Electronic Supplementary Material (ESI) for New Journal of Chemistry.

SUPPLEMENTARY INFORMATION

Synthesis, optical, antioxidant and anticancer activity of benzoheterazole dendrimers with triazole bridging unit

Kannan Rajavelu,[a] Mamangam Subaraja[b] and Perumal Rajakumar*[a]

[a]Department of Organic Chemistry, University of Madras, Guindy Campus, Chennai 600 025 and

[b]Department of Biochemistry, University of Madras, Guindy Campus, Chennai 600 025, Tamilnadu, India.

Content

S-1
Abbreviations

BPA	:	Bisphenol A
$CuSO_4$. $5H_2O$	:	Copper sulphate pentahydrate
NaAsc	:	Sodium ascorbate
$CDCl_3$	:	Chloroform-d
DCM	:	Dichloromethane
DMSO-d_6	:	Dimethyl sulfoxide-d_6
UV	:	Ultraviolet
CV	:	Cyclic Voltammetry
NMR	:	Nuclear Magnetic Resonance
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
ABTS	:	2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonate)
$K_2S_2O_8$	:	Potassium persulfate
MTT	:	(3-[4,5-methylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide)
MPP	:	Mitochondrial Membrane Potential
ROS	:	Reactive oxygen species

General procedure for dendritic chlorides

A mixture of 2-(4-(azidomethyl)phenyl)benzoheterazole **9** and **10** (2.2 equiv.) and 3,5-bis(propargloxy) benzyl chloride **11** (1.0 equiv.) was dissolved in a mixture of t-BuOH and water (1:1; 20 mL) and sodium ascorbate (10 mol%) was added followed by the addition of $CuSO_4.5H_2O$ (5 mol%). The reaction mixture was stirred for 12 h at room temperature and then the solvent was evaporated, the crude product was dissolved with $CHCl_3$ (3 x100 mL), washed with water (200 mL) and brine (50 mL), dried over Na_2SO_4 and concentrated to give the crude triazole, which was purified by column chromatography (SiO_2) with $CHCl_3$ or $CHCl_3$-MeOH as eluent to give the corresponding dendritic chlorides.

Dendritic chloride 12 (G1-Cl)

Colorless solid; yield: 90%; mp: 154-156 ^oC; ^1H NMR (300 MHz, $CDCl_3$): δ_H 4.47 (s, 2H); 5.16 (s, 4H); 5.55 (s, 4H); 6.56 (s, 4H); 6.61 (s, 2H); 7.07 (d, *J* = 16.2 Hz, 2H); 7.30 (t, *J* = 8.7 Hz, 8H); 7.53 (t, *J* = 5.1 Hz, 2H); 7.59 (d, *J* = 7.2 Hz, 6H); 7.71 (t, *J* = 4.8 Hz, 3H); 7.75 (d, *J* = 16.5 Hz, 1H).^{13}C NMR (75 MHz, $CDCl_3$): δc 46.9, 53.8, 62.1, 101.9, 108.0, 110.3, 115.0, 119.9, 123.0, 124.6, 125.4, 128.2, 128.6, 135.7, 135.8, 138.2, 140.0, 142.1, 144.3, 150.4, 159.4, 162.4.

Dendritic chloride 13 (G1-Cl)

Colorless solid; yield: 87%; mp: 184-188 ^oC; ^1H NMR (300 MHz, DMSO-d_6): δ_H 4.66 (s, 2H); 5.15 (s, 4H); 5.66(s, 4H); 6.72 (s, 2H); 7.37 (d, *J* = 7.5 Hz, 4H); 7.45 (d, *J* = 7.5 Hz, 2H); 7.52 (t, *J* = 7.8 Hz, 2H); 7.64 (s, 4H); 7.79 (d, *J* = 7.5 Hz, 4H); 7.98 (d, *J* = 7.8 Hz, 2H); 8.09 (d, *J* = 7.5 Hz, 2H); 8.33 (s, 2H).^{13}C NMR (75 MHz, DMSO-d_6): δc 45.9, 52.5, 61.2, 101.4, 107.9, 122.2, 122.5, 124.7, 125.4, 126.5, 128.0, 128.4, 134.0, 135.0, 136.6, 137.1, 139.7, 142.8, 153.4, 159.1, 166.2.

General procedure for dendritic azides

To the corresponding dendritic chloride **12** and **13** (1.0 equiv.) in dry DMF (10 mL), sodium azide (1.5 equiv.) was added and stirred at room temperature for 24 h. The reaction mixture was poured into the water (50 mL) and extracted with $CHCl_3$ (3 x100 mL). The organic layer was washed with water (100 mL) and brine (50 mL), dried over Na_2SO_4. The solvent was evaporated under reduced pressure to afford the crude product, which was purified by column chromatography (SiO_2), using $CHCl_3$ or $CHCl_3$-MeOH as eluent to give the corresponding first generation dendritic azides.

Dendritic azide 14 (G1-N_3)

Colorless solid; yield: 87%; mp: 168-172 ^oC; ^1H NMR (300 MHz, DMSO-d_6): δ 4.34 (s, 2H); 5.14 (s, 4H); 5.65 (s, 4H); 6.64 (s, 2H); 6.72 (s, 1H); 7.36 (t, *J* = 7.2 Hz, 9 H); 7.70-7.80 (m, 10 H); 8.23 (s, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6): δc 40.1, 52.5, 61.1, 101.1, 107.4, 110.5, 114.1, 119.5, 124.7, 124.8, 125.5, 128.2, 128.4, 134.7, 137.4, 137.8, 138.7, 141.5, 142.8, 149.7, 159.2, 162.2.

Dendritic azide 15 (G1-N_3)

Colorless solid; yield: 86%; mp: 164-168 ^oC; ^1H NMR (300 MHz, $CDCl_3$): δ 4.22 (s, 2H); 5.15 (s, 4H); 5.52 (s, 4H); 6.53 (s, 2H); 6.57 (s, 1H); 7.27 (d, *J* = 7.8 Hz, 4H); 7.35 (d, *J* = 5.7 Hz, 2H); 7.40 (s, 2H); 7.44 (d, *J* = 5.9 Hz, 3H); 7.50 (d, *J* = 5.7 Hz, 1H); 7.56 (t, *J* = 8.1 Hz, 6H); 7.84 (d, *J* = 7.8 Hz, 2H), 7.98 (d, *J* = 8.1Hz, 2H).^{13}C NMR (75 MHz, $CDCl_3$): δc 53.8, 54.6, 62.1, 101.7, 107.5, 121.5, 122.8, 123.0, 125.5, 126.4, 128.0, 128.6, 134.4, 135.4, 136.0, 136.3, 137.8, 144.3, 153.8, 159.6, 166.5.

General procedure for synthesis of bis(propargyloxy) core

A mixture of the corresponding phenol (1.0 equiv.), propargyl bromide (2.1 equiv.) and anhydrous K_2CO_3 or Cs_2CO_3 (5.0 equiv.) in dry DMF (10 mL) was stirred for 48 h at

room temperature. The reaction mixture was then poured into ice water (50 mL). The resulting precipitate was filtered, washed thoroughly with water (100 mL) and residue was dissolved in $CHCl_3$ (3 x 100 mL). The combined organic layer was washed with brine (50 mL), dried over Na_2SO_4 and evaporated to give the crude product, which was purified by column chromatography (SiO_2) using the eluent as mentioned under each compound.

Compound 17

Colorless liquid; yield: 90%; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.65 (s, 6H); 2.48 (t, J = 2.1 Hz, 2H); 4.63 (s, 4H); 6.86 (d, J = 8.4 Hz, 4H); 7.14 (d, J = 6.9 Hz, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δc 31.0, 41.8, 55.8, 75.3, 78.8, 114.3, 127.8, 143.9, 155.5.

Compound 19

Colorless solid; yield: 80%; mp: 98-100 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 2.45 (s, 2H); 3.85 (s, 6H); 4.51 (d, J = 2.1 Hz, 4H); 6.55 (s, 2H); 6.58 (s, 2H); 7.54 (d, J = 8.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δc 55.5, 56.5, 75.8, 78.3, 100.1, 105.7, 124.4, 132.4, 158.0, 163.2, 192.3.

DPPH radical scavenging activity

The antioxidant properties of the benzoheterazole dendrimers were determined on the basis of their free radical scavenging activity. This was measured in vitro by using the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH). DPPH generates stable free radical containing an odd electron and is usually utilized for the detection of radical scavenging properties in chemical analysis. A 200 μM solution of DPPH in methanol was prepared and added to test tubes containing the samples to be analysed at different concentrations (5–25 μg/mL). All the tubes were incubated for 30 min and then the absorbance was measured at 517 nm by using a Beckman UV/Vis spectrophotometer. The percentage of the free radical scavenging activity at different concentrations of the compounds was determined with ascorbic acid as the

standard. The DPPH absorbs at 517 nm, and its concentration is reduced by the existence of an antioxidant, which is reflected in the reduction of absorbance. The following formula is used to calculate the percentage of radical scavenging [Eq. (1)].

DPPH Radical scavenging (%) = $[(A_{control} - A_{sample})/ A_{control} \times 100$ ----------Eq. (1)

ABTS radical scavenging assay

A stock solution of the ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) radical cation was prepared by dissolving ABTS (7 mM) with potassium persulfate ($K_2S_2O_8$, 2.45 mM). The mixture was left to stand in the dark at room temperature for 16 h (the time required for formation of the radical) before use. For the evaluation of ABTS radical scavenging activity, the working solution was prepared by diluting the previous solution in ethanol to obtain the absorbance of 0.700 ± 0.02 at 734 nm. The solvent extracts (0.1 mL) at different concentrations were mixed with the ABTS working solution (1.9 mL) and the reaction mixture was allowed to stand at 30 °C for 6 min and then the absorbance was measured by using a UV-visible spectrophotometer at 734 nm, at which point the antioxidants present in the extracts began to inhibit the radical, producing a reduction in absorbance, with a quantitative relationship between the reduction and the concentration of antioxidants present in the tested sample. The radical scavenging activities of the extracts were compared with that of the vitamins C and percentage inhibition is calculated [Eq. (2)].

ABTS Radical scavenging (%) = $[(A_{control} - A_{sample})/ A_{control} \times 100$ ---------- Eq. (2)

Evaluation of Cell proliferation by MTT assay

The number of the viable A549 lung cancer cells benzothiazole dendrimer **8** was treated by the MTT (3-[4,5-methylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay. A549 lung cancer cells(1×10^4 cells/well) were seeded in a 96-well plate and kept overnight for attachment. The next day the medium was replaced with fresh medium with various concentrations of the benzothiazole dendrimer **8** (100–300 μM) and cells were allowed to

grow for 12 and 24 h. Four hours before completion of incubation, 10 µl of MTT (10 mg/ml) was added in each well. After completing the incubation, 100 µl of the solubilisation buffer (10% SDS with 0.01 N HCl) was added to each well and incubated overnight at room temperature. Colour appeared after the reaction was measured at 550 nm using Bio-Tek microplate reader.

Evaluation of A549 cell count and viability by trypan blue dye

A549 lung cancer cells (5×10^5) were seeded in each well of the 6-well plate and kept overnight for attachment. Next day old medium was replaced with fresh medium containing 0, 100 and 200 µM of the benzothiazole dendrimer **8** and incubated for 24 h. Following incubation, the supernatant pool was collected and adherent cells were trypsin zed and collected. Cell viability was performed by the dye exclusion test with 0.5% trypan blue using a hemocytometer.

Clonogenic assay

A549 lung cancer cells (5×10^5) were seeded per well in a 6-well plate and allowed to attach overnight. The next day medium was replaced with fresh medium containing different concentration (0, 50, 100, 200 µM) of the benzothiazole dendrimer **8** and incubated for 2 h. Following incubation, cells were harvested by trypsinisation and counted. For clonogenic assay of A549 cells in 4 mL medium were seeded in 6-well plate and were incubated at 37°C for 12 days without any disturbances. Following incubation, the medium was removed and colonies were fixed and stained with 0.5% methylene blue (dissolved in 50% ethanol). Colony counting was performed with cell and Analyst software (AssaySoft, Inc., CA). The experiment was carried out twice in triplicates.

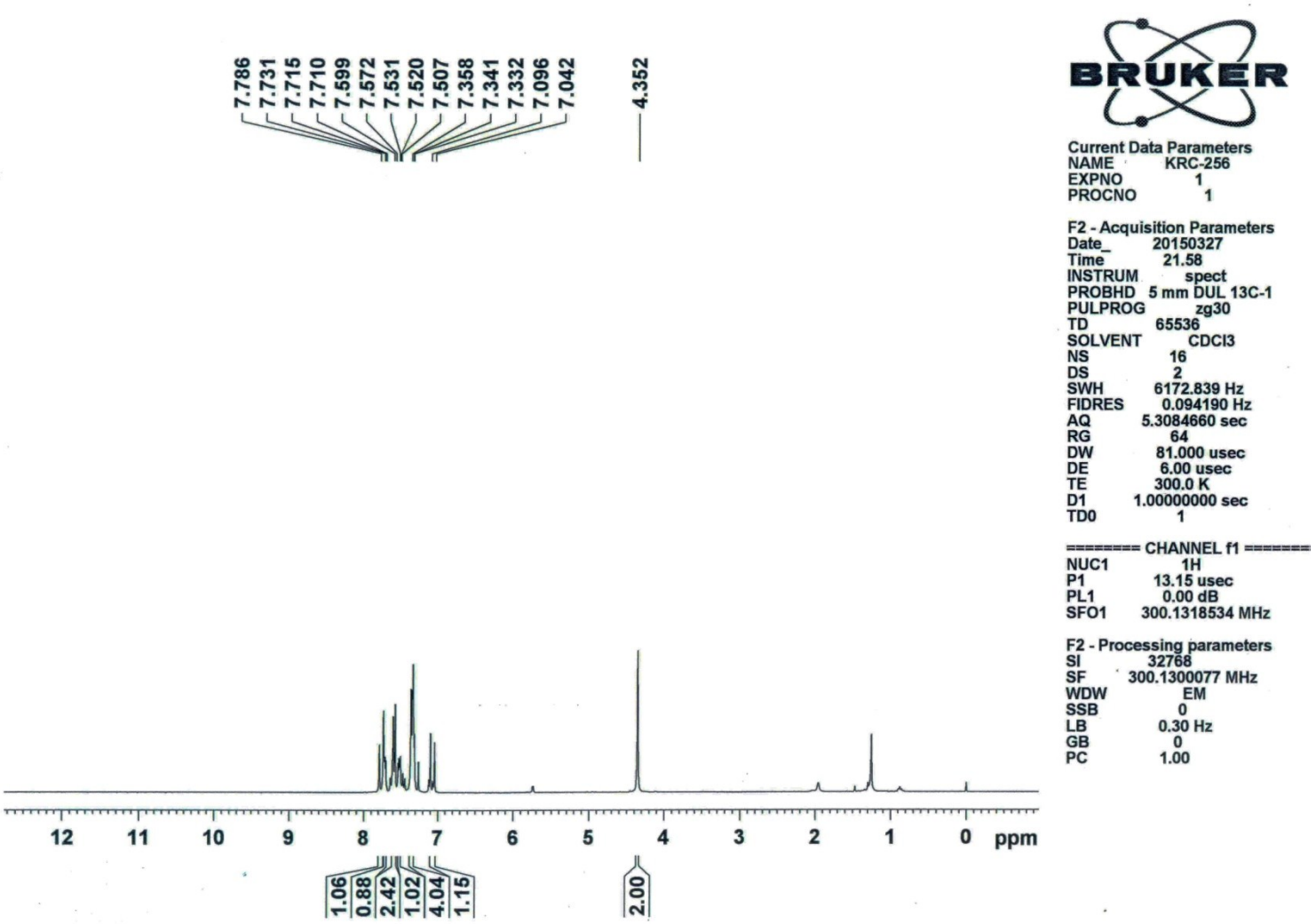
Detection of intracellular ROS levels

For measuring total ROS level in the cells, 5×10^5 cells were seeded in a 6-well plate and allowed to attach overnight. The next day, medium was replaced by fresh medium containing 0, 100 and 200 μM of the benzothiazole dendrimer **8** and allowed to incubate for 12 and 24 h. Following incubations, the cells were harvested by trypsinization and washed with PBS and re suspended in PBS containing 10 μM DCFH-DA. Equal number of the cells in 100 μl of DCFH-DA containing PBS were distributed in each well of 96-well fluorimetry plate and incubated at 37°C for 45 min. The plate was read using an excitation of 485 nm and emission at 520 nm and graph was plotted denoting the change in ROS level.

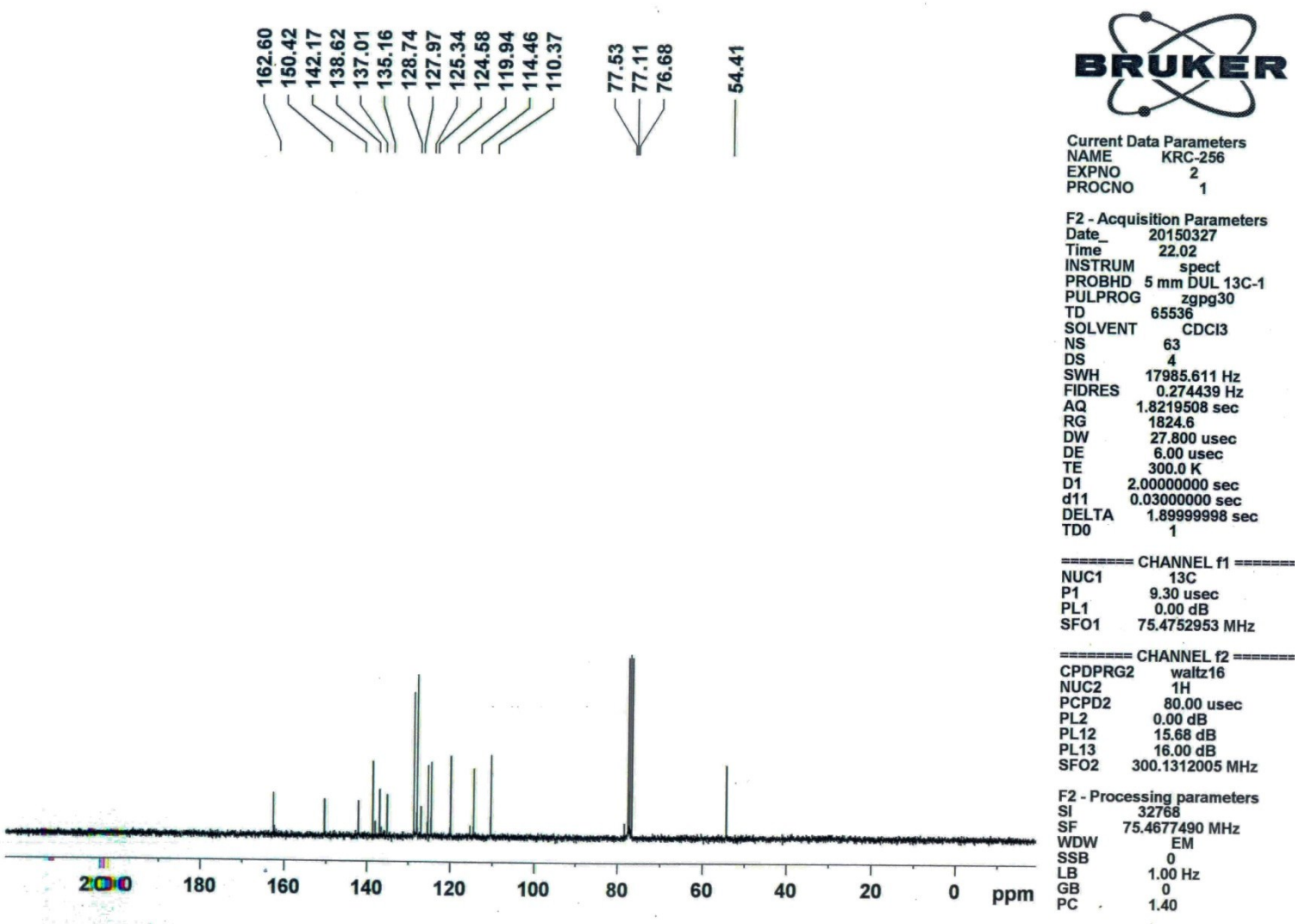
Assessment of mitochondrial membrane potential

For assessing the mitochondrial membrane potential, A549 lung cancer cells (5×10^5) were seeded in 6-well plates and incubated overnight for attachment. Next day old medium was replaced with fresh medium containing 200 μM of the benzothiazole dendrimer **8** and incubated for 12 h. Cells were collected by trypsinization and resuspended in 1.0 mL of DiO6 (3,3'-dihexyloxacarboxyanine iodide) solution (40 nM DiO6 in 1 × PBS) and incubated for 30 min at room temperature. Fluorescence intensity (Ex/Em: 488/525) was measured using microtiter plate reader.

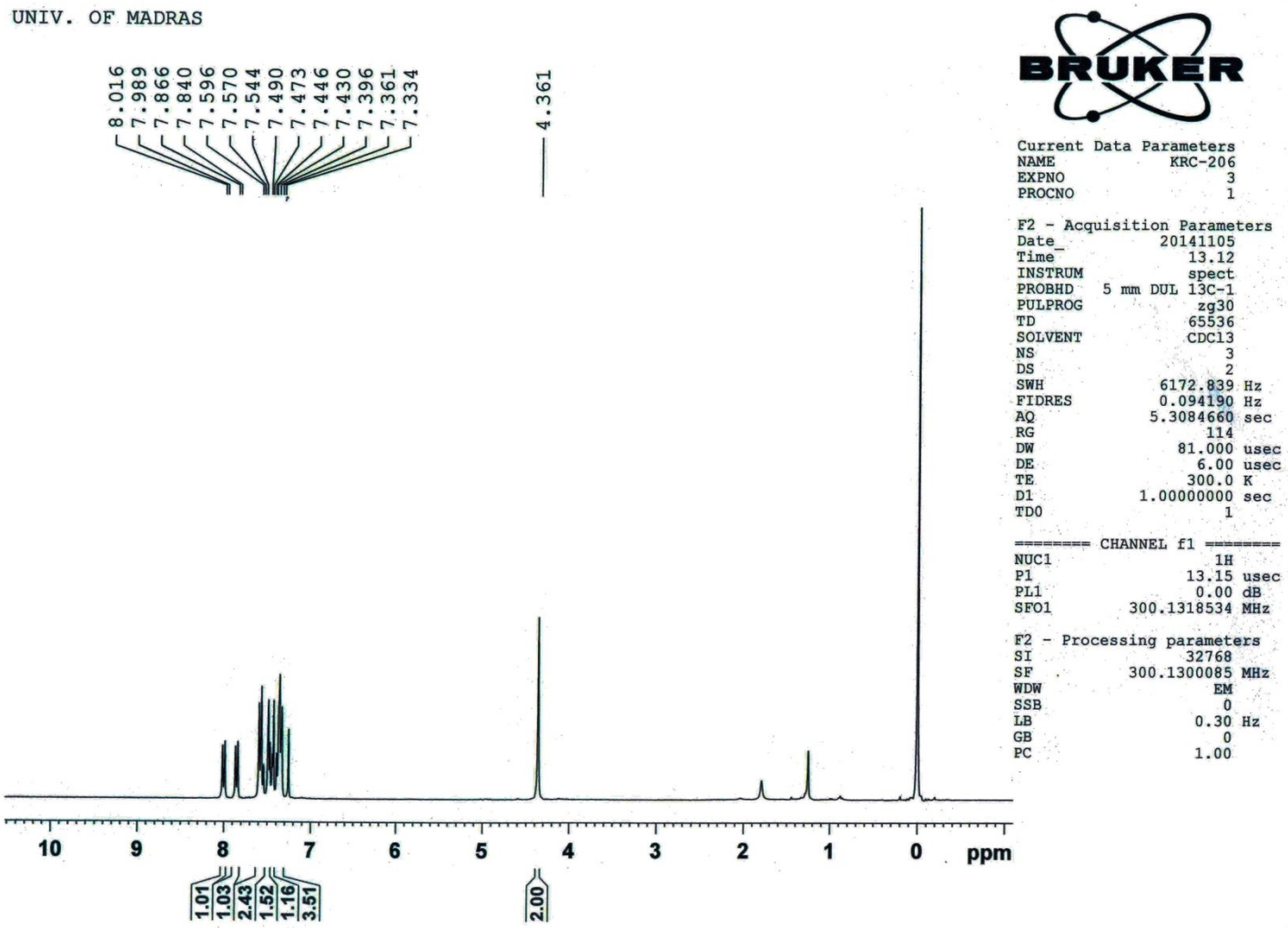
Spectroscopic data



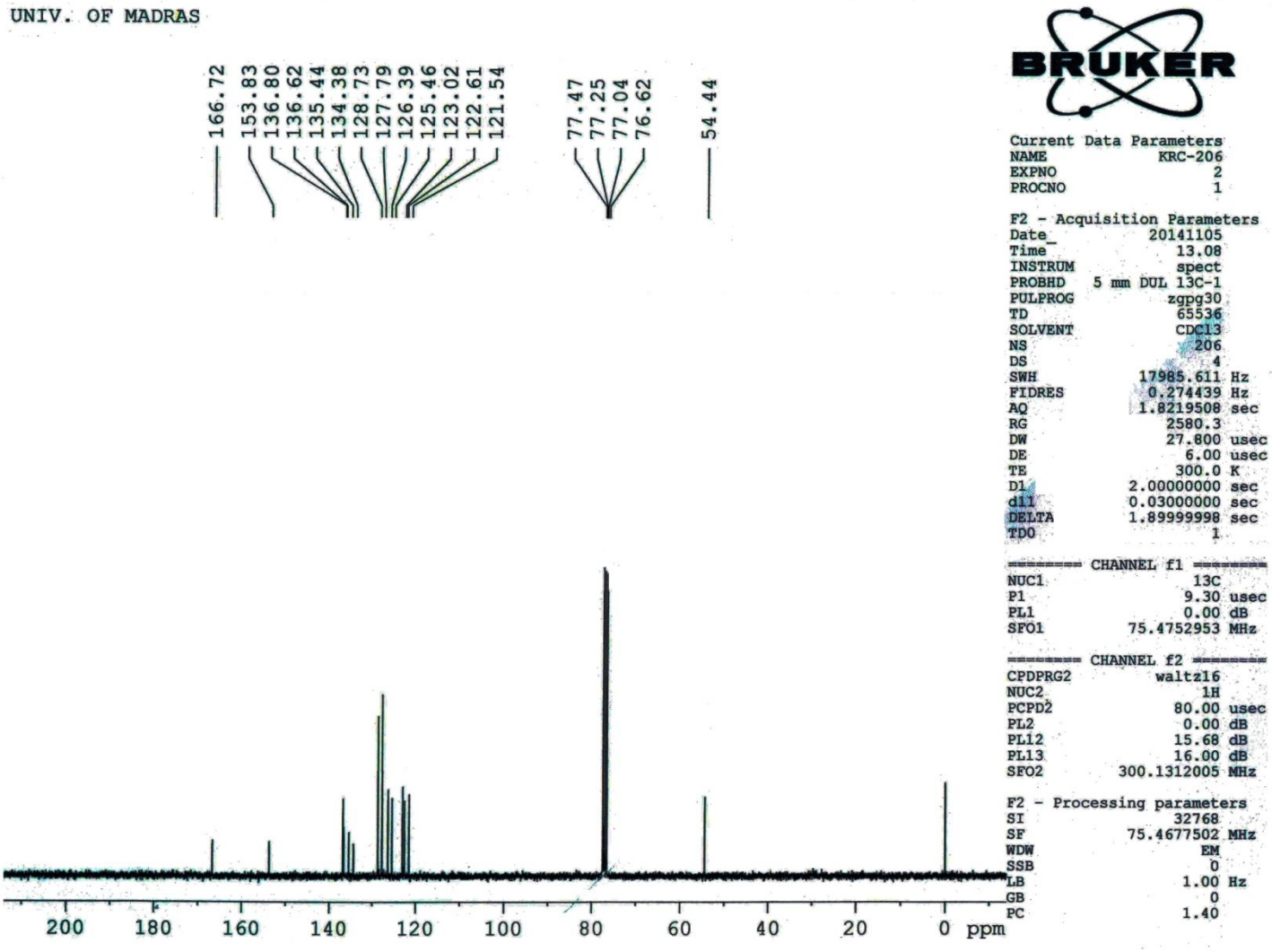
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the compound **9**



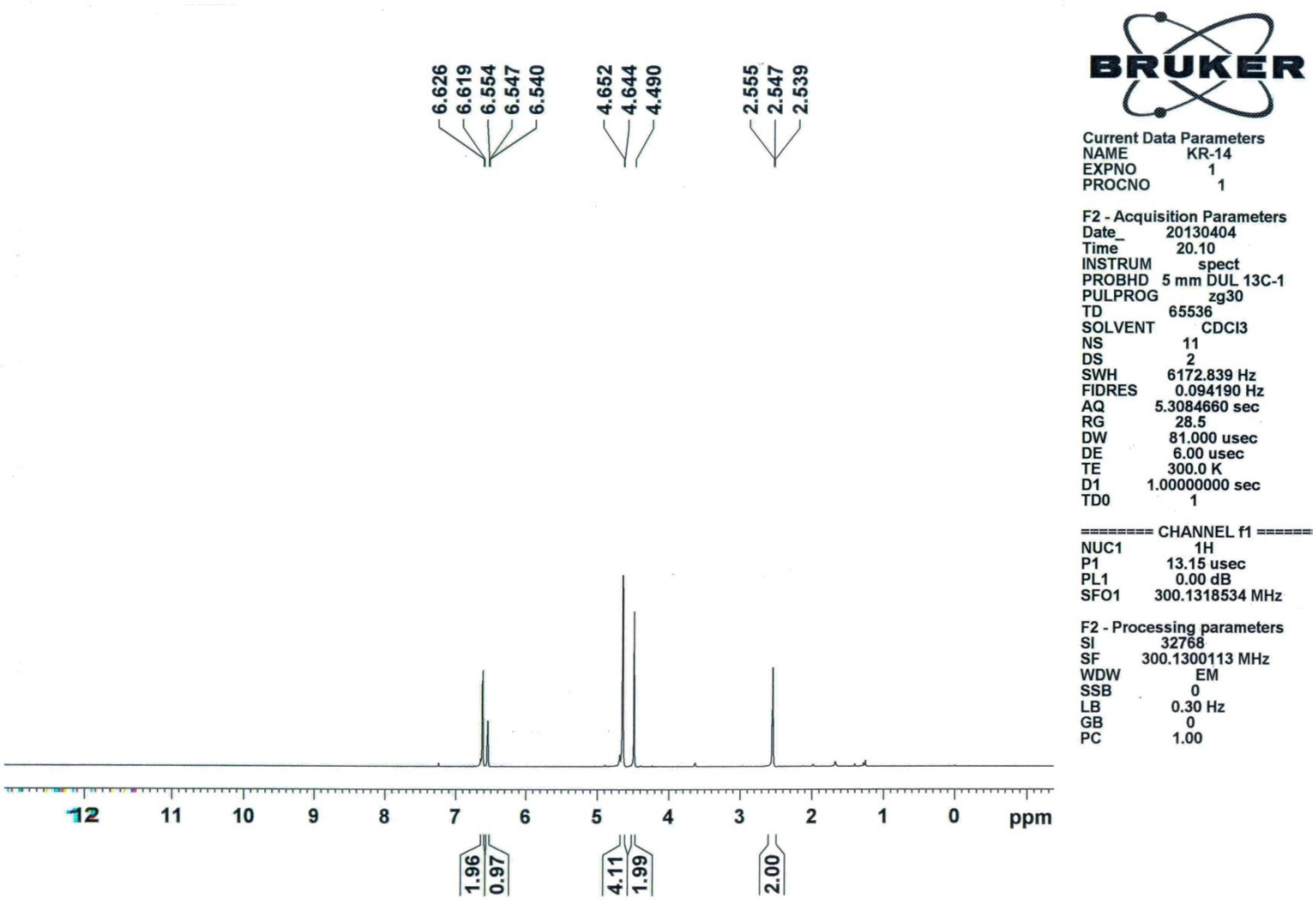
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the compound **9**



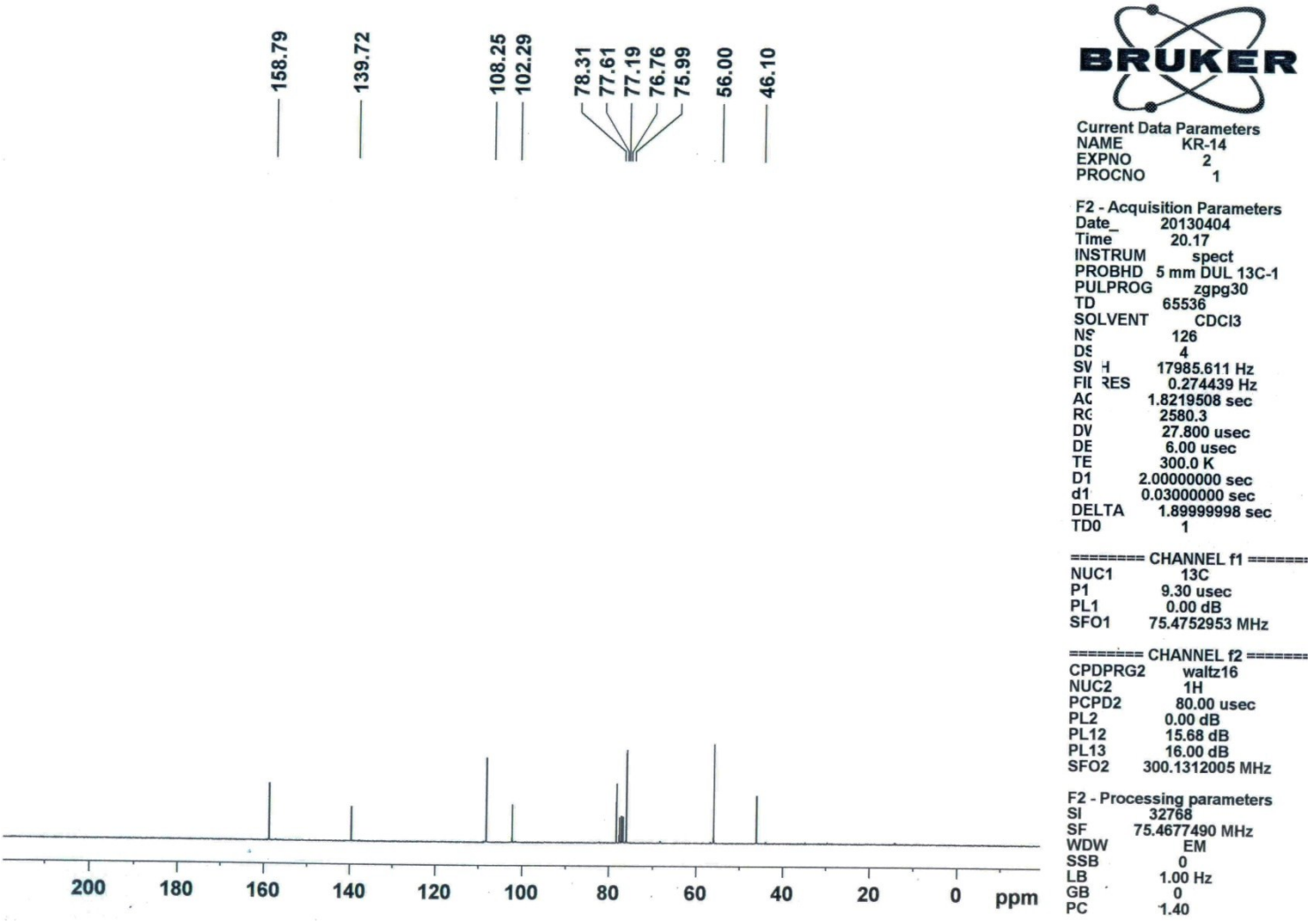
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the compound **10**



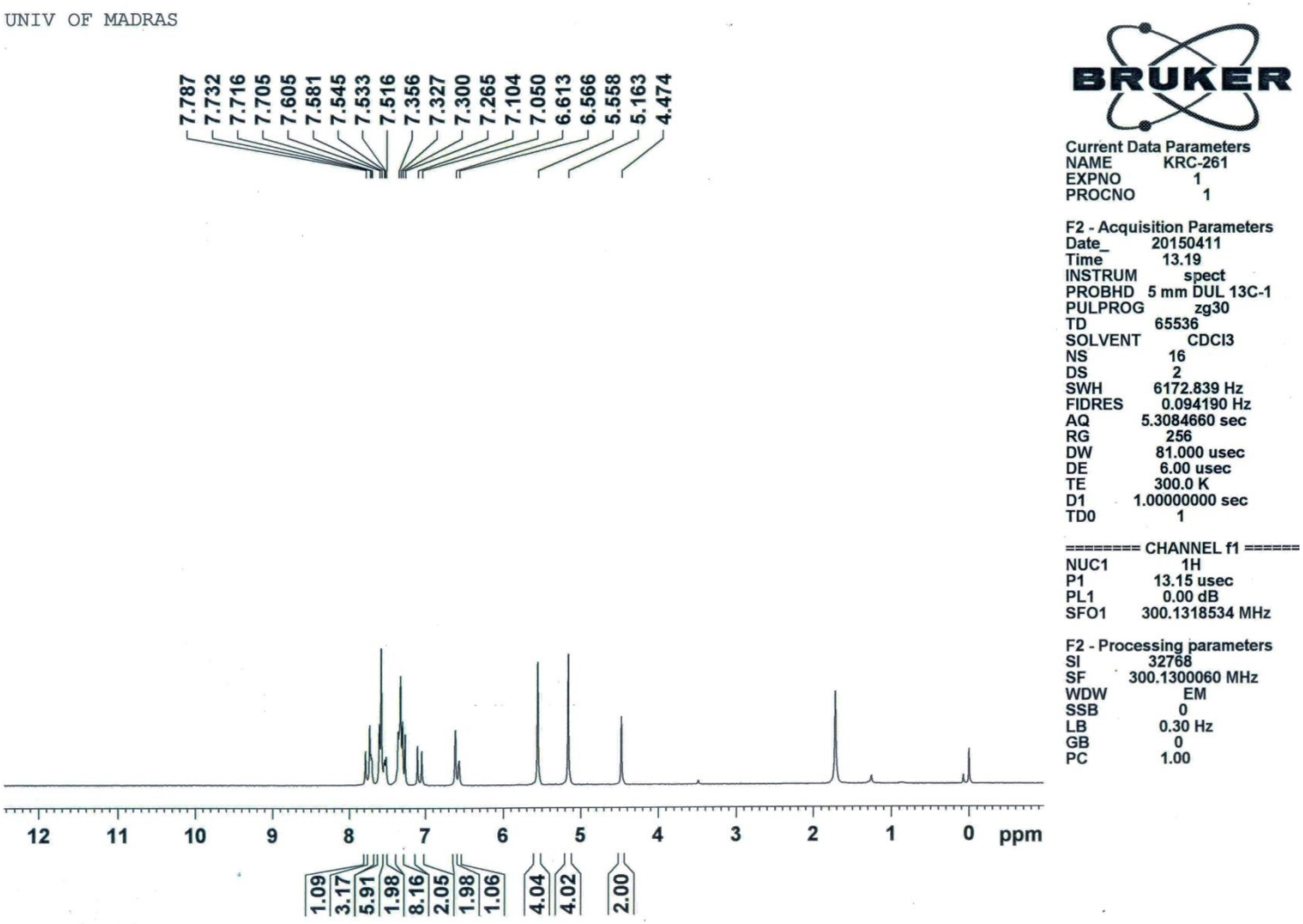
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the compound **10**



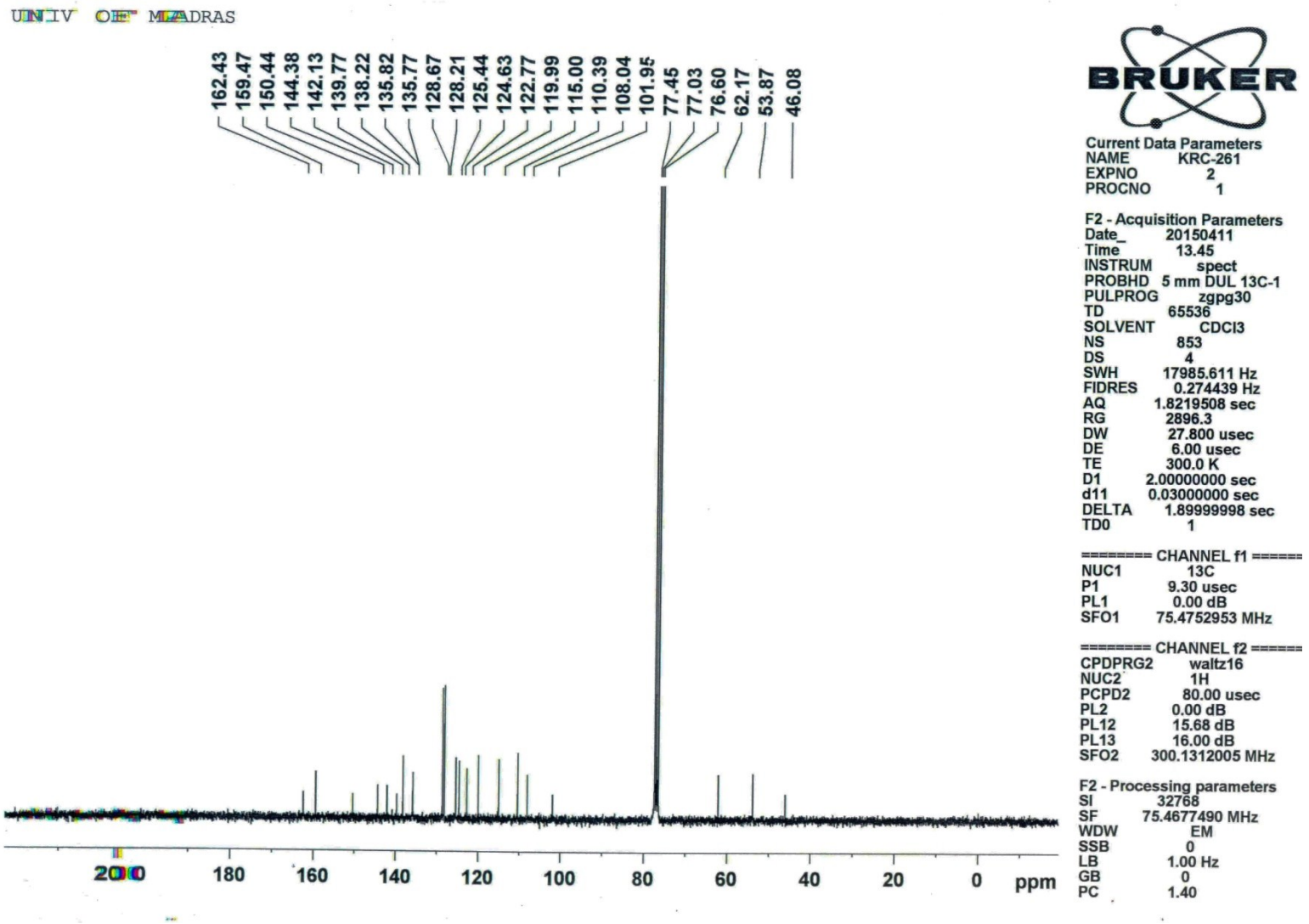
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the compound **11**



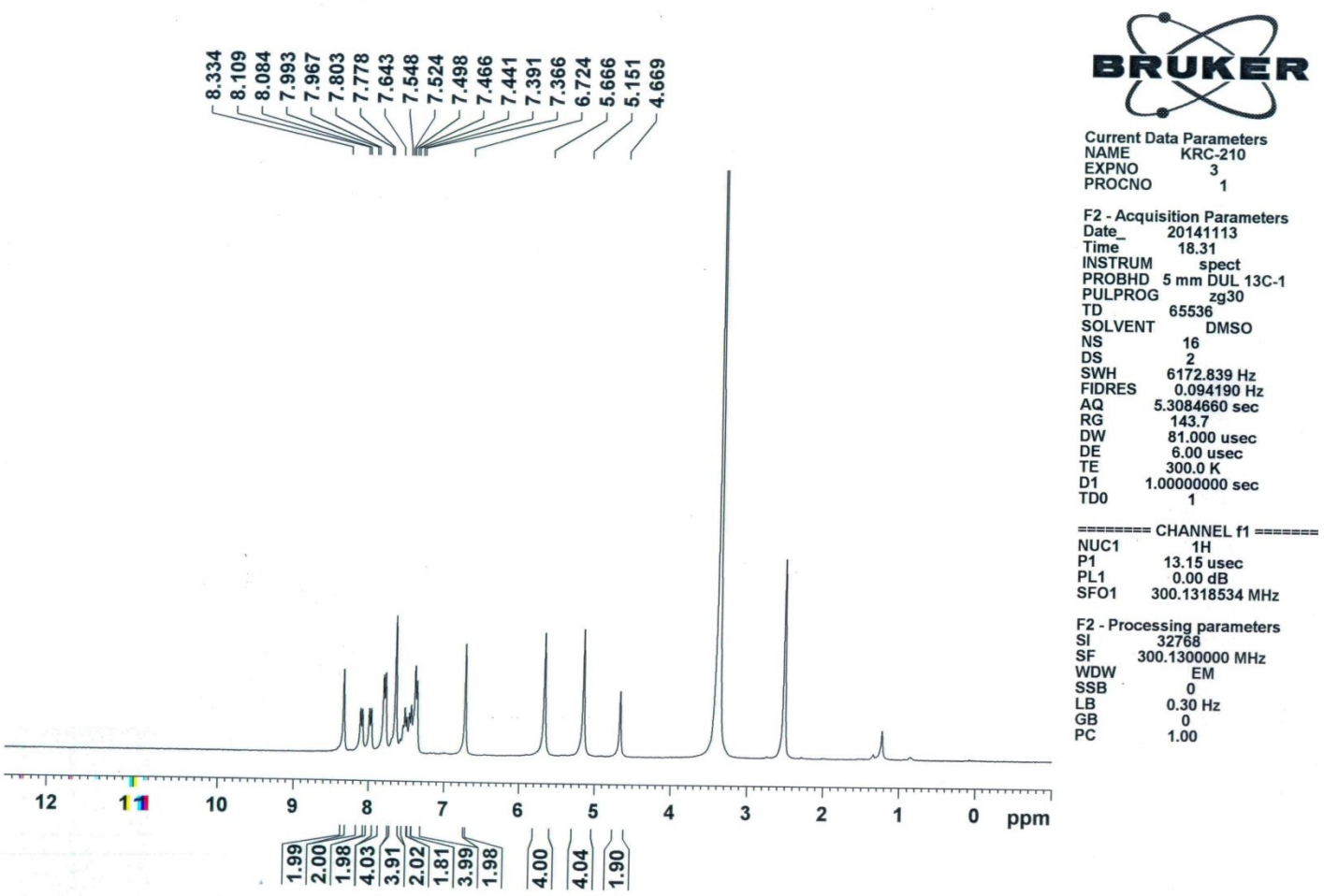
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the compound **11**



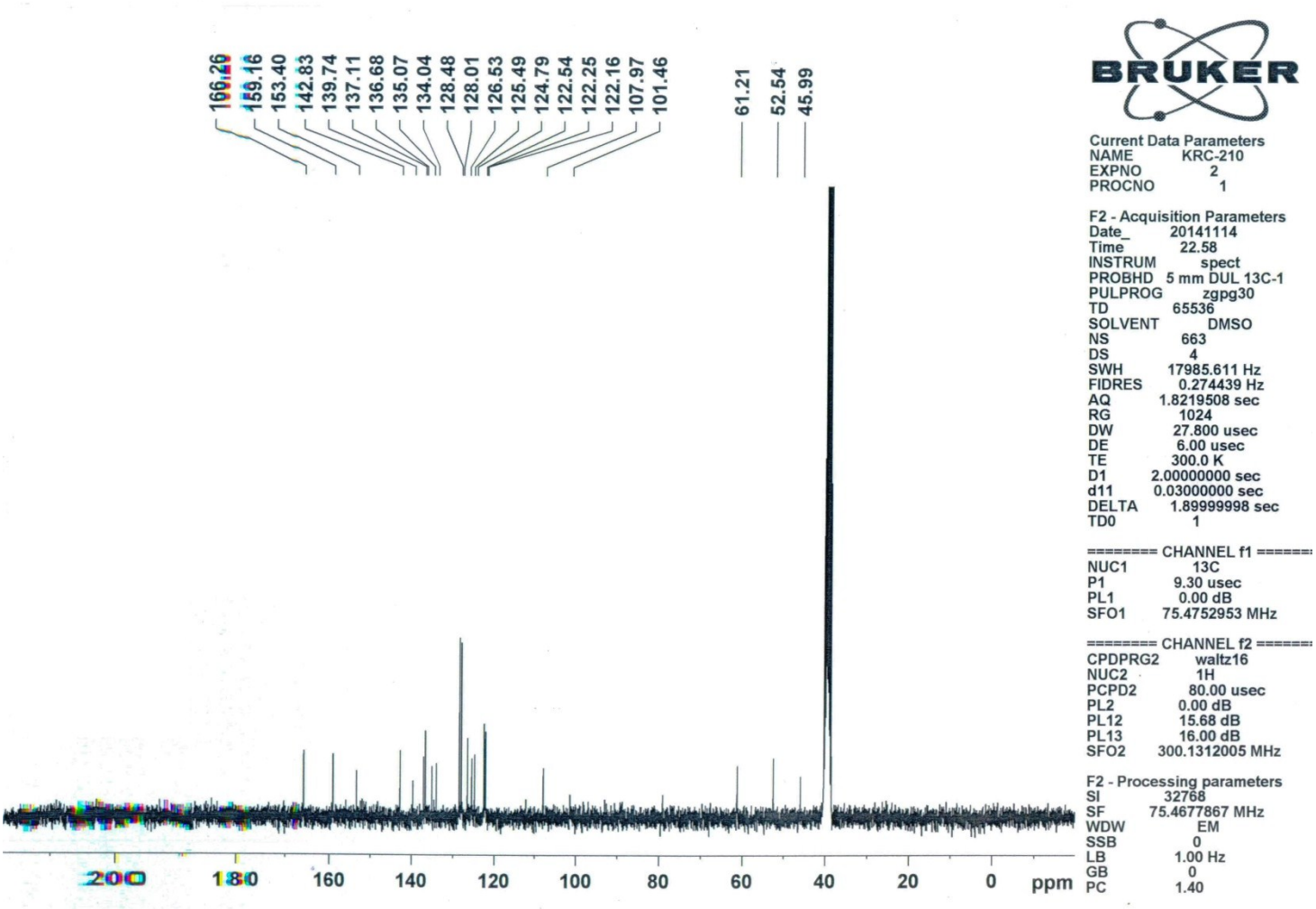
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the dendritic chloride **12**



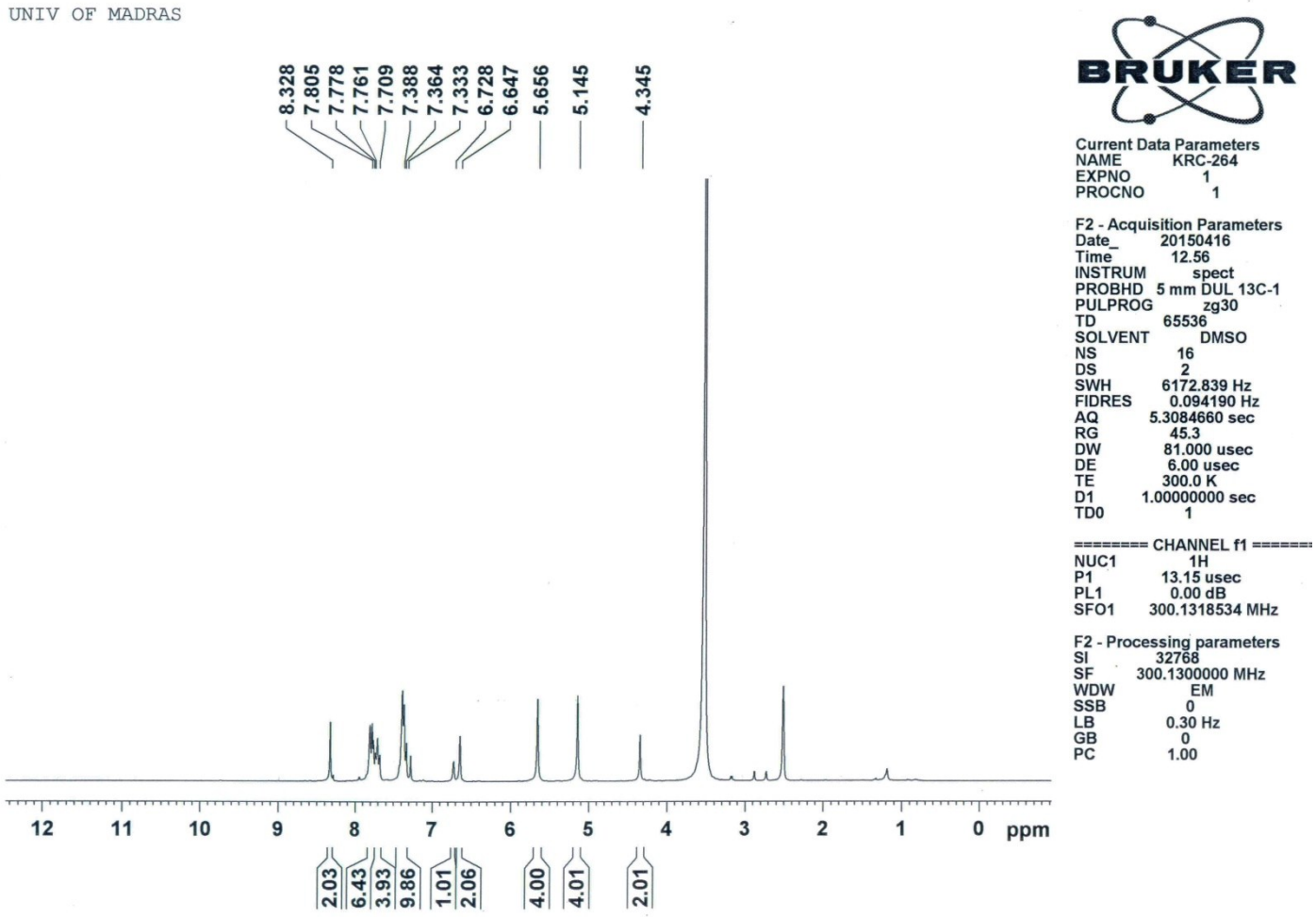
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the dendritic chloride **12**



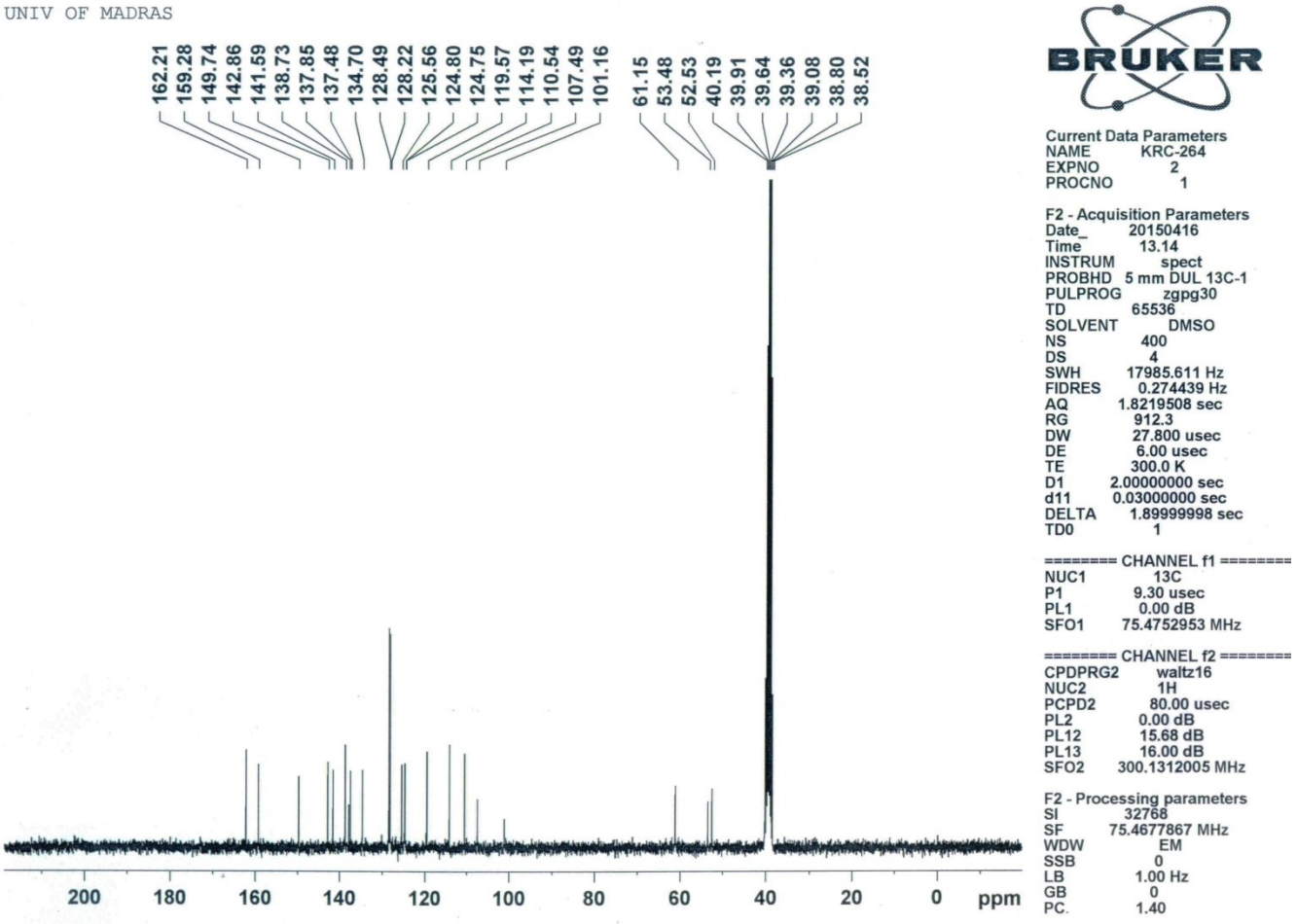
^{1}H NMR (300 MHz, DMSO-d_6) spectrum of the dendritic chloride **13**



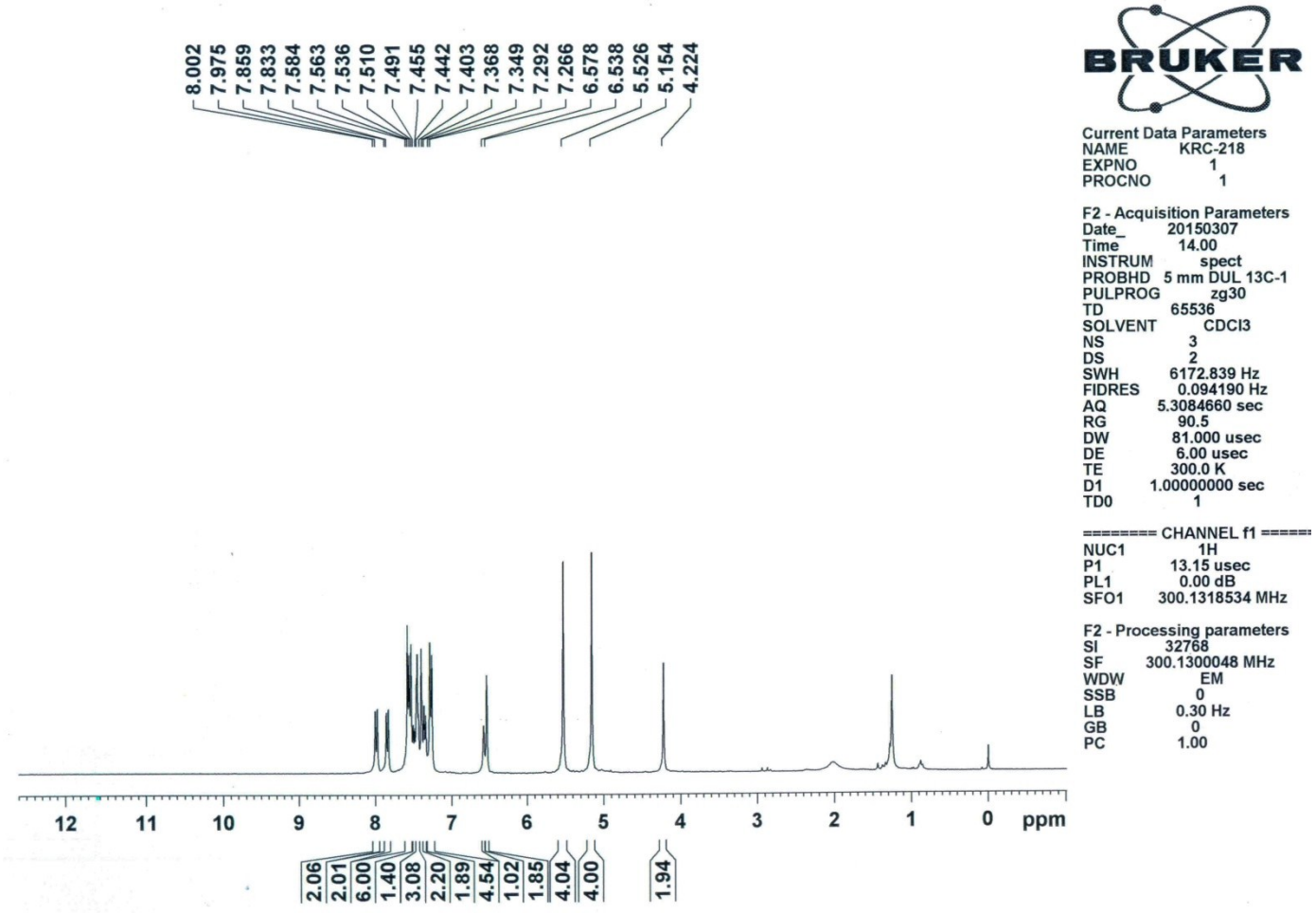
^{13}C NMR (75 MHz, DMSO-d_6) spectrum of the dendritic chloride **13**



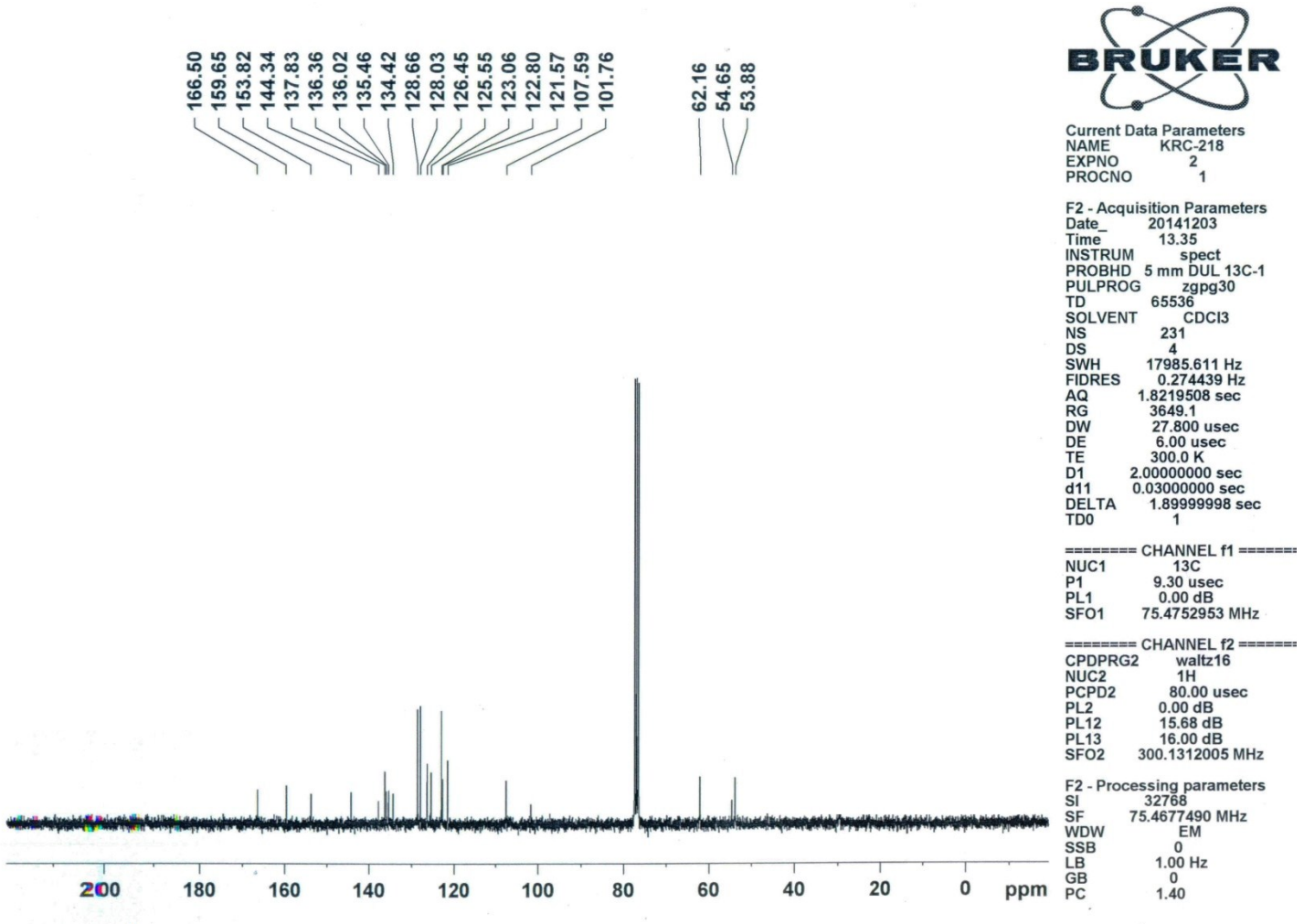
^{1}H NMR (300 MHz, DMSO-d_6) spectrum of the dendritic azide **14**



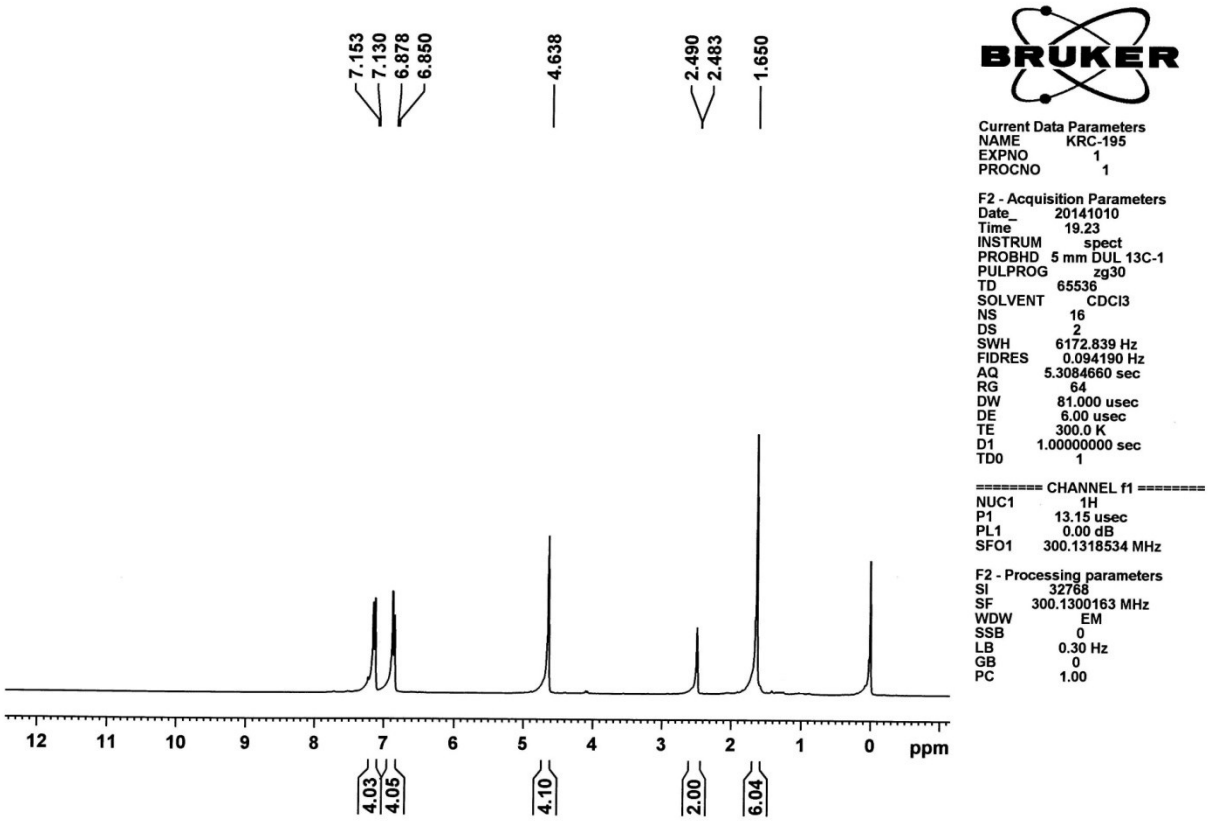
^{13}C NMR (75 MHz, DMSO-d_6) spectrum of the dendritic azide **14**



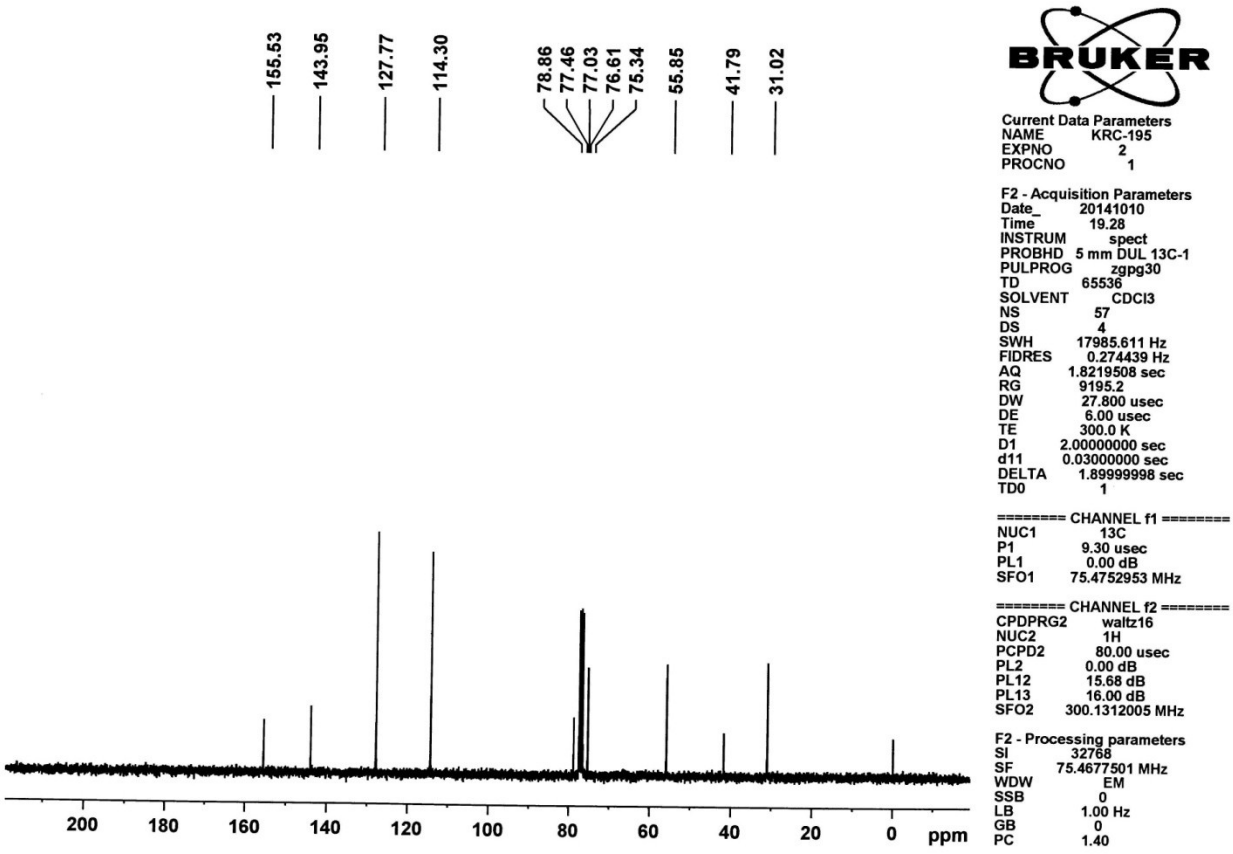
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the dendritic azide **15**



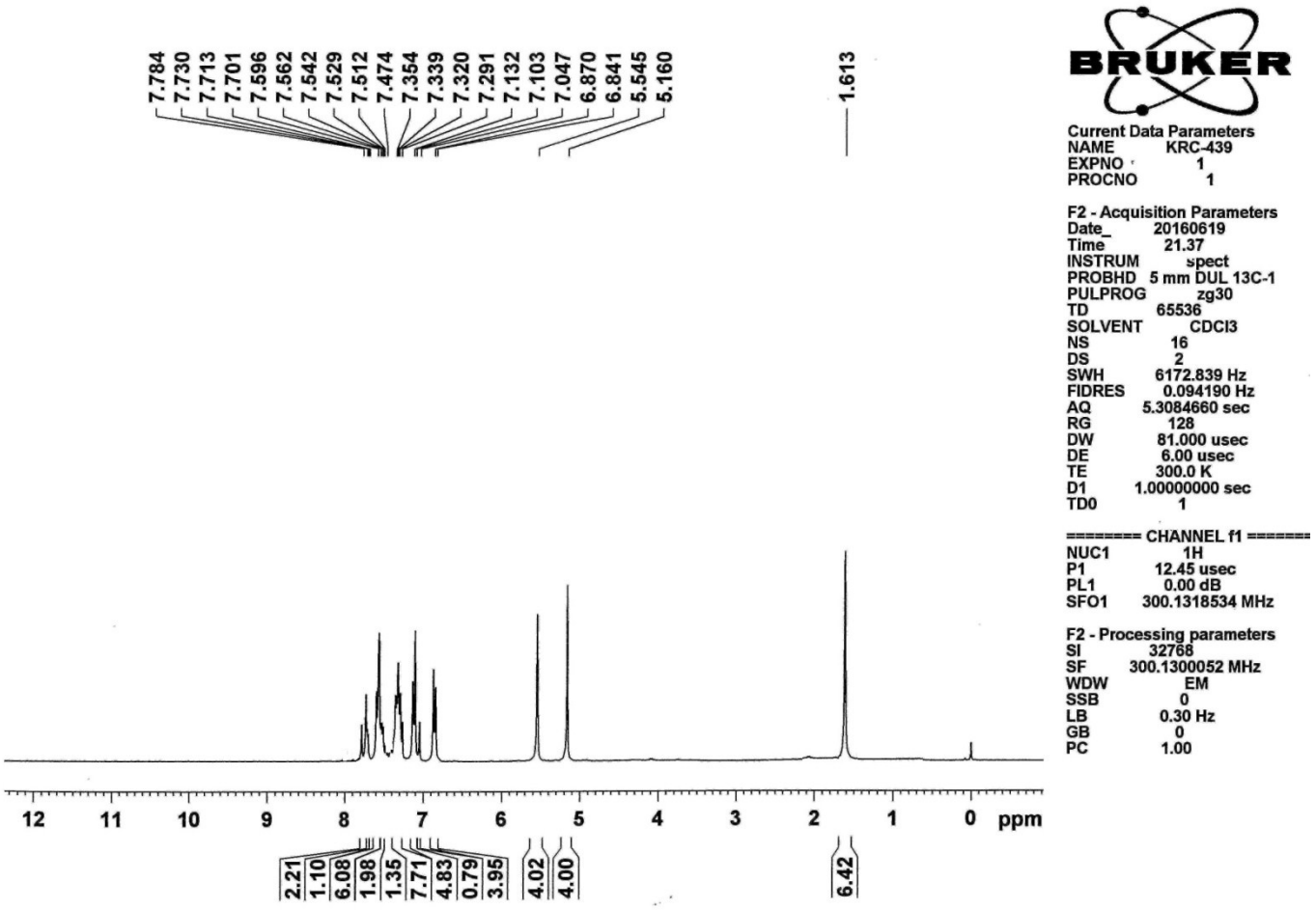
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the dendritic azide **15**



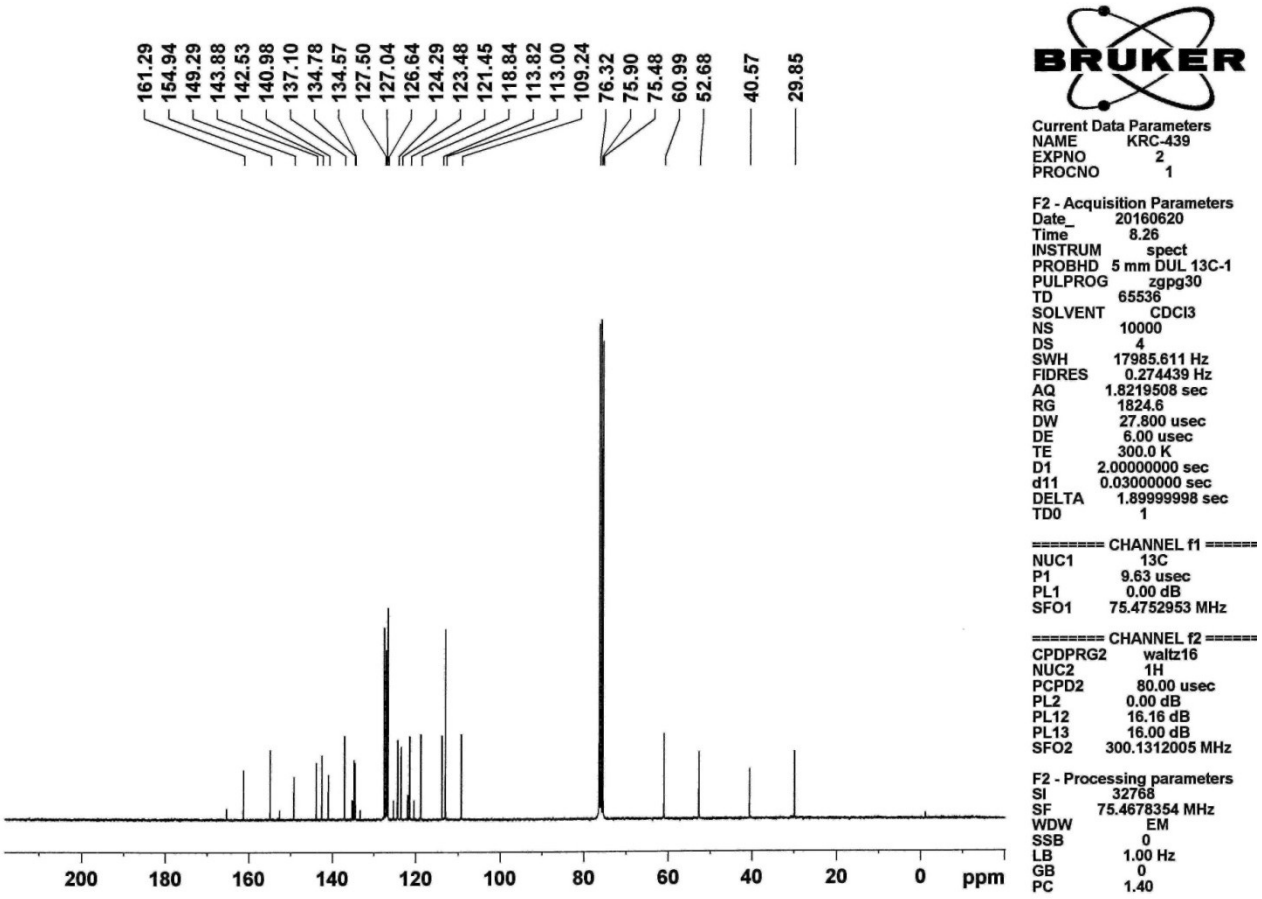
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the compound **17**



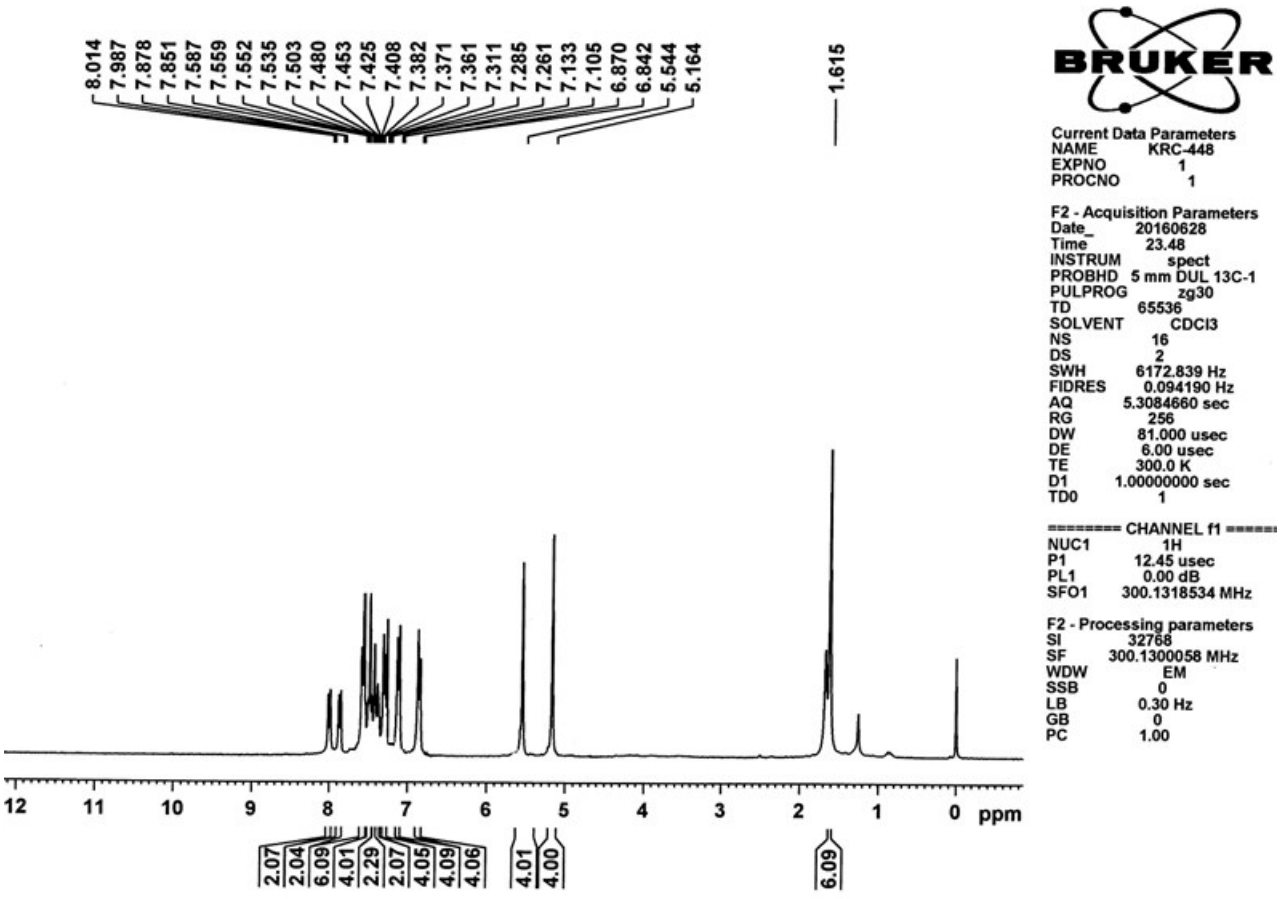
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the compound **17**



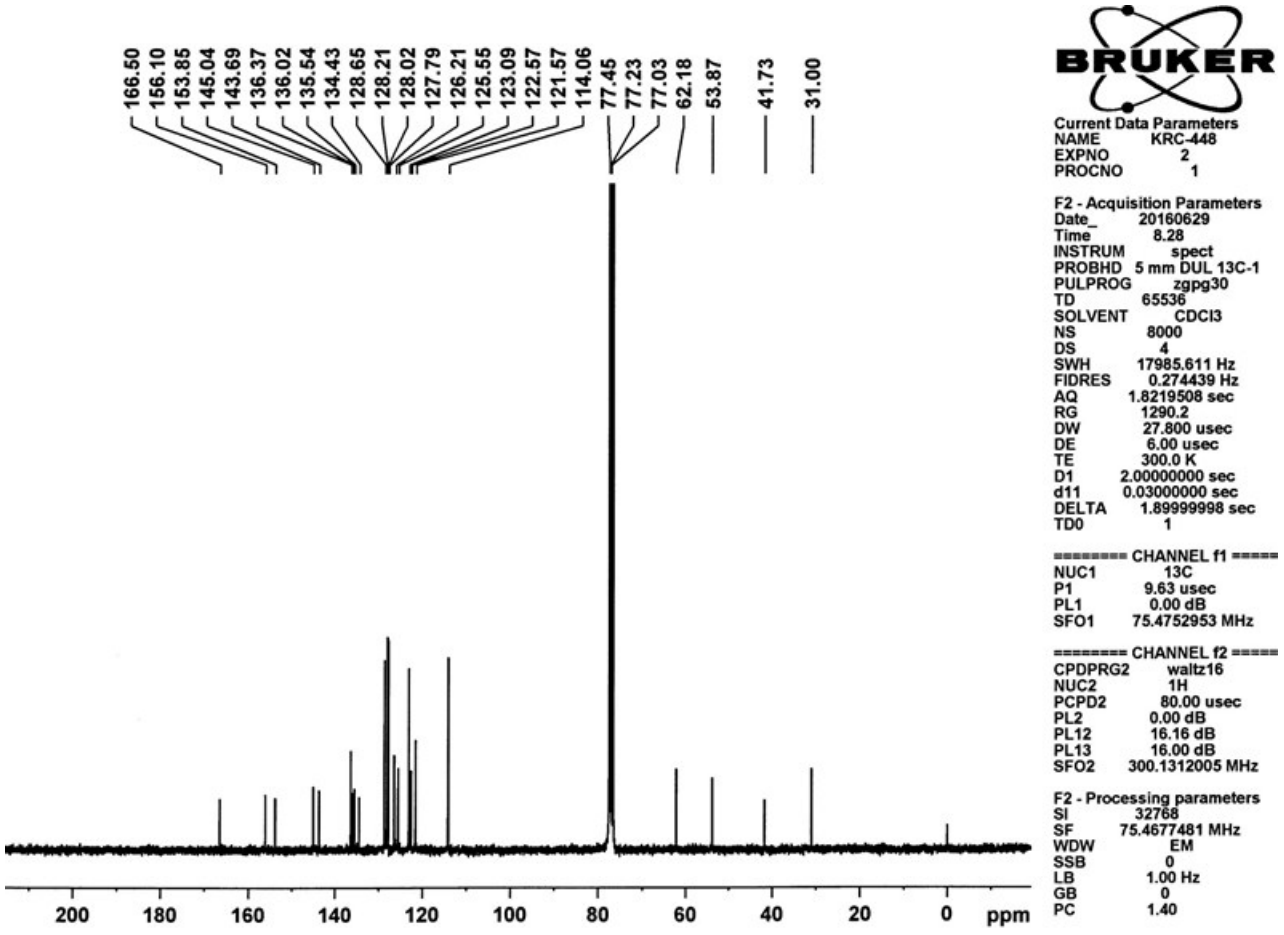
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the dendrimer **1**



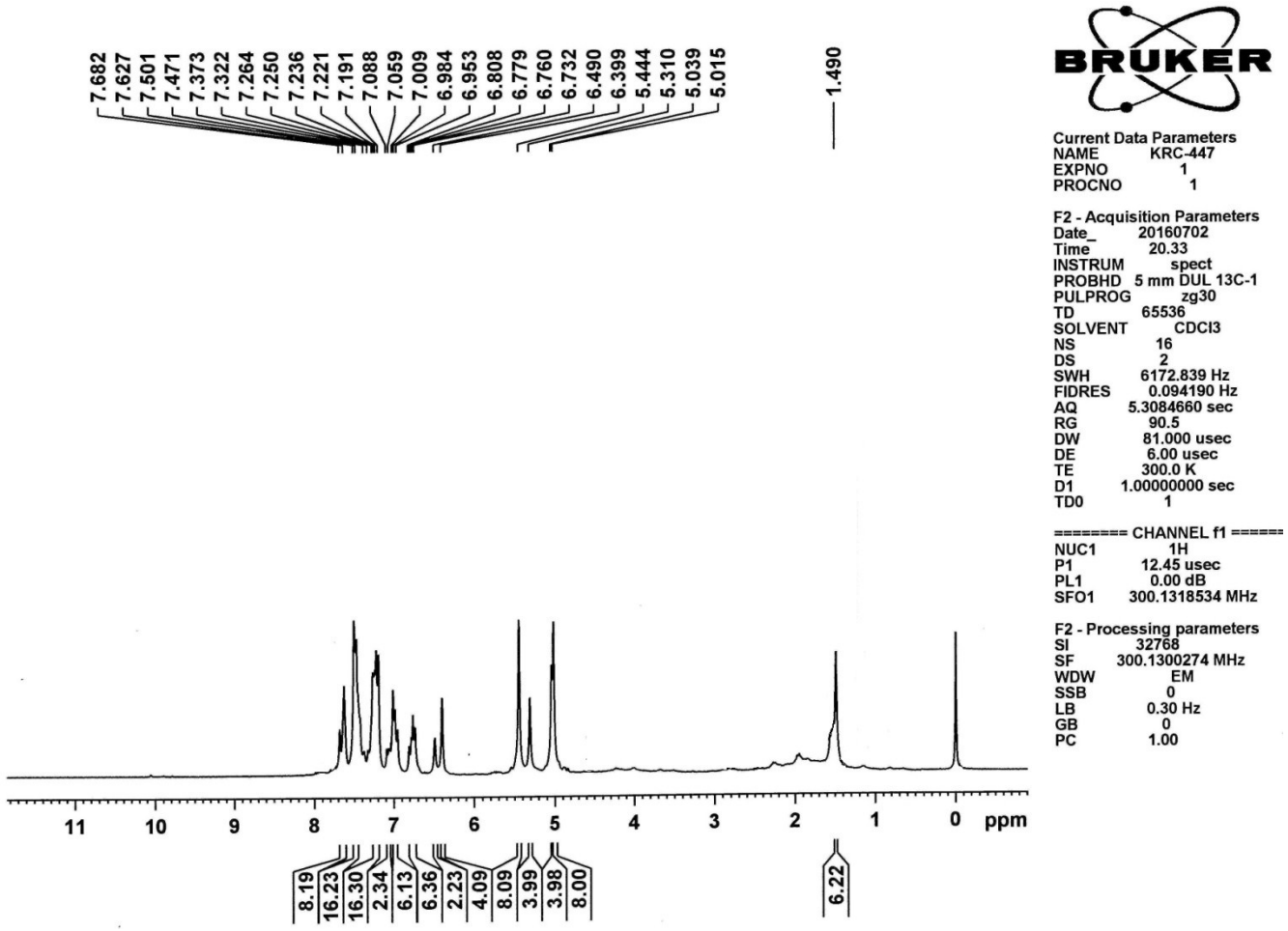
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the dendrimer **1**



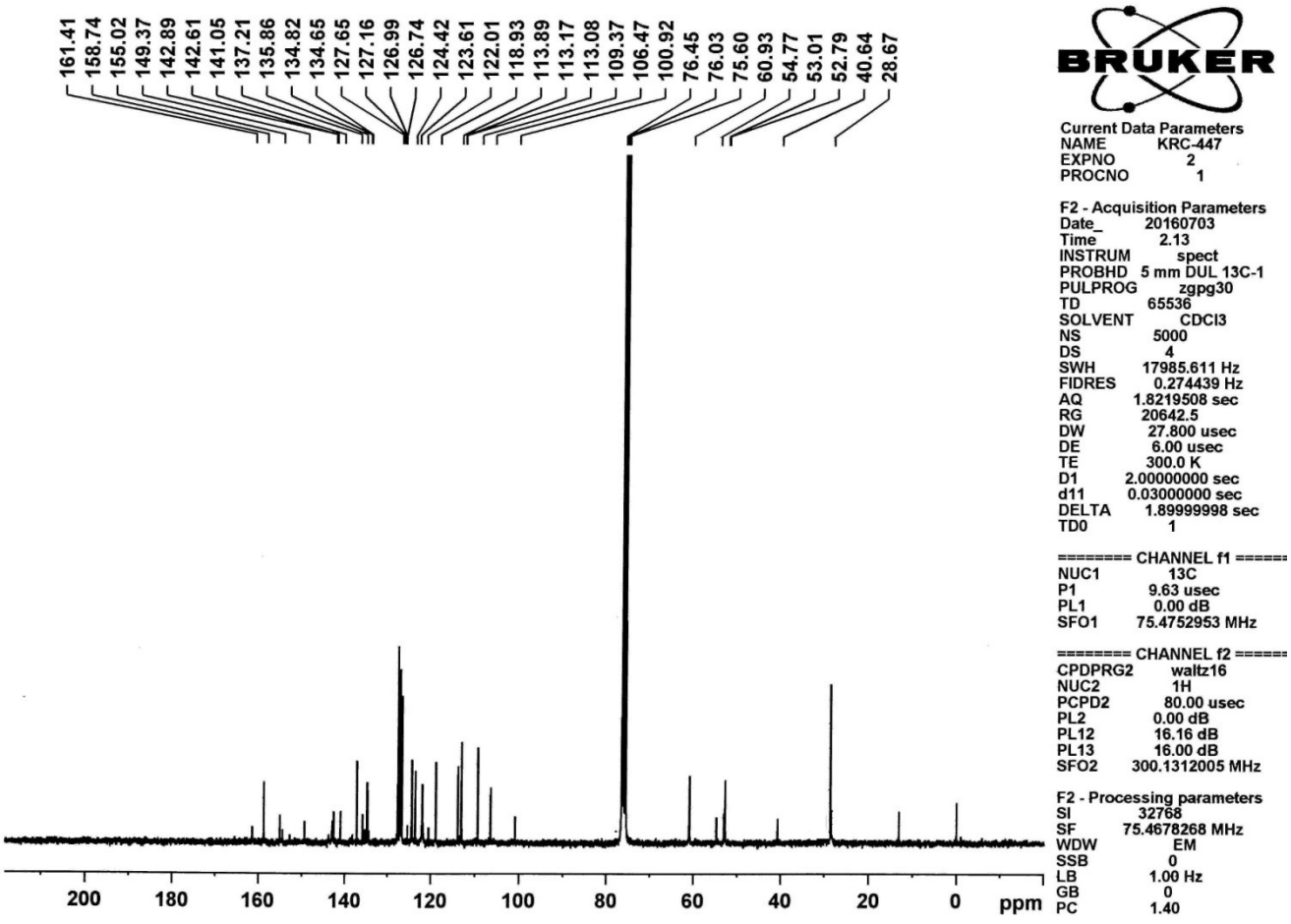
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the dendrimer **2**



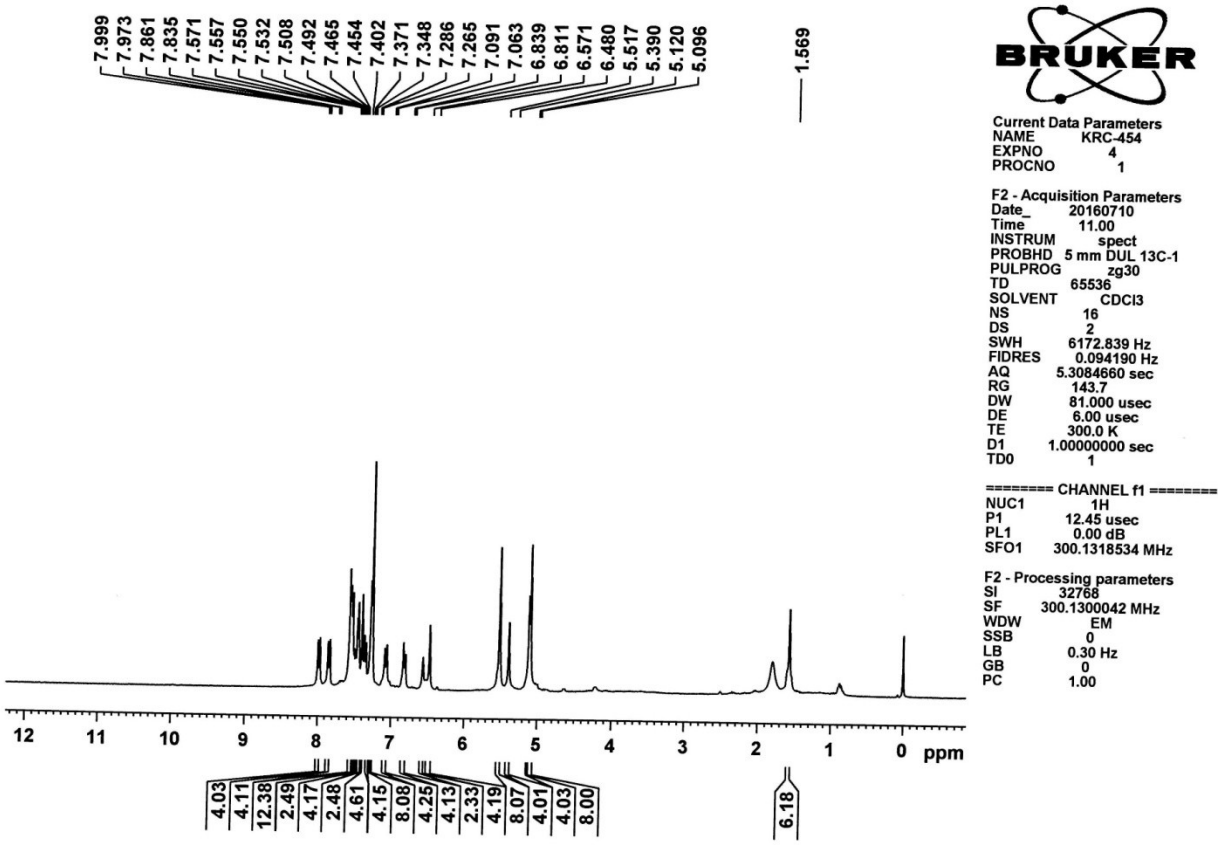
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the dendrimer **2**



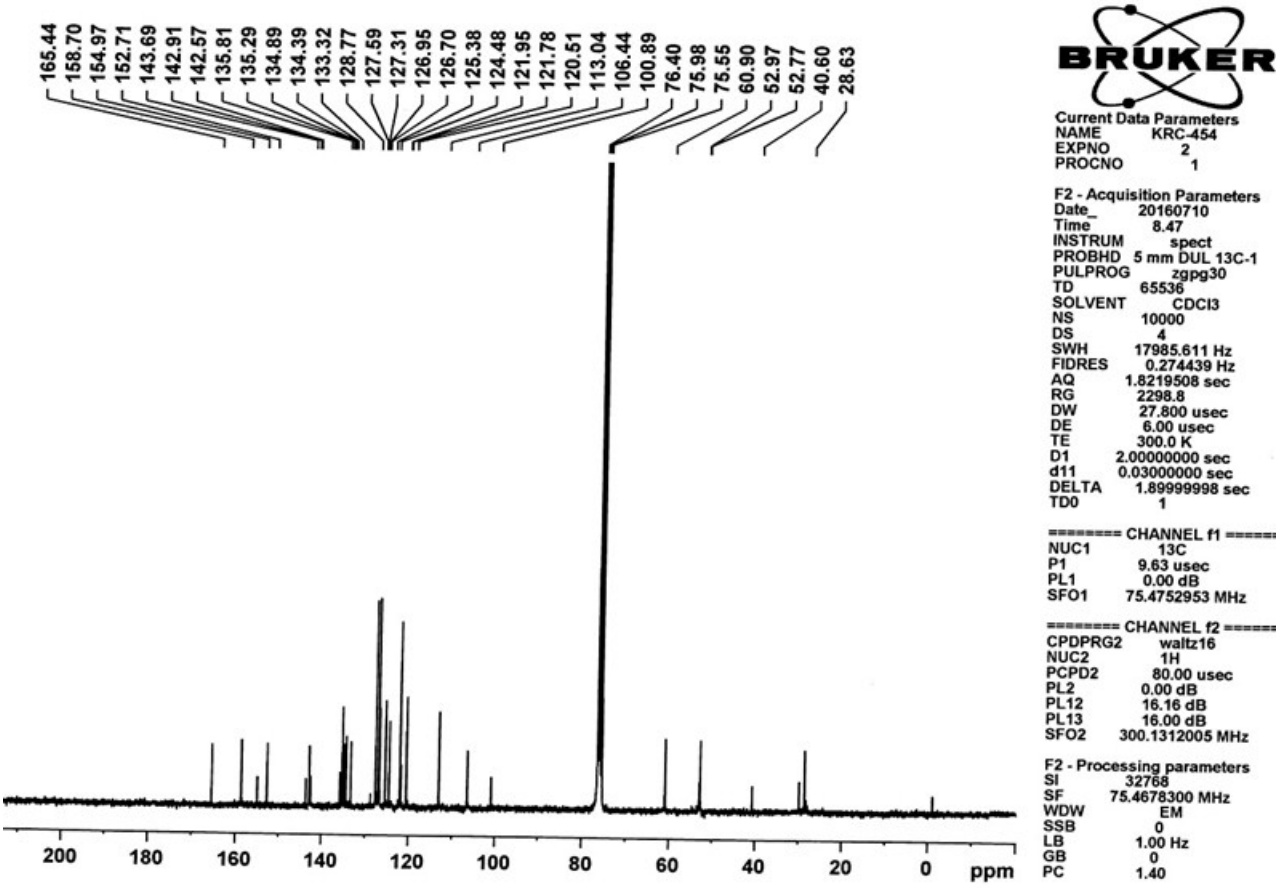
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the dendrimer **3**



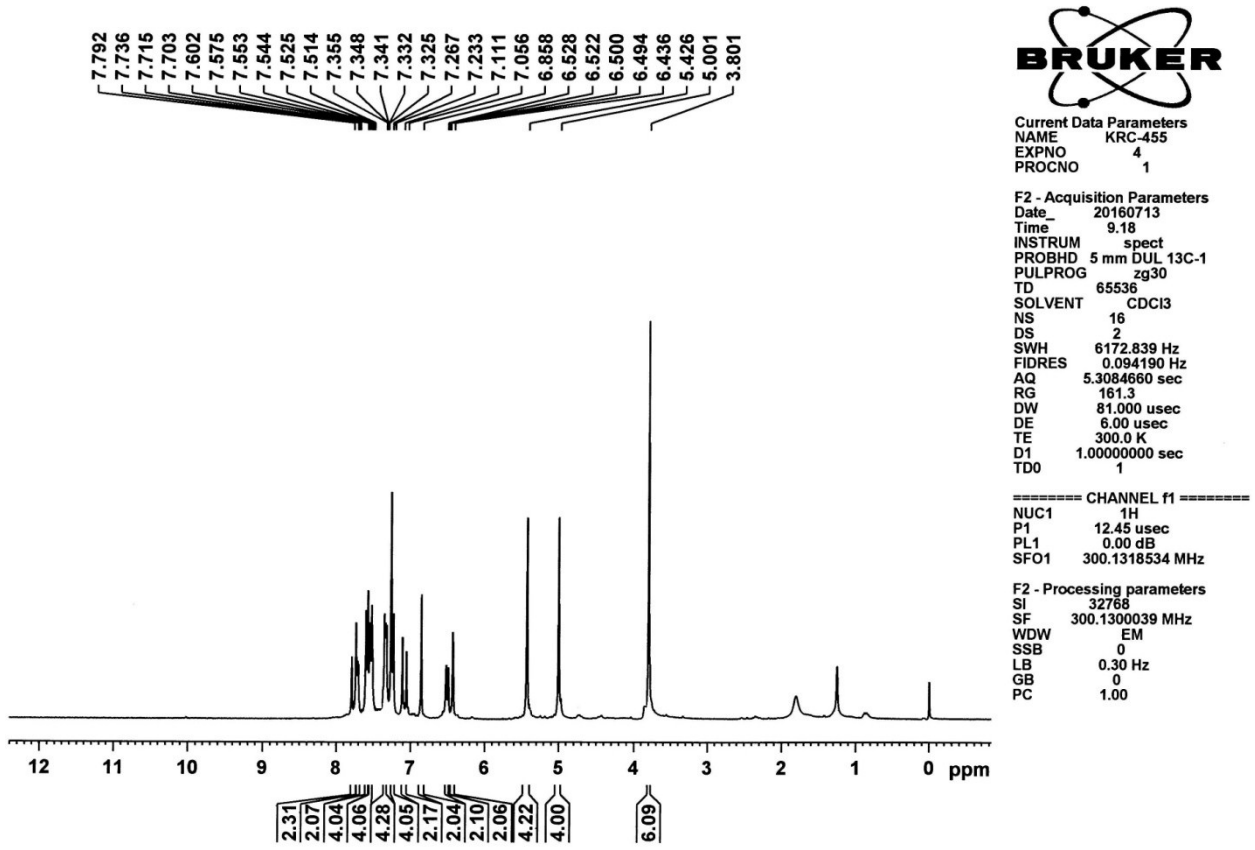
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the dendrimer **3**



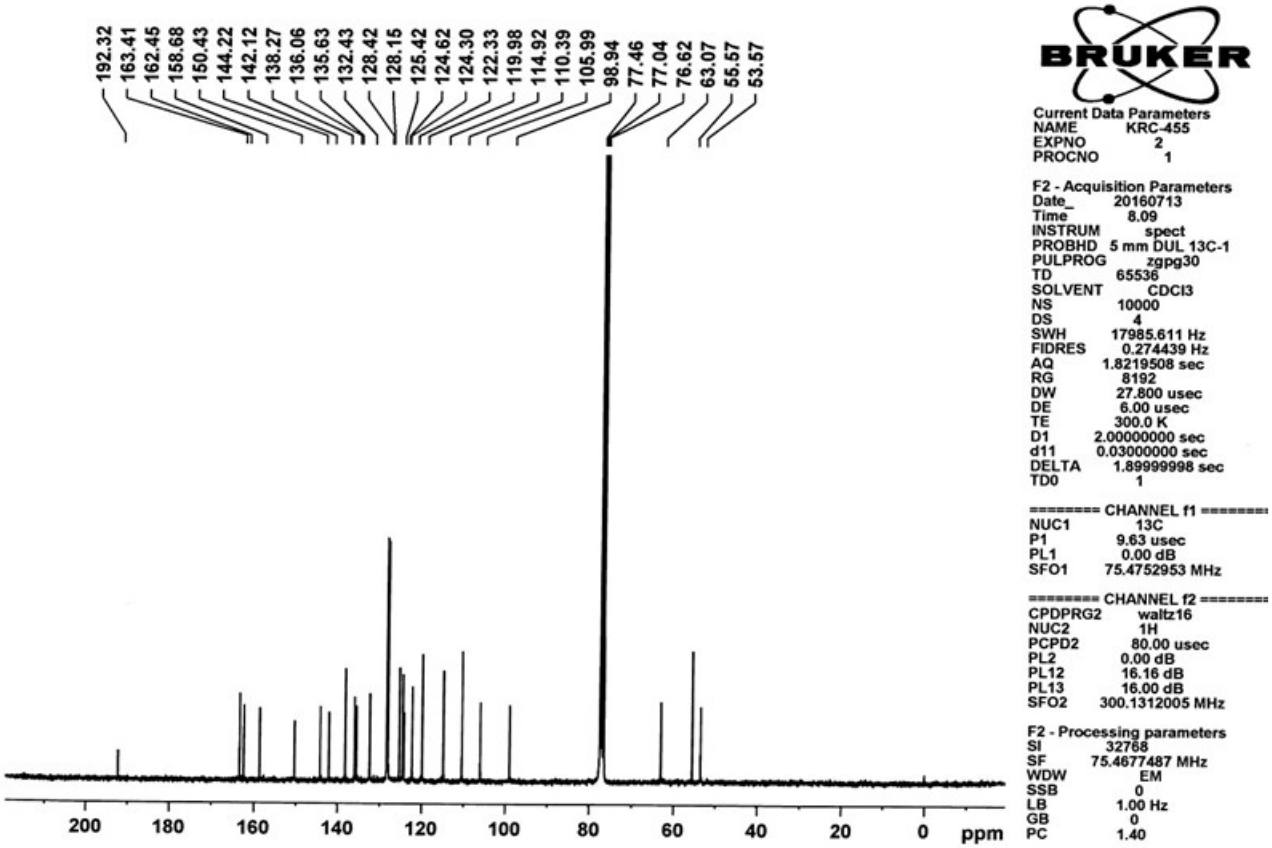
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the dendrimer **4**



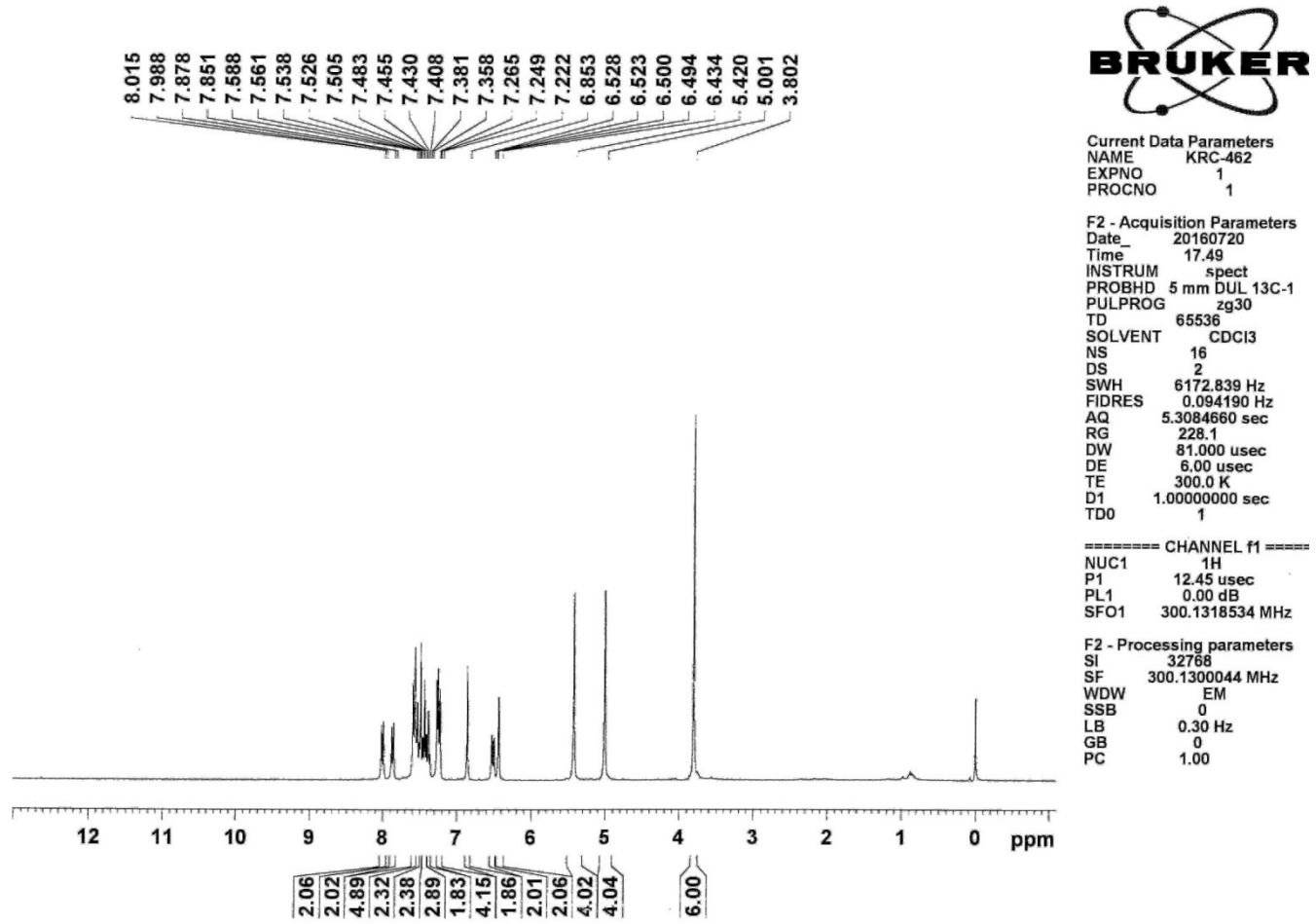
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the dendrimer **4**



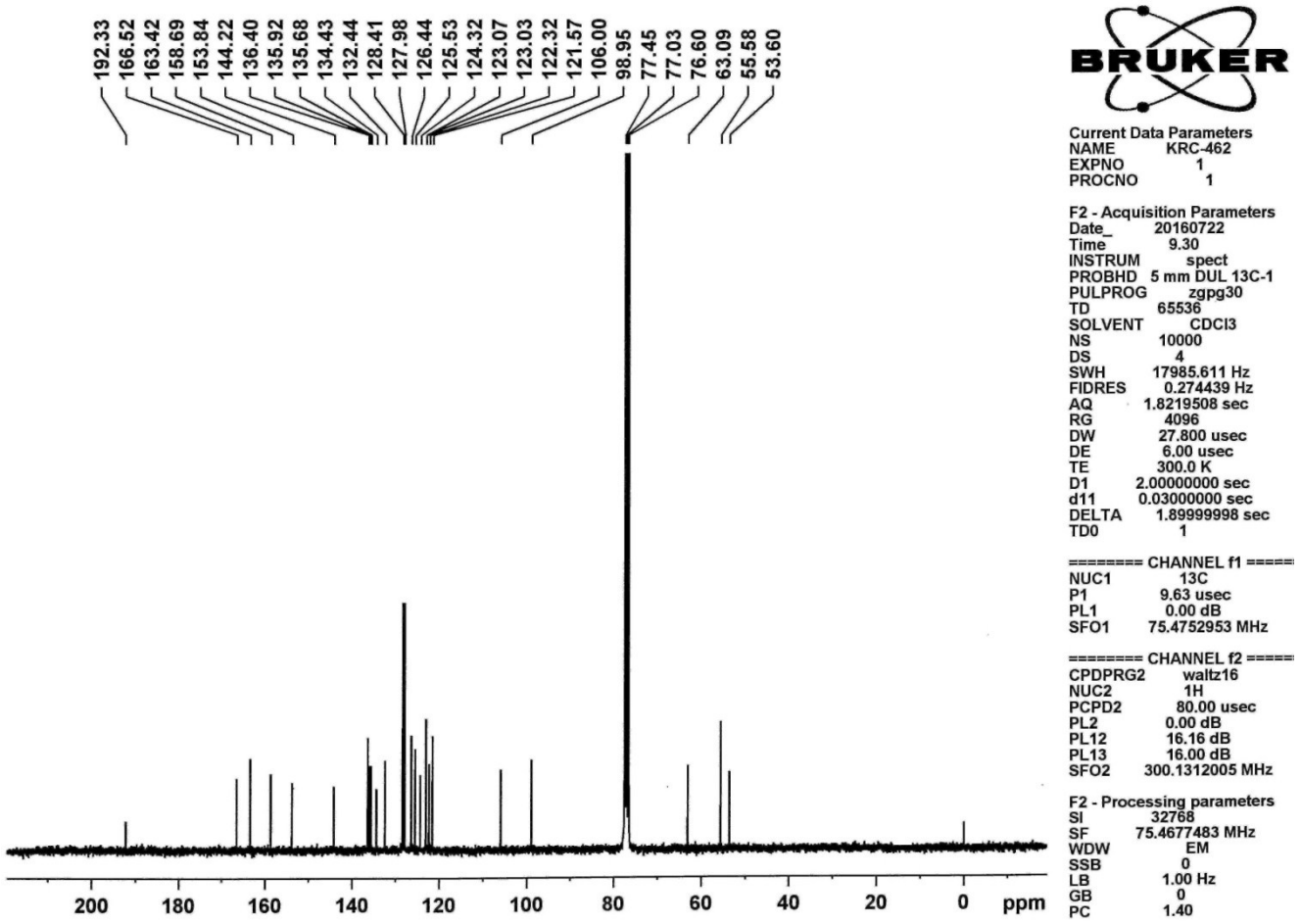
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the dendrimer **5**



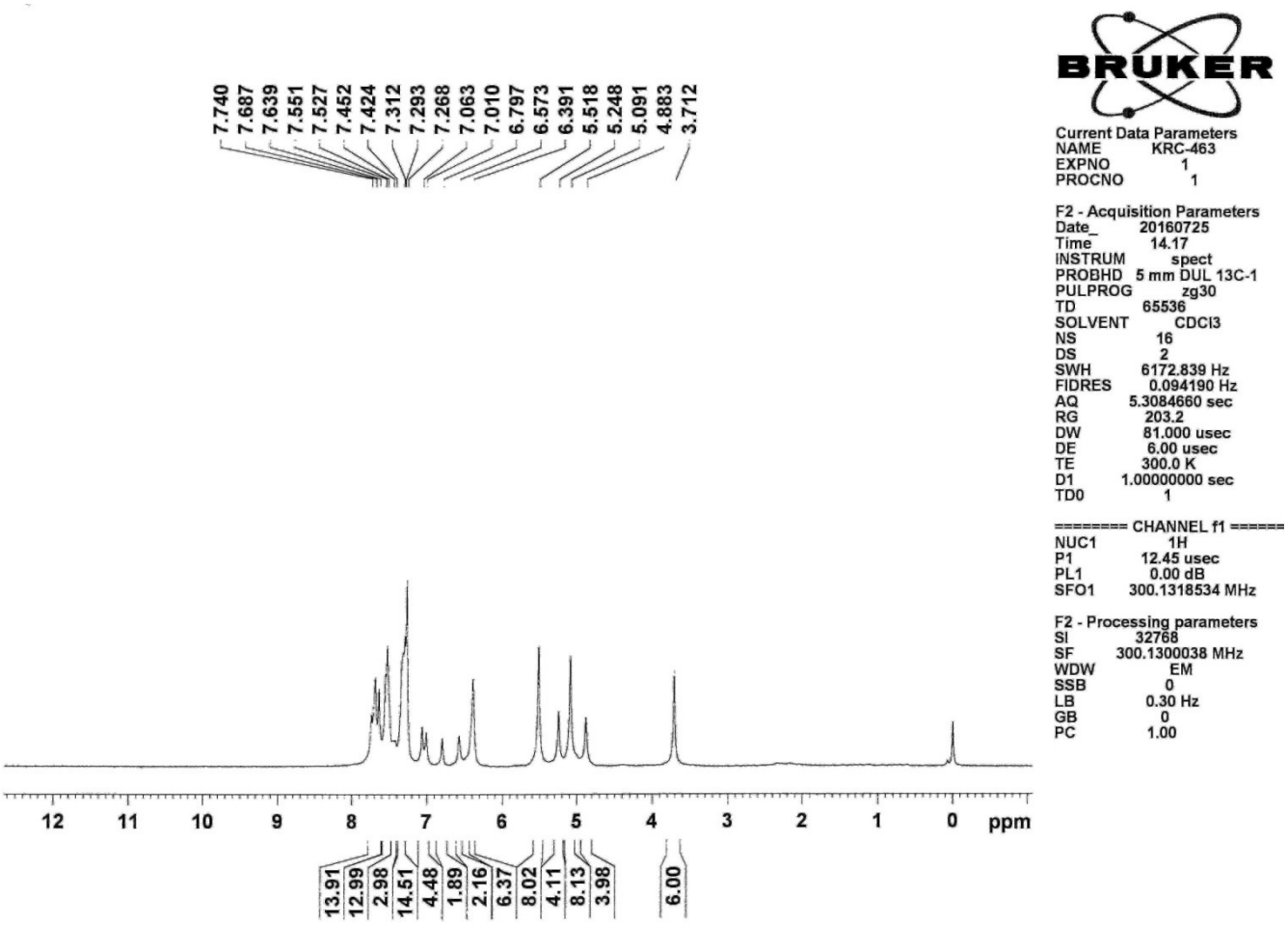
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the dendrimer **5**



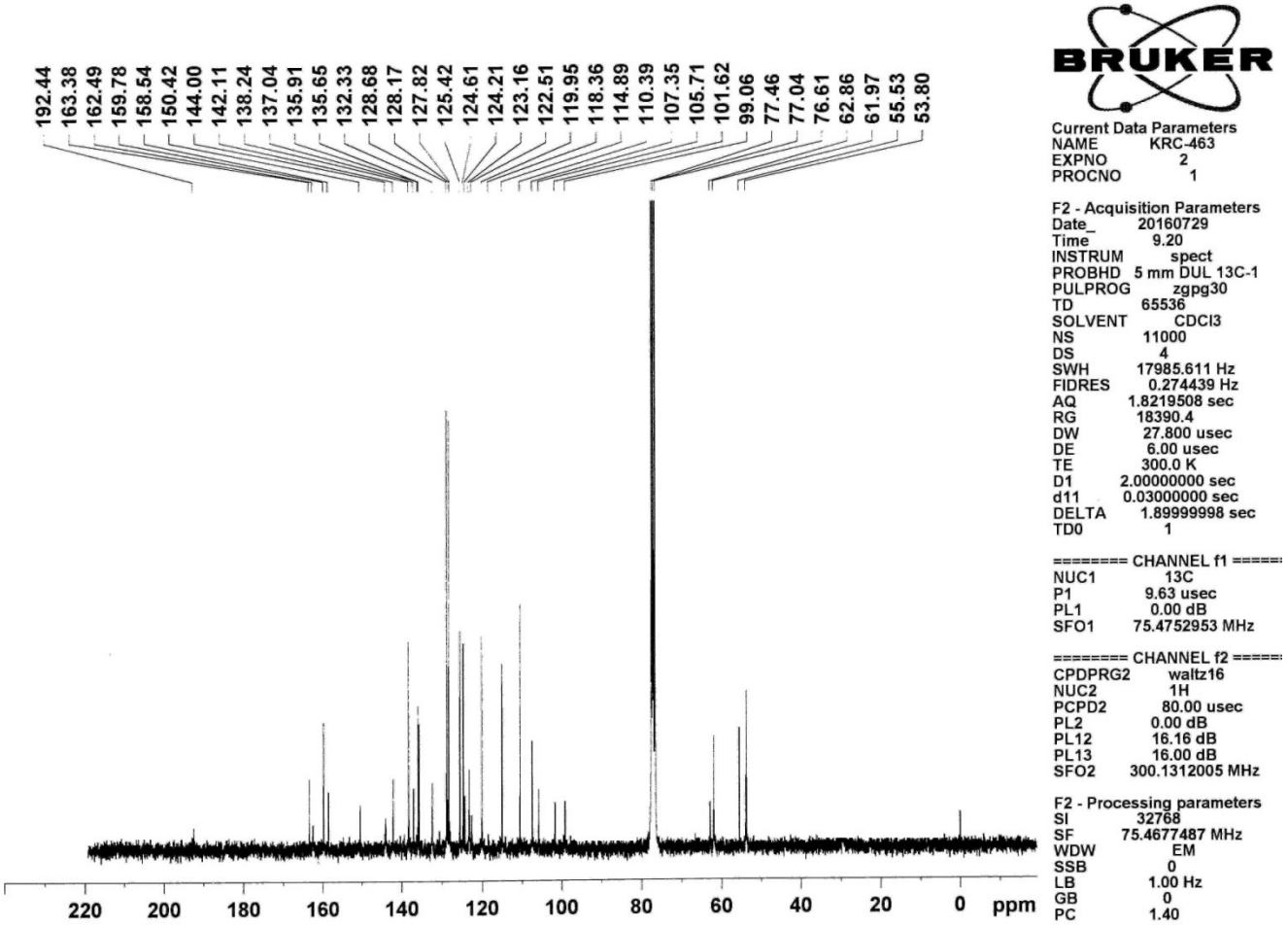
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the dendrimer **6**



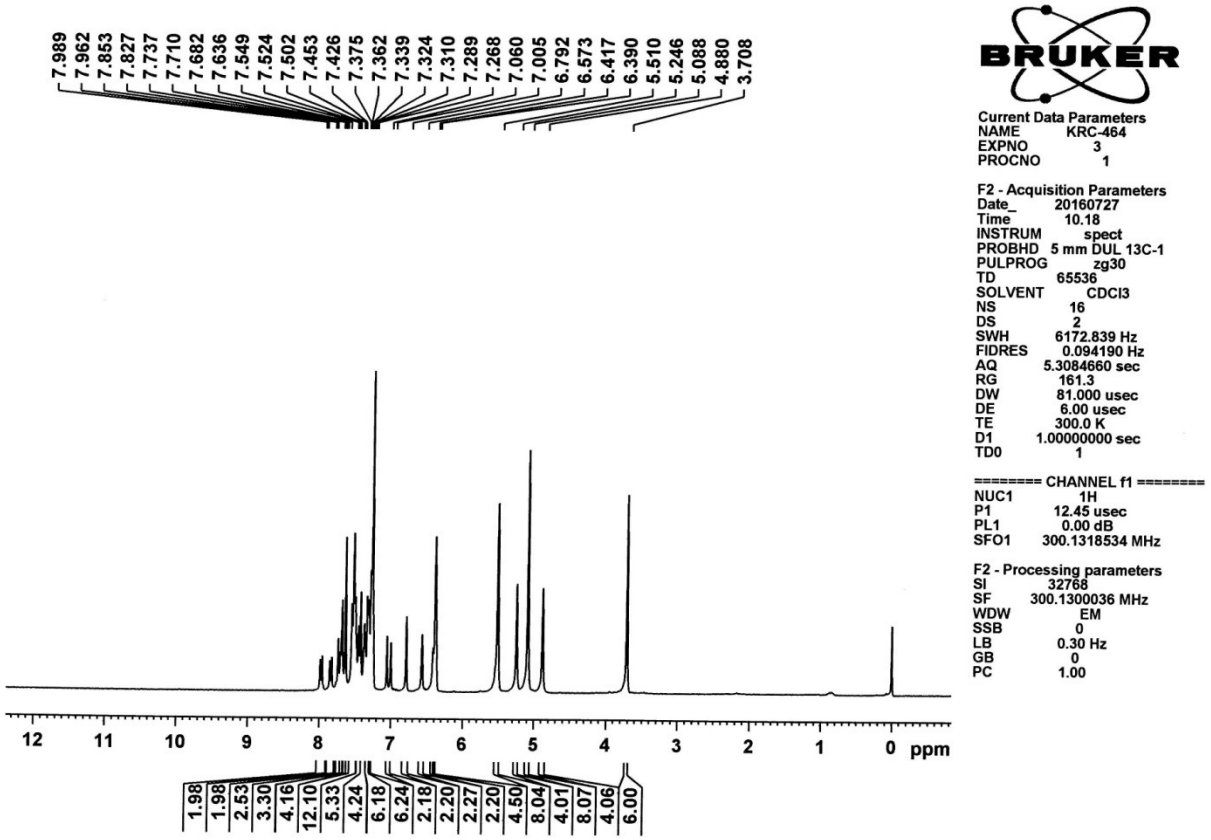
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the dendrimer **6**



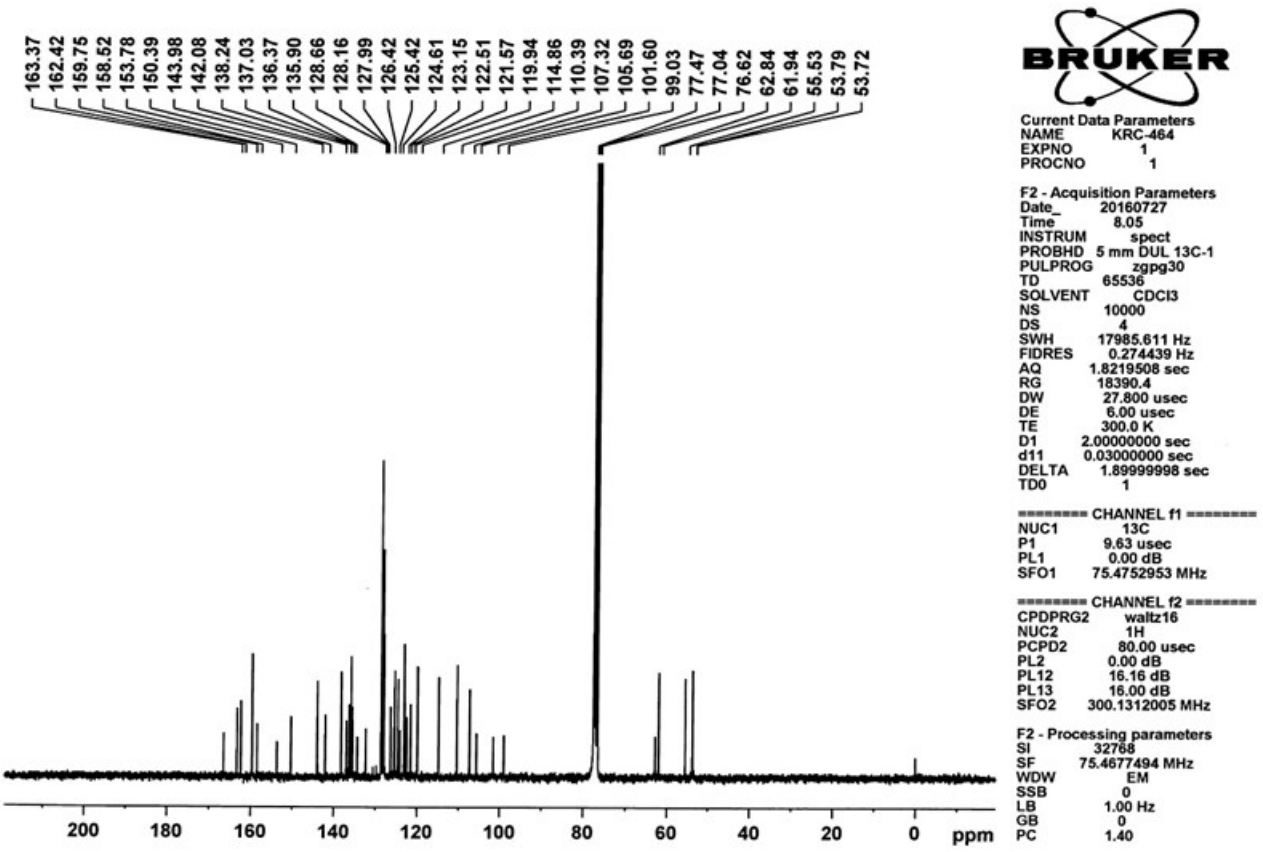
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the dendrimer **7**



^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the dendrimer **7**



^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the dendrimer **8**



^{13}C NMR (75 MHz, $CDCl_3$) spectrum of the dendrimer **8**

Antioxidant activity

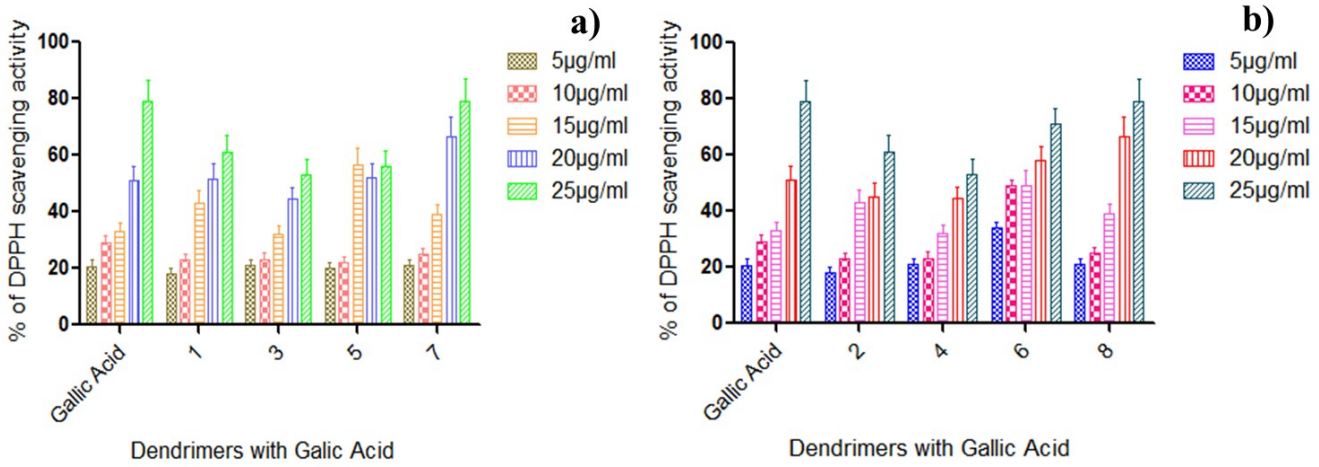


Fig. 1 a) DPPH scavenging activity (IC_{50}) of the benzoxazole dendrimers **1, 3, 5** and **7** and **b)** benzothiazole dendrimers **2, 4, 6** and **8** with Gallic acid standard

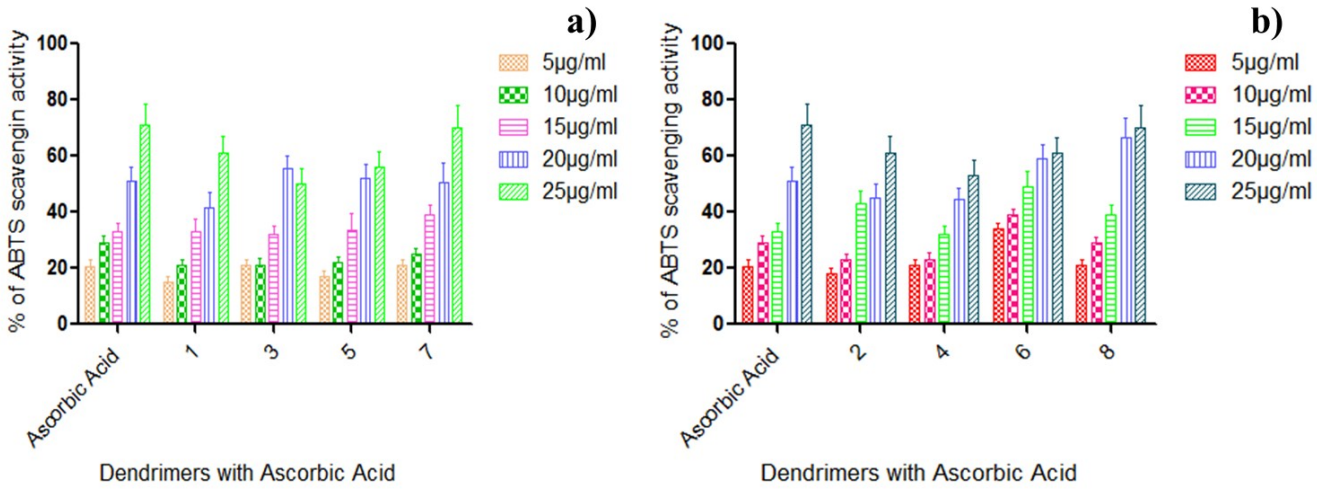


Fig. 2 a) ABTS scavenging activity (IC_{50}) of the benzoxazole dendrimers **1, 3, 5** and **7** and **b)** benzothiazole dendrimers **2, 4, 6** and **8 (**B and D) with Ascorbic acid standard