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中草藥標準品

Gentianine, Honokiol, Wogonin

之研究、開發及檢驗技術之研究

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摘 要

合成中草藥標準品 gentianine, honokiol, wogonin 及開發檢驗技術。中藥材品種混淆，就是同一種藥材主要成分的含量有時能相差數倍甚至數十倍。另外，由於中藥的成分複雜，再加上真正的有效成分，及藥效和作用機制之間的關係尚不明確，造成藥材及製劑的規格難以規範，以致於製程、品管上無法有效管控，安全性及有效性均受質疑，因此，急需在研究的基礎上，建立從中藥原料到成品的統一規格標準體系，進而提升品質和中藥製劑的評價。因部分成份在藥材中含量不高，分離不易，本計畫主要是以化學合成製備中草藥標準品。

Wogonin 之合成：可以苦味酸起始物，用疊氮甲烷處理，產生 2,4,6-三硝基甲氧基苯，或將 2,4-dinitroanisole 硝化得化合物⁽²⁾，將硝基還原成胺基，產生 2,4,6-三胺基甲氧基苯，在鹽酸的醋酸溶液中加熱即可生成 2,4,6-三羥基甲氧基苯，將之與苯甲醯基乙酸乙酯一起加熱至 200°C 即可生成 Wogonin 和 oroxylin A (是主要產物)。改良方法，將化合物⁽⁴⁾，cinnamoyl chloride 和 dichloroethane，置於 85°C 油浴中，再慢慢滴加 BF₃/Et₂O，用 TLC 偵測反應，約 1 至 1.5 小時原料消失。將反應溶液倒入冰水中，以管柱層析純化，產率 88%。將產物溶於無水 DMSO 中，再加入碘，從室溫緩慢加熱至 85°C，用 TLC 偵測反應，若原料漸漸消失且在 Oroxylin A 上方出現另一新點，則在升高溫度至 120°C，用 TLC 偵測反應，待新點消失產生 Oroxylin A 及 Wogonin 時 (約需 2 小時)，終止反應，以管柱層析純化，得 Oroxylin A，產率 13.6% 及 Wogonin，產率 16%。Wogonin 總產率 4.95%。Oroxylin A 總產率 4.21%。

Honokiol 之合成：取 4-Allylanisole 置於 -78°C ，加入 1M BBr_3 ，加完之後，讓溫度從 -78°C 慢慢回室溫，約 5 個小時回到 15°C ，小心加入冰水，以管柱層析純化，得產物 8，產率 68%。將醋酸鉛 ($\text{Pb}(\text{OAc})_4$) 加到 4-allylphenol 8 的醋酸溶液中，在室溫下攪拌 15 分鐘後，倒入冰水中並以乙醚萃取，以管柱層析純化，得產物 9，產率 8%。若改用 $\text{PhI}(\text{OAc})_2$ 替代醋酸鉛產率提升至 23%。取酚溶於甲醇，加入碘化鈉和氫氧化鈉，將此混合液冷卻至 0°C ，再將 1% 漂白水慢慢滴入，滴完控溫在 $0-20^{\circ}\text{C}$ 攪拌 1 小時，加入 10% $\text{Na}_2\text{S}_2\text{O}_3$ ，用 5% 鹽酸水溶液調到 $\text{pH}=7$ ，會有結晶發生，若沒有結晶產生，加入乙醚，有機層以飽合食鹽水洗，以無水硫酸鎂乾燥，過濾濃縮，得產物 10，產率 88%。取 4-Iodo-phenol 10 溶於丙酮，加入碳酸鉀和 Allylbromide，室溫下反應兩天，純化得產物 11，產率 90%。將鎂懸浮於乙醚中，再將少量之 4-allyloxyiodobenzene 11 和 methyl iodide 的乙醚溶液加入，在氫氣下迴流約 1 小時，將剩餘之 4-allyloxyiodobenzene 的乙醚溶液一滴一滴的加入，加完後迴流約 4 小時直到鎂消失。在 -15°C 氫氣下，將 9 的乙醚溶液一滴一滴的加到剛製備好之 Grignard reagent 中，在 -15°C 下攪拌 30 分鐘，將此溶液倒入冰的飽和氯化銨水溶液中，以乙醚萃取，純化得產物 12，產率 67%。在 -15°C 氫氣下，將 12 的 chlorobenzene 溶液慢慢加到 BCl_3 的 chlorobenzene 溶液中，在 -15°C 下攪拌 1 小時，將此溶液倒入冰的甲醇中，以乙醚萃取，純化得產物 13，產率 80%。總產率 8.38%。

Gentianine 之合成：取 Ethyl cyanoacetate 和 α -acetobutyro lactone 14 混合後，再加入氨水，並攪拌之，開始會放熱並有固體產生，隨著氨水加入固體會再溶解，加完之後靜置隔夜會有沉澱產生，過濾收集固體，得 15，產率 42%。取 15 置於密閉管中，加入 DMF 再加入 POCl_3 ，將瓶蓋鎖緊，於密閉管中加熱至 180°C ，持續加熱 8 小時，冷卻後加入水，再以乙酸乙酯萃取純化得固體 16，產率 53%。將 16 溶於甲醇，並加入醋酸鈉和 10% Pd/C ，在氫氣下反應 2 天，純化得 17，產率 59%。取 17 和 KOH 溶於酒精，在室溫下攪拌 1 小時後，加水稀釋並以乙醚萃取，純化得產物 18，產率 54%。取 18 和碳酸鈉置於密閉管中，將稀釋的甲醛加到密閉管之中，鎖緊瓶蓋，加熱至 $100^{\circ}\text{C}-120^{\circ}\text{C}$ 迴流 10 小時，冷卻後，加入 10% 鹽酸，攪拌 5 小時，再加入 10% 氨水，以二氯甲烷萃取純化，得 19 產率 23%。總產率 1.63%。

合成 gentianine、honokiol、wogonin，至少 3 克。收集其熔點、紅外光譜、氫核磁共振光譜、碳核磁共振光譜、質譜。氣相層析質譜和高效液相層析圖譜。將藥材之萃取物以高效液相層析圖譜與合成標準品比對，並標定藥材中標準品的含量。

關鍵詞：gentianine、honokiol、wogonin

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The Preparations of Chinese Herbal Standard, Gentianine, Honokiol and Wogonin, and the Studies of Their Examining Methods

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ABSTRACT

The preparations of Chinese herbal standard, gentianine, honokiol and wogonin, and the development of their examine methods. Chinese herbs have various species. Sometimes the amount of their major component in same medicinal materials have different amount. The components in Chinese herbs are very complicated. The relationship between active component, efficacy and mechanism is not clear. Therefore, the medicinal materials and preparations are very difficult to regulate, and their manufacture process and quality control can not be investigated efficiently. The safety and efficacy may be not trustable. It is very urgent to create a consistent regulation system from Chinese herb material to products, and promote the quality and the valuation of Chinese herb preparations. Because some components contain in medicinal materials very few amount and very difficult to isolated, in this project three Chinese herb standard were synthesized.

Synthesis of wogonin started from picric acid by treatment with diazomethane to give 2,4,6-trinitroanisole, or prepared from the nitration of 2,4-dinitroanisole. 2,4,6-Trinitroanisole was converted to 2,4,6-triaminoanisole by hydrogenation, and then treated with conc hydrochloric acid in acetic acid under refluxing to yield

2,4,6-trihydroxyanisole. 2,4,6-Trihydroxyanisole was heated with ethyl 2-phenyl acetate to 200°C to produce wogonin and oroxylin A, but oroxylin A was the major product. Alternatively, 2,4,6-trihydroxyanisole was treated with cinnamoyl chloride in 1,2-dichloroethane at 85°C with $\text{BF}_3/\text{Et}_2\text{O}$ after 1.5 h to give an intermediate in 88% yield. The intermediate was treated with iodine in DMSO at 85°C, and then heated up to 120°C when a spot above the spot of oroxylin A was disappeared on TLC. After purification, we obtained oroxylin A in 13.6% yield and wogonin in 16%. The total yield of wogonin is 4.95% and oroxylin A is 4.21%.

Honokiol synthesis: 4-Allylanisole was cooled under -78°C, and then added 1 M BBr_3 . After addition was finished, the temperature was increased to room temperature it took 5 hours. The reaction was quenched by addition of ice-water, and purified by column chromatogram to produce compound 8 in 68% yield. To a solution of 4-allylphenol in acetic acid was added $\text{Pb}(\text{OAc})_4$, and stirred at room temperature for 15 min. Then the reaction mixture was poured into ice-water, and extracted with ether. After purification, gave product 9 in 8% yield. If instead of $\text{Pb}(\text{OAc})_4$ with $\text{PhI}(\text{OAc})_2$ the yield was increased to 23%. To a solution of phenol in methanol was added sodium iodide and sodium hydroxide, and then cooled to 0°C, and followed a 1% bleach was added dropwise. After addition, the reaction mixture was stirred at 0-20°C for one hour. After added 10% $\text{Na}_2\text{S}_2\text{O}_3$ and 5% hydrochloric acid to the solution became neutral, there have some crystalline formation. If the crystalline did not form, some ether was added and the organic phase was washed with brine. After dried, filtration and concentration, gave compound 10 in 88% yield. To a solution of 4-iodo-phenol in acetone was added potassium carbonate and allylbromide, and stirred at room temperature for two days to give compound 11 in 90% yield. To a suspended solution of magnesium in ether was added a solution of 4-allyloxyiodobenzene and methyl iodide in ether. After refluxing under argon for one hour, the remained 4-allyloxyiodobenzene in ether was added dropwise, and then refluxed for four hours. A solution of 9 in ether was added dropwise to the prepared Grignard reagent, and stirred at -15°C for 30 minutes. The reaction mixture was poured into an ice cold of ammonium chloride solution, and extracted with ether. After purification gave compound 12 in 67% yield. To a solution of BCl_3 in chlorobenzene was added a solution of 12 in chlorobenzene at -15°C under argon for one hour. The reaction mixture was poured into a cold methanol, and extracted with ether. After purification, gave compound 13 in 80% yield. The total yield was 8.38%.

Gentianine synthesis: To a mixture of ethyl cyanoacetate and α -acetobutyrolactone was added ammonia water and stirred. An exothermic reaction occurred with formation of solid. When more ammonia water was added the solid dissolved. After addition, the reaction was standed overnight and precipitate was formed. The solid was collected by filtration to give compound 15 in 42% yield. A solution of compound 15 in DMF and POCl_3 was sealed in a tube, and heated up to 180°C for 8h. After cold, water was added and extracted with ethyl acetate. The crude product was purified to give compound 16 in 53% yield. To a solution of 16 in methanol was added sodium acetate and 10% Pd/C, and under hydrogen for two days to give compound 17 in 59% yield. A solution of 17 and KOH in ethanol was stirred at room temperature for one hour. The reaction mixture was diluted by water and extracted with ether. After purification gave compound 18 in 54% yield. A solution of 18 and sodium carbonate in water was sealed in a tube, and heated up to $100\text{-}120^\circ\text{C}$ for 10 hours. After cooled to room temperature, 10% hydrochloric acid was added and stirred for 5 hours, and then 10% ammonia water was added. After extraction with dichloromethane and purification, gave compound 19 in 23% yield. The total yield was 1.63%.

Gentianine, honokiol and wogonin were prepared three grams each of them. Their melting point, IR, ^1H NMR, ^{13}C NMR, MS, GC-MS and HPLC spectra were collected. The extracts of medicinal materials were analyzed by HPLC, and compared to the corresponding synthesized standard samples.

Keywords: gentianine, honokiol, wogonin

壹、前言

本計畫產生之背景是依據行政院衛生署中醫藥委員會九十五年度中醫藥健康安全防護網促進中草藥製備品質研究類徵求研究重點（案號：0950006938）徵求重點 1-1 中草藥標準品之研究、開發及檢驗技術之研究（一）。

穩定中藥品質，建立完善標準是促進中藥現代化非常重要的一環。中藥材品種混淆，存在同名異物、同物異名現象，就是同一種藥材主要成分的含量有時能相差數倍甚至數十倍。另外，由於中藥的成分複雜，再加上真正的有效成分，及藥效和作用機制之間的關係尚不明確，造成藥材及製劑的規格難以規範，以致於製程、品管上無法有效管控，安全性及有效性均受質疑，因此，急需在研究的基礎上，建立從中藥原料到成品的統一規格標準體系，進而提升品質和中藥製劑的評價。本研究計畫合成標準品作為檢驗的比較標準。

國內外相關研究有，中國 Feng YL⁽¹⁾等人用 HPLC 分析鑑定 Sanhuang 錠中之五種成份，包括 emodin, chrysophanol, baicalin, wogonin and berberine hydrochloride，這種準確又快速的方法可以應用於其他產品製程之品管。日本 Miyaichi Y⁽²⁾等人從 *Scutellaria amabilis* HARA 的根部分離鑑定七種 flavonoids，其中包括 wogonin。中國 Zhou XN⁽³⁾等人，發展 HPLC-電化學偵測法（HPLC-ECD）鑑定 *Scutellaria baicalensis* 中之 baicalin, baicalein, wogonin 三種成份。長庚醫院 Lin IH⁽⁴⁾等人發展一種以 HPLC 結合光電二極體陣列偵測和 MS 的方法，作為分析以中藥材製備 Gan-Lu-Yin 之定性和定量的指紋光譜，鑑定 *Radix Glycyrrhizae* 中之 14 種成份，包括 baicalin, baicalein, oroxylin A-7-O-glucuronide, wogonin-7-O-glucuronide, wogonin, and oroxylin A。中國之 Du CF⁽⁵⁾等人以管柱層析和製備型薄層層析法，從 *Dictamnus dasycarpus* 的皮中分離鑑定出 12 種成份，包括 dictamnine, fraxinellone, skimmianine, gamma-fagarine, beta-sitosterol, obacunone, limonin disophenol, fraxinellonone, wogonin, rutevin, kihadinin B, dasycarine。台大藥學院 Chang LC 等人為了評估中藥製備過程中之生物活性成份，發展一種毛細管膠束電動色譜法，可以鑑定含有三種草藥 *Coptidis Rhizoma*, *Scutellariae Radix*, and *Rhei Rhizoma* 之中藥的 8-13 種生物活性成份。中國 Li HB⁽⁷⁾等人發展一種高速逆流色譜法（HSCCC）方法，製備分離純化 *S. baicalensis* Georgi 中之 baicalein, wogonin and oroxylin A 三種成

份。美國 Bonham M⁽⁸⁾ 等人，以 HPLC 分離鑑定出 *S. baicalensis* 中之四種成份包括 baicalein, wogonin, neobaicalein, and skullcapflavone。中國 Li C⁽⁹⁾ 等人發展一種簡單而靈敏的非水溶液毛細管電泳法，分析傳統中藥製備時的三種具有生物活性成份 puerarin, daidzein and wogonin。中國 Su YL⁽¹⁰⁾ 等人，以管柱層析法分離以光譜鑑定出 *S. rehderiana* 中含有的五種成份，包括 oroxylin A, wogonin, baicalein, ganhuangenin, ganhuangemin。香港 Li HB⁽¹¹⁾ 等人，應用 HPLC, high-speed counter-current chromatography, TLC, 毛細管電泳，and micellar electrokinetic capillary chromatography 等方法從 *Scutellaria baicalensis* Georgi 分離出 baicalin, baicalein, wogonin and oroxylin A 等成份。日本 Ohtake N⁽¹²⁾ 等人，以 three-dimensional (3D) HPLC 分析鑑定出 Sho-saiko-to (SST) 中之 baicalin, wogonin-7-O-glucuronide, liquiritin, their three aglycons, liquiritin apioside, glycyrrhizin, saikosaponin b1, saikosaponin b2, ginsenoside Rg1, ginsenoside Rb1, (6)-gingerol, (6)-shogaol and arginine 等 15 種成份。捷克 Bochorakova H⁽¹³⁾ 等人以 RP-HPLC 方法，分離鑑定 *Scutellaria baicalensis* Georgi 之根部之 baicalin and wogonin glucuronide 成份。中國 Li XD⁽¹⁴⁾ 等人以層析法分離鑑定 *Sorbaria sorbifolia* 中間部份的成份，包括 noreugenin, wogonin, 5,7,3',4'-tetrahydroxy-3-methoxyflavone, protocatechuic acid, benzoic acid, p-hydroxy benzoic acid, emodin and daucosterol 等八種成份。中國 Liu YP⁽¹⁵⁾ 等人以 HPLC 分離鑑定出 Huangqin (*Scutellaria baicalensis*) 中之四種成份，包括 baicalin, baicalein, wogonin and wogonin-7-O-glucuronide。中國 Yang LX⁽¹⁶⁾ 等人以 HPLC 分離鑑定本地與非本地草藥中的 baicalin, wogonin, baicalein, neobaicalein, wogonin, visidulin and oroxylin，結果發現沒有明顯的差異。中國 Du Y⁽¹⁷⁾ 等人以靈敏又快速的 LC/MS/MS 方法，從血漿中鑑定 wogonin。中國 Xie LH [18] 等人，發展毛細管膠束電動色譜 (MEKC) 分析 *Scutellariae Radix* (SR) 和 *Scutellaria baicalensis* 根部之 baicalin, baicalein and wogonin 三種成份。中國 Zuo F⁽¹⁹⁾ 等人以 HPLC 方法鑑定 Huangqin-Tang 中之 baicalin, wogonoside, oroxylin-A-glucoside, baicalein, wogonin, oroxylin-A, paeoniflorin, glycyrrhizic acid, glycyrrhetic acid, liquiritin, isoliquiritin, liquiritigenin, isoliquiritigenin and ononin 等成份。中國 Qi L⁽²⁰⁾ 等人發展毛細管膠束電動色譜法鑑定 *Scutellariae Radix* 中之 baicalin, baicalein, wogonin 7-O-glucuronide, wogonin, oroxylin A 7-O-glucuronide, and oroxylin A 等六種成份。日本 Nagashima S⁽²¹⁾ 等人從 *Scutellaria baicalensis* Georgi 的細胞培養液中以不同的層析法純化出 UDP-glucuronate。日本

Okamura N⁽²²⁾等人以離子對 HPLC 法從 Kampo medicines (固有藥劑) 鑑定出 baicalin, wogonoside, baicalein, wogonin, berberine, coptisine, palmatine, jateorrhizine, and glycyrrhizin 等成份。食品及藥物國家實驗室之 Lin SJ⁽²³⁾等人，應用 HPLC 鑑定 Sann-Joong-Kuey-Jian-Tang 中之 gentiopicroside, mangiferin, palmatine, berberine, baicalin, wogonin and glycyrrhizin 等成份。日本 Ikegami F⁽²⁴⁾等人以 DEAE-Sepharose Fast Flow and chromatofocusing used PBETM94 and Polybuffer 74 方法純化 *Scutellaria* 根部，作為固有藥劑品管標準。日本 Ishimaru K⁽²⁵⁾等人從 *Scutellaria baicalensis* 的根部分離出 5,7,2',6'-terahydroxyflavone, 5,7,2',5'-tetrahydroxy-8,6'-dimethoxyflavone, skullcapflavone II, baicalin, baicalin methyl ester, wogonin 7-glucuronide and 3,5,7,2',6'-pentahydroxyflavanone 等成份。日本 Inagaki I⁽²⁶⁾等人從 *Anodendron affine* 的莖部分離出 wogonin, dambonitol 等成份。

中藥在中國文化所及的地區已有數千年的使用記載，國內使用中藥的人數持續增加中，對國人身心健康具有深遠的影響，如何提高檢驗之效率與準確性是亟待加強的重點，尤其種類繁多的中草藥物若無法即時建立篩選檢驗方法，將是藥物檢驗的一大漏洞，因此合成出這些中藥所含有效成份之標準品，將可快速又準確的檢驗出中藥內的有效成份，進而監控其品質，對國人身心健康之維護將有莫大之幫助。

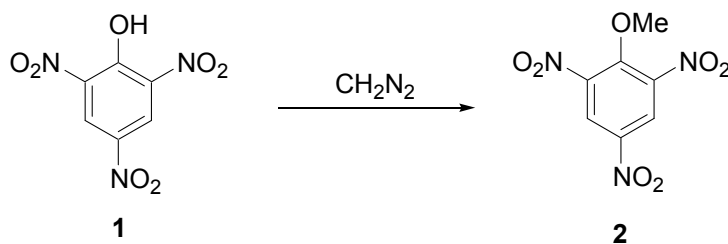
貳、材料與方法

一、一般實驗方法

- (一) 熔點 ($^{\circ}\text{C}$)：將固體樣品置於毛細管中，使用 Yanagimoto Micro Melting Point Apparatus Model-S3 型熔點測定器測定之。
- (二) 紅外光譜 (IR)：使用 JASCO FT/IR 410 型紅外線光譜儀測定之。
- (三) 氫核磁共振光譜 (^1H NMR)：測定係使用 Bruker AVANCE DPX-300 型 (300 MHz) 核磁共振光譜儀。碳核磁共振光譜 (^{13}C NMR)：請清華大學貴儀中心以 VARIAN UNIYTINOVA-500 型 (125.7 MHz) 核磁共振光譜儀代測。使用的溶劑為氘-氯仿 (CDCl_3)，氫光譜化學位移係以三氯甲烷 (CHCl_3) $\delta = 7.26\text{ppm}$ 為內標準；碳光譜化學位移係以氘-氯仿 (CDCl_3) $\delta = 77\text{ppm}$ 為內標準；光譜單位 (δ) ppm，耦合常數 (J) 單位為 Hz。s 表單峰 (singlet)；d 雙峰 (doublet)；t 表三重峰 (triplet)；m 表多重峰 (multiplet)。
- (四) 質譜 (Mass Spectrum)：低解析度質譜 (MS)：係請東華大學何彥鵬師實驗室代測；高解析度質譜 (HRMS)：係請中央大學貴儀中心使用 JMS-700 型高解析度質譜儀代測。
- (五) 減壓濃縮：使用 RIKA Rotary vacuum evaporator SN series 型旋轉濃機；抽氣幫浦使用 SIBATA Circulating Aspirator WJ-20 型幫浦。
- (六) 氫化反應器：Parr shaker type hydrogenator (3911)。
- (七) 所使用的乾燥溶劑，tetrahydrofuran (THF) 及 ether (Et_2O) 以鈉迴流並加入二苯甲酮 (benzophenone) 當指示劑經由蒸餾而得；二氯甲烷 (dichloromethane, CH_2Cl_2)，DMF，苯 (benzene) 皆以氫化鈣 (CaH_2) 迴流蒸餾而得。

二、合成 Wogonin (漢黃芩素)

(一) 合成 2-Methoxy-1,3,5-trinitro-benzene ⁽²⁾



秤取苦味酸 **1** (picric acid) (含 40%水) (82.0g), 用最少量的乙醚完全溶掉苦味酸, 將水層以分液漏斗分離, 乙醚層加入無水硫酸鎂乾燥, 過濾濃縮後, 秤重得 44.0g (19.2mmol)。另外製備疊氮甲烷 (diazomethane), 在冰浴下緩緩將加入疊氮甲烷加至苦味酸的乙醚溶液中, 直到不再產生氣泡為止, 再加入少量醋酸分解多餘的疊氮甲烷, 減壓濃縮得橘紅色固體, 以管柱層析純化, 乙酸乙酯/正己烷 (1:20) 為沖提液, 得黃色固體 **2** (28.2g, 116 mmol), 產率 60%。

產物 2:

MP: 68°C.

^1H NMR (300MHz, CDCl_3 , δ): 8.89 (s, 2H), 4.17 (s, 3H).

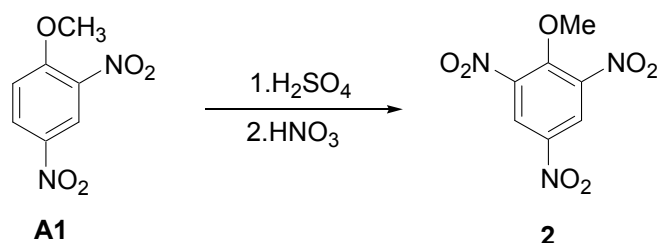
^{13}C NMR (75MHz, CDCl_3 , δ): 152.2, 144.3, 141.4, 124.6, 65.2.

IR (KBr): 3097, 1540, 1351, 1269, 1085 cm^{-1} .

MS m/z : 244 ($\text{M}^+ + 1$, 2), 299 (23), 213 (100), 197 (1), 75 (26).

HRMS-FAB(m/z): $[\text{M}+1]^+$ calcd for $\text{C}_7\text{H}_5\text{N}_3\text{O}_7$, 243.0126; found, 243.0128.

(二) 改良合成 2-Methoxy-1,3,5-trinitro-benzene ⁽²⁾



秤取 2,4-dinitroanisole (19.8g, 0.1mol), 溶在 100mL 濃硫酸中, 放置在冰浴, 然後慢慢滴加 10mL 濃硝酸, 滴完後在冰浴下攪拌 3 小時, 溶液由黃色澄清變成乳黃色黏稠。將反應溶液慢慢倒入 200g 冰水中, 終止反應, 濾出固體並用水洗掉多餘的酸, 收集固體並乾燥, 得黃白色固體 **2** (22.2g, 91.4mmol), 產率 91%。

產物 2:

MP: 68°C.

^1H NMR (300MHz, CDCl_3 , δ): 8.89 (s, 2H), 4.17 (s, 3H).

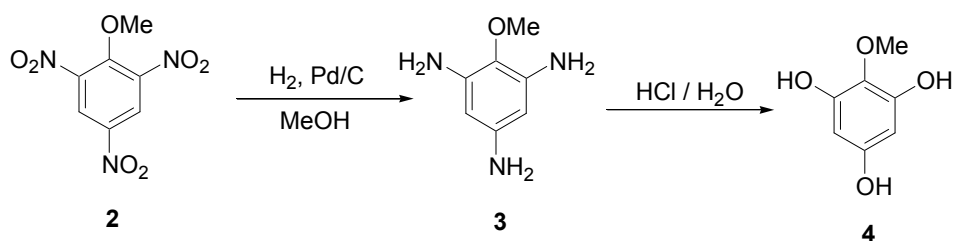
^{13}C NMR (75MHz, CDCl_3 , δ): 152.2, 144.3, 141.4, 124.6, 65.2.

IR (KBr): 3097, 1540, 1351, 1269, 1085 cm^{-1} .

MS m/z : 244 ($M^+ + 1$, 2), 299 (23), 213 (100), 197 (1), 75 (26).

HRMS-FAB(m/z): $[M+1]^+$ calcd for $\text{C}_7\text{H}_5\text{N}_3\text{O}_7$, 243.0126; found, 243.0128.

(三) 合成 2-Methoxy-benzene-1,3,5-triol 4



秤取化合物 **2** (28.2g, 116.0mmol) 加入乙酸乙酯 20mL 溶解之，再加入甲醇 180mL 及 10% Pd/C 2.82g，在氫氣壓力 60psi 下進行氫化反應，直到壓力不再下降後，並用 TLC 偵測待反應完成；將反應溶液用矽藻土過濾，將濾液濃縮後，得黑色固體。直接加入水 500mL 及 12M 鹽酸 29mL，加熱迴流 20 小時，待反應溶液降溫到室溫後，將反應溶液濃縮，得黑色黏稠液體。分次加入乙醚洗黑色黏稠液體，直到變成酥鬆狀，收集洗液濃縮，再以管柱層析純化，乙酸乙酯/正己烷 (3:10) 為沖提液，得米白色固體 **4** (6.2g, 39.7mmol)，二步產率 34%。

產物 **3**：

MP: 117-118°C.

^1H NMR (300MHz, CDCl_3 , δ): 5.57 (s, 2H), 3.70 (s, 3H), 3.60 (s, 6H).

^{13}C NMR (75MHz, CDCl_3 , δ): 143.6, 140.4, 128.3, 93.7, 58.9.

IR (KBr): 3394, 3142, 1621, 1216, 1001 cm^{-1} .

MS m/z : 154 ($M^+ + 1$, 4), 138 (100), 111 (23), 82 (5), 69 (3).

HRMS-FAB(m/z): $[M+1]^+$ calcd for $\text{C}_7\text{H}_{11}\text{N}_3\text{O}$, 153.0904; found, 153.0902.

產物 4：

MP: 190-191°C.

^1H NMR (300MHz, CDCl_3 , δ): 6.03 (s, 2H), 5.29 (s, 2H), 3.81 (s, 3H).

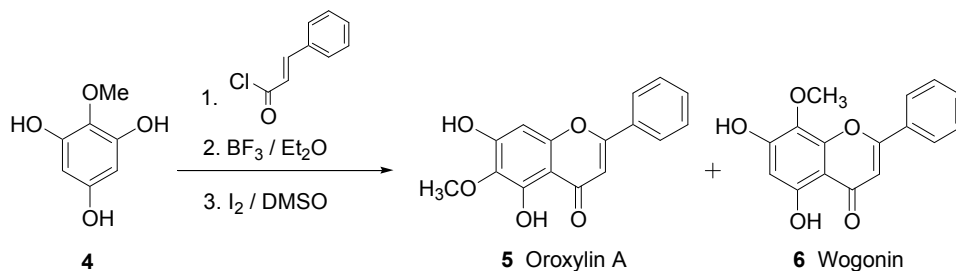
^{13}C NMR (75MHz, CDCl_3 , δ): 153.4, 150.5, 129.0, 94.6, 59.7.

IR (KBr): 3273, 3088, 1288, 1156, 1051 cm^{-1} .

MS m/z : 157 ($\text{M}^+ + 1$, 5), 141 (100), 113 (62), 95 (4), 69 (10).

HRMS-FAB(m/z): $[\text{M} + 1]^+$ calcd for $\text{C}_7\text{H}_8\text{O}_4$, 156.0422; found, 156.0423.

(四) 改良合成 Wogonin⁽⁶⁾



取烘過 100 mL 雙口瓶 (120°C, 3 小時以上), 加入化合物 4 (3.12g, 20mmol), cinnamoyl chloride (3.34g, 20mmol) 和 dichloroethane (40mL), 將反應瓶置於 85°C 油浴中, 再在慢慢滴加 $\text{BF}_3/\text{Et}_2\text{O}$ (10.14mL, 80mmol), 剛開始反應會劇烈冒泡, 待滴加完後用 TLC 偵測反應, 約 1 至 1.5 小時原料消失。將反應溶液倒入約 80mL 冰水中, 再用乙酸乙酯萃取 2 次, 用無水硫酸鎂乾燥, 過濾濃縮後, 以管柱層析純化, 用乙酸乙酯/正己烷 (1:1) 溶解粗產物, 以乙酸乙酯/正己烷 (1:1) 為沖提液, 得紅黃色固體 5.04g (17.6mmol), 88%。秤取紅黃色固體 (5.04g, 17.6mmol), 溶於無水 DMSO (57mL) 中, 再加入碘 (89.4mg, 0.35mmol, 2%), 將反應置於油浴中, 從室溫緩慢加熱至 85°C, 用 TLC 偵測反應, 若原料漸漸消失且在 Oroxylin A 上方出現另一新點, 則在升高溫度至 120°C, 用 TLC 偵測反應, 待新點消失產生 Oroxylin A 及 Wogonin 時 (約需 2 小時), 終止反應。加入 150mL 乙酸乙酯, 再用 100mL 1N HCl 洗有機層 2 次, 用 50mL 水洗有機層 1 次, 有機層以無水硫酸鎂乾燥, 過濾濃縮後, 以管柱層析純化,

用 230~400mesh 的 silicon gel 填充在管徑 4cm，長度 34cm 的玻璃管柱中，用丙酮溶解粗的產物，取 6mL 丙酮溶液加至管柱上，以乙酸乙酯/正己烷（1：5）為沖提液，得 Oroxylin A (0.69g, 2.4 mmol)，產率 13.6%及 Wogonin (0.80g, 2.8mmol)，產率 16%。Wogonin 總產率 4.95%。Oroxylin A 總產率 4.21%。(所需藥品價格合成每克臺幣 7,271 元，加上人事費和雜支 50%，約 10,907 元/克，購得標準品 20mg/1,760 元，即 88,000 元/克)。

產物 5 (Oroxylin A)：

MP: 192-193°C.

^1H NMR (300.13MHz, CDCl_3 , δ): 13.01 (s, 1H), 7.90-7.87 (m, 2H), 7.56-7.50 (m, 3H), 6.66 (s, 1H), 6.62 (s, 1H), 6.53 (s, 1H), 4.05 (s, 3H).

^{13}C NMR (75.0MHz, CDCl_3 , δ): 183.0, 164.1, 155.2, 153.2, 152.1, 131.8, 131.3, 130.4, 129.1, 126.3, 105.9, 105.2, 93.5, 60.9.

IR (KBr): 3442, 3055, 1653, 1606, 1580, 1276, 1095 cm^{-1} .

MS m/z : 285 ($\text{M}^+ + 1$, 16), 269 (66), 266 (38), 241 (56), 238 (9), 167 (8), 139 (16), 103 (9), 69 (41).

HRMS-FAB(m/z): $[\text{M}]^+$ calcd for $\text{C}_{16}\text{H}_{12}\text{O}_5$, 284.0681; found, 284.0685.

產物 6 (Wogonin)：

MP: 197.5-198.5°C

^1H NMR (300.13MHz, CDCl_3 , δ): 12.50 (s, 1H), 7.93-7.90 (m, 2H), 7.57-7.55 (m, 3H), 6.69 (s, 1H), 6.45 (s, 1H), 4.04 (s, 3H).

^{13}C NMR (75.0MHz, CDCl_3 , δ): 182.5, 163.5, 157.7, 155.3, 148.9, 132.0, 131.2, 129.2, 126.9, 126.2, 105.9, 105.2, 98.9, 62.0.

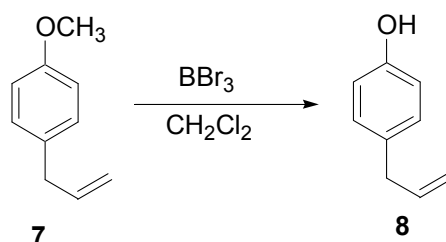
IR (KBr): 3390, 3073, 1660, 1613, 1580, 1272, 1100, 1023 cm^{-1} .

MS m/z : 285 ($\text{M}^+ + 1$, 12), 269 (100), 241 (26), 167 (8), 139 (40), 111 (7), 77 (4), 69 (11).

HRMS-FAB(m/z): $[\text{M}]^+$ calcd for $\text{C}_{16}\text{H}_{12}\text{O}_5$, 284.0688; found, 284.0685.

三、合成 Honokiol (和厚朴酚)

(一) 合成 4-Allyl-phenol 8



取 4-Allylanisole **7** (1.53mL, 10mmol) 加入乾燥的二氯甲烷 (25mL)，將反應瓶置於冷浴並控溫在 -78°C ，慢慢以針加入 1M BBr_3 (10mL)，加完之後，讓溫度從 -78°C 慢慢回室溫，約 5 個小時回到 15°C ，小心加入冰水，有機層加入 5% NaOH (10mL)，水層以 1N HCl 酸化至 $\text{pH} = 6$ ，再以乙醚萃取，濃縮後以乙酸乙酯/正己烷 (1:9, v/v) 為移動相，得產物 **8** (0.91g, 6.8mmol)，產率 68%。

^1H NMR (400MHz, CDCl_3 , δ): 7.08-7.04 (d, $J = 17.6\text{Hz}$, 2H), 6.79-6.74 (d, $J = 16.7\text{ Hz}$, 2H), 5.99-5.89 (m, 1H), 5.07-5.02 (m, 2H), 3.32-3.31 (d, $J = 6.7\text{Hz}$, 2H).

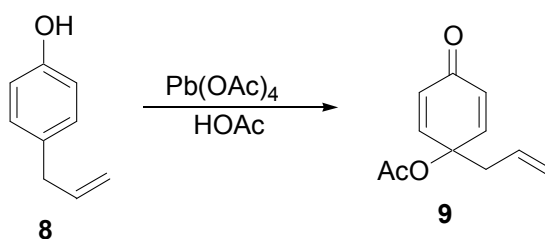
^{13}C NMR(100MHz, CDCl_3 , δ): 153.8, 137.8, 132.3, 129.7, 115.5, 115.2, 39.3.

IR(KBr, thin film): 3435, 2983, 2922, 1639, 1513, 1242, 912, 735 cm^{-1} .

MS-EI m/z : 134 (M^+ , 45), 133 (58), 132 (31), 114 (21), 106 (100), 91 (18), 77 (39).

HRMS-EI (m/z): $[\text{M}]^+$ calcd for $\text{C}_9\text{H}_{10}\text{O}$, 134.0732; found, 134.0734.

(二) 合成 Acetic acid 1-allyl-4-oxo-cyclohexa-2,5-dienyl ester 9



將醋酸鉛 ($\text{Pb}(\text{OAc})_4$, 8.6g, 19.3mmol) 加到攪拌中之 4-Allylphenol **8** (1.8g, 13.4mmol) 的醋酸溶液中 (40mL), 在室溫下攪拌 15 分鐘後, 倒入冰水中並以乙醚萃取, 有機層以飽和碳酸氫鈉水溶液洗, 再以水洗, 以無水硫酸鎂乾燥, 過濾濃縮後, 以管柱層析純化, alumina (Al_2O_3) 為固定相, 正己烷/氯仿 (1:1, v/v) 為移動相, 得產物 **9** (215mg, 1.1mmol), 產率 8.3%。

取 4-allyl-phenol (5.06g, 0.0377mol), 加入醋酸鉍 (23.20g, 0.302mol), 在加入醋酸 (125mL) 和二氯甲烷 (125mL) 此為 A 瓶, 另取一瓶裝有 Iodo benzene diacetate (98%, 13.32g, 0.041mol) 溶於二氯甲烷 (125mL) 此為 B 瓶, 將 B 瓶以雙頭針注入 A 瓶室溫反應下反應 12 小時, 加水 200mL 洗 2 次, 無水硫酸鎂除水, 濃縮後, 以 silica gel 管柱層析純化, 以 Ethyl acetate/Hexane (1:9, v/v) 為移動相, 得產物 1.53g, 產率 21%。

^1H NMR (400MHz, CDCl_3 , δ): 6.84-6.82 (d, $J = 9.9\text{Hz}$, 2H), 6.28-6.26 (d, $J = 9.9\text{Hz}$, 2H), 5.71-5.65 (m, 1H), 5.19-5.09 (m, 2H), 2.58-2.56 (d, $J = 7.2\text{Hz}$, 2H), 2.07 (s, 3H).

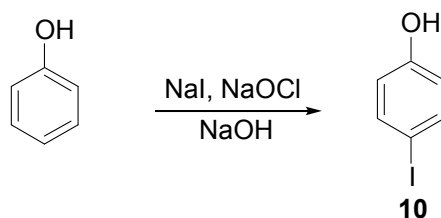
^{13}C NMR (100MHz, CDCl_3 , δ):

$^{\text{IR}}$ (KBr, thin film):

MS-EI m/z : 192 (M^+ , 5), 166 (18), 151 (27), 150 (100), 134 (38), 133 (40), 131 (21), 109 (44), 107 (64), 105 (45), 103 (49), 91 (65), 81 (37), 79 (50), 78 (69), 77 (99), 51 (59).

HRMS-EI (m/z): $[\text{M}]^+$ calcd for $\text{C}_{11}\text{H}_{12}\text{O}_3$, 192.0786; found, 192.0794.

(三) 合成 4-Iodophenol **10**



取酚 (10.0g, 106.3mmol) 溶於甲醇 (250mL), 加入碘化鈉 (15.92g, 0.1mol) 和氫氧化鈉 (4.25g, 0.1mol), 將此混合液冷卻至 0°C , 再將 1% 漂白水 (172.5g) 慢慢滴入, 並控溫在 $0-3^\circ\text{C}$,

每一滴滴入會出現紅色，並馬上退掉，滴完控溫在 0-20°C 攪拌 1 小時，加入 10% Na₂S₂O₃ (100mL)，用 5% 鹽酸水溶液調到 pH = 7，會有結晶發生，若沒有結晶產生，加入乙醚 (375mL)，有機層以飽合食鹽水 (350mL) 洗，以無水硫酸鎂乾燥，過濾濃縮，得產物 **10** (20.65g, 93.9mmol)，產率 88%。

¹H NMR (400MHz, CDCl₃, δ): 7.52-7.50 (d, J = 8.0Hz, 2H), 6.63-6.61(d, J = 7.9Hz, 2H).

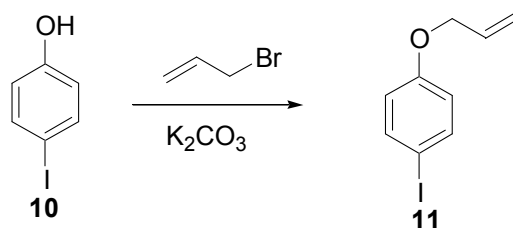
¹³C NMR(100MHz, CDCl₃, δ): 155.4, 138.5, 117.8, 82.6.

IR(KBr, thin film): 3360, 1699, 1581, 1487, 1233, 1005, 822 cm⁻¹.

MS-EI m/z : 220 (M⁺, 100), 191 (4), 127 (18), 93 (28), 65 (64), 63 (34).

HRMS-EI (m/z): [M]⁺ calcd for C₆H₅IO, 219.9385; found, 219.9382.

(四) 合成 1-Allyloxy-4-iodo-benzene **11**



取 4-Iodo-phenol **10** (2.0g, 9.1mmol) 溶於丙酮 (30mL)，加入碳酸鉀 (1.25g, 9.1mmol) 和 Allylbromide (0.86mL, 10mmol)，室溫下反應兩天，減壓濃縮將丙酮抽乾，加水並以乙酸乙酯萃取，以無水硫酸鎂乾燥，過濾濃縮後，以管柱層析純化，以乙酸乙酯/正己烷 (0.5 : 9.5, v/v) 為移動相，得產物 **11** (2.12g, 8.15 mmol)，產率 90%。

¹H NMR (400MHz, CDCl₃, δ): 7.55-7.53 (d, J = 6.7Hz, 2H), 6.70-6.69 (d, J = 6.8Hz, 2H), 6.07-5.97 (m, 1H), 5.42-5.37 (d, J = 17.2Hz, 1H), 5.30-5.27 (d, J = 10.5Hz, 1H), 4.51-4.49 (d, J = 8.2Hz, 2H).

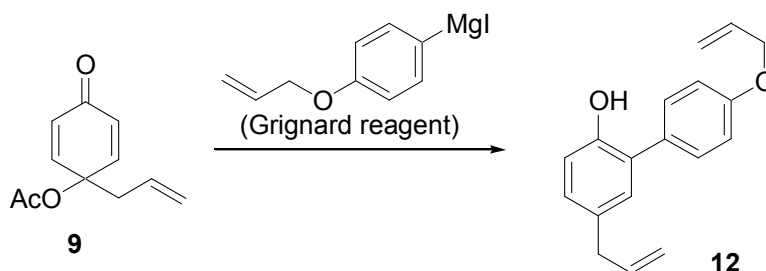
IR(KBr, thin film): 3082, 2920, 2866, 1585, 1485, 1282, 1242, 819 cm⁻¹.

MS-EI m/z : 260 (M⁺, 100), 219 (36), 191 (41), 133 (22), 127 (30),

105 (37), 92 (21), 64 (42), 63 (77), 62 (25).

HRMS-EI (m/z): $[M]^+$ calcd for C_9H_9IO , 259.9698; found, 259.9699.

(五) 合成 5-Allyl-4'-allyloxy-biphenyl-2-ol



將鎂 (182.2mg, 7.60mmol) 懸浮在四氫呋喃 (20mL) 溶液，再加入催化量碘和少量的 4-allyloxyiodobenzene **11** 的四氫呋喃溶液，在氬氣下迴流 1 小時，再將剩餘的 4-allyloxyiodobenzene **11** (1.52g, 5.85mmol) 的四氫呋喃 (20mL) 溶液，一滴一滴的加入，加完後迴流 2 小時，在冰浴氬氣下將 **9** (282mg, 1.47mmol) 的四氫呋喃 (20mL) 溶液一滴一滴的加入剛製備之 Grignard reagent 中，在冰浴下攪拌 2 小時，將此溶液倒入冰的飽和氯化銨水溶液中，以乙醚萃取，乙醚層以水洗，並以無水硫酸鎂乾燥，過濾濃縮後，以管柱層析純化，以 silica gel 為固定相，以乙酸乙酯/正己烷 (2:8, v/v) 為移動相，得產物 **12** (221mg, 0.83mmol)，產率 56%。

1H NMR (400MHz, $CDCl_3$, δ): 7.38-7.36 (d, $J = 8.6Hz$, 2H), 7.06-7.01 (t, $J = 11.2Hz$, 4H), 6.91-6.89 (d, $J = 8.1Hz$, 1H), 6.11-5.92 (m, 2H), 5.46-5.42 (d, $J = 17.4Hz$, 1H) 5.33-5.30 (d, $J = 10.6 Hz$, 1H), 5.06-5.03 (d, $J = 9.3Hz$, 2H), 4.59-4.58 (d, $J = 5.2Hz$, 2H), 3.35-3.34 (d, $J = 6.7Hz$, 2H).

^{13}C NMR (100MHz, $CDCl_3$, δ): 158.3, 150.8, 137.8, 133.1, 132.3, , 130.2, 129.5, 128.8, 127.7, 117.8 115.6, 115.6, 115.5, 68.9, 39.4.

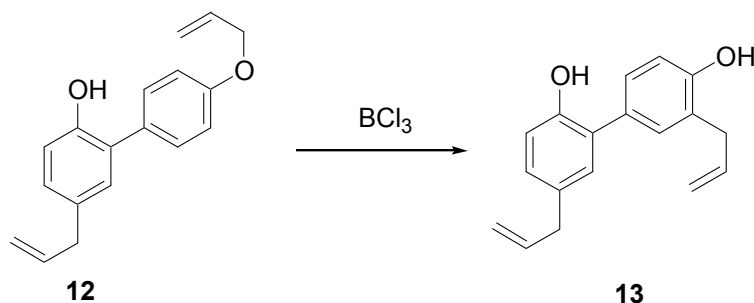
IR (KBr, thin film): 3336, 2918, 2849, 1607, 1506, 1241, 1179 cm^{-1} .

MS-EI m/z: 266 (M^+ , 51), 226 (18), 225 (100), 165 (11), 161 (20), 156 (13), 155 (15), 152 (13), 141 (13), 128 (19), 115 (16), 77 (14).

HRMS-EI (m/z): $[M]^+$ calcd for $C_{18}H_{18}O_2$, 266.1307; found,

266.1314.

(六) 合成 5,3'-Diallyl-biphenyl-2,4'-diol (Honokiol) 13



在 -15°C 下，將 5-Allyl-4'-allyloxy-biphenyl-2-ol **12** (221mg, 0.83mmol) 的 chloroenezene (5mL) 溶液，慢慢加到 BCl_3 (1M in *p*-xylene, 0.83mL, 0.83mmol) 的 chloroenezene (10mL) 溶液中，在 -15°C 攪拌 1 小時，將此溶液倒入冰的甲醇中，以乙醚萃取，乙醚層以飽和碳酸氫鈉水溶液洗，再以水洗，以無水硫酸鎂乾燥，過濾濃縮後，以管柱層析純化，以 silica gel 為固定相，以乙酸乙酯/正己烷(3:10, v/v)為移動相，得產物 **13** (150 mg, 0.56 mmol)，產率 67%。(所需藥品價格合成每克臺幣 12,087 元，加上人事費和雜支 50%，約 18,130 元/克，購得標準品 10 mg/2,400 元，即 240,000 元/克)。

m.p.: $85\sim 87^{\circ}\text{C}$.

^1H NMR (400 MHz, CDCl_3 , δ): 7.23-7.21 (d, $J = 9.5\text{Hz}$, 2H), 7.06-7.02 (d, $J = 14.8\text{Hz}$, 2H), 6.93-6.89 (m, 2H), 6.07-5.93 (m, 2H), 5.23-5.04 (m, 6H), 3.47-3.45 (d, $J = 6.3\text{Hz}$, 2H), 3.35-3.33 (d, $J = 6.7\text{Hz}$, 2H).

^{13}C NMR (100MHz, CDCl_3 , δ): 153.9, 150.7, 137.8, 135.9, 132.2, 131.1, 130.2, 129.6, 128.8, 128.5, 128.5, 127.7, 126.3, 116.9, 115.6, 115.5, 39.4, 35.1

IR(KBr, thin film): 3493, 1638, 1494, 1433, 1224, 1120, 995, 914, 822 cm^{-1} .

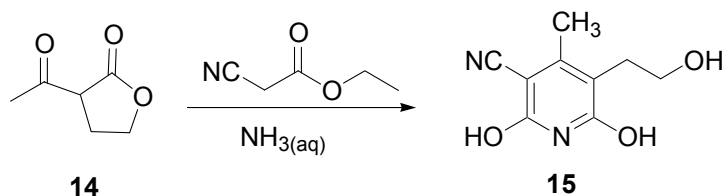
MS m/z : 266(M^+ , 100), 237 (17), 197 (18), 181 (15), 165 (23), 152 (18), 115 (30), 77 (17).

HRMS-EI (m/z): $[\text{M}]^+$ calcd for $\text{C}_{18}\text{H}_{18}\text{O}_2$, 266.1307; found,

266.1304.

四、合成 Gentianine (龍膽鹼)

(一) 合成 2,6-Dihydroxy-5-(2-hydroxy-ethyl)-4-methyl-nicotinonitrile 34



取 Ethyl cyanoacetate (10.36mL, 0.1mol) 和 α -acetobutyrolactone **14** (10.75mL, 0.1mol) 混合後，再加入氨水 (28%-30%, 30 mL)，並攪拌之，開始會放熱並有固體產生，隨著氨水加入固體會再溶解，加完之後靜置隔夜會有沉澱產生，過濾收集固體，並以酒精洗之，得 **15** (8.2g, 42.2mmol)，產率 42%。

^1H NMR (400MHz, D_2O , δ): 3.51-3.48 (t, $J = 7.0\text{Hz}$, 2H), 2.57-2.53 (t, $J = 7.0\text{Hz}$, 2H), 2.16 (s, 3H).

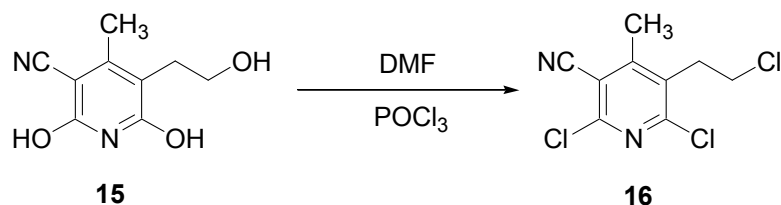
^{13}C NMR (100MHz, D_2O , δ): 165.3, 164.9, 154.8, 121.5, 108.2, 79.6, 60.6, 28.3, 17.8.

IR (KBr, thin film): 3196, 3077, 2908, 2193, 1615, 1376, 1219 cm^{-1} .

MS m/z : 194(M^+ , 16), 176 (76), 175 (55), 164 (22), 163 (100), 105 (17), 92 (22), 78 (22), 77 (24), 76 (28), 70 (26), 66 (33), 65 (40), 64 (37), 63 (32) 52 (35).

HRMS-EI (m/z): [M] $^+$ calcd for $\text{C}_9\text{H}_{10}\text{N}_2\text{O}_3$, 194.0691; found, 194.0698.

(二) 合成 2,6-Dichloro-5-(2-chloro-ethyl)-4-methyl-nicotinonitrile 16



取 **15** (1.5g, 7.7mmol) 置於密閉管中，加入 DMF (0.6mL, 7.7 mol) 再加入 POCl_3 (2.65mL, 23.1mmol)，將瓶蓋鎖緊，於密閉管

中加熱至 180°C，持續加熱 8 小時，冷卻後加入水 15mL，再以乙酸乙酯萃取，以無水硫酸鎂乾燥，過濾濃縮後，以管柱層析純化，以乙酸乙酯/正己烷(1:9, v/v)為移動相，得固體 **16** (1.02g, 4.1 mmol)，產率 53%

^1H NMR (400MHz, CDCl_3 , δ): 3.78-3.75 (t, $J = 7\text{Hz}$, 2H), 3.31-3.27 (t, $J = 7.0\text{Hz}$, 2H), 2.68 (s, 3H).

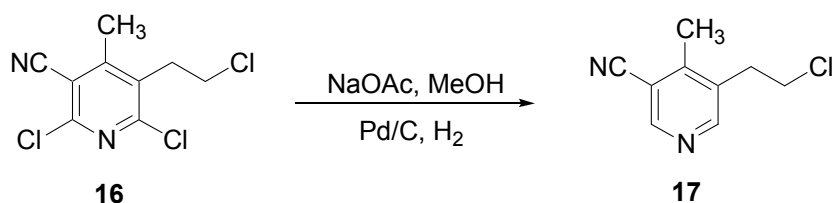
^{13}C NMR (100MHz, CDCl_3 , δ): 155.8, 154.0, 150.6, 130.8, 113.7, 111.3, 41.2, 32.4, 19.8.

IR (KBr, thin film): 2965, 2233, 1543, 1436, 1355, 1200, 1145, 794 cm^{-1} .

MS m/z : 252 ($\text{M}^+ + 4$, 6), 250 ($\text{M}^+ + 2$, 14), 248 (M^+ , 12), 201 (62), 199 (100), 163 (13), 127 (10), 76 (16), 75 (12), 51 (14), 49 (16).

HRMS-EI (m/z): $[\text{M}]^+$ calcd for $\text{C}_9\text{H}_7\text{Cl}_3\text{N}_2$, 247.9675; found, 247.9666.

(三) 合成 5-(2-Chloro-ethyl)-4-methyl-nicotinonitrile **17**



將 **16** (1.02g, 4.1mmol) 溶於甲醇 (20mL)，並加入醋酸鈉 (0.88g) 和 10% Pd/C (0.1g)，在氫氣 (1atm) 下反應 2 天，以矽藻土過濾除去催化劑，並以甲醇洗之，濃縮後加入碳酸氫鈉水溶液，再以乙醚萃取，以無水硫酸鈉乾燥，過濾濃縮後，以管柱層析純化，以乙酸乙酯/正己烷(2:8, v/v)為移動相，得 **17** (0.44g, 2.4mmol)，產率 59%。

^1H NMR (400MHz, CDCl_3 , δ): 8.71 (s, 1H), 8.57 (s, 1H), 3.74-3.70 (t, $J = 7.0\text{Hz}$, 2H), 3.18-3.15 (t, $J = 6.9\text{Hz}$, 2H), 2.57 (s, 3H).

^{13}C NMR (100 MHz, CDCl_3 , δ): 153.5, 151.6, 149.1, 132.8, 116.1, 111.5, 42.9, 33.2, 27.3.

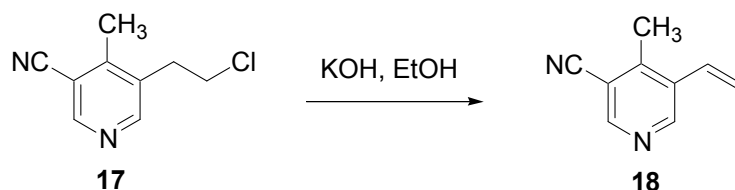
IR (KBr, thin film): 2923, 2853, 2233, 1579, 1413, 1334, 1155, 775,

717 cm^{-1} .

MS m/z : 182 ($M^+ + 2$, 9), 180 (M^+ , 28), 131 (100), 104 (11), 77 (14), 63 (11), 51 (16), 49 (19).

HRMS-EI (m/z): $[M]^+$ calcd for $\text{C}_9\text{H}_9\text{ClN}_2$, 180.0454; found, 180.0451.

(四) 合成 4-Methyl-5-vinyl-nicotinonitrile **18**



取 **17** (0.4436g, 2.4mmol) 和 KOH (100mg) 溶於酒精 (5mL)，在室溫下攪拌 1 小時後，加水稀釋並以乙醚萃取，以硫酸鈉乾燥，濃縮後以管柱層析純化，以乙酸乙酯/正己烷 (2:8, v/v) 為移動相，得 **18** (184.2mg, 1.3mmol)，產率 54%

^1H NMR (400MHz, CDCl_3 , δ): 8.75 (s, 1H), 8.69 (s, 1H), 6.87-6.80 (m, 1H), 5.79-5.75 (d, $J = 17.5\text{Hz}$, 1H), 5.58-5.55 (d, $J = 11.2\text{Hz}$, 1H), 2.55 (s, 3H).

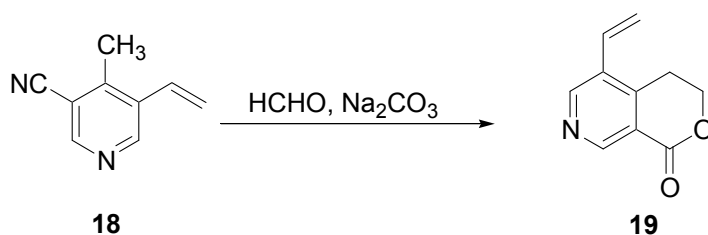
^{13}C NMR (100MHz, CDCl_3 , δ): 151.5, 150.1, 147.3, 133.7, 130.4, 120.2, 116.1, 111.7, 17.5.

IR (KBr, thin film): 2964, 2235, 1579, 1468, 1247, 1158 cm^{-1} .

MS m/z : 144 (M^+ , 3), 143 (6), 132 (12), 131 (100), 77 (14), 63 (11), 51 (16), 49 (19).

HRMS-EI (m/z): $[M]^+$ calcd for $\text{C}_9\text{H}_8\text{N}_2$, 144.0687; found, 144.0684.

(五) 合成 5-Vinyl-3,4-dihydro-pyrano[3,4-c]pyridin-1-one **19**
(Gentianine)



取 18 (72.4mg, 0.5mmol) 和碳酸鈉 (63.8mg, 0.6mmol) 置於密閉管中，再配製稀釋的甲醛溶液 [HCOH (35%, 1mL) 加上水 (8.5mL)]，將此稀釋的甲醛加到密閉管之中，鎖緊瓶蓋，加熱至 100°C-120°C 迴流 10 小時，冷卻後，加入 10% 鹽酸 (5mL)，攪拌 5 小時，再加入 10% 氨水 (5mL)，以二氯甲烷萃取，濃縮後，以管柱層析純化，以 Ethyl acetate/Hexane (3:7, v/v) 為移動相，得 19 (20mg, 114.2mmol)，產率 23%。(所需藥品價格合成每克臺幣 49,050 元，加上人事費和雜支 50%，約 73,575 元/克)。

m.p.: 80~82°C

^1H NMR (400MHz, CDCl_3 , δ): 9.17 (s, 1H), 8.84 (s, 1H), 6.82-6.75 (m, 1H), 5.82-5.78 (d, $J = 17.5\text{Hz}$, 1H), 5.60-5.57 (d, $J = 11.2\text{Hz}$, 1H), 4.57-4.54 (t, $J = 6.0\text{Hz}$, 2H), 3.10-3.07 (t, $J = 6.0\text{Hz}$, 2H).

^{13}C NMR (100MHz, CDCl_3 , δ): 163.5, 151.2, 150.7, 144.6, 130.8, 129.7, 121.1, 120.4, 66.22, 24.4.

IR (KBr, thin film): 2923, 1723, 1394, 1299, 1129, 1044, 778 cm^{-1} .

MS m/z : 175 (M^+ , 29), 147 (20), 123 (15), 119 (17), 117 (53), 111 (919), 109 (20), 97 (35), 95 (32), 90 (26), 89 (24), 85 (30), 83 (40), 81 (34), 71 (49), 69 (59), 67 (22), 57 (100), 56 (922), 55 (61).

HRMS-EI (m/z): $[\text{M}]^+$ calcd for $\text{C}_{10}\text{H}_9\text{NO}_2$, 175.0633; found, 175.0626.

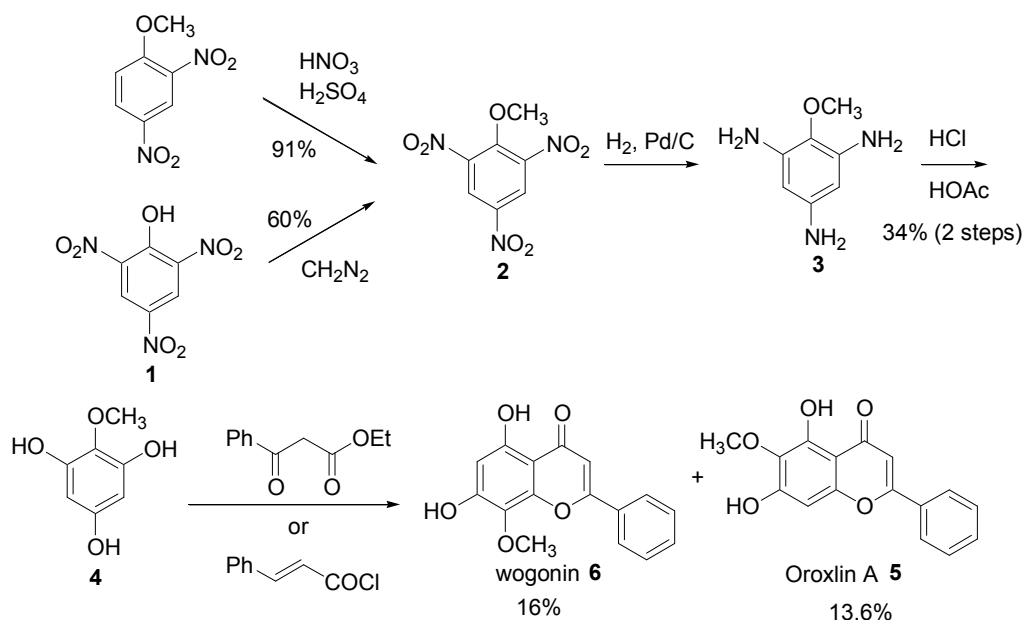
五、中藥成分之萃取和定量：龍膽

將由中藥店購得之龍膽以咖啡磨豆機磨成粉末，秤取龍膽粉末 10 g，加濃氨水 (25-30%) 50mL，在室溫下 (約 25°C) 攪拌 4 小時，然後加氯仿 50mL，加熱迴流 12 小時 (油浴溫度約 100°C)，冷卻至室溫，加入二氯甲烷 50mL，攪拌 5 分鐘，以棉花塞住漏斗，抽氣過濾除去龍膽粉末殘渣，並以 50mL 二氯甲烷洗龍膽粉末殘渣，二氯甲烷濾液呈黃色，將此濾液以 10% 硫酸萃取三次 (20mL x 3)，硫酸萃取液以濃氨水調至鹼性 (pH 約為 9)，以二氯甲烷萃取三次 (30mL x 3)，二氯甲烷萃取液以無水硫酸鎂乾燥，過濾濃縮後得黃棕固體 (13.7mg, 13.2mg, 10.0mg)，將之以製備型薄層色層分析 (PTLC) 純化，得白色固體。

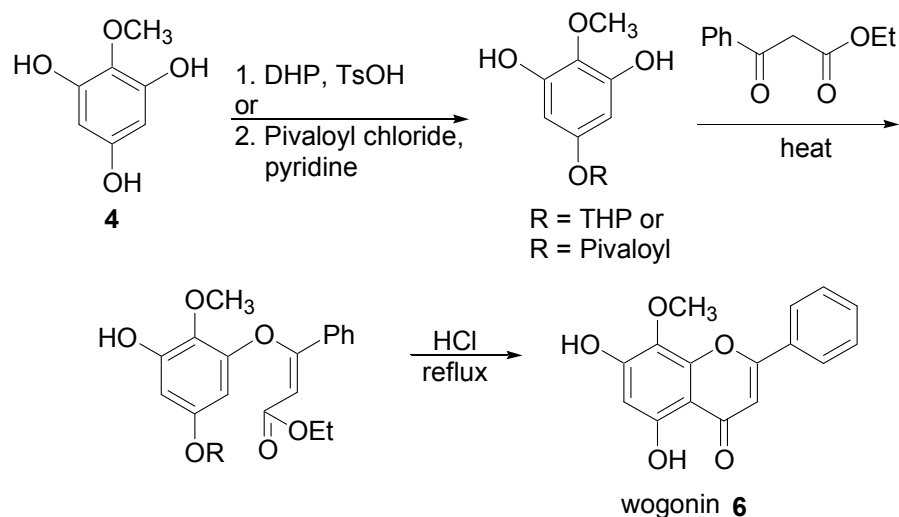
參、結果

一、合成 Wogonin (漢黃芩素)

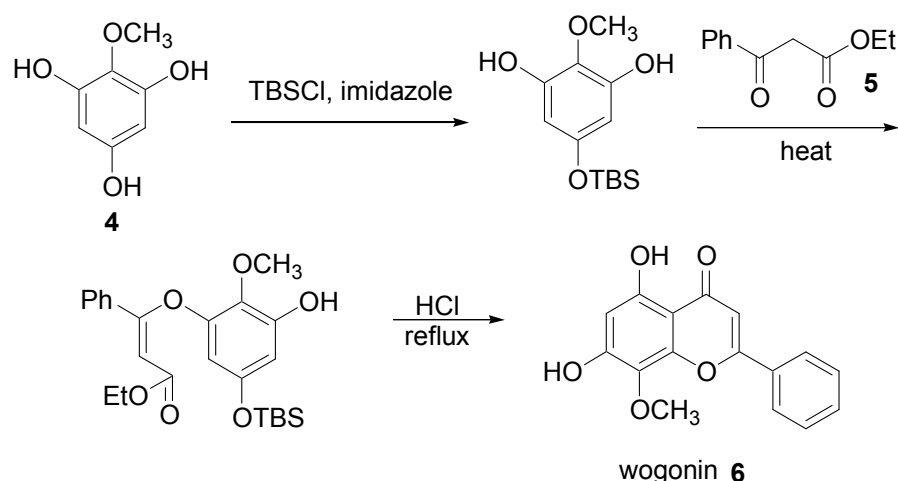
漢黃芩素 (wogonin **6**) 可以苦味酸 **1** 起始物，用疊氮甲烷處理，產生 2,4,6-三硝基甲氧基苯 **2**，另一方法是將 2,4-dinitroanisole 硝化得化合物 **2**，以鈀/碳為催化劑，在 60 psi 氫氣中將硝基還原成胺基，產生 2,4,6-三胺基甲氧基苯 **3**，在鹽酸的醋酸溶液中加熱即可生成 2,4,6-三羥基甲氧基苯 **4**，將之與苯甲醯基乙酸乙酯一起加熱至 200°C 即可生成 Wogonin (漢黃芩素) **6** 和 oroxylin A (**5**) (千層紙黃素)。上述方法雖可得到 Wogonin (漢黃芩素) **6**，但主要產物是 Oroxylin A (千層紙黃素)，因此改用另一種方法。



上述方法雖可得到漢黃芩素 **6** 和 oroxylin A (**5**) (千層紙黃素)，但主要產物是千層紙黃素，而漢黃芩素只有微量，改善方式，先將化合物 **4** 之 4-OH 以 THP 或 Pivaloyl 保護再與苯甲醯基乙酸乙酯反應，預期此方法將可產生漢黃芩素 **6** 為主要產物，但還是沒有拿到 wogonin。



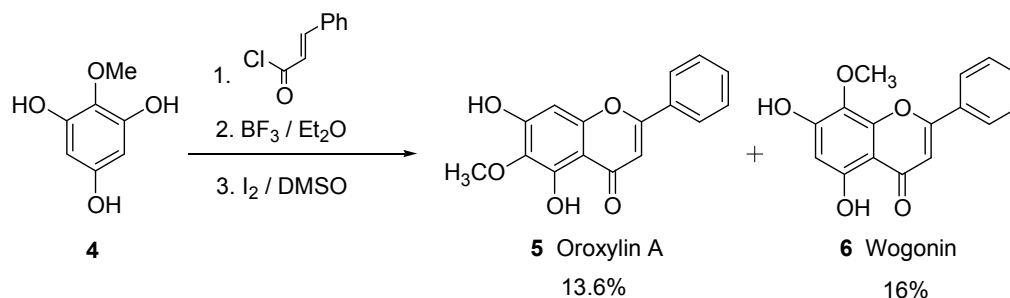
當 OH 以 THP 保護時，並不能完成控制只保護 4-OH，有一部分保護二個 OH，有一部分與苯環反應。因此改換成 pivalate 保護，發生相似的問題。因此改用 TBS ether 保護基，可以得到只保護一個 OH 的產物，但還是沒有拿到 wogonin。



二、改良合成 Wogonin (漢黃芩素)

取雙口瓶，加入化合物 4，cinnamoyl chloride 和 dichloroethane，將反應瓶置於 85°C 油浴中，再在慢慢滴加 $\text{BF}_3/\text{Et}_2\text{O}$ ，剛開始反應會劇烈冒泡，待滴加完後用 TLC 偵測反應，約 1 至 1.5 小時原料消失。將反應溶液倒入冰水中，再用乙酸乙酯萃取，用無水硫酸鎂乾燥，過濾濃縮後，以管柱層析純化，得紅黃色固體，產率 88%。將紅黃色固體，溶於無水 DMSO 中，再加入碘，將反應置於油浴中，從室溫緩慢加熱至 85°C，用 TLC 偵測反應，若原料漸漸消失且在 Oroxylin A 上方出現另一新點，則在升高溫度至 120°C，用 TLC 偵測反應，待新

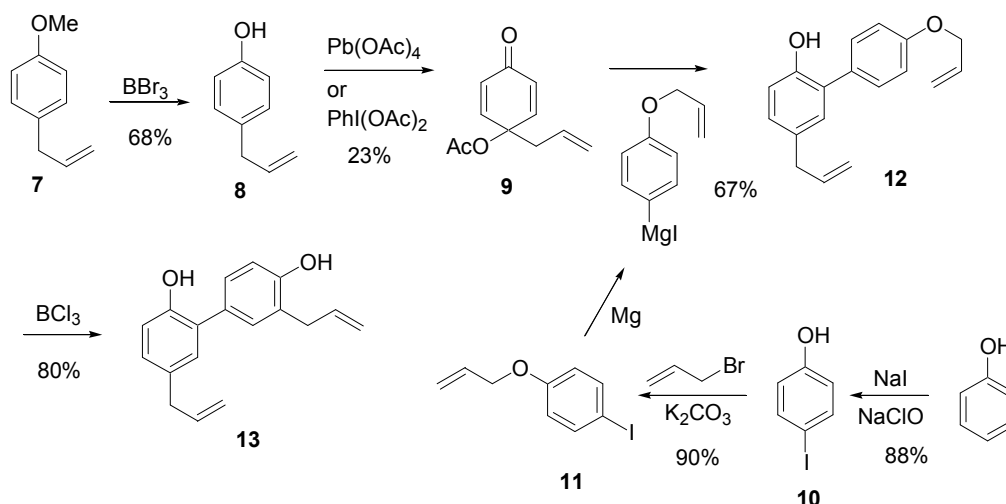
點消失產生 Oroxylin A 及 Wogonin 時（約需 2 小時），終止反應。加入乙酸乙酯，再用 1N HCl 洗有機層 2 次，用水洗有機層 1 次，以管柱層析純化，得 Oroxylin A，產率 13.6% 及 Wogonin，產率 16%。Wogonin 總產率 4.95%。Oroxylin A 總產率 4.21%。



三、合成 Honokiol（和厚朴酚）

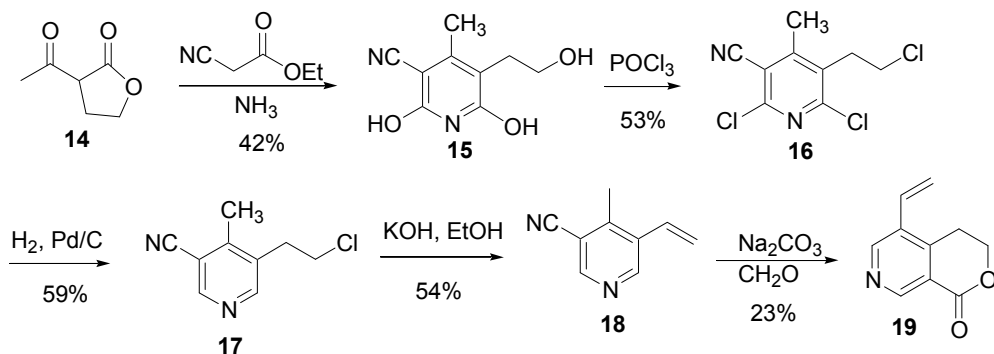
取 4-Allylanisole 7 加入乾燥的二氯甲烷，將反應瓶置於冷浴並控溫在 -78°C ，慢慢以針加入 1M BBr_3 ，加完之後，讓溫度從 -78°C 慢慢回室溫，約 5 個小時回到 15°C ，小心加入冰水，有機層加入 5% NaOH ，水層以 1N HCl 酸化至 $\text{pH} = 6$ ，再以乙醚萃取，濃縮後以管柱層析純化，得產物 8，產率 68%。將醋酸鉛 ($\text{Pb}(\text{OAc})_4$) 加到攪拌中之 4-allylphenol 8 的醋酸溶液中，在室溫下攪拌 15 分鐘後，倒入冰水中並以乙醚萃取，以管柱層析純化，得產物 9，產率 8.3%。若改用 $\text{PhI}(\text{OAc})_2$ 替代醋酸鉛產率提升至 23%，且反應較乾淨易純化。取酚溶於甲醇，加入碘化鈉和氫氧化鈉，將此混合液冷卻至 0°C ，再將 1% 漂白水慢慢滴入，並控溫在 $0-3^\circ\text{C}$ ，每一滴滴入會出現紅色，並馬上退掉，滴完控溫在 $0-20^\circ\text{C}$ 攪拌 1 小時，加入 10% $\text{Na}_2\text{S}_2\text{O}_3$ ，用 5% 鹽酸水溶液調到 $\text{pH} = 7$ ，會有結晶發生，若沒有結晶產生，加入乙醚，有機層以飽合食鹽水洗，以無水硫酸鎂乾燥，過濾濃縮，得產物 10，產率 88%。取 4-Iodo-phenol 10 溶於丙酮，加入碳酸鉀和 Allylbromide，室溫下反應兩天，純化得產物 11，產率 90%。將鎂懸浮於乙醚中，再將少量之 4-allyloxyiodobenzene 11 和 methyl iodide 的乙醚溶液加入，在氫氣下迴流約 1 小時，將剩餘之 4-allyloxyiodobenzene 的乙醚溶液一滴一滴的加入，加完後迴流約 4 小時直到鎂消失。在 -15°C 氫氣下，將 9 的乙醚溶液一滴一滴的加到剛製備好之 Grignard reagent 中，在 -15°C 下攪拌 30 分鐘，將此溶液倒入冰的飽和氯化銨水溶液中，以乙醚萃取，純化得產物 12，產率 67%。在 -15°C 氫氣下，將 12 的 chlorobenzene 溶液慢慢加到 BCl_3 的 chlorobenzene 溶液中，

在 -15°C 下攪拌 1 小時，將此溶液倒入冰的甲醇中，以乙醚萃取，純化得產物 13，產率 80%。總產率 8.38%。



四、合成 Gentianine (龍膽鹼)

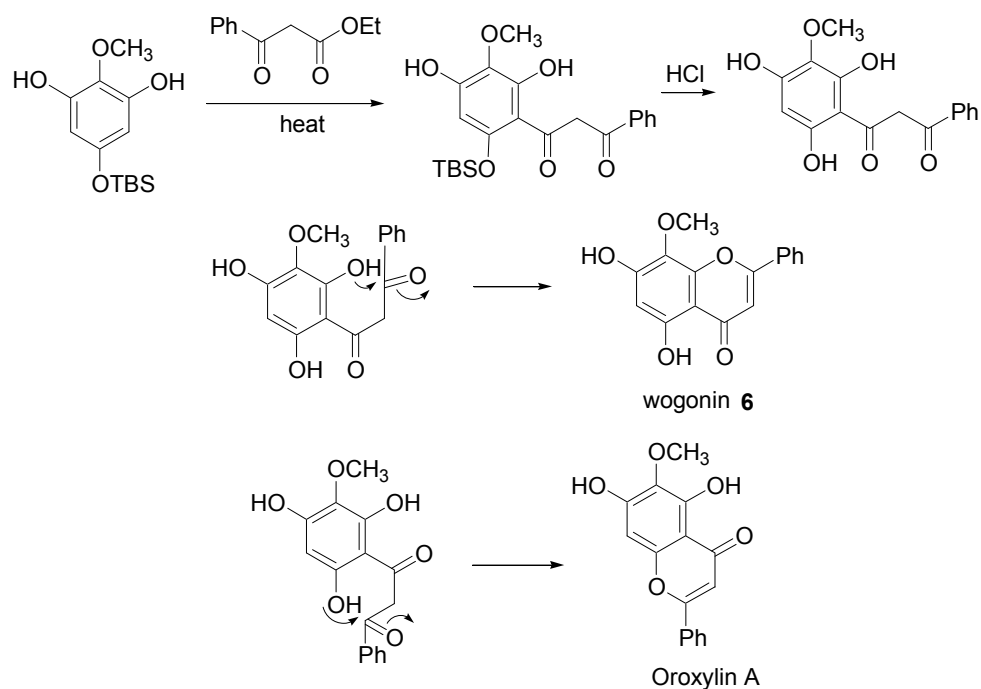
取 Ethyl cyanoacetate 和 α -acetobutyro lactone 14 混合後，再加入氨水，並攪拌之，開始會放熱並有固體產生，隨著氨水加入固體會再溶解，加完之後靜置隔夜會有沉澱產生，過濾收集固體，得 15，產率 42%。取 15 置於密閉管中，加入 DMF 再加入 POCl_3 ，將瓶蓋鎖緊，於密閉管中加熱至 180°C ，持續加熱 8 小時，冷卻後加入水，再以乙酸乙酯萃取純化得固體 16，產率 53%。將 16 溶於甲醇，並加入醋酸鈉和 10% Pd/C，在氫氣下反應 2 天，純化得 17，產率 59%。取 17 和 KOH 溶於酒精，在室溫下攪拌 1 小時後，加水稀釋並以乙醚萃取，純化得產物 18，產率 54%。取 18 和碳酸鈉置於密閉管中，將稀釋的甲醛加到密閉管之中，鎖緊瓶蓋，加熱至 100°C - 120°C 迴流 10 小時，冷卻後，加入 10% 鹽酸，攪拌 5 小時，再加入 10% 氨水，以二氯甲烷萃取純化，得 19 產率 23%。總產率 1.63%。



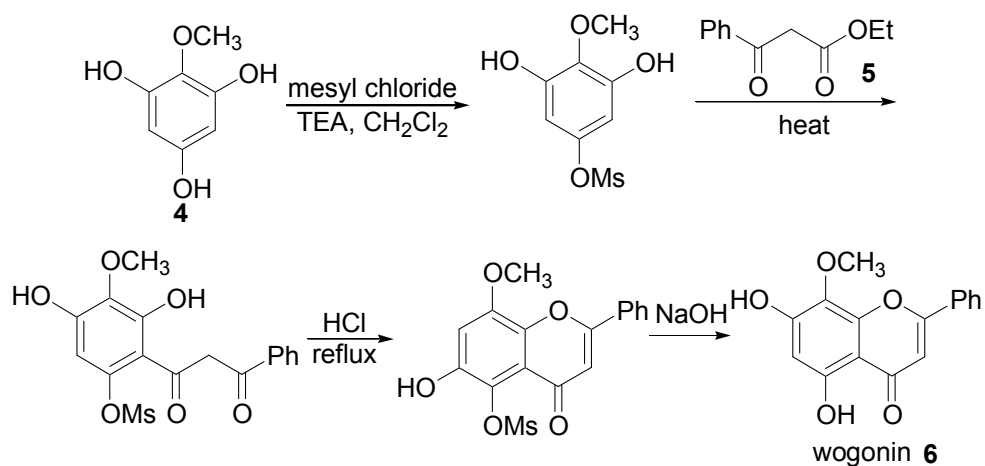
肆、討論

一、Wogonin 的合成

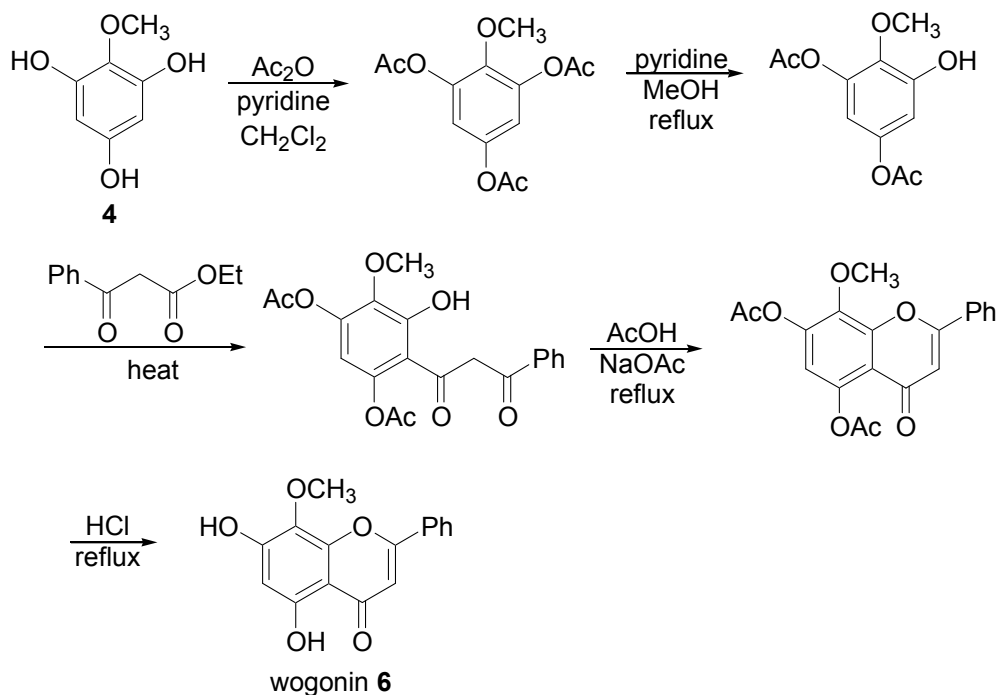
當 OH 以 TBS ether 保護時，雖可以得到只保護一個 OH 的產物，但進行與 ethyl benzoylacetate 接合反應後再進行合環，結果還是 oroxylin A 為主要產物，因此其反應機制預測如下圖。



由上圖之機制推測，該保護基必須在強酸又高溫下能穩定存在，因此改用 methanesulfonate 保護基，可以達到此需求。當合環成所需之結構後，必須將 methanesulfonate 切除，須用強鹼條件，可能會有副反應，試反應結果副產物很多，因此放棄此途徑。

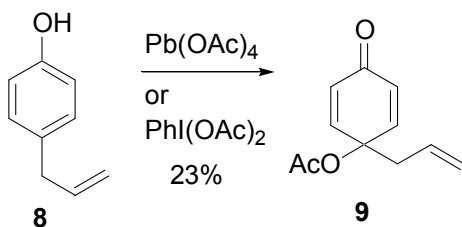


另闢途徑，將化合物 4 先以醋酸酐處理產生 3 個醋酸酯保護基，接著在甲醇和吡啶溶液中迴流即可得 2 個醋酸酯保護基的產物，將 2 個醋酸酯保護基的產物與化合物 ethyl benzoylacetate 一起加熱，緊接著在醋酸和醋酸鈉溶液中迴流即可產生 2 個醋酸酯保護基的 wogonin，再於濃鹽酸中迴流，即可得 wogonin 6，此法也沒拿到預期產物。

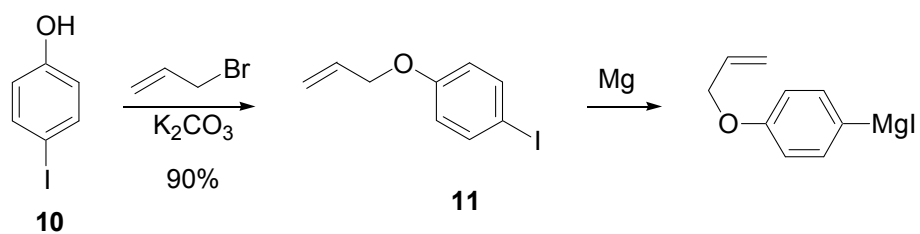


二、Honokiol 的合成

在合成化合物 9 時，起初用醋酸鉛當氧化劑，但產率只有 8.3%，且副產物不易分離，在第二次專業諮詢會議時，委員建議改用 $\text{PhI}(\text{OAc})_2$ 當氧化劑，產率提升至 23%。

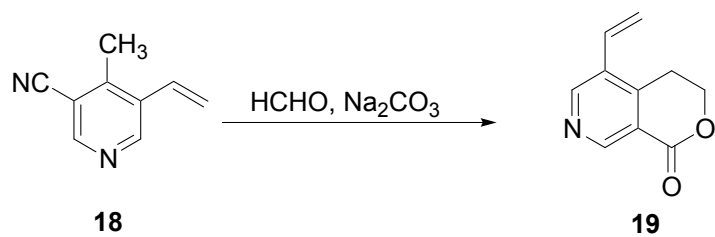


化合物 11 合成出來後，應馬上與鎂作用製備成 Grignard reagent，放置隔夜即很難得到 Grignard 反應之產物。



三、Gentianine 的合成

Gentianine 之合成在最後一步，文獻報導用碳酸氫鈉與甲醛水溶液一起加熱，產率偏低，改用碳酸氫鈉產率提升至 23%。



伍、結論與建議

在合成 wogonin, honokiol, gentianine 時都有一些產率偏低的反應，對總產率的影響甚大，雖然總產率 wogonin (4.95%), honokiol (8.38%), gentianine (1.63%)，但相對於由藥材中提取，應是更經濟有效率。

合成 wogonin 費用 10,907 元/克，購得標準品 88,000 元/克。合成 honokiol 費用 18,130 元/克，購得標準品 240,000 元/克。合成 gentianine 費用 73,575 元/克。由合成方法取得標準品有其經濟價值。

誌謝

本研究計畫承蒙行政院衛生署中醫藥委員會，計畫編號 CCMP95-TP-011 提供經費贊助，使本計畫得以順利完成，特此誌謝。

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柒、圖表

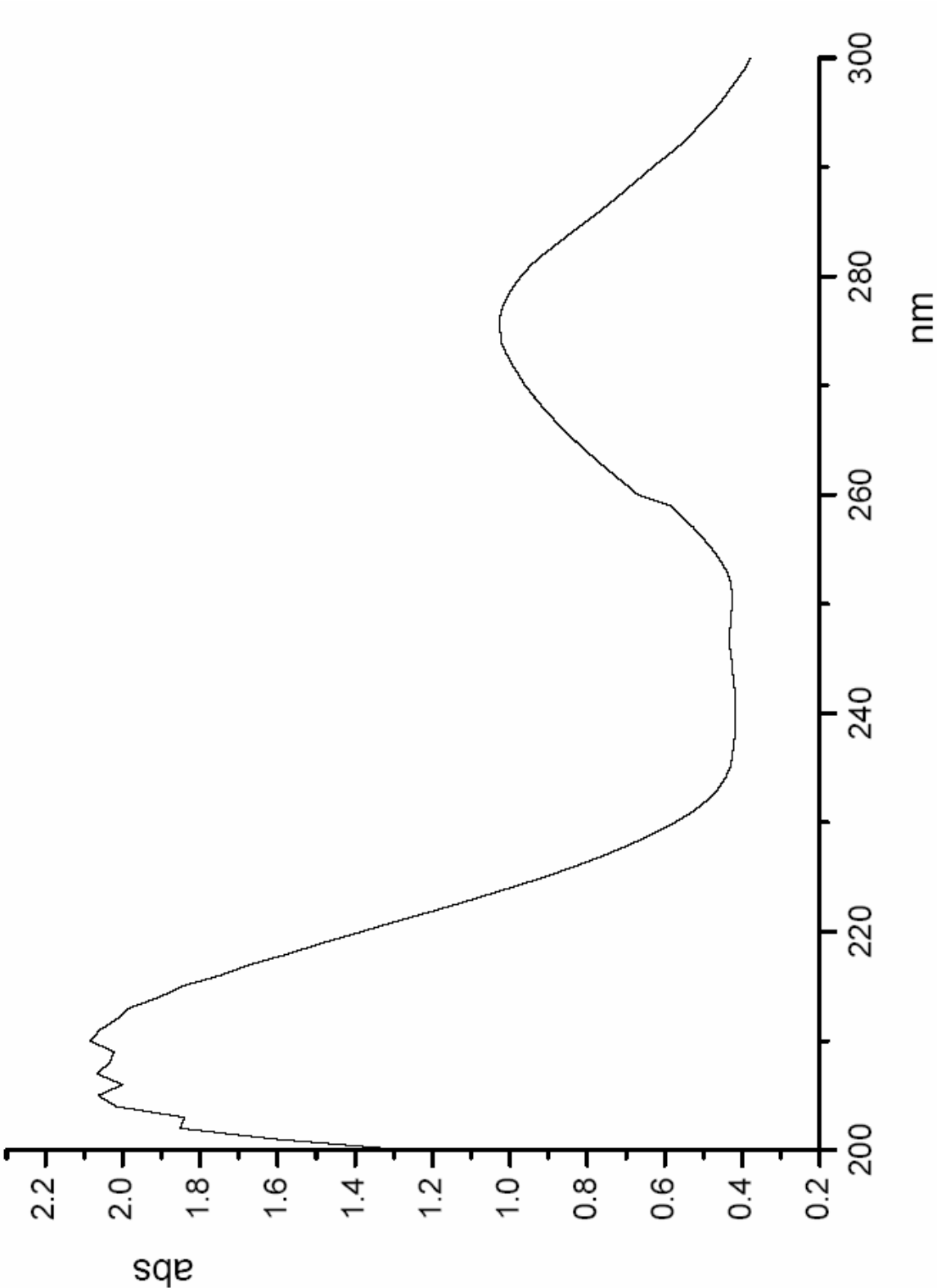
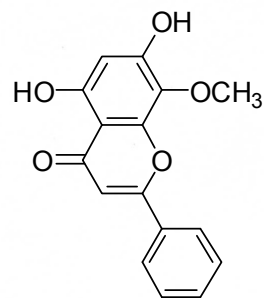
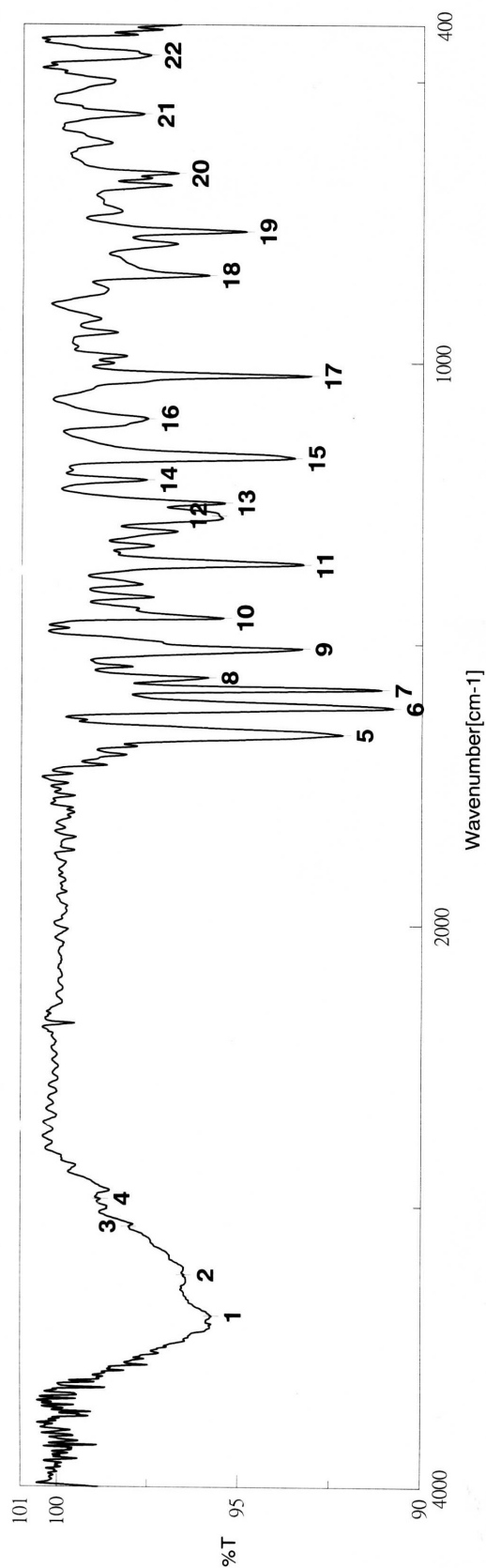


圖 1 W-UV



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Operator	2006/11/12 11:20AM		
File Name	lauchun Cheng		
Sample Name	big-a6		
Comment	big a6		

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9: 1508.06, 9.33E+01	11: 1358.60, 9.33E+01	12: 1272.79, 9.56E+01
13: 1249.65, 9.54E+01	15: 1169.62, 9.35E+01	16: 1100.19, 9.75E+01
17: 1023.05, 9.31E+01	19: 766.57, 9.49E+01	20: 663.39, 9.67E+01
21: 558.29, 9.77E+01	22: 454.15, 9.75E+01	

圖 2 W-IR

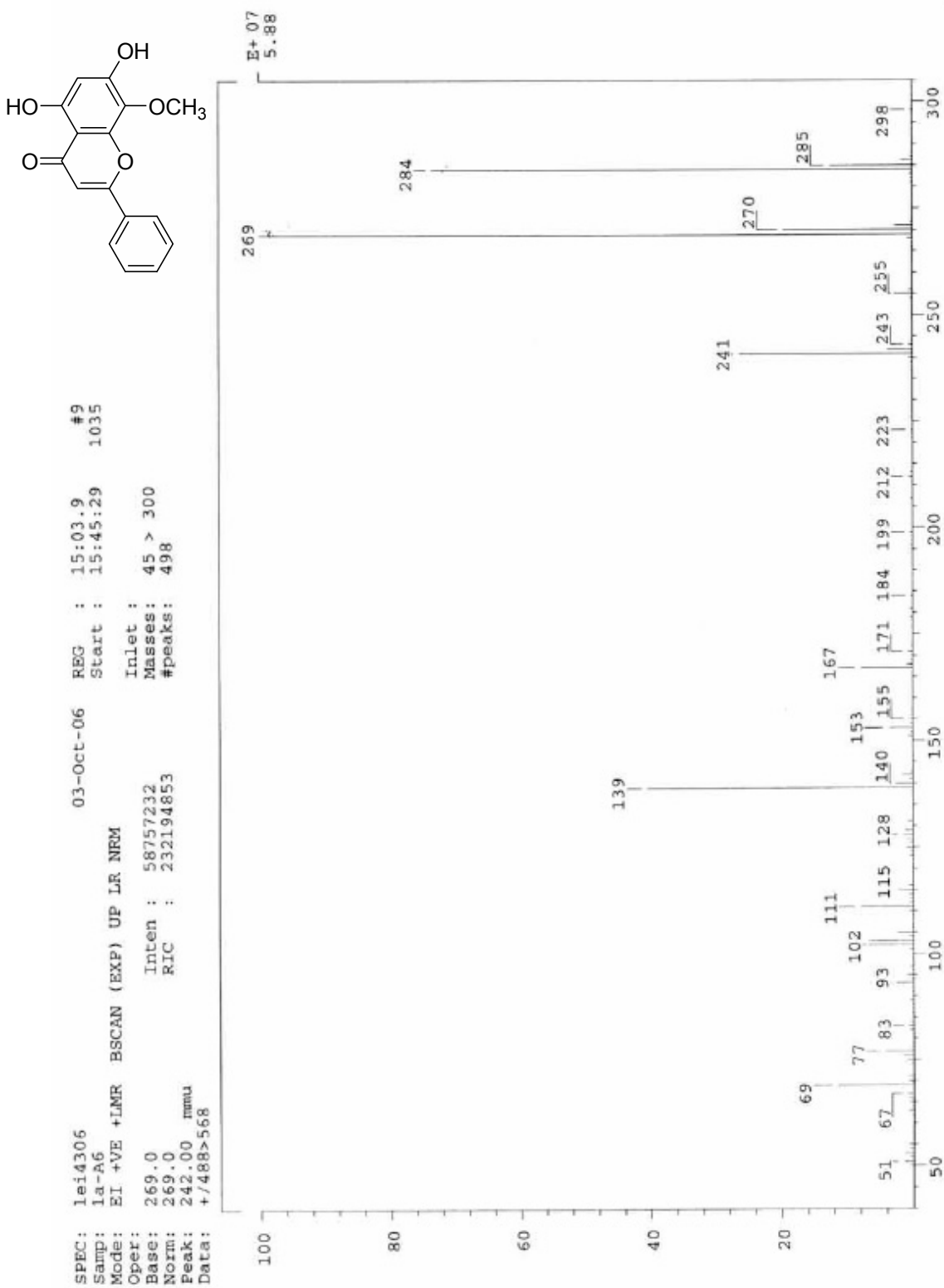


圖 3 W-MS

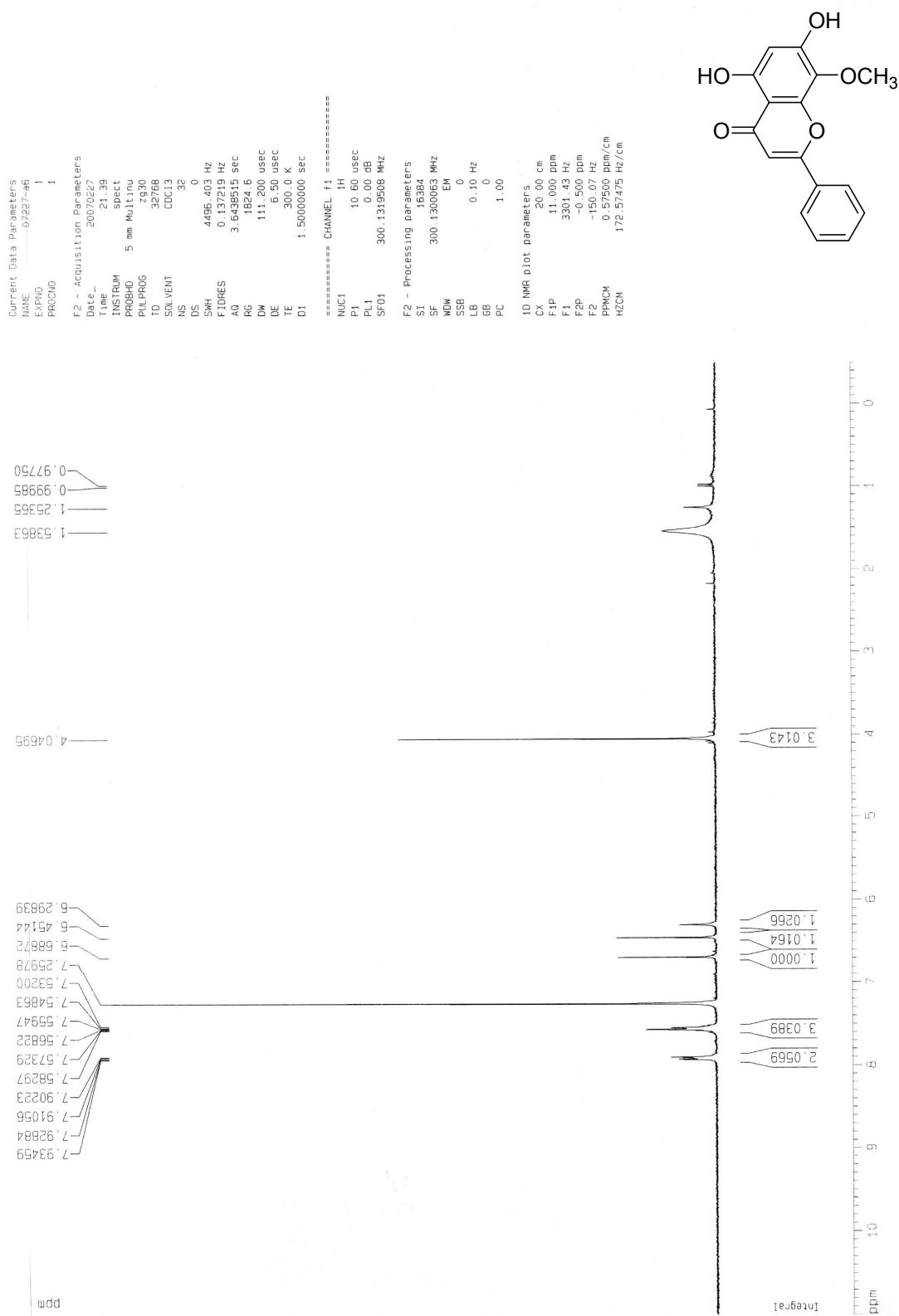


圖 4 W-H NMR

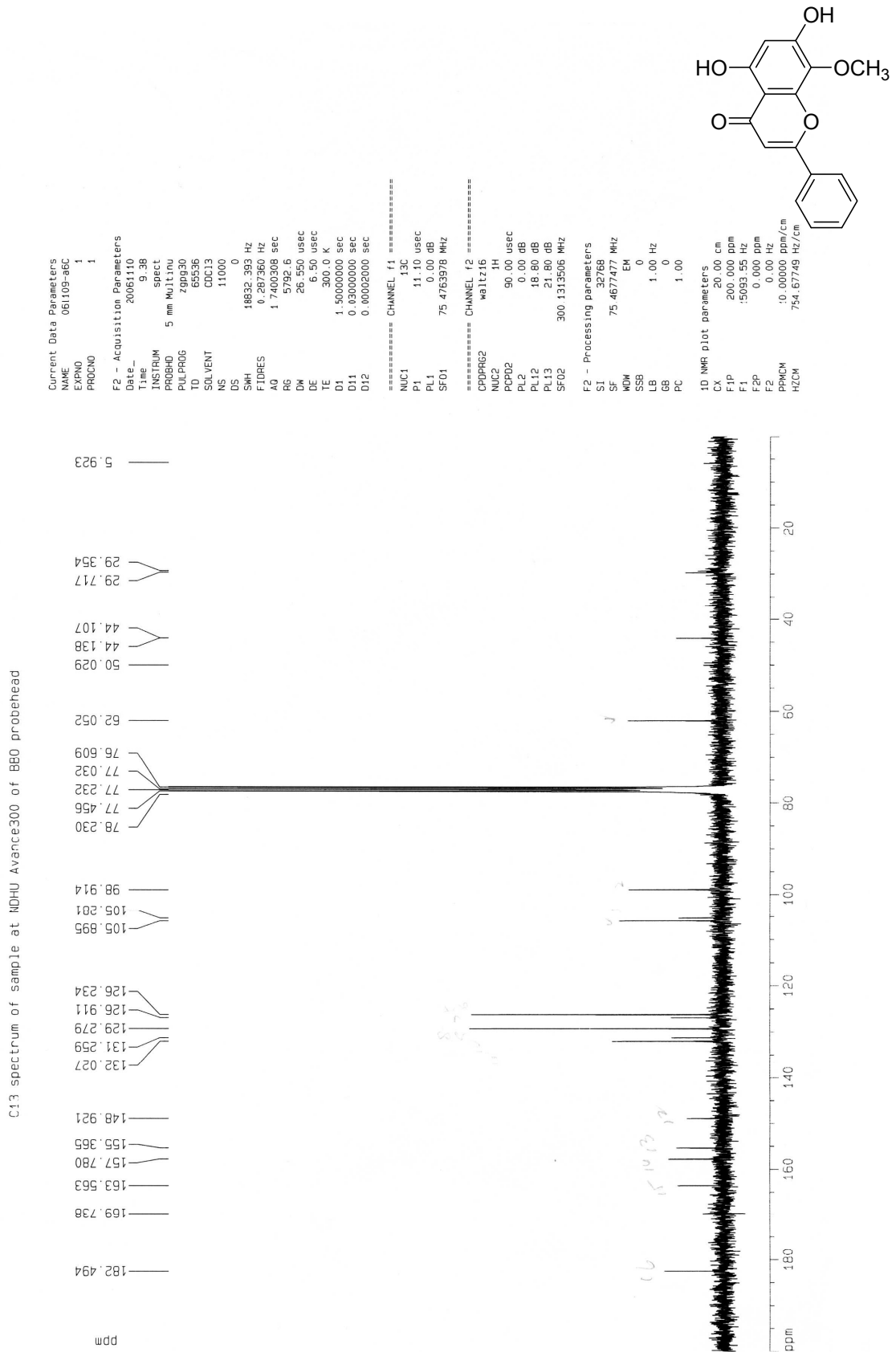


圖 5 W-C NMR

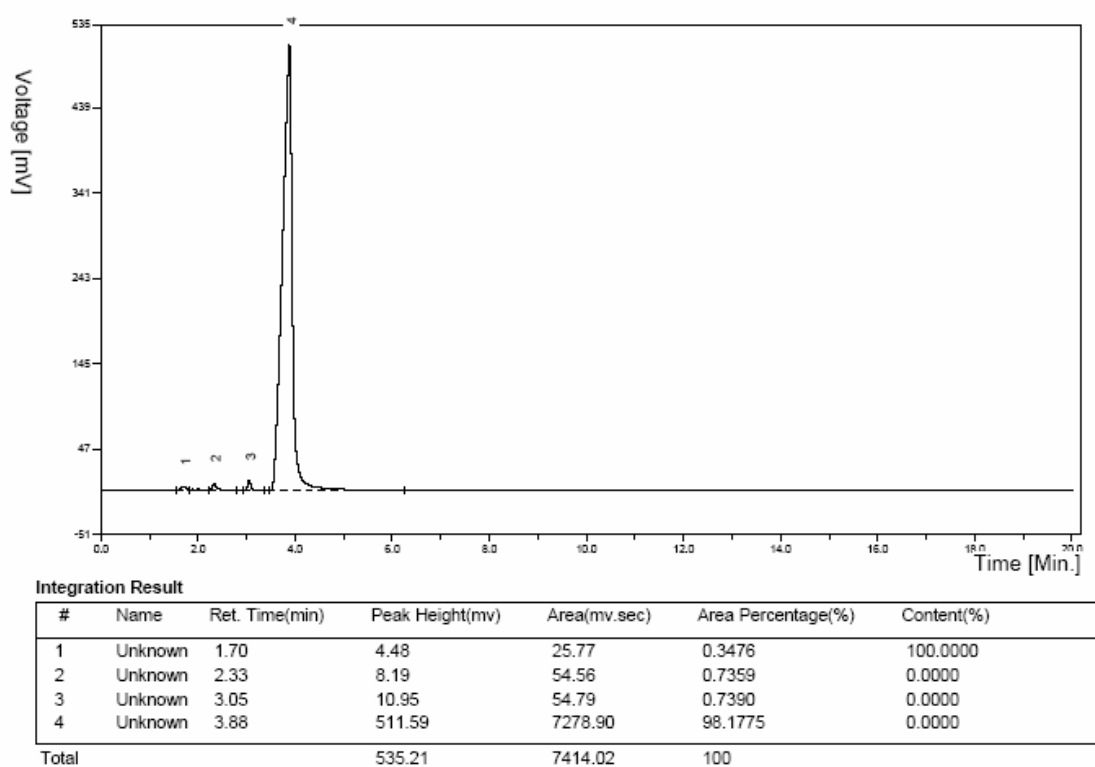


圖 6 W-HPLC

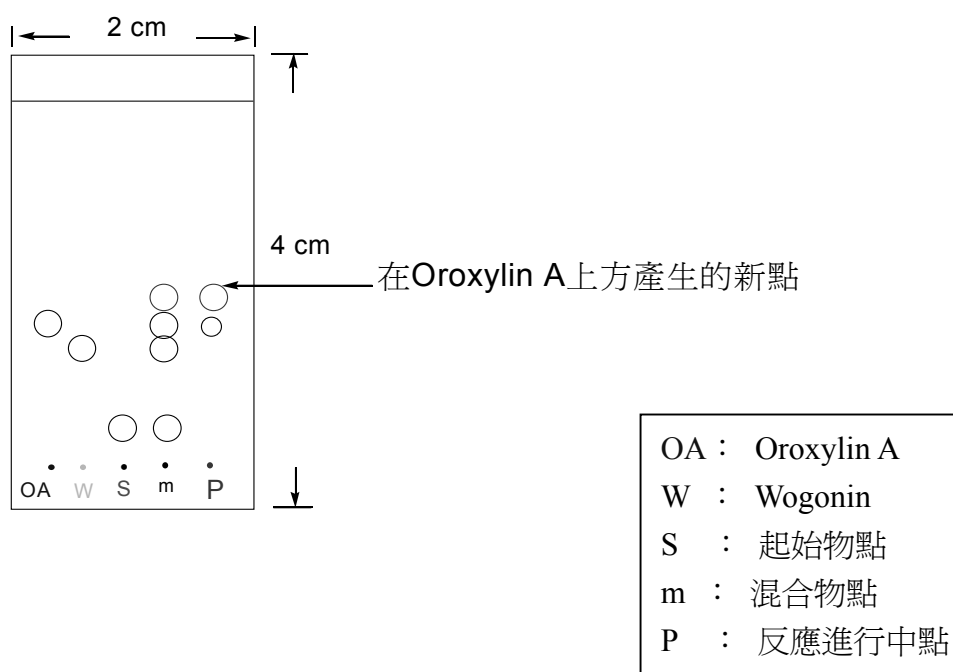
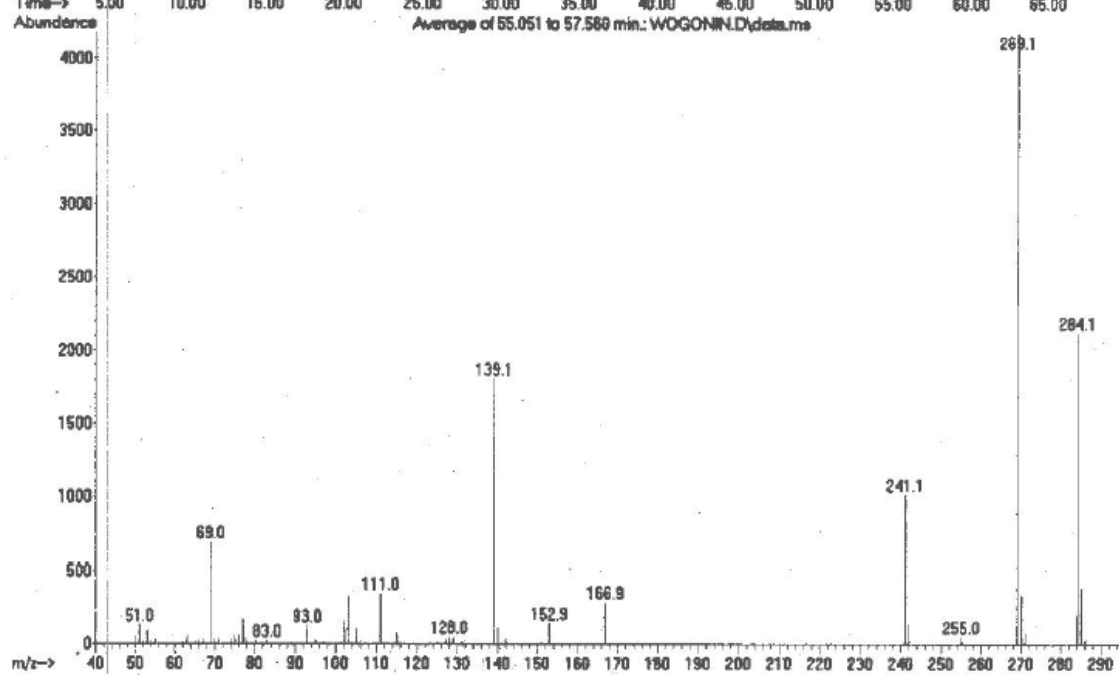
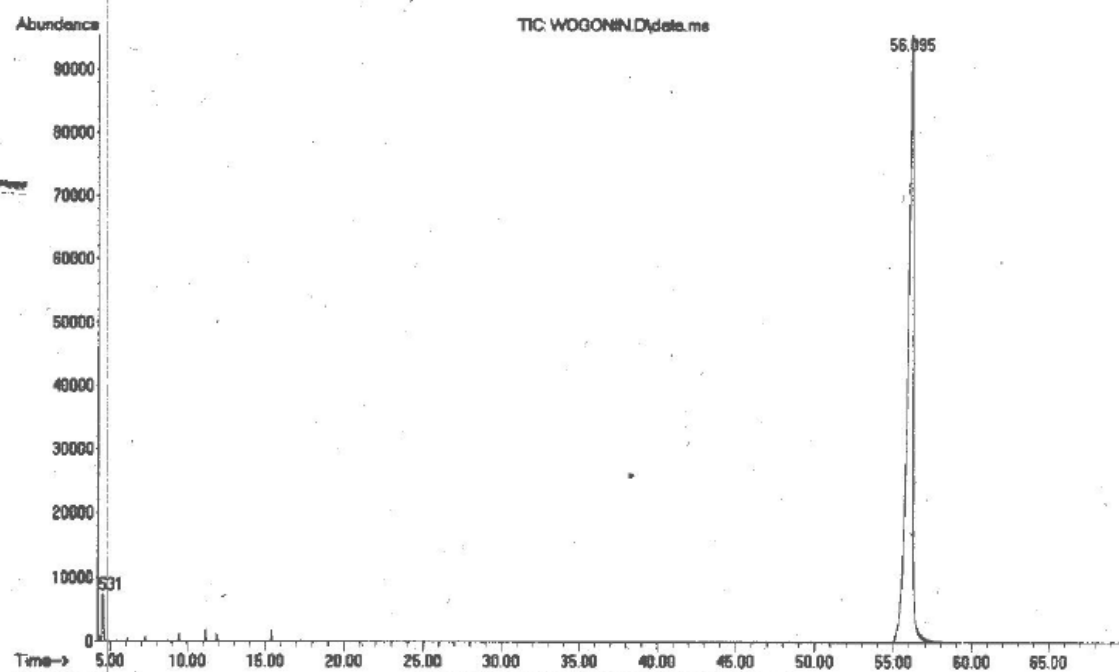


圖 7 W-TLC

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 Instrument: 5975C MSD
 Sample Name:
 Misc Info:
 Vial Number: 1



Area Percent Report

Data Path : C:\msdchem\1\DATA\G1.D\
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 Acq On : 13 Nov 2007 18:24
 Operator :
 Sample :
 Misc :
 ALS Vial : 1 Sample Multiplier: 1

Integration Parameters: autoint1.e
 Integrator: ChemStation

Method : C:\msdchem\1\METHODS\TFA-SCAN.M
 Title : PURE MA IN MEOH

Signal : TIC: WOGONIN.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.469	375	379	423	M5	7497	427833	1.92%	1.882%
2	4.872	489	490	493	M	167	840	0.00%	0.004%
3	4.893	495	496	497	M	181	368	0.00%	0.002%
4	4.904	499	499	501	M	171	278	0.00%	0.001%
5	5.381	629	630	634	M	189	1370	0.01%	0.006%
6	5.451	646	649	651	M	211	1137	0.01%	0.005%
7	6.137	826	837	843	M	579	12028	0.05%	0.053%
8	6.634	973	974	975	M	151	150	0.00%	0.001%
9	7.275	1149	1150	1155	M	865	5420	0.02%	0.024%
10	8.737	1549	1551	1554	M	179	905	0.00%	0.004%
11	9.286	1702	1702	1707	M	146	157	0.00%	0.001%
12	9.487	1752	1757	1761	M2	1287	13673	0.06%	0.060%
13	56.105	14248	14558	15088	M5	95911	22267252	100.00%	97.958%

Sum of corrected areas: 22731408

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圖 8 W-GC

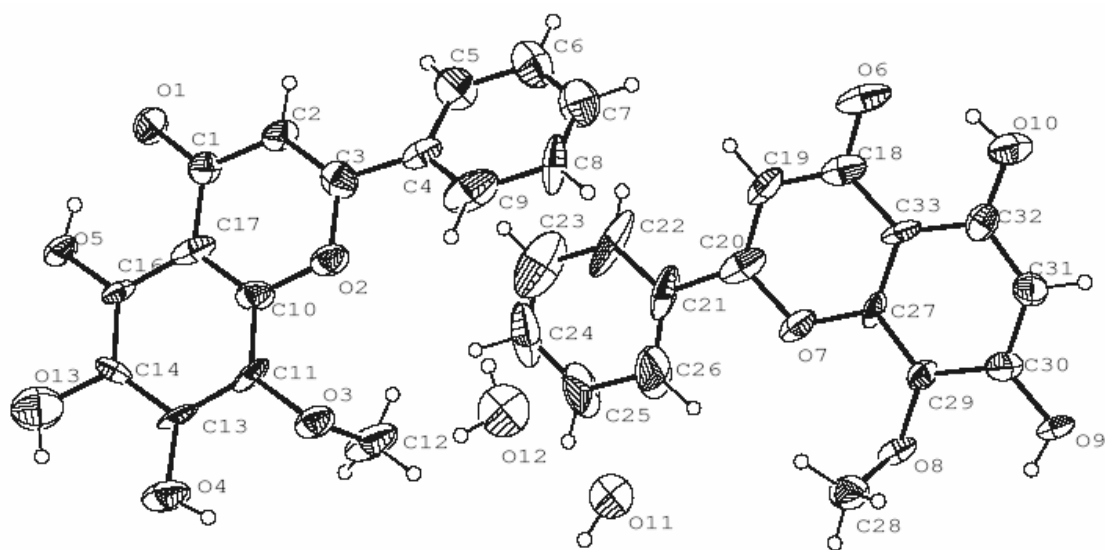


圖 9 Wogonin X-ray 單晶繞射結構圖

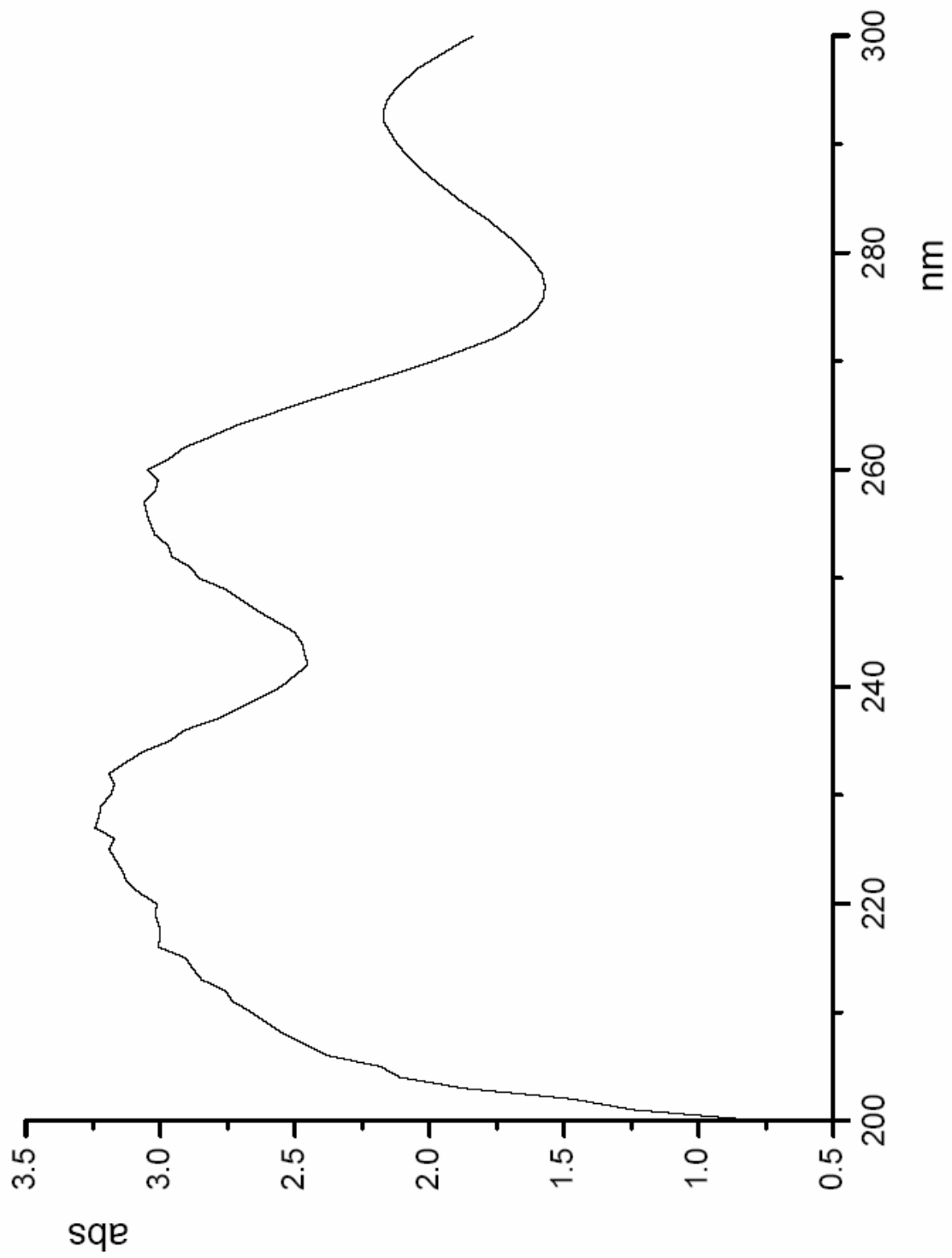


圖 10 H-UV

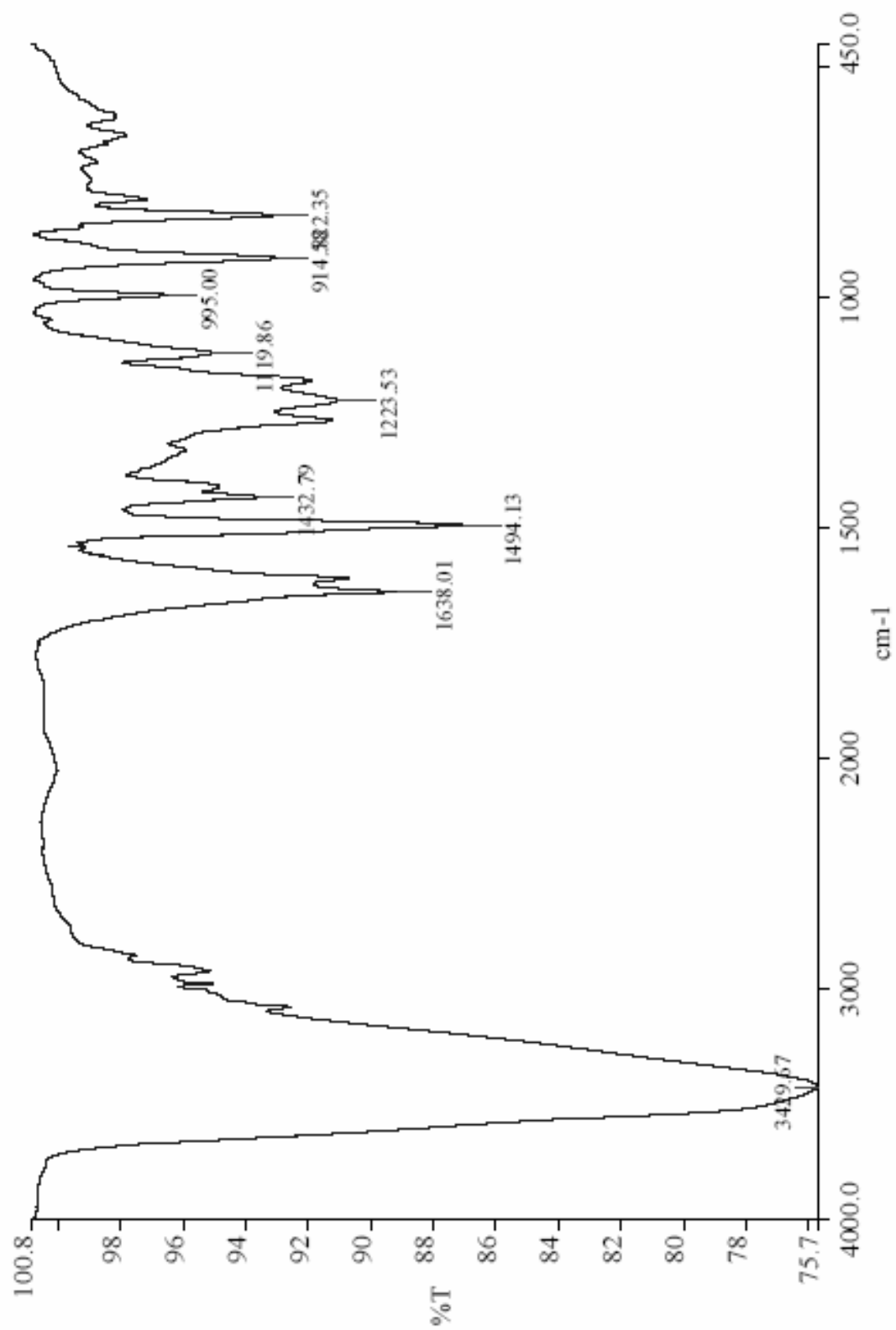


圖 11 H-IR

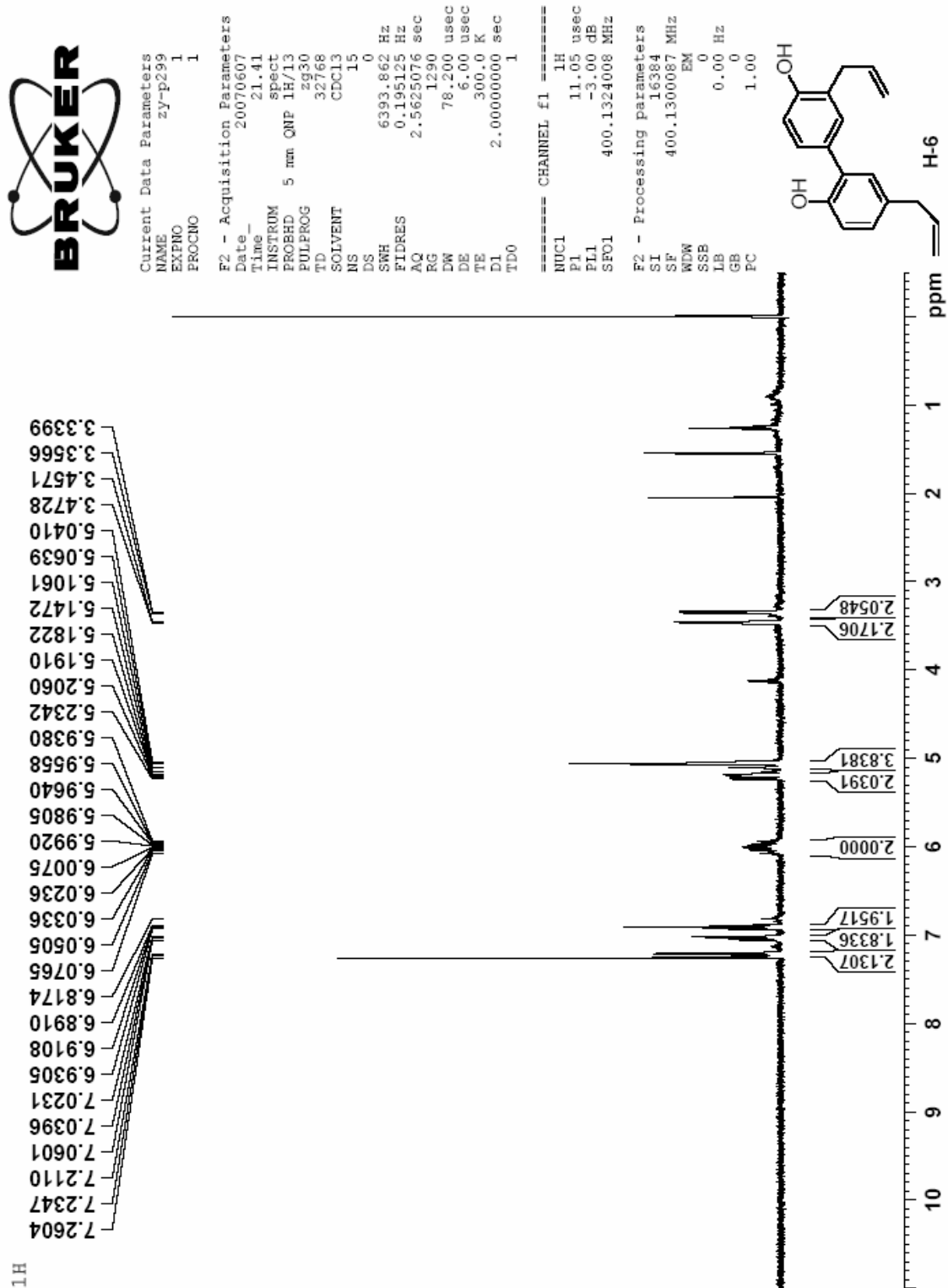
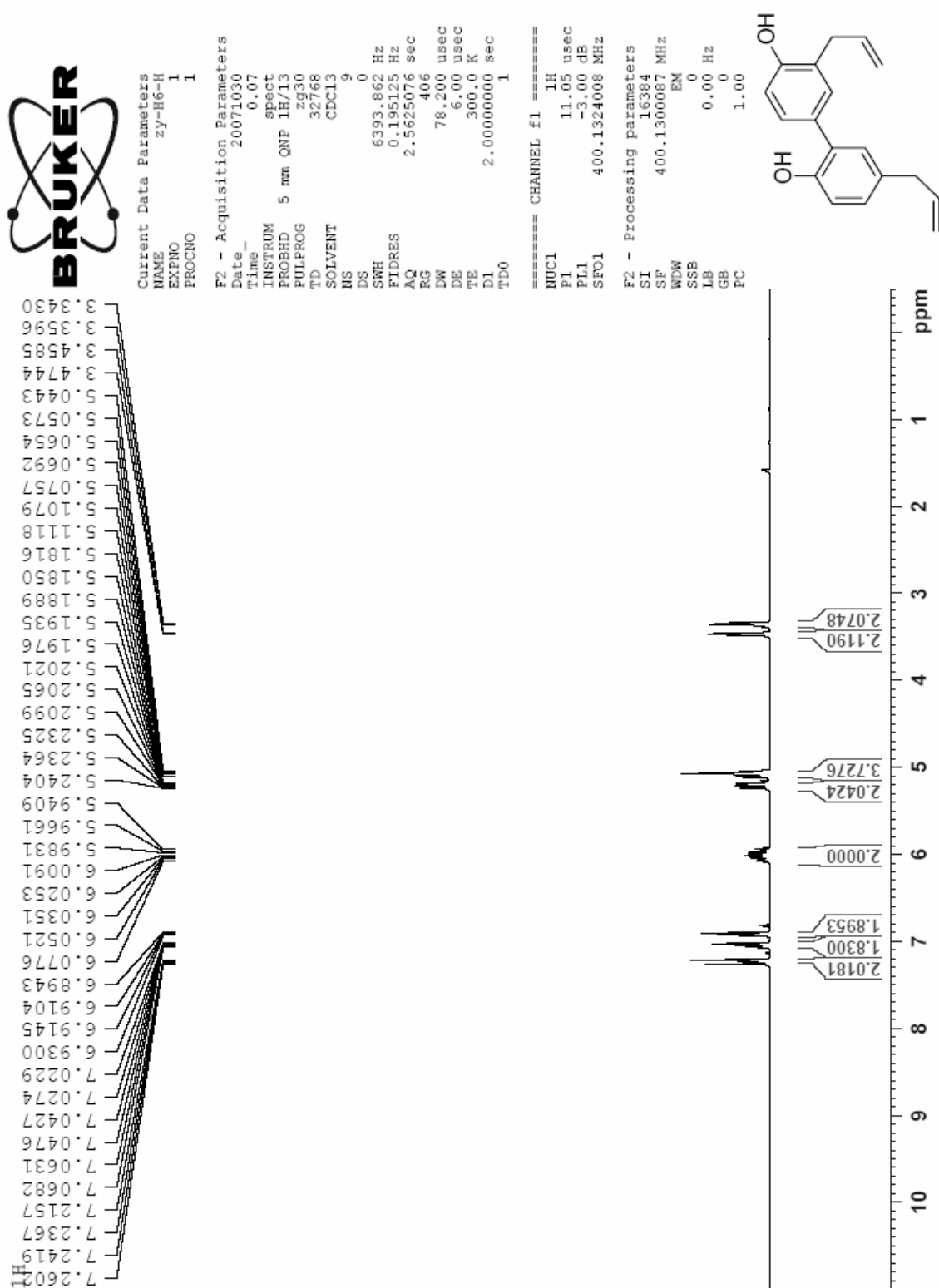


圖 12 H-MS

圖 13 ¹H-NMR

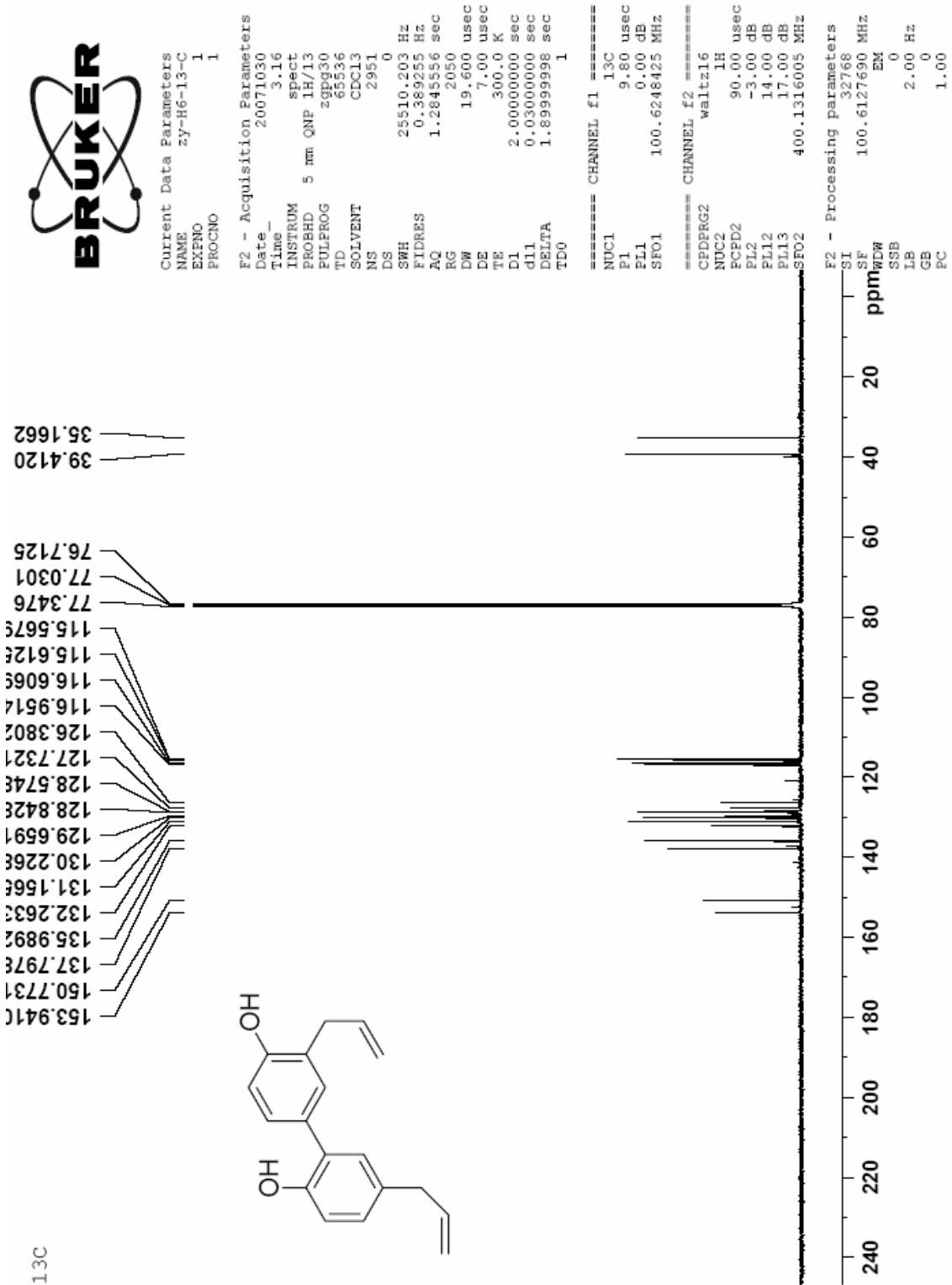


圖 14 H-C NMR

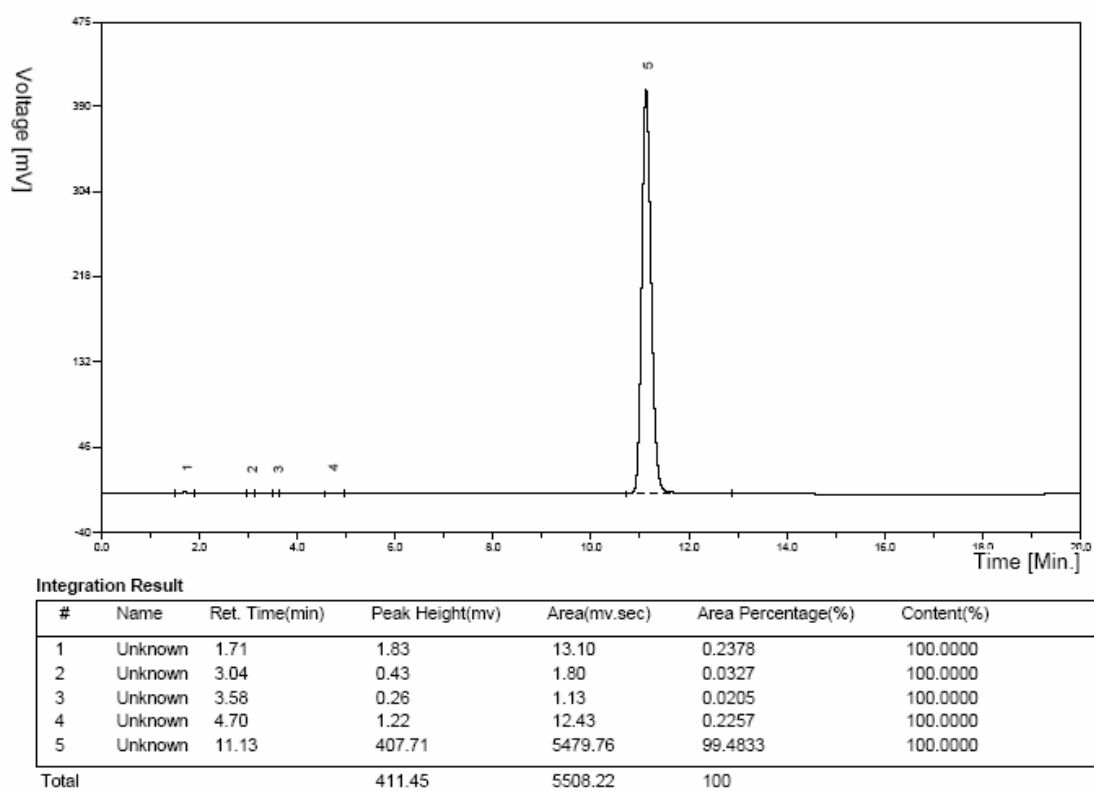


圖 15 H-HPLC

展開液乙酸乙酯：正丙烷= 3: 7
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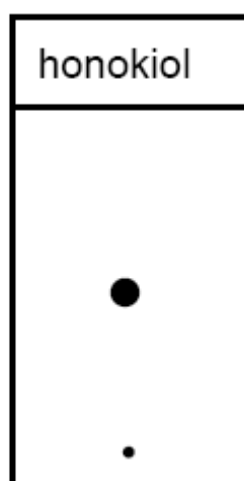


圖 16 H-TLC

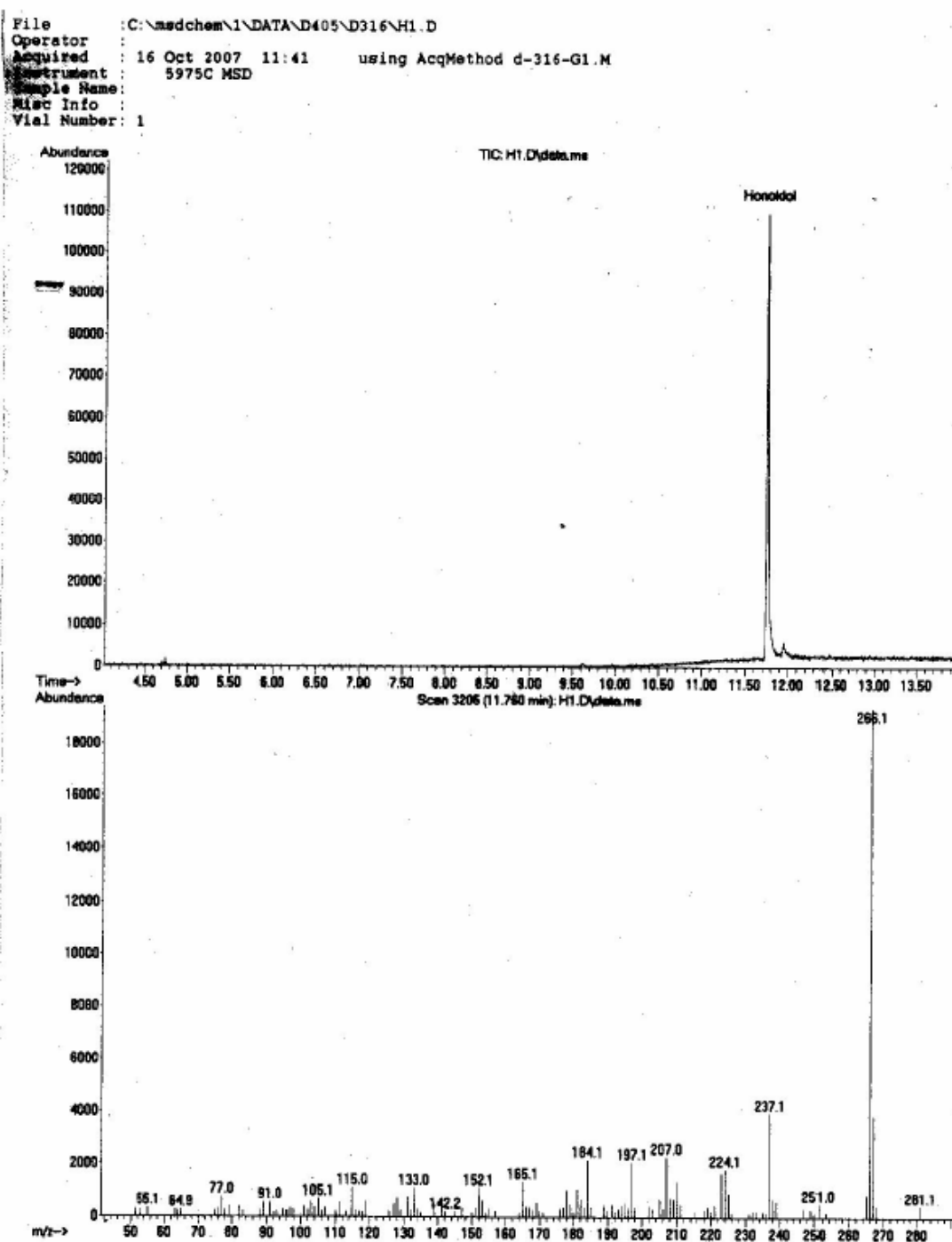


圖 17 H-GC

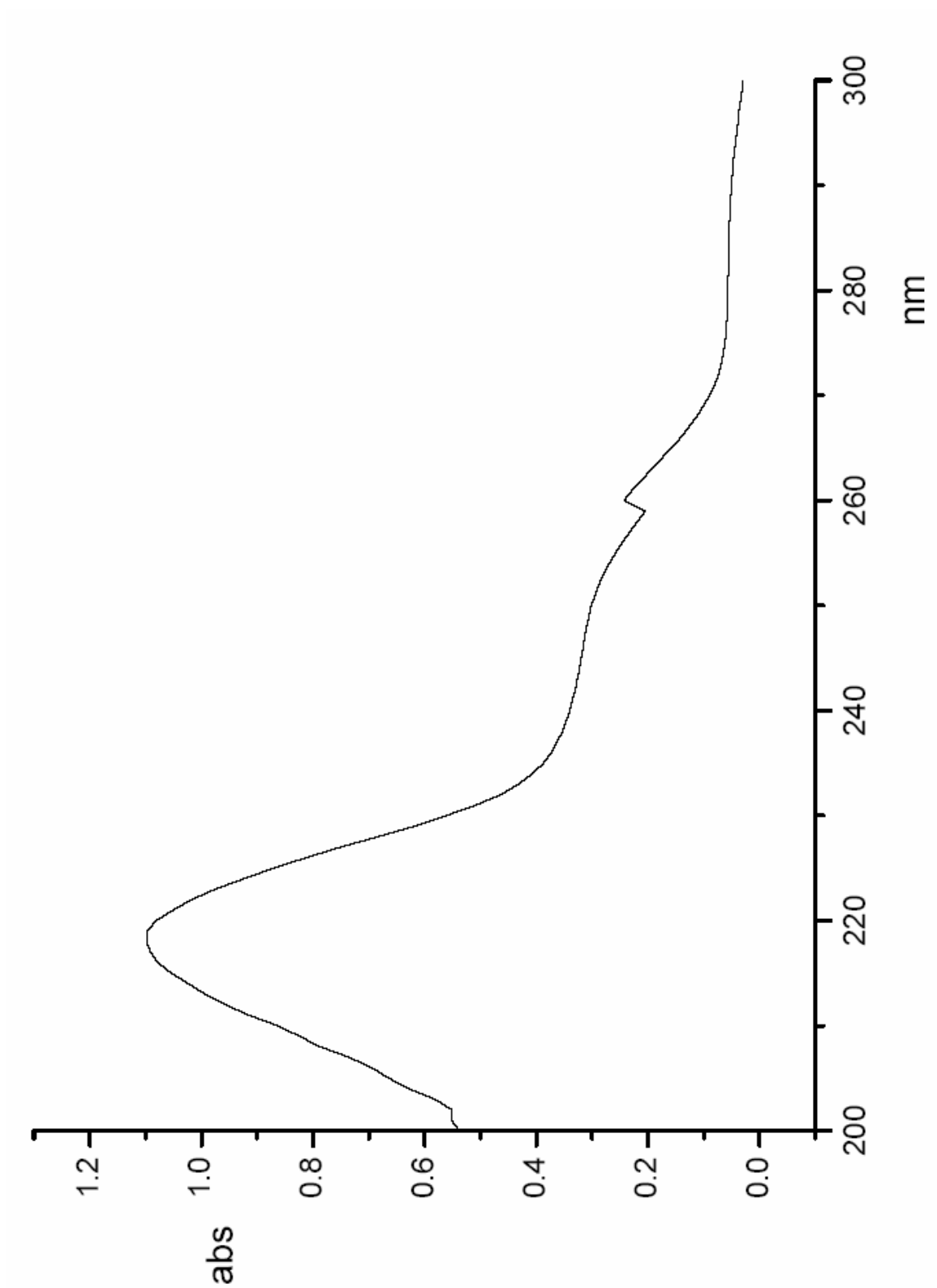


圖 18 G-UV

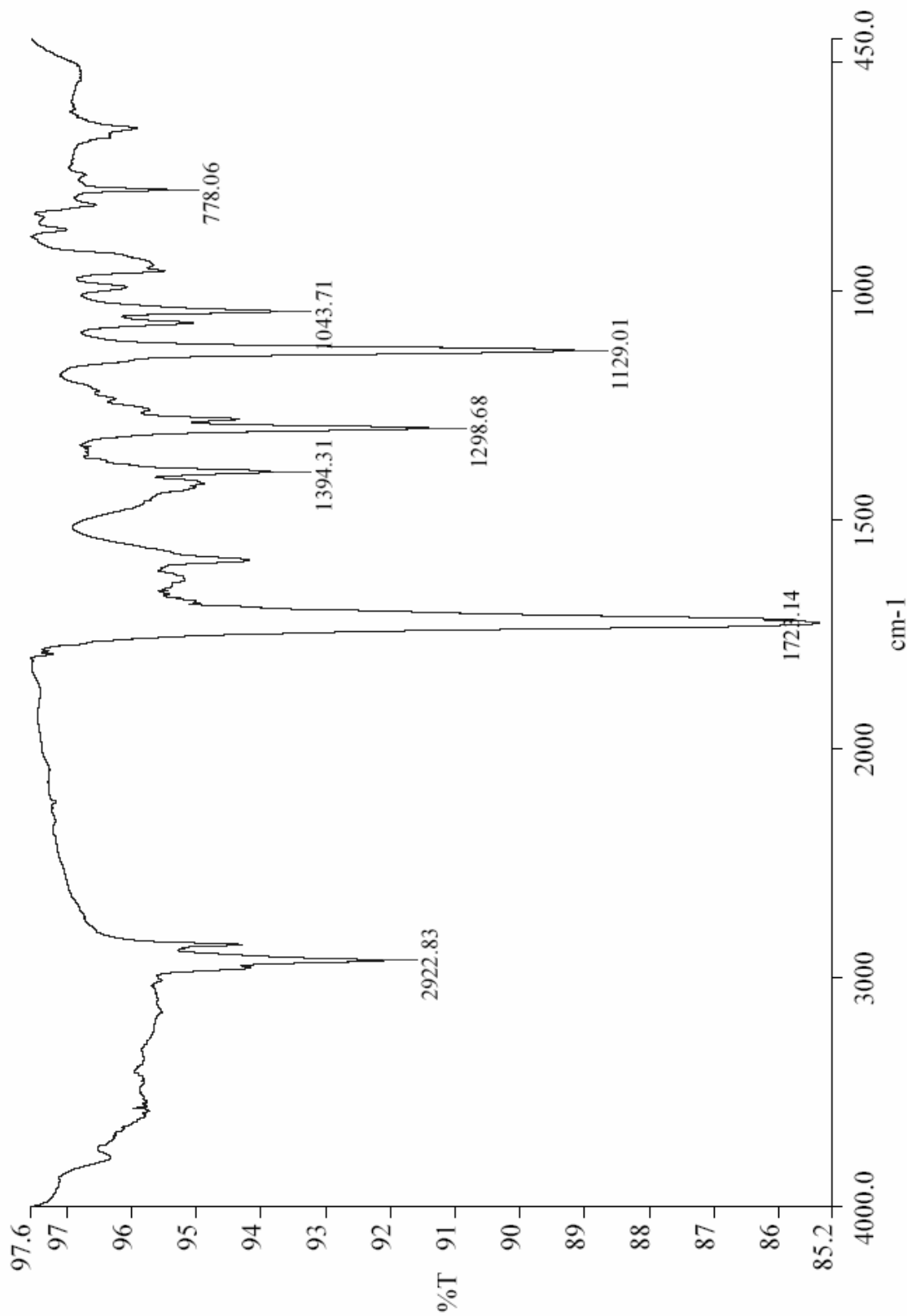


圖 19 G-IR

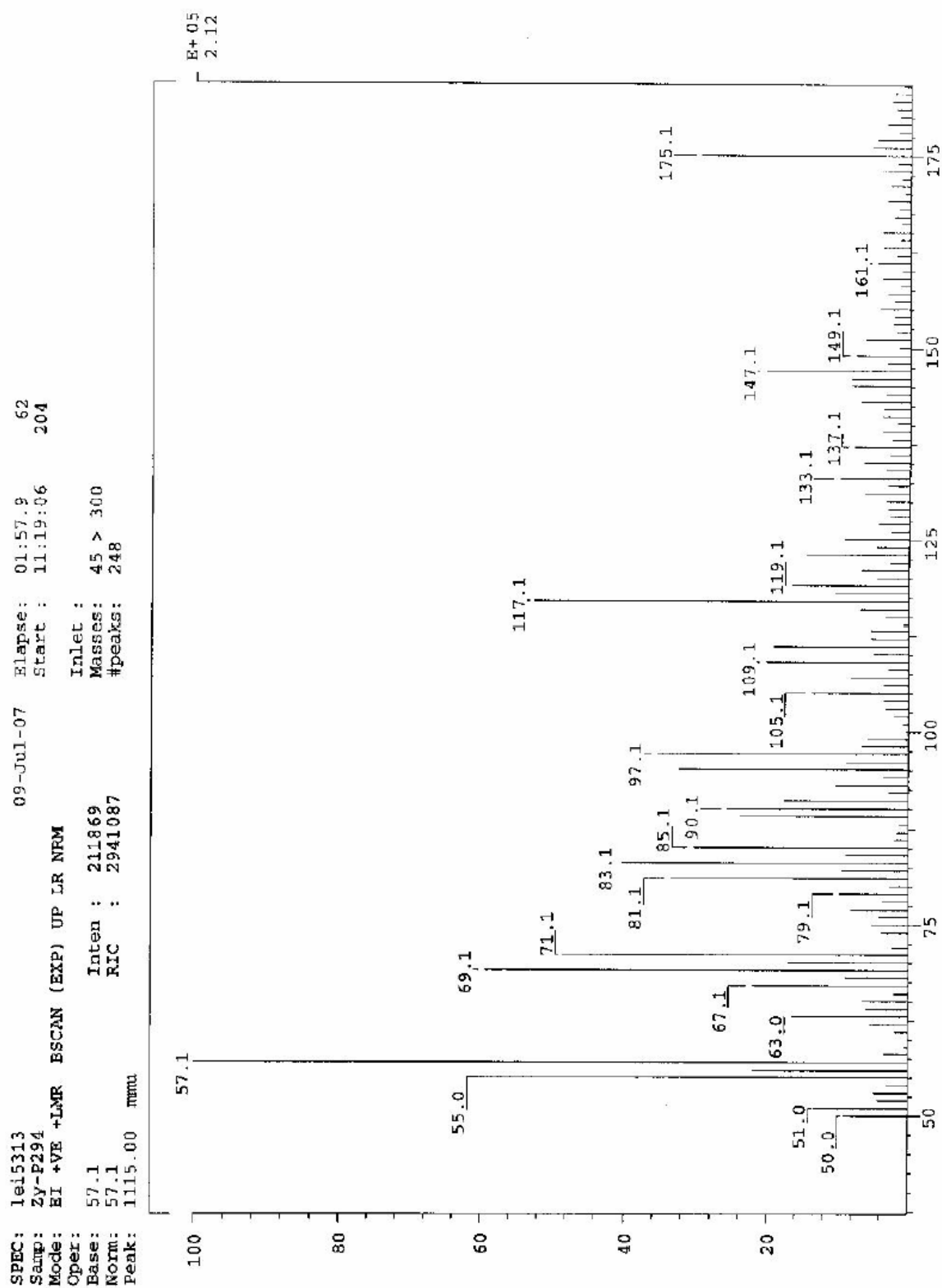


圖 20 G-MS

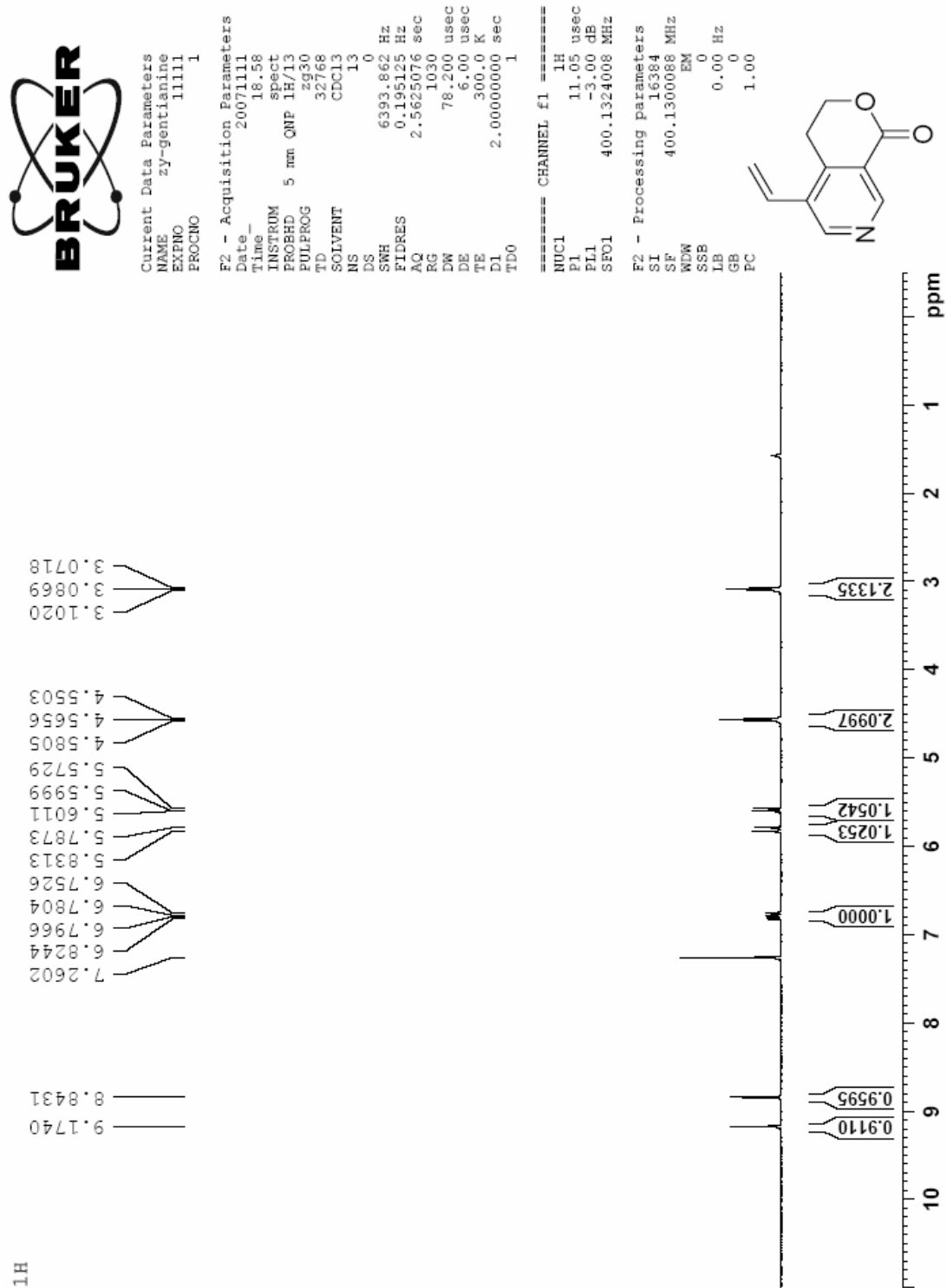


圖 21 G-H NMR

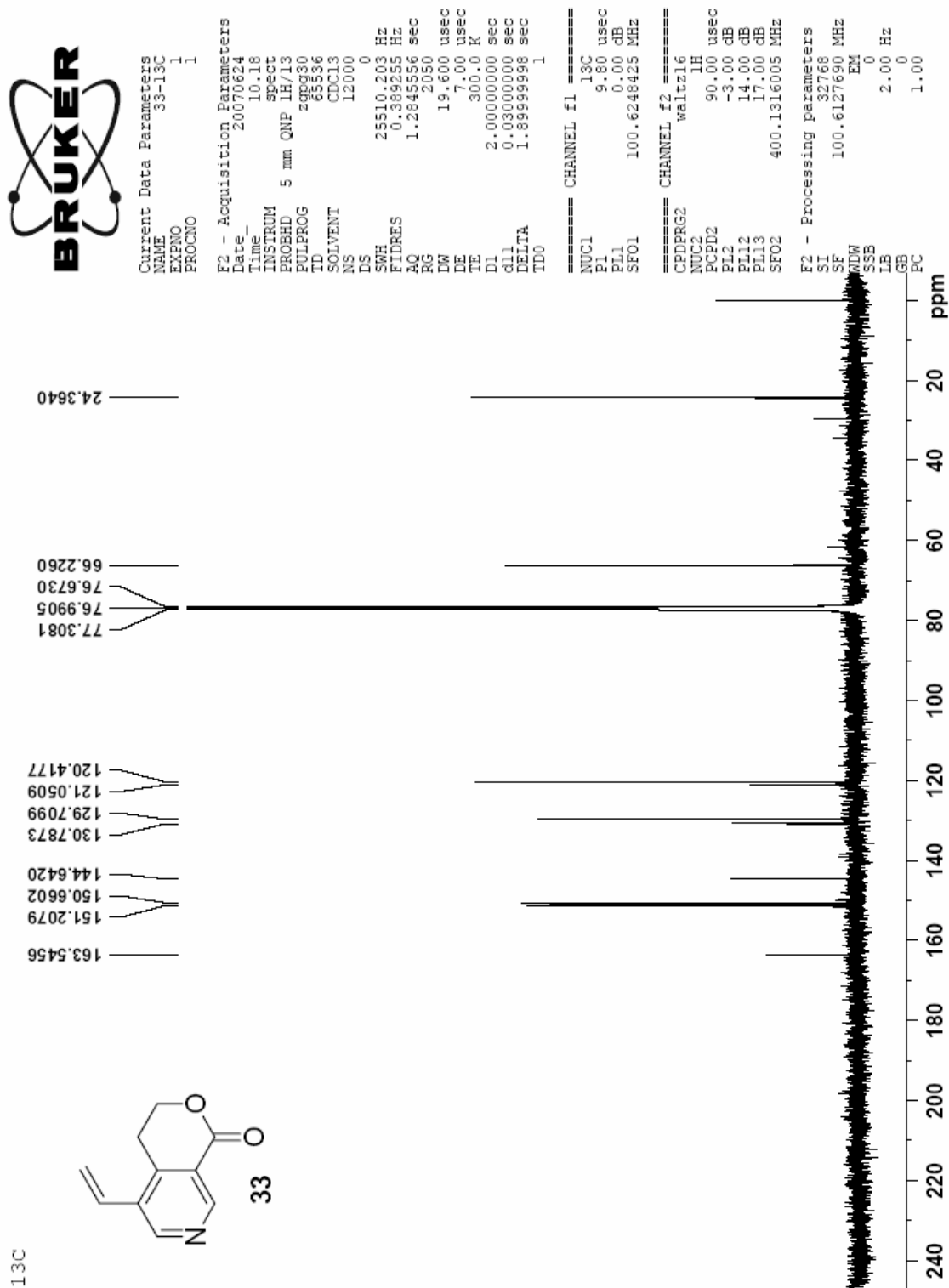


圖 22 G-C NMR

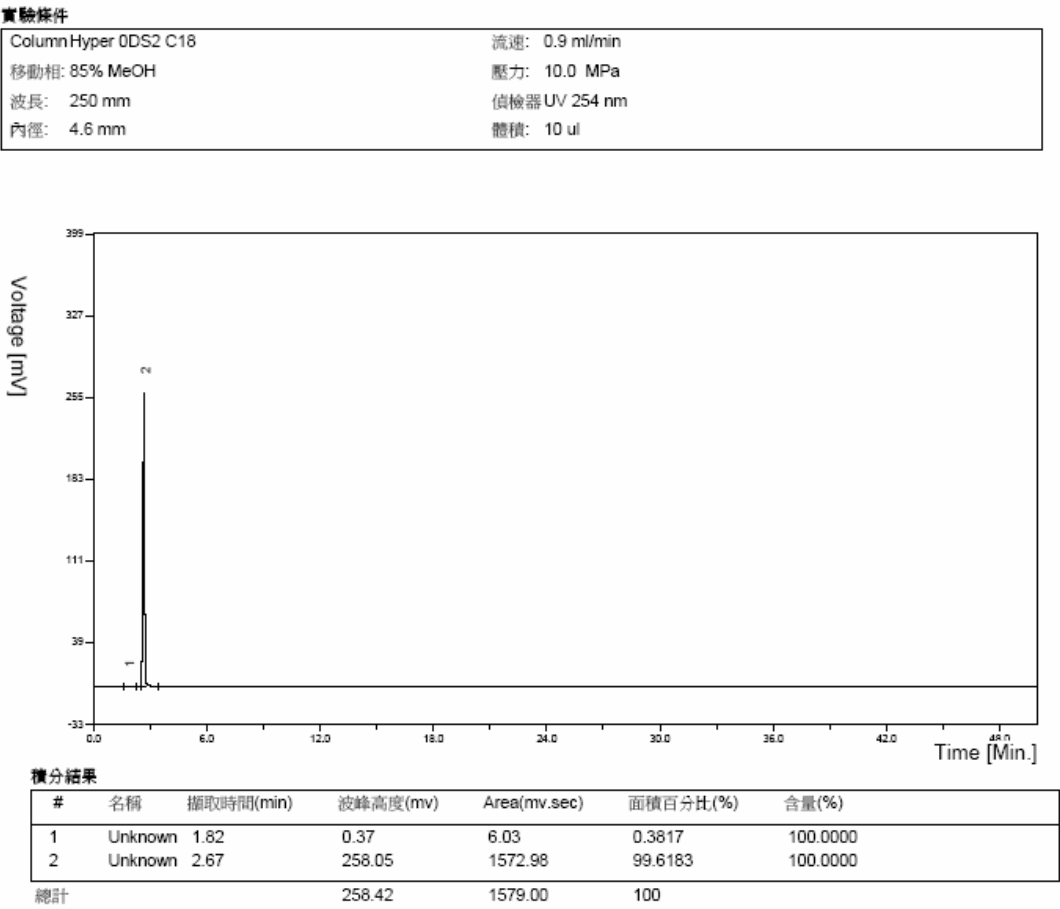


圖 23 G-HPLC

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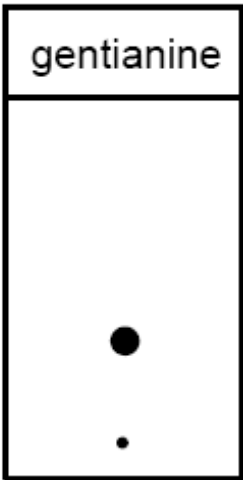


圖 24 H-TLC

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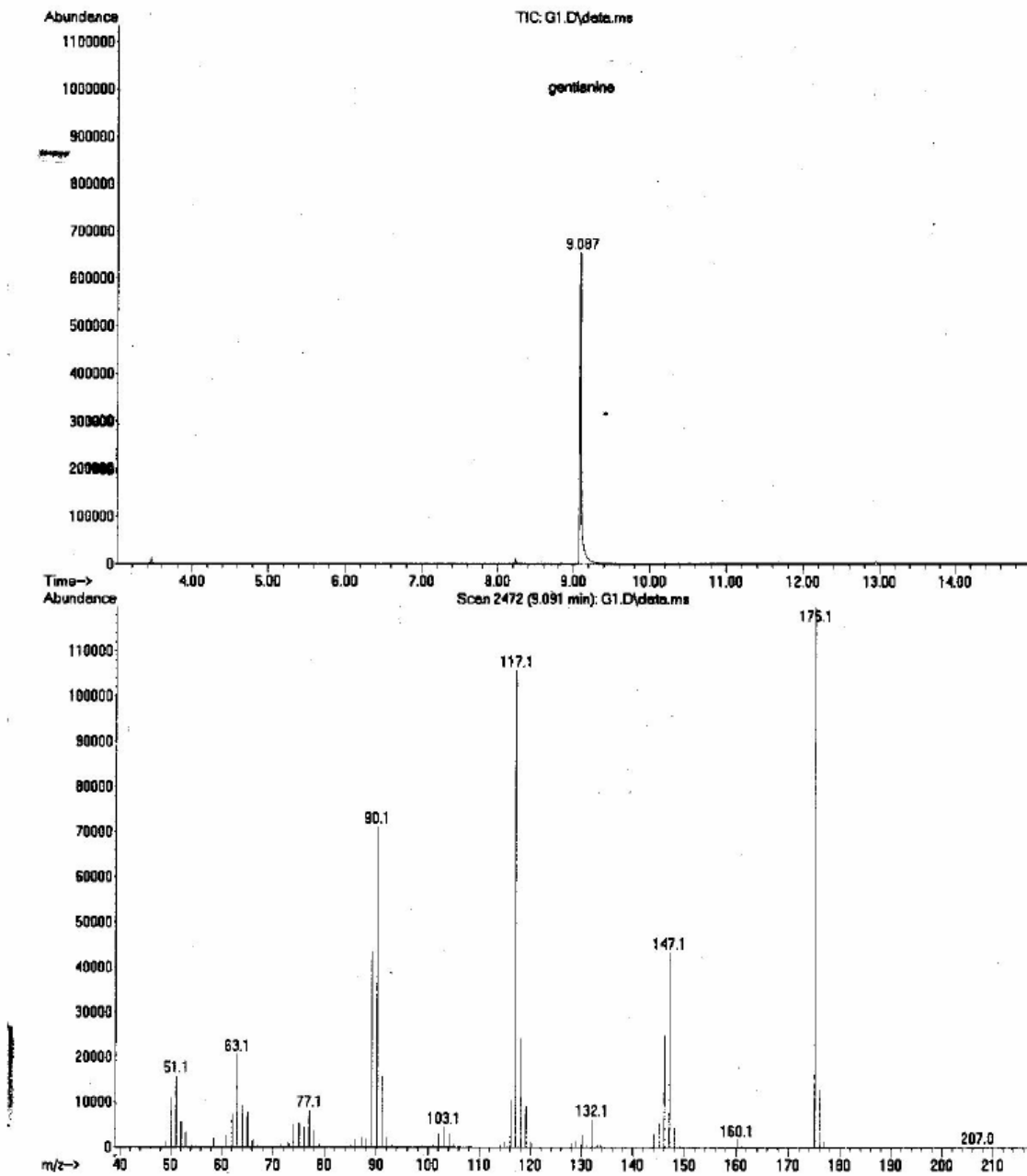
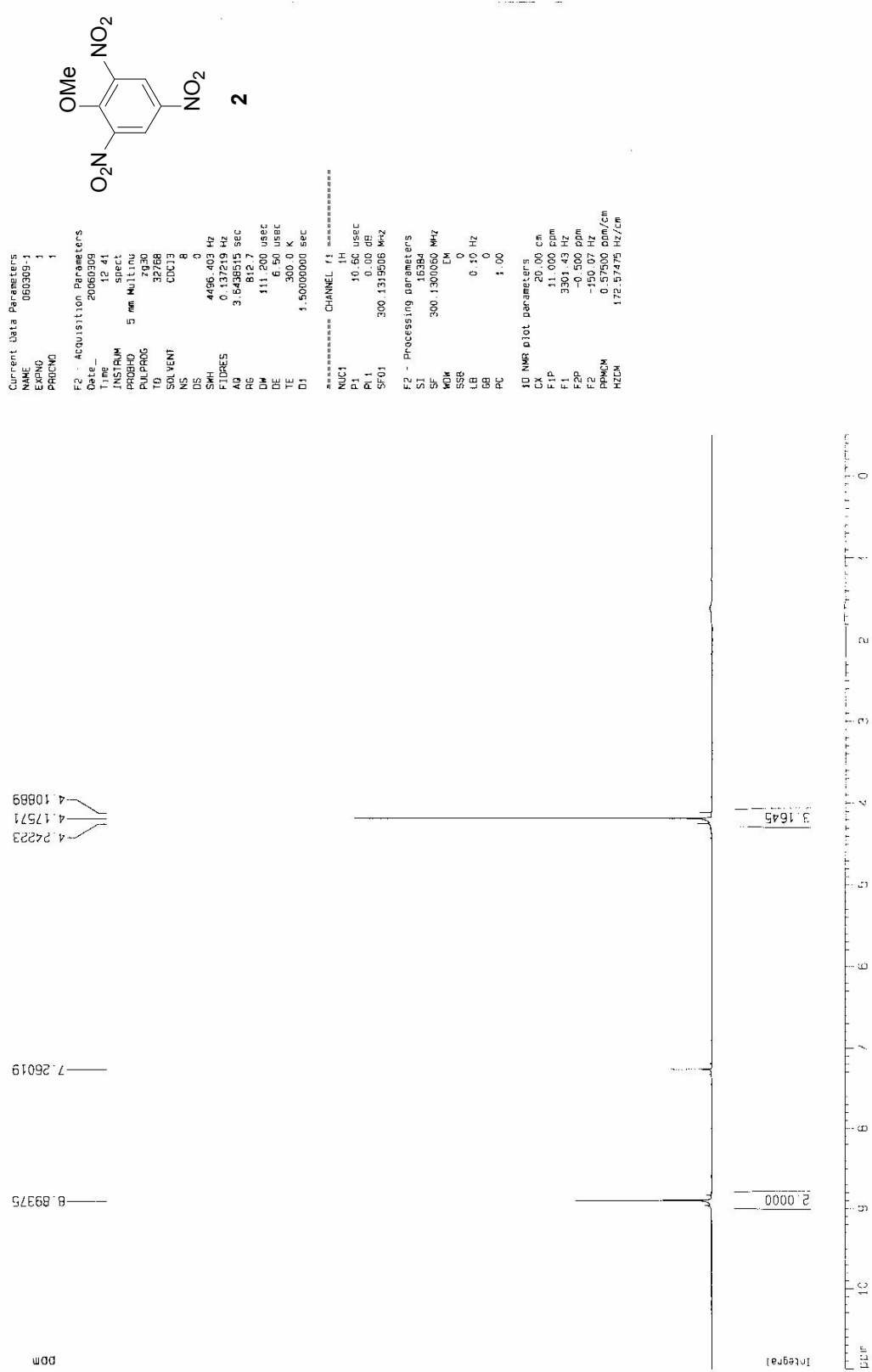
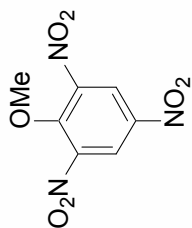


圖 25 H-GC

捌、附錄：光譜

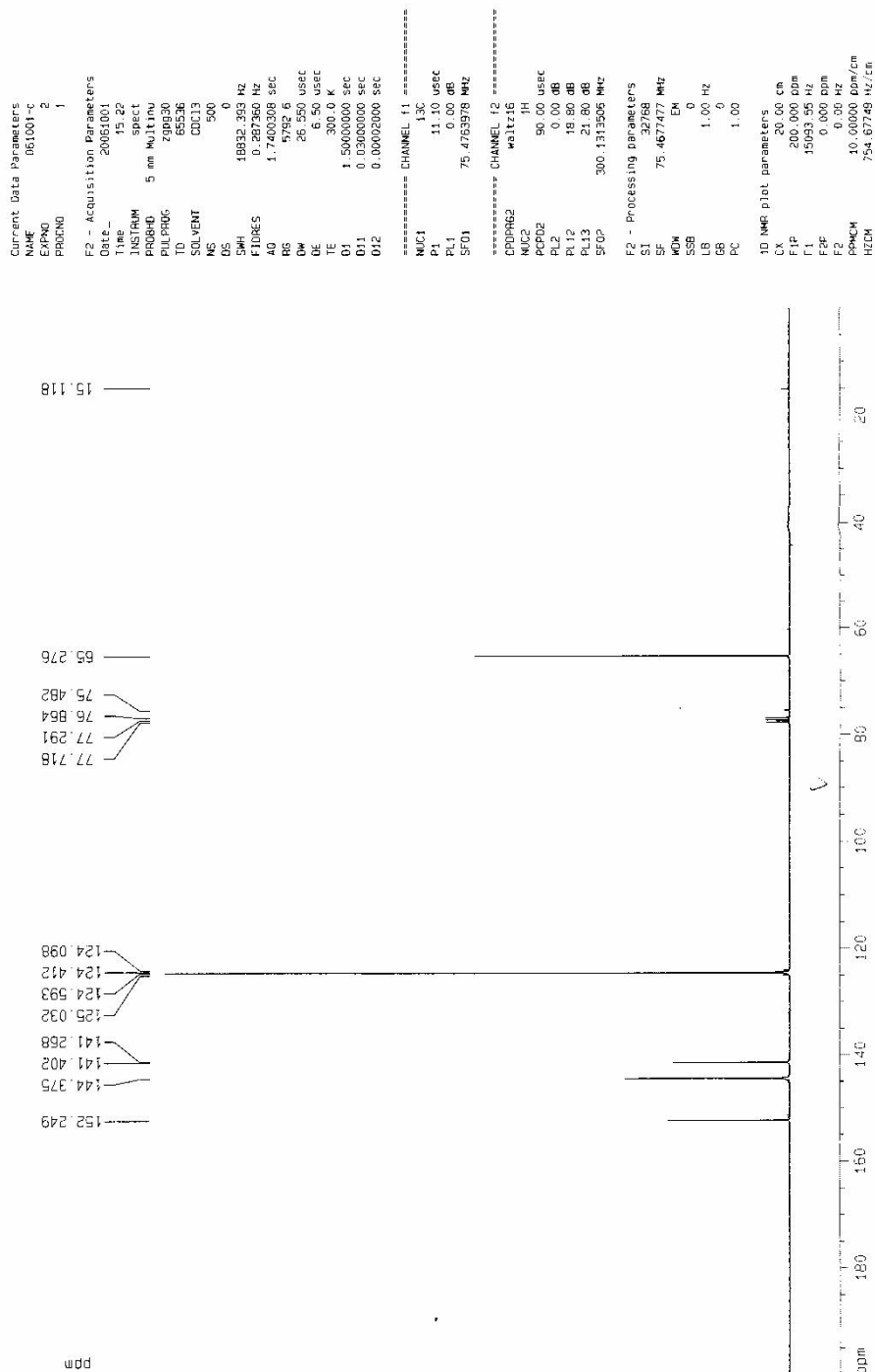


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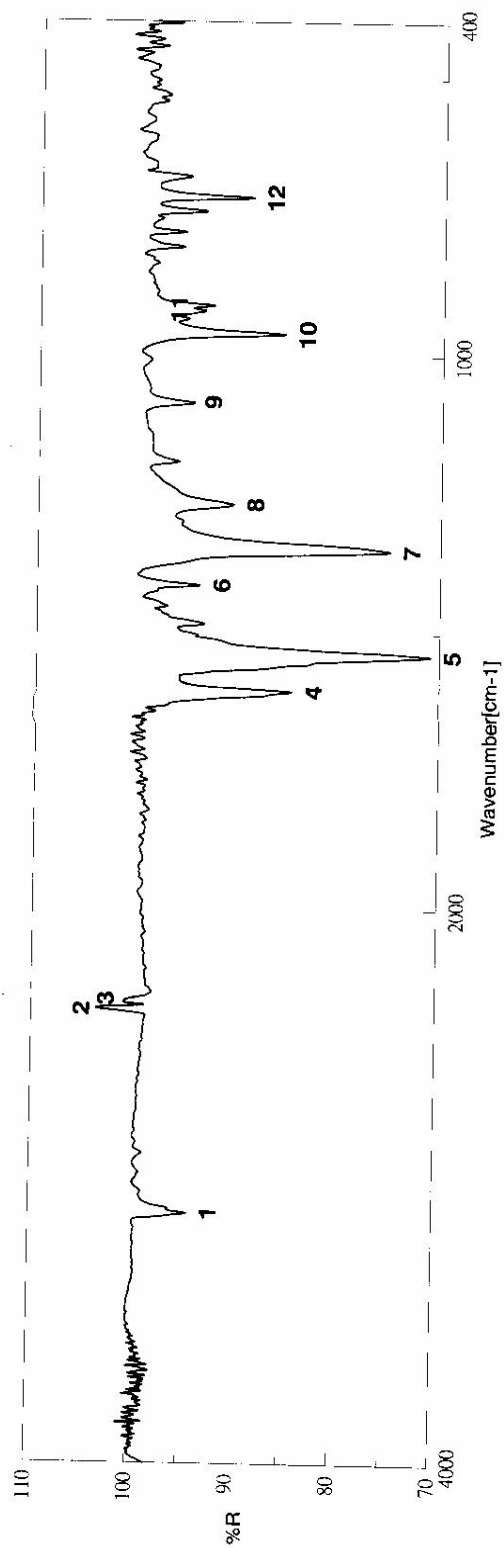


2

C13 spectrum of sample at NDUH Avance300 of BBO protenead



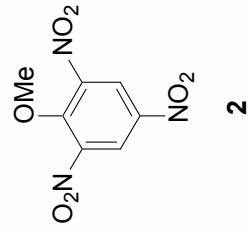
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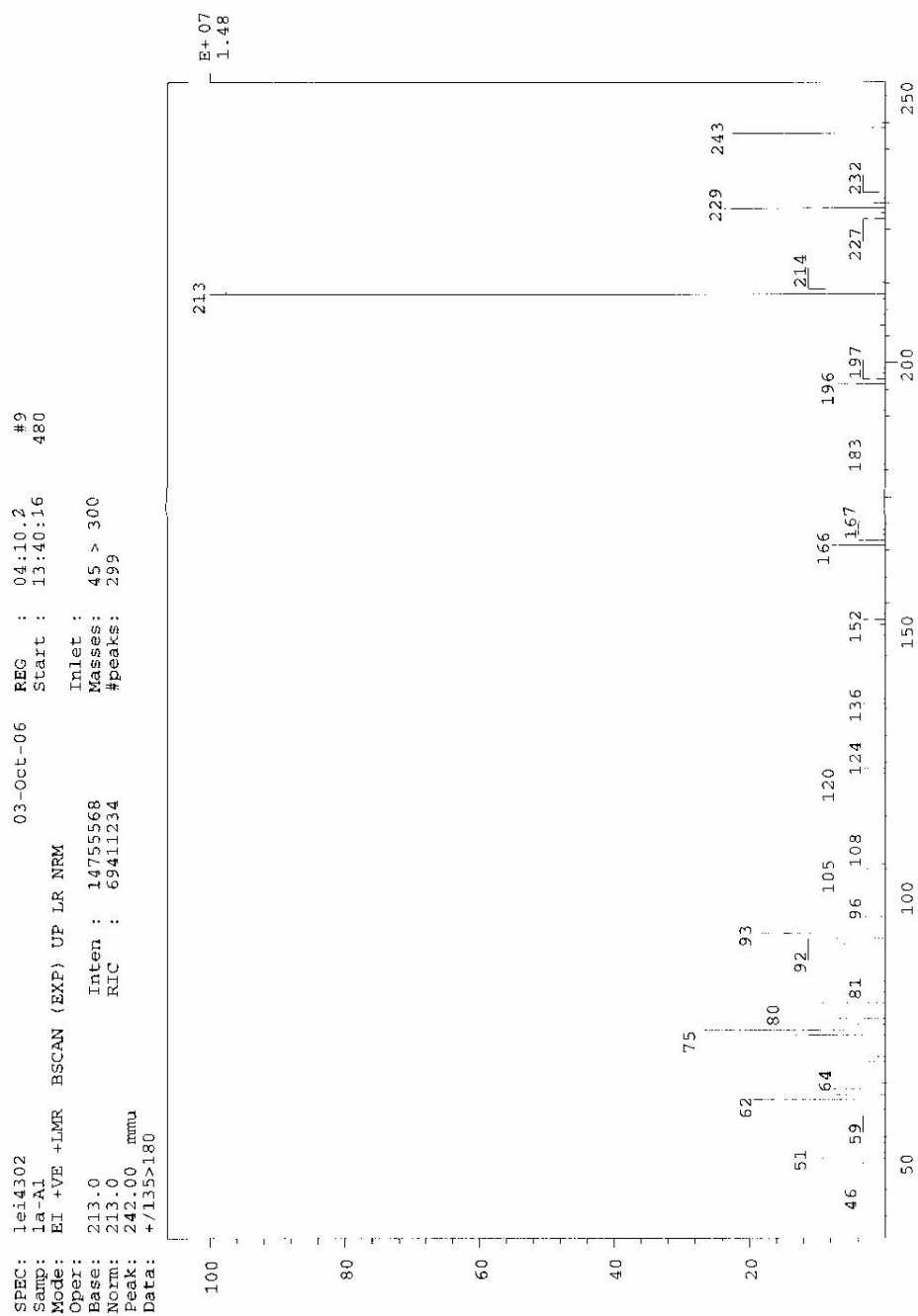
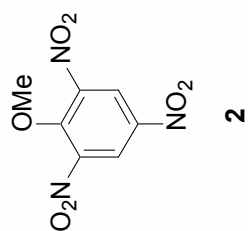
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Operator huchun/Cheng
File Name big-91
Sample Name big-91
Comment

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Scanning Speed 2 mm/sec

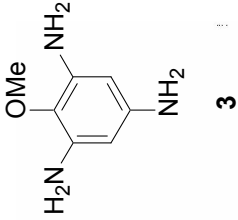
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6: 1100.00, 100.483
7: 1062.30, 100.483
8: 1038.91, 94.2328
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10: 846.29, 84.6292
11: 715.46, 89.0264
12: 629.29, 89.0264



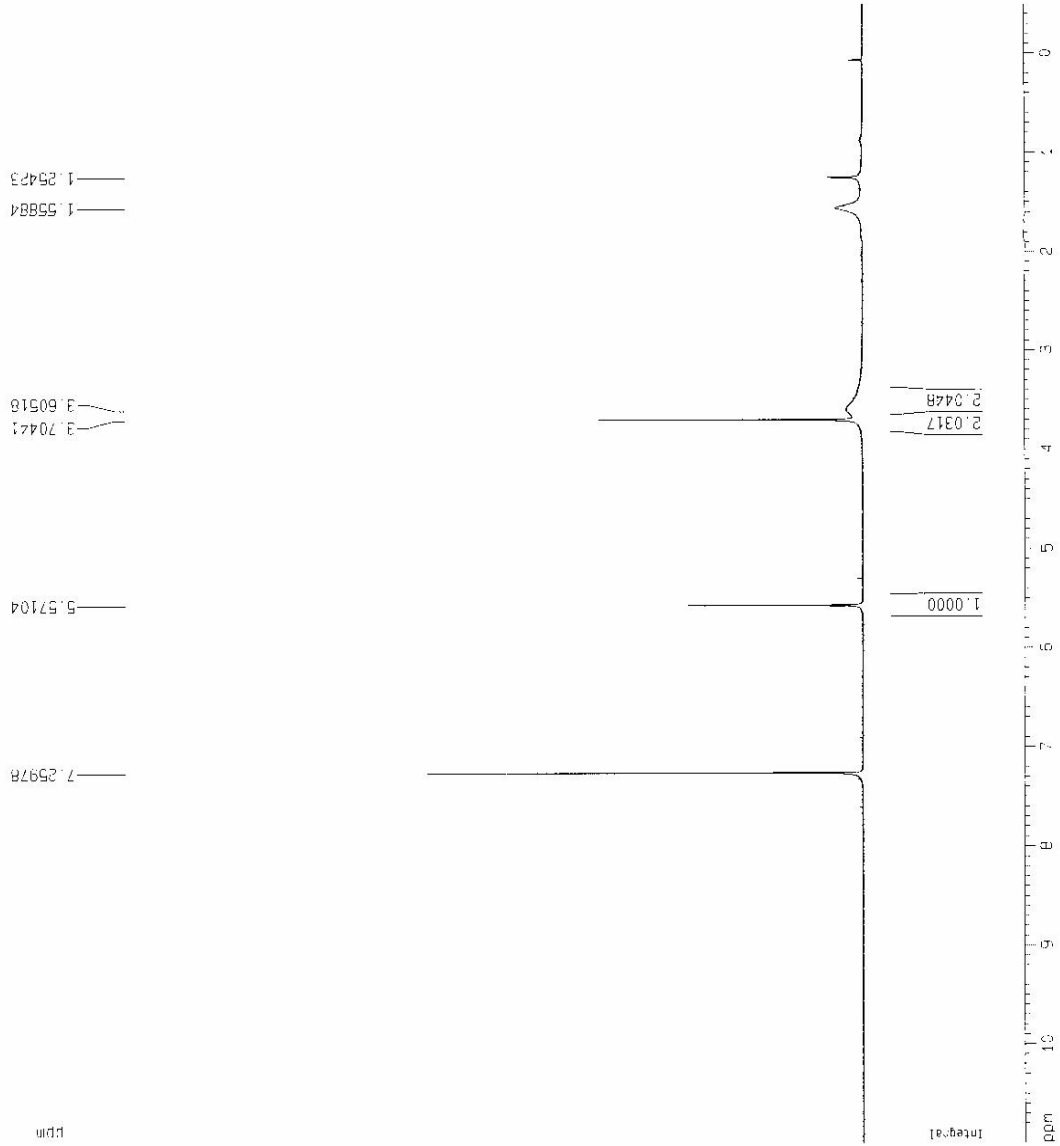
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2: MS



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DE 6.50 usec
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NUC1 1H
P1 10.00 usec
PL1 0.00 dB
SFO1 300.1319508 MHz
F2 - Processing parameters
SI 16384
SF 300.130060 MHz
WDW EN
SSB 0
LB 0.10 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
FIP 11.000 ppm
F1 3301.43 Hz
F2 -150.07 Hz
PPM0 0.57500 ppm/cm
HZ0 172.57475 Hz/cm



3: ¹H NMR



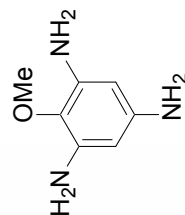
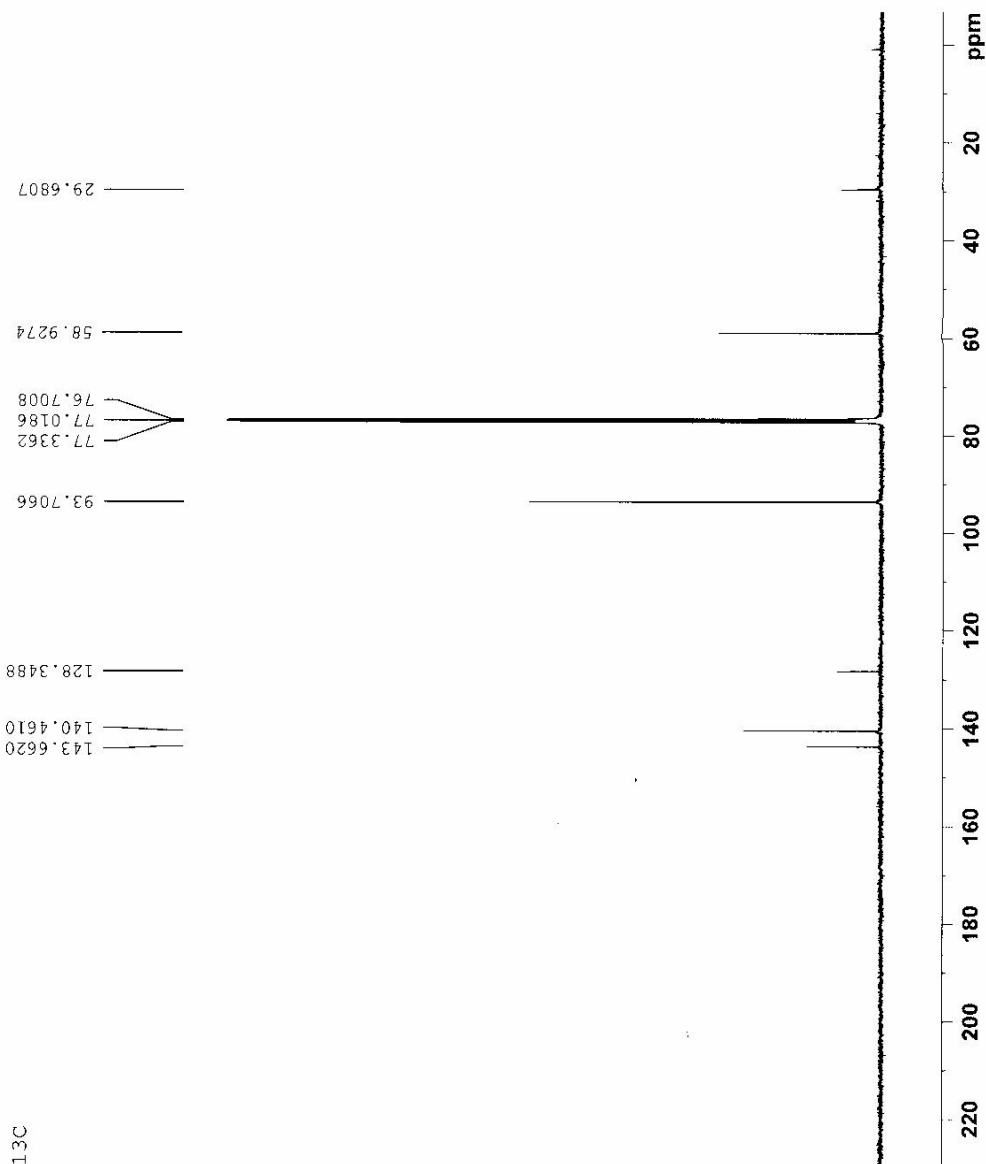
Current Data Parameters
NAME big0925-1
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters
Date_ 20060926
Time 4.12
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 5000
DS 0
SWH 25510.203 Hz
FIDRES 0.389255 Hz
AQ 1.2845556 sec
RG 1620
DW 19.600 usec
DE 7.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999999 sec
TD0 1

===== CHANNEL f1 =====
NUC1 13C
P1 9.80 usec
PL1 0.00 dB
SFO1 100.6248425 MHz

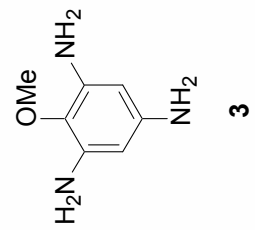
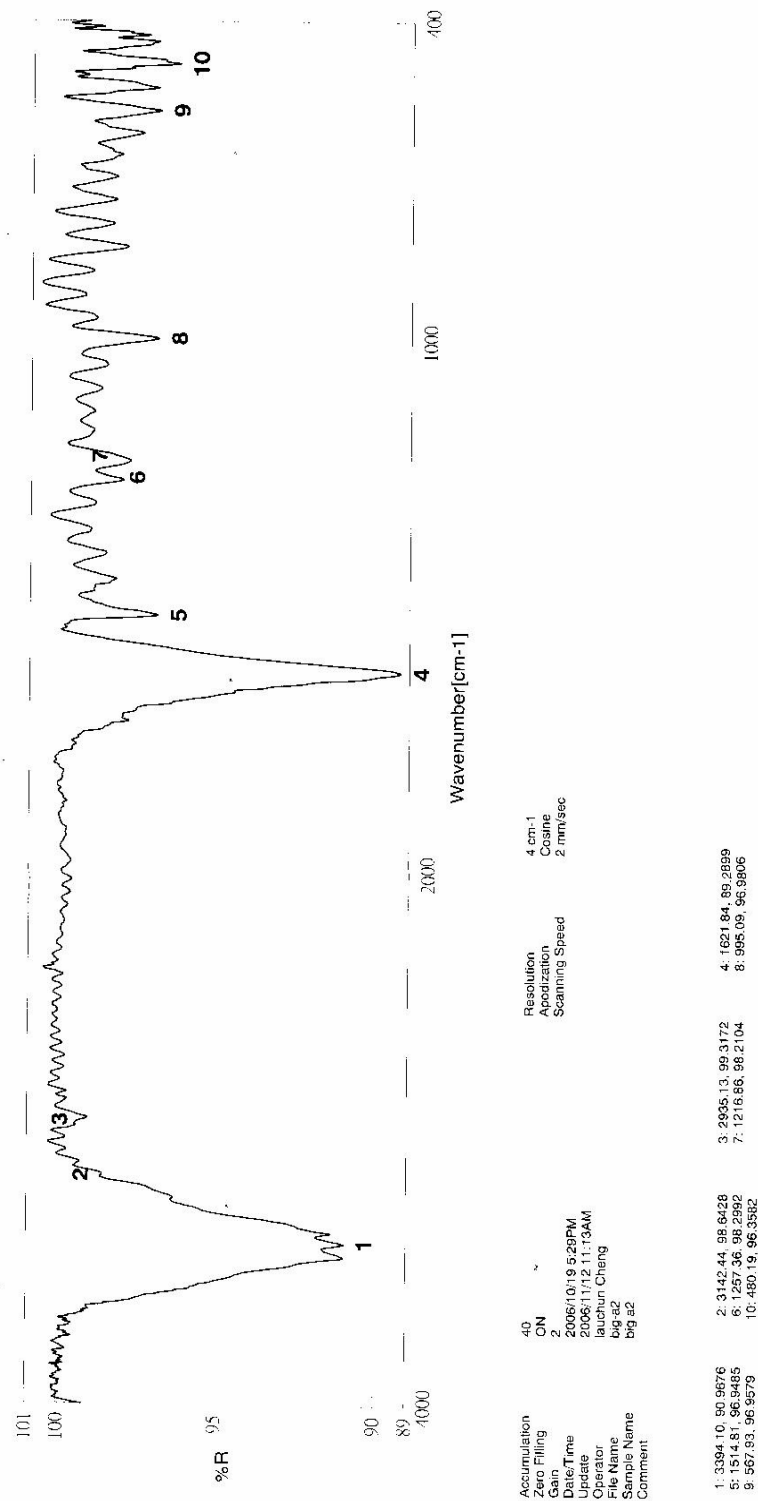
===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -3.00 dB
PL12 14.00 dB
PL13 17.00 dB
SFO2 400.1316005 MHz

F2 - Processing parameters
SI 32768
SF 100.6127690 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.00



3

3: C NMR



3: IR

SPEC: lei4303
 Samp: 1a-A2
 Mode: EI +VE +LMR BSCAN (EXP) UP LR NRM
 Oper: Inlet :
 Base: 138.0 Inter: 40179060
 Norm: 138.0 RIC : 121946061
 Peak: 242.00 mmu
 Data: +/224>335

03-Oct-06

REG : 06:56.4

#9

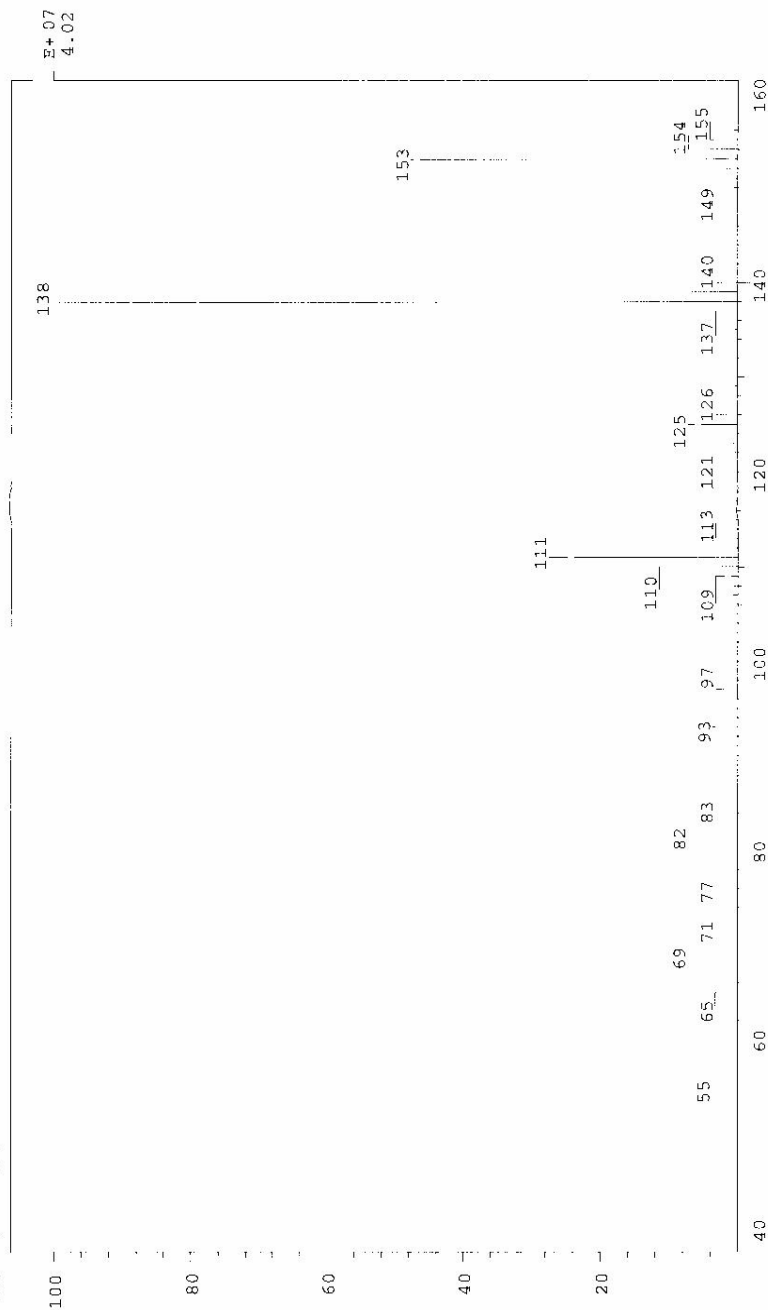
Start : 14:08:08

591

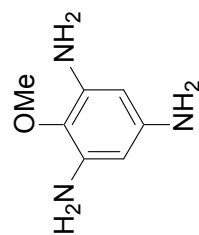
Inlet :

Masses: 45 > 300

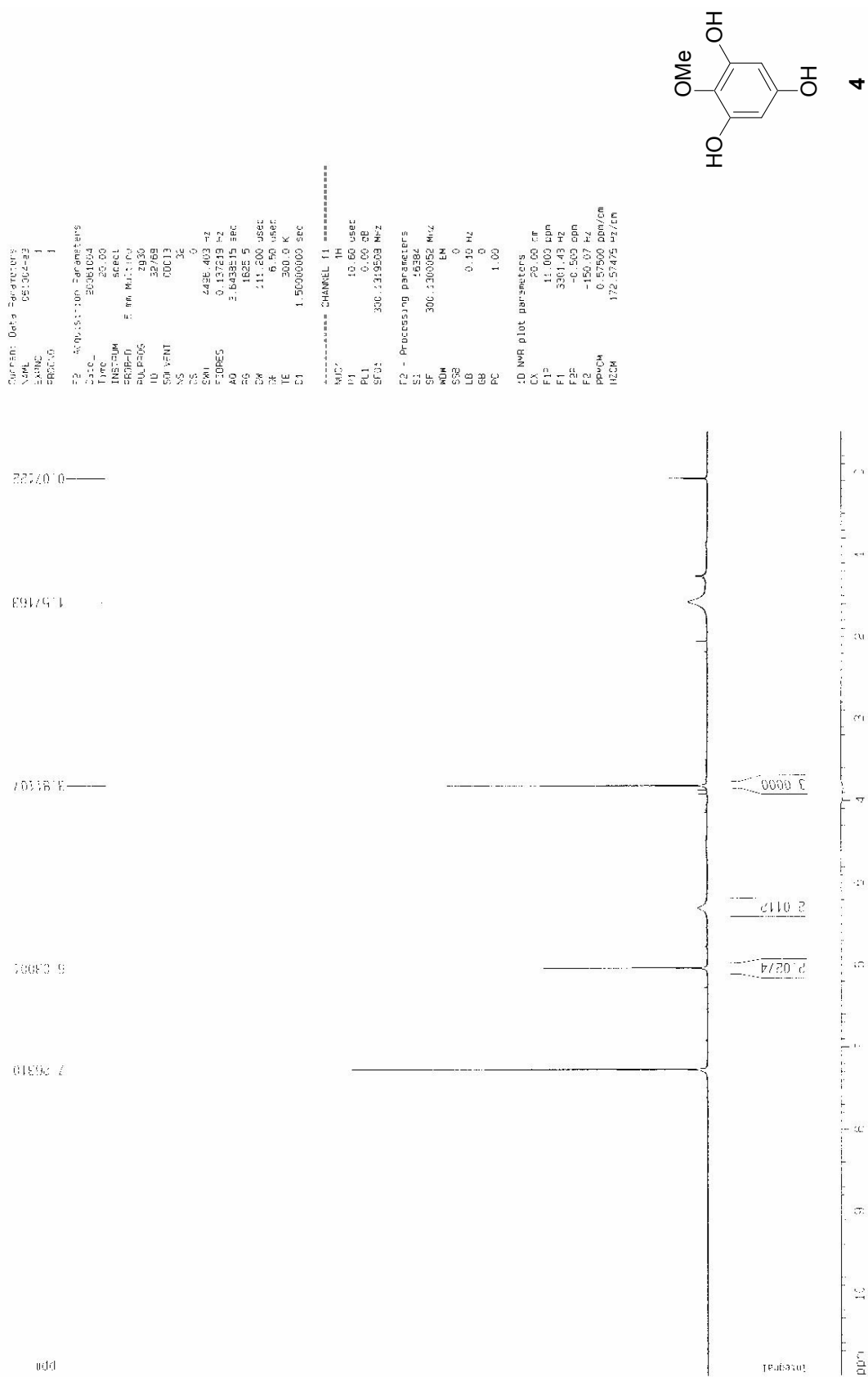
#Peaks: 505



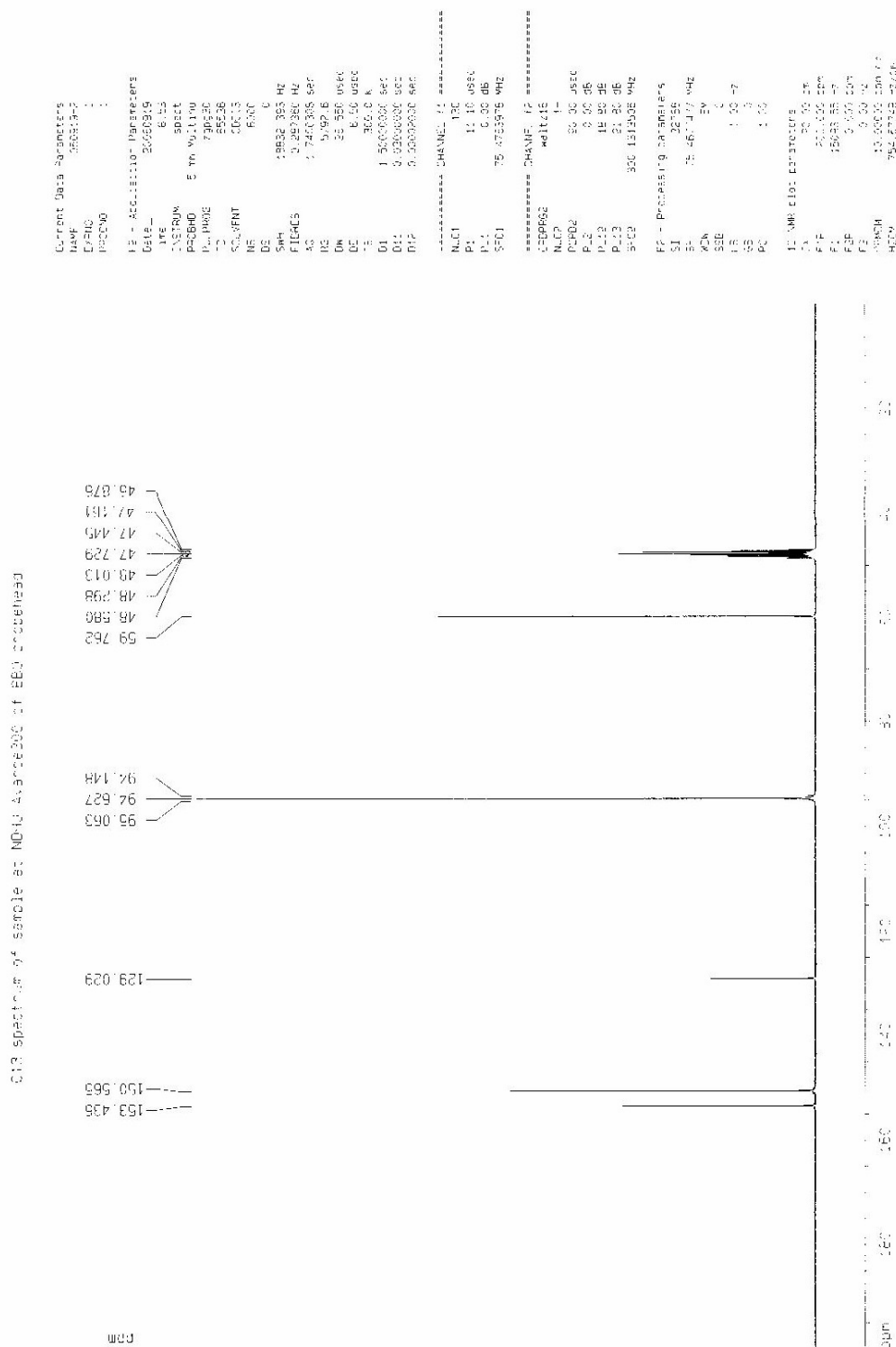
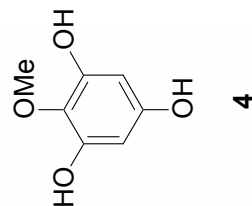
3: MS



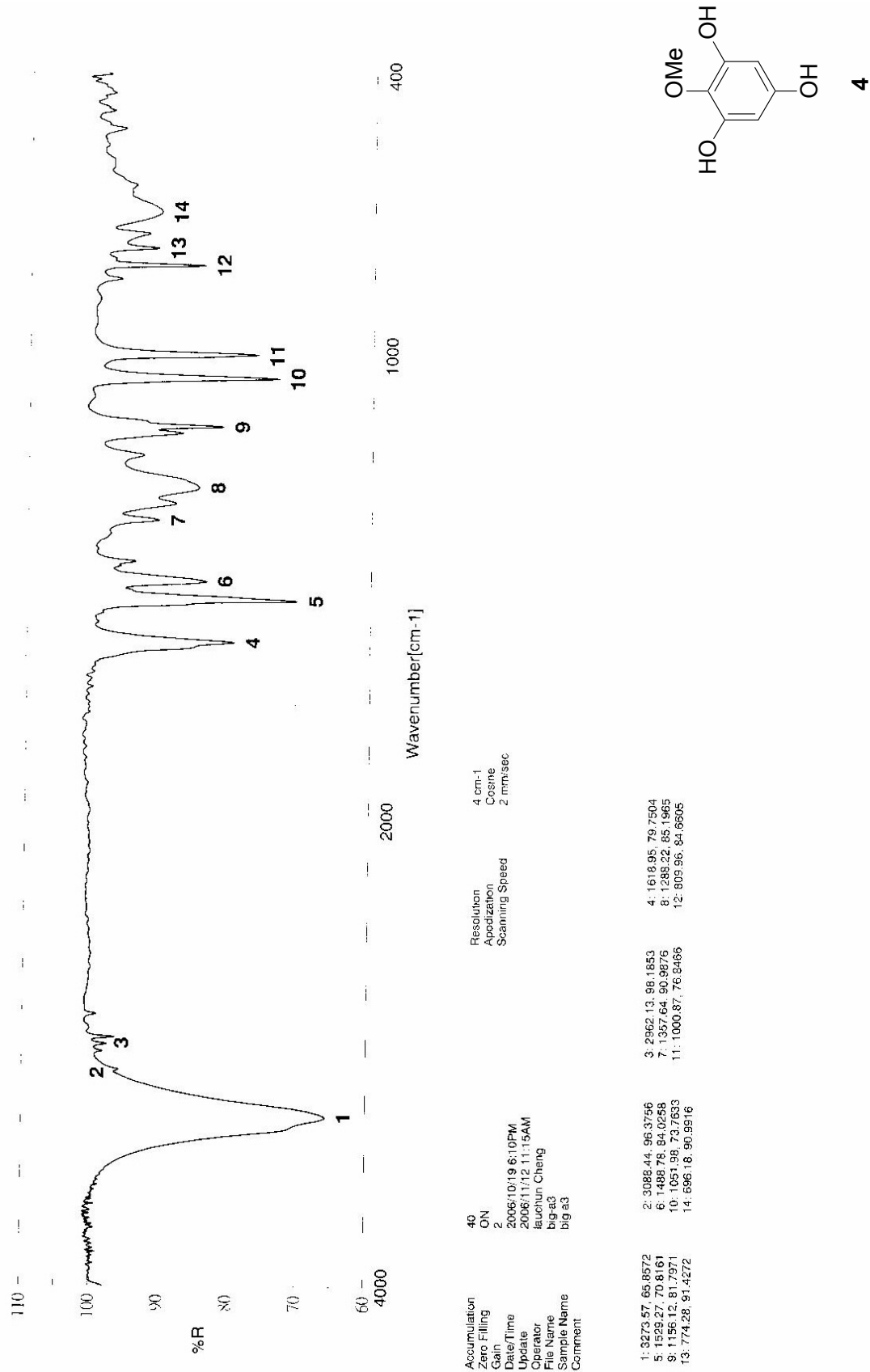
3



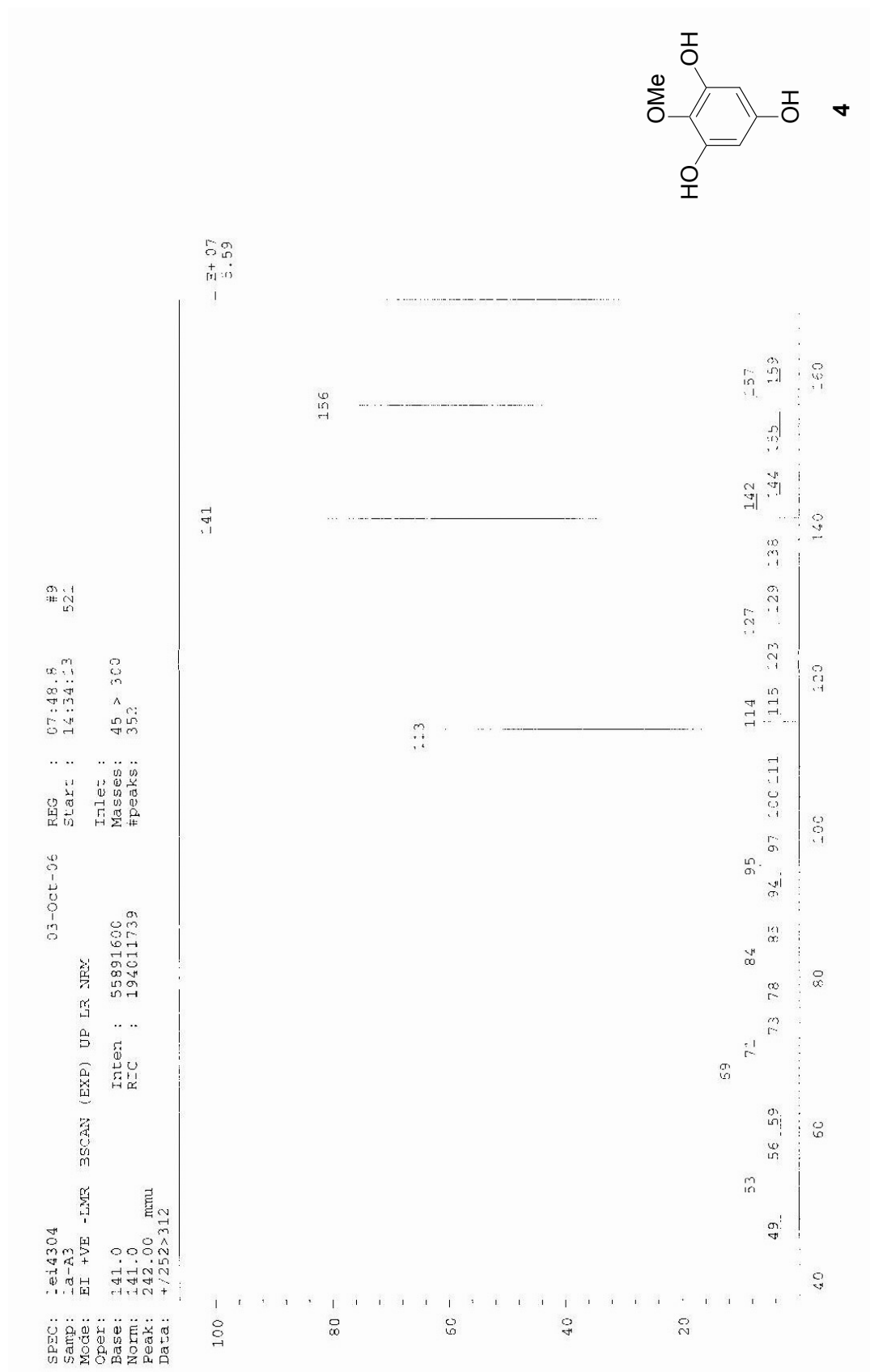
4: H NMR



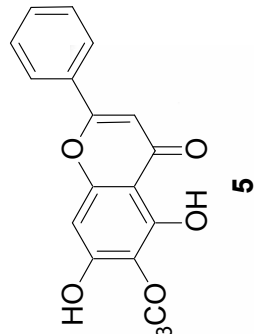
4: C NMR



4: IR



4: MS



Current Data Parameters
NAME 061109-a5H
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20061108
Time 23.13
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 8
DS 0
SWH 4496.403 Hz
FIDRES 0.137219 Hz
AQ 3.6438515 sec
RG 1290.2
DM 111.200 usec
DE 6.50 usec
TE 300.0 K
D1 1.50000000 sec
===== CHANNEL f1 =====
NUC1 1H
P1 10.60 usec
PL1 0.00 dB
SFO1 300.1319508 MHz
F2 - Processing parameters
SI 16384
SF 300.1300060 MHz
WDW EM
SSB 0
LB 0.10 Hz
GB 0
PC 1.00
10 NMR plot parameters
CX 20.00 cm
F4 14.00 ppm
F1 420.52 Hz
F2 650.00 ppm
F3 -150.06 Hz
PPH4M 0.72500 ppm/cm
HZCM 217.59425 Hz/cm

2.0454
1.5614
1.2528

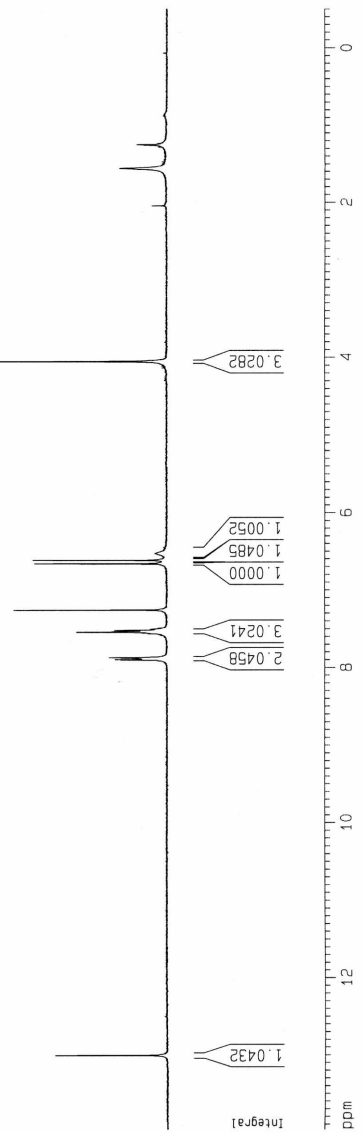
4.0523

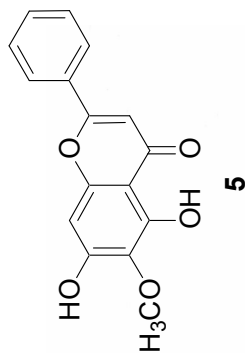
7.9027
7.8971
7.8910
7.8782
7.8704
7.8634
7.8566
7.8453
7.8416
7.8202
7.8092
7.8032
7.7603
7.6634
6.6182
6.5294

13.0107

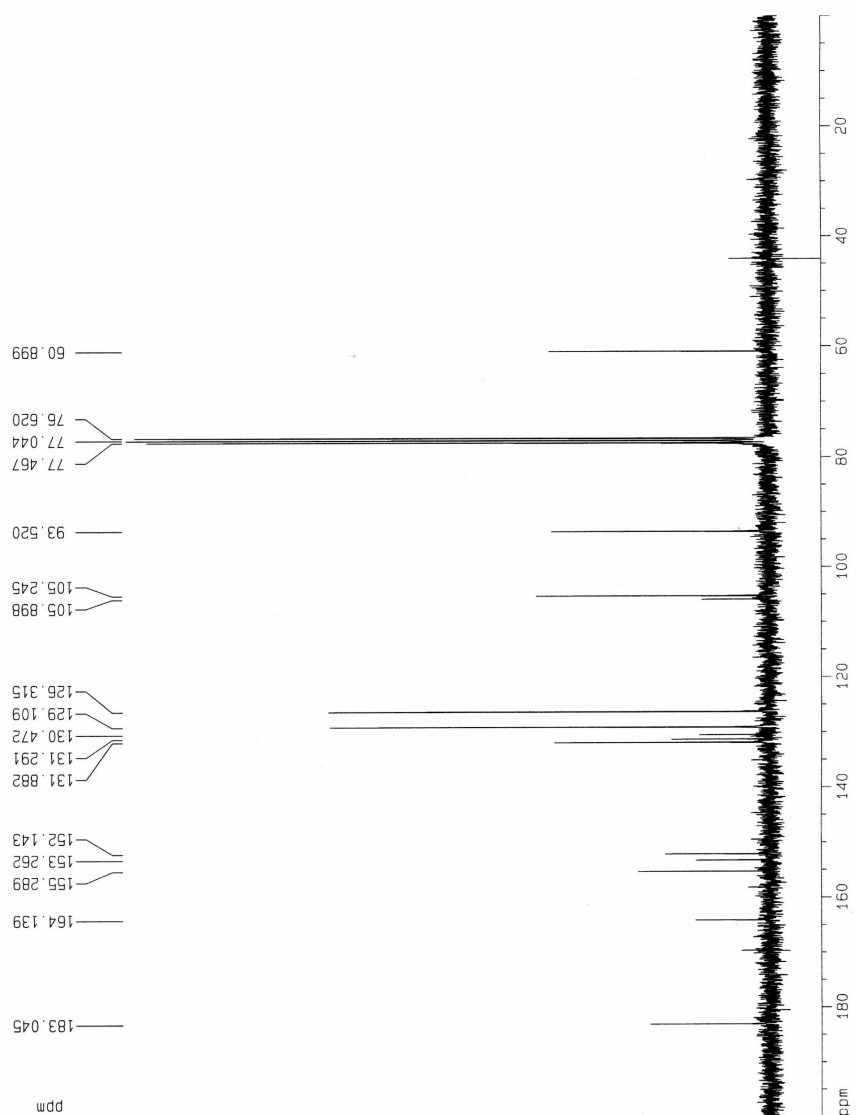
ppm

5: ¹H NMR





C13 spectrum of sample at NDHU Avance300 of BBO probehead



```

Current Data Parameters
NAME      06110-55a
EXPNO    1
PROCNO    1

F2 - Acquisition Parameters
Date_     20061109
Time      1.05
INSTRUM   spect
PROBHD    5 mm Multinu
PULPROG   zgpg30
TD         65536
SOLVENT   CDCl3
NS         1000
DS         0
SWH        18832.393 Hz
FIDRES     0.287360 Hz
AQ         1.7400308 sec
RG         5792.6
DM         26.550 usec
DE         6.50 usec
TE         300.0 K
O1         1.50000000 sec
O11        0.03000000 sec
O12        0.00002000 sec

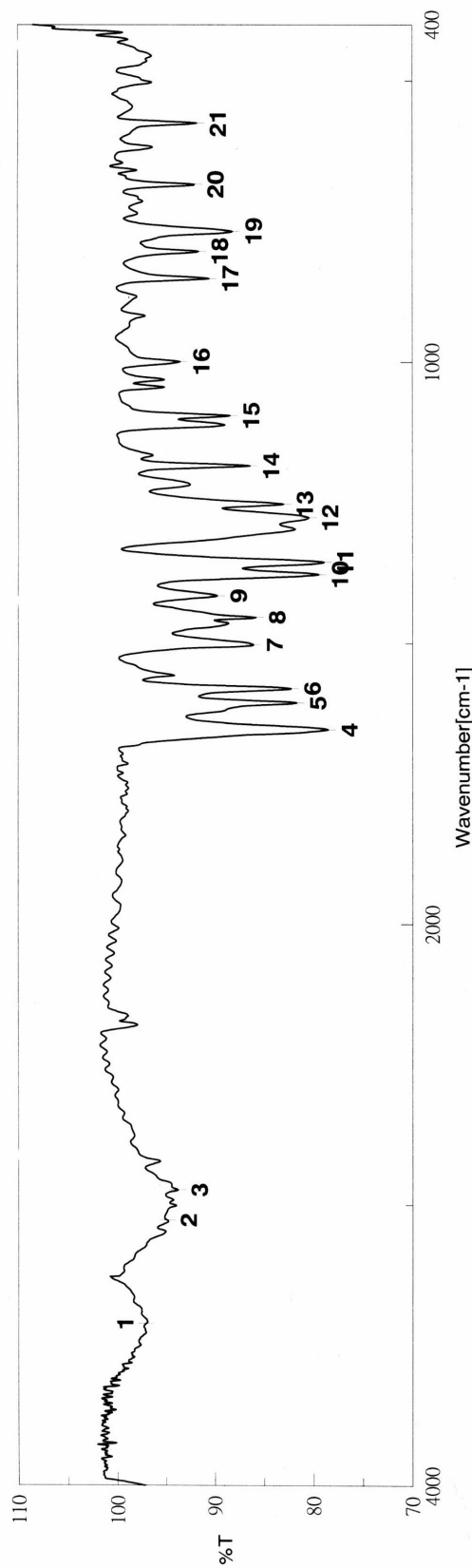
===== CHANNEL f1 =====
NUC1       13C
P1         11.10 usec
PL1        0.00 dB
SFO1       75.4763978 MHz

===== CHANNEL f2 =====
CPDPRG2   waltz16
NUC2       1H
PCPD2      90.00 usec
PL2        0.00 dB
PL12       18.80 dB
PL13       21.80 dB
SFO2       300.1313506 MHz

F2 - Processing parameters
SI         32768
SF         75.4677477 MHz
WDW        EM
SSB        0
LB         1.00 Hz
GB         0
PC         1.00

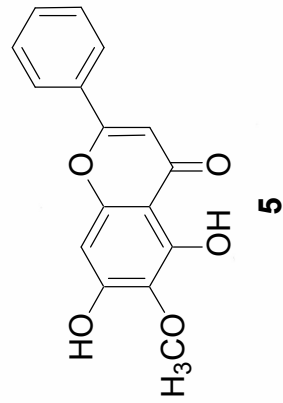
1D NMR plot parameters
CX         20.00 cm
F1P        200.000 ppm
F1         15093.55 Hz
F2P        0.000 ppm
F2         0.00 Hz
PPMCM      10.00000 ppm/cm
HZCM       754.67749 Hz/cm
    
```

5: C NMR



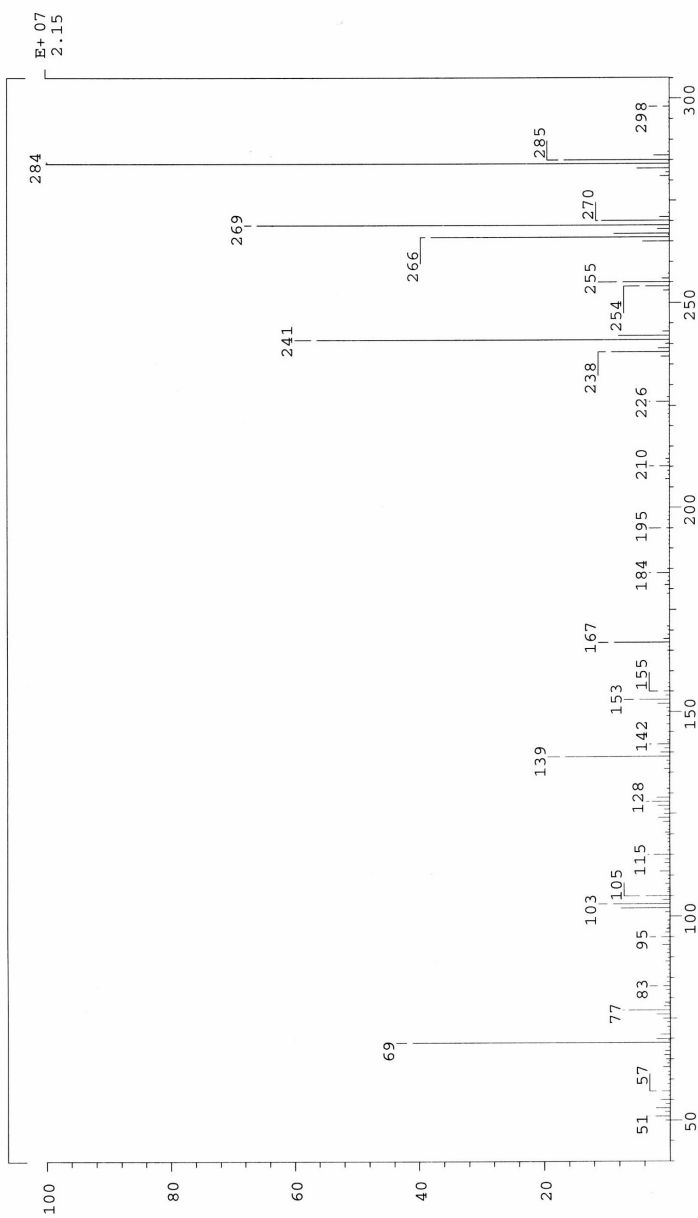
Accumulation	50	Resolution	4 cm-1
Zero Filling	ON	Apodization	Cosine
Gain	2	Scanning Speed	2 mm/sec
Date/Time	2006/10/27 4:53PM		
Update	2006/11/12 11:18AM		
Operator	lauchun Cheng		
File Name	big-a5-11		
Sample Name	big a5		
Comment			

1: 3423.99, 9.70E+01	3: 2947.66, 9.37E+01	4: 1653.66, 7.86E+01
5: 1606.41, 8.18E+01	7: 1502.28, 8.61E+01	8: 1454.06, 8.59E+01
9: 1415.49, 8.98E+01	11: 1355.71, 7.90E+01	12: 1276.65, 8.05E+01
13: 1252.54, 8.30E+01	15: 1095.37, 8.85E+01	16: 998.95, 9.35E+01
17: 850.45, 9.06E+01	18: 802.24, 9.16E+01	20: 683.64, 9.21E+01
21: 574.68, 9.18E+01		

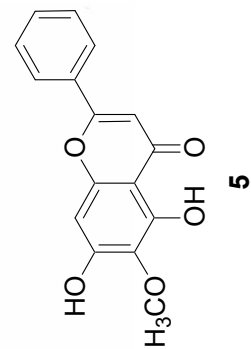


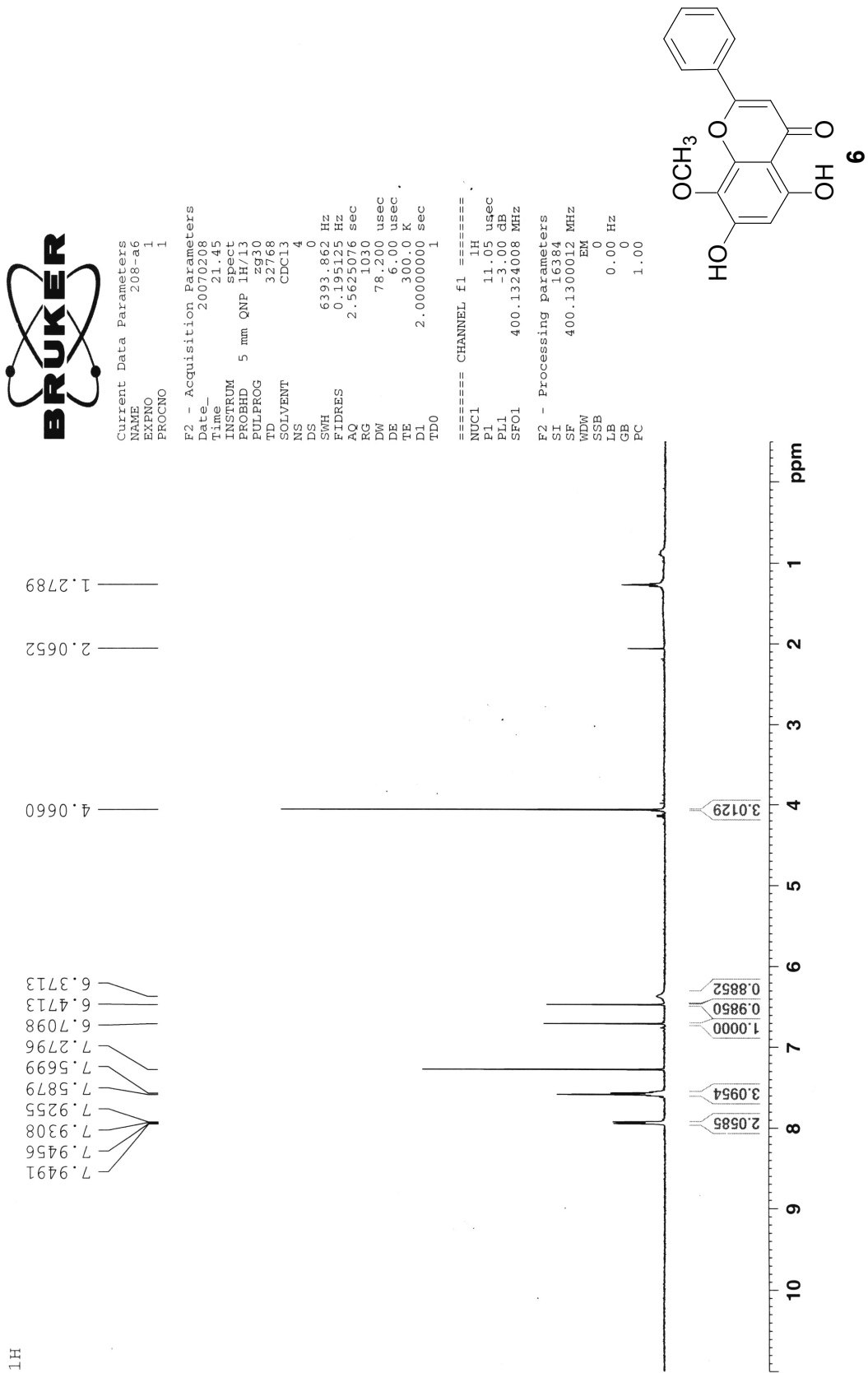
5: IR

SPEC: lei4305
 Samp: 1a-A6
 Mode: EI +VE +LMR BSCAN (EXP) UP LR NRM
 Oper: 03-Oct-06 REG : 09:56.9 #9
 Base: 284.0 Inten : 21503854 Start : 15:21:01 784
 Norm: 284.0 RIC : 119109782 Inlet :
 Peak: 242.00 mmu Masses: 45 > 300
 Data: +/-322>409 #peaks: 547



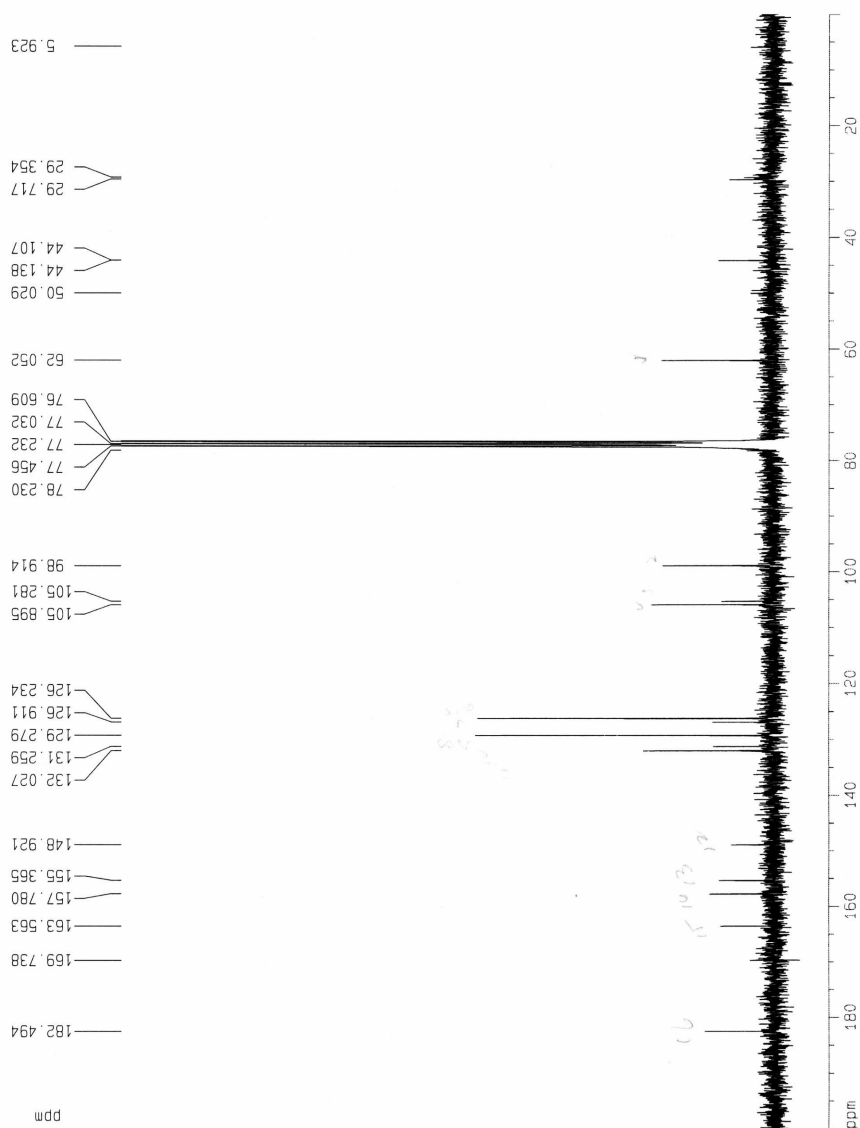
5: MS



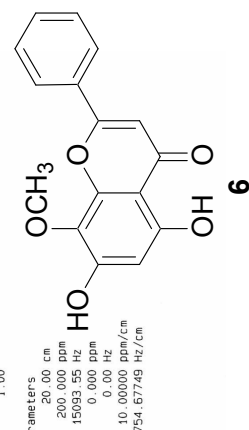


6: 1H NMR

C13 spectrum of sample at NDHU Avance300 of BBO probehead



6: CNMR



```

Current Data Parameters
NAME      061105-a6C
EXPNO     1
PROCNO    1

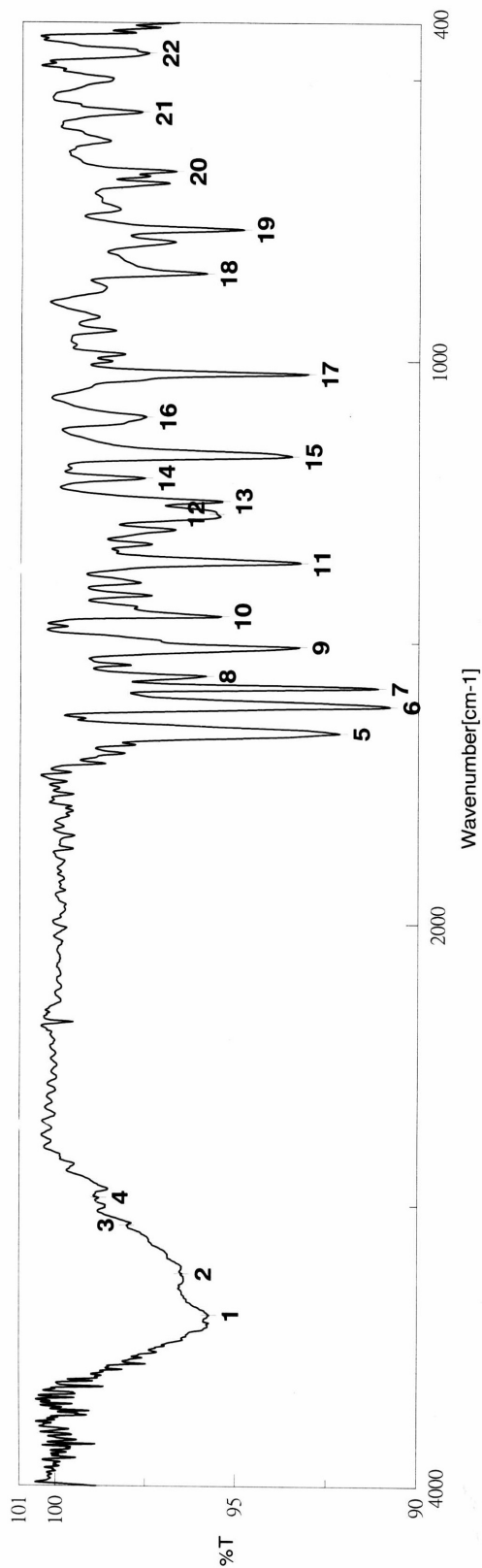
F2 - Acquisition Parameters
Date_     20061110
Time      9.38
INSTRUM   spect
PROBHD    5 mm Multinu
PULPROG   zgpg30
TD         65536
SOLVENT   CDCl3
NS         11000
DS         0
SWH        18832.393 Hz
FIDRES     0.287360 Hz
AQ          1.7400308 sec
RG          5792.6
DM          26.550 usec
DE          6.50 usec
TE          300.0 K
D1          1.50000000 sec
D11         0.03000000 sec
D12         0.00002000 sec

===== CHANNEL f1 =====
NUC1       13C
P1         11.10 usec
PL1        0.00 dB
SFO1       75.4763978 MHz

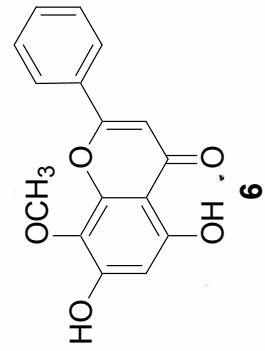
===== CHANNEL f2 =====
CPDPRG2   waltz16
NUC2       1H
P2         90.00 usec
PL2        0.00 dB
PL12       18.80 dB
PL13       21.80 dB
SFO2       300.1313505 MHz

F2 - Processing parameters
SI         32768
SF         75.4677477 MHz
WDW        EM
SSB        0
LB         1.00 Hz
GB         0
PC         1.00

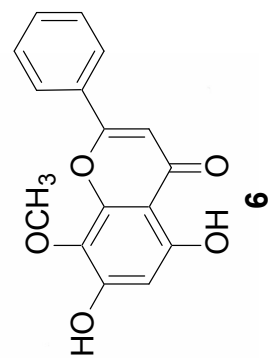
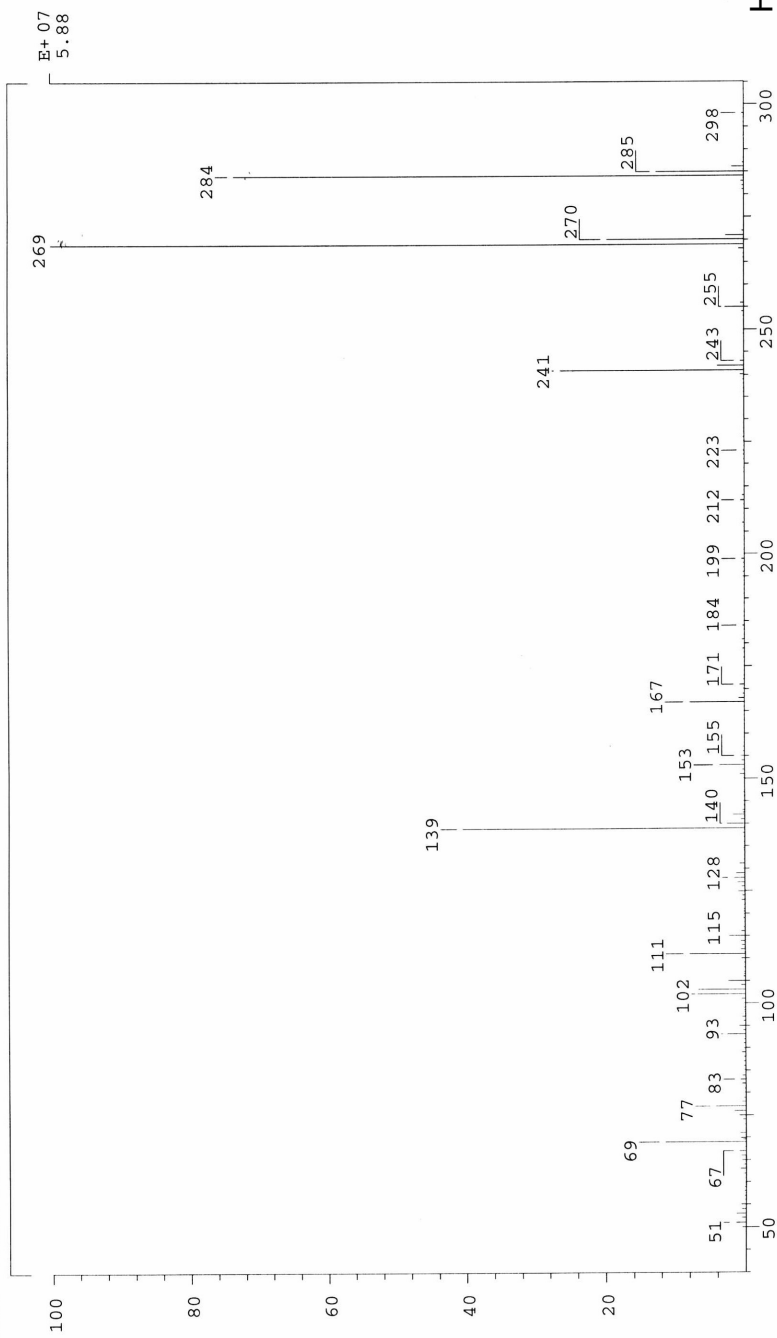
1D NMR plot parameters
CX          0.00 cm
CY          0.00 cm
F1P        15093.55 Hz
F2P         0.00 Hz
F2          0.00000 ppm/cm
PPMCM       10.00000 ppm/cm
HZCM        754.67749 Hz/cm
    
```



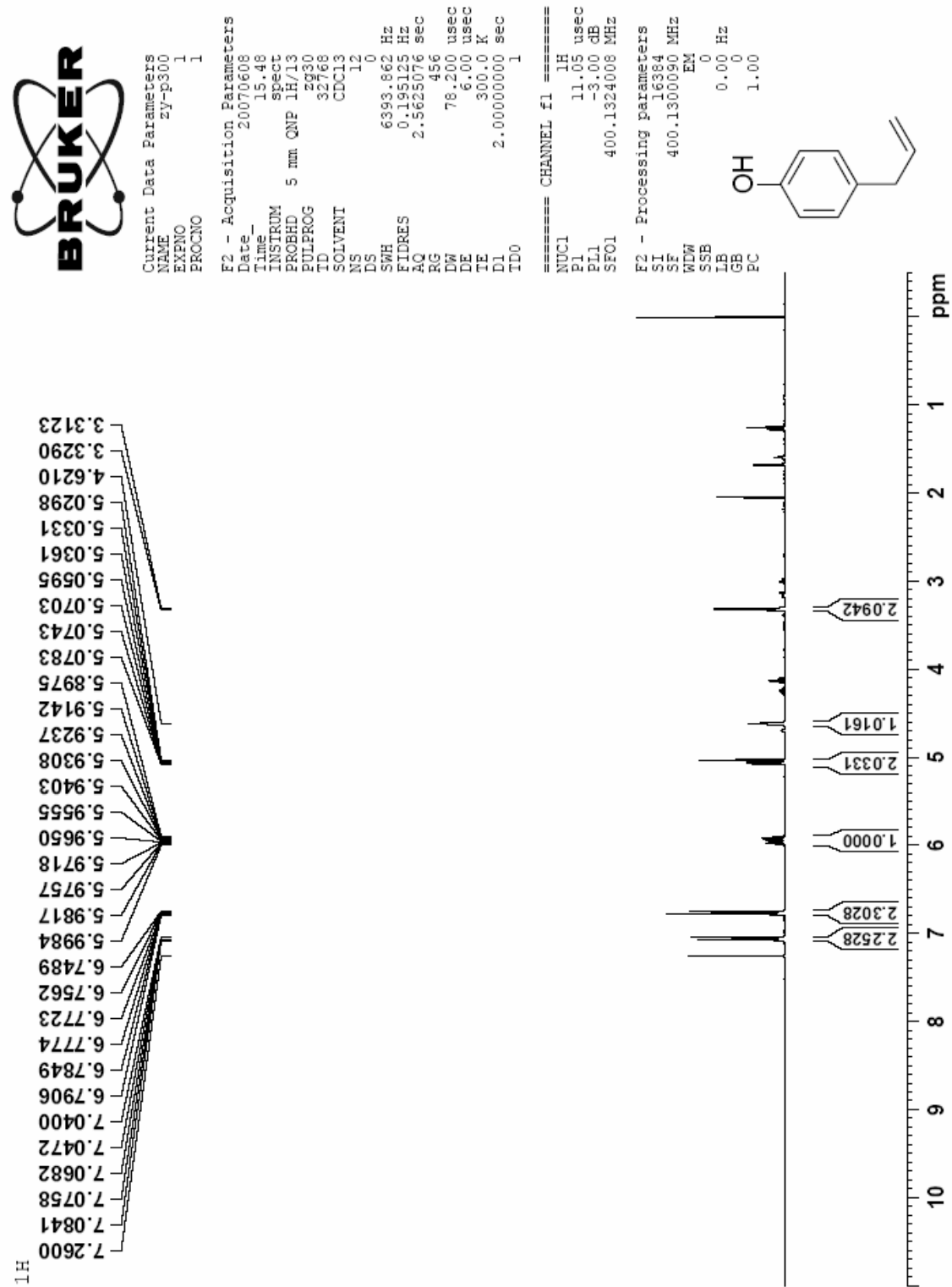
6: IR



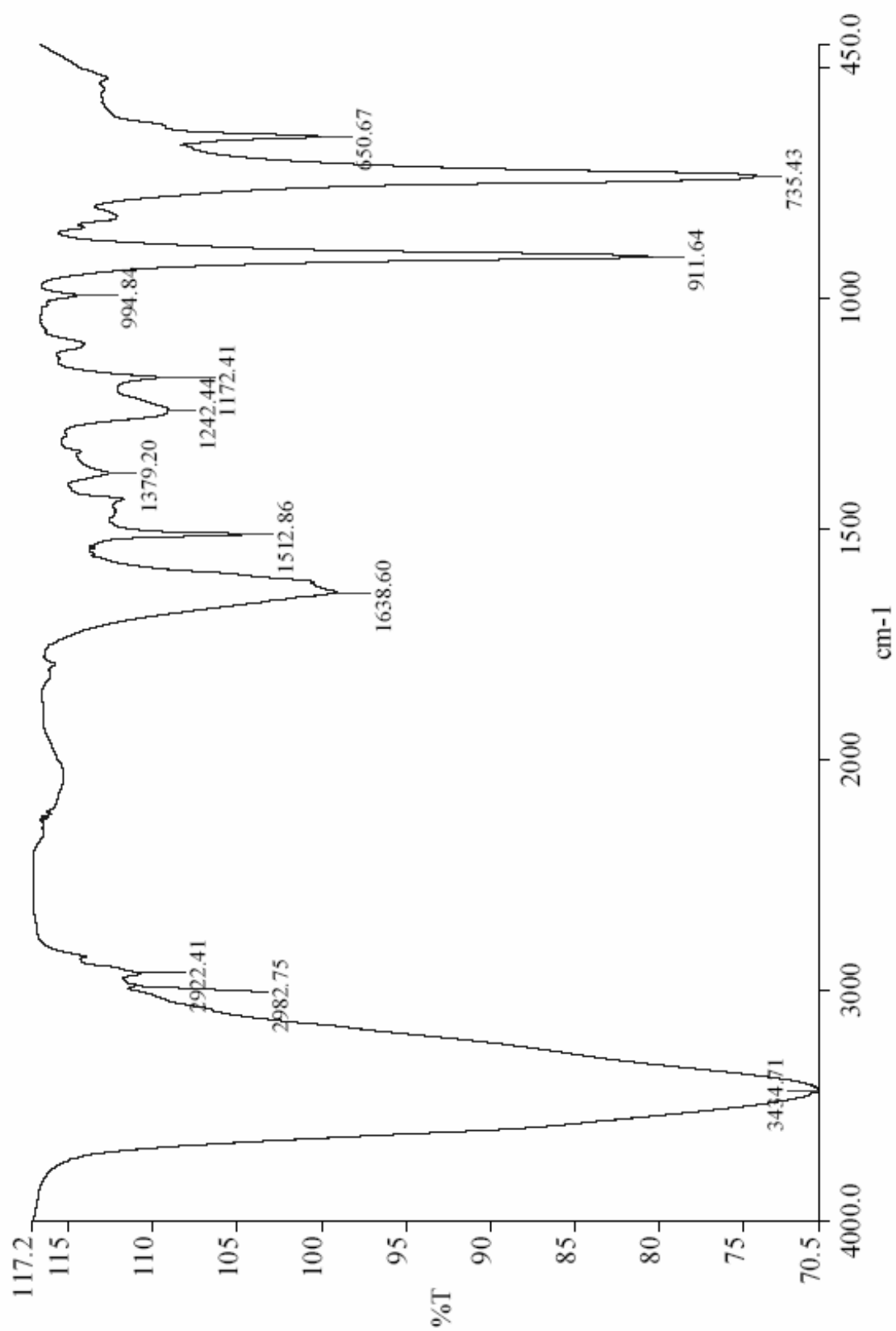
SPEC: lei4306
 Samp: 1a-A6
 Mode: EI +VE +LMR BSCAN (EXP) UP LR NFM
 Oper: 269.0
 Base: 269.0
 Norm: 269.0
 Peak: 242.00 mmu
 Data: +/-488>568
 03-Oct-06 REG : 15:03.9 #9
 Start : 15:45:29 1035
 Inlet :
 Masses: 45 > 300
 #peaks: 498



6: MS

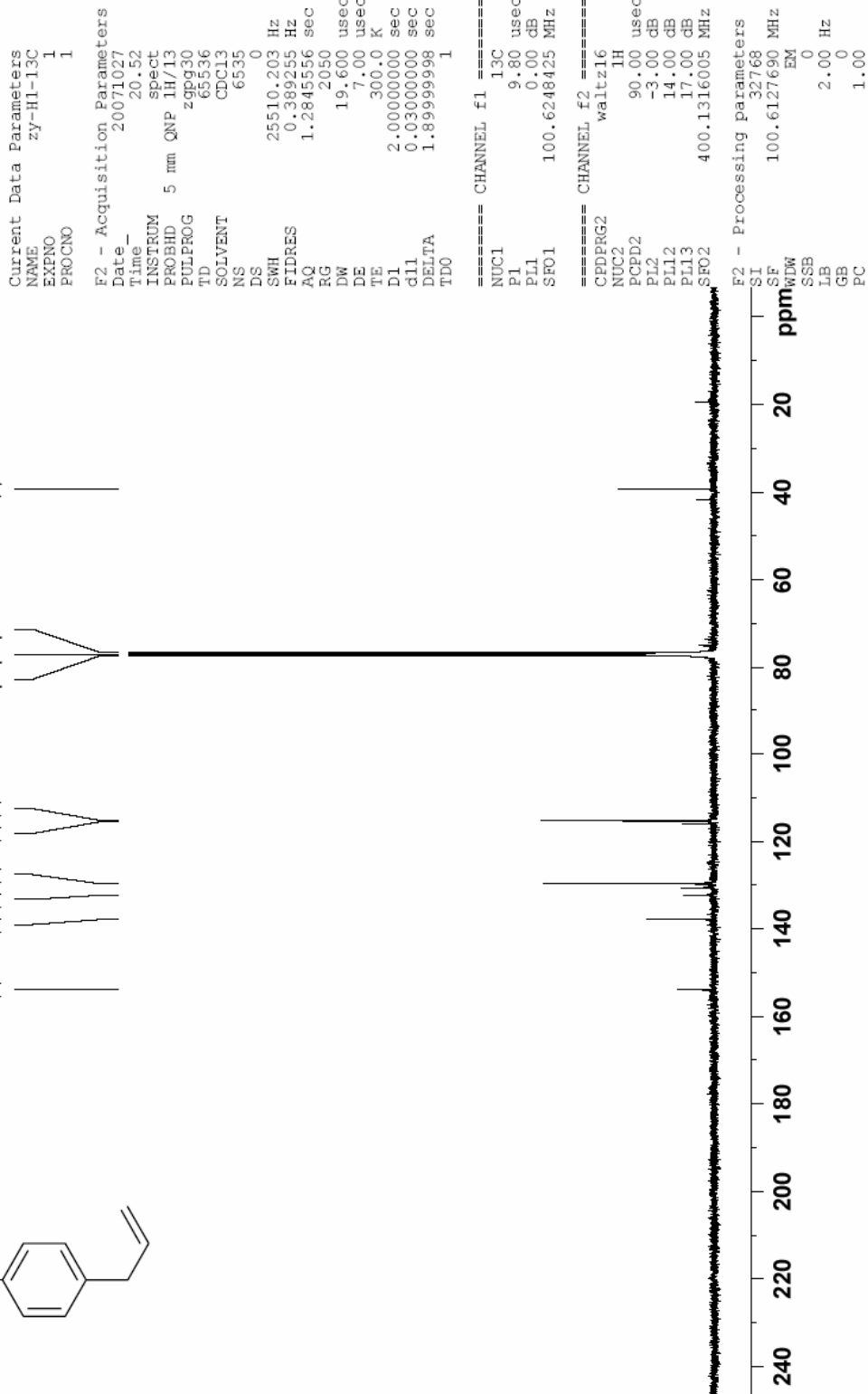
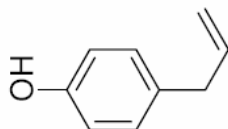


8: H NMR

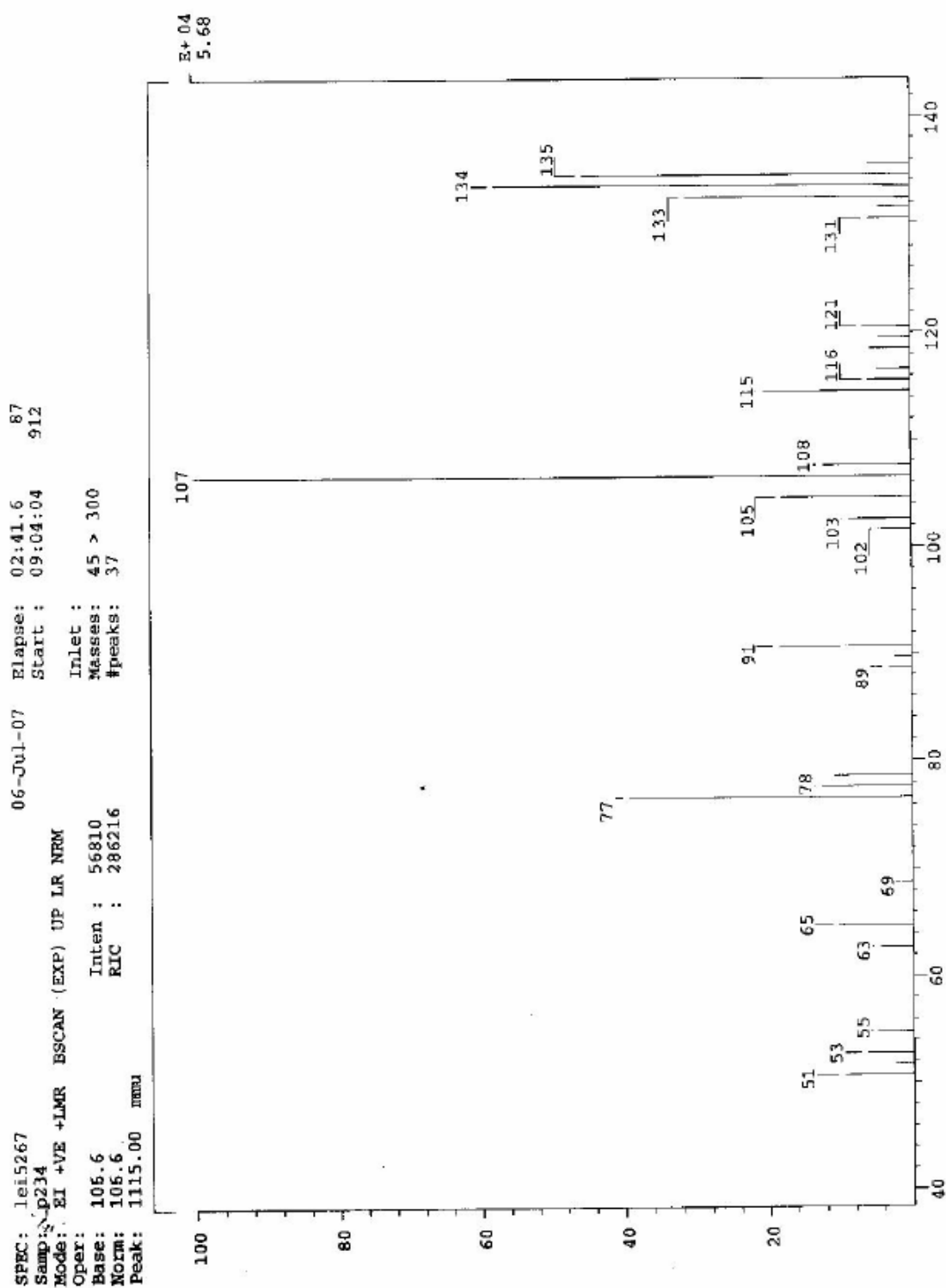


8: IR

¹³C



8: C NMR



8: MS

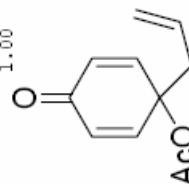


Current Data Parameters
NAME H2
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20070604
Time 22.08
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 11
DS 0
SWH 6393.862 Hz
FIDRES 0.195125 Hz
AQ 2.5625076 sec
RG 724
DW 78.200 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
TD0 1

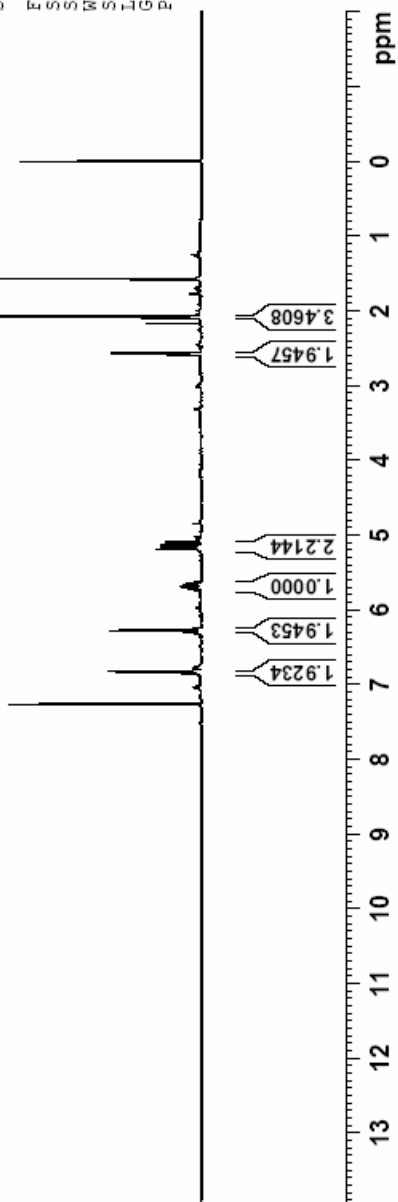
===== CHANNEL f1 =====
NUC1 1H
P1 11.05 usec
PL1 -3.00 dB
SFO1 400.1324008 MHz

F2 - Processing parameters
SI 16384
SF 400.1300085 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00



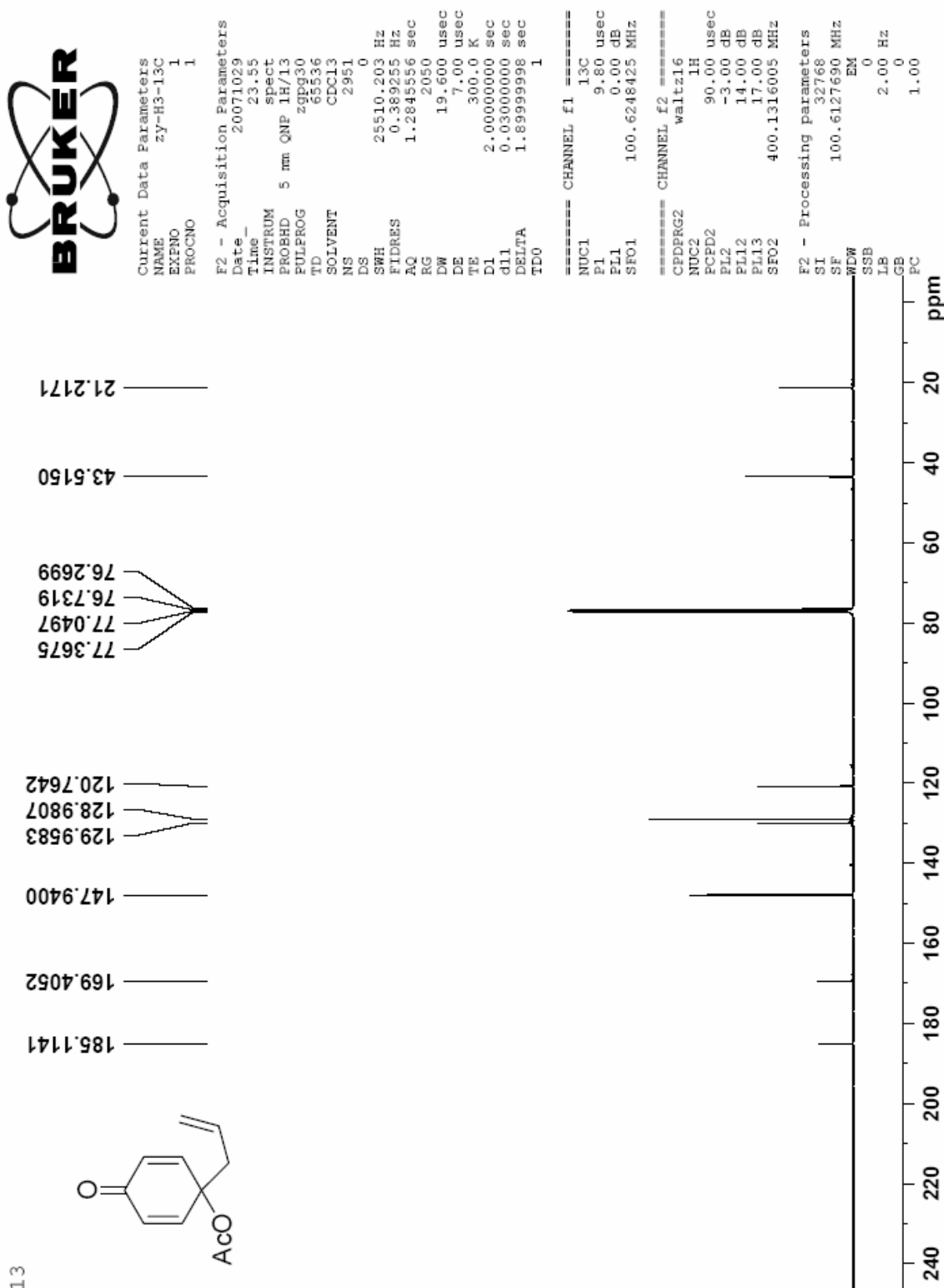
¹H

7.2611
6.8460
6.8213
6.2873
6.2626
5.7186
5.6943
5.6767
5.6516
5.1912
5.1659
5.1397
5.0969
2.5831
2.5649
2.0743

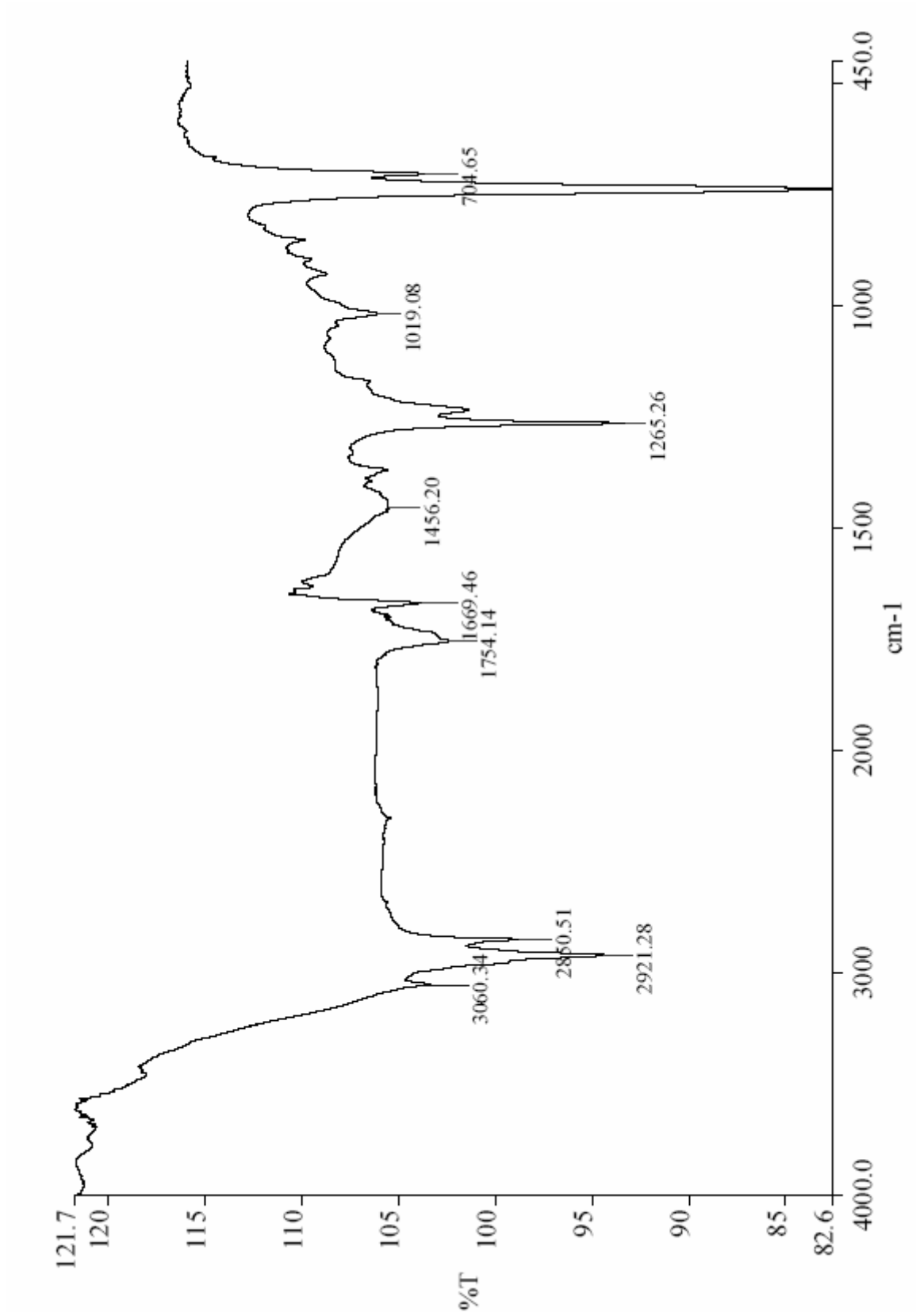


9: H NMR

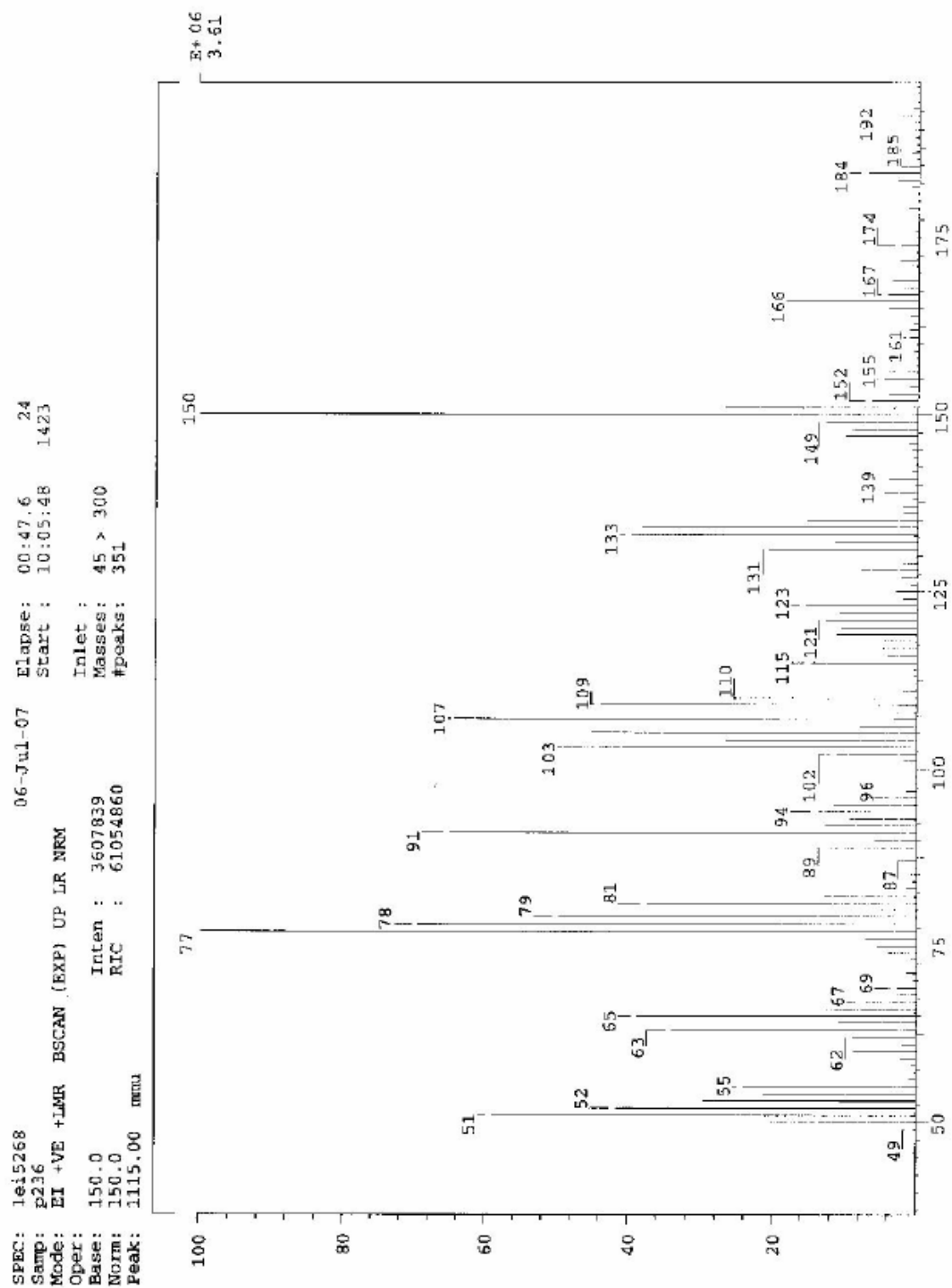
13



9: C NMR



9: IR



9: MS

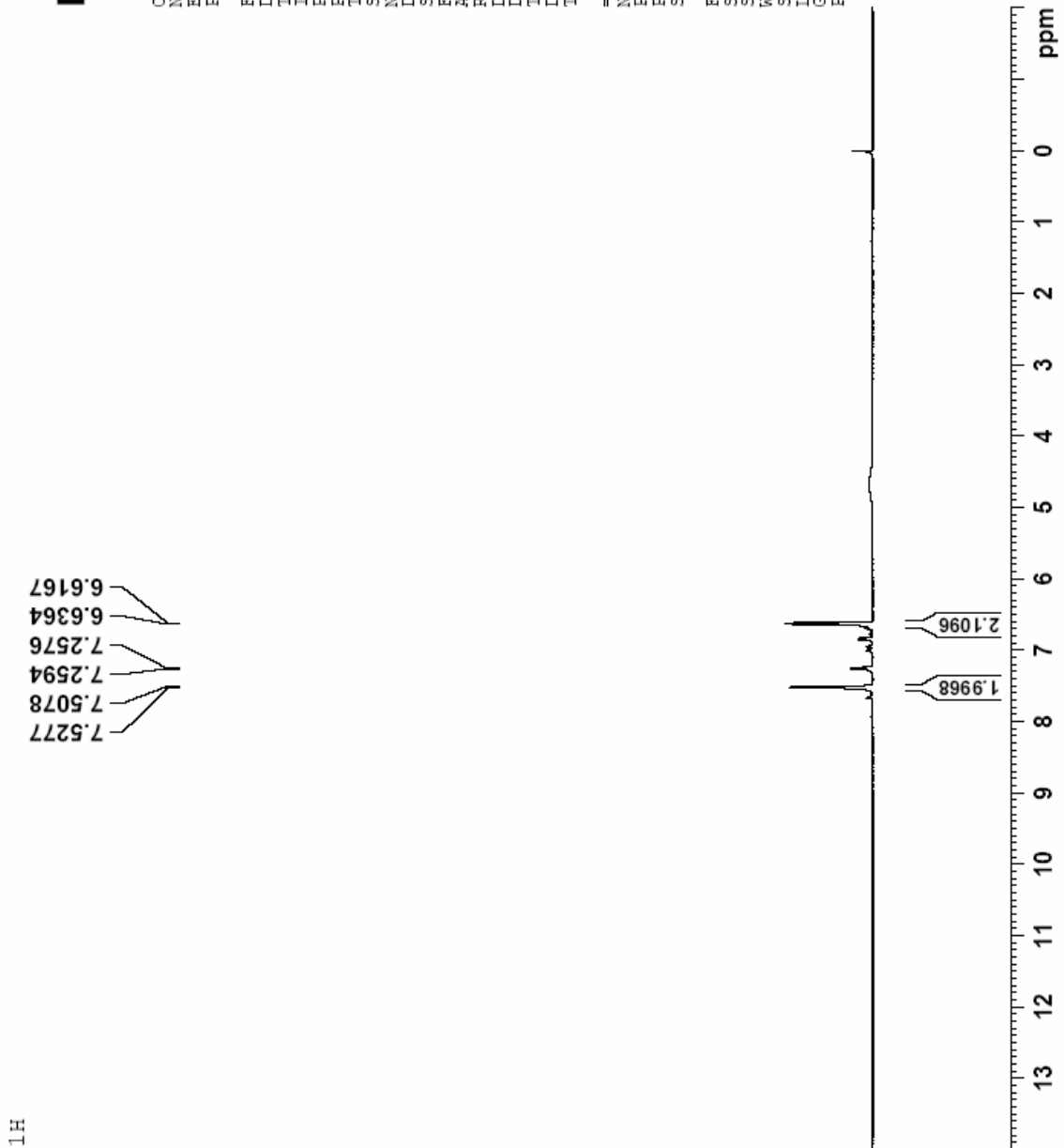
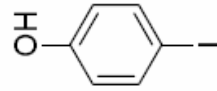


Current Data Parameters
NAME H3
EXPNO 1
PROCNO 1

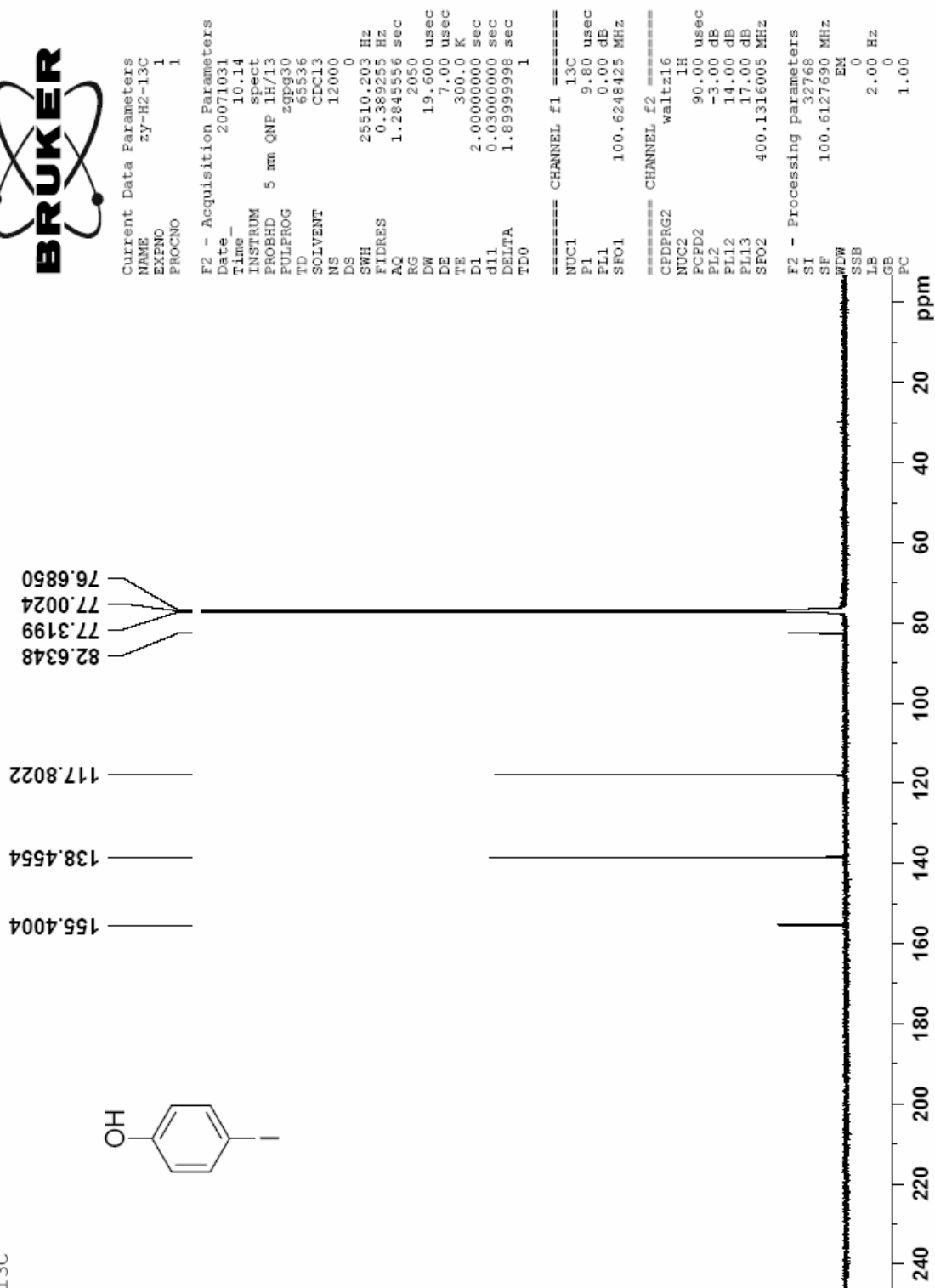
F2 - Acquisition Parameters
Date_ 20070621
Time 20.50
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 11
DS 0
SWH 6393.862 Hz
FIDRES 0.195125 Hz
AQ 2.5628076 sec
RG 203
DW 78.200 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
TD0 1

===== CHANNEL f1 =====
NUC1 1H
P1 11.05 usec
PL1 -3.00 dB
SFO1 400.1324008 MHz

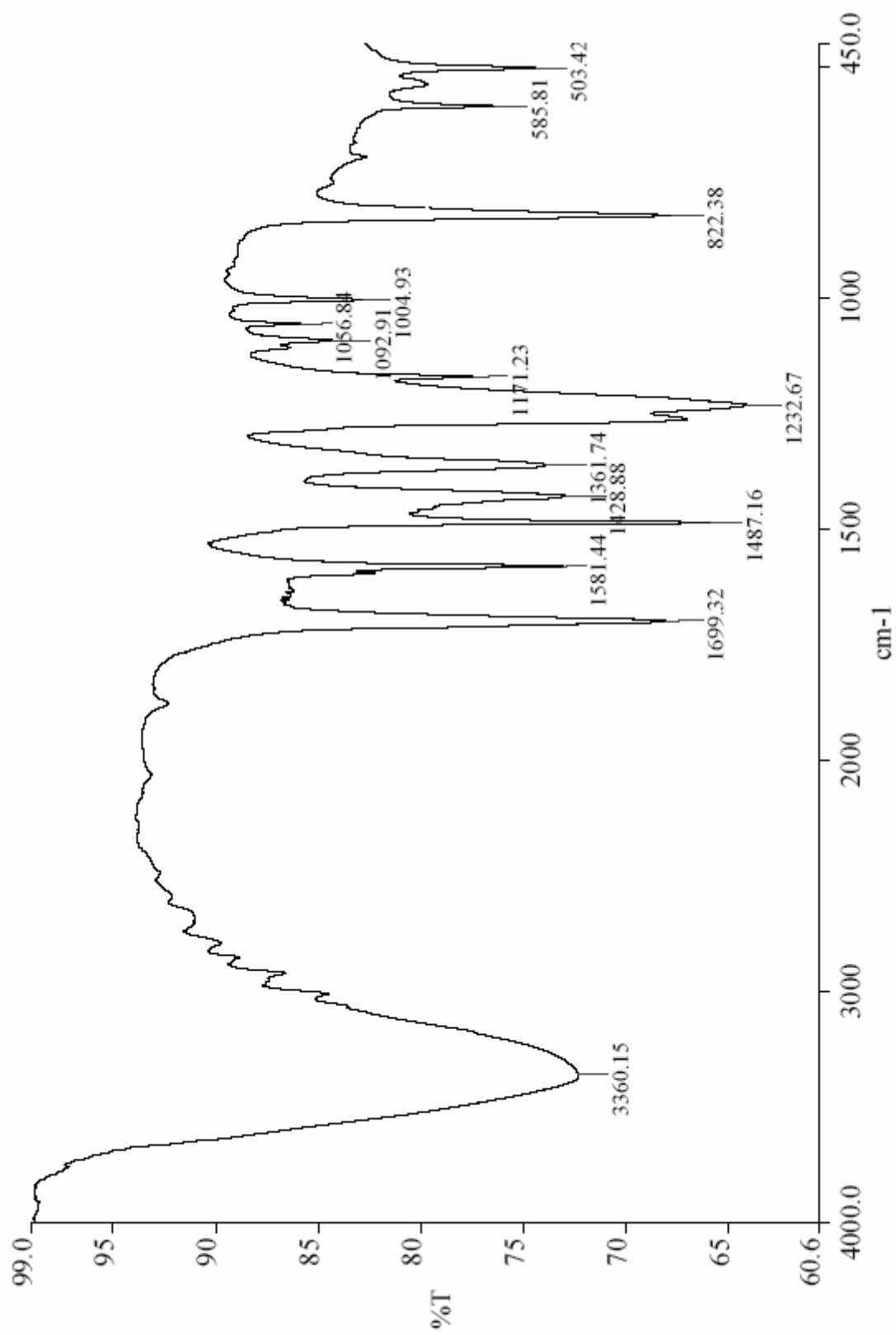
F2 - Processing parameters
SI 16384
SF 400.1300082 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00



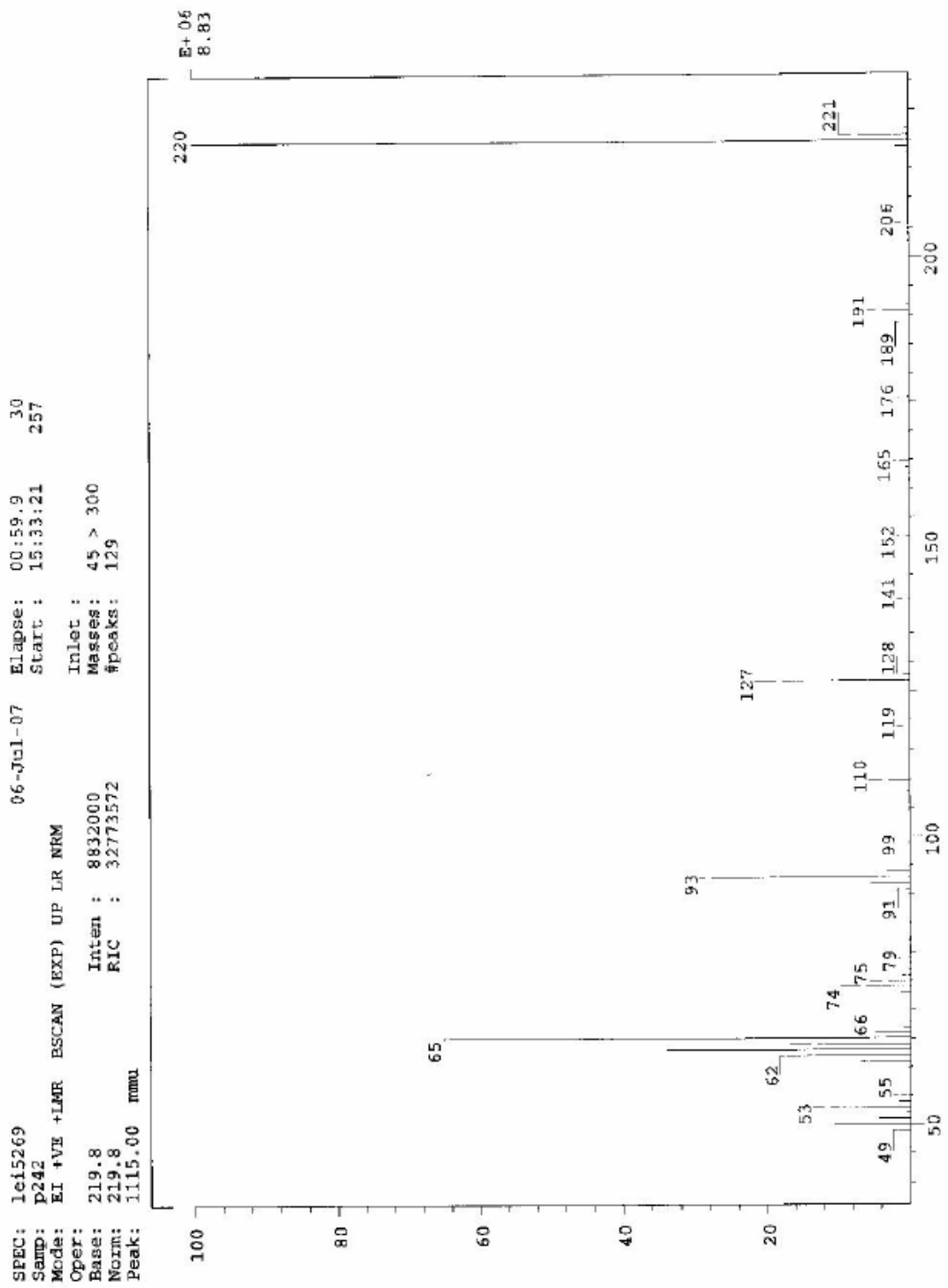
10: ¹H NMR

¹³C

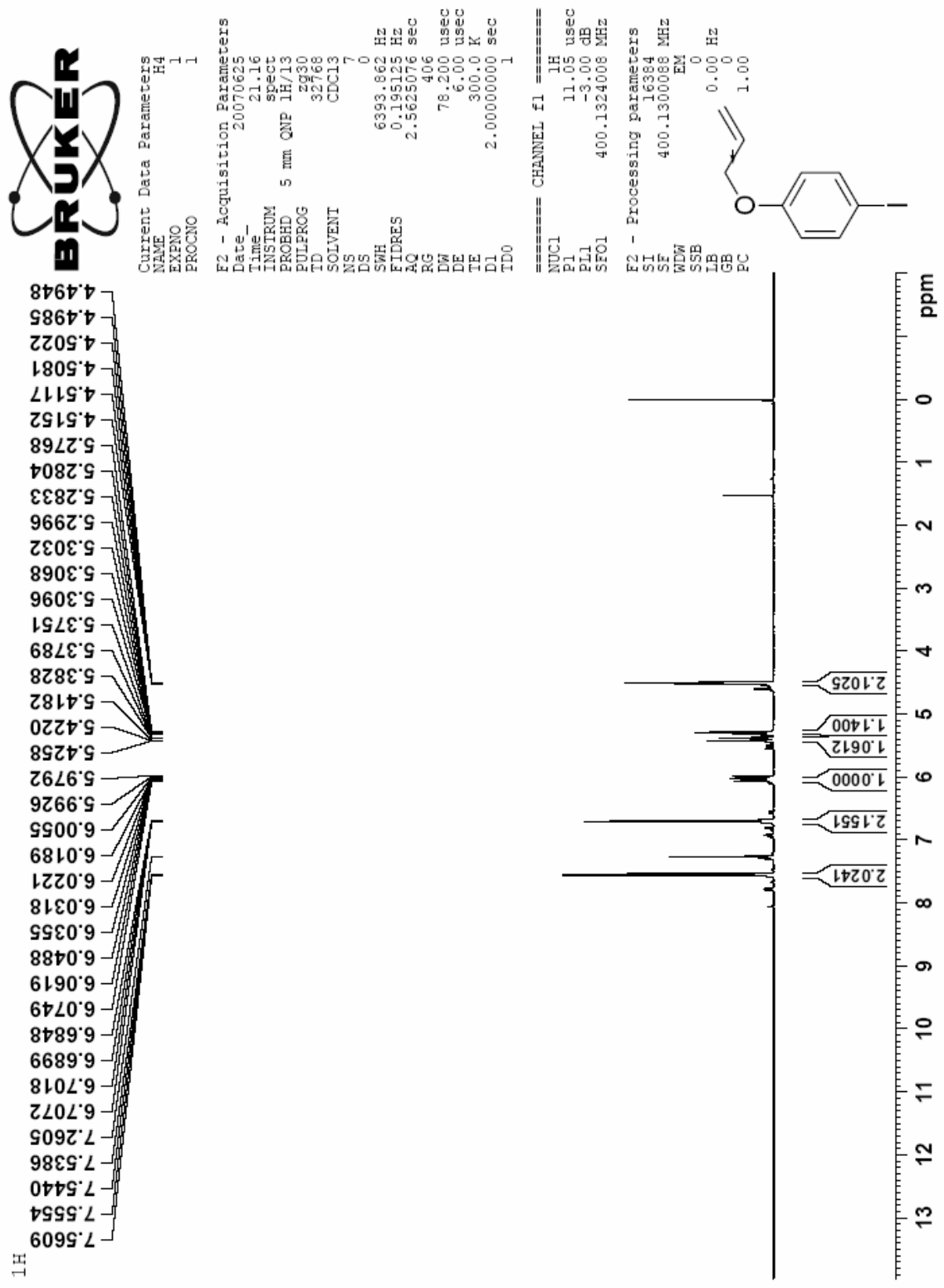
10: C NMR



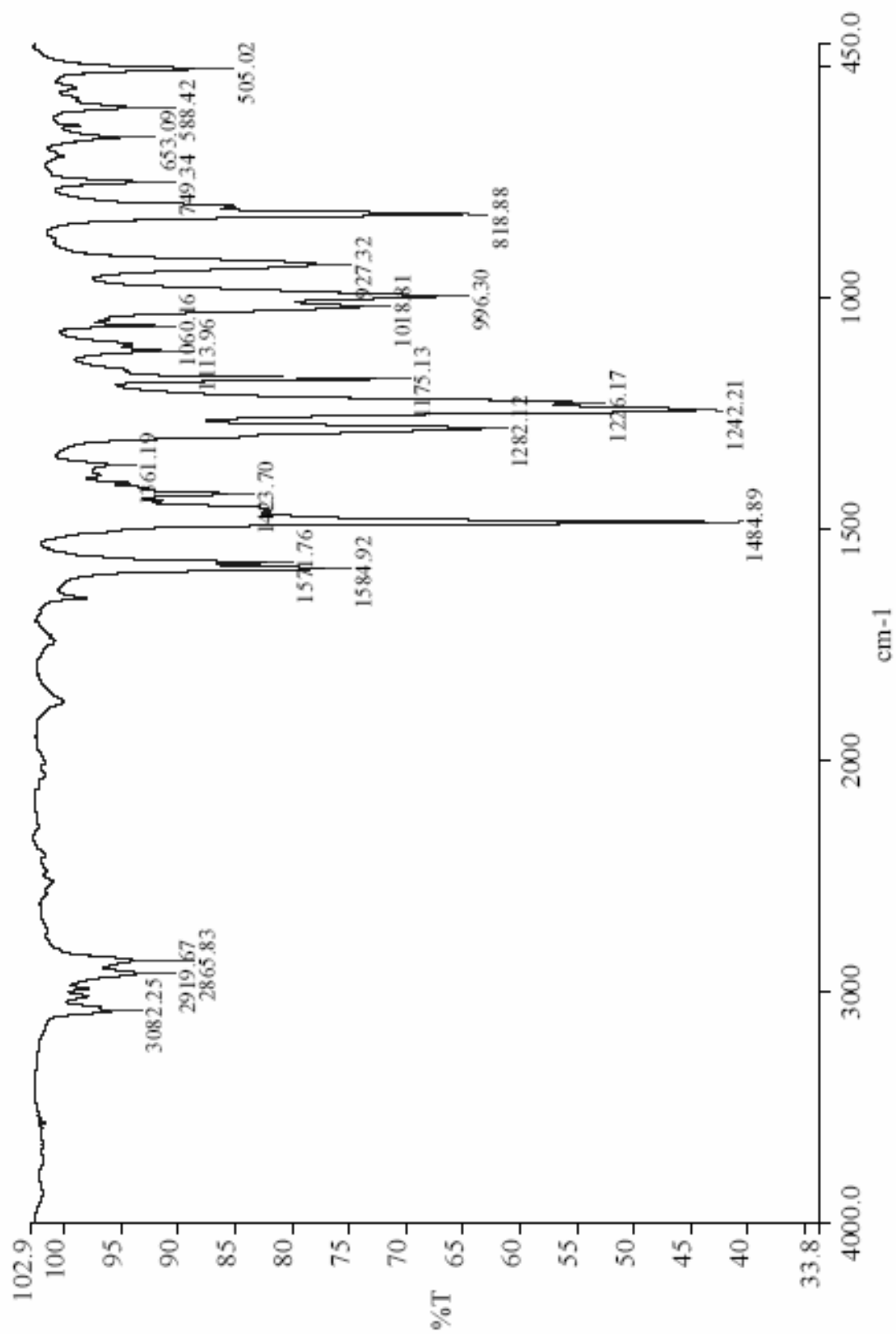
10: IR



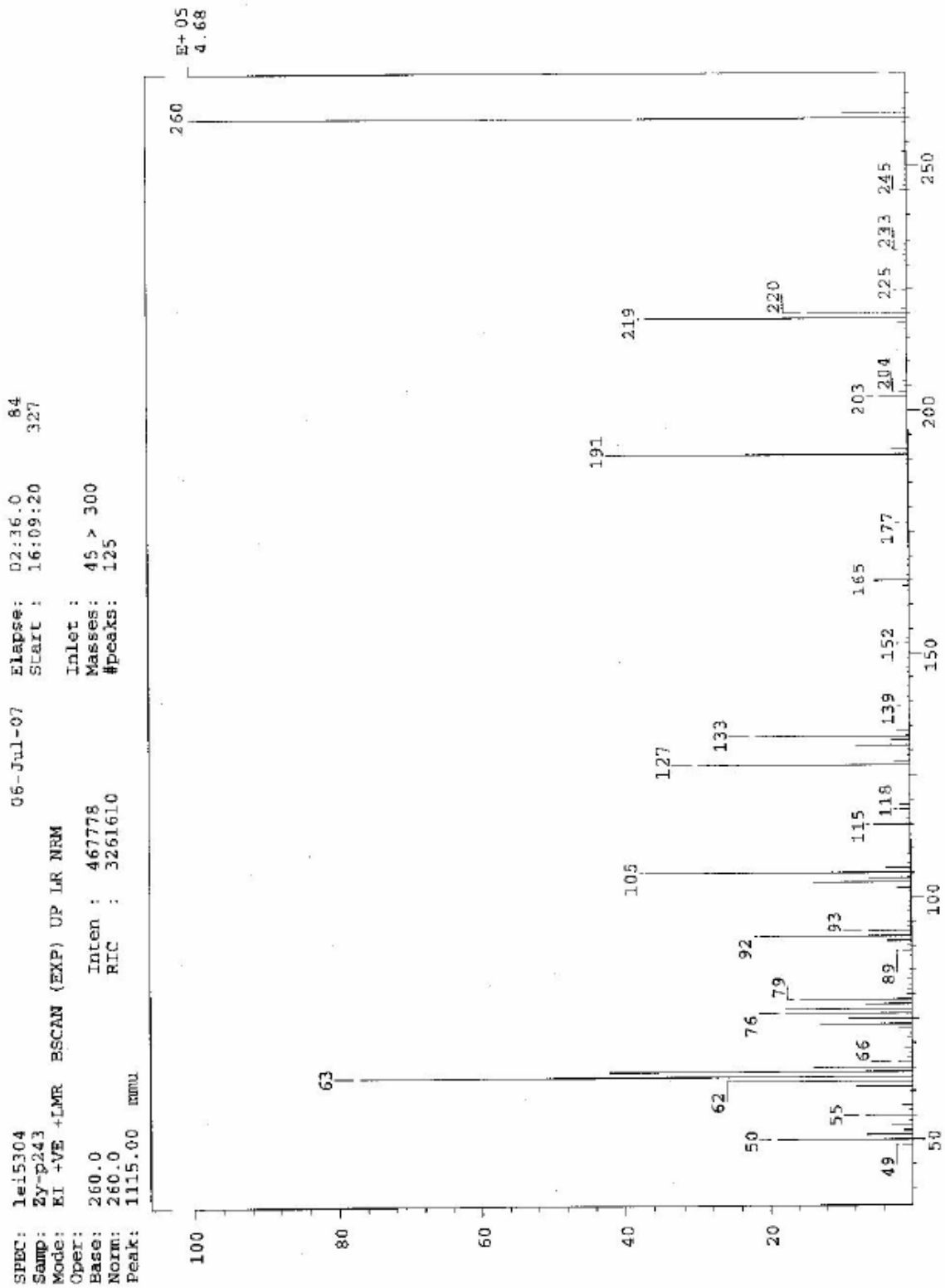
10: MS



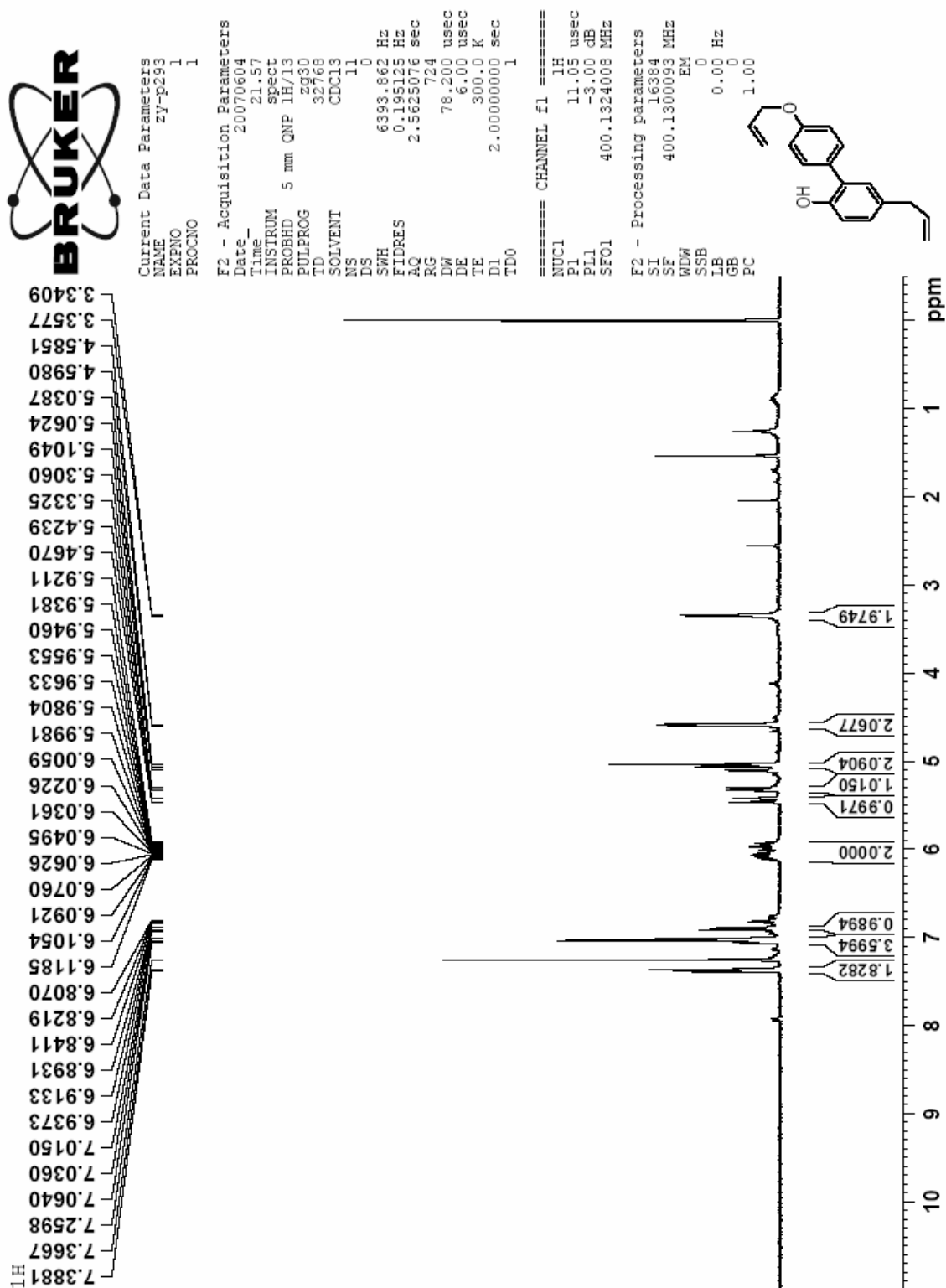
11: ¹H NMR

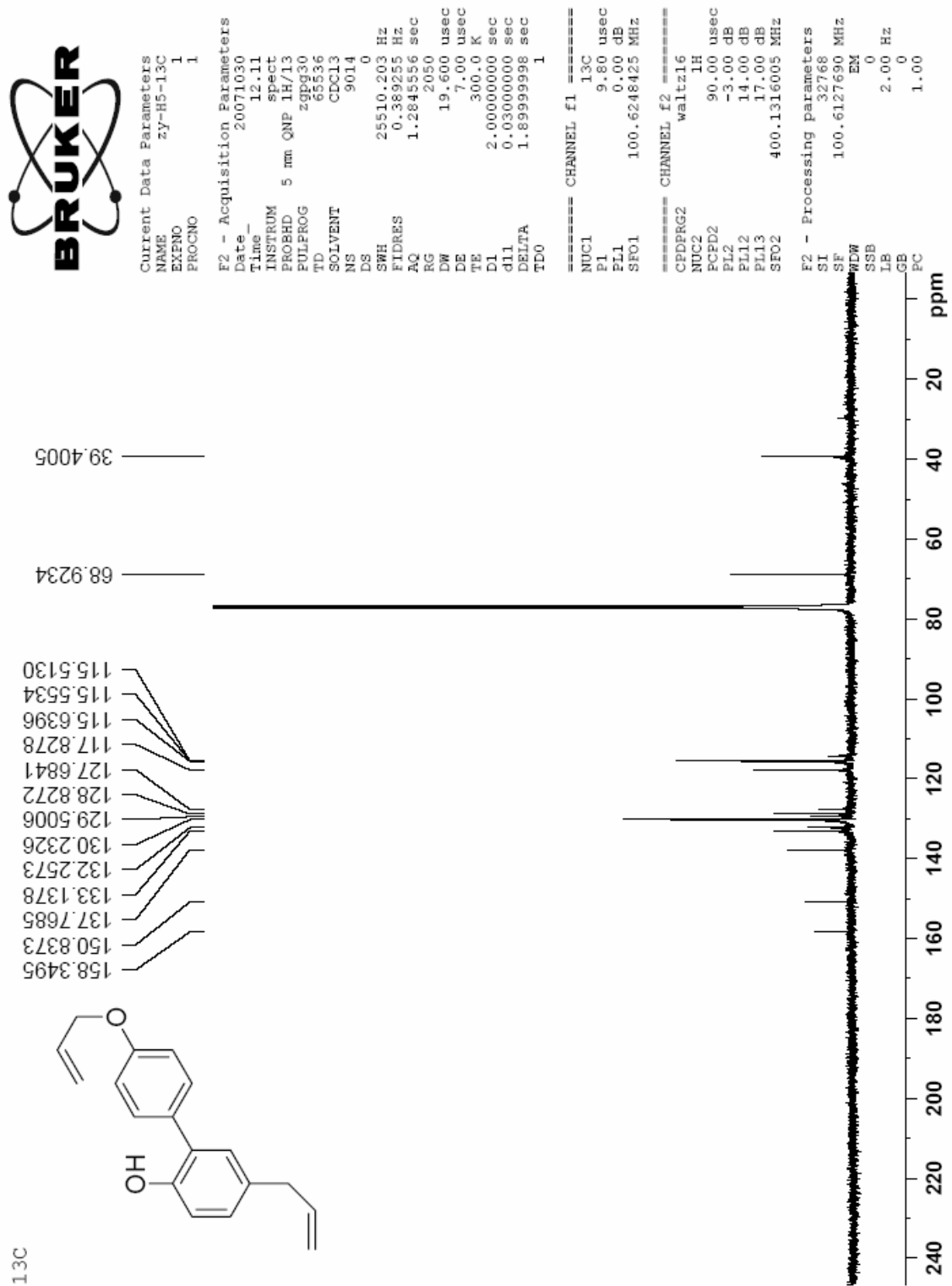


11: IR

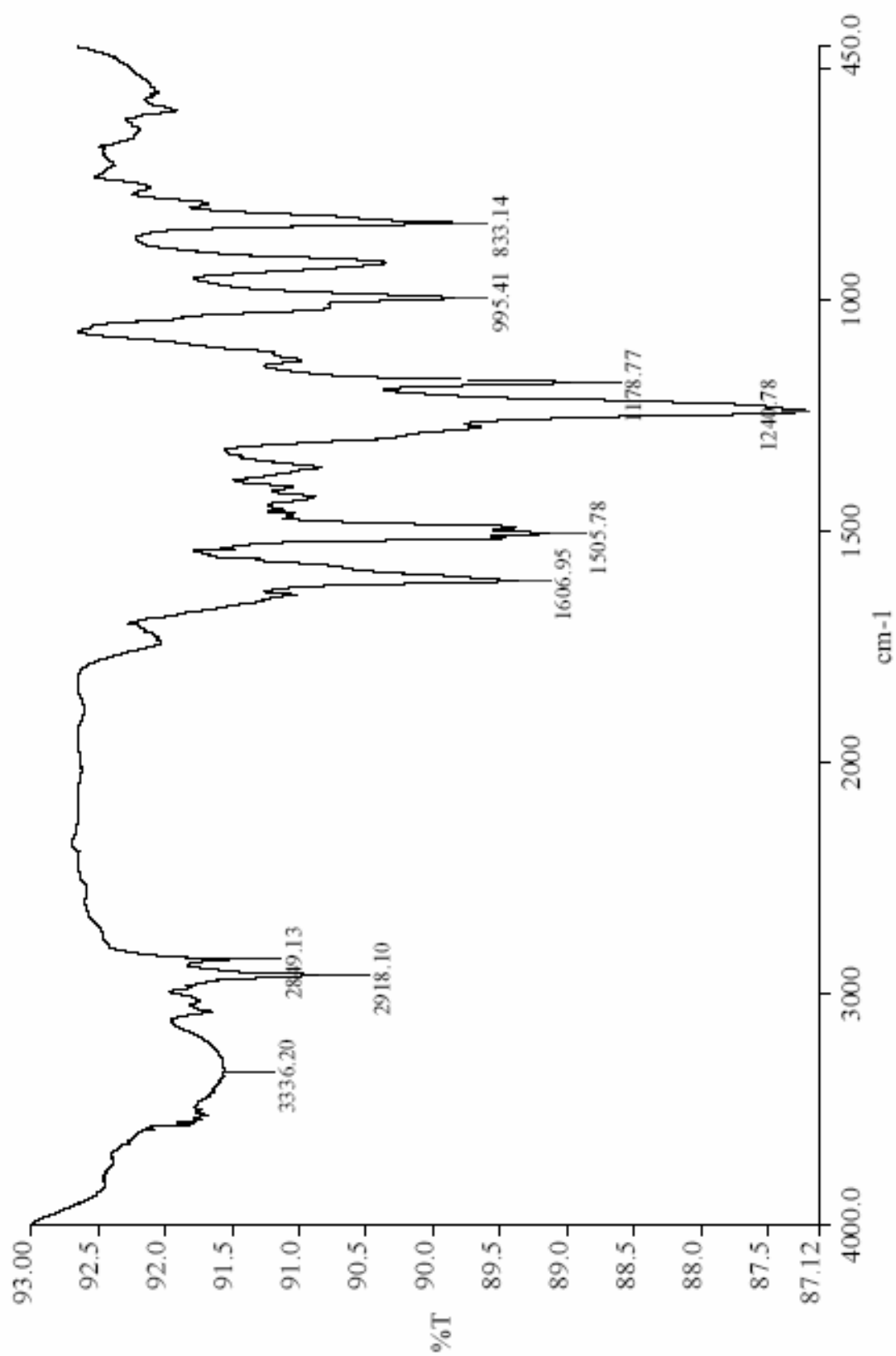


11: MS

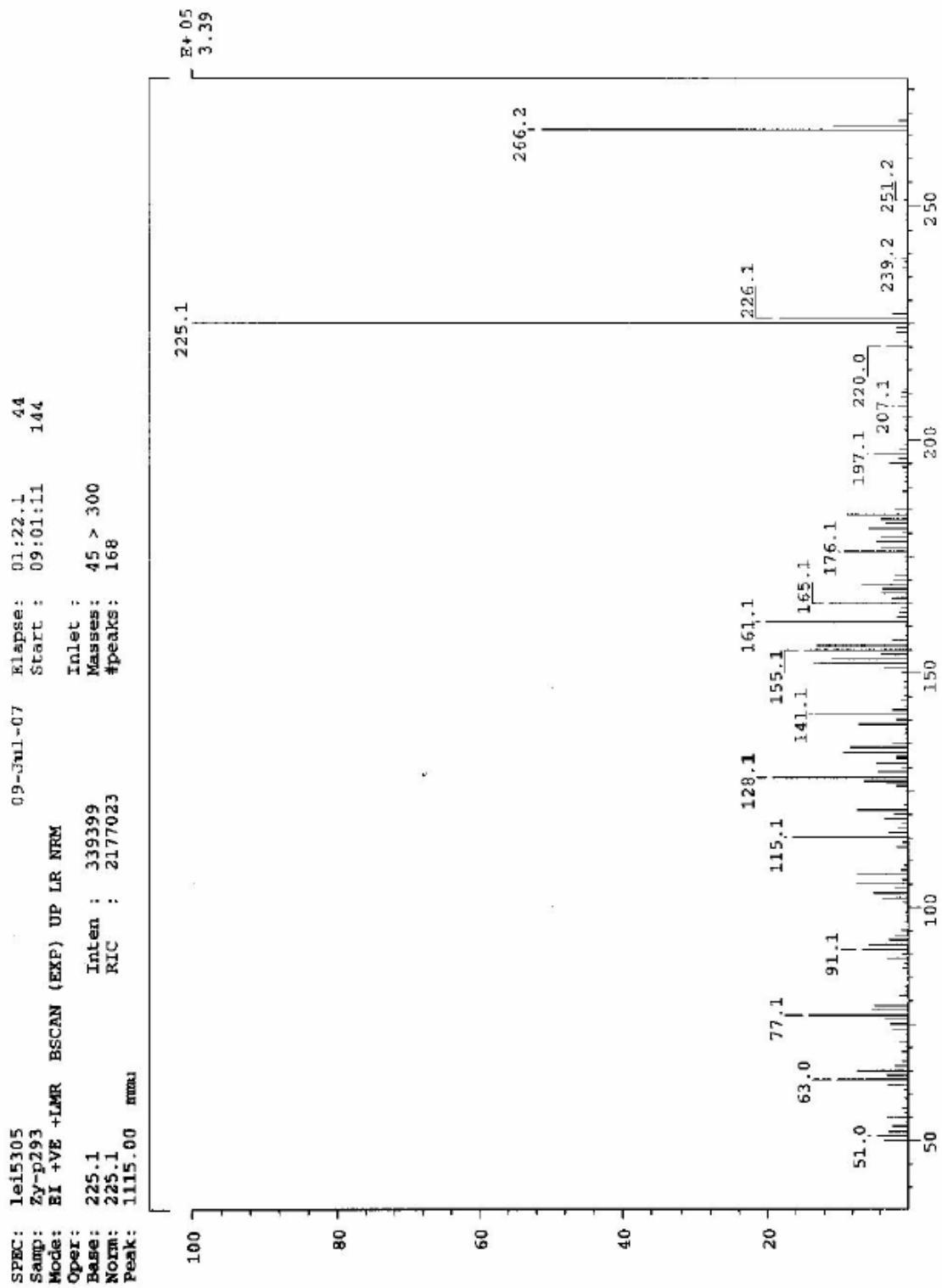
12: ¹H NMR



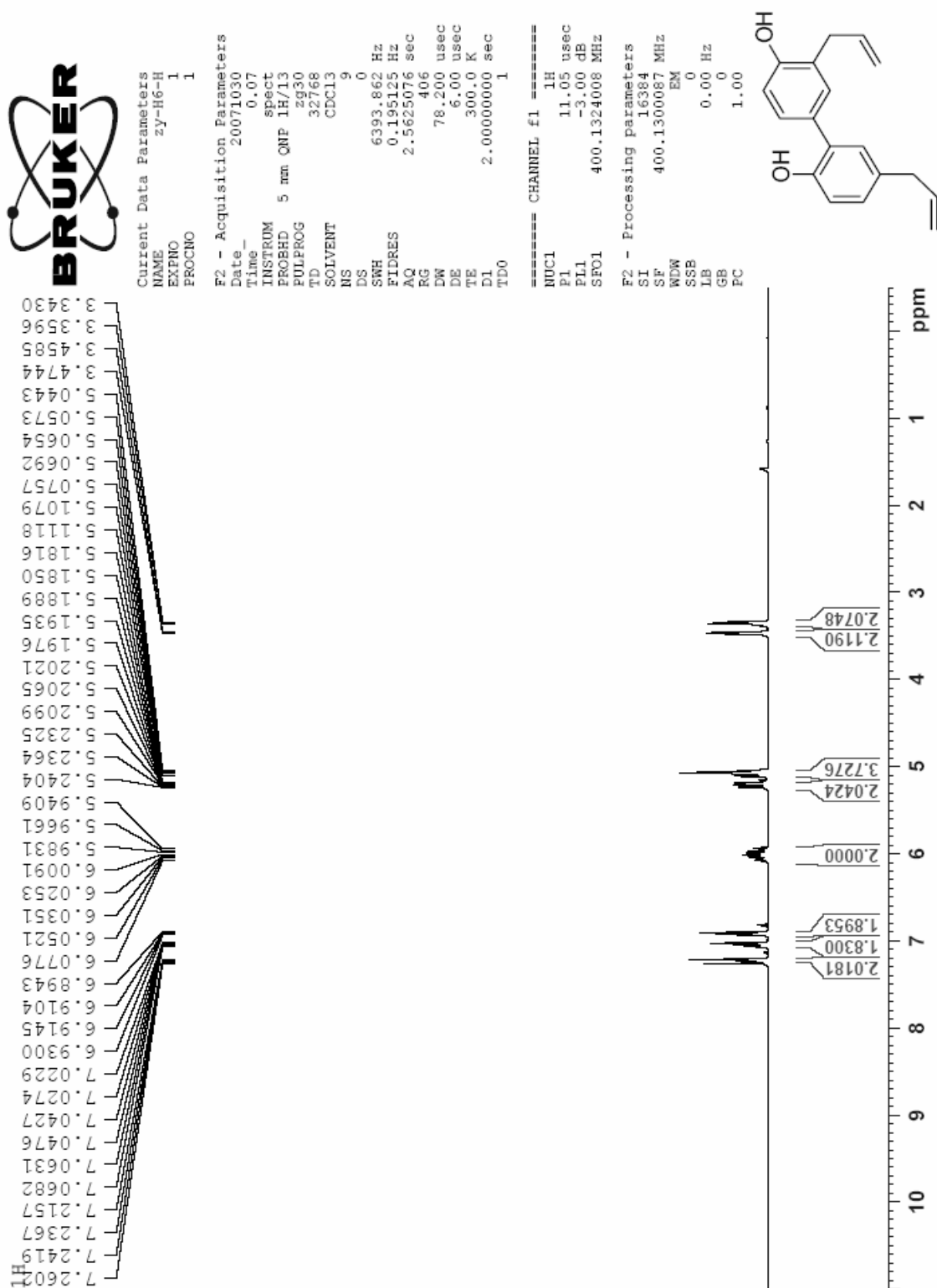
12: C NMR

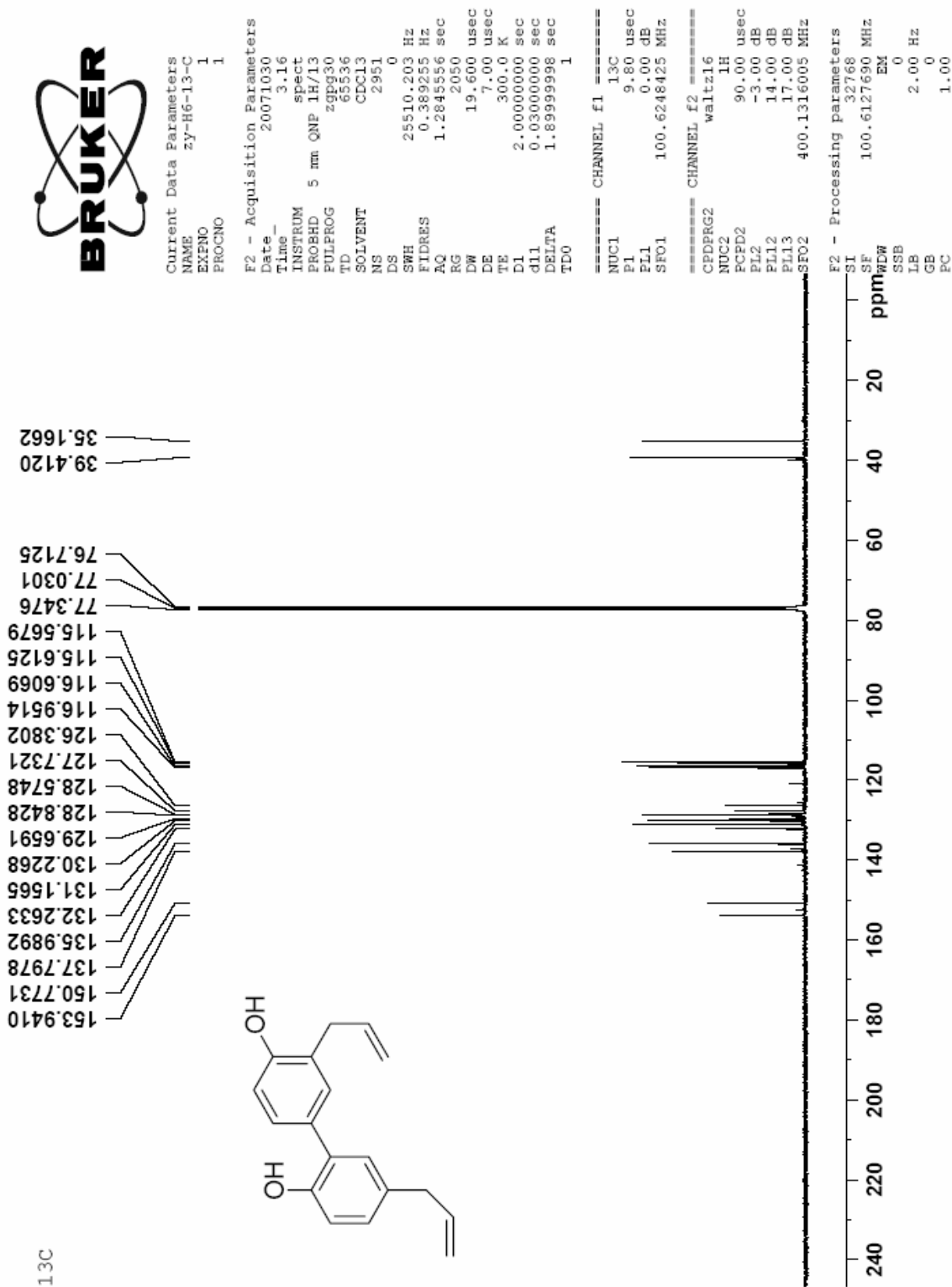


12: IR

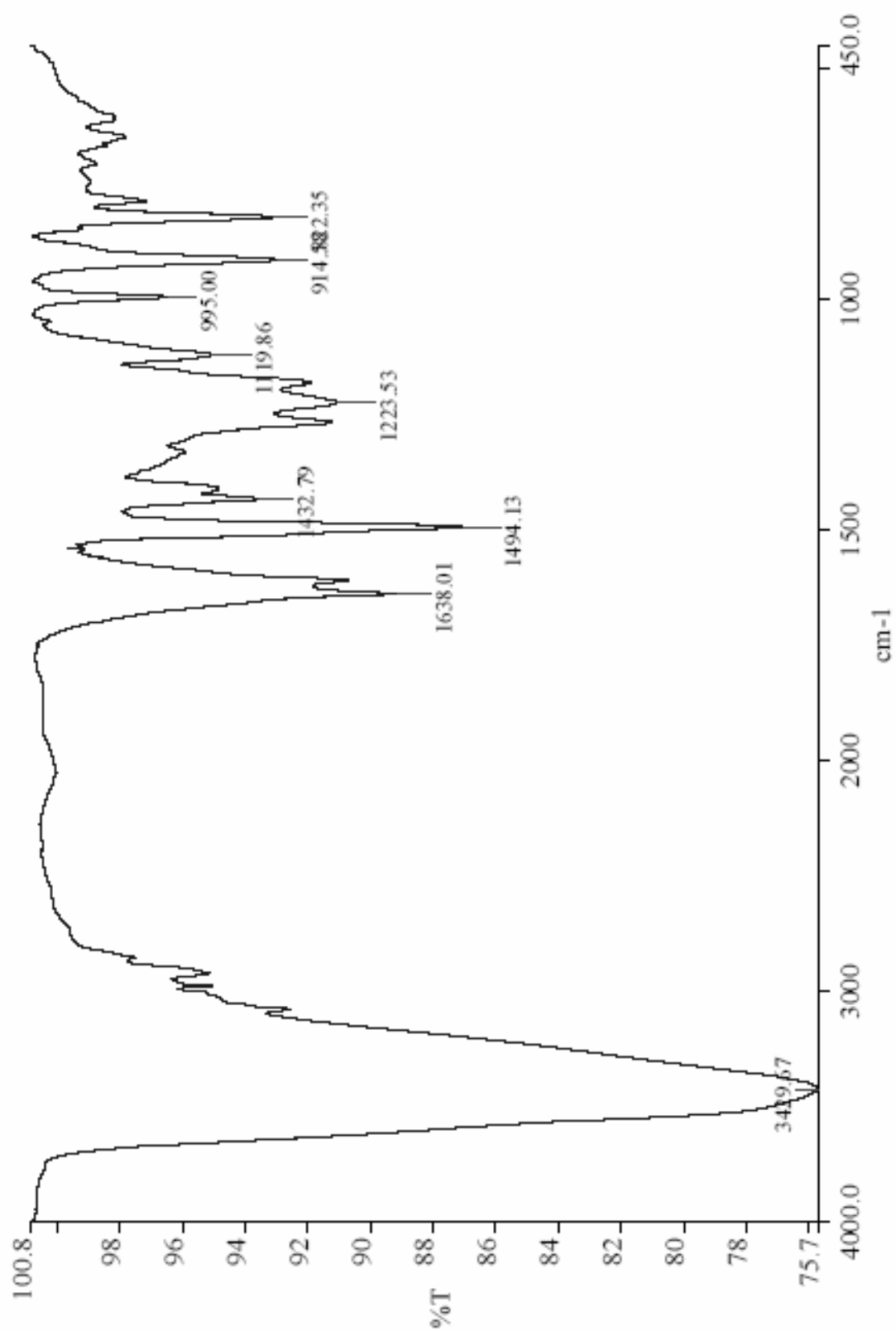


12: MS

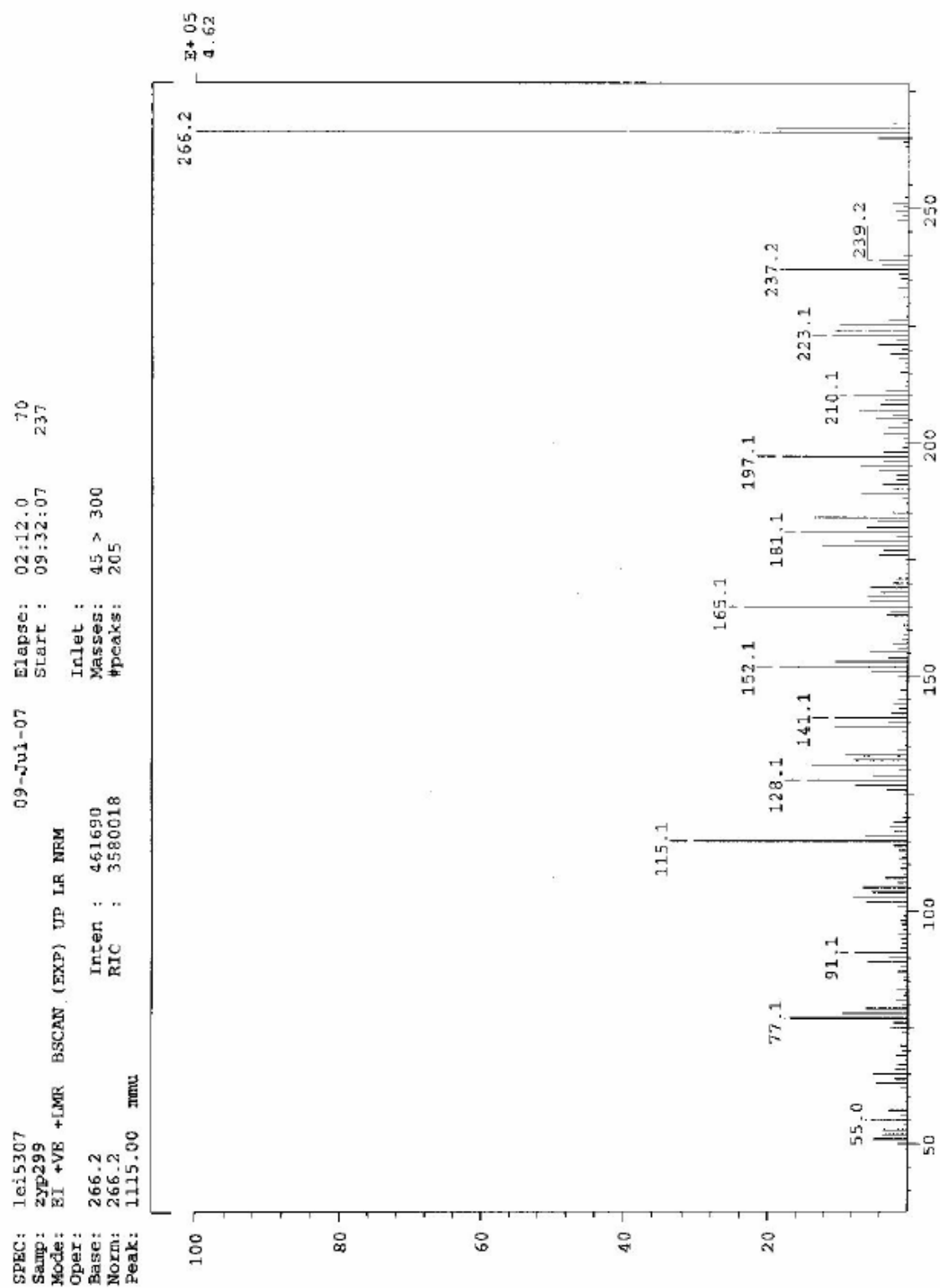
13: ¹H NMR



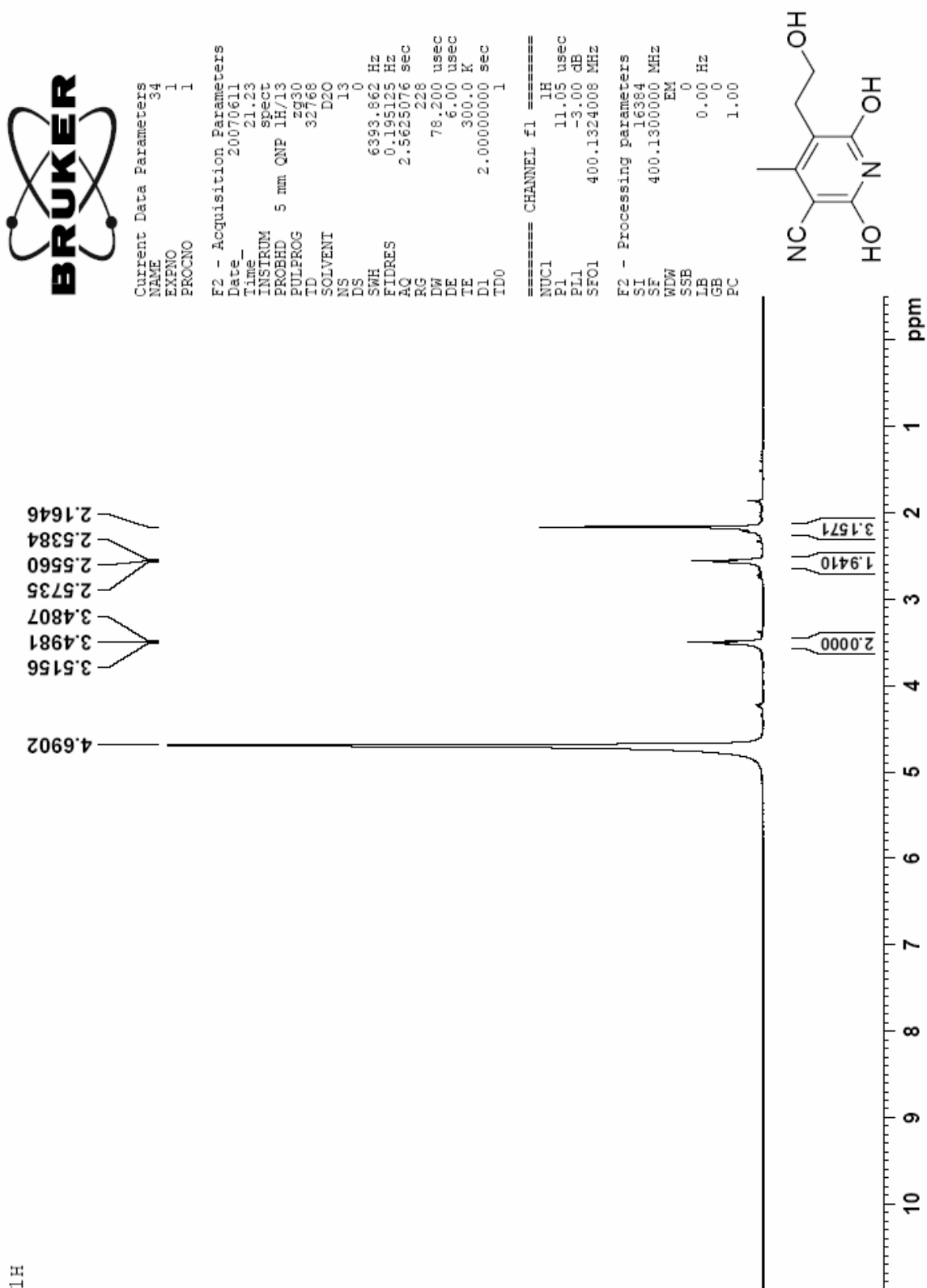
13: C NMR



13: IR

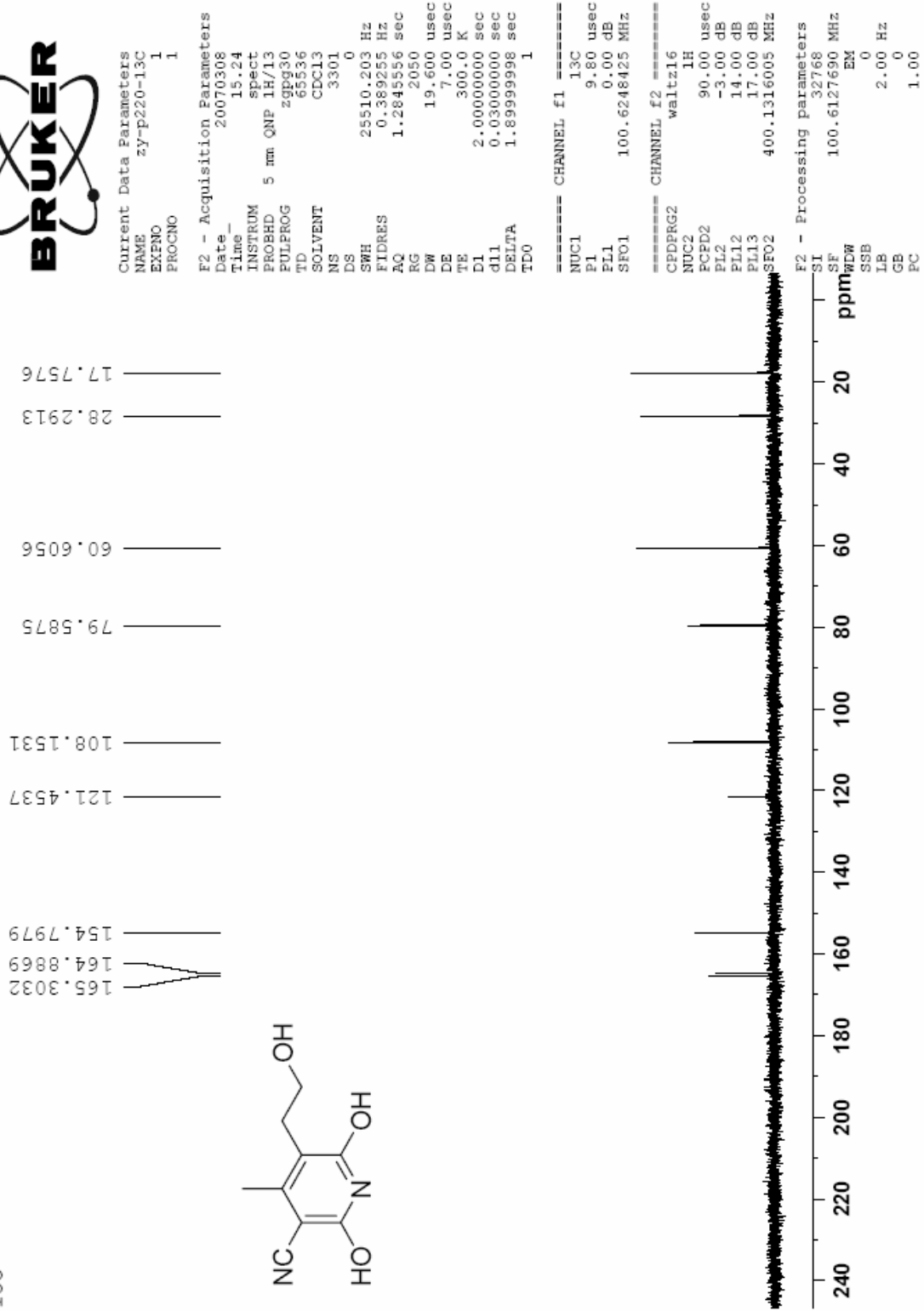


13: MS

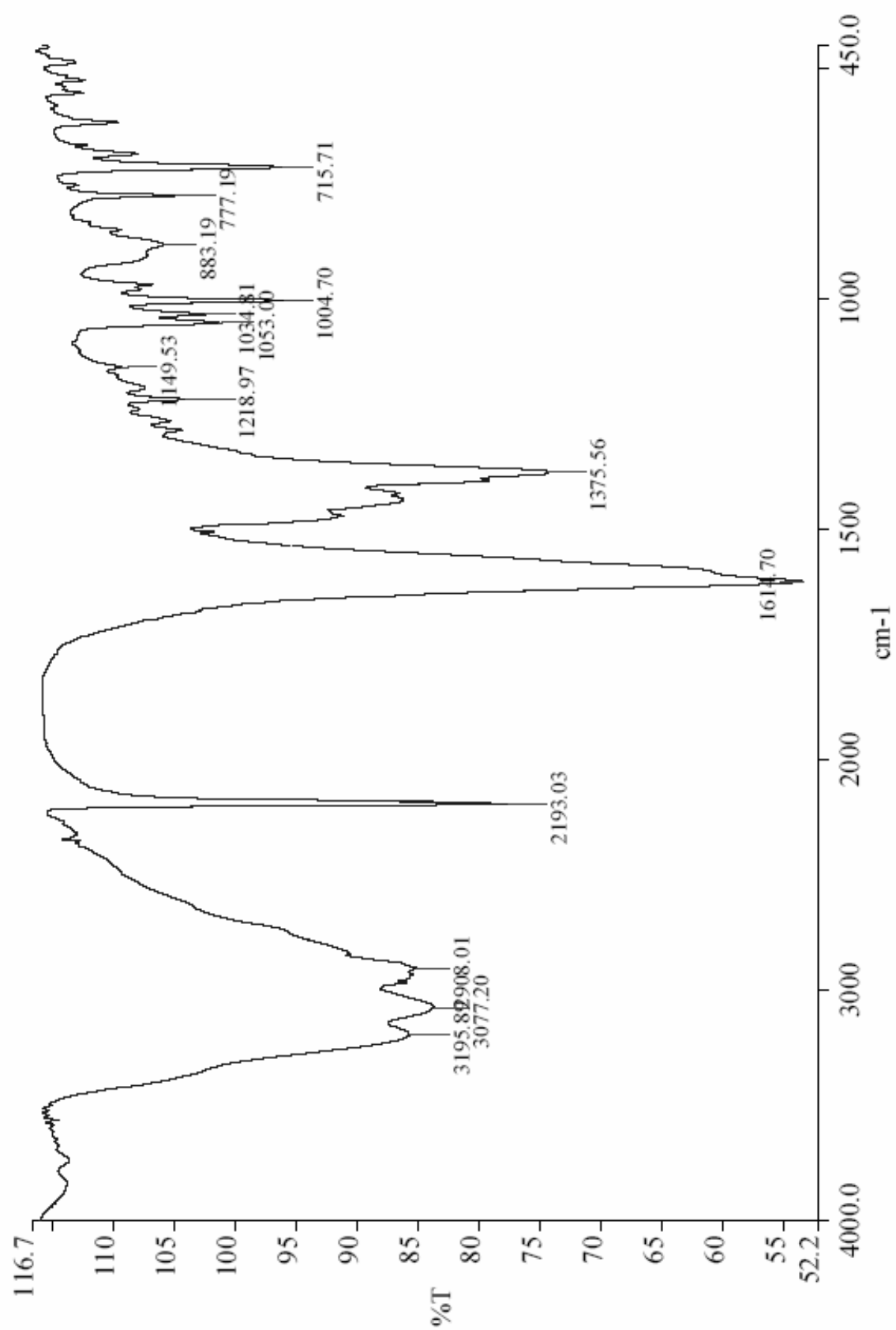
15: ¹H NMR



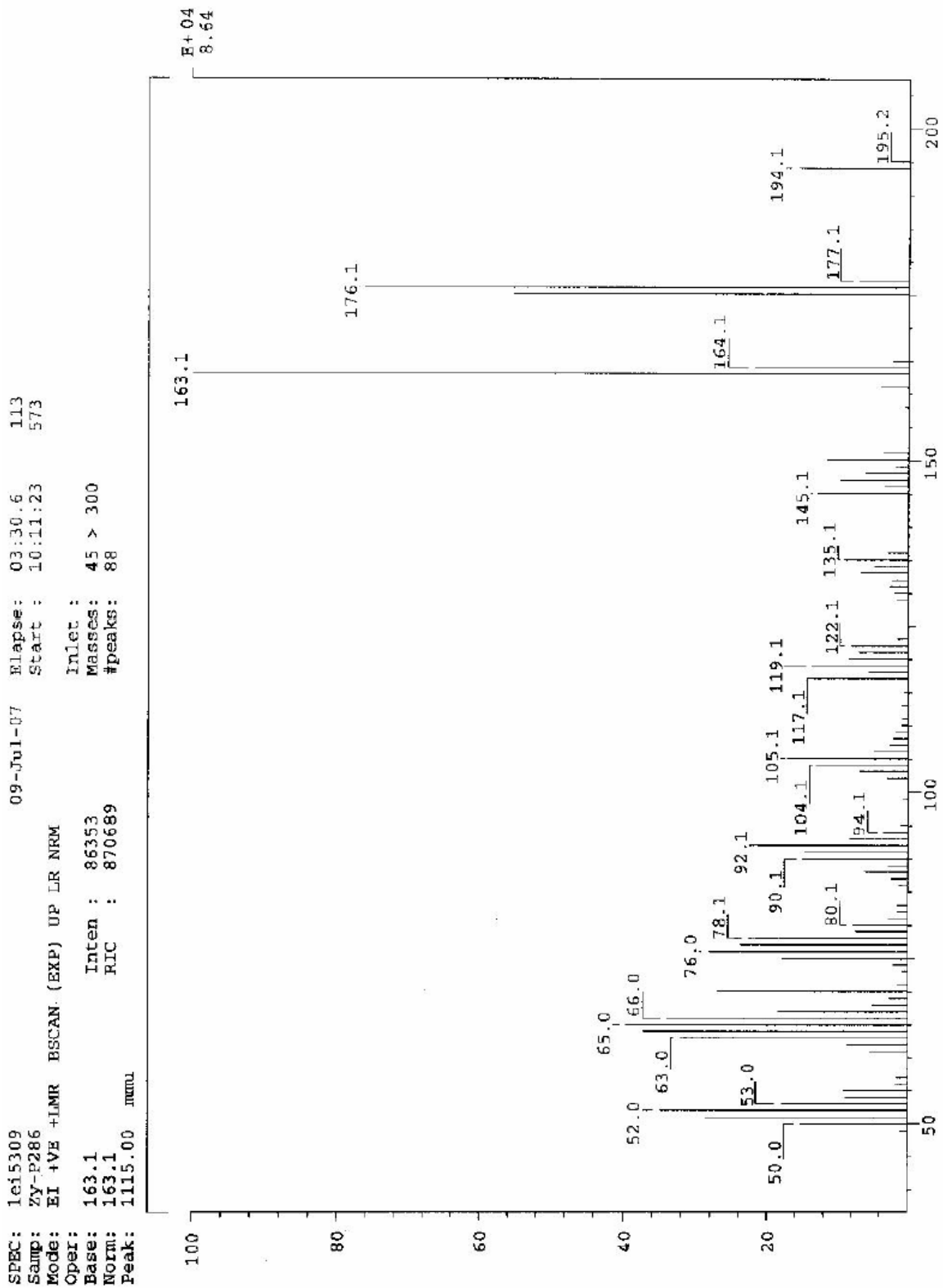
¹³C



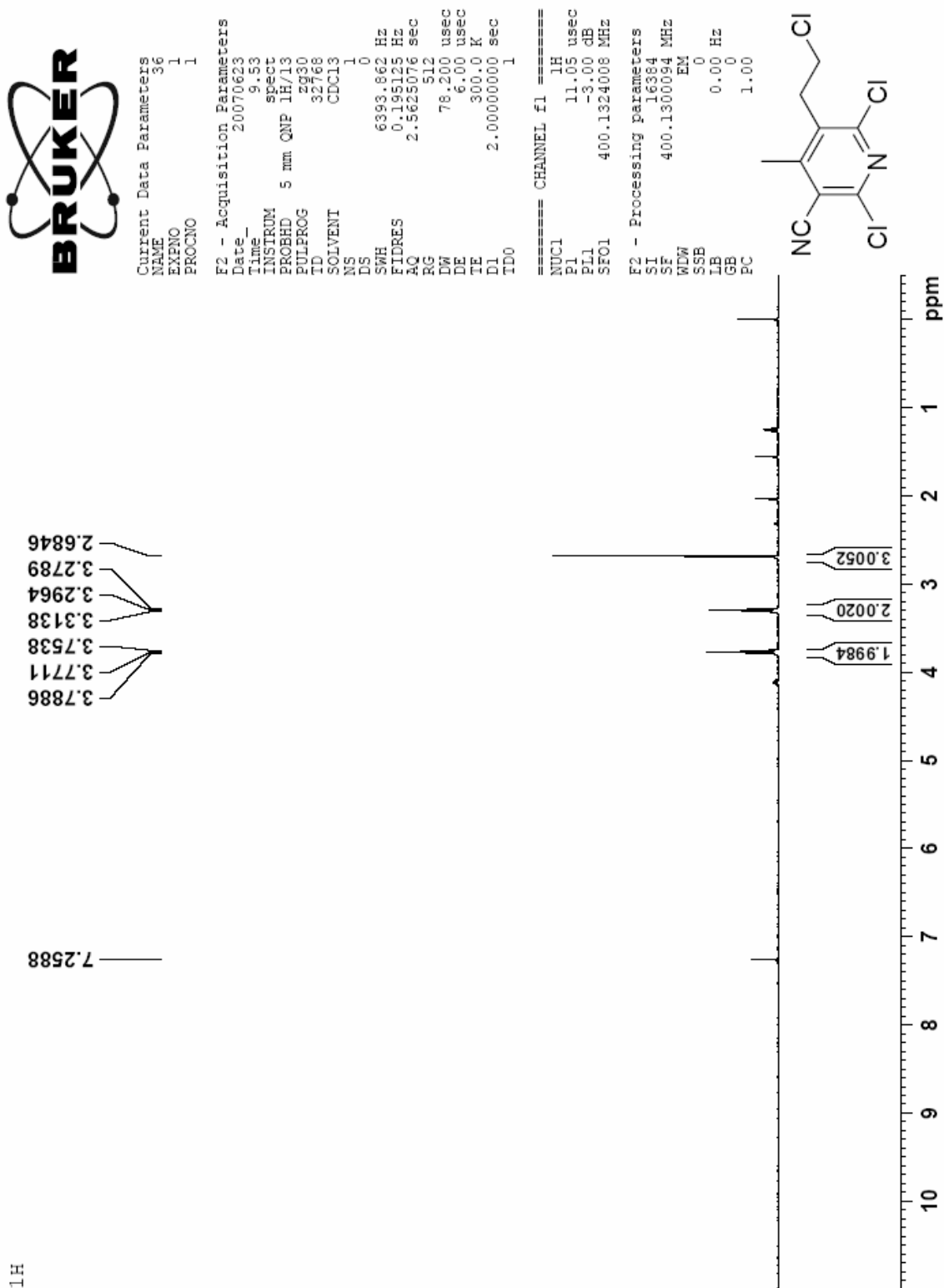
15: C NMR



15: IR



15: MS

16: ¹H NMR



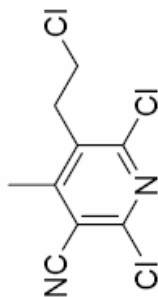
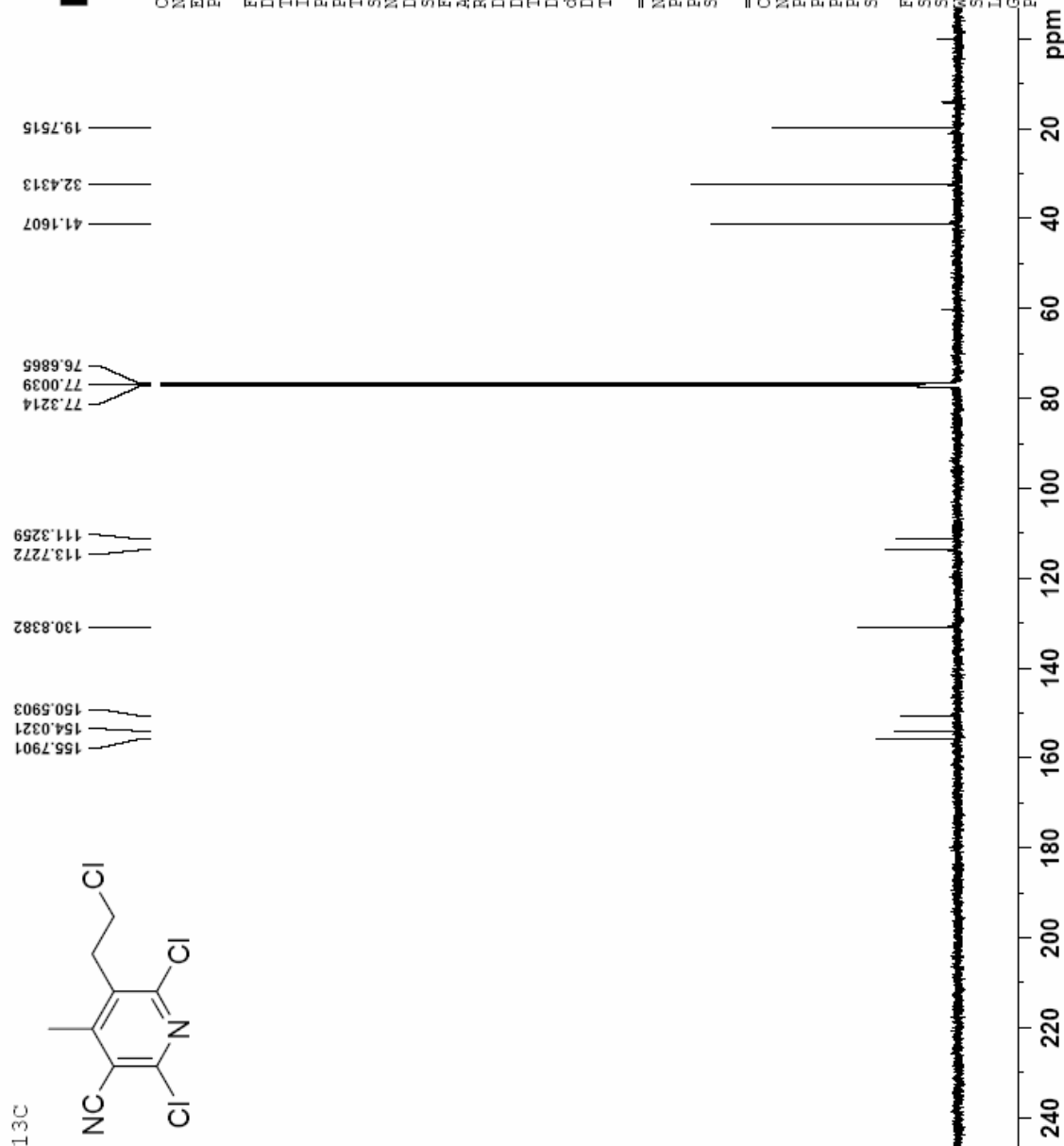
Current Data Parameters
 NAME zy-E314-13C
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20070623
 Time 11.29
 INSTRUM spect
 PROBHD 5 mm QNP 1H/13
 PULPROG zgpg30
 ID 65536
 SOLVENT CDCl3
 NS 1500
 DS 0
 SWH 25510.203 Hz
 FIDRES 0.389255 Hz
 AQ 1.2845556 sec
 RG 2050
 DW 19.600 usec
 DE 7.00 usec
 TE 300.0 K
 D1 2.00000000 sec
 d11 0.03000000 sec
 DELTA 1.89999998 sec
 TD0 1

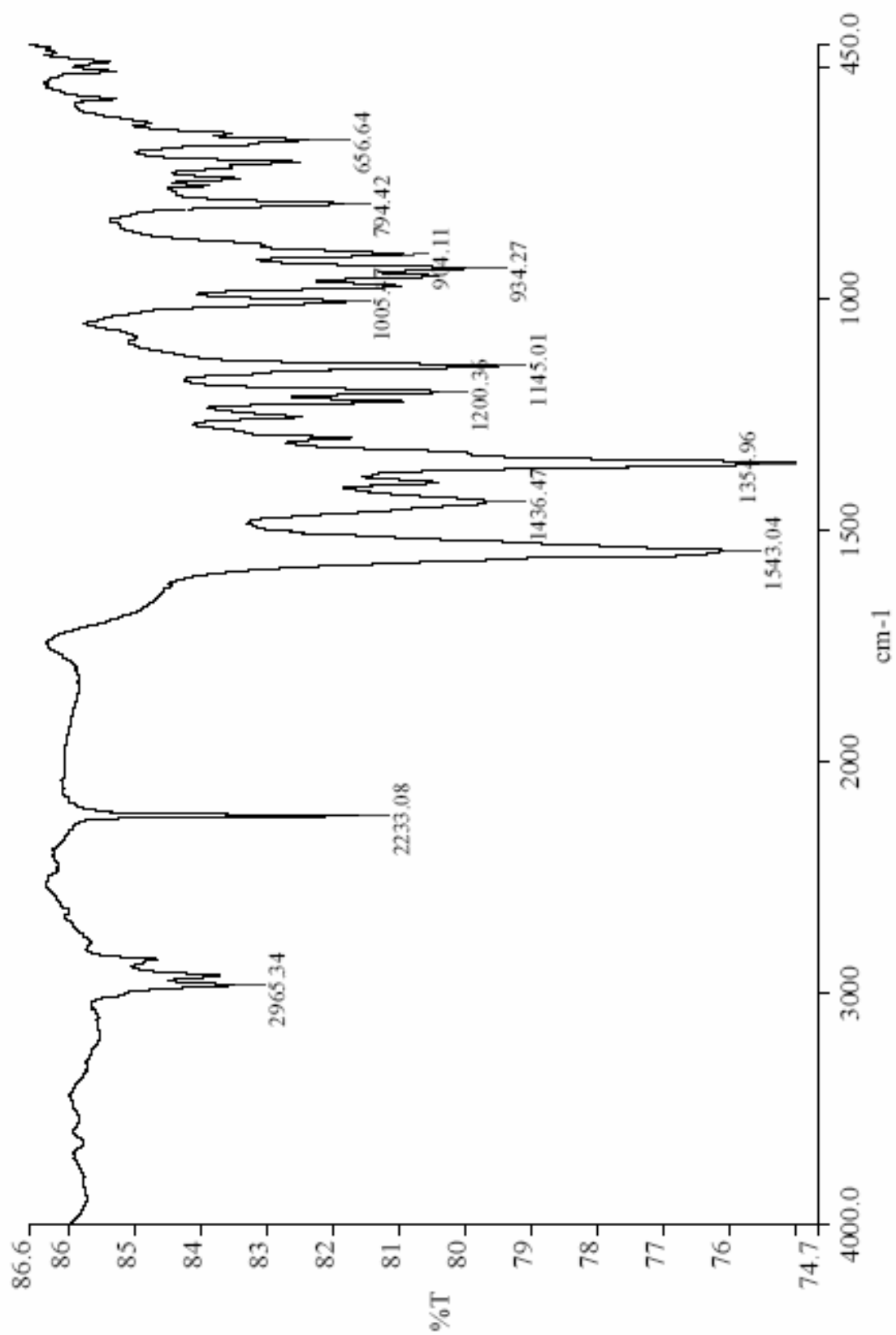
===== CHANNEL f1 =====
 NUC1 13C
 P1 9.80 usec
 PL1 0.00 dB
 SFO1 100.6248425 MHz

===== CHANNEL f2 =====
 CDEPRG2 waltz16
 NUC2 1H
 PCPD2 90.00 usec
 PL2 -3.00 dB
 PLI2 14.00 dB
 PLI3 17.00 dB
 SFO2 400.1316005 MHz

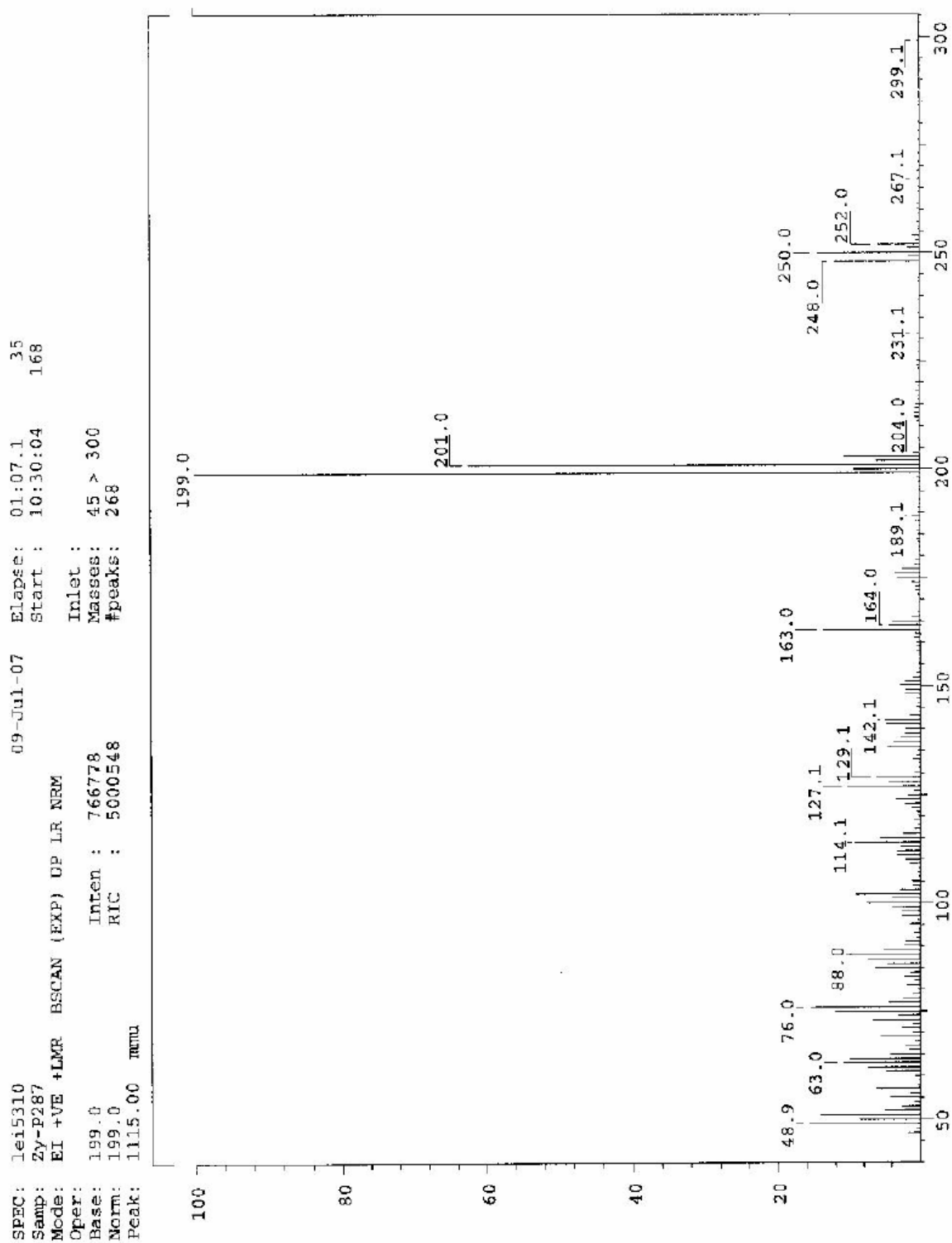
F2 - Processing parameters
 SI 32768
 SF 100.6127690 MHz
 WDW EM
 SSB 0
 LB 2.00 Hz
 GB 0
 FC 1.00



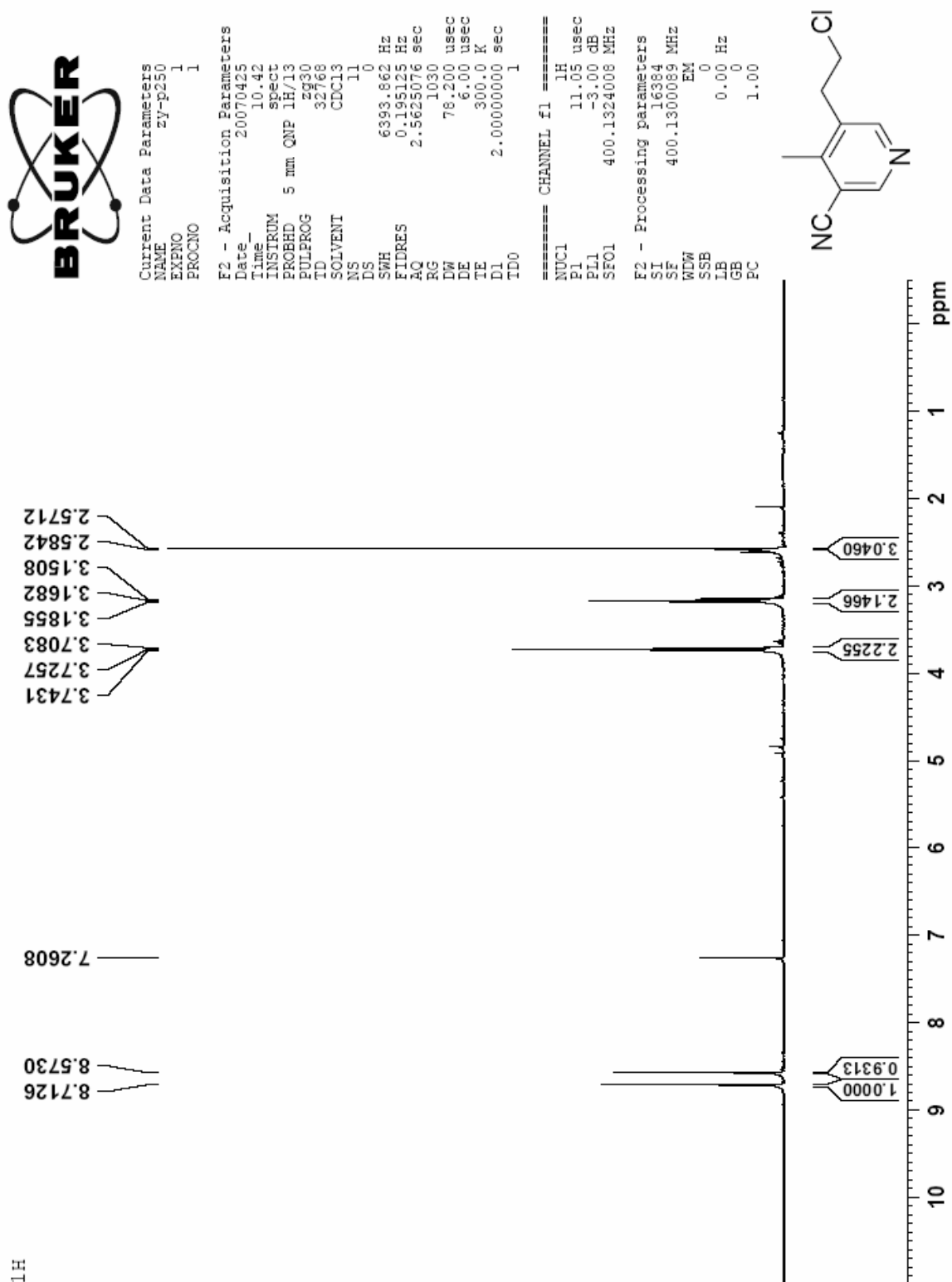
16: C NMR



16: IR



16: MS

17: ¹H NMR



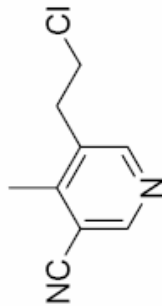
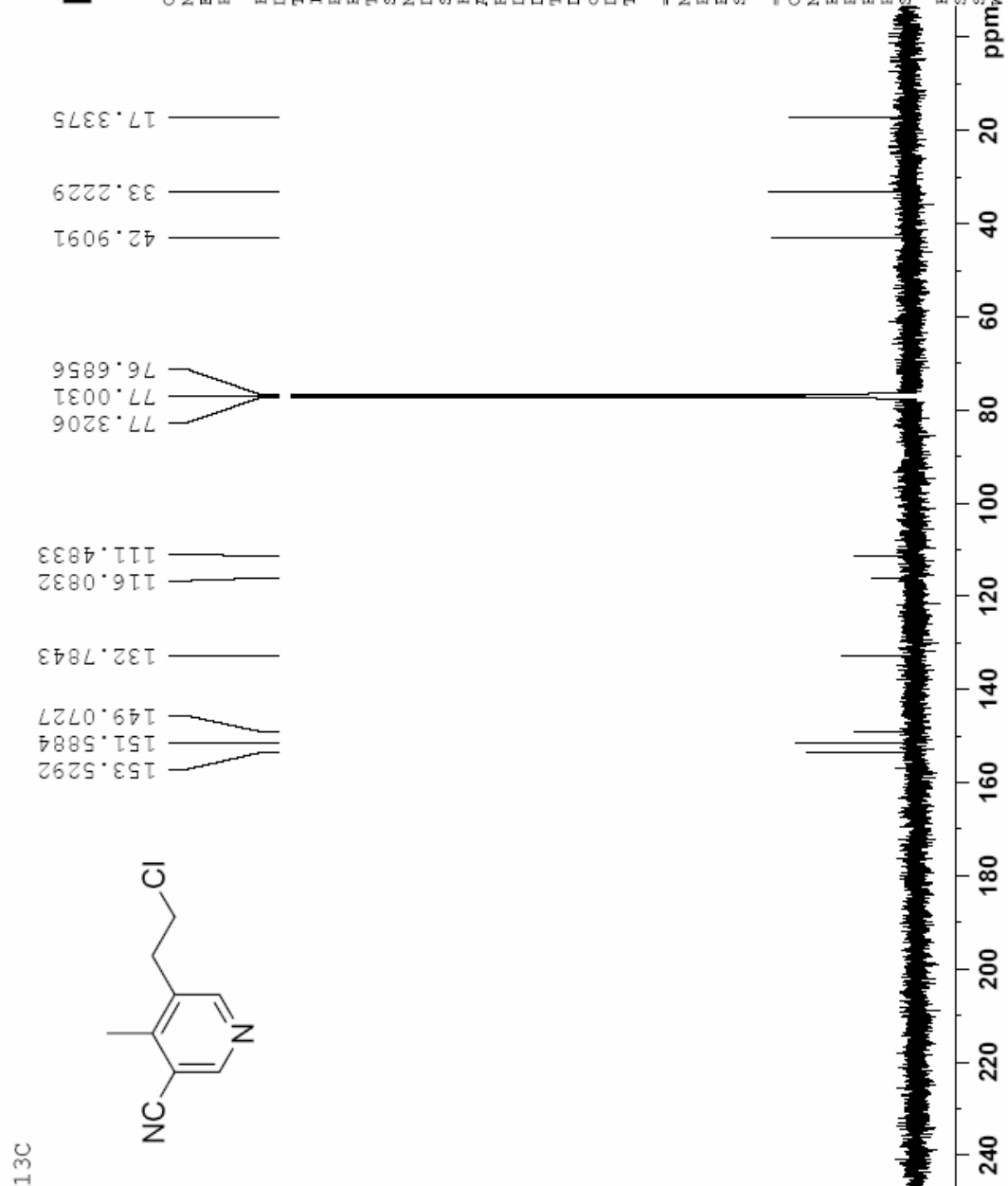
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NAME zy-p241-13C
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20070412
Time_ 8.53
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 10000
DS 0
SWH 25510.203 Hz
FIDRES 0.389255 Hz
AQ 1.2845556 sec
RG 2050
DW 19.600 usec
DE 7.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1

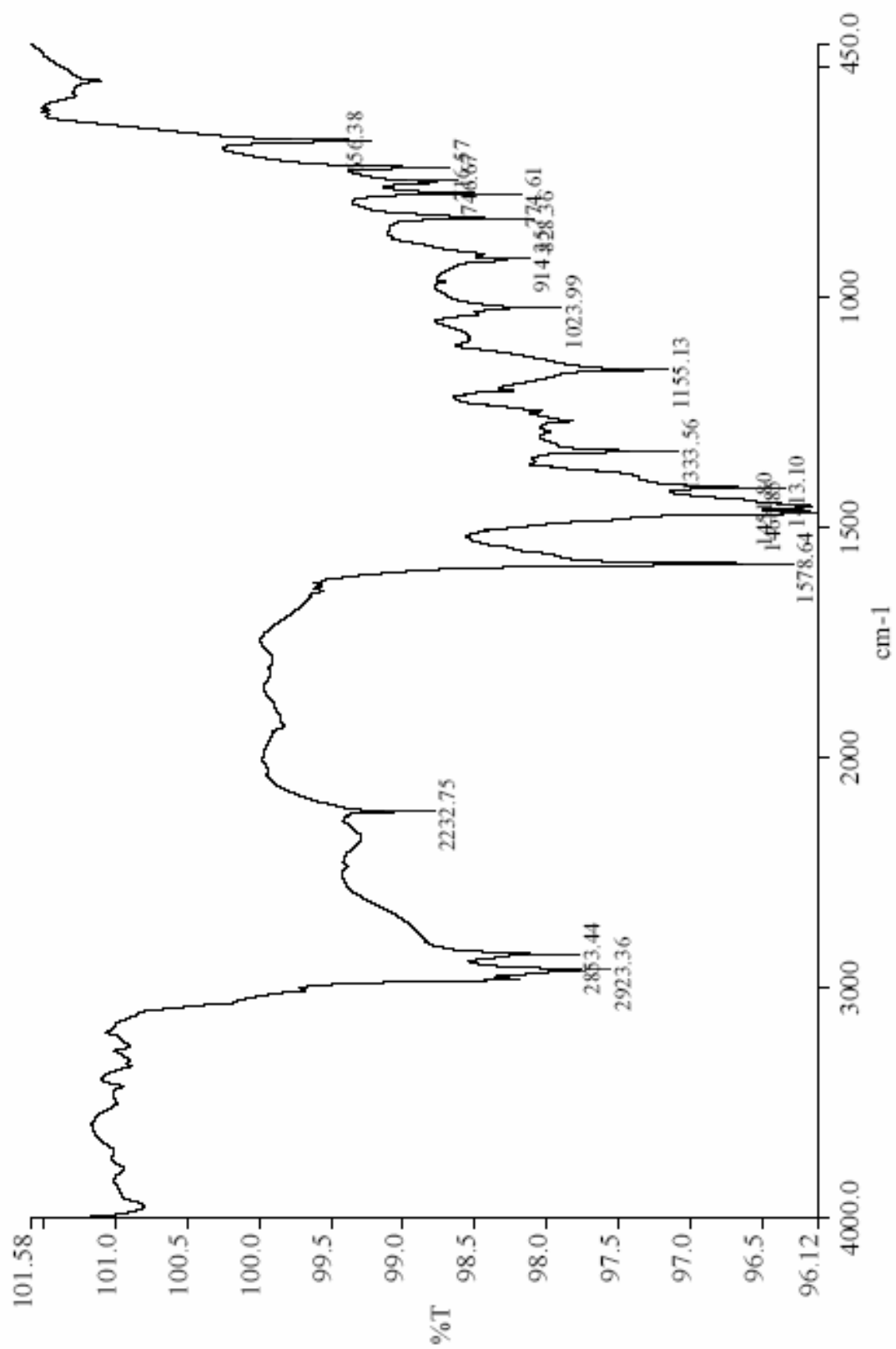
===== CHANNEL f1 =====
NUC1 13C
P1 9.80 usec
PL1 0.00 dB
SFO1 100.6248425 MHz

===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -3.00 dB
PL12 14.00 dB
PL13 17.00 dB
SFO2 400.1316005 MHz

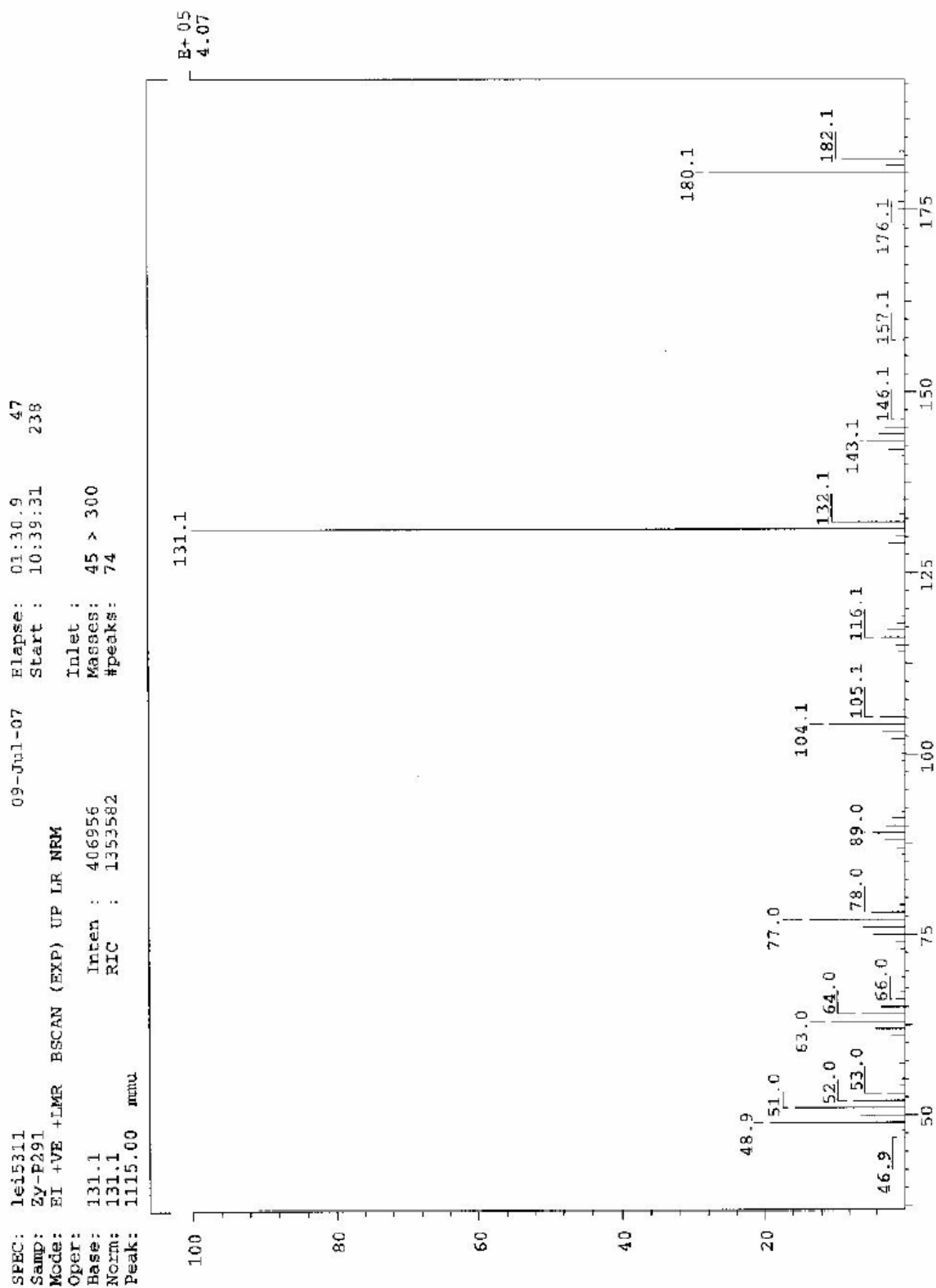
F2 - Processing parameters
SI 32768
SF 100.6127690 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.00



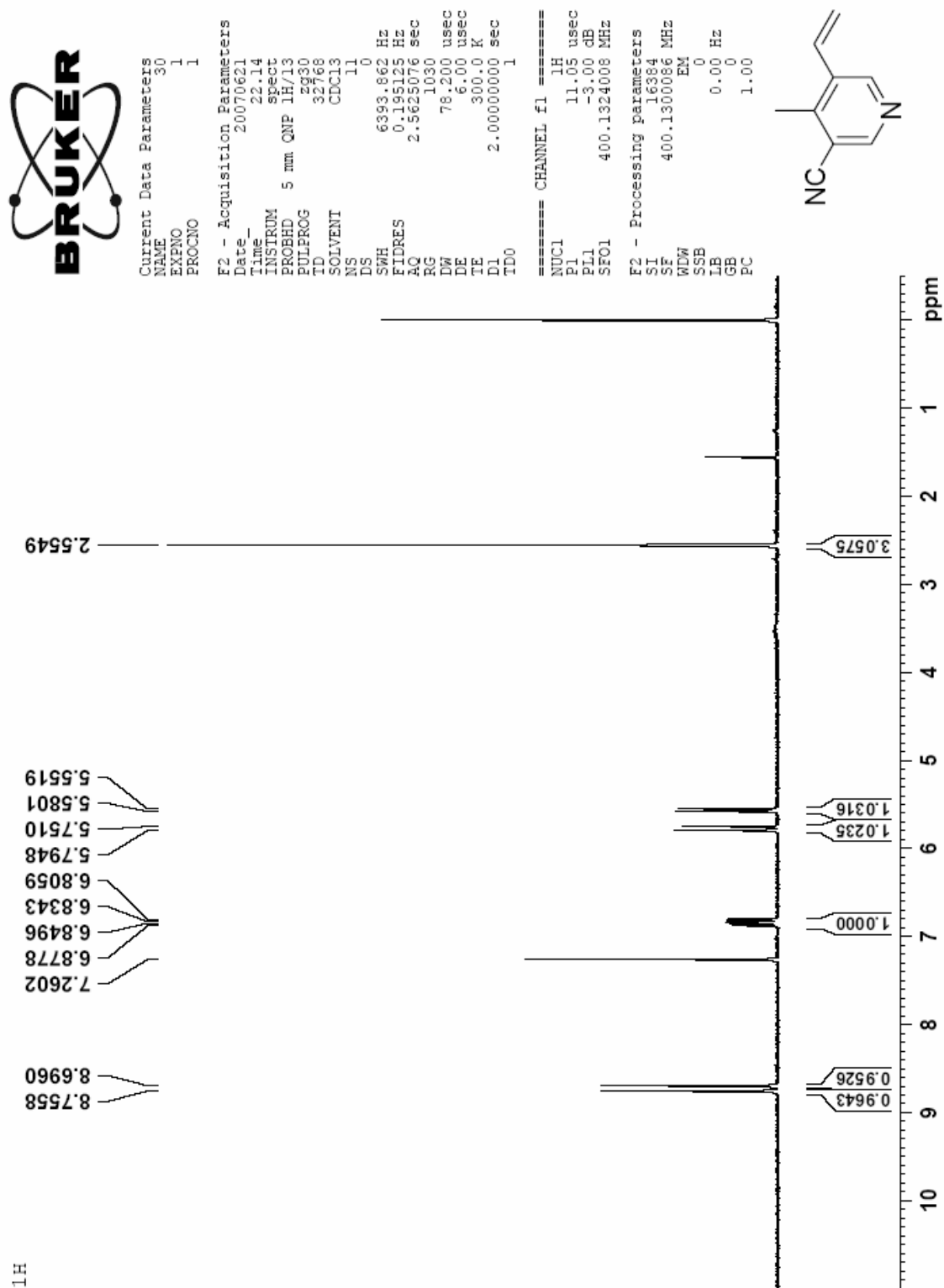
17: C NMR

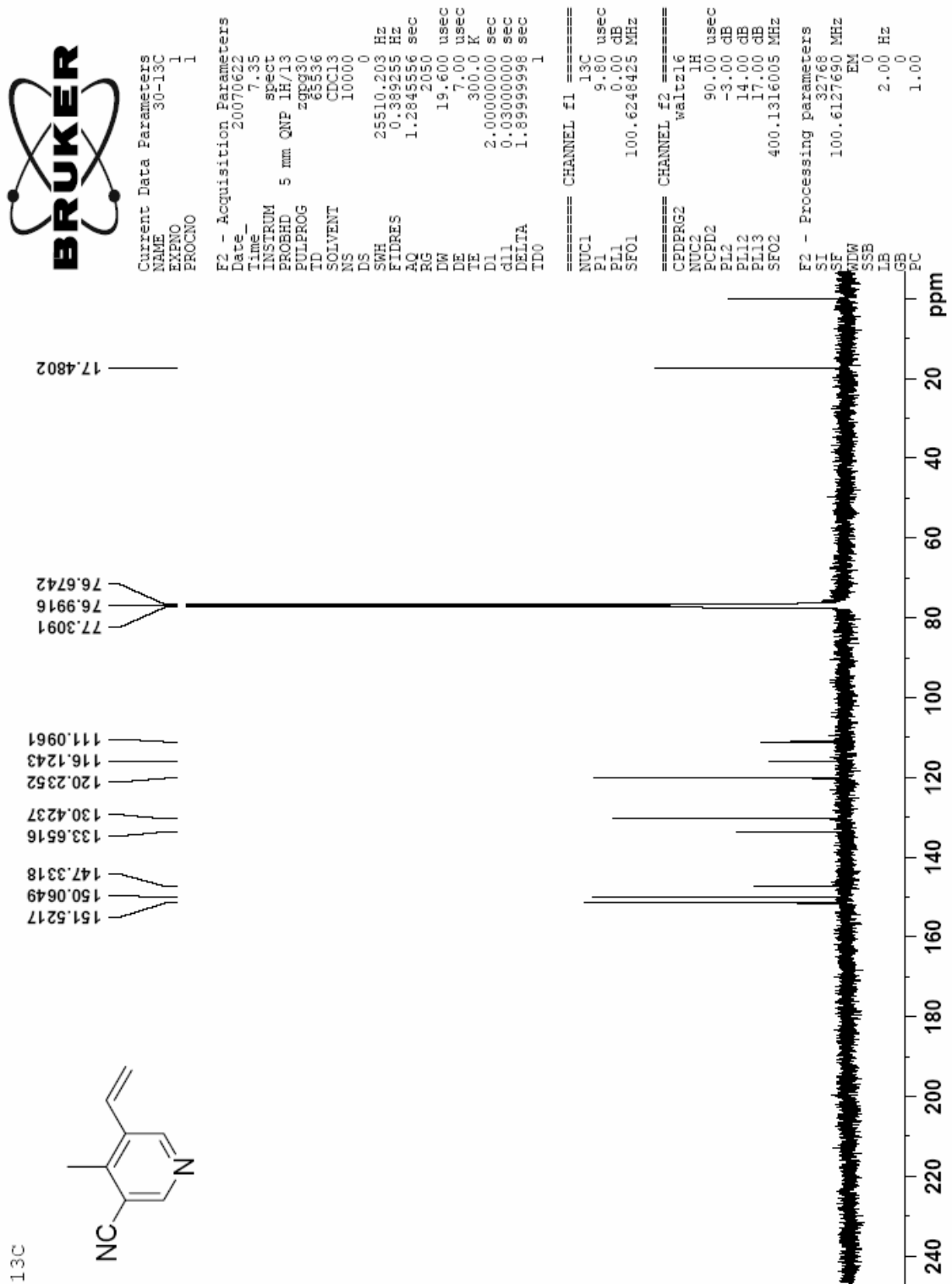


17: IR

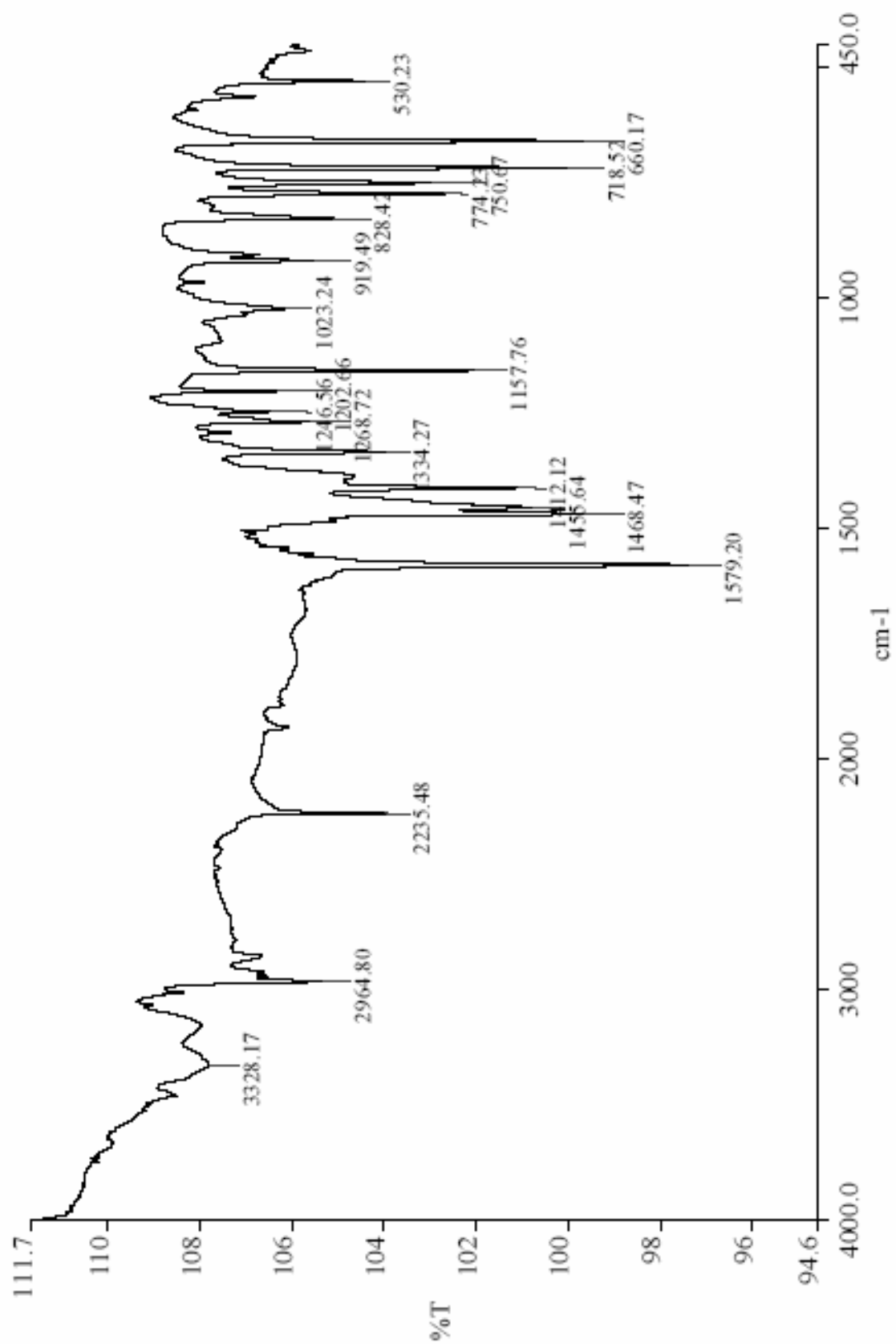


17: MS

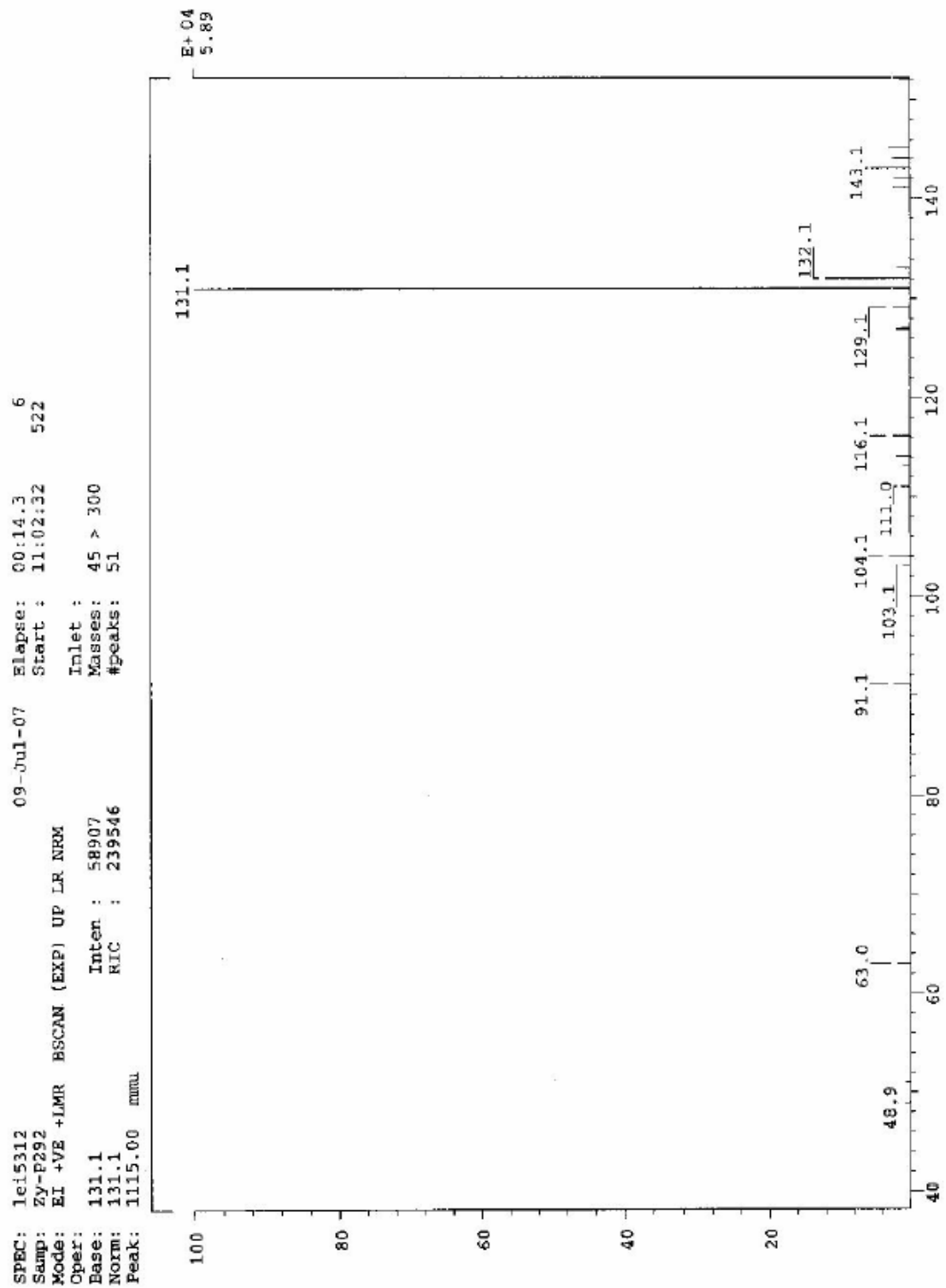
18: ¹H NMR



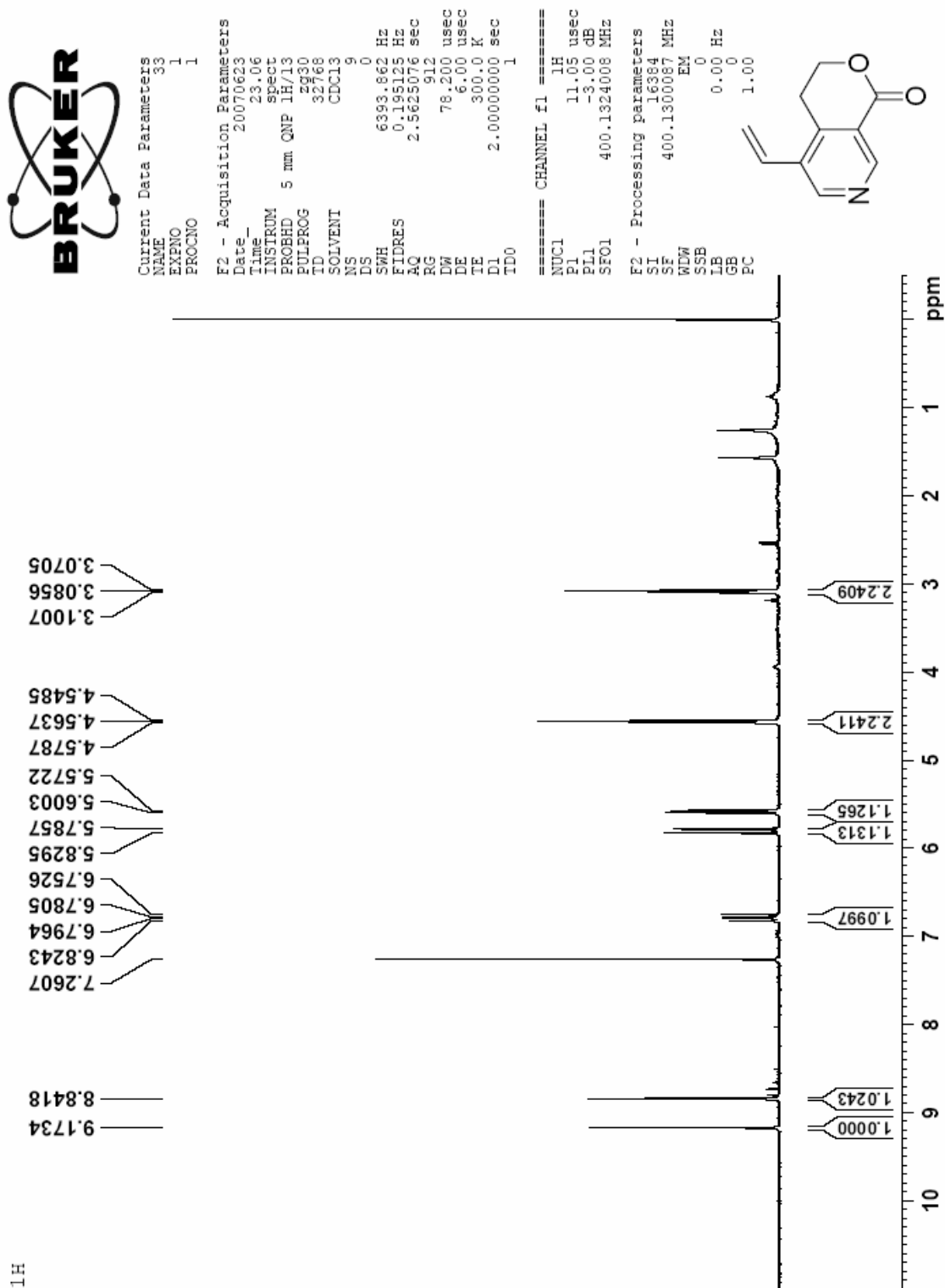
18: C NMR

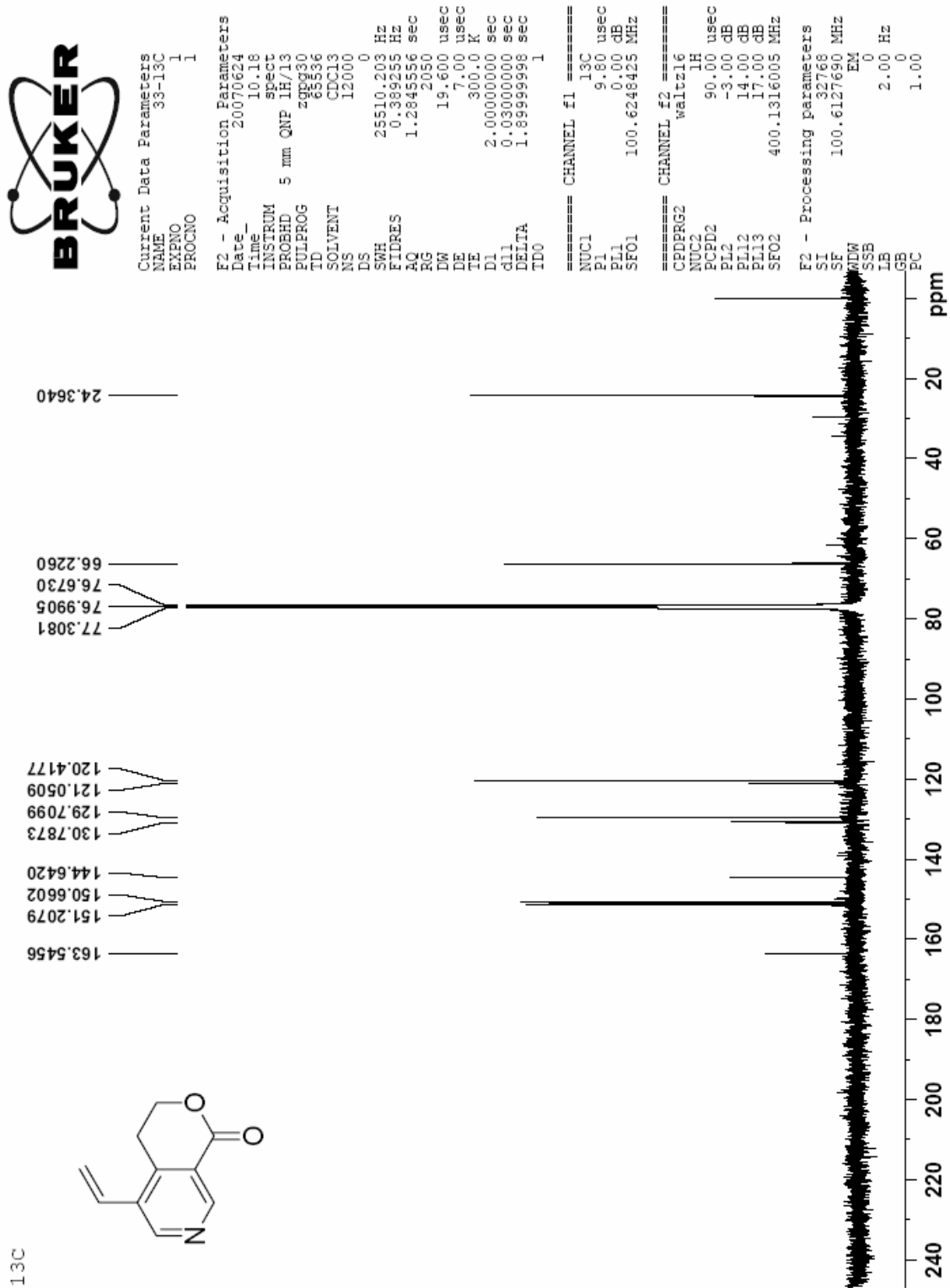


18: IR

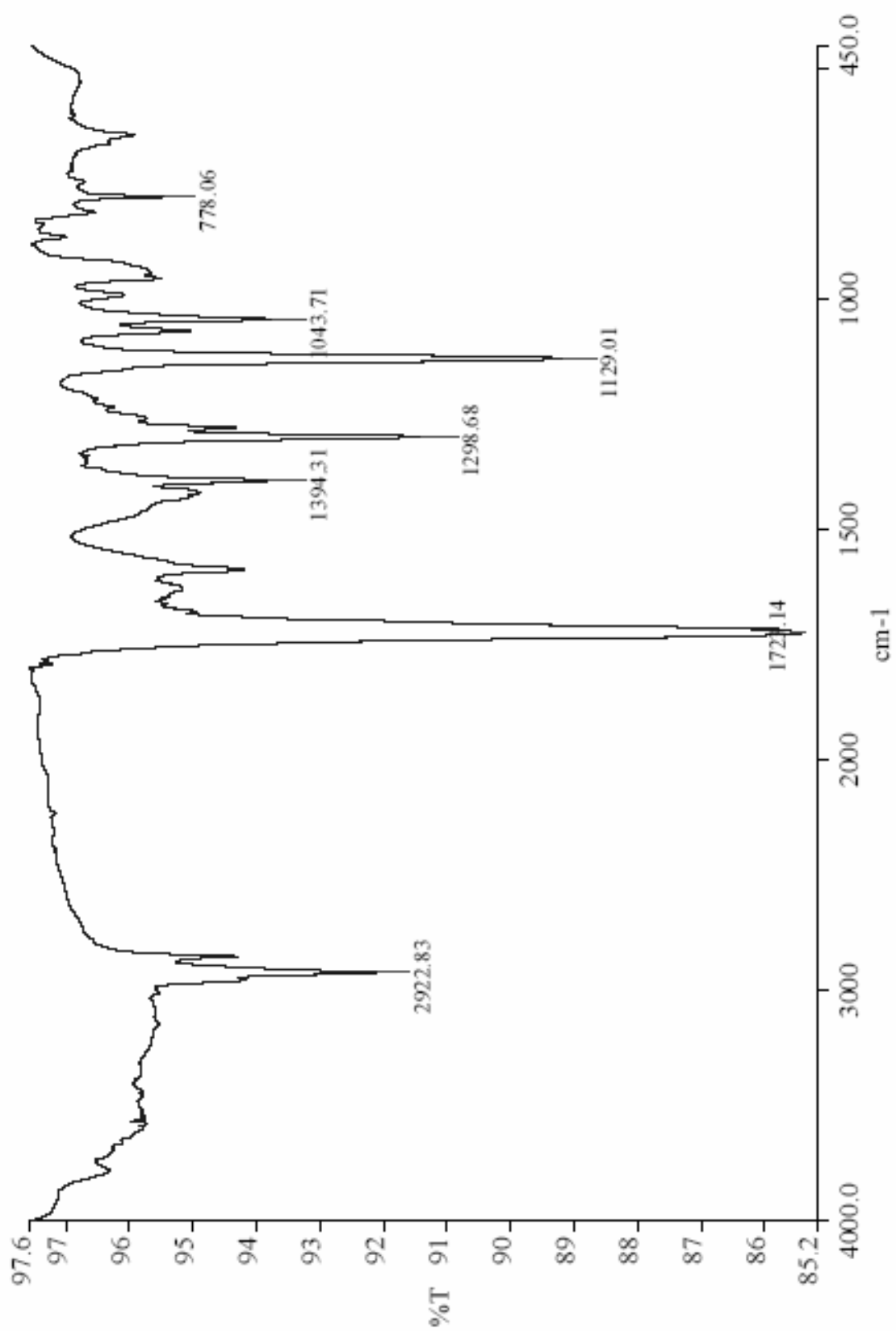


18: MS

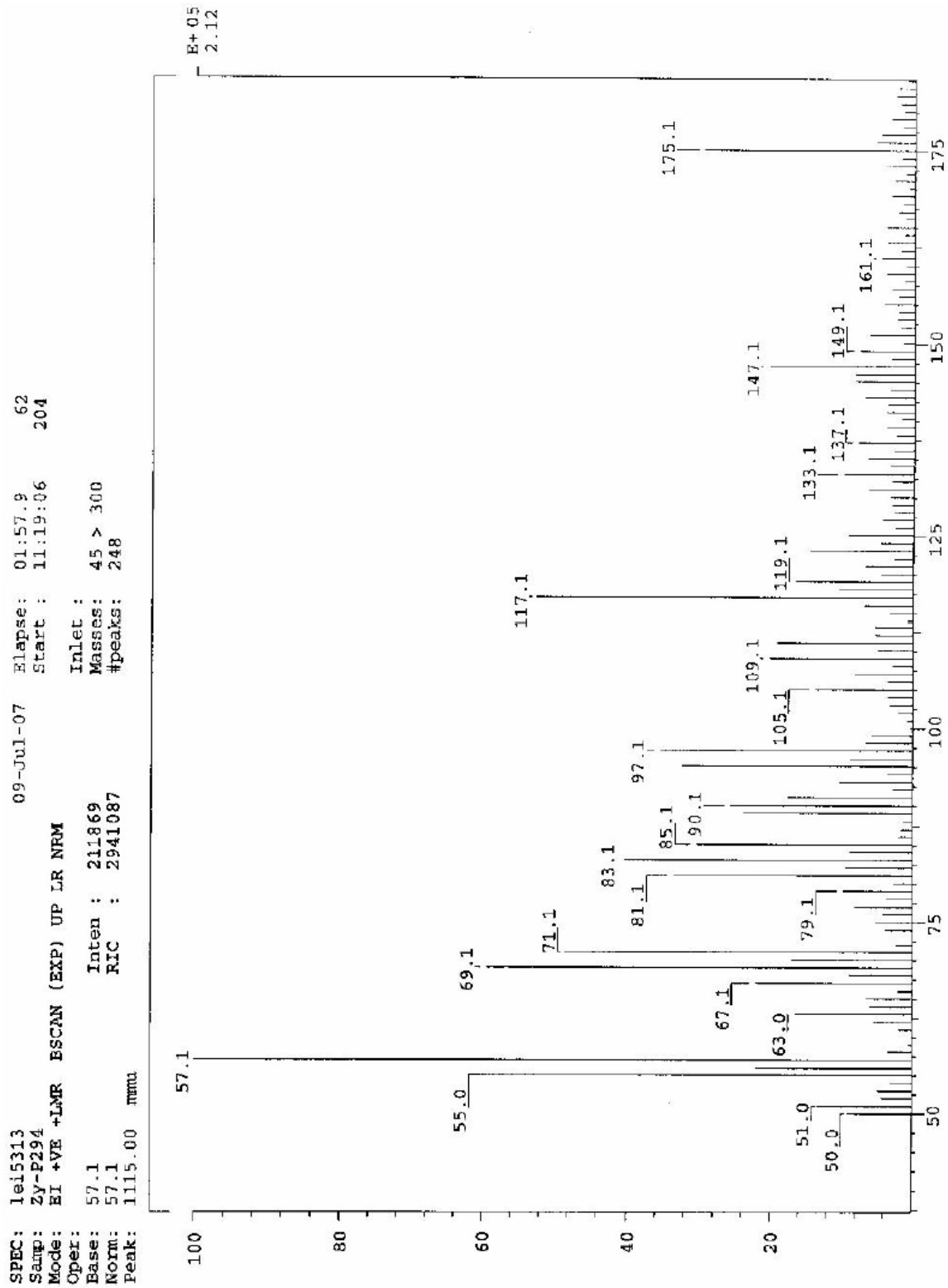
19: ¹H NMR



19: C NMR



19: IR



19: MS