SYNTHESIS OF CARBON-14 AND TRITIUM LABELED GLYBURIDE.

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SUMMARY

N-{4-[2-(5-Chloro-2-methoxybenzamido) ethyl] phenylsulfonyl} N'-cyclohexylurea, IXc, or glyburide, has been labeled with tritium and carbon-14. The tritium label is located in the C-3 position of the 5-chloro-2-methoxybenzoyl portion of the molecule, while the carbon-14 label is incorporated into the C-2 position of the 2-phenylethylamine moiety in the compound.

The specific activities of the tritium and carbon-14 labeled products are 8.50 mCi/mM and 3.66 mCi/mM, respectively.

INTRODUCTION

N-{4-[2-(5-Chloro-2-methoxybenzamido)ethyl]phenylsulfonyl}-N'-cyclohexylurea[1], IXc, or glyburide, is a potent oral hypoglycemic agent. Pharmacokinetic and metabolism studies with this compound in animals and man have been reported[2-5]. In order to conduct additional absorption and metabolism studies with IXc in our laboratories, radioactive forms of the drug were required. Based on information already available on the metabolic fate of other

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sulfonylureas such as tolbutamide[6] and acetohexamide[7,8], as well as glyburide[4,5], it appeared that double-labeling IXc with tritium and carbon-14 would be desirable. The hydrogen at the C-3 position in the 5-chloro-2-methoxybenzoic acid portion of IXc, being *ortho* to a methoxy group, appeared to be a good candidate for exchange with tritium. Carbon-14 should be readily introduced into the C-2 position of phenylethylamine portion of IXc by means of carbonation of phenylmagnesium bromide followed by the elaboration of the side chain.

DISCUSSION AND RESULTS

Tritium Labeled Glyburide

In order to find the appropriate reaction conditions for preparing tritium labeled 5-chloro-2-methoxybenzoic acid (IIb, Figure 2) *via* ionic exchange, the deuteration of II with monitoring by NMR spectroscopy was studied to serve as a model. In 9:1 (v/v) $^2H_2SO_4$-^2H_2O at 90°C, the C-3 hydrogen readily exchanged with deuterium with a half-time ($T_{\frac{1}{2}}$) of 42 minutes (Figure 3). However the exchange was accompanied by demethylation ($T_{\frac{1}{2}}$=9.1 hrs., Figure 4) as well as the formation of a third product, probably III. The NMR data suggested the reaction sequence shown in Figure 1.

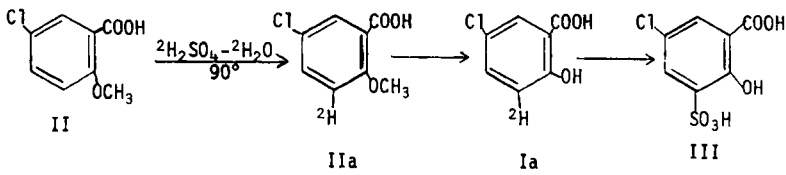
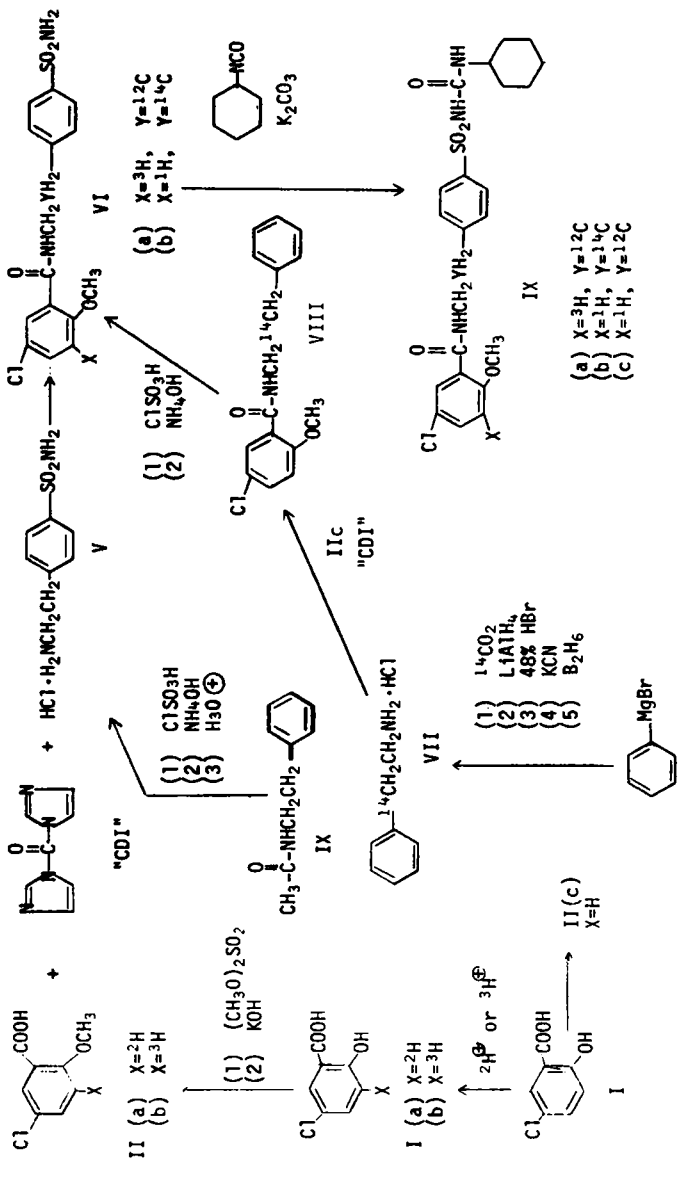


Figure 1

Deuteration of 5-Chloro-2-methoxybenzoic Acid

Figure 2

Reaction Sequences for the Preparation of Carbon-14 and Tritium Labeled Glyburide



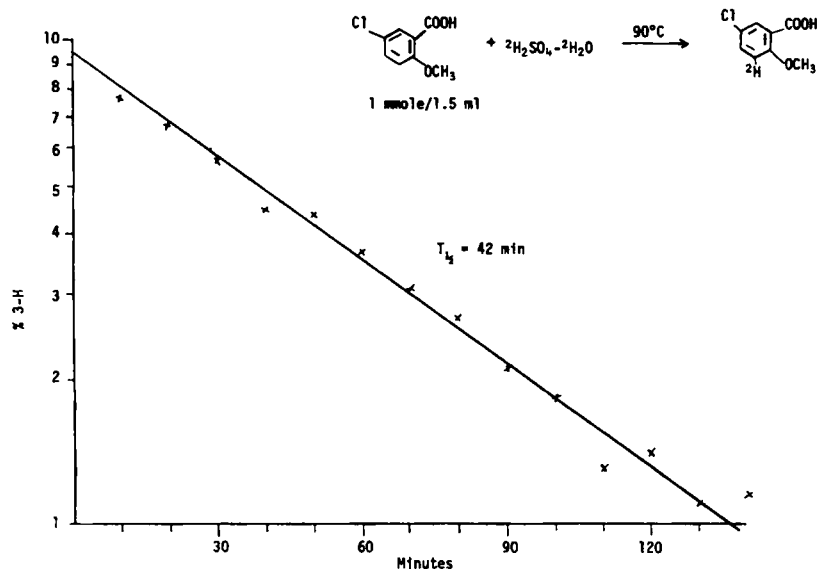


Figure 3
Deuteration of 5-Chloro-2-methoxybenzoic Acid
in 9:1 $^2H_2SO_4$-^2H_2O at 90°C

The incorporation of deuterium into 5-chlorosalicylic acid (I) proved to be much more facile than in the case of II. The exchange of the C-3 hydrogen with deuterium proceeded with a half-time of 22.5 minutes (Figure 5) in 9:1 (v/v) $^2H_2SO_4$-^2H_2O at 60°C. Formation of the same by-product III was negligible under these conditions and it was easily removed from the desired product Ia by recrystallization. Methylation of Ia afforded 5-chloro-3-deutero-2-methoxybenzoic acid (IIa) with full retention of deuterium (Figure 2).

To help assess the stability of labels at the C-3 position, other exchange

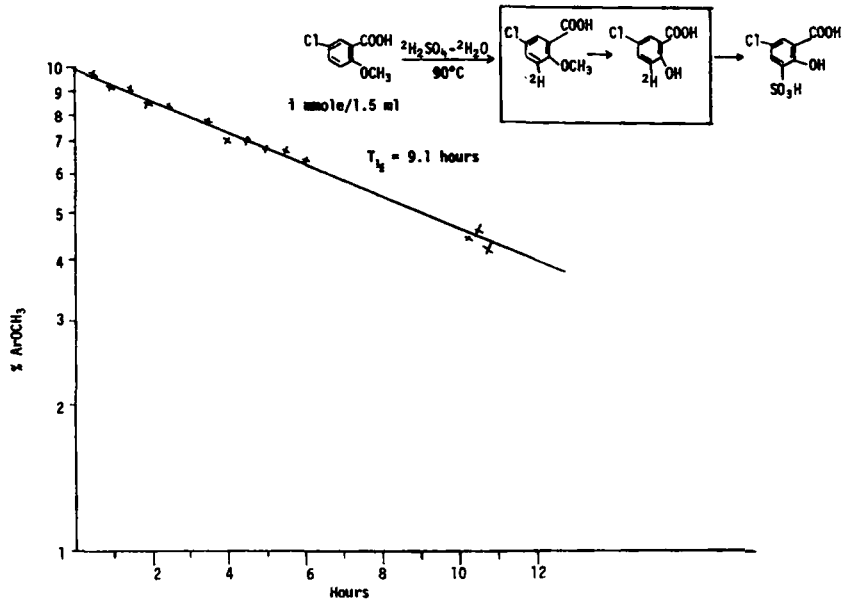


Figure 4

Demethylation of 5-Chloro-2-methoxybenzoic Acid in 9:1 $^2H_2SO_4$-^2H_2O at 90°C

reactions were carried out. In 9:1 (v/v) $^2H_2SO_4$-^2H_2O deuteration of 1 had a half-time of 30 hours (Figure 6) at 25°C., but at 90°C. the reaction was too rapid to follow. Removal of deuterium from Ia in 9:1 (v/v) H_2SO_4-H_2O at 60°C. proceeded with a half-time of 17 minutes (Figure 7). The faster dedeuteration rate at C-3 in comparison to deuteration suggested that carbon-deuterium or carbon-hydrogen bond formation was the rate determining step in the exchange. If so, tritiation would be expected to be slower than deuteration because of the isotope effect. Also, detritiation could be expected to be faster than tritiation. Nevertheless the deuteration conditions should serve as useful guides for tritiation. Furthermore the stability of the C-3 tritium label should be enhanced

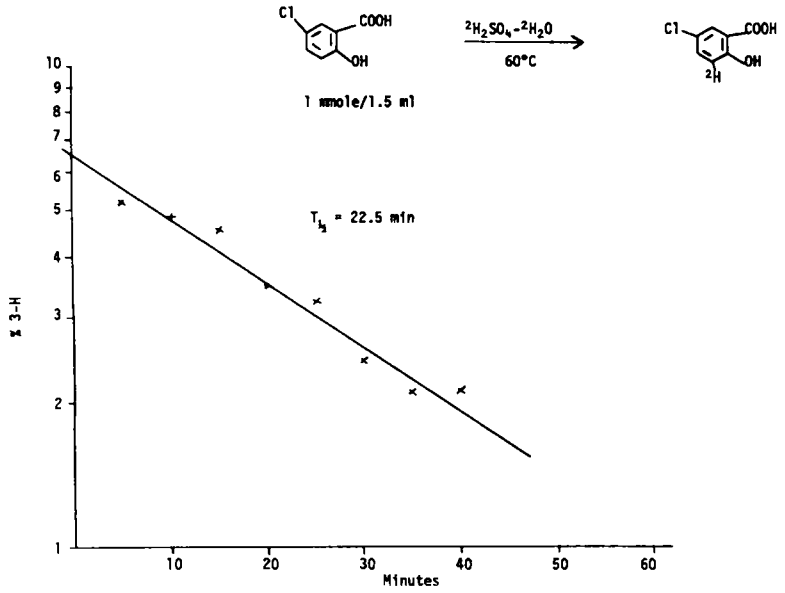


Figure 5
Deuteration of 5-Chlorosalicylic Acid in 9:1 $^2H_2SO_4$-^2H_2O at 60°C

by the methylation of the 2-hydroxy function since the deuterium exchange rate at C-3 was found to be vastly slower in II than in I.

5-Chlorosalicylic acid was heated in 9:1 (v/v) H_2SO_4-H_2O containing tritiated water at 60°C. for 2.5 hours (6.6 deuteration half-times). Probably because of a change in the work-up procedure necessitated by safety reasons, or possibly because of isotope effect, or both, the extent of tritium incorporation was substantially less than expected. However, an ample amount of Ib was obtained. The reaction sequence for preparing tritium-labeled glyburide (IXa)

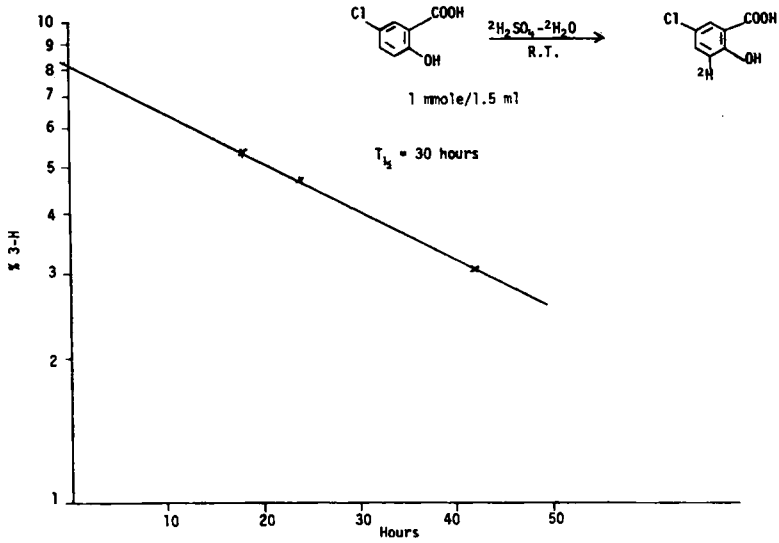


Figure 6

Deuteration of 5-Chlorosalicylic Acid in 9:1 $^2H_2SO_4$-^2H_2O at Room Temperature

from 5-chloro-3-tritiosalicylic acid (Ib) is shown in Figure 2. Methylation of Ib gave IIb, which was converted to VIa by treating its imidazolide with 4-(2-aminoethyl)benzenesulfonamide hydrochloride (V). Compound VIa was condensed with cyclohexylisocyanate to give IXa with a specific activity of 8.5 mCi/mM.

Carbon-14 Labeled Glyburide

The reaction sequence for the synthesis of carbon-14 labeled glyburide (IXb) is shown in Figure 2. Benzoic acid-α-^{14}C was reduced with lithium aluminum

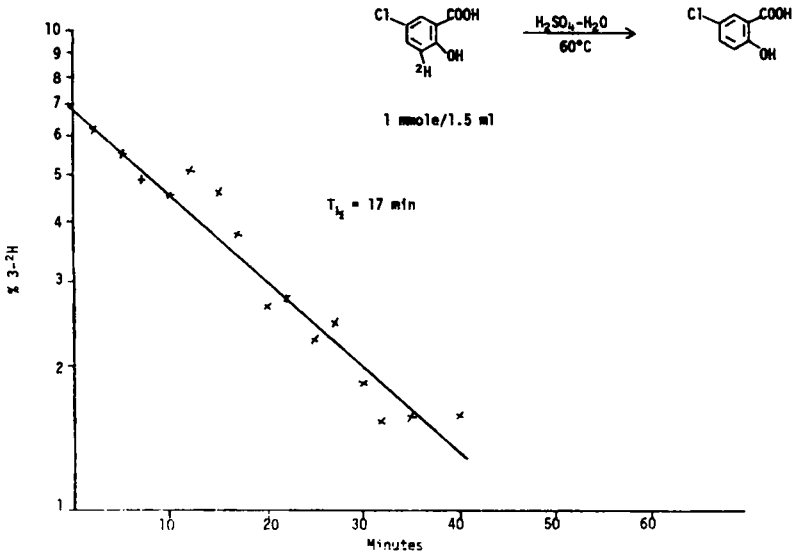


Figure 7

Dedeuteration of 5-Chlorosalicylic Acid-3-^{2}H in 9:1 H_2SO_4-H_2O at 60°C

hydride to benzyl alcohol-α-^{14}C which was converted to phenylacetonitrile-2-^{14}C *via* benzyl bromide-α-^{14}C. The nitrile was reduced with diborane to give 2-phenylethylamine-2-^{14}C hydrochloride (VII). Compound VII was treated with 5-chloro-2-methoxybenzoyl imidazole to give N-(5-chloro-2-methoxybenzoyl)-2-phenylethylamine-2-^{14}C (VIII) which on chlorosulfonation followed by treatment with ammonia yielded the sulfonamide VIb. Reaction of VIb with cyclohexylisocyanate afforded IXb. The overall yield of IXb, with a specific activity of 3.66 mCi/mM, was 10.5% based on benzoic acid-α-^{14}C.

EXPERIMENTAL

NMR spectra were obtained with a Varian A-60-A spectrometer. Radioactivity determinations were carried out with a Packard Tri-Carb Model 314 liquid scintillation spectrometer. Thin layer chromatography plates were scanned with a Vanguard Model 880 Autoscanner equipped with a Vanguard Model 885 glass plate scanner. Thin layer chromatography analyses were carried out on silica gel coated glass plates. The solvent systems used were as follows: A) 45:35:10:10 v/v ethyl acetate, isopropanol, conc.ammonium hydroxide and water: B) 95:4:1 v/v chloroform, methanol and formic acid: C) 6:1 v/v methanol and chloroform: D) 1:3 v/v ethyl acetate and cyclohexane. Melting points were uncorrected.

Deuterium Exchange Rate Studies

The compound under study was dissolved in an appropriate solvent mixture, 9:1 (v/v) $^2H_2SO_4$-^2H_2O in the case of deuteration and 9:1 (v/v) H_2SO_4-H_2O in the case of dedeuteration. The solution (1 mmole compound/1.5 ml solvent) was placed in the probe of the NMR spectrometer which had been preheated to the desired temperature. Spectra were taken periodically and integrated at indicated times. The C-3 proton integral was measured against the C-4 and C-6 proton integrals as standards. The appropriate chemical shifts of the compounds examined were as follows (compound, 3-H, 4-H, 6-H, CH_3): I, δ 7.10 (d, J=9 Hz), 7.72 (dd, J=2.5,9), 7.97 (d, J=2.5): II, 6.44 (d, J=9), 7.39 (dd, J=2.5,9), 7.26 (d, J=2.5), 4.20 (s). The CH_3 signal at δ 3.98, from dimethyl sulfate present in $^2H_2SO_4$, was taken as the reference frequency for the chemical shifts. The % starting material *vs.* time plots are shown in Figures 3, 5, 6 and 7, from which the exchange half-times were determined.

Demethylation of 5-chloro-2-methoxybenzoic Acid (II) under Exchange Conditions

The NMR spectrum of a solution of 94 mg (0.5 mmole) of II in 0.75 ml of 9:1 (v/v) $^2H_2SO_4$-^2H_2O at ambient temperature was scanned and integrated to

determine the CH_3 integral contribution by the dimethyl sulfate present in the $^2H_2SO_4$. The sample was removed from the instrument and the probe heated to 90°C. The sample tube was put back in the probe and the aromatic (δ 7.08 - 8.75) and methyl (δ 3.75 - 4.83)[a] regions of the spectrum were scanned and integrated periodically. The extent of demethylation was calculated as follows:

A = dimethyl sulfate CH_3 integral at ambient temperature before start of reaction

B = dimethyl sulfate CH_3 integral at time t at 90°C

C = B - A = dimethyl sulfate CH_3 integral due to dimethyl sulfate arising from demethylation at time t at 90°C

D = methoxy CH_3 integral at time t at 90°C

% II at time $t = \frac{D}{C+D} \times 100$

The % II *vs.* time plot is shown in Figure 4, from which demethylation half-time was obtained.

5-Chlorosalicylic Acid-3-^2H (Ia)

A solution of 1.728 g (10 mmoles) of 5-chlorosalicylic acid in ∿12 ml of 9:1 $^2H_2SO_4$-^2H_2O was heated in an oil bath at 60°C for 2.5 hours. The mixture was cooled to room temperature and added dropwise to ∿70 g of crushed ice. The resulting mixture was stirred and filtered. The solids were washed with cold water, dried and recrystallized from a mixture of methanol and water to give an 88% yield of product. NMR spectrum of this material in acetone-$^2H_6$ showed that it was essentially fully deuterated in the C-3 position.

5-Chloro-2-methoxybenzoic Acid-3-^2H (IIa)

5-Chloro-2-methoxybenzoic acid-3-^2H was prepared according to a modified

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[a] This region contains signals arising from CH_3 groups of both dimethyl sulfate (δ 3.98) and compound II (δ 4.20).

procedure of Huffman[9]. A solution of 1.0 g (5.76 mmoles) of 5-chlorosalicylic acid-3-^{2}H (Ia) in 6.5 ml of acetone was added to a warm suspension of 9.1 g (51.2 mmoles) of potassium carbonate in 10 ml of acetone. The mixture was heated to reflux with stirring and 3.6 g (28.6 mmoles) of dimethyl sulfate in 4.5 ml of acetone was added dropwise. After the mixture was refluxed with stirring for 20 hours, the inorganic salts were filtered and the filtrate was evaporated. The remaining oil was refluxed in methanolic KOH for 1 hour and the solution concentrated . The residue was stirred with dilute HCl to give 1.03 g (95% yield) of IIa, m.p. 80-83°C. The NMR spectrum of the product in acetone-$^2H_6$ showed there was no loss of deuterium during the reaction or work-up. The material was identical to an authentic sample of 5-chloro-2-methoxybenzoic acid by TLC (solvent system A).

5-Chlorosalicylic Acid-3-^{3}H (Ib)

The exchange phase of this preparation was carried out at New England Nuclear Corporation according to instructions supplied by us. A mixture of recrystallized 5-chlorosalicylic acid (863 mg, 5 mmoles) and 5 ml of 9:1 H_2SO_4-H_2O containing 25 Ci of tritium was heated at 60°C under N_2 with stirring for 2.5 hours. To the cooled mixture was added[a] cold water and the resulting mixture was filtered and the solids were washed with cold water and dried. The crude product was dissolved in methanol and the solution was evaporated to dryness to remove labile tritium. The residue (746 mg) was purified in our laboratories by recrystallization with dilution (1.600 g unlabeled 5-chlorosalicylic acid added) to give 2.30 g (98% recovery) of 5-chlorosalicylic acid-3-^{3}H, m.p. 174-175°C, sp. act. 8.20 mCi/mM.

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[a]This reverse mode of addition, necessitated by safety reasons, undoubtedly caused a rise in temperature in the mixture, and probably facilitated back-exchange and thus lowered the radiochemical yield.

5-Chloro-2-methoxybenzoic Acid-3-^3H (IIb)

The procedure for preparing IIa was followed. From 1.913 g (11.0 mmoles) of 5-chlorosalicylic acid-3-^3H was obtained 1.963 g (95.7% yield) of IIIb, m.p. 80-83°C, sp. act. 8.31 mCi/mM. Radiochemical purity of the product was established by TLC (solvent system A).

4-[2-(5-Chloro-2-methoxy-3-tritiobenzamido)ethyl]benzenesulfonamide (VIa)

4-(2-Aminoethyl)benzenesulfonamide hydrochloride (v), m.p. 233-235°C, was prepared in 25% yield from N-acetyl-2-phenylethylamine (IV) according to the procedure of Miller *et al*[10].

A mixture of 1.680 g (9 mmoles) of IIb and 1.752 g (10.8 mmoles) of carbonyl diimidazole (CDI) in 15 ml of dry dimethyl formamide (DMF) was stirred at room temperature until evolution of gases subsided. The solution was warmed on steam bath for 15 min and 2.604 g (11 mmoles) of V in 5 ml of DMF was added in one portion. The resulting mixture was heated on steam bath for 25 min, filtered and the filtrate was mixed with 75 ml of water. The precipitates were collected, washed with water followed by 100 ml of methanol, and dried to give 2.627 g (79.1% yield) of VIa, m.p. 210-213°C, sp. act. 8.21 mCi/mM, radiochemically pure by TLC (solvent system B).

Glyburide-^3H (IXa)

IXa was prepared according to the procedure of Weber *et al*[11]. The crude product obtained from refluxing 1.844 g (5.0 mmoles) of VIa with 729 mg (5.8 mmoles) of cyclohexylisocyanate in 80 ml of acetone in the presence of 3.7 g of potassium carbonate was recrystallized from acetone to give 1.477 g (60% yield) of IXa, m.p. 171-173.5°C; sp. act. 8.50 mCi/mM; UV and IR spectra conformed with those of an authentic sample of IXc; radiochemically pure by TLC (solvent system B).

Anal. Calcd. for $C_{23}H_{28}ClN_3O_5S$ (M.W. 493.99):
C, 55.92; H, 5.71; Cl, 7.18; N, 8.51; S, 6.49
Found: C, 55.70; H, 5.79; Cl, 7.27; N, 8.50; S, 6.71

2-Phenylethylamine-2-^{14}C Hydrochloride (VII)

Benzoic acid-α-^{14}C was prepared by treating an ethereal solution of phenylmagnesium bromide with $^{14}CO_2$ according to the procedure of Dauben *et al*[12].

From 150 mCi of $Ba^{14}CO_3$, 84.9 mCi of benzoic acid-α-^{14}C was obtained in two lots, 36.7 mCi at 5.11 mCi/mM and 48.2 mCi at 2.95 mCi/mM. These materials were combined (2.856 g, 23.4 mmoles) and reduced with lithium aluminum hydride (2.0 g, 52.6 mmoles) in ether to give benzyl alcohol-α-^{14}C in 64% yield. A mixture of 1.621 g (15 mmoles) of benzyl alcohol-α-^{14}C and 10 ml of 48% HBr was refluxed with stirring under N_2 for 2.5 hours, diluted with 30 ml of water and extracted with ether. The extracts were washed with saturated NaCl solution and concentrated to give 2.507 g (97.5% yield) of benzyl bromide-α-^{14}C.

To a warm solution of 1.320 g (20 mmoles) of KCN in 2 ml of water was added 2.507 g (14.65 mmoles) of benzyl bromide-α-^{14}C in 5 ml of ethanol. The mixture was refluxed with stirring under N_2 for 1.5 hours and concentrated at reduced pressure. The residual mixture of oil, water and inorganic salts was extracted with 70 ml of ether. The extracts were washed with water followed by saturated NaCl solution. Removal of ether gave 1.636 g of crude product which was chromatographed on a 13 x 1.5 cm column of silica gel eluted with methylene chloride to give 1.634 g phenylacetonitrile-2-^{14}C.

The nitrile was reduced with diborane according to a modified procedure of Brown and Rao[13]. A 1 *M* solution of diborane in dry tetrahydrofuran (15 ml, 15 mmoles) was added dropwise with stirring to 1.634 g (14 mmoles) of phenylacetonitrile-2-^{14}C in 12 ml of dry THF at 0°C in 20 min. The mixture was stirred at 0°C for 1.5 hours and kept at room temperature for 2 hours. The mixture was cooled and the excess diborane was decomposed by cautious addition of

5 ml of absolute ethanol. The resulting mixture was stirred at room temperature for 1 hour, cooled to 0°C, and anydrous HCl was passed into the solution. After 1 hour at room temperature the mixture was concentrated at reduced pressure. The residue was recrystallized from acetone to yield 0.814 g of VII, m.p. 214-219°C, sp. act. 3.28 mCi/mM. From the mother liquid was obtained another 0.324 g of product, sp. act. 3.13 mCi/mM. The two crops of crystals were combined for use in the subsequent step. Radiochemical purity of both crops was established by TLC (solvent system C).

N-(5-Chloro-2-methoxybenzoyl)-2-phenylethylamine-2-^{14}C (VIII)

5-Chloro-2-methoxybenzoic acid (1.567 g, 8.4 mmoles) was treated with 1.362 g (8.4 mmoles) of CDI in 5 ml of dry DMF at room temperature until evolution of CO_2 subsided. The mixture was warmed on steam bath for 15 min and added to 1.095 g (7.0 mmoles) of VII. The resulting mixture was warmed on steam bath for 15 min, diluted with 50 ml of water, and extracted with ether. The extracts were washed with *N* NaOH, *N* HCl and saturated NaCl solution in that order. The ether solution was dried over $MgSO_4$ and concentrated at reduced pressure to give 1.966 g (97% yield) of VIII, a colorless viscous oil which was shown by TLC (solvent system D) to be radiochemically pure.

4-[2-(5-Chloro-2-methoxybenzamido)ethyl-1-^{14}C]benzenesulfonamide (VIb)

A solution of 1.966 g (6.78 mmoles) of VIII in 10 ml of carbon tetrachloride was added with stirring under N_2 to 5 ml of chlorosulfonic acid at -15° to -5°C in 45 min. The mixture was stirred at -10°C for 15 min and then at room temperature for 1 hour. The heavier $ClSO_3H$ layer was cautiously added dropwise to 50 g of crushed ice. The crude sulfonyl chloride was repeatedly washed with ice water, stirred with a mixture of 20 ml conc NH_4OH and 20 ml of dioxane first at 0°C for 15 min, then at room temperature for 1 hour and finally at 95°C for 30 min. The mixture was diluted with water, chilled in ice bath and filtered to give 1.614 g (63.5% yield) of VIb, m.p. 211-213°C, sp. act. 3.27 mCi/mM,

radiochemically pure by TLC (solvent system B).

Glyburide-^{14}C (IXb)

The procedure for the preparation of IXa was followed. From 1.600 g (4.33 mmoles) of VIb was obtained 1.290 g (60.2% yield) of IXb, m.p. 171-173.5°C, sp. act. 3.66 mCi/mM, radiochemically pure by TLC (solvent system B). The IR and UV spectra of the product conformed with those of an authentic sample of IXc.

Anal. Calcd. for $C_{23}H_{28}ClN_3O_5S$ (493.99):

C, 55.92: H, 5.71: Cl, 7.18: N, 8.51; S, 6.49

Found: C, 56.04: H, 6.10; Cl, 7.27: N, 8.45; S, 6.53

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