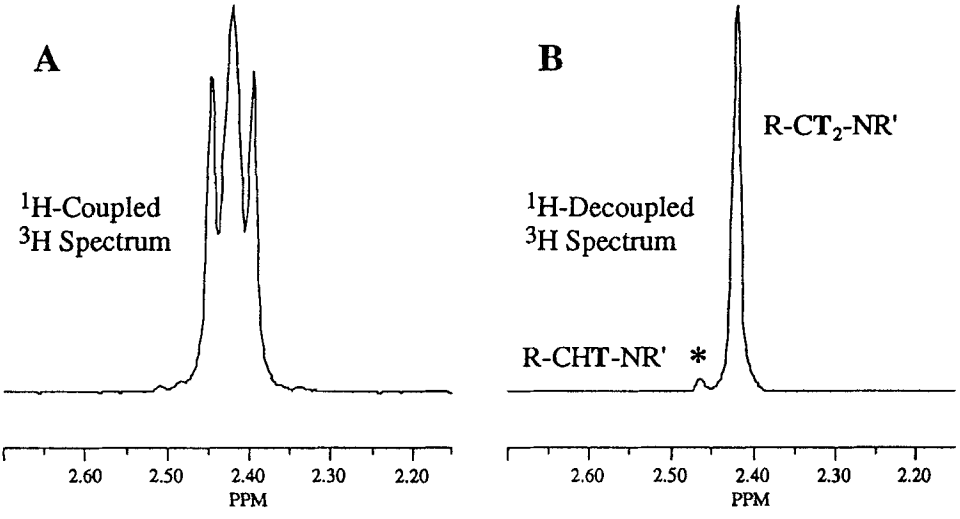
Tritium NMR analysis of the HPLC purified product, **1b**, confirmed the unique location of the label (Figure 1). The use of high specific activity $LiAlT_4$ for the reduction of the amide (R-CO-NR') resulted in the formation of the desired product, in which two tritium atoms were incorporated into the methylene unit (R-CT_2-NR'). The proton-decoupled tritium NMR spectrum also showed a small peak (*) from the product which has a single tritium in the methylene unit (*i.e.* R-CHT-NR'). Inspection of the proton spectra showed no signals at 2.42 ppm, and hence we assumed that the percentage of R-CH_2-NR' species was negligible. A specific activity (S.A.) of 55.6 Ci/mmol was calculated from the tritium NMR spectra.[3] Determination of the specific activity of **1b** *via* HPLC analysis and comparison of the UV absorbance to a standard curve gave a specific activity of 53.0 Ci/mmol. The difference between these two calculated S.A.'s is less than 5%, and is a fair reflection of the experimental errors associated with obtaining specific activity values *via* these two techniques.

Experimental

Materials. — All experimental conditions were optimized using non-radioactive materials. The identity of the final product **1b** was confirmed by co-elution *via* HPLC of the radiolabeled substance with authentic unlabeled compound from Bristol-Myers Squibb Company (New Brunswick, N.J.), and by tritium and proton NMR spectroscopy.

Tritium NMR Spectroscopy. — Samples were made to a volume of 250 μL in Teflon tubes (Wilmad #6005), which were then placed inside 5 mm glass NMR tubes having a screw-cap (Wilmad 507-TR-8"). NMR spectroscopy was carried out on an IBM Instrument Inc. AF-300 spectrometer (^3H at 320MHz, ^1H at 300 MHz), using a $^3H/^1H$ 5 mm dual probe. A high quality ^3H band stop, ^1H band pass filter (Cir-Q-Tel Inc., FBT/20-300/3-6/50-3A/3A) was placed in the proton decoupling line of the instrument. Referencing of chemical shifts was achieved by generation of a ghost ^3H TMS signal from internal TMS in the ^1H NMR spectrum.[4]

Figure 1. 320 MHz Tritium NMR spectra of **1b** in CD_3OD (2.15 — 2.70 ppm). A). Proton-coupled tritium spectrum, and B). Proton-decoupled tritium spectrum.



HPLC Analysis. — HPLC was performed using an Inertsil ODS-2 4.6 x 250 mm column, Waters Model 510 pumps, a Hewlett Packard 1040A diode array spectrophotometer for UV analysis and a IN/US Ramona-5-LS radiometric detector for radioactivity measurements. The HPLC conditions were established using unlabeled reference samples with a mobile phase of CH_3CN : 0.02 M NaOAc (pH 7.2) in the ratio of 45:55. The flowrate of the mobile phase was 1 mL/min. The chemical yields and specific activity were determined by UV analysis and radioactivity measurements.

Chemical Syntheses. —

5-Fluoro-1H-indole-3-propanoic acid, **4** [5]

A solution of 5-fluoroindole, **3**, (5 g, 0.037 mol) in 37 mL of glacial acetic acid containing acrylic acid (5.6 mL, 0.082 mol) and acetic anhydride (7 mL, 0.074 mol) was allowed to stir at 90 °C for 42 h under N_2. The solution was then concentrated *in vacuo* and the residue taken up in 3N NaOH. The insoluble material was removed by filtration and the filtrate was acidified with conc. HCl and then extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered and then evaporated to yield **4** (4.347 g, 57%) as a solid. ^{1}H NMR (DMSO-d_6/D_2O) (ppm) 2.50 (t, 2H), 2.87 (t, 2H), 6.89 (dt, 1H), 7.18 (s, 1H), 7.28 (m, 2H). DCI-M.S. m/e 208 $(M+H)^+$. Anal. calc'd for $C_{11}H_{10}NO_2F$: C, 62.67; H, 4.98; N, 6.65. Found: C, 62.61; H, 4.71; N, 6.41.

5-Fluoro-3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-piperazinyl]-3-oxopropyl]-1H-indole, **2**

To a solution of **4** (0.756 g, 0.0037 mol) in 15 mL of DMF was added diphenylphosphoryl

azide (0.87 mL, 0.004 mol), Et_3N (0.76 mL, 0.0055 mol) and 1-(5-methoxy-4-pyrimidinyl)-piperazine (0.780g, 0.0040 mol).[1] The solution was allowed to stir at 23 °C for 17 h under N_2, after which time the DMF was removed *via* a Kugelrohr oven with heat. The crude product was crystallized from hot methanol to yield **2** (0.697 g, 52%) as a white solid (m.p. 180-181 °C). ^1H NMR (DMSO-d_6) (ppm) 2.66 (t, 2H), 2.88 (t, 2H), 3.53 (m, 8H), 3.82 (s, 3H), 6.87 (dt, 1H), 7.22 (d, 1H), 7.27(m, 2H), 8.04 (s, 1H), 8.24 (s, 1H), 10.86 (br s, 1H). DCI-M.S. m/e 384 $(M+H)^+$. Anal. calc'd for $C_{20}H_{22}N_5O_2F$: C, 62.65; H, 5.78; N, 18.27. Found: C, 62.64; H, 5.55; N, 18.15.

Tritium labeled 5-fluoro-3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-piperazinyl]propyl]-1H-indole, **1b.**

A 7.6 mg (20 μmol) sample of **2** was placed in a 5 mL round bottom flask and put under high vacuum for 1 h to remove residual solvents. One mL of anhydrous THF was then added to the flask and the suspension warmed briefly until a solution was obtained. The solution was cooled to RT and then immediately added to a freshly prepared sample of $LiAlT_4$[2] (20 μmol, 2.27 Ci) and the reaction allowed to stir at RT for 1 h. The reaction was then quenched with 500 μL of 1N HCl/MeOH, the solvent was removed and the residue extracted with 5 mL of Et_2O and 2 mL of 1N KOH. The organic layer was removed and the aqueous layer was extracted with fresh Et_2O (4X). The combined ether layers were then rinsed with a few mL of a saturated NaCl solution, the organic layer was separated and the ether removed *via* a stream of N_2 to yield 405 mCi of H-3 labeled **1b** (18% radiochemical yield). A sample of this crude radiolabeled material was then dissolved in 1 mL of the HPLC mobile phase (45% CH_3CN, 55% 0.02 M NaOAc, pH 7.2) and purified by HPLC to yield 150 mCi of **1b** with a radiochemical purity of 98.9%. ^3H NMR (CD_3OD, 320 MHz, proton-decoupled) (ppm) 2.42.

Acknowledgements

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References

1. Smith, D.W., Yocca, F.D., Yevich, J.P., Mattson, R.J. — U.S. Patent No. 5,077,293, (1991).

2. Andres, H., Morimoto, H., Williams, P.G. — J. Chem. Soc., Chem. Commun., 627 (1990).

3. Callaway, J.C., Morimoto, H., Gynther, J., Airaksinen, M.M., Williams, P.G. — J. Labelled Compd. Radiopharm., 31: 355 (1992).

4. Bloxsidge J.P., Elvidge J.A., Jones J.R., Mane R.B., Saljoughian M. — Org. Magn. Reson., 12: 574 (1979).

5. Smith, D.W., Yocca, F.D., Yevich, J.P., Mattson, R.J., Williams, A., Ruediger, E.H., Combrink, K.D., Pearce, B.C. — Eur. Pat. Appl. 0 548 813 A1, (1993).