CHEMBIOCHEM

Supporting Information

CHEMBIOCHEM

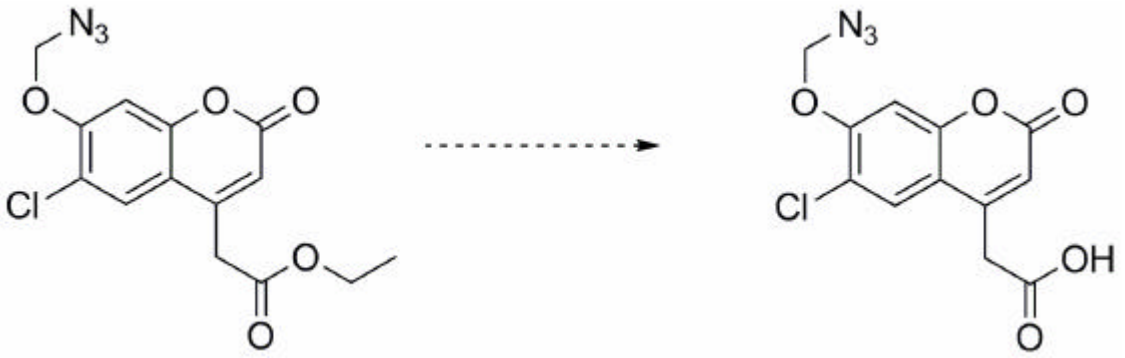
Supporting Information

for

7-Azidomethoxy Coumarins as Profluorophores for Templated Nucleic Acid Detection

Raphael M. Franzini and Eric T. Kool*

Table of Contents



***Scheme S1*:** Attempted ester-hydrolysis of Ethyl 6-chloro-7-azidomethoxycoumarin-4-acetate. Hydrolysis failed because of inertness of the ester and decarboxylation of the acid.

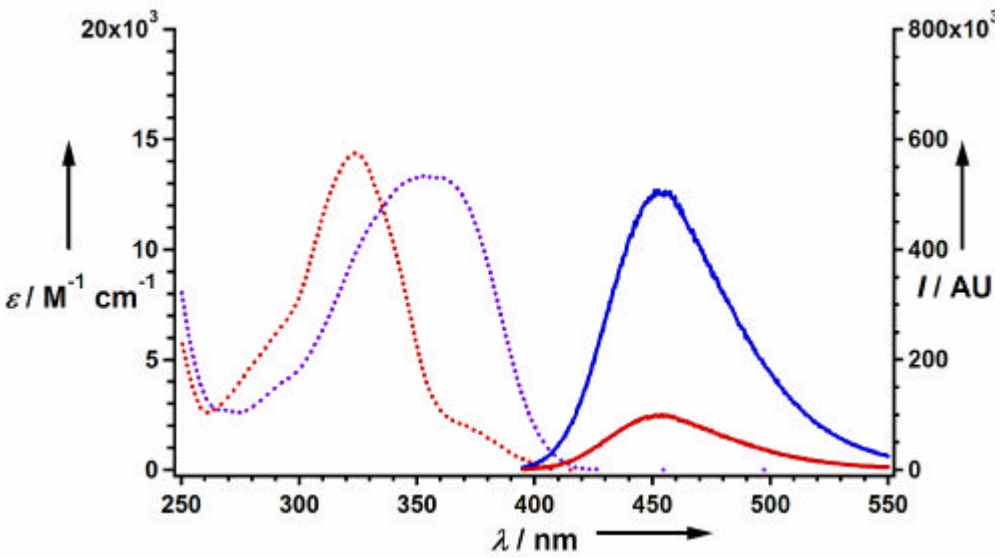


***Figure S1*:** pH dependence of absorbance and emission spectra of 7-AzMeOHCoum (**8a**). Absorbance (dotted line) and emission (solid line) spectra of 7-AzMeOHCoum at pH 7.55 (red; 0.1 M HEPES buffer) and pH 8.5 (blue; 70 mM tris-borate + 10 mM $MgCl_2$). Probe concentrations for fluorescence experiments were 2 µM; excitation wavelength was $?_{ex}$ = 375 nm.

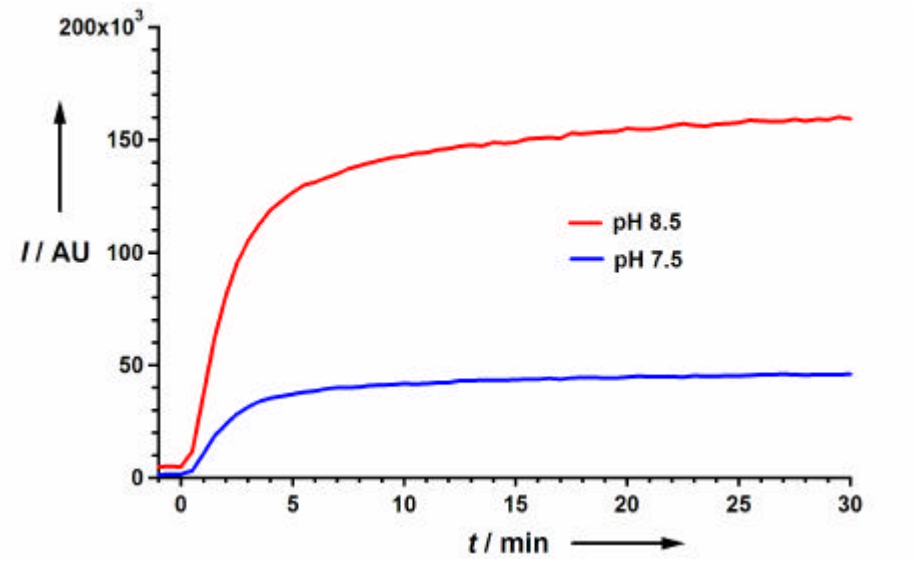


Figure S2: Template mediated uncaging of AzMeOCoum-DNA at variable pH. AzMeOCoum-DNA and *mut A* (both 200 nM) were incubated in buffer (70 mM tris-borate + 10 mM $MgCl_2$, pH 7.55 or 8.5 as indicated) at 37°C and the change in fluorescence ($?_{em}$ = 375 nm and $?_{ex}$ = 450 nM) was measured as a function of time after addition of TPP-DNA (400 nM).

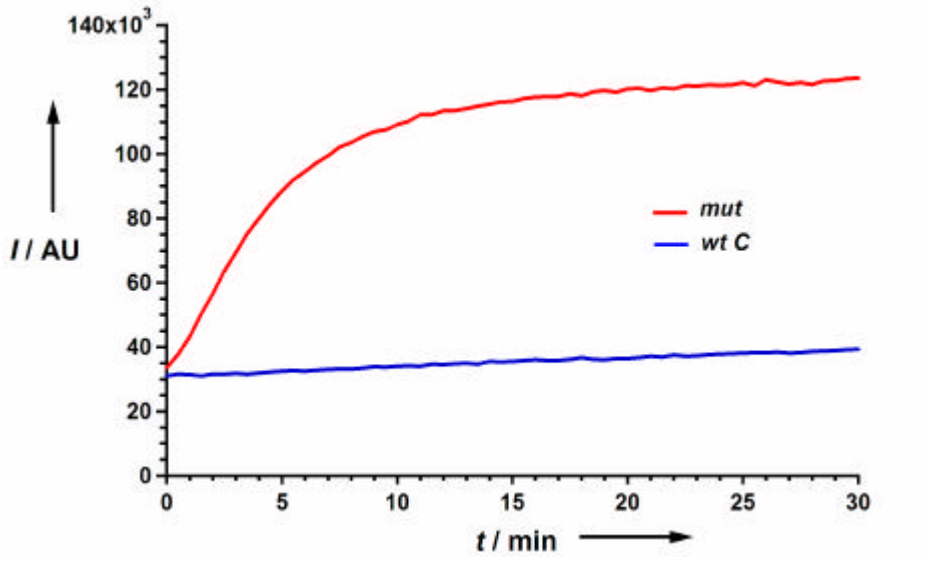
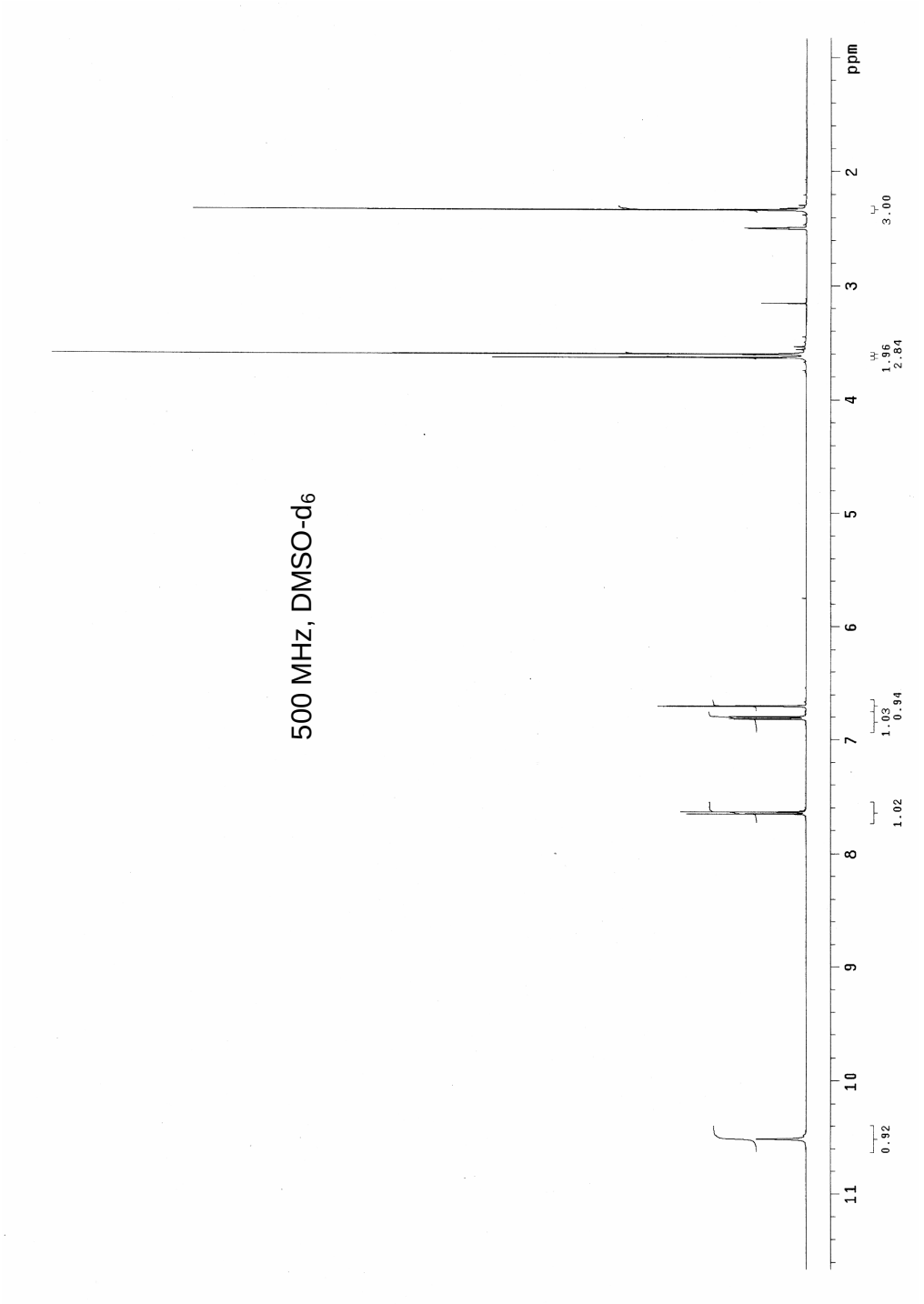


Figure S3: Time courses of fluorescence activation of AzMeOCoum-FRET-DNA by TPP-DNA. AzMeOCoum-FRET-DNA (200 nM) was incubated with either *mut A* or *wt C* (200 nM) in buffer (70 mM tris-borate + 10 mM $MgCl_2$, pH 8.5) at 25°C and the change in fluorescence ($?_{em}$ = 521 nm and $?_{ex}$ = 360 nM) was measured as a function of time after addition of TPP-DNA (400 nM).

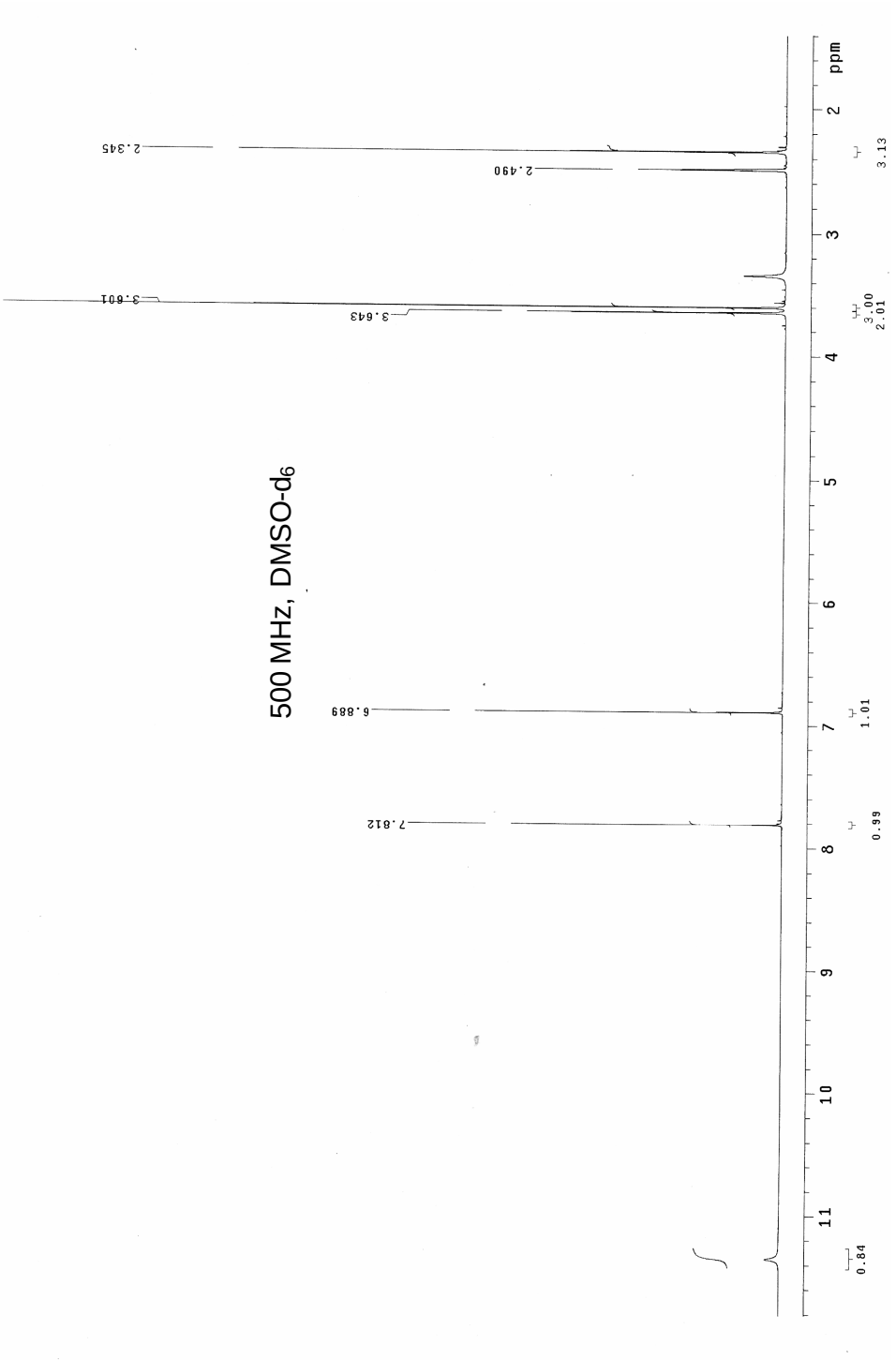
^{1}H NMR Spectra

---

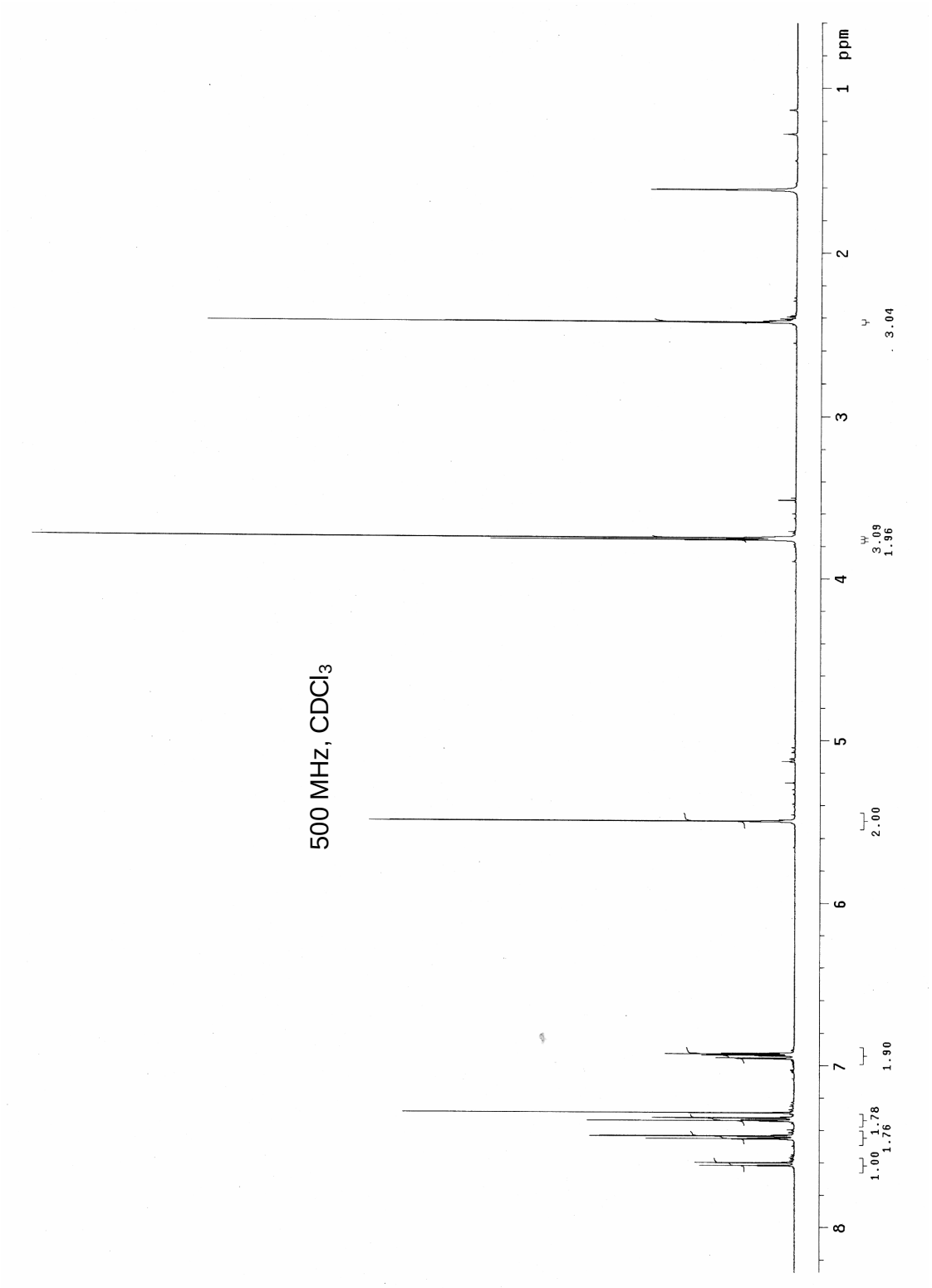
^{1}H NMR spectrum of Methyl 7-hydroxy-4-methylcoumarin-3-acetate (**3a**)



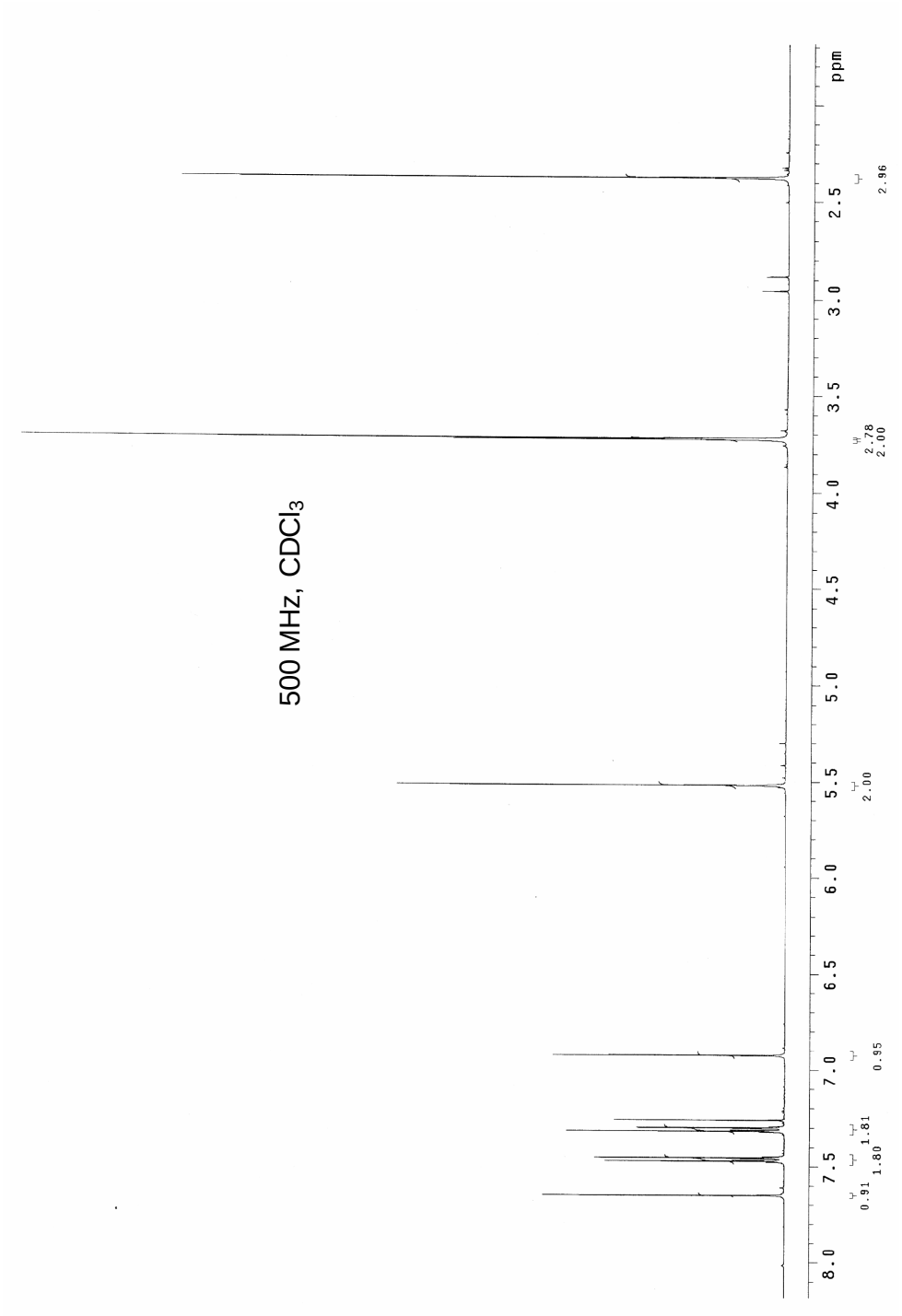
^{1}H NMR spectrum of Methyl 6-chloro-7-hydroxy-4-methylcoumarin-3-acetate (**3b**)



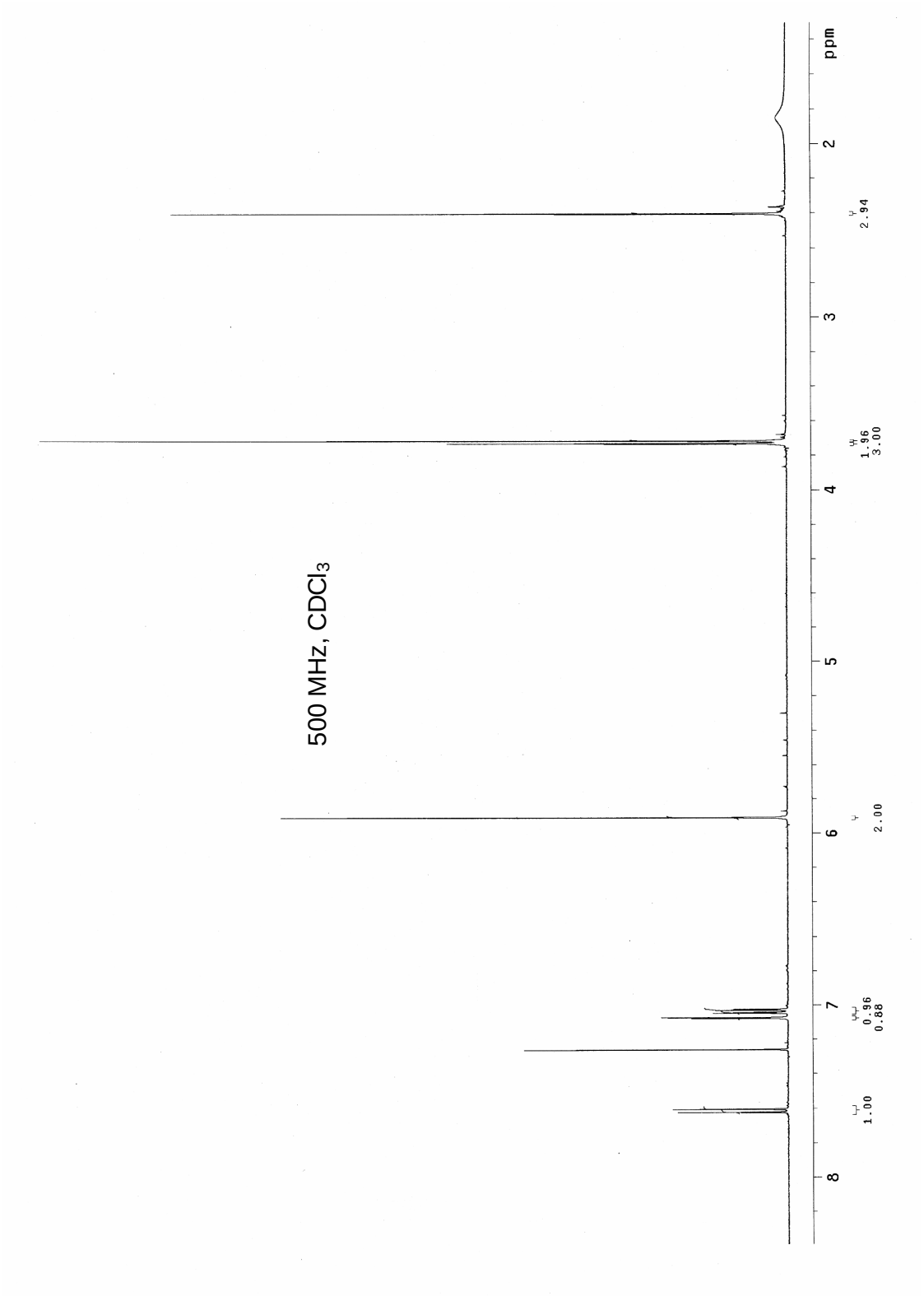
^{1}H NMR spectrum of 7-(4-chlorophenyl)thio-4-methylcoumarin-3-acetic acid (**4a**)



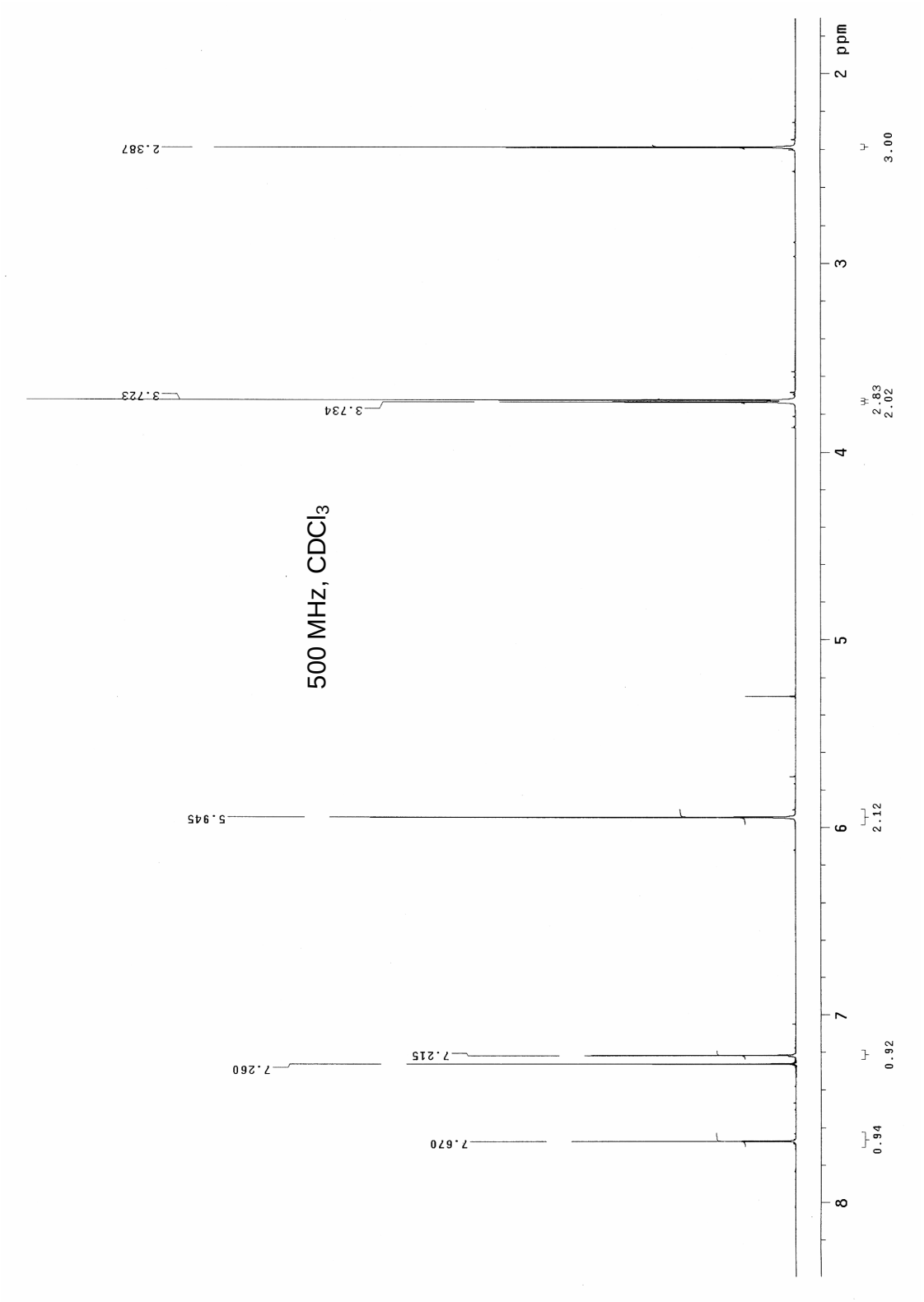
^{1}H NMR spectrum Methyl-6-chloro-7-(4-chlorophenyl)thio-4-methylcoumarin-3-acetic acid (**4b**)



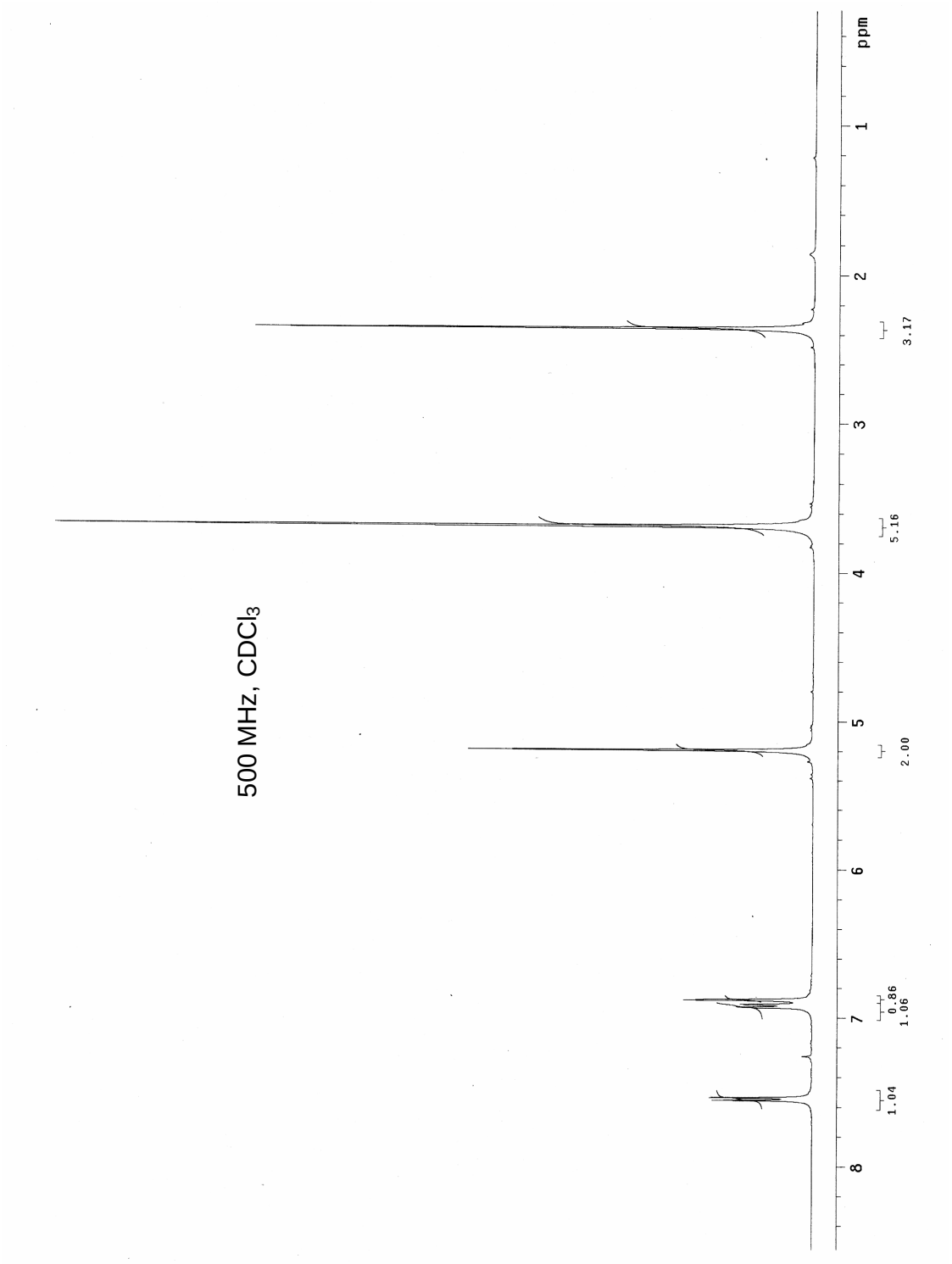
^{1}H NMR spectrum of Methyl 7-chloromethoxy-4-methylcoumarin-3-acetate (**5a**)



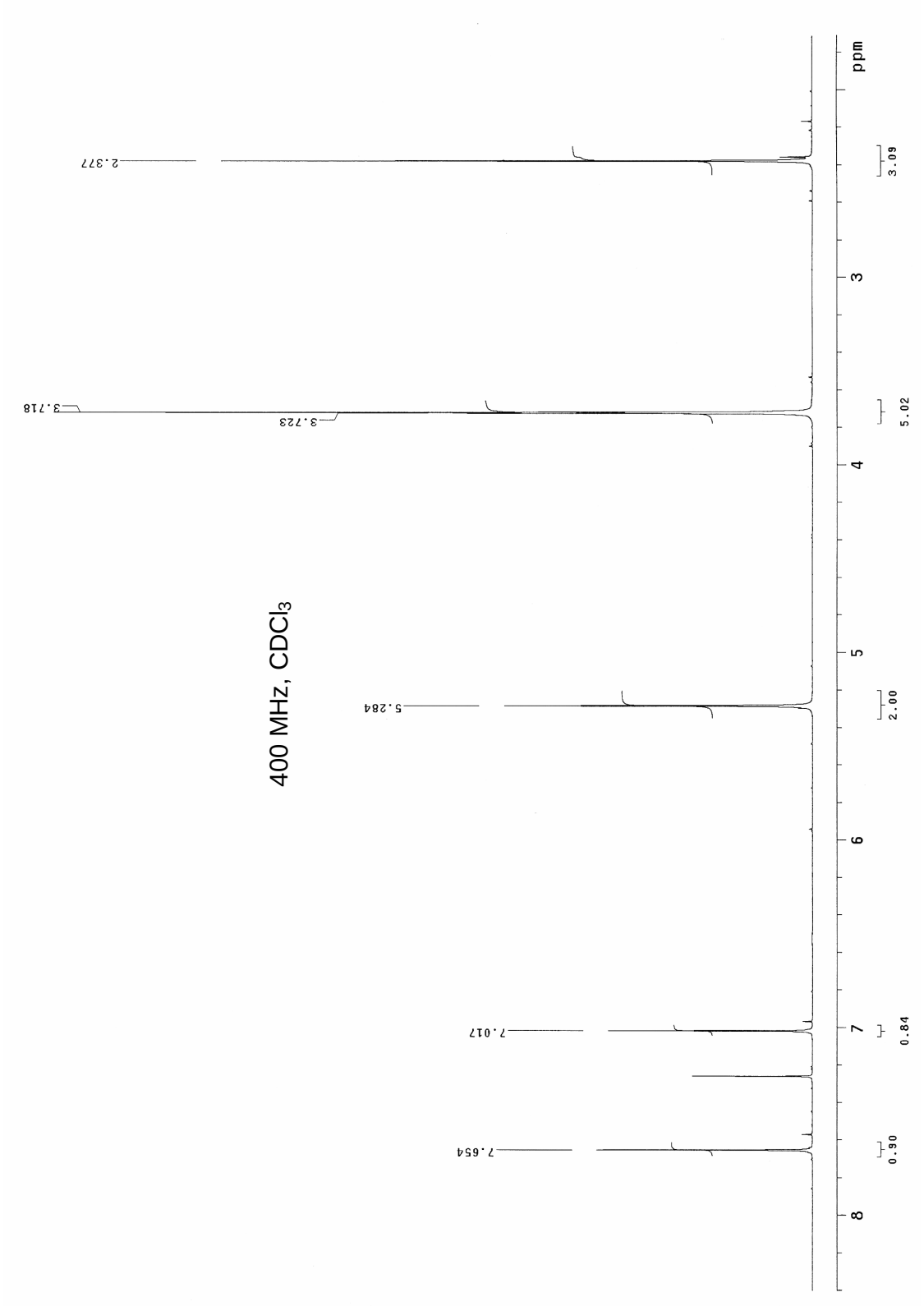
^{1}H NMR spectrum of Methyl 6-chloro-7-chloromethoxy-4-methylcoumarin-3-acetate (**5b**)



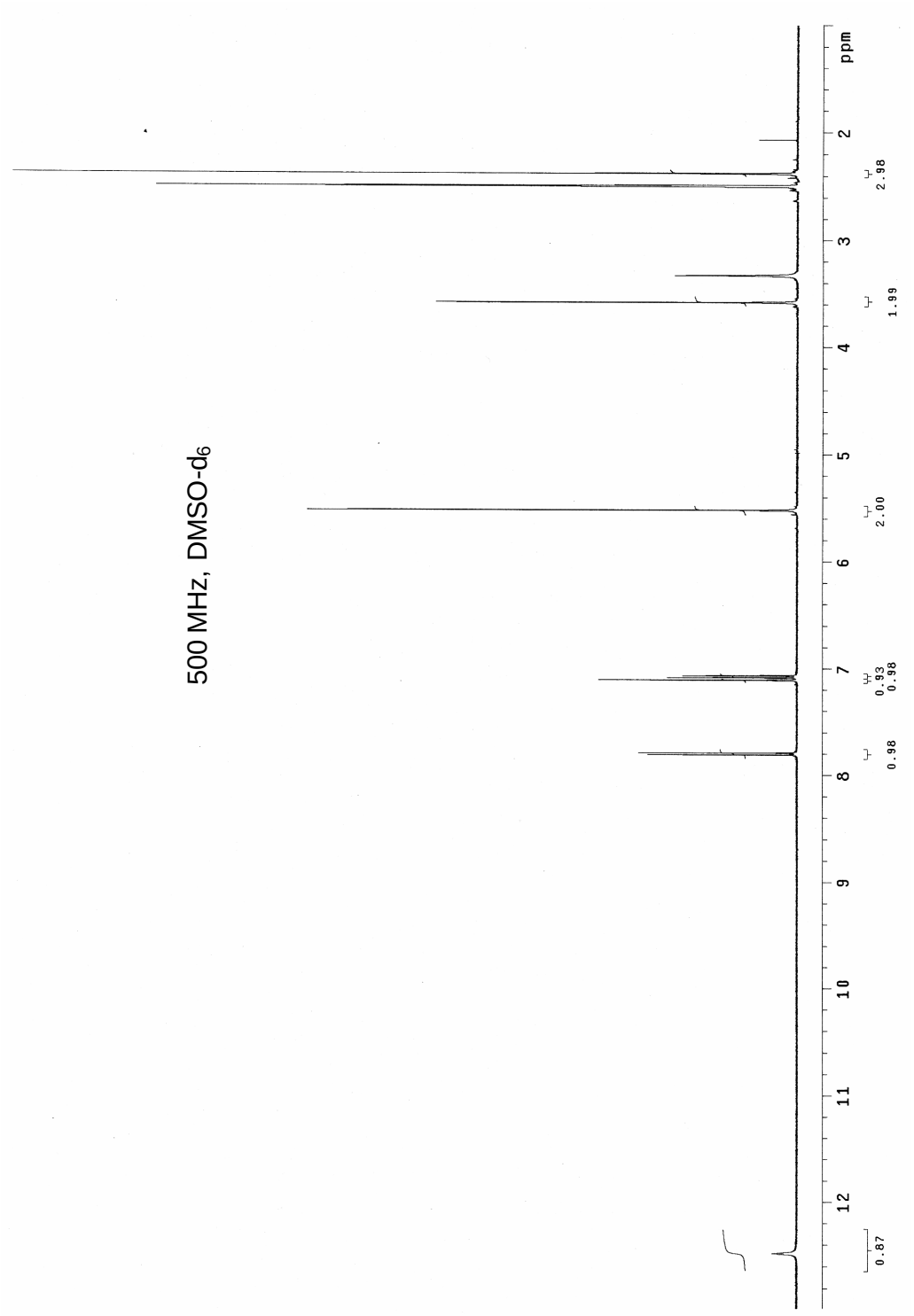
^{1}H NMR spectrum of Methyl 7-azidomethoxy-4-methylcoumarin-3-acetate (**6a**)



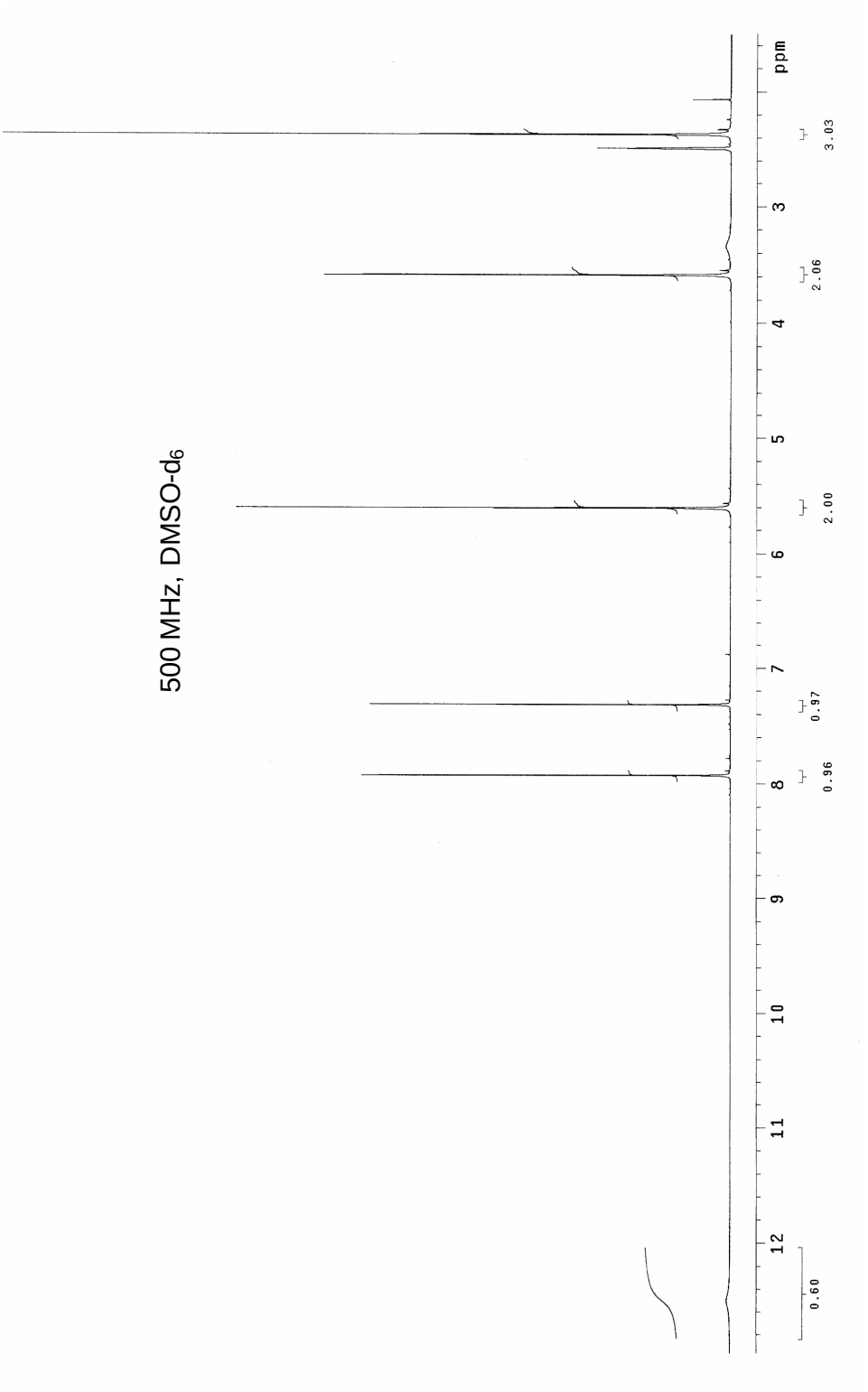
^{1}H NMR spectrum of Methyl 7-azidomethoxy-6-chloro-4-methylcoumarin-3-acetate (**6b**)



^{1}H NMR spectrum of 7-Azidomethoxy-4-methylcoumarin-3-acetic acid (**7a**)

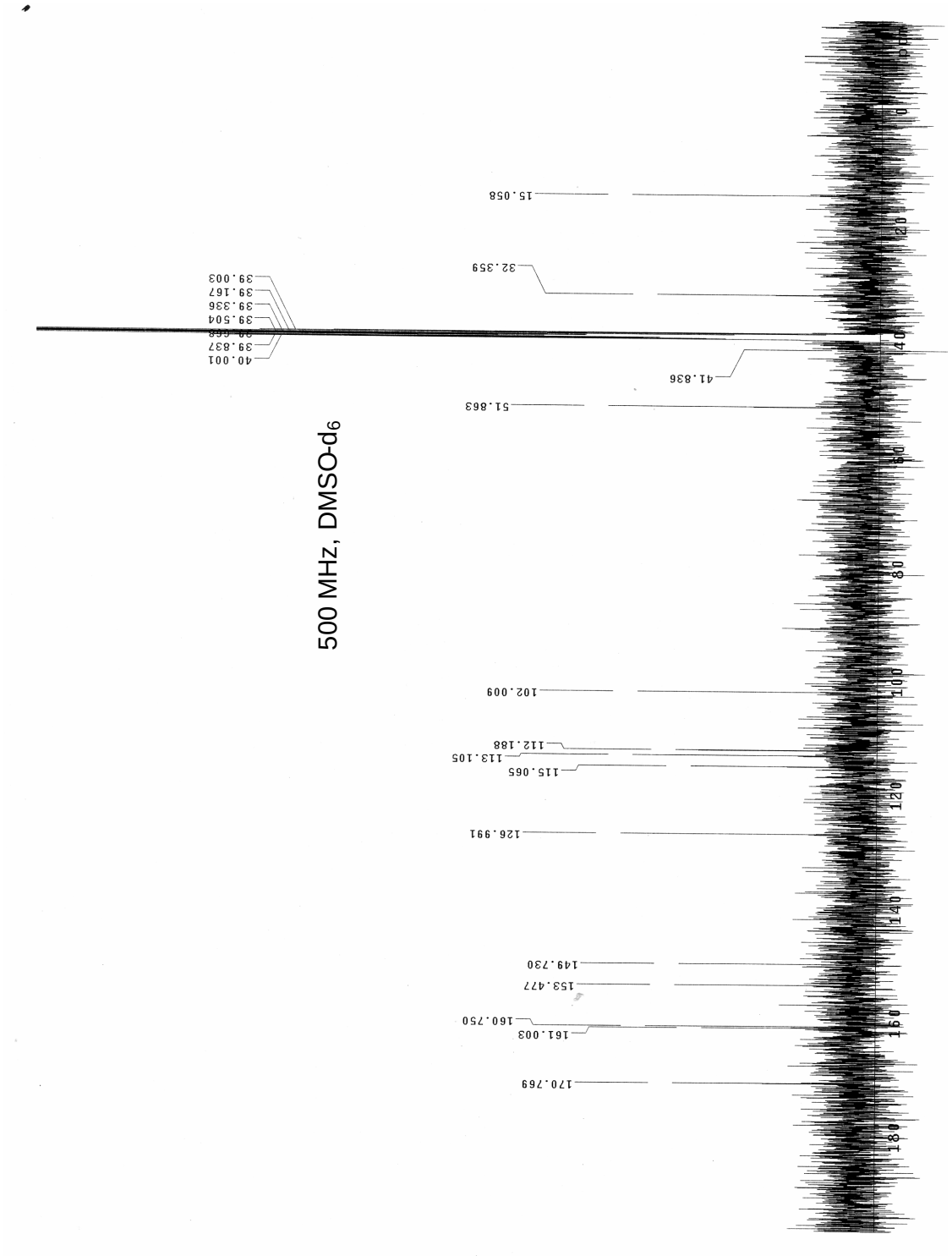


^{1}H NMR spectrum of 7-Azidomethoxy-6-chloro-4-methylcoumarin-3-acetic acid (**7b**)

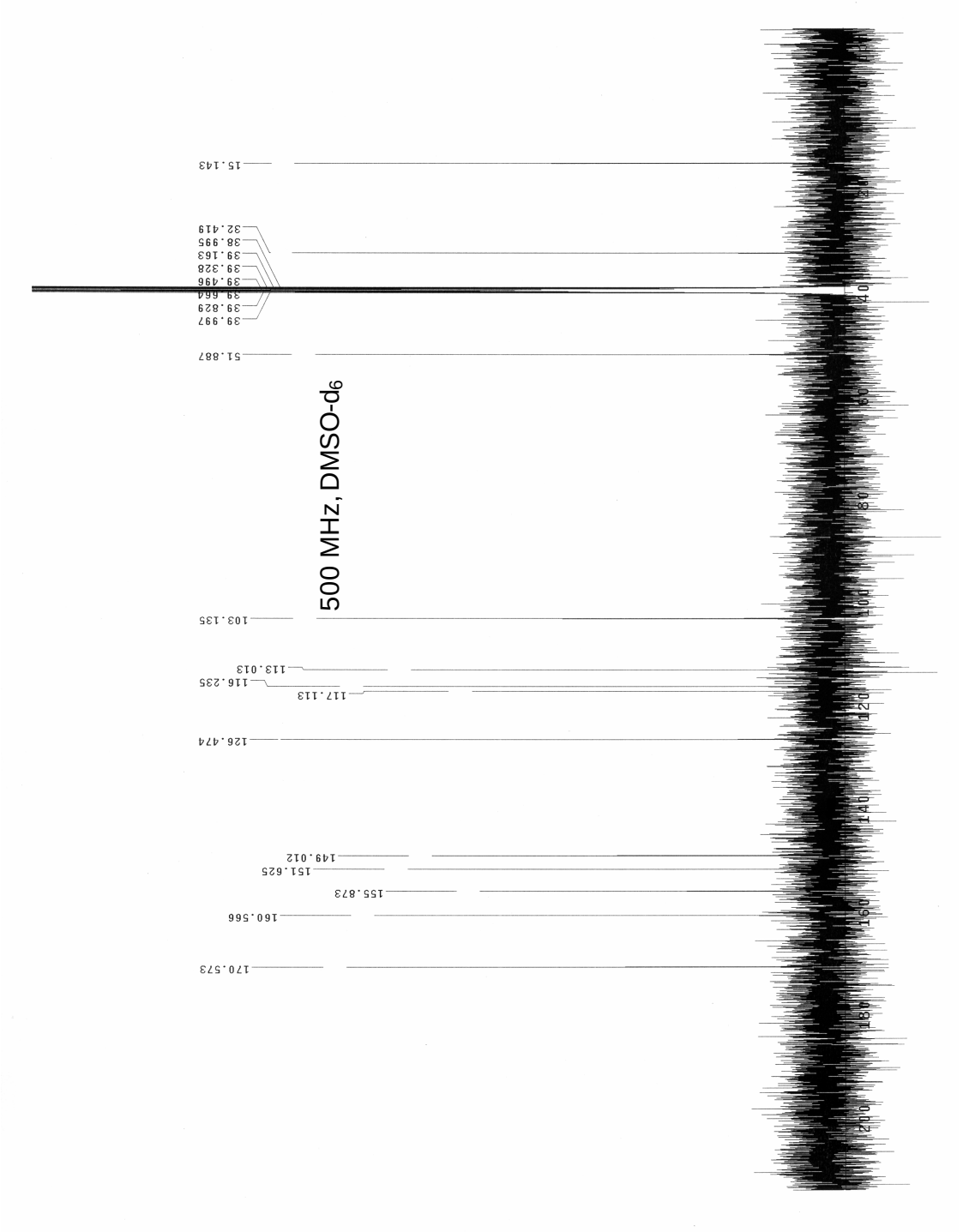


^{13}C NMR Spectra

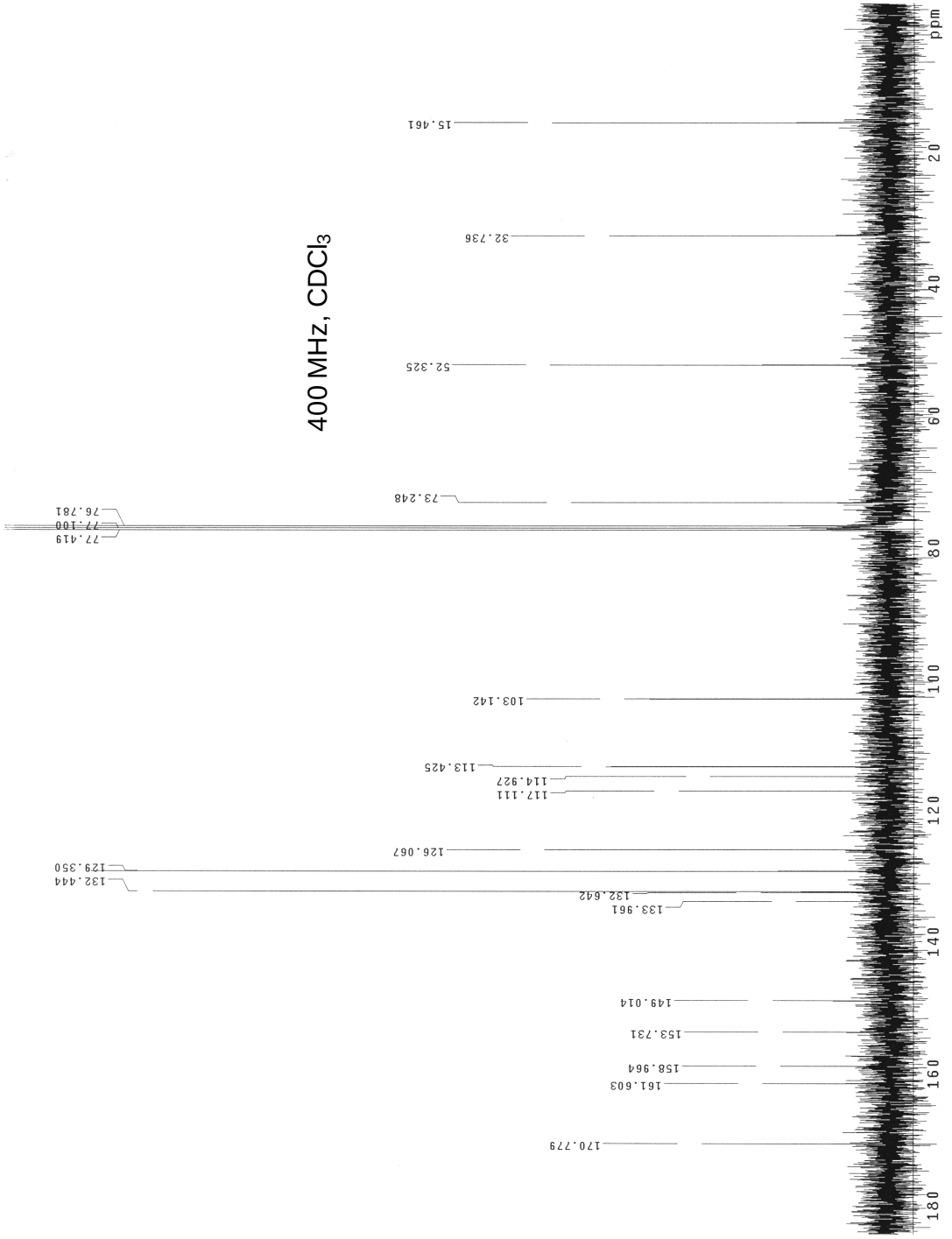
^{13}C NMR spectrum of Methyl 7-hydroxy-4-methylcoumarin-3-acetate (**3a**)



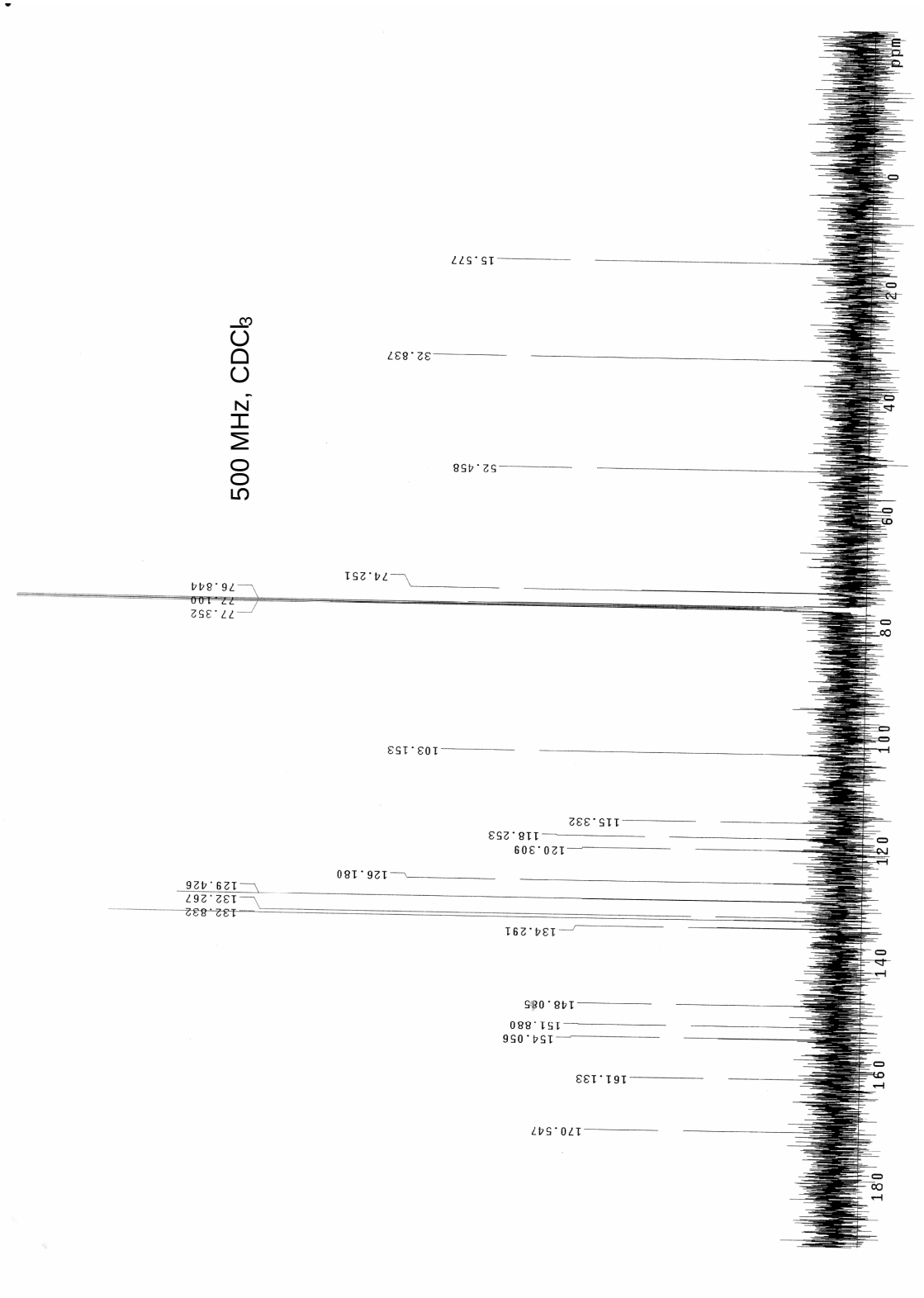
^{13}C NMR spectrum of Methyl 6-chloro-7-hydroxy-4-methylcoumarin-3-acetate (**3b**)



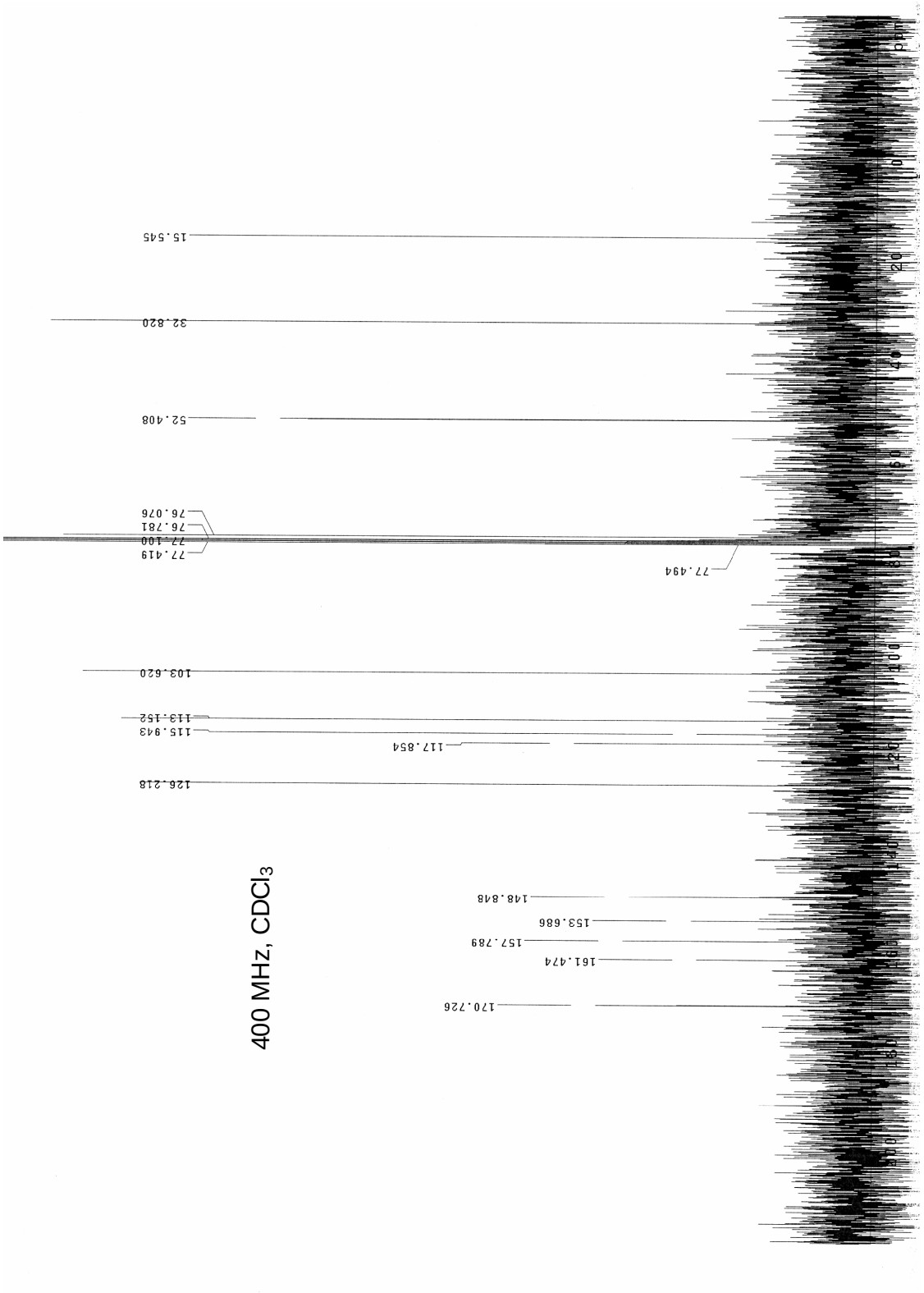
^{13}C NMR spectrum of Methyl 7-(4-chlorophenyl)thio-4-methylcoumarin-3-acetate (**4a**)



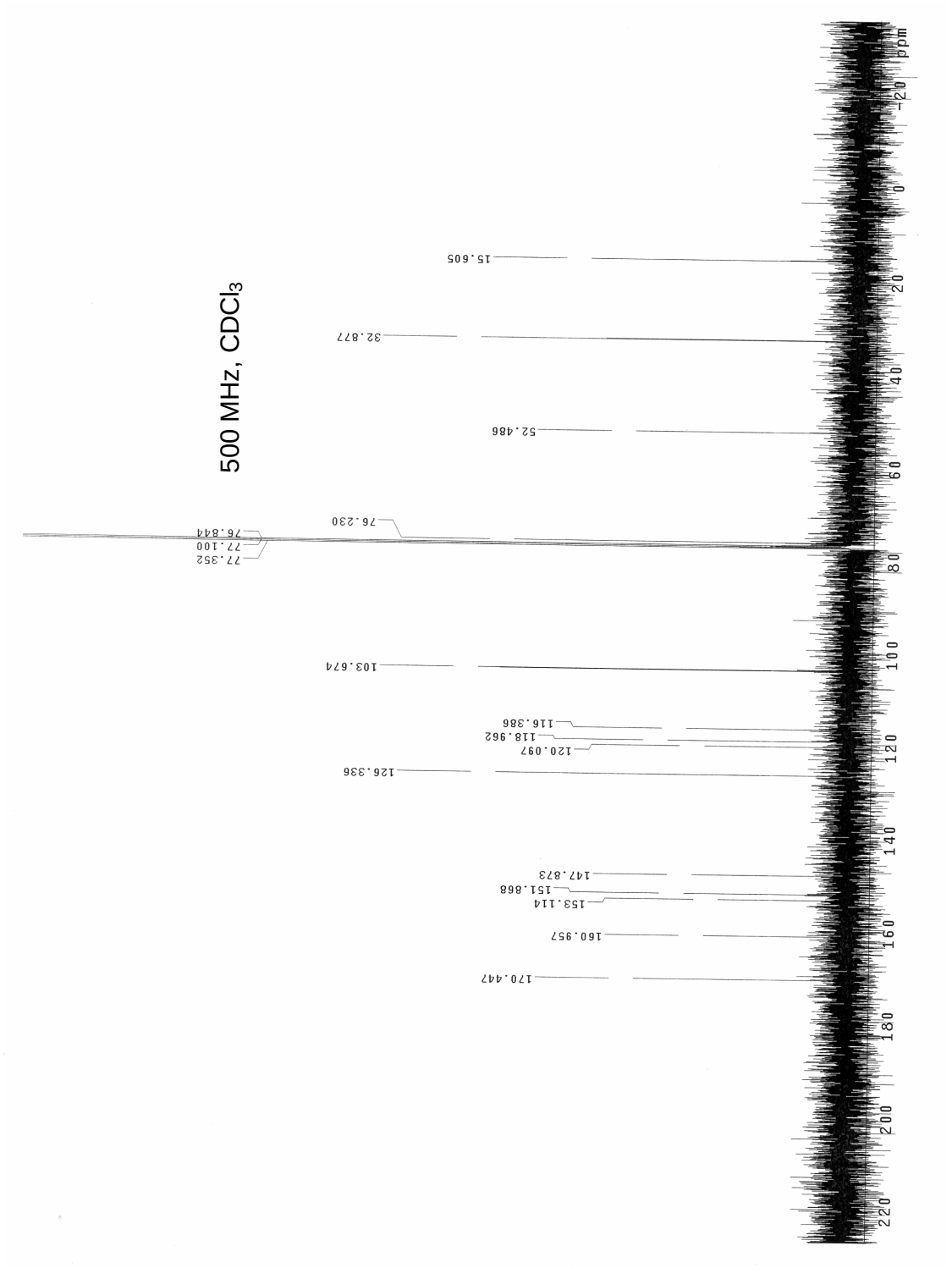
^{13}C NMR spectrum of Methyl 6-Chloro-7-(4-chlorophenyl)thio-4-methylcoumarin-3-acetate (**4b**)



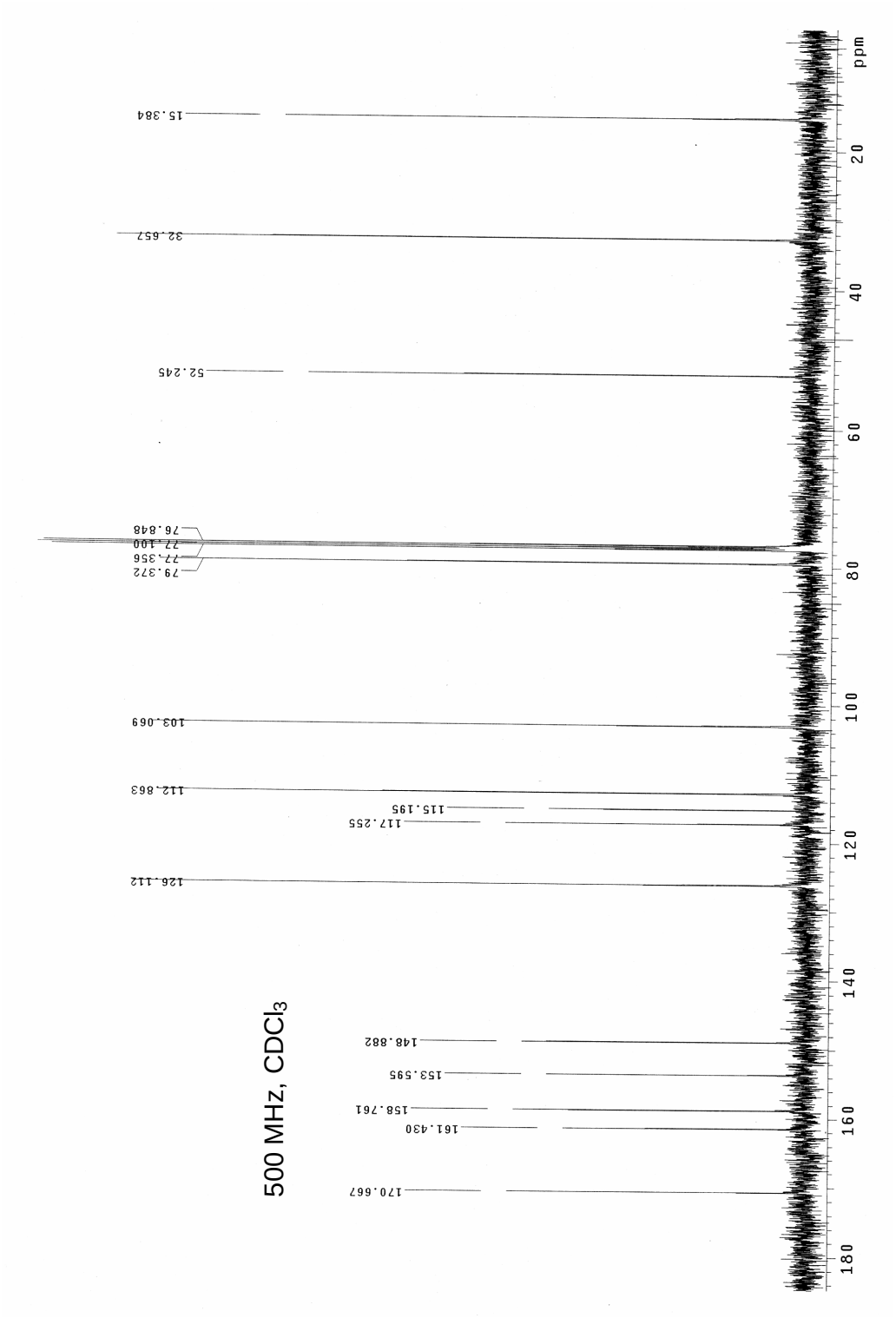
^{13}C NMR spectrum of Methyl 7-chloromethoxy-4-methylcoumarin-3-acetate (**5a**)



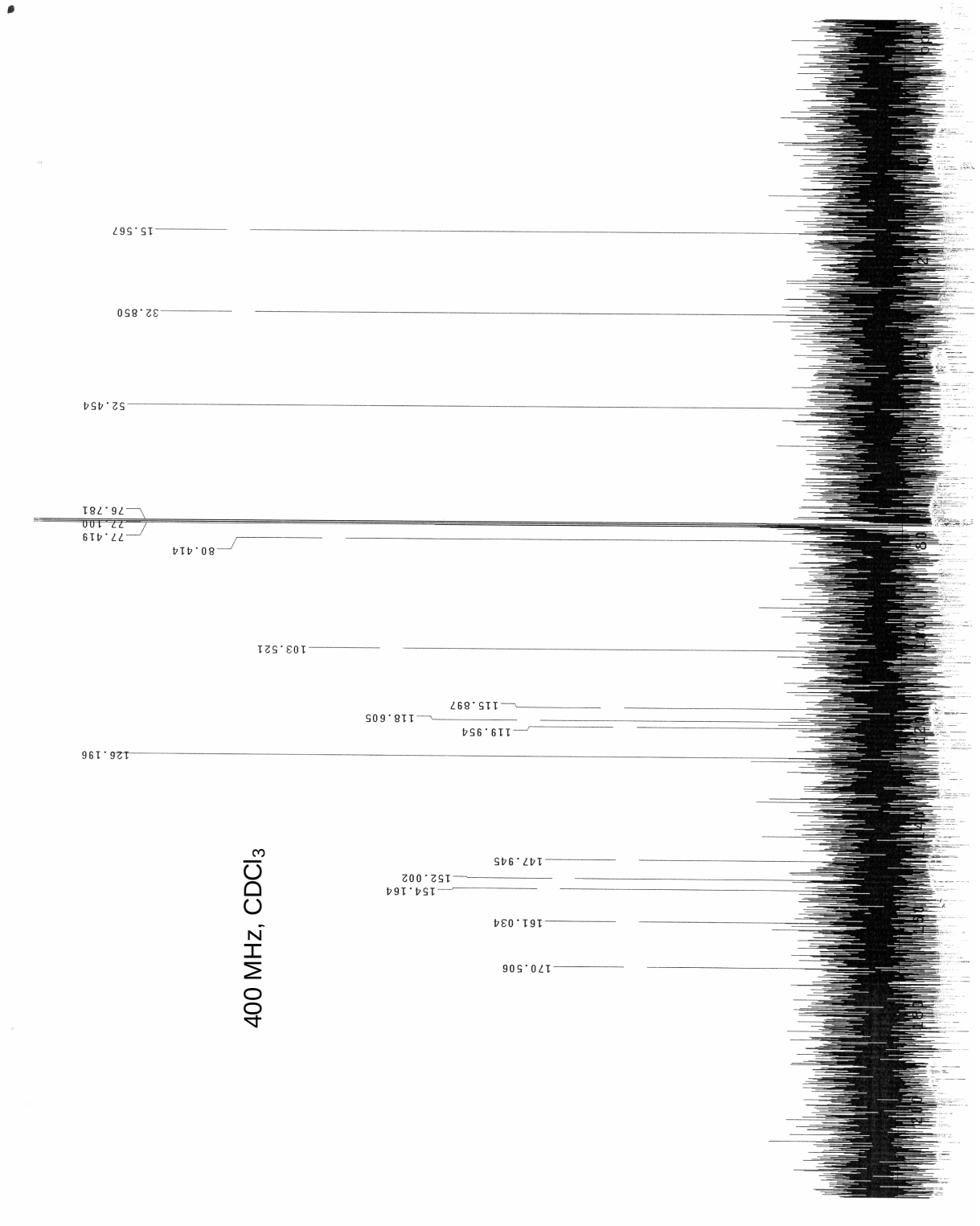
^{13}C NMR spectrum of Methyl 6-chloro-7-chloromethoxy-4-methylcoumarin-3-acetate (**5b**)



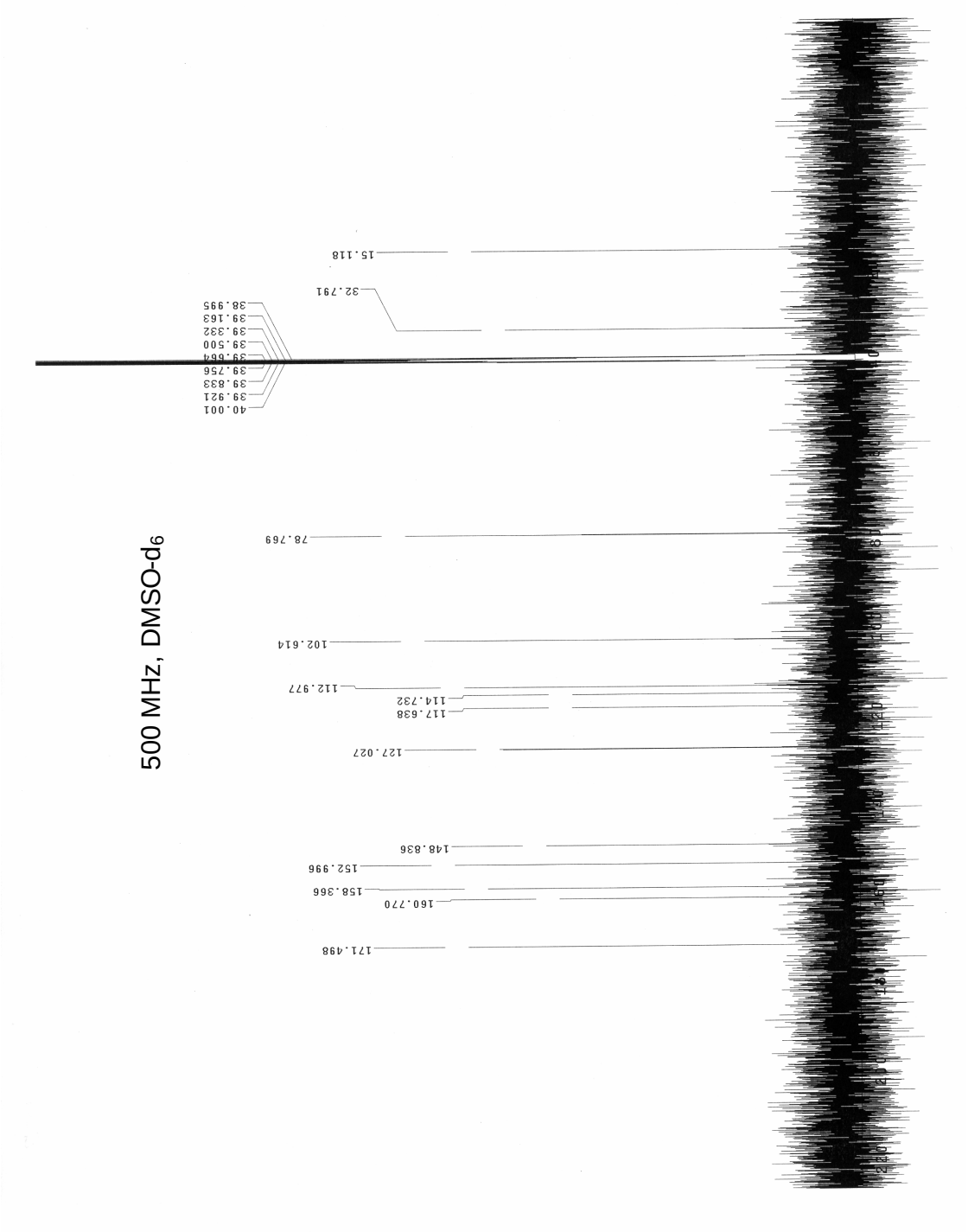
^{13}C NMR spectrum of Methyl 7-azidomethoxy-4-methylcoumarin-3-acetate (**6a**)



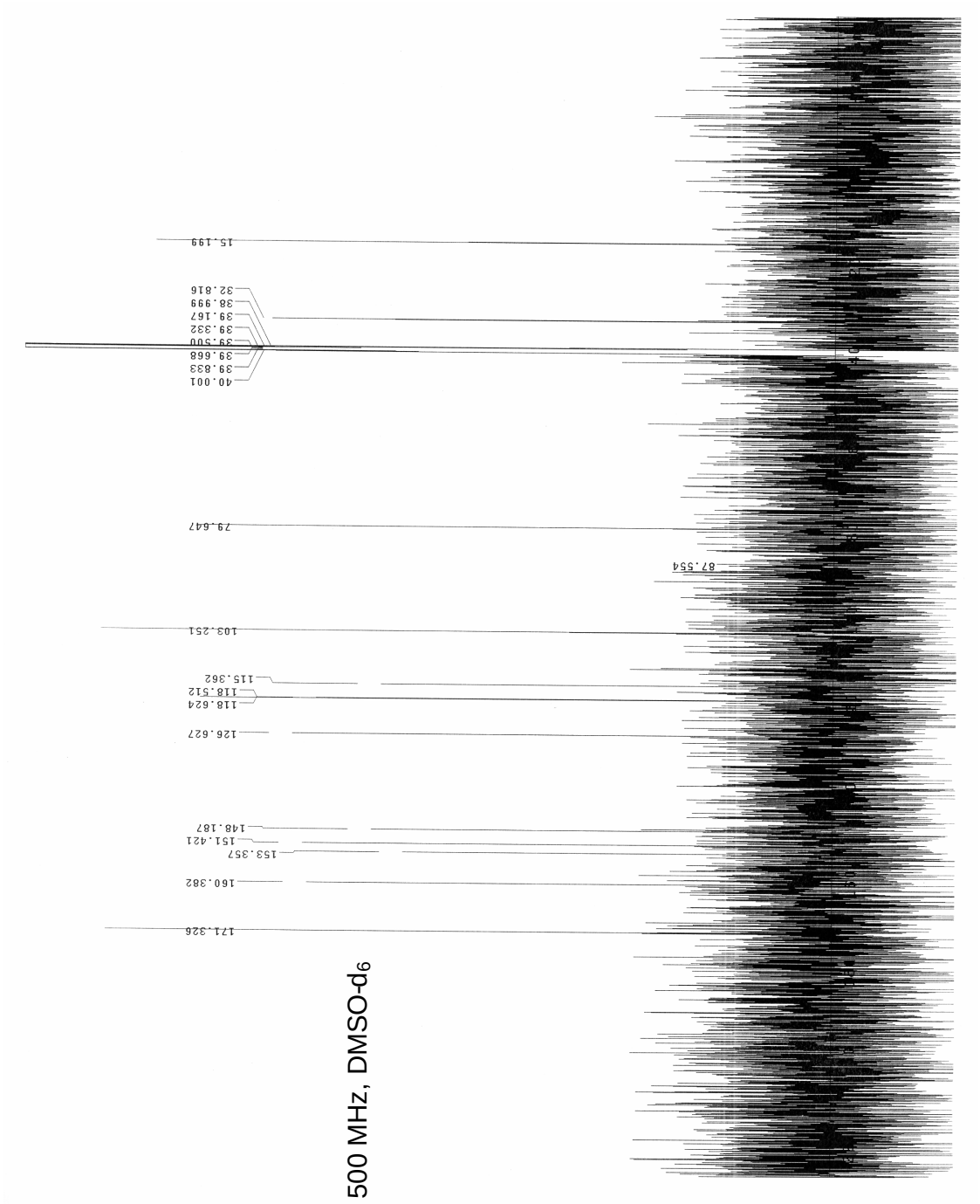
^{13}C NMR spectrum of Methyl 7-azidomethoxy-6-chloro-4-methylcoumarin-3-acetate (**6b**)



^{13}C NMR spectrum of 7-azidomethoxy-4-methylcoumarin-3-acetic acid (**7a**)

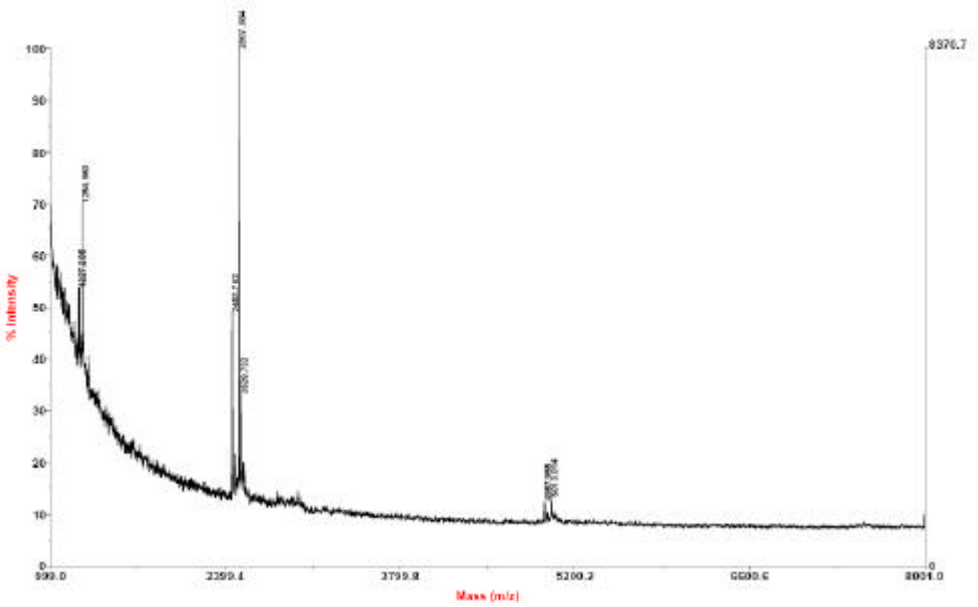


^{13}C NMR spectrum of 6-chloro-7-azidomethoxy-4-methylcoumarin-3-acetic acid (**7b**)

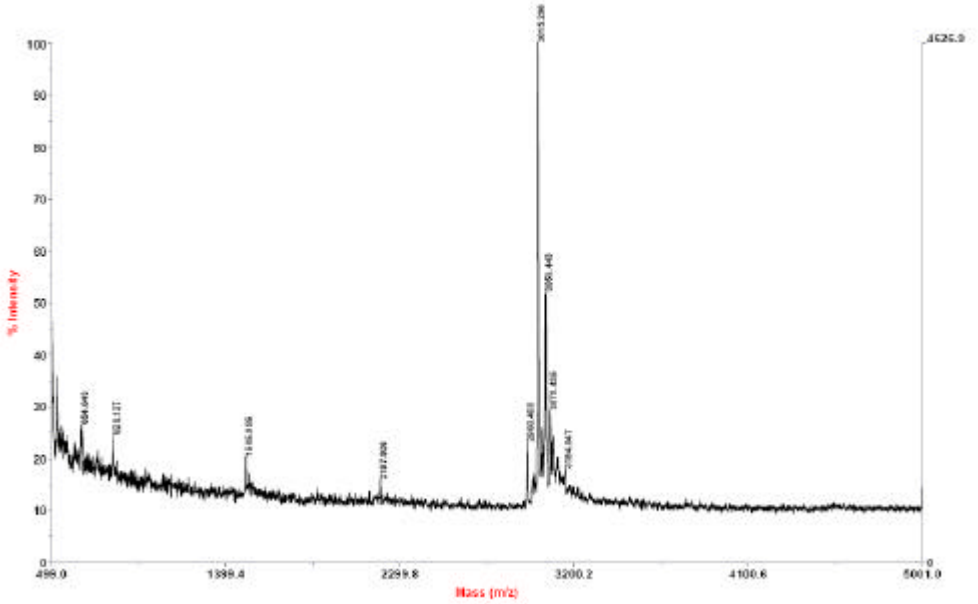


MALDI-TOF Mass Spectra

AzMeOCoum-DNA:[1] calc. = 2505.5 m/z; exp. = 2507.8 m/z.

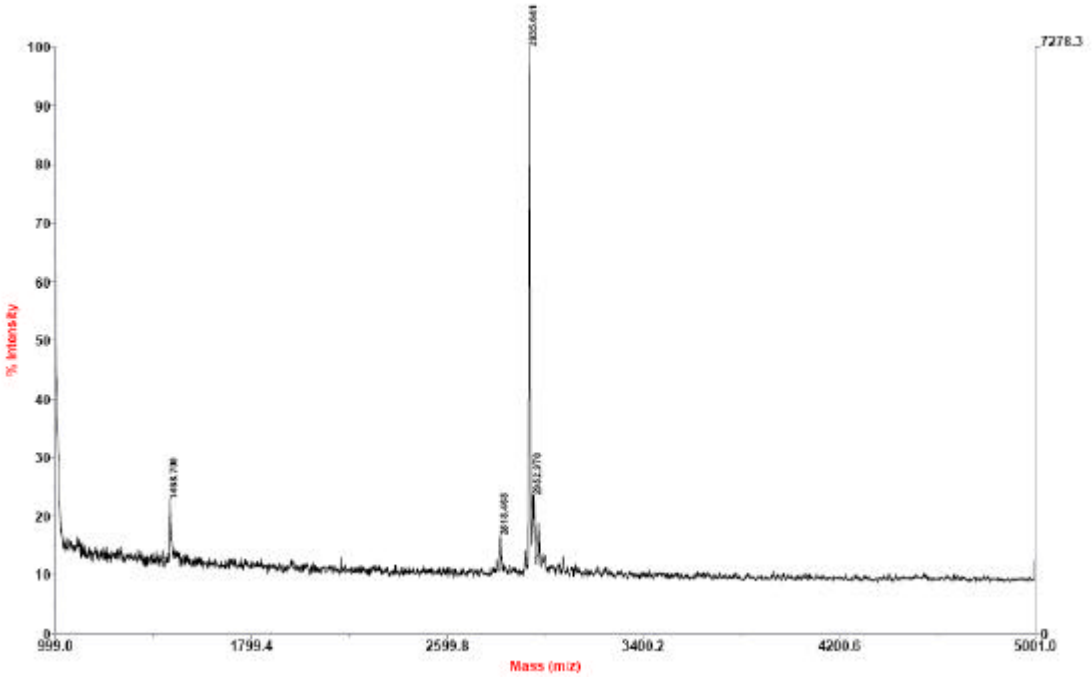


AzMeOCoum-FRET-DNA:[1] calc. = 3017.6 m/z; exp. = 3015.3 m/z.



[1] Besides the major peak corresponding to the expected isotopic mass, a minor peak was present in the mass spectrum with m– 56 m/z corresponding to the product without the AzMe-group.

TPP-DNA:[2] calc. = 2919.6 m/z, calc. for oxidized probe = 2935.6 m/z; exp. = 2935.6 m/z.



[2] Phosphine oxidation during preparation and analysis of MALDI-TOF samples was inevitable.