Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

- **Biological screening information**

- **^{1}H and ^{13}C NMR of most active LasR-antagonists**

Bacterial strains and growth conditions

The *P. aeruginosa* strains; PA01 wild type (B.Iglewski) and PAO-JP1 (Δ*lasI* mutant)[1] were used in this study. Colonies of the desired strain were grown on a Luria-Bertani (LB) agar plate at 37 ^{o}C overnight. The colonies were used to inoculate 10 mL fresh LB media and grown overnight at 37 ^{o}C. The overnight cultures were used to inoculate fresh LB media to an initial OD_{600} of 0.05 and cultures were grown with good aeration (300 rpm in an orbital shaker) at 37 ^{o}C. Cultures were then used as a source of bacteria for various phenotypic assays. A Jenway 6705 spectrometer 1cm path-length cuvettes were used for all spectrophotometric assays. AHL analogues were stored as 10 mM stock solutions in DMSO at – 20 ^{o}C. The AHL analogues in DMSO were added at the desired concentration to the culture at the start of growth. The final DMSO volume remained below 1% and an equivalent volume of DMSO with no AHL analogue was added to control cultures.

Pyocyanin assay

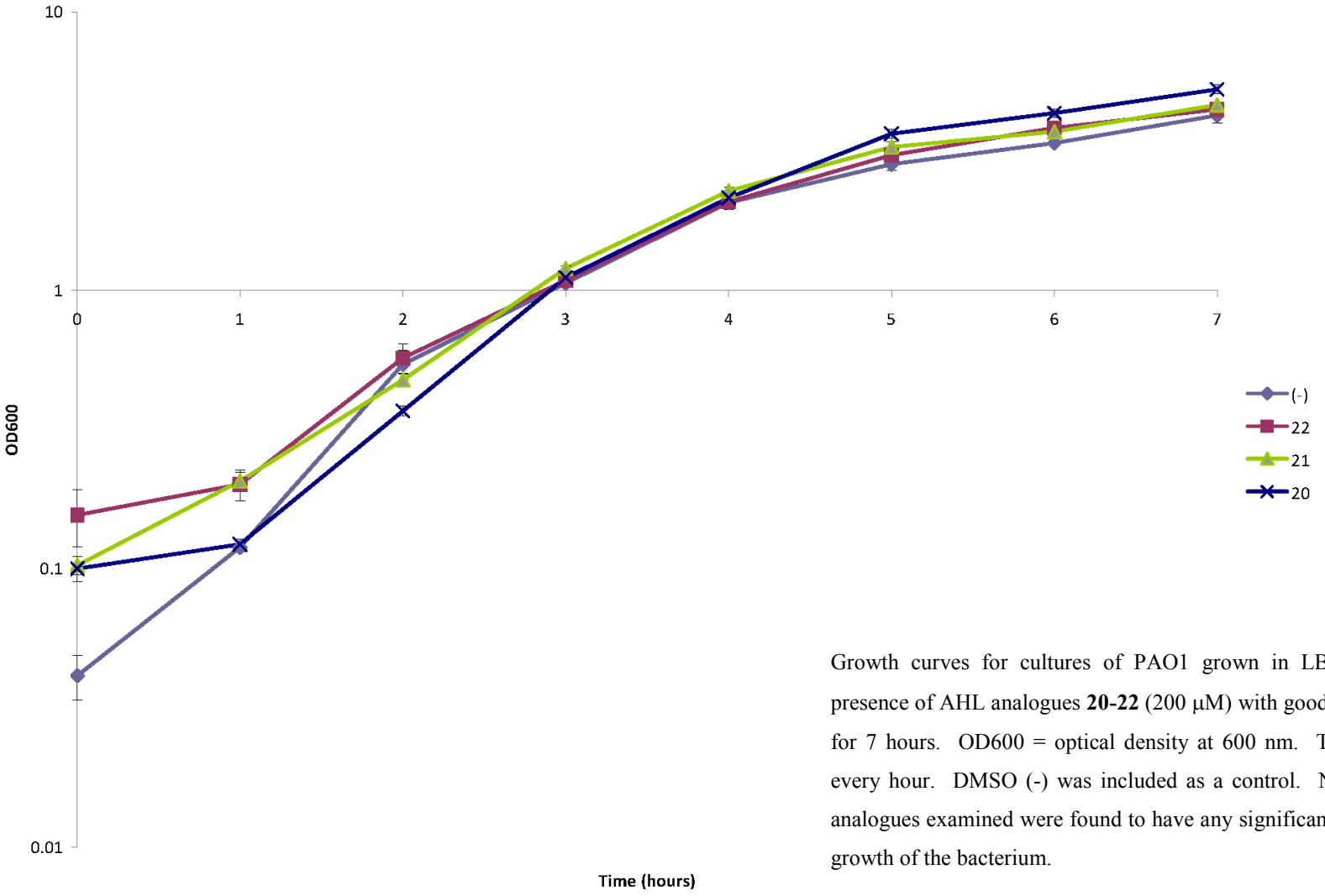
Pyocyanin in the culture supernatant was quantified as previously described.[2] After growth the OD_{600} was recorded, and the samples were clarified by centrifugation (3,150 x*g*, 10 min, 20 ^{o}C) to remove cell debris. The supernatant (5 mL) was then extracted with chloroform (3 mL) by vortexing. The phases were separated by centrifugation (3,150 x*g*, 10 min, 20 ^{o}C) and the chloroform phase was transferred to a fresh tube and extracted with HCl aq (0.2 N, 1 mL). The phases were separated by centrifugation (3,150 x*g*, 10 min, 20 ^{o}C) and the absorption of the aqueous phase was measured at 520 nm and corrected for the culture OD_{600}.

Elastase assay

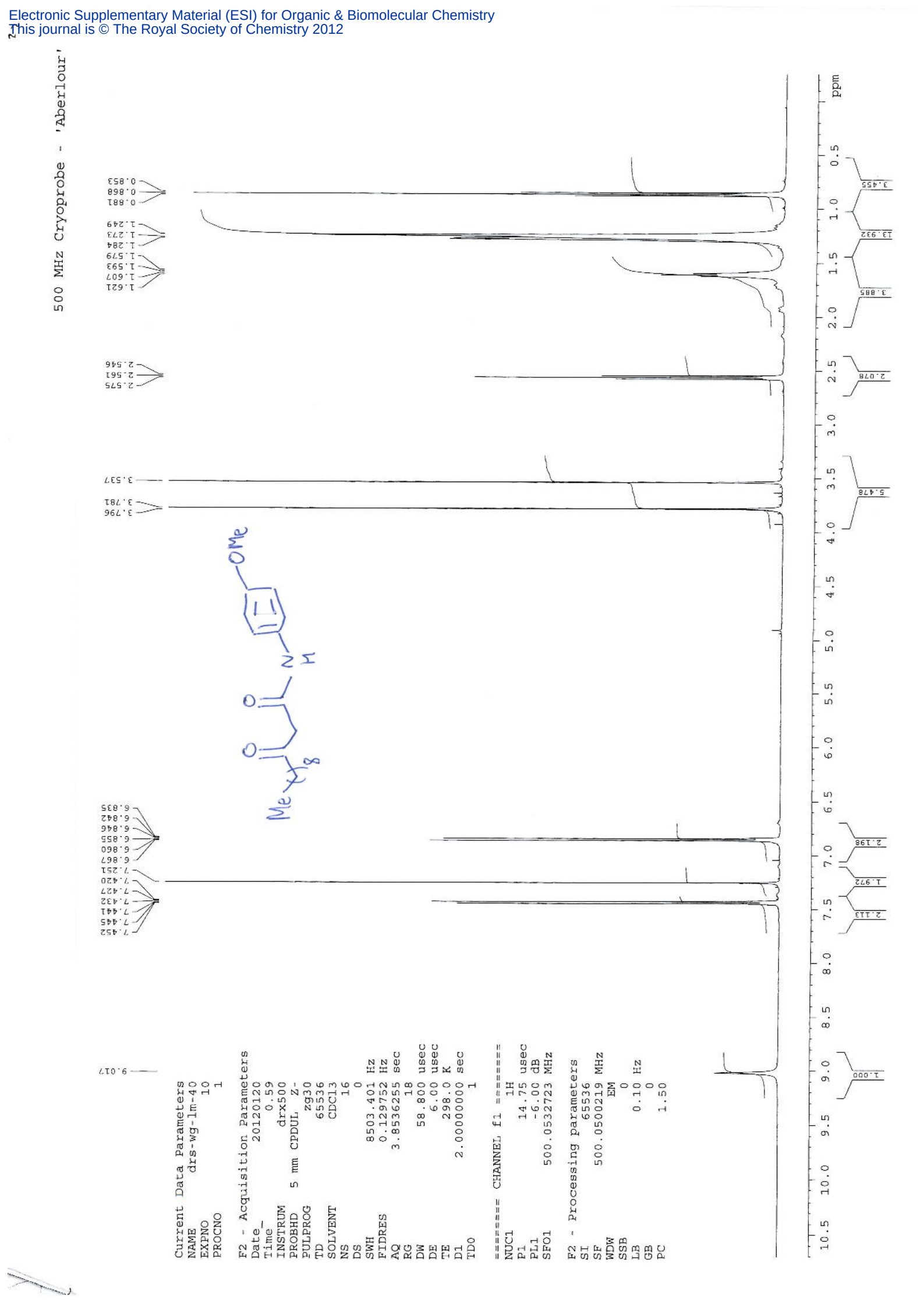
Elastase in culture supernatant was quantified as previously described,[3] with minor modifications. Elastin congo red (5 mg) purchased from Sigma Aldrich was weighed out into 2 mL eppendorf tubes. Elastase congo red buffer (1 mM $CaCl_2$, 100 mM tris base, pH 7.5) and supernatant (0.1 mL) from the bacterial culture was then added to the eppendorf tube. The resulting suspension was then left to incubate for 13 hours with shaking at 37 ^{o}C. The suspension was clarified by centrifugation (3,150 x*g*, 10 min, 20 ^{o}C) and the aqueous phase was separated from the solid residue by pipetting. The absorbance at 492nm was measured and corrected for the culture OD_{600}.

References:

1. J. P. Pearson, E. C. Pesci and B. H. Iglewski, *J Bacteriol*, 1997, **179**, 5756-5767.
2. D. W. Essar, L. Eberly, A. Hadero and I. P. Crawford, *J Bacteriol*, 1990, **172**, 884-900.
3. D. E. Ohman, S. J. Cryz and B. H. Iglewski, J Bacteriol, 1980, 142, 836-842.



Growth curves for cultures of PAO1 grown in LB medium in the presence of AHL analogues **20-22** (200 μM) with good aeration at 37 ºC for 7 hours. OD600 = optical density at 600 nm. Time points taken every hour. DMSO (-) was included as a control. None of the AHL analogues examined were found to have any significant effects upon the growth of the bacterium.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PROBHD 5 mm CPDUL Z-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 0
SWH 8503.401 Hz
FIDRES 0.129752 Hz
AQ 3.8536255 sec
RG 18
DW 58.800 usec
DE 6.00 usec
TE 298.0 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 14.75 usec
PL1 -6.00 dB
SFO1 500.0532723 MHz
F2 - Processing parameters
SI 65536
SF 500.0500219 MHz
WDW EM
SSB 0
LB 0.10 Hz
GB 0
PC 1.50
9.017
7.452
7.445
7.441
7.432
7.427
7.420
7.251
6.867
6.860
6.855
6.846
6.842
6.835
3.796
3.781
3.537
2.575
2.561
2.546
1.621
1.607
1.593
1.579
1.284
1.273
1.249
0.881
0.868
0.853
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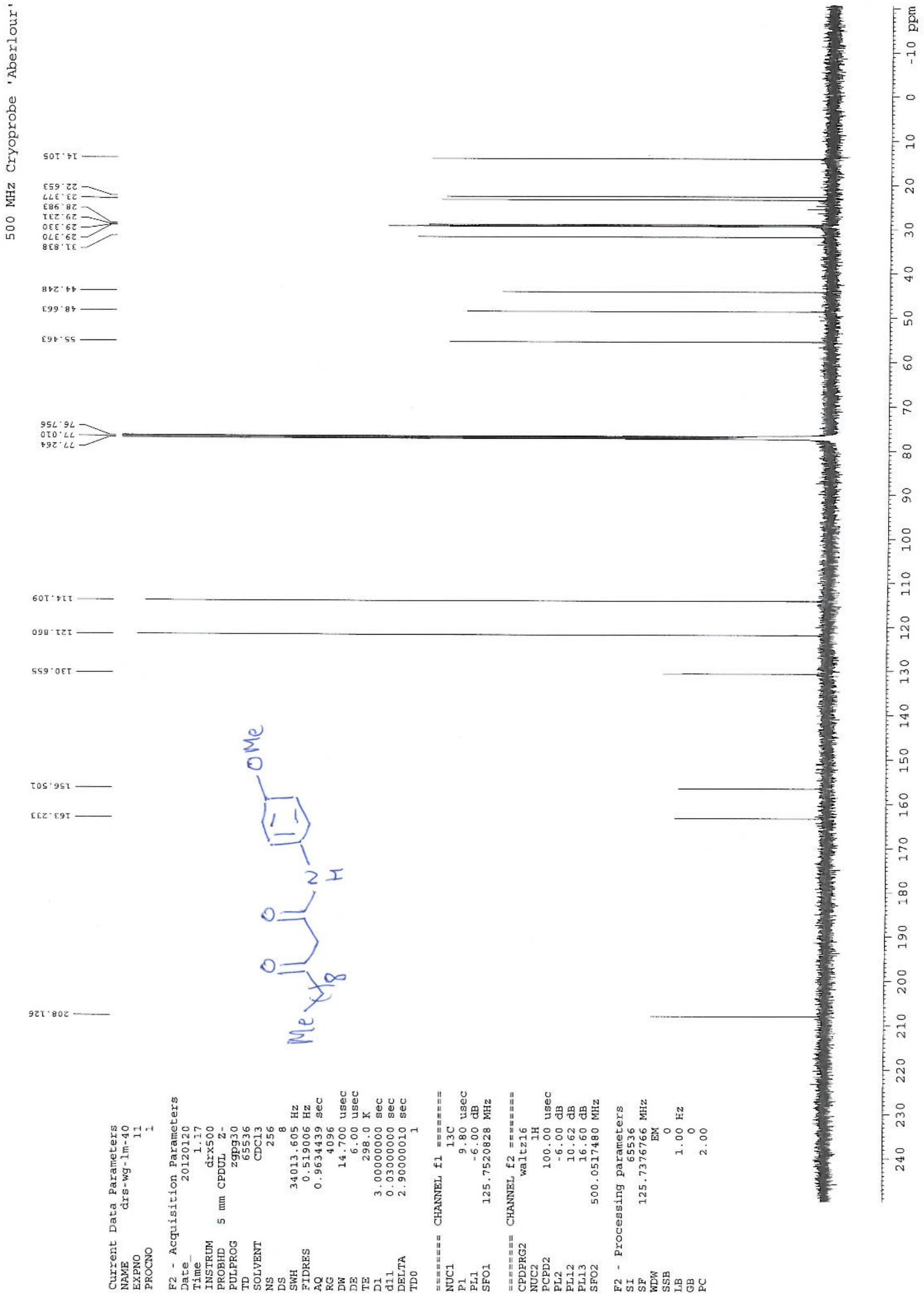

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F2 - Acquisition Parameters
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FIDRES 0.519006 Hz
AQ 0.9634439 sec
RG 4096
DW 14.700 usec
DE 6.00 usec
TE 298.0 K
D1 3.00000000 sec
d11 0.03000000 sec
DELTA 2.90000010 sec
TD0 1

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NUC1 13C
P1 9.80 usec
PL1 -6.00 dB
SFO1 125.7520828 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 -6.00 dB
PL12 10.62 dB
PL13 16.60 dB
SFO2 500.0517480 MHz

F2 - Processing parameters
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SF 125.7376766 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 2.00