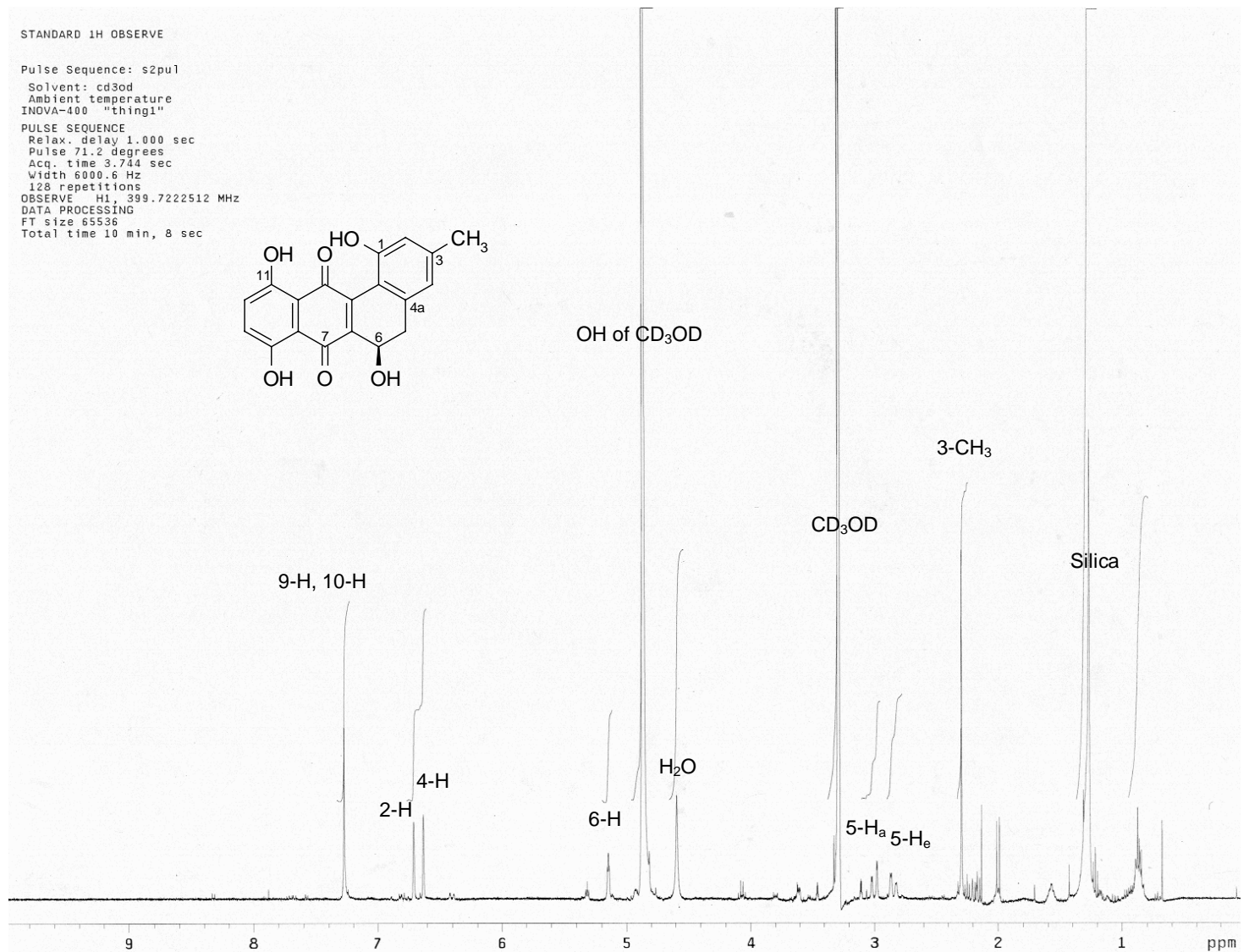


## Supporting Information for

### **The Identification of the Function of Gene *IndM2* Encoding a Bifunctional Oxygenase-Reductase Involved in the Biosynthesis of the Antitumor Antibiotic Landomycin E by *Streptomyces globisporus* 1912 Supports the Originally Assigned Structure for Landomycinone**

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New  $^1\text{H}$  NMR spectrum of natural landomycinone in  $\text{CD}_3\text{OD}$  (400 MHz)

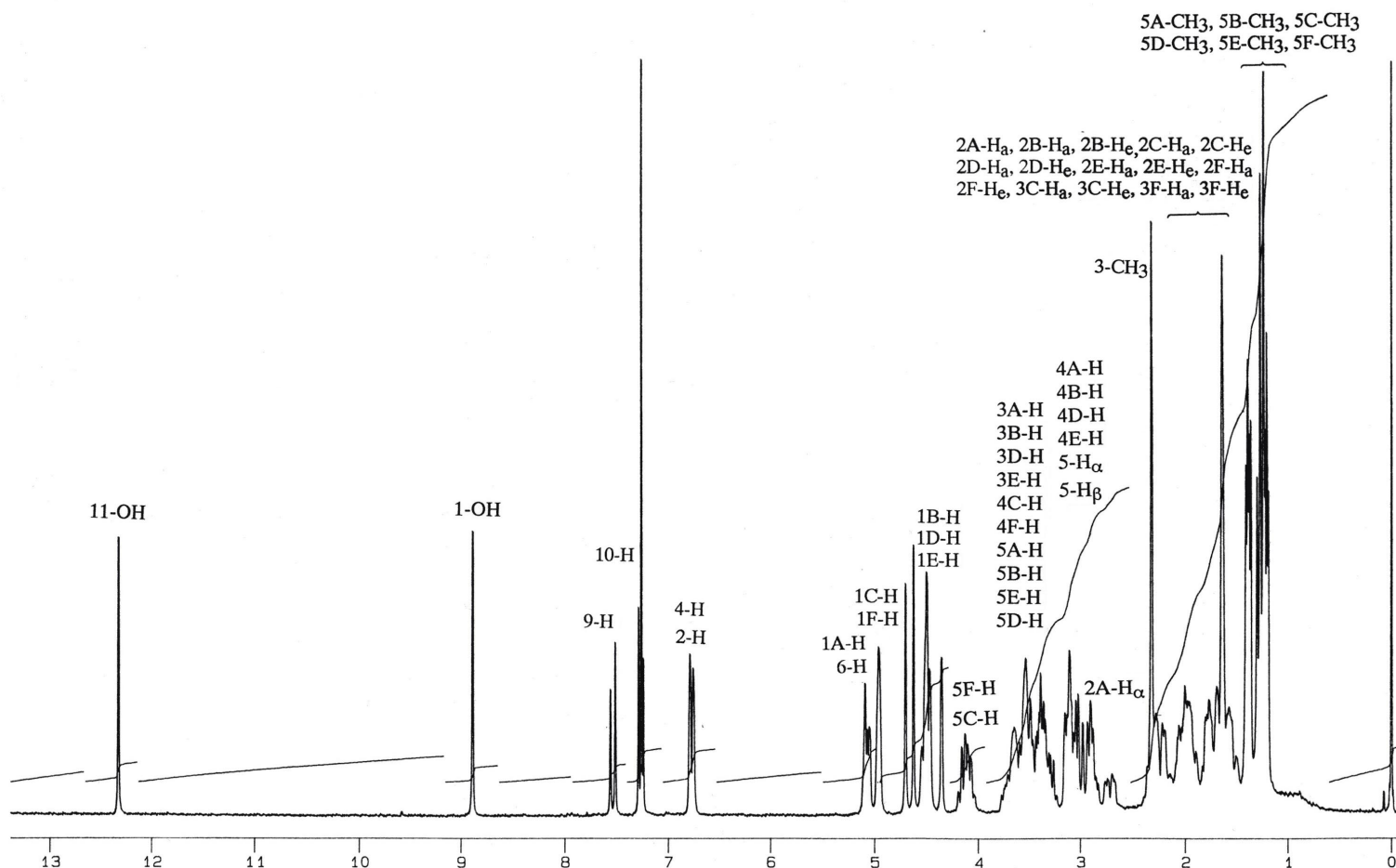


The feeding experiment with 1-<sup>13</sup>C-acetate labeled nine carbons: C-1, C-3, C-4a, C-6, C-7, C-8, C-10, C-11a, and C-12a

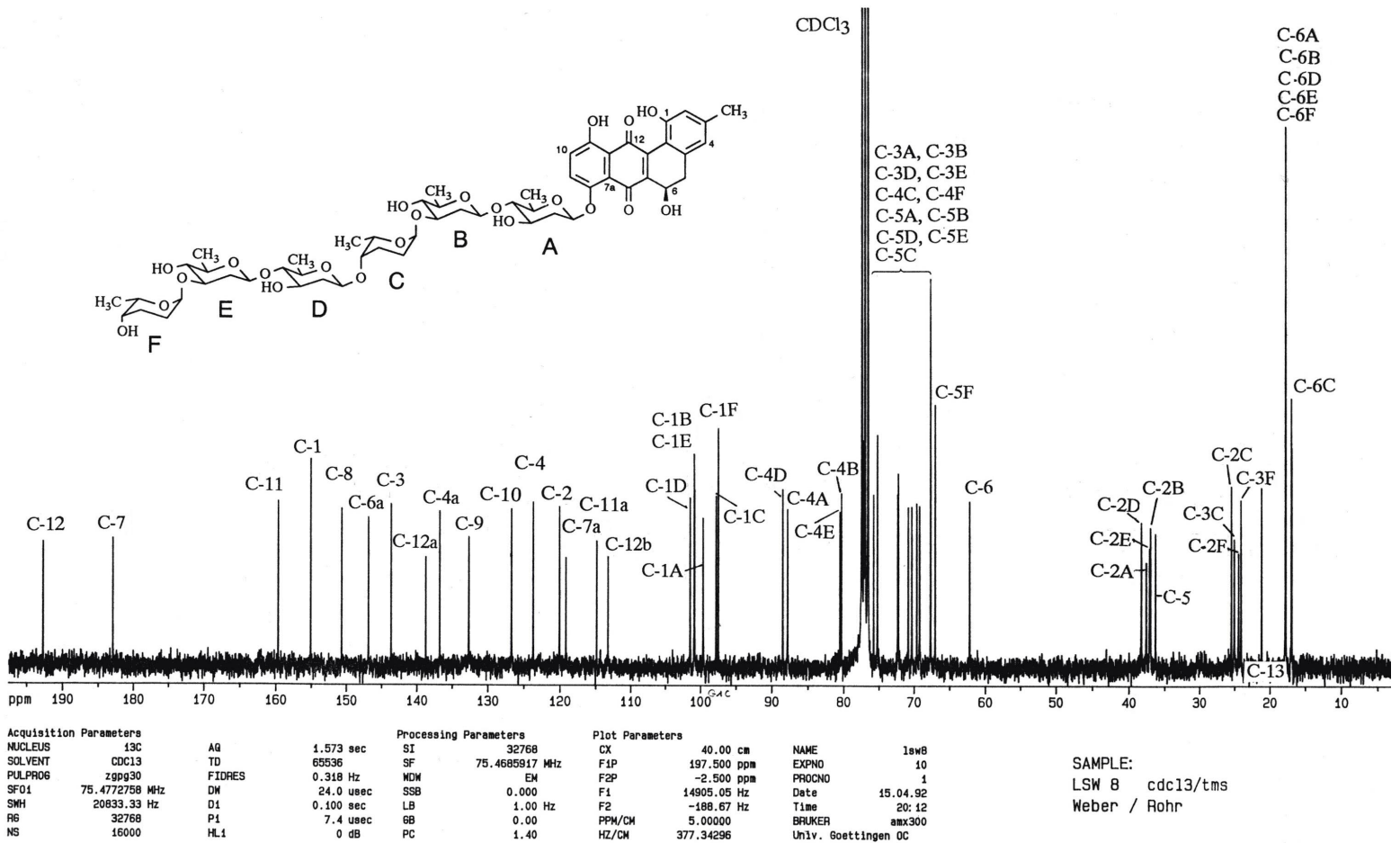
The assignment of all the carbons were confirmed by various 2D-NMR methods, including a 2D-INADEQUATE NMR upon feeding with 1,2-<sup>13</sup>C<sub>2</sub>-acetate. The latter clearly proves that C-5 and C-6 derive from the same acetate unit ( $J_{C-5/C-6} = 37$  Hz). Since the signal at  $\delta$  62.0, and not the other one of this pair ( $\delta$  36.3) is enriched from the feeding with 1-<sup>13</sup>C-acetate, and the chemical shift clearly proves that the oxygen is attached at this 1-<sup>13</sup>C-acetate-enriched carbon. Thus, there cannot be any doubt that C-6 (and not C-5) carries the oxygen. If Roush's 'revised' structure (oxygen at C-5) were correct then the third acetate building block would have been incorporated against the direction of all other acetates, which is biochemically impossible. Our assignments are further confirmed by the long-range couplings between 6-H and C-12a, between 5-H<sub>2</sub> and C-4, and between 4-H and C-5 ( $\delta$  36.3) observable in the HMBC spectrum.

Original NMR spectra of landomycin A

## Landomycin A, <sup>1</sup>H NMR (CDCl<sub>3</sub>, TMS, 300 MHz)

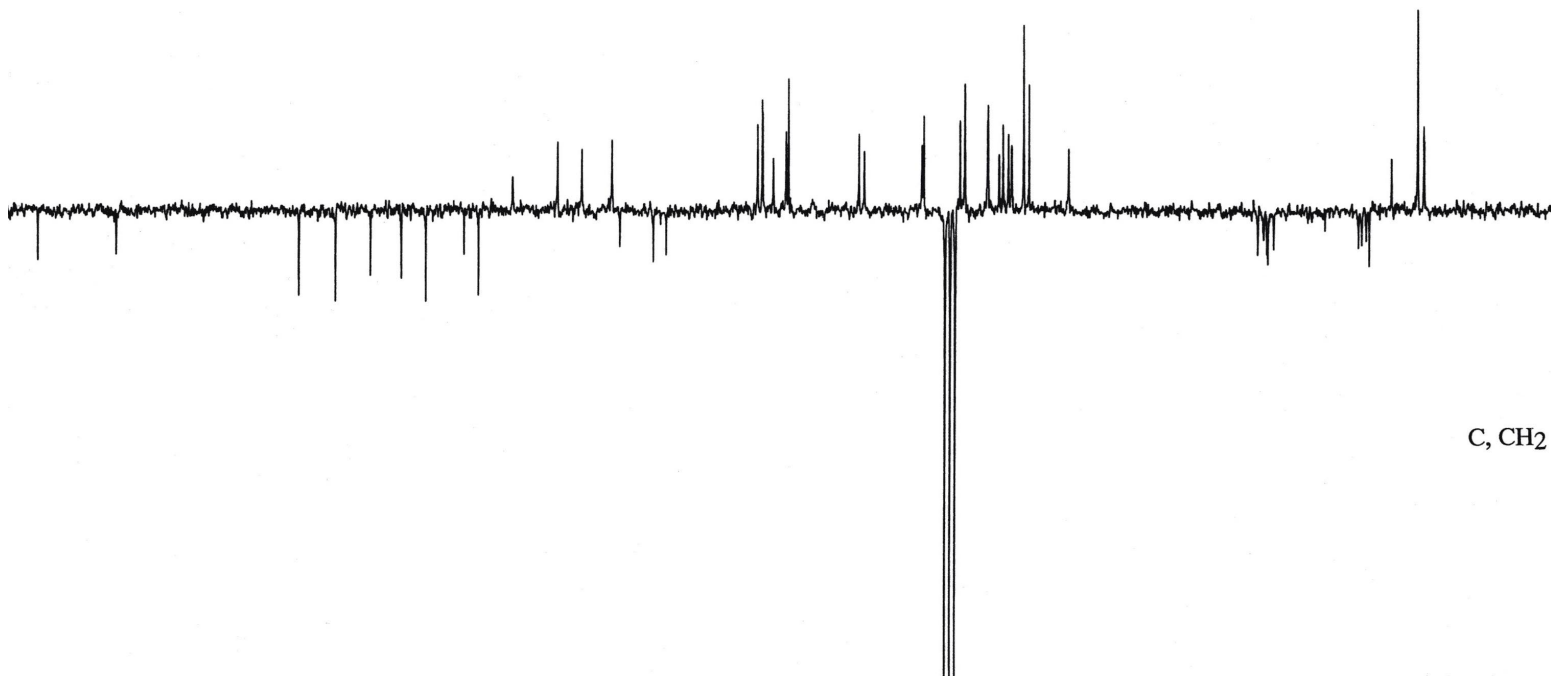


# Landomycin A, $^{13}\text{C}$ NMR ( $\text{CDCl}_3$ , TMS, 75.5 MHz)



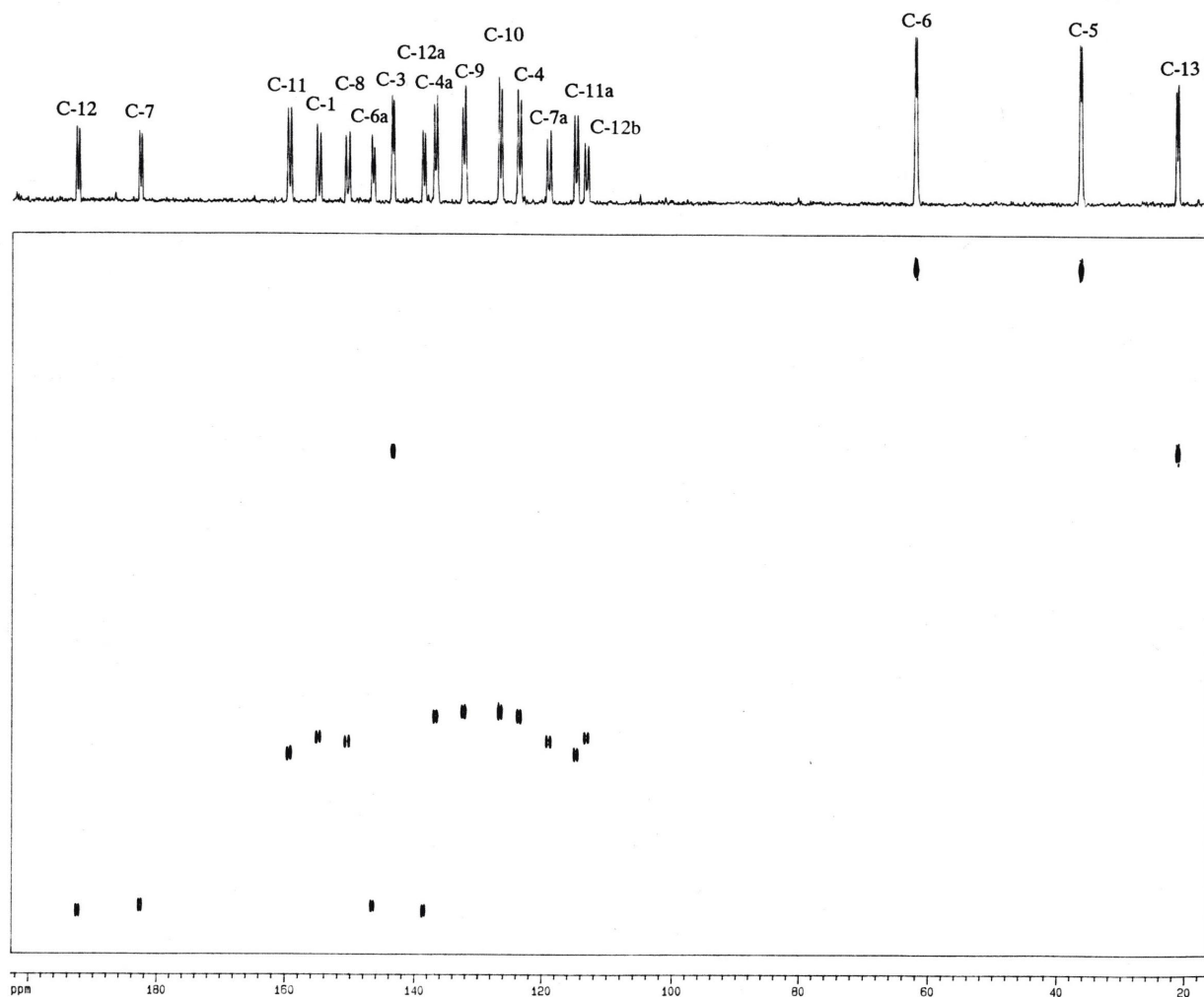
Landomycin A, APT- $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , TMS, 75.5 MHz)

CH,  $\text{CH}_3$



C,  $\text{CH}_2$

# Landomycin A, $^{13}\text{C}$ NMR-INADEQUATE after $1,2^{13}\text{C}_2$ -acetate incorporation



```

Current Data Parameter
NAME      landinad.ser
EXPNO    1
PROCNO   1

F2 - Acquisition Parameter
Date      921021
Time      21.55
PULPROG   Inad
SOLVENT   CDCl3
AQ        0.065560 sec
FIDRES    7.629395 Hz
DW        16.0 usec
RG         4096
NUCLEUS   13C
D1        1.600000 sec
S2        26 dB
HL1       26 dB
P1        7.8 usec
D4        0.0041666 sec
P2        15.5 usec
D0        0.000030 sec
DE        20.0 usec
SF01     125.706852 MHz
SMH       31250.00 Hz
TD         4096
NS         128
DS         4
IN0       0.0000160 sec

F1 - Acquisition parameter
ND0       1
TD        256
SF01     125.7068 MHz
FIDRES    244.140625 Hz
SW        497.189 ppm

F2 - Processing parameter
SI        4096
SF        125.6936373 MHz
WDW       GSINE
SSB       3
LB        0.00 Hz
GB        0
PC        1.00

F1 - Processing parameter
SI        1024
MC2       GF
SF        125.7068964 MHz
WDW       GSINE
SSB       3
LB        0.00 Hz
GB        0

2D NMR plot parameter
CX2       30.00 cm
CX1       18.00 cm
F2PLO     202.603 ppm
F2LO      25465.90 Hz
F2PHI     16.198 ppm
F2PHI     2036.03 Hz
F1PLO     136.435 ppm
F1LO      17150.76 Hz
F1PHI     -124.784 ppm
F1HI      -15686.15 Hz
F2PPMCM   6.21349 ppm/ci
F2HZCM    780.99567 Hz/cm
F1PPMCM   14.51212 ppm/ci
F1HZCM    1824.27295 Hz/cm
    
```

Landomycin A,  $^{13}\text{C}$  NMR after  $1\text{-}^{13}\text{C}$ -acetate incorporation (upper spectrum) in comparison with natural abundance  $^{13}\text{C}$  NMR (lower spectrum, mirror imaged)

