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Photocleavable Linker for Protein Affinity Labeling to Identify the Binding Target of KCN-1

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PHOTOCLEAVABLE LINKER FOR PROTEIN AFFINITY LABELING TO IDENTIFY THE BINDING TARGET OF KCN-1

by

HANG T. TRAN

Under the Direction of Binghe Wang

ABSTRACT

KCN-1 is known to reduce tumor growth 6-fold in mice implanted with LN229 glioma cells. Although this inhibitor is effective, the mechanism of action for KCN-1 is not well understood. Based on preliminary studies, KCN-1 reduces tumor growth by disrupting the HIF-1 (hypoxia-induced factor-1) pathway. The binding target of KCN-1 needs to be investigated in order to develop KCN-1 or its analogs for therapeutic applications. In this research, a molecule was designed and synthesized for the identification of the binding target of KCN-1. Specifically, this molecule contains the inhibitor (KCN-1), a photocleavable linker,

beads, and the affinity label (L-DOPA). When UV light shines on the linker, the *trans*-alkene isomerizes to *cis*-alkene and undergoes intramolecular ring-closing reaction, which helps cleave the immobilized bead from the linker. The immobilized bead is used to separate the binding fragment attached to the photocleavable linker from the solution after enzyme digestion. The affinity label (L-DOPA) reacts with a nucleophile from the binding target and creates a covalent bond. If the design is successful, this method is able to analyze the mass of the peptide sequence and determine the binding target of KCN-1.

INDEX WORDS: Photocleavable linker, Affinity label, KCN-1, HIF-1 pathway

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1. INTRODUCTION

1.1 Hypoxia

Hypoxia is a condition of oxygen deprivation. When oxygen level decreases, an adaptive response occurs that is mediated by hypoxia-inducible factor-1 (HIF-1)[1]. HIF-1 activates transcription of genes that help cells survive in low oxygen conditions. These genes code for proteins that promote angiogenesis to form new blood vessels, glucose metabolism to provide more energy, and cell proliferation.

Hypoxia commonly occurs in solid tumors because oxygen is limited in the interior of the tumor mass[2]. The blood vessel is too far from the hypoxic area to deliver adequate amount of oxygen. Thus, there is an increase of HIF-1 activity and stabilization in those areas. As a result, the activation of genes helps solid tumor cells survive and expand. Hypoxia is a major driving force of tumor progression because cells undergo adaptive genetic changes that prevent hypoxia-induced cell death and necrosis[2].

1.2 HIF-1 Pathway

Hypoxia-inducible factor-1 (HIF-1) is a transcription factor that responds to changes in oxygen and is up-regulated in hypoxia[3]. It is a heterodimeric protein that consists of HIF-1α and HIF-1β subunits. HIF-1α is oxygen-sensitive consisting of an oxygen-dependent degradation (ODD) domain. HIF-1β completes the heterodimeric protein and binds with HIF-1α for the translocation to the nucleus.

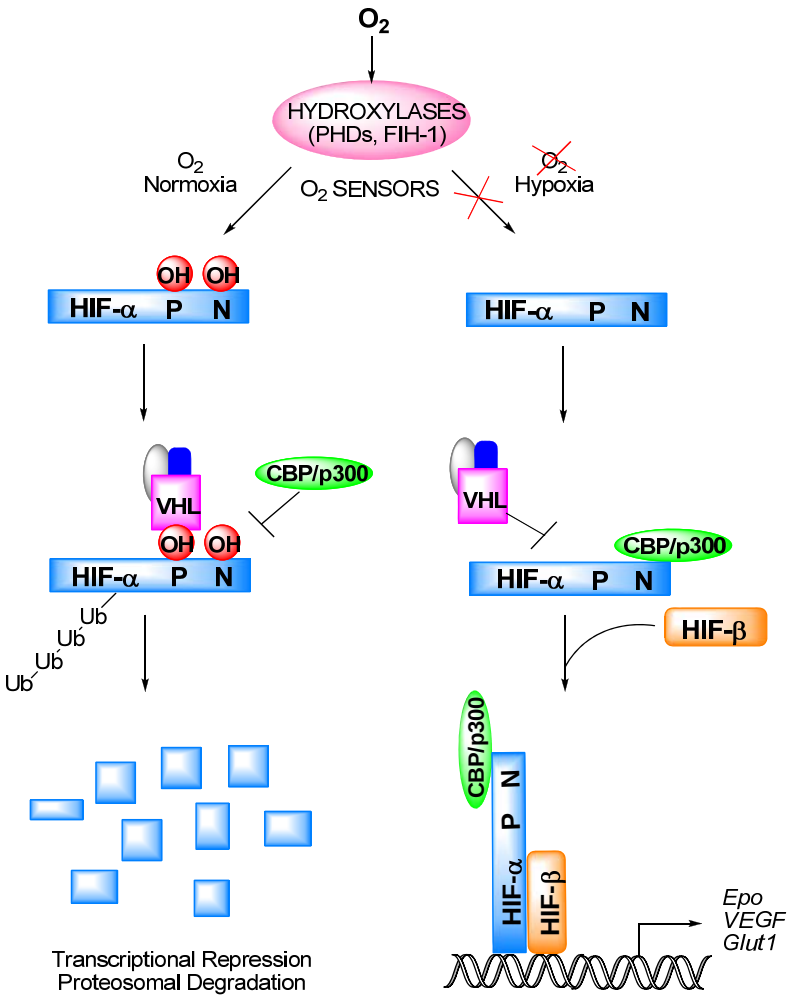


Figure 1.1: A comparison of hypoxia-inducible factor-1 pathway in normoxia and hypoxia[4]

Under normoxia, as shown in Figure 1.1, HIF-1α subunit has an exceptionally short half-life because it is subjected to rapid turnover and degradation by the ubiquitin proteasome pathway[5]. Since oxygen is available, the oxygen-dependent degradation (ODD) domain undergoes hydroxylation by prolyl hydoxylases (PHDs) and factor inhibiting HIF-1 (FIH-1). The PHDs and FIH-1 are iron-dependent enzymes requiring oxygen to hydroxylate prolines and asparagines, respectively, in the ODD domain. As a result of the hydroxylated prolines, the von Hippel-Lindau protein (VHL) binds to the HIF-1α subunit, which leads to ubiquitination. Since

the asparagines are hydroxylated, the transcription co-activator (CBP/p300) cannot bind to the HIF-1α subunit, which leads to transcriptional repression. Thus, transcriptional activity is reduced and HIF-1α subunit undergoes proteasomal degradation.

Under hypoxic condition, as shown in Figure 1.1, HIF-1α subunit cannot be hydroxylated because of decreased oxygen availability. This means that VHL cannot bind to HIF-1α subunit and cannot lead to proteosomal degradation. HIF-1α subunit is abundant and binds to the transcription co-activator (CBP/p300), which initiates the transcriptional activity. Then, HIF-1β subunit binds to the HIF-1α subunit and the heterodimeric protein translocates to the nucleus for the transcription of genes. These genes include erythropoiesis (Epo) to produce red blood cells, vascular endothelial growth factor A (VEGF) to form more blood vessels, and glucose metabolism (Glut1) to provide energy for cells[5].

1.3 KCN-1 Inhibitor

HIF-1 is overly expressed in human cancer. Since more genes are expressed, as well as altered, many tumors undergo genetic changes that facilitate adaptation and survival of the cells[2]. Thus, these adaptations and genetic changes make tumor cells more resistance to conventional treatments, such as chemotherapy[2]. Therefore, inhibition of HIF-1 leads to anti-tumor effects and can be a target for drug development.

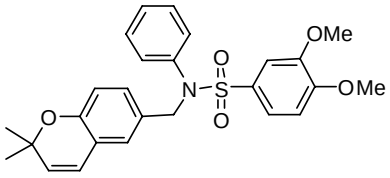


Figure 1.2: Molecular structure of KCN-1.

The interest of finding small molecules that disrupt the HIF-1 pathway led to an inhibitor known as KCN-1. KCN-1, shown in Figure 1.2, is a lead compound identified by screening a

10,000 member combinatorial library from Nicolaou[6]. It was chosen for evaluation in Dr. Van Meir's laboratory at Emory University. Preliminary *in vivo* experiments of KCN-1 showed significant anti-tumor activity. Nude mice were implanted with LN229 glioma tumor cells on both hind flanks. After one week, one group of mice was injected with KCN-1 (60 mg/kg; 5 days/week) and the vehicle. The other group received the vehicle alone, the control. The mice injected with KCN-1 showed 6-fold less in tumor mass than the control. Based on the results, KCN-1 reduces and slows tumor growth. In addition, the KCN-1 injected mice displayed no apparent signs of toxicity or other negative effects.

Although KCN-1 is a potent inhibitor that reduces tumor growth dramatically, the binding target of KCN-1 is not known. However, there is evidence to support that KCN-1 binds to component of the HIF-1 pathway. Based on other preliminary studies done in collaboration with the Van Meir lab, KCN-1 may bind to transcription co-activator p300. This study was performed by Oregon Green fluorophore modified KCN-1 for cell imaging. Although results showed that KCN-1 binds to transcription co-activator p300, further experiments are needed to confirm this mechanism of KCN-1 interacting in tumor cells. Therefore, protein affinity labeling experiments will be used to identify the binding target of KCN-1.

1.4 Affinity Labeling

There are many techniques for tagging proteins. One of these techniques is affinity labeling. This method uses the property of recognition between two different molecules to react and form a chemical bond. It is widely used to identify proteins, localize binding sites, and determine targets of drugs. The affinity label molecule binds specifically and reversibly to the target protein[7]. However, when it is photo-activated or chemically activated, it forms a highly reactive intermediate and reacts irreversibly with the nucleophilic residue of the target protein[7].

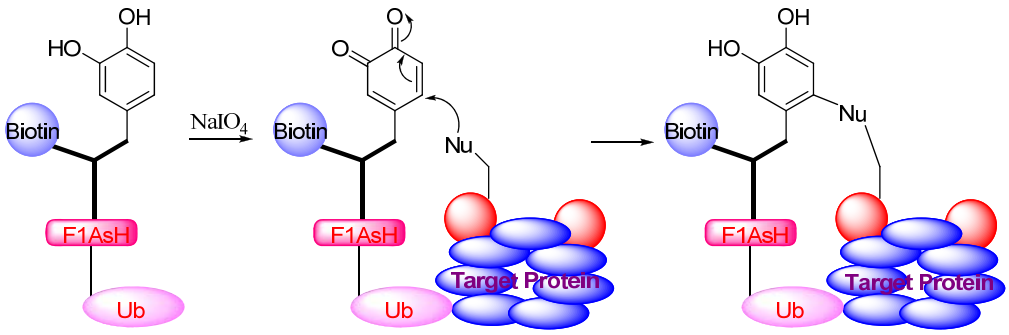


Figure 1.3: Kodadek laboratory's novel type chemically activated molecule used to determine the binding target of free ubiquitin[8]

In recent years, the Kodadek laboratory developed a novel type of chemically activated molecule that formed an extremely reactive electrophilic intermediate. This method was used to determine the binding target of free ubiquitin (Ub). The molecule contained ubiquitin tagged with a biarsenical fluorescein derivative (F1AsH), a biotin moiety, and a catechol of the dihydroxyphenylalanine (L-DOPA)[8]. The most important aspect of this molecule was the catechol of L-DOPA as shown in Figure 1.3. The catechol ring of the L-DOPA subunit can be oxidized to an *ortho*-quinone selectively with sodium periodate ($NaIO_4$)[8]. At close proximity between the ubiquitin and its binding target protein, the electrophilic quinone intermediate reacts with the nucleophilic residue of the protein[8]. The results showed that the proteasome function was not affected by the chemical activator $NaIO_4$. Since the Kodadek method was very effective in their studies, it will be used for the affinity labeling work in this research. The affinity label (L-DOPA) will be utilized to investigate the binding of KCN-1 to its target protein.

1.5 Purpose of the Study

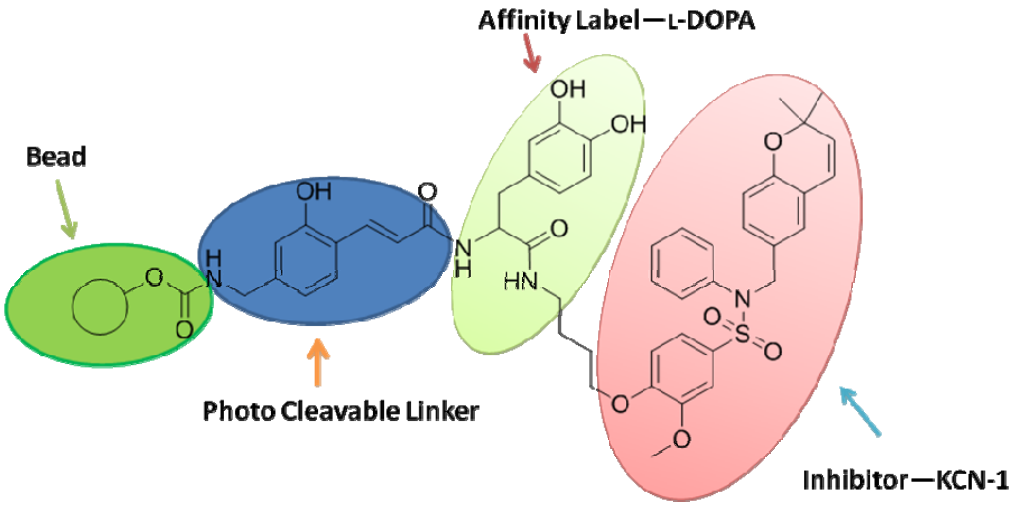


Figure 1.4: Photocleavable linker attached to the immobilized bead, L-DOPA, and KCN-1.

The purpose of this study is to design a small molecule that will identify the binding target of KCN-1. Thus, the objective is to synthesize the molecule shown in Figure 1.4. This molecule contains inhibitor KCN-1, a chemically activatable affinity label L-DOPA, the bead, and a photocleavable linker.

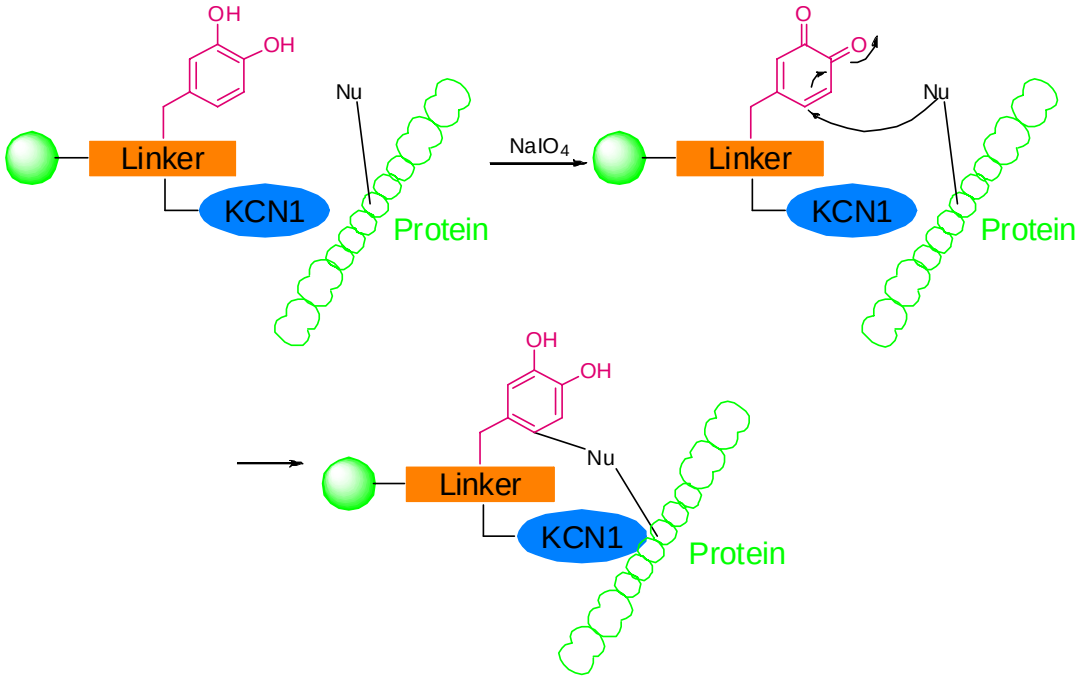


Figure 1.5: Proposed mechanism for the affinity label, L-DOPA, to covalently link to the target protein.

The proposed mechanism of this project consists of KCN-1 binding to a protein at a specific site. As mentioned above, the L-DOPA is the chemically activatable affinity label molecule. It is used to form a covalent bond with the binding target. When the target protein and the KCN-1 are closely associated, the chemical activator $NaIO_4$ converts the catechol of L-DOPA to a very reactive electrophile. As shown in Figure 1.5, sodium periodate ($NaIO_4$) oxidizes the catechol of L-DOPA to form 1,2-benzoquinone[9]. Thus, a nucleophile from the receptor of the target protein reacts with this electrophilic quinone. This reaction converts the quinone back to the catechol[9]. Overall, this procedure forms a covalent bond between the affinity label molecule L-DOPA and the binding target.

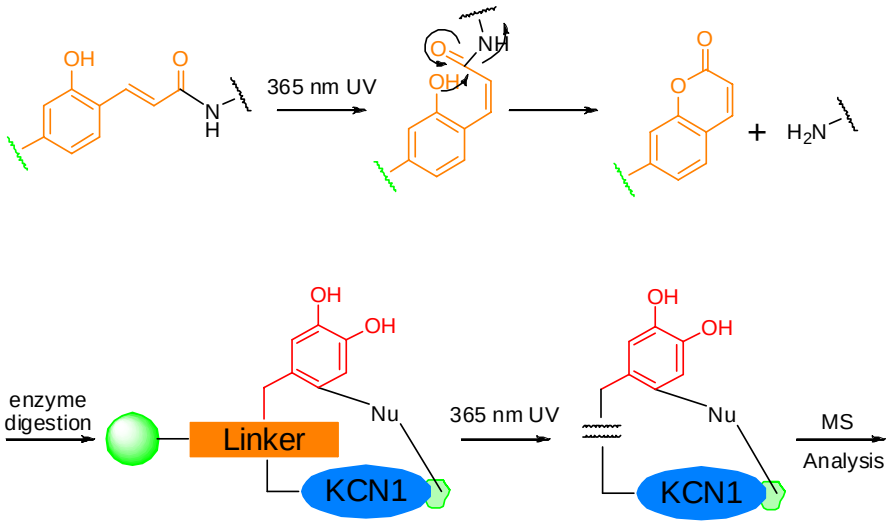


Figure 1.6: Proposed mechanism of the photocleavable linker.

After the protein undergoes enzyme digestion, as shown in Figure 1.6, the fragment covalently bound to the photocleavable linker is pulled out through the use of the bead, which is insoluble so it can easily separate from the solution and other fragments. The linker and immobilized bead are cleaved from the rest of the molecule once separated from the solution. This process occurs when UV light of 365 nm excites the *trans*-alkene, leading to isomerization and formation of the *cis*-alkene[10]. The linker then undergoes intramolecular cyclization reaction. The carbonyl, adjacent to the double bond carbon, reacts with the hydroxyl, on the aromatic ring, to form a chromanone and cleaves the KCN-1, L-DOPA, and the residue covalently bond to the L-DOPA[10]. Then, the covalently linked residue is analyzed through mass spectroscopy by ESI or MALDI. The molecular weight is used to determine the sequence of the binding target. If the design is successful, this method is able to analyze the mass of the binding sequence and identify the target protein.

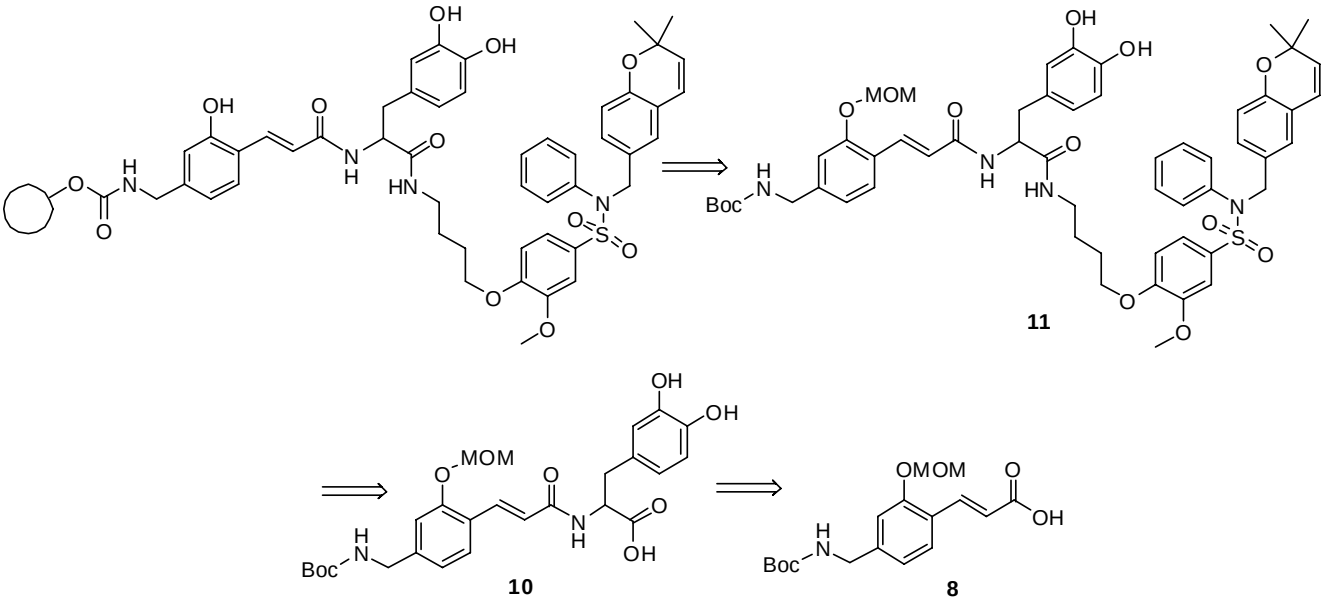
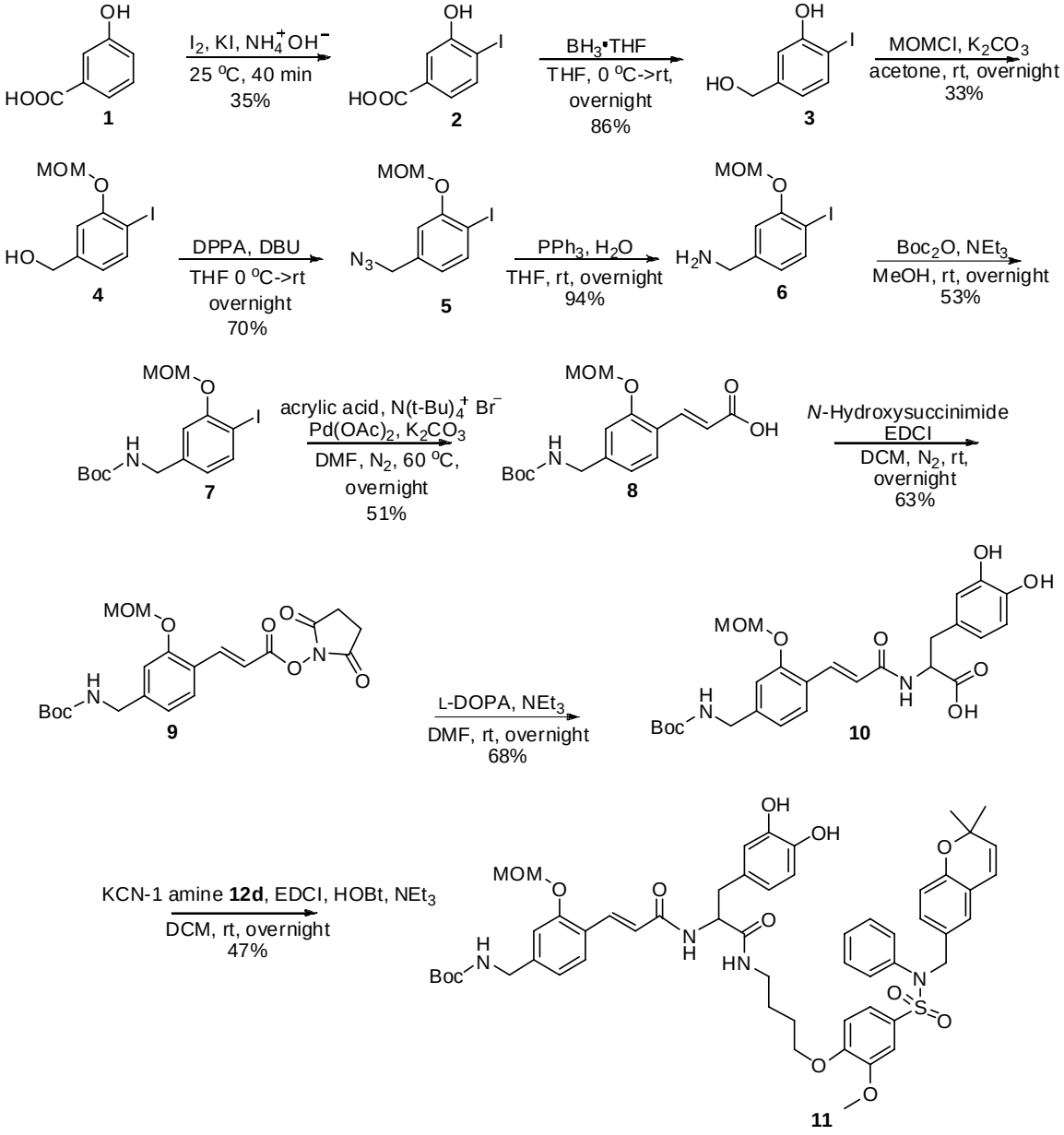


Figure 1.7: The retrosynthetic analysis of the photocleavable linker.

The retrosynthesis of the photocleavable linker is shown in Figure 1.7. Amidation is the key component, which makes this synthetic route possible. Deprotection of Boc- and MOM-group make it possible for the amidation with the immobilized bead. Compound **11** is formed by reacting KCN-1 free amine **12d** and carboxylic acid of the L-DOPA **10**. The L-DOPA fragment is commercially available and is added by yet another amidation with the linker **8**.

2. DISCUSSION

2.1 Synthesis of Compound **11**

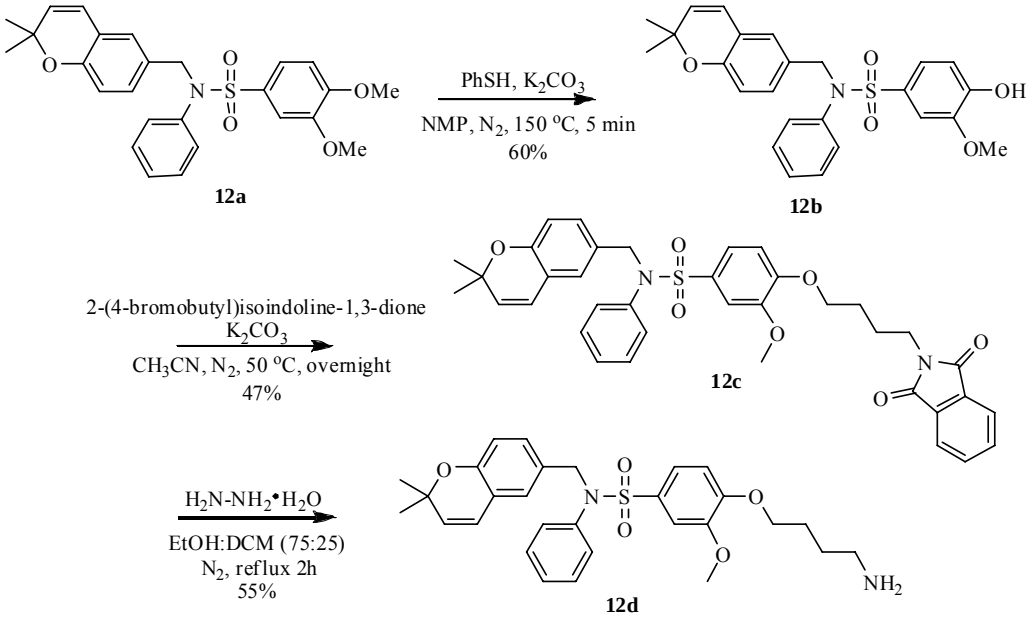


Scheme 1. Synthetic route of compound 11

The synthesis of the photocleavable linker was accomplished in 3.6% overall yield, as shown in Scheme 1. The first step was the iodination of commercially available *m*-hydroxybenzoic acid **1** by the use of iodine and potassium iodide in aqueous ammonia. The

aromatic carboxylic acid **2** was reduced with borane in tetrahydrofuran to generate primary alkyl benzylic alcohol **3**. Protection of the hydroxyl was needed for the conversion of the alcohol **4** to azide **5**. Thus, the hydroxyl on the aromatic ring **3** was protected with chloromethyl methyl ether (MOMCl). The next step was azidation of the primary alkyl benzylic alcohol **4** using diphenylphosphoryl azide (DPPA) in the presence of strong organic base diazabicycloundecane (DBU). This reaction was preceded by *O*-phosphorylation followed by S_N2 displacement to generate the azide compound **5**. This compound was reduced to amine by a mild method of Staudinger reaction, which used triphenylphosphine as the reducing agent[11]. The final reaction yielded triphenylphosphine oxide as the side product and the amine compound **6**. The amine needed to be preserved for the attachment with the bead at the end. Thus, the Boc-protected compound **7** was generated using di-*tert*-butyl dicarbonate in the presence of base triethylamine in methanol. The next step was to create the photochemical esterase. This step was a simple Heck reaction using palladium catalyze carbon-carbon coupling between aryl iodide and activated alkene, acrylic acid, in the presence of K_2CO_3 base[12]. The carboxylic acid **8** was activated with *N*-hydroxysuccinimide (NHS) and coupling reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (EDCI) in dichloromethane (DCM). The activated ester **9** reacted with commercially available *(S)*-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid (L-DOPA) and triethylamine in DMF to generate compound **10**. Compound **11** was successfully synthesized by amidation of compound **10** with KCN-1 amine **12d** in the presence of coupling agents EDCI and hydroxybenzotriazole (HOBt). The final protected compound **11** was purified by HPLC.

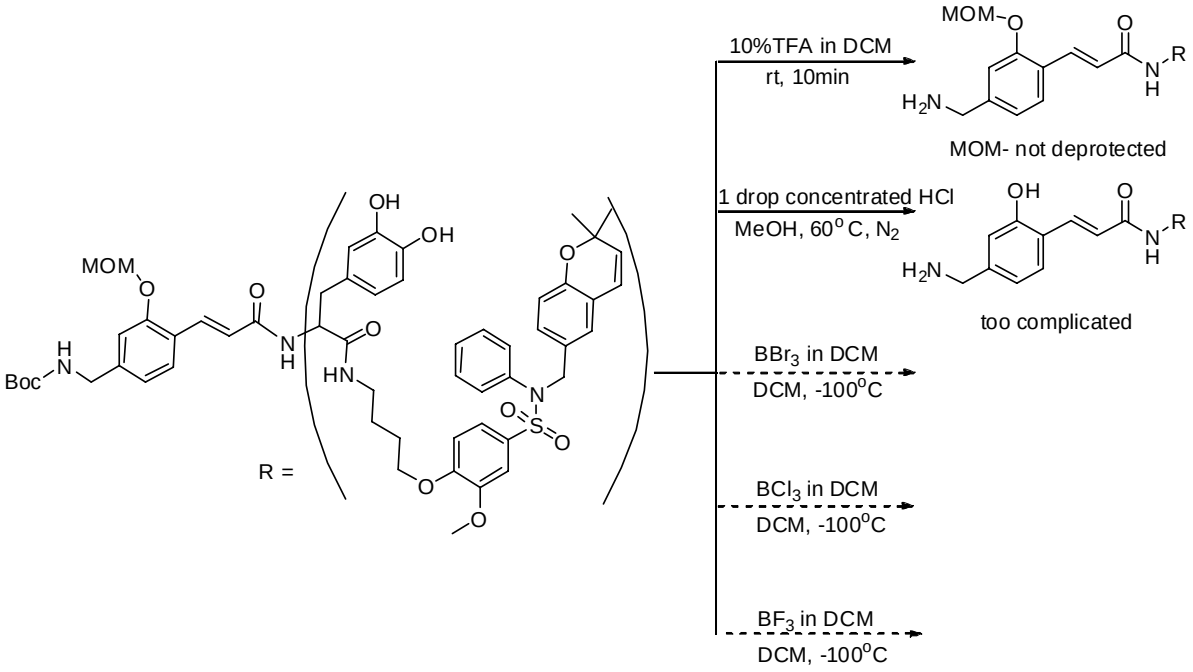
2.2 Synthesis of KCN-1 amine **12d**



Scheme 2. Synthetic route of compound 12d (KCN-1 amine).

The KCN-1 amine **12d** was synthesized as shown in Scheme 2. The KCN-1 **12a** was made available by the Van Meir Laboratory at Emory University. The methoxy on the *para*-position was selectively demethylated with thiophenol and K_2CO_3 base in *N*-Methyl-2-pyrrolidone (NMP) at temperature of 150 ºC. The time was extremely important because if the reaction ran more than five minutes, the methoxy on the *meta*-position would be demethylated also. Then the hydroxyl compound **12b** was alkylated with *N*-(4-bromobutyl) phthalimide and K_2CO_3 base in dimethylformamide (DMF). The phthalimide KCN-1 **12c** was reduced to the final KCN-1 amine **12d** by refluxing with hydrazine in a mixture of ethanol and dichloromethane.

2.3 Approaches to Compound **13**

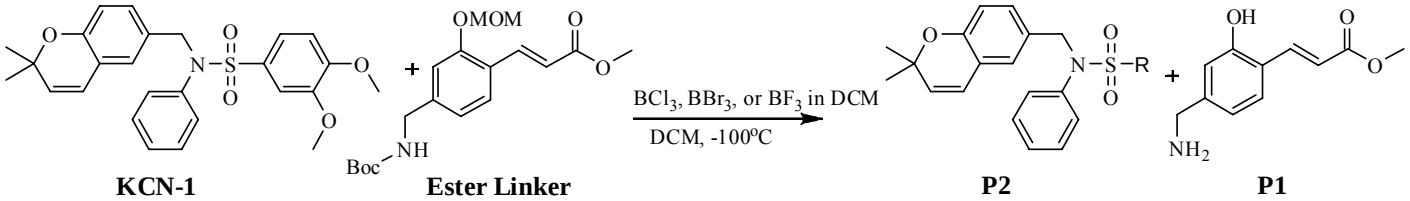


Scheme 3. Synthetic routes of final deprotected compound 13.

Although compound **11** was successfully synthesized, there were many complications for the deprotection of MOM- and Boc- groups, as shown in Scheme 3. The molecule was very fragile to harsh conditions. The trifluoroacetic acid (TFA), p*K*a of ~0.3, was first used to remove the MOM- and Boc- groups, which was unsuccessful. At 10% TFA in DCM, only the Boc-group was cleaved and MOM-group was intact. At 50% TFA in DCM, the products were too complicated to separate and the residue, left after the reaction, had overlapping peaks on the HPLC. Apparently, TFA was not a good reagent for the removal of the MOM- and Boc- groups. Thus, other methods were considered.

It was reported that trace amount of concentrated HCl in MeOH at 62 ºC for 15 minutes can cleave the MOM-group[13]. There was also an experiment that reported 4 M HCl in MeOH at room temperature for 30 minutes can cleave the Boc-group[14]. In addition, water in the

concentrated HCl has been used to prevent double bond isomerization that occur under anhydrous acidic condition[15]. Therefore, the second route was to use a trace concentrated HCl in MeOH at 60 ºC for 15 minutes. Unfortunately, this experiment was not successful either. KCN-1 was found to be sensitive in acidic environment and decomposed easily based on ^{1}H NMR.



KCN-1 **Ester Linker** **P2** **P1**

Scheme 4. Test model reactions of boron trihalides in DCM under -100 ºC.

Since HCl was too acidic for compound **11**, the search for better reagents led to boron trihalides. Some boron trihalides, such as boron trichloride (BCl_3), boron tribromide (BBr_3), and boron trifluoride (BF_3) were considered to be good candidates. Test model reactions were conducted to determine the effectiveness of the boron trihalides. These tests consisted of one equivalent of KCN-1, one equivalent of photocleavable linker derivative ester, and two equivalents of the boron trihalides in DCM under -100 ºC, as shown in Scheme 4. The products were the deprotected ester linker (P1) and the decomposed KCN-1 (P2). The R in P2 meant that portion of KCN-1 had decomposed. After the reaction was completed, the product was separated by flash chromatography and analyzed by ^{1}H NMR.

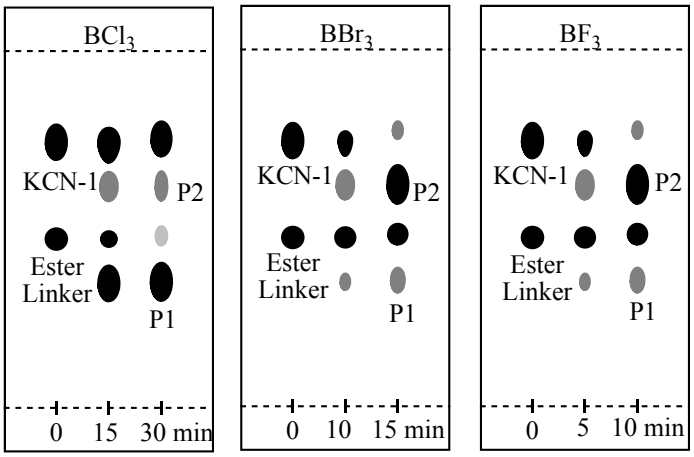


Figure 2.1: Test model reactions monitored by TLC.

Thin layer chromatography (TLC) was used to monitor the overall reaction as shown in Figure 2.1. For boron trichloride, the ester linker was deprotected completely within 30 minutes and KCN-1 decomposed slightly. However, for boron tribromide, the ester linker was not fully deprotected and KCN-1 decomposed entirely at 15 minutes. Boron trifluoride was even worse than BBr_3. For BF_3, KCN-1 had decomposed at 10 minutes. Based on these results, BCl_3 was a better candidate because BF_3 and BBr_3 are too acidic for the reaction. A milligram test of compound **11** will be used to determine the reactivity of BCl_3 soon. Hopefully, the deprotection of compound **11** will be resolved as expected.

3. CONCLUSION

Compound **11** was synthesized in 10 steps with 3.6% overall yield for now, but the step of deprotection seems more difficult than expected. Searching for an effective deprotecting reagent for compound **11** is underway now. If an effective reagent cannot be found in the near future, the design of the research has to be modified to replace the MOM- group with a different protecting group. Hopefully, the deprotection of compound **11** will be resolved soon. Then this research will be complete once compound **11** is deprotected and attached to the immobilized bead. After the small molecule is completely synthesized, it will be sent off for affinity labeling and analysis of the mass of the peptide sequence to determine the binding target of KCN-1.

4. EXPERIMENTAL

4.1 General Methods

NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a Bruker 400 Avance instrument. Chemical shifts (δ values) and coupling constants (J values) are reported in parts per million (ppm) and hertz (Hz), respectively, using TMS (^{1}H NMR) or deuterated solvent (^{13}C NMR) as the internal standard (s = singlet, d =doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet, br = broad). Unless otherwise noted, all reagents and the authentic samples were purchased from commercial sources and were used without further purification. Flash chromatography was carried out on flash silica gel (Sorbent 230–400 mesh). TLC analysis was conducted on silica gel plates (Sorbent Silica G UV254). Mass spectroscopy was recorded on API 3200 LC/MS/MS system.

4.2 Photocleavable Linker

3-Hydroxy-4-iodobenzoic acid 2. To a mixture of a solution of 3-hydroxybenzoic acid **1** (13.8 g, 0.10 mol) in concentrated aqueous ammonia was slowly added a mixture of I_2 (23.4 g, 0.09 mol) and potassium iodide (18.6 g, 0.11 mol) in de-ionized water (100 mL) (to prevent from generating NI_3). The reaction mixture was stirred at room temperature for 40 minutes, and then acidified by concentrated HCl (180 mL). The precipitate was collected by filtration. The filtrate was extracted with EtOAc (250 mL × 2). The precipitate was combined with the organic layer and the EtOAc was removed under reduced pressure. The crude compound was purified by recrystalization (H_2O/MeOH=5/1) to yield white crystal (9.24 g, 35%). ^{1}H NMR (MeOD): δ 7.79 (d, J=8.0 Hz, 1H), 7.46 (d, J=2.0 Hz, 1H), 7.22 (dd, J=8.0, 2.0 Hz, 1H). ^{13}C NMR (MeOD): δ 169.5, 158.3, 140.6, 133.4, 123.1, 116.3, 93.4. ESI-MS (−): Calcd for $C_7H_5IO_3$: 263.93 m/z. Found: 262.9 m/z $[M-H]^-$.

5-(Hydroxymethyl)-2-iodophenol 3. Under N_2 atmosphere at 0 ºC, to a solution of 3-hydroxy-4-iodobenzoic acid **2** (9.30 g, 0.04 mol) in anhydrous THF (100 mL) was slowly added borane in THF (70.0 mL, 0.07 mol). The reaction mixture was stirred at room temperature overnight. Then reaction mixture was quenched with 2 M Na_2CO_3 (10 mL). The aqueous solution was extracted with Et_2O (20 mL × 2). The organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by flash chromatography on silica gel (hexane/EtOAc=5/1) to yield pale yellow crystal (8.60 g, 86%). ^{1}H NMR (CD_3OD): δ 7.61 (d, J=8.0 Hz, 1H), 6.86 (s, 1H), 6.59 (dd, J=8.0 Hz, 1H), 4.50 (s, 2H). ^{13}C NMR (CD_3OD): δ 158.2, 145.0, 140.3, 120.9, 114.4, 82.9, 64.6. ESI-MS (−): Calcd for $C_7H_7IO_2$: 249.95 m/z. Found: 248.4 m/z $[M-H]^-$.

(4-Iodo-3-(methoxymethoxy)phenyl)methanol 4. To a mixture of 5-(hydroxymethyl)-2-iodophenol **3** (5.00 g, 0.02 mol) and K_2CO_3 (5.60 g, 0.04 mol) in acetone (4 mL) was added methoxymethyl chloride (4 mL, 0.53 mol) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was filtered and washed with acetone. The filtrate was concentrated under reduced pressure. The residue was added H_2O (20 mL) and EtOAc (20 mL). Then the aqueous solution was further extracted with EtOAc (20 mL × 2). The organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel (hexane/EtOAc=7/1 (200 mL), 2/1 (400 mL), 3/2 (200 mL)) to yield yellow liquid (1.94 g, yield 33%). ESI-MS (−): Calcd for $C_9H_{11}IO_3$: 293.98 m/z. Found: 292.9 m/z $[M-H]^-$.

4-(Azidomethyl)-1-iodo-2-(methoxymethoxy)benzene 5. Under N_2 atmosphere at 0 ºC, to a solution of (4-iodo-3-(methoxymethoxy)phenyl) methanol **4** (104 mg, 0.35 mmol) in

anhydrous THF (5 mL) was added diphenyl phosphorazidate (DPPA) (91 μL, 0.42 mmol) and the resulting mixture was stirred for ten minutes. Then diazabicycloundecene (DBU) (64 μL, 0.42 mmol) was added into the solution. The reaction was warmed to room temperature and stirred overnight under N_2. Then reaction mixture was concentrated under reduced pressure. The residue was added H_2O (10 mL) and EtOAc (10 mL). The aqueous solution was further extracted with EtOAc (10 mL × 2). The organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel (hexane/EtOAc=20/1) to yield yellow liquid (78 mg, yield 70%). ^{1}H NMR ($CDCl_3$): δ 7.74 (d, J=8.0 Hz, 1H), 7.01 (d, J=2.0 Hz, 1H), 6.71 (dd, J=8.0, 2.0 Hz, 1H), 5.23 (s, 2H), 4.27 (s, 2H), 3.49 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 156.3, 139.7, 137.3, 123.2, 114.4, 94.9, 86.8, 56.5, 54.2. ESI-MS (−): Calcd for $C_9H_{10}IN_3O_2$: 318.98 m/z. Found: 317.7 m/z $[M-H]^-$.

(4-Iodo-3-(methoxymethoxy)phenyl)methanamine 6. To a solution of 4-(azidomethyl)-1-iodo-2-(methoxymethoxy)benzene **5** (1.60 g, 4.9 mmol) and triphenylphosphine (PPh_3) (2.60 g, 9.9 mmol) in THF (49 mL, 10 mL per mmol) was added de-ionized water (2 mL, 0.04 mL per mmol) and the reaction was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure. The residue was added brine (10 mL) and EtOAc (10 mL). The aqueous solution was further extracted with EtOAc (15 mL × 2). The organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel (hexane/EtOAc=20/1 (200 mL) to elute out PPh_3, then pure EtOAc (100 mL) once the triphenylphosphine oxide came out, finally pure MeOH (300 mL) for the product) to yield yellow liquid (1.35 g, yield 94%). ESI-MS (+): Calcd for $C_9H_{12}INO_2$: 292.99 m/z. Found: 293.9 m/z $[M+H]^+$.

***tert*-Butyl 4-iodo-3-(methoxymethoxy)benzylcarbamate 7**. To a solution of (4-iodo-3-(methoxymethoxy)phenyl)methanamine **6** (25 mg, 0.08 mmol) and di-*tert*-butyl dicarbonate (23 mg, 0.10 mmol) in MeOH (5 mL) was added triethylamine (14 μL, 0.10 mmol) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel (hexane/EtOAc=10/1) to yield yellowish white crystal (17 mg, yield 53%). ^{1}H NMR (D_2O): δ 7.71 (d, *J*=8.0 Hz, 1H), 6.98 (d, *J*=2.0 Hz, 1H), 6.71 (d, *J*=8.0 Hz, 1H), 5.23 (s, 2H), 4.89 (s, 1H), 4.25 (d, *J*=8 Hz, 2H), 3.51 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (D_2O): δ 156.4, 156.1, 141.4, 139.7, 122.8, 114.1, 95.2, 85.7, 79.9, 56.7, 44.5, 28.6, 27.6. ESI-MS (+): Calcd for $C_{14}H_{20}INO_4$: 393.04 m/z. Found: 394.4 m/z $[M+H]^+$.

(*E*)-3-(4-((*tert*-Butoxy carbonylamino) methyl)-2- (methoxymethoxy) phenyl) acrylic acid 8. Under N_2 atmosphere at room temperature, to a mixture of $(t\text{-Bu})_4N^+$ Br □ (188 mg, 0.58 mmol), $Pd(OAc)_2$ (5.4 mg, 0.02 mmol), and K_2CO_3 (642 mg, 4.7 mmol) was added a solution of *tert*-butyl 4-iodo-3-(methoxymethoxy)benzylcarbamate **7** (183 mg, 0.47 mmol) in DMF (2 mL). Then acrylic acid (48 μL, 0.70 mmol) was added into the mixture. The reaction mixture was heated to 60^o C and stirred overnight. Then the reaction mixture was cooled to room temperature. Cold H_2O (5 mL) and 1 M HCl (1 mL) were added to the mixture. The reaction mixture was added brine (10 mL) and EtOAc (15 mL). The aqueous solution was further extracted with EtOAc (15 mL × 2). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel (hexane/EtOAc=20/1 (200 mL), 4/1 (400 mL), 1/1 (200 mL)) to yield yellow crystal (81 mg, 51%). ^{1}H NMR (D_2O): δ 8.09 (d, *J*=16.0 Hz, 1H), 7.51 (d, *J*=8.0 Hz, 1H), 7.08 (s, 1H), 6.95 (d, *J*=8.0 Hz, 1H), 6.51 (d, *J*=16 Hz, 1H), 5.26 (s, 2H), 4.96 (s, 1H), 4.31 (d, J=8 Hz, 2H), 3.51 (s,

3H), 1.47 (s, 9H). ^{13}C NMR (D_2O): δ 172.5, 156.6, 156.2, 143.8, 141.9, 129.2, 123.0, 120.9, 117.9, 113.8, 94.8, 80.0, 56.6, 44.7, 28.6. ESI-MS (−): Calcd for $C_{17}H_{23}NO_6$: 337.15 m/z. Found: 336.2 m/z $[M-H]^-$.

(*E*)-2,5-Dioxopyrrolidin-1-yl 3-(4-((*tert*-butoxycarbonylamino)methyl)-2-(methoxy methoxy) phenyl)acrylate 9. Under N_2 atmosphere at room temperature, to a solution of (*E*)-3-(4-((*tert*-butoxycarbonylamino)methyl)-2-(methoxymethoxy)phenyl)acrylic acid **8** (70 mg, 0.21 mmol) and EDCI (39 mg, 0.21 mmol) in anhydrous DCM (3 mL) was added *N*-hydroxysuccinimide (24 mg, 0.21 mmol) dissolved in anhydrous DCM (1 mL) and the resulting mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure. The residue was added brine (10 mL) and EtOAc (10 mL). The aqueous solution was further extracted with EtOAc (10 mL × 2). The organic layer was dried in $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel (hexane/EtOAc=4/1 (100 mL), 3/1 (400 mL), and 1/1 (300 mL)) to yield colorless crystals (57 mg, 63%). ESI-MS (+): Calcd for $C_{21}H_{26}N_2O_8$: 434.17 m/z. Found: 435.1 m/z $[M+H]^+$.

(*E*)-2-(3-(4-((*tert*-Butoxycarbonylamino)methyl)-2-(methoxymethoxy) phenyl) acryl amido) -3-(3,4-dihydroxyphenyl)propanoic acid 10. To a solution of (*E*)-2,5-dioxopyrrolidin-1-yl 3-(4-((*tert*-butoxycarbonylamino)methyl)-2-(methoxymethoxy)phenyl) acrylate **9** (57 mg, 0.13 mmol) and (*S*)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid (L-DOPA) (22 mg, 0.11 mmol) in anhydrous DMF (1.5 mL) was added triethyl amine (NEt_3) (18 μL, 0.13 mmol) and the reaction was stirred at room temperature overnight. Then the pH was adjusted from 8 to 3 by adding 1 M HCl (~10 mL) to the mixture. The reaction mixture was added brine (10 mL) and EtOAc (15 mL). The aqueous solution was further extracted with EtOAc (10 mL × 2). The organic layer was dried in $MgSO_4$ and concentrated under reduced pressure. The crude was

purified by flash chromatography on silica gel (DCM/MeOH=14/1 (200 mL), 10/1 (400 mL), and 5/1 (100 mL)) to yield yellow crystals (46 mg, 68%). ^{1}H NMR (MeOD): δ 7.82 (d, *J*=16 Hz, 1H), 7.47 (d, *J*=8 Hz, 1H), 7.07 (s, 1H), 6.89 (d, *J*=8 Hz, 1H), 6.70 (d, *J*=16 Hz, 1H), 6.67 (s, 1H), 6.66 (d, *J*=8 Hz, 1H), 6.57 (d, *J*=8 Hz, 1H), 5.22 (s, 2H), 4.71 (dd, *J*=7.2, 5.6 Hz, 1H), 4.19 (s, 2H), 3.43 (s, 3H), 3.10 (dd, *J*=14, 6 Hz, 1H), 2.91 (dd, *J*=14, 6 Hz, 1H), 1.45 (s, 9H). ^{13}C NMR (MeOD): δ 175.9, 168.8, 158.7, 157.3, 146.2, 145.2, 144.6, 137.0, 130.1, 129.3, 124.4, 121.9, 121.8, 121.6, 117.5, 116.4, 114.6, 95.8, 80.4, 56.9, 56.1, 44.9, 38.2, 28.9. Calcd for $C_{26}H_{32}N_2O_9$: 516.21 m/z. Found: 515.1 m/z $[M-H]^-$.

(*E*)-*tert*-Butyl 4-(3-(3-(3,4-dihydroxyphenyl)-1-(4-(4-(*N*-((2,2-dimethyl-2H-chromen-6-yl)methyl)-*N*-phenylsulfamoyl)-2-methoxyphenoxy)butylamino)-1-oxopropan-2-ylamino)-3-oxoprop-1-enyl)-3-(methoxymethoxy)benzylcarbamate 11. Under N_2 atmosphere at room temperature, to a solution of (*E*)-2-(3-(4-((*tert*-butoxycarbonylamino) methyl)-2-(methoxy methoxy)phenyl) acrylamido)-3-(3,4-dihydroxy phenyl) propanoic acid **10** (21 mg, 0.04 mmol), EDCI hydrochloride (8, 0.04 mmol), and HOBt (5 mg, 0.04 mmol) in anhydrous DCM (2 mL) was added KCN-1 amine **12d** (20 mg, 0.04 mmol) dissolved in anhydrous DCM (0.2 mL) by a syringe. Two equivalent of anhydrous DCM (0.2 mL) was used to wash the vial and syringe used to add the KCN-1 amine. The reaction was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel (DCM/MeOH=20/1 (100 mL), 10/1 (100 mL), 5/1 (150 mL), pure MeOH (50 mL)) to yield white crystal (19 mg, 47%). ^{1}H NMR (MeOD): δ 7.85 (d, *J*=16 Hz, 1H), 7.51 (d, *J*=8 Hz, 1H), 7.29 (m, 2H), 7.23 (m, 3H), 7.08 (m, 3H), 7.00 (m, 2H), 6.93 (m, 2H), 6.82 (m, 1H), 6.69 (m, 2H), 6.60 (m, 1H), 6.53 (m, 1H), 6.22 (d, *J*=10 Hz, 1H), 5.62 (d, *J*=10 Hz, 1H), 5.25 (s, 3H), 4.61 (s, 3H), 4.21 (s, 2H), 4.02 (t, 2H), 3.69 (s, 3H), 3.44 (s,

3H), 3.14 (dd, *J*=12, 6 Hz, 1H), 2.96 (dd, *J*=12, 6 Hz, 1H), 2.86 (dd, *J*=12, 6 Hz, 1H), 1.65 (m, 5H). ^{13}C NMR (MeOD): δ 173.7, 168.8, 157.5, 153.9, 150.6, 146.4, 145.4, 140.6, 137.3, 132.2, 131.1, 130.6, 129.9, 129.1, 127.9, 124.5, 123.2, 122.6, 121.75, 117.6, 117.1, 116.6, 114.7, 113.2, 111.9, 95.9, 80.5, 77.5, 70.0, 57.1, 56.7, 55.1, 49.8, 48.5, 45.0, 40.0, 38.9, 30.9, 28.9, 28.3, 27.3, 27.0. ESI-MS (−): Calcd for $C_{55}H_{64}N_4O_{13}S$: 1020.42 m/z. Found: 1019.8 m/z $[M-H]^-$.

4.3 KCN-1 AMINE (**12d**)

***N*-((2,2-Dimethyl-2H-chromen-6-yl)methyl)-4-hydroxy-3-methoxy-*N*-phenyl benzene sulfonamide 12b**. Under N_2 atmosphere at 150 ºC, to a mixture of K_2CO_3 (149 mg, 1.07 mmol) in *N*-Methyl-2-pyrrolidone (NMP) (2 mL) was added thiophenol (PhSH) (0.11 mL, 1.07 mmol) and the reaction mixture was stirred for 15 minutes. Then a solution of *N*-((2,2-dimethyl-2H-chromen-6-yl)methyl)-3,4-dimethoxy-*N*-phenylbenzene-sulfonamide **12a** (100 mg, 0.21 mmol) in NMP (1 mL) was added into the reaction mixture. The reaction was stirred for 5 minutes (no longer than 5 minutes) and immediately poured into cold de-ionized H_2O (25 mL). The aqueous solution was extracted with EtOAc (50 mL × 4). Brine (~10 mL) was added as needed for better separation. The organic layer was washed with de-ionized water (20 mL × 6) to get rid of most of NMP. The organic layer was dried in $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel (DCM/EtOAc=50/1 (500 mL), 30/1 (200 mL)) to yield white crystal (57 mg, 60%). ESI-MS (−): Calcd for $C_{25}H_{25}NO_5S$: 451.15 m/z. Found: 450.1 m/z $[M-H]^-$.

***N*-((2,2-Dimethyl-2H-chromen-6-yl)methyl)-4-(4-(1,3-dioxoisoindolin-2-yl)butoxy)-3-methoxy-*N*-phenylbenzenesulfonamide 12c**. Under N_2 atmosphere at 50 º C, a solution of 2-(4-bromobutyl) isoindoline-1,3-dione (90 mg, 0.32 mmol), *N*-((2,2-dimethyl-2H-chromen-6-yl)methyl)-4-hydroxy-3-methoxy-*N*-phenyl benzenesulfonamide **12b** (130 mg, 0.29 mmol), and

K_2CO_3 (44 mg, 0.32 mmol) in (DMF) (2 mL) was stirred overnight. The reaction mixture was added de-ionized H_2O (20 mL) and EtOAc (20 mL). The aqueous solution was further extracted with EtOAc (100 mL × 2). The organic layer was dried in $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel (DCM/EtOAc=50/1) to yield colorless solid (89 mg, 47%). ^{1}H NMR (MeOD): δ 7.75 (m, 2H), 7.65 (m, 2H), 7.32 (m, 1H), 7.27 (m, 3H), 6.99 (m, 2H), 6.97 (m, 4H), 6.59 (d, *J*=8 Hz, 1H), 6.21 (d, *J*=8 Hz, 1 H), 5.55 (d, *J*=8 Hz, 1H), 4.59 (s, 2H), 4.12 (m, 3H), 3.88 (m, 2H), 3.73 (s, 3H), 2.04 (s, 1H), 1.92 (s, 4H), 1.27 (s, 6H), 1.24 (m, 2H). ESI-MS (+): Calcd for $C_{37}H_{36}N_2O_7S$: 652.22 m/z. Found: 653.3 m/z $[M+H]^+$.

4-**(4-Aminobutoxy)-*N*-((2,2-dimethyl-2H-chromen-6-yl)methyl)-3-methoxy-*N*-phenyl benzenesulfonamide (KCN-1 amine) 12d**. Under N_2 atmosphere at refluxed, to a solution of *N*-((2,2-dimethyl-2H-chromen-6-yl)methyl)-4-(4-(1,3-dioxoisoindolin-2-yl)butoxy)-3-methoxy-*N*-phenylbenzenesulfonamide **12c** (48 mg, 0.07 mmol) in EtOH/DCM (75/25) (3 mL) was added hydrazine monohydrate (H_2N-NH_2·H_2O) (7.1 μL, 0.15 mmol) and the resulting mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure and then vacuum filtrate with DCM. The filtrate organic layer was concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel (DCM/MeOH=20/1 (200 mL), 10/1 (500 mL), 5/1 (300 mL)) to yield colorless solid (20 mg, 55%). ^{1}H NMR (MeOD): δ 7.29 (m, 1H), 7.27 (m, 3H), 6.98 (m, 2H), 6.94 (m, 1H), 6.91 (m, 2H), 6.85 (m, 1H), 6.57 (d, J=8 Hz, 1H), 6.20 (d, J=8 Hz, 1H), 5.55 (d, J=8 Hz, 1H), 4.60 (s, 2H), 4.05 (t, 2H), 3.69 (s, 3H), 2.74 (t, 2H), 1.87 (m, 5H), 1.70 (s, 3H), 1.61 (m, 2H), 1.35 (s, 6H). ESI-MS (+): Calcd for $C_{29}H_{34}N_2O_5S$: 522.22 m/z. Found: 523.3 m/z $[M+H]^+$.

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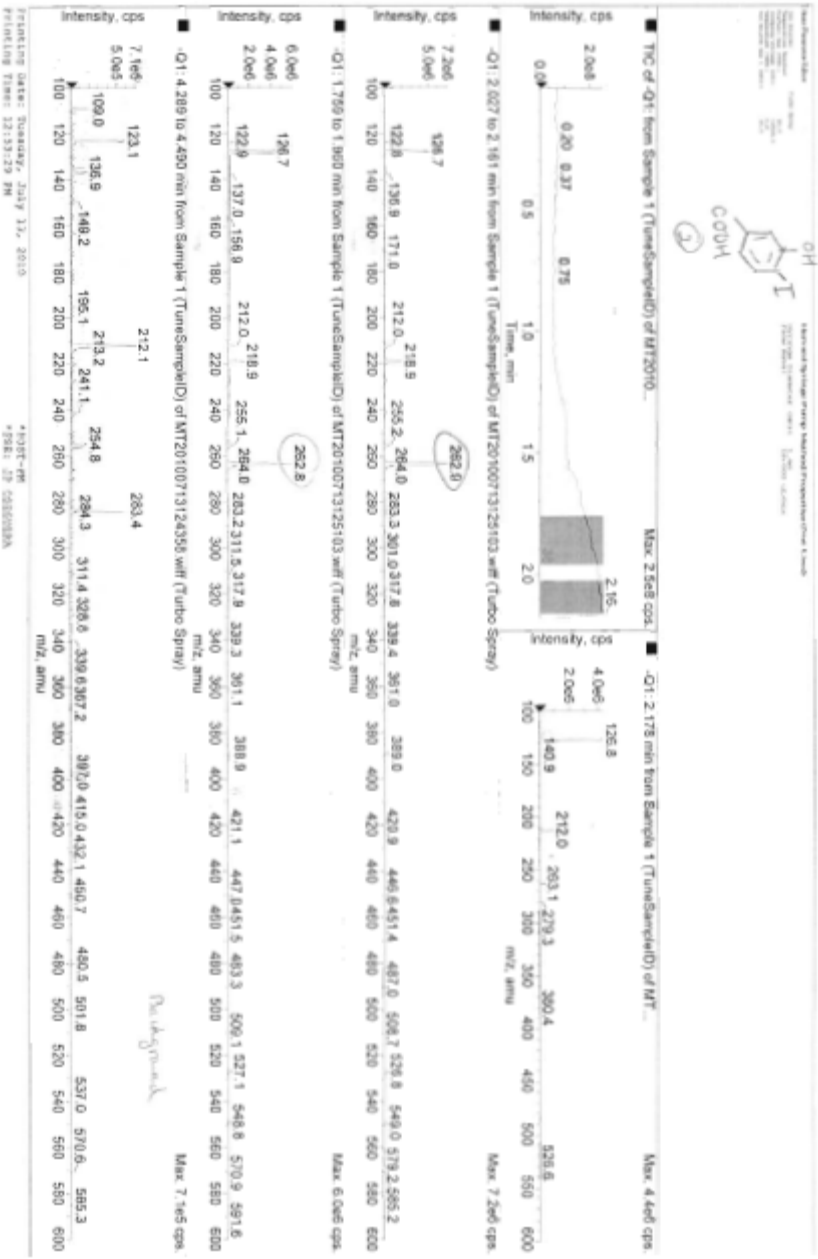
APPENDICES



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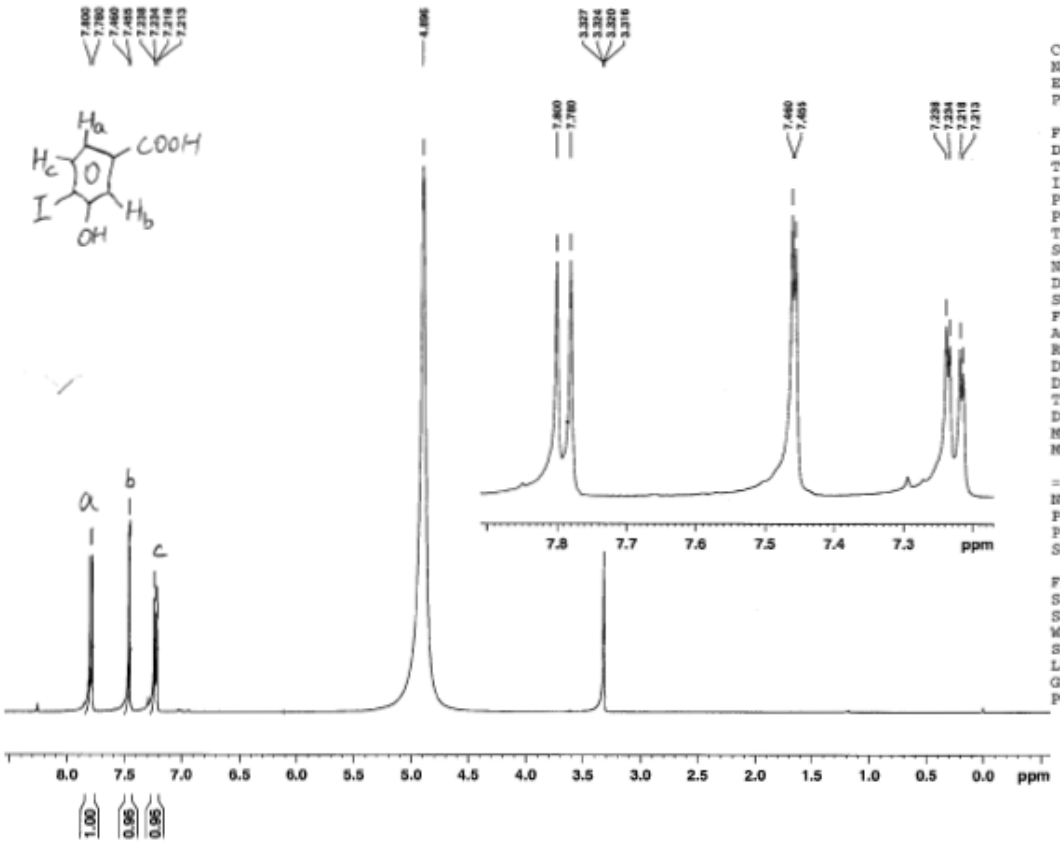


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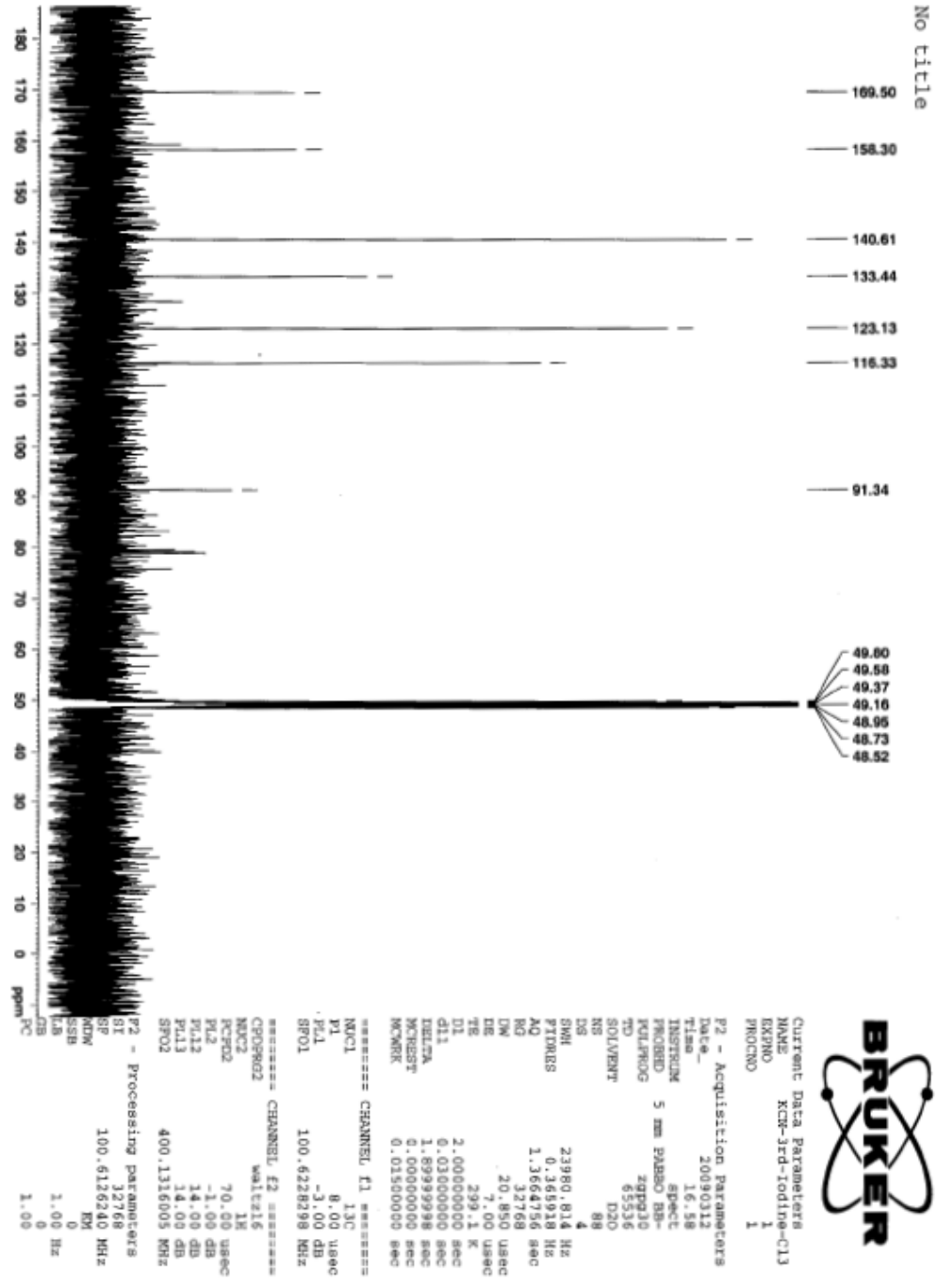
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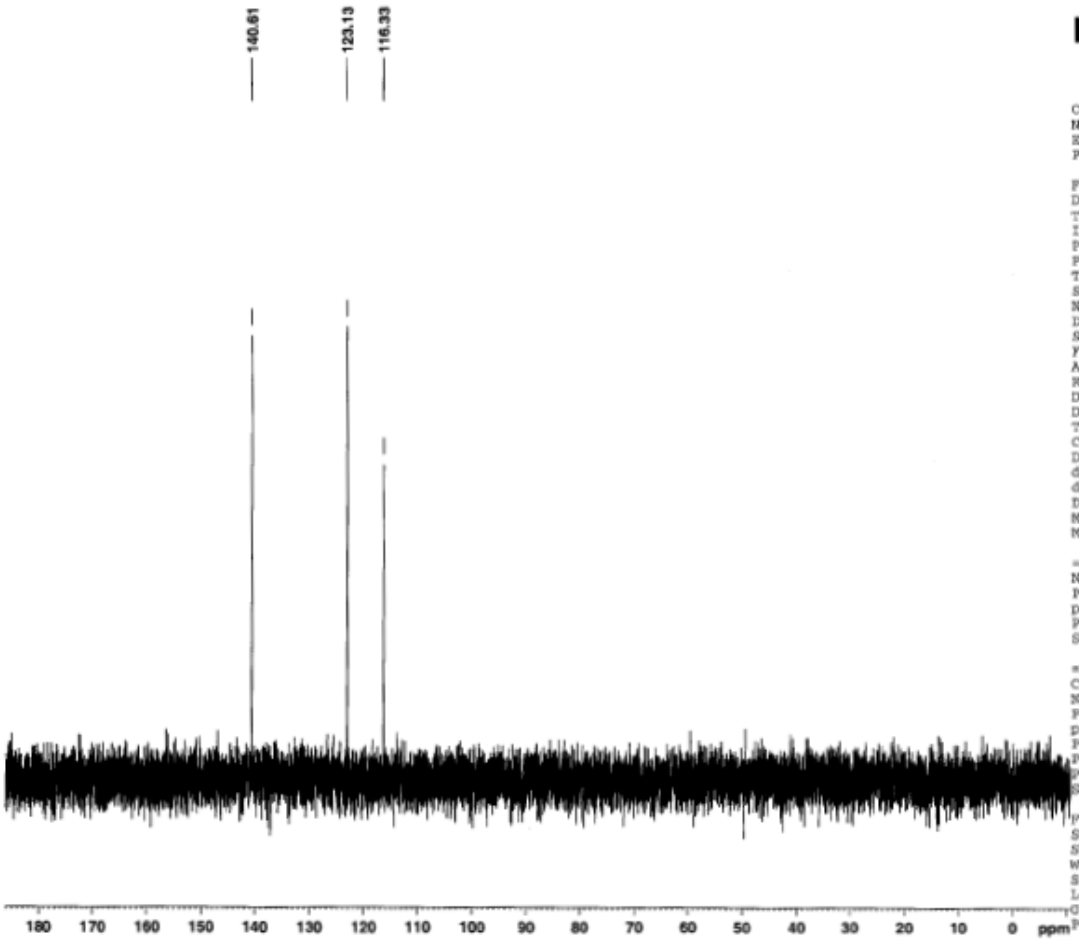

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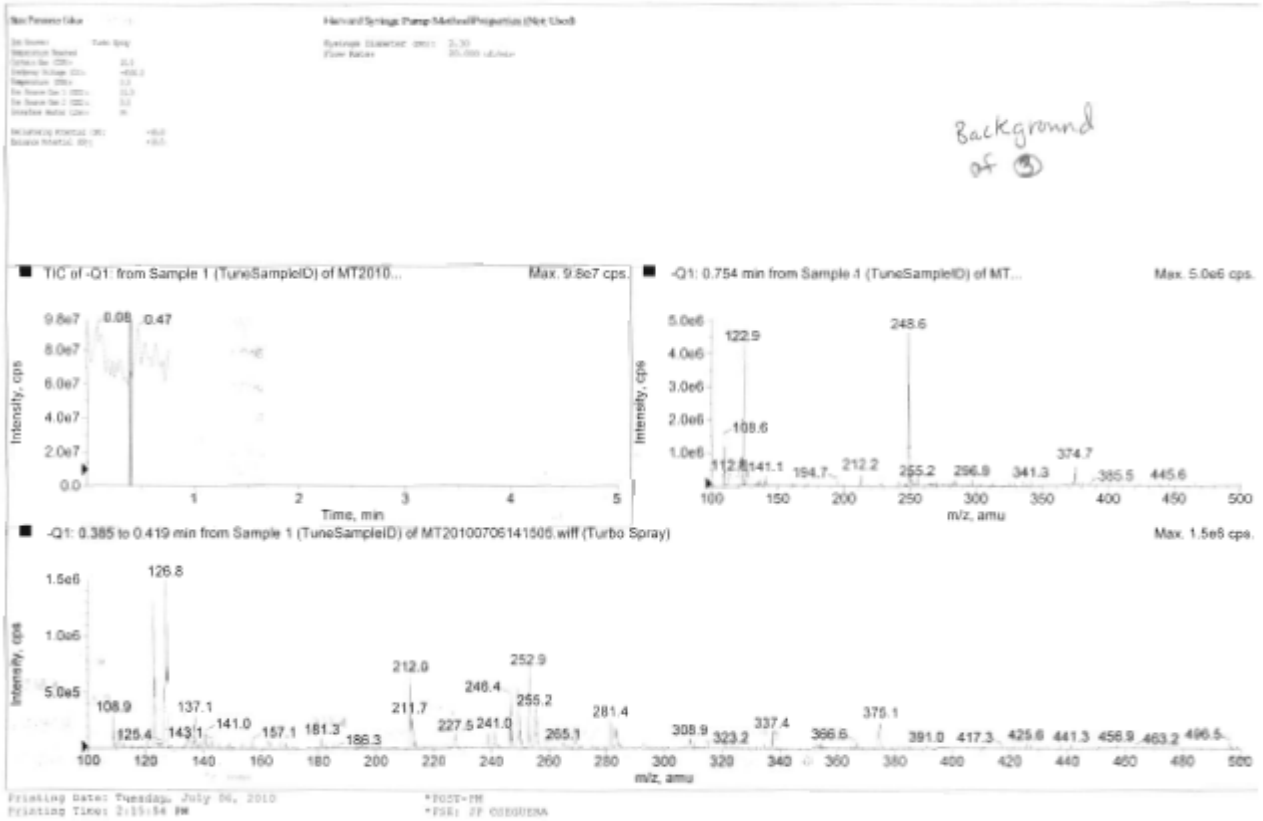
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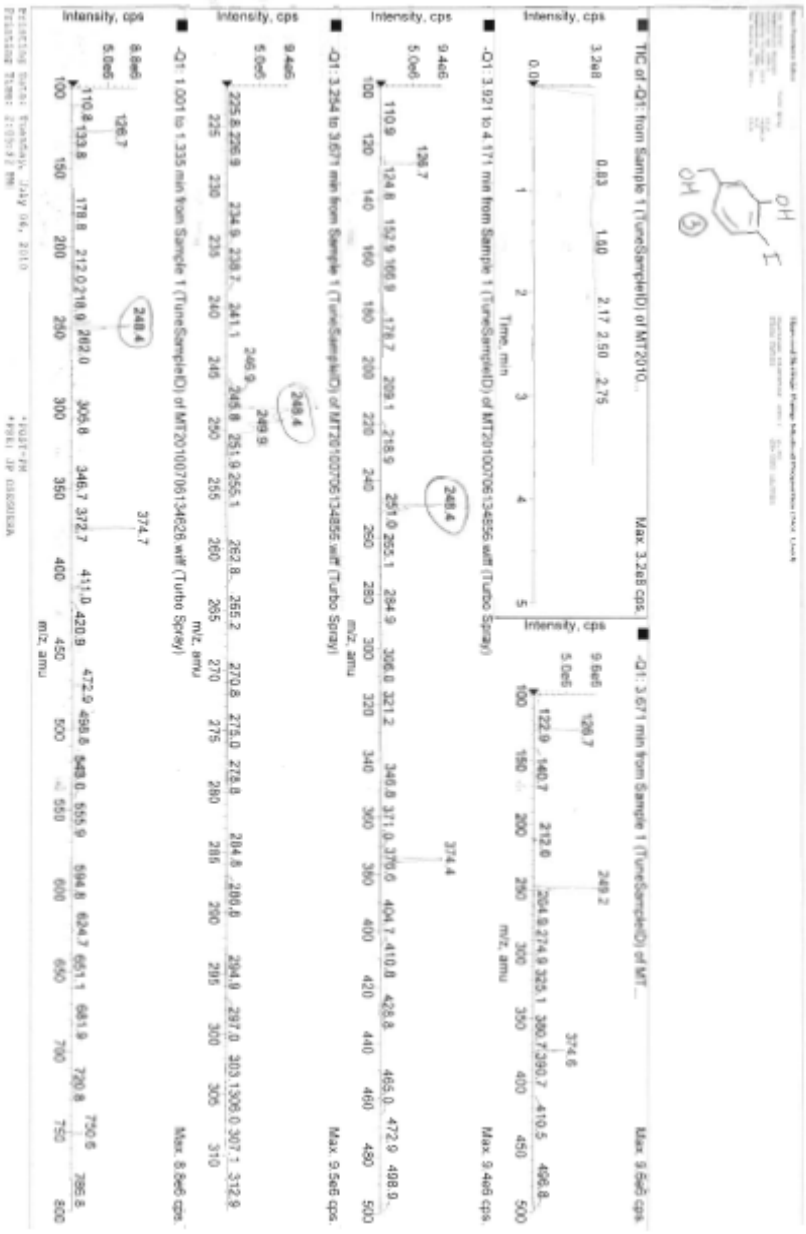
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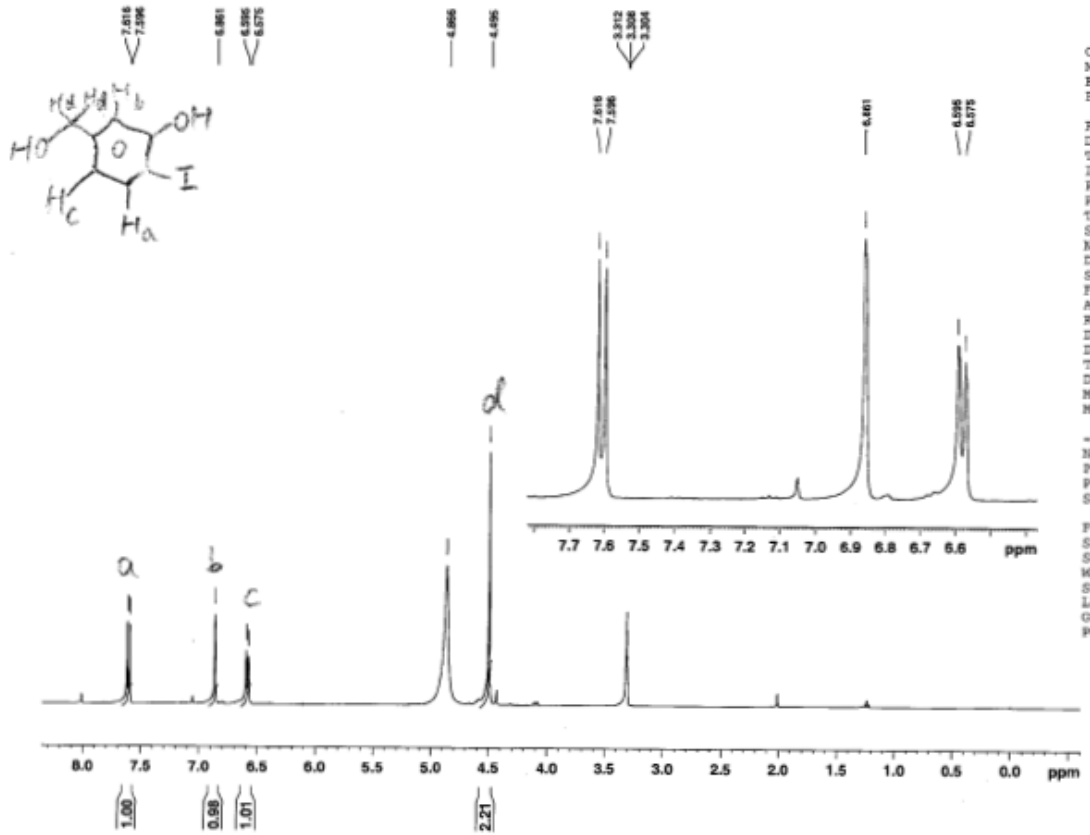
Max. 3.2e8 cps.
Intensity, cps
Time, min
-Q1: 3.671 min from Sample 1 (TuneSampleID) of MT...
Max. 9.5e6 cps.
-Q1: 3.921 to 4.171 min from Sample 1 (TuneSampleID) of MT20100706134856.wiff (Turbo Spray)
Max. 9.4e6 cps.
-Q1: 3.254 to 3.671 min from Sample 1 (TuneSampleID) of MT20100706134855.wiff (Turbo Spray)
Max. 9.5e6 cps.
-Q1: 1.001 to 1.335 min from Sample 1 (TuneSampleID) of MT20100706134826.wiff (Turbo Spray)
Max. 8.8e6 cps.
m/z, amu

No title



Current Data Parameters

NAME	KCN-3rd-Iodine-Raduc-H
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

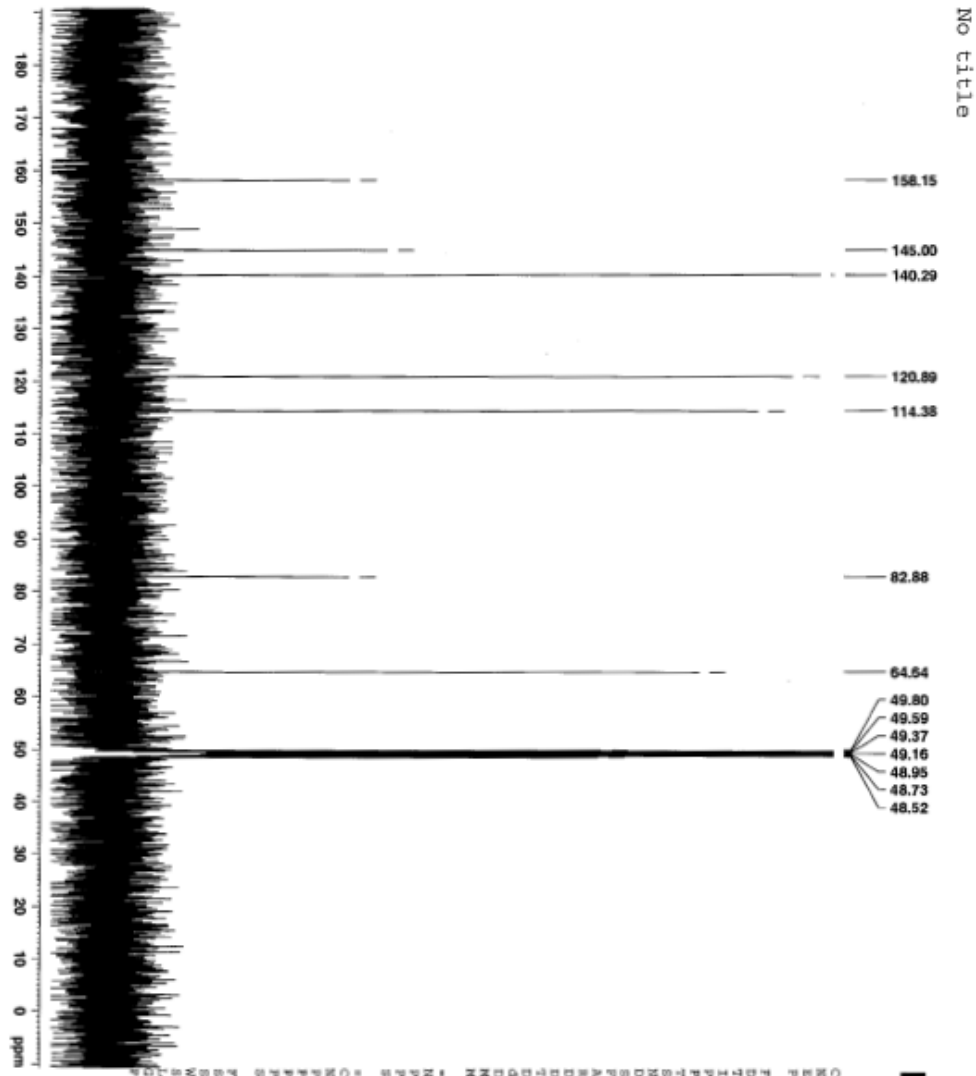
Date_	20090313	
Time	18.47	
INSTRUM	spect	
PROBHD	5 mm PABBO BB-	
PULPROG	zg30	
TD	65536	
SOLVENT	MeOD	
NS	16	
DS	2	
SWH	8278.146	Hz
FIDRES	0.126314	Hz
AQ	3.9584243	sec
RG	181	
DW	60.400	usec
DE	7.00	usec
TE	298.9	K
D1	1.00000000	sec
MCREST	0.00000000	sec
MCWRK	0.01500000	sec

-------- CHANNEL f1 --------

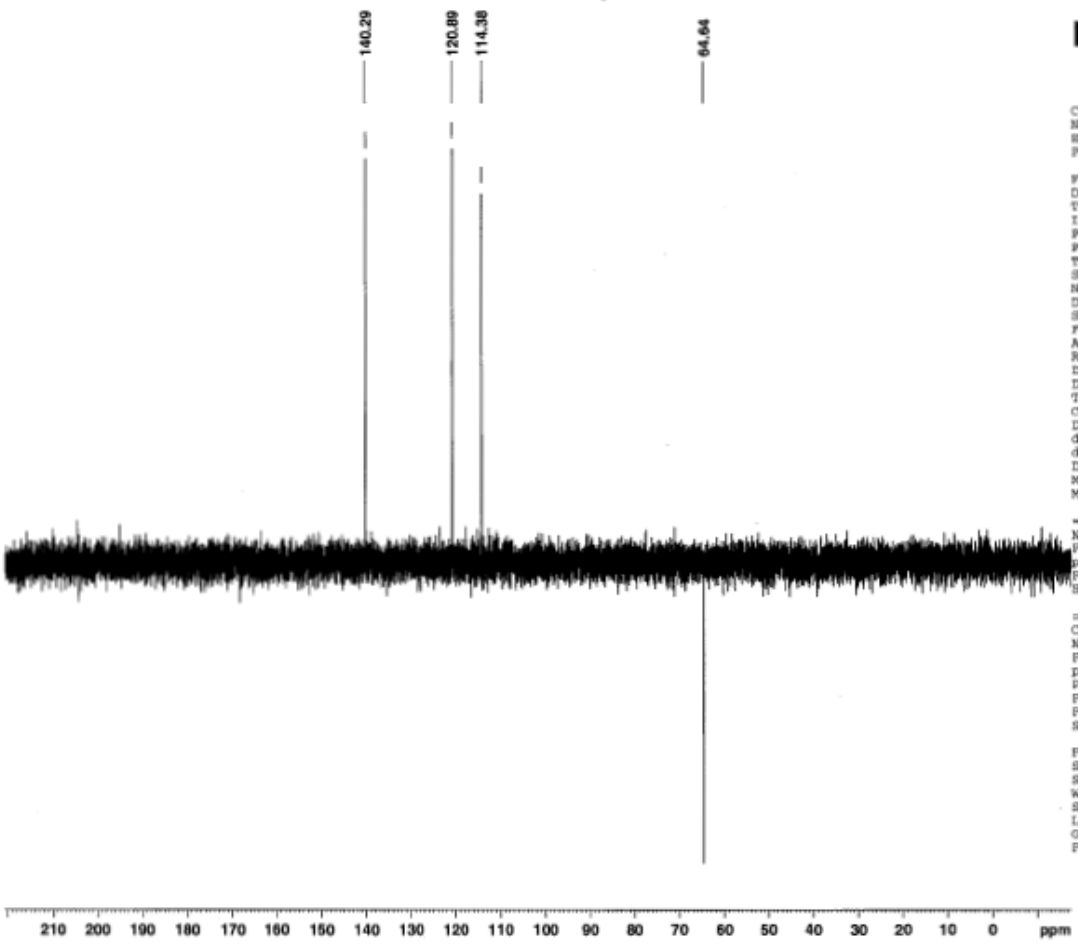
NUC1	1H	
P1	12.80	usec
PL1	0.00	dB
SFO1	400.1324710	MHz

F2 - Processing parameters

SI	32768	
SF	400.1300086	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.40	

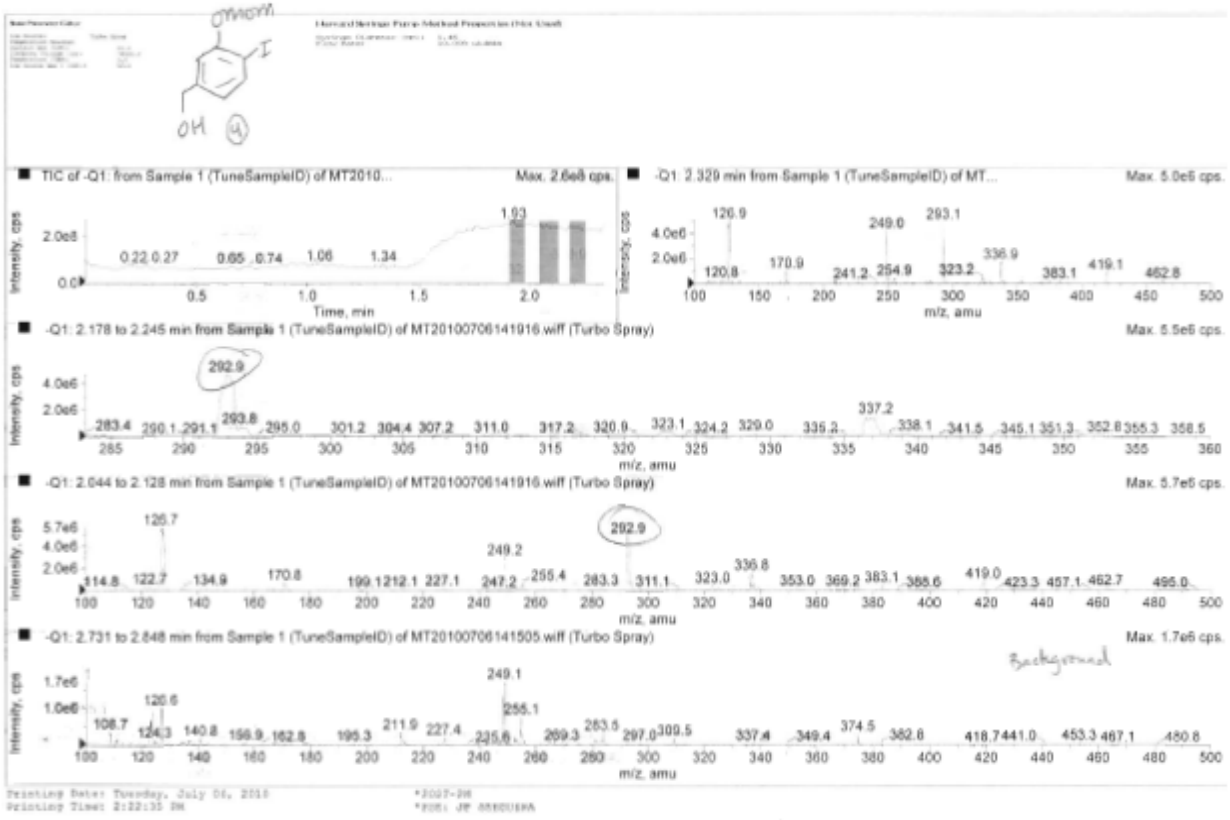

No title
158.15
145.00
140.29
120.89
114.38
82.88
64.54
49.80
49.59
49.37
49.16
48.95
48.73
48.52
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
BRUKER
Current Data Parameters
NAME MCN-3rd-Iodine-ReDuc-C13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20090313
Time 18.52
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 46
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 32768
DW 20.850 usec
DE 7.00 usec
TE 299.1 K
D1 2.00000000 sec
D11 0.03000000 sec
DELTA 1.89999998 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 8.00 usec
PL1 -3.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -1.00 dB
PL12 14.00 dB
PL13 14.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6126152 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00

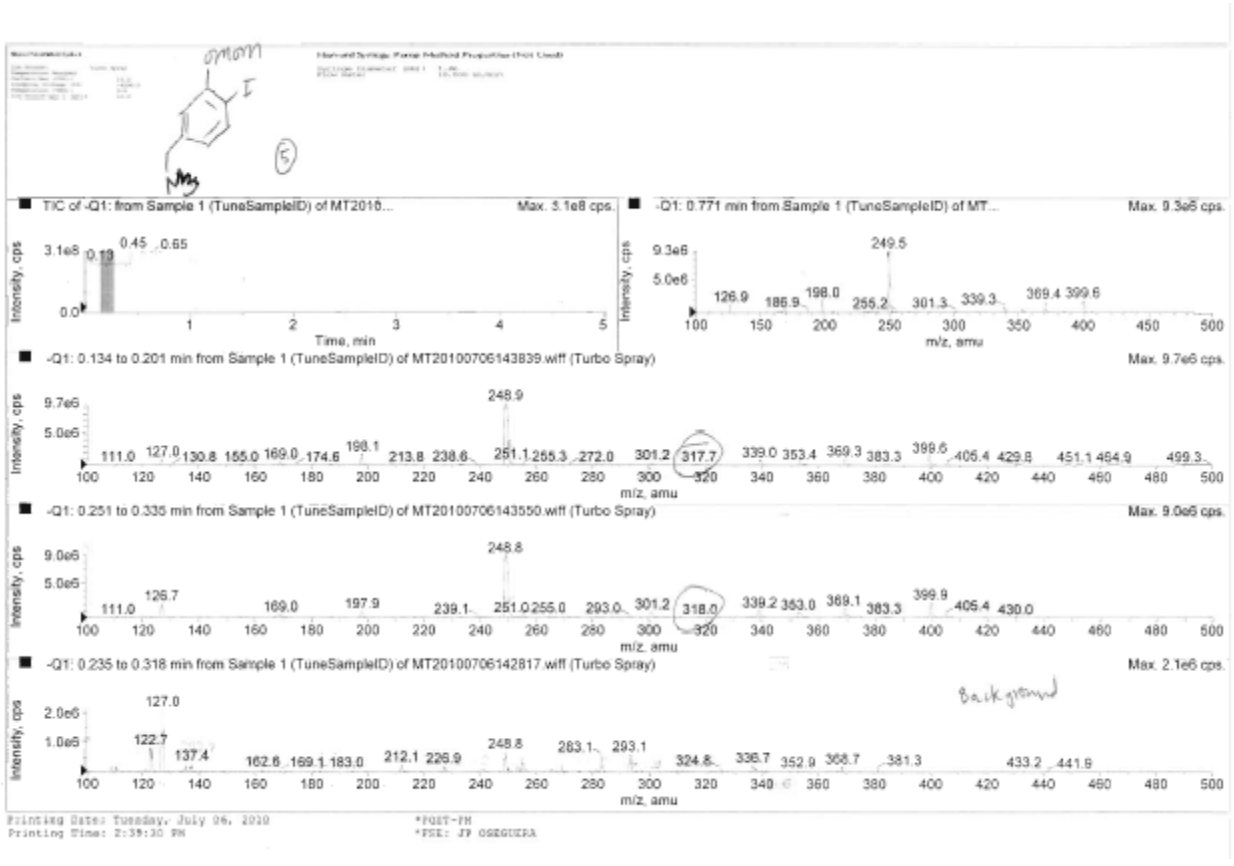
No title



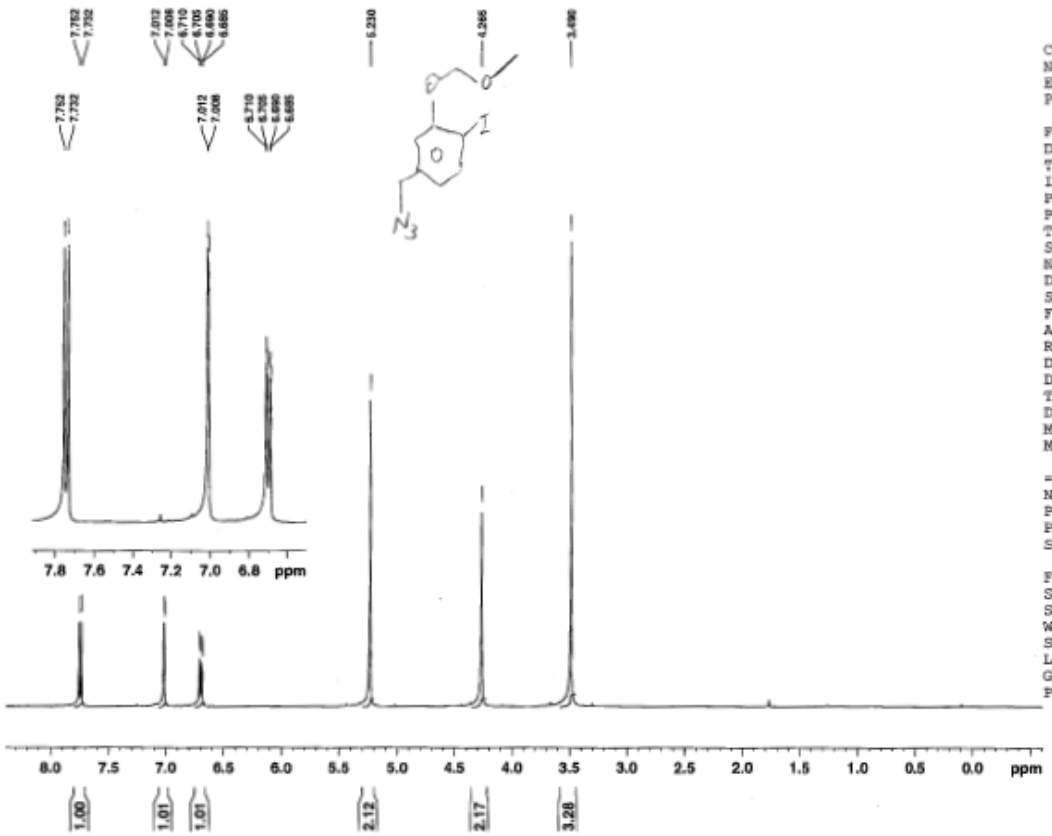
BRUKER

```
Current Data Parameters
NAME       KCN-3rd-Iodine-Reduc-Dept
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20090313
Time              18.57
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG         dept135
TD                65536
SOLVENT             D2O
NS                    40
DS                     4
SWH           23980.814 Hz
FIDRES        0.365918 Hz
AQ            1.3664756 sec
RG                 32768
DW                20.850 usec
DE                 7.00 usec
TE                299.2 K
CNST2         145.0000000
D1             2.00000000 sec
d2             0.00344828 sec
d12            0.00002000 sec
DELTA          0.00001019 sec
MCREST         0.00000000 sec
MCWRK          0.01500000 sec

======== CHANNEL f1 ========
NUC1                  13C
P1                 8.00 usec
p2                16.00 usec
PL1               -1.00 dB
SFO1         100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
P3                12.38 usec
p4                24.75 usec
PCPD2             70.00 usec
PL2               -1.00 dB
PL12              14.00 dB
SFO2         400.1316005 MHz

F2 - Processing parameters
SI                 32768
SF           100.6126152 MHz
WDW                   EM
SSB                    0
LB                1.00 Hz
GB                     0
PC                1.40
```


TIC of -Q1: from Sample 1 (TuneSampleID) of MT2010...
Max. 2.6e8 cps.
Intensity, cps
Time, min
-Q1: 2.329 min from Sample 1 (TuneSampleID) of MT...
Max. 5.0e6 cps.
m/z, amu
-Q1: 2.178 to 2.245 min from Sample 1 (TuneSampleID) of MT20100706141916.wiff (Turbo Spray)
Max. 5.5e6 cps.
-Q1: 2.044 to 2.128 min from Sample 1 (TuneSampleID) of MT20100706141916.wiff (Turbo Spray)
Max. 5.7e6 cps.
-Q1: 2.731 to 2.848 min from Sample 1 (TuneSampleID) of MT20100706141505.wiff (Turbo Spray)
Max. 1.7e6 cps.
Background
Printing Date: Tuesday, July 06, 2010


TIC of -Q1: from Sample 1 (TuneSampleID) of MT2010...
Max. 3.1e8 cps.
Intensity, cps
Time, min
-Q1: 0.771 min from Sample 1 (TuneSampleID) of MT...
Max. 9.3e6 cps.
m/z, amu
-Q1: 0.134 to 0.201 min from Sample 1 (TuneSampleID) of MT20100706143839.wiff (Turbo Spray)
Max. 9.7e6 cps.
-Q1: 0.251 to 0.335 min from Sample 1 (TuneSampleID) of MT20100706143550.wiff (Turbo Spray)
Max. 9.0e6 cps.
-Q1: 0.235 to 0.318 min from Sample 1 (TuneSampleID) of MT20100706142817.wiff (Turbo Spray)
Max. 2.1e6 cps.
Printing Date: Tuesday, July 06, 2010
Printing Time: 2:39:30 PM
*FGET-FM
*FSE: JP OSEGUEPA

No title



Current Data Parameters

NAME	KCN-3rd-OMOM-N3-H
EXPNO	4
PROCNO	1

F2 - Acquisition Parameters

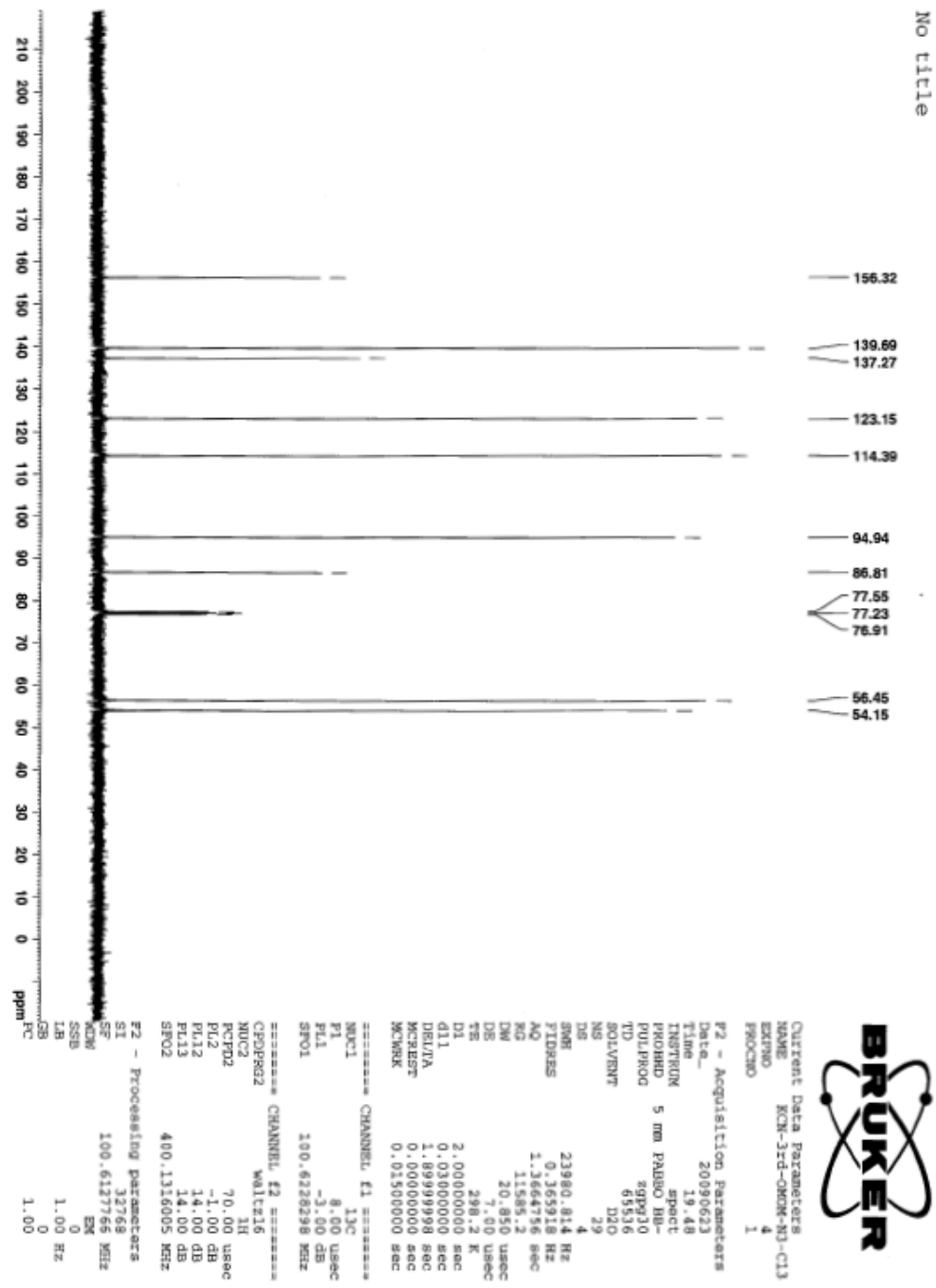
Date_	20090623
Time	19.44
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	4
DS	2
SWH	8278.146 Hz
FIDRES	0.126314 Hz
AQ	3.9584243 sec
RG	28.5
DW	60.400 usec
DE	7.00 usec
TE	297.9 K
D1	1.00000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec

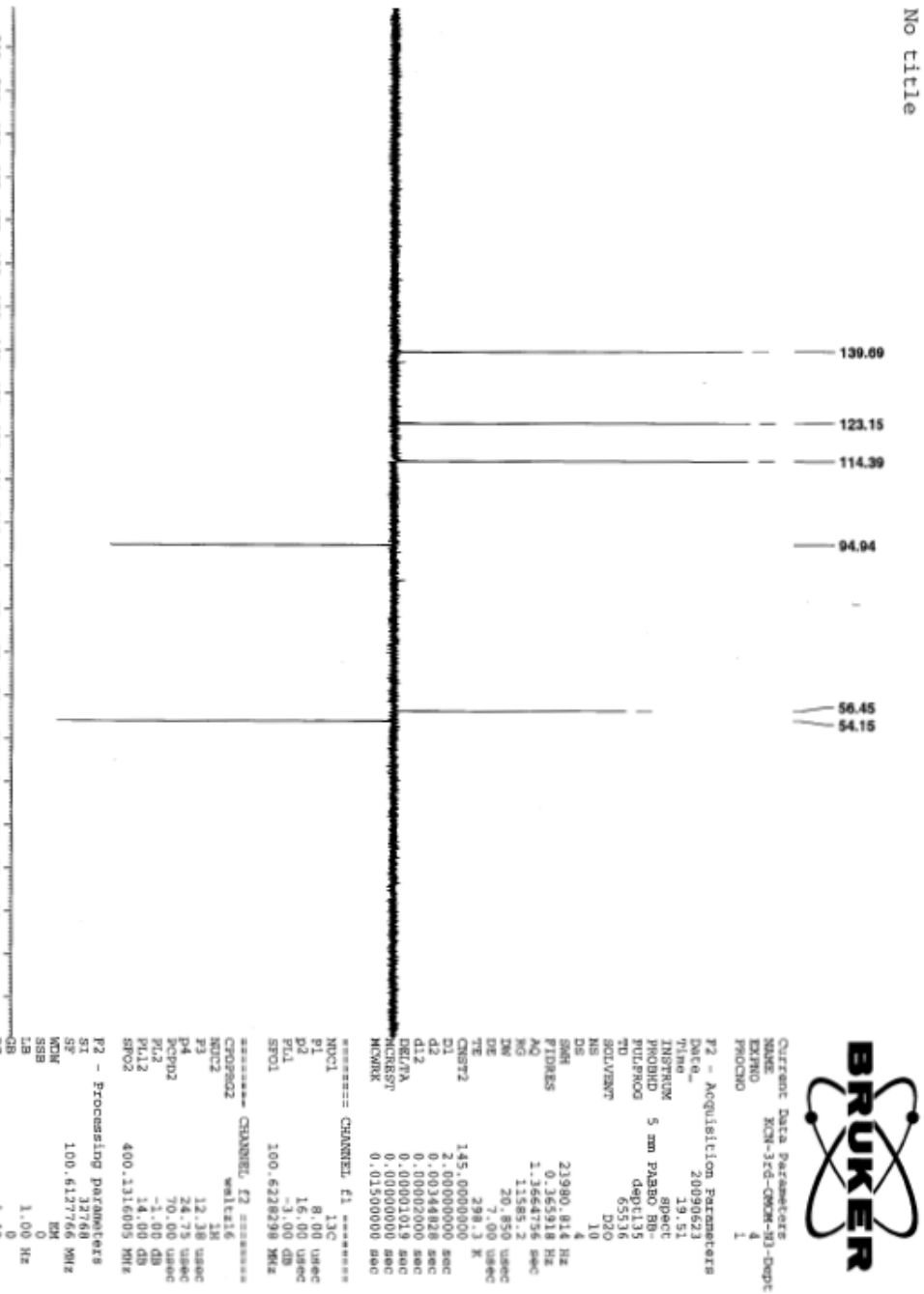
======== CHANNEL f1 ========

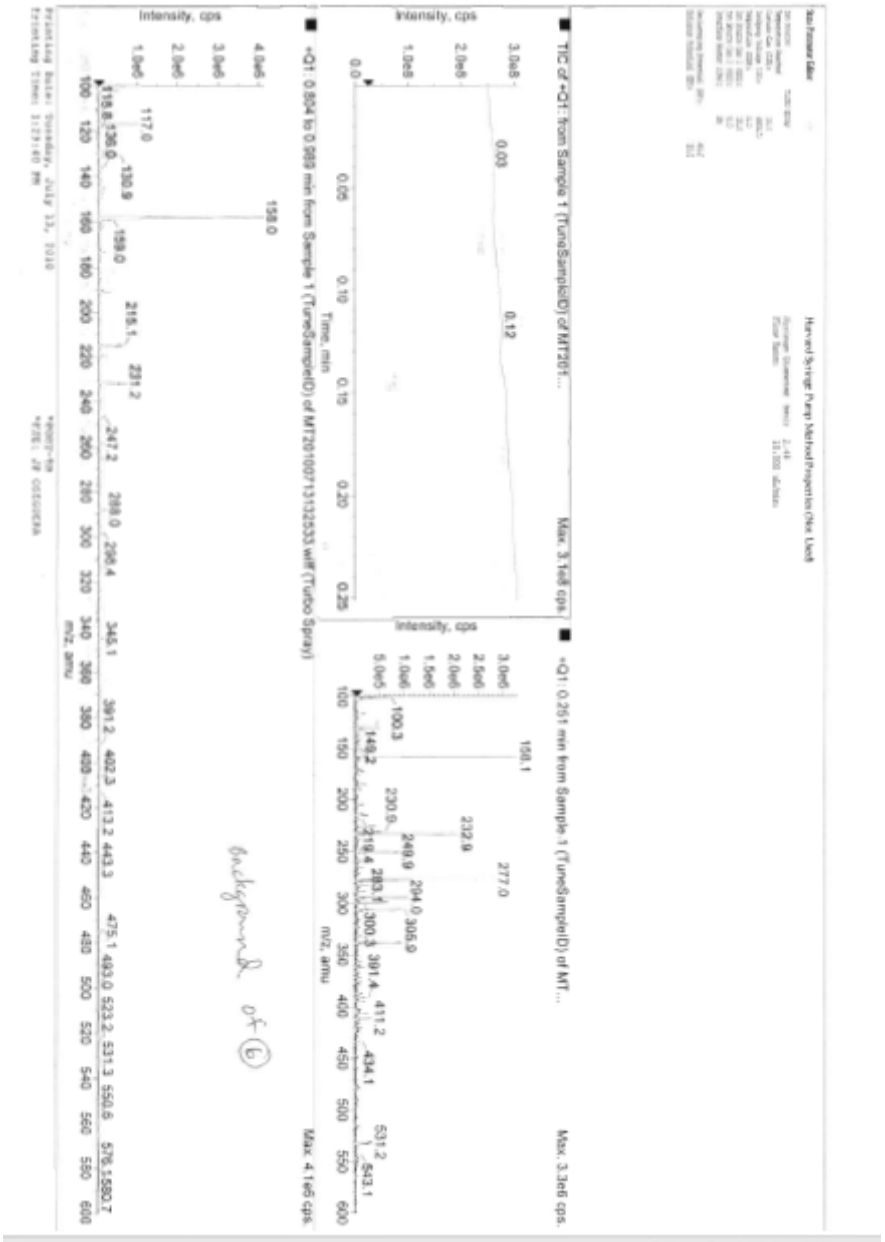
NUC1	1H
P1	12.80 usec
PL1	0.00 dB
SFO1	400.1324710 MHz

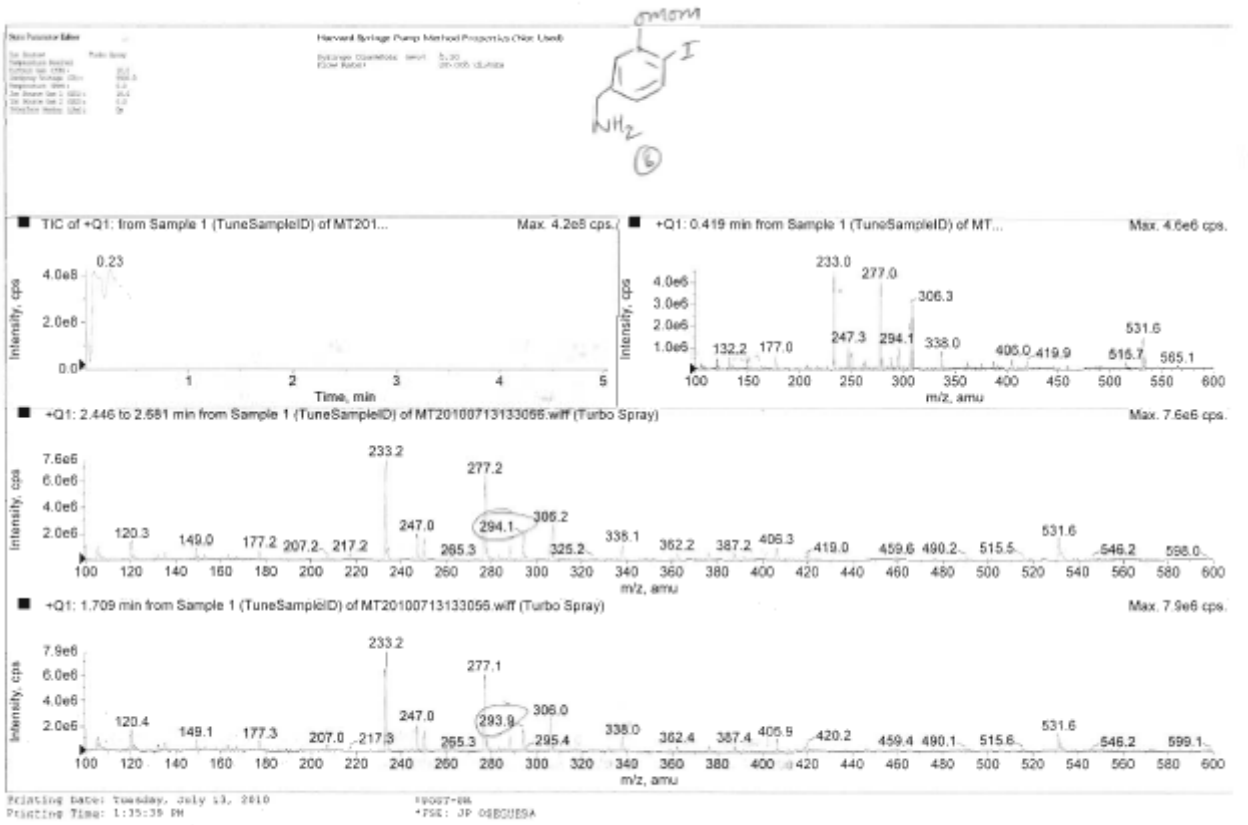
F2 - Processing parameters

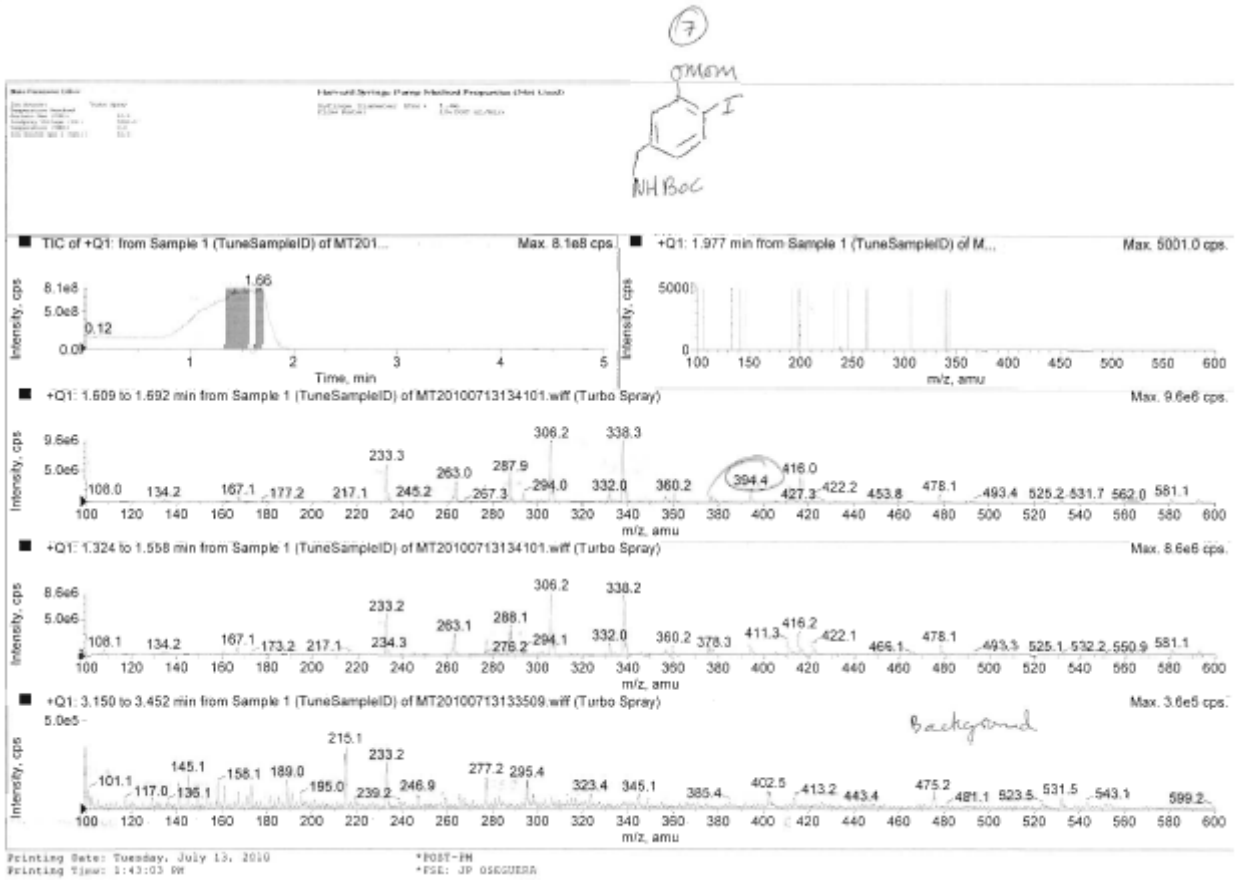
SI	32768
SF	400.1300124 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

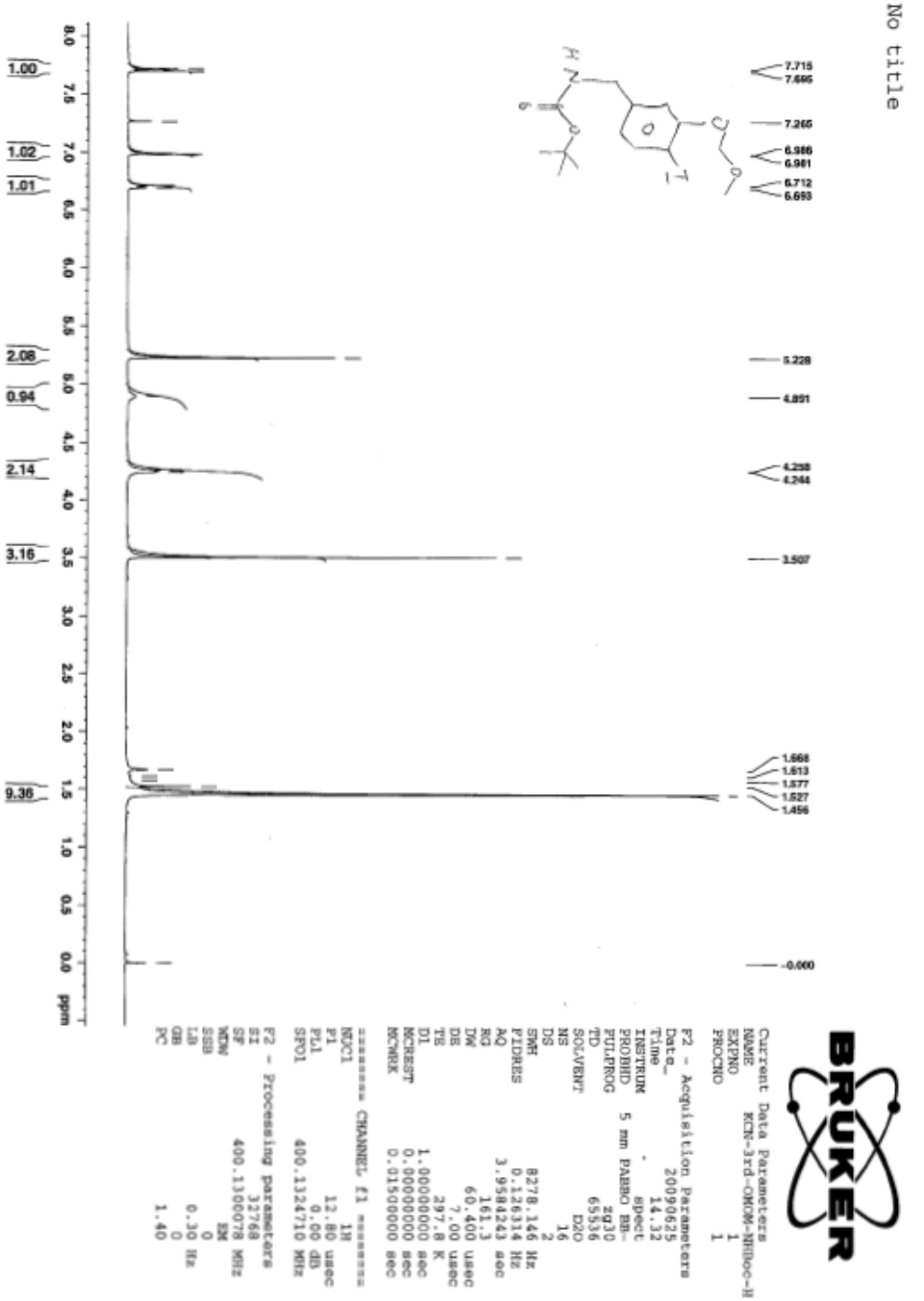

No title
156.32
139.69
137.27
123.15
114.39
94.94
86.81
77.55
77.23
76.91
56.45
54.15
BRUKER
Current Data Parameters
NAME KCN-3rd-OMOM-N3-C13
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20090623
Time 19.48
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 29
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 11585.2
DW 20.850 usec
DE 7.00 usec
TE 298.2 K
D1 2.00000000 sec
D11 0.03000000 sec
DELTA 1.89999998 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13c
P1 8.00 usec
PL1 -3.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -1.00 dB
PL12 14.00 dB
PL13 14.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127766 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00
ppm
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

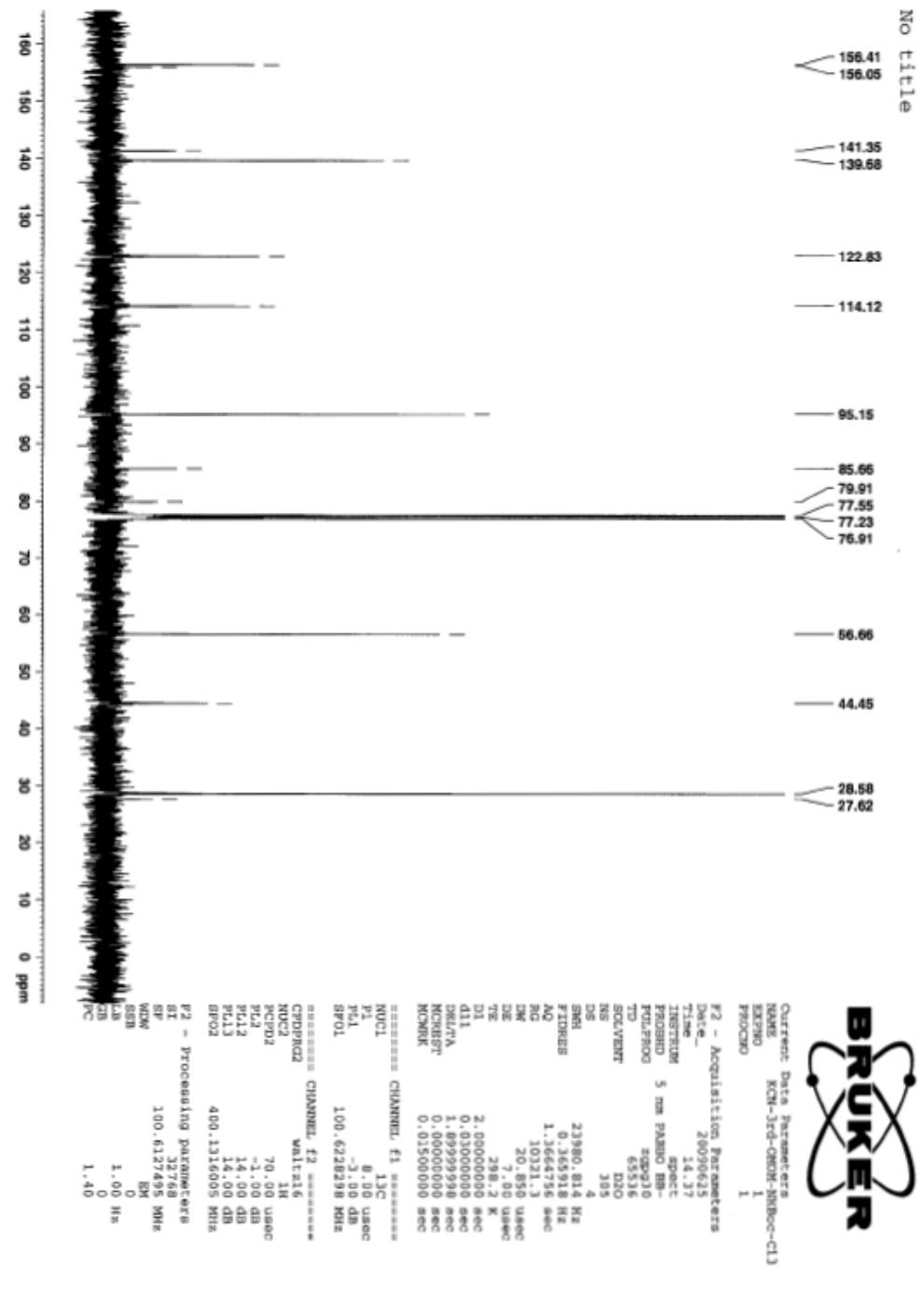

No title
139.69
123.15
114.39
94.94
56.45
54.15
BRUKER
Current Data Parameters
NAME KCN-3rd-CMCM-M3-Dept
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20090623
Time 19.51
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG dept135
TD 65536
SOLVENT D2O
NS 10
DS 4
SWH 21980.814 Hz
FIDRES 0.335918 Hz
AQ 1.3664756 sec
RG 11585.2
DW 20.850 usec
DE 7.00 usec
TE 298.3 K
CNST2 145.0000000
D1 2.00000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
DELTA 0.00001019 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 8.00 usec
P2 16.00 usec
PL1 -3.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
P3 12.30 usec
P4 24.75 usec
PCPD2 70.00 usec
PL2 -1.00 dB
PL12 14.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127766 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
ppm
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

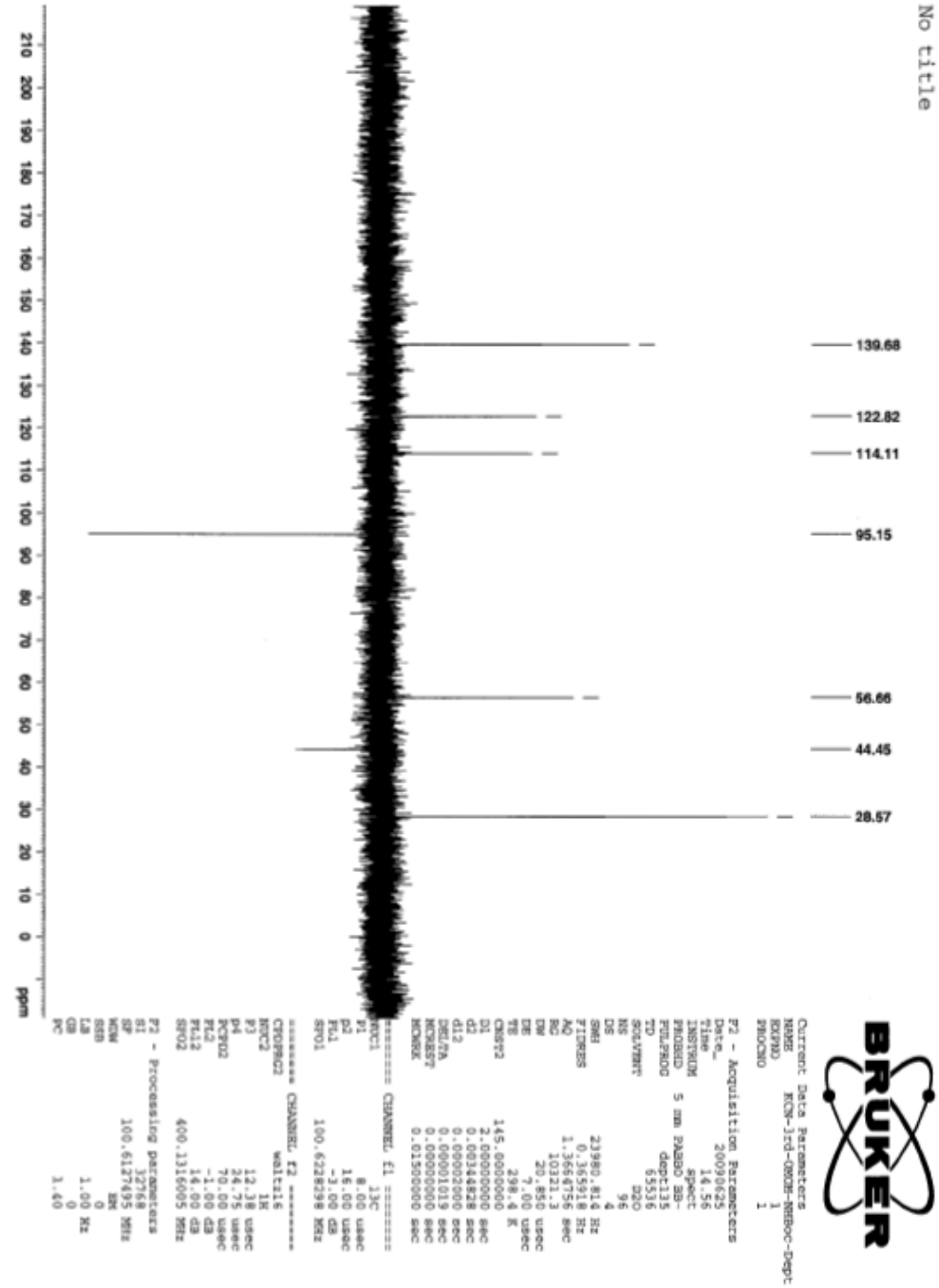

Harvard Syringe Pump Method Properties (New Used)
TIC of +Q1 from Sample 1 (TuneSampleID) of MT201...
Max. 3.1e8 cps.
Intensity, cps
Time, min
+Q1 0.804 to 0.989 min from Sample 1 (TuneSampleID) of MT20100713132533.wiff (Turbo Spray)
Max. 4.1e6 cps.
m/z, amu
+Q1 0.251 min from Sample 1 (TuneSampleID) of MT...
Max. 3.3e6 cps.
Printing Date: Tuesday, July 13, 2010
Printing Time: 1:29:40 PM

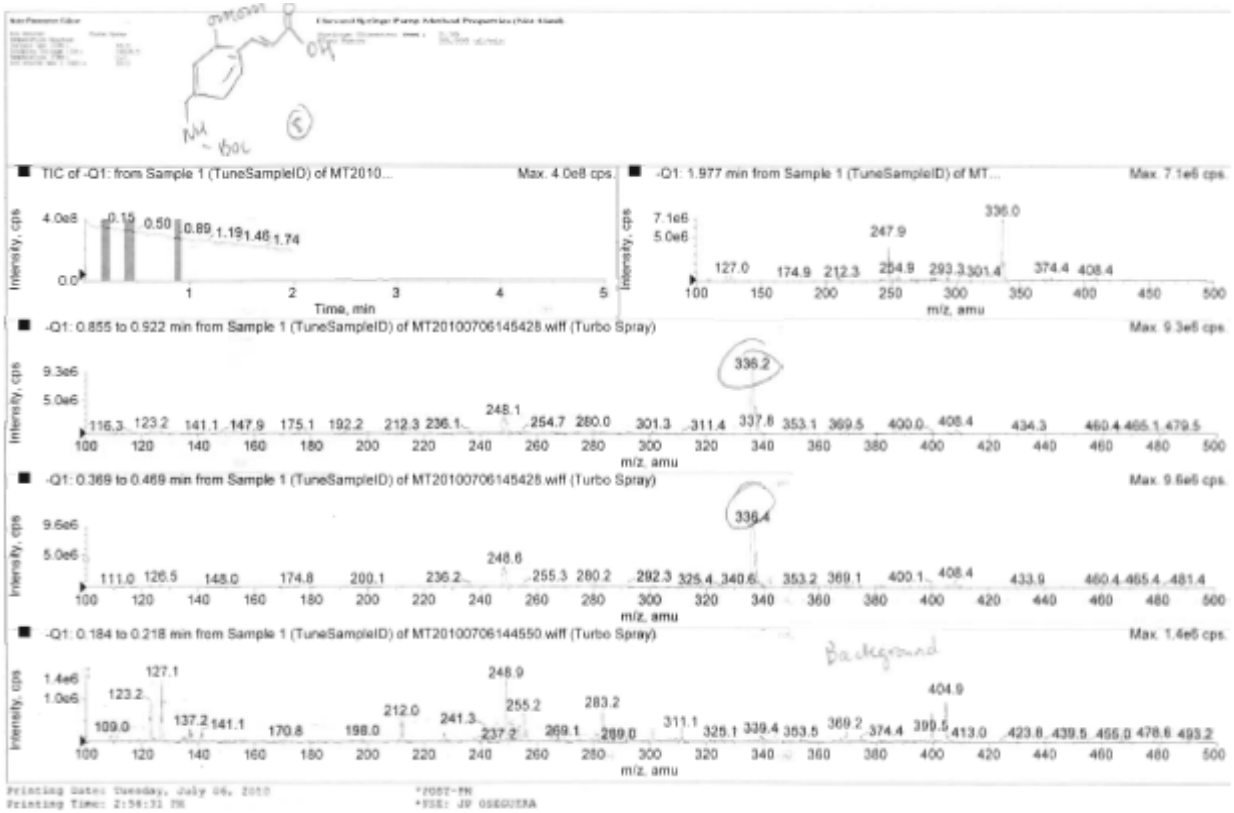

Harvard Syringe Pump Method Properties (Not Used)
TIC of +Q1: from Sample 1 (TuneSampleID) of MT201...
Max. 4.2e8 cps.
Intensity, cps
Time, min
+Q1: 0.419 min from Sample 1 (TuneSampleID) of MT...
Max. 4.6e6 cps.
m/z, amu
+Q1: 2.446 to 2.681 min from Sample 1 (TuneSampleID) of MT20100713133056.wiff (Turbo Spray)
Max. 7.6e6 cps.
+Q1: 1.709 min from Sample 1 (TuneSampleID) of MT20100713133056.wiff (Turbo Spray)
Max. 7.9e6 cps.
Printing Date: Tuesday, July 13, 2010
Printing Time: 1:35:39 PM

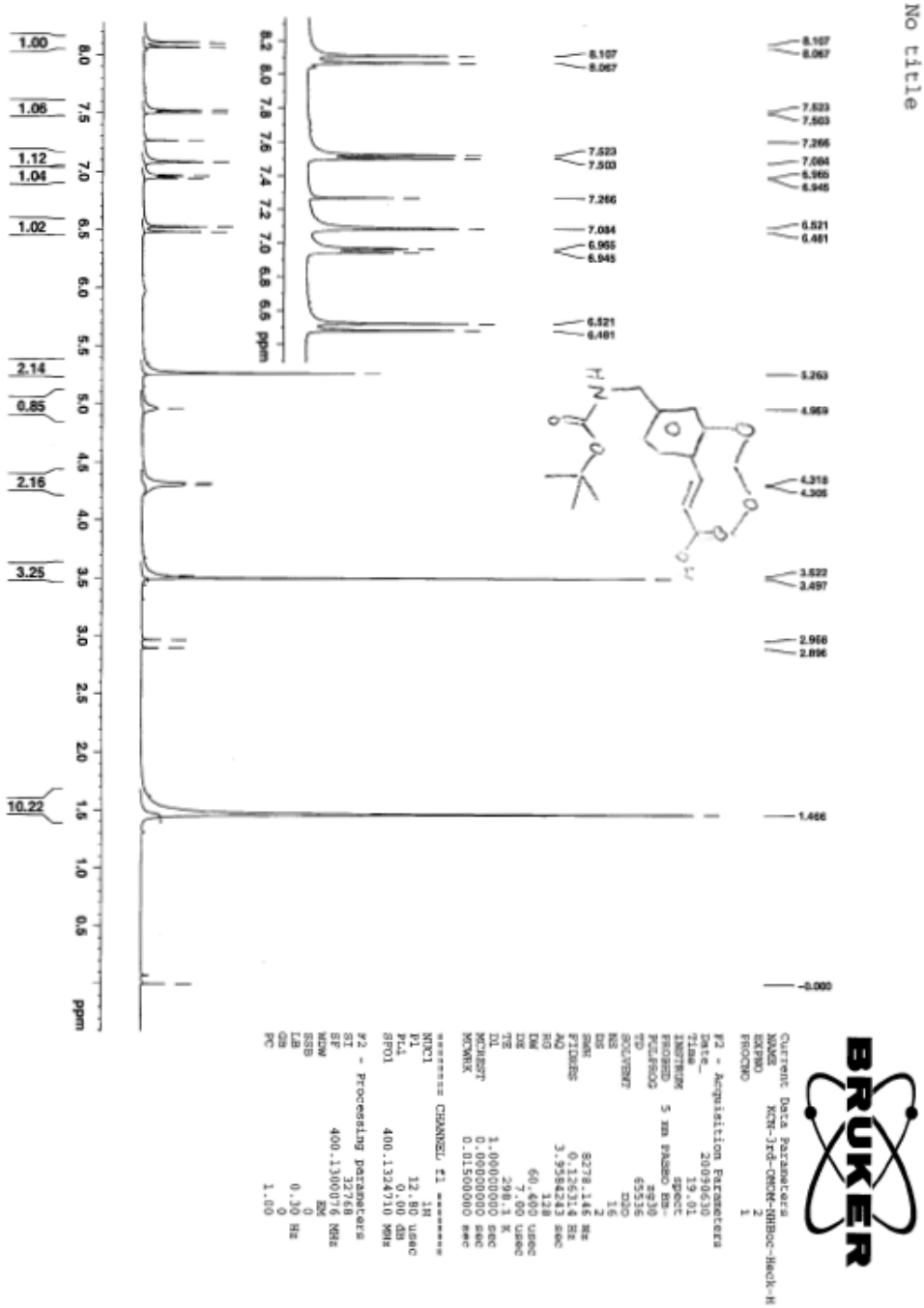

(7)
OMOM
I
NHBoc
TIC of +Q1: from Sample 1 (TuneSampleID) of MT201...
Max. 8.1e8 cps.
Intensity, cps
Time, min
+Q1: 1.977 min from Sample 1 (TuneSampleID) of M...
Max. 5001.0 cps.
m/z, amu
+Q1: 1.609 to 1.692 min from Sample 1 (TuneSampleID) of MT20100713134101.wiff (Turbo Spray)
Max. 9.6e6 cps.
+Q1: 1.324 to 1.558 min from Sample 1 (TuneSampleID) of MT20100713134101.wiff (Turbo Spray)
Max. 8.6e6 cps.
+Q1: 3.150 to 3.452 min from Sample 1 (TuneSampleID) of MT20100713133509.wiff (Turbo Spray)
Max. 3.6e5 cps.
Background
Printing Date: Tuesday, July 13, 2010
Printing Time: 1:43:03 PM
*POST-PM
*FSE: JP OSEGUERA

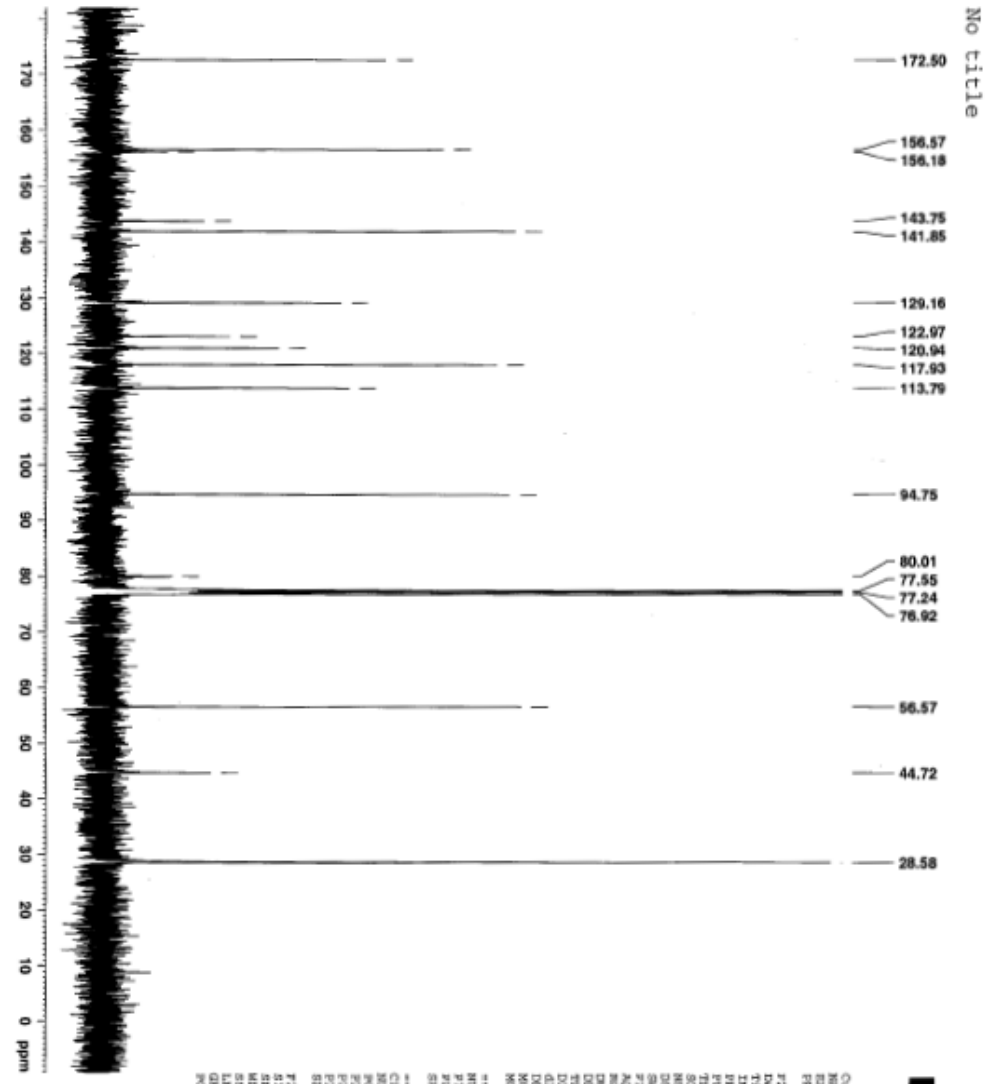

No title
7.715
7.695
7.265
6.986
6.981
6.712
6.693
5.228
4.891
4.258
4.244
3.507
1.668
1.613
1.577
1.527
1.456
-0.000
1.00
1.02
1.01
2.08
0.94
2.14
3.16
9.36
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
BRUKER
Current Data Parameters
NAME KCN-3rd-OMOM-NHBoc-H
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20090625
Time 14.32
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT D2O
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 161.3
DW 60.400 usec
DE 7.00 usec
TE 297.8 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 12.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 32768
SF 400.1300078 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

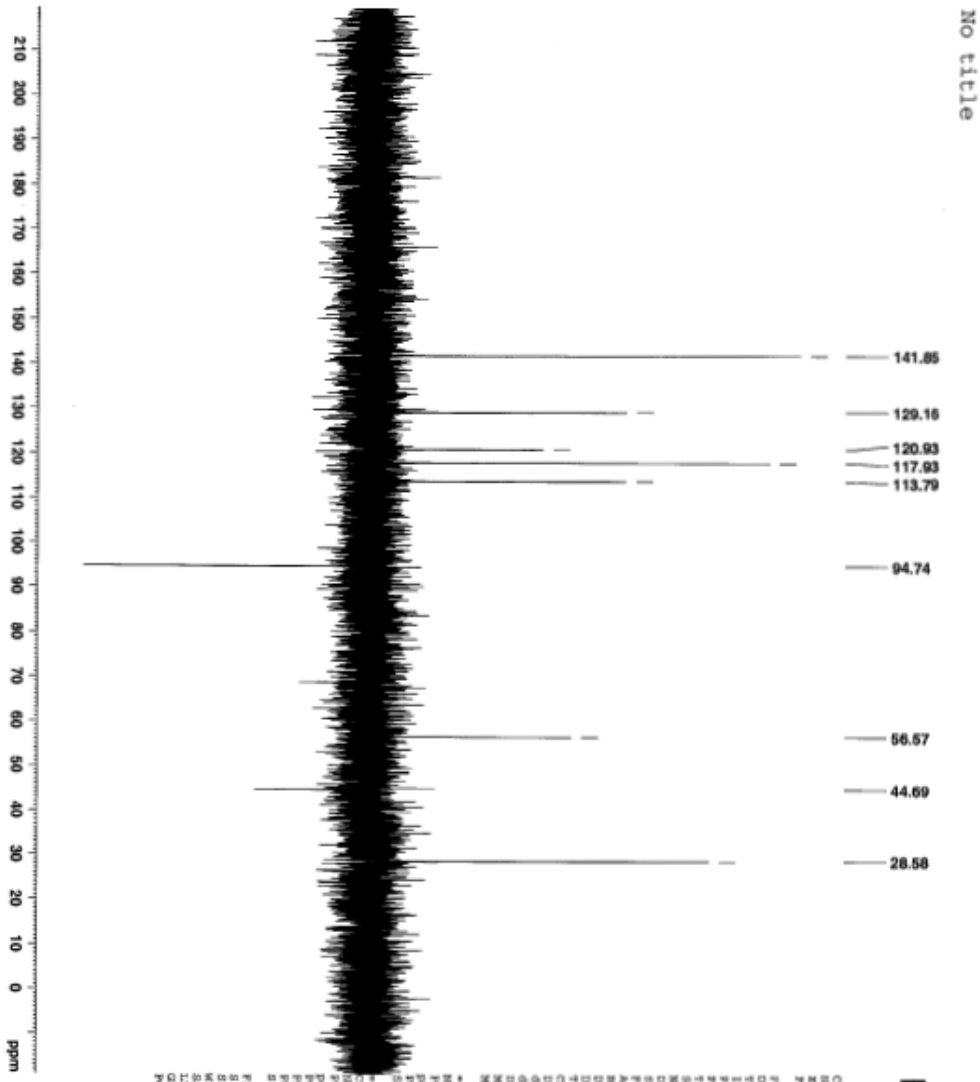

No title
156.41
156.05
141.35
139.68
122.83
114.12
95.15
85.66
79.91
77.55
77.23
76.91
56.66
44.45
28.58
27.62
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
BRUKER
Current Data Parameters
NAME KCN-3rd-CHCM-NXBoc-Cl3
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20090625
Time 14.37
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 305
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664796 sec
RG 10321.3
DW 20.850 usec
DE 7.00 usec
TE 298.2 K
D1 2.00000000 sec
D11 0.03000000 sec
DELTA 1.89999998 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 8.00 usec
PL1 -3.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -1.00 dB
PL12 14.00 dB
PL13 14.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127495 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

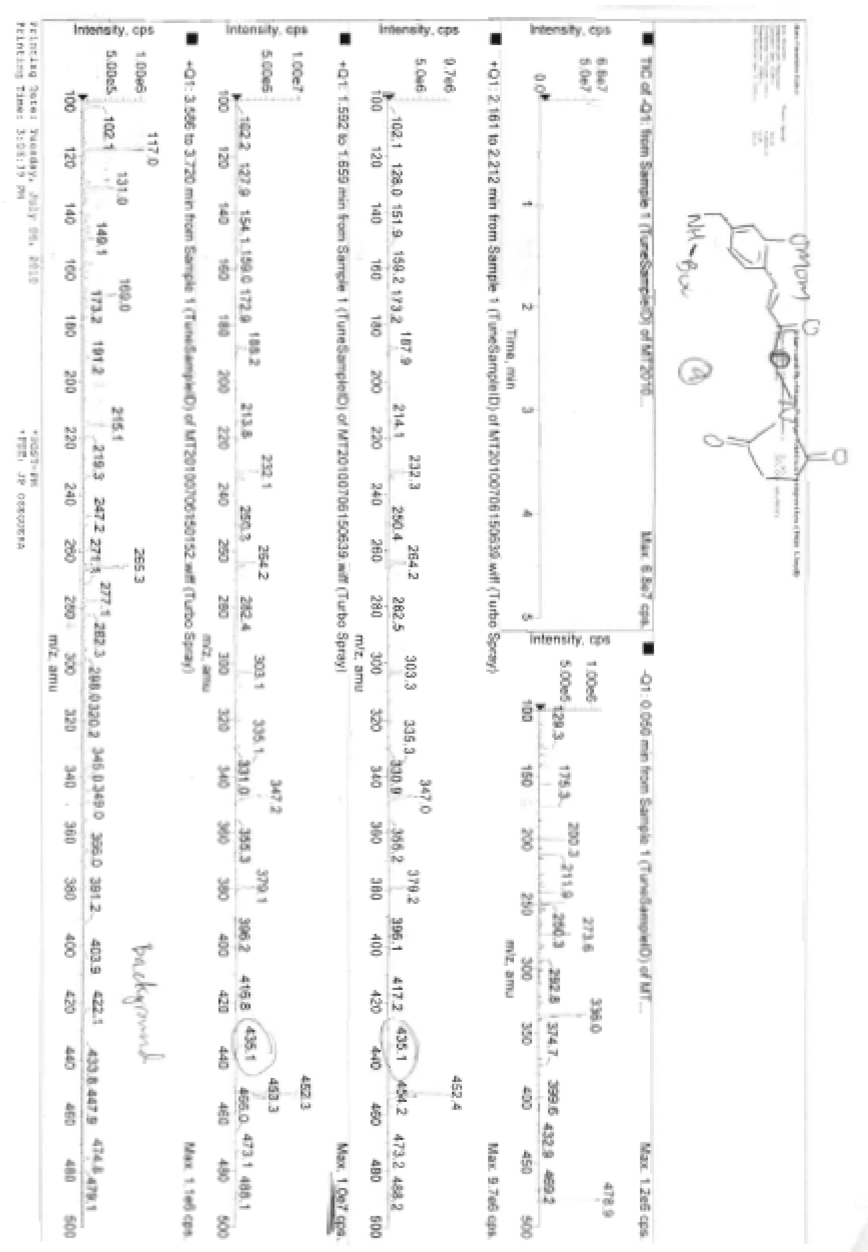

No title
139.68
122.82
114.11
95.15
56.66
44.45
28.57
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
BRUKER
Current Data Parameters
NAME KCN-Jrd-OMDM-NMRboc-Dept
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20090625
Time 14.56
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG dept135
TD 65536
SOLVENT D2O
NS 96
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 101321.2
DW 20.850 usec
DE 7.00 usec
TE 298.1 K
CNST2 145.0000000
D1 2.0000000 sec
d2 0.0034828 sec
d12 0.0000200 sec
DELTA 0.0000019 sec
MCNEST 0.0000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 8.00 usec
p2 16.00 usec
PL1 -2.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
P3 12.38 usec
P4 24.75 usec
PCPD2 70.00 usec
PL2 -1.00 dB
PL12 14.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127435 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

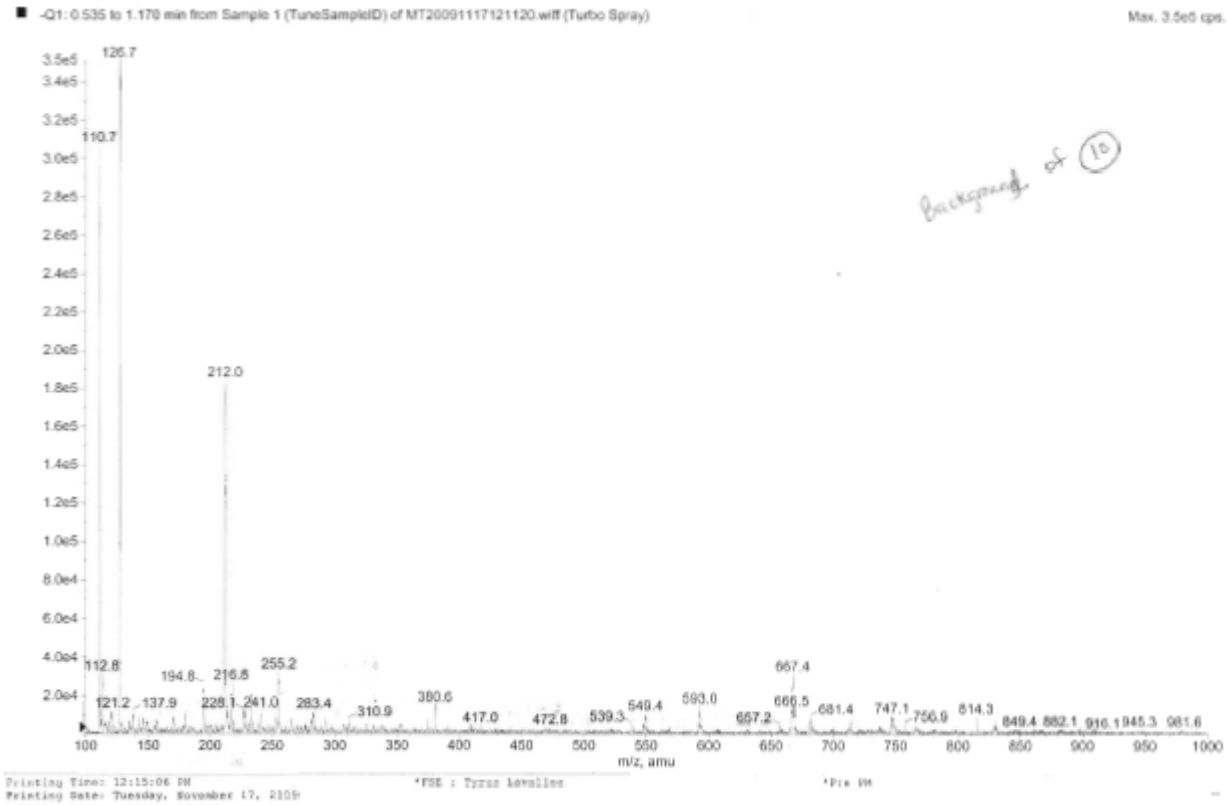

TIC of -Q1: from Sample 1 (TuneSampleID) of MT2010...
Max. 4.0e8 cps.
Intensity, cps
Time, min
-Q1: 1.977 min from Sample 1 (TuneSampleID) of MT...
Max. 7.1e6 cps.
m/z, amu
-Q1: 0.855 to 0.922 min from Sample 1 (TuneSampleID) of MT20100706145428.wiff (Turbo Spray)
Max. 9.3e6 cps.
-Q1: 0.369 to 0.469 min from Sample 1 (TuneSampleID) of MT20100706145428.wiff (Turbo Spray)
Max. 9.6e6 cps.
-Q1: 0.184 to 0.218 min from Sample 1 (TuneSampleID) of MT20100706144550.wiff (Turbo Spray)
Max. 1.4e6 cps.
Background
Printing Date: Tuesday, July 06, 2010
Printing Time: 2:58:31 PM
*POST-PM
*USE: JP OSEGUERA

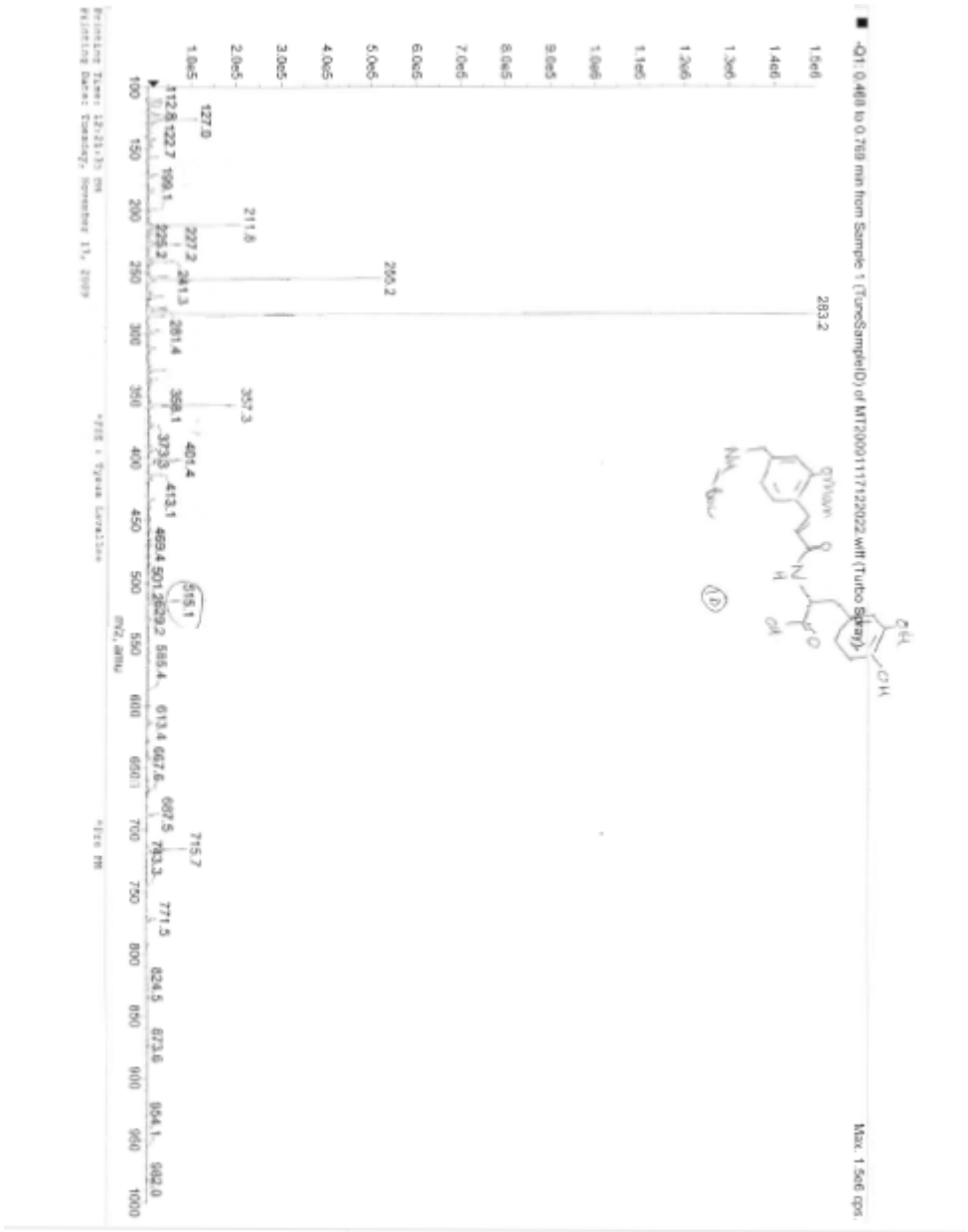

No title
8.107
8.067
7.523
7.503
7.266
7.084
6.965
6.945
6.521
6.481
5.263
4.969
4.318
4.305
3.522
3.497
2.968
2.896
1.466
-0.000
1.00
1.06
1.12
1.04
1.02
2.14
0.85
2.16
3.25
10.22
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.5 ppm
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
BRUKER
Current Data Parameters
NAME KCM-Jed-ONOM-BNBoc-Heck-H
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20090630
Time 19.01
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9586243 sec
RG 128
DW 60.400 usec
DE 7.00 usec
TE 298.1 K
D1 1.00000000 sec
D11 0.03000000 sec
MCREST 0.01500000 sec
MCWRK
======== CHANNEL f1 ========
NUC1 1H
P1 12.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 32768
SF 400.1300076 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

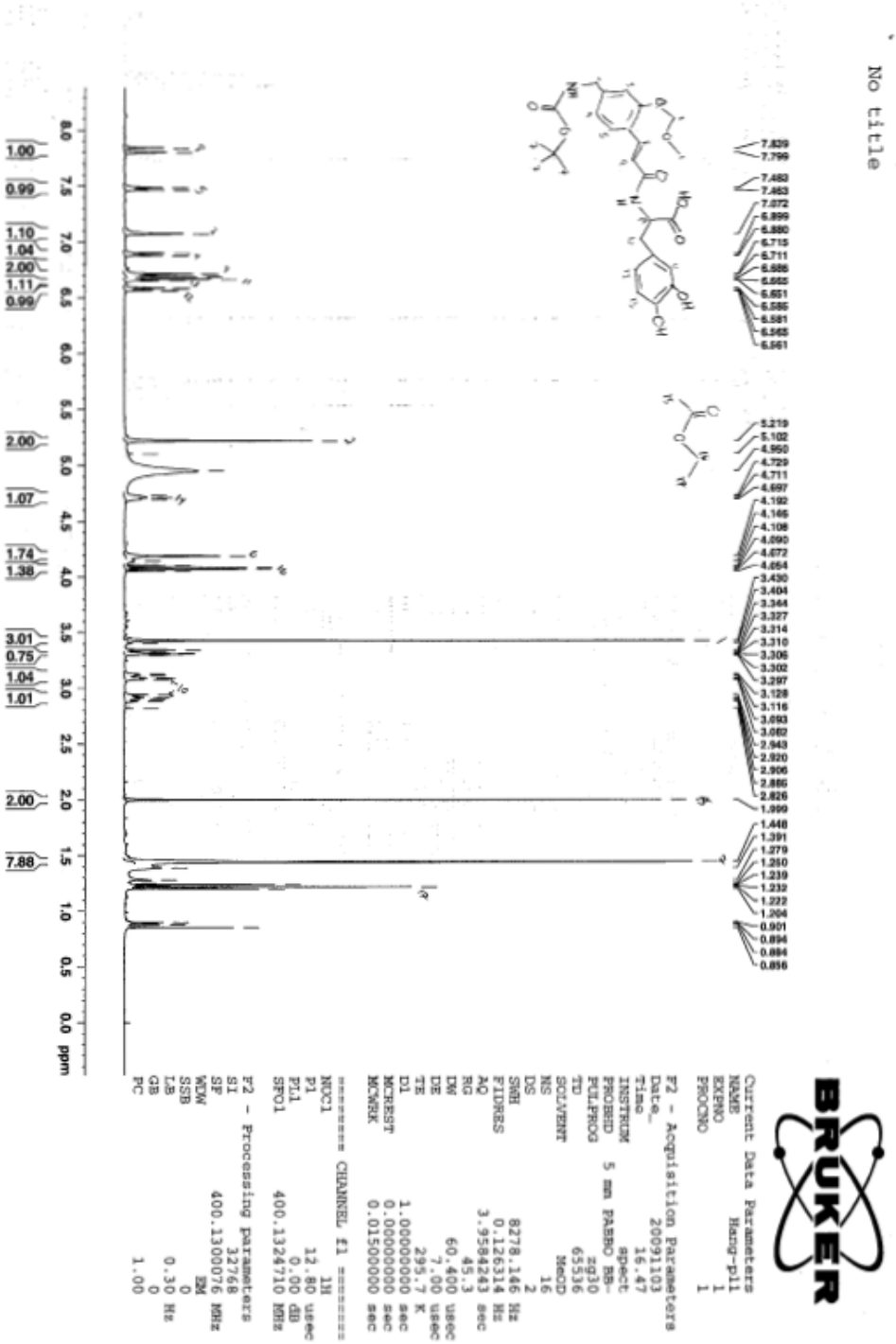

No title
172.50
156.57
156.18
143.75
141.85
129.16
122.97
120.94
117.93
113.79
94.75
80.01
77.55
77.24
76.92
56.57
44.72
28.58
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
BRUKER
Current Data Parameters
NAME RCH-3rd-CMEM-2-DMSOc-Rech-C13
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20090630
Time 19.07
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 434
DS 4
SWH 31980.814 Hz
FIDRES 0.365518 Hz
AQ 1.1565756 sec
RG 11585.2
DW 20.850 usec
DE 7.00 usec
TE 298.4 K
D1 2.00000000 sec
D11 0.03000000 sec
DELTA 1.89999998 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13c
P1 8.00 usec
PL1 -3.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -1.00 dB
PL12 14.00 dB
PL13 14.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127488 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

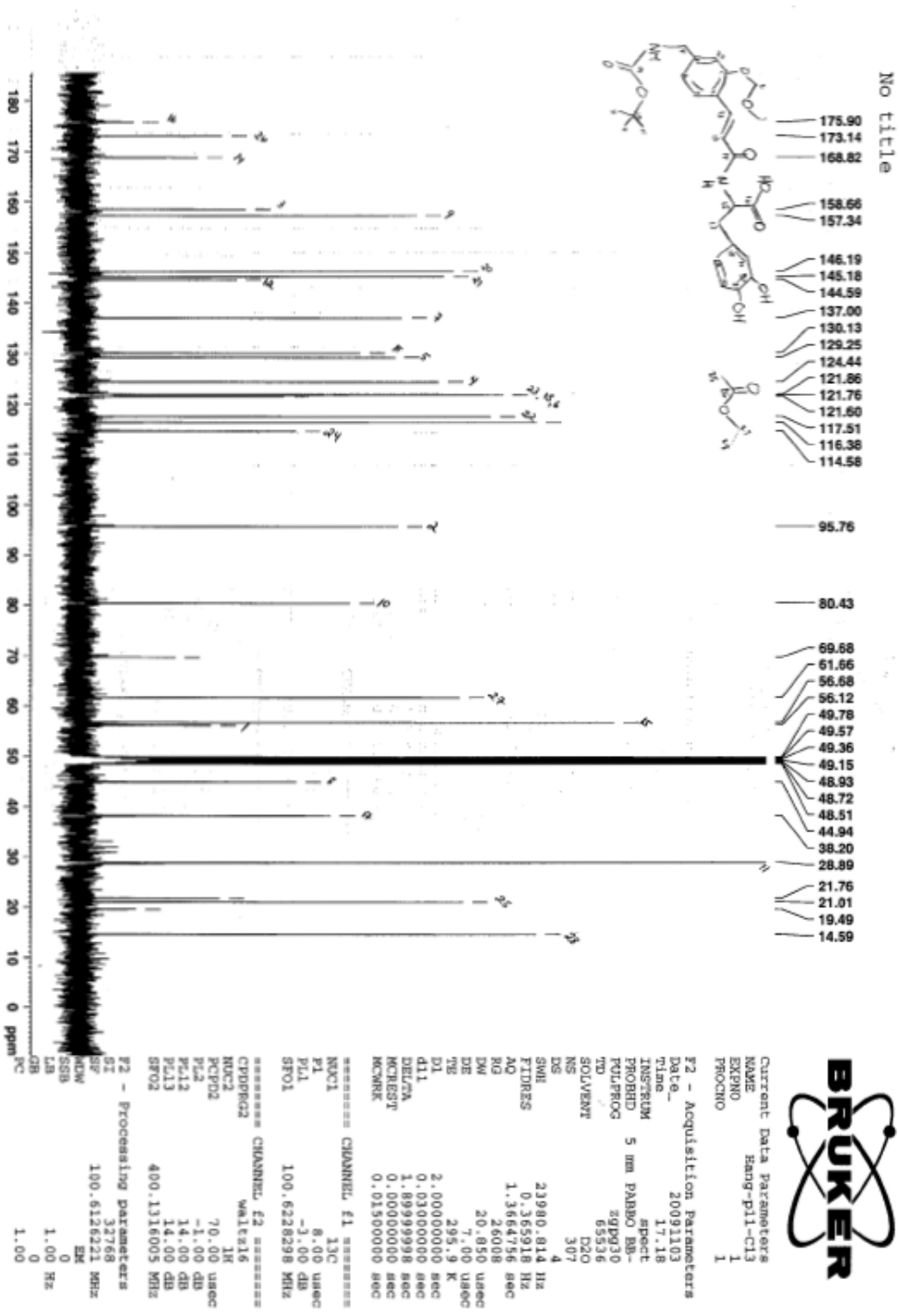

No title
141.85
129.16
120.93
117.93
113.79
94.74
56.57
44.69
28.58
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

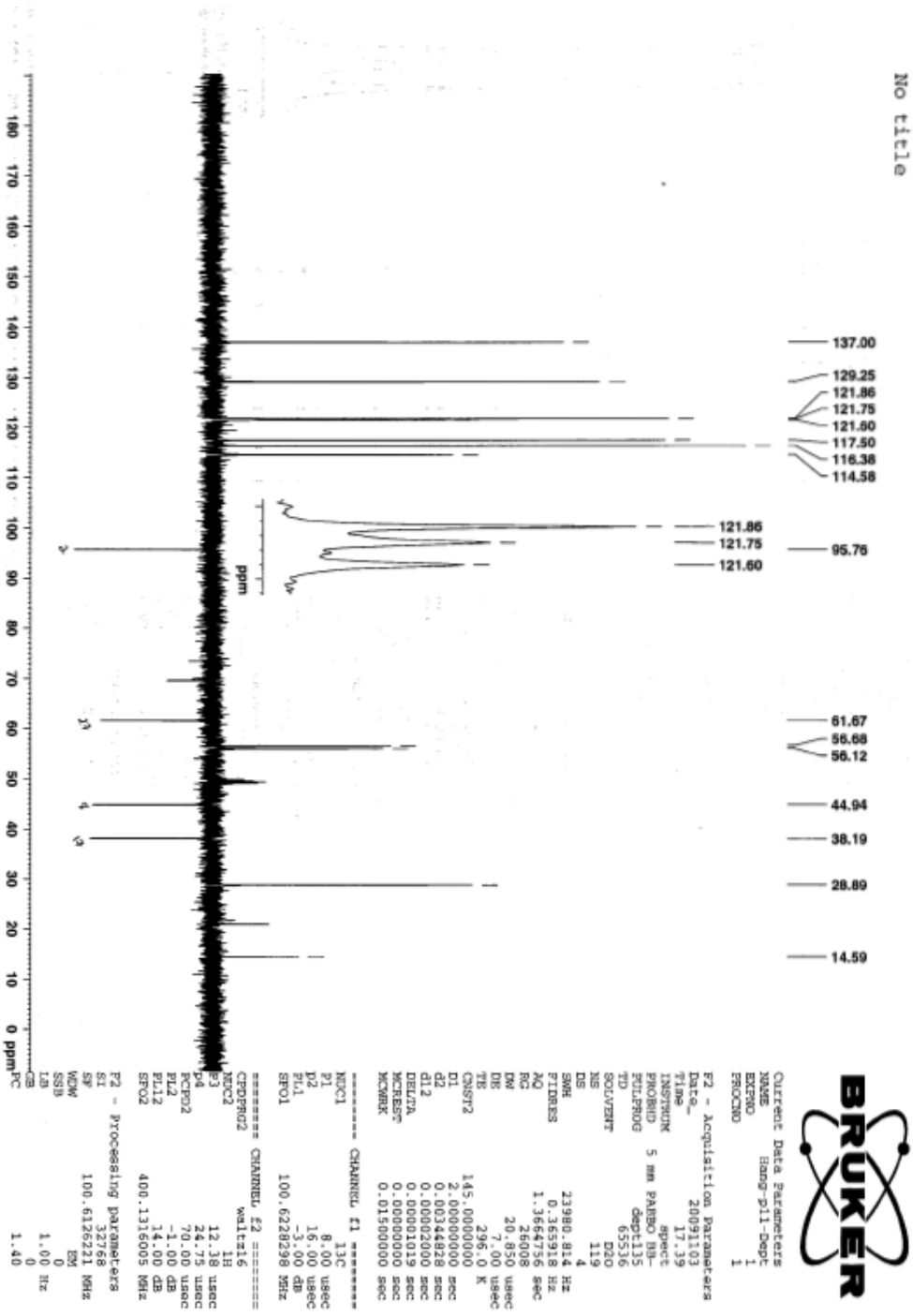


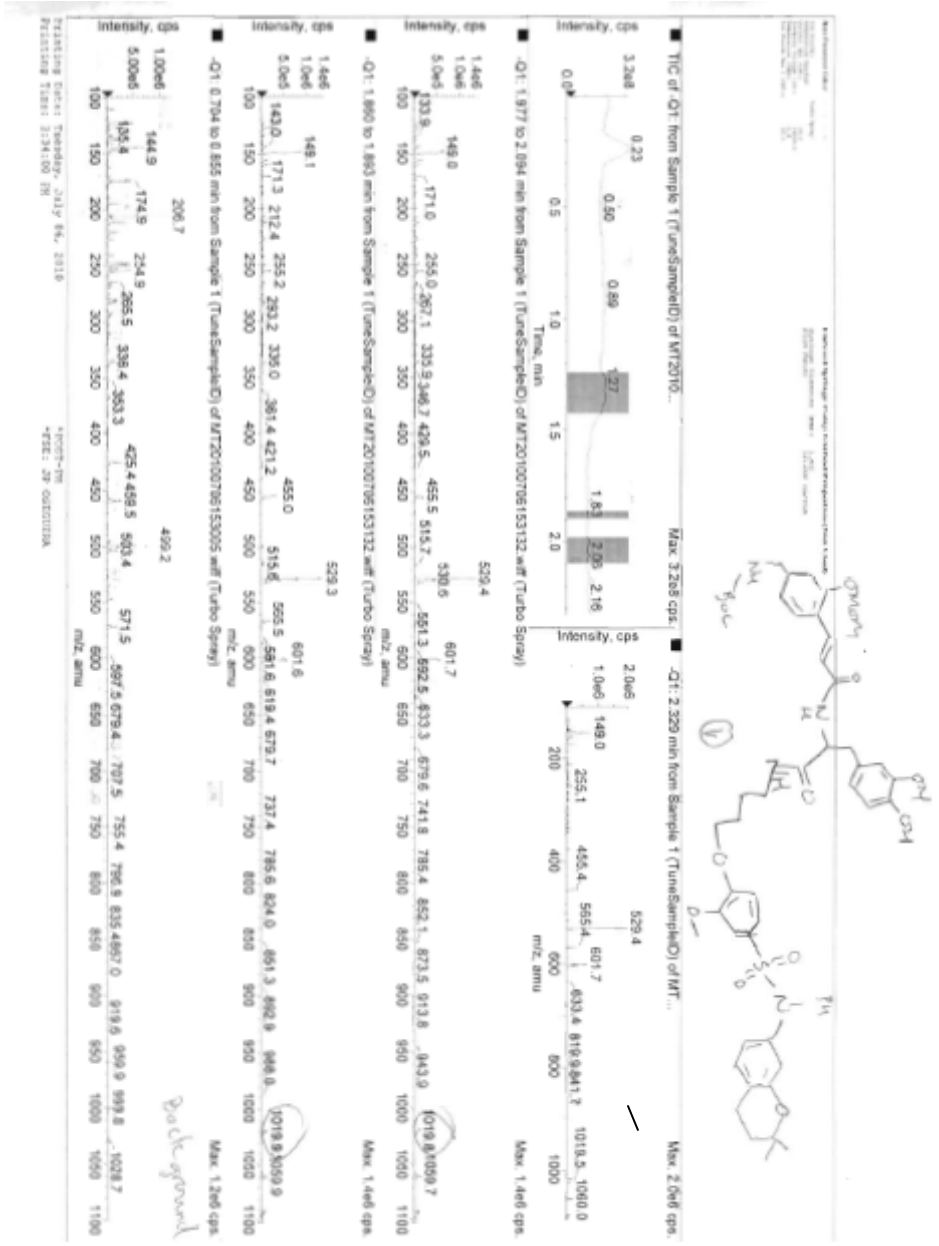

-Q1: 0.535 to 1.170 min from Sample 1 (TuneSampleID) of MT20091117121120.wiff (Turbo Spray)
Max. 3.5e6 cps.
Background of (10)
126.7
110.7
212.0
112.8
194.8
216.8
255.2
667.4
121.2
137.9
228.1
241.0
283.4
310.9
380.6
417.0
472.8
539.3
549.4
593.0
657.2
666.5
681.4
747.1
756.9
814.3
849.4
882.1
916.1
945.3
981.6
m/z, amu
Printing Time: 12:15:06 PM
Printing Date: Tuesday, November 17, 2009
*ESE : Tyrus Lavallee
*Pre PM

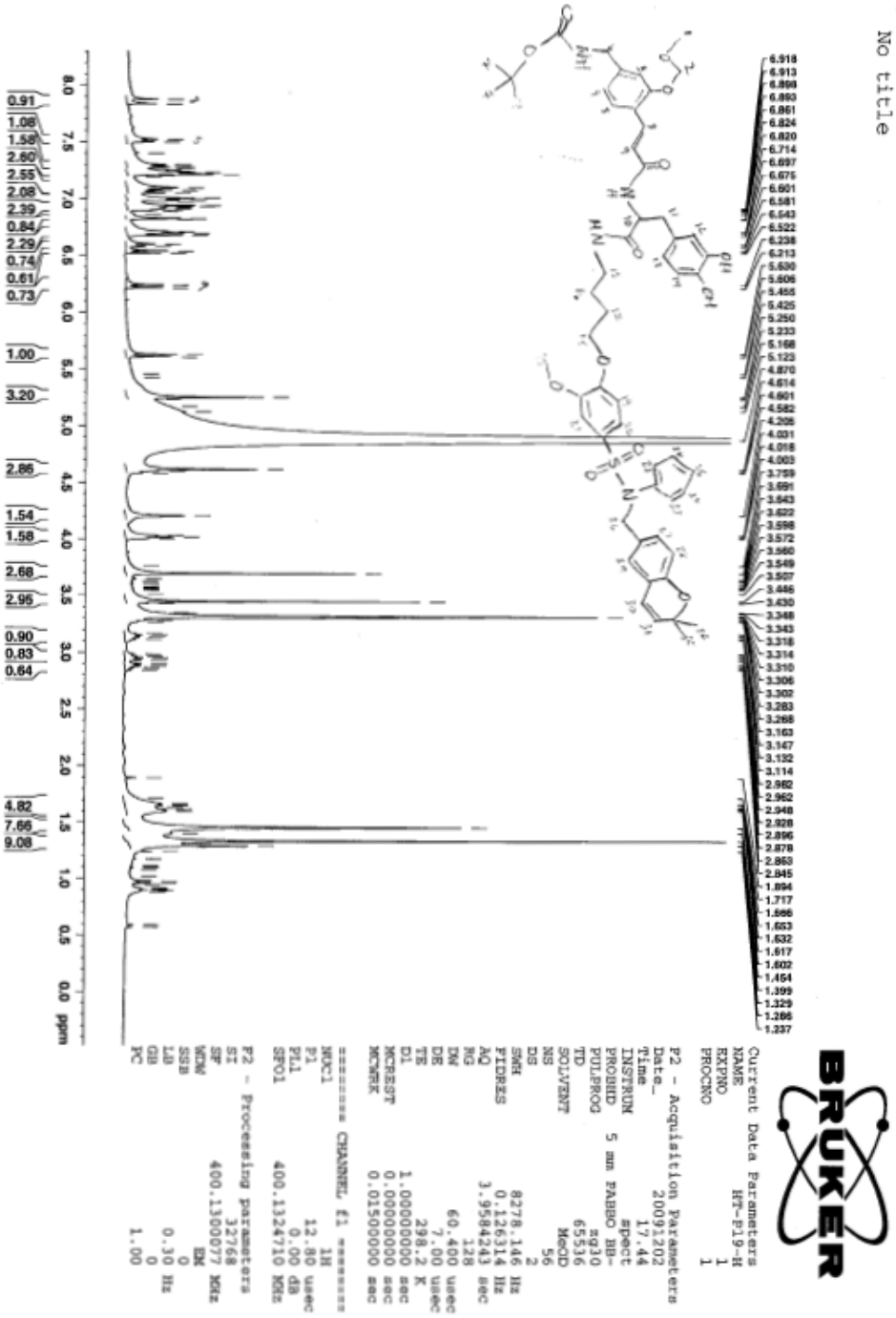

-Q1: 0.468 to 0.769 min from Sample 1 (TuneSampleID) of MT20091117122022.wiff (Turbo Spray)
Max. 1.5e6 cps
283.2
265.2
211.8
357.3
515.1
127.0
m/z, amu

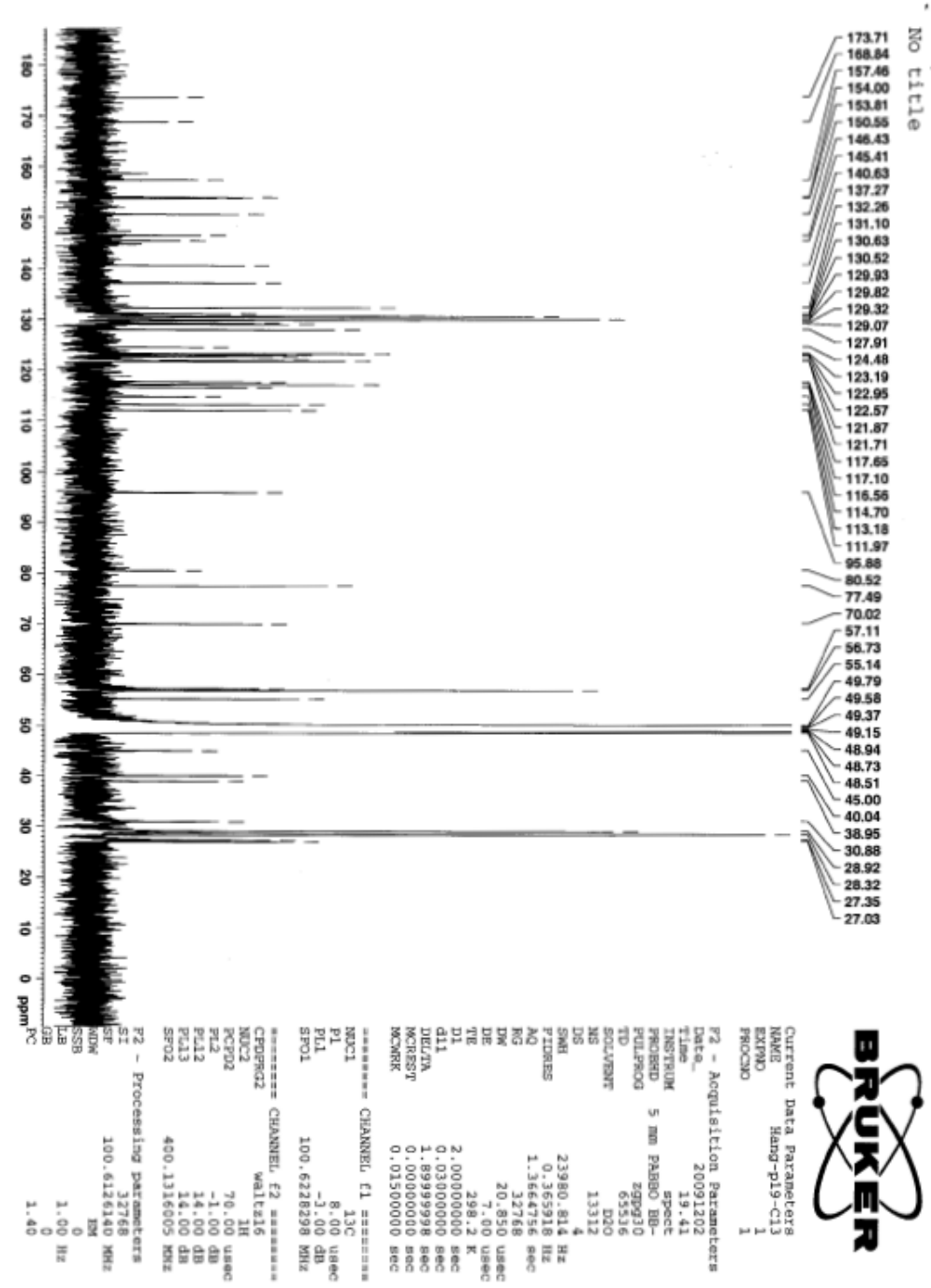

No title
Current Data Parameters
NAME Hang-pll
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20091103
Time 16.47
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 45.3
DW 60.400 usec
DE 7.00 usec
TE 299.7 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 12.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 32768
SF 400.1300076 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.839
7.799
7.482
7.463
7.072
6.899
6.880
6.715
6.711
6.686
6.682
6.651
6.585
6.581
6.565
6.561
5.219
5.102
4.950
4.729
4.711
4.697
4.192
4.165
4.108
4.090
4.072
4.054
3.430
3.404
3.344
3.327
3.314
3.310
3.306
3.302
3.297
3.128
3.116
3.093
3.082
2.943
2.920
2.906
2.886
2.826
1.999
1.448
1.391
1.279
1.250
1.239
1.232
1.222
1.204
0.901
0.894
0.884
0.856
1.00
0.99
1.10
1.04
2.00
1.11
0.99
2.00
1.07
1.74
1.38
3.01
0.75
1.04
1.01
2.00
7.88
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

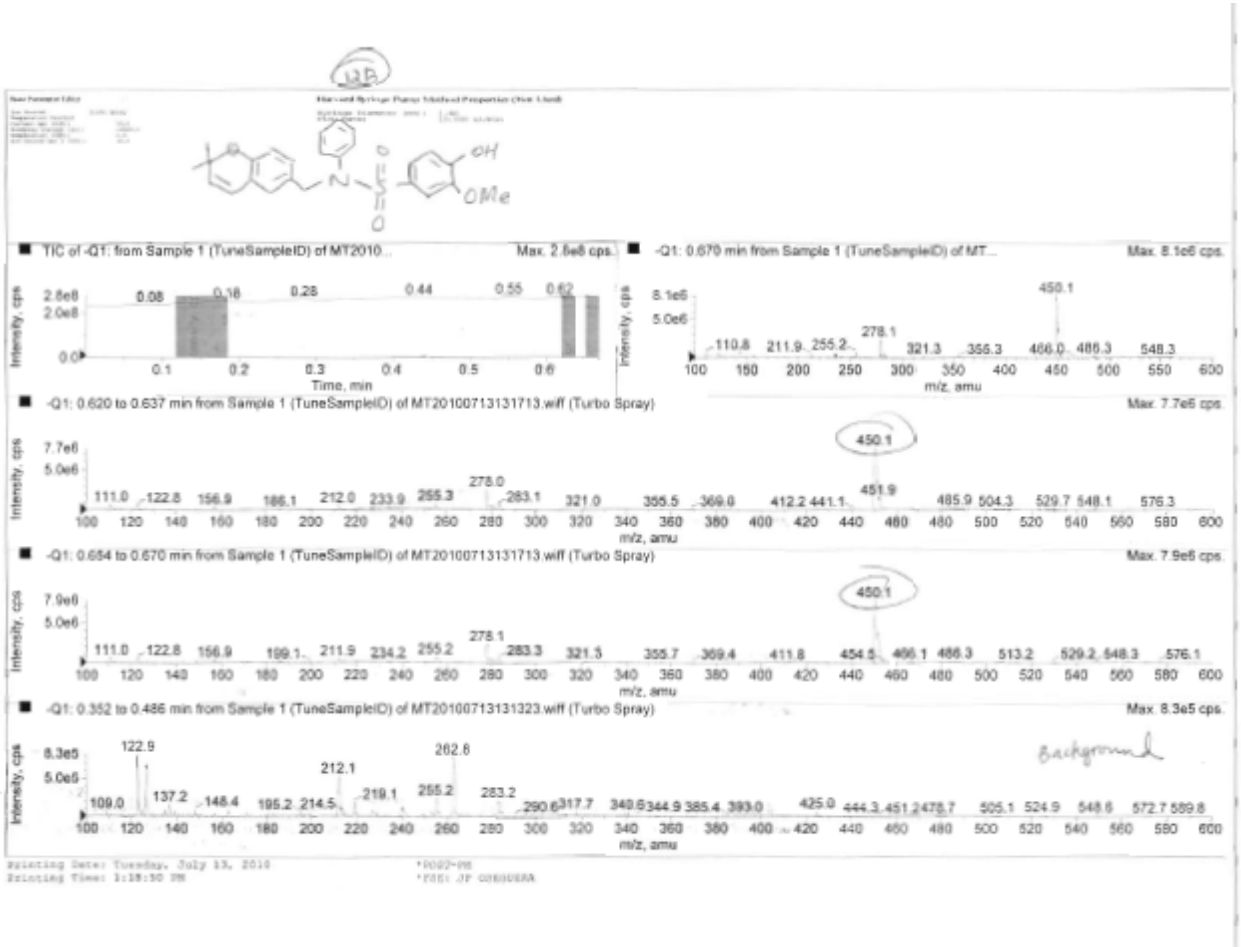

No title
175.90
173.14
168.82
158.66
157.34
146.19
145.18
144.59
137.00
130.13
129.25
124.44
121.86
121.76
121.60
117.51
116.38
114.58
95.76
80.43
69.68
61.66
56.68
56.12
49.78
49.57
49.36
49.15
48.93
48.72
48.51
44.94
38.20
28.89
21.76
21.01
19.49
14.59
ppm
Current Data Parameters
NAME Hang-pli-c13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20091103
Time 17.18
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 307
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 26008
DW 20.850 usec
DE 7.00 usec
TE 295.9 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 8.00 usec
PL1 -3.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -1.00 dB
PL12 14.00 dB
PL13 14.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6126221 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00

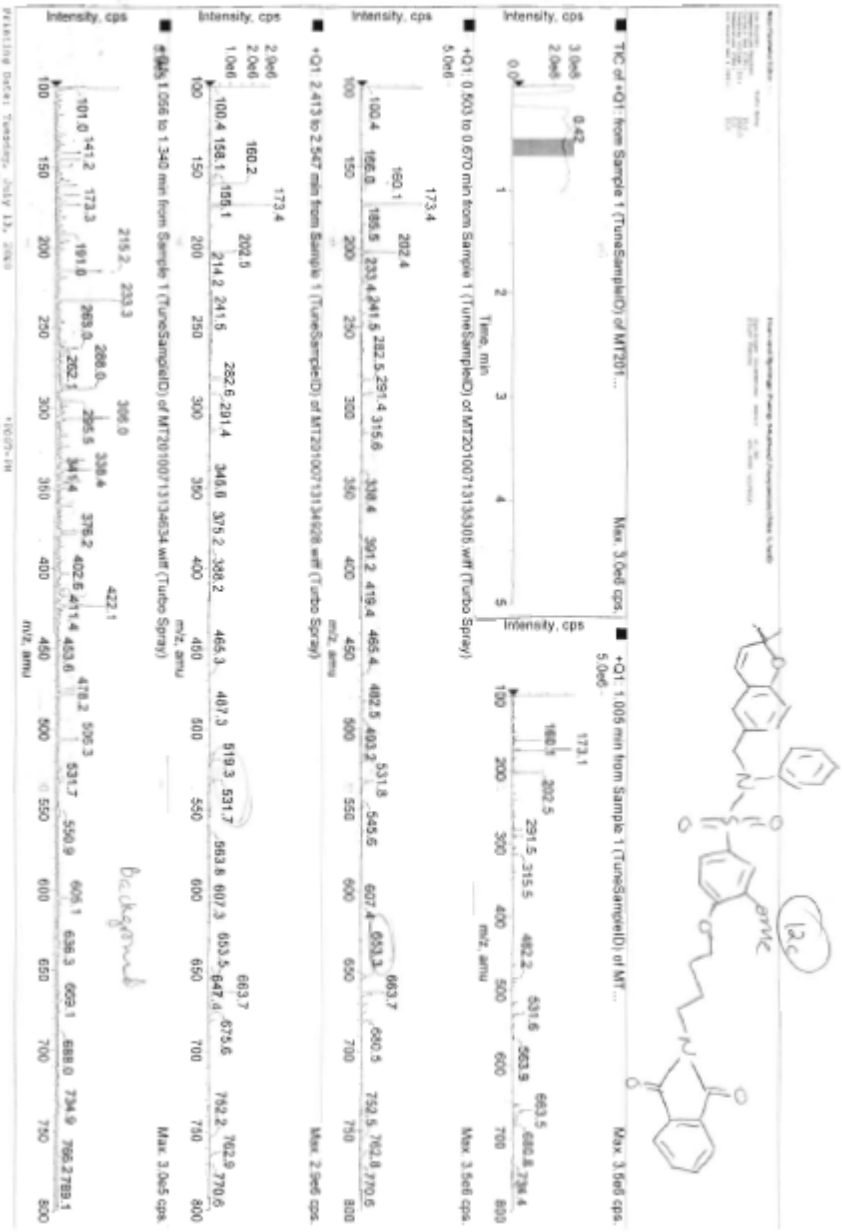

No title
137.00
129.25
121.86
121.75
121.60
117.50
116.38
114.58
121.86
121.75
121.60
95.76
61.67
56.68
56.12
44.94
38.19
29.89
14.59
ppm
0 ppm
Current Data Parameters
NAME Hsmg-p11-Dept
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20091103
Time 17.39
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG dept135
TD 65536
SOLVENT D2O
NS 119
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 26008
DW 20.850 usec
DE 7.00 usec
TE 296.0 K
CNST2 145.0000000
D1 2.00000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
DELTA 0.00001019 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 8.00 usec
p2 16.00 usec
PL1 -3.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
P3 12.38 usec
p4 24.75 usec
PCPD2 70.00 usec
PL2 -1.00 dB
PL12 14.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6126221 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40


TIC of -Q1: from Sample 1 (TuneSampleID) of MT2010...
Max. 3.2e8 cps.
Intensity, cps
Time, min
-Q1: 1.977 to 2.094 min from Sample 1 (TuneSampleID) of MT20100706153132.wiff (Turbo Spray)
Max. 1.4e6 cps.
-Q1: 1.860 to 1.893 min from Sample 1 (TuneSampleID) of MT20100706153132.wiff (Turbo Spray)
Max. 1.4e6 cps.
-Q1: 0.764 to 0.855 min from Sample 1 (TuneSampleID) of MT20100706153005.wiff (Turbo Spray)
Max. 1.2e6 cps.
-Q1: 2.329 min from Sample 1 (TuneSampleID) of MT...
Max. 2.0e6 cps.
m/z, amu

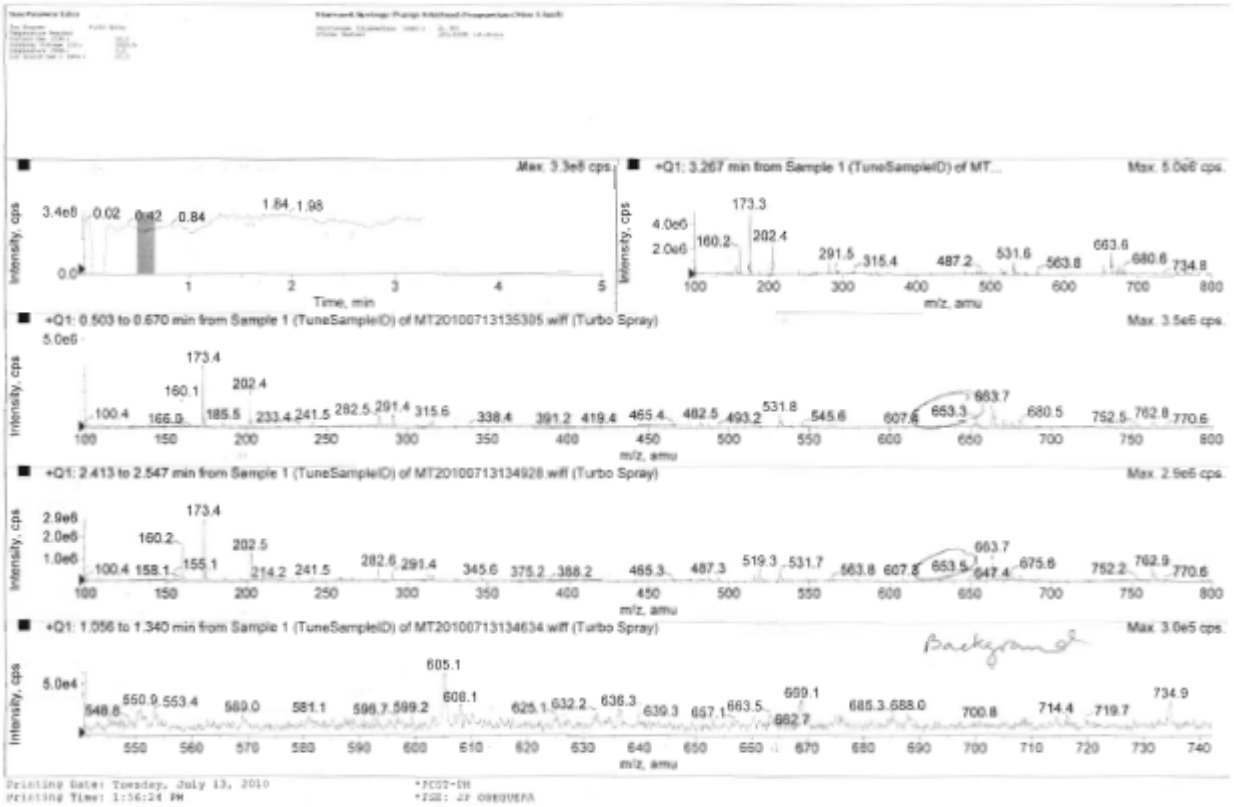

No title
Current Data Parameters
NAME HT-P19-H
EXPNO 1
PROCNO 1
P2 - Acquisition Parameters
Date_ 20091202
Time 17.44
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 56
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 128
DW 60.400 usec
DE 7.00 usec
TE 298.2 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 12.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 32768
SF 400.1300077 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
0.91
1.08
1.58
2.60
2.55
2.08
2.39
0.84
2.29
0.74
0.61
0.73
1.00
3.20
2.86
1.54
1.58
2.68
2.95
0.90
0.83
0.64
4.82
7.66
9.08
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

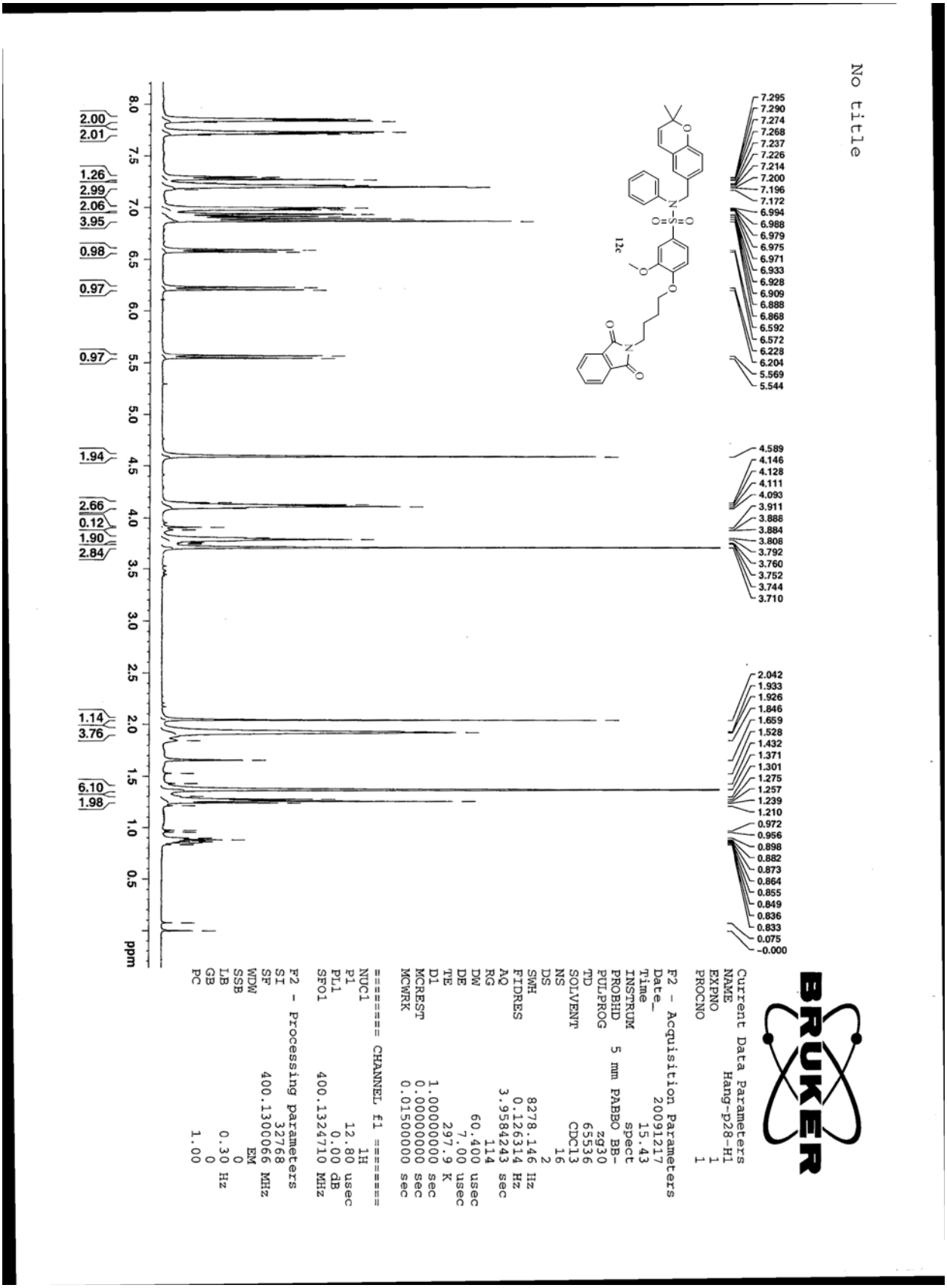

No title
173.71
168.84
157.46
154.00
153.81
150.55
146.43
145.41
140.63
137.27
132.26
131.10
130.63
130.52
129.93
129.82
129.32
129.07
127.91
124.48
123.19
122.95
122.57
121.87
121.71
117.65
117.10
116.56
114.70
113.18
111.97
95.88
80.52
77.49
70.02
57.11
56.73
55.14
49.79
49.58
49.37
49.15
48.94
48.73
48.51
45.00
40.04
38.95
30.88
28.92
28.32
27.35
27.03
BRUKER
Current Data Parameters
NAME Wang-p19-c13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20091202
Time 19.41
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 13312
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 32768
DW 20.850 usec
DE 7.00 usec
TE 298.2 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 8.00 usec
PL1 -3.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -1.00 dB
PL12 14.00 dB
PL13 14.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6126140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

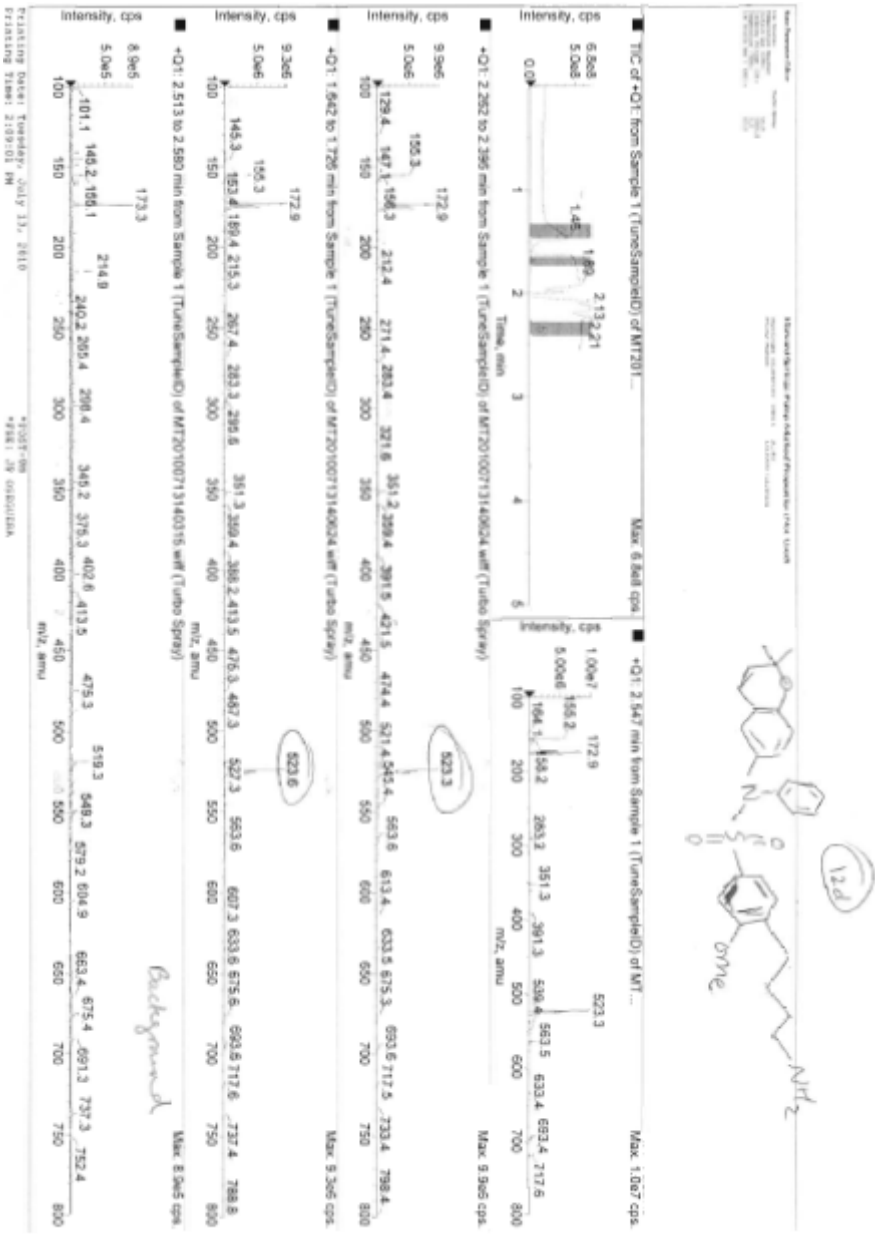

13P
OH
OMe
TIC of -Q1: from Sample 1 (TuneSampleID) of MT2010...
Max. 2.6e8 cps.
Intensity, cps
Time, min
-Q1: 0.679 min from Sample 1 (TuneSampleID) of MT...
Max. 8.1e6 cps.
m/z, amu
-Q1: 0.620 to 0.637 min from Sample 1 (TuneSampleID) of MT20100713131713.wiff (Turbo Spray)
Max. 7.7e6 cps.
-Q1: 0.654 to 0.670 min from Sample 1 (TuneSampleID) of MT20100713131713.wiff (Turbo Spray)
Max. 7.9e6 cps.
-Q1: 0.352 to 0.486 min from Sample 1 (TuneSampleID) of MT20100713131323.wiff (Turbo Spray)
Max. 8.3e5 cps.
Background
Printing Date: Tuesday, July 13, 2010
Printing Time: 1:18:50 PM


TIC of +Q1 from Sample 1 (TuneSampleID) of MT201...
Max. 3.0e6 cps.
Intensity, cps
Time, min
+Q1 0.503 to 0.670 min from Sample 1 (TuneSampleID) of MT20100713135305.wiff (Turbo Spray)
Max. 3.5e6 cps.
+Q1 1.005 min from Sample 1 (TuneSampleID) of MT...
Max. 3.6e6 cps.
+Q1 2.413 to 2.547 min from Sample 1 (TuneSampleID) of MT20100713134928.wiff (Turbo Spray)
Max. 2.9e6 cps.
+Q1 1.056 to 1.340 min from Sample 1 (TuneSampleID) of MT20100713134834.wiff (Turbo Spray)
Max. 3.0e5 cps.
m/z, amu
background

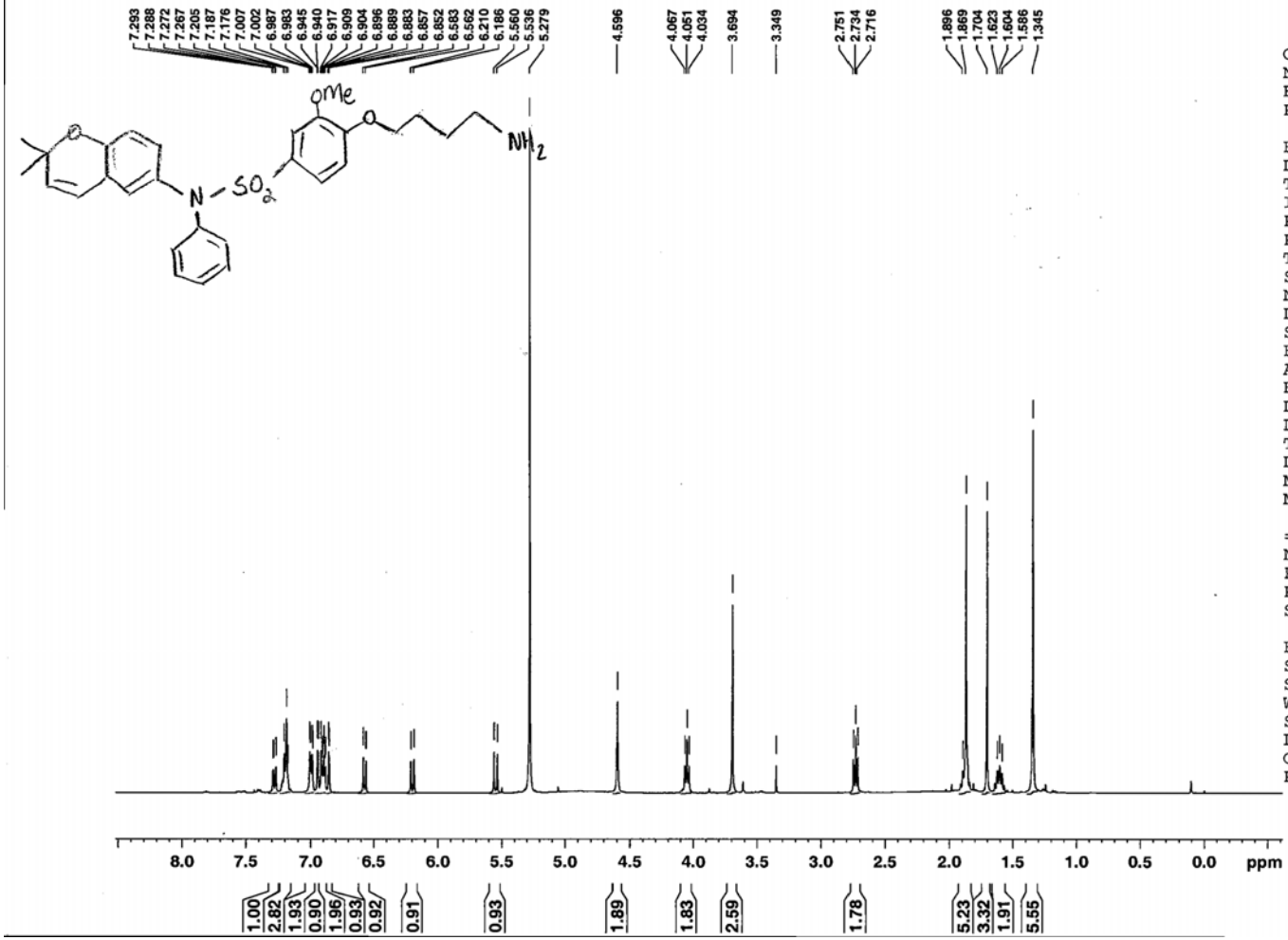
Re zoom in


Max. 3.3e8 cps.
Intensity, cps
Time, min
+Q1: 3.267 min from Sample 1 (TuneSampleID) of MT...
Max. 5.0e6 cps.
m/z, amu
+Q1: 0.503 to 0.670 min from Sample 1 (TuneSampleID) of MT20100713135305.wiff (Turbo Spray)
Max. 3.5e6 cps.
+Q1: 2.413 to 2.547 min from Sample 1 (TuneSampleID) of MT20100713134928.wiff (Turbo Spray)
Max. 2.9e6 cps.
+Q1: 1.056 to 1.340 min from Sample 1 (TuneSampleID) of MT20100713134634.wiff (Turbo Spray)
Max. 3.0e5 cps.
Background
Printing Date: Tuesday, July 13, 2010
Printing Time: 1:56:24 PM


No title
12c
7.295
7.290
7.274
7.268
7.237
7.226
7.214
7.200
7.196
7.172
6.994
6.988
6.979
6.975
6.971
6.933
6.928
6.909
6.888
6.868
6.592
6.572
6.228
6.204
5.569
5.544
4.589
4.146
4.128
4.111
4.093
3.911
3.888
3.884
3.808
3.792
3.760
3.752
3.744
3.710
2.042
1.933
1.926
1.846
1.659
1.528
1.432
1.371
1.301
1.275
1.257
1.239
1.210
0.972
0.956
0.898
0.882
0.873
0.864
0.855
0.849
0.836
0.833
0.075
-0.000
2.00
2.01
1.26
2.99
2.06
3.95
0.98
0.97
0.97
1.94
2.66
0.12
1.90
2.84
1.14
3.76
6.10
1.98
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
BRUKER
Current Data Parameters
NAME Hang-p28-H1
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20091217
Time 15.43
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDC13
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 114
DW 60.400 usec
DE 7.00 usec
TE 297.9 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
========== CHANNEL f1 ==========
NUC1 1H
P1 12.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 32768
SF 400.1300066 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


12d
NH2
OMe
TIC of +Q1: from Sample 1 (TuneSampleID) of MT201...
Max: 6.8e8 cps.
Intensity, cps
Time, min
+Q1: 2.547 min from Sample 1 (TuneSampleID) of MT...
Max: 1.0e7 cps.
+Q1: 2.262 to 2.396 min from Sample 1 (TuneSampleID) of MT20100713140824.wiff (Turbo Spray)
Max: 9.9e6 cps.
+Q1: 1.642 to 1.726 min from Sample 1 (TuneSampleID) of MT20100713140824.wiff (Turbo Spray)
Max: 9.3e6 cps.
+Q1: 2.513 to 2.580 min from Sample 1 (TuneSampleID) of MT20100713140315.wiff (Turbo Spray)
Max: 8.9e6 cps.
m/z, amu
Background
Printing Date: Tuesday, July 13, 2010
Printing Time: 2:09:01 PM

No title



BRUKER

```
Current Data Parameters
NAME        KCN1-amine-H
EXPNO                  4
PROCNO                 1

F2 - Acquisition Parameters
Date_           20090528
Time               11.07
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ              3.9584243 sec
RG                  12.7
DW                 60.400 usec
DE                  7.00 usec
TE                 297.3 K
D1           1.00000000 sec
MCREST       0.00000000 sec
MCWRK        0.01500000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 12.80 usec
PL1                 0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF           400.1299409 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```