**Supplementary material**

**Synthesis of nucleotide analogues, EFdA, EdA and EdAP, and the effect of EdAP on hepatitis B virus replication**

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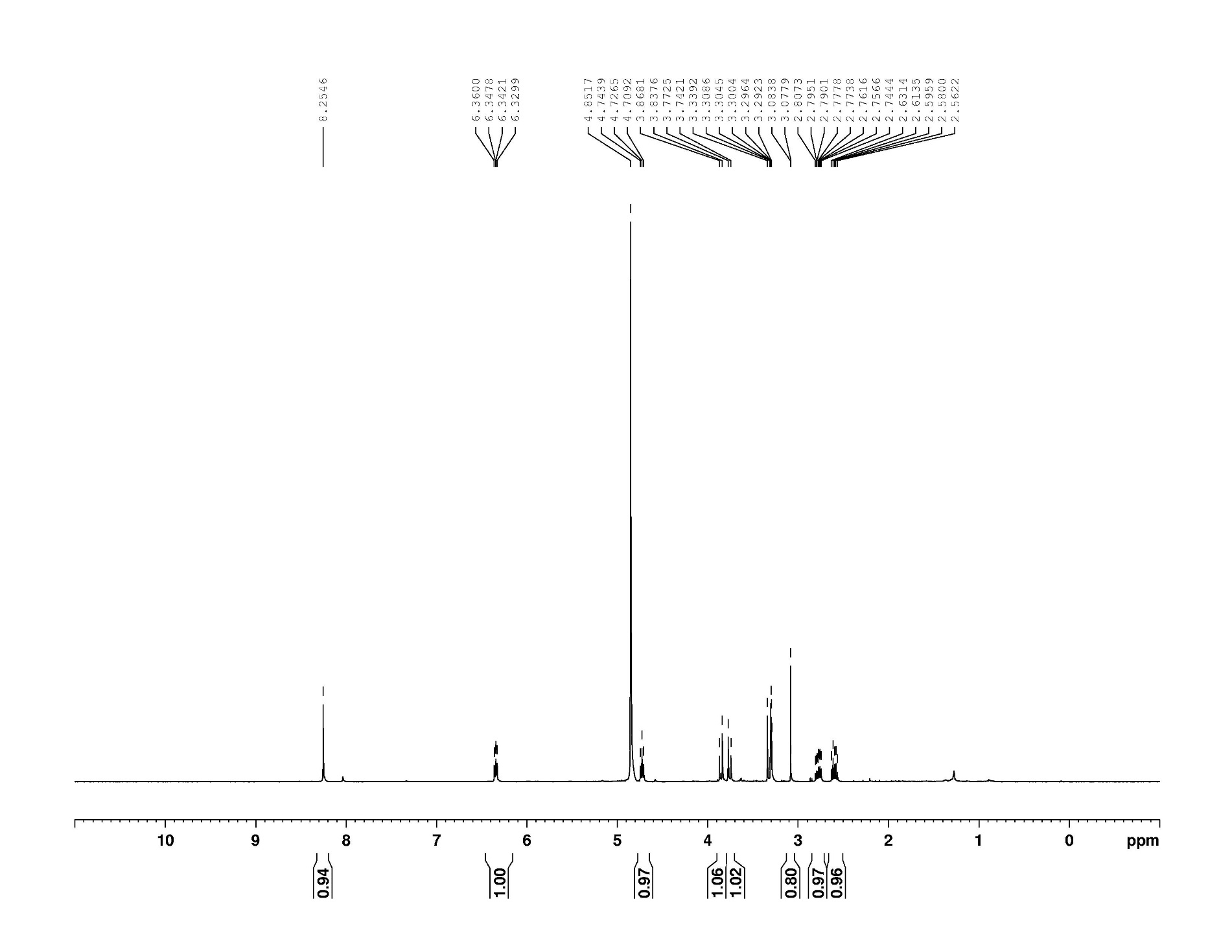
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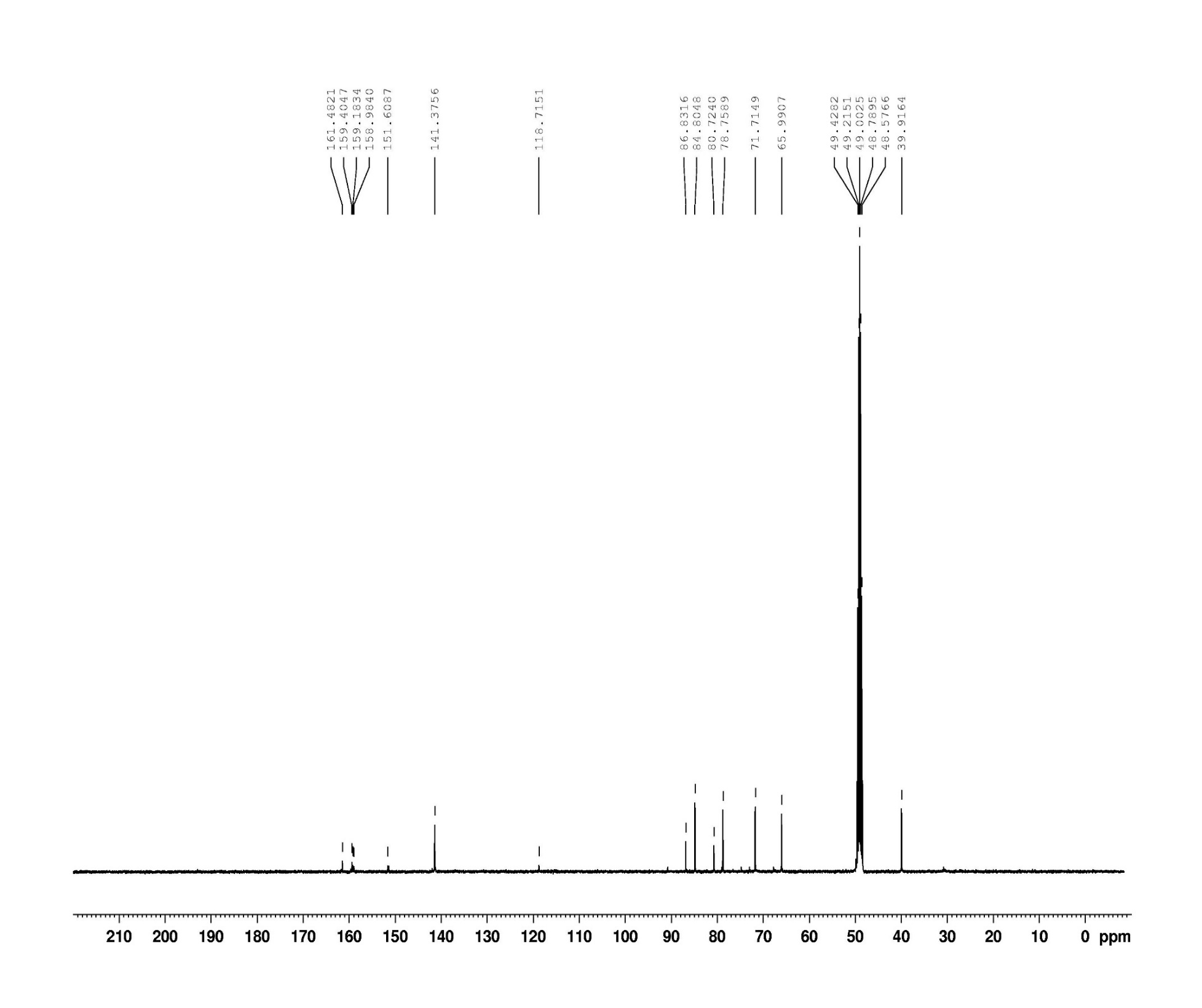
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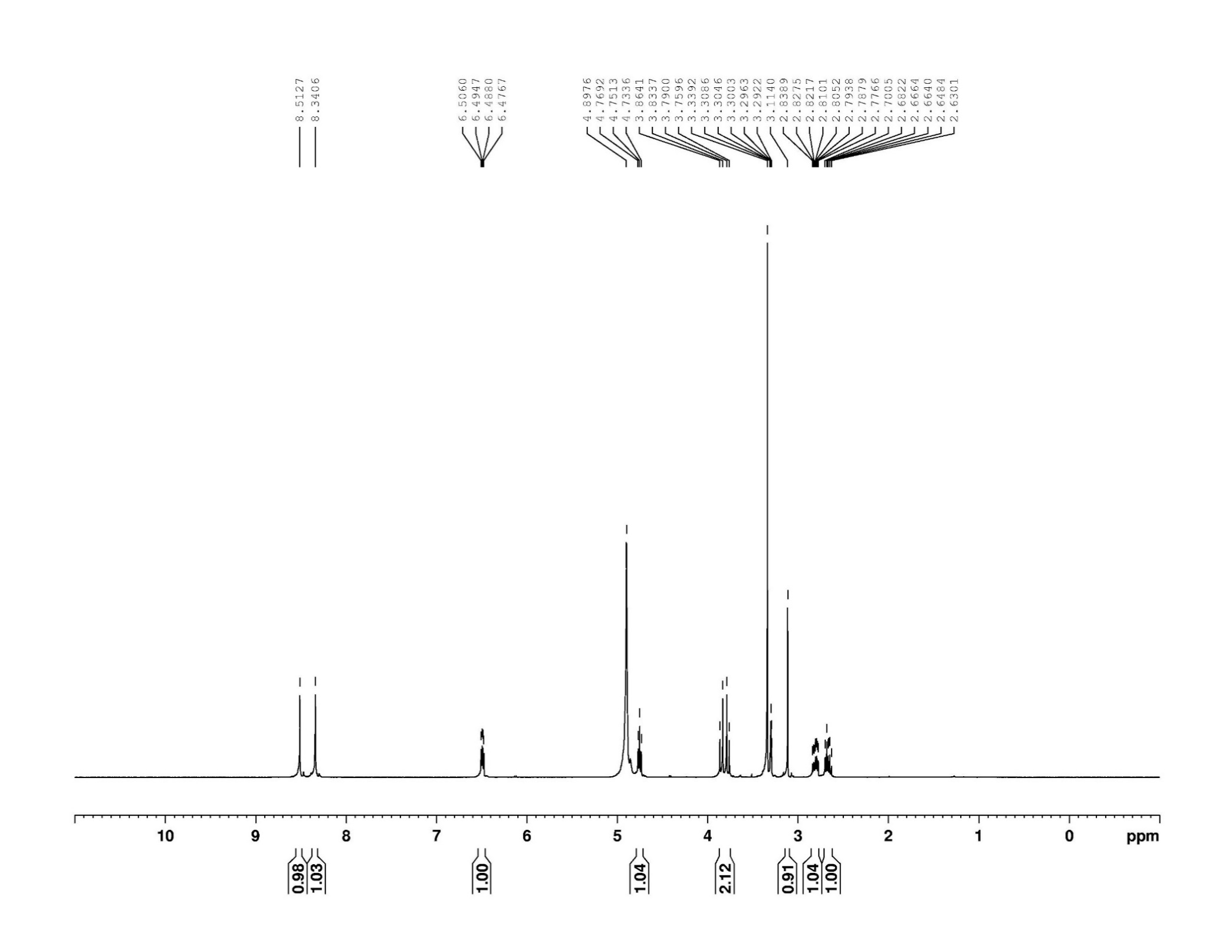
**Figure S1.** 1H NMR spectrum (400 MHz, methanol-*d*4) of EFdA (**1**).



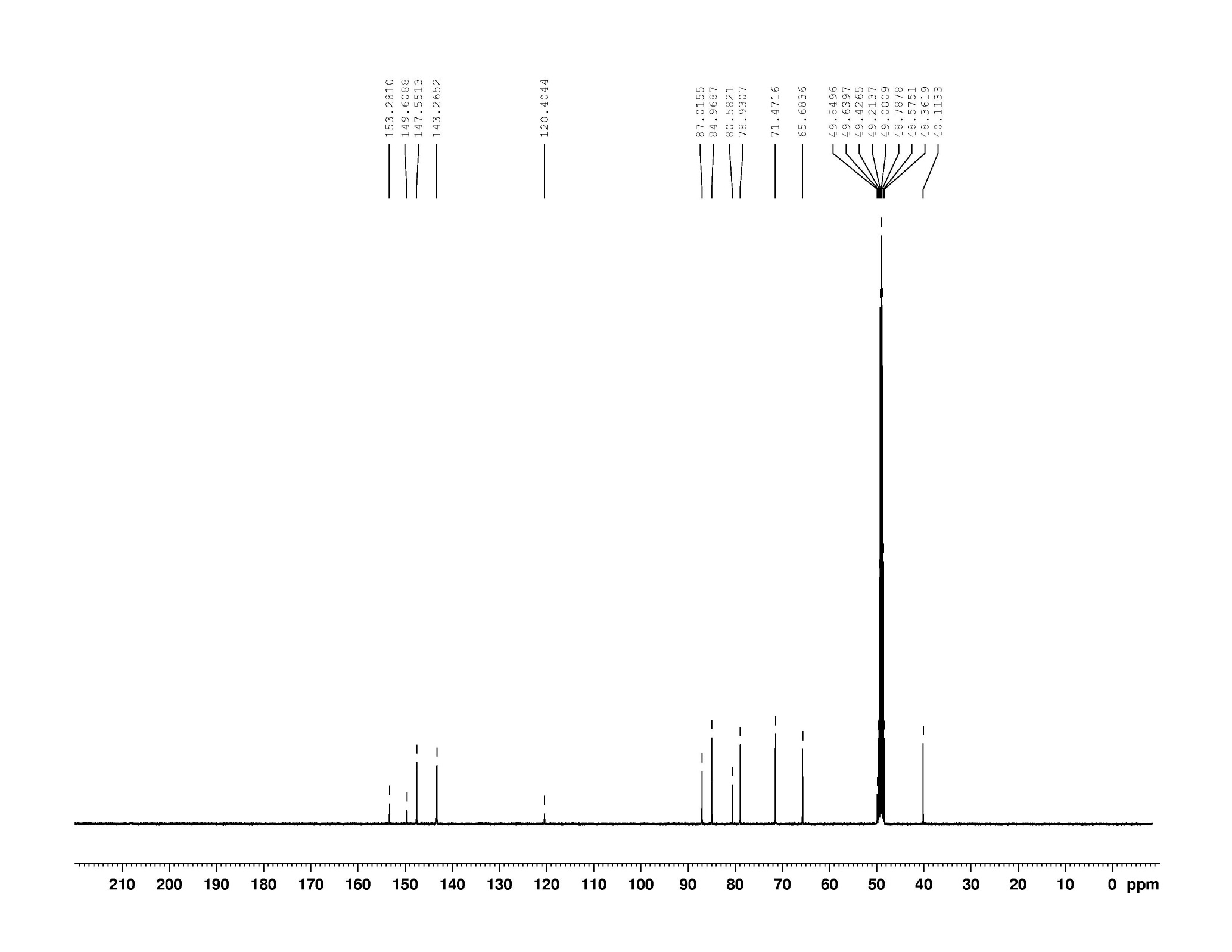
**Figure S2.** 13C NMR spectrum (100 MHz, methanol-*d*4) of EFdA (**1**).



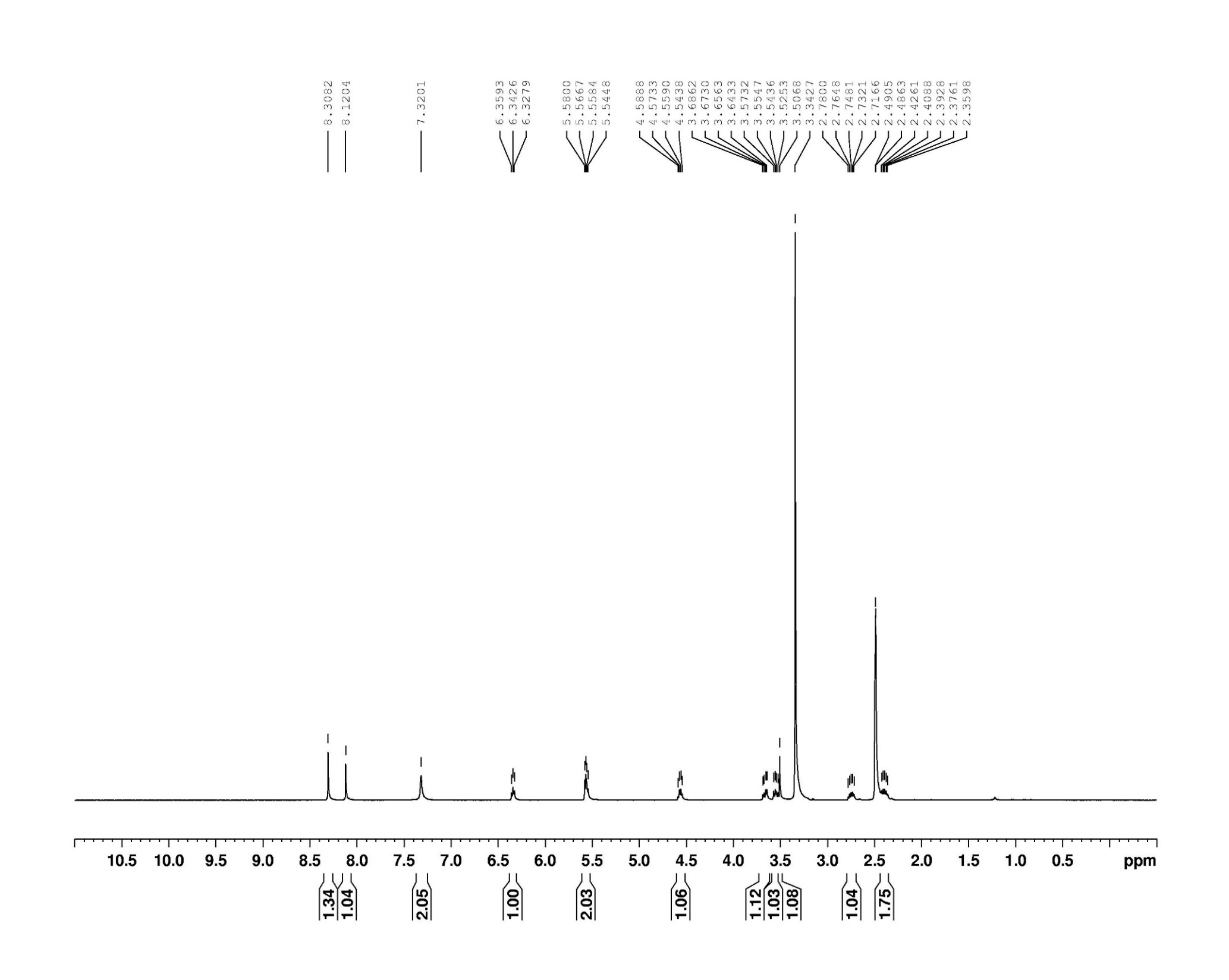
**Figure S3.** 1H NMR spectrum (400 MHz, methanol-*d*4) of EdA (**2**).



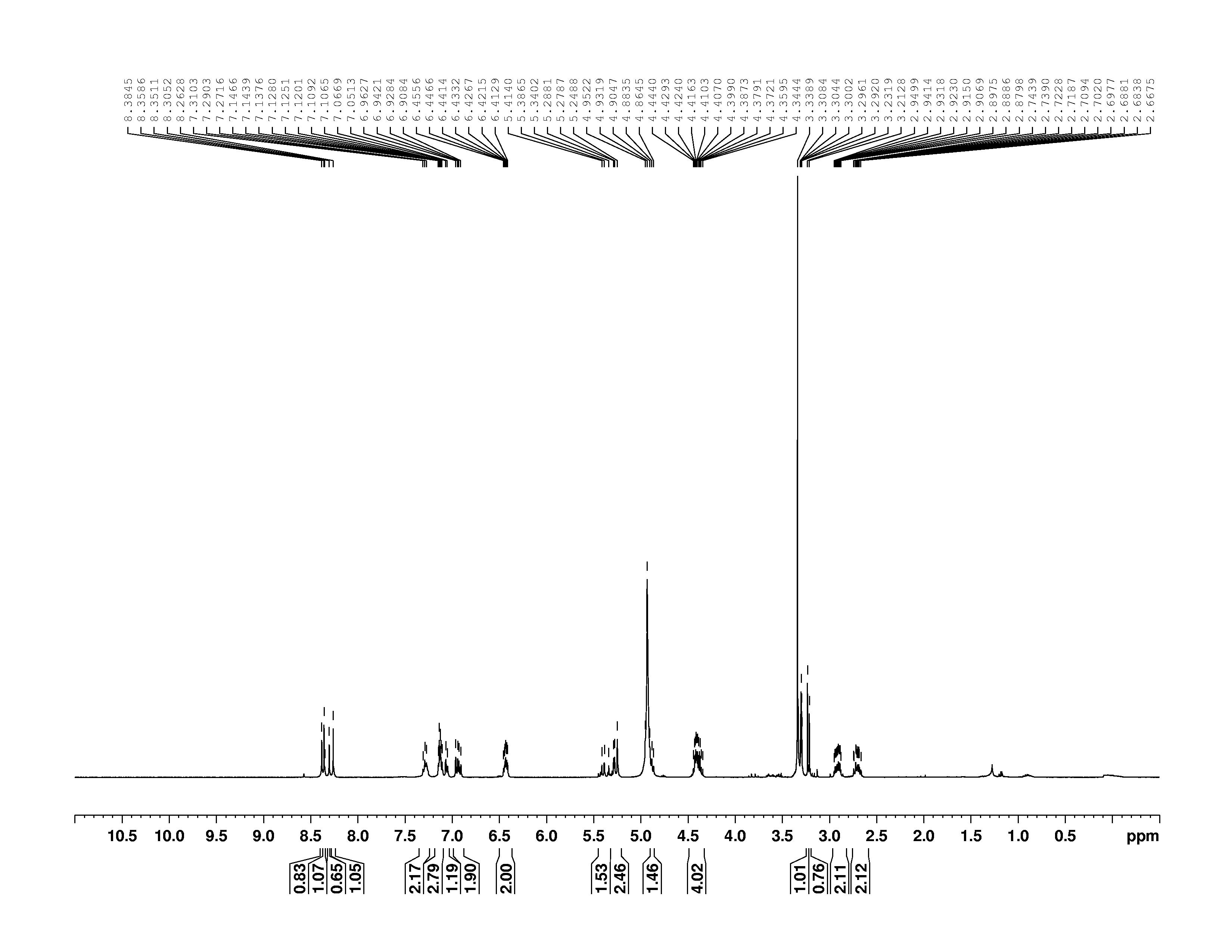
**Figure S4.** 13C NMR spectrum (100 MHz, methanol-*d*4) of EdA (**2**).



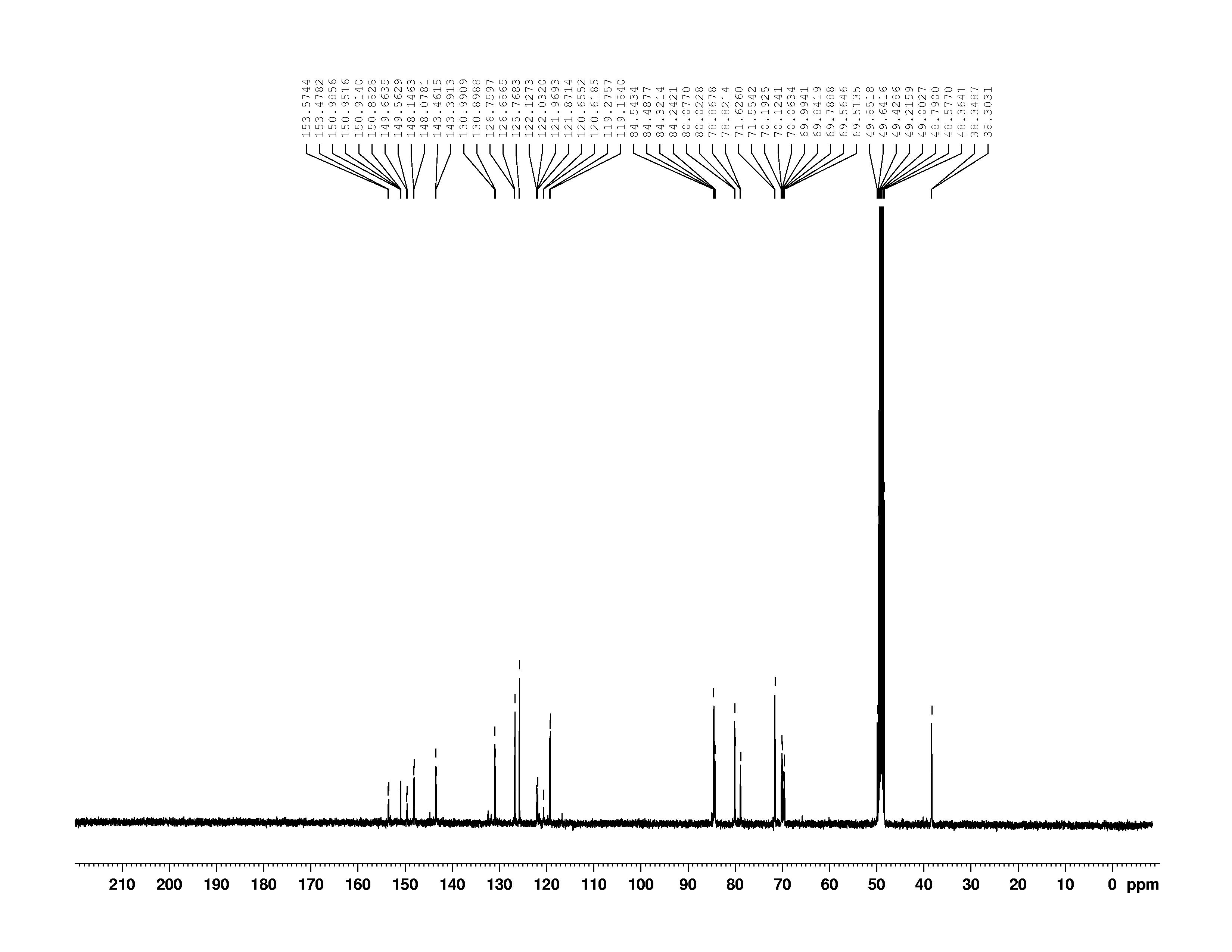
**Figure S5.** 1H NMR spectrum (400 MHz, DMSO-*d*6) of EdA (**2**).



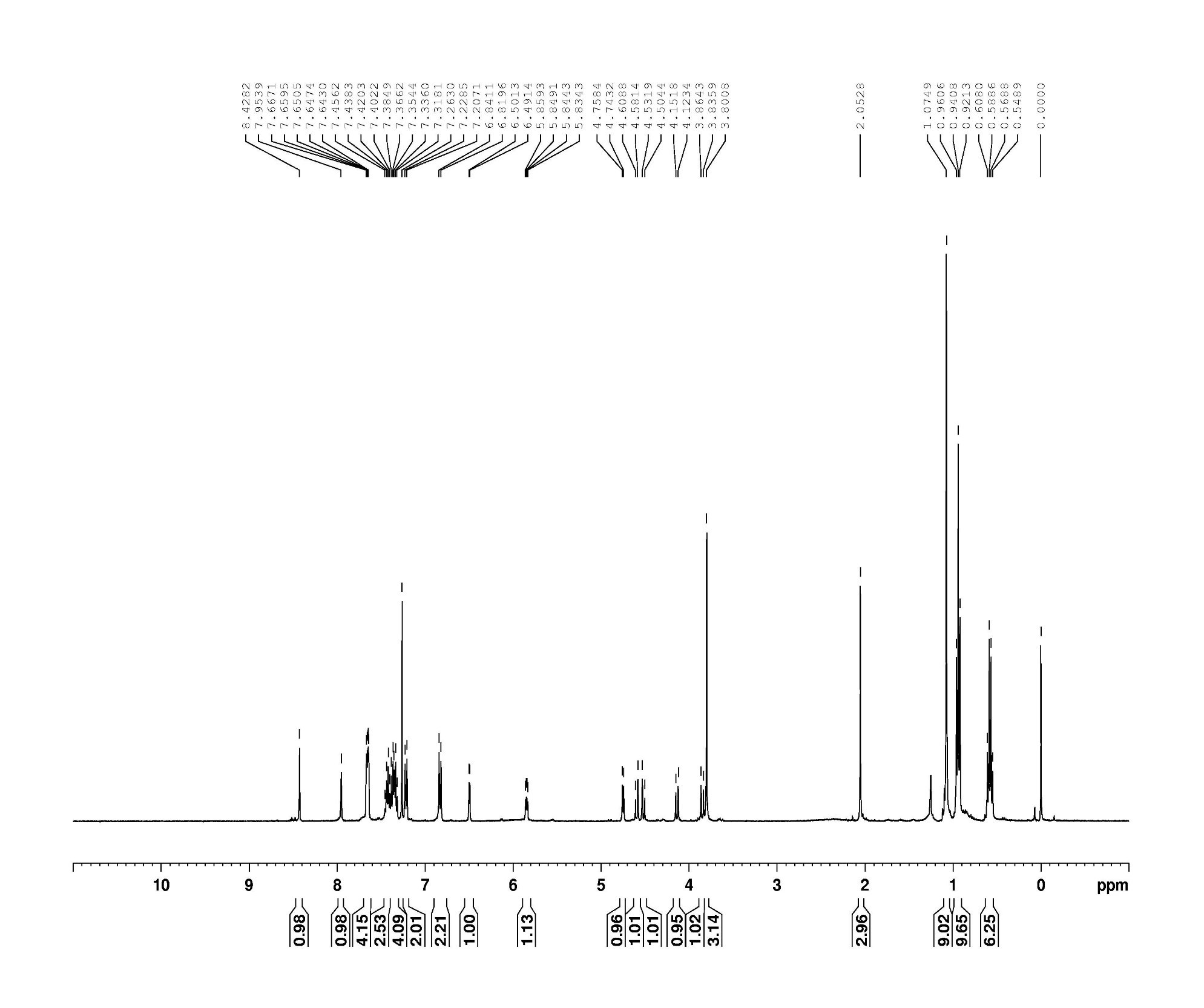
**Figure S6**. 1H NMR spectrum (400 MHz, methanol-*d*4) of EdAP (**3**).



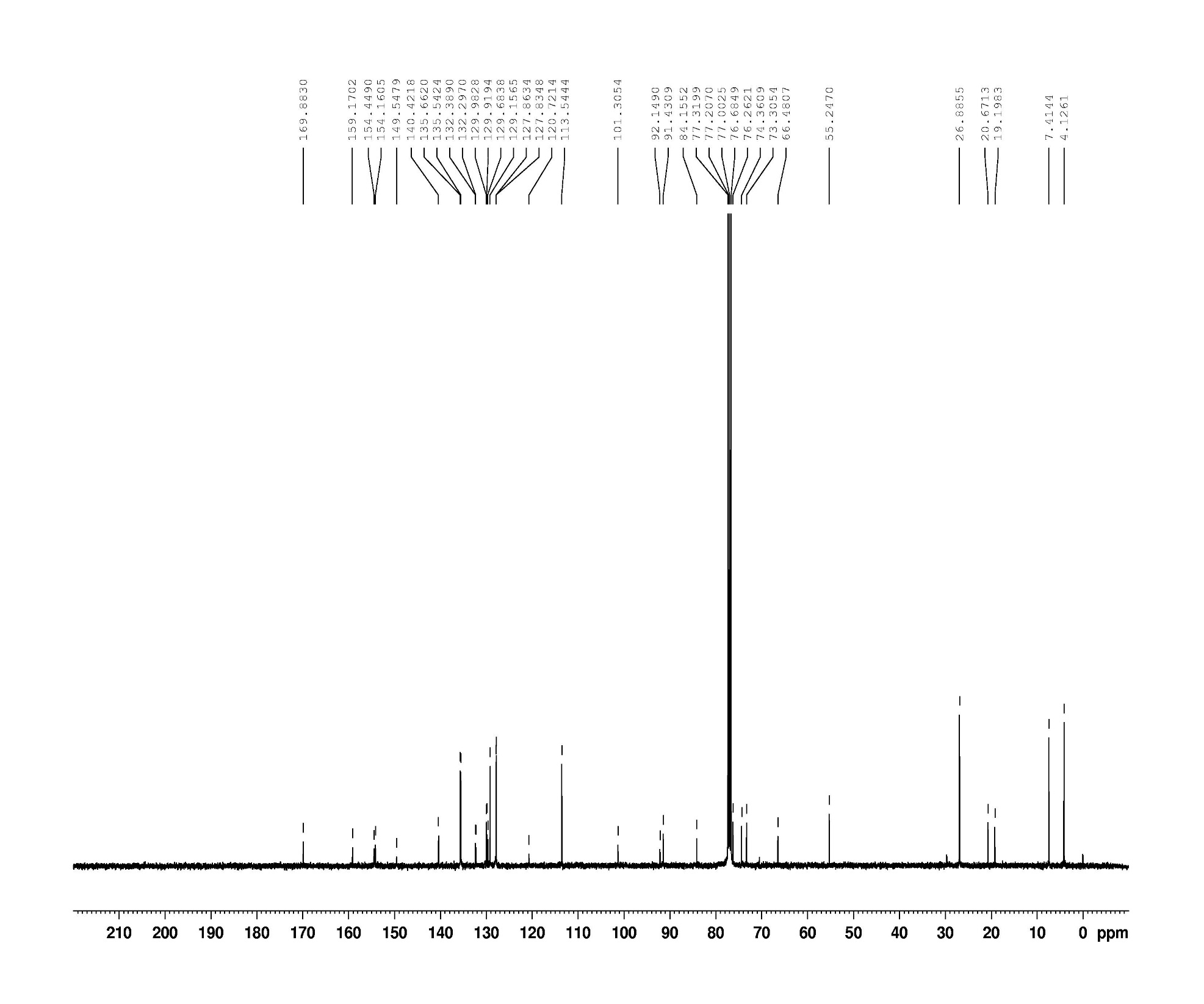
**Figure S7**. 13C NMR spectrum (100 MHz, methanol-*d*4) of EdAP (**3**).



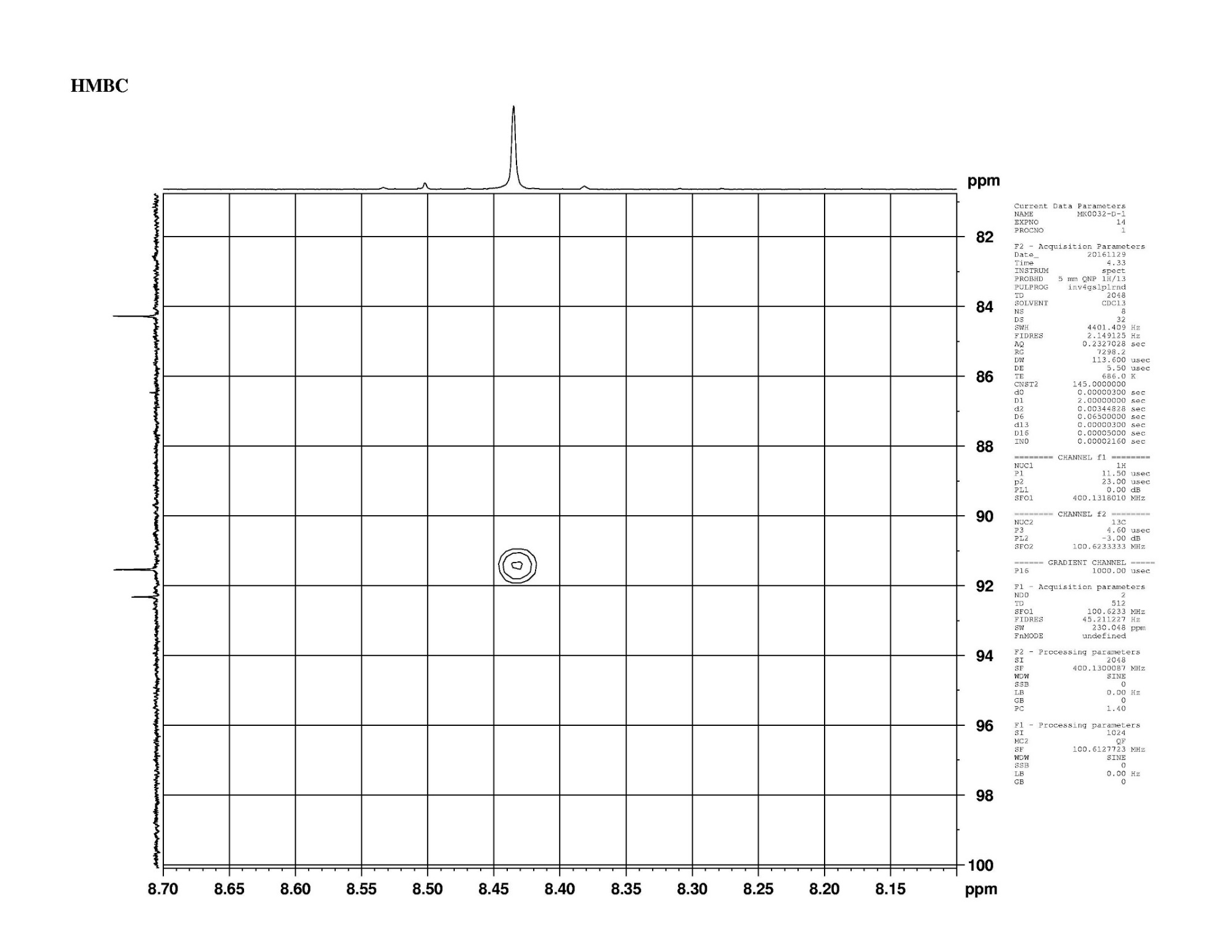
**Figure S8.** 1H NMR spectrum (400 MHz, chloroform-*d*) of compound **21**.

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**Figure S9.** 13C NMR spectrum (100 MHz, chloroform-*d*) of compound **21**.



**Figure S10.** Amagnified view of HMBC spectrum of compound **21**.





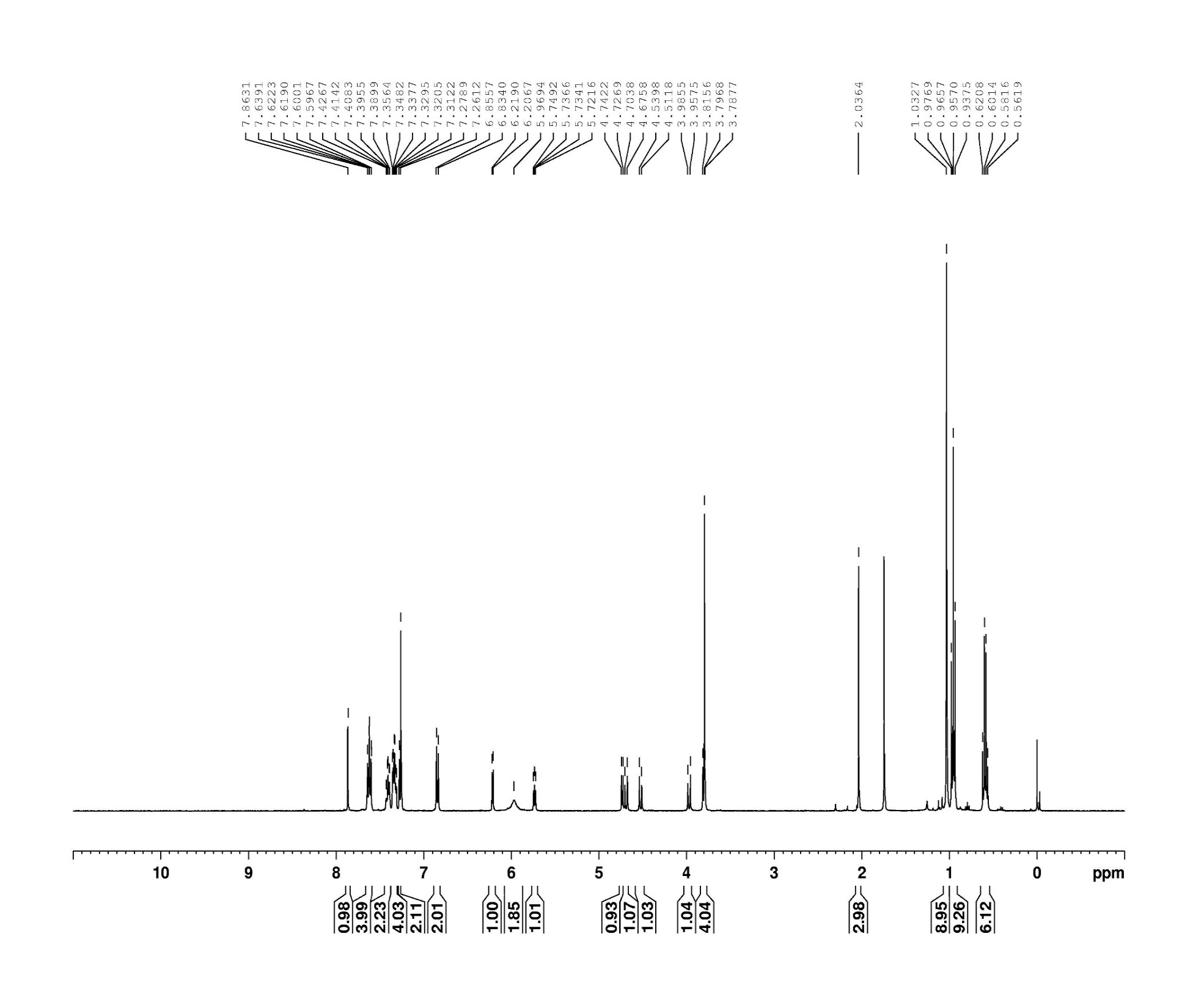
C-1ʹ

H-2

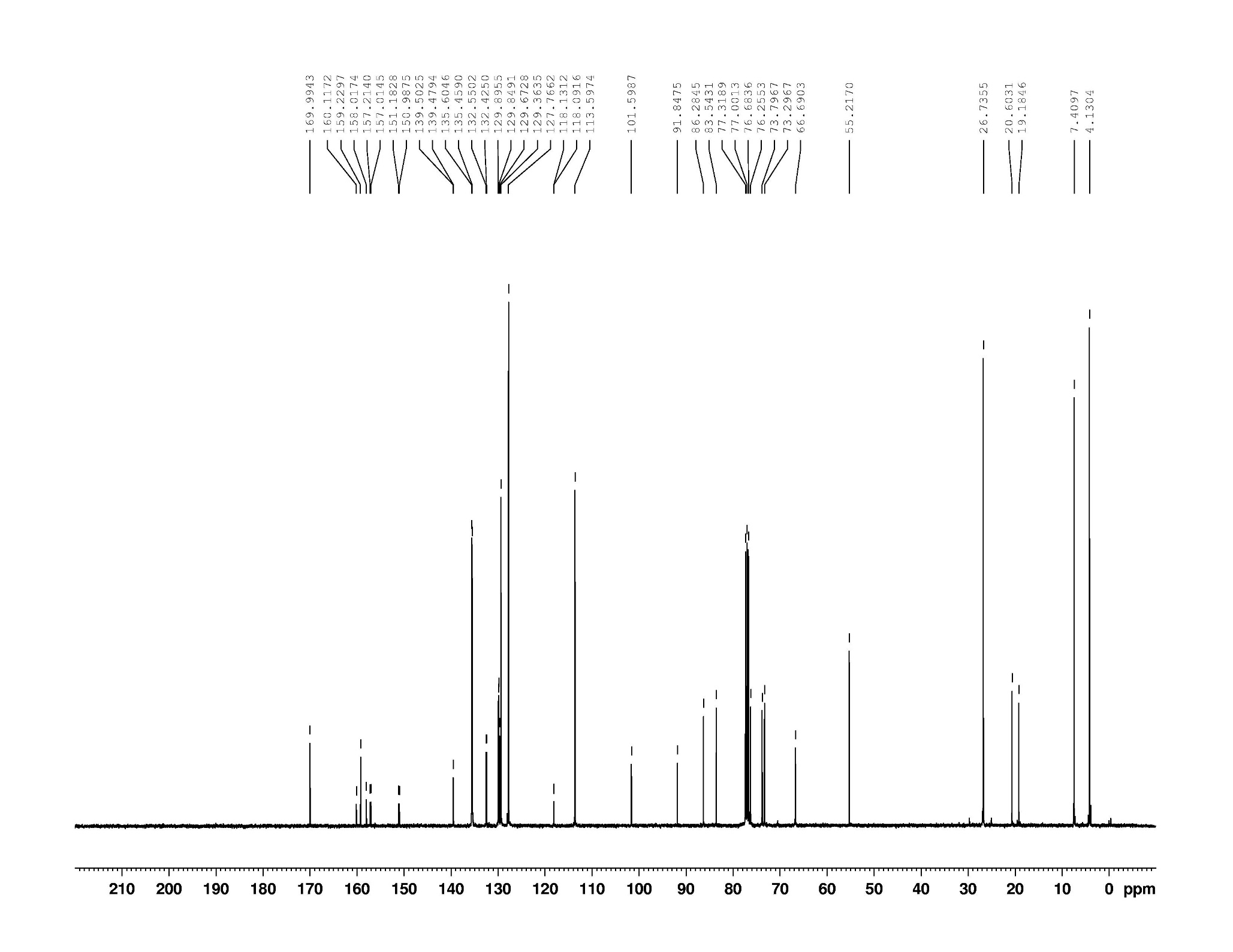


C-4ʹ

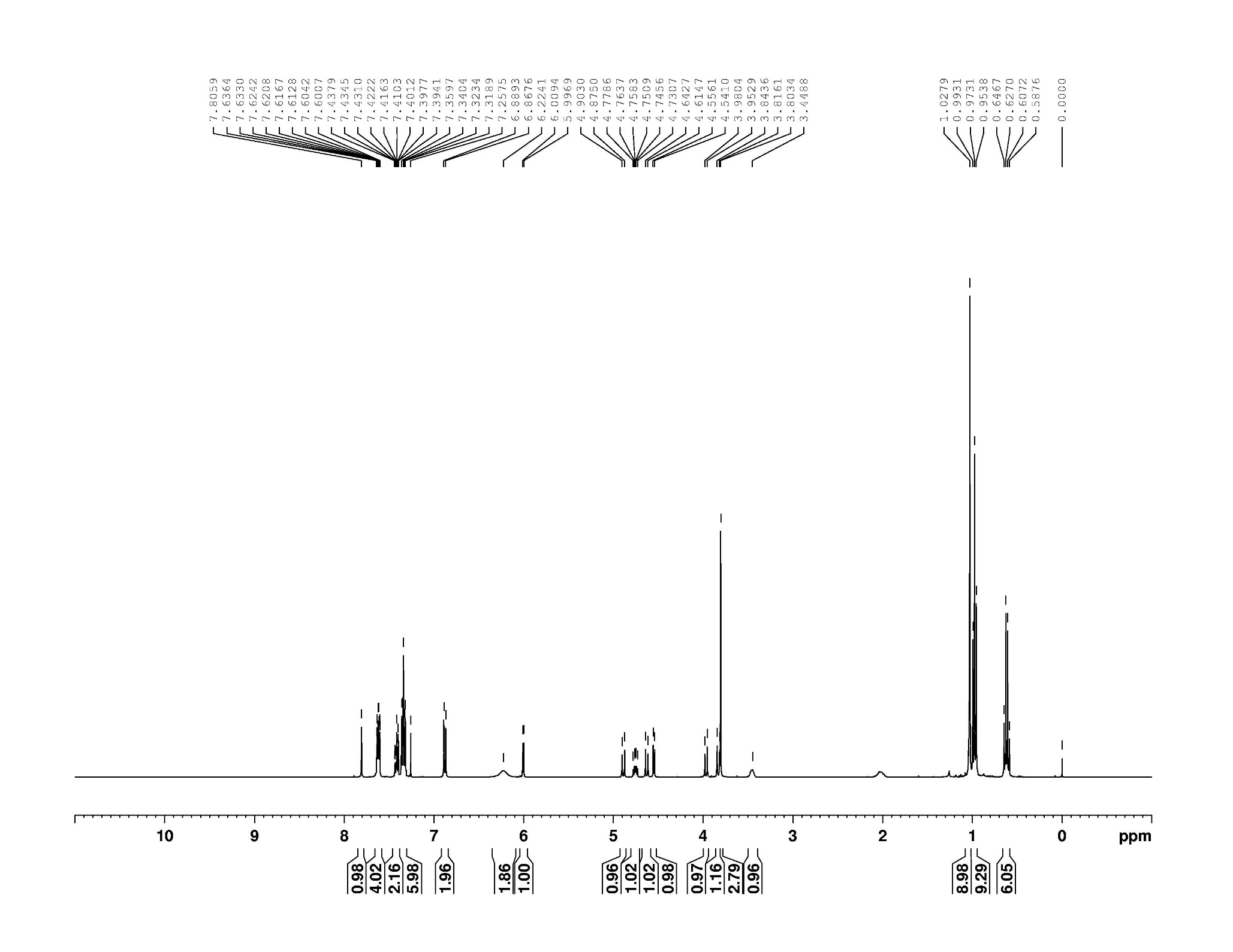
**Figure S11.** 1H NMR spectrum (400 MHz, chloroform-*d*) of compound **25**.

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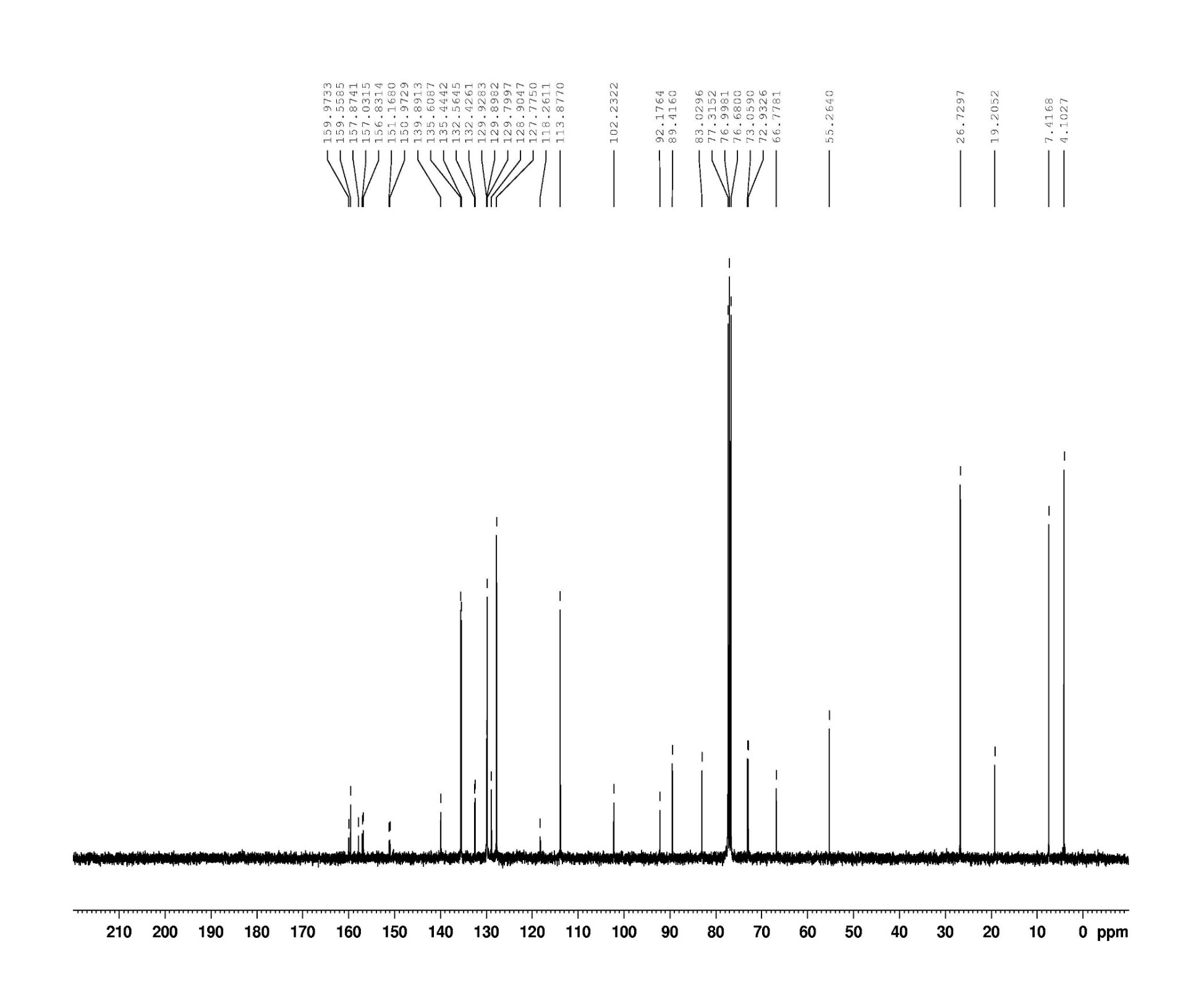
**Figure S12.** 13C NMR spectrum (100 MHz, chloroform-*d*) of compound **25**.

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**Figure S13.** 1H NMR spectrum (400 MHz, chloroform-*d*) of compound **26**.

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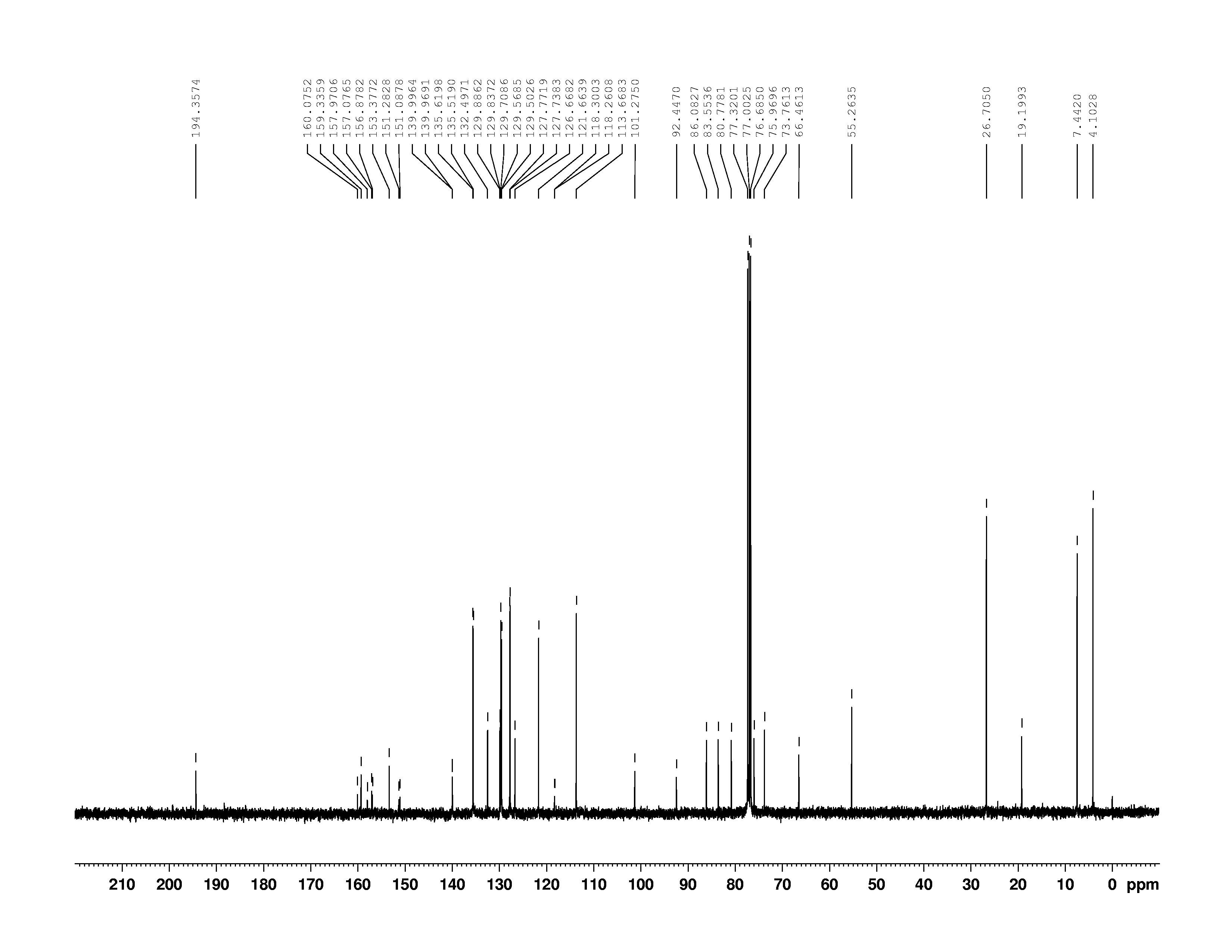
**Figure S14.** 13C NMR spectrum (100 MHz, chloroform-*d*) of compound **26**.

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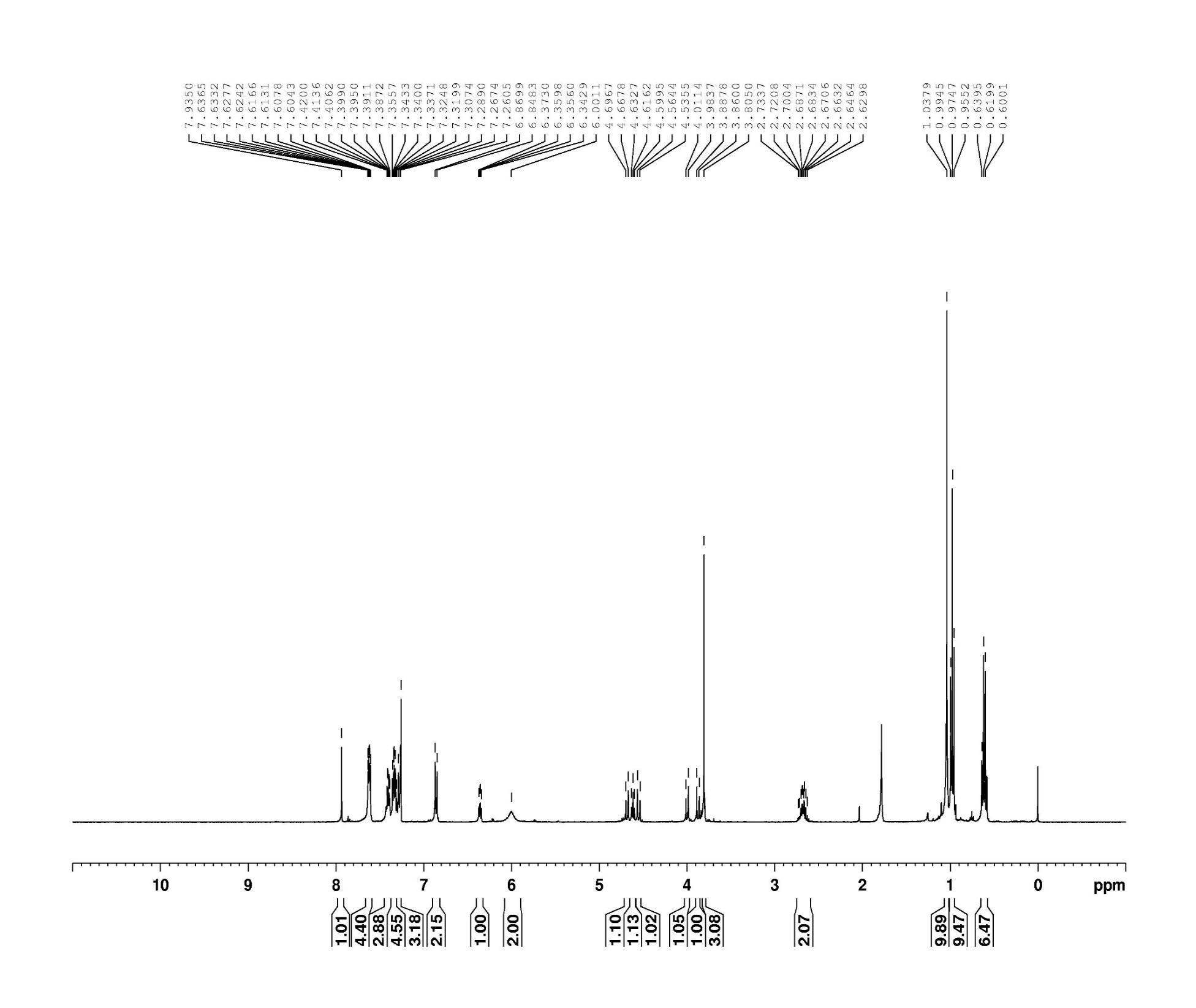
**Figure S15.** 1H NMR spectrum (400 MHz, chloroform-*d*) of compound **27**.



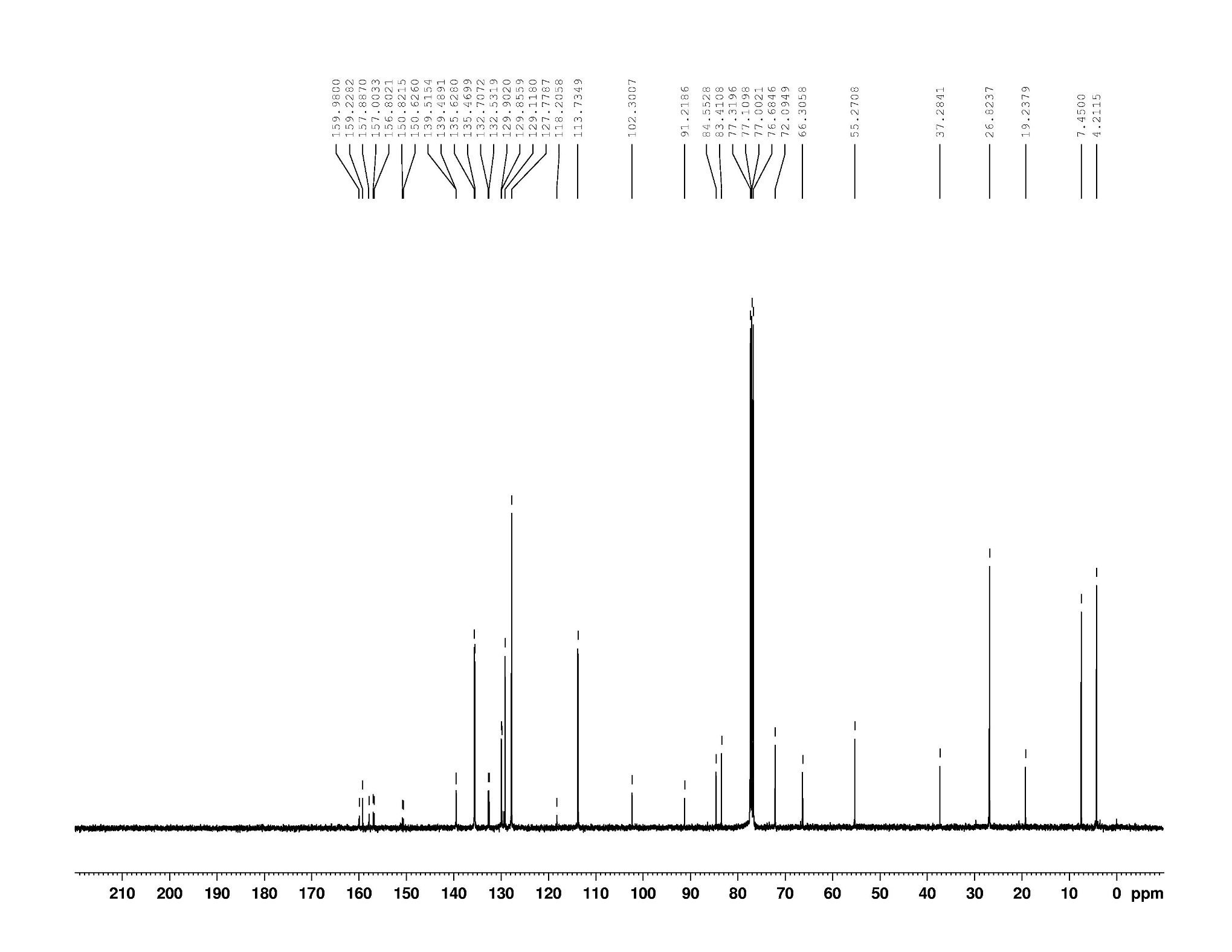
**Figure S16.** 13C NMR spectrum (100 MHz, chloroform-*d*) of compound **27**.

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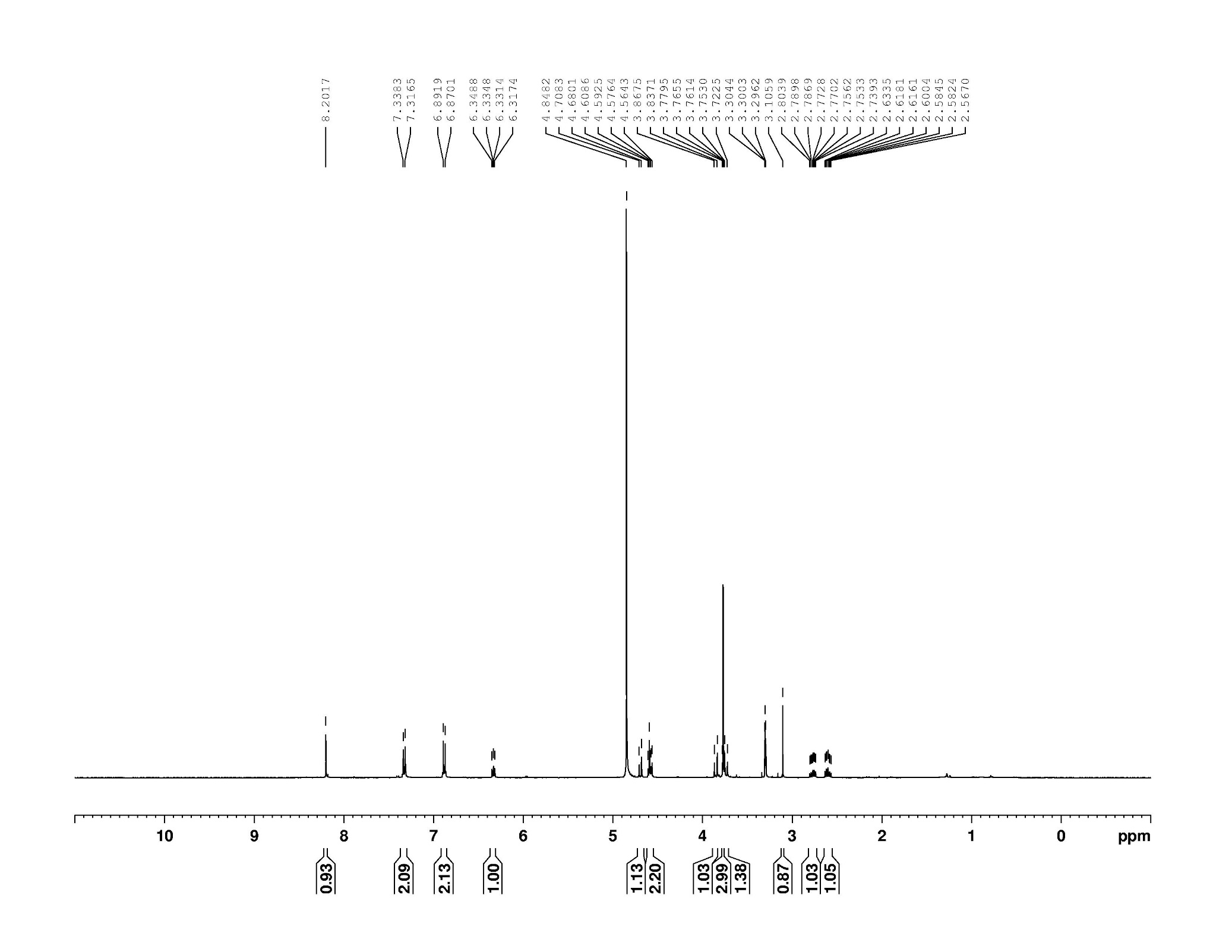
**Figure S17.** 1H NMR spectrum (400 MHz, chloroform-*d*) of compound **28**.

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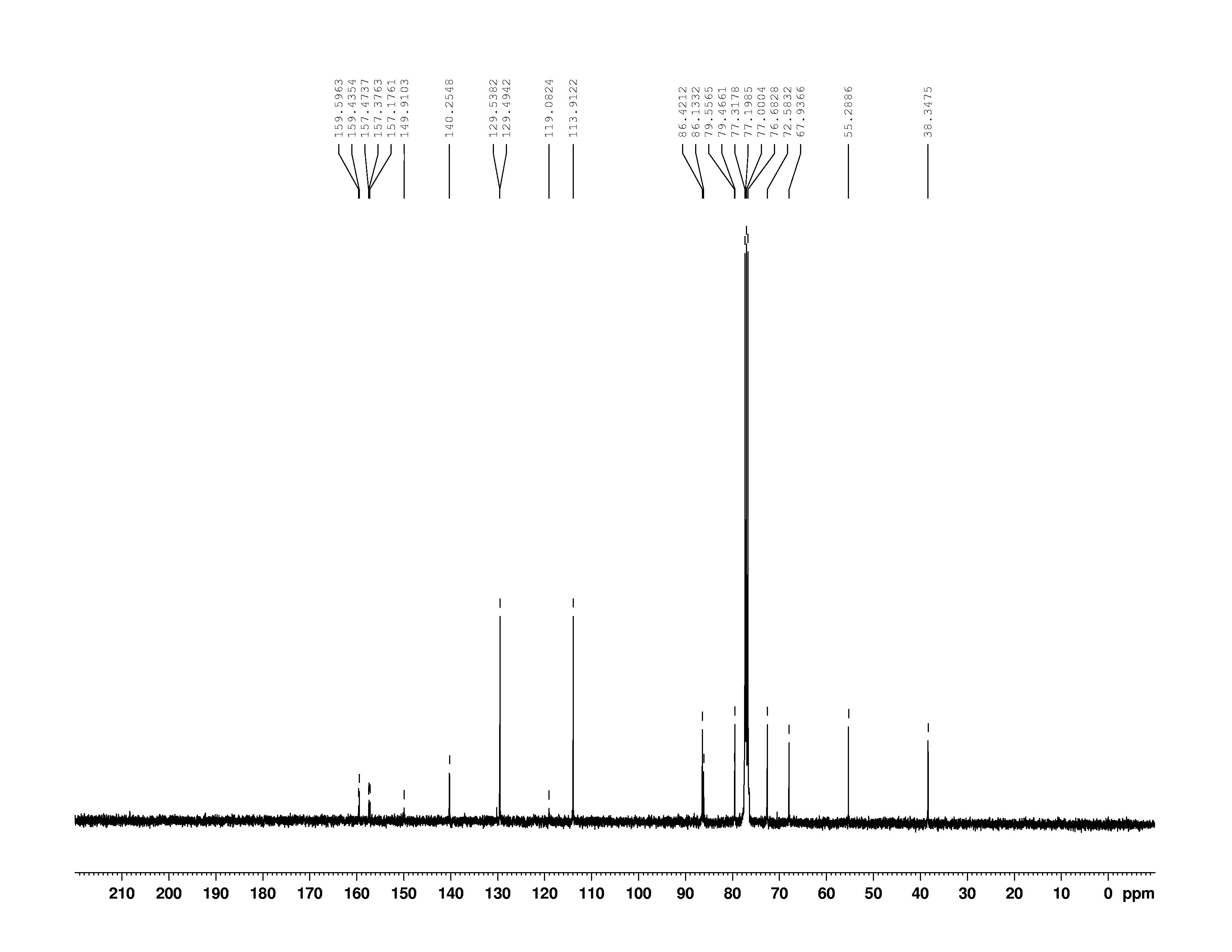
**Figure S18.** 13C NMR spectrum (100 MHz, chloroform-*d*) of compound **28**.

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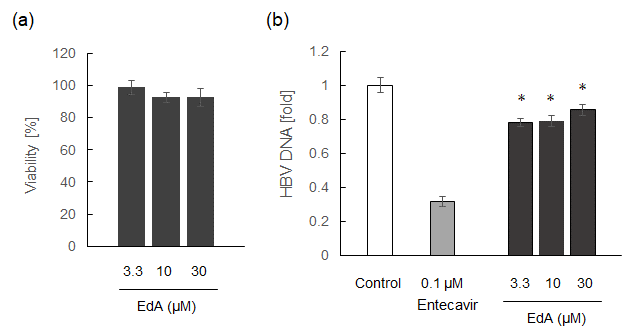
**Figure S19.** 1H NMR spectrum (400 MHz, methanol-*d*4) of compound **29**.



**Figure S20.** 13C NMR spectrum (100 MHz, chloroform-*d*) of compound **29**.



**Figure S21.** Effect of EdA on the replication of HBV.



(a) Cytotoxicity of EdP against Hep38.7-Tet cells after six days was determined by XTT assay [S1]. Gray bar, various concentrations of EdA. The viability of vehicle-treated control cells was defined as 100%. (b) Anti-HBV activity was evaluated by real-time PCR quantifying HBV DNA in the culture supernatant of Hep38.7-Tet cells treated with the indicated compounds for six days [S2]. White bar, control, 0.3% DMSO; Light gray bar, 0.1 μM entecavir [S3]; Gray bar, various concentrations of EdA. The data indicate the means ± SD of five samples from an experiment. Statistical significance was determined by Student's *t* test (\*, *p* < 0.05).

Reference

[S1] Roehm NW, Rodgers GH, Hatfield SM, et al. An improved colorimetric assay for cell proliferation and viability utilizing the tetrazolium salt XTT. J Immunol Methods. 1991;142:257–265.

[S2] Ogura N, Watashi K, Noguchi T, et al. Formation of covalently closed circular DNA in Hep38.7-Tet cells, a tetracycline inducible hepatitis B virus expression cell line. Biochem Biophys Res Commun. 2014;452:315–321.

[S3] Keating GM, Entecavir: a review of its use in the treatment of chronic hepatitis B in patients with decompensated liver disease. Drugs. 2011;71:2511–2529.