Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Benzaldehyde lyase catalyzed enantioselective self and cross condensation reactions of acetaldehyde derivatives

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General

NMR spectra were recorded with Brucker-Spectrospin DPX-400, Ultra Shield, High Performance Digital FT-NMR spectrometer using tetramethylsilane (TMS) as an internal standard and deuterated chloroform ($CDCl_3$) as the solvent. The data were reported in the following order: chemical shift in ppm (δ) (multiplicity were indicated by s (singlet), d (doublet), t (triplet), m (multiplet); coupling constants (*J*, Hz).

The mass spectra were recorded with Thermo Quest GC-MS equipped with a Phenomenex Zebron capillary GC column (60 m length, 0.25 mm ID, 0.25 μm film thickness). HRMS analysis was performed with Waters Synapt. Flash column chromatography was performed by using Merck Silica Gel 60 (particle size: 40-63 μm, 230-400 mesh ASTM).

Enantiomeric excess values were determined by Agilent 1100 series HPLC device using a Chiralpak OA column and Chiralpak OD column

The *E.coli* BL21 (DE3) pLysS strain purchased from Invitrogen® was used as a host to produce the recombinant enzyme (BAL_{HIS}). Enzyme production was performed in New Brunswick BioFlo110 Fermentor equipped with pH and temperature probes as well stirring rate controls. The purification of the hexa-histidine tagged enzyme was performed with an Ni^{2+}-NTA affinity column (Invitrogen®).

Preparation of benzaldehyde lyase

E.coli BL21(DE3)pLysS carrying the pUC19-BAL_{HIS} plasmid was used for BAL (EC. 4.1.2.38) production. Cultures were maintained on LB agar plates. Cells from the freshly prepared plates were inoculated into the preculture in Luria broth (LB) where they were grown for 8 hours at 37^{o}C, then transferred to the production (LB) medium with an inoculation ratio of 1/10 (1.65 L) in a 2 L fermenter). BAL production was induced with 1 mM IPTG (isopropyl-β-D-thiogalacto-pyranoside) after 3 h at 37^{o}C. 6 h after the induction cells were harvested by centrifugation. The enzyme was either used in its crude form without purification (Pelleted cells were transferred to a Petri dish and lyophilized for 36 h) or as a purified enzyme.

One unit (U) of BAL activity is defined as the amount of enzyme that catalyzes the cleavage of 1 μmol benzoin in potassium phosphate buffer (50 mM, pH 7) containing $MgSO_4$ (2.5 mmol L^{-1}), ThDP (0.15 mmol L^{-1}) and DMSO (20%, v/v) at 30°C per minute at 30°C.

General procedure for BAL catalyzed self condensation of benzyloxyacetaldehyde

Screening of solvents

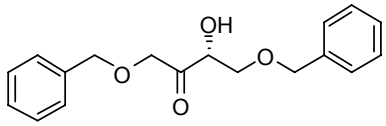
Benzyloxyacetaldehyde **1** (150 mg, 1 mmoL) was dissolved in 10 mL of corresponding solvent (25 vol-%), and then 30 mL (75 vol-%) phosphate buffer (50mM, pH 7) containing 0.15 mM TPP and 2.5 mM $MgSO_4$ was added to this solution. By the addition of BAL (50 U), the reaction was started at 30^{o}C. The same amount of enzyme was added on a daily basis. The reaction was monitored by TLC and GC-MS. After 96 h, no further increase of the product concentration was observed and the reaction was stopped. The reaction mixture was extracted with chloroform (3 x 50 mL) and the combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The product was purified with flash column chromatography. Enantiomeric excess (ee) values were determined by HPLC equipped with chiral OA column at 220 nm (80:20/ hexane: isopropanol, 0.8 mL/min, retention times were 12.70 min and 13.78 min, respectively).
The solvents that were screened are as follows; diisopropyl ether (DIPE), dimethyl sulfoxide (DMSO), dioxan, toluene, benzene, hexane, xylene, isopropanol, diethyl ether and ethanol.

Screening of buffers

Benzyloxyacetaldehyde **1** (150 mg, 1 mmoL) was dissolved in 10 mL of DIPE (25 vol-%), and then 30 mL (75 vol-%) of corresponding buffer solution (50mM, containing 0.15 mM TPP and 2.5 mM $MgSO_4$) was added to this solution. By the addition of BAL (50 U), the reaction was started at 30°C. The same amount of enzyme was added on a daily basis. The reaction was monitored by TLC and GC-MS. After 96 h, no further increase of the product concentration was observed and the reaction was stopped. The reaction mixture was extracted with chloroform (3 x 50 mL) and the combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The product was purified with flash column chromatography. Enantiomeric excess (ee) values were determined by HPLC equipped with chiral OA column at 220 nm (80:20/ hexane: isopropanol, 0.8 mL/min, retention times were 12.70 min and 13.78 min, respectively).

Buffers were employed within the enzyme pH range (pH 6-8). The pH values of the buffers were set to the following values: Phosphate buffer – pH 7 & pH 8; 2-(*N*-morpholino)ethanesulfonic acid (MES) - pH 6.5 & pH 7; 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) pH 7 & pH 8; 3-(*N*-morpholino)propanesulfonic acid (MOPS) pH 7 & pH 8 ; Tris(hydroxymethyl)aminomethane (TRIS) pH 7 & pH 8.

***(R)*-1,4-Bis(Benzyloxy)-3-hydroxybutan-2-one (5)**



Benzyloxyacetaldehyde **4** (150 mg, 1 mmoL) was dissolved in 10 mL of DIPE (25 vol-%), and then 30 mL (75 vol-%) of corresponding buffer solution (50mM, containing 0.15 mM TPP and 2.5 mM $MgSO_4$) was added to this solution. By the addition of BAL (50 U), the reaction was started at 30°C. The same amount of enzyme was added on a daily basis. The reaction was monitored by TLC and GC-MS. The reaction was concluded after 96 h. The reaction mixture was extracted with chloroform (3 x 50 mL) and the combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The product was purified with flash column chromatography and obtained as a yellowish oil, $[\alpha]_D^{25}$= -1.3 (c= 0.9, CH_2Cl_2, 95% ee). Ee value (chiral OA column , 80:20/ hexane: isopropanol, 0.8 mL/min, 220 nm, Rt: 12.036 min for *(S)*, 13.934 min for *(R)*) was determined as 95%.

^{1}H-NMR (400 MHz, 2.5/1: $CDCl_3/CCl_4$): δ =3.35 (s, 1H, OH), 3.72 (dd, J=3.7, 10.1, Hz, 1H), 3,8 (dd, J= 3.7, 10.1 Hz, 1H), 4.21 (d, J=17.2 Hz, 1H), 4,28 (d, J=17.2 Hz, 1H), 4.58-4.30 (m, 5H), 7.37-7.09 (m, 10H). ^{13}C-NMR (100 MHz, 2.5/1: $CDCl_3/CCl_4$): δ ppm 70.86, 73.21, 73.53, 73.63, 75.07, 127.80, 127.93, 128.12, 128.45, 128.54, 132.75, 134.12, 204.90. HRMS for $C_{18}H_{20}NaO_4$ ($M+Na^+$): calcd. 323.1259, found: 323.1263.

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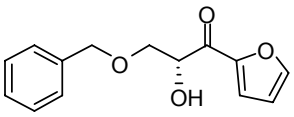
General procedure for BAL catalyzed cross condensation reactions of benzyloxyacetaldehyde

Benzyloxyacetaldehyde **4** (150 mg, 1 mmoL) and the corresponding aldehyde (1 mmoL**, 10** or **12**) were dissolved in 10 mL diisopropylether (25 vol-%), and then 30 mL (75 vol-%) MOPS buffer (50mM, pH 7) containing 0.15 mM TPP and 2.5 mM $MgSO_4$ was added to this solution. By the addition of BAL (50 U), the reaction was started at 30^{o}C. The same amount of enzyme was added on a daily basis. The reaction was monitored by TLC and GC-MS. The reaction was concluded after 96 h. The reaction mixture was extracted with chloroform (3 x 50 mL) and the combined organic layers were dried over $MgSO_4$, the solvent was removed under reduced pressure. The product was purified with flash column chromatography.

***(R)*-3-(benzyloxy)-1-(furan-2-yl)-2-hydroxypropan-1-one (11)**



Benzyloxyacetaldehyde **4** (150 mg, 1 mmoL) and furan-2-carbaldehyde **6** (96 mg, 1 mmoL) were dissolved in 10 mL diisopropylether (25 vol-%), and then 30 mL (75 vol-%) MOPS buffer (50mM, pH 7) containing 0.15 mM TPP and 2.5 mM $MgSO_4$ was added to this solution. The reaction was started with the addition of BAL (50 U) at 30°C (120 rpm). BAL was added (50 U) on a daily basis. The reaction was concluded after 96 h. The reaction mixture was extracted with chloroform (3 x 50 mL) and the combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The product was purified with flash column chromatography and obtained as a yellow oil, $[\alpha]^{25}_{D}$: +70.3 (c 0.4, $CHCl_3$, 80% ee). Ee value (chiral OD column, 98:2/ hexane: isopropanol, 1 mL/min, 254 nm, Rt: 68.688 min for *(S)*, 75.499 min *(R)*) was determined as 90%.

^{1}H NMR(400 MHz, 2,5/1: $CDCl_3/CCl_4$): 3.72 (dd, J= 3.26, 10.24, Hz, 1H), 3.81 (dd, J=4.12, 10.24 Hz, 1H), 4.38 (d, J=12.3 , , 1H), 4.48 (d, J=12.3 Hz, 1H), 4.82 (t, J= 3.57 Hz, 1H), 6.47 (m, 1H), 7.06-7.09 (m, 2H), 7.11-7.19 (m, 3H), 7.24 (d, 1H), 7.47 (m, 1H). ^{13}C

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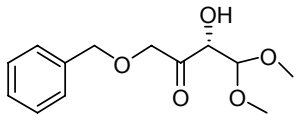
NMR (100 MHz, 2,5/1: $CDCl_3/CCl_4$): 72.26, 75.92, 95.07, 111.36, 117.70, 126, 127, 128, 137, 145.52, 145.59, 149.59, 186.32. HRMS for $C_{14}H_{14}NaO_4$ ($M+Na^+$): calcd. 269.0790; found: 269.0784.

***(R)*-1-(benzyloxy)-3-hydroxy-4,4-dimethoxybutan-2-one (13)**



Benzyloxyacetaldehyde **4** (150 mg, 1mmoL) and dimethoxy-acetaldehyde **10** (104 mg, 1 mmol) were dissolved in 10 mL diisopropylether (25 vol-%), and then 30 mL (75 vol-%) MOPS buffer (50mM, pH 7) containing 0.15 mM TPP and 2.5 mM $MgSO_4$ was added to this solution. The reaction was started with the addition of BAL (50 U) at 30°C (120 rpm). BAL was added (50 U) on a daily basis. The reaction was concluded after 96 h. The reaction mixture was extracted with chloroform (3 x 50 mL) and the combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The product was purified with flash column chromatography and obtained as a yellow oil, $[\alpha]^{25}_D$: -14.5 (c 0.4, $CHCl_3$). Ee value (chiral OJ column, 90:10/ hexane: isopropanol, 0,8 mL/min, 220 nm, Rt: 18.833 min for *(S)*, 65.387 min *(R)*) was determined as 93%.

^{1}H-NMR(400 MHz, 2.5/1: $CDCl_3/CCl_4$): δ =3.37 (s, 6H), 4.18 (d, J=18.2 Hz, 2H), 4.35 (m, 2H),4.49 (dd, J=11.8 Hz, 1H), 4.55 (dd, J= 11.8, Hz, 1H), 7.22-7.26 (m, 5H). ^{13}C-NMR (100 MHz, 2.5/1: $CDCl_3/CCl_4$): δ ppm 54.69, 55.971, 72.482, 72.561, 104.397, 127.009, 127.281, 127.465, 136.201, 205.274. HRMS for $C_{13}H_{18}NaO_5$ ($M+Na^+$): calcd. 277.1052; found: 277.1045.

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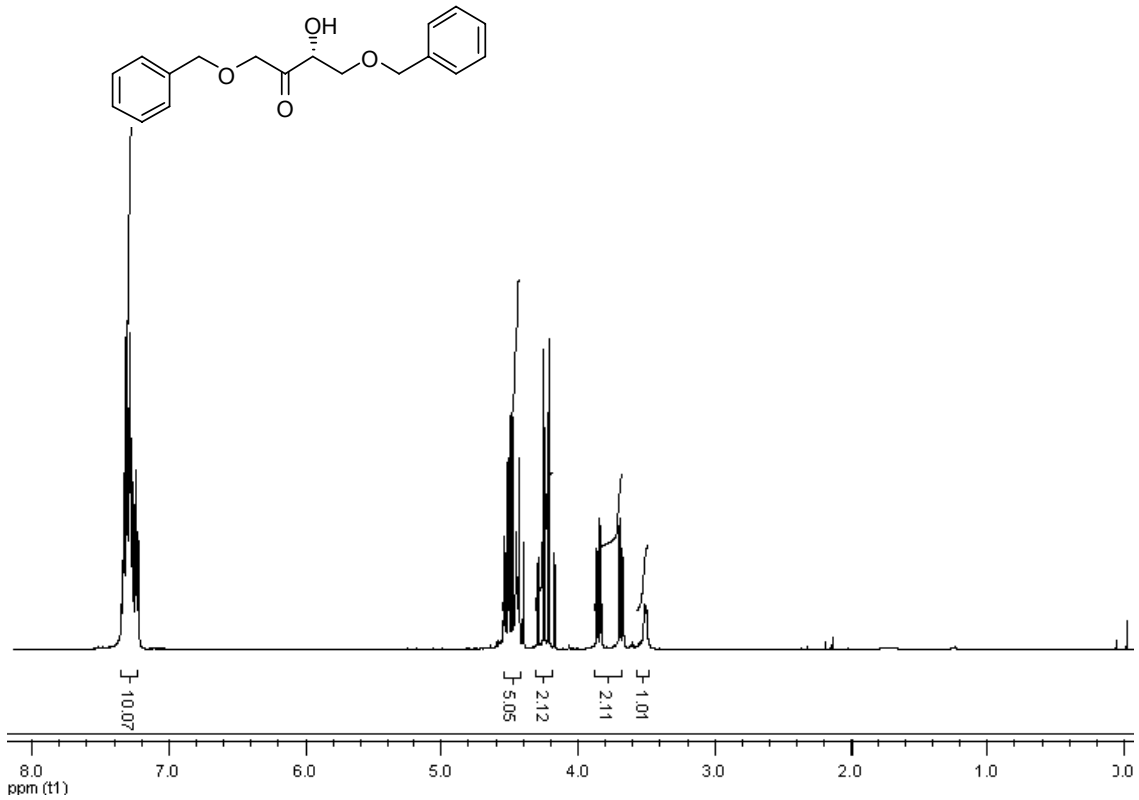
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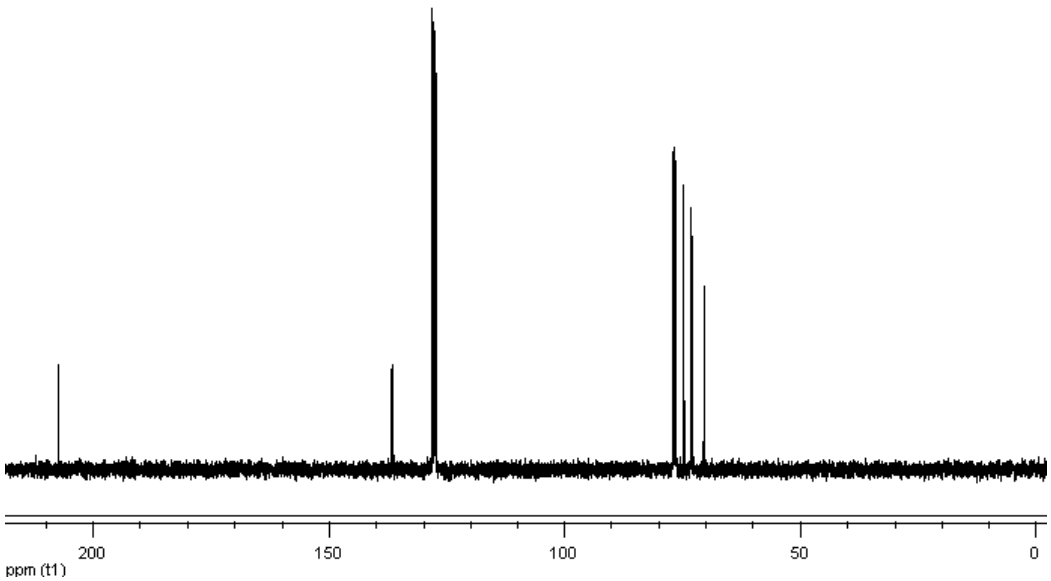
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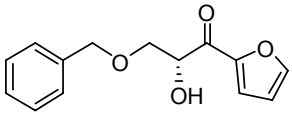
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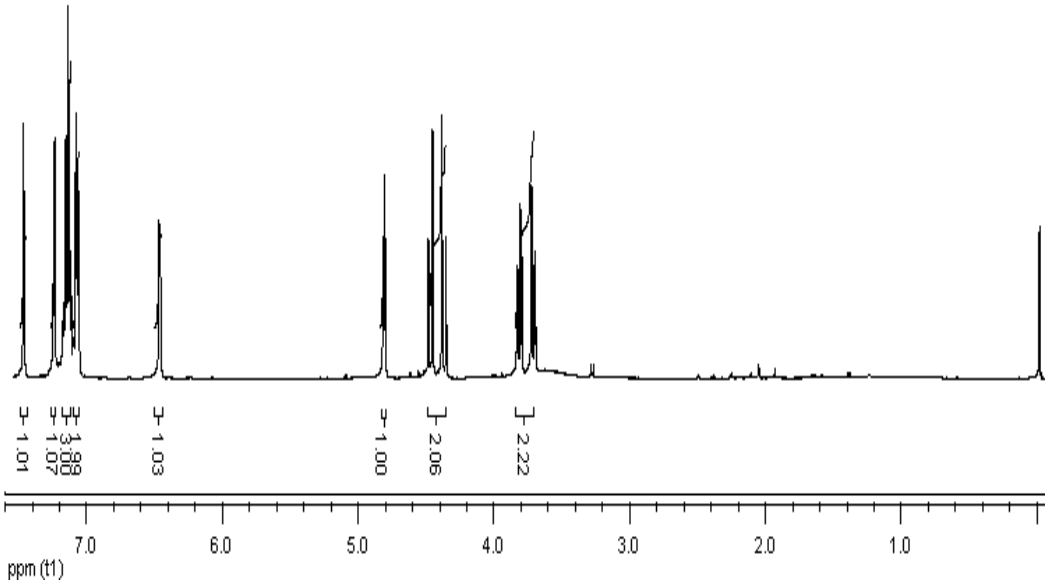
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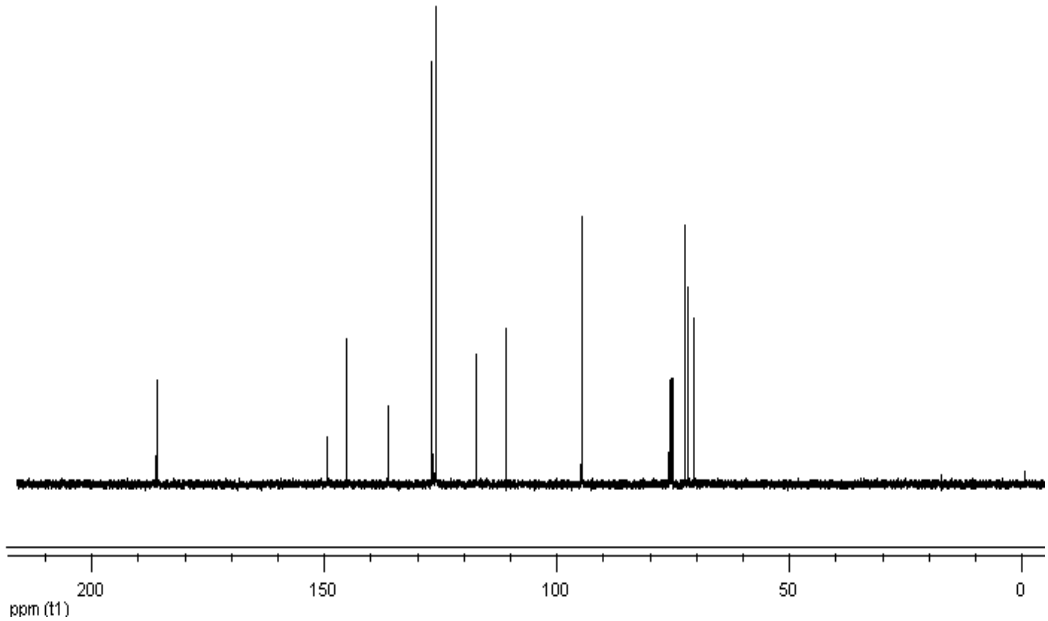
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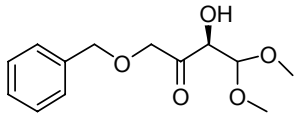
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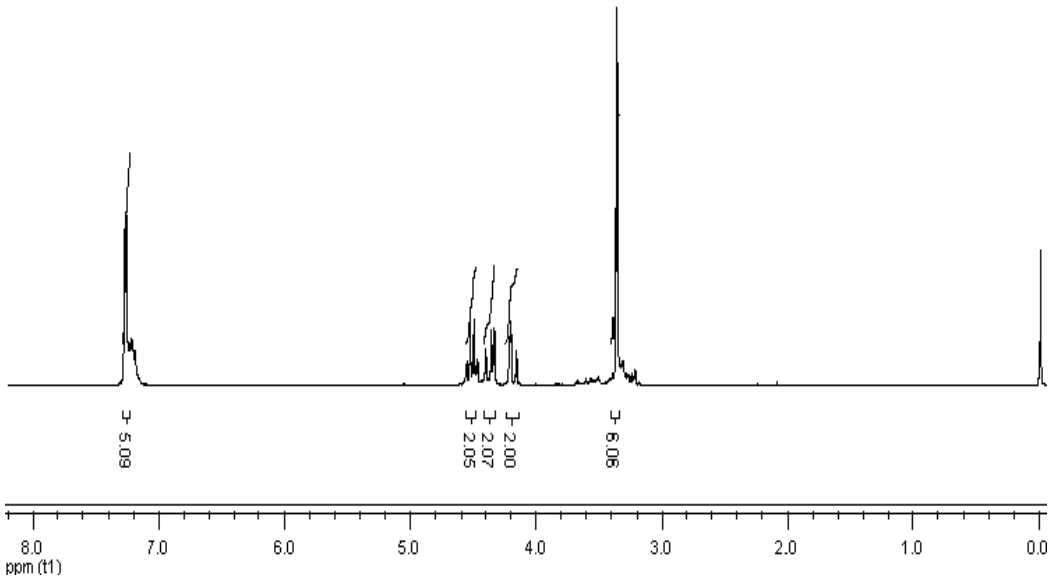
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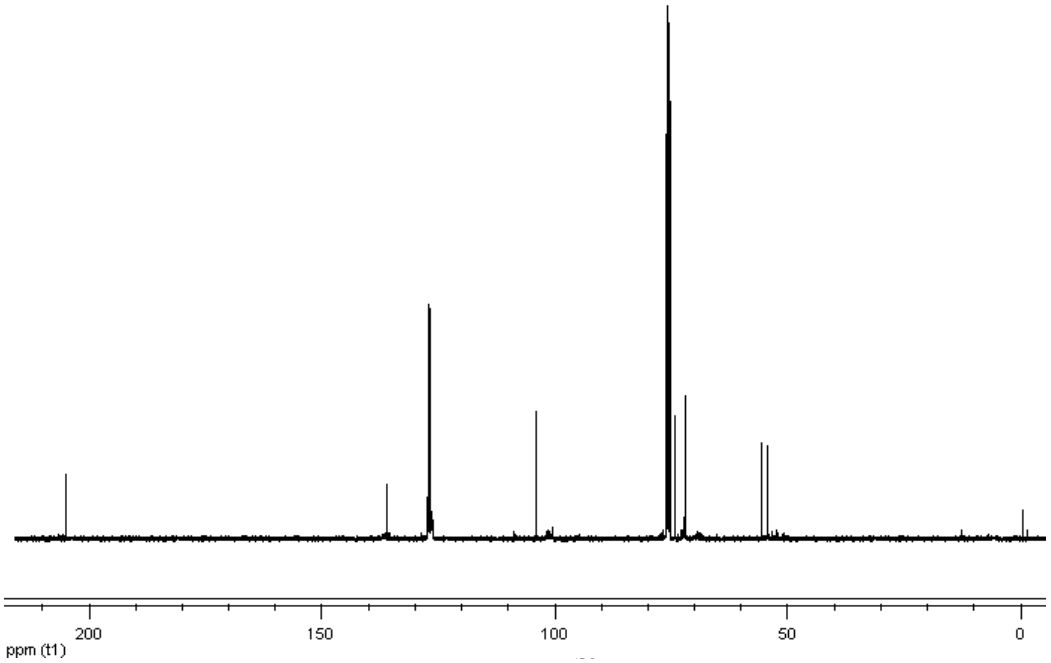
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