Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 3

SYNTHESIS OF ADINAZOLAM MESYLATE MULTIPLY LABELED WITH CARBON-13 AND DEUTERIUM

Richard S.P. Hsi and Lindsay S. Stelzer, Drug Metabolism Research, The Upjohn Company, Kalamazoo, MI 49001 U.S.A.

SUMMARY

Adinazolam mesylate labeled with stable isotopes was synthesized for conducting bioavailability studies. Adinazolam labeled uniformly with carbon-13, >99% enrichment, in the unsubstituted phenyl ring was prepared for administration as drug. In addition, adinazolam was also dual-labeled with carbon-13, as in the drug, and with deuterium, six atoms per molecule, in the N-methyl groups. The double-labeled material was prepared for use as an internal standard in the mass spectral assay procedure to be employed in the study.

Keywords: Adinazolam mesylate, benzodiazepine, carbon-13, deuterium, Friedel-Crafts acylation, Girard Reagent "D", N,N-dimethyl-aminoacetyl hydrazine, stable isotope, synthesis

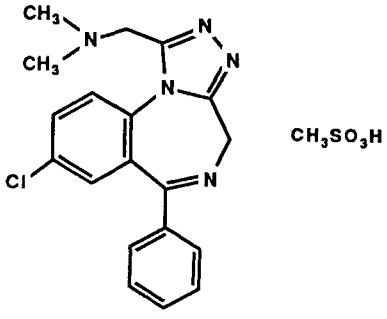
INTRODUCTION

Adinazolam mesylate is a triazolobenzodiazepine which, in addition to possessing anxiolytic activity, exhibits antidepressant activity as well (1-3). Earlier we synthesized radioactive adinazolam labeled with carbon-14 (4) for conducting drug disposition studies. Recently the need arose for stable isotope labeled adinazolam to carry out bioavailability and other pharmacokinetic studies with the drug using mass spectral assay procedures. For best results in such studies, labeled and unlabeled drugs will be administered concurrently, and the labeled drug should preferably produce distinct molecular or fragment mass ions in the mass spectra cleanly removed from those arising from the unlabeled drug. This report describes the synthesis of adinazolam mesylate uniformly labeled with carbon-13 at all six positions, each with 99% isotopic enrichment, of the unsubstituted phenyl ring. This material will produce molecular or fragment ions which are six mass units higher than and cleanly separated from the ion

0362–4803/89/030287–09$05.00

Received April 20, 1988
Revised July 8, 1988

cluster* arising from unlabeled drug, therefore enabling an accurate measurement. We also prepared adinazolam labeled with carbon-13 at these same six positions, as well as with six deuterium atoms per molecule in the N-methyl groups in the "side chain." This material, which is 12 mass units above the unlabeled drug, is intended for use as an internal standard in the assay procedure.



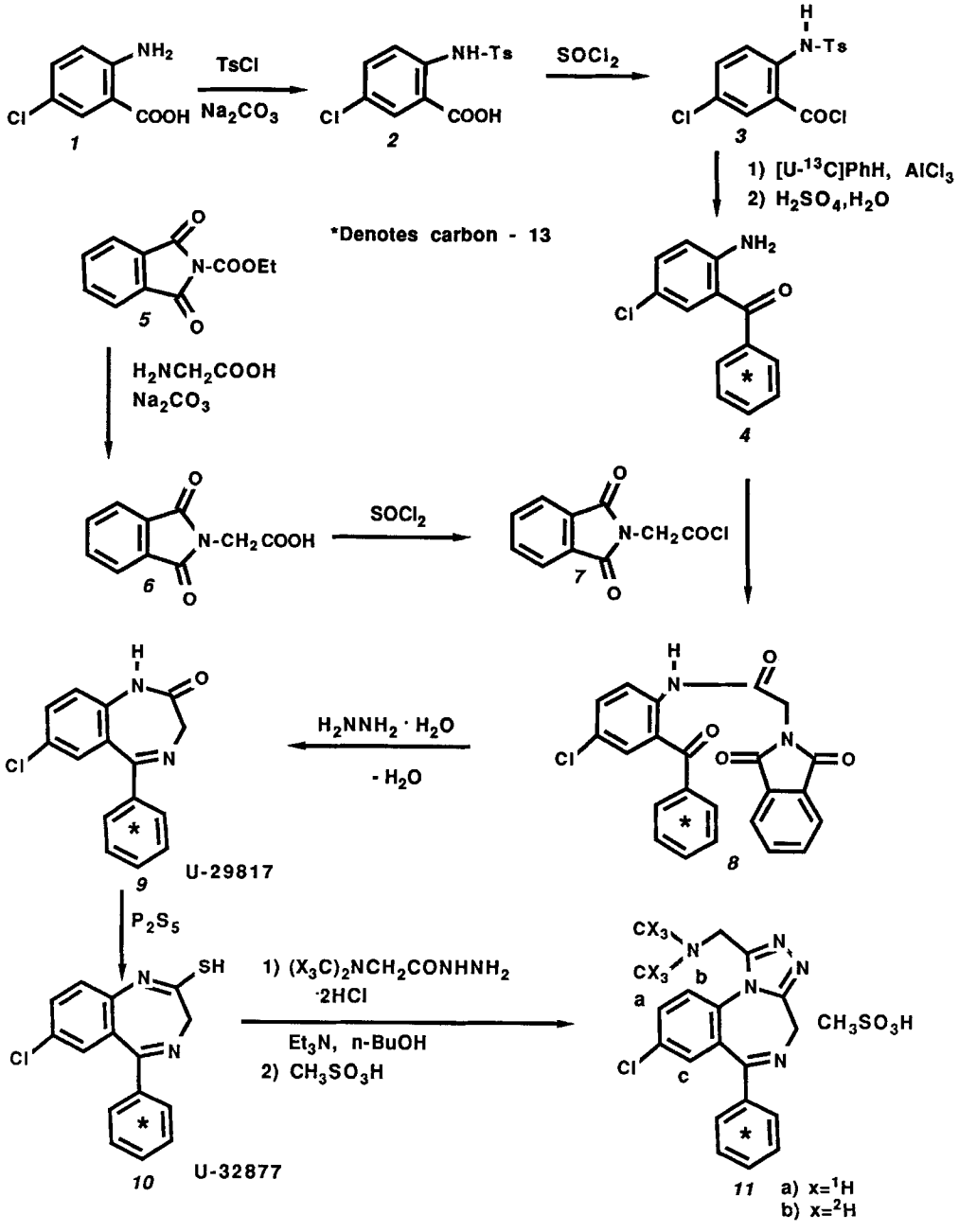
Adinazolam Mesylate

DISCUSSION

The synthesis of carbon-13 and deuterium labeled adinazolam mesylate shown in Scheme 1, follows the same route which was used to synthesize [^{14}C] adinazolam (4). 2-(N-*p*-Toluenesulfonylamino)-5-chlorobenzoic acid (***2***), prepared from 2-amino-5-chlorobenzoic acid (***1***), was converted to 2-(N-*p*-toluenesulfonylamino)-5-chlorobenzoyl chloride (***3***) by refluxing with thionyl chloride. Friedel-Craft acylation (5) of benzene, uniformly labeled with carbon-13, with the acid chloride ***3***, followed by removal of the tosyl protecting group by hydrolysis, afforded [U-^{13}C]phenyl 2-amino-5-chlorophenyl ketone (***4***). Phthaloyl glycine (***6***) was prepared from N-carbethoxyphthalimide [***5***, Nefken's reagent (***6***)] and converted to the corresponding acid chloride ***7***. Acylation of the labeled aminobenzophenone ***4*** with ***7*** gave compound ***8*** which upon removal of the phthaloyl protecting group undergoes cyclization to produce carbon-13 labeled N-demethyldiazepam (***9***). Treatment of ***9*** with phosphorous pentasulfide gave 7-chloro-1,3-dihydro-5-[U-^{13}C]phenyl-2H-1,4-benzodiazepin-2-thione (***10***). Cycloaddition of N,N-dimethyl-acetyl hydrazine to ***10***

*Because of the presence of naturally occurring carbon-13 at all carbon positions as well as other isotopes, e.g., ^{36}Cl, etc., in a molecule, a molecular or fragment ion is usually flanked by a group of ions one or more mass units above and below the mass of the ion of interest.

Scheme 1. Synthesis of Carbon 13 and Deuterium Labeled Adinazolam Mesylate



followed by mesylate salt formation afforded the [$^{13}C_6$]adinazolam mesylate (***11a***) in 16% overall yield based on [$^{13}C_6$]benzene. Similarly, addition to ***10*** of N,N-dimethylaminoacetyl hydrazine (Girard Reagent "D") fully labeled with deuterium in the N-methyl groups led to [$^{13}C_6{}^2H_6$]adinazolam mesylate (***11b***) in 22% overall yield based on

[$^{13}C_6$]benzene, and 87% overall yield based on N,N-di[$^{2}H_3$]methyl-aminoacetyl hydrazine.

EXPERIMENTAL SECTION

Thin-layer chromatographic (TLC) analysis was done on 2.5 x 10 cm glass plates precoated with a 250 μm layer of silica gel GF (Analtech). Developed zones were visualized under UV light (254 nm). Melting points were determined in capillary tubes with a Thomas-Hoover Unimelt and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AM300 spectrometer. Ultra violet spectra were taken on a Perkin-Elmer Lambda 7 instrument. Mass spectral analyses were performed on a Finnigan MAT 8230 spectrometer. Elemental analyses were performed on a Heraeus CHN-O-Rapid Unit.

2-(N-*p*-Toluenesulfonylamino)-5-chlorobenzoic acid (*2*)

A solution of Na_2CO_3 (69.96 g, 660 mmol) in 500 ml H_2O was warmed to 50°C in a 1-liter, 3-neck flask equipped with a reflux condenser, magnetic bar, and thermometer. 2-Amino-5-chlorobenzoic acid (47.2 g, 275 mmol) was dissolved in the mixture after warming to 77°C. After cooling to 70°C, *p*-toluene-sulfonyl chloride (62.91 g, 330 mmol) was added carefully, with evolution of HCl gas and precipitation of solids. A homogeneous mixture was obtained upon warming to 88°C, with more gas evolution. Heating at 88°C was continued for 30 minutes, and the contents cooled to 60°C. Dropwise addition of 150 ml of 6N HCl precipitated an oily mass from the pH 1 solution, which turned into a greenish brown solid. The solids were filtered, washed with 2 x 500 ml H_20 and air dried. Recrystallization from 850 ml hot 95% EtOH and H_20 gave ***2*** as pale yellow crystals, 71 g (79% yield), mp 195-8°C, homogeneous by TLC [5:30:65:1 v/v MeOH:Et_2O:hexane (HX):HOAc, Rf 0.20].

2-(N-*p*-Toluenesulfonylamino)-5-chlorobenzoic acid (*3*)

In a 200-ml flask equipped with a magnetic bar and condenser, ***2*** (15.00 g, 46.04 mmol) was refluxed in 50 ml of thionyl chloride for 6 hours. After cooling, 40 ml of $SOCl_2$ was removed by distillation. Fifty ml of benzene was added to the remaining contents and

an additional 50 ml distilled. Concentration *in vacuuo* gave an oil, which was triturated with 100 ml hexane. The resulting solid was filtered, thoroughly washed with hexane and dried. The yield of off-white crystalline *3* was 14.09 g (89% yield), mp. 137-8°C. A sample of ***3*** heated in H_2O, and extracted into Et_2O gave one spot on TLC (5:30:65:1 v/v MeOH:Et_2O:HX:HOAc, Rf 0.20, identical to starting material ***2***). A sample of ***3*** heated in diethylamine, treated with H_2O and extracted into Et_2O, gave one spot (4:6 v/v EtOAc:CH_2Cl_2, Rf 0.64, diethylamide of ***2***).

2-Amino-5-chlorophenyl [U-^{13}C]Phenyl Ketone (***4***)

Into a 1-liter, 3-neck flask equipped with a reflux condenser and magnetic bar were placed 38.98 g of ***3*** (113.2 mmol), 450 ml of CS_2, and 10 g of [$^{13}C_6$]benzene (118.9 mmol, MSD isotopes, nominally 99.0 atom% ^{13}C, Lot # 713L). All materials were in solution after 20 min of reflux under N_2. After the mixture was cooled to room temperature anhydrous $AlCl_3$ (65.53 g, 491.5 mmol) was added in small portions over a one-hour period with good stirring. The brown reaction mixture was refluxed under N_2 for 1 hour. After cooling, the mixture containing grainy brown precipitates was carefully added to a mixture of 500 ml of crushed ice and 160 ml of 1N HCl with stirring. The resulting mixture was partitioned with 100 ml of CH_2Cl_2, and the aqueous phase extracted with 2 x 100 ml CH_2Cl_2. The combined organic layers were washed with 500 ml of brine and concentrated. The brown, grainy residue was heated in 350 ml of concentrated H_2SO_4 on a steam bath for 15 minutes. After cooling, the black liquid was poured into 2 separate 2-liter beakers, and 250 ml of crushed ice was carefully added to each beaker with stirring, causing the separation of red crystals. After cooling to 30°C, the acidic mixture was basified by dropwise addition with stirring of 575 ml of 12 N NaOH, to each beaker, to afford *4* as yellow crystals. The temperature was maintained at 30-35°C during the neutralization by the periodic addition of small amounts of crushed ice. The resulting solids were filtered, washed with 3 x 500 ml of H_2O, air-dried and dissolved in 300 ml of hot 95% EtOH with stirring. An oily residue which formed upon dropwise addition of 150 ml of H_2O was filtered off, and an additional 250 ml of H_2O was added. The resulting mixture was filtered, and the solids washed with H_2O and dried to give 21.1 g of yellow crystals. An additional 1.10 g of material was recovered from the mother liquor. Recrystallization from 300 ml of hot

95% EtOH and 550 ml of H_2O (brown precipitates removed after addition of 150 ml H_2O) gave ***4*** as bright yellow crystals, 18.10 g. An additional 0.68 g was recovered from the mother liquor and combined with the main crop, 19.78 g total (73.5% yield), corresponds to an authentic sample of 2-amino-5-chlorobenzophenone by TLC (2:7:1 v/v Et_2O:HX:CH_2Cl_2, Rf 0.29, faint impurity at origin).

2-(2-Phthalimidoacetamido)-5-chlorophenyl [U-^{13}C]Phenyl Ketone (***8***)

Phthaloylglycine (***6***) was prepared as reported earlier (4). A stirred solution of glycine (18.83 g, 0.304 mole) in 340 ml of 0.89 M Na_2CO_3 was chilled in an icebath for 15 minutes. The bath was removed and to the solution was added 50.00 g (0.228 mole) of N-carbethoxyphthalimide. The mixture was stirred at room temperature for one hour, filtered and solids washed with 40 ml of cold water. The filtrate and washings were cooled and 127 ml of 6N HCl was added dropwise with stirring. The resulting precipitates were filtered, washed with cold water and dried to give ***6***, 32.4 g (69.3% yield).

Phthaloylglycine (***6***) (17.84 g, 86.93 mmol) was cautiously added to 113 ml of cold thionyl chloride. After the initial evolution of gases had subsided, the mixture was refluxed with stirring for 1 hour. To the resulting clear solution was added 110 ml of toluene and the mixture distilled with simultaneous addition of another 110 ml of toluene until 220 ml of distillated was collected and the boiling point reached 106^{0}C. To the remaining solution of 2-phthalimidoacetyl chloride (***7***) (~86.9 mmol) was added 175 ml of toluene and 18.78 g of ***4*** (79.0 mmol). The solids dissolved rapidly, and the product began precipitating from the red solution after 45 minutes. The mixture was stirred at room temperature overnight. The resulting paste was diluted with 200 ml hexane. The product was filtered, washed with 2 x 200 ml of hexane, and dried to give ***8*** as white crystals, 26.5 g (79% yield), homogeneous by TLC analysis, (2% v/v MeOH in CH_2Cl_2, Rf 0.51), identical to an authentic sample of unlabeled ***8*** (4).

7-Chloro-1,3-dihydro-5-[U-^{13}C]phenyl-2H-1,4-benzodiazepin-2-one (***9***)

Compound ***9*** was prepared according to a modified version of the previously described

method (4). To a solution of ***8*** (26.50 g, 62.38 mmol) in 500 ml of absolute ethanol was added 3.2 ml of hydrazine hydrate (65.5 mmol). The mixture was refluxed with stirring under N_2 for 1 hour, cooled, and an additional 0.3 ml (6.27 mmol) of hydrazine hydrate added. After 21 hours of reflux with stirring under N_2, the mixture was cooled slightly and transferred to a 2-liter Erlenmeyer flask. To the hot mixture was added with stirring 1100 ml of water until the solution became cloudy. The solution was decanted from brown, oily precipitates and the resulting clear solution was allowed to stir overnight. The precipitated solids were filtered, washed with water, and dried to give a first crop of ***9*** as yellow crystals, 8.74 g. The mother liquor was concentrated to remove ethanol, and the aqueous remainder was extracted with 3 x 100 ml of CH_2Cl_2. The combined organic layers were used to dissolve the brown oily precipitates from above and the solution was washed with 175 ml of brine, dried over $MgSO_4$, and concentrated to 8.3 g. Recrystallization of this crude material from 260 ml of CH_2Cl_2 and 520 ml of hexane gave a second crop of ***9*** as yellow crystals, 2.58 g. The concentrated mother liquor was chromatographed on 300 g of silica gel packed in and eluted with 6:4 v/v CH_2Cl_2:EtOAC. After a forerun of 500 ml, 85 fractions of 16 ml each were collected at a flow rate of 4 ml per minute. Fractions 45-80 were pooled, concentrated, and the residue was recrystallized from 43 ml of CH_2Cl_2 and 100 ml of hexane to give 1.61 g of ***9*** as white crystals. The total yield of ***9*** was 12.93 g (75% yield) homogeneous by TLC (6:4 v/v CH_2Cl_2:EtOAc, Rf 0.47), identical to an authentic sample of unlabeled N-demethyldiazepam (***9***).

7-Chloro-1,3-dihydro-5-[U-^{13}C]phenyl-2H-1,4-benzodiazepin-2-thione (***10***)

A suspension of P_2S_5 (11.74 g, 52.83 mmol) in 280 ml of pyridine was stirred under N_2 at 85-90^{0}C for 45 min and a solution of 13.29 g of ***9*** (48.03 mmol) in 127 ml of pyridine was added.The mixture was refluxed with stirring under N_2 for 70 minutes, and cooled to room temperature. To the mixture was added dropwise with stirring 30 ml of water to give precipitates which were filtered, washed with H_2O, and dried The yield of light brown powdered ***10*** was 8.33 g (59%), homogeneous by TLC (5% v/v MeOH in CH_2Cl_2), identical to an authentic sample of the unlabeled thiolactam ***10*** (4).

[$^{13}C_6$]Adinazolam Mesylate (***11a***)

A mixture of 6.52 g of ***10*** (22.20 mmol), 4.43 g of N,N-dimethylglycinehydrazide dihydrochloride (23.3 mmol, American Tokyo Kasei, Inc.) 7.2 ml of triethylamine (51.3 mmol) and 67 ml of n-butanol was refluxed with stirring under N_2 for 6 hours. After cooling, the precipitated triethyamine hydrochloride salt was filtered and washed with 2 x 15 ml of n-butanol. The combined filtrate and washings were concentrated at 50°C and 2 torr. The residue was chromatographed on 300 g of silica gel packed in and eluted with 97:3:0.5 v/v CH_2Cl_2:MeOH:NH_4OH. After a forerun of 400 ml, 200 fractions of 16 ml each were collected at a flow rate of 4 ml per minute. Fractions 93-189 were pooled and concentrated to an oil which crystallized upon trituration with 20 ml of hexane. The white crystals were filtered, washed with hexane and dried, 5.54 g. Fractions 31-93 were rechromatographed on 160 g of silica gel, using the same solvent system. Collected were 70, 20-ml fractions at a flow rate of 5 ml per minute. From fractions 39-60 there was obtained another 1.14 g of white crystalline [^{13}C]adinazolam free base, total 6.68 g (84% yield) homogeneous by TLC (95:7:1 v/v CH_2Cl_2:MeOH:NH_4OH, Rf 0.25), same as an authentic sample of unlabeled adinazolam free base. To a stirred solution of 5.92 g of [^{13}C]adinazolam free base (16.55 mmol) in 115 ml of absolute ethanol was added in one portion with stirring 15.75 ml (15.72 mmol) of 0.998 M methanesulfonic acid in absolute ethanol. There was instant precipitation. After 10 minutes of stirring, the mixture was diluted with 115 ml of Et_2O, and filtered. The collected crystals were washed with Et_2O and dried to give 6.76 g of ***11a*** (90% yield), mp. 241-243.5°C, homogeneous by TLC (95:7:1 v/v CH_2Cl_2:MeOH:NH_4OH, Rf 0.25, [^{13}C]adinazolam free base generated by dissolving ***11a*** in this solvent mixture) identical to an authentic sample of unlabeled adinazolam mesylate treated in the same manner, λ_{max} in EtOH nm (ε):222 (38,360), 245 (15,970); mass spectrum: m/z (rel. intensity), 316 (32.8) M^+-[CH_3SO_3H + $(CH_3)_2N$] + H_2, 314 (100) M^+ - [CH_3SO_3H + $(CH_3)_2N$], 313 (20.0) M^+-[CH_3SO_3H + $(CH_3)_2N$] -H, 315 (19.9) M^+-[CH_3SO_3H + $(CH_3)_2N$] + H, 58 (28.8) $(CH_3)_2N^+ = CH_2$; ^{1}H-NMR δ (D_2O): 2.71 (s, 3H, $C\underline{H}_3SO_3$), 2.82 (s, 6H, N ($C\underline{H}_3)_2$), 4.34 and 5.30 (ab, 2H, J = 13Hz, ring $C\underline{H}_2$), 4.60 and 4.69 (ab, 2H, J = 15Hz, chain $C\underline{H}_2$), 4.82 (s, 1H, $\underline{H}DO$), 7.09-7.42 (m, 5H, [^{13}C]Ar-$\underline{H}$) 7.57 (d, 1H, J_{ac} = 3Hz, H_c), 7.62-7.95 (m, 5H, ^{13}CAr-$\underline{H}$), 7.71 (d, 1H, J_{ab} = 9Hz, H_b), 7.88 (dd, 1H, J_{ab} = 9Hz, Jac = 2Hz, H_a); anal: calcd.

for $^{13}C_6$ C_{14} H_{22} ClN_5O_3S (mol. wt 453.94): C, 54.2; H, 4.89; Cl 7.81; N, 15.4; S, 7.06, found: C, 54.1; H, 4.74; Cl, 7.46; N, 15.5, S, 6.73.

[$^{13}C_6{}^2H_6$] Adinazolam Mesylate Acid Salt (***11b***)

Similarly, from 1.76 g of *10* (6.0 mmol) and 1.24 g of N,N-di[$^2H_3$]methyl-aminoacetyl hydrazine (6.30 mmol, MSD isotopes, nominally 99.8 atom % ^2H, lot 926-L), there was obtained 2.0 g of [$^{13}C_6{}^2H_6$] adinazolam free base, from which 2.39 g of the methanesulfonic acid salt ***11b*** (87.4% yield) was obtained, mp 241-243 .5°C, homogenous by TLC (95:7:1 v/v CH_2Cl_2:MeOH:NH_4OH, Rf 0.25, [$^{13}C_6{}^2H_6$] adinazolam free base generated by dissolving ***11b*** in the eluent solvent mixture); λ_{max} in EtOH nm (ε): 222 (38,270), 245 (16,100); mass spectrum: m/z (rel. intensity), 317 (31.9) M^+-[CH_3SO_3H + $(CD_3)_2N$] + H_2, 316 (19.5) M^+-[CH_3SO_3H + $(CD_3)_2N$] + H, 315 (100) M^+-[CH_3SO_3H + $(CD_3)_2N$], 64 (26.0) $(CD_3)_2 = N^+ = CH_2$; ^1H-NMR δ (D_2O): 2.71 (s, 3H, CH_3SO_3), 4.33 and 5.30 (ab, 2H, J = 13H_z, ring C<u>H</u>$_2$), 4.57 and 4.64 (ab, 2H, J = 15Hz, chain C<u>H</u>$_2$), 4.83 (s, 1H, <u>H</u>DO), 7.12-7.40 (m, 5H, ^{13}CAr-H), 7.58 (d, 1H, J_{ac} = 2Hz, Hc),, 7.65-7.94 (m, 5H, ^{13}CAr-<u>H</u>), 7.72 (d, 1H, J_{ab} = 9Hz, H_b), 7.88 (dd, 1H, J_{ab} = 9Hz, J_{ac} = 2Hz, H_a); anal: calcd. for $^{13}C_6$ C_{14} $^2H_6$ H_{16} Cl N_5O_3 S (mol wt 459.95): C, 53.5; H, 6.13; Cl, 7.70; N, 15.2; S, 6.97, Found: C, 53.8; H, 6.25; Cl, 7.65; N, 15.3; S, 6.95.

Acknowledgement

We gratefully acknowledge the interpretation of NMR spectra in this report by S.A. Mizsak and D.A. Kloosterman. Assistance in the preparation of this manuscript by D.J. de Castro is much appreciated.

REFERENCES

1. Hester J.B.Jr., Rudzik A.D., and VonVoigtlander P.F.-J. Med. Chem., 23:392 (1980).
2. Hester J.B.Jr.-J. Heterocycl. Chem., 17:575 (1980).
3. Hester J.B.Jr.-J. Org. Chem., 44:4165 (1979).
4. Hsi R.S.P. and Johnson T.D.-J. Labelled Compds. and Radiopharm., 12:613 (1976).
5. Scheifele H.J.Jr. and De Tar D.F.-Org. Syn., Coll., Vol.4:34 (1963), N. Rabjohn Editor-in-Chief, John Wiley and Sons, New York.
6. Nefkens G.H.L.-Nature, 4709:309 (1960).