Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry


# Rational substrate and enzyme engineering of transketolase for aromatics

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## Supplementary Information

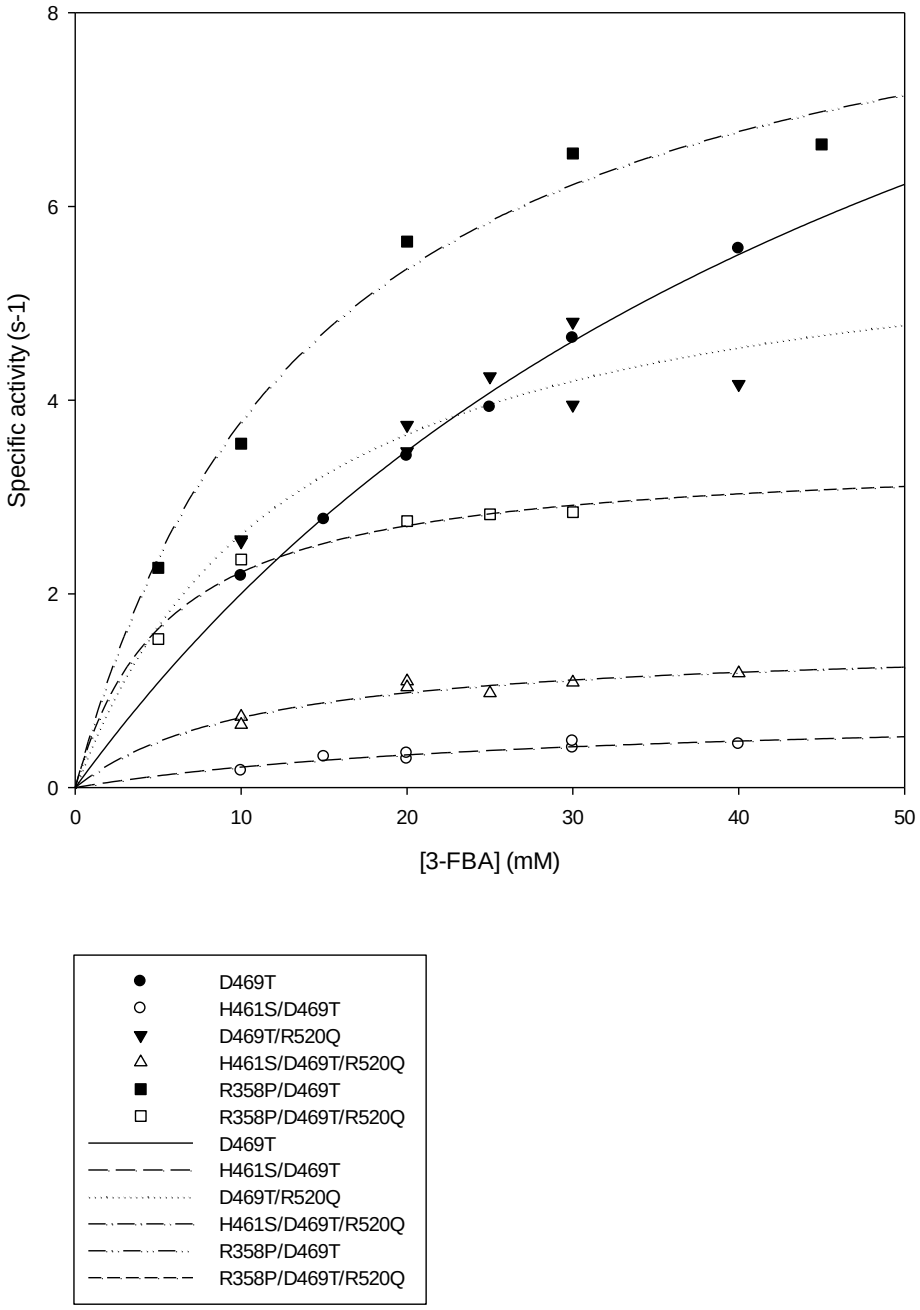



**Figure S1.** The Michaelis-Menten plot between 3-FBA concentration and the specific activities of each mutant.

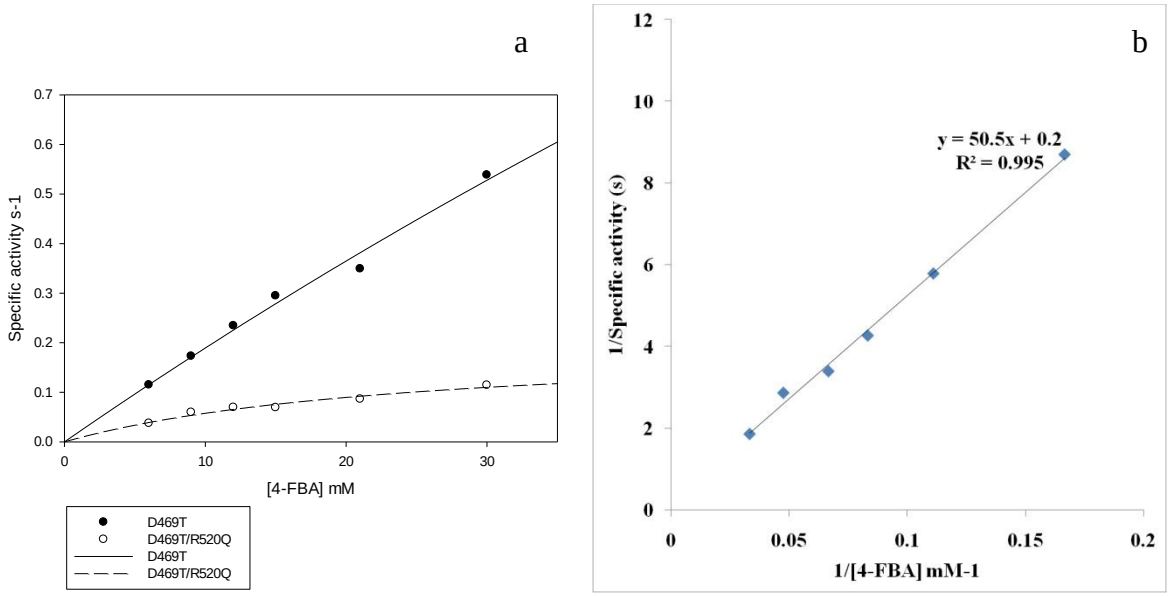



**Figure S2.** a) The Michaelis-Menten plot between 4-FBA concentrations and the specific activities of D469T and D469T/R520Q. b) Lineweaver-Burk plot between 1/[4-FBA] and 1/specific activity of D469T was also added for comparison.

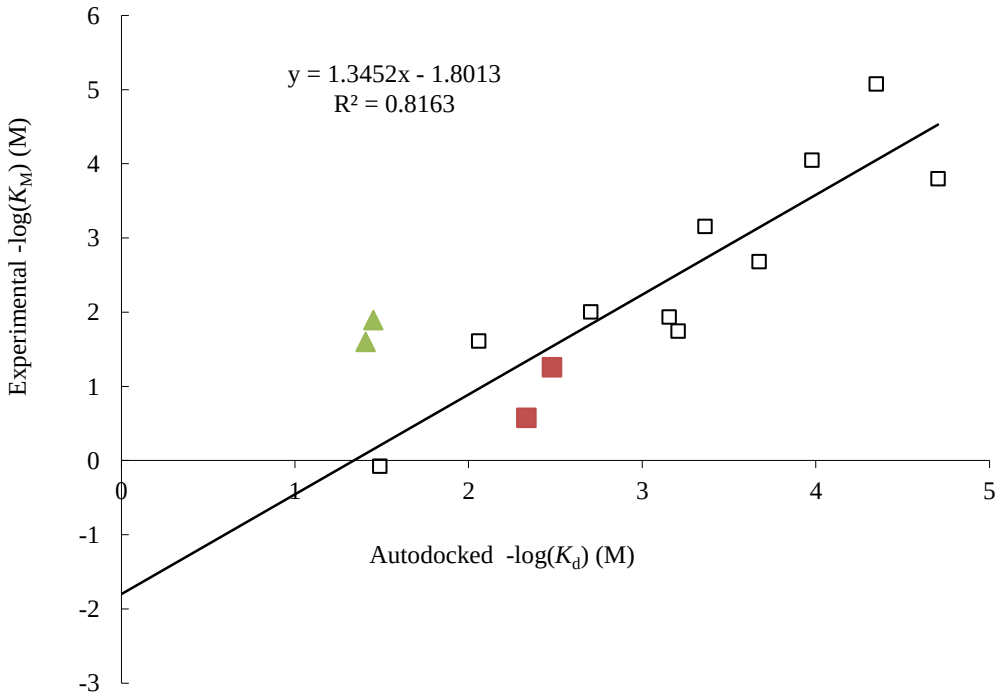
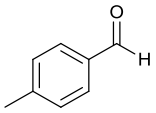
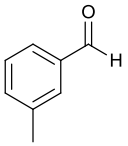
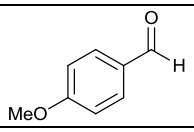
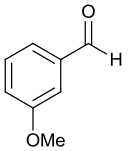
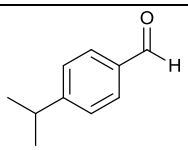
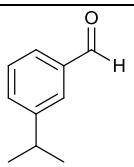



**Figure S3**. Comparison of binding constants calculated using AutoDOCK, to those determined experimentally for (□) *E. coli*wild-type, (■) D469T and (▲) D469T/R520Q transketolases with a range of natural and non-natural substrates. Line of best fit shown for wild-type TK values only.

**Table S1.** The 24-hour Bioconversion yield of other aldehyde substrates by D469T. The reaction was performed at 20 mL reaction scale with 50 mM of aldehyde, 50 mM HPA, 1 mg/mL D469T, in water, pH 7.0 similar to the bioconversion of 3-FBA **2b** and 4-FBA **2c**.

| Substrate | D469T |
|---|---|
|  | No conversion |
|  | No conversion |
|  | No conversion |
|  | No conversion |
|  | No conversion |
|  | No conversion |

**HPA degradation study**

**Table S2**. The percentage of HPA left after 18 hours with 50 mM HPA in various control conditions. Various lysates, and also a no lysate sample, were assessed both with and without ThDP, after 18 hours in 50 mM Tris buffer pH 7.0. Aldehyde acceptors were not added to any of the reactions. HPA concentrations were analysed by HPLC with the same method used for screening for 3-FBA **2b** and 4-FBA **2c**.

| **Reaction conditions** | **% HPA left** |
|---|---|
| D469T with ThDP | 5.7 |
| D469T/R520Q with ThDP | 5.7 |
| 50 mM Tris | 92 |
| 50 mM Tris with ThDP | 95 |
| D469T without ThDP | 98 |
| D469T/R520Q without ThDP | 89 |
| WT with ThDP | 86 |
| WT without ThDP | 92 |
| XL10 Gold with ThDP | 91 |
| XL10 Gold without ThDP | 97 |

**Table S3.** Comparison of *E. coli* TK $K_d$ values obtained by docking with $K_M$ from experiments.

| **Substrate** | $K_d$ (calc) (mM) | $K_M$ (exp) [a] (mM) | Distance to nucleophile [g] (Å) |
|---|---|---|---|
| D-Ribose | 0.47 | 0.7 [b] | 2.64 |
| D-Ribose-5-phosphate | 0.05 | 0.0084 [c] | 3.13 |
| D-Erythrose | 0.67 | 18.15 [d] | 3.93 |
| D-Erythrose-4-phosphate | 0.12 | 0.09 | 4.28 |
| D-Glyceraldehyde | 2.10 | 10 [e] | 2.33 |
| D-Glyceraldehyde-3-phosphate | 0.23 | 2.1 [e] | 5.01 |
| Glycolaldehyde | 9.14 | 24.5 [f] | 2.66 |
| Acetaldehyde | 33.6 | 1200 | 2.69 |
| Hydroxypyruvate | 3.61 | 11.65 [f] | 3.14 |
| D-Xylulose-5-phosphate | 0.022 | 0.16 | 4.73 |

[a] Experimental data obtained by Sprenger et al [1]
[b] adjusted by equilibrium for 0.05% acyclic form [2]
[c] adjusted by equilibrium for 0.6% acyclic form [3]
[d] adjusted by equilibrium for 12.1% acyclic form [4]
[e] Experimental data obtained with D,L racemate.
[f] average of independent values from Sprenger et al [1] and Hibbert et al [5]
[g] distances measured between either a) aldehyde carbon atom of aldol acceptor and the ThDP-enamine nucleophile, or b) carbonyl carbon atom of ketol donor and the ThDP thiazolium C2 nucleophile.

## Computational Docking Method

*AutoDock 3.0.5*. The open source AutoDock software version 3.0.5 was used for all the automated docking reported in this paper. AutoDock combines a Lamarckian Genetic algorithm with an empirical free energy function to obtain ligand docked conformations (*13*). Substrate docking models were obtained using the *E. coli* TK structure 1qgd.pdb with a cubic grid in the active site of sides 80 Å. Defaults were used for docking each substrate except for the following: the maximum number of energy evaluations was increased to 1 million, the number of genetic algorithm runs was increased from 10 to 200, and the grid spacing used was 0.375 Å. AutoDock performed a cluster analysis to each final conformation obtained from the 200 GA runs such that two conformations with an RMSD less than 0.5Å are stored in the same cluster. Clusters are output in ranked order of increasing energy following completion of analysis. Manual visual analysis of docked conformations and further analysis of the docked conformations was carried out with Pymol and Ligplot.

*Docking of D-erythrose 4-phosphate in yeast TK*. D-erythrose 4-phosphate (DE4P) was removed from the yeast TK PDB file 1NGS. AutoDock was used to re-dock the substrate back into the binding site. Grid centre and size used for AutoDock run: (-12.645, 56.02, 19.419) 80Åx80Åx80Å.

*Docking of D-erythrose 4-phosphate in E. coli TK*. DE4P was docked into the binding site of *E. coli* TK (1QGD). Grid centre and size used for AutoDock run: (-10.6, 27.6, 36.4) 80Åx80Åx80Å.

*Creation of a model of the ThDP-enamine intermediate in E. coli TK.* The ThDP-enamine intermediate was docked into *E. coli* TK (1QGD). Grid centre and size for AutoDock run: (-10.0, 28.1, 36.0) 80Åx80Åx80Å.

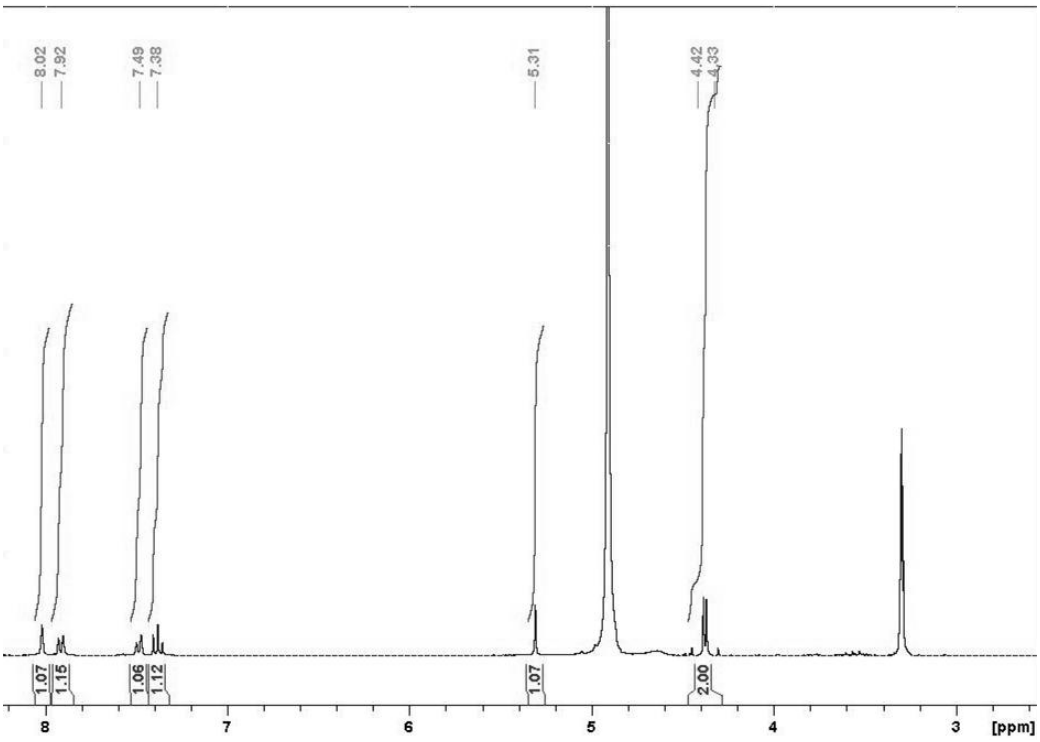
*Docking DE4P and glycolaldehyde in ThDP-enamine complexed forms of yeast and E. coli TK.* DE4P was docked into the yeast ThDP-enamine-TK complex (1GPU) and in the modelled *E. coli* ThDP-enamine-TK complex. Glycolaldehyde (GA) was docked into the modelled *E. coli* ThDP-enamine-TK complex. Grid centres and sizes were (-6.6, 56.7, 18.4) 60Åx60Åx60Å for DE4P in yeast ThDP-enamine-TK, and (-11.4, 26.3, 36.4) 60Åx60Åx60Å for DE4P and GA in *E. coli* ThDP-enamine-TK.

*Docking of natural and non-natural aldehydes substrates into E. coli TK.* PDB files for the ten TK substrates for which there are published $K_m$ values, and also fluoropyruvate, found to be a potential inhibitor (unpublished data), were generated using the Dundee PRODRG server (*14*). Each substrate was docked into the active site of *E. coli* TK. Preliminary docking identified two docking regions within the binding funnel of *E. coli* TK for some of these substrates. Grid sizes and positions were altered to obtain docked conformations for each substrate in the binding region closest to the ThDP cofactor. For some substrates the grid centres were adapted to avoid inaccessible pocket "traps" within the protein. Grid centres and sizes were as follows (grid centres in brackets):

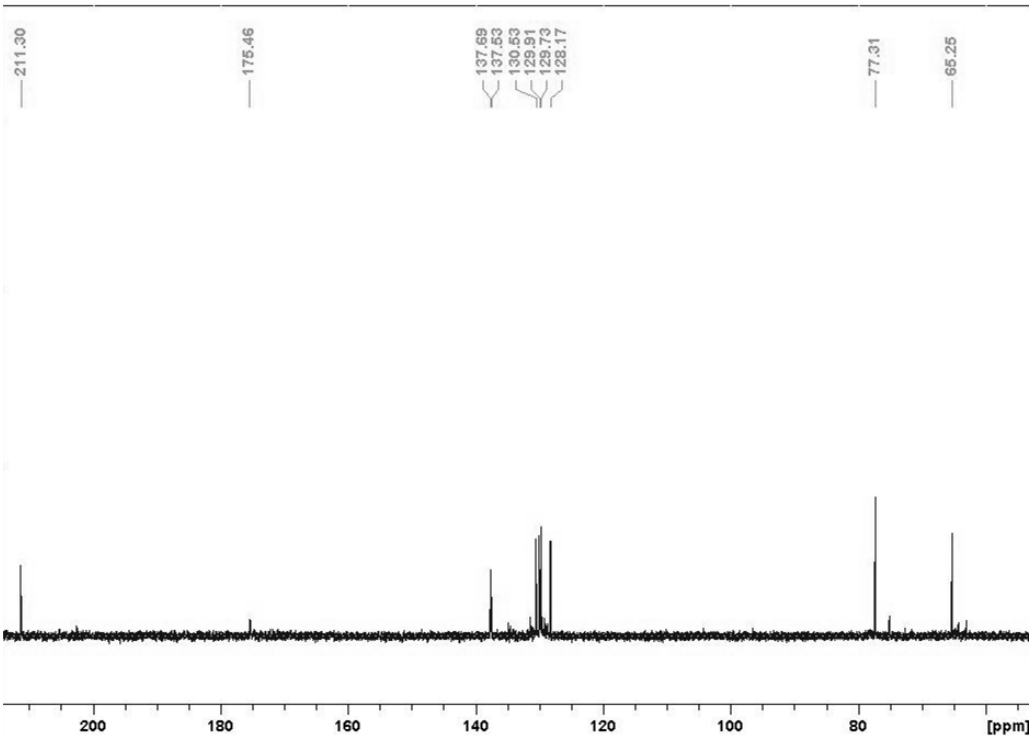
| | |
|---|---|
| Hydroxypyruvate: | (-15.991 21.945 37.096) 60Åx60Åx60Å |
| Acetaldehyde: | (-18.344 24.016 40.547) 60Åx60Åx60Å |
| D-erythrose 4-phosphate: | (-18.344 24.016 40.547) 40Åx40Åx40Å |
| D-erythrose: | (-18.344 24.016 40.547) 40Åx40Åx40Å |
| D-glyceraldehyde 3-phosphate: | (-10.586 27.153 35.586) 80Åx80Åx80Å |
| D-glyceraldehyde: | (-18.344 24.016 40.547) 40Åx40Åx40Å |
| D-ribose 5-phosphate: | (-10.586 27.153 35.586) 80Åx80Åx80Å |
| D-ribose: | (-10.586 27.153 35.586) 80Åx80Åx80Å |
| Glycolaldehyde: | (-15.991 21.945 37.096) 60Åx60Åx60Å |
| Xylulose 5-phosphate: | (-10.586 27.153 35.586) 80Åx80Åx80Å |
| Fluoropyruvate: | (-15.991 21.945 37.096) 60Åx60Åx60Å |

## 3-(1,3-Dihydroxy-2-oxopropyl)benzoic acid 3b

$^{1}$H NMR spectrum




$^{13}$C NMR spectrum



## 4-(1,3-Dihydroxy-2-oxopropyl)benzoic acid 3c

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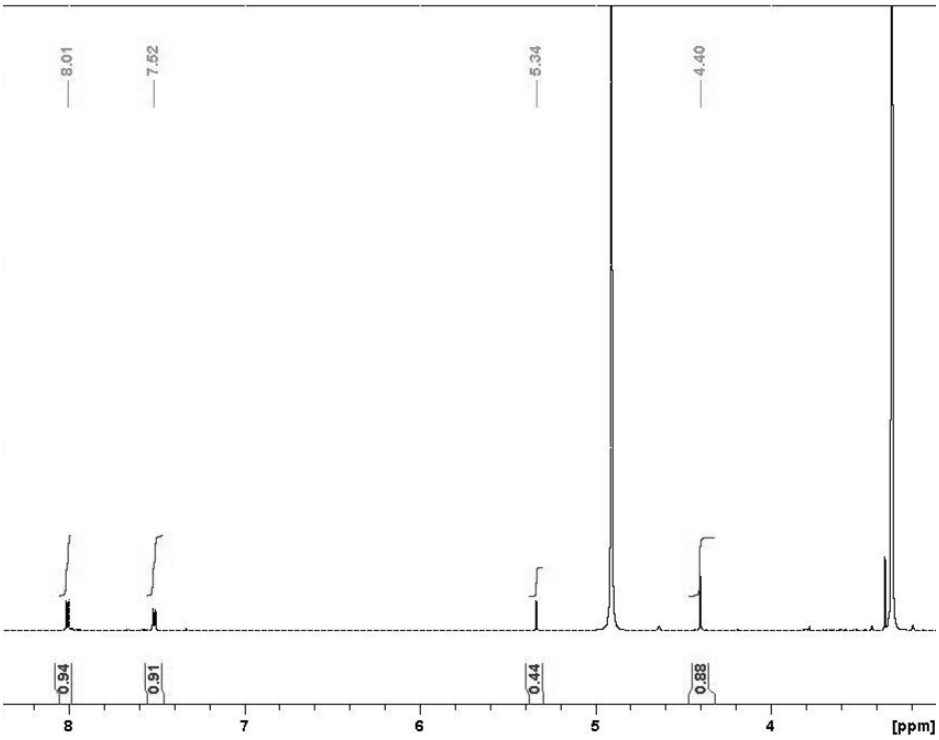
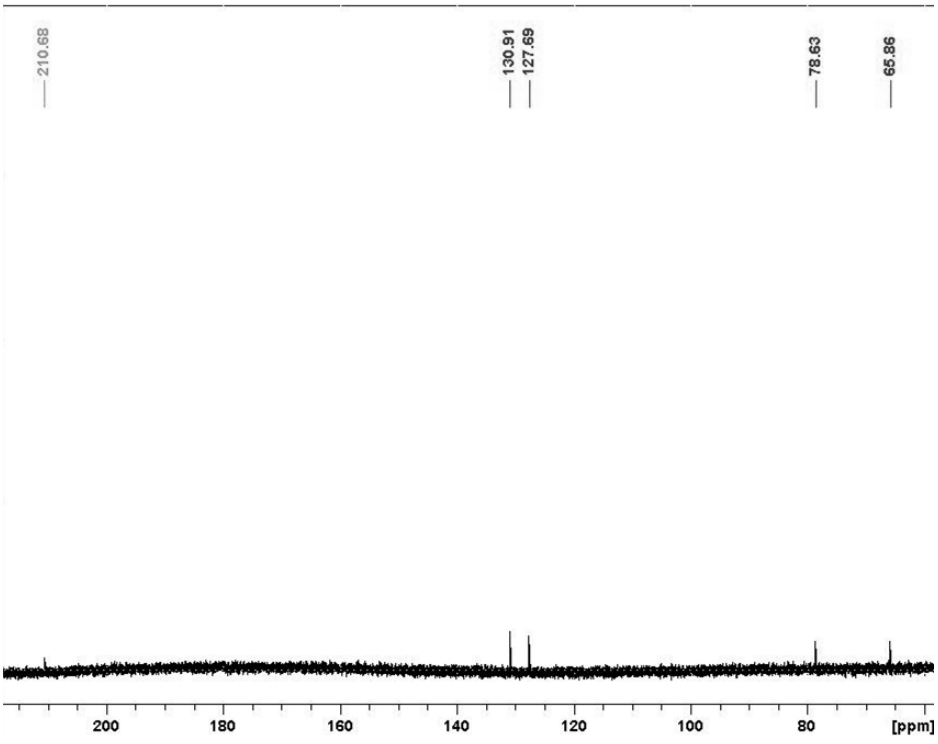



$^{13}$C NMR spectrum



References

1. G. A. Sprenger, U. Schörken, G. Sprenger and H. Sahm, *European journal of biochemistry / FEBS*, 1995, **230**, 525-532.
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