

Supporting Information for:

The First Biosynthetic Studies of the Azinomycins – Acetate Incorporation into Azinomycin B

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Contents:

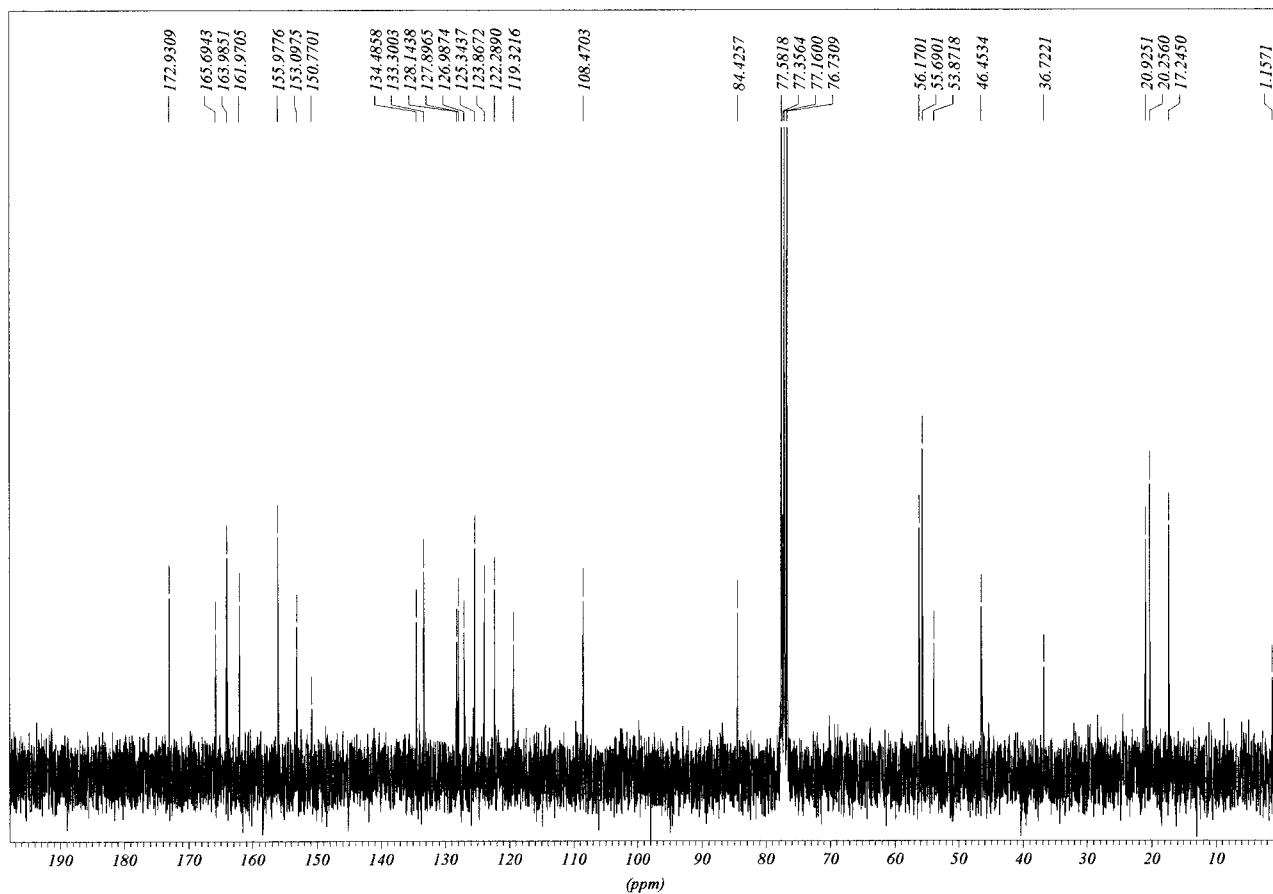
Experimental procedures

NMR spectra:

1. ¹³C NMR spectrum in CDCl₃ of unlabeled azinomycin B.
2. ¹³C NMR spectrum in CD₂Cl₂ of unlabeled azinomycin B.
3. HMBC ¹H-¹³C correlation spectrum of the naphthoate fragment of azinomycin B, used to reassign C4'a and C5'.
4. ¹³C NMR spectrum in d₆-DMSO of the naphthoate fragment of azinomycin B after feeding 1-¹³C acetate.
5. ¹³C NMR spectrum in CDCl₃ of azinomycin B after feeding 1-¹³C acetate.
6. ¹³C NMR spectrum in CD₂Cl₂ of azinomycin B after feeding 2-¹³C acetate.
7. ¹³C NMR spectrum in CDCl₃ of azinomycin B after feeding 1,2-¹³C₂ acetate.
8. ¹³C NMR spectrum in CD₂Cl₂ of azinomycin B after feeding 1,2-¹³C₂ acetate.
9. ¹³C NMR spectrum in CDCl₃ of azinomycin B after feeding methyl-¹³C-methionine.
10. ESMS spectrum of azinomycin B after feeding methyl-¹³C-methionine.

Experimental procedures: A 100 ml seed culture of *S. sahachiroi* (NRRL 2485), maintained on GYM agar plates (glucose monohydrate, 4 g/L; yeast extract, 4 g/L; malt extract, 10 g/L; CaCO₃, 2 g/L; agar, 12 g/L; tap water to balance; adjusted to pH 6.8 with NaOH, 1 M before sterilisation) at 28 °C, was grown in PS5 medium (Pharmamedia, 5 g/L; starch, 5 g/L; tap water to balance; adjusted to pH 6.0) at 30 °C, 200 rpm for 24 hours then 25 ml was used to inoculate 500 ml of PS5 medium, which was grown at 30 °C, 200 rpm for 72 hours. Aqueous solutions of labeled precursors (purchased from Cambridge Isotope Labs) were added through a 0.2 µm filter at concentrations and times as outlined in the text. After centrifugation of the cultures, azinomycin B was isolated by extraction of the supernatant (pH 8.0) with an equal volume of chloroform at 4°C, concentration and then a series of precipitations. For each 100 ml of culture, the residue was precipitated from 600 µl chloroform/hexane (1:29), centrifuged at 2000 rpm and the supernatant discarded. This was repeated and then the residue dissolved in 600 µl chloroform/hexane (2:1), centrifuged and the supernatant retained. This residue was then dissolved in 600 µl chloroform/diethyl ether (1:4), centrifuged and the supernatant concentrated to give pure azinomycin B (~1.5 mg per 100 ml). ¹³C NMR spectra were obtained on a Bruker Advance 400 at 100.6 MHz in CDCl₃ or CD₂Cl₂, referenced to solvent.

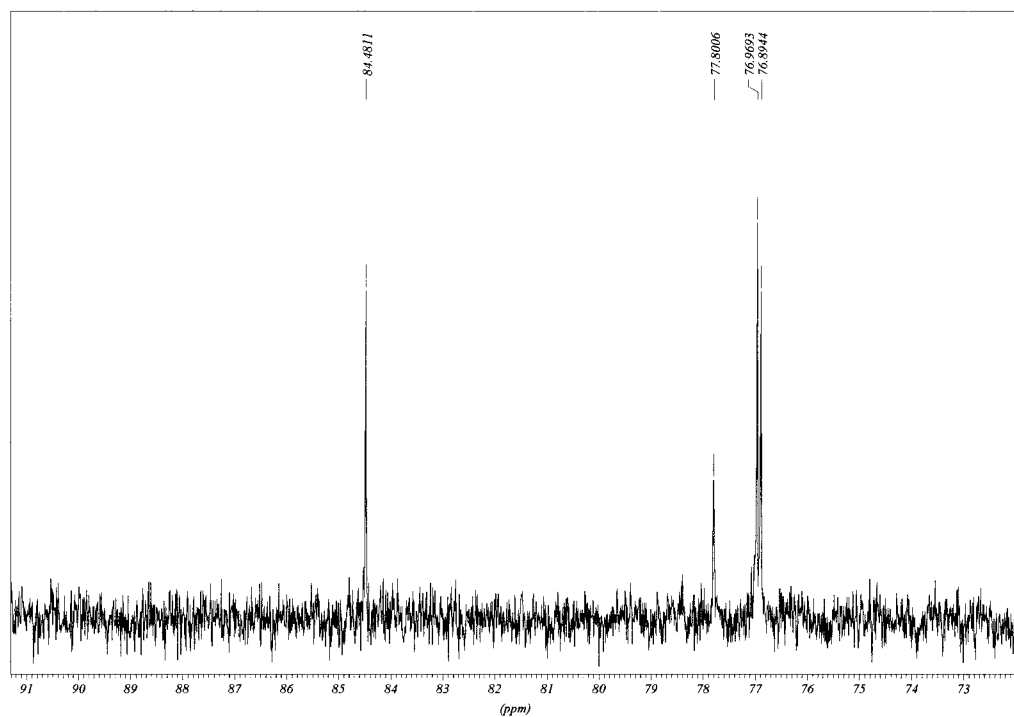
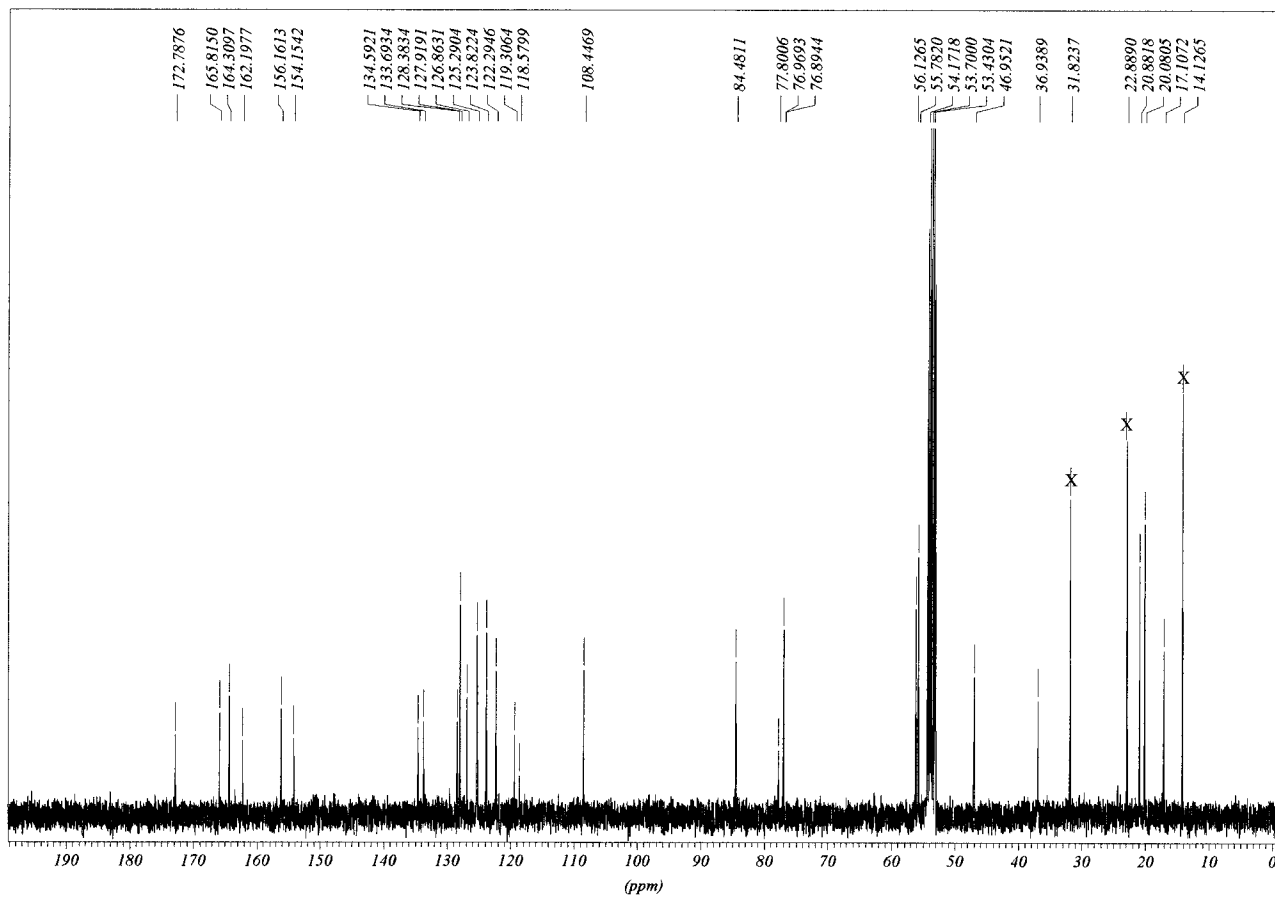
1. ^{13}C NMR spectrum in CDCl_3 of unlabeled azinomycin B.



2. ^{13}C NMR spectrum in CD_2Cl_2 of unlabeled azinomycin B.

Resonances from an unidentified impurity are marked with X.

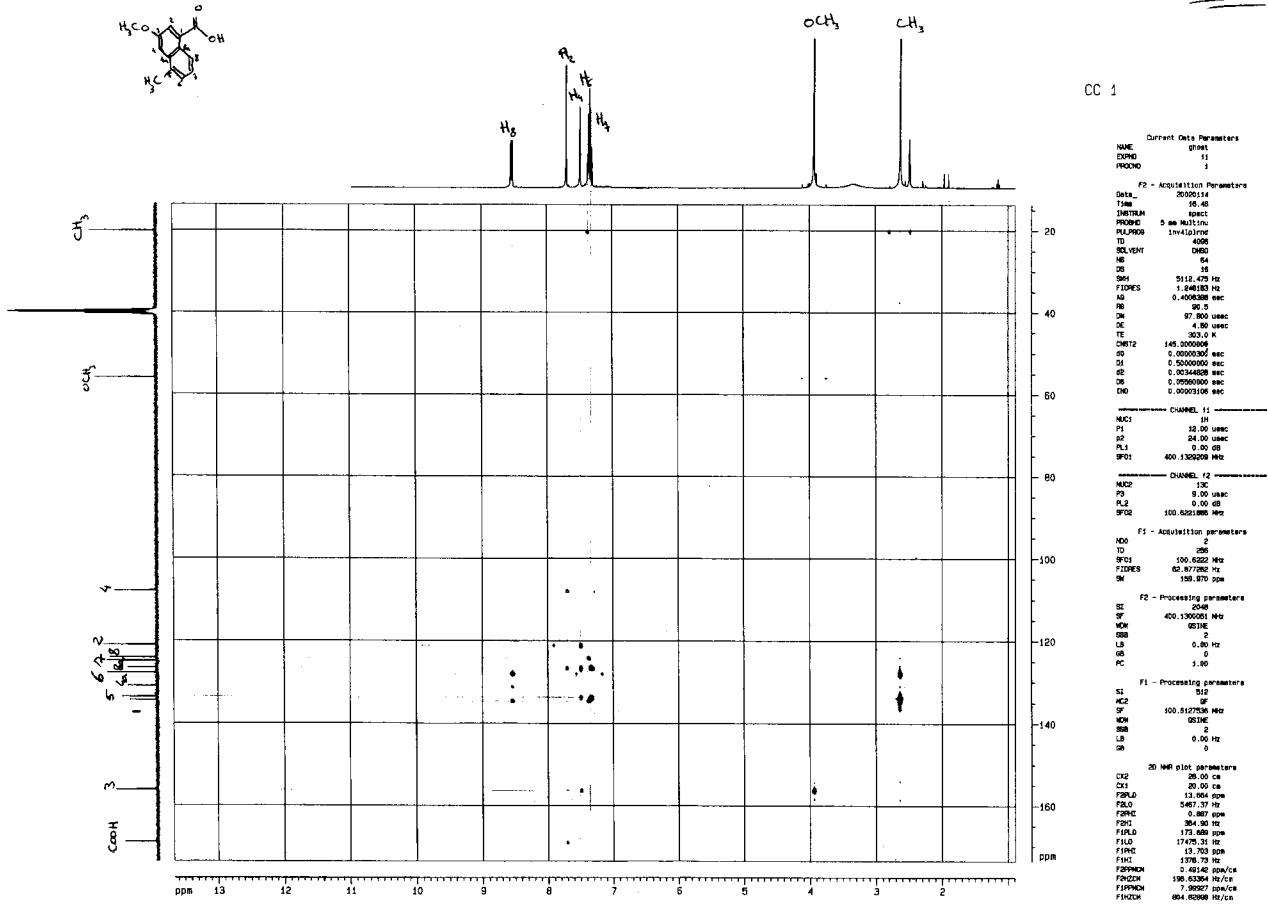
The expansion shows C12, C13, C18 and residual chloroform.



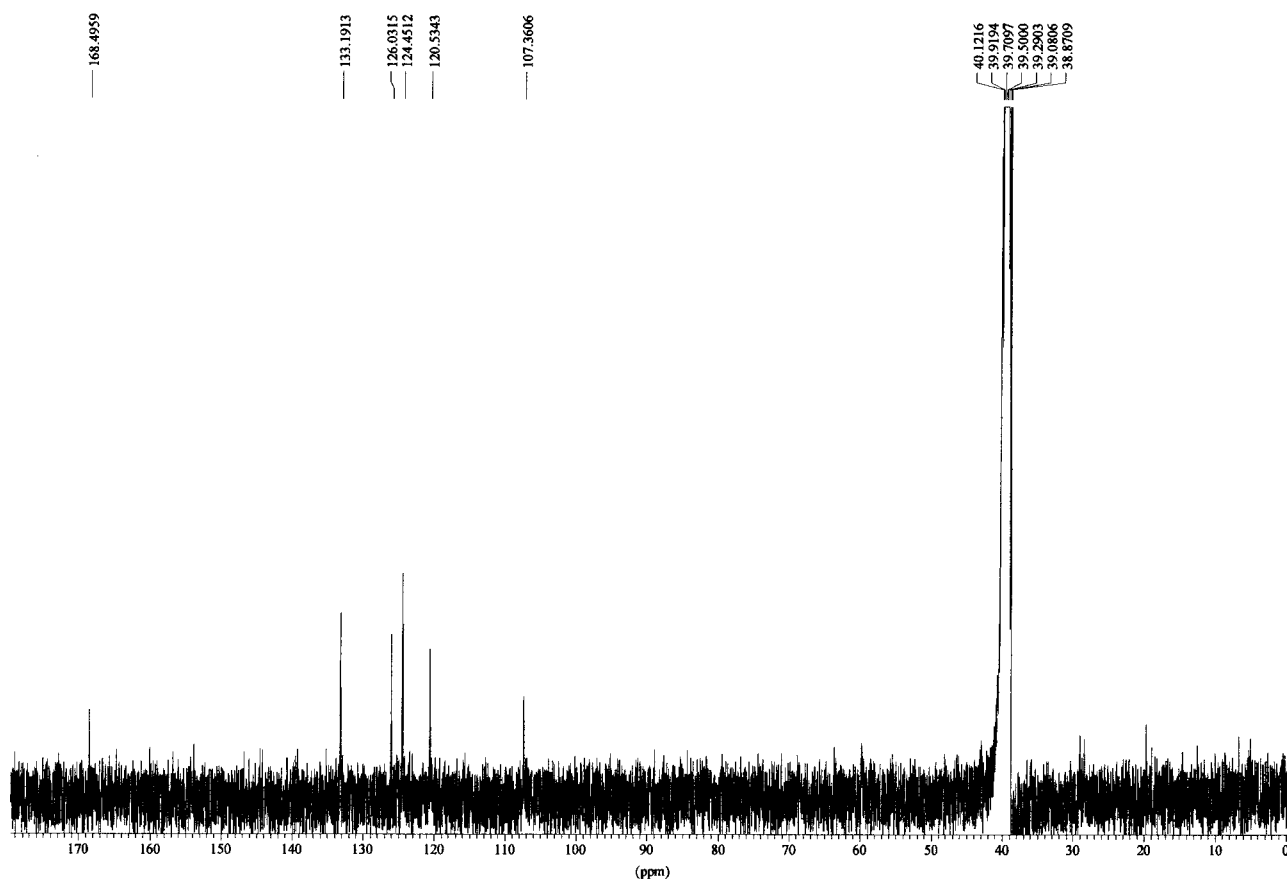
3. HMBC ^1H - ^{13}C correlation spectrum of the naphthoate fragment of azinomycin B.

These data were used to reassign C4'a and C5'.

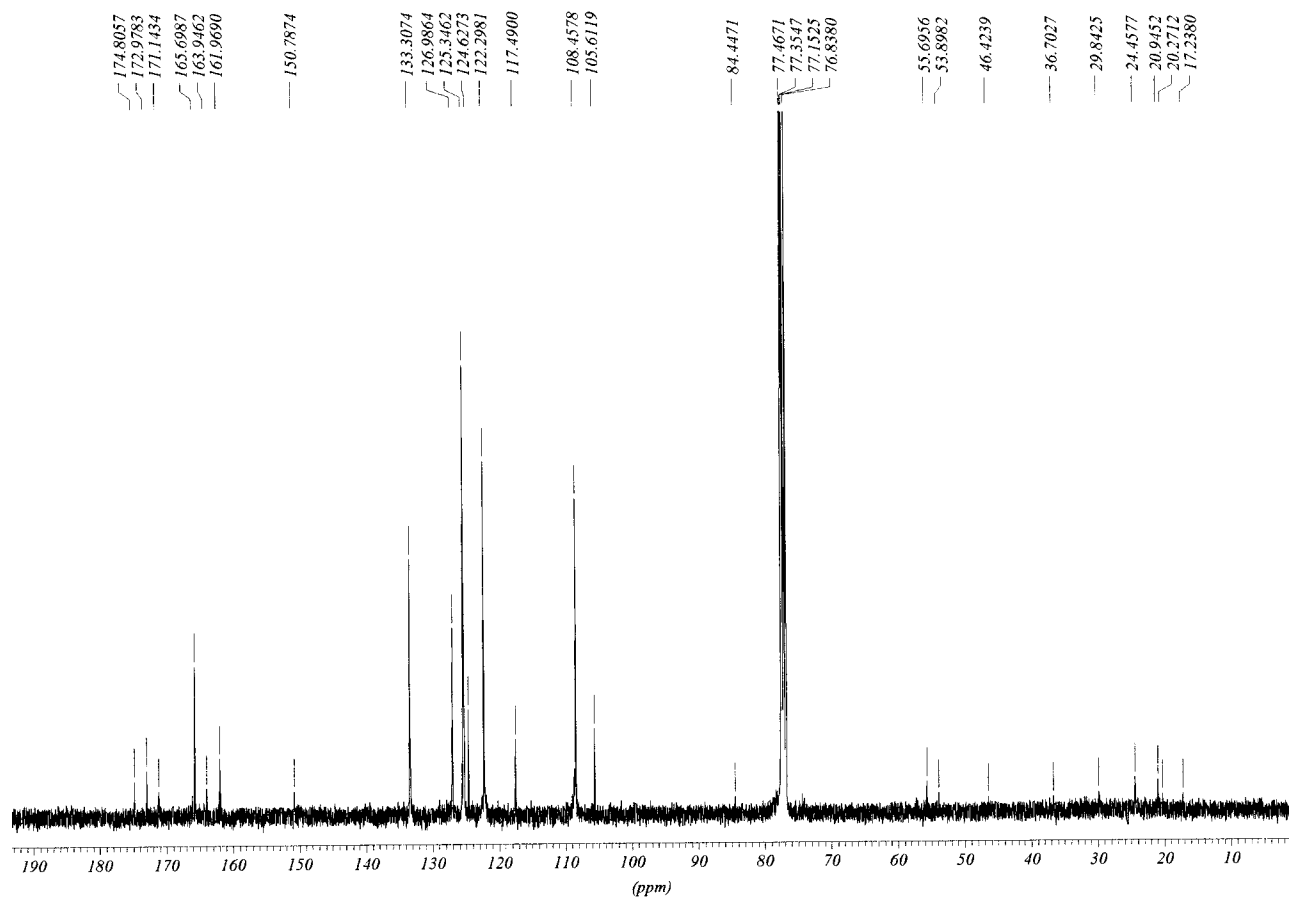
HMBC



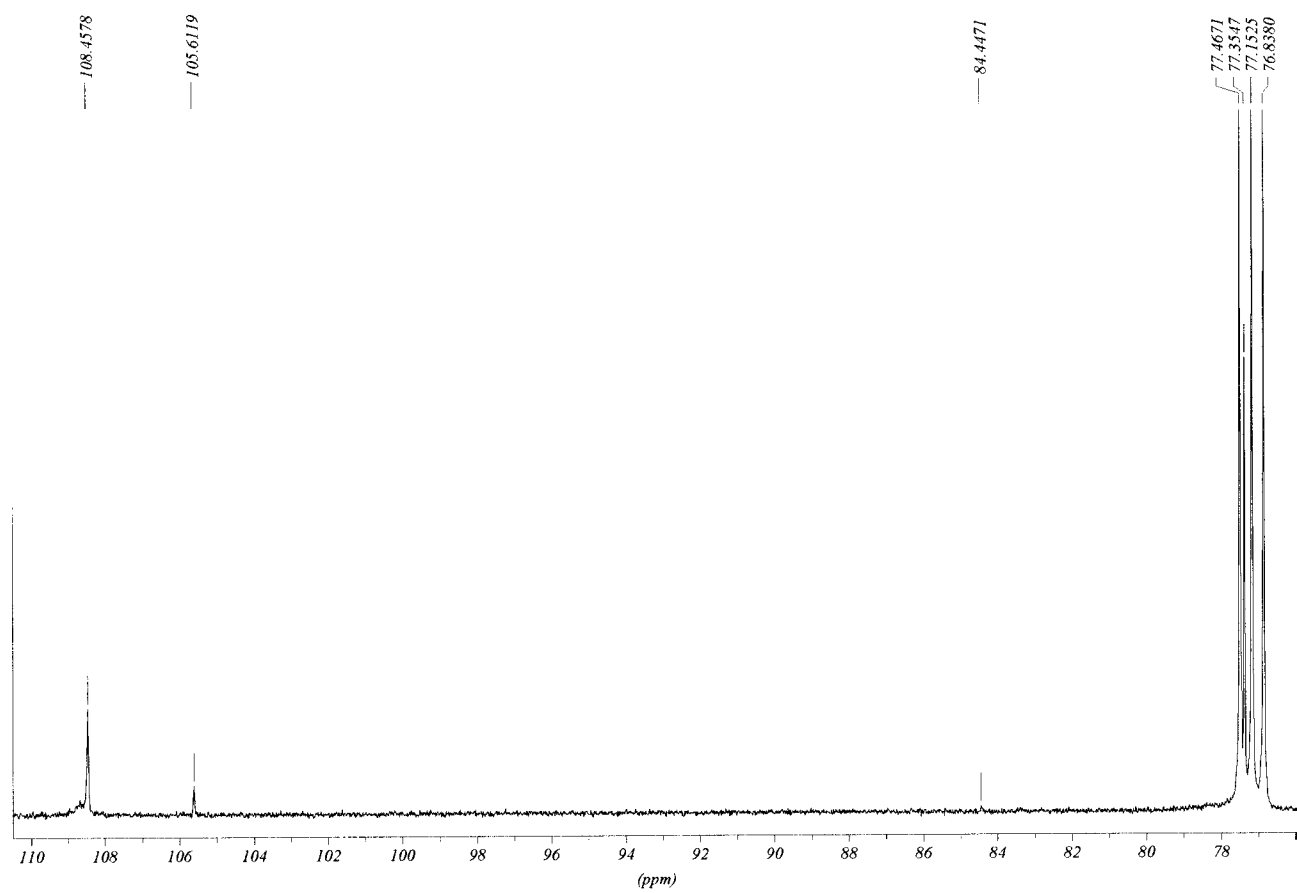
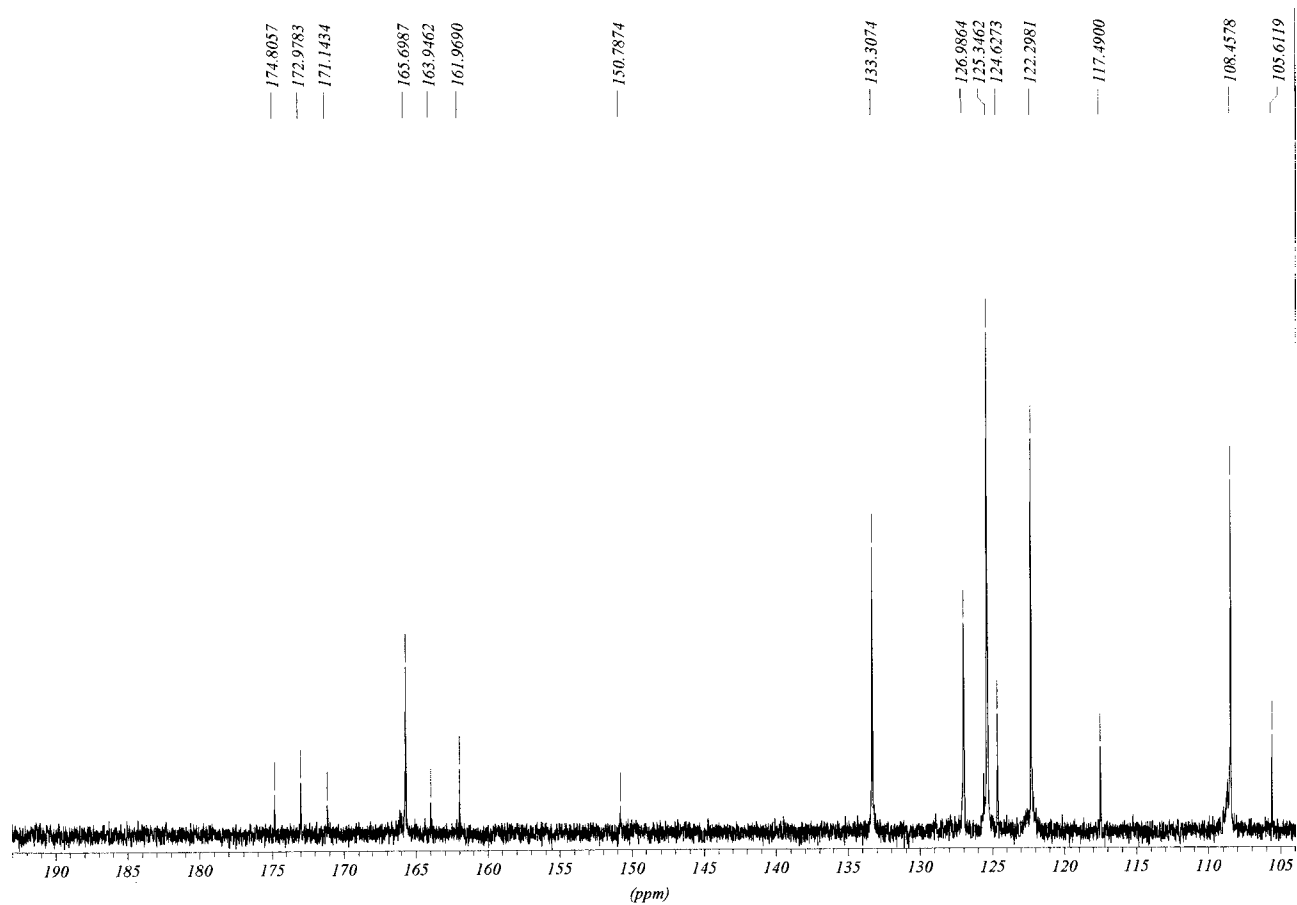
4. ^{13}C NMR spectrum in $\text{d}_6\text{-DMSO}$ of the naphthoate fragment of azinomycin B after feeding $1\text{-}^{13}\text{C}$ acetate.



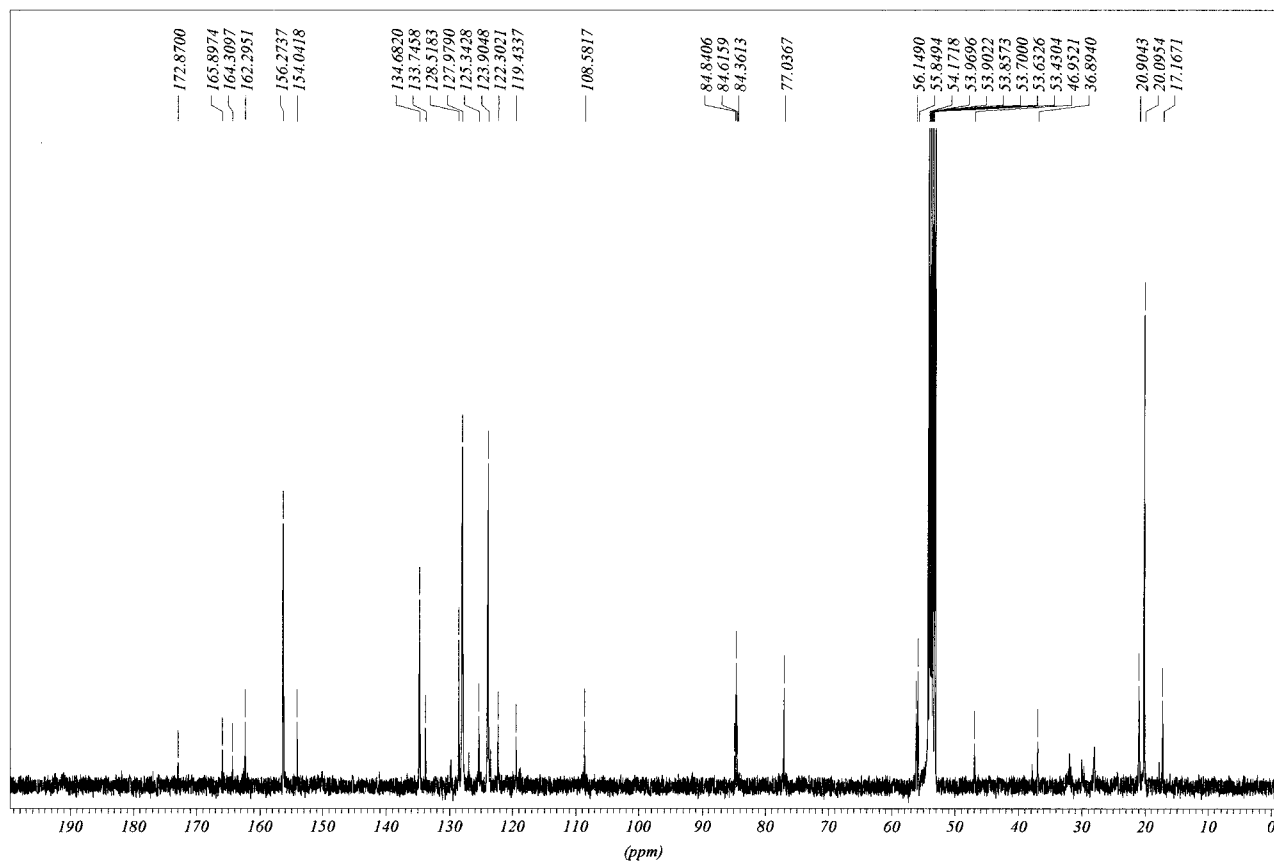
5. ^{13}C NMR spectrum in CDCl_3 of azinomycin B after feeding $1\text{-}^{13}\text{C}$ acetate.



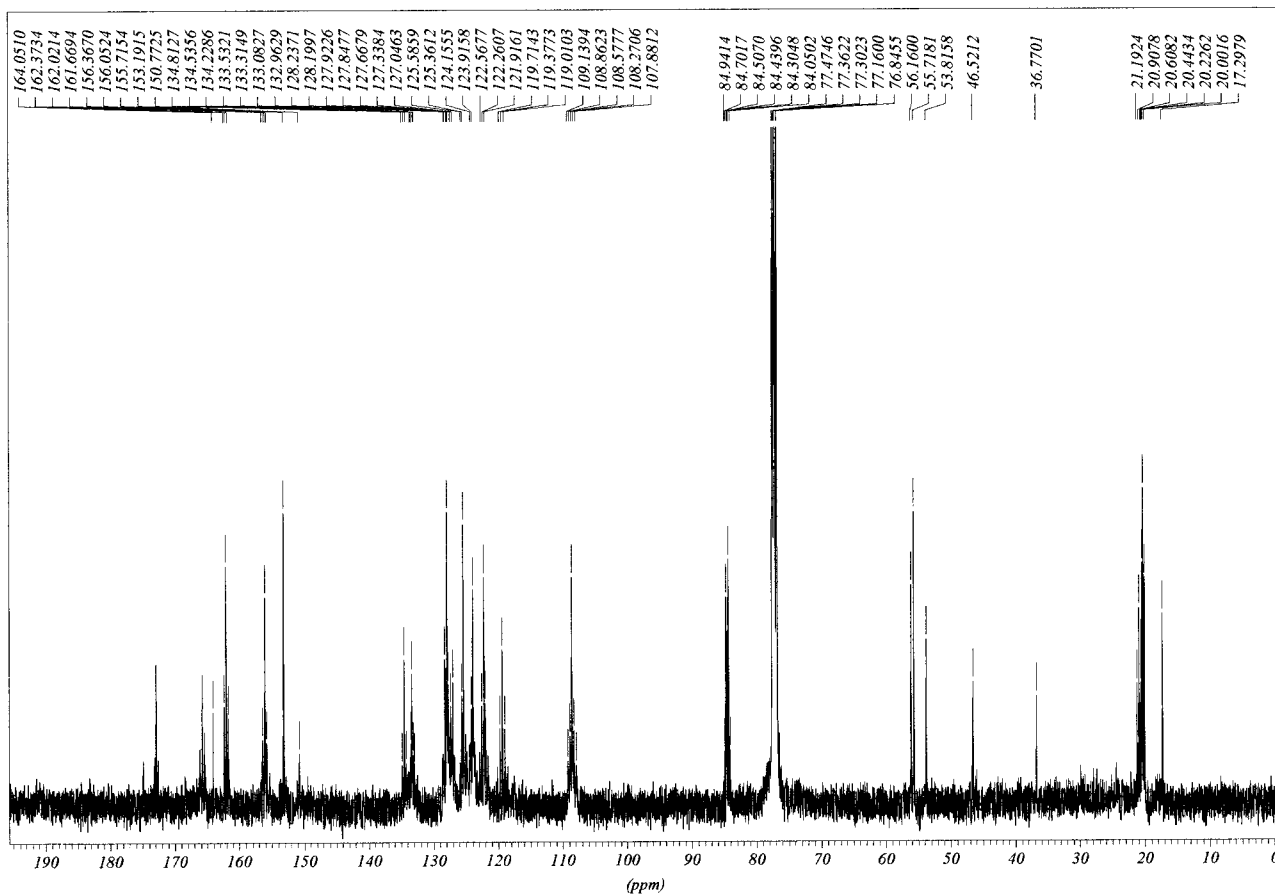
Expansions of ^{13}C NMR spectrum in CDCl_3 of azinomycin B after feeding $1\text{-}^{13}\text{C}$ acetate.



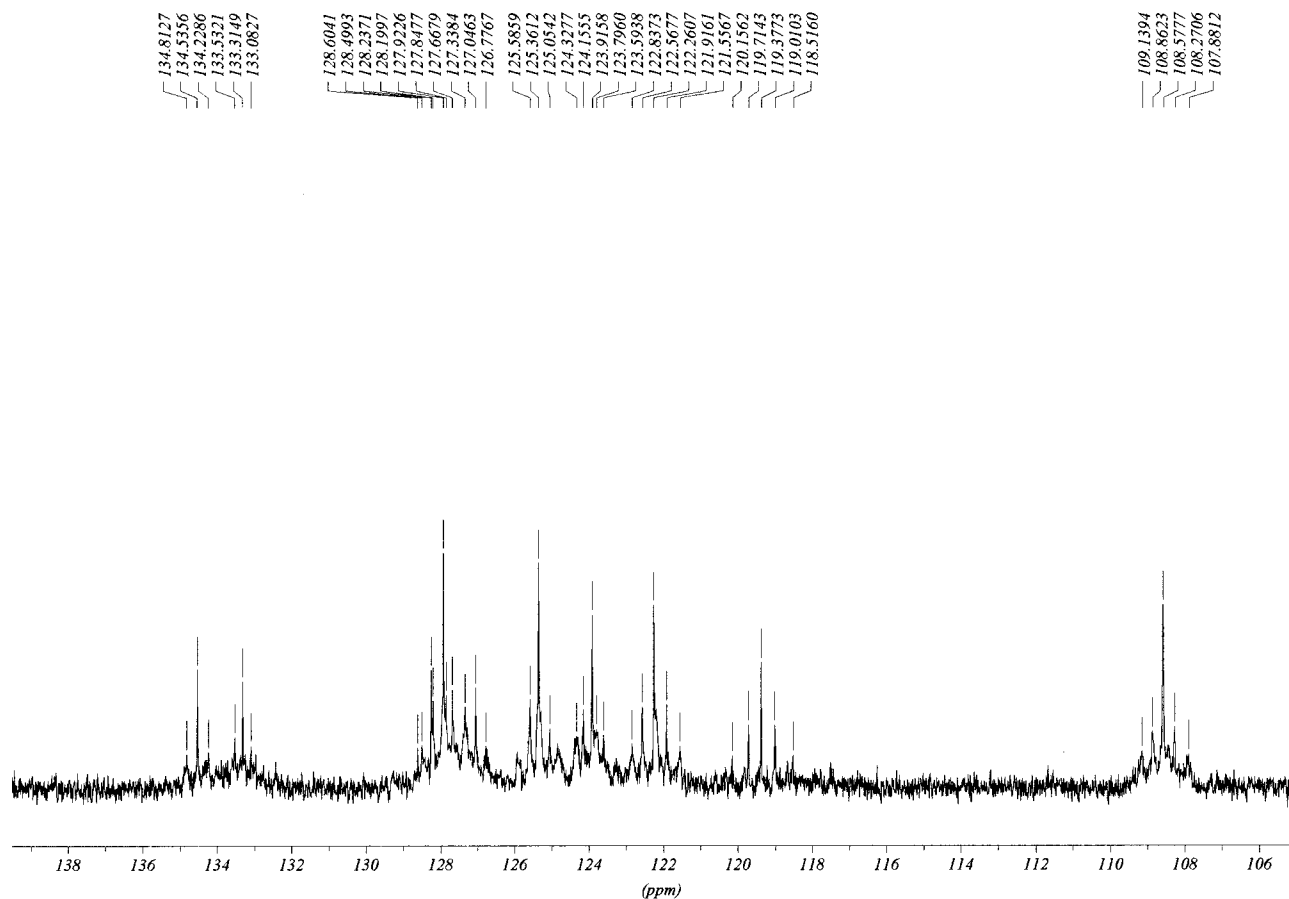
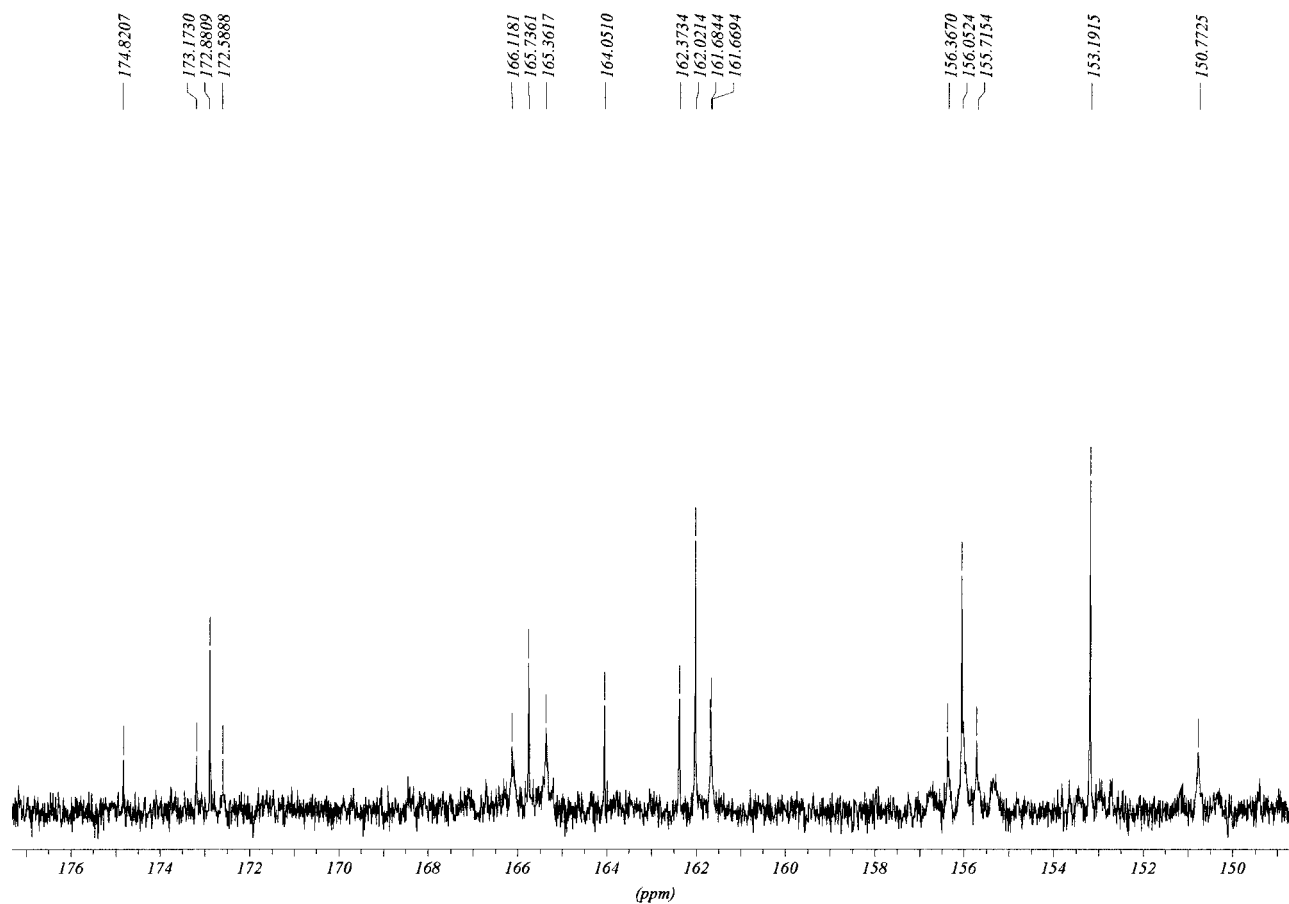
6. ^{13}C NMR spectrum in CD_2Cl_2 of azinomycin B after feeding $2\text{-}^{13}\text{C}$ acetate.

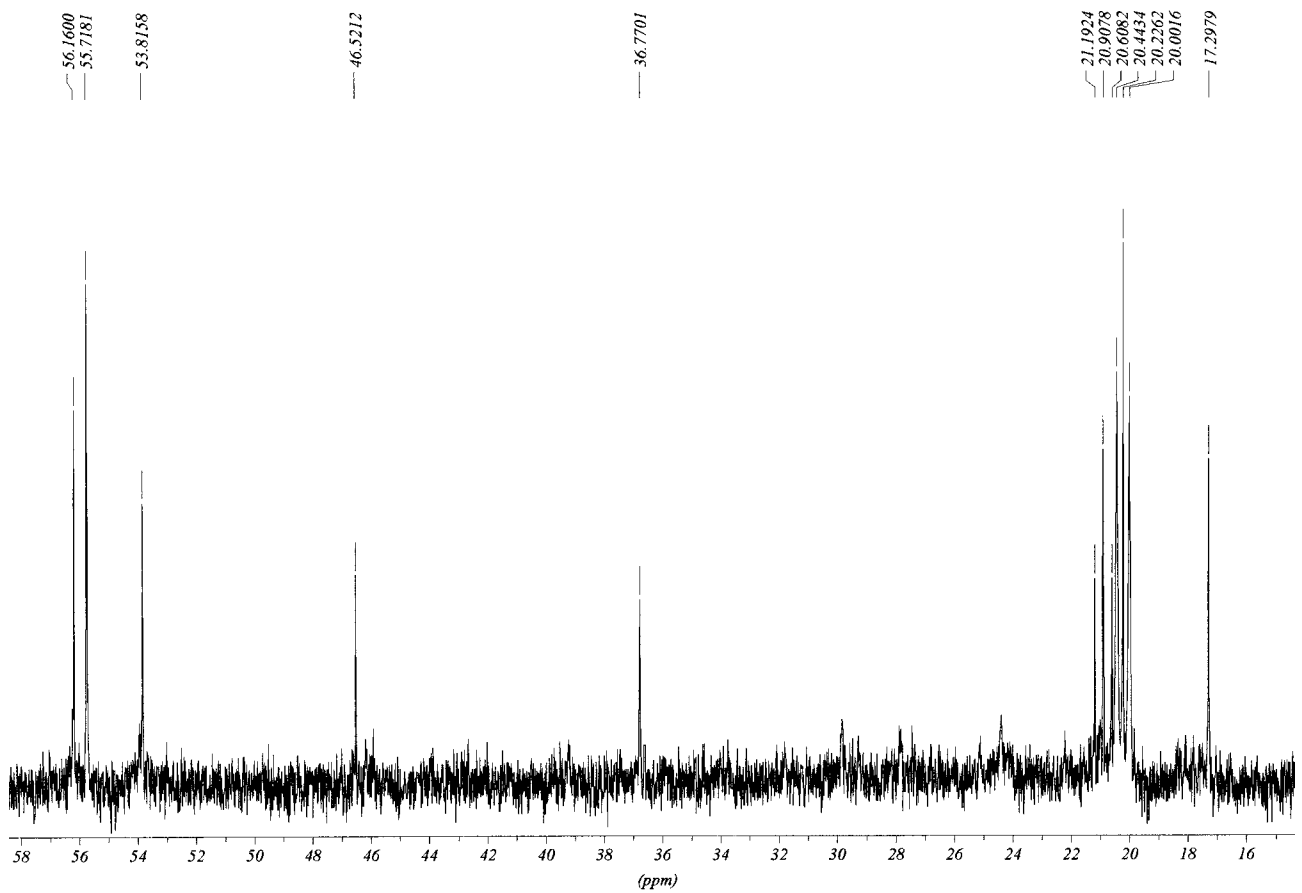
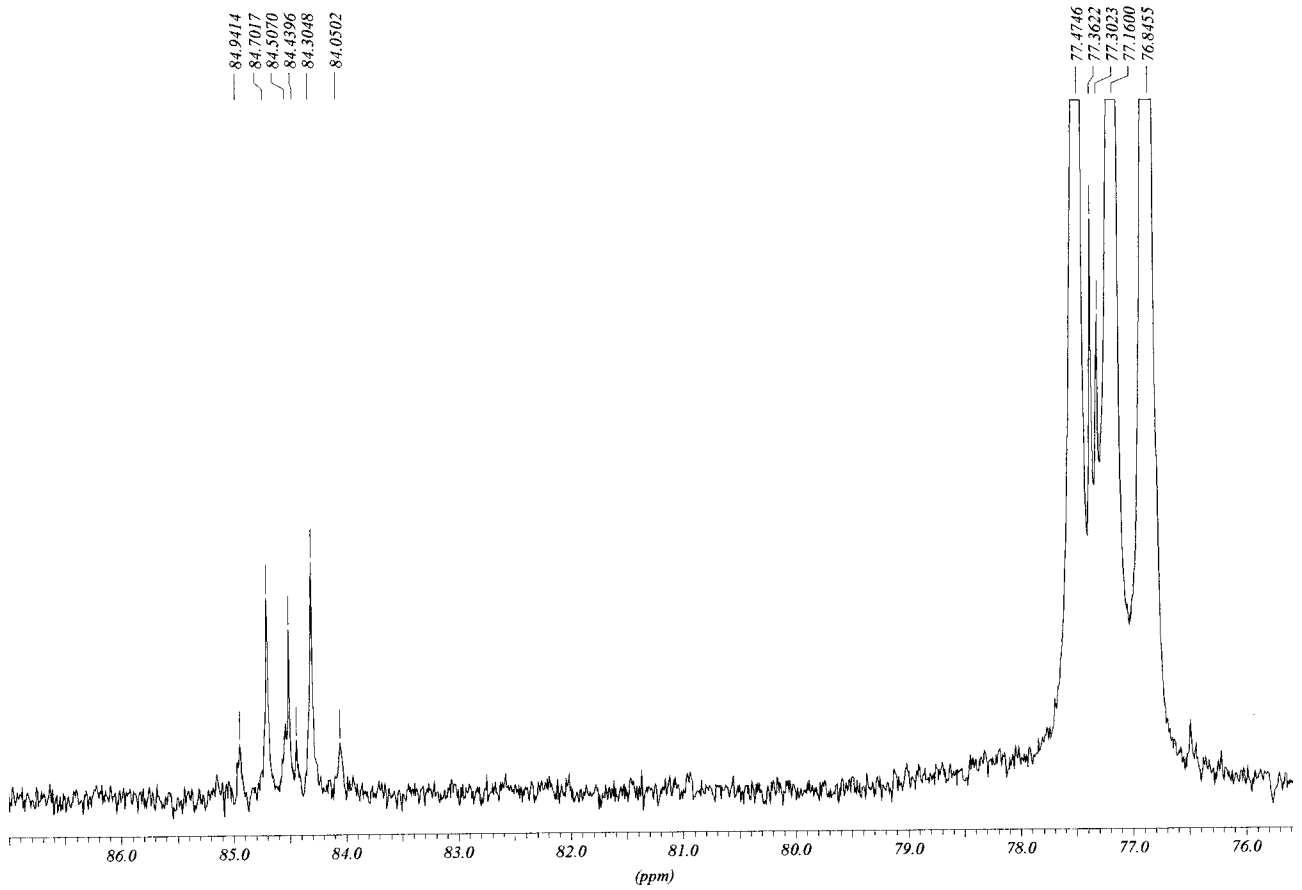


7. ^{13}C NMR spectrum in CDCl_3 of azinomycin B after feeding $1,2\text{-}^{13}\text{C}_2$ acetate.



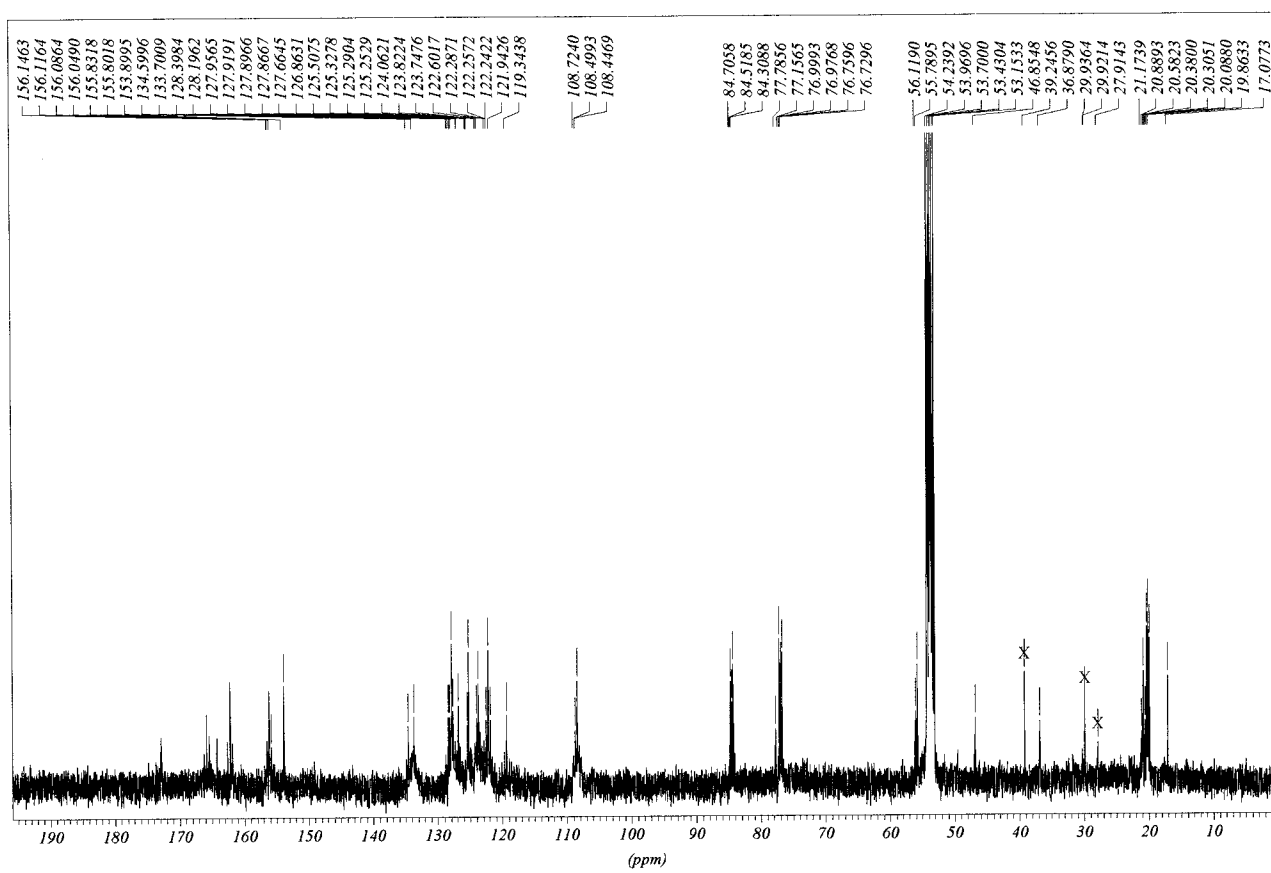
Expansions of ^{13}C NMR spectrum in CDCl_3 of azinomycin B after feeding $1,2\text{-}^{13}\text{C}_2$ acetate.



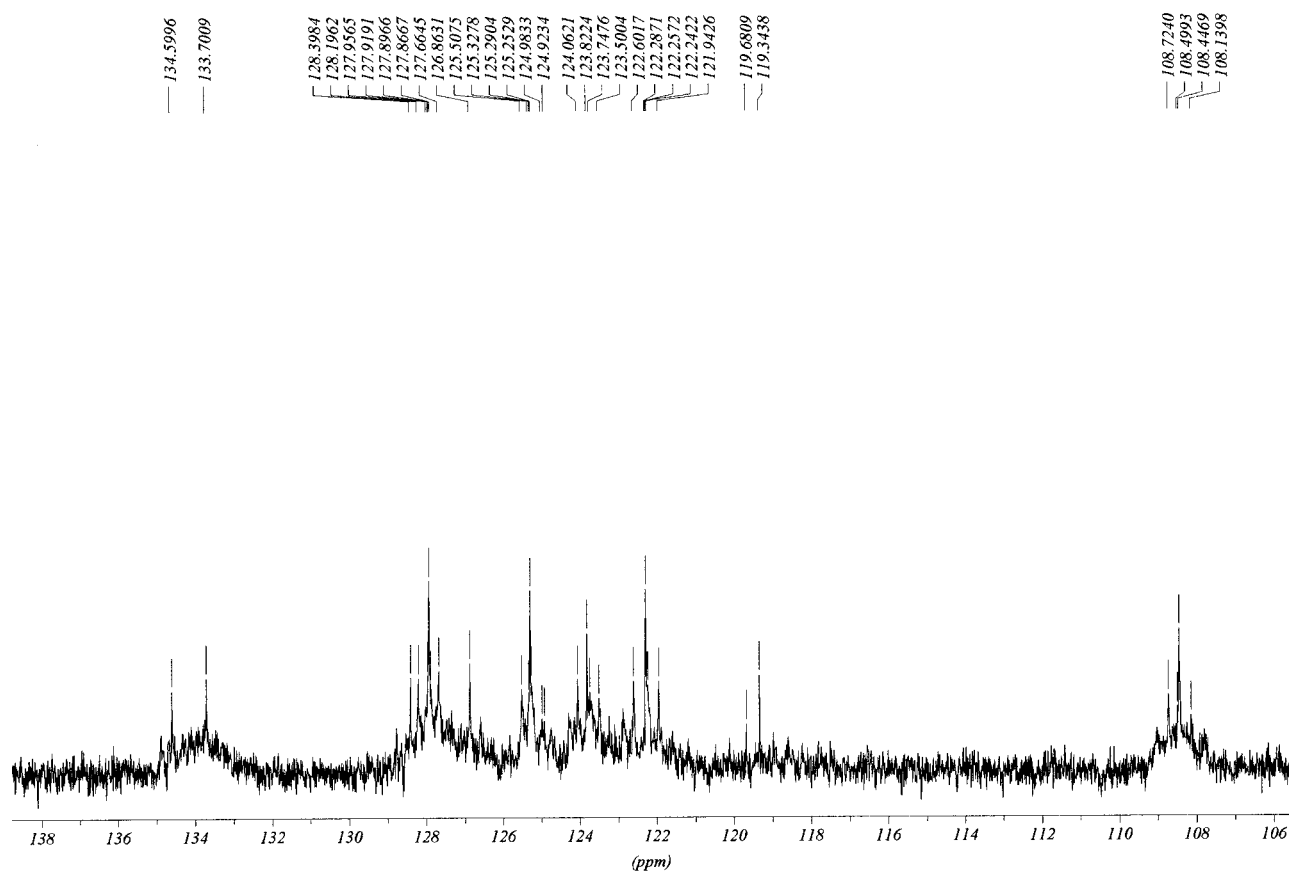
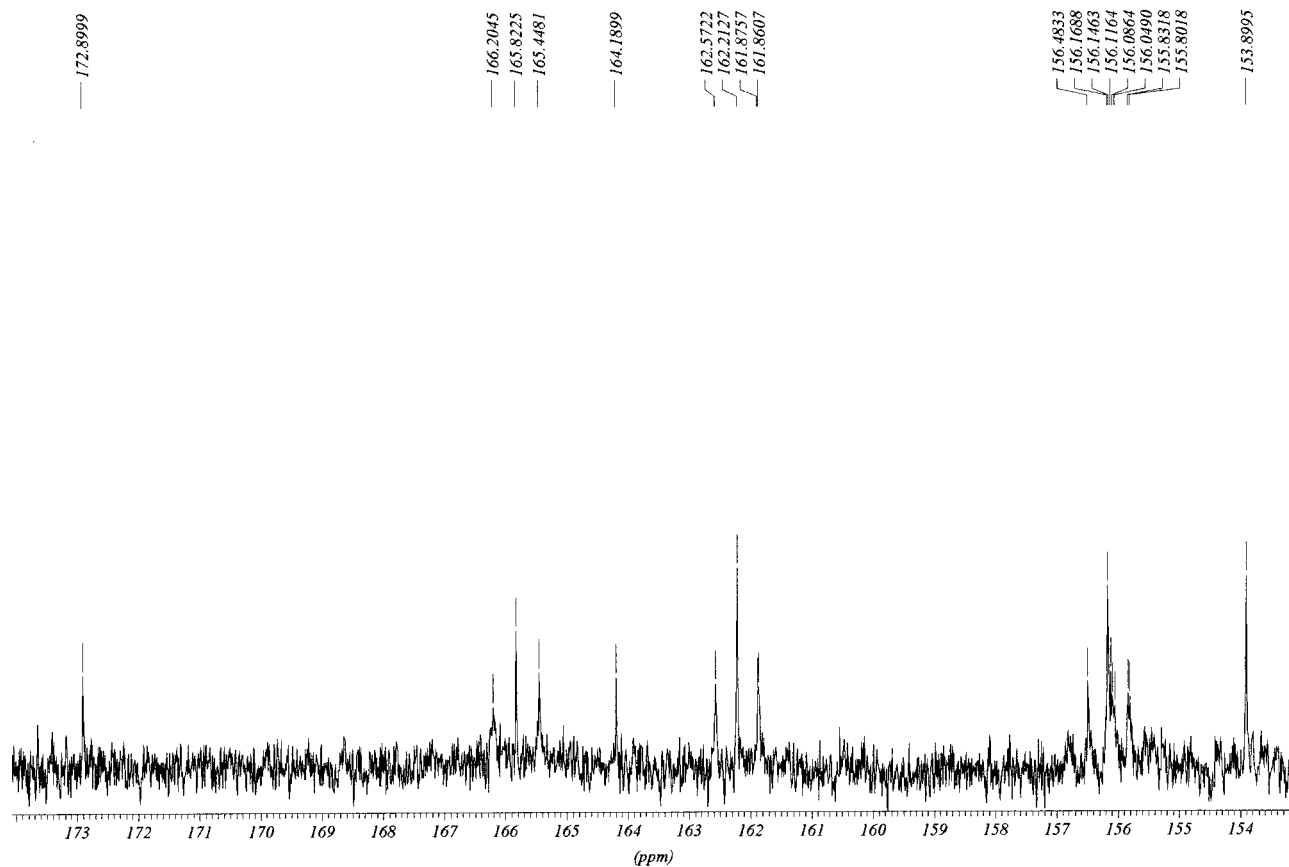


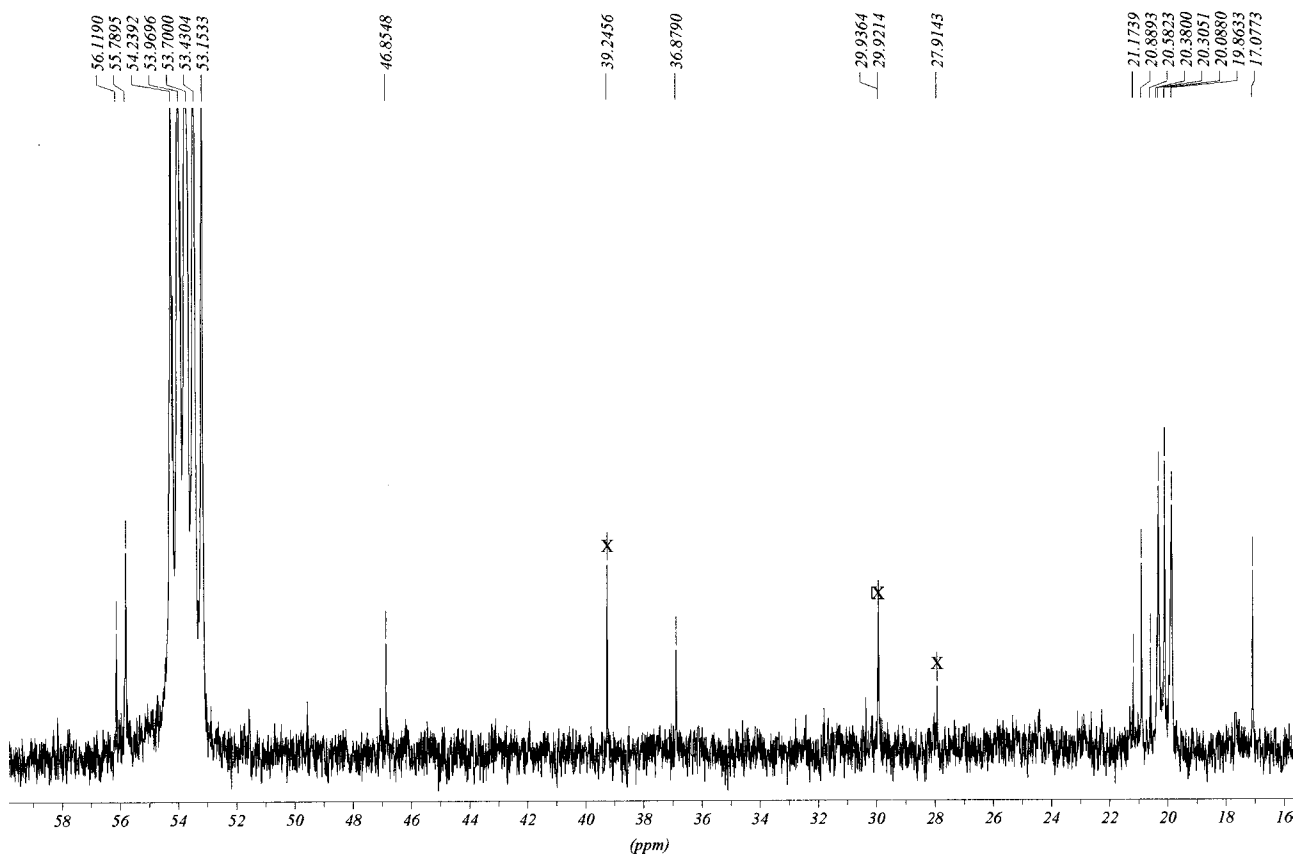
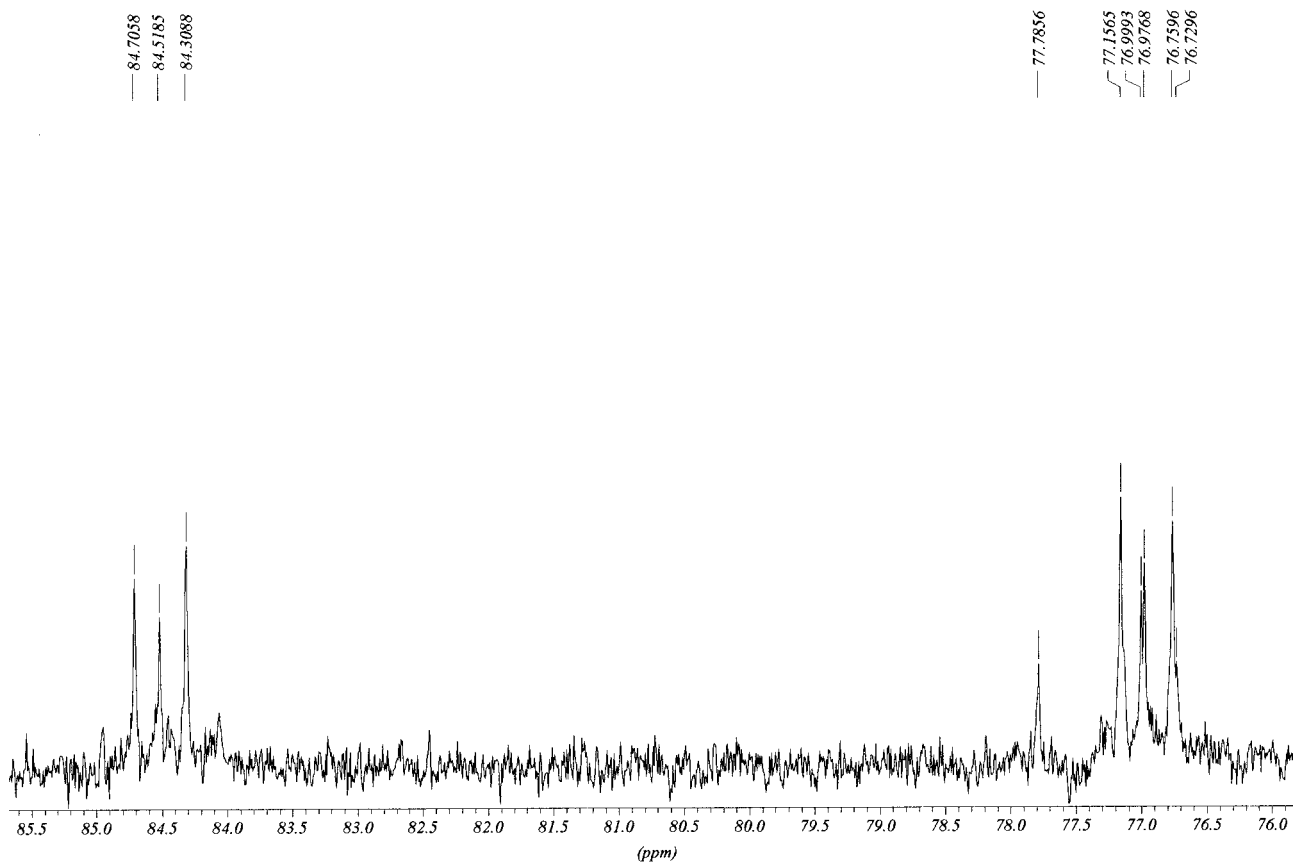
8. ^{13}C NMR spectrum in CD_2Cl_2 of azinomycin B after feeding $1,2\text{-}^{13}\text{C}_2$ acetate.

Resonances from an unidentified impurity are marked with X.

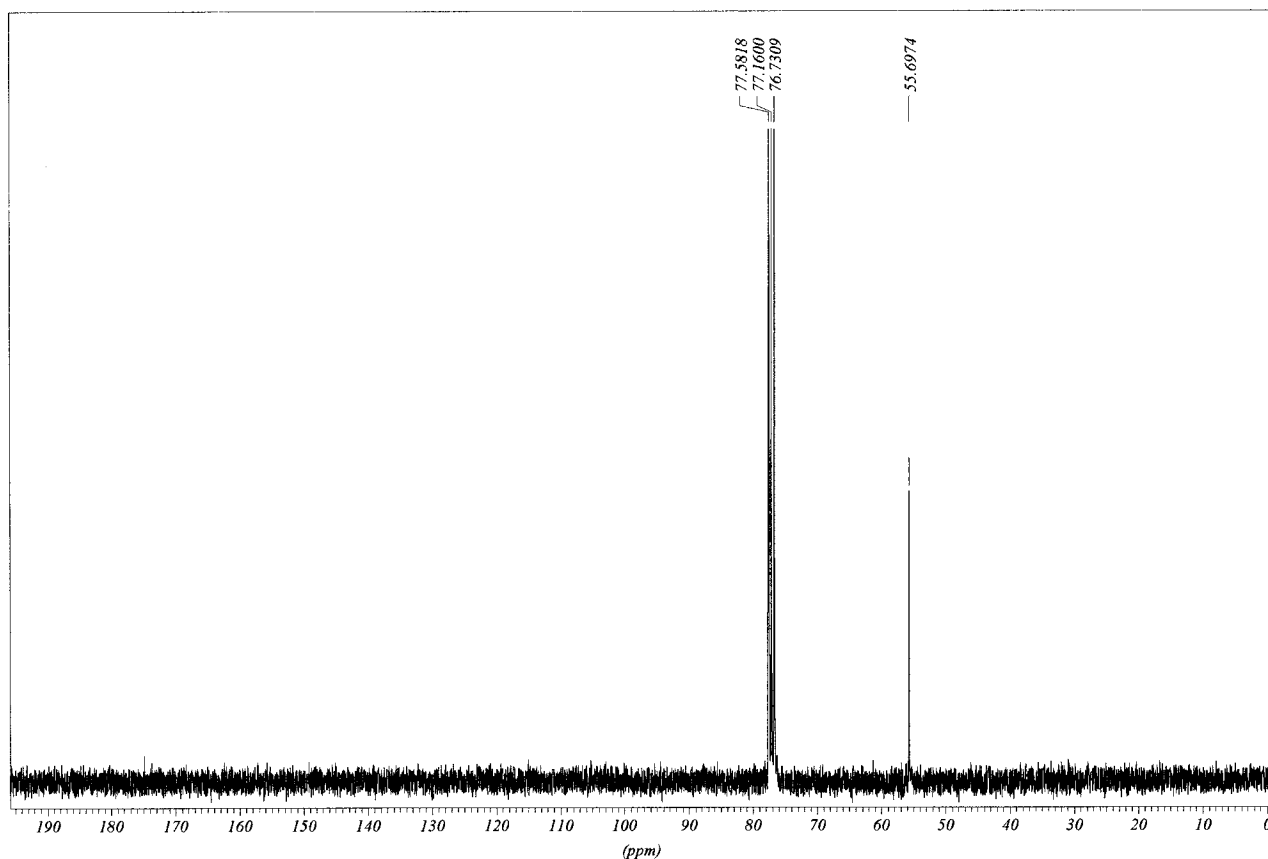


Expansions of ^{13}C NMR spectrum in CD_2Cl_2 of azinomycin B after feeding $1,2\text{-}^{13}\text{C}_2$ acetate.





9. ^{13}C NMR spectrum in CDCl_3 of azinomycin B after feeding methyl- ^{13}C -methionine.



10. ESMS spectrum of azinomycin B after feeding methyl- ^{13}C -methionine.

