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Synthesis of Carbon-14 and Tritium Labelled Analogs of the Novel Antischizophrenic Agent Clozapine

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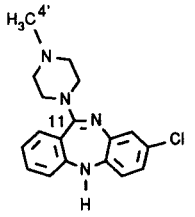
SUMMARY

8-Chlor-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b,e*][1,4]diazepine (SDZ 100-129, Clozapine) labeled with carbon-14 in the 11-position was prepared from 2-aminobenzonitrile-[*cyano*-^{14}C]. In addition, SDZ 100-129 was also labeled with C^3H_3 in the methyl group of the 4-methyl-piperazine ring.

Key Words : Clozapine, carbon-14, tritium, Jourdan-Ullmann coupling.

INTRODUCTION

The dibenzo-epine system containing a seven-membered central ring has been a rich source of biologically active molecular entities[1]. Of these, SDZ 100-129 (Clozapine), **1**, has proven to be a novel neuroleptic which is distinguished from other antipsychotic agents in its lack of extrapyramidal reactions[2]. To further elucidate the mechanism of action and to support ongoing drug metabolism studies, there arose a need for an isotopomer labelled in a biologically stable site. To this end, the synthesis of [11-^{14}C] Clozapine was undertaken.



1 SDZ 100-129

2 [11-^{14}C] SDZ 100-129

3 (4'-C^3H_3) SDZ 100-129

DISCUSSION

The initial approach to the carbon-14 analog, **2**, was to have followed the published method utilizing Jourdan-Ullmann coupling[3] of 2-bromo-5-chloronitrobenzene, **4**, with anthranilic acid, **5** :

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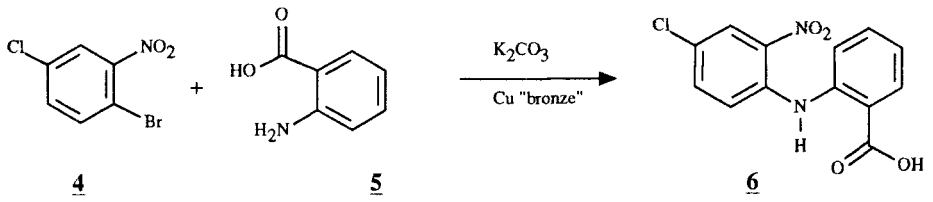
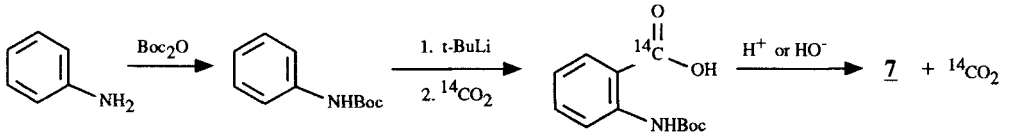


4 5 6

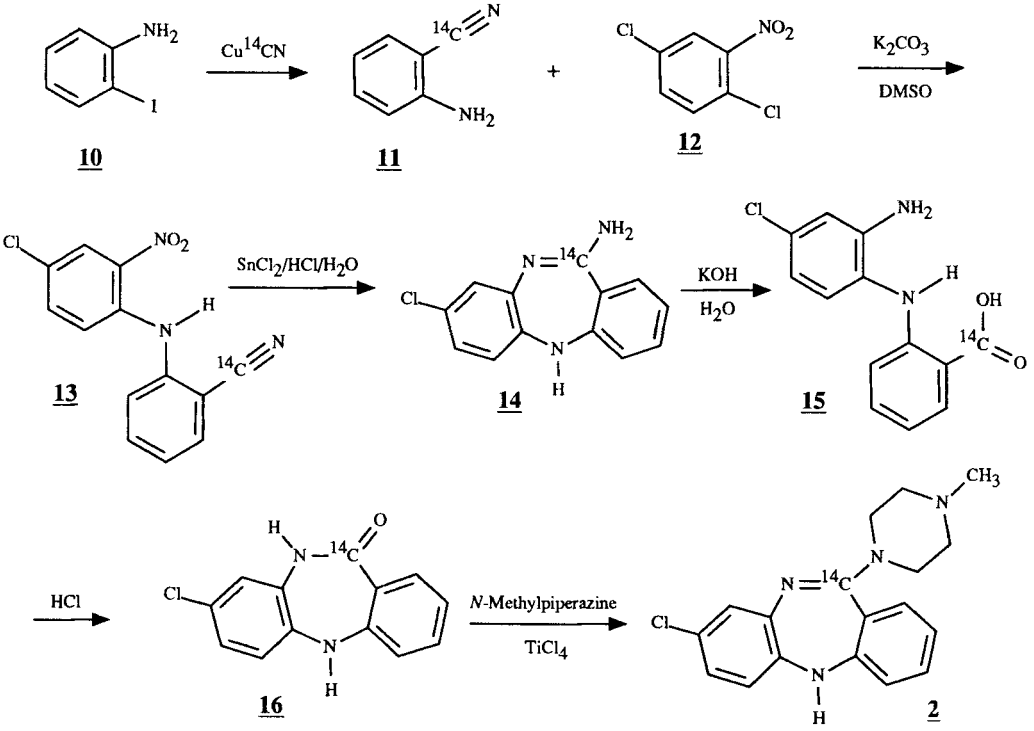
However, attempted synthesis of anthranilic acid-[*carboxy*-^{14}C] proved to be highly problematical. While access to the labelled Boc-protected analog **9** could be achieved in excellent yield *via ortho*-lithiation and $^{14}CO_2$ trapping of **8**, attempted deprotection (under acidic or basic conditions) afforded only aniline. The loss of $^{14}CO_2$ could not be avoided under all conditions studied.



7 8 9

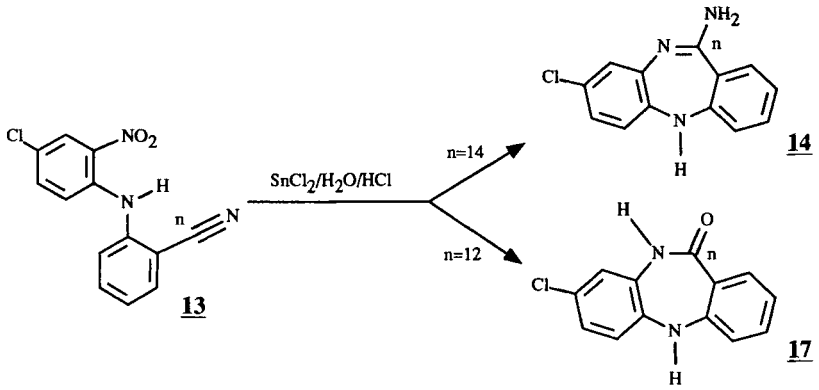
As an alternative approach (Scheme 1), the aminobenzonitrile **11** (derived from the addition of $Cu^{14}CN$ to 2-iodo-benzamine, **10**) was coupled with 1,4-dichloro-2-nitrobenzene, **12**, in the presence

Scheme 1



10 11 12 13 14 15 16 2

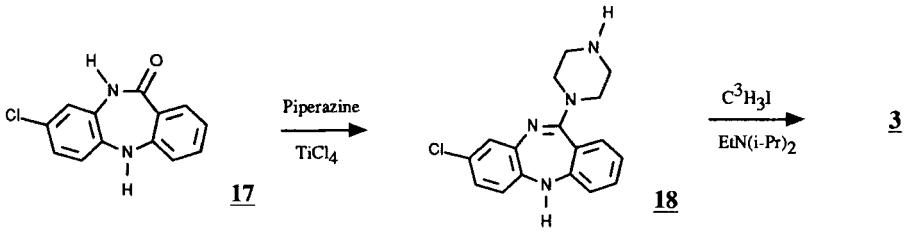
of potassium carbonate in hot DMSO. The product, **13**, when exposed to aqueous stannous chloride and HCl gave the cyclic amino-imine **14** as the major product. This result was surprising, since under identical conditions the unlabeled material smoothly proceeded to the cyclic lactam **17**[4] :



This unexpected turn of events, however, did not block access to the title material. Reaction of **14** with aqueous base for a prolonged period gave the amino acid **15**, which when treated with acid provided the key diazapin-11-one **16**. This material could be converted to [^{14}C] Clozapine, **2**, in a one-pot operation by reaction with the complex of *N*-methylpiperazine and titanium tetrachloride[5,6]. Chromatographic purification then led to drug substance of > 97% radiochemical purity.

In connection with protein binding and receptor mapping studies, an analog of SDZ 100-129 that possessed a much higher specific activity was also required. To meet this demand, compound **3** was synthesized (Scheme 2). Lactam **17** was accessed by literature methodology[7] (Jourdan-Ullmann coupling of 2-bromo-5-chloronitrobenzene, **4**, and anthranilic acid, **5**, followed by reduction of the nitro group with sodium dithionate and thermal cyclization in refluxing xylene) and was subjected to the complex formed between piperazine and titanium tetrachloride to give des-methyl clozapine, **18**. This material was reacted with tritiated iodomethane in the presence of N,N-diisopropylethyl amine and the reaction mixture was purified by high performance liquid chromatography to afford the title compound **3** in > 95% radiochemical purity and a specific activity of *ca.* 250 mCi/mg[8].

Scheme 2



EXPERIMENTAL

Potassium ^{14}C-cyanide was purchased from Amersham Corporation and iodo-^{3}H-methane was obtained from American Radiolabeled Chemicals, Inc. Chemical ionization mass spectroscopy was performed on a Finnigan 4600 mass spectrometer utilizing ammonia as the reagent gas. Radio-TLC chromatograms were recorded on 5 x 20 cm E. Merck silica gel F-254 plates (250 micron thickness). Radiochemical purities were determined by scanning the chromatograms for radioactivity with a Vanguard gas proportional scanner equipped with a 1 mm x 10 mm collimator, as well as radio-HPLC for compounds **2** and **3**. Identities of the intermediates were determined by comparative TLC versus unlabelled standards and isotopic dilution experiments where feasible. Specific activities were determined by the "weight-in-volume assay" method, as well as mass spectroscopy where indicated. ^{1}H-NMR data were recorded on a Bruker AC 300 instrument.

Copper(I) ^{14}C-cyanide

To a solution of potassium ^{14}C-cyanide (5.59 mmol, 295 mCi, 52.7 mCi/mmol) in 14 mL of water was added dropwise an aqueous solution of copper sulfite (14 mL, 0.22 M). The grayish suspension was stirred at room temperature for sixteen hours and then neutralized to pH 7 with aqueous 1.0 N sodium hydroxide (2.2 mL). The aqueous phase was decanted and the white precipitate was washed with two 20-mL portions of water followed by two 20-mL portions of acetone to give 271 mCi of the title compound.

2-Amino-benzonitrile-[*cyano*-^{14}C], **11**

A mixture of 2-iodo-benzamine, **10**, (2448 mg, 11.2 mmol) and copper(I) [^{14}C]-cyanide (5.14 mmol, 271 mCi) in 20 mL of N,N-dimethylformamide was stirred at reflux for seven hours. The solution was allowed to cool to room temperature and extracted into 100 mL of ethyl acetate. The extract was washed with water followed by brine and dried over anhydrous sodium sulfate. Concentration under reduced pressure afforded a brown residue which was purified by column chromatography on silica gel using 20% ethyl acetate-hexane as eluant to give 4.65 mmol, 240 mCi of the title compound. Specific activity determination at this stage revealed a value of 51.6 mCi/mmol. Radiochemical purity was determined to be > 95% *via* radio-TLC (10% ethyl ether:chloroform; R_f= 0.48).

2-[(4-Chloro-2-nitrophenyl)amino]-benzonitrile-[*cyano*-^{14}C], **13**

To a slurry of 2-amino-benzonitrile-[*cyano*-^{14}C], **11**, (4.65 mmol, 240 mCi) and potassium carbonate (5.12 mmol, 707.1 mg) in 10 mL of dimethyl sulfoxide was added 1,4-dichloro-2-nitro benzene, **12**, (5.12 mmol, 982.1 mg), and the resulting mixture was stirred at 110°C for sixteen hours, at which time an additional equivalent of 1,4-dichloro-2-nitro benzene was added and heating was continued for thirty-nine hours. The solution was allowed to cool to room temperature and diluted with 100 mL of ethyl acetate. This mixture was washed with water, followed by brine and then dried over anhydrous sodium sulfate. Concentration of this solution under reduced pressure gave a black residue

which was purified by silica gel chromatography using toluene as eluant to give 2.77 mmol, 143 mCi, of the title compound, **13**. Radiochemical purity was determined to be > 97 % *via* radio-TLC (10% ethyl ether:chloroform; $R_f = 0.68$).

8-Chloro-5*H*-dibenzo[b,e][1,4]diazapin-11-amine-[*11-^{14}C*], 14

To a solution of 2-[4-chloro-2-nitrophenyl)amino]-benzonitrile-*cyano*-^{14}C, **13**, (2.77.mmol, 143 mCi) and stannous chloride (8.31 mmol, 1872.2 mg) in 10 mL of absolute ethanol was added slowly 1.0 mL of concentrated hydrochloric acid. The solution was heated at reflux for thirty-six hours, cooled to room temperature and neutralized with ammonium hydroxide. The mixture was extracted into 200 mL of ethyl acetate and the organic phase was washed with two 100-mL portions of water and two 50-mL portions of brine, and then dried over anhydrous sodium sulfate. Concentration *in vacuo* gave a black residue which was purified by silica gel column chromatography using ethyl acetate as eluant to give 42.6 mCi of the title compound, **14**, as a solid. Radiochemical purity was determined to be > 97% *via* radio-TLC (ethyl acetate; $R_f = 0.16$). Mass spec. : $MH^+ = 243$.

2-[(2-Amino-4-chlorophenyl)amino]-benzoic-[*carboxy-^{14}C*] acid, 15

To a slurry of 8-chloro-5H-dibenzo[b,e][1,4]diazapin-11-amine-[*11-^{14}C*], **14**, (0.82 mmol, 42.6 mCi) in 10 mL of absolute ethanol was added an aqueous solution of potassium hydroxide (2.46 mmol, 0.5 N) and the resulting mixture was stirred at reflux for seventy-two hours. After cooling to room temperature, the reaction mixture was neutralized with 2N hydrochloric acid and extracted with 200 mL of ethyl acetate. The organic phase was washed with water, followed by brine and then dried over sodium sulfate. Concentration under reduced pressure gave the title compound, **15**, as a brown residue (0.81 mmol, 41.8 mCi). Radio-chemical purity was determined to be > 97% *via* radio-TLC (10% ethyl ether:chloroform with 0.1% acetic acid; $R_f = 0.38$).

8-Chloro-5,10-dihydro-11*H*-dibenzo[b,e][1,4]diazapin-11-one-[*11-^{14}C*], 16

An aqueous solution of hydrochloric acid (18 mmol, 9N) was added to a solution of 2-[(2-amino-4-chlorophenyl)amino]-benzoic-[*carboxy-^{14}C*] acid, **15**, (0.81 mmol, 41.8 mCi) in 10 mL of absolute ethanol and allowed to stir at reflux for sixteen hours. At this point, the mixture was cooled to room temperature and concentrated under reduced pressure. The residue was taken up in 250 mL of ethyl acetate and washed with two 100-mL portions of water and two 100-mL portions of brine. The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo to give a brown residue. This was purified by silica gel column chromatography using chloroform as eluant to give 33.3 mCi of the title compound, **16**. Radiochemical purity was determined to be > 98% *via* radio-TLC (10% methanol:chloroform; $R_f = 0.49$).

8-Chloro-11-(4-methylpiperazinyl)-5*H*-dibenzo[b,e][1,4]diazapin-[*11-^{14}C*], 2

To a slurry of 8-chloro-5,10-dihydro-11H-dibenzo[b,e][1,4]diazapin-11-one-*[11-^{14}C]*, **16**, (0.64 mmol, 33.3 mCi), 1-methylpiperazine (0.14 mL, 1.27 mmol) and triethylamine (0.18 mL, 1.27

mmol) in 50 mL of toluene was added dropwise a solution of titanium tetrachloride (0.10 mL, 0.95 mmol) in 10 mL of toluene. The slurry was heated at reflux for 48 hours and then allowed to cool to room temperature. The heterogeneous reaction mixture was quenched by the addition of 10 mL of water and passed through a short pad of Celite®. The filtrate was extracted with three 50-mL portions of ethyl acetate, and the combined organic layers were washed with two 25-mL portions of brine, dried over anhydrous sodium sulfate and concentrated in vacuo to give a dark brown residue. This material was purified by chromatography on silica gel using 20% ethyl acetate-toluene as eluant. Concentration of the fractions of interest yielded 20.5 mCi of the title product that was recrystallized from isopropanol:water (3:1, v/v) with seeding to give [^{14}C] 100-129, 2, as pale yellow crystals (42.0 mg, 50.7 mCi/mmol, 6.5 mCi). The mother liquor was purified by column chromatography, and the isolated pure product was stored as a solution in 100 mL of ethanol (0.1178 mCi/mL). The radiochemical purity of the crystals was determined by radio-TLC (three solvent systems : ethyl acetate:acetic acid:methanol, 5:2:2; methanol:chloroform 1:9; and n-heptane:chloroform:ethanol:conc. ammonia, 30:30:30:1. All systems indicated a radiochemical purity of > 97%) and radio-HPLC (The analysis was carried out with a Waters HPLC system consisting of a 600E pump module, a WISP 712A autosampler and a 484 UV/VIS variable wavelength detector. The radioactivity was monitored with a Raytest Ramona-90 flow-through detector and its data base collected both radioactive and UV data. The Ramona-90 was equipped with a 100 µL calcium fluoride solid scintillator cell. A LiChrosorb RP-8 column with a particle size of 10 µm and dimensions of 250 mm x 4.6 mm ID was used. The mobile phase consisted of methanol:water:triethylamine 800:200:0.75, v:v:v. The flow rate was set at 1.0 mL/min. and UV detection was at 257 nm. Five identical injections indicated a radiochemical purity of > 97%). Mass spec. MH^+ = 329. Specific activity was determined to be 50.7 mCi/mmol, both by the weight-in-volume assay and mass spectroscopy.

8-Chloro-11-piperazinyl-5*H*-dibenzo[b,e][1,4]diazepine, 18

To a solution of 2.75 mL (25 mmol) of titanium tetrachloride in 25 mL of anisole was added 7.24 g (83.8 mmol) of piperazine and the mixture was warmed to 80 °C. To this was added in one portion 5.1 g (20.9 mmol) of 8-chloro-5,10-dihydro-11*H*-dibenzo[b,e][1,4]diazapin-11-one[7] and an additional 3.64 g (42.1 mmol) of piperazine. The reaction mixture was then heated to 140 °C and stirred for 3 hours. At this time, the solution was allowed to cool to room temperature and concentrated under reduced pressure. The residue was purified by silica gel chromatography (5% methanol-methylene chloride). The fractions of interest were pooled and concentrated and partitioned between ethyl acetate and 2N HCl. The aqueous layer was separated and basified with 20% NaOH solution and extracted with ethyl acetate. The organic phase was concentrated *in vacuo* and the residue obtained was crystallized from ethyl acetate-hexane to give 2.23 g of the title compound. ^{1}H-NMR ($CDCl_3$, TMS, 300 MHz) : δ7.25, m, 2H; δ7.05, m, 2H; δ6.80, m, 2H; δ6.60, m, 1H; δ4.90, s, 1H; δ3.41, m, 4H; δ2.95, m, 4H; δ1.62, bs, 1H. Mass spec. MH^+ = 313; Elemental analysis, Calc. : C=65.28%, H=5.48 %, N=17.61%, Cl=11.33%; Found : C=65.67%, H=5.95%; N=16.87%, Cl=12.01%.

8-Chloro-11-[4-(^{3}*H*-methylpiperazinyl)]-5*H*-dibenzo[b,e][1,4]diazepine, 3

To a solution of 8-chloro-11-piperazinyl-5*H*-dibenzo[b,e][1,4]diazadiazepine, **18**, (3.3 μg, 10.56 μmol) and N,N-diisopropylethyl amine (5.0 μL, 28.7 μmol) in 1000 μL of dimethylformamide at -78 °C was added *via* high vacuum 390 μg (1.18 μmol, 85 Ci/mmol[8]) of tritiated methyl iodide in 500 μL of toluene. The solution was allowed to warm to room temperature and stirred for 12 hours. The reaction mixture was placed on a bed of silica gel and eluted with 10% methanol:chloroform and the fractions of interest were pooled and concentrated *in vacuo*. This material was further purified by preparative-HPLC utilizing the conditions described for compound **2** to give 14 mCi of **3**. Radio-HPLC evaluation (same conditions as used for **2**) revealed a radiochemical purity of > 95%.

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4. It has been our observation that there exists a significant isotope effect in the reactions of carbon-14 labelled nitriles. Many simple hydrolyses require extended reaction times (under acidic and basic conditions) in the case of the heavier isotopes and in the presence of other functional groups, such as the case here, different reaction products are isolated. These observations and possible explanations will be the subject of a future paper.

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