Synthesis and Evaluation of [^{18}F]Fluorobutyl Ethacrynic Amide: A Potential PET Tracer for Studying Glutathione Transferase

Ho-Lien Huang,[a,†] Chun-Nan Yeh,[b,†] Kang-Wei Chang,[c] Jenn-Tzong Chen,[c] Kun-Ju Lin,[d] Li-Wu Chiang,[a] Kee-Ching Jeng,[e] Wei-Ting Wang,[f] Ken-Hong Lim,[g] Caleb Gonshen Chen,[g] Kun-I Lin,[a,h] Ying-Cheng Huang,[i] Wuu-Jyh Lin,[c] Tzu-Chen Yen[d] and Chung-Shan Yu[a,j*]

[†] *Equal contribution*

[a] *Department of Biomedical Engineering and Environmental Sciences, National Tsing-Hua University, Hsinchu 300, Taiwan*

[b] *Department of Surgery, Chang Gung Memorial Hospital at Linkou, Chang Gung University, Taiwan*

[c] *Institute of Nuclear Energy Research, Taoyuan 32546, Taiwan.*

[d] *Department of Nuclear Medicine, Chang Gung Memorial Hospital at Linkou, Chang Gung University, Taiwan*

[e] *Taichung Veterans General Hospital, Taichung 40705, Taiwan*

[f] *Good Clinical Research Center, Department of Medical Research, Mackay Memorial Hospital, Taipei 104, Taiwan*

[g] *Division of Hematology and Oncology, Department of Internal Medicine, Mackay Memorial Hospital, and Department of Medicine, Mackay Medical College, Taipei 104, Taiwan*

[h] *Departments of Obstetrics & Gynecology, Chang Bing Show Chwan Memorial Hospital, Lukang Zhen, Changhua County, Taiwan*

[i] *Department of Neurosurgery, Chang Gung Memorial Hospital at Linkou, Chang Gung University, Taiwan*

[j] *Institute of Nuclear Engineering and Science, National Tsing-Hua University, Hsinchu, 300, Taiwan*

Fax: (+886)3-5718649

Tel: (+886)3-5751922

E-mail: csyu@mx.nthu.edu.tw

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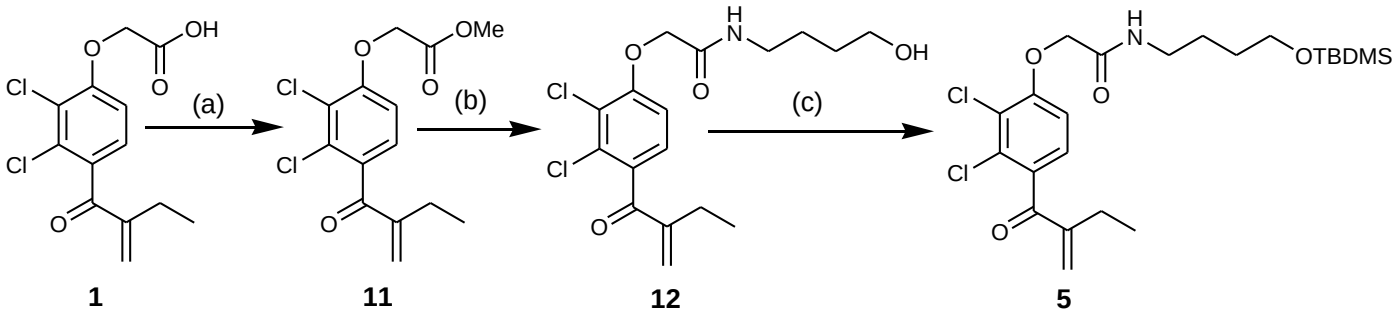
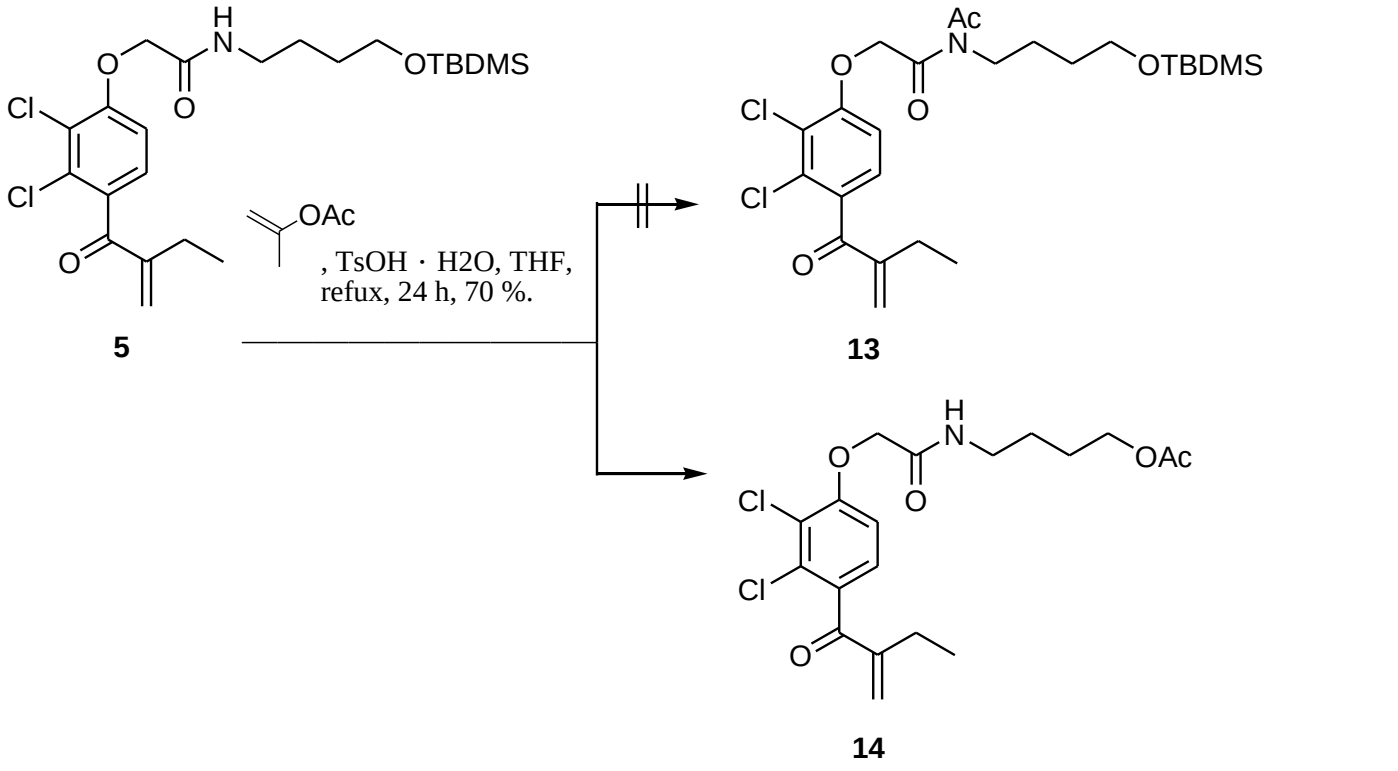
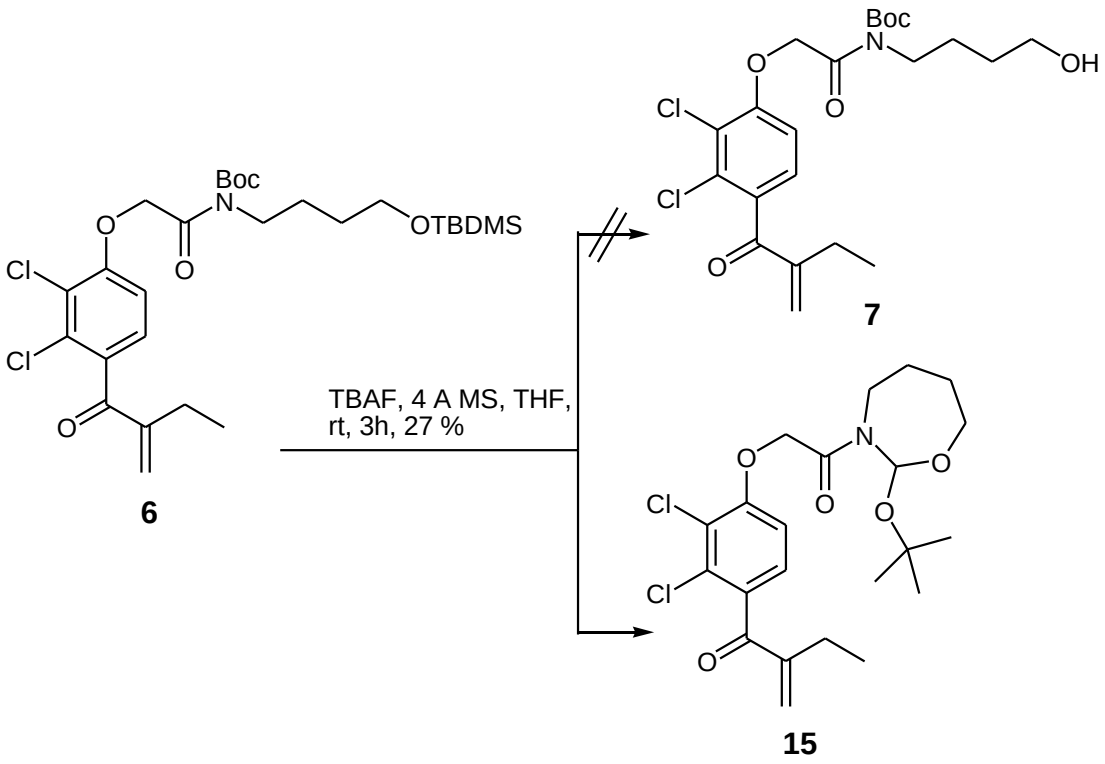
1. The schemes of side reactions



Scheme 1. Attempt to introduce 4-aminobutanol: low yield due to no regioselectivity. Reaction conditions: (a) CH_2N_2, ether, EtOAc, 91 %; (b) 4-amino-1-butanol, Et_3N, DMF, 60 ^oC, 8 h, 20 %; (c) TBDMSCl, Py, DMAP, CH_2Cl_2, rt, 8 h, 70 %.



Scheme 2. Attempt of introduction of acetyl group leads to the cleavage of *t*-butyl dimethylsilyl group and undesired *O*-acetylation.



Scheme 3. Attempt to remove the silyl group in the presence of 4Å MS lead to an undesired ring closure.

2. Experimental section

2.1 General

All reagents and solvents were purchased from Sigma-Aldrich, Malingkrodt, Acros, Alfa, Tedia, or Fluka. All preparations for nonradioactive compounds were routinely conducted in dried glassware under a positive pressure of nitrogen at room temperature unless otherwise noted. CH_2Cl_2, toluene, CH_3CN, and pyridine were dried over CaH_2 and MeOH was dried over Mg and distilled prior to reaction. DMF and NEt_3 were distilled under reduced pressure. Reagents and solvents were of reagent grade. Dimethyl amino pyridine (DMAP) was purified through recrystallization from the combination of EtOAc and *n*-hexane before use. The eluents for chromatography: EtOAc, acetone, and *n*-hexane were reagent grade and distilled prior to use; MeOH and $CHCl_3$ were reagent grade and used without further purification. 0.22 μM filters

(Nylone) were supplied from Waters. NMR spectroscopy including ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125MHz, DEPT-135) was measured on Varian UnityInova 500 MHz. D-solvents employed for NMR including CD_3OD, $CDCl_3$, C_6D_6, and DMSO-d^6 were purchased from Cambridge Isotope Laboratories, Inc. Low-resolution mass spectrometry (LRMS) was performed on a ESI-MS spectrometry employing VARIAN 901-MS Liquid Chromatography Tandem Mass Q-Tof Spectrometer was performed at the department of chemistry of National Tsing-Hua University (NTHU). High-resolution mass spectrometry (HRMS) was performed using a varian HPLC (prostar series ESI/APCI) coupled mass detector of Varian 901-MS (FT-ICR Mass) and triple quadrapole. Elemental analysis was performed using Foss Heraeus elemental analysis; CHN-O-RAPID. Thin layer chromatography (TLC) was performed with MERCK TLC Silica gel 60 F_{254} precoated plates. The starting materials and products were visualized with UV light (254 nm). Further confirmation was carried out by using staining with 5% *p*-anisaldehyde, ninhydrin or ceric ammonium molybdate under heating. Flash chromatography was performed using Geduran Si 60 silica gel (230-400 mesh). Melting points were measured with MEL-TEMP and were uncorrected.

Recombinant human glutathione S-transferase alpha-1 (GSTA1 human, 50 μg/50 μL) was purchased from ProSpec-Tany TechnoGene Ltd (ENZ-469). Recombinant human glutathione S-transferase Pi-1 (GSTP1 human, two packages of 25μg/10 μL and 25μg/25 μL) were purchased from Alpha Diagnostic International Inc (GST P35-R-25). All the three enzyme products were unpacked freshly followed by enzymatic assay immediately.

[^{18}F]HF was produced on a GE PET tracer Cyclotron via the ^{18}O(p,n) nuclear reaction (NERI, Taiwan). The radiolabeling was performed on a GE TracerLAB FX_{FN} synthesis module (GE medical Systems,

Milwaukee, WI). The intermediate product labeled with fluorine-18 was analyzed by HPLC, consisting of a Waters 510 pump, a linear UVIS detector (254 nm) in series with a Berthhold γ-flow detector, on a ZORBAX SILcolumn (250 mm×9.4 mm, 5 μm) at 3 mL/min with EtOAc/*n*-hexane 1:9 as the mobil phase. [^{18}F]FBuEA was purified by HPLC separated from TracerLAB FX_{FN} synthesis module, consisting of the pump, the UV detector, the γ-flow detector and ZORBAX SILcolumn (250 mm×9.4 mm, 5 μm) at 3 mL/min with EtOAc/*n*-hexane 1:2 as the mobil phase as described above. Quality control analysis of [^{18}F]FBuEA was performed on the same HPLC conditions as described above. The identity of the labeled compound was confirmed by coinjection with the authentic compound on HPLC. The area of the UV absorbance peak measured at 260 nm corresponding to carrier product was measured and compared to standard curve relating mass to UV absorbance. Only a specific activity below 40 GBq/μmol can be measured accurately. Radioactivity was measured with a Capintec R15C dose calibrator.

In-vivo stability studies were performed on the system as described, excepting the column CHEMCOSORB 7-ODS-H, 10×250 mm, 5 μm; eluent was set isocratically from CH_3CN/0.05% trifluoracetic acid =20/80 at 0 min to CH_3CN /0.05% trifluoracetic acid =95/5 at 10 min and a further gradient to CH_3CN (100%) at 20 min. Flowrate = 3 mL/min, t_R = 14.8 min (Radio).

All experiments were performed in compliance with the NHMRC Taiwan Code of Practice for the care and use of animals for scientific purposes. Female Balb/c rats (5 weeks) were obtained from the Animal Research Center (ChangGung, Taiwan). Rats were housed under constant environmental conditions and were allowed free access to food and water throughout the experimental period. In vivo studies were performed in TTA-induced CCA rats.

The rats were anaesthetized via inhalant isoflurane (Forthane, Abott) in 200 mL/min oxygen during the imaging study. PET imaging [^{18}F]FBuEA and [^{18}F]FDG were performed with microPET (microPET R4; Concorde Microsystems Inc.) in Nuclear Energy Research Institute and Inveon™ system (Siemens Medical Solutions, USA, Inc.) in Chang-Gung Memorial Hospital, respectively. Both were manufactured by Siemens Medical Solutions, Knoxville, United States.

2.2 Chemical synthesis

2.2.1 4-(tert-butyldimethylsilanyloxy)butan-1-amine **4**. Preparation of this compound was according to the procedure reported by Krivickas S. J. *et al*.[19] TBDMSCl (8.2 g, 54 mmol, 1.2 eq) was added to a mixture of 4-aminobutanol (4 g, 45 mmol) and pyridine (8 mL). Stirring was allowed for 12 h. TLC (MeOH/$CHCl_3$ = 5/5) indicated the consumption of 4-aminobutanol (R_f = 0.13) and formation of product **4** (R_f = 0.40). The mixture was then concentrated under reduced pressure at 40 ^oC to provide a residue, which was dissolved by CH_2Cl_2 (50 mL). After extraction with satd. $NaHCO_3$ (aq), the organic layer was dried over Na_2SO_4 and filtered through celite pad to provide the filtrate, which was concentrated under reduced pressure. The residue obtained was purified by flash chromatography using silica gel (50 g) with eluents of Et_3N/MeOH/$CHCl_3$=2/10/90 to provide colorless oil **4** in quantitative yield (8.8 g). Spectroscopic data is available in the literature.[19] Anal. $C_{10}H_{25}NOSi$, MW: 203.4, ESI+ Q-TOF MS, M = 203.2 (m/z), $[M+H]^+$ = 204.2; ^{1}H-NMR (500 MHz, CD_3OD): δ 0.06 (s, 6H, H_{TBDMS}), 0.90 (s, 9H, H_{TBDMS}), 1.55-1.59 (m, 4H), 2.74 (dd, 2H), 3.66 (dd, 2H).

2.2.2 *N*-[4-(*t*-butyldimethylsilanyloxy)butyl]ethacrynic amide **5**. Ethacrynic acid **1** (1.2 g, 4 mmol) was dried through azeotropical distillation with toluene three times. After mixing with DMF (2 mL), the mixture was

transferred to a two-neck round-bottom flask followed by charging with HBTU (1.65 g, 4.4 mmol, 1.1 eq), DIEA (0.76 mL, 4.4 mmol, 1.1 eq) and compound **4** (805 mg, 4 mmol, 1 eq) sequentially. Stirring was allowed for 15 min. TLC (MeOH/$CHCl_3$ = 2/8) indicated the consumption of EA **1** (R_f = 0.13) and formation of the product **5** (R_f = 0.40). The mixture was then concentrated at 40 ^oC under reduced pressure and the residue obtained was purified further by flash chromatography using silica gel (100 g) with eluents of EtOAc/*n*-hexane 3/7 to provide colorless oil **5** in 70% yield (1.35 g).

Anal. $C_{23}H_{35}Cl_2NO_4Si$, MW: 488.5, ESI+Q-TOF MS, M = 487.2 (m/z), $[M+H]^+$ = 488.2, $[M+Na]^+$ = 510.1, $[2M+Na]^+$ = 997.4; the isotope clusters agree with the presence of Cl×2. ^{1}H-NMR (500 MHz, C_6D_6): δ 0.04 (s, 6 H, H_{TBDMS}), 0.96 (s, 9H, H_{TBDMS}), 1.02 (dd, *J* = 7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.36-1.48 (m, 4 H, CH_2), 2.42 (q, *J* = 7.5 Hz, 2 H, $CC\underline{H}_2CH_3$), 3.18 (q, *J* = 6.5 Hz, 2H, (CONH)$C\underline{H}_2CH_2$), 3.45 (t, *J* = 6.0 Hz, 2H, $CH_2C\underline{H}_2$OTBDMS), 3.91 (d, *J* = 5.0 Hz, 2H, O($C\underline{H}_2$)CONH), 5.26 (s, 1H, C=$C\underline{H}_2$), 5.43 (s, 1H, C=$C\underline{H}_2$), 5.85-5.90 (m, 1H, H_{arom}), 6.36 (bs, 1H, NH), 6.63 (dd, *J* = 8.5, 2.0 Hz, 1H, H_{arom}). ^{13}C-NMR (125 MHz, C_6D_6): δ -5.24 (CH_3, TBDMS), 12.63 ($CH_2\underline{C}H_3$), 18.44 (C, TBDMS), 23.90 ($\underline{C}H_2CH_3$), 26.10 (CH_3, TBDMS), 26.60 (CH_2), 30.16 (CH_2), 38.89 (CH_2), 62.70 (CH_2), 68.50 (CH_2), 111.13 (CH, arom), 122.83 (C, $\underline{C}$=CH_2), 127.29 (CH, arom), 127.57 (CH_2, C=$\underline{C}H_2$), 131.42 (C, arom), 134.42 (C, arom), 150.64 (C, arom), 154.72 (C, arom), 165.87 (C, C=O), 194.76 (C, C=O).

2.2.3 *N*-Boc-*N*-[4-(*t*-butyldimethylsilanyloxy)butyl]ethacrynic amide **6**. The starting material **5** (682 mg, 1.40 mmol) was dried through azeotropical distillation with toluene at 40 ^oC three times. After mixing with CH_3CN (10 mL), the mixture was transferred to a two-neck round-bottom flask followed by charging with Boc_2O (2 mL, 2.80 mmol, 2 eq), Et_3N (0.27 mL, 1.96 mmol, 1.4 eq), and dimethyl aminopyridine (273 mg ,

2.24 mmol, 1.6 eq) sequentially. Stirring was allowed for 6 h. TLC (EtOAc/*n*-hexane 3/7) indicated the consumption of compound **5** (R_f = 0.40) and formation of the product **6** (R_f = 0.73). The mixture was then concentrated at 40 ^{o}C under reduced pressure followed by purification by flash chromatography using silica gel (80 g) with eluents of EtOAc/*n*-hexane 1/9 to provide colorless oil **6** in 76% yield (626 mg). Anal. $C_{28}H_{43}Cl_2NO_6Si$, MW: 588.6, ESI+Q-TOF MS, M = 587.2 (m/z), $[M\text{-Boc}+H]^+$ = 488.2, $[M+H]^+$ = 588.2, $[M+Na]^+$ = 610.3; the isotope clusters agree with the presence of Cl×2. HRMS-ESI, Calcd. $C_{28}H_{43}Cl_2NO_6Si$ $[M]^+$: 587.22367; found: 587.21601. ^{1}H-NMR (500 MHz, C_6D_6): δ 0.00 (s, 6H, H_{TBDMS}), 0.95 (s, 9H, H_{TBDMS}), 0.98 (t, *J* = 7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.30 (s, 9H, H_{Boc}), 1.38-1.42 (m, $C\underline{H}_2CH_2OTBDMS$), 1.59-1.63 (m, $(CON)CH_2C\underline{H}_2$), 2.43 (ddd, *J* = 7.5 Hz, 2H, $CC\underline{H}_2CH_3$), 3.45 (t, *J* = 7.0 Hz, 2H, $(CON)C\underline{H}_2CH_2$), 3.62 (t, *J* = 6.0 Hz, 2H, $CH_2C\underline{H}_2OTBDMS$), 5.00 (s, 2H, $O(C\underline{H}_2)CON$), 5.25 (s, 1H, $C=C\underline{H}_2$), 5.42 (s, 1H, $C=C\underline{H}_2$), 6.24 (d, *J* = 8.5 Hz, 1H, H_{arom}), 6.75 (d, *J* = 8.5 Hz, 1H, H_{arom}). ^{13}C-NMR (125 MHz, C_6D_6): δ -5.24 (CH_3, TBDMS), 12.62 ($CH_2\underline{C}H_3$), 18.41 (C, TBDMS), 23.93 ($\underline{C}H_2CH_3$), 25.63 (CH_2), 26.09 (CH_3, TBDMS), 27.70 (CH_3, Boc), 30.42 (CH_2), 44.26 (CH_2), 62.69 (CH_2), 70.52 (CH_2), 83.24 (C, Boc), 111.22 (C, $\underline{C}=CH_2$), 123.46 (CH, arom), 127.55 (CH, arom), 127.80 (CH_2, $C=\underline{C}H_2$), 131.55 (C, arom), 133.72 (C, arom), 150.47 (C, arom), 153.10 (C, arom), 169.67 (C, C=O), 195.17 (C, C=O).

2.2.4 *N*-Boc-*N*-(4-hydroxybutyl)ethacrynic amide **7**. A solution of TBAF/THF (1.16 mL, 1M, 2 eq), AcOH (0.066 mL, 1.16 mmol, 2 eq) in THF (10 mL) was added to a solution of the starting material **6** (340mg, 0.58 mmol) in THF (10 mL). Stirring was allowed for 8 h. TLC (EtOAc/*n*-hexane 3/7) indicated the consumption of compound **6** (R_f = 0.77) and formation of the product **7** (R_f = 0.27). The mixture was then concentrated at 40 ^{o}C under reduced pressure, followed by purification with flash chromatography using

silica gel (50 g) with eluents of EtOAc/*n*-hexane = 3/7 to provide colorless oil **7** in quantitative yield (270 mg). Anal. $C_{22}H_{29}Cl_2NO_6$, MW: 474.4, ESI+ Q-TOF MS, M = 473.1 (m/z), $[2M+Na]^+$ = 970.9; the isotope clusters agree with the presence of Cl×2. HRMS-ESI, Calcd. $C_{22}H_{29}Cl_2NO_6$ $[M]^+$:473.13719; found: 473.13166. ^{1}H-NMR (500 MHz, C_6D_6): δ 0.99 (t, *J* = 7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.28 (s, 9H, H_{Boc}), 1.28.1.32 (m, $C\underline{H}_2CH_2OH$), 1.52 (q, *J* = 7.5 Hz, *J* = 7.0 Hz, $(CON)CH_2C\underline{H}_2$), 2.43 (q, *J* = 7.5 Hz, 2H, $CC\underline{H}_2CH_3$), 3.25 (t, *J* = 6.0 Hz, 2H, $CH_2C\underline{H}_2OH$), 3.58 (t, *J* = 7.0 Hz, 2H, $(CON)C\underline{H}_2CH_2$), 5.01 (s, 2H, $O(C\underline{H}_2)CON$), 5.26 (s, 1H, $C=C\underline{H}_2$), 5.42 (s, 1H, $C=C\underline{H}_2$), 6.27 (d, *J* = 9.0 Hz, 1H, H_{arom}), 6.77 (d, *J* = 9.0 Hz, 1H, H_{arom}). ^{13}C-NMR (125 MHz, C_6D_6): δ 12.60 ($CH_2\underline{C}H_3$), 23.90 ($\underline{C}H_2CH_3$), 25.30 (CH_2), 27.67 (CH_3, Boc), 29.99 (CH_2), 44.18 (CH_2), 61.94 (CH_2), 70.50 (CH_2), 83.36 (C, Boc), 111.23 (CH, arom), 123.39 (C, $\underline{C}=CH_2$), 126.97 (CH, arom), 127.80 (CH_2, $C=\underline{C}H_2$), 131.53 (C, arom), 133.69 (C, arom), 150.45 (C, arom), 153.06 (C, arom), 156.45 (C, Boc), 169.84 (C, C=O), 195.33 (C, C=O).

2.2.5 *N*-Boc-*N*-[4-(toluenesulfonyloxy)butyl)ethacrynic amide **8**. The starting material **7** (270 mg, 0.57 mmol) was dried through azeotropical distillation with toluene (1 mL×3) at 40 ^{o}C three times. After mixing with CH_2Cl_2 (10 mL), the mixture was moved to an ice bath and stirred for 5 min. A solution of TsCl (162 mg, 0.85 mmol, 1.5 eq) in CH_2Cl_2 (1 mL) and DMAP (139 mg, 1.13 mmol, 2 eq) were added sequentially. Stirring was allowed for 12 h. TLC (EtOAc/*n*-hexane = 5/5) indicated the consumption of compound **7** (R_f = 0.45) and formation of product **8** (R_f = 0.75). The mixture was then concentrated at 40 ^{o}C under reduced pressure followed by purification with flash chromatography using silica gel (50 g) with eluents of EtOAc/*n*-hexane 1/4 to provide colorless oil **8** in 76% yield (271 mg). Anal. $C_{29}H_{35}Cl_2NO_8S$, MW: 628.6, ESI+Q-TOF MS, M = 627.2 (m/z), $[M+Na]^+$ = 650.4. HRMS-ESI, Calcd. $C_{29}H_{35}Cl_2NO_8S$ $[M]^+$:627.14604;

found: 627.14733. Anal. ($C_{29}H_{35}Cl_2NO_8S$) C, H, N; ^{1}H-NMR (500 MHz, C_6D_6): δ 0.98 (tt, *J* = 7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.23-1.25 (m , $C\underline{H}_2CH_2OTs$), 1.28 (s, 9H, H_{Boc}), 1.38-1.44 (m, (CON)$CH_2C\underline{H}_2$), 1.84 (s, 3H, $C\underline{H}_3$, OTs), 2.43 (q, *J* = 7.5 Hz, 2H, $CC\underline{H}_2CH_3$), 3.44 (t, *J* = 7.0 Hz, 2H, (CON)$C\underline{H}_2CH_2$), 3.75 (dd, *J* = 6.0 Hz, 2H, $CH_2C\underline{H}_2OTs$), 4.99 (s, 2H, O($C\underline{H}_2$)CON), 5.27 (s, 1H, C=$C\underline{H}_2$), 5.43 (s, 1H, C=$C\underline{H}_2$), 6.27 (d, *J* = 8.5 Hz, 1H, H_{arom}), 6.70 (d, *J* = 8.5 Hz, 2H, CH, OTs), 6.79 (d, *J* = 8 Hz, 1H, H_{arom}), 7.72 (d, *J* = 8.5 Hz, 2H, CH, OTs). ^{13}C-NMR (125 MHz, C_6D_6): δ 12.60 ($CH_2\underline{C}H_3$), 21.09 ($C\underline{H}_3$, OTs), 23.93 ($\underline{C}H_2CH_3$), 24.79 (CH_2), 26.45 (CH_2), 27.70 (CH_3, Boc), 43.44 (CH_2), 69.66 (CH_2), 70.44 (CH_2), 83.67 (C, Boc), 111.19 (CH, arom), 123.47 (C, $\underline{C}$=CH_2), 126.97 (CH, arom), 127.80 (CH_2, C=$\underline{C}H_2$), 128.00 (CH, arom), 129.83 (CH, arom), 131.63 (C, arom), 133.85 (C, arom), 134.27 (C, arom), 144.31 (C, arom), 150.51 (C, arom), 152.87 (C, arom), 156.43 (C, Boc), 169.76 (C, C=O), 195.18 (C, C=O).

2.2.6 *N*-Boc-*N*-(4-fluorobutyl)ethacrynic amide **9**. The starting material **7** (100 mg, 0.21 mmol) was dried through azeotropic distillation with toluene (1 mL) at 50 ^{o}C three times. After mixing with CH_2Cl_2 (5 mL), the mixture was stirred at -78 ^{o}C for 5 min. Diethylamino sulfur trifluoride (40 μL, 0.30 mmol, 1.5 eq) was then added and the mixture was stirred for 30 min. TLC (EtOAc/*n*-hexane 3/7) indicated the consumption of compound **7** (R_f = 0.40) and formation of the product **9** (R_f = 0.60). After addition of satd. aqueous $NaHCO_3$ (10 mL), the organic layer was separated, and the aqueous layer was further extracted with CH_2Cl_2 twice. The organic layers were combined and dried over Na_2SO_4, followed by filtration through celite pad. The filtrate obtained was concentrated under reduced pressure at 40 ^{o}C. The residue obtained was purified by flash chromatography using silica gel (30 g) with eluents of EtOAc/*n*-hexane 1/4 to provide colorless oil **9** in 35% yield (35 mg). Anal. $C_{22}H_{28}Cl_2FNO_5$, MW: 476.4, ESI+ Q-TOF MS, M = 475.1 (m/z), $[M+Na]^+$ =

498.0; the isotopic clusters agree with the presence of Cl×2. HRMS-ESI, Calcd. $C_{22}H_{28}Cl_2FNO_5$ $[M]^+$:475.13286; found: 475.13207. ^{1}H-NMR (500 MHz, C_6D_6): δ 0.92 (did, *J* = 7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.25 (s, 9H, H_{BOS}), 1.28 (dt, *J* = 7.5 Hz, *J* = 6.0 Hz, $J_{H,F}$ = 25.4 Hz, $C\underline{H}_2CH_2F$), 1.48 (tt, *J* = 7.5 Hz, *J* = 7.0 Hz, $(CON)CH_2C\underline{H}_2$), 2.43 (q, *J* = 7.5 Hz, 2H, $CC\underline{H}_2CH_3$), 3.51 (dd, *J* = 6.0 Hz, $J_{H,F}$ = 48.0 Hz, 2H, $CH_2C\underline{H}_2F$), 4.00 (t, *J* = 7.0 Hz, 2H, $(CON)C\underline{H}_2CH_2$), 4.99 (s, 2H, $O(C\underline{H}_2)CON$), 5.25 (s, 1H, $C=C\underline{H}_2$), 5.41 (s, 1H, $C=C\underline{H}_2$), 6.24 (d, *J* = 9.0 Hz, 1H, H_{arom}), 6.77 (d, *J* = 9.0 Hz, 1H, H_{arom}). ^{13}C-NMR (125 MHz, C_6D_6): δ 12.61 ($CH_2\underline{C}H_3$), 23.92 ($\underline{C}H_2CH_3$), 24.72 ($J_{C,F}$ = 3.8 Hz, $\underline{C}H_2CH_2CH_2F$), 27.66 ($CH_3$, Boc), 27.90 ($J_{C,F}$ = 20.0 Hz, $\underline{C}H_2CH_2F$), 43.75 (CH_2), 70.48 (CH_2), 82.41 (C, Boc), 83.42 ($J_{C,F}$ = 166.4 Hz, CH_2F), 111.21 (CH, arom), 123.49 (C, $\underline{C}=CH_2$), 126.90 (CH, arom), 127.58 (CH_2, $C=\underline{C}H_2$), 131.62 (C, arom), 133.85 (C, arom), 150.47 (C, arom), 152.92 (C, arom), 156.43 (C, Boc), 169.70 (C, C=O), 195.18 (C, C=O). ^{19}F-NMR (470 MHz, C_6D_6): δ -218.23 (dd, $J_{F,H}$ = 25.4, $J_{F,H}$ = 48.0 Hz, 1F).

2.2.7 *N*-(4-fluorobutyl)ethacrynic amide **3**. A solution of trifluoro acetic acid (250 μL) was added to a two-necked round-bottomed flask containing starting material **9** (30 mg, 0.063 mmol) in CH_2Cl_2 (2 mL). Stirring was allowed for 1 h. TLC (EtOAc/*n*-hexane 5/5) indicated the consumption of compound **9** (R_f = 0.70) and formation of product **3** (R_f = 0.30). After addition of saturated aqueous $NaHCO_3$ (10 mL), the organic layer was collected and the aqueous layer was extracted with CH_2Cl_2 (2 mL×2). The organic layers combined were dried over Na_2SO_4 and filtered through celite pad. The filtrates were concentrated under reduced pressure, and the residue obtained was further purified by flash chromatography using silica gel (20 g) with eluents of EtOAc/*n*-hexane = 5/5 to provide white solids **3** in 70% yield (16 mg). Mp: 94-95 ^oC. Anal. $C_{17}H_{20}Cl_2FNO_3$, MW: 376.3, ESI+Q-TOF MS, M = 375.1 (m/z), $[M+Na]^+$ = 398.0; the isotope

clusters agree with the presence of Cl. HRMS-ESI, Calcd. $C_{17}H_{20}Cl_2FNO_3$ $[M]^+$:375.08043; found: 375.07974. Anal. ($C_{17}H_{20}Cl_2FNO_3$) C, H, N; ^{1}H-NMR (500 MHz, CD_3OD): δ 1.12 (t, *J* = 7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.65-1.73 (m, 4H, $C\underline{H}_2CH_2F$ and $(CONH)CH_2C\underline{H}_2$), 2.44 (q, *J* = 7.5 Hz, 2H, $CC\underline{H}_2CH_3$), 3.33 (td, *J* = 6.5 Hz, 2H, $(CONH)C\underline{H}_2CH_2$), 4.37 (dt, *J* = 5.5 Hz, $J_{H,F}$ = 48.9 Hz, 1H, $CH_2C\underline{H}_2F$), 4.49 (dt, *J* = 5.5 Hz, $J_{H,F}$ = 48.9 Hz, 1H, $CH_2C\underline{H}_2F$ s, 1H, $C=C\underline{H}_2$), 4.69 (s, 2H, $O(C\underline{H}_2)CONH$), 6.03(s, 1H, $C=C\underline{H}_2$), 6.59 (s, 1H, $C=C\underline{H}_2$), 7.00 (d, *J* = 8.5 Hz, 1H, H_{arom}), 7.24 (d, *J* = 8.5 Hz, 1H, H_{arom}). ^{13}C-NMR (125 MHz, C_6D_6): δ 12.62 ($CH_2\underline{C}H_3$), 23.89 ($\underline{C}H_2CH_3$), 25.81 ($J_{C,F}$ = 3.9 Hz, $\underline{C}H_2CH_2CH_2F$), 27.79 ($J_{C,F}$ = 22.5 Hz, $\underline{C}H_2CH_2F$), 38.53 (CH_2), 68.44 (CH_2), 83.05 ($J_{C,F}$ = 165.0 Hz, CH_2F), 111.12 (CH, arom), 122.82 (C, $\underline{C}=CH_2$), 127.29 (CH, arom), 127.62 (CH_2, $C=\underline{C}H_2$), 131.46 (C, arom), 134.50 (C, arom), 150.63 (C, arom), 154.65 (C, arom), 165.95 (C, C=O), 194.75 (C, C=O). ^{19}F-NMR (470 MHz, C_6D_6): δ -217.82 (tt, $J_{F,H}$ = 25.9, $J_{F,H}$ = 48.9 Hz, 1F).

2.2.8 Methyl ethacrynic ester **11**. Preparation of **11** was the methylation of acyl group using diazomethane. To the suspension of pale yellow nitrosomethylurea* (1.6 g, 9.9 mmol, 1.5 eq) in Et_2O (80 mL) was added aqueous KOH (1.1 M, 16 mL). Upon stirring for 5 min, diazomethane (CH_2N_2, bp -23 °C) was formed in the organic layer. After collecting the Et_2O layer (80 mL), the solution was moved to ice bath. KOH pellet was added until compact pellet of KOH precipitated. The resultant diazomethane in Et_2O was added to the solution of ethacrynic acid **3** (2 g, 6.6 mmol, 1 eq) in EtOAc (20 mL) in one portion. Product **10** (R_f = 0.46) was observed by TLC (EtOAc/n-hexane = 1/4) and ethacrynic acid **3** (R_f = 0) has been consumed. Before concentrating the reaction mixture with rotavapor at 40 °C, a drop of AcOH (0.02 mL) was added to quench the reaction and the solution of reaction became clear. The crude product **11** was purified by flash

chromatography with EtOAc/*n*-hexane = 3/17 and the semitranslucent, soft-solid product **11** was obtained in 91% yield (1.9 g). Anal. $C_{14}H_{14}Cl_2O_4$, MW: 317.2, ESI+ Q-TOF MS, M = 316.0 (m/z), $[M+H]^+$ = 317.0, $[M+Na]^+$ = 339.0, $[M+K]^+$ = 355.0, $[2M+H]^+$ = 635.1, $[2M+Na]^+$ = 655.0; the isotopic clusters agree with the presence of Cl×2. Element analysis: Calcd. C, 53.02; H, 4.45. Found C, 52.90; H, 4.81. ^{1}H-NMR (500 MHz, C_6D_6): δ 0.99 (dd, *J* = 7.5 Hz, 3H, $CH_2C\underline{H}_3$), 2.43 (ddd, *J* = 7.5 Hz, 2H, $C\underline{H}_2CH_3$), 3.19 (s, 3H, COOMe), 3.99 (s, 2H, $O(C\underline{H}_2)COOMe$), 5.23 (s, 1H, $C=C\underline{H}_2$), 5.42 (s, 1H, $C=C\underline{H}_2$), 6.06 (d, *J* = 8.5 Hz, 1H, H_{arom}), 6.73 (d, *J* = 8.5 Hz, 1H, H_{arom}). ^{13}C-NMR (125 MHz, C_6D_6): δ 12.61 ($CH_2\underline{C}H_3$), 23.89 ($\underline{C}H_2CH_3$), 51.58 ($COO\underline{C}H_3$), 65.76 ($O(\underline{C}H_2)COOCH_3$), 110.85 (CH, arom), 123.53 (C, $\underline{C}=CH_2$), 126.85 (CH, arom), 127.65 (CH_2, $C=\underline{C}H_2$), 131.75 (C, arom), 134.36 (C, arom), 150.48 (C, arom), 155.69 (C, arom), 167.70 (C, C=O), 195.02 (C, C=O).

* Warning: Diazomethane (CH_2N_2) should be dissolved in the solution; explosion may occur when drying the solvent.

2.2.9 *N*-(4-hydroxy-butyl)ethacrynic amide **12**. Starting material **11** (1.1 g, 2.94 mmol) was dissolved in dried DMF (40 mL) under nitrogen. To the solution of **11** was added Et_3N (5 mL, 29.4 mmol, 10 eq) and 4-amino-1-butanol (1.5 mL, 162 mmol, 5.5 eq). The stirring was allowed at 60 ^{o}C for 8 h and the drying tube of blue silica gel was installed on the round bottom flask. TLC (acetone/*n*-hexane = 1/3) indicated the consumption of **11** (R_f = 0.44) and formation of the product **12** (R_f = 0.23). DMF was removed under high vacuo at 55 ^{o}C. The residue was purified by flash chromatography with acetone/*n*-hexane 1/3 → 3/7 to obtain white foam product **12** in 20% yield (255 mg). mp = 92-94 ^{o}C. Anal. $C_{18}H_{25}Cl_2NO_4$, MW: 374.3, ESI+ Q-TOF MS, M = 373.1 (m/z), $[M+H]^+$ = 374.1, $[2M+H]^+$ = 749.3; the isotopic clusters agree with the

presence of Cl×2. Element analysis: Calcd. C, 54.56; H, 5.66; N, 3.74. Found C, 54.54; H, 5.94; N, 3.64. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.26 (d, *J* = 7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.60 (d, *J* = 6.5 Hz, 2H, CH_2), 1.63-1.72 (m, 2H, CH_2), 1.68 (bs, 1H, OH), 2.45 (ddd, *J* = 6.5 Hz, 2H, $CC\underline{H}_2CH_3$), 3.41 (ddd, *J* = 6.5 Hz, 2H, CH_2), 3.67 (dd, *J* = 6.0 Hz, 2H, CH_2), 4.55 (s, 2H, $O(C\underline{H}_2)CONH$), 5.57 (s, 1H, $C=C\underline{H}_2$), 5.94 (s, 1H, $C=C\underline{H}_2$), 6.84 (d, *J* = 8.5 Hz, 1H, H_{arom}), 6.90 (bs, 1H, NH), 7.17 (d, *J* = 8.5 Hz, 1H, H_{arom}). ^{1}H-NMR (500 MHz, C_6D_6): δ 1.01 (dd, *J* = 7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.06 (bs, 1H, OH), 1.25 (dd, *J* = 6.0 Hz, *J* = 6.5 Hz, 2H, $C\underline{H}_2CH_2OH$), 1.33 (dd, *J* = 6.5 Hz, *J* = 7.5 Hz, 2H, $(CONH)CH_2C\underline{H}_2$), 2.45 (d, *J* = 6.5 Hz, 2H, $CC\underline{H}_2CH_3$), 3.16 (d, *J* = 7.5 Hz, 2H, $(CONH)C\underline{H}_2CH_2$), 3.26 (d, *J* = 6.0 Hz, 2H, $CH_2C\underline{H}_2OH$), 3.96 (s, 2H, $O(C\underline{H}_2)CONH$), 5.30 (s, 1H, $C=C\underline{H}_2$), 5.47 (s, 1H, $C=C\underline{H}_2$), 5.94 (d, *J* = 8.5 Hz, 1H, H_{arom}), 6.48 (bs, 1H, NH), 6.67 (d, *J* = 8.5 Hz, 1H, H_{arom}). ^{13}C-NMR (125 MHz, C_6D_6): δ 12.61 ($CH_2\underline{C}H_3$), 23.88 ($\underline{C}H_2CH_3$), 26.52 (CH_2), 29.83 (CH_2), 38.89 (CH_2), 61.99 (CH_2), 68.46 (CH_2), 111.15 (CH, arom), 122.84 (C, $\underline{C}=CH_2$), 127.29 (CH, arom), 127.81 (CH_2, $C=\underline{C}H_2$), 131.41 (C, arom), 134.40 (C, arom), 150.60 (C, arom), 154.74 (C, arom), 166.21 (C, C=O), 194.86 (C, C=O).

2.2.10 *N*-[4-(*t*-butyldimethylsilanyloxy)butyl]ethacrynic amide **5**. Starting material **11** (220 mg, 0.45 mmol,) was dissolved in toluene (4 mL) and the solution was distilled under reduced pressure. The residue was dissolved in CH_2Cl_2 (11 mL) under nitrogen. To the mixture of **11** were added a solution of TBDMSCl (243 mg, 1.62 mmol, 3.6 eq) in CH_2Cl_2 (11 mL), pyridine (0.5 mL), and DMAP (90 mg, 0.73 mmol, 1.6 eq), sequentially. The stirring was allowed at 60 ^{o}C for 8 h. TLC (acetone/*n*-hexane = 1) indicated the consumption of **11** (R_f = 0.43) and formation of the product **5** (R_f = 0.78). The solvent was distilled under reduced pressure at 40 ^{o}C. The residue obtained was purified by flash chromatography with EtOAc/*n*-hexane

= 3/7 to provide colorless oil product **5** in 20% yield (57 mg). Spectroscopic data is consistent with the above described.

2.2.11 *N*-[4-(*O*-acetyl)butyl]ethacrynic amide **14**. Starting material **5** (5 mg, 0.01 mmol) was dissolved in THF (1 mL). TsOH · H_2O (10 mg, 0.0502 mmol, 3.6 eq) was dissolved at 70 ^oC and diluted in isopropenyl acetate (7.5 mL). To the solution of **5** in THF was added the above diluted mixture of TsOH · H_2O (1 mg, 0.3 eq) and isopropenyl acetate (0.5 mL). The stirring was allowed under reflux for 24 h and the drying tube of blue silica gel was installed on the round bottom flask. TLC (EtOAc/*n*-hexane = 1) indicated the consumption of **5** (R_f = 0.63) and formation of the product **14** (R_f = 0.27). The reaction was quenched with Et_3N. The mixture was concentrated under reduced pressure and the residue obtained was purified by flash chromatography with EtOAc/*n*-hexane = 1 to provide colorless oil product **14** in 70% yield (3 mg). Anal. $C_{19}H_{23}Cl_2NO_5$, MW: 416.3, ESI+ Q-TOF MS, M = 415.1 (m/z), $[M+H]^+$ = 416.1, $[M+Na]^+$ = 438.1, $[M+K]^+$ = 454.1, $[2M+Na]^+$ = 853.2; the isotopic clusters agree with the presence of Cl×2. ^{1}H-NMR (500 MHz, C_6D_6): δ 1.02 (dd, *J* = 7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.21 (dddd, *J* = 7.5 Hz, *J* = 7.0 Hz, 2H, CH_2), 1.30 (dddd, *J* = 6.5 Hz, *J* = 7.5 Hz, 2H,CH_2), 1.67 (s, 3H, OAc), 2.45 (ddd, *J* = 7.5 Hz, 2H, $CC\underline{H}_2CH_3$), 3.05 (ddd, *J* = 7.0 Hz, *J* = 6.5 Hz, 2H, (CONH)$C\underline{H}_2CH_2$), 3.86 (dd, *J* = 6.5 Hz, 2H, $CH_2C\underline{H}_2OAc$), 3.93 (s, 2H, $O(C\underline{H}_2)CONH$), 5.30 (s, 1H, $C=C\underline{H}_2$), 5.47 (s, 1H, $C=C\underline{H}_2$), 5.89 (d, *J* = 8.5 Hz, 1H, H_{arom}), 6.27 (bs, 1H, NH), 6.67 (d, *J* = 8.5 Hz, 1H, H_{arom}). ^{13}C-NMR (125 MHz, C_6D_6): δ 12.62 ($CH_2\underline{C}H_3$), 20.44 (CH_3, OAc), 23.89 ($\underline{C}H_2CH_3$), 26.13 (CH_2), 26.39 (CH_2), 38.57 (CH_2), 63.70 (CH_2), 68.43 (CH_2), 111.28 (CH, arom), 122.78(C, $\underline{C}=CH_2$), 127.30 (CH, arom), 127.81 (CH_2, $C=\underline{C}H_2$), 131.44 (C, arom), 134.49 (C, arom), 150.62 (C, arom), 154.63 (C, arom), 165.90 (C, C=O), 194.74 (C, C=O).

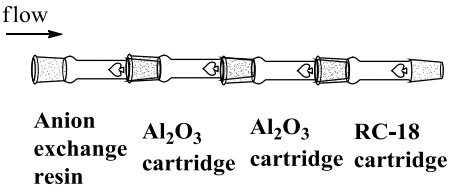
2.2.12 *N*-(2-tert-Butoxy-2-hydroxy-[1,3]oxazepan-3-yl)ethacrynic amide **15**. 4 Å molecular sieve was dried at 200 ^{o}C under high vacuo for 8 h. TBAF (0.08 mL, 1M, 1.9 eq) in 5% water/THF solution was diluted with dried THF (3 mL). The above diluted solution of TBAF was added to the round bottom flask (10 mL) containing starting material **6** (25 mg, 0.0425 mmol) followed by addition of dried 4 Å molecular sieve (30 mg). The stirring was allowed at rt for 3h. TLC (EtOAc/*n*-hexane = 1) indicated the consumption of **6** and formation of the product **15** (R_f = 0.56). The solvent was distilled under reduced pressure at 40 ^{o}C. The crude product **15** was purified by flash chromatography with EtOAc/n-hexane = 3/7 to obtain colorless oil product **15** in 27% yield (5.5 mg). Anal. $C_{22}H_{29}Cl_2NO_6$, MW: 474.4, ESI+ Q-TOF MS, M = 473.1 (m/z), $[M\text{-Boc}+H]^+$ = 374.2, $[M+H]^+$ = 474.3, $[M+Na]^+$ = 496.3; the isotopic clusters agree with the presence of Cl×2. ^{1}H-NMR (500 MHz, C_6D_6): δ 0.98-1.05 (m, 2H, CH_2), 1.00 (dd, *J* = 7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.49 (dddd, *J* = 7.5 Hz, *J* = 6.5 Hz, CH_2), 1.45 (s, 9H, $H_{t\text{-}Bu}$), 2.41 (ddd, *J* = 7.5 Hz, 2H, $CC\underline{H}_2CH_3$), 2.75-2.83 (m, 2H, $CH_2C\underline{H}_2O$), 3.79 (dd, *J* = 6.5 Hz, *J* = 6.0 Hz, 2H, $NC\underline{H}_2CH_2$), 4.03 (bs, 1H, OH), 4.07 (s, 2H, $O(C\underline{H}_2)CON$), 5.27 (s, 1H, $C=C\underline{H}_2$), 5.45 (s, 1H, $C=C\underline{H}_2$), 6.16 (d, *J* = 8.5 Hz, 1H, H_{arom}), 6.77 (d, *J* = 8.5 Hz, 1H, H_{arom}); the hydrogen bond may occur at 4.03 ppm. ^{13}C-NMR (125 MHz, C_6D_6): δ 12.60 ($CH_2\underline{C}H_3$), 20.43 ($CH_2\underline{C}H_3$), 23.88 ($\underline{C}H_2CH_3$), 23.93 ($\underline{C}H_2CH_3$), 25.71 (CH_2), 27.70 (CH_3, t-Bu), 30.43 (CH_2), 44.28 (CH_2), 62.70 (CH_2), 70.57 (CH_2), 83.19 (C, t-Bu), 95.09 (C, $\underline{C}=CH_2$), 111.28 (CH, arom), 127.29 (CH, arom), 127.44 (CH_2, $C=\underline{C}H_2$), 131.64 (C, arom), 133.82 (C, arom), 150.53 (C, arom), 153.11 (C, arom), 169.95 (C, C=O), 195.16 (C, C=O).

2.3 Radiosynthesis of [^{18}F]-*N*-(4-fluorobutyl)ethacrynic amide {[^{18}F]FBuEA **3**}

The radiolabeling of [^{18}F]FBuEA **3** was performed on a GEMS TracerLAB FX_{FN} synthesis module using an

in-house reaction sequence. On the GE TracerLAB FX_{FN} synthesis module, aqueous [^{18}F]fluoride solution (0.5 – 1 Ci/1.8 mL) was loaded on a QMA-Light Sep-Pak cartridge (waters) first activated with Bu_4NHCO_3 (0.6 mL, 0.075M). The concentrated [^{18}F]fluoride was eluted into the reactor with CH_3CN (1 mL). The mixture was purged by a fume of helium gas for 2 min followed by concentration under vacuo at 100 ^{o}C for 2 min. After terminating the helium purge, the mixture was cooled down to 70 ^{o}C. CH_3CN (1 mL) was added and the mixture was heated to 100 ^{o}C under the purge of a fume of helium gas for 2 min. After concentration under vacuo for 5 min, the mixture was cooled down to 70 ^{o}C. The mixture was measured to be 710 mCi (71 % yield). A solution of tosylate **8** (10 mg) in CH_3CN (1 mL) was added, and the mixture was heated to 100 ^{o}C for 5 min. A fume of He gas was purged at 100 ^{o}C over 2 min followed by concentration under reduced pressure at 100 ^{o}C for 2 min. After cooling down to 30 ^{o}C, a solution of TFA and CH_2Cl_2 (2 mL, v/v 1:9) was added to the mixture of [^{18}F]**9**, and stirring was allowed at 50 ^{o}C for 15 min. After cooling down to 30 ^{o}C, EtOH (1 mL, 95%) was added and the stirring was allowed for 20 s. The solution was loaded onto a cartridge setting comprising anionic exchange resin (Dowex, OH^- form, 4-5g), neutral alumina supporting (Waters) and RC-18 plus (Waters), followed by eluting with acetone/H_2O (1 mL, 9/1, v/v) .The fractions were collected. Repeating the washing procedures twice and the fractions were combined for subsequent concentration at 40 ^{o}C for 5 min. CH_3CN (1mL) was added followed by concentration at 40 ^{o}C for 3 min. The procedure was repeated twice. After addition of CH_3CN (0.2 mL), the mixture was purified using HPLC as described below. According to the experimental purposes, [^{18}F]FBuEA **3** was purified by two types of HPLC conditions. HPLC settings: (A) ZORBAX SIL, 9.4×250 mm, 5 μm, EtOAc/*n*-hexane 1/2, Flowrate = 3 mL/min, t_R = 39.6 min (Radio); (B) CHEMCOSORB 7-ODS-H, 10×250 mm, 5 μm; eluent was

set isocratically from CH_3CN/0.05% trifluoracetic acid =20/80 at 0 min to CH_3CN /0.05% trifluoracetic acid =95/5 at 10 min and a further gradient to CH_3CN (100%) at 20 min. Flowrate = 3 mL/min, t_R = 14.8 min (Radio). Fractions to [^{18}F]FBuEA **3** isolated from several injections were combined and concentrated to provide the purified [^{18}F] FBuEA **3** in a radiochemical yield of 2.3% (7.8 mCi, based on E.O.B., decay corrected). Specific radioactivity and radiochemical purity were 48 GBq/μmole and 98%, respectively.



2.4 Conjugating Experiment

2.4.1 Conjugation of [^{18}F]FBuEA **3** with GST under catalysis of GST-π and GST-α. Enzymatic transformation of GSH to [^{18}F]FBuEA was performed according to the protocol reported by Lo et al.[25] The fractions of [^{18}F]FBuEA (220 μCi or 76 μCi for GST-π or 600 μCi for GST-α) isolated from HPLC purification was concentrated under reduced pressure at 50 ^{o}C for 20 min and the resultant residue was mixed with MeOH (0.1 mL). The following were added sequentially: an aliquot (0. 1 mL) drawn from a solution of GSH (1 mg) in saline (1 mL), Na_3PO_4 buffer (1 mL, pH 7.0, 10 mM), and an aliquot (0.1 mL) drawn from a solution of GST-**π** protein (25 μg) in Na_3PO_4 buffer (0.2 mL). For the case of GST-α, an aliquot (0.1 mL) was drawn from a solution of 50 μg of GST-α in 0.4 mL was used. The mixture was stirred at room temperature for 2 h followed by addition of the quenching agent acetone (2 mL), and stirring was allowed for a further 2 min. After concentration under reduced pressure at 40 ^{o}C for 10 min, the mixture was washed with CH_2Cl_2 (2 mL) two times to collect the aqueous layer, and H_2O (1 mL) was used to backextract the organic layer. The aqueous layers combined were submitted to HPLC analysis using eluting condition (B)

as described above for preparation of both the nonradioactive and radioactive FBuEA-GSH complex. t_R = 16.5 min (radio). In the case of GST-π, the organic layer containing the most radioactivity (70 μCi) implied an incomplete consumption of the starting [^{18}F]FBuEA. The complex [^{18}F]FBuEA-GSH **10** was obtained in a radiochemical yield of 16% (13.5 μCi, decay corrected) and 5% for the second set of experiment. For the case of GST-α, no radioactivity in the organic layer could be found. The aqueous layer containing 203 μCi (92%, crude yield) was further purified using HPLC to give [^{18}F]FBuEA-GSH **10** in a radiochemical yield of 85%, decay corrected.

2.4.2 Conjugation of nonradioactive FBuEA **3** with GSH. The conjugation method was as previously described in the literature.[22] A solution of GSH (22 mg, 72 μmol, 1.5 eq) in distilled H_2O (1 mL) was added to a solution of FBuEA **3** (18 mg, 48 μmol, 1 eq) in CH_3CN (1 mL). NaOH (50 mM, 1.5 mL) was added to adjust the pH value to 8. Stirring was allowed for 15 min. TLC indicated the consumption of the starting material **3** (R_f = 0.9) and the formation of the product complex FBuEA-GSH **10** (R_f = 0.4). The mixture was filtered through Nylon (0.20 μM, National Scientific), and the resulting filtrate (3 mL) was purified using HPLC. The eluting condition was set at constant CH_3CN/0.05% trifluoracetic acid = 20/80 for the first 1 min and then isocratically to a ratio of CH_3CN/0.05% trifluoracetic acid = 40/60 at 11 min and a further gradient to CH_3CN (100%) at 20 min. Flowrate = 3 mL/min, t_R = 16.3 min (UV). The isolated fractions from a number of injections of HPLC were collected, followed by precipitation under the addition of CH_3CN (1 mL) to provide solids. The solid mixture was further filtered through gravity filtration followed by washing with cold CH_3CN. The residue thus obtained was dried under high vacuo at 40 ^{o}C to provide a white solid of FBuEA-GSH **10** complex in 72% yield (21 mg). Cocrystalized solvents (e.g., H_2O or MeOH) were

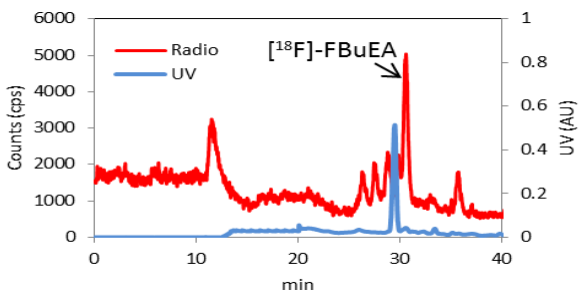
estimated to contribute a weight percent of 30% to 40%. Anal. $C_{27}H_{37}Cl_2FN_4O_9S$, MW: 682.2, LRMS, ESI+ Q-TOF MS, M = 682.2 (m/z), $[M+H]^+$ = 683.2, $[M+Na]^+$ = 705.1, $[M+K]^+$ = 721.1; the isotopic clusters agree with the presence of Cl. Melting point: 127-128 ^oC. HRMS-ESI, Calcd. $C_{27}H_{37}Cl_2FN_4O_9S$ $[M]^+$:682.16423; found: 682.16389. ^{1}H-NMR (500 MHz, CD_3OD:D_2O = 1:3, 50 ^oC): δ 0.87 (bs, 3H, CH_3), 1.65 (bs, 4H, CH_2CH_2), 1.71 (bs, 2H, CH_2), 2.14 (bs, 2H, CH_2), 2.52 (bs, 2H, CH_2), 2.75-3.04 (m, 4H, (CH_2SCH_2), 3.33 (bs, 2H, CH_2), 3.52 (d, *J* = 5.0 Hz, 1H, HCCO), 3.70 (bs, 1H, NCHCO), 3.78 (d, *J* = 5.0 Hz, 2H, CH_2), 4.43 (d, *J* = 3.5 Hz, 1H, CH_2F), 4.74 (bs, 2H, OCH_2CO), 7.12 (d, *J* = 5.0 Hz, 1H, H_{arom}) , 7.59-7.62 (m, 1H, H_{arom}); ^{13}C-NMR (125 MHz, CD_3OD:D_2O=1:3, 50 ^oC): 11.25 (CH_3), 25.18 (CH_2), 27.24 (CH_2), , 28.00 (d, CH_2CH_2F, $J_{C,F}$ = 18.8 Hz), 32.59 (CH_2), 33.27 (CH_2), 34.90 (CH_2), 39.62 (CH_2), 44.31 (CH_2), 52.63 (CH), 54.14 (CH), 54.19 (CH), 55.22 (CH), 68.89 (CH_2), 85.63 (d, CH_2F, $J_{C,F}$ = 158 Hz), 112.57 (CH, arom), 124.17 (C, arom), 129.44 (CH, arom), 131.84 (C, arom), 134.07 (C, arom), 134.11 (C, arom), 156.84 (C, CO), 170.11 (C, CO), 172.31 (C, CO), 175.47 (C, CO), 175.51 (C, CO), 206.75 (C, CO). ^{19}F-NMR (470 MHz, CD_3OD:D_2O=1:3, 50 ^oC): δ -218.16 (heptet, $J_{F,H}$ = 46.5, $J_{F,H}$ = 25.9 Hz, 1F).

2.4.3 [^{18}F]**10** prepared from conjugation of [^{18}F]FBuEA **3** with GSH at pH = 8.0. The conjugation method was according to the nonradioactive conjugation protocol as described above. HPLC-isolated [^{18}F]FBuEA **3** (1.1 MBq) in a round-bottom flask (25 mL) was concentrated under reduced pressure at 50 ^oC for 3 min. CH_3CN (1 mL) was added, and the azeotropic distillation was allowed for 5 min. CH_3CN (1 mL) and a solution of GSH (20 mg, 65 μmol) in distilled water (1 mL) were added sequentially. An aqueous solution of NaOH (50 mM, 0.6 mL) was added to adjust the pH to 8.0. Stirring was allowed for 15 min followed by HPLC analysis. A portion (0.4 mL) of the mixture (0.93 MBq, 3 mL), obtained from filtration through a 0.45

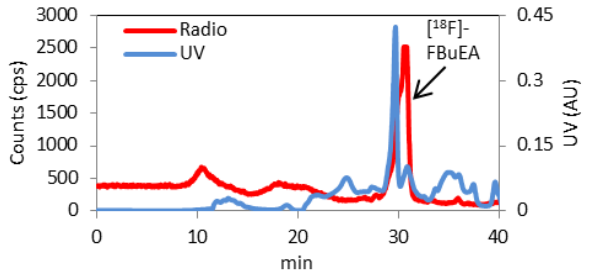
μM membrane filter, was drawn for HPLC injection. The eluting condition was the same as that described above for the nonradioactive preparation. The radiochemical yield of [^{18}F]**10** was 41% according to the calculation of the peak areas (Fig. 4). Specific activity was 10 GBq/μmol.

2.5 Metabolite analysis

The protocol used was modified from that in the literature.[26,27] Fractions of [^{18}F]FBuEA **3** (2 mL) isolated from reverse phase HPLC using condition (B) was added to the vial (10 mL). The mixture was concentrated under reduced pressure at 50 ^{o}C for 10 min. Toluene (1 mL) was added and distilled azeotropically two times. The cosolvent EtOH/saline (0.8 mL, 1:4 v/v) was added. The solution of [^{18}F]FBuEA **3** (6.3 MBq) was injected through the tail vein, and each of the blood samples (2 mL) was drawn from the femoral artery at 10, 30, 60 and 90 min. Following centrifugation at 3500 rpm for 5 min, the supernatant (0.5 mL) was mixed with $CHCl_3$ (2 mL) and H_2O (2 mL) under ultrasonic vibration for 5 min. The organic layer collected was eluted through a RC-18 cartridge (Waters) and washed with a cosolvent of MeOH/H_2O (3 mL, 1:4 v/v) to remove the undesired polar solutes, followed by eluting with CH_3CN (4 mL). The mixture obtained from each time point was then concentrated under reduced pressure at 40 ^{o}C for 10 min, and the residue obtained from each was mixed with a solution of authentic FBuEA in MeCN (200 μL drawn from 1 mg/2 mL) followed by filtration through a filter (Milipore, PTFE, 0.45 μm) for HPLC investigation. HPLC purification condition (B) as described above was adopted.



(a)



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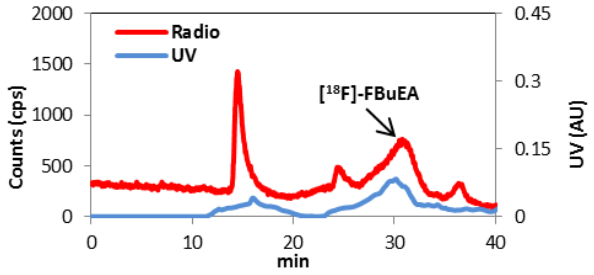


(c)

Figure Study of the stability of [^{18}F]FBuEA *in vivo*. Panel (a)-(c): HPLC chromatogram for plasma samples drawn from femoral artery at 10, 30 and 60 min postinjection of [^{18}F]FBuEA. The plasma sample at 90 min displayed no radioactivity.

2.6 Octanol/Water Partition Coefficient

A lipophilicity test was carried out by measuring the log P value using $P = C_{n\text{-octanol}}/C_{water}$ with the "Shake-flask method" (O. J. L. 383A) according to the official Journal of the European Community. *n*-octanol (2.5 mL) was added to a sample vial containing the isolated fraction of [^{18}F]FBuEA (12.6 μCi) obtained from HPLC after concentration under reduced pressure. Stirring was allowed for 1 min, and an aqueous solution of PBS (0.01M, pH 7.3, 2.5 mL) was added. Vigorous stirring was continued for 15 min. The two layers were then separated, and three aliquots (0.5 mL×3) from each layer were drawn for counting in a gamma counter. The partition coefficient was logP = 1.47±0.04.

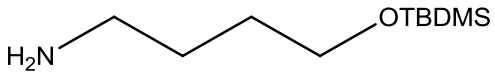
2.7 Preparation of animal model

The protocol is according to the previous published work.[8] The experimental animal ethics committee of Chang Gung Memorial Hospital approved all animal protocols in this study. Furthermore, the investigation conformed to the US National Institute of Health (NIH) guidelines for the care and use of laboratory animals (Publication No. 85-23, revised 1996). Three adult male Sprague-Dawley (SD) rats (330-370 g) were used in these experiments. The animals were divided into two groups, including a control group and an experiment group. The rats were housed in an animal room with a 12:12-h light-dark cycle (light from 08:00 to 20:00) at an ambient temperature of 22 $\pm$ 1 ^{o}C, with food and water available *ad libitum*. The experimental group rats were administered 300 mg TAA/1L in their drinking water every day up to the time they were killed (as described below). In the experiment group, the animals were harvested weekly during the study to examine the effect of TAA and establish the natural history of the animal model.

2.8 Small Animal MicroPET Studies

The animals were anesthetized using 1 L/min 2% isoflurane (100% oxygen). After they had been anesthetized, the rats were given ^{18}F-FBuEA (265 - 801 μCi) via the lateral tail vein. After the injection, the rats were fixed in the prone position on a carbon bed. Dynamic imaging (0-120 min) single-frame scans were acquired with a small-animal PET camera (microPET R4; Concorde Microsystems Inc.).

3. ^{1}H- and ^{13}C-NMR, ESI-MS and element analysis of the compounds



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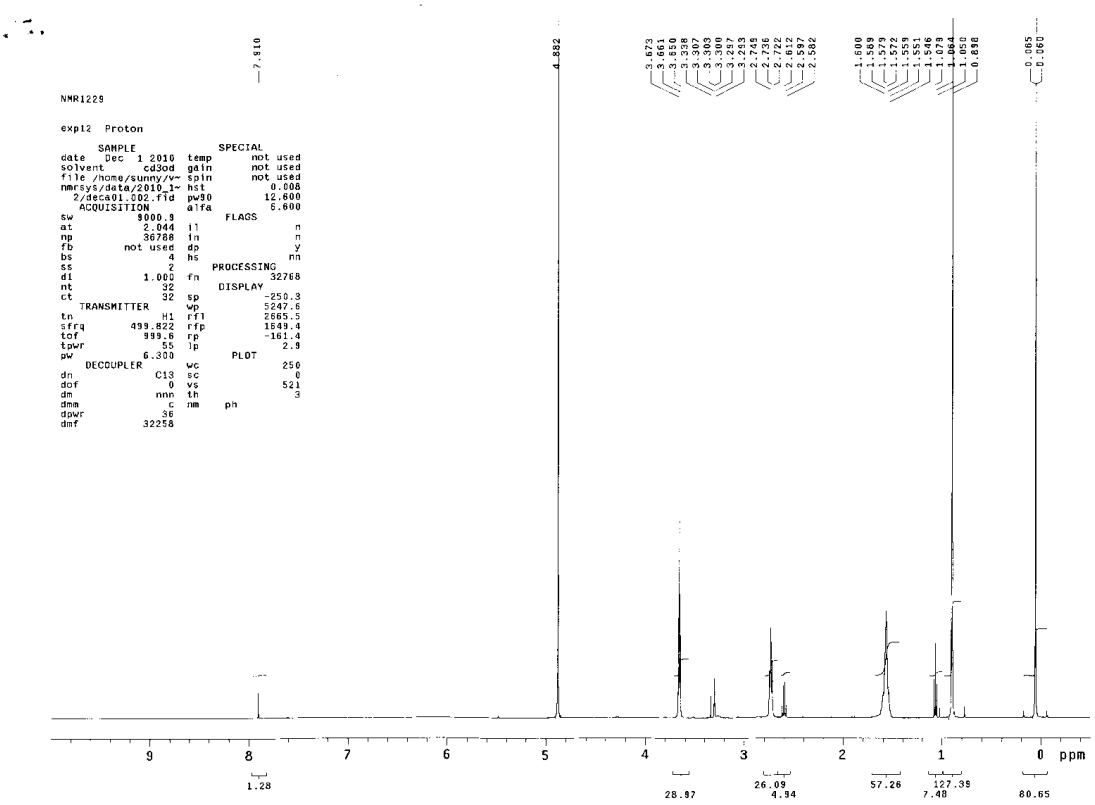

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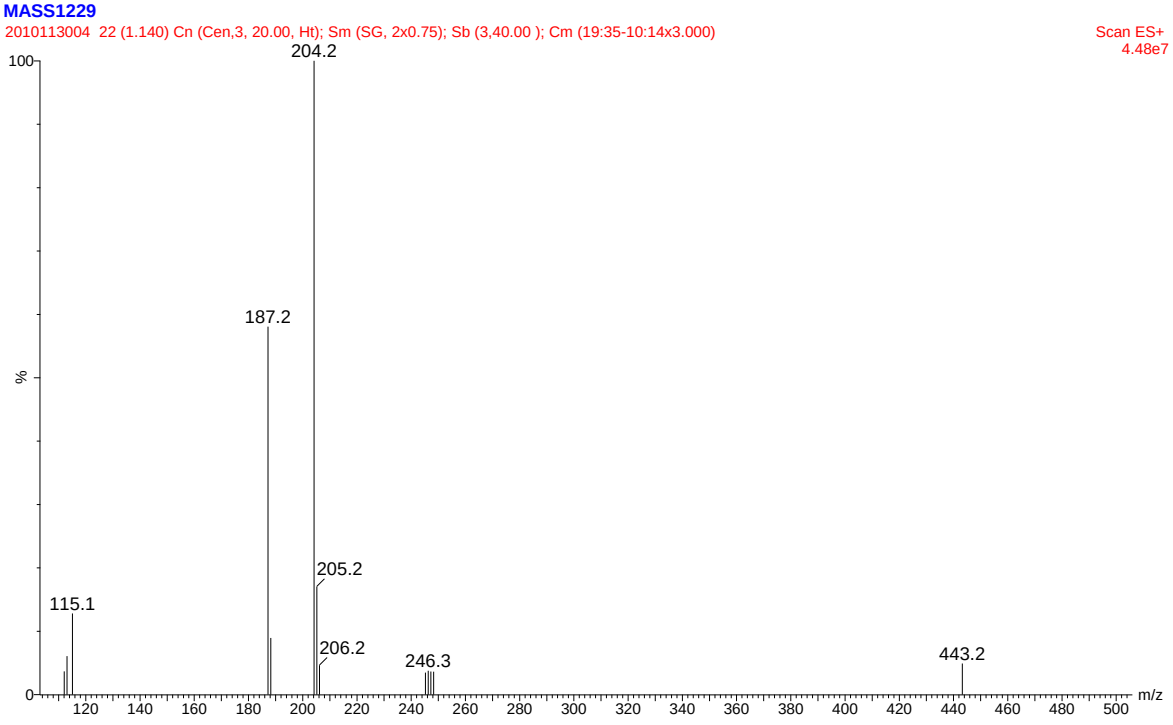

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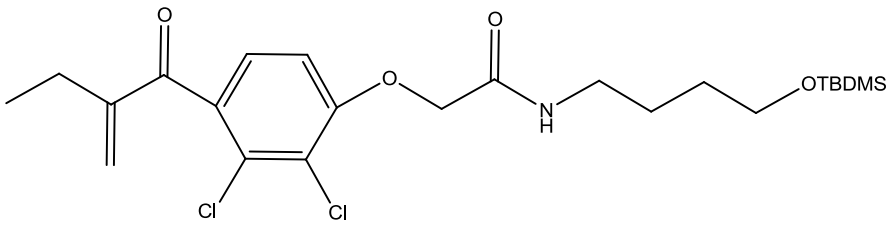
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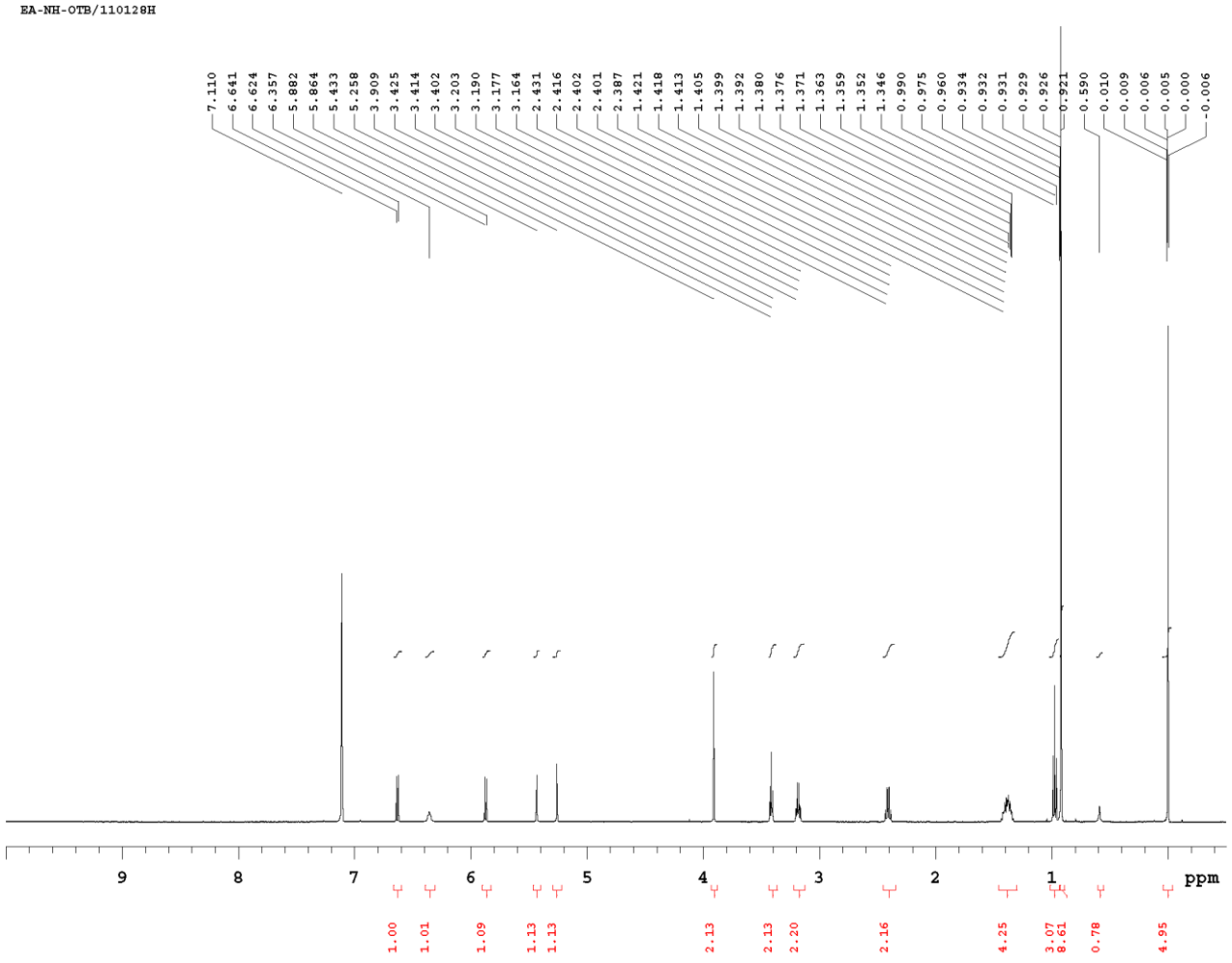
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2.402
2.401
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1.418
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7
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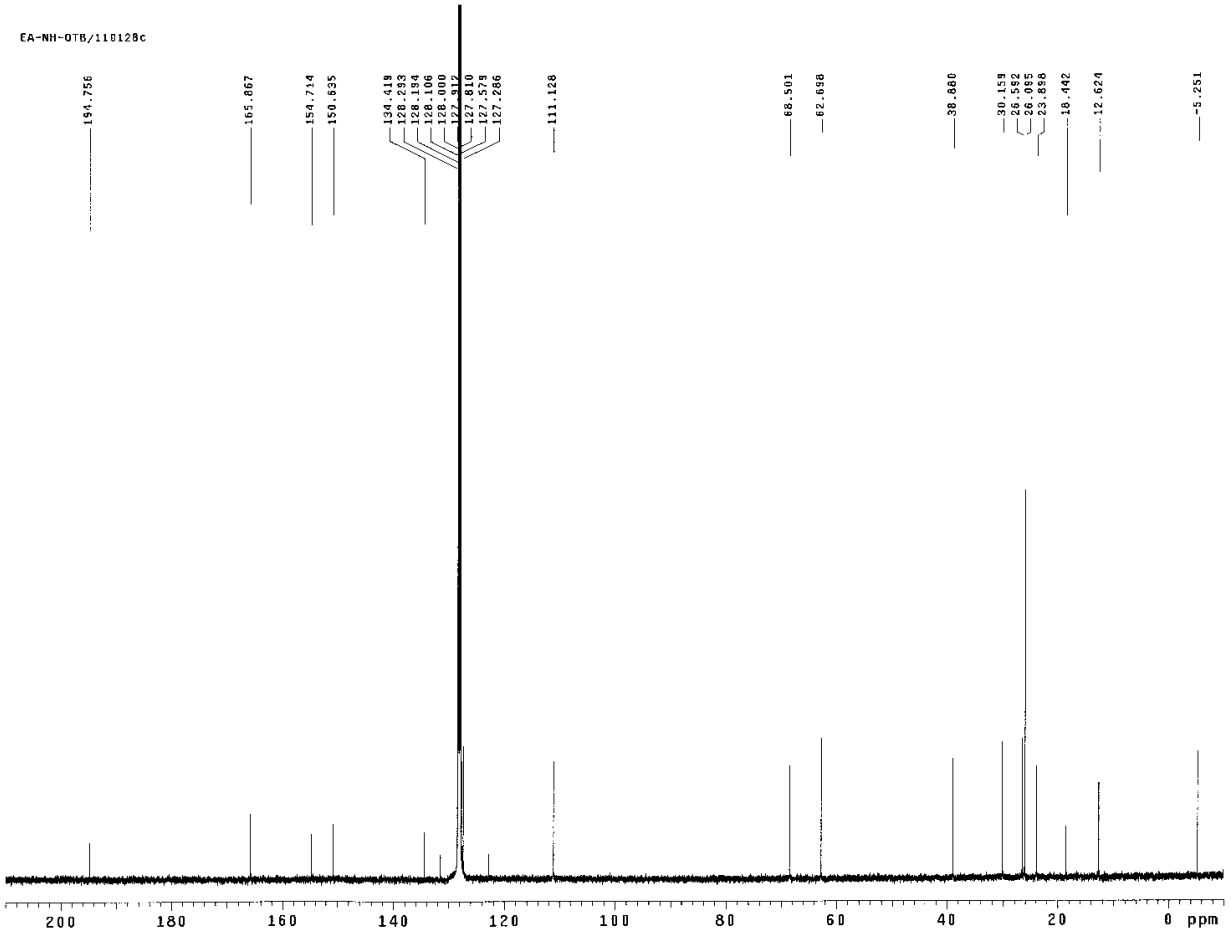
4.95

^{1}H-NMR



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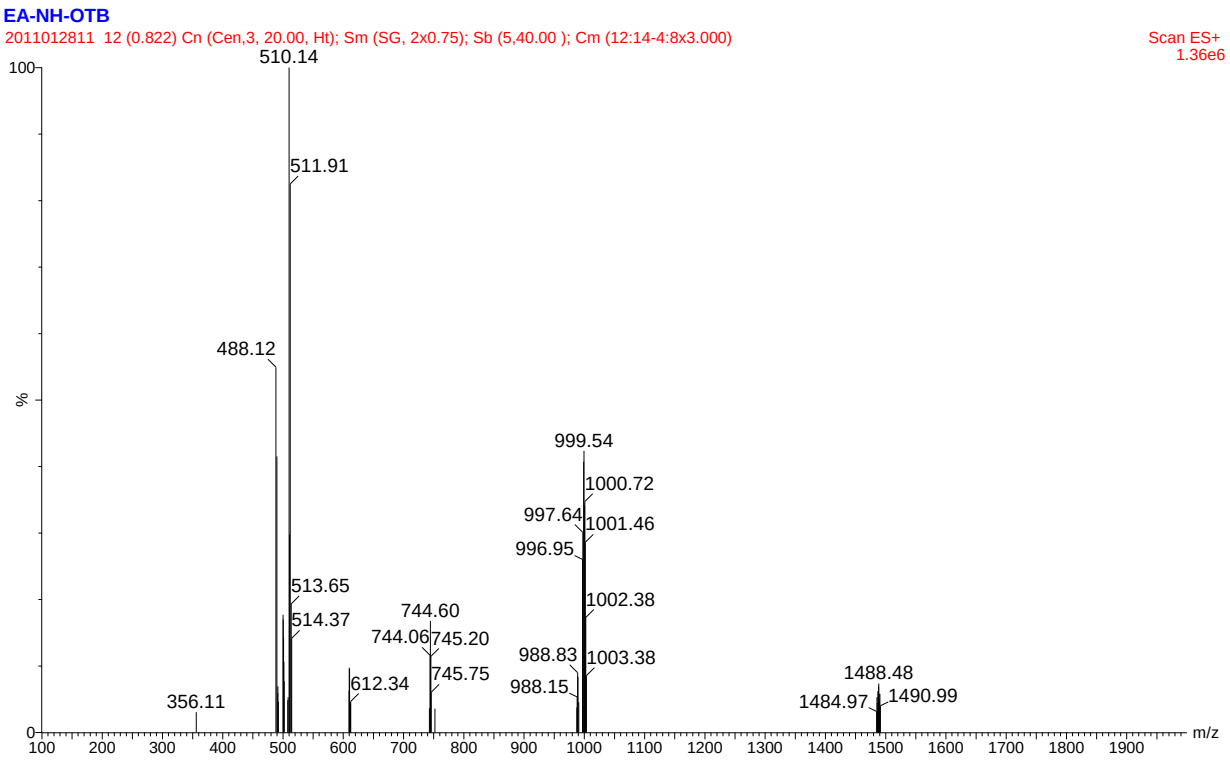

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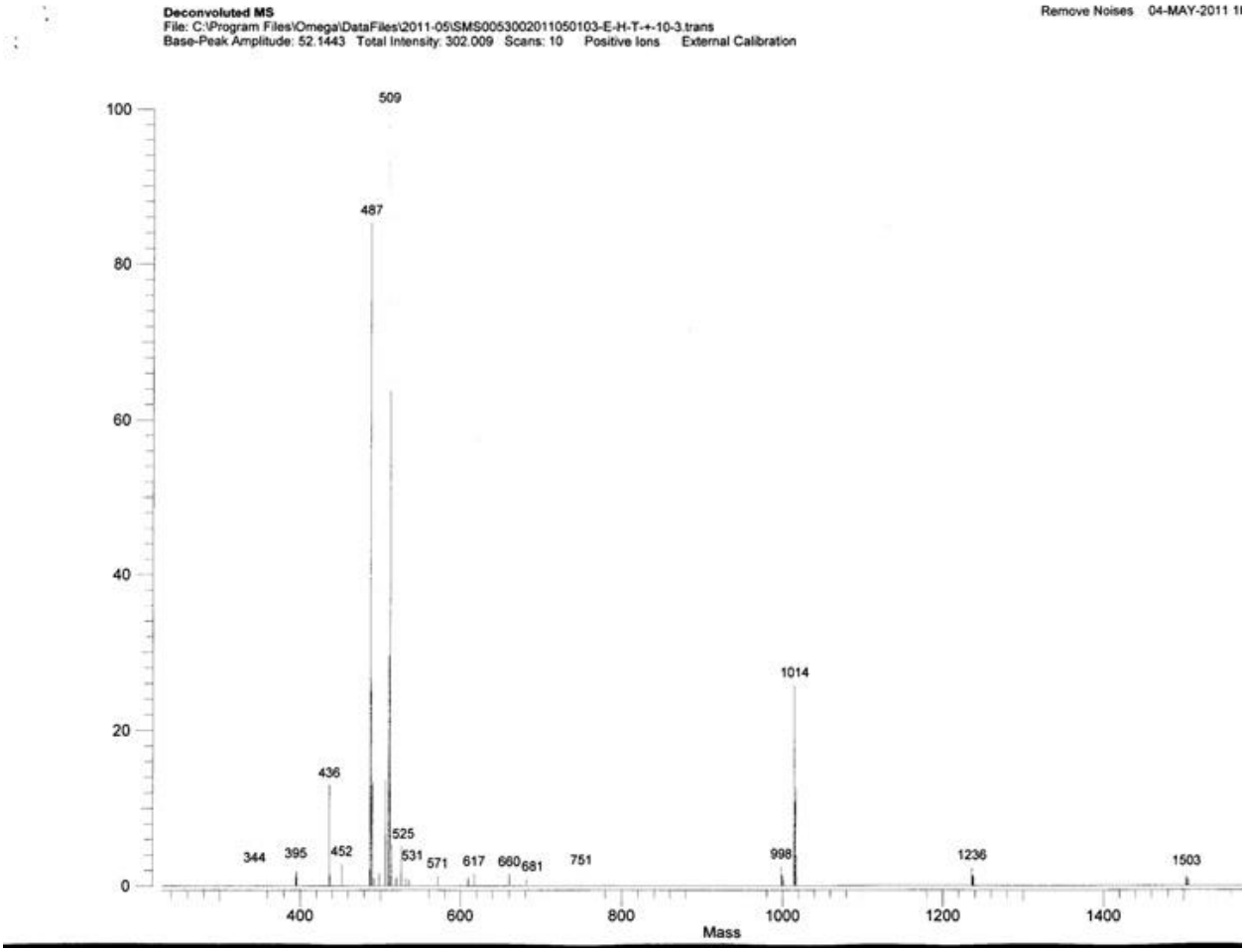
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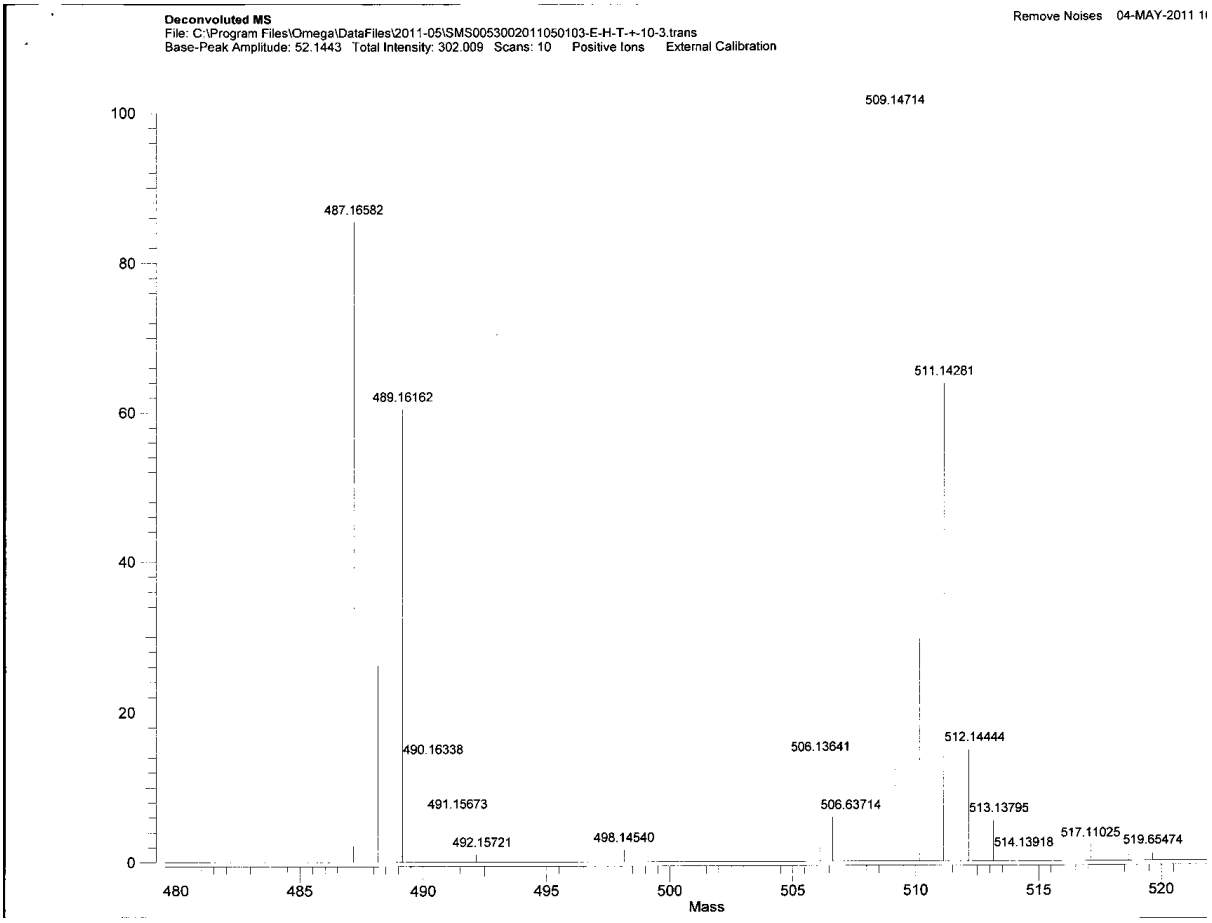
Mass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Remove Noises 04-MAY-2011 1
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490.16338
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Mass

Average Mass List - Varian ESI FTMS with Omega v9.1.20

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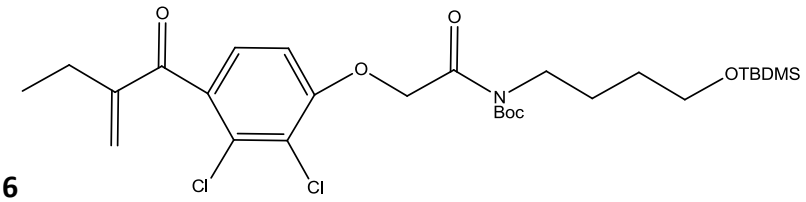
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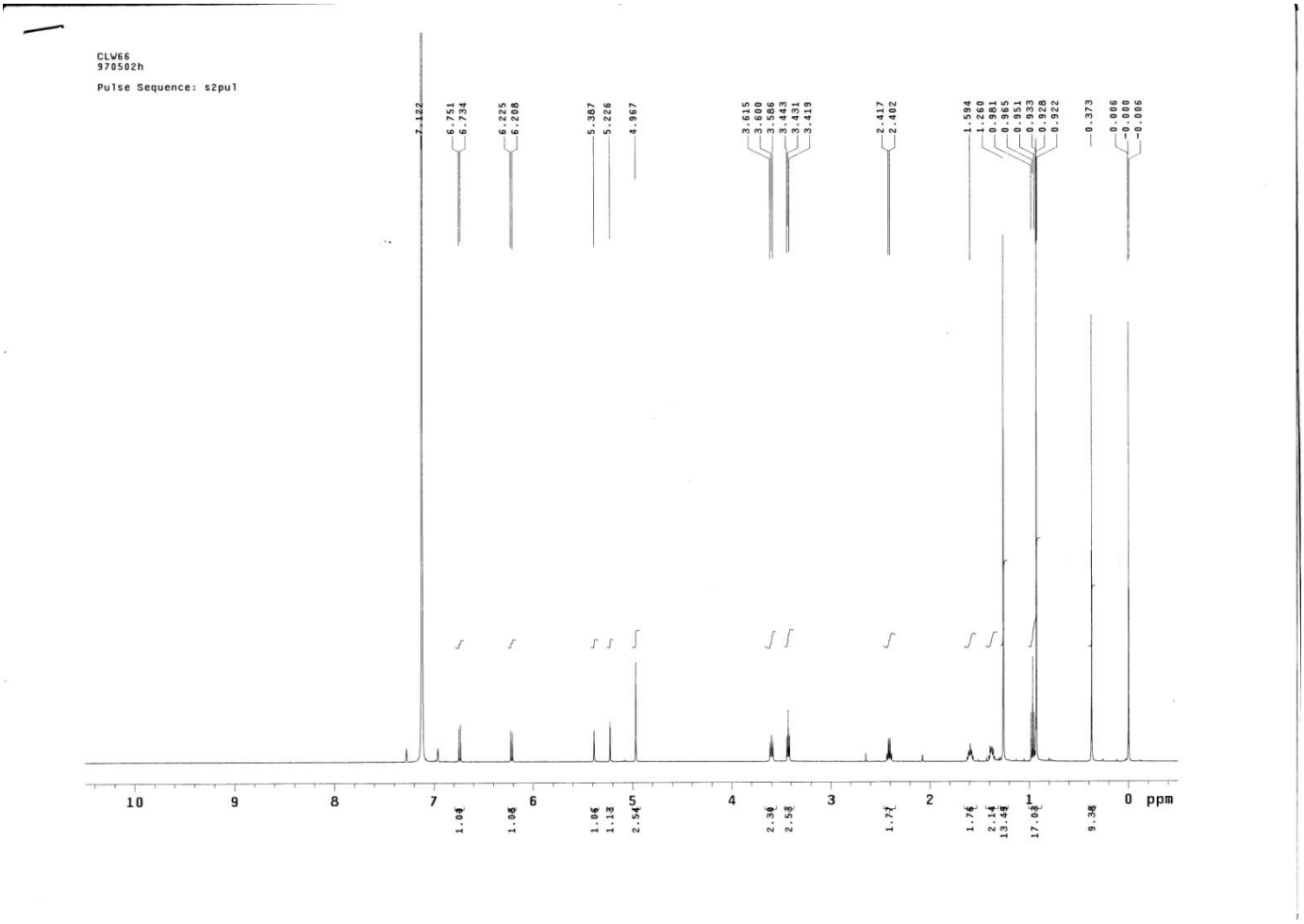
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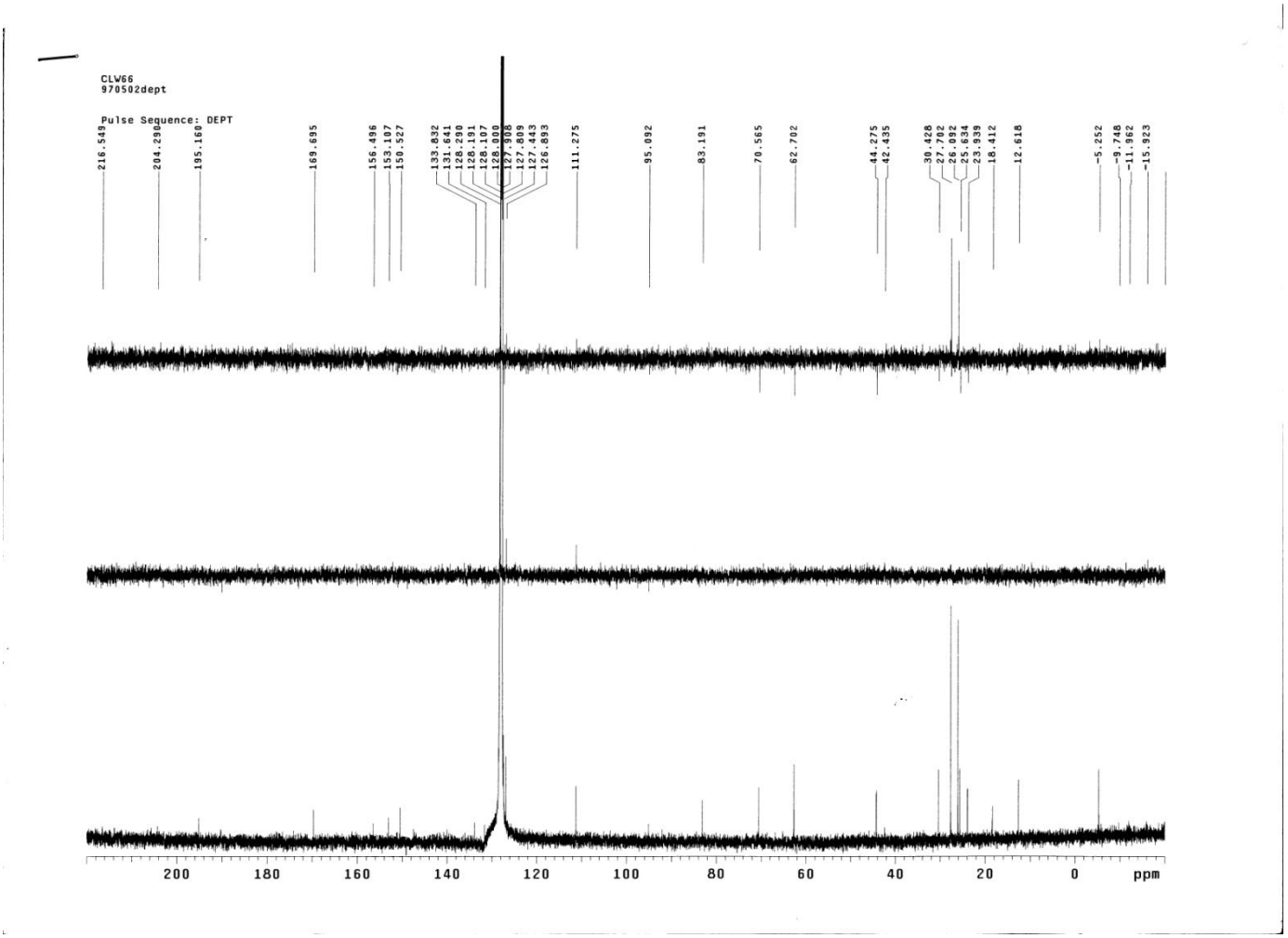
^{1}H-NMR



^{13}C-NMR

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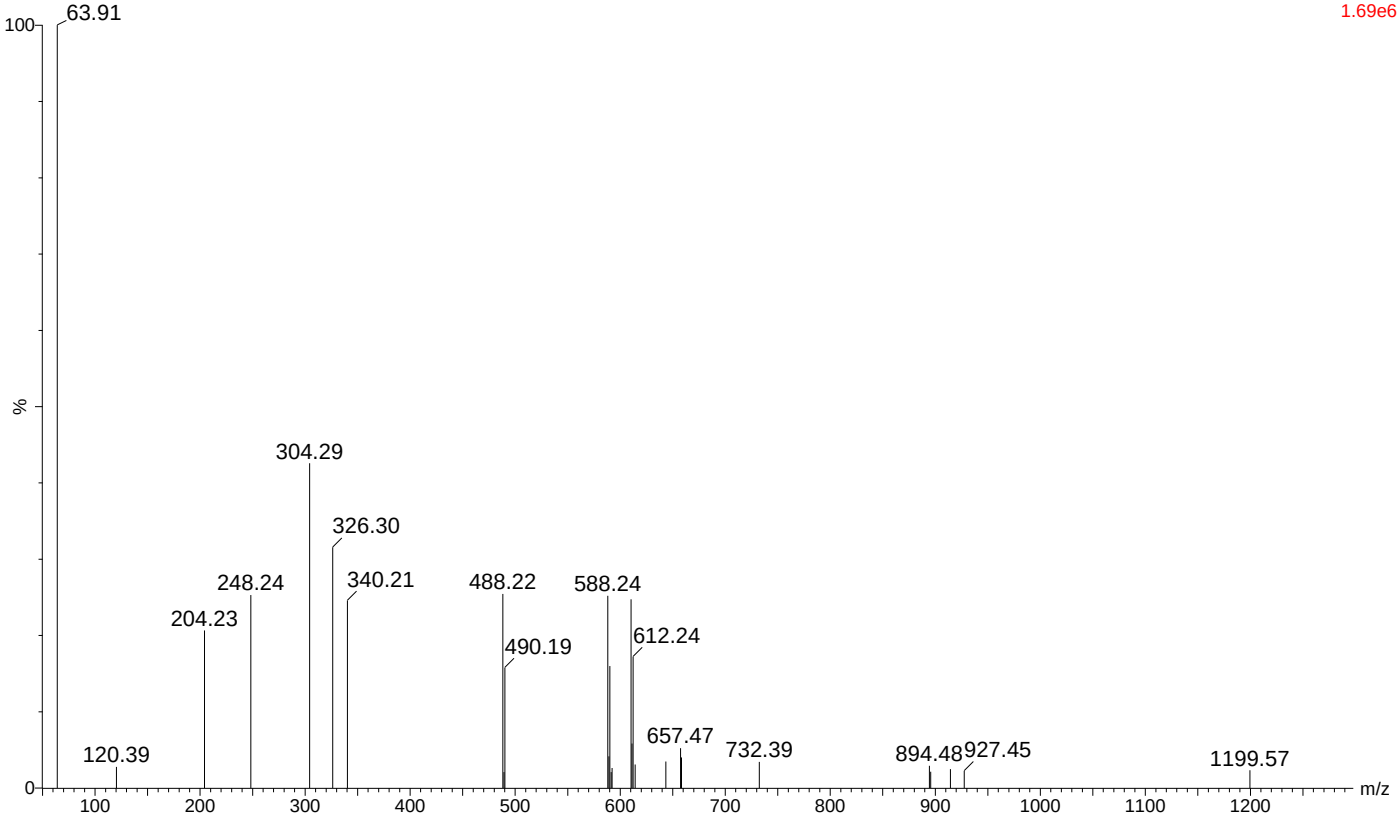

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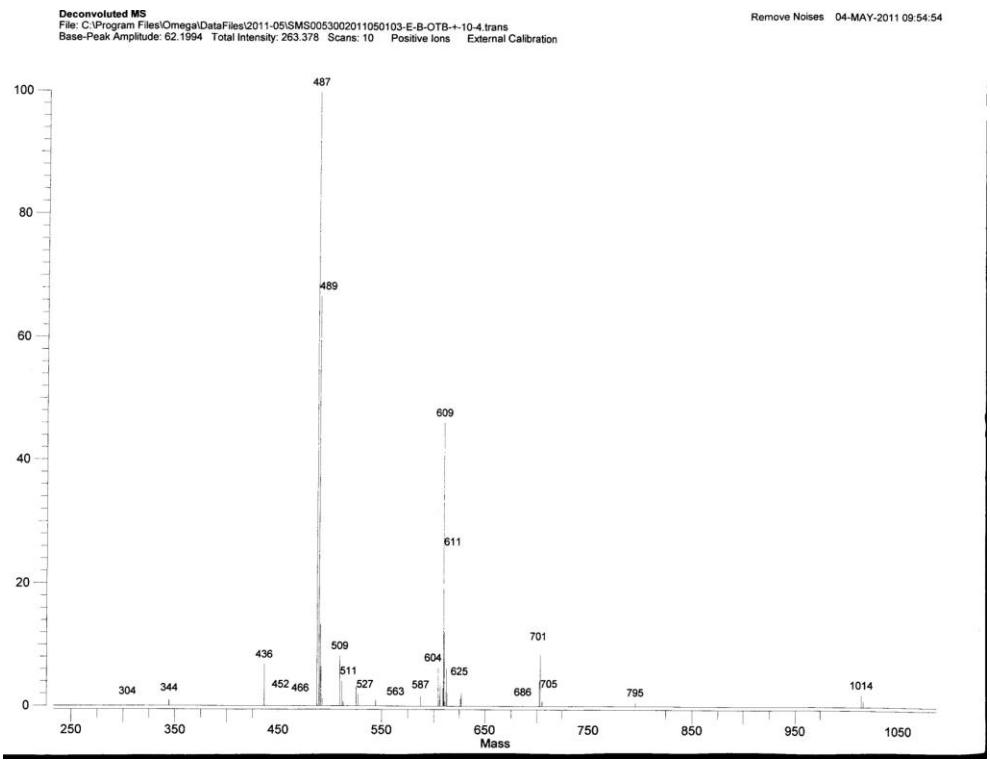

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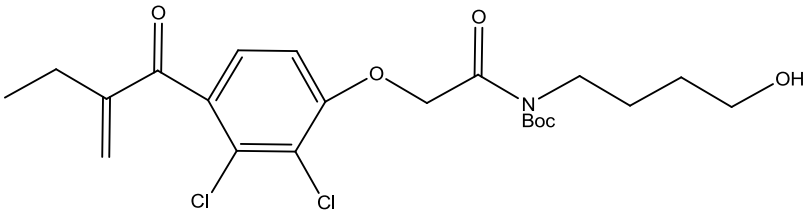
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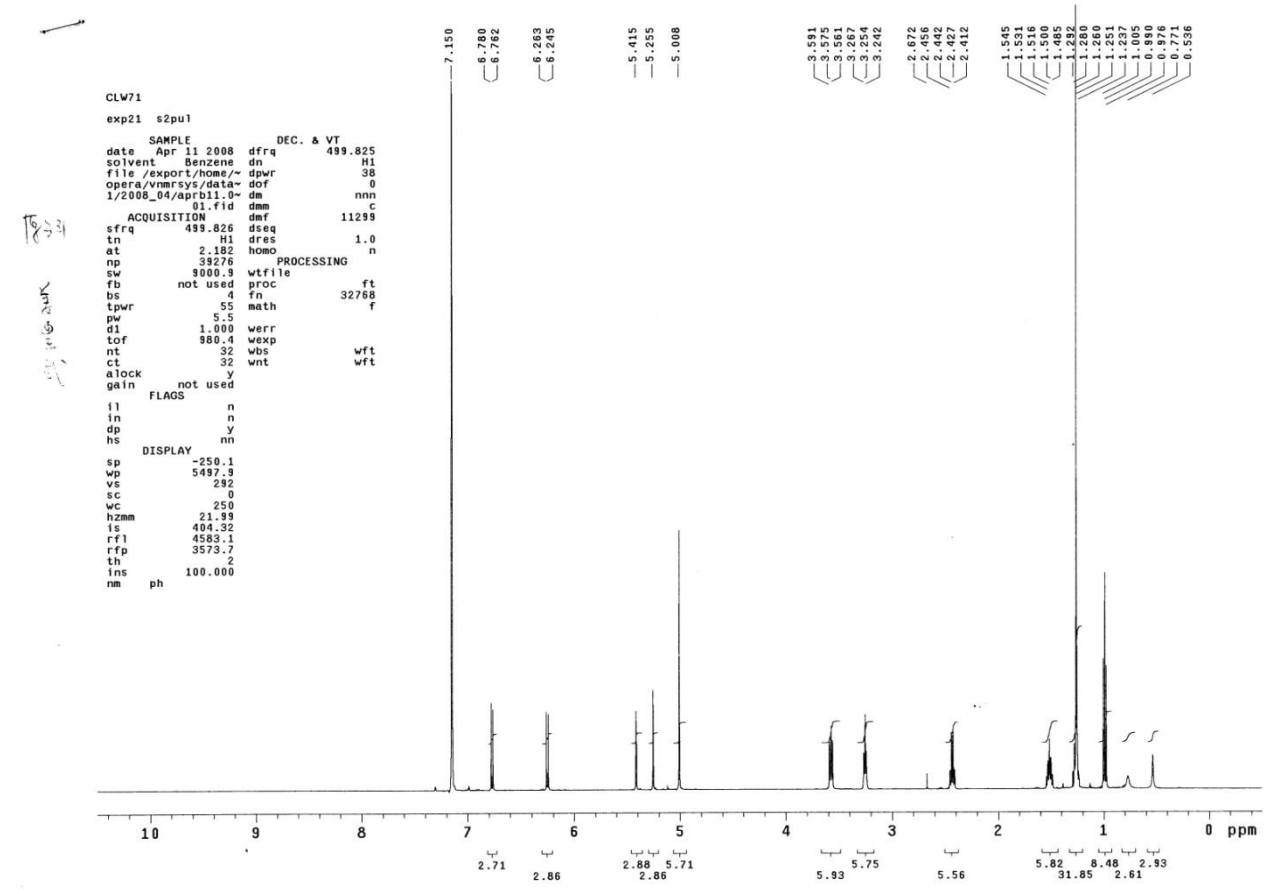
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9
8
7
6
5
4
3
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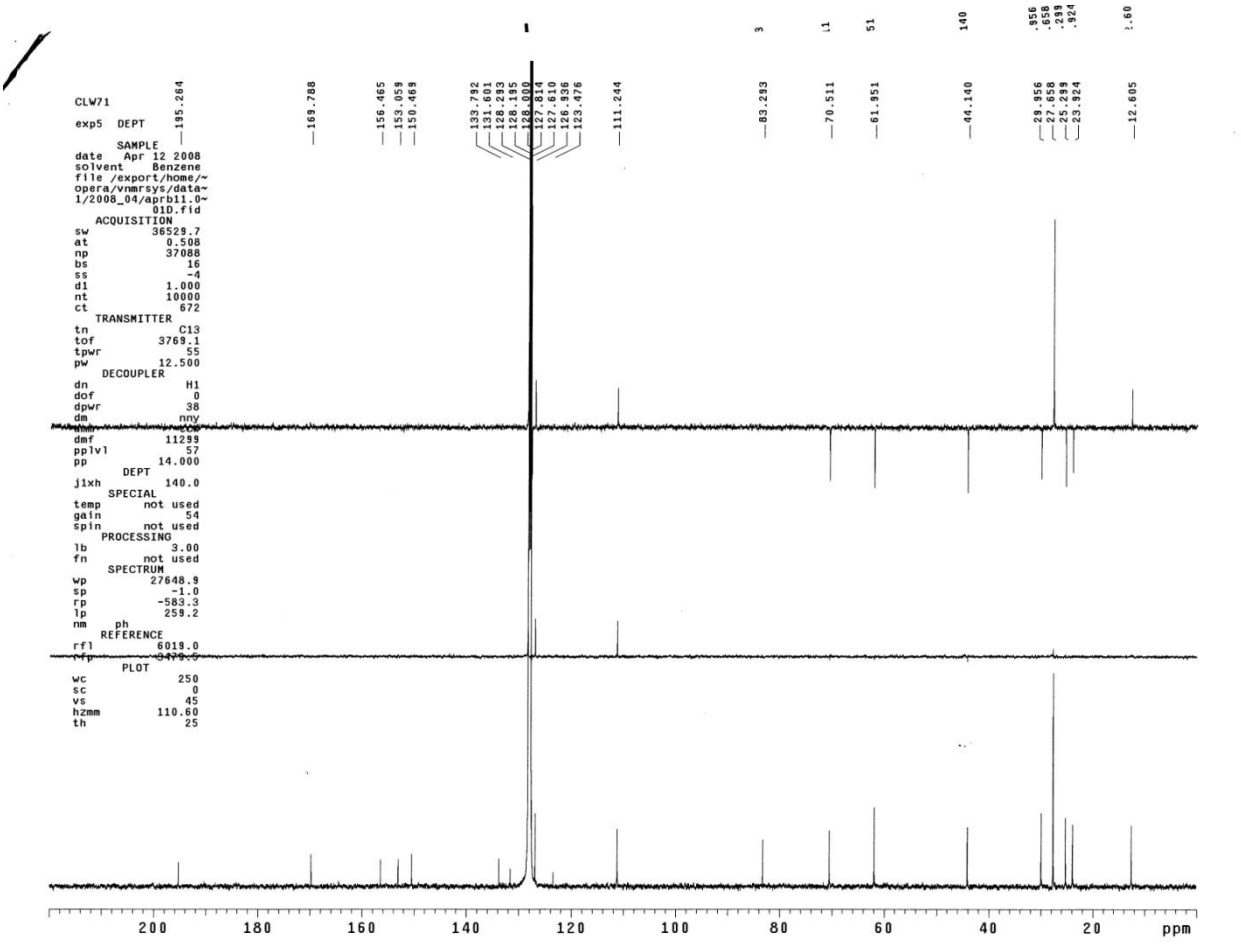
^{1}H-NMR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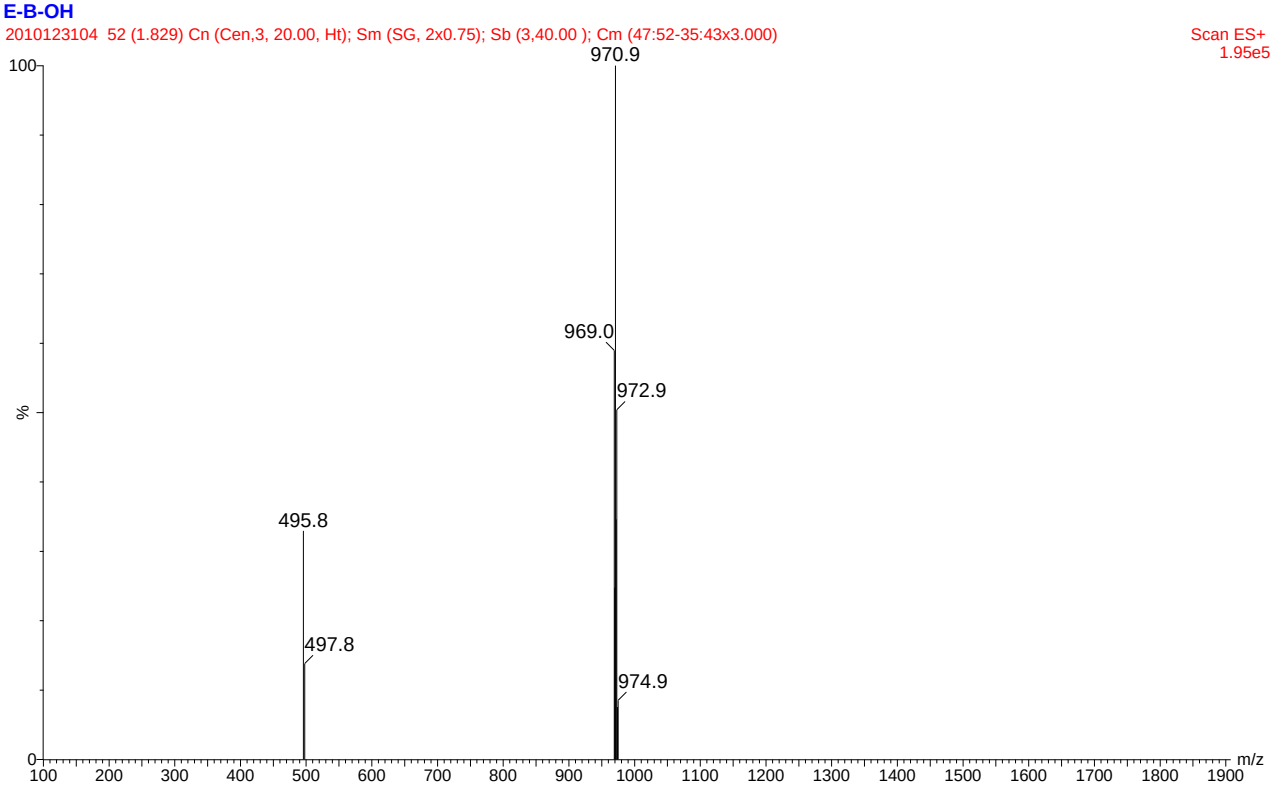
wp 27648.9
sp -1.0
rp -583.3
lp 259.2
nm ph
REFERENCE
rfl 6019.0
PLOT
wc 250
sc 0
vs 45
hzmm 110.60
th 25
195.264
169.788
156.465
153.059
150.469
133.792
131.601
128.293
128.195
128.000
127.814
127.610
126.856
123.476
111.244
83.293
70.511
61.951
44.140
29.956
27.658
25.299
23.924
12.605
200
180
160
140
120
100
80
60
40
20
ppm

^{13}C-NMR

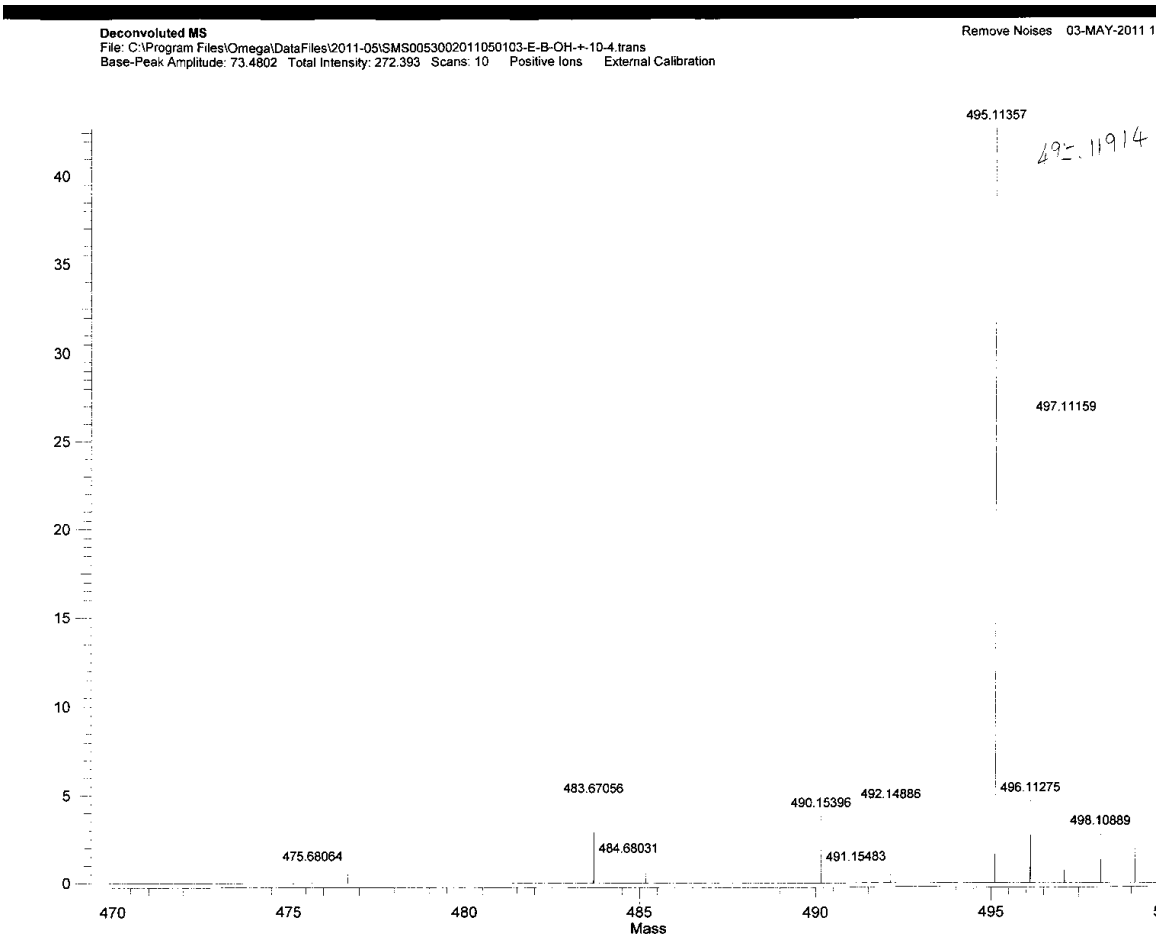

E-B-OH
2010123104 52 (1.829) Cn (Cen,3, 20.00, Ht); Sm (SG, 2x0.75); Sb (3,40.00); Cm (47:52-35:43x3.000)
Scan ES+
1.95e5
100
%
0
970.9
969.0
972.9
495.8
497.8
974.9
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
m/z

E-B-OH

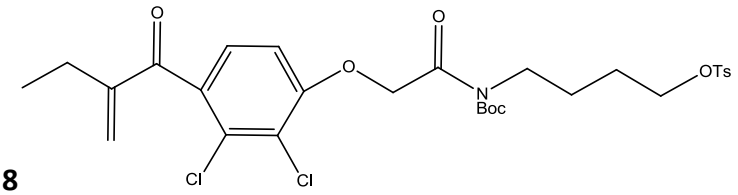
2010123104 52 (1.829) Cn (Cen,3, 20.00, Ht); Sm (SG, 2x0.75); Sb (3,40.00); Cm (47:52-35:43x3.000) Scan ES+

No	Mass	Inten	%BPI	%TIC	No	Mass	Inten	%BPI	%TIC	No	Mass	Inten	%BPI	%TIC	No	Mass	Inten	%BPI	%TIC	No
1:	495.8	6.43e4	32.93	9.75																
2:	497.8	2.69e4	13.79	4.08																
3:	969.0	1.15e5	58.93	17.45																
4:	969.9	4.83e4	24.74	7.32																
5:	970.9	1.95e5	100.00	29.61																
6:	971.9	6.75e4	34.58	10.24																
7:	972.9	9.84e4	50.36	14.91																
8:	974.0	1.47e4	7.54	2.23																
9:	974.9	1.66e4	8.49	2.51																

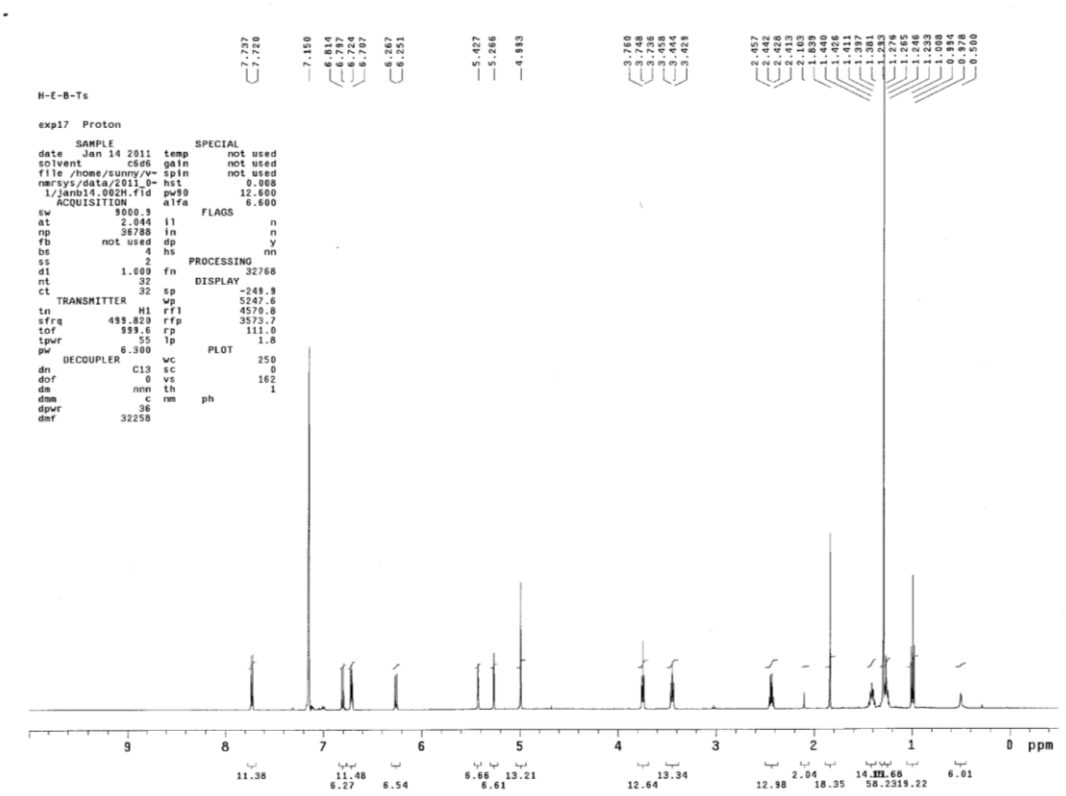
ESI-MS



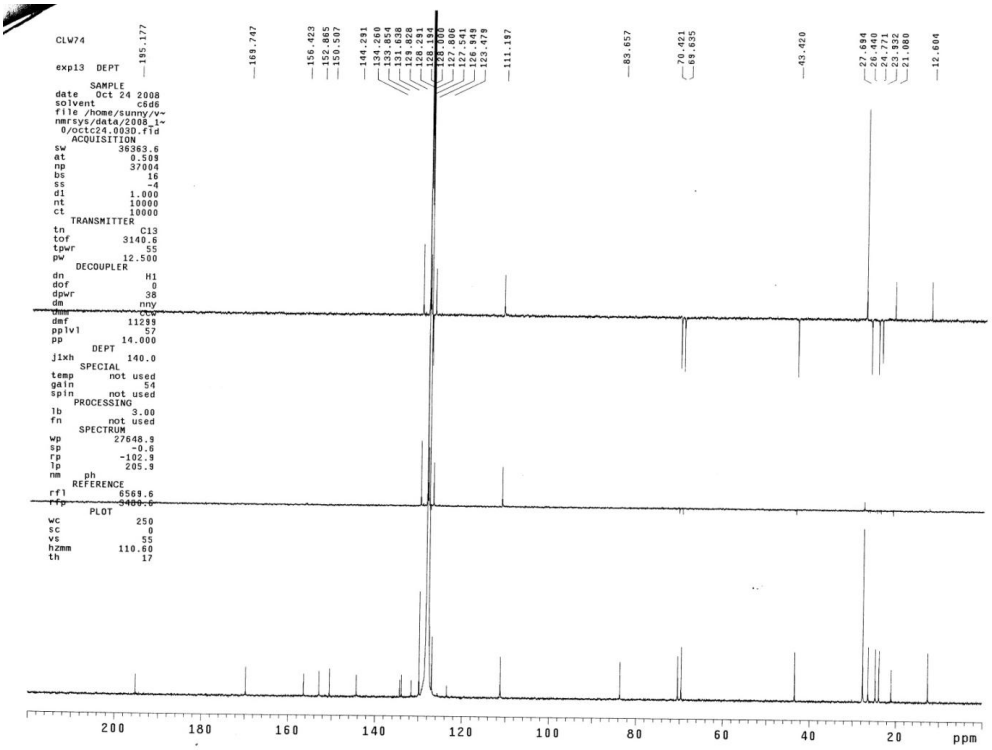
ESI-HRMS


O
O
O
N
Boc
OTs
Cl
Cl

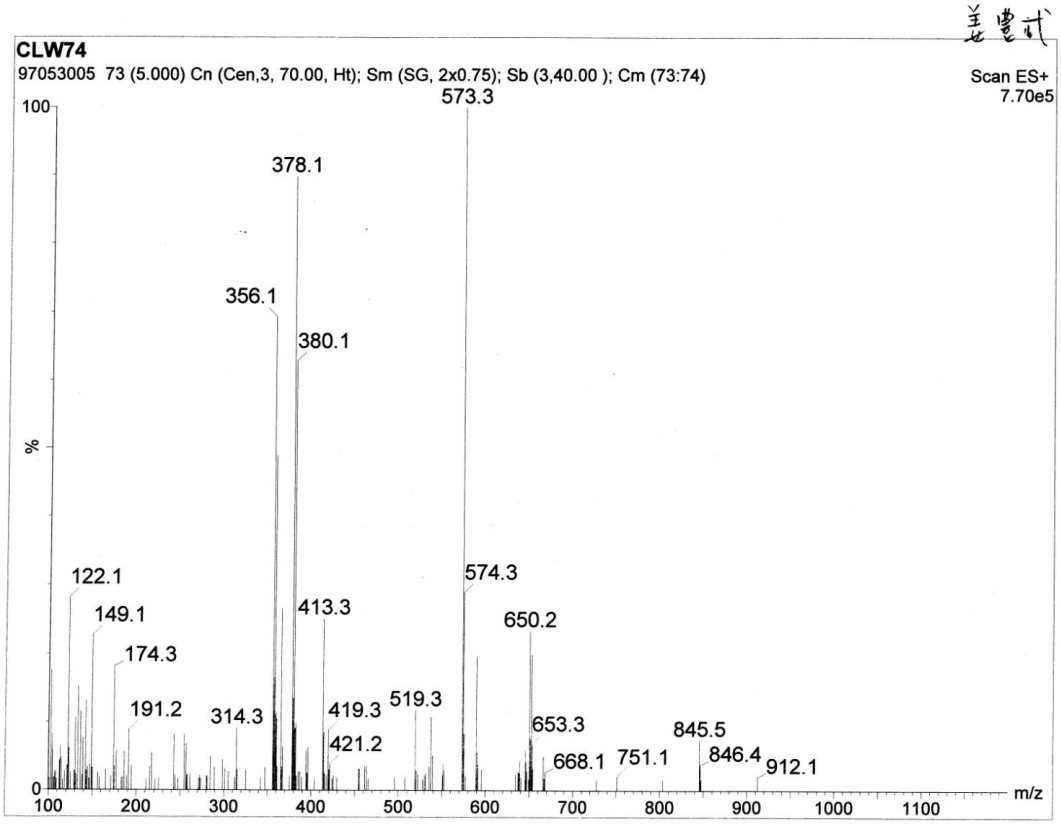
8


H-E-B-Ts
exp17 Proton
SAMPLE
date Jan 14 2011
solvent c6d6
file /home/sunny/v~
nmrsys/data/2011_0~
1/janb14.002H.fid
ACQUISITION
sw 9000.9
at 2.044
np 36788
fb not used
bs 4
ss 2
d1 1.000
nt 32
ct 32
TRANSMITTER
tn H1
sfrq 499.820
tof 999.6
tpwr 55
pw 6.300
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dpwr 36
dmf 32258
SPECIAL
temp not used
gain not used
spin not used
hst 0.008
pw90 12.600
alfa 6.600
FLAGS
il n
in n
dp y
hs nn
PROCESSING
fn 32768
DISPLAY
sp -248.9
wp 5247.6
rfl 4570.8
rfp 3573.7
rp 111.0
lp 1.8
PLOT
wc 250
sc 0
vs 162
th 1
nm ph
7.737
7.720
7.150
6.814
6.797
6.724
6.707
6.267
6.251
5.427
5.266
4.893
3.760
3.748
3.736
3.458
3.444
3.429
2.457
2.442
2.428
2.413
2.163
1.639
1.446
1.426
1.411
1.397
1.381
1.253
1.246
1.265
1.246
1.233
1.008
0.854
0.838
0.500
9
8
7
6
5
4
3
2
1
0 ppm
11.38
11.48
6.27
6.54
6.66
6.61
13.21
12.64
13.34
12.98
2.04
18.35
14.68
58.23
19.22
6.01

^{1}H-NMR



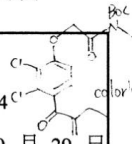
^{13}C-NMR



ESI-MS

國立交通大學應用化學系

元素分析儀 Heraeus CHN-O Rapid 服務報告書



使用者姓名：姜豐武　　中心編號：971035

服務單位：清大原科 俞鐘山實驗室　樣品名稱或代號：CLW74

收件日期： 97 年 10 月 25 日完成日期： 97 年 10 月 29 日

分析結果：

實驗值：	N%	C%	H%
1.	2.27	55.55	5.80
2.	2.18	55.56	5.75
3.			
4.			
推測值.：	2.23	55.41	5.61

本日所使用之 Standard：A

(A)Acetanilide (B)Atropin (C)N-Anilin

	N%	C%	H%
理論值：	10.36	71.09	6.71
測出值：	10.24	70.99	6.69

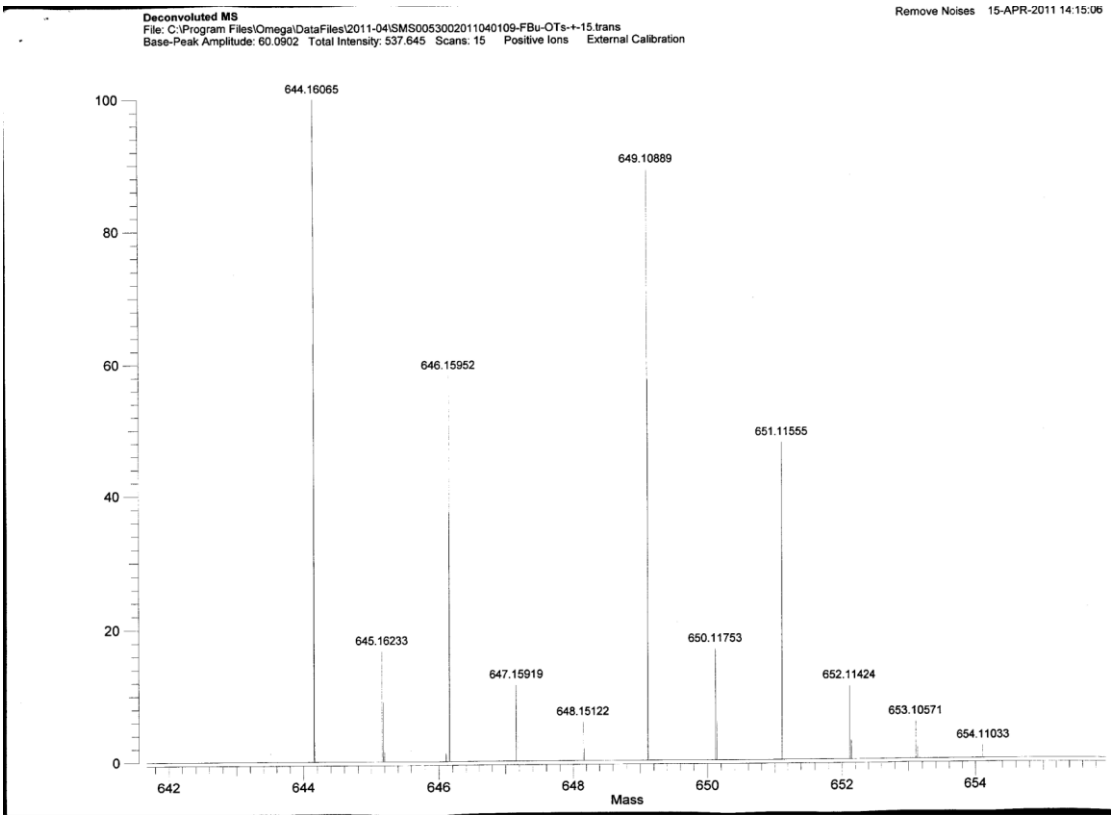
建議：

費用核算：NCH：800 元

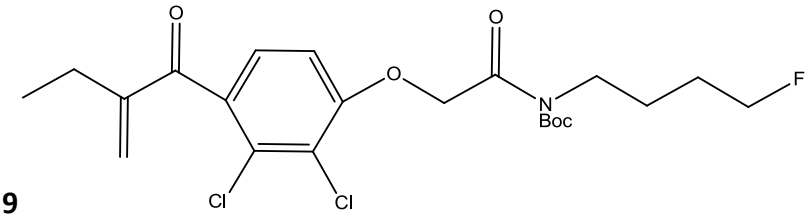
報告日期： 97 年 11 月 3 日

儀器負責人簽章：　　技術員簽章：

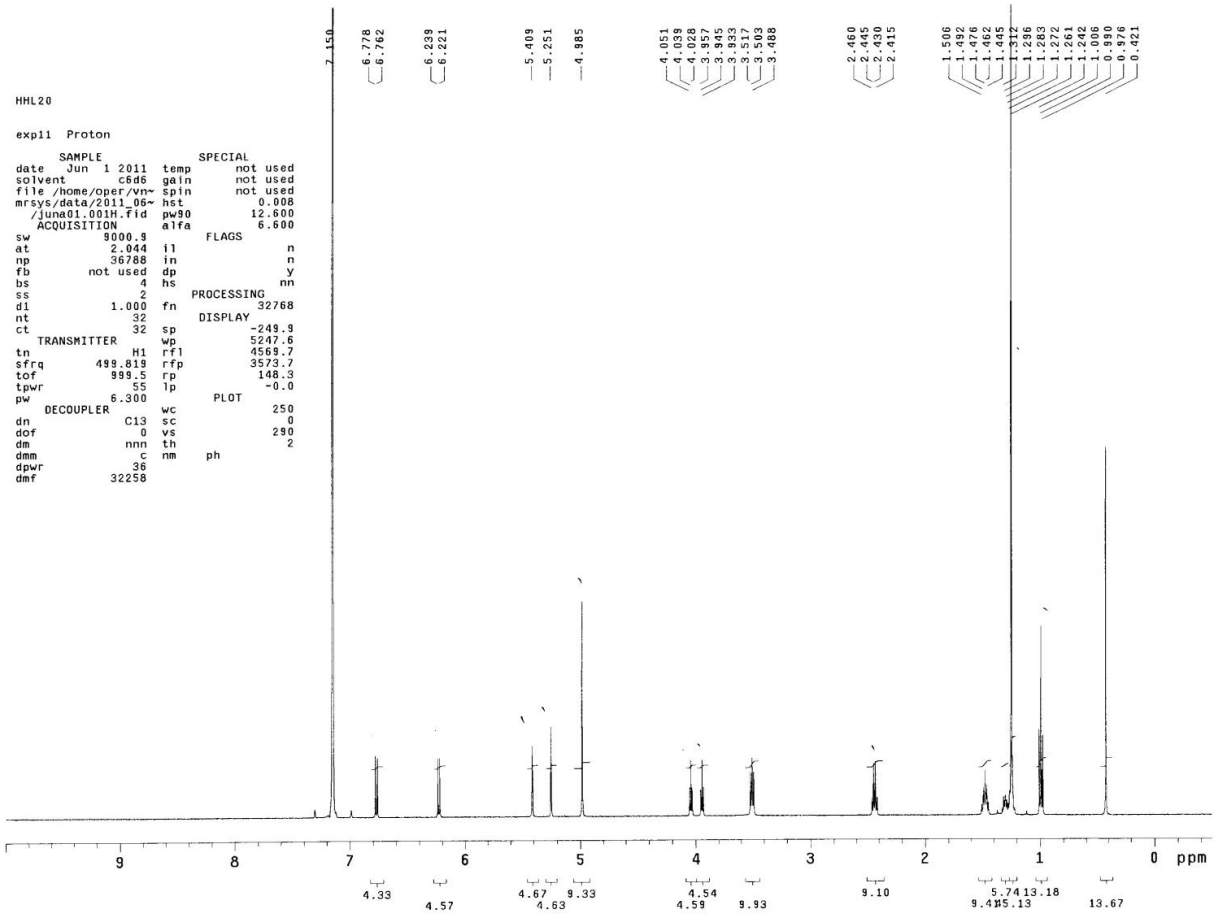
Elemental analysis


Deconvoluted MS
File: C:\Program Files\Omega\DataFiles\2011-04\SMS005300201104O109-FBu-OTs-+-15.trans
Base-Peak Amplitude: 60.0902 Total Intensity: 537.645 Scans: 15 Positive Ions External Calibration
Remove Noises 15-APR-2011 14:15:06
644.16065
649.10889
646.15952
651.11555
645.16233
650.11753
647.15919
652.11424
648.15122
653.10571
654.11033
100
80
60
40
20
0
642
644
646
648
650
652
654
Mass

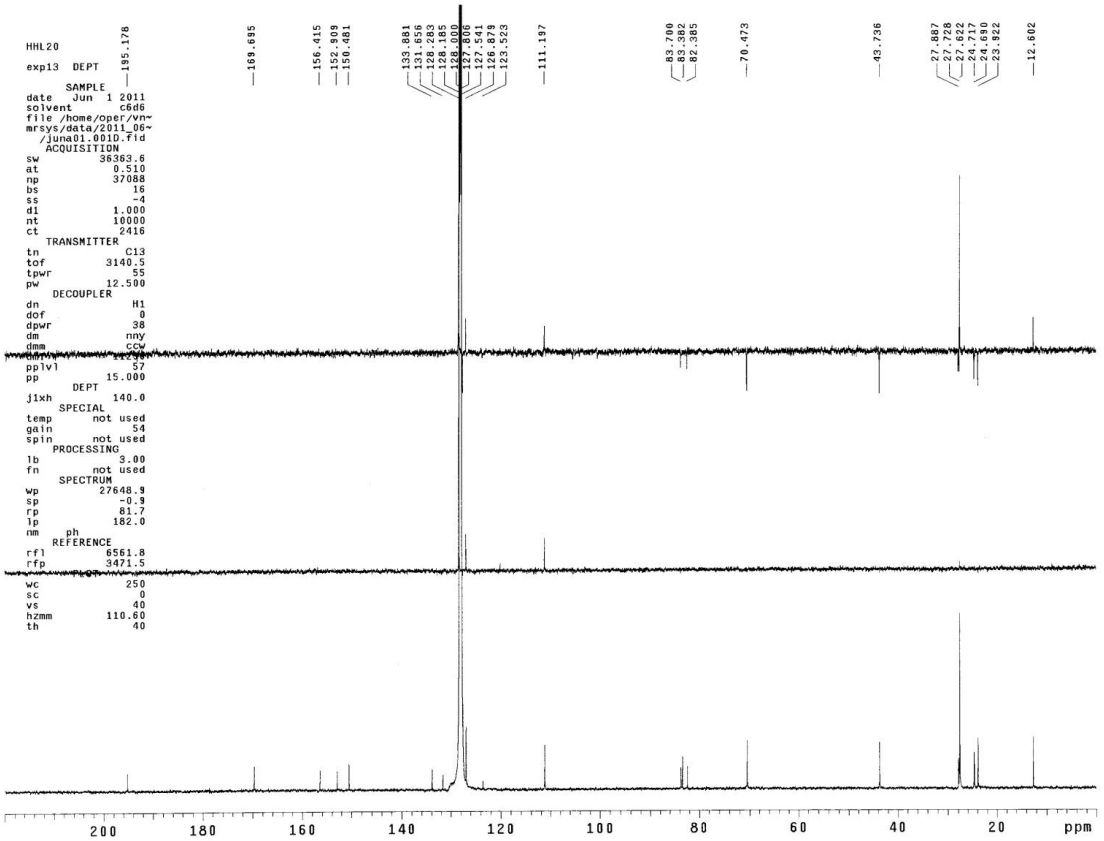
ESI-HRMS


O
O
O
N
Boc
F
Cl
Cl

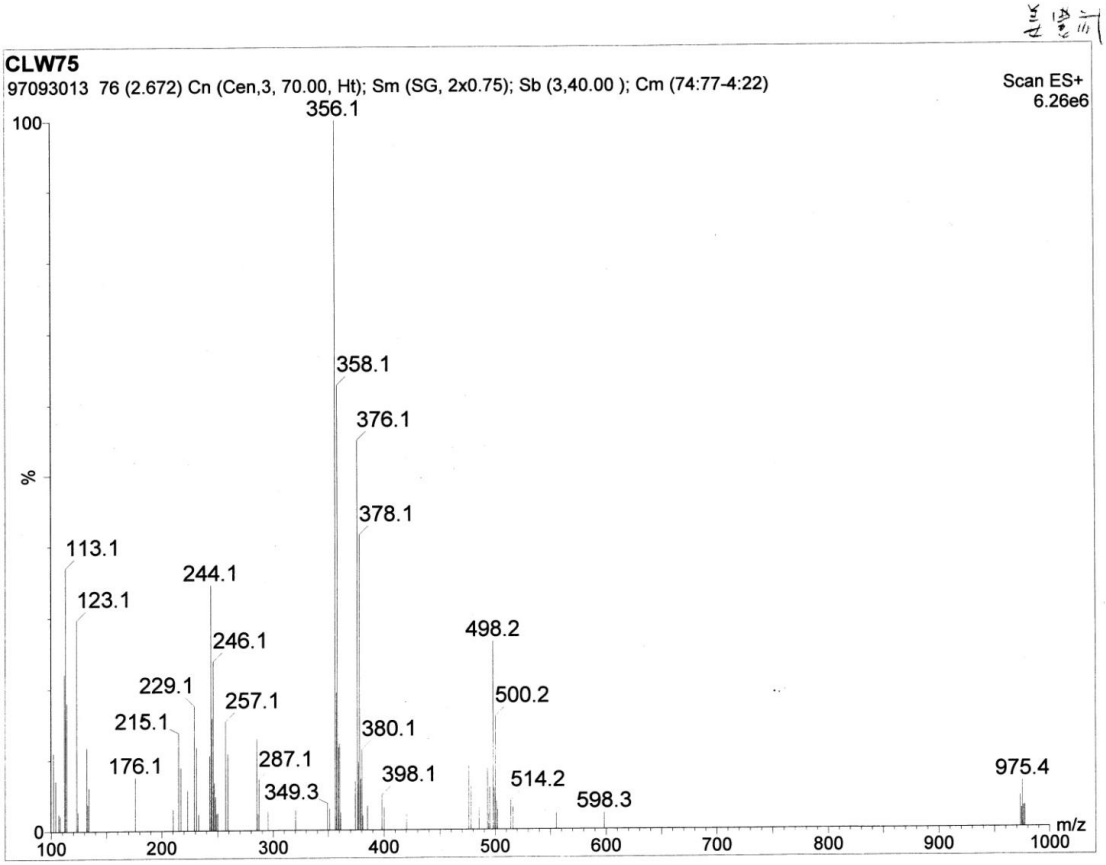
9


HHL20
exp11 Proton
SAMPLE
date Jun 1 2011
solvent c6d6
file /home/oper/vn~
mrsys/data/2011_06~
/juna01.001H.fid
ACQUISITION
sw 9000.9
at 2.044
np 36788
fb not used
bs 4
ss 2
d1 1.000
nt 32
ct 32
TRANSMITTER
tn H1
sfrq 499.819
tof 999.5
tpwr 55
pw 6.300
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dpwr 36
dmf 32258
SPECIAL
temp not used
gain not used
spin not used
hst 0.008
pw90 12.600
alfa 6.600
FLAGS
il n
in n
dp y
hs nn
PROCESSING
fn 32768
DISPLAY
sp -249.9
wp 5247.6
rfl 4569.7
rfp 3573.7
rp 148.3
lp -0.0
PLOT
wc 250
sc 0
vs 290
th 2
nm ph
7.150
6.778
6.762
6.239
6.221
5.409
5.251
4.985
4.051
4.039
4.028
3.957
3.945
3.933
3.517
3.503
3.488
2.460
2.445
2.430
2.415
1.506
1.492
1.476
1.462
1.445
1.312
1.296
1.283
1.272
1.261
1.242
1.006
0.990
0.976
0.421
9
8
7
6
5
4
3
2
1
0 ppm
4.33
4.57
4.67
4.63
9.33
4.54
4.59
9.93
9.10
9.41
5.74
5.13
13.18
13.67

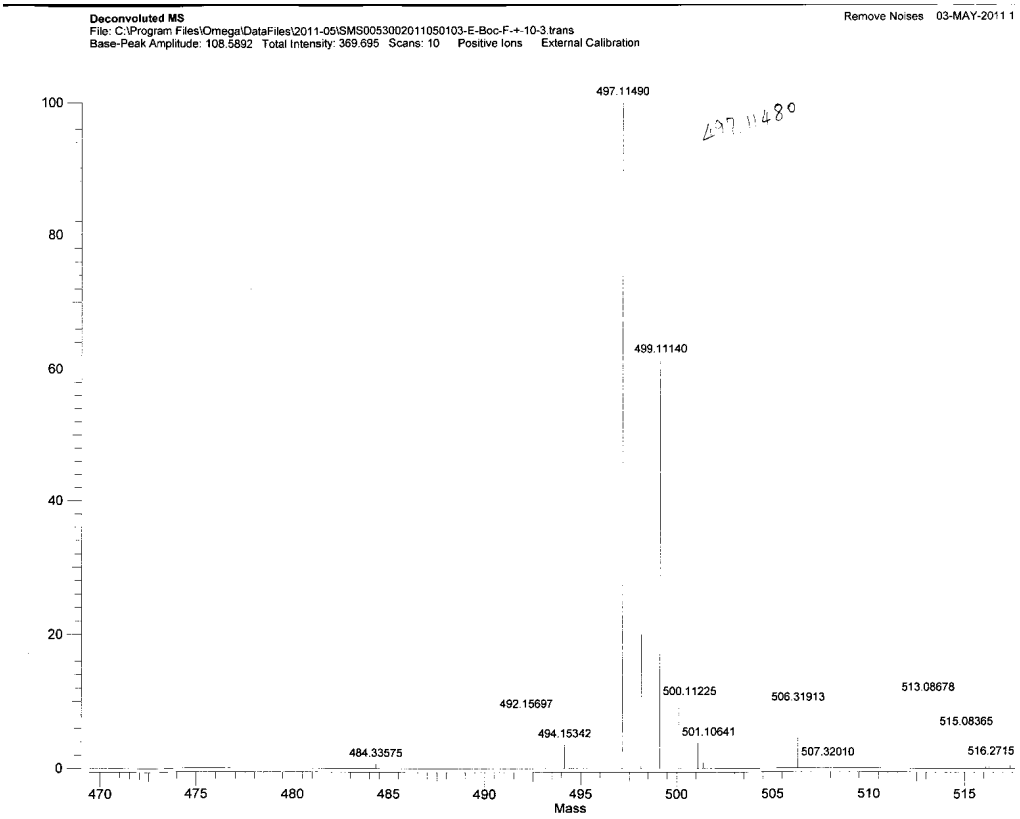
^{1}H-NMR



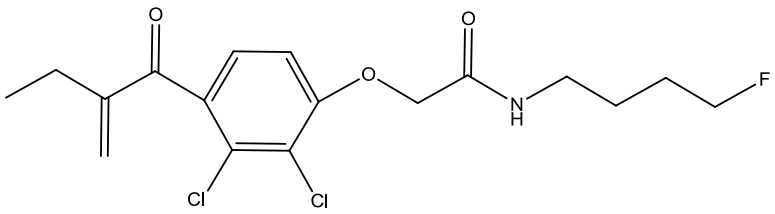
^{13}C-NMR



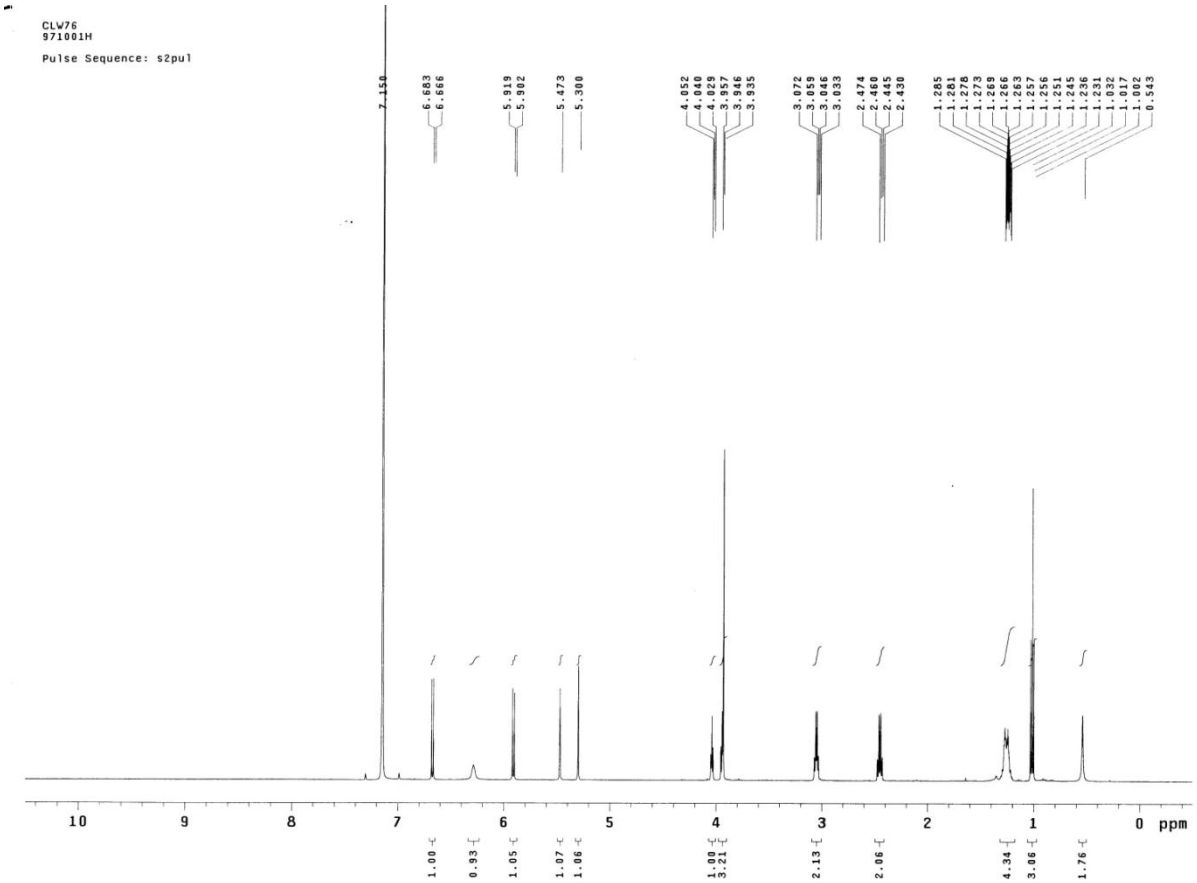
ESI-MS


Deconvoluted MS
File: C:\Program Files\Omega\DataFiles\2011-05\SMS00530020110501O3-E-Boc-F-+-10-3.trans
Base-Peak Amplitude: 108.5892 Total Intensity: 369.695 Scans: 10 Positive Ions External Calibration
Remove Noises 03-MAY-2011 1
497.11490
499.11140
500.11225
506.31913
513.08678
492.15697
494.15342
501.10641
515.08365
484.33575
507.32010
516.2715
100
80
60
40
20
0
470
475
480
485
490
495
500
505
510
515
Mass

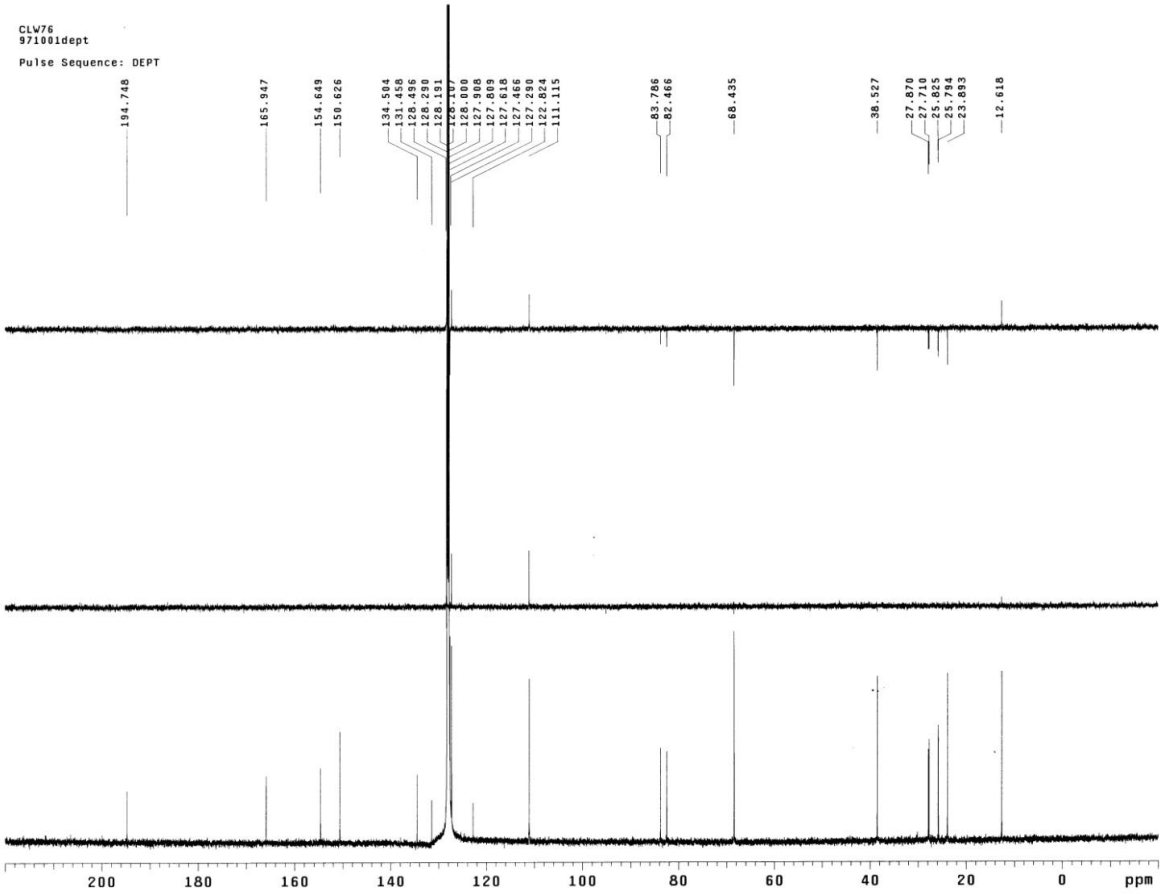
ESI-HRMS



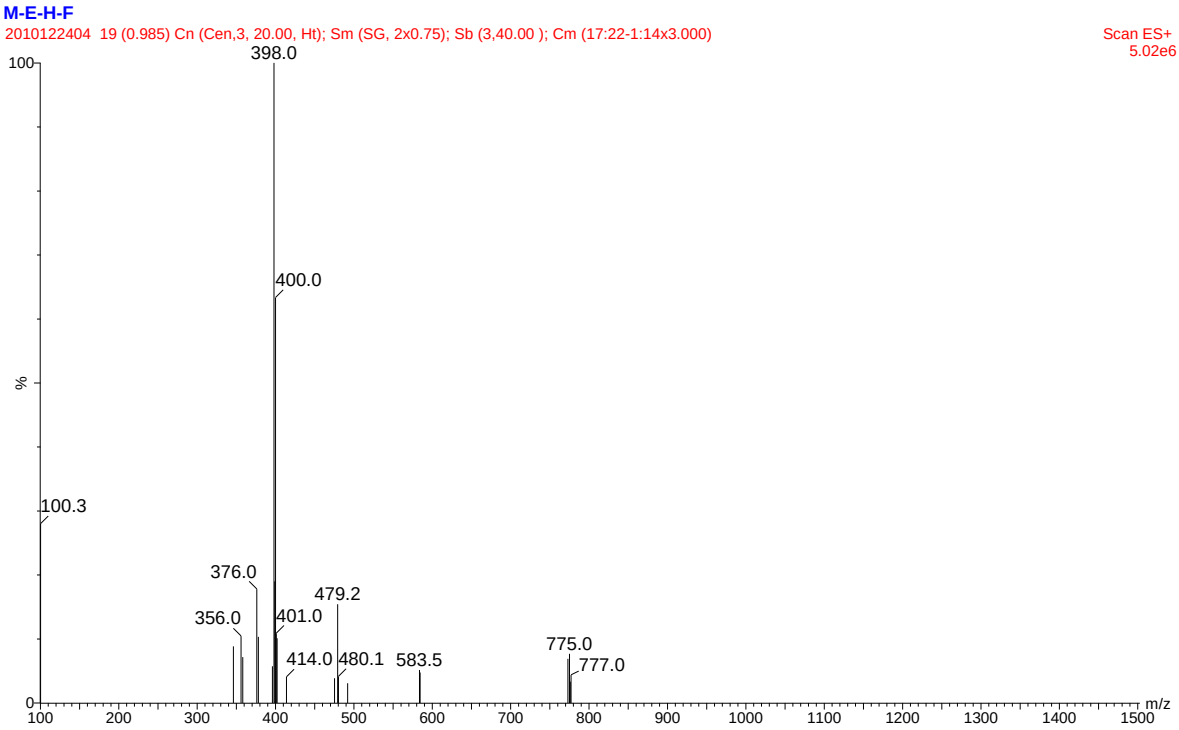
FBuEA 3



^{1}H-NMR



^{13}C-NMR



ESI-MS

國立交通大學應用化學系

元素分析儀 Heraeus CHN-O Rapid 服務報告書

使用者姓名：黃鶴聯　　中心編號：1000159

服務單位：清大原科 俞鐘山實驗室　樣品名稱或代號：FBuEA

收件日期： 100 年 1 月 17 日 完成日期： 100 年 1 月 20 日

分析結果：

實驗值：	N%	C%	H%
1.	3.95	53.95	5.57
2.	3.91	54.17	5.45
3.			
4.			

推測值：

本日所使用之 Standard：A

(A)Acetanilide (B)Atropin (C)N-Anilin

	N%	C%	H%
理論值：	10.36	71.09	6.71
測出值：	10.38	71.05	6.70

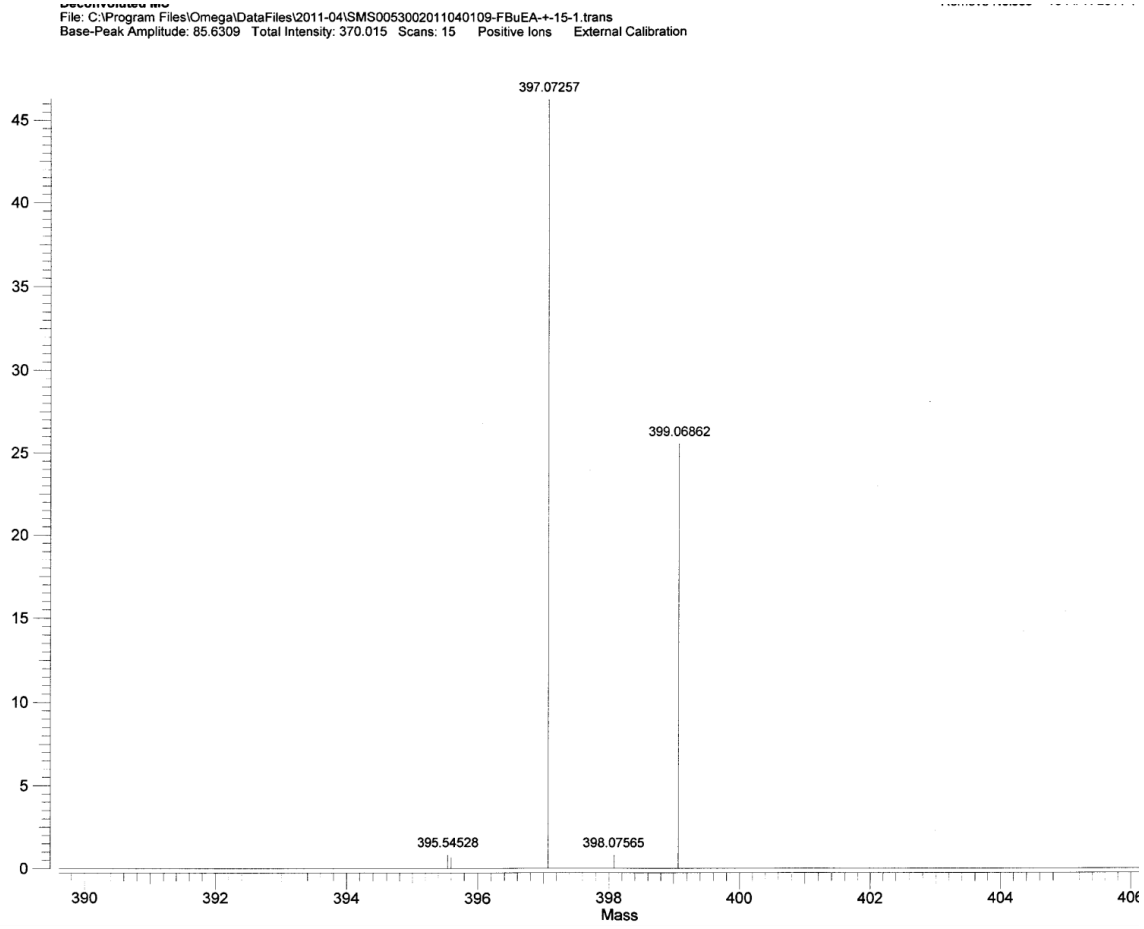
建議：

費用核算：NCH：800 元

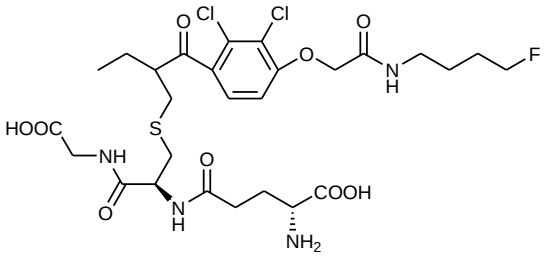
報告日期： 100 年 1 月 21 日

儀器負責人簽章：　　技術員簽章：

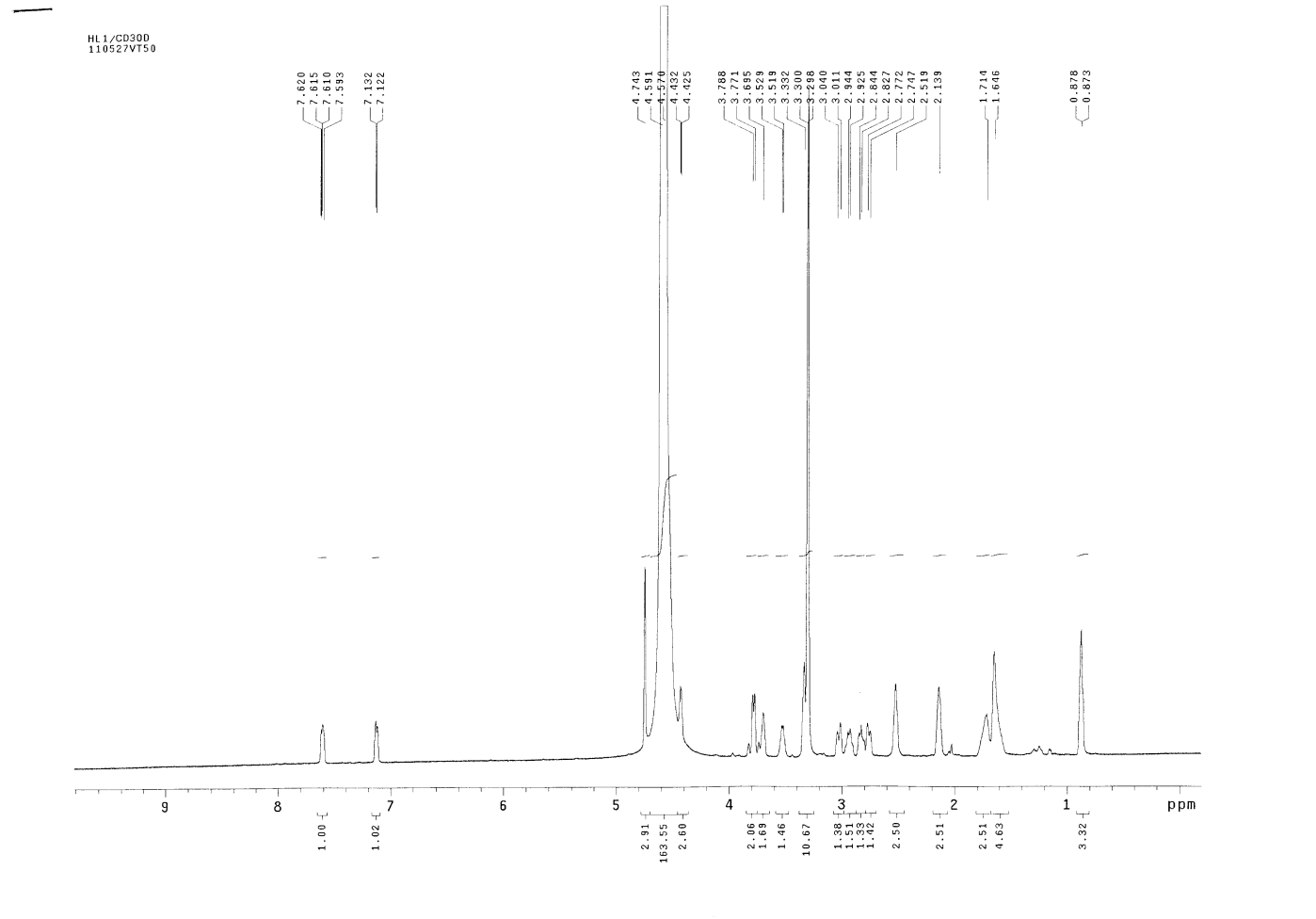
Elemental analysis


File: C:\Program Files\Omega\DataFiles\2011-04\SMS005300201104O109-FBuEA-+-15-1.trans
Base-Peak Amplitude: 85.6309 Total Intensity: 370.015 Scans: 15 Positive Ions External Calibration
397.07257
399.06862
395.54528
398.07565
45
40
35
30
25
20
15
10
5
0
390
392
394
396
398
400
402
404
Mass

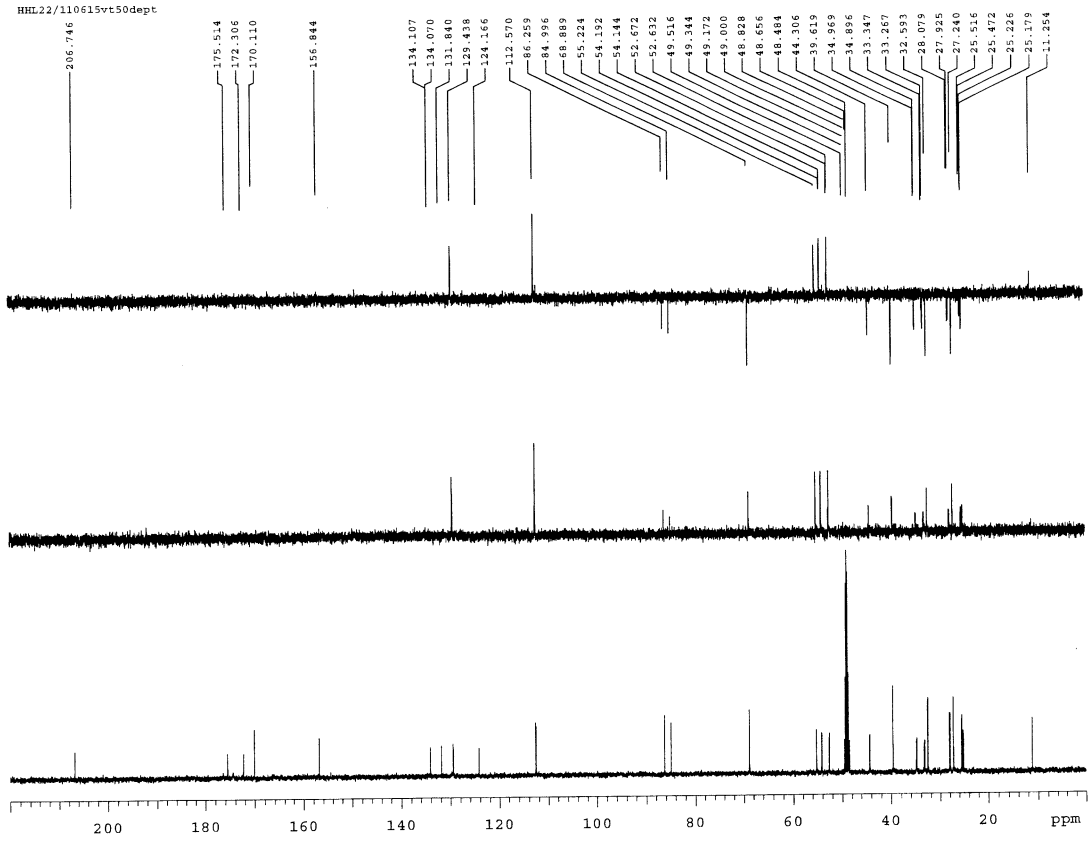
ESI-HRMS


O
Cl
Cl
O
O
N
H
F
HOOC
NH
S
O
N
H
O
COOH
NH2

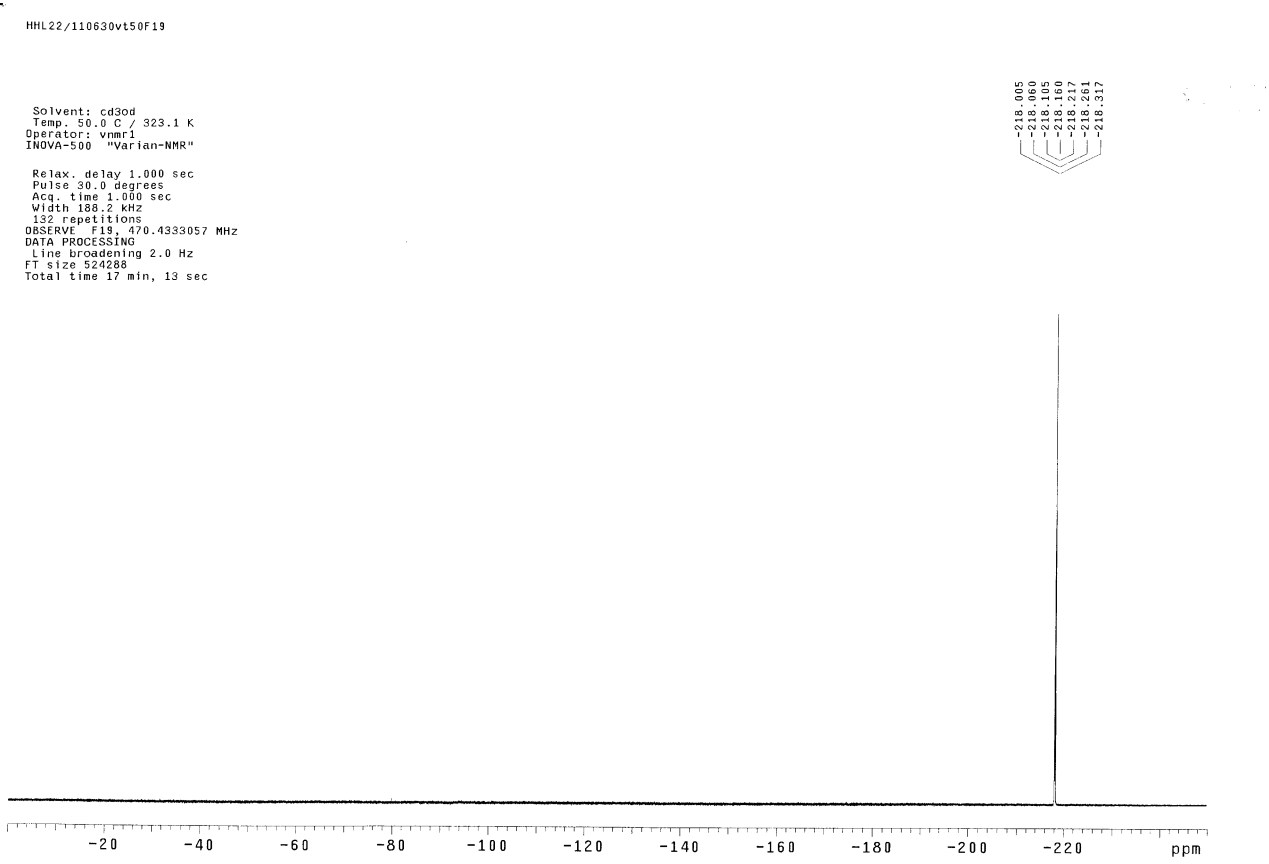
10


HL1/CD3OD
110527VT50
7.620
7.615
7.610
7.593
7.132
7.122
4.743
4.591
4.580
4.432
4.425
3.788
3.771
3.695
3.529
3.519
3.332
3.300
3.288
3.040
3.011
2.944
2.925
2.804
2.627
2.772
2.707
2.519
2.139
1.714
1.646
0.878
0.873
9
8
7
6
5
4
3
2
1
ppm
1.00
1.02
2.91
163.55
2.60
2.06
1.69
1.46
10.67
1.38
1.51
1.33
1.02
2.50
2.51
2.51
4.63
3.32

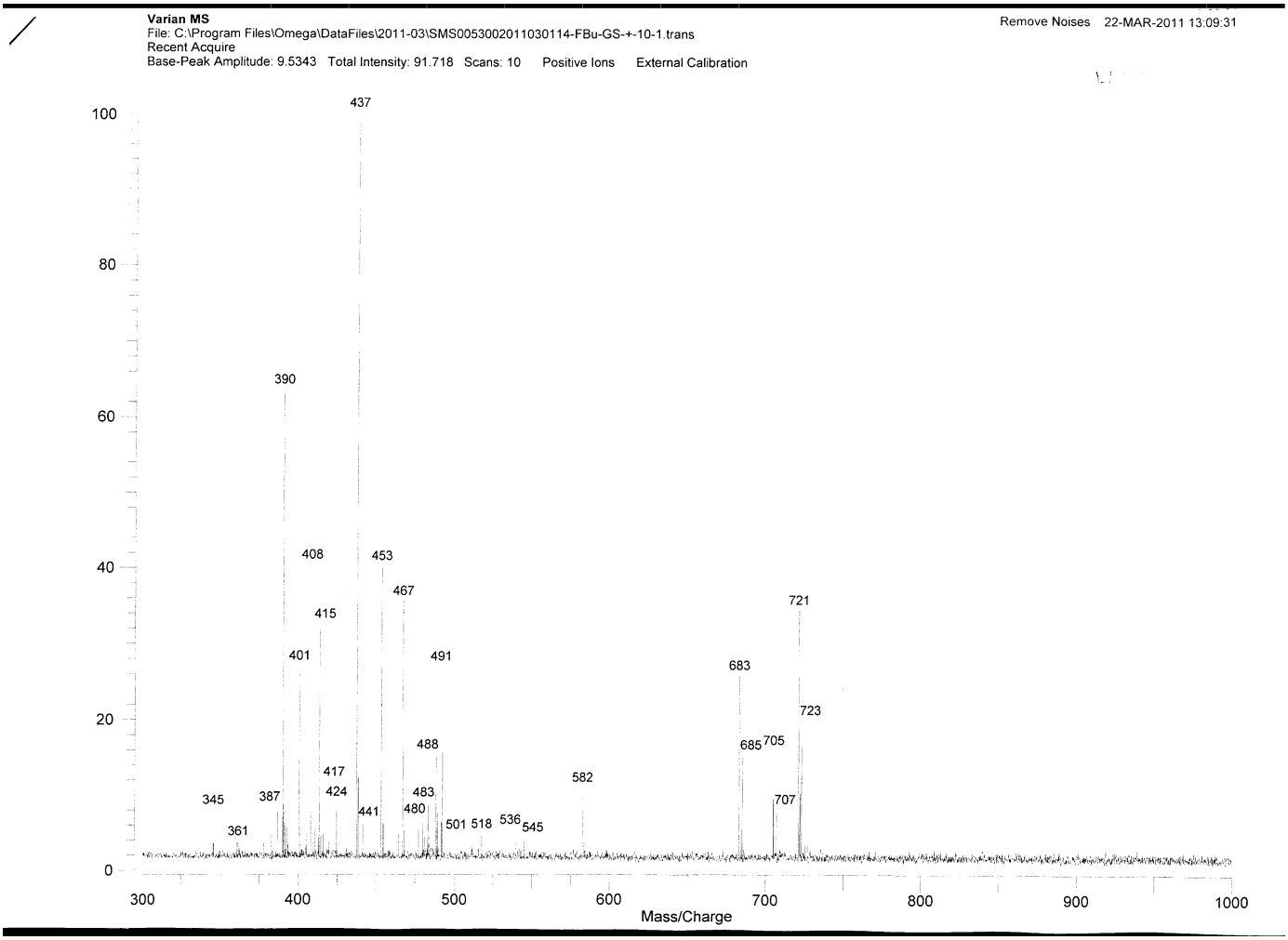
^{1}H-NMR


HHL22/110615vt50dept
206.746
175.514
172.306
170.110
156.844
134.107
134.070
131.640
129.438
124.166
112.570
86.259
84.996
68.889
55.224
54.192
54.144
52.672
52.632
49.516
49.344
49.172
49.000
48.828
48.656
48.484
44.306
39.619
34.969
34.896
33.347
33.267
32.593
28.079
27.925
27.240
25.516
25.472
25.226
25.179
11.254
200
180
160
140
120
100
80
60
40
20
ppm

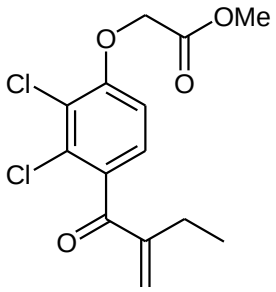
^{13}C-NMR


HHL22/110630vt50F19
Solvent: cd3od
Temp. 50.0 C / 323.1 K
Operator: vnmr1
INOVA-500 "Varian-NMR"
Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 1.000 sec
Width 188.2 kHz
132 repetitions
OBSERVE F19, 470.4333057 MHz
DATA PROCESSING
Line broadening 2.0 Hz
FT size 524288
Total time 17 min, 13 sec
-218.005
-218.060
-218.105
-218.160
-218.217
-218.261
-218.317
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
ppm

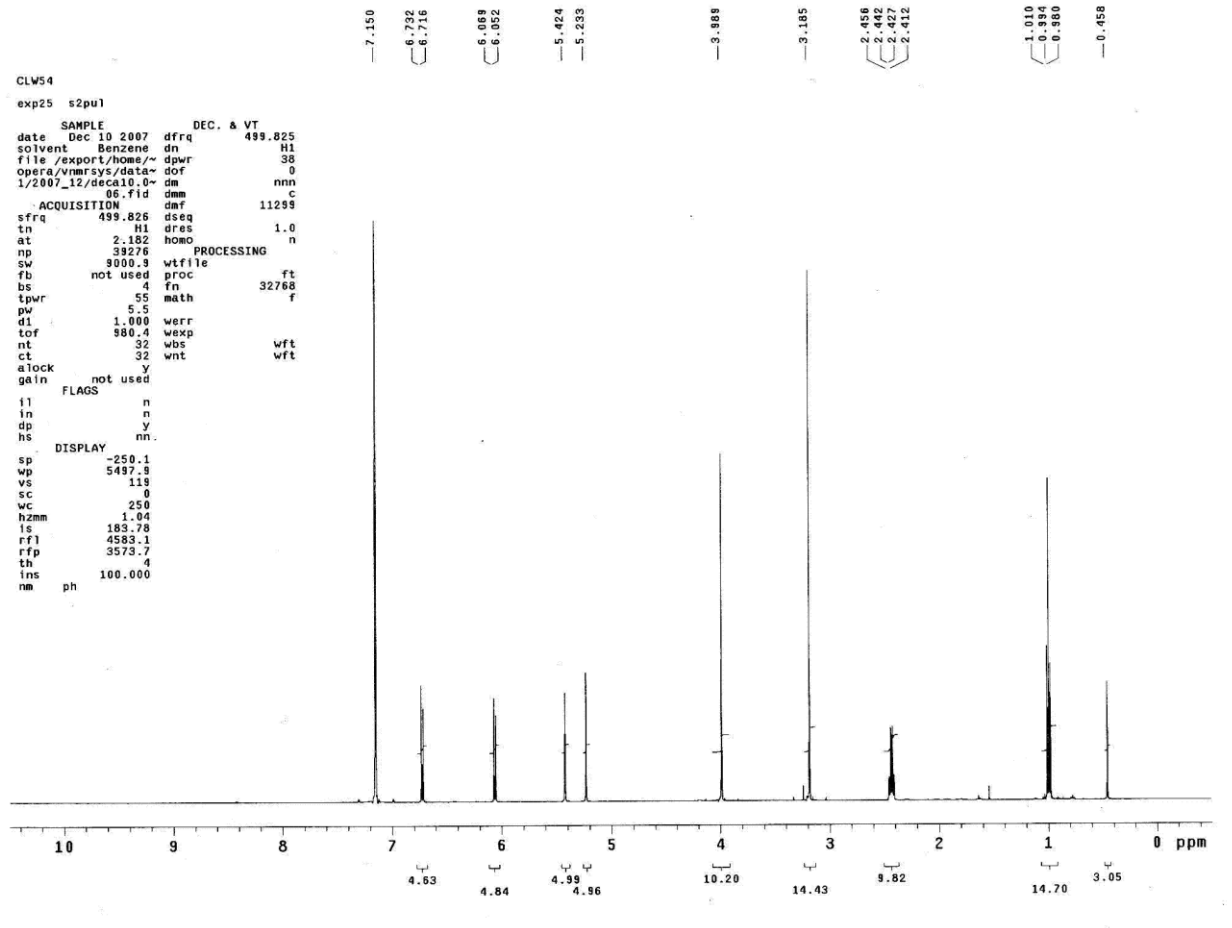
^{19}F-NMR


Varian MS
File: C:\Program Files\Omega\DataFiles\2011-03\SMS0053002011030114-FBu-GS-+-10-1.trans
Recent Acquire
Base-Peak Amplitude: 9.5343 Total Intensity: 91.718 Scans: 10 Positive Ions External Calibration
Remove Noises 22-MAR-2011 13:09:31
437
390
408
453
467
415
401
491
721
683
723
488
685
705
417
582
424
483
345
387
441
480
707
501
518
536
545
361
100
80
60
40
20
0
300
400
500
600
700
800
900
1000
Mass/Charge

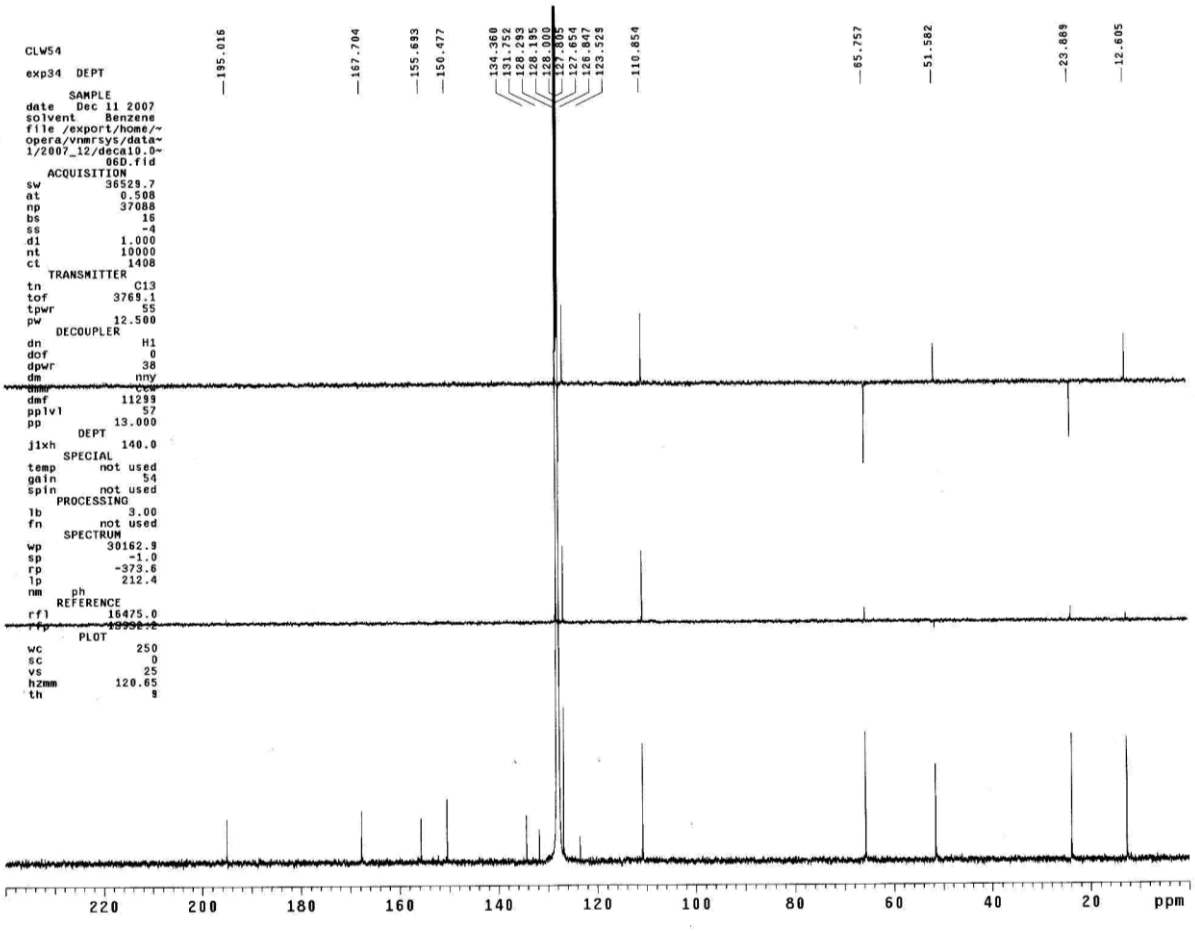
ESI-MS


OMe
O
O
Cl
Cl
O

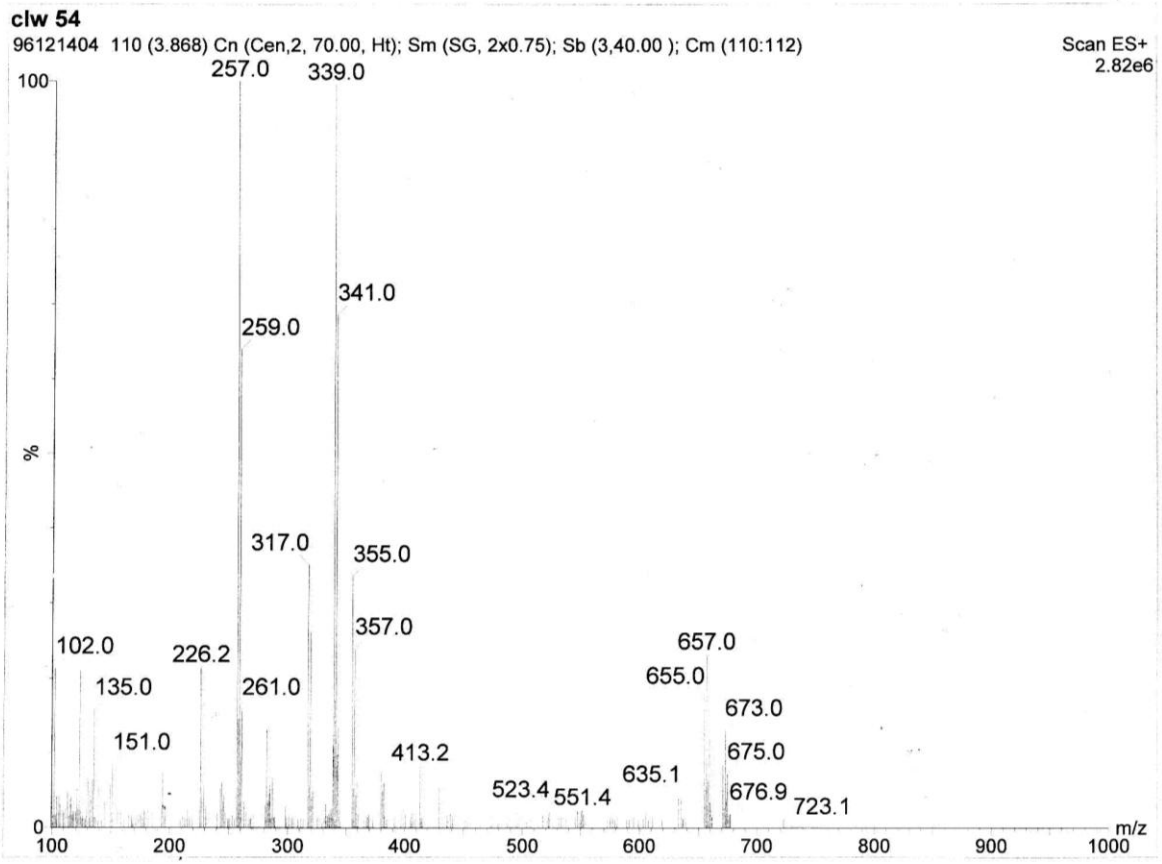
11


7.150
6.732
6.716
6.069
6.052
5.424
5.233
3.988
3.185
2.456
2.442
2.427
2.412
1.010
0.994
0.980
0.458
CLW54
exp25 s2pul
SAMPLE
date Dec 10 2007
solvent Benzene
file /export/home/~
opera/vnmrsys/data~
1/2007_12/deca10.0~
06.fid
ACQUISITION
sfrq 499.826
tn H1
at 2.182
np 39276
sw 9000.9
fb not used
bs 4
tpwr 55
pw 5.5
d1 1.000
tof 980.4
nt 32
ct 32
alock y
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -250.1
wp 5497.9
vs 119
sc 0
wc 250
hzmm 1.04
is 183.78
rfl 4583.1
rfp 3573.7
th 4
ins 100.000
nm ph
DEC. & VT
dfrq 499.825
dn H1
dpwr 38
dof 0
dm nnn
dmm c
dmf 11299
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp
wbs wft
wnt wft
10
9
8
7
6
5
4
3
2
1
0 ppm
4.63
4.84
4.99
4.96
10.20
14.43
9.82
14.70
3.05

^{1}H-NMR


CLW54
exp34 DEPT
SAMPLE
date Dec 11 2007
solvent Benzene
file /export/home/~
opera/vnmrsys/data~
1/2007_12/deca10.0~
06D.fid
ACQUISITION
sw 36529.7
at 0.508
np 37088
bs 16
ss -4
d1 1.000
nt 10000
ct 1408
TRANSMITTER
tn C13
tof 3769.1
tpwr 55
pw 12.500
DECOUPLER
dn H1
dof 0
dpwr 38
dm nny
dmf 11299
pplvl 57
pp 13.000
DEPT
j1xh 140.0
SPECIAL
temp not used
gain 54
spin not used
PROCESSING
lb 3.00
fn not used
SPECTRUM
wp 30162.9
sp -1.0
rp -373.6
lp 212.4
nm ph
REFERENCE
rfl 16475.0
PLOT
wc 250
sc 0
vs 25
hzmm 120.65
th 9
195.016
167.704
155.693
150.477
134.360
131.752
128.293
128.195
128.000
127.805
127.654
126.847
123.529
110.854
65.757
51.582
23.889
12.605
220
200
180
160
140
120
100
80
60
40
20
ppm

^{13}C-NMR


clw 54
96121404 110 (3.868) Cn (Cen,2, 70.00, Ht); Sm (SG, 2x0.75); Sb (3,40.00); Cm (110:112)
Scan ES+
2.82e6
100
%
0
257.0
339.0
341.0
259.0
317.0
355.0
357.0
102.0
226.2
135.0
261.0
151.0
413.2
523.4
551.4
635.1
655.0
657.0
673.0
675.0
676.9
723.1
m/z
100
200
300
400
500
600
700
800
900
1000

ESI-MS

國立交通大學應用化學系

元素分析儀 Heraeus CHN-O Rapid 服務報告書

使用者姓名：姜豐武　　中心編號：971033

服務單位：清大原科 俞鐘山實驗室　樣品名稱或代號：CLW54

收件日期： 97 年 10 月 25 日 完成日期： 97 年 10 月 29 日

分析結果：

實驗值：	N%	C%	H%
1.	—	52.92	4.91
2.	—	52.90	4.81
3.			
4.			
推測值.：	—	53.02	4.45

本日所使用之 Standard：A

(A)Acetanilide (B)Atropin (C)N-Anilin

	N%	C%	H%
理論值：	10.36	71.09	6.71
測出值：	10.24	70.99	6.69

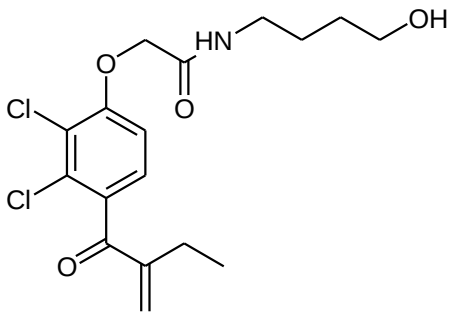
建議：

費用核算：NCH：800 元

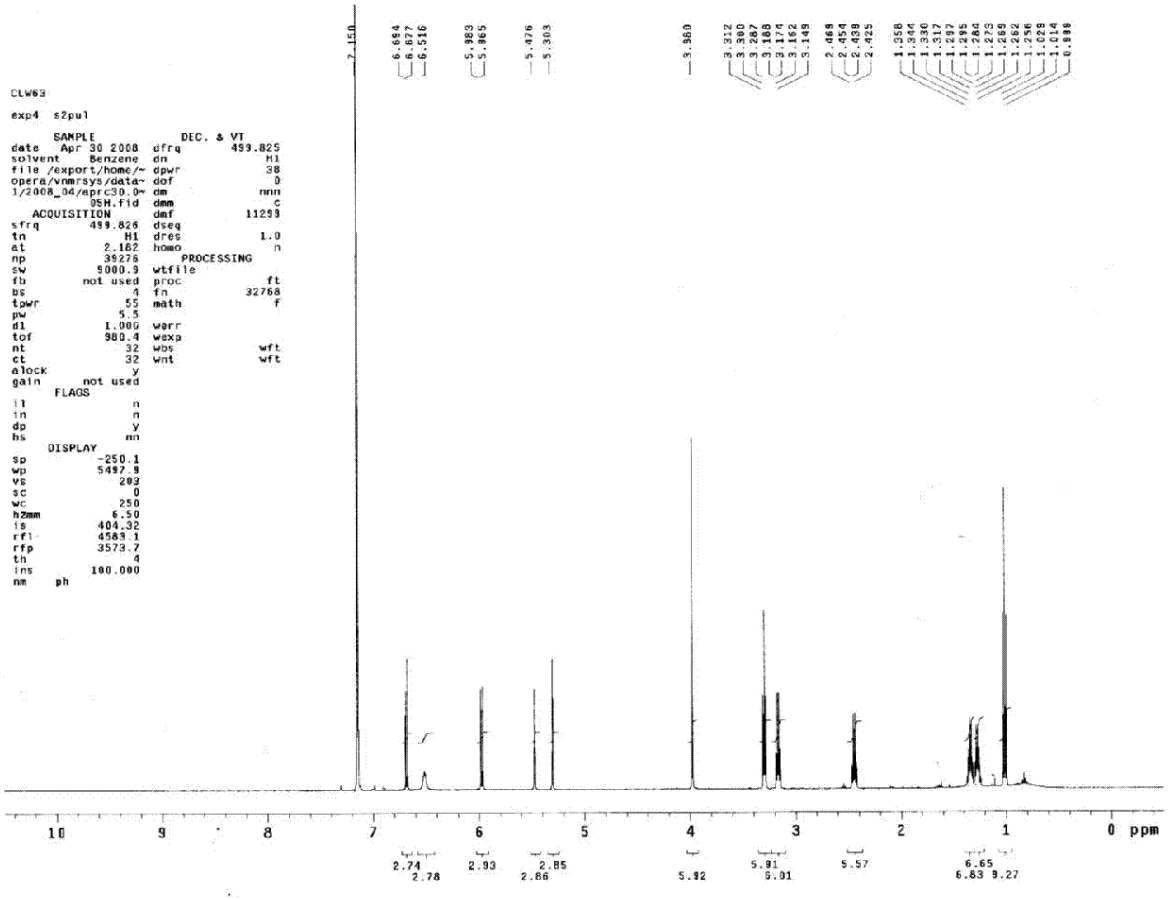
報告日期： 97 年 11 月 3 日

儀器負責人簽章：　　技術員簽章： 技士李蘊明

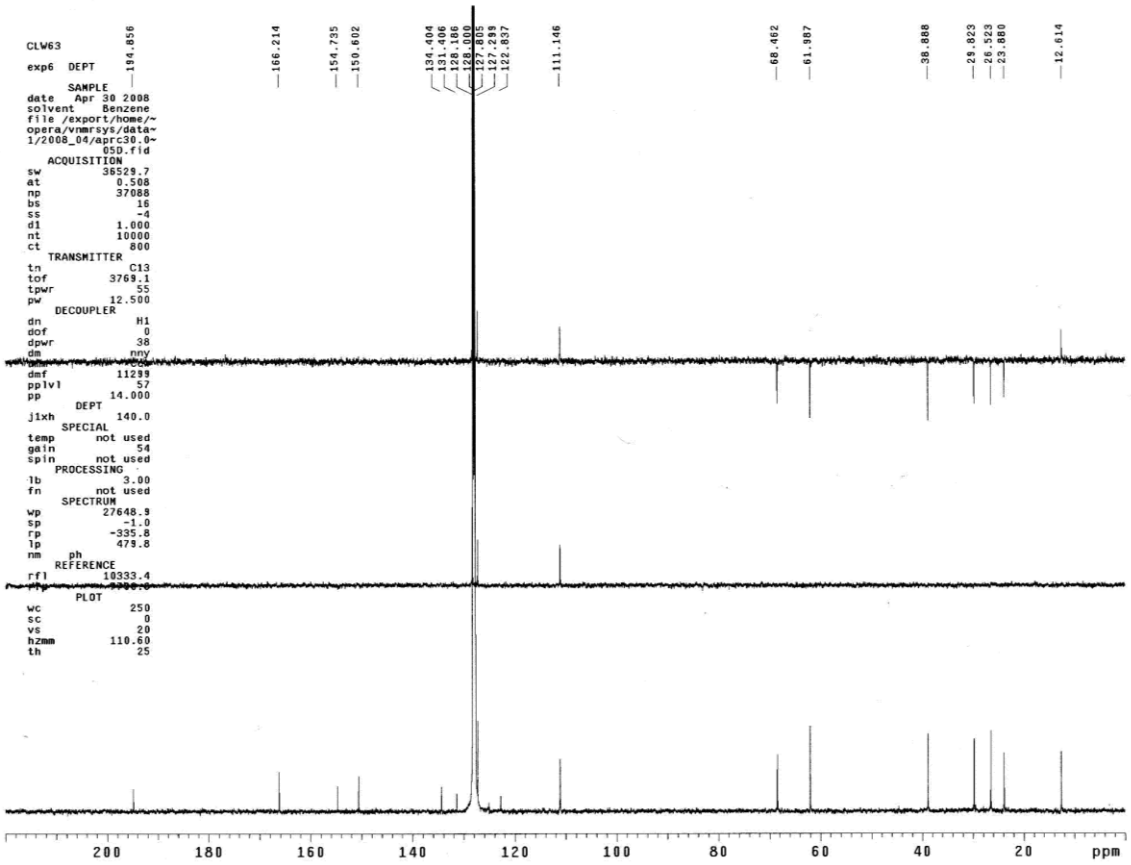
Elemental analysis


HN
OH
O
O
Cl
Cl
O

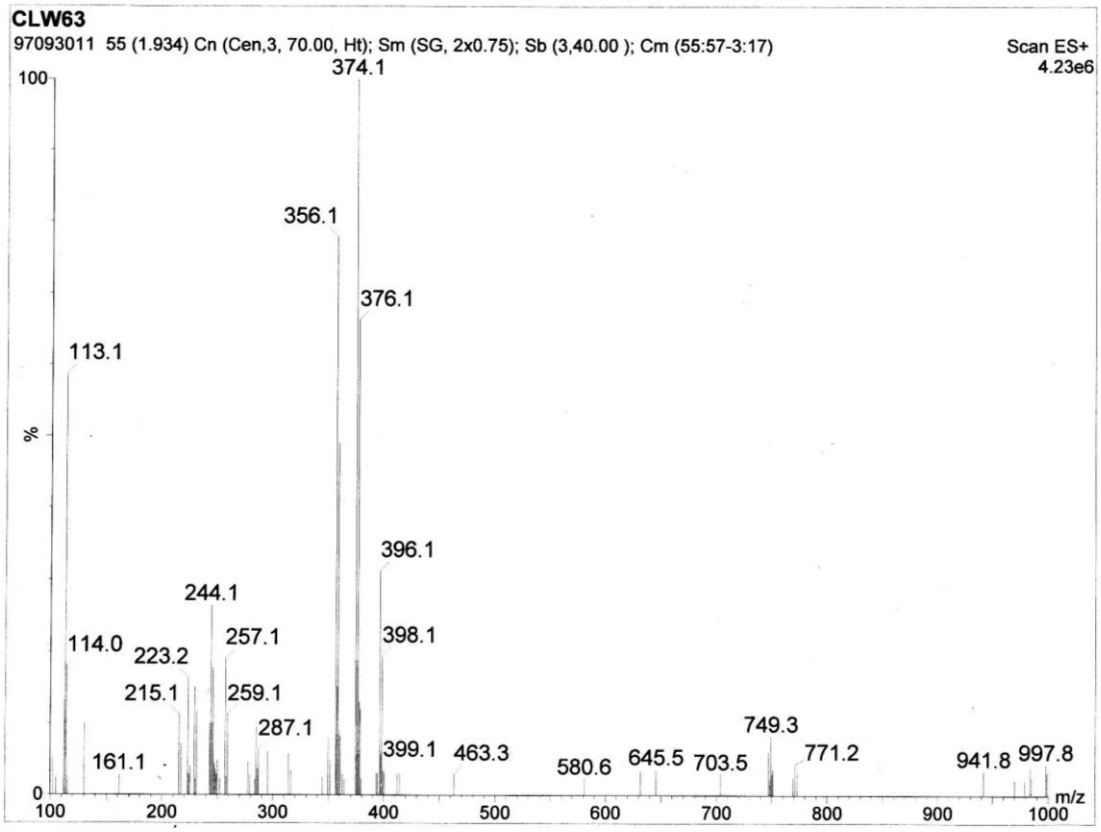
12


CLW63
exp4 s2pul
SAMPLE
date Apr 30 2008
solvent Benzene
file /export/home/~
opera/vnmrsys/data~
1/2008_04/eprc30.0~
05H.fid
ACQUISITION
sfrq 499.826
tn H1
at 2.182
np 35278
sw 5000.9
fb not used
bs 4
tpwr 55
pw 5.5
d1 1.000
tof 980.4
nt 32
ct 32
alock y
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -250.1
wp 5497.9
vs 203
sc 0
wc 250
hzmm 6.50
is 404.32
rf1 4583.1
rfp 3573.7
th 4
ins 100.000
nm ph
DEC. & VT
dfrq 499.825
dn H1
dpwr 38
dof 0
dm nnn
dmm c
dmf 11299
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp
wbs wft
wnt wft
7.150
6.684
6.677
6.516
5.983
5.965
5.476
5.363
3.960
3.312
3.300
3.287
3.168
3.174
3.162
3.149
2.468
2.454
2.439
2.425
1.356
1.346
1.336
1.317
1.297
1.295
1.284
1.273
1.269
1.262
1.256
1.029
1.014
0.998
10
9
8
7
6
5
4
3
2
1
0 ppm
2.74
2.78
2.93
2.85
2.86
5.92
5.91
9.91
5.57
6.65
6.83 9.27

^{1}H-NMR



^{13}C-NMR



ESI-MS

國立交通大學應用化學系

元素分析儀 Heraeus CHN-O Rapid 服務報告書

使用者姓名：姜豐武　　中心編號：980475

服務單位：清大原科 俞鐘山實驗室　樣品名稱或代號：HoBuEA

收件日期： 98 年 4 月 23 日 完成日期： 98 年 4 月 23 日

分析結果：

實驗值：	N%	C%	H%
1.	3.80	54.57	6.02
2.	3.71	54.23	5.47
3.			
4.			
推測值.：	3.74	54.56	5.66

本日所使用之 Standard：A

(A)Acetanilide (B)Atropin (C)N-Anilin

	N%	C%	H%
理論值：	10.36	71.09	6.71
測出值：	10.38	71.07	6.89

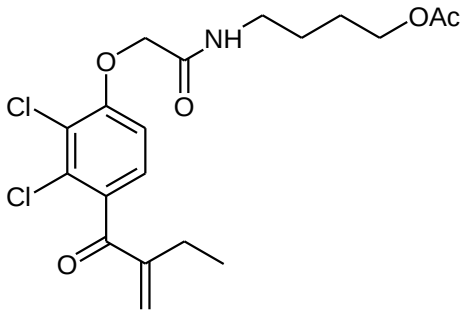
建議：

費用核算：NCH：800 元

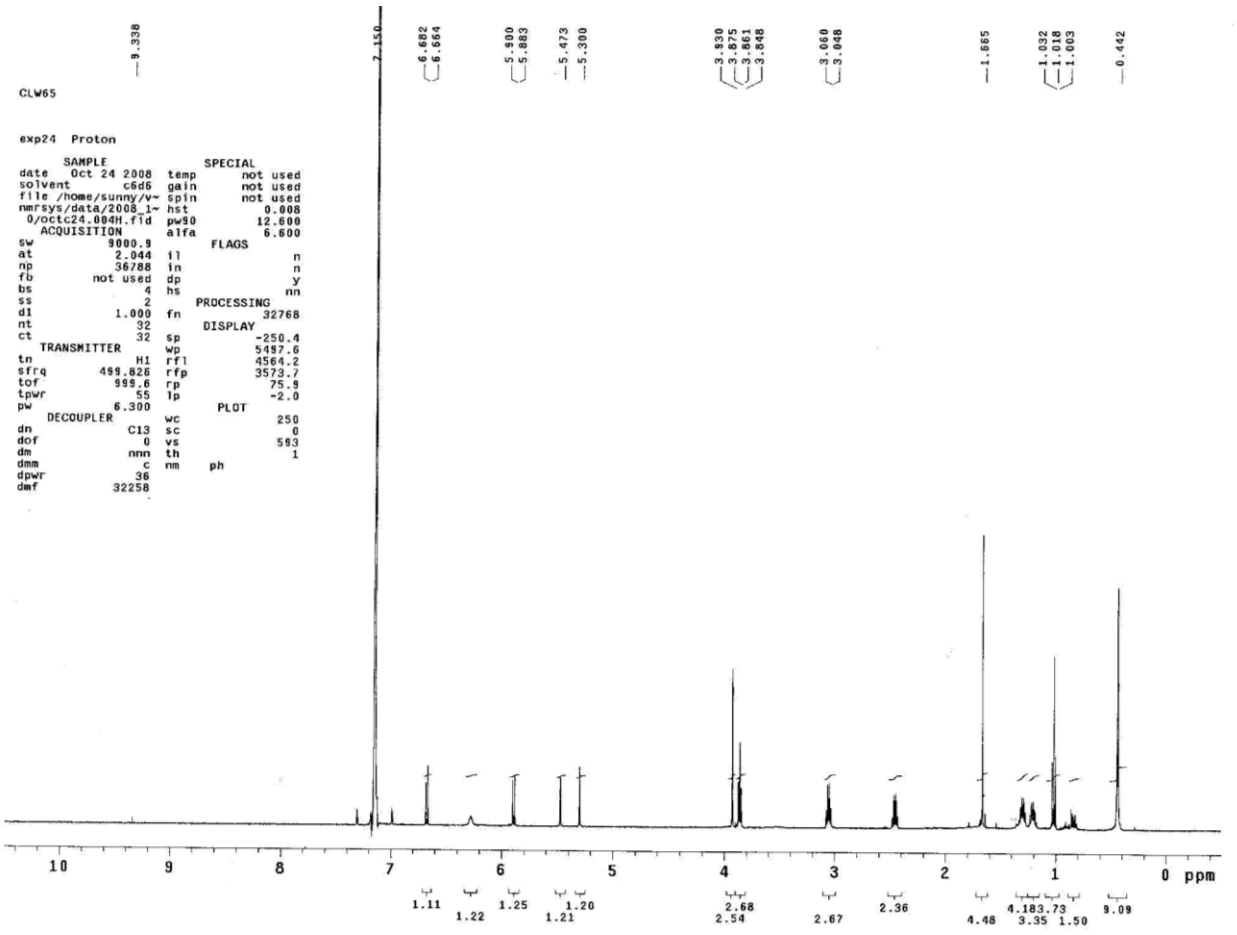
報告日期： 98 年 4 月 24 日

儀器負責人簽章：　　技術員簽章：技士李蘊明

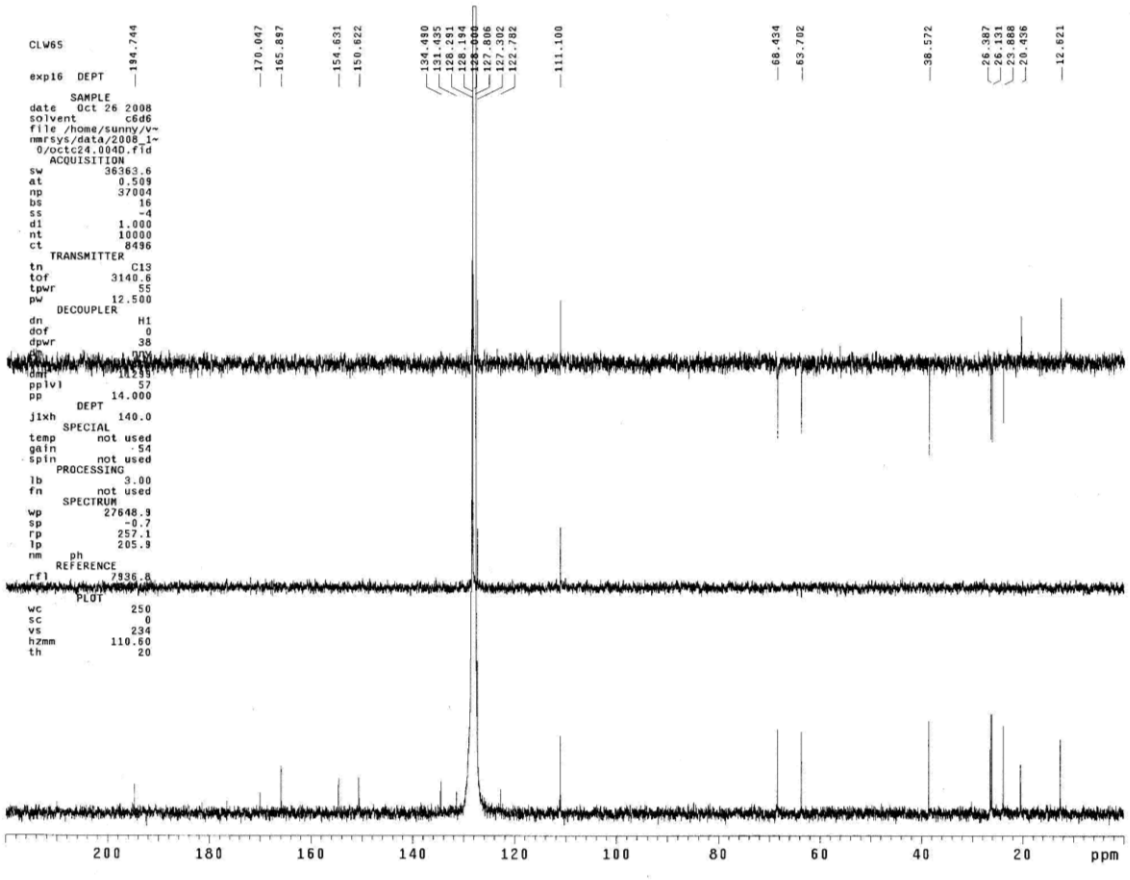
Elemental analysis


OAc
NH
O
O
Cl
Cl
O

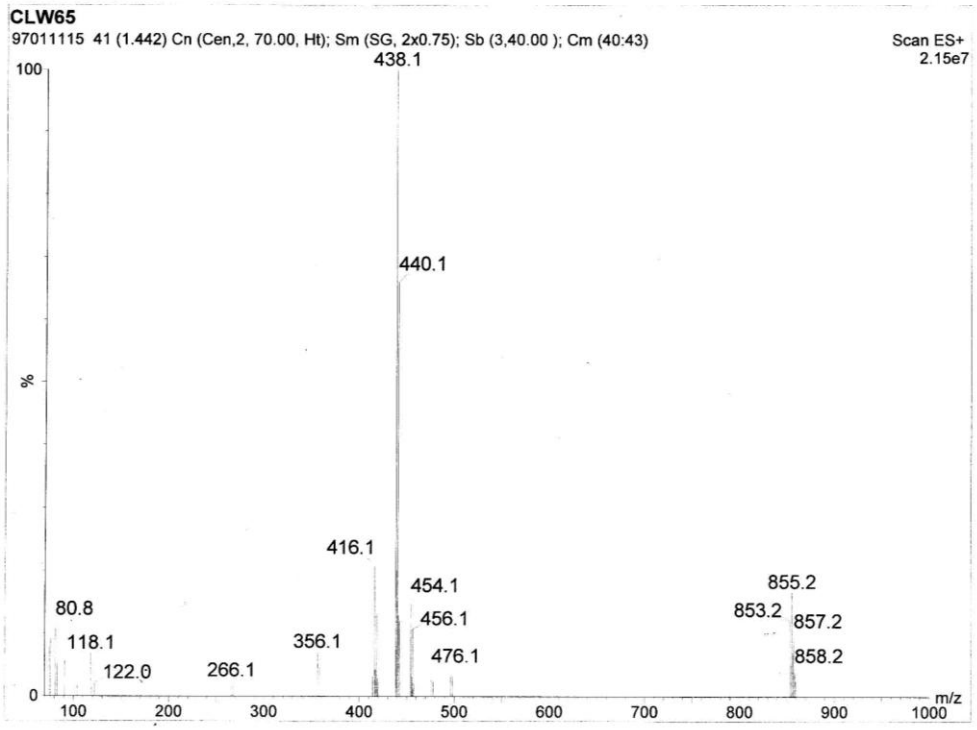
14


9.338
7.150
6.682
6.664
5.900
5.883
5.473
5.300
3.930
3.875
3.861
3.848
3.060
3.048
1.665
1.032
1.018
1.003
0.442
CLW65
exp24 Proton
SAMPLE
date Oct 24 2008
solvent c6d6
file /home/sunny/v~
nmrsys/data/2008_1~
0/octc24.004H.fid
ACQUISITION
sw 9000.9
at 2.044
np 36788
fb not used
bs 4
ss 2
d1 1.000
nt 32
ct 32
TRANSMITTER
tn H1
sfrq 499.826
tof 999.6
tpwr 55
pw 6.300
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dpwr 36
dmf 32258
SPECIAL
temp not used
gain not used
spin not used
hst 0.008
pw90 12.600
alfa 6.600
FLAGS
il n
in n
dp y
hs nn
PROCESSING
fn 32768
DISPLAY
sp -250.4
wp 5497.6
rfl 4564.2
rfp 3573.7
rp 75.9
lp -2.0
PLOT
wc 250
sc 0
vs 593
th 1
nm ph
10
9
8
7
6
5
4
3
2
1
0 ppm
1.11
1.22
1.25
1.21
1.20
2.68
2.54
2.67
2.36
4.48
4.18
3.35
3.73
1.50
9.09

^{1}H-NMR



^{13}C-NMR



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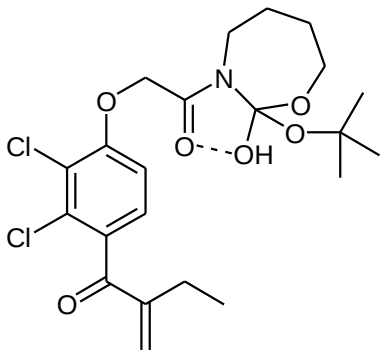
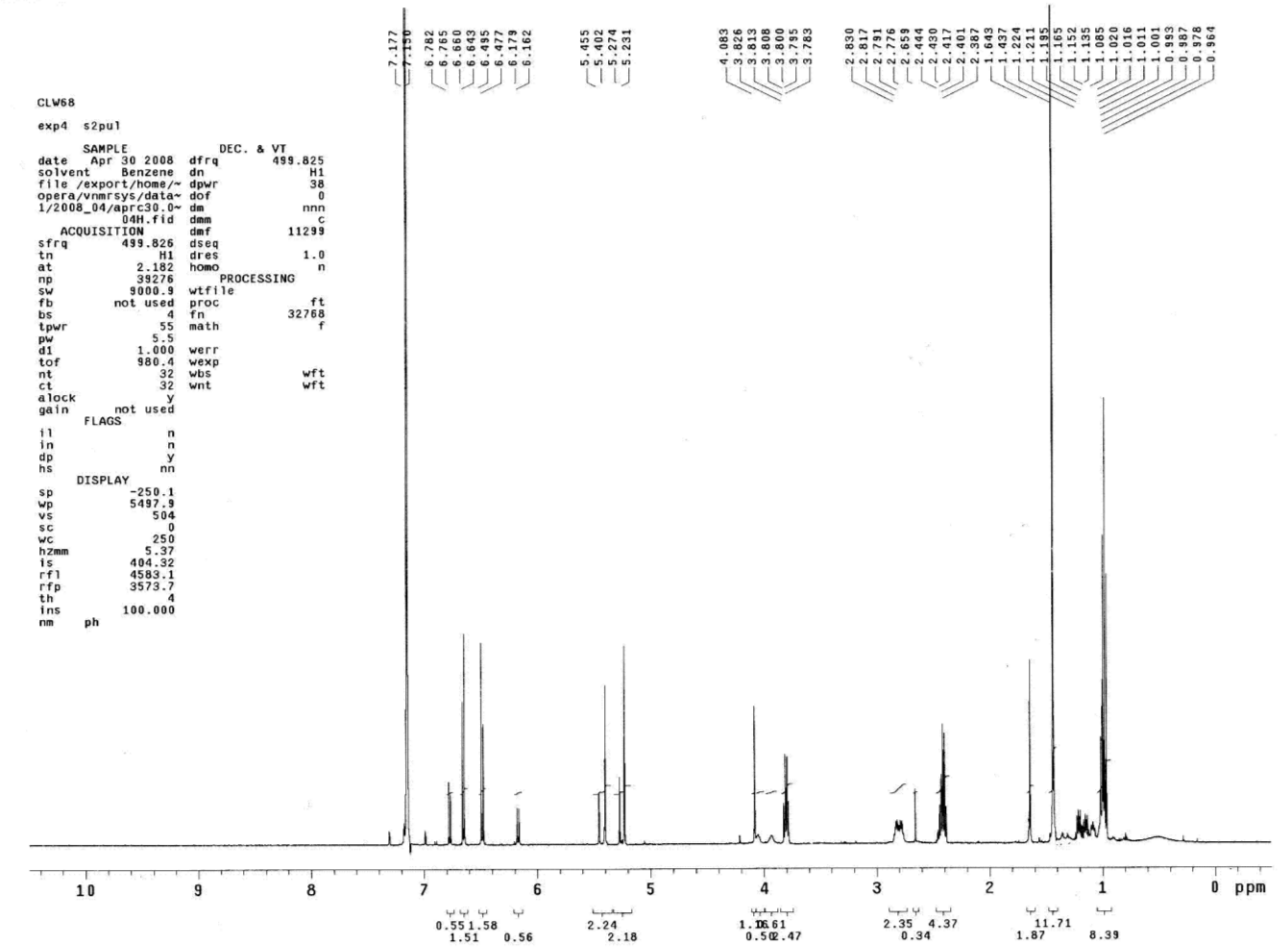
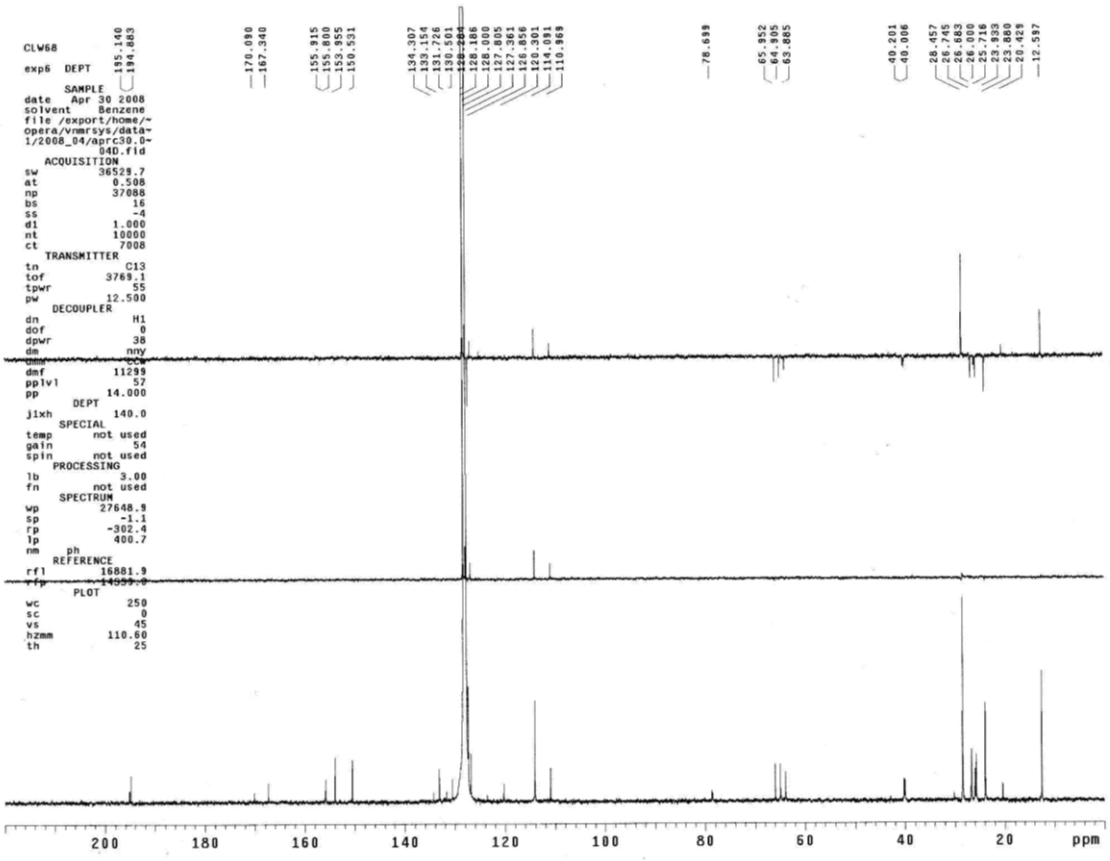

Cl
Cl
O
N
O
O
O
OH
O

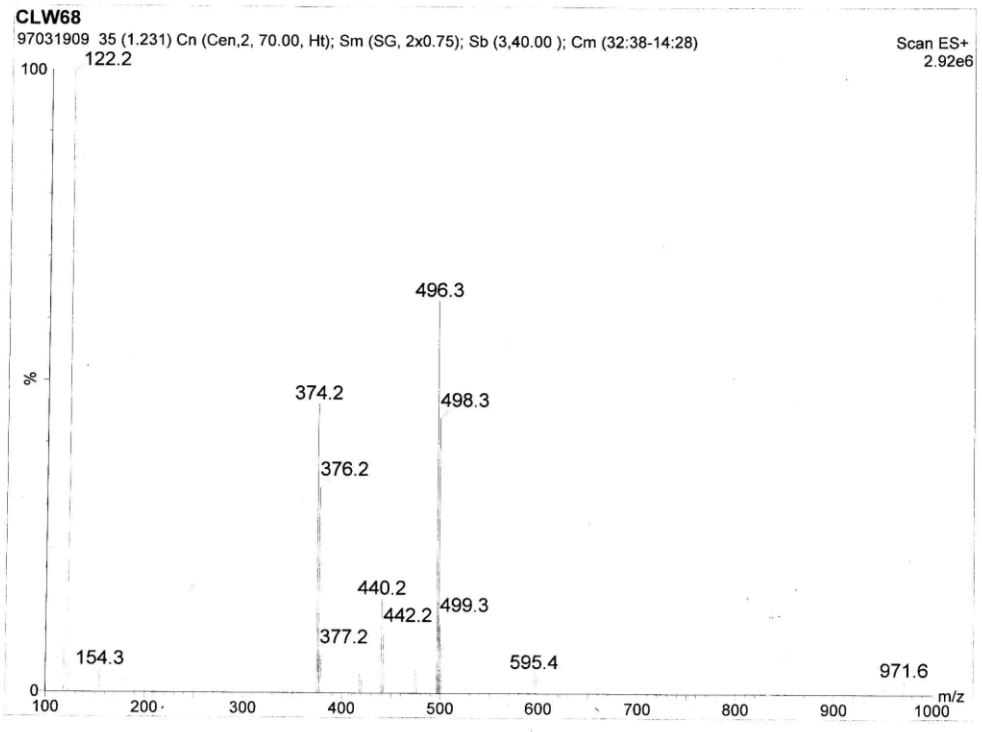
15


CLW68
exp4 s2pul
SAMPLE
date Apr 30 2008
solvent Benzene
file /export/home/~
opera/vnmrsys/data~
1/2008_04/aprc30.0~
D4N.fid
ACQUISITION
sfrq 499.826
tn H1
at 2.182
np 39276
sw 9000.9
fb not used
bs 4
tpwr 55
pw 5.5
d1 1.000
tof 980.4
nt 32
ct 32
alock y
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -250.1
wp 5497.9
vs 504
sc 0
wc 250
hzmm 5.37
is 404.32
rfl 4583.1
rfp 3573.7
th 4
ins 100.000
nm ph
DEC. & VT
dfrq 499.825
dn H1
dpwr 38
dof 0
dm nnn
dmm c
dmf 11299
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 32768
math f
werr
wexp
wbs wft
wnt wft
7.177
7.150
6.782
6.765
6.660
6.643
6.495
6.477
6.179
6.162
5.455
5.402
5.274
5.231
4.083
3.826
3.813
3.808
3.800
3.795
3.783
2.830
2.817
2.791
2.776
2.659
2.444
2.430
2.417
2.401
2.387
1.643
1.437
1.224
1.211
1.195
1.165
1.152
1.135
1.085
1.020
1.016
1.011
1.001
0.993
0.987
0.978
0.964
10
9
8
7
6
5
4
3
2
1
0 ppm
0.55 1.58
1.51
0.56
2.24
2.18
1.16 1.61
0.56 2.47
2.35 4.37
0.34
11.71
1.87
8.39

^{1}H-NMR



^{13}C-NMR



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