SYNTHESIS OF [^{3}H]CLOZAPINE

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SUMMARY

[^{3}H]Clozapine was prepared with a specific activity of 9.9 Ci/mmol by reaction of 8-chloro-11-(methylthio)-5*H*-dibenzo[*b*,*e*][1,4]diazepine with an excess of [^{3}H]*N*-methylpiperazine. The latter was prepared from *N*-methylpyrazinium bromide in ethanolic hydrogen chloride by reduction at room temperature with tritium over 5% rhodium on aluminum oxide.

Key Words: [^{3}H]Clozapine, [^{3}H]*N*-Methylpiperazine, Atypical Neuroleptic Agent, Antipsychotic Agent

INTRODUCTION

Reports that clozapine [8-chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*b*,*e*][1,4]diazepine, **1**] is an antipsychotic drug which in clinical use produces practically no neurological side effects (1) suggest that the pharmacological separation of these side effects from antipsychotic activity seems feasible. Clinical use of this atypical neuroleptic agent, however, displayed agranulocytosis as an unacceptable toxic effect (2). There is therefore a need to find new analogues of clozapine lacking this side effect but with a retained neuropharmacological profile.

It has also been observed that [^{3}H]clozapine ([^{3}H]**1**) binds strongly to sites in rat forebrain that appear not to be dopaminergic in nature (3,4). The displacement of [^{3}H]**1** from these sites by

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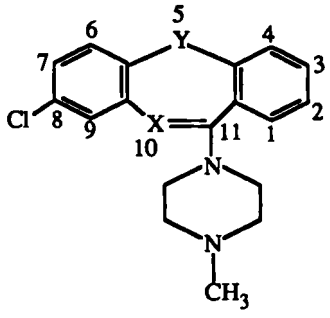


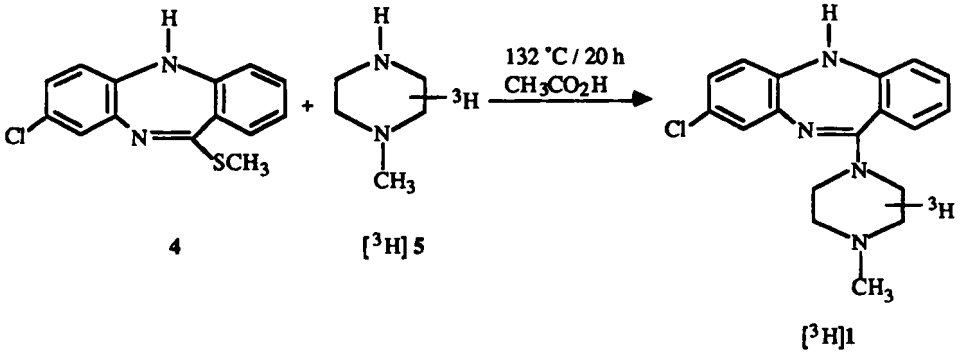
1, X = N; Y = NH
2, X = CH; Y = CH_2
3, X = CH; Y = O

antipsychotic drugs (3,4) suggests the use of these binding sites for the evaluation of analogues of clozapine as potential antipsychotic agents without extrapyramidal effects. These analogues should retain the receptor-binding profile of clozapine but with structural changes to eliminate the toxic effect of clozapine. Thus, *5H*-dibenzo[*a*,*b*]cycloheptene (**2**) and dibenz[*b*,*f*]oxepin (**3**) analogues of clozapine (**1**) were prepared and were compared with **1** in the displacement of [^{3}H]clozapine from nondopaminergic and noncholinergic binding sites in rat forebrain (5,6). The results with the carbocyclic and oxygen-heterocyclic clozapine analogues demonstrated that the anticholinergic property of clozapine can be substantially reduced while retaining full affinity to the nondopaminergic clozapine binding sites (5,6). For a continuation of this work, additional quantities of [^{3}H]clozapine are required, and we now report its synthesis.

RESULTS AND DISCUSSION

As shown in Scheme I, [^{3}H]clozapine ([^{3}H]**1**), labeled on the piperazino ring, was prepared

Scheme I

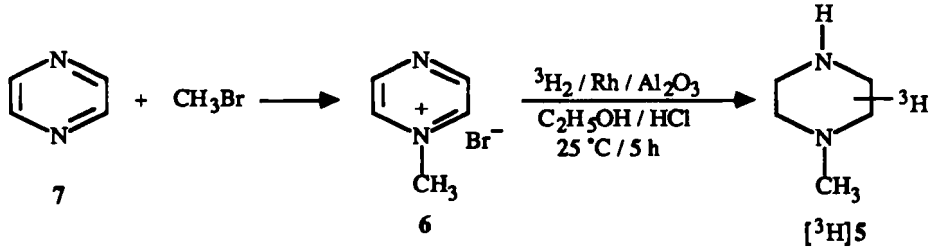


4 [^{3}H]**5** [^{3}H]**1**

by reaction of 8-chloro-11-(methylthio)-*5H*-dibenzo[*b*,*e*][1,4]diazepine (**4**) with an eight-fold excess of [^{3}H]*N*-methylpiperazine ([^{3}H]**5**) in the presence of a catalytic amount of acetic acid (7). In [^{3}H]**1**, the 4-methylpiperazino group is the moiety of choice for both introduction of the tritium label as well as being the label carrier. While the chemistry involved in the synthesis of **4** is substantial (Scheme

III), none of the synthetic steps in the preparation of **4** presents as convenient an opportunity for high specific activity tritium labeling as does the reduction with tritium of *N*-methylpyrazinium bromide (**6**) (8). This reduction was done in ethanolic hydrogen chloride at room temperature over 5% rhodium on aluminum oxide (Scheme II). The bromide **6** was prepared by methylation of pyrazine (**7**) using

Scheme II



7 **6** [^{3}H]**5**

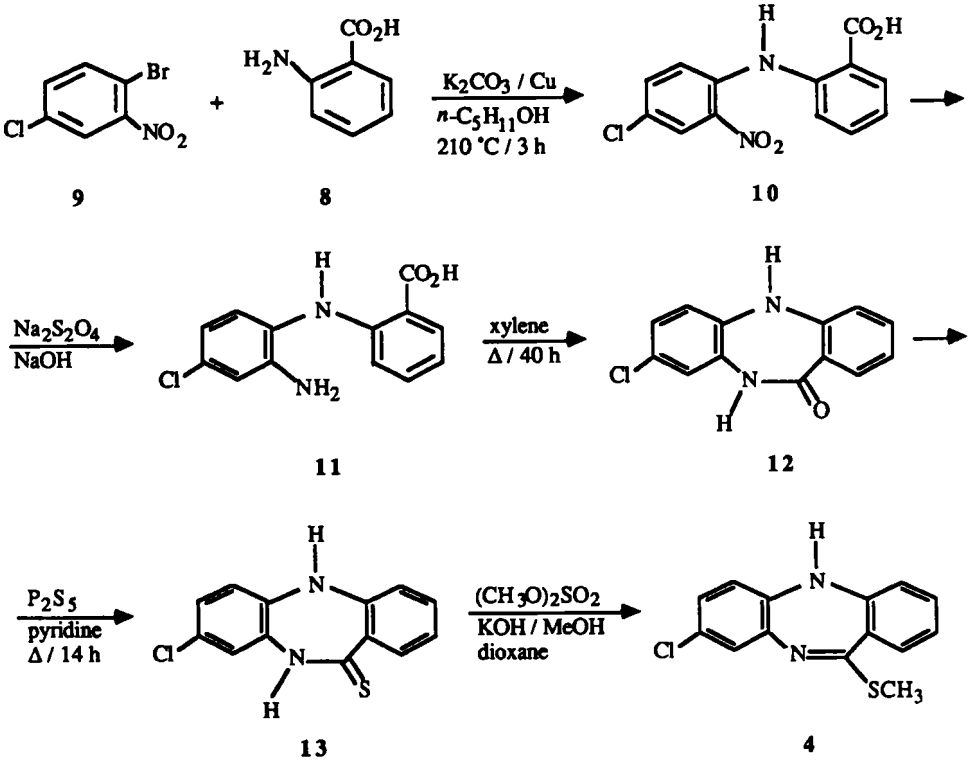
methyl bromide (9). The salt **6**, however, is somewhat unstable as the crystalline solid and should be freshly prepared a few days before use in the formation of [^{3}H]**5**. In preliminary reduction experiments using freshly prepared **6** and hydrogen, the yield of **5** was only 20 to 40% of the theoretical, and it was assumed that the same poor yield was obtained with tritium and the reaction condition shown in Scheme II.

For the formation of [^{3}H]**5**, reduction of **6** was continued until consumption of tritium ceased. The ether-extractable product contained 6.4 Ci of tritium, and on the basis of a yield of 30% of [^{3}H]**5**, each molecule of [^{3}H]**5** incorporated on average 1.4 atoms of tritium, substantial tritium exchange having occured with the solvent (10). Thus, an aspect of this procedure is that on average less than two atoms of tritium are incorporated in a particular piperazine ring. The sample of [^{3}H]**5** with estimated specific activity of 40 Ci/mmol was diluted with unlabeled **5** to an estimated maximum specific activity of 16 Ci/mmol before condensation with **4**.

As shown in Scheme III, the preparation of **4** begins with nucleophilic addition (11) of anthranilic acid (**8**) to 2-bromo-5-chloro-nitrobenzene (**9**) (12) to give *N*-(4-chloro-2-nitrophenyl)-anthranilic acid (**10**) (13). In our hands, the reported (13) use of 2,5-dichloronitrobenzene for the formation of **10** failed. Compound **9** was prepared from 4-chloro-2-nitroaniline by the Sandmeyer reaction (12).

The subsequent conversion of **10** to 8-chloro-10,11-dihydro-11-oxo-5*H*-dibenzo[*b*,*e*][1,4]-diazepine (**12**) by way of *N*-(2-amino-4-chlorophenyl)anthranilic acid (**11**) was done as outlined for the formation of substituted 5*H*-dibenzo[*b*,*e*][1,4]diazepines (13). Treatment of **12** with phosphorous pentasulfide (14) gave the corresponding 8-chloro-10,11-dihydro-11-thione-5-dibenzo[*b*,*e*][1,4] diazepine (**13**) (7). The latter was converted to **4** using dimethylsulfate (7).

Scheme III



Since the coupling of **4** with [^{3}H]**5** was done with only a catalytic amount of acetic acid, excess [^{3}H]**5** was the reaction solvent. Final purification of [^{3}H]**1** was accomplished by column chromatography giving [^{3}H]**1** with a radiochemical purity greater than 96%. The identity of [^{3}H]**1** was confirmed, and its specific activity of 9.9 Ci/mmol was determined by analytical high performance liquid chromatography, the amount and identity of the substances in the various fractions determined using a calibrated flow through ultraviolet spectrometer.

EXPERIMENTAL

Earlier experiments, including the preparation of tritium-labeled clozapine, were done at the Chemistry Research Department, Hoffmann-La Roche. The synthetic operations reported here leading to nontritiated products were done at Vanderbilt University, and those for the formation of [^{3}H]*N*-methylpiperazine ([^{3}H]**5**) by reduction of *N*-methylpyrazinium bromide (**6**) with tritium and the condensation of [^{3}H]**5** with 8-chloro-11-(methylthio)-5*H*-dibenzo[*b*,*e*]diazepine (**4**) were done at the National Tritium Labeling Facility (NTLF), Lawrence Berkeley Laboratory, University of California, Berkeley, California. Purification and characterization of [^{3}H]clozapine ([^{3}H]**1**) were also done at the

NTLF. At Vanderbilt University, melting points were taken in open capillary tubes and are corrected. Thin layer chromatography (TLC) was done on silica gel-coated plates (silica gel 60 F-254) using isopropyl ether-methanol, 20:1, as eluant, and visualization was done with ultraviolet light or iodine vapor.

Clozapine (1) was formed by reaction of 8-chloro-11-(methylthio)-5*H*-dibenzo[*b*,*e*]-diazepine (**4**) (137 mg, 0.499 mmol) with a 4-fold excess of *N*-methylpiperazine (**5**) (200 mg, 2.02 mmol) in the presence of a catalytic amount of acetic acid (6 mg, 0.1 mmol) as reported earlier (7). Recrystallization from isopropyl ether gave 80 mg (49%) of **1**: mp 178-180 °C [lit. (7) mp 183-184 °C]; R_f (TLC) 0.10. A reaction employing only a 3-fold excess of **5** gave a much lower yield.

[^{3}H]Clozapine ([^{3}H]1). A mixture of 8-chloro-11-(methylthio)-5*H*-dibenzo[*b*,*e*][1,4]-diazepine (**4**) (14.5 mg, 0.053 mmol), [^{3}H]*N*-methylpiperazine ([^{3}H]**5**) (0.41 mmol, 16 Ci/mmol), and acetic acid (1 μL) was heated at 128-132 °C with stirring for 20 h. The cooled reaction mixture was dissolved in chloroform (3 mL) and washed with 1 N ammonium hydroxide (2 x 2 mL) and water (1 x 2 mL) and then dried ($MgSO_4$). Thin layer chromatography (chloroform-isopropyl alcohol-concentrated ammonium hydroxide, 1000:50:1) showed no spot corresponding to **4** (R_f 0.85), a major spot assigned to [^{3}H]**1** (R_f 0.05), and some material at R_f 0.00. The solvent was removed by lyophilization, and the crystalline residue was transfered with chloroform (2 x 1 mL) to a 15-mL chromatography column (SiO_2, 230-400 mesh). Elution with chloroform-isopropyl alcohol-concentrated ammonium hydroxide (500:50:1) gave a series of 10-mL fractions (F): F 1-5, background radioactivity; F 6, 7.7 mCi; F 7, 8.7; F 8, 64.5; F 9, 90.3; F 10, 58.6; F 11, 38.6; F 12, 23.3; F 13, background radioactivity. The amount of tritium was determined using a Hewlett-Packard 1500 liquid scintillation counter operating at 65% efficiency. Fractions 7-12 on TLC showed a single spot assigned to [^{3}H]**1**. Fractions 8-11 were combined and diluted with chloroform (50.0 mL). An aliquot (400 μL) of this solution was subjected to HPLC on an analytical silica gel column calibrated with 50 μg of clozapine (**1**) using chloroform-isopropyl alcohol-concentrated ammonium hydroxide as eluant (500:50:1). The effluent was monitored using a flow-through Hewlett-Packard 1040A diode array ultraviolet spectrometer. The HPLC peak corresponding to **1** (retention time 7.2 min) contained 64.8 μg of [^{3}H]**1** and thus the total chloroform solution contained 8.10 mg (0.0246 mmol) of [^{3}H]**1**. The radioactivity in an aliquot (2 μL) of the HPLC peak was also determined. Thus, the total [^{3}H]**1** radioactivity in the chloroform solution above was 244 mCi with a specific radioactivity of 9.9 Ci/mmol. The chloroform was removed by lyophilization. The residue was diluted with benzene, (10.0 mL), and this solution frozen at 4 °C for storage until use.

8-Chloro-11-(methylthio)-5*H*-dibenzo[*b*,*e*]diazepine (4) was prepared from **13** as reported earlier (7) and outlined in Scheme III and was recrystallized from isopropyl ether: mp 117-118 °C [lit. (7) mp 116-121 °C]; R_f (TLC) 0.79.

[^{3}H]*N*-Methylpiperazine ([^{3}H]5). Hydrochloric acid (1.1 mL of 1.0 *N*, 1.1 mmol) was added to *N*-methylpyrazinium bromide (92.3 mg, 0.53 mmol) in 95% ethanol (3.0 mL). The mixture in a closed system was cooled in liquid nitrogen. The air was removed by pumping (4.0 Pa), and nitrogen was introduced (0.10 MPa). After a second evacuation and nitrogen addition, 5% rhodium on aluminum oxide (32.8 mg) was brought into contact with the aqueous ethanol solution using an internal glass spoon. After again removal of the nitrogen atmosphere, tritium was introduced, and the reaction mixture allowed to warm to room temperature (ca. 25 °C) and then stirred until consumption of tritium ceased (5 h, 33 mL at 0.10 MPa, 1.3 mmol, 76 Ci, 82% of theory). The reaction mixture was frozen in liquid nitrogen. The untreated tritium was removed by pumping (4.0 Pa), and nitrogen was introduced. After a second evacuation and addition of nitrogen, methanol (1 mL) was added, and the reaction mixture was filtered through Celite. The Celite was washed with methanol (2 x 0.5 mL). The reaction mixture was lyophilized, and 50% aqueous potassium hydroxide (0.50 mL) was added to the yellow residual oil. This mixture was extracted with ether (5 x 10 mL), and the ether solution was dried ($MgSO_4$, 14 min) and filtered through glass wool. The drying agent and glass wool were washed with ether (1 x 5 mL), and the combined ether solutions were evaporated through a 15-cm Vigreux distilling column using a bath temperature of 60 °C. The residual yellow oil was dissolved in ether (5.0 mL), and an aliquot (1.0 μL) was dissolved in toluene (1.0 mL). Counting of an aliquot (1.0 μL) of this solution showed that the ether extractable product contained 6.4 Ci of tritium. Assuming a yield of 30% of theory (0.16 mmol) and 1.4 g-atom of tritium per mole, the specific activity of [^{3}H]4 was 40 Ci/mmol. This sample was diluted with *N*-methylpiperazine (25 mg, 0.25 mmol) to give [^{3}H]4 (0.41 mmol) with an estimated maximum specific activity of 16 Ci/mmol.

***N*-Methylpyrazinium Bromide (6).** Methyl bromide (3.4 g, 0.036 mol) at 0 °C was added to pyrazine (1.6 g, 0.020 mol) in methylene chloride (10 mL) also cooled to 0 °C. The mixture was kept over night at -5 °C and then tightly closed and allowed to warm to 25 °C. After an additional 39 h, colorless crystals had formed. Filtration gave 1.4 g (40%) of **6**, mp 170-180 °C dec [lit. (8) mp 171 °C]. In a similar preparation, the yield was lower (0.62g, 18%), and the melting point was higher: mp 174-176 °C dec.

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REFERENCES

1. Small, J.G., Milstein, V., Marhenke, J.D., Hall, D.D. and Kellams, J.J. - J. Clin. Psychiat. *48*: 263 (1987)

2. Idänpään-Heikkilä, J., Alhava, E., Olkinuora, M. and Palva, I.P. - Eur. J. Clin. Pharmacol. *11*: 193 (1977)

3. Hauser, D. and Closse, A. - Life Sci. *23*: 557 (1978)

4. Bürki, H.R. - Life Sci. *26*: 2187 (1980)

5. de Paulis, T., Betts, C.R., Smith, H.E., Mobley, P.L., Mainer, D.H. and Sulser, F. - J. Med. Chem. *24*: 1021 (1981).

6. Harris, T.W., Smith, H.E., Mobley, P. L., Mainer, D.H. and Sulser, F. - J. Med. Chem. *25*: 855 (1982)

7. Hunziker, F., Fischer, E. and Schmutz, J. - Helv. Chim. Acta *50*: 1588 (1967)

8. Darby, W. L. and Vallarino, L. M. - Inorg. Chim. Acta *75*: 65 (1983)

9. Bahner, C.T. and Norton, L. L.- J. Am. Chem. Soc. *72*: 2881 (1950)

10. Černý, B. and Hanuš, J. - J. Label. Compounds Radiopharm. *18*: 947 (1981)

11. Hanze, A. R., Strube, R. E. and Greig, M. E. - J. Med. Chem. *6*: 767 (1963)

12. Hammond, G. S. and Modic, F. J. - J. Am. Chem. Soc. *75*: 1385 (1953)

13. Hunziker, F., Lauener, H. and Schmutz, J. - Arzneim.-Forsch. *13*: 324 (1963)

14. Hunziker, F., Künzle, F., Schindler, O., Schmutz, J. - Helv. Chem. Acta *47*: 1163 (1964)