Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information for:

Characterization of DcsC, a PLP-independent racemase involved in the biosynthesis of D-cycloserine

David Dietrich, Marco J. van Belkum, John C. Vederas

Department of Chemistry, University of Alberta
11227 Saskatchewan Dr.
Edmonton, AB, Canada, T6G 2G2

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1. Sequence alignment of DcsC with DapF genes

Sequences for the following enzymes were submitted to ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/), for alignment:

Protein accession code: AAM42769 [DapF, *Xanthomonas campestris* (X.c)]
AAM83223.1 [DapF, *Arabidopsis thaliana* (A.t)]
BAI70377 [DcsC, *Streptomyces lavenduale* (S.l)]

```
DapF (X.c)      ------------------------------------------------------------
DapF (A.t)      MEIAAVSTVSVAPQSRRVSNAFSRNLGSVSSLSFGFFEKEYCFKSPSLRVSAAASMDAVT 60
DcsC (S.l)      ------------------------------------------------------------

DapF (X.c)      --------MSADVHTGRLRFTKMHGAGNDFVVLDLRNGT-PPPDASLAARLADRHFGVGC 51
DapF (A.t)      AEKFSPASFLDKKETGVLHFVKYHGLGNDFILVDNRDSSEPKITQEQAAKLCDRNFGVGA 120
DcsC (S.l)      --------MIRMRTPSTLPFTKMHGAGNDFVVLDLRDG--PDPSPELCRALADRHKGVGC 50

DapF (X.c)      DQILTIETPRSAEAVAAYRIWNSDGSHSQQCGNGARCVAAWLVREGTAQGDV-FTIDSPF 110
DapF (A.t)      DGVI-FAMPGVNGTDYAMRIFNSDGSEPEMCGNGVRCFARFIAELENLQGKHSFTIHTGA 179
DcsC (S.l)      DLVLGIREPRSARAVAAFDIWTADGSRSAQCGNGARCVAAWAVRAGLARGPR-FALDSPS 109


DapF (X.c)      TAHRVERLDAGTYSVAMGVPQFEPTQIP--LAGFAHARDEYALPV-HGETVRFGAVSMGN 167
DapF (A.t)      GLIVPEIQDDGQVKVDMGTPILKAQDVPTKLSGNKGEAVVEAELVVDGVSWNVTCVSMGN 239
DcsC (S.l)      GTHEVDVLDADTFRVALAVPRFAPESIP--LFGHDGEQDLYEADLGDGTRVRFAAVSMGN 167

DapF (X.c)      PHAVVEVG------RVDAAPVERVGALLQQNAAFPESVNVGFAQVVDPAHVRLRVYERGV 221
DapF (A.t)      PHCITFGKKGGPNLKVDDLNLPEIGPKFEHHEMFPARTNTEFVEVLSRSHLKMRVWERGA 299
DcsC (S.l)      PHAVIEVD------DTATAPVARVGRAVQASGLFLPTVNVGFARVESRDRVHLRVHEYGA 221

Dap-F (X.c)      GETLACGSGACAAAVVLMHRGRVERDVRVSLPGGELRIRWAGEQAQVVMSGPAVFVFDGE 281
Dap-F (A.t)      GATLACGTGACALVVAAVLEGRADRKCTVDLPGGPLEIEWKQEDNHIYMTGPAEAVFYGS 359
Dcs-C (S.l)      GETLACGSGACAAAVLMRRGRVDRNVSVVLPGGELRISWPDDAADVLMTGPAAFVYEGT 281

Dap-F (X.c)      WNQ--- 284
Dap-F (A.t)      ALL--- 36
Dcs-C (S.l)      FLHASV 287
```

Fig. S1. Amino acid sequence alignment of DcsC and DapF from two organisms. Identical residues are marked in yellow; active site cysteine residues are marked in red.

2. SDS-PAGE purification of DcsC

Lane: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

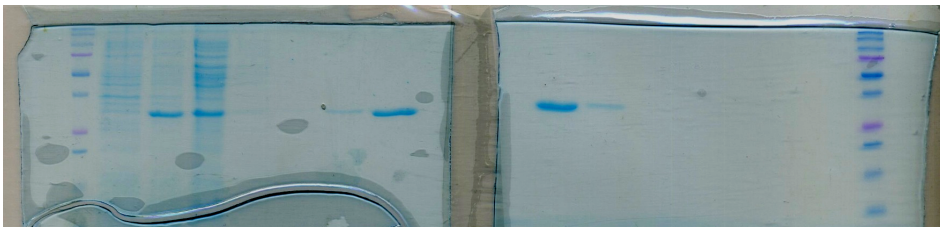

Fig. S2. Following elution from Ni-NTA column, an SDS-PAGE gel was used to analyze the purification. This is a typical get that was the result of the purification of DcsC. Lanes 8 and 9, containing pure DcsC, were collected and dialyzed as described in the text.

LEGEND:

Lane 1 and 16: Molecular weight markers
Lane 2: Cell lysate, pre-induction
Lane 3: Cell lysate, post-induction
Lane 4: Ni-NTA column flow-through, following binding
Lane 5: 1st Ni-NTA column wash with lysis buffer with 10 mM imidazole
Lane 6: 2nd Ni-NTA column wash with lysis buffer with 10 mM imidazole
Lane 7: lysis buffer with 25 mM imidazole
Lane 8: lysis buffer with 50 mM imidazole
Lane 9: lysis buffer with 75 mM imidazole
Lane 10: lysis buffer with 100 mM imidazole
Lane 11: lysis buffer with 125 mM imidazole
Lane 12: lysis buffer with 150 mM imidazole
Lane 13: lysis buffer with 200 mM imidazole
Lane 14: lysis buffer with 250 mM imidazole
Lane 15: lysis buffer with 300 mM imidazole

3. MS-MS sequencing of DcsC

Protocol:
Samples of DcsC (25 μg) were run on a 15 % SDS-PAGE gel, and stained using Coomassie Blue. The bands corresponding to DcsC were excised from the gel (~10 μg/band), and digested in-gel for analysis. Samples were dehydrated by consecutive washes with MeCN (500 μL/wash), and dried in a speed vac. Any disulfides in the enzyme were reduced by treatment with DTT (4.5 mM) in NH_4HCO_3 (0.1 M) at 50 °C for forty-five minutes. Free thiols were labeled by treatment with iodoacetic acid (11 mM final) for thirty minutes at room temperature in the dark. The gel slices were then dehydrated following the same protocol described above. Digestion of the enzyme was carried out by incubation of the dried gel slice in a solution of trypsin (10 ng/mL) in NH_4HCO_3 (0.1 M) at 37°C for three hours. Fragments were recovered from solution, and the gel band was washed twice with MeCN-H_2O-TFA (300 μL, 1:1:0.001). Extracts were combined and dried in a speed vac. Finally, the fragments were suspended 0.1% aqueous formic acid (15 μL), and submitted for MS-MS analysis. Results were analyzed using MASCOT (Matrix Science).

Sequence Coverage: 75%

```
   1 MIRMRTPSTL PFTKMHGAGN DFVVLDLRDG PDPSPELCRA LADRHKGVGC
  51 DLVLGIREPR SARAVAAFDI WTADGSRSAQ CGNGARCVAA WAVRAGLARG
 101 PRFALDSPSG THEVDVLDAD TFRVALAVPR FAPESIPLFG HDGEQDLYEA
 151 DLGDGTRVRF AAVSMGNPHA VIEVDDTATA PVARVGRAVQ ASGLFLPTVN
 201 VGFARVESRD RVHLRVHEYG AGETLACGSG ACAAAAVLMR RGRVDRNVSV
 251 VLPGGELRIS WPDDAADVLM TGPAAFVYEG TFLHASV
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Fig. S3. The following sequence was generated based on the fragments that were identified in MS-MS (shown in red):

4. ^{1}H-NMR assay of DcsC activity with *O*-ureido-D-serine

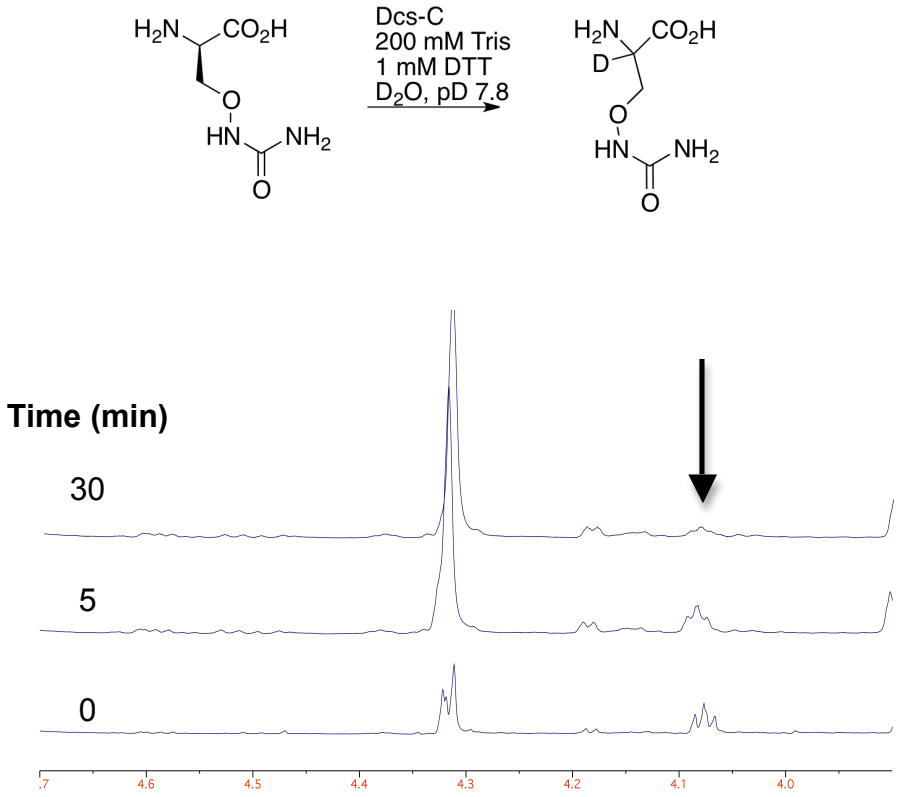


Fig. S4. To a solution of **1-D** (10 mg, 0.06 mmol), in Tris buffer (700 μL, 200 mM, pD 8.0) containing DTT (1 mM), was added DcsC (18 μg). The ^{1}H-NMR spectrum was recorded periodically, and the disappearance of the α-proton at ~ 4.1 ppm was noted.

5. ^{2}H-NMR of *O*-ureido-D-serine after racemization in deuterated buffer

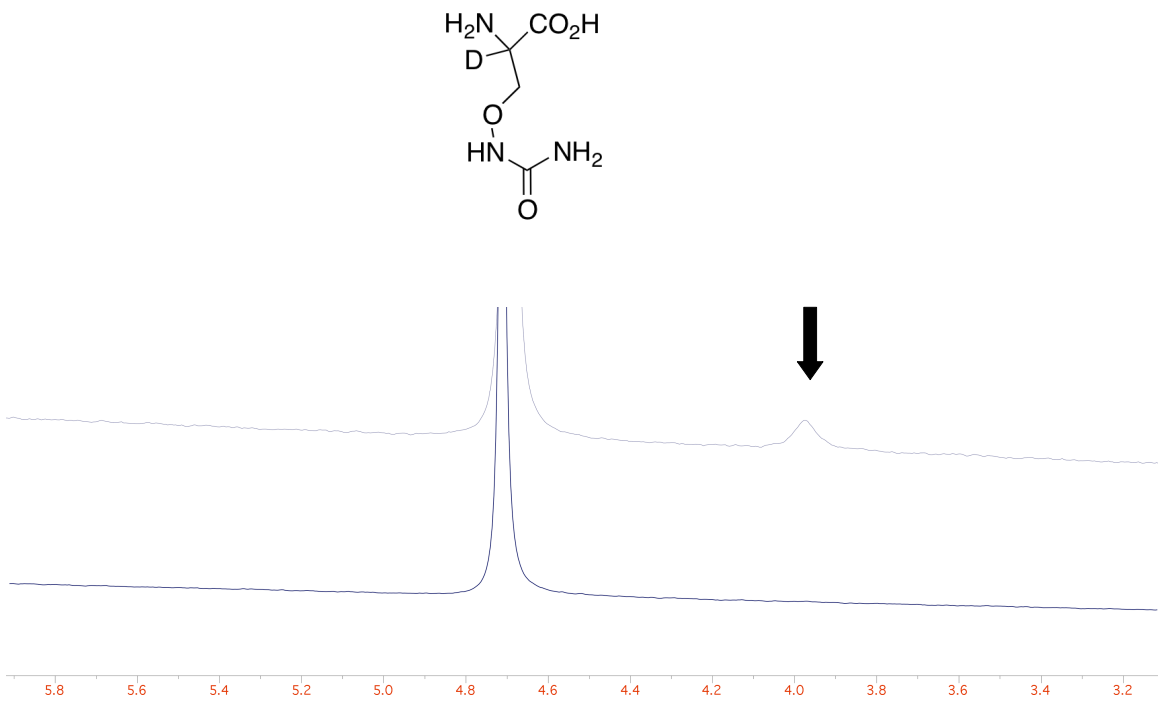


Fig. S5. Following reaction of **1-D** with DcsC in deuterated buffer, the ^{2}H-NMR was recorded (top). The α-deuterium peak is noted at ~ 4.0 ppm. When the enzyme was inhibited with iodoacetamide, no incorporation of deuterium was observed (bottom).

6. ^{1}H-NMR assay of DcsC substrate selectivity

Amino acids were dissolved in deuterated Tris buffer (670 μL, 20 mM, pD 7.8) containing DTT (1 mM) to a final concentration of 0.1 M. Stock solutions of DcsC (30 μL, 0.1 μg/μL) were added to initiate the reaction, and the ^{1}H-NMR spectra were recorded after thirty minutes. The following amino acids were tested: **1-L**, L-Hse, L-Asn, L-Lys, L-Arg and L-Ser.

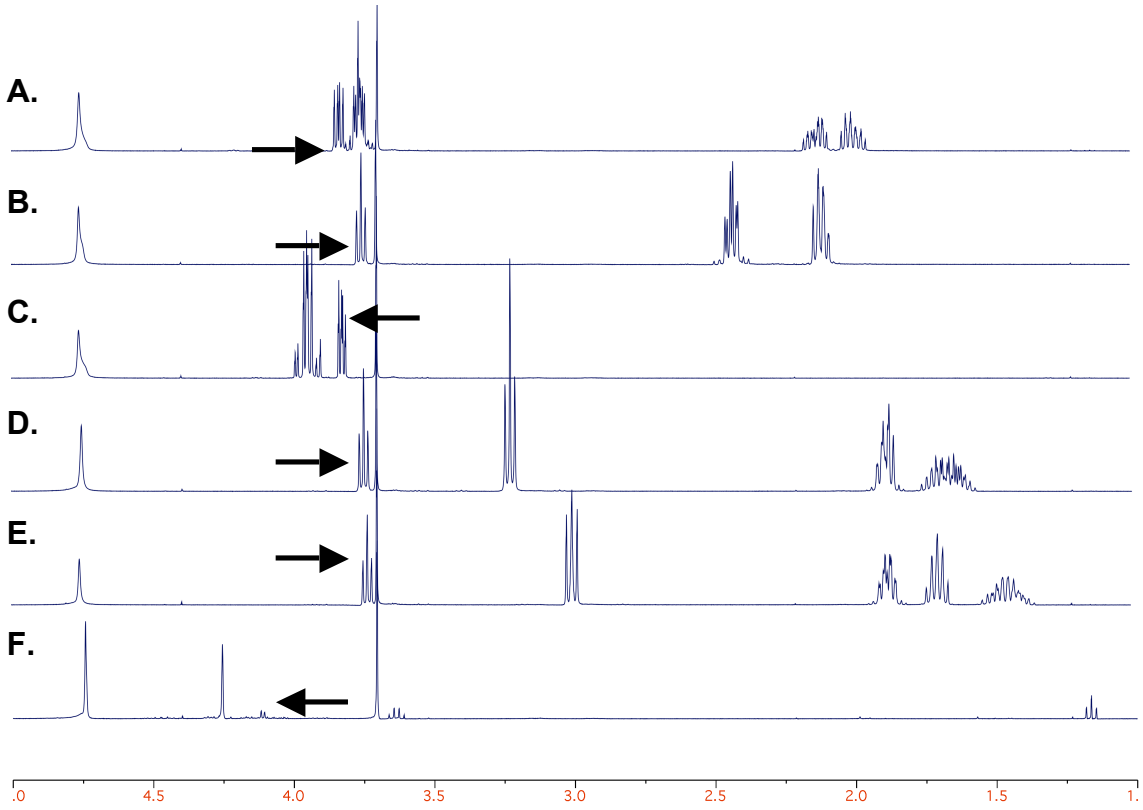


Fig. S6. The ^{1}H-NMR spectra that were obtained following incubation of various amino acids with DcsC. The α-proton for each amino acid is indicated with an arrow. A. L-Hse. B. L-Gln. C. L-Ser. D. L-Arg. E. L-Lys. F. **1-D**.

7. ^{1}H-NMR assay of DcsC reaction with 1-D in the presence of inhibitor 6

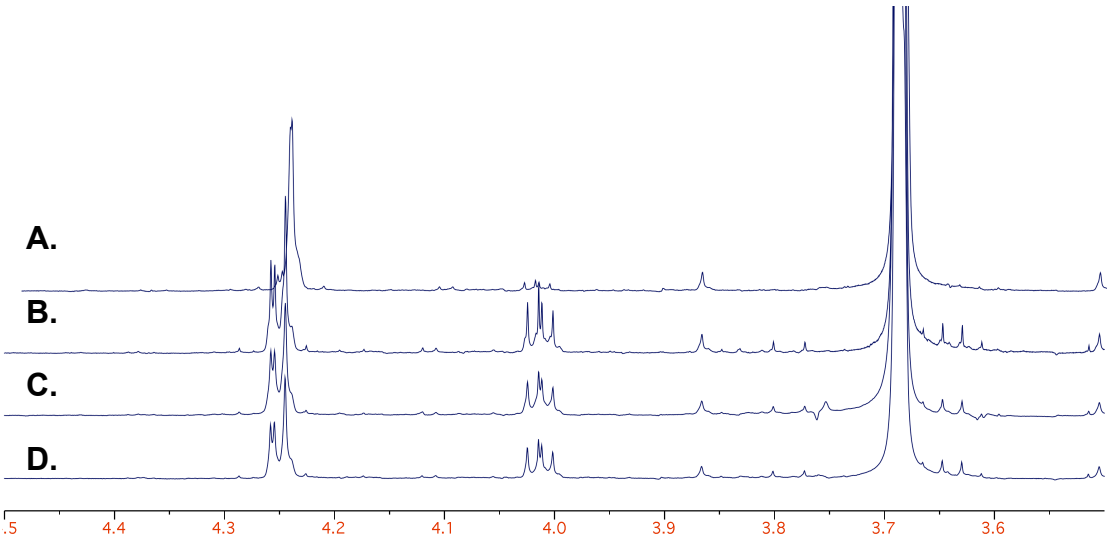
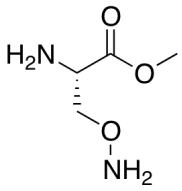


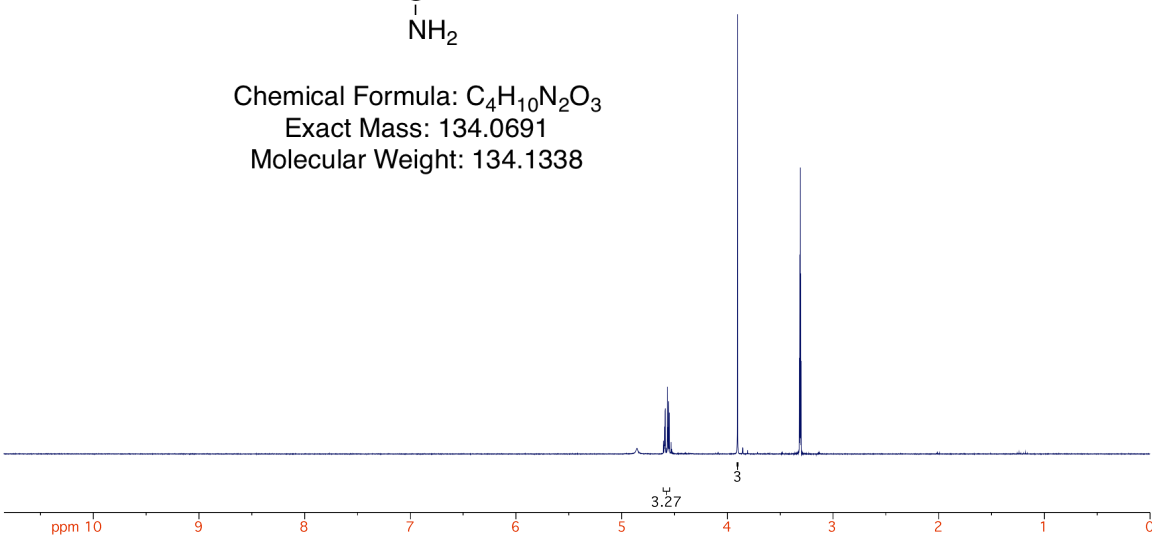
Fig S7. ^{1}H-NMR spectra obtained following incubation of **1-D** with DcsC. A. Spectrum obtained following thirty-six hour incubation with untreated DcsC. B. Spectrum obtained following five-minute incubation with DcsC, which had been pre-treated with inhibitor **6**. C. Spectrum obtained following one hour incubation with DcsC, which had been treated with inhibitor **6**. D. Spectrum obtained following thirty-six hour incubation with DcsC, which had been treated with inhibitor **6**.

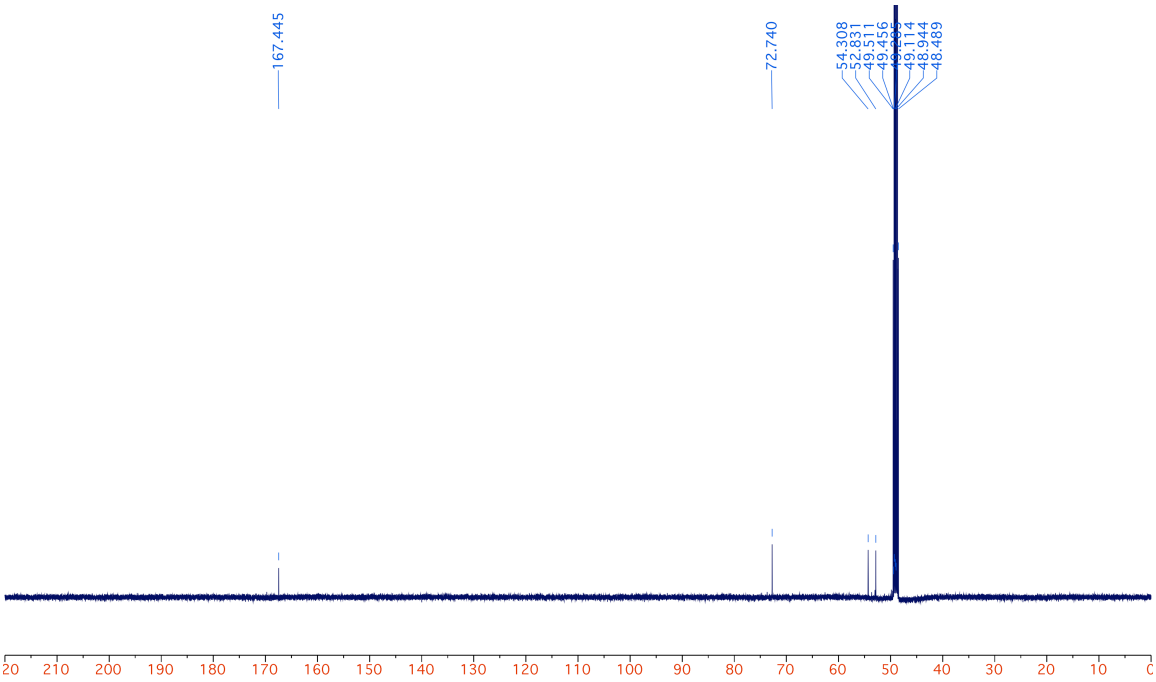
8. ^{1}H- and ^{13}C-NMR characterization

L-aminoserine methyl ester dihydrochloride (**5-L**)

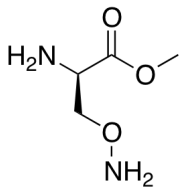


Chemical Formula: $C_4H_{10}N_2O_3$
Exact Mass: 134.0691
Molecular Weight: 134.1338





D-aminoserine methyl ester dihydrochloride (**5-D**)



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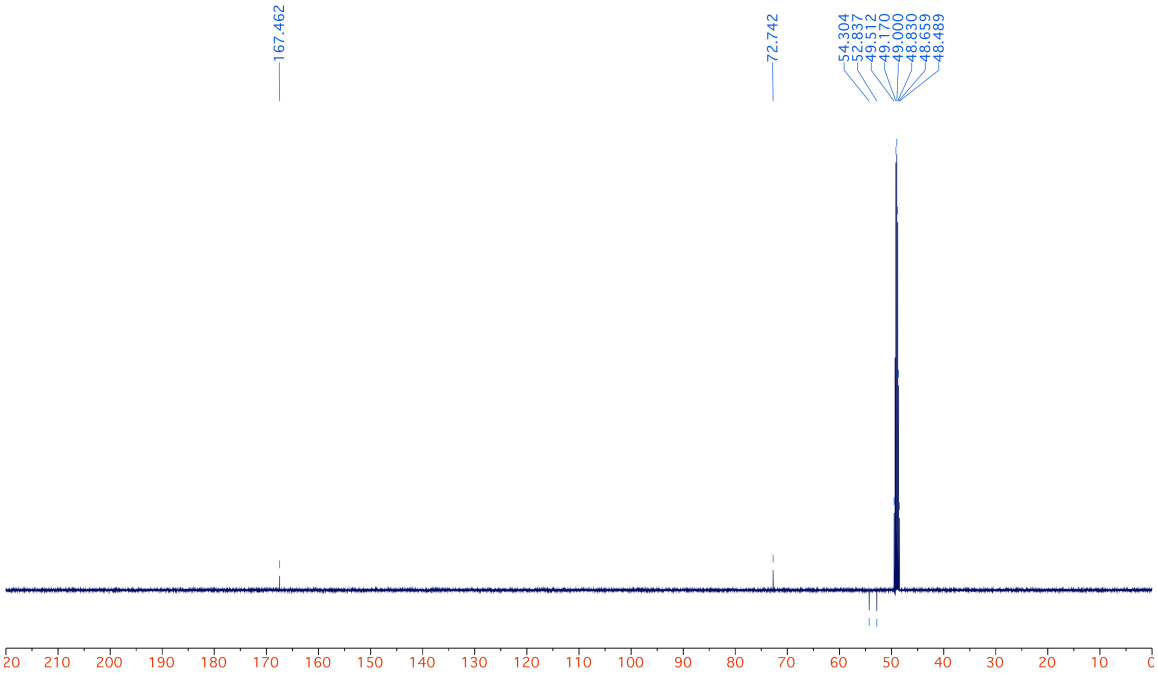
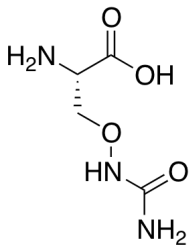
Exact Mass: 134.0691
Molecular Weight: 134.1338



L-ureidoserine (**1-L**)



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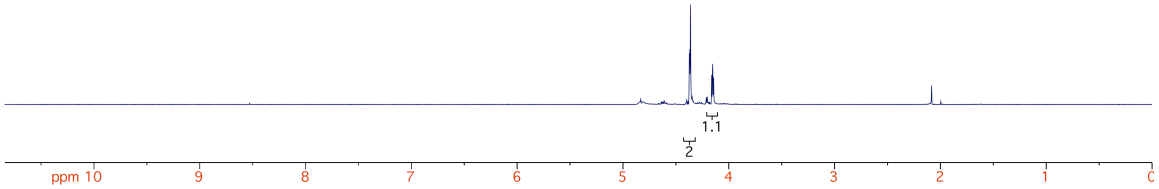
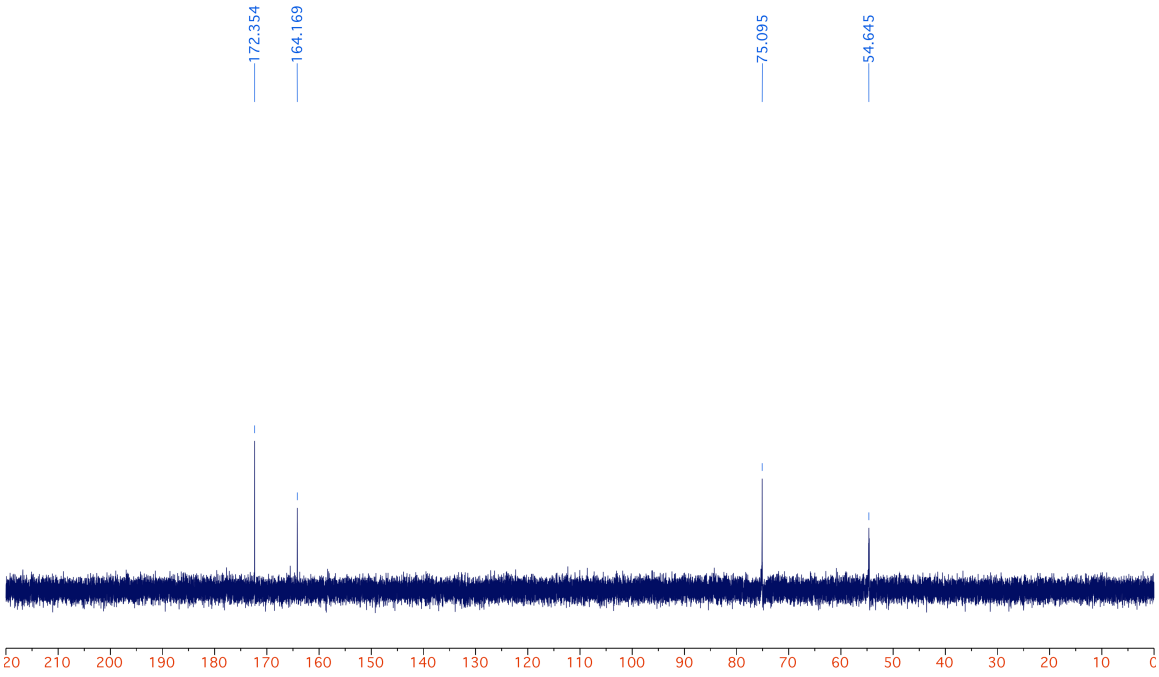
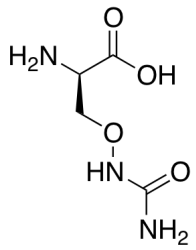




D-ureidoserine (**1-D**)



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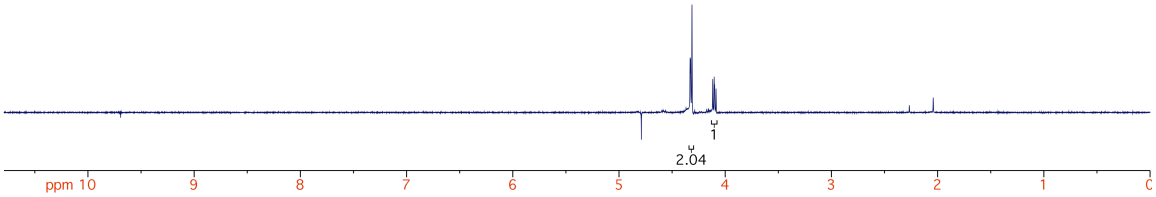
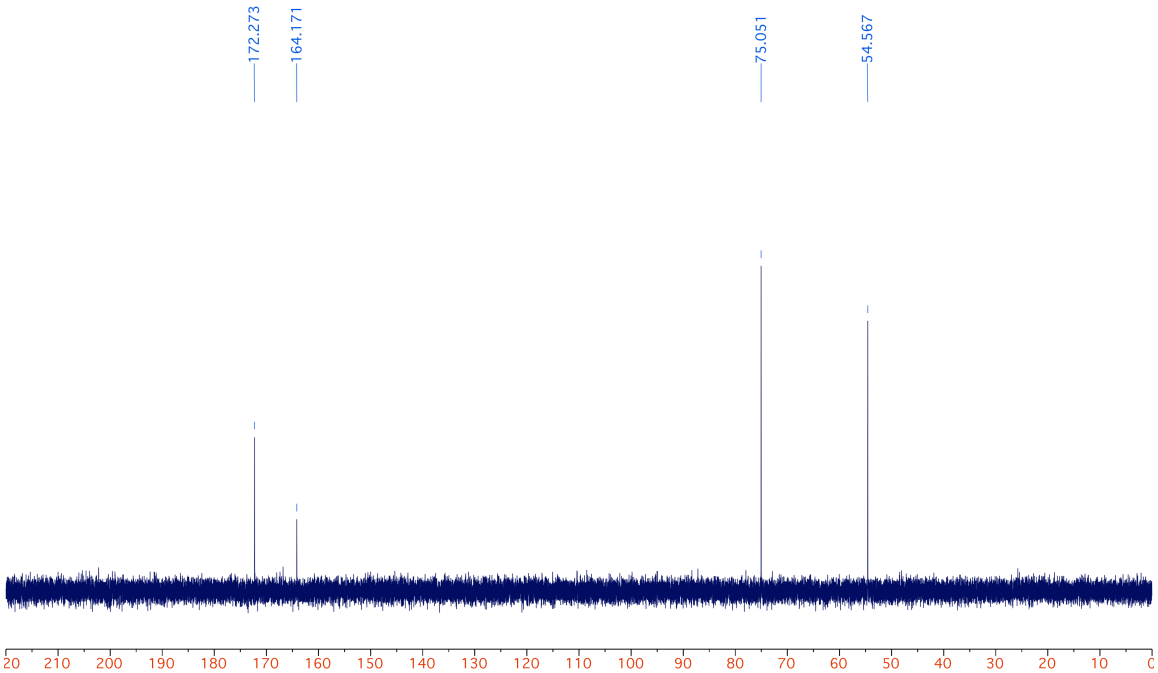
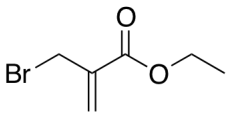




ethyl-2-bromomethyl acrylate (**7**)



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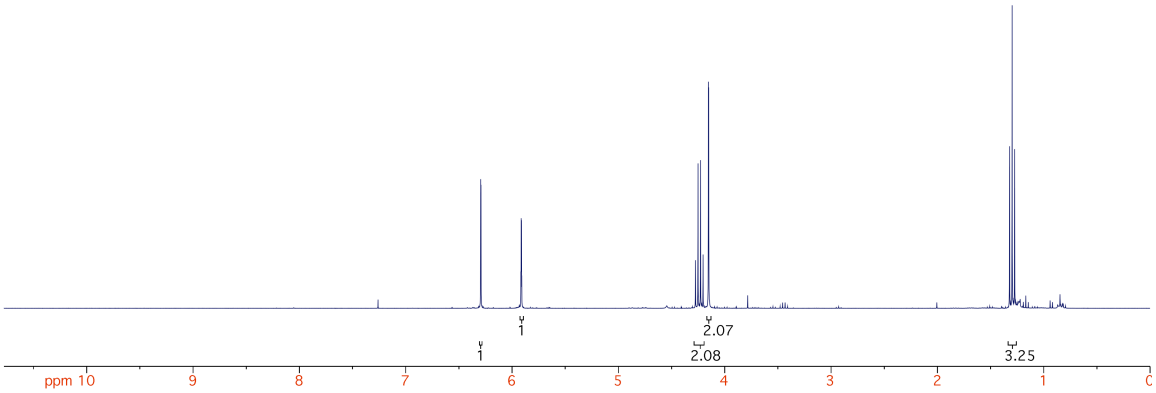
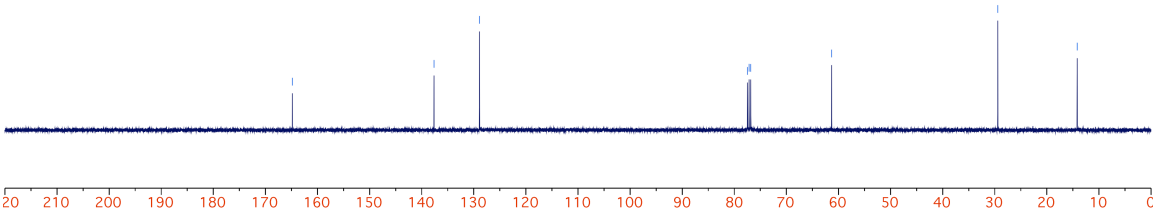
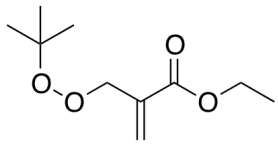
Molecular Weight: 193.0385





ethyl 2-*t*-butylperoxomethyl acrylate (**8**)



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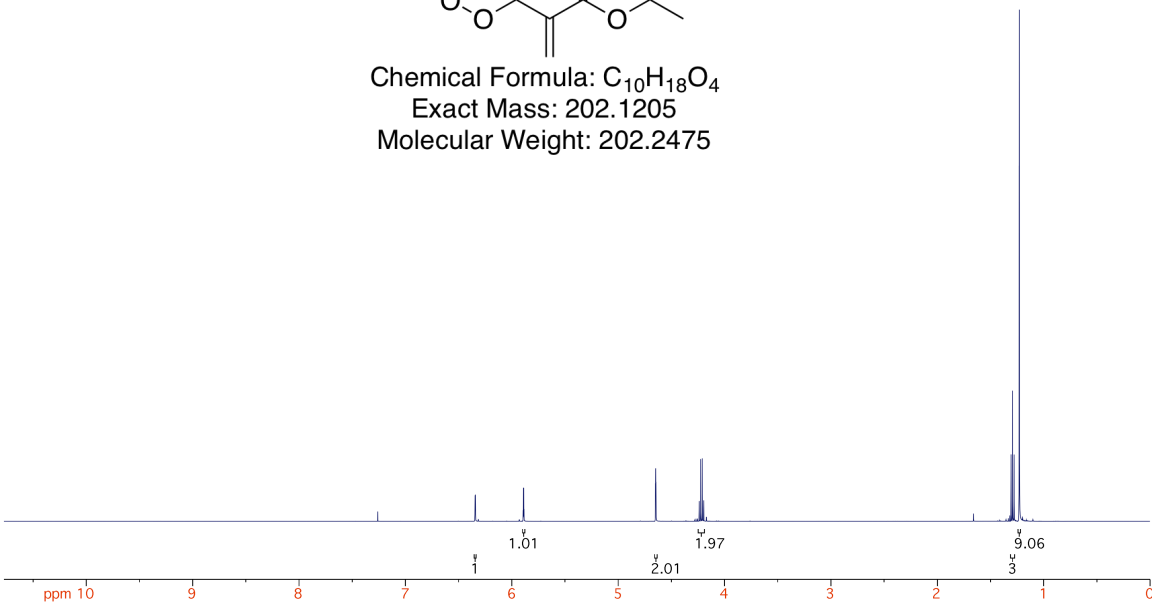
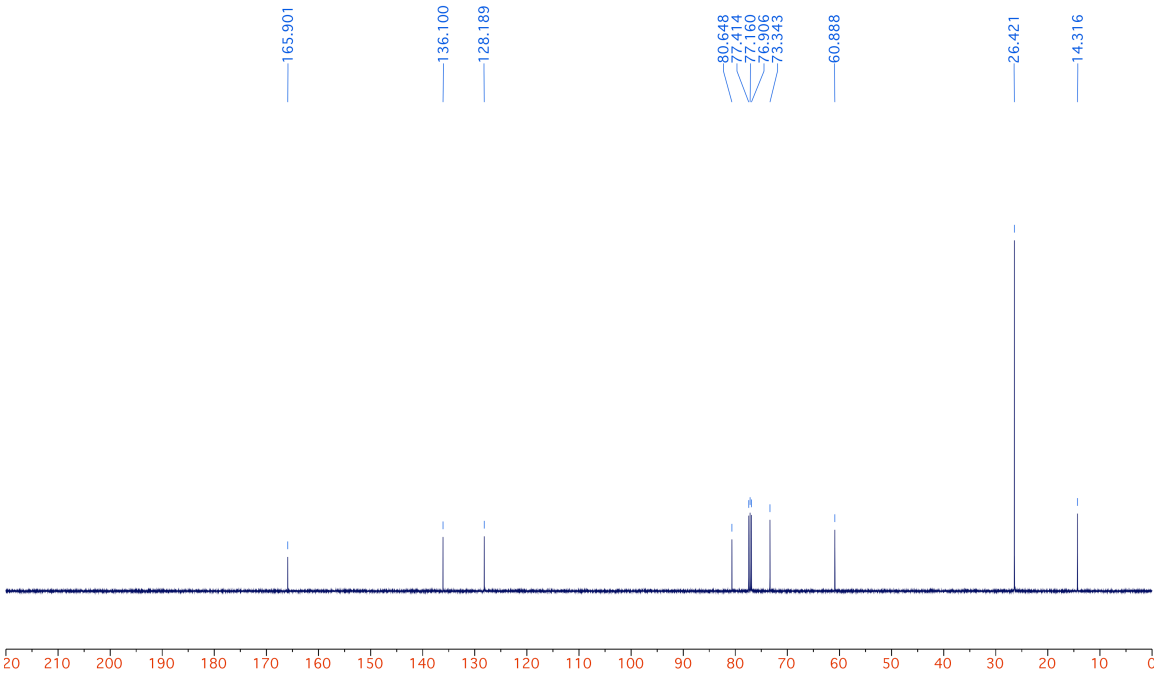
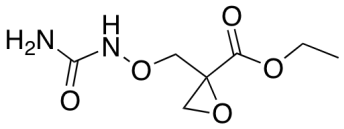




1-carboxyethyl-1-*O*-ureido-methoxy oxirane (**9**)



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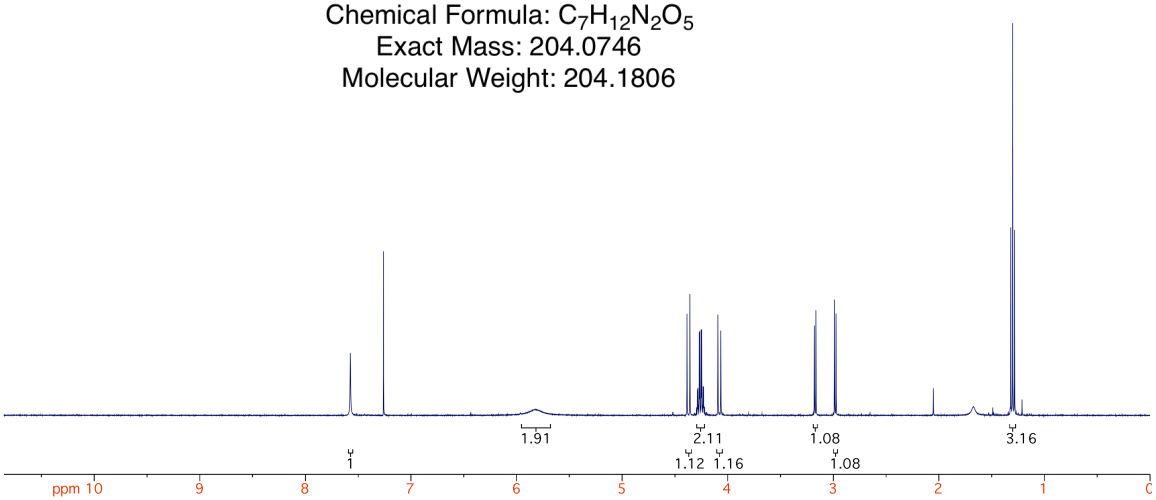
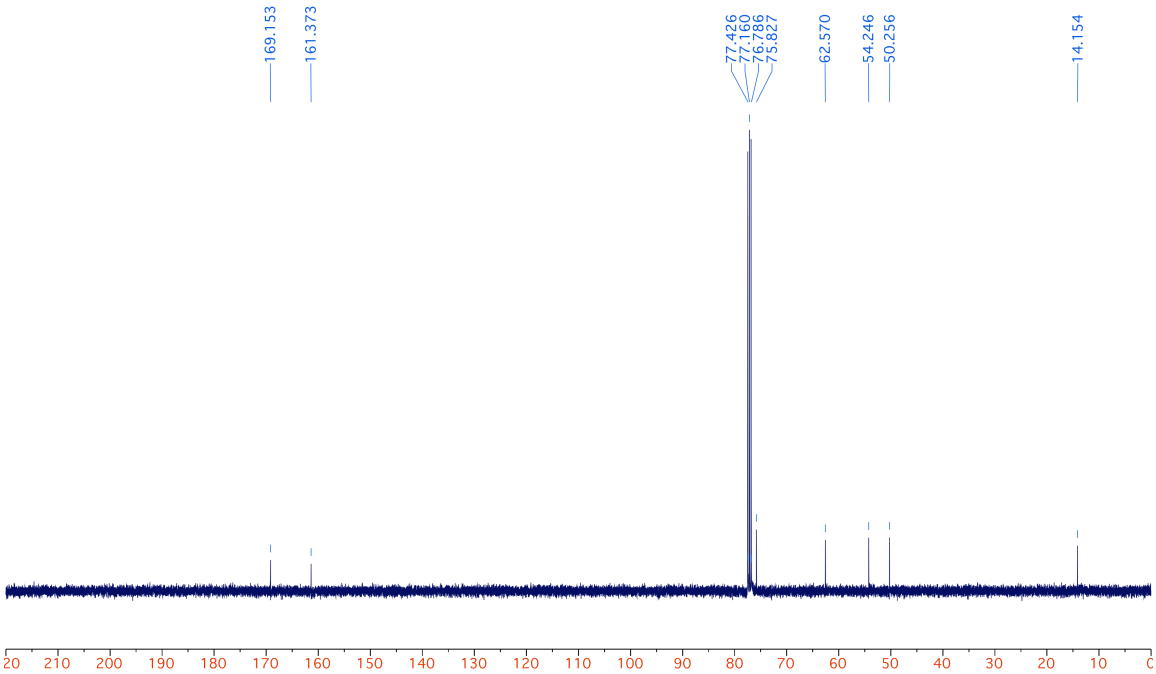
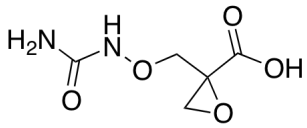




1-carboxy-1-*O*-ureido-methoxy oxirane (**6**)



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