

Syntheses of [2-¹³C] and [1,2-¹³C] Labelled α-Ketoglutaric Acid

Jack E. Baldwin,* Robert M. Adlington, Mark A. Russell, Christopher J. Schofield and Mark E. Wood.

The Dyson Perrins Laboratory, South Parks Road,
Oxford, OX1 3QY.

SUMMARY

The syntheses of α-keto[2-¹³C]glutaric acid and α-keto[1,2-¹³C]glutaric acid were each achieved from commercial ¹³CH₃I in 5 steps. ¹³CH₃NO₂, prepared *in situ* from ¹³CH₃I and AgNO₂, was treated with magnesium methyl carbonate, followed by acidified methanol to give O₂N¹³CH₂CO₂CH₃. Michael addition to methyl acrylate, followed by *in situ* nitronate formation and ozonolysis gave the dimethyl ester of α-keto [2-¹³C]glutaric acid, which was deprotected with hydrochloric acid. O₂N¹³CH₂¹³CO₂CH₃ was prepared from ¹³CH₃NO₂ by reaction with aqueous KOH, followed by treatment with acidified methanol, and was converted to 1,2-¹³C-α-ketoglutaric acid in a similar fashion to that used for mono-labelled material.

Keywords: α-keto[2-¹³C]glutaric acid, α-keto[1,2-¹³C]glutaric acid, α-keto acid, oxygenase, [¹³C]nitromethane.

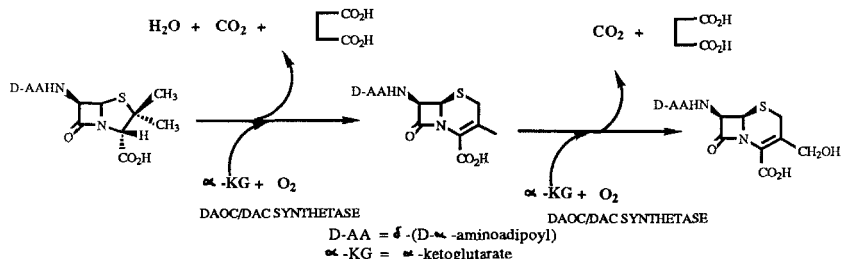
INTRODUCTION

α-Ketoglutaric acid is a co-substrate in many reactions catalysed by non haem iron-dependent oxygenases.^{1a} Currently we are investigating deacetoxy/deacetyl-cephalosporin C synthetase from Cephalosporium acremonium which catalyses the biosynthesis of cephalosporin from penicillin N (Scheme 1).^{1b} In order to investigate the role of α-ketoglutarate in these biotransformations we wanted to synthesise and incubate [2-¹³C]- and [1,2-¹³C]-α-ketoglutarate with deacetoxy/deacetyl cephalosporin C synthetase. In this publication we describe short syntheses of these labelled compounds.²

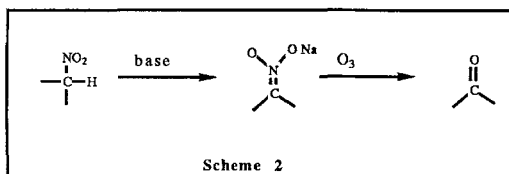
The key step in our strategy was the ozonolysis of a nitronate, generated by the treatment of a precursor nitro group with base as has

been reported by McMurry *et al.*³ (Scheme 2). Thus $^{13}\text{CH}_3\text{NO}_2$ (1) was prepared,⁴ and used *in situ*, from $^{13}\text{CH}_3\text{I}$ ⁵ (silver nitrite, diethyl ether). Reaction of (1) with an excess of magnesium methyl carbonate (4 equiv.)⁶ gave a magnesium chelate which was treated with acidified methanol to yield the desired methyl nitroacetate (2) [17% from methyl iodide (2.0 g)]. Michael addition of (2) to methyl acrylate proceeded smoothly to give dimethyl α -nitroglutarate (3) (79%).⁷ Reaction of (3) with NaOMe generated the nitronate (4), which was ozonolysed³ *in situ*, to give after work up dimethyl α -keto[2- ^{13}C]glutarate (5). Deprotection (hydrochloric acid, reflux) gave the desired [2- ^{13}C] α -ketoglutaric acid [5% from $^{13}\text{CH}_3\text{I}$]. The only problematic steps in this sequence were the preparation and reaction of $^{13}\text{CH}_3\text{NO}_2$ and the low yield [for (1) \rightarrow (2)] obtained may reflect the small scale on which the reaction was carried out.

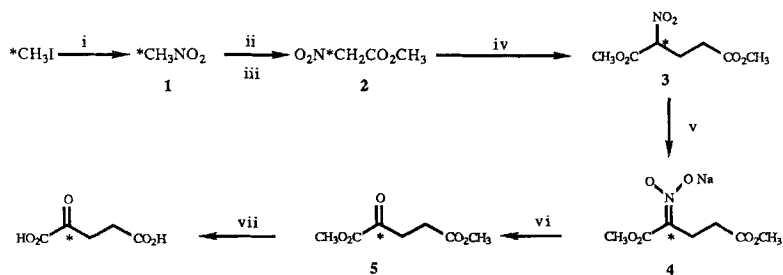
The synthesis of α -keto[1,2- ^{13}C]glutaric acid was achieved from methylnitro[1,2- ^{13}C]acetate which was prepared using the classical method from nitromethane.⁸ Thus reaction of $^{13}\text{CH}_3\text{NO}_2$ (1) with aqueous KOH (reflux) gave the di-potassium salt of nitro[1,2- ^{13}C]acetic acid (6) which was treated with acidified methanol to give methyl ester (7) [49% from $^{13}\text{CH}_3\text{I}$ (2.3 g)]. Conversion to α -keto[1,2- ^{13}C]glutarate was achieved in an analogous manner to that used for the monolabelled material [13 % from $^{13}\text{CH}_3\text{I}$]. (Scheme 4).



Scheme 1

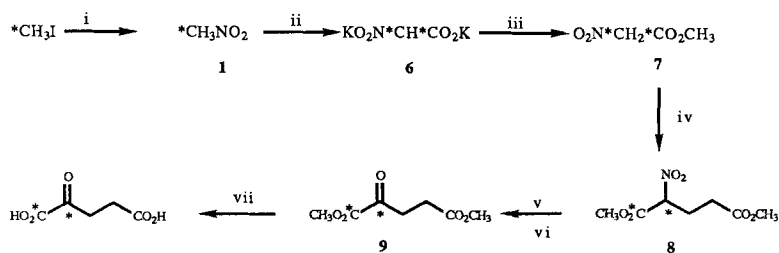


Scheme 2



Scheme 3

Reagents (i) $\text{AgNO}_2/\text{Et}_2\text{O}$, (ii) $\text{CH}_3\text{OCO}_2\text{MgOCH}_3 \cdot x\text{CO}_2/\text{HCONMe}_2$, (iii) $\text{CH}_3\text{OH}/\text{HCl}$,
 (iv) $\text{PhCH}_2(\text{Me})_2\text{NOH}/\text{CH}_2=\text{CHCO}_2\text{CH}_3/\text{dioxan}/\text{CH}_3\text{OH}$, (v) $\text{NaOCH}_3/\text{CH}_3\text{OH}$, (vi) O_3 , (vii) $\text{HCl}/\text{H}_2\text{O}$



Scheme 4

Reagents (i) $\text{AgNO}_2/\text{Et}_2\text{O}$, (ii) $\text{KOH}/\text{H}_2\text{O}$, (iii) $\text{CH}_3\text{OH}/\text{H}_2\text{SO}_4$, (iv) $\text{PhCH}_2(\text{Me})_2\text{NOH}/\text{CH}_2=\text{CHCO}_2\text{CH}_3/\text{dioxan}/\text{CH}_3\text{OH}$, (v) $\text{NaOCH}_3/\text{CH}_3\text{OH}$, (vi) O_3 , (vii) $\text{HCl}/\text{H}_2\text{O}$

* indicates position of ^{13}C atoms

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage or Buchi 510 apparatus and are quoted uncorrected. Infra red spectra were recorded on Perkin-Elmer 681 or Perkin-Elmer 1750-FT IR machines (s strong, m medium, w weak). Proton n.m.r. spectra were recorded on either Bruker WH 300 or AM 500 machines operating at 300 and 500 MHz respectively. Carbon-13 n.m.r. spectra were recorded on either Bruker AM 250 or AM 500 machines operating at 62.9 and 125.8 MHz respectively. Mass spectra were recorded on a V.G. Micromass 30 F or VG 20-250. Thin layer chromatography was carried out on Merck DC-Alufolien Kieselgel 60 F_{254} 0.2 mm pre-coated plates with spot detection by iodine vapour, quenching of u.v. fluorescence or 10% ammonium molybdate in 1M sulphuric acid. Flash chromatography was carried out on Merck Kieselgel 60. Solvents were removed by rotary film evaporation under reduced pressure. All reactions were carried out at room temperature unless otherwise stated. Reaction times are recorded in hours (h) and minutes (min.). Magnesium methyl

carbonate was prepared by the method of Finkbeiner et al.⁶ Standard synthetic procedure as previously reported was employed.⁹

[¹³C] NITROMETHANE (1)

[¹³C]Methyl iodide (Aldrich 99.5 atom % ¹³C, 2.00 g, 14 mmol) was added dropwise to a vigorously stirred suspension of silver nitrite (3.02 g, 19.6 mmol) in diethyl ether (8 ml) at 0°C. The flask was sealed and protected from light and stirring was continued at 0°C for 10 h and at room temperature for 14 h. The crude product was used directly without isolation.

METHYL NITRO[2-¹³C]ACETATE (2)

The ethereal solution of crude [¹³C]nitromethane was filtered through a celite plug into a stirred 2.1 M solution of magnesium methyl carbonate in N,N-dimethylformamide (30 ml). After stirring for 10 min. the temperature was increased to 50°C, and the diethyl ether was removed by distillation. After 6 h at 50°C, the mixture was cooled to room temperature and added dropwise to vigorously stirred anhydrous methanol (250 ml) containing acetyl chloride (40 ml, 0.56 mol) at -40° to -50°C. The reaction mixture was allowed to warm to room temperature and stirring was continued for 15 h. The mixture was poured into diethyl ether (400 ml) and the resulting solution was extracted with water (5 x 150 ml), dried (magnesium sulphate), filtered and evaporated to give (2) as a yellow, mobile oil (292 mg, 17% from methyl iodide) (c.f. 58% from unlabelled nitroacetate, see ref. 6); R_f (CH₂Cl₂) 0.55; $\gamma_{\max}/\text{cm}^{-1}$ (liquid film) 3030(m), 2970(m), 1760(s), 1565(s), 1442(s), 1406(s), 1385(s), 1332(s), 1275(s), 1225(s), 1010(m), 780(m), 685(s); δ_H (300 MHz, CDCl₃) 3.86 (3H, s, CH₃O₂C-), 5.18 (2H, d, $J_{\text{HC}} = 149$ Hz, -¹³CH₂-); δ_C (62.9 MHz, CDCl₃ broad band decoupled) 53.6 (OCH₃), 76.1 (O₂N¹³CH₂-); m/e (direct chemical ionisation [NH₃]) 138 (100 %, M + NH₄⁺), 137 (2%) 45 (24%).

METHYL NITRO[1,2- ^{13}C]ACETATE (7)

An ethereal solution of [^{13}C] nitromethane was prepared (vide supra) using [^{13}C]methyl iodide (Aldrich 99.5 atom % ^{13}C , 2.28 g, 16 mmol) and silver nitrite (3.02 g, 19.6 mmol). This solution was filtered through a celite plug into a stirred solution of potassium hydroxide (3.75 g, 67 mmol) in water (1.7 ml). Stirring was continued for 10 min. and the temperature was raised to 50°C to distil off the diethyl ether. Stirring was stopped and the reaction mixture was heated under reflux (oil bath temperature 160°C) for 70 min. After cooling to room temperature, the buff-coloured crystalline solid produced was filtered off, washed with anhydrous methanol and dried in vacuo. The solid was suspended in stirred methanol (10 ml) at -30°C and concentrated sulphuric acid (1.3 ml) was added dropwise. The reaction mixture was allowed to slowly attain room temperature with stirring at this temperature being continued for 20 h. The white solid produced was filtered off, the methanol evaporated and the residue was partitioned between dichloromethane (40 ml) and water (20 ml). The separated aqueous phase was further extracted with dichloromethane (3 x 30 ml) and the combined organic extracts were dried (magnesium sulphate), filtered and evaporated to give (7) as an orange, mobile oil (414 mg, 49% from methyl iodide); R_f (CH_2Cl_2) 0.55; $\gamma_{\text{max}}/\text{cm}^{-1}$ (liquid film) 3020(w), 2960(w), 1720(s), 1565(s), 1440(m), 1405(w), 1385(m), 1322(s), 1255(m), 1215(s) 1178(s), 1010(w), 780(m), 685(m); δ_{H} (300 MHz, CDCl_3) 3.88 (3H, d, $J = 4$ Hz, $\text{CH}_3\text{O}_2^{13}\text{C}-$), 5.19 (2H, dd, $J_{\text{HC}} = 7$, 149 Hz, $^{-13}\text{CH}_2-$); δ_{C} (125.8 MHz, CDCl_3 , broad band decoupled) 53.7 (OCH_3), 76.1 ($\text{O}_2\text{N}^{13}\text{CH}_2-$), 162.2 ($^{13}\text{CO}_2\text{CH}_3$); m/e (direct chemical ionisation [NH_3]) 139 (100 %, $\text{M}+\text{NH}_4^+$), 45 (60%).

DIMETHYL α -NITRO[2- ^{13}C]GLUTARATE (3)

To a stirred solution of methyl nitro[2- ^{13}C]acetate (2) (249 mg, 2.1 mmol) in 1,4-dioxan (2 ml) containing a 40 % solution of benzyl-trimethylammonium hydroxide in methanol (60 μl) at 60-70°C was added dropwise, methyl acrylate (185 μl , 2.1 mmol). After 15 h at 60-70°C,

dichloromethane (5 ml) was added to the cooled solution which was then vigorously stirred with 2M hydrochloric acid (8 ml) for 5 min. More dichloromethane (20 ml) was added and the separated organic phase was washed with water (2 x 20 ml), dried (magnesium sulphate), filtered and evaporated to give an orange oil which was purified by flash chromatography on silica gel (150 ml silica, 3 cm diameter column, eluting with dichloromethane) to give (3) as a pale yellow oil (336 mg, 79%); R_f (CH_2Cl_2) 0.5; $\gamma_{\text{max}}/\text{cm}^{-1}$ (liquid film); 2960(m), 1750(s), 1560(s), 1440(s), 1105(w), 1020(w), 985(w), 830(w); δ_{H} (300 MHz, CDCl_3); 2.38 - 2.55 (4H, m, $-\text{CH}_2\text{CH}_2-$), 3.71 (3H, s, $\text{CH}_3\text{O}_2\text{CCH}_2-$), 3.85 (3H, s, $\text{CH}_3\text{O}_2\text{C}-^{13}\text{C}$), 5.04 - 5.14 and 5.53 - 5.65 (1H, 2 x m $^{13}\text{CH}-\text{NO}_2$); δ_{C} (62.9 MHz, CDCl_3 , broad band decoupled) 86.5 ($^{-13}\text{CHNO}_2$)-enriched signal only; m/e (direct chemical ionisation [NH_3]) 224 (100 %, $\text{M}+\text{NH}_4^+$), 223 (1%), 162 (25%).

DIMETHYL α -NITRO[1,2- ^{13}C]GLUTARATE (8)

The procedure described above was repeated using the following quantities of reagents; methyl nitro[1,2- ^{13}C]acetate (7) (551 mg, 4.6 mmol), 40 % benzyltrimethylammonium hydroxide in methanol (140 μl) and methyl acrylate (410 μl , 4.6 mmol). The crude product was purified by flash chromatography on silica gel (250 ml silica, 3 cm diameter column, eluting with dichloromethane) to give (8) as a pale yellow oil (528 mg, 56%); R_f (CH_2Cl_2) 0.50; $\gamma_{\text{max}}/\text{cm}^{-1}$ (liquid film) 2960(m), 1740(s), 1710(s), 1560(s), 1440(w), 1370(m), 1260(m), 1205(s), 1175(m); δ_{H} (300 MHz, CDCl_3) 2.38 - 2.66 (4H, m, $-\text{CH}_2\text{CH}_2-$), 3.71 (3H, s, $\text{CH}_3\text{O}_2\text{CCH}_2-$), 3.85 (3H, d, $J_{\text{HC}}=4$ Hz, $\text{CH}_3\text{O}_2^{13}\text{C}-$), 5.04 - 5.14 and 5.53 - 5.65 (1H, 2 x m, $^{13}\text{CH}-\text{NO}_2$); δ_{C} (125.8 MHz, CDCl_3 , broad band decoupled) 86.5 ($^{13}\text{CH}-\text{NO}_2$), 164.6 ($^{-13}\text{CO}_2\text{CH}_3$)-enriched signals only; m/e (direct chemical ionisation [NH_3]) 225 (100 %, $\text{M}+\text{NH}_4^+$), 224 (1%) 163 (55%).

DIMETHYL α -KETO[2- ^{13}C]GLUTARATE (5)

Sodium (31 mg, 1.3 mmol) was added to anhydrous methanol (14 ml) at

0°C. A solution of α -nitro[2- ^{13}C]glutaric acid dimethyl ester (3) (262 mg, 1.3 mmol) in anhydrous methanol (15 ml) was added and stirring at 0°C was continued for 10 min. The stirred mixture was then cooled to -78°C and ozone was passed through the solution for 75 min. After purging the solution with oxygen for 20 min, the methanol was evaporated in vacuo to give a pale yellow oil containing a white solid. This residue was partitioned between dichloromethane (30 ml) and water (30 ml) and the separated aqueous layer was further extracted with dichloromethane (3 x 30 ml), the combined organic extracts being dried (magnesium sulphate), filtered and evaporated to give a pale yellow oil which was purified by flash chromatography on silica gel (100 ml silica, 3 cm diameter column, eluting with dichloromethane). This gave (5) as a pale yellow oil (153 mg, 69 %); R_F (CH_2Cl_2) 0.2; $\gamma_{\text{max}}/\text{cm}^{-1}$ (liquid film) 2960(m), 1740(s), 1697(s), 1440(s), 1260(s), 1205(s), 1175(m), 1075(m), 1038(m); δ_{H} (300 MHz, CDCl_3) 2.63 - 2.73 (2H, $\text{CH}_2\text{CO}_2\text{CH}_3$), 3.13 - 3.20 (2H, m, $^{13}\text{CO}-\text{CH}_2-$), 3.68 (3H, s, $\text{CH}_3\text{O}_2\text{CCH}_2-$), 3.87 (3H, s, $\text{CH}_3\text{O}_2\text{C}-^{13}\text{CO}$); δ_{C} (62.9 MHz, CDCl_3 , broad band decoupled) 192.3 ($^{13}\text{CO}-$)-enriched signal only; m/e (chemical ionisation [NH_3]) 193 (100%, $\text{M}+\text{NH}_4^+$), 133 (50%), 116 (38%), 101 (40%).

DIMETHYL α -KETO[1,2- ^{13}C]GLUTARATE (9)

The procedure described above was repeated using the following quantities of reagents; sodium (54 mg, 2.4 mmol) and nitro[1,2- ^{13}O] α -glutaric acid di-methyl ester (8) (481 mg, 2.3 mmol). The crude product was purified by flash chromatography on silica gel (100 ml silica, 3 cm diameter column, eluting with dichloromethane) to give (9) as a pale yellow oil (316 mg, 77%); R_F (CH_2Cl_2) 0.2; $\gamma_{\text{max}}/\text{cm}^{-1}$ (liquid film) 2960(m), 1740(s), 1692(s), 1440(m), 1245(s), 1075(m), 1038(m); δ_{H} (300 MHz, CDCl_3) 2.63 - 2.70 (2H, $\text{CH}_2\text{CO}_2\text{CH}_3$), 3.11 - 3.21 (2H, m, $^{13}\text{CO}-\text{CH}_2-$), 3.67 (3H, s, $\text{CH}_2\text{CO}_2\text{CH}_3$), 3.89 (3H, d, $J=4$ Hz, $\text{CH}_3\text{O}_2^{13}\text{C}$); δ_{C} (125.8 MHz, CDCl_3 , broad band decoupled) 160.9 ($\text{CH}_3\text{O}_2^{13}\text{C}-$), 192.2 ($\text{CH}_3\text{O}_2^{13}\text{C}^{13}\text{CO}$); m/e

(chemical ionisation $[\text{NH}_3]$) 194 (100 %, $\text{M}+\text{NH}_4^+$), 193 (1%), 133 (27%), 116 (80%), 101 (45%).

α -KETO[2- ^{13}C]GLUTARIC ACID

A stirred mixture of α -keto[2- ^{13}C]glutaric acid dimethyl ester (5) (56 mg, 0.3 mmol), water (250 μl) and concentrated hydrochloric acid (150 μl) was heated under reflux for 2 h. The reaction mixture was evaporated to dryness to give a buff-coloured crystalline solid which was recrystallised twice from ethyl acetate/hexane to give the title material as a white, crystalline solid (26.1 mg, 55%); m.p. 108 - 109°C (commercial unlabelled α -ketoglutaric acid m.p. 113 - 115°C; δ_{H} (500 MHz, $(\text{CD}_3)_2\text{SO}$) 2.37 - 2.43 (2H, m, $-\text{CH}_2\text{CO}_2\text{H}$), 2.89 - 2.95 (2H, m, $^{-13}\text{CO}-\text{CH}_2-$); δ_{C} (125.8 MHz, $(\text{CD}_3)_2\text{SO}$, broad band decoupled) 27.6 ($-\text{CH}_2\text{CO}_2\text{H}$), 34.9 ($^{-13}\text{CO}-\text{CH}_2$), 195.3 (s, $^{-13}\text{CO}-$, enriched), other (natural abundance) carboxyl carbon signals not visible; m/e (fast atom bombardment, Ar^+) 192 ($\text{M} = \text{H}^+$ - disodium salt); H.P.L.C. retention time (Hypersil ODS reverse-phase column, eluting with 0.2% aqueous formic acid) identical to commercial unlabelled material.

α -KETO[1,2- ^{13}C]GLUTARIC ACID

A stirred mixture of α -keto[1,2- ^{13}C]glutaric acid dimethyl ester (9) (44 mg, 0.25 mmol), water (200 μl) and hydrochloric acid (100 μl) was heated under reflux for 2 h. The reaction mixture was evaporated to dryness to give a buff-coloured crystalline solid which was recrystallised twice from ethyl acetate/hexane to give the title material as a white, crystalline solid (21.5 mg, 59%); m.p. 111°C (commercial unlabelled α -ketoglutaric acid m.p. 113-115°C); (21.5 mg, 59%); m.p. 111°C (commercial unlabelled α -ketoglutaric acid m.p. 113 - 115°C); $\nu_{\text{max}}/\text{cm}^{-1}$ (KBr disc) 3097 (broad, s), 1710 (s), 1693 (s), 1668 (s), 1445 (m), 1426 (m), 1407 (m), 1324 (m), 1275 (s), 1225 (m), 1087 (m), 1032 (m), 878 (m), 639 (s); δ_{H} (300 MHz, $(\text{CD}_3)_2\text{SO}$) 2.36 - 2.42 (2H, m, $-\text{CH}_2\text{CO}_2\text{H}$), 2.88 - 2.94 (2H, m, $^{-13}\text{CO}-\text{CH}_2-$); δ_{C} (125.8 MHz, $(\text{CD}_3)_2\text{SO}$, broad band decoupled)

27.6 ($-\text{CH}_2\text{CO}_2\text{H}$), 34.9 ($-\text{}^{13}\text{CO}-\text{CH}_2-$), 162.4 ($-\text{}^{13}\text{CO}_2\text{H}$, enriched), 175.7 ($-\text{CH}_2\text{CO}_2\text{H}$), 195.3 ($-\text{}^{13}\text{CO}-\text{CH}_2-$, enriched); m/e (fast atom bombardment, Ar^+) 193 ($\text{M} + \text{H}^+$ -disodium salt); H.P.L.C. retention time (Hypersil ODS reverse-phase column, eluting with 0.2% aqueous formic acid) identical to commercial unlabelled material.

Acknowledgement

We thank Eli Lilly and Co., Indianapolis for financial and collaborative support.

REFERENCES

- 1(a) Kivirikko K.I., and Myllyla R., Methods Enzymol., **82**: 245 (1982).
- 1(b) Baldwin J.E., Adlington R.M., Aplin R.T., Crouch N.P., Knight G. and Schofield C.J. - J.Chem.Soc., Chem. Commun., 1651 (1987); Baldwin J.E., Adlington R.M., Crouch N.P., Schofield C.J. and Ting H-H. - ibid, 1556 (1987).
2. Recently an enzymatic synthesis of [$2-^{13}\text{C}$] α -ketoglutarate from [$2-^{13}\text{C}$] -glutamate has been reported: Ehrlich R.S. and Colman R.F. - Biochemistry **26**:3461 (1987).
3. McMurry J.E., Melton J., and Padgett H., J.Org.Chem., **39**:259 (1974).
4. Kornblum N., Taub B. and Ungnade H.E., J.Am.Chem.Soc., **76**:3209 (1954).
5. 99.5% $^{13}\text{CH}_3\text{I}$ was purchased from Aldrich Chemical Co. Ltd., The Old Brickyard, New Road, Gillingham, Dorset, SP8 4JL, U.K.
6. Finkbeiner H.L. and Wagner G.W., J.Org.Chem., **28**: 215 (1963).
7. Leonard N.J. and Shoemaker G.L., J.Am.Chem.Soc., **71**:1762 (1949).
8. Zen S., Koyama M. and Koto S., Organic Syntheses Coll. Vol. VI: 797 (1988).
9. Baldwin J.E., Adlington R.M., Greengrass C.W., Jones R.H., Schofield C.J. and Zaracostas C., Tetrahedron, **42**:4879 (1986).