Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Electronic Supplementary Information

α-Hydroxy-β-keto Acid Rearrangement-Decarboxylation: Impact on Thiamine Diphosphate-Dependent Enzymatic Transformations

Maryam Beigi,[a] *Sabrina Loschonsky*,[a] *Patrizia Lehwald*,[a] *Volker Brecht*,[a] *Susana L. A. Andrade*,[b] *Finian J. Leeper*,[c] *Werner Hummel*,[d] *Michael Müller*[a,*]

[a]Institute of Pharmaceutical Sciences, Albert-Ludwigs-Universität, Albertstraße 25, 79104 Freiburg, Germany
[b]Institute of Organic Chemistry and Biochemistry, Albert-Ludwigs-Universität, Albertstraße 21, 79104 Freiburg, Germany,
[c]University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge, UK CB2 1EW,
[d]Institute of Molecular Enzyme Technology at the Heinrich-Heine-University of Düsseldorf, Research Centre Jülich, Stetternicher Forst, 52426 Jülich, Germany

michael.mueller@pharmazie.uni-freiburg.de

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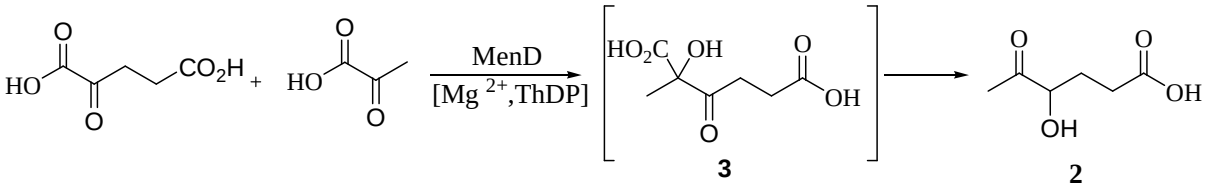
Additional general experimental procedures:

Sodium salts of [1,2-^{13}C]- and [2-^{13}C]-pyruvate were purchased from *Sigma-Aldrich Co Ltd* . Sodium salt of [1-^{13}C]-pyruvate and L-[1,2-^{13}C]-glutamic acid were purchased from *Cambridge Isotope Labs*.

The *MenD* gene from *Escherichia coli* K12 and *yerE* gene from *Yersinia pseudotuberculosis* were expressed and purified following standard molecular biology techniques as explained elsewhere.[1,2]
The *sucA* gene of *Escherichia coli* K12 was cloned into a pET-28b (+) (*Novagen*) at *NcoI* and *HindIII* restriction sites. The synthetic gene of *Azoarcus sp.* 22LIN *CDH* (*GeneArt, Invitrogen*) was cloned into pET-21a vector (*Novagen*) at *NdeI* and *XhoI* restriction sides. The resulting constructs were full length sequenced to confirm in-frame cloning. The SucA and CDH recombinant proteins with C-terminal 6 x histidine tag were over-expressed in *E. coli* BL21(DE3) cells and were consequently purified following standard molecular biology techniques.[1]
For the coupled enzymatic system[3] the NADH oxidase from *Lactobacillus brevis* and L-glutamate dehydrogenase (L-GluDH) from *Clostridium sp.* were used.

^{13}C NMR studies with α-KG and ^{13}C-labeled pyruvate



α-KG + [1-^{13}C]-pyruvate

[1-^{13}C]-Pyruvate: ^{13}C NMR (D_2O): δ = 170.1 (s), 178.5 ppm (s, hydrate).

2-Hydroxy-2-methyl-3-oxohexanedioic acid (3): ^{13}C NMR (D_2O): δ = 176.8 ppm (s).

Carbonate: ^{13}C NMR (D_2O): δ = 160.2 ppm (s).

Carbon dioxide: ^{13}C NMR (D_2O): δ = 124.6 ppm (s).

α-KG + [1,2-^{13}C]-pyruvate

[1,2-^{13}C]-Pyruvate: ^{13}C NMR (D_2O): δ = 93.9 (d, *J* = 63 Hz, hydrate), 170.2 (d, *J* = 62 Hz), 178.5 (d, *J* = 63 Hz, hydrate), 205.1 ppm (d, *J* = 62 Hz).

2-Hydroxy-2-methyl-3-oxohexanedioic acid (3): ^{13}C NMR (D_2O): δ = 82.7 (d, *J* = 51 Hz), 176.8 ppm (d, *J* = 51 Hz).

4-Hydroxy-5-oxohexanoic acid (2): ^{13}C NMR (D_2O): δ = 214.8 ppm (s).

Carbonate: ^{13}C NMR (D_2O): δ = 160.2 ppm (s).

Acetic acid as side product ^{13}C NMR (D_2O): δ = 181.4 ppm (s).

α-KG + [2-^{13}C]-pyruvate

[2-^{13}C]-Pyruvate: ^{13}C NMR (D_2O): δ = 93.8 (s, hydrate), 205.1 ppm (s).

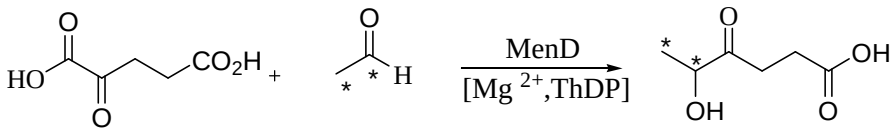
2-Hydroxy-2-methyl-3-oxohexanedioic acid (3): ^{13}C NMR (D_2O): δ = 82.7 ppm (s).

4-Hydroxy-5-oxohexanoic acid (2): ^{13}C NMR (D_2O): δ = 214.8 ppm (s).

Carbonate: ^{13}C NMR (D_2O): δ = 160.2 ppm (s).

Acetic acid as side product ^{13}C NMR (D_2O): δ = 181.4 ppm (s).

^{13}C NMR studies with α-KG and ^{13}C-labeled acetaldehyde



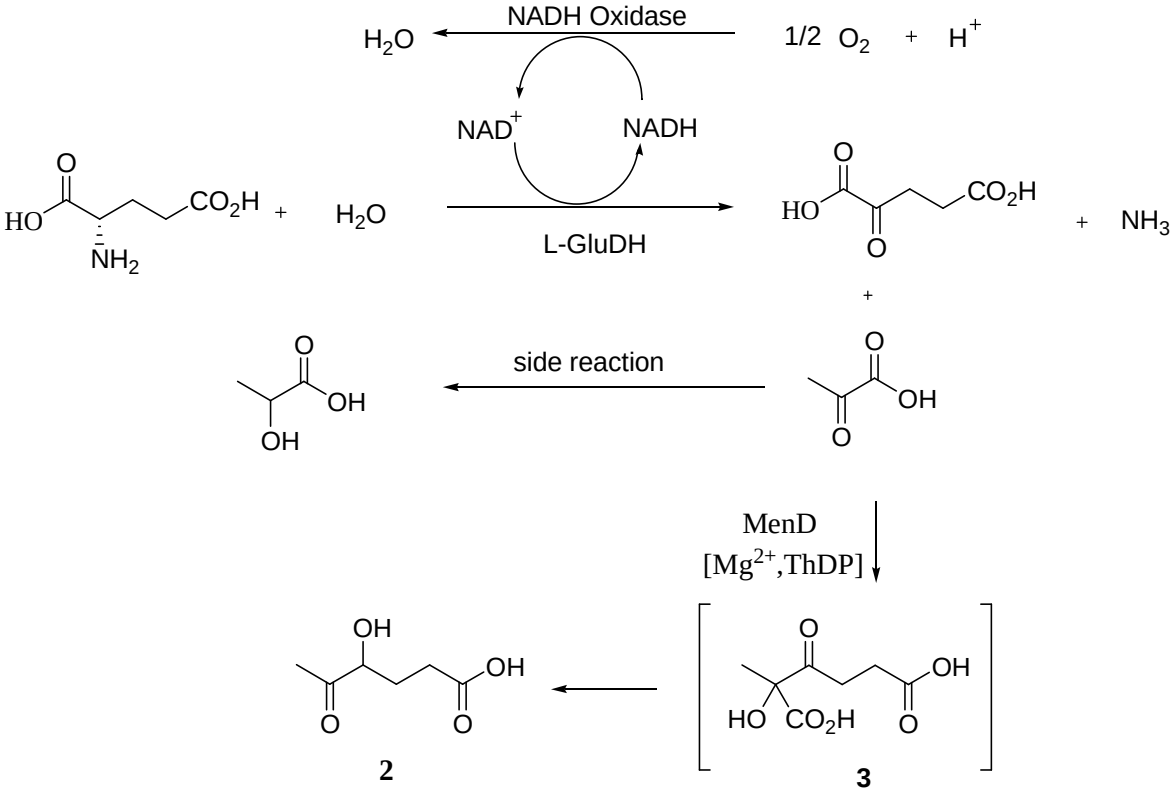
1

α-KG + [1,2-^{13}C]-acetaldehyde

[1, 2-^{13}C]Acetaldehyde: ^{13}C NMR (D_2O): δ = 23.3 (d, *J* = 44 Hz, hydrate), 30.2(d, *J* = 39 Hz), 88.3 (d, *J* = 44 Hz, hydrate), 206.9 ppm (d, *J* = 39 Hz).

5-Hydroxy-4-oxohexanoic acid (1): ^{13}C NMR (D_2O): δ = 18.5 (d, *J* = 36 Hz), 72.8 ppm (d, *J* = 36 Hz).

^{13}C NMR kinetic studies with in situ production of ^{13}C-labeled α-KG



2 **3**

L-[1,2-^{13}C]-Glutamic acid: ^{13}C NMR (D_2O): δ = 54.8 (d, *J* = 53 Hz), 174.6 ppm (d, *J* = 53 Hz).

L-[1,2-^{13}C]-Glutamic acid + [1-^{13}C]-pyruvate

2-Hydroxy-2-methyl-3-oxohexanedioic acid (3): ^{13}C NMR (D_2O): δ = 176.8 (s), 212.1 ppm (s).

4-Hydroxy-5-oxohexanoic acid (2): ^{13}C NMR (D_2O): δ = 76.5 ppm (s).

Carbonate: **^{13}C NMR** (D_2O): δ = 160.2 ppm (s).

Carbon dioxide: **^{13}C NMR** (D_2O): δ = 124.6 ppm (s).

Lactate: **^{13}C NMR** (D_2O): δ = 182.4 ppm (s).

L-[1,2-^{13}C]-Glutamic acid + [1,2-^{13}C]-pyruvate

2-Hydroxy-2-methyl-3-oxohexanedioic acid (3): **^{13}C NMR** (D_2O): δ = 82.7 (dd, *J* = 51, 42 Hz), 176.8 (d, *J* = 51 Hz), 212.0 ppm (d, *J* = 42 Hz).

4-Hydroxy-5-oxohexanoic acid (2): **^{13}C NMR** (D_2O): δ = 76.5 (d, *J* = 41 Hz) 214.8 ppm (d, *J* = 41 Hz).

Carbonate: **^{13}C NMR** (D_2O): δ = 160.2 ppm (s).

Carbon dioxide: **^{13}C NMR** (D_2O): δ = 124.6 ppm (s).

Lactate: **^{13}C NMR** (D_2O): δ =68.5 (d, *J* = 55 Hz), 182.4 ppm (d, *J* = 55 Hz).

L-[1,2-^{13}C]-Glutamic acid + [2-^{13}C]-pyruvate

2-Hydroxy-2-methyl-3-oxohexanedioic acid (3): **^{13}C NMR** (D_2O): δ =82.7 (d, *J* = 42 Hz), 212.0 ppm (d, *J* = 42 Hz).

4-Hydroxy-5-oxohexanoic acid (2): **^{13}C NMR** (D_2O): δ =76.5 (d, *J* = 41 Hz), 214.8 ppm (d, *J* = 41 Hz).

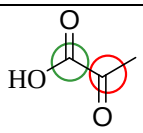
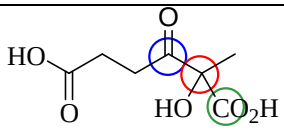
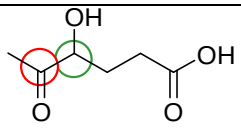
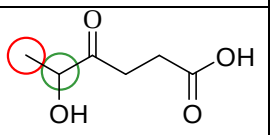
Carbonate: **^{13}C NMR** (D_2O): δ = 160.2 ppm (s).

Carbon dioxide: **^{13}C NMR** (D_2O): δ = 124.6 ppm (s).

Lactate: **^{13}C NMR** (D_2O): δ = 68.5 ppm (s).

Acetic acid as side product **^{13}C NMR** (D_2O): δ = 181.4 ppm (s).

Summary table for the chemical shifts of ^{13}C-labeled compounds

MenD	Pyruvate		3			2		1	
									
	C-1	C-2	C-1	C-2	C-3	C-4	C-5	C-5	C-6
	ppm		ppm			ppm		ppm	
α-KG + [1-^{13}C]-pyruvate	170.1(s)	-	176.8(s)	-	-	-	-	-	-
α-KG + [1,2-^{13}C]-pyruvate	170.2(d)	205.1(d)	176.8(d)	82.7(d)	-	-	214.8(s)	-	-
α-KG + [2-^{13}C]-pyruvate	-	205.1(s)	-	82.7(s)	-	-	214.8(s)	-	-
α-KG + [1,2-^{13}C]-acetaldehyde	-	-	-	-	-	-	-	72.8(d)	18.5(d)
L-[1,2-^{13}C]-Glutamic acid + [1-^{13}C]-pyruvate	170.1(s)	-	176.8(s)	-	212.1(s)	76.5(s)	-	-	-
L-[1,2-^{13}C]-Glutamic acid+[1,2-^{13}C]-pyruvate	170.2(d)	205.1(d)	176.8(d)	82.7(dd)	212.1(d)	76.5(d)	214.8(d)	-	-
L-[1,2-^{13}C]-Glutamic acid + [2-^{13}C]-pyruvate	-	205.1(s)	-	82.7(d)	212.1(d)	76.5(d)	214.8(d)	-	-

Enzyme separation by ultrafiltration

To follow the intermediate **3** in the absence of the enzyme, the reaction was started with [1,2-^{13}C]-pyruvate and MenD under the standard reaction condition mentioned above (1.5 mL reaction volume). After 5 hours, the ^{13}C-NMR experiment proved the formation of the intermediate. Then the enzyme was removed by ultrafiltration using Vivaspin 20 [10 MWCO] concentrator (Sartorius). Qualitative screening by Bradford assay in 96 well plates as well as SDS-PAGE confirmed complete removal of the protein from the solution. Then the solution was filled in NMR tube and the reaction was directly followed by sequential ^{13}C-NMR experiments.

^{13}C NMR studies for YerE-catalyzed carboligation of pyruvate and 2-oxobutyrate:

2-oxobutyrate + [2-^{13}C]-pyruvate

[2, 3-^{13}C]-acetolactate (**4**)**: ^{13}C NMR** (D_2O): δ = 82.7 (d, *J* = 43 Hz), 211.7 ppm (d, *J* = 43 Hz).

[3-^{13}C]-acetohydroxybutyrate (**5**)**: ^{13}C NMR** (D_2O): δ = 212.0 ppm (s).

[1, 2-^{13}C]-acetoin (**6**)**: ^{13}C NMR** (D_2O): δ = 73.1 (d, *J* = 41 Hz), 215.5 ppm (d, *J* = 41 Hz).

3-Hydroxypentan-2-one (7): ^{13}C NMR (D_2O): δ = 215.4 ppm (s).

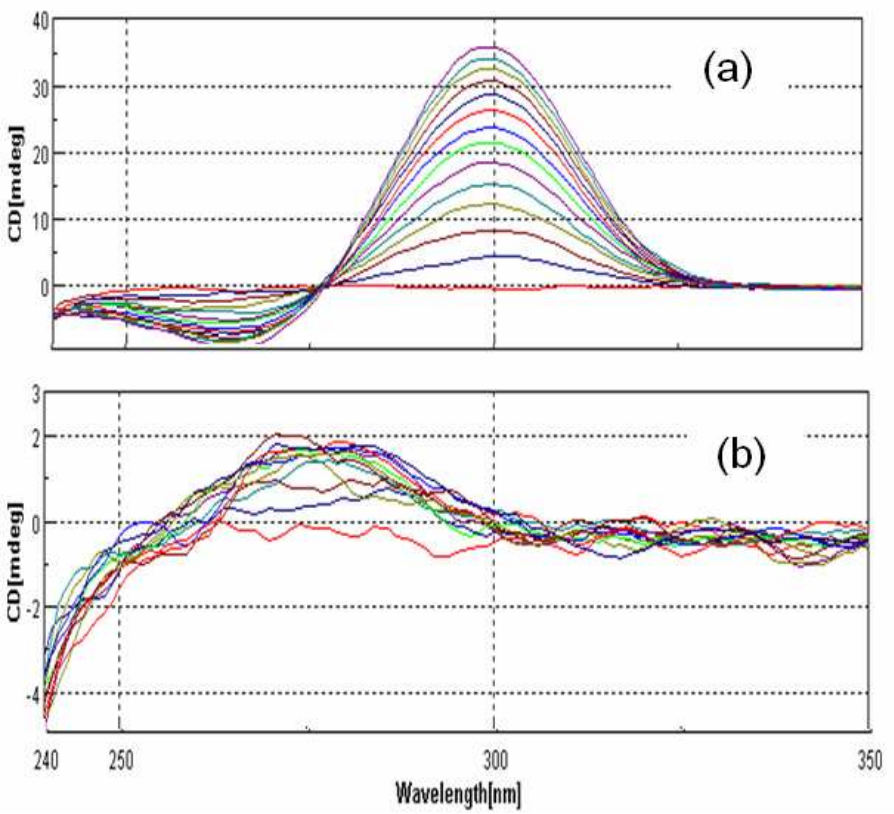
2- Hydroxypentan-3-one (8): ^{13}C NMR (D_2O): δ = 72.6 ppm (s).

References:

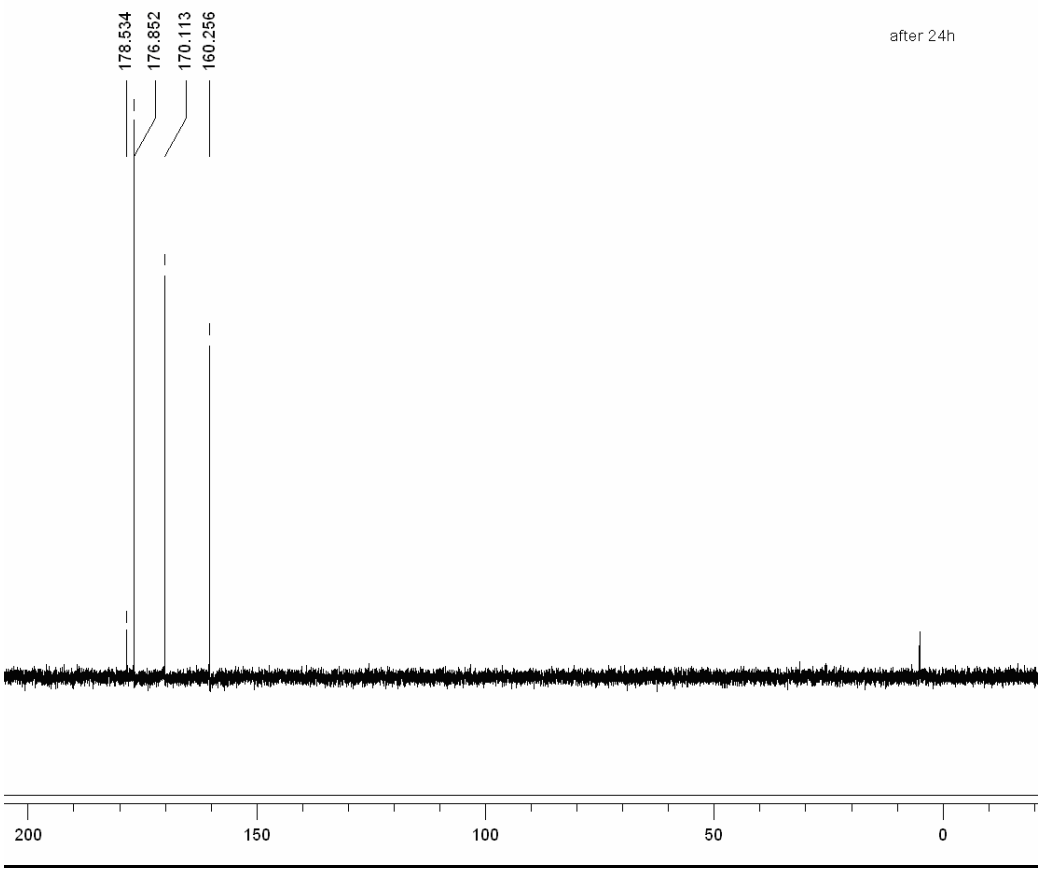
[1] Kurutsch, A.; Richter, M.; Brecht, V.; Sprenger, G. A.; Müller, M. *J. Mol. Catal. B: Enzym.* **2009**, *61*, 56.
[2] Lehwald, P.; Richter, M.; Röhr, C.; Liu, H. W.; Müller, M. *Angew. Chem., Int. Ed.* **2010**, *49*, 2389.
[3] Ödman, P.; Wellborn, W. B.; Bommarius, A. S. *Tetrahedron: Asymmetry* **2004**, *15*, 2933.

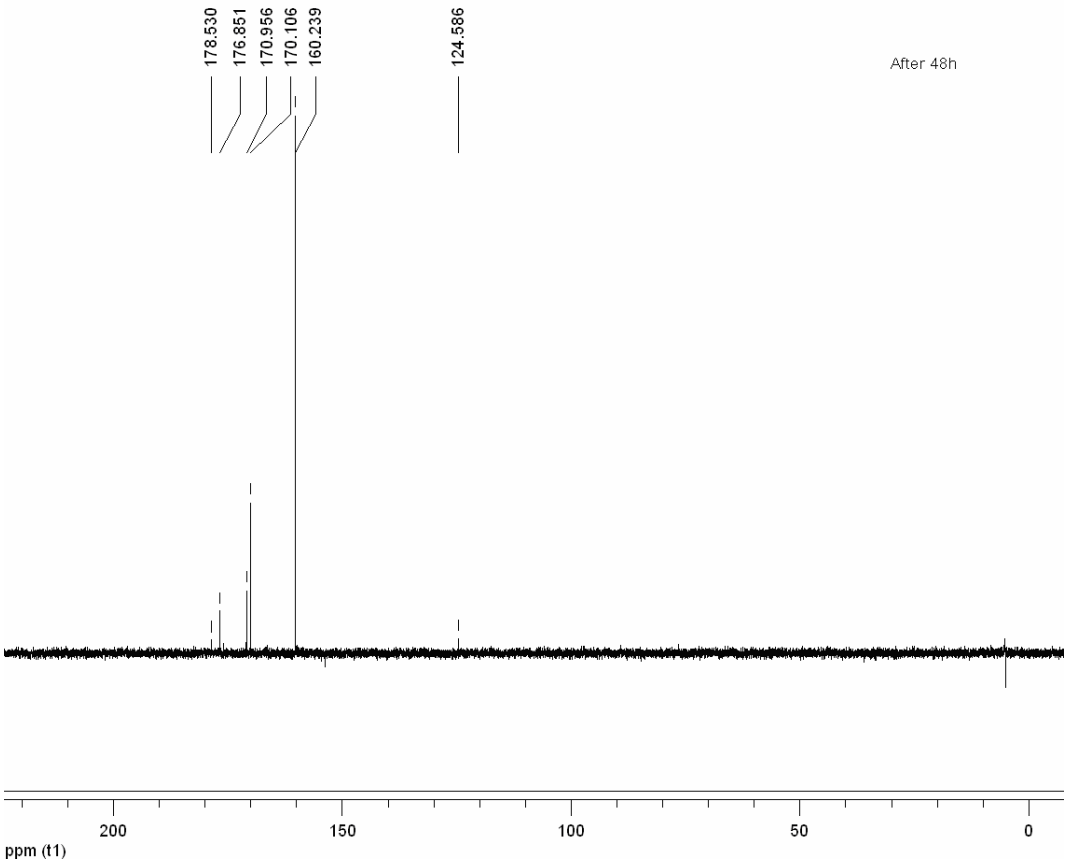
CD spectra:

MenD-catalyzed reaction of α-KG with (a) pyruvate or (b) acetaldehyde over time. Time delay between each scan was set to 60 minutes.

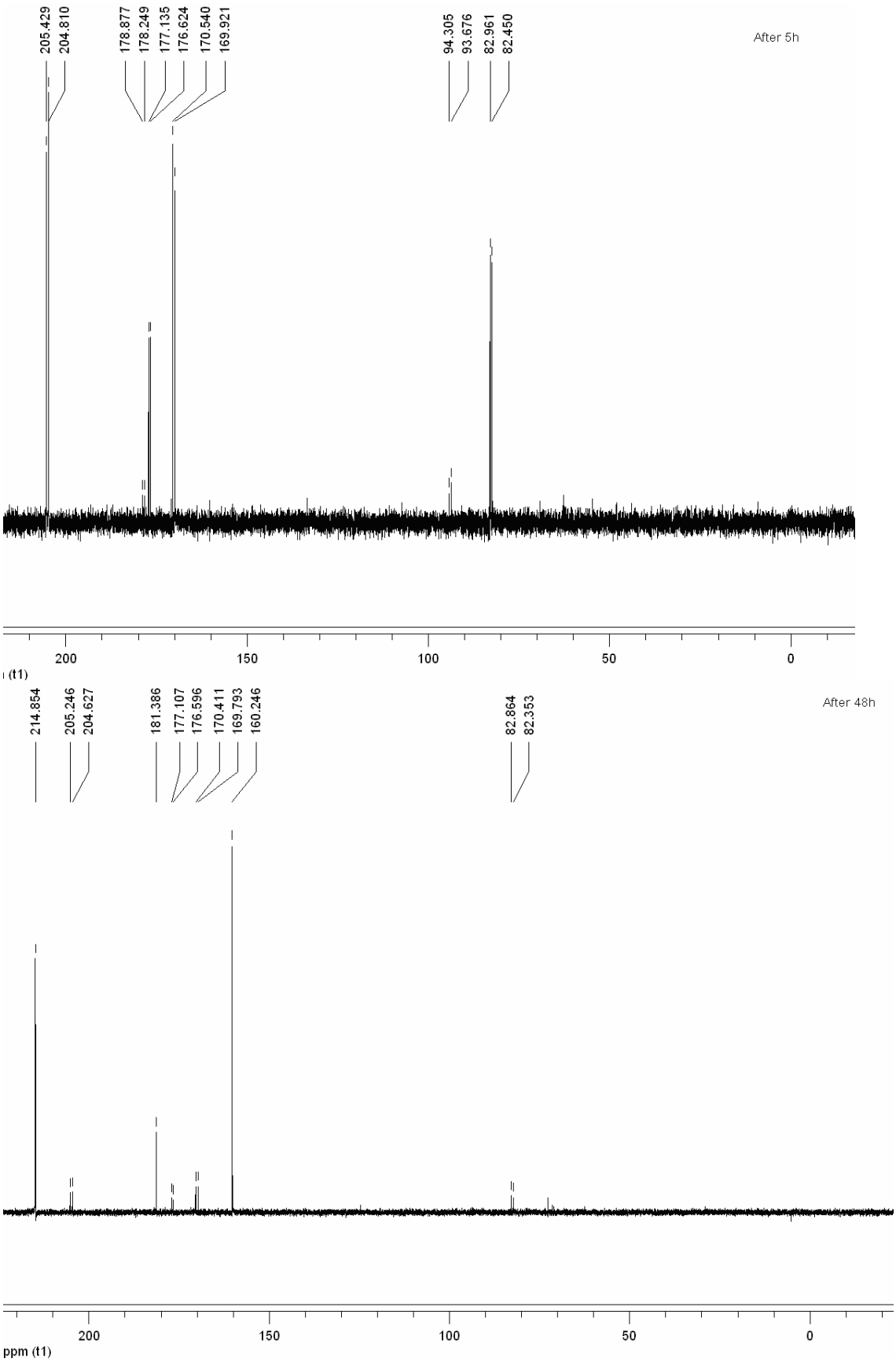


NMR spectra: **α-KG + [1-^{13}C]-pyruvate**

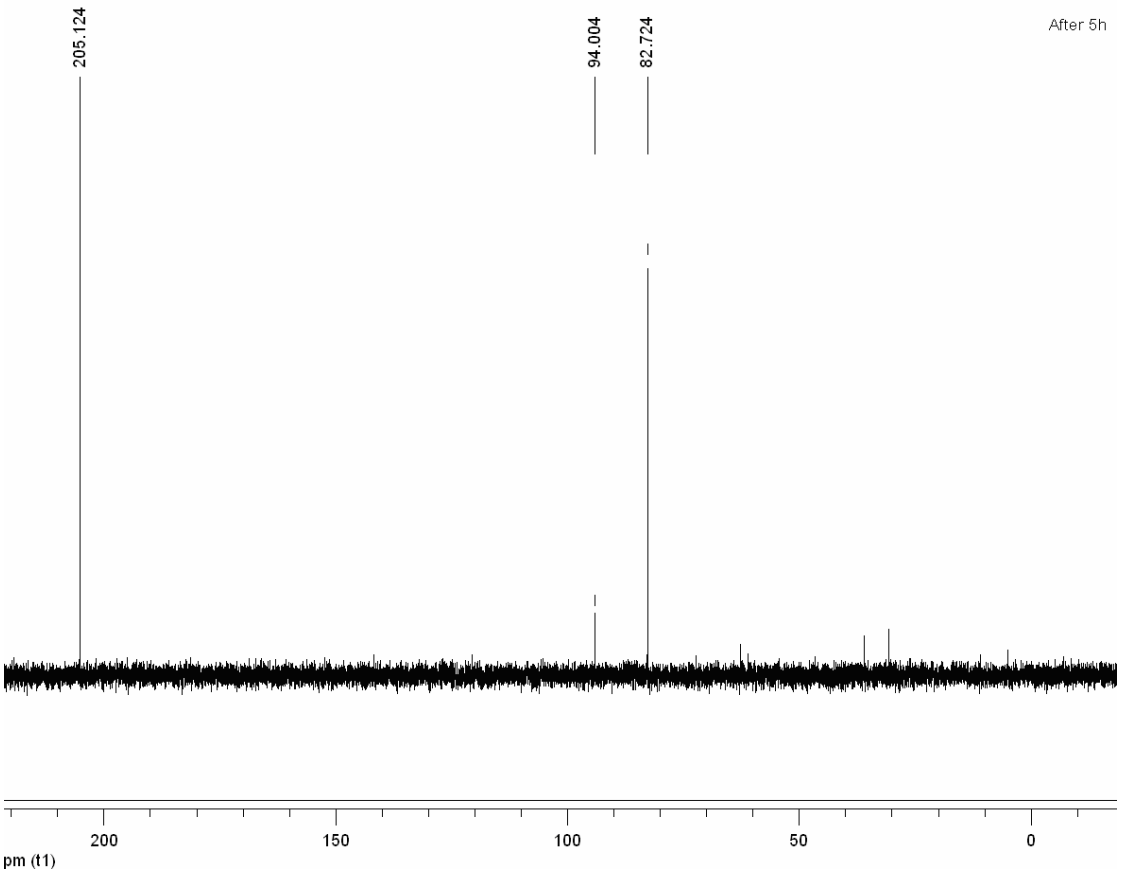


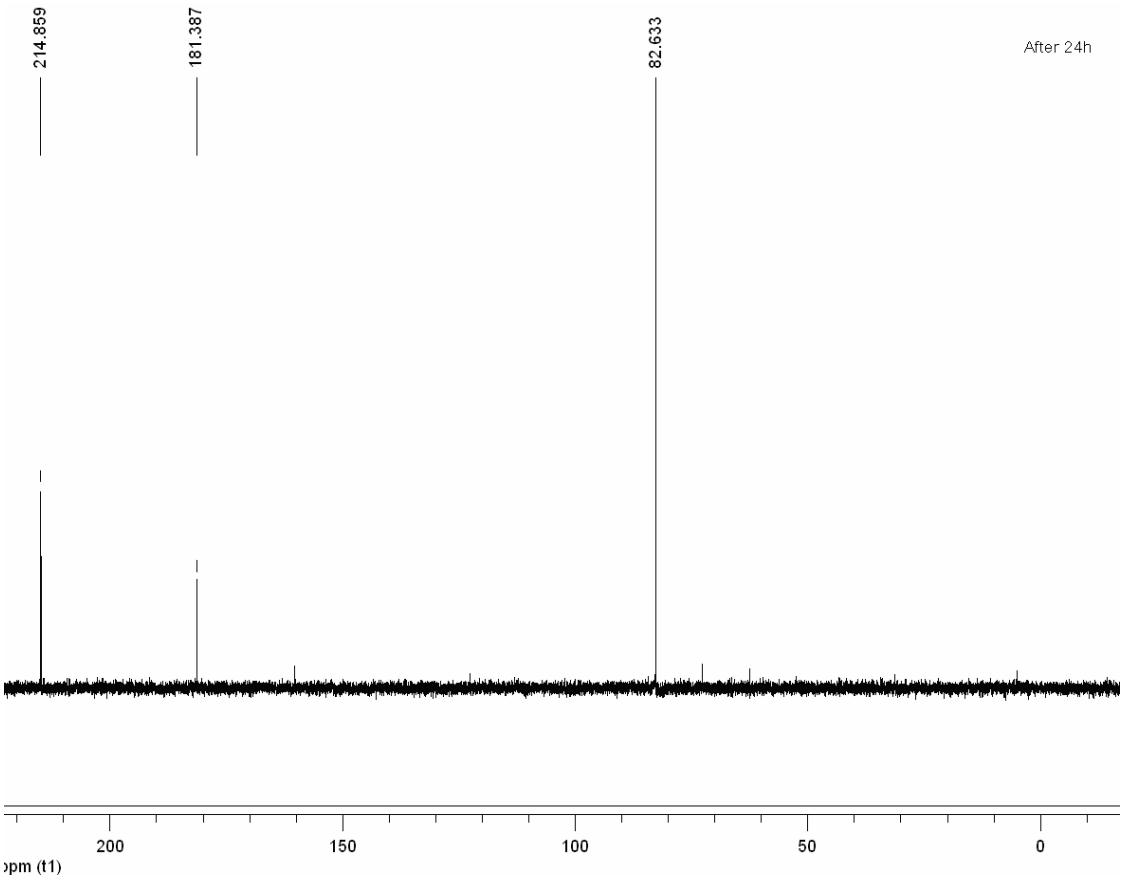


α-KG + [1,2-^{13}C]-pyruvate

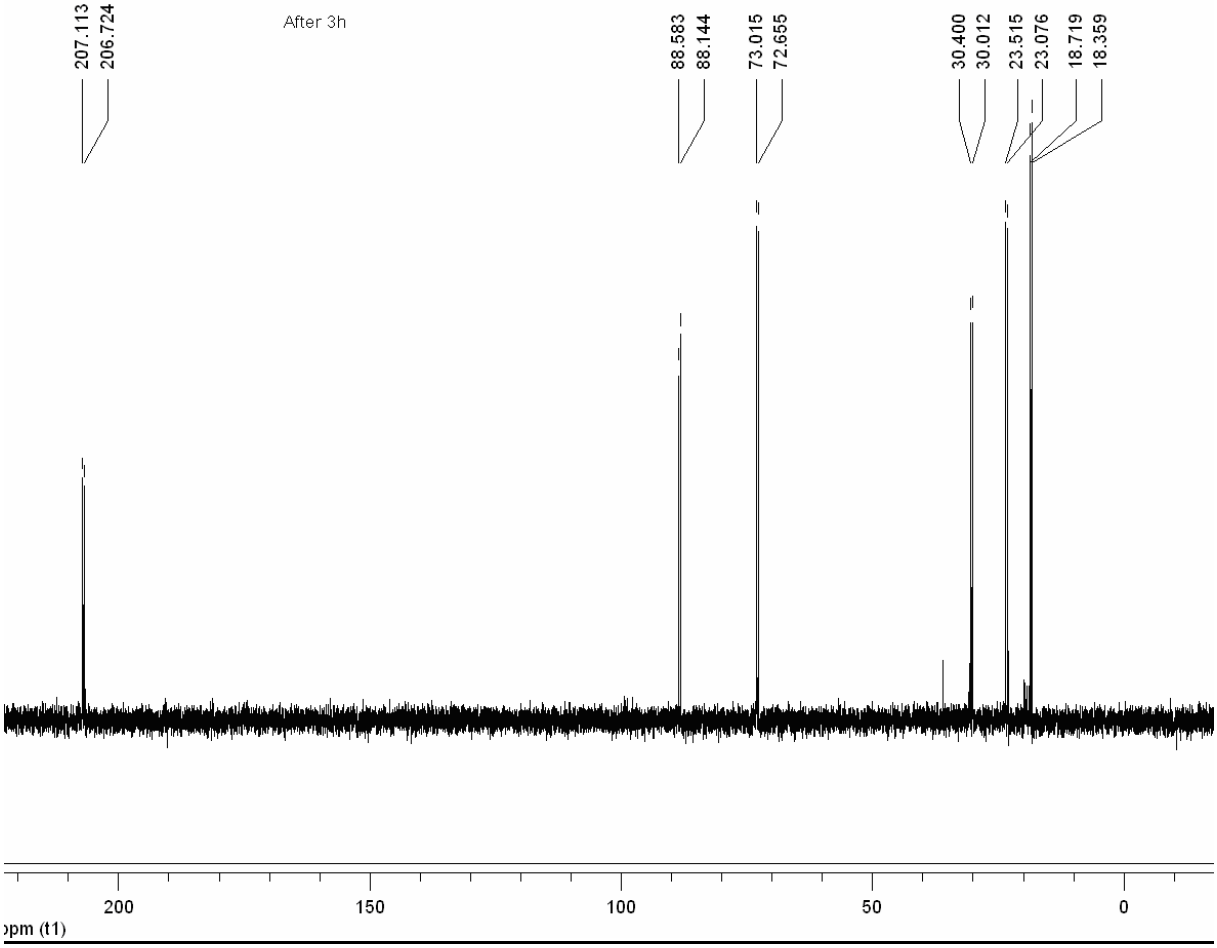


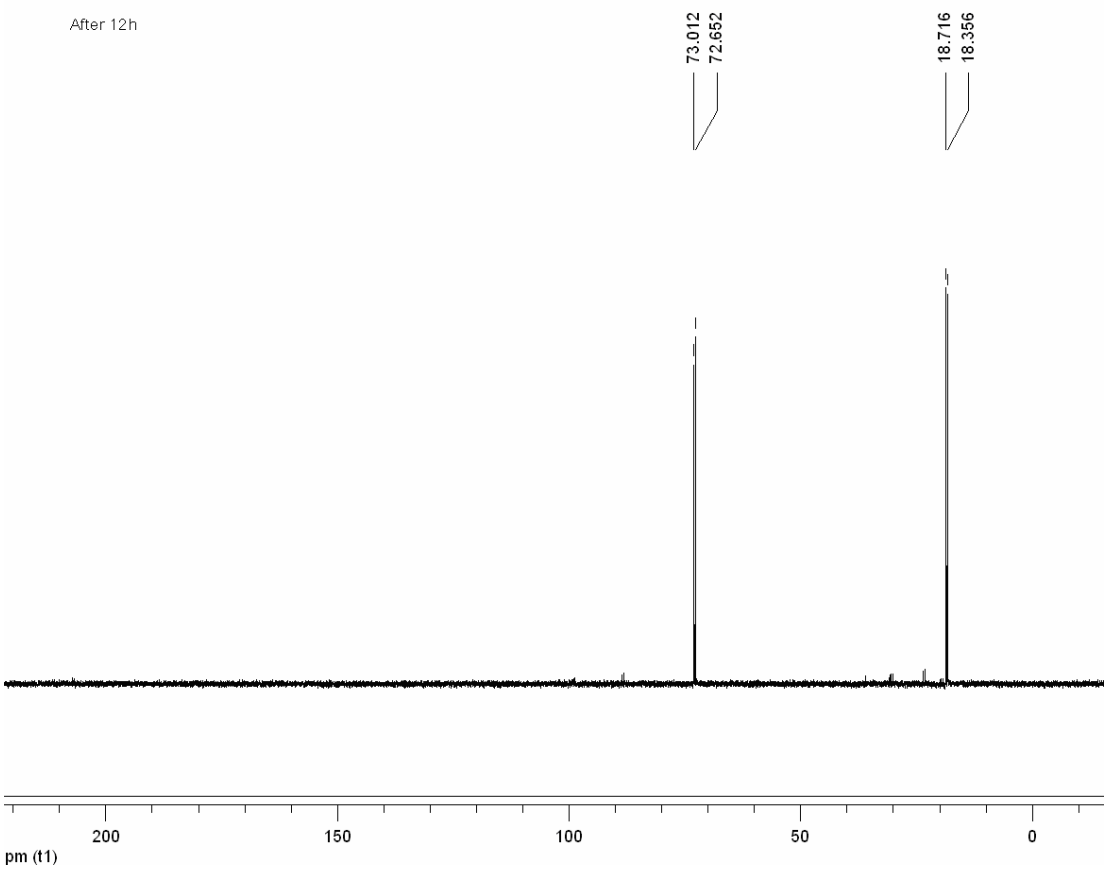
α-KG + [2-^{13}C]-pyruvate



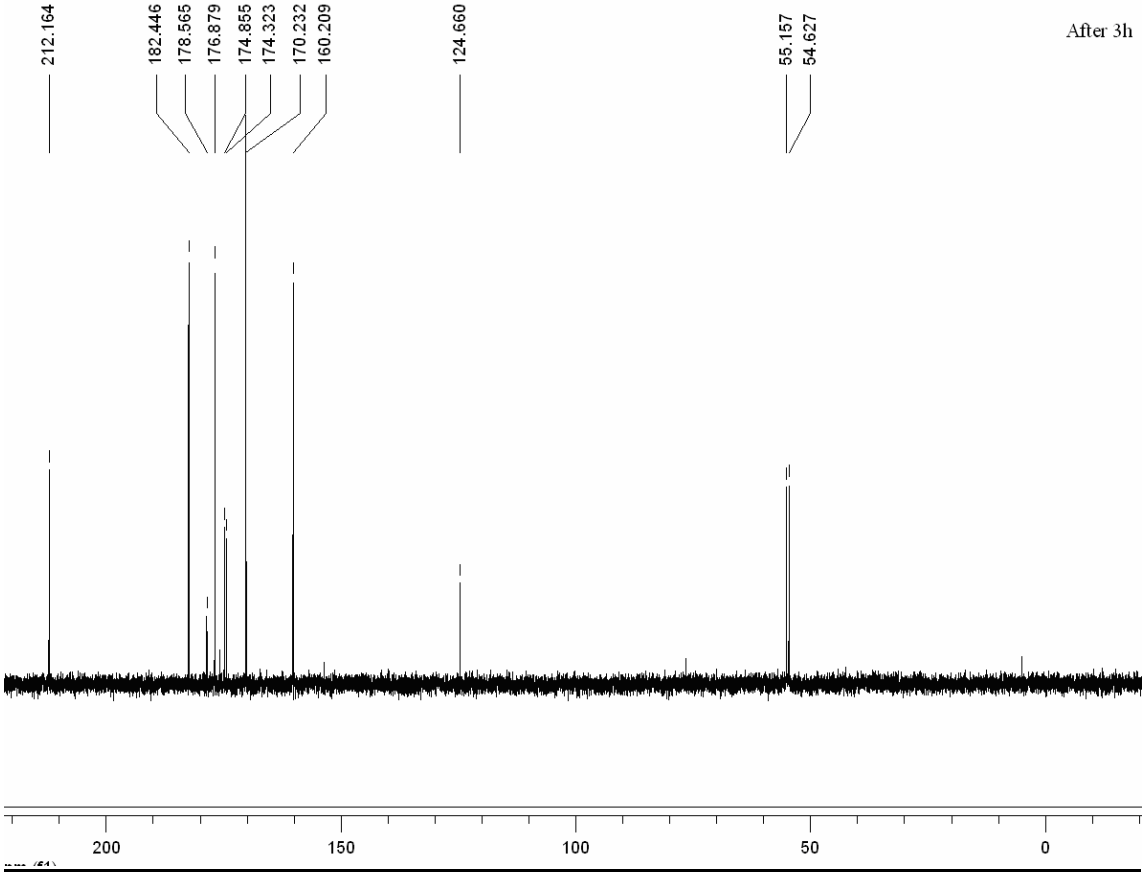


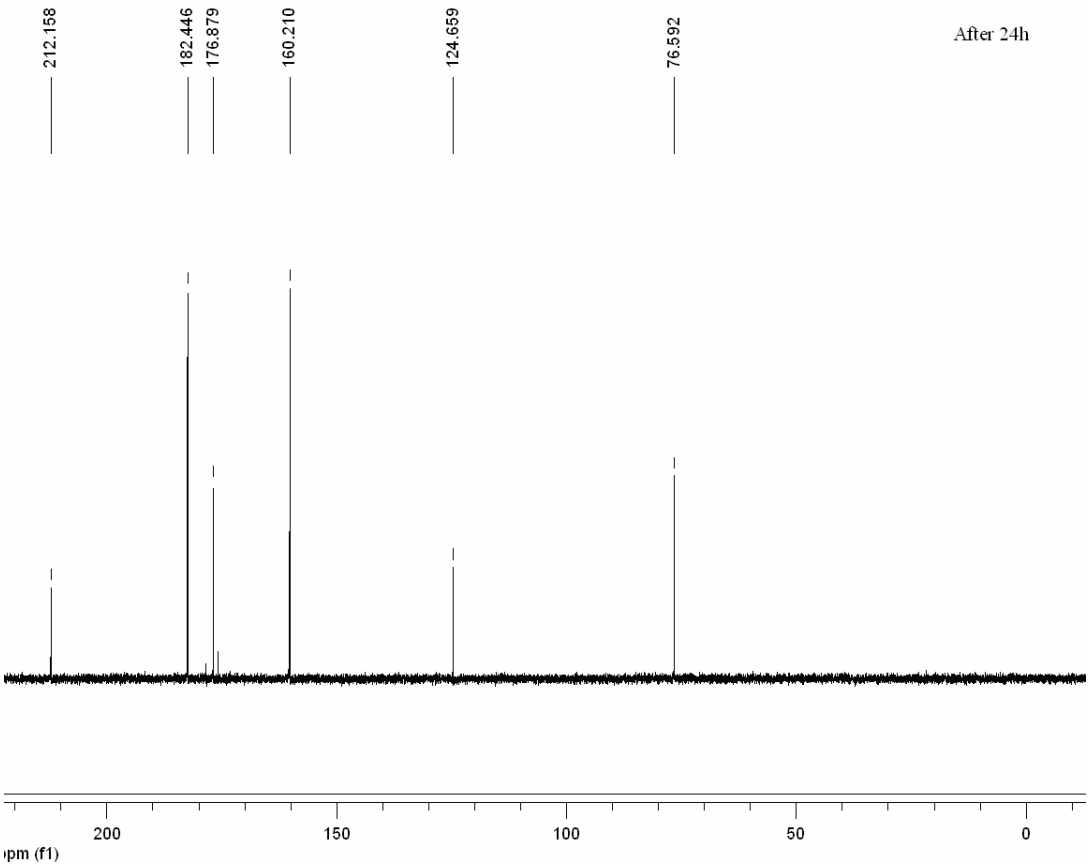
α-KG + [1,2-^{13}C]-acetaldehyde



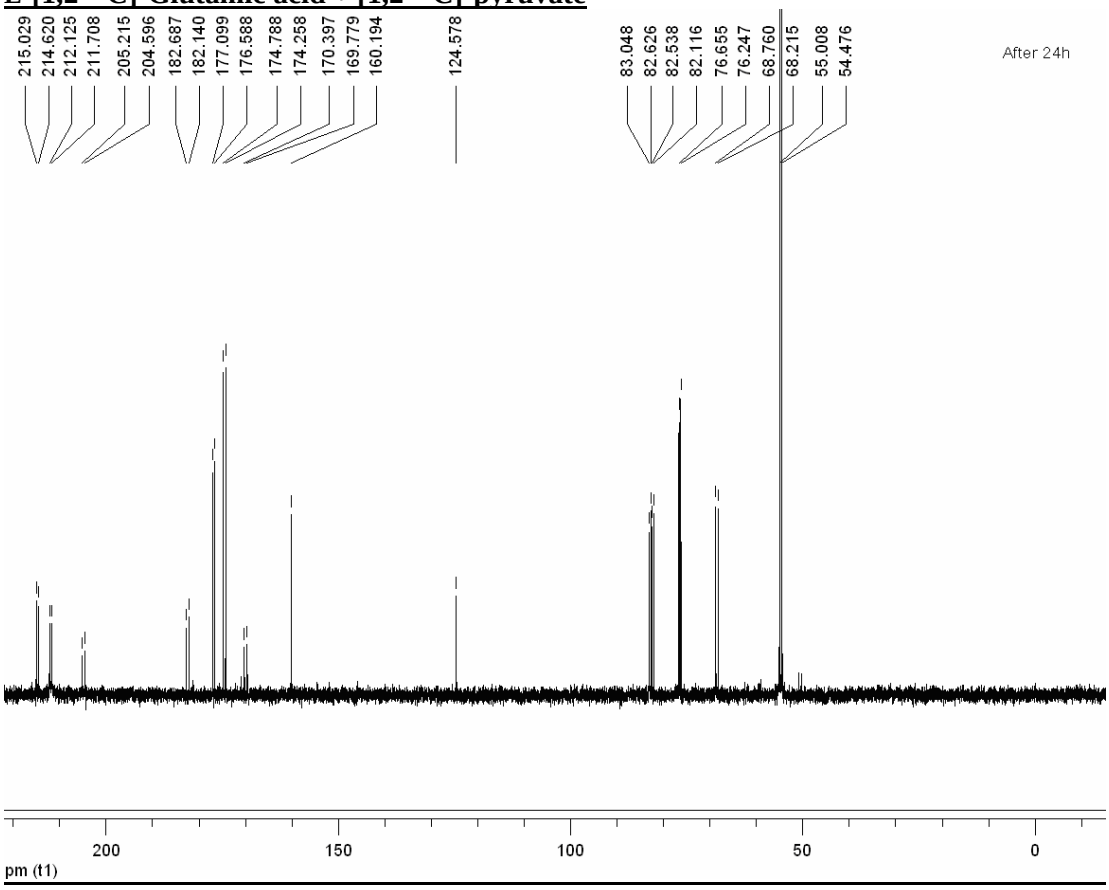


L-[1,2-^{13}C]-Glutamic acid + [1-^{13}C]-pyruvate

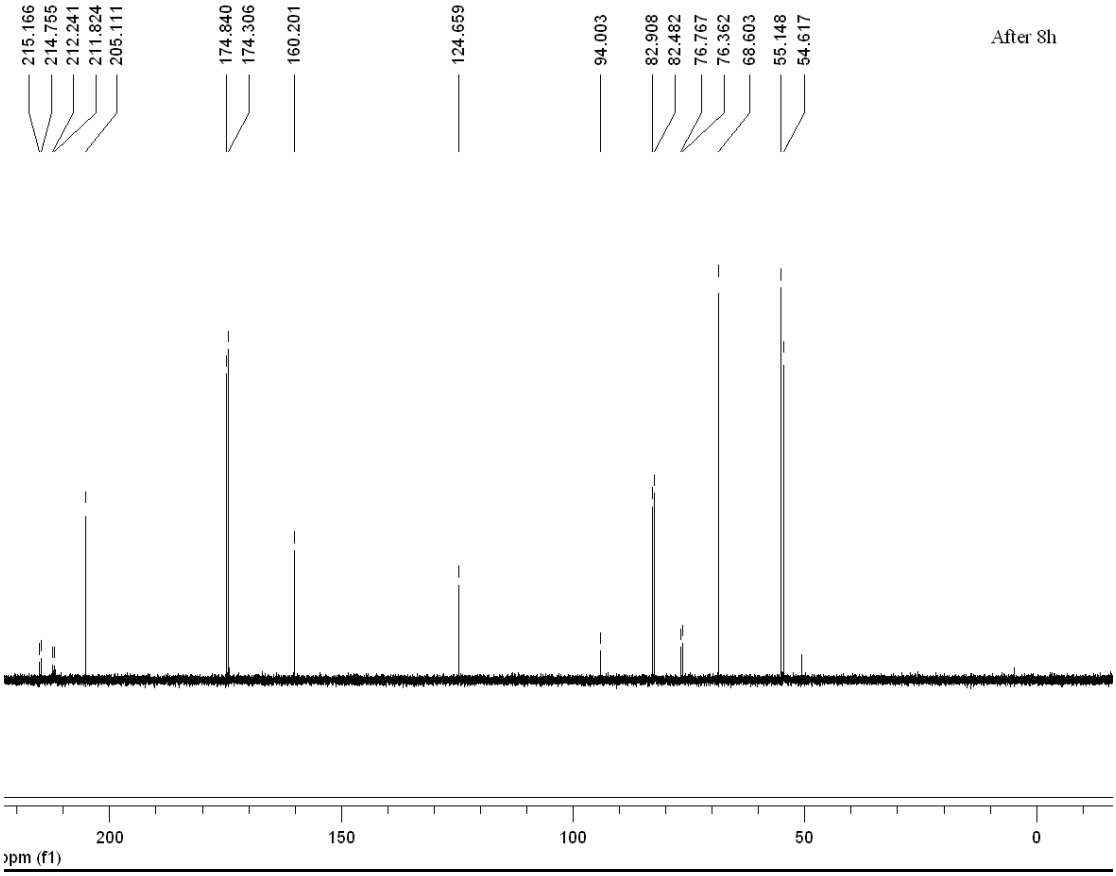


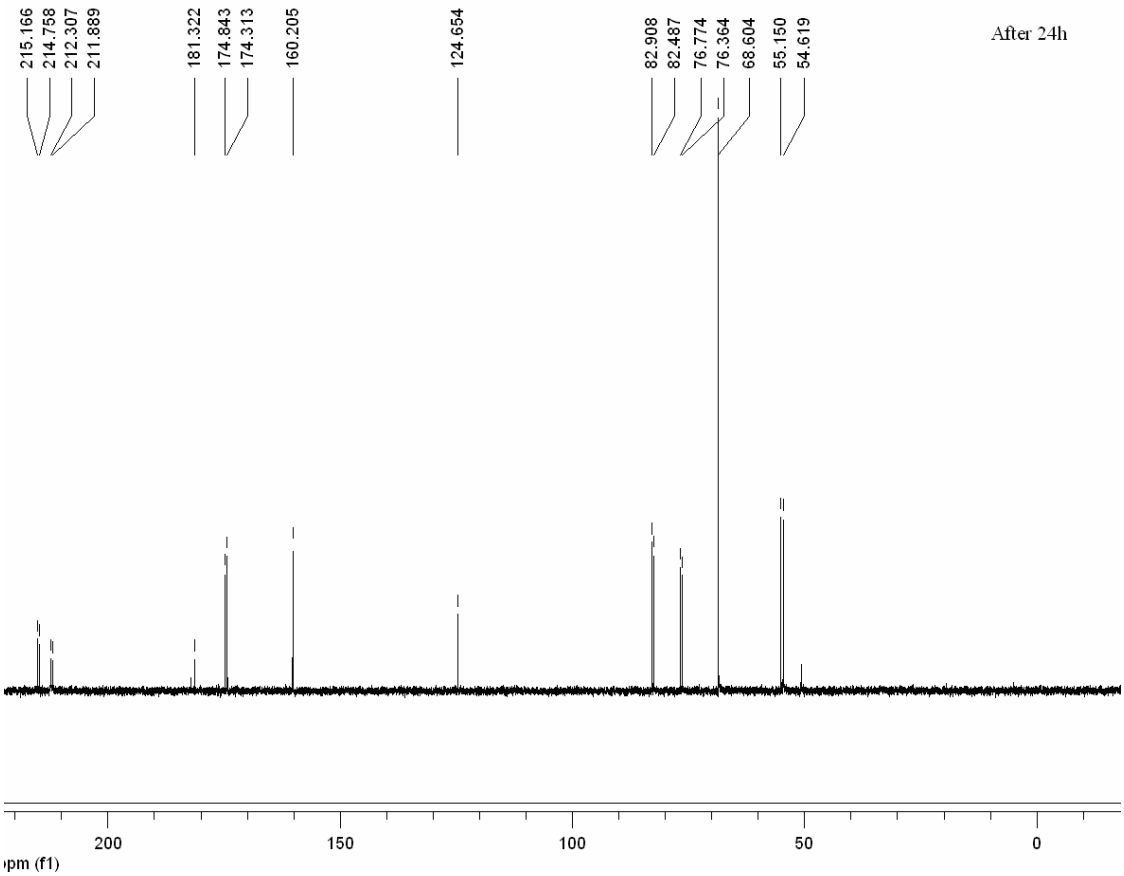


L-[1,2-^{13}C]-Glutamic acid + [1,2-^{13}C]-pyruvate

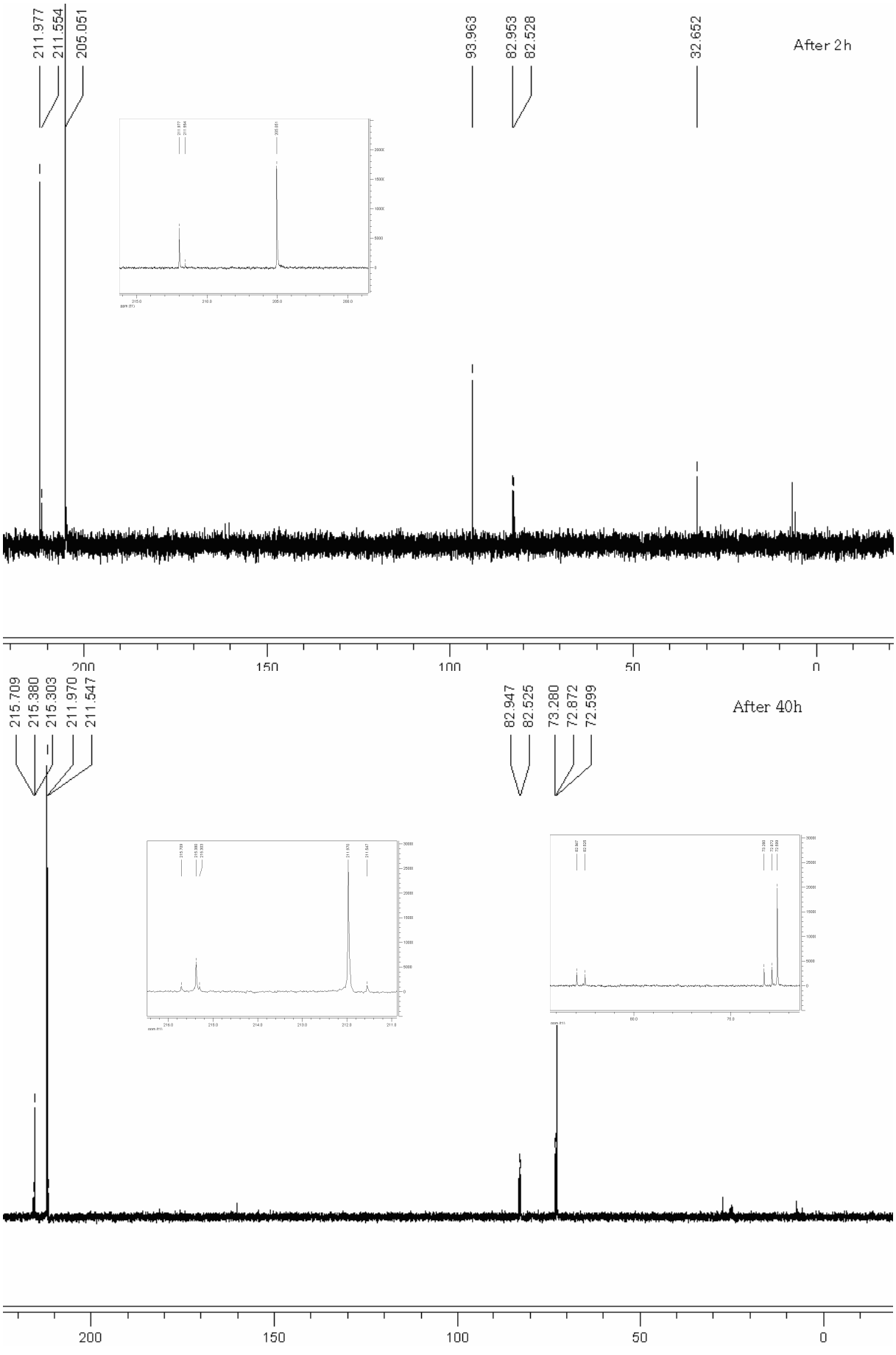


L-[1,2-^{13}C]-Glutamic acid + [2-^{13}C]-pyruvate



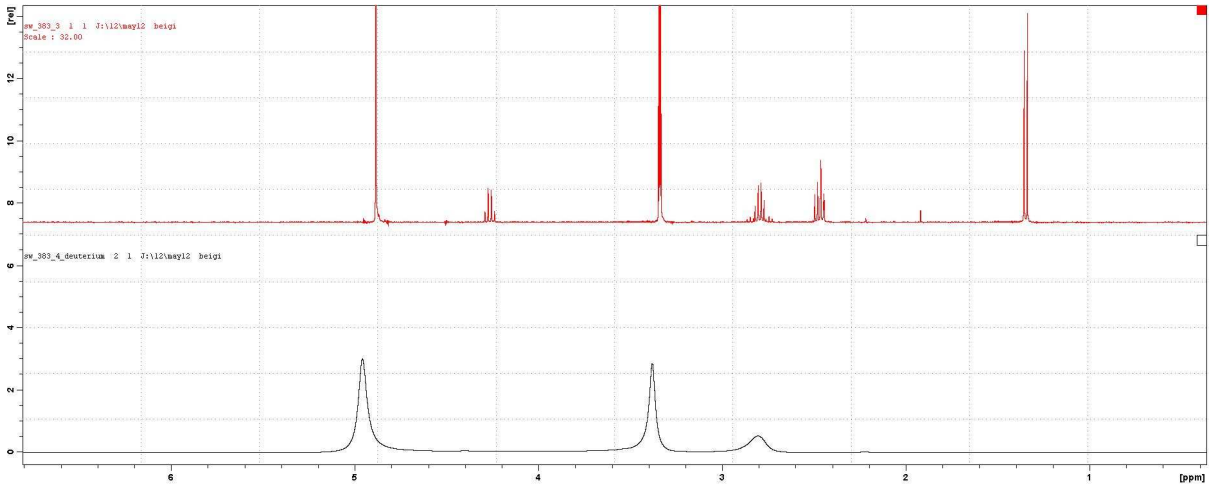


2-Oxobutyrate + [2-^{13}C]-pyruvate



Spectra of Deuterium labeling experiments

^{1}H NMR of **1** in CD_3OD (up)
^{2}H NMR of **1** in CH_3OH after incubation in D_2O for 48 h (bottom)



^{13}C NMR of **1** in CD_3OD after incubation in D_2O for 48 h