

Synthesis of 2,3-Diyne-1,4-naphthoquinone Derivatives and Evaluation of Cytotoxic Activity against Tumor Cell Lines

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Uma série de derivados 2,3-diino-1,4-naftoquinona foi sintetizada a partir do 2,3-dibromo-1,4-naftoquinona e diversos alquinos terminais funcionalizados usando a reação de acoplamento de Sonogashira catalisada por paládio. Os compostos foram submetidos à avaliação do potencial citotóxico em três linhagens de células tumorais, OVCAR-8 (ovário), PC-3M (próstata) e NCI-H358M (pulmão), apresentando, no geral, resultados satisfatórios para inibição do crescimento celular.

A series of 2,3-diyne-1,4-naphthoquinone derivatives was synthesized from 2,3-dibromo-1,4-naphthoquinone and various functionalized terminal alkynes using palladium-catalyzed Sonogashira cross-coupling reaction. The diynes were evaluated as potential cytotoxic agents against three tumor cell lines: human ovarian adenocarcinoma (OVCAR-8), human metastatic prostate cancer (PC-3M) and human bronchoalveolar lung carcinoma (NCI-H358M), presenting, in general, satisfactory results for inhibition of cell growth.

Keywords: 1,4-naphthoquinone, diyne, palladium, Sonogashira, cytotoxicity

Introduction

Naphthoquinones are substances of great pharmacological interest as they exhibit a wide variety of biological activities,¹ such as antitumor,^{2,3} antibacterial,⁴ antifungal,⁵ molluscicides,⁶⁻⁸ antileishmanial⁹ and anti-inflammatory.¹⁰ The mechanism of action of naphthoquinones involves oxidants and electrophile properties influenced by their chemical structure.¹¹ There are two important mechanisms of quinone cytotoxicity: stimulation of oxidative stress and alkylation of cellular nucleophiles, which encompass a large range of biomolecules. Reactive oxygen species (ROS) may react directly with DNA, lipids and proteins, leading to cell damage^{12,13} and shunting electrons toward oxygen, a futile pathway for reduction equivalents otherwise used for cytochrome P450 reductase-dependent reactions. Cellular damage can also occur through the alkylation of crucial proteins and nucleic acids.¹⁴⁻¹⁶

Naphthoquinones containing acetylenic substituents on the quinone ring are promising intermediates in the synthesis of heterocyclic quinones and highly reactive unsaturated compounds.¹⁷⁻¹⁹ 2,3-Diyne-1,4-naphthoquinones and related compounds exhibit an enediyne system that is capable of causing damage to cellular DNA through cycloaromatization, with the formation of diradical benzenoid intermediates that remove hydrogen atoms from the sugar skeleton-phosphate of the DNA double helix.²⁰ Enediyne compounds are among the most effective known chemotherapy agents, highly potent for cleavage of DNA. In recent years, considerable efforts have been invested in the development of drugs such as enediyne showing a maximum activity against tumor cells and minimal toxicity in normal cells.²¹

In this work, we report the synthesis of 2,3-diyne-1,4-naphthoquinones employing Sonogashira cross-coupling reaction²² between 2,3-dibromo-1,4-naphthoquinone and various functionalized terminal alkynes. We also report the cytotoxic activity of these derivatives in three tumor cell lines: human ovarian adenocarcinoma (OVCAR-8),

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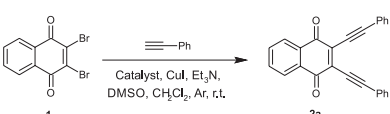
human metastatic prostate cancer (PC-3M) and human bronchoalveolar lung carcinoma (NCI-H358M), presenting, in general, satisfactory results for cell growth inhibition.

Results and Discussion

Synthesis of the compounds

The diyne derivatives (**2a-i**) were synthesized following a procedure described in the literature,¹⁷ via cross-coupling between 2,3-dibromo-1,4-naphthoquinone (**1**) and several terminal alkynes catalyzed by palladium complexes and co-catalyzed by copper(I) iodide.²² Initially, the synthesis of compound **2a** from the phenylacetylene alkyne and (**1**) was performed in a mixture of solvents, DMSO (dimethylsulfoxide) and DCM (dichloromethane) (1:1), using triethylamine, CuI and a palladium complex (Pd(PPh₃)₂Cl₂) as catalyst. The product (**2a**) was obtained in 45% yield after isolation on silica gel column chromatography. In virtue of the importance of the catalyst to the formation of the coupling product, other palladium complexes were also tested under the same reaction conditions. Using Pd(PPh₃)₄, compound **2a** was obtained in only 27% yield (after consumption of **1** by TLC inspection). Other catalysts tested were unable to provide the product of interest (**2a**) (Table 1).

Table 1. Catalytic conditions tested to obtain **2a**



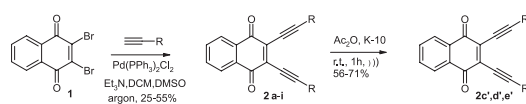
entry	Catalyst	time / min	Yield / %
1	PdCl ₂ + PPh ₃ ^a	not formed	–
2	Pd(PPh ₃) ₂ Cl ₂	120	45
3	Pd(PPh ₃) ₄	50	27
4	Pd(OAc) ₂	–	–
5	Pd(CH ₃ CN) ₂ Cl ₂	–	–

^aIncluded in the reaction mixture.

The preformed complex Pd(PPh₃)₂Cl₂ (Table 1, entry 2) was chosen as catalyst in the coupling reaction of (**1**) with several terminal alkynes due to its higher stability and solubility and better performance for **2a** formation. As a result, compounds **2a-i** were obtained with 25-55% yields, as shown in Table 2. Compounds **2c-e** were acetylated using acetic anhydride and montmorillonite clay K-10 under ultrasound treatment,²³ producing three new derivatives (**2c'-e'**) in yields ranging from 56 to 71%. All compounds were characterized by ¹H and ¹³C nuclear

magnetic resonance (NMR), liquid chromatography-mass spectrometry (LC-MS) and Fourier transform infrared spectroscopy (FTIR). The characterization of **2a** by ¹H and ¹³C NMR (300 and 75 MHz, CDCl₃) analyses showed a simplified spectrum due to a planar symmetry. A signal centered at δ 8.16 ppm (dd, 2H, *J* 3.3 and 5.7 Hz) and at δ 7.77 ppm (dd, 2H, 3.3 and 5.7 Hz) were attributed to *ortho*- (5/8) and *meta*-benzenoid hydrogens. A multiplet centered at δ 7.68 ppm (4H) and another at δ 7.41 ppm (6H) were attributed to phenyl hydrogens. The ¹³C NMR spectra presents a single carbonyl peak at δ 181.1 ppm, and the sp carbons of triple bond at δ 85.4 and 109.7 ppm, among the expected eight sp² signals.

Table 2. 2,3-Diyne-1,4-naphthoquinone derivatives



entry	Compound	Yield ^a / %	time / h / Temperature / °C
1	2a	45	2 / 28
2	2b	46	1 / 28
3	2c	42	4 / 40
4	2d	30	2.5 / 40
5	2e	25	12 / 28
6	2f	38	1 / 28
7	2g	55	1 / 28
8	2h	38	1 / 28
9	2i	30	2 / 28

2c': 56%, **2d'**: 65% and **2e'**: 71%; ^aAll yields after column chromatography.

DMSO was the solvent of choice for the preparation of compounds **2a-i** due to the relatively good stability of **1** in this solvent, as reported by Romanov *et al.*¹⁷ in contrast to what is observed in other solvents (pyridine, Dimethylformamide-DMF, Et₃N, etc.). CH₂Cl₂ was previously reported as a suitable co-solvent to reduce resinification produced in reaction.¹⁷ The use of THF (tetrahydrofuran) as solvent in the synthesis of **2a** was unsuccessful, resulting in a rather complex mixture of products, not allowing isolation. Alternatively, when this reaction was performed in acetonitrile, **2a** was produced in 20 min, but with 36% yield after column chromatography purification procedure.

The low yields in Sonogashira coupling products (**2a-i**) are related to several factors, e.g., low reactivity of 2,3-dibromo-1,4-naphthoquinone (**1**) in performing coupling reactions. In addition, vinyl bromides are less

reactive than their iodide and triflate analogs in the Sonogashira type coupling reactions.²⁴ Another problem is the undesirable formation of alkyne homocoupling due to reaction conditions and exposure to oxidizing agents.²⁴

Several terminal alkynes with different substituents can be submitted to Sonogashira coupling, but some specific alkynes have a low reactivity or even do not react.²⁵ The reaction with 4-methoxyphenylacetylene afforded compound **2b** with 46% yield. It is possible to observe that the electron donor methoxy substituent increased the reactivity, and this reaction occurred two times faster than that from unsubstituted phenyl (entry 2, Table 2). Even though, the yields were not significantly different. The coupling with alkynes containing the alcohol function resulted in products **2c**, **2d** and **2e** with yields of 42, 30 and 25%, respectively. It was observed that the hydroxyl group influences the yield of the coupling, so that the less hindered the hydroxyl group, the lower the reaction yields. The coupling of **1** with different alkyne alkyl (1-pentyne, 1-hexyne, 1-octyne and 1-decyne) resulted in products **2f**, **2g**, **2h** and **2i** yields with 38, 55, 38 and 30%, respectively. No obvious relation between the size of the alkyne chain and the reaction yield could be noticed.

Using the same above reaction conditions, the coupling between 2,3-dibromo-1,4-naphthoquinone (**1**) and some terminal alkynes were not successful. The reaction with alkyne prop-2-yn-1-ol (propargyl alcohol) resulted in a complex mixture of products which could not be isolated. The reaction with ethyl propiolate also did not result in the desired coupling product, probably due to electron-

withdrawing alkyne conjugation, thus lowering the reactivity of the alkyne to Sonogashira coupling reaction.²⁶ Attempts to react **1** with trimethylsilylacetylene were also unsuccessful, confirming previously reported results.²⁷

Cytotoxicity assay

The cytotoxicity of the 2,3-diyne-1,4-naphthoquinone derivatives (**2a-i**; **2c'-e'**) were evaluated *in vitro* against three tumor cell lines of different histotypes, PC-3M, OVCAR-8 and NCI-H358M, using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric assay.²⁸ Doxorubicin was used as a positive control. The cytotoxic activity of the samples is shown in Table 3, with their respective percentage of cell growth inhibition (GI in %). Substances which showed inhibition of tumor cell growth greater than 75% in at least two tumor cell lines tested in the preliminary test with a single dose of 25 $\mu\text{g mL}^{-1}$ were selected to determine the IC₅₀ value.

Among the tested samples, ten (**2a-f**, **2h** and **2c'-e'**) were selected as the most active as cytotoxic agents, as they presented cell growth inhibition greater than 75% in at least two tested tumor cell lines. In addition, compound **2g**, 2,3-di(hex-1-yn-1-yl)-1,4-naphthoquinone, was also selected for IC₅₀ determination as it presented high specificity (95.83% inhibition) towards OVCAR-8 cell line. Only compound 2,3-di(dec-1-yn-1-yl)-naphthoquinone **2i** showed no cytotoxic potential higher than 75% in any of the three tested tumor cell lines. In general, compounds having aliphatic side chains had lower percentage of cell

Table 3. Percent inhibition of cell growth obtained with the samples in three tumor cell lines in a single dose of 25 $\mu\text{g mL}^{-1}$

Compound	OVCAR-8		PC-3M		NCI-H358M	
	GI / %	SD	GI / %	SD	GI / %	SD
2a	100	0.08	72.52	3.05	85.46	2.55
2b	99.28	0.39	71.12	4.86	77.77	3.97
2c	100.50	0.08	99.63	0.05	98.21	0.13
2c'	97.67	1.41	98.55	2.39	100.84	3.91
2d	104.70	0.00	110.69	1.19	112.75	0.07
2d'	98.61	0.39	90.71	3.26	103.24	1.19
2e	100.89	0.79	100.07	0.00	98.25	0.19
2e'	101.72	3.36	96.85	2.17	99.88	1.87
2f	102.35	1.53	93.89	4.41	84.02	2.98
2g	95.83	1.49	55.51	2.61	59.39	3.82
2h	93.76	3.03	52.17	3.76	98.80	3.06
2i	50.01	3.63	50.82	2.41	32.25	0.70
Doxorubicin	98	1.1	97.8	2.4	99.8	0.89

GI: growth inhibition percentage; SD: standard deviation.

Table 4. Cytotoxic activity expressed as IC₅₀ of compounds **2a-h** against tumor cell lines

Compound	Cell lines IC ₅₀ / μM (confident range 95%)			
	PBMC	NCI-H358M	OVCAR-8	PC-3M
2a	0.388 (0.307-0.490)	6.55 (4.93-8.70)	4.49 (4.04-4.96)	14.26 (13.11-15.50)
2b	0.323 (0.232-0.449)	4.57 (3.67-5.69)	3.90 (2.69-5.65)	9.03 (5.62-14.49)
2c	ND	2.98 (2.43-3.64)	2.28 (2.02-2.57)	4.28 (3.44-5.33)
2c'	ND	4.95 (4.71-5.20)	5.63 (3.77-8.39)	5.74 (3.91-8.41)
2d	0.549 (0.410-0.736)	5.07 (4.15-5.42)	5.98 (5.32-6.71)	6.56 (5.72-7.52)
2d'	0.045 (0.038-0.053)	2.74 (2.57-2.92)	3.09 (2.08-4.58)	5.26 (4.13-6.68)
2e	0.213 (0.161-0.281)	6.23 (4.72-8.21)	6.56 (5.96-7.21)	8.94 (7.51-10.63)
2e'	0.310 (0.270-0.360)	5.48 (5.10-5.75)	7.40 (6.25-8.75)	6.92 (5.35-8.94)
2f	0.361 (0.289-0.451)	8.03 (6.82-9.44)	9.01 (7.94-10.22)	9.17 (8.10-10.37)
2g	0.568 (0.123-2.64)	6.42 (4.38-9.41)	9.70 (7.48-12.57)	20.23 (15.48-26.42)
2h	0.307 (0.270-0.349)	3.99 (3.62-4.38)	7.53 (5.31-10.66)	17.70 (13.17-23.78)
Doxorubicin	0.970 (0.520-1.80)	0.258 (0.173-0.385)	0.357 (0.278-0.463)	0.476 (0.322-0.705)

Data are presented as IC₅₀ values and 95% confidence intervals obtained by nonlinear regression for all cell lines. Doxorubicin was used as positive control. Only compounds with an IC₅₀ value lower than 5 $\mu\text{g mL}^{-1}$ for at least one cell line were considered active. ND: not determined.

growth inhibition. The calculation of IC₅₀ was measured by MTT assay after 72 h of incubation at concentrations from 0.01 to 25 $\mu\text{g mL}^{-1}$. Peripheral mononuclear blood cells (PBMC) were tested to confront the data in normal cells. The results are shown in Table 4.

From the eleven selected samples for determination of IC₅₀, eight (**2b-f**, **2c'-e'**) showed inhibitory concentration smaller than 10 μM for the three tested tumor cell lines, characterizing them as potent cytotoxic agents. The compounds (**2c**, **2d** and **2e**) which bear hydroxyl groups have approximately the same cell growth inhibition (IC₅₀) of acylated compounds (**2c'**, **2d'** and **2e'**). Thus, it is possible to see that the hydroxyl group in these compounds did not significantly influence the cytotoxic action for the three analyzed tumor cell lines. Samples **2a**, **2g** and **2h** exhibited IC₅₀ higher than 10 μM for PC-3M, showing lower cytotoxic action for this cell line and higher selectivity for inhibitory action against OVCAR-8 and NCI-H358M lines. The increase of the aliphatic chain reduced the cytotoxic effect on PC-3M cell line. The 2,3-di(3-hydroxy-3-methylbut-1-in-yl)-1,4-naphthoquinone (**2c**) presented the lower IC₅₀ (2.28-4.28 μM) for all three tested cell lines, characterizing this substance as a potent cytotoxic agent. Although the excellent cytotoxicity in cancer cell lines, all the compounds presented high inhibitory effect in peripheral blood mononuclear cells, which means non-selectivity effect and high cytotoxicity. Further investigation aiming at mechanisms of action and hemolytic activity are required for a better understanding of the cytotoxic effects and for determining the potential of the compounds reported in this work as future antitumor agents.

Conclusions

In this work, new potential antitumor active diynaphthoquinones were synthesized (twelve derivatives), nine of them new entities. In general, they exhibited inhibitory concentrations (IC₅₀) smaller than 10 μM for all three tested tumor cell lines, characterizing potent cytotoxic action. These results are significant for continuing studies of cytotoxicity in this class of bioactive compounds.

Experimental

General remarks

¹H and ¹³C NMR spectra were recorded on a Varian unity plus-300 or 400 MHz spectrometer in CDCl₃ or DMSO-*d*₆. All IR spectra were recorded on a Bruker IFS66 spectrophotometer using KBr pellets. High resolution mass spectra were obtained by electrospray on a Shimadzu LC-MS-IT-TOF spectrometer. Sonication was performed using a Model USC-1400A ultracleaner with a frequency of 40 kHz. Melting points are uncorrected and were determined with an electrically heated block apparatus. Monitoring of the course of the reactions was performed by GF₂₅₄ thin layer chromatography (TLC) and the column chromatography was performed on silica gel G₆₀ (70-230 mesh, Merck). All solvents and reagents were purchased from commercial suppliers (Sigma-Aldrich, Tedia, Merck, Cinética, Fluka), and used as received or purified by standard procedures.

Synthesis of the 2,3-diyne-1,4-naphthoquinone derivatives (**2**)

A mixture of terminal alkynes (1.0 mmol), CuI (0.13 g, 0.68 mmol) and triethylamine (0.07 g, 0.8 mmol) in anhydrous DMSO (4 mL) and CH₂Cl₂ (3 mL) was stirred for 2 min under argon atmosphere, then 2,3-dibromo-1,4-naphthoquinone (0.100 g, 0.32 mmol) and Pd(PPh₃)₂Cl₂ (0.005 g, 0.007 mmol) were added to the reaction mixture. The stirring was continued until total consumption of the starting material (**1**), after that, CH₂Cl₂ was removed by reduced pressure and water was added to the reaction mixture. The precipitate formed was separated by vacuum filtration and the compounds were isolated by silica gel column chromatography.

2,3-di(Phenylethynyl)-1,4-naphthoquinone (**2a**)

Using dichloromethane/hexane 30:70 as eluent, **2a** was obtained as a red solid (mp 151-152)¹⁷ in 45% (51 mg, 0.143 mmol) yield.

2,3-di(4-Methoxyphenylethynyl)-1,4-naphthoquinone (**2b**)

Using dichloromethane/hexane 50:50 as eluent, **2b** was obtained as a red-winesh solid (mp 162-163 °C) in 46% (61 mg, 0.146 mmol) yield; IR (KBr) ν_{\max} /cm⁻¹ 2175, 1664, 1605, 1531, 1376, 1330, 1302, 1257, 1156, 1026, 904, 826, 711, 535; ¹H NMR (CDCl₃, 300 MHz) δ 8.15 (dd, 2H, 5.7/3.3 Hz), 7.76 (dd, 2H, 5.7/3.3 Hz), 7.63 (m, 4H), 6.92 (m, 4H), 3.86 (s, 6H); ¹³C NMR (CDCl₃, 75 MHz) δ 180.9, 161.1, 134.2, 133.9, 133.1, 132.1, 126.8, 114.3, 109.9, 84.8, 55.4. LC-MS calcd. for [C₂₈H₁₉O₄]⁺, 419.1278; found: 419.1261.

2,3-di(3-Hydroxy-3-methylbutynyl)-1,4-naphthoquinone (**2c**)

Using ethyl acetate/hexane 30:70 as eluent, **2c** was obtained as a dark yellow solid (mp 138-139 °C)¹⁷ in 42% (43 mg, 0.134 mmol) yield.

2,3-di[(1-Hydroxycyclohexyl)ethynyl]-1,4-naphthoquinone (**2d**)

Using ethyl acetate/hexane 25:75 as eluent, **2d** was obtained as a yellow solid (mp 109-110 °C) in 30% (38 mg, 0.095 mmol) yield; IR (KBr) ν_{\max} /cm⁻¹ 3227, 2934, 2856, 2208, 1666, 1594, 1550, 1449, 1345, 1277, 1185, 1073, 986, 966, 788, 712; ¹H NMR (CDCl₃, 400 MHz) δ 8.07 (d, 2H, 3.2 Hz), 7.73 (d, 2H, 3.6 Hz), 2.07 (s, 4H), 1.71 (m, 16H); ¹³C NMR (CDCl₃, 100 MHz) δ 180.7, 134.1, 133.9, 131.8, 126.8, 113.6, 79.1, 69.5, 39.5, 25.2, 23.2. LC-MS calcd. for [C₂₆H₂₆O₄Na]⁺, 425.1729; found: 425.1705.

2,3-di(5-Hydroxypent-1-yn-1-yl)-1,4-naphthoquinone (**2e**)

Using ethyl acetate/dichloromethane 80:20 as eluent, **2e** was obtained as a pale yellow solid (mp 130-131 °C) in

25% (25 mg, 0.078 mmol) yield; IR (KBr) ν_{\max} /cm⁻¹ 3328, 3237, 2949, 2213, 1667, 1597, 1358, 1322, 1292, 1194, 1031, 954, 796, 713, 642; ¹H NMR (DMSO-*d*₆, 300 MHz) δ 7.96 (dd, 2H, 6.0/3.3 Hz), 7.85 (dd, 2H, 6.0/3.3 Hz), 4.60 (t, 2H, 5.2 Hz), 3.57 (dd, 4H, 6.3/5.1 Hz), 2.65 (t, 4H, 7.0 Hz), 1.73 (quin, 4H, 6.6 Hz); ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 180.6, 134.3, 133.9, 131.5, 126.3, 110.6, 76.3, 59.2, 31.5, 16.3. LC-MS calcd. for [C₂₀H₁₉O₄]⁺, 323.1284; found: 323.1295.

2,3-di(Pent-1-yn-1-yl)-1,4-naphthoquinone (**2f**)

Using dichloromethane/hexane 40:60 as eluent, **2f** was obtained as a yellow solid (mp 92-93 °C) in 38% (35.4 mg, 0.122 mmol) yield; IR (KBr) ν_{\max} /cm⁻¹ 2962, 2932, 2870, 2212, 1665, 1595, 1547, 1456, 1356, 1321, 1292, 1188, 991, 794, 713, 642; ¹H NMR (CDCl₃, 300 MHz) δ 8.09 (dd, 2H, 5.7/3.3 Hz), 7.72 (dd, 2H, 5.7/3.3 Hz), 2.59 (t, 4H, 7.2 Hz), 1.69 (sex, 4H, 7.2 Hz), 1.10 (t, 6H, 7.5 Hz); ¹³C NMR (CDCl₃, 75 MHz) δ 181.3, 134.6, 133.9, 131.9, 126.5, 111.4, 76.6, 22.5, 21.79, 13.5. LC-MS calcd. for [C₂₀H₁₉O₂]⁺, 291.1385; found: 291.1419.

2,3-di(Hex-1-yn-1-yl)-1,4-naphthoquinone (**2g**)

Using dichloromethane/hexane 25:75 as eluent, **2g** was obtained as a pale yellow solid (mp 68-69 °C) in 55% (55 mg, 0.173 mmol) yield; IR (KBr) ν_{\max} /cm⁻¹ 3314, 3370, 2954, 2928, 2867, 2212, 1670, 1595, 1548, 1455, 1420, 1357, 1320, 1292, 796, 711, 641; ¹H NMR (CDCl₃, 300 MHz) δ 8.09 (dd, 2H, 5.7/3.3 Hz), 7.72 (dd, 2H, 5.7/3.3 Hz), 2.62 (t, 4H, 7.0 Hz), 1.60 (m, 8H), 0.96 (t, 6H, 7.2 Hz); ¹³C NMR (CDCl₃, 75 MHz) δ 181.3, 134.5, 133.8, 131.8, 126.8, 111.5, 76.1, 30.3, 21.9, 20.2, 13.8. LC-MS calcd. for [C₂₂H₂₃O₂]⁺, 319.1698; found: 319.1731.

2,3-di(Oct-1-yn-1-yl)-1,4-naphthoquinone (**2h**)

Using dichloromethane/hexane 30:70 as eluent, **2h** was obtained as a red solid (mp 54-55 °C) in 38% (45 mg, 0.120 mmol) yield; IR (KBr) ν_{\max} /cm⁻¹ 2924, 2853, 2213, 1673, 1595, 1538, 1463, 1359, 1320, 1294, 1193, 1072, 989, 795, 711, 644; ¹H NMR (CDCl₃, 400 MHz) δ 8.09 (2H), 7.72 (2H), 2.60 (t, 4H, 7.0 Hz), 1.68 (quin, 4H, 7.2 Hz), 1.50 (m, 4H), 1.33 (bs, 8H), 0.90 (t, 6H, 6.0 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 181.3, 134.6, 133.9, 131.9, 126.8, 111.6, 76.2, 31.3, 28.6, 28.3, 22.5, 20.6, 14.0. LC-MS calcd. for [C₂₆H₃₁O₂]⁺, 375.2325; found: 375.2363.

2,3-di(Dec-1-yn-1-yl)-1,4-naphthoquinone (**2i**)

Using dichloromethane/hexane 30:70 as eluent, **2i** was obtained as a red solid (mp 52-53 °C) in 30% (40 mg, 0.093 mmol) yield; IR (KBr) ν_{\max} /cm⁻¹ 2954, 2917, 2848, 2213, 1670, 1594, 1544, 1362, 1320, 1295, 1196, 712, 642;

^1H NMR (CDCl_3 , 300 MHz) δ 8.09 (dd, 2H, 5.7/3.3 Hz), 7.72 (dd, 2H, 5.7/3.3 Hz), 2.59 (t, 4H, 7.0 Hz), 1.66 (m, 4H), 1.29 (m, 16H), 1.49 (m, 4H), 0.87 (t, 6H, 6.7 Hz); ^{13}C NMR (CDCl_3 , 75 MHz) δ 181.3, 134, 133.9, 131.8, 126.8, 111.6, 76.1, 31.8, 29.2, 29.1, 28.9, 28.4, 22.6, 20.6, 14.1. LC-MS calcd. for $[\text{C}_{30}\text{H}_{39}\text{O}_2]^+$, 431.2951; found: 431.2909.

Acetylation of diyne derivatives²³

Diol (**2c**, **2d** or **2e**) (0.125 mmol) was mixed with montmorillonite K-10 (200% by weight of substrate) and 1 mL of acetic anhydride. The reaction was performed under ultrasound irradiation at room temperature for 60 min. After this time, 15 mL of dichloromethane were added and montmorillonite K-10 was removed by simple filtration. The filtrate was neutralized with a concentrated solution of NaHCO_3 (3 \times 20 mL) in a separatory funnel and the organic phase dried with anhydrous sodium sulfate. The product was purified by column chromatography on silica gel using a mixture of ethyl acetate/hexane.

Compound **2c'**

Compound was obtained as a pale yellow solid (mp 123-124 °C) in 56% yield; IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 2992, 2937, 2211, 1739, 1673, 1587, 1547, 1351, 1254, 1131, 1016, 956, 790, 711, 608; ^1H NMR (CDCl_3 , 300 MHz) δ 8.06 (dd, 2H, 5.7/3.3 Hz), 7.73 (dd, 2H, 5.7/3.3 Hz), 2.06 (s, 6H), 1.80 (s, 12H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 180.4, 169.0, 134.1, 133.7, 131.7, 126.8, 109.6, 78.5, 71.9, 28.6, 21.7. LC-MS calcd. for $[\text{C}_{24}\text{H}_{22}\text{O}_6\text{Na}]^+$, 429.1314; found: 429.1325.

Compound **2d'**

Compound was obtained as a pale yellow solid (mp 120-121 °C) in 65% yield; IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 2954, 2932, 2860, 2205, 1741, 1671, 1550, 1444, 1345, 1276, 1223, 1031, 994, 966, 712, 607; ^1H NMR (CDCl_3 , 300 MHz) δ 8.07 (dd, 2H, 5.7/3.3 Hz), 7.72 (dd, 2H, 5.7/3.3 Hz), 2.29 (m, 4H), 2.08 (s, 6H), 1.94 (m, 4H), 1.71 (m, 12H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 180.5, 168.8, 133.9, 133.3, 131.8, 126.8, 109.3, 80.9, 75.5, 36.7, 25.2, 22.6, 21.7. LC-MS calcd. for $[\text{C}_{30}\text{H}_{30}\text{O}_6\text{Na}]^+$, 509.1941; found: 509.1947.

Compound **2e'**

Compound was obtained as a pale yellow solid (mp 91-92 °C) in 71% yield; IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 2215, 1735, 1667, 1548, 1357, 1296, 1246, 1036, 713; ^1H NMR (CDCl_3 , 300 MHz) δ 8.09 (dd, 2H, 6.0/3.0 Hz), 7.74 (dd, 2H, 6.0/3.0 Hz), 4.27 (t, 4H, 6.3 Hz), 2.73 (t, 4H, 7.0 Hz), 2.07 (s, 6H), 2.01 (m, 4H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 181.1, 171.0, 134.3, 134.0, 131.8, 126.9, 109.6, 62.9, 27.4, 20.9, 17.3. LC-MS calcd. for $[\text{C}_{24}\text{H}_{22}\text{O}_6\text{Na}]^+$, 429.1314; found: 429.1317.

Biological

Cytotoxicity against cancer cell lines

The cytotoxic effects of the synthesized compounds were evaluated against the following human cancer cell lines and non-tumor cells (PBMC), all transformed cell lines were obtained from the National Cancer Institute, Bethesda, MD, USA: PC-3M (prostate carcinoma), OVCAR-8 (ovarian carcinoma) and NCI-H358M (bronchoalveolar lung carcinoma). The cell lines were maintained in RPMI-1640 medium (cancer cells) supplemented with 10% fetal bovine serum, 2 mmol L^{-1} glutamine, 100 $\mu\text{g mL}^{-1}$ penicillin and 100 $\mu\text{g mL}^{-1}$ streptomycin at 37 °C with 5% CO_2 .

Cell growth was quantified by the ability of living cells to reduce a yellow dye (MTT) to a purple formazan product.²⁸ For all of the experiments, the cells were seeded in 96-well plates (0.1 \times 10⁵ cells *per* well for adherent cells). After 24 h, the compounds (0.01 to 25 mg mL^{-1}), dissolved in DMSO, were added to each well (using an HTS (high-throughput screening) Biomek 3000, Beckman Coulter, Inc. Fullerton, California, USA) and incubated for 72 h. Doxorubicin (Sigma-Aldrich Co., St. Louis, MO, USA) was used as a positive control. At the end of the incubation, the plates were centrifuged, and the medium was replaced by fresh medium (200 μL) containing 0.5 mg mL^{-1} MTT. After 3 h, the formazan product was dissolved in 150 μL DMSO, and the absorbance was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter, Inc., Fullerton, California, USA). The substance effect was quantified as the percentage of the control absorbance at 595 nm.

Supplementary Information

Supplementary information (spectral data and figures containing IR, HRMS, ^1H and ^{13}C NMR) are available free of charge at <http://jbc.sbq.org.br> as a PDF file.

Acknowledgments

We are grateful to CNPq, CAPES, FUNCAP and FACEPE (PRONEM 1232.1.06/10) for financial support. Our thanks are also due to Analytical Centers DQF-UFPE and CENAPESQ-UFRPE. Special thanks to Prof. Patricia L. B. Araujo (DCM-UFRPE) for kind suggestions.

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Supplementary Information

Synthesis of 2,3-Diyne-1,4-naphthoquinone Derivatives and Evaluation of Cytotoxic Activity against Tumor Cell Lines

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Synthesis of 2,3-dibromo-1,4-naphthoquinone (**1**)

Bromine (16.0 g, 0.1 mol) was added dropwise over 5 h to a stirred solution of 1,4-naphthoquinone (7.91 g, 0.05 mol) in CH₂Cl₂ (80 mL), and during this time, hydrogen bromide was evolved. After, the solution was stirred overnight, N₂ was blown through the mixture, and the dark yellow precipitate was filtered off and washed with CH₂Cl₂. Recrystallization from CH₂Cl₂ afforded 10.27 g (65%) of the dibromo derivative, mp 220-222 °C (219-221, recrystallized from EtOAc).¹

2,3-di(Phenylethynyl)-1,4-naphthoquinone (**2a**)

Using dichloromethane/hexane 30:70 as eluent, **2a** was obtained as a red solid (mp 151-152)² in 45% (51 mg, 0.143 mmol) yield; IR (KBr) ν_{\max} /cm⁻¹ 2198, 1667, 1591, 1534, 1490, 1370, 1328, 1309, 1258, 1159, 936, 748, 712, 682, 528; ¹H NMR (CDCl₃, 300 MHz) δ 8.16 (dd, 2H, 5.7/3.3 Hz), 7.77 (dd, 2H, 5.7 and 3.3 Hz), 7.68 (m, 4H), 7.42 (m, 6H); ¹³C NMR (CDCl₃, 75 MHz) δ 181.1, 134.5,

134.2, 132.8, 132.4, 130.5, 129.0, 127.4, 122.6, 109.8, 85.4. LC-MS calcd. for [C₂₆H₁₅O₂]⁺, 359.1072; found: 359.1111.

2,3-di(3-Hydroxy-3-methylbutynyl)-1,4-naphthoquinone (**2c**)

Using ethyl acetate/hexane 30:70 as eluent, **2c** was obtained as a dark yellow solid (mp 138-139 °C)¹⁷ in 42% (43 mg, 0.134 mmol) yield; IR (KBr) ν_{\max} /cm⁻¹ 3322, 2979, 2931, 2202, 1671, 1591, 1540, 1459, 1349, 1300, 1234, 1164, 1133, 984, 951, 790, 713; ¹H NMR (CDCl₃, 400 MHz) δ 8.06 (t, 2H, 3.2 Hz), 7.73 (d, 2H, 3.2 Hz), 1.67(s, 12H), 3.74 (s, 2H); ¹³C NMR (CDCl₃, 100 MHz) δ 180.6, 134.4, 134.2, 131.6, 126.9, 113.9, 76.9, 65.9, 30.9. LC-MS calcd. for [C₂₀H₁₈O₄]⁺, 322.1205; found: 322.1271.

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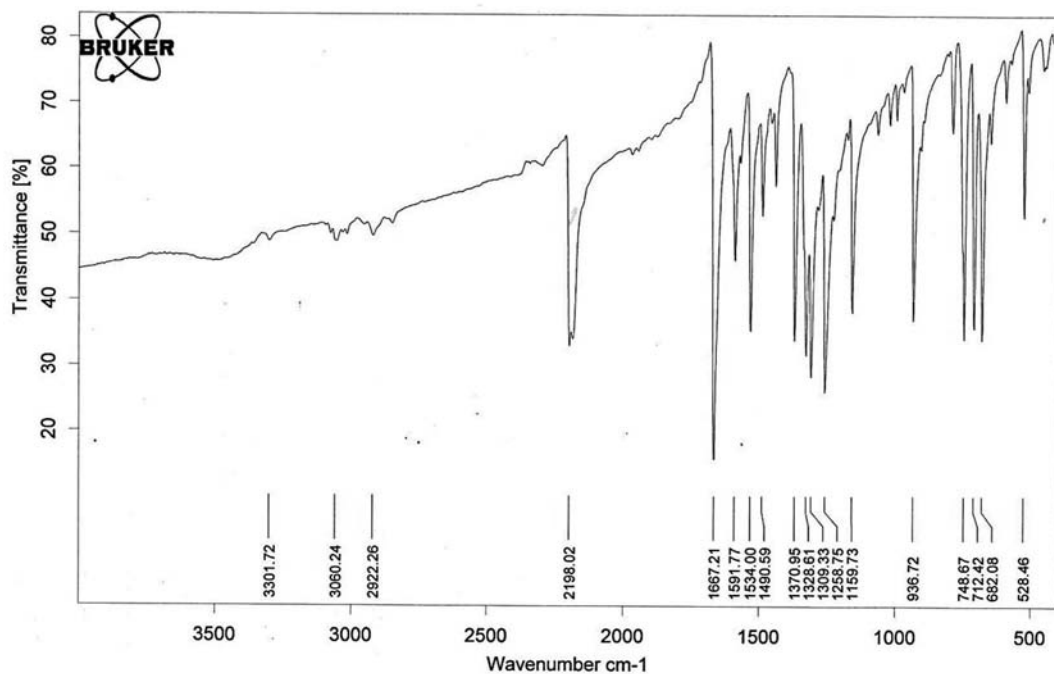


Figure S1. Infrared spectrum of **2a** (KBr).

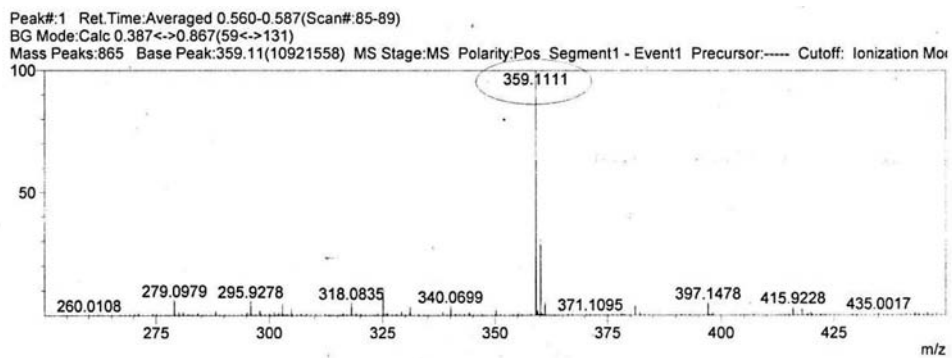


Figure S2. High resolution mass spectra (ESI-MS) of **2a**.

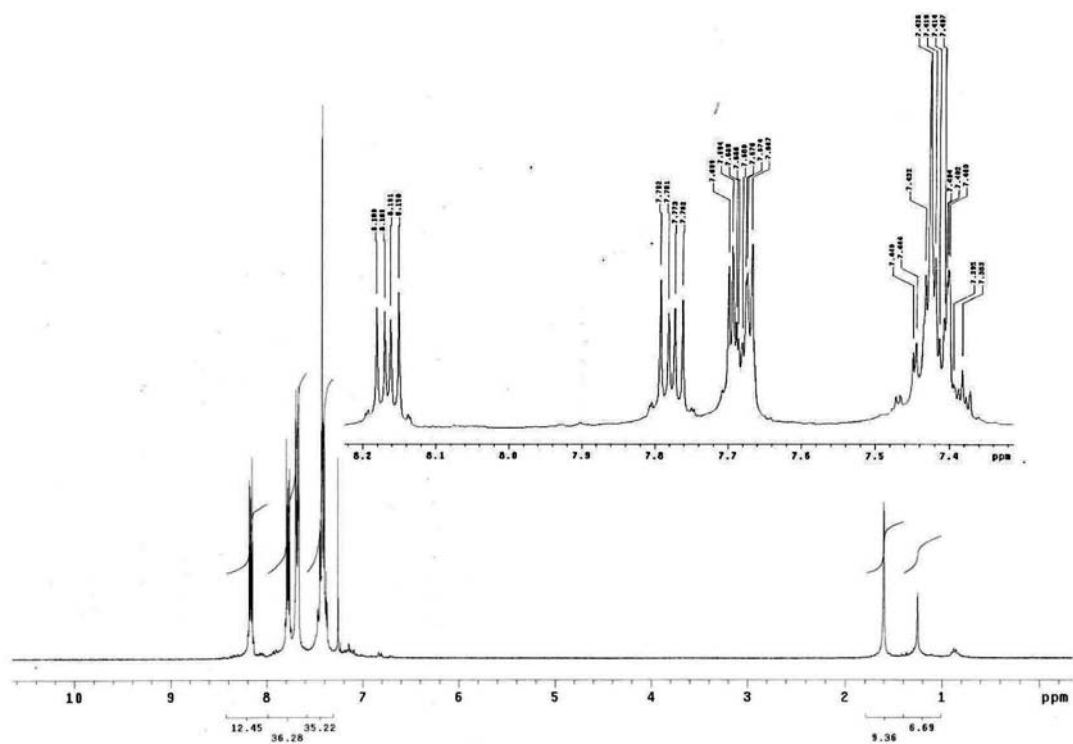


Figure S3. ¹H NMR spectrum (300 MHz, CDCl₃) of 2a.

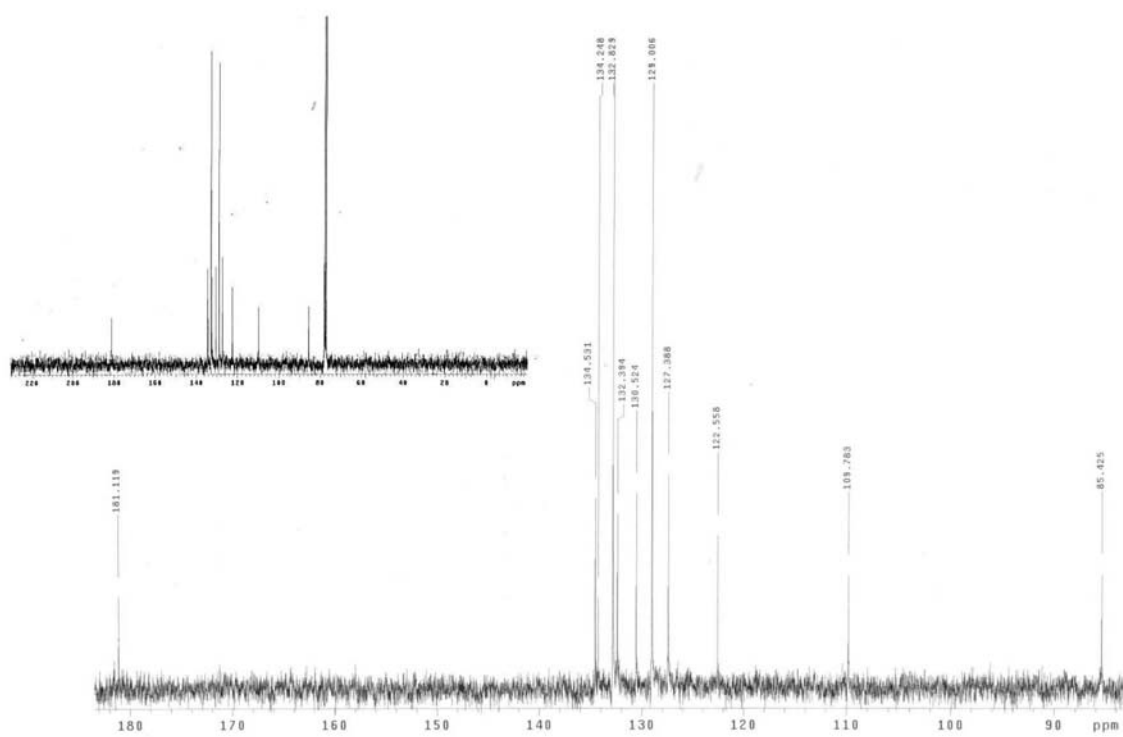


Figure S4. ¹³C NMR spectrum (75 MHz, CDCl₃) of 2a.

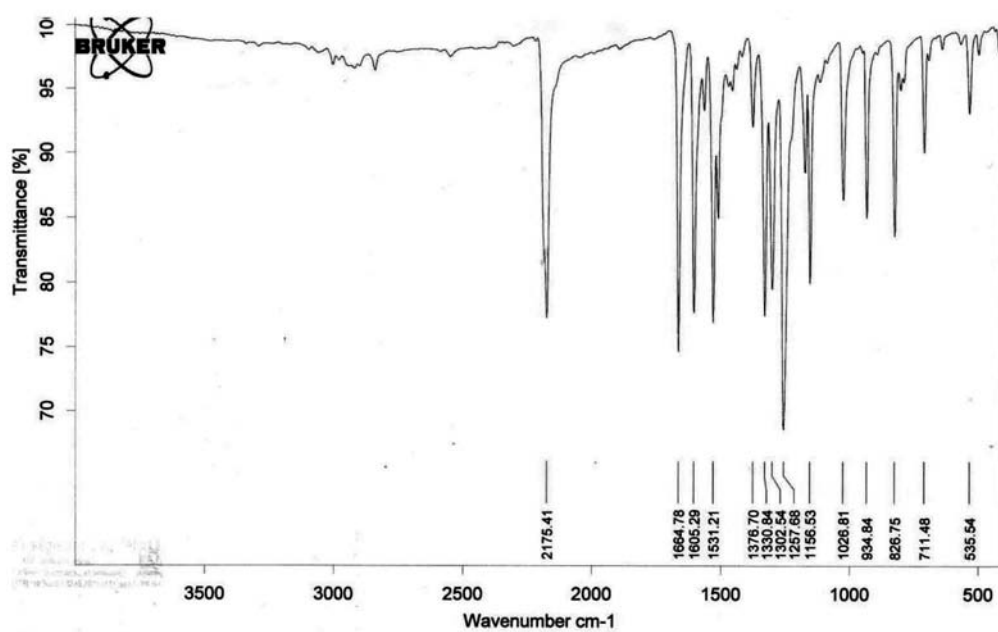


Figure S5. Infrared spectrum of **2b** (KBr).

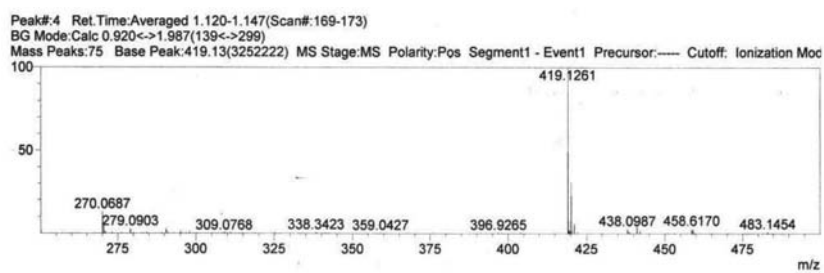


Figure S6. High resolution mass spectra (ESI-MS) of **2b**.

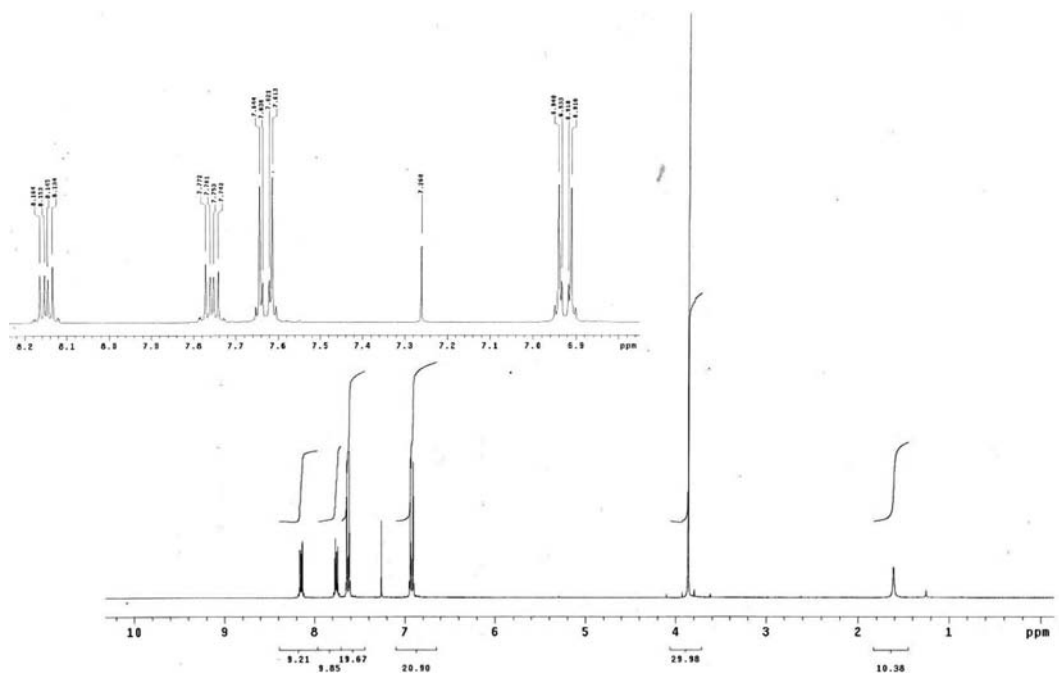


Figure S7. ¹H NMR spectrum (300 MHz, CDCl₃) of **2b**.

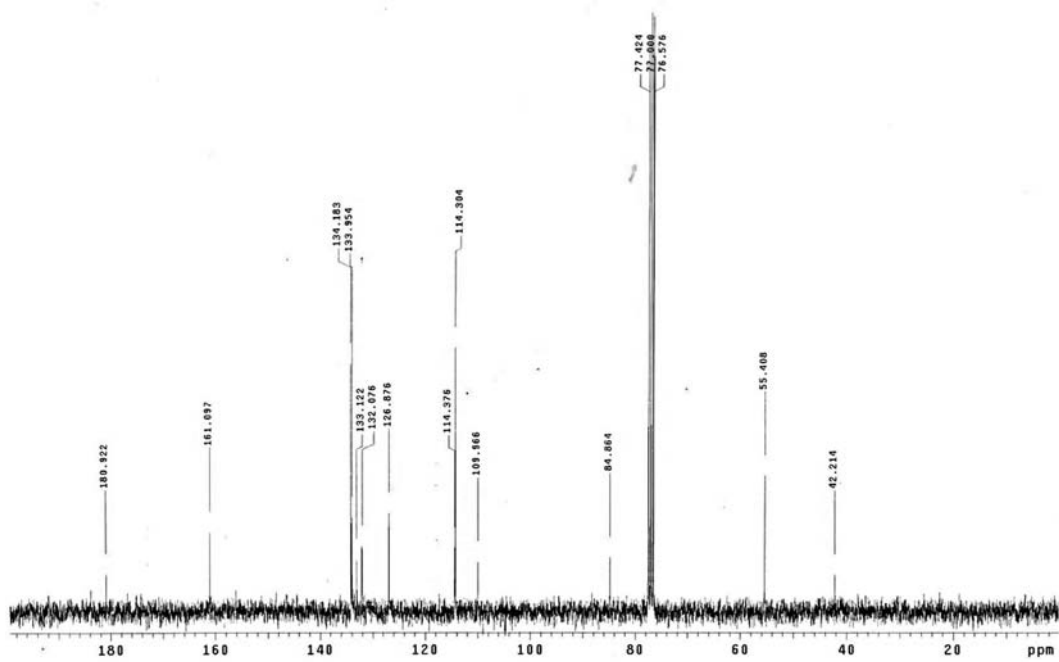


Figure S8. ¹³C NMR spectrum (75 MHz, CDCl₃) of **2b**.

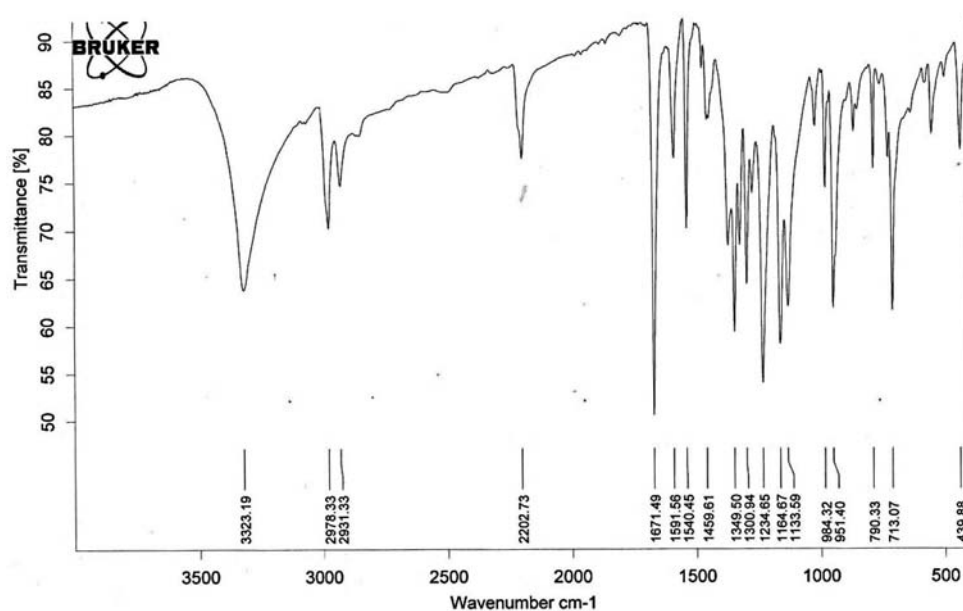


Figure S9. Infrared spectrum of 2c (KBr).

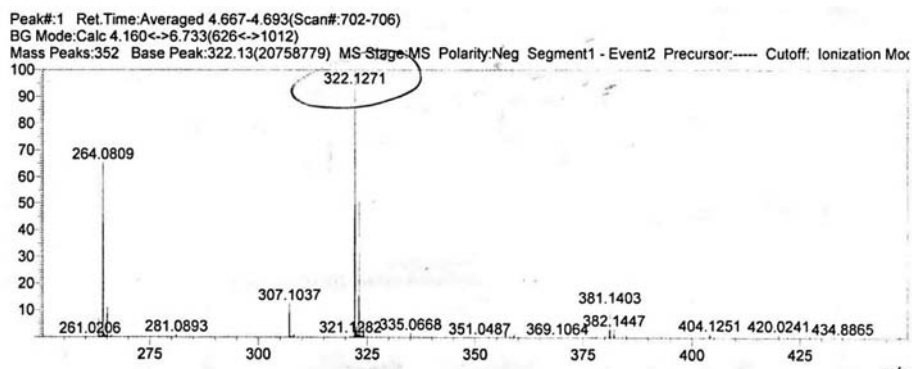


Figure S10. High resolution mass spectra (ESI-MS) of 2c.

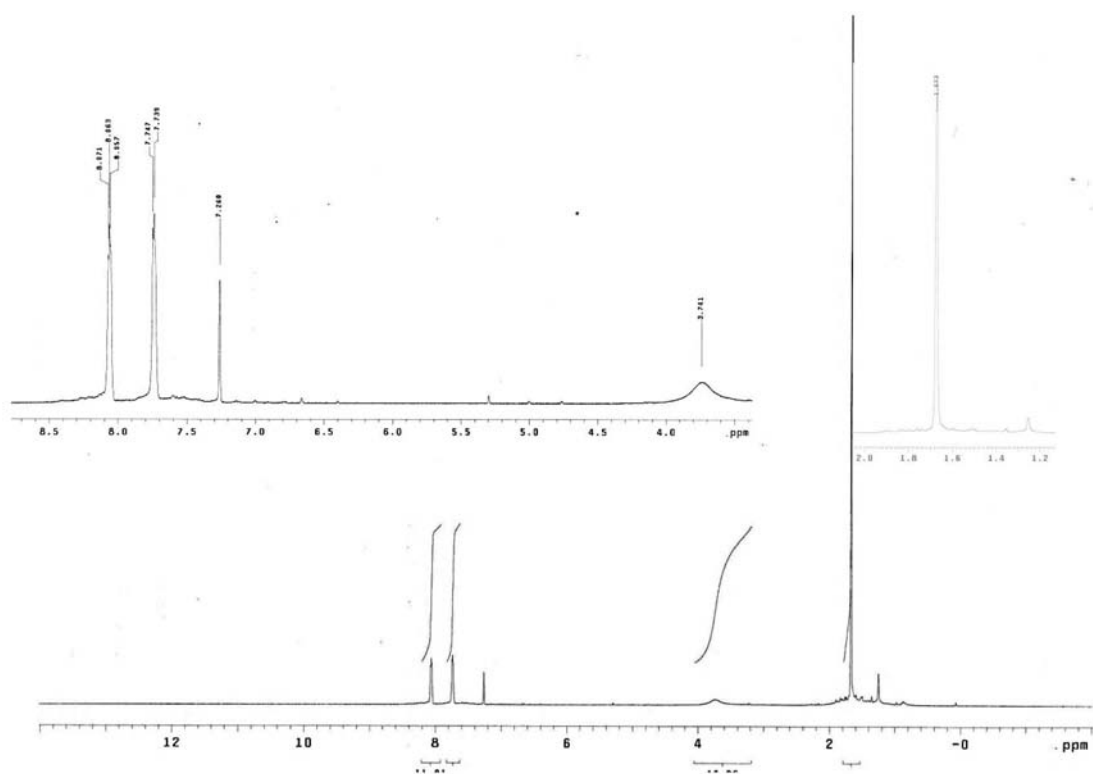


Figure S11. ¹H NMR spectrum (400 MHz, CDCl₃) of 2c.

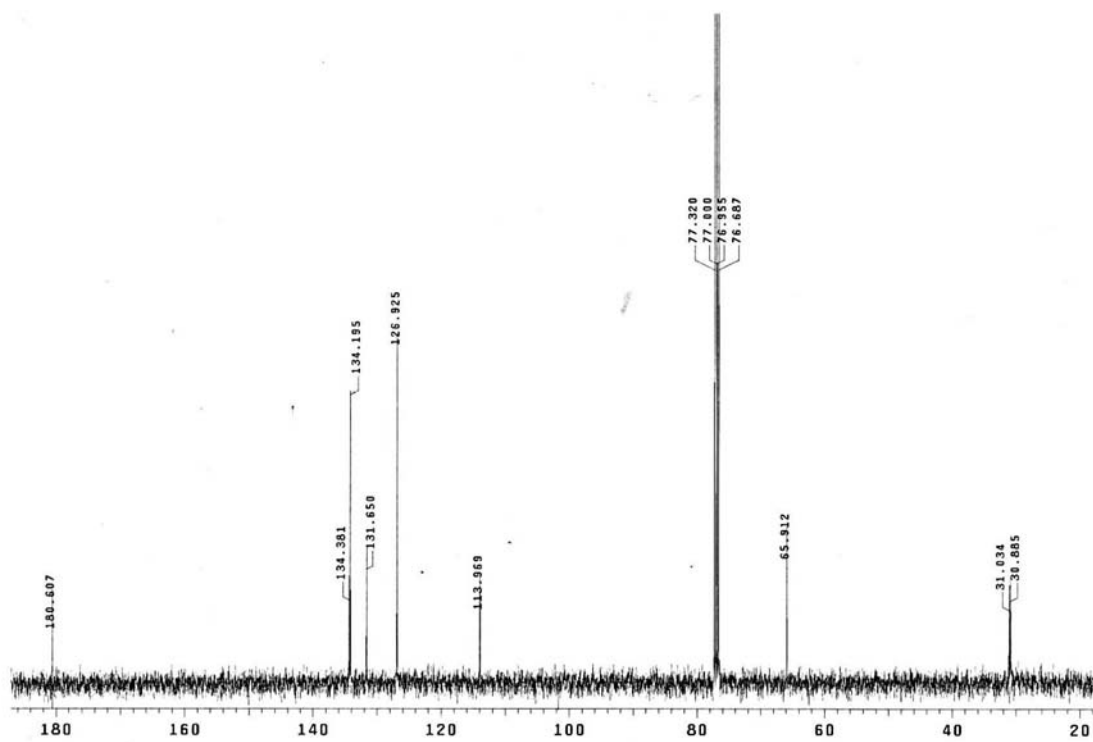


Figure S12. ¹³C NMR spectrum (100 MHz, CDCl₃) of 2c.

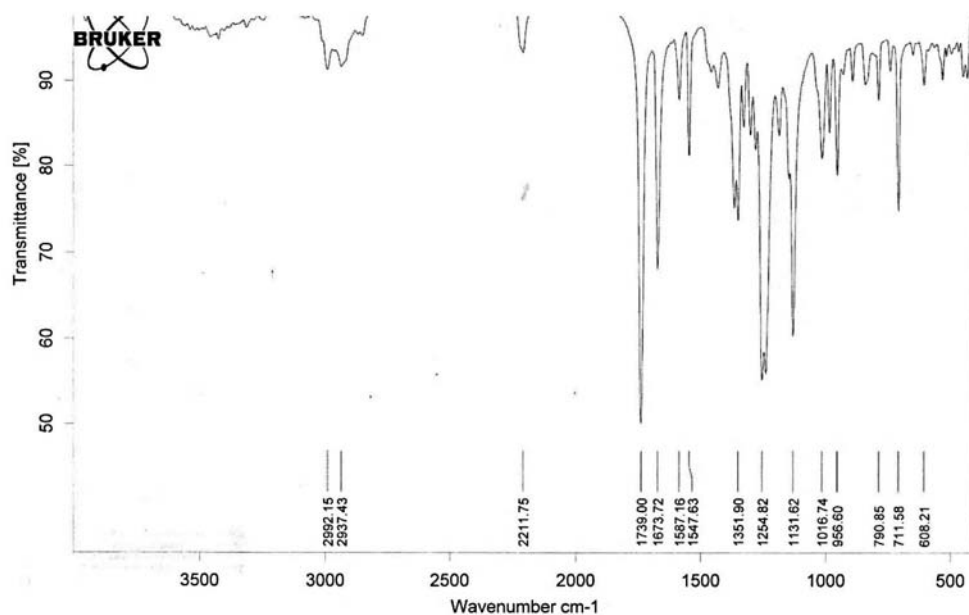


Figure S13. Infrared spectrum of 2c' (KBr).

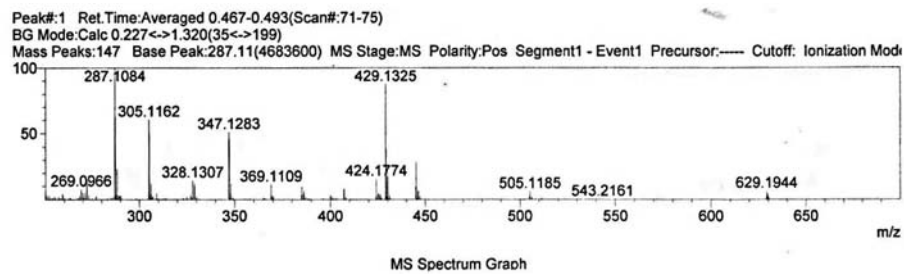


Figure S14. High resolution mass spectra (ESI-MS) of 2c'.

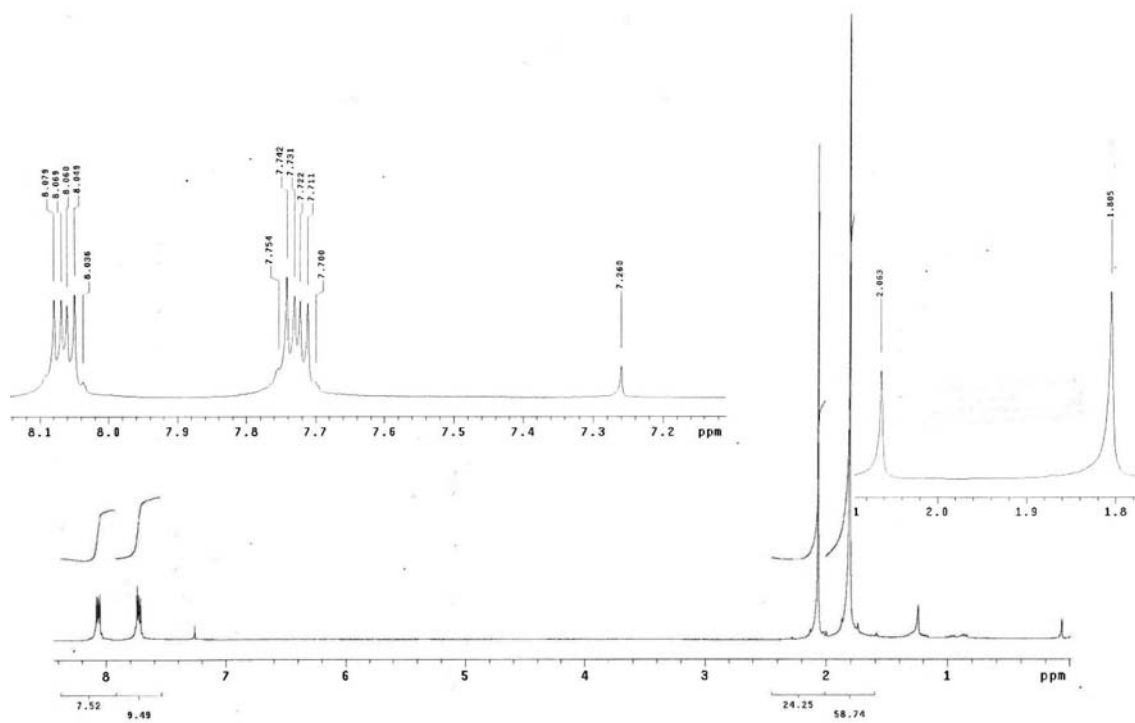


Figure S15. ¹H NMR spectrum (300 MHz, CDCl₃) of 2c'.

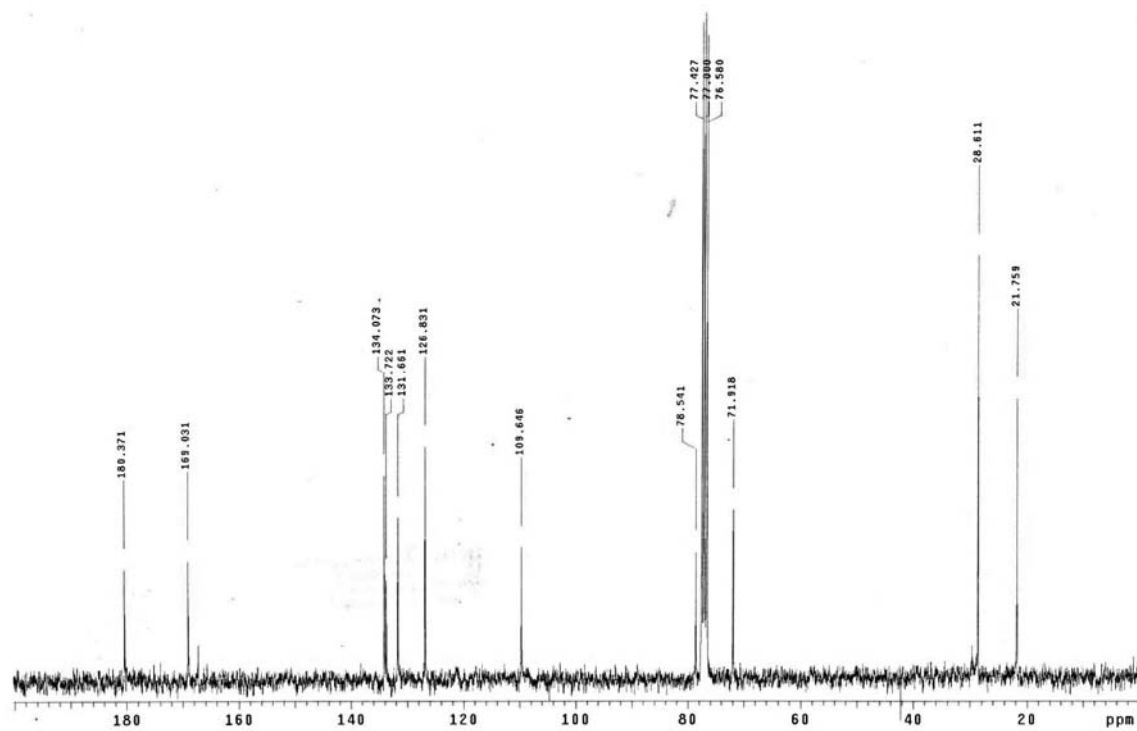
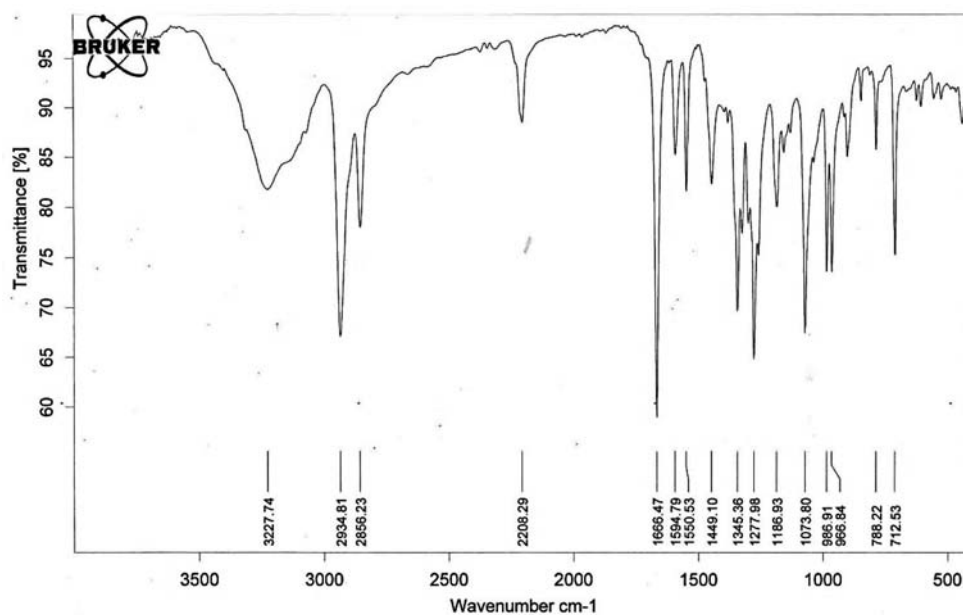
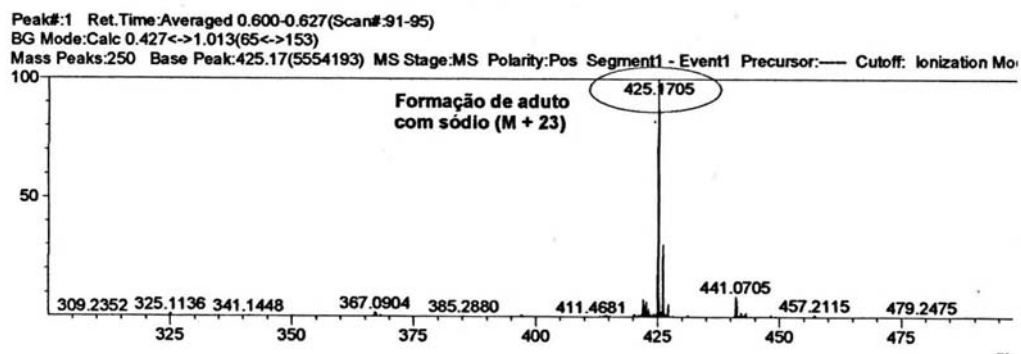


Figure S16. ¹³C NMR spectrum (75 MHz, CDCl₃) of 2c'.

Figure S17. Infrared spectrum of **2d** (KBr).Figure S18. High resolution mass spectra (ESI-MS) of **2d**.

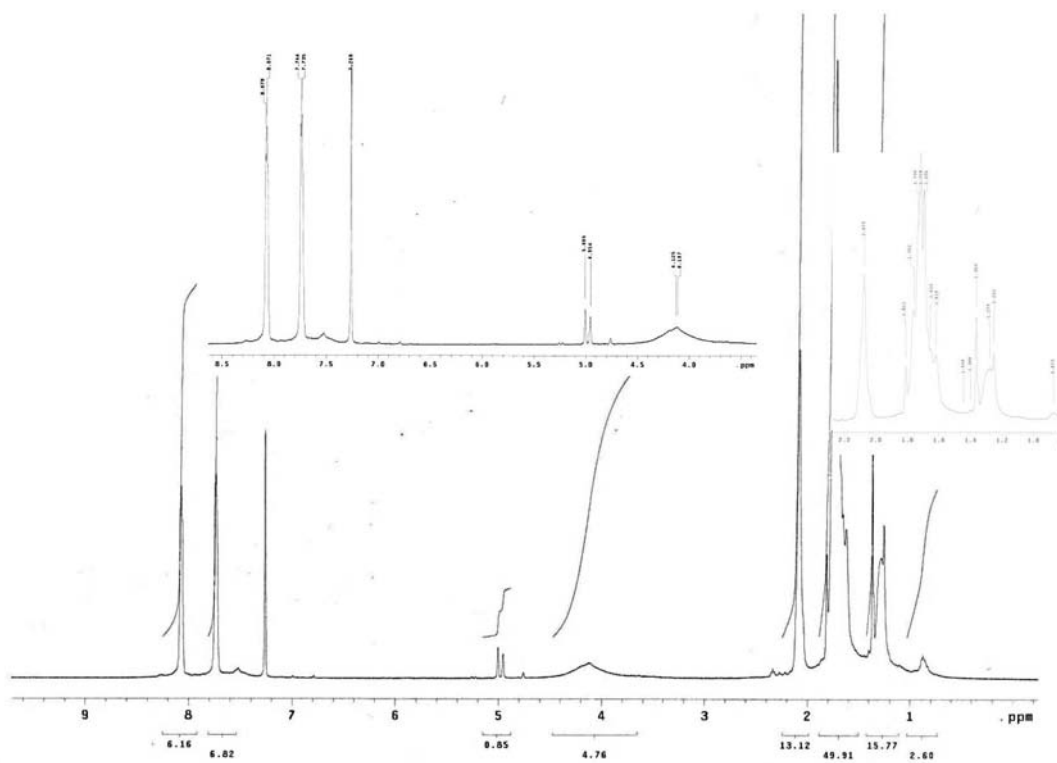


Figure S19. ^1H NMR spectrum (400 MHz, CDCl_3) of **2d**.

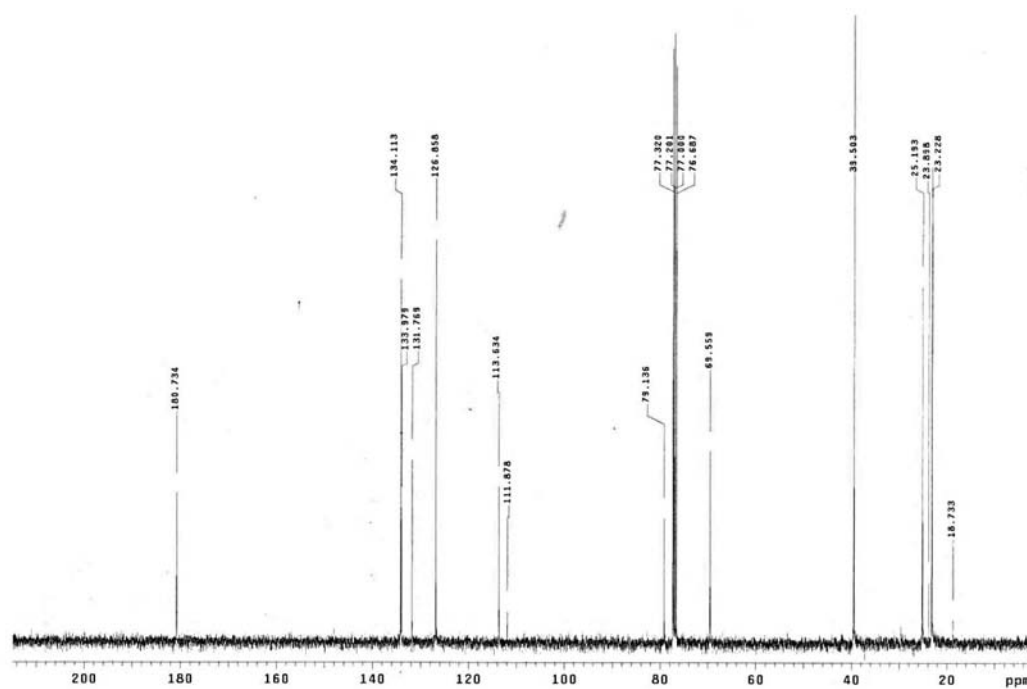


Figure S20. ^{13}C NMR spectrum (100 MHz, CDCl_3) of **2d**.

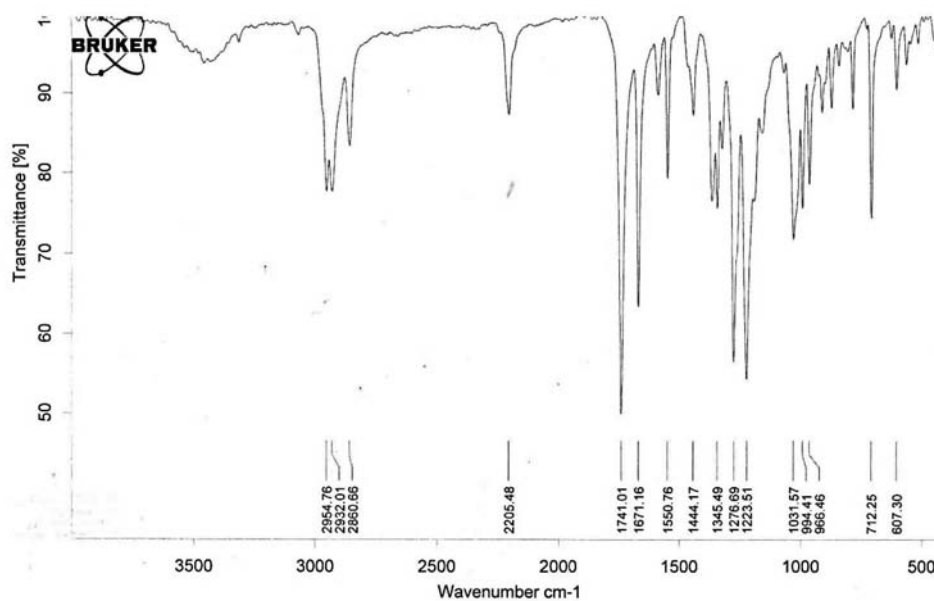


Figure S21. Infrared spectrum of **2d'** (KBr).

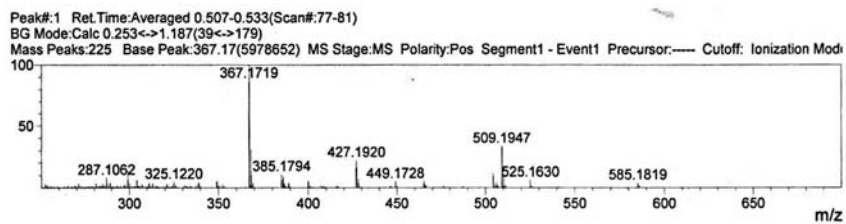


Figure S22. High resolution mass spectra (ESI-MS) of **2d'**.

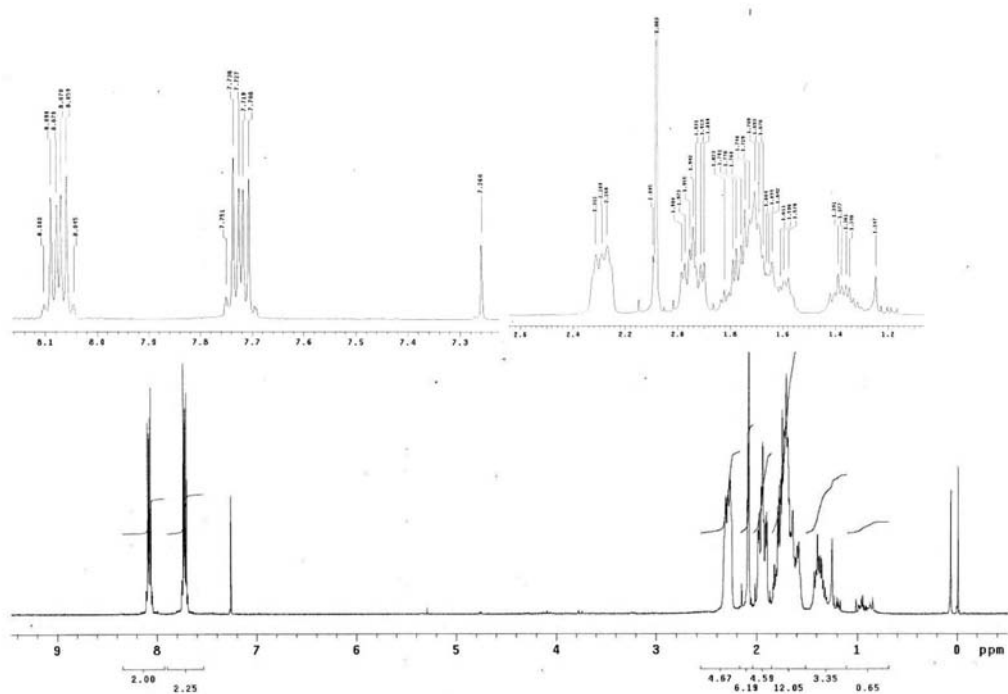


Figure S23. ¹H NMR spectrum (300 MHz, CDCl₃) of 2d'.

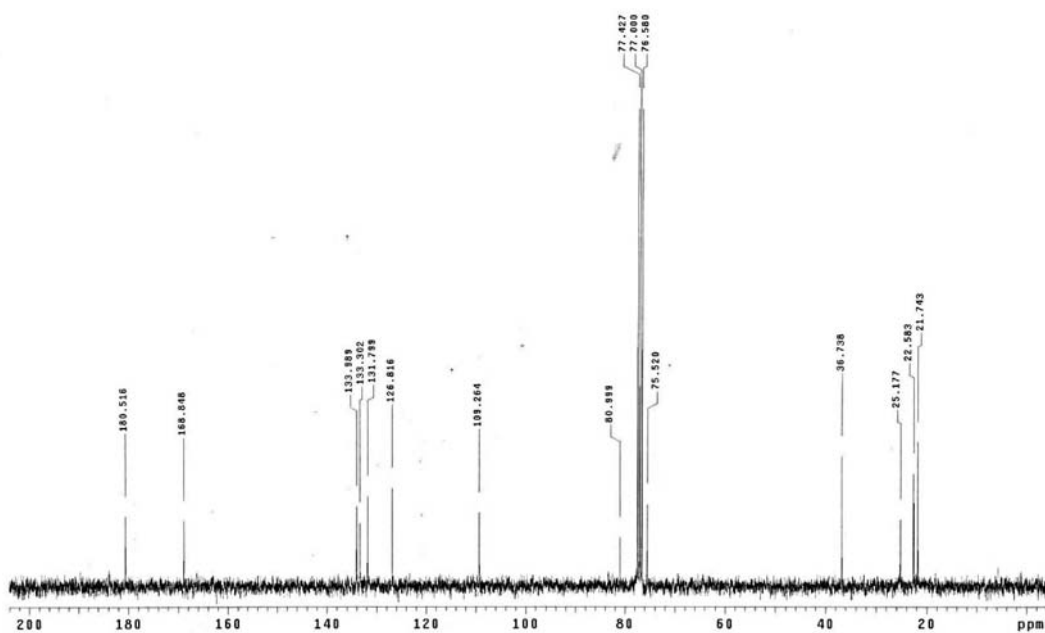


Figure S24. ¹³C NMR spectrum (75 MHz, CDCl₃) of 2d'.

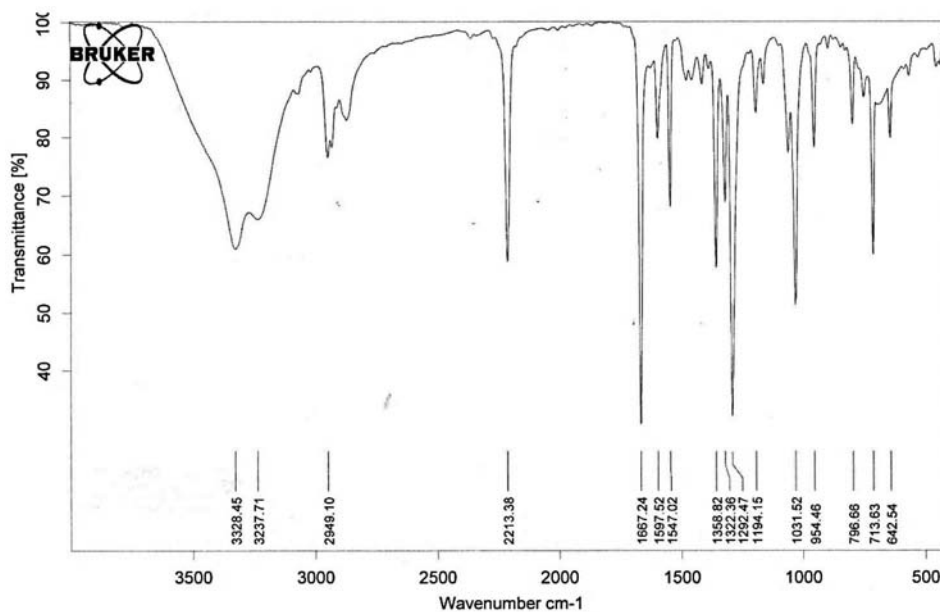


Figure S25. Infrared spectrum of **2e** (KBr).

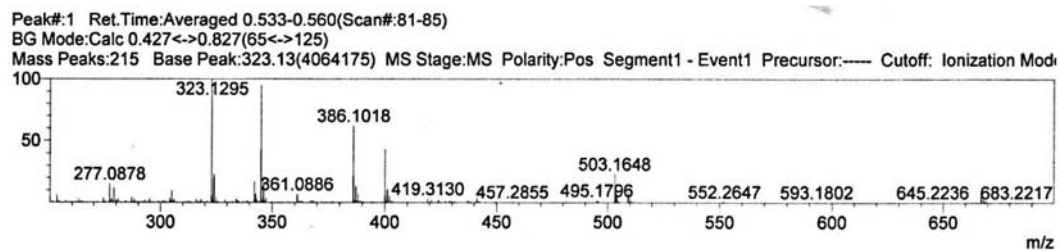
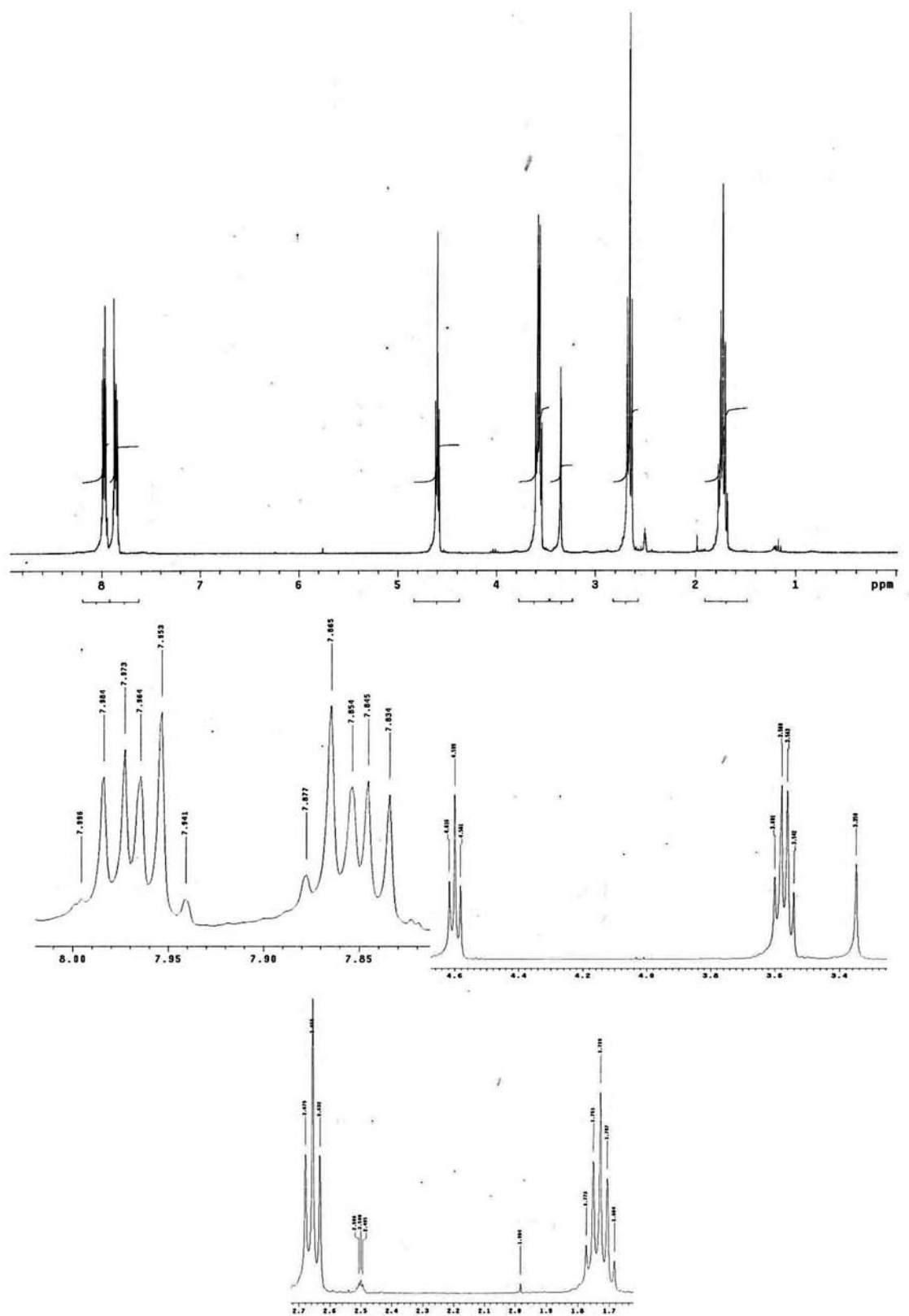


Figure S26. High resolution mass spectra (ESI-MS) of **2e**.

**Figure S27.** ¹H NMR spectrum (300 MHz, DMSO-d₆) of 2e.

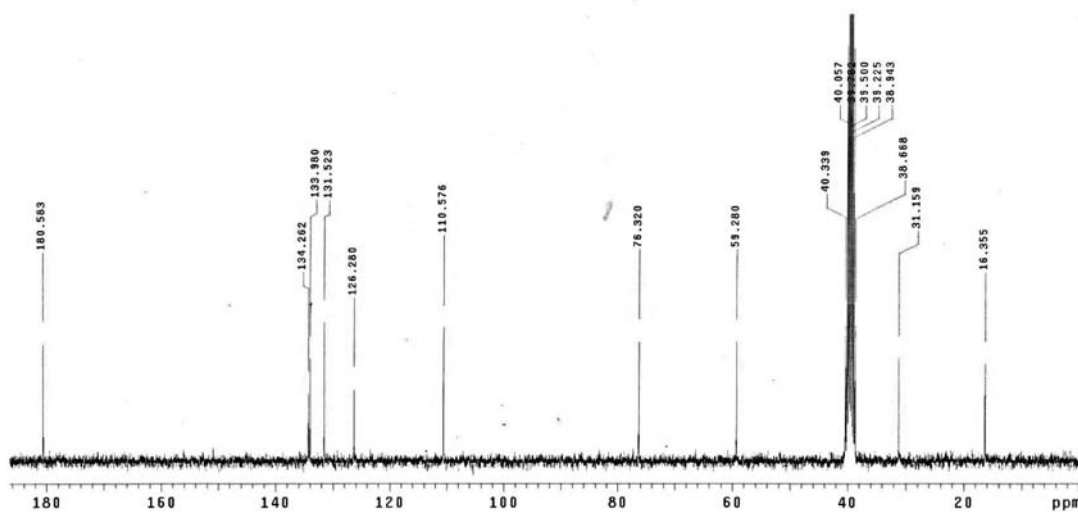


Figure S28. ^{13}C NMR spectrum (75 MHz, $\text{DMSO-}d_6$) of **2e**.

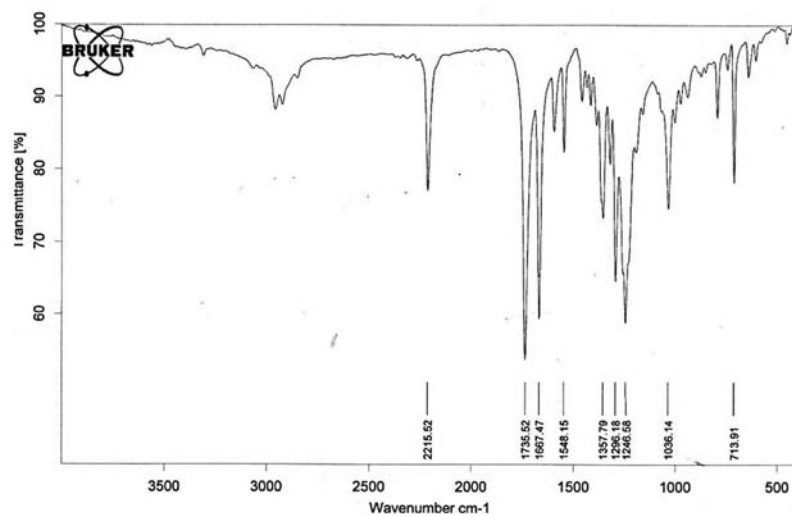


Figure S29. Infrared spectrum of **2e'** (KBr).

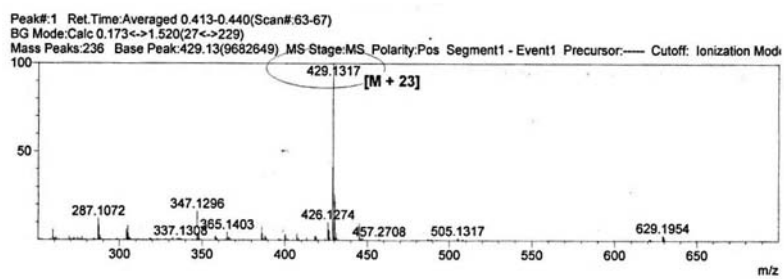


Figure S30. High resolution mass spectra (ESI-MS) of $2e'$.

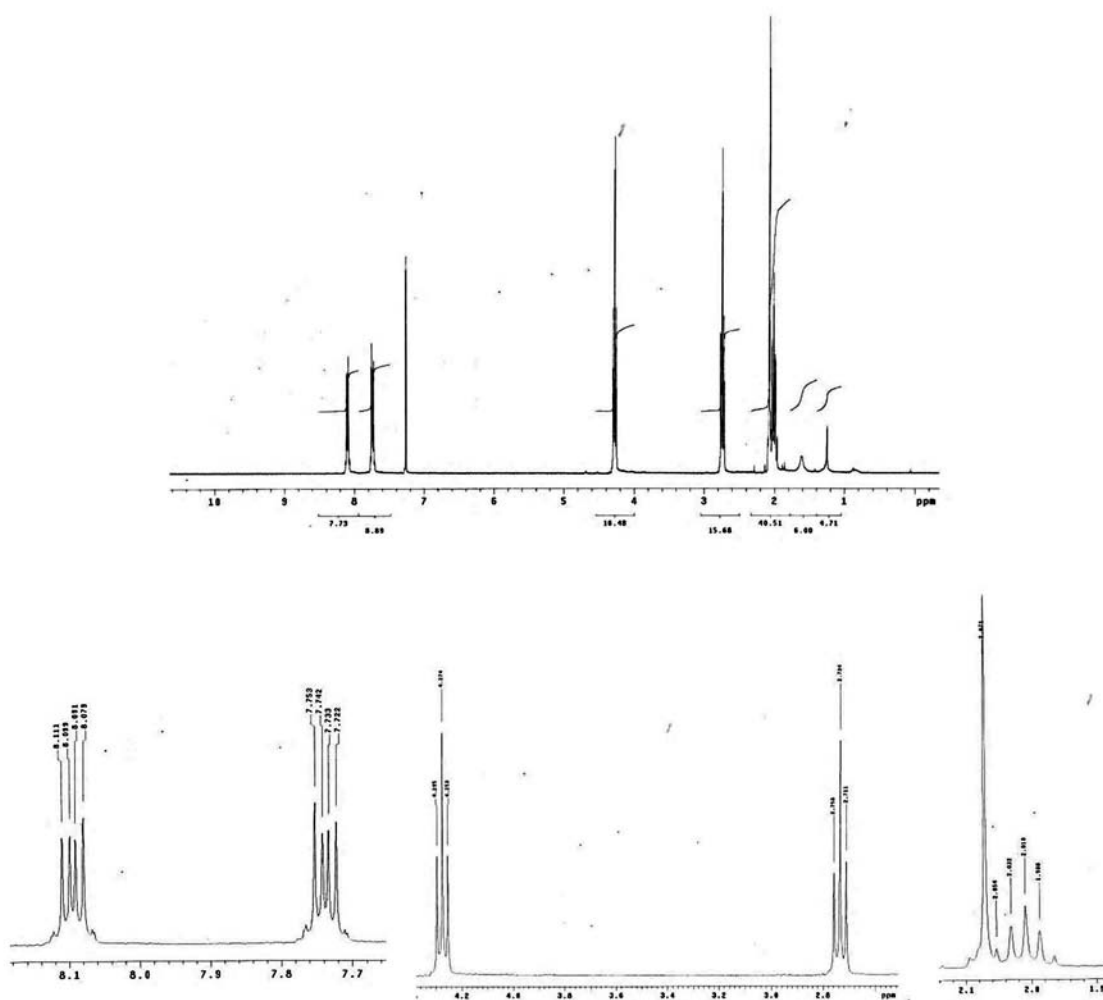


Figure S31. ^1H NMR spectrum (300 MHz, CDCl_3) of $2e'$.

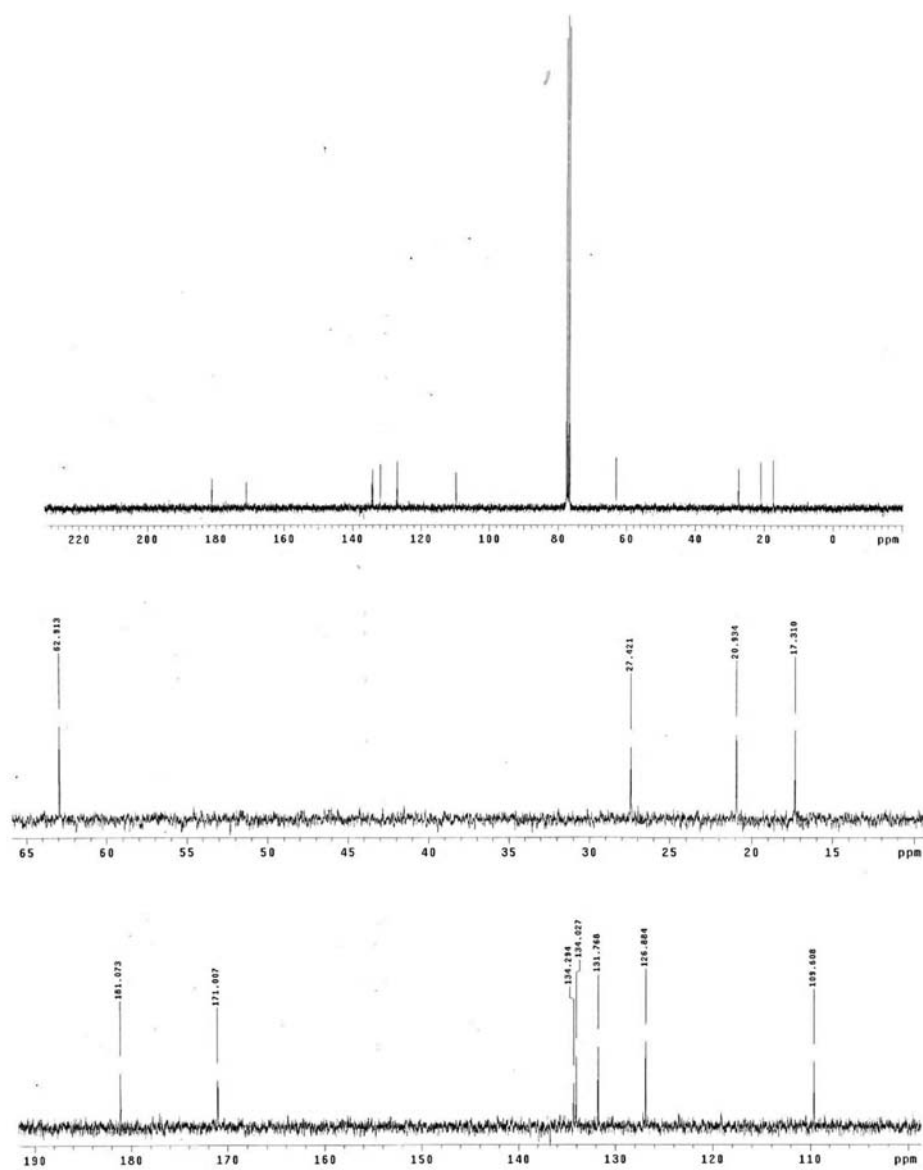


Figure S32. ^{13}C NMR spectrum (75 MHz, CDCl_3) of $2e$.

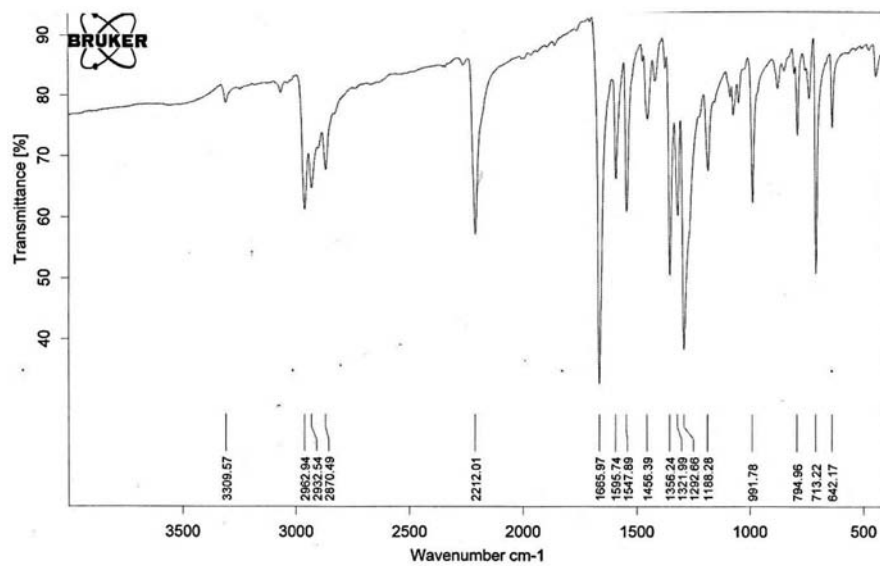


Figure S33. Infrared spectrum of **2f** (KBr).

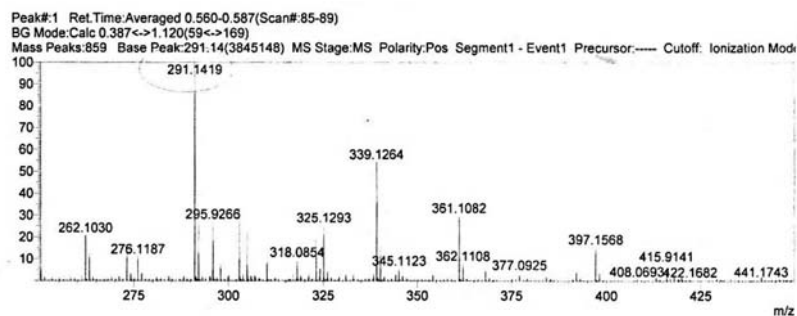


Figure S34. High resolution mass spectra (ESI-MS) of **2f**.

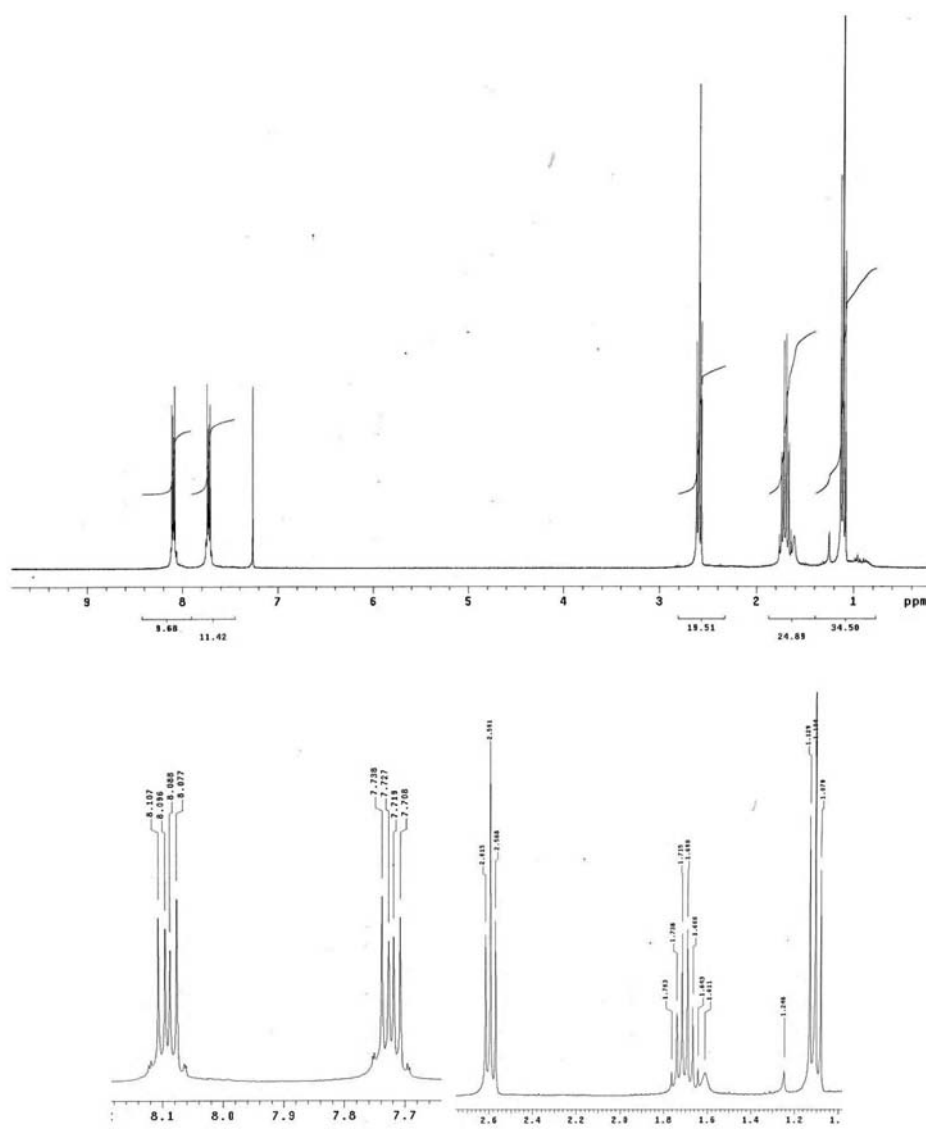


Figure S35. ¹H NMR spectrum (300 MHz, CDCl₃) of 2f.

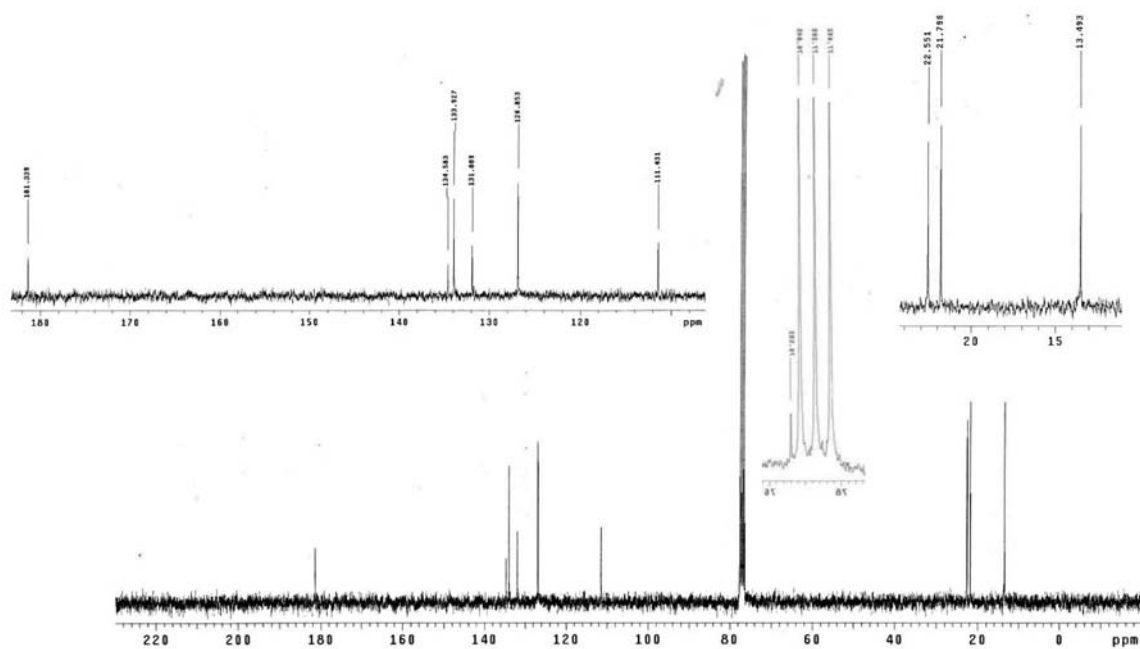


Figure S36. ¹³C NMR spectrum (75 MHz, CDCl₃) of 2f.

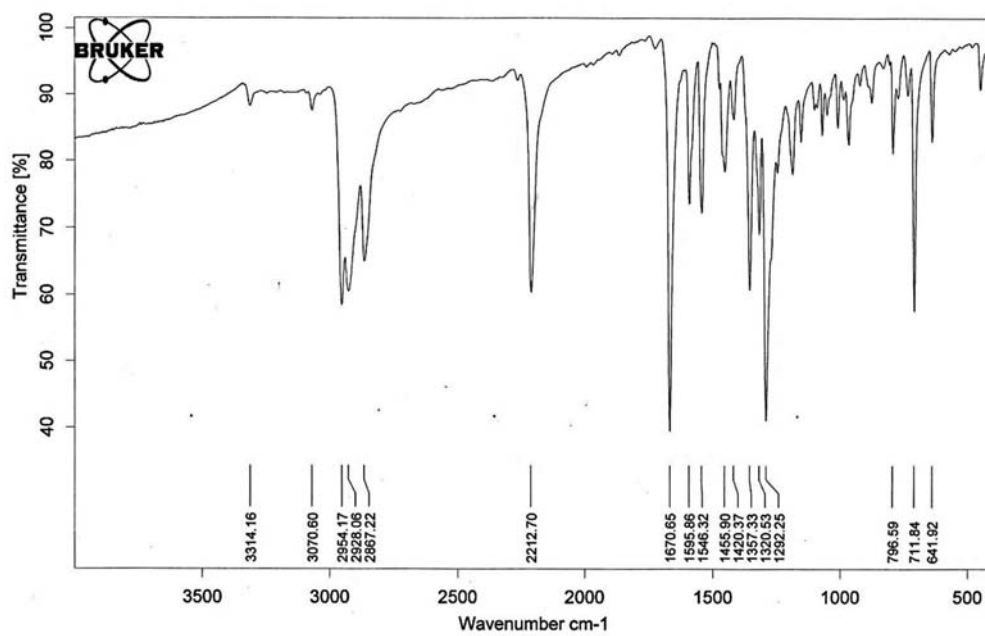


Figure S37. Infrared spectrum of 2g (KBr).

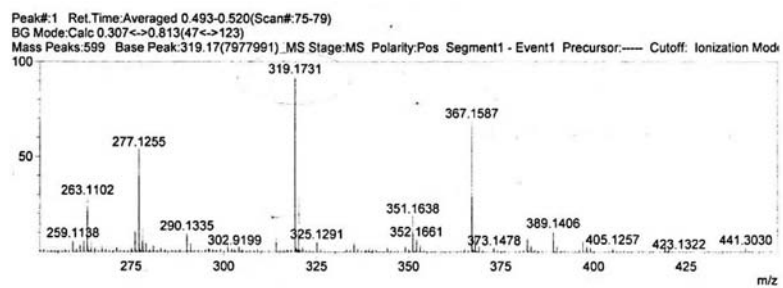


Figure S38. High resolution mass spectra (ESI-MS) of **2g**.

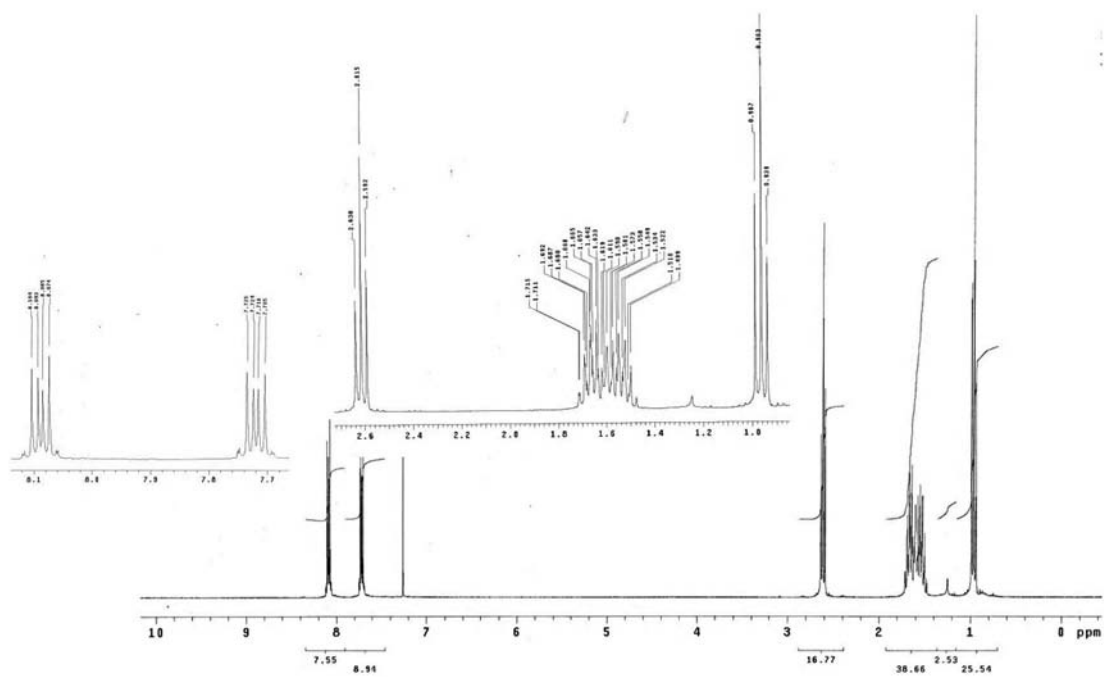


Figure S39. ^1H NMR spectrum (300 MHz, CDCl_3) of **2g**.

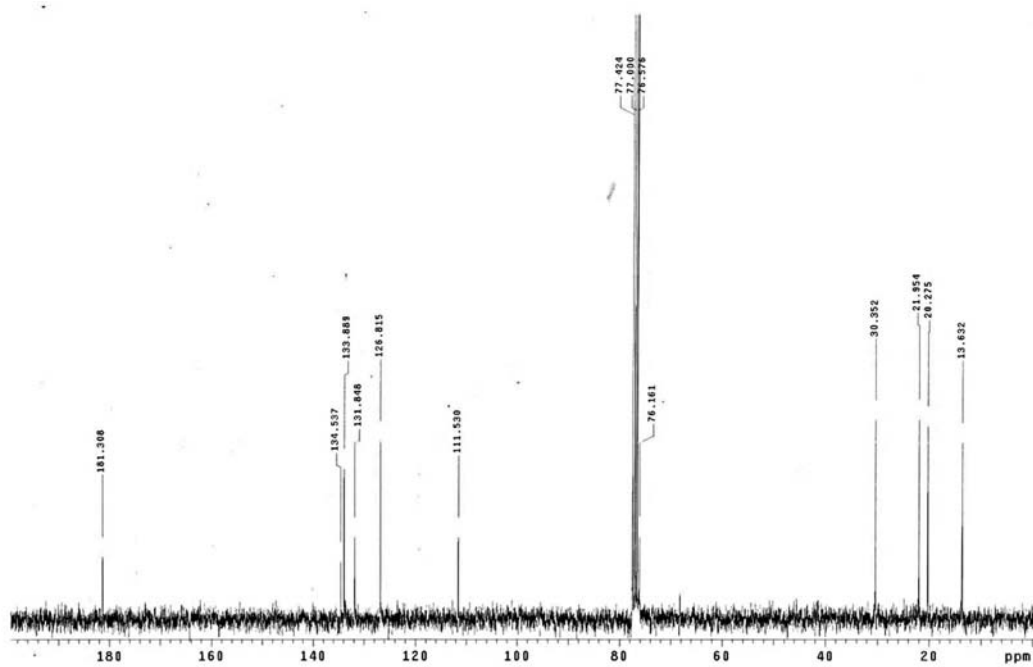


Figure S40. ¹³C NMR spectrum (75 MHz, CDCl₃) of **2g**.

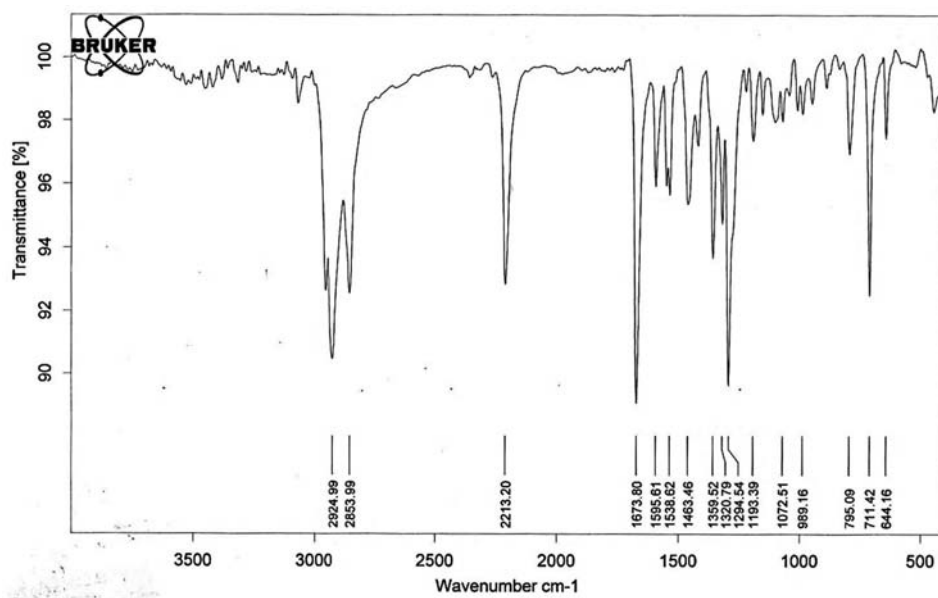


Figure S41. Infrared spectrum of **2h** (KBr).

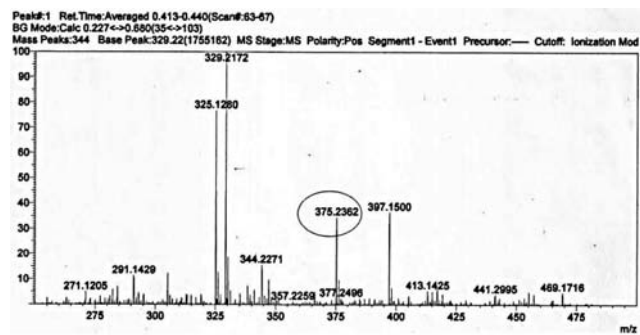


Figure S42. High resolution mass spectra (ESI-MS) of **2h**.

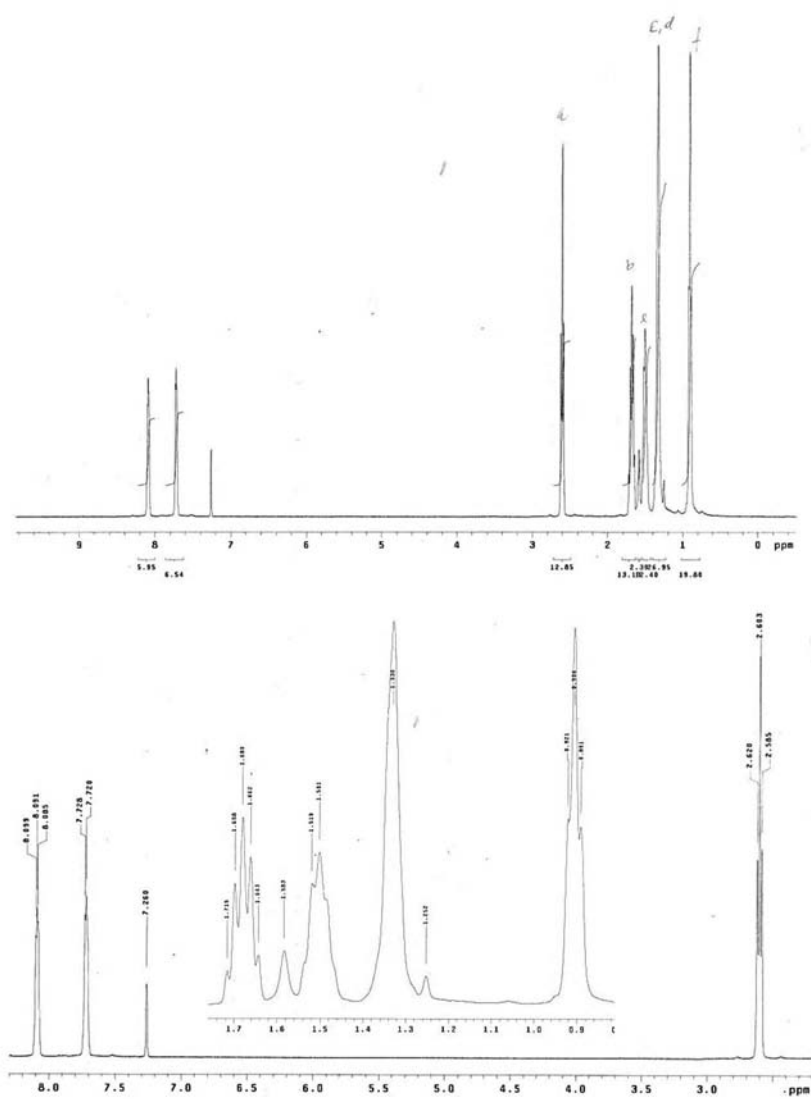


Figure S43. ^1H NMR spectrum (400 MHz, CDCl_3) of **2h**.

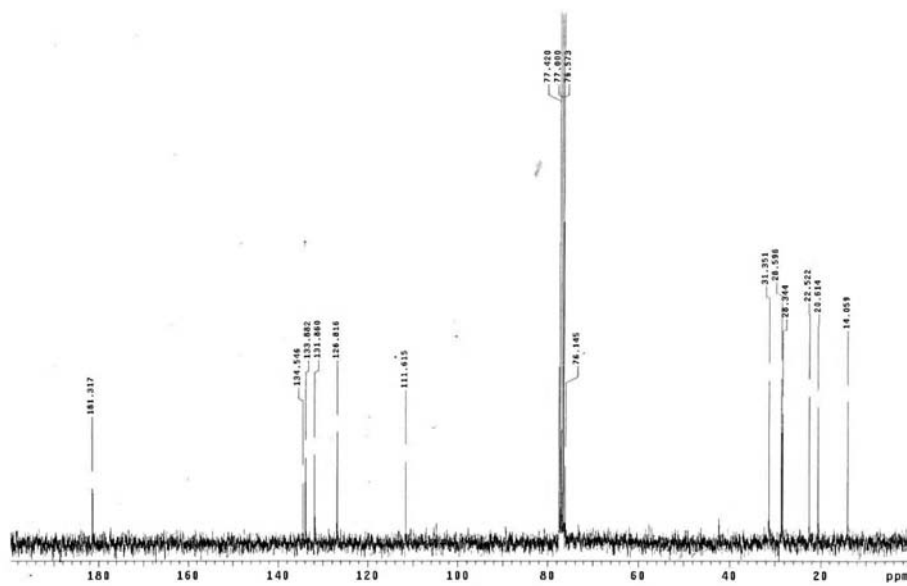


Figure S44. ^{13}C NMR spectrum (100 MHz, CDCl_3) of **2h**.

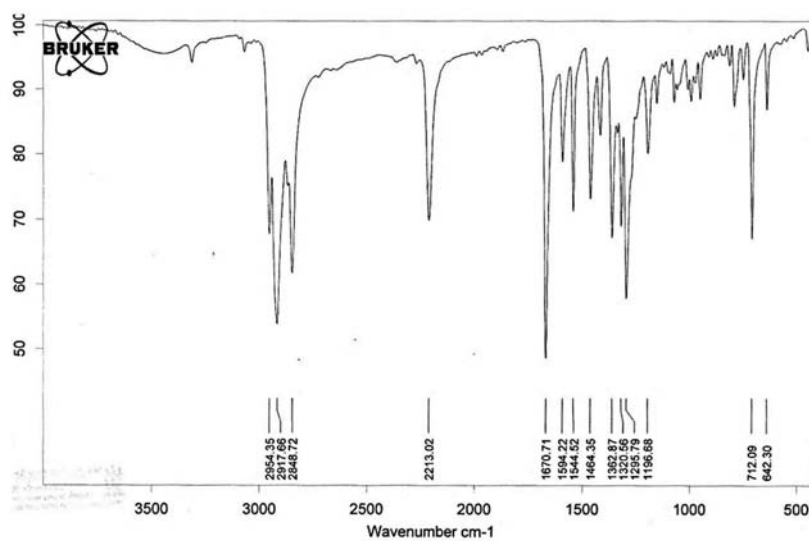


Figure S45. Infrared spectrum of **2i** (KBr).

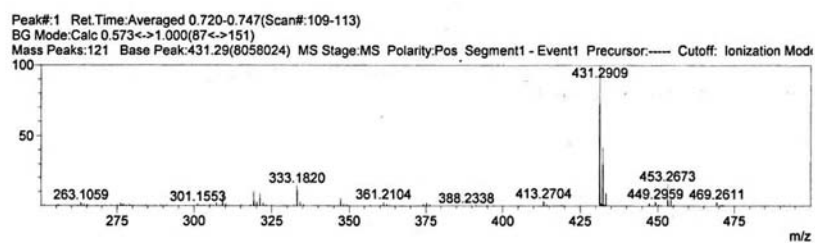


Figure S46. High resolution mass spectra (ESI-MS) of **2i**.

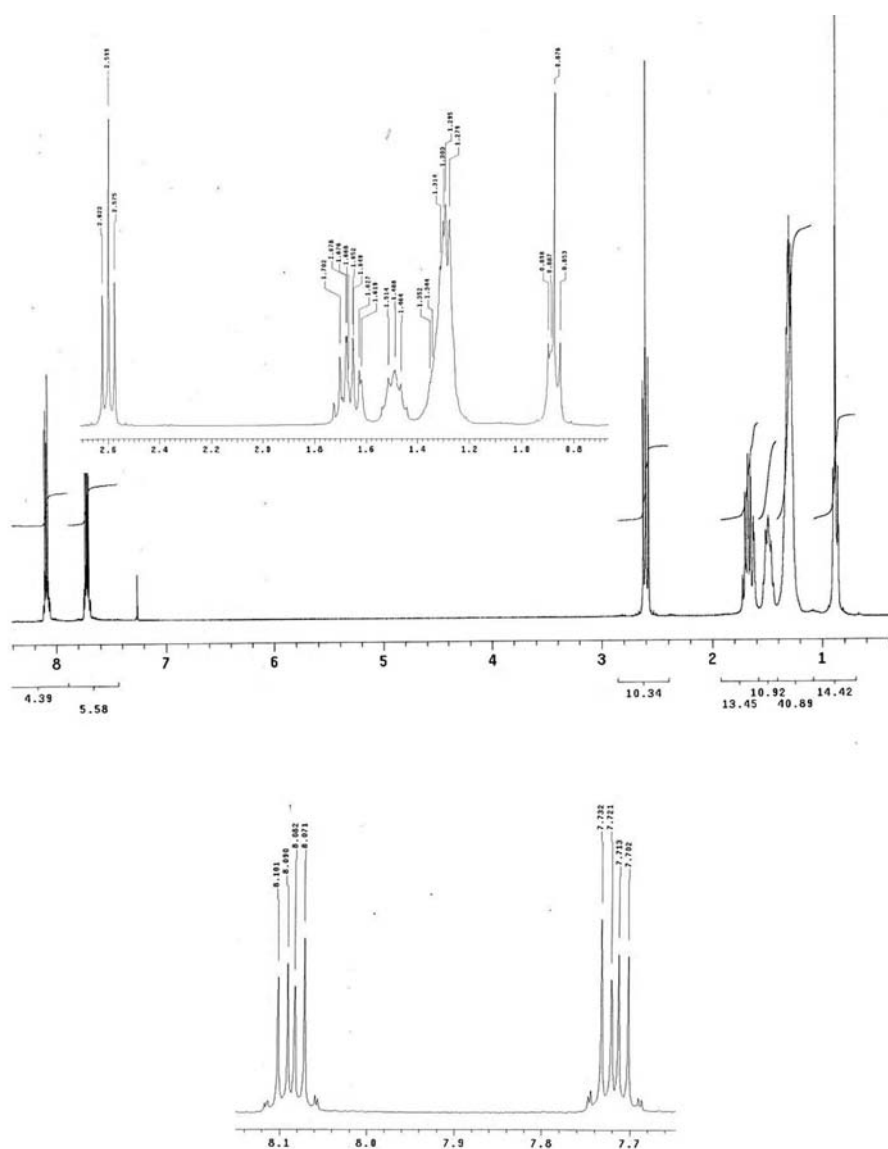


Figure S47. ^1H NMR spectrum (300 MHz, CDCl_3) of **2i**.

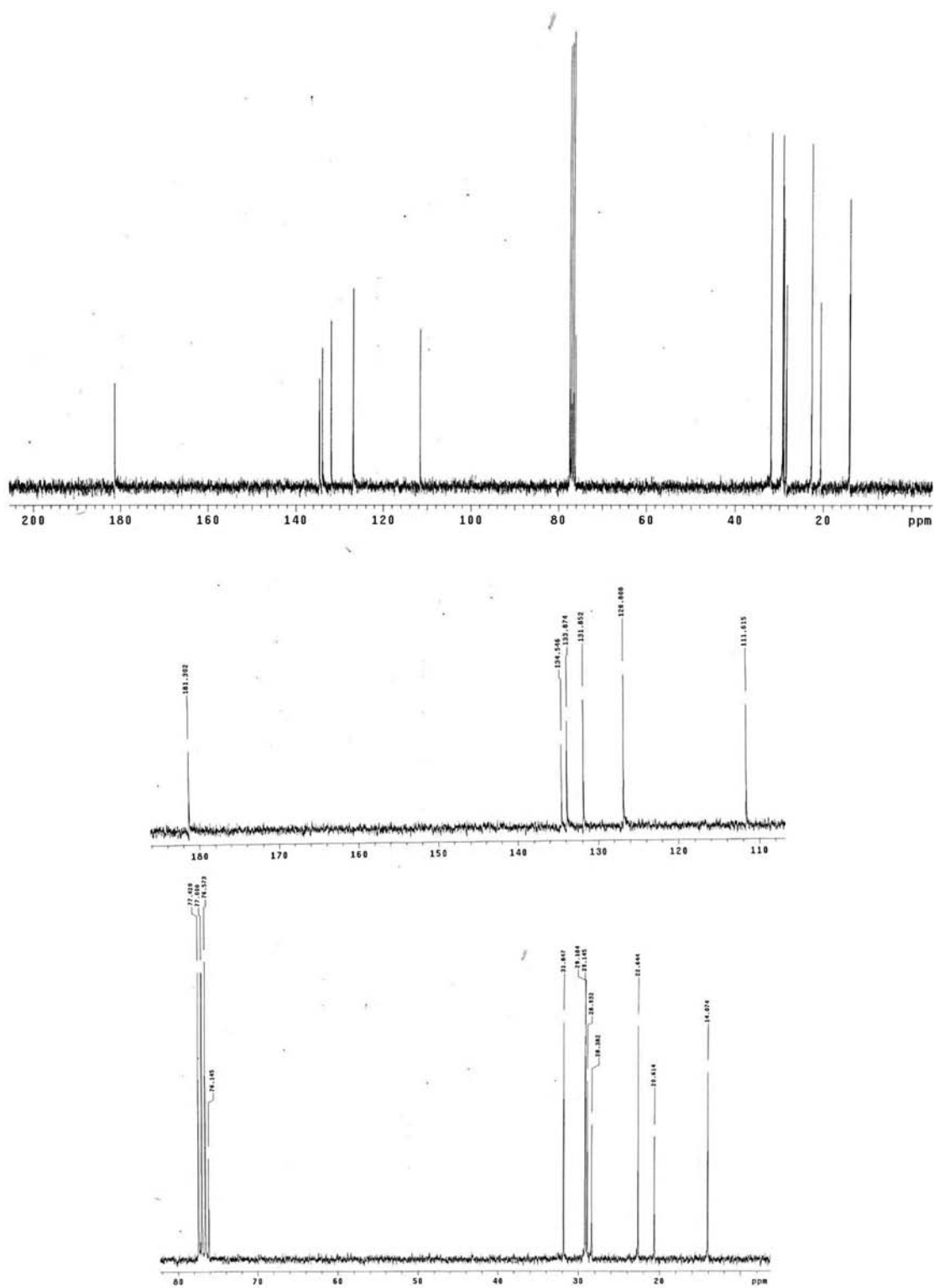


Figure S48. ^{13}C NMR spectrum (75 MHz, CDCl_3) of **2i**.