Supplementary information for

Design, synthesis and evaluation of acetylcholine-antitumor lipids hybrids lead to identification of a potential anticancer agent disrupting CDK4/6-Rb pathway in lung cancer

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1. Chemistry

1.1. General

Reagents and solvents used in the synthesis were purchased from companies such as Sigma-Aldrich, Alfa Aesar, Acros Organics, or TCI. Most reagents were used without a separate purification process. When additional purification was required, they were purified using generally known methods (distillation, silica gel column chromatography, recrystallization). To confirm the reaction, thin-layer chromatography (TLC) with silica gel (E. Merck silica gel 60 F_{254} pre-coated plates, 0.25 mm) was used. To confirm the compound on TLC, a UV lamp (365, 254 nm) was used, or the color was developed with p-anisaldehyde solution or ninhydrin solution.NMR and HR-MS spectra were measured to elucidate the structure of the compound. 1H and 13C NMR spectra were measured at 500 MHz and 125 MHz, respectively, using JEOL JNM-ECZ500R. Chemical shifts were expressed as parts per million (ppm, δ) from the downfield using tetramethylsilane (0 ppm) as an internal reference. HR-MS spectra were acquired using a JEOL accuTOF (JMS-T100TD), and direct analysis in real time (DART-SVP) ion source (Ion sense) was used.

1.2. General procedure for preparation of Methyl hydroxyphenylacetates 27–29

To an ice cooled solution of hydroxyphenylacetic acids **24–26** (1 equivalent) in methanol under an inert atmosphere, acetyl chloride (2.5 equivalents) was added slowly, was stirred at 0 °C for 1 hour, and then refluxed at 60 °C for 2 hours. After consumption of starting material (TLC; EtOAc/*n*-Hexane = 1:2), the mixture was evaporated under reduced pressure, dissolved in dichloromethane and treated with saturated aqueous $NaHCO_3$ solution. The organic layer was extracted three times with dichloromethane, washed with saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification (column chromatography, silica gel, EtOAc/*n*-Hexane = 1:5) afforded the desired compounds.

Methyl 2-(2-hydroxyphenyl)acetate (27)

Compound **27** (99%) was obtained from compound **24** and acetyl chloride following general procedure 1.2. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24-7.19 (1H, m), 7.13–7.11 (1H, m), 6.97–6.88 (2H, m), 3.77 (3H, s), 3.71 (2H, s).

Methyl 2-(3-hydroxyphenyl)acetate (28)

Compound **28** (96%) was obtained from compound **25** and acetyl chloride following general procedure 1.2. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (1H, t, *J* = 7.8), 6.85–6.75 (3H, m), 5.76 (1H, s), 3.73 (3H, s), 3.61 (2H, s).

Methyl 2-(4-hydroxyphenyl)acetate (29)

Compound **29** (99%) was obtained from compound **26** and acetyl chloride following general procedure 1.2. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (2H, d, *J* = 8.5 Hz), 6.78 (2H, d, *J* = 8.5 Hz), 5.31 (1H, s), 3.72 (3H, s), 3.58 (2H, s).

1.3. General procedure for preparation of methyl stearoxyphenylacetates 30–32

To a solution of methyl hydroxyphenylacetates **27–29** (1 equivalent) in DMF, potassium carbonate (3 equivalents) was added, stirred at room temperature for 60 minutes, then potassium iodide (0.2 equivalents) and stearyl tosylate (1.1 equivalents) were added. The mixture was heated with stirring at 60 °C for 20 hours. After complete reaction (TLC; EtOAc/*n*-Hexane = 1:7), the reaction was cooled to ambient temperature, quenched with distilled water and extracted three times with ethyl acetate, washed with brine and saturated aqueous $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification (column chromatography, silica gel, EtOAc/*n*-Hexane = 1:20) afforded desired compounds **30–32**.

Methyl 2-(2-(octadecyloxy)phenyl)acetate (30)

Compound **30** (54%) was obtained following general procedure 1.3 using compound **27**, potassium carbonate, potassium iodide, and stearyl tosylate. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.21–7.18 (2H, m), 6.94–6.81 (2H, m), 3.96 (2H, t, *J* = 6.6 Hz), 3.69 (3H, s), 3.64 (2H, s), 1.76–1.73 (2H, m), 1.45–1.24 (30H, m), 0.87 (3H, t, *J* = 6.9 Hz).

Methyl 2-(3-(octadecyloxy)phenyl)acetate (31)

Compound **31** (47%) was obtained following general procedure 1.3 using compound **28**, potassium carbonate, potassium iodide, and stearyl tosylate. ^{1}H-NMR (500 MHz, $CDCl_3$) 7.22 (1H, t, *J* = 8.0 Hz), 6.84-6.81 (3H, m), 3.96 (2H, t, *J* = 6.6 Hz), 3.68 (3H, s), 3.64 (2H, s), 1.76-1.73 (2H, m), 1.45–1.24 (30H, m), 0.87 (3H, t, *J* = 6.9 Hz).

Methyl 2-(4-(octadecyloxy)phenyl)acetate (32)

Compound **32** (57%) was obtained following general procedure 1.3 using compound **29**, potassium carbonate, potassium iodide, and stearyl tosylate. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.19 (2H, *J* = 8.6 Hz, d), 6.86 (2H, *J* = 8.6 Hz, d), 3.93 (2H, *J* = 6.6 Hz, t), 3.70 (3H, s), 3.59 (2H, s), 1.78-1.74 (2H, m), 1.47–1.38 (30H, m), 0.89 (3H, *J* = 6.9 Hz, t).

1.4. General procedure for preparation of stearoxyphenylacetic acids 33–35

To a solution of methyl stearoxyphenylacetates **30–32** (1 equivalent) in a mixture of THF/Methanol (1:1) was added NaOH (1.8 equivalent, 3N, aqueous solution). The mixture was heated with stirring at 60 °C and stir for 2 hours. Upon reaction completion (TLC; EtOAc/*n*-Hexane = 1:5), the organic solvents were evaporated under reduced pressure. The remaining aqueous solution was acidified with HCl (5N, aqueous solution), extracted three times with dichloromethane, washed with brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to afford the desired pure compounds **33–35**.

2-Stearoxyphenylacetic acid (33)

Compound **33** (97%) was obtained following general procedure 1.4 using compound **30**. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.20-7.18 (2H, m), 6.94-6.81 (2H, m), 3.96 (2H, t, *J* = 6.6 Hz), 3.64 (2H, s), 1.76-1.73 (2H, m), 1.24-1.45 (30H, m), 0.87 (3H, t, *J* = 6.9 Hz).

3-Stearoxyphenylacetic acid (34)

Compound **34** (95%) was obtained following general procedure 1.4 using compound **31**. ^{1}H-NMR (500 MHz, $CDCl_3$) 7.22 (1H, t, *J* = 8.0 Hz), 6.84–6.81 (3H, m), 3.94 (2H, t, *J* = 6.6 Hz), 3.62 (2H, s), 1.74–1.71 (2H, m), 1.44–1.24 (30H, m), 0.87 (3H, t, *J* = 6.9 Hz).

4-Stearoxyphenylacetic acid (35)

Compound **35** (97%) was obtained following general procedure 1.4 using compound **32**. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.19 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 3.93 (t, *J* = 6.6 Hz, 2H), 3.59 (s, 2H), 1.74–1.78 (m, 2H), 1.38–1.47 (m, 30H), 0.89 (t, *J* = 6.9 Hz, 3H).

1.5. General procedure for preparation of aminoalkyl stearoxyphenylacetates 36–53

To an ice-cooled solution of the appropriate stearoxyphenylacetic acid derivative **33–35** (1 equivalent) in dichloromethane was added DMF (catalytic) followed by slow addition of oxalyl chloride (2 equivalents) then the reaction mixture was stirred at room temperature for 3 hours. Upon the reaction completion of the reaction (TLC; EtOAc/*n*-Hexane = 1:1), the mixture was evaporated under reduced pressure distillation. The crude residue was dissolved in dichloromethane (10 ml), cooled in ice bath followed by slow addition of the appropriate alkanolamine derivative (3 equivalents) then stirred at ambient temperature (5 hours). Upon reaction completion (TLC; DCM/MeOH = 10:1), $NaHCO_3$ (saturated aqueous solution) was added, stirred for 30 minutes, extracted three times with dichloromethane, washed with brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification of the residue (column chromatography, silica gel, DCM/MeOH = 25:1) afforded aminoalkyl stearoxyphenylacetates **36–53**.

2-(Dimethylamino)ethyl 2-stearoxyphenylacetate (36)

Compound **36** (87.5 %) was obtained following general procedure 1.5 using compound **33**, oxalyl chloride, and 2-dimethylaminoethanol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-7.18 (2H, m), 6.94-6.84 (2H, m), 4.22 (2H, t, *J* = 5.9 Hz), 3.96 (2H, t, *J* = 6.4 Hz), 3.67 (2H, s), 2.58 (2H, t, *J* = 5.9 Hz), 2.27 (6H, s), 1.81-1.74 (2H, m), 1.49-1.42 (2H, m), 1.38-1.26 (28H, m), 0.90 (3H, t, *J* = 6.8 Hz).

3-(Dimethylamino)propyl 2-stearoxyphenylacetate (37)

Compound **37** (79%) was obtained following general procedure 1.5 using compound **33**, oxalyl chloride, and 3-dimethylamino-1-propanol. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.24-7.21 (2H, m), 6.89 (1H, t, J = 7.4 Hz), 6.84 (1H, d, J = 8.0 Hz), 4.12 (2H, t, *J* = 6.6 Hz), 3.92 (2H, t, *J* = 6.6 Hz), 3.55 (2H, s), 2.28 (2H, t, *J* = 7.4 Hz), 2.20 (6H, s), 1.81-1.73 (4H, m), 1.46-1.25 (30H, m), 0.88 (3H, t, *J* = 6.9 Hz).

2-(Diethylamino)ethyl 2-stearoxyphenylacetate (38)

Compound **38** (83%) was obtained following general procedure 1.5 using compound **33**, oxalyl chloride, and 2-(diethylamino)ethanol. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.24-7.21 (2H, m), 6.89 (1H, t, J = 7.4 Hz), 6.84 (1H, d, J = 8.0 Hz), 4.22 (2H, t, *J* = 5.7 Hz), 3.92 (2H, t, *J* = 6.6 Hz), 3.60 (2H, s), 2.96 (4H, q, *J* = 7.1 Hz), 2.59 (2H, d, *J* = 5.7 Hz), 2.26 (6H, s), 1.79-1.73 (2H, m), 1.45-1.08 (36H, m), 0.88 (3H, t, *J* = 6.9 Hz).

2-(Pyrrolidin-1-yl)ethyl 2-stearoxyphenylacetate (39)

Compound **39** (87%) was obtained following general procedure 1.5 using compound **33**, oxalyl chloride, and 1-(2-hydroxyethyl)pyrrolidine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.24-7.21 (2H, m), 6.89 (1H, t, J = 7.4 Hz), 6.84 (1H, d, J = 8.0 Hz), 4.22 (2H, d, *J* = 12.0 Hz), 3.95 (2H, d, *J* = 6.9 Hz), 3.59 (2H, s), 2.72 (2H, d, *J* = 12.0 Hz), 2.53 (4H, s), 1.78-1.71 (6H, m), 1.48-1.23 (30H, m), 0.88 (3H, t, *J* = 7.2 Hz).

2-(Piperidin-1-yl)ethyl 2-stearoxyphenylacetate (40)

Compound **40** (76%) was obtained following general procedure 1.5 using compound **33**, oxalyl chloride, and 1-(2-hydroxyethyl)piperidine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.24-7.21 (2H, m), 6.89 (1H, t, J = 7.4 Hz), 6.84 (1H, d, J = 8.0 Hz), 4.22 (2H, t, *J* = 6.0 Hz), 3.94 (2H, t, *J* = 6.6 Hz), 3.57 (2H, s), 2.58 (2H, t, *J* = 6.0 Hz), 2.39 (4H, s), 1.80-1.73 (2H, m), 1.57-1.53 (4H, m), 1.45-1.21 (30H, m), 0.88 (3H, t, *J* = 7.2 Hz).

2-Morpholinoethyl 2-stearoxyphenylacetate (41)

Compound **41** (83%) was obtained following general procedure 1.5 using compound **33**, oxalyl chloride, and 4-(2-hydroxyethyl)morpholine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.24-7.21 (2H, m), 6.89 (1H, t, J = 7.4 Hz), 6.84 (1H, d, J = 8.0 Hz), 4.23 (2H, t, *J* = 5.7 Hz), 3.90 (2H, t, *J* = 6.6 Hz), 3.70 (4H, t, *J* = 4.9 Hz), 3.61 (2H, s), 2.65 (2H, t, *J* = 5.7 Hz), 2.45 (4H, t, *J* = 4.6 Hz), 1.83-1.76 (2H), 1.48-1.21 (30H, m), 0.90 (3H, t, *J* = 7.2 Hz).

2-(Dimethylamino)ethyl 3-stearoxyphenylacetate (42)

Compound **42** (77%) was obtained following general procedure 1.5 using compound **34**, oxalyl chloride, and 2-dimethylaminoethanol. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.21 (1H, t, *J* = 7.7 Hz), 6.78-6.85 (3H, m), 4.19 (2H, t, *J* = 5.7 Hz), 3.93 (2H, t, *J* = 6.6 Hz), 3.59 (2H, s), 2.56 (2H, d, *J* = 5.7 Hz), 2.26 (6H, s), 1.79-1.73 (2H, m), 1.46-1.26 (30H, m), 0.88 (3H, t, *J* = 6.9 Hz).

3-(Dimethylamino)propyl 3-stearoxyphenylacetate (43)

Compound **43** (73%) was obtained following general procedure 1.5 using compound **34**, oxalyl chloride, and 3-(dimethylamino)-1-propanol. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.21 (1H, t, *J* = 7.7 Hz), 6.78-6.85 (3H, m), 4.12 (2H, t, *J* = 6.6 Hz), 3.92 (2H, t, *J* = 6.6 Hz), 3.55 (2H, s), 2.28 (2H, t, *J* = 7.4 Hz), 2.20 (6H, s), 1.81-1.73 (4H, m), 1.46-1.25 (30H, m), 0.88 (3H, t, *J* = 6.9 Hz).

2-(Diethylamino)ethyl 3-stearoxyphenylacetate (44)

Compound **44** (70%) was obtained following general procedure 1.5 using compound **34**, oxalyl chloride, and 2-(diethylamino)ethanol. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.21 (1H, t, *J* = 7.7 Hz), 6.78-6.85 (3H, m), 4.23 (2H, t, *J* = 5.7 Hz), 3.94 (2H, t, *J* = 6.6 Hz), 3.60 (2H, s), 2.96 (4H, q, *J* = 7.1 Hz), 2.58 (2H, d, *J* = 5.7 Hz), 2.26 (6H, s), 1.79-1.73 (2H, m), 1.46-1.10 (36H, m), 0.88 (3H, t, *J* = 6.9 Hz).

2-(Pyrrolidin-1-yl)ethyl 3-stearoxyphenylacetate (45)

Compound **45** (58%) was obtained following general procedure 1.5 using compound **34**, oxalyl chloride, and 1-(2-hydroxyethyl)pyrrolidine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.21 (1H, t, *J* = 7.7 Hz), 6.78-6.85 (3H, m), 4.23 (2H, d, *J* = 12.0 Hz), 3.95 (2H, d, *J* = 6.9 Hz), 3.59 (2H, s), 2.72 (2H, d, *J* = 12.0 Hz), 2.53 (4H, s), 1.79-1.73 (6H, m), 1.45-1.26 (30H, m), 0.88 (3H, t, *J* = 7.2 Hz).

2-(Piperidin-1-yl)ethyl 3-stearoxyphenylacetate (46)

Compound **46** (75%) was obtained following general procedure 1.5 using compound **34**, oxalyl chloride, and 1-(2-hydroxyethyl)piperidine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.21 (1H, t, *J* = 7.7 Hz), 6.78-6.85 (3H, m), 4.22 (2H, t, *J* = 6.0 Hz), 3.94 (2H, t, *J* = 6.6 Hz), 3.57 (2H, s), 2.58 (2H, t, *J* = 6.0 Hz), 2.39 (4H, s), 1.80-1.73 (2H, m), 1.57-1.53 (4H, m), 1.45-1.21 (30H, m), 0.88 (3H, t, *J* = 7.2 Hz).

2-Morpholinoethyl 3-stearoxyphenylacetate (47)

Compound **47** (62%) was obtained following general procedure 1.5 using compound **34**, oxalyl chloride, and 4-(2-hydroxyethyl)morpholine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.21 (1H, t, *J* = 7.7 Hz), 6.78-6.85 (3H, m), 4.24 (2H, t, *J* = 5.7 Hz), 3.93 (2H, t, *J* = 6.6 Hz), 3.66 (4H, t, *J* = 4.9 Hz), 3.56 (2H, s), 2.61 (2H, t, *J* = 5.7 Hz), 2.44 (4H, t, *J* = 4.6 Hz), 1.83-1.71 (2H), 1.46-1.26 (30H, m), 0.88 (3H, t, *J* = 7.2 Hz).

2-(Dimethylamino)ethyl 4-stearoxyphenylacetate (48)

Compound **48** (71%) was obtained following general procedure 1.5 using compound **35**, oxalyl chloride, and 2-dimethylaminoethanol. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.18 (2H, d, *J* = 8.6 Hz), 6.84 (2H, d, *J* = 8.6 Hz), 4.18 (2H, t, *J* = 5.7 Hz), 3.93 (2H, t, *J* = 6.6 Hz), 3.58 (2H, s), 2.56 (2H, d, *J* = 5.7 Hz), 2.26 (6H, s), 1.79-1.73 (2H, m), 1.46-1.26 (30H, m), 0.88 (3H, t, *J* = 6.9 Hz).

3-(Dimethylamino)propyl 4-stearoxyphenylacetate (49)

Compound **49** (71%) was obtained following general procedure 1.5 using compound **35**, oxalyl chloride, and 3-(dimethylamino)-1-propanol. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.18 (2H, d, *J* = 8.6 Hz), 6.84 (2H, d, *J* = 8.6 Hz), 4.13 (2H, t, *J* = 6.6 Hz), 3.93 (2H, t, *J* = 6.6 Hz), 3.54 (2H, s), 2.28 (2H, t, *J* = 7.4 Hz), 2.20 (6H, s), 1.81-1.73 (4H, m), 1.46-1.25 (30H, m), 0.88 (3H, t, *J* = 6.9 Hz).

2-(Diethylamino)ethyl 4-stearoxyphenylacetate (50)

Compound **50** (73%) was obtained following general procedure 1.5 using compound **35**, oxalyl chloride, and 2-(diethylamino)ethanol. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.18 (2H, d, *J* = 8.6 Hz), 6.85 (2H, d, *J* = 8.6 Hz), 4.23 (2H, t, *J* = 5.7 Hz), 3.93 (2H, t, *J* = 6.6 Hz), 3.59 (2H, s), 2.96 (4H, q, *J* = 7.1 Hz), 2.58 (2H, d, *J* = 5.7 Hz), 2.26 (6H, s), 1.79-1.73 (2H, m), 1.46-1.10 (36H, m), 0.88 (3H, t, *J* = 6.9 Hz).

2-(Pyrrolidin-1-yl)ethyl 4-stearoxyphenylacetate (51)

Compound **51** (74%) was obtained following general procedure 1.5 using compound **35**, oxalyl chloride, and 1-(2-hydroxyethyl)pyrrolidine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.18 (2H, d, *J* = 8.6 Hz), 6.84 (2H, d, *J* = 9.2 Hz), 4.22 (2H, d, *J* = 12.0 Hz), 3.93 (2H, d, *J* = 6.9 Hz), 3.58 (2H, s), 2.72 (2H, d, *J* = 12.0 Hz), 2.53 (4H, s), 1.79-1.73 (6H, m), 1.45-1.26 (30H, m), 0.88 (3H, t, *J* = 7.2 Hz).

2-(Piperidin-1-yl)ethyl 4-stearoxyphenylacetate (52)

Compound **52** (85%) was obtained following general procedure 1.5 using compound **35**, oxalyl chloride, and 1-(2-hydroxyethyl)piperidine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.18 (2H, d, *J* = 8.6 Hz), 6.84 (2H, d, *J* = 8.6 Hz), 4.21 (2H, t, *J* = 6.0 Hz), 3.93 (2H, t, *J* = 6.6 Hz), 3.56 (2H, s), 2.58 (2H, t, *J* = 6.0 Hz), 2.39 (4H, s), 1.79-1.73 (2H, m), 1.57-1.53 (4H, m), 1.45-1.22 (30H, m), 0.88 (3H, t, *J* = 7.2 Hz).

2-Morpholinoethyl 4-stearoxyphenylacetate (53)

Compound **53** (59%) was obtained following general procedure 1.5 using compound **35**, oxalyl chloride, and 4-(2-hydroxyethyl)morpholine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.18 (2H, d, *J* = 8.6 Hz), 6.84 (2H, d, *J* = 8.6 Hz), 4.22 (2H, t, *J* = 5.7 Hz), 3.92 (2H, t, *J* = 6.6 Hz), 3.66 (4H, t, *J* = 4.9 Hz), 3.56 (2H, s), 2.59 (2H, t, *J* = 5.7 Hz), 2.44 (4H, t, *J* = 4.6 Hz), 1.83-1.71 (2H), 1.46-1.26 (30H, m), 0.88 (3H, t, *J* = 7.2 Hz).

1.6. General procedure for preparation of *N*,*N*,*N*-trialkyl (stearoxyphenylacetoxy)alkan-1-aminium iodide derivatives 6–23

To a stirred ice cooled solution of the appropriate aminoalkyl stearoxyphenylacetate derivative **36–53** in acetonitrile/ethyl acetate (1:1) was added the appropriate iodoalkane. Stirring was continued at room temperature for 12 hours. The formed precipitate was filtered, washed with diethyl ether and dried to obtain the desired targeted compounds **6–23**.

***N*,*N*,*N*-Trimethyl-2-(2-stearoxyphenylacetoxy)ethan-1-aminium iodide (6)**

Compound **6** (96.8 %) was obtained following general procedure 1.6 using compound **36** and iodomethane. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27-7.18 (2H, m), 6.98-6.86 (2H, m), 4.46-4.40 (2H, m), 3.94 (2H, t, *J* = 6.4 Hz), 3.65 (2H, s), 3.63-3.60 (2H, m), 3.04 (9H, s), 1.71-1.64 (2H, m), 1.72-1.36 (2H, m), 1.32-1.22 (28H, m), 0.85 (3H, t, *J* = 6.8 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) *δ* 170.9, 157.0, 131.4, 129.0, 123.0, 120.6, 111.9, 68.0, 64.0, 58.5, 53.4, 35.8, 31.7,

29.6-29.4 (12C), 29.18, 29.15, 29.1, 25.9, 22.5, 14.4; HR-MS found 476.4088 (calcd for $C_{31}H_{56}INO_3$ $[M\text{-}CH_3+H]^+$ 476.4098).

***N,N,N*-Trimethyl-3-(2-stearoxyphenylacetoxy)propan-1-aminium iodide (7)**

Compound **7** (92%) was obtained following general procedure 1.6 using compound **37** and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.26-7.19 (2H, m), 6.97 (1H, d, J = 8.0 Hz), 6.89 (1H, t, J = 7.4 Hz), 4.08 (2H, t, J = 6.3 Hz), 3.94 (2H, t, J = 6.3 Hz), 3.61 (2H, s), 3.32 (2H, dd, J = 11.7, 4.9 Hz), 3.05 (9H, s), 2.03 (2H, td, J = 11.2, 5.7 Hz), 1.67 (2H, q, J = 7.1 Hz), 1.39-1.23 (30H, m), 0.85 (3H, t, J = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 170.89, 156.48, 130.82, 128.41, 122.78, 119.94, 111.28, 67.25, 62.41, 60.90, 52.09 (3C), 35.29, 31.20, 28.95-28.57 (12C), 25.28, 22.01, 21.96, 13.88; HR-MS: *m/z* found 490.4247 (calcd for $C_{31}H_{56}N_1O_3$ $[M\text{-}CH_3+H]^+$ 490.4260).

***N,N,N*-Triethyl-2-(2-stearoxyphenylacetoxy)ethan-1-aminium iodide (8)**

Compound **8** (89%) was obtained following general procedure 1.6 using compound **38** and iodoethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.27-7.20 (2H, m), 6.97 (1H, d, J = 8.0 Hz), 6.89 (1H, td, J = 7.4, 1.1 Hz), 4.36 (2H, t, J = 4.9 Hz), 3.95 (2H, t, J = 6.3 Hz), 3.64 (2H, s), 3.48 (2H, t, J = 4.9 Hz), 3.21 (6H, q, J = 7.1 Hz), 1.70-1.64 (2H, m), 1.40-1.24 (30H, m), 1.09 (9H, t, J = 7.2 Hz), 0.85 (3H, t, J = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 171.03, 157.08, 131.55, 129.19, 123.03, 120.67, 111.95, 68.01, 58.09, 54.75, 53.20 (3C), 36.10, 31.83, 29.58-29.25 (13C), 25.99, 22.63, 14.50, 7.60 (3C); HR-MS: *m/z* found 504.4399 (calcd for $C_{32}H_{58}N_1O_3$ $[M\text{-}C_2H_5+H]^+$ 504.4417).

1-Methyl-1-(2-(2-stearoxyphenylacetoxy)ethyl)pyrrolidin-1-ium iodide (9)

Compound **9** (72%) was obtained following general procedure 1.6 using compound **39** and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.26-7.21 (2H, m), 6.97 (1H, d, J = 8.0 Hz), 6.89 (1H, t, J = 7.4 Hz), 4.42 (2H, t, J = 4.6 Hz), 3.94 (2H, t, J = 6.3 Hz), 3.64 (4H, q, J = 4.6

Hz), 3.42-3.38 (4H, m), 2.94 (3H, s), 2.05-2.00 (4H, m), 1.70-1.64 (2H, m), 1.39-1.23 (30H, m), 0.85 (3H, t, J = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 170.35, 156.41, 130.89, 128.51, 122.45, 119.98, 111.28, 67.33, 63.98 (2C), 60.95, 58.44, 47.36, 35.41, 31.20, 28.95-28.57 (13C), 25.33, 22.01, 20.66 (2C), 13.88; HR-MS: *m/z* found 502.4238 (calcd for $C_{32}H_{56}N_1O_3$ $[M\text{-}CH_3+H]^+$ 502.4260).

1-Methyl-1-(2-(2-stearoxyphenylacetoxy)ethyl) piperidin-1-ium iodide (10)

Compound **10** (66%) was obtained following general procedure 1.6 using compound **40** and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.26-7.20 (2H, m), 6.97 (1H, d, J = 8.6 Hz), 6.89 (1H, t, J = 7.4 Hz), 4.44 (2H, t, J = 4.6 Hz), 3.94 (2H, t, J = 6.6 Hz), 3.63 (4H, d, J = 8.6 Hz), 3.27 (4H, t, J = 5.7 Hz), 2.98 (3H, s), 1.74-1.66 (6H, m), 1.54-1.23 (32H, m), 0.85 (3H, t, J = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 170.35, 156.39, 130.87, 128.52, 122.42, 119.99, 111.28, 67.34, 60.59 (2C), 57.41, 35.40, 31.20, 28.94-28.57 (13C), 25.34, 22.00, 20.37, 19.09 (2C), 13.87; HR-MS: *m/z* found 516.4392 (calcd for $C_{33}H_{58}N_1O_3$ $[M\text{-}CH_3+H]^+$ 516.4417).

4-Methyl-4-(2-(2-stearoxyphenylacetoxy)ethyl) morpholin-4-ium iodide (11)

Compound **11** (35%) was obtained following general procedure 1.6 using compound **41** and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.27-7.20 (2H, m), 6.97 (1H, d, J = 8.6 Hz), 6.89 (1H, t, J = 7.4 Hz), 4.46 (2H, t, J = 4.6 Hz), 3.96-3.76 (8H, m), 3.65 (2H, s), 3.11 (3H, s), 1.70-1.65 (2H, m), 1.39-1.23 (30H, m), 0.85 (3H, t, J = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 170.27, 156.39, 130.87, 128.53, 122.41, 119.99, 111.32, 67.35, 59.54 (2C), 59.43 (2C), 57.28, 35.36, 31.20, 28.94-28.56 (13C), 25.32, 22.00, 13.87; HR-MS: *m/z* found 518.4177 (calcd for $C_{32}H_{56}N_1O_4$ $[M\text{-}CH_3+H]^+$ 518.4209).

***N,N,N*-Trimethyl-2-(3-stearoxyphenylacetoxy)ethan-1-aminium iodide (12)**

Compound **12** (92%) was obtained following general procedure 1.6 using compound **42** and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.22 (1H, t, J = 8.0 Hz), 6.84-6.81 (3H, m),

4.46 (2H, s), 3.92 (2H, t, *J* = 6.3 Hz), 3.69-3.64 (4H, m), 3.08 (9H, s), 1.72-1.66 (2H, m), 1.42-1.24 (30H, m), 0.85 (3H, t, *J* = 7.2 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 170.99, 159.18, 135.79, 129.90, 116.34, 113.26, 67.83, 64.11, 58.75, 53.41 (3C), 31.83, 29.56-29.25 (13C), 26.07, 22.64, 14.50; HR-MS: *m/z* found 476.4072 (calcd for $C_{30}H_{54}N_1O_3$ $[M\text{-}CH_3+H]^+$ 476.4104).

***N,N,N*-Trimethyl-3-(3-stearoxyphenylacetoxy)propan-1-aminium iodide (13)**

Compound **13** (89%) was obtained following general procedure 1.6 using compound **43** and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.23 (1H, t, *J* = 7.7 Hz), 6.83 (3H, d, *J* = 8.0 Hz), 4.11 (2H, t, *J* = 6.0 Hz), 3.93 (2H, t, *J* = 6.6 Hz), 3.66 (2H, s), 3.34 (2H, t, *J* = 4.3 Hz), 3.05 (9H, s), 2.04 (2H, td, *J* = 11.2, 5.7 Hz), 1.72-1.66 (2H, m), 1.42-1.24 (30H, m), 0.85 (3H, t, *J* = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 171.49, 159.17, 136.10, 129.94, 121.99, 116.24, 113.16, 67.81, 63.06, 61.78, 52.76 (3C), 31.84, 29.57-29.26 (13C), 26.08, 22.65, 22.57, 14.51; HR-MS: *m/z* found 490.4248 (calcd for $C_{31}H_{56}N_1O_3$ $[M\text{-}CH_3+H]^+$ 490.4260).

***N,N,N*-Triethyl-2-(3-stearoxyphenylacetoxy)ethan-1-aminium iodide (14)**

Compound **14** (82%) was obtained following general procedure 1.6 using compound **44** and iodoethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.22 (1H, t, J = 8.0 Hz), 6.84-6.82 (3H, m), 4.40 (2H, t, J = 4.9 Hz), 3.93 (2H, t, J = 6.3 Hz), 3.69 (2H, s), 3.52 (2H, t, J = 4.9 Hz), 3.26 (6H, q, J = 7.1 Hz), 1.72-1.66 (2H, m), 1.41-1.24 (30H, m), 1.13 (9H, t, J = 7.4 Hz), 0.85 (3H, t, J = 7.2 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 171.00, 159.23, 135.76, 129.95, 122.07, 116.36, 113.32, 67.86, 58.21, 54.91, 53.24 (2C), 31.82, 29.55-29.23 (13C), 26.06, 22.62, 14.49, 7.67 (3C); HR-MS: *m/z* found 504.4417 (calcd for $C_{32}H_{58}N_1O_3$ $[M\text{-}C_2H_5+H]^+$ 504.4417).

1-Methyl-1-(2-(3-stearoxyphenylacetoxy)ethyl) pyrrolidin-1-ium iodide (15)

Compound **15** (59%) was obtained following general procedure 1.6 using compound **45** and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.22 (1H, t, *J* = 7.7 Hz), 6.84-6.81 (3H, m),

4.46 (2H, t, *J* = 4.6 Hz), 3.92 (2H, t, *J* = 6.3 Hz), 3.70-3.66 (4H, m), 3.49-3.38 (4H, m), 2.91-3.05 (3H), 2.08-1.99 (4H, m), 1.72-1.66 (2H, m), 1.41-1.24 (30H, m), 0.85 (3H, t, *J* = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 170.98, 159.19, 135.81, 129.92, 122.11, 116.36, 113.25, 67.83, 64.61 (2C), 61.72, 59.28, 48.13, 31.83, 29.56-29.25 (13C), 26.08, 22.64, 21.32 (2C), 14.50; HR-MS: *m/z* found 502.4216 (calcd for $C_{32}H_{56}N_1O_3$ $[M\text{-}CH_3+H]^+$ 502.4260).

1-Methyl-1-(2-(3-stearoxyphenylacetoxy)ethyl) piperidin-1-ium iodide (16)

Compound **16** (78%) was obtained following general procedure 1.6 using compound **46** (200 mg, 0.39 mmol) and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.22 (1H, t, *J* = 7.7 Hz), 6.84-6.81 (3H, m), 4.47 (2H, t, *J* = 4.6 Hz), 3.92 (2H, t, *J* = 6.3 Hz), 3.69-3.66 (4H, m), 3.31 (4H, t, *J* = 4.9 Hz), 3.02 (3H, s), 1.72 (6H, ddd, *J* = 36.2, 14.7, 5.9 Hz), 1.54-1.24 (32H, m), 0.85 (3H, t, *J* = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 170.95, 159.18, 135.80, 129.92, 122.10, 116.32, 113.25, 67.82, 61.19 (2C), 58.23, 31.83, 29.56-29.25 (13C), 26.08, 22.64, 21.04, 19.75 (2C), 14.51; HR-MS: *m/z* found 516.4365 (calcd for $C_{33}H_{58}N_1O_3$ $[M\text{-}CH_3+H]^+$ 516.4417).

4-Methyl-4-(2-(3-stearoxyphenylacetoxy)ethyl) morpholin-4-ium iodide (17)

Compound **17** (54%) was obtained following general procedure 1.6 using compound **47** and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.23 (1H, t, *J* = 8.0 Hz), 6.84-6.82 (3H, m), 4.50-4.48 (2H, m), 3.94-3.80 (8H, m), 3.70 (2H, s), 3.43 (4H, td, *J* = 7.6, 3.8 Hz), 3.16 (3H, s), 1.72-1.66 (2H, m), 1.42-1.24 (30H, m), 0.85 (3H, t, *J* = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 170.92, 159.18, 135.79, 129.93, 122.12, 116.38, 113.24, 67.83, 60.22 (2C), 60.07 (2C), 58.08, 31.83, 29.56-29.25 (13C), 26.07, 22.64, 14.50; HR-MS: *m/z* found 518.4180 (calcd for $C_{32}H_{56}N_1O_4$ $[M\text{-}CH_3+H]^+$ 518.4209).

***N,N,N*-Trimethyl-2-(4-stearoxyphenylacetoxy)ethan-1-aminium iodide (18)**

Compound **18** (87%) was obtained following general procedure 1.6 using compound **48** and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.18 (2H, d, J = 8.6 Hz), 6.87 (2H, d, J = 8.6 Hz), 4.45 (2H, s), 3.92 (2H, t, J = 6.6 Hz), 3.64 (4H, d, J = 6.9 Hz), 3.08 (9H, s), 1.71-1.66 (2H, m), 1.42-1.23 (30H, m), 0.85 (3H, t, J = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 171.40, 158.20, 131.06 (2C), 126.15, 114.77 (2C), 67.89, 64.13, 58.68, 53.42 (3C), 31.83, 29.56-29.25 (13C), 26.06, 22.64, 14.50; HR-MS: *m/z* found 476.4073 (calcd for $C_{30}H_{54}N_1O_3$ $[M\text{-}CH_3+H]^+$ 476.4104).

***N,N,N*-Trimethyl-3-(4-stearoxyphenylacetoxy)propan-1-aminium iodide (19)**

Compound **19** (88%) was obtained following general procedure 1.6 using compound **49** (200 mg, 0.41 mmol) and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.17 (2H, d, J = 8.6 Hz), 6.87 (2H, d, J = 8.6 Hz), 4.09 (2H, t, J = 6.0 Hz), 3.92 (2H, t, J = 6.3 Hz), 3.61 (2H, s), 3.34 (2H, q, J = 4.0 Hz), 3.05 (9H, s), 2.03 (2H, td, J = 11.3, 5.7 Hz), 1.71-1.66 (2H, m), 1.42-1.23 (30H, m), 0.85 (3H, t, J = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 171.26, 157.51, 130.32 (2C), 125.78, 114.13 (2C), 67.22, 62.43, 61.07, 52.11 (3C), 31.20, 28.92- 28.58 (13C), 25.43, 22.01, 21.93, 13.87; HR-MS: *m/z* found 490.4251 (calcd for $C_{31}H_{56}N_1O_3$ $[M\text{-}CH_3+H]^+$ 490.4260).

***N,N,N*-Triethyl-2-(4-stearoxyphenylacetoxy)ethan-1-aminium iodide (20)**

Compound **20** (61%) was obtained following general procedure 1.6 using compound **50** and iodoethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.17 (2H, d, J = 8.6 Hz), 6.87 (2H, d, J = 8.6 Hz), 4.39 (2H, t, J = 4.9 Hz), 3.92 (2H, t, J = 6.3 Hz), 3.65 (2H, s), 3.51 (2H, t, J = 5.2 Hz), 3.27 (6H, q, J = 7.1 Hz), 1.71-1.66 (2H, m), 1.42-1.12 (39H, m), 0.85 (3H, t, J = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 171.42, 158.24, 131.03 (2C), 126.13, 114.86 (2C), 67.94, 58.11, 54.93, 53.25 (3C), 31.82, 29.54-29.22 (13C), 26.05, 22.62, 14.49, 7.68 (3C); HR-MS: *m/z* found 504.4398 (calcd for $C_{32}H_{58}N_1O_3$ $[M\text{-}C_2H_5+H]^+$ 504.4417).

1-Methyl-1-(2-(4-stearoxyphenylacetoxy)ethyl) pyrrolidin-1-ium iodide (21)

Compound **21** (68%) was obtained following general procedure 1.6 using compound **51** and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.18 (2H, d, J = 8.6 Hz), 6.87 (2H, d, J = 9.2 Hz), 4.45 (2H, t, J = 4.9 Hz), 3.92 (2H, t, J = 6.6 Hz), 3.67-3.65 (4H, m), 3.49-3.40 (4H, m), 2.98 (3H, s), 2.07-2.03 (4H, m), 1.71-1.66 (2H, m), 1.40-1.23 (30H, m), 0.85 (3H, t, J = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 171.39, 158.21, 131.08 (2C), 126.17, 114.77 (2C), 67.88, 64.62 (2C), 61.76, 59.21, 48.13, 31.83, 29.56-29.25 (13C), 26.07, 22.64, 21.32 (2C), 14.50; HR-MS: *m/z* found 502.4218 (calcd for $C_{32}H_{56}N_1O_3$ $[M\text{-}CH_3+H]^+$ 502.4260).

1-Methyl-1-(2-(4-stearoxyphenylacetoxy)ethyl) piperidin-1-ium iodide (22)

Compound **22** (81%) was obtained following general procedure 1.6 using compound **52** and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.18 (2H, d, J = 8.6 Hz), 6.87 (2H, d, J = 8.6 Hz), 4.46 (2H, t, J = 4.9 Hz), 3.92 (2H, t, J = 6.6 Hz), 3.67-3.65 (4H, m), 3.31 (4H, t, J = 6.0 Hz), 3.02 (3H, s), 1.77-1.66 (6H, m), 1.55-1.47 (2H, m), 1.40-1.24 (30H, m), 0.85 (3H, t, J = 7.2 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 171.37, 158.20, 131.06 (2C), 126.15, 114.78 (2C), 67.88, 61.20 (2C), 58.12, 47.98, 31.83, 29.56-29.25, 29.21, 26.06, 22.64, 21.43, 21.04, 19.75 (2C), 14.50; HR-MS: *m/z* found 516.4366 (calcd for $C_{33}H_{58}N_1O_3$ $[M\text{-}CH_3+H]^+$ 516.4417).

4-Methyl-4-(2-(4-stearoxyphenylacetoxy)ethyl) morpholin-4-ium iodide (23)

Compound **23** (61%) was obtained following general procedure 1.6 using compound **53** and iodomethane. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.18 (2H, d, J = 9.2 Hz), 6.87 (2H, d, J = 8.6 Hz), 4.48 (2H, dd, J = 5.4, 3.7 Hz), 3.93-3.79 (8H, m), 3.65 (2H, s), 3.45-3.40 (4H, m), 3.16 (3H, s), 1.71-1.66 (2H, m), 1.41-1.24 (30H, m), 0.85 (3H, t, J = 6.9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 171.3, 158.2, 131.1 (2C), 126.1, 114.8 (2C), 67.9, 60.2 (2C), 60.1 (2C), 58.0, 31.8, 29.6-29.2 (13C), 26.1, 22.6, 14.5; HR-MS: *m/z* found 518.4156 (calcd for $C_{32}H_{56}N_1O_4$ $[M\text{-}CH_3+H]^+$ 518.4209).

2. Biological evaluation

2.1. Cells growth inhibition assays

The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96-well microtiter plates in 100 μL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (T_z). Experimental drugs are solubilized in dimethylsulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. Additional four, 10-fold, or 1/2 log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated

plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μL of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements (time zero [T_z], control growth [C], and test growth in the presence of drug at the five concentration levels [T_i]), the percentage growth is calculated at each of the drug concentration levels. Percentage growth inhibition is calculated as:

- $[(T_i - T_z)/(C - T_z)] \times 100$ for concentrations for which $T_i \geq T_z$.
- $[(T_i - T_z)/ T_z] \times 100$ for concentrations for which $T_i < T_z$.

2.2. General

Roswell Park Memorial Institute (RPMI) 1640 medium, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and trypsin-EDTA were purchased from Invitrogen (Grand Island, NY). Dimethyl sulfoxide (DMSO), bovine serum albumin (BSA), and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). All the tested compounds were dissolved in 100% DMSO and the final concentration of DMSO was under 0.1% *in vitro* and *in vivo* condition. The anti-β actin, was purchased Santa Cruz Biotechnology, Inc. (Dallas, TX). The anti-Cyclin D1, anti-CDK2, anti-CDK4, anti-CDK6, anticleaved PARP, and anticleaved caspase 8 were purchased from Cell Signaling Technology (Danvers, MA). The test compound solutions were dissolved in 100% DMSO.

2.3. Cell Culture

The A549 cancer cell line was obtained from the American Type Culture Collection (Manassas, VA, USA). The MRC-5 cell line was provided by the Korean Cell Line Bank (Seoul, Korea). Cells were cultivated in medium (RPMI-1640 media for A549; DMEM media for MRC-5 cells) containing 10 % fetal bovine serum (FBS) and 1% of penicillin-streptomycin solution. (Thermo-Scientific, MA) in a humidified incubator containing 5% CO_2 at 37 °C.

Cell Cycle Distribution Analysis

Cells were seeded and treated with test compound for 24 h and the cell pellets were fixed with 70% ethanol overnight at −20°C. Fixed cells were trypsinized and washed with PBS. The cells were resuspended in 50 μg/mL RNase A and 20 μg/mL of propidium iodide (PI) in the dark for 10 min at room temperature. The fluorescence binding cells were analyzed DNA content using a FACScalibur flow cytometer (BD Bioscience, Franklin Lakes, NJ). The distribution of cell contents was measured with 10,000 cells in each group and the results were represented as histograms of the DNA content.

Annexin V-FITC/Propidium Iodide (PI) Double Staining Analysis

Cells were treated with test compound for 48 h and then stained with Annexin V-FITC and PI using an annexin V-FITC apoptosis detection kit (BD Biosciences, San Diego, CA) according to the manufacturer's instruction. Briefly, the incubated cells were harvested and resuspended with 1× binding buffer. Annexin V-FITC and PI (5 μL) were added to cell suspensions and further incubated in the dark for 15 min at room temperature. Stained cells were immediately analyzed with 1× binding buffer using a flow cytometer.

Western Blot Analysis

Cells were seeded and treated with test compound for 24 h. After 24 h incubation, total cell lysates were mixed with lysis buffer, and boiled for 12 min at 100°C. Each protein sample was quantified with BCA reagents and equal amounts of protein samples were subjected to SDS-polyacrylamide gel electrophoresis (PAGE). The separated proteins were electrically transferred to poly(vinylidene fluoride) membranes (PVDF) activated with 100% methanol (Millipore, Bedford, MA). The membranes were blocked using 5% bovine serum albumin in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 30 min at room temperature. The desired primary antibodies were diluted in 5% BSA (1:200–1:2000) and incubated overnight

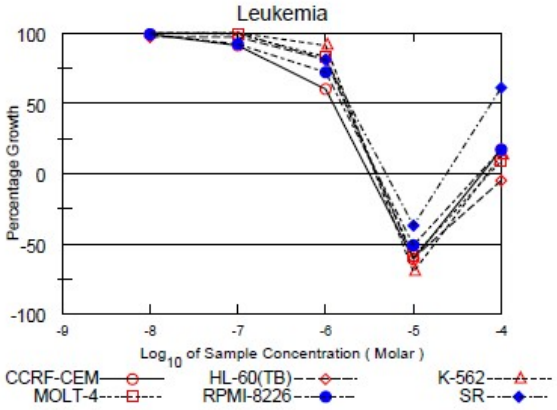
at 4 °C. The membranes were washed three times with TBST and then incubated with the appropriate secondary antibodies diluted in TBST for 2 h at room temperature. The membranes were washed three times with TBST and then visualized using an enhanced chemiluminescence detection kit (iNtRON Biotechnology, Seongnam, Korea). The membranes were analyzed using an ImageQuant LAS 4000 imager (GE Healthcare, Chicago, IL).

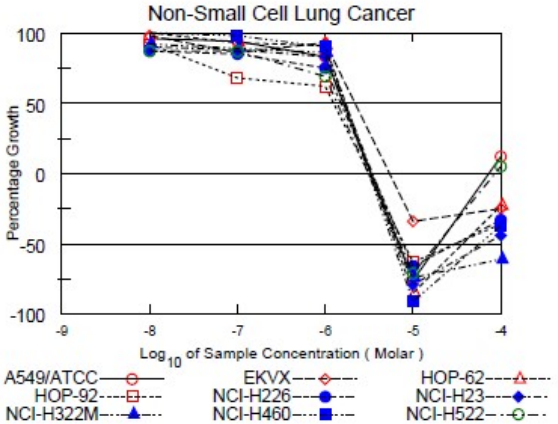
Table S1. Anticancer activity expressed as % growth inhibition of synthesized compounds **6–23** against diverse NSCLC cell lines at 10 µM concentration in comparison with stPEPC and edelfosine.

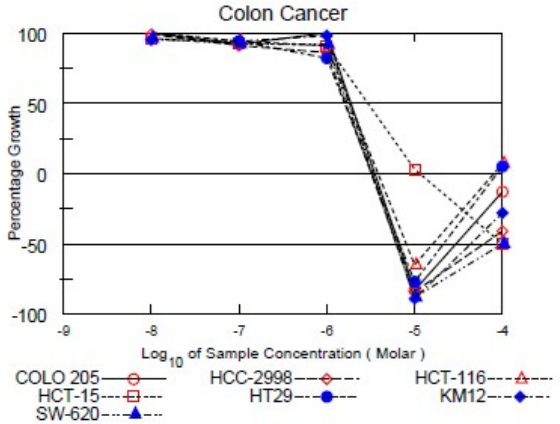
Compound	Non-small cell lung cancer cell line									Mean % growth inhibition
	Adenocarcinoma						Metastatic adenocarcinoma	large cell carcinoma	squamous carcinoma	
	A549	EKVX	HOP62	HOP92	H23	H522	H322M	H460	H226	
6	**88.49**	**53.13**	**95.07**	**114.01**	**59.76**	**97.81**	33.49	**86.52**	**71.34**	**77.74**
7	45.81	20.99	32.85	**60.39**	**53.60**	**63.73**	15.69	19.86	22.02	37.22
8	**70.67**	**56.59**	**58.96**	**93.19**	**86.93**	**89.48**	29.45	**65.44**	46.32	**66.34**
9	**76.98**	46.15	46.50	**67.33**	**91.55**	**93.15**	31.90	**72.54**	**86.56**	**68.07**
10	**57.82**	36.88	29.97	**109.25**	**70.17**	**66.73**	15.40	37.81	**71.86**	**55.10**
11	37.37	13.30	44.42	**55.77**	33.52	**60.11**	20.24	30.76	**50.18**	38.41
12	8.12	1.89	3.94	12.15	11.03	13.33	0.32	2.40	5.79	6.55
13	16.20	4.64	15.91	22.40	24.08	21.79	4.07	4.47	14.96	14.28
14	40.03	22.26	30.94	47.72	**64.35**	**61.52**	22.31	32.99	40.45	40.29
15	13.89	1.14	9.94	30.01	24.64	36.56	NI[a]	4.17	14.63	16.87
16	9.38	3.05	NI	18.99	16.11	28.53	NI	0.88	10.80	12.53
17	7.62	NI	9.38	13.92	4.14	16.94	1.47	1.00	6.29	7.60
18	5.41	NI	NI	NI	6.67	4.28	NI	NI	4.87	5.31
19	21.86	3.92	8.58	22.15	18.90	21.55	9.97	26.72	22.11	17.31
20	21.16	12.53	2.95	13.34	39.51	26.64	13.29	15.83	16.15	17.93
21	2.78	NI	NI	NI	6.84	3.23	NI	0.31	7.36	4.11
22	11.09	2.11	NI	2.18	13.33	10.83	3.63	NI	NI	7.19
23	NI	NI	NI	NI	5.91	5.79	3.14	0.33	NI	3.79
stPEPC	38.60	0.19	44.74	42.96	3.81	42.46	NI	37.88	10.86	27.69
Edelfosine	2.5	NI	**56**	40.1	10.9	27	NI	**53.6**	6.1	28.03
NSC43067	NI	17.7	NI	NI	NI	6.5	ND[b]	NI	6.2	3.80

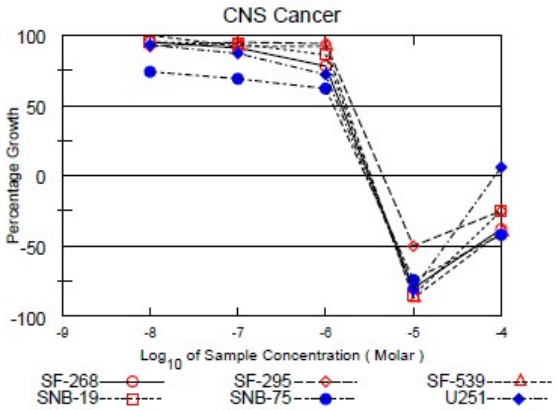
[a] NI: no inhibition
[b] ND: not determined

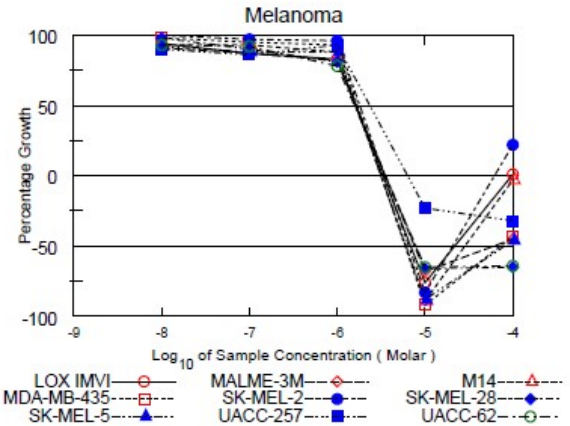
Dose-Response Curves for compound 6

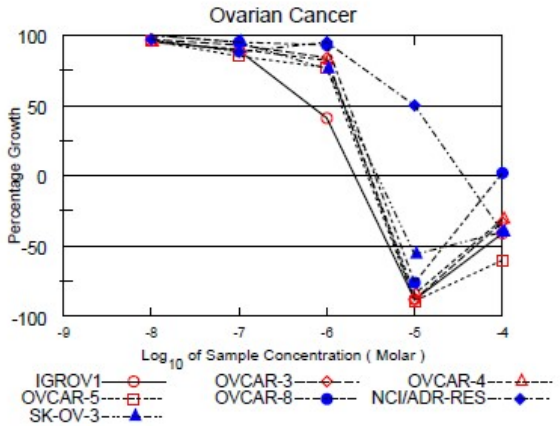


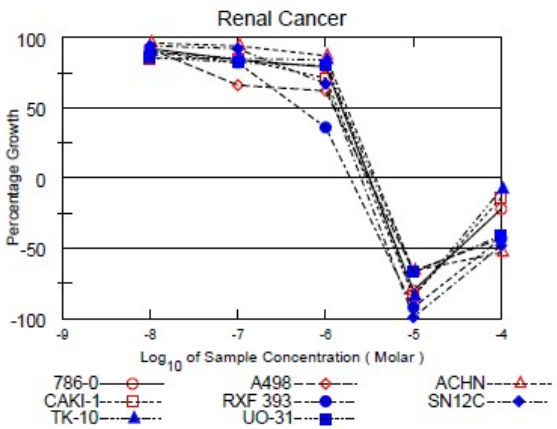


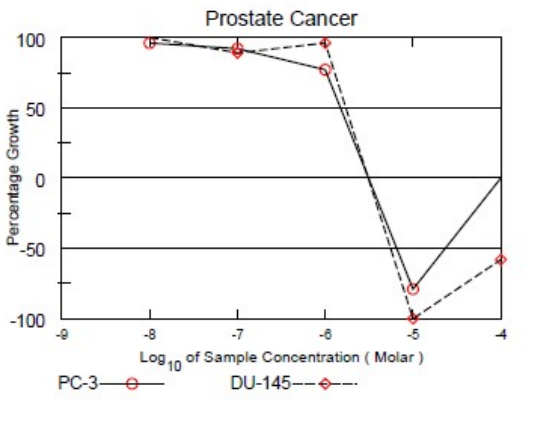


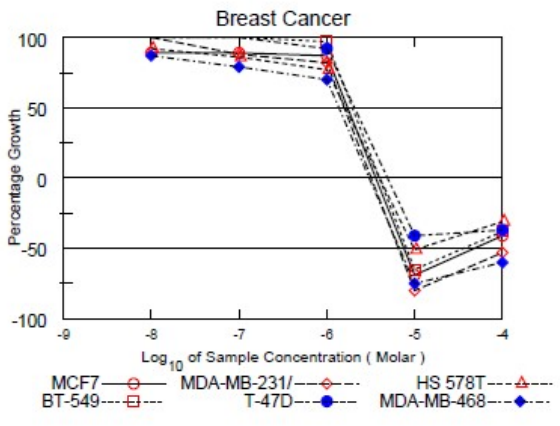




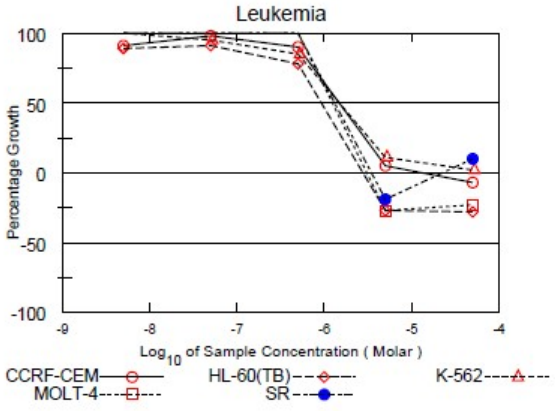


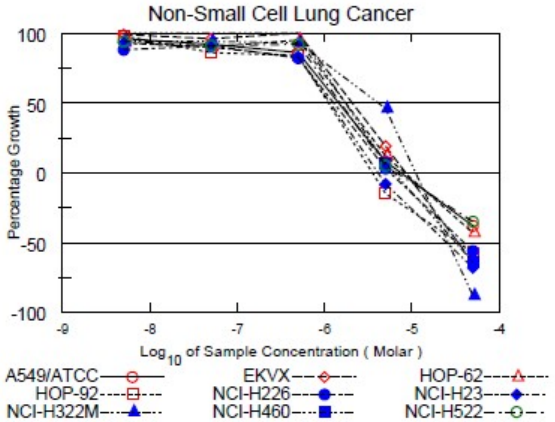


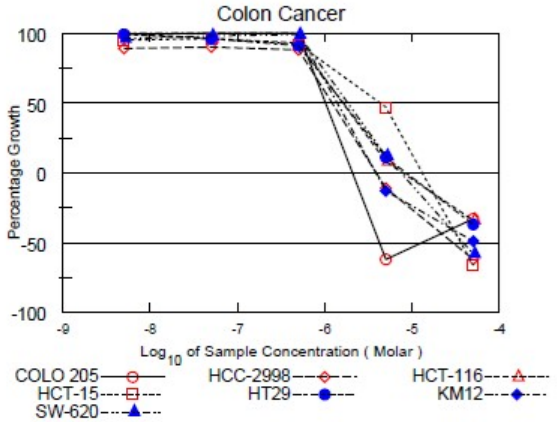


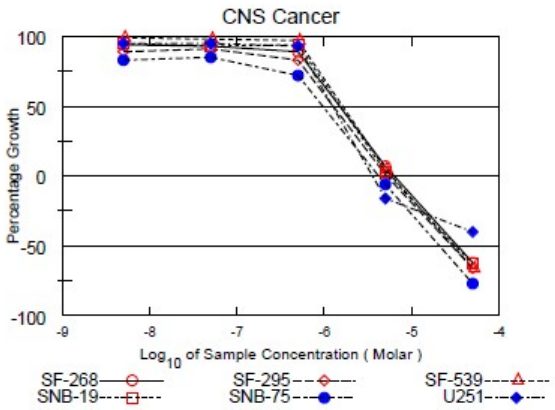


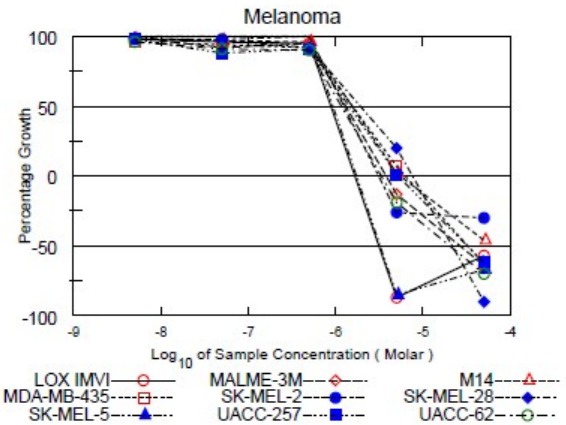
Dose-Response Curves for compound 8

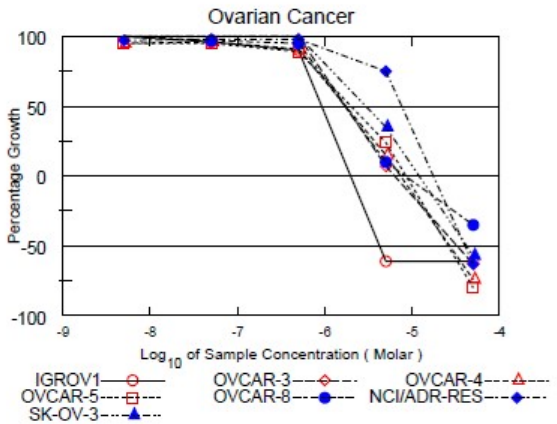


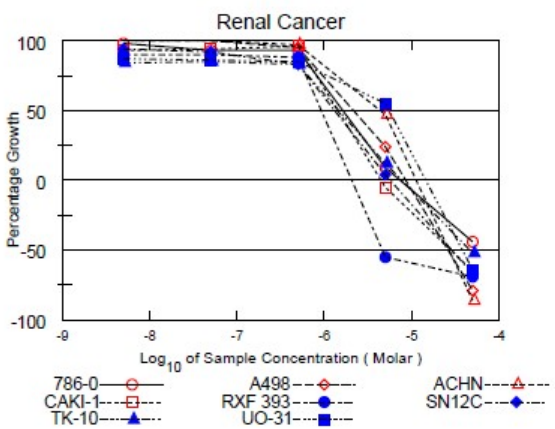


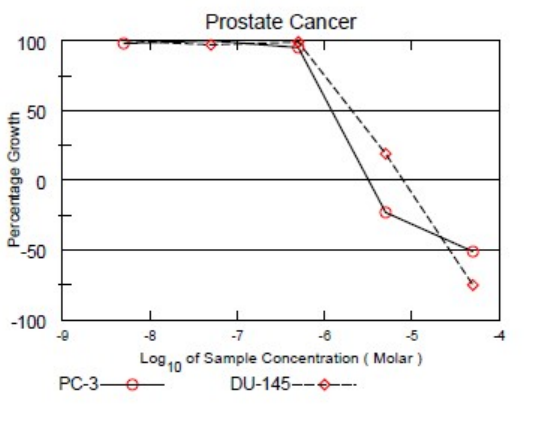


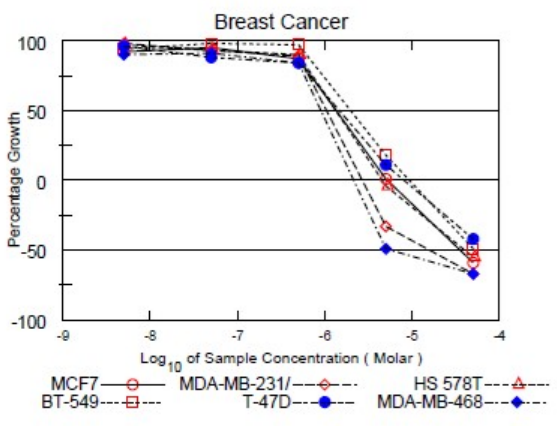




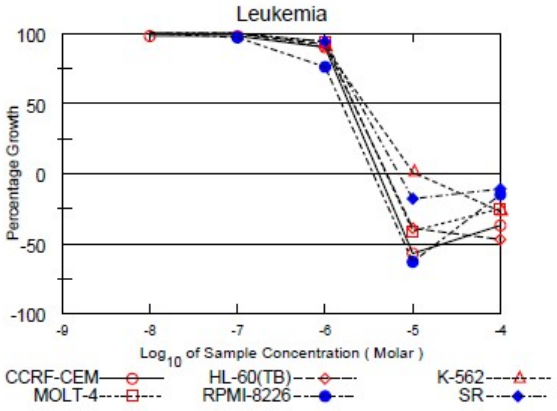


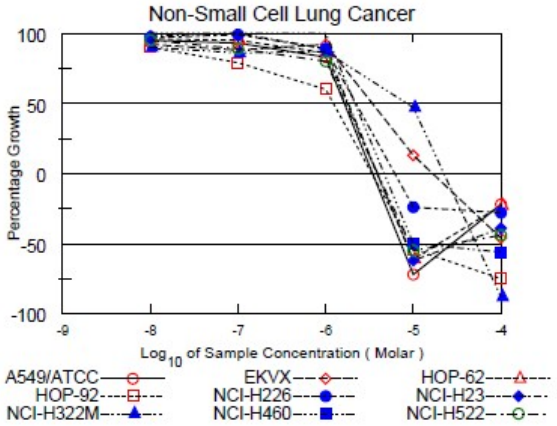


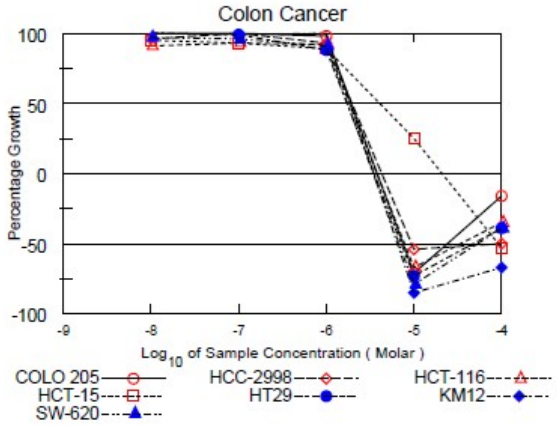


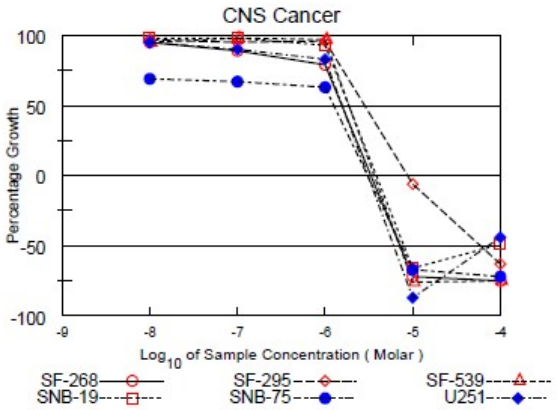


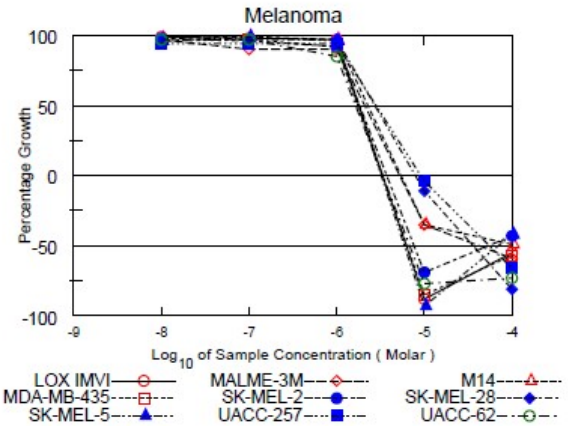
Dose-Response Curves for compound 9

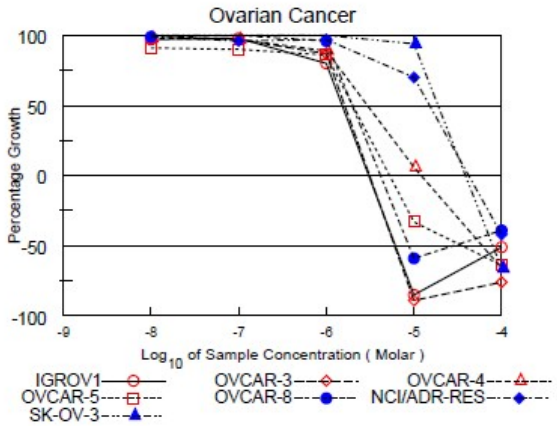


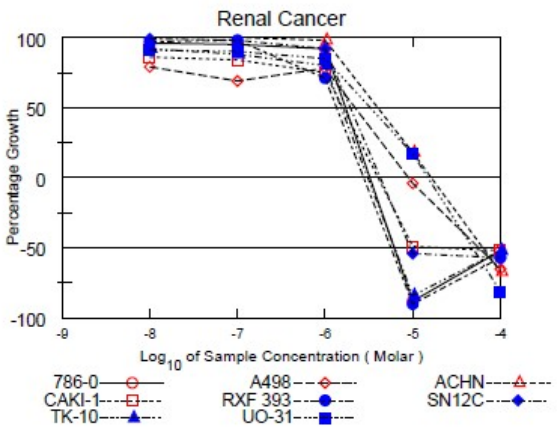


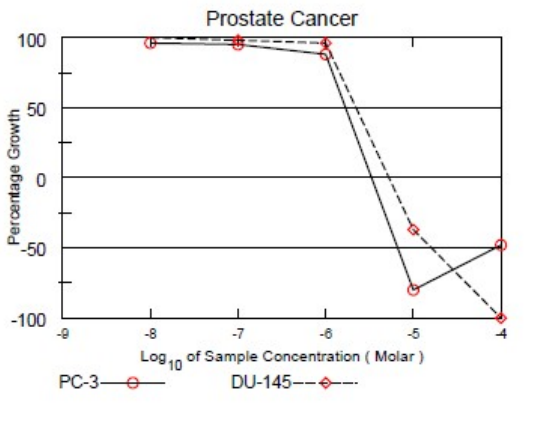


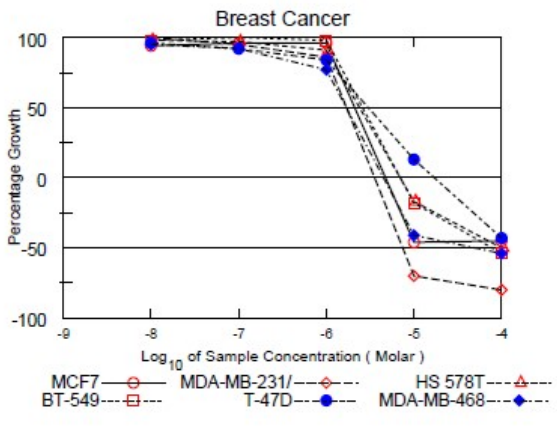




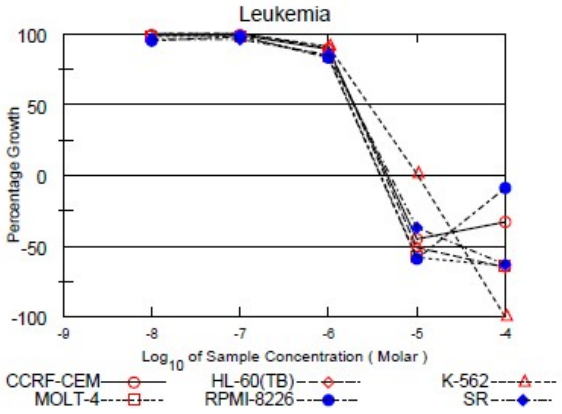


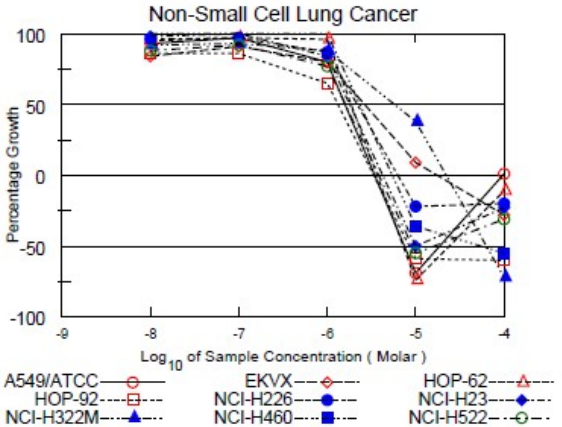


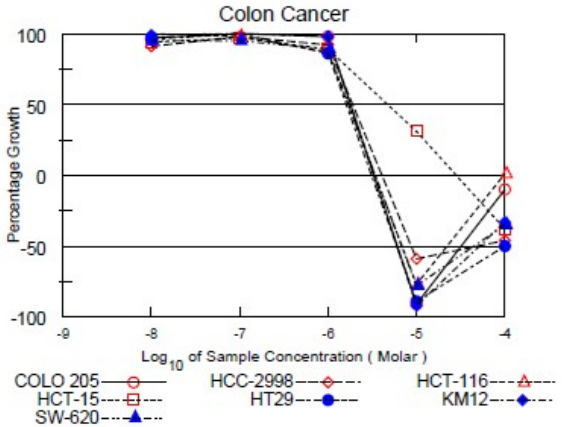


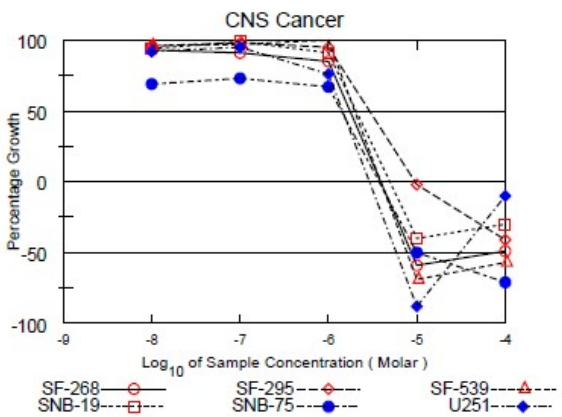


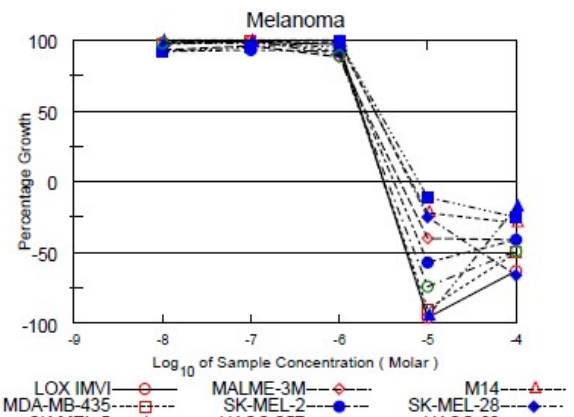
Dose-Response Curves for compound 10

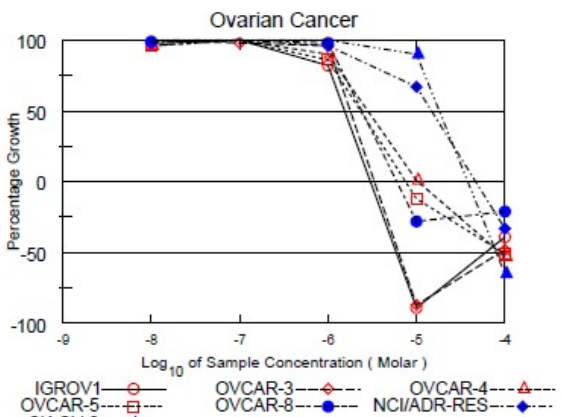


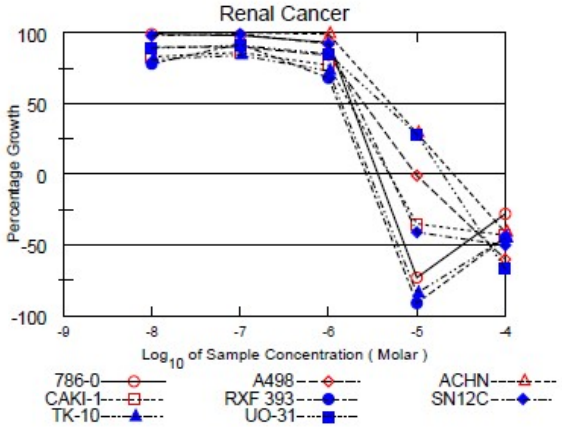


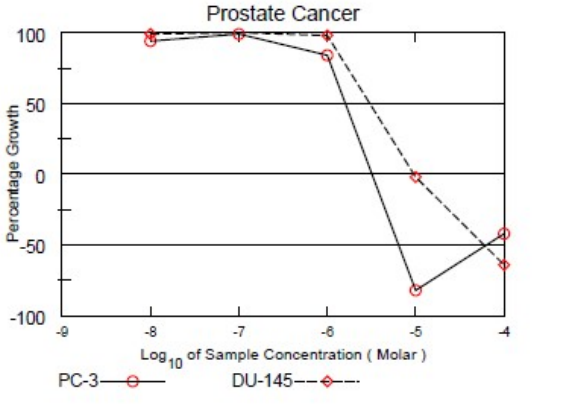


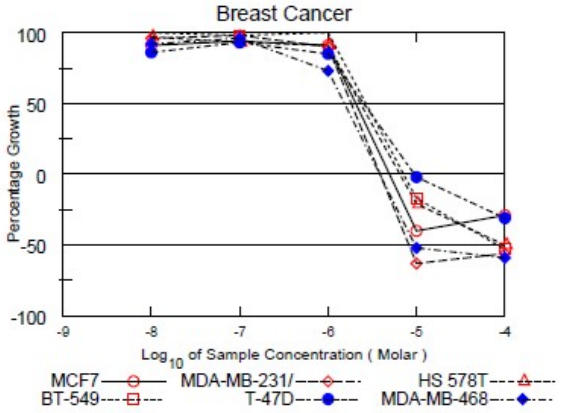












Western blot analysis of CDK1, CDK7 and CDK8

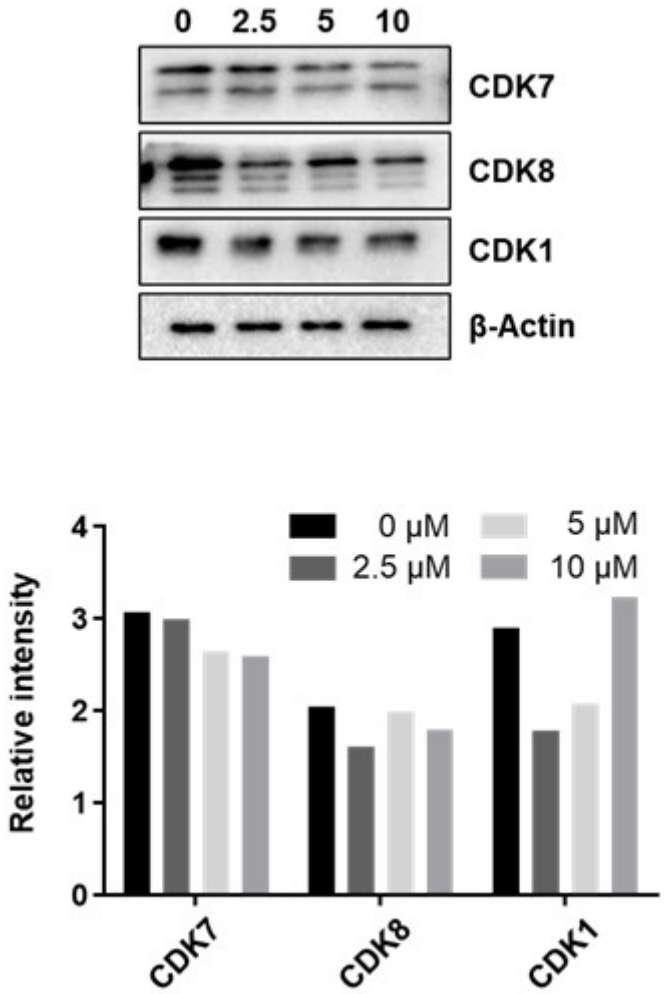
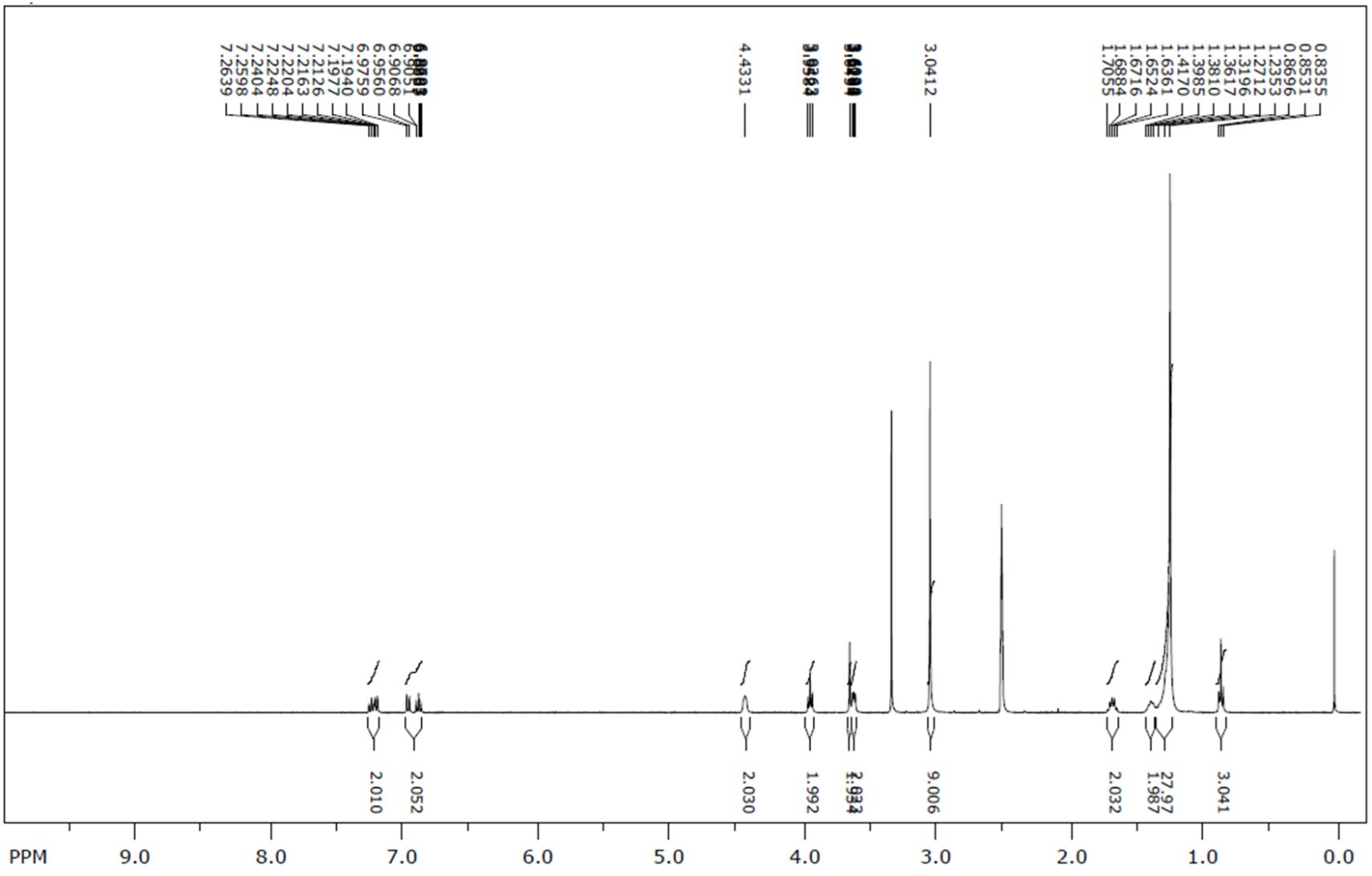
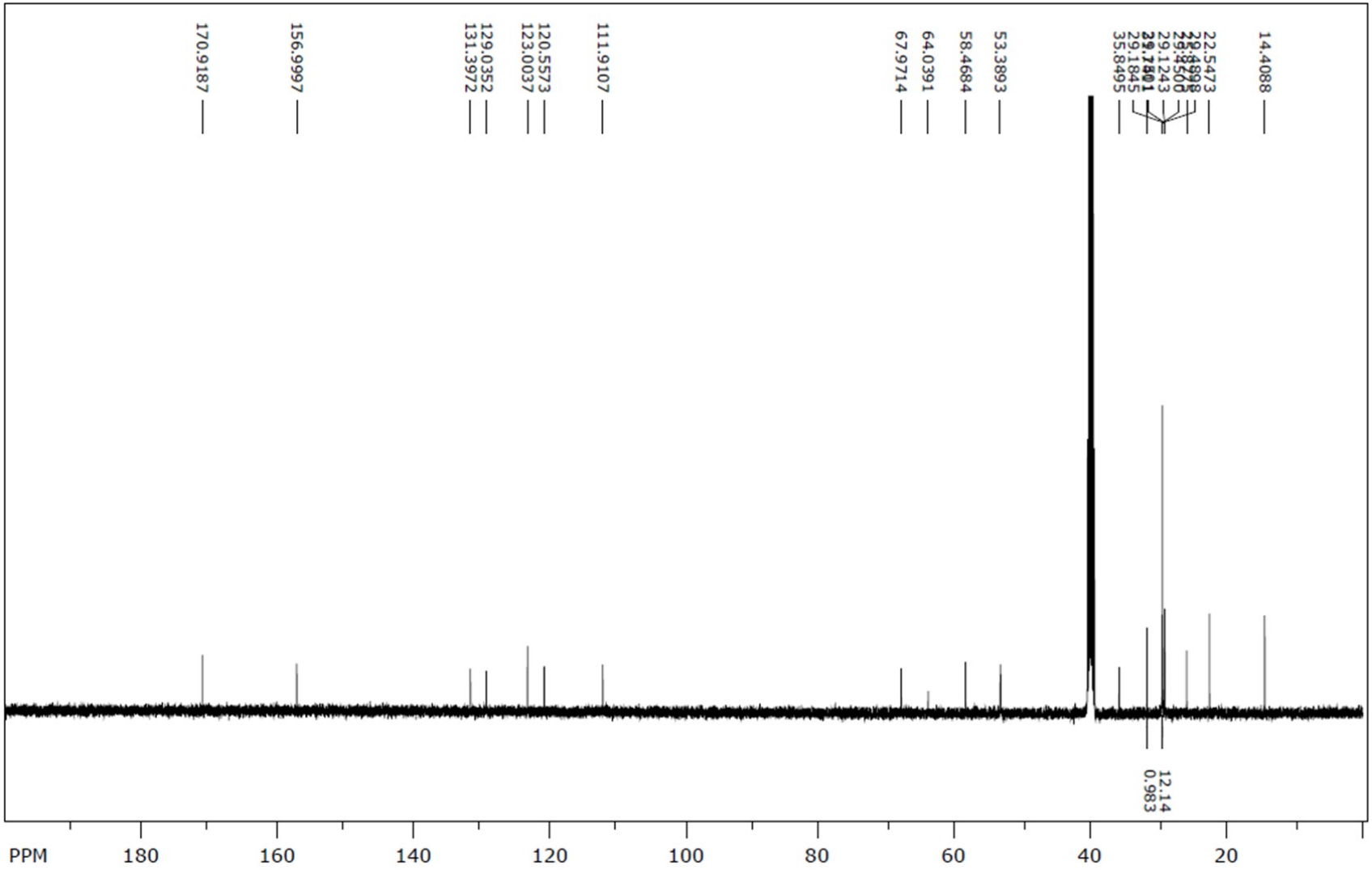


Figure S1. Western blot analysis of CDK1, CDK7 and CDK8 levels in A549 cells upon treatment with different concentrations of compound **6**.

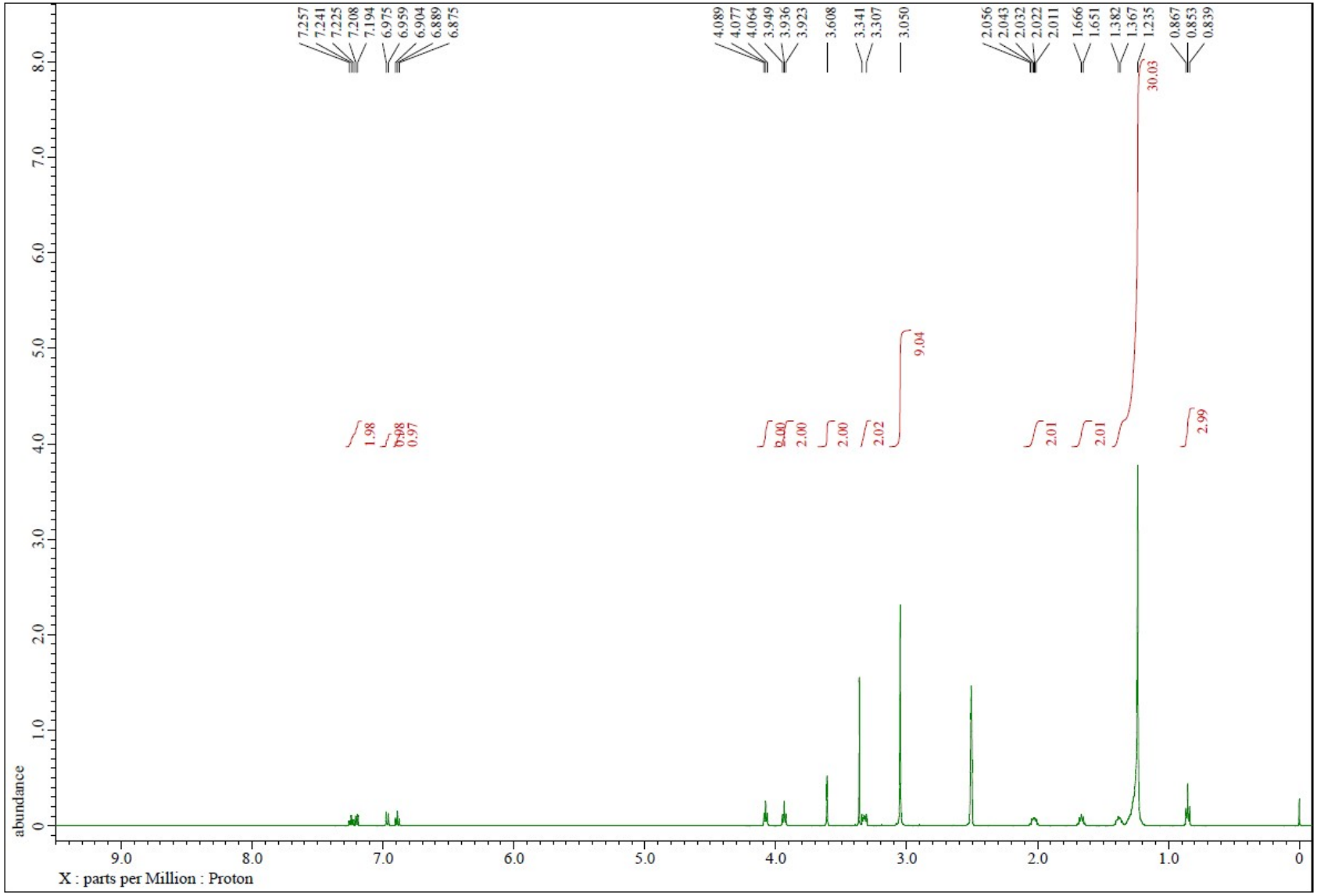
NMR Spectra



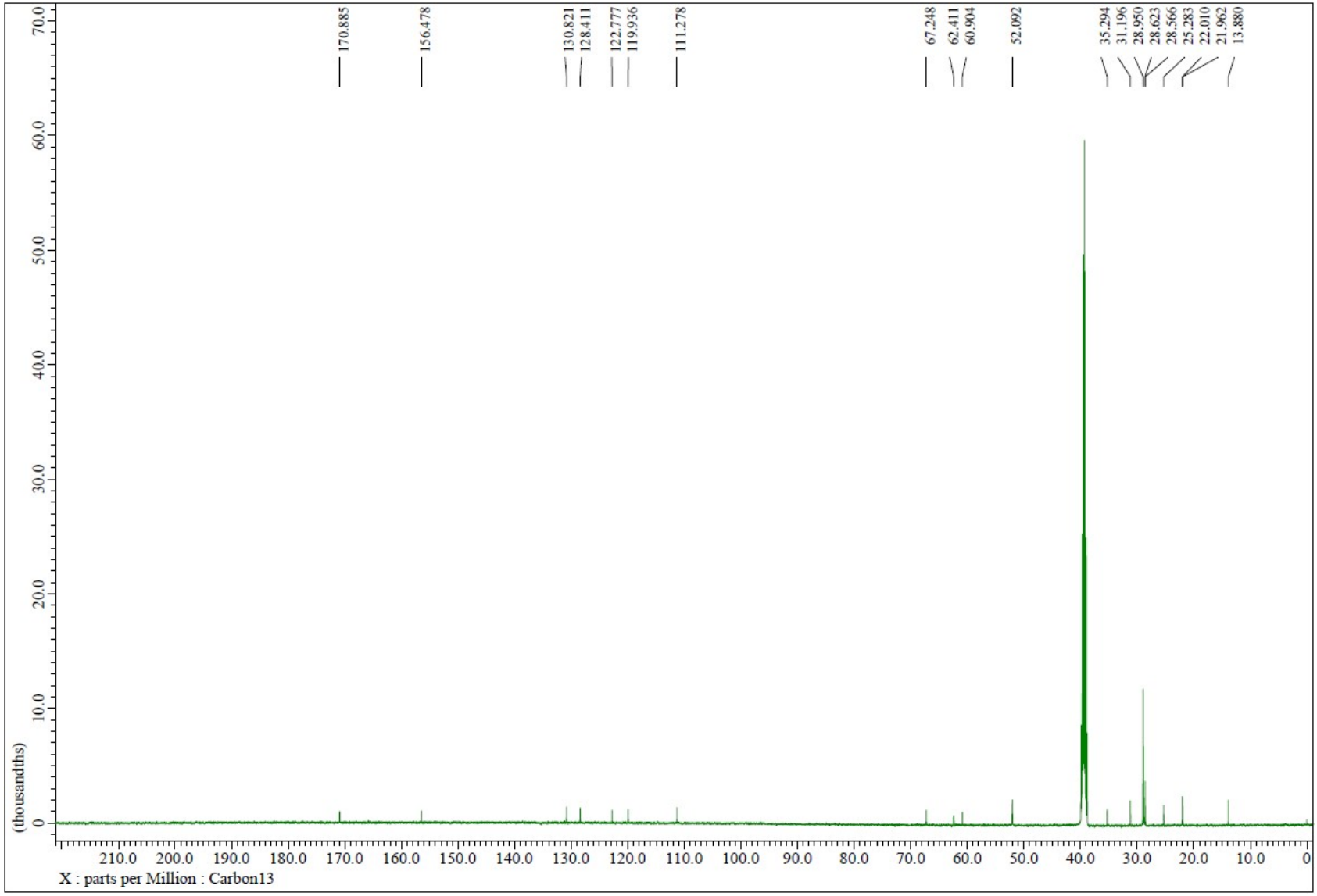
^{1}H NMR spectrum of compound **6**



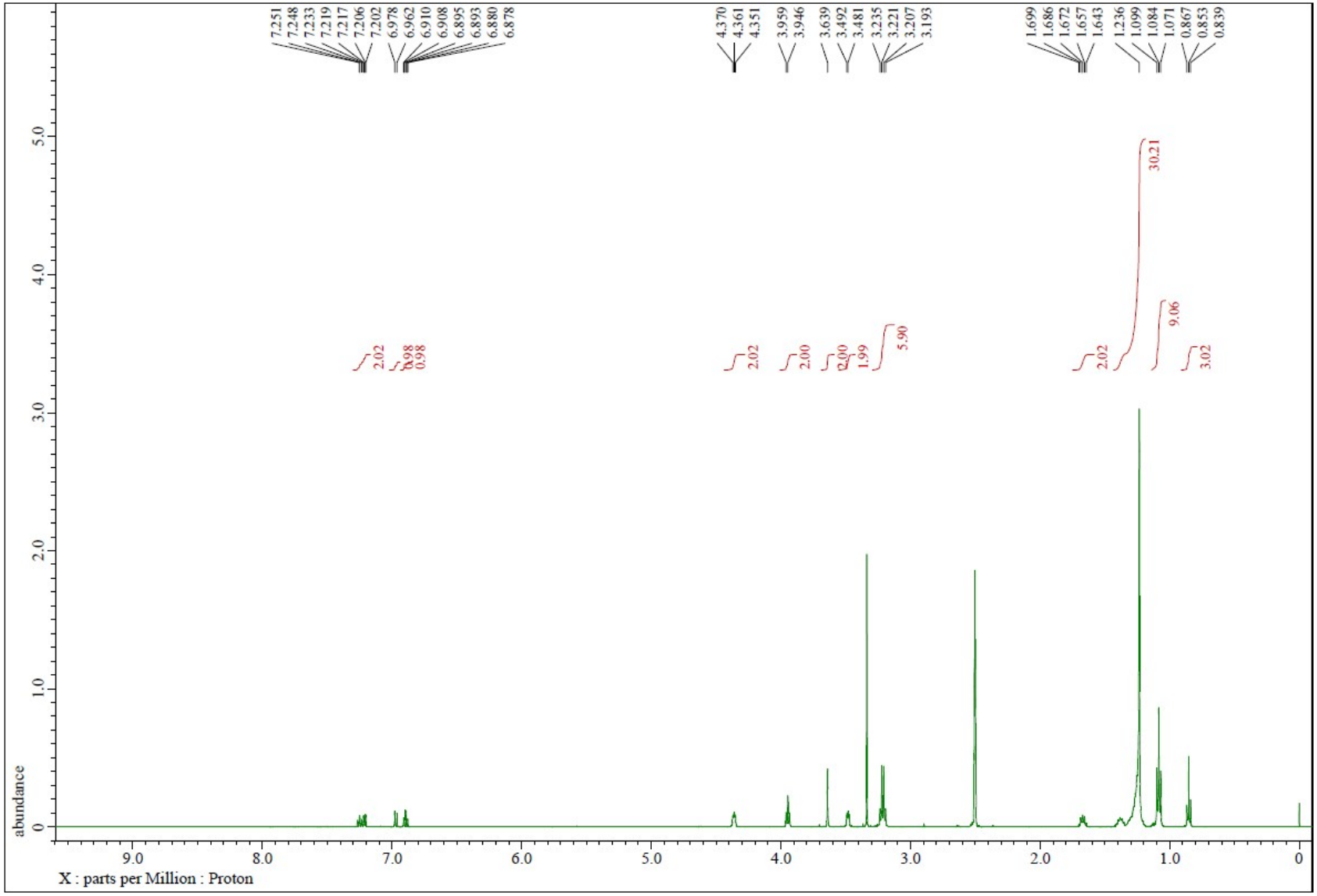
^{13}C NMR spectrum of compound **6**



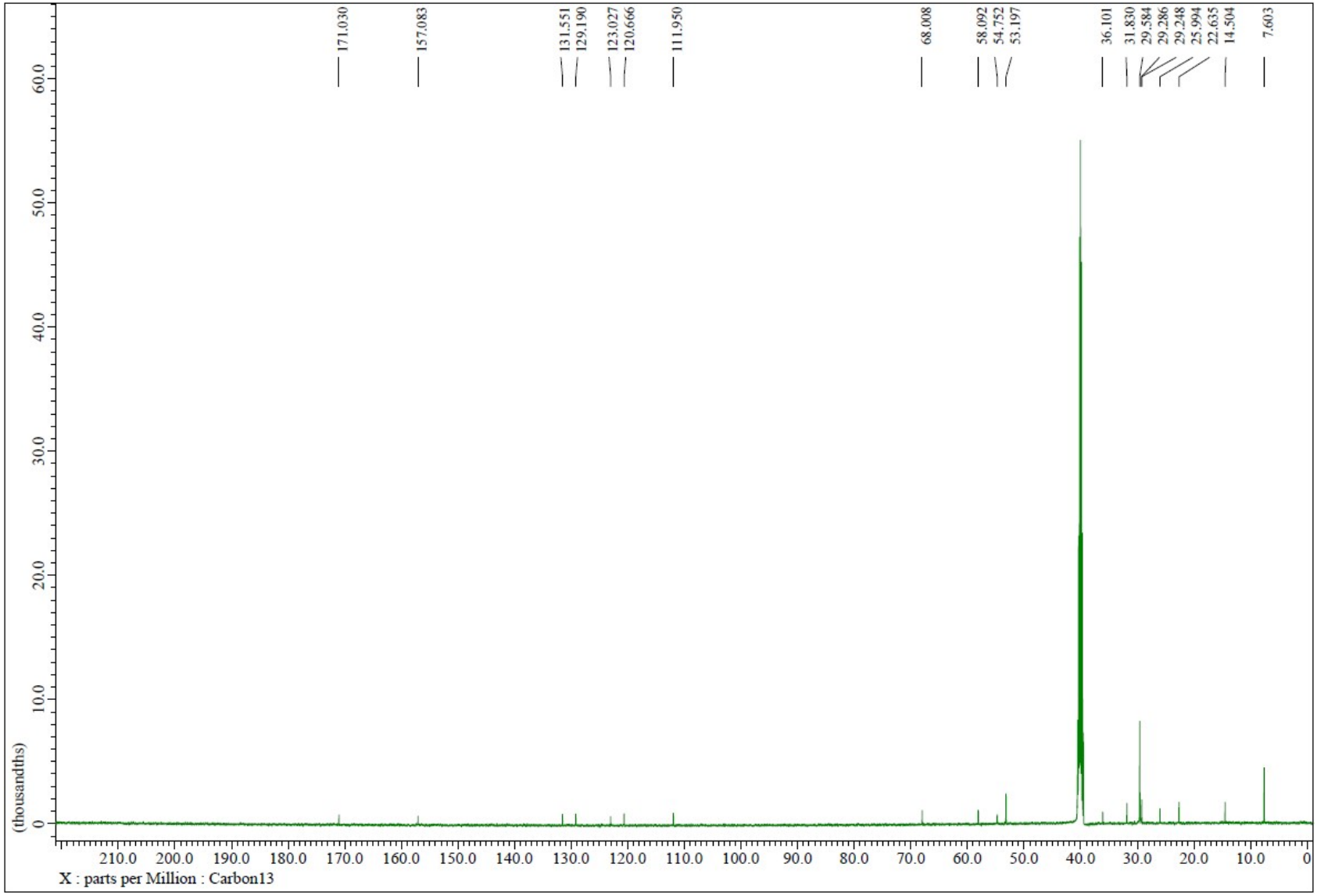
^{1}H NMR spectrum of compound **7**



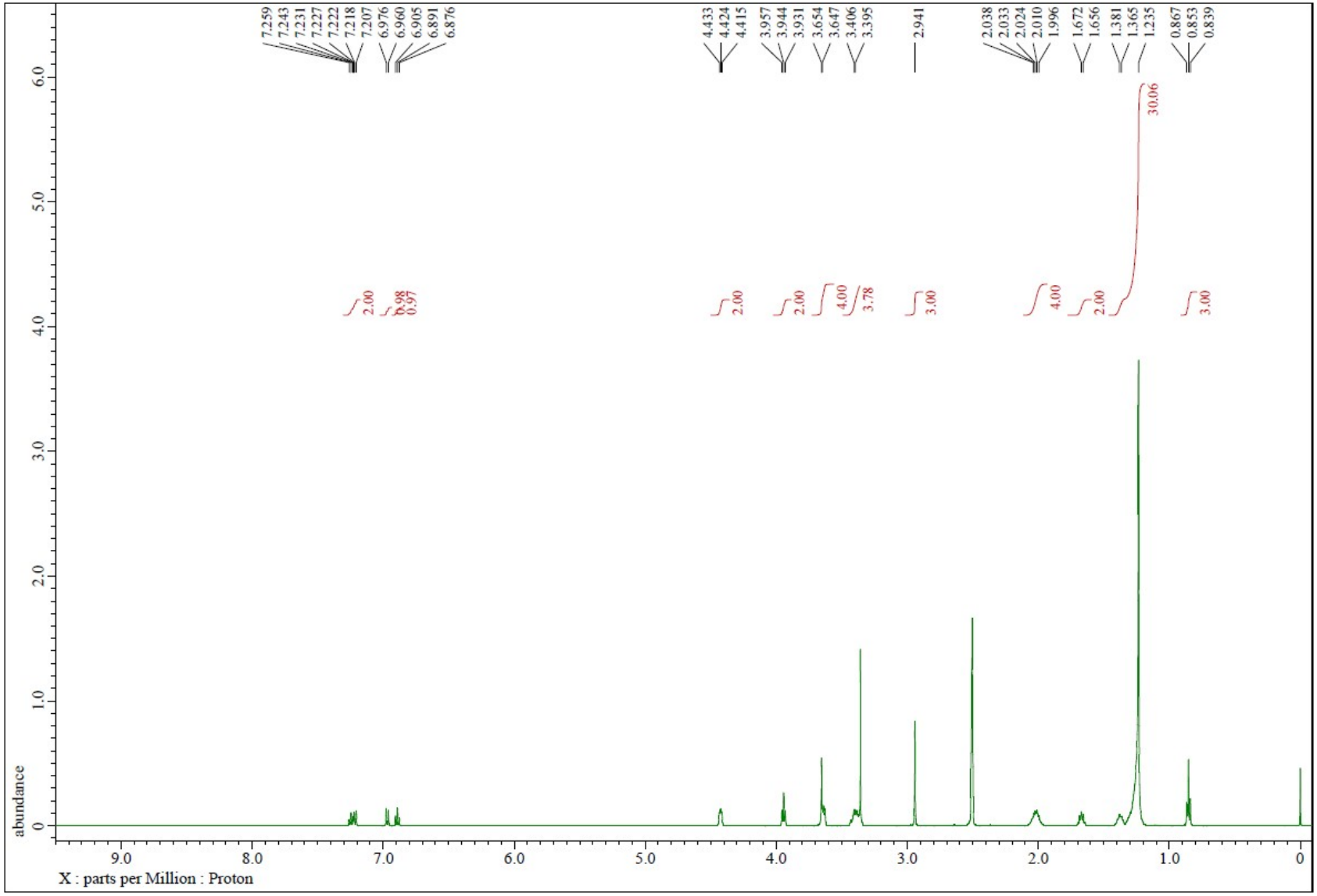
^{13}C NMR spectrum of compound **7**



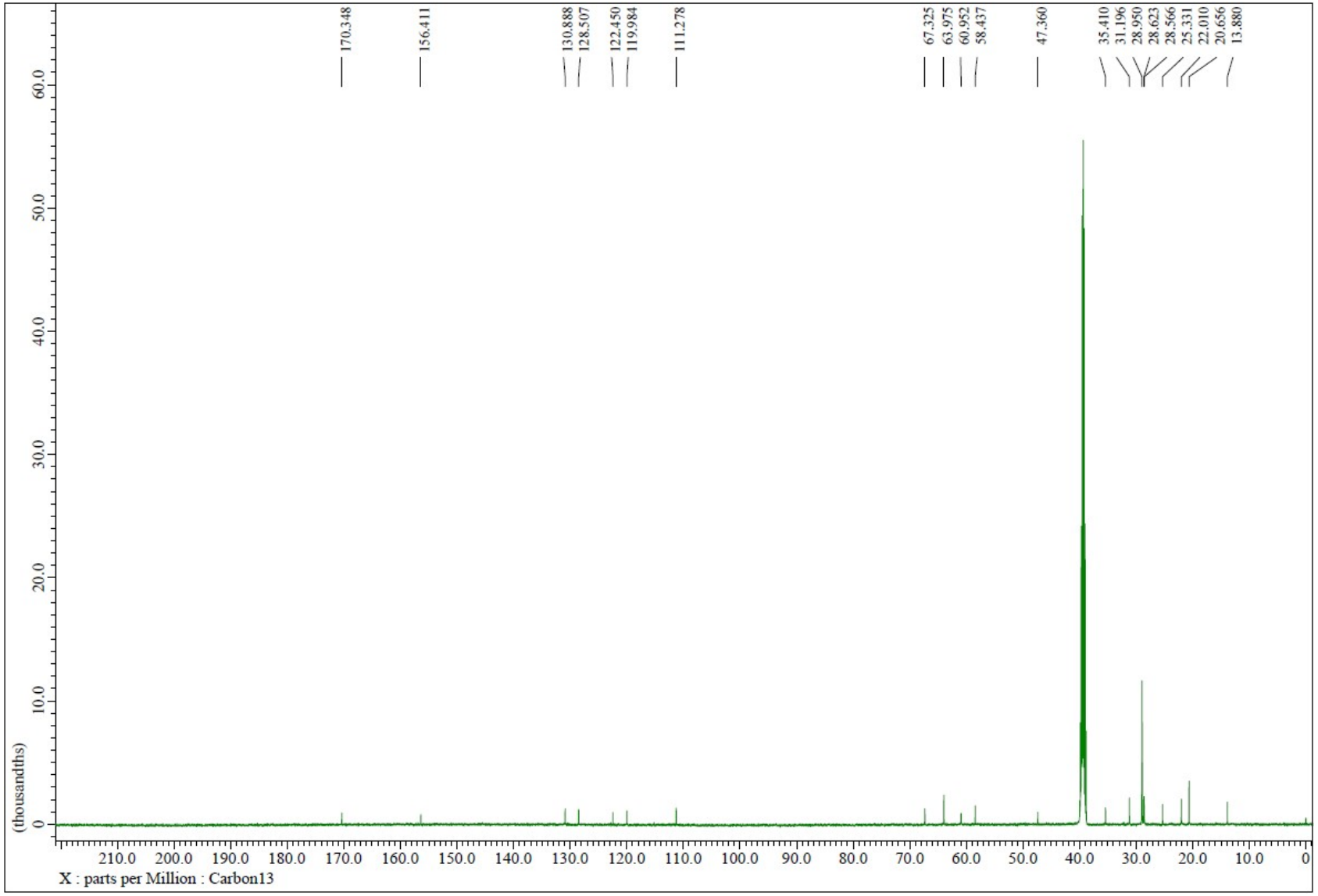
^{1}H NMR spectrum of compound **8**



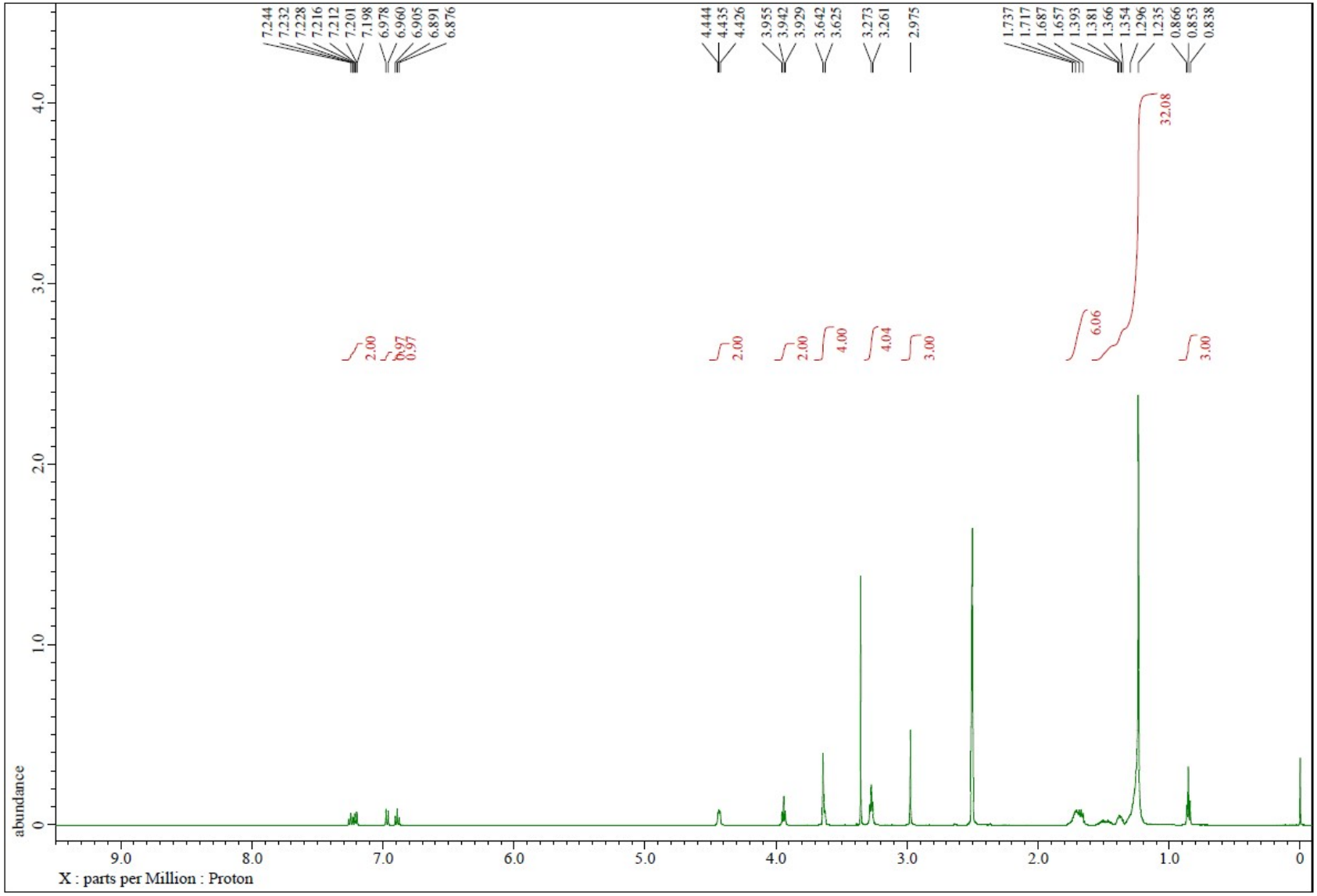
^{13}C NMR spectrum of compound **8**



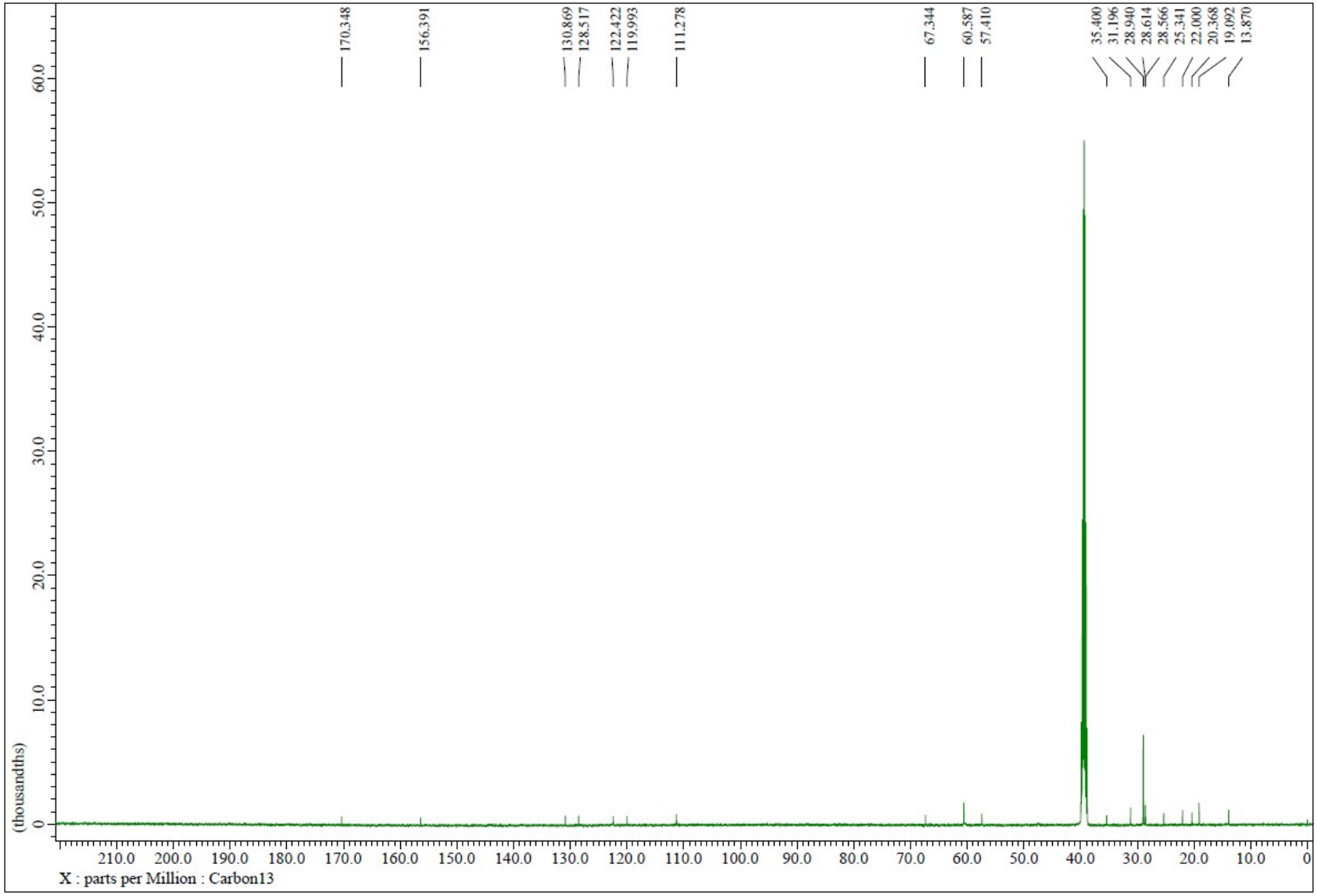
^{1}H NMR spectrum of compound **9**



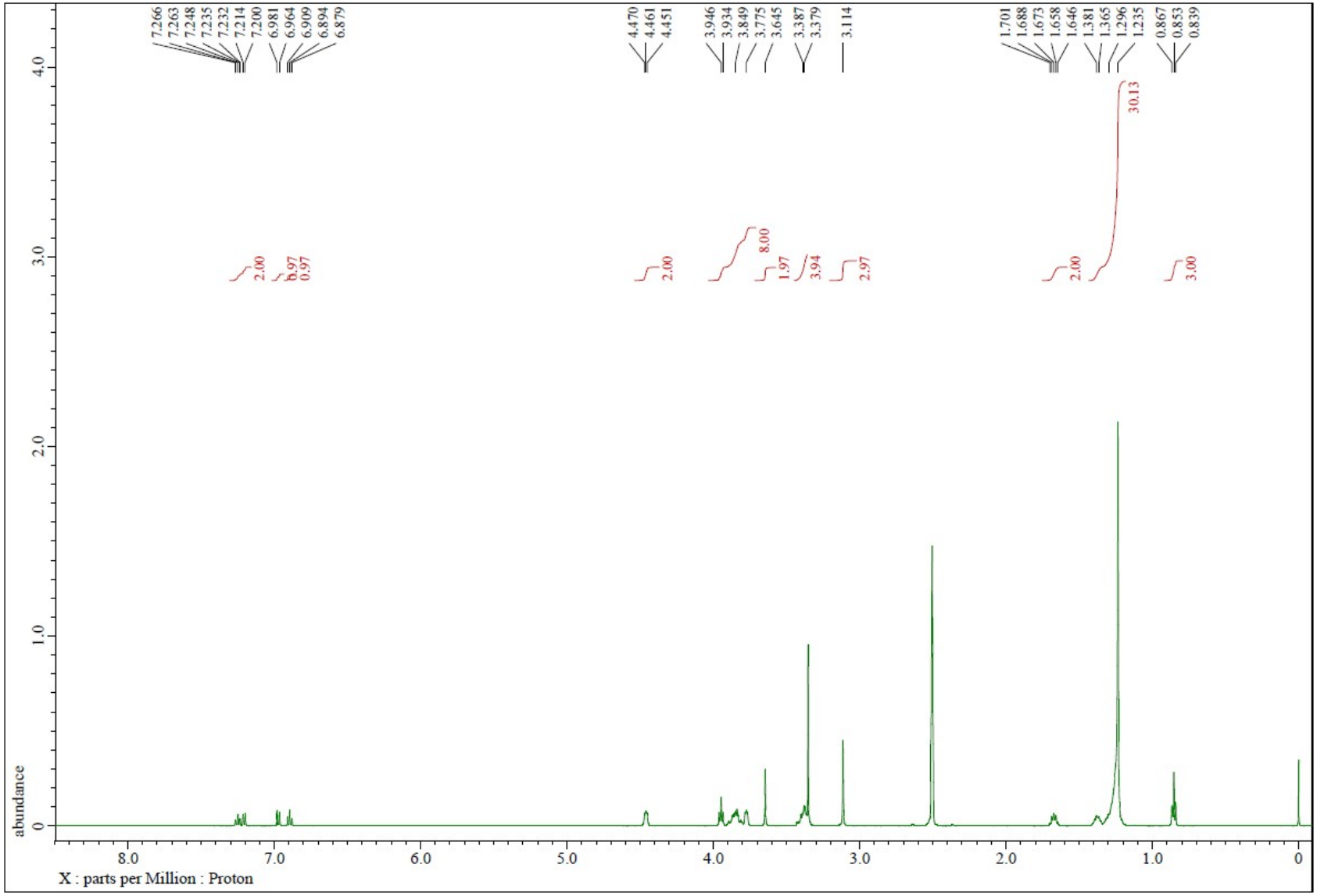
^{13}C NMR spectrum of compound **9**



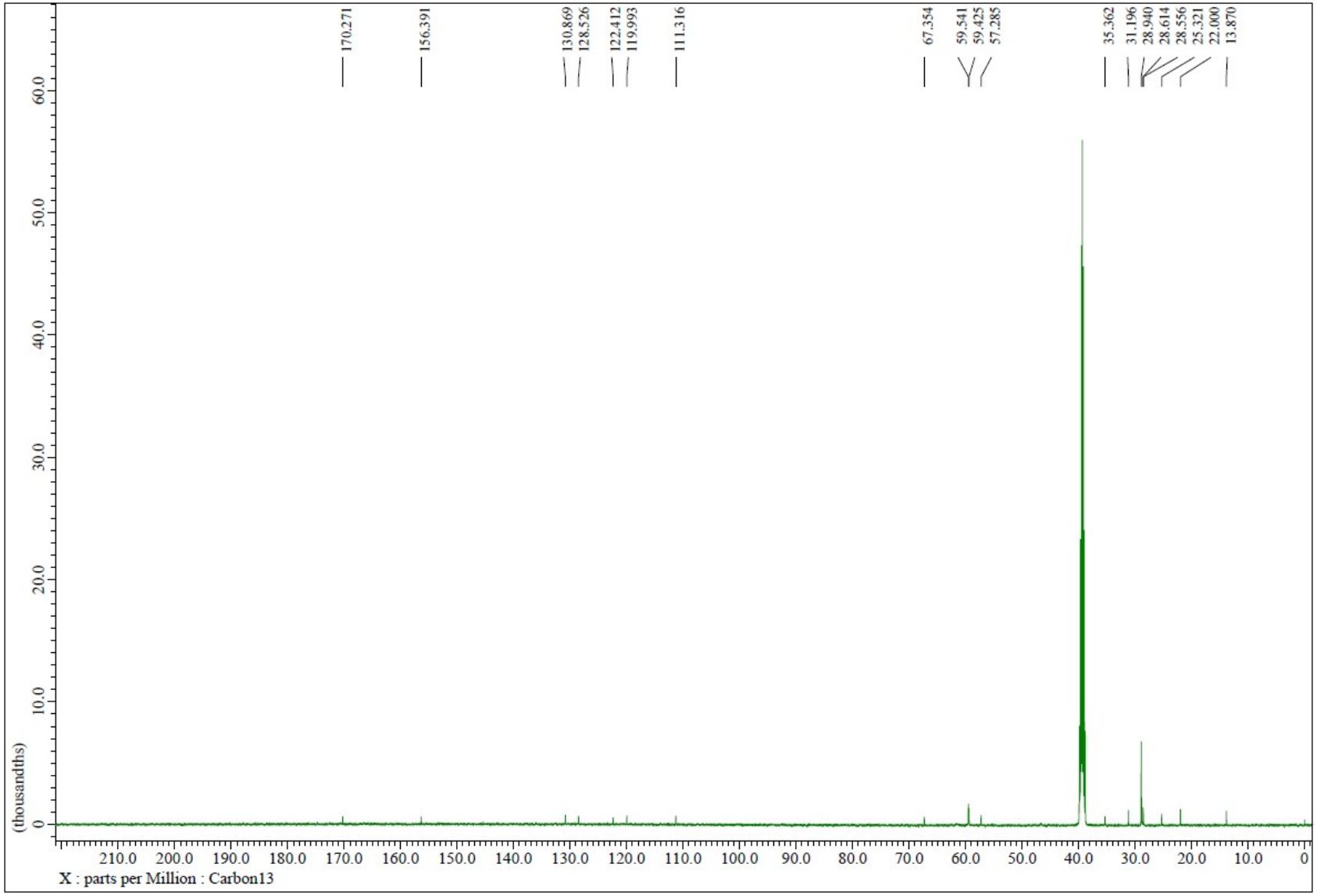
^{1}H NMR spectrum of compound **10**



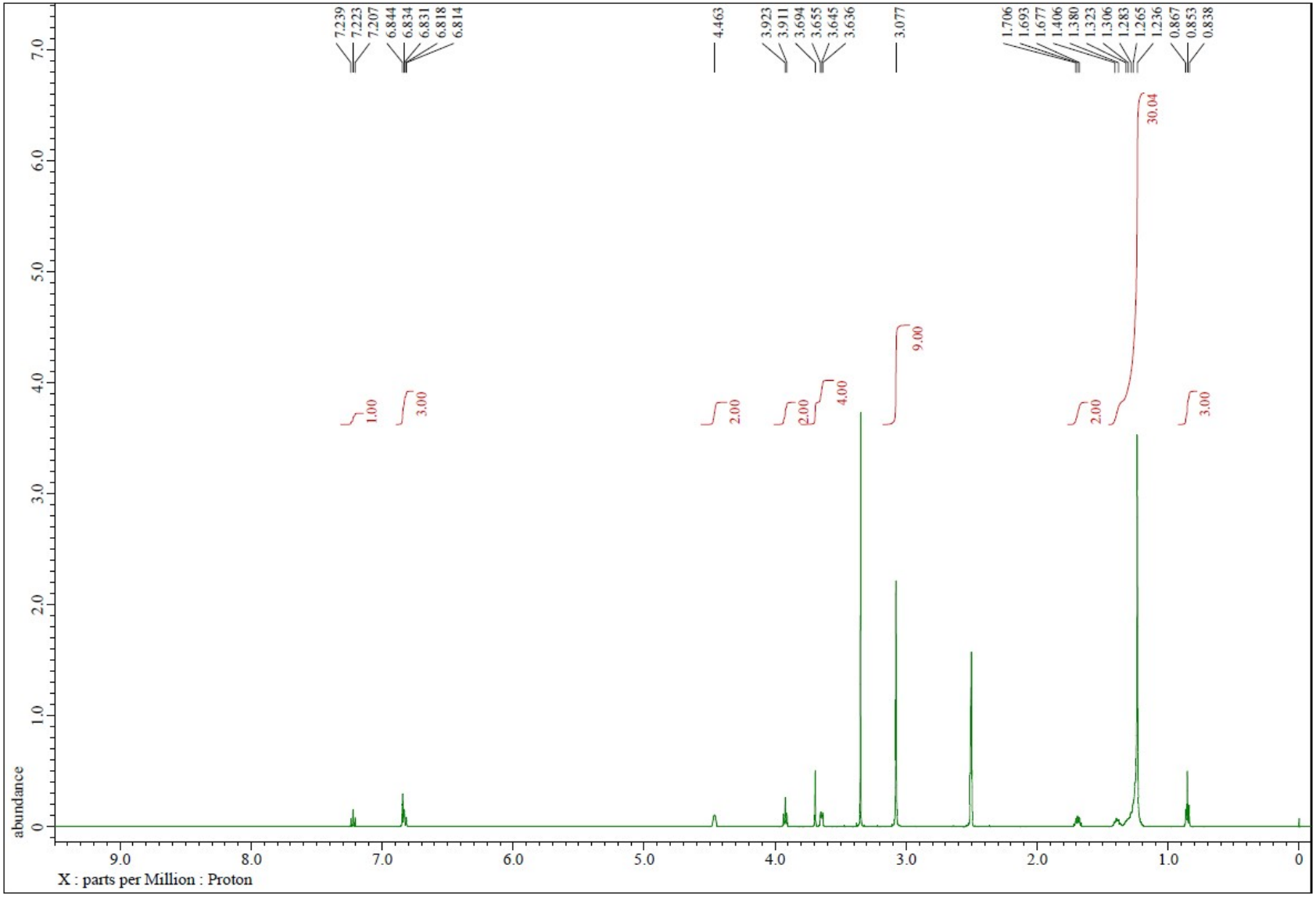
^{13}C NMR spectrum of compound **10**



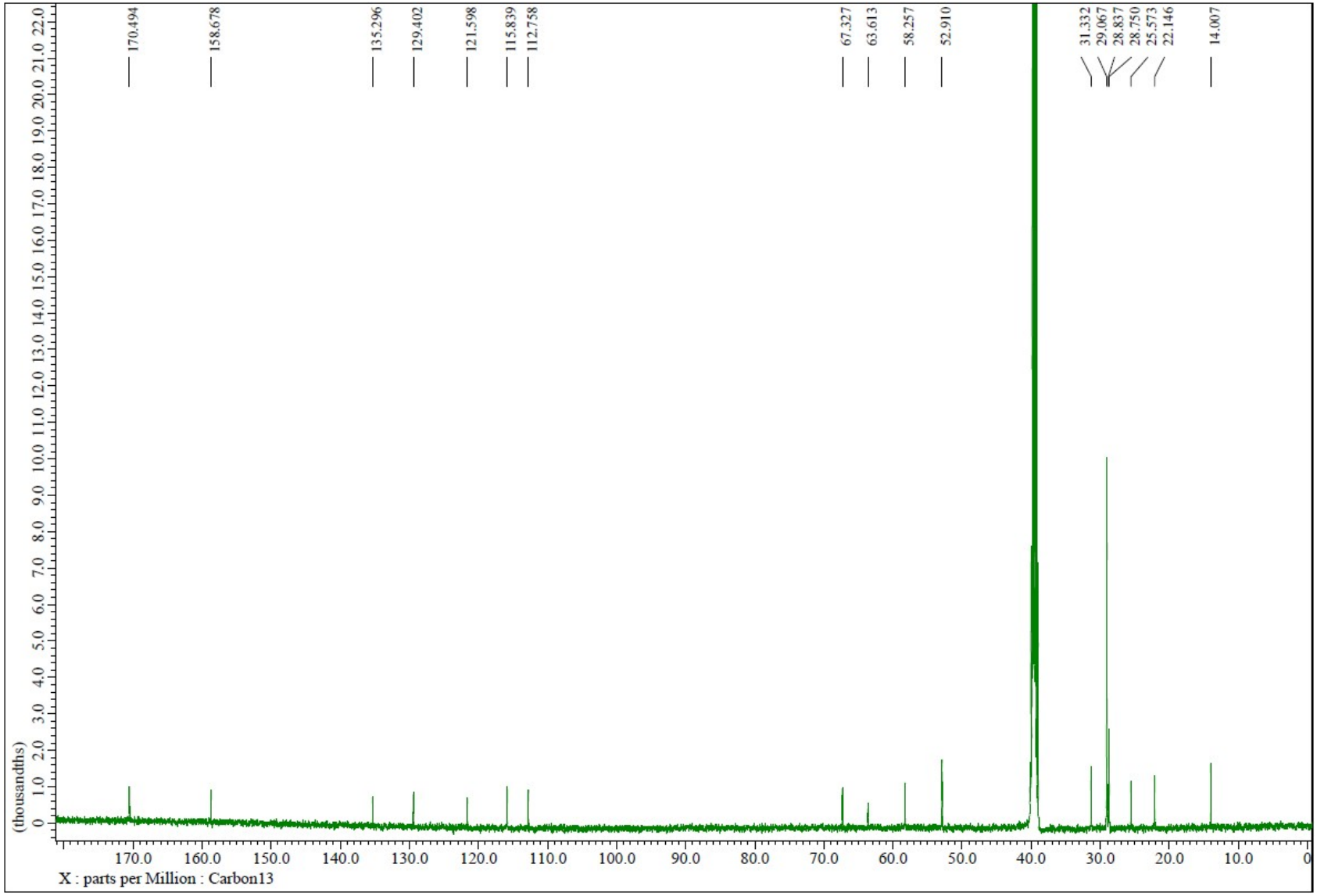
^{1}H NMR spectrum of compound **11**



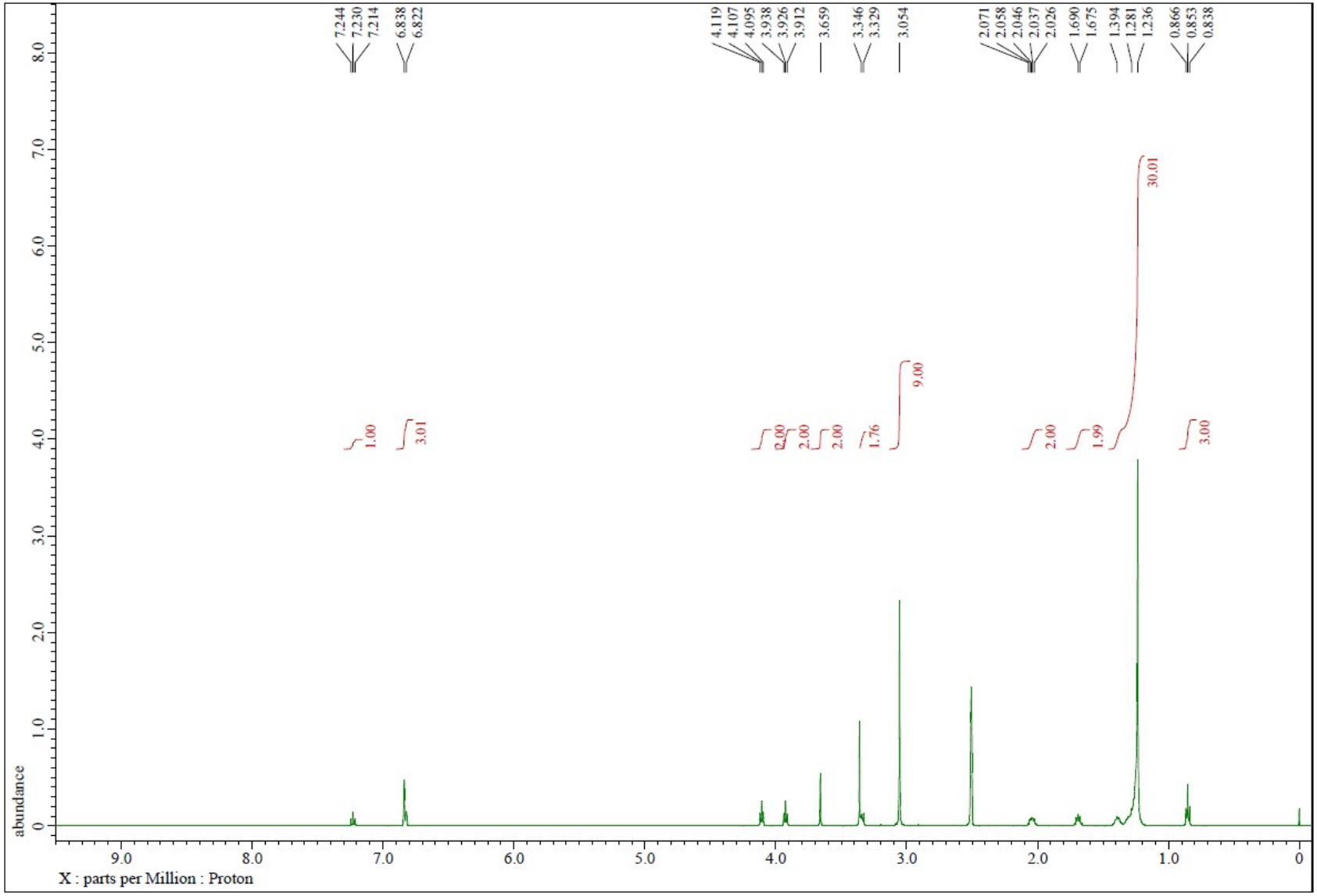
^{13}C NMR spectrum of compound **11**



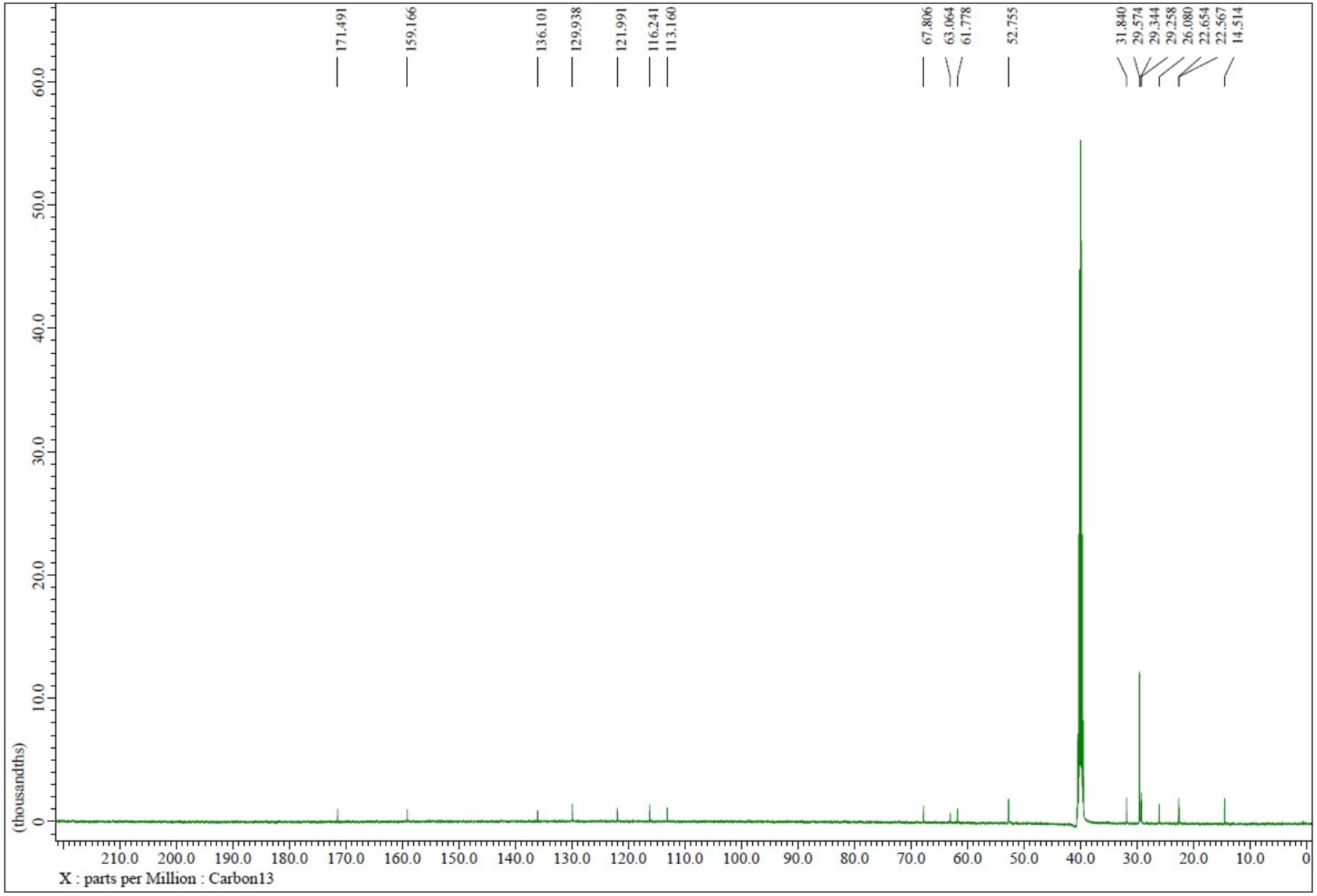
^{1}H NMR spectrum of compound **12**



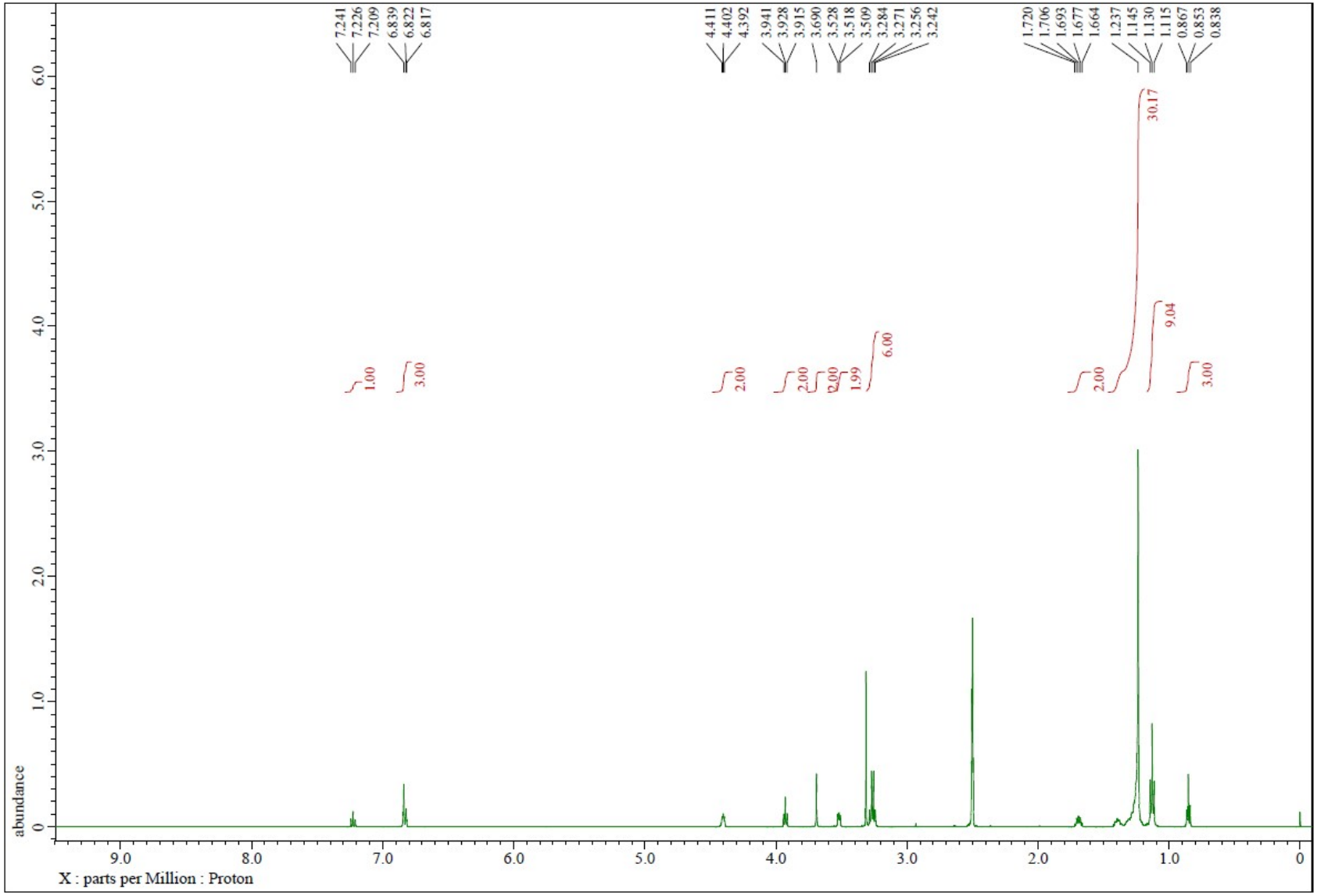
^{13}C NMR spectrum of compound **12**



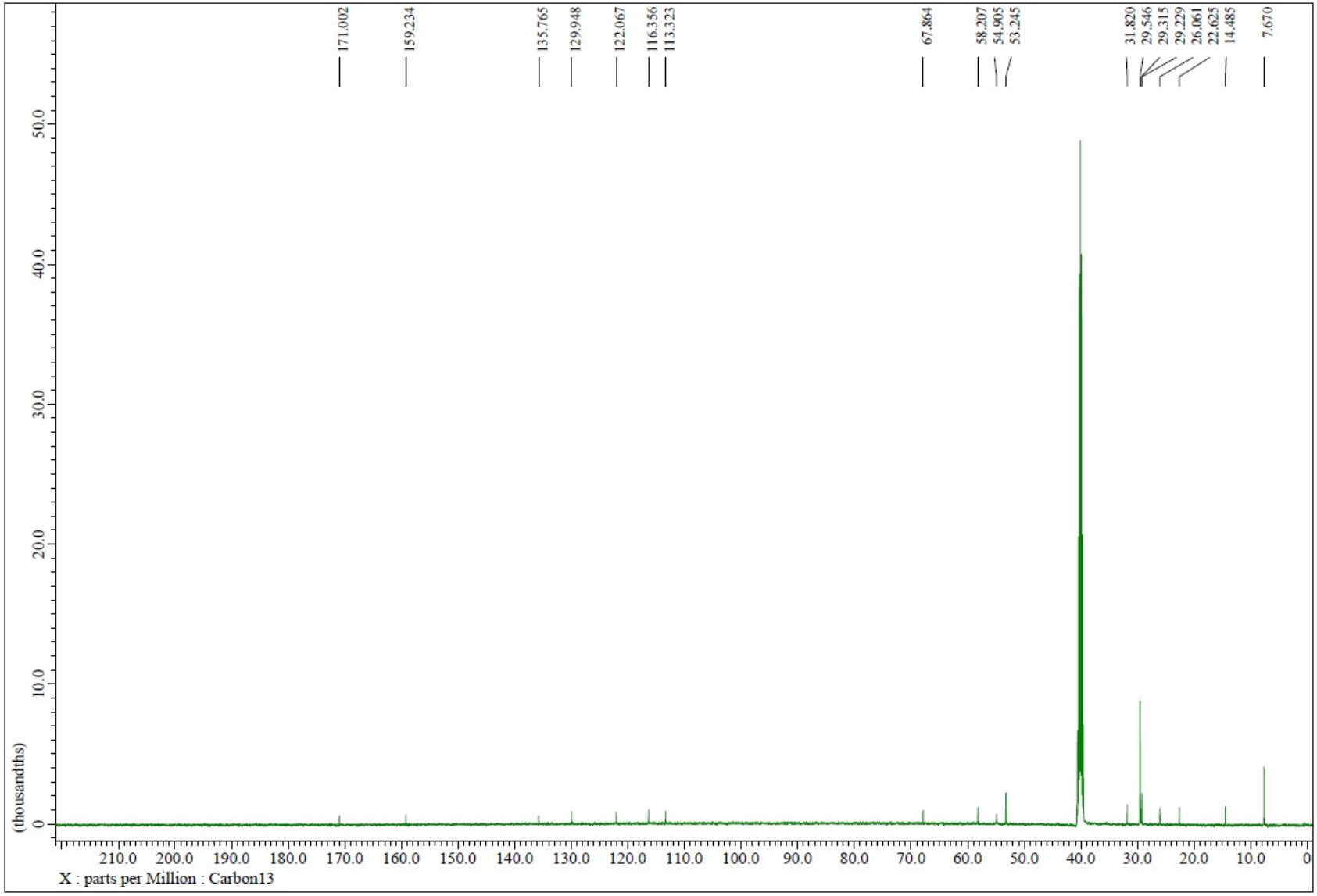
^{1}H NMR spectrum of compound **13**



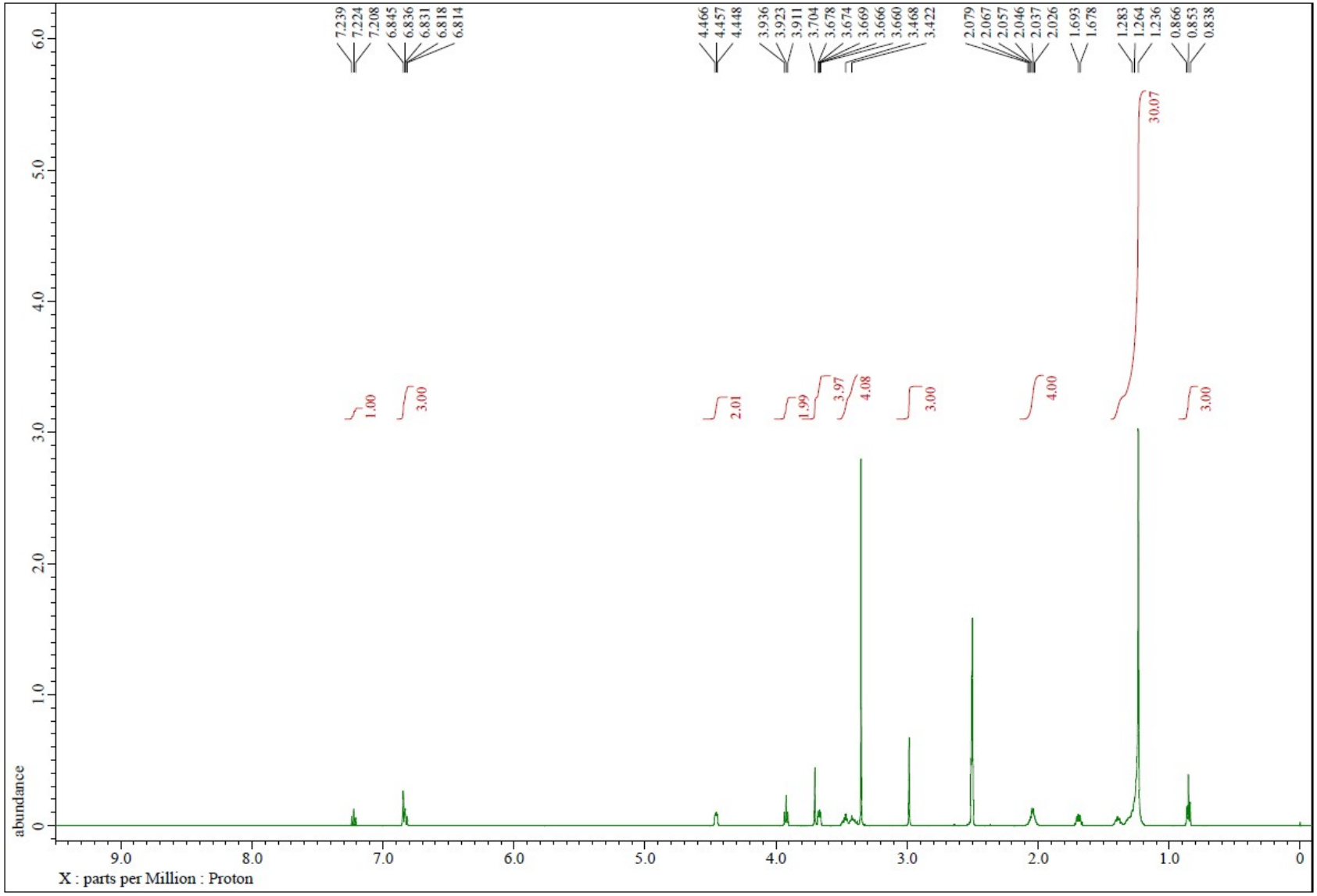
^{13}C NMR spectrum of compound **13**



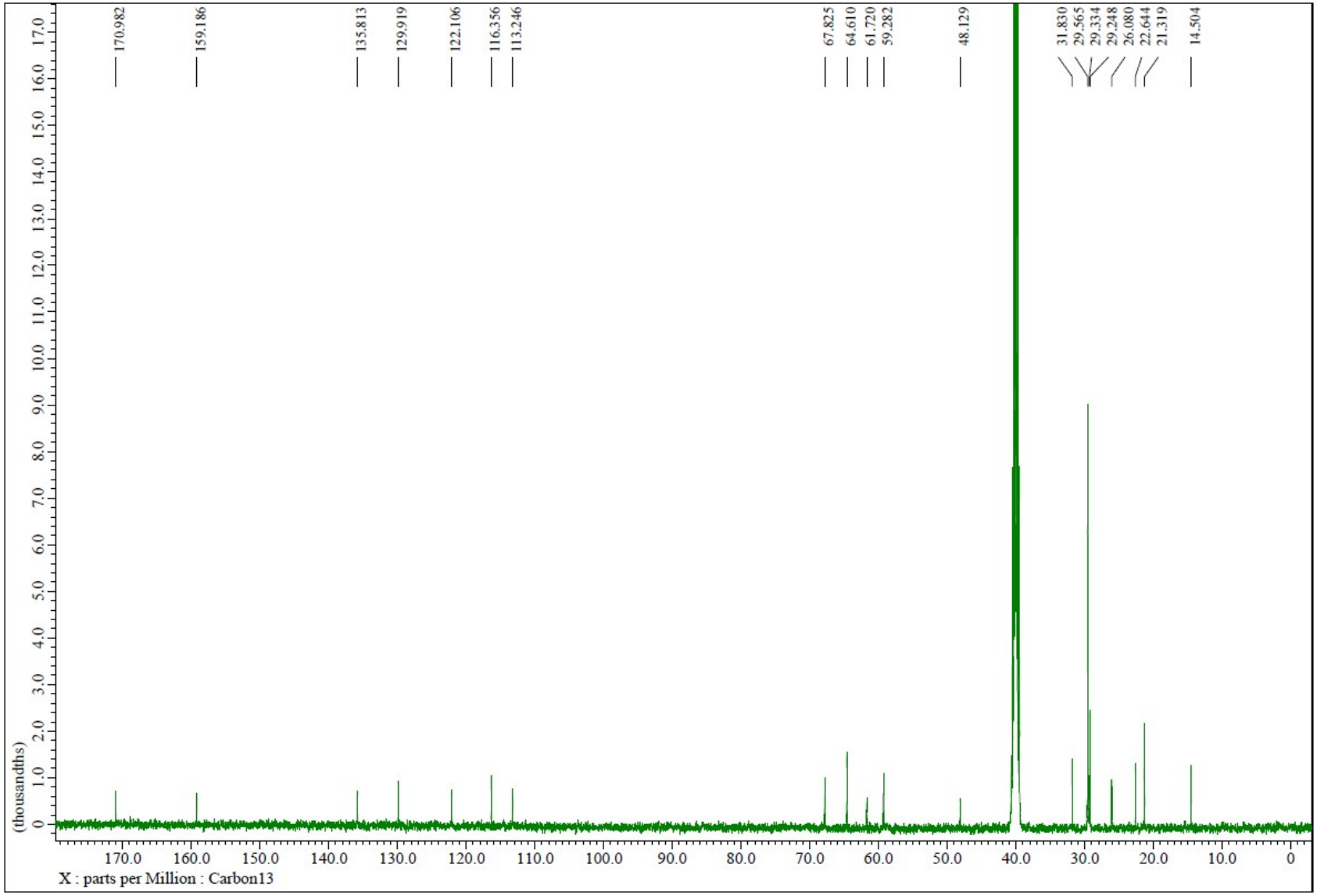
^{1}H NMR spectrum of compound **14**



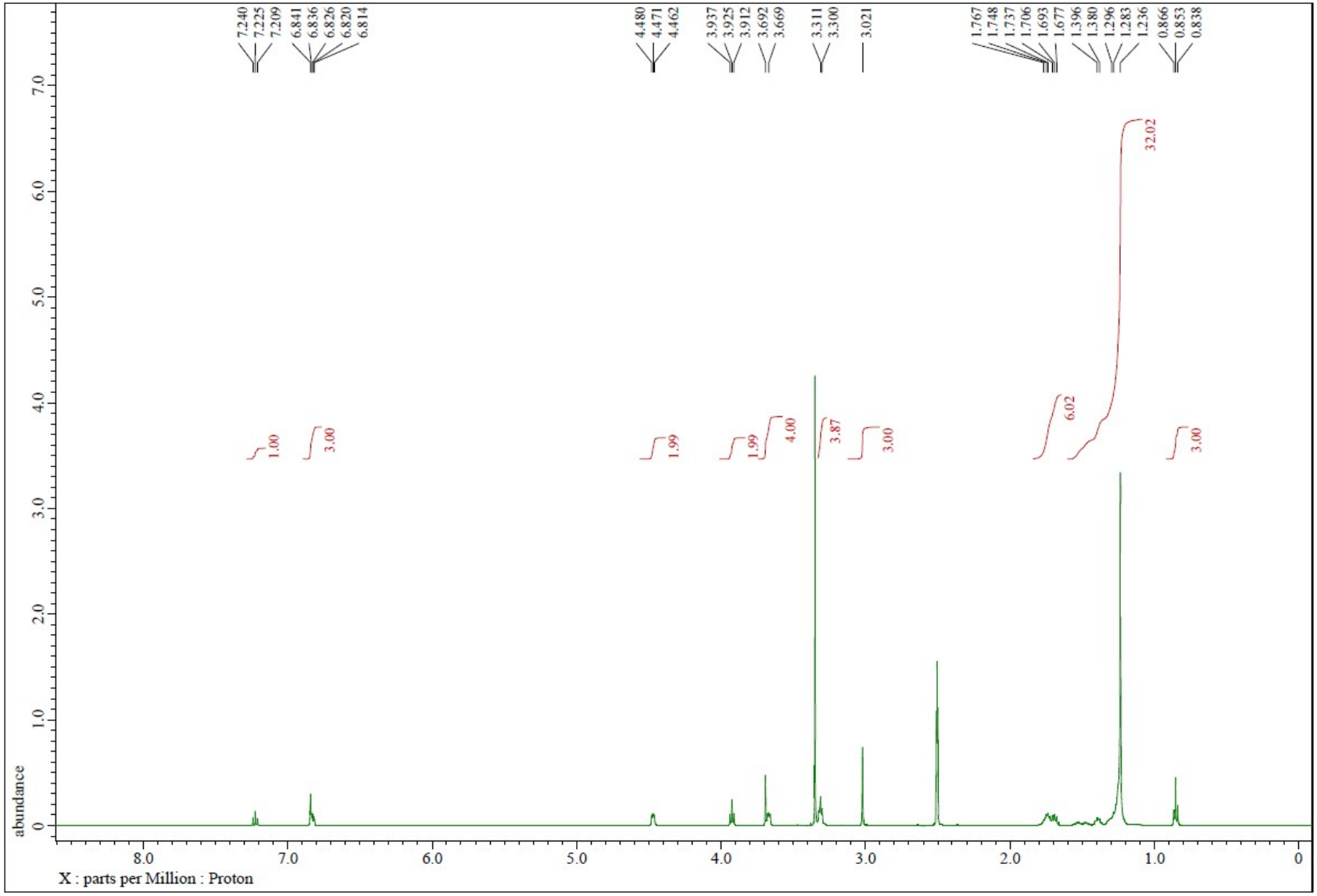
^{13}C NMR spectrum of compound **14**



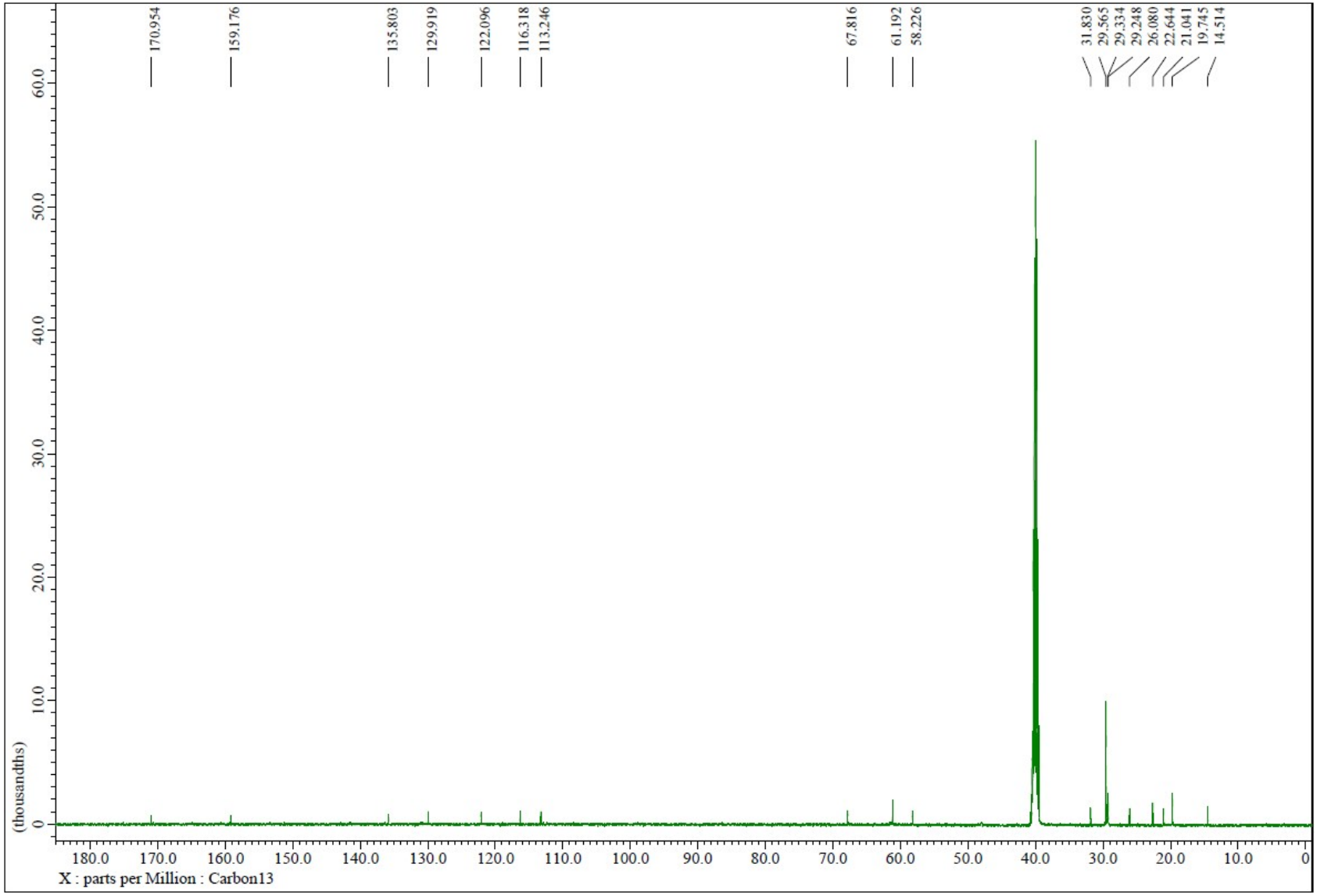
^{1}H NMR spectrum of compound **15**



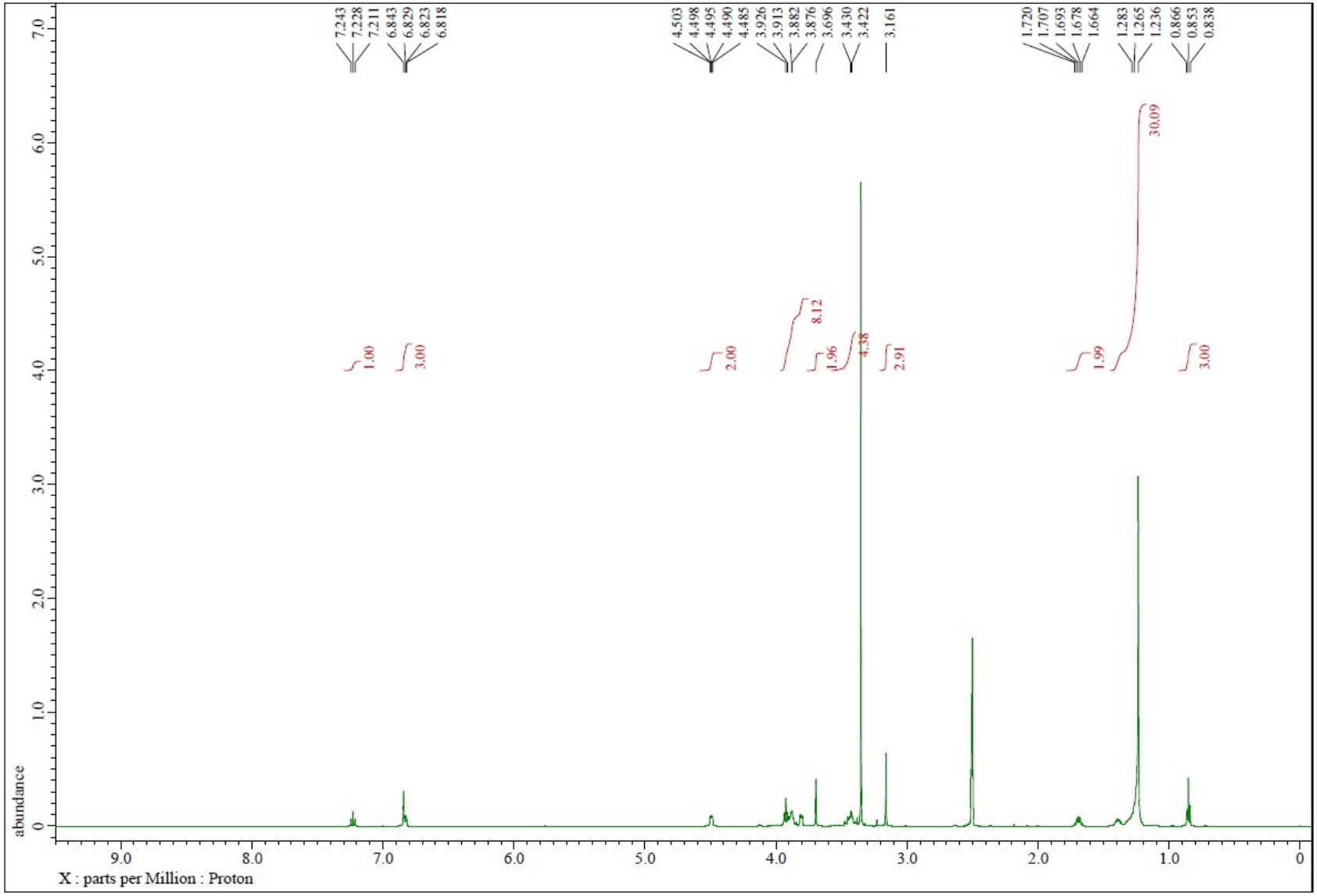
^{13}C NMR spectrum of compound **15**



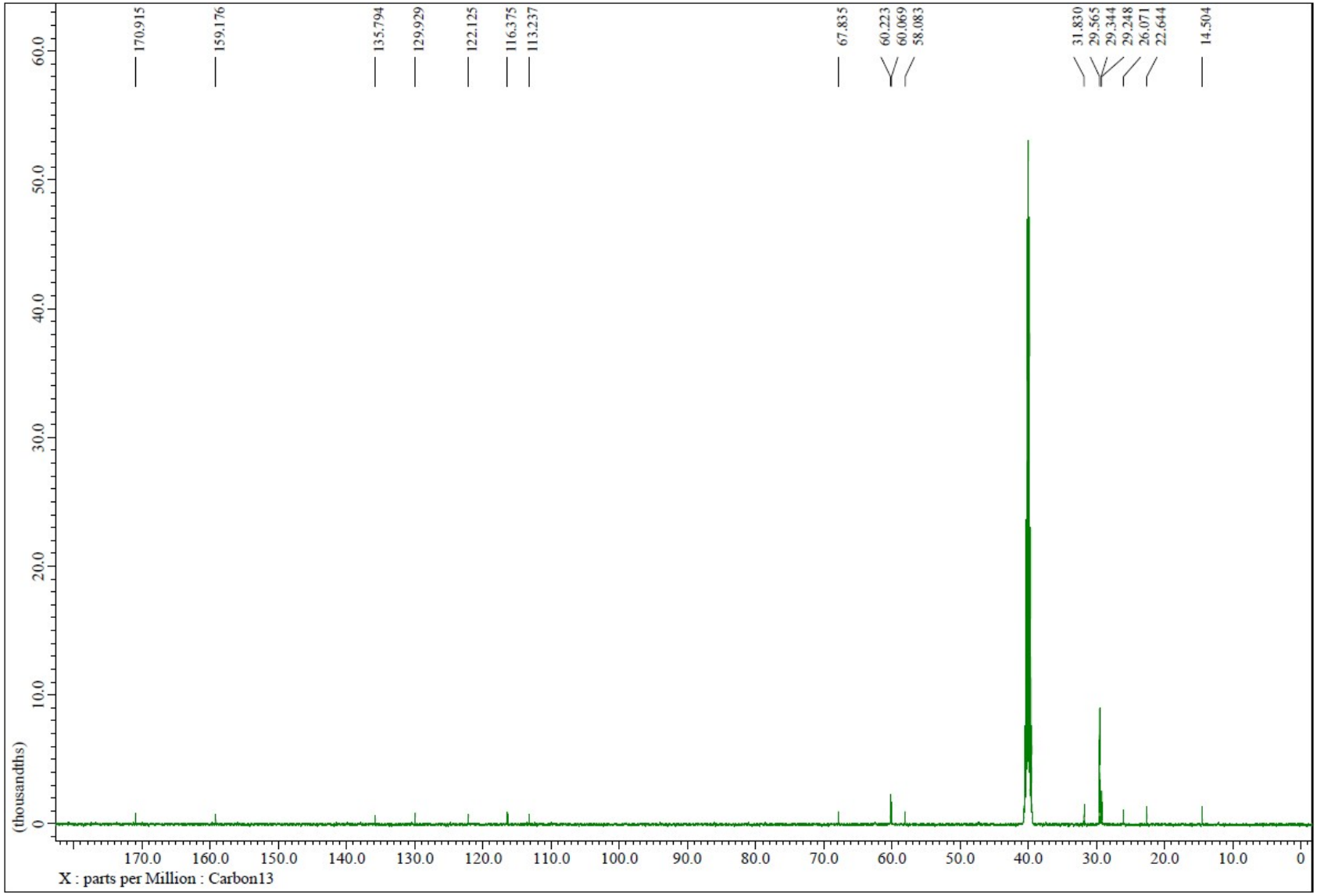
^{1}H NMR spectrum of compound **16**



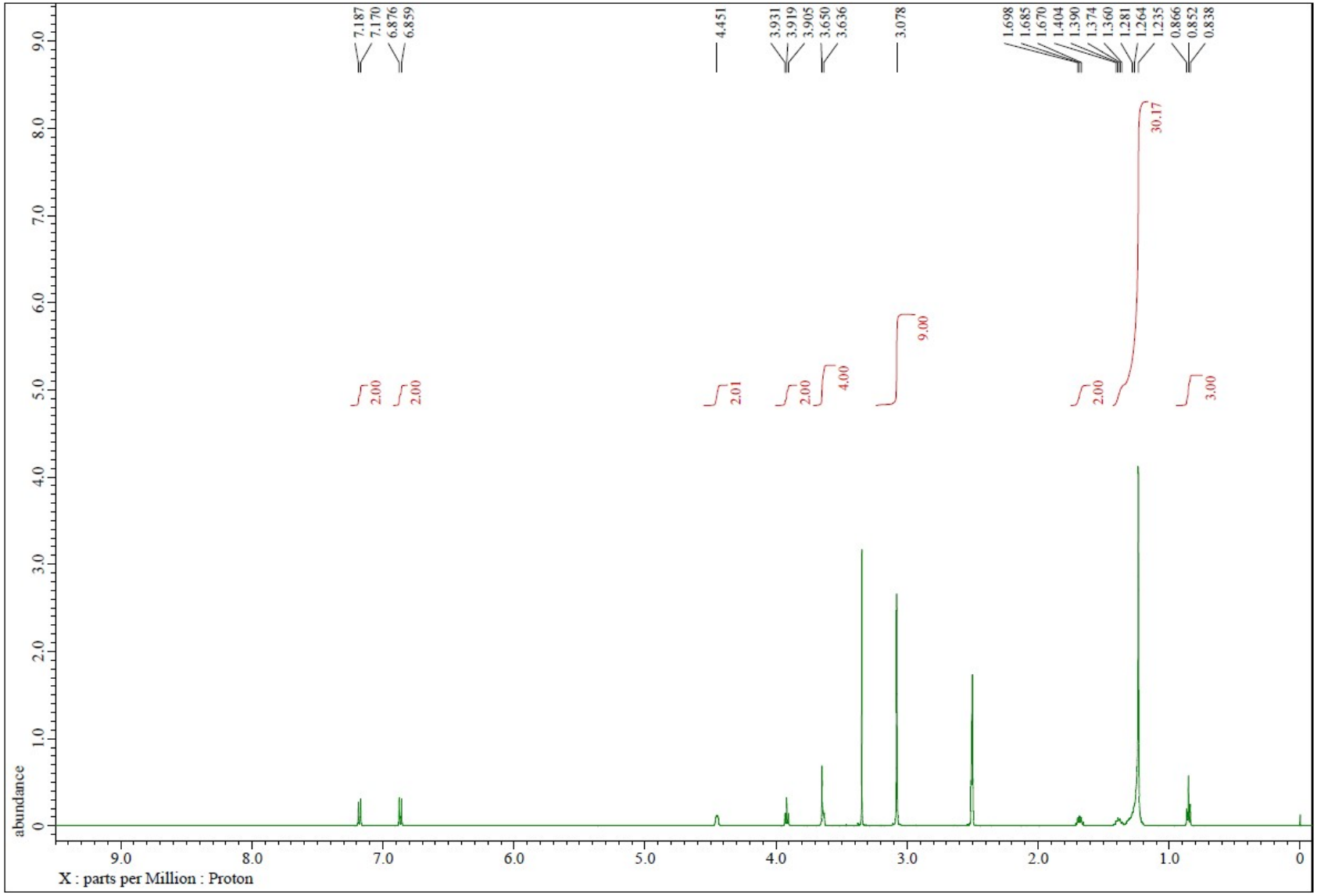
^{13}C NMR spectrum of compound **16**



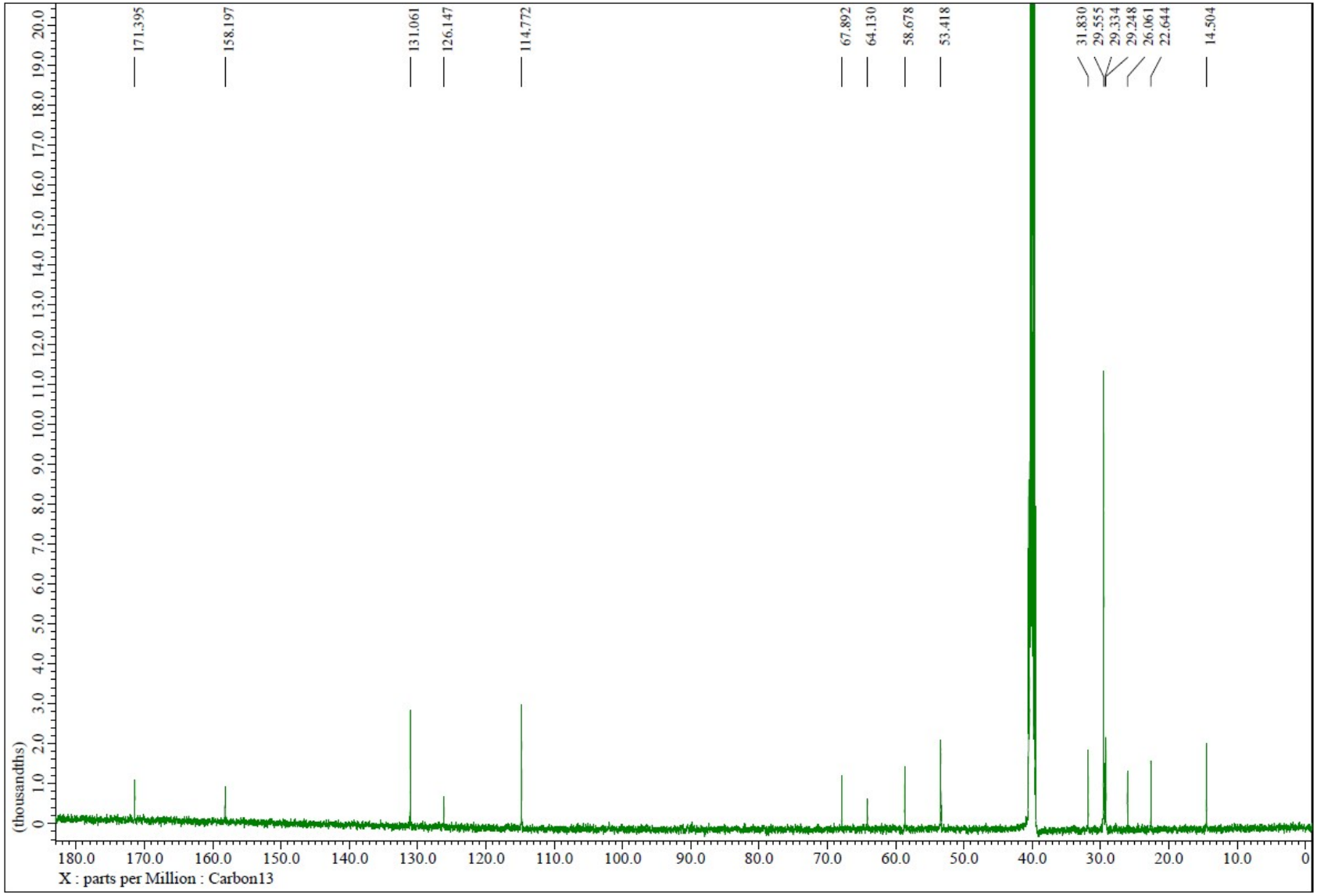
^{1}H NMR spectrum of compound **17**



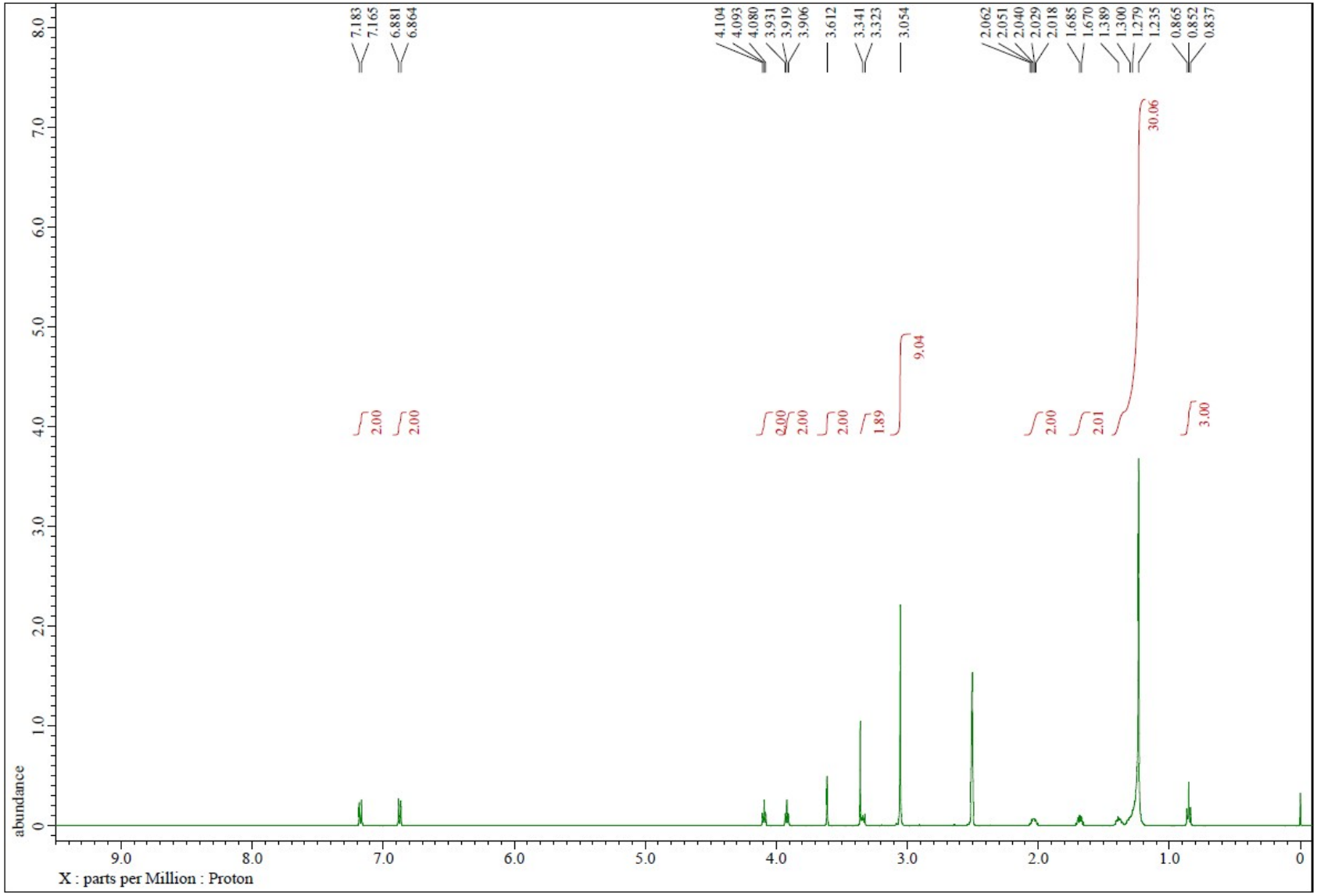
^{13}C NMR spectrum of compound **17**



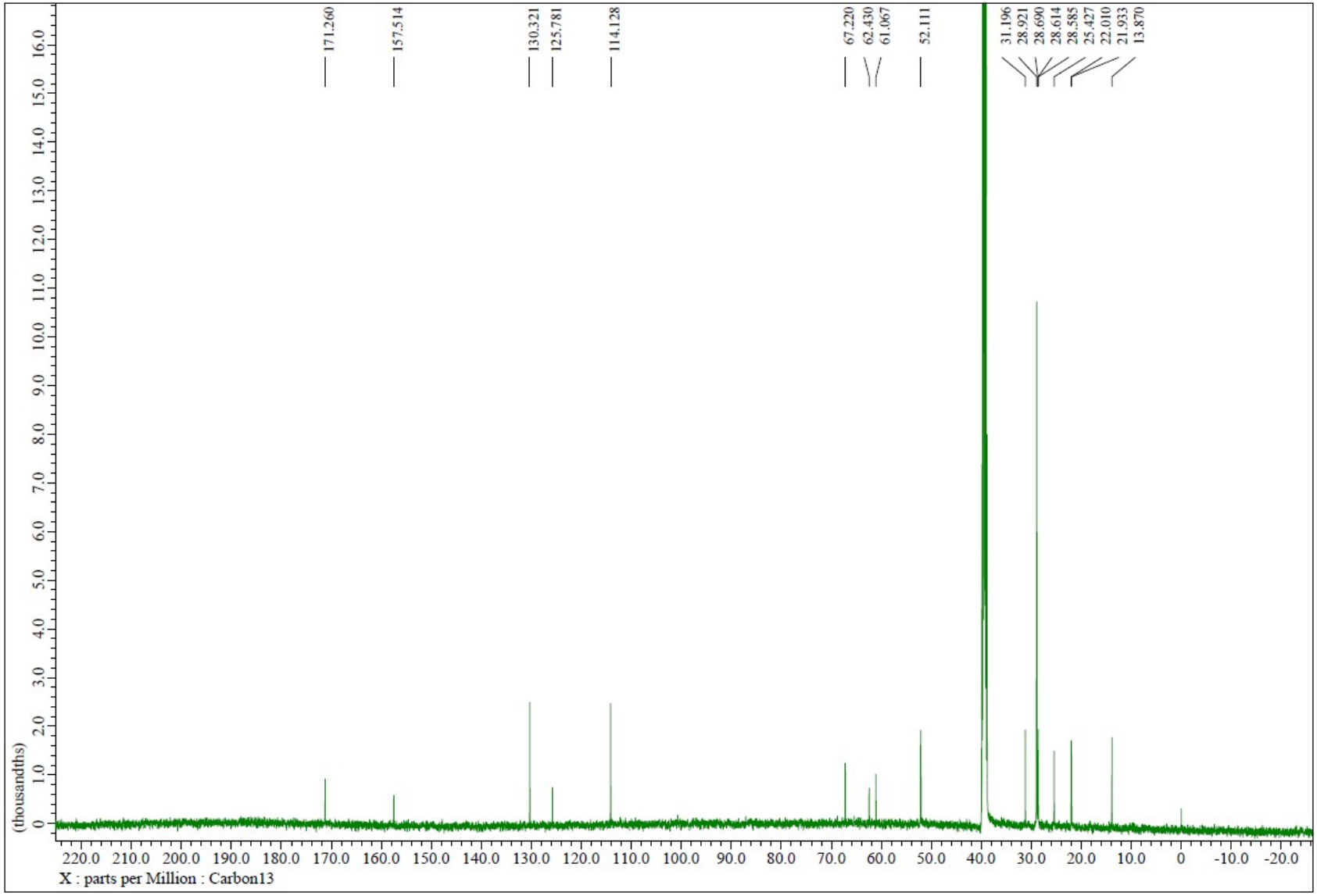
^{1}H NMR spectrum of compound **18**



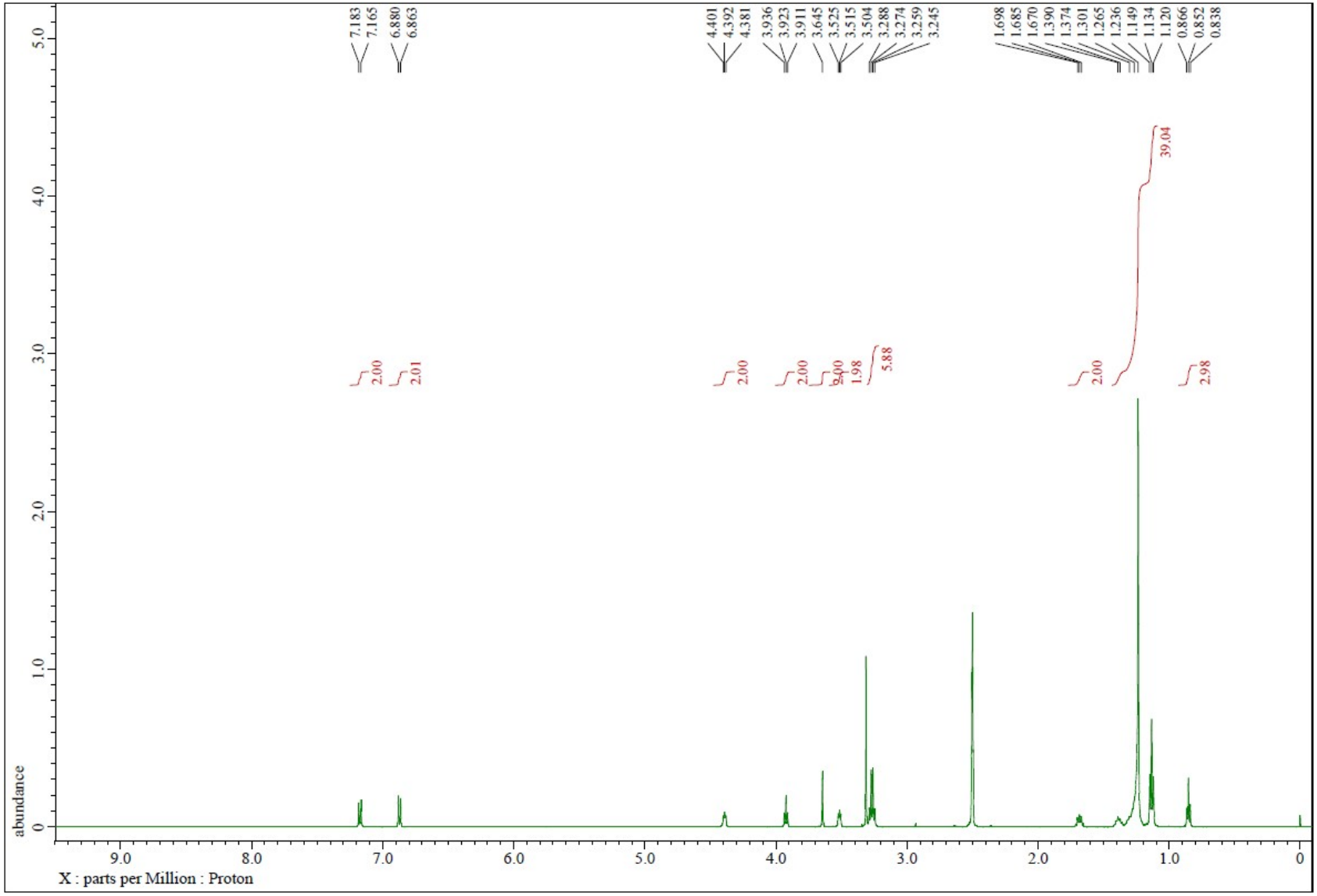
^{13}C NMR spectrum of compound **18**



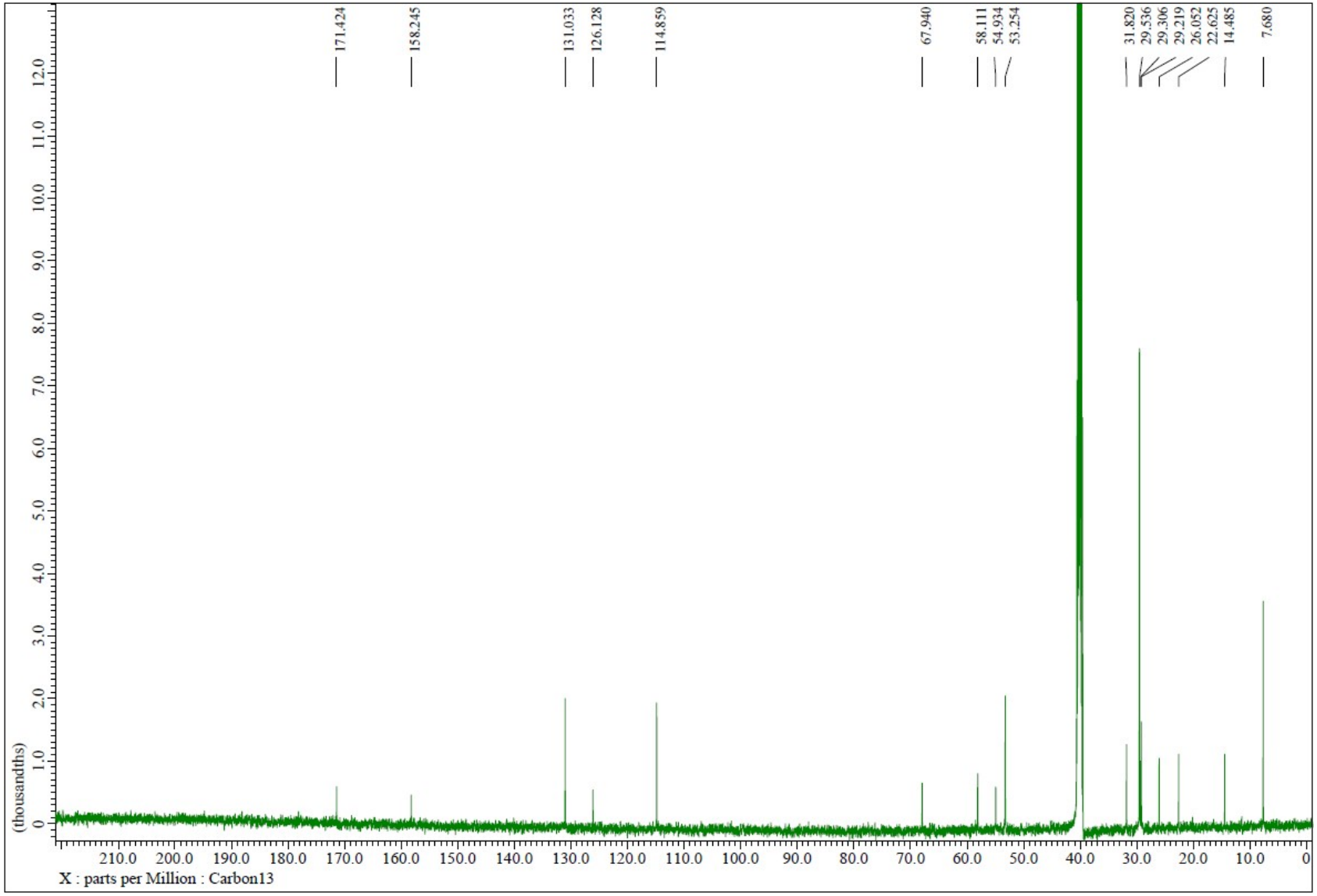
^{1}H NMR spectrum of compound **19**



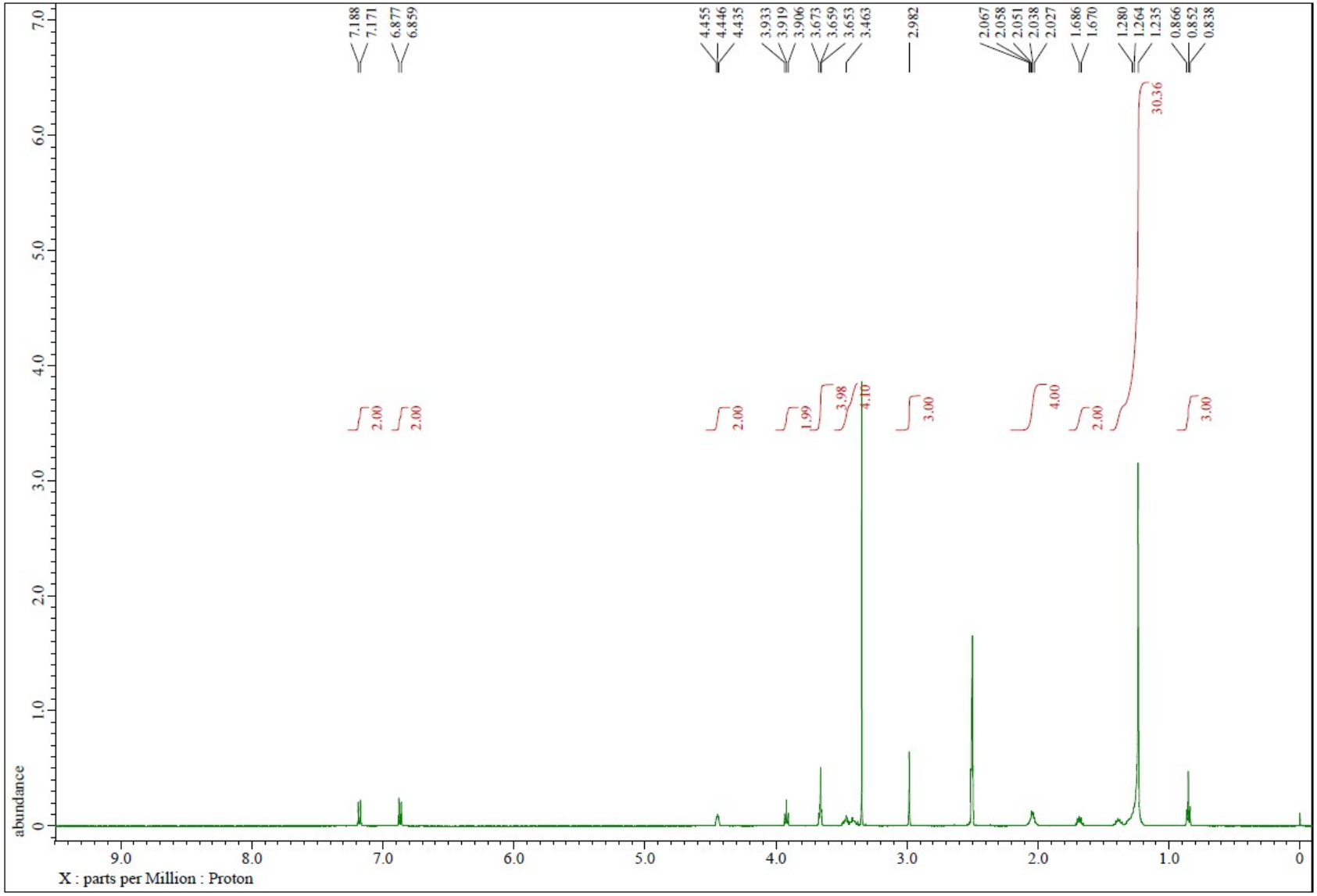
^{13}C NMR spectrum of compound **19**



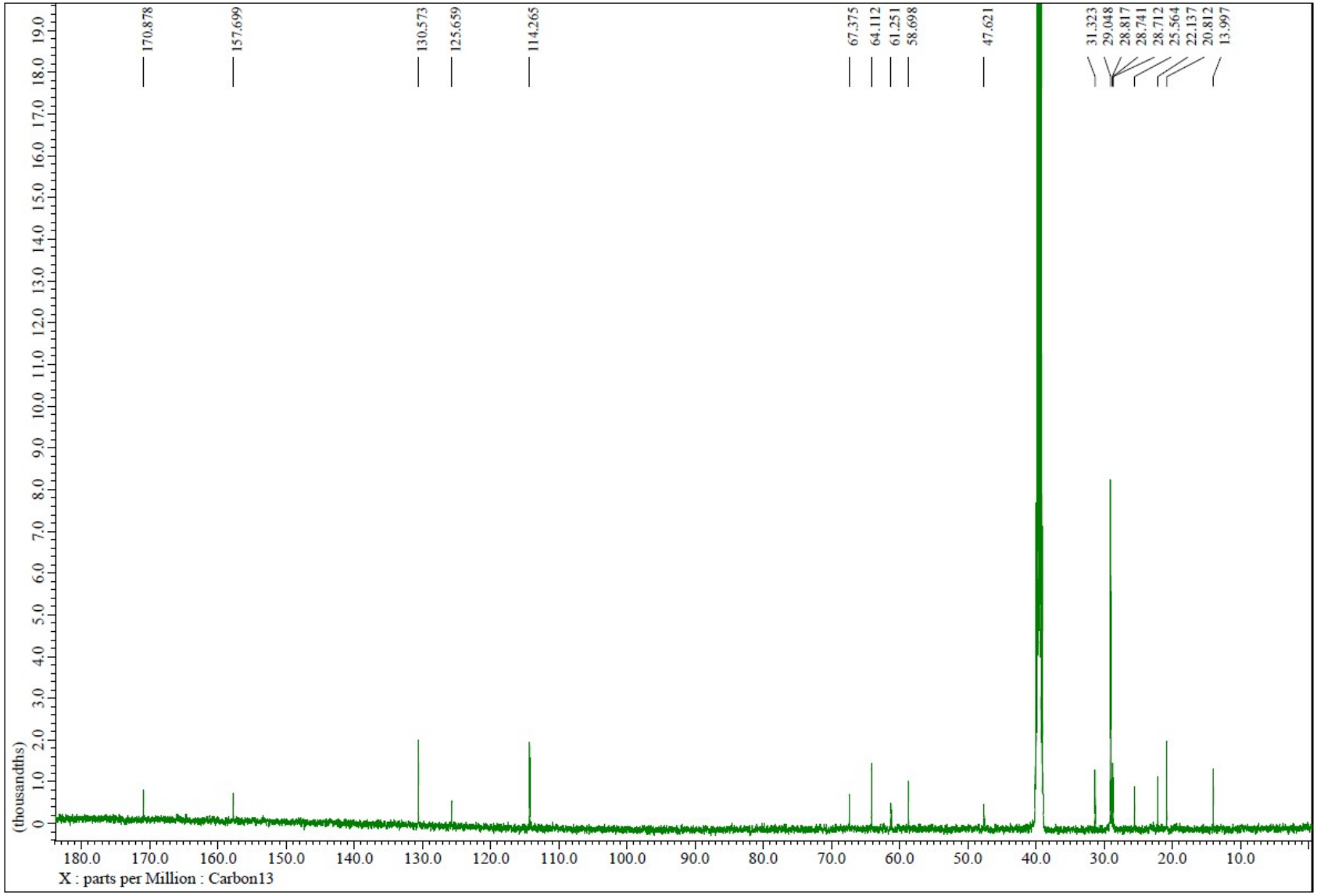
^{1}H NMR spectrum of compound **20**



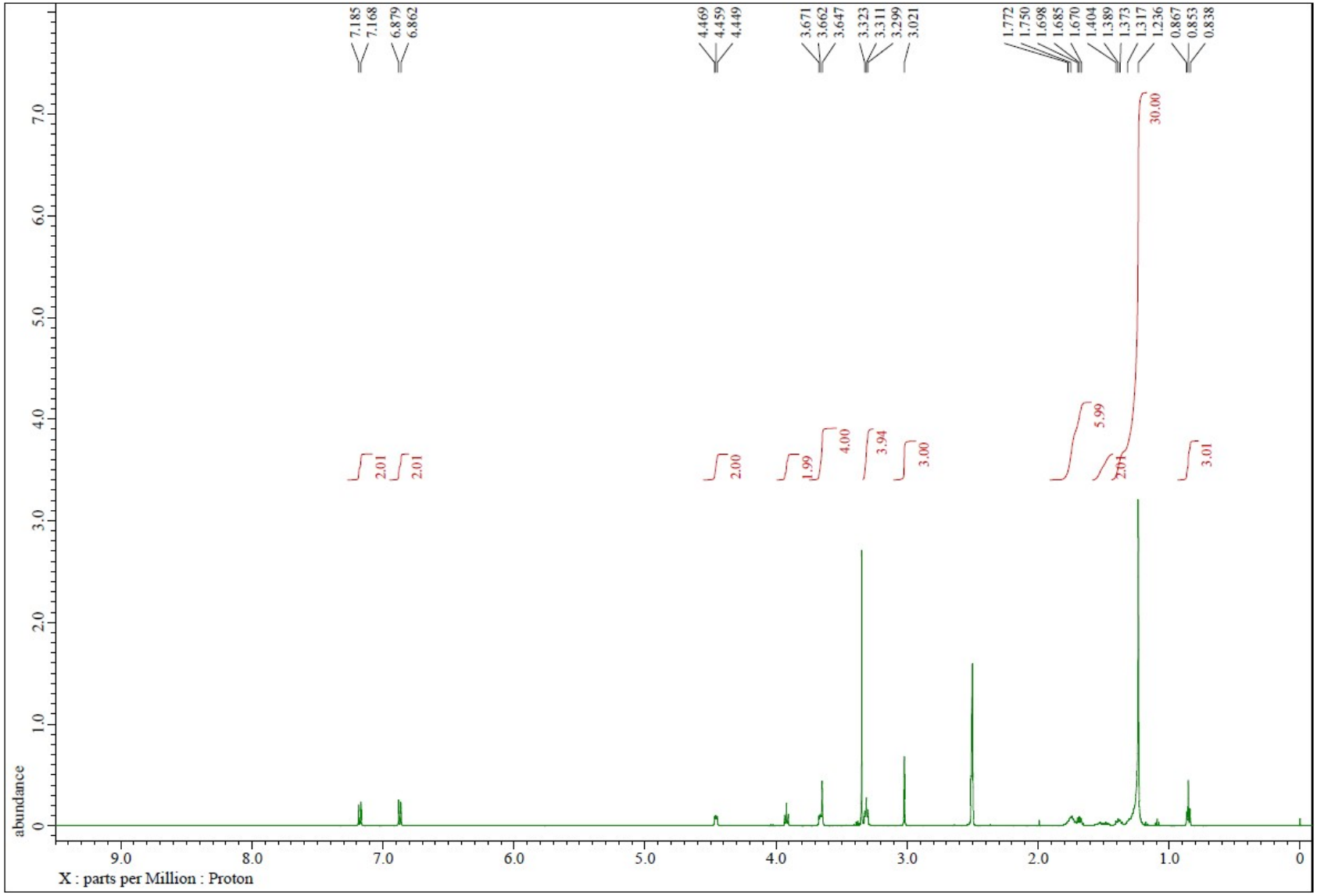
^{13}C NMR spectrum of compound **20**



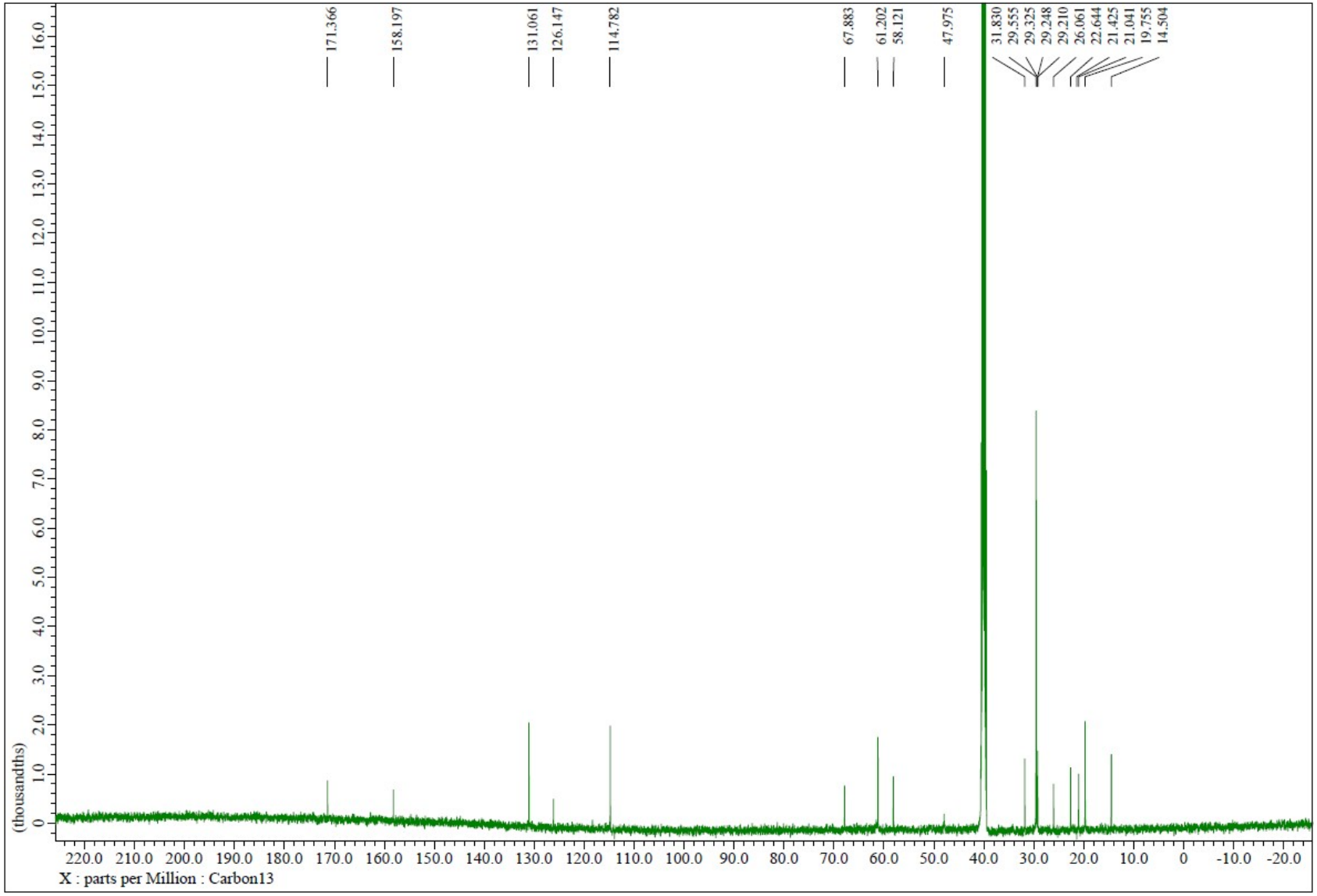
^{1}H NMR spectrum of compound **21**



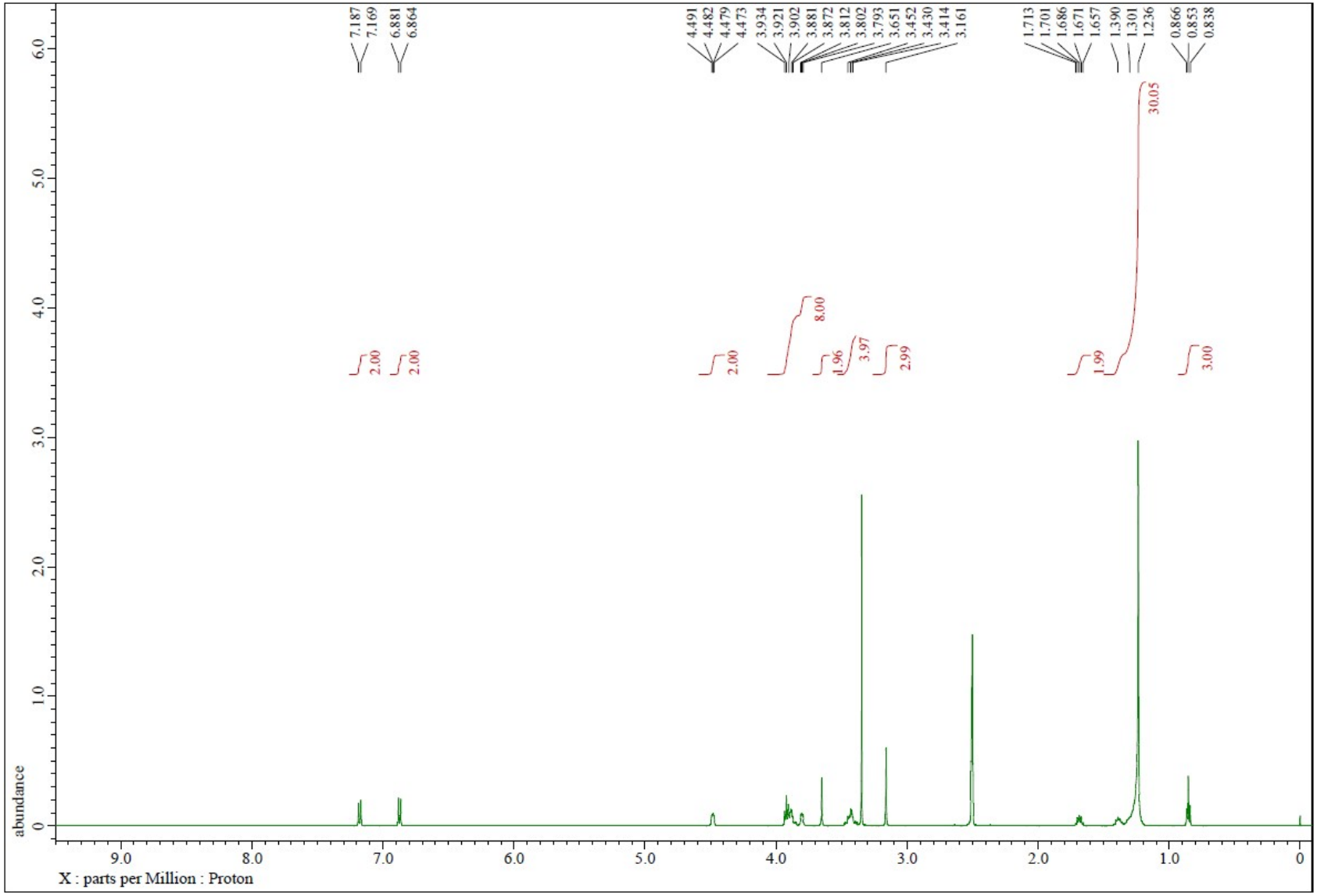
^{13}C NMR spectrum of compound **21**



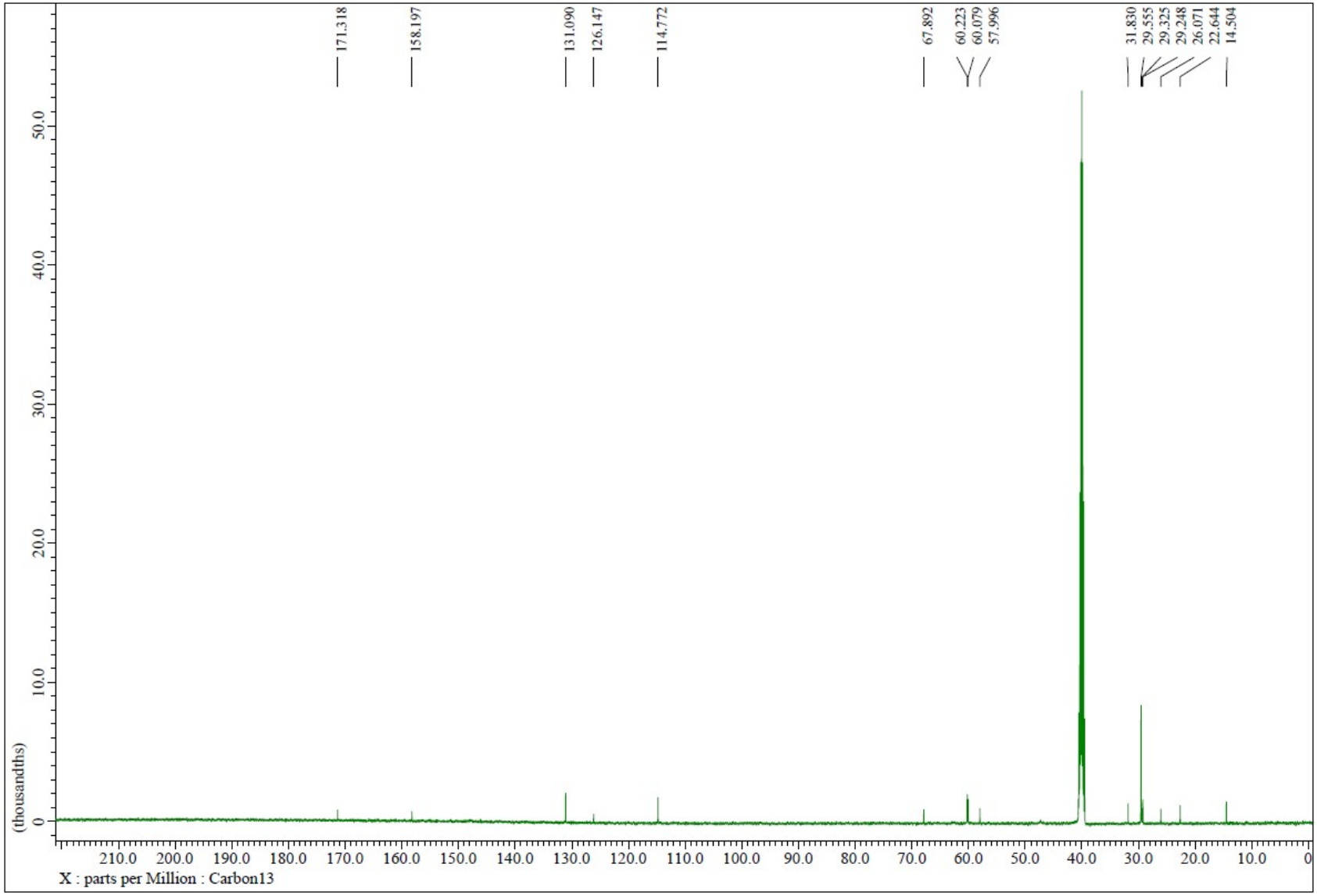
^{1}H NMR spectrum of compound **22**



^{13}C NMR spectrum of compound **22**

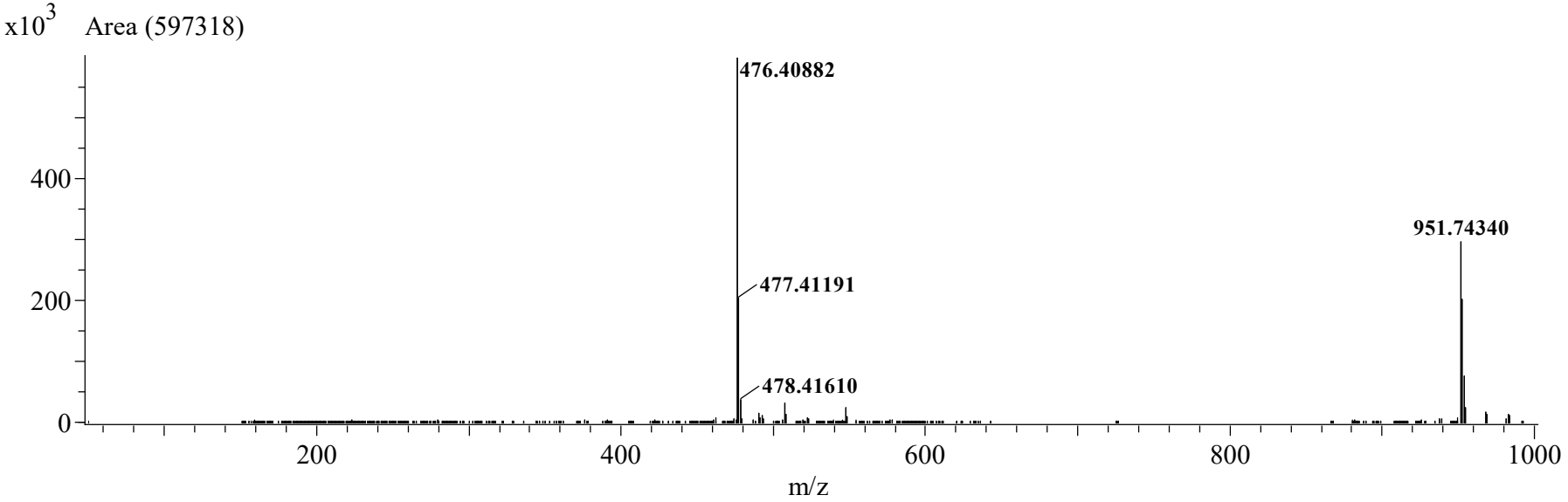


^{1}H NMR spectrum of compound **23**

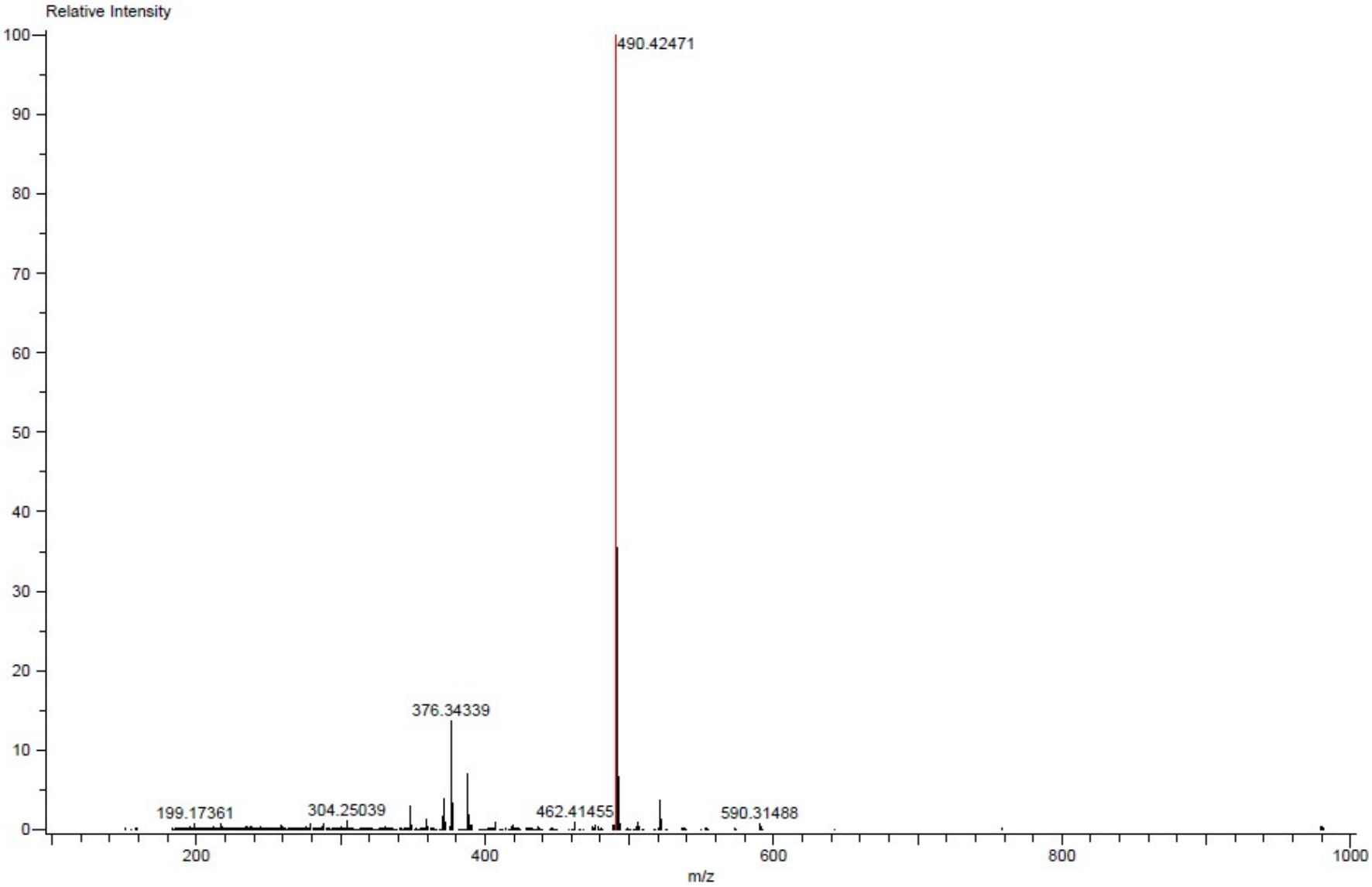


^{13}C NMR spectrum of compound **23**

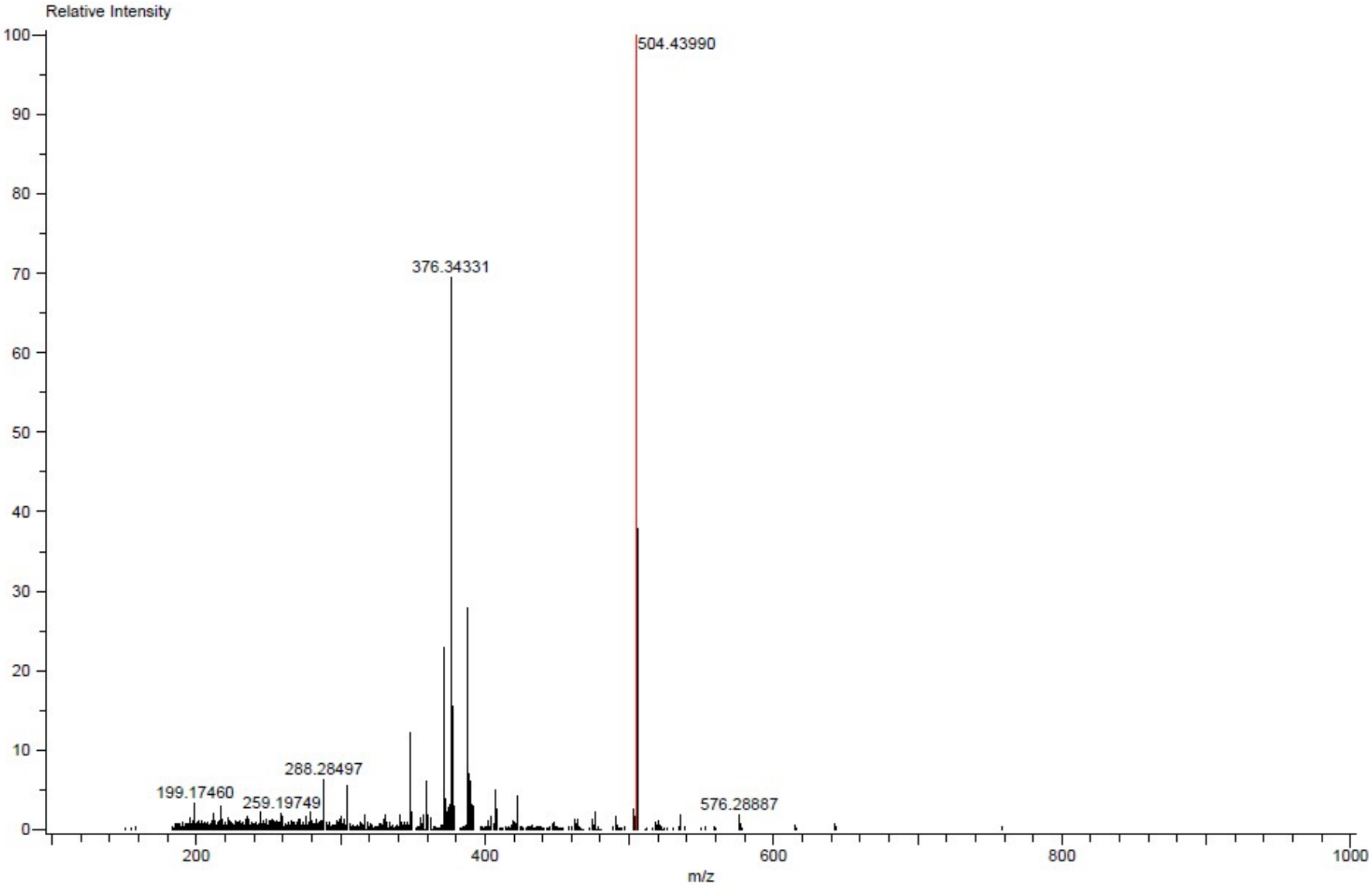
HR-MS Spectra



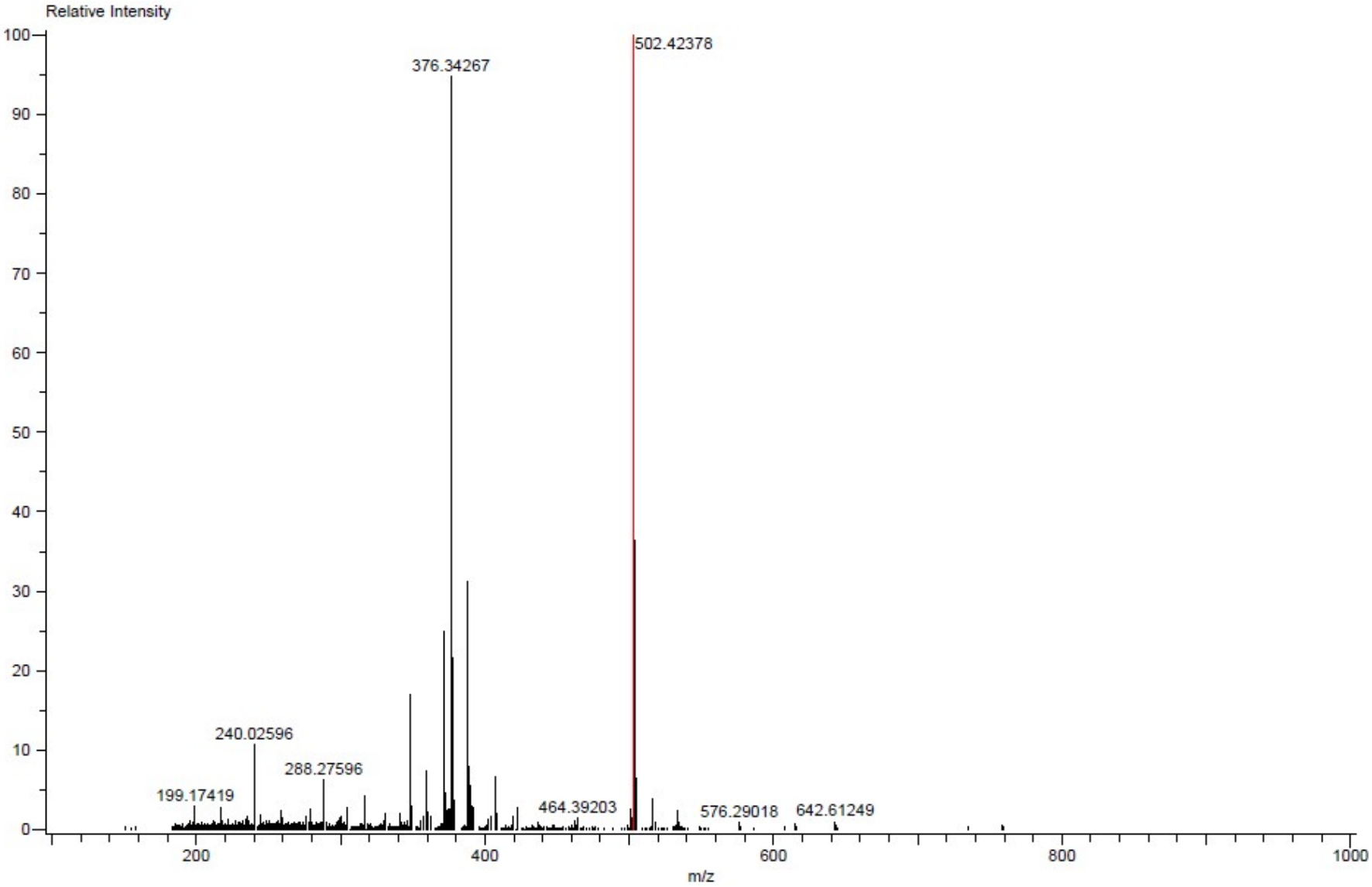
HR-MS Spectrum of compound **6**



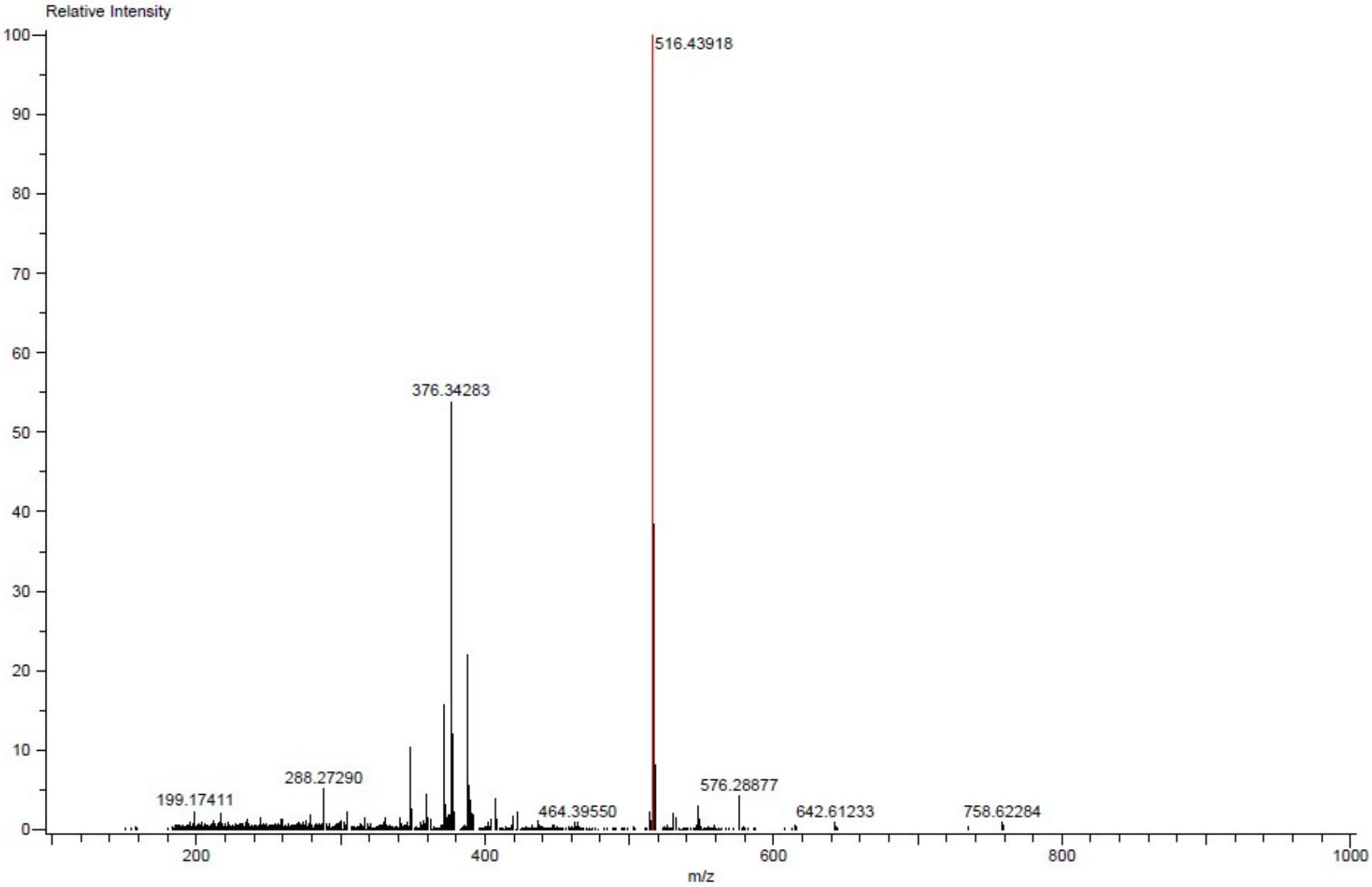
HR-MS spectrum of compound **7**



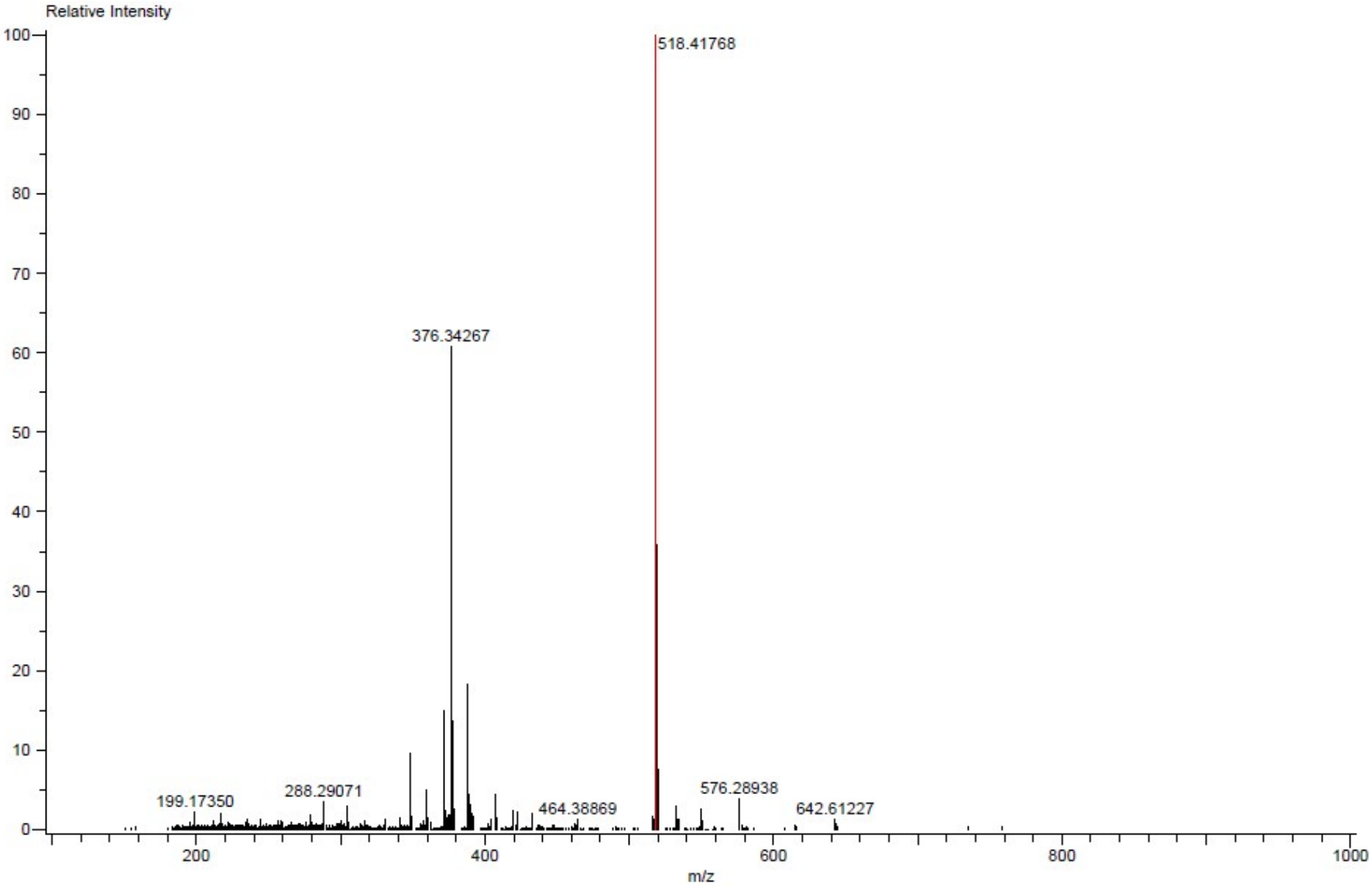
HR-MS spectrum of compound **8**



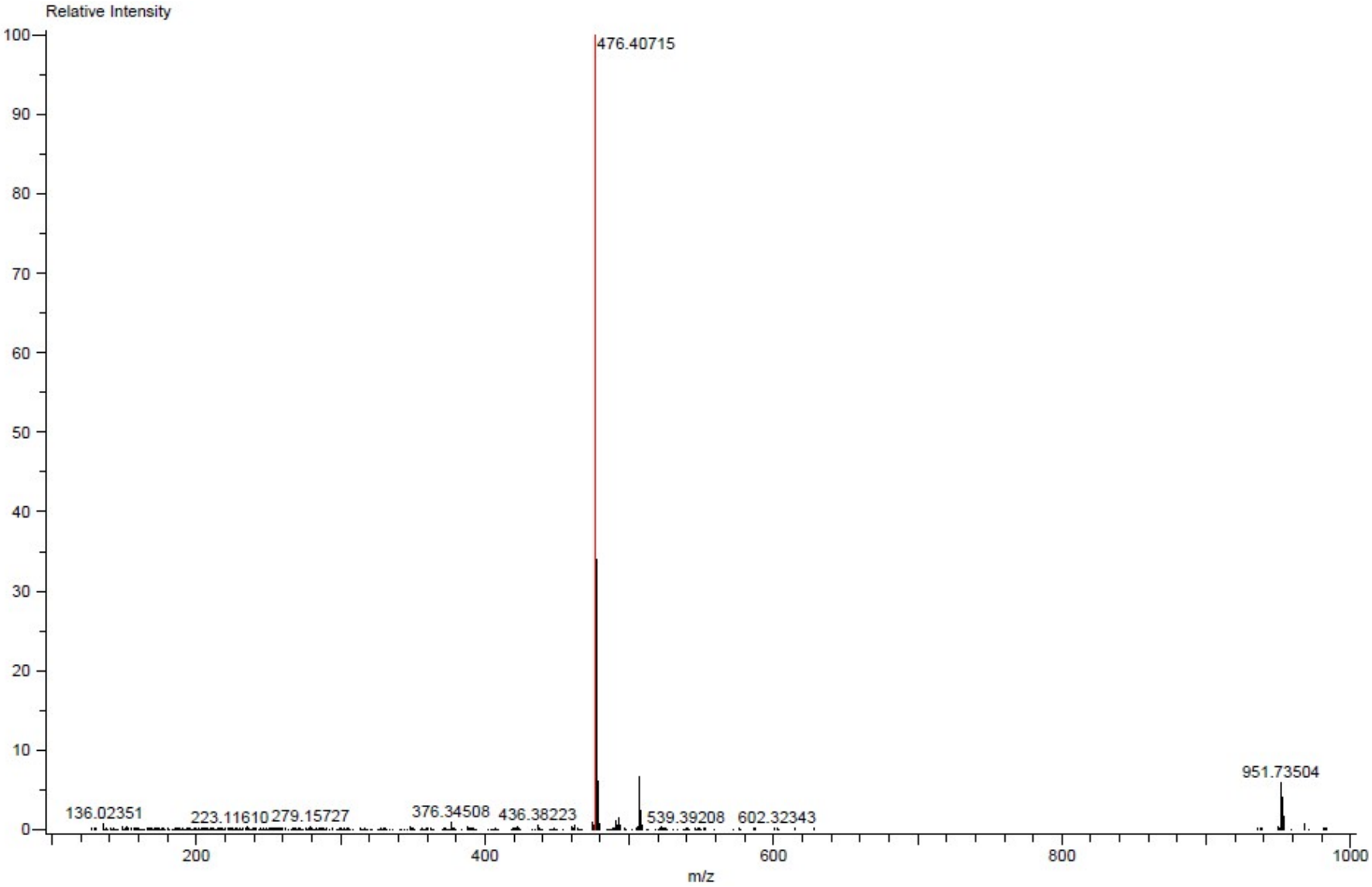
HR-MS spectrum of compound **9**



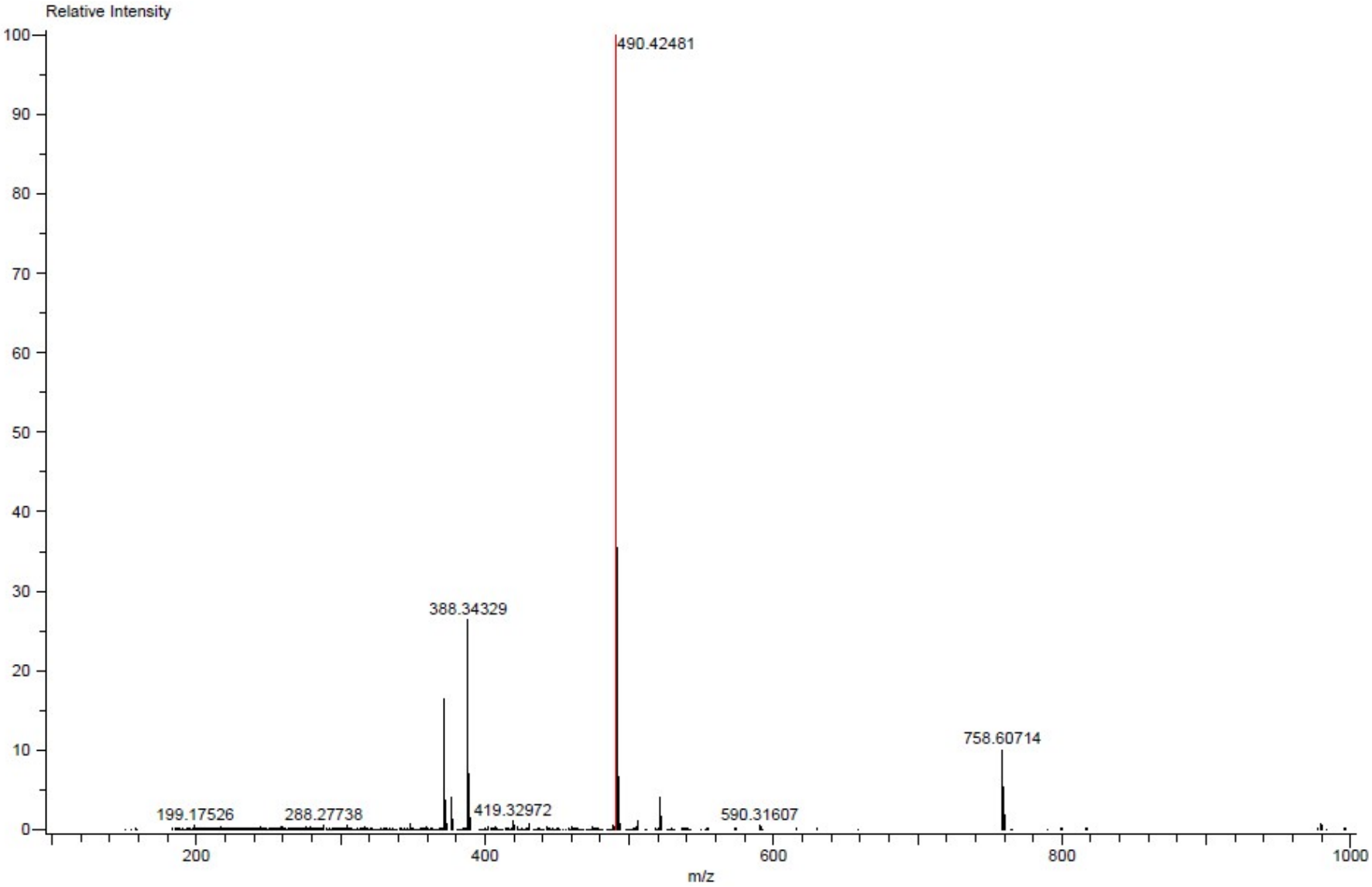
HR-MS spectrum of compound **10**



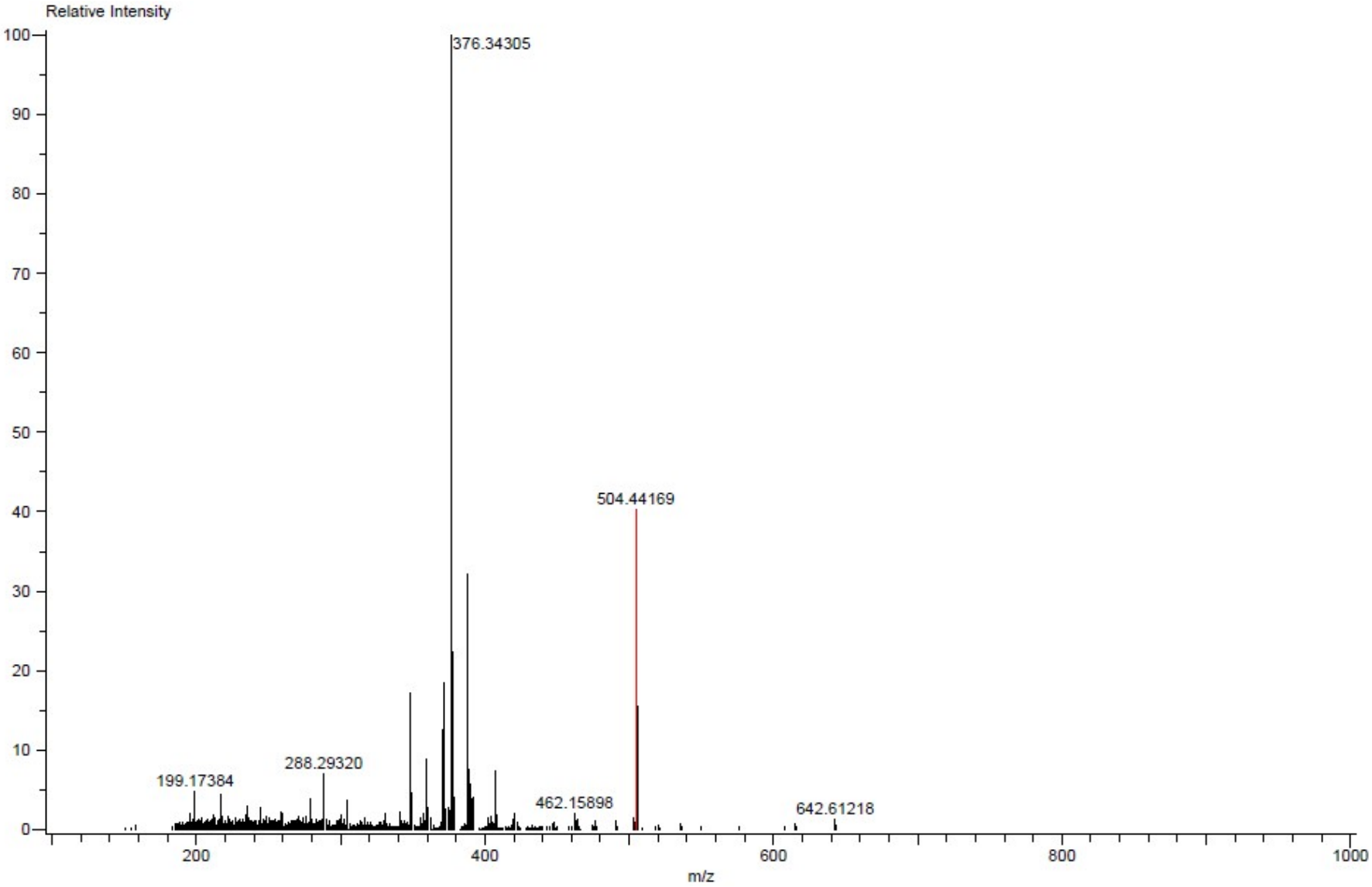
HR-MS spectrum of compound **11**



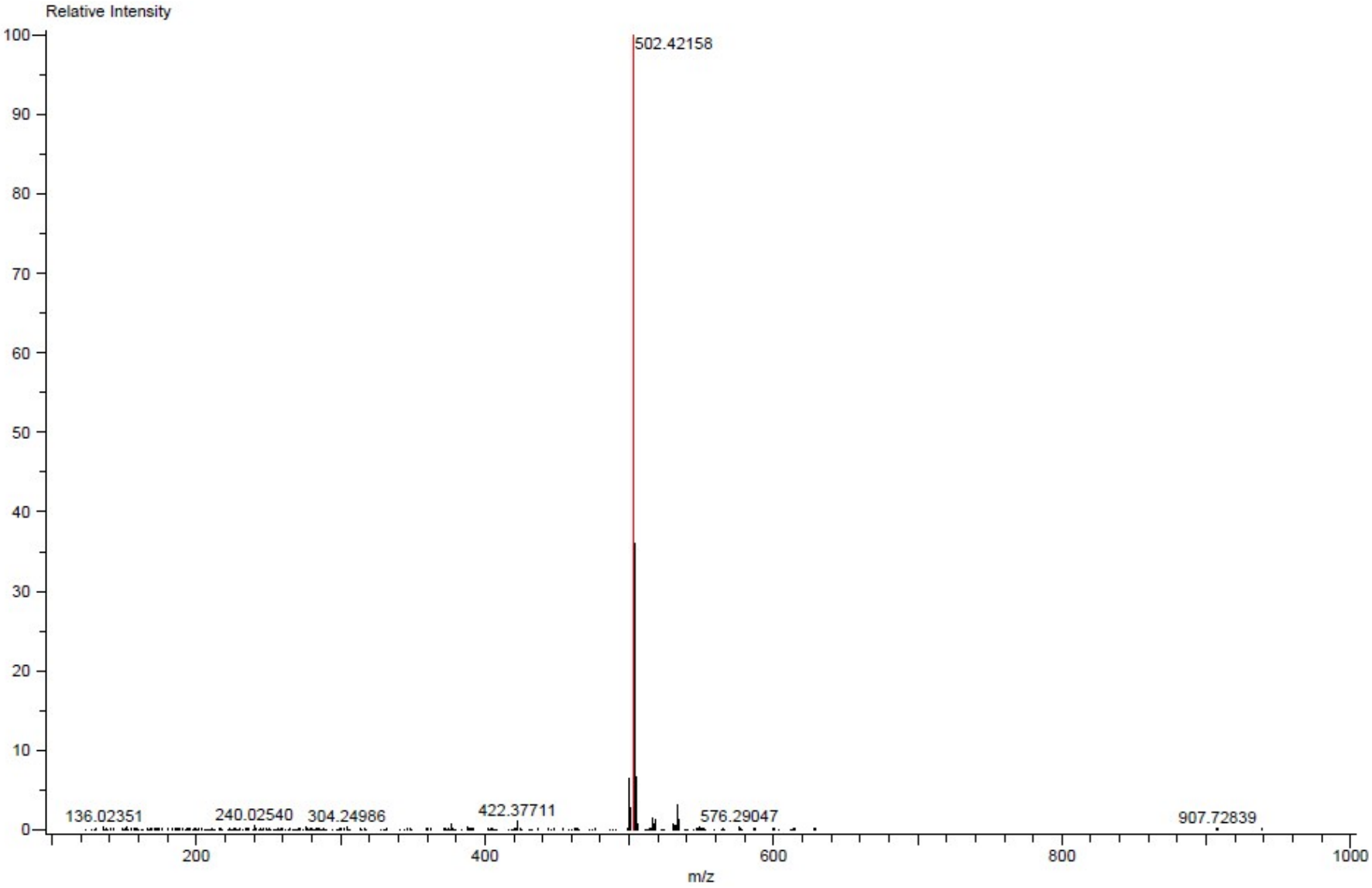
HR-MS spectrum of compound **12**



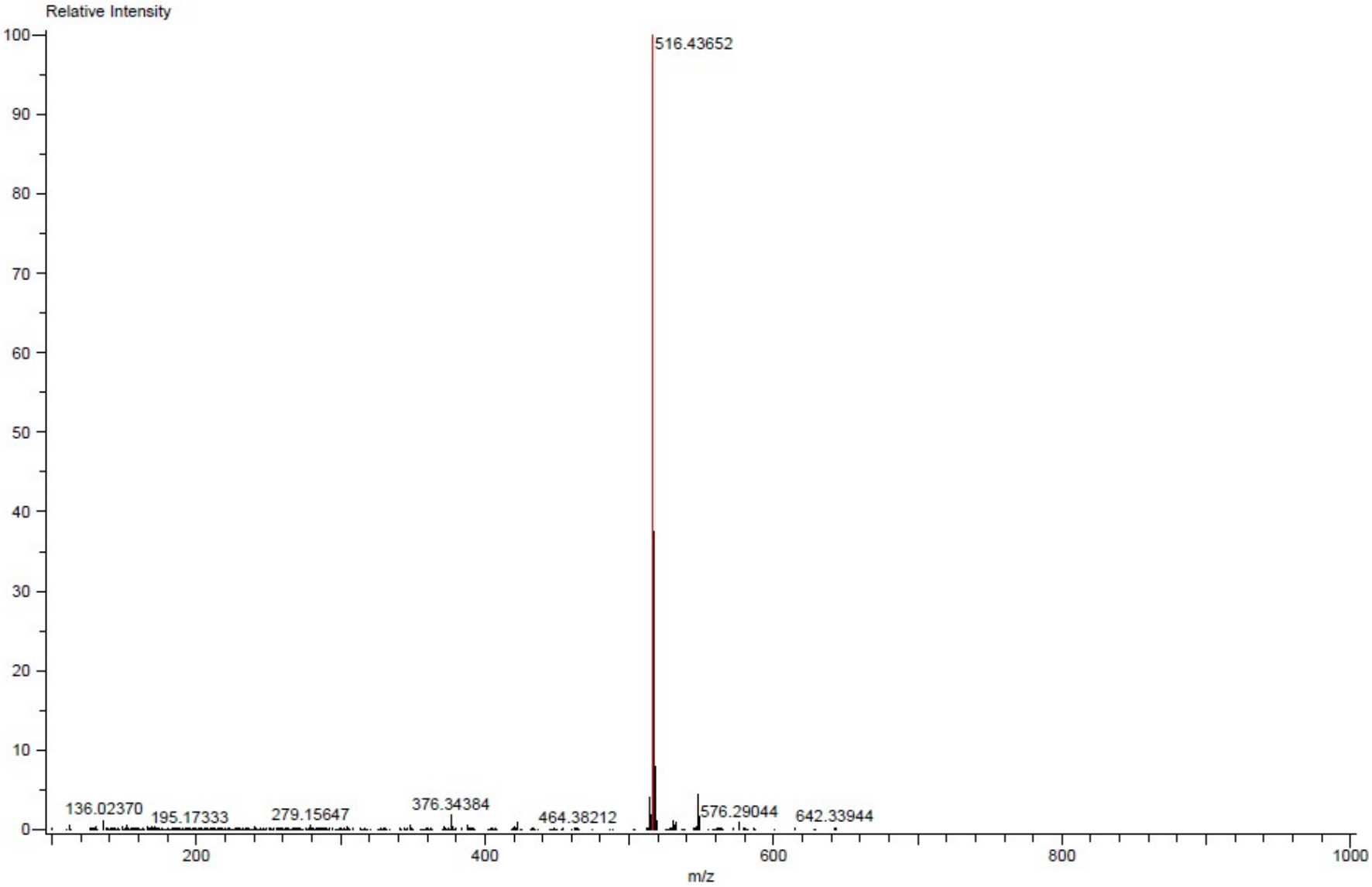
HR-MS spectrum of compound **13**



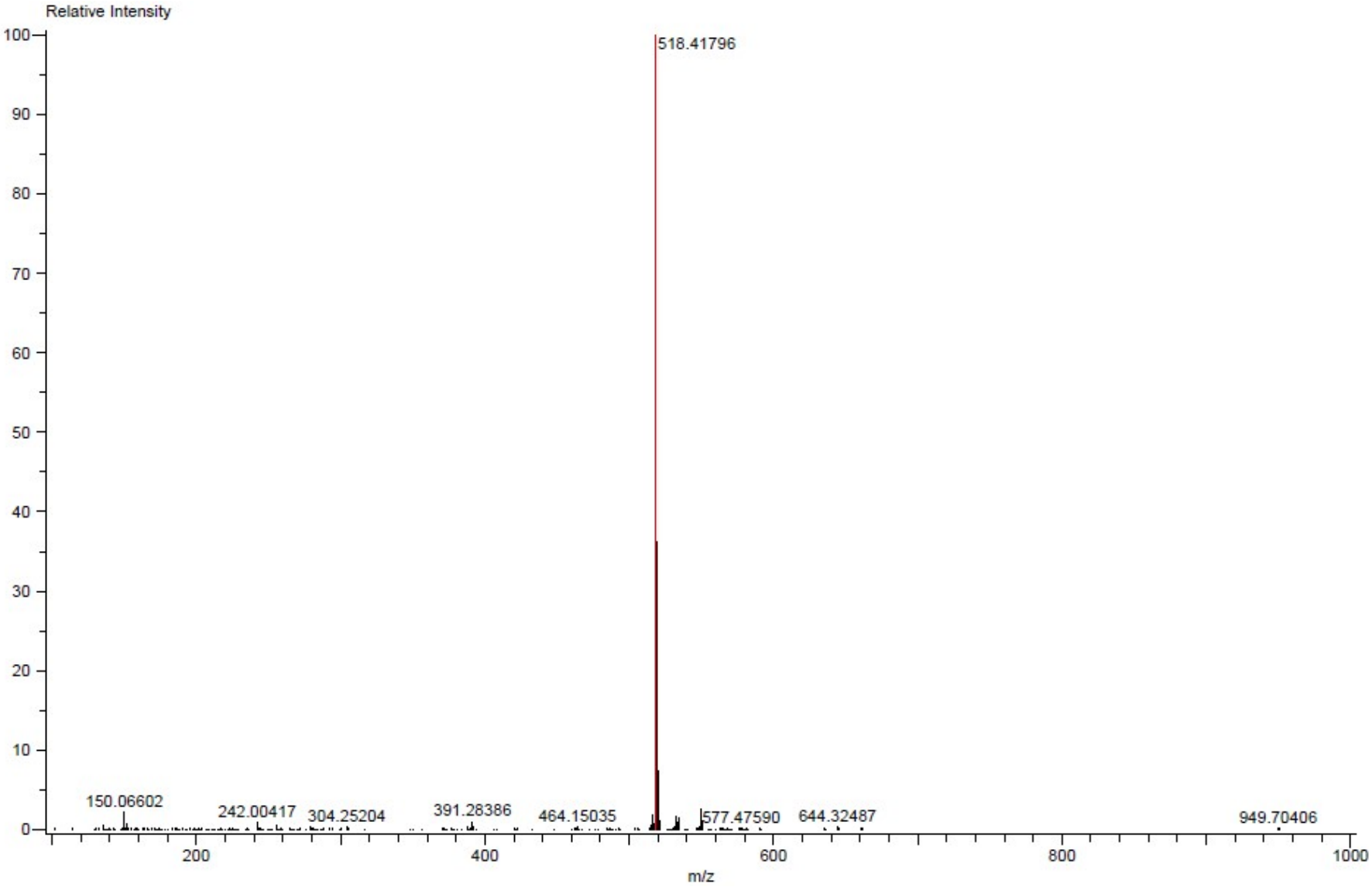
HR-MS spectrum of compound **14**



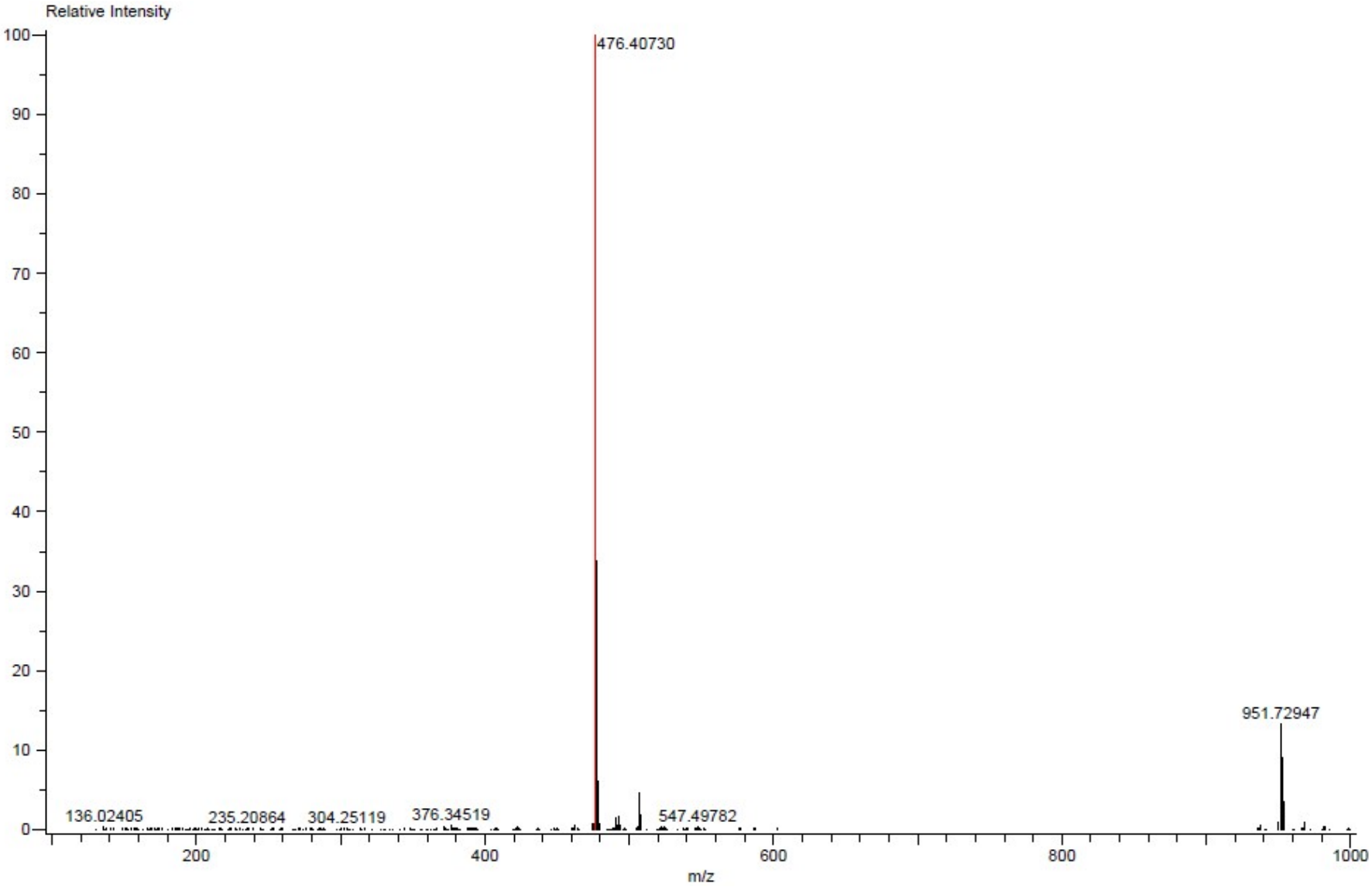
HR-MS spectrum of compound **15**



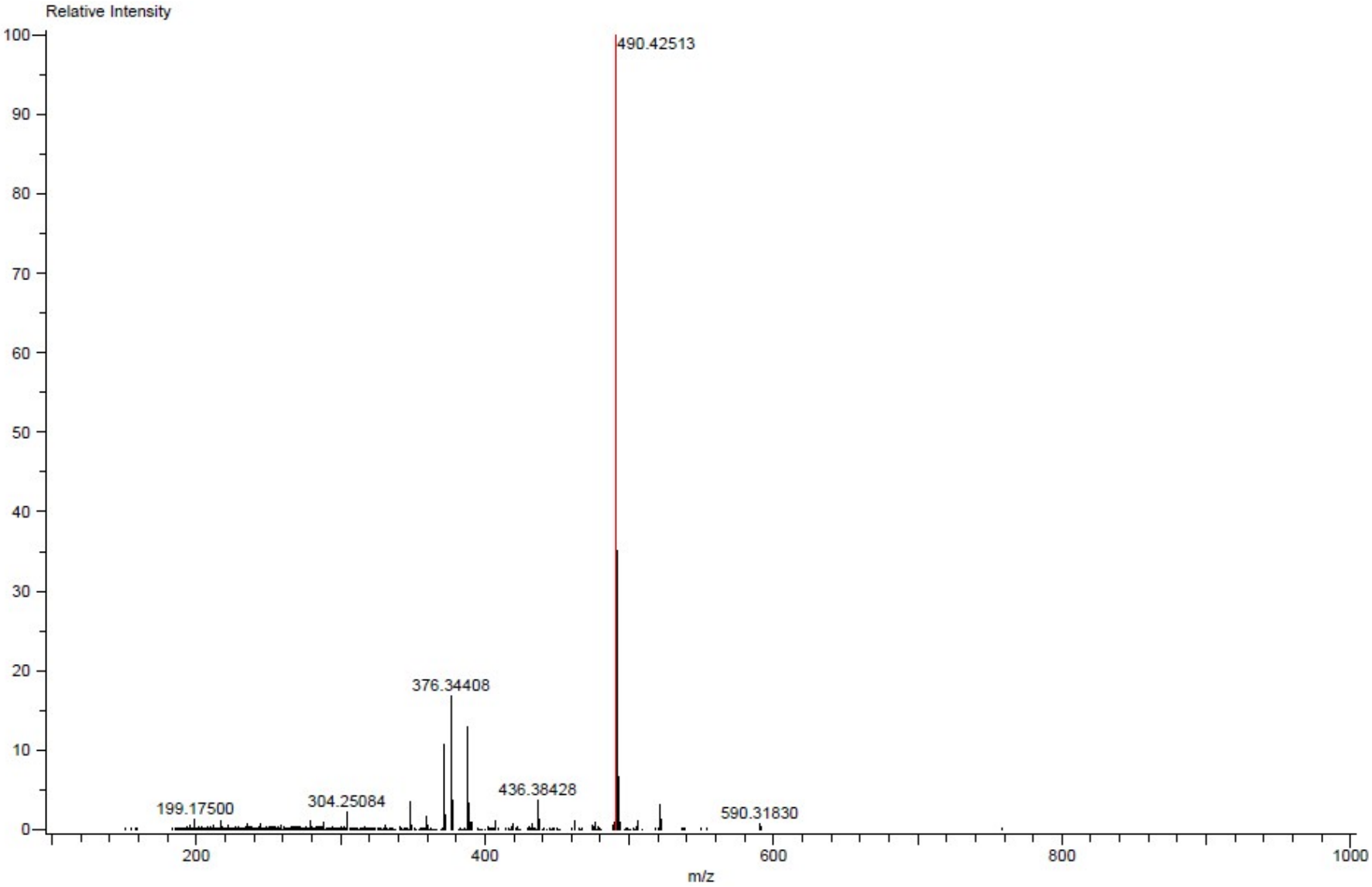
HR-MS spectrum of compound **16**



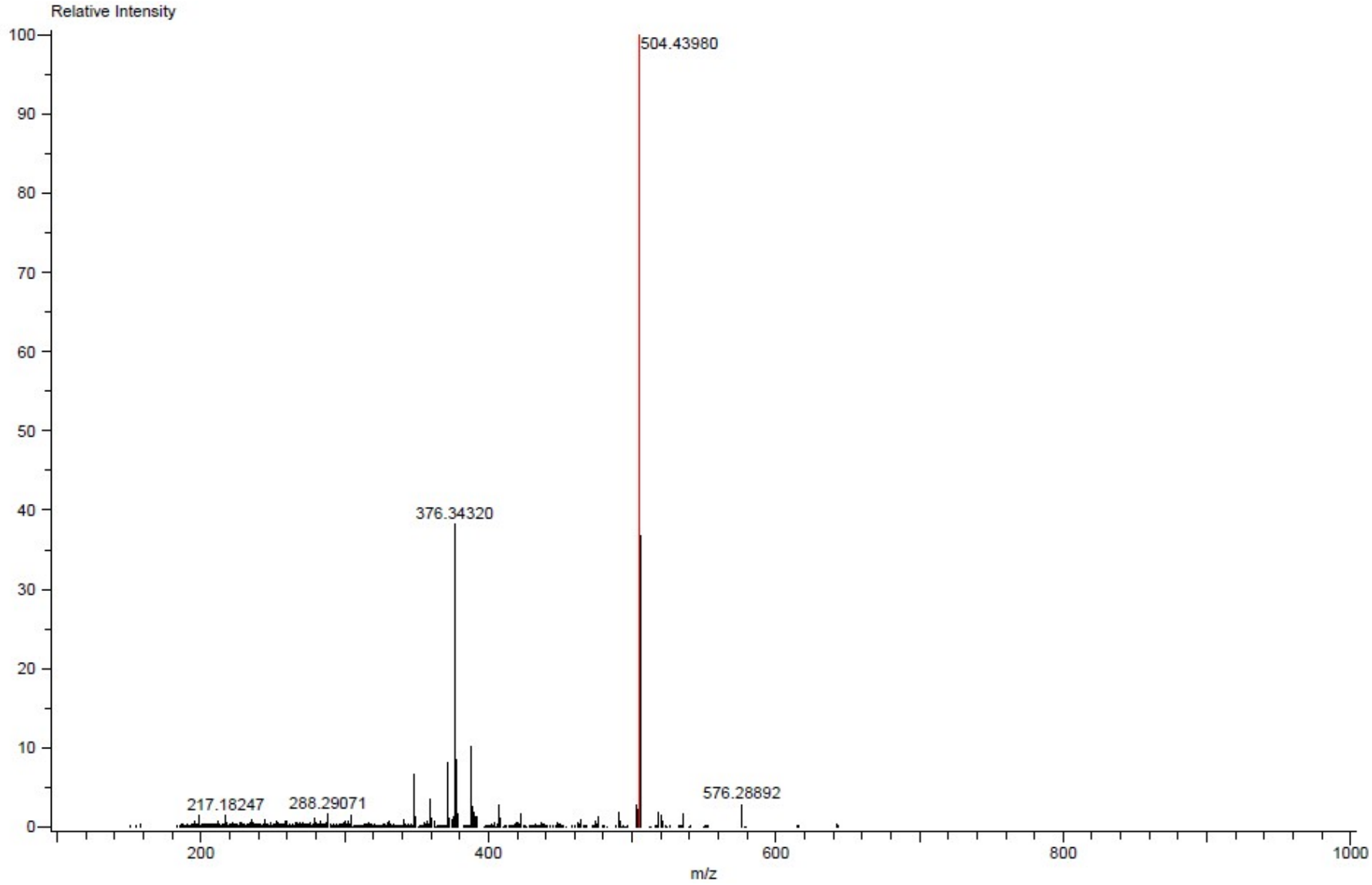
HR-MS spectrum of compound **17**



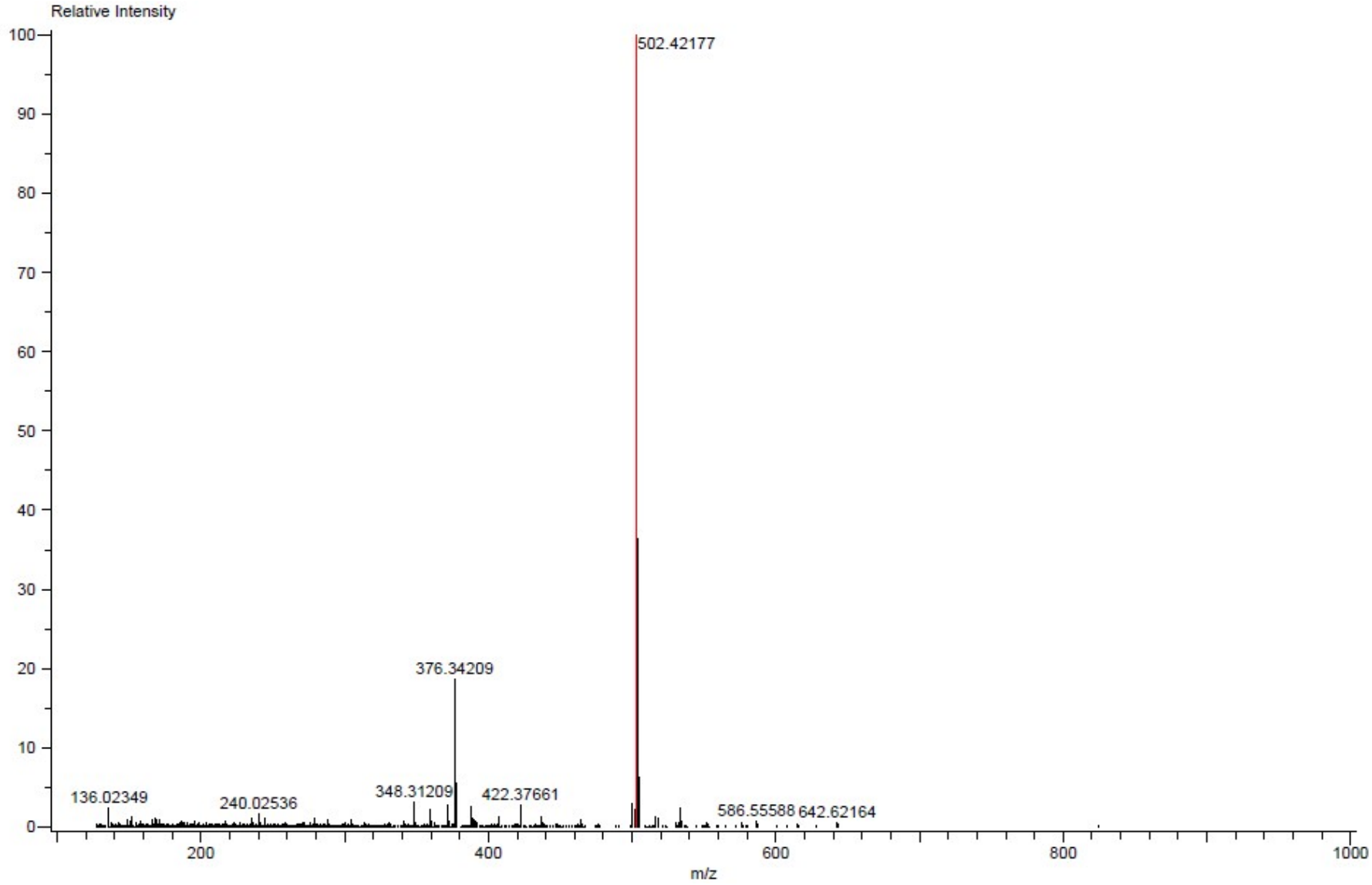
HR-MS spectrum of compound **18**



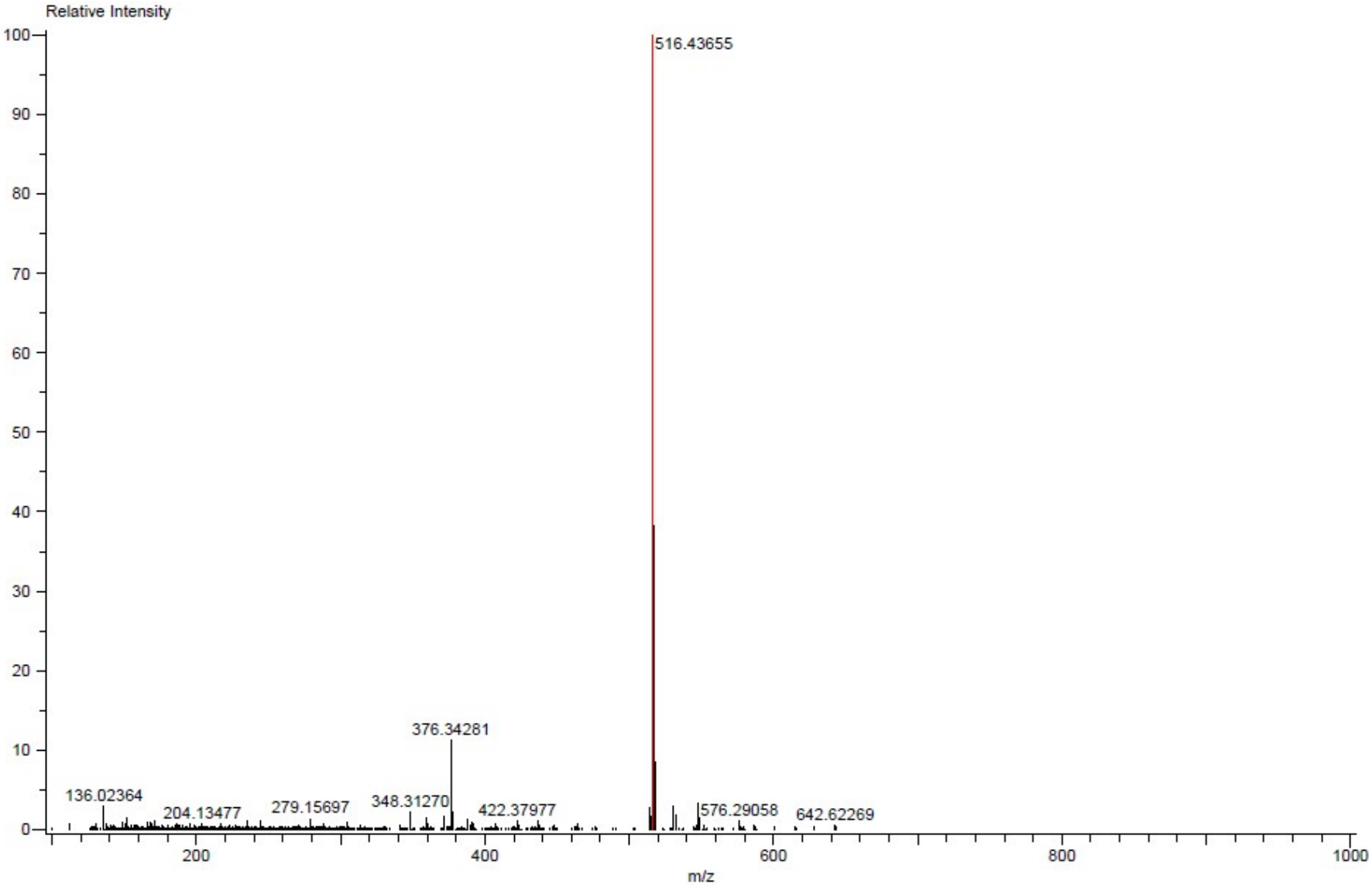
HR-MS spectrum of compound **19**



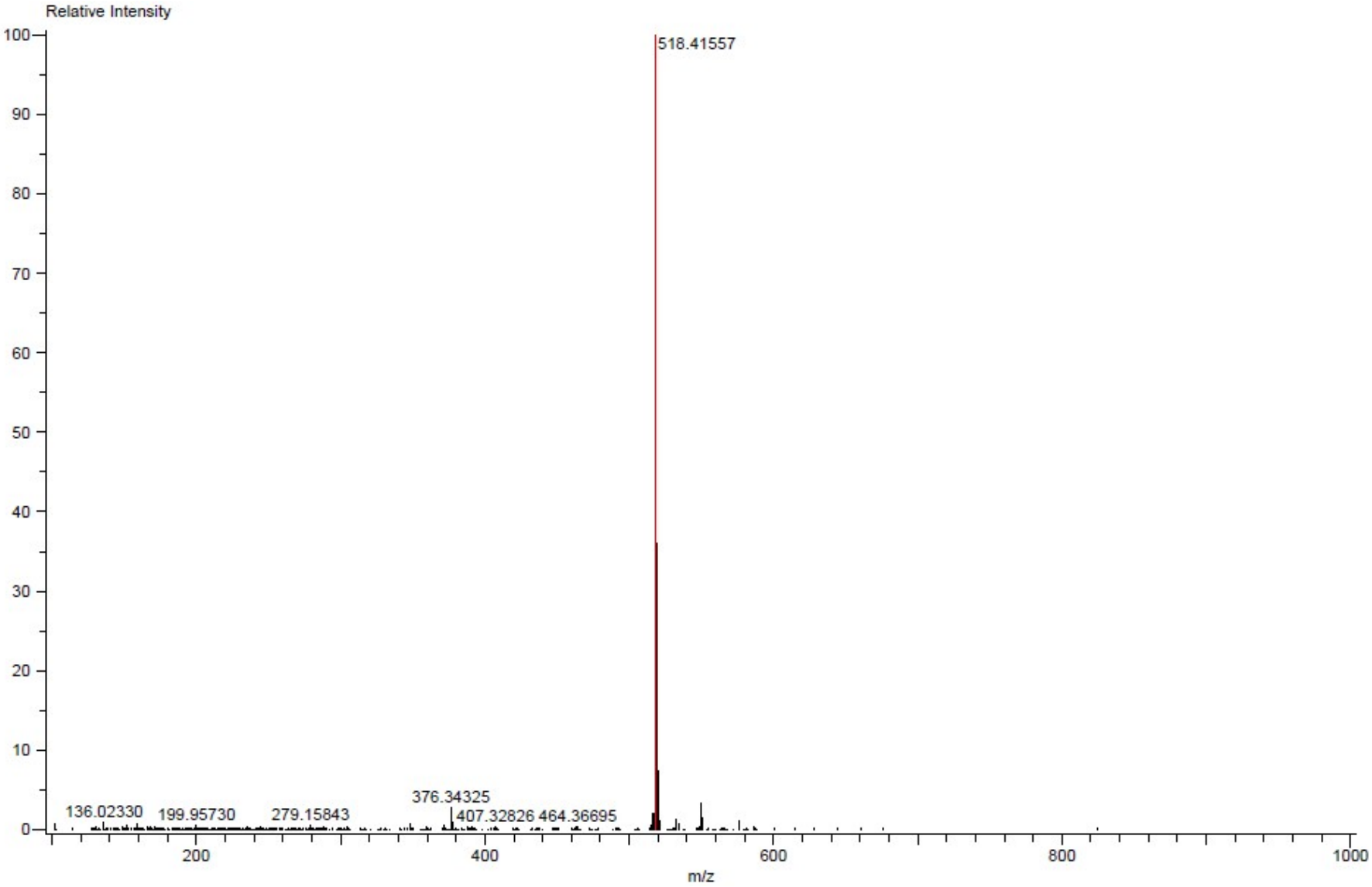
HR-MS spectrum of compound **20**



HR-MS spectrum of compound **21**



HR-MS spectrum of compound **22**



HR-MS spectrum of compound **23**