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DESIGN, SYNTHESIS AND MECHANISTIC STUDIES OF SMALL MOLECULE INHIBITORS OF
THE HYPOXIA INDUCIBLE FACTOR PATHWAY

by

SUAZETTE REID MOORING

Under the Direction of Professor Binghe Wang

ABSTRACT

Cancer accounts for nearly one-quarter of deaths in the United States, exceeded only by heart diseases. In 2006, there were 559,888 cancer deaths in the US. Finding effective treatments for cancer is a major challenge among researchers. In solid tumor, hypoxia increases the progression of malignancy and metastasis by promoting angiogenesis. The transcription factor HIF-1 is responsible for the regulation of cellular processes, including glycolysis and angiogenesis. Clinical evidence has determined that expression of HIF-1 is strongly associated with poor patient prognosis. Also, activation of HIF-1 contributes to malignant behavior and therapeutic resistance. In view of these observations, there is a need for anti-cancer treatments that addresses hypoxic related tumors. HIF-1 presents a viable target for inhibition of tumor growth with small molecules. Herein, we describe the design and synthesis of small molecules that inhibit the

HIF-1 pathway, as well as mechanistic studies involved in the investigation of the mode of action of these compounds.

INDEX WORDS: HIF-1, Hypoxia, Small-molecule inhibitors

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SUAZETTE REID MOORING

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy
in the College of Arts and Sciences
Georgia State University

2010

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Suazette Reid Mooring
2010

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THE HYPOXIA INDUCIBLE FACTOR PATHWAY

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College of Arts and Sciences
Georgia State University
May 2010

DEDICATION

For Ray and Sariah

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First, I would like to thank the Author and Finisher of my Faith –I owe all to you Lord Jesus! Thank you for your Amazing Grace! I could not have done this without You!

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1 INTRODUCTION

Cancer accounts for nearly one-quarter of deaths in the United States, exceeded only by heart disease.¹ Although there have been many new treatments, cancer still claims many lives. As a result, there is a need for new therapeutics that address this problem. Hypoxia is characterized by a reduction in the partial oxygen pressure in cells and/or tissue and is characteristic of solid tumors. Tumor hypoxia has been shown to reduce the effectiveness of radiation and chemotherapy.^{2,3} According to Powis and Kilpatrick, oxygen can diffuse 100–180 μM from the end of the nearest capillary to the cells before it is used up completely.⁴ Consequently, in solid tumors, when the existing vascular system is unable to supply the growing tumor with adequate amounts of oxygen, it results in hypoxia, low pH and a lack of sufficient nutrients^{5,6}

Hypoxia Inducible Factor (HIF) is the primary transcription factor activated by hypoxia and is responsible for orchestrating a number of cellular responses (such as angiogenesis and glycolysis) that help tumor cells adapt to hypoxic conditions.⁷ HIF-1 is a basic helix-loop-helix heterodimeric transcription factor and is composed of two subunits: HIF-1 α and the constitutively expressed HIF-1 β .

The levels of HIF-1 α are determined by intracellular oxygen concentration (Figure 1). Under normoxic conditions, HIF-1 α is continually degraded by ubiquitination and proteasomal degradation. Degradation occurs when HIF-1 α is hydroxylated at Pro 564 and Pro 402 located at its oxygen-dependent degradation domain (ODDD). This process is facilitated by the prolyl hydroxylase enzyme that requires oxygen, iron and 2-oxoglutarate as the co-substrate to hydroxylate the specific amino acid residues.⁸⁻¹⁰ After hydroxylation, HIF-1 α binds to Von Hippel-Lindau protein (pVHL) and becomes a part of the E3-ubiquitin ligase complex, such that, HIF-1 is ubiquitinated and thereby marked for proteasomal degradation.^{11,12} Oxygen is required for the function of the prolyl hydroxylase enzyme. Therefore, under hypoxic conditions, HIF-1 α is stabilized. Once stabilized, HIF-1 α accumulates and translocates to the nucleus where it inte-

racts with HIF-1 β to form the active transcription factor, HIF-1.^{11, 12} HIF-1 then activates a number of cellular genes by binding to the DNA sequence 5'-ACGTG-3'. So far, more than 100 genes have been identified that are activated by HIF.^{13, 14} The activated genes includes those for proteins that carry out anaerobic glycolysis, for erythropoietin (red blood cell production), and for vascular endothelial cell growth factor (VEGF). VEGF is believed to be a powerful stimulus to new capillary formation, is a major driver of tumor angiogenesis and is the primary transcription factor activated by hypoxia.¹⁵ The overexpression of HIF-1 α is indicated in a number of human cancers¹⁶⁻¹⁸ and is associated with poor response to treatment and patient mortality.^{16, 19-22} As a result, HIF-1 has been investigated for its potential as an anti-tumor therapeutic target with small molecules.^{23, 24}

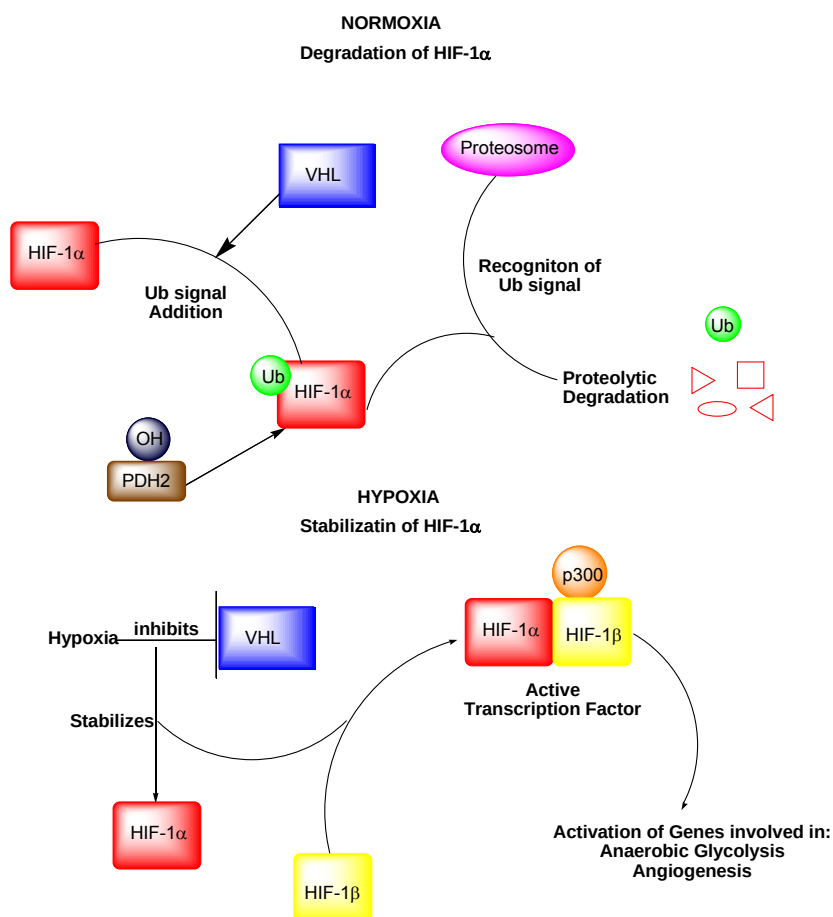


Figure 1. Hypoxia-Inducible Factor (HIF) Pathway of Hypoxia Control

1.1 Effect of Hypoxia on Tumors

In order for cells to survive in a hypoxic environment, they adopt a number of mechanisms that trigger apoptotic resistance, angiogenesis, and glycolysis. Cell proliferation and metastasis is also promoted.

Tumors are able to grow uncontrollably because the balance of cell growth versus cell death is disrupted. Some of the antiapoptotic activity of cancer cells may be the result of hypoxic conditions.²⁵ The adaptive mechanisms that cancer cells develop in response to hypoxia causes them to multiply faster than normal cells.³ The expression of various proteins in response to hypoxia may have an effect on apoptosis activity. For example, hypoxia can induce the production of anti-apoptosis protein IAP-2.²⁶ Another protein, periostin, can significantly increase metastasis of colon cancer by preventing apoptosis.²⁷ Also, osteopontin (OPN) has been identified as a protein that plays an important role in tumor development.^{28, 29} OPN is thought to promote tumor development through Akt activation that enables cell survival under stress related conditions.^{30, 31}

As cancer cells proliferate and tumors increase in size, a larger blood supply is needed to provide adequate oxygen and nutrients.²⁹ Hypoxia can induce factors such as VEGF and angiogenin that promote angiogenesis. VEGF has an especially important role in angiogenesis in tumors.^{32, 33} It is well established that hypoxic conditions will cause an increase in HIF-1 activity, which in turn enhances VEGF levels leading to increased angiogenesis³⁴⁻³⁶.

There are two schools of thought on the role of hypoxia in inducing angiogenesis. The first is known as the hypoxic crisis model,³⁷ which suggests that hypoxia leads to angiogenesis. This model proposes that initial overexpression of angiopoietin 2 leads to hypoxia, which in turn causes upregulation of VEGF. VEGF then promotes angiogenesis. The second model, the acceleration model³⁸ proposes that hypoxia is not responsible for promoting angiogenesis, but instead, angiogenesis is initiated by VEGF-promoting oncogenes. In essence, angiogenesis

leads to tumor cell proliferation. The poorly formed blood vessels will promote hypoxia. Because of hypoxia, HIF-1 is upregulated. These two models may be at work in angiogenesis and proliferation of tumor cells.

Hypoxia can also have an effect on glycolysis. Under hypoxic conditions cancer cells tend to move their ATP production from aerobic to anaerobic via glycolysis. Hypoxic cancer cells use glycolysis as a primary mechanism of ATP production, and HIFs are master regulators of glucose metabolism during hypoxia.^{3, 5, 39, 40}

Given the discussed roles of hypoxia in solid tumors and the fact that HIF-regulated genes are linked to cancer progression, inhibition of HIF activity may have therapeutic potential for cancer.

1.2 HIF-1 inhibitors

Several research groups have identified compounds that have been known to inhibit the HIF-1 pathway. These compounds affect HIF-1 levels by directly inhibiting HIF-1 signaling or by indirectly inhibiting signal pathways that affect HIF-1 expression. The mechanisms of action for HIF-1 inhibitors involve reduction in HIF1- α mRNA levels or protein levels, HIF-1 DNA-binding activity or HIF-1 mediated transactivation of target genes. Compounds may also reduce protein levels by decreasing the rate of HIF-1 α synthesis or by increasing the rate of HIF-1 α degradation. Several reviews have been published that describe HIF-1 inhibitors.^{24, 35, 41, 42}

Hsp90 inhibitors are a class of compounds that can inhibit the HIF-1 pathway. Hsp90 plays an important role in the stabilization of HIF-1 α under hypoxic conditions.⁴³⁻⁴⁵ Ansamycin derivatives such as Geldanamycin (GA)⁴⁶ and its analogues 17-allylamino-17-demethoxygeldanamycin (17-AAG) or the water-soluble 17-dimethylaminoethylamino-17-demethoxy-geldanamycin(17-DMAG) have been developed. These inhibitors work by binding to Hsp90 and interfering with its function.⁴⁷ GA binds to the N-terminal ATP binding domain of Hsp90 with high affinity and causes the destabilization and degradation of many Hsp90 client

proteins.⁴⁸ Structural analyses show that the N-terminal domain of Hsp90 binds to GA and its derivatives in the N-terminal high-affinity ATP-binding site.⁴⁹ Hsp90 binds to the HIF-1 α PAS domain, and the Hsp90 inhibitors geldanamycin and 17-allylaminogeldanamycin (17-AAG) induce proteasomal degradation of HIF-1 α even in renal carcinoma cells that lack functional VHL.^{45, 50, 51} 17-AAG is currently in phase I and phase II clinical trials.^{52, 53}

Inhibitors of topoisomerase (Top I) have also been identified as HIF inhibitors. Topoisomerases are enzymes that unwind and wind DNA, thereby controlling the synthesis of proteins for replication of DNA. One such inhibitor, topotecan (TPT),^{54, 55} a camptothecin analogue⁵⁶ was shown to inhibit the accumulation of HIF-1 α protein. Exploration of the mechanism of action of topotecan revealed that its inhibitory activity is independent of proteasomal degradation. TPT inhibited HIF-1 α even in the presence of protease inhibitors. TPT also did not affect the HIF-1 α mRNA accumulation but did decrease HIF-1 α protein translation. To address that Top I is required for inhibitory activity of HIF-1 of TPT, cells sensitive to camptothecins (CEM) and cell resistant to camptothecins (CEM-C2) cells were tested for transcription and protein accumulation of HIF-1 in a luciferase assay. Addition of TPT to CEM cells showed a decrease in luciferase levels, whereas in CEM-C2 cells TPT had no effect on luciferase levels. The researchers concluded that Top I was required for inhibition of HIF-1 activity by TPT. In addition, it was shown that RNA transcription (not DNA replication) was required for inhibition of HIF-1 α protein accumulation by TPT. In essence, topotecan forms a stable covalent complex with the DNA/topoisomerase I complex, which leads to breaks in the DNA strand resulting in apoptosis. In 2007, GlaxoSmithKline was approved for hycamtin® by the FDA and TPT became the first Top I inhibitor approved for oral use.

Also included in HIF-1 inhibitors are compounds that affect microtubule formation such as 2-methoxyestradiol (2ME2).⁵⁷ 2ME2 inhibits tumor growth and angiogenesis by disrupting tumor microtubules (MTs) in vivo. 2ME2 downregulates hypoxia-inducible factor-1 (HIF) at the

posttranscriptional level and inhibits HIF-1-induced transcriptional activation of VEGF expression. Inhibition of HIF-1 occurs downstream of the 2ME2/tubulin interaction, as disruption of interphase MTs is required for HIF-1 α downregulation. The effects of 2ME2 on human prostate cancer cells PC-3 and human breast cancer cells MDA-MB-231 were examined. Both PC-3 and MDA-MB-231 cells showed a reduction in nuclear and total HIF-1 α protein. The inhibition of HIF-1 α was dose dependent and seen under hypoxic and normoxic conditions. It was also noted that VEGF protein levels were also reduced by 2ME2 in a dose-dependent manner. To determine if the inhibition of VEGF was related to a direct effect on HIF-1 α , cells were transfected with luciferase gene and under the control of hypoxia response elements from the VEGF promoter. The result showed that 2ME2 treatment activity also blocked the hypoxia-induced transcriptional activity of HIF-1.

Examination of the effect of 2ME2 on HIF-1 α on the post-transcriptional level using cycloheximide (a post transcriptional inhibitor), showed that new protein synthesis was inhibited. Therefore, the decrease in HIF-1 α levels in the presence of 2ME2 is due to degradation of HIF-1 α protein. Lastly, the role of 2ME2 in microtubule depolymerization was also investigated. 2ME2 treated PC-3 cells were labeled with antibodies against tubulin and HIF-1 α and using a laser scanning confocal microscope, a dose dependent depolymerization of microtubules was observed in the 2ME2 treated cells compared to untreated control cells. 2ME2 displays a novel mechanism in which microtubules are depolymerized and HIF-1 α protein levels as well as HIF transcriptional activity are downregulated independent of oxygen. Also, at concentrations of 2ME2 that inhibit tumor growth and vascularization, tumor microtubules are depolymerized. 2ME2 has been established as a small molecule inhibitor of HIF-1.

Thioredoxin (Trx-1) inhibitors PX-12 and pleurotin have also been identified as inhibitors of HIF-1 α and VEGF.⁵⁸ Welsh and colleagues investigated the effect of PX-12 and pleurotin on HIF-1 α . These inhibitors decreased HIF-1 α protein, HIF-1 trans-activation and expression of

HIF-1, VEGF and inducible nitric oxide synthase (*iNOS*) *in vitro*. PX-12 and pleurotin inhibited the growth of MCF-7 cells with an IC_{50} of 1.9 ± 0.8 and 0.9 ± 1.0 μ M, respectively and in HT-29 cells of 2.9 ± 2.0 and 0.9 ± 1.2 μ M, respectively. Dose dependent decreases in HIF-1 α protein in MCF-7 cells under hypoxic conditions when exposed to PX-12 and pleurotin were also observed. These inhibitors also decreased the expression of HIF-1 α and VEGF proteins *in vivo*. The mechanism of the decrease in HIF-1 α protein by these Trx-1 inhibitors is unknown. However, it is clear that the mechanism does not involve pVHL since PX-12 and pleurotin decreased HIF-1 α in RCC4 cells that lack pVHL. The authors suggest that either a Hsp90 or PI3K/AKT pathway may be involved, but were not investigated.

Echinomycin⁵⁹ has been shown to affect HIF-1 DNA binding. Echinomycin inhibits HIF-1 α and HIF-1 β DNA binding activity in a dose dependent manner. Echinomycin was previously determined to be a sequence-specific DNA binding agent.⁶⁰ In that, it binds to the sequence 5'-CGTG-3' of HRE consensus sequence 5'-R(A/G)CGTG-3'. It was also shown that echinomycin inhibited hypoxic induction of luciferase in U251-HRE cells and VEGF mRNA expression in U251 cells.

PX-478 is a HIF-1 transactivation inhibitors that reduces HIF-1 α protein levels. PX-478 (S-2-amino-3-[4V-N,N-bis(2-chloroethyl)amino]phenylpropionic acid N-oxide dihydrochloride) is an inhibitor of constitutive and hypoxia-induced HIF-1 α levels (and thus HIF-1 activity). Welsh et al. initially reported that PX-478 suppresses hypoxia induction of HIF-1 α in cancer cell lines and suppresses constitutive HIF-1 α in cells that have lost pVHL.⁶¹ PX-478 decreased hypoxia-induced HIF-1 α protein levels in many cancer cell lines. The levels of constitutively elevated HIF-1 α in Panc-1 pancreatic cancer and PC-3 prostate cancer and RCC4 renal cancer were also decreased. The elevated HIF in Panc-1 and PC-3 cells are due to increase in phosphatidylinositol 3-kinase/Akt signaling and HIF-1 α gene amplification. In RCC4 cells, elevated HIF is a result of loss of pVHL. Therefore, the decrease in HIF-1 α by PX-478 does not require oxygen

or pVHL. The decrease in HIF-1 α protein is accompanied by a decrease in HIF-1 transactivating activity and decrease in VEGF expression.

Researchers also found that PX-478 inhibits HIF-1 α in many levels by decreasing the level of HIF-1 α mRNA, decreased translation of HIF-1 α protein in normoxic and hypoxic conditions and increased ubiquitination of HIF-1 α . It was suggested that the specificity of PX-478 on HIF-1 α may be the result of its cumulative effects on transcription, translation and ubiquitination. PX-478 is a potent anti-tumor activity against HIF-1 α expressing tumors.

Some inhibitors of HIF-1 do not affect the mRNA levels or protein levels but inhibit the binding of HIF-1 to DNA and prevent the activation of transcription. One such compound is DJ12. DJ12 was identified as a HIF-1 α inhibitor by the screening of 15,000 compounds.⁶² The compounds were screened using Chinese hamster ovary cells, which were designed to stably express luciferase reporter construct under the control of a hypoxia response element. DJ12 inhibited VEGF in breast cancer cell lines MDA-468 and ZR-75, melanoma cell line MDA-435, and pVHL mutant renal cancer cell lines RCC4 and 786-0. DJ12 down-regulates mRNA of downstream targets of HIF-1 α , and significantly inhibited HIF-1 α transactivation activity by blocking HIF-1 α hypoxia response element-DNA binding. The researchers suggested that DJ12 may work by inhibition of HIF-1 α transactivation through the blocking of HIF-1 α HRE DNA binding. DJ12 also showed 45% inhibition of HIF-1 α DNA binding from nuclear extracts of hypoxic MDA-468 cells treated with 100 μ mol/L of DJ12. Direct addition of DJ12 to nuclear extracts containing constitutive expression of HIF-1 α had no effect in blocking HIF-1 α HRE-DNA binding, suggesting that DJ12 does not directly interfere with the formation of protein-DNA complex but may inhibit the formation of HIF-1 α , HIF-1 β , and CBP/p300 transcription complex or folding of HIF-1 α . Hence, DJ12 has been introduced as a novel HIF inhibitor that is effective against the HIF pathway for breast, renal and melanoma cancer cell lines. However, as with many HIF inhibitors, long term (16 hours) toxicity assay studies revealed that DJ12 was toxic to MDA-468 cells

at IC₅₀ of 191 μmol/L in normoxic cells and greater than 250 μmol/L in hypoxic cells. Toxicity was attributed to multiple HIF targets by these compounds.

Other mechanisms that decrease HIF-1α protein levels, include cyclin dependent kinase inhibitor, Flavopiridol.⁶³ Flavopiridol is a potent cyclin-dependent kinase inhibitor that has an effect on VEGF. As previously discussed, VEGF secretion is important for the angiogenesis and vascular proliferation in cancer cells and VEGF is under the control of HIF-1 gene expression. Newcomb and co-workers has investigated the effect of flavopiridol on U87MG and T98G gliomas cells lines.⁶³ Flavopiridol has shown anti-angiogenic properties by inhibition of VEGF, decreased tumor cell migration, decreased hypoxia HIF-1α expression and reduced vascularity in GL261 glioma cells in animals treated with Flavopiridol.

Another inhibitor, chetomin is a member of the epidithiodiketopiperazine (ETP) family, (specifically from the fungus *Chaetominum* species) and was initially shown to have antimicrobial activity.^{64, 65} CBP/p300 is required for the coactivation of HIF.⁶⁶⁻⁶⁹ Chetomin has been shown to be a disrupter of HIF binding to p300 and works by disrupting the structure of the cysteine-histidine-rich domain 1(CH1) domain of p300 and interferes with its interaction with HIF and therefore HIF-1 transcription and inhibited tumor growth.⁷⁰ More recently, it was shown that chetomin reacts with p300, causing zinc ion ejection.⁷¹ It was proposed that compounds such as chetomin cause zinc ion ejection via a mechanism related to other known zinc binding disrupting compounds⁷² in which a zinc-binding cysteinyl thiol reacts with the torsionally strained disulfide of the ETP core to generate a transient protein-ETP disulfide. This disulfide can then rearrange to form an intramolecular protein disulfide with consequent reduction in zinc ion affinity. The ejected zinc ion (or zinc ETP complex) can then complex with a second ETP core to form a stable complex.

Other inhibitors include the histone deacetylase (HDAC) inhibitor, FK228, a bicyclic peptide, has also been shown to inhibit HIF-1 activity under hypoxic conditions, as well as inhibit tumor

angiogenesis.⁷³ Bortezomib, which functions by interfering with the carboxyl-terminal transactivation domain of HIF-1 α ⁷⁴ and the antifungal drug amphoteric B that inhibits HIF-1 by inducing the interaction of HIF-1 α with (FIH-1), leading to decreased recruitment of p300.⁷⁵

It is clear from the large number of HIF inhibitors in the literature that HIF-1 is a viable target for anti-tumor therapy. Thus far, there has been no small molecule that directly inhibits HIF-1. Therefore, our goal is to design and synthesize small molecule inhibitors of HIF-1.

2 DESIGN AND SYNTHESIS OF SMALL MOLECULE INHIBITORS OF HIF-1

2.1 Background

It has already been established in the literature that inhibition of HIF-1 with small molecules can have an anti-tumor effect (briefly reviewed in chapter 1.2). However, a specific HIF-1 pathway inhibitor was yet to be discovered. Therefore, the Van Meir laboratory at Emory University initiated a random screen of 10,000 compounds from a 2,2-dimethylbenzopyran combinatorial library.⁷⁶ The benzopyran moiety was chosen because it appears in more than 4000 natural compounds and is considered to be lipophilic enough to cross the blood-brain barrier.⁷⁶ The library was screened using an HRE-alkaline phosphatase assay developed by the Van Meir lab.⁷⁷⁻⁷⁹ In this assay, LN229 tumor cells were stably transfected with the alkaline phosphatase reporter, driven by 6 copies of the HRE (hypoxia response element) for the VEGF gene. This initial screening provided a few hit compounds, from which one compound, KCN-1 (Figure 2) was chosen for further evaluation. KCN-1 had an average IC_{50} of 4 μ M using the HRE-alkaline phosphatase assay.

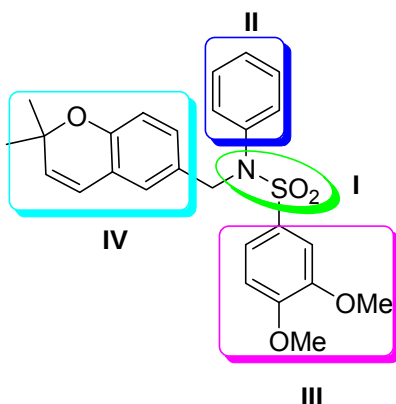


Figure 2. Four regions for synthetic modification of KCN-1

Next, preliminary *in vivo* experiments were performed to determine the anti-tumor effectiveness of KCN-1 (Narra Devi, Emory University). Nude mice (8 per group) were implanted

with LN229 tumor cells on both hind flanks. After 1 week, one group of mice was subjected to peritoneal injection of KCN-1 (60 mg/kg; 5 days/week), while the other group received the vehicle. The mice injected with KCN-1 showed tumors that weighed 6-fold less than that of the mice treated with vehicle (Figure 3). In addition, the KCN-1 injected mice displayed no apparent signs of toxicity or other negative effects.

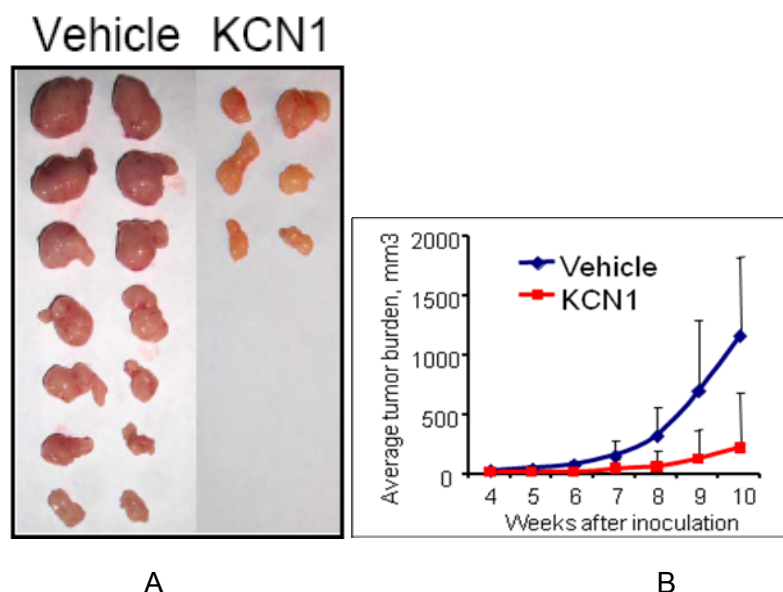


Figure 3. KCN1 inhibits LN229 glioma xenograft growth. LN-229 was implanted in both flanks of nude mice (8/group) and 1 week later started intraperitoneal injections of KCN1 (60 mg/kg; 5 days/week). A. Tumor volume over time. B. Tumors weighed 6-fold less at termination in KCN1 group. (This figure courtesy of the Van Meir lab).

Motivated by the encouraging results from both the *in vitro* inhibition assay and the *in vivo* experiments with mice, optimization of the structure of KCN-1 molecule was an obvious next step. Our goal was to improve the potency and the physiochemical properties of these inhibitors through design and synthesis of analogues. That is, the new analogues should possess properties that are predicted to lead to oral bioavailability, chemical and metabolic stability and minimal toxicity.

The first property to consider is solubility. All compounds have to be water-soluble (to various degrees) in order to be active. This is in addition to the need for solubility for reasons such as formulation, delivery, and membrane penetration. For membrane permeation considerations, compounds should possess an optimal solubility in both water and fat. It needs to be lipophilic enough to cross the cell membrane and enter into the cell, as well as water soluble enough to be transported in the aqueous media such as blood and cellular fluid. In addition, low water solubility can hinder absorption and activity. The water solubility is related to polarity, charge, molecular size, and the number of hydrogen bond donors in the molecule. Too many hydrogen bond donors can lead to low lipophilicity such that the drug cannot penetrate the cell membrane. LogP is a measure of the lipophilicity of a molecule. LogP is determined by the ratio of the solubility of the compound in 1-octanol versus water. The more positive the logP is the more lipophilic compound is. Ionization is also a factor in lipophilicity, since at physiological pH (7.4), amines are normally protonated and carboxylic acid deprotonated. Such ionizations may hinder the drug from crossing the cell membrane. However, this problem can be solved by adding electron-withdrawing groups (to lower pK_a) or electron-donating groups (to increase pK_a) of the molecule.

There are other important features that are desirable in an orally bioavailable drug. Christopher Lipinski has compiled some of these properties in "the rule of 5"⁸⁰. This rule of thumb is used by medicinal chemists to evaluate the drug-likeness of a molecule. It is based on the statistical analysis of a large database of known drugs. According to the Lipinski "rule of 5," compounds that are most likely to become a drug have the following features: Not more than 5 hydrogen bond donors (oxygen and nitrogen molecule attached to at least one hydrogen), no more than 10 hydrogen bond acceptors, a molecular weight under 500 and a logP of less than 5. These properties are associated with 90% of orally active drugs that have achieved phase II clinical status. Although following this rule is no guarantee that a compound will be active, it

provides a good starting point. There are many other sophisticated studies that allow for evaluation of the drug-like properties of a compound.⁸¹

Toxicity is also an important consideration in drug design as many drug candidates are withdrawn for unforeseen toxicity. The metabolism of certain functional groups can lead to products that result in toxicity. For example, *N*-hydroxylation on the amine nitrogen of anilines can lead to the formation of *N*-hydroxylamine metabolites that eventually form reactive nitrosamine intermediates. Also, hydrazines can lead to metabolites that can cause hepatotoxicity. Benzene rings can also present a problem, since they can potentially undergo enzymatic epoxidation to form an electrophilic arene oxide that is toxic. Michael acceptors such as α - β unsaturated carbonyls are also avoided because they are very reactive and toxic. Compounds containing alkyl halide are also problematic because halides are good leaving groups and are susceptible to nucleophilic attack. Alkenes can also lead to toxic products after they undergo metabolic epoxidation. These are only some of the functional groups that can cause toxicity. However, the presence of some of these functional groups does not necessarily mean that a compound will not make a good drug candidate.⁸²

Keeping all of these general considerations about physiochemical properties and toxicity in mind, our goal was to design and synthesize analogues of KCN-1. Our approach was to systematically modify KCN-1 along four regions of the molecule as shown in Figure 2. Each region of KCN-1 was modified separately to see the effect that these modifications will have on activity. As a result, we hoped to generate potent compounds, as well as develop a structure-activity relationship (SAR).

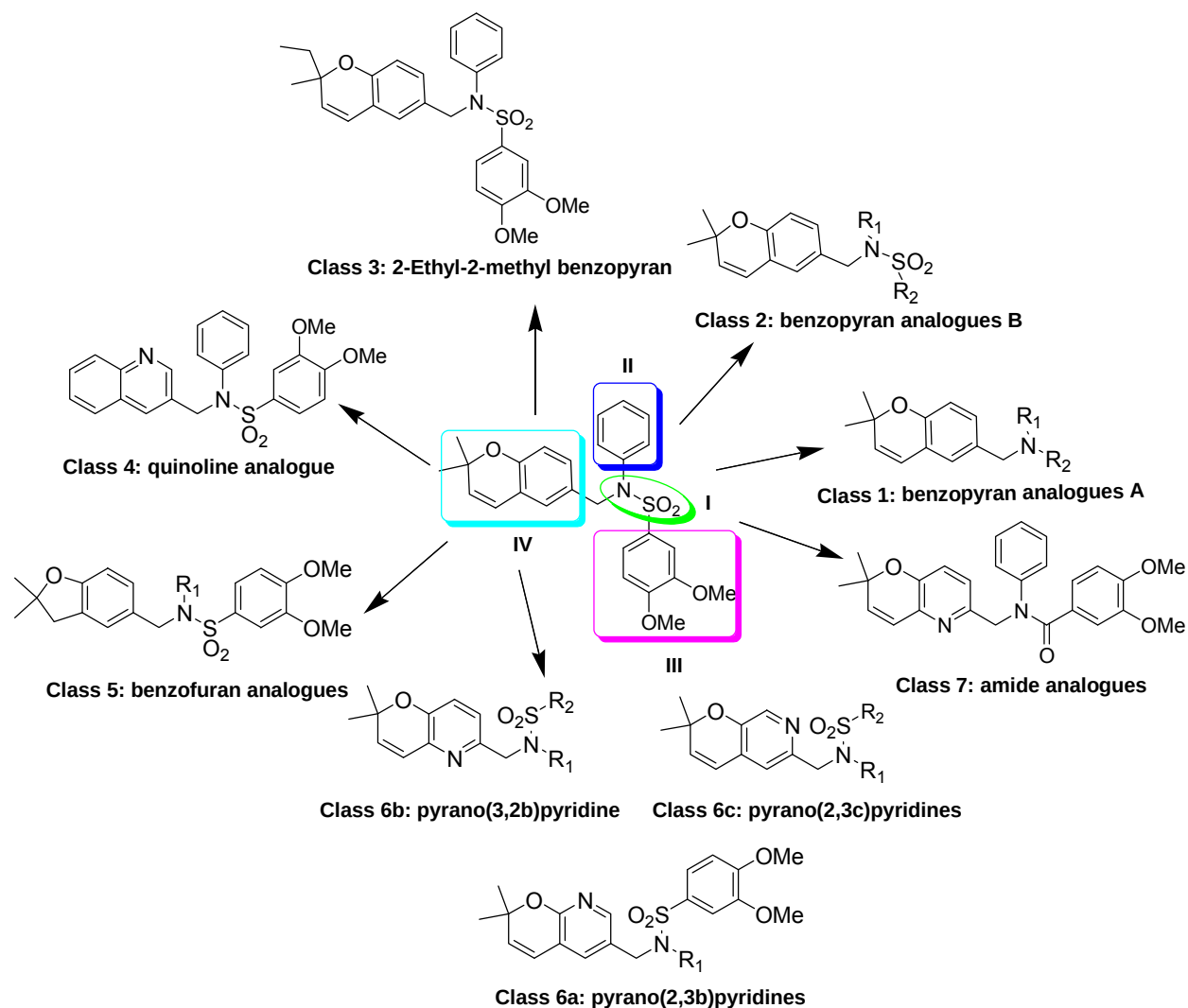


Figure 4. Analogues designed and synthesized

In all, seven classes of compounds were synthesized (Figure 4). The sulphonamide group of Region I was either eliminated (Class 1) or replaced by an amide group (Class 7). Region II and III was modified with several alkyl and aryl substitutions using known synthetic procedures (Class 2). Namely, the aldehyde derivative of the core structure of region I underwent reductive amination with a variety of alkyl and aryl primary amines to provide modifications to region II. Then, sulfonylation of the resulting secondary amines with various sulfonyl chlorides allowed modifications to region III (Figure 4). The benzopyran ring of region IV was probed to

determine the influence of subtle and major modifications of this region on activity. The first modification was the replacement of the gem-dimethyl group of region IV with a 2-Ethyl-2-methyl group (class 3). The benzopyran ring of region IV was also replaced with a quinoline ring (class 4) and benzofuran ring (class 5). Finally, the benzopyran ring of region IV was replaced with a pyranopyridine ring to generate classes 6a, 6b and 6c.

2.2 Results and Discussion

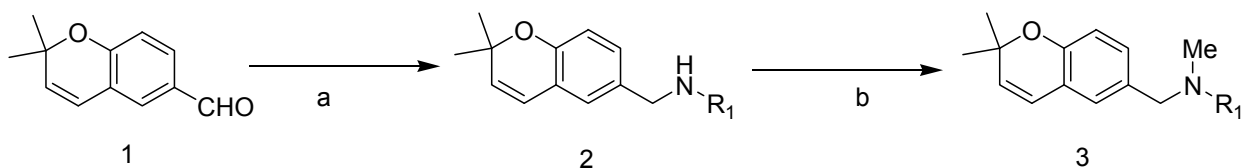
2.2.1 Chemistry

Class 1: Benzopyran analogues A

For the synthesis of the class I analogues, the benzopyran moiety was retained, while the sulphone group as well as the 3,4-dimethoxyphenyl group was eliminated. To afford these analogues, the aldehyde derivative of the benzopyran moiety was synthesized followed by reductive amination and methylation of the resulting secondary amine (in some cases).

The synthesis of class I analogues began with compound **1** that was synthesized according to literature procedures.⁸³ Reductive amination of **1** with several primary amines gave analogues

2. Methylation of secondary amine **2** with MeI and NaH generated analogues **3** (Scheme 1)



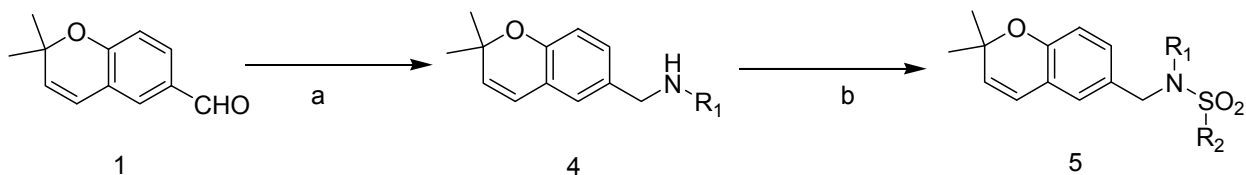
R_1 = 3,4-dimethoxyphenyl (**2a**, **3a**), 2-pyridinyl (**2b**, **3b**), 2,4-dimethylphenyl (**2c**, **3c**), 4-carboxyphenyl (**2d**), 2-bromophenyl (**2e**), 2-fluorophenyl (**2f**)

Reagents and Conditions: (a) $R_2\text{NH}_2$, ZnCl_2 , NaCNBH_3 , r.t.; (b) MeI, NaH, THF, r.t.

Scheme 1. Synthesis of benzopyran analogues A

Class 2: Benzopyran analogues B

Next, modifications were separately made to region II (**5a – 5j**) and then to region III (**5k – 5q**) of KCN-1 with various alkyl and aryl substituent, in order to probe their effect on activity. Reductive amination of aldehyde **1** with various aryl or alkyl amines afforded compound **4** that was subsequently sulphonated with various sulfonylchlorides to give analogues **5** (Scheme 2).



R_2 = 3,4-dimethoxyphenyl and

R_1 = isopropyl (**5a**), propargyl (**5b**), butyl (**5c**), *t*-butyl (**5d**), allyl (**5e**), 2-pyridinyl (**5f**), isobutyl (**5g**), cyclopentyl (**5h**), cyclopropyl (**5i**), cyclohexyl (**5j**)

R_1 = phenyl and

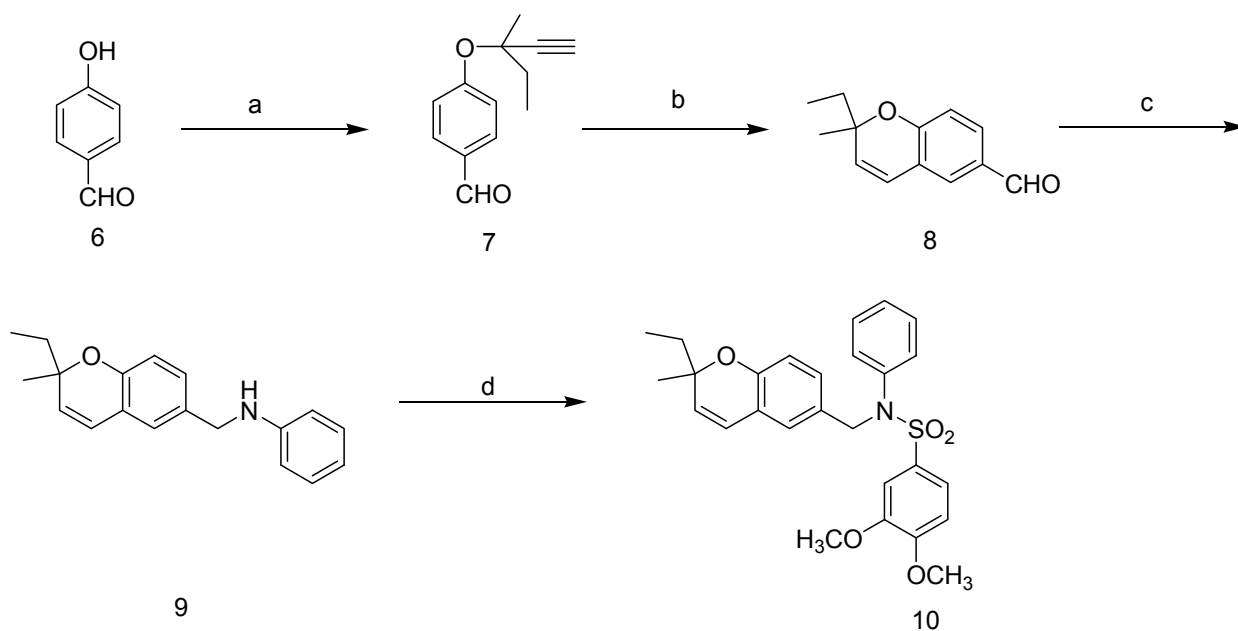
R_2 = 4-methoxyphenyl (**5k**), 2,4-dimethylphenyl (**5l**), 2,4-dichlorophenyl (**5m**), 2-trifluoromethoxy-4-bromophenyl (**5n**)

Reagents and Conditions: (a) R_1NH_2 , $ZnCl_2$, $NaCNBH_3$, r.t.; (b) R_2SO_2Cl , Et_3N , DCM, r.t.

Scheme 2. Synthesis of benzopyran analogues B

Class 3: 2-Ethylmethyl benzopyran analogue

Next, class 3 analogues were generated that involved a slight modification to the benzopyran portion (Region IV) of KCN-1. The ethyl group replaced one of the gem-dimethyl group on the benzopyran ring (Scheme 3). For the synthesis of these analogues, O-alkylation of 4-hydroxybenzophenone **6** with 3-methylpentyn-3-ol afforded compound **7**. Claisen rearrangement and re-aromatization of **7** by microwave irradiation yielded compound **8**. Reductive amination of aldehyde **8** gave the secondary amine **9** that was sulfonated with 3, 4-dimethoxybenzenesulfonyl chloride to give the final product **10**.

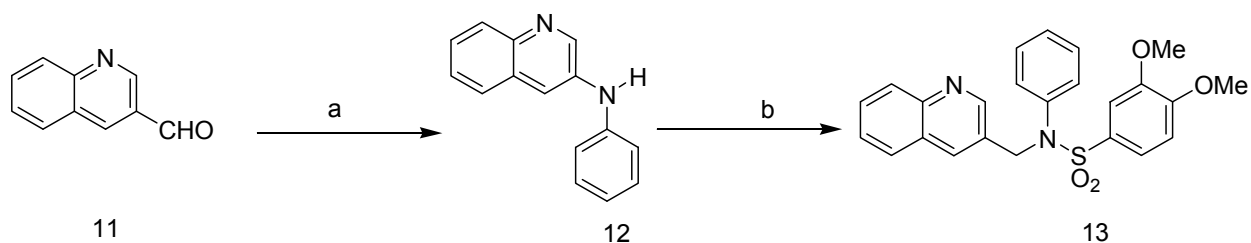


Reagents and Conditions: (a) 3-Methyl-pent-1-yn-3-ol DBU, TFAA, CuCl, CH₃CN, 0 °C to r.t., 32%; (b) xylene, microwave (220 W, 200 torr, 120 °C, 100 min; (c) aniline, ZnCl₂, NaCNBH₃, r.t., overnight, 40%; d) 3,4-dimethoxybenzylsulfonyl chloride, Et₃N, DCM, r.t., 24 h.

Scheme 3. Synthesis of 2-ethyl-2-methylbenzopyran analogue

Class 4: Quinoline analogues

The next modification to region IV was the replacement of the benzopyran ring with another fused ring system - quinoline (**Scheme 4**). Commercially available quinoline aldehyde **11** was subjected to reductive amination, followed by sulfonylation to afford compound **13**.

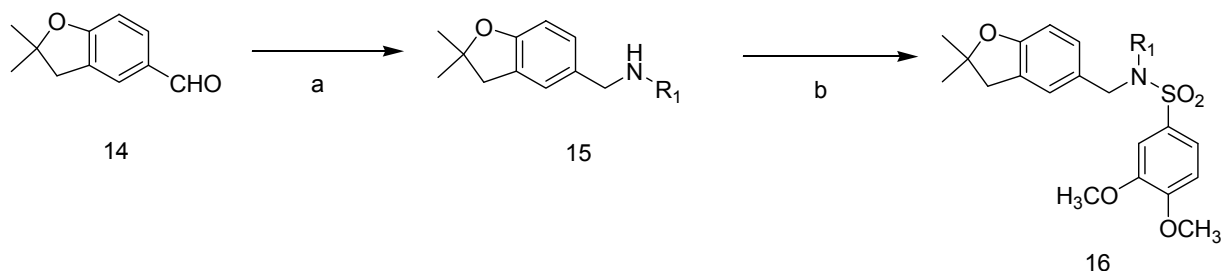


Reagents and Conditions: (a) aniline, ZnCl_2 , NaCNBH_3 , r.t.; (b) 3,4-dimethoxybenzylsulfonyl chloride, pyridine, r.t.

Scheme 4. Synthesis of quinoline analogue

Class 5: Benzofuran analogues

Additionally, the 2,2-dimethylbenzopyran ring (Region IV) of KCN-1 was replaced with a 2,2-dimethylbenzofuran ring (Scheme 5). Commercially available 2,2-dimethyl-2,3-dihydrobenzofuran-5-carbaldehyde **14** was treated by reductive amination with various primary amines to give compound **15** and then sulfonylation with 3,4-dimethoxybenzenesulfonyl chloride to give analogues **16**.



R_1 = phenyl (**16a**), cycloheptyl (**16b**), isopropyl (**16c**), butyl (**16d**), cyclohexyl (**16e**), cyclopentyl (**16f**)

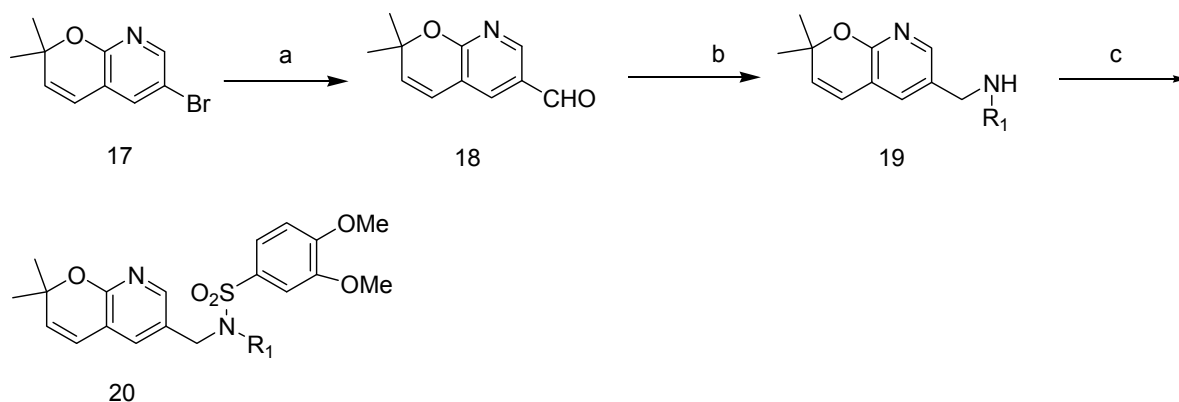
Reagents and Conditions: (a) R_1NH_2 , ZnCl_2 , NaCNBH_3 , r.t., 2 h; (b) 3,4-dimethoxybenzenesulfonyl chloride, Et_3N , DCM, r.t.

Scheme 5. Synthesis of benzofuran analogues

Class 6: Pyranopyridines

We also replaced one of the carbons on the aromatic portion of the benzopyran ring with nitrogen to afford pyranopyridine analogues. The pyridine nitrogen was separately placed in each of the three available positions on the benzopyran ring. It was envisioned that these compounds would provide increased water solubility and additional interaction points and therefore increased activity.

The first of these compounds was the pyrano(2,3b)pyridines **20**. The 2H-pyrano-[2,3b]-pyridine core **17** was synthesized as previously described.⁸⁴ Formylation of **17** with BuLi and DMF gave compound **18**. Reductive amination with aniline (**19a**) or cyclohexylamine (**19b**) followed by sulfonylation with 3,4-dimethoxybenzenesulfonyl chloride afforded compounds **20a** and **20b** (Scheme 6).

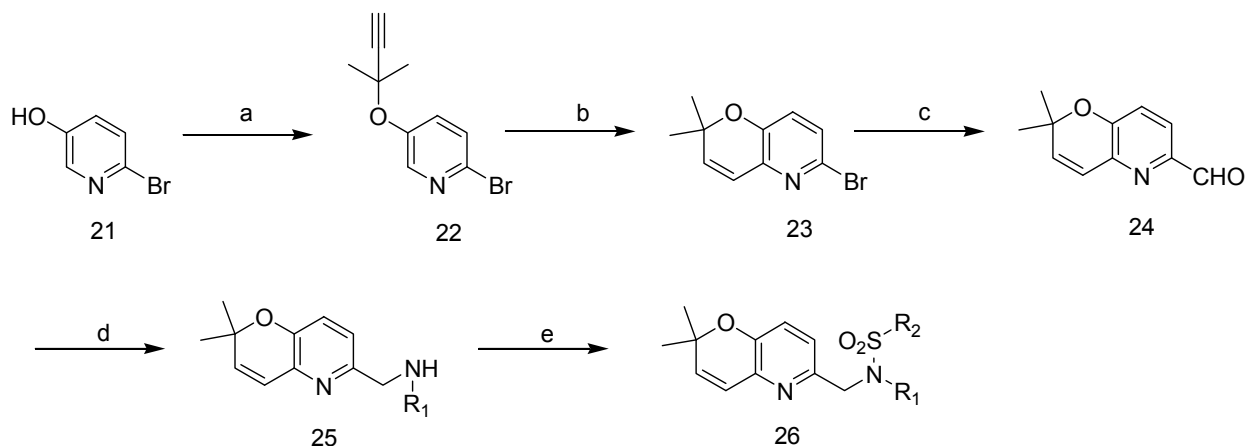


R₁ = phenyl (**20a**), cyclohexyl (**20b**)

Reagents and Conditions: (a) (i) BuLi, -78 °C (ii). DMF, anhydrous ether; (b) R₁NH₂, ZnCl₂, NaCNBH₃, MeOH; (c) 3,4-diethoxybenzenesulfonyl chloride, Et₃N, DCM, r.t.

Scheme 6. Synthesis of pyrano(2,3b)pyridine analogues

The second set of analogues in this class was the pyrano(3,2b)pyridines that were prepared using the following procedure. O-alkylation of commercially available 2-bromo-5-hydroxypyridine **22** followed by Claisen rearrangement and formylation gave compound **24** in 23% overall yield for two steps. Subsequent reductive amination of **24** and then reaction of secondary amine **25** with various sulfonyl chlorides gave analogues **26** (Scheme 7).



R_2 = 3,4-dimethoxyphenyl and

R_1 = phenyl (**26a**), butyl (**26b**), 3,4-dimethoxyphenyl (**26c**), cyclopentyl (**26d**), cyclohexyl (**26e**), tetrahydronaphthyl (**26f**), cycloheptyl (**26g**), cyclooctyl (**26h**), cyclobutyl (**26i**)

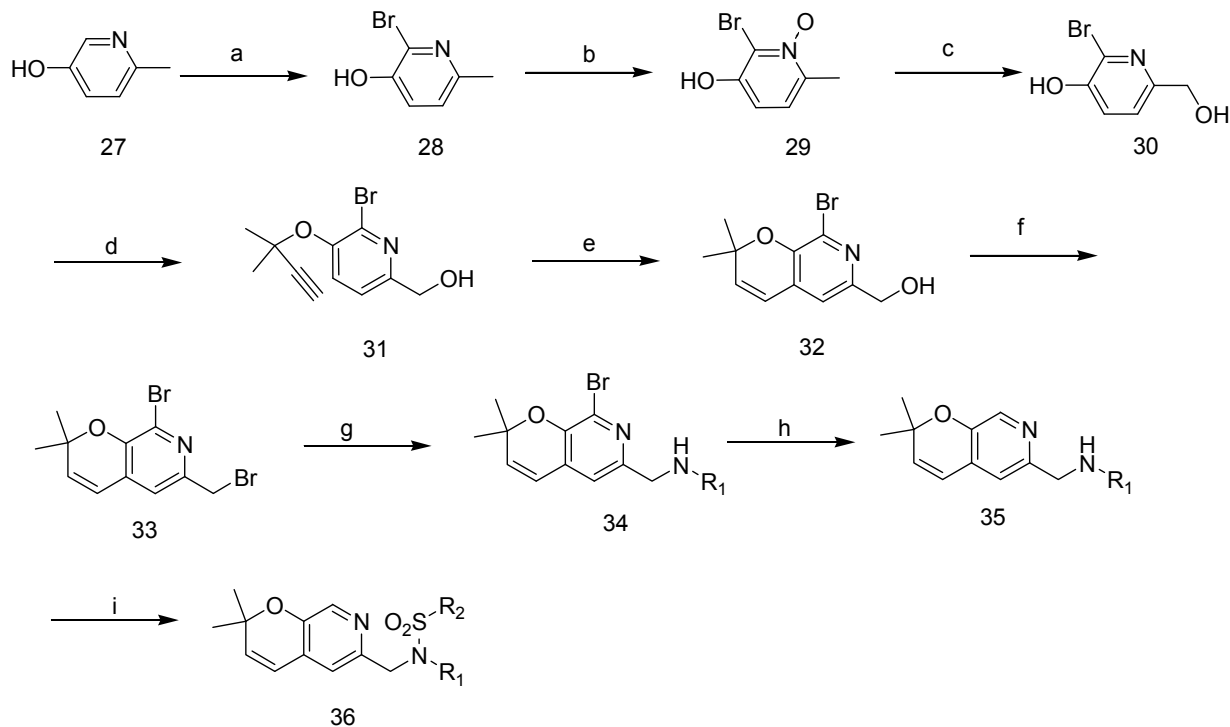
R_1 = phenyl and

R_2 = cyclohexyl (**26j**), isopropyl (**26k**), butyl (**26l**), propyl (**26m**), isobutyl (**26n**), 4-biphenyl (**26o**), propyl (**26p**), benzodioxolyl (**26q**), 2,3-dihydrobenzo(1,4)dioxinyl (**26r**), 4-fluorophenyl (**26s**)

Reagents and Conditions: (a) 2-methylbut-3-yn-2-ol, TFAA, DBU, CH_3CN ; (b) xylene, microwave heating 120°C , 30 min; (c) 1. BuLi, 2. DMF, anhydrous THF; (d) R_1NH_2 , ZnCl_2 , NaCNBH_3 , MeOH; (e) $\text{R}_2\text{SO}_2\text{Cl}$, Et_3N , DCM

Scheme 7. Synthesis of pyrano(3,2b)pyridine analogues

The final pyranopyridine derivative was the pyrano(2,3c)pyridines (class 6c). These analogues were synthesized by Hui Jin. To synthesize these analogues, 2-hydroxy-5-methylpyridine **28** was brominated to afford compound **28**. The *N*-oxidation of **28** with *m*-CPBA gave product **29** in 70% yield. Rearrangement of **29**, facilitated by TFAA afforded compound **30**. O-alkylation of **30** with 3-chloro-3-methyl-1-butene followed by Claisen rearrangement gave compound **32**. Substitution of the primary alcohol **32** with bromine followed to give **33**. Subsequent nucleophilic substitution of **33** with various primary amines followed by sulfonylation with appropriate aryl sulfonylchlorides resulted in analogues **36** (Scheme 8).



R_1 = phenyl and R_2 = 4-methoxyphenyl (**36a**), 4-nitrophenyl (**36b**)

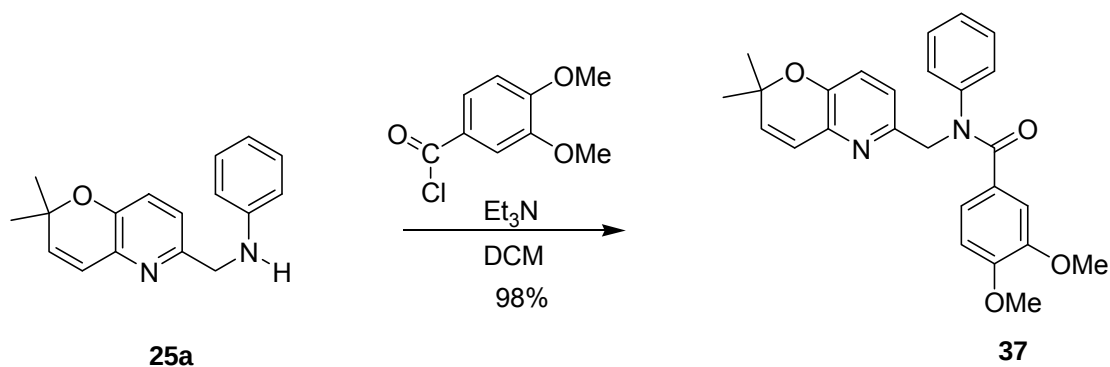
R_1 = cyclohexyl and R_2 = 4-isopropylphenyl (**36c**), 3,4-dimethoxyphenyl (**36d**)

Reagents and Conditions: (a) Br_2 , pyridine, $0^\circ C$, 74%; (b) m-CPBA, THF, 70%; (c) 1. TFAA, 2. MeOH, 30%; (d) 3-chloro-3-methyl-1-butene, K_2CO_3 , KI, $CuCl_2$, acetone (e) $CuCl$, toluene, microwave heating (200W, $120^\circ C$, 1h); (f) CBr_4 , PPh_3 , DCM, 40 %; (g) DIEA, DMF; (h) BuLi, THF, $-78^\circ C$, (i) pyridine, r.t.

Scheme 8. Synthesis of pyrano(2,3c) pyridine analogues

Class 7: Amide analogue

Finally, we replaced the sulphonamide of compound **26a** with an amide group. An amide group is common bioisostere for sulfonamide and may enhance activity. In this case, the previously synthesized **25a** was reacted with 3,4-dimethoxybenzoyl chloride in the presence of triethylamine to give the product **37** in 98% yield (Scheme 9).



Scheme 9. Synthesis of amide analogue **37**

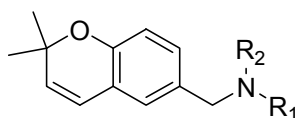
2.2.2 Biology

The generated analogues of KCN-1 were screened using a human glioma cell line LN229-HRE-AP, which stably expresses a hypoxia-responsive luciferase reporter gene. The IC_{50} values of all compounds were calculated based on a concentration curve testing of compounds at 0, 1, 5, 10 and 25 μM . The compounds were tested in triplicate. KCN-1 was tested along with the other analogues. Since the compounds were not all tested in a single experiment, the value of KCN-1 ranged from 0.3 -1.3 μM , with an average IC_{50} of $0.7 \pm 0.4 \mu\text{M}$.

Class I (benzopyran A) analogues were designed to probe the importance of the sulphone group. In general, the loss of the sulphone group in compound **2a** - **2f** and **3a** - **3c** resulted in a marked decrease in activity (Table 1). For secondary amine compounds **2a** - **2f**, only **2a** and **2b** had IC_{50} values below 10 μM , the others were higher than 25 μM (beyond the scope of the experiment). The best compound in that series was the 3,4-dimethoxyphenyl derivative **2a** with an IC_{50} of 3.0 μM . Analogues **3a** - **3c** showed similar IC_{50} as their secondary amine counterparts **2a** - **2c**, with the exception of the 2,4-dimethoxyphenyl derivative **3c** that had an IC_{50} of 2.6 μM . Therefore, methylation of the secondary amine did not seem to make much of a

difference. As a result, it was concluded that the sulfone group was essential to the activity of these compounds and was retained in future modifications of KCN-1.

Table 1. Structures and activities of analogues **2** and **3**^a



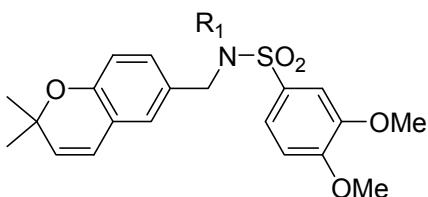
compound	R ₁	R ₂	IC ₅₀ (μM)	compound	R ₁	R ₂	IC ₅₀ (μM)
2a	H		3.0	2f	H		>25
2b	H		8.5	3a	Me		5.0
2c	H		>25.	3b	Me		8.4
2d	H		>25	3c	Me		2.6
2e	H		>25				

^aResults were from single runs

Next, Region II of the molecule was probed with various alkyl and aryl substituent (**5a** – **5k**). All the compounds were active to some extent (Table 2). The best of this group was the pro-

pargyl derivative **5b**, *iso*-butyl derivative **5g** and the cyclopropyl derivative **5i** with IC₅₀ values of 1.3, 1.6 and 1.5 μ M, respectively. In general, longer branched alkyl chains such as the *iso*-butyl group of **5g** (1.6 μ M) tended to do better than long unbranched chains such as the butyl group of **5c** (3.3 μ M) or shorter branched chains as the *tert*-butyl group of **5d** (3.5 μ M). Surprisingly, the pyridinyl substituted compound **5f** was almost more than 5 fold lower in activity than KCN-1, which is substituted by a phenyl group at the same position. Also, alkyl rings smaller than 6 carbons were better tolerated.

Table 2. Structures and activities of analogues **5a** – **5j**^a

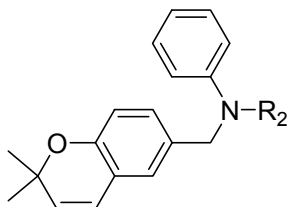


compound	R ₁	IC ₅₀ (μ M)	compound	R ₁	IC ₅₀ (μ M)
5a		3.1	5f		5.0
5b		1.3	5g		1.6
5c		3.3	5h		0.5
5d		3.5	5i		1.5
5e		3.4	5j		4.0

^aResults were from single runs

Compound **5k** – **5u**, were modified at region III of KCN-1 with various aryl substitutions (Table 3). The best compound in this group was the 4-methoxyphenyl substituted **5k** and 2,4-dimethylphenylsubstituted **5i** with IC_{50} values of 0.6 and 0.5 μ M, respectively. The 2-trifluoroester 4-bromo phenyl substitution (**5p**) resulted in a significant decrease in activity as compared to **5m** that was only a 4-bromosubstituted that had relatively better activity with an IC_{50} of 5.5 μ M. .

Table 3. Structures and activities of analogues **5k** – **5o**^a



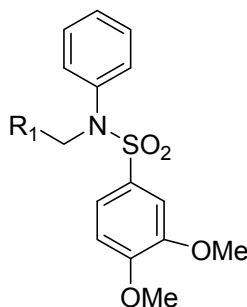
compound	R ₂	IC ₅₀ (μ M)
5k		0.6
5l		0.5
5m		2.1
5n		>25

^aResults were from single runs

Compound **10** represented a subtle change to region IV of the KCN-1 molecule. In this case, simply substituting one of the gemdimethyls of the benzopyran ring system of KCN-1 with an ethyl group resulted in a decrease in activity with an IC_{50} of 2.2 μ M (Table 4).

In the case of compound **13**, replacement of the benzopyran ring of KCN-1 with a quinoline ring led to a reduction in activity against HIF-1 with an IC_{50} of 3.5 μ M (Table 4).

Table 4. Structures and activities of analogues **10** and **13**^a



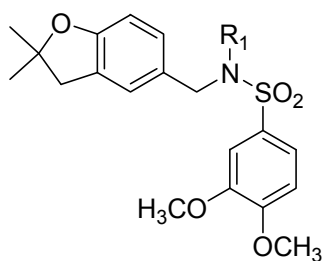
compound	R ₁	IC ₅₀ (μ M)
10		3.1
13		1.3

^aResults were from single runs

The benzofuran derivatives **16** afforded some potent compounds (Table 5). A comparison of compound **16a** (IC_{50} = 0.5 μ M) to KCN-1 shows that the substitution of the benzopyran ring with benzofuran did not necessarily result in a more potent compound than KCN-1, but the benzofuran analogue was comparable to that of KCN-1. The foreseeable benefit of the benzofuran structure of **16** is that it eliminates the double bond on the pyran ring of KCN-1. Since that

double bond may be susceptible to substitution in vivo and thereby introduce toxicity, the benzo-furan ring may be a better alternative. The ring size of the cycloalkyl derivatives seems to have an effect on activity. A comparison of the cycloheptyl ring of **16b** (9.1 μM), the cyclohexyl ring of **16e** (8.2 μM) and the cyclopentyl ring of **16f** (0.4 μM) seems to suggest that smaller rings (ring size 5 or smaller), tend to be more favorable than large rings (6 carbons or more). This is similar to the trend seen with the benzopyran analogues B (class 2).

Table 5. Structures and activities of analogues **16**^a



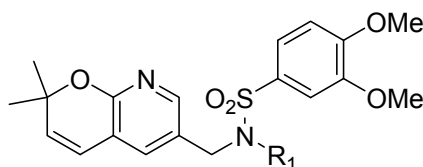
compound	R ₁	IC ₅₀	compound	R ₁	IC ₅₀
16a		0.5	16d		0.6
16b		9.1	16e		8.2
16c		1.5	16f		0.4

^aResults were from single runs

The first of the pyranopyridine analogues was Class 6a, the pyrano(2,3b)pyridines. Only two compounds were designed in this class. Compound **20a** had the same substitutions as KCN-1 with the exception of the pyranopyridine core. This compound showed some activity

with an IC_{50} of 2.5 μM . However, it was not as active as KCN-1. Replacing the phenyl ring by the cyclohexyl ring (**20b**) resulted in a loss of activity, that is, the IC_{50} was higher than 25 μM (Table 6).

Table 6. Structures and activities analogues **20**^a



compound	R ₁	IC ₅₀ (μM)
20a		2.5
20b		>25

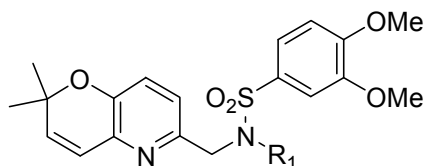
^aResults were from single runs

The next group of compounds in this class was the pyrano(3,2b)pyridine (class 6b). Compounds **26a** – **26k** were modified at region I with various alkyl and aryl amines. This class of compounds was among the best in the pyranopyridine class (Table 7). All of these compounds showed inhibitory activity against HIF-1 with the exception of the 2,4-dimethoxy derivative **26c**. Phenyl derivative **26a** is similar in structure to KCN-1 with the exception of the pyrano(3,2b)pyridine core. This compound had an IC_{50} of 1.3 μM , which is within the range of activity for KCN-1. The best compound in this group (**26a** – **26t**) was the cyclobutyl derivative **26i** with an IC_{50} of 0.25 μM . Comparing all the cycloalkyl analogues, the general trend remained about the same as that of other series, in that smaller rings (< 6 carbons) tend to have better activity than larger ring derivatives (> 6 carbons). The tetrahydronaphthalene derivatives **26f**

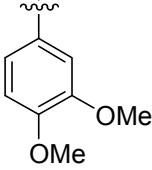
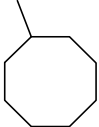
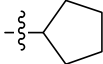
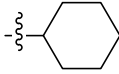
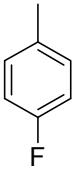
and the 4-fluorophenyl derivative **26j** had the lowest activity in this series with IC_{50} of 6.15 and 7.7 μ M, respectively.

Additionally, these pyrano(3,2b)pyridines **26k** – **26t** was also modified at region III with alkyl and aryl sulphonyl derivatives. Generally, this group did not produce very active compounds (Table 8). The cyclohexyl derivative **26i** was well tolerated in this position, with an IC_{50} of 0.4 μ M. In this case, the smaller cyclopropyl ring showed almost no activity within the experimental conditions. Compound **26r** and **26t** was an attempt to fix the conformation of the 2,4 dimethoxybenzene to see if it would enhance activity. Compound **26r** that separated the oxygen atoms by one carbon showed a decrease in activity (IC_{50} = 6.5 μ M) compared to the 3,4 dimethoxy substituted compound **26a** (1.25 μ M). However **26t**, in which the oxygen atoms are separated by 2 carbons showed an IC_{50} of 0.85 μ M. Also noted is the quinoline derivative **26s** that showed relatively good activity with an IC_{50} of 0.9 μ M.

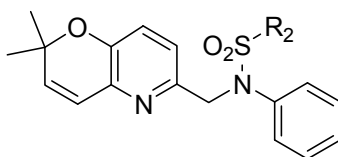
Table 7. Structures and activities of analogues **26a** – **26j**^a



Compound	R ₁	IC ₅₀ (μ M)	Compound	R ₁	IC ₅₀ (μ M)
26a		1.3	26f		6.2
26b		0.9	26g		6.6

26c		>25	26h		0.7
26d		0.6	26i		0.25
26e		0.8	26j		5.7

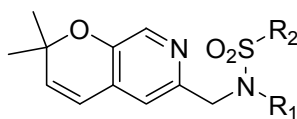
^aResults were from single runs

Table 8. Structure and activities of analogues **26k** – **26t**^a

Compound	R ₂	IC ₅₀ (μM)	Compound	R ₂	IC ₅₀ (μM)
26k		0.4	26p		0.88
26l		13.4	26q		3.4
26m		>25	26r		6.5
26n		5.0	26s		0.9
26o		6.4	26t		0.9

^aResults were from single runs

The third group of compounds in class 6 was the pyrano(2,3c)pyridinyl derivatives (class 6c). These compounds also showed good activity and were comparable to KCN-1 (Table 9). Derivatives **36a**, **36b** and **36c** all showed similar IC₅₀ values of 1.42, 1.80 and 1.08 μM, respectively. The cyclohexyl derivative **36d** was similar to other series of compounds with a much lower IC₅₀ of 5.8 μM. Comparing **36c** to **36d**, the isopropylphenyl derivative (**36c**) allowed for improvement in activity compared to the 3,4 dimethoxy substitution of **36d**.

Table 9. Structures and activities analogues **36**^a

compound	R ₁	R ₂	IC ₅₀ (μM)
36a			1.4
36b			1.8
36c			1.1
36d			5.8

^aResults were from single runs

Because the pyranopyridine analogues were among the best compounds, we decided to replace the sulphonamide group with an amide group to see what effect this modification will have on activity. The amide derivative showed a 2-fold increase in activity over the sulphonamide derivative (Figure 5). This amide group can be incorporated in future modifications of the compound.

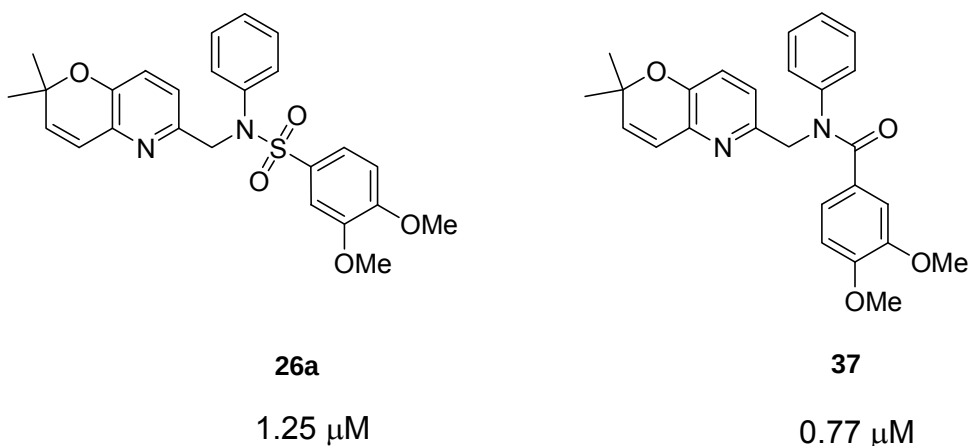


Figure 5. Comparison of structures and activities of **26a** and **37**

2.3 Summary of analogues designed and synthesized

Several potent analogues of the lead compound KCN-1 were synthesized. These analogues were able to yield information on the important functional groups at each of the four regions indentified in Figure 1. This has allowed us to make some general conclusion about the SAR of this molecule (Figure 4). Analogues **2** showed that the sulphone group was required for the activity of these compounds. Also, alkyl rings 5 carbons or shorter, as well as longer branched chains were well tolerated at region II of KCN-1. At region III, aryl substitutions seem to be better than alkyl substitutions. The benzofuran analogues (**16**) were also successful in that the analogues in this series of analogues showed activity comparable to that of KCN-1, but may be a good future alternative to the benzopyran derivatives, since it does not contain an electrophilic double bond. To date, the pyrano(3,2b)pyridine analogues provided the most improvement in activity as compared to KCN-1. The best overall compound came from this group – the cyclobutyl derivative **26i**, that had an IC_{50} of 0.25 μ M. The improvement in activity of these analogues over KCN-1 may be a result of increased hydrophilicity and/or hydrogen bond interactions from the addition of the pyridine ring.

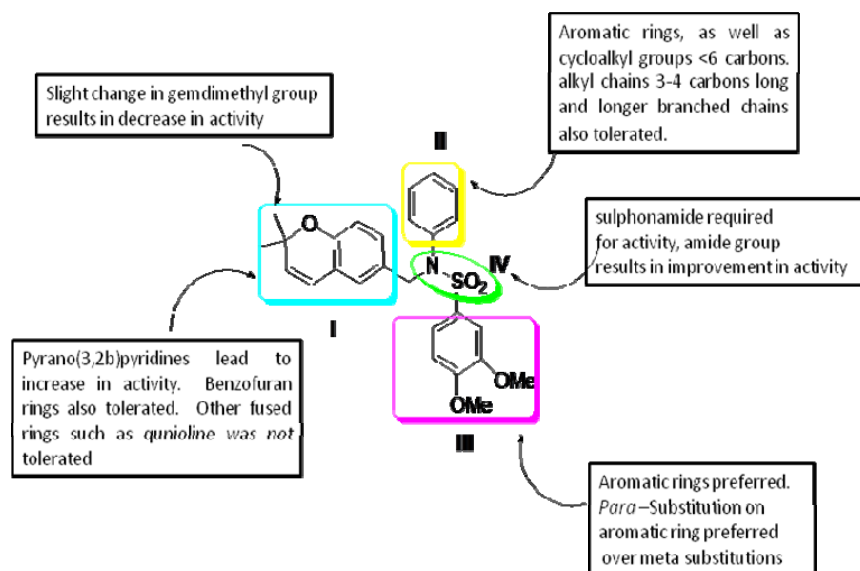


Figure 6. Structure-activity relationship of KCN-1

3 MECHANISTIC STUDIES

In addition to the synthesis of more potent analogues of KCN-1, another goal was to determine the mechanism of action of these compounds. The following discussions will emphasize our synthetic efforts toward this goal, along with biological work by the Van Meir laboratory.

Initial investigation into the mechanism of action of KCN-1 by the Van Meir lab suggested that KCN-1 specifically affects the HIF pathway without affecting common signal transduction pathways in the cell (Figure 7). Cell extracts of normoxic and hypoxic cells were analyzed for changes in total protein expression levels in the presence of KCN-1. The extracts were analyzed by Western blot at 5 hours (when strong anti-HIF-1 α activity is detected). No changes were observed for signal transduction proteins (Akt, p85 PI3K subunit, Erk1/2), short-lived proteins (p53, I κ Ba, cyclin D1) or control proteins (HIF-1 α and actin). Western blot analysis also showed that KCN-1 inhibits the synthesis of HIF-1 α under hypoxic but not normoxic conditions (data not shown). This suggests that KCN-1 has a unique mechanism of action targeting a component of the HIF translation machinery operating specifically under hypoxia. KCN-1 is shown to prevent HIF-1 α stabilization under hypoxia within 5 hours, while they do not perturb main cellular signaling events or stability of other short-lived proteins in the cells during the same time frame.

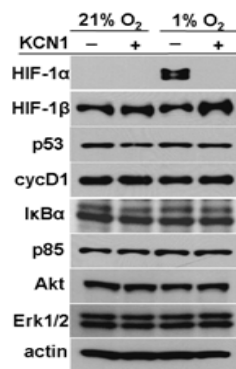


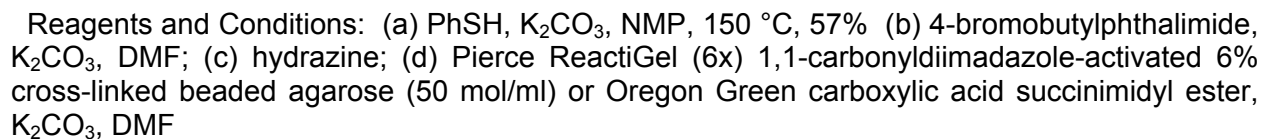
Figure 7. Analysis of specificity of KCN1. LN229 cells were pre-treated with KCN1 (25 μ M for 1h and then put in 1% O₂ for 5 hrs). Cell extracts were analyzed by Western blot for levels of total proteins. (*This figure, courtesy of the Van Meir lab*)

To further investigate the mechanism of action of KCN-1, we attached a linker to KCN-1 that can be coupled to a variety of amine reactive moieties such as an agarose matrix or a fluorophore. These linker-modified versions of KCN-1 can then be used in pull-down assays to identify any interacting proteins from cell lysates.

Next, we probed the structure of KCN-1 for a convenient place of attachment to the linker that will not significantly affect its activity. KCN-1 was selectively demethylated at the para position in the presence of thiophenol. The resulting derivative **38** was shown to be approximately 80% as active as the unmodified KCN1 in the HRE-AP assay, suggesting that this modification allowed the molecule to retain much of its activity. We then attached a 4-carbon linker with a terminal Boc-protected amine. The Boc-modified compound also retained the majority of the activity of KCN-1. The next step was simply to attach the linker to an immobile solid phase after removal of the Boc protecting group. However, removal of the Boc group using TFA resulted in cleavage and/or alteration of the KCN-1 molecule. Therefore, a different strategy for successful synthesis of the primary amine was employed (Scheme 8).

The hydroxyl group of the demethylated compound **38** was alkylated with 1-bromo-4-phthalimidebutane to give **39**. Deprotection of the phthalimide group with hydrazine afforded

For coupling to the agarose bead, the linker modified compound was mixed with Pierce ReactiGel (6x) 1,1'-carbonyldiimidazole-activated 6% cross-linked beaded agarose (50 $\mu\text{mol/ml}$) and attached through the amine group (Scheme 8). The remaining carbonylimidazole residues of the agarose beads were subsequently quenched with ethanolamine. A control matrix was also generated through the reaction of the activated agarose with ethanolamine alone.



Scheme 10. Synthesis of four-carbon linker modified KCN-1 and its derivatives

The KCN-1 linker coupled to agarose beads was used to pull down any interacting proteins in cell lysates. One of the major components of the pull down experiments was a protein called plectin-1. Plectin-1 is a large protein of approximately 500 kDa in size. It acts as a link between the three major components of the cytoplasm: actin filaments, microtubules and intermediate filaments.⁸⁵ As a result, plectin plays an important role in maintaining the mechanical strength and elasticity of tissues.⁸⁶

In order to investigate if there is a direct interaction between plectin-1 and KCN-1, the proteins bound to the KCN-1 resin and the control resin was probed with plectin-1 antibody (BD bioscience) (Figure 4). Plectin-1 was shown to bind to the KCN-1 affinity resin but not the control resin.

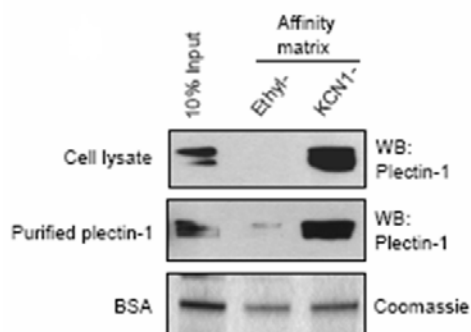


Figure 8. Gel electrophoresis of proteins from immobilized KCN1 and control matrix. (*This figure, courtesy of the Van Meir lab*)

Further studies into the mechanism of inhibition of KCN-1 on HIF-1, a comparison of the gene expression signatures of KCN1 and those of known Hsp90 inhibitors geldanamycin, 17-AAG, and 17-DMAG were very similar. Therefore, the possibility existed that KCN-1 could act via an identical mechanism. To assess the binding of Hsp90 to KCN-1, the KCN-1 bound and the control matrices were incubated with the lysates of tumor cells, followed by the Western blot

analysis of the unbound and matrix-bound proteins. Hsp90 was undetectable in the eluates, suggesting that KCN-1 does not bind to the chaperone in cell extracts (Figure 5).

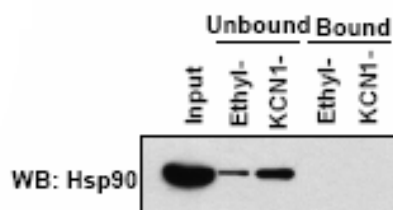


Figure 9. Western blot analysis of the unbound and matrix-bound proteins. (*This figure, courtesy of the Van Meir lab*)

Based on these results and other experiments (not discussed here) by the Van Meir lab, it was hypothesized that KCN-1 may bind to plectin-1, but not to Hsp90. A possible mechanism of action was suggested: KCN-1 binds to plectin-1 and perturbs its 3-dimensional conformation. This plectin-1-KCN-1 complex binds to Hsp90 and traps it in the cytoplasm and therefore lowers its concentration in the nucleus. As a result, Hsp90 is less available to HIF-1, where it is needed to maintain its transcriptionally active conformation of HIF

This initial hypothesis that KCN-1 binds to plectin-1 initiated some cell imaging experiments to determine if KCN-1 did indeed bind to plectin. KCN-1 was coupled to a green fluorescent dye - Oregon Green (Scheme 7) and used in fluorescent cell imaging studies. Oregon Green dye was chosen because it is not easily quenched and has a very bright green fluorescence as compared to its fluorescein counterpart. The cells were also co-stained with plectin antibody (Texas Red) to see if any overlap would result. The Oregon Green linked KCN-1 (KCN-OG) was incubated with Hela cells (fixed with methanol) for 1 hour then washed to remove excess compound and observed under a fluorescent microscope. The results showed the bright green color of KCN-OG in these cells (Figure 6A). Under the confocal microscope, there

was indication that there may be significant overlap of the green KCN-OG and the Texas Red plectin antibody and therefore, they are perhaps binding to the same structure (Figure 6).

In addition, we were able to obtain plectin^{-/-} (plectin negative) mouse fibroblasts⁸⁷ from the Van Meir lab to further explore the immunofluorescence experiments. KCN-OG stained both the plectin ^{-/-} and the plectin ^{+/+} cells. These results indicated that the binding of KCN-OG may not be exclusive to plectin, but may bind to another cellular component as well (Figure 7).

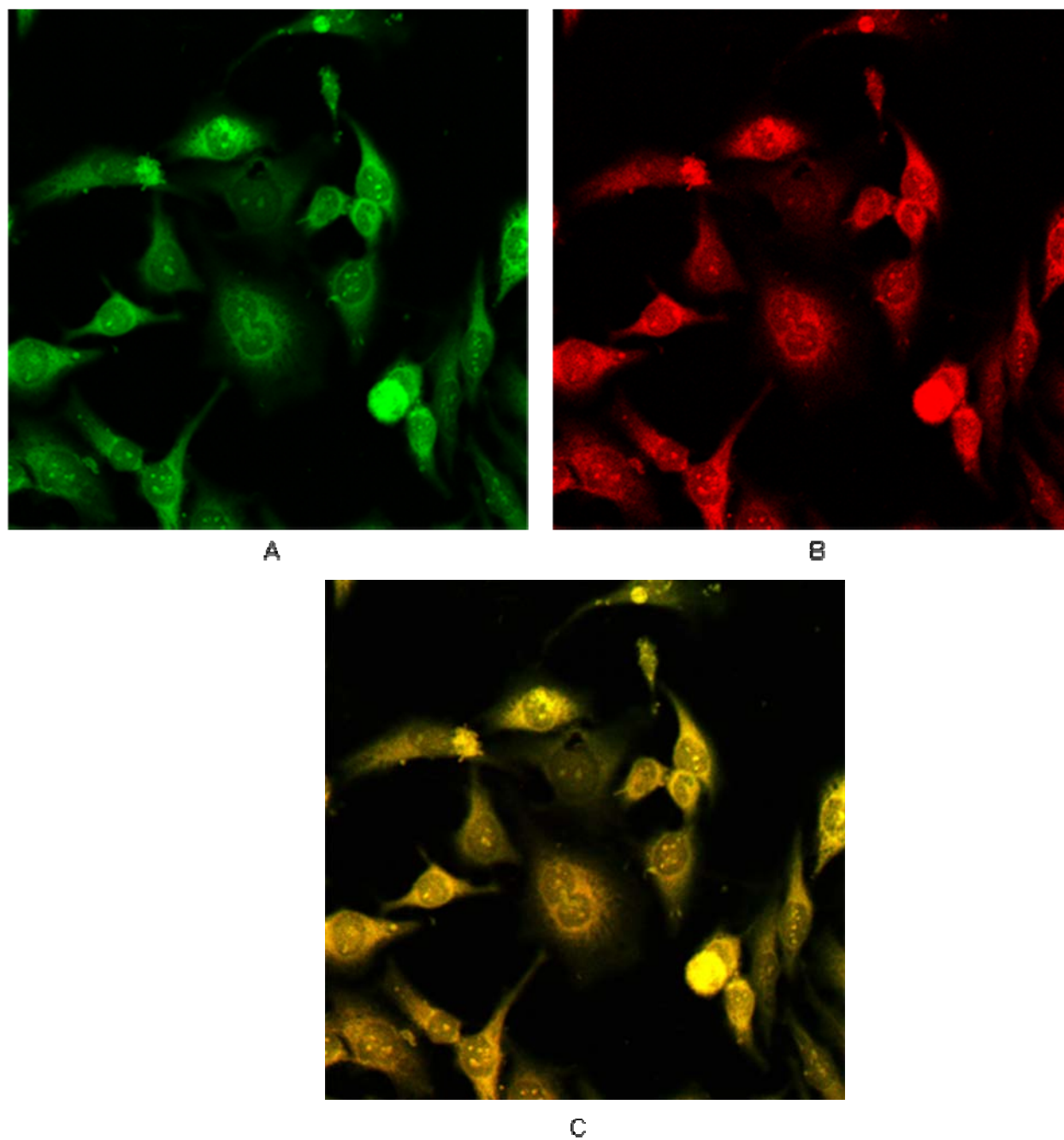


Figure 10. Fluorescent cell images of hela cells. A. Cells labeled with KCN-OG. B. Plectin antibody (Texas Red). C. Overlap of A and B. (Confocal microscope $\times 100$)

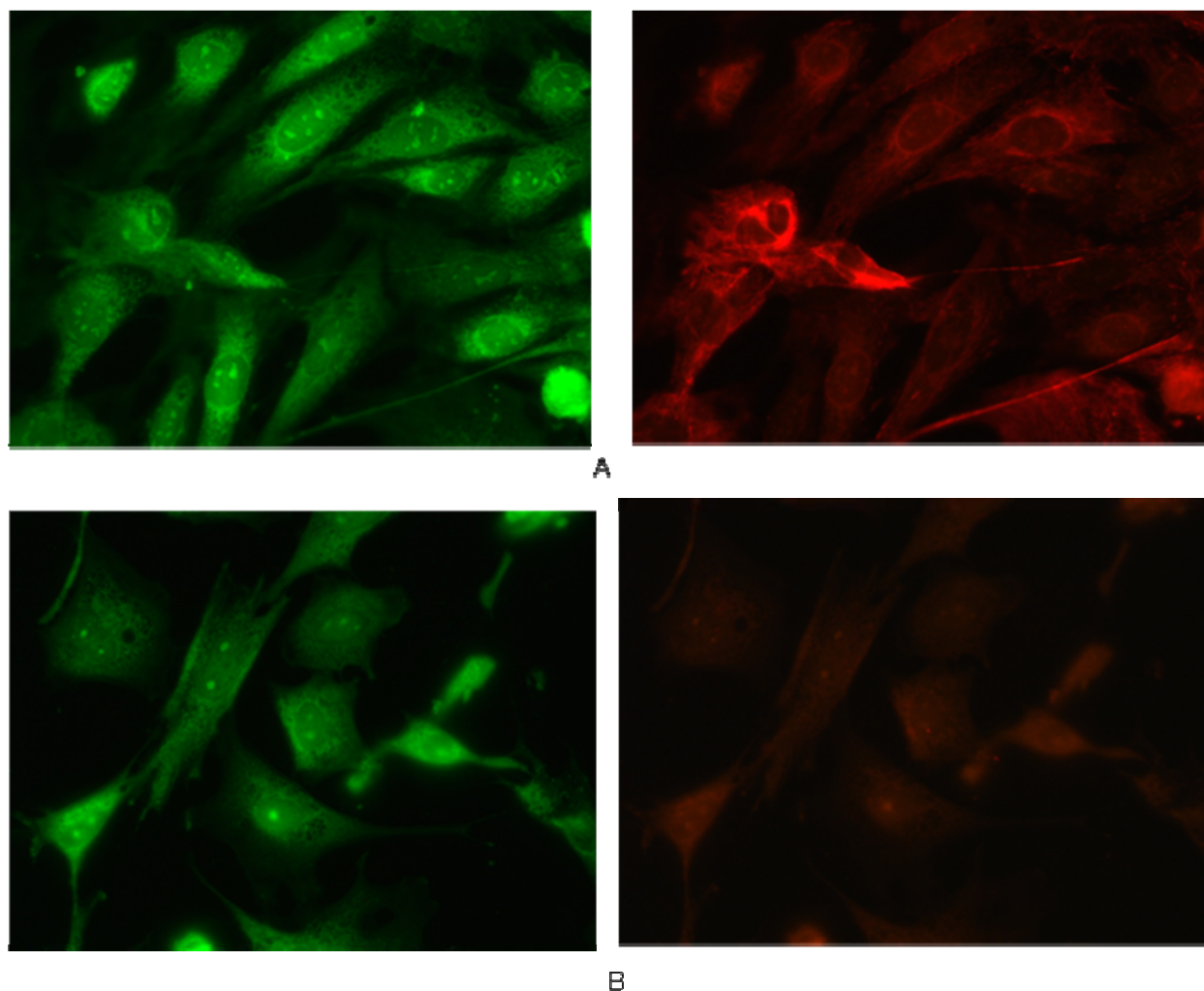


Figure 11. Fluorescent cell images of mouse fibroblasts with KCN-OG and plectin antibody A. Comparison of plectin positive cells B. Comparison of plectin negative cells. (Epifluorescent microscope $\times 25$)

Further probing into the mechanism by the Van Meir lab confirmed this result. They observed that although KCN-1 did indeed bind to plectin-1 it was not the main reason for the anti-tumor activity of the compound. Investigation into the components of the HIF-1 pathway revealed that KCN-1 may be binding to the transcription co-factor p300. More recently, the use of KCN-OG provided further clues about the interaction of KCN-1 with p300. To explore the interaction of KCN-1 with p300, the CH1 domain of p300 was amplified by PCR and cloned into a bacterial expression vector and GST/p300CH1 fusion protein was expressed.

Glutathione S-transferase (GST) binds to glutathione with very high affinity and GST fusion proteins can be purified on immobilized glutathione (agarose beads coupled to glutathione). GST fusion proteins can then be mixed with various concentrations of KCN-OG and the fluorescence measured with a plate-reading fluorimeter (Stefan Kaluz – Emory University). Figure 6A shows the binding of KCN-OG to 3 proteins: GST only, GST galectin and GST-p300CH1. From this figure, it appears that KCN-OG binds the best to the p300. In order to verify these results, a control compound of Oregon Green was prepared by blocking the amine reactive portion of the Oregon green adduct with ethylamine. As shown in Figure 6B, this Oregon green control showed almost no binding to p300, but KCN-OG did. Therefore, it was concluded that the binding of KCN-OG to p300 was a result of KCN-1 and not the Oregon Green portion of the molecule.

An additional experiment involved a competition assay with KCN-OG and EDTA, as well as KCN-OG and the known p300 CH1 inhibitor chetomin. As previously stated in chapter 1.2, chetomin has been shown to be a disrupter of HIF binding to p300. It works by disrupting the structure of the cysteine-histidine-rich domain 1(CH1) domain of p300 and interferes with its interaction with HIF. Therefore, HIF-1 transcription is inhibited.⁷⁰ Chetomin reacts with p300, causing zinc ion ejection.⁷¹ EDTA has been shown to chelate to the zinc ions coordinated by CH1 and disrupt its structure.⁸⁸ Therefore, competition experiments with EDTA (Figure 10A) and chetomin (Figure 10B) were conducted. In both cases, KCN-OG was used at 20 μ M. It seems evident from this experiment that even in the presence of vast excess of EDTA and a moderate concentration of chetomin there is still significant KCN-OG binding. This result shows that KCN-OG does not act in the same way or at the same point on the CH1 domain as chetomin or EDTA. This result is not surprising, since the structure of KCN-1 does not in any way indicate that it can bind to zinc and disrupt the CH1 domain of p300.

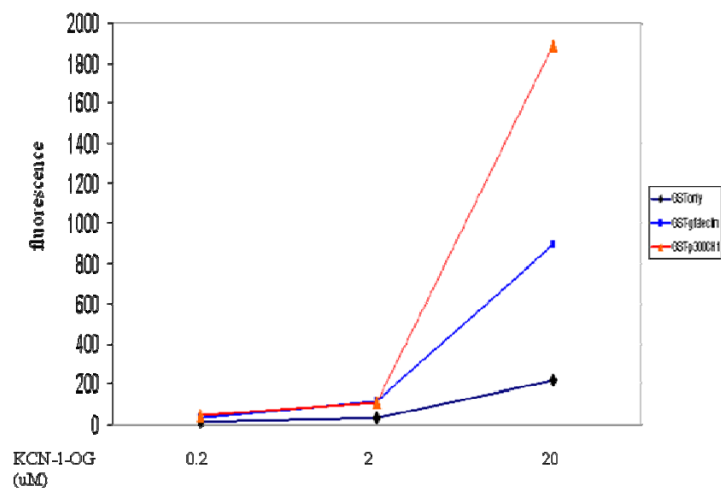


Figure 12. Binding of KCN-1-OG to GST-proteins (*This figure, courtesy of the Van Meir lab*)

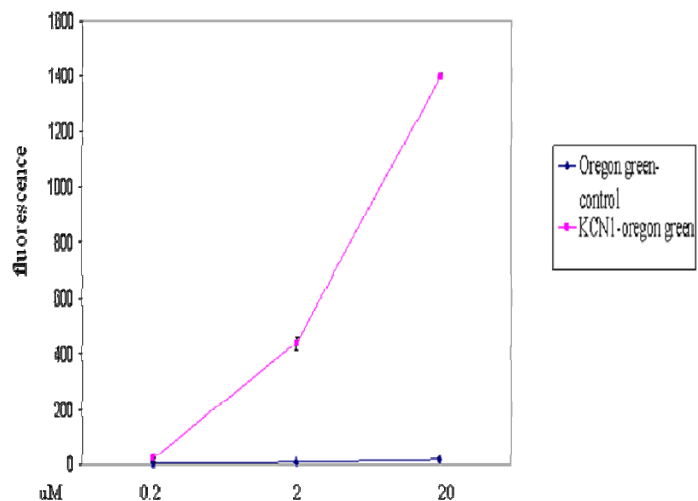


Figure 13. Binding of KCN-1-OG and OG control to GST-p300CH1 (*This figure, courtesy of the Van Meir lab*)

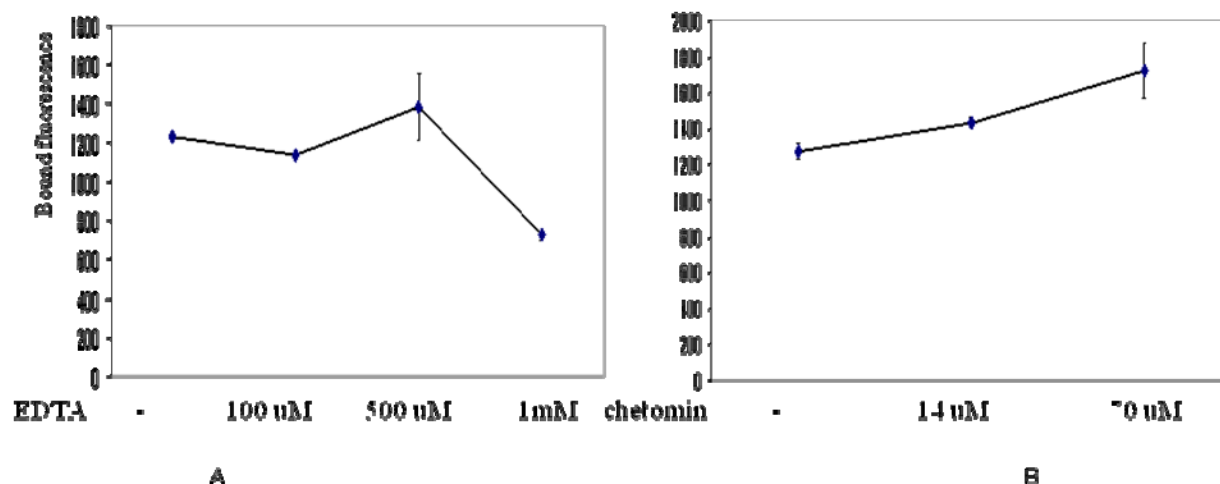


Figure 14. Binding of KCN-OG to GST-p300CH1. (This figure, courtesy of the Van Meir lab)

Summary of mechanistic studies

KCN-1 modified with a 4-carbon linker with a terminal amine was used to couple to agarose beads as well as an Oregon Green fluorophore. These compounds have provided important clues into the mechanism of action of KCN-1, especially as it related to its binding to the transcriptional co-activator, p300. Further experiments will be necessary to verify that binding of KCN-1 to p300 is responsible for the inhibitory and anti-tumor activity of KCN-1.

4 CONCLUSIONS AND FUTURE OUTLOOK

Several potent analogues of KCN-1 were synthesized. Many of these compounds have activity against HIF-1 in the submicromolar range. The best analogues were the pyrano(3,2b)pyridines, of which compound **26i** was the best overall compound with an IC_{50} of 0.25 μ M. The benzofuran derivatives also showed good potential, especially since they were void of the alkene that is present in the KCN-1 molecule.

The analogues synthesized allowed for an understanding of the structure- activity relationship profile of KCN-1: A sulfonamide or amide group is preferred at Region I of KCN-1. Also, alkyl rings of 4 or 5 carbons are preferred in Region II of KCN-1. Aromatic rings are preferred in Region III and cyclohexyl rings are also tolerated. The pyrano(3,2b)pyridines were best in Region IV of KCN-1. More potent analogues can be generated in the future, that take all of these structural features into consideration.

KCN-1 modified with the 4-carbon linker and attached to either a fluorophore or agarose beads was very useful in understanding the anti-tumor mechanism of KCN-1. Since p300 is now being investigated as the possible binding partner for KCN-1, molecular modeling studies of this interaction will be very useful in generating more potent analogues. Molecular modeling studies can take advantage of interaction between KCN-1 and the p300 protein that are important for activity.

These HIF-1 inhibitors have good potential to be drug candidates for the treatment of cancer. These compounds have the potential to be especially effective in tumors that are resistant to chemotherapeutic and radiation therapies because of hypoxia. Combination therapy of KCN-1 analogues, together with chemotherapeutic drugs already in use may produce the best results.

5 EXPERIMENTAL

5.1 Biology

HRE-Luciferase assay. The Inhibition constants (IC_{50}), the inhibitor concentration that produces 50% inhibition in the presence of substrate [S] was determined in the laboratory of Dr. Erwin Van Meir of Emory University. LN229-HRE-luciferase glioblastoma cell was stably integrated with a reporter construct made of 6 copies of the HIF responsive element derived from the VEGF gene cloned in front of a luciferase gene. The cells were treated with drugs of choice (KCN1 or analogs) for 1 hour, and then transferred to a hypoxia incubator (1% hypoxia) and 24 hours later the luciferase activity measured.

Expression and purification of glutathione-S-transferase (GST)/p300CH1 fusion protein.

CH1 domain of p300 was amplified by PCR and cloned in frame into a pGEX-2T vector (bacterial expression vector). This construct, upon induction with 0.1 M IPTG for 4 hrs, expressed GST/p300CH1 fusion protein. GST fusion proteins were purified on immobilized glutathione (agarose beads coupled to glutathione). This procedure typically yields 95-100% pure GST fusion protein.

KCN-OG binding assay. GST fusion proteins (approx. 3 μ g, still on immobilized glutathione) were mixed with various concentrations of KCN-OG in 1 ml of Dulbecco's Phosphate Buffered Saline (DPBS), rocked overnight at 4° C. Tubes were centrifuged for 5 seconds, supernatant carefully removed, and the beads were washed 3 times with 1 ml of DPBS (simple inverting 5-10x, centrifugation 5s). Beads with bound KCN-OG were mixed with elution buffer (120 μ l, 10 mM dithiothreitol), boiled at 100 °C for 10 min, centrifuged 5s, and supernatants was transferred into a 96-well plate and the fluorescence was measured in plate-reading fluorimeter. Competi-

tion with EDTA, chetomin, or KCN1 was performed similarly, except for the pre-treatment of GST proteins with these agents for 1 hour, when KCN-OG was added.

5.2 Chemistry

All reagents were purchased from Acros, Aldrich and Matrix Scientific and were used as received without further purification. Microwave heating was performed in a single-mode microwave cavity of a Discover Synthesis System (CEM corp.) and all microwave-irradiated reactions were conducted in a heavy walled glass vials sealed with Teflon septa. ^1H NMR and ^{13}C NMR were recorded at 400 MHz on a Bruker 400 NMR spectrometer. Mass Spectra was performed by the mass spectrometry facilities at Georgia State University.

General procedure for reductive amination for synthesis of 2a - 2f

To a solution of 2,2-dimethyl-2H-chromene-6-carbaldehyde **1** (1 eq) in methanol was added the amine (2 eq), sodium cyanoborohydride (2 eq) and zinc chloride (dried) (2 eq.). The reaction was stirred overnight. Then the solvent was removed by rotary evaporation and a 1M NaOH solution was added to the residue. The organic layer was extracted with ethyl acetate or DCM ($\times$ 2), dried over magnesium sulfate and concentrated *in vacuo*. The compound was purified by flash column chromatography.

(3,4-Dimethoxy-phenyl)-(2,2-dimethyl-2H-chromen-6-ylmethyl)-amine (2a). Yield: 60%. ^1H NMR (CDCl_3): δ 7.09 (dd, J = 8.2, 2.1 Hz, 1H), 6.99 (d, J = 2.0 Hz, 1H), 6.74 (d, J = 8.4 Hz, 2H), 6.30 (s, 1H), 6.30 – 6.24 (m, 1H), 6.17 (dd, J = 8.5, 2.6 Hz, 1H), 5.61 (d, J = 9.8 Hz, 1H), 4.15 (s, 2H), 3.81 (t, J = 6.2 Hz, 6H), 1.43 (s, 6H). ^{13}C NMR (CDCl_3): δ 152.2, 150.0, 143.3, 141.6, 131.6, 131.0, 128.4, 125.7, 122.2, 121.3, 116.4, 113.3, 103.6, 99.0, 76.2, 56.7, 55.7, 48.8, 28.0. HRMS (ESI +): $\text{C}_{20}\text{H}_{23}\text{NO}_3$ + H: observed: 326.1750, calculated: 326.1756.

(2,2-Dimethyl-2H-chromen-6-ylmethyl)-methyl-pyridin-2-yl-amine (2b). Yield: 60%. ^1H NMR (CDCl_3): δ 8.10 - 8.08 (m, 1H), 7.77- 7.35 (m, 1H), 7.08 – 7.07 (m, 1H), 7.00 – 6.96 (m, 1H), 6.77 – 6.72 (m, 1H), 6.50 – 6.58 (m, 1H), 6.36 (d, J = 8.4 Hz, 1H), 6.28 (d, J = 9.6 Hz, 1H), 4.80 (s, br, 1H), 4.40 (s, 2H), 1.41 (s, 1H). HRMS (ESI⁺): $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}$ + H: observed: 267.1505, calculated: 267.1497.

(2,2-Dimethyl-2H-chromen-6-ylmethyl)-(2,4-dimethyl-phenyl)-amine (2c). Yield: 69%. ^1H NMR (CDCl_3): δ 7.10 (dd, J = 6.0 Hz, 2.4 Hz, 1H), 6.99 (d, J = 8.0 Hz), 6.92 – 6.90 (m, 1H), 6.74 (d, J = 8.4 Hz, 1H), 6.54 (d, J = 7.6 Hz, 1H), 6.30 (d, J = 9.6 Hz, 1H), 4.21 (s, 2H), 2.23 (s, 3H), 2.12 (s, 3H), 1.43 (s, 6H). MS (ESI⁺): $M + H = 292$.

4-[(2,2-Dimethyl-2H-chromen-6-ylmethyl)-amino]-benzoicacid (2d). ^1H NMR ($(\text{CD}_3)_2\text{SO}$): δ 1.35 (s, 6H), 4.17 (d, J = 5.5 Hz, 2H), 5.72 (d, J = 9.8 Hz, 1H), 6.37 (d, J = 9.8 Hz, 1H), 6.54 (d, J = 7.7 Hz, 2H), 6.68 (d, J = 8.2 Hz, 1H), 7.03 (s, 1H), 7.08 (s, 1H), 7.71 (s, 2H). HRMS (ESI⁻): $\text{C}_{19}\text{H}_{19}\text{NO}_3 - \text{H}$: observed: 308.1276, calculated: 308.1287.

(2-Bromo-phenyl)-(2,2-dimethyl-2H-chromen-6-ylmethyl)-amine (2e). Yield: 11%. ^1H NMR (CDCl_3) δ 7.43 (d, J = 7.8 Hz, 1H), 7.20 – 7.04 (m, 2H), 6.97 (s, 1H), 6.75 (d, J = 8.2 Hz, 1H), 6.62 (d, J = 8.0 Hz, 1H), 6.58 (d, J = 7.1 Hz, 1H), 6.30 (d, J = 9.8 Hz, 1H), 5.61 (d, J = 9.8 Hz, 1H), 4.63 (s, 1H), 4.26 (d, J = 5.3 Hz, 2H), 1.43 (s, 6H). ^{13}C NMR (CDCl_3) δ 152.3, 144.9, 132.4, 131.1, 130.7, 128.1, 125.4, 122.2, 121.4, 117.9, 116.5, 111.6, 109.6, 76.3, 47.6, 28.0. HRMS (ESI⁺): $\text{C}_{18}\text{H}_{18}\text{NOBr} + \text{H}$: observed: 344.0663, calculated: 344.0650.

(2,2-Dimethyl-2H-chromen-6-ylmethyl)-(2-fluoro-phenyl)-amine (2f). Yield: 71%. ^1H NMR (CDCl_3): δ 7.10 (dd, J = 6.0, 2.0 Hz, 1H), 6.99 – 6.94 (m, 3H), 6.76 – 6.56 (m, 3H), 6.29 (d, J =

9.6 Hz, 1H), 5.61 (d, $J = 9.6$ Hz, 1H), 4.23 (s, 3H), 1.43 (s, 6H). ^{13}C NMR (CDCl_3): 152.4, 143.9, 138.1, 136.3, 130.9, 128.3, 128.2, 127.5, 127.1, 124.7, 123.5, 122.3, 121.1, 116.2, 77.3, 28.1, 21.5. HRMS (ESI +): $\text{C}_{18}\text{H}_{18}\text{NOF} + \text{H}$: observed: 284.1442, calculated: 284.1451.

General Procedure for synthesis of 3a - 3c by methylation of secondary amines 2a, 2b and 2c, respectively. To a solution of secondary amine **2** (1 eq) in THF was added to a flask containing NaH (2 eq) in THF. After 5 minutes, MeI (2 eq) was added and the reaction allowed to stir overnight. The reaction mixture is quenched with water and diluted with ethyl acetate. The organic layer was washed with water and brine, dried over MgSO_4 and concentrated *in vacuo*.

(3,4-Dimethoxy-phenyl)-(2,2-dimethyl-2H-chromen-6-ylmethyl)-methyl-amine (3a). ^1H NMR (CDCl_3) δ 6.98 (dt, $J = 7.2, 3.6$ Hz, 1H), 6.87 (s, 1H), 6.78 (d, $J = 8.7$ Hz, 1H), 6.73 (s, 1H), 6.43 (s, 1H), 6.27 (d, $J = 10.1$ Hz, 2H), 5.59 (d, $J = 9.8$ Hz, 1H), 4.31 (s, 2H), 3.82 (d, $J = 2.2$ Hz, 6H), 2.89 (s, 3H), 1.42 (s, 6H). ^{13}C NMR (CDCl_3) δ 149.7, 145.6, 131.2, 130.9, 127.9, 125.1, 122.3, 121.3, 116.3, 113.0, 104.8, 99.5, 77.4, 77.0, 76.7, 76.2, 57.6, 56.7, 55.8, 38.9, 28.0. HRMS (ESI +): $\text{C}_{21}\text{H}_{25}\text{NO}_3 + \text{H}$: observed: 340.1900, calculated: 340.1913.

(2,2-Dimethyl-2H-chromen-6-ylmethyl)-methyl-pyridin-2-yl-amine (3b). ^1H NMR (CDCl_3) δ 8.21 (m, 1H), 7.45 (m, 1H), 6.99 (d, $J = 8.5$ Hz, 1H), 6.86 (s, 1H), 6.72 (d, $J = 8.0$ Hz, 1H), 6.52-6.59 (m, 2H), 6.28 (d, $J = 10$ Hz, 1H), 5.60 (d, $J = 10$ Hz, 1H), 4.70 (s, 2H), 3.06 (s, 3H), 1.44 (s, 6H). ^{13}C NMR (CDCl_3) δ 151.9, 148.0, 137.2, 130.9, 130.8, 127.8, 125.0, 122.4, 121.3, 116.3, 11.7, 105.8, 76.1, 52.6, 36.0, 28.0. HRMS (ESI +): $\text{C}_{18}\text{H}_{20}\text{N}_2\text{O}_3 + \text{H}$ observed: 281.1659, calculated: 281.1654.

(2,2-Dimethyl-2H-chromen-6-ylmethyl)-(2,4-dimethyl-phenyl)-methyl-amine(3c). Yield: 48%. ^1H NMR (CDCl_3) δ 7.13 (dd, $J = 2.0, 6.0$ Hz, 1H), 7.05 – 7.00 (m, 4H), 6.75 (d, $J = 8.0$ Hz,

1H), 6.34 (d, $J = 9.6$ Hz, 1H), 5.63 (d, $J = 9.2$ Hz, 1H), 3.88 (s, 2H), 2.55 (s, 3H), 2.40 (s, 3H), 2.31 (s, 3H), 1.45 (s, 6H). MS (ESI+): $M + H = 308$.

N-Benzyl(2,2-dimethyl-2H-chromen-6-yl)methanamine (4q). Follows general procedure for reductive amination as for compound **2** using benzylamine. ^1H NMR (CDCl_3): 7.37–7.36 (m, 5H), 7.10 (d, $J = 2.0$ Hz, 1H), 6.77 (d, $J = 9.0$ Hz, 1H), 6.34 (d, $J = 9.6$ Hz, 1H), 5.63 (d, $J = 9.6$ Hz, 1H), 3.84 (s, 1H), 3.73 (s, 1H), 1.46 (s, 6H).

General Procedure for synthesis of 5a–5r by alkyl sulfonation. To a solution of the secondary amine **4** (1 eq) in dichloromethane was added triethylamine (3 eq) and sulfonylchloride (1.5 eq), the reaction was allowed to stir for 24 to 48 hours, then water was added and the organic layer extracted with dichloromethane, dried over magnesium sulfate and concentrated *in vacuo* and purified by flash column chromatography.

N-(2,2-Dimethyl-2H-chromen-6-ylmethyl)-N-isopropyl-3,4-dimethoxy-benzenesulfonamide (5a). Yield: 58%. ^1H NMR (CDCl_3) δ 7.39 (dd, $J = 6.4, 2.0$ Hz, 1H), 7.22 (d, $J = 2.0$ Hz, 1H), 7.07 (dd, $J = 6.0, 2.0$ Hz, 1H), 7.00 (d, $J = 2.0$ Hz, 1H), 6.90 (t, $J = 8.6$ Hz, 1H), 6.69 (d, $J = 8.2$ Hz, 1H), 6.28 (d, $J = 9.8$ Hz, 1H), 5.60 (d, $J = 9.8$ Hz, 1H), 4.39–4.19 (m, 2H), 4.23–4.02 (m, 1H), 4.04–3.73 (m, 6H), 1.41 (s, 6H), 1.05 (d, $J = 7.2$ Hz, 6H). ^{13}C NMR (CDCl_3): δ 152.2, 152.2, 149.0, 133.2, 131.0, 130.8, 128.6, 126.0, 122.3, 121.2, 120.8, 116.1, 110.5, 109.6, 76.34, 56.2, 56.1, 50.0, 46.0, 27.9, 21.3. HRMS (ESI +): $\text{C}_{23}\text{H}_{29}\text{NO}_5\text{S} + \text{Na}$ observed: 451.1651 Calc: 451.1664.

N-(2,2-Dimethyl-2H-chromen-6-ylmethyl)-3,4-dimethoxy-N-prop-2-ynyl-benzenesulfonamide (5b). Yield: 95%. 7.53 (dd, $J = 2.0$ Hz, 1H), 7.38 (d, $J = 2.0$ Hz, 1H), 7.09

– 7.07 (m, 1H), 7.00 – 6.96 (m, 2H), 6.74 (d, J = 8.0 Hz, 1H), 6.31 (d, J = 9.6 Hz, 1H), 5.64 (d, J = 9.6 Hz, 1H), 4.24 (s, 1H), 4.01 – 3.96 (m, 7 H), 1.59 (s, 2H), 1.43 (s, 6H). HRMS (ESI +): $C_{23}H_{25}NO_5S$ + Na: observed: 450.1352, calc: 450.1351.

(N-Butyl-N-(2,2-dimethyl-2H-chromen-6-ylmethyl)-3,4-dimethoxy-benzenesulfonamide

(5c). Yield: 55%. ^1H NMR ($CDCl_3$): δ 7.46 (dd, J = 6.4, 2.1 Hz, 1H), 7.28 (m, 1H), 7.00 – 6.89 (m, 2H), 6.71 (d, J = 8.0 Hz, 1H), 6.27 (d, J = 10.0 Hz, 1H), 5.63 (d, J = 9.6 Hz, 1H), 4.23 (s, 1H), 3.97 – 3.92 (m, 6H), 3.10 (t, J = 7.6 Hz, 2H), 1.43(s, 6H), 1.38 – 1.16 (m, 6H), 0.79 (t, J = 7.6 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 152.3, 149.0, 132.2, 131.1, 129.1, 128.5, 126.4, 122.1, 121.3, 121.0, 116.2, 110.5, 109.8, 76.3, 56.2, 56.2, 51.1, 47.4, 30.0, 27.9, 19.8, 13.6. HRMS (ESI +): $C_{24}H_{31}NO_5S$ + Na: observed: 468.1815, calculated: 468.1821.

(N-tert-Butyl-N-(2,2-dimethyl-2H-chromen-6-ylmethyl)-3,4-dimethoxy-benzenesulfonamide

(5d). Yield: 49%. ^1H NMR ($CDCl_3$) δ 7.41 (dd, J = 8.5, 2.1 Hz, 1H), 7.22 – 7.12 (m, 2H), 7.06 (d, J = 2.1 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 6.74 (t, J = 9.7 Hz, 1H), 6.30 (t, J = 8.9 Hz, 1H), 5.62 (t, J = 9.5 Hz, 1H), 4.56 (s, 2H), 3.94 (d, J = 13.4 Hz, 3H), 3.85 (d, J = 14.6 Hz, 3H), 1.48 – 1.39 (m, 6H), 1.33 (s, 9H).

(N-Allyl-N-(2,2-dimethyl-2H-chromen-6-ylmethyl)-3,4-dimethoxy-benzenesulfonamide (5e).

Yield: 53%. ^1H NMR ($CDCl_3$) δ 7.48 (dd, J = 8.4, 2.2 Hz, 1H), 7.29 (t, J = 2.3 Hz, 1H), 7.01 – 6.91 (m, 2H), 6.88 (d, J = 2.1 Hz, 1H), 6.72 (t, J = 9.7 Hz, 1H), 6.29 (d, J = 6.4 Hz, 1H), 5.64 (t, J = 10.6 Hz, 1H), 5.53 (ddt, J = 16.7, 10.2, 6.5 Hz, 1H), 5.09 (ddd, J = 18.4, 13.6, 1.3 Hz, 2H), 4.28 (d, J = 25.4 Hz, 2H), 4.03 – 3.95 (m, 3H), 3.95 – 3.88 (m, 3H), 3.84 – 3.72 (m, 2H), 1.44 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 152.6, 152.4, 149.1, 132.4, 132.4, 131.9, 129.4, 127.9, 126.6, 122.1, 121.3, 121.1, 199.2, 116.2, 110.6, 109.8, 76.4, 56.2, 56.2, 49.6, 49.2, 28.0. EI probe: $M^+ = 429$.

HRMS (ESI +): $C_{23}H_{27}NO_5 + Na$, observed: 452.1505, calculated.: 452.1508.

Pyridine-2-sulfonic acid (2,2-dimethyl-2H-chromen-6-ylmethyl)-isopropyl-amide (5f). ^1H NMR ($CDCl_3$): δ 8.33 (d, $J = 4.8$, 1H), 7.69 – 7.61 (m, 1H), 7.50 (dd, $J = 1.2$, 7.2 Hz, 1H), 7.34 (dd, $J = 2.4$, 6.4 Hz, 1H), 7.27 -7.00 (m, 3 H), 6.97 (d, $J = 2.0$ Hz, 1H), 6.90 (d, $J = 8.4$ Hz, 1H), 6.24 (d, $J = 8.0$ Hz, 1H), 5.57 (d, $J = 10$ Hz, 1H), 4.87 (s, 2H), 3.95 (s, 3H), 3.77 (s, 3H), 1.59 (s, 6 H). ESI (+): $M+H = 467$.

(N-(2,2-Dimethyl-2H-chromen-6-ylmethyl)-3,4-dimethoxy-N-(3-methyl-butyl)-benzenesulfonamide (5g). Yield: 31%. ^1H NMR ($CDCl_3$): δ 7.44 (dd, $J = 6.4$, 2.0 Hz), 7.29 – 7.28 (m, 1H), 6.96 – 6.94 (m, 2H), 6.83 (s, 1H), 6.24 (d, $J = 10.0$ Hz, 1H), 5.62 (d, $J = 10.0$ Hz, 1H), 3.97 – 3.92 (m, 6H), 2.90 (d, $J = 7.6$ Hz, 2H), 1.75 (sep, $J = 6.8$ Hz, 1H), 1.43 (s, 6H), 0.792 – 0.779 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 152.5, 152.2, 149.0, 132.1, 131.1, 129.2, 128.5, 126.5, 122.1, 121.1, 116.1, 110.5, 109.9, 76.3, 56.2, 56.2, 55.8, 52.1, 27.9, 26.8, 20.0. HRMS (ESI +): $C_{24}H_{31}NO_5S + Na$: observed: 468.1801, calculated: 468.1821.

(N-Cyclopentyl-N-(2,2-dimethyl-2H-chromen-6-ylmethyl)-3,4-dimethoxy-benzenesulfonamide (5h). Yield: 58%. ^1H NMR ($CDCl_3$) δ 7.39 (d, $J = 6.4$ Hz, 1H), 7.21 (d, $J = 2.1$ Hz, 1H), 7.10 – 6.82 (m, 3H), 6.67 (d, $J = 8.2$ Hz, 1H), 6.26 (d, $J = 9.8$ Hz, 1H), 5.57 (d, $J = 9.8$ Hz, 1H), 4.22 (s, 3H), 3.88 (d, $J = 20.2$ Hz, 6H), 1.70 – 1.18 (m, 15H). ^{13}C NMR ($CDCl_3$): δ 152.2, 152.1, 149.0, 132.7, 131.0, 130.9, 127.9, 152.3, 122.3, 121.2, 121.0, 116.1, 110.5, 109.9, 76.2, 59.5, 56.2, 56.1, 46.8, 29.3, 28.0, 23.5. HRMS (ESI +): $C_{25}H_{31}NO_5S + Na$: observed: 480.1822, calculated: 480.1832

Cyclopropyl-N-(2,2-dimethyl-2H-chromen-6-ylmethyl)-3,4-dimethoxy-benzenesulfonamide

(5i). Yield: 47%. ^1H NMR (CDCl_3): δ 7.46(dd, J = 2.0, 6.4 Hz, 1H), 7.27(d, J = 2.0Hz, 1H), 7.06 (dd, J = 2.0, 6.4 Hz, 1H), 6.97- 6.93 (m, 2H), 6.70 (d, J = 8.0 Hz, 1H), 6.29 (d, J = 9.6 Hz, 1H), 4.27 (s, 2H), 5.62 (d, J = 9.6 Hz, 1H), 2.01(quin, J = 4.0, 1H), 1.44(s, 6H), 0.720 (q, J = 3.2 Hz, 2H), 0.591(q, J = 3.2 Hz, 2H). ^{13}C NMR (CDCl_3) δ 152.5, 148.9, 131.0, 130.5, 129.7, 129.0, 126.9, 122.2, 121.6, 121.1, 116.0, 110.4, 110.2, 76.3, 56.2, 56.2, 54.2, 30.6, 28.0, 7.3. HRMS (ESI +): $\text{C}_{23}\text{H}_{27}\text{NO}_5\text{S} + \text{Na}$: observed: 452.1489, calculated: 452.1508

N-Cyclohexyl-N-(2,2-dimethyl-2H-chromen-6-ylmethyl)-3,4-dimethoxy-

benzenesulfonamide (5j). Yield: 79%. ^1H NMR: δ 7.42 (dd, J = 2.0 Hz, 6.4 Hz, 1H), 7.30 – 7.15 (m, 1H), 7.16 (d, J = 8.4 Hz, 1H), 7.10 – 7.08 (m, 1H), 7.02 (d, J = 2.0 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.71 (d, J = 8.0 Hz, 1H), 6.31 (d, J = 9.6 Hz, 1H), 5.63 (d, J = 9.6 Hz, 1H), 4.31 (s, 2H), 3.95 (s, 3H), 3.90 (s, 3H), 1.70 – 1.54 (m, 4H), 1.43 (s, 6H), 1.27 – 1.20 (m, 6H). Yield: 79%. HRMS (ESI+): $\text{C}_{26}\text{H}_{33}\text{NO}_5\text{S} + \text{Na}$: observed: 494.1998, calculated: 44.1977.

General Procedure for synthesis of 5k - 5p

To a solution of secondary amine **4** (1 eq) in DCM was added triethylamine (3 eq), then appropriate sulfonylchloride (2 eq). The reaction was allowed to stir at room temperature for 24 hours after which sat. NH_4Cl was added to the reaction mixture and extracted with DCM ($\times$ 2), dried over MgSO_4 and concentrated under vacuum. The crude product was purified by flash column chromatography.

N-((2,2-Dimethyl-2H-chromen-6-yl)methyl)-4-methoxy-N-phenylbenzenesulfonamide (5k).

Yield: 30 %. ^1H NMR (CDCl_3): δ 7.60 (d, J = 9.2 Hz, 1H), 7.28-7.22 (m, 3H), 7.00 – 6.90 (m, 4H), 6.62 (d, J = 9.2 Hz, 1H), 6.24 (d, J = 10.0 Hz, 1H), 5.58 (d, J = 10 Hz, 1H), 4.62 (s, 2H),

3.90 (s, 3H), 1.40 (s, 6H). ^{13}C NMR: 162.90, 139.1, 130.9, 129.8, 129.3, 129.1, 128.8, 128.1, 127.6, 126.6, 122.3, 116.0, 114.0, 76.3, 55.6, 54.3, 28.0. MS: ESI (+): $\text{M} + \text{Na} = 458$

N-((2,2-Dimethyl-2H-chromen-6-yl)methyl)-3,5-dimethyl-N-phenylbenzenesulfonamide (5l).

^1H NMR: δ 7.54 (s, 2H), 7.30 – 7.23 (m, 3H), 7.00 – 6.97 (m, 1H), 6.90 – 6.88 (m, 2H), 6.60 (d, $J = 8.8$ Hz, 1H), 6.24 (d, $J = 10$ Hz, 1H), 5.58 (d, $J = 9.6$ Hz, 1H), 4.63 (s, 2H), 2.41 (s, 3H), 2.36 (s, 3H), 1.42 (s, 6H).

2,5-Dichloro-N-((2,2-dimethyl-2H-chromen-6-yl)methyl)-N-phenylbenzenesulfonamide

(5m). Yield: 23%. ^1H NMR (CDCl_3): δ 7.84 (d, $J = 2.4$ Hz, 1H), 7.48 (d, $J = 8.4$ Hz, 1H), 7.44 – 7.40 (m, 1H), 7.23 – 7.21 (m, 3H), 7.06 – 7.04 (m, 2H), 6.92 – 6.90 (m, 2H), 6.64 (d, $J = 7.6$ Hz, 1H), 6.27 (d, $J = 10.0$ Hz, 1H), 5.60 (d, $J = 9.6$ Hz, 1H), 4.92 (s, 2H), 1.42 (s, 6H).

4-Bromo-N-((2,2-dimethyl-2H-chromen-6-yl)methyl)-N-phenyl-2-

(trifluoromethoxy)benzenesulfonamide (5n). Yield: 23 %. ^1H NMR (CDCl_3): δ 1.45 (s, 6H), 4.95 (s, 2H), 5.60 (d, 1H, $J = 9.6$), 6.27 (d, 1H, $J = 10$), 6.64 (s, 1H, $J = 7.6$), 6.91 (m, 2H), 7.05 (m, 2H), 7.21 (m, 3H), 7.43 (m, 1H), 7.48 (m, 1H), 7.84 (d, 1H, 2.4).

4-(3-Ethylpent-1-yn-3-yloxy)benzaldehyde (7). To a solution of 3-methyl-1-pentyn-3-ol **6** (0.319 mL, 2.83 mmol) in acetonitrile (3ml) at 0°C was added DBU (0.55 mL, 3.69 mmol). Then, TFAA (0.34 mL, 2.46 mmol) was added drop wise and stirred at 0°C for 30 minutes. To a solution of 4- hydroxybenzaldehyde (300 mg, 2.46 mmol) in acetonitrile at 0°C was added DBU (0.55 mL, 3.69 mmol) and $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.42 mg, 0.0025 mmol). The first mixture was added to the second mixture over a period of five minutes. The reaction was stirred overnight. The solvent was removed by rotary evaporation and the residue diluted with DCM and the organic layer

washed with 1M HCl, 1M NaOH, sat NaHCO₃ and brine. The organic layer was dried over MgSO₄ and concentrated *in vacuo* to give 170 mg (32%) of product as a brown oil. ¹H NMR (CDCl₃): δ 9.91(s, 1H), 7.83 – 7.81 (m, 2H), 7.36 -7.34 (m, 2H), 2.69 (s, 1H), 2.06 – 1.92 (m, 2H), 1.66 (s, 3H), 1.12 (t, J = 7.2 Hz, 3H).

2-Ethyl-methyl-2H-chromene-6-carbaldehyde (8). A solution of **7** (170 mg) in xylene (3 mL) was subjected to microwave irradiation for 100 minutes at 220 W, 200 torr, 120 °C. The solvent was removed *in vacuo* to give a quantitative yield of product (170 mg). ¹H NMR(CDCl₃): δ 9.81 (s, 1H), 7.63 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 7.50 (d, J = 2.0 Hz, 1H), 6.85 (d, J = 8.4 Hz, 1H), 6.42 (d, J = 10.0 Hz, 1H), 5.62 (d, J = 10.0 Hz, 1H), 1.81 – 1.66 (m, 3H), 1.43 (s, 3H), 0.97 (t, J = 7.6 Hz, 3H).

N-((2-Ethyl-2-methyl-2H-chromen-6-yl)methyl)benzenamine (9). Reaction was carried out with the same procedure as for **2a - 2f** using 170 mg of **8** to give 90.9 mg (41%) of product. ¹H NMR (CDCl₃): δ 7.22- 7.18 (m, 5H), 7.11(dd, J = 6.0 Hz, 2.4 Hz, 1H), 6.99 (d, J = 2.0 Hz, 2H), 6.77 -6.74 (m, 3H), 6.68 – 6.66 (m, 2H), 6.36 (d, J = 10.0 Hz, 1H), 5.58 (d, J = 10.0 Hz, 1H), 4.21 (s, 2H), 1.76 – 1.71 (m, 3H), 1.33(s, 3H), 0.96 (t, J = 7.6 Hz, 3H).

N-((2-Ethyl-2-methyl-2H-chromen-6-yl)methyl)-3,4-dimethoxy-N-phenylbenzenesulfonamide (10). To a solution of **9** (80 mg, 0.29 mmol) in DCM (3mL) was added Et₃N (0.12 ml, 0.85 mmol) and 3,4-dimethoxybenzenesulfonyl chloride (135mg, 0.573 mmol). After 24 hours, sat.NH₄Cl was added to the reaction mixture and the aqueous layer was extracted with DCM (5 × 2 ml), dried over MgSO₄ and concentrated under vacuum. The crude product was purified by column (silica gel, 5: 1 Hx/EtOAc) to give a white solid. ¹H NMR(CDCl₃): δ 7.35 (dd, J = 6.4 Hz, 2.0 Hz, 1H), 7.25 – 7.23 (m, 3H), 7.02 – 6.98 (m, 3H), 6.94 (d, J = 8.4 Hz,

1H), 6.91 – 6.88 (m, 2H), 4.62 (s, 2H), 3.98 (s, 3H), 3.77 (s, 3H), 1.71 – 1.65 (m, 2H), 1.43 (s, 3H), 0.94 (t, J = 7.6 Hz, 3H). ¹³C NMR (CDCl₃): δ 152.8, 152.5, 148.7, 139.2, 130.5, 129.8, 129.3, 129.2, 128.8, 127.9, 127.8, 126.6, 122.8, 121.4, 121.1, 115.8, 110.4, 79.0, 56.2, 56.1, 54.3, 34.0, 26.0, 8.2. MS (ESI +): M + Na = 502

N-Phenylquinolin-3-amine (12). To a solution of quinoline-3-carbaldehyde **11** (79 mg, 0.5 mmol) in MeOH (5mL) was added aniline (0.05 mL, 0.55 mmol) and ZnCl₂ (136 mg, 2.0 mmol) and allowed to stir at room temperature for 15 minutes. Then, NaCNBH₃ (62.84 mg, 2.0 mmol) was added and allowed to stir overnight at room temperature. The solvent was removed by rotary evaporation and the residue suspended in EtOAc. The organic layers were washed with NaHCO₃, water and brine and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The crude product was used in the next step without further purification. ¹H NMR (CDCl₃): 9.05 (s, 1H), 8.23 (d, J = 7.6 Hz, 2H), 7.77 (d, J = 8.0 Hz, 1H), 7.71 – 7.66 (m, 1H), 7.54 – 7.51 (m, 1H), 7.21 – 7.16 (m, 2H), 6.76 – 6.72 (m, 1H), 6.68 – 6.60 (m, 2H), 4.54 (s, 2H), 4.16 (s, br, 1H).

3,4-Dimethoxy-N-phenyl-N-(quinolin-3-ylmethyl)benzenesulfonamide (13). To a solution of **12** (75 mg, 0.320 mmol) in DCM was added 3,4 dimethoxy benzylsulfonyl chloride (83mg, 0.35 mmol) and triethylamine(0.09 ml, 0.640 mmol). The reaction was allowed to stir overnight at room temperature and the reaction mixture was washed with water and brine and the combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. Purified by column chromatography: silica gel, 2:1 Hexane/EtOAc to give a white powder. ¹H NMR(CDCl₃): 8.86 (s, 1H), 8.14(d, J = 8.0 Hz, 2H), 7.84 (dd, J =7.2 Hz, 1.2 Hz, 1H), 7.80 – 7.76 (m, 1H), 7.64 -7.60 (m, 1H), 7.49 (dd, J = 6.0 Hz, 2.4 Hz, 1H), 7.38 – 7.30 (m, 3H), 7.17 – 7.14 (m, 2H), 7.08 – 7.05 (m, 2H), 5.03 (s, 2H), 4.08 (s, 3H), 3.85 (s, 3H). ¹³C NMR (CDCl₃): 152.8, 150.8, 148.8, 147.6, 138.7,

135.7, 129.6, 129.2, 129.1, 129.0, 128.2, 127.7, 127.7, 126.9, 121.6, 110.5, 110.4, 56.2, 56.1, 52.4. MS (ESI⁺): M + H = 435.

N-((2,2-Dimethyl-2H-chromen-6-yl)methyl)benzenamine (15a). To a solution of 2,3-Dihydro-2,2-dimethylbenzofuran-5-carboxaldehyde **14** (250 mg, 1.42 mmol) in MeOH(10ml) was added aniline (0.14 ml, 1.022 mmol), NaCNBH₃ (178 mg, 2.84 mmol) and ZnCl₂ (dried in oven) (387mg, 2.84 mmol). The reaction was allowed to stir at room temperature overnight, and then the solvent was removed by rotary evaporation. 0.1M NaOH was added to the resulting residue and extracted with EtOAc (× 2), dried over MgSO₄ and concentrated *in vacuo* to give 301 mg of product (84 %). ¹H NMR (CDCl₃): d 7.29 – 7.22 (m, 3H), 7.17 (d, J = 8.0 Hz, 1H), 6.95 – 6.70 (m, 4H), 4.28 (s, 2H), 3.06 (s, 2H), 1.55 (s, 6H).

N-((2,2-Dimethyl-2,3-dihydrobenzofuran-5-yl)methyl)-3,4-dimethoxy-N-phenylbenzenesulfonamide (16a). To a solution of **15a** (100 mg, 0.395 mmol) in DCM (5 ml) was added triethylamine (0.17 mL, 0.790 mmol) and 3,4 dimethoxybenzenesulfonylchloride (187 mg, 0.790 mmol) dissolved in 1mL of DCM and allowed to stir for 72 hours. Ammonium chloride was added to the reaction mixture, then extracted with DCM (× 2), dried over MgSO₄ and concentrated *in vacuo*. The crude reaction mixture was purified by column (silica gel, 3:1 Hx/EtOAc) to give product as a white solid – 76 mg, 42 %. ¹H NMR (CDCl₃) d 7.33 (dd J = 2.13, 8.44, 1H), 7.26 - 7.21 (m, 3H), 7.09 (s, 1H) 7.00 - 6.96 (m, 3H), 6.92 (d, J = 8.5, 1H), 6.83 (d, J = 8.1, 1H), 6.52 (d, J = 8.1, 1H), 4.62 (s, 2H), 3.95 (s, 1H), 3.75 (s, 1H), 2.92 (s, 2H), 1.42 (s, 1H). ¹³C NMR (CDCl₃) 158.4, 152.5, 148.7, 139.3, 129.2, 127.5, 125.6, 121.4, 110.4, 108.9, 86.9, 56.2, 56.0, 54.6, 42.7, 28.2. MS (ESI⁺): M + Na = 476.

General Procedure for Synthesis of compound 16b – 16f

To a solution of 1 in methanol was added amine (1.1 eq.), zinc chloride (2 eq.) and allowed to stir for 2 hours before adding NaCNBH_3 (2 eq.). The reaction was allowed to stir at room temperature overnight. The solvent was removed by rotary evaporation and the residue diluted with EtOAc and washed with Na_2CO_3 (sat) and brine. The organic layers were dried over MgSO_4 and concentrated *in vacuo*. The product was used without further purification in the next step. The resulting secondary amine **15** (1 eq) in DCM was added Et_3N (2 eq.) and the appropriate aryl or alkyl sulfonyl chloride (1.1 eq) and the reaction allowed to stir at room temperature overnight. The reaction mixture was diluted with DCM and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash chromatography.

N-Cycloheptyl-N-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)methyl)-3,4-

dimethoxybenzenesulfonamide (16b). Yield: 14%. ^1H NMR (CDCl_3) δ 7.43 (dd, J = 2.14, 8.44, 1H), 7.25 – 7.24 (m, 2H), 7.05 (d, J = 8.13, 1H), 6.92 (d, J = 8.5, 1H), 6.65 (d, J = 8.1, 1H), 4.28 (s, 2H), 3.96 (s, 3H), 3.90 (s, 3H), 3.00 (s, 2H), 1.63 -1.51 (m, 8H), 1.48 (s, 6H), 1.45 -1.27 (m, 7H). HRMS (ESI +): $\text{C}_{26}\text{H}_{35}\text{NO}_5\text{S} + \text{Na}$: observed: 496.2122, calculated: 496.2134,

N-((2,2-Dimethyl-2,3-dihydrobenzofuran-5-yl)methyl)-N-isopropyl-

3,4dimethoxybenzenesulfonamide (16c). Yield: 84%. ^1H NMR (CDCl_3) δ 7.45 (d, J = 8.2 Hz, 1H), 7.27 (s, 1H), 7.10 (d, J = 15.6 Hz, 1H), 6.94 (t, J = 7.5 Hz, 2H), 6.63 (d, J = 7.6 Hz, 1H), 4.24 (s, 2H), 3.94 (d, J = 19.3 Hz, 6H), 3.05 – 2.93 (m, 2H), 2.91 (d, J = 6.9 Hz, 2H), 1.82 – 1.66 (m, 1H), 1.48 (s, 6H), 0.77 (d, J = 5.7 Hz, 6H). ^{13}C NMR (CDCl_3) δ 158.5, 152.2, 149.0, 132.1, 128.4, 127.9, 127.7, 125.6, 121.1, 110.5, 109.9, 109.0, 87.1, 77.4, 77.1, 76.7, 56.2, 56.2, 55.8,

52.4, 42.7, 28.1, 26.9, 20.0. HRMS (ESI+): $C_{23}H_{31}NO_5S + Na$: observed: 456.1833, calculated: 456.1821,

N-Butyl-N-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)methyl)-3,4-

dimethoxybenzenesulfonamide (16d). Yield: 18%. ^1H NMR ($CDCl_3$) δ 7.47 (dd, $J = 8.4$, 1.5 Hz, 1H), 7.34 – 7.22 (m, 2H), 7.13 (s, 1H), 6.96 (d, $J = 8.4$ Hz, 2H), 6.65 (d, $J = 8.1$ Hz, 1H), 4.25 (s, 2H), 3.95 (d, $J = 16.7$ Hz, 6H), 3.22 – 3.03 (m, 2H), 2.99 (s, 2H), 1.57 (d, $J = 21.8$ Hz, 1H), 1.49 (s, 6H), 1.41 – 1.24 (m, 3H), 1.23 – 1.08 (m, 2H), 0.79 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 158.6, 152.2, 149.2, 143.0, 132.3, 128.3, 127.4, 125.5, 120.9, 110.5, 109.8, 109.0, 87.1, 77.4, 77.0, 76.7, 56.2, 56.2, 51.3, 47.3, 42.7, 29.9, 28.1, 19.9, 13.7. HRMS (ESI +): $C_{23}H_{31}NO_5S + Na$: observed: 456.1812, calculated: 456.1821

N-Cyclohexyl-N-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)methyl)-3,4-

dimethoxybenzenesulfonamide(16e). Yield: 36%. ^1H NMR ($CDCl_3$) δ 7.41 (d, $J = 8.4$, 1H), 7.22 (s, 2H), 7.03 (d, $J = 7.9$, 1H), 6.90 (d, $J = 8.3$, 1H), 6.63 (d, $J = 7.9$, 1H), 4.31 (s, 2H), 3.70 (1H) 3.94 (s, 3H) 3.88 (s, 3H), 2.98 (s, 1H), 1.69 – 1.52 (m, 7H), 1.47(s, 6H), 1.27-1.22 (m, 4H). HRMS (ESI +): $C_{25}H_{33}NO_5S + Na$: observed: 482.1981, calculated: 482.1977.

N-Cyclopentyl-N-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)methyl)-3,4-

dimethoxybenzenesulfonamide (16f). Yield: 31% ^1H NMR ($CDCl_3$) δ 7.44 (d, $J = 8.5$, 1H), 7.27 (s, 2H), 7.04 (d, $J = 8.10$, 1H), 6.92 (d, $J = 8.4$, 1H), 6.65 (d, $J = 8.1$, 1H), 4.29 (s, 3H), 3.95 (s, 3H), 3.90 (s, 3H), 2.99 (s, 2H), 1.85 -1.58 (m, 3H), 1.60 – 1.22 (m, 12 H). HRMS (ESI +): $C_{24}H_{31}NO_5S + Na$: observed: 468.1817, calculated: 468.1821.

2,2-Dimethyl-2H-pyrano[2,3-b]pyridine-6-carbaldehyde (18). To a solution of **17**(100 mg, 0.390 mmol) in dry ether (2 ml) was added BuLi (0.25 mL, 2.5 M solution in THF) dropwise at -65 °C and stirred for 15 minutes. Then DMF was added dropwise and stirred at -65 °C for 1.5

hours. Water was added to quench the reaction and extracted with EtOAc ($\times 2$). The organic layers were washed with water ($\times 1$), brine ($\times 1$), dried over MgSO_4 and concentrated *in vacuo* to give a yellow oil. Purification by column chromatography 6:1 Hx/EtOAc to give white solid 23 mg (31 %). ^1H NMR (CDCl_3) δ 1.58 (s, 1H); 5.79 (d, 1H, $J = 8$), 6.36 (d, 1H, $J = 9.6$), 7.76 (s, 1H), 8.50 (s, 1H), 9.92 (s, 1H).

N-((2,2-Dimethyl-2H-pyrano[2,3-b]pyridin-6-yl)methyl)benzenamine (19a). To a solution of 2,2-dimethyl-2H-pyrano[2,3-b]pyridine-6-carbaldehyde **18** (20 mg, 0.106 mmol) in methanol (1 ml) was added aniline (0.01 ml, 0.12 mmol), NaCNBH_3 (13 mg, 0.212 mmol) and ZnCl_2 (29 mg, 0.212 mmol). The reaction was allowed to stir for 30 minutes after which the solvent was removed by rotary evaporation and the 1M NaOH added to the residue, extracted with DCM, dried over MgSO_4 and concentrated *in vacuo*. Purified by column chromatography (3:1 Hx/EtOAc) to give a white solid 20 mg (72%). ^1H NMR (CDCl_3): δ 8.01 (s, 1H), 7.29 (s, 1H), 7.26 – 7.16 (m, 2H), 6.74 (t, $J = 7.2$ Hz, 1H), 6.63 (d, $J = 8.0$ Hz, 1H), 6.26 (d, $J = 9.6$ Hz, 1H), 5.67 (d, $J = 9.6$ Hz, 1H), 4.21 (s, 2H), 1.51 (s, 6H). ^{13}C NMR (CDCl_3): δ 159.6, 147.8, 146.4, 133.8, 132.2, 129.3, 128.4, 120.9, 118.0, 115.4, 113.0, 79.2, 45.4, 28.8.

N-((2,2-Dimethyl-2H-pyrano[2,3-b]pyridin-6-yl)methyl)cyclohexanamine (19b). To a solution of 2,2-dimethyl-2H-pyrano[2,3-b]pyridine-6-carbaldehyde **18** (23 mg, 0.121 mmol) in MeOH (1 mL) was added cyclohexylamine (0.014 ml, 0.121 mmol), NaCNBH_3 (15 mg, 0.242 mmol) and Zinc chloride (33 mg, 0.242 mmol) and stirred overnight. The solvent was removed by rotary evaporation and the residue dissolved in EtOAc and washed with 1M NaOH, water and brine, dried over MgSO_4 and concentrated *in vacuo*. The product was used in the next step without further purification.

N-((2,2-Dimethyl-2H-pyrano[2,3-b]pyridin-6-yl)methyl)-3,4-dimethoxy-N-

phenylbenzenesulfonamide (20a). To a solution of **19a** (20 mg, 0.075 mmol) in DCM (1mL) was added 3,4-dimethoxybenzenesulfonyl chloride (36 mg, 0.150 mmol) and triethylamine (0.021 ml, 0.150 mmol). The reaction was allowed to stir for 24 hours at room temperature. The reaction mixture was washed with water ($\times$ 2) and the organic layer dried over MgSO_4 and concentrated *in vacuo*. Column chromatography (2:1 hexane/EtOAc) gave a white solid. Yield 43%. ^1H NMR (CDCl_3) δ 1.47 (s, 6H), 3.76 (s, 3H), 3.97 (s, 3H), 4.60 (s, 2H), 5.66 (d, 1H, $J = 9.6$), 6.26 (d, 1H, $J = 10$), 6.93 – 6.99 (m, 4H), 7.23 – 7.25 (m, 3H), 7.32 – 7.36 (m, 2H), 7.63 (d, 1H, $J = 2.4$). HRMS (ESI +): $\text{C}_{25}\text{H}_{25}\text{N}_2\text{O}_5\text{S} + \text{Na}$: observed: 467.1636, calculated: 467.1641.

N-Cyclohexyl-N-((2,2-dimethyl-2H-pyrano[2,3-b]pyridin-6-yl)methyl)-3,4-

dimethoxybenzenesulfonamide (20b). Yield: 60%. ^1H NMR (CDCl_3): δ 7.89 (s, 1H), 7.49 (s, 1H), 7.44 (d, $J = 2.0$ Hz, 1H), 7.28 (s, 1H), 6.93 (d, $J = 8.8$ Hz, 1H), 6.31 (d, $J = 10$ Hz, 1H), 5.69 (d, $J = 10$ Hz, 1H), 4.30 (s, 2H), 3.95 (s, 3H), 3.91 (s, 3H), 1.71 – 1.52 (m, 10 H), 1.29 – 1.21 (m, 6H). HRMS (ESI +): $\text{C}_{25}\text{H}_{32}\text{N}_2\text{O}_5\text{S} + \text{H}$: observed: 473.2127, calculated: 473.2110.

6-Bromo-2,2-dimethyl-2H-pyrano[3,2-b]pyridine(23). To a solution 2-methyl-3-butyn-2-ol (**21**) in acetonitrile (6 mL) was added DBU (0.80 mL, 6.61 mmol) at 0°C , then TFAA was added dropwise, also at 0°C . The reaction was allowed to stir for 30 minutes. In another round bottom flask, DBU (0.80 ml, 6.61 mmol) was added to a solution of 2-bromo-5-hydroxy pyridine (1.0 g, 5.75 mmol) in 6 ml of acetonitrile at 0°C . The 2-methyl-3-butyn-2-ol reaction was then added dropwise into this reaction and allowed to stir for 30 additional minutes. The solvent was removed by rotary evaporation and the residue diluted with DCM and the organic layer washed with 1M HCl, 1M NaOH, sat NaHCO_3 and brine. The organic layer was dried over MgSO_4 and concentrated *in vacuo*. The crude product was dissolved in 2 mL of xylene and subjected to

microwave irradiation (130 °C, 220 W) for 30 minutes. The solvent was removed by rotary evaporation and the product concentrated *in vacuo*. The crude product was purified by column chromatography (10:1 Hx/EtOAc) to give a 300 mg of a yellow solid (22% for two steps). ¹H-NMR: 1.45 (s, 6H), 5.86 (d, 1H, J = 10.4), 6.44 (d, 1H, J = 10), 6.90 (d, 1H, J = 8.8), 7.14 (s, 1H, J = 8.4). HRMS (ESI+): C₁₀H₁₁NOBr: observed: 240.0026, calculated: 240.0024,

2,2-Dimethyl-2H-pyrano[3,2-b]pyridine-6-carbaldehyde(24). To a solution of **23** (200 mg, 0.83 mmol) in anhydrous THF (5 mL) at -78 °C was added BuLi (2.5 M, 0.35 ml) and allowed to stir for 35 minutes, then DMF (0.08 mL, 0.1 mmol) was added dropwise. The reaction was stirred at -78°C for 30 additional minutes. Water (3 mL) was added to quench the reaction and was extracted with EtOAc. The organic layer was washed with water, brine, dried over MgSO₄ and concentrated *in vacuo*. Column chromatography 10:1 Hx/EtOAc gave the product as yellowish solid (23% yield). ¹H NMR: 1.53 (s, 6H), 6.01 (d 1H, J = 10.4), 6.58 (d, 1H, J = 10.4), 7.13 (d, 1H, J = 8.4), 7.77 (d, 1H, J = 8.4), 9.93 (s, 1H). ¹³C NMR: 28.8, 78.7, 123.0, 123.3, 123.4, 136.3, 145.8, 153.7, 191.2.

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)aniline (25a). To a solution of 2,2-dimethyl-2H-pyrano[3,2-b]pyridine-6-carbaldehyde (434 mg, 2.28 mmol) in methanol (3 mL) was added aniline (0.3 mL, 2.52 mmol) and zinc chloride (621 mg, 4.56 mmol) and stirred at room temperature for 2 hours. Then NaCNBH₃ (287 mg, 4.56 mmol) was added and stirred overnight. Purification by column: 4: 1 Hx/ EtOAc to give an off-white solid. Yield: (203 mg, 48%). ¹H NMR (CDCl₃): 1.49 (s, 6H), 4.361 (s, 2H), 5.91 (d, 1H, J = 10), 6.55 (d, 1H, J = 10), 6.68 – 6.71 (m, 3H), 7.01 (d, 1H, J = 8.4), 7.08 (d, 1H, J = 8.4), 7.18 – 7.38 (m, 2H).

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-3,4-dimethoxy-N-phenylbenzenesulfonamide (26a). To a solution of **25a** (60 mg, 0.237 mmol) in diclorome-

thane (2.5 ml) was added triethylamine (0.07 mL, 0.474 mmol) and the 3,4 dimethoxybenzenesulfonyl chloride (84 mg, 0.355 mmol)), the reaction was allowed to stir for 24 hours. The reaction mixture diluted with DCM and the organic layer washed with then water and brine and dried over magnesium sulfate and concentrated *in vacuo*. The crude product was purified by column chromatography: silica gel; 3:1 Hexane/EtOAc to 1:1 Hexane/EtOAc to give an off-white solid 55.8 mg (50 %). ^1H NMR (CDCl_3) δ 7.29 (dd, J = 8.4, 2.1 Hz, 2H), 7.25 – 7.17 (m, 3H), 7.14 – 7.09 (m, 2H), 6.96 (d, J = 8.4 Hz, 1H), 6.92 – 6.86 (m, 2H), 6.32 (d, J = 10.1 Hz, 1H), 5.80 (d, J = 10.1 Hz, 1H), 4.78 (s, 2H), 3.94 (s, 3H), 3.72 (s, 3H), 1.40 (s, 6H). HRMS (ESI +): $\text{C}_{25}\text{H}_{26}\text{N}_2\text{O}_5\text{S}$: observed 467.1641, calculated: 467.1641.

General Procedure for synthesis of 26b – 26j by alkyl sulfonation . To a solution of **25** (1 eq) in methanol was added the primary amine (1 eq), ZnCl_2 (2 eq) and NaCNBH_3 (2 eq) and allowed to stir at room temperature for 4 hours to overnight. The solvent was removed by rotary evaporation, and then the residue was dissolved in EtOAc, which was washed with 1M NaOH, water ad brine. The organic layer was dried over MgSO_4 and concentrated *in vacuo*. The crude secondary amine was used without further purification.

To a solution of the secondary amine (1 eq) in dichloromethane was added triethylamine (3 eq) and the sulfonylchloride (1.5 eq), the reaction was allowed to stir for 24 to 48 hours, then water was added and the organic layer extracted with dichloromethane, dried over magnesium sulfate and concentrated *in vacuo* and purified by flash column chromatography.

N-Butyl-N-((2,2-dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-3,4-

dimethoxybenzenesulfonamide (26b). Yield: 42%. ^1H NMR (CDCl_3) δ 7.47 (d, J = 2.4 Hz, 1H), 7.29 – 7.26 (m, 2H), 7.04 (d, J = 8.4, 1H), 6.94 (d, J = 8.8, 1H), 6.40 (d, J = 8.0, 1H), 5.88 (d, J = 10, 1H), 4.37 (s, 1H), 3.96 (s, 3H), 3.93 (s, 3H), 3.18 (t, J = 7.6, 2H), 1.48 (s, 1H), 1.38

(m, 2H), 1.18 (sx, $J = 7.2$, 2H), 0.79 (t, $J = 7.2$, 3H). ^{13}C NMR (CDCl_3) δ 152.4, 149.0, 148.8, 148.7, 135.4, 131.6, 123.7, 123.7, 122.7, 121.0, 110.5, 109.8, 56.2, 56.2, 53.3, 48.8, 30.2, 28.2, 19.9, 13.6. HRMS (ESI +): $\text{C}_{23}\text{H}_{31}\text{N}_2\text{O}_5\text{S}$: observed: 447.1937, calculated: 447.1954

N-(3,4-Dimethoxyphenyl)-N-((2,2-dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-3,4-

dimethoxybenzenesulfonamide (26c). Yield: 51%. ^1H NMR (CDCl_3) δ 7.32 – 7.38 (m, 2H), 7.01 – 6.98 (m, 2H), 6.91 (d, $J = 8.4$ Hz, 1H), 6.66 (d, $J = 8.4$ Hz, 1H), 6.68 – 6.62 (m, 2H), 6.34 (dd, $J = 12.0$, 0.4 Hz), 5.82 (d, $J = 10.0$ Hz, 1H), 4.76 (s, 2H), 3.96 (s, 3H), 3.83 (s, 3H), 3.79 (s, 3H), 3.72 (s, 3H), 1.43 (s, 6H). ^{13}C NMR (CDCl_3) δ 152.6, 148.7, 148.7, 148.6, 148.5, 147.8, 140.2, 135.3, 132.2, 129.6, 123.6, 123.6, 122.6, 121.8, 121.0, 112.5, 110.5, 110.3, 56.2, 56.1, 56.1, 55.9, 28.2. HRMS (ESI +): $\text{C}_{27}\text{H}_{31}\text{N}_2\text{O}_7\text{S}$: observed: 527.1866, calculated: 527.1852.

N-Cyclopentyl-N-((2,2-dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-3,4-

dimethoxybenzenesulfonamide (26d). Yield: 31%. ^1H NMR (CDCl_3) δ 7.55 – 7.35 (m, 2H), 7.35 – 7.22 (m, 1H), 7.07 (d, $J = 8.4$ Hz, 1H), 6.98 – 6.86 (m, 1H), 6.46 (dd, $J = 14.7$, 10.2 Hz, 1H), 5.89 (d, $J = 10.1$ Hz, 1H), 4.44 – 4.24 (m, 3H), 3.96 (s, 3H), 3.93 (s, 3H), 1.76 – 1.15 (m, 15H). ^{13}C NMR (CDCl_3) δ 152.4, 150.7, 148.5, 135.4, 132.2, 123.8, 123.7, 121.9, 121.2, 110.5, 109.8, 59.4, 56.3, 56.2, 48.6, 29.1, 28.2, 23.4. HRMS (ESI+): $\text{C}_{24}\text{H}_{31}\text{N}_2\text{O}_5$: observed: 459.1938, calculated: 459.1954.

N-Cyclohexyl-N-((2,2-dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-3,4-

dimethoxybenzenesulfonamide (26e). Yield: 46%. ^1H NMR (CDCl_3) 7.47 (dd, $J = 2.4$, 6.4 Hz, 1H), 7.42 (d, $J = 8.4$ Hz, 1H), 7.30 (d, $J = 2.4$ Hz, 1H), 7.06 (d, $J = 8.4$, 1H), 6.93 (d, $J = 8.8$ Hz, 1H), 6.45 (d, $J = 10$ Hz, 1H), 5.88 (d, $J = 10$ Hz, 1H), 3.96 (s, 3H), 3.92 (s, 3H), 3.80 (m, 1H), 1.64 (m, 3H), 1.48 (m, 9H), 1.25 -1.20 (m, 4H). ^{13}C NMR (CDCl_3) δ 152.2, 150.7, 149.1, 148.5,

139.9, 135.2, 133.2, 123.7, 123.7, 122.5, 120.7, 110.6, 109.5, 58.4, 56.2, 56.1, 48.5, 31.3, 28.2, 26.1, 25.2. HRMS (ESI +): $C_{25}H_{33}N_2O_5S$: observed: 473.2118, calculated: 473.2110.

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-3,4-dimethoxy-N-(5,6,7,8-tetrahydronaphthalen-2-yl)benzenesulfonamide (26f). ^1H NMR ($CDCl_3$) δ 7.39 – 7.31 (m, 2H), 6.99 (dd, J = 13.4, 5.2 Hz, 2H), 6.92 (dd, J = 8.1, 2.8 Hz, 2H), 6.81 (d, J = 7.6 Hz, 2H), 6.35 (d, J = 10.1 Hz, 1H), 5.94 – 5.71 (m, 1H), 4.78 (d, J = 25.5 Hz, 2H), 3.97 (s, 3H), 3.79 (d, J = 5.3 Hz, 3H), 2.65 (dd, J = 25.6, 13.3 Hz, 4H), 1.83 – 1.66 (m, 4H), 1.41 (d, J = 16.1 Hz, 6H). MS (ESI +): $M + H = 521$

N-Cycloheptyl-N-((2,2-dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-3,4-dimethoxybenzenesulfonamide (26g). Yield: 18%. ^1H NMR ($CDCl_3$) δ 7.47 (dt, J = 4.4, 2.2 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.31 – 7.27 (m, 1H), 7.07 (d, J = 8.4 Hz, 1H), 6.94 (dd, J = 8.5, 4.9 Hz, 1H), 6.45 (dd, J = 10.1, 0.5 Hz, 1H), 5.88 (d, J = 10.1 Hz, 1H), 4.44 – 4.29 (m, 2H), 3.96 (s, 3H), 3.94 (s, 1H), 3.93 (s, 3H), 1.62 – 1.29 (m, 18H).

N-Cyclooctyl-N-((2,2-dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-3,4-dimethoxybenzenesulfonamide (26h). Yield: 45%. ^1H NMR ($CDCl_3$) δ 7.49 (d, J = 6.4, 1H), 7.46 (d, J = 10, 1H), 7.32 – 7.29 (m, 1H), 7.07 (d, J = 8.0, 1H), 6.94 (d, J = 8.4, 1H), 6.45 (d, J = 10, 1H), 5.89 (d, J = 10, 1H), 4.38 (s, 2H), 3.97 – 3.93 (m, 7H), 1.61 – 1.42 (m, 20H). MS (ESI+): $M + H = 501.2$

N-Cyclobutyl-N-((2,2-dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-3,4-dimethoxybenzenesulfonamide (26i). Yield: 40%. ^1H NMR ($CDCl_3$) δ 7.44 (dd, J = 8.4, 2.2 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.31 – 7.21 (m, 2H), 7.07 (d, J = 8.4 Hz, 1H), 6.94 (t, J = 6.9 Hz, 1H), 6.40 (dd, J = 32.8, 10.0 Hz, 1H), 5.88 (t, J = 10.6 Hz, 1H), 4.49 – 4.28 (m, 3H), 3.96 (s,

3H), 3.93 (d, J = 3.0 Hz, 3H), 2.08 – 1.83 (m, 4H), 1.60 – 1.40 (m, 8H). ^{13}C NMR (CDCl_3) δ 152.5, 150.3, 149.0, 148.6, 140.1, 135.4, 131.7, 123.8, 123.7, 121.8, 121.0, 110.5, 109.6, 77.3, 77.0, 76.7, 56.2, 56.2, 52.7, 49.3, 28.9, 28.2, 15.0. $M + H = 445$.

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-N-(4-fluorophenyl)-3,4-

dimethoxybenzenesulfonamide (26j). Yield: 12%. ^1H NMR (CDCl_3) δ 7.55 – 7.40 (m, 1H), 7.38 – 7.23 (m, 3H), 7.16 – 7.01 (m, 4H), 7.00 – 6.87 (m, 5H), 6.40 (t, J = 11.7 Hz, 1H), 5.88 (t, J = 13.4 Hz, 1H), 4.83 (d, J = 14.0 Hz, 2H), 3.97 (s, 3H), 3.86 – 3.73 (m, 3H), 1.44 (s, 6H). HRMS (ESI +) $\text{C}_{25}\text{H}_{25}\text{FN}_2\text{O}_5\text{S} + \text{H}$: observed: 485.1531, calculated: 485.1546.

General Procedure for 26k – 26t. To a solution of **25a** (1 eq) in pyridine at 0°C , was added the appropriate sulfonyl chloride dropwise. The reaction was allowed to warm up to room temperature overnight. The reaction mixture was then diluted with EtOAc and the organic layer washed with 10% citric acid and sat. NaHCO_3 , water and brine. The organic layer was dried over MgSO_4 , concentrated *in vacuo*, and purified by column chromatography (silica gel).

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-N-phenylcyclohexanesulfonamide

(26k). Yield: 60%. ^1H NMR (CDCl_3): δ 7.30 – 7.22 (m, 4H), 7.07 – 7.01 (m, 2H), 6.90 (dd, J = 24.2, 1.8 Hz, 1H), 6.46 (dd, J = 29.3, 2.1 Hz, 1H), 5.86 (d, J = 29.3 Hz, 1H), 5.05 (d, J = 48.2 Hz, 1H), 4.90 (d, J = 48.2 Hz, 1H), 2.13 – 1.96 (m, 4H), 1.78– 1.70 (m, 5H), 1.44 (d, J = 6.3 Hz, 6H), 1.27 (m, 2H). ^{13}C NMR (CDCl_3): δ 148.5, 148.3, 143.6, 140.4, 135.2, 129.1, 123.9, 123.8, 123.6, 122.0, 121.8, 92.4, 34.5, 31.8, 28.3, 28.2, 24.7, 21.7, 21.3.

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-N-phenylpropane-2-sulfonamide

(26l). Yield: 58%. ^1H NMR (CDCl_3): δ 7.37 – 7.19 (m, 4H), 7.16 – 6.99 (m, 2H), 6.93 (t, J = 9.4 Hz, 1H), 6.46 (dd, J = 10.1, 0.5 Hz, 1H), 5.87 (d, J = 10.1 Hz, 1H), 4.92 (dd, J = 36.5, 16.5 Hz, 2H), 1.81 (s, 3H), 1.75 (d, J = 11.8 Hz, 4H), 1.45 (t, J = 5.8 Hz, 6H). ^{13}C NMR (CDCl_3): δ 148.5,

148.2, 143.1, 140.5, 135.3, 129.1, 124.2, 123.7, 123.6, 122.2, 122.1, 86.0, 28.3, 28.2, 27.4, 25.4.

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-N-phenylcyclopropanesulfonamide (26m). Yield: 46%. ^1H NMR (CDCl_3) δ 7.44 – 7.42 (m, 2H), 7.34 – 7.23 (m, 4H), 6.99 (d, J = 8 Hz, 1H), 6.40 (d, J = 10, 1H), 5.85 (dd, J = 10, 1H), 4.98 (s, 2H), 2.55 (m, 1H), 1.44 (d, J = 16.1 Hz, 6H), 1.13- 1.11 (m, 2H), 0.98-0.95 (m, 2H). ^{13}C NMR (CDCl_3) δ 148.7, 148.1, 140.4, 139.7, 135.3, 129.1, 128.8, 127.7, 123.7, 123.6, 122.4, 56.2, 28.6, 28.2, 5.16. HRMS (ESI+) for $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_3\text{S} + \text{Na}$: observed: 393.1231, calculated: 393.1249.

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-N-phenylbutane-1-sulfonamide (26n). Yield: 48%. ^1H NMR (CDCl_3) δ 7.41 – 7.29 (m, 4H), 7.27 – 7.22 (m, 1H), 7.18 (d, J = 8.3 Hz, 1H), 7.00 (dd, J = 12.4, 5.5 Hz, 1H), 6.41 (d, J = 10.1 Hz, 1H), 5.86 (d, J = 10.1 Hz, 1H), 4.94 (s, 2H), 3.38 – 2.79 (m, 2H), 1.96 – 1.77 (m, 2H), 1.54 – 1.38 (m, 8H), 1.12 – 0.77 (m, 3H). ^{13}C NMR (CDCl_3) 170.9, 148.8, 148.2, 139.6, 135.4, 129.3, 128.3, 127.6, 123.7, 123.2, 122.5, 56.3, 51.3, 28.2, 25.3, 21.7, 13.6. MS (ESI +): $M + H = 387.2$

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-N-phenylpropane-1-sulfonamide (26o). Yield: 20%. ^1H NMR (CDCl_3) δ 7.40 – 7.21 (m, 6H), 7.17 (d, J = 8.3 Hz, 1H), 6.98 (t, J = 7.2 Hz, 1H), 6.40 (d, J = 10.1 Hz, 1H), 5.85 (d, J = 10.1 Hz, 1H), 4.93 (s, 2H), 3.20 – 2.99 (m, 2H), 1.98 – 1.79 (m, 2H), 1.43 (s, 6H), 1.03 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CDCl_3) δ 148.8, 148.2, 139.6, 135.4, 129.3, 128.4, 127.6, 123.7, 123.6, 122.5, 77.3, 77.0, 76.7, 56.2, 53.2, 28.2, 17.1, 13.1. MS (ESI+): $M + H = 373.0$

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-2-methyl-N-phenylpropane-1-sulfonamide (26p). Yield: 28%. ^1H NMR (CDCl_3) δ 7.40 – 7.30 (m, 4H), 7.27 – 7.21 (m, 1H),

7.19 (d, $J = 8.3$ Hz, 1H), 6.99 (d, $J = 8.3$ Hz, 1H), 6.42 (d, $J = 10.1$ Hz, 1H), 5.89 (dd, $J = 21.3$, 10.0 Hz, 1H), 4.93 (s, 2H), 3.01 (dd, $J = 14.1$, 6.5 Hz, 2H), 2.34 (dp, $J = 13.3$, 6.7 Hz, 1H), 1.47 (d, $J = 14.5$ Hz, 6H), 1.10 (d, $J = 6.7$ Hz, 6H). MS (ESI⁺): $M + H = 387$

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-N-phenylbiphenyl-4-sulfonamide

(26q). Yield: 17%. ^1H NMR (CDCl_3) δ 7.69 (s, 4H), 7.67 – 7.60 (m, 2H), 7.57 – 7.41 (m, 3H), 7.36 – 7.22 (m, 5H), 7.21 – 7.13 (m, 2H), 7.00 (t, $J = 7.3$ Hz, 1H), 6.34 (d, $J = 10.1$ Hz, 1H), 5.83 (t, $J = 9.5$ Hz, 1H), 4.86 (d, $J = 5.9$ Hz, 2H), 1.43 (s, 6H). HRMS (ESI⁺): $\text{C}_{29}\text{H}_{27}\text{N}_2\text{O}_3\text{S}$: observed: 483.1723, calculated: 483.1742,

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-N-phenylbenzo[d][1,3]dioxole-4-sulfonamide (26r)

Yield: 65%. ^1H NMR (CDCl_3) δ 7.35 – 7.21 (m, 4H), 7.21 – 7.12 (m, 3H), 7.08 (t, $J = 3.9$ Hz, 1H), 7.02 – 6.93 (m, 1H), 6.88 – 6.78 (m, 1H), 6.36 (dd, $J = 10.1$, 0.5 Hz, 1H), 6.09 (s, 2H), 5.84 (dd, $J = 14.0$, 9.0 Hz, 1H), 4.82 (s, 2H), 1.68 (s, 2H), 1.43 (s, 6H). HRMS (ESI⁺): $\text{C}_{24}\text{H}_{23}\text{N}_2\text{O}_5\text{S}$: observed: 451.1316. calculated: 451.1328,

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-N-phenylquinoline-8-sulfonamide

(26s). Yield: 65%. ^1H NMR (CDCl_3) δ 9.19 (dd, $J = 4.2$, 1.8 Hz, 1H), 8.43 – 8.16 (m, 2H), 8.00 (dt, $J = 10.5$, 5.3 Hz, 1H), 7.66 – 7.56 (m, 2H), 7.54 – 7.47 (m, 1H), 7.14 – 6.94 (m, 6H), 6.40 (d, $J = 10.1$ Hz, 1H), 5.85 (t, $J = 15.1$ Hz, 1H), 5.58 (d, $J = 35.1$ Hz, 2H), 1.45 (s, 6H). ^{13}C NMR (CDCl_3) δ 151.3, 150.0, 148.5, 144.2, 140.1, 139.6, 137.1, 136.5, 135.1, 133.7, 133.5, 128.8, 128.8, 128.2, 127.2, 125.4, 123.9, 123.8, 122.4, 122.1, 77.4, 77.1, 76.9, 76.7, 58.8, 28.2. HRMS (ESI⁺): $\text{C}_{26}\text{H}_{24}\text{N}_3\text{O}_3\text{S}$: observed: 485.1543, calculated: 485.1538.

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-N-phenyl-2,3-

dihydrobenzo[b][1,4]dioxine-6-sulfonamide (26t). Yield: 57%. ^1H NMR (CDCl_3): 1.419 (s,

6H), 4.33-4.28 (m, 2H), 4.81 (s, 2H), 5.82 (d, 1H, J=10), 6.35 (d, 1H, J = 10), 6.89 (d, 1H, J = 8.4), 6.97 (d, 1H, J = 8.4), 7.06 (dd, 1H), 7.12 -7.15 (m, 2H), 7.20 – 7.3 (m, 5H). ^{13}C NMR (101 MHz, CDCl_3) δ 148.6, 147.7, 147.5, 143.4, 140.4, 139.4, 135.2, 130.4, 128.8, 128.5, 127.6, 123.7, 123.6, 122.4, 121.6, 117.4, 117.4, 77.4, 77.1, 77.0, 76.7, 64.6, 64.1, 60.4, 55.7, 28.2, 21.1, 14.2. HRMS (ESI+): $\text{C}_{15}\text{H}_{15}\text{N}_2\text{O}_5\text{S}$: observed: 465.1489, calculated: 465.1484.

N-((2,2-Dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-3,4-dimethoxy-N-phenylbenzamide

(27). To a solution of N-((2,2-dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)aniline **25a** (60 mg, 0.226 mmol) in DCM (3 mL) was added triethylamine (0.06 mL, 0.452 mmol) and 3,4-dimethoxybenzoyl chloride (54 mg, 0.271 mmol). The reaction was stirred overnight at room temperature. The reaction mixture was washed with dH_2O ($\times 2$) and sat. NaHCO_3 ($\times 2$) dried over MgSO_4 and concentrated *in vacuo*. Purification by column: silica gel: 3:1 DCM/EtOAc to give a light yellow oil (98 mg, 100%). ^1H NMR (CDCl_3) δ 7.26 – 7.17 (m, 3H), 7.17 – 7.08 (m, 3H), 7.06 – 6.97 (m, 2H), 6.95 (t, J = 5.0 Hz, 1H), 6.66 (d, J = 8.4 Hz, 1H), 6.53 – 6.40 (m, 1H), 5.92 – 5.79 (m, 1H), 5.16 (s, 2H), 3.83 (s, 3H), 3.66 (s, 3H), 1.46 (s, 6H). ^{13}C NMR (CDCl_3) δ 169.9, 150.3, 149.0, 148.6, 147.9, 144.7, 140.6, 135.1, 129.0, 127.8, 127.2, 126.3, 124.0, 123.6, 123.0, 122.3, 112.6, 109.9, 77.4, 77.0, 77.0, 76.7, 55.8, 55.7, 55.6, 28.2. HRMS: $\text{C}_{26}\text{H}_{27}\text{N}_2\text{O}_4$: observed: 431.1951, calculated: 431.1971;

2-Bromo-6-(hydroxymethyl)pyridin-3-ol (30). A solution of 2-bromo-3-hydroxy-6-methylpyridine 1-oxide **29** (15g, 0.075 mol) in TFAA (50 mL, 0.375 mol) was stirred at 40 °C and stirred for 24 hours. The solvent was removed under vacuum. The residue was purified by column chromatography, silica gel: EA:Hex (2:1). Yield: 4.5g, 30%. δ 7.32 (d, J = 8.0 Hz, 1H), 7.25 (d, J = 8.5 Hz, 1H), 4.56 (s, 2H).

(6-Bromo-5-(2-methylbut-3-yn-2-yloxy)pyridin-2-yl)methanol (31). Compound **30** was dissolved in acetone (20 ml) and K_2CO_3 (166 mg, 7 mmol), KI (33 mg, 0.2 mmol), $CuCl_2 \cdot 2H_2O$ (33 mg, 0.2 mmol). The suspension was stirred at 60 °C for 10 minutes. The solution of 3-chloro-3-methylbutyne (1.02 g, 5 mmol) in acetone (5 ml) was added dropwise to the solution of **30**. The reaction mixture was cooled to room temperature and the suspension filtered. The solid residue was washed with MeOH. The filtrate was concentrated under vacuum and purified with column chromatography: silica gel (EA: Hexane, 1:1). Yield: 700 mg, 57%. ^1H NMR ($CDCl_3$): δ 7.88 (d, J = 8.0 Hz, 1H), 7.23 (d, J = 8.0 Hz, 1H), 4.72 (s, 2H), 1.73 (s, 6H). ^{13}C NMR: δ 154.7, 149.6, 137.0, 129.5, 120.4, 85.7, 75.8, 75.7, 64.6, 30.1.

(8-Bromo-2,2-dimethyl-2H-pyrano[2,3-c]pyridin-6-yl)methanol (32). A solution of **31** (700 mg, 2.5 mmol) in toluene (10ml) was subjected to microwave irradiation (200 W, 120 °C) for 1 hour. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under vacuum and the residue purified by column chromatography: silica gel (EA:Hex 1:3 – 1:2). Yield: 500 mg, 70%. ^1H NMR ($CDCl_3$): δ 6.88 (s, 1H), 6.28 (d, J = 10 Hz, 1H), 5.90 (d, J = 9.6 Hz, 1H), 4.63 (s, 2H), 1.51 (s, 6H).

8-Bromo-6-(bromomethyl)-2,2-dimethyl-2H-pyrano[2,3-c]pyridine (33). To a solution of **32** in DCM (2 ml) was added CBr_4 (66 mg, 0.2 mmol) and PPh_3 (264 mg, 0.2 mmol). The reaction mixture was stirred at room temperature for 1 hour. The solvent was removed under vacuum and the residue purified by column chromatography: silica gel (EA:Hex, 1:4). Yield 260 mg, 40%. ^1H NMR ($CDCl_3$): δ 6.28 (d, J = 10 Hz, 1H), 5.91 (d, J = 10 Hz, 1H), 4.47 (s, 2H), 1.53 (s, 6H).

General Procedure for synthesis of 34. To a degassed flask with **33** (1 eq) was added DMF, aniline (1.5 eq) and DIEA (1.5 eq). The mixture was stirred at room temperature overnight. Water (50 ml) was added to the reaction mixture and the resulting solution was extracted with ethyl acetate (3 $\times$ 25ml). The combined organic layer was washed with 0.5N HCl (50 ml), 40 %

NaHCO₃ (50 ml) , water (50 ml) and brine, dried over Na₂SO₄ ad concentrated under vacuum. The residue was purified by column chromatography.

N-((8-Bromo-2,2-dimethyl-2H-pyrano[2,3-c]pyridin-6-yl)methyl)benzenamine 34a. Yield: 78%. ¹H NMR (CDCl₃) δ 7.26 - 7.21 (m, 3H), 6.97 -6.95 (m, 3H), 6.23 (d, J = 9.6 Hz, 1H), 5.85 (d, J = 9.6 Hz, 1H), 4.40 (s, 2H), 1.48 (s, 6 H).

N-((8-Bromo-2,2-dimethyl-2H-pyrano[2,3-c]pyridin-6-yl)methyl)cyclohexanamine (34b). Yield: 60%. ¹³C NMR (CD₃OD) δ 152.0, 145.2, 136.9, 129.7, 129.1, 119.8, 118.7, 78.5, 56.0, 49.8, 32.4, 26.8, 25.8, 24.7.

General Procedure for synthesis of compound 35. A flask of secondary amine 34 (1 eq) was degassed and THF (anh) was added under nitrogen. The solution was cooled to -78 °C and stirred for 1 hour. BuLi (2.5 eq) was the added to the solution dropwise at -78 °C. The resulting solution was stirred for 1 hour. Water (10ml) was added to the solution and diluted with ethyl acetate (25ml). After separation, the aqueous layer was extracted with Ethyl acetate and washed with water (25 mL × 3), brine (25 ml), dried over Na₂SO₄ ad concentrated under vacuum. The residue was purified by column chromatography (EA: Hex, 1:4)

N-((2,2-Dimethyl-2H-pyrano[2,3-c]pyridin-6-yl)methyl)benzenamine (35a). Yield: 70%. ¹H NMR (CD₃OD) δ 7.91 (s, 1H), 7.08 -7.04 (m, 3H), 6.59 – 6.57 (m, 3H), 6.28 (d, J = 9.6 Hz, 1H), 5.92 (s, J = 10 Hz, 1H), 4.88 (s, 2H), 1.41 (s, 6H). ¹³C NMR (D₂O) δ 152.3, 148.3, 148.2, 136.7, 135.2, 128.9, 128.7, 119.9, 117.8, 116.8, 112.6, 76.9, 26.8.

N-((2,2-Dimethyl-2H-pyrano[2,3-c]pyridin-6-yl)methyl)cyclohexanamine (35b). Yield: 50%. ¹H NMR (CD₃OD) δ 7.95 (s, 1H), 7.08 (s, 1H), 6.44 (d, J = 9.6 Hz, 1H), 6.03 (d, J = 10 Hz, 1H), 3.78 (s, 2H), 2.46 (m, 1H), 1.97 – 1.75 (m, 5 H), 1.46 (s, 6H, 1.27 -1.16 (m, 5H). ¹³C NMR

(CD₃OD) δ 151.5, 148.3, 136.7, 136.5, 128.7, 119.9, 119.0, 77.0, 56.0, 50.3, 32.4, 26.8, 25.8, 24.7.

General procedure for Synthesis of 36. A mixture of compound **35** (1 eq.) and the appropriate sulfonyl chloride (2 eq.) in pyridine was stirred overnight at room temperature. 1 M HCl was added to the reaction mixture and the solution extracted with Ethyl Acetate (3 $\times$ 15 ml). The combined organic layer was washed with water (3 $\times$ 20 ml), brine and dried over Na₂SO₄. The solvent was removed under vacuum and the residue purified by column chromatography: silica gel (EA: Hex; 1:4).

N-((2,2-Dimethyl-2H-pyrano[2,3-c]pyridin-6-yl)methyl)-4-methoxy-N-

phenylbenzenesulfonamide (36a). Yield: 50%. ¹H NMR (CD₃OD) δ 7.76 (s, 1H), 7.59 – 7.56 (m, 2H), 7.25 – 7.18 (m, 3H), 7.09 – 7.04 (m, 4H), 6.37 (d, 1H, 10 Hz) 5.97 (d, 1H, 10 Hz), 4.88 (s, 3H), 4.78 (s, 2H), 3.084 (s, 3H), 1.40 (s, 6H). ¹³C NMR (CD₃OD), 163.5, 148.6, 139.3, 136.7, 136.1, 129.7, 129.3, 128.8, 128.5, 128.5, 127.6, 119.7, 119.4, 113.9, 77.1, 55.0, 54.9, 26.8. MS (ESI⁺): M + H = 437

N-((2,2-Dimethyl-2H-pyrano[2,3-c]pyridin-6-yl)methyl)-4-nitro-N-

phenylbenzenesulfonamide (36b). Yield: 59%. ¹H NMR (CD₃OD) δ 8.41 (dd, J = 2.0 Hz, 4.8 Hz, 2H), 7.89 (dd, J = 2.0 Hz, 4.8 Hz), 7.78 (s, 1H), 7.29 – 7.28 (m, 4H), 7.10 – 7.08 (m, 2H), 6.39 (d, J = 10 Hz, 1H), 6.00 (d, J = 10 Hz, 1H), 1.42 (s, 6H). MS(ESI⁺): M + H = 452.1

N-Cyclohexyl-N-((2,2-dimethyl-2H-pyrano[2,3-c]pyridin-6-yl)methyl)-4-

isopropylbenzenesulfonamide (36c). Yield: 28%. ¹H NMR (CDCl₃) δ 7.98 (s, 1H), 7.77 – 7.75 (m, 2H), 7.37 – 7.34 (m, 1H), 7.28 (d, J = 8.8 Hz, 1H), 6.34 (d, J = 9.6 Hz, 1H), 5.84 (d, J = 10 Hz, 1H), 4.42 (s, 2H), 3.80 (m, 1H), 2.99 (m, 1H), 1.75 – 1.59 (m, 3H), 1.55 – 1.39 (m, 9 H), 1.29 – 1.27 (m, 6H), 1.23 – 1.19 (m, 4H). ¹³C NMR (CDCl₃) δ 153.8, 151.8, 148.1, 138.7, 136.7,

135.8, 128.1, 127.1, 127.0, 120.9, 118.9, 58.5, 48.7, 34.1, 28.1, 26.1, 25.1, 23.7. MS (ESI⁺): M + H = 455.

N-Cyclohexyl-N-((2,2-dimethyl-2H-pyrano[2,3-c]pyridin-6-yl)methyl)-3,4-

dimethoxybenzenesulfonamide (36d). Yield: 28%. ¹H NMR (CDCl₃) δ 8.00 (s, 1H), 7.50 – 7.47 (m, 1H), 7.33 – 7.29 (m, 2H), 6.94 (d, J = 8.4 Hz, 1H), 6.36 (d, J = 9.6 Hz, 1H), 5.86 (d, J = 9.6 Hz, 1H), 5.32 (s, 2H), 3.967 (s, 3H), 3.94 (s, 3H), 3.97 (m, 1H), 1.68 -1.52 (m, 4H), 1.48 (s, 6H), 1.28 -1.20 (m, 4H). ¹³C NMR (CDCl₃): δ 151.7, 149.1, 136.7, 135.9, 133.2, 128.1, 120.8, 120.7, 118.9, 110.6, 109.5, 83.1, 58.5, 56.2, 56.2, 48.6, 31.4, 28.0, 26.1, 25.1. MS (ESI⁺): M + H = 473.3

N-((2,2-dimethyl-2H-pyrano[3,2-b]pyridin-6-yl)methyl)-3,4-dimethoxy-N-phenylbenzamide

(37). To a solution of **25a** (60 mg, 0.226 mmol) in DCM (3 ml) was added Et₃N (0.06 ml, 0.452 mmol) and 3,4dimethoxybenzenesulfonyl chloride (54 mg, 1.2 mmol) and allowed to sitr at room temperature overnight. The reaction mixture was washed with d. H₂O (× 2) and brine, dried over MgSO₄ and concentrated *in vacuo*. The crude product was purified by column chromatography silica gel: DCM/EtOAc (3:1). To give a yellow oil (98mg, 98 %). ¹H NMR (CDCl₃) δ 7.26 – 7.17 (m, 3H), 7.17 – 7.08 (m, 3H), 7.06 – 6.97 (m, 2H), 6.95 (t, J = 5.0 Hz, 1H), 6.66 (d, J = 8.4 Hz, 1H), 6.53 – 6.40 (m, 1H), 5.92 – 5.79 (m, 1H), 5.16 (s, 2H), 3.83 (s, 3H), 3.66 (s, 3H), 1.46 (s, 6H). ¹³C NMR (CDCl₃) δ 169.91, 150.31, 149.00, 148.56, 147.88, 144.66, 140.55, 135.09, 129.03, 127.77, 127.24, 126.32, 123.99, 123.55, 123.02, 122.28, 112.56, 109.86, 77.36, 77.04, 76.98, 76.73, 55.78, 55.70, 55.62, 28.22. HRMS (ESI⁺): C₂₆H₂₇N₂O₄: observed: 431.1951, calculated: 431.1971.

N-((2,2-Dimethyl-2H-chromen-6-yl)methyl)-4-hydroxy-3-methoxy-N-

phenylbenzenesulfonamide (38). To a flask containing potassium carbonate was added NMP

and then placed in an oil bath at 150 °C. Benzenethiol was added and the mixture allowed to stir for 10 minutes, after which a solution of KCN-1 in NMP was added and the reaction allowed to stir at 150°C for exactly 5 minutes. The flask was removed from the heat and immediately poured into ice water, then extracted with EtOAc ($\times 3$) and the combined organic layer washed with water ($\times 3$), dried over MgSO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography: silica gel 50:1 (DCM: EtOAc) to give an off-white solid. Yield: 53%. ^1H NMR: δ 7.32 – 7.23 (m, 1H), 7.03 – 7.00 (m, 3H), 6.99 – 6.89 (m, 2H), 6.90 (d, $J = 8.4$ Hz, 1H), 6.61 (d, $J = 7.6$ Hz, 1H), 5.58 (d, $J = 10$ Hz, 1H), 4.62 (s, 2H), 3.81 (s, 3H), 1.40 (s, 6H).

N-((2,2-Dimethyl-2H-chromen-6-yl)methyl)-4-(4-(1,3-dioxoisindolin-2-yl)butoxy)-3-methoxy-N-phenylbenzenesulfonamide (39). To a solution of **38** (50.2 mg, 0.11 mmol) in DMF (2 mL) was added 4-bromophthalimide (34 mg, 0.12 mmol). The reaction mixture was stirred at 50 °C for 3 hours. Water was added to the reaction mixture and extracted with EtOAc (3×10 ml). The combined organic layers was washed with brine (10 ml), dried over Na_2SO_4 and concentrated under vacuum. The crude mixture was purified by column chromatography: silica gel, DCM: EtOAc, 50:1. Yield: 55 mg (76%). CDCl_3 7.87 – 7.85 (m, 2H), 7.75 – 7.73 (m, 2H), 7.31 – 7.21 (m, 5H), 7.01 – 6.89 (m, 6H), 6.60 (d, $J = 7.6$ Hz, 1H), 6.24 (d, $J = 9.6$ Hz, 1H), 5.57 (d, $J = 10$ Hz, 1H), 4.60 (s, 2H), 4.13 (s, 2H), 3.83 – 3.81 (m, 2H), 3.75 (m, 2H), 3.72 (s, 3H), 1.95 (m, 4H), 1.40 (s, 6H).

4-(4-Aminobutoxy)-N-((2,2-dimethyl-2H-chromen-6-yl)methyl)-3-methoxy-N-phenylbenzenesulfonamide (40). To a solution of **39** (55.1 mg, 0.085 mmol) in 4 ml of EtOH:DCM (3:1) was added 51% hydrazine. The reaction was refluxed for 2 hours, and then allowed to stir at r.t. overnight. The solvent was removed, and DCM was added to the residue. The white insoluble material was filtered and the filtrate was collected. The solvent was removed under vacuum. The crude product was purified by column chromatography: silica gel, DCM: MeOH: Et_3N (100:5:1) to give an off white solid. Yield: 30.2 mg (67%). ^1H NMR δ 7.33 –

7.22 (m, 4H), 7.02 -6.98 (m, 3H), 6.92 – 6.89 (m, 3H), 6.60 (d, J = 8.0 Hz, 1H), 6.24 (d, J = 9.6 Hz, 1H), 5.58 (d, J = 10 Hz, 1H), 4.15 (s, 2H), 3.82 (s, 3H), 3.22 – 3.11 (m, 4H), 1.45 – 1.39 (m, 10H).

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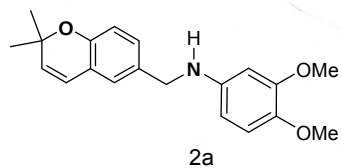
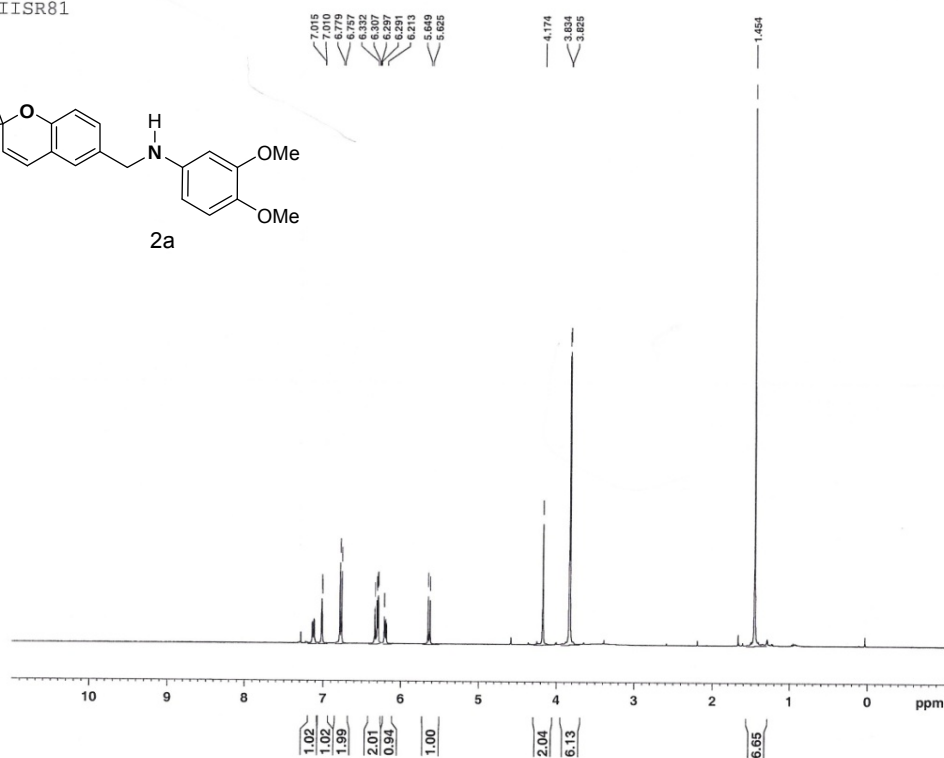
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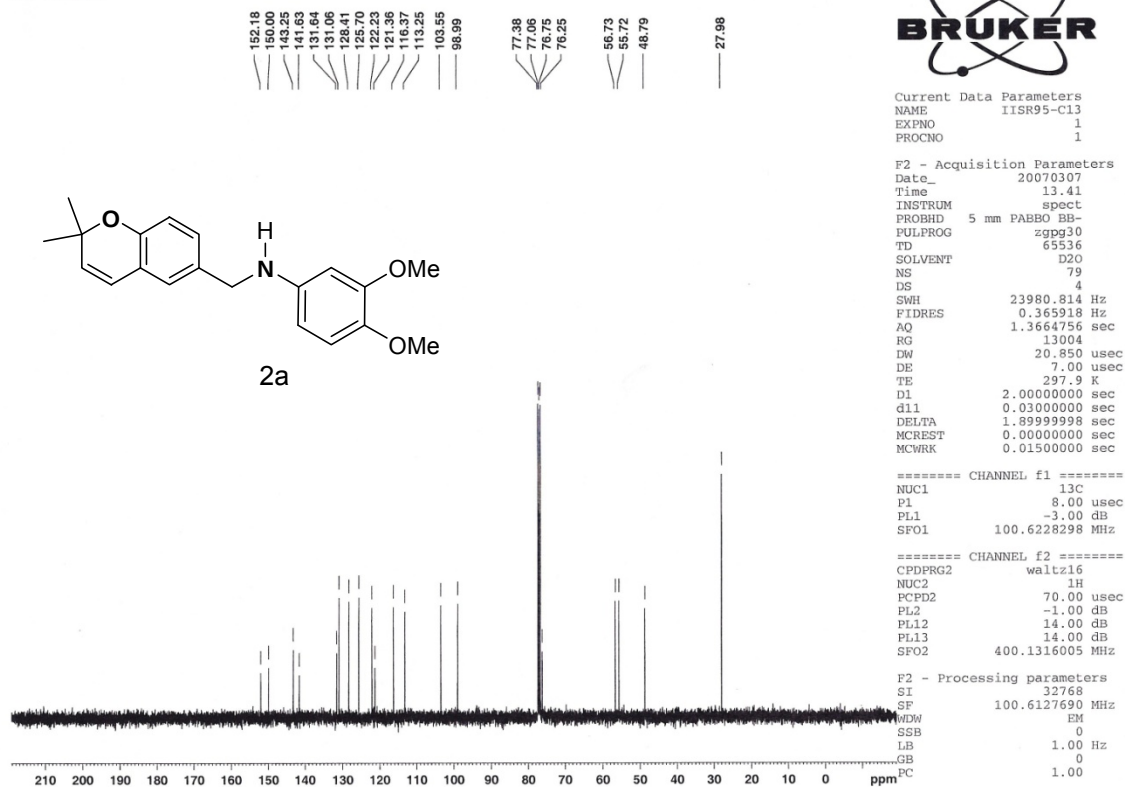
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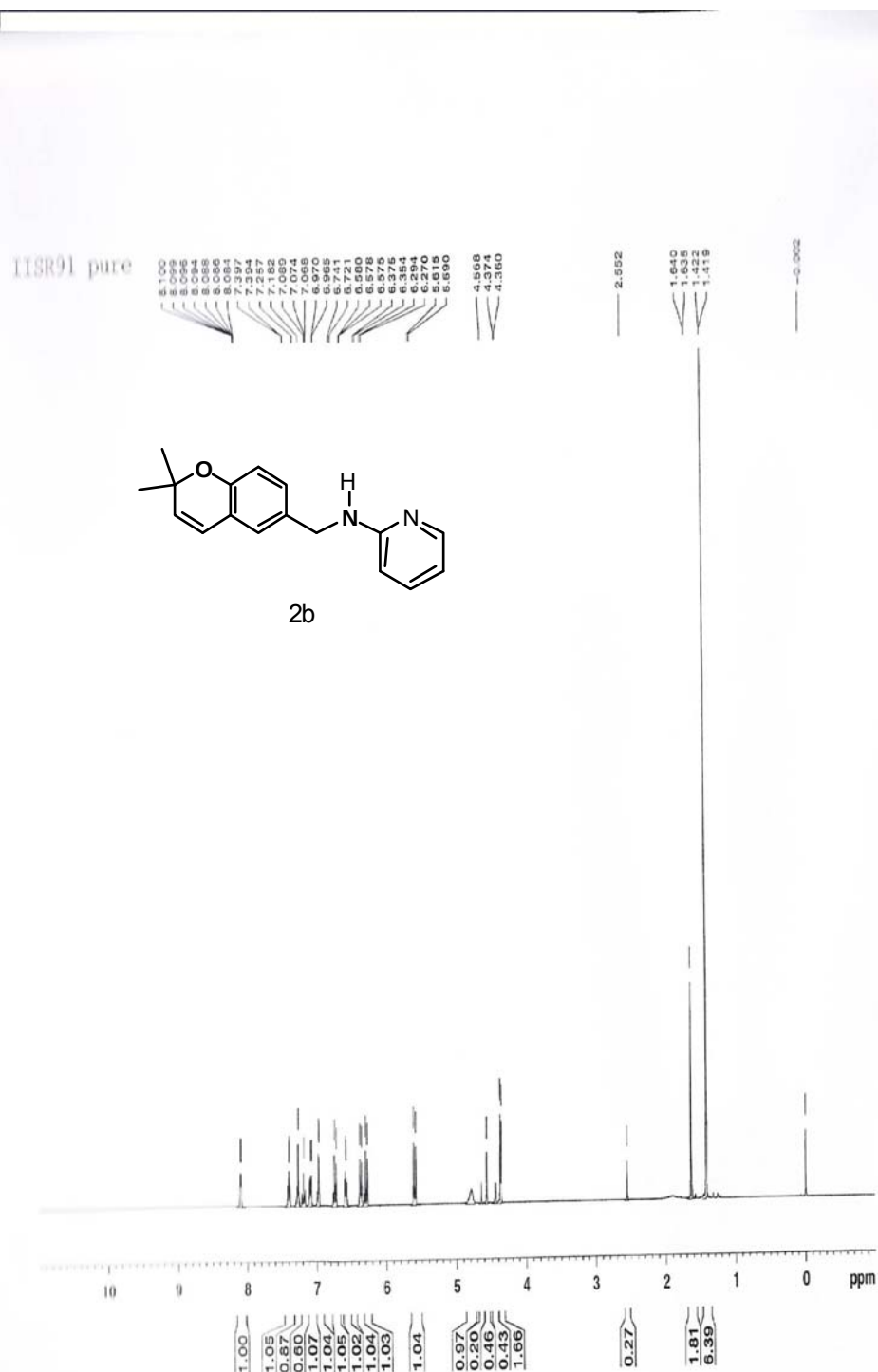
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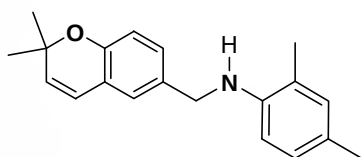
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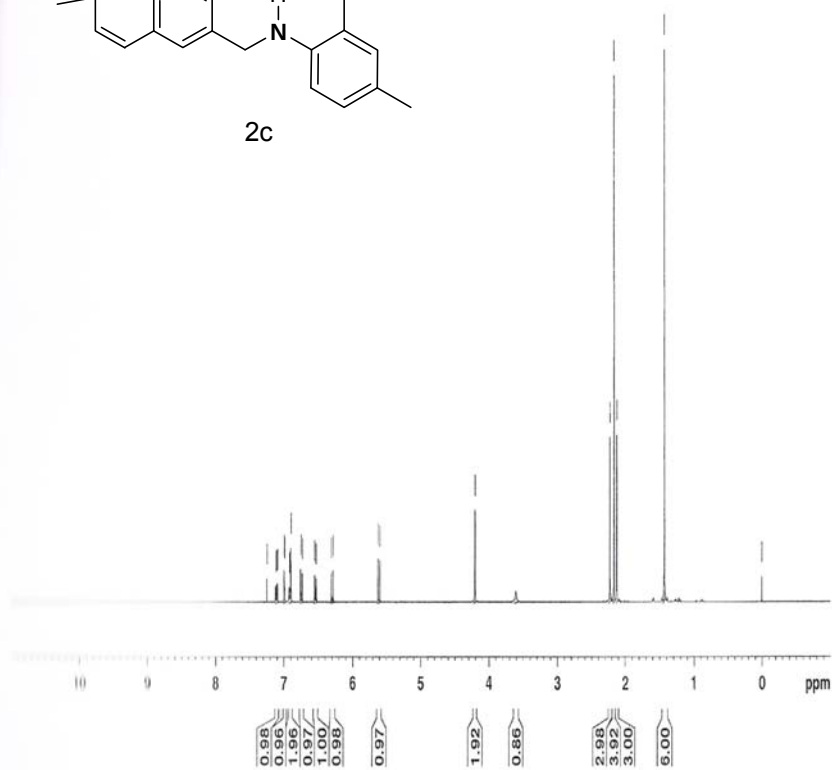
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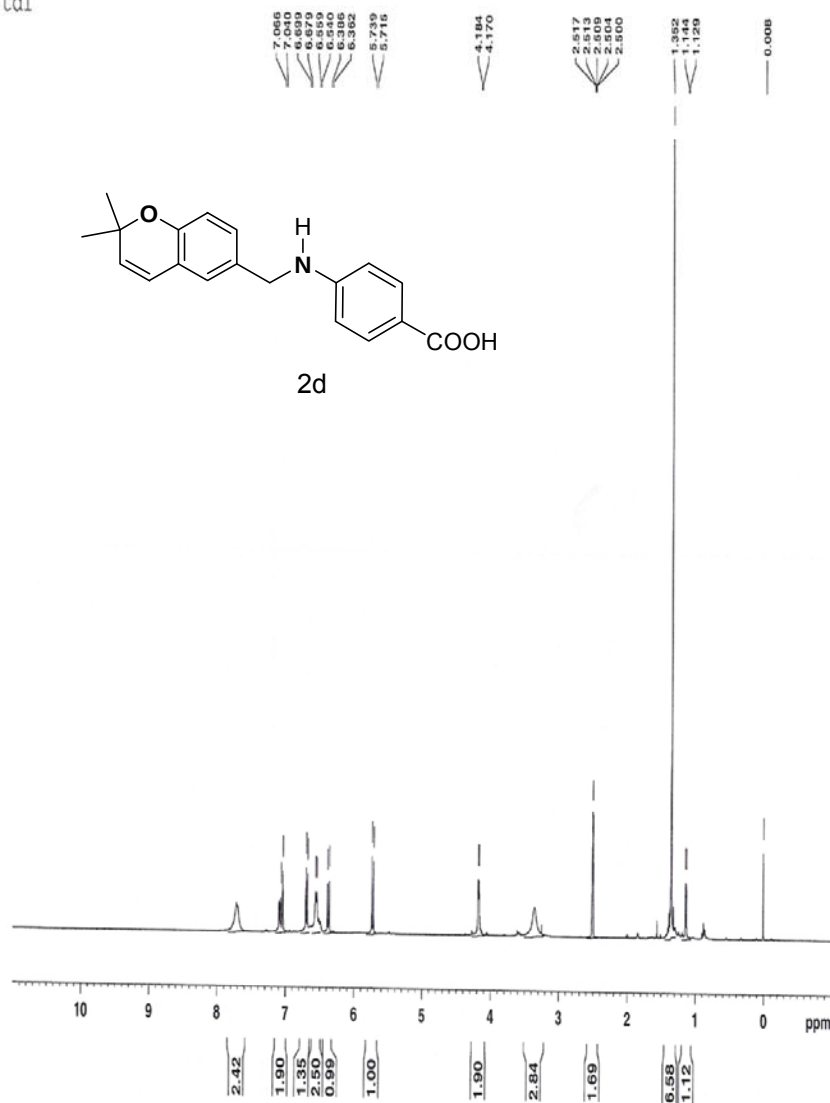


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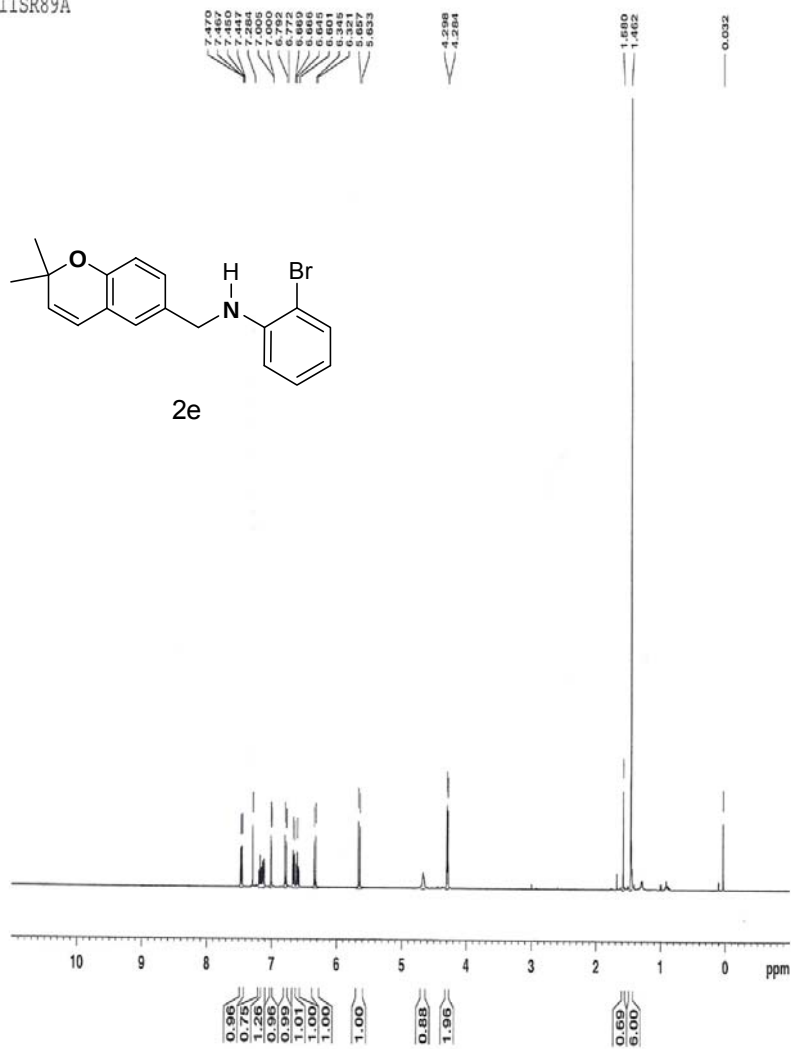
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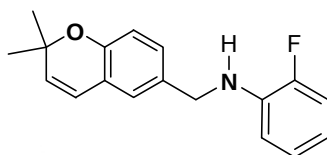


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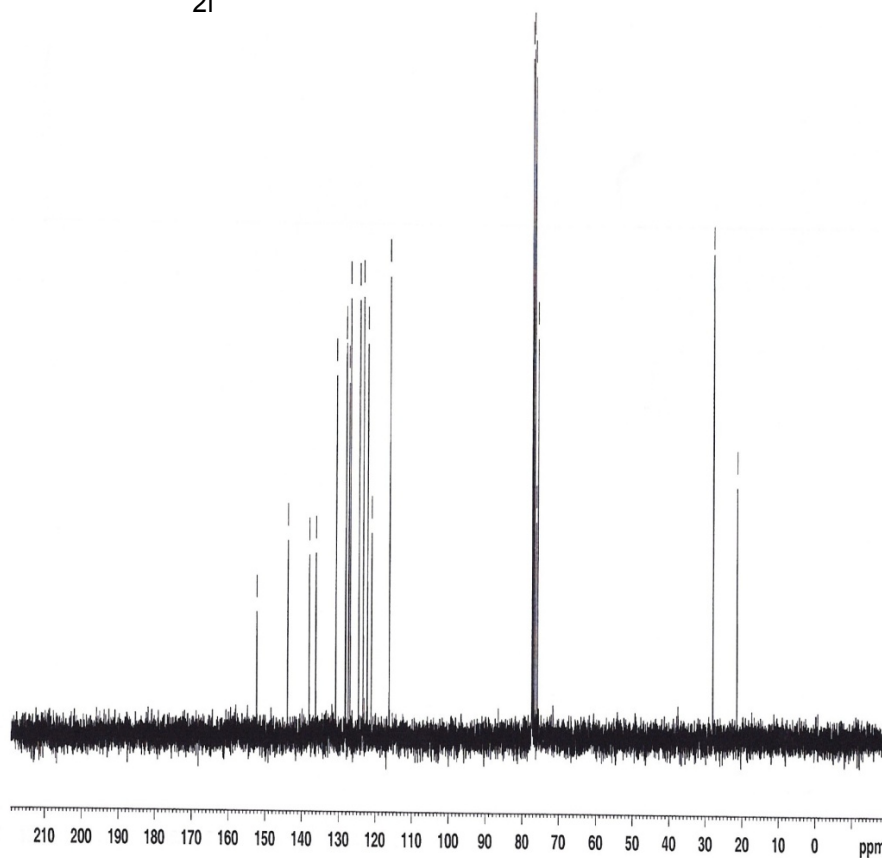
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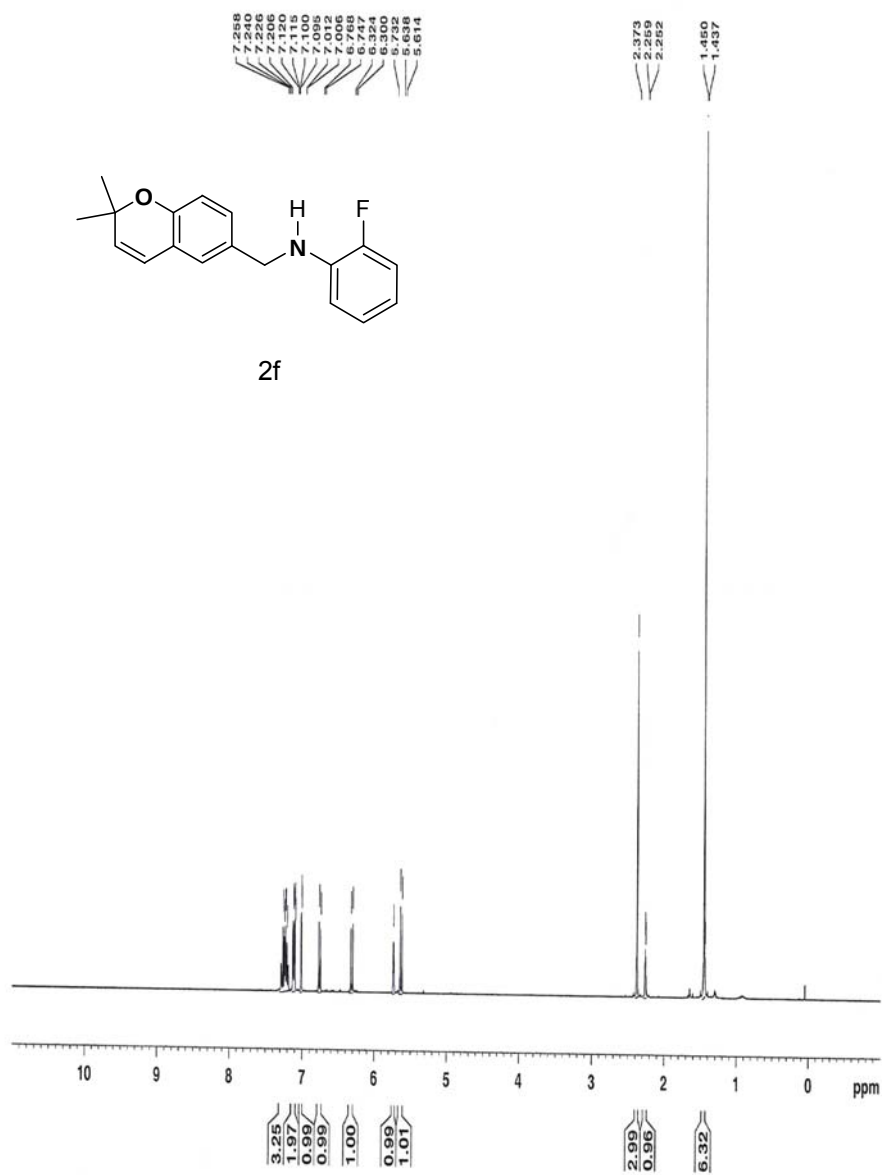
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3a

3a

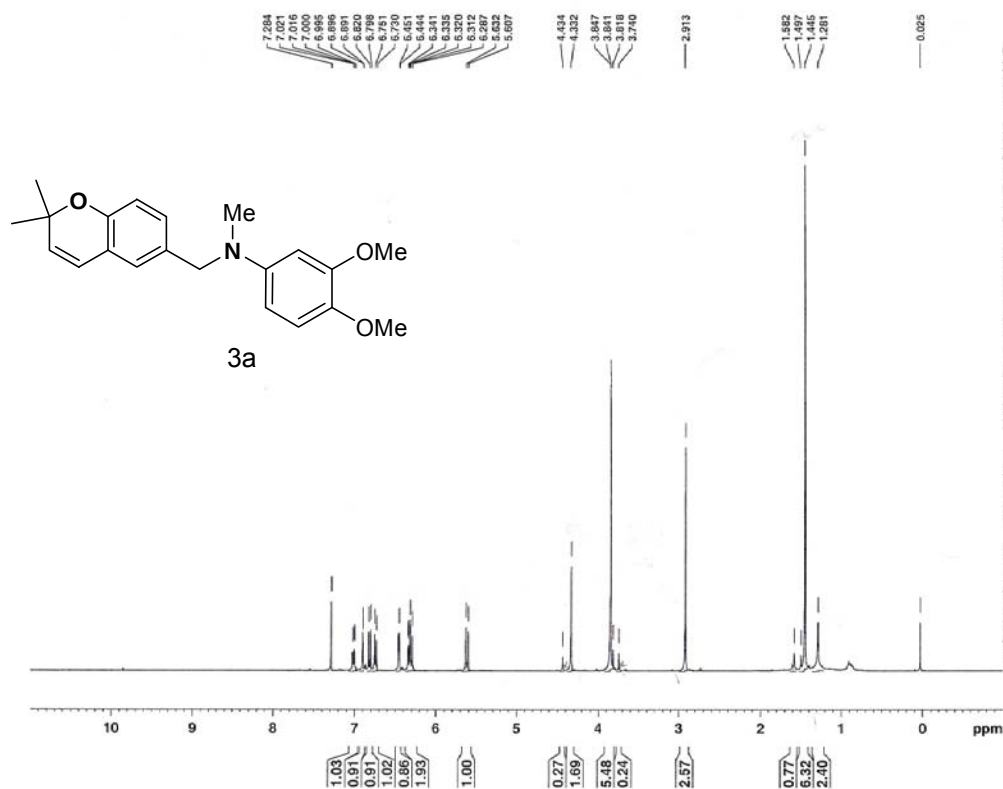


Current Data Parameters
 NAME LISRI00 452101
 EXPNO 1
 PROCNO 1

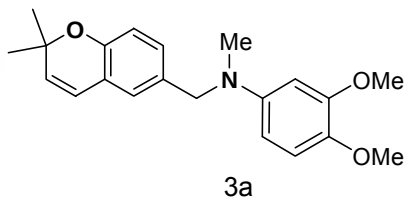
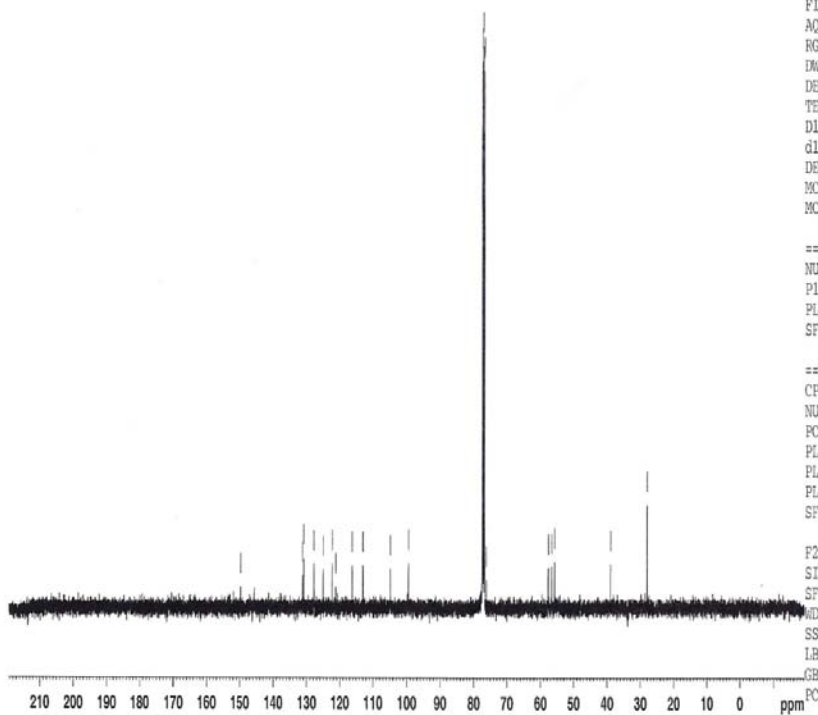
F2 - Acquisition Parameters
 Date_ 20070317
 Time 14.53
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT CDC13
 NS 16
 DS 2
 SWH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 228.1
 DW 60.400 usec
 DE 7.00 usec
 TE 297.6 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRK 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 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



No title



Current Data Parameters
 NAME MSB100-C13
 EXPNO 10 1
 PROCNO 1

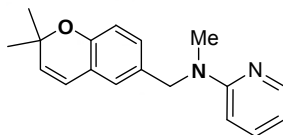
F2 - Acquisition Parameters
 Date_ 20070317
 Time 15.58
 INSTRUM spect
 PROBED 5 mm PABBO BB-
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 512
 DS 4
 SWH 23980.814 Hz
 FIDRES 0.365918 Hz
 AQ 1.3664756 sec
 RG 32768
 DW 20.850 usec
 DE 7.00 usec
 TE 298.0 K
 D1 2.0000000 sec
 d11 0.0300000 sec
 DELTA 1.8999999 sec
 MCREST 0.0000000 sec
 MCWPK 0.0150000 sec

===== CHANNEL f1 =====
 NUC1 13C
 P1 8.00 usec
 PL1 -3.00 dB
 SFO1 100.6226298 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 100.6127690 MHz
 MDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40

No title

3b

3b

151.92
147.97
137.27
130.87
130.76
127.82
125.00
122.35
121.26
116.27
111.72
105.76

77.35
77.03
76.71
76.14

52.63

35.95

27.98



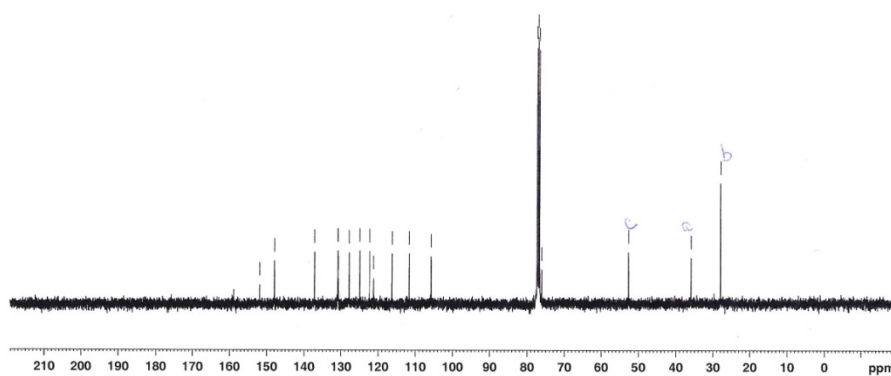
Current Data Parameters
NAME 15R100-C3
EXPNO 1
PROCNO 1

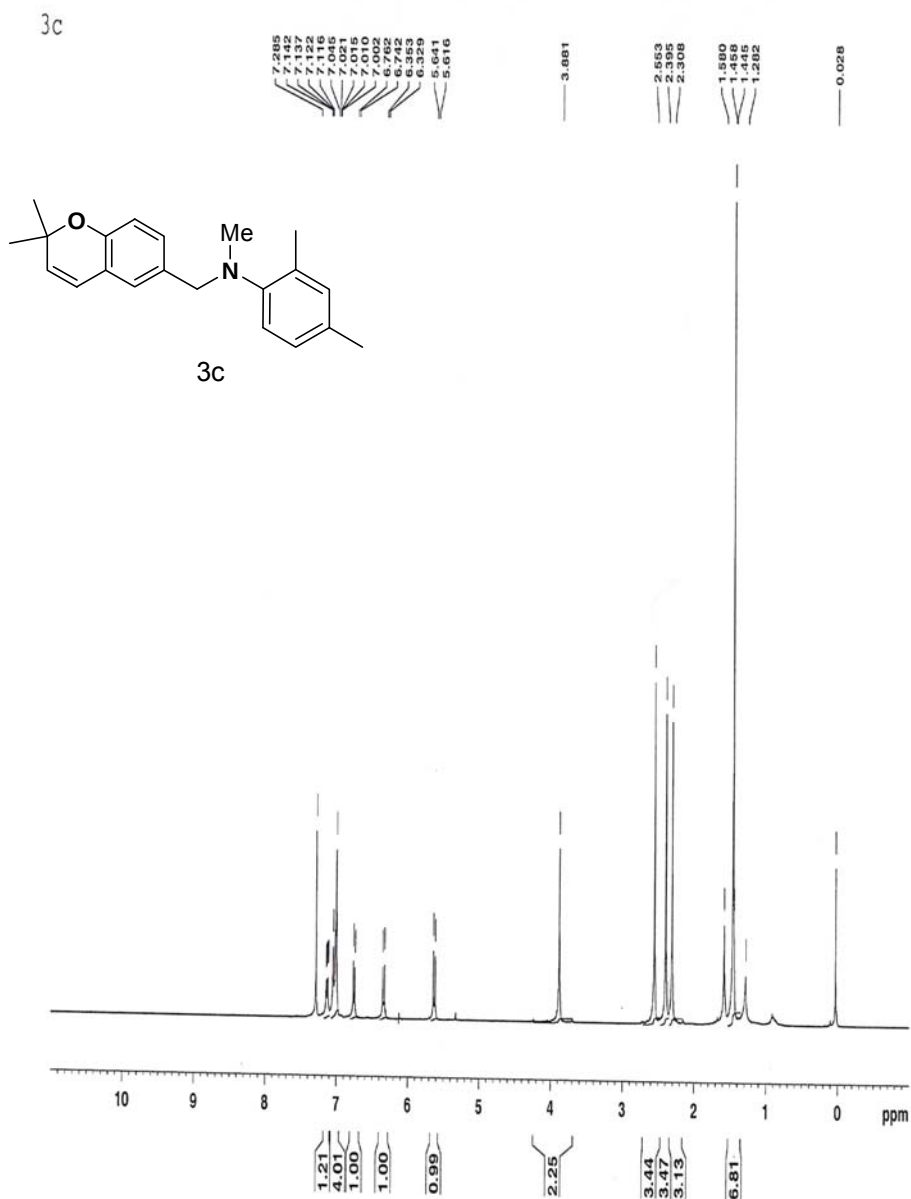
F2 - Acquisition Parameters
Date_ 20070317
Time 15.15
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 121
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 32768
DW 20.850 usec
DE 7.00 usec
TE 297.7 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
MCREST 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

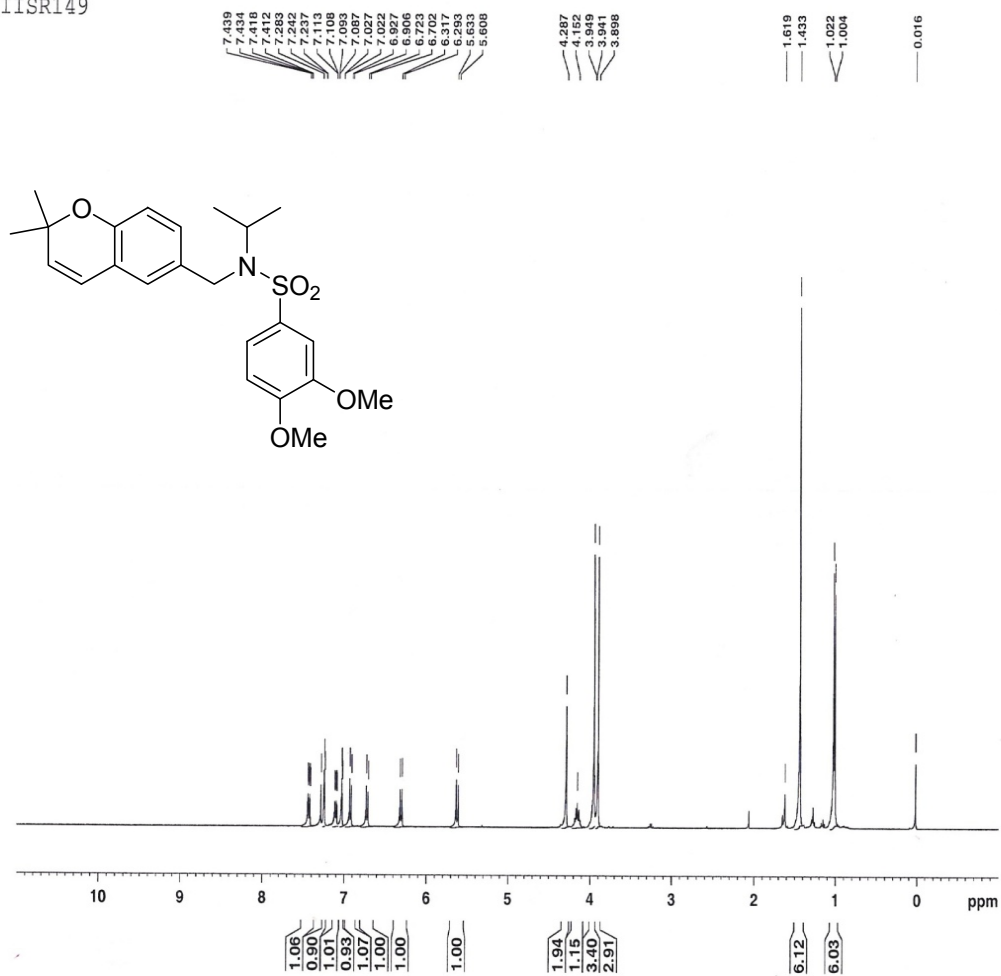
F2 - Processing parameters
SI 32768
SF 100.6127690 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



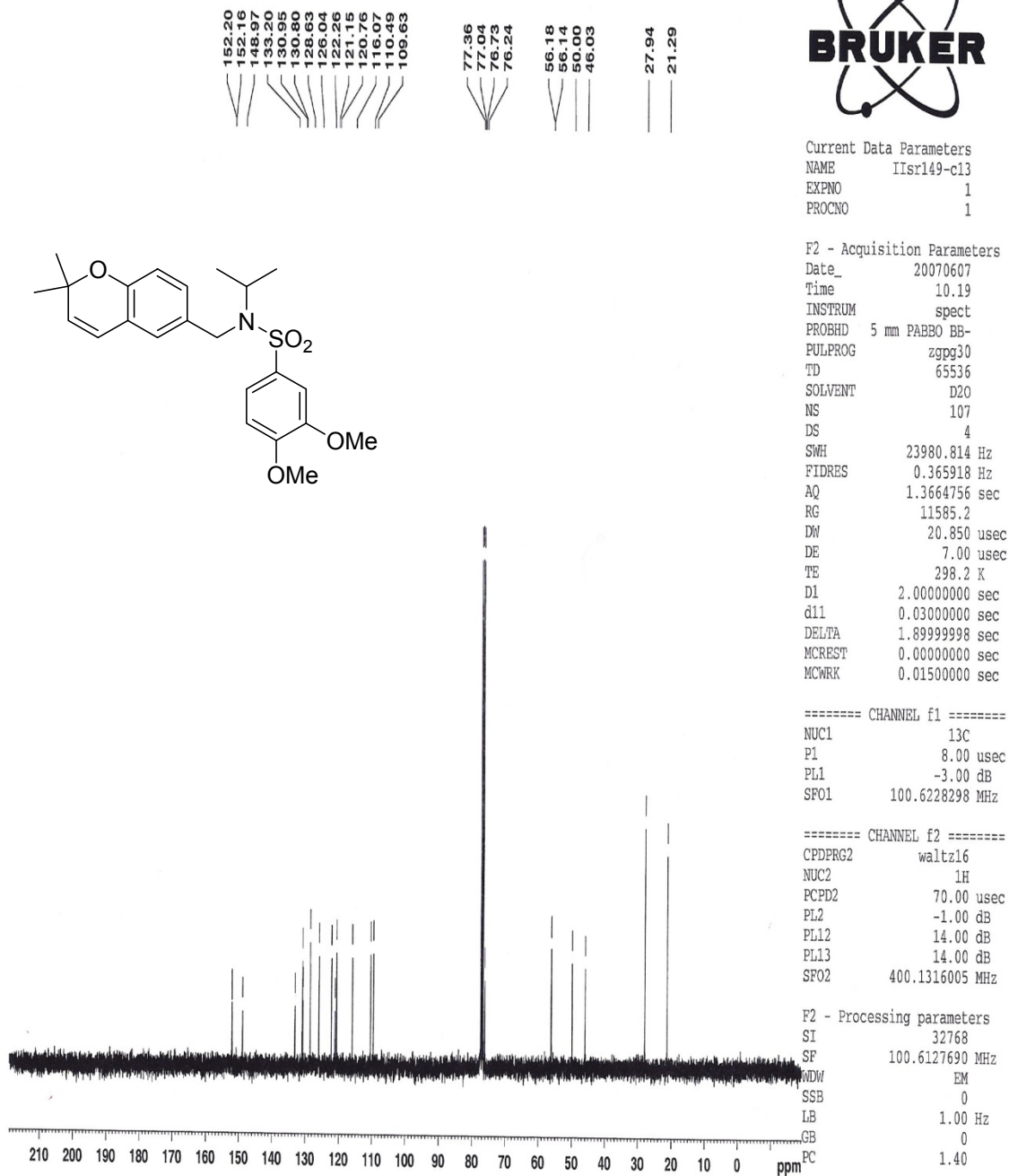


5a

IISR149



No title



5b

No title

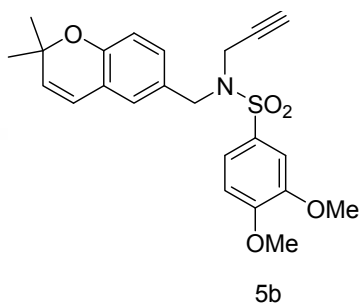
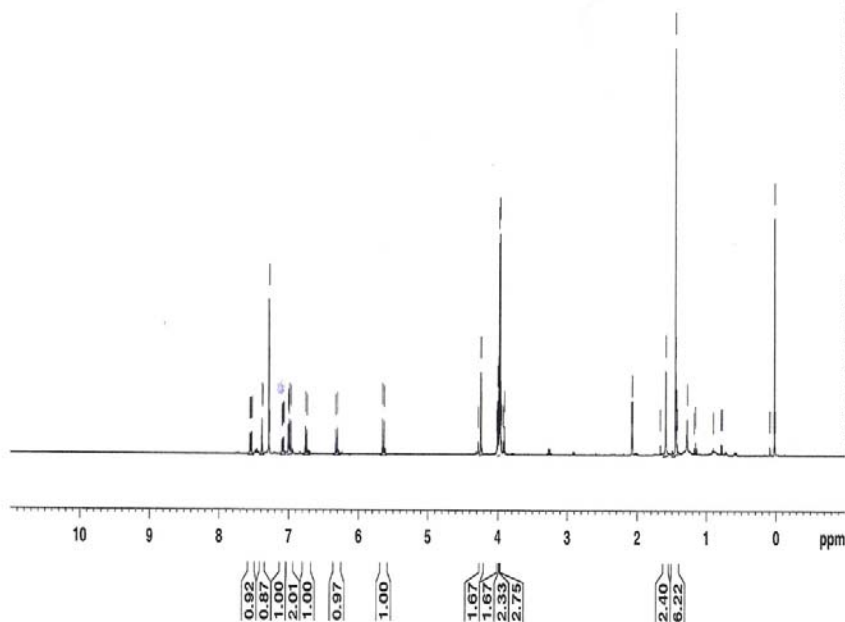


Current Data Parameters
 NAME IIsr143p
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20070612
 Time 14.39
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 16
 DS 2
 SMH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 362
 DW 60.400 usec
 DE 7.00 usec
 TE 298.2 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRR 0.01500000 sec

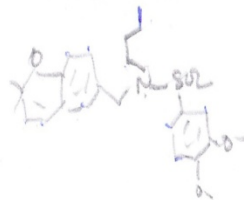
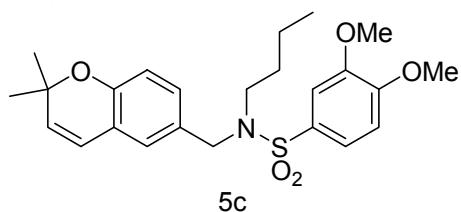
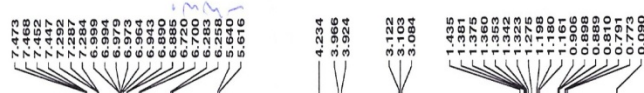
===== CHANNEL f1 =====
 NUC1 1H
 P1 12.80 usec
 PL1 0.00 dB
 SFO1 400.1324710 MHz

F2 - Processing parameters
 SI 32768
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



No title

5c

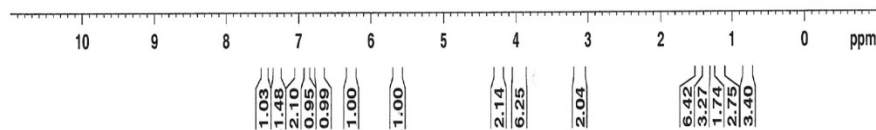


Current Data Parameters
 NAME IIsr138
 EXPNO 1
 PROCNO 1

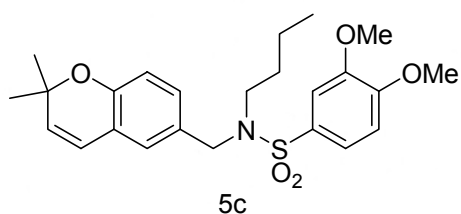
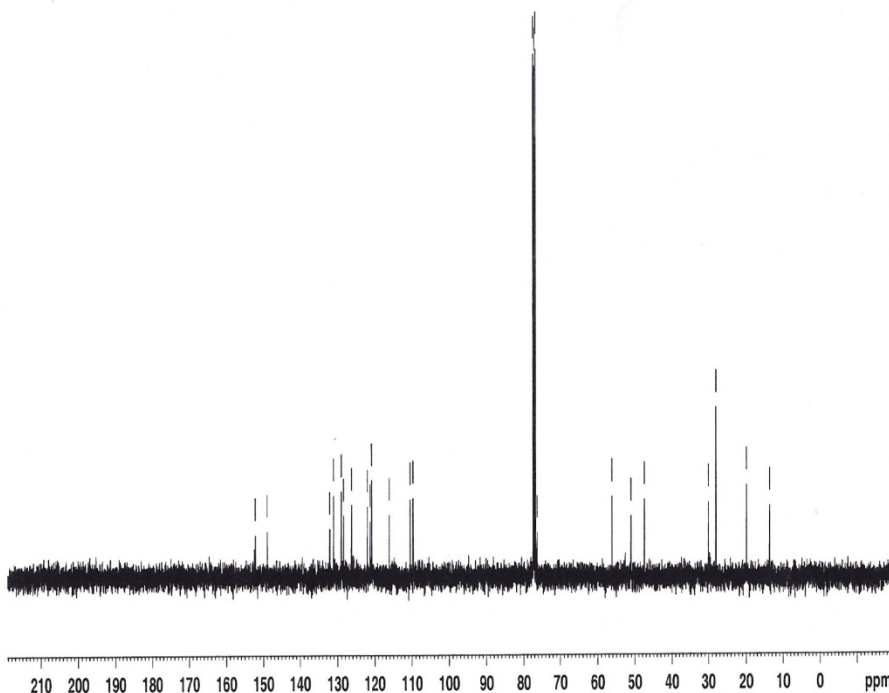
F2 - Acquisition Parameters
 Date_ 20070516
 Time 13.01
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT D2O
 NS 16
 DS 2
 SWH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 128
 DW 60.400 usec
 DE 7.00 usec
 TE 298.2 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRK 0.01500000 sec

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

F2 - Processing parameters
 SI 32768
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



No title



Current Data Parameters
 NAME IIsr138-cl3
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20070516
 Time 13.10
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 55
 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

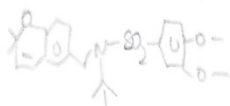
===== CHANNEL f1 =====
 NUC1 13C
 P1 8.00 usec
 PL1 -3.00 dB
 SFO1 100.6228298 MHz

===== 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 100.6127690 MHz
 ADW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40

No title

5d

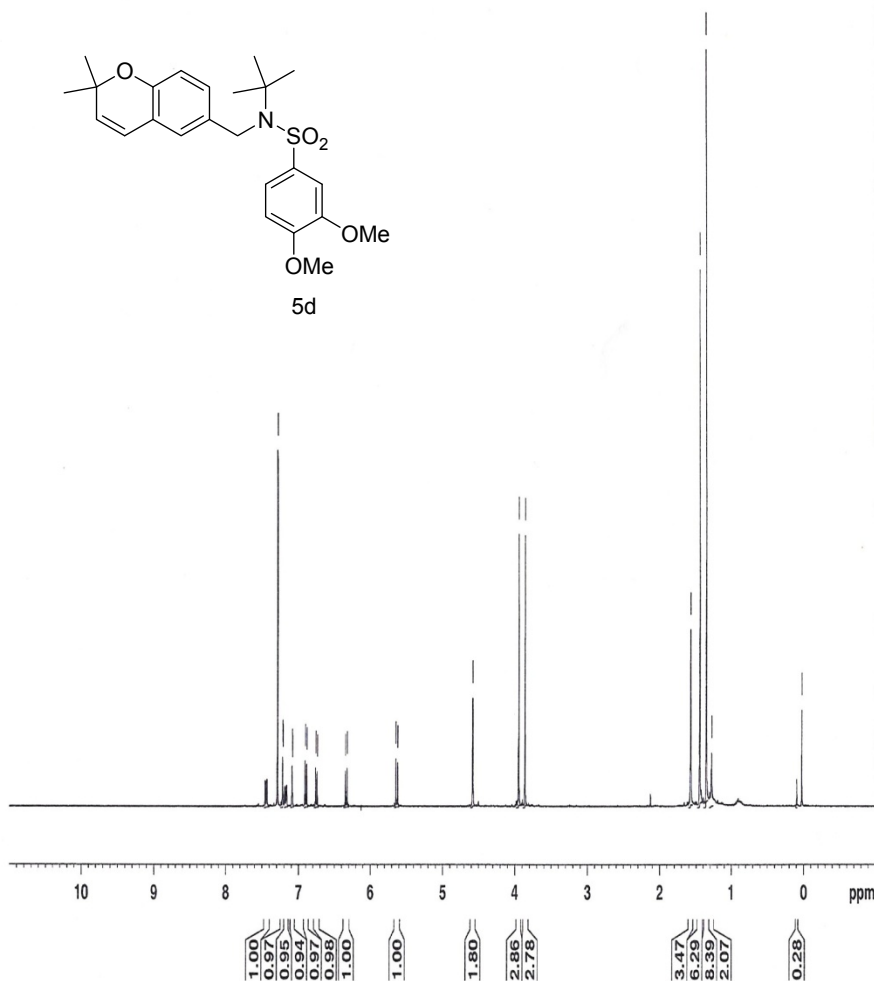


Current Data Parameters
 NAME IIsr137
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20070529
 Time 9.54
 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 3.9584243 sec
 RG 406.4
 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 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



No title

Be



```
Current Data Parameters
NAME          IIsr133pure
EXPNO          1
PROCNO         1
```

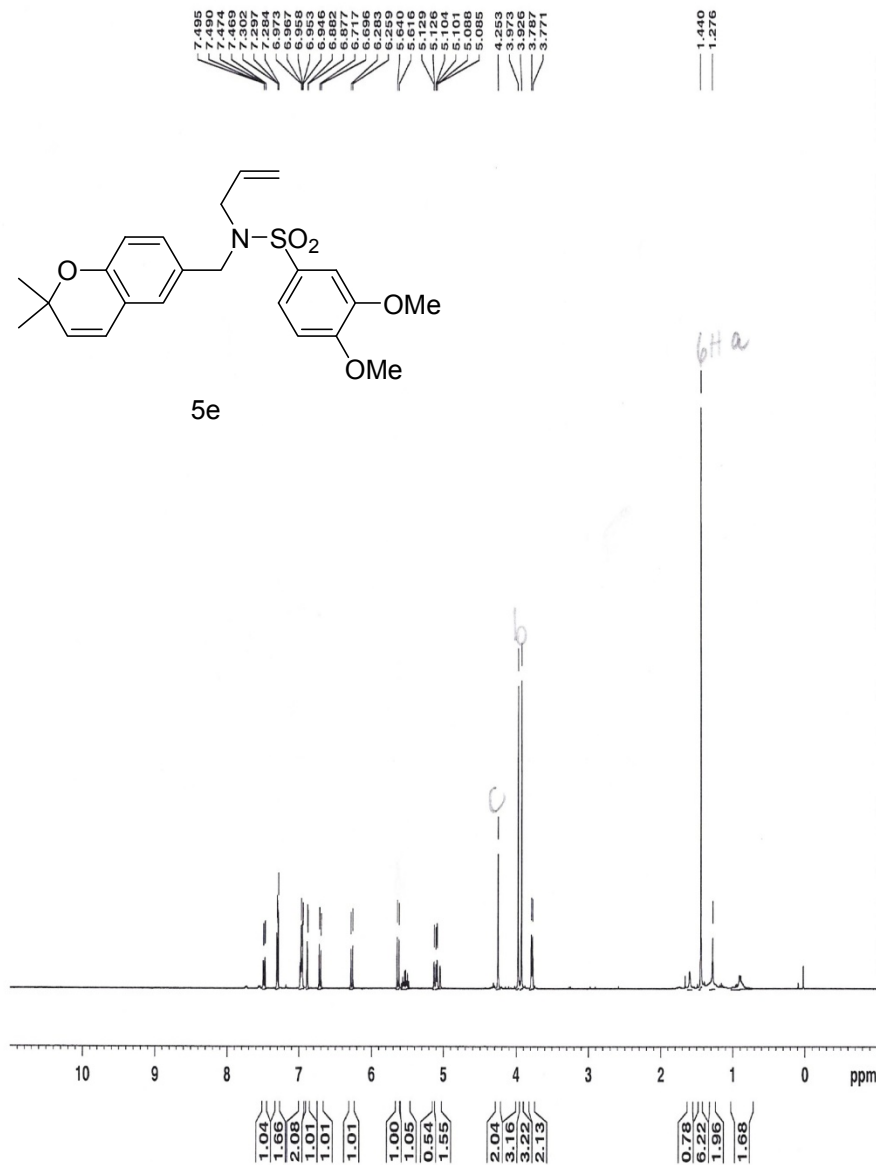
```

F2 - Acquisition Parameters
Date_          20070522
Time           14.13
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            3.9584243 sec
RG             181
DW            60.400 usec
DE             7.00 usec
TE            298.2 K
D1            1.00000000 sec
MCREST         0.00000000 sec
MCWRK         0.01500000 sec

```

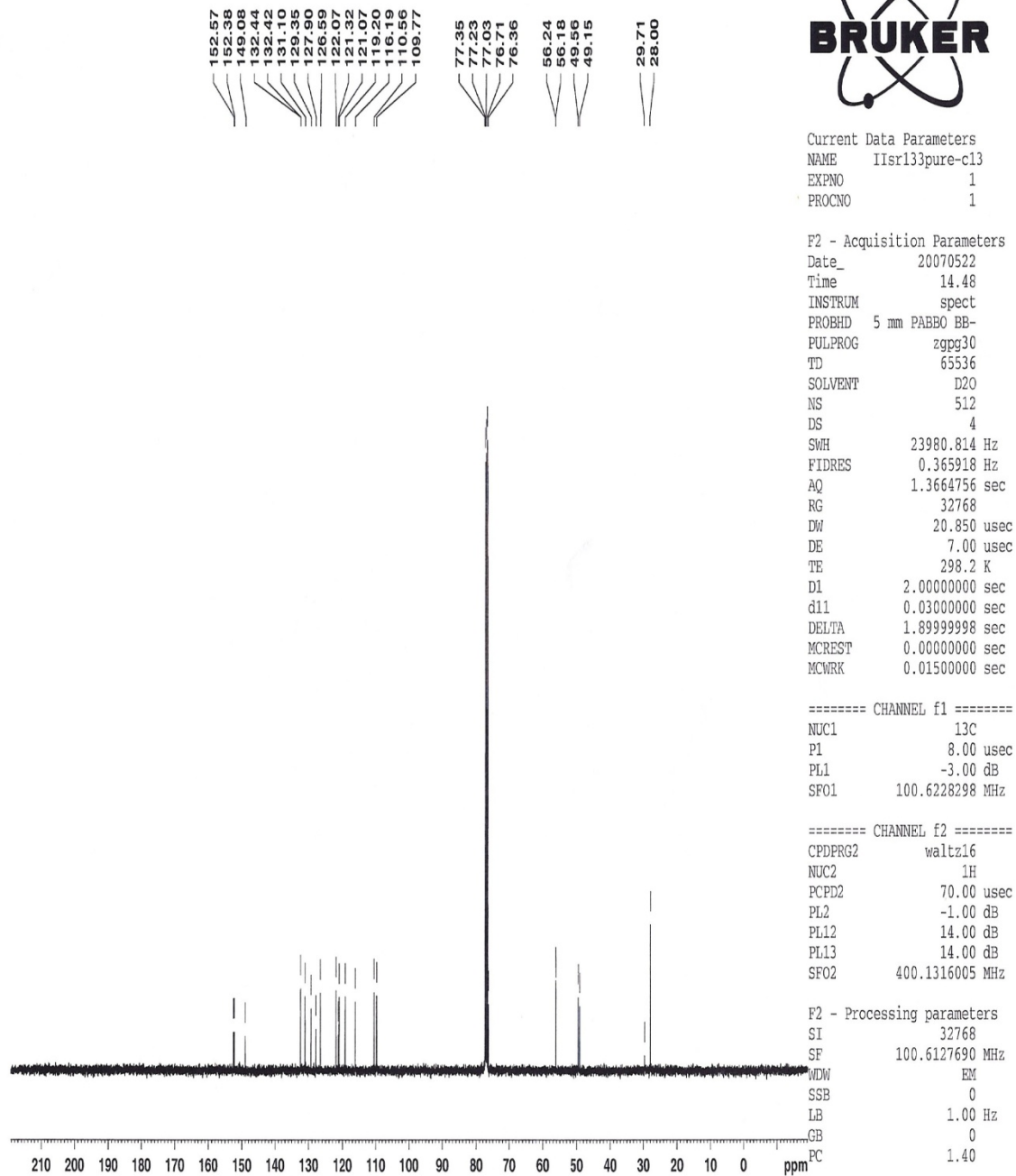
```
===== CHANNEL f1 =====
NUC1          1H
P1            12.80 usec
PL1           0.00 dB
SF01         400.1324710 MHz
```

```
F2 - Processing parameters
SI              32768
SF             400.1300000 MHz
WDW             EM
SSB             0
LB             0.30 Hz
GB             0
PC             1.00
```



5e

No title



No title

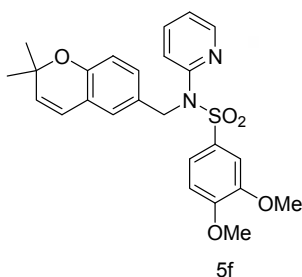
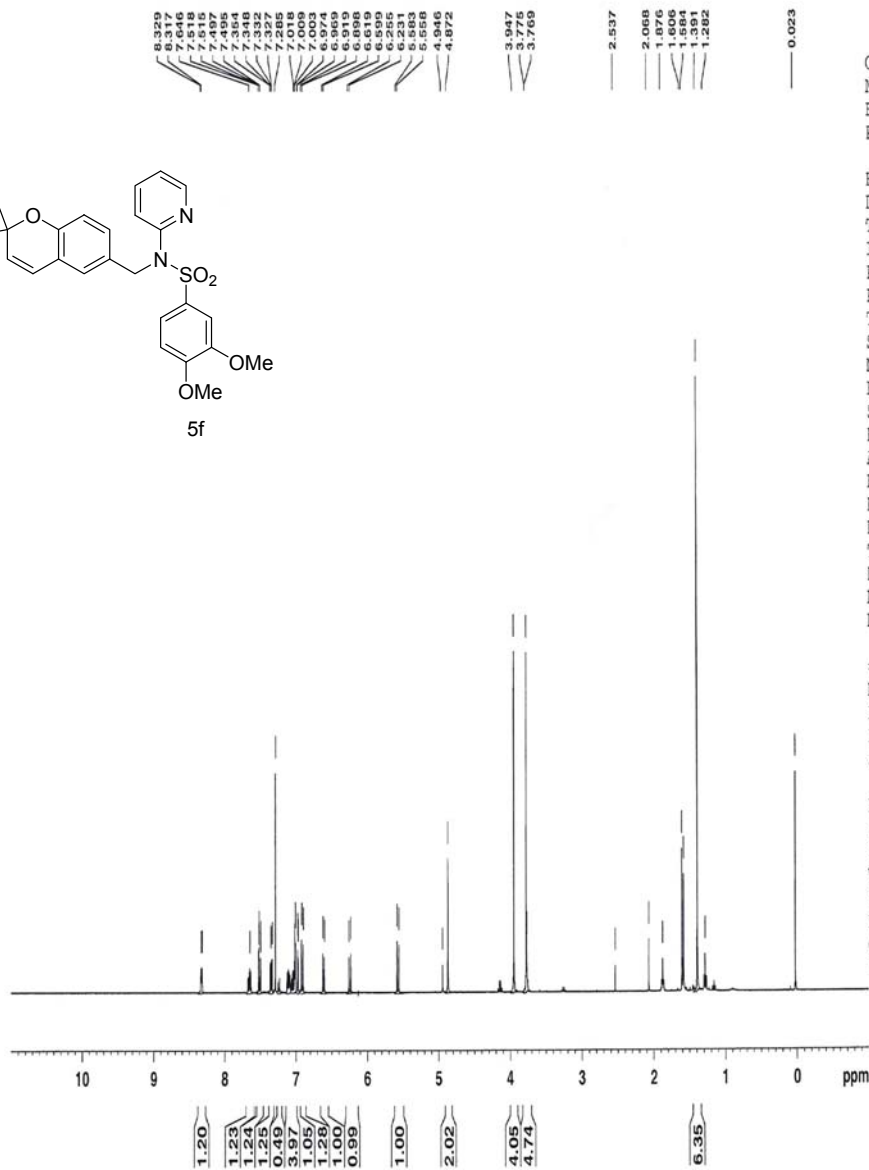


Current Data Parameters
 NAME IISR94p
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20100222
 Time 14.09
 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 3.9584243 sec
 RG 362
 DW 60.400 usec
 DE 7.00 usec
 TE 299.3 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRK 0.01500000 sec

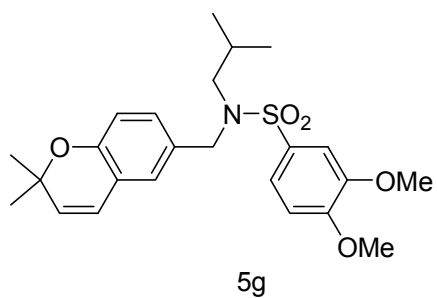
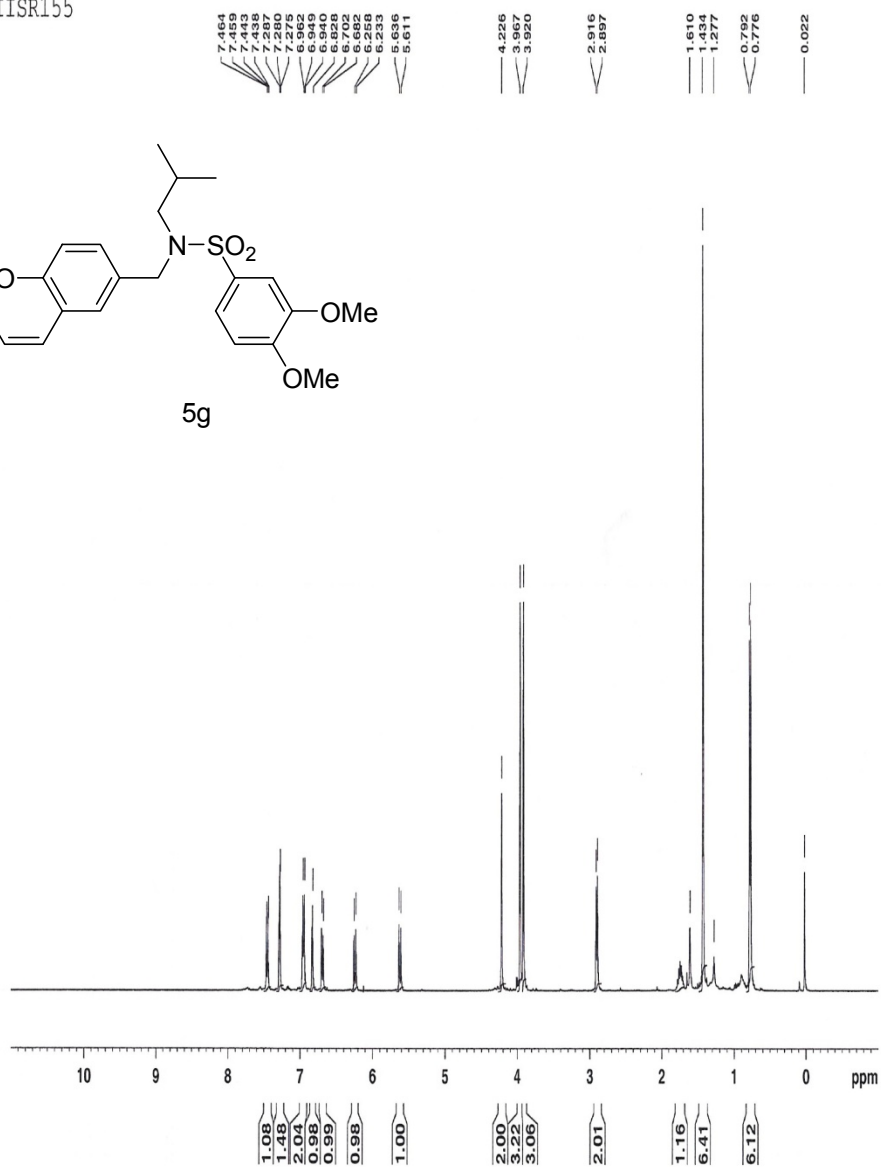
===== CHANNEL f1 =====
 NUC1 1H
 P1 12.80 usec
 PL1 0.00 dB
 SFO1 400.1324710 MHz

F2 - Processing parameters
 SI 32768
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



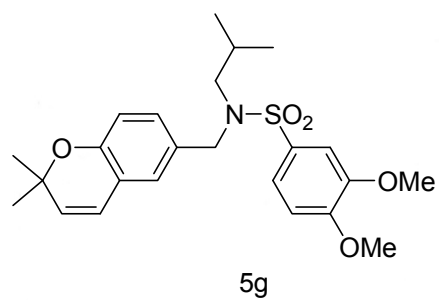
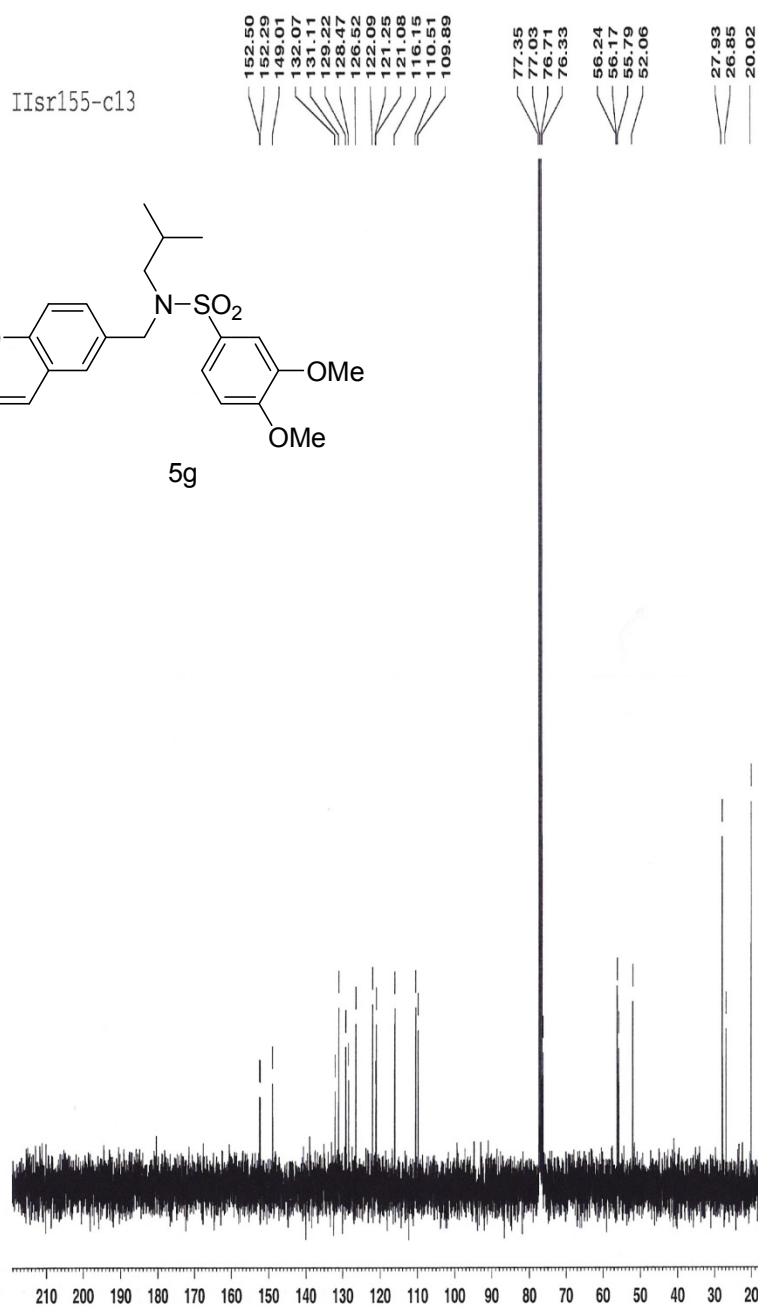
58

IISR155



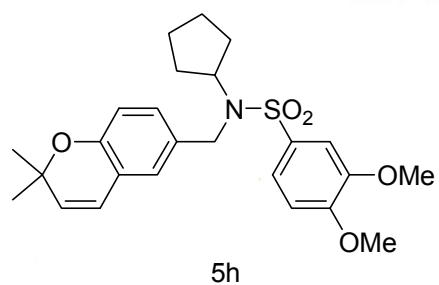
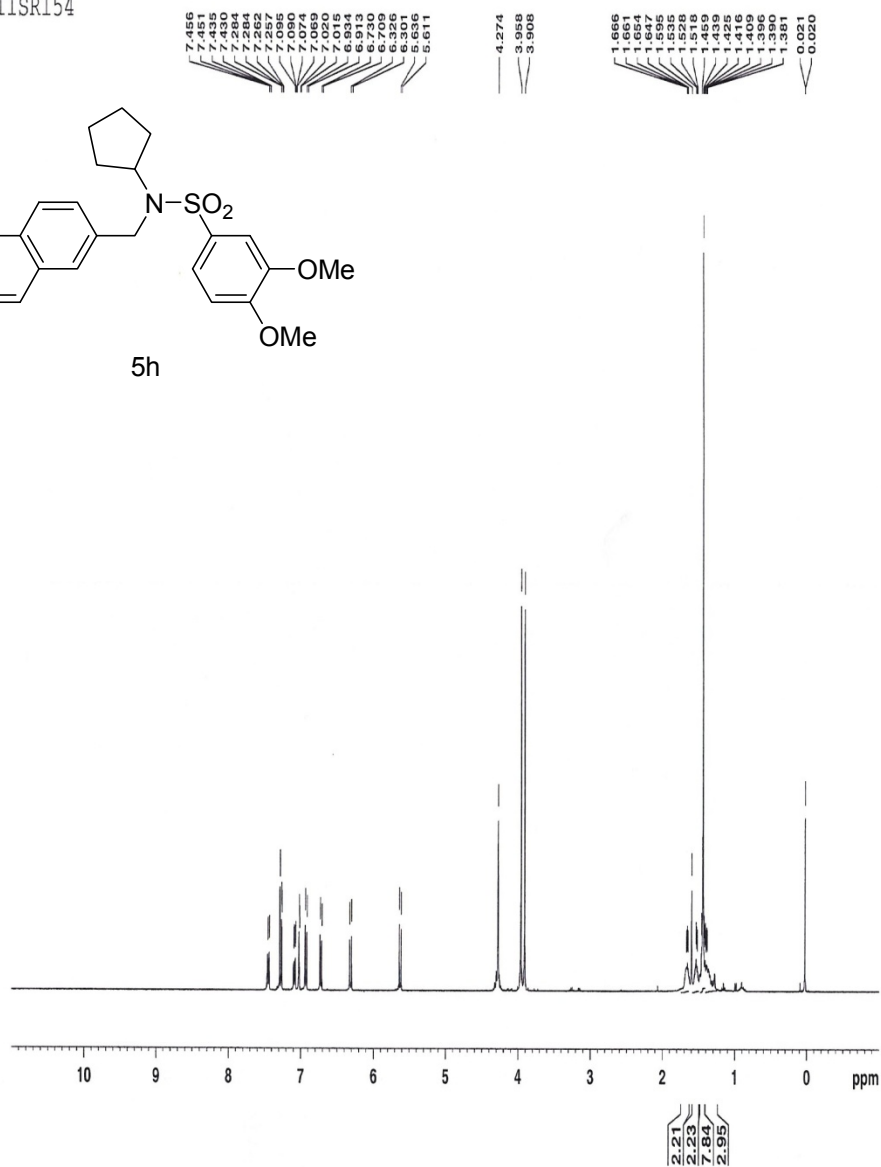
5g

IIIsr155-c13



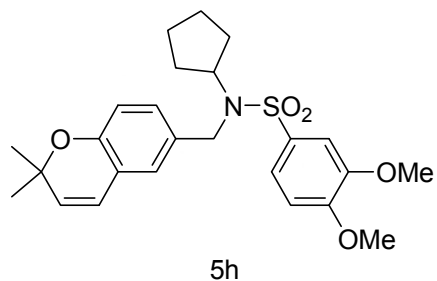
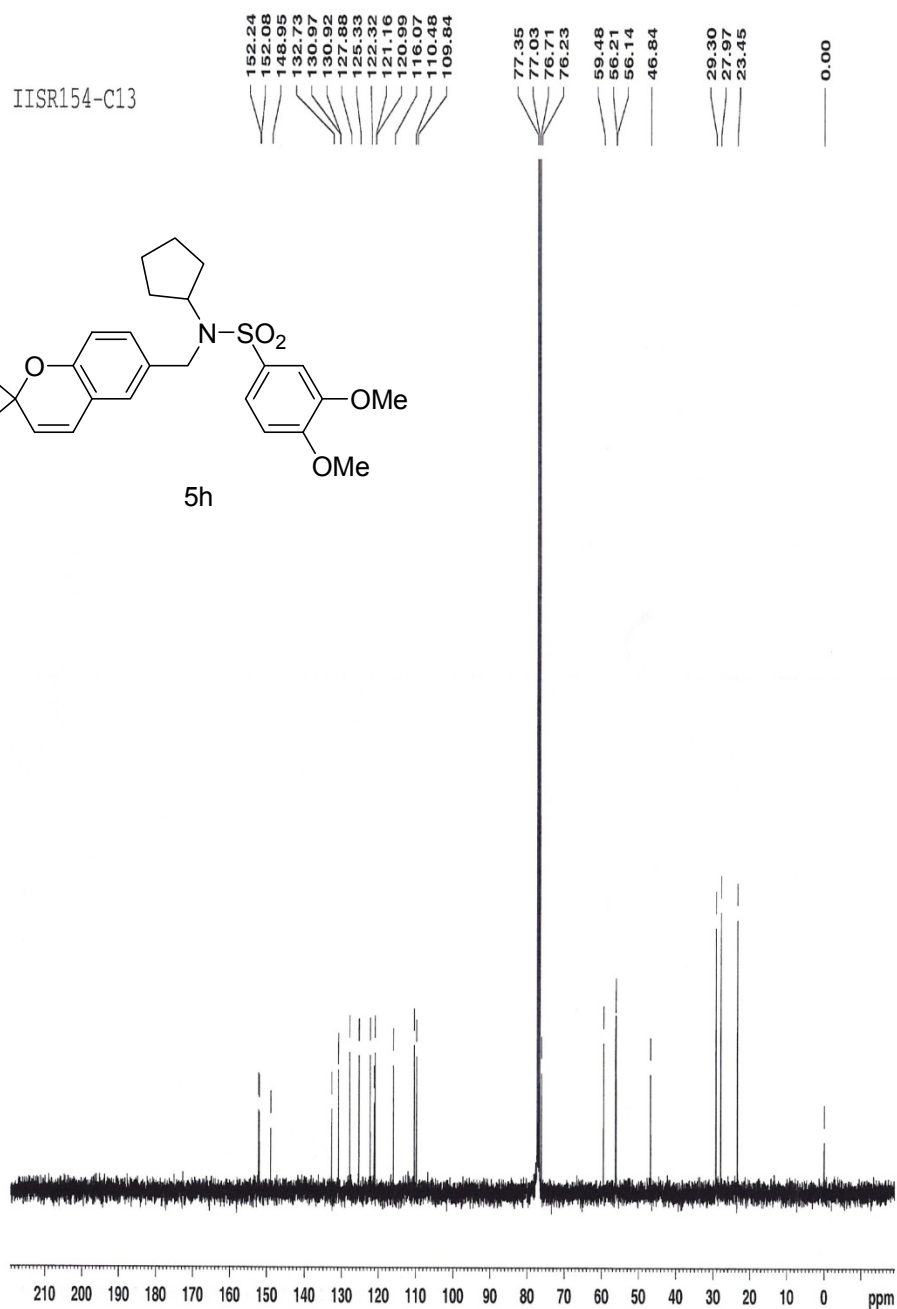
5h

IISR154



5h

IISR154-C13



No title

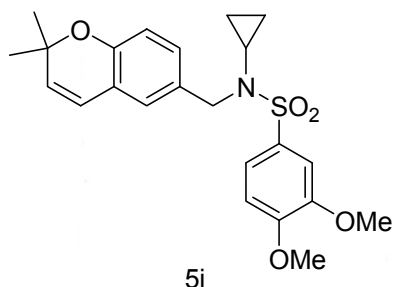
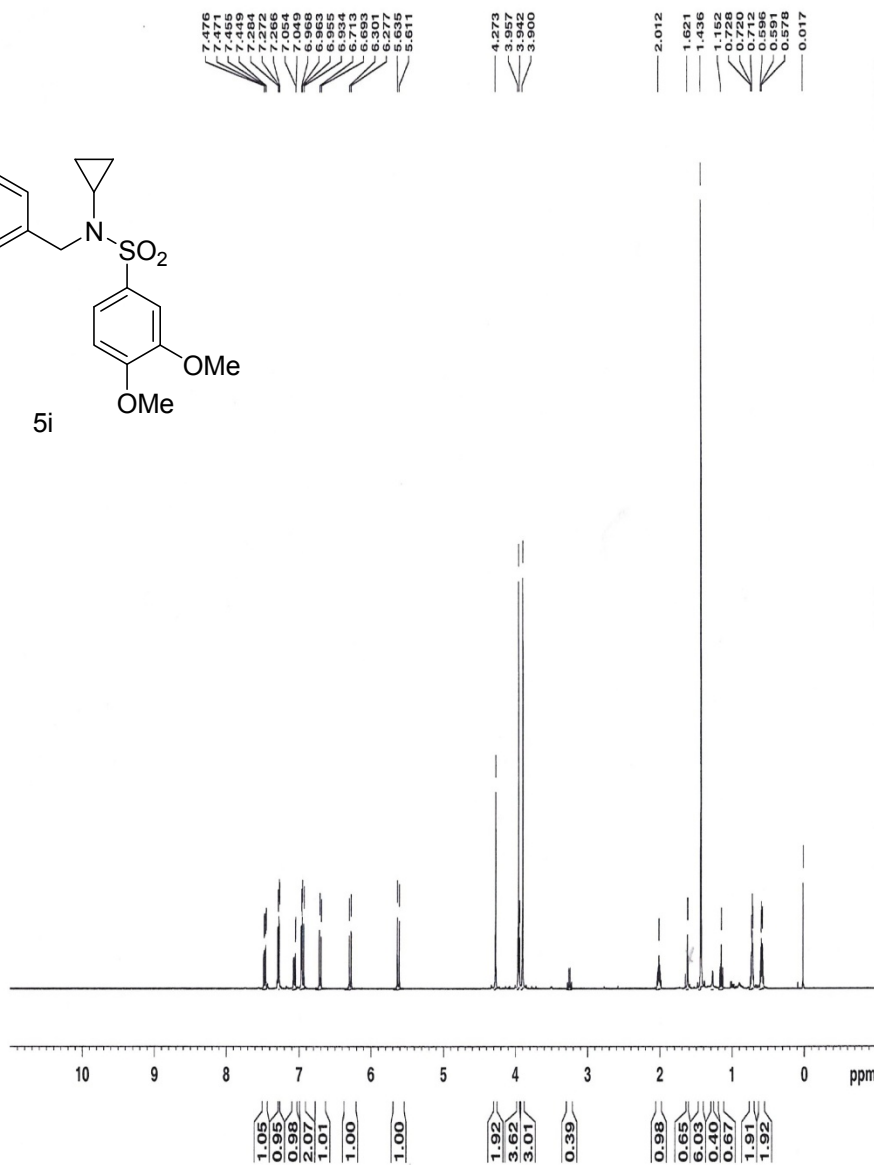


Current Data Parameters
 NAME IIsr151p
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20070607
 Time 10.31
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 11
 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

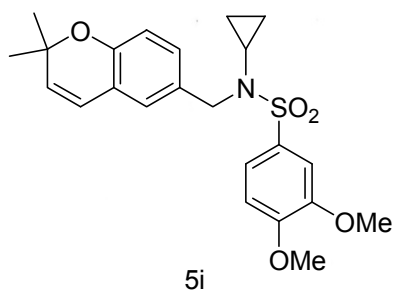
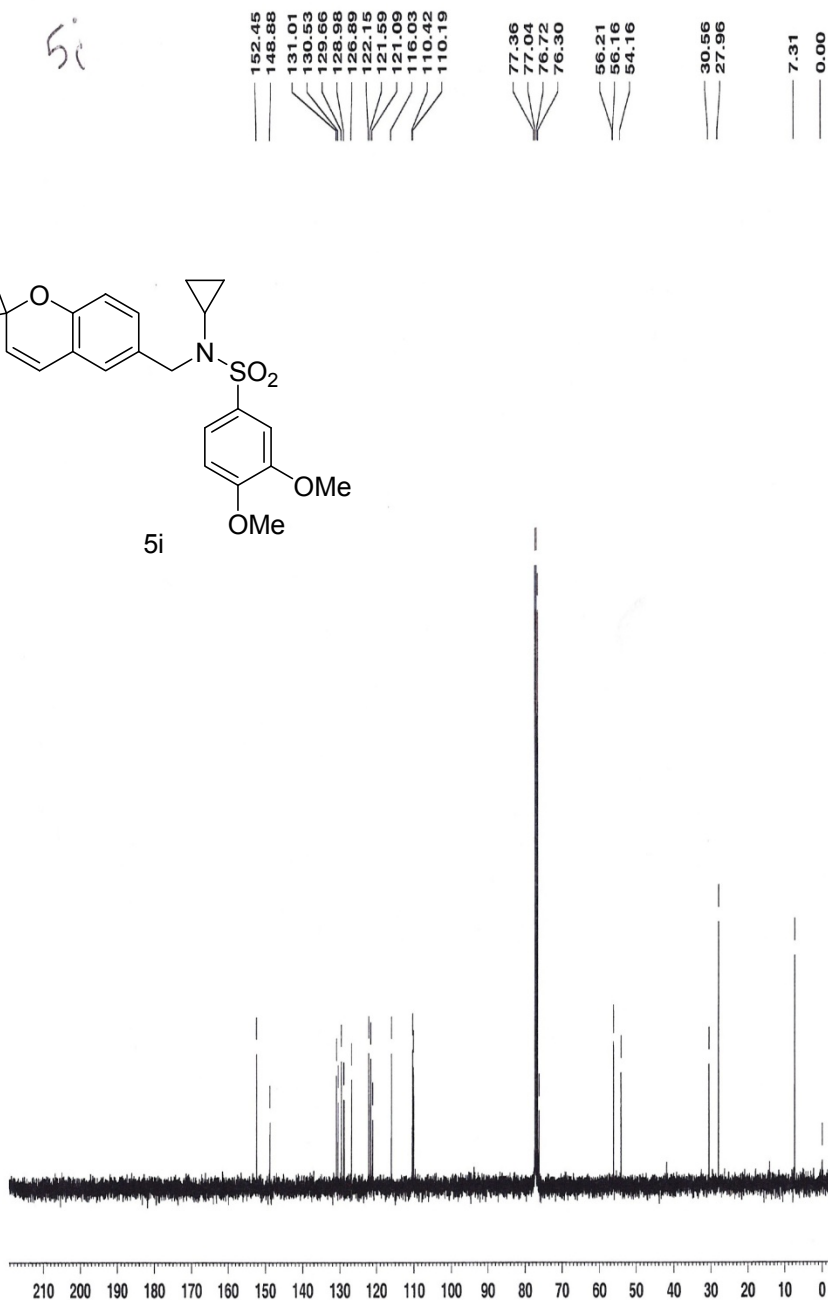
===== CHANNEL f1 =====
 NUC1 1H
 P1 12.80 usec
 PL1 0.00 dB
 SFO1 400.1324710 MHz

F2 - Processing parameters
 SI 32768
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



No title

5i



Current Data Parameters
 NAME IIsr151p-cl3
 EXPNO 1
 PROCNO 1

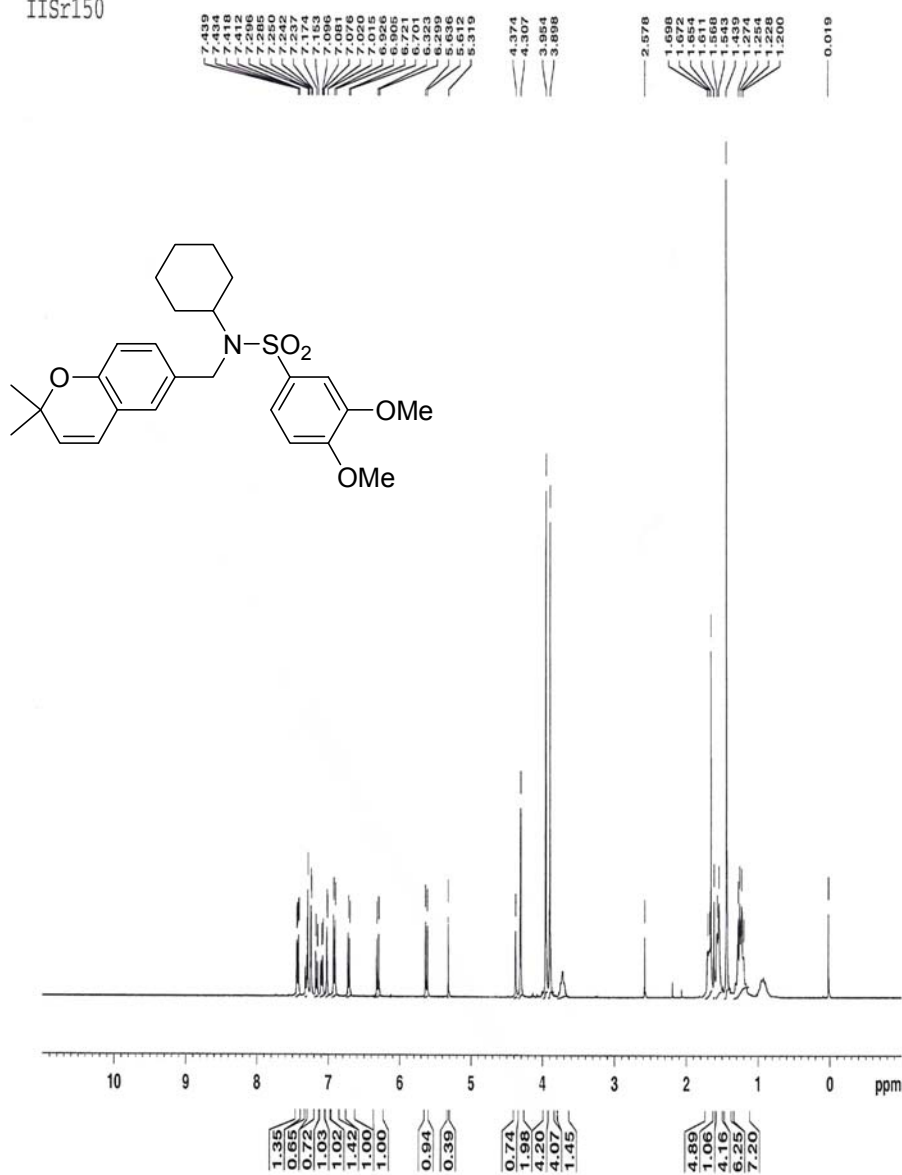
F2 - Acquisition Parameters
 Date_ 20070607
 Time 10.38
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 87
 DS 4
 SWH 23980.814 Hz
 FIDRES 0.365918 Hz
 AQ 1.3664756 sec
 RG 8192
 DW 20.850 usec
 DE 7.00 usec
 TE 298.2 K
 D1 2.0000000 sec
 d11 0.0300000 sec
 DELTA 1.89999998 sec
 MCREST 0.0000000 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

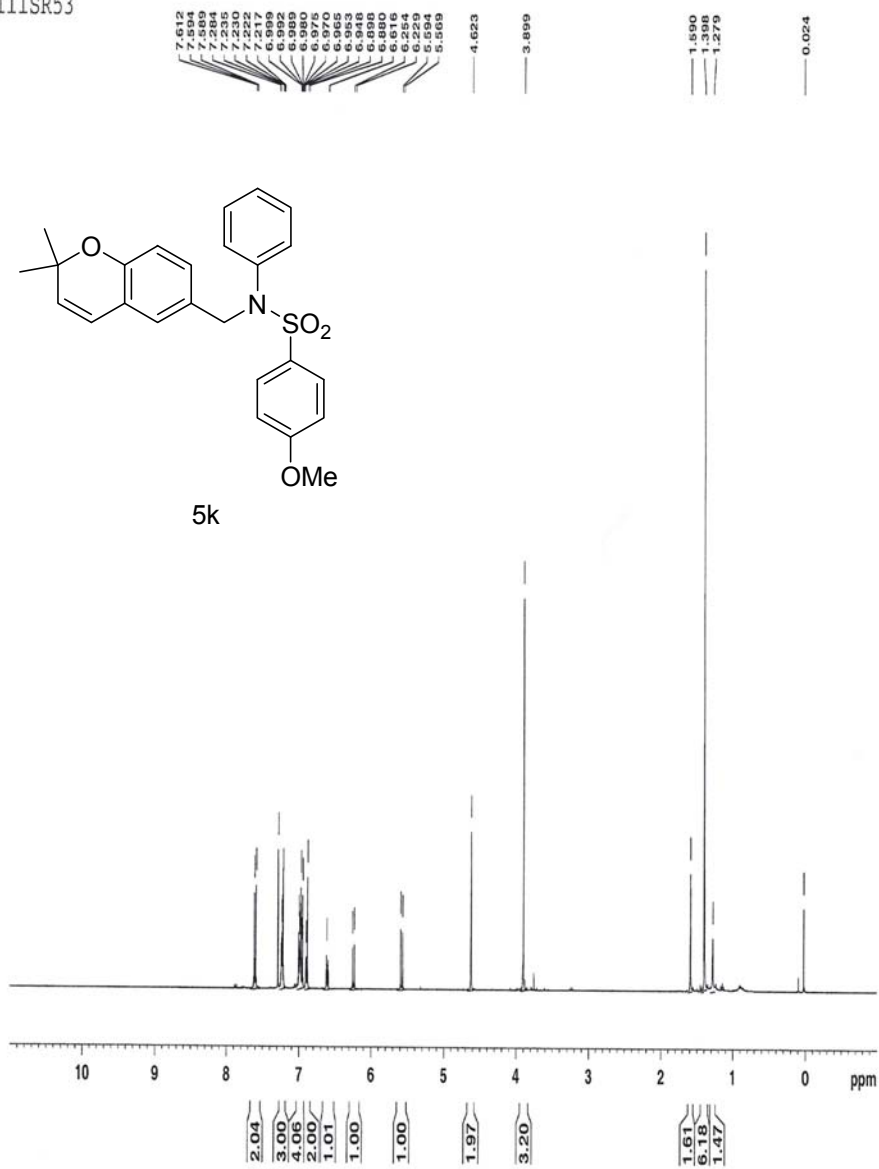
F2 - Processing parameters
 SI 32768
 SF 100.6127690 MHz
 ADW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40

IISr150

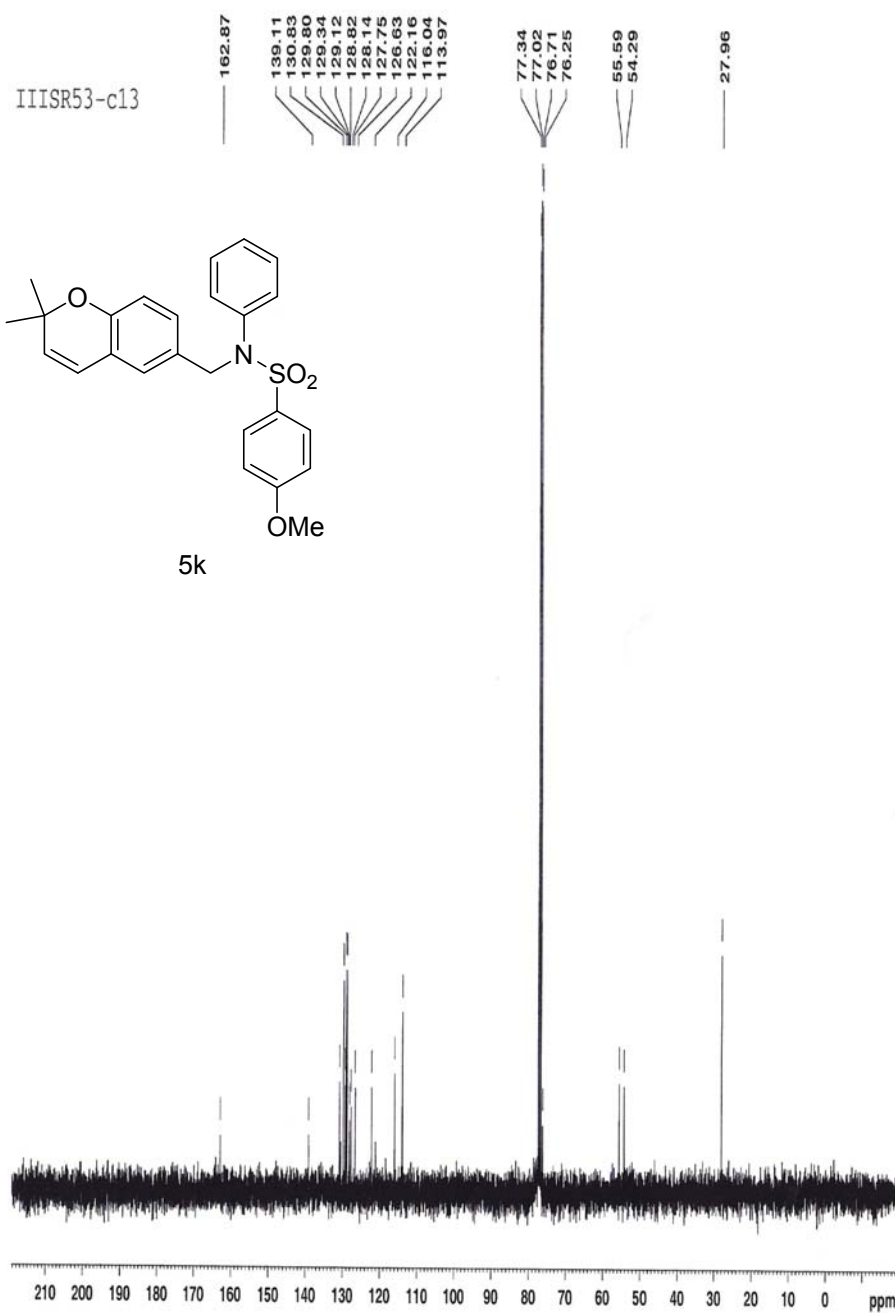


5k

IIISR53

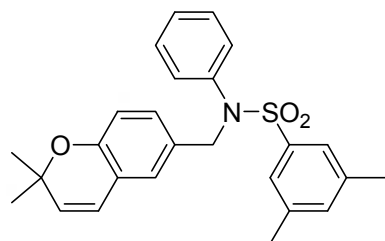


5k

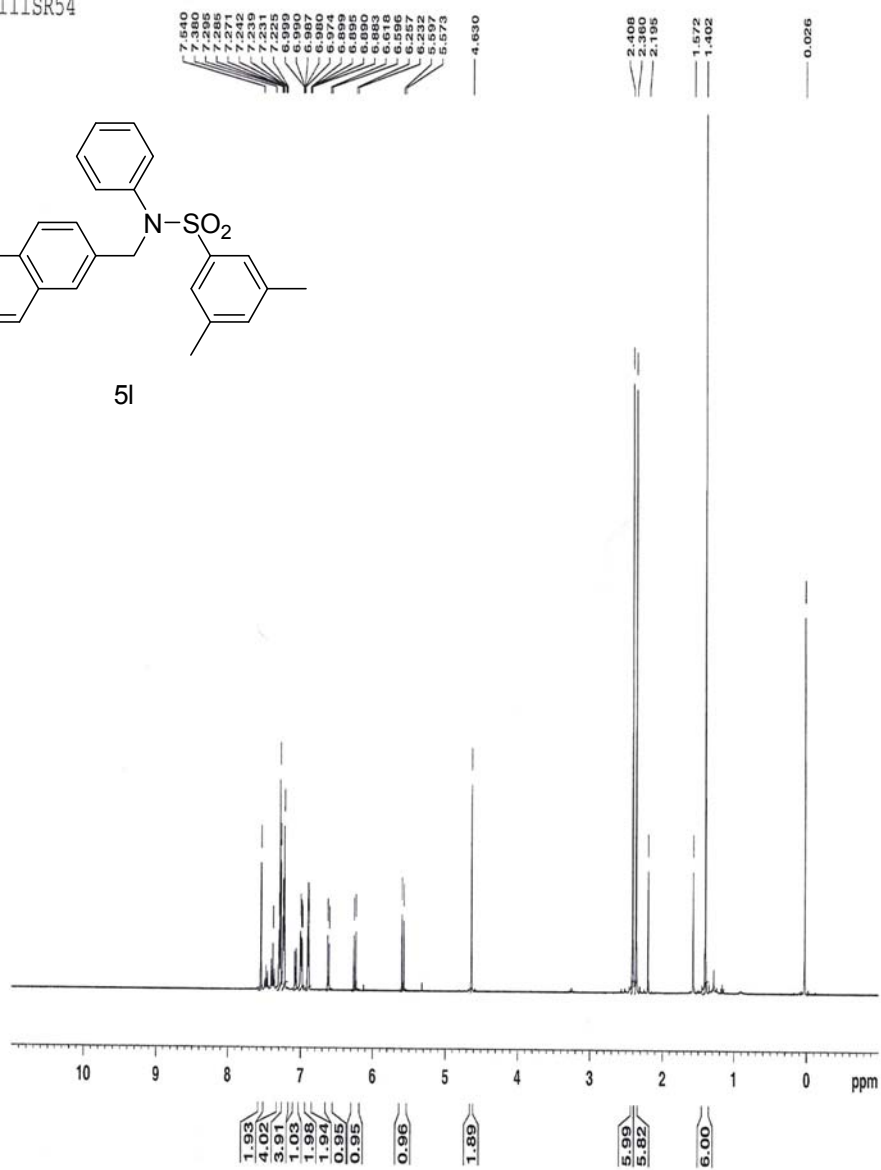


5l

IIISR54



5l

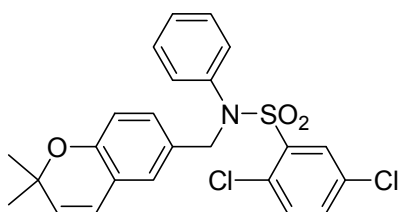


IIISR69b

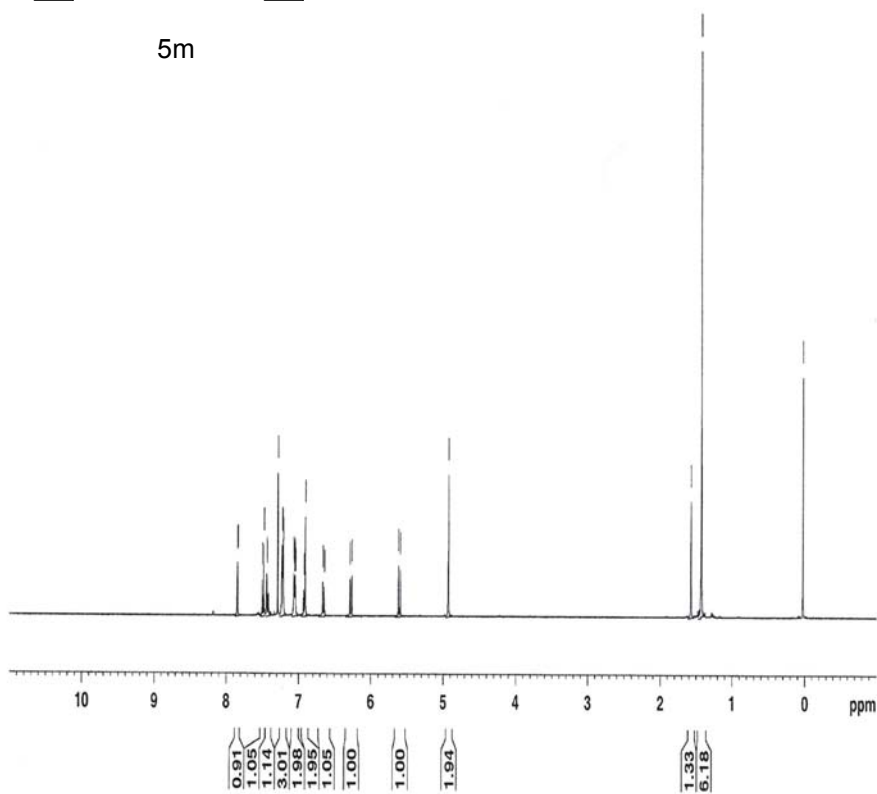
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7.835
7.830
7.474
7.436
7.386
7.285
7.226
7.214
7.209
7.062
7.047
7.050
6.985
6.921
6.902
6.857
6.836
6.263
6.258
5.995
4.925

1.569
1.424

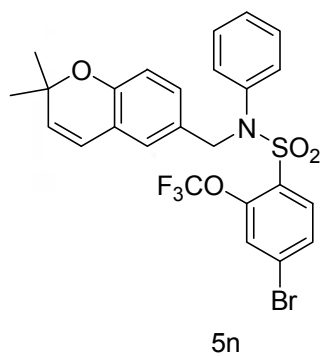
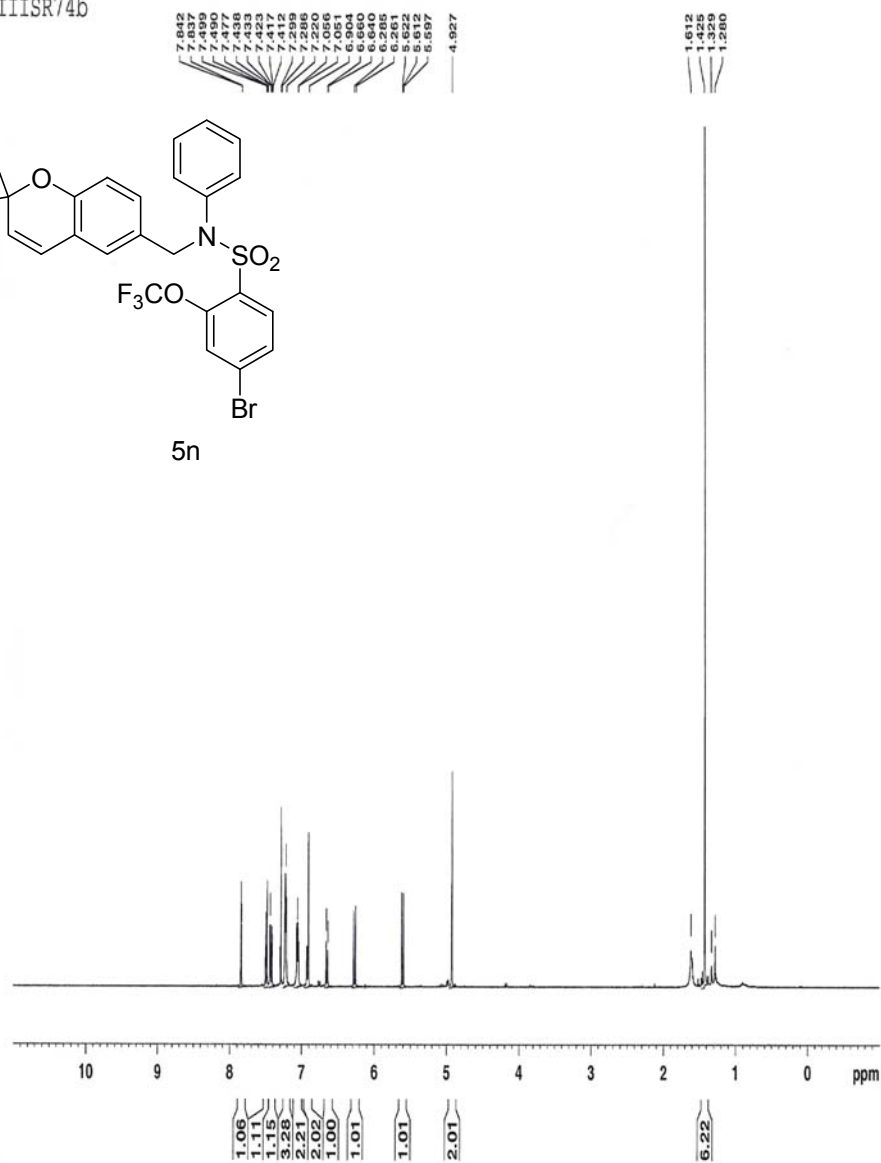
0.025

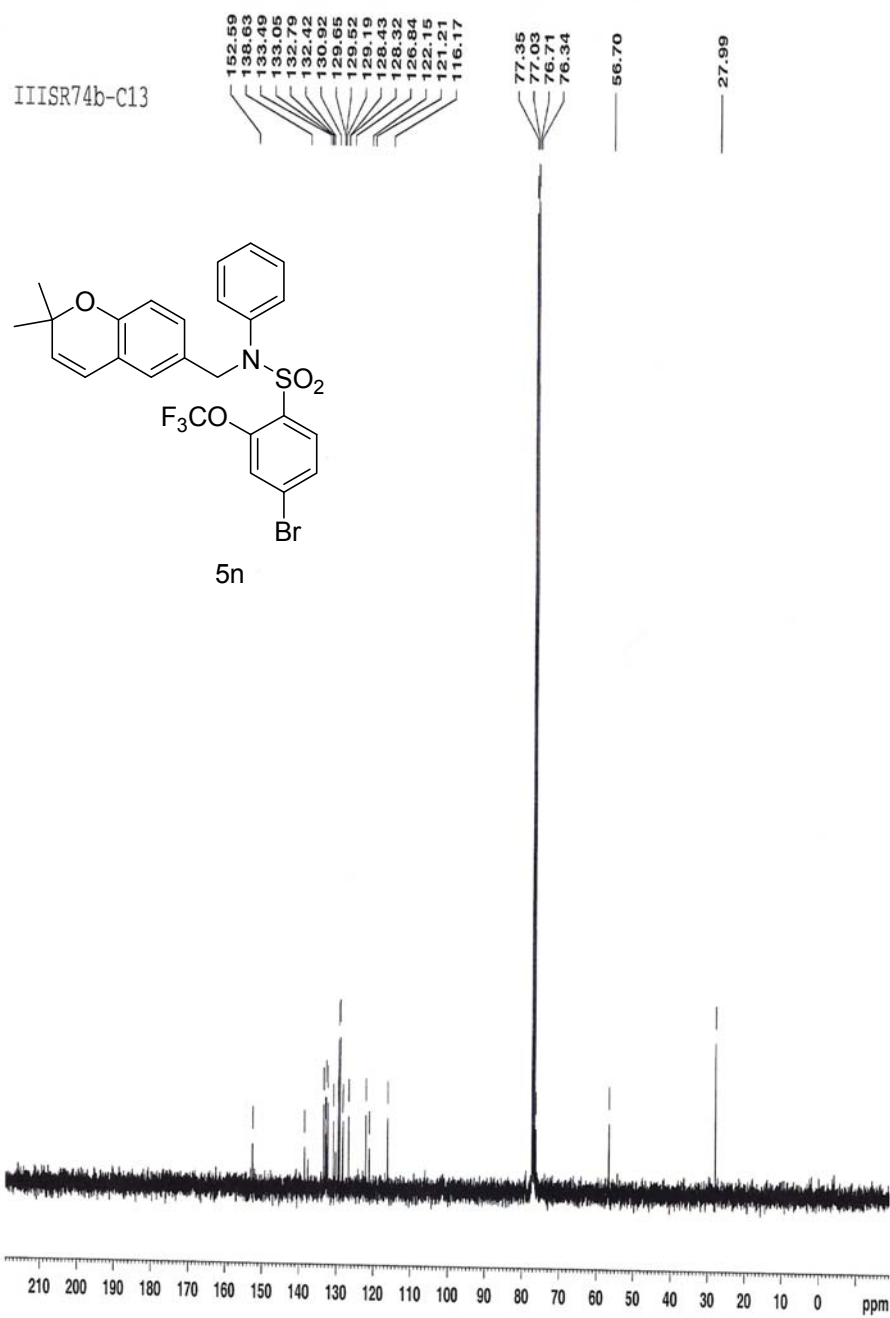


5m



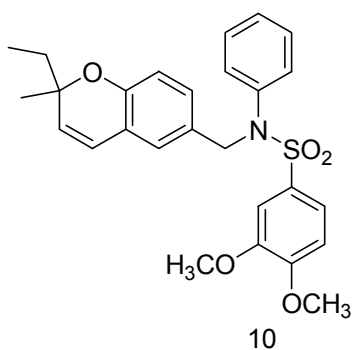
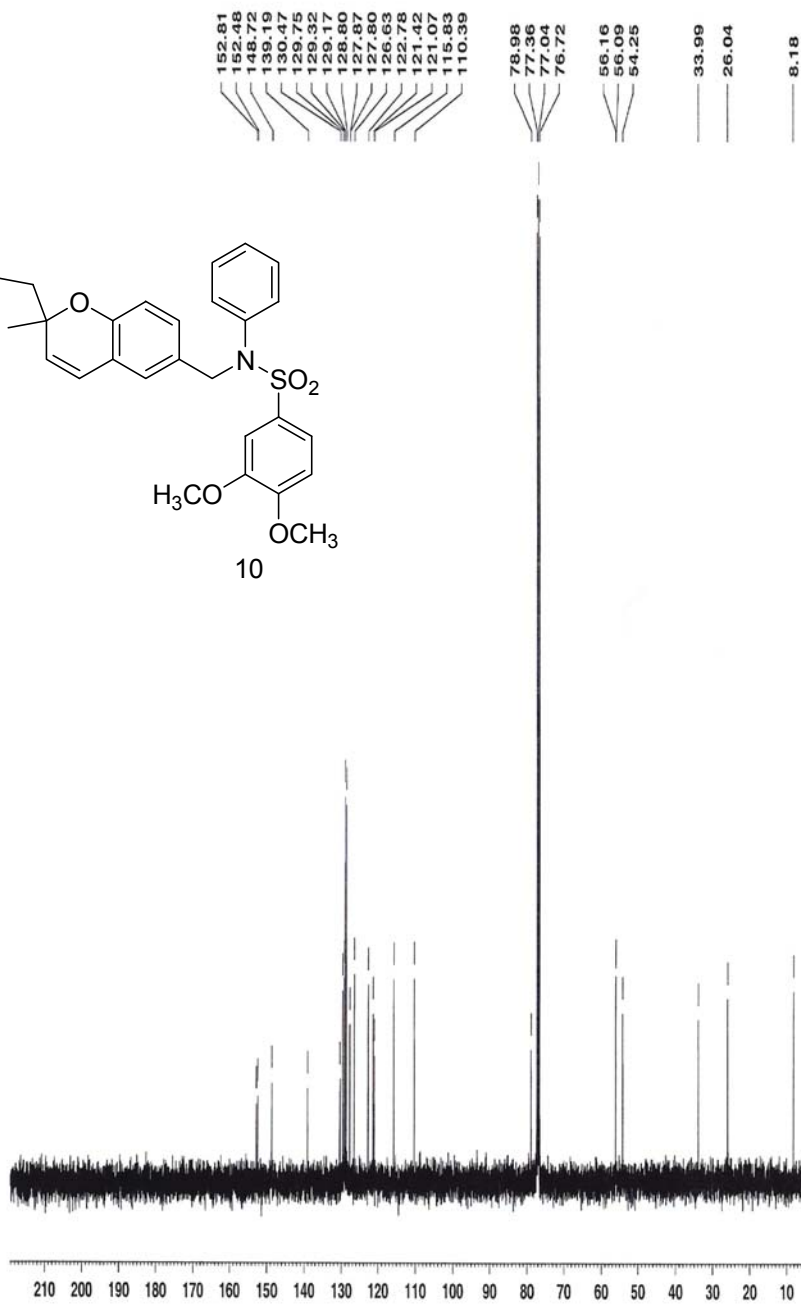
IIISR74b





10

No title



Current Data Parameters
 NAME IISR37b-cl3
 EXPNO 1
 PROCNO 1

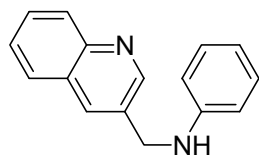
F2 - Acquisition Parameters
 Date_ 20070813
 Time 15.15
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 99
 DS 4
 SWH 23980.814 Hz
 FIDRES 0.365918 Hz
 AQ 1.3664756 sec
 RG 32768
 DW 20.850 usec
 DE 7.00 usec
 TE 299.2 K
 D1 2.0000000 sec
 d11 0.0300000 sec
 DELTA 1.89999998 sec
 MCREST 0.0000000 sec
 MCWRK 0.0150000 sec

===== CHANNEL f1 =====
 NUC1 13C
 P1 8.00 usec
 PL1 -3.00 dB
 SF01 100.6228298 MHz

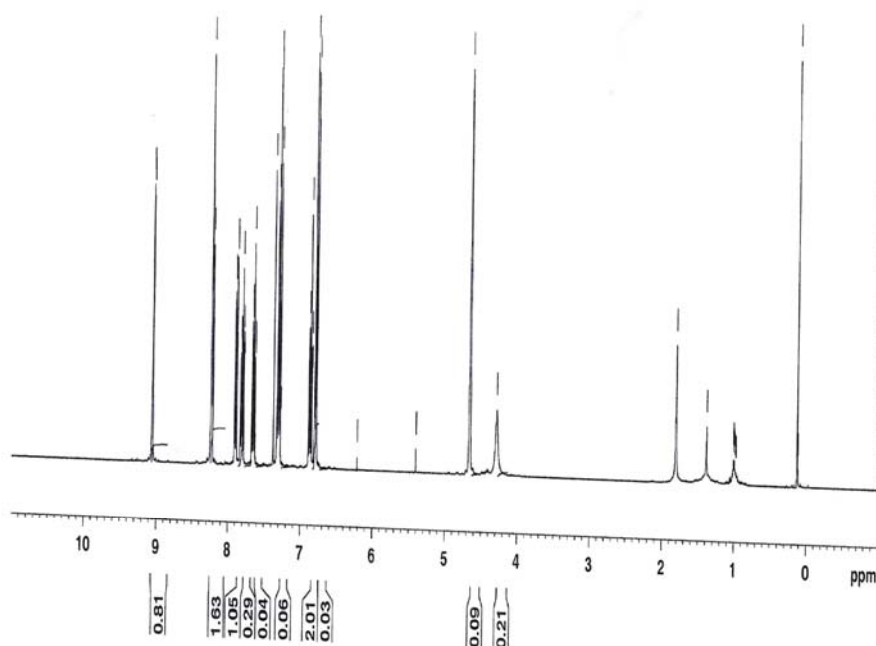
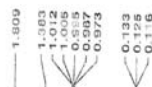
===== CHANNEL f2 =====
 CPDPRG2 waltz16
 NUC2 1H
 PCPD2 70.00 usec
 PL2 -1.00 dB
 PL12 14.00 dB
 PL13 14.00 dB
 SF02 400.1316005 MHz

F2 - Processing parameters
 SI 32768
 SF 100.6127690 MHz
 ROW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40

No title



12



Current Data Parameters
 NAME V-SR10
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20080813
 Time 10.47
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 10
 DS 2
 SWH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 228.1
 DW 60.400 usec
 DE 7.00 usec
 TE 308.2 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRK 0.01500000 sec

===== CHANNEL f1 =====
 NUC1 1H
 P1 12.60 usec
 PL1 0.00 dB
 SFO1 400.1324710 MHz

F2 - Processing parameters
 SI 32768
 SF 400.1299636 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

13

No title

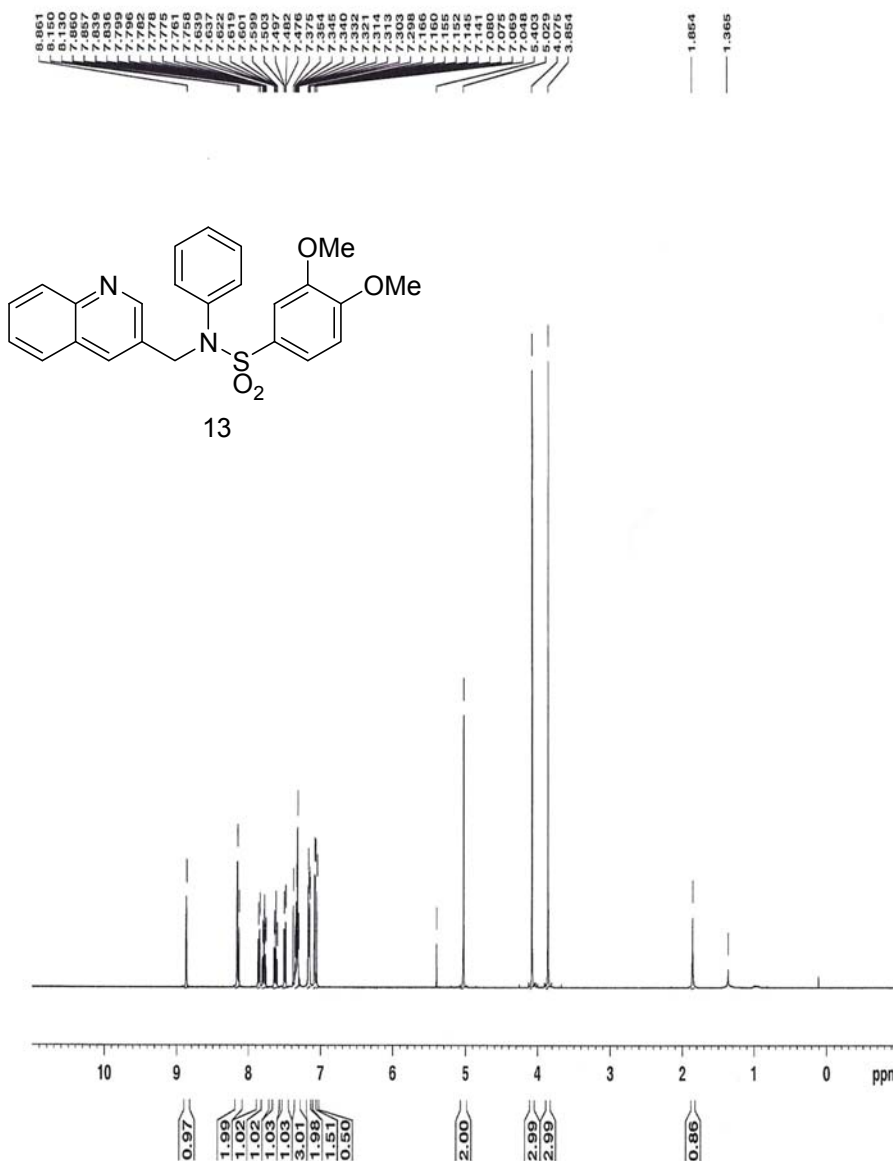


Current Data Parameters
 NAME V-SR16
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20080822
 Time 11.38
 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 228.1
 DW 60.400 usec
 DE 7.00 usec
 TE 297.9 K
 D1 1.0000000 sec
 MCREST 0.0000000 sec
 MCWRK 0.0150000 sec

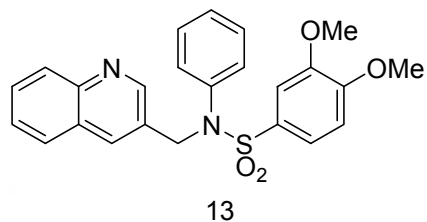
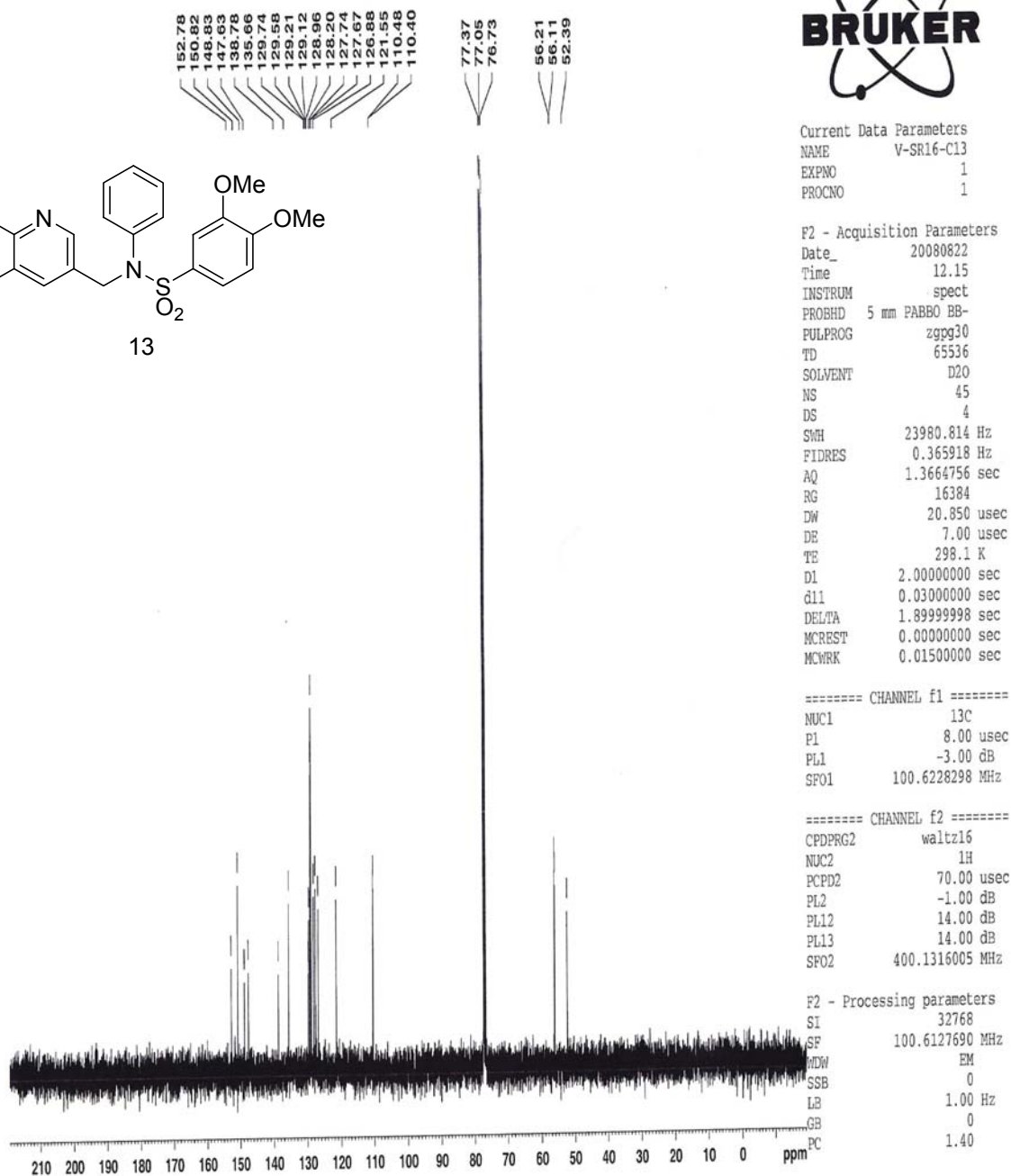
===== CHANNEL f1 =====
 NUC1 1H
 P1 12.80 usec
 PL1 0.00 dB
 SFO1 400.1324710 MHz

F2 - Processing parameters
 SI 32768
 SF 400.1299636 MHz
 MDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

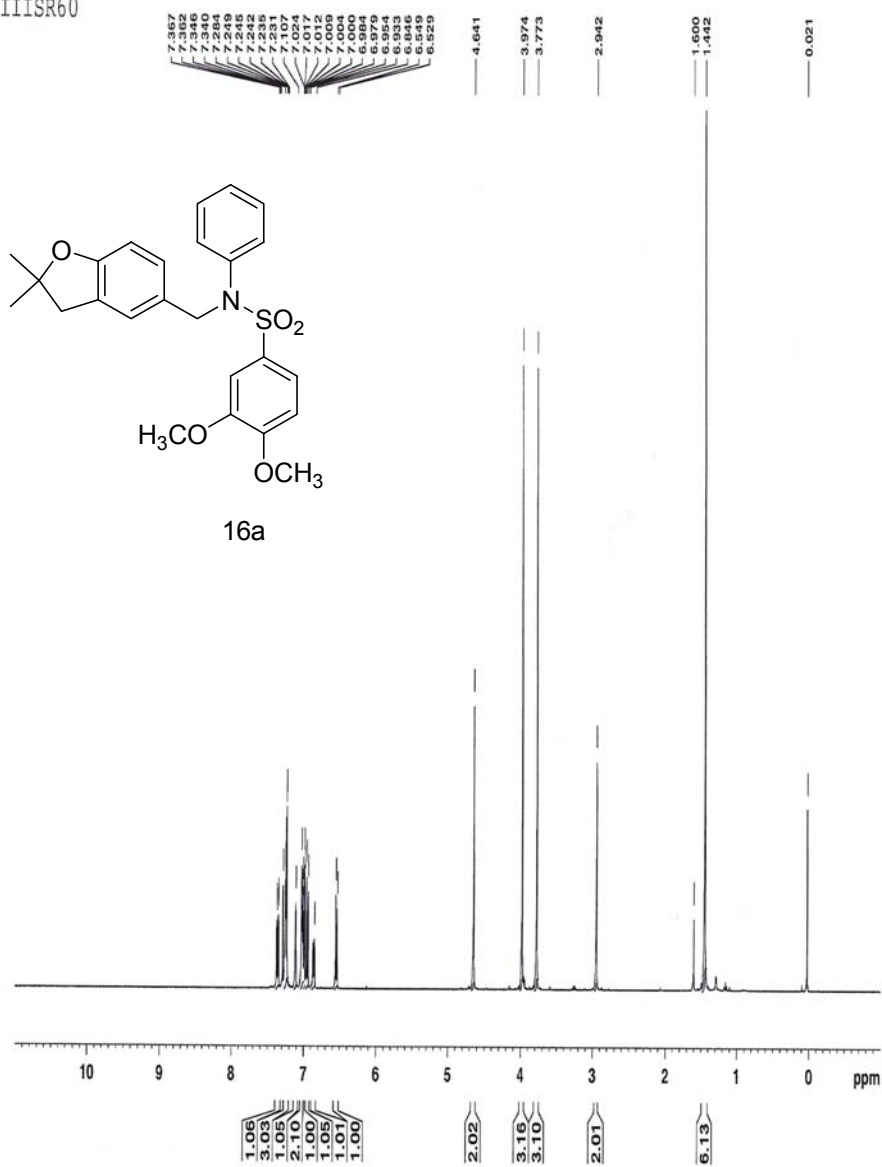


13

No title



IIISR60



No title



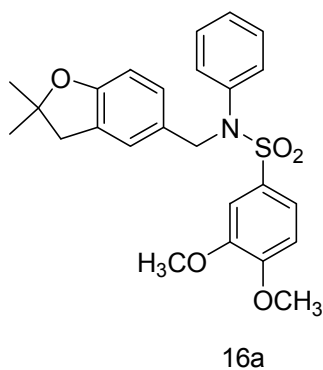
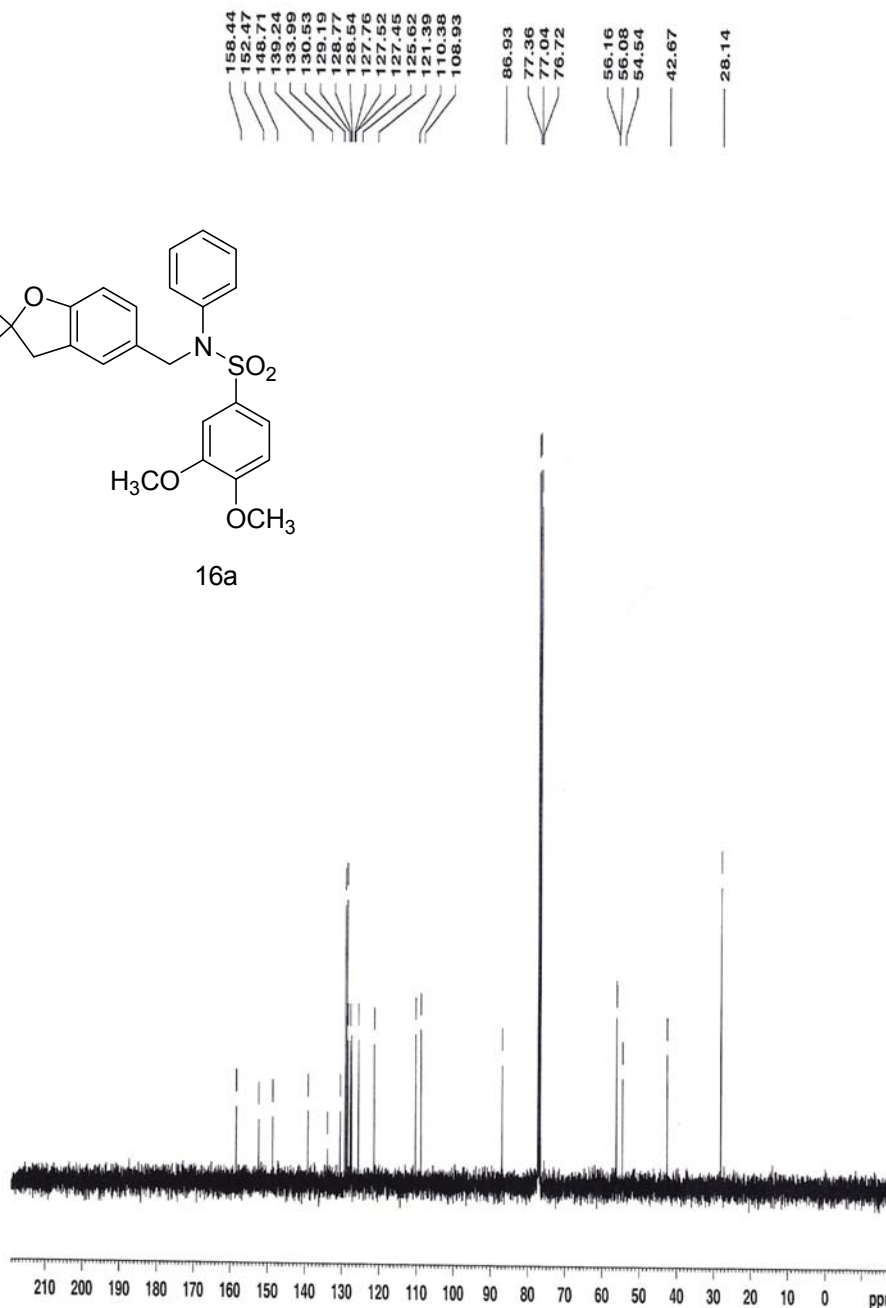
Current Data Parameters
 NAME III-SR60-c13
 EXPNO 1
 PROCNO 1

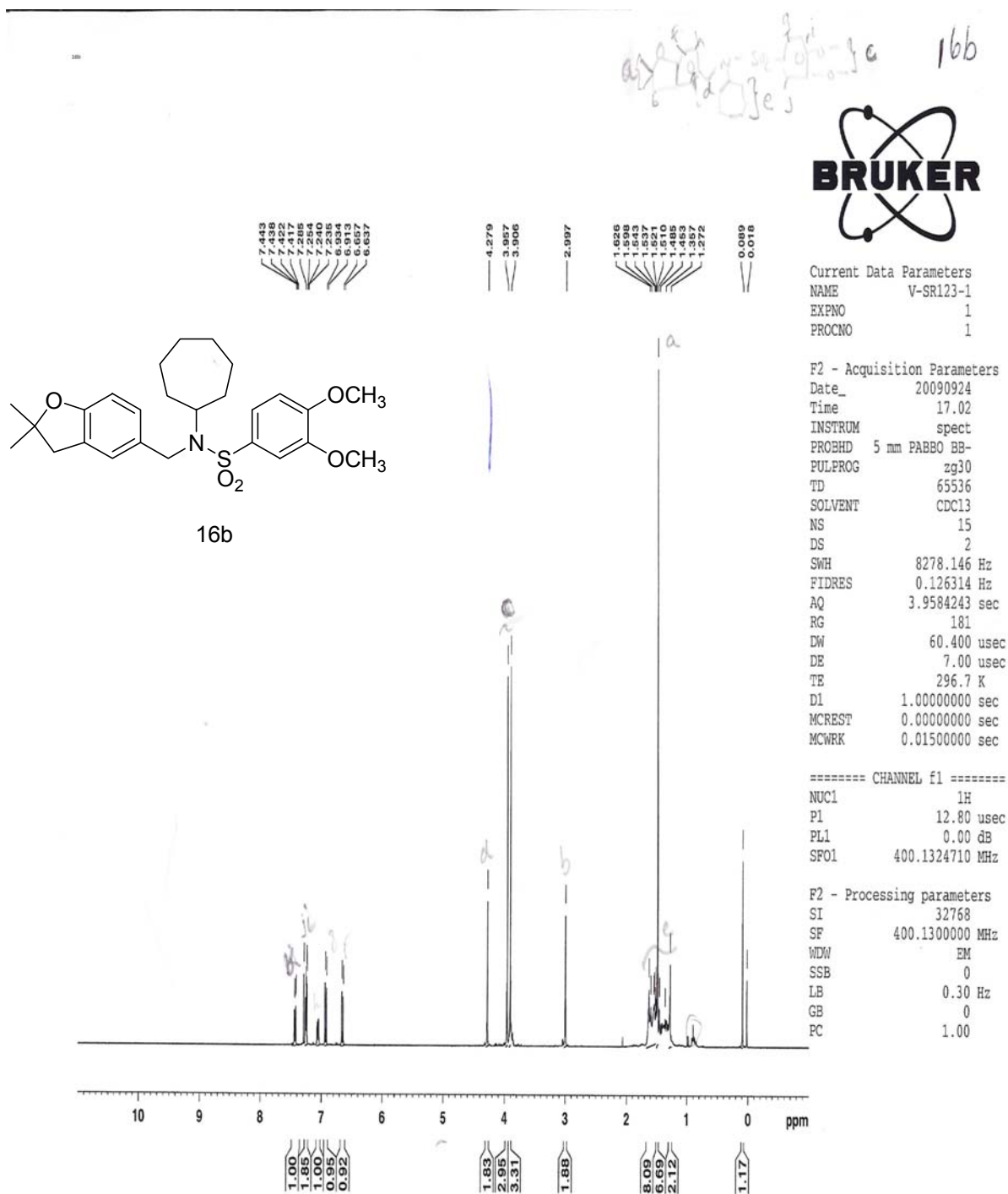
F2 - Acquisition Parameters
 Date_ 20070830
 Time 14.09
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 78
 DS 4
 SWH 23980.814 Hz
 FIDRES 0.365918 Hz
 AQ 1.3664756 sec
 RG 14596.5
 DW 20.850 usec
 DE 7.00 usec
 TE 299.4 K
 D1 2.00000000 sec
 d11 0.03000000 sec
 DELTA 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 100.6127690 MHz
 WDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40





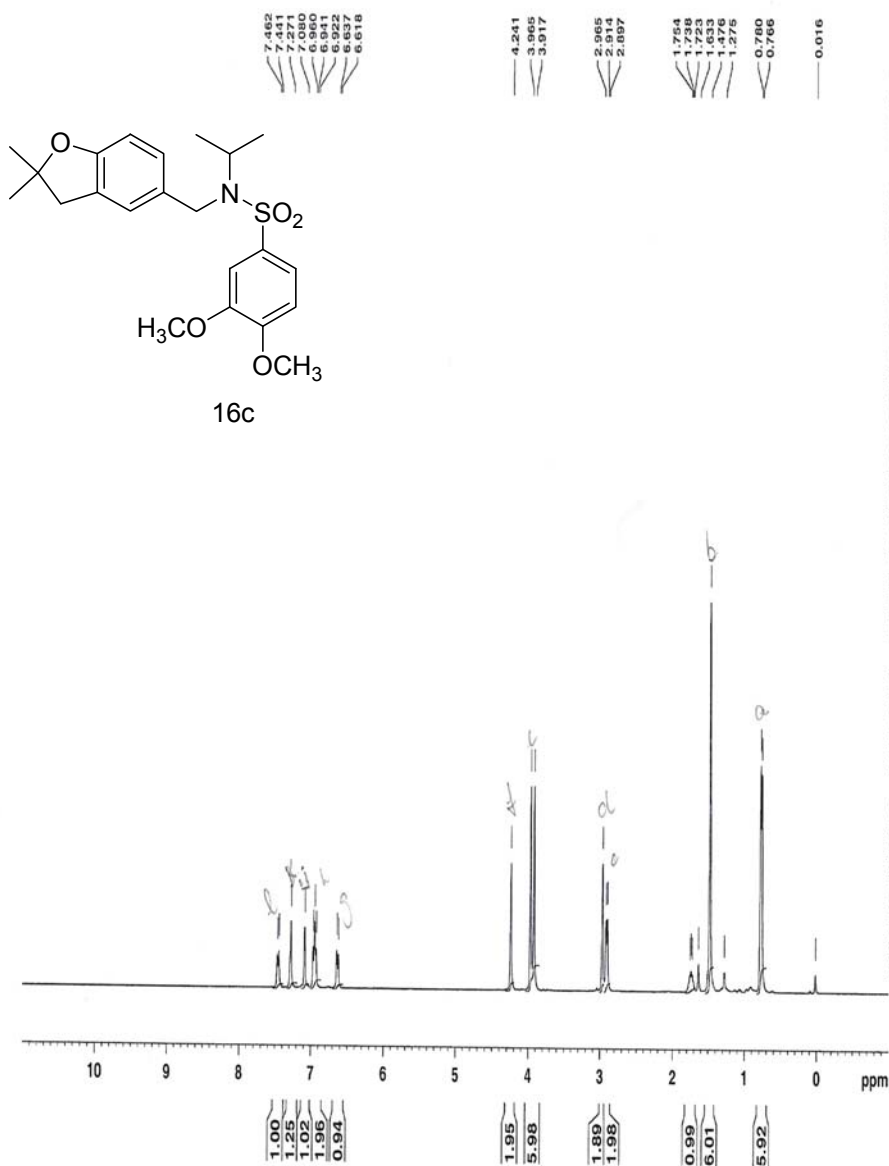


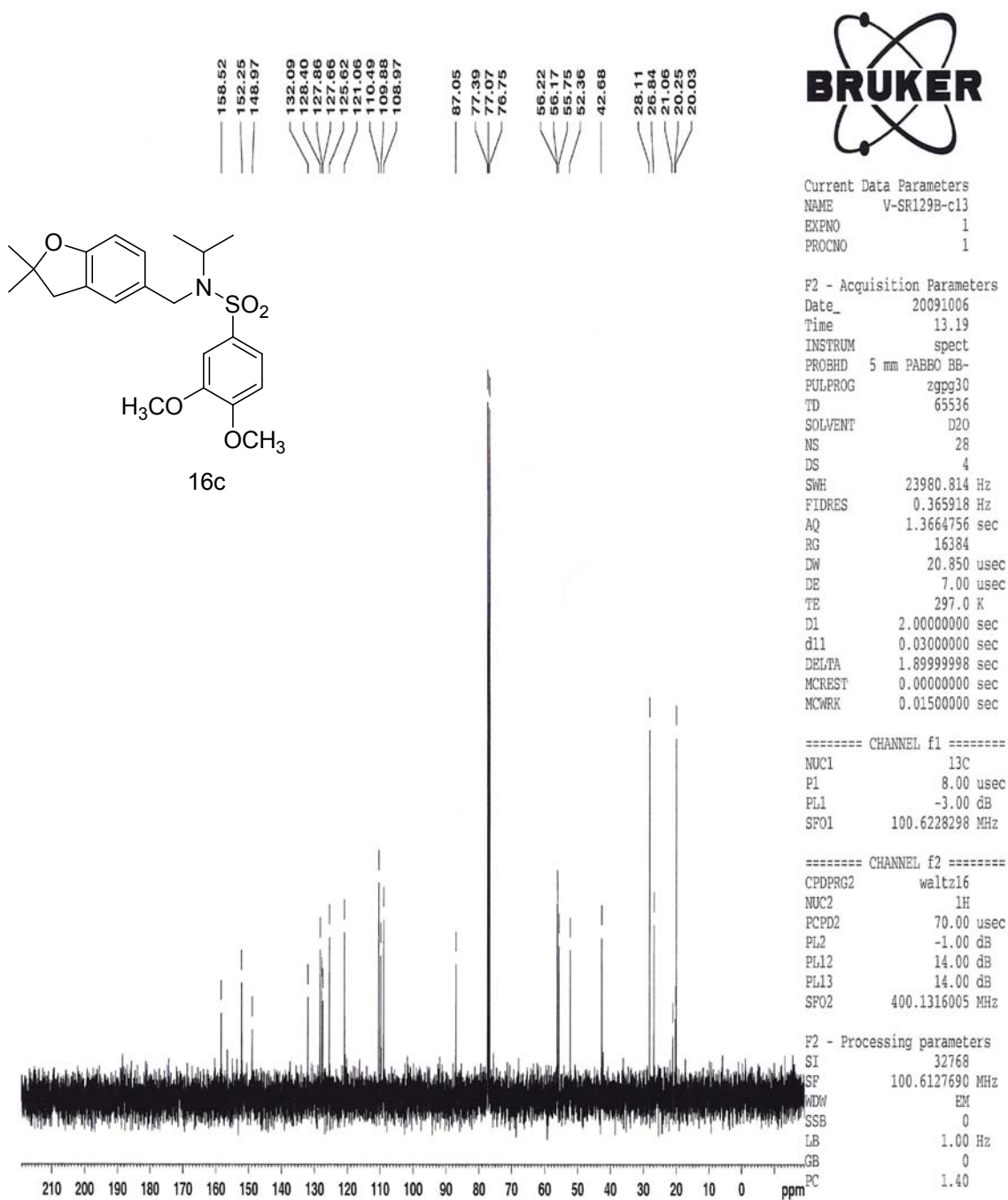
Current Data Parameters
 NAME V-SR129B2
 EXPNO 1
 PROCNO 1

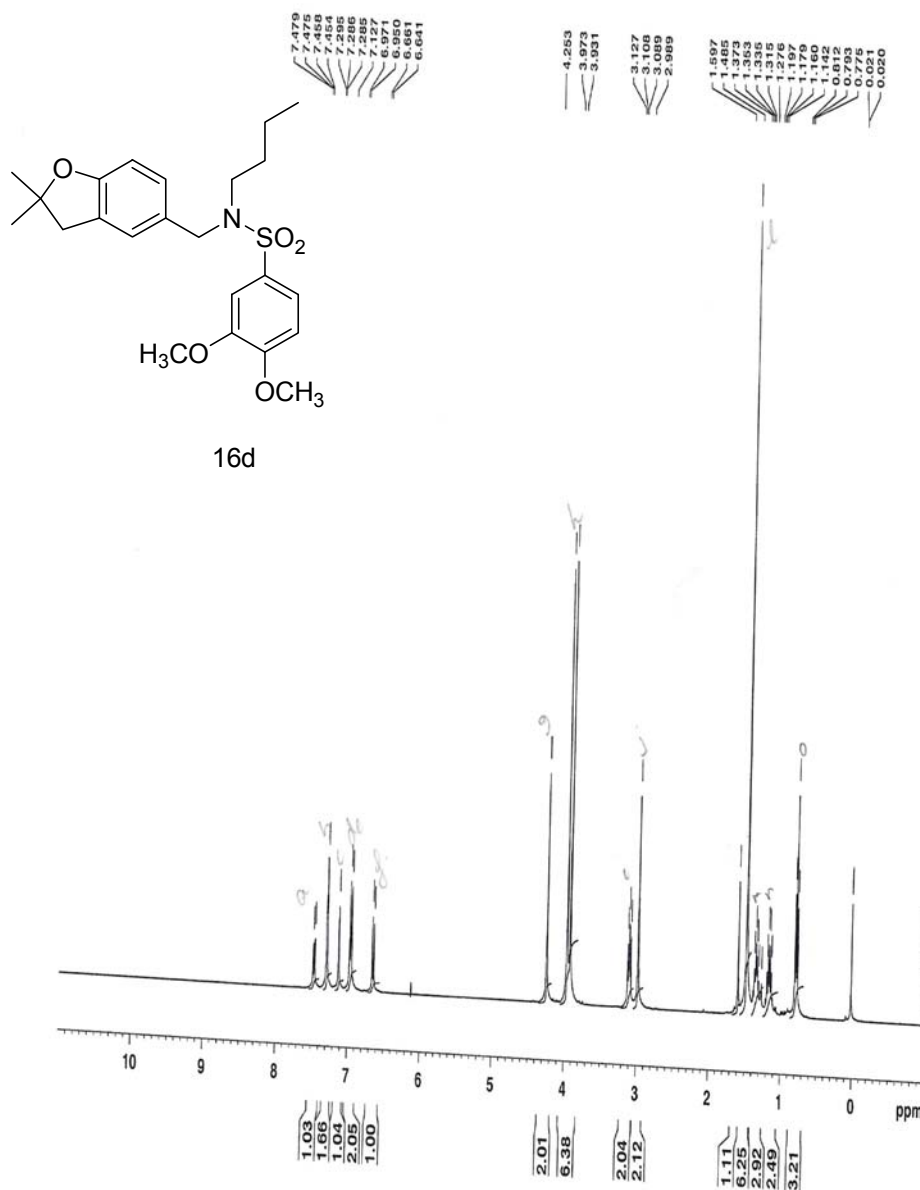
F2 - Acquisition Parameters
 Date_ 20091008
 Time 12.20
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 16
 DS 2
 SMH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 128
 DW 60.400 usec
 DE 7.00 usec
 TE 296.8 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRK 0.01500000 sec

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

F2 - Processing parameters
 SI 32768
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00





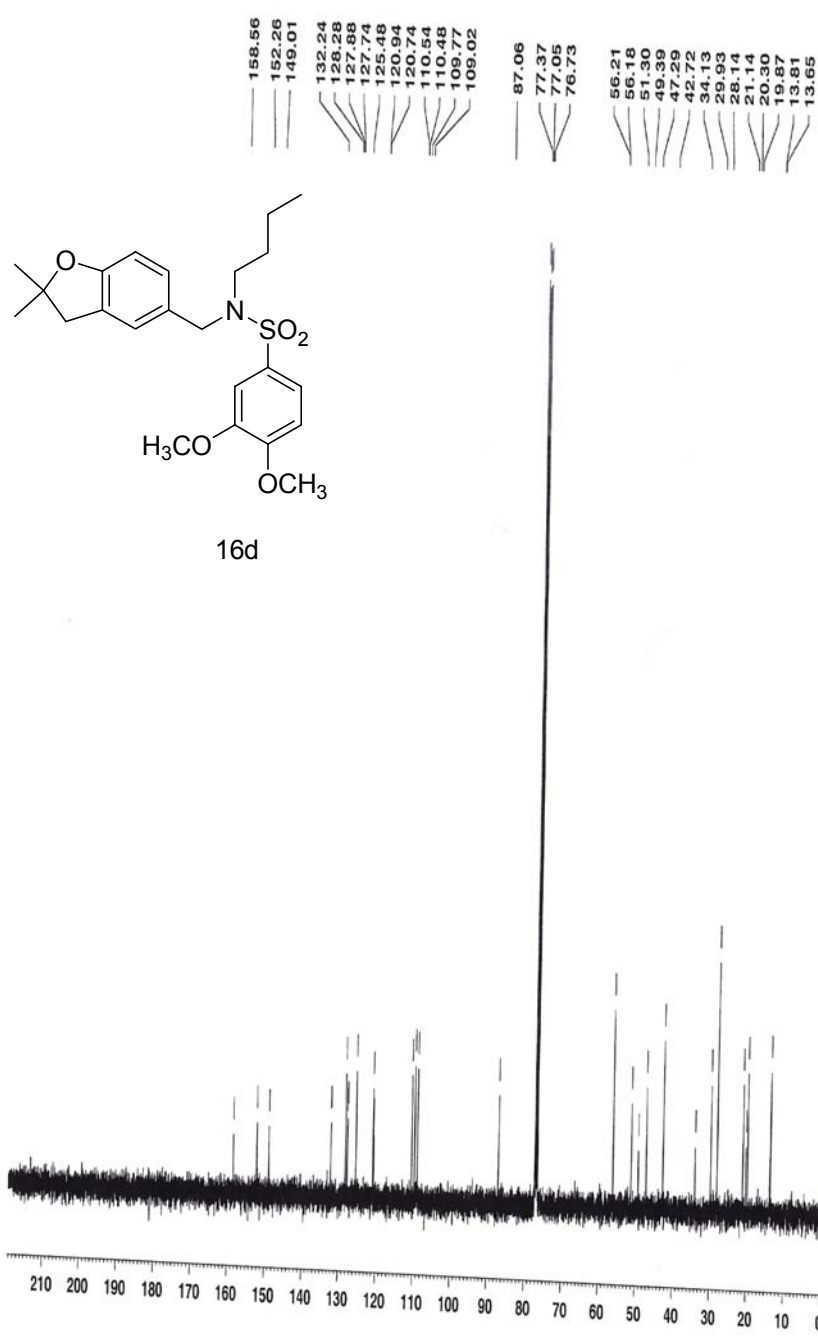


Current Data Parameters
 NAME V-SR129A2
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20091009
 Time 11.13
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 7
 DS 2
 SMH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 256
 DW 60.400 usec
 DE 7.00 usec
 TE 296.0 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 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



Current Data Parameters
 NAME V-SR129A-C13
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20091006
 Time 13.05
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 124
 DS 4
 SMH 23980.814 Hz
 FIDRES 0.365918 Hz
 AQ 1.3664756 sec
 RG 11585.2
 DW 20.850 usec
 DE 7.00 usec
 TE 297.1 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 100.6127690 MHz
 MDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40

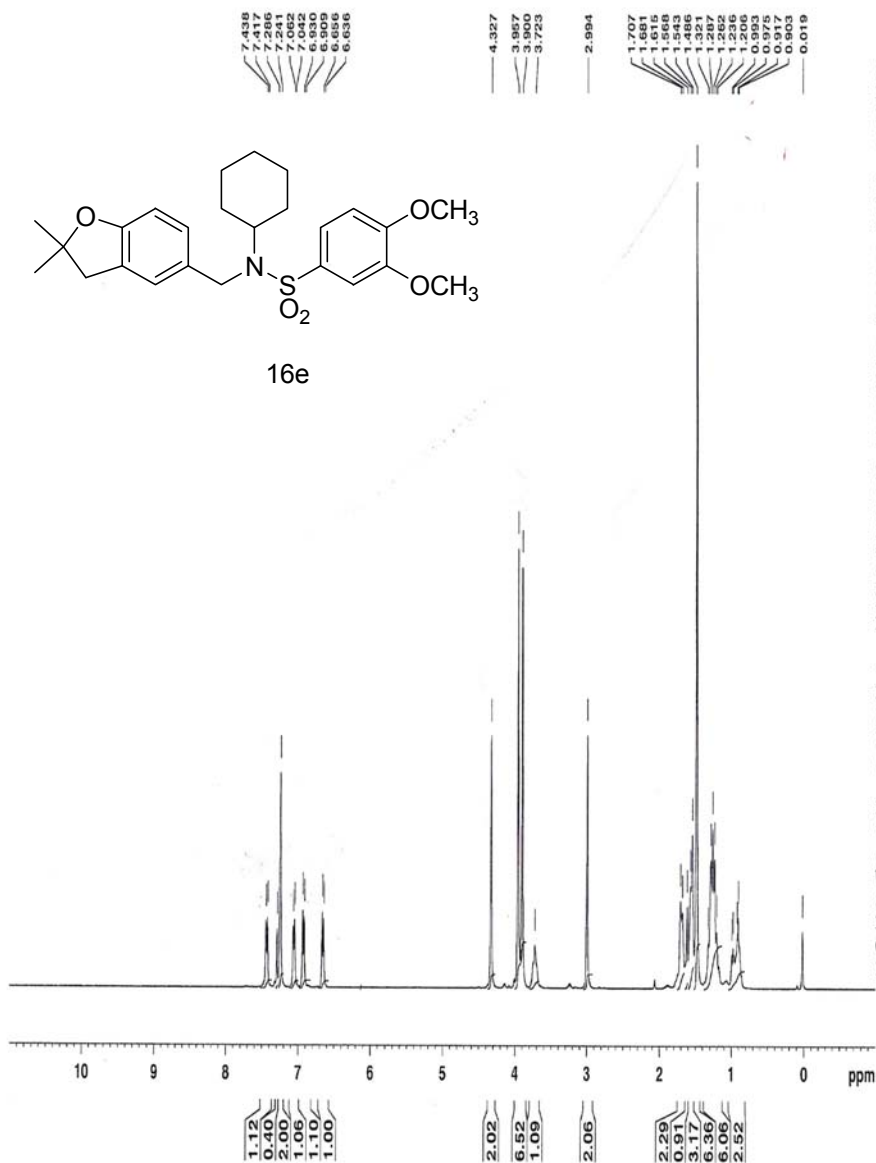


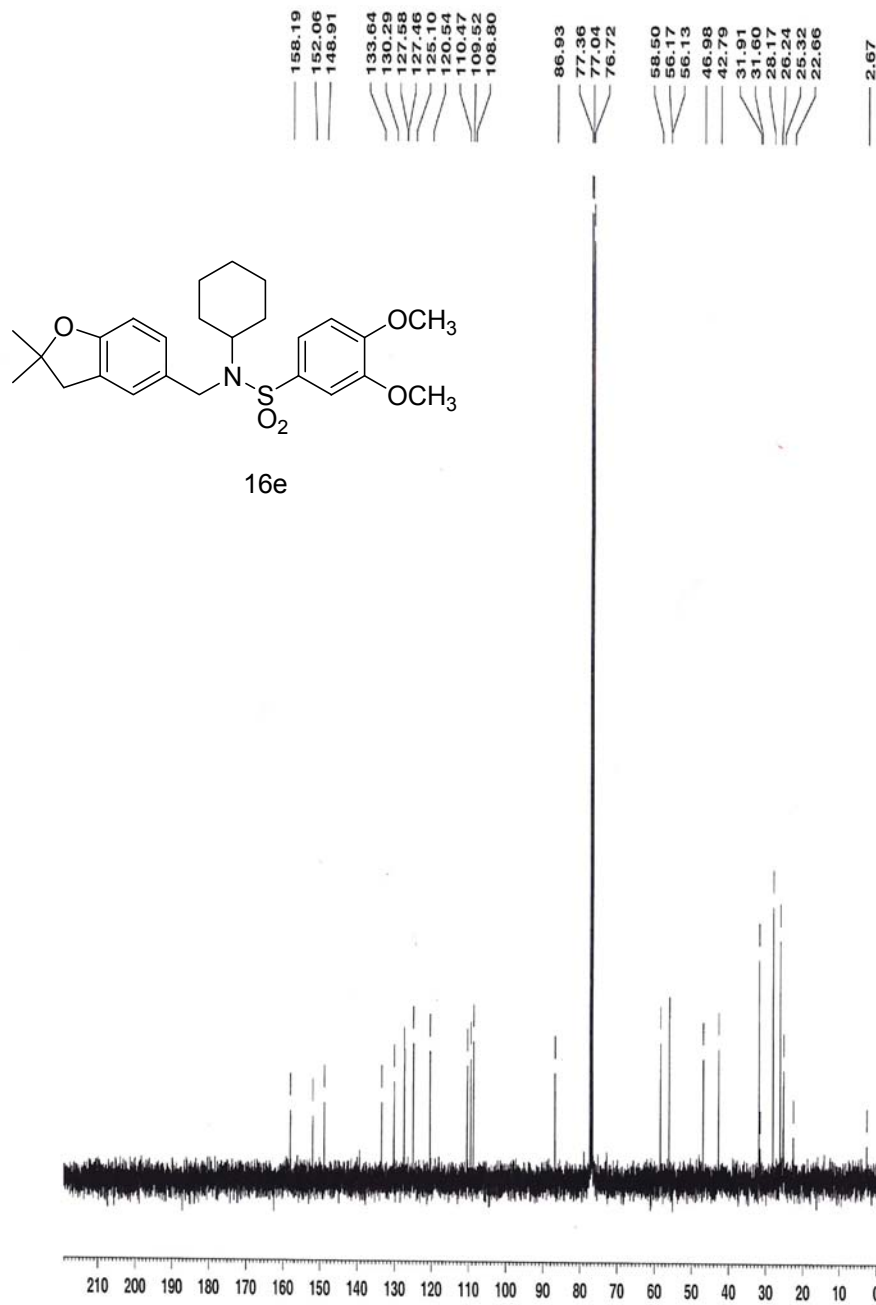
Current Data Parameters
 NAME V-SR132
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20091008
 Time 12.46
 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
 DM 60.400 usec
 DE 7.00 usec
 TE 296.9 K
 DI 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 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00





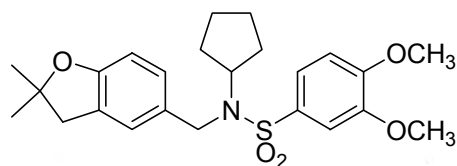
Current Data Parameters
 NAME V-SR132-C13
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20091008
 Time 12.37
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 100
 DS 4
 SMH 23980.814 Hz
 FIDRES 0.365918 Hz
 AQ 1.3664756 sec
 RG 8192
 DW 20.850 usec
 DE 7.00 usec
 TE 296.9 K
 D1 2.00000000 sec
 d11 0.03000000 sec
 DELTA 1.89999998 sec
 MCREST 0.00000000 sec
 MCWRK 0.01500000 sec

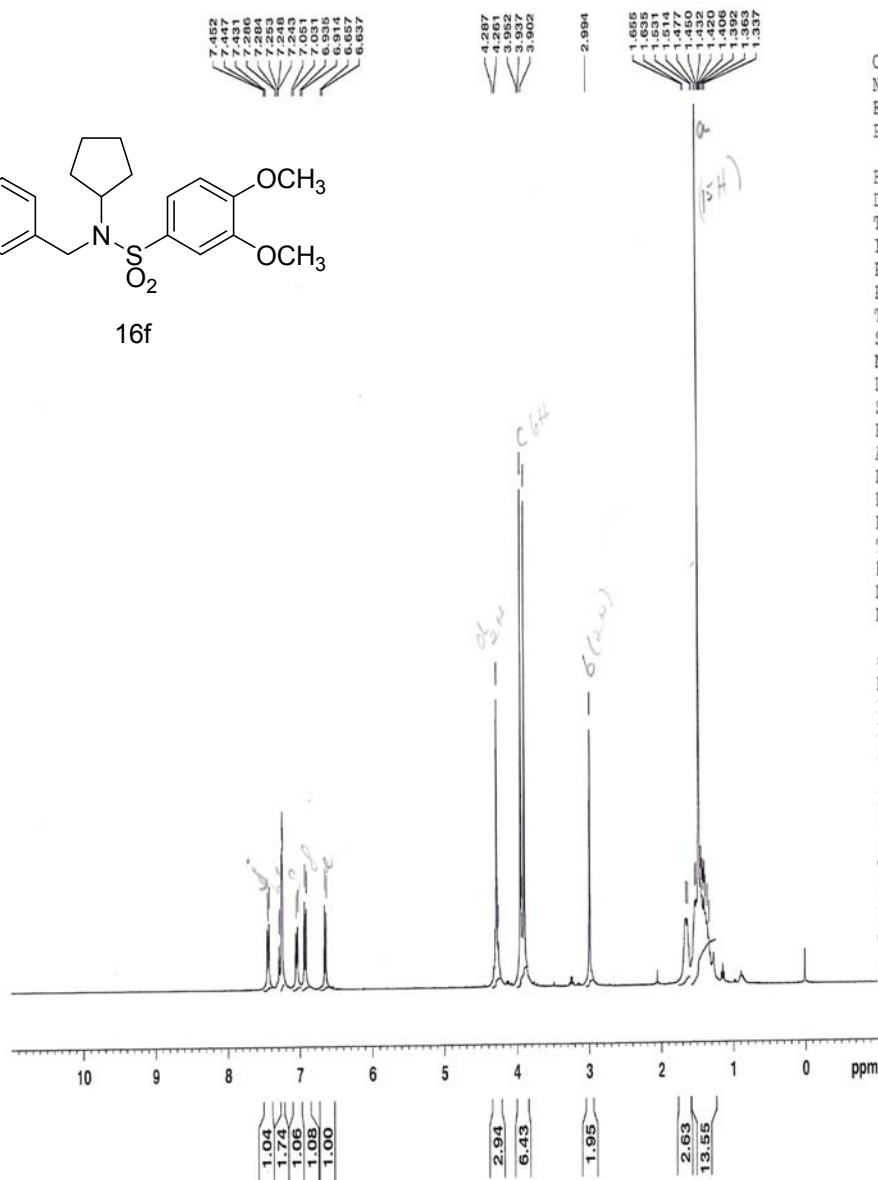
===== CHANNEL f1 =====
 NUC1 13C
 P1 8.00 usec
 PL1 -3.00 dB
 SFO1 100.6228298 MHz

===== 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 100.6127690 MHz
 MDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40



16f

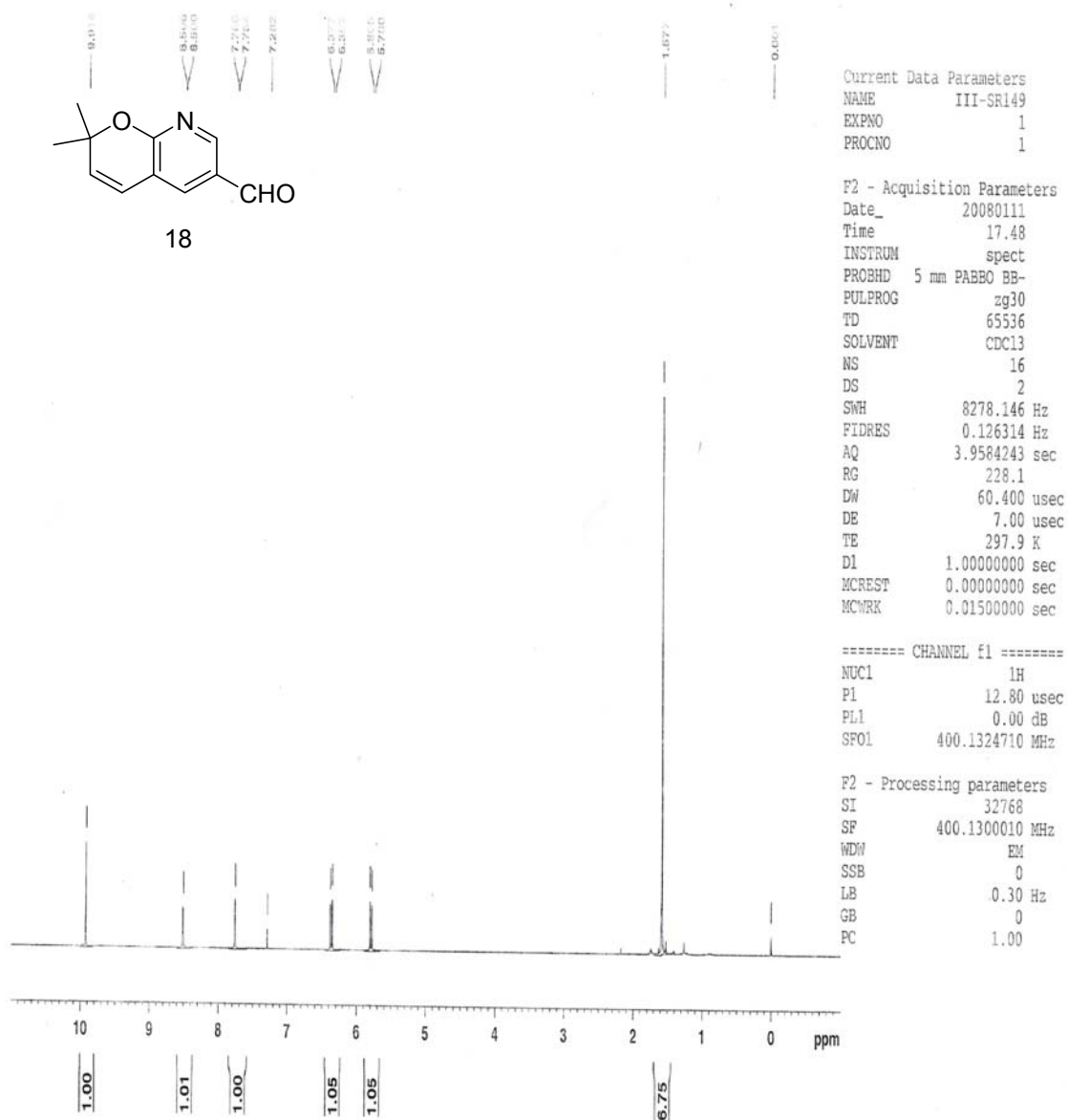


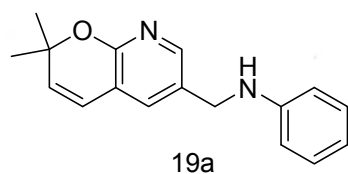
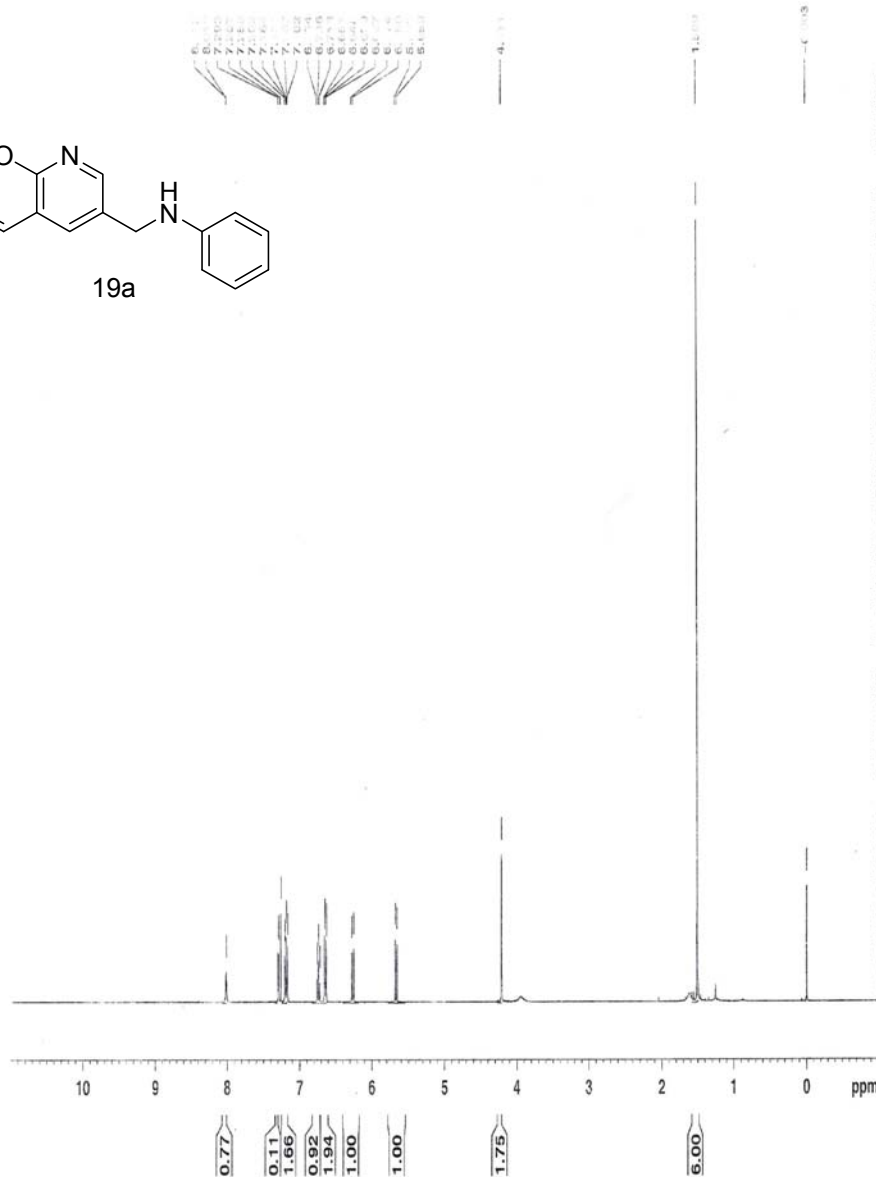
Current Data Parameters
 NAME V-SR133
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20091009
 Time 11.37
 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 90.5
 DW 60.400 usec
 DE 7.00 usec
 TE 296.1 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 400.1300000 MHz
 WDM EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.40





Current Data Parameters

NAME III-SR150
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters

Date_ 20080115
Time 13.34
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 3.9584243 sec
RG 256
DW 60.400 usec
DE 7.00 usec
TE 297.6 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

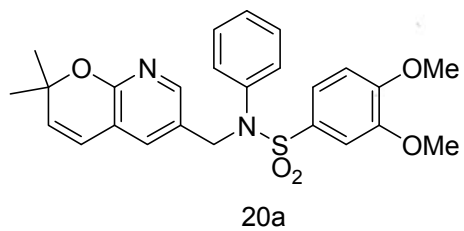
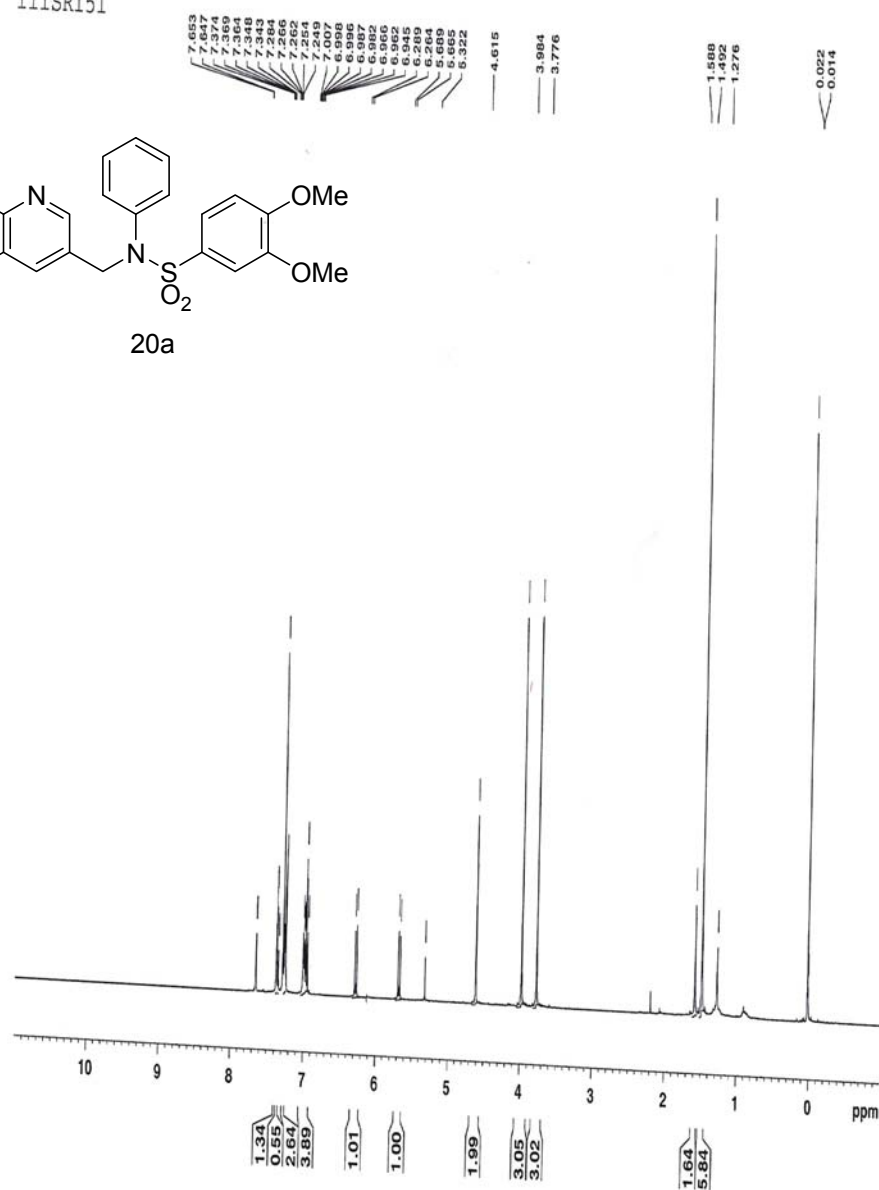
===== CHANNEL f1 =====

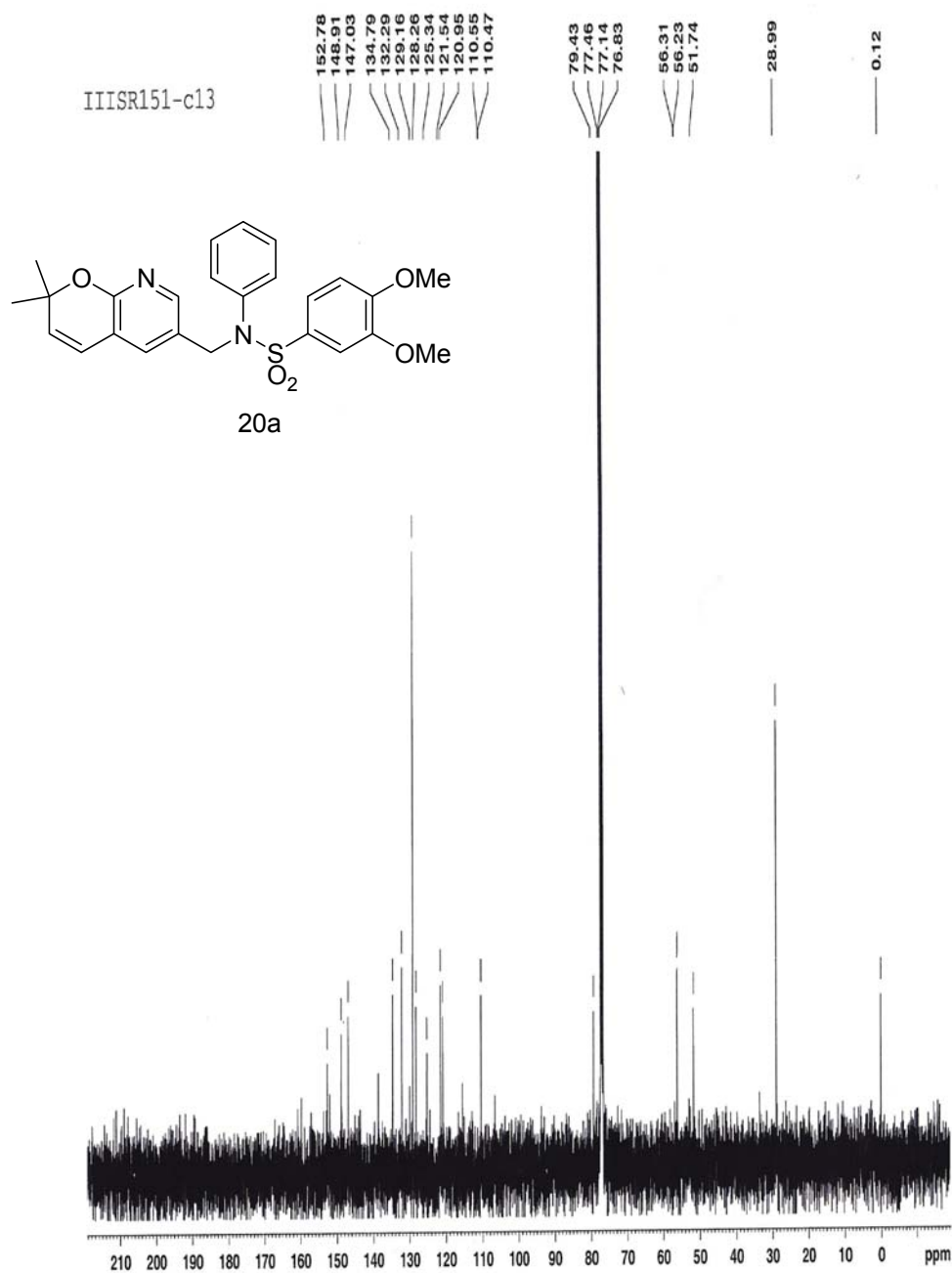
NUC1 1H
P1 12.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz

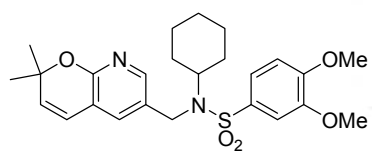
F2 - Processing parameters

SI 32768
SF 400.1300106 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

IIISR151

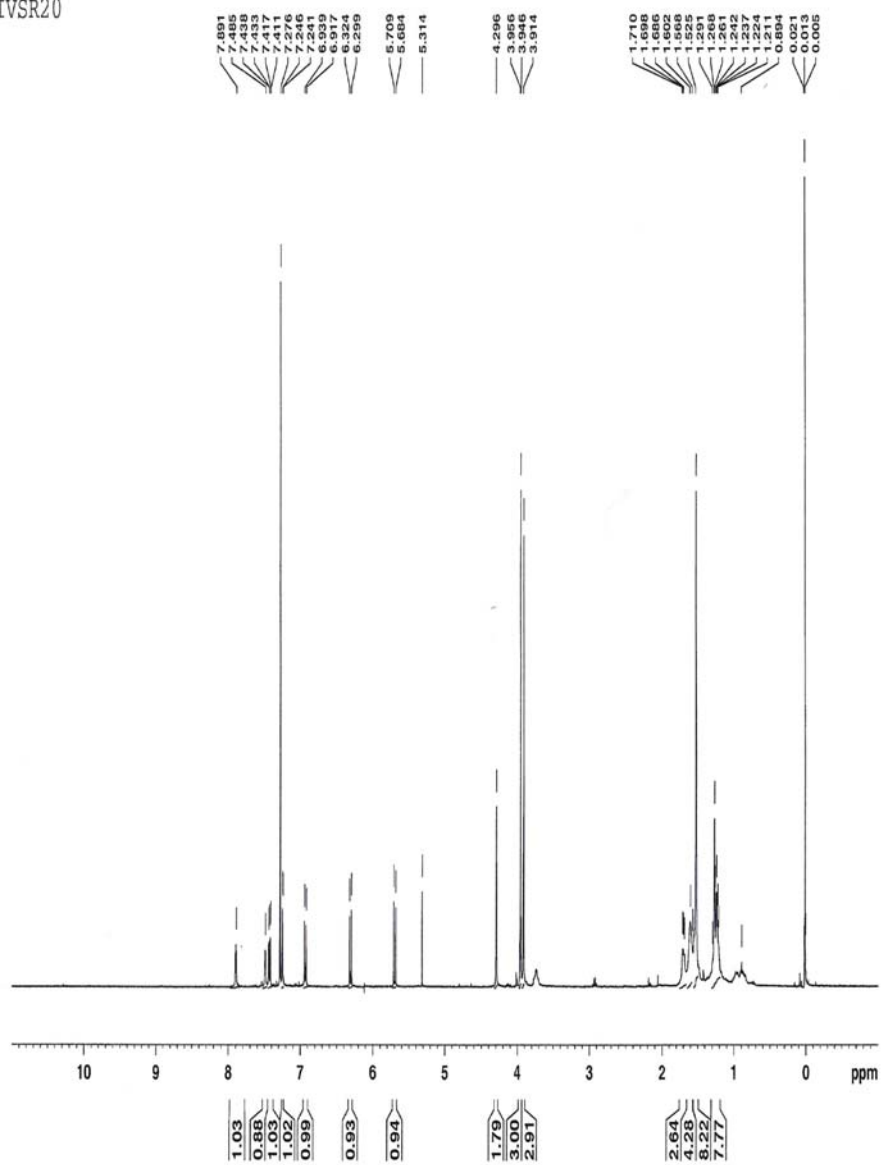


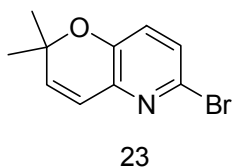




20b

IVSR20





7.159
7.138
6.919
6.897
6.463
6.438
5.885
5.859

848810 104

1.456
1.445

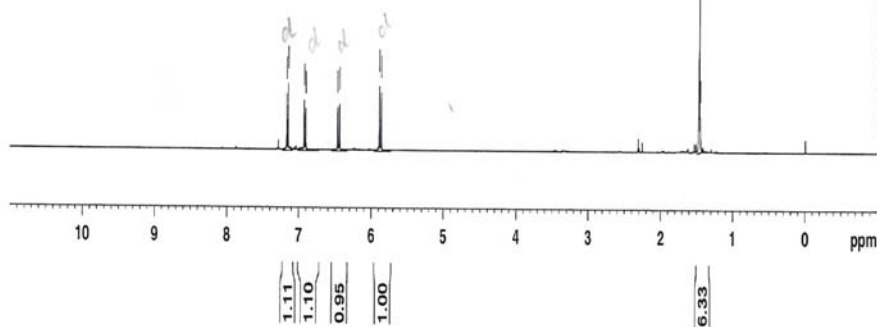


Current Data Parameters
NAME IV-SR12-1V-S613
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20080213
Time 10.08
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 15
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 57
DW 60.400 usec
DE 7.00 usec
TE 297.8 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

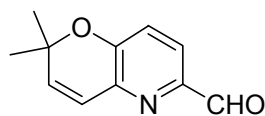
===== CHANNEL f1 =====
NUC1 1H
P1 12.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1300028 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

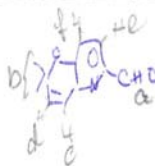


24

No title



24



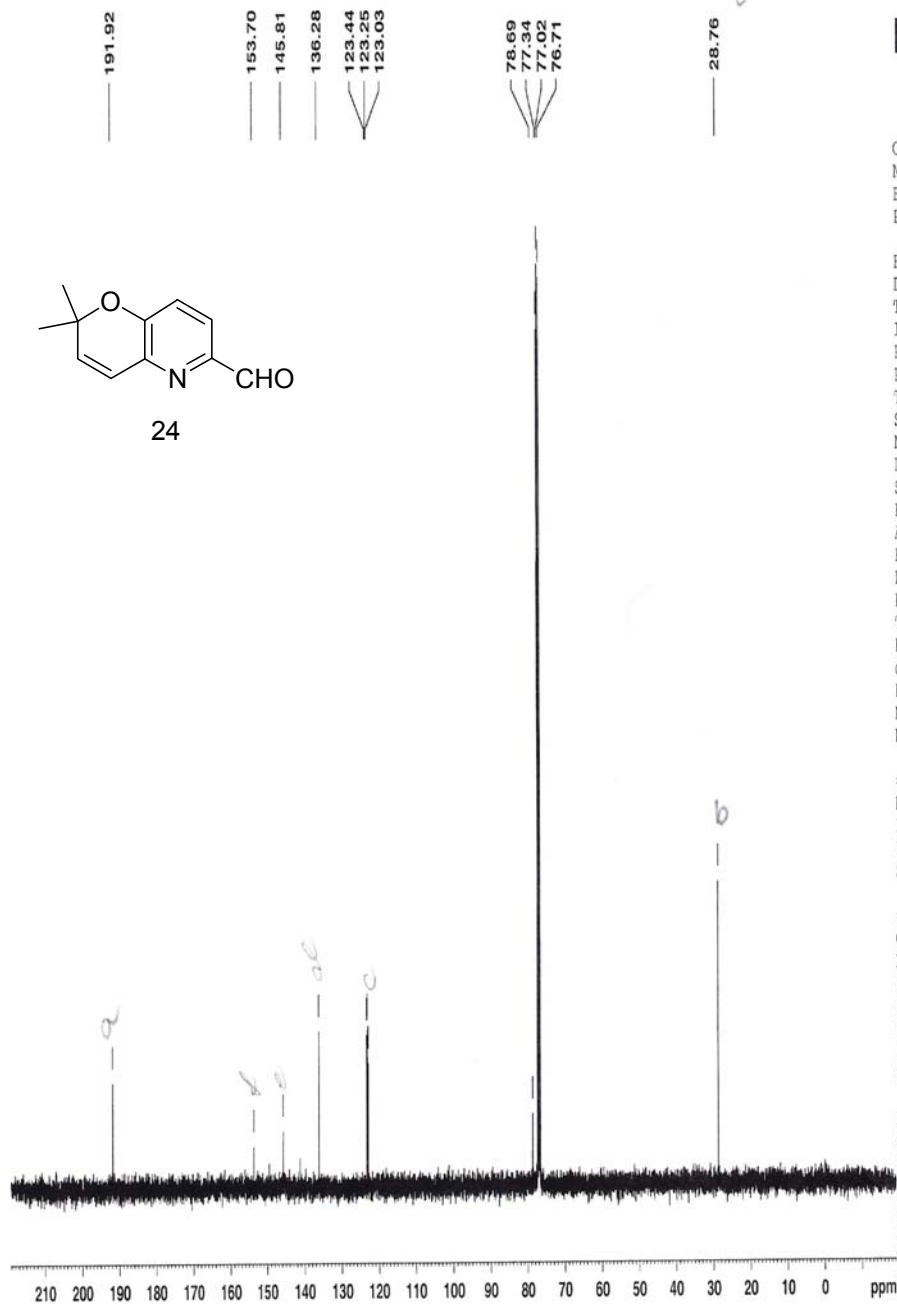
Current Data Parameters
 NAME IV-SR13-C13
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20080214
 Time 11.20
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 146
 DS 4
 SWH 23980.814 Hz
 FIDRES 0.365918 Hz
 AQ 1.3664756 sec
 RG 14596.5
 DW 20.850 usec
 DE 7.00 usec
 TE 297.7 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

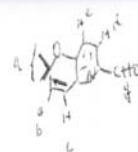
===== CHANNEL f2 =====
 CPDPRG2 waltz16
 NUC2 1H
 PCPD2 70.00 usec
 PL2 -1.00 dB
 PL12 14.00 dB
 PL13 14.00 dB
 SFO2 400.1316005 MHz

F2 - Processing parameters
 SI 32768
 SF 100.6127690 MHz
 WDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40



94

No title



Current Data Parameters

NAME IV-SR13-1
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters

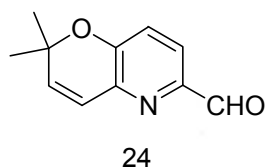
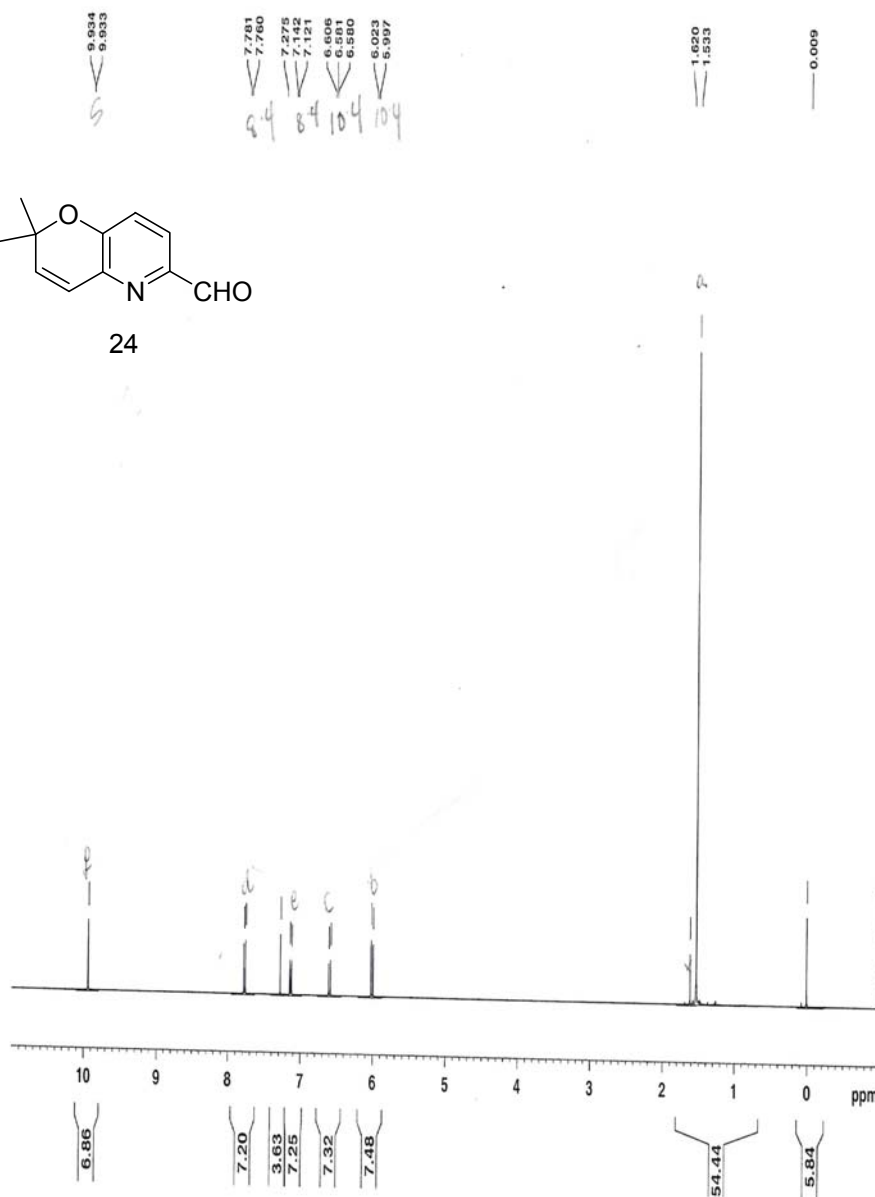
Date_ 20080214
Time 11.15
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 19
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 362
DM 60.400 usec
DE 7.00 usec
TE 297.5 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

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

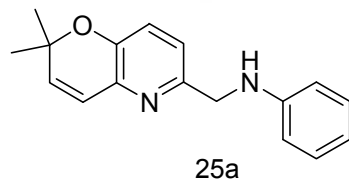
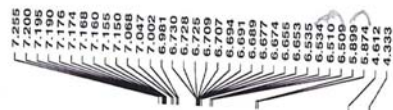
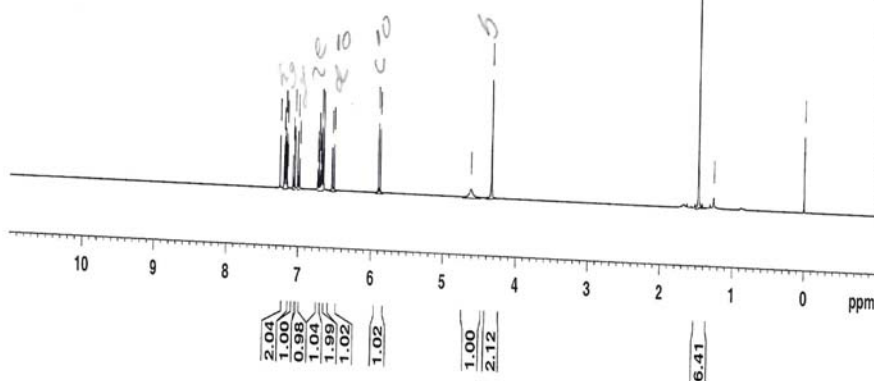
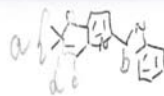
NUC1 1H
P1 12.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters

SI 32768
SF 400.1300038 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



No title



25a



Current Data Parameters

NAME	IV-SR16
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20080218
Time	10.07
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	181
DW	60.400 usec
DE	7.00 usec
TE	297.7 K
D1	1.00000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec

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

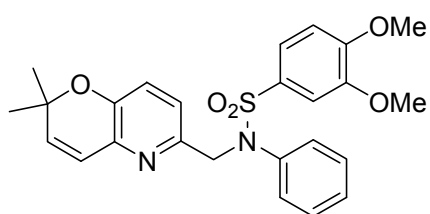
NUC1	1H
P1	12.80 usec
PL1	0.00 dB
SFO1	400.1324710 MHz

F2 - Processing parameters

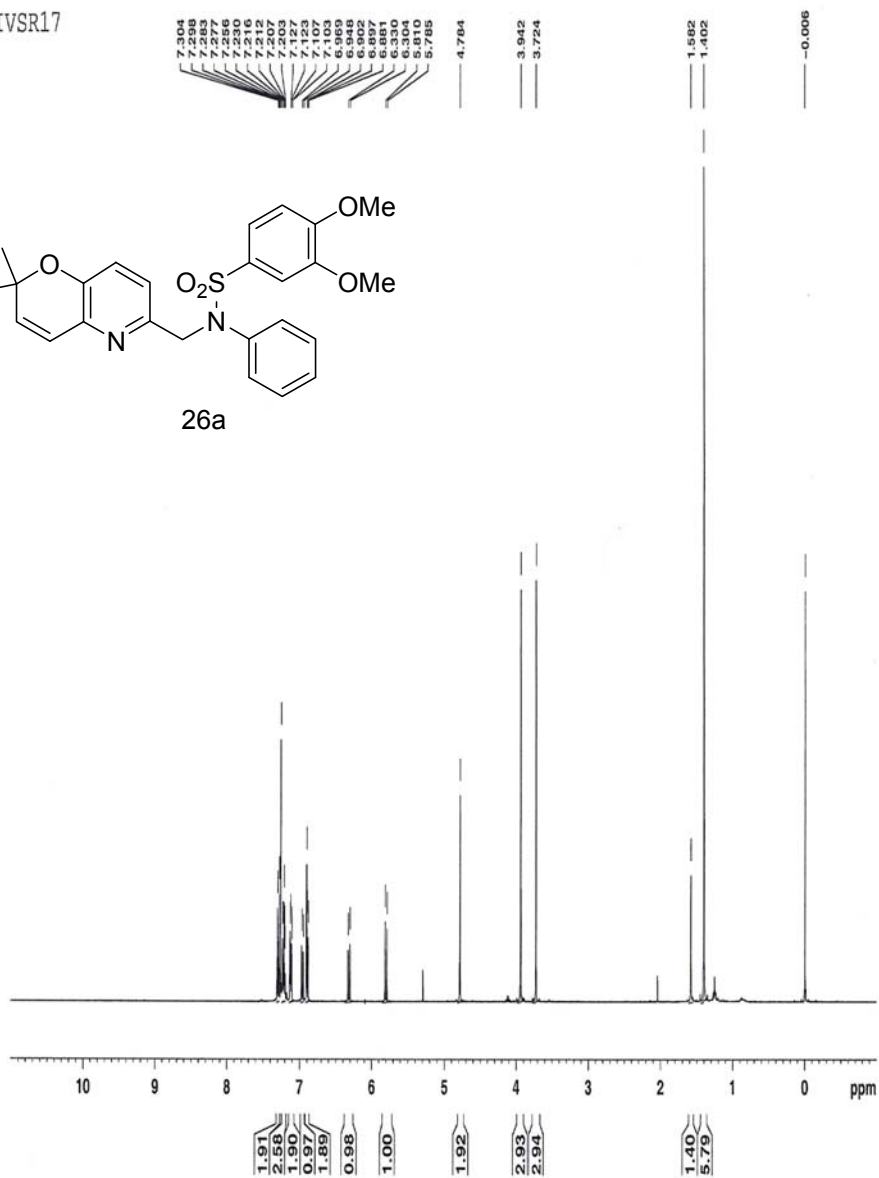
SI	32768
SF	400.1300116 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

26a

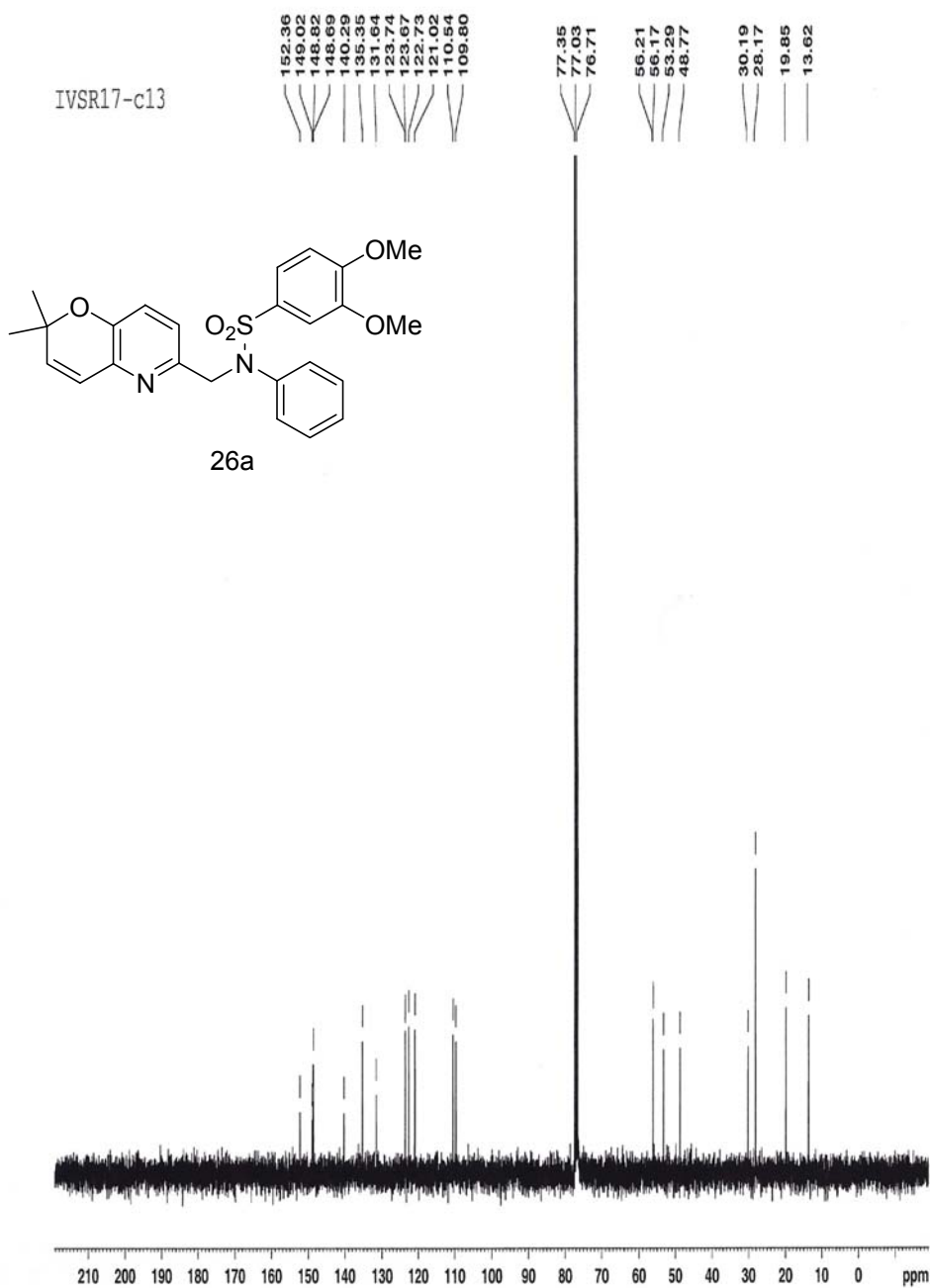
IVSR17



26a

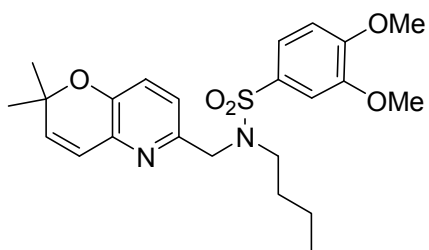


26a.

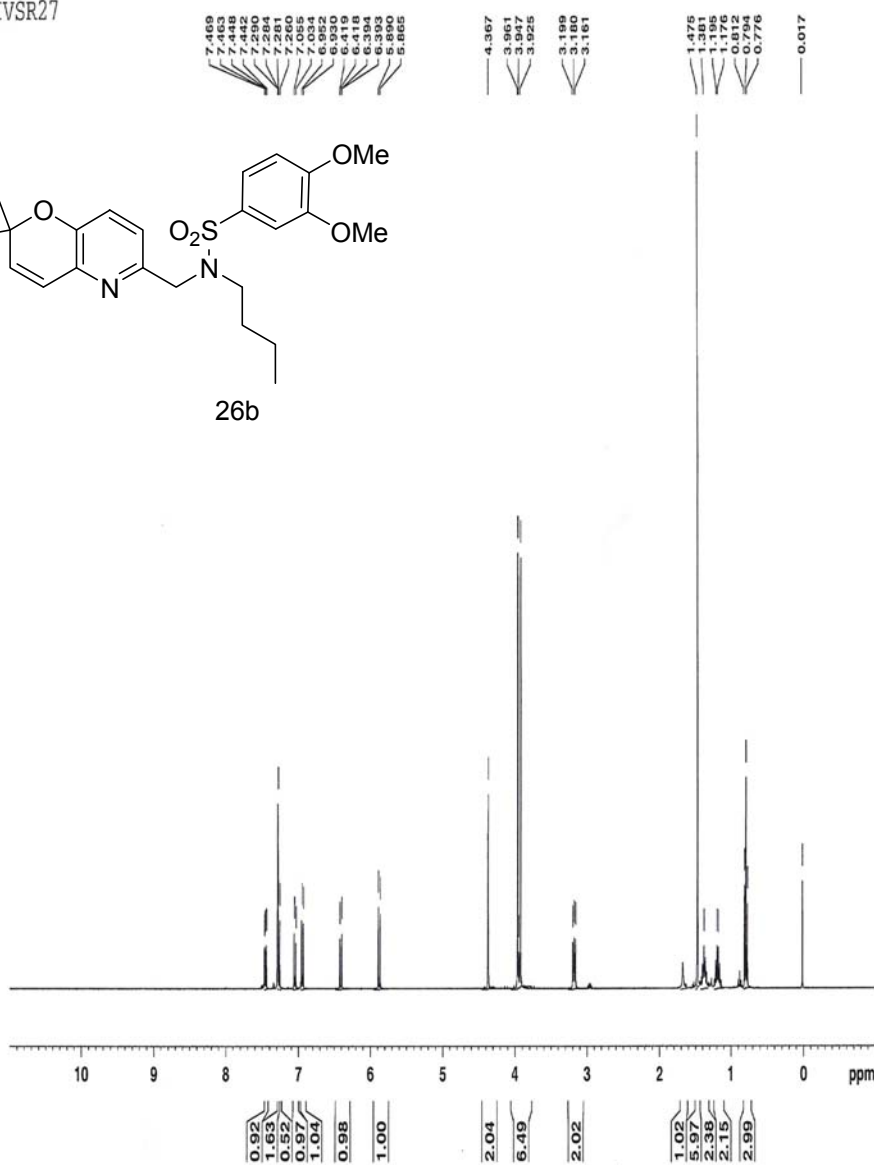


26b

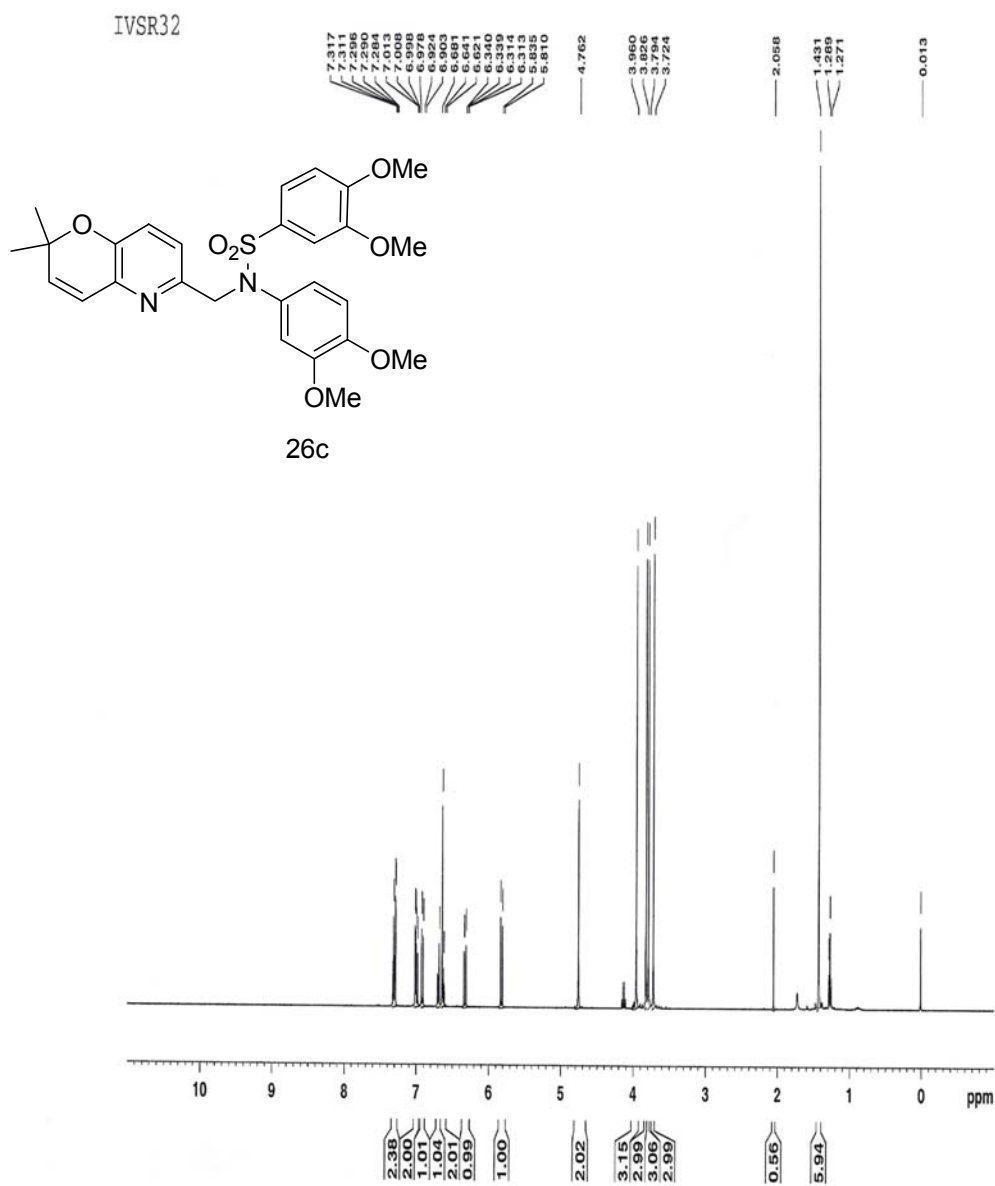
IVSR27



26b

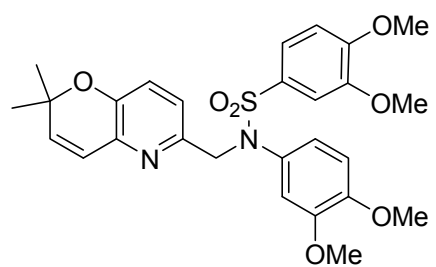


26c

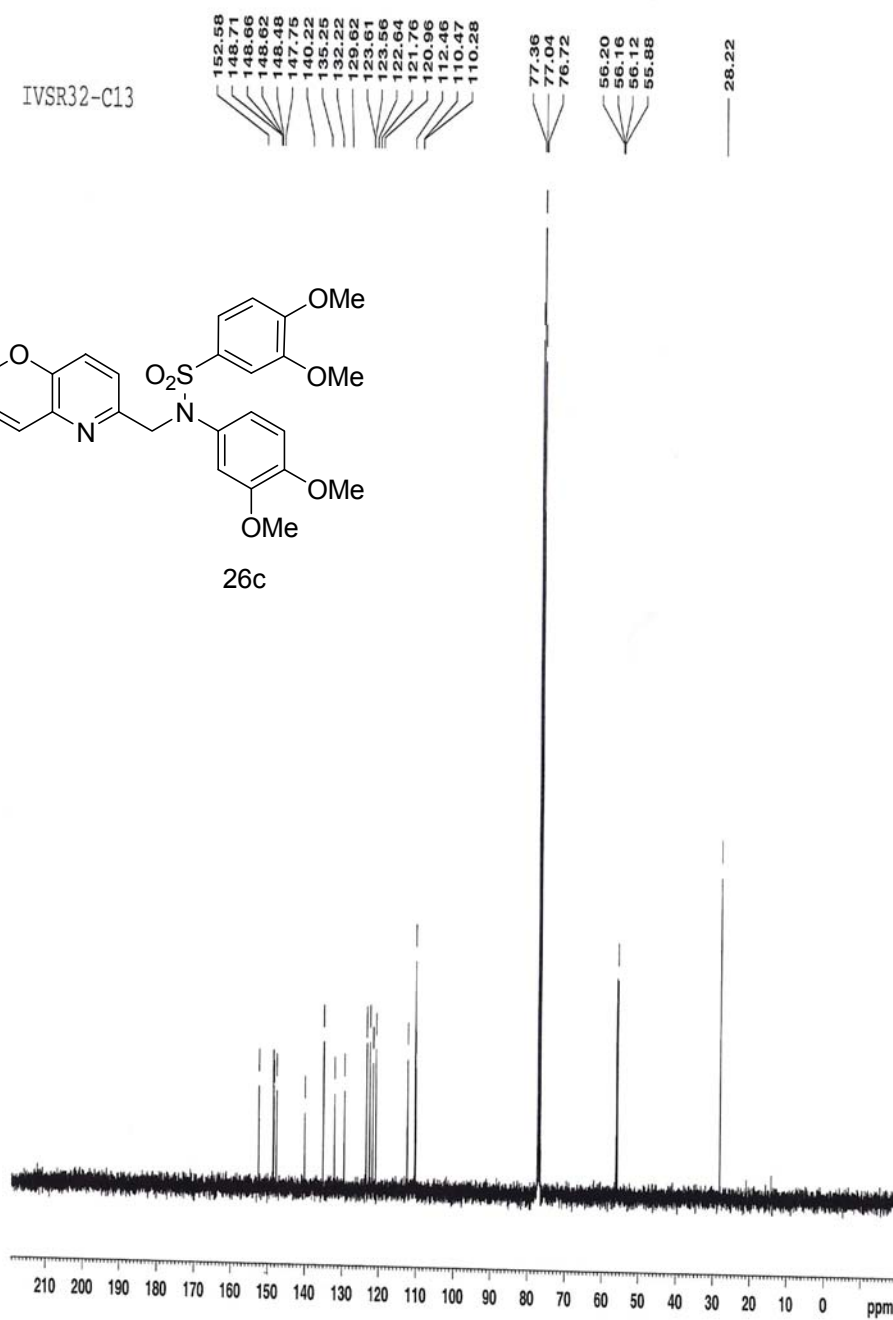


26c

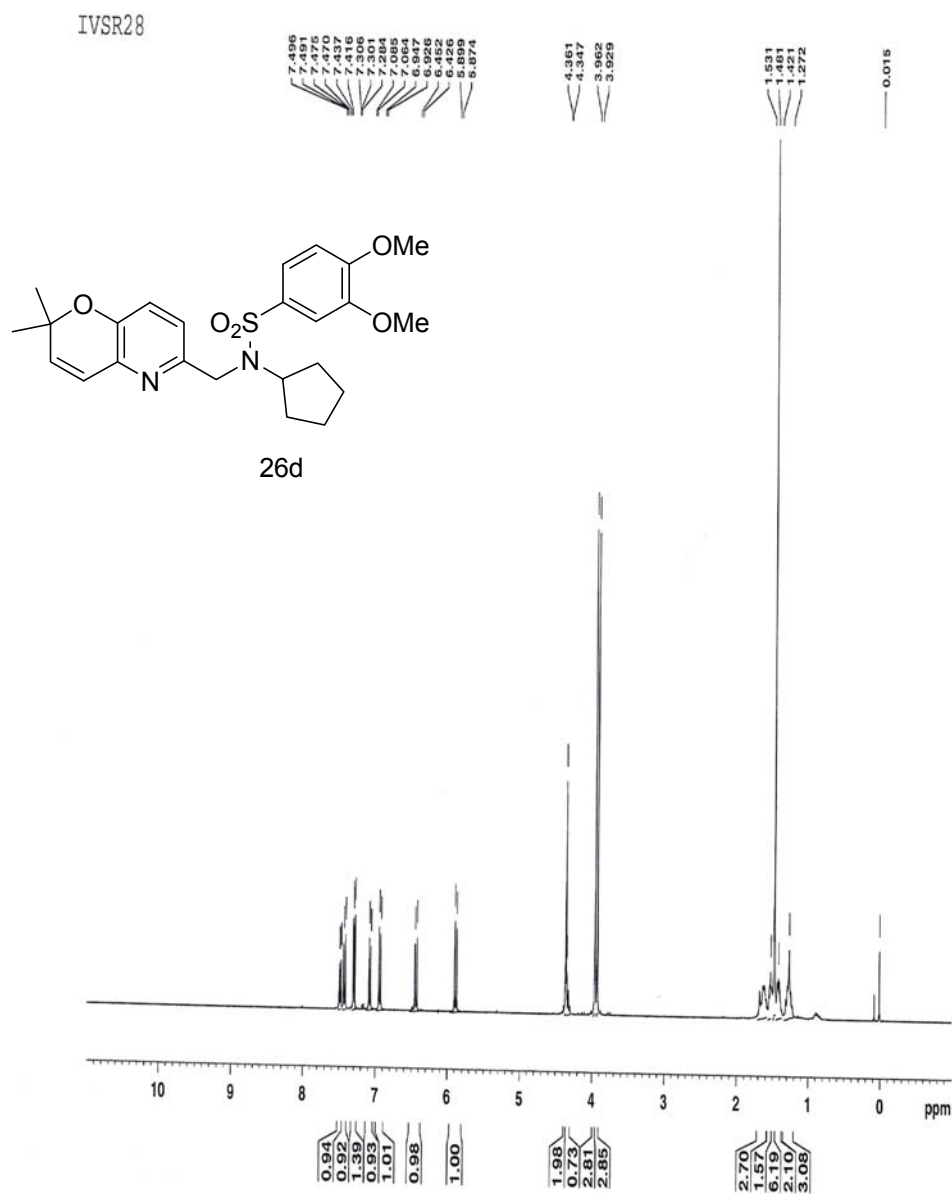
IVSR32-C13

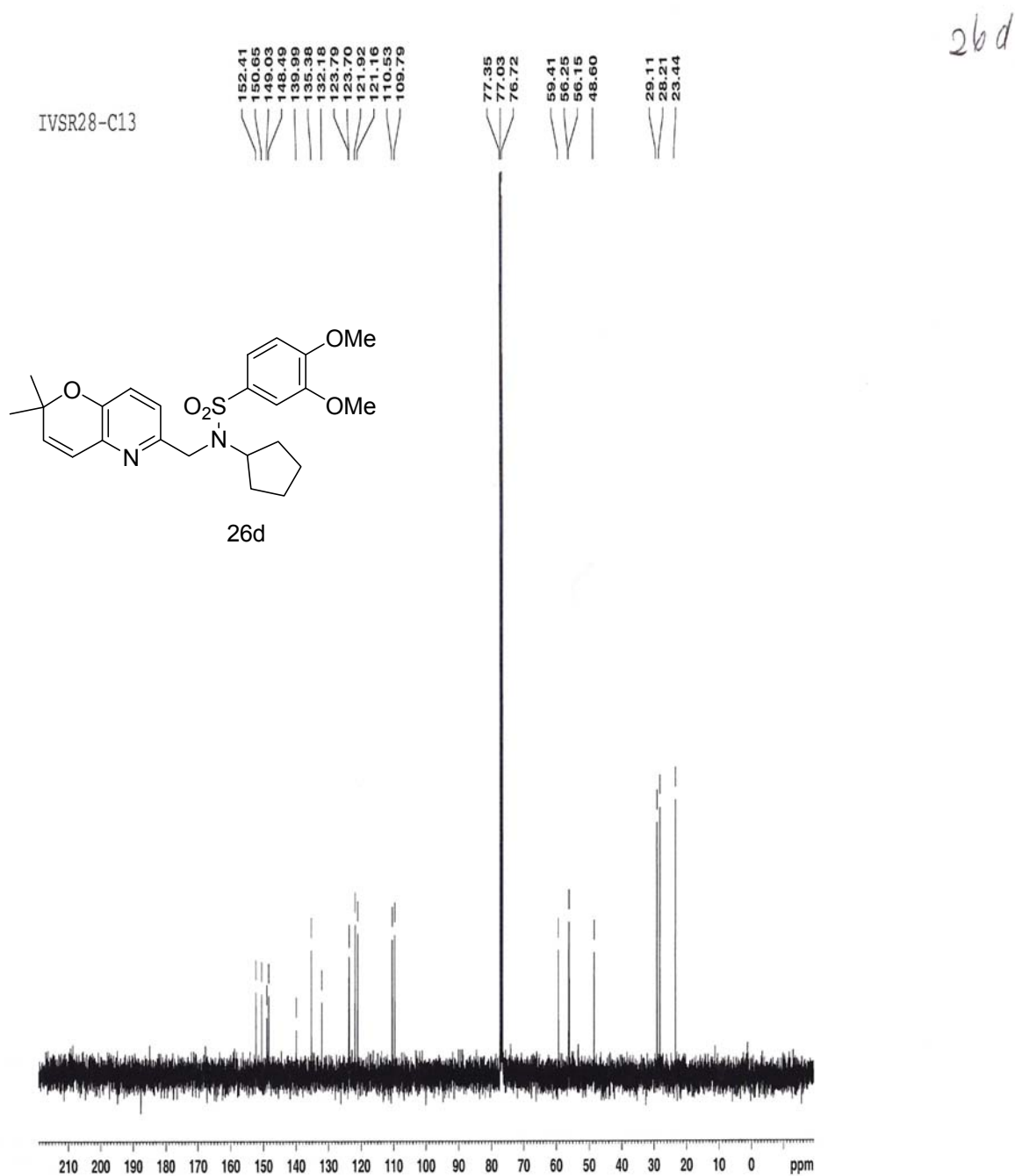


26c



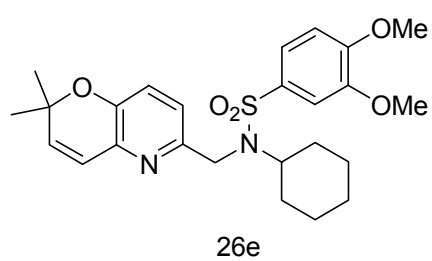
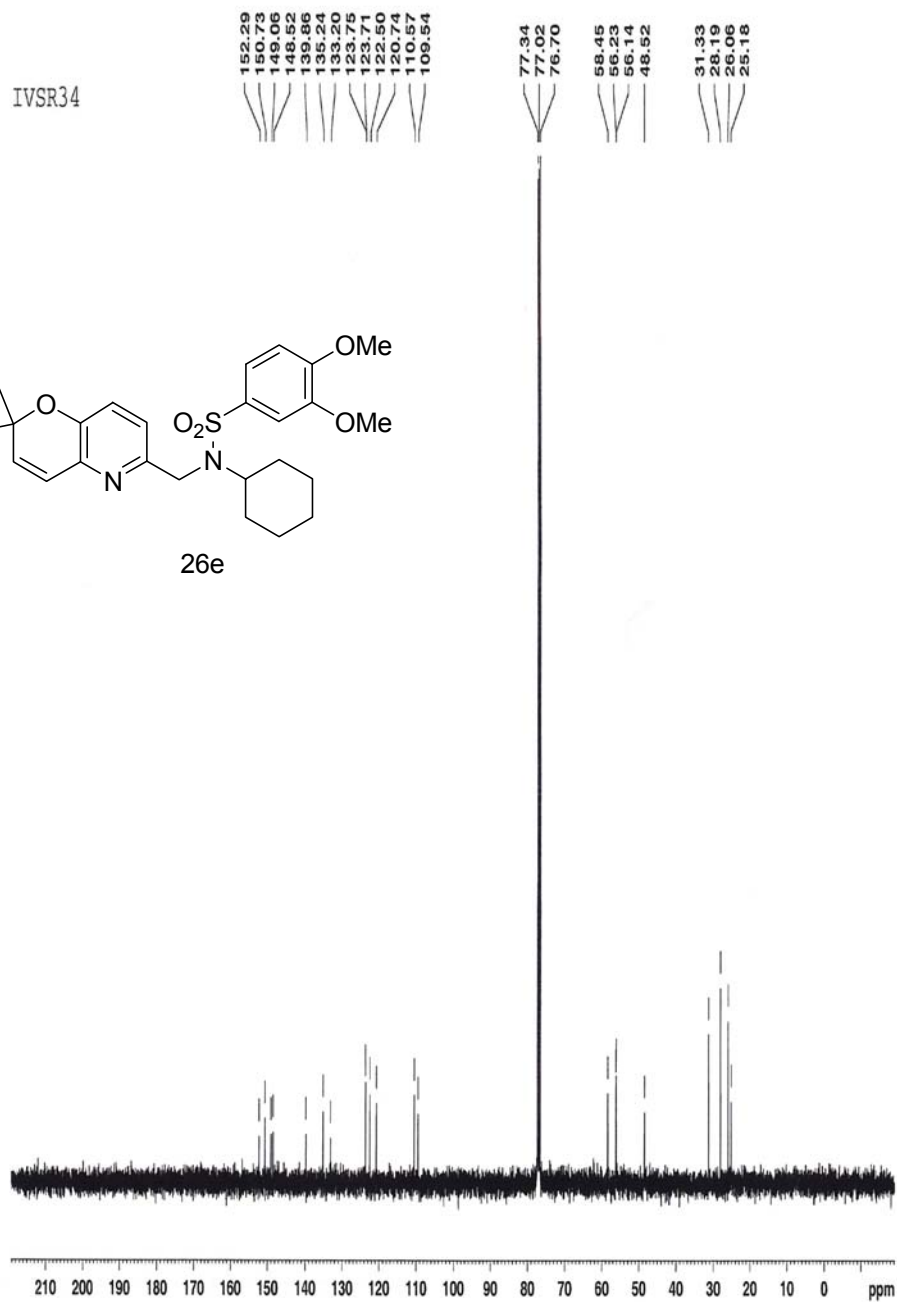
26d





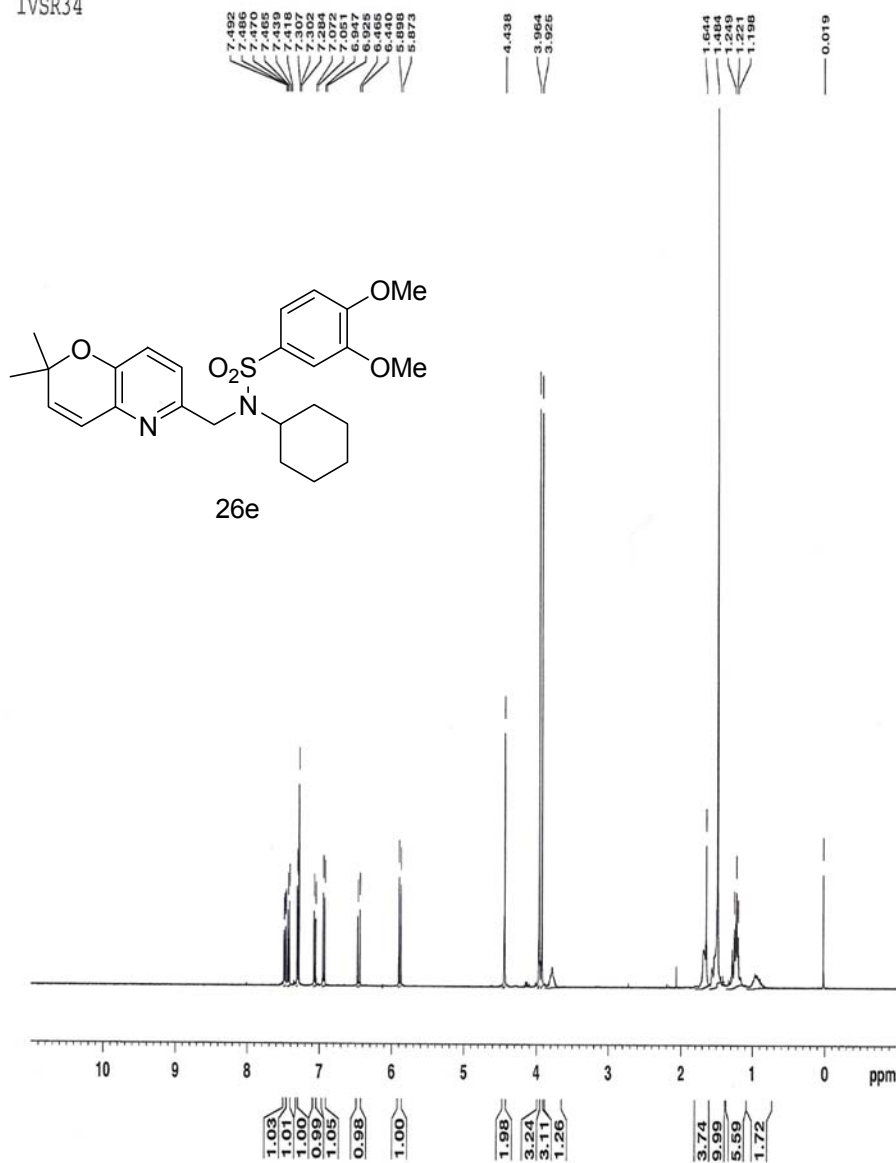
26e

IVSR34



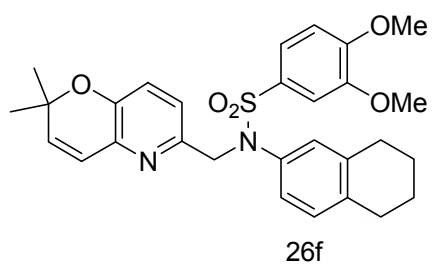
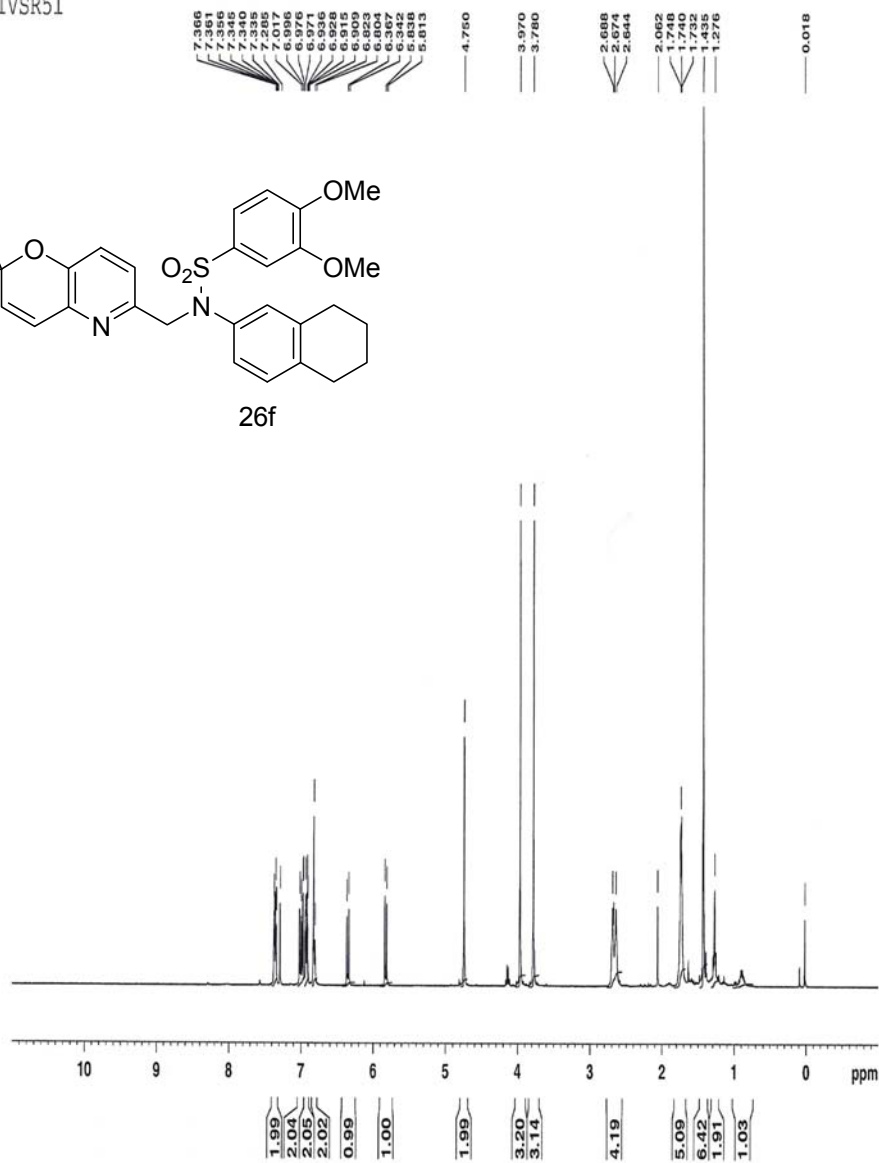
26e

IVSR34

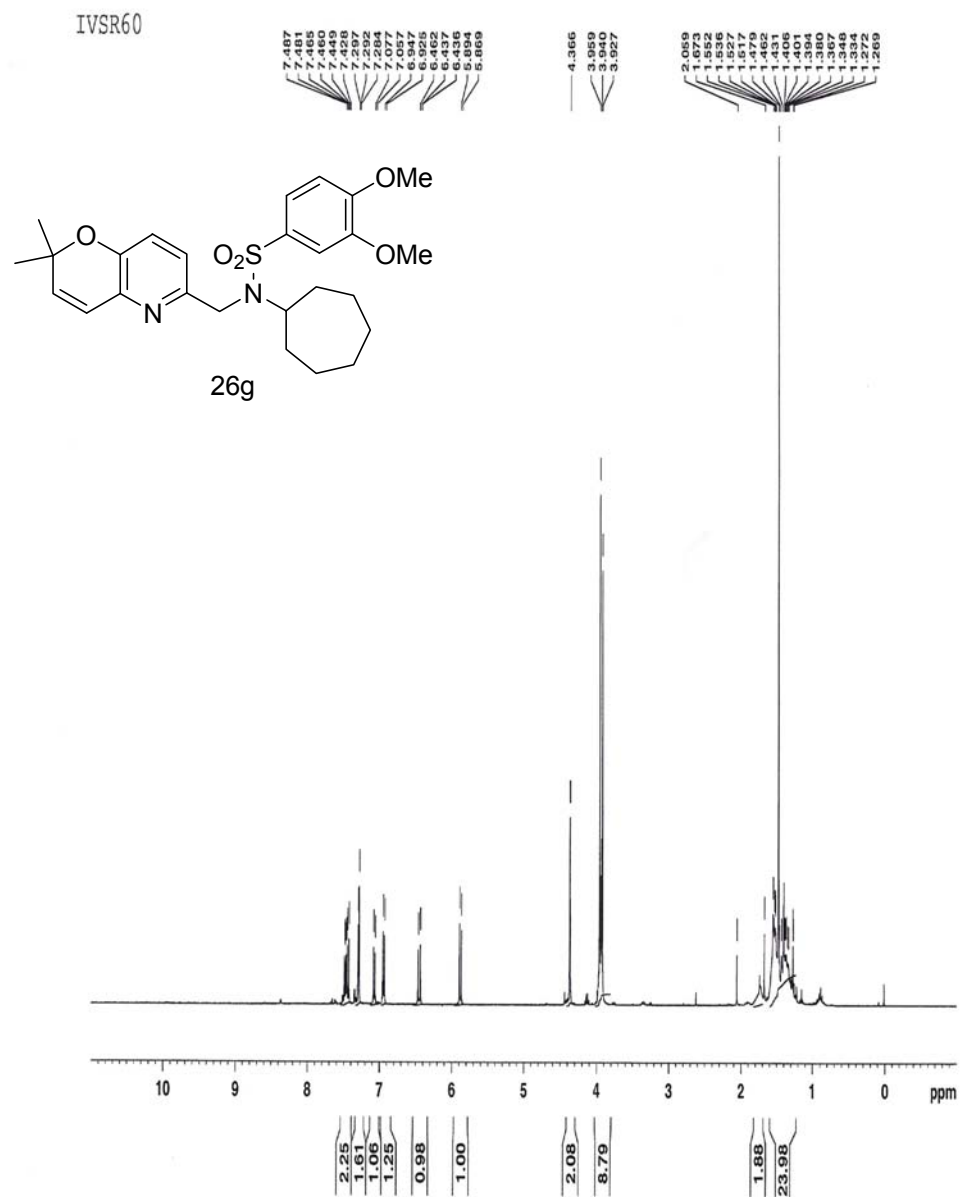


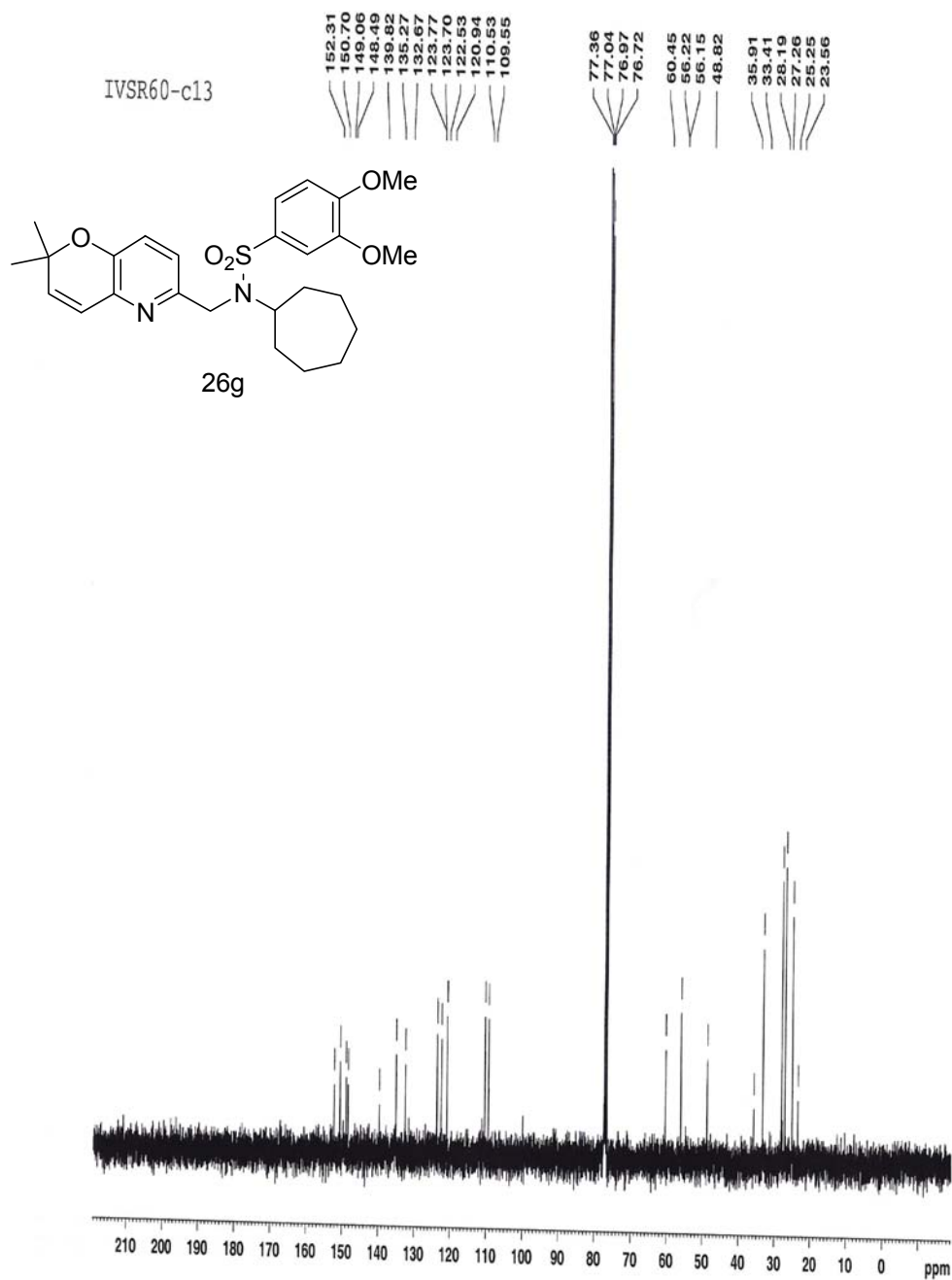
26f

IVSR51



26g





26h

No title



Current Data Parameters

NAME IVSR64a
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters

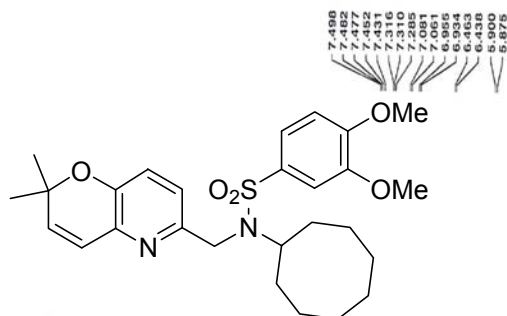
Date_ 20080506
Time 10.51
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 12
DS 2
SMH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 256
DW 60.400 usec
DE 7.00 usec
TE 297.8 K
D1 1.0000000 sec
MCREST 0.0000000 sec
MCWRK 0.0150000 sec

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

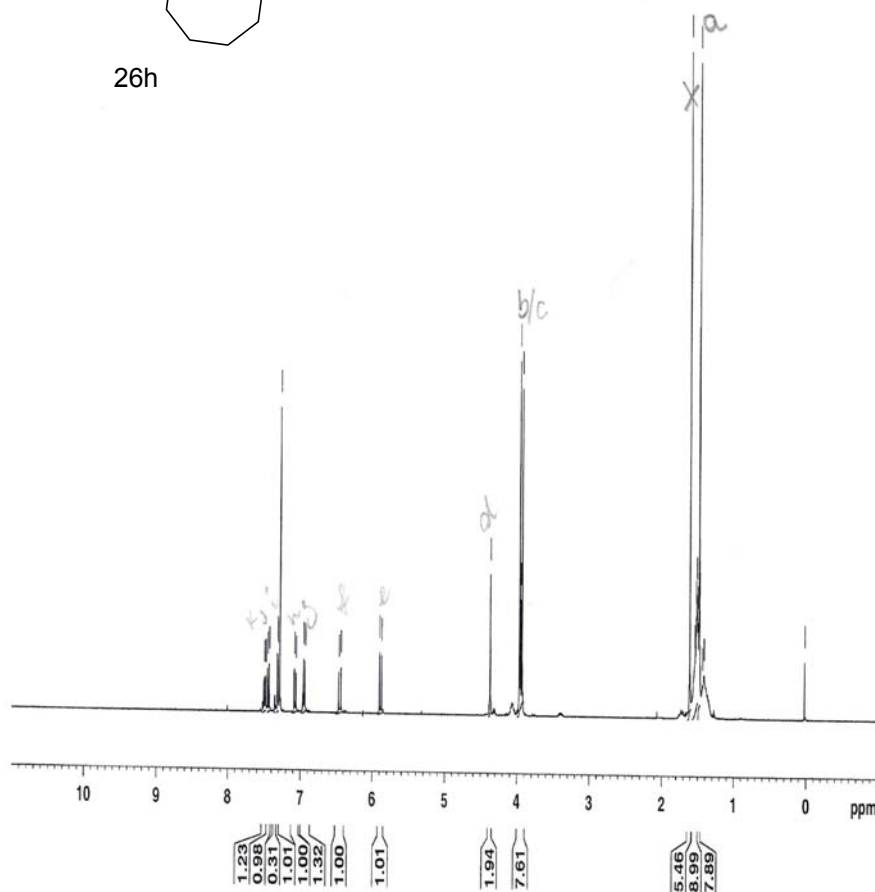
NUC1 1H
P1 12.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters

SI 32768
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

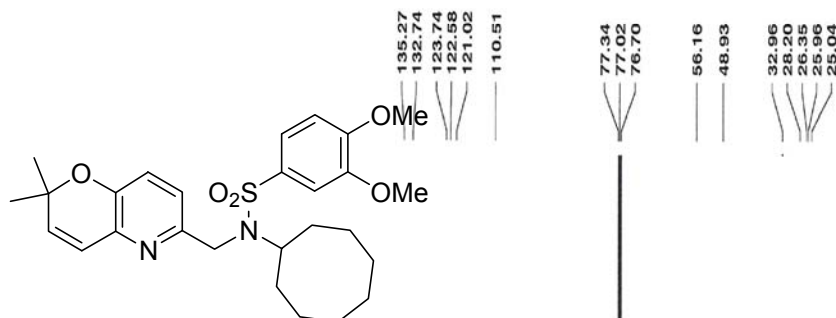


26h



26h

No title



26h



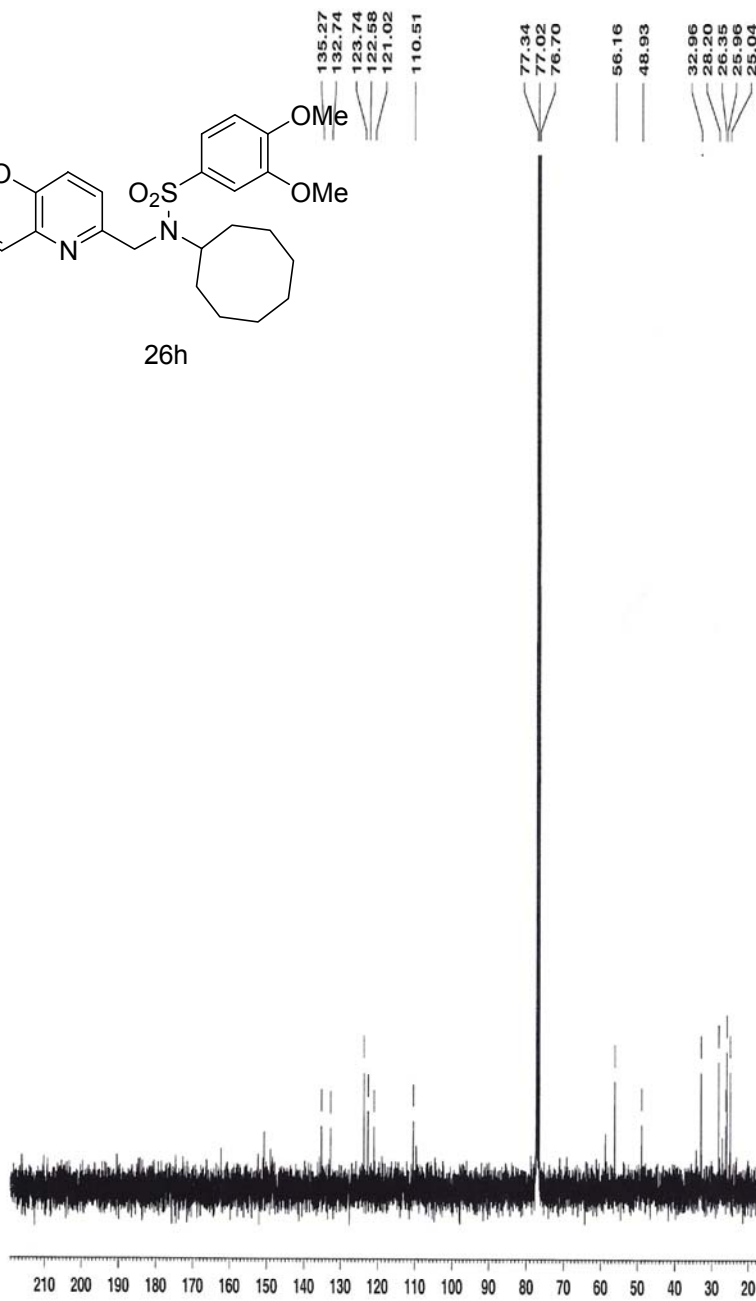
Current Data Parameters
 NAME IVSR64a-C13
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20080606
 Time 10.57
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 276
 DS 4
 SWH 23980.814 Hz
 FIDRES 0.365918 Hz
 AQ 1.3664756 sec
 RG 14596.5
 DW 20.850 usec
 DE 7.00 usec
 TE 298.1 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

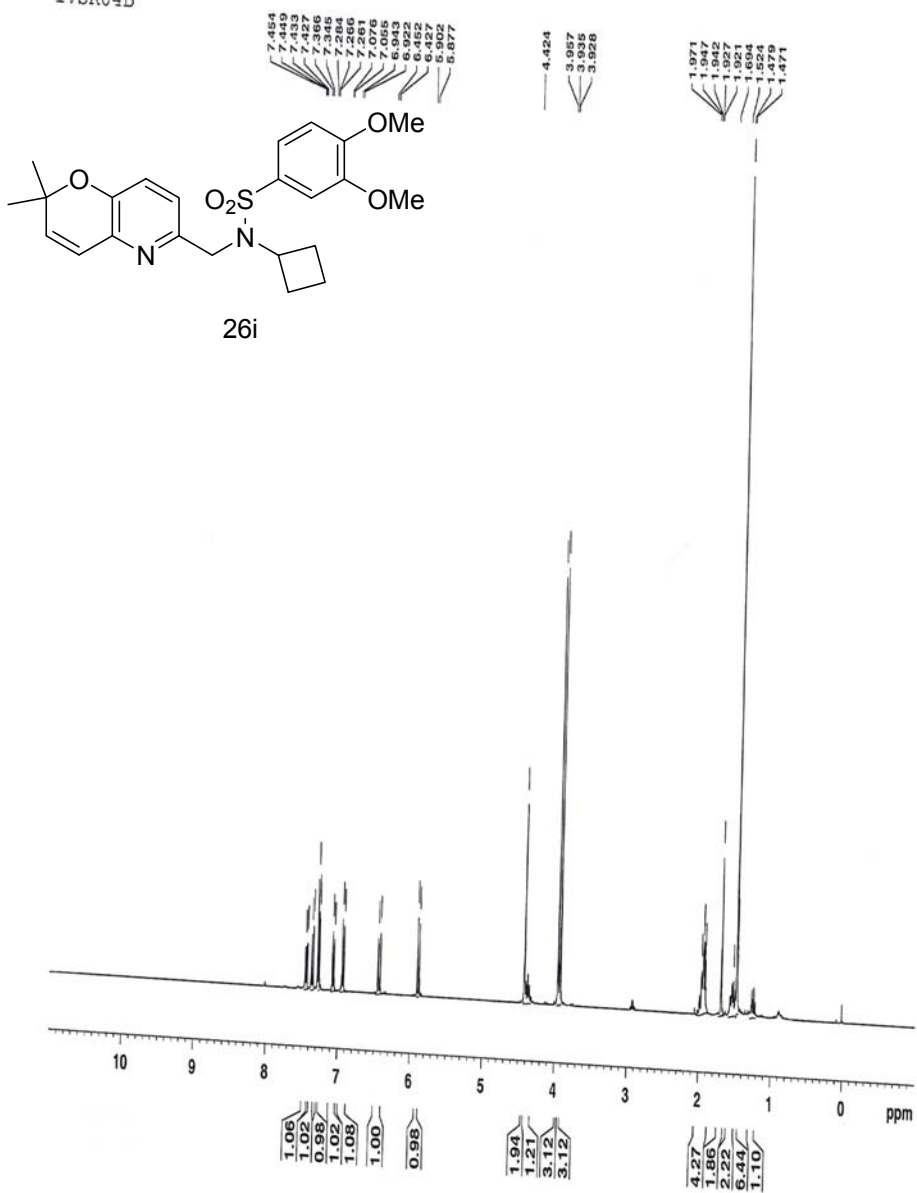
===== CHANNEL f2 =====
 CPDPRG2 waltz16
 NUC2 1H
 PCPD2 70.00 usec
 PL2 -1.00 dB
 PL12 14.00 dB
 PL13 14.00 dB
 SFO2 400.1316005 MHz

F2 - Processing parameters
 SI 32768
 SF 100.6127690 MHz
 MDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40



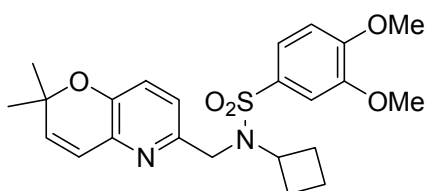
26i

IVSR64B

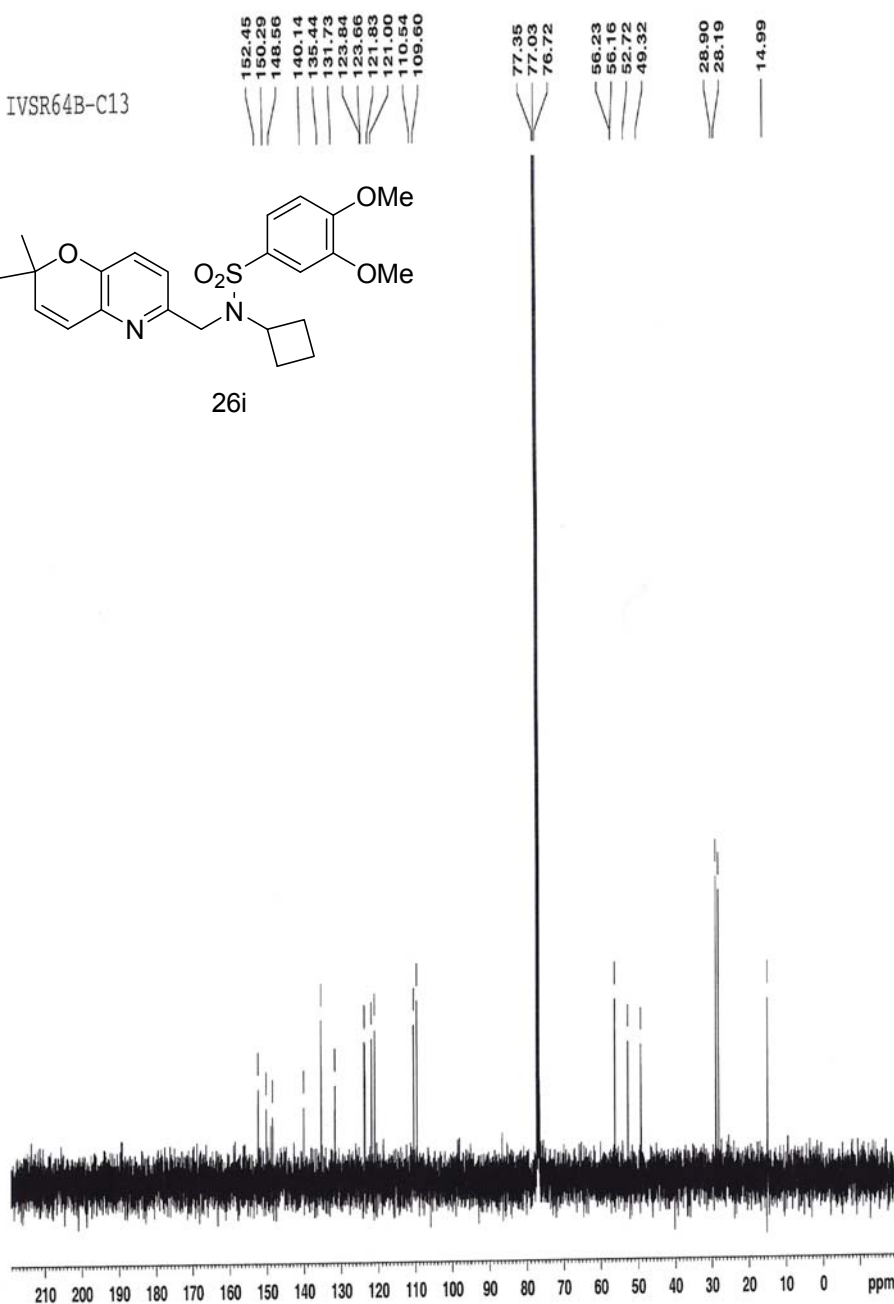


26i

IVSR64B-C13



26i



No title

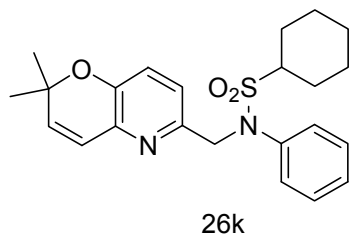
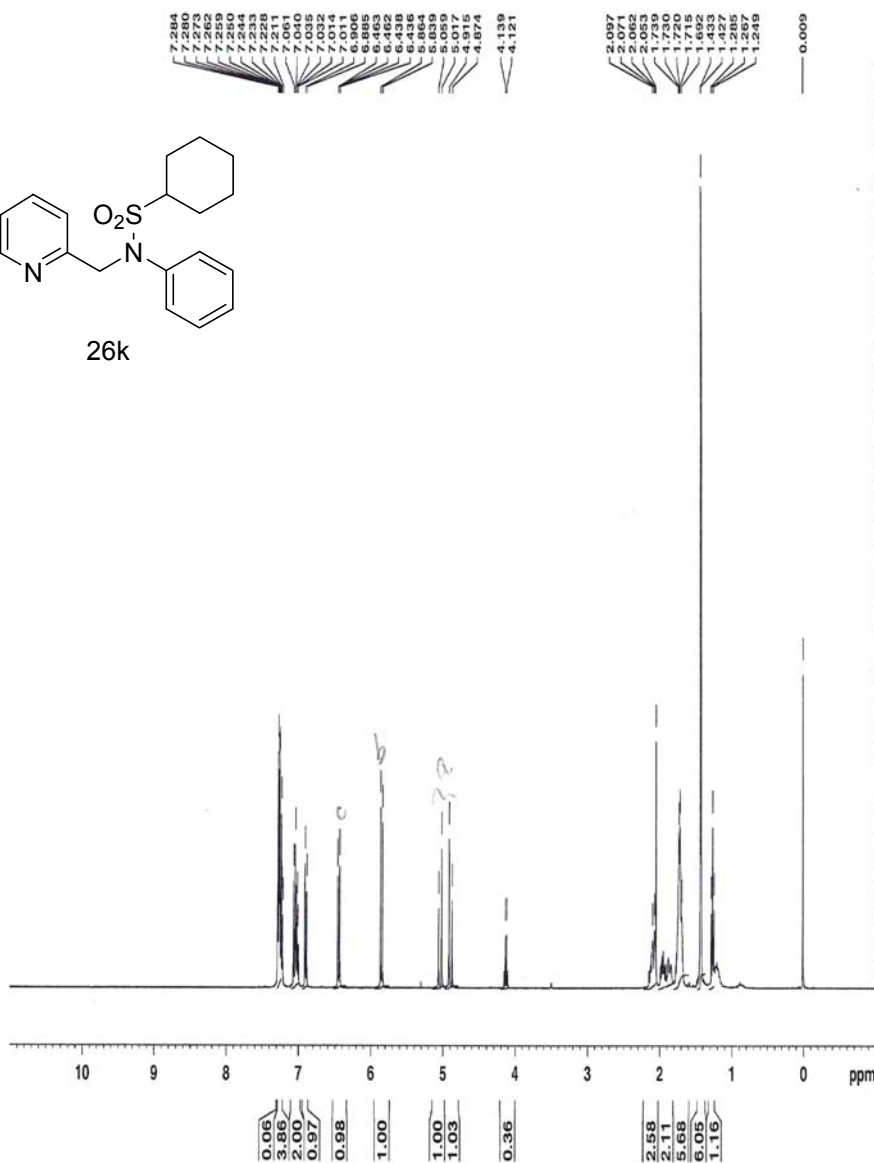


Current Data Parameters
 NAME IV-SR73
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20080617
 Time 9.47
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 17
 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 297.9 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRK 0.01500000 sec

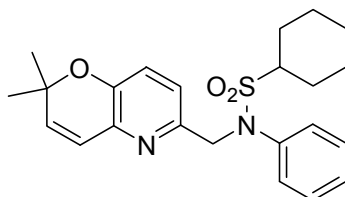
===== CHANNEL f1 =====
 NUC1 1H
 P1 12.80 usec
 PL1 0.00 dB
 SFO1 400.1324710 MHz

F2 - Processing parameters
 SI 32768
 SF 400.1300046 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



26k

No title



26k



Current Data Parameters
 NAME IV-SR73-C13
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20080617
 Time 10.05
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 24
 DS 4
 SWH 23980.814 Hz
 FIDRES 0.365918 Hz
 AQ 1.3664756 sec
 RG 7298.2
 DW 20.850 usec
 DE 7.00 usec
 TE 298.1 K
 D1 2.0000000 sec
 d11 0.0300000 sec
 DELTA 1.89999998 sec
 MCREST 0.0000000 sec
 MCWPK 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 100.6127690 MHz
 MDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.00



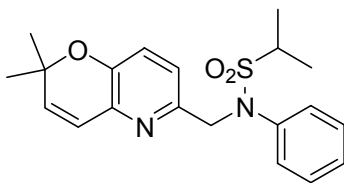
262

IVSR75

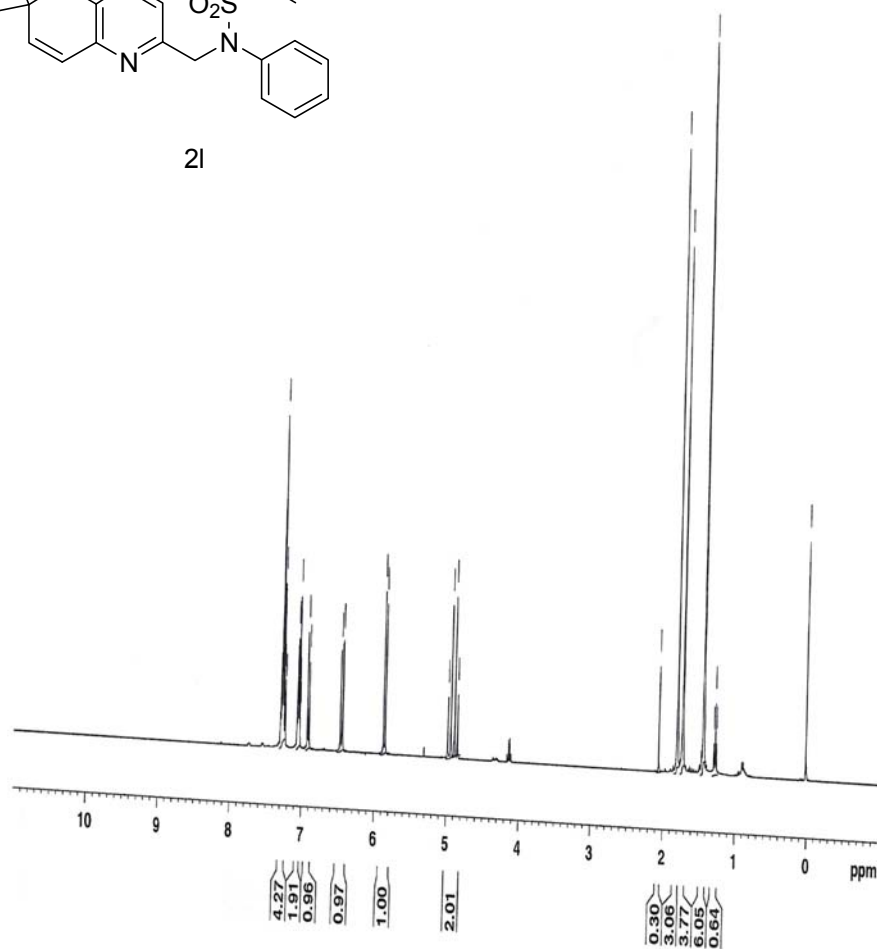
7.303
7.298
7.292
7.287
7.280
7.275
7.268
7.261
7.254
7.236
7.230
7.060
7.055
7.047
7.039
7.030
6.925
6.918
6.472
6.465
6.447
5.001
4.896
4.886
4.855

2.054
1.910
1.734
1.447
1.296
1.278
1.261

0.020

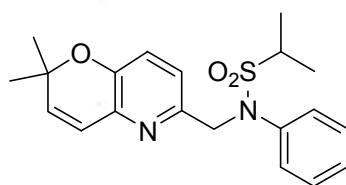


2l

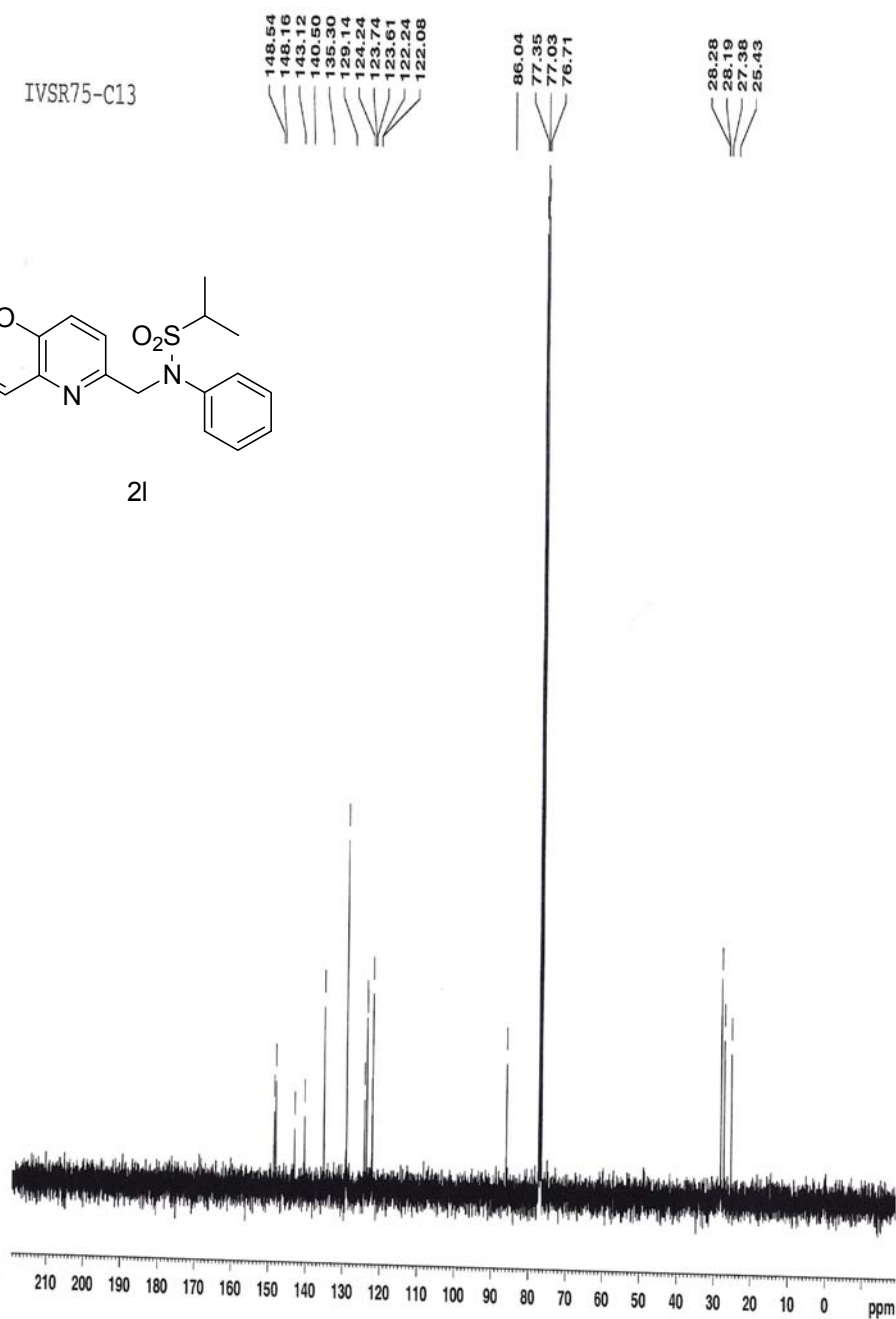


26d.

IVSR75-C13

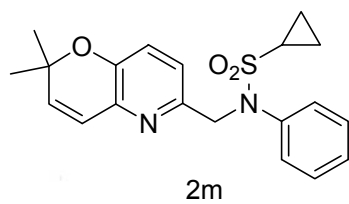
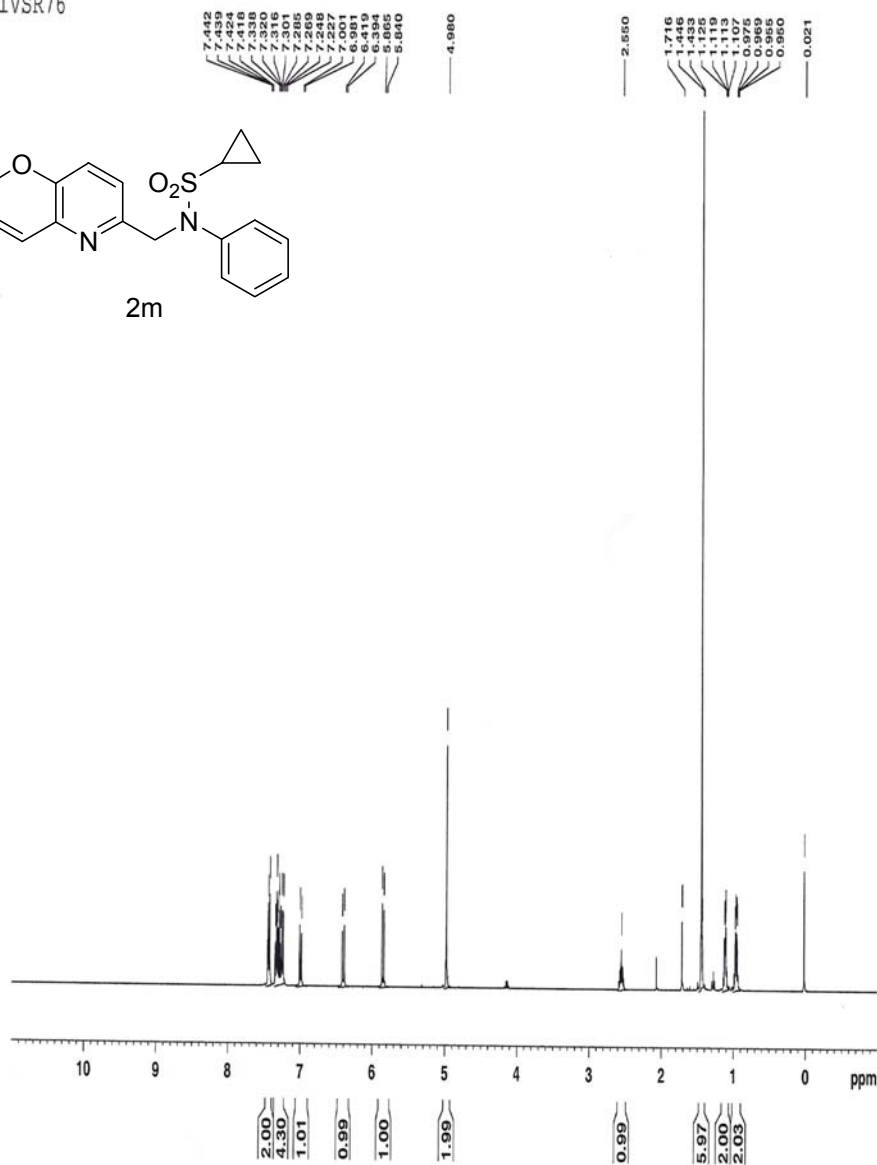


2l



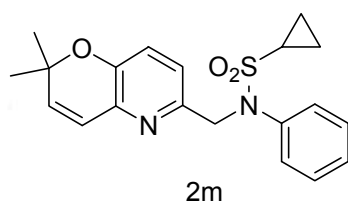
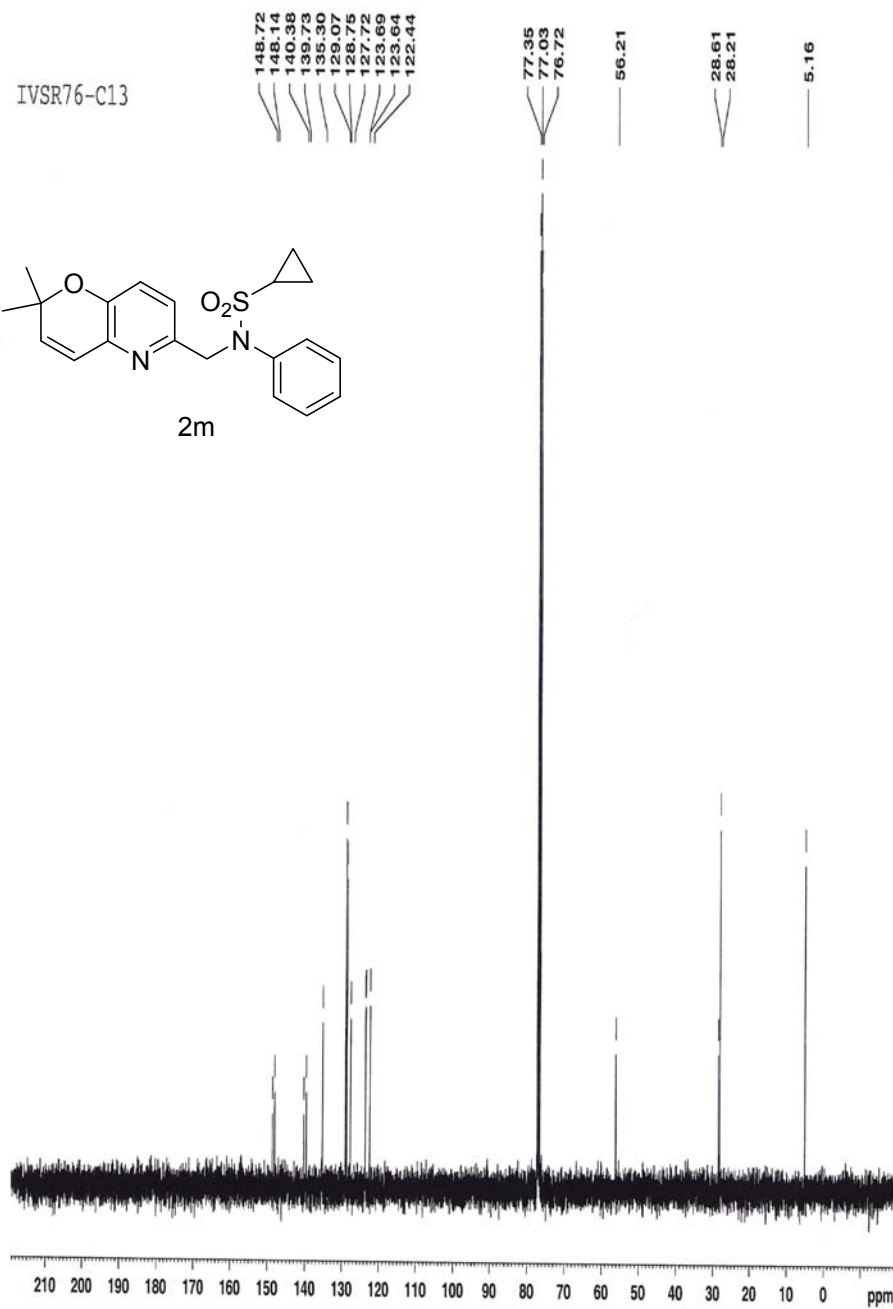
26 m

IVSR76



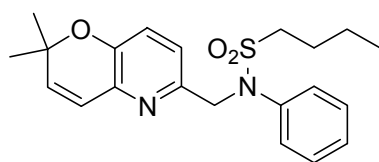
26m.

IVSR76-C13

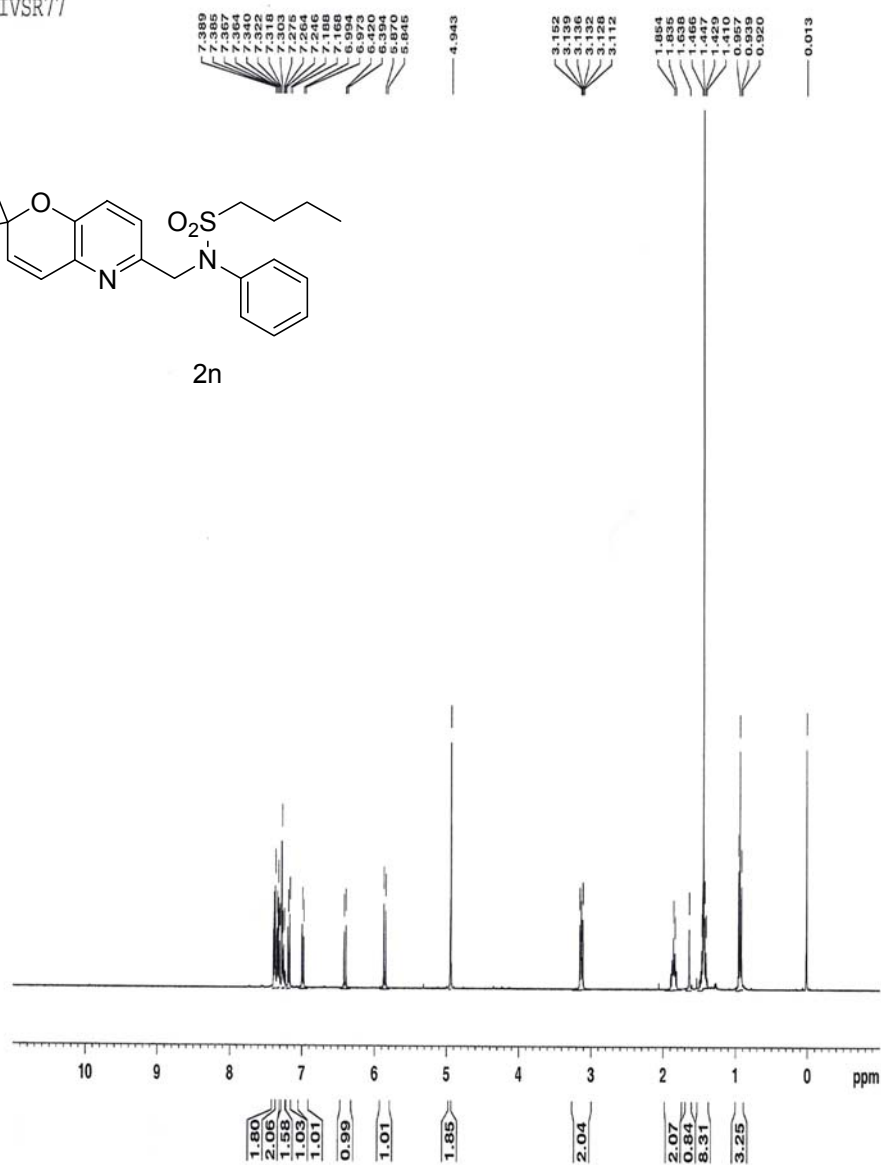


26n

IVSR77



2n

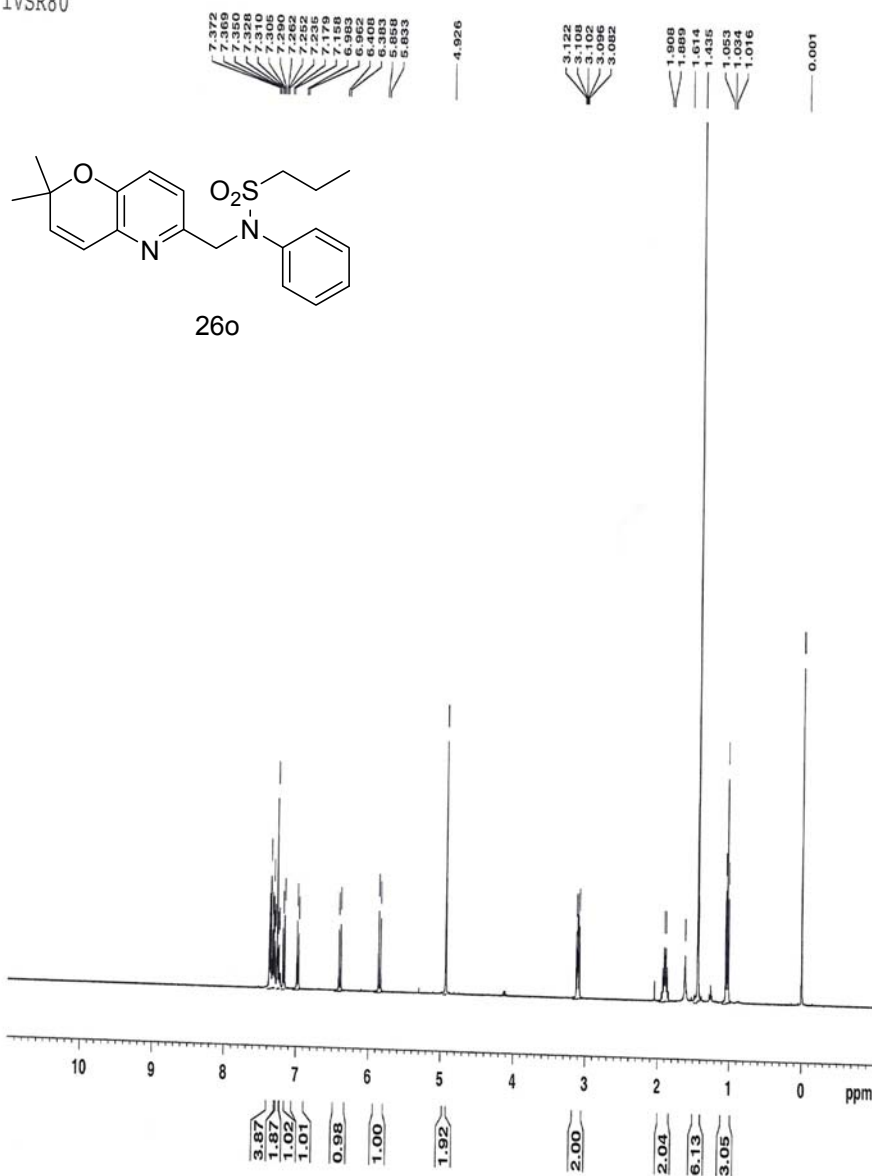


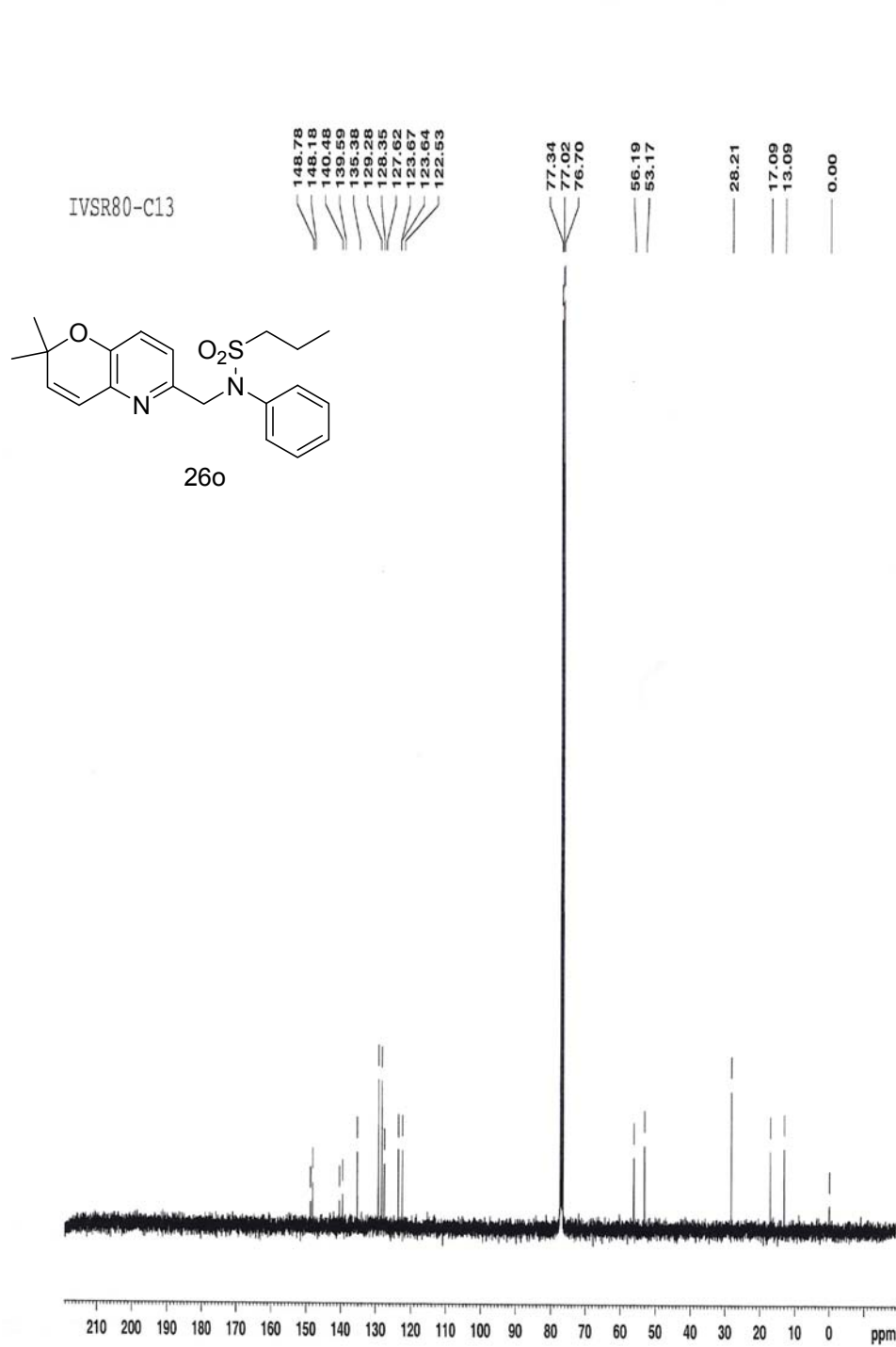
26n



260

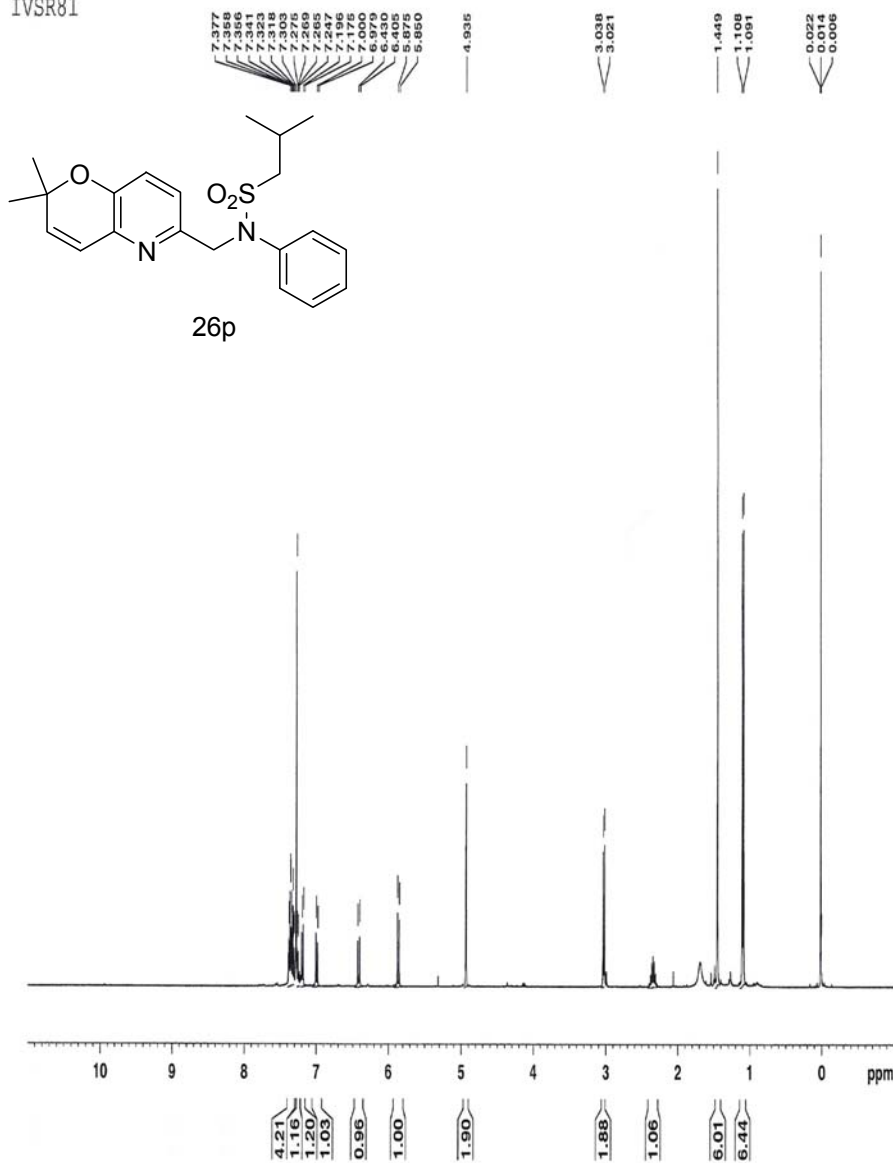
IVSR80





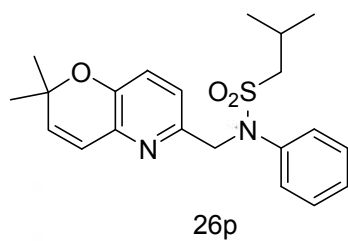
26p

IVSR81

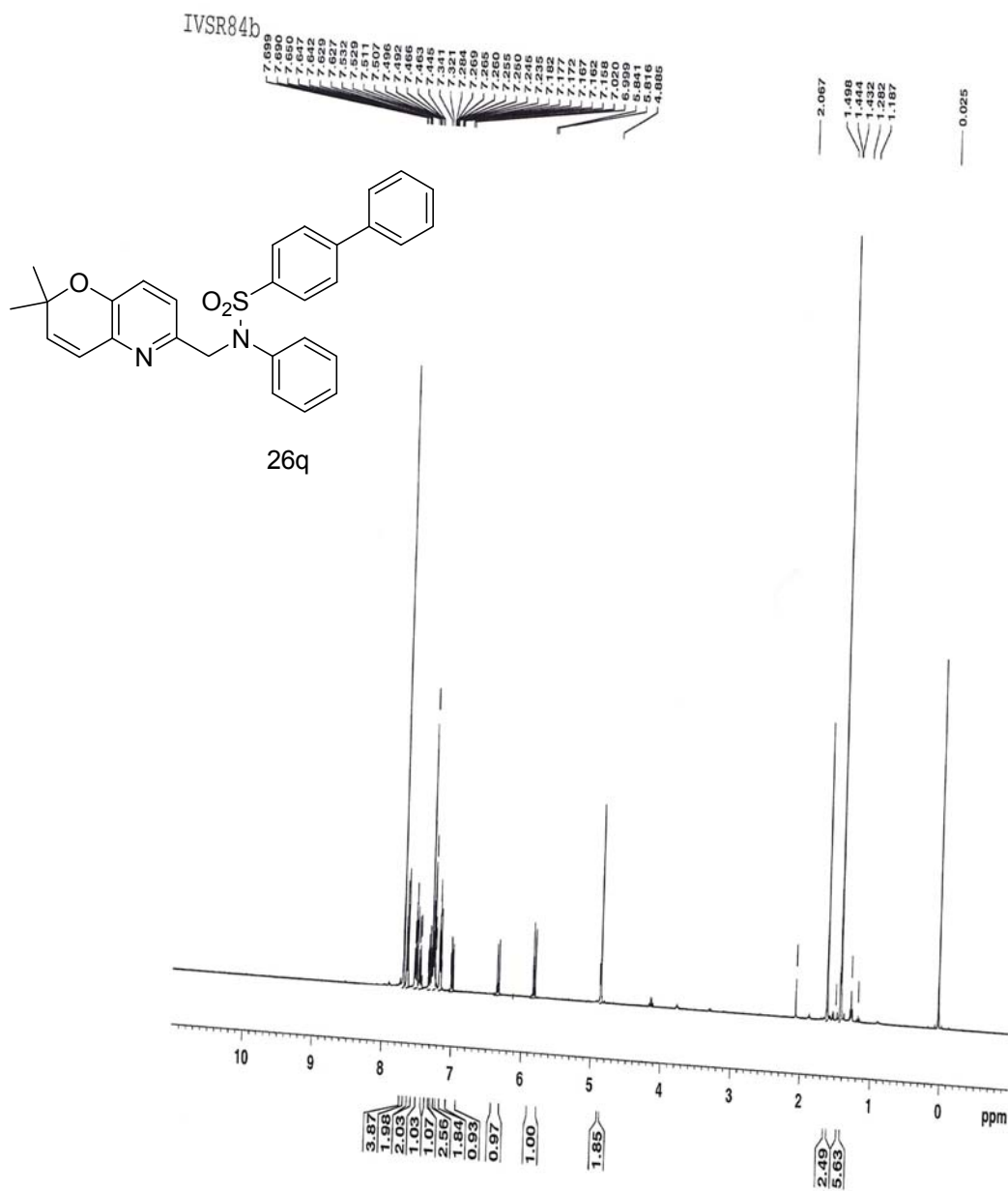


26p

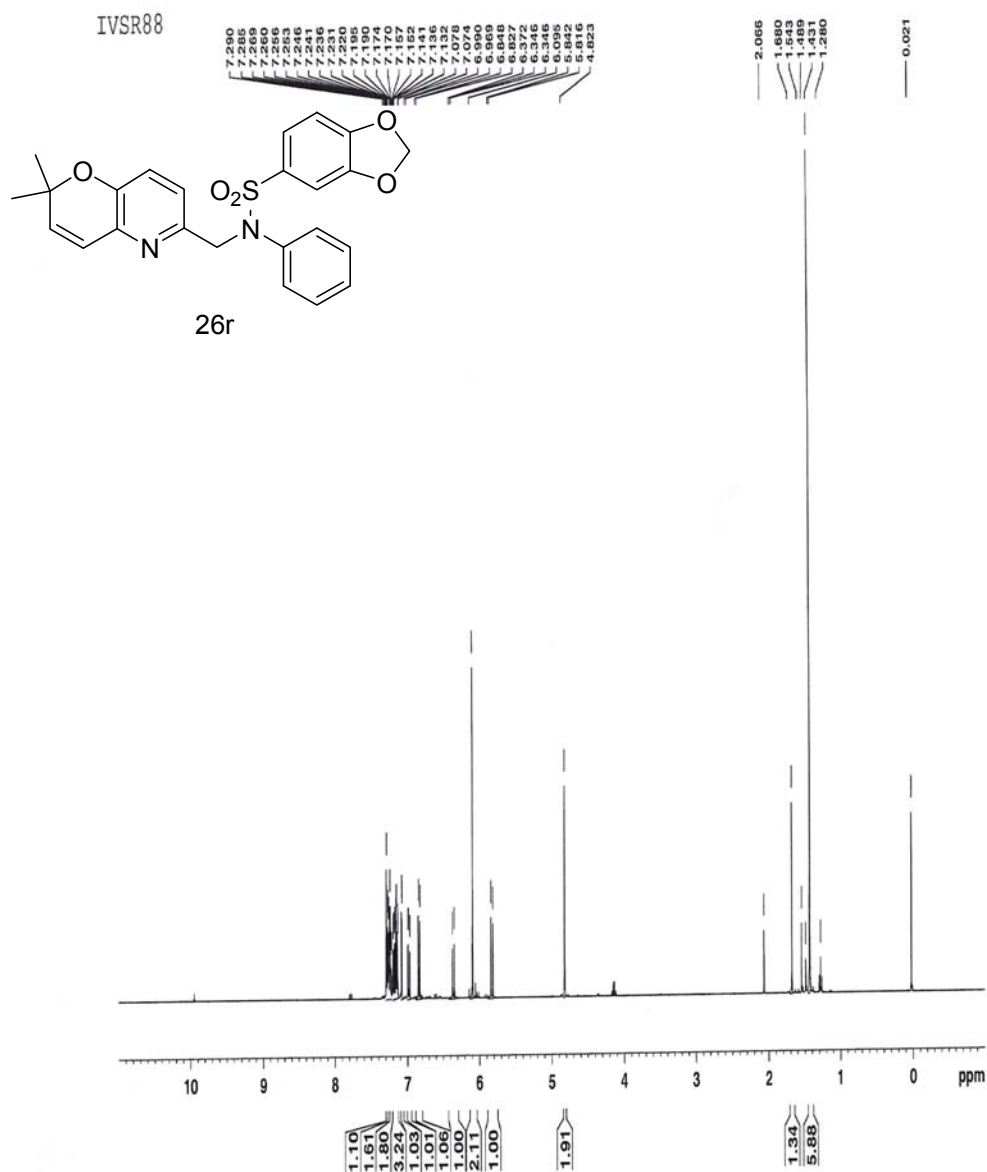
IVSR81-C13



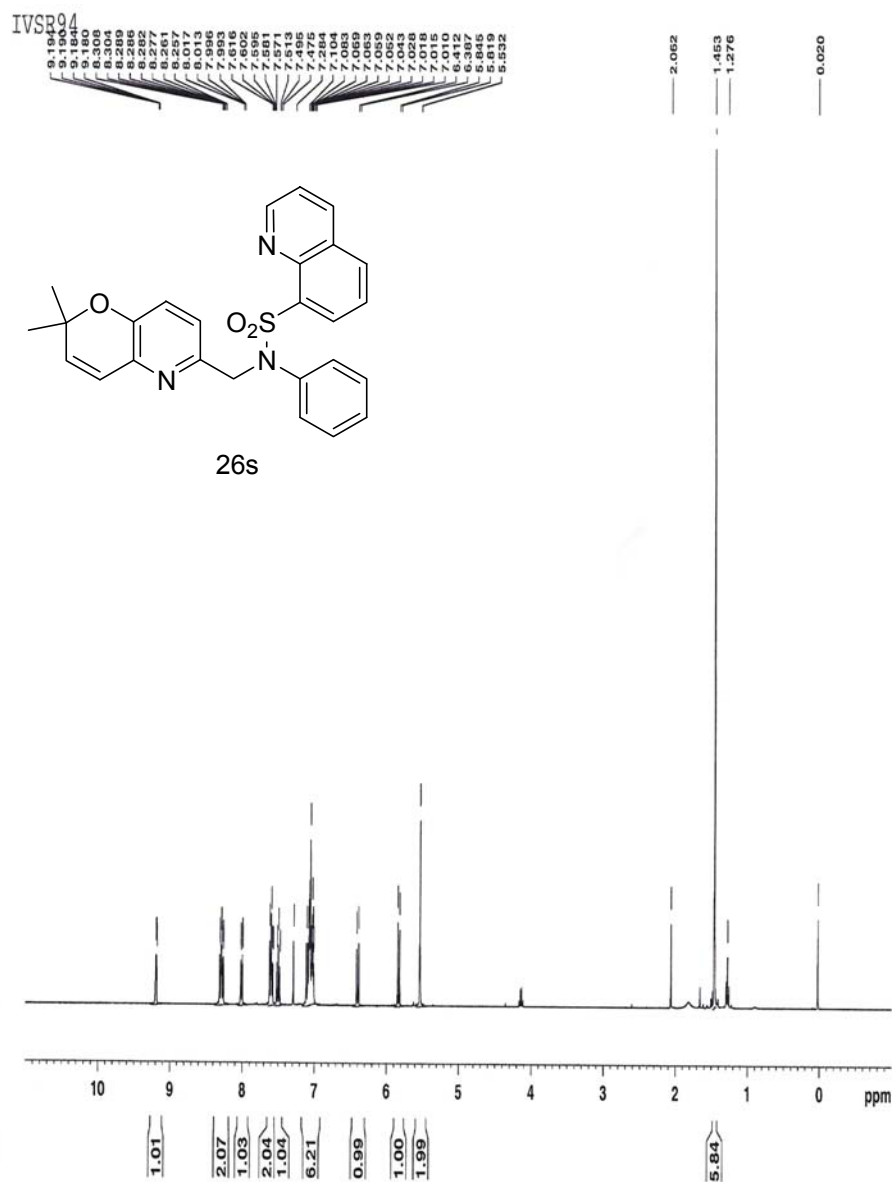
26q



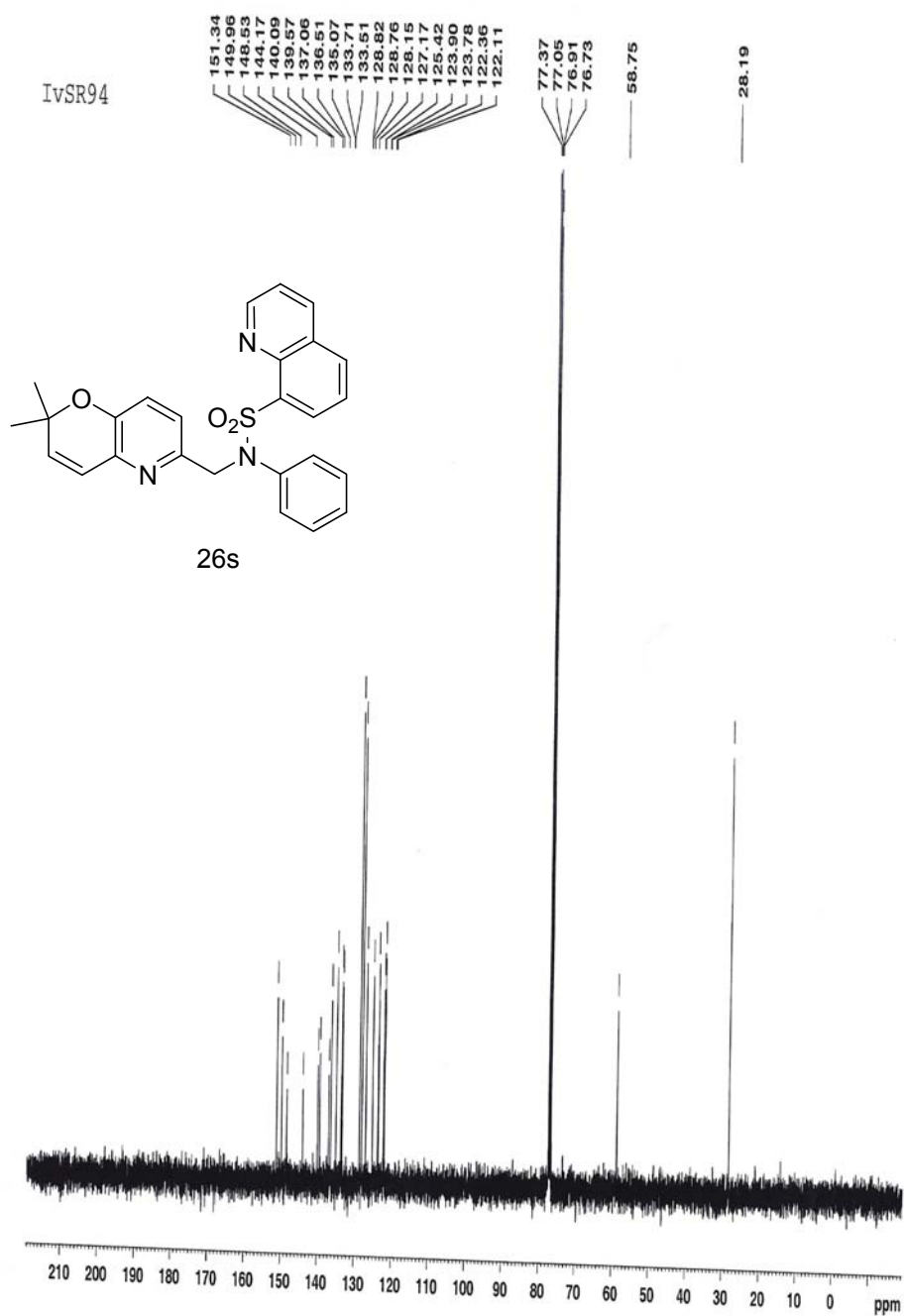
26r



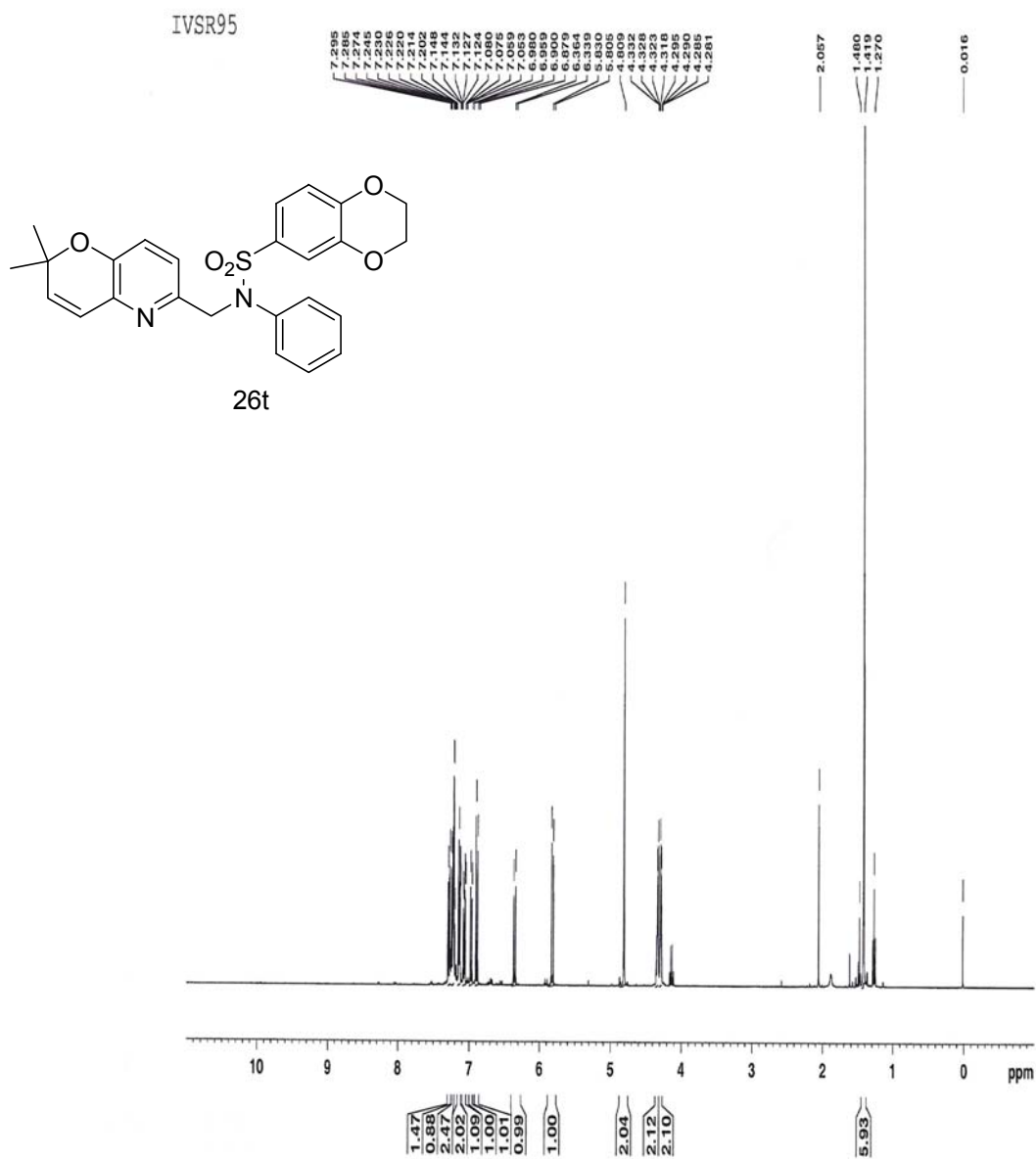
26s



26s.

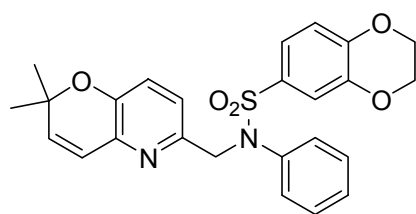


26t

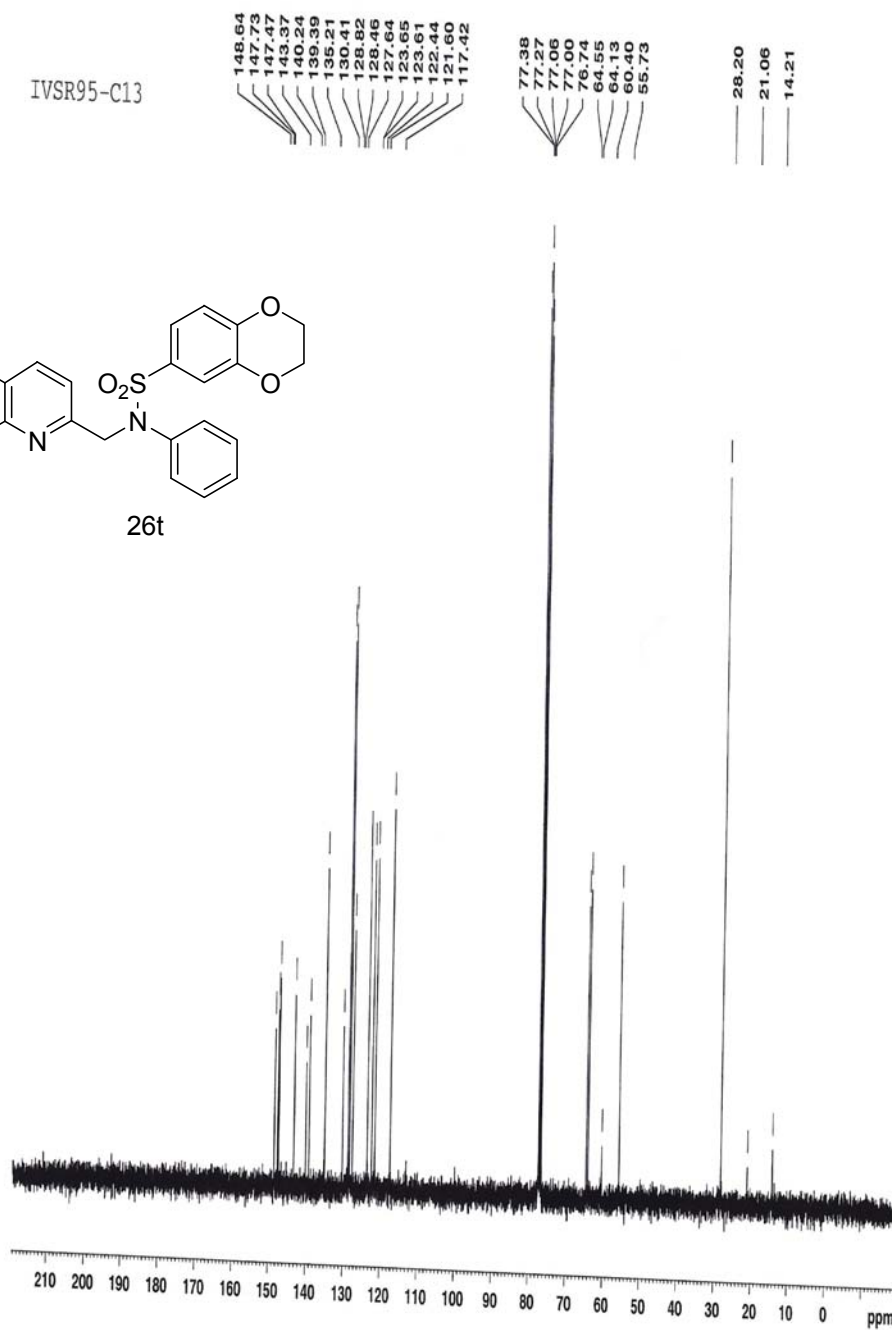


26t

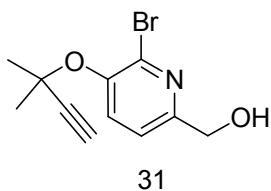
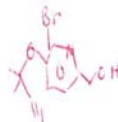
IVSR95-C13



26t



No title



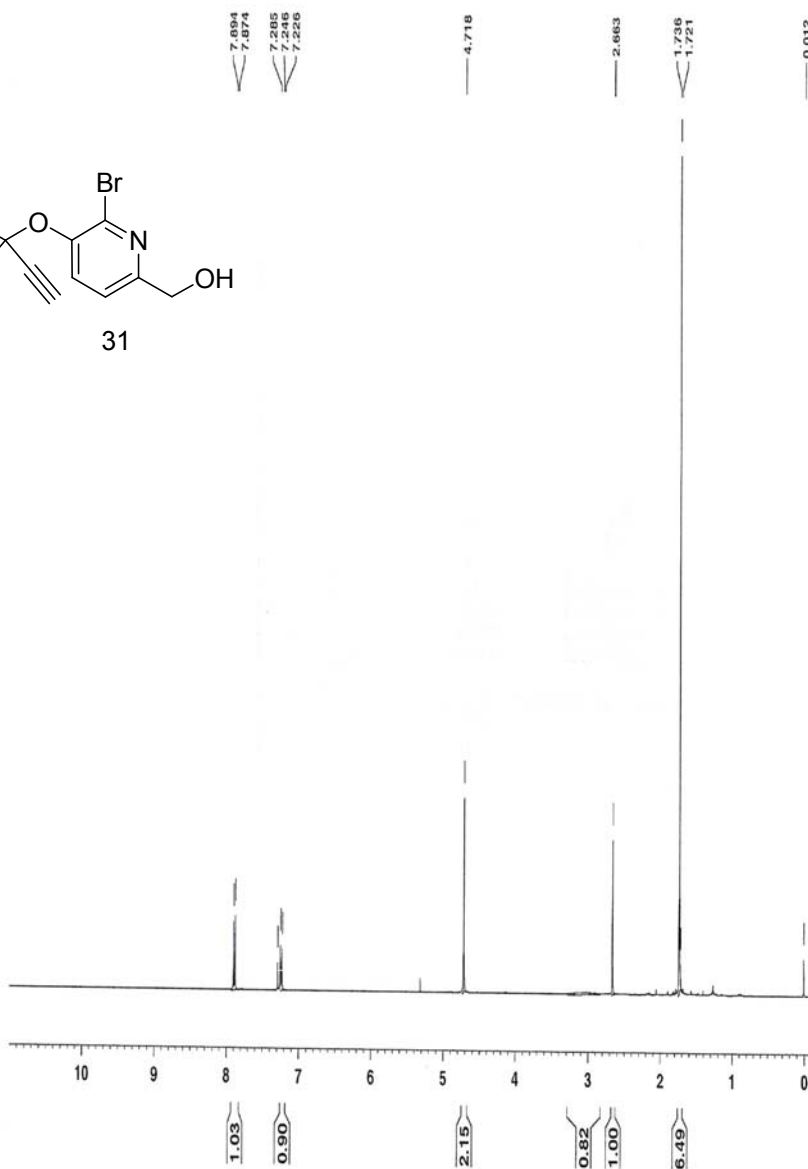
Current Data Parameters
 NAME JH133 H1
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20080423
 Time 17.32
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 1
 DS 4
 SWH 23980.814 Hz
 FIDRES 0.365918 Hz
 AQ 1.3664756 sec
 RG 16384
 DW 20.850 usec
 DE 7.00 usec
 TE 298.3 K
 D1 2.00000000 sec
 d11 0.03000000 sec
 DELTA 1.89999998 sec
 MCREST 0.00000000 sec
 MCWRK 0.01500000 sec

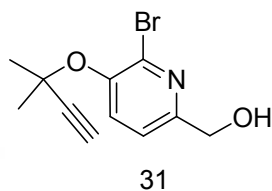
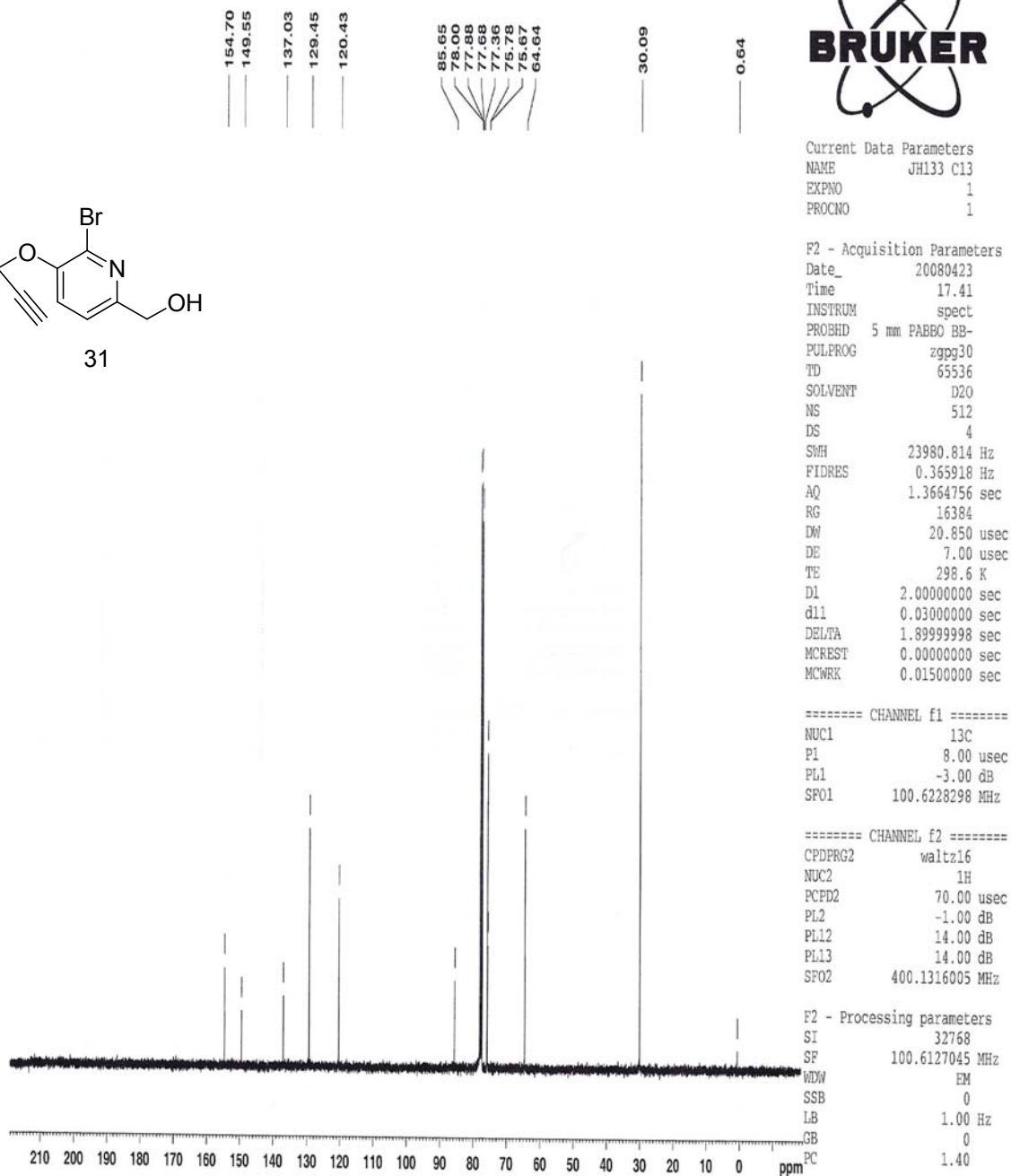
===== CHANNEL f1 =====
 NUC1 13C
 P1 8.00 usec
 PL1 -3.00 dB
 SFO1 100.6228298 MHz

===== 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

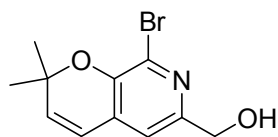
F2 - Processing parameters
 SI 32768
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.40



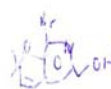
No title



32
No title



32

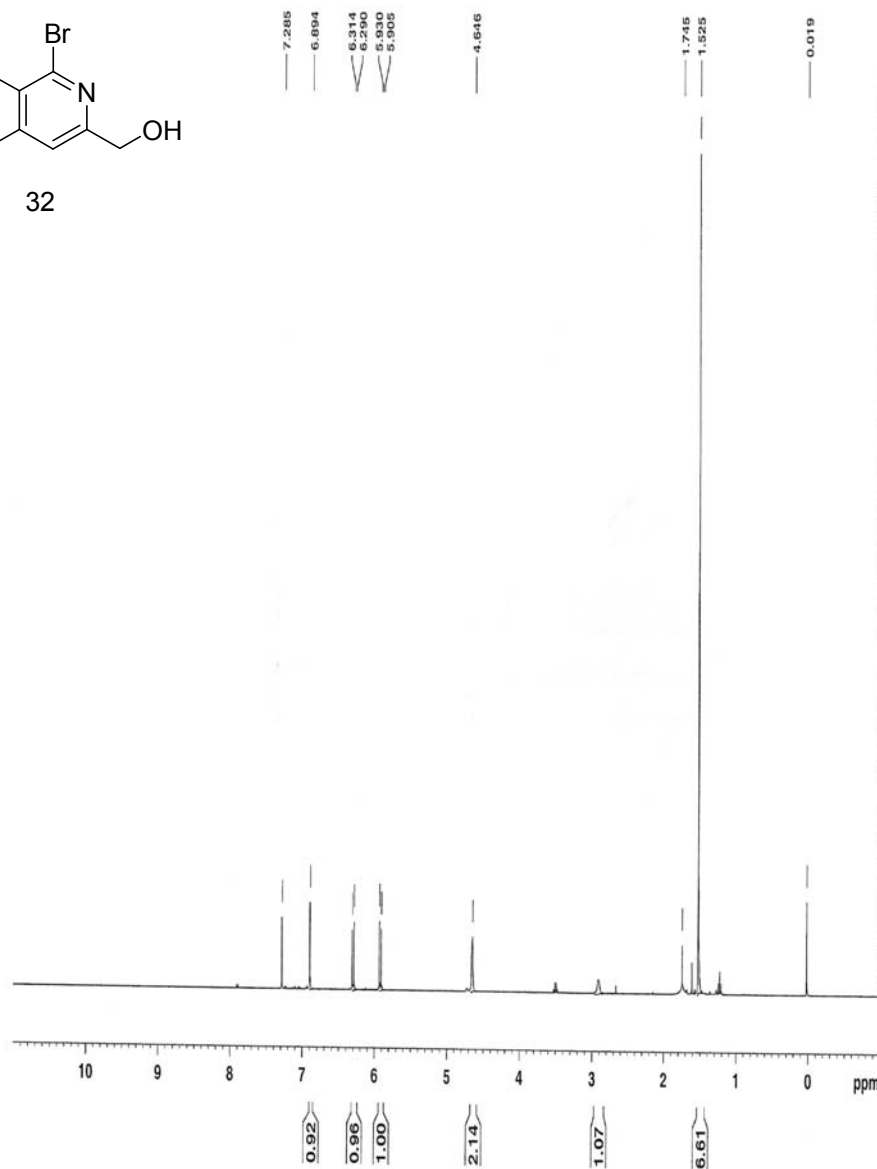


Current Data Parameters
NAME JH136 H1
EXPNO 1
PROCNO 1

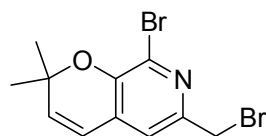
F2 - Acquisition Parameters
Date_ 20080428
Time 9.56
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 228.1
DW 60.400 usec
DE 7.00 usec
TE 298.0 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

===== CHANNEL f1 =====
NUC1 1H
P1 12.80 usec
PL1 0.00 dB
SF01 400.1324710 MHz

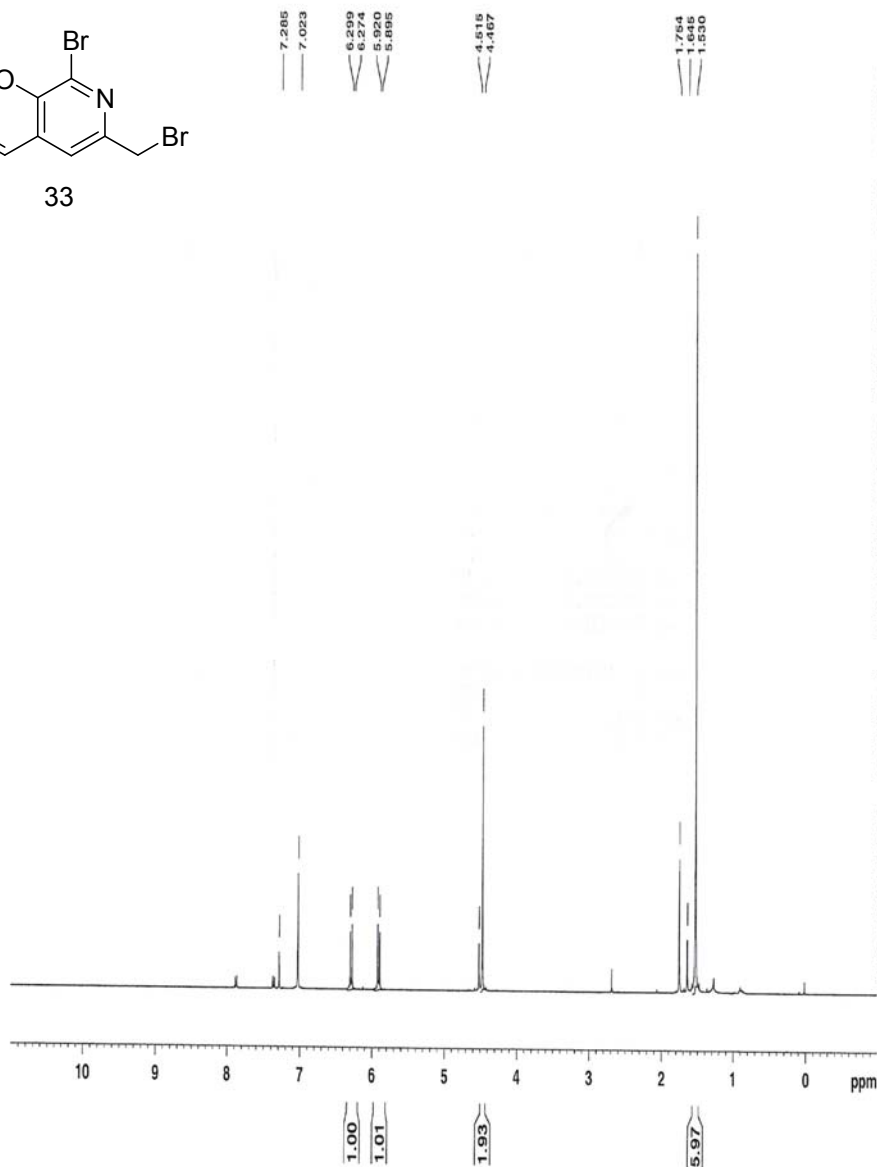
F2 - Processing parameters
SI 32768
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



No title



33



Current Data Parameters
NAME JH-II-37 H1
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20080723
Time 14.52
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT D2O
NS 16
DS 2
SMH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 203.2
DW 60.400 usec
DE 7.00 usec
TE 298.7 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 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

No title

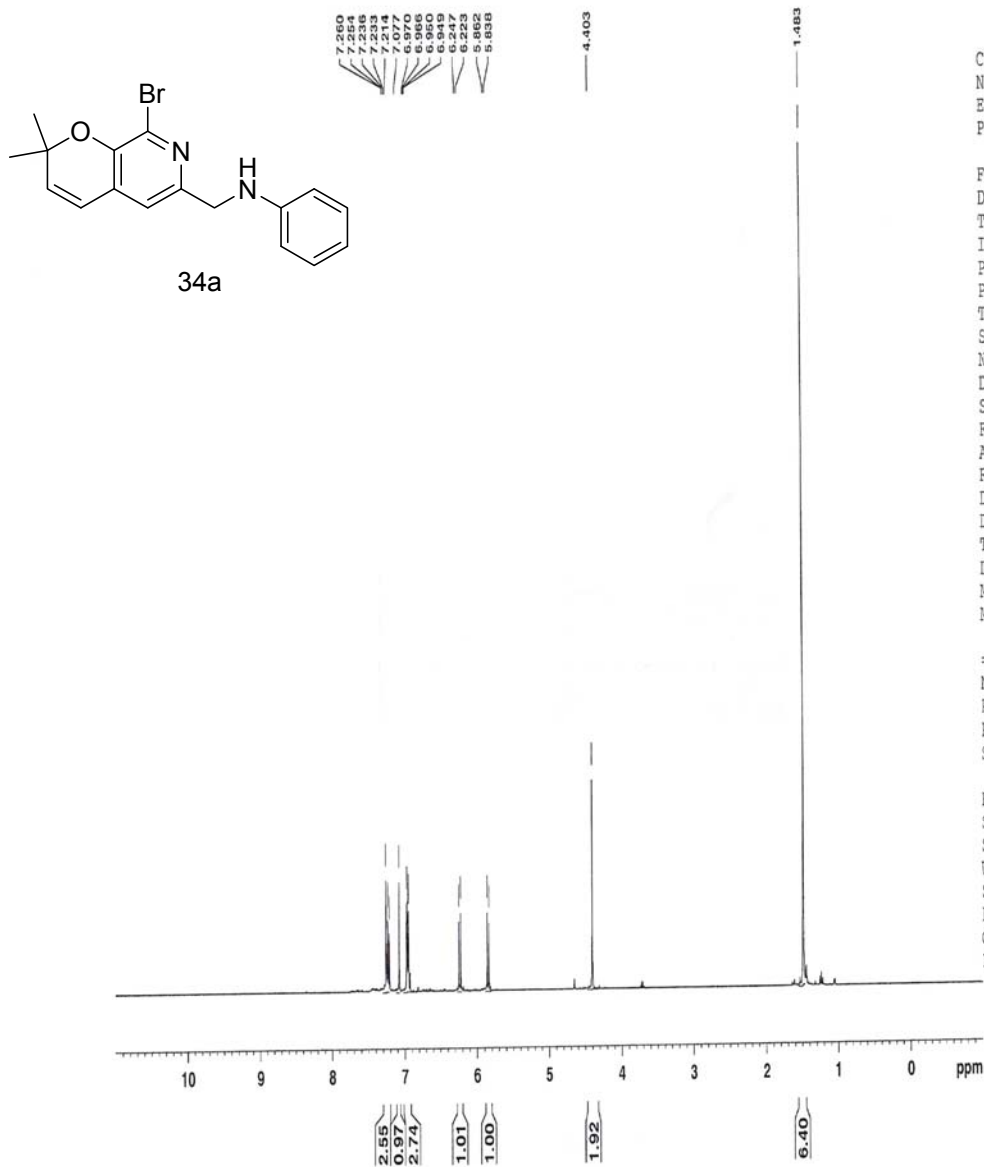


Current Data Parameters
 NAME JH-II-66 H1
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20080821
 Time 11.32
 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 3.9584243 sec
 RG 287.4
 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 400.1300099 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



No title

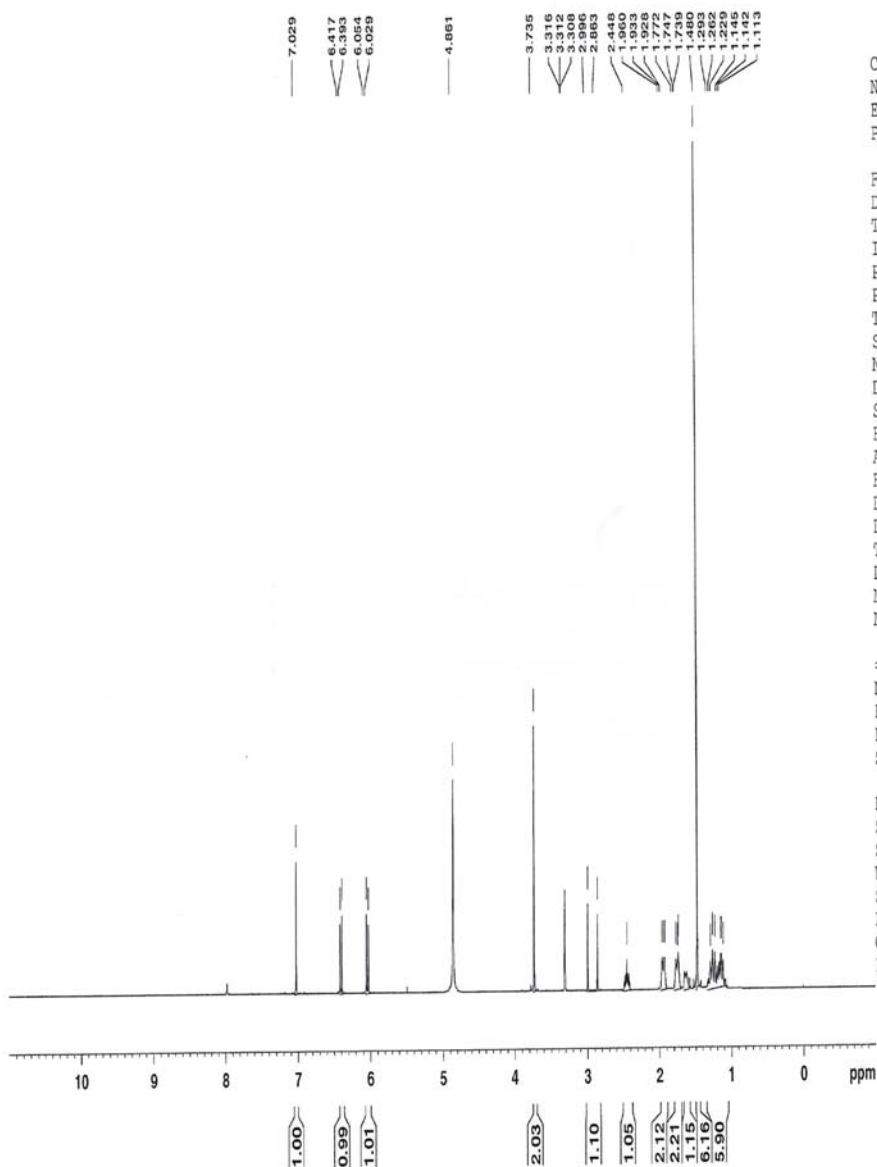


Current Data Parameters
 NAME JH-II-73 H1
 EXPNO 1
 PROCNO 1

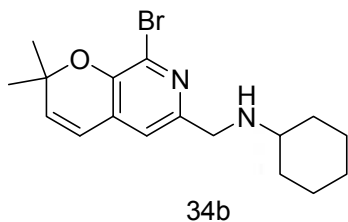
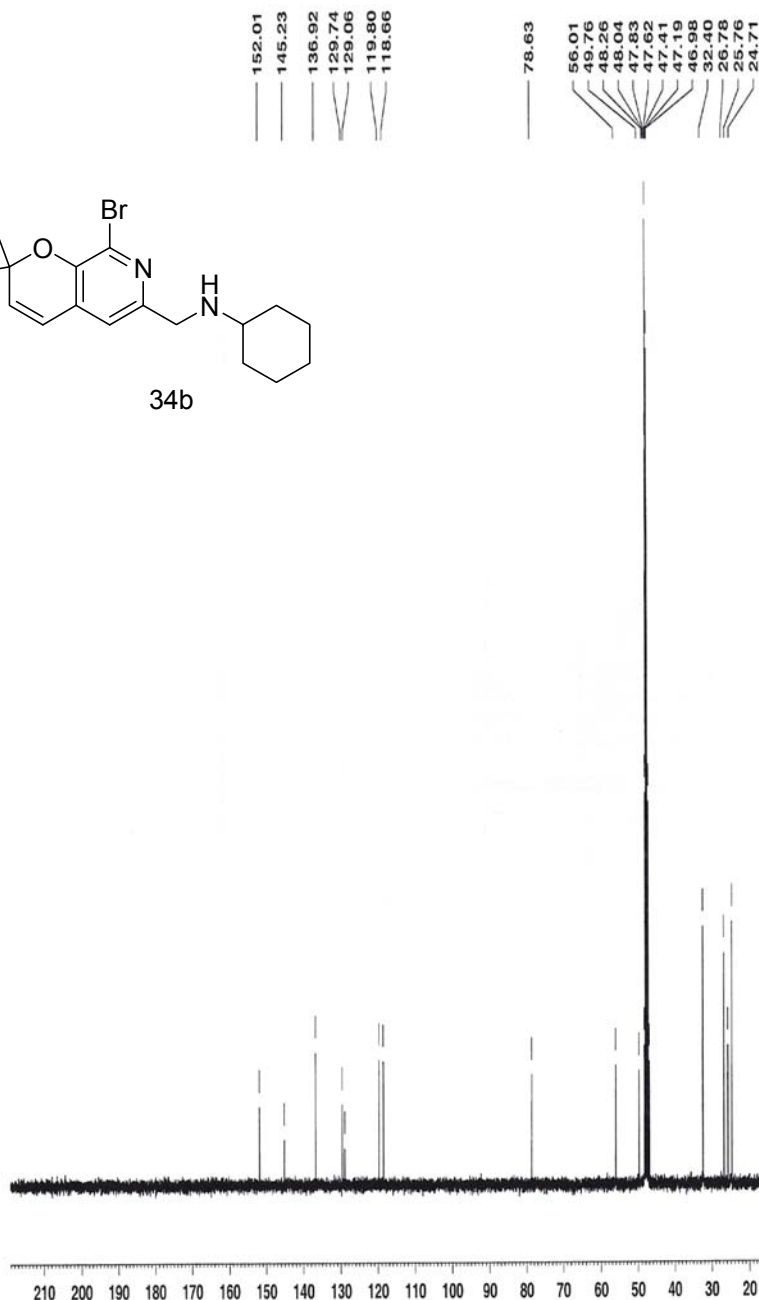
F2 - Acquisition Parameters
 Date_ 20080904
 Time 10.44
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 16
 DS 2
 SMH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 80.6
 DW 60.400 usec
 DE 7.00 usec
 TE 298.0 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRK 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 400.1315833 MHz
 WDM EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



No title



Current Data Parameters

NAME JH-II-73 C13
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters

Date_ 20080904
Time 10.54
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 67
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 298.1 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 100.6127690 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

No title



Current Data Parameters

NAME JH-II-68 H1
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters

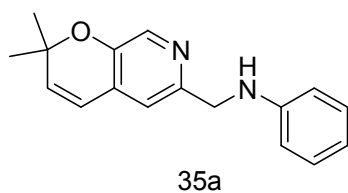
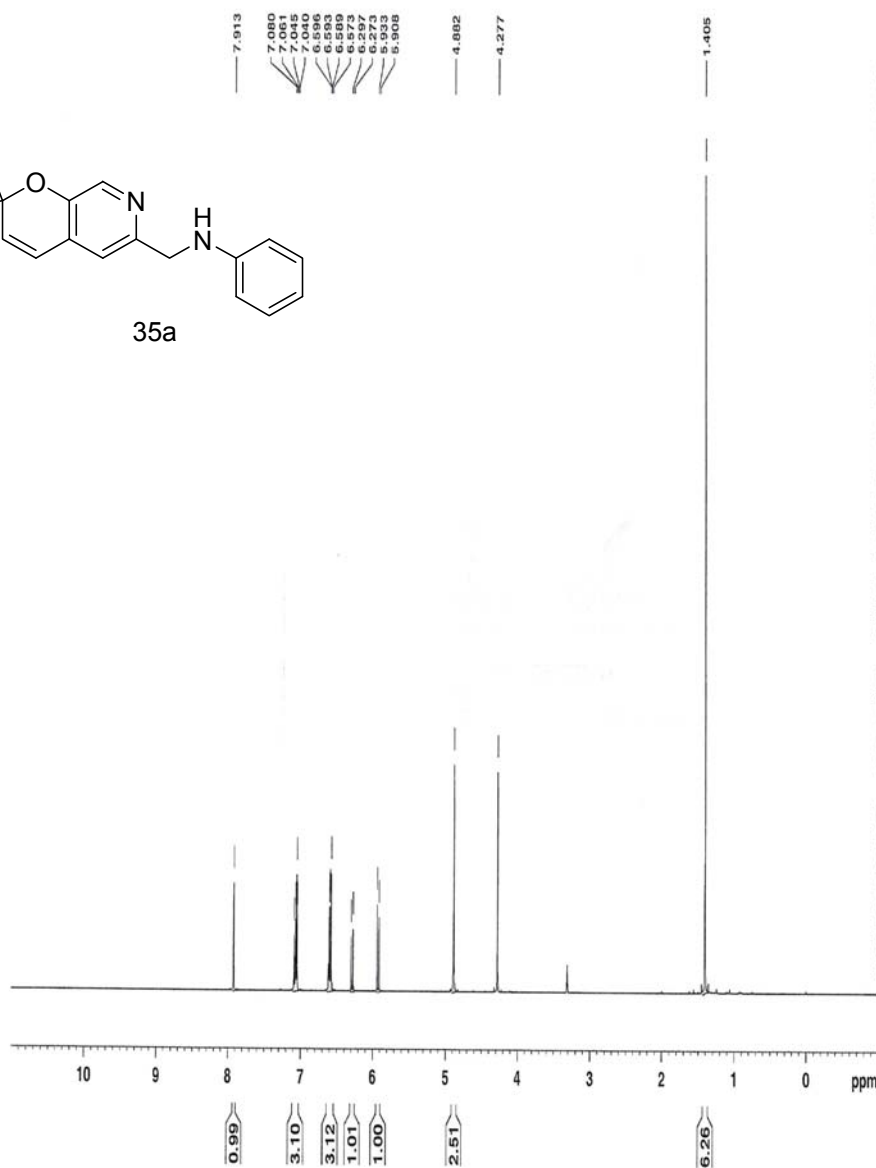
Date_ 20080822
Time 15.23
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 64
DW 60.400 usec
DE 7.00 usec
TE 297.9 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

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

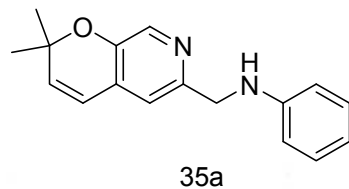
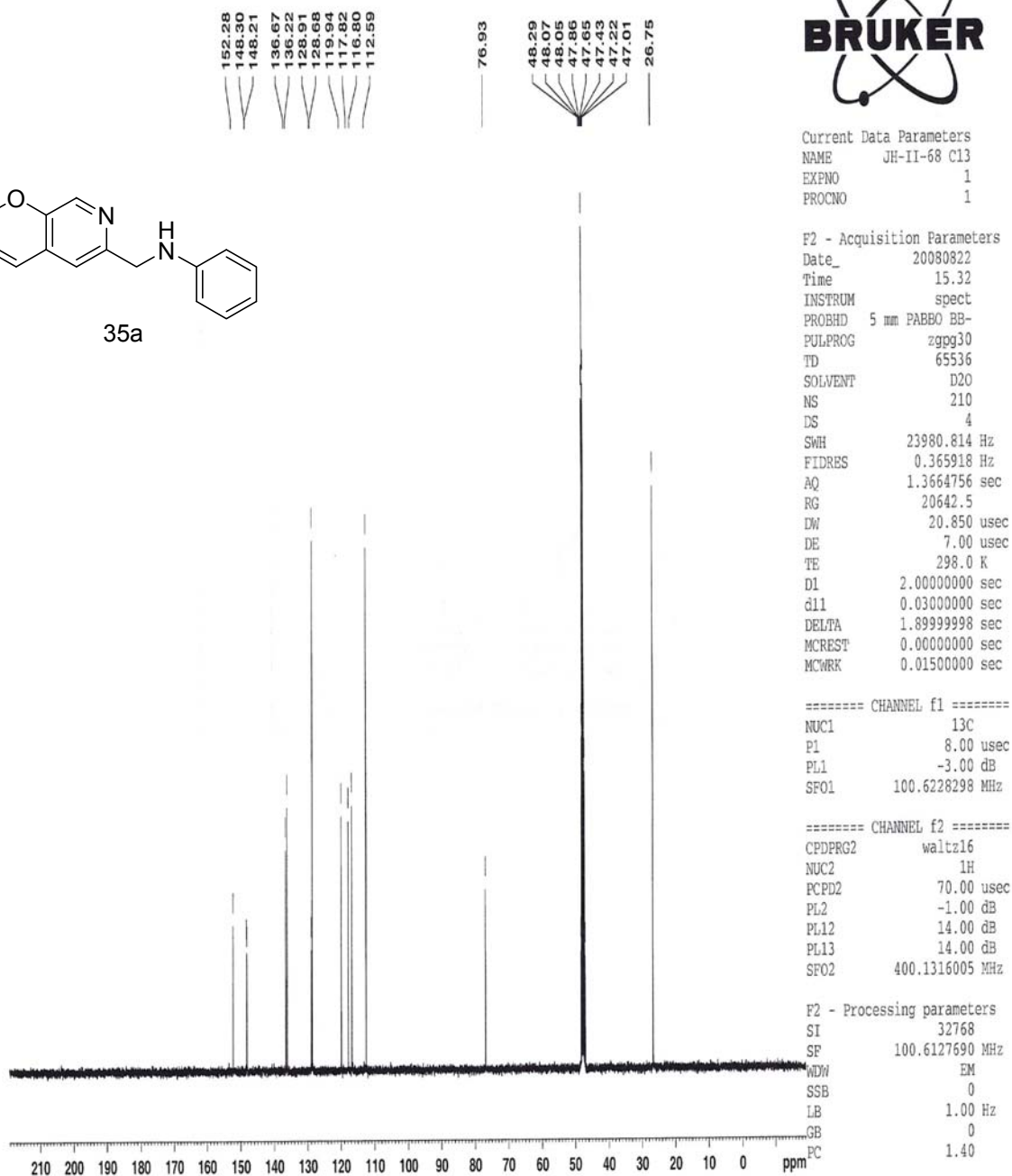
NUC1 1H
P1 12.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters

SI 32768
SF 400.1300073 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



No title



No title

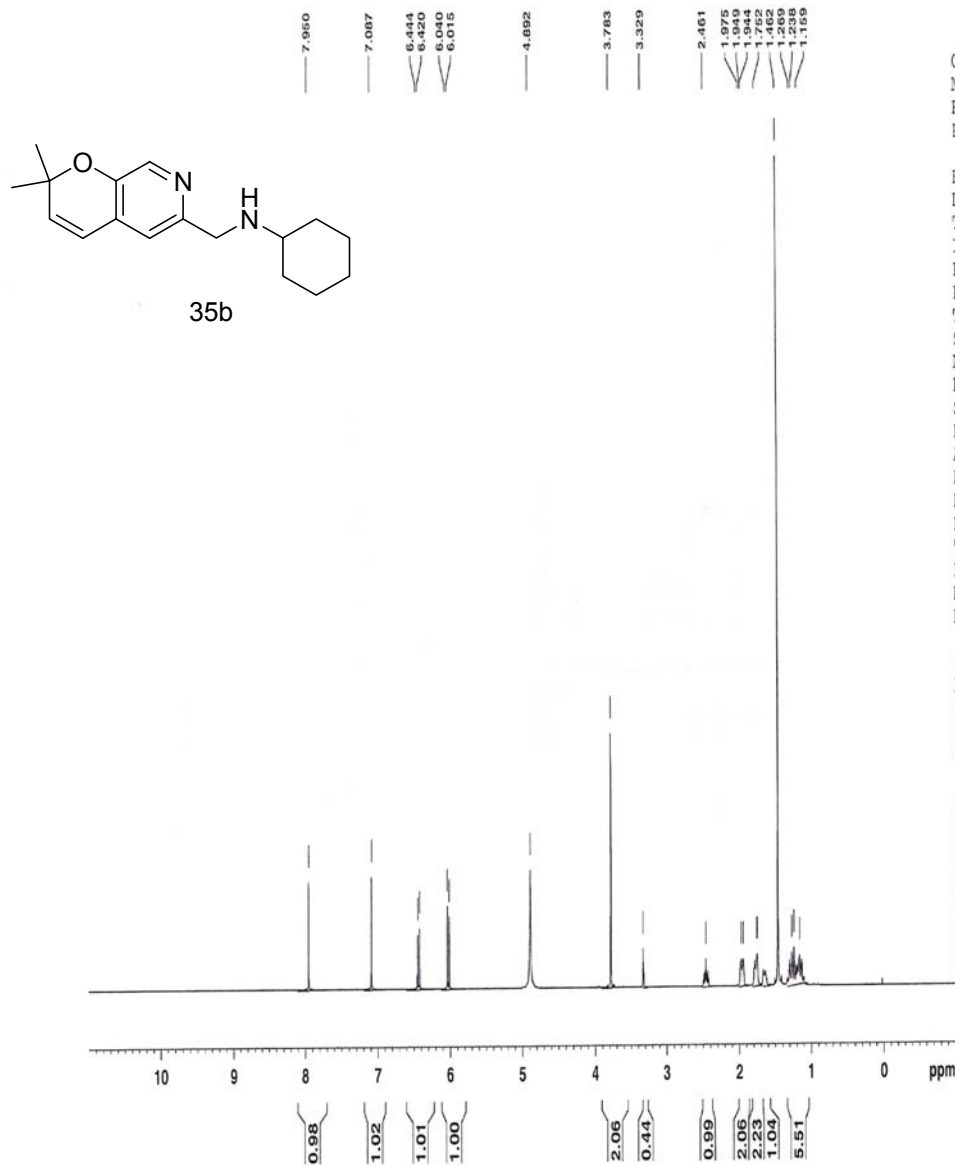


Current Data Parameters
 NAME JH-II-75 H1
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20080908
 Time 11.02
 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 50.8
 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 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



No title

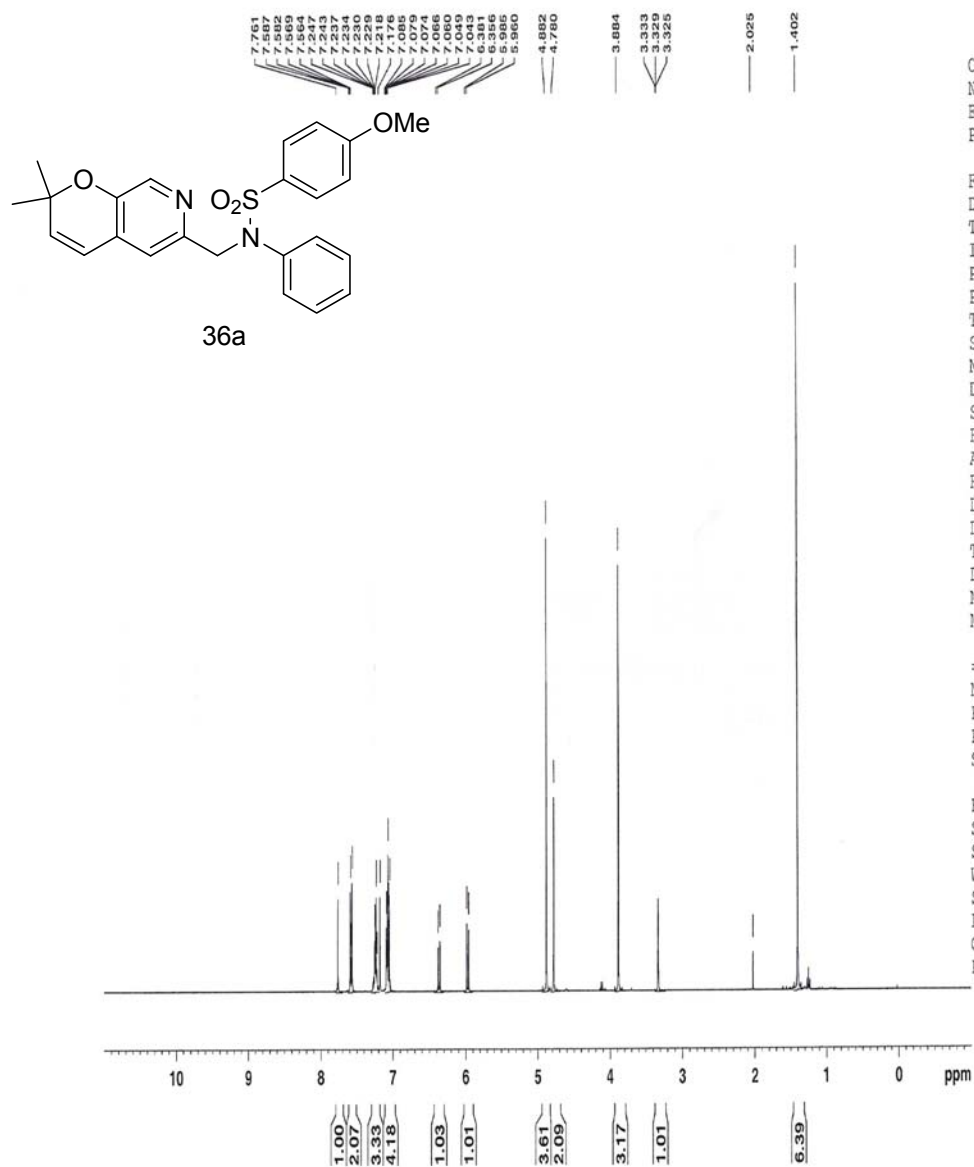


Current Data Parameters
 NAME JH-II-69 H1
 EXPNO 1
 PROCNO 1

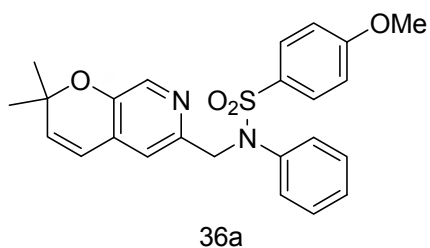
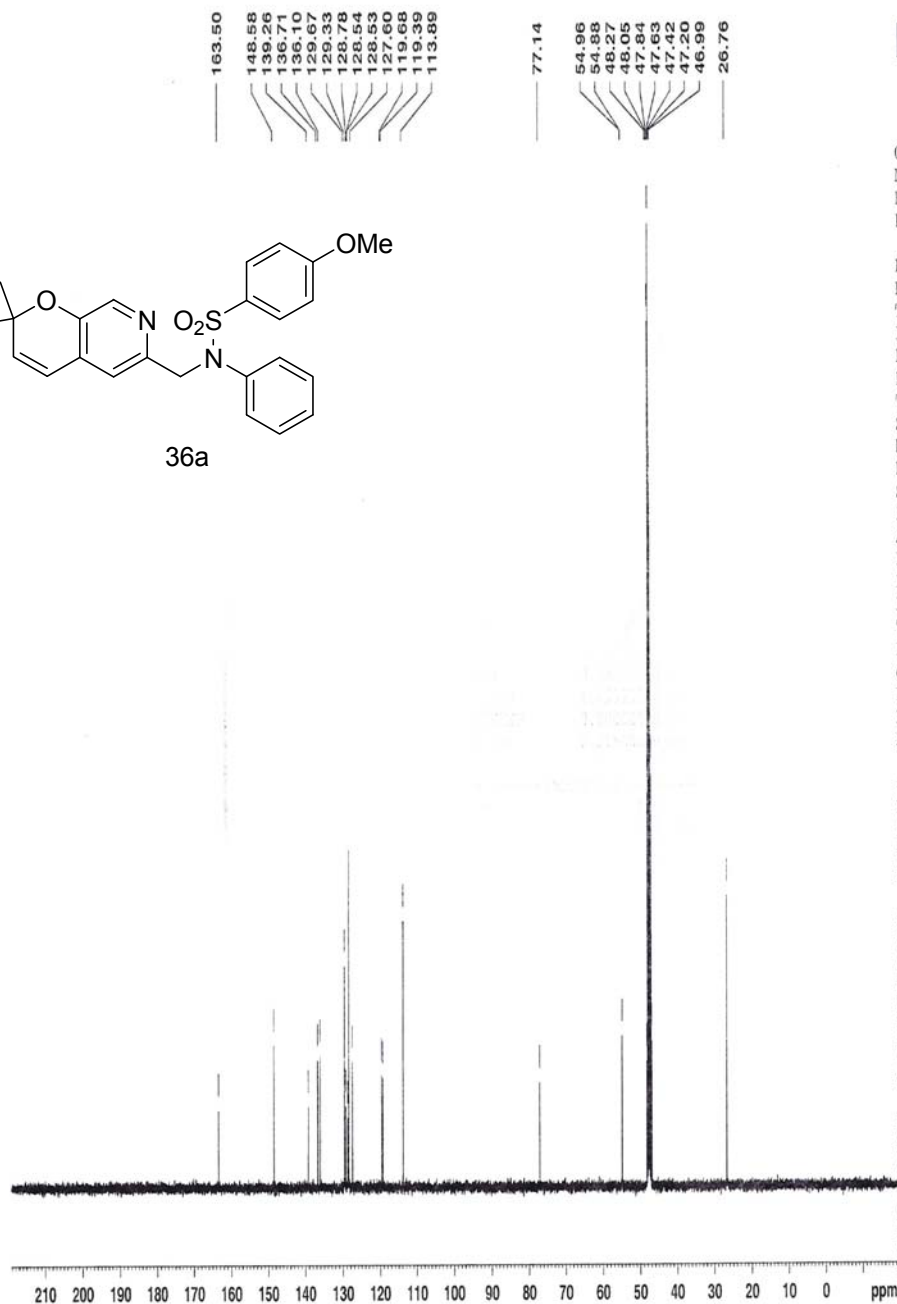
F2 - Acquisition Parameters
 Date_ 20080823
 Time 14.45
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT MeOD
 NS 16
 DS 2
 SWH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 90.5
 DW 60.400 usec
 DE 7.00 usec
 TE 297.9 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRK 0.01500000 sec

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

F2 - Processing parameters
 SI 32768
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



No title



Current Data Parameters
 NAME JH-II-69 C13
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20080823
 Time 14.53
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 159
 DS 4
 SWH 23980.814 Hz
 FIDRES 0.365918 Hz
 AQ 1.3664756 sec
 RG 13004
 DW 20.850 usec
 DE 7.00 usec
 TE 298.0 K
 D1 2.00000000 sec
 d11 0.03000000 sec
 DELTA 1.89999998 sec
 MCREST 0.00000000 sec
 MCWET 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 100.6127690 MHz
 MDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40

No title

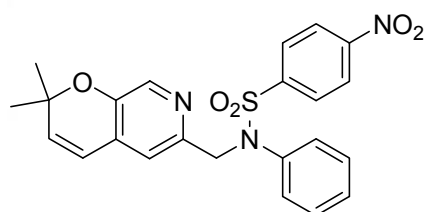


Current Data Parameters
 NAME JH-II-70 H1
 EXPNO 1
 PROCNO 1

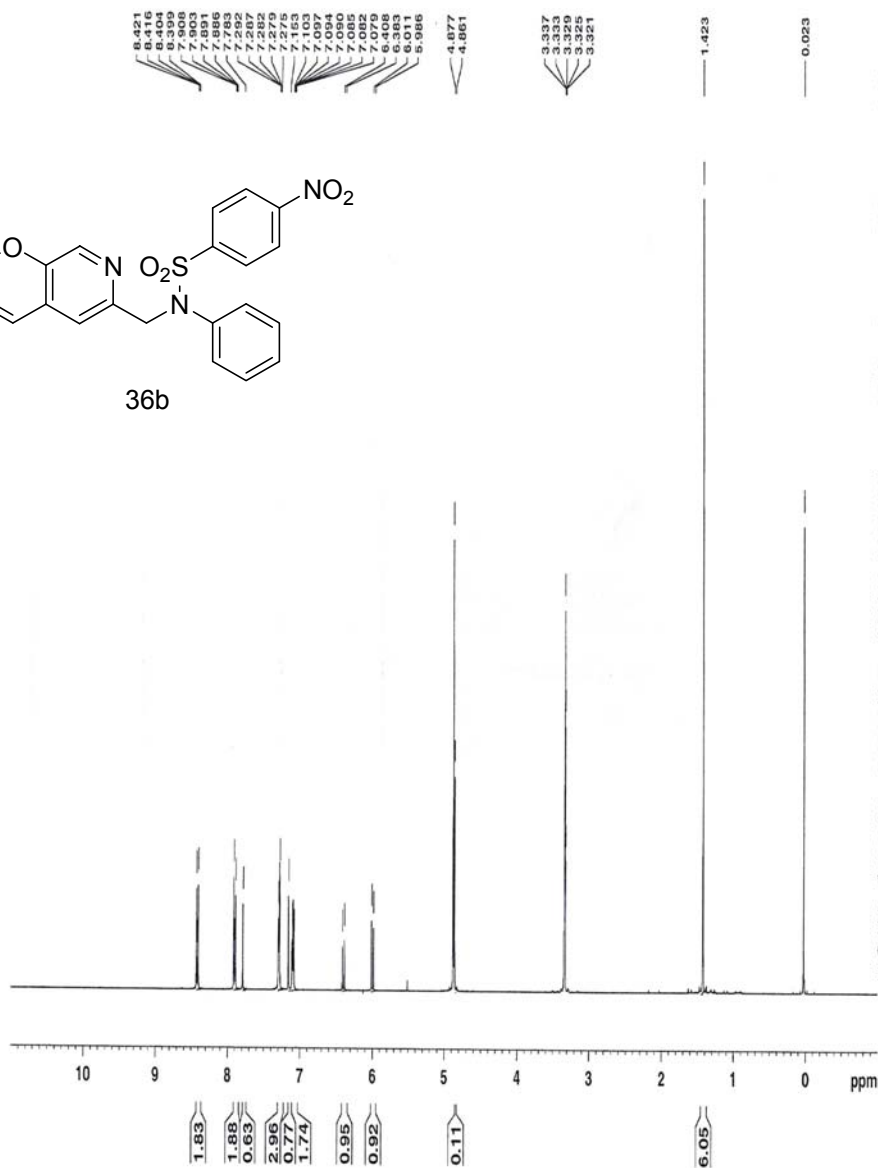
F2 - Acquisition Parameters
 Date_ 20080909
 Time 17.47
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT MeOD
 NS 16
 DS 2
 SMH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 406.4
 DN 60.400 usec
 DE 7.00 usec
 TE 298.3 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCMRK 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 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



36b



36b

No title

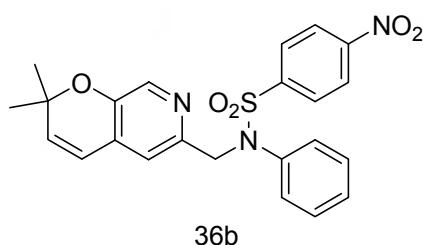
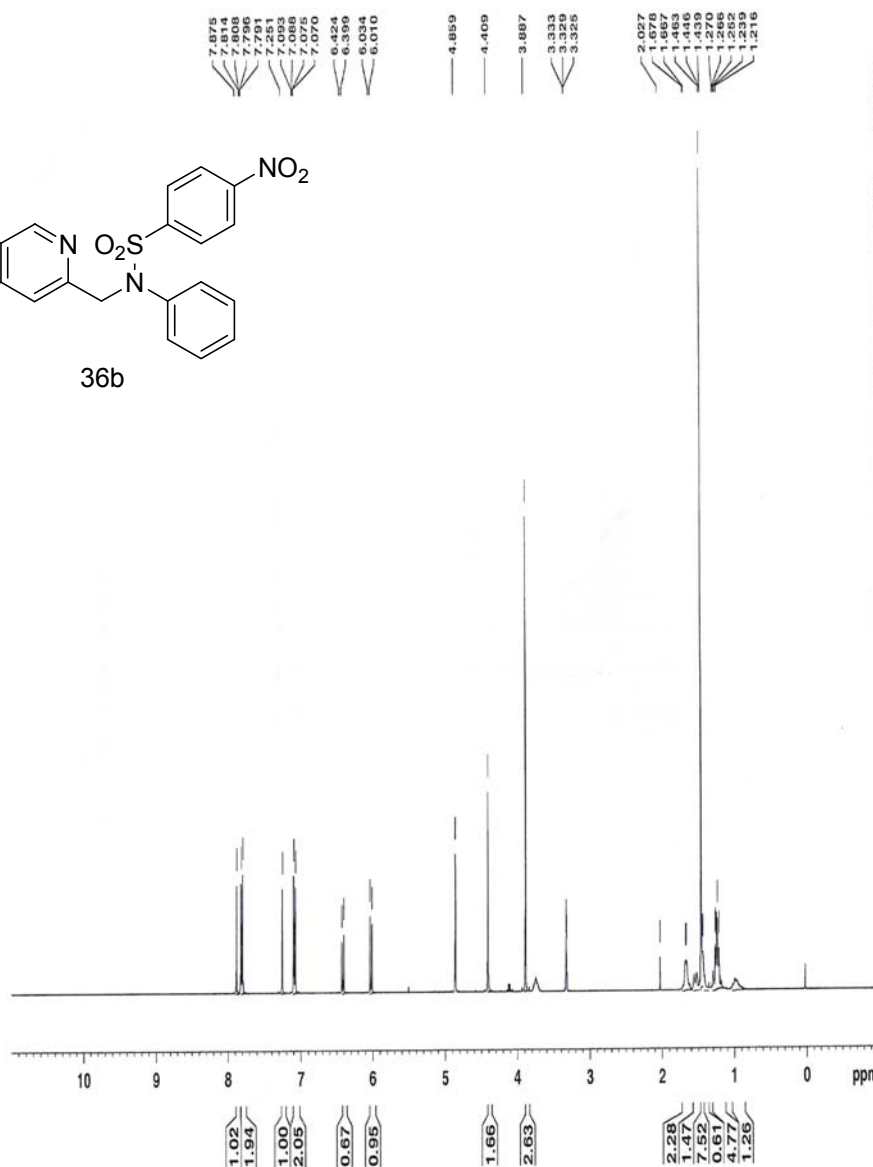


Current Data Parameters
 NAME JH-II-70 C13
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20081018
 Time 14.46
 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 80.6
 DW 60.400 usec
 DE 7.00 usec
 TE 299.8 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
 SF01 400.1324710 MHz

F2 - Processing parameters
 SI 32768
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.40



No title

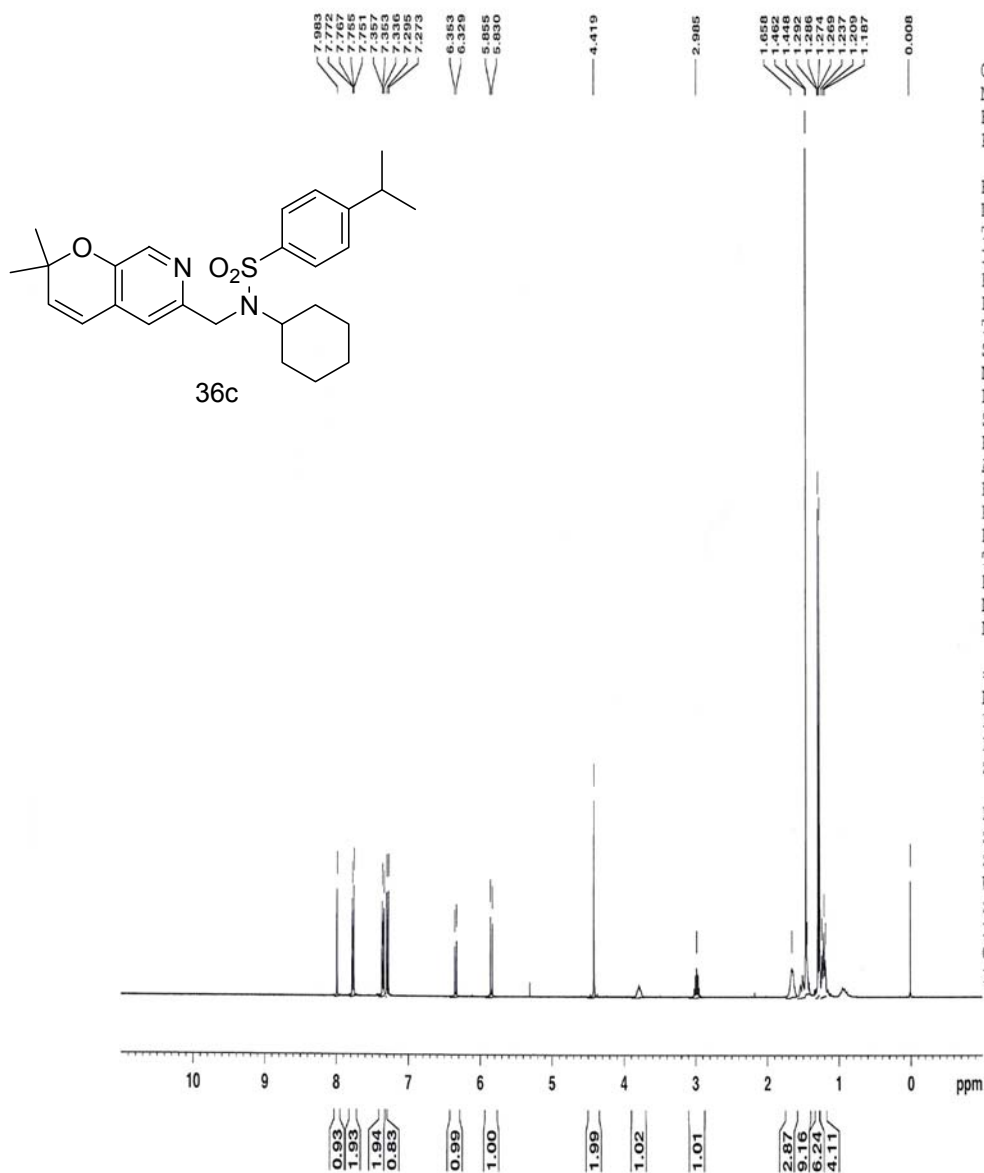


Current Data Parameters
 NAME JH-II-78
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20100223
 Time 11.29
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT D2O
 NS 13
 DS 2
 SWH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 228.1
 DW 60.400 usec
 DE 7.00 usec
 TE 299.6 K
 DI 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRK 0.01500000 sec

===== CHANNEL f1 =====
 NUC1 1H
 P1 12.80 usec
 PL1 0.00 dB
 SF01 400.1324710 MHz

F2 - Processing parameters
 SI 32768
 SF 400.1300046 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



No title



Current Data Parameters

NAME JH-II-78-C13
EXPNO 1
PROCNO 1

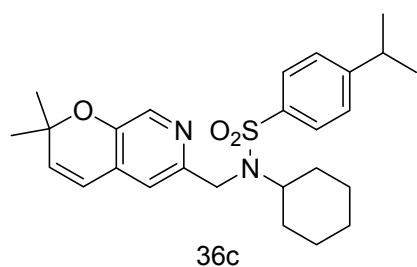
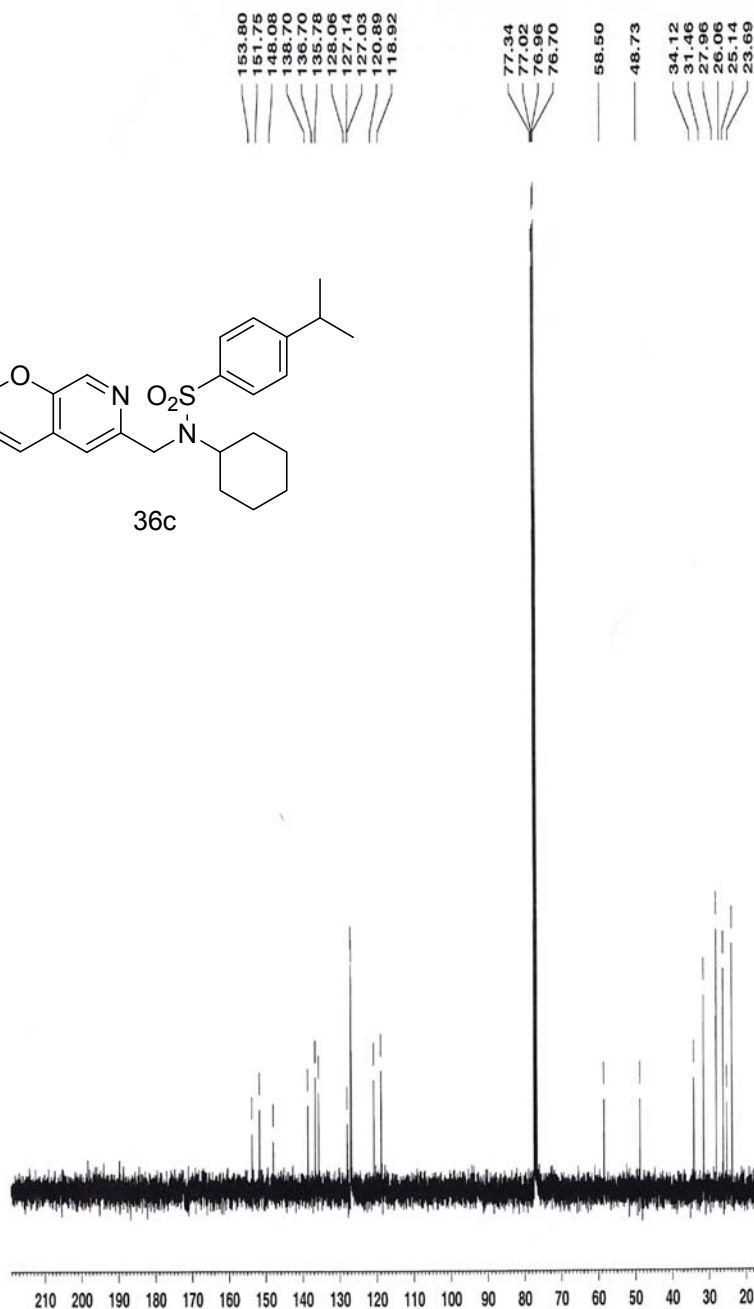
F2 - Acquisition Parameters

Date_ 20100223
Time 11.20
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 85
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 16384
DW 20.850 usec
DE 7.00 usec
TE 299.7 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 100.6127690 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



No title

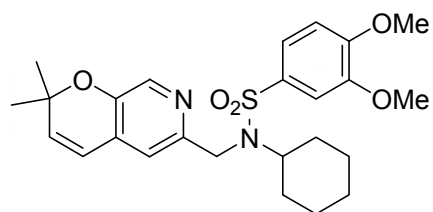


Current Data Parameters
 NAME JH-II-76
 EXPNO 1
 PROCNO 1

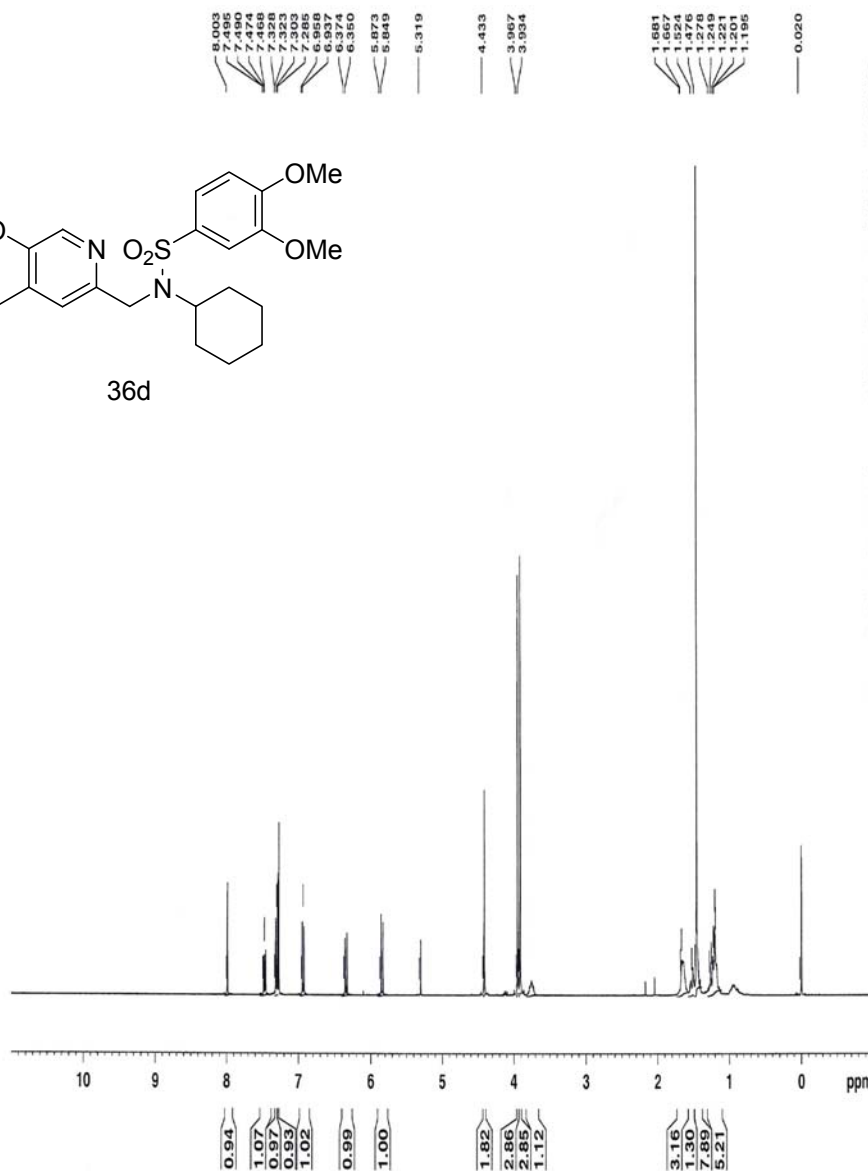
F2 - Acquisition Parameters
 Date_ 20100223
 Time 11.05
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 11
 DS 2
 SWH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 322.5
 DW 60.400 usec
 DE 7.00 usec
 TE 299.6 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRK 0.01500000 sec

===== CHANNEL f1 =====
 NUC1 1H
 P1 12.80 usec
 PL1 0.00 dB
 SF01 400.1324710 MHz

F2 - Processing parameters
 SI 32768
 SF 400.1300000 MHz
 WDM EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



36d



No title

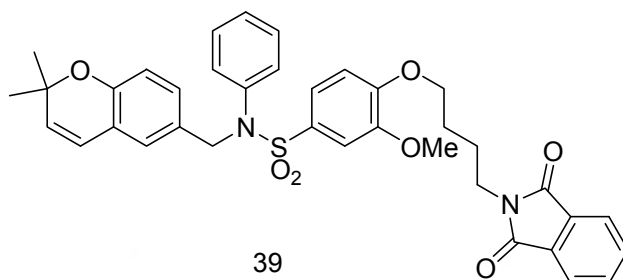


Current Data Parameters
 NAME VISR9
 EXPNO 1
 PROCNO 1

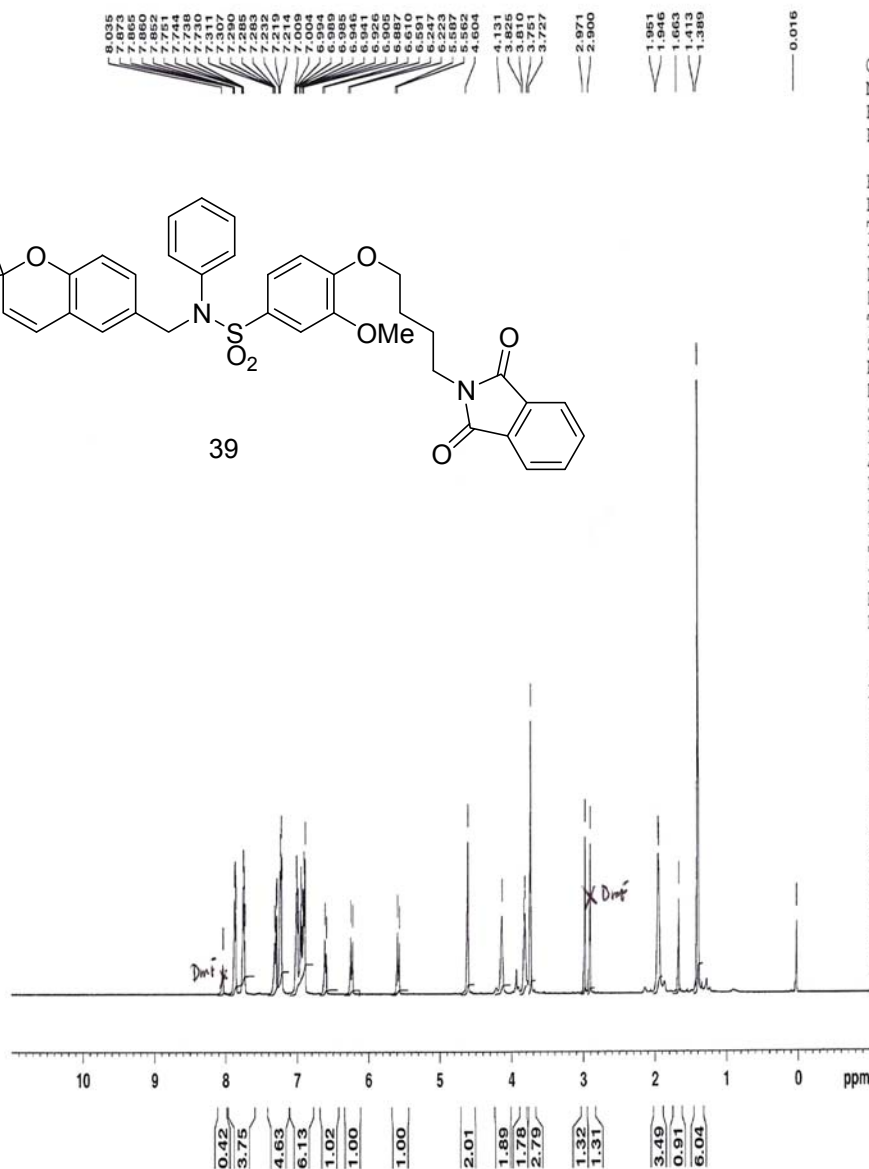
F2 - Acquisition Parameters
 Date_ 20091102
 Time 10.01
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 16
 DS 2
 SWH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 161.3
 DW 60.400 usec
 DE 7.00 usec
 TE 295.5 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRK 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 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



39



No title



Current Data Parameters
 NAME IV-SR83b
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20080625
 Time 19.13
 INSTRUM spect
 PROBHD 5 mm PABBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 34
 DS 2
 SWH 8278.146 Hz
 FIDRES 0.126314 Hz
 AQ 3.9584243 sec
 RG 322.5
 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 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

