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Synthesis of 123-Iodine Labeled Rofecoxib Analogues: Potential Nuclear Medicine Imaging Agents

Brandy U. Belue

University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Brandy U. Belue entitled "Synthesis of 123-Iodine Labeled Rofecoxib Analogues: Potential Nuclear Medicine Imaging Agents." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemistry.

George W. Kabalka, Major Professor

We have read this thesis and recommend its acceptance:

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Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Brandy Underwood Belue entitled "Synthesis of 123-Iodine Labeled Rofecoxib Analogues: Potential Nuclear Medicine Imaging Agents." I have examined the final paper copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemistry.

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Vice Chancellor and Dean of
Graduate Studies

SYNTHESIS OF 123-IODINE LABELED ROFECOXIB ANALOGUES: POTENTIAL NUCLEAR MEDICINE IMAGING AGENTS

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Brandy Underwood Belue
December 2005

DEDICATION

To my husband, Brian,

You have been there all along.

No time nor distance could quiet the infinite love we share.

To my parents,

For showing me your unconditional love

And understanding throughout all of my life.

ACKNOWLEDGMENTS

A special thanks and expressions of gratitude are due to members of the Chemistry Department for their help and friendship throughout my work:

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ABSTRACT

Single photon emission computed tomography (SPECT) is a medical imaging technique which provides three-dimensional images of living systems after introduction of a radiolabeled pharmaceutical. Rofecoxib (4-[4-methylsulphonylphenyl]-3-phenylfuran-2(5*H*)-one) is an inhibitor of the cycloxygenase-2 enzyme, an enzyme which has been found to promote cancer cell growth. The synthesis of a no-carrier-added iodine-123 labeled rofecoxib derivative is potentially of value for the detection of cancer utilizing SPECT.

Iodine-123 labeled 3-(3-Iodophenyl)-4-(methanesulfonylphenyl)-5*H*-furan-2-one, **1**, and 3-(4-Iodophenyl)-4-(methanesulfonylphenyl)-5*H*-furan-2-one, **2**, were prepared from the respective potassium organotrifluoroborate salts. The trifluoroborates were prepared from a simple six step pathway.

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	Description
°C	Degree Celsius
α	Alpha
γ	Weak gamma radiation
μL	Microliter
J	Coupling constant

Abbreviation	Description
$AlCl_3$	Aluminum chloride
cm	Centimeters
COX	Cyclooxygenase
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
DMF	*N,N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
ECT	Emission computed tomography
ED_{50}	Effective Dose 50
g	Grams
h	Hours
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry
Hz	Hertz

KHF_2	Potassium hydrogen fluoride
M	Molarity
MBq	Megabecquerels
MeOH	Methanol
MeV	Mega electron volt
MHz	Megahertz
min	Minutes
mL	Milliliters
mmol	Millimoles
MMPP	Magnesium (bis)monoperoxyphthalate hexahydrate
mol	Moles
N	Normality
NMR	Nuclear magnetic resonance
NSAID	Nonsteroidal anti-inflammatory drug
PET	Positron emission tomography
PMA	Phosphomolybdic acid
ppm	parts per million
R_f	Retention factor
SPECT	Single photon emission computed tomography
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilane

Chapter 1 Introduction

1.1 NUCLEAR MEDICINE IMAGING

Utilization of boron chemistry as a means of incorporating radioactive isotopes into pharmacologically active compounds for exploration in nuclear medicine imaging has long been of interest in this laboratory.[1] Significant breakthroughs in radiotracer development have resulted since the discovery of radioactivity by Henri Becquerel in 1896.[2] Radiochemical applications can be found in biology, chemistry, geology, medicine, and pharmacology. In 1913, Hevesy pioneered the use of radiolabeled compounds in medicine and biology.[3] Since then, radiopharmaceuticals have become very important in medicine. A radiopharmaceutical can be defined as a compound whose medical application is dependent upon the radioactive emission of a constituent radionuclide.[4] The chemistry of radiolabeled agents parallels that of their nonradioactive isomers, and therefore, their synthesis can be accomplished using similar preparative chemistry. The use of short-lived isotopes helps to alleviate problems related to the storage and disposal of radioactive compounds.

Nuclear medicine imaging techniques have led to the development of diagnostic tools which provide valuable insight into physiological processes that would otherwise be unobservable. Nuclear imaging provides a non-invasive technique in which the use of computers, detectors, and radioactive pharmaceuticals are combined to study physiological processes in the human body. The technique requires the use of radionuclides contained in reagents that are administered to the patient.[5] The

radionuclide decay is then observed by a series of radiographic and computed tomographic techniques.

Positron emission tomography (PET) and single photon emission computer tomography (SPECT) are two tomography techniques that are now accepted as diagnostic tools for a variety of tumors, illnesses, blood flow problems, aneurysms, and organ dysfunctions.[6] A detailed discussion of nuclear imaging theory is beyond the scope of this thesis. However, some insight into the principles of both PET and SPECT is necessary to understand the choice of the target molecules that will be further discussed in this thesis.

PET is a nuclear medicine procedure in which the presence of positron emitters such as ^{11}C, ^{13}N, ^{15}O, and ^{18}F are detected by a tomographic imaging system. A low dose of a radiopharmaceutical labeled with one of these positron emitters is injected into the patient and the resultant radioactive decay is observed in an emission scan, either dynamic or static. SPECT is a nuclear imaging technique that uses nuclei that decay via a single photon disintegration. These include iodine-123, technetium-99m, thallium-201, gallium-67, and indium-111. Iodine-123 is readily available.

Positrons are formed when neutron-deficient nuclei of low atomic number decay to more stable nuclei.[7] This decay occurs via two possible pathways: positron emission or electron capture. PET utilizes positron emission via positron annihilation, while SPECT utilizes photons via electron capture. Positrons have a finite lifetime with a short mean free path, typically a few millimeters. The positron annihilates by colliding with an electron. This annihilation reaction between a positron and an electron produces two 0.511 MeV gamma rays, that travel in opposite directions, and are detected by two

detectors in parallel planes. PET utilizes opposed detectors to count the photons which must reach the detectors within a certain time frame of the annihilation. SPECT uses only one detector, thus eliminating the time relationship. The raw data sinograms are reconstructed into a cross-sectional image which provides regional concentration measurements of the radiopharmaceutical.

Many factors affect emission computed tomography (ECT). Radionuclide decay, biokinetics of the pharmaceuticals, imaging systems, organ motion and location are just a few of these factors which consequently affect the determination of nuclide concentrations.[8] Physical and mathematical factors arising from the algorithms used to reconstruct data also affect ECT.[9] Future developments to alleviate some of these challenges include better detectors, more efficient algorithms, and other compensatory techniques such as reduction of scatter.

Researchers are aggressively exploring the developments of radiopharmaceuticals for ECT. Initial experiments in nuclear imaging focused on the uptake of radionuclides in the brain that exhibited little retention. Radiolabeled agents that crossed the blood-brain barrier were a subsequent focus of development.[4] Once that goal was reached, evolution of radiopharmaceutical development occurred rapidly. Classes of compounds that have emerged from development in ECT since the pioneering studies involving placement of radionuclides in the brain continue to increase on a daily basis and include a broad spectrum of body organs to which they can be applied. The specific action of the carrier molecule is thought to be the same as the corresponding non-labeled molecule, thus creating a versatile avenue for the introduction of radionuclides into a wide variety

of compounds.[10] One class of compounds, the cyclooxygenase (COX) inhibitors, has gained intense interest.

1.2 COX INHIBITORS

1.2.1 Non-steroidal Anti-inflammatory Drugs (NSAIDs)

Inflammation can be defined as the first response of the immune system to infection or irritation.[11] Clinically the features of inflammation are heat, redness, swelling, and pain. The host attempts to limit the extent of damage, counteract infection, and promote healing as well as recovery of function.[12] The inflammation process involves blood cells from bone marrow, and many organs are effected by inflammation to varying degrees. These can include the central nervous system, cardiovascular system, liver, and the endocrine system. Inflammation can be acute or chronic, and it contributes to all disease processes.

Anti-inflammatory agents' origins can be traced back to ancient times when people chewed on willow tree bark for pain relief. Hippocrates described the use of salicin for pain relief in the 5th century B.C.E. Aspirin, a derivative of salicylic acid, was discovered in 1899.[13] Aspirin has been noted to have elevated risks when taken long term such as the development of gastrointestinal bleeding, ulcers, and kidney damage. Other anti-inflammatory drugs like ibuprofen and naproxen produce some of the same destructive effects.

1.2.2 COX Enzymes

To understand why anti-inflammatory drugs caused the effects observed, research was conducted to determine exactly how aspirin and other NSAIDs functioned. A British

pharmacologist by the name of John R. Vane proposed that prostaglandins are produced within the body's cells by the enzyme cyclooxygenase (COX).[14] NSAIDs work to inhibit the production of prostaglandins by blocking the COX enzyme. With the COX enzyme inhibited, inflammation, pain, and fever subside. Two COX enzymes exist, COX-1 and COX-2.[15] Both enzymes produce prostaglandins that promote pain, inflammation, and fever. They differ in that COX-1 produces prostaglandins that protect the stomach and support platelets; inhibition of COX-I enzymes by NSAIDs reduces platelets, thereby causing ulcerative bleeding.

COX-2 inhibitors gained interest with the finding that COX-2 is associated with inflammatory conditions and COX-1 is expressed as a constitutive enzyme.[15] COX-1 synthesizes prostaglandins for physiological functions such as renal blood flow regulation and cell division. COX-2 is induced by two types of cellular messengers: inflammatory cytokines and growth factors. Both of these processes promote the initiation of cancer cell growth and survival.[16,17] The inhibition of COX-2 is believed to induce the anti-inflammatory effects, while COX-1 inhibition causes the destructive effects to the stomach and liver.[18]

The above factors led pharmaceutical companies to invest vast resources into developing classes of compounds that targeted COX-2 while allowing expression of COX-1. In 1999, Searle/Monsanto brought celecoxib onto the market for the treatment of arthritis. Soon after the introduction of celecoxib (celebrex®), Merck introduced rofecoxib (vioxx®), which has recently been the subject of many investigations. Pfizer marketed valdecoxib (bextra®). Many other pharmaceutical companies followed with the introduction of various COX-2 inhibitors. Among these are Glaxo Smith Kline,

Novartis, Roche, Parke Davis, Boeringer-Ingelheim, Abbott Laboratories, Procter and Gamble, DuPont and others. There are many classes of compounds that make up the COX-2 inhibitor family[15,19]: thiazoles, oxazoles, 1,5-diarylpyrazoles, ethers, thioethers, cyclopentenes, cyclobutenones, cyclopentones, fused heterocycles, pyridazinones, 2,3-diarylbenzopyrans, 1,1-dihalo-2,3-diphenylcyclopropanones, pyrimidin-2-amines, and 3,4-diarylfuranones. The advantage of these drugs is that they reduce inflammation without the harsh side effects of earlier NSAIDs such as ulcerative bleeding and liver damage.

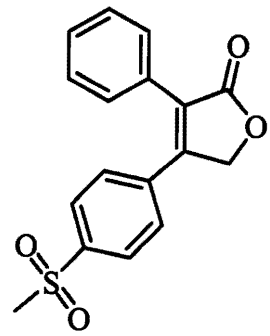
Since both COX-1 and COX-2 catalyze the conversion of arachidonic acid to prostaglandin, understanding what differentiates these enzymes on the molecular level aided in the development of the selective inhibitors. The COX enzymes have a specific orientation within the cell membrane. Four amphipathic helices form a hydrophobic surface that floats these enzymes in the upright position on the membrane.[20] The helices make up the base of the molecule, and form the opening to a hydrophobic pocket on the inner membrane surface of the enzyme, which is the COX active site.[20] As these helices are embedded within the membrane, fatty acids and NSAIDs must cross the lipid bilayer to reach the COX active site entrance.

Arginine, in the charged form, is present in the COX-1 active site position 120.[21] COX-1 activity is dependent on the lock and key fitting of fatty acid substrates with Arg^{120} within the site. Replacing arginine with lysine changes the activity of COX-1.[21] This illustrates that a single amino acid can affect the activity of a particular enzyme, thereby opening a gateway for designing selective COX inhibitors.

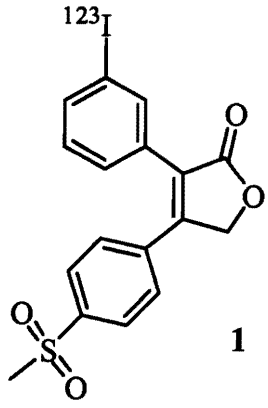
1.2.3 COX-2 Inhibitors and Carcinogenesis

Kune discovered that individuals who consumed aspirin on a daily basis had a 40% lower risk of colon cancer than those who used no aspirin.[22] Later, the expression of the COX-2 enzyme was noted in 85% human colorectal carcinomas.[23] Following this observation, sulindac (a NSAID) was found to eliminate rectal polyps, thus giving insight to the possibility that NSAIDs might be a possible treatment for tumors.[24]

In vitro studies with cell lines derived from adenocarcinomas of the lungs, pancreas, colon, stomach, breast and prostate, as well as relevant animal studies and epidemiological studies in humans, have provided evidence that cyclooxygenase inhibitors (both COX-1 and COX-2) can significantly reduce the risk for development of this cancer family.[25-36] Cyclooxygenase inhibitors are thus being evaluated in cancer prevention and treatment trials.[36-39] Recent reports have revealed that the chronic use of COX-2 inhibitors may increase the rate of cardiovascular morbidity.[40] Monitoring patients undergoing chronic treatment with COX-2 inhibitors prior to and during therapy could become important in the future. Nuclear medicine imaging utilizing SPECT or PET might help in this regard. We have developed a rapid and convenient method for preparing two iodine-123 labeled rofecoxib analogues (**Figure 1**, page 8). The synthetic methods utilized in the preparation of these radiopharmaceuticals are outlined in this thesis.



Rofecoxib



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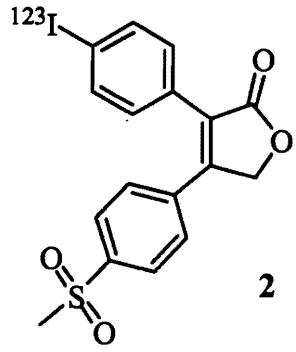

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Figure 1. Rofecoxib and Iodine-123 labeled rofecoxib analogues

Chapter 2 Approach to the Study

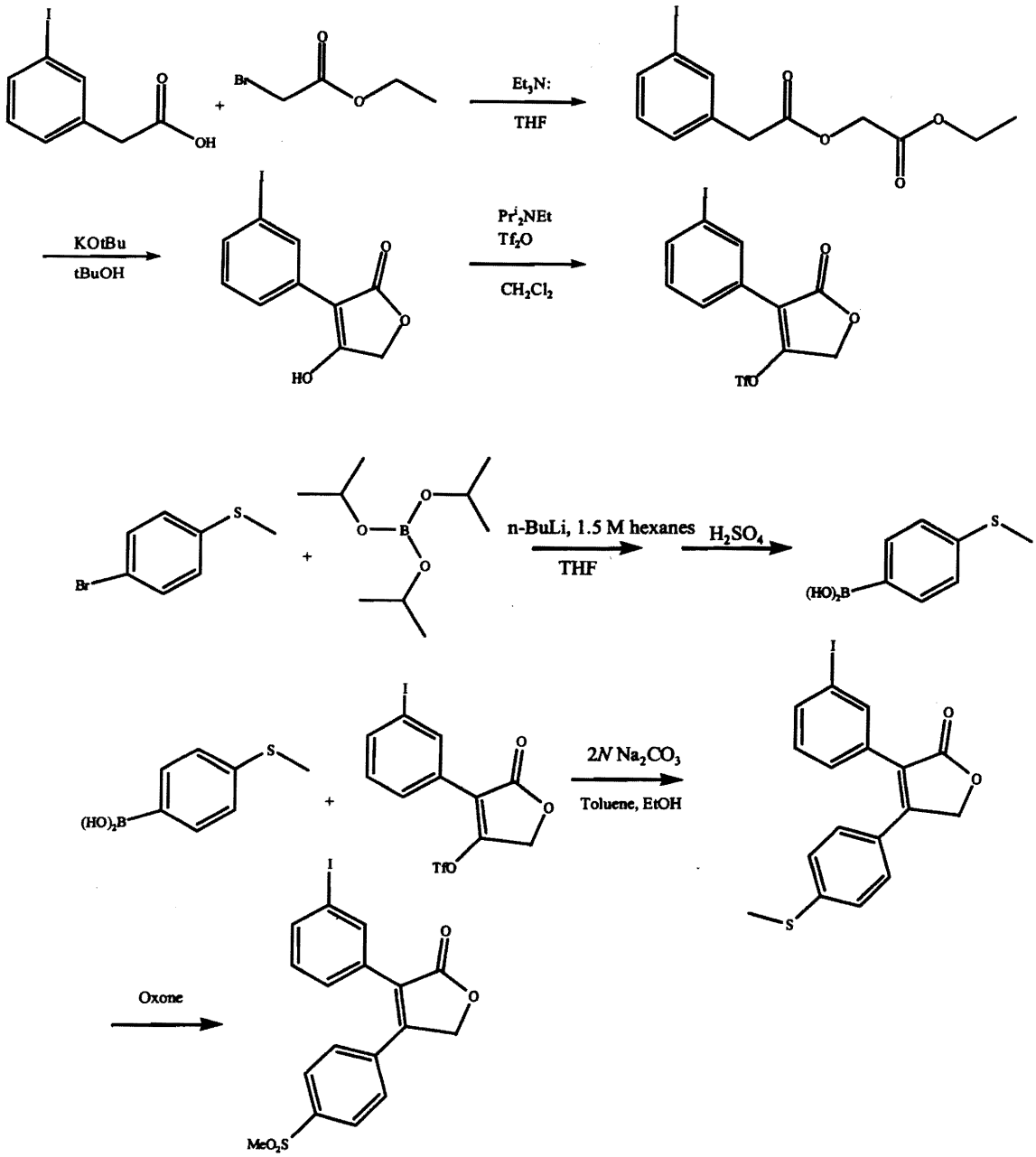
2.1 RATIONALE FOR SYNTHESIS OF ROFECOXIB DERIVATIVES

The iodine-123-labeled 3-iodo analogue of rofecoxib **1** was chosen for the study because the related 3-chloro derivative was well-tolerated in a rat paw edema assay, with ED_{50} values comparable to rofecoxib.[41]

The iodine-123-labeled 4-iodo analogue of rofecoxib **2** was chosen for the study based on the fact that substitution of a halogen (including bromine and chlorine as well as other large electron withdrawing groups) at the *para* position does not significantly alter COX-2/COX-1 selectivity.[41-45] In a rat paw edema assay, *para*-substituted bromo and chloro derivatives displayed ED_{50} values comparable to rofecoxib itself.[42,43] A *para*-substituted sulfonamide derivative proved to be even more effective than rofecoxib.[45]

2.2 PREVIOUS SYNTHETIC ATTEMPTS AT ROFECOXIB DERIVATIVES

Two paths were explored prior to arriving at the current method for preparing the prerequisite iodinated precursors to the targeted *meta* and *para* iodine-123 rofecoxib analogues. They differ in the sequence in which the trifluorborate rofecoxib precursor is assembled. One path followed the synthetic pathway developed by Desmond, Dolling, Marcune, Tillyer, and Taschaen,[42] and is outlined in **Scheme 1**, page 10. This route was abandoned early on in the study as many difficulties were encountered in achieving acceptable yields of the necessary furanone. Many reaction conditions were explored in which the stoichiometric amounts of base used and the reaction time were varied.



Scheme 1. Rofecoxib patent synthetic pathway

However, none of these methods resulted in improved yields. Furthermore, no correlation could be made as to what conditions were ideal.

A second potential route involved building the molecule as shown in **Scheme 2**, page 12. This pathway involved conversion of *meta*-iodophenylacetic acid to a methyl ester, from which the boronic ester moiety was formed. However, upon cleavage of the methyl ester, the boronic ester was also converted to the boronic acid giving undesired results. This pathway was also abandoned.

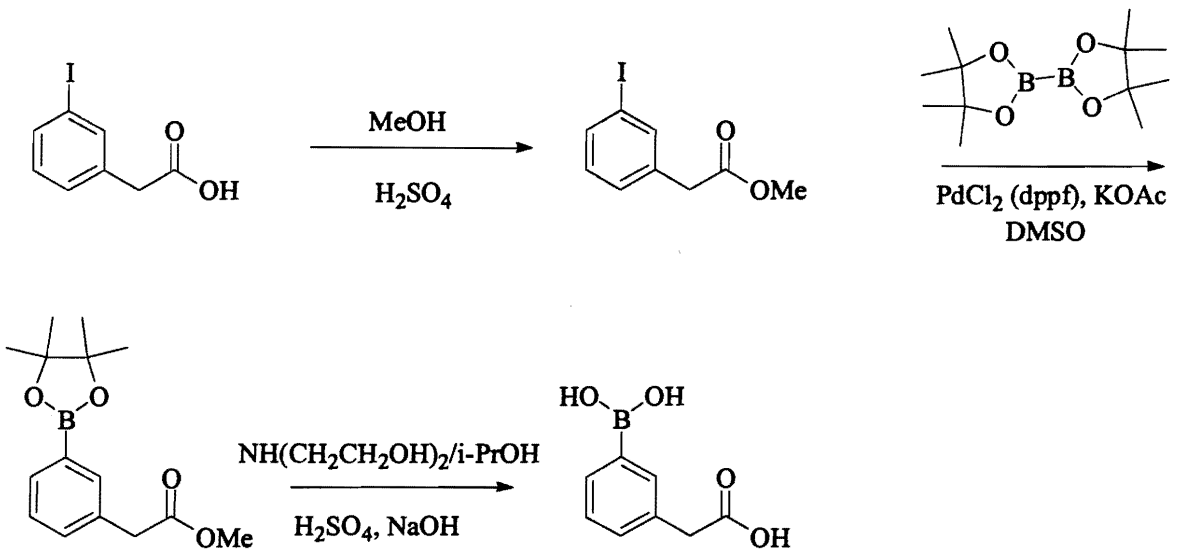
A more simplistic, shorter pathway was sought and found in the literature.[46] This pathway, with few deviations was followed to arrive at the trifluoroborate rofecoxib precursors which were suitable for radioiodination. The synthetic pathway is discussed in the following section.

2.3 BACKGROUND

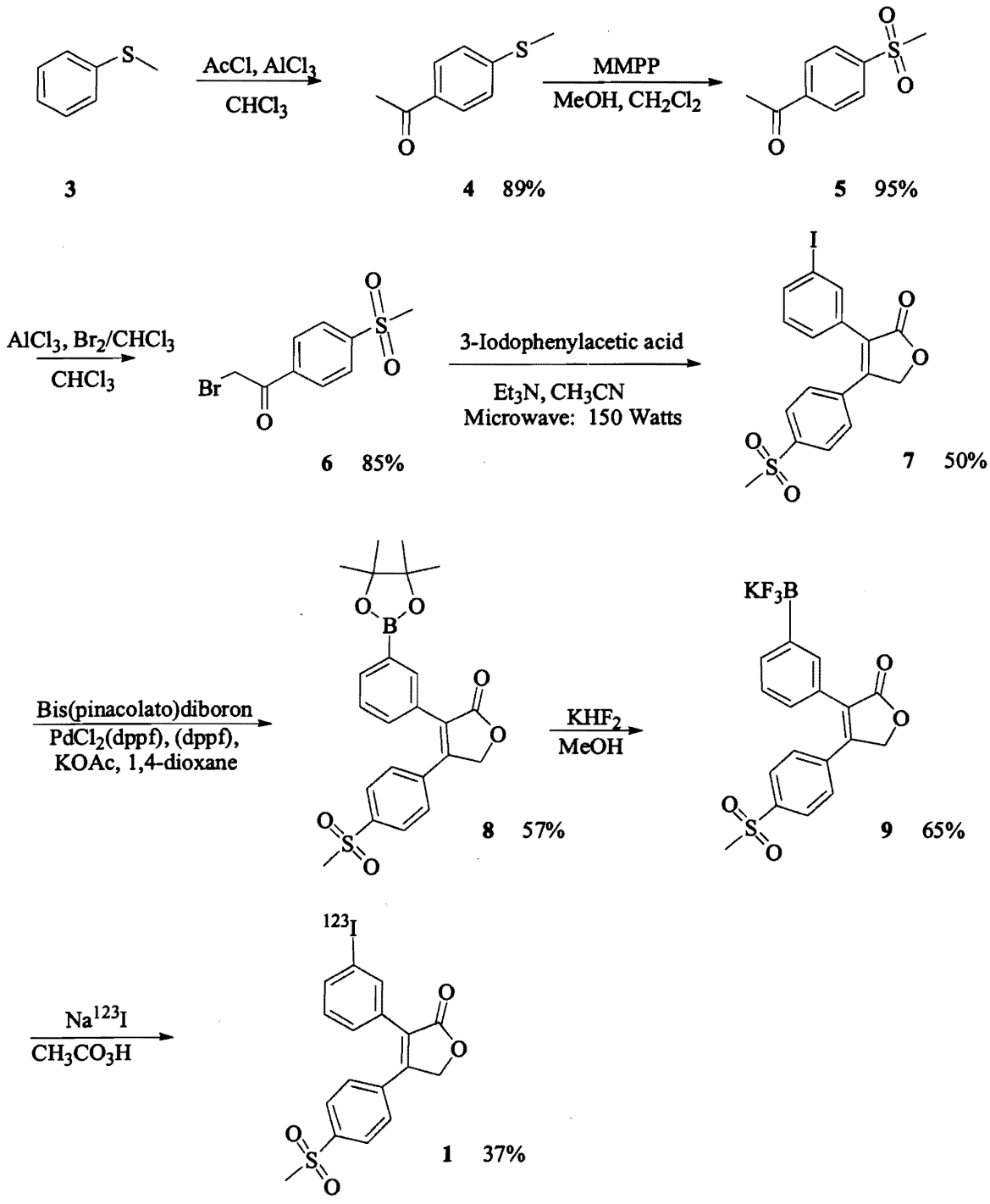
The successful route to the *meta* iodine-123 radiolabeled rofecoxib analogue is shown in **Scheme 3** on page 13.[47] The *para* iodine-123 radiolabeled rofecoxib analogue is readily prepared in the same manner, using 4-iodophenylacetic acid in the coupling reaction along with **6**. This is shown in **Scheme 4** on page 14. The following discussion provides the background to each synthetic step.

2.3.1 Friedel Crafts Acylation

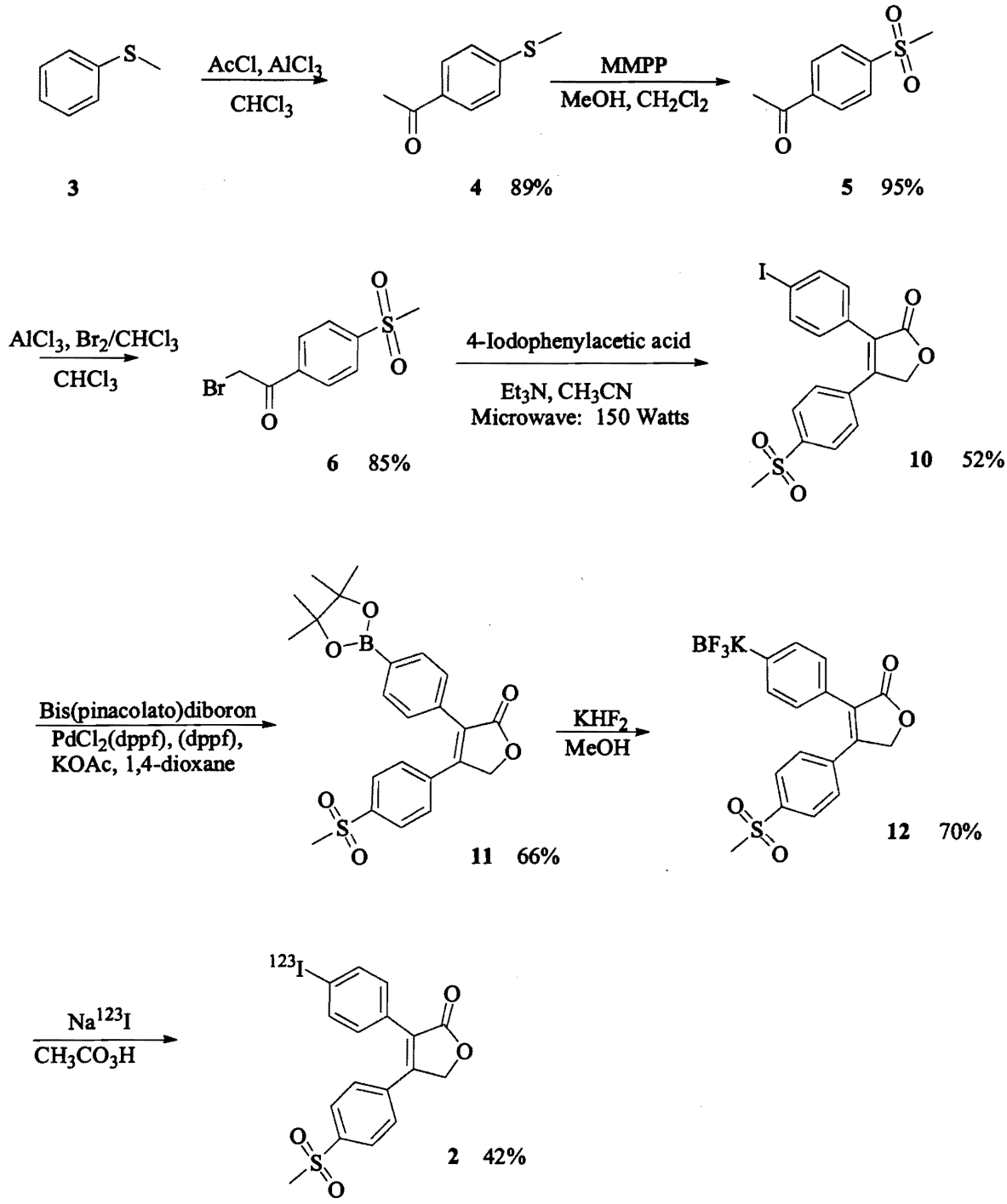
The first step in the synthesis of the trifluoroborate rofecoxib precursor **9** utilizes a Friedel-Crafts acylation. This involves the formation of an aryl ketone via electrophilic substitution utilizing an aryl compound, an acid halide, and a Lewis acid as a catalyst.



Scheme 2. Second partial synthesis of rofecoxib analogue



Scheme 3. Successful pathway to *meta*-iodinated rofecoxib analogue



Scheme 4. Successful pathway to *para*-iodinated rofecoxib analogue

Since the reagent used here is thioanisole, the sulfur acts as an activating group, enhancing the electrophilic substitution reaction. The sulfur group is an *ortho-para* directing group, therefore the *para* product is formed in relatively high yields.[48]

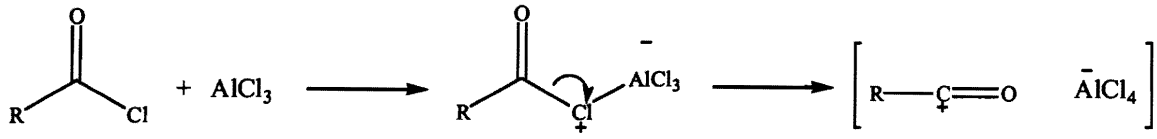
The desired reaction is achieved by cooling the Lewis acid in chloroform to 0 °C, slowly adding the acid chloride and the aryl reagent, and allowing the reaction to proceed while warming to room temperature. The reaction is quenched by cooling it to 0 °C, adding water while stirring, and subsequent washing with water, sodium bicarbonate, and brine to remove residual starting materials. Common reagents for Friedel-Crafts acylations are used: thioanisole as the aryl component, acetyl chloride, and aluminum chloride as the Lewis acid catalyst.

The mechanism involves the formation of an acylium ion (*Step 1*, **Scheme 5**, page 16) rather than a carbocation, which is common in carbon-carbon bond formations. The acylium ion then adds to the aromatic ring to produce an intermediate arenium cation, which is resonance stabilized (*Step 2*, **Scheme 5**). Removal of the proton from the arenium cation is the final step in the reaction, which is achieved by the base (*Step 3*, **Scheme 5**).

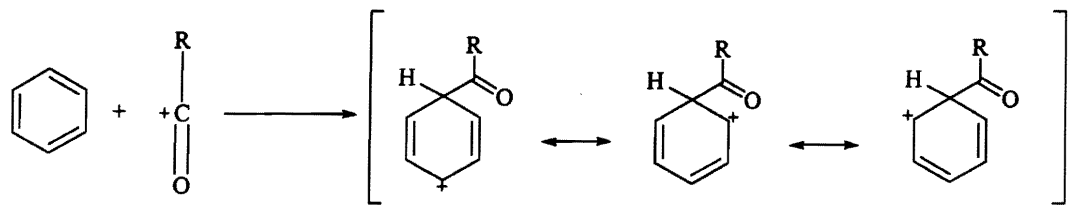
2.3.2 Oxidation of the Sulfur Substituent

The second step in the reaction sequence involves oxidation of the sulfur atom to the requisite sulfone. In order to achieve the desired degree of oxidation, an organometallic peroxide is used which coordinates to the sulfur, and efficiently oxidizes it. One can envision that this occurs via a coordination mechanism.

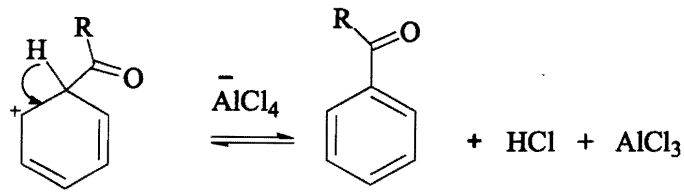
Step 1



Step 2



Step 3



Scheme 5. Friedel Crafts acylation mechanism

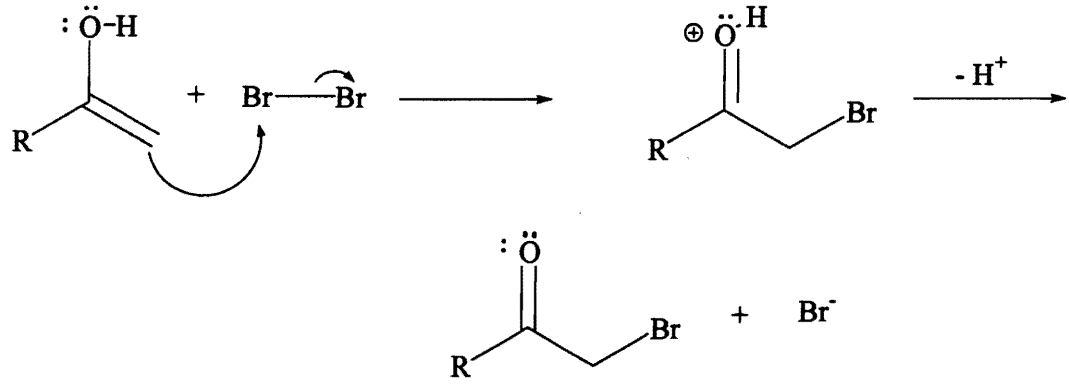
2.3.3 Bromination Reactions of Ketones

The third step in the sequence occurs via α-bromination of the ketone. Halogenations at the alpha position of aldehydes and ketones are possible with bromine, iodine, and chlorine.[49] Generally, the order of halogenation in an asymmetric ketone is $CH > CH_2 > CH_3$. Halogenations can be difficult to control, as they proceed until all of the halogen is used in base. Therefore, careful consideration much be taken in calculating the stoichiometric amounts of both the ketone and halogen. A 1:1 molar ratio must be used, or by-products such as the dihalogenated analogues contaminate the product. In a bromination involving a methyl ketone, the reaction can proceed via a haloform reaction. Haloform reactions usually produce a carboxylic acid. However, in this particular reaction sequence, low temperatures efficiently provide a method to halt the reaction at the desired point. An acid catalyst, $AlCl_3$, and cold temperatures initiate the formation of the enol. The reaction is allowed to warm to room temperature and proceeds via the mechanism shown in **Scheme 6**, page 18.

The reaction progress is monitored by thin layer chromatography (TLC), and upon disappearance of the starting material, the reaction is cooled to 0 °C. Water is added to quench the reaction. Subsequent washings with brine ensure completion of the workup.

2.3.4 Furanone Ring Formations

The most difficult step in the synthesis involves the furanone ring formation. Typically, these rings are cyclized from a diketo ester moiety in the presence of a strong base.[50] Yields are generally on the order of ~50%. The reaction involving the furanone



Scheme 6. Bromination reaction mechanism

ring formation is accomplished via an *in situ* coupling of the α-bromo ketone precursor and iodophenylacetic acid, followed by cyclization. The reaction can be accomplished in a one pot, two step synthesis, using a strong base, triethylamine (to initialize the coupling) and an additional base, 1,8-diazabicyclo[5,4,0]-undec-7-ene (DBU), to afford the furanone moiety.[47] However, with hindered starting materials, such as 3-iodophenylacetic acid, ring closure is somewhat inhibited.

This barrier can be overcome with the use of microwave chemistry. Microwave chemistry can be utilized in a variety of organic transformations. It provides a facile means of transforming organic compounds in short reaction times. Many variables can be controlled in microwave synthesis such as temperature, reaction times, and power. Reactions can even be accomplished in solvent-free environments. Microwave chemistry systems are so advanced that they can be programmed to control power, ramp, time, and other variables such that complex syntheses can be readily achieved.

The reaction to form the desired furanones proceeds in good (50%) yields. The two starting materials are dissolved in a solvent and an excess of base is added. The reaction vial is microwaved for a short period of time and then allowed to cool to room temperature. Acidification initiates precipitation of the product, which is then purified with column chromatography.

2.3.5 Suzuki Coupling

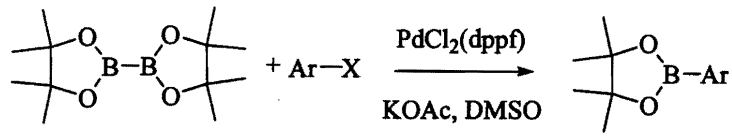
Boronic esters have become quite useful in organic synthesis.[51] They are readily coupled to aryl electrophiles via organometallic chemistry employing palladium-catalyzed cross-couplings.[52] A simple one pot synthesis is available for converting aryl

halides to arylboronic esters via a palladium cross-coupling reaction utilizing bis(pinacolato)diboron, an aryl halide, and a base as shown in **Scheme 7**, page 21.[52]

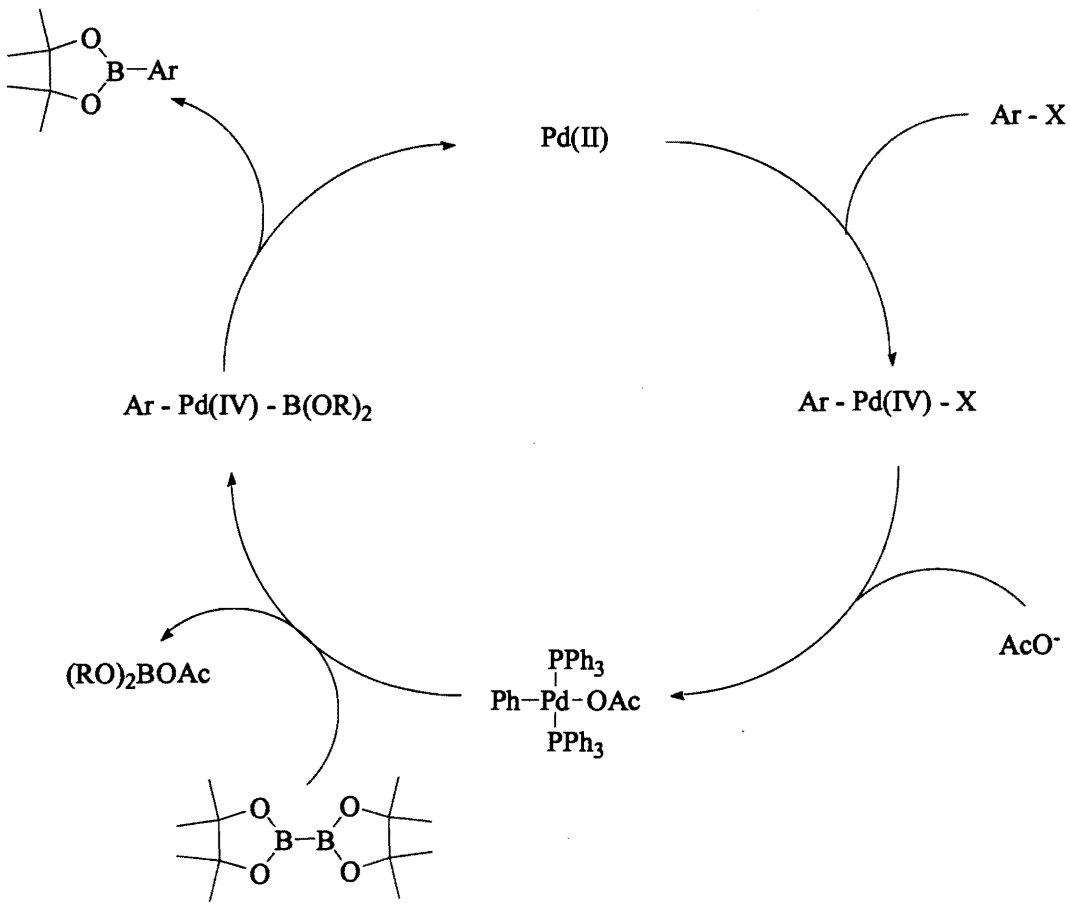
Refluxing temperatures are required for the reaction to proceed. A weak base such as potassium acetate provides the best yields while bases such as potassium phosphate and potassium carbonate give byproducts such as biphenyl. Polar solvents work best and increasing the solvent polarity accelerates the reactions. The reaction rates in various solvents decrease in the following order: DMSO > DMF > dioxane > toluene. Benzene may also be used as a solvent.

1,1-Bis(diphenylphosphino)ferrocene dichloro palladium is the most efficient catalyst for arenes possessing electron-withdrawing or electron-donating substituents. Electron-withdrawing substituents accelerate the rate of reaction, while substituents with electron-donating substituents require longer reaction times. Reaction times may be as short as one hour or as long as 24 hours. Grignard or lithium reagents require functional group protection, but many functional groups are tolerated in the coupling reaction itself, e.g., nitriles, ethers, and esters. Steric hindrance does not pose a barrier to successful coupling. Heteroaromatics reagents also readily react.

Suzuki coupling is believed to occur via the oxidative-reductive palladium cycle shown in **Scheme 8**, page 22. The haloarene undergoes oxidative addition to the palladium(0) complex giving a ArPd(II)X adduct. Transmetallation occurs between the diboron substrate and the palladium adduct to give a $ArPd(II)B(OR)_2$ intermediate. Reductive elimination produces the recycled palladium(0) complex and the desired aryl boronic ester. While the mechanism of the transmetallation is not completely understood,



Scheme 7. Suzuki coupling reaction



Scheme 8. Palladium cycle of Suzuki coupling

it is believed that the base generates the acetoxopalladium(II) species by displacing the palladium halide; it is this species that coordinates with the diboron substrate.

2.3.6 Conversion of Arylboronic Esters to Potassium Trifluoroborates

Potassium aryltrifluoroborates are now widely used as intermediates in organic synthesis.[53] They are chemically reactive salts that are both air and water stable.[54] Potassium trifluoroborates are readily prepared from the corresponding boronic ester and KHF_2 in methanol. The preparative reaction tolerates a variety of functional groups.

The reactions of trifluoroborates proceed via ligand exchange in weakly acidic media. It has been suggested by Thierig and Umland that the fluoroborate anion is thermodynamically more stable than the neutral boron species, and therefore, the product culminates from "ate" dissociation and hydrolytic cleavage.[55]

The preparation of trifluoroborates is straightforward. A solution of the boronic ester is dissolved in methanol along with saturated aqueous KHF_2. Precipitation of the potassium trifluoroborate product immediately occurs. The solvent is removed, and the salt is recrystallized from acetonitrile and acetone, producing a powdery solid.

2.3.7 Radioiodinations of Trifluoroborate Salts

Our laboratory has been interested in radiohalogenated pharmaceuticals for over 20 years[56-59] Previously, boronic acids, organoboronates and organometallics were utilized to synthesize radiolabeled compounds. The boronic acids were used under highly basic conditions, which proved detrimental to substitutents like esters and ketones.[1] Many organometallic reagents are undesirable since they can be difficult to separate from the desired radiohalogenated product. Furthermore, organometallics, such as organotin reagents, involve the use of heavy metals that have toxic effects on

humans.[60] Boronic esters have been successfully utilized, but their preparation is often difficult. Trifluoroborate salts are readily separated from reaction mixtures due to their ionic nature. This, combined with their stability, makes them attractive for use in radiohalogenation reactions.

We developed a radiohalogenation method utilizing boron reagents in 1984.[59] Since that time, the method has been improved and now can be applied to a wide variety of organic compounds. Reactions of substrates with electron-donating substituents are rapid and produce yields above 70%, while reactions of substrates with electron-withdrawing substituents require longer reaction times and produce more modest yields.[56] A wide variety of functional groups are tolerated, however, the presence of a nitro group has been found to be detrimental. Heterocycles are also tolerated, as are vinyltrifluoroborates.[56]

The reaction itself is straightforward. Radiohalogenations are carried out in aqueous tetrahydrofuran (THF) using no-carrier-added sodium [^{123}I]iodide and an oxidant. A solution of trifluoroborate in aqueous THF is added to the no-carrier-added Na^{123}I in aqueous sodium hydroxide. A solution of peracetic acid is added, and the reaction is allowed to stir in the dark until completion of the reaction, normally less than 15 minutes. Sodium thiosulfate is added to decompose the excess iodine, and the product is isolated using Sep-Pak filtration.

Chapter 3 Results and Discussion

The synthesis of a no-carrier-added iodine-123 labeled analogue of rofecoxib, **1**, is outlined in **Scheme 3**. Thioanisole, **2**, was converted to the requisite potassium trifluoroborate salt **8** in six steps. This synthetic pathway provided the *meta* and *para* analogues of rofecoxib. A total of six previously unknown compounds were synthesized: 3-(3-iodophenyl)-4-(methanesulfonylphenyl)-5*H*-furan-2-one, 4-(4-methanesulfonyl-phenyl)-3-[3-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2-yl)phenyl]-5*H*-furan-2-one, potassium 3-(3-trifluoroboro)-4-(4-methanesulfonylphenyl)phenyl-5*H*-furan-2-one, 3-(4-iodophenyl)-4-(methanesulfonylphenyl)-5*H*-furan-2-one, 4-(4-methanesulfonylphenyl)-3-[4-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2yl)phenyl]-5*H*-furan-2-one, and potassium 3-(4-trifluoroboro)-4-(4-methanesulfonylphenyl)phenyl-5*H*-furan-2-one. Both 3-(3-iodophenyl)-4-(methanesulfonylphenyl)-5*H*-furan-2-one and 3-(4-iodophenyl)-4-(methanesulfonylphenyl)-5*H*-furan-2-one are iodinated analogues of rofecoxib which, when labeled form with iodine-123, could be used as an imaging agent in nuclear medicine.

The first half of the synthesis was straightforward, with only recrystallization required to arrive at pure products. Washing the reaction mixtures with water, saturated sodium bicarbonate, and brine provided the desired products. Ketone **4** was prepared according to the literature procedure[46] by Friedel-Crafts acylation of thioanisole **3**. Distinct color changes signaled the reaction's progress. The cold reaction mixture developed a deep blue color upon the addition of all the reagents. The reaction was cooled to 0 °C and quenched by the addition of water. This produced a yellow color in

the organic phase. Further sequential washings with water, aqueous sodium bicarbonate, and brine lightened the yellow color, indicating removal of residual starting material. Yields greater than 80% were achieved after recrystallization. Proton (^{1}H) and carbon (^{13}C) NMR spectra were obtained and are presented in Appendix 1. The ^{1}H NMR assignments are shown in **Figure 2** on page 27; the ^{13}C NMR assignments are shown in **Figure 3** on page 28.

Oxidation of **4** using MMPP (magnesium (bis)monoperoxyphthalate hexahydrate) afforded sulfone **5** in 95% yield. This reaction provided a very clean product that needed no recrystallization,. Its ^{1}H and ^{13}C NMR spectra are presented in Appendix 1. The ^{1}H NMR assignments are shown in **Figure 2** on page 27; the ^{13}C NMR assignments are shown in **Figure 3** on page 28.

Sulfone **5** was allowed to react with bromine in chloroform at 0 °C in the presence of a trace amount of $AlCl_3$. A distinct color change, from dark red to light orange, signaled the end of the reaction. The reaction was quenched with water and a color change occurred (light orange to clear and colorless) to generate **6** in 85% yields. It was noted that allowing this reaction to run longer than 1 hour led to increased contamination of the product with the dibromoketone. The ^{1}H and ^{13}C NMR spectra of the product obtained are presented in Appendix 1. The ^{1}H NMR assignments are shown in **Figure 2** on page 27; the ^{13}C NMR assignments are shown in **Figure 3** on page 28.

During the second stage of the synthesis, column purification was a necessity at every step. However, the columns were run in a short time frame, to achieve rapid separation. Bromoketone **6** was coupled with 3-iodophenylacetic acid and then cyclized *in situ* in the presence of 3 molar equivalents of triethylamine using microwave

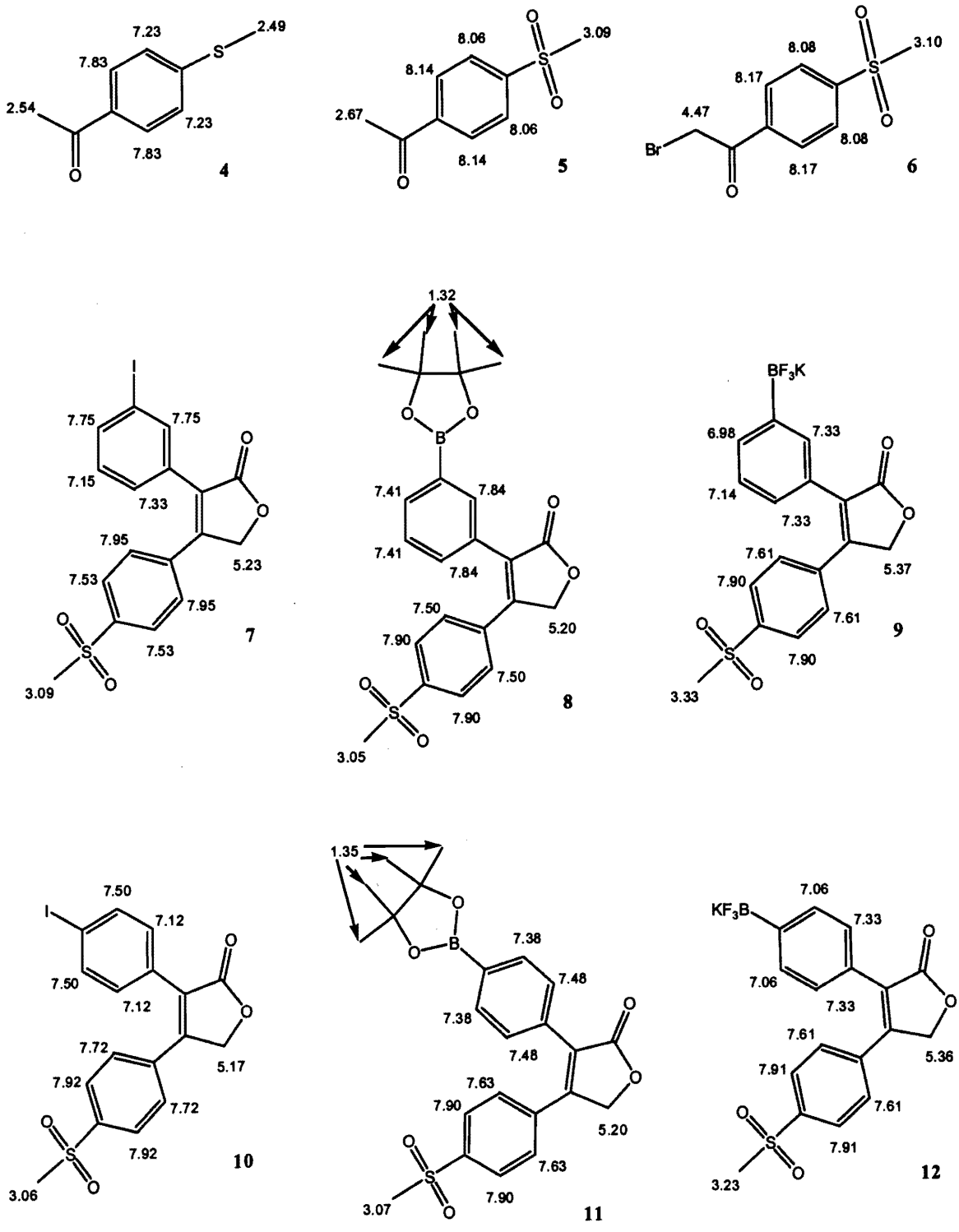


Figure 2. ^{1}H NMR assignments

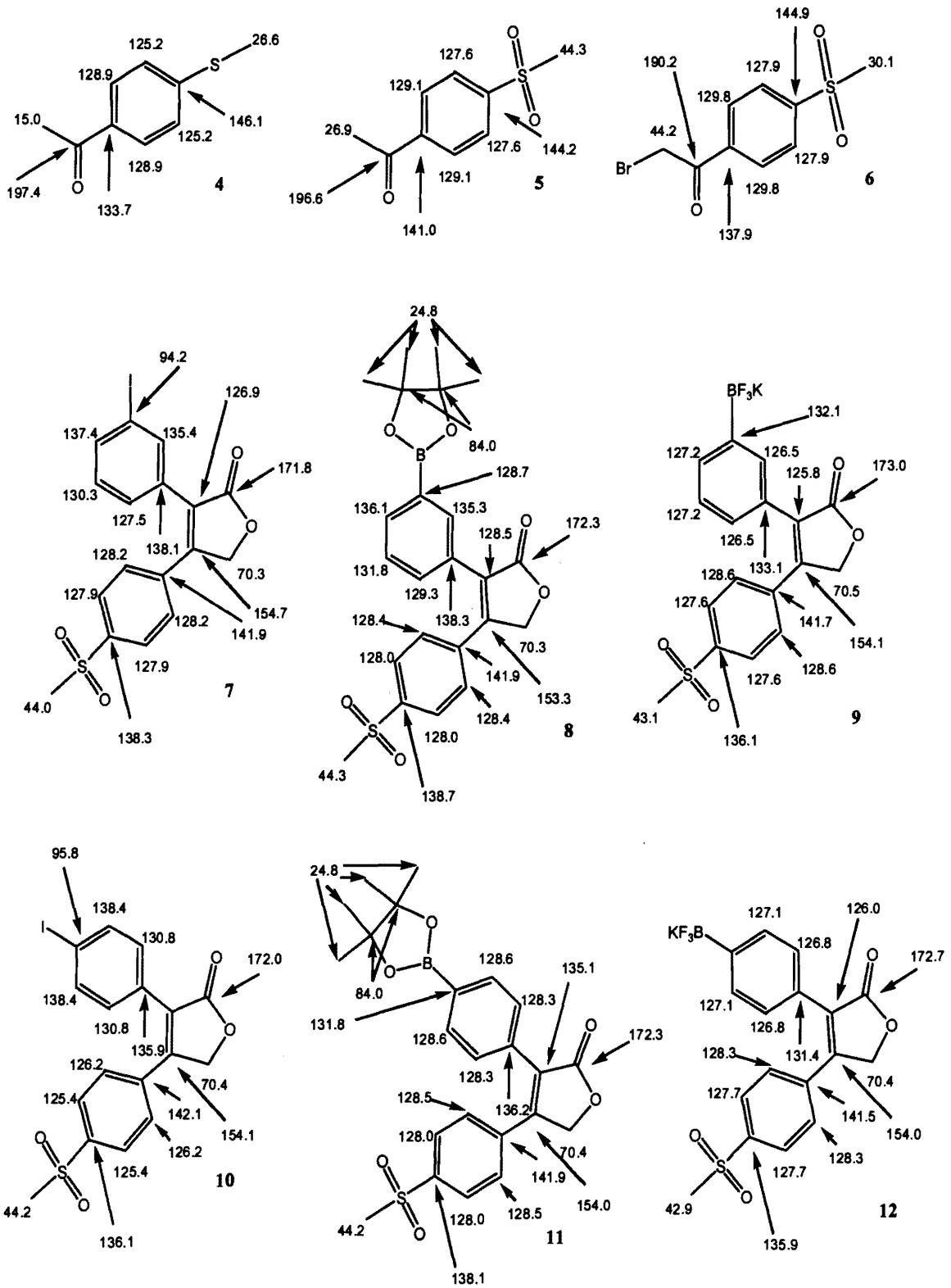


Figure 3. ^{13}C NMR assignments

irradiation to afford **7** (the non radioactive analogue of the target molecule **1**). The product was precipitated out of the reaction mixture by acidification. Completion of the reaction was evidenced by a color change from a dark brown mixture to a bright yellow precipitate. Column purification provided pure crystalline product in 50% yield. The presence of the dibromoketone impurity in the bromoketone did not markedly affect the coupling reaction, and it was removed during column purification.

A sample of the product was submitted for high resolution mass spectroscopy (HRMS); the results of which are presented in Appendix 2. The sample gave a molecular ion peak at 439.9572 mass units, 0.0002% difference from the calculated value of 439.9579 for the *meta*-iodo rofecoxib analogue. The ^{1}H and ^{13}C NMR spectra are presented in Appendix 1. The ^{1}H NMR assignments are shown in **Figure 2** on page 27; the ^{13}C NMR assignments are shown in **Figure 3** on page 28.

Compound **7** was converted to boronic ester **8** using Suzuki-Miyaura chemistry.[25] Thin layer chromatographic analysis revealed 3 spots, indicating that byproducts were formed during the coupling. Careful purification via column chromatography produced **8** in 57% yield. The reaction time was longer than expected, and it is suspected that the hindered position of the coupling site was a contributing factor.

A sample of the boronic ester **8** was submitted for elemental analysis. The carbon analysis showed 63.17%, a difference of 0.43% from the calculated value of 62.74%. Hydrogen analysis revealed 5.92%, a difference of 0.2% from the calculated value of 5.72%. Sulfur analysis indicated the compound contained 7.03% sulfur, a difference of 0.25% from the calculated value of 7.28%. This confirmed that **8** had been successfully prepared and characterized. The ^{1}H and ^{13}C NMR spectra are shown in Appendix 1. The

^{1}H NMR assignments are presented in **Figure 2** on page 27; the ^{13}C NMR assignments are shown in **Figure 3** on page 28.

Addition of KHF_2 to **8** then generated **9**.[55] Recrystallization afforded a 65% yield of the desired product. A sample of **9** was submitted for elemental analysis and high resolution mass spectrometry after drying at elevated temperatures *in vacuo*. The elemental analysis revealed that the compound contained water of hydration. The HRMS (shown in Appendix 2) revealed a peak at 381.2 mass units, a difference of 0.03% from the calculated value of 381.1 for the peak of the trifluoroborate salt. This confirmed that the compound was formed. The ^{1}H, ^{13}C, and ^{19}F NMR spectra are presented in Appendix 1. The ^{1}H NMR assignments are shown in **Figure 2** on page 27; the ^{13}C NMR assignments are shown in **Figure 3** on page 28.

The trifluoroborate salt of the *para* rofecoxib analogue was also synthesized in a parallel manner. 4-Iodophenylacetic acid was coupled to bromoketone **6** and then cyclized *in situ* using microwave irradiation to afford **10**, the *para*-iodo rofecoxib analogue (the non radioactive analogue of the target molecule **1**) in 52% yield. Its HRMS is presented in Appendix 2, with a mass ion peak at 439.9584 mass units, a 0.05% difference from the calculated value of 439.9579. The ^{1}H and ^{13}C NMR spectra are shown in Appendix 1. The ^{1}H NMR assignments are presented in **Figure 2** on page 27; the ^{13}C NMR assignments are shown in **Figure 3** on page 28.

The *para*-iodo rofecoxib analogue was converted to the *para* boronic ester analogue, **11** using Suzuki-Miyaura chemistry.[52] This reaction proceeded more rapidly and efficiently than that of the *meta* analogue in 67% yields. A sample of the pure compound was submitted for HRMS, with a mass ion peak at 440.1461, a difference of

0.0002% from the calculated value of 440.1469. The ^{1}H and ^{13}C NMR spectra are shown in Appendix 1. The ^{1}H NMR assignments are shown in **Figure 2** on page 27; the ^{13}C NMR assignments are shown in **Figure 3** on page 28.

The trifluoroborate salt, **12** was prepared by the addition of KHF_2 in 70% yield.[55] The compound was submitted for elemental analysis. The elemental analysis revealed that the compound contained water of hydration. The carbon analysis showed 46.36%, a difference of 0.23% from the calculated value of 46.59%. Hydrogen analysis revealed 3.39%, a difference of 0.06% from the calculated value of 3.45%. Sulfur analysis indicated the compound contained 7.22% sulfur, a difference of 0.10% from the calculated value of 7.32%. This confirmed the identity of the new compound. The ^{1}H, ^{13}C, and ^{19}F NMR spectra are presented in Appendix 1. The ^{1}H NMR assignments are shown in **Figure 2** on page 27; the ^{13}C NMR assignments are shown in **Figure 3** on page 28.

We then utilized our recently developed radioiodination procedure to generate the no-carrier-added radioiodination products.[56] The radiochemical purity of **1** ($R_f = 0.36$) determined by radio-TLC, shown in **Figure 4**, page 32, was 92% with a decay corrected yield of 37%. The radiochemical purity of **2** ($R_f = 0.36$) determined by radio-TLC, shown in **Figure 5**, page 33, was 98% with a decay corrected yield of 42%. Decay corrected yield is a radiochemical yield, in which the yield is corrected for the time dependent decay of the radionuclide. As was expected, the *meta* analogue showed lower yields throughout the synthesis than the *para* derivative.

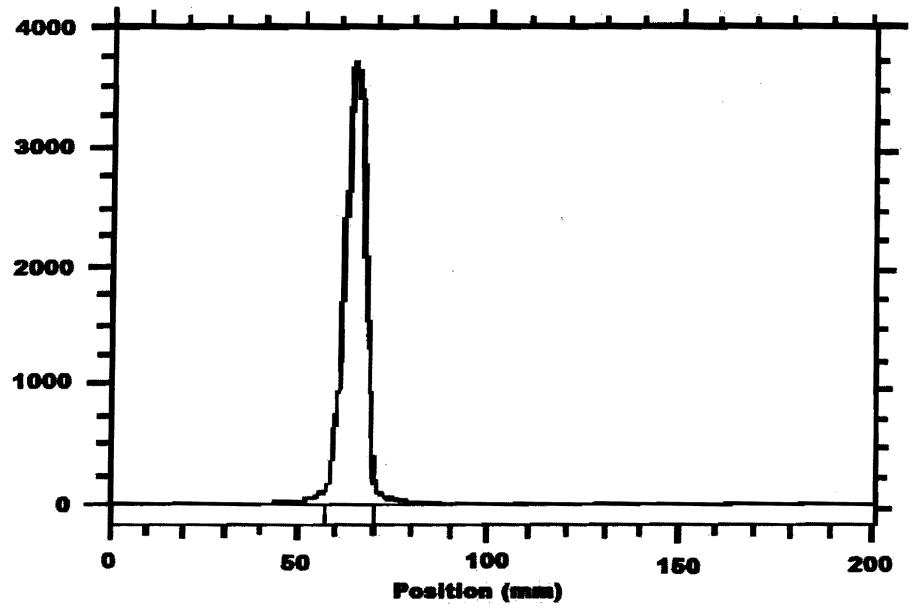


Figure 4. Radio-TLC of 3-(3-[^{123}I]Iodophenyl)-4-(methanesulfonylphenyl) -5*H*-furan-2-one, 1

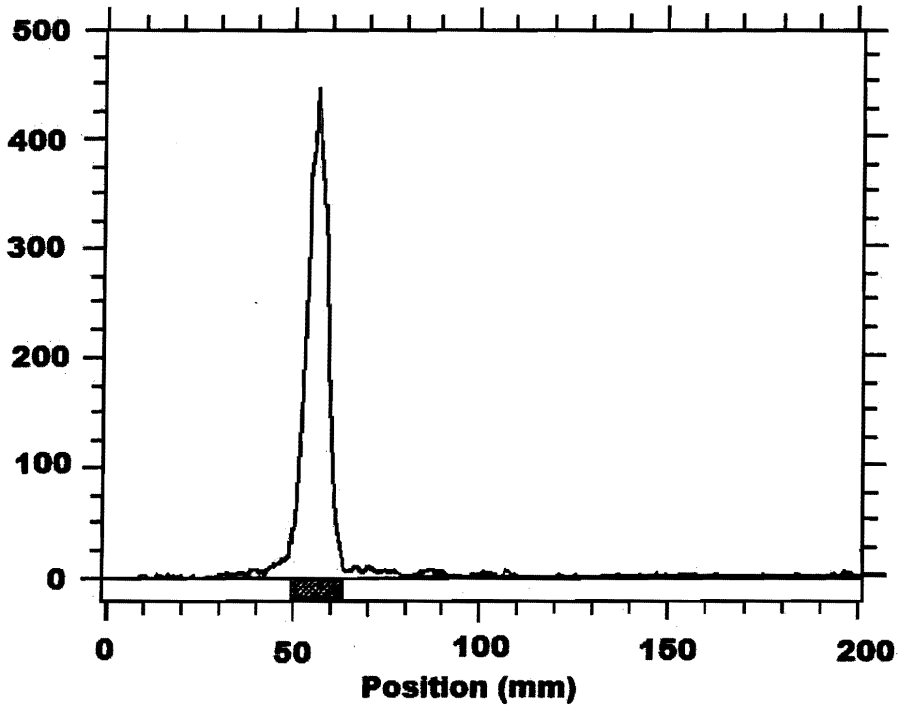


Figure 5. Radio-TLC of 3-(4-[^{123}I]Iodophenyl)-4-(methanesulfonylphenyl) -5*H*-furan-2-one, 2

The procedure is unique in that the reactive intermediates (**8** and **12** in this study) are ionic in contrast to the lipophilic radiolabeled products. This facilitates product purification. Indeed, separation of the ionic trifluoroborate precursors from **1** and **2** is readily achieved using simple Sep-Pak filtration which greatly facilitates product recovery.

HPLC separation of the radiolabeled product was deemed unnecessary since TLC analysis of the eluent obtained after Sep-Pak separation showed no evidence of starting material or hydrolyzed byproducts using ultraviolet light, phosphomolybdic acid, or iodine visualization. In addition, unlike the situation in which the goal is to detect limited numbers of neuroreceptors, cyclooxygenase enzymes are over expressed obviating the need for extraordininarily high specific activity levels. It is noteworthy that the radiochemical purity of the products exceeds 92% as revealed by radio TLC.

Chapter 4 Experimental Section

4.1 GENERAL METHODS

Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were acquired either on a Bruker AC250 at 250.13 and 62.89 MHz, or on a Varian Mercury 300 at 300.09 and 75.46 MHz respectively. Chemical shifts are reported in parts per million (ppm) referenced to trimethylsilane (TMS) using residual protons in deuterated solvents for measurement. The splitting patterns are abbreviated as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, and m = multiplet. Fluorine (^{19}F) NMR spectra were obtained on a Varian Mercury 300 at 282.33 MHz. The mass spectral analyses were obtained on a VG Quattro II electrospray mass spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Melting points were determined on a Barnstead Thermolyne melting point apparatus and are uncorrected.

All commercial reagents were purchased from Aldrich Chemical Co., Milwaukee, WI, and used as received without further purification unless otherwise noted. All solvents were reagent grade and were distilled from appropriate drying agents under a nitrogen atmosphere prior to use. A CEM Discover microwave was utilized in the coupling and cyclization synthesis.

Column chromatography was performed using silica gel (60 Å, 32-63 mesh) obtained from Bodman Chemical, Aston, PA. Analytical thin layer chromatography (TLC) was performed using 250 μm silica gel plates obtained from Analtech, Newark,

DE, which were visualized with ultraviolet illumination at 254 nm, phosphomolybdic acid, or development in an iodine chamber. The pH of solutions was determined using Whatman pH Indicator Paper.

Radio-TLC was carried out using a Bioscan Autochanger AR-2000 imaging scanner. $Na^{123}I$ (specific activity = 1.93 x 10^6 Ci/g) was obtained from Nordion Inc., Vancouver, Canada.

All glassware, syringes, and needles were dried in an oven and cooled under nitrogen prior to use.

4.2 EXPERIMENTAL PROCEDURES FOR SYNTHESIS OF POTASSIUM 3-(3-TRIFLUOROBORO)-4-(4-METHANESULFONYLPHENYL)PHENYL-5*H*-FURAN-2-ONE, 9

4.2.1 Synthesis of 1-(4-Methylsulfanylphenyl)ethanone, 4.[47]

To a cold (0 °C) solution of $AlCl_3$ (6.8 g, 51 mmol) in $CHCl_3$ (50 mL) was added acetyl chloride (4.0 mL, 55 mmol) while maintaining the temperature below 10 °C. Thioanisole **3** (5.2 g, 5.0 mL, 42 mmol) was then added while maintaining the temperature below 5 °C; additional $CHCl_3$ (20 mL) was added to facilitate stirring. The mixture was allowed to warm to room temperature while stirring. After 2 hours, the mixture was cooled to 10 °C. Water (20 mL) was slowly added and the layers separated. The organic layer was washed sequentially with water (2 x 30 mL), saturated aqueous $NaHCO_3$ (2 x 50 mL) and brine, and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was recrystallized from a hexane/ethyl acetate solution to afford an off-white solid, 6.32 g (89.3%); m.p. 81-82 °C; ^1H NMR

(300 MHz, $CDCl_3$): δ 2.49 (s, 3H), 2.54 (s, 3H), 7.23 (d, 2H, J = 8.7 Hz), 7.83 (d, 2H, J = 8.7 Hz); ^{13}C NMR (300 MHz, $CDCl_3$): δ 15.0, 26.6, 125.2, 128.9, 133.7, 146.1, 197.4.

4.2.2 Synthesis of 1-(4-Methanesulfonylphenyl)ethanone, 5.[47]

To a solution of **4** (1.7 g, 10 mmol) in a mixture of MeOH (10 mL) and CH_2Cl_2 (30 mL) was added magnesium bis(monoperoxyphthalate) hexahydrate (MMPP) (7.5 g, 15 mmol) over a 30 min period. The mixture was stirred at room temperature for 2.5 h, filtered, and the filtrate washed with saturated aqueous $NaHCO_3$ and then brine. The organic layer was removed under reduced pressure to give 1.89 g (95%) of **5** as a white solid; m.p. 129-130 °C; ^1H NMR (250 MHz, $CDCl_3$): δ 2.67 (s, 3H), 3.09 (s, 3H), 8.06 (d, 2H, J = 8.6 Hz), 8.14 (d, 2H, J = 8.6 Hz); ^{13}C NMR (250MHz, $CDCl_3$): δ 26.9, 44.3, 127.6, 129.1, 141.0, 144.2, 196.6.

4.2.3 Synthesis of 2-Bromo-1-(4-methanesulfonylphenyl)ethanone, 6.[47]

Ketone **5** (2.0 g, 10 mmol) was dissolved in $CHCl_3$ (20 mL) and cooled to 0 °C. $AlCl_3$ (2.0 g, 10 mg) was added, followed by a solution of bromine (0.5 mL, 10 mmol) in $CHCl_3$ (10 mL). The ice bath was removed and the solution stirred at room temperature until completion of the reaction (TLC). Water (50 mL) was added to the reaction mixture, and the organic layer was separated. After washing the organic layer with brine and drying over Na_2SO_4, the solvent was removed under reduced pressure. The residue was recrystallized from ethyl acetate-hexane (1:1) to give pure product (2.4 g, 85%); m.p. 127-129 °C; ^1H NMR (250 MHz, $CDCl_3$): δ 3.10 (s, 3H), 4.47 (s, 2H), 8.08 (d, 2H, J = 8.5 Hz), 8.17 (d, 2H, J = 8.5 Hz); ^{13}C NMR (CDCl3): δ 30.1, 44.2, 127.9, 129.8, 137.9, 144.9, 190.2.

4.2.4 Synthesis of 3-(3-Iodophenyl)-4-(methanesulfonylphenyl)-5*H*-furan-2-one, 7.

Ketone **6** (0.29 g, 1.05 mmol) and 3-iodophenyl acetic acid (0.22 g, 1.0 mmol) were dissolved in acetonitrile (10 mL) in a clean, dry microwave tube. Triethylamine (0.42 mL, 3.0 mmol) was slowly added with stirring. The tube was then placed in the microwave and irradiated at 150 Watts for 20 minutes. The mixture was acidified with aqueous 1*N* HCl (color changed from dark brown to light yellow). A mixture of ice and water (50 mL) was added with stirring. The precipitate was filtered, rinsed with water, dissolved in CH_2Cl_2 (20 mL) and then dried over anhydrous $MgSO_4$. The filtrate was concentrated to near dryness and chromatographed over silica gel using ethyl acetate and hexane (1:1) to generate pure product **7** (0.23 g, 50%); m.p. 152-154 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.09 (s, 3H), 5.23 (s, 2H), 7.15 (t, 1H, J = 7.8 Hz), 7.33 (m, 1H, J = 8.1 Hz), 7.53 (d, 2H, J = 8.1 Hz), 7.75 (d, 2H, J = 9.9 Hz), 7.95 (d, 2H, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$): δ 44.0, 70.3, 94.2, 126.9, 127.5, 127.9, 128.2, 130.3, 135.4, 137.4, 138.1, 138.3, 141.9, 154.7, 171.8. Exact mass calculated for $C_{17}H_{13}IO_4S$ *m/z* 439.9579; found *m/z* 439.9572.

4.2.5 Synthesis of 4-(4-Methanesulfonylphenyl)-3-[3-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2-yl)phenyl]-5*H*-furan-2-one, 8.[47]

A 2-necked, 25 mL round-bottom flask was charged with 1,1-bis-(diphenylphosphino)ferrocene dichloropalladium [$PdCl_2$(dppf)] (23 mg, 0.03 mmol), dppf (33 mg, 0.06 mmol), potassium acetate (0.30 g, 3.0 mmol), bis(pinacolato)diboron (0.38g, 1.5 mmol) and flushed with nitrogen. A solution of **7** (0.44 g, 1.0 mmol) in 1,4-dioxane (5 mL) was added and the solution stirred for 24 h at 100 °C. The mixture was cooled to room temperature, 10 g silica gel was added and the mixture was concentrated

to dryness. The residue was subjected to column chromatography over silica gel using ethyl acetate and hexane (6:4) to obtain pure product **8** (0.26 g, 57.2%), m.p. 181-183 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32 (s, 12H), 3.05 (s, 3H), 5.20 (s, 2H), 7.41 (m, 2H), 7.50 (d, 2H, J = 8.4 Hz), 7.84 (m, 2H), 7.90 (d, 2H, J = 8.4 Hz); ^{13}C NMR (300 MHz, $CDCl_3$): δ 24.8, 44.3, 70.3, 84.0, 128.0, 128.4, 128.5, 128.7, 129.3, 131.8, 135.3, 136.1, 138.3, 138.7, 141.9, 153.3, 172.3. Analytically calculated for $C_{23}H_{25}BO_6S$: C, 62.74; H, 5.72, S, 7.28. Found: 63.17, 5.92, 7.03.

4.2.6 Synthesis of Potassium 3-(3-trifluoroboro)-4-(4-methanesulfonylphenyl) phenyl-5*H*-furan-2-one, 9.[47]

Compound **8** (0.44 g, 1.0 mmol) was dissolved in MeOH (5 mL) and KHF_2 (0.47 g, 6.0 mmol) dissolved in minimal water was added with stirring at room temperature. After one hour, the mixture was concentrated to near dryness, dissolved in acetone, and filtered. The filtrate was concentrated; and the product was recrystallized from CH_3CN to produce pure **9** (0.28 g, 65.3%); m.p. 281-283 °C; ^{1}H NMR (DMSO-d_6): δ 3.33 (s, 3H), 5.37 (s, 2H), 6.98 (bs, 1H), 7.14 (bs, 1H), 7.33 (bs, 2H), 7.61 (d, 2H, J = 6.6 Hz), 7.90 (d, 2H, J = 6.6 Hz); ^{13}C NMR (DMSO-d_6): δ 43.1, 70.5, 125.8, 126.5, 127.2, 127.6, 128.5, 128.6, 131.9, 132.1, 133.1, 136.1, 141.7, 154.1, 173.0; ^{19}F NMR (DMSO-d_6): δ -136.6. Exact mass calculated for $[C_{17}H_{15}BF_3O_5S]^-$ *m/z* 381.1; found *m/z* 381.2. Analytically calculated for $C_{17}H_{19}BF_3KO_7S$: C, 43.05; H, 4.04; S, 6.76. Found C, 43.84; H, 3.18; S, 6.59.

4.3 EXPERIMENTAL PROCEDURES FOR SYNTHESIS OF POTASSIUM 3-(4-TRIFLUOROBORO)-4-(4-METHANESULFONYLPHENYL)PHENYL-5*H*-FURAN-2-ONE, 12

4.3.1 Synthesis of 3-(4-Iodophenyl)-4-(methanesulfonylphenyl)-5*H*-furan-2-one, 10.

Ketone **6** (2.90 g, 10.5 mmol) and 4-iodophenyl acetic acid (2.20 g, 10.0 mmol) were dissolved in acetonitrile (25 mL) in a clean, dry microwave tube. Triethylamine (4.2 mL, 30.0 mmol) was slowly added with stirring. The tube was then placed in the microwave and irradiated at 150 Watts for 20 minutes. The mixture was acidified with aqueous 1*N* HCl (color changed from dark brown to light yellow). A mixture of ice and water (200 mL) was added with stirring. The precipitate was filtered, rinsed with water, dissolved in CH_2Cl_2 (20 mL) and then dried over anhydrous $MgSO_4$. The filtrate was concentrated to near dryness and chromatographed over silica gel using ethyl acetate and hexane (1:1) to generate pure **10** (2.28 g, 52%); m.p. 154-156 °C; ^{1}H NMR (250 MHz, $CDCl_3$): 3.06 (s, 3H), 5.17 (s, 2H), 7.12 (d, 2H, J = 8.4 Hz), 7.50 (d, 2H, J = 8.4 Hz), 7.72 (d, 2H, J = 8.4 Hz), 7.92 (d, 2H, J = 8.4 Hz); ^{13}C NMR (250 MHz, $CDCl_3$): δ 44.2, 70.4, 95.8, 125.4, 126.2, 130.8, 135.9, 136.1, 138.4, 142.1, 154.1, 172.0. Exact mass calculated for $C_{17}H_{13}IO_4S$ *m/z* 439.9579; found *m/z* 439.9584.

4.3.2 Synthesis of 4-(4-Methanesulfonylphenyl)-3-[4-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2yl)phenyl]-5*H*-furan-2-one, 11.[47]

A 2-necked, 25 mL round-bottomed flask was charged with 1,1-bis-(diphenylphosphino)ferrocene dichloropalladium [$PdCl_2$(dppf)] (23 mg, 0.03 mmol), dppf (33 mg, 0.06 mmol), potassium acetate (0.30 g, 3.0 mmol), bis(pinacolato)diboron

(0.38g, 1.5 mmol) and flushed with nitrogen. A solution of **10** (0.44 g, 1.0 mmol) in 1,4-dioxane (5 mL) was added and the solution stirred for 16 h at 100 °C. The mixture was cooled to room temperature, 10 g silica gel was added and the solvent removed under reduced pressure to provide a powder. The powder was subjected to column chromatography over silica gel using ethyl acetate and hexane (6:4) to obtain pure product **11** (0.30 g, 66%), m.p. 182-184 °C; ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.35(s, 12H), 3.07 (s, 3H), 5.20 (s, 2H), 7.38 (d, 2H, J = 7.6 Hz), 7.48 (d, 2H, J = 8.4 Hz), 7.63 (d, 2H, J = 8.0 Hz), 7.90 (d, 2H, J = 8.4 Hz); ^{13}C NMR (250 MHz, $CDCl_3$): δ 24.8, 44.2, 70.4, 84.0, 128.0, 128.3, 128.5, 128.6, 131.8, 135.1, 136.2, 138.1, 141.9, 154.0, 172.3. Exact mass calculated for $C_{23}H_{25}BO_6S$: *m/z* 440.1469; Found: *m/z* 440.1461.

4.3.3 Synthesis of Potassium 3-(4-trifluoroboro)-4-(4-methanesulfonylphenyl) phenyl-5*H*-furan-2-one, 12.[47]

Compound **11** (0.44 g, 1.0 mmol) was dissolved in MeOH (5 mL) and KHF_2 (0.47 g, 6.0 mmol) dissolved in minimal waterwas added with stirring at room temperature. After one hour, the mixture was concentrated to near dryness, dissolved in acetone, and filtered. The filtrate was concentrated and the product was recrystallized from CH_3CN to produce pure **12** (0.30 g, 70.0%); m.p. 283-285 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.23 (s, 3H), 5.36 (s, 2H), 7.06 (d, 2H, J = 7.8Hz), 7.33 (d, 2H, J = 7.5 Hz), 7.61 (d, 2H, J = 8.4 Hz), 7.91 (d, 2H, J = 8.1 Hz); ^{13}C NMR (250 MHz, DMSO-d_6): δ 42.9, 70.4, 126.0, 126.8, 127.1, 127.7, 128.3, 131.4, 135.9, 141.5, 154.0, 172.7; ^{19}F NMR (300 MHz, DMSO-d_6): δ -139.0. Analytically calculated for $C_{17}H_{15}BF_3KO_5S$: C, 46.59; H, 3.45; S, 7.32. Found C, 46.36; H, 3.39; S, 7.22.

4.4 EXPERIMENTAL PROCEDURES FOR RADIOLABELING

4.4.1 Synthesis of 3-(3-[^{123}I]Iodophenyl)-4-(methanesulfonylphenyl) -5*H*-furan-2-one, 1.[47]

Trifluoroborate **9** (100 μL of 5.2 x 10^{-2} solution in 50% aqueous tetrahydrofuran) was placed in a 2 mL Wheaton vial containing no-carrier-added Na^{123}I (37 MBq in 0.1% aqueous NaOH). To this was added peracetic acid (100 μL, 0.3% solution in methanol). The reaction vial was sealed, covered with aluminum foil, and the mixture stirred for 30 min at room temperature. A drop of 10% aqueous sodium thiosulfate was added to decompose excess iodine and the radioiodinated product was isolated by passing it through a silica gel Sep-Pak cartridge using petroleum ether: ethyl acetate (40:60) as eluent. The radiochemical purity of **1** was determined by radio-TLC (aluminum backed silica gel plate, petroleum ether:ethyl acetate = 40:60); R_f = 0.36. The TLC retention time was identical to that of an authentic non radioiodinated sample. The radiochemical purity was 92% and the decay corrected yield was 37%. HPLC separation of the radiolabeled product was deemed unnecessary since TLC analysis of the eluent obtained after Sep-pak separation showed no evidence of starting material or hydrolyzed by products using ultraviolet light, phosphomolybdic acid. The total synthesis time was 40 min.

4.4.2 Synthesis of 3-(4-[^{123}I]Iodophenyl)-4-(methanesulfonylphenyl) -5*H*-furan-2-one, 2.[47]

Trifluoroborate **12** (100 μL of 5.2 x 10^{-2} solution in 50% aqueous tetrahydrofuran) was placed in a 2 mL Wheaton vial containing no-carrier-added Na^{123}I (37 MBq in 0.1% aqueous NaOH). To this was added peracetic acid (100 μL, 0.3% solution in methanol).

The reaction vial was sealed, covered with aluminum foil, and the mixture stirred for 30 min at room temperature. A drop of 10% aqueous sodium thiosulfate was added to decompose excess iodine and the radioiodinated product was isolated by passing it through a silica gel Sep-Pak cartridge using petroleum ether: ethyl acetate (40:60) as eluent. The radiochemical purity of **2** was determined by radio-TLC (aluminum backed silica gel plate, petroleum ether:ethyl acetate = 40:60); $R_f = 0.36$. The TLC retention time was identical to that of an authentic non radioiodinated sample. The radiochemical purity was 98% and the radiochemical yield was 42%. HPLC separation of the radiolabeled product was deemed unnecessary since TLC analysis of the eluent obtained after Sep-pak separation showed no evidence of starting material or hydrolyzed by products using ultraviolet light, phosphomolybdic acid, or iodine visualization the radiochemical purity of the product exceeds 98% as revealed by radio TLC. The total synthesis time was 40 min.

Chapter 5 Conclusions and Future Work

5.1 CONCLUSIONS

In conclusion, two no-carrier-added iodine-123 labeled rofecoxib analogues, **1** and **2**, were synthesized as potential SPECT agents for imaging COX-2 receptor sites. The syntheses involved an iododeboronation sequence. The high yield preparation has the advantage that the reactive intermediates are hydrolytically and chemically stable under normal laboratory conditions. Furthermore, because of the ionic nature of the intermediates, they are readily separated from the lipophilic target molecules by simple filtration using commercially available silica gel Sep-paks.

5.2 FUTURE WORK

Future work should focus on preparation of the ortho-derivative of the no-carrier-added iodine-123 labeled rofecoxib analogue using the iododeboronation sequence. The evaluation of both the prepared rofecoxib analogues **1** and **2** both *in vitro* and *in vivo* should be investigated. Results from these studies could be correlated with their parent structures and aid in the design of additional target molecules.

The syntheses of more complex rofecoxib analogues should also be explored, as well as *in vivo* biodistribution studies of these target molecules.

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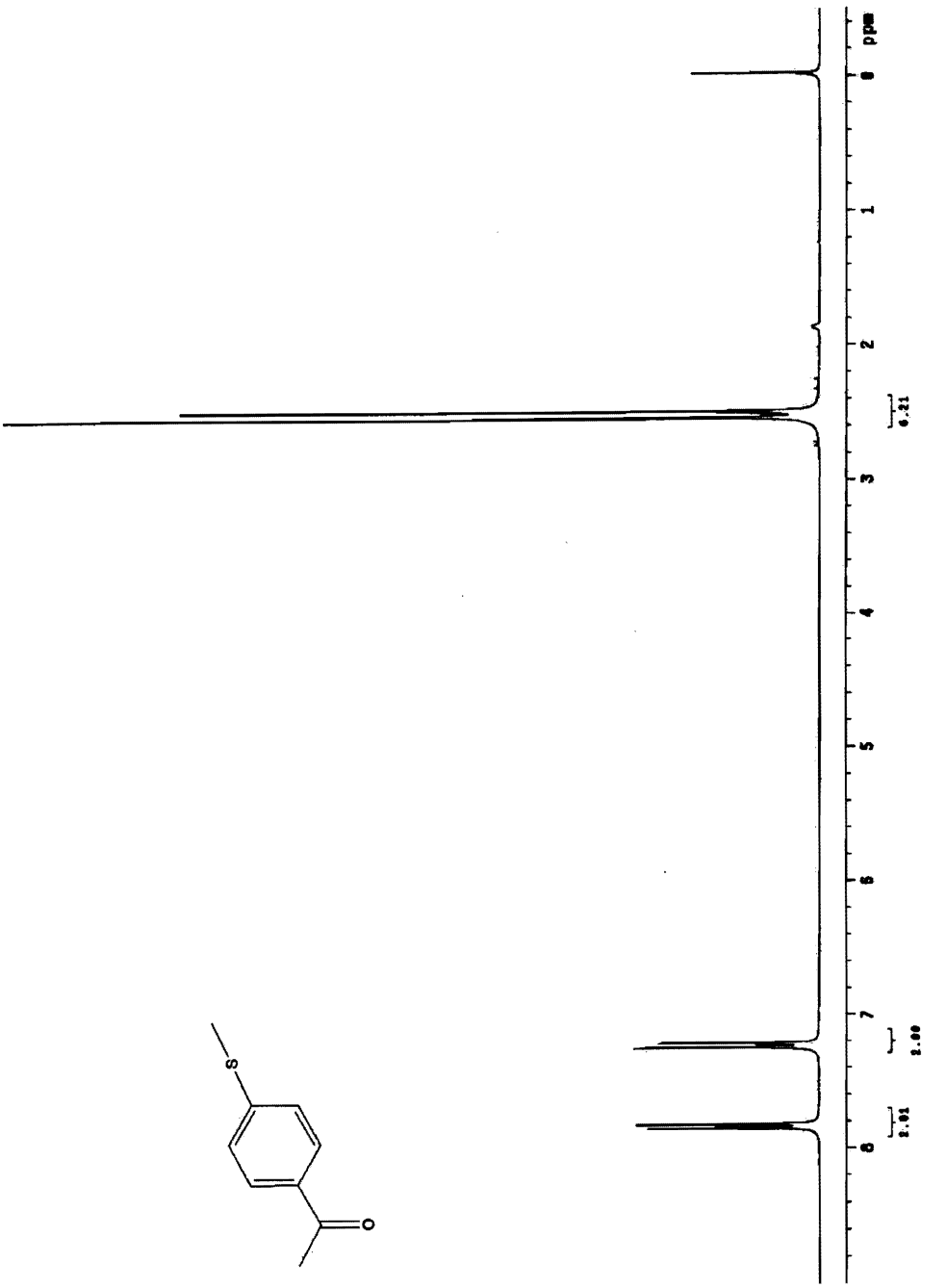
APPENDIX

APPENDIX 1

^{1}H, ^{13}C, and ^{19}F NMR Spectra of Intermediate and Target Compounds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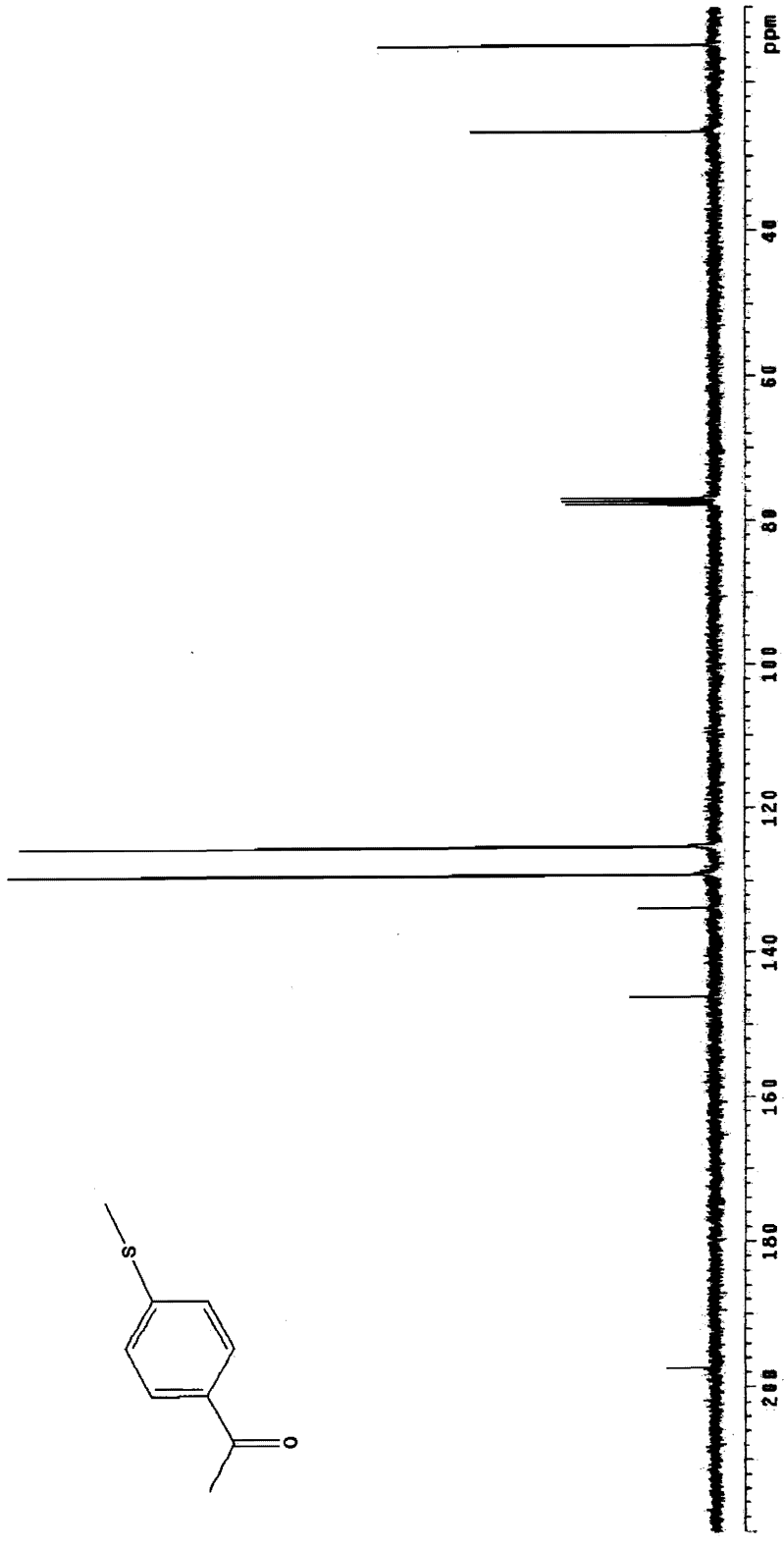
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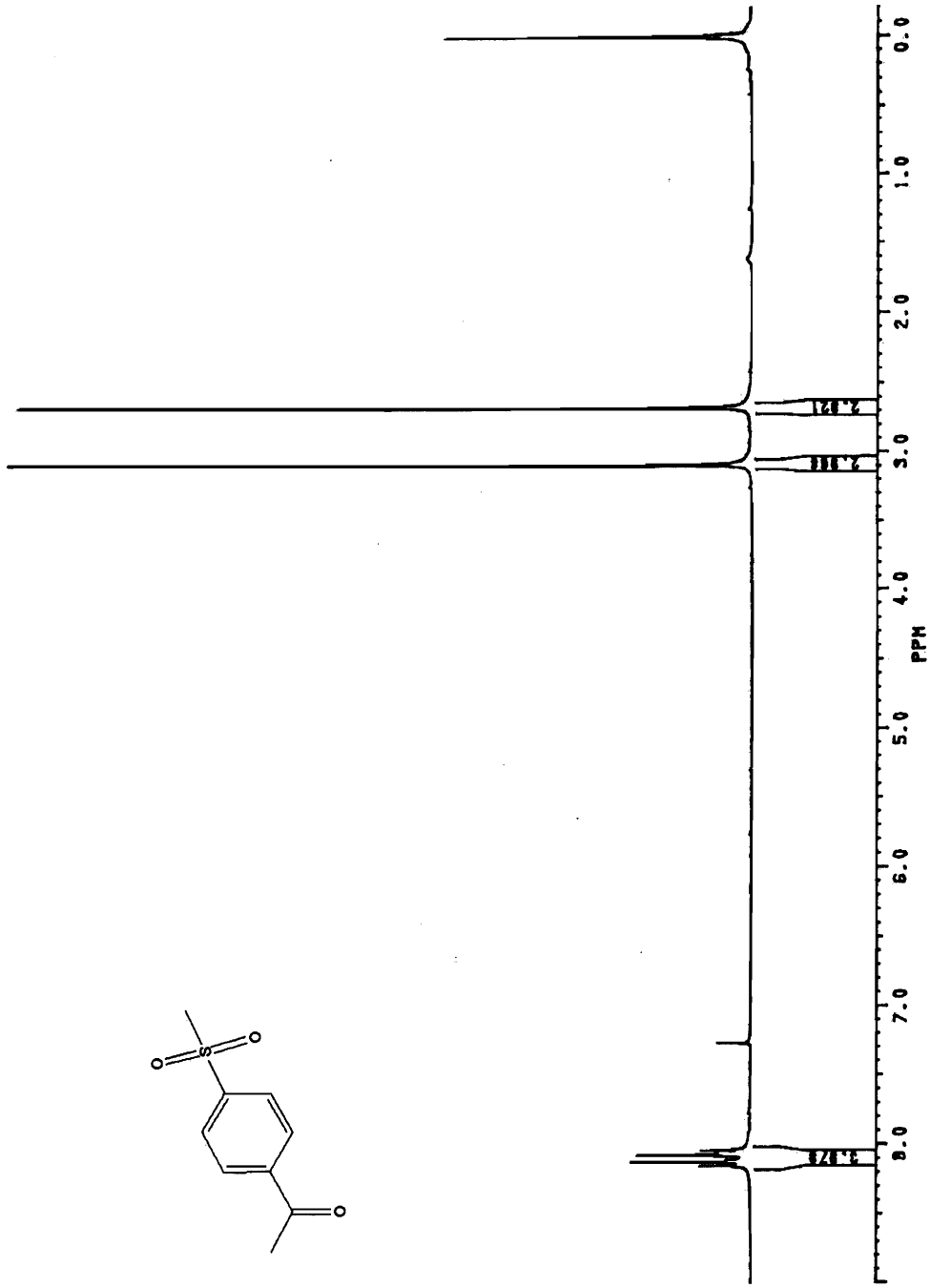
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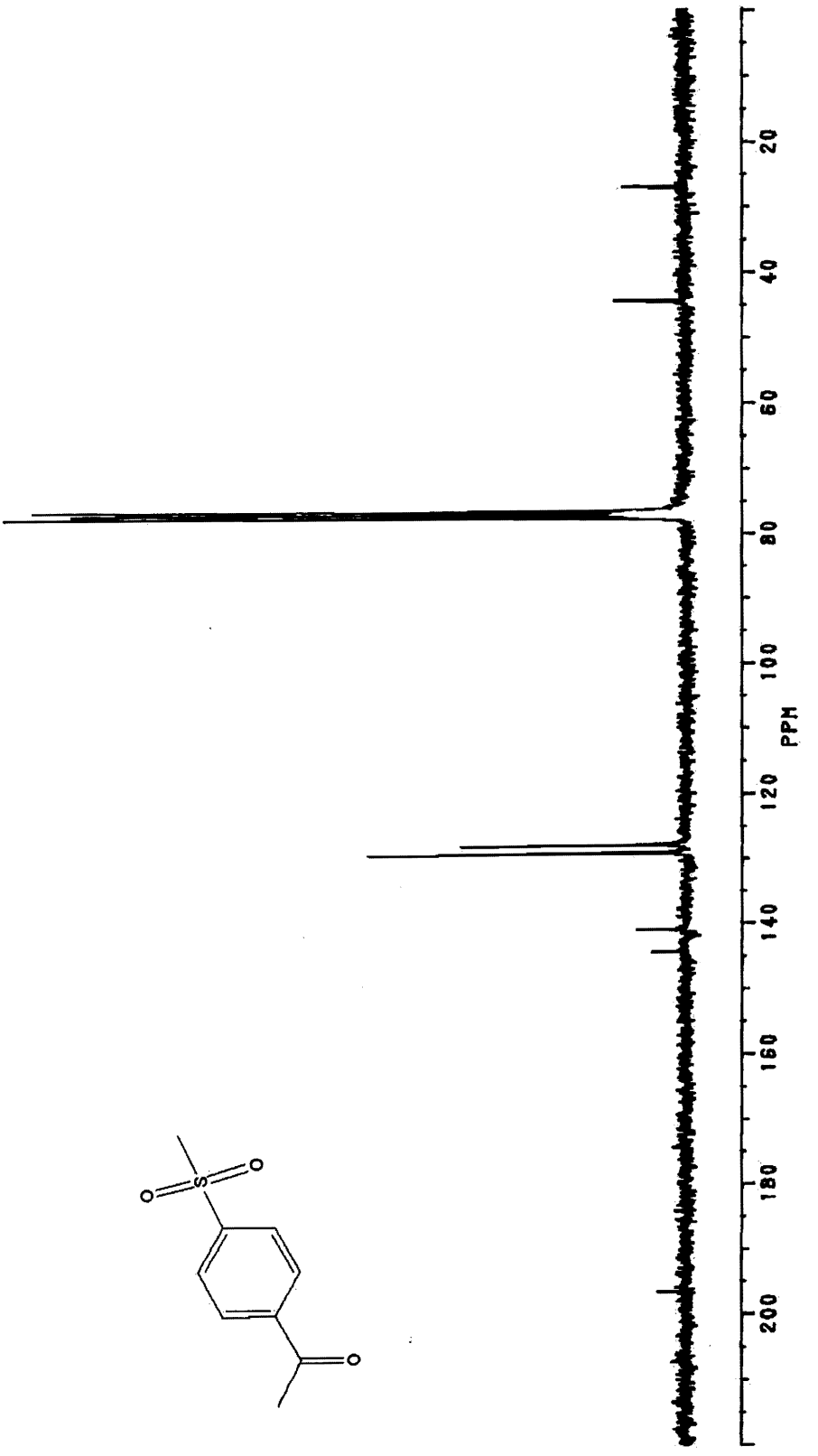
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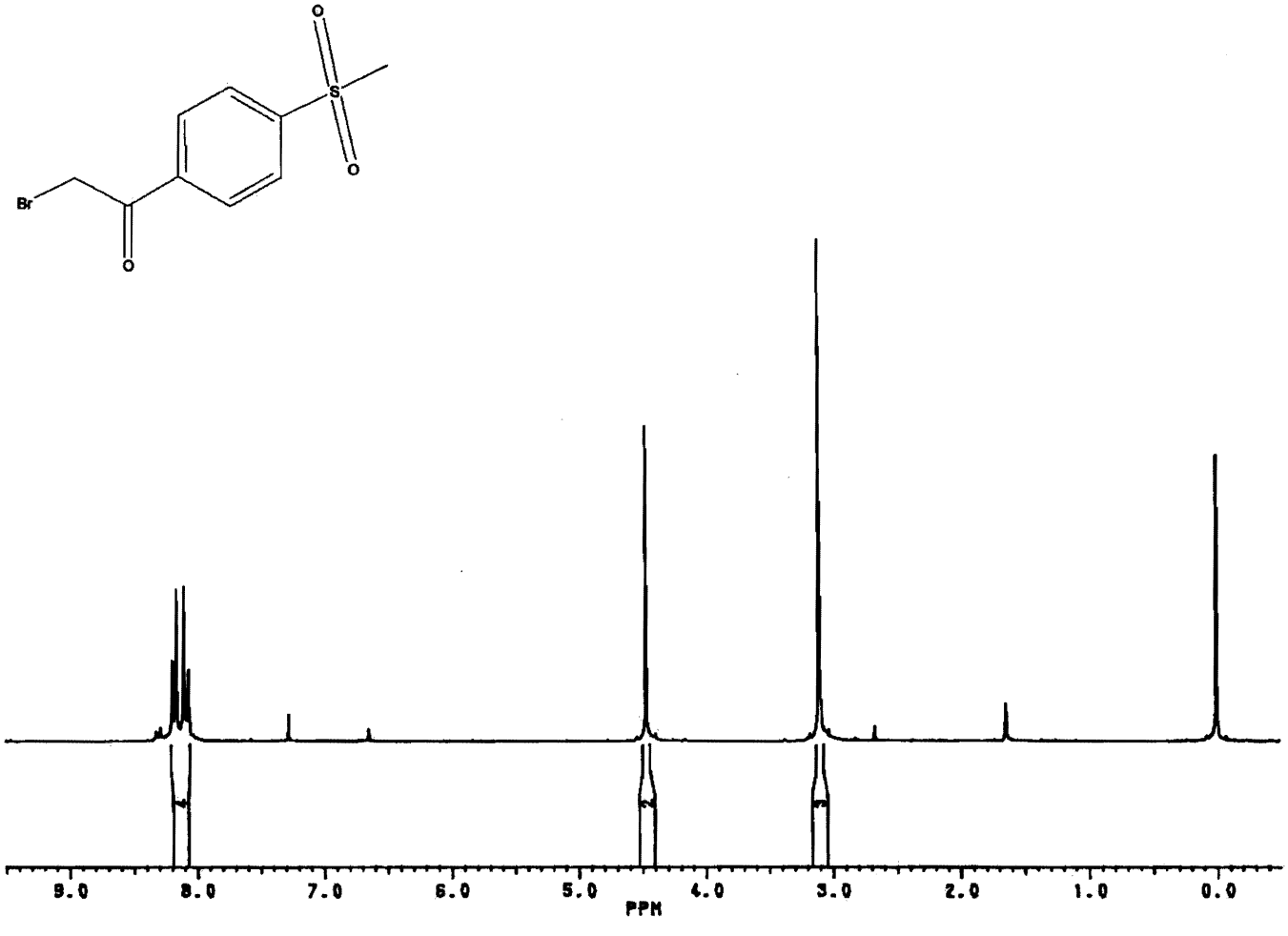
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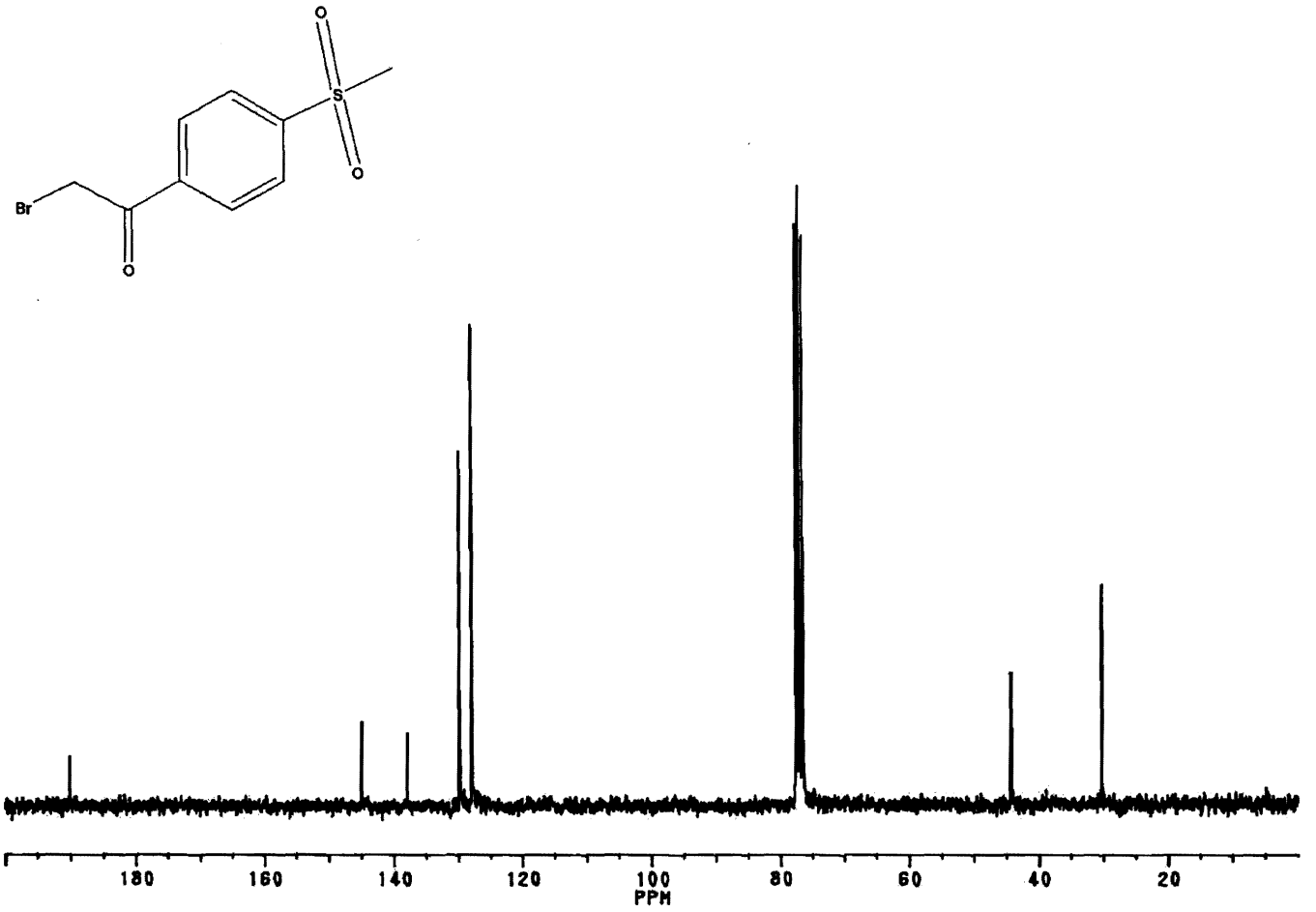
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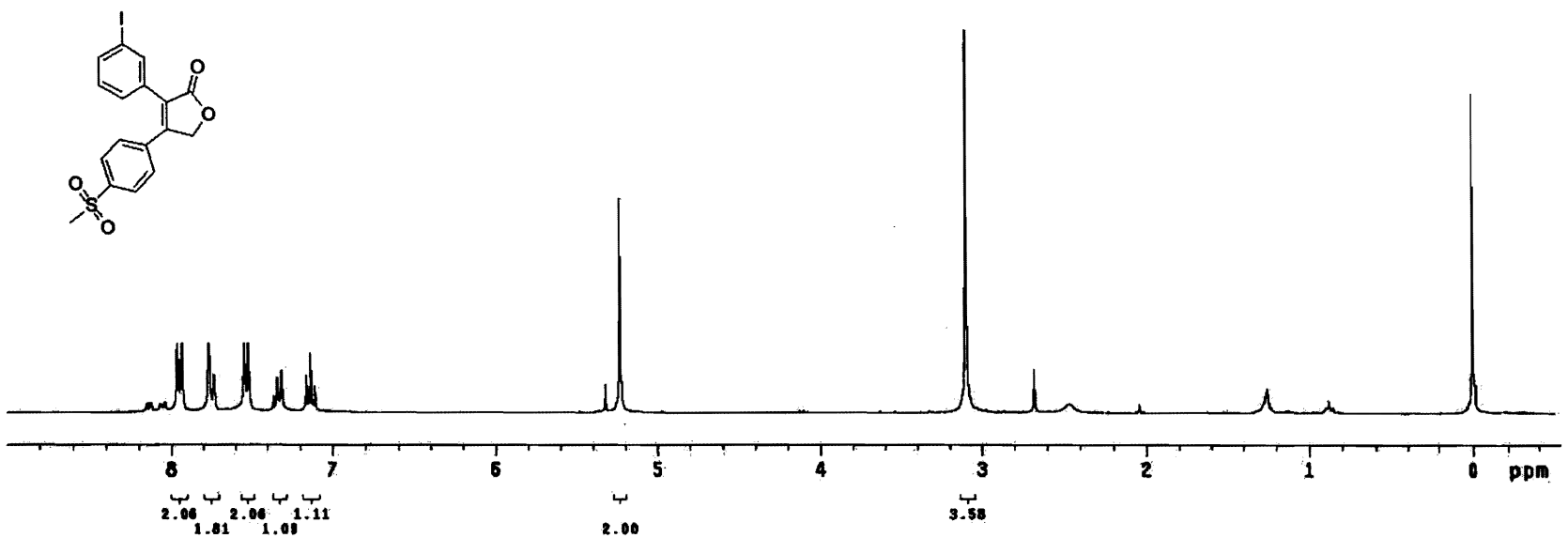
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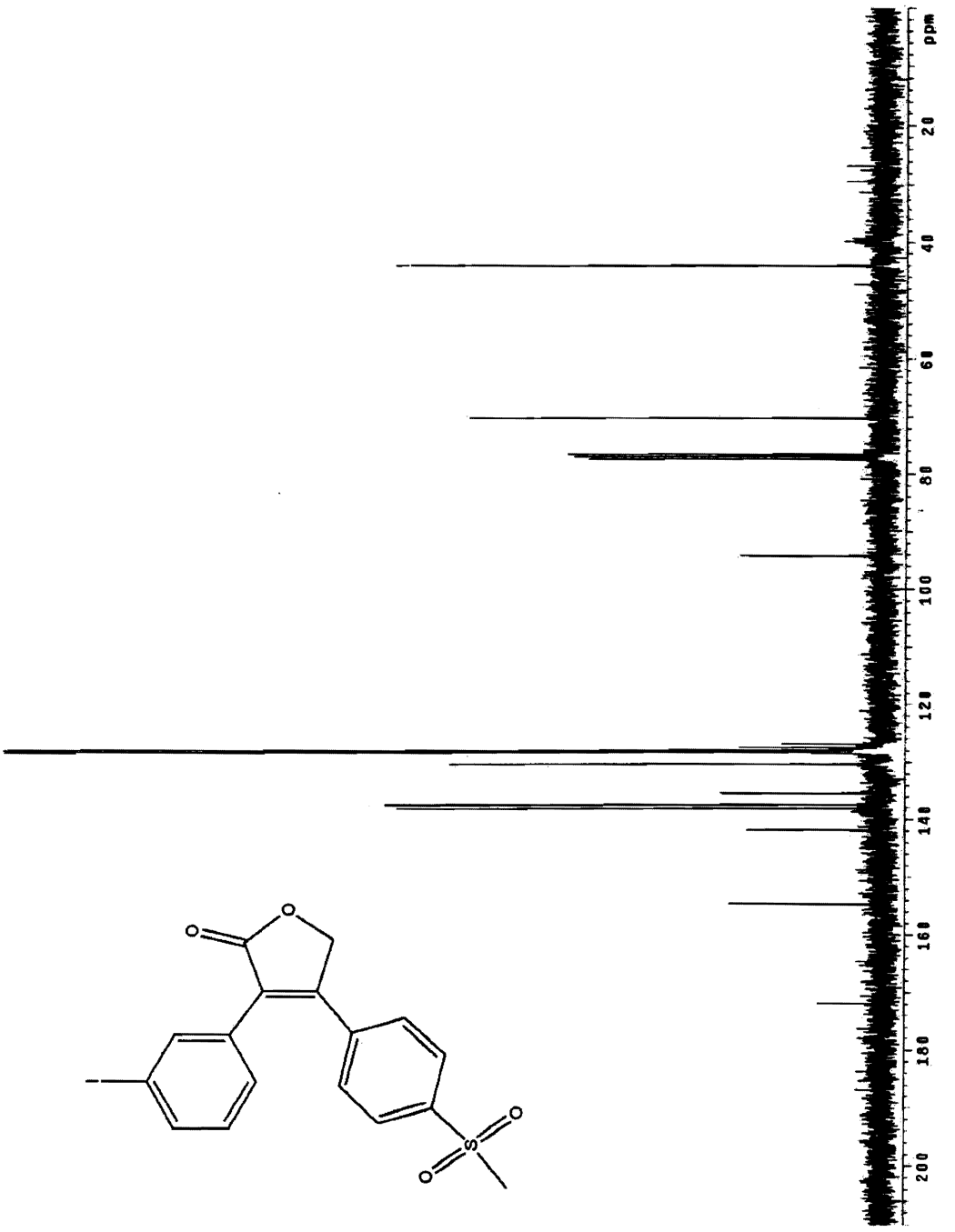
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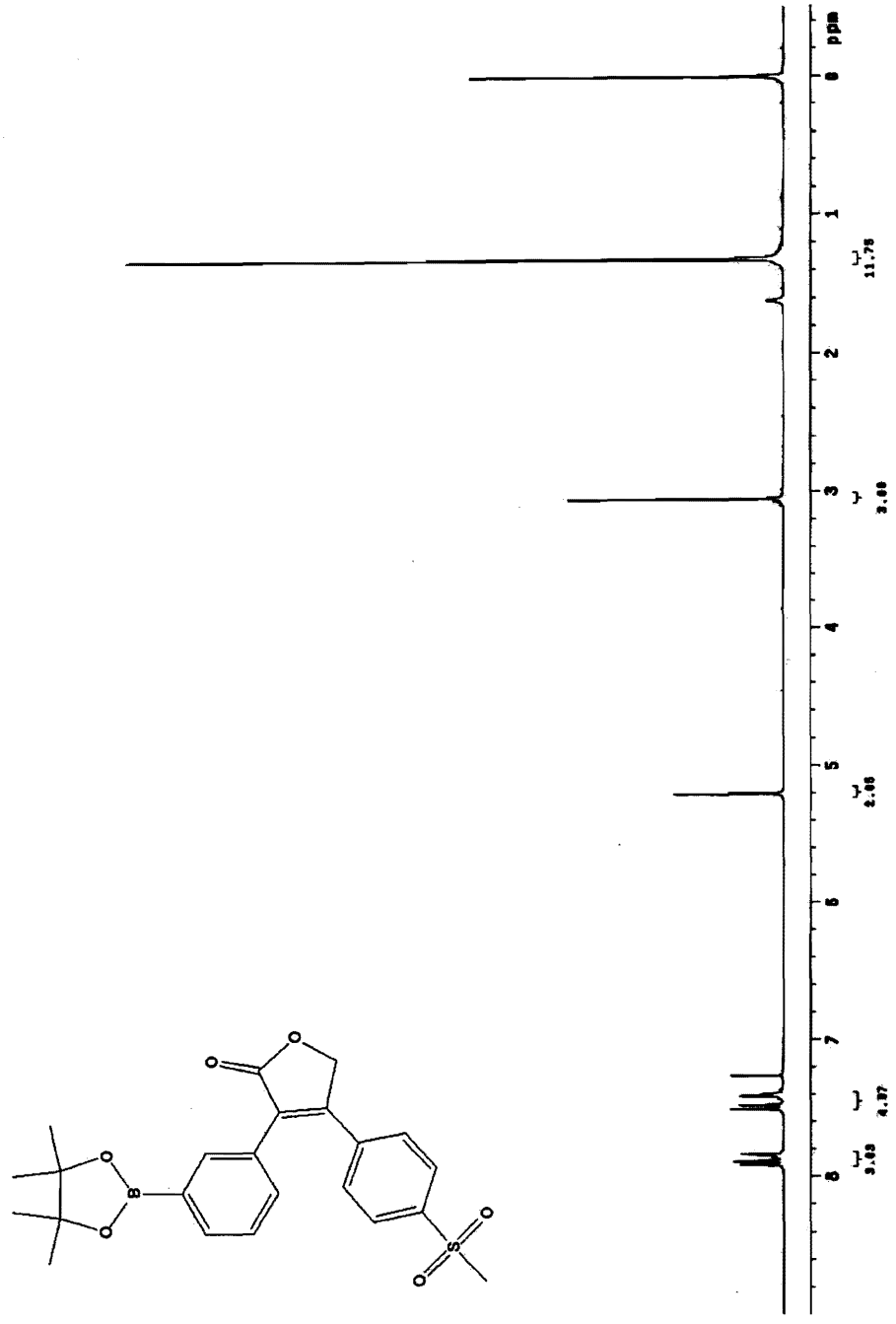
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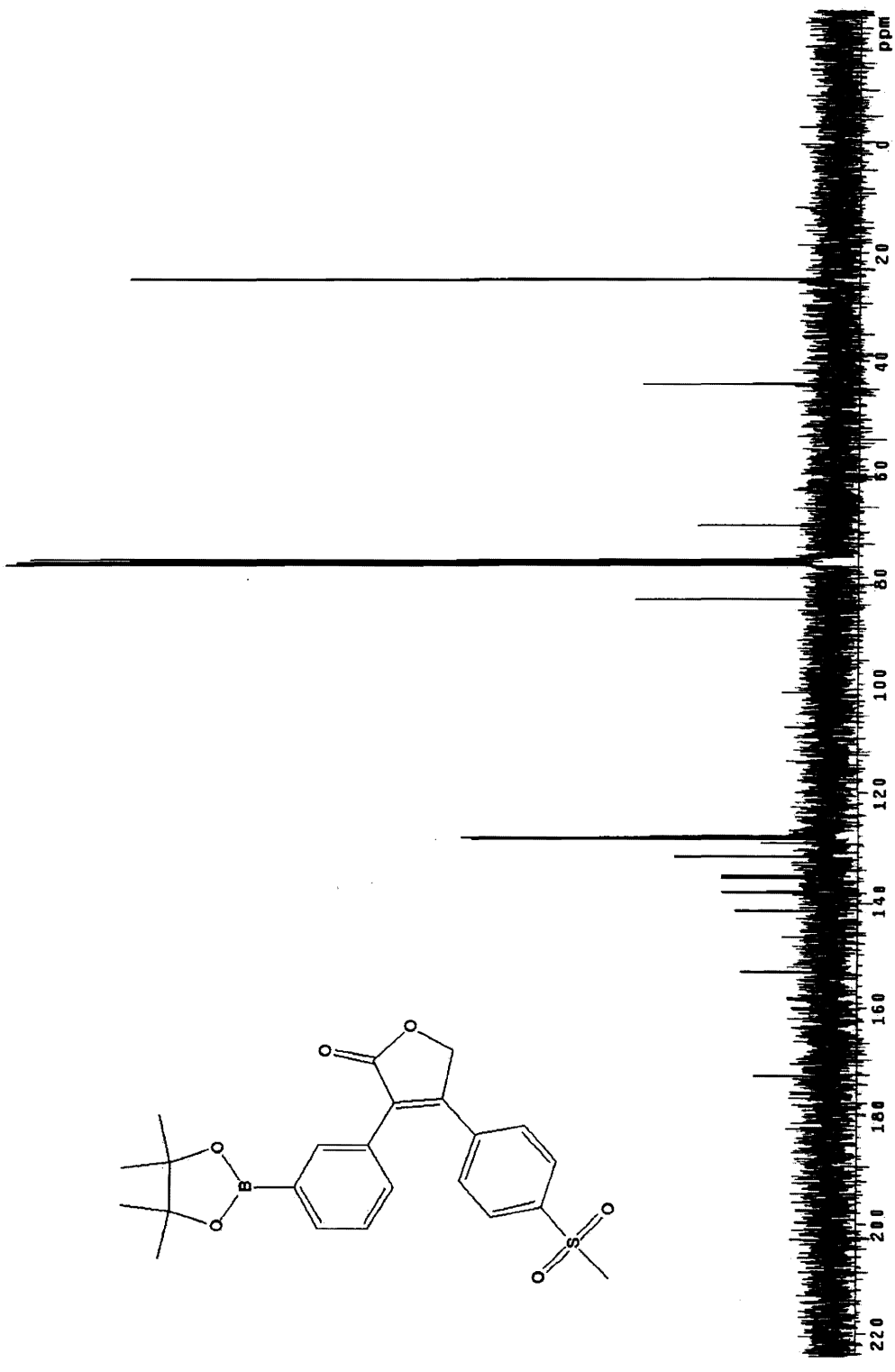
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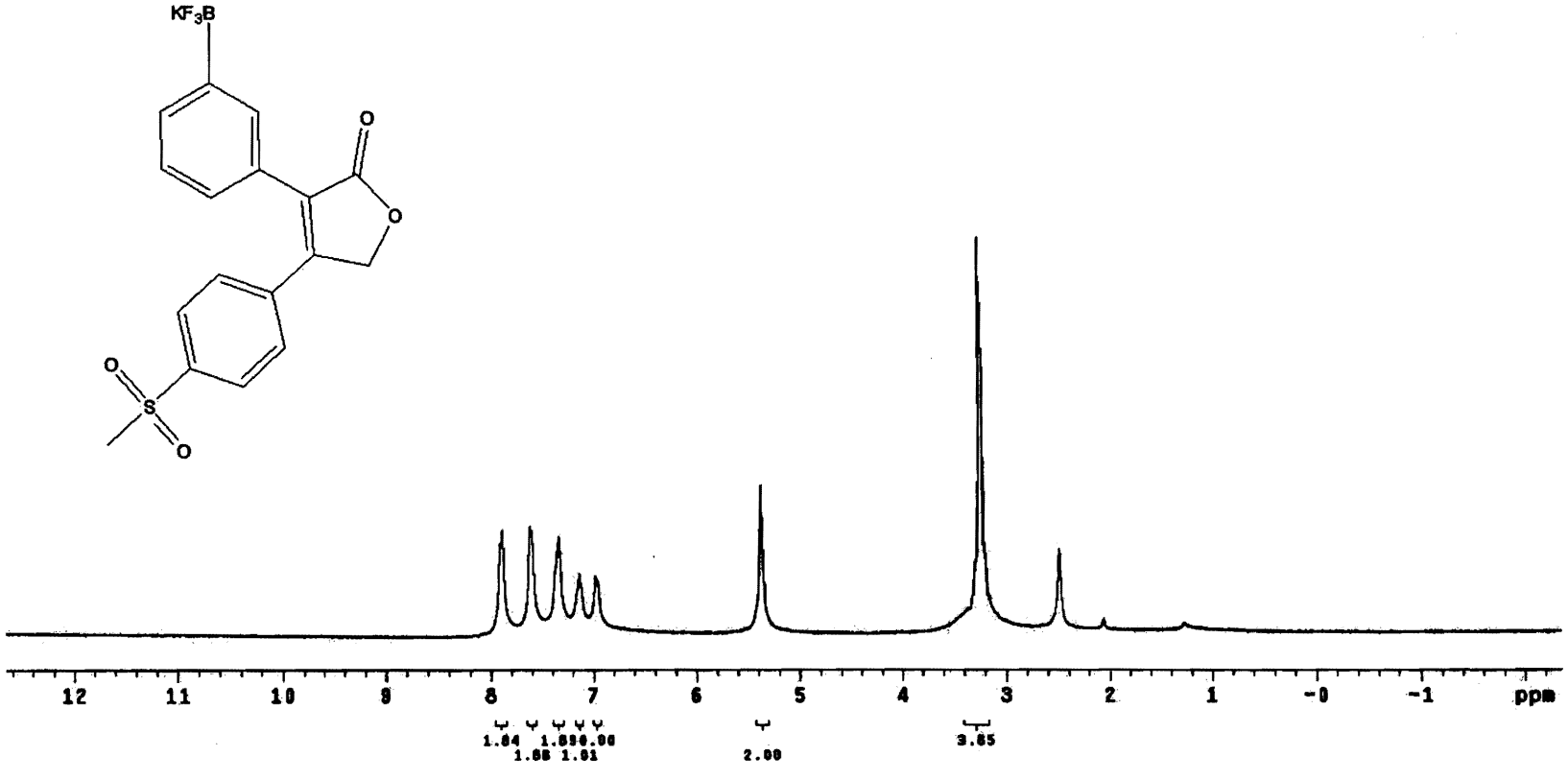
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KF3B
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O
S
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100
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20
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ppm

KF3B
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O
O
S
O
O

-20
-40
-60
-80
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-140
-160
-180 ppm

9.0
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7.0
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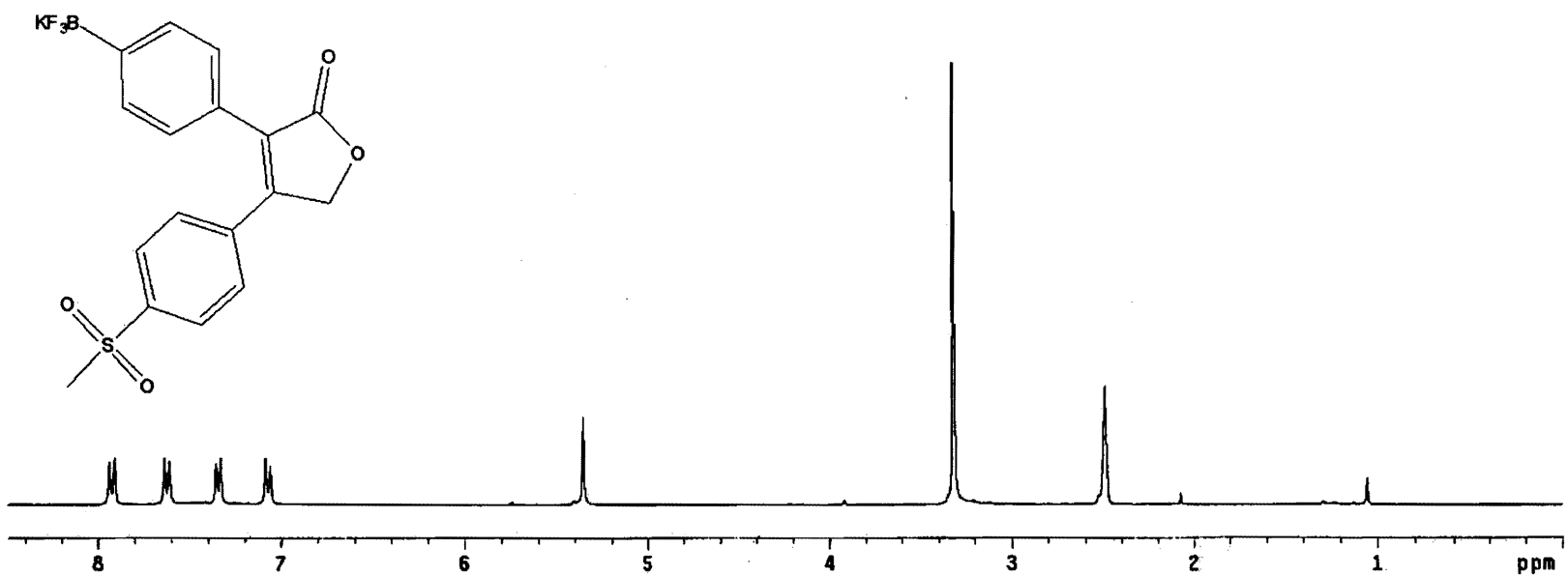
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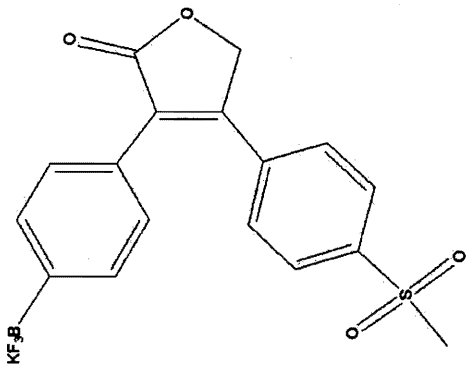
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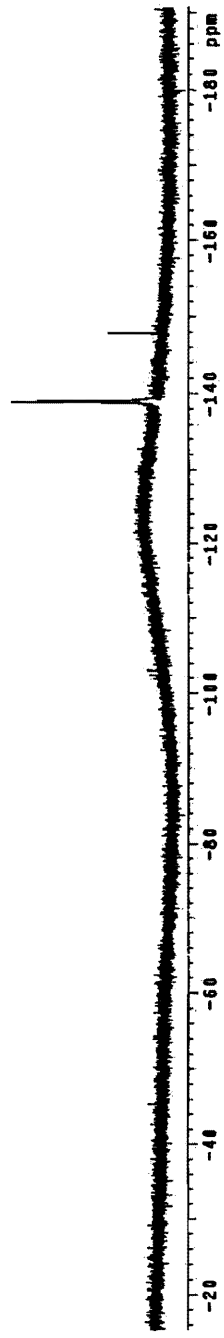
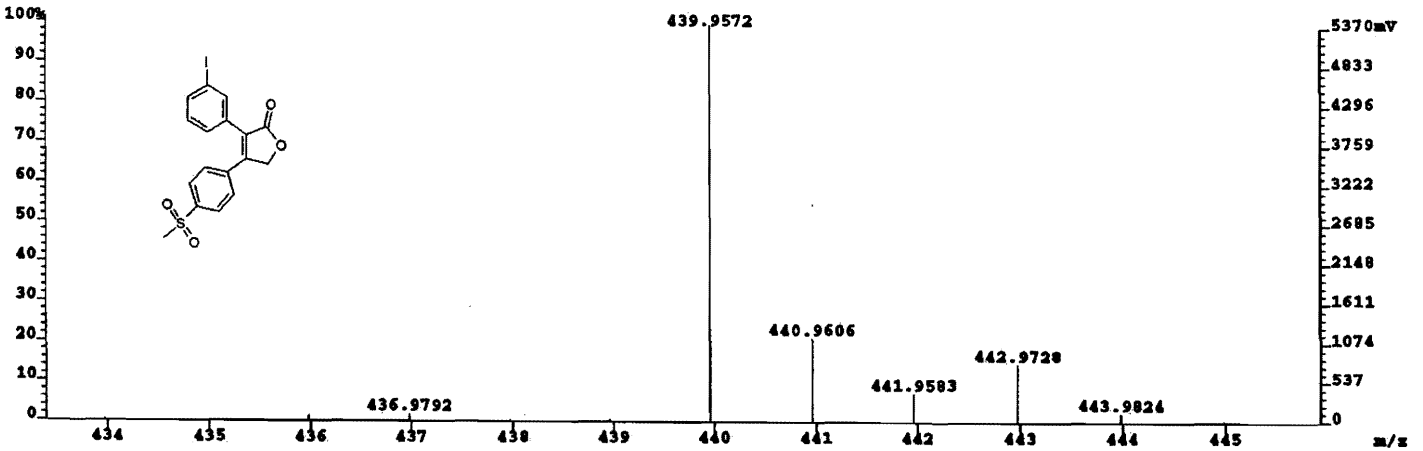
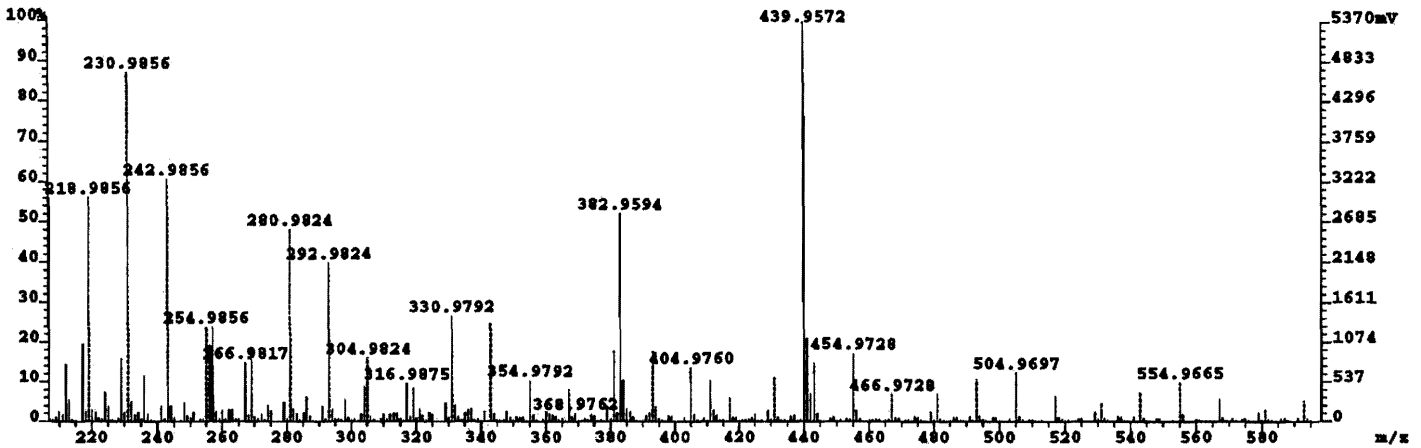
S
O
O

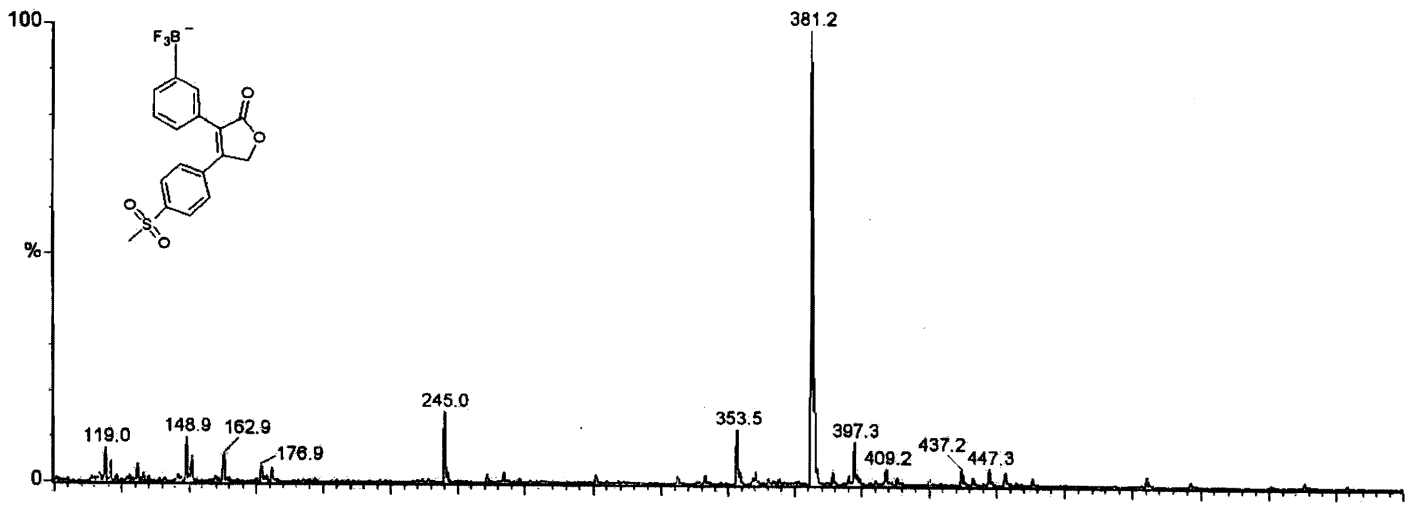

-20
-40
-60
-80
-100
-120
-140
-160
-180 ppm

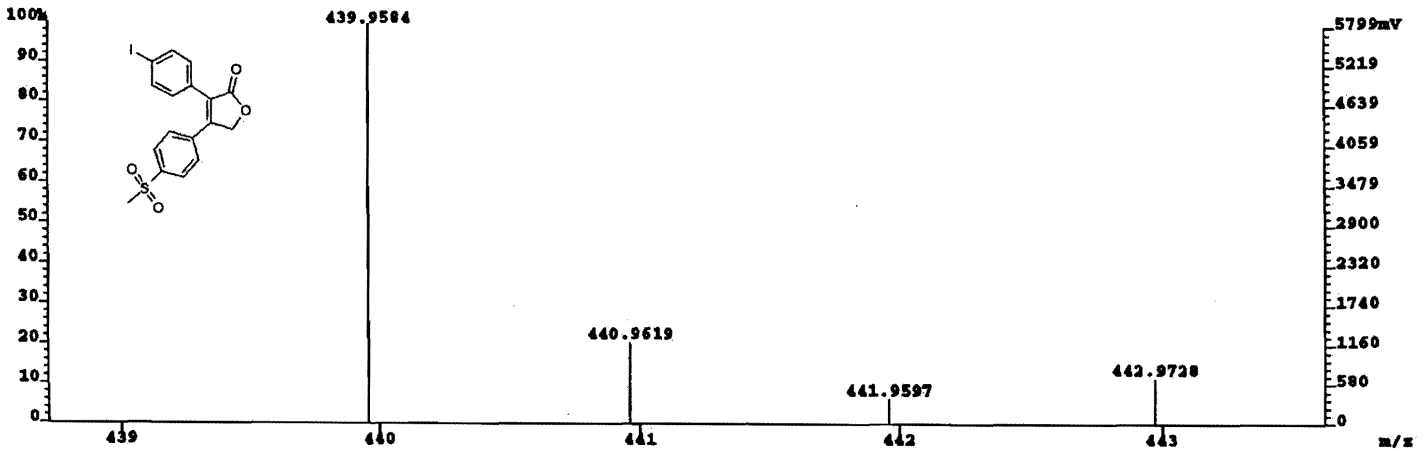
APPENDIX 2

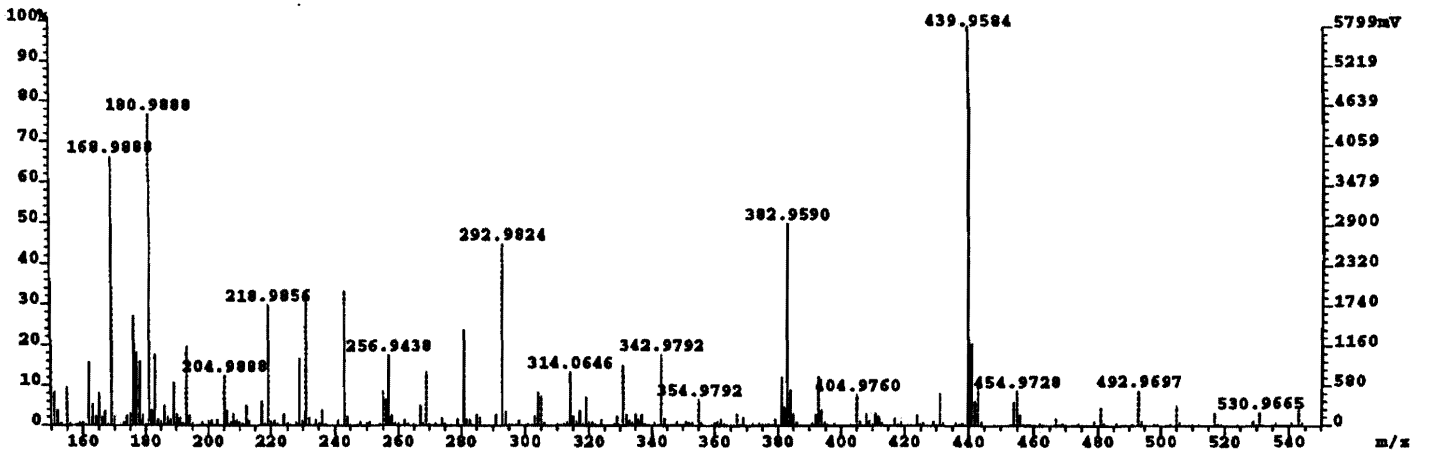
HRMS Spectra of Intermediate and Target Compounds

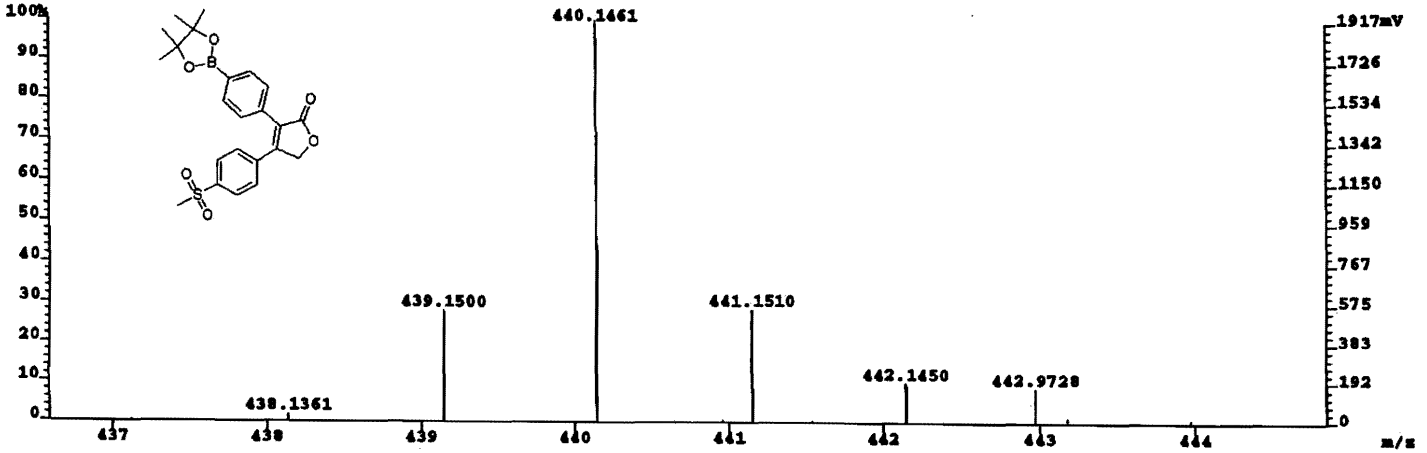

100%
90
80
70
60
50
40
30
20
10
0
439.9572
440.9606
442.9728
441.9583
436.9792
443.9824
434
435
436
437
438
439
440
441
442
443
444
445
m/z
5370mV
4833
4296
3759
3222
2685
2148
1611
1074
537
0

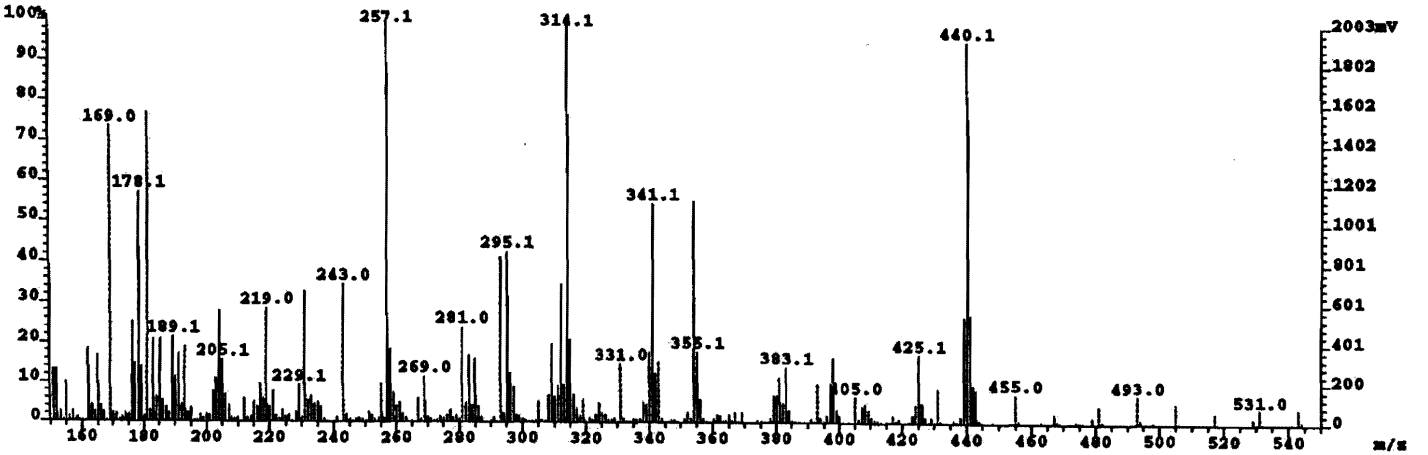

100%
90
80
70
60
50
40
30
20
10
0
439.9572
230.9856
242.9856
218.9856
280.9824
292.9824
382.9594
330.9792
254.9856
266.9817
304.9824
316.9875
354.9792
368.9762
404.9760
454.9728
504.9697
466.9728
554.9665
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
540
560
580
m/z
5370mV
4833
4296
3759
3222
2685
2148
1611
1074
537
0


100
%
0
381.2
245.0
353.5
119.0
148.9
162.9
176.9
397.3
409.2
437.2
447.3


439.9584
440.9619
441.9597
442.9728
5799mV
m/z


439.9584
180.9888
169.9888
382.9590
292.9824
218.9856
256.9438
342.9792
314.0646
204.9888
354.9792
404.9760
454.9728
492.9697
530.9665
5799mV
m/z


440.1461
439.1500
441.1510
438.1361
442.1450
442.9728
1917mV
m/z


257.1
314.1
440.1
169.0
178.1
341.1
295.1
243.0
219.0
189.1
281.0
205.1
331.0
355.1
383.1
425.1
229.1
269.0
455.0
493.0
531.0
2003mV
m/z

VITA

Brandy Underwood Belue was born in Tupelo, Mississippi on December 30, 1977. She attended high school at Tishomingo County Magnet High School in Iuka, MS, graduating with highest honors in 1996. In 1998, she earned the Associate of Science degree at Northeast Mississippi Community College. She received the Bachelor of Science degree in 2001 from the University of North Alabama, with a major in Professional Chemistry, with American Chemical Society accreditation. In August 2001, she joined the University of Tennessee, Knoxville and received the Master of Science in Chemistry in 2005.