Supporting Information

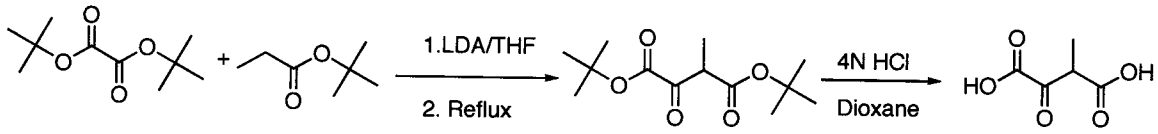
Structure and function of PA4872 from *Pseudomonas aeruginosa*, a novel oxaloacetate decarboxylase from the PEP mutase / isocitrate lyase superfamily[†‡]

Buvaneswari Narayanan[1#], Weiling Nu[2#], Ying Han[2], Jiwen Zou[2], Patrick Mariano[2], Debra Dunaway-Mariano[2*] and Osnat Herzberg[1*]

[1]Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville, Maryland

[2] Department of Chemistry, University of New Mexico, Albuquerque, New Mexico

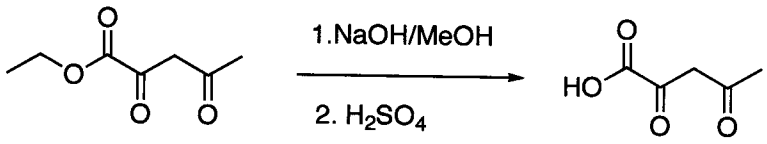
Table of Contents



Di-t-butyl 3-Methyloxalacetate. To a solution of LDA, made from diisopropylamine (7.0 g, 0.07 mol) in THF (150 mL) and n-butyllithium (28 mL, 0.07 mol, 2.5 M in hexane), at -78 °C was added a solution of t-butyl propionate (8.5 g, 0.065 mol) in THF (50 mL). After stirring at -78 °C for 1 h, a solution of di-t-butyl oxalate (13.2 g, 0.065 mol) in THF (50 ml) was added. The resulting mixture was stirred overnight at room temperature, at reflux for 3 h and concentrated *in vacuo.* A solution of the residue in ethyl acetate was acidified and washed with water, dried over Na_2SO_4 and concentrated *in vacuo* to give a residue, which was subjected to chromatography on silica gel (1:5 acetone-hexane) to provide di-t-butyl 3-methyloxalacetate (12.6 g, 75%). Spectroscopic data for the major keto tautomer ^{1}H NMR ($CDCl_3$) 1.33 (d, 3H, J = 7.0 Hz), 1.42 (s, 9H), 1.53 (s, 9H), 3.92 (q, 1H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) 11.7, 27.6, 27.7, 49.2, 82.2, 84.1, 159.6, 168.9, 190.5; HRMS (ES) m/z 281.1367, calcd for $C_{13}H_{22}O_5Na$ 281.1365.

3-Methyloxalacetate. To a solution of 4N HCl in dioxane (20 mL) was added di-t-butyl 3-methyloxalacetate (1.0 g, 4 mmol) and $MgCl_2 \cdot 6H_2O$ (0.79 g, 4 mmol) at 0 °C, The mixture was stirred for 48 h at room temperature and filtered. The filtrate was concentrated *in vacuo* to afford 3-methyloxalacetate as a colorless crystalline solid, which was recrystallized from benzene to afford 0.57 g (100%), mp 128 °C (lit mp (1) 144 °C). Spectroscopic data for the major enol tautomer: ^{1}H NMR ($CDCl_3$) 1.97 (s, 3H), 7.25(s, 1H); ^{13}C NMR ($CDCl_3$) 6.8, 112.0, 151.6, 163.3, 165.1.

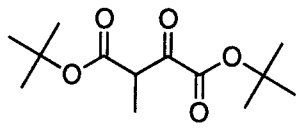
(1) Kabala, G.; Martell, A. E. (1981) *J. Am. Chem. Soc.*, **103**, 7609-7615.



Acetopyruvate. A modification of the procedure described by Brecker *et al.* (2) was used. A solution of ethyl acetopyruvate (4.0 g, 0.025 mol) in methanol (50 mL) at 0 °C containing 5N NaOH (10 mL) was stirred for 2 h at room temperature. Filtration gave a precipitate which was washed with methanol (20 mL) and dissolved in 2 N H_2SO_4 (40 mL). The resulting solution was extracted with chloroform. The chloroform extract were dried over Na_2SO_4 and concentrated *in vacuo* to afford acetopyruvate as a yellow crystalline solid (1.7 g, 52 %). ^{1}H NMR ($CDCl_3$) 2.26 (s, 3H), 6.45 (s, 1H); ^{13}C NMR ($CDCl_3$) 26.3, 100.7, 163.3, 169.8, 197.5. the spectroscopic data of this substance matched those previously reported (2).

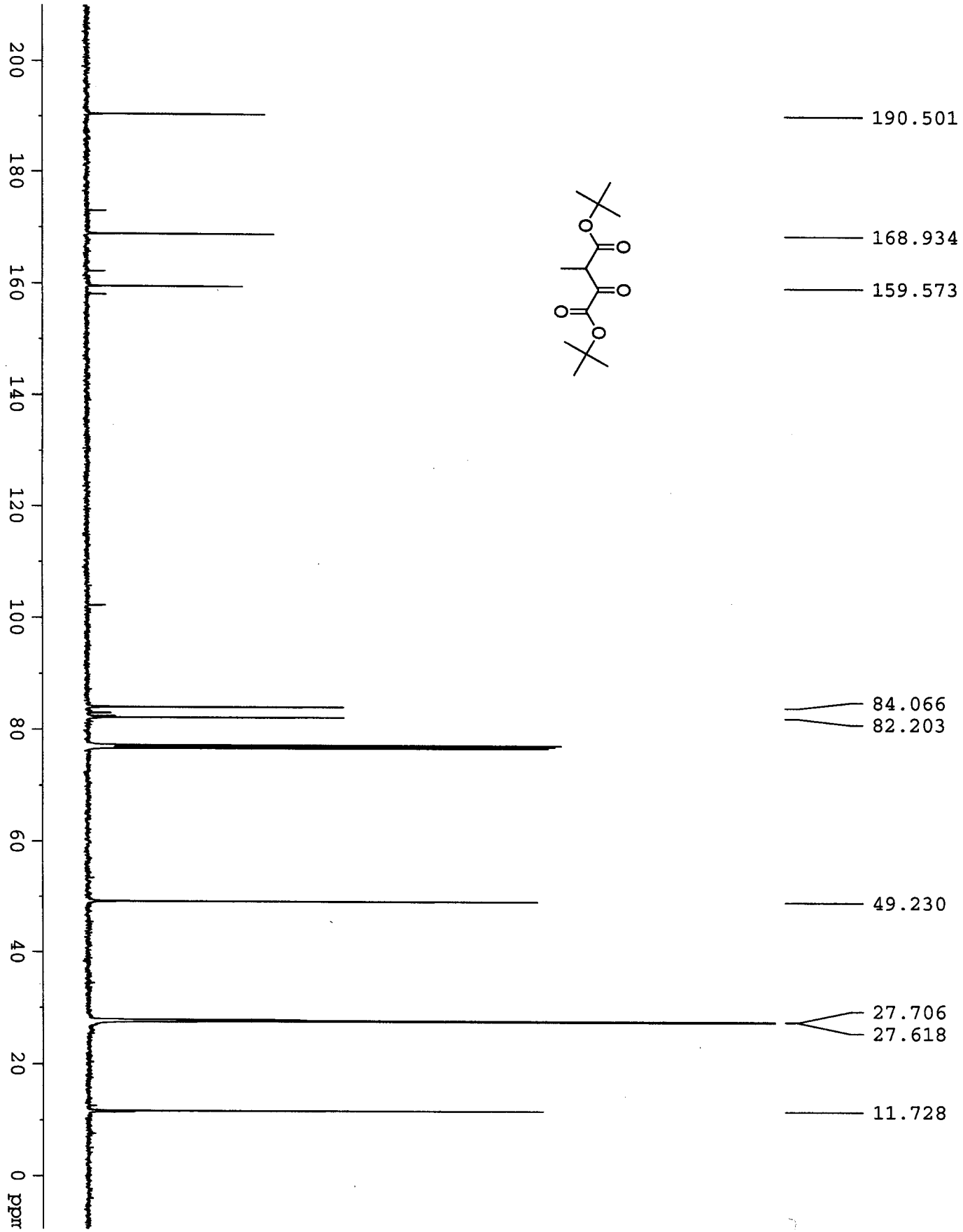
(2) Brecker, L., Pogorevc, M., Griengl H., Steiner, W., Kappe, T. and Ribbons,D.W. (1999) *New J. Chem.* **23**,437-446.

S-4

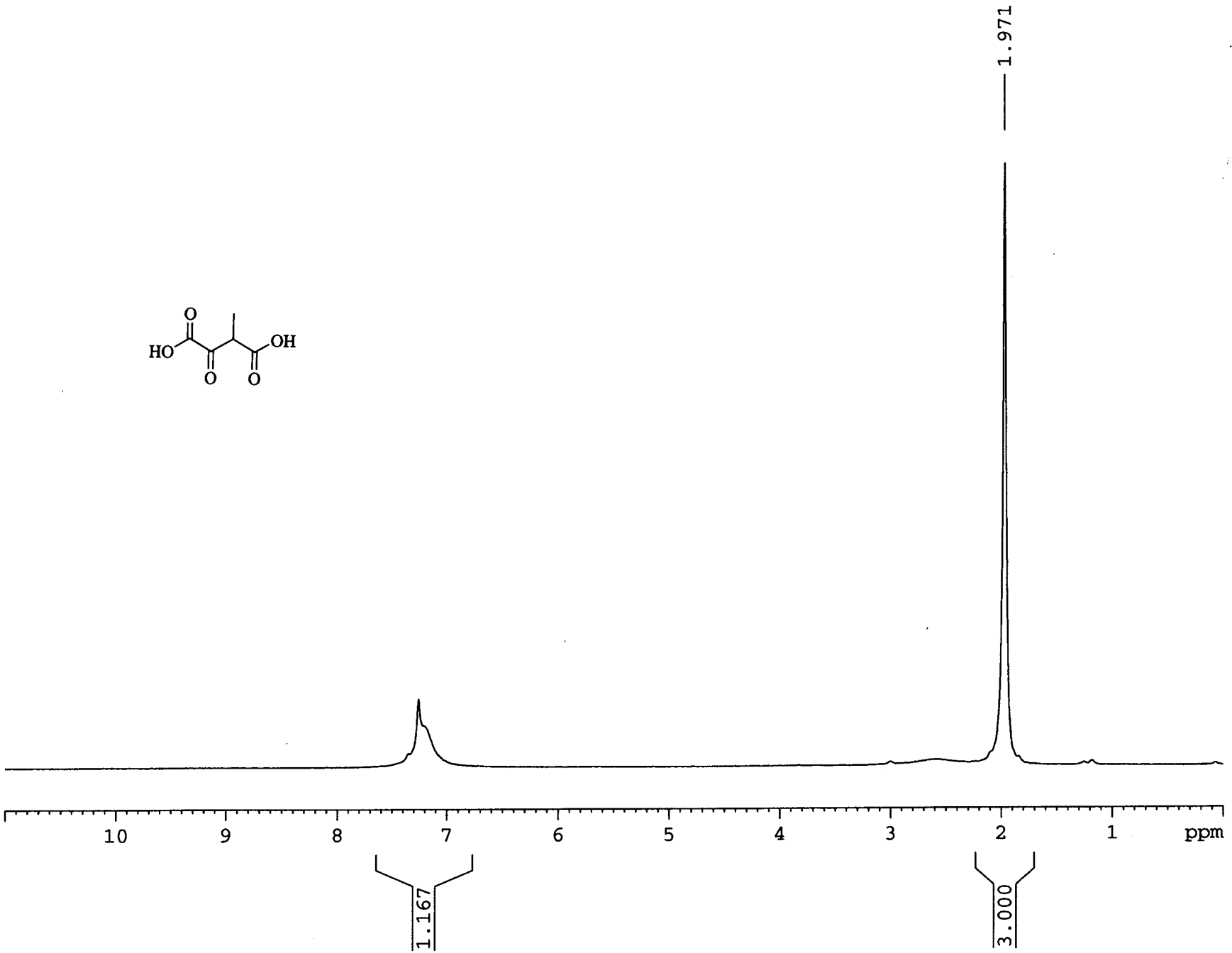


3.931
3.921
3.917
1.532
1.419
1.339
1.330
1.325
10
9
8
7
6
5
4
3
2
1
ppm
0.856
8.858
9.000
2.927

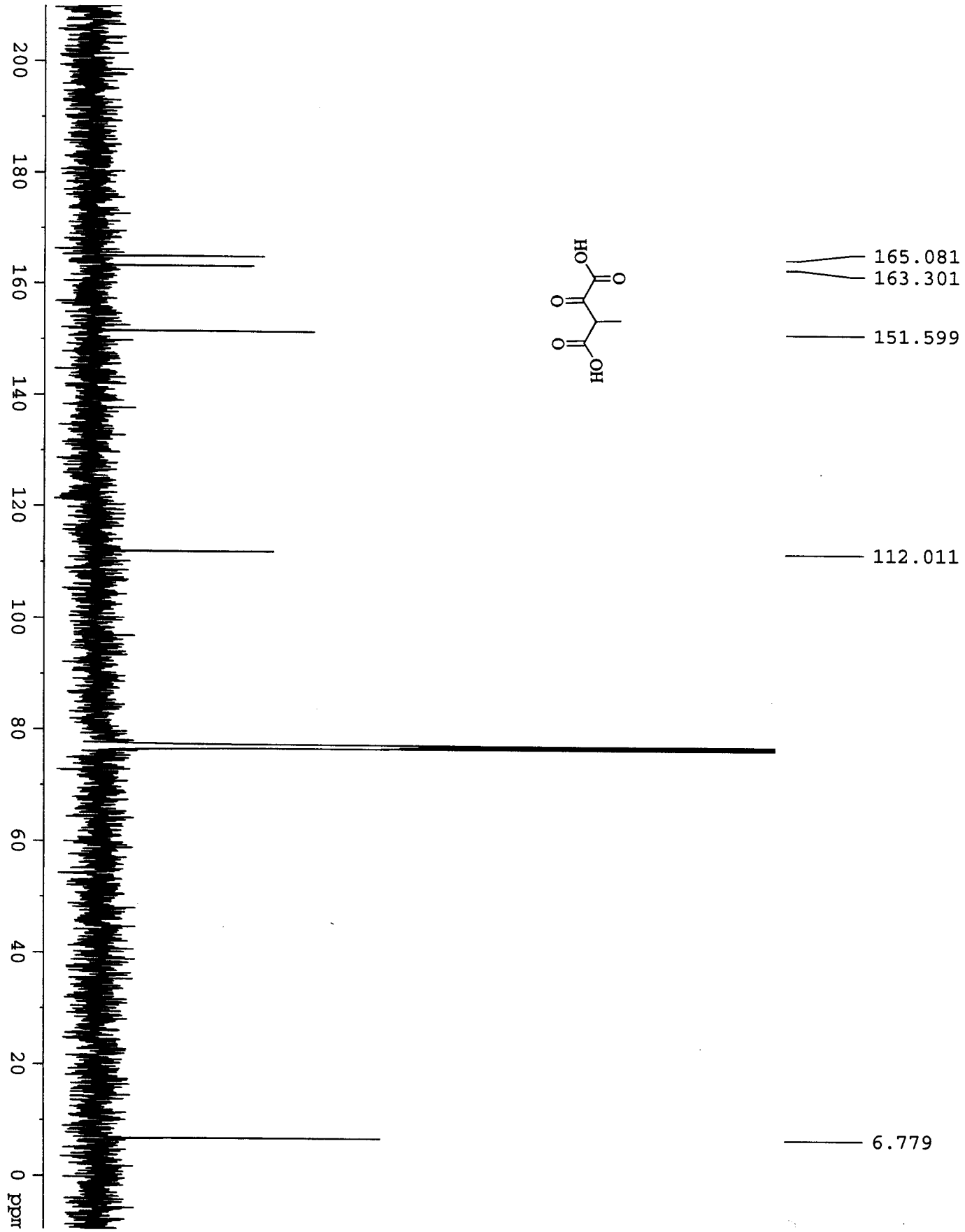
S-S


200
180
160
140
120
100
80
60
40
20
0 ppm
190.501
168.934
159.573
84.066
82.203
49.230
27.706
27.618
11.728

S-9



L-S


200
180
160
140
120
100
80
60
40
20
0 ppm
OH
O
O
O
OH
165.081
163.301
151.599
112.011
6.779