

Synthesis of Serotonin Agonists as 5-HT_{2A} and 5-HT_{2C} Receptor Molecular Probes

Dani M. Schultz

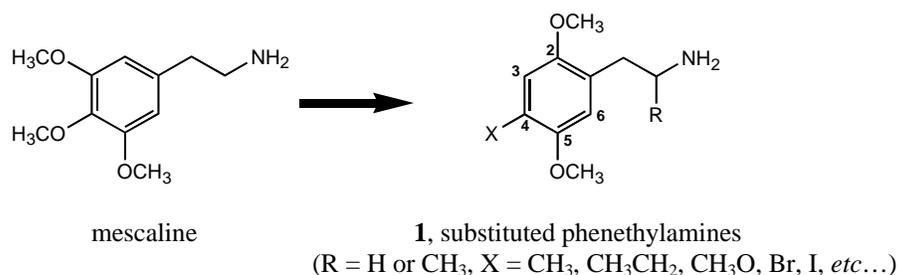
Faculty Sponsor: Aaron Monte, Department of Chemistry

ABSTRACT

The 5-HT₂ receptor is one member of the serotonin (5-HT) receptor family and consists of at least three distinct subclasses, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}, each being linked to different physiological/behavioral effects when activated by serotonin. In previous experiments performed in this laboratory, it was found that one specific chemical structure showed selectivity for the 5-HT_{2A} and 5-HT_{2C} receptor proteins but did not show a strong preference for binding to either one of these receptors selectively. The project described here involves the synthesis of two related molecules that have an altered structure, which may prove to be specific for the 5-HT_{2A} or 5-HT_{2C} receptor subtype. By synthesizing these two molecules, a more complete picture of the 5-HT_{2A/2C} agonist binding sites can be developed and may ultimately lead to drugs that selectively bind to and better define these receptors.

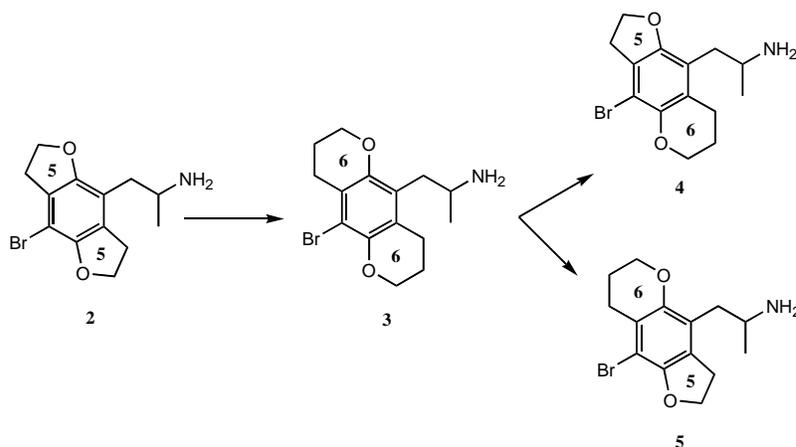
INTRODUCTION

Because serotonin is responsible for many of the body's vital functions, a good deal of research has been aimed at determining how serotonin causes different specific responses when it binds to the different 5-HT receptors. With these insights, pharmacological advances have been made in which drugs have been engineered to specifically bind to an appropriate 5-HT receptor to bring about a desired response or correct a dysfunction. One chemical class of serotonergic drugs, known as the phenethylamines, are selective agonists of 5-HT_{2A/2C} receptors.¹ This class has been the subject of years of structure-activity relationship (SAR) studies to determine which chemical/physical properties are essential for activity and yield the highest affinity for 5-HT_{2A/2C} receptors. Mescaline, the first hallucinogenic phenethylamine discovered, was structurally modified to prepare numerous analogs to determine which structural features produced optimal binding to the serotonin 5-HT_{2A} and 5-HT_{2C} receptors. From this research, the basic phenethylamine scaffold **1** was constructed that had characteristics that yielded the highest affinity for the 5-HT_{2A/2C} receptors.^{2, 3}



Essential structural characteristics of **1** are the two methoxy substituents located at positions 2 and 5 on the aromatic ring. It is believed that the relative positions of the methoxy groups is crucial for hydrogen-bonding within the 5-HT₂ receptor and ultimately affords the highest *in vivo* and *in vitro* activity.⁴ In addition, a hydrophobic substituent at the 4 position also is essential for tight binding to the 5-HT₂ receptor populations. Previous work in this laboratory altered the phenethylamine structure so that the alkoxy substituents at the 2 and 5 positions of **1** were locked in place, ultimately making a rigid structure that could not freely rotate. By securing these positions, one new structure **2** showed a dramatic increase in affinity for the 5-HT_{2A/2C} receptors. The oxygen atoms at the 2 and 5 positions oriented *syn* and *anti* relative the alkylamine side chain, respectively, allowed optimal hydrogen-bonding to occur between the oxygen atom lone-pair electrons and putative hydrogen-bond donors within the 5-HT₂

receptor.^{4,5} Today, structure **2** remains to be one of the most potent and selective 5-HT_{2A/2C} receptor agonist yet reported, and thus, a good avenue to explore other rigid phenethylamine structures. To test the steric limitations of the 5-HT₂ receptor, rigid analog **3** was created. Similar to **2**, analog **3** consisted of two 6-membered rings as opposed to the dual 5-membered ring system present in **2**. After both *in vivo* and *in vitro* pharmacological assays were performed, it was shown that **3** had a decreased affinity for the 5-HT₂ receptor.⁶ Thus, to further probe the topography of the 5-HT₂ receptors, a pair of mixed heterocycles, **4** and **5**, were designed that would possess structural characteristics of both **2** and **3**.

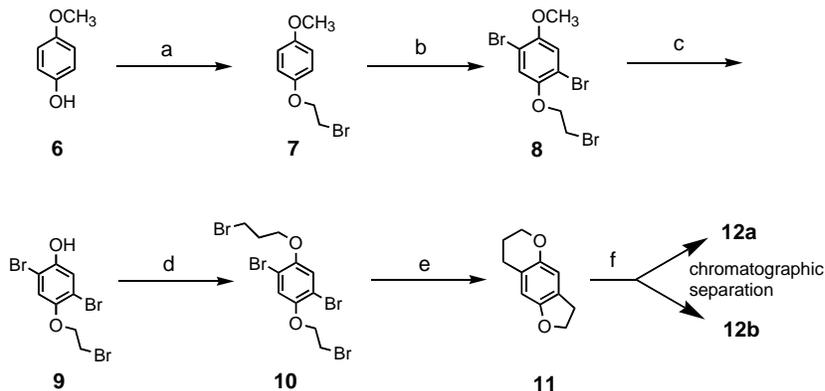


Once the synthesis of **4** and **5** is complete, the knowledge regarding the topography of the serotonin 5-HT₂ receptor will be further advanced and will ultimately lead to a more complete picture of the agonist binding site. In the end, molecules **4** and **5** may prove to be the first selective agonists for either the 5-HT_{2A} and/or 5-HT_{2C} receptor.

METHODS: CHEMISTRY

Due to the asymmetry of the two target molecules **4** and **5**, each ring system had to be constructed independently. Following previous research, the best synthetic route to the key tricyclic intermediate **11** was begun with commercially available *para*-methoxy phenol **6** as outlined in Scheme 1.⁷

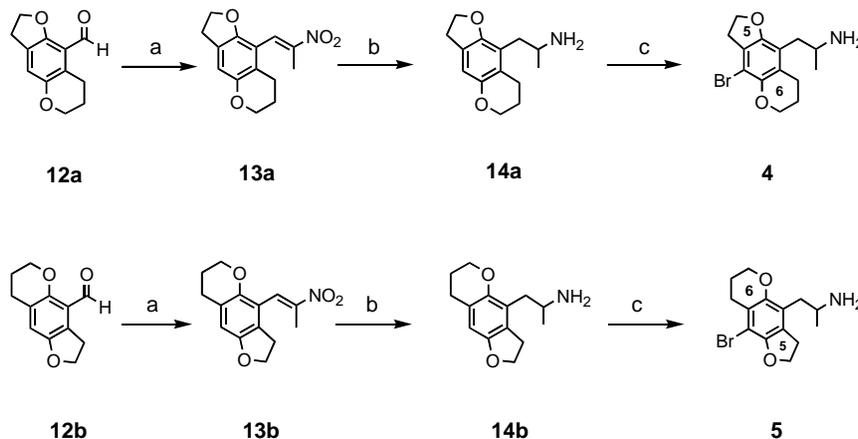
Scheme 1^a



^a (a) 1,2-Dibromoethane, K₂CO₃, acetone, Δ; (b) Br₂, CH₂Cl₂, Fe; (c) *B*-bromo-9-*BBN*, CH₂Cl₂, 14 days Δ; (d) 1,3-dibromopropane, K₂CO₃, acetone, Δ; (e) *n*-butyllithium, THF, 0 °C; (f) dichloromethyl methyl ether, SnCl₄, CH₂Cl₂, 0 °C.

To prepare the mono-alkylated product **7**, a solution of acetone, 1,2 dibromoethane and potassium carbonate were heated and stirred. *Para*-methoxy phenol **6** was added over a 24 hour period to the heated and stirred solution. After purification and recrystallization with ethyl acetate and hexanes, **7** was obtained as white plate-like crystals. The brominated product **8** was obtained in relatively good yield as white, needle-like crystals by the addition of elemental bromine to a solution of **7** dissolved in CH₂Cl₂. In order to attain the phenolic product **9** a challenging selective methyl ether cleavage was performed on **8** using the reagent *B*-bromo-9-BBN. Due to the unstable nature of the phenol, immediately after **9** was isolated and purified using flash-column chromatography, it was alkylated with 1,3-dibromopropane in the same manner as **6**, to afford the asymmetric bis-ether, **10**. The key tricyclic intermediate **11** was constructed by the addition of **10** to a stirred solution of anhydrous tetrahydrofuran (THF) under nitrogen and cooled over an ice bath. Using a needle and syringe, *n*-butyllithium was quickly added to the reaction mixture, and was quenched after 10 minutes with the cautious addition of water. The desired product **11** was purified by recrystallization from ethyl acetate and hexanes to afford white powdery crystals.

Once the synthesis of **11** was complete, it was apparent that the formylation reaction (step f) would create two regioisomers, **12a** and **12b**, which would have to be separated chromatographically. A solution of **11** in anhydrous CH₂Cl₂ was stirred over an ice bath and under nitrogen. Tin (IV) chloride was added to the reaction mixture, followed by the addition of dichloromethyl methyl ether. After the reaction was complete, the desired products, **12a** and **12b**, were isolated as a bright yellow oil. It was found that a selective recrystallization could be performed on the oil using ethyl acetate and hexanes to obtain pure **12a** in relatively good yield. The remaining oil, which contained both isomers, was separated by flash-column chromatography eluting with 95% CH₂Cl₂-5% hexane mixture. Each isomer, **12a** and **12b**, was isolated in pure form in a 5:1 ratio, respectively, as yellow, needle-like crystals. Isomers **12a** and **12b** were then carried through a parallel sequence (Scheme 2), allowing construction of the desired alkylamine side chains that would ultimately afford molecules **4** and **5**. Since the aldehydes, **12a** and **12b**, were isolated in different ratios, the condensation reaction (step a) was first performed on the more abundant isomer **12a** before moving on to isomer **12b**. In order to attain nitropropene **13a**, ammonium acetate and **12a** were added to nitroethane and allowed to react. Once the reaction was complete, the organic phase was purified to leave **13a** as a bright orange oil. Unfortunately, direct recrystallization of the oil using hot methanol failed, so further purification of **13a** was achieved using flash-column chromatography, N₂ pressure, and CH₂Cl₂ as an eluant. Recrystallization was then successfully performed using hot methanol to afford orange needle-like crystals of **13a** in good yield.

Scheme 2^a

^a (a) CH₃CH₂NO₂, NH₄OAc, Δ; (b) LiAlH₄, THF, reflux; (c) Br₂, CH₂Cl₂.

CONCLUSIONS/FUTURE WORK

The rest of the outlined synthesis will be completed during the summer of 2006 at Purdue University. Once the proposed synthesis is complete, pharmacological analysis of molecules **4** and **5** will be carried out to assess their biological activities and affinity for the human 5-HT_{2A} and 5-HT_{2C} receptors. Ultimately, the binding profiles of these molecules will provide further knowledge about serotonin and the detailed structure of the receptors to which it binds.

EXPERIMENTAL

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. ¹H-NMR spectra were obtained using a 300 MHz Bruker AC 300 NMR spectrometer with TMS as the internal standard. Abbreviations used in NMR analyses are as follows: b = broad, s = singlet, d = doublet, t = triplet, p = pentet, m = multiplet, Ar = aromatic. Infrared spectra were recorded on a Midac Prospect FT-IR spectrometer. Thin-layer chromatography (TLC) was performed using Baker-flex silica gel plastic-backed plates with fluorescent indicator, developing with CH₂Cl₂, and visualizing with UV light. All solvents (acetone, diethyl ether, CH₂Cl₂, THF, ethyl acetate, hexanes, acetic acid) were obtained from Fisher Scientific and used as received. Most inorganic reagents, along with 4-nitrophenol, 4-methoxyphenol, 1,3-dibromopropane, 1,2-dibromoethane, 1.0 M *B*-bromo-9-BBN in CH₂Cl₂, and dichloromethyl methyl ether were used as received from Aldrich.

1-Bromo-2-(4-methoxyphenoxy)ethane (7). A stirred solution of 750 mL of acetone, 100 g (0.724 mol) of anhydrous potassium carbonate, and 156 mL (1.81 mol) of 1,2-dibromoethane was brought to reflux in a 2,000 mL three-neck reaction flask equipped with a water-cooled condenser. Commercially available *para*-methoxy phenol **6** (50.0 g, 0.403 mol) dissolved in 250 mL of acetone was slowly added to the refluxing mixture over a 36 h period. The reaction was halted after 8 days when TLC analysis indicated that minimal starting material remained. The reaction was allowed to cool and then filtered through Celite. The solvent was removed from the filtrate *in vacuo* to yield a yellow oil. The yellow oil was taken up in CH₂Cl₂, and was washed with 3 x 100 mL of 2.5M NaOH, 3 x 100 mL of 3M HCl, and 100 mL of brine. The organic layer was dried using MgSO₄, filtered, and the solvent was removed *in vacuo* to yield a yellow oil. Recrystallization of the oil was accomplished using hexanes and ethyl acetate to afford 15.2 g (16.3%) of **7** as white plate-like crystals: mp 53-55 °C. ¹H NMR (CDCl₃) δ 3.76 (s, 3, ArOCH₃), 4.64 (s, 1, ArOH), 6.80 (pair of superimposed doublets, 4, ArH).

1-Bromo-2-(2,5-dibromo-4-methoxyphenoxy)ethane (8). To a stirred solution of 500 mL of CH₂Cl₂ and a catalytic amount of Fe filings, 14.9 g (0.0646 mol) of **7** was added. Bromine (21.8 g, 0.137 mol), in 50 mL of CH₂Cl₂, was added dropwise to the stirred solution over a 6 h period at room temperature. The reaction continued for an additional 24 h before being halted. The reaction mixture was placed in a separatory funnel, and was washed with 4 x 100 mL of 3M NaOH, 3 x 100 mL of 3M HCl, and 75 mL of brine. The organic phase was dried with MgSO₄ before being filtered. The solvent was removed *in vacuo* to give an orange solid that was recrystallized from ethyl acetate and hexanes to yield 21.5 g (85.3%) of **8** as off-white crystals: mp 64-65 °C. ¹H NMR (CDCl₃) δ 3.66 (t, 2, ArOCH₂CH₂Br), 3.87 (s, 3, ArOCH₃), 4.30 (t, 2, ArOCH₂CH₂Br), 7.11 (s, 1, ArH), 7.17 (s, 1, ArH).

2,5-Dibromo-4-(2-bromoethoxy)phenol (9). To a dry, three-neck flask equipped with a stir bar, 45.0 g (0.116 mol) of **8** in 1,000 mL of CH₂Cl₂ was added with stirring. A water-cooled condenser with a N₂ inlet was attached, and the flask was flushed with N₂ for 10 min. The reaction flask was then sealed with a glass stopcock and addition funnel. A solution of 1.0 M *B*-Br-9-BBN in hexanes (522 mL, 0.522 mol) was placed in the addition funnel. A needle and syringe, purged several times with N₂, was used to deliver the *B*-Br-9-BBN to the addition funnel. *B*-Br-9-BBN solution was introduced to the reaction flask dropwise over a 2 min period, and the mixture was heated to reflux. The reaction was monitored by TLC and was halted after 19 days when TLC indicated all starting material was consumed. The volatiles were removed *in vacuo* to give a yellow oil (that emitted white fumes). The yellow oil was stirred under N₂ for 2 h until the white fumes subsided and the oil was taken up in 500 mL of Et₂O and brought to 0 °C in an ice bath. Ethanamine (37.9 mL, 1.2 equiv relative to *B*-Br-9-BBN) was cautiously added to the mixture with a needle and syringe. Once the addition was complete, a white precipitate formed and the flask was sealed and stored at 4 °C for 24 h. The white precipitate that formed was removed by filtration thru Celite, using Et₂O as the solvent. The solvent was removed from the filtrate *in vacuo* to yield a yellow oil. The oil was taken up in 1,000 mL of CH₂Cl₂ and washed with 3 x 150 mL of 3M HCl, and was then extracted with 4 x 200 mL of 3M NaOH. The base extracts were pooled and re-acidified with 75 mL of 12M HCl. The acidic solution was then extracted with 3 x 200 mL of CH₂Cl₂, the organic layers were combined and washed once with 150 mL of brine, and dried with MgSO₄ and filtered. The solvent was removed *in vacuo* to give a yellow oil that was loaded onto a dry flash column. The column was run using CH₂Cl₂, with each 100 mL fraction being collected. The fractions that contained the desired product **9** were combined, and the solvent was removed *in vacuo* to afford 29.52 g (73.7%) of **9** as fine white powder: mp 106-107 °C. ¹H NMR (CDCl₃) δ 3.65 (t, 2, ArOCH₂CH₂Br), 4.27 (t, 2, ArOCH₂CH₂Br), 5.29 (s, 1, ArOH), 7.07 (s, 1, ArH), 7.28 (s, 1, ArH).

1-Bromo-3-(2,5-dibromo-4-(2-bromoethoxy)phenoxy)propane (10). A stirred solution of 19.4 g (0.14 mol) of anhydrous potassium carbonate, 29 mL (0.280 mol) of 1,3-dibromoethane, and 1,500 mL of acetone was heated to reflux. The phenol **9** (17.5 g, 0.0466 mol) was dissolved in 250 mL of acetone and introduced to the mixture

dropwise over a 48 hour period. The reaction was halted after 3 days when TLC analysis indicated that no more starting material remained. The reaction mixture was allowed to cool and was filtered thru Celite. The solvent was removed *in vacuo*, and the yellow oil that remained was taken up in 500 mL of CH₂Cl₂ and washed with 3 x 150 mL of 2.5M NaOH, 3 x 150 mL of 3M HCl, and 100 mL of brine. The organic phase was dried with MgSO₄ and filtered. The solvent was removed *in vacuo* to yield a yellow oil. The yellow oil was recrystallized from hexanes to yield 18.3 g (78%) of **10** as a white needles: 67-68 °C. ¹H NMR (CDCl₃) δ 2.34 (p, 2, ArOCH₂CH₂CH₂Br), 3.64 (t, 2, ArOCH₂CH₂CH₂Br), 3.67 (t, 2, ArOCH₂CH₂Br), 4.11 (t, 2, ArOCH₂CH₂CH₂Br), 4.26 (t, 2, ArOCH₂CH₂Br), 7.17 (s, 1, ArH), 7.28 (s, 1, ArH).

2,3-Dihydrofurano[2,3-g]chroman (11). To a dry flask containing a magnetic stir bar, 17.6 g (0.035 mol) of **10** was added. The flask was flushed with N₂ for 5 minutes, and sealed off with a rubber septum. Using a needle and syringe, 75 mL of anhydrous THF was delivered to the flask and the reaction was stirred at room temperature until all of **10** had dissolved. The reaction was cooled to 0 °C over an ice bath. To the stirred solution, 10.9 mL (0.109 mol) of 10M *n*-butyllithium in hexanes was added to the flask. The reaction was held at 0 °C for 4 h, and was quenched with the careful addition of 10 mL of H₂O. The reaction was allowed to warm to room temperature before the solvent was removed *in vacuo*. The red oil that remained was taken up in 250 mL of Et₂O, and washed with 4 x 75 mL of H₂O and 50 mL of brine. The organic phase was dried with MgSO₄ and filtered. The solvent was removed *in vacuo* to afford a red-orange oil that was recrystallized from ethyl acetate and hexanes to yield 4.3 g (69%) of **11** as an orange crystalline solid: mp 75-76 °C. ¹H NMR (CDCl₃) δ 1.96 (p, 2, ArOCH₂CH₂CH₂), 2.72 (t, 2, ArOCH₂CH₂CH₂), 3.12 (t, 2, ArOCH₂CH₂), 4.12 (t, 2, ArOCH₂CH₂CH₂), 4.56 (t, 2, ArOCH₂CH₂), 6.46 (s, 1, ArH), 6.65 (s, 1, ArH).

Formylated Isomers (12a and 12b). To a clean and dry 500 mL round bottom flask containing a magnetic stir bar, 8.294 g (0.0471 mol) of **11** was added and dissolved in 250 mL of CH₂Cl₂. The flask and contents were stirred over an ice bath and under N₂ for 30 minutes before a rubber septum was placed over the flask and the N₂ line was reintroduced. The reaction solution was stirred for an additional 15 minutes before a dry and N₂ purged syringe was used to deliver 6.07 mL (0.0518 mol) of SnCl₄ to the reaction mixture. Upon the addition of the SnCl₄, the reaction mixture turned a dark brown color. The reaction was stirred at 0 °C for 15 minutes. Using a dry and N₂ purged syringe, 5.9 mL (0.0518 mol) of dichloromethyl-methyl ether was delivered to the flask over an 8-minute period resulting in a dark purple solution once the addition was complete. The reaction was halted by the cautious addition of 50 mL of H₂O after 3 h when TLC analysis indicated that the reaction had proceeded to completion. The reaction mixture was transferred to a 500 mL separatory funnel where the organic layer was partitioned and saved. The aqueous layer was extracted with 3 x 50 mL of CH₂Cl₂. The organic fractions were combined and washed with 3 x 50 mL of 3M HCl and once with 50 mL of brine. The organic phase was dried with MgSO₄ and filtered through Celite. The solvent was removed *in vacuo* to yield a dark red oil (10.29 g) that contained both isomers, **12a** and **12b**. The red oil was recrystallized using ethyl acetate and hexanes to afford 3.91 g (40.6%) of **11a**. The remaining oil, containing **12a** and **12b**, was placed onto a flash column containing silica gel. The column was eluted using 95% CH₂Cl₂-5% hexane mixture and N₂ pressure. Fractions were collected in 10 mL test tubes and monitored by TLC analysis. The fractions containing **12b**, the first to elute from the column, were combined and the solvent was removed *in vacuo* to afford a yellow solid: mp 69-70 °C. ¹H NMR (CDCl₃) δ 1.96 (p, 2, ArOCH₂CH₂CH₂), 2.79 (t, 2, ArOCH₂CH₂CH₂), 3.45 (t, 2, ArOCH₂CH₂), 4.22 (t, 2, ArOCH₂CH₂CH₂), 4.55 (t, 2, ArOCH₂CH₂), 6.70 (s, 1, ArH), 10.47 (s, 1, ArCHO).

The fractions containing **12a**, the second to elute from the column, were combined and the solvent was removed *in vacuo* to yield a bright yellow solid: mp 70-71 °C. ¹H NMR (CDCl₃) δ 1.96 (p, 2, ArOCH₂CH₂CH₂), 3.15 (pair of superimposed triplets, 4, ArOCH₂CH₂, ArOCH₂CH₂CH₂), 4.11 (t, 2, ArOCH₂CH₂CH₂), 4.65 (t, 2, ArOCH₂CH₂), 6.92 (s, 1, ArH), 10.37 (s, 1, ArCHO).

Nitroisopropene-substituted dihydrofuranochroman (13a). To a dry, 250 mL, round bottom flask equipped with magnetic stir bar, 3.50 g (0.0171 mol) of aldehyde **12a** and 1.449 g (0.0188 mol) of ammonium acetate were added with stirring. A water-cooled condenser with a N₂ inlet was attached, and N₂ was allowed to flush the reaction vessel for 15 minutes before the remaining necks were sealed off with a thermometer, adapter, and rubber septum. With needle and syringe, 15 mL (excess) of nitroethane was added to the stirred solution, and the reaction was heated to 80 °C. The reaction was halted after 3 h when TLC analysis indicated that the reaction had proceeded to completion. The reaction was allowed to cool to warm temperature before solvent removal *in vacuo*. The bright orange oil that remained was taken up in 300 mL of CH₂Cl₂ and transferred to a 500 mL separatory funnel. The organic phase was washed with 3 x 50 mL of 3M HCl, 2 x 50 mL of H₂O, and once with brine. The organic phase was dried with MgSO₄ and filtered. Excess solvent was removed *in vacuo* to afford a bright orange oil that contained the desired final product **13a**. The orange oil was placed on a flash column containing silica gel and was

eluted using CH_2Cl_2 . The fractions containing **13a** were combined, and the solvent was removed *in vacuo* to afford a bright orange solid that was recrystallized from methanol to yield 2.90 g (64.7%) of orange needle-like crystals: mp 89-90 °C, $^1\text{H NMR}$ (CDCl_3) δ 1.96 (p, 2, $\text{ArOCH}_2\text{CH}_2\text{CH}_2$), 2.16 (s, 3, CH_3), 2.63 (t, 2, $\text{ArOCH}_2\text{CH}_2\text{CH}_2$), 3.19 (t, 2, $\text{ArOCH}_2\text{CH}_2$), 4.11 (t, 2, $\text{ArOCH}_2\text{CH}_2\text{CH}_2$), 4.55 (t, 2, $\text{ArOCH}_2\text{CH}_2$), 6.76 (s, 1, ArH), 7.84 (s, 1, $\text{ArCH}=\text{C}$).

REFERENCES

1. Teitler, M., Lyon, R. A., and Glennon, R. A., "Radioligand binding evidence implicates the brain the brain 5-HT₂ receptor as a site of action for LSD and phenylisopropylamine hallucinogens." *Psychopharmacology*, **1988**, *94*, 213.
2. Glennon, R. A.; Young, R.; Benington, F.; Morin, R. D. "Behavioral and Serotonin Receptor Properties of 4-Substituted Derivatives of the Hallucinogen 1-(2,5-Dimethoxyphenyl)-2aminopropane." *J. Med. Chem.*, **1982**, *25*, 1163-1168.
3. Glennon, R. A.; Raghupathl, R.; Bartyzel, P.; Teitler, M.; Leonhardt, S. "Binding of Phenylalkylamine Derivatives at the 5-HT_{1C} and 5-HT₂ Serotonin Receptors: Evidence for a Lack of Selectivity." *J. Med. Chem.*, **1992**, *35*, 734-740.
4. Westkaemper, R. B.; Glennon, R. A. Molecular Modeling of the Interaction of LSD and Other Hallucinogens With 5-HT₂ Receptors. In *NIDA Research Monograph 146, Hallucinogens: An Update*; Lin, G. C., Glennon, R. A., Eds.; NIH Publication No. 94-3872; NIDA: Rockville, **1994**; pp 263-283.
5. Monte, A. P., Marona-Lewicka, D., Parker, M. A., Wainscott, D. B., Nelson, D. L., Nichols, D. E., "Dihydrobenzofuran analogues of hallucinogens. 3. Models of 4-substituted (2,5-Dimethylphenyl) alkylamine derivatives with rigidified methoxy groups." *J. Med. Chem.*, **1996**, *39*, 2953-2961.
6. Whiteside, M. S.; Kurrasch, D.; Nichols, D. E.; Monte, A. P. "Substituted Hexahydrobenzodipyran as 5-HT_{2A/2C} Receptor Probes." *Bioorganic & Medicinal Chemistry*, **2002**, *10*, 3301-3307.
7. Prescher, J. A.; Monte, A.P. "Design and Synthesis of Substituted Furobenzopyrans as New Serotonergic Molecular Probes." *UW-L Journal of Undergraduate Research*, **2003**, 143-152.