SYNTHESIS OF ^{14}C-LABELED AND STABLE ISOTOPE-LABELED CGS 16617

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SUMMARY

The synthesis of a ^{14}C-labeled and two stable isotope-labeled analogs of CGS 16617 is described. The synthetic method involved the preparation of tetrahydro-3--bromo-1-benzazepin-2-one, labeled with a ^{14}C or four deuterium atoms, followed by introduction of two side chains at 1- and 3-positions. The labeled bromobenzazepinones were prepared by Beckmann rearrangement of bromooximes of α-tetralones, obtained by cyclization of labeled benzenebutanoic acids. The ^{14}C-labeled acid was prepared by hydrolysis of the nitrile, prepared by reaction of 3-bromopropylbenzene and K^{14}CN. The tetradeutero acid was prepared from ethyl phenylpropynoate by catalytic reduction of the triple bond with deuterium gas, followed by reduction of the deuterated ester with lithium aluminum hydride and conversion of the resulting alcohol into the carboxylic acid. The acetic acid side chain was introduced by N-alkylation with ethyl bromoacetate or ethyl bromoacetate-1,2-^{13}C$_2$ followed by hydrolysis, and the L-lysine side chain, by reaction with L-(-)-3-amino-ε-caprolactam followed by hydrolysis of the caprolactam ring.

Key Words: [^{14}C]CGS 16617, 1,3,4,5-tetrahydro-3-bromo--2H-1-benzazepine-2-oxo-2-^{14}C, CGS 16617-d$_4$

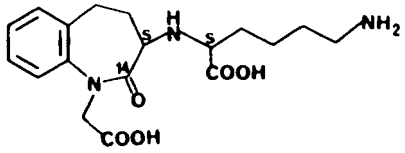
INTRODUCTION

Recent introduction of captopril and enalapril as therapeutic agents for the treatment of hypertension and congestive heart failure has led to the synthesis of a number of compounds which, like captopril and enalapril, inhibit the angiotensin-converting enzyme. 3-[(5-Amino-1-carboxy-1S-pentyl)-amino]-2-oxo-3S-1H-1-benzazepine-1-acetic acid (CGS 16617) is one such compound, synthesized in our Research Department (1). We have now synthesized ^{14}C-labeled CGS 16617 (1) for preclinical metabolism and pharmacokinetic studies. We have also synthesized two stable isotope-labeled analogs 2 and 3.

0362–4803/88/070739–10$05.00

Received August 31, 1987
Revised November 16, 1987

Compound 2, a tetradeutero analog, was needed for clinical studies, and compound 3, labeled with four deuterium and two ^{13}C atoms, for use as a reference material for analysis of 2 in biological fluids by GC-MS. Details of these syntheses are described in this paper.



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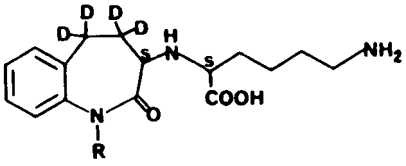
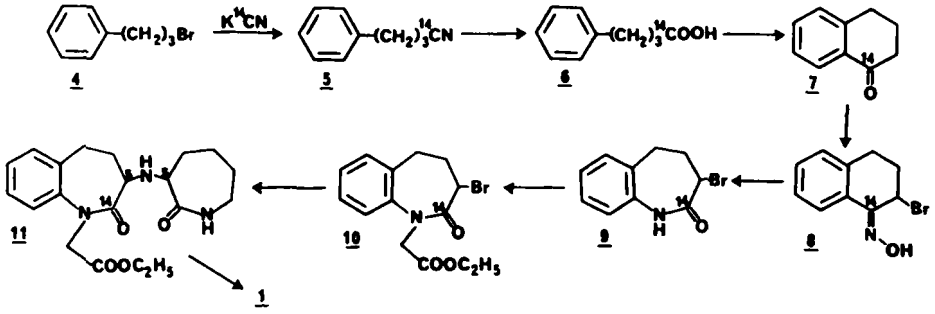


2, R = $-CH_2COOH$
3, R = $-^{13}CH_2^{13}COOH$

Since CGS 16617 is a derivative of 1-benzazepin-2-one with an acetic acid chain at 1-position and a L-lysine chain at 3-position, our strategy for the synthesis of these labeled compounds was first to synthesize the benzazepinone moiety labeled with the required isotopes, and then to introduce the side chains. For the synthesis of 3 however, the acetic acid side chain was also labeled with two ^{13}C atoms.

The synthesis of 1 (Scheme 1) by this approach required ^{14}C-labeled tetrahydro-3-bromo-1-benzazepin-2-one 9. It was synthesized in five steps starting with 3-bromopropylbenzene and $K^{14}CN$ following our previously

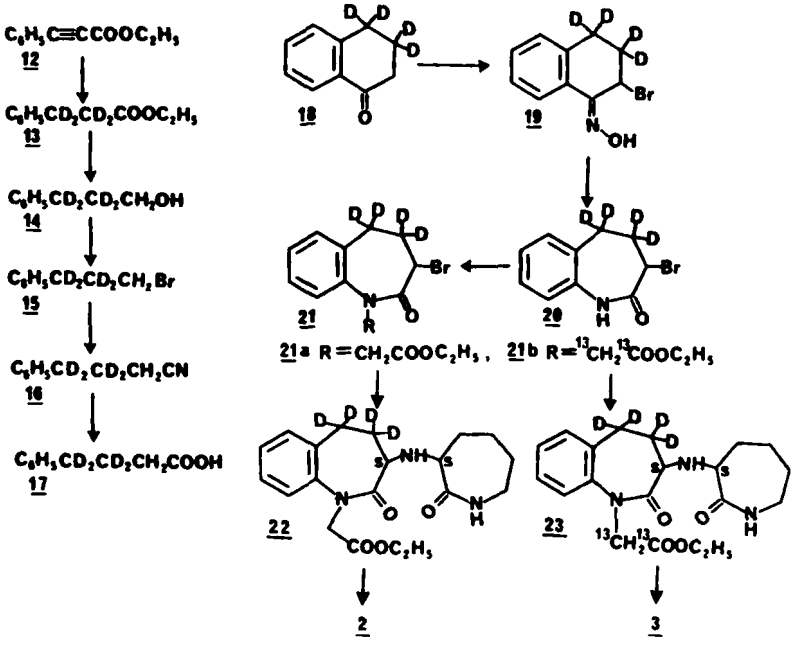
Scheme 1



published procedure (2). The acetic ester side chain was then introduced by N-alkylation of 8 with ethyl bromoacetate in the presence of potassium carbonate. The resulting bromoester 10 was then condensed with L-(-)-3-amino-ε-caprolactam, and the condensation product, which was a mixture of RS and SS

diastereoisomers, was crystallized from methanol for the separation of isomers. Compound 11, which crystallized out, had the optical rotation of the required SS-isomer. On heating with hydrochloric acid, the caprolactam ring and the acetic ester group of 11 were hydrolyzed to give ^{14}C-labeled CGS 16617 (1), identical with unlabeled CGS 16617 having the SS configuration.

The synthesis of the stable isotope-labeled compounds 2 and 3 (Scheme 2) by the above method required the synthesis of 4,4,5,5-tetradeutero-3-bromo-1-benzazepin-2-one (20). This was accomplished in eight steps starting with ethyl 3-phenyl-2-propynoate (12). Catalytic reduction of 12 with deuterium gave the tetradeutero compound 13. Similar reduction of 3-phenyl-2-propynoic acid, however, yielded a (2:3) mixture of tri and tetradeuterated compounds. The ester group of 13 was then reduced with lithium aluminum hydride, and the resulting alcohol 14 was converted to the carboxylic acid 17 via the bromide 15 and the nitrile 16. Cyclization of 17 by heating with polyphosphoric acid furnished 18, the tetraduetero analog of α-tetralone. Bromination of 18 followed by reaction in situ with hydroxylamine produced the bromo oxime 19, which on heating with polyphosphoric acid underwent Beckmann rearrangement to give the ring



Scheme 2

expansion product 20. The bromo lactam 20 was then N-alkylated with ethyl bromoacetate for the introduction of the acetic ester chain at 1-position.

Alkylation of 20 with ordinary ethyl bromoacetate gave 21a, and alkylation with ethyl bromoacetate-1,2-$^{13}C_2$ gave 21b. The L-lysine side chain was then introduced at 3-position by the condensation of 21a and 21b with L-(-)-3-amino-ϵ-caprolactam, followed by isomer separation by the procedures described above for the ^{14}C-compound. The condensation products from 21a and 21b, upon crystallization from methanol, gave compounds 22 and 23 respectively having the required optical rotations. Acid hydrolysis of 22 and 23 gave compounds 2 and 3 respectively.

EXPERIMENTAL

All melting points were taken on a Kofler hot stage apparatus. Thin layer chromatography (TLC) was performed on 0.25 mm silica gel 60F-254 plates. Potassium cyanide-^{14}C was purchased from Du Pont NEN Products, ethyl bromoacetate-1,2-$^{13}C_2$ from Aldrich Chemical Co. and deuterium gas (99.5%) from Matheson.

3-Bromo-1,3,4,5-tetrahydro-2H-1-benzazepine-2-oxo-2-^{14}C (9). This was prepared by the published procedure (2). Starting with 400 mCi of potassium cyanide-^{14}C, about 168 mCi of 9 with a specific activity of 7.61 mCi/mmol was obtained; yield, 42% based on $K^{14}CN$.

Ethyl 3-bromo-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-2-^{14}C-1-acetate (10). To 1.3 g (40 mCi) of the above material, dissolved in 25 ml of dry acetone, was added with stirring 1.1 g of anhydrous potassium carbonate followed by 0.65 ml of ethyl bromoacetate. Stirring was continued overnight at room temperature. The mixture was filtered and washed with acetone. The filtrate and the washings were evaporated to give 1.77 g of an oil; TLC in ethyl acetate/petroleum ether (2/8, v/v) showed one major radioactive spot which was different from that of the starting material.

Ethyl 3-[(hexahydro-2-oxo-3S-1H-azepin-3-yl)amino]-2,3,4,5-tetrahydro-2-oxo-3S-1H-1-benzazepine-2-^{14}C-1-acetate (11). A mixture of the above oil and 3.5 g of L-(-)-3-amino-ϵ-caprolactam in 35 ml of acetonitrile was

heated under reflux in a nitrogen atmosphere for 72 hr. The mixture was then cooled, filtered, and washed with acetonitrile. The filtrate was evaporated to remove acetonitrile, and the residue was dissolved in dichloromethane. The solution was then washed with water, dried with $MgSO_4$, and evaporated to dryness. The residue was dissolved in 25 ml of methanol by heating under reflux, and the solution was allowed to crystallize by stirring at room temperature for 16 hr. The crystallized solid was filtered, and 500 mg of unlabeled compound 11 was added to the filtrate which was then stirred overnight at room temperature. The crystallized material was filtered and combined with the crystals obtained before. The combined solid was dissolved in acetonitrile and chromatographed on a column of silica gel using acetonitrile, and acetonitrile/methanol (95/5, v/v) as eluents. The acetonitrile eluate gave a small amount of an impurity which was discarded. The acetonitrile/methanol eluate was evaporated, and the residue triturated with methanol to give a solid; yield, 864 mg (8.4 mCi); m.p. 143 - 145°C; $[\alpha]_D^{25} = -201°$ (1% in $CHCl_3$).

3-[(5-Amino-1-carboxy-1S-pentyl)amino]-2,3,4,5-tetrahydro-2-oxo-3S-1H-1-benzazepine-2-^{14}C-1-acetic acid (1) ([^{14}C]CGS 16617). The above solid (864 mg) was heated with 16 ml of 6N HCl at 95°C for 40 hr. The solution was then evaporated to dryness under high vacuum and the residue was dissolved in water. The acidic solution was neutralized (pH 6.5) and evaporated under high vacuum. The residue was stirred with a 1:1 mixture of methanol and dichloromethane for 1 hr and filtered. The filtrate was evaporated to give an oily residue which was crystallized from a mixture of 7 ml of ethanol and 1.5 ml of water. The crystallized material was filtered, washed with cold ethanol, and dried at 60°C under vacuum for 24 hr; m.p. 210 - 212°C; $[\alpha]_D^{25} = -179°$ (1% in water); yield, 385 mg. The specific activity was 10.5 μCi/mg and the total radioactivity was 4.04 mCi. The radiochemical yield was approximately 10% from 10 and 4.2% from K^{14}CN. The m.p. and specific rotation of the labeled material were identical to those of unlabeled CGS 16617.

Ethyl benzenepropanoate-α,α,β,β-d_4 (13). To a solution of 75 g of ethyl 3-phenyl-2-propynoate in 350 ml of ethyl acetate was added 3 g of 10% palladium-on-charcoal. The mixture was then shaken overnight in an atmo-

sphere of deuterium gas in a Paar shaking machine. Catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was distilled (b.p. 120 - 123°C/16 mm) to yield 75 g of an oil; mass spectrum (CI): m/z 183 and 182 (M^++ 1 ions); the percentage of the d_4 compound, as assayed by corrected mass peak intensities, was 95.

Benzenepropanol-β,β,γ,γ-d_4 (14). To 350 ml of 1M lithium aluminum hydride solution in ether, was added dropwise 40 g of the above compound 13 under gentle reflux. The mixture was then heated under reflux for 8 hr. After cooling to room temperature, the mixture was decomposed by careful addition of 13.3 ml of water, 13.3 ml of 15% NaOH and 40 ml of water. The mixture was filtered and the filtrate, after drying with $MgSO_4$, was distilled (b.p. 118 - 120°/16 mm) to yield 30 g of an oil. The reaction was repeated with 35 g of 13 to yield 25.5 g. IR showed the presence of a OH band and absence of a carbonyl band; mass spectrum (EI): m/z 140 (M^+), 121, 122, 93 and 94; ^1H NMR ($CDCl_3$): 3.9 (s, 2H, CH_2) and 7.4 - 7.6 (m, 5H, aromatic) ppm.

3-Bromopropyl-1,1,2,2-d_4-benzene (15). To a stirred solution of 55.5 g of the above alcohol 14 in 30 ml of chloroform, was added dropwise 16 ml of phosphorus tribromide. The mixture was heated overnight under reflux, cooled, and poured onto ice with stirring. The chloroform layer was separated from the aqueous layer which was extracted once with chloroform. The combined chloroform solution was washed with sodium carbonate solution and then dried with $MgSO_4$. The dried solution was evaporated to remove chloroform, and the residue was distilled (b.p. 105 - 110°/16 mm) to yield 76 g of an oil; mass spectrum (EI): m/z 202 and 204 (M^+), 123 (M^+-Br) and 93. IR spectrum showed the absence of a OH peak.

Benzenebutanenitrile-β,β,γ,γ-d_4 (16). To a solution of 76 g of the above compound 15 in 500 ml of ethanol was added a solution of 33 g of potassium cyanide in 200 ml of water. The mixture was heated overnight under reflux, and then evaporated under reduced pressure in a rotary evaporator. The residue was extracted with ether and the ether extract, after drying with $MgSO_4$, was evaporated to yield 50 g of an oil.

Benzenebutanoic-β,β,γ,γ-d_4 acid (17). A mixture of 50 g of the above nitrile

16, 112 ml of acetic acid, 105 ml of water and 33.5 ml of sulfuric acid was heated under reflux until the hydrolysis was complete (30 hr) by TLC. The mixture was diluted with water and extracted with ether. The ether extract was washed with water and saturated sodium chloride solution. After drying with $MgSO_4$, the ether solution was evaporated, and traces of acetic acid were removed by distillation under reduced pressure to yield 56.6 g of a white solid upon cooling; m.p. 48 - 50°C [cf. m.p. of the corresponding hydrogen compound, 51 - 52°C (3)].

3,4-Dihydro-1(2H)-naphthalen-3,3,4,4-d_4-one (18). To 150 g of polyphosphoric acid heated to 85°C, was added 29.5 g of the above acid 17 with hand stirring. The mixture was kept at 85 - 90°C for 15 min, and then poured onto ice. The aqueous solution was extracted with ether, and the ether extract was washed with 1N sodium hydroxide solution. After drying with $MgSO_4$, the ether solution was evaporated to dryness to yield 26.2 g of an oil. The reaction was repeated with 29.1 g of 17 to obtain 26 g more of the oil. TLC of the oil in ethyl acetate/petroleum ether (1/2, v/v) was identical to that of α-tetralone (Aldrich). A small quantity of the oil was distilled for analysis; b.p. 96-98°C/3mm; mass spectrum (CI): m/z 151 (M^++1); ^1H NMR ($CDCl_3$): 2.6 (s, 2H, CH_2), 7.2 (m, 2H, aromatic) 7.4 (m, 1H, aromatic) and 8.0 (d, 1H, aromatic) ppm. Anal. Calcd. for $C_{10}H_6D_4O$: C, 80.0; Found C, 79.83.

2-Bromo-3,4-dihydro-N-hydroxy-1(2H)-naphthalen-3,3,4,4-d_4-imine (19). To a solution of 52 g of compound 18 in 450 ml of methanol was added dropwise 18 ml of bromine with stirring. The mixture was stirred at room temperature for 1.5 hr, and 63 g of hydroxylamine hydrochloride was added. Stirring was continued for 4 days at room temperature. The reaction flask was kept covered with aluminum foil during this time to protect against exposure to light. The mixture was then evaporated to dryness and diluted with 400 ml of water. After stirring for 1 hr at room temperature, the mixture was extracted with ethyl acetate. The extract was washed with saturated sodium chloride solution, dried with $MgSO_4$, and evaporated to give a yellow solid. The solid was triturated with ether/petroleum ether (1/1, v/v) and filtered. The filtrate, which was

shown by TLC to contain unreacted 18, was evaporated and the residue was recycled to give some more solid. The yield of the combined solid (19) was 64.7 g (77% yield); m.p. 128-130°C; mass spectrum (CI): m/z 244 and 246 (M^++1 ions), 226 and 228 (M^+-OH) and 164 (M^+-Br); ^{1}H NMR ($CDCl_3$): 7.15 - 7.95 (m, 5H, 4 aromatic and OH) and 5.75 (s, 1H, CHBr) ppm.

3-Bromo-1,3,4,5-tetrahydro-2H-1-benzazepin-4,4,5,5-d_4-2-one (20). To 350 g of polyphosphoric acid heated to 90°C, was added the above solid (64.7 g) in small portions, maintaining the reaction temperature at 95 - 100°C. The mixture was heated at this temperature for 15 min longer, cooled to 60°C, and ice chips were added to it with hand-stirring. The aqueous mixture was extracted with chloroform, and the extract was washed with sodium carbonate solution, dried with $MgSO_4$, and evaporated. The residue was triturated with ether and filtered. The filtrate, which was found to contain unreacted 19 by TLC, was evaporated and the residue was recycled once more to yield some more product. The yield of the combined solid (20) was 34.5 g, m.p. 169 - 173°C [cf. m.p. of the hydrogen compound, 170 - 174°C (2)]; mass spectrum (CI); m/z 244 and 246 (M^++1 ions), 226 and 228 (M^+-OH) and 164 (M^+-Br); ^{1}H NMR ($CDCl_3$): 4.55 (s, 1H, CHBr), 7.0 - 7.3 (m, 4H, aromatic) and 7.9 (s, 1H, NH) ppm.

Ethyl 3-bromo-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-4,4,5,5-d_4-1-acetate (21). To a stirred mixture of 26 g of anhydrous potassium carbonate in 380 ml of acetone was added 30 g of compound 20, followed by 12 ml of ethyl bromoacetate. Stirring was continued at room temperature until the reaction was found to be complete by TLC (48 hr). The mixture was filtered and the filtrate evaporated under reduced pressure. The residue, on trituration with petroleum ether, yielded 35 g of 21a as a solid; m.p. 121 - 125°C [cf. m.p. of the hydrogen compound 120 - 123°C (2)]; mass spectrum (CI): m/z 330 and 332 (M^++1 ions) and 250 (M^+-Br); ^{1}H NMR ($CDCl_3$): 1.25 (t, 3H, CH_3), 4.20 (m, 2H, ester CH_2), 4.35 and 4.7 (d, 2H, N-CH_2), 4.50 (s, 1H, CHBr) and 7.10 - 7.30 (m, 4H, aromatic) ppm.

3-[(5-Amino-1-carboxy-1S-pentyl)amino]-2,3,4,5-tetrahydro-2-oxo-3S-1H-1-benzazepine-4,4,5,5-d_4-1-acetic acid (2). A mixture of 35 g of compound 21a, 57.6 g of L-(-)-3-amino-ϵ-caprolactam in 700 ml of acetonitrile was heated under

reflux in a nitrogen atmosphere for 84 hr. The mixture was cooled and filtered. The filtrate was evaporated under reduced pressure, and the residue dissolved in dichloromethane. The dichloromethane solution was washed with water, dried with $MgSO_4$ and evaporated. The residue was dissolved in 300 ml of methanol by heating, and the solution was cooled at -5°C. After 24 hr, the crystallized solid was filtered, washed with cold methanol and dried to yield 15.7 g compound 22; m.p. 143 - 147°C; $[\alpha]_D^{25}$ = -203° (1% in $CHCl_3$), [m.p. and specific rotation of the hydrogen compound, 145 - 147°C and -201° respectively (2)]; mass spectrum (EI): m/z 377 (M^+), 305 (M^+-72), 265 (M^+-caprolactam ring), 251, 237, 222 and 192. ^{1}H NMR ($CDCl_3$): 1.25 (t, 3H, CH_3), 1.3 - 2.2 (m, 8H, $3CH_2$ and 2 NH), 3.0 (m, 2H, CH_2NH), 3.30 (t, 3H, CH_3), 3.40 (s, 1H, $CO.CH.CD_2$), 4.20 (m, 2H, ester CH_2), 4.2 and 4.7 (d, 2H, N-CH_2) and 7.0 - 7.3 (m, 4H, aromatic). The solid was mixed with 270 ml of 6N HCl and heated at 95°C for 40 hr. It was then worked up, following the procedure described above for the preparation of the ^{14}C-compound, to yield 8.5 g of compound 2 as a white, hygroscopic solid; m.p. 212 - 215°C; $[\alpha]_D^{25}$ = -177° (1% in water). [m.p. and specific rotation of the hydrogen compound are 210 - 212°C, and -179° respectively); ^{1}H NMR ($CDCl_3$): 1.50 (m, 2H, CH_2), 1.70 (m, 2H, CH_2), 1.90 (m, 2H, CH_2), 3.0 (t, 2H, $\underline{CH}_2NH_2$), 3.58 (t, 1H, $\underline{CH}$-COOH), 3.90 (s, 1H, $\underline{CH}$-NH), 4.2 and 4.5 (d, 2H, $\underline{CH}_2$.COOH), and 7.20 - 7.40 (m, 4H, aromatic); mass spectrum (CI): m/z 368 and 367 (M^++1). The percentage of the compound with 3D was 6.3 and of that with 4D was 93.5, as assayed by corrected mass peak intensities. Anal. Calculated for $C_{18}H_{21}D_4N_3O_5.H_2O$: C, 56.10; N, 10.91. Found: C, 55.86; N, 10.66.

3-[(5-Amino-1-carboxy-1S-pentyl)amino]-2,3,4,5-tetrahydro-2-oxo-3S-1H-benzazepine-4,4,5,5-d_4-1-acetic acid-1,2-^{13}C (3). Compound 20 (3 g) was reacted with 2 g of ethyl bromoacetate-1,2-^{13}C following the above procedure to yield 3.9 g of 21b, m.p. 120 - 124°C; mass spectrum (CI): m/z 332 and 334 (M^++1 ions) and 252 (M^+-Br). Compound 21b (3.9 g) was then condensed with 7.6 g of L-(-)-3-amino-ε-caprolactam, and the condensation product was crystallized from methanol to yield 1.78 g of 23; m.p. 143 - 147°C, mass spectrum (CI): m/z 380 (M^++1), $[\alpha]_D^{25}$ = -201.7° (1% in $CHCl_3$). The crystalline material was

hydrolyzed with HCl to yield 650 mg of 3 as a crystalline solid; m.p. 216 - 219°C; $[\alpha]_D^{25}$ = -163° (1% in water); ^{13}C NMR ($CDCl_3$): 56 (d, J_{cc} = 60 Hz, $^{13}CH_2$) and 178 (d, J_{cc} = 60 Hz, $^{13}CO_2C_2H_5$) ppm; mass spectrum (CI): m/z 369 and 370 (M^++1 ions). The percentage of the species with 4D + $2^{13}C$ was 93.9 and of that with 3D + $2^{13}C$ was 5.6, as assayed by corrected mass peak intensities.

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