Electronic Supplementary Material (ESI) for RSC Advances.

Green synthesis and pharmacological screening of polyhydroquinoline derivatives bearing a fluorinated 5-aryloxypyrazole nucleus

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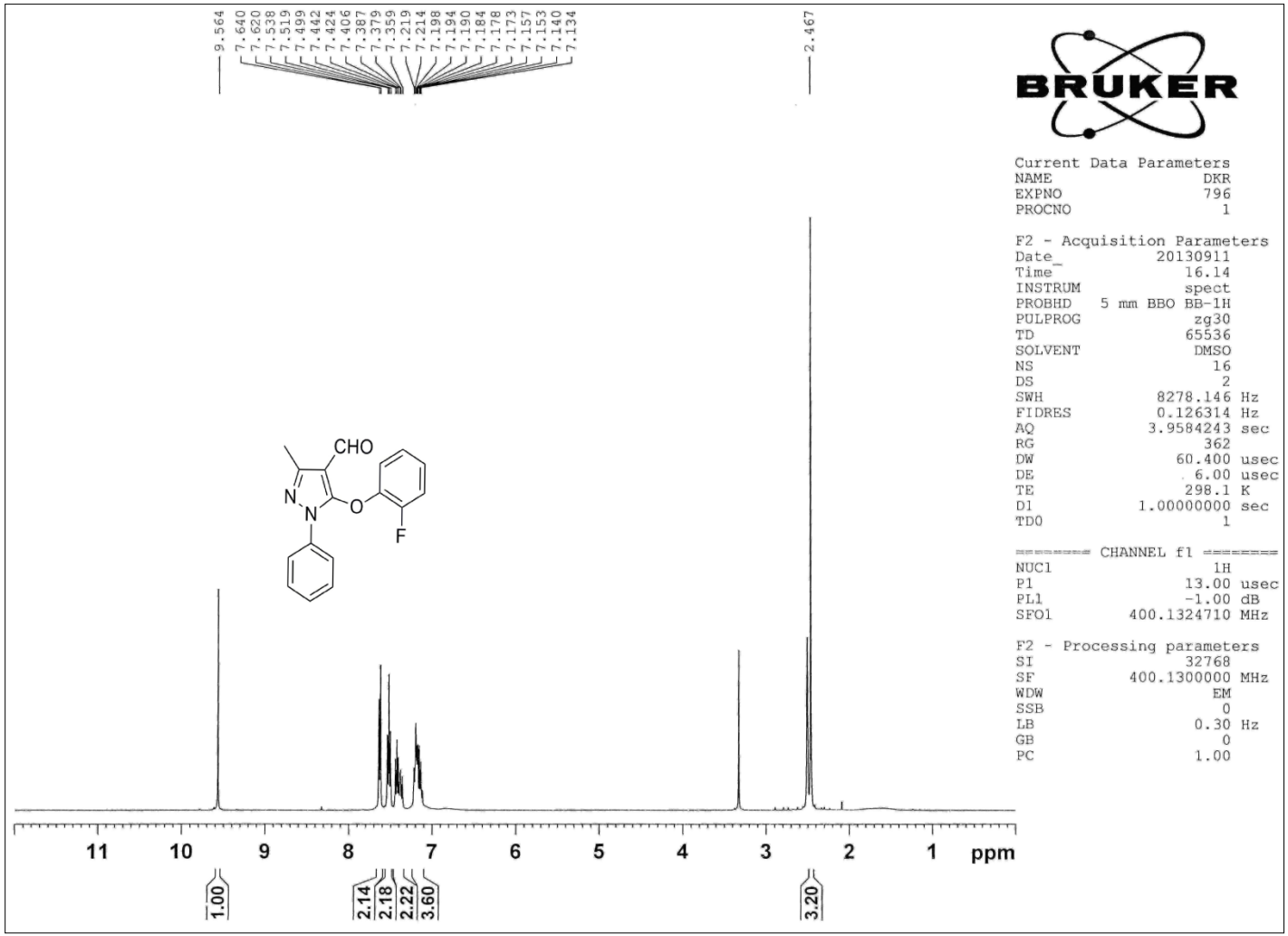
Supplementary Information

^{1}H NMR spectra of compound **3a**



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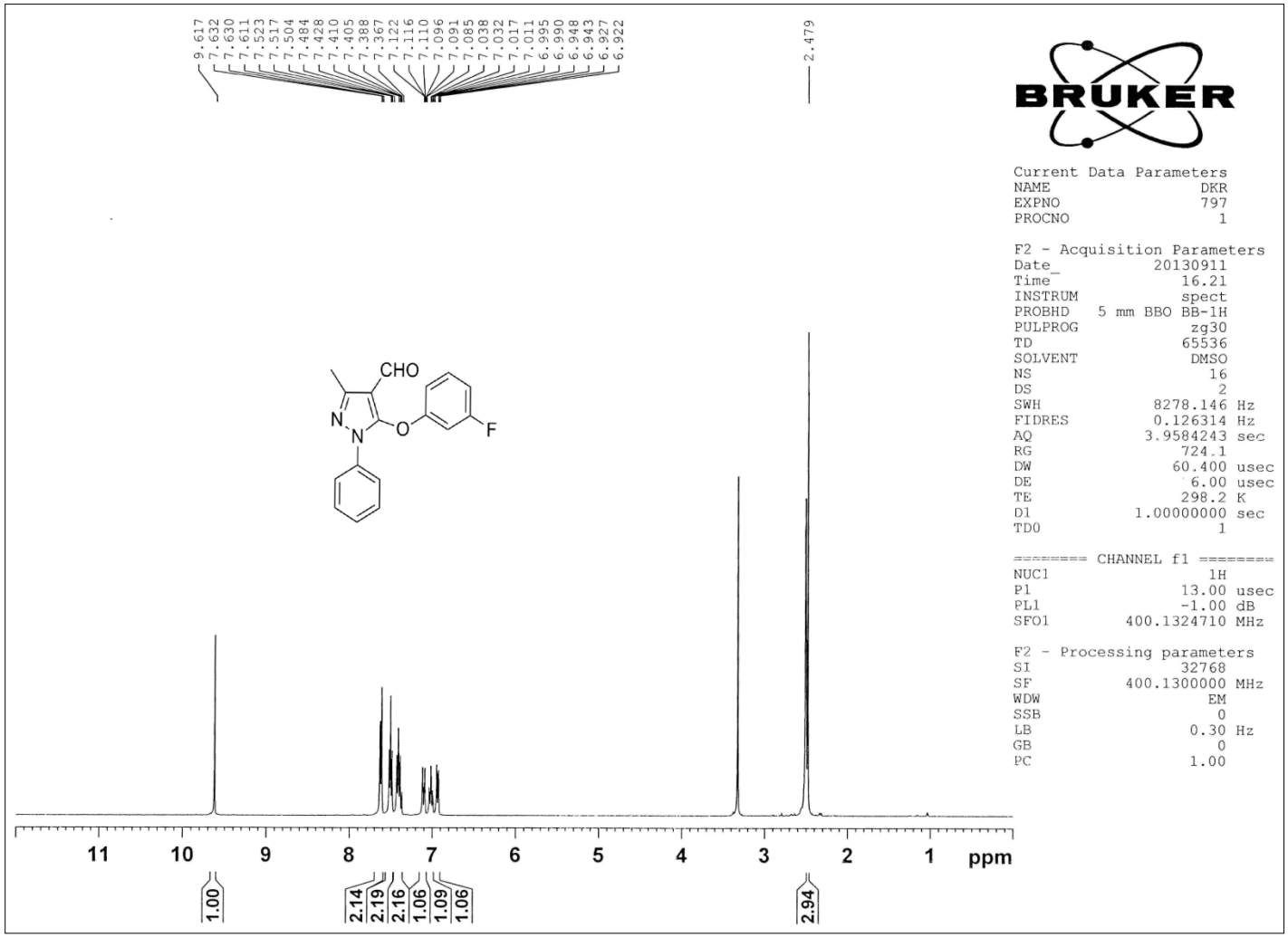

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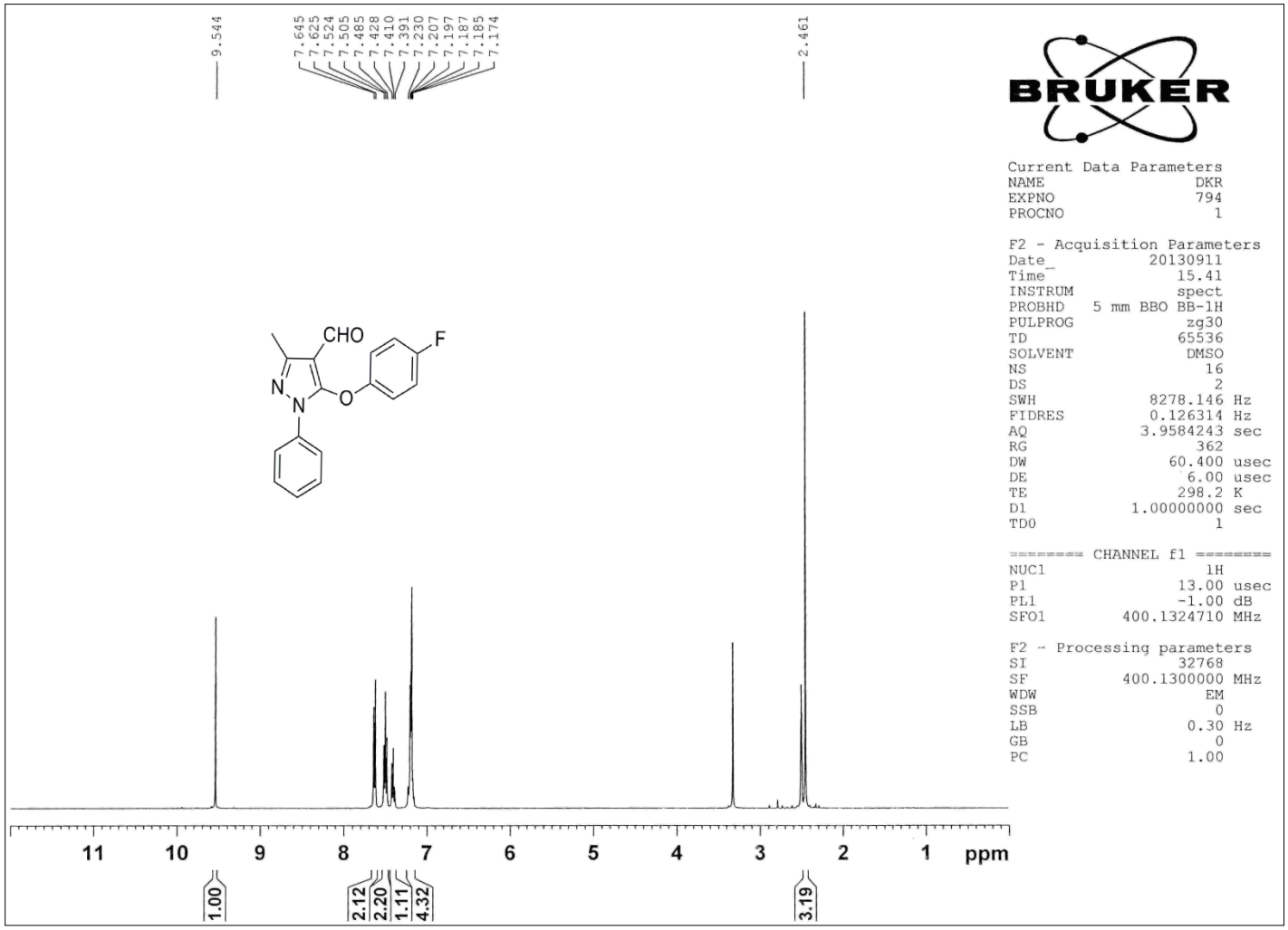

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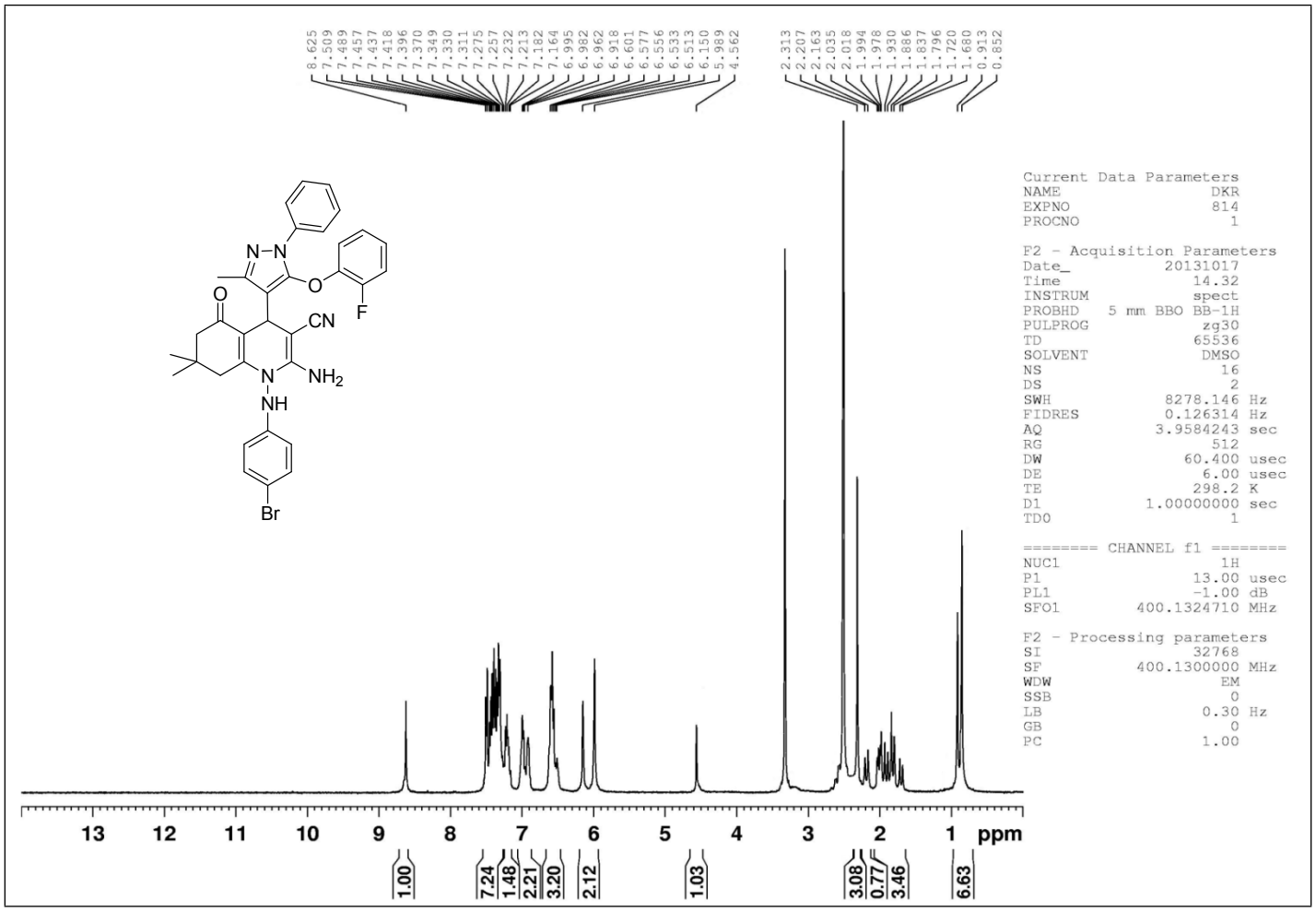

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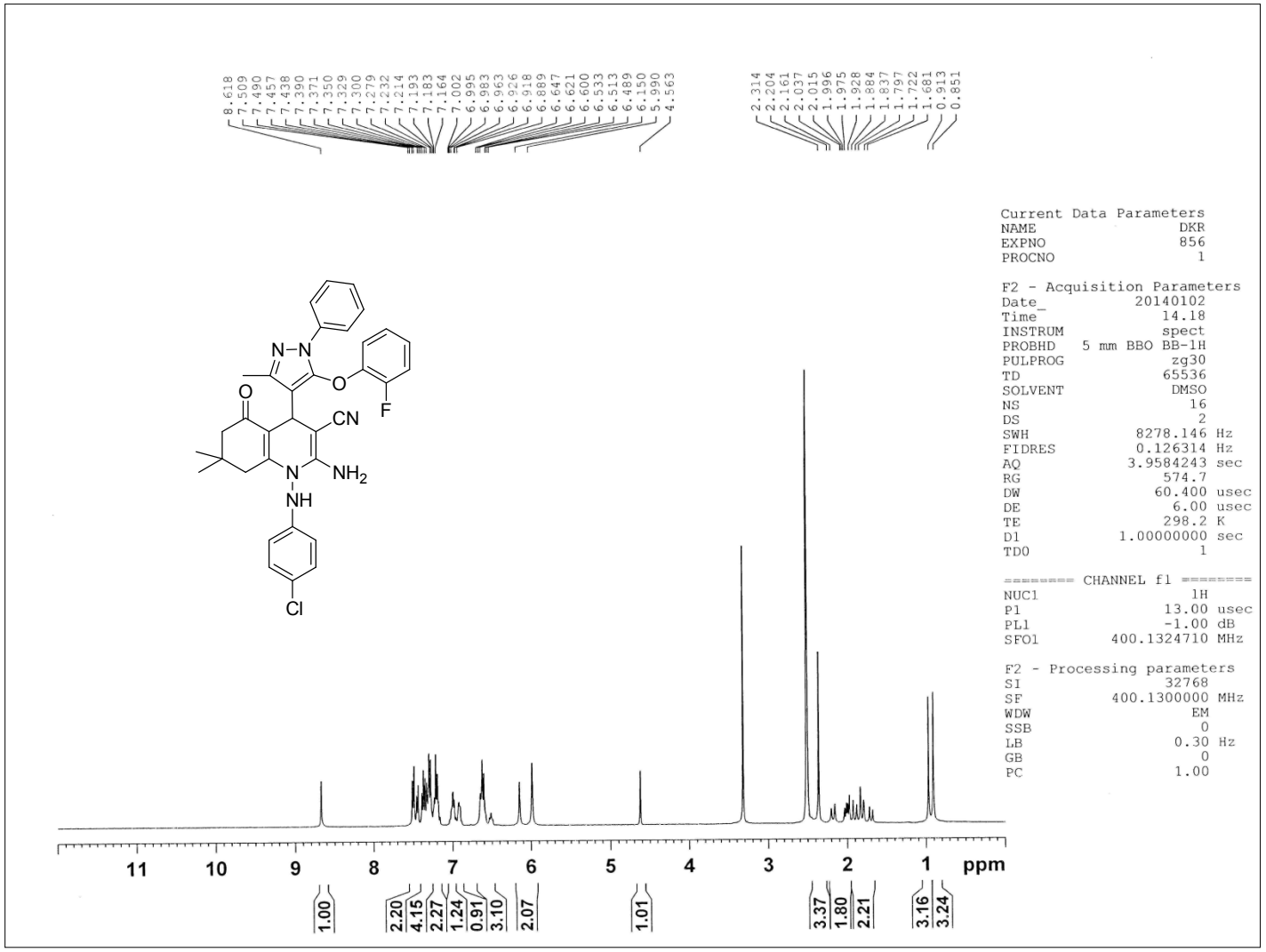

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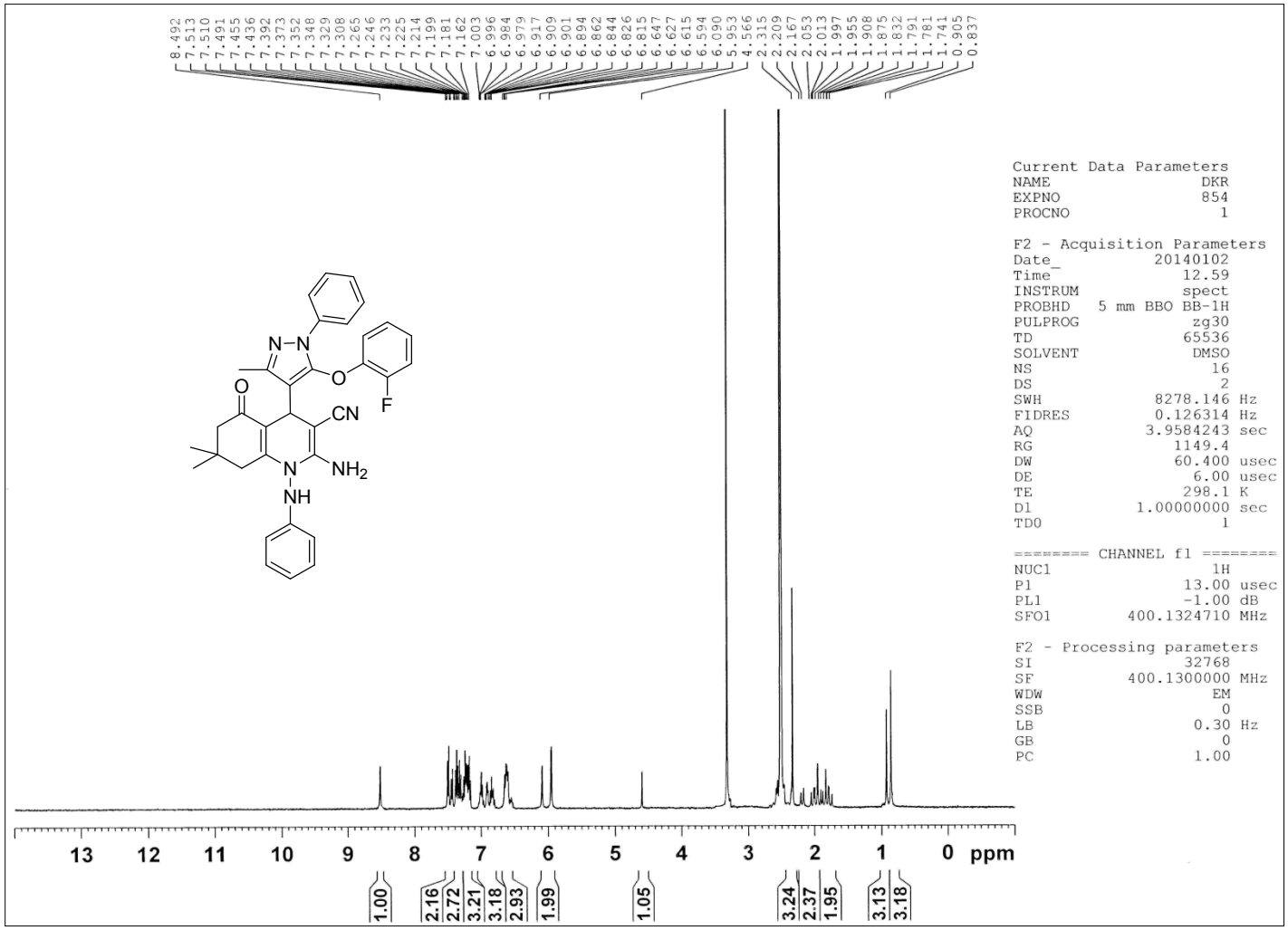

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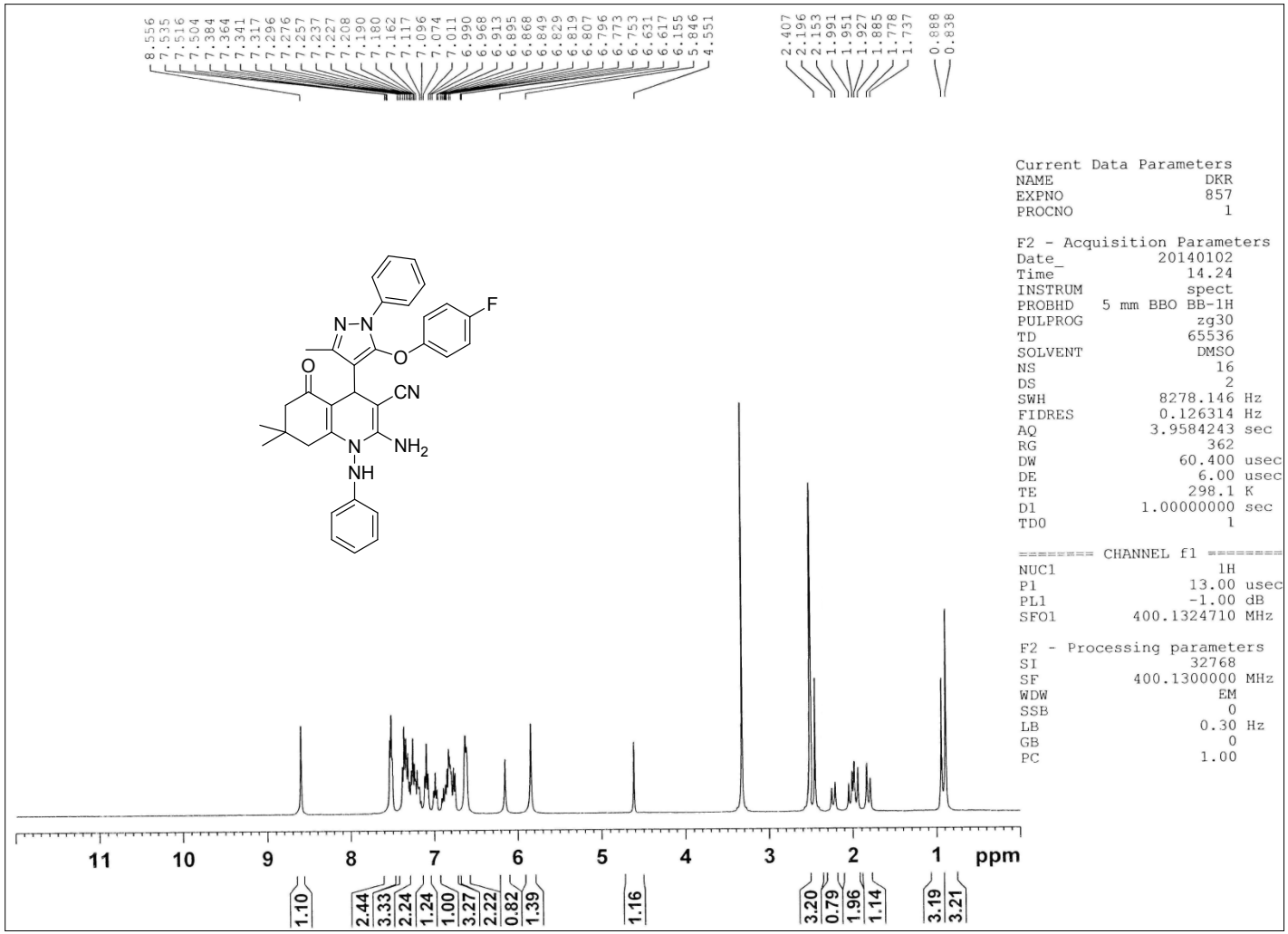

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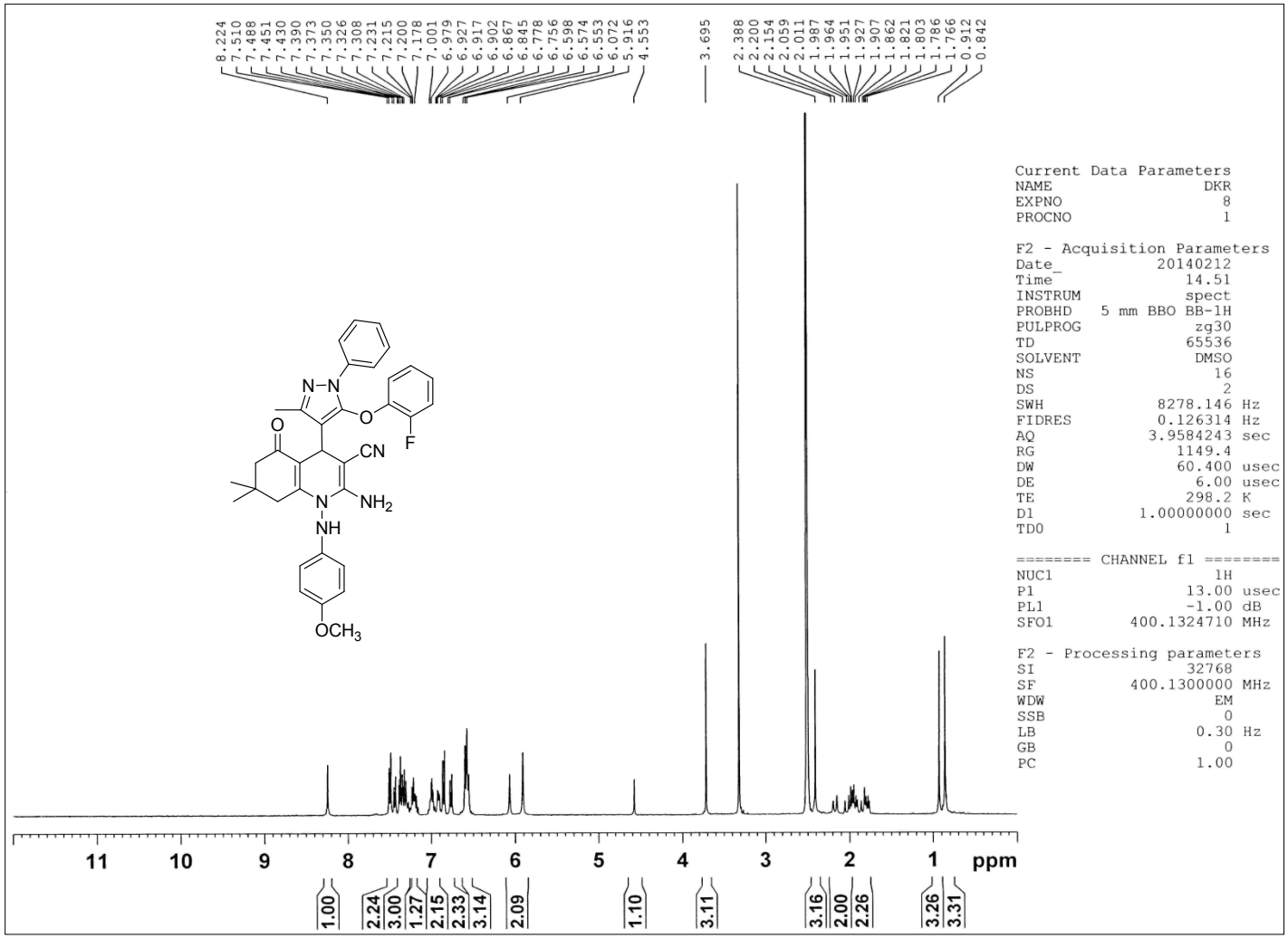

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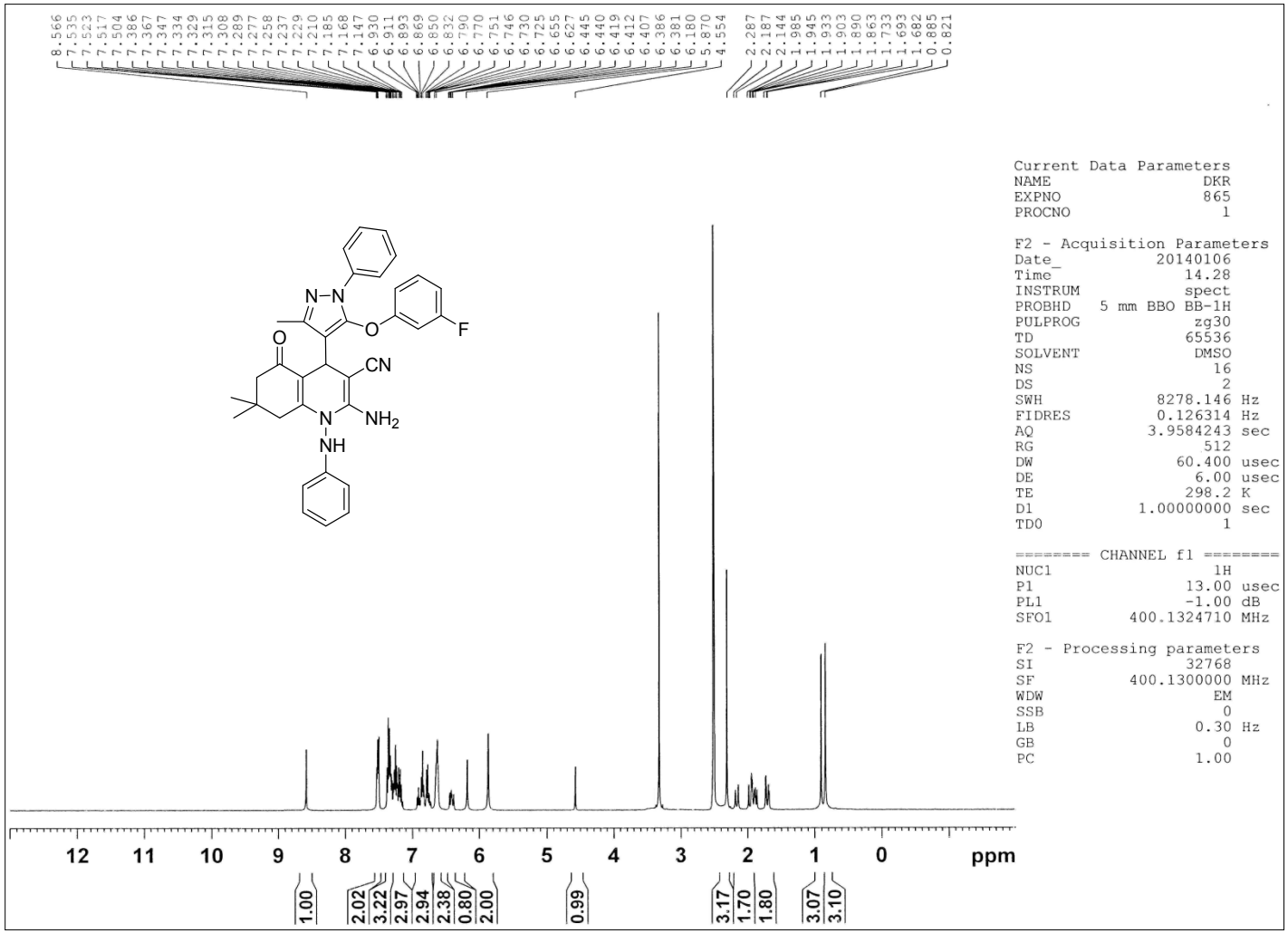

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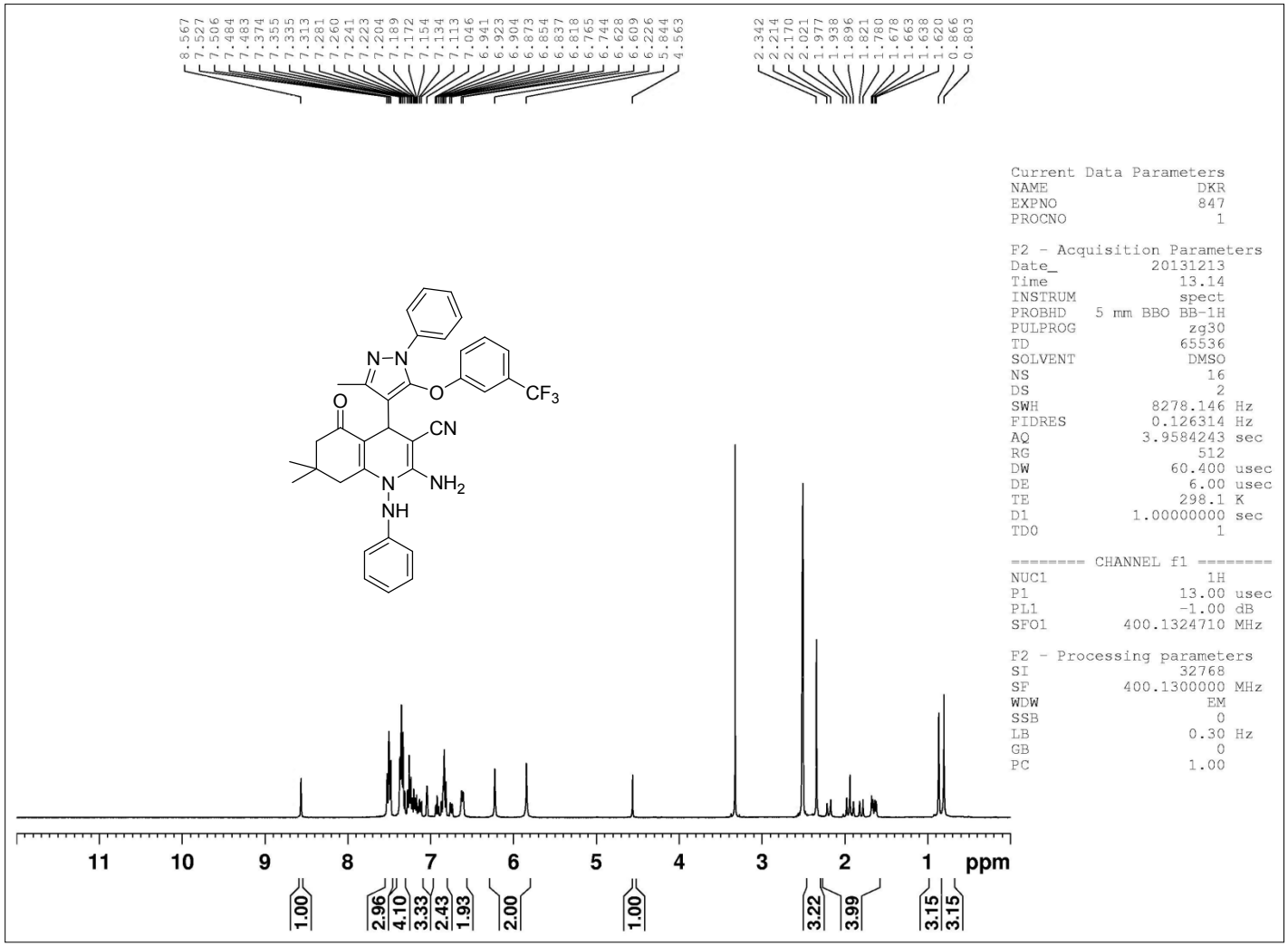

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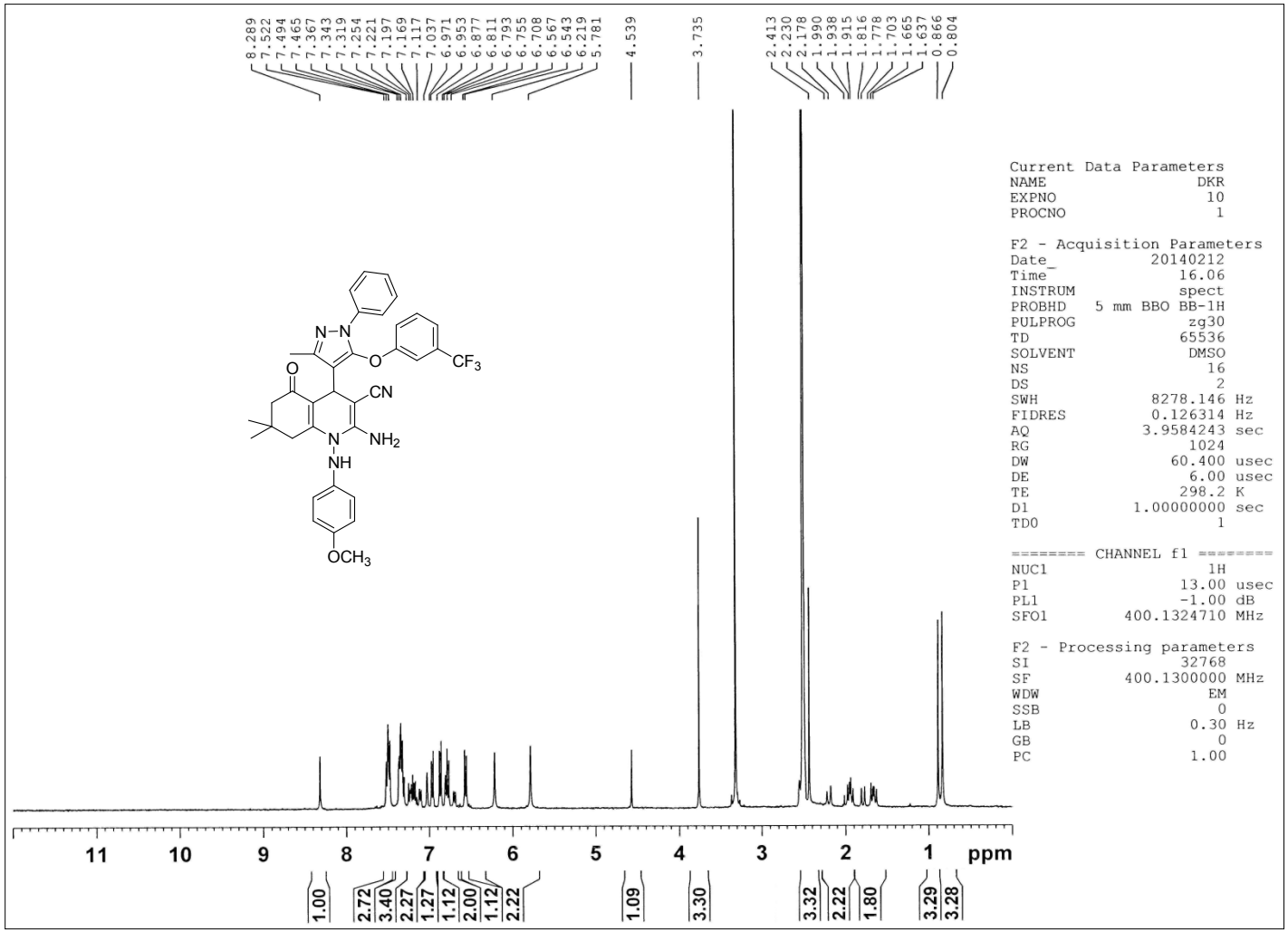

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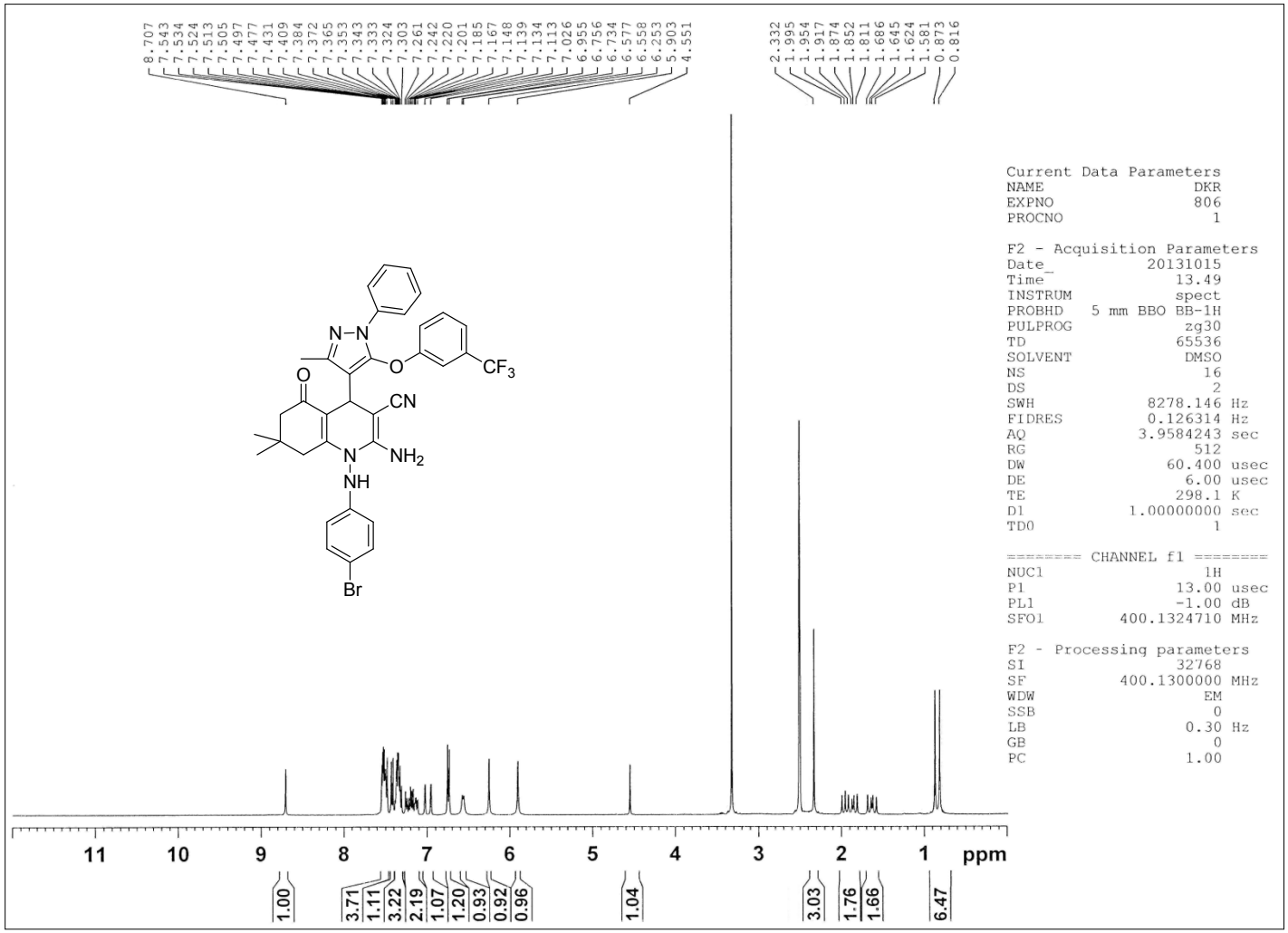

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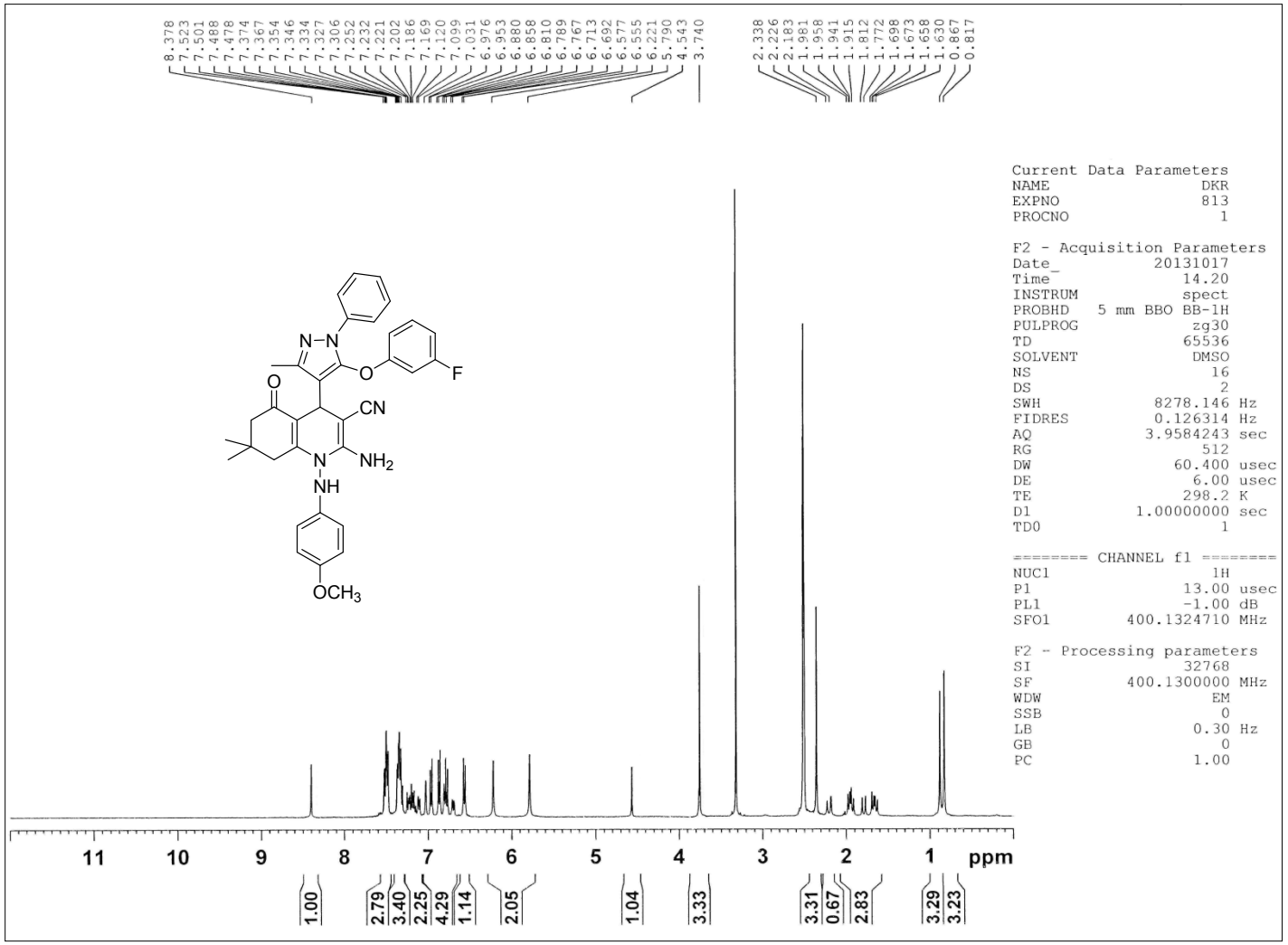

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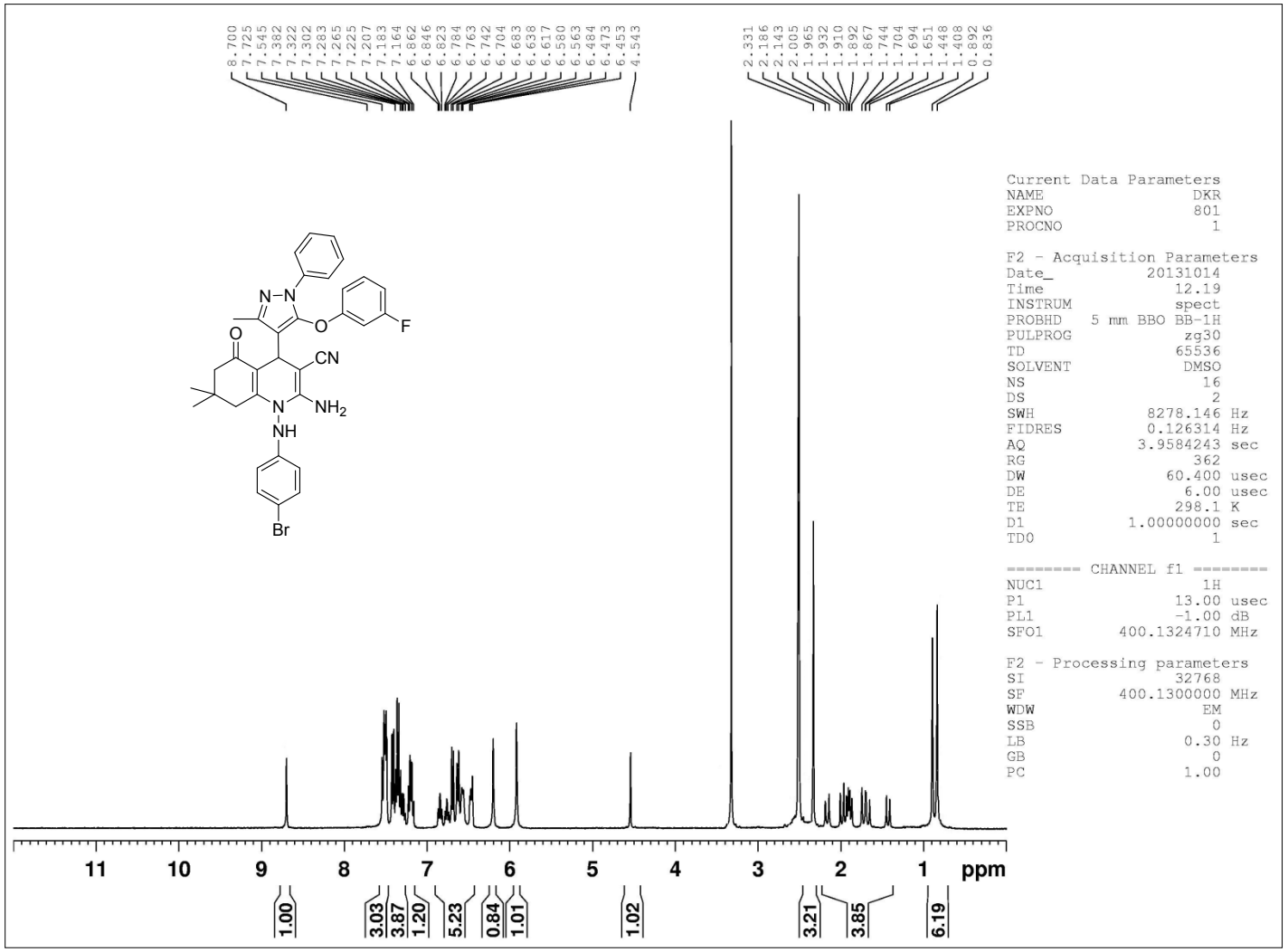

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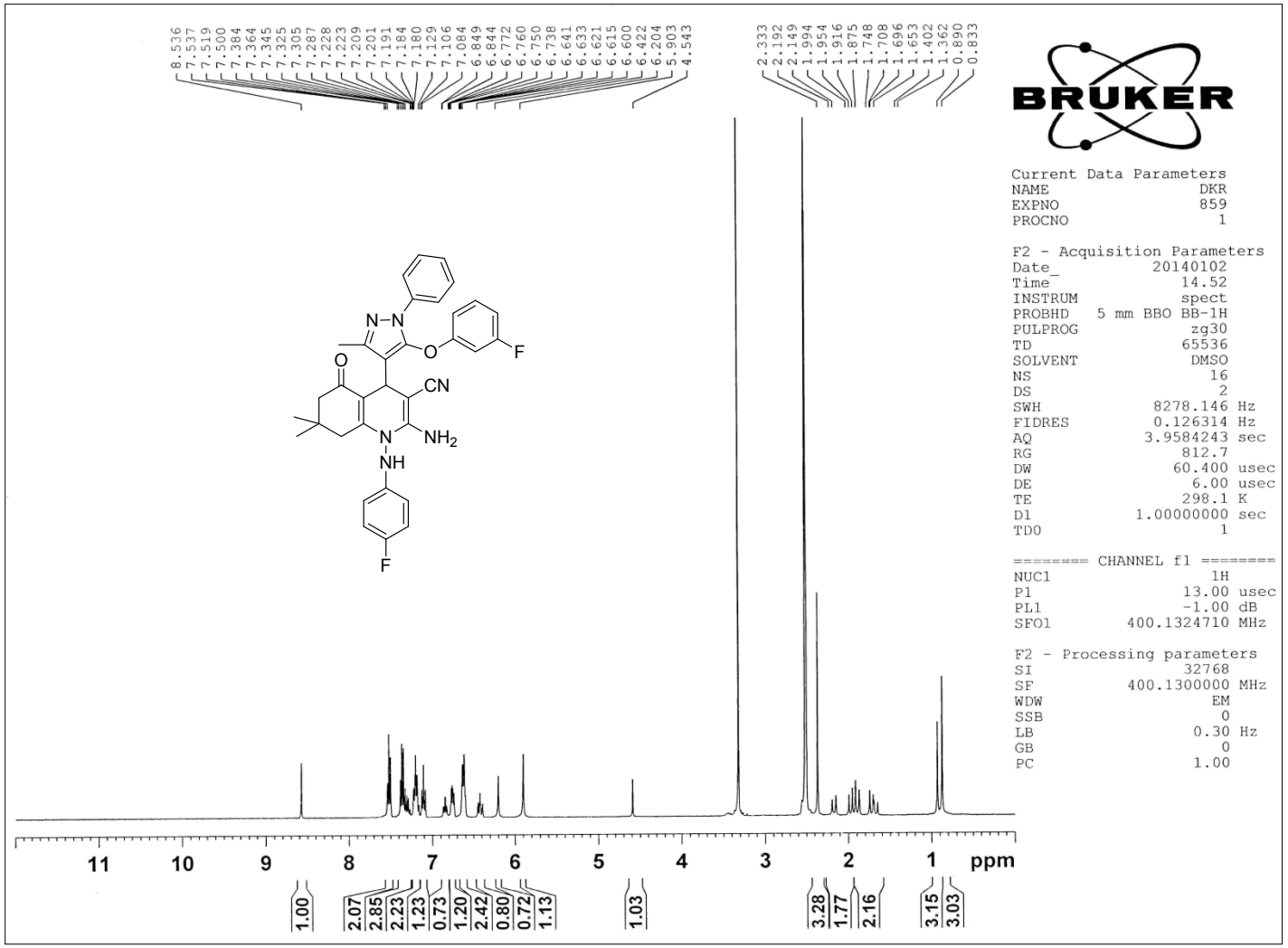

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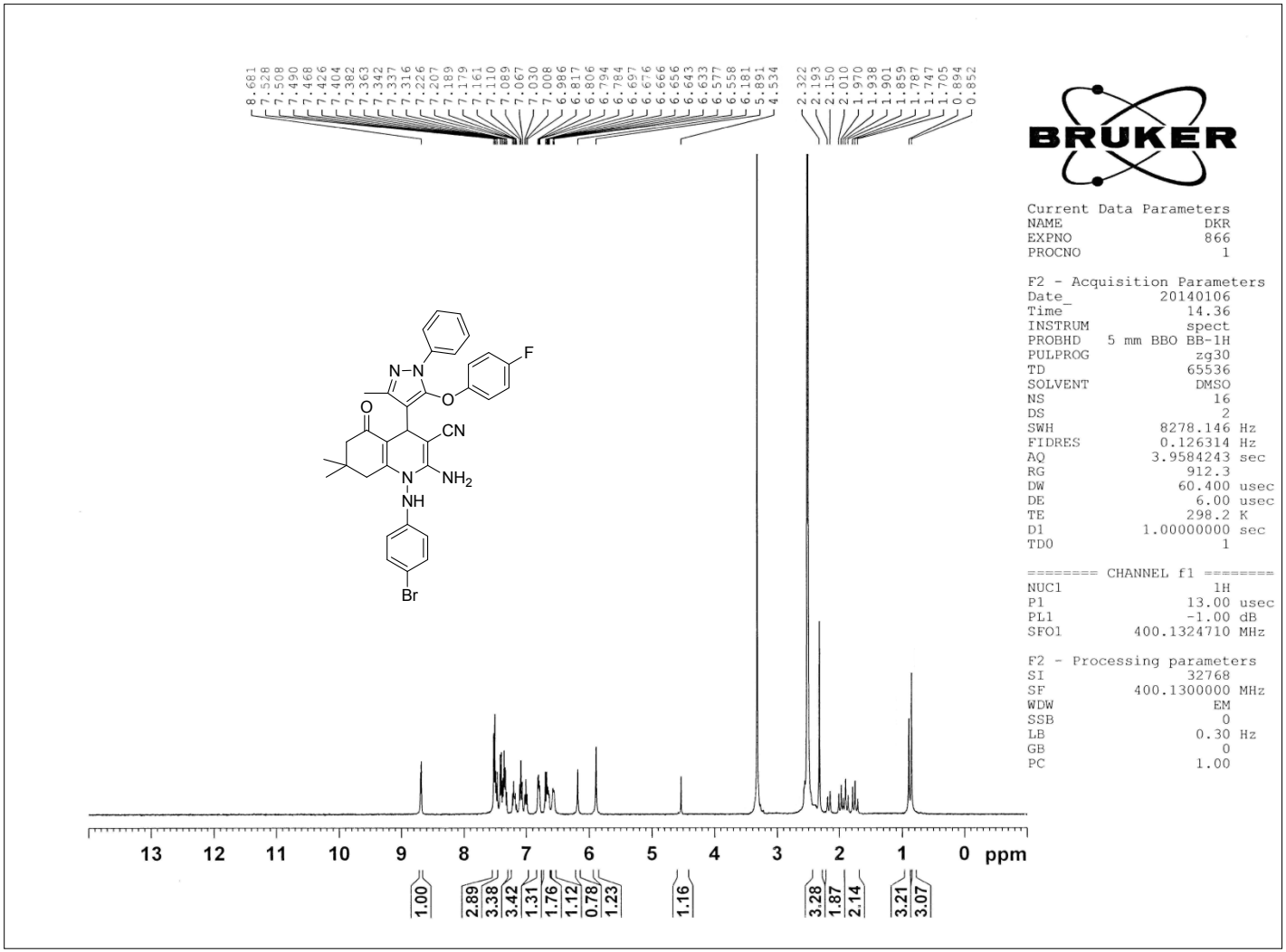

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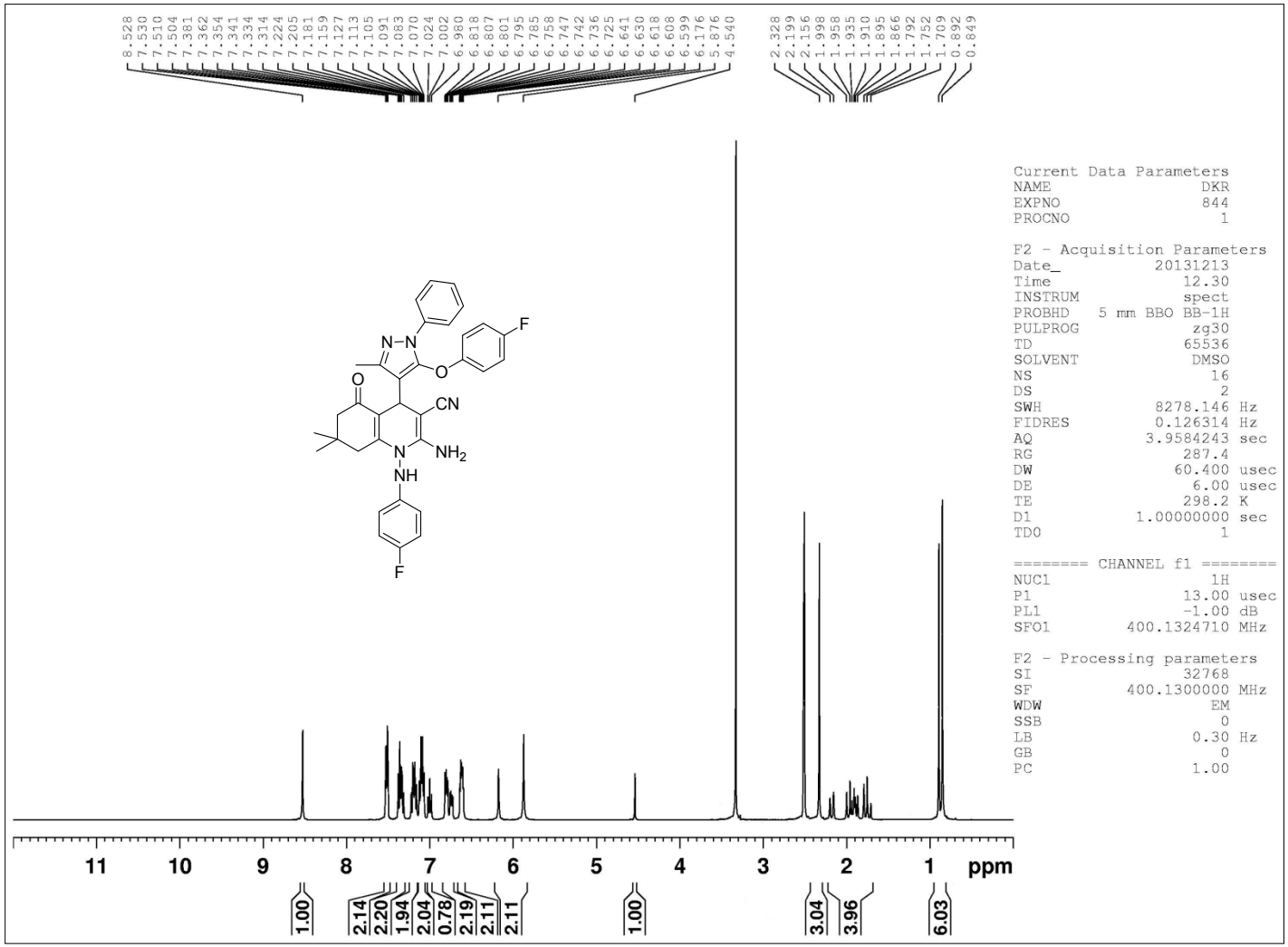

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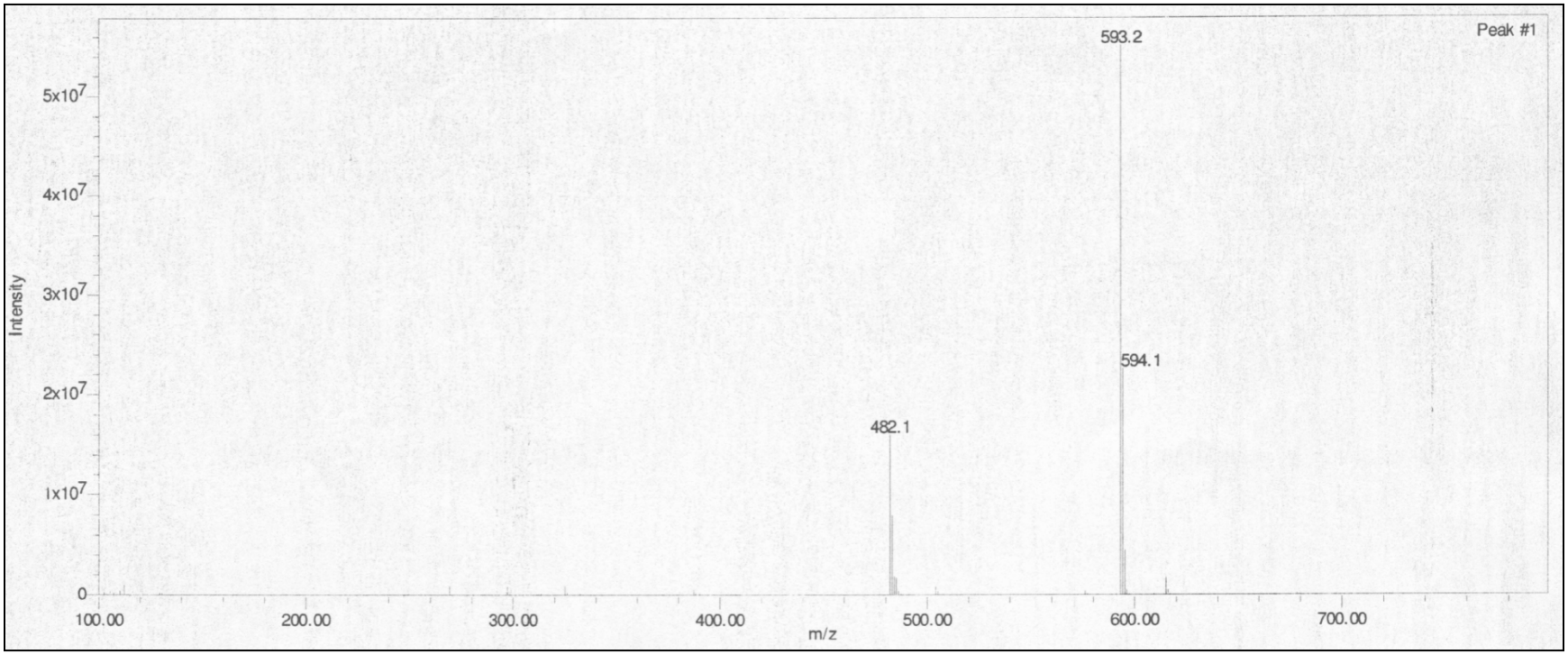

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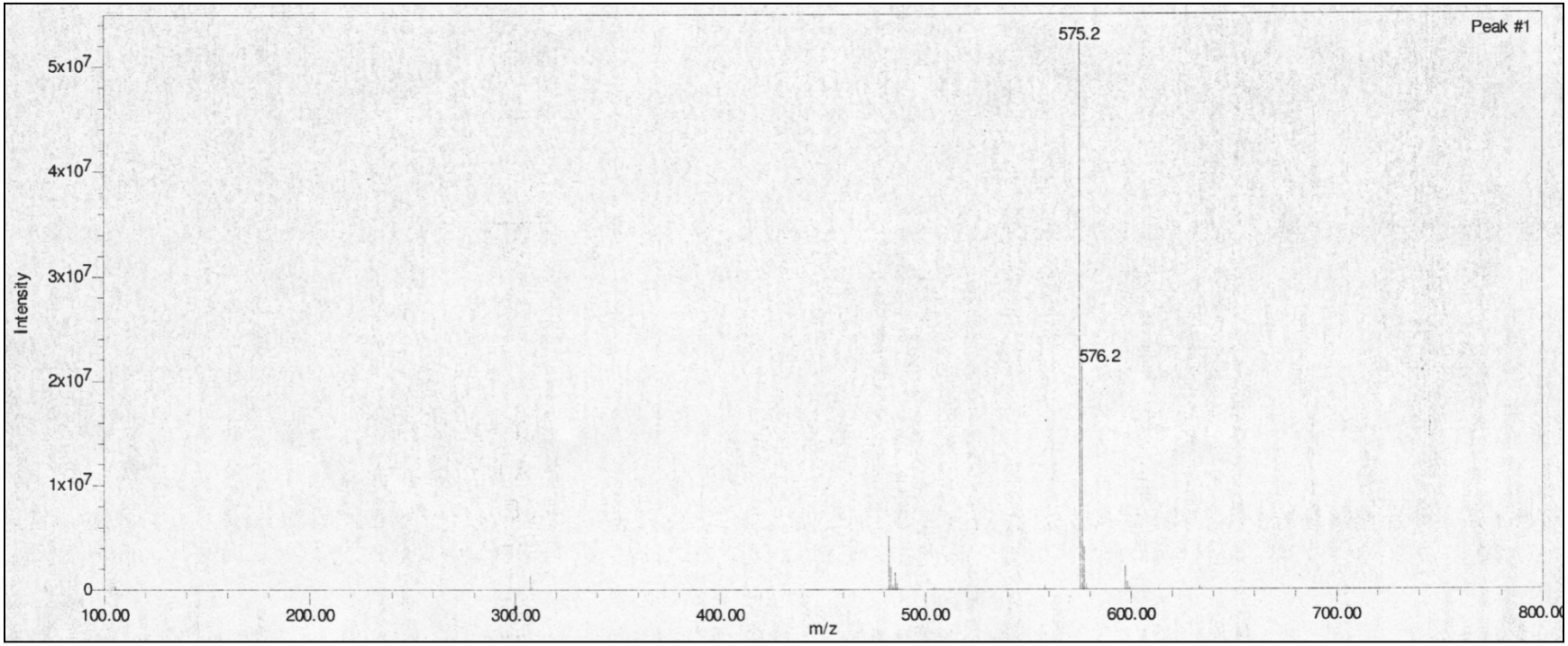

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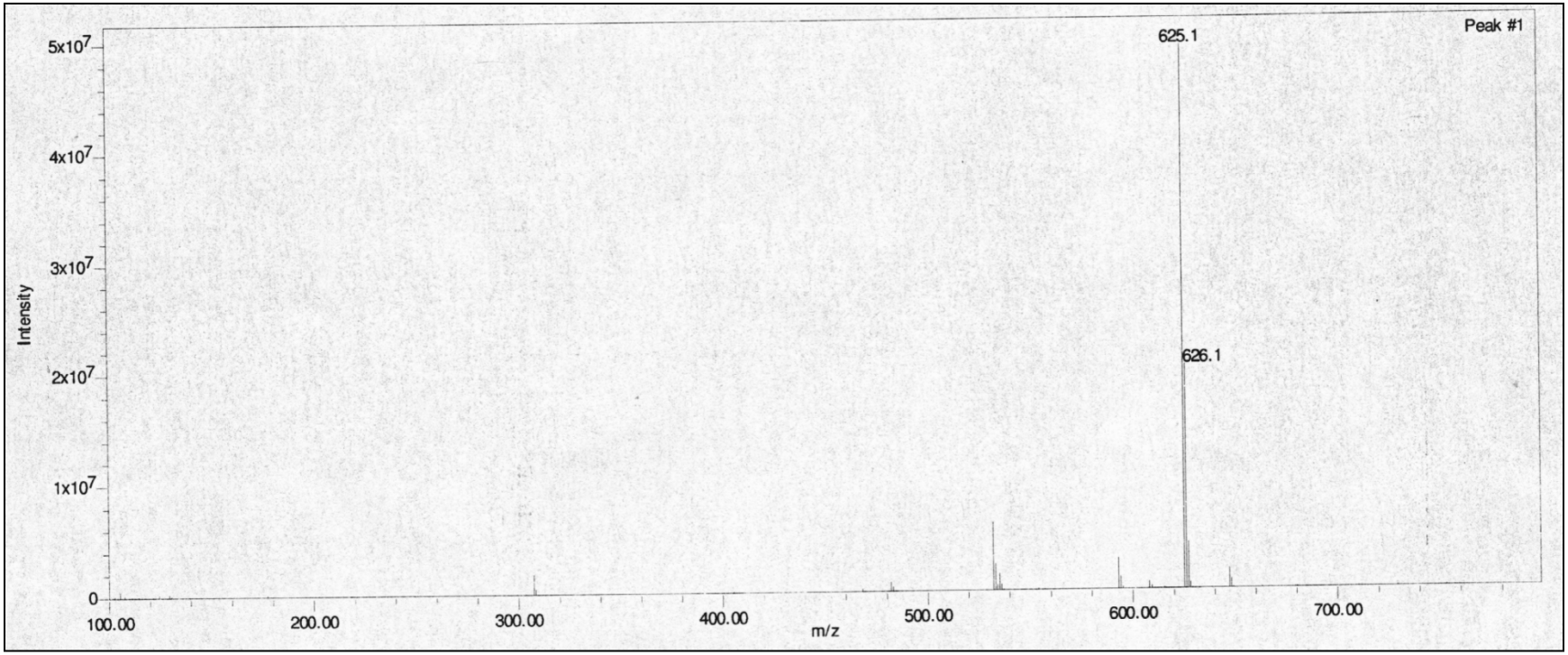

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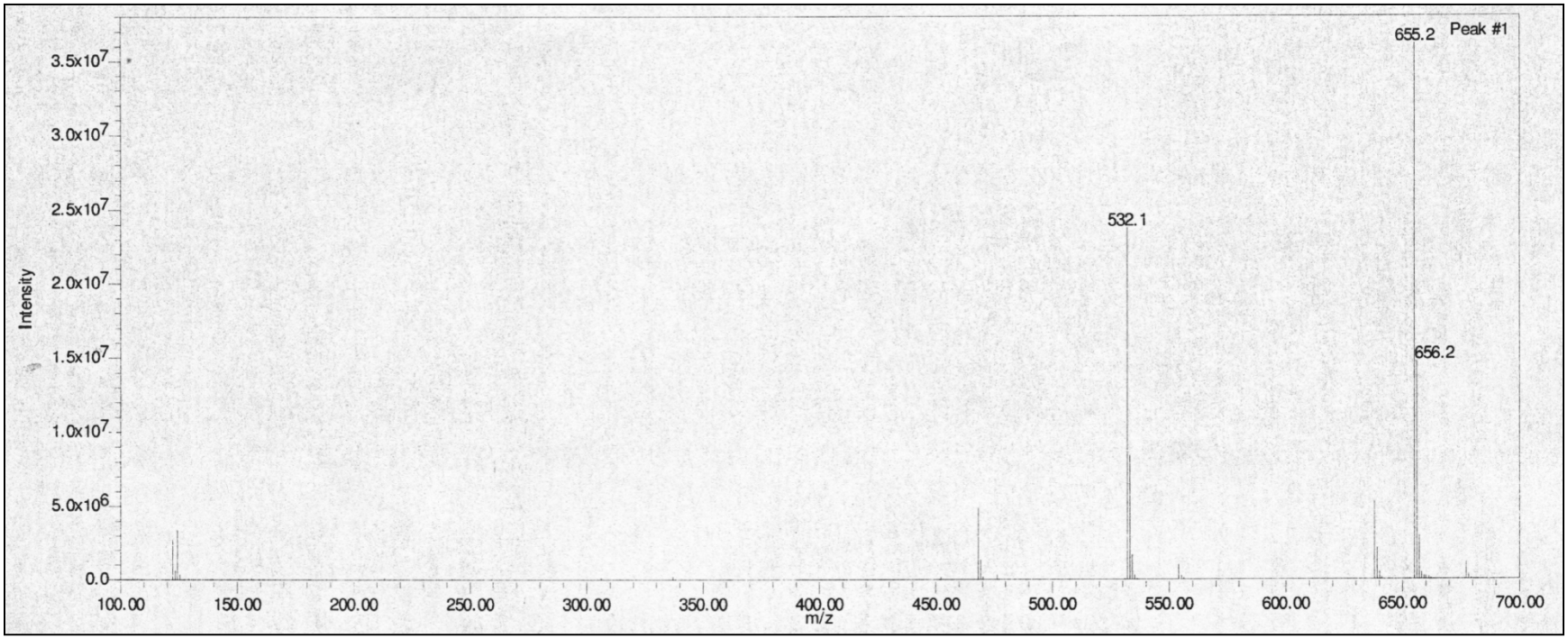

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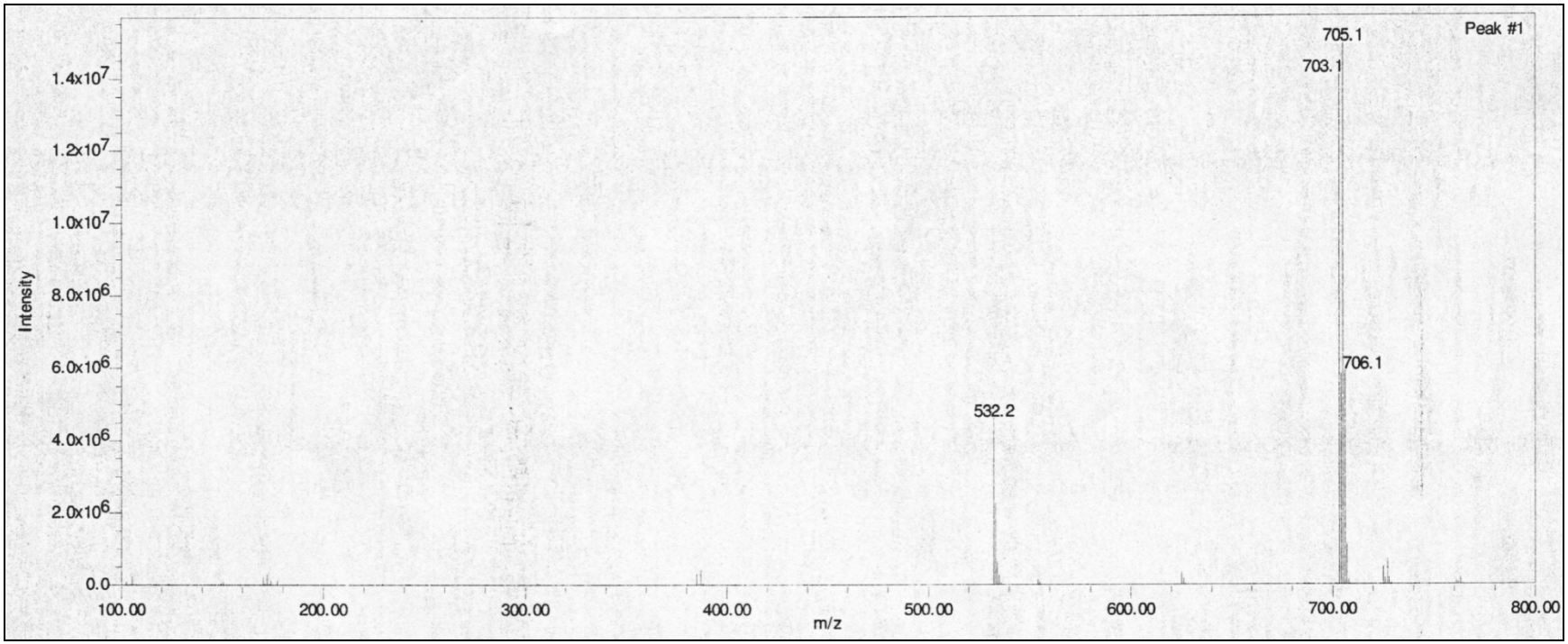

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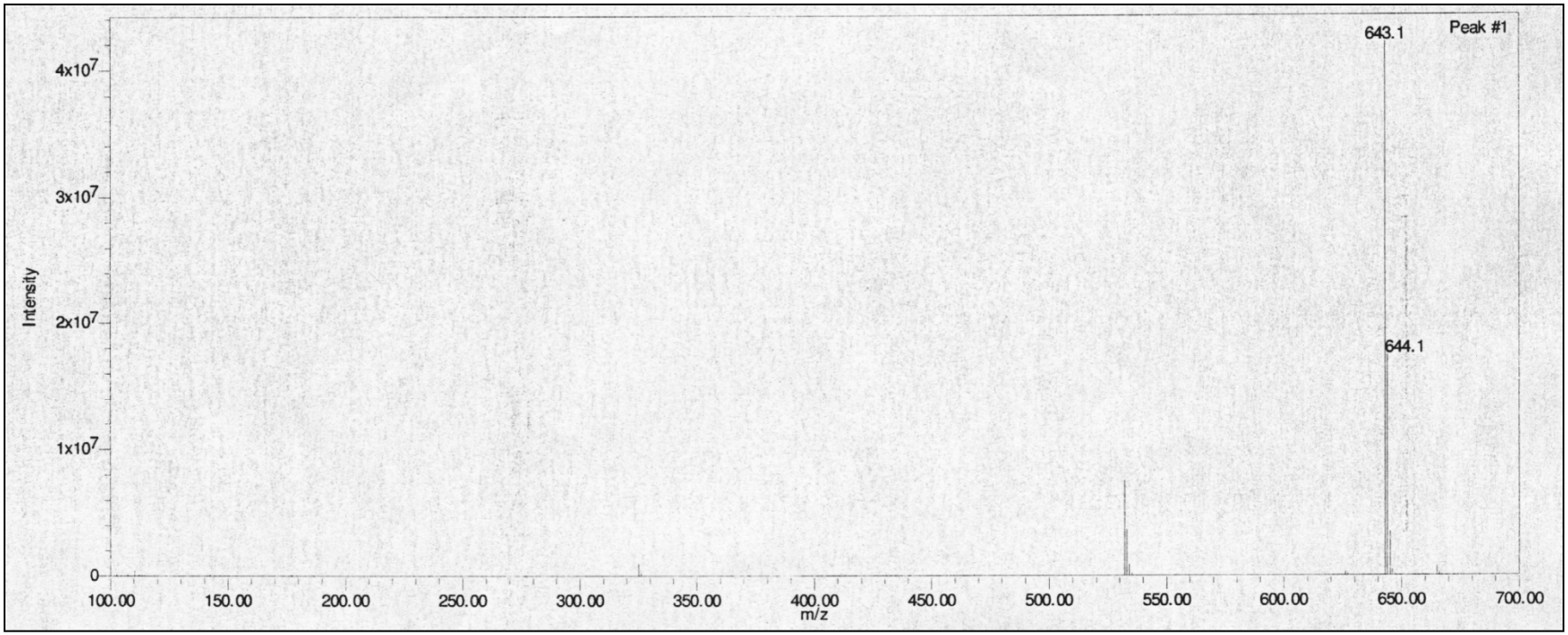

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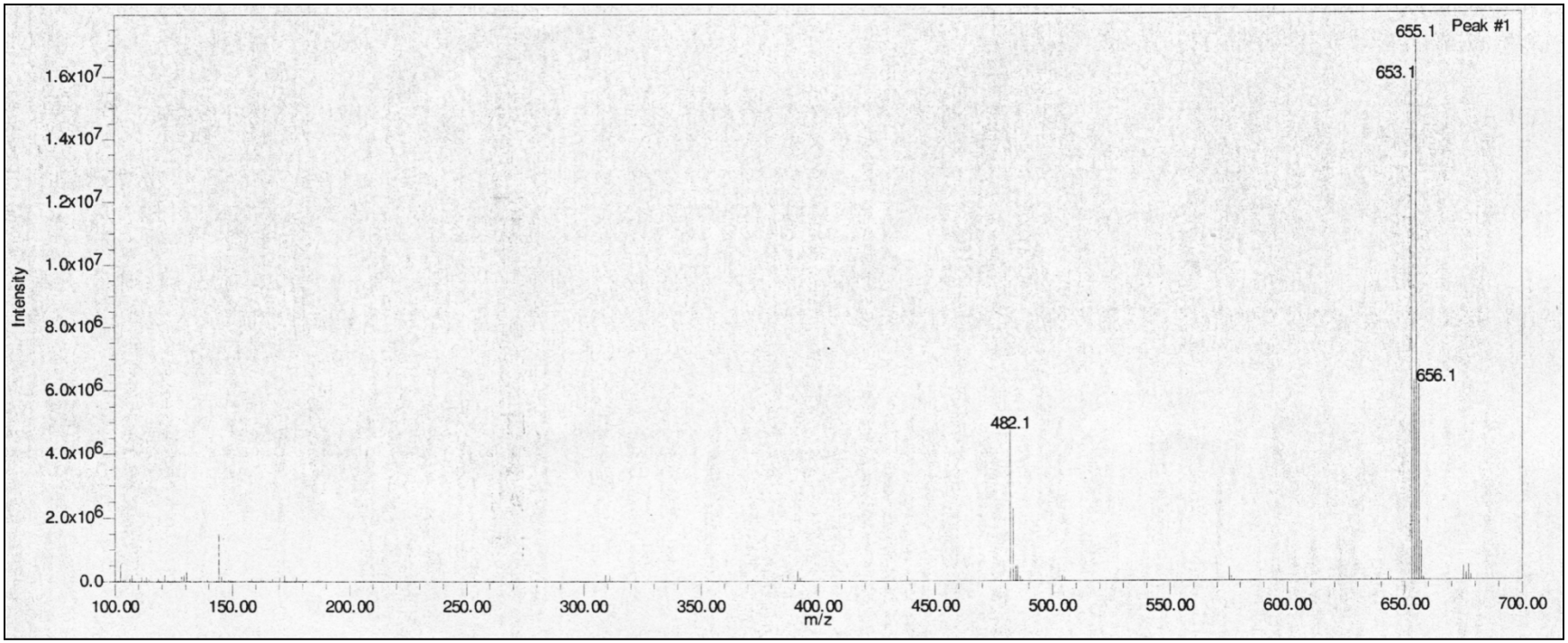

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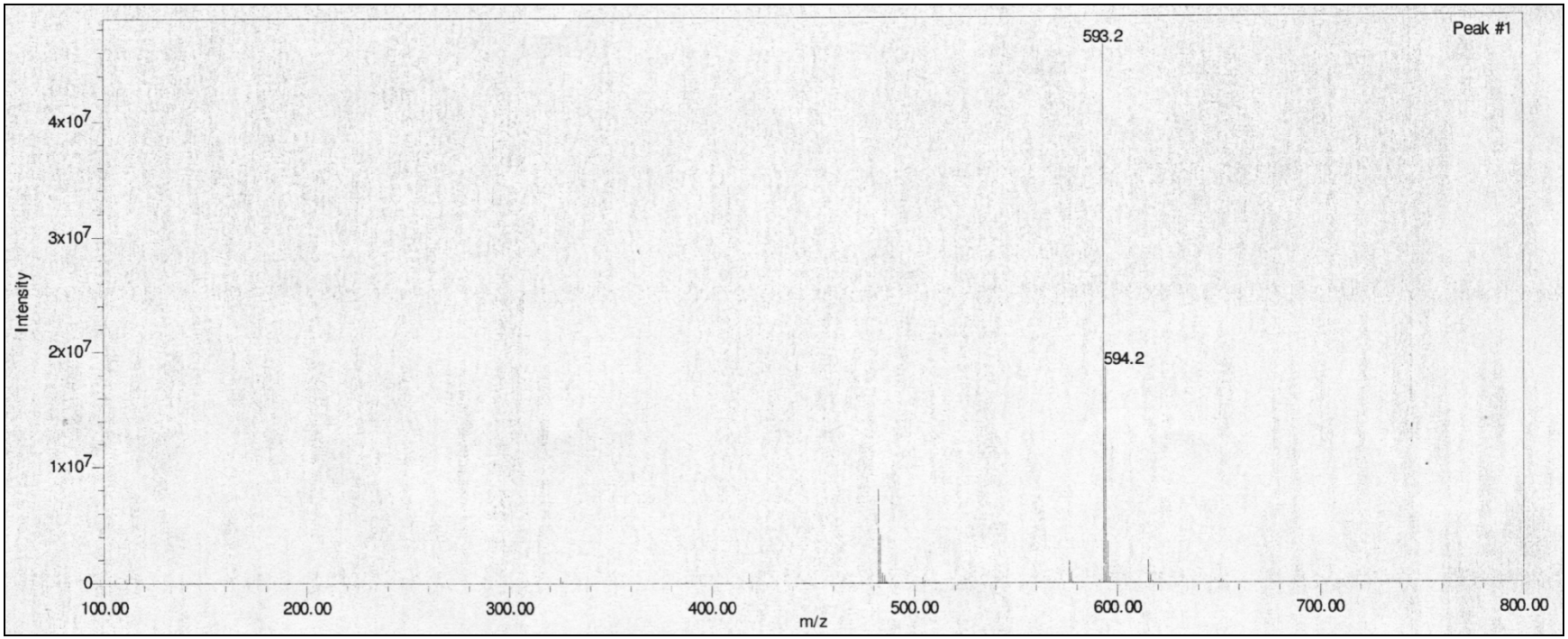
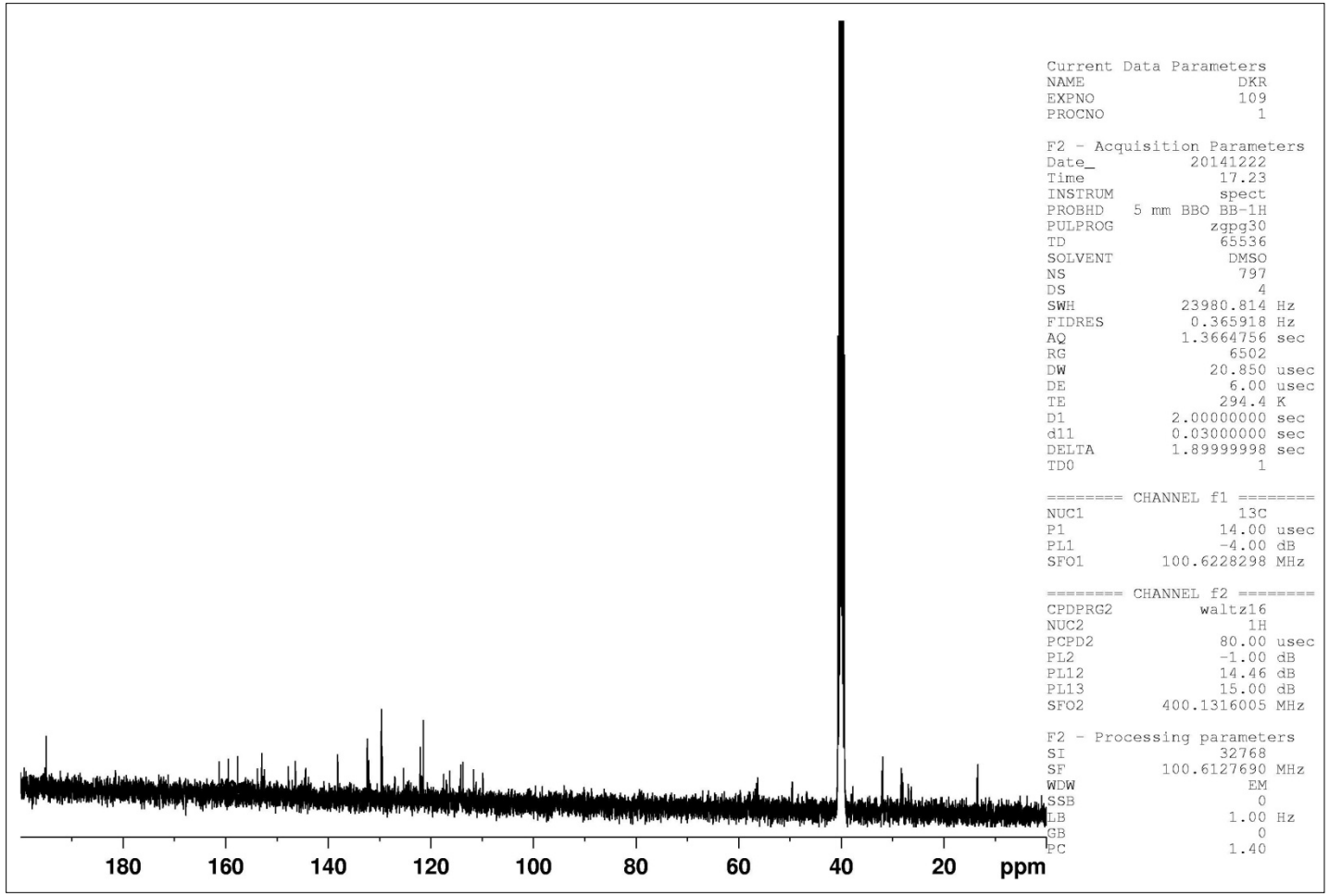


^{13}C NMR spectra of compound **8a**



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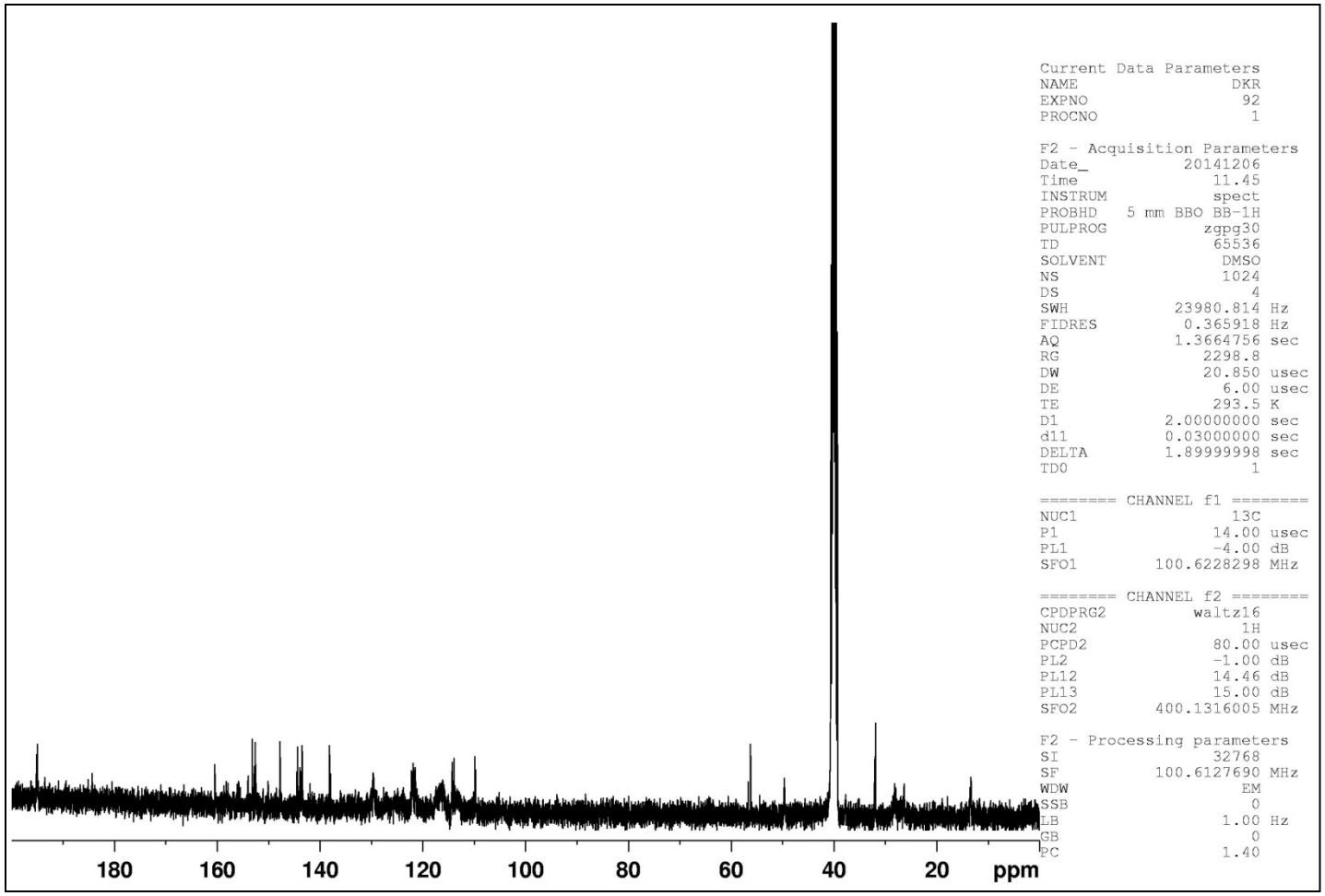

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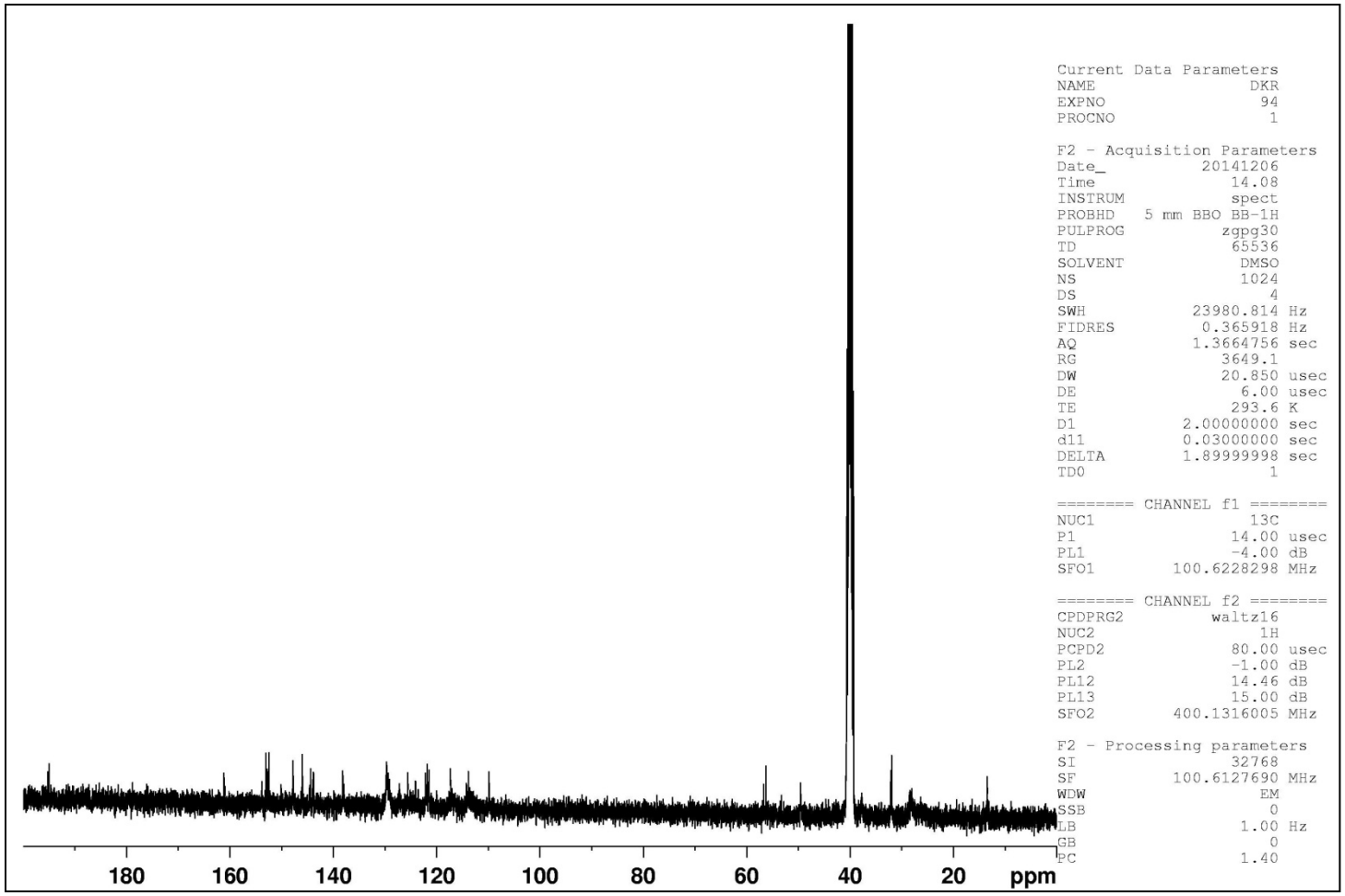

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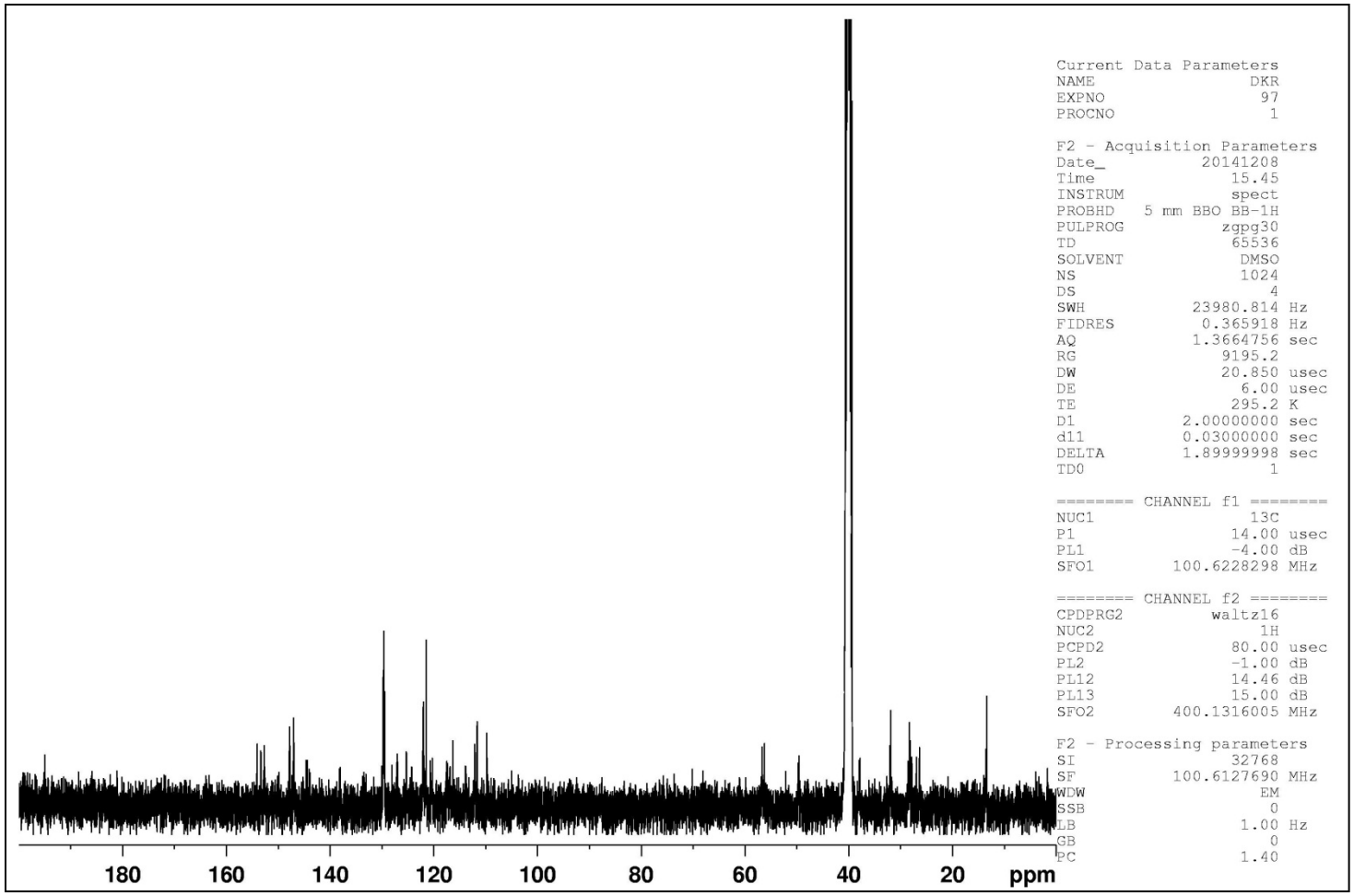

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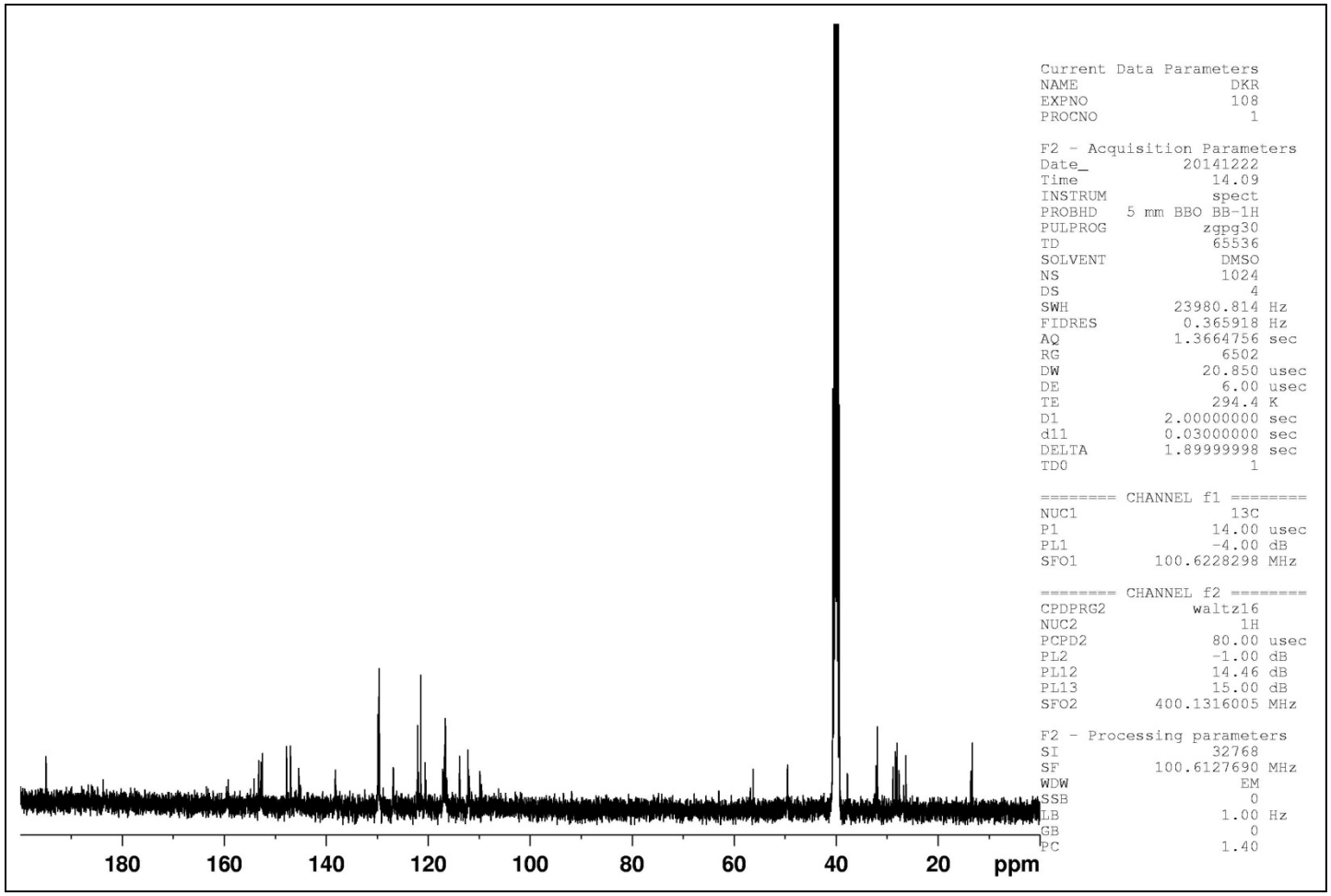

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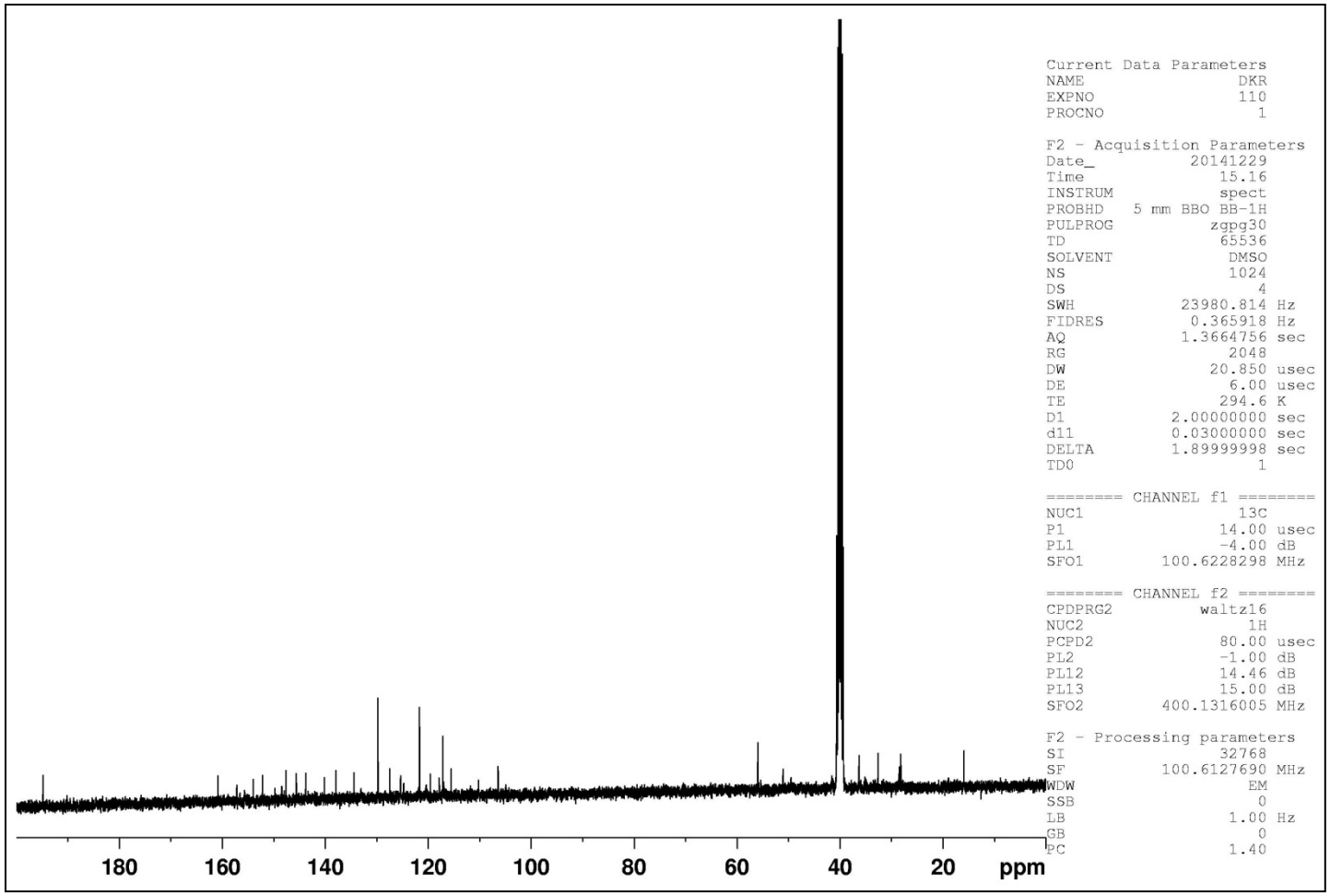

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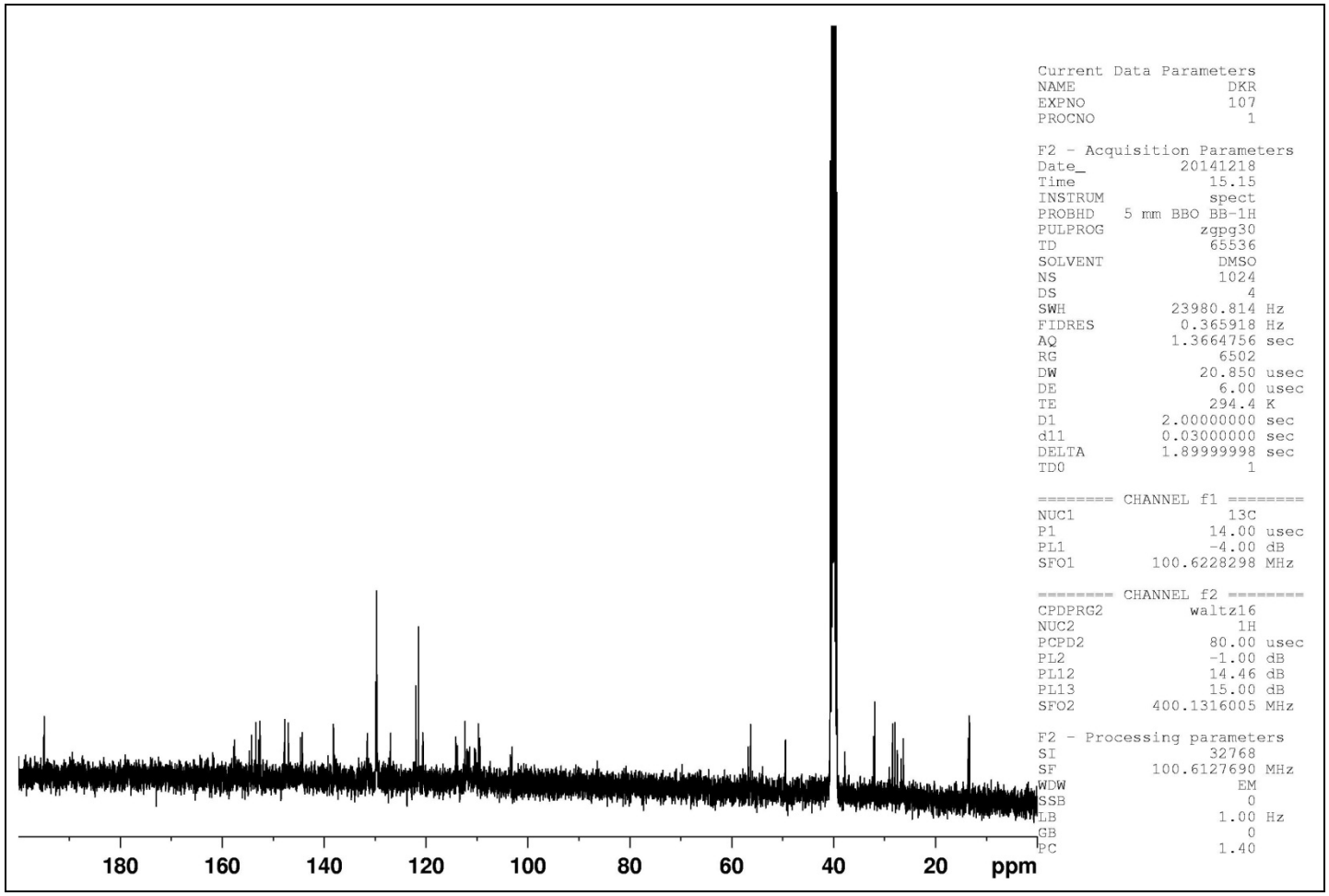

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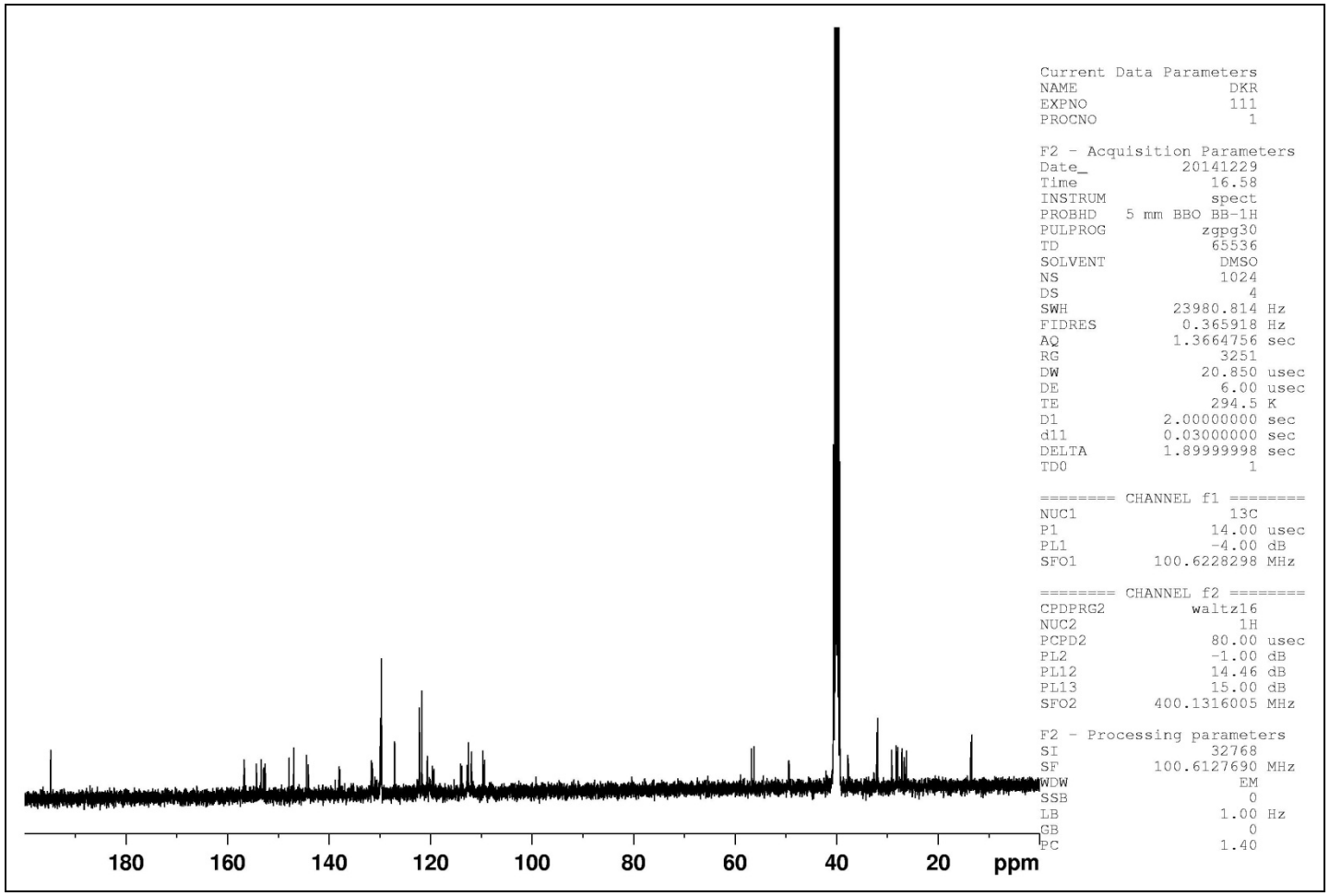

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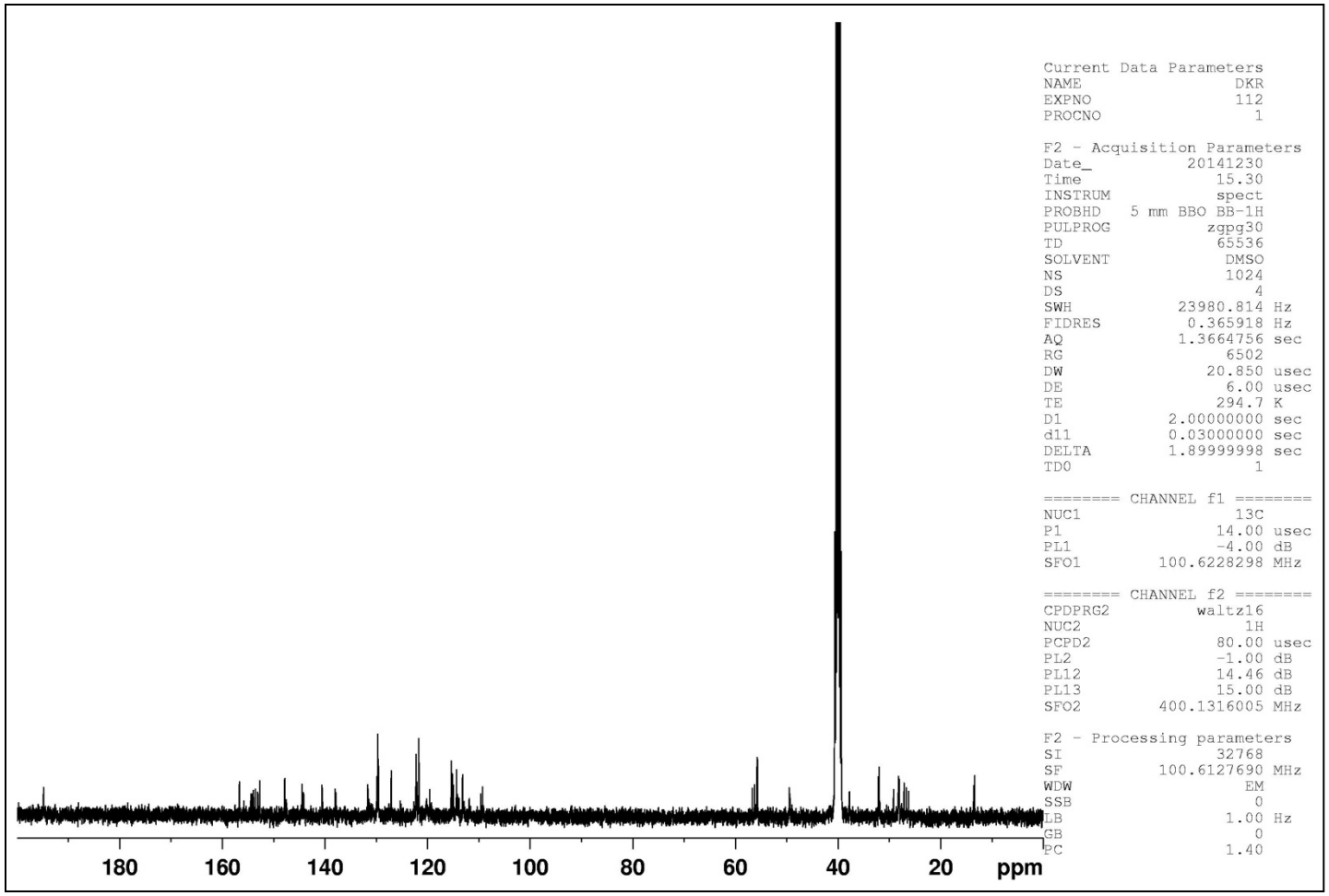

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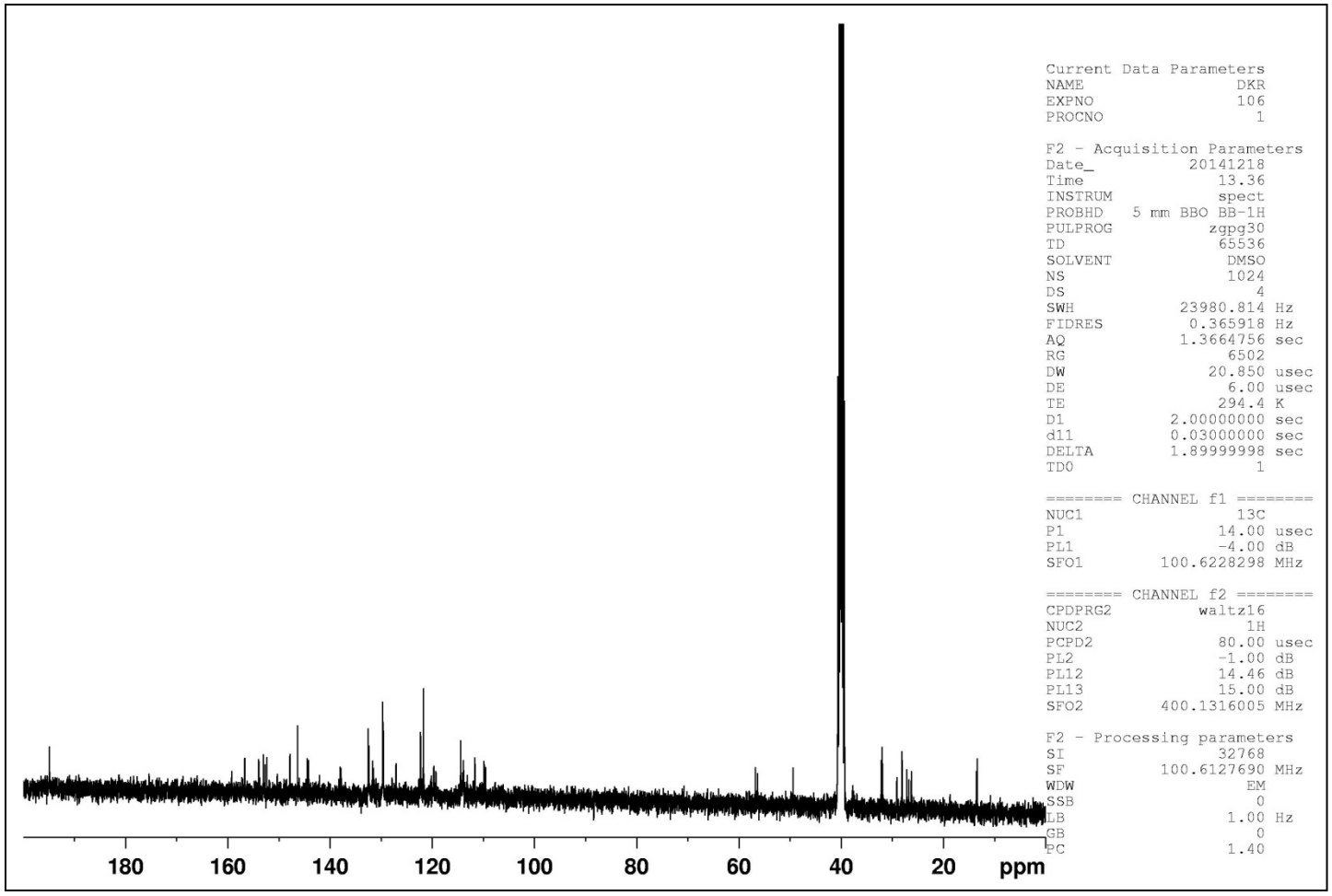

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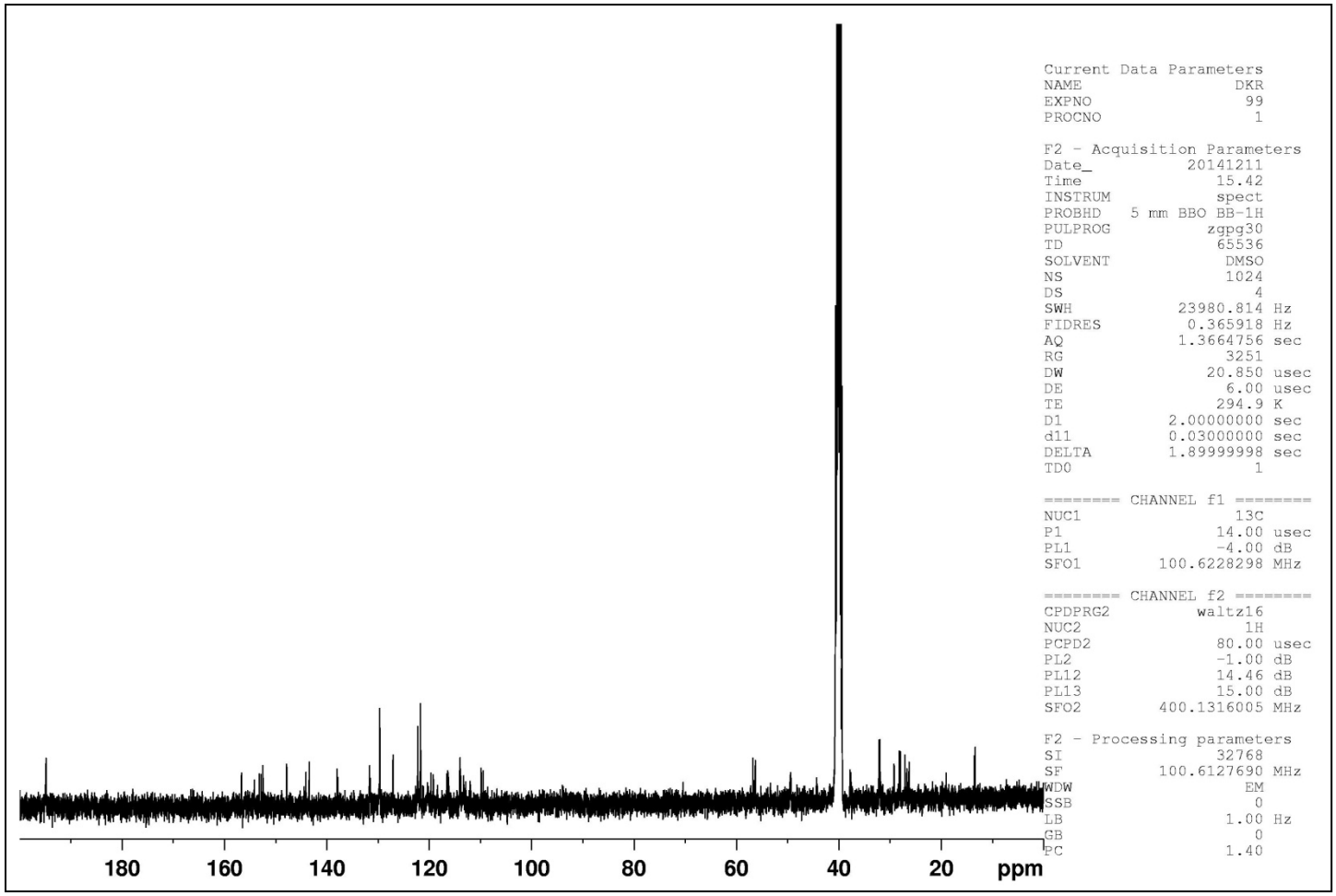

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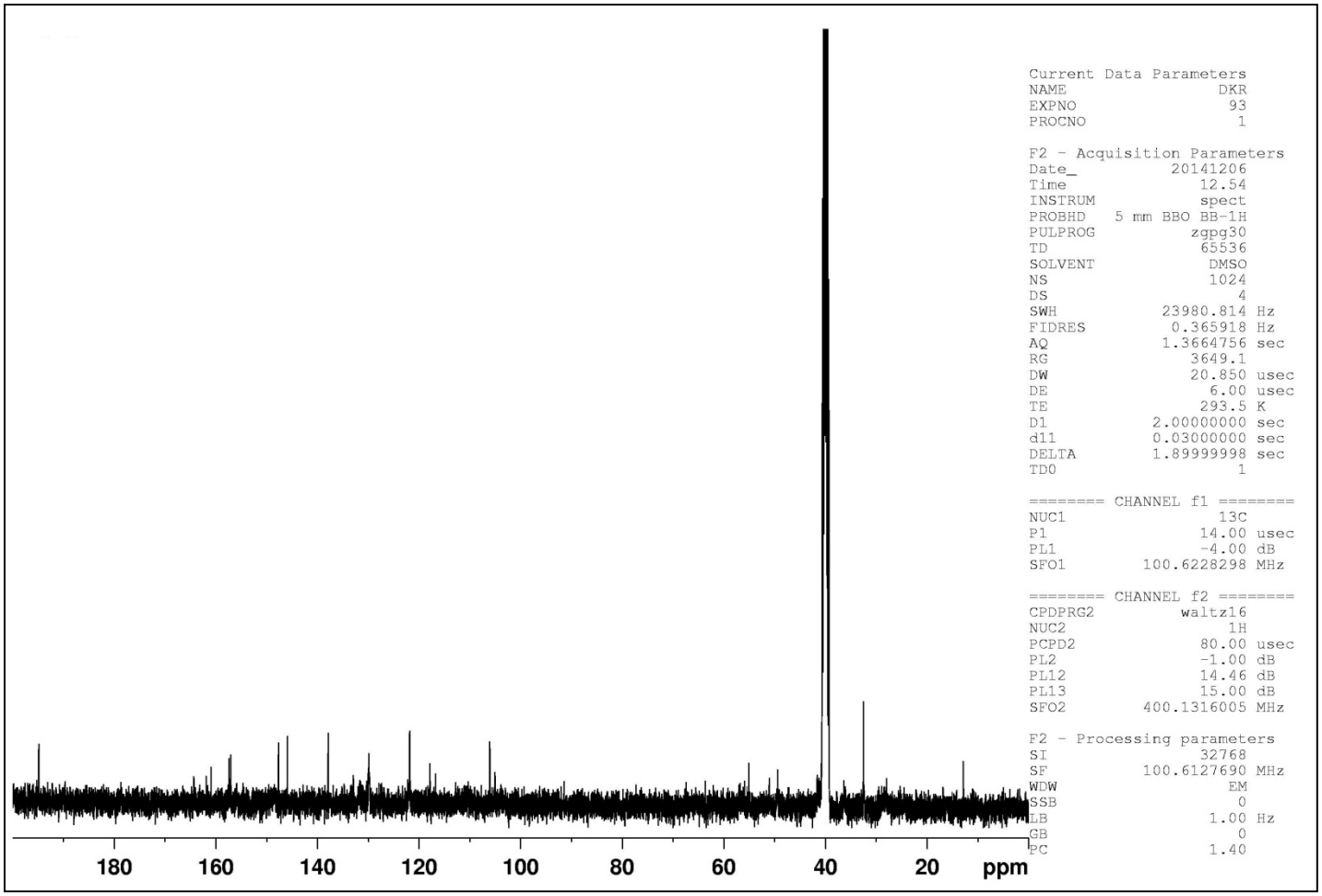

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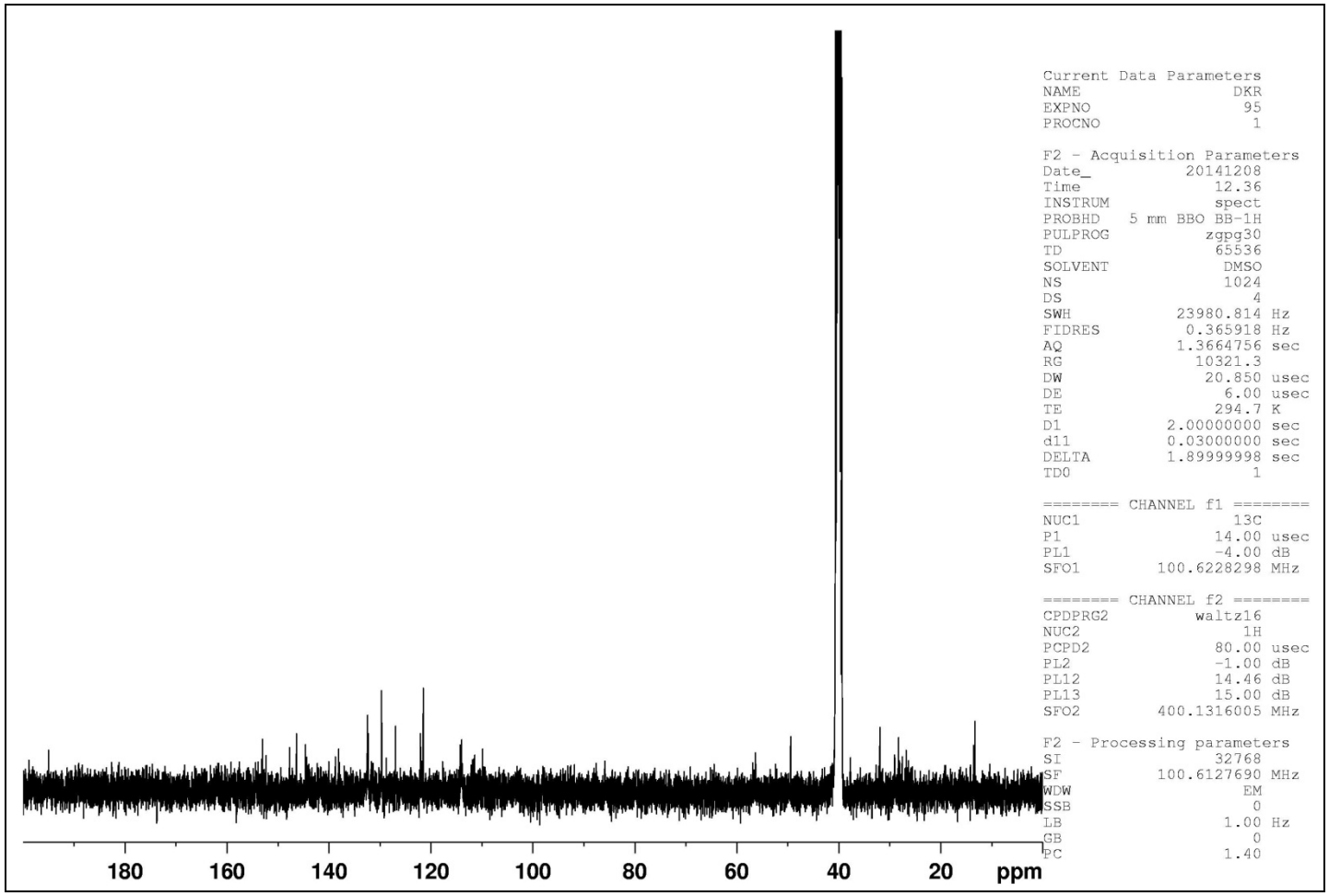

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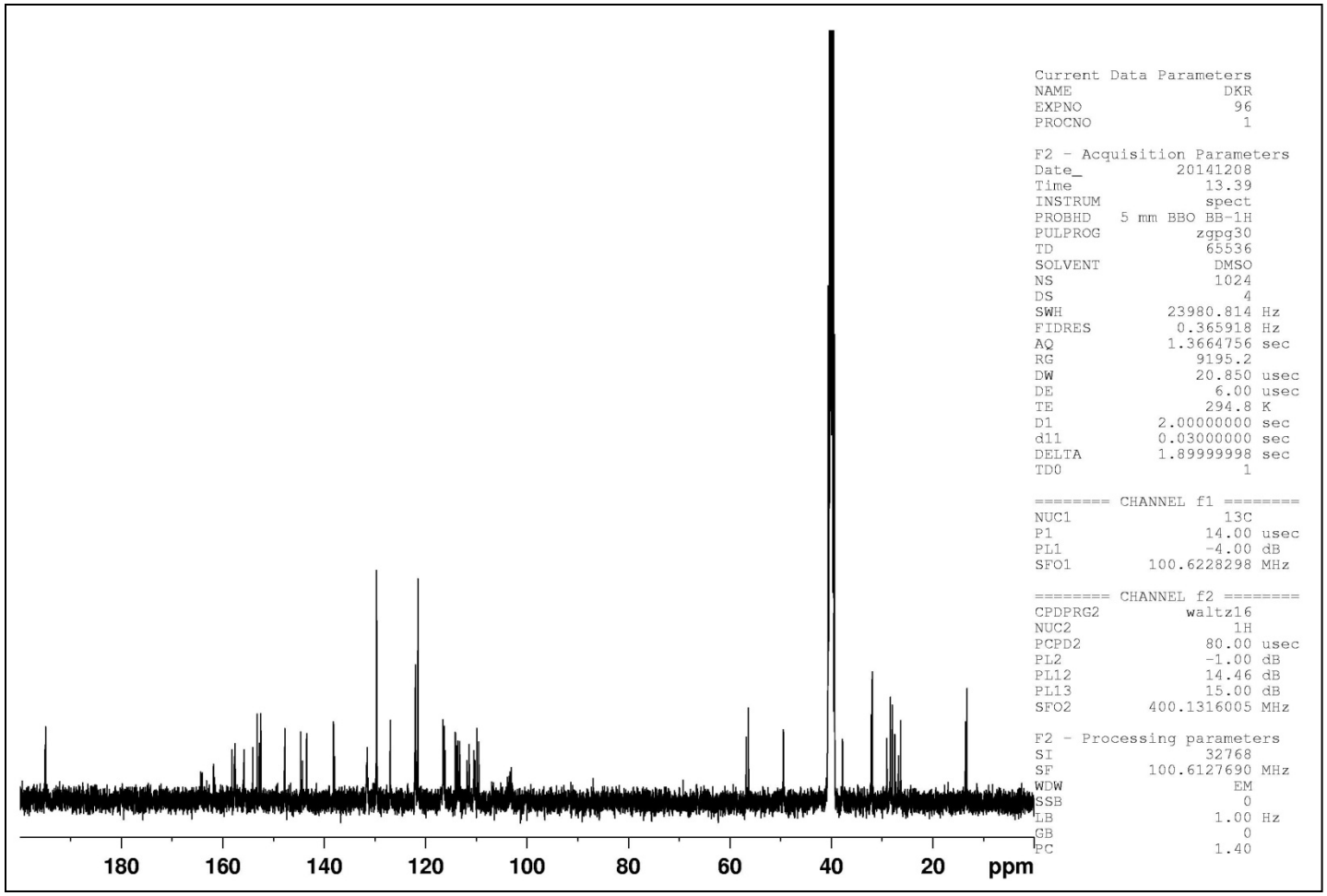

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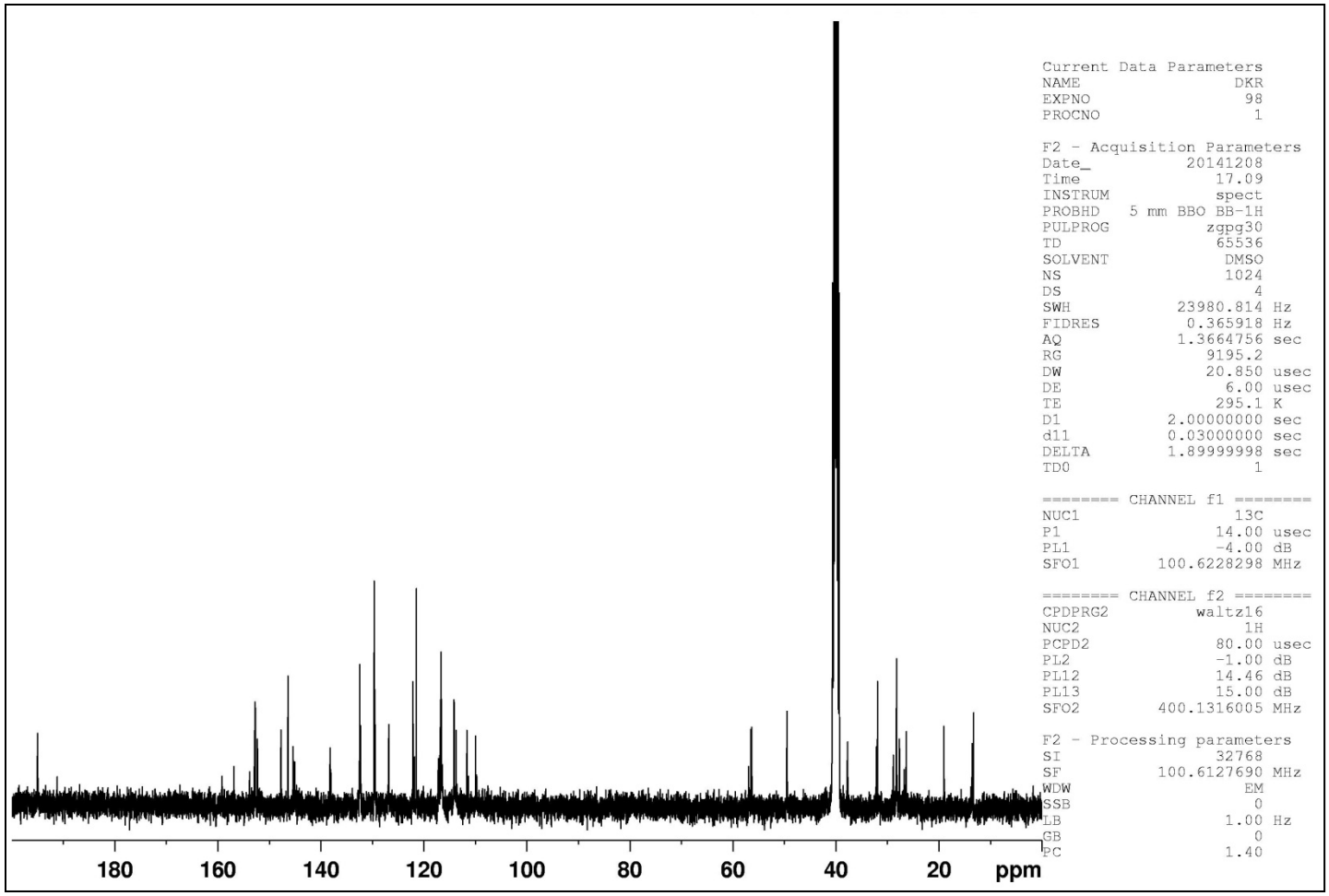

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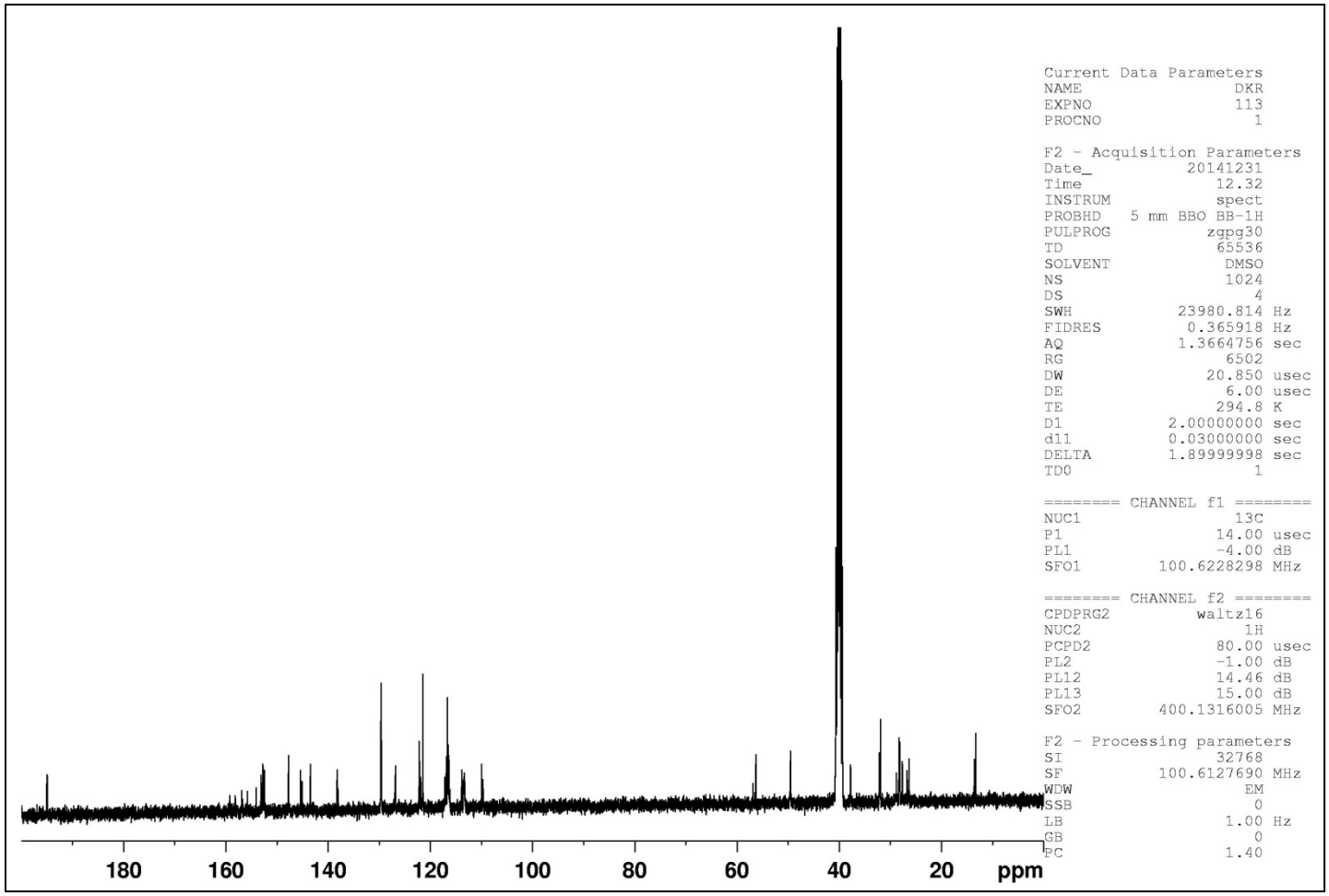

1. Biological evaluation

1.1. *In vitro* antimicrobial assay

The antimicrobial activity of newly synthesized compounds **8a-p** was carried out by broth micro dilution method. DMSO was used as the diluent to get desired concentration of compounds to test upon standard bacterial strains. Mueller – Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria. Sabouraud Dextrose broth was used for fungal nutrition. Inoculum size for test strain was adjusted to 10^8 CFU mL^{-1} by comparing the turbidity. Serial dilutions were prepared in primary and secondary screening. Each synthesized compound and the standard drugs were diluted obtaining 2000 µg/mL concentration as the stock solution. The drugs which were found to be active in primary screening (i.e. 500, 250 and 200 µg/mL concentrations) were further screened in their second set of dilution at 100, 50, 25 and 12.5 µg/mL concentration against all microorganisms. 10 micro liter suspensions were further inoculated on appropriate media and growth was noted after 24 and 48 h. The control tube containing no antibiotic was instantaneously subcultured (before inoculation) by evenly spreading a loopful over an area of medium in a plate suitable for growth of the test organism. The tubes were then put overnight for incubation at 37°C. The highest dilution preventing appearance of turbidity after spot subculture was considered as minimal inhibitory concentration (MIC, µg/mL). All the tubes showing no visible growth (same as control tube) were subcultured and incubated overnight at 37°C. The amount of growth from the control tube before incubation was compared. In this study ampicillin, norfloxacin and chloramphenicol were used as the standard antibacterial drugs. Nystatin and Griseofulvin were used as the standard antifungal drugs.

1.2. *In vitro* antituberculosis assay

All the synthesized compounds **8a-p** were screened for their antitubercular activity against *Mycobacterium tuberculosis* H37Rv by Lowensteine-Jensen method with minor modification where 250 µg/mL dilution of each compound was added to Lowensteine-Jensen medium and then media was uncontaminated by inspissation method. A culture of *Mycobacterium tuberculosis* H37Rv growing on Lowensteine-Jensen medium was harvested in 0.85% saline in bijou bottle. The stock solutions of the title compounds were prepared

in DMSO i.e. 250 µg/mL. These tubes were then incubated at 37 °C for 24 h followed by streaking of *Mycobacterium tuberculosis* H37Rv (5×10^4 bacilli per tube). The growth of bacilli was observed after 2 weeks, 3 weeks and finally after 4 weeks of incubation. The tubes having the compounds were compared with control tubes where medium alone was incubated with *Mycobacterium tuberculosis* H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of the tested compound. The standard strain *Mycobacterium tuberculosis* H37Rv was also tested with known drug isoniazid and rifampicin for comparison purpose.

1.3. *In vitro* antimalarial assay

In vitro antimalarial activity of the synthesized compounds **8a-p** was screened against *P. falciparum* strain. The *P. falciparum* strain was acquired from Shree R. B Shah Mahavir Super-speciality hospital, Surat, Gujarat, India and was used in the *in vitro* tests. The *P. falciparum* strains were cultivated by a modified method described by Trager and Jensen.[43] Compounds were dissolved in DMSO. The final concentration of DMSO used was not toxic and did not interfere with the assay. The antiparasitic effect of the compounds was measured by growth inhibition percentage as described by Carvalho and Krettli.[44] For experimental purposes, the cultures were synchronized with 5% D-sorbitol when the parasites were in the ring stage.[45] The parasite suspension, predominately in the ring stage, was adjusted to a 1-2 % parasitaemia and 2.5 % haematocrit in hypoxanthine-free RPMI-1640 culture medium with 10% human plasma and was exposed to 7 concentrations of each compound for a single cycle of parasitic growth for 48 h at 37 °C. A positive control with reference to antimalarial drugs in standard concentrations was used for each experiment. The stock solutions were additionally diluted in whole medium (RPMI 1640 plus 10% human serum) to each of the used concentrations. The concentration that inhibited 50% of parasite growth (IC_{50} value) was determined by interpolation using Microcal Origin software. The standard drugs chloroquine and quinine were used as the reference antimalarial agents, blood smears were read blind and each duplicate experiment was repeated three times.

1.4 Cytotoxicity

MATERIALS AND METHODS

Schizosaccharomyces pombe

S. pombe Var. Paul Linder 3360 was obtained from IMTECH, Chandigarh. It was maintained on yeast extract:glucose medium having composition 30:5 gm.l^{-1}. [46]

Bioassay

In vitro bioassay was performed using *S. pombe* cells. *S. pombe* has become an important tool to study cell biology due to its eukaryotic and fairly big size characteristics. *S. pombe* cells were grown in 50 ml liquid yeast extract media in 150 ml Erlenmeyer flask. Flask was incubated at 30ºC on shaker at 150 rpm till the exponential growth of *S. pombe* was obtained (24 to 30 hrs). Then the cell culture was treated with the different concentrations (2.5, 5, 7.5, 10, 12.5 µg/ml) of synthesized compounds abbreviated as 8C, 8M, 8E, 8I, 8J, 8K, 8L (**Table 5**) and with Dimethylsulphoxide (DMSO) as the control. It was further allowed to grow for 16-18 hrs. Next day, by centrifugation at 10,000 rpm for 10 min; treated cells were collected and dissolved in 500 µl of phosphate buffer saline solution (PBS). 80 µl of yeast culture dissolved in PBS and 20 µl of 0.4 % trypan blue prepared in PBS were mixed and cells were observed in a compound microscope (40X). Live cells resisted the entry of dye whereas dead cells appeared blue. The number of dead cells and number of live cells were counted in one field. Cell counting was repeated in two more of the microscopic fields and percentage of cells died due to synthesized compounds was averaged out.[47]

References

43. W. Trager and J. Jensen, *Science*, 1976, **193**, 673.
44. A. U. K. L.H. Carvalho, Mem. Inst. Oswaldo. , *Cruz. 86 (Suppl. II)*, 181.
45. J. P. v. d. B. C. Lambros, J., *Parasitol.* , (1979), **65** 418.
46. Pinaki B. Patel and Vasudev Thakkar Cell proliferation and DNA damage study by SCGE in fission yeast exposed to curcumin and 5-fluorouracil. *Asian Journal of Cell Biology* (2013), **8** (1): 22-32.

47. Vasudev R. Thakkar, Disha D. Patel, Pinaki B. Patel, Ravi R. Patel and Vaidehi J. Oza (2012). Detection and isolation of a novel toxic metabolite from a plant pathogenic fungus, Lasiodiplodia theobromae. *PRAJÑÂ - Journal of Pure and Applied Sciences* (2012), **20** 15 – 18.