Electronic Supplementary Material (ESI) for New Journal of Chemistry

Synthesis, characterization and biological screening of novel 5-imidazopyrazole incorporated fused pyran motifs

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Supporting information

1. Experimental part of biological evaluation

1.1. In vitro antimicrobial assay

The *in vitro* antimicrobial activity of all synthesized fused pyran derivatives **6a-x** was carried out by broth microdilution method. 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. DMSO was used as the diluent to get desired concentration of compounds to test upon standard bacterial strains. 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 a 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 1 and 2 days. The control tube containing no antibiotic was instantaneously subcultured (before inoculation) by spreading a loopful evenly over an area of plate of medium suitable for the growth of the test organism. The tubes were then put for incubation at 37°C overnight. The highest dilution preventing

appearance of turbidity after spot subculture was considered as minimal inhibitory concentration (MIC, µ/L). 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 standard antifungal drugs. The results are summarized in Table 2.

1.2. In vitro antituberculosis assay

The antitubercular activity of all synthesized compound against *Mycobacterium tuberculosis* H37Rv was performed by Lowensteine-Jensen method [30] with minor modification where 250 µg/mL and 100 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 all compounds were prepared in DMSO i.e. 250 µg/mL and 100 µg/mL. These tubes were then incubated at 37 °C for 24 h followed by streaking of *Mycobacterium tuberculosis* H37Rv (5 _ 104 bacilli per tube). The growth of bacilli was seen after 14 days, 21 days and finally after 28 days 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 tested with known drug isoniazid and rifampicin for comparison purpose. The results are summarized in Table 3.

1.3. In vitro antimalarial assay

All the synthesized fused pyran derivatives **6a-x** were screened for their antimalarial activity against the *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 vitro tests. The *P. falciparum* strains were cultivated by a modified method described by Trager and Jensen [1]. 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 [2]. For experimental purposes, the cultures were synchronized with 5% D-sorbitol when the parasites were in the ring stage [3]. The parasite suspension, consisting of predominately 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 parasite growth of 48 h at 37 °C. A positive control with reference to antimalarial drugs in standard concentrations was used in 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. The results are summarized in Table 4.

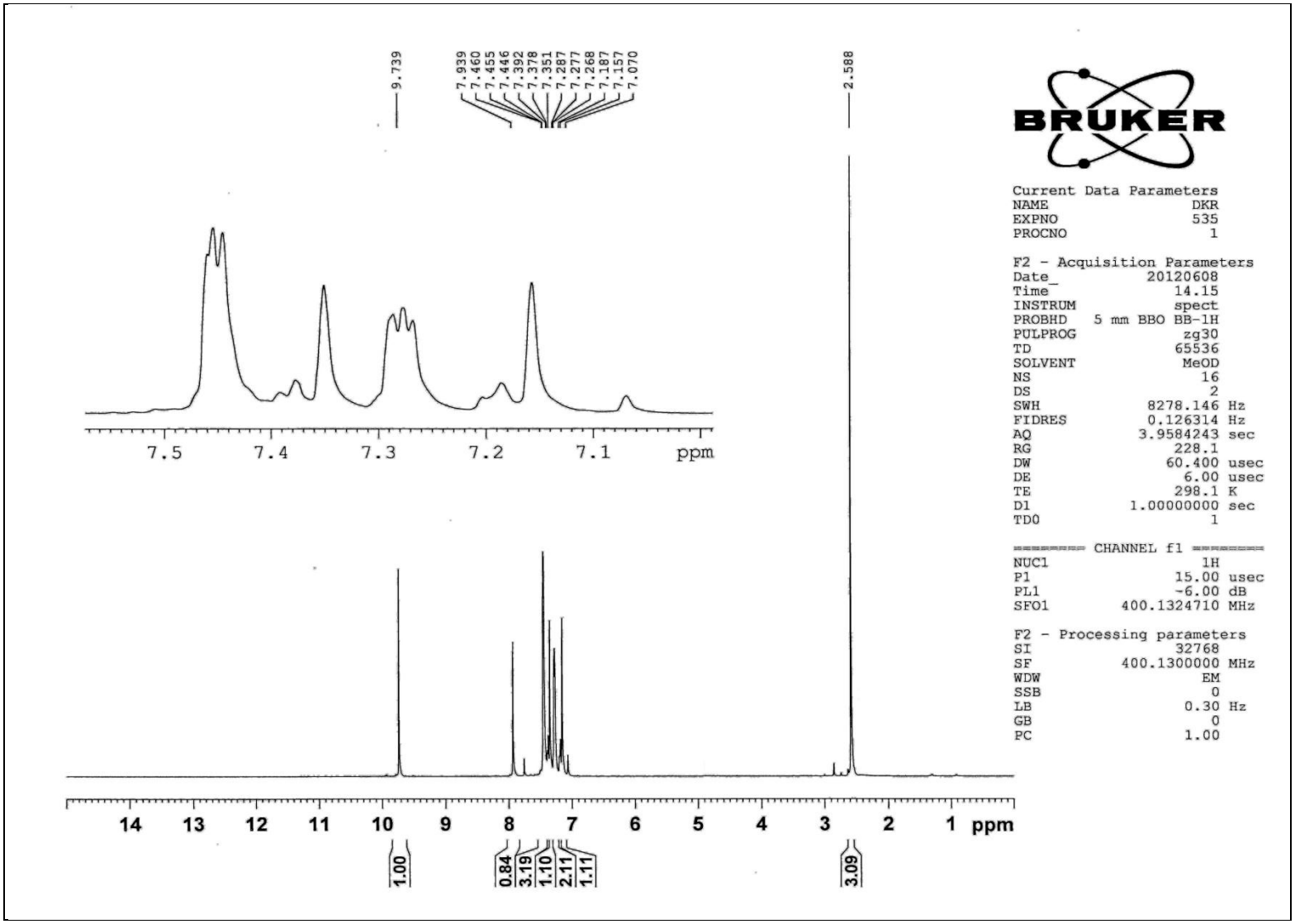
References

[1] W. Trager, J.B. Jensen, *Science,* 1976, 193, 673.

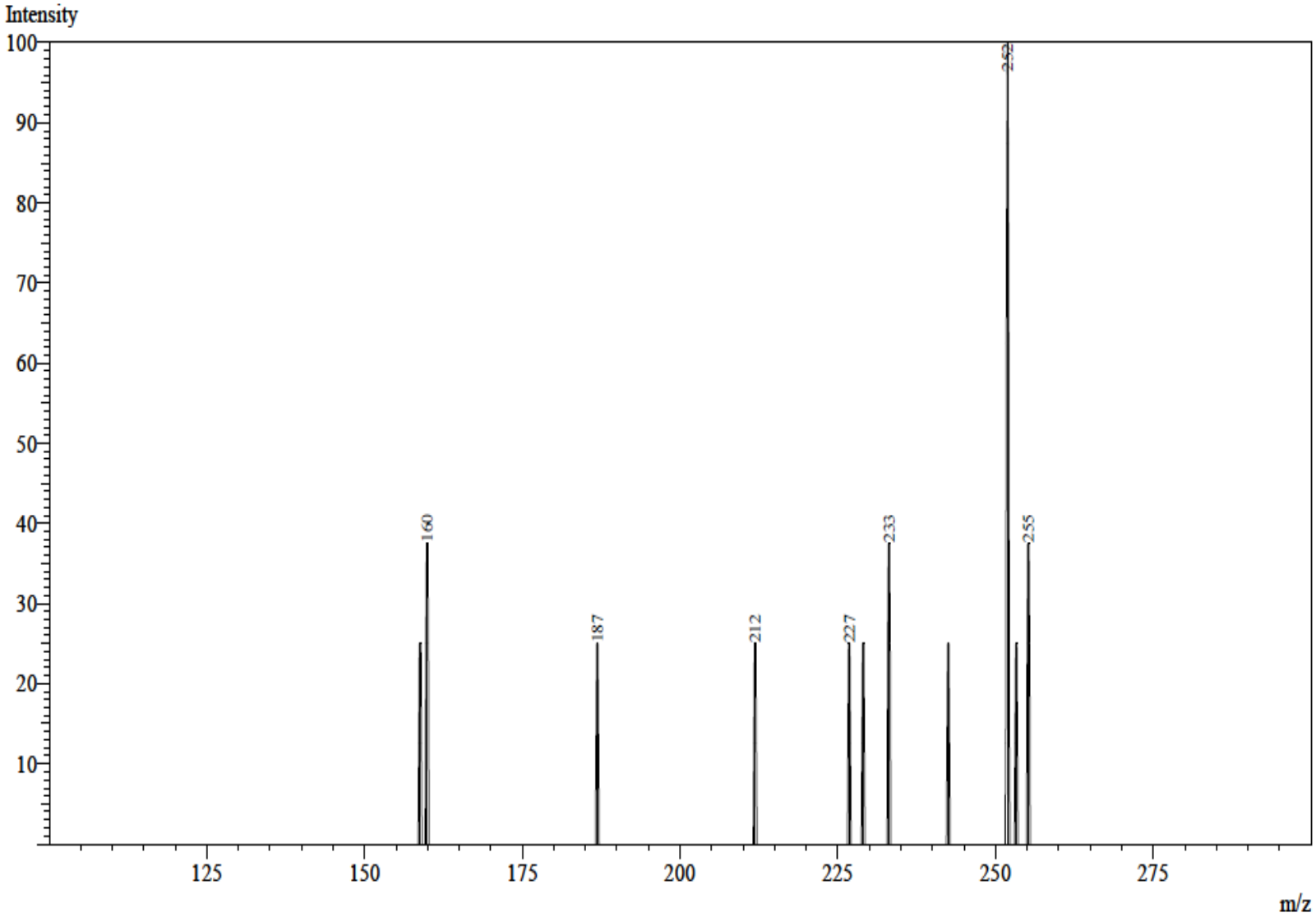
[2] L.H. Carvalho, A.U. Krettli, *Mem. Inst. Oswaldo. Cruz. (Suppl. II)* 86, 181.

[3] C. Lambros, J.P. van der Berg, J. Parasitol. 1979, 65, 418.

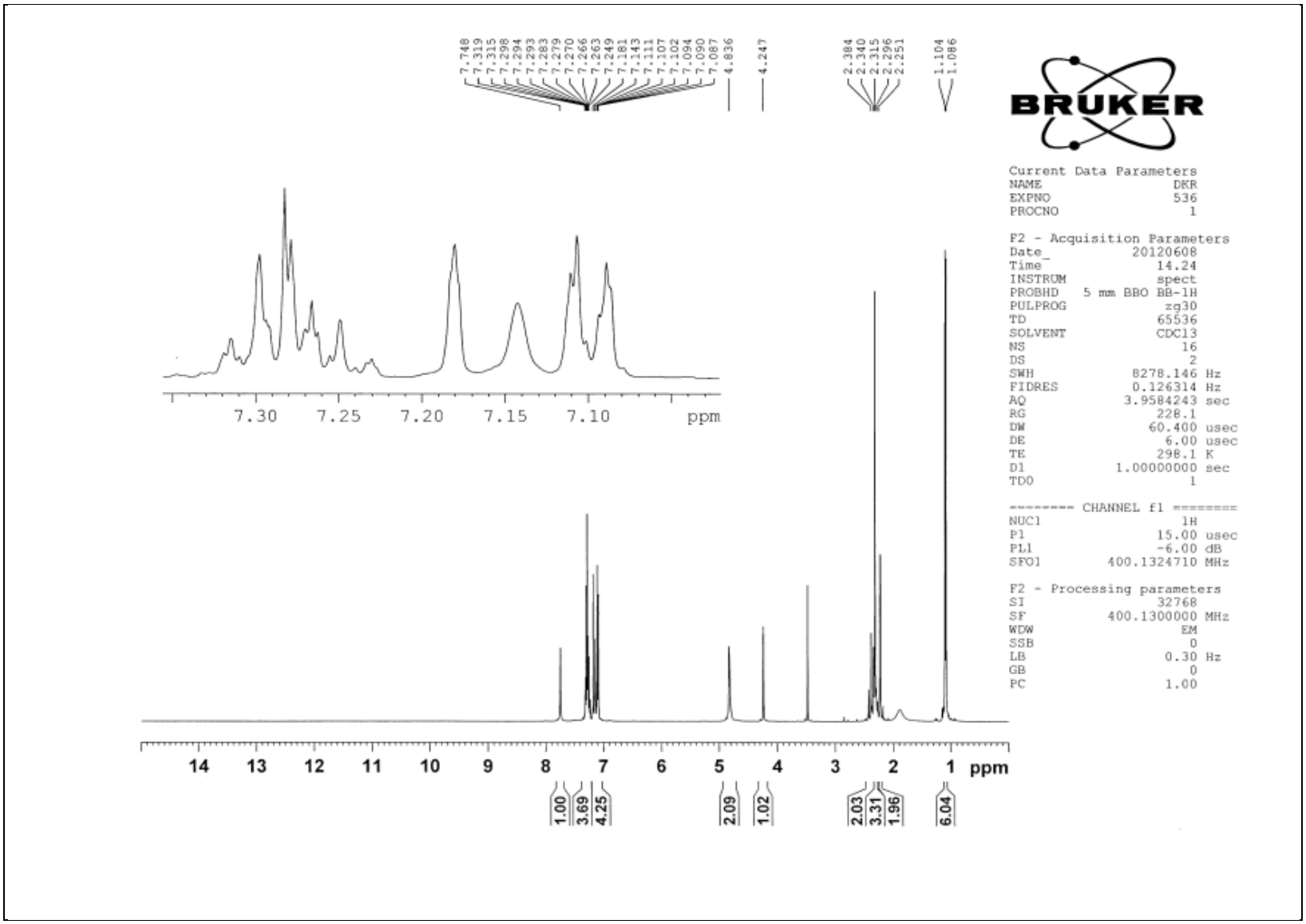
NMR spectra of compound **3**



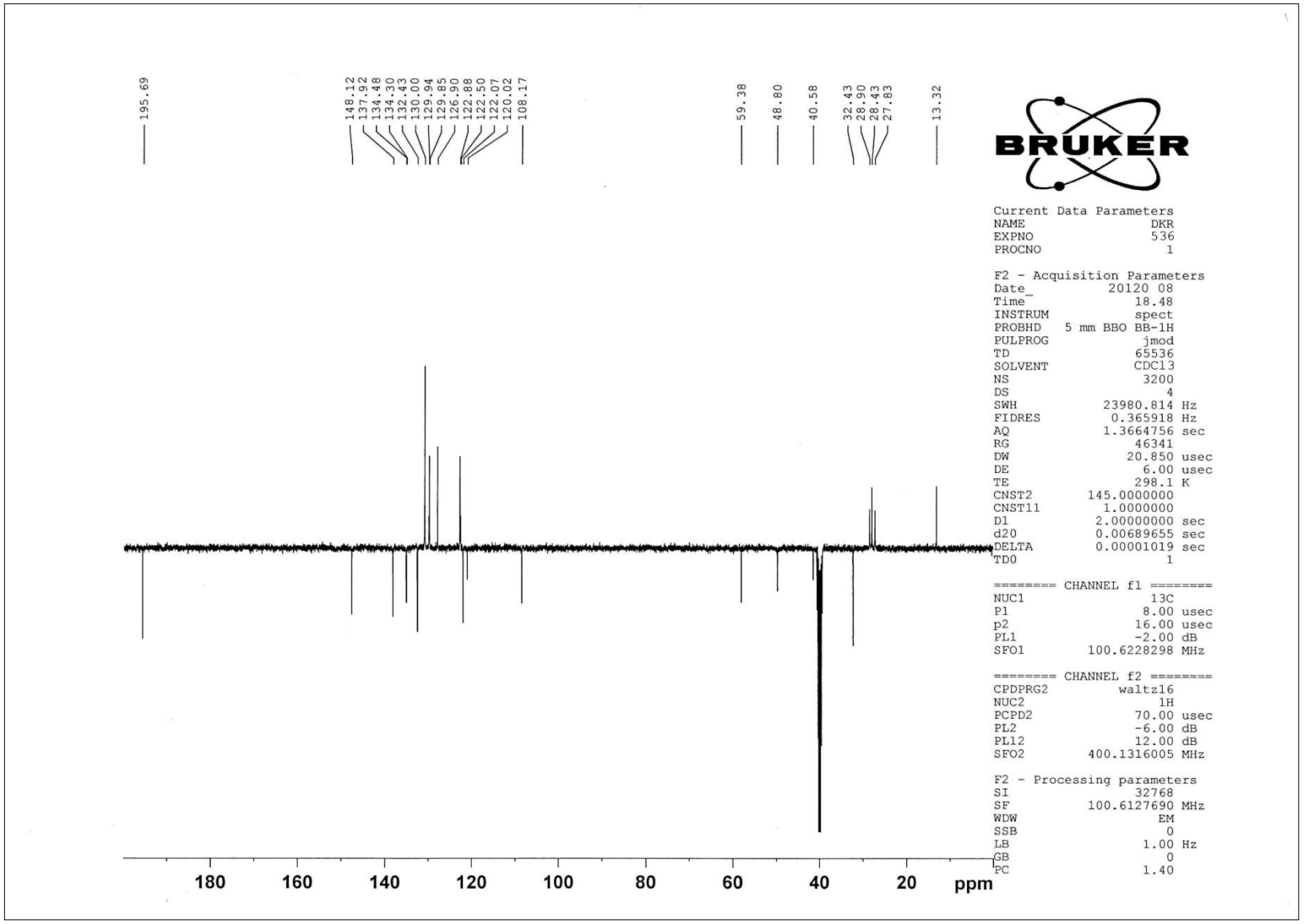
Mass spectra of compound **3**



NMR spectra of compound **6a**



CMR spectra of compound **6a**



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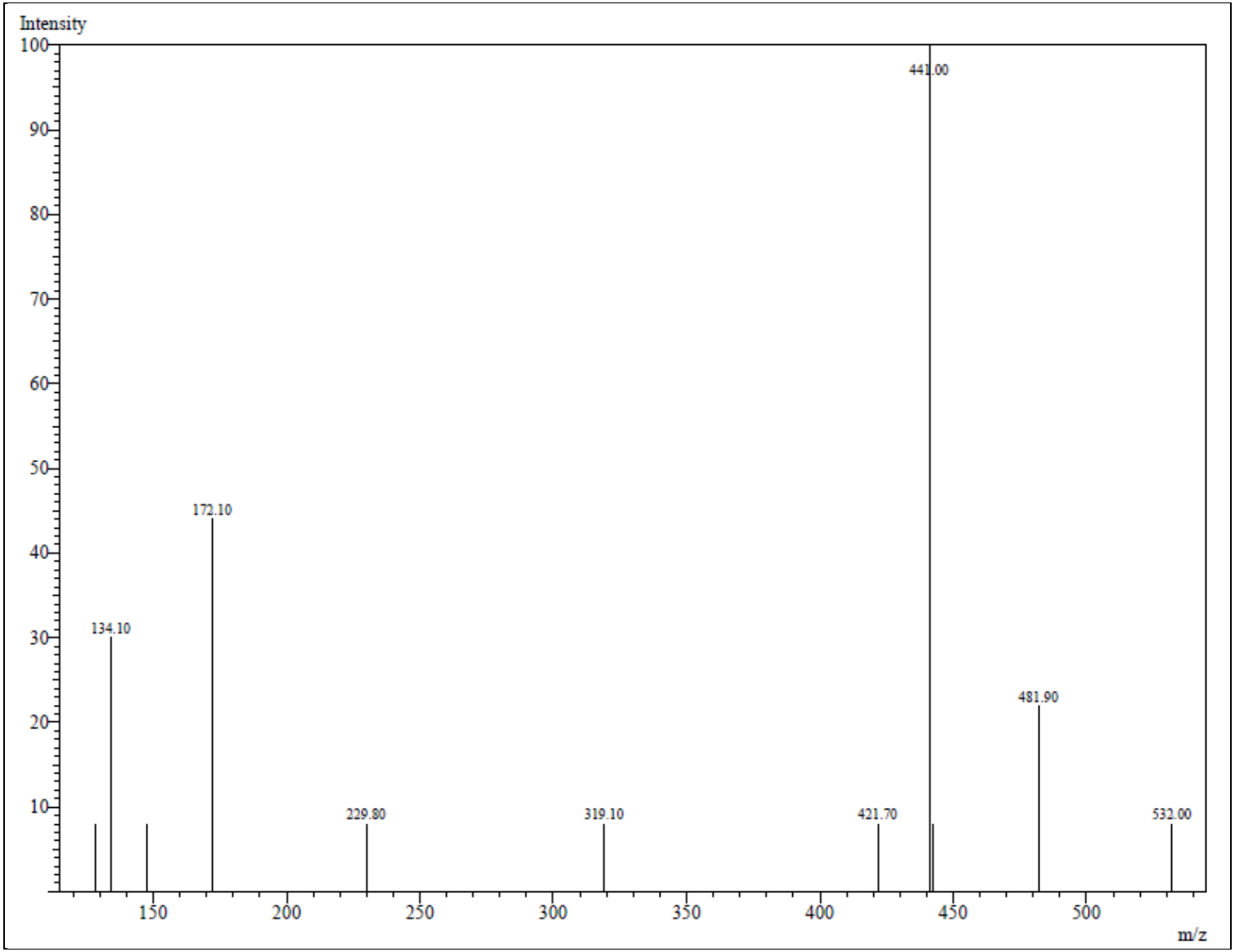

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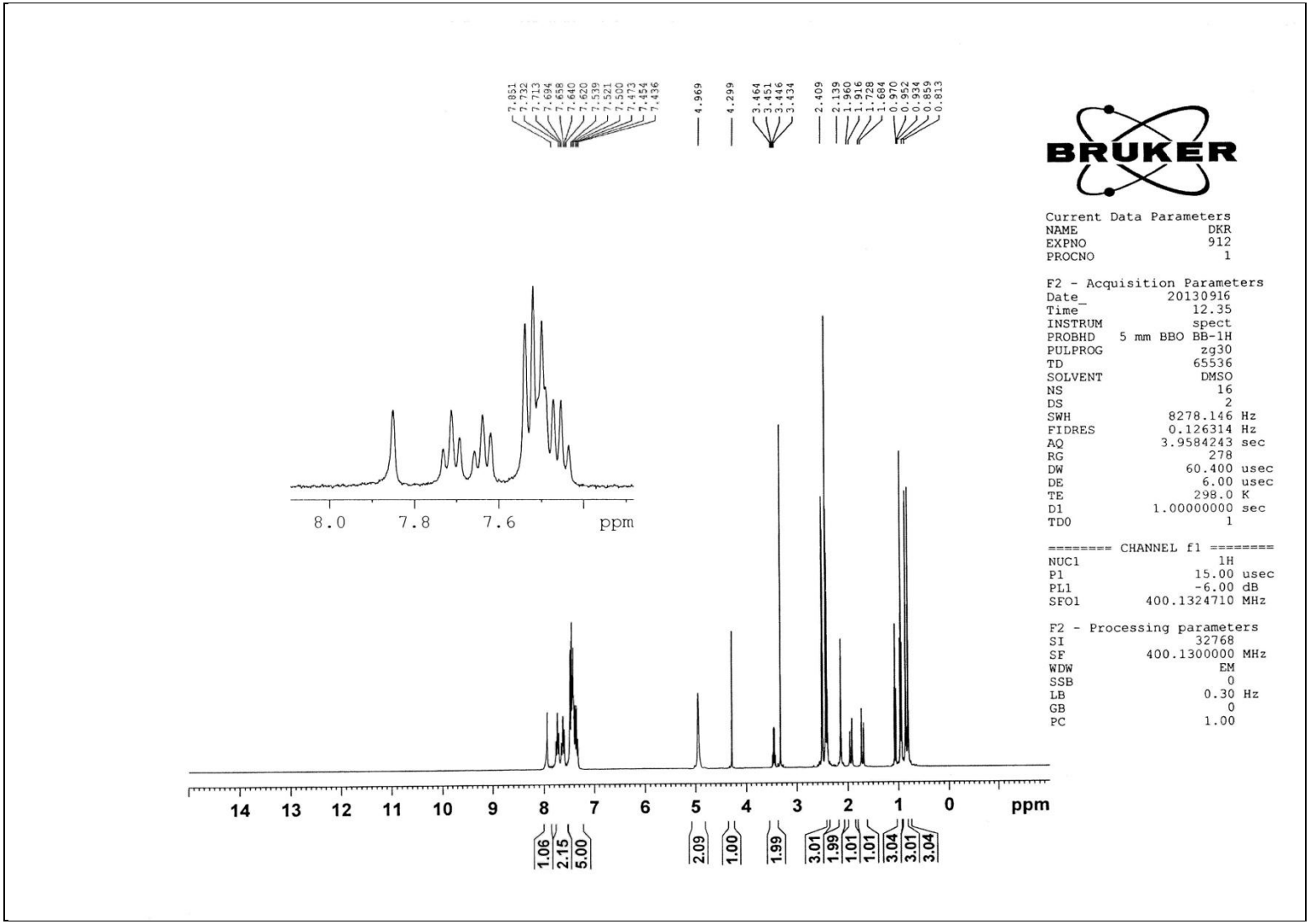

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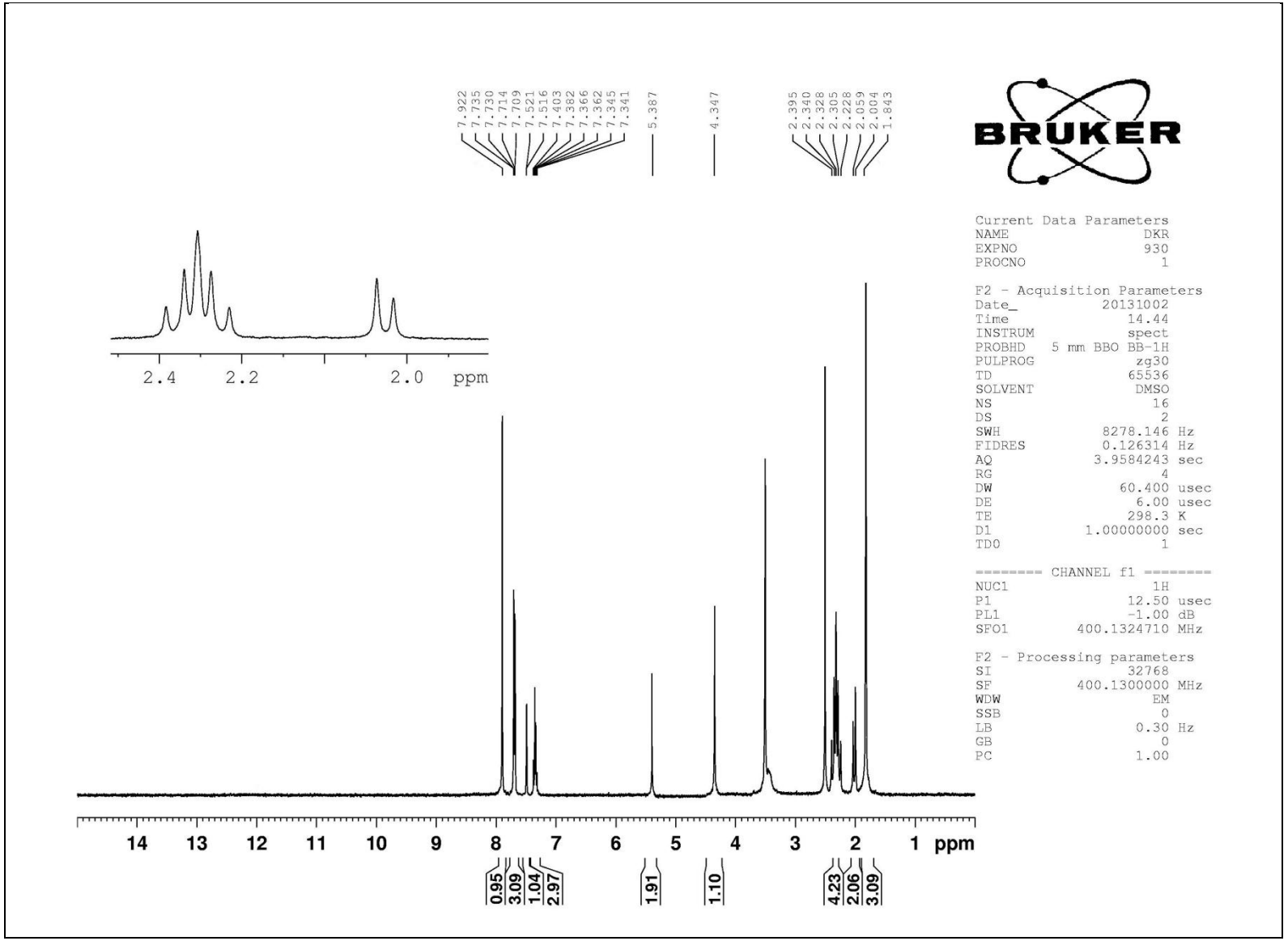

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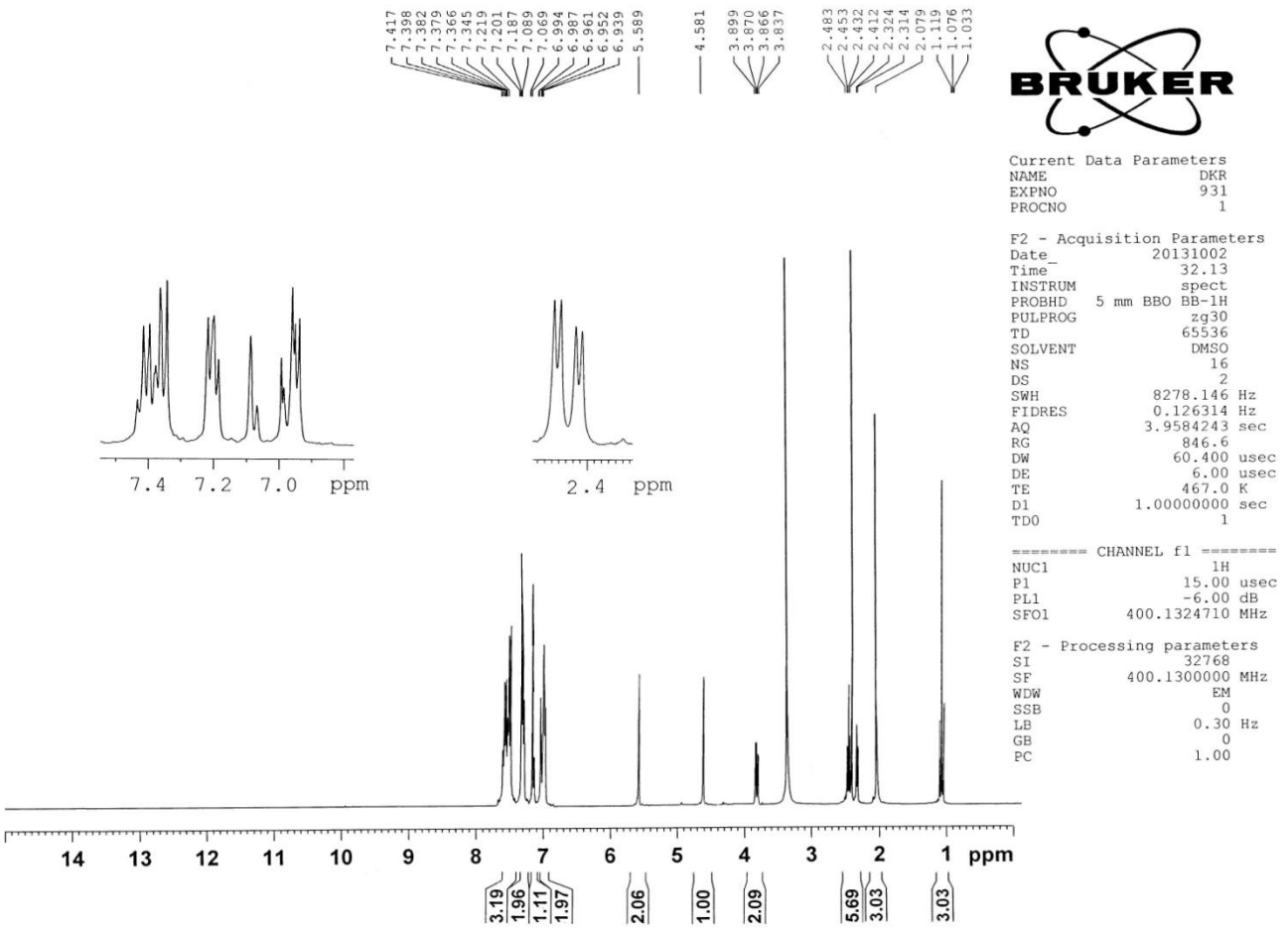

BRUKER

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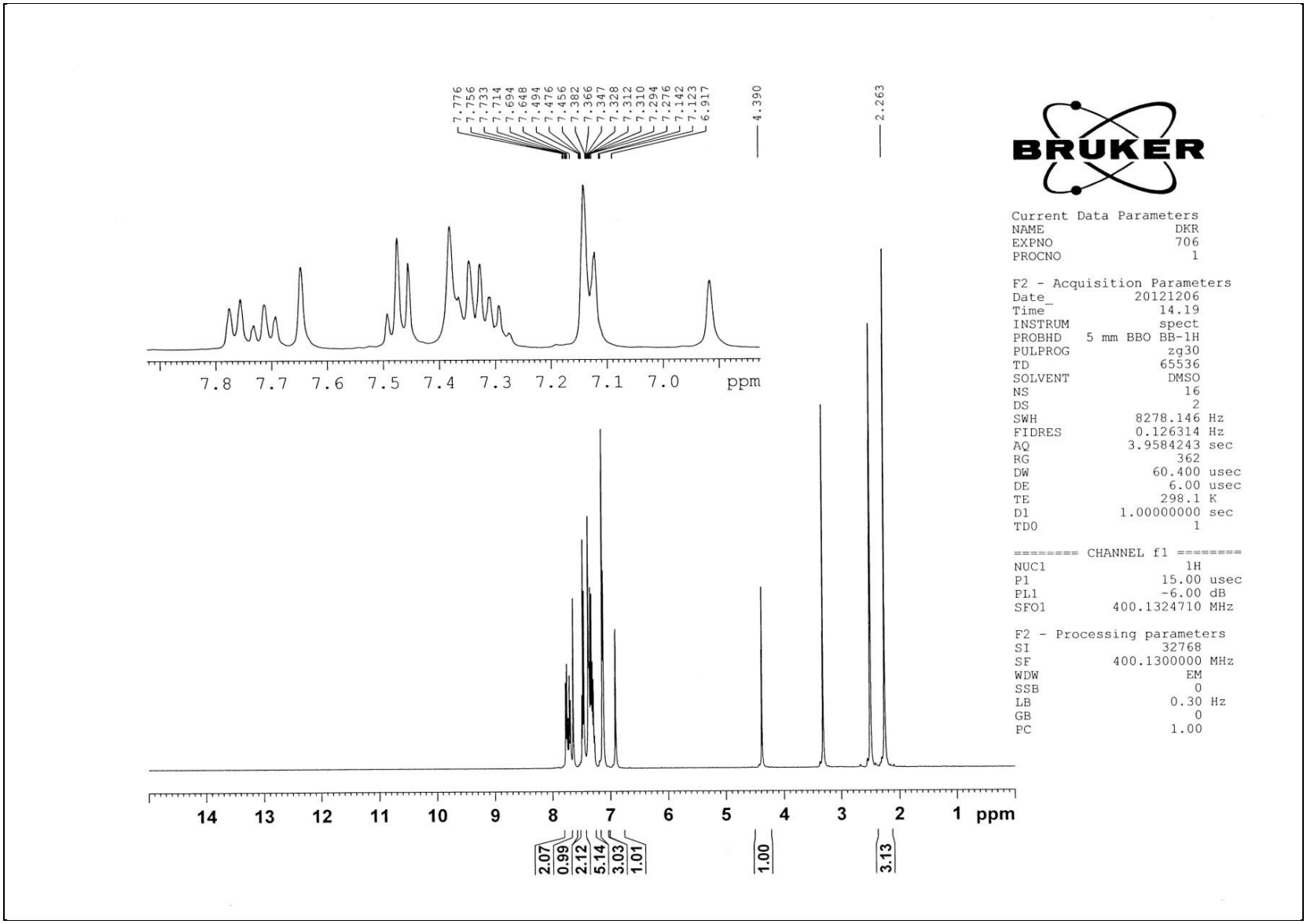

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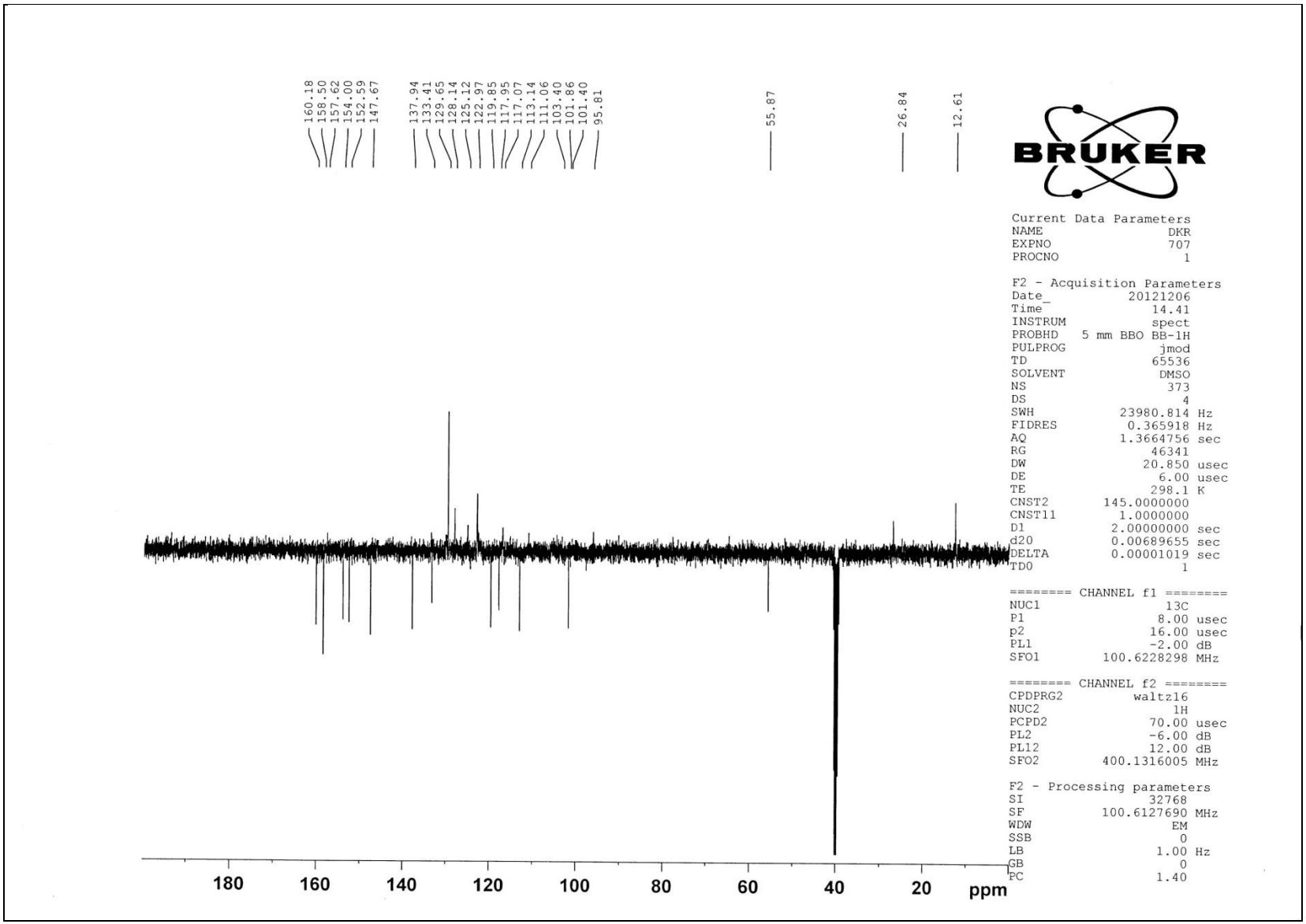

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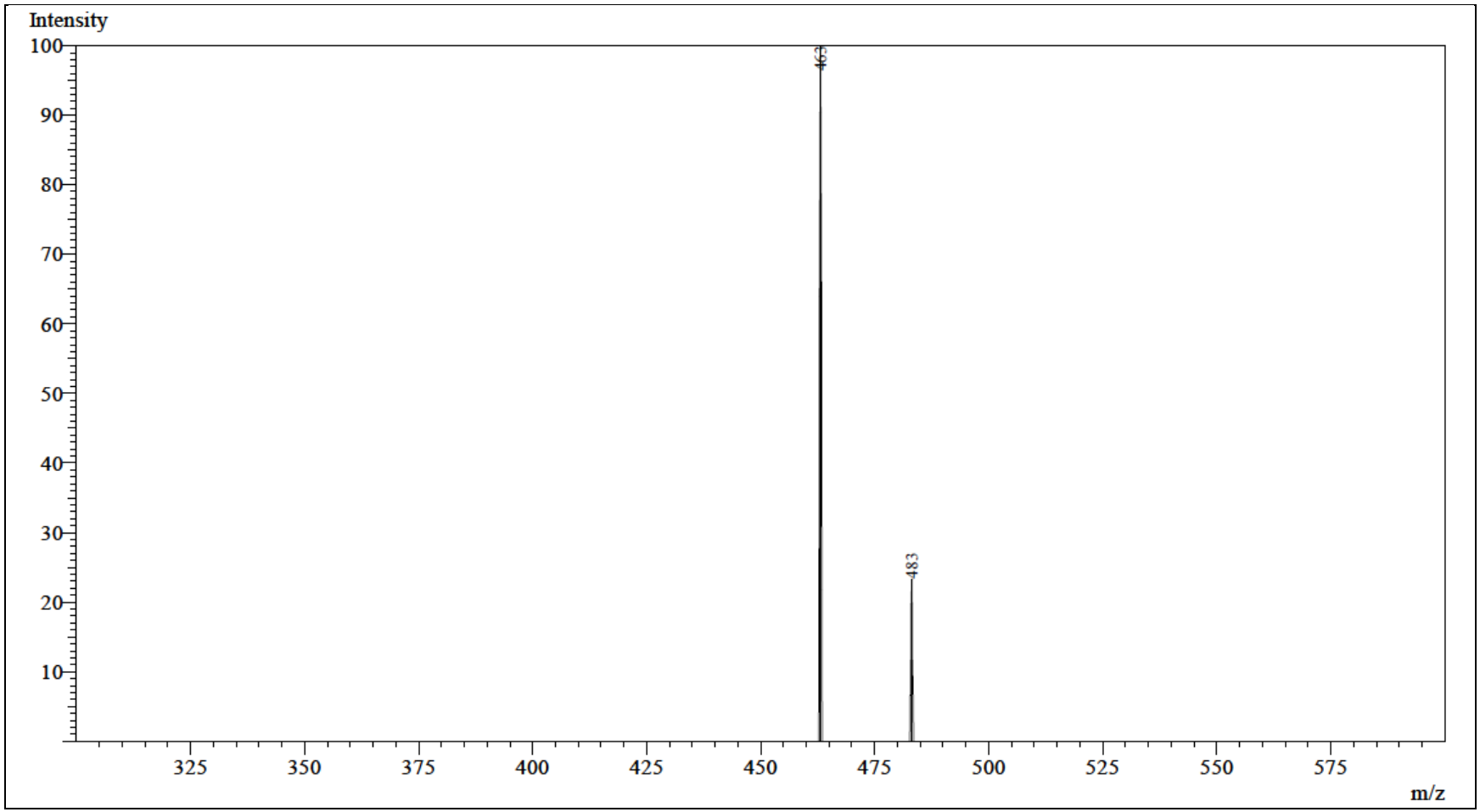

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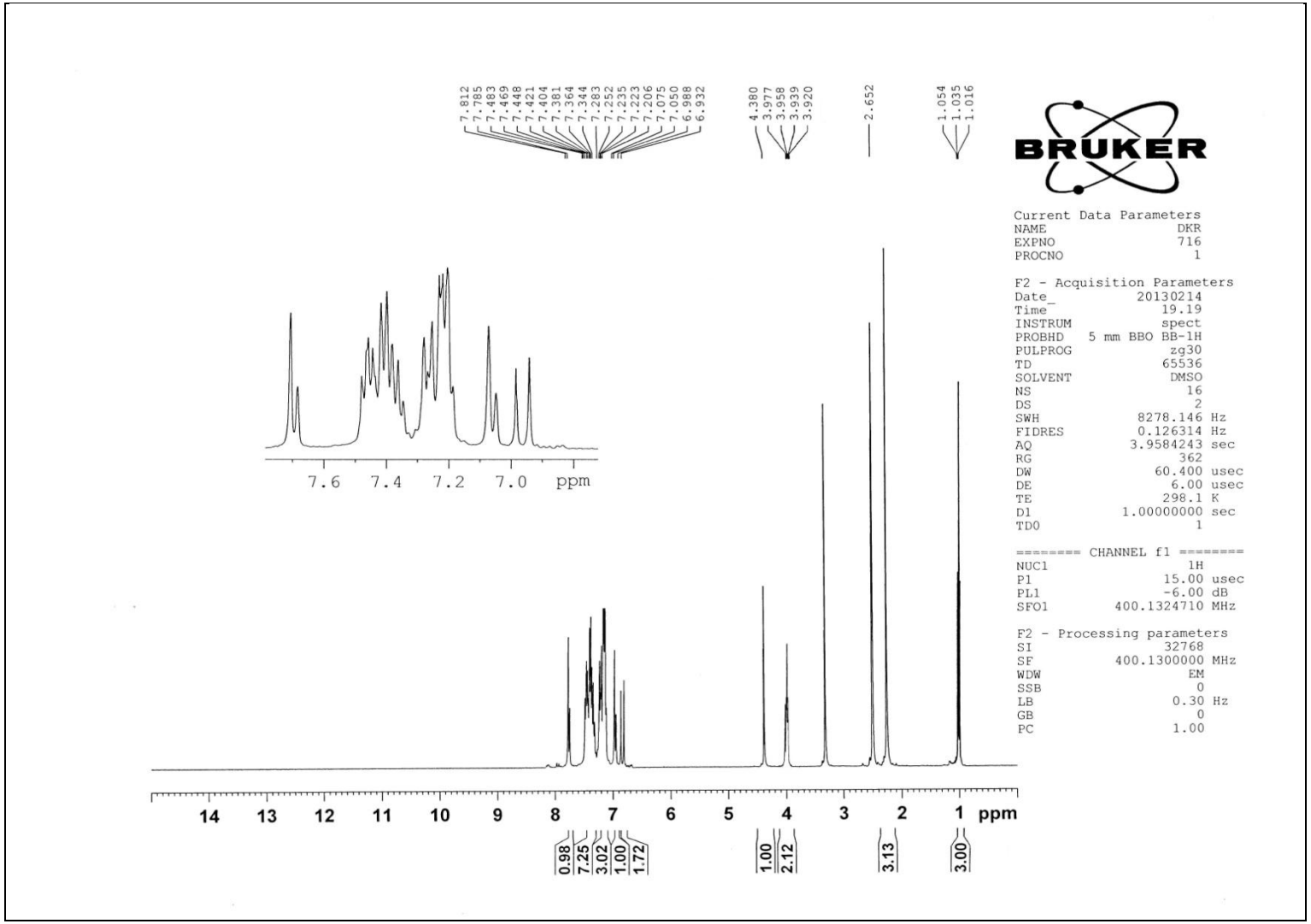

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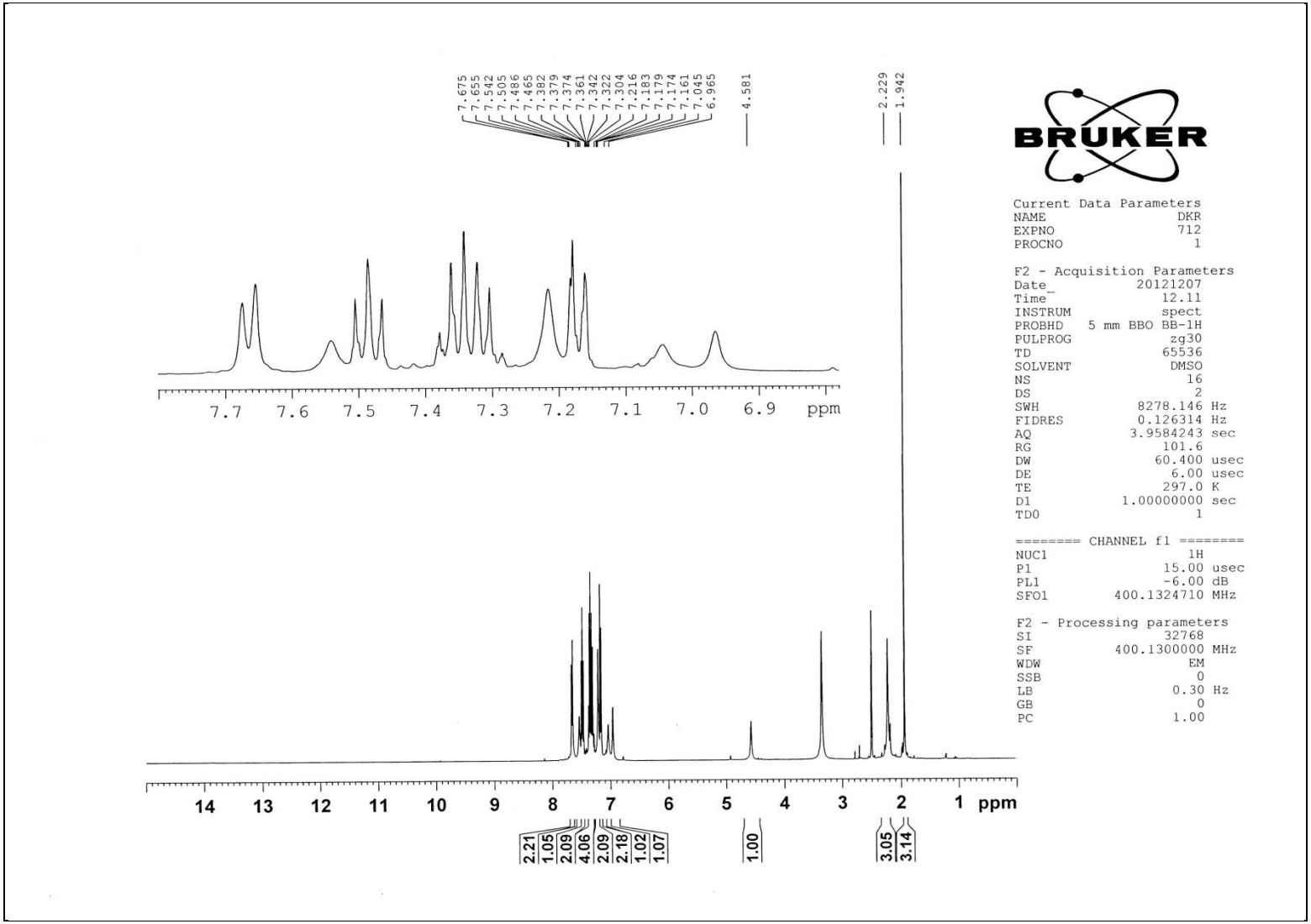

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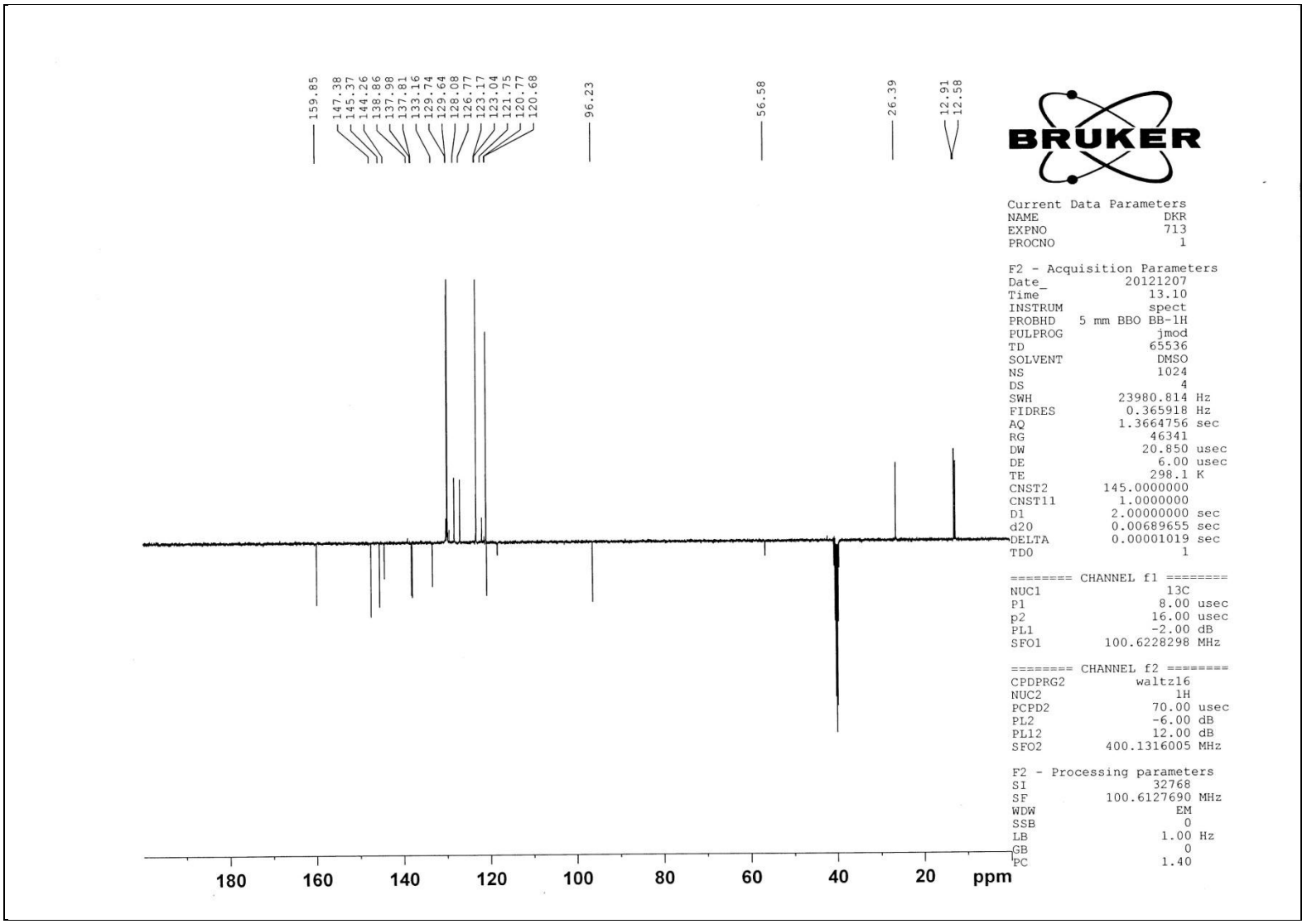
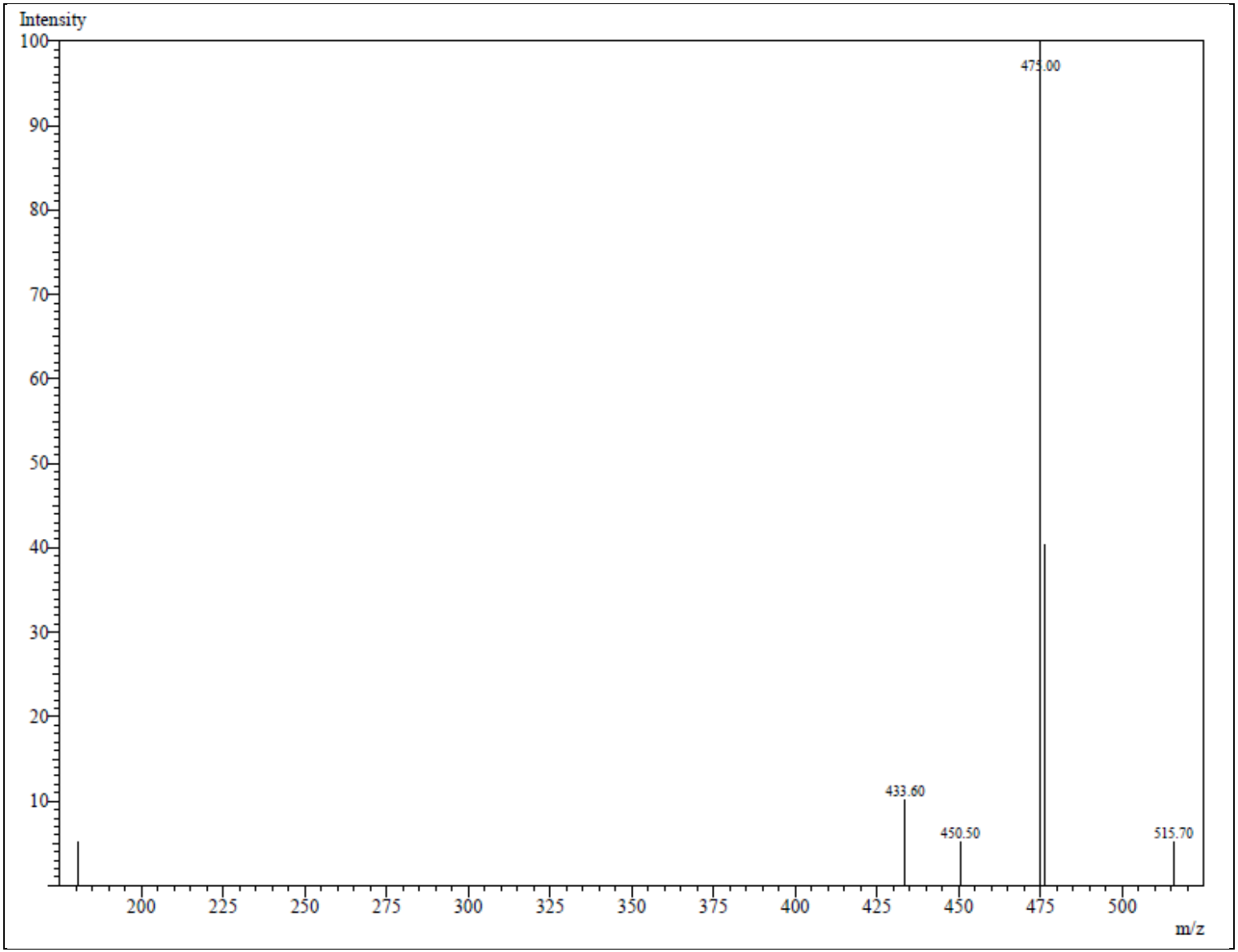


Mass spectra of compound **6g**



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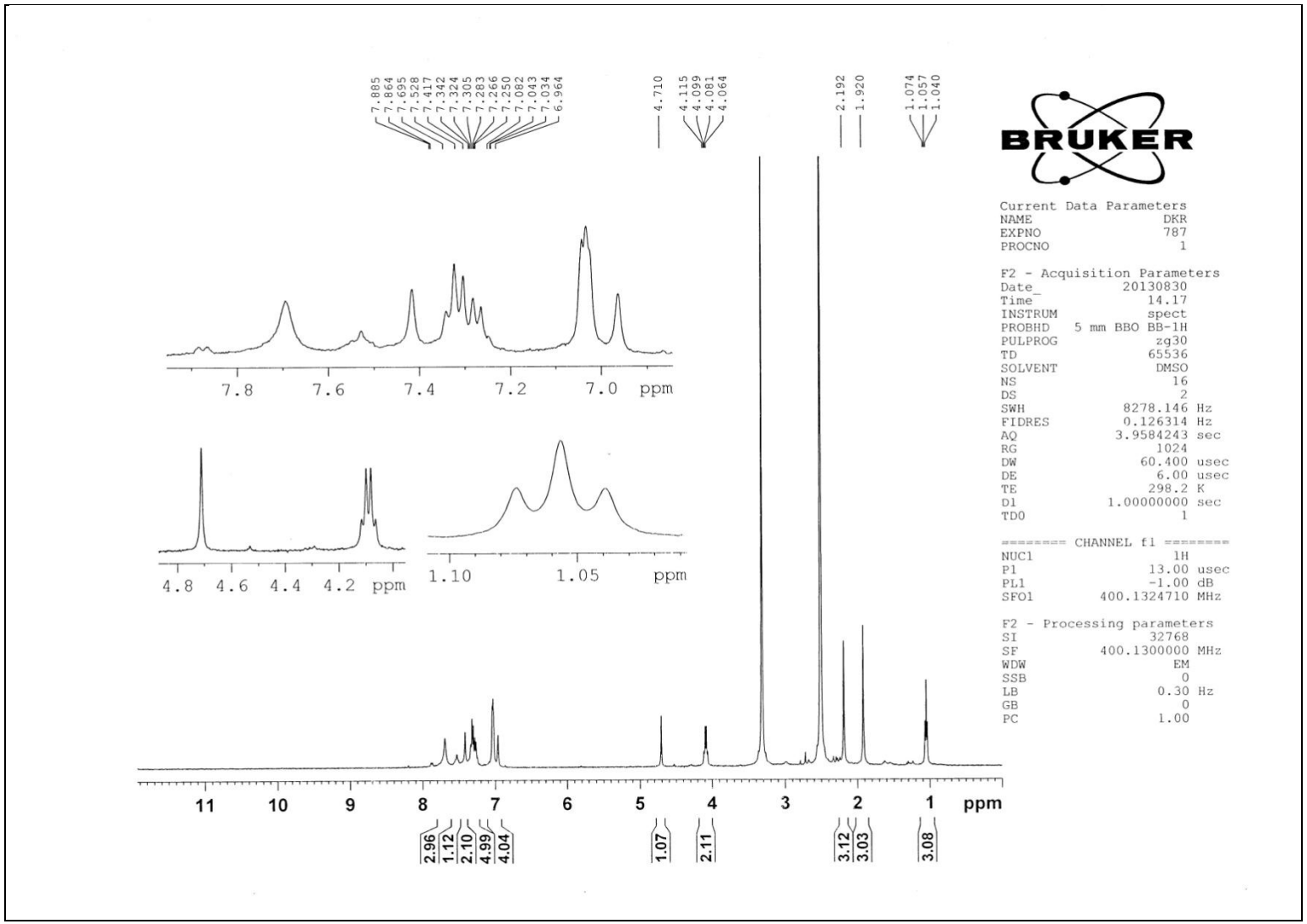

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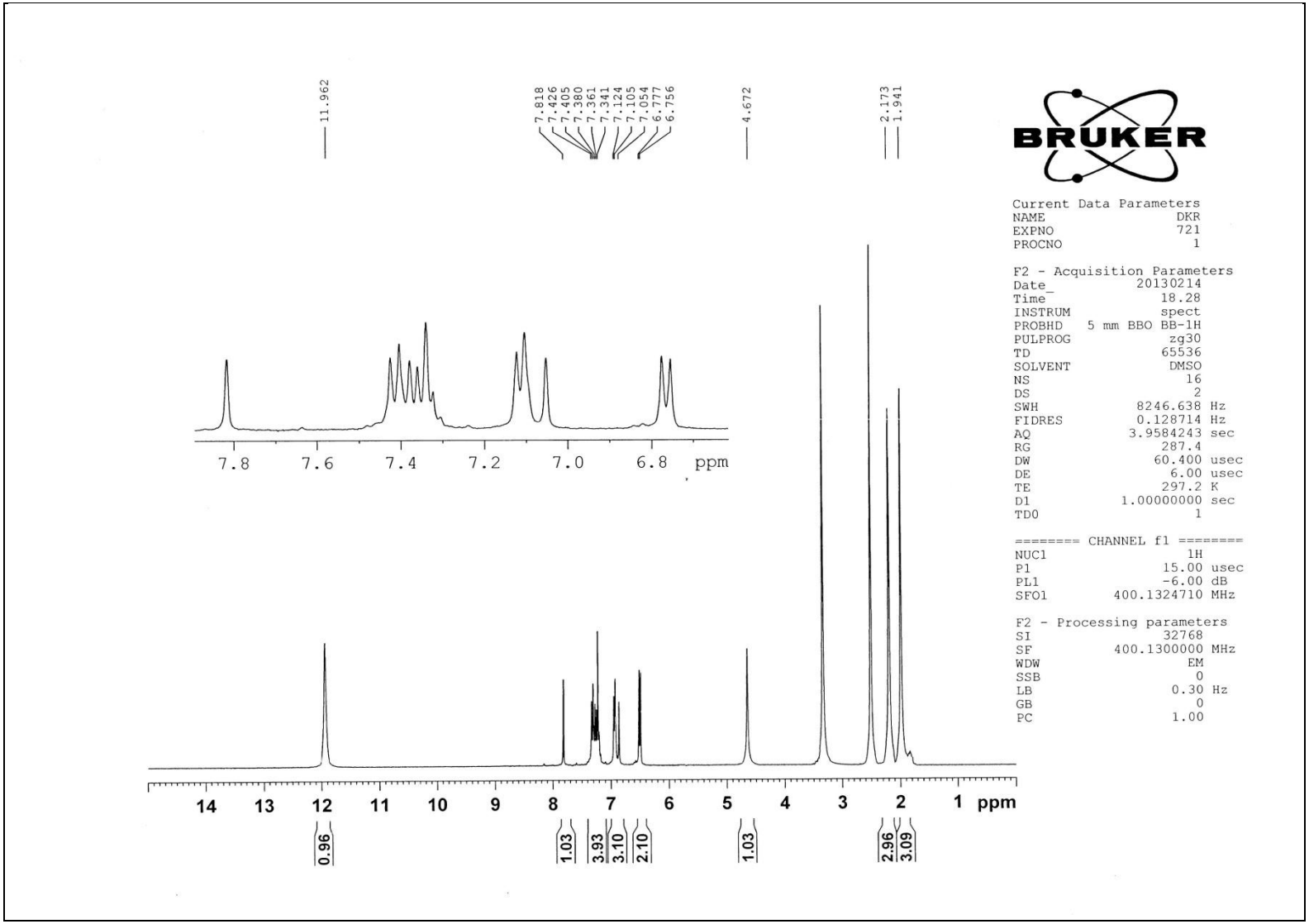

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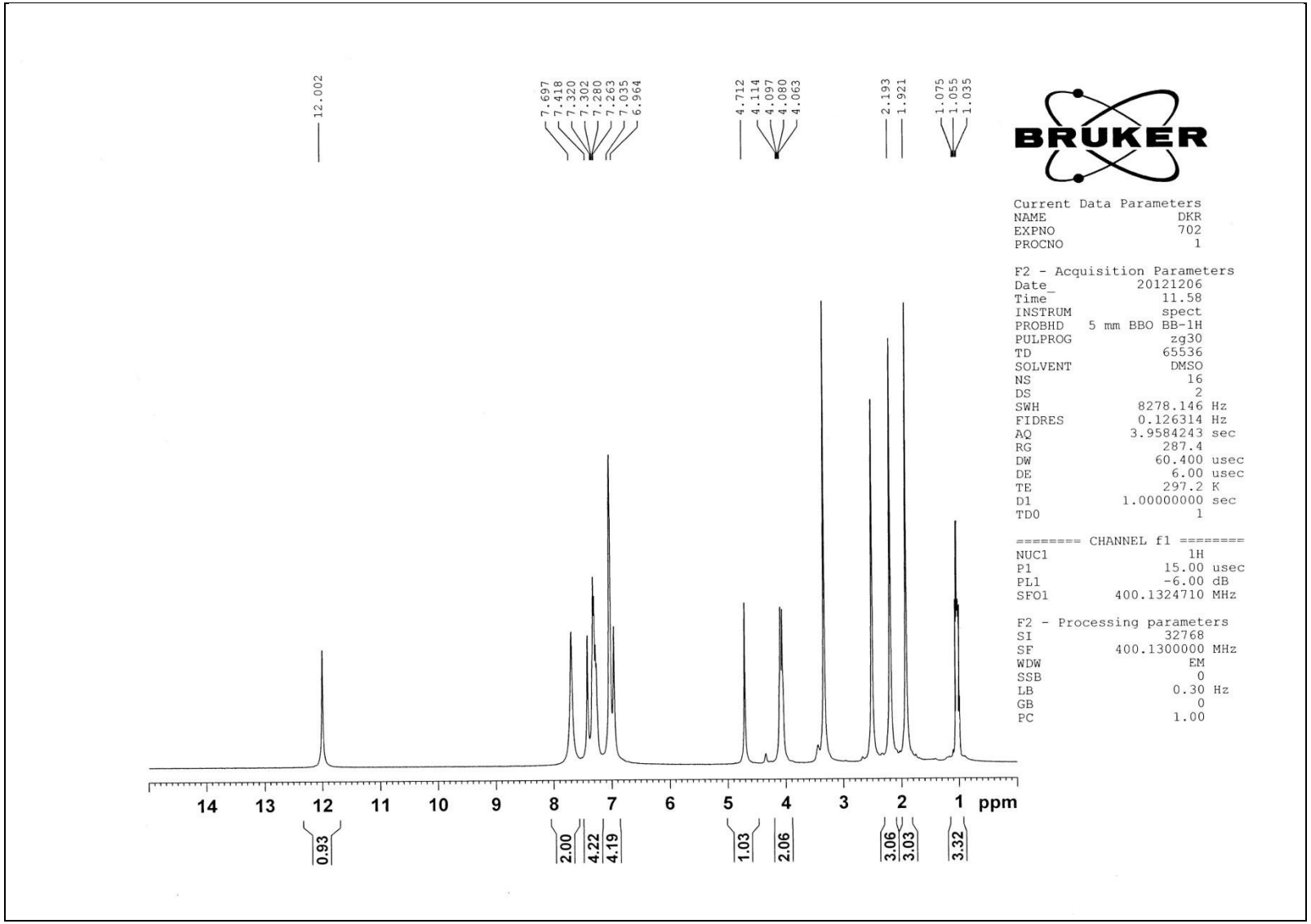

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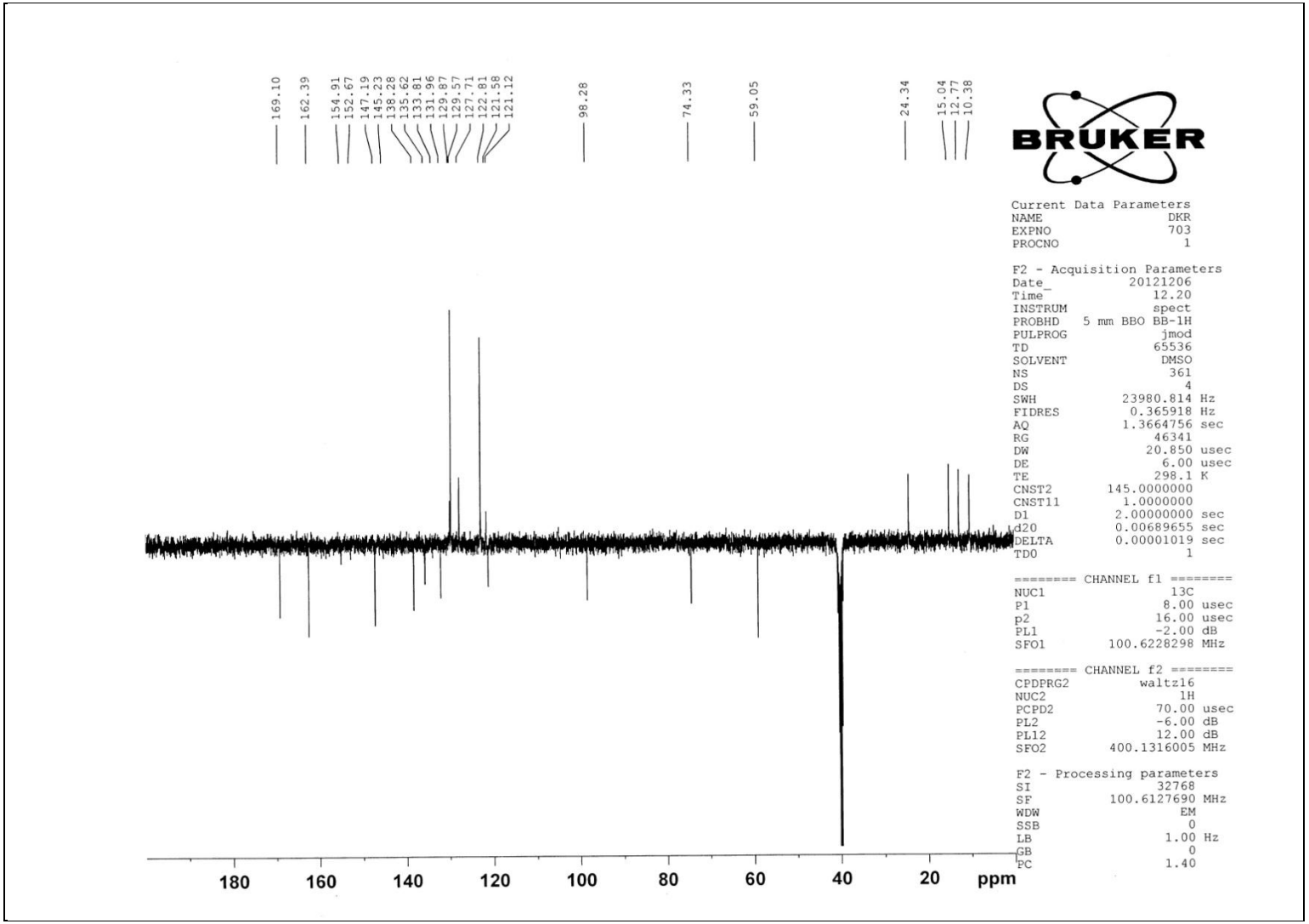

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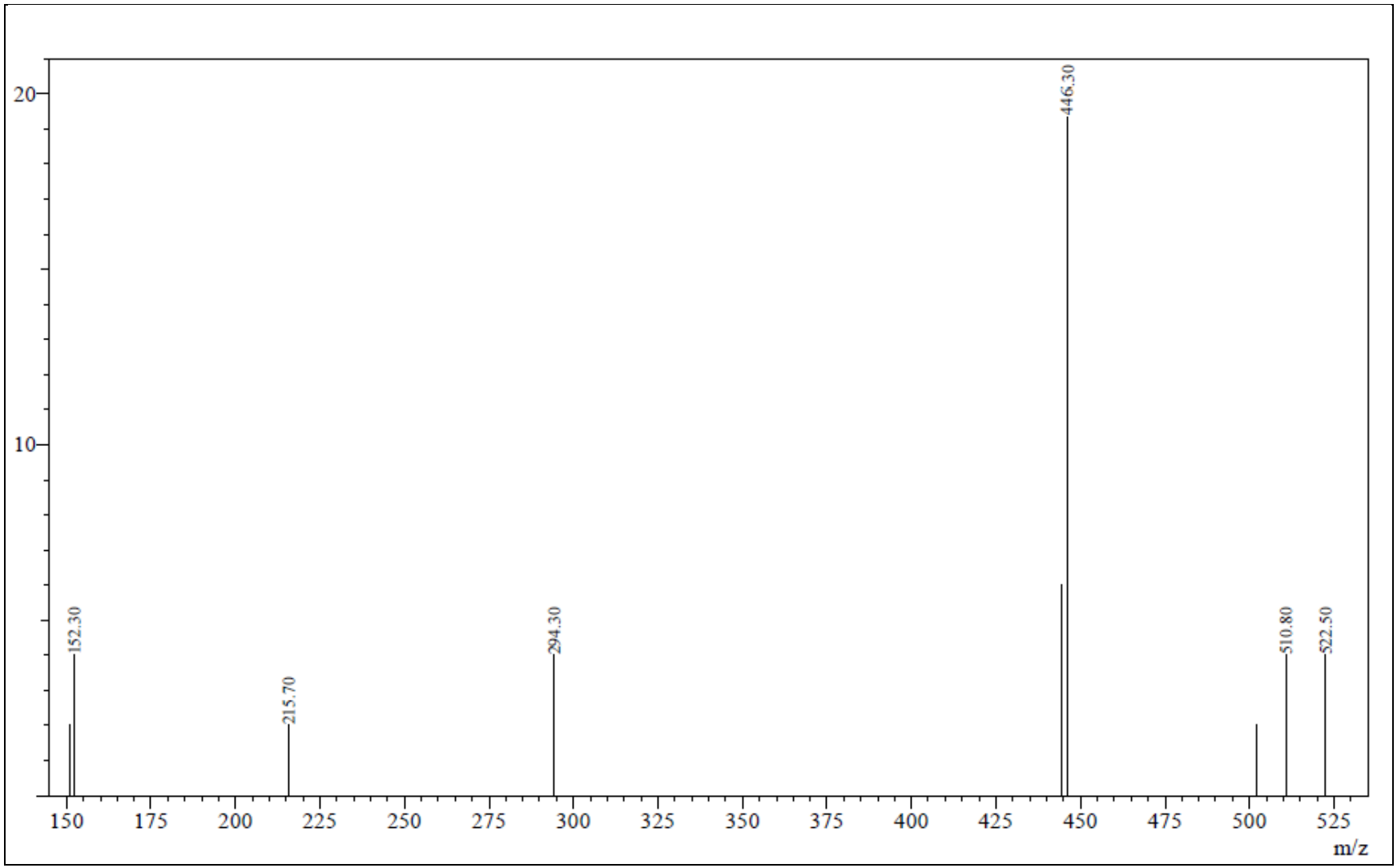

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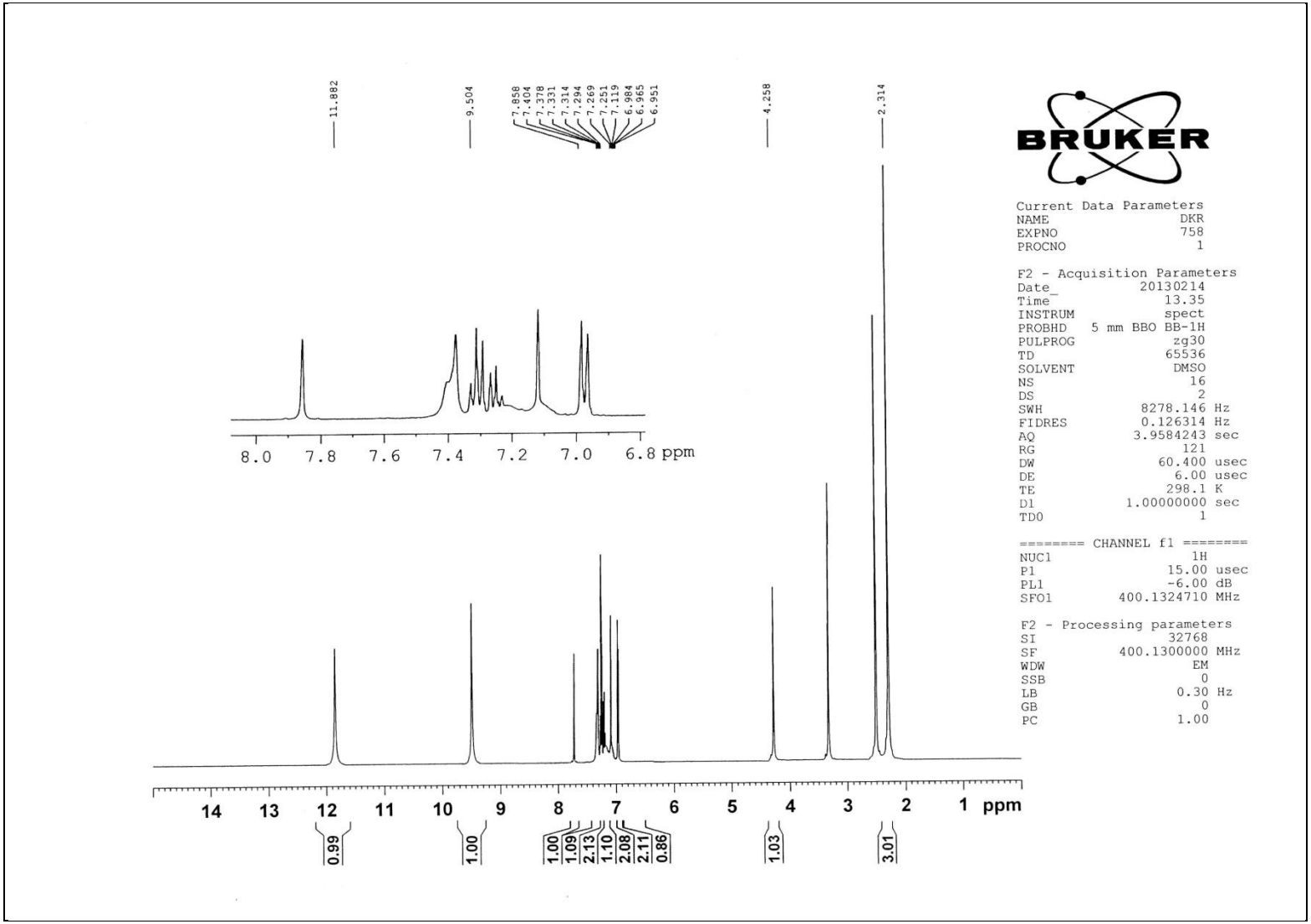
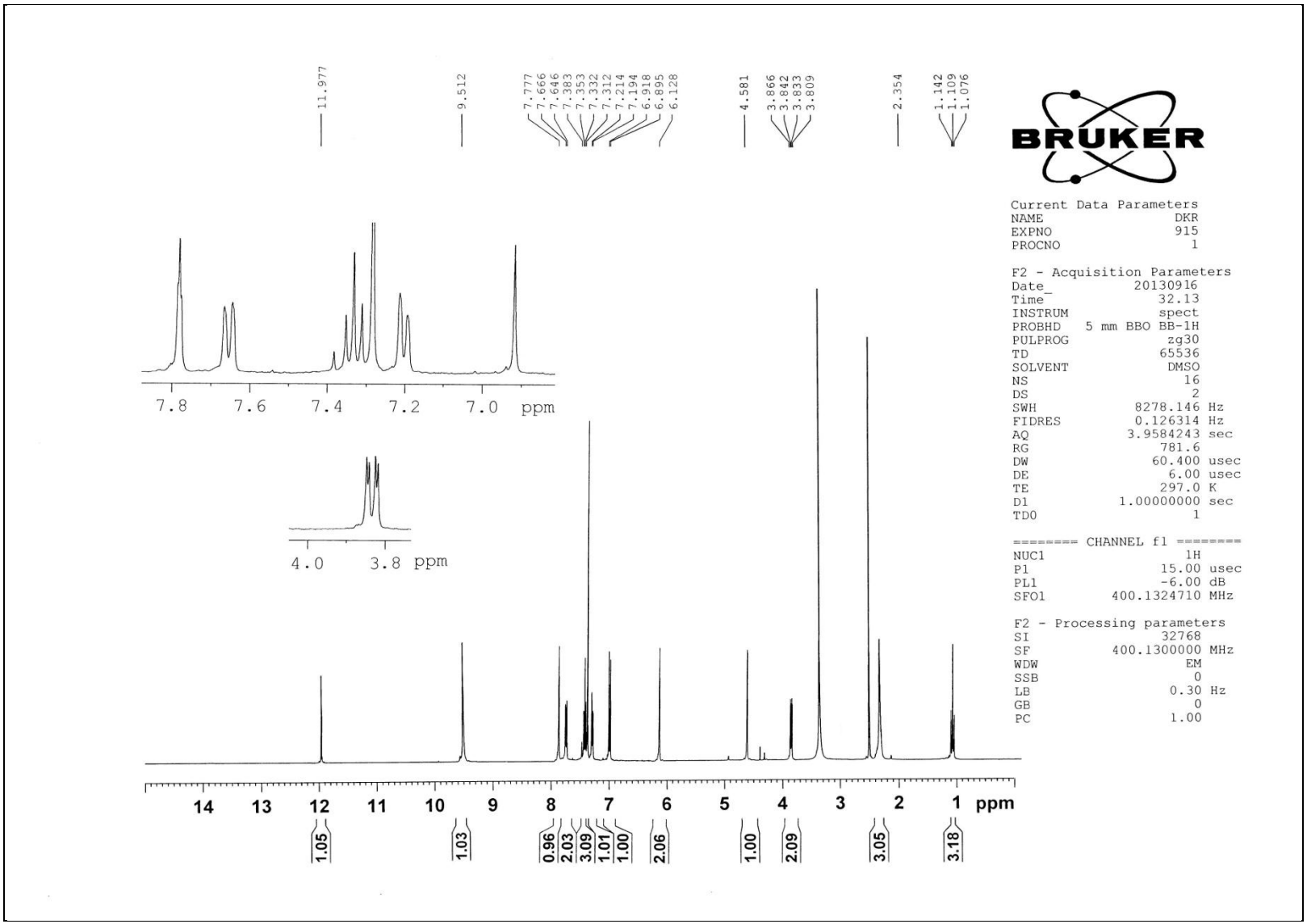
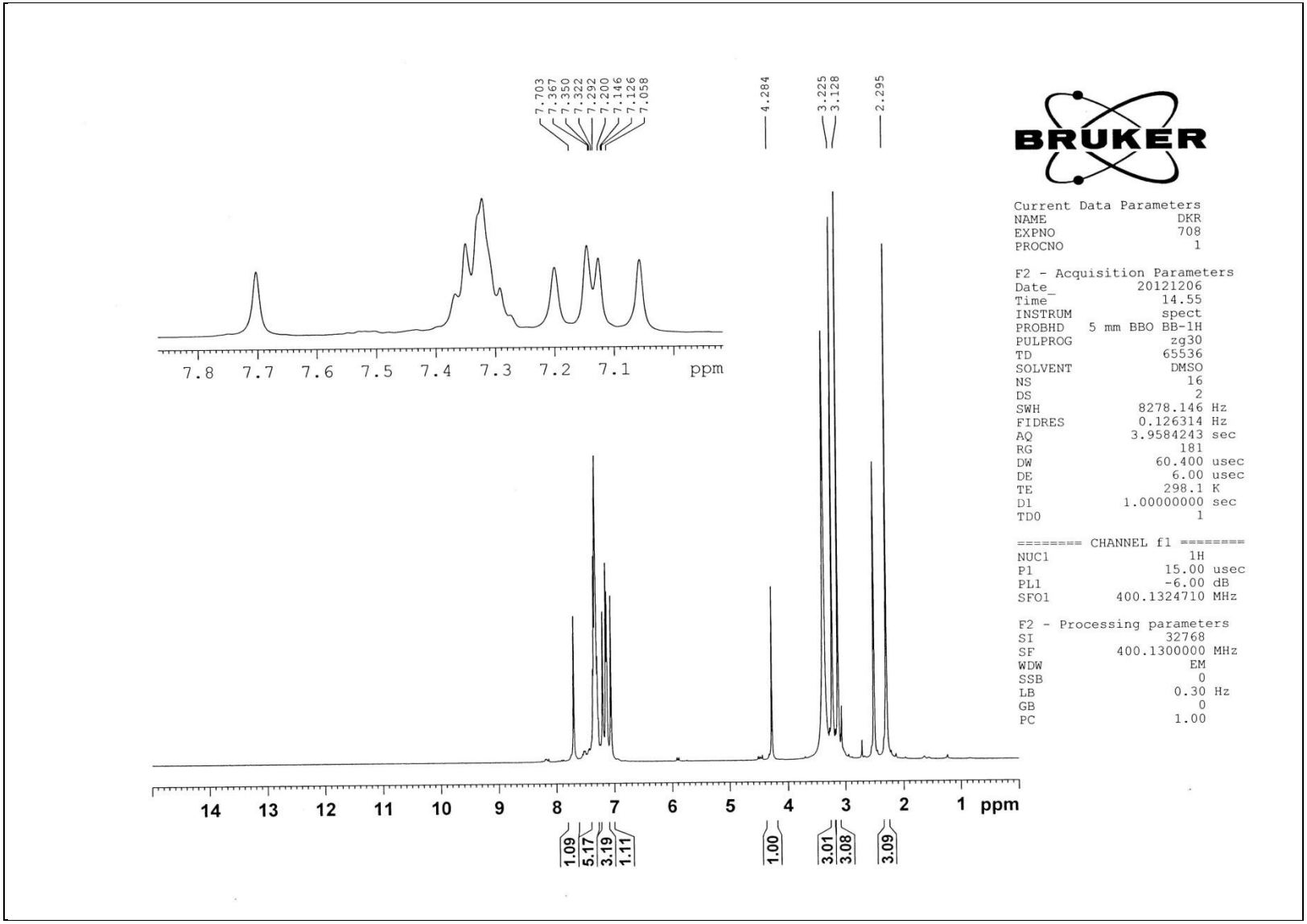


NMR spectra of compound **6l**



NMR spectra of compound **6m**



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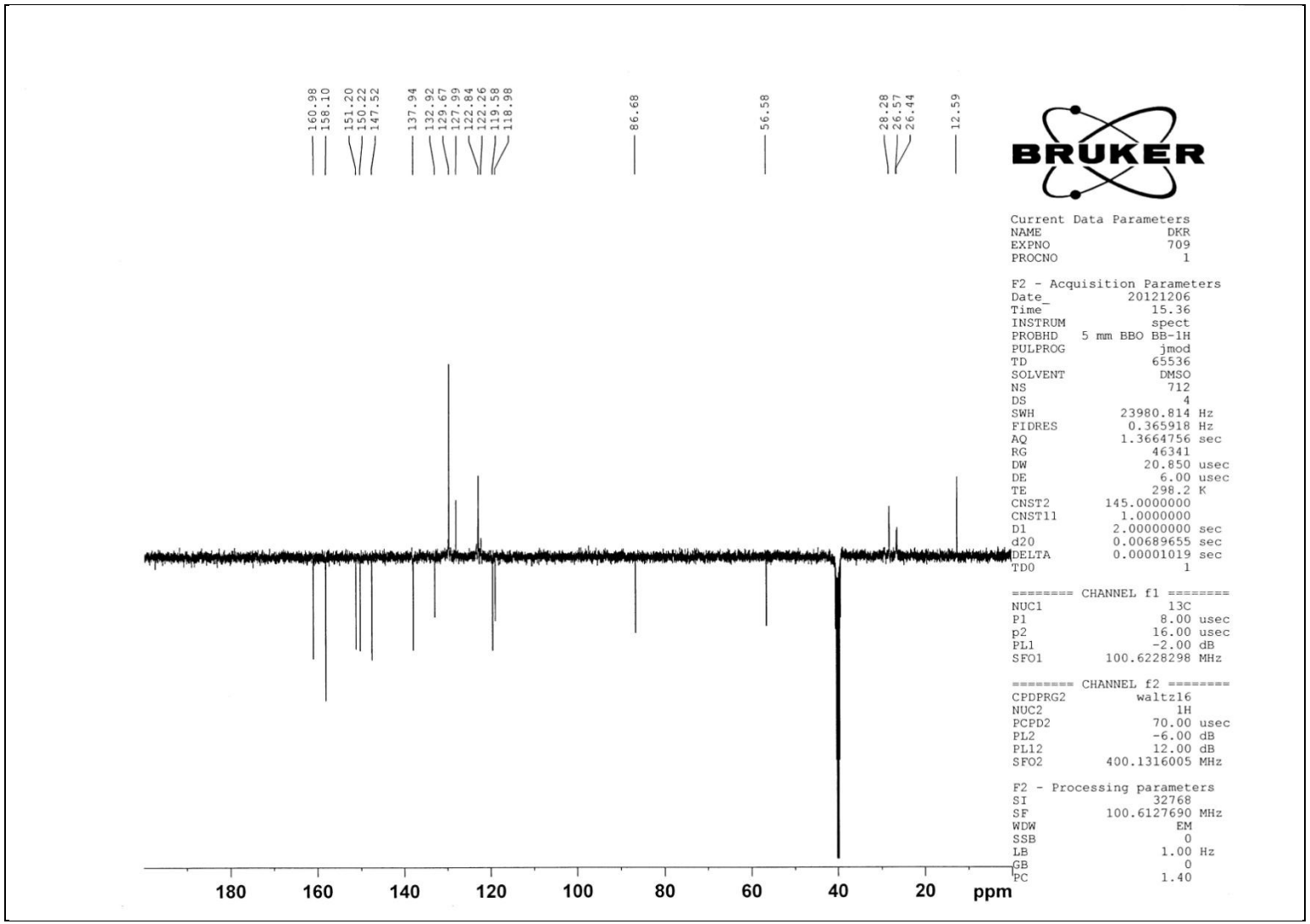

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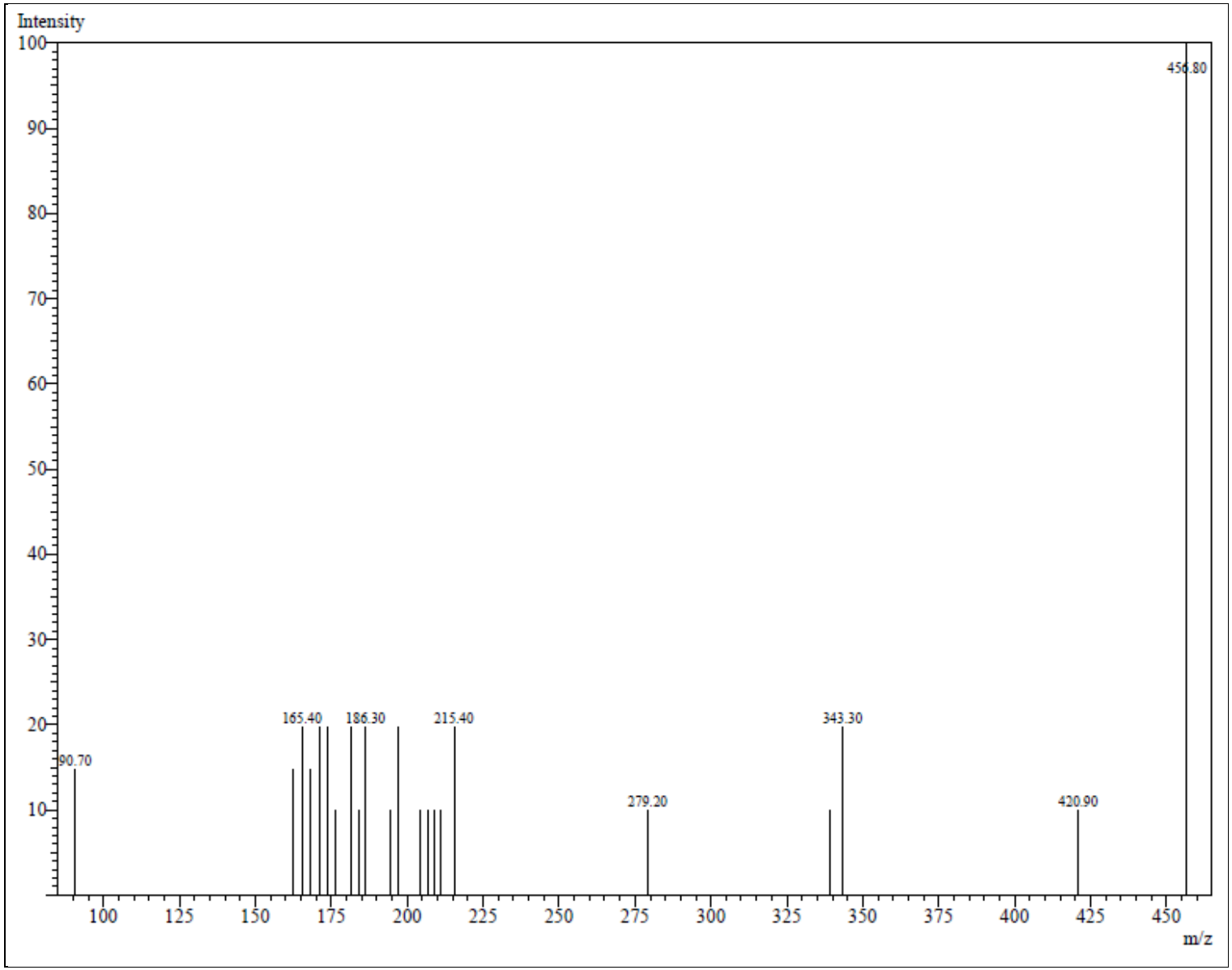
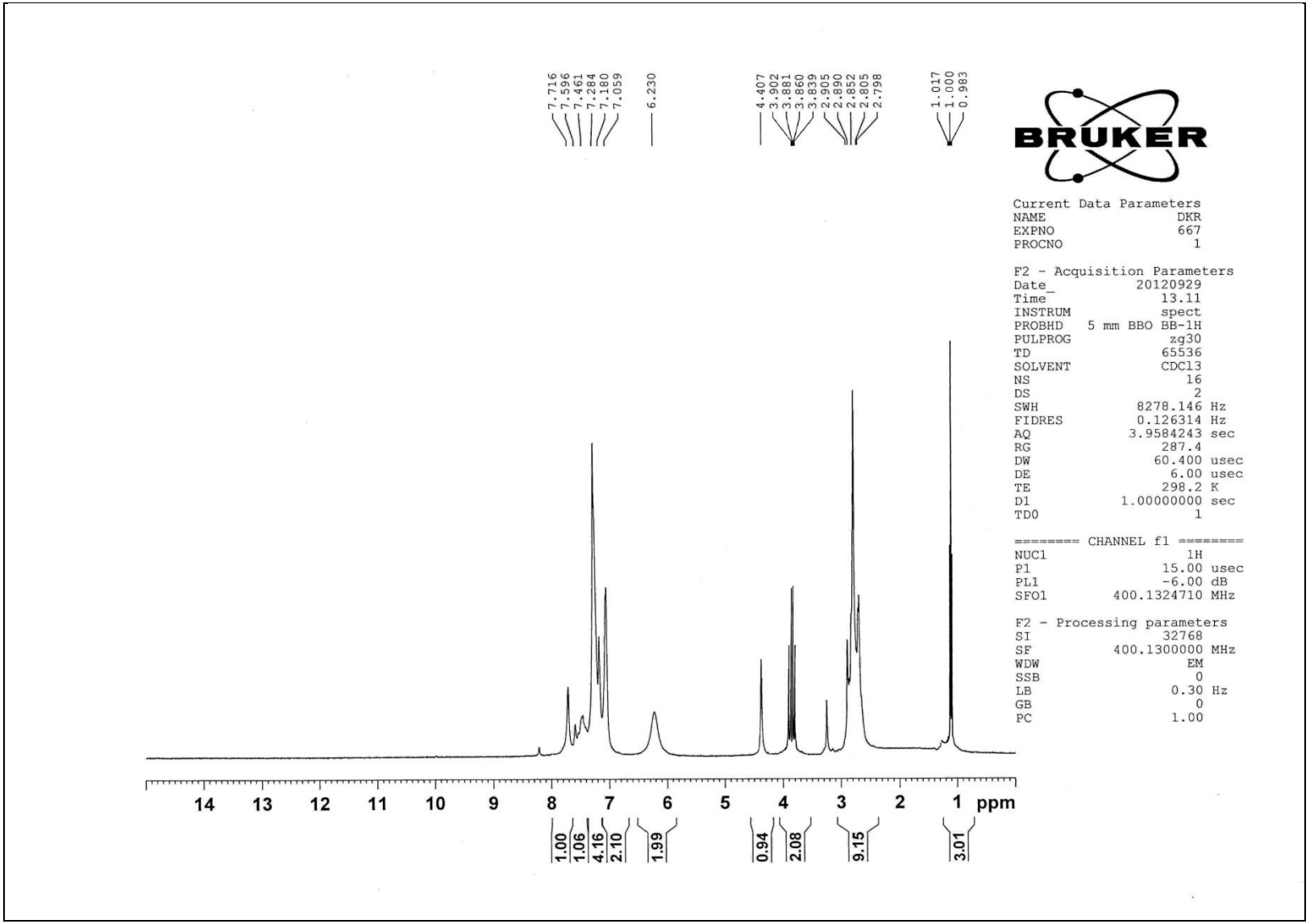


NMR spectra of compound **6n**



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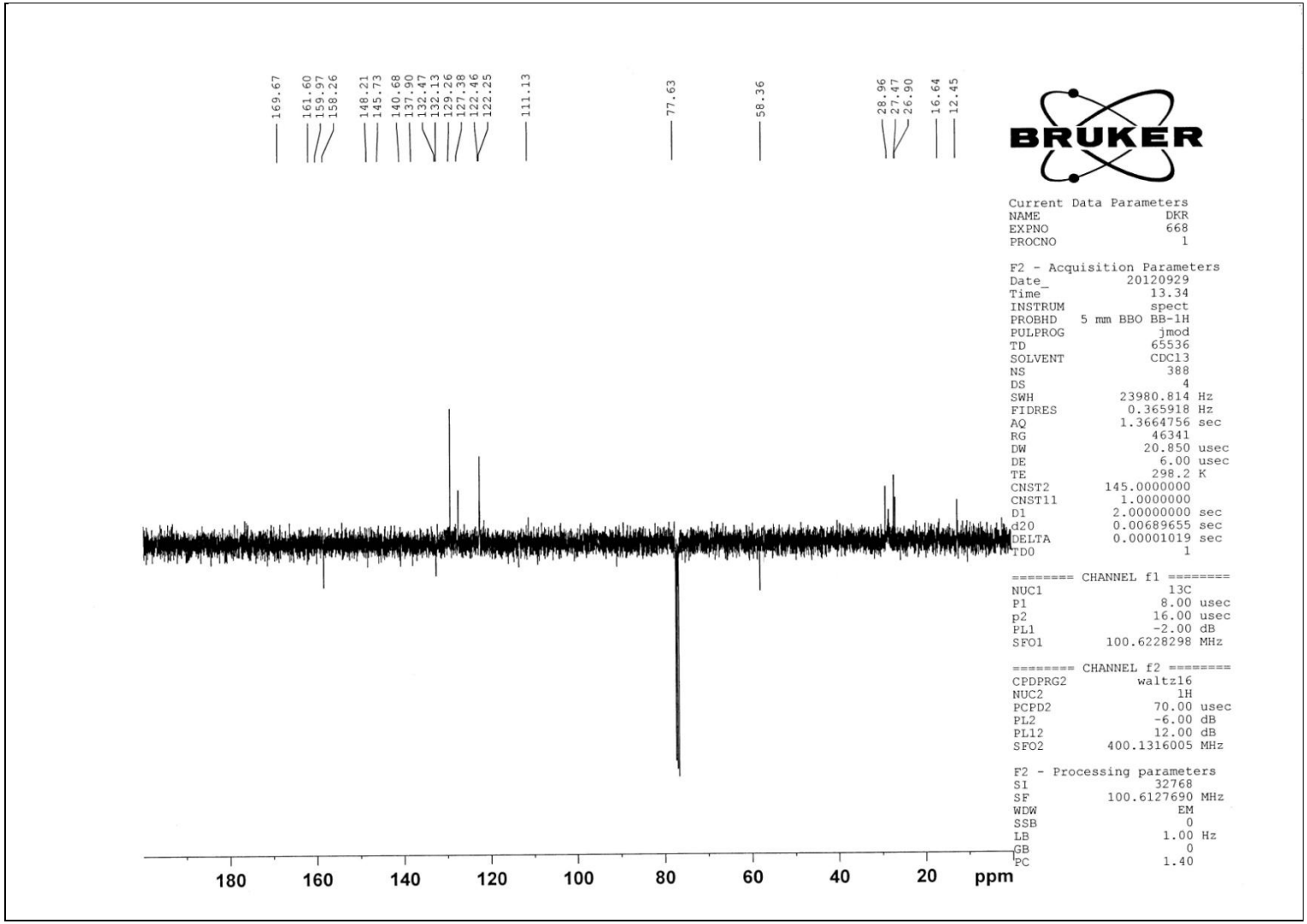
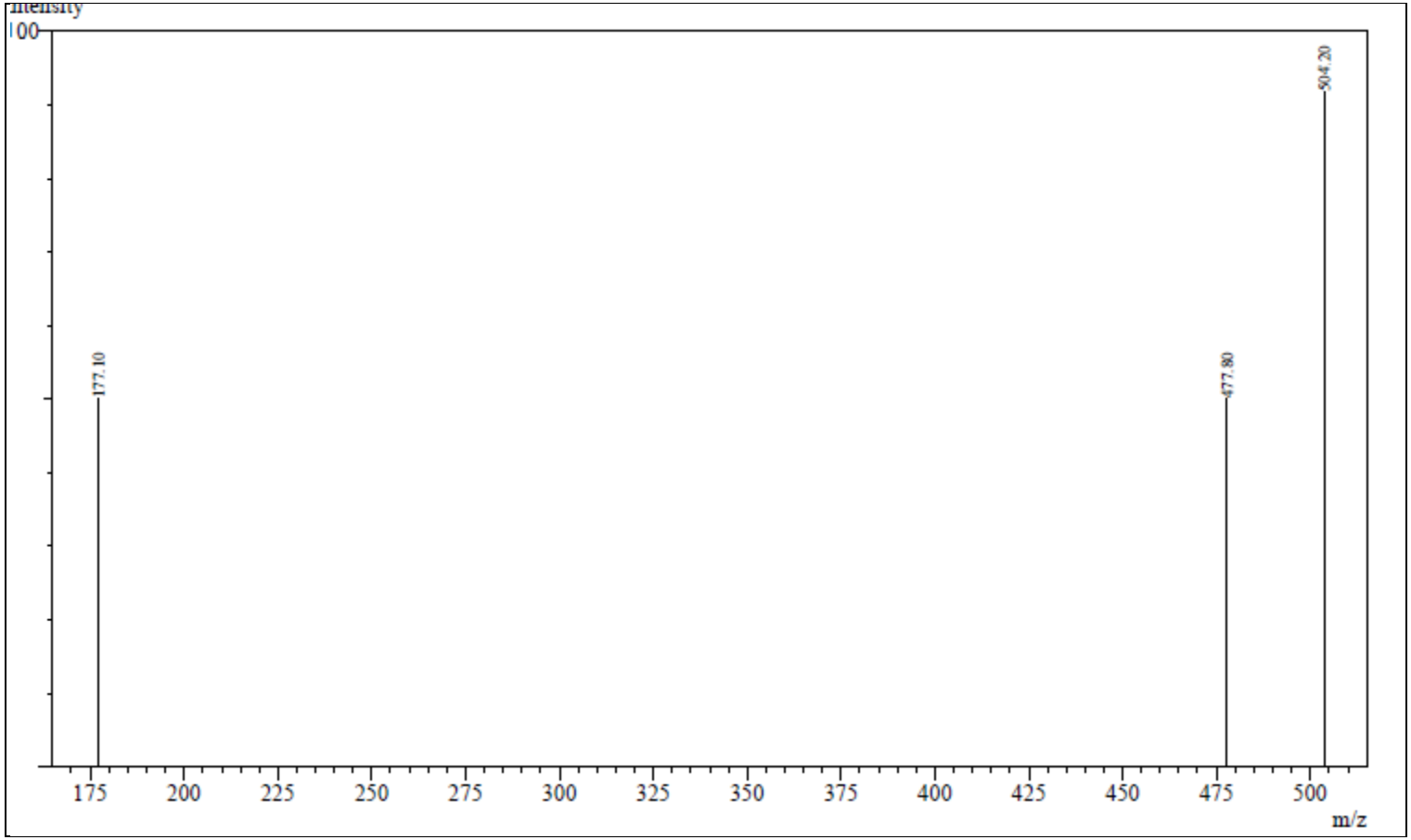


Mass spectra of compound **6n**



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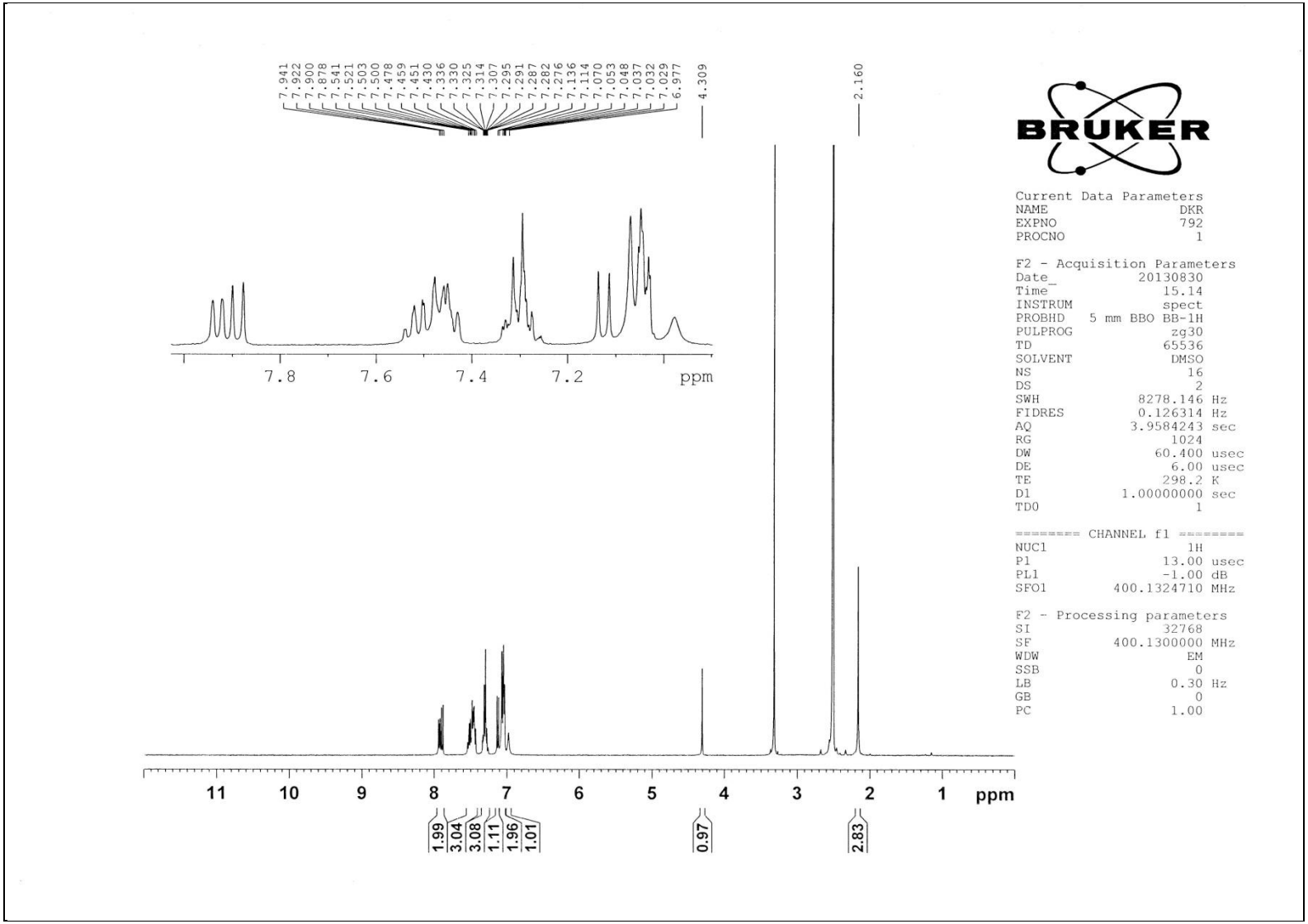

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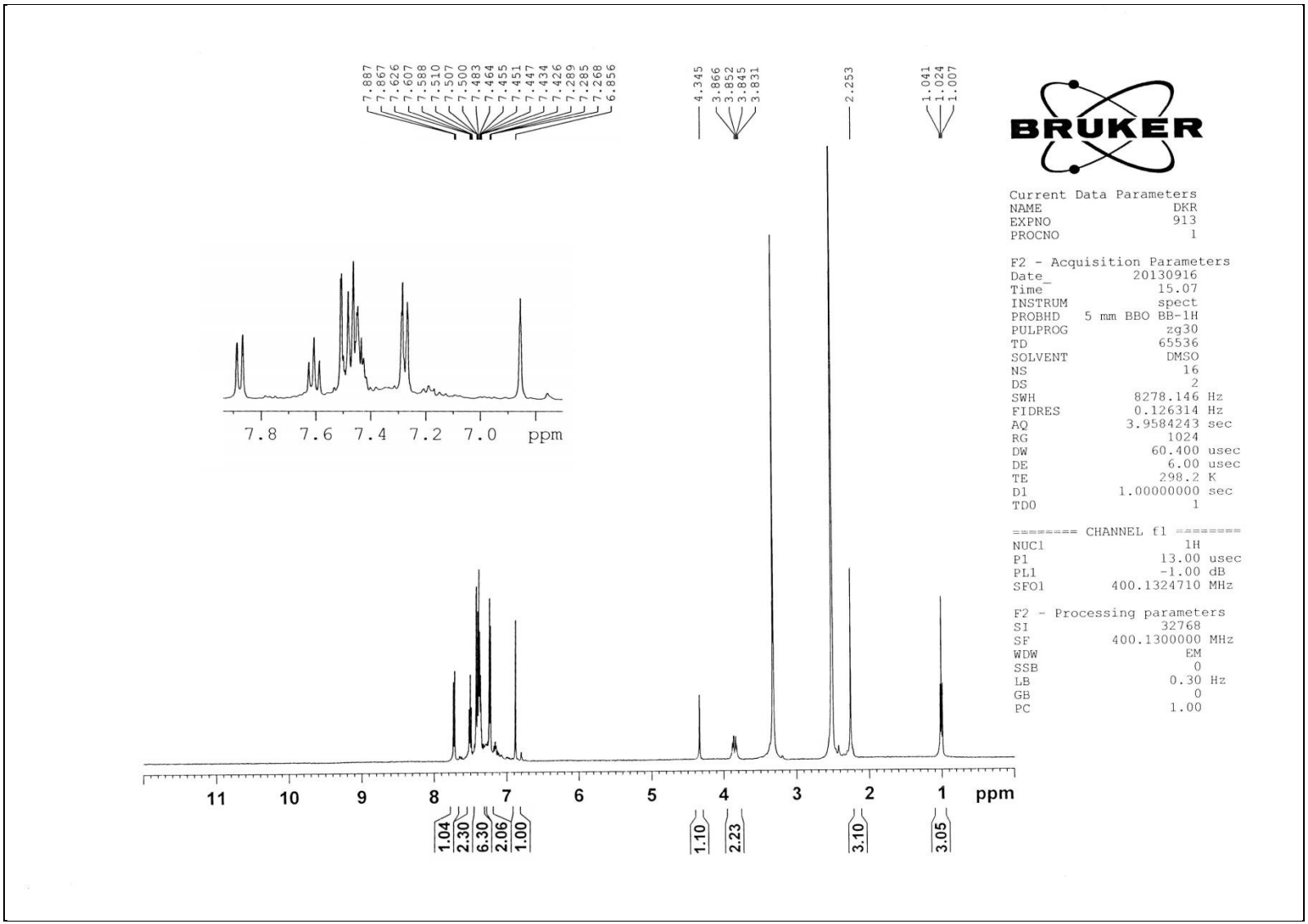
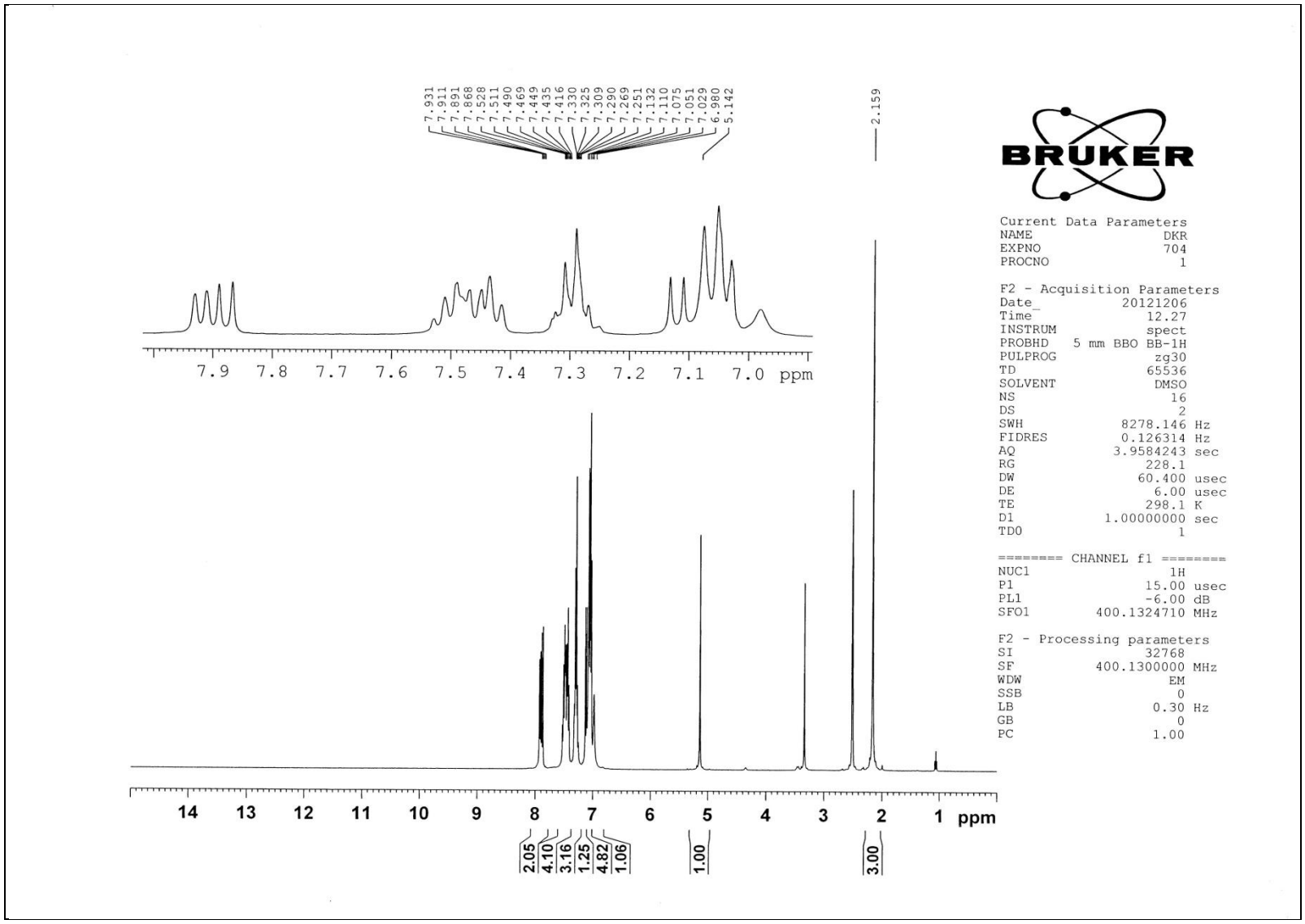
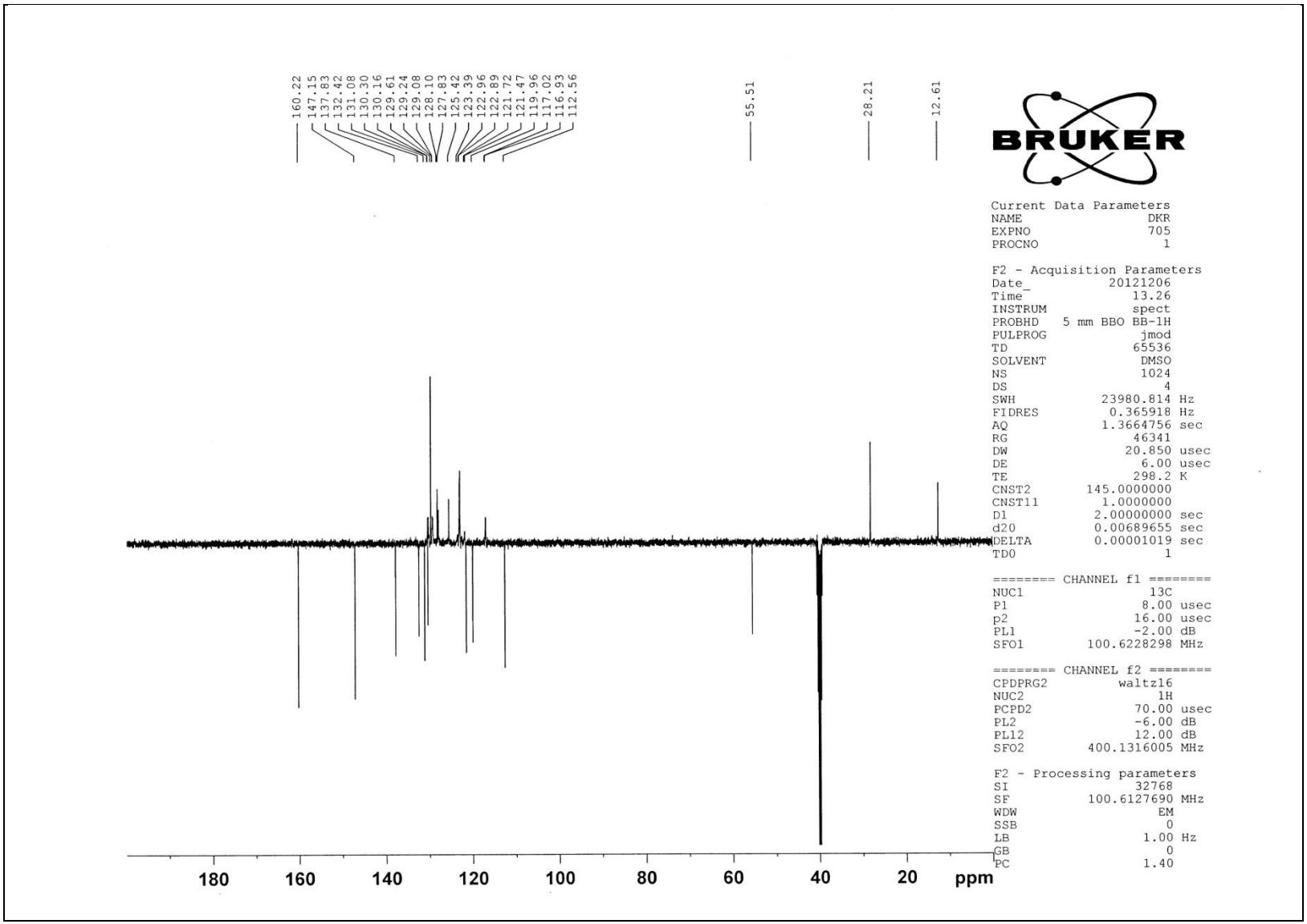


NMR spectra of compound **6q**



CMR spectra of compound **6q**



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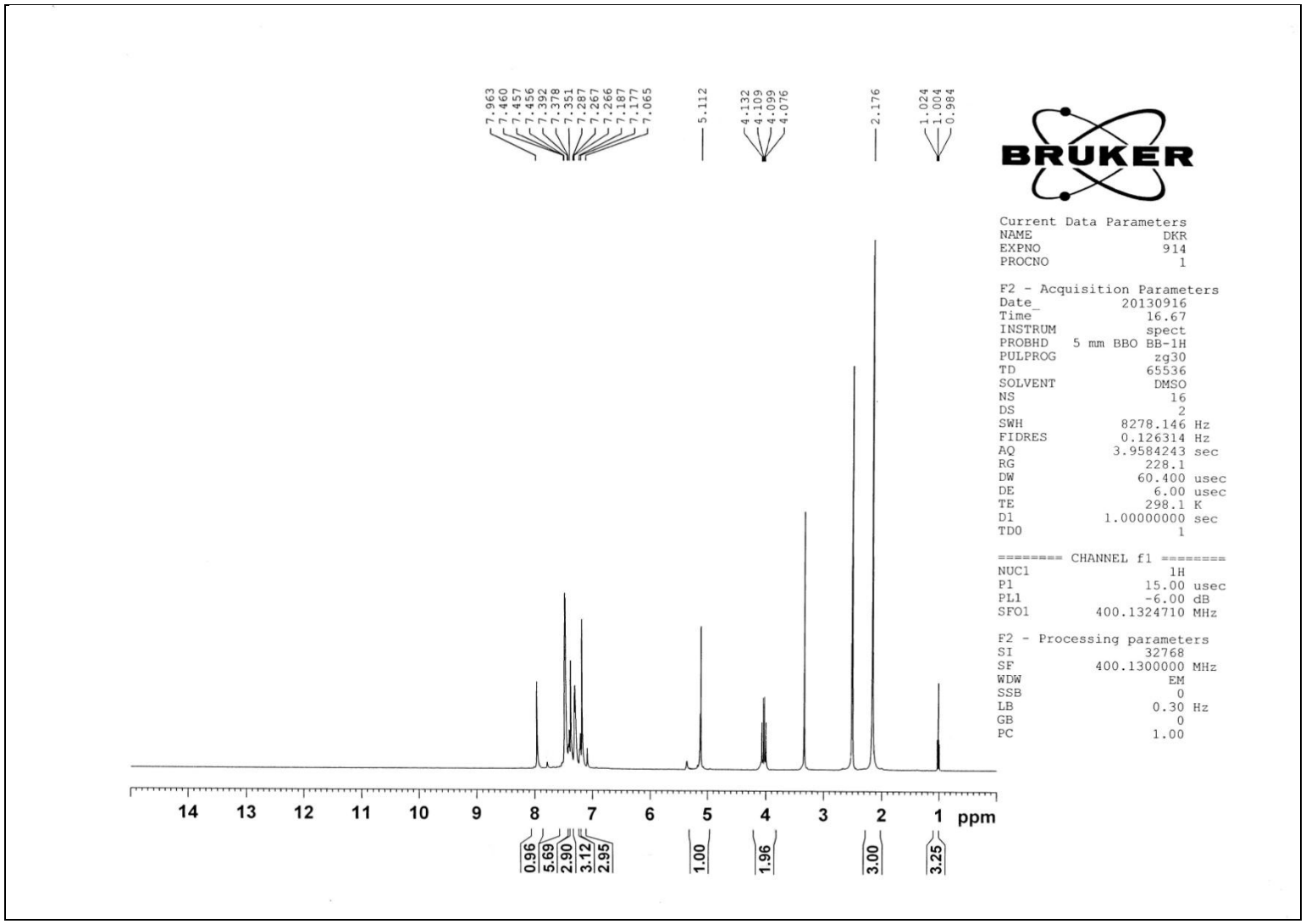

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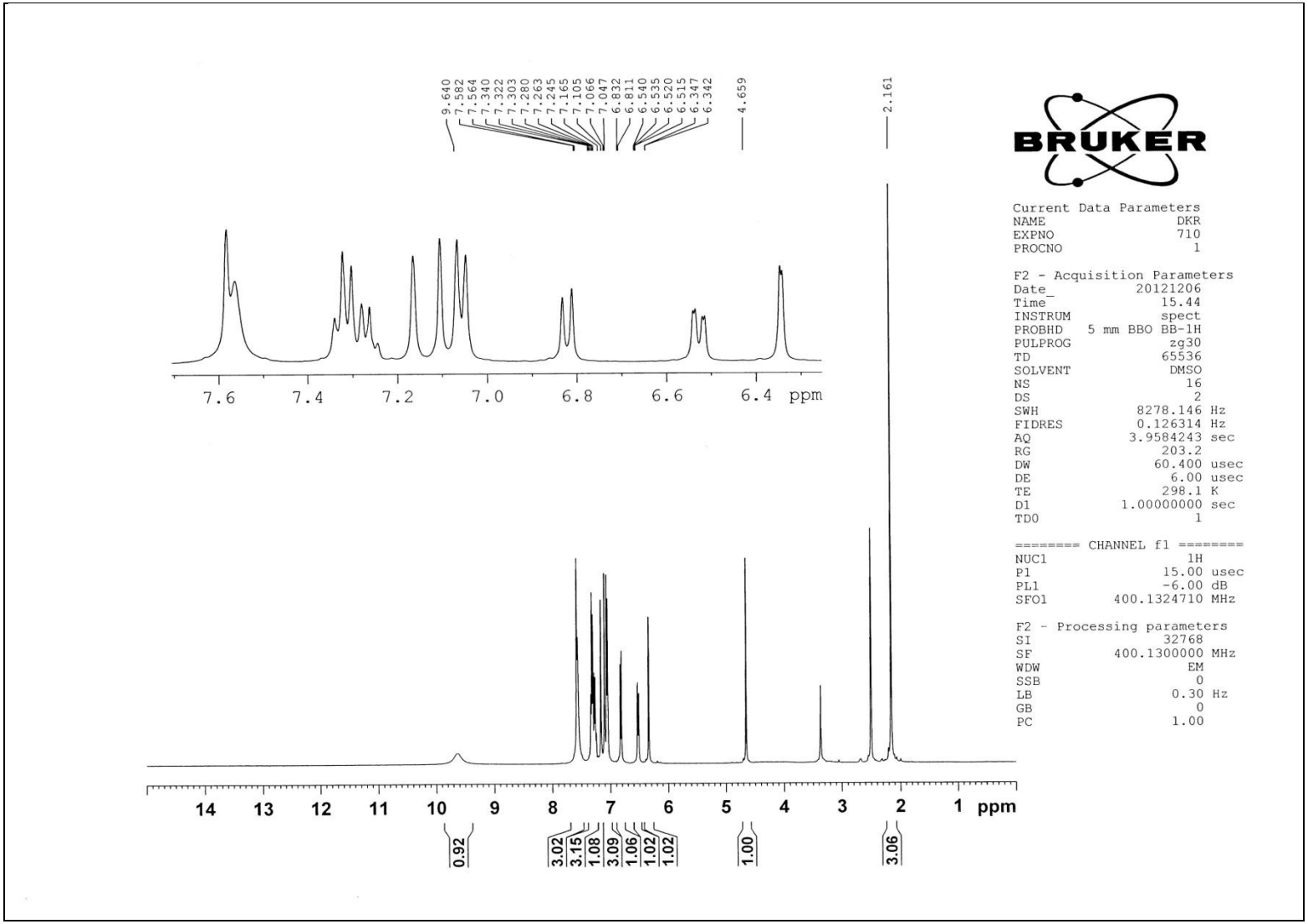

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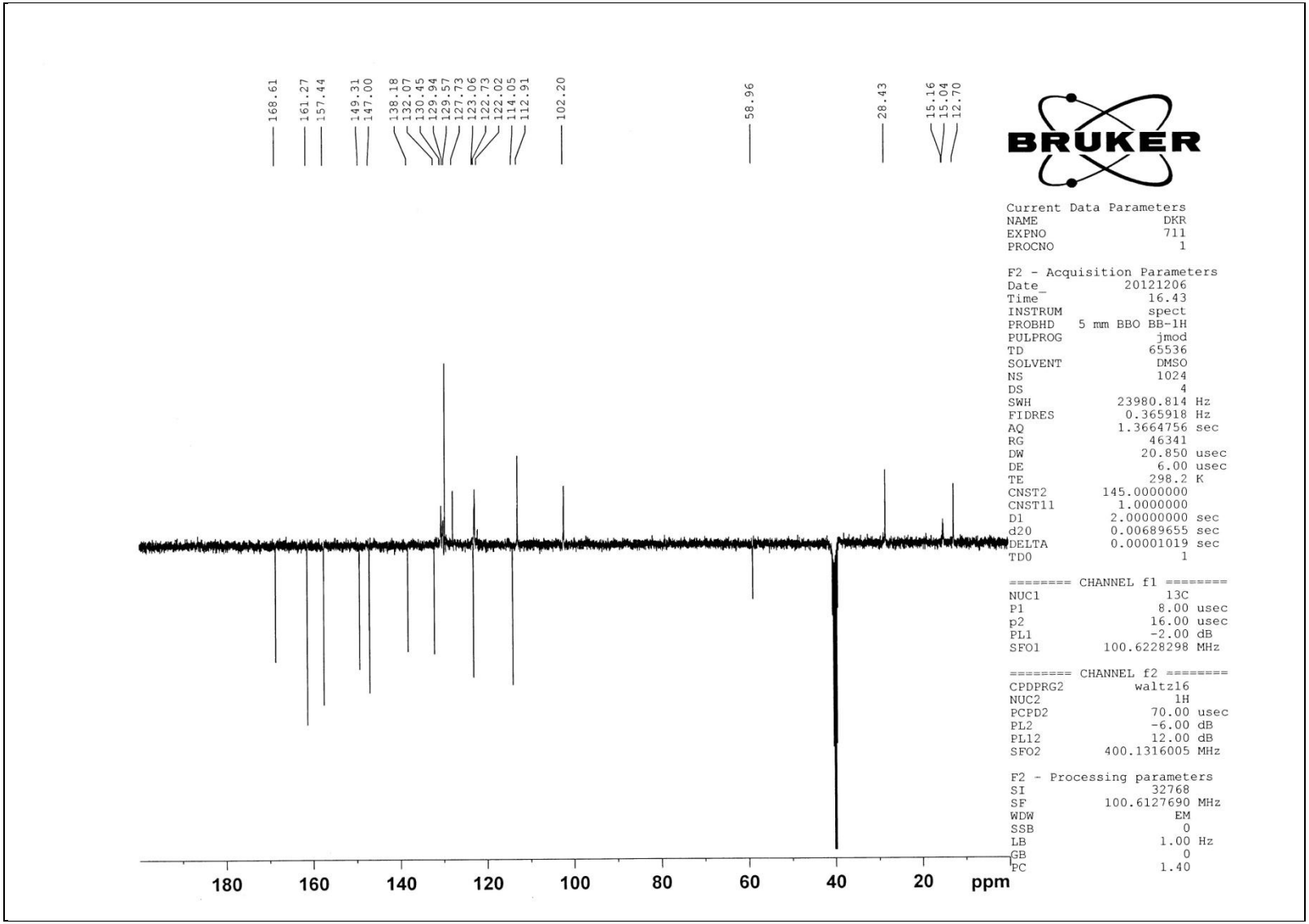

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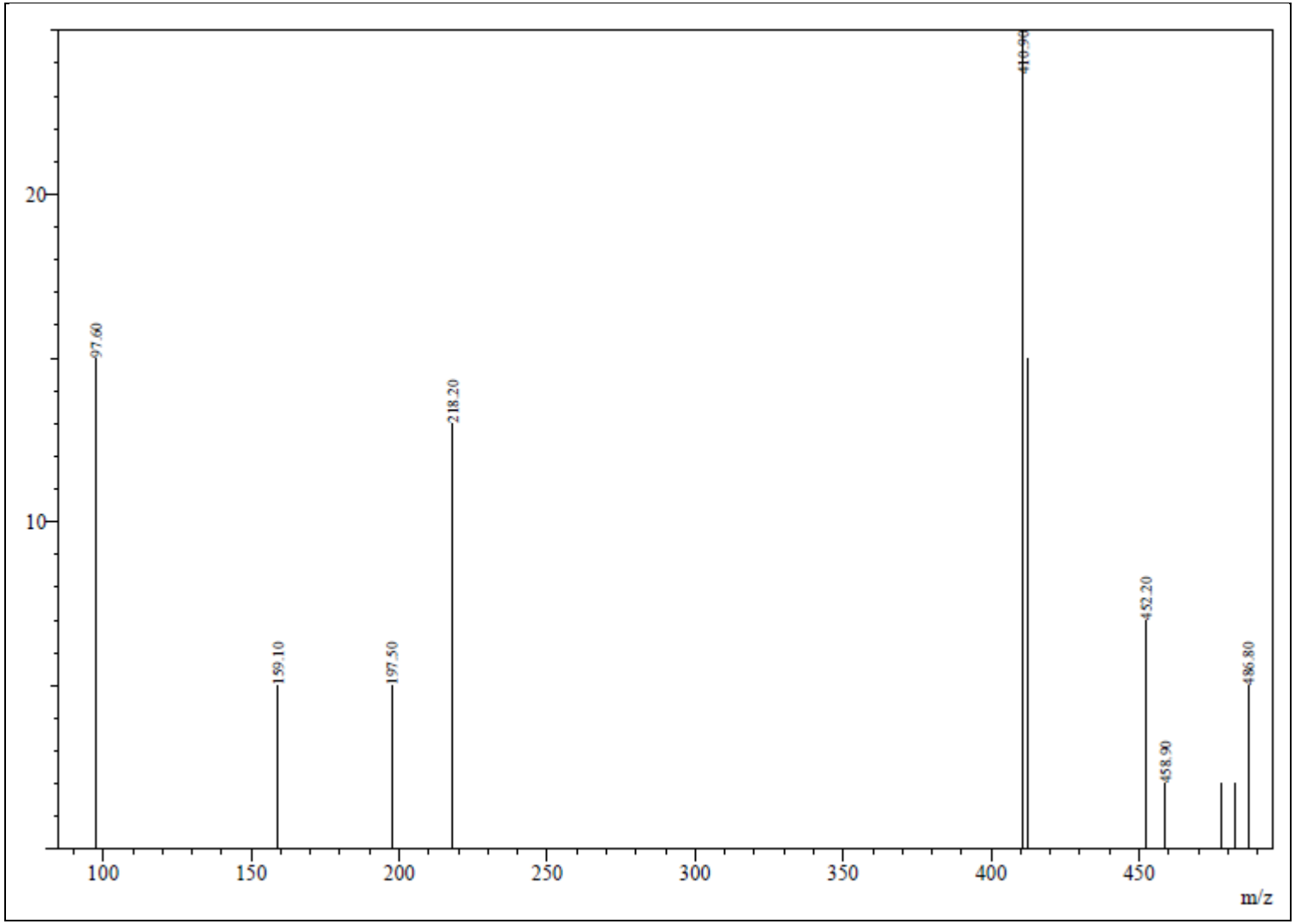

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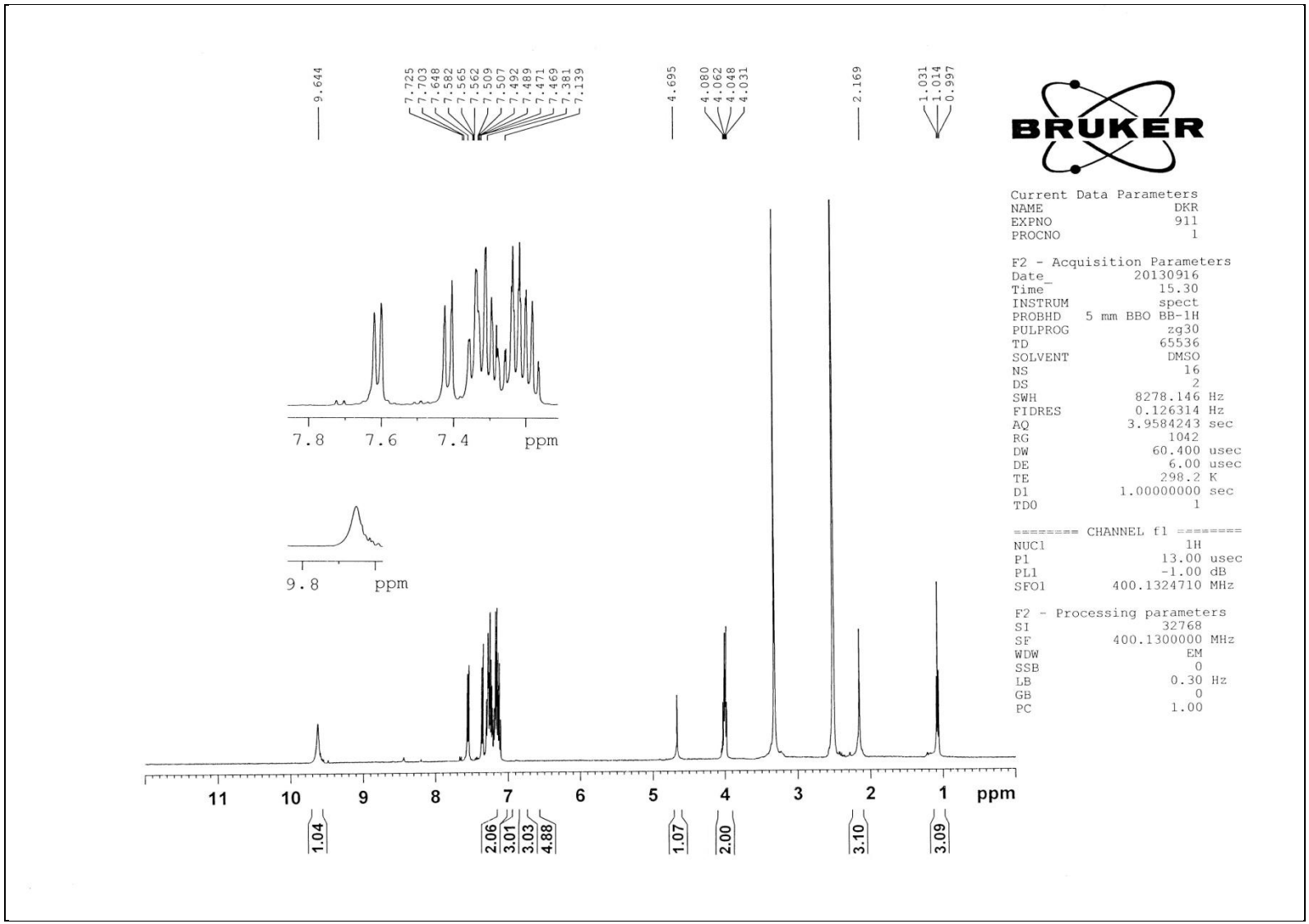

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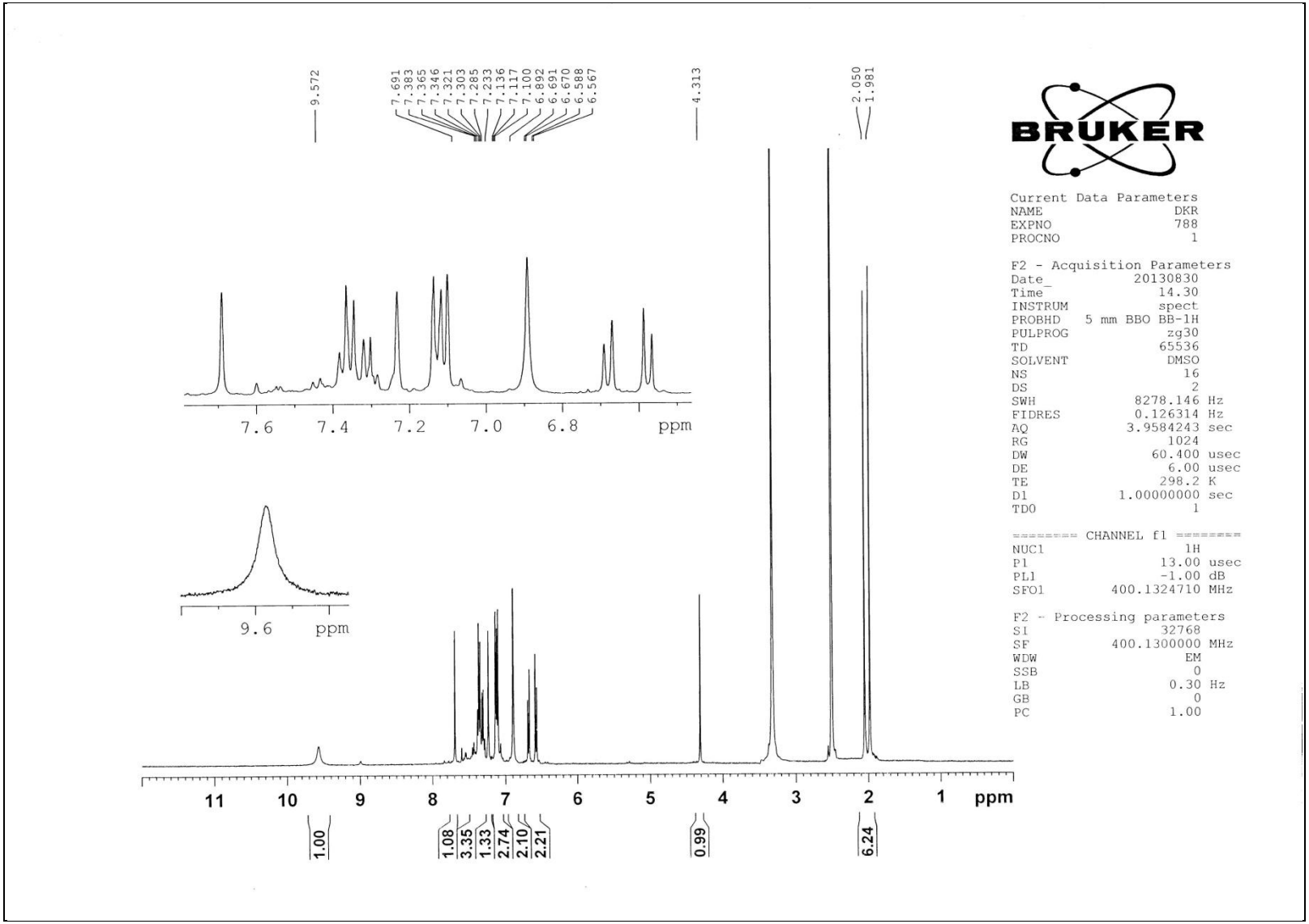

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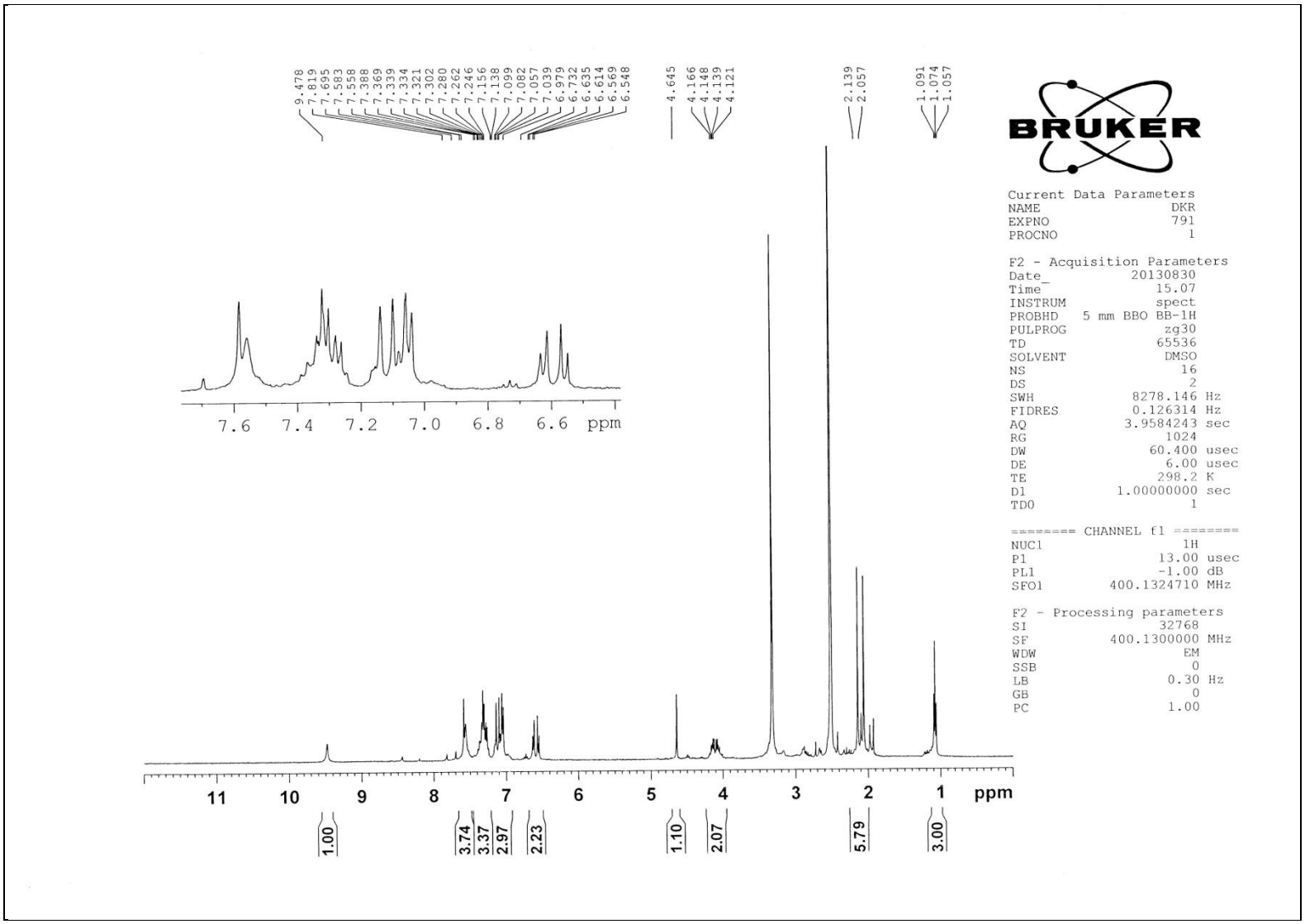

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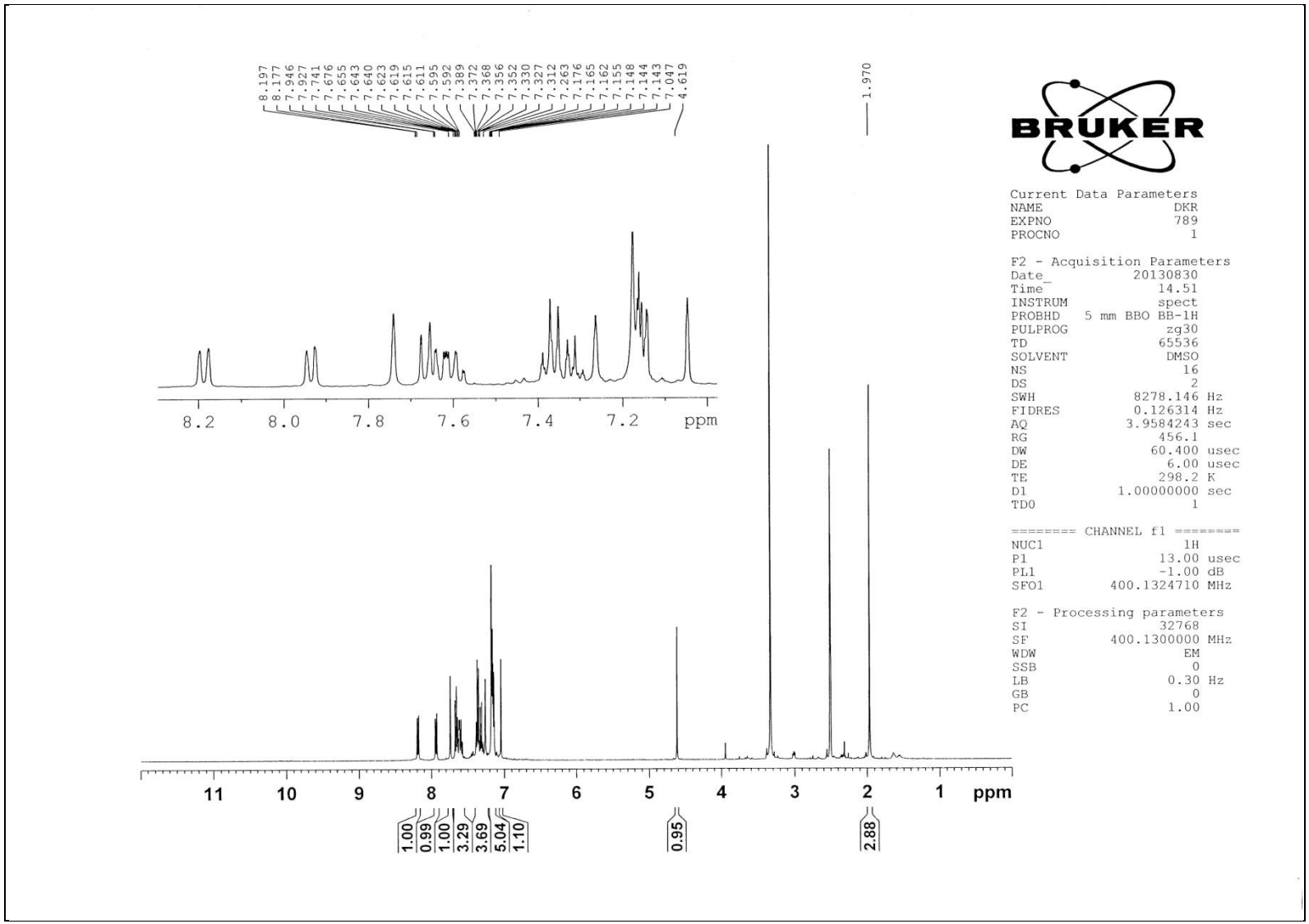

NMR spectra of compound **6x**