Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVIII, No. 9

SYNTHESIS OF 2,2-DIMETHYL-$^{13}C_2$-PROPIONITRILE-1-^{13}C AND ITS CONVERSION TO TRIPLY CARBON-13 LABELED DOWCO 429 INSECTICIDE

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SUMMARY

A convenient synthesis of triply carbon-13 labeled 2,2-dimethyl-$^{13}C_2$-propionitrile-1-^{13}C is described. The synthetic sequence begins with commercially available propionic acid-1-^{13}C and methyl iodide-^{13}C with the application of a sequential dianion alkylation. The 2,2-dimethyl-$^{13}C_2$-propionitrile-1-^{13}C was carried on to triply labeled DOWCO 429 insecticide for use in residue and metabolism studies.

Key Words: Triply carbon-13 labeled 2,2-dimethyl-$^{13}C_2$-propionitrile-1-^{13}C, triply carbon-13 labeled trimethyl^2 acetonitrile, DOWCO 429 insecticide, carboxylic acid dianion alkylation.

INTRODUCTION

Residue and metabolism studies on the phosphorothioate insecticide DOWCO 429 (O,O-Diethyl-O-(1,1-dimethylethyl)-5-pyrimidinyl-phosphorothioate) instigated a synthetic study directed at carbon-13 labeling of the molecule. The carbon-13 labeled sample will be used as an internal standard for gas chromatography/mass spectrometry (GC/MS) determinations and also in stable isotope metabolism studies. This communication describes a synthetic route to DOWCO 429 which incorporates three carbon-13 labels.

DISCUSSION

The requirements for the carbon-13 labeled DOWCO 429 were: 1) It contains at least three carbon-13 atoms per molecule at 99+% enrichment and 2) the position of the carbon-13 atoms be in the aromatic ring and/or in the alkyl side chain.

The synthesis of DOWCO 429 has been thoroughly investigated in our laboratories[1a,b]. Utilizing trimethylacetonitrile as the starting material, a five step synthesis can be carried out in high yield. Carbon-13 labeled trimethylacetonitrile is unavailable commercially, thus we began to

0362–4803/90/091073–08$05.00

Received February 14, 1990
Revised March 23, 1990

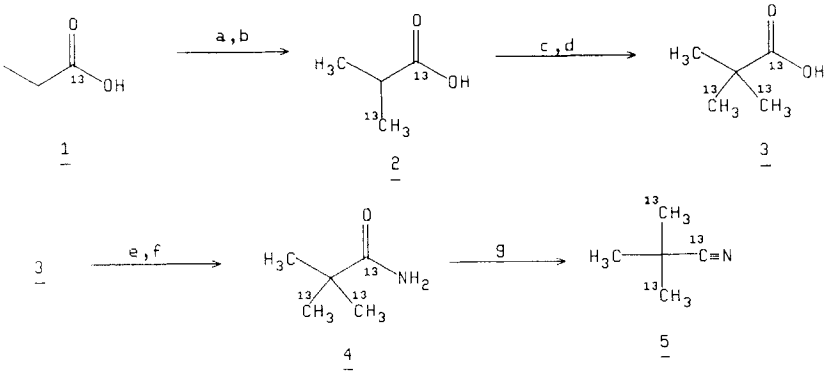
investigate the synthesis starting with inexpensive and readily available carbon-13 precursors.

A 1967 communication by Creger[2a] describes the dianion alkylation of propionic acid under mild reaction conditions. A similar synthetic study revealed that α-substituted carboxylic acids also undergo dianion formation and alkylation[2b,c]. Prompted by these results, we set out to investigate the sequential dianion alkylation of propionic acid using methyl iodide as the electrophile. Our choice of propionic acid as the starting material was dictated by the fact that propionic acid-1-^{13}C and methyl iodide-^{13}C are both commercially available at relatively low cost.

Upon manipulation of several experimental parameters[3], it was discovered that 2-methylpropionic acid-1,3-^{13}C (2) could be prepared in high yield from propionic acid-1-^{13}C (1, Scheme I).

A second dianion alkylation starting with 2 provided 2,2-(dimethyl-$^{13}C_2$) propionic acid-1-^{13}C (3) of sufficient purity for use in the next step. Conversion of 3 to 5 was effected by treatment with thionyl chloride followed by reaction of the acid chloride with aqueous ammonia in methylene chloride to provide 2,2-(dimethyl-$^{13}C_2$)propionamide (4). Dehydration of 4 was accomplished using solid phosphorous pentoxide with removal of 2,2-dimethyl-$^{13}C_2$ propionitrile-1-^{13}C (5) via distillation.

Scheme I



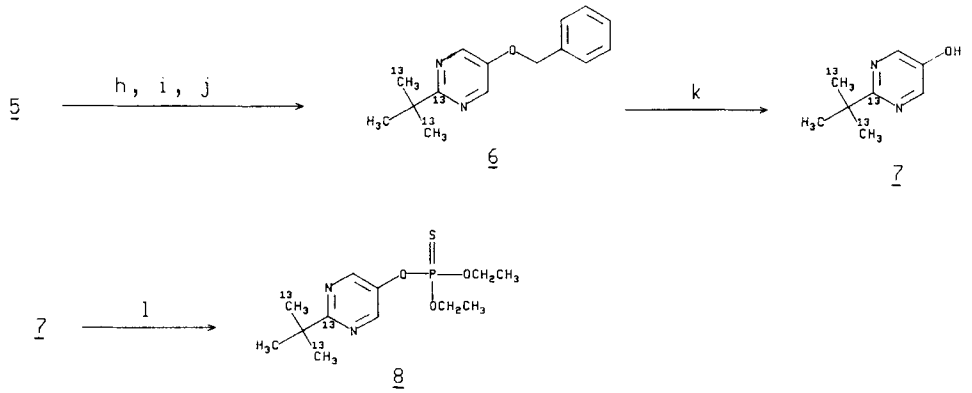
1 2 3 4 5

a) lithium diisopropylamide, THF, hexamethylphosphoramide; b) $^{13}CH_3I$;
c) lithium diisopropylamide, THF; d) $^{13}CH_3I$; e) $SOCl_2$; f) NH_3, H_2O,CH_2Cl_2;
g) P_2O_5

With the triple labeled 5 in hand, conversion to the desired labeled DOWCO 429 was carried out using a reaction sequence that had been developed previously in our laboratories[1a,b] (Scheme II). Treatment of 5 with dry hydrogen

chloride in ethanol/toluene provided the imino ester. It has been observed that much higher yields of imino ester are achieved when the reaction is conducted under sealed conditions in the presence of excess HCl than under atmospheric conditions[1b]. Reaction of the imino ester with ammonia in methanol gave the corresponding amidine. Reaction of the amidine with the three carbon bis-electrophilic reagent N-(3-(dimethylamino)-2-benzyloxy-2-propylidene)-N-methylmethaminium perchlorate [Me_2N^+=CHC(OBn)=CHNMe$_2$ C^-lO_4] provided pyrimidine 6 in excellent yield. Hydrogenolysis of the benzyl group in 6 followed by reaction of the pyrimidinol with diethyl-chlorothiophosphate afforded the triply labeled DOWCO 429 (8).

Scheme II



h) HCl, EtOH, toluene; i) NH_3, CH_3OH; j) N-(3-(dimethylamino)-2-benzyloxy-2-propylidene)-N-methyl-methaminium perchlorate; k) cyclohexene, $Pd(OH)_2/C$, EtOH; l) diethylchlorothiophosphate, CH_3CN, K_2CO_3

EXPERIMENTAL

Thin layer chromatography (TLC) was routinely used to monitor reactions and to check purities. TLC was conducted using 2.5 x 10 cm Analtech silica gel GF (UV 254) plates. ^1H NMR spectra were recorded using a Varian EM-390 (90 MHZ) instrument using the solvent noted and tetramethylsilane (TMS) as an internal standard. Chemical shifts are recorded in parts per million (delta scale) downfield from TMS. Significant ^1H NMR data is tabulated as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant in Hertz and assignment. Mass spectra were obtained using a Finnigan 4615 instrument operated in the positive ion mode. All mass spectra were obtained at 70 eV ionization potential using a direct exposure probe or GC inlet. Melting points were

determined using a Thomas Hoover capillary melting point apparatus and are uncorrected. Gas chromatographic analysis was conducted using a Hewlett Packard 5890A instrument containing a J&W Scientific DB-5 (15m x 0.32 mm, 0.25 μm film) column. Analyses were performed as follows: Temp 1 = 100°C, Time 1 = 2 min, rate = 20°C/min, Temp 2 = 280°C, Time 2 = 5 min at 2 mL/min He flow using an FID detector. All reactions were magnetically stirred unless noted.

2-Methylpropionic acid-1,3-^{13}C (2)

To a stirred, cooled (0°C) solution of diisopropylamine (10.3 mL, 73 mmol) in THF (40 mL) under N_2 was added dropwise butyllithium (45 mL of 1.6M in hexane, 72 mmol). Upon completion of the addition, the mixture was stirred at 0°C for one hour. Propionic acid-1-^{13}C (2.5 mL, 34.7 mmol, 99% isotopic purity) was added dropwise resulting in a white precipitate. Hexamethylphosphoramide (45 mL) was added and the mixture was stirred at 0°C for 5 minutes, then warmed to room temperature and stirred for 0.5 hours. The resulting yellow homogeneous solution was cooled to 0°C and $^{13}CH_3I$ (2.2 mL, 35 mmol, 99% isotopic purity) was added. The mixture was warmed slowly to room temperature and allowed to stir for 1.5 hours. The resulting mixture was poured into ice cold 10% HCl (150 mL) and extracted with Et_2O (1 x 200 mL, 2 x 75 mL). The combined organic extracts were washed with cold 10% HCl/brine (3 x 150 mL of a 1:1 solution), then brine (2 x 150 mL). The mixture was dried ($MgSO_4$), and filtered. The solvents were removed on the rotary evaporator (35 mm Hg, room temperature bath) to provide a dark amber liquid. The liquid was filtered through silica gel (5g, 230-400 mesh) using Et_2O (250 mL) as the eluent. The solvent was removed on the rotary evaporator to provide a dark amber liquid (≈ 3.5g). The silica gel filtration was repeated and the solvent was removed on the rotary evaporator with additional drying at 35 mmHg (room temperature) for 20 minutes to provide the product as a dark amber liquid (2.77 g, 88%). The liquid was dissolved in Et_2O (8 mL) and washed with a 1:1 solution of saturated sodium bisulfite/brine (8 mL). The Et_2O phase was filtered through layers of Na_2SO_4, SiO_2, and $MgSO_4$. The solvent was removed on the rotary evaporator to provide a yellow liquid (2.24 g). ^{1}H NMR (90MHz, $CDCl_3$) 1.2 (d,d,d,J = 126 Hz, 10Hz and 5Hz, 3H, $^{13}C\underline{H}_3$), 1.2 (dt, J = 7Hz and 4Hz, 3H, $^{12}C\underline{H}_3$), 2.55 (m, 1H, C$\underline{H}$), 11.4 (bs, 1H, OH).

2,2-(Dimethyl-$^{13}C_2$)-propionic acid-1-^{13}C (3)

n-Butyllithium (32 mL of 1.6M in hexane, 51 mmol) was added dropwise to a solution of diisopropylamine (7.4 mL, 53 mmol) in THF (25 mL) at 0°C under N_2. The resultant mixture was stirred at 0°C for 15 minutes. The double labeled propionic acid (2) (2.22 g, 24.6 mmol) in THF (5 mL) was added and the mixture stirred at 0°C for 5 minutes. The mixture was allowed to warm to room temperature and then heated at 45-50°C for 2 hours. The mixture was cooled

to 0°C and $^{13}CH_3I$ (1.64 mL, 26 mmol) was added. The mixture was stirred at 0°C for 5 minutes, then warmed to room temperature and stirred for 1.5 hours. The mixture was poured into ice cold 10% HCl (125 mL) and extracted with Et_2O (3 x 75 mL). The combined organic extracts were washed with brine (2 x 100 mL), dried ($MgSO_4$) and filtered. The solvent was removed on the rotary evaporator to leave a dark amber liquid. The liquid was filtered through silica gel (5 g, 230-400 mesh) using Et_2O (250 mL) as the eluent. The solvent was removed on the rotary evaporator to provide a light amber liquid. The liquid was filtered into a tared flask using Et_2O (5 x 2 mL) and the solvent was removed on the rotary evaporator. The product was dried at 35 mm Hg (room temperature) for 0.5 hours to yield a light amber liquid (2.3 g, 89%). ^1H NMR (90MHz, $CDCl_3$) 1.25 (dt, J = 127 Hz and 4Hz, 6H, 2 x $^{13}C\underline{H}_3$), 1.25 (q, J = 5Hz, 3H, $^{12}C\underline{H}_3$), 11.1 (bs, 1H, O<u>H</u>).

2,2-(Dimethyl-$^{13}C_2$)propionamide-1-^{13}C (4)

Thionyl chloride (1.75 mL, 24 mmol) was slowly added to the triple-^{13}C labeled 3 (2.25 g, 21.4 mmol) at 0°C under N_2. The mixture was stirred at 0°C for 5 minutes, warmed to room temperature, and stirred for 45 minutes. The mixture was warmed to 55°C and stirred under a $CaSO_4$ drying tube for 15 hours. The resulting brown liquid was dissolved in CH_2Cl_2 (15 mL) and added dropwise over 15 minutes to a stirred, cooled (0°) mixture of CH_2Cl_2 (15 mL) and concentrated NH_4OH (15 mL). The resultant mixture was stirred at 0°C for 15 minutes, warmed to room temperature, and stirred for 3 hours. The CH_2Cl_2 layer was removed, dried ($MgSO_4$), and filtered. The aqueous layer was evaporated to dryness on the rotary evaporator (35 mm Hg, 60°C) followed by additional drying at 0.5 mm Hg (room temperature) for 0.5 hours to leave a white solid. The solid was boiled with ethyl acetate (3 x 50 mL) and each of the extracts filtered hot. The ethyl acetate filtrates were combined with the previously dried CH_2Cl_2 extract and the solvents were removed on the rotary evaporator with final drying at 0.5 mm Hg (room temperature) for 20 minutes to leave a light brown solid (1.94g). The solid was dissolved in refluxing ethyl acetate (14 mL), and hexane (4 mL) added. The mixture was cooled slowly to room temperature and filtered. The precipitate was washed with hexane and dried at 0.5 mm Hg (room temperature) for 0.5 hours affording the product as white platelets (1.6 g, 15.4 mmol, 72%): m.p. 150-151°C, lit. m.p. 154-157°C[4]; MS (*M/e*) 104 (M^+, 6%), 88 (6.1%), 59 (100%).

2,2-(Dimethyl-$^{13}C_2$) propionitrile-1-^{13}C (5)

The amide (4) was ground by mortar and pestle and transferred to a flask. P_2O_5 (2.8 g, 19.7 mmol) was added and the solids were mixed well. The flask was fitted with a short path distillation column and the mixture was heated at

180°C - 210°C. The receiving flask was cooled with dry ice/acetone. Vacuum (35 mm Hg) was periodically applied at the distillation head. The reaction was complete in approximately 15 minutes. The product was collected as a yellow liquid distillate (1.07 g, 83%): ^{1}H NMR (90MHz, $CDCl_3$) 1.35 (dt, J = 128 Hz and 4Hz, 6H, 2 x $^{13}C\underline{H}_3$), 1.35 (q, J = 5 Hz, 3H, $^{12}C\underline{H}_3$).

(1,1-Dimethyl-$^{13}C_2$-ethyl)-5-benzyloxypyrimidine-2-^{13}C (6)

The nitrile (5, 1.0 g, 11.6 mmol) was transferred to an ampule with toluene (3 x 2 mL). EtOH (820 μL, 14 mmol) was added followed by additional toluene (2 mL). The mixture was cooled to -78°C (dry ice/CH_2Cl_2) and anhydrous HCl gas was sparged in for 7 minutes. The ampule was sealed and allowed to warm slowly to room temperature. The ampule was heated at 50°C and stirred for 14.5 hours. The ampule was cooled to -78°C, opened, and allowed to stir slowly to room temperature. The contents of the ampule were transferred to a 250 mL round bottom flask with toluene (4 x 2 ml). The solvent was removed on the rotary evaporator (35 mm Hg, 40°C water bath) to leave an off-white solid. The solid was dissolved in CH_3OH (20 mL) and anhydrous gaseous ammonia was sparged into the solution for 10 minutes. The mixture was stirred an additional 3 hours at room temperature. The volatiles were removed on the rotary evaporator (35 mm Hg, room temperature) with final drying at 0.5 mm Hg (room temperature) for 30 minutes to provide a pale yellow solid. To the dry solid was added CH_3CN (30 mL), K_2CO_3 (8.0 g, 58 mmol), and N-(3-(dimethylamino)-2-benzyloxy-2- propylidene)-N-methylmethaminium perchlorate[5] (4.1 g, 12.3 mmol). The mixture was stirred at reflux under N_2 for 3 hours, cooled to room temperature, and stirred for an additional 15 hours. The solvent was removed on the rotary evaporator leaving a white-yellow solid. The solid was partitioned between H_2O (60mL) and Et_2O (50 mL). The aqueous phase was extracted twice with Et_2O (2 x 50 mL). The combined Et_2O extracts were dried ($MgSO_4$) and filtered through silica gel (50 g, 230-400 mesh) and the silica gel column washed with Et_2O (150 mL). The solvent was removed on the rotary evaporator to leave a yellow oil. The oil was dissolved in Et_2O and filtered through layers of Na_2SO_4 and $MgSO_4$. The solvent was removed on the rotary evaporator with final drying at 0.5 mm Hg to provide a yellow oil. The oil was flash chromatographed on silica gel (100g, 230-400 mesh, slurry packed in 10:1 hexane/ethyl acetate) using hexane/ethyl acetate (10:1) to load the oil onto the column (3 x 3 mL) and as the eluent. The pure fractions (as determined by TLC) were combined and the solvents were removed on the rotary evaporator affording a colorless oil. The oil was dissolved in Et_2O and filtered into a tared flask. The solvent was removed on the rotary evaporator to provide the product as a clear colorless oil (2.2 g, 77%): ^{1}H NMR (90 MHz, $CDCl_3$) 1.35 (dt, J = 125Hz and 4Hz, 6H, 2 x $^{13}C\underline{H}_3$), 1.35 (q, J = 5Hz, 3H, $^{12}C\underline{H}_3$), 5.1 (s, 2H, $OC\underline{H}_2Ph$), 7.35 (s, 5H, Ph$\underline{H}$'s), 8.35 (d, J = 10Hz, 2H, pyrimidine $\underline{H}$'s).

(1,1-Dimethyl-$^{13}C_2$-ethyl)-5-pyrimidinol-2-^{13}C (7)

The benzyloxypyrimidine (6, 2.2g, 8.97 mmol) was combined with EtOH (12 mL), cyclohexene (1.9 mL, 18.1 mmol), and $Pd(OH)_2/C$ (300 mg, 20% Pd). The mixture was heated under N_2 at reflux for 10 minutes, cooled to room temperature, and additional $Pd(OH)_2/C$ (200 mg) was added. The mixture was heated at reflux for 0.5 hours. The mixture was cooled to room temperature and filtered through celite (5g). The filter was rinsed with EtOH (50 mL). The solvent was removed on the rotary evaporator to provide a very pale yellow solid. The solid was dissolved in CH_2Cl_2/ethyl acetate (5 x 5 mL) and filtered through silica gel (50 g, 230-400 mesh) which was eluted with CH_2Cl_2/ethyl acetate (200 mL). The solvent was removed on the rotary evaporator to leave a white solid. The solid was dissolved in CH_2Cl_2 (25 mL) and filtered into a tared flask. The solvent was removed by a stream of N_2 and the residue dried at 0.5 mm Hg (room temperature) to provide the pyrimidinol (7) as a white crystalline solid (1.3 g, 93%): m.p. 131-132°C, lit. m.p. 127-129°C[6]; ^1H NMR (90 MHz, $CDCl_3$), 1.4 (dt, J = 125 Hz and 4Hz, 6H, 2 x $^{13}C\underline{H}_3$), 1.4 (q, J = 5Hz, 3H, $^{12}C\underline{H}_3$), 8.25 (d, J = 10Hz, 2H, Pyrimidine H's), 9.3 (bs, 1H, O<u>H</u>); capillary gas chromatography (one peak, retention time = 4.542 minutes, 99 area %); TLC (2:1 hexane/ethyl acetate (5% acetic acid), one spot, Rf = 0.55); reverse phase HPLC (one peak, retention time = 14.593 min, retention time of unlabeled standard = 14.426 min); mass spectrometry/gas chromatography (70 eV, electron impact) 155 (M+, 58.3%), 140 (57.1), 139 (100%).

O,O-Diethyl-O-(1,1-dimethyl-$^{13}C_2$-ethyl)-5-pyrimidinyl phosphorothioate-2-^{13}C (8)

The labeled pyrimidinol (32 mg, 0.206 mmol) was dissolved in CH_3CN (2 mL) and K_2CO_3 (170 mg, 0.22 mmol) was added. Diethyl chlorothiophosphate (35 μL, 0.22 mmol) was added and the mixture was stirred under N_2 at 50°C for 2.5 hours and at 90°C for 1.5 hours. The CH_3CN was removed on the rotary evaporator leaving a residue which was partitioned between CH_2Cl_2 (6 mL) and H_2O (6 mL). The aqueous phase was extracted with CH_2Cl_2 (6 mL). The combined organic extracts were filtered through layers of Na_2SO_4 and $MgSO_4$. The solvent was removed from the filtrate on the rotary evaporator to leave a clear colorless oil. The oil was dissolved in CH_2Cl_2 and flash chromatographed on a column of silica gel (30 g, 230-400 mesh, slurry packed with 15:1 hexane/ethyl acetate) using 15:1 hexane/ethyl acetate as the eluent. The pure fractions (as determined by TLC) were combined and the solvents were removed on the rotary evaporator with final drying at 0.5 mm Hg (room temperature) for 1 hour to provide the product as a clear colorless oil (57 mg, 90%): TLC (15:1 hexane/ethyl acetate, one spot, Rf = 0.308); GLC (one peak, retention time = 7.592 minutes, retention time of standard = 7.637 minutes); MS (70eV, DEP) 307

(M+, 98.5%), 292 (34.6%), 291 (46.9%), 264 (100%), 263 (43.4%), 155 (22.1%), 139 (33.9%), 97 (80.2%).

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