ABSTRACT

HUNTER WILSON
8-Chloro-7-hydroxyquinoline and xanthone acetic acid derivatives as photoremovable protecting groups with susceptibility to two-photon excitation
(Under the Direction of TIMOTHY M. DORE.)

Photochemistry offers scientists a powerful method of exploring biological processes. Photoremovable protecting groups (PPGs) are quickly removed with a flash of light, allowing researchers to explore how the timing and location of events triggered by messengers, such as nucleotides, neurotransmitters, peptides, drugs, etc., impact cellular function. Although most caging groups rely on single-photon excitation (1PE) mechanisms to govern their release, caging groups susceptible to two-photon excitation (2PE) enable precise control of uncaging events and are better-adapted to the study of biological systems. Recently, the synthesis and photochemical analyses of 8-bromo-7-hydroxyquinoline (BHQ), 4-xanthone acetic acid (4-XAA) and 2-xanthone acetic (2-XAA) acid have indicated their photochemical sensitivity. To enhance understanding of quinoline photochemistry, 8-chloro-7-hydroxyquinoline (CHQ) was synthesized and its photochemistry measured based on cleavage of an acetyl group. Irradiation of CHQ with a UV lamp afforded the quantum efficiency (Q_u), and the 2PE cross-section (δ_u) was determined with a Ti:Sapphire laser. δ_u values for 4-XAA and 2-XAA in aqueous phosphate buffer were measured. The stability of CHQ was measured in an aqueous buffer solution of high ionic strength, which mimicked biological conditions. Results show that CHQ demonstrates a lower rate of both 1PE and 2PE cleavage than BHQ. However, δ_u measurements for 4-XAA and 2-XAA indicate excellent susceptibility to 2PE. Future work will involve mechanistic studies of quinoline photorelease and will examine 2-XAA and 4-XAA as 2PE cages for biomolecules.

INDEX WORDS: Caged Compounds, Quinoline, Photochemistry, Two-photon Excitation, 8-Chloro-7-hydroxyquinoline, 8-Bromo-7-hydroxyquinoline, 2-Xanthone Acetic Acid, 4-Xanthone Acetic Acid

8-CHLORO-7-HYDROXYQUINOLINE AND XANTHONE ACETIC ACID DERIVATIVES AS PHOTOREMOVABLE PROTECTING GROUPS WITH SUSCEPTIBILITY TO TWO-PHOTON EXCITATION

by

HUNTER C. WILSON

A Thesis Submitted to the Honors Council of the University of Georgia
in Partial Fulfillment of the Requirements for the Degree

BACHELOR OF SCIENCE

in CHEMISTRY

and

BACHELOR OF SCIENCE

in GENETICS

with HIGHEST HONORS

and CURO SCHOLAR DISTINCTION

Athens, Georgia

2009

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HUNTER C. WILSON

Approved:

Timothy M. Dore — May 6, 2009
Dr. Timothy M. Dore — Date
Faculty Research Mentor

Approved:

Vladimir V. Popik — May 6, 2009
Dr. Vladimir V. Popik — Date
Reader

Approved:

David S. Williams — May 8, 2009
Dr. David S. Williams — Date
Director, Honors Program, Foundation Fellows and
Center for Undergraduate Research Opportunities

Approved:

Pamela B. Kleiber — May 8, 2009
Dr. Pamela B. Kleiber — Date
Associate Director, Honors Program and
Center for Undergraduate Research Opportunities

DEDICATION

I would like to dedicate this work to my parents. Thank you for all of the opportunities that you have given me and for being wicked awesome.

ACKNOWLEDGEMENTS

I would first and foremost like to thank Dr. Dore, without whom none of this would have been possible; I am fortunate to have had such an excellent teacher and mentor over the past few years. I will always be grateful to Dr. Dore for letting me work in his lab and for all of his help and (most notably) patience.

I would like to thank Dr. Tito Sciano and Jessie Blake for sending us samples of XAA compounds and the Center for Undergraduate Research Opportunities (CURO) for awarding me a CURO Summer Fellowship in 2008 to support my research over the summer. Thanks also to the Center for Advanced Ultrastructural Research (CAUR), Urbauer laboratories for assistance with fluorescence measurements, and my reader, Dr. Popik, for his insight and comments on my thesis.

Finally, I would like to thank Yue Zhu, Kyle Harris, and the rest of Dore laboratories for all of your help over the past few years. I don't know how I would have survived were it not for all of the helpful tips on how to best accomplish whatever task I had at hand.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

PPG ..... photoremovable protecting group
1PE ..... one-photon excitation
2PE ..... two-photon excitation
VES ..... virtual excited state
NP 2-nitrobenzyl
DMNB 4,5-dimethoxy-3-nitrobenzyl
CNB α-carboxy-2-nitrobenzyl
NPE ..... 1-(2-nitrophenyl)ethyl
*p*HP ..... phenacyl
NI ..... nitroindolyl
MNI ..... methoxy nitroindolyl
Bnz ..... benzoin
Bhc 6-bromo-7-hydroxycoumarin-4-yl-methyl
DMNPD ..... 3-(4,5-dimethoxy-2-nitrophenyl)-2-butyl
BCMACM ..... 7-[bis(carboxymethyl)amino]coumarin-4-yl}methyl
PMNB ..... 3-(2-propyl)-4-methoxy-4-nitrobiphenyl
o-HCA ..... *o*-hydroxycinnamic acid
BNSMB ..... 4,4′-bis-{8-[4-nitro-3-(2-propyl)-styryl]}-3,3′-di-methoxybiphenyl
BNSF ..... 2,7-bis-{4-nitro-8-[3-(2-propyl)-styryl]}-9,9-bis-[1-(3,6-dioxaheptyl)]-fluorene
NDBF ..... nitrodibenzofuran
CDNI 4-carboxymethoxy-5,7-dinitro-indolinyl
BHQ 8-bromo-7-hydroxyquinoline
EWG ..... electron withdrawing group
HQ 7-hydroxyquinoline
CHQ 8-chloro-7-hydroxyquinoline
MOM-CHQ 8-chloro-7-methoxymethylquinoline
MOM-CHQ-CHO 8-chloro-2-formyl-7-methoxymethylquinoline
MOM-CHQ-OH 8-chloro-2-hydroxymethyl-7-methxymethylquinoline
MOM-CHQ-OAc ..... 8-chloro-7-(methoxymethylquinoline-2-yl)methyl acetate
CHQ-OAc ..... 8-chloro-7-hydroxyquinolin-2-ylmethyl acetate
4-XAA ..... 4-xanthone acetic acid
2-XAA ..... 2-xanthone acetic acid
4-MeX ..... 4-methyl xanthone
2-MeX ..... 2-methyl xanthone

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SCHEMES

Page

CHAPTER 1
INTRODUCTION

Introduction to Photochemistry and Caged Compounds

Over the past few decades, "caged compounds" have emerged as an important tool for the study of biological systems.[1-3] A "caged compound," or photoremovable protecting group (PPG), is one that may be attached to various molecules, called effectors, and released upon irradiation with light (Figure 1). The utility of these PPGs is derived from their ability to inactivate the molecules that they cage. It is important to note that the term cage does not refer to a physical encapsulation of the effector molecule; rather, a covalent bond between the effector and its cage is sufficient to render inactivity. Upon irradiation, photons provide the energy to cleave the covalent bond binding the cage to its effector. The effector regains its activity and the system may be studied under the conditions of a sudden influx of active effector.

To be effective as tools for biological study, cages must first and foremost release the effector rapidly, efficiently, and in high yield. In addition, a cage should fulfill the following criteria: (1) The system under study must not be affected by the cage; (2) the system under study should not be affected by any photoproducts; (3) the effector should be rapidly released by the cage at wavelengths that are not detrimental to the system; (4) the caged compound must be stable in the dark in physiological buffers with high ionic strength; (5) the caged compound should have adequate solubility in an aqueous solution; and (6) synthesis of the caged compound should be accessible. Compounds possessing all of these characteristics are rare, but the satisfaction of each of these requirements is essential for any PPG that is to be used for biological study.[4]

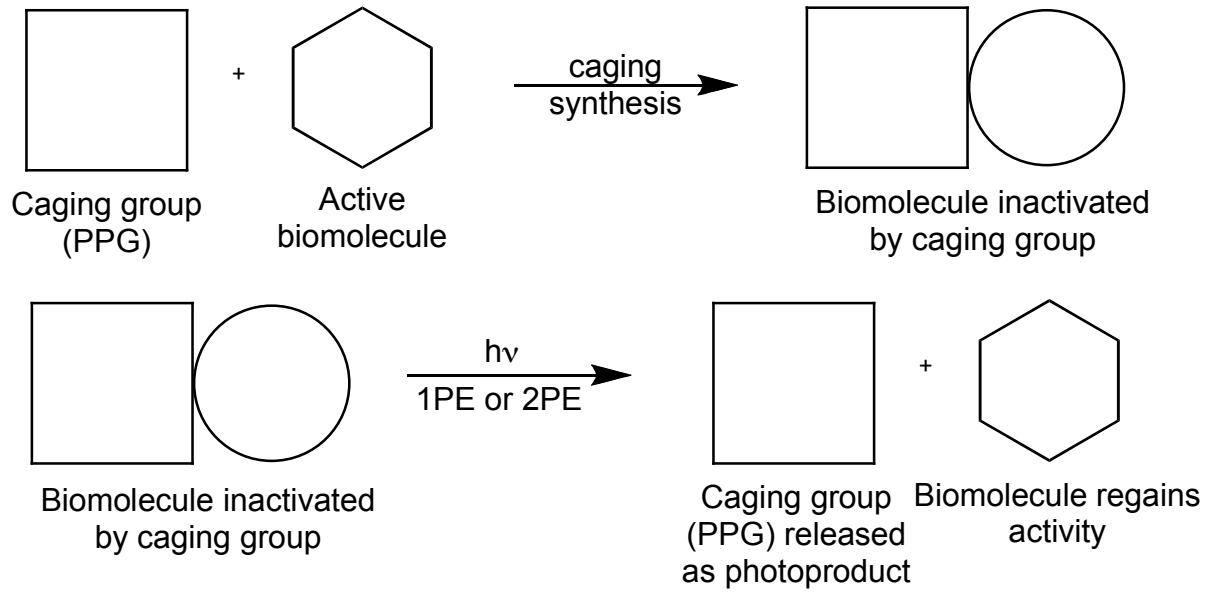


Figure 1: Representation of an uncaging event. The PPG is first bound to a biological molecule (effector) through a covalent bond to inactivate effector activity. Upon irradiation with light, the cage is released and the effector regains function.

Over the past few decades, a number of different caging groups have been developed for the study of physiology. Currently, some of the most widely employed PPGs are based upon the well-studied 2-nitrobenzyl (NB) group (Figure 2).[5,6] The caging and release of the energetically important biomolecule ATP by Kaplan et al. using NB helped in great part to usher in photochemistry as a tool for the dynamic study of biological systems.[7] Since its inception, numerous variations of NB have been created to modify core characteristics of the molecule, including solubility, wavelength of maximum absorption, and molar absorptivity. Although by far the most widely used PPG to date, NB derivatives have a number of properties that limit their usefulness as tools for biological study. In general, the NB derived cages are characterized by absorption in the UVB range of light, which is often detrimental to biological systems. The release kinetics demonstrated by NB and its derivatives are also slow, often occurring on the second or minute time scale, and rates of photorelease are very sensitive to reaction conditions

and pH.[3,8] Additionally, NB photoproducts possess a nitroso group which is toxic to biological preparations, thereby limiting the range of processes that may be studied.

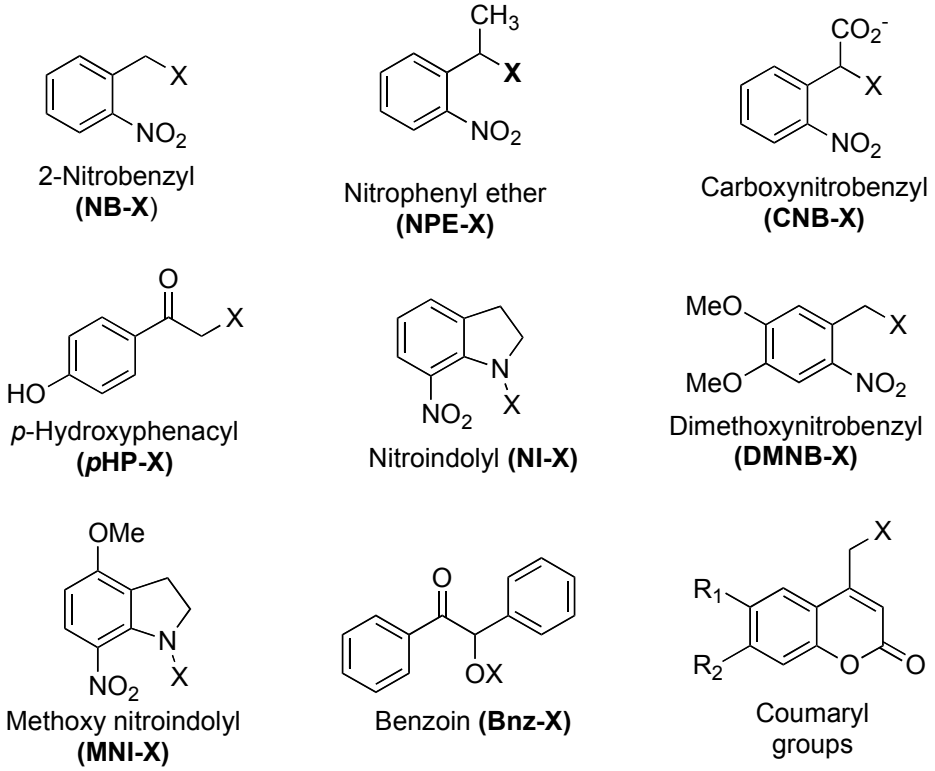


Figure 2: A number of common 1PE PPGs. X represents a caged effector.

The phenacyl (*p*HP) classes of compounds have been introduced relatively recently, and have been used to release effectors such as ATP and the neurotransmitters γ-aminobutyric acid (GABA) and glutamate.[9,10] The release kinetics of *p*HP occur on the nanosecond timescale. Nitroindolyl (NI) has been used to release neurotransmitters as well as the biologically important Ca^{2+} ion.[11,12] Addition of a methoxy group to the NI chromophore gives methoxy nitroindolyl (MNI), which possesses increased photolysis efficiency when compared with NI. Both NI and MNI undergo photorelease with kinetics on the order of microseconds. The PPG benzoin (Bnz) is limited to carboxylic acids and phosphates, but generates inert photoproducts and possesses fast rates of photolysis. However, aqueous solubility for Bnz-caged effectors is somewhat of an issue.[13,14] The coumaryl chromophore has been used as a base for a wide variety of PPGs.

Although the photochemical data regarding coumaryl-based PPGs vary in accordance with the types of substituents attached to the base, they are generally characterized by bright fluorescence.[3] The properties of coumaryl compounds and a few specific coumaryl-based PPGs are discussed in greater detail later.

Although the previously mentioned PPGs have enjoyed wide use in the realm of photochemistry, they are all generally cleaved most effectively through a process called single-photon excitation (1PE). During 1PE, one photon of light is absorbed and used to promote the chromophore to the excited state. The chromophore may then use its excited state energy to cleave the covalent bond that binds the PPG to its effector. Unfortunately, molecules that release through 1PE have a number of properties that limit their usefulness as tools for the study of biological preparations. First, photochemical effector release that proceeds most efficiently through a 1PE mechanism often demands UVB light around 300 nm. These wavelengths are often absorbed by and cause damage to biological tissue. A notable exception to this 1PE wavelength correlation is the widely used dimethoxynitrobenzyl (DMNB) chromophore, which absorbs light most efficiently at wavelengths greater than 350 nm.[15] In regard to the body of known 1PE chromophores, however, this compound is the exception rather than the rule. Second, the tendency of tissue to absorb UV light severely limits the depth of penetration; biological compounds in the pathway of the light may take up light that is meant for the cage, reducing efficiency. Also, light with shorter UV wavelengths is generally scattered more than light possessing higher wavelengths. Finally, 1PE is a linear process. Excitation is proportional to the light intensity; thus, all photons used to irradiate the sample will have sufficient energy to promote excitation. The result of this characteristic is that all caged molecules in the pathway of

light are uncaged, which imposes a sharp limit upon the extent of three-dimensional resolution and precision with which these chromophores may be employed.

2-Photon Excitation Photochemistry

More recently, PPGs that undergo release governed by 2-photon excitation (2PE) have been explored as tools for the study of biological systems. A PPG that undergoes 2PE is promoted to the singlet excited state by the absorption of two photons within a very short time period rather than the absorption of one photon, as occurs during 1PE (Figure 3). During 2PE, the first photon is absorbed and its energy is used to promote the chromophore to the virtual excited state (VES). The lifetime of the VES is very short (often approximately one femtosecond) and if left alone, the chromophore in the VES will release its energy and decay back to the unexcited ground state. Conversely, if another photon is absorbed by the chromophore before the decay of the VES, then the chromophore will be promoted to the singlet excited state and 2PE will have occurred. A tightly focused laser is needed to elicit 2PE because only a laser can deliver a sufficient photon flux to promote absorption of two photons within the time frame mandated by the lifetime of the VES.

Once the singlet excited state has been achieved, the system may then undergo a photochemical reaction, fluoresce, cross to the triplet exited state and phosphoresce, or release energy through non-radiative decay to the ground state through internal conversion. Photochemical reaction may proceed through either the triplet or singlet excited state, depending on the chromophore. For PPGs, it is desired that as much of the excited state energy as possible is used to promote a photochemical reaction; decay through fluorescence and phosphorescence decreases efficiency. A law of nature dictates that if the wavelength of light doubles, then the energy of the light is halved. Thus, wavelengths used for 2PE are usually twice the wavelengths

of a compound's maximum absorption – that is, the wavelength at which 1PE proceeds most rapidly – since 2PE requires the absorption of two photons half the energy of the photon absorbed during 1PE to reach an energetically equivalent excited state. For example, if a chromophore undergoes 1PE at 350 nm, 2PE will usually occur most efficiently at 700 nm.

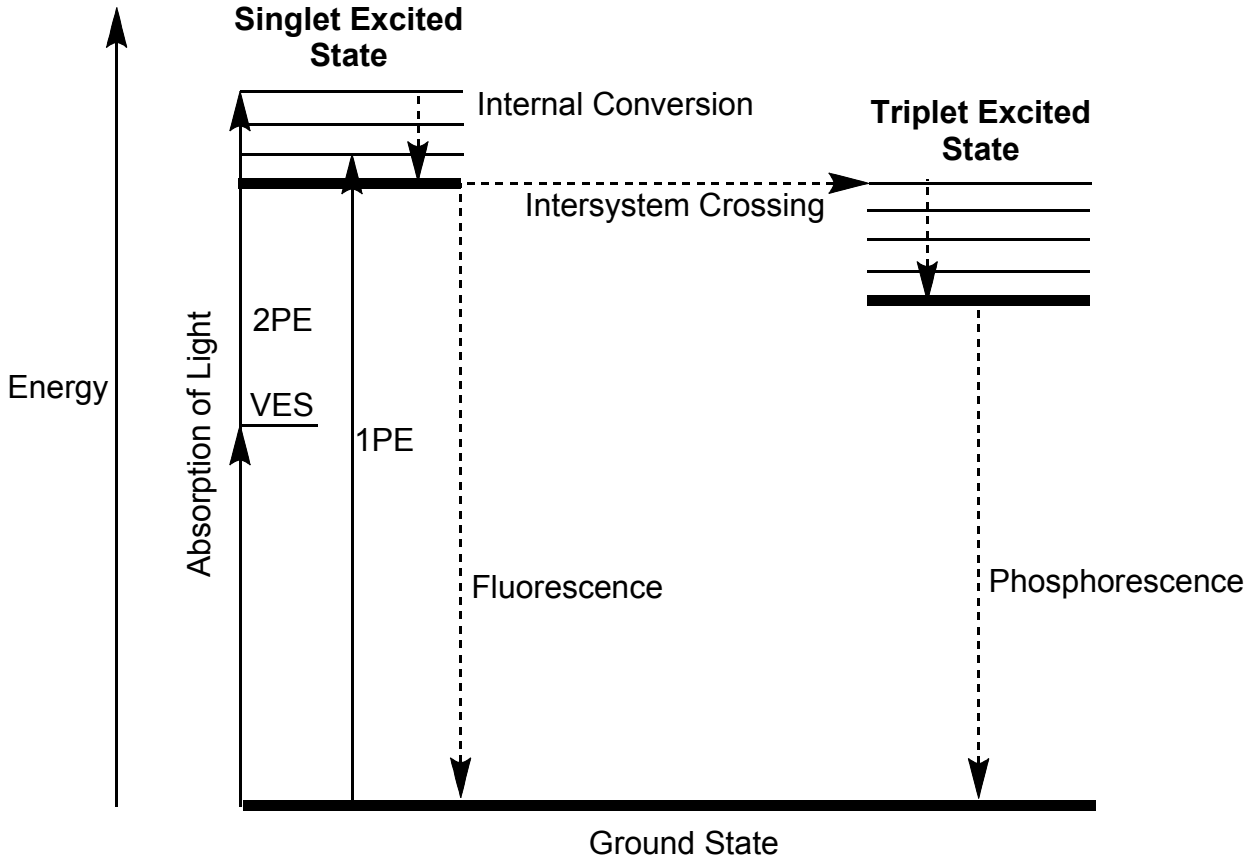


Figure 3: Jablonski diagram summarizing possible outcomes of chromophore excitation. For 2PE, the first photon promotes the chromophore from the low energy ground state to the virtual excited state (VES) and the second promotes the chromophore from the VES to the singlet excited state. Internal conversion represents the release of energy as heat to surroundings as the chromophore lowers its vibrational level in the singlet and triplet excited states.

PPGs that undergo release through 2PE have numerous advantages over their 1PE predecessors that make them favorable to the study of biological systems.[4] First, wavelengths used to initiate 2PE often fall in the near-IR (700 – 1100 nm) region of the electromagnetic spectrum. This light is not generally absorbed by biological preparations. Thus, the damage to the system is decreased since light from the infrared (IR) region cannot be absorbed and used to

promote damage and degradation of native biomolecules outside of the focal volume. It is important to note that biomolecules within the focal volume will likely be damaged to some degree as a result of 2PE-mediated excitation promoted by laser intensity; however, this damage is negligible when compared to damage that is elicited by 1PE processes and is of minimal importance with regard to the preparation as a whole. Keeping the average power of the laser below 10 mW has been shown to minimize collateral damage.[16] 2PE can also be used to study processes at greater tissue depth than 1PE since the wavelengths used for 2PE are not scattered or absorbed by biomolecules in its path. Finally, in contrast to 1PE, 2PE is a non-linear process; that is, the rate of 2PE uncaging varies according to the square of light intensity. A very intense light source is needed to promote absorption of two photons within the lifetime of the VES and will only occur at the focal point of a laser (Figure 4). These requirements enable tight control of the volume of excitation. Depending on the degree of focus demonstrated by the laser, volumes of excitation might be as small as one femtoliter, roughly the size of an *E. coli* bacterium. To summarize, when compared with 1PE, 2PE allows cages to release effectors at greater depths with increased 3-dimensional resolution and less damage to surrounding tissues.

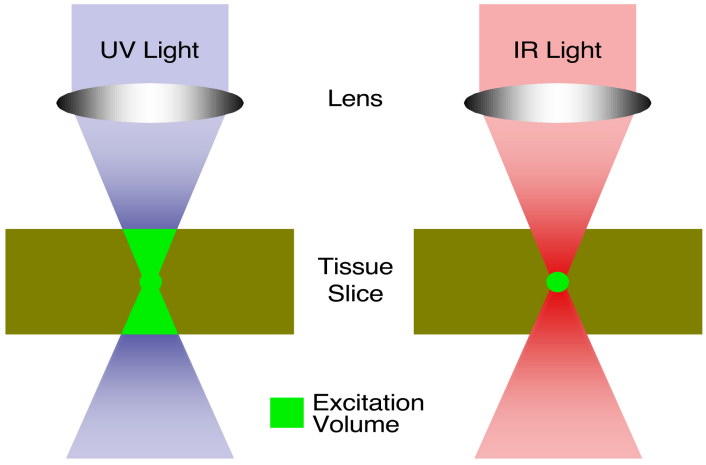


Figure 4: 2-photon excitation enables greater spatial control of uncaging events.[17]

Measuring 2-Photon Excitation Susceptibility

The measurement of the susceptibility of a PPG to photoreaction is known as its two-photon action cross-section, δ_u. This sensitivity is quantified by a unit called the Goeppert-Mayer (GM, 10^{-50} (cm^4·s)/photon), named in honor of the scientist who first hypothesized the phenomenon of 2PE.[18] The two-photon action cross-section δ_u is a product of two values, the two-photon absorbance cross-section, δ_a, and the quantum yield of the photolysis reaction, Q_R. Since δ_a represents the probability that two photons will be absorbed by the PPG and Q_R indicates the efficiency of the reaction (that is, what portion of the excited state energy is used to promote photoreaction as opposed to fluorescence, phosphorescence, etc.), δ_u is a measure of the probability that a PPG will both absorb two photons and undergo productive photocleavage from its effector. Typically, a PPG relevant to the study of biological systems should have a δ_u value exceeding 0.10 GM.[15]

A method developed by Tsien enables δ_u of the PPG or chromophore in question to be determined by comparison with known photochemical parameters of the chromophore fluorescein and measurement of the decay of cage-bound effector by HPLC.[15] These variables are related by the following equation:

$$\delta_u = \frac{N_p \phi Q_{F2} \delta_{aF} C_F}{< F(t) > C_s}$$

Here, N_p represents the number of molecules that undergo photoreaction (as determined by HPLC), ϕ is the detector collection efficiency, Q_{F2} is the fluorescence quantum yield of fluorescein, δ_{aF} is the fluorescein two-photon absorbance cross-section, $<F(t)>$ represents the time averaged fluorescent photon flux from fluorescein, and C_F and C_s are the respective concentrations of the fluorescein standard and the caged molecule to be studied. Although this

method gives only moderately accurate absolute δ_u values (due in part to slight variations in the numerous variables in the equation), it is well-suited to measure relative rates of 2PE photolysis between PPGs. Indeed, if a compound has a known δ_u value, then this compound and an unknown compound may be photolyzed under the same conditions and the unknown δ_u may be found by simply comparing the decay rates, which are represented by N_p.[19]

2-Photon Excitation Cages

The inadequacy of most of the well-studied cages developed over the past years has spawned the introduction of novel caging groups that have been built with high 2PE susceptibility as a primary objective. For example, one of the most popular NB-derived caging groups, DMNB, has a δ_u value of only 0.03 GM at 740 nm, well below the desired physiological criteria of 0.10 GM.[15,20] Only one NB variant, 3-(4,5-dimethoxy-2-nitrophenyl)-2-butyl (DMNPB, Figure 5) has been found to possess adequate 2PE characteristics in the near IR. DMNPB has a δ_u value of 0.17 at 720 nm and has been used to release glutamate *ex vivo*.[21]

Furuta et al. have utilized a coumaryl base group to develop 6-bromo-7-hydroxycoumarin (Bhc), a cage suitable for the protection of functional groups including alcohols, amines, carboxylates, phosphates, and diols.[4,15,22] A derivation called Bhc-diol has been shown to protect aldehydes and ketones.[23,24] The sensitivities of Bhc 2PE photolysis vary according to the effector, but in general are quite high; release of an acetyl group, used as a test effector for many cages, has a δ_u value of 0.72 at 740 nm in physiological buffer. Bhc also possesses fast kinetics; photolysis generally proceeds on the submicrosecond timescale.[4] Some drawbacks of Bhc as a PPG include low levels of water solubility and high fluorescence, which might limit the compound's use in certain biological studies that rely upon fluorescent indicators.

Another coumarin-based PPG, {7-[bis(carboxymethyl)amino]coumarin-4-yl}methyl (BCMACM), has been shown to possess favorable 2PE sensitivities, with δ_u values ranging from 0.4 – 2.6 GM for various effectors. The BCMACM chromophore also has excellent water solubility and exhibits low levels of fluorescence.[25] The PPG 8-bromo-7-hydroxyquinoline (BHQ) has increased aqueous solubility and decreased fluorescence compared to Bhc but measurement of the photorelease of an acetyl group gave a slightly lower δ_u value of 0.59. BHQ is discussed in greater detail in chapter 2.[17]

The 3-(2-propyl)-4-methoxy-4-nitrobiphenyl (PMNB) group has been recently introduced and releases glutamate with δ_u values slightly higher than those of Bhc at 800 nm (δ_u = 0.45 GM for PMNB vs. 0.42 GM for Bhc). *Ex vivo* deployment of PMNB in neurological tissue has been successful, although aqueous solubility might prove problematic at higher concentrations.[26]

Derivatives of *o*-hydroxycinnamic acid (*o*-HCA) have been explored by Jullien and coworkers for 2PE photochemistry. Findings show that *o*-HCA and various derivatives releases effectors with δ_u values in excess of 1 GM. Authors have used a fluorescent indicator to monitor *in vivo* 2PE uncaging of *o*-HCA in zebrafish embryos.[27,28]

Recent work by Gug et al. has produced the compounds 4,4′-bis-{8-[4-nitro-3-(2-propyl)-styryl]}-3,3′-di-methoxybiphenyl (BNSMB) and 2,7-bis-{4-nitro-8-[3-(2-propyl)-styryl]}-9,9-bis-[1-(3,6-dioxaheptyl)]-fluorene (BNSF), which posses δ_u values of 0.9 GM and 5.0 GM, respectively, based upon the photorelease of glutamate at 800 nm. These PPGs have the ability to cage two effectors each and have good water solubility. The δ_u value of 5.0 GM for BNSF is one of the largest ever measured for a PPG.[29]

Ellis-Davies and colleagues have explored the chromophore nitrodibenzofuran (NDBF), which has shown favorable photolysis through both 1PE (Q_u = 0.7) and 2PE. The δ_u value for NDBF was measured to be 0.6 GM based upon the release of calcium ions. NDBF has been used *ex vivo* to stimulate cardiac muscle contractions.[30] Also introduced by Ellis-Davies and coworkers is the PPG 4-carboxymethoxy-5,7-dinitro-indolinyl (CDNI). CDNI has been used to effectively release the neurotransmitter glutamate in biological preparations to elicit neuronal action potentials. Although no δ_u value is reported by the authors, direct comparisons of the photorelease of glutamate by CDNI and MNI, a popular cage currently used by the neuroscience community, indicate the superiority of CDNI with regard to efficiency of photorelease and axon response in neurological tissue.[31] A number of PPGs with susceptibility to 2PE are summarized in Figure 5.

Conclusions

The emergence of 2PE caging groups as tools for the study of biological systems demonstrates an improvement upon the 1PE cages that have to date comprised the bulk of literature concerning PPGs as tools for biological study. In contrast to 1PE, the PPGs that are highly susceptible to 2PE release are characterized by deep penetration and high precision. These compounds will prove an invaluable tool for the study of local signaling dependence by small molecules. Additionally, 2PE processes generally use light from the near-IR, which is not absorbed by relevant species in most biological preparations. The introduction and characterization of novel PPGs that are efficiently removed by multiphoton processes will provide high precision tools for the study of physiological systems.

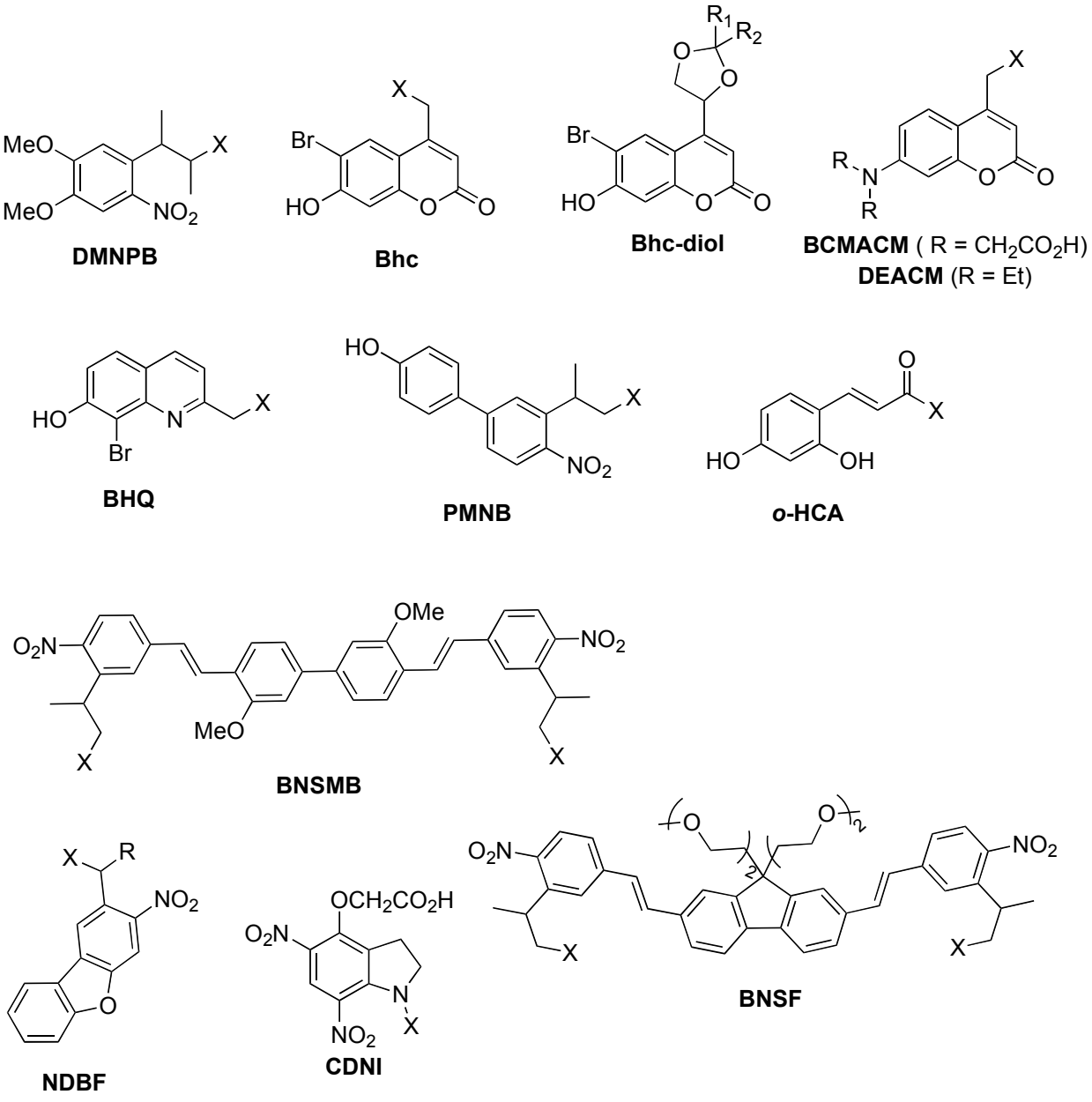


Figure 5: Cages with susceptibility to 2PE. For each cage, X represents the effector molecule. For Bhc-diol, the effector is released as a ketone with groups R_1 and R_2.

CHAPTER 2
SYNTHESIS AND PHOTOCHEMISTRY OF CHQ-OAC

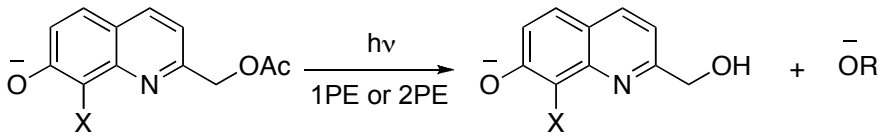
Introduction

Recent synthesis and subsequent characterization of 8-bromo-7-hydroxyquinoline (BHQ) have indicated its potential utility as a PPG in biological systems. BHQ is formed by adding a bromide substituent to the 8-position of the chromophore 7-hydroxyquinoline (HQ) and may be used to cage a wide variety of functional groups found on biomolecules, including carboxylic acids, diols, phosphates, and phenols.[17,32] In addition to having high quantum efficiency and substantial 2PE sensitivity, BHQ is very soluble in aqueous buffer and has significant dark stability, all of which are desirable qualities for compounds that are built with physiological deployment in mind. In general, BHQ has been shown to satisfy with adequate measure the six criteria established in the previous chapter that are necessary for compounds to be useful as tools for biological study.[4] In an effort to increase understanding of how various substituents impact quinoline 1PE and 2PE photochemistry, and to potentially find a quinoline chromophore with photochemical properties greater than those of BHQ, a number of quinoline derivatives with different substituents have been synthesized and studied.

An electron-withdrawing group (EWG) is necessary for the BHQ base compound HQ to retain its desirable photochemical properties. An EWG lowers the pK_a of the hydroxyl group, which increases the phenolate to phenol ratio at physiological pH. The phenolic form has a λ_{max} of 320 nm, whereas the λ_{max} of the phenolate form is 370 nm. A larger λ_{max} is desirable for two reasons: first, higher wavelengths are less damaging to tissue, so 1PE release may be used with fewer ill effects on biological preparations; second, 2PE excitation usually occurs at twice the

wavelength of 1PE, and a λ_{max} of 740 nm places the compound well within the range of the Ti:Sapphire laser, which is often used to elicit 2PE release. Thus, increased concentrations of the phenolate form of HQ at physiological pH translate into more efficient and accessible 1PE and 2PE uncaging processes (Scheme 1). A recent study has shown that ground state BHQ in aqueous solution consists primarily of its neutral, or phenolic, form.[33] Replacing the bromide substituent in BHQ with a more electron-withdrawing group might lower the pK_a of the hydroxy group further and increase the proportion of phenolate present at physiological pH.

Scheme 1: Release of a model biological effector from various 7-hydroxyquinolines. A number of different substituents have been tested at the 8-position (represented by X).

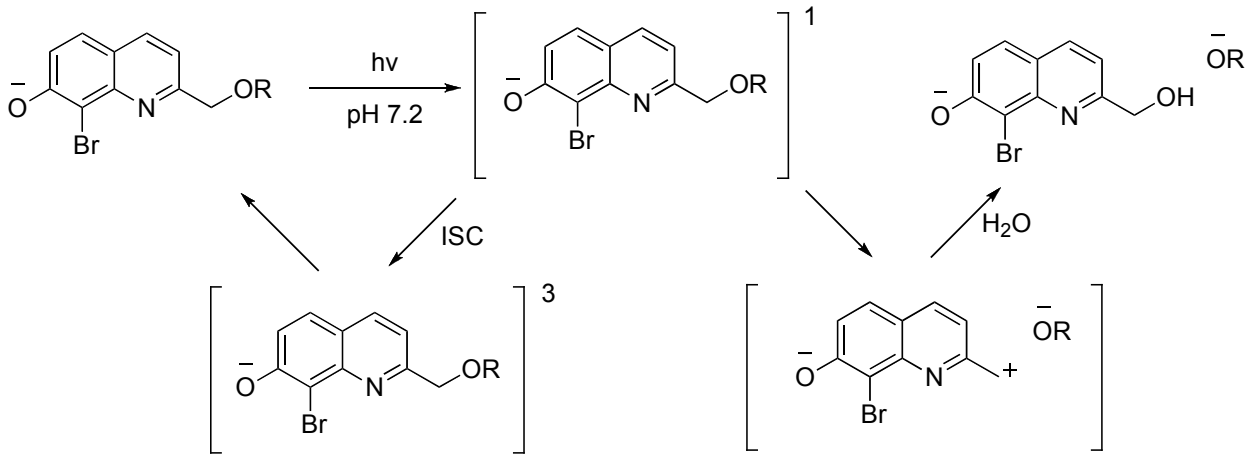


BHQ: X = Br
CyHQ: X = CN
NHQ: X=NO_2
CHQ: X = Cl

Past research in Dore laboratories has shown that building quinoline chromophores with strong electron-withdrawing groups, such as the cyano and nitro groups, in the 8-position has not resulted in increased 2PE susceptibility relative to BHQ. Addition of the cyano group to the 8-position of HQ has substantially increased the fluorescence of the chromophore through extensive π-bond conjugation with the aromatic ring system. Florescence occurs as the singlet excited state falls back to ground state and results in a loss of energy available for photochemical reaction. Although the quantum efficiency of 8-cyano-7-hydroxyquinoline (CyHQ) was similar to that of BHQ, the δ_u value was reduced. A decrease in fluorescence might increase the 2PE sensitivity of HQ.[34] Replacement of the bromine group with a nitro group gave the opposite effect: fluorescence was inhibited. Yet, the chromophore was demonstrated to be photoinert with regard to both 1PE and 2PE photorelease. This result may be rationalized by considering

how the low-lying triplet state that is manifest by the nitro group might promote non-radiative decay over photoreaction.[8,35]

Scheme 2: Proposed mechanism of BHQ photolysis. Intersystem crossing (ISC) from the singlet excited state gives the triplet excited state, which decays down to the ground state.[32]



Studies undertaken by Dore and colleagues have suggested that productive photochemistry for BHQ proceeds through the singlet excited state (Scheme 2).[32] It was with this working hypothesis that chloride was chosen to replace bromide as a substituent at the 8-position of HQ. The chloride group is more strongly electron-withdrawing than bromide. Thus, it was hypothesized that replacing the bromide with the chloride would result in a higher portion of the phenolate form of HQ at physiological pH. In light of singlet state evidence, the chloride was also expected to increase the efficiency of photoreaction since it does not promote intersystem crossing. Through the heavy atom effect, bromine facilitates intersystem crossing to the triplet excited state, which evidence has shown to be unproductive toward photochemical reaction.[8,32] Additionally, the molecular orbitals of chlorine and carbon overlap poorly due to disparities in size. Poor orbital overlap was expected limit the extensive π-bond conjugation and resulting fluorescence that was shown by adding the cyano group to the 8-position. In short, the properties of chlorine were hypothesized to reduce competing photophysical processes so that quantum

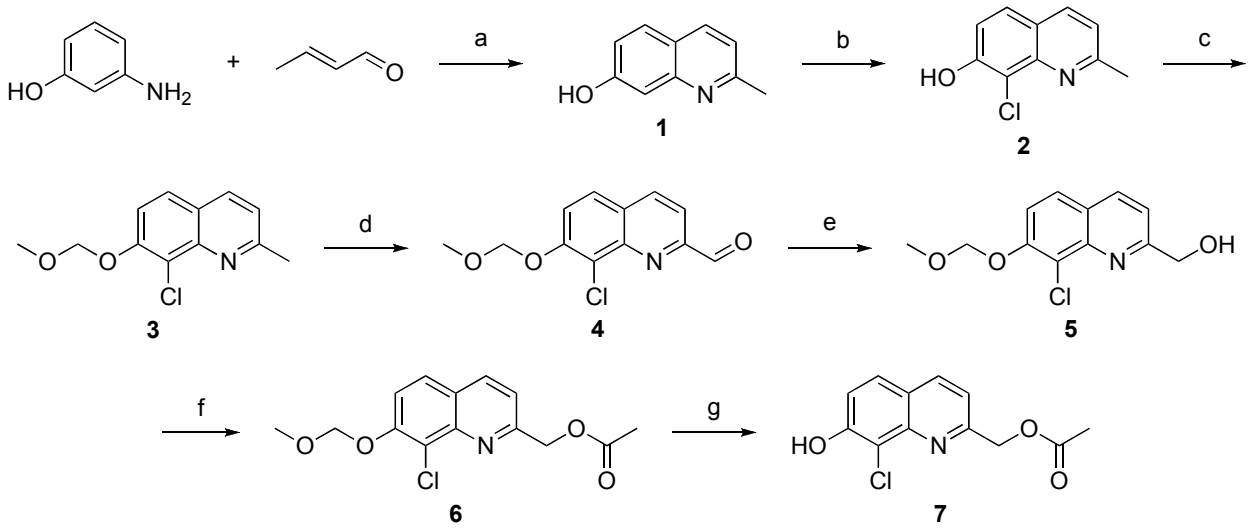
efficiency of the photochemical reaction would be improved. Currently, evidence has accumulated that supports an alternate mechanistic view of BHQ photolysis as proceeding through a short-lived triplet excited state. Work is currently underway to elucidate with more certainty the mechanism of BHQ photolysis.[36]

Synthesis

A seven-step procedure was utilized to synthesize CHQ-OAc (Scheme 3). A modified Skraup reaction using tetrachloro-1,4-benzoquinone as an oxidizing agent combined *m*-aminophenol and crotanaldehyde to afford 7-hydroxyquinaldine (**1**, HQ).[37] *N*-chlorosuccinimide was then used as a chlorinating agent along with the Lewis acid catalyst zirconium chloride in a solution of dichloromethane to afford 8-chloro-7-hydroxyquinaldine (**2,** CHQ).[38] As expected for electrophilic aromatic substitutions of 7-hydroxyquinolines, the chlorination was highly regioselective for the correct position and no regioisomers were detected.[39] The four downfield doublets and disappearance of the downfield HQ singlet demonstrated by ^{1}H NMR are only consistent with chlorination at the 8-position. After the chlorination, chloromethyl ether was used to protect the hydroxyl group to afford 8-chloro-7-methoxymethylquinoline (**3**, MOM-CHQ). The methyl group was then oxidized using selenium (IV) oxide as an oxidizing agent to give 8-chloro-2-formyl-7-methoxymethylquinoline (**4**, MOM-CHQ-CHO). Conveniently, reduction of the aldehyde using $NaBH_4$ afforded 8-chloro-2-hydroxymethyl-7-methoxymethylquinoline (**5**, MOM-CHQ-OH) in high yields without need for further purification after solvent evaporation. The hydroxy group of **5** was then acetylated to generate (8-chloro-7-(methoxymethoxy)quinolin-2-yl)methyl acetate (**6**, MOM-CHQ-OAc). Reaction of **6** in methanol using acid catalysis removed the MOM group with greater preference than the acetyl group to afford (8-chloro-7-hydroxyquinolin-2-yl)methyl acetate (**7**, CHQ-OAc)

in low yields. For all deprotection reactions performed, two product spots were always visible on TLC plates, suggesting that a substantial portion of **6** lost both the acetyl group and the MOM group during the reaction.

Scheme 3: Synthesis of CHQ-OAc.



Reagents and conditions: (a) *p*-chloranil, *n*-butanol, conc. HCl, 90 °C, 1 h; (b) NCS, dichloromethane, $ZrCl_4$, 23 h, 55%; (c) MOMCl, triethylamine, THF, 2 h, 72%; (d) SeO_2, *p*-dioxane, 85 °C, 2 h, 56%; (e) $NaBH_4$, EtOH, 20 min, 99%; (f) Ac_2O, pyridine, 2 h, 87%; (g) MeOH, HCl, 10 min, 35%.

UV and Fluorescence Spectra

The UV-vis spectrum of CHQ-OAc in KMOPS is similar in appearance to the spectrum of an equimolar portion of BHQ-OAc. A distinguishing difference between the two chromophores, however, is manifest upon juxtaposition of the two spectra: absorption of CHQ-OAc is slightly higher around the 370 nm band and lower at the 320 nm band when compared with BHQ-OAc (Figure 6). The extinction coefficient for CHQ-OAc was measured in KMOPS buffer and found to be 2,800 $M^{-1}cm^{-1}$ at λ_{max} 350 nm. This value is only slightly higher than the extinction coefficient for BHQ-OAc, which indicates a similar degree of photon absorption for the two compounds. Determination of the extinction coefficient allowed for the creation of a standard curve on the HPLC so that detector response could be correlated to concentration.

Results of fluorescence measurements show that CHQ is much more fluorescent than BHQ (Figure 7).

Dark Hydrolysis

To test the stability of CHQ-OAc in the dark under physiological conditions (aqueous, neutral, high salt buffer), a portion of CHQ-OAc was dissolved in KMOPS and stored in the dark. Periodically, aliquots of solution were drawn and analyzed via HPLC to gauge the rate of decay. The time constant τ for dark hydrolysis was found to be 49 h, which is less than the 79 h time constant for BHQ-OAc dark hydrolysis.

1PE and 2PE Photochemistry

The 1PE quantum efficiency (Q_u) of CHQ-OAc was measured in KMOPS buffer calibrated to a pH of 7.2 and was determined to be 0.10, which is almost one third the value calculated for BHQ-OAc (Figure 8). The two-photon action cross-section of CHQ-OAc was then measured in KMOPS at 740 nm at a power of 350 mW and found to be 0.12 GM, which is significantly less than the measured 0.59 GM δ_u value for BHQ-OAc under similar conditions (Figure 9). CHQ-OAc 2PE data points were corrected for dark hydrolysis that occurred while samples were waiting to be analyzed on the HPLC.

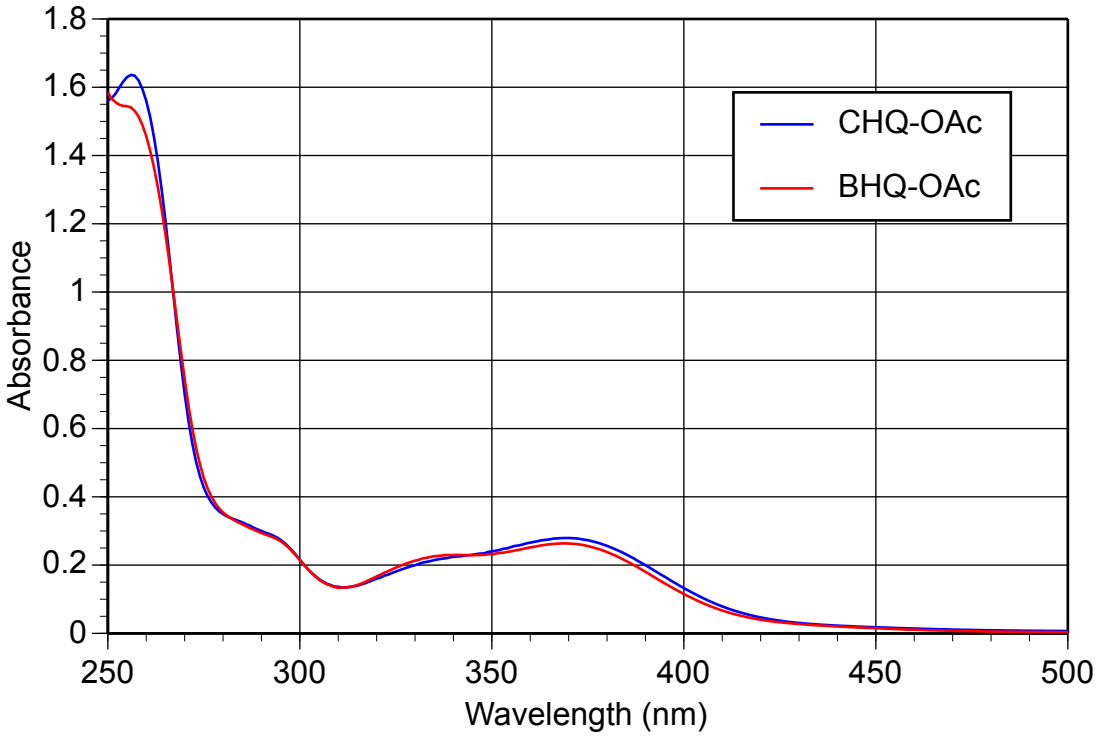


Figure 6: UV Spectra of 100 μM solutions of CHQ-OAc and BHQ-OAc. The band corresponding to the phenolate at 370 nm is slightly higher for CHQ-OAc.

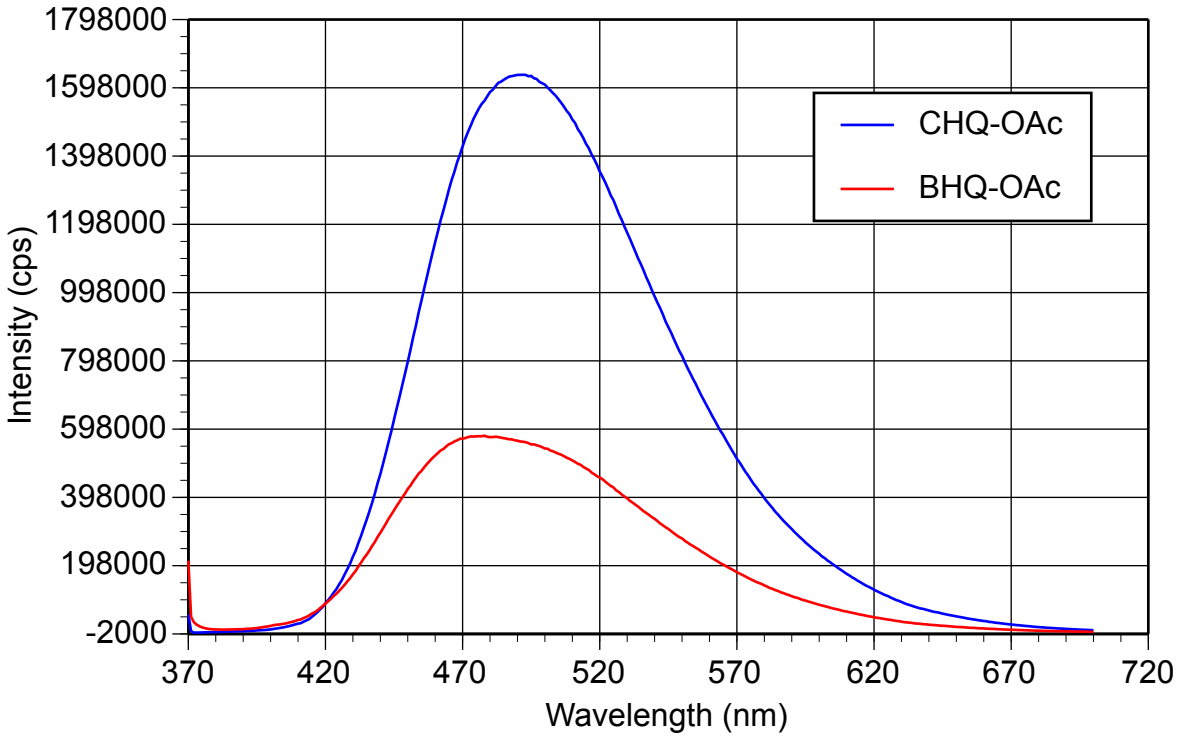


Figure 7: Fluorescence of 15 μM samples of CHQ-OAc and BHQ-OAc in KMOPS at λ_{ex} = 370 nm.

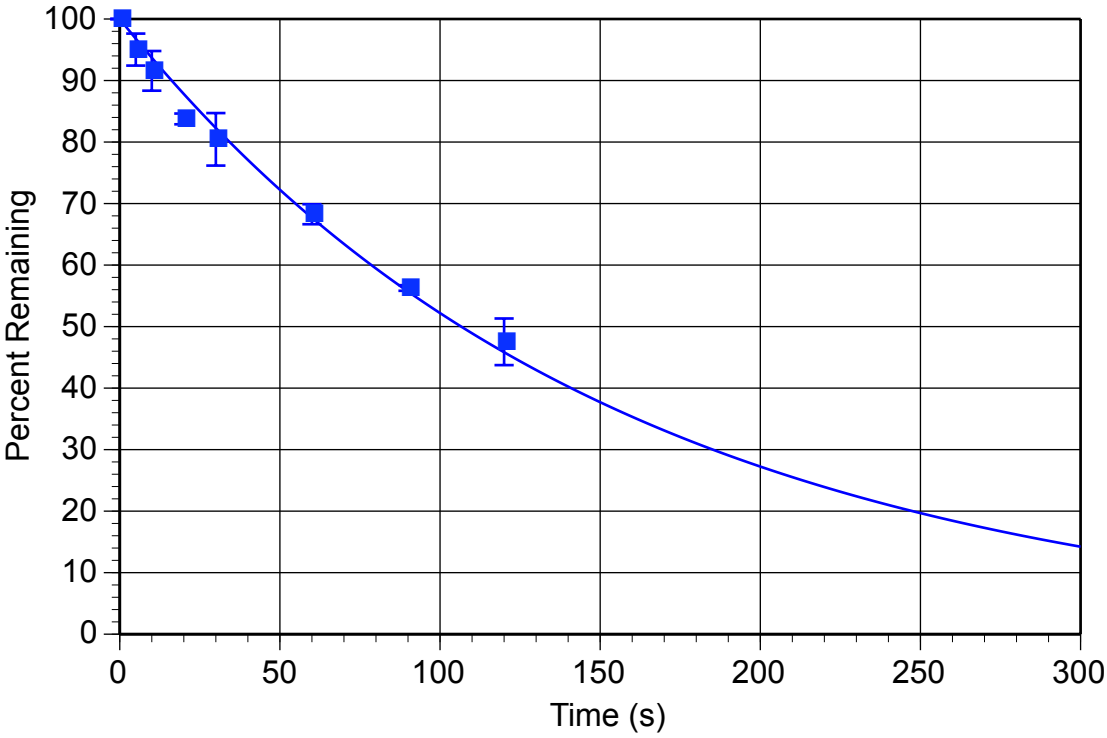


Figure 8: Time course for 1-photon photolysis of CHQ-OAc. Error bars represent standard deviation of 3 concentration measurements. A single exponential decay equation provided the line of best fit.

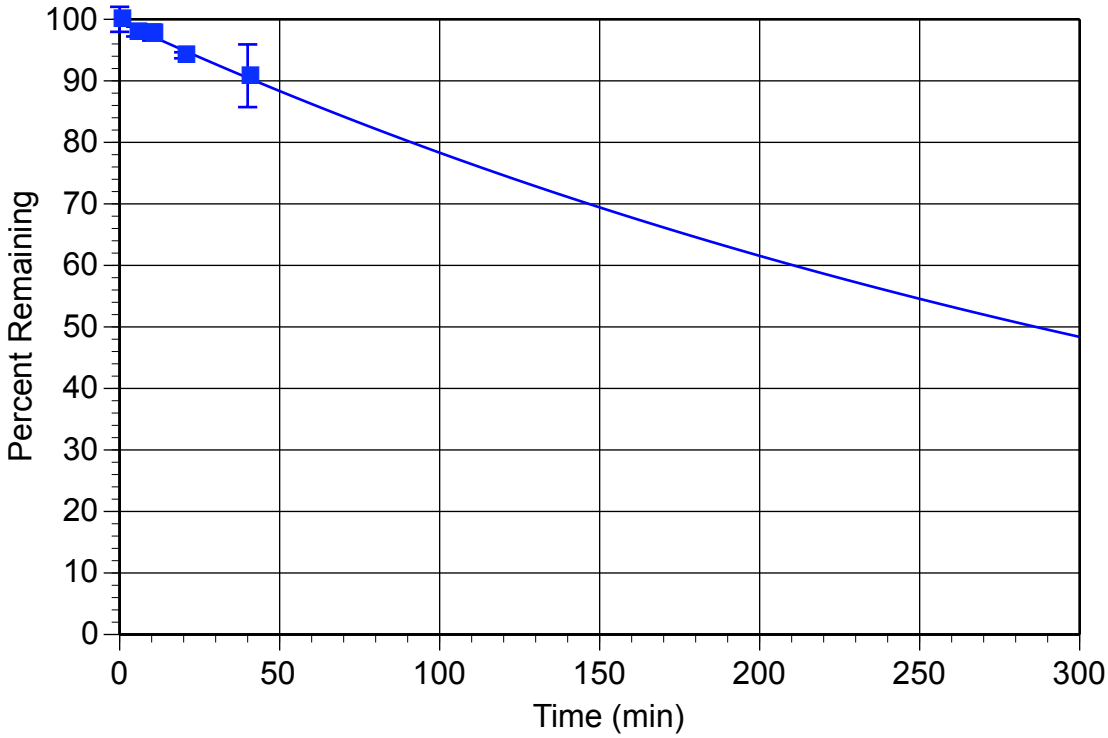


Figure 9: Time course for 2-photon photolysis of CHQ-OAc at 740 nm. Error bars represent standard deviation of 3 concentration measurements. A single exponential decay equation provided the line of best fit.

Discussion

Unfortunately, photochemical measurements of CHQ-OAc have demonstrated its poor suitability as a PPG when compared to BHQ. The uncaging quantum efficiency (Q_u) is almost one third that of BHQ, and the two-photon action cross-section is just above the 0.10 GM that is widely accepted to be the minimum δ_u value needed for a PPG to be useful for biological study.[15] Finally, the time constant for dark hydrolysis τ is 49 h, which shows that CHQ-OAc is more subject to non-photoinduced cleavage in dark physiological settings than BHQ-OAc, which has a time constant of 71 h. In fact, the CHQ-OAc rate of dark hydrolysis occurred at a rate sufficient to necessitate corrections for the 2PE time points since the HPLC analysis took a number of hours to complete. To explain the increased rate of CHQ-OAc dark hydrolysis relative to BHQ-OAc, it is useful to consider both the relative electronegativities and the electron-donating abilities of chloride and bromide substituents used for the two chromophores as well as the stabilities of HQ derivatives that are substituted with the cyano and nitro groups in the 8-position. Data in Table 1 show that cyano- and nitro-substituted HQ derivatives (CyHQ-OAc and NHQ-OAc, respectively) are remarkably resistant toward dark hydrolysis. The respective time constants of dark hydrolysis τ for CyHQ-OAc and NHQ-OAc are 500 h and 278 h.[34,35] The stabilities demonstrated by CyHQ-OAc and NHQ-OAc are likely a result of their electron-withdrawing abilities; by withdrawing electrons via resonance and, to a lesser degree, induction, these substituents decrease electron density of the heterocyclic nitrogen lone pair and inhibit the lone pair's ability to act as a general base catalyst for the hydrolysis of the acetyl group. Based upon induction alone, it would seem that the more electronegative chloride in CHQ-OAc would withdraw electrons more strongly than the bromide in BHQ-OAc and would as a result confer a greater degree of dark stability. However, both the chloride and the bromide substituent, in

contrast to the cyano and nitro groups, have the ability to donate one of their lone pairs of electrons into the ring, resulting in an increase in ring electron density. Electron donation from chloride in CHQ-OAc is more pronounced than the bromide of BHQ-OAc due to better relative orbital overlap with the ring carbon. These donation effects might mitigate the inductive effects and result in an increase in electron density of the heterocyclic nitrogen lone pair relative to BHQ-OAc, which would increase the rate of dark hydrolysis. When compared to BHQ and other chromophores such as Bhc, the photochemical parameters of CHQ suggest that it is not worthy of pursuit as a PPG.

Table 1: Photochemical parameters of a number of quinoline-based PPGs.

Chromophore	λ_{max} (nm)	ε ($M^{-1}\cdot cm^{-1}$)	λ_{em} (nm)	Q_u	Sensitivity $Q_u \times \varepsilon$	δ_u (GM)	δ_a (GM)	τ_{dark} (h)
BHQ-OAc	369	2600	478	0.29	754	0.59	2.0	71
NHQ-OAc	350	6500	-	0.00	0	0.00	-	278
CyHQ-OAc	364	7700	436	0.31	2387	0.32	1.0	500
CHQ-OAc	**370**	**2800**	**492**	**0.10**	**280**	**0.12**	**1.2**	**49**
DMAQ-OAc	368	4600	496	0.046	211	0.13	2.8	31
DMAQ-Cl-OAc	386	3300	493	0.090	234	0.47	5.2	34
TQ-OAc	369	5200	-	0.063	328	0.42	6.7	29

A recent study reports the photochemical parameters of a number of quinoline-based chromophores with similarity to BHQ-OAc (Table 1).[35] The photochemistry of CHQ-OAc adds to the knowledge of how substituents impact quinoline photochemistry. A halogen, chloride is the most chemically similar substituent added to the 8-position of HQ in place of the bromide in BHQ. Spectroscopic studies have shown that BHQ exists primarily in the neutral form at physiological pH,[33] and it was hoped that the more electronegative chloride would increase the acidity of the HQ hydroxy group and thereby increase the amount of phenolate at physiological pH. The 370 nm wavelength of the phenolate (as opposed to the wavelength of around 320 nm

for the neutral form of the HQ chromophore) is more suitable for biological study using both 1PE and 2PE mediated photorelease. In fact, Figure 6 shows that at physiological pH, CHQ-OAc has a higher portion of phenolate than BHQ-OAc. As would be expected given the UV spectra, the value of the molar extinction coefficient for CHQ-OAc is slightly greater than that of BHQ-OAc at a wavelength of 370 nm (2,800 $M^{-1}cm^{-1}$ for CHQ-OAc and 2,600 $M^{-1}cm^{-1}$ for BHQ-OAc),[17] indicating a greater degree of photon absorption. Unfortunately, these increases do not appear to be significant, and both 1PE and 2PE processes were inferior to those demonstrated by BHQ-OAc. Currently, spectroscopic and computational studies are underway to help elucidate the nature and relative concentrations of the prototropic equilibria demonstrated by CHQ-OAc, CyHQ-OAc, and NHQ-OAc at physiological pH.

To help assign reasonable explanations for the inferior photochemistry demonstrated by CHQ-OAc despite spectral and absorptive similarities with BHQ-OAc, fluorescence measurements were taken. Equimolar samples of CHQ-OAc and BHQ-OAc were monitored for fluorescence under identical conditions and showed that CHQ-OAc fluoresces more strongly than BHQ-OAc (Figure 7). This result suggests that a larger ratio of singlet state energy in CHQ-OAc is lost through fluorescence or other photophysical processes rather than being used to promote photoreaction.

A survey of numerous quinoline derivations in Table 1 shows that there is little correlation between 1PE and 2PE sensitivity other than an absence of 2PE when 1PE is not present, as shown by NHQ-OAc. No other compound was found to exceed BHQ in terms of susceptibility to 2PE excitation. Together, the synthesis and photochemistry of CHQ-OAc and numerous other quinoline analogues suggest that the bromide substituent in HQ is needed to promote 2PE photochemistry for this class of chromophores. Although this study was

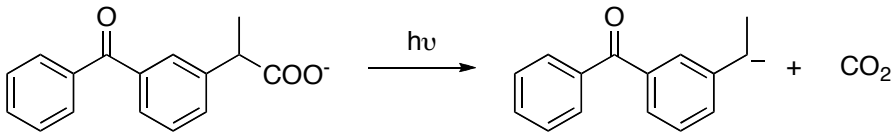
undertaken with the working hypothesis that the mechanism of BHQ photorelease proceeds through the singlet excited state,[8,32] recent work using ultrafast spectroscopy has pointed toward a mechanistic route that utilizes the triplet excited state.[36] If this is found to be the case, then the intersystem crossing from the singlet to the triplet excited state facilitated by the bromide substutuent in BHQ might in fact promote photoreaction. Future work will focus on BHQ and other HQ derivatives using computational and mechanistic studies to help determine the role that bromine plays in promoting 2PE susceptibility.

CHAPTER 3
PHOTOCHEMISTRY OF XANTHONE ACETIC ACID DERIVATIVES

Introduction

Recent studies have indicated ketoprofen derivatives as favorable PPGs for carboxylates and amines. Ketoprofen release from effector molecules is characterized by clean photochemistry, high quantum efficiency, high yield, and rapid kinetics.[40] With ketoprofens, the mechanism of photoreaction has been shown to rely upon the formation of a carbanion after decarboxylation (Scheme 4). The carbanion is then rapidly reprotonated from solvent, or may be manipulated to elicit elimination reactions that release various leaving groups.[41,42] One of the disadvantages of ketoprofen as a core molecule for various PPGs is that the ketoprofen 1PE photochemistry is promoted by light from the UVB region around 300 nm. This light is damaging to biological tissues and provides a severe limit to the deployment of ketoprofen derivatives in physiological settings.

Scheme 4: Decarboxylation of a ketoprofen upon irradiation with UVB light.



In hopes of overcoming the unfavorable wavelengths of absorption posed by the ketoprofens, Sciano et al. explored the photochemistry of the structurally similar xanthone series of compounds. A series of xanthone acetic acids was synthesized with carboxylic acid groups at the 2-, 3-, and 4-positions and their photochemistry was tested by monitoring rates of decarboxylation (Figure 10). Compounds with carboxylic acid groups at the 2- and 4-positions, (2-xanthone acetic acid (2-XAA) and 4-xanthone acetic acid (4-XAA), respectively) were found

to exhibit favorable quantum efficiencies (Q_u = 0.67 for 2-XAA and 0.64 for 4-XAA) similar to chromophores from the ketoprofen family while possessing more biologically-suitable wavelengths of absorption around 350 nm. Mechanistic work showed that photochemical reaction of xanthone acetic acids likely proceeds through the singlet excited state.[43] In an interesting contrast, 3-xanthone acetic acid (3-XAA) showed almost no photochemistry. This result may be explained by consideration of a molecular orbital theory prediction called the "*meta* effect," which states that substituents positioned *meta* to the activating center of an aromatic compound will have a more pronounced effect on the compound's chemistry. In the cases of 2-XAA and 4-XAA, the carboxylic acid groups are both *meta* to the carbonyl, whereas the substituent in 3-XAA is in the *para* position.[44]

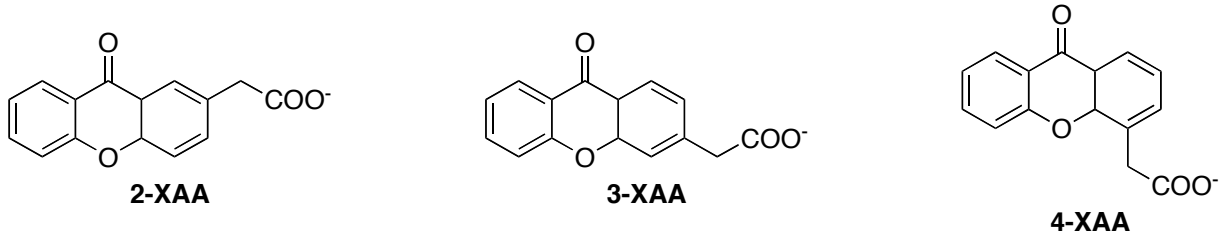
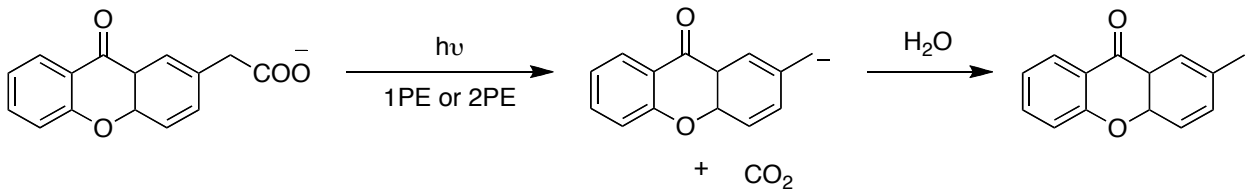


Figure 10: Various xanthone acetic acids. These xanthone acetic acids were synthesized and tested for their photochemistry. 3-XAA was found to be photoinert.

Given the excellent quantum efficiencies of 2-XAA and 4-XAA, the decision was made to measure the two-photon action cross-sections of both compounds. Past work has in fact attempted to measure 2PE susceptibility of these compounds, but the wavelengths of irradiation used were around 730 nm, the lowest threshold of the instrument used for the measurement, and the results were inconclusive.[45] The maximum absorption for both 2-XAA and 4-XAA was 348 nm, which suggested that a measurement at 700 nm, approximately twice the 1PE wavelength, would give more insight into the photoreactivity of 2-XAA and 4-XAA through 2-photon

processes. The procurement of new instrumentation enabled the examination of the 2PE cross-sections of these compounds at this wavelength with sufficient power (Scheme 5).

Scheme 5: Decarboxylation of 2-XAA occurs through both 1PE and 2PE.



Measurement of 2-XAA and 4-XAA Two-photon Action Cross-Sections

Measurements of the 2PE action cross-sections of 2-XAA and 4-XAA were carried out in 100 mM phosphate buffer at pH 7.4 to replicate conditions used for the establishment of the 1PE quantum efficiencies of both compounds.[43] 2-XAA and 4-XAA were irradiated at 700 nm and 250 mW power to give two-photon action cross-sections of 2.1 GM each (Figures 11 and 12, respectively). Rises of the methylated photoproducts, 2-methyl xanthone and 4-methyl xanthone (2-MeX and 4-MeX, respectively) were also monitored.

Discussion

At 2.1 GM, these δ_u values are some of the largest ever reported for PPGs and greatly exceed the threshold of 0.10 GM that is generally agreed upon to be the lower limit for 2PE sensitive compounds to be useful in biological applications.[15] Notably, the two-photon action cross-section values for 2-XAA and 4-XAA were found to be the same. This result is reasonable given the structural similarities of the two compounds and their similar quantum efficiencies (Q_u = 0.67 for 2-XAA and 0.64 for 4-XAA); however, further studies are needed to establish the mechanistic rational for this result. Future work will endeavor to apply 2-XAA or 4-XAA to a relevant biomolecule such as glutamate and measure the rate of uncaging through 2PE processes.

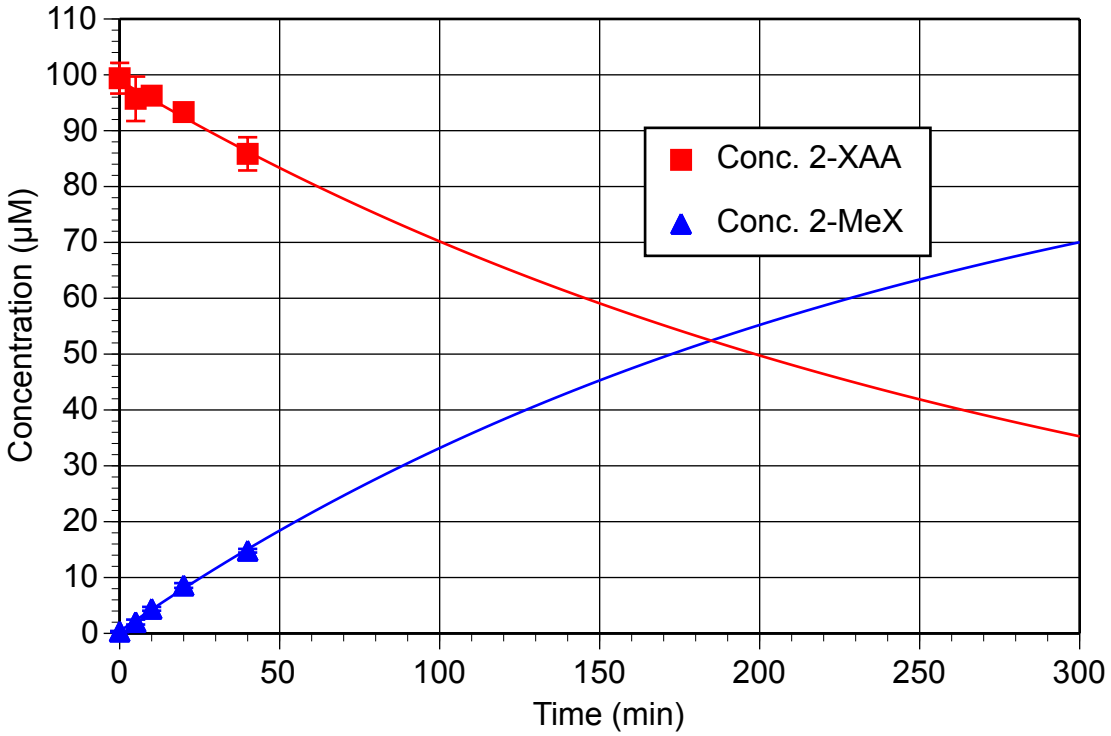


Figure 11: Time course for 2-photon photolysis of 2-XAA at 700nm. Error bars represent standard deviation of 3 or 4 concentration measurements. Single exponential decay and rise to max equations provided lines of best fit.

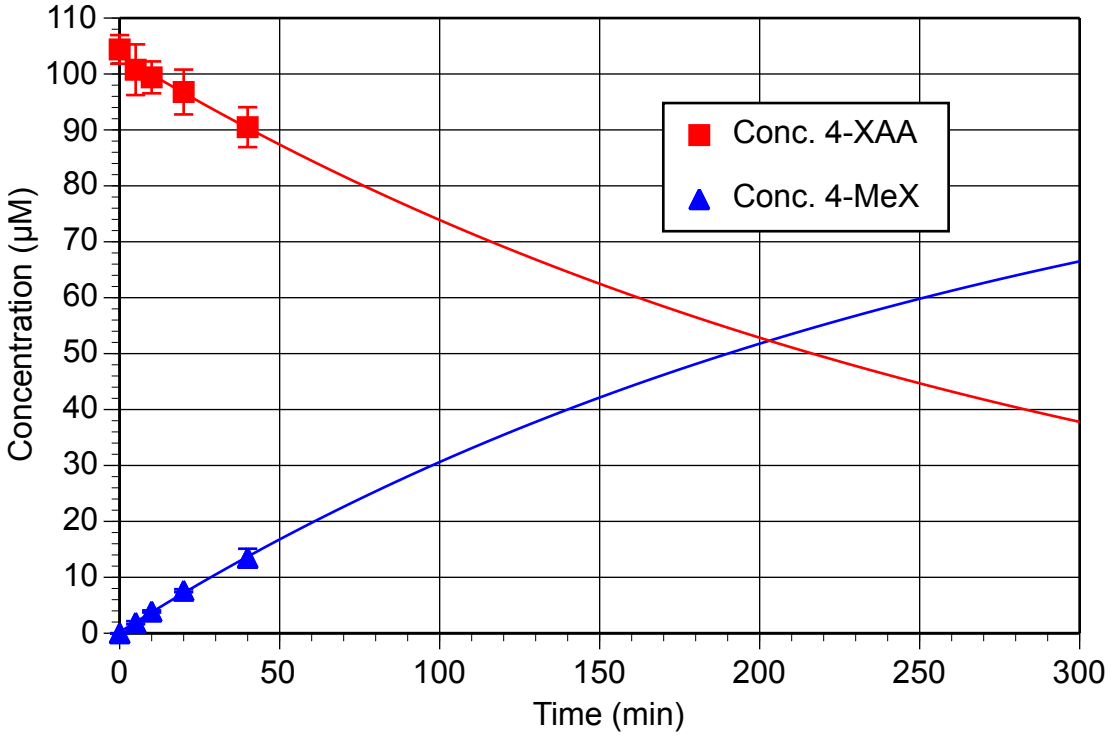


Figure 12: Time course for 2-photon photolysis of 4-XAA at 700 nm. Error bars represent standard deviation of 3 or 4 concentration measurements. Single exponential decay and rise to max equations provided the lines of best fit.

CHAPTER 4
EXPERIMENTAL

General

All solvents and reagents used in synthesis were purchased from commercial sources and were used without further purification unless noted otherwise. Moisture sensitive reactions were carried out using flame or oven-dried glassware and inert atmospheres were maintained using gas bubblers. Tetrahydrofuran (THF), benzene, and toluene were dried by passing the solvents through activated alumina under positive nitrogen pressure before use. All ^{1}H NMR values were taken on a Varian Mercury Plus 400 MHz spectrometer and chemical shift data were reported in parts per million (δ) relative to TMS (0.00 δ). FTIR spectra were recorded on a Shimadzu IR Prestige-21 spectrophotometer. UV spectra were recorded on a Varian Cary 300 Bio UV-Visible spectrophotometer. HPLC analyses were performed on a Varian ProStar HPLC system with an autosampler and diode array detector using Microsorb C-18 reverse phase columns. A Sciex API-1 Plus quadropole mass spectrometer with an electrospray ionization source was used for mass spectrometry. Electrospray ionization was used to obtain HRMS spectra. Analytical thin layer chromatography took place on precoated silica gel 60 F254 plates (Sorbent Technologies). 230-400 mesh silica gel 60 (Sorbent Technologies) was used for column chromatography. Evaporation of solvents was carried out with a rotary evaporator with an aspirator pump and heated water bath followed by evaporation at < 1 torr before spectral data was obtained.

The standard fluorescein solution used for measurement of two-photon action cross-sections was created by dissolving 10 μL of 1 M aq. NaOH solution and fluorescein (1mg) in 10 mL of deionized water. A 300 μL aliquot of this solution was dissolved in 3 mL of water. UV-

Vis spectroscopy was used to measure the absorbance of fluorescein at 488 nm, and the final concentration of the solution was determined using the fluorescein's molar extinction coefficient (ε = 88,000 $M^{-1} \cdot cm^{-1}$ at 488 nm).

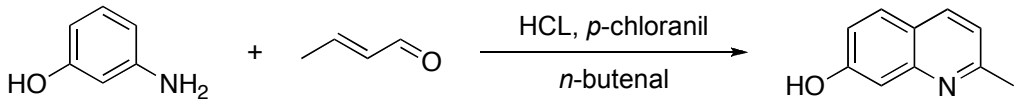
The KMOPS buffer consisted of 100 mM KCl and 10 mM MOPS titrated to pH 7.2 with KOH. HPLC analysis (40% acetonitrile, 60% H_2O [0.1% TFA]) was used to measure the concentrations of CHQ-OAc for 1PE, 2PE, and dark hydrolysis experiments. An external standard of CHQ-OAc allowed for the conversion of peak area to concentrations.

HPLC analysis (70% acetonitrile, 30% H_20 [0.1% TFA]) was used to measure the concentrations of 2-XAA and 4-XAA for 2PE experiments. External standards were created for 2-XAA and 4-XAA as well as their photochemical products, 2-MeX and 4-MeX.

The potassium ferrioxalate solution was prepared by dissolving 2.947g $K_3Fe(C_2O_4)_3$ in H_2O (800 mL), adding 1.0 N H_2SO_4 (100 mL), and diluting the resulting solution to 1 L. The phenanthroline solution was prepared by dissolving 10 mg phenanthroline in deionized water (100 mL). The aqueous buffer was created by combining 1.0 N sodium acetate (600 mL) with 1.0 N H_2SO_4 (360 mL) and diluting to 1 L.

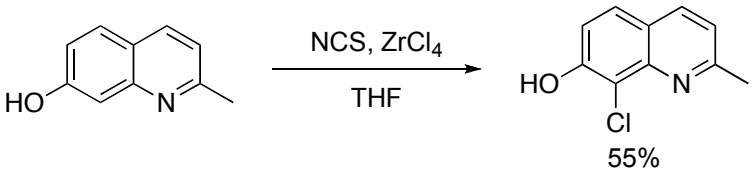
Synthesis of CHQ-OAc

7-hydroxyquinaldine (1).



m-Aminophenol (6.9982 g, 64.2 mmol) was added to conc. HCl (45 mL, minimal). *p*-Chloranil (16.2755 g 66.4 mmol) was added to the gray solution followed by *n*-butanol (5 mL). Water (10 mL) was added to bring reagents into solution. The solution was heated to 90 °C and *n*-butenal (5.85 g, 83.6 mmol) was slowly added dropwise. The solution turned red and then brown. After 70 min of stirring, the reaction was quenched with water and the solution was hot-filtered into a vacuum flask. The brown solid was washed with deionized water and the solution was titrated to pH 6 with 10% NaOH solution. The brown solution was vacuum filtered and a brown solid was collected. The solid was air-dried for two days under a hood and then purified with column chromatography (EtOAc/hexanes 5:5 followed by EtOAc). The solvent was evaporated to afford light brown solid **1**. ^{1}H NMR spectroscopic data was identical to previously reported literature values.[7]

8-Chloro-7-hydroxyquinaldine (2).



Under a nitrogen atmosphere, 7-hydroxyquinaldine **1** (0.3900 g, 2.452 mmol) was added to a solution of NCS (0.3283 g, 2.459 mmol) and zirconium chloride (0.0285 g, 0.122 mmol) in dichloromethane (35 mL). After stirring for 23 h, the solution was diluted with chloroform and washed successively with saturated sodium carbonate solution, water, and brine. The solvent was evaporated to give a brown residue, which was purified by column chromatography (1:3 EtOAc/hexane) to yield **2** (0.2612 g, 1.349 mmol, 55%) as an oily yellow residue.

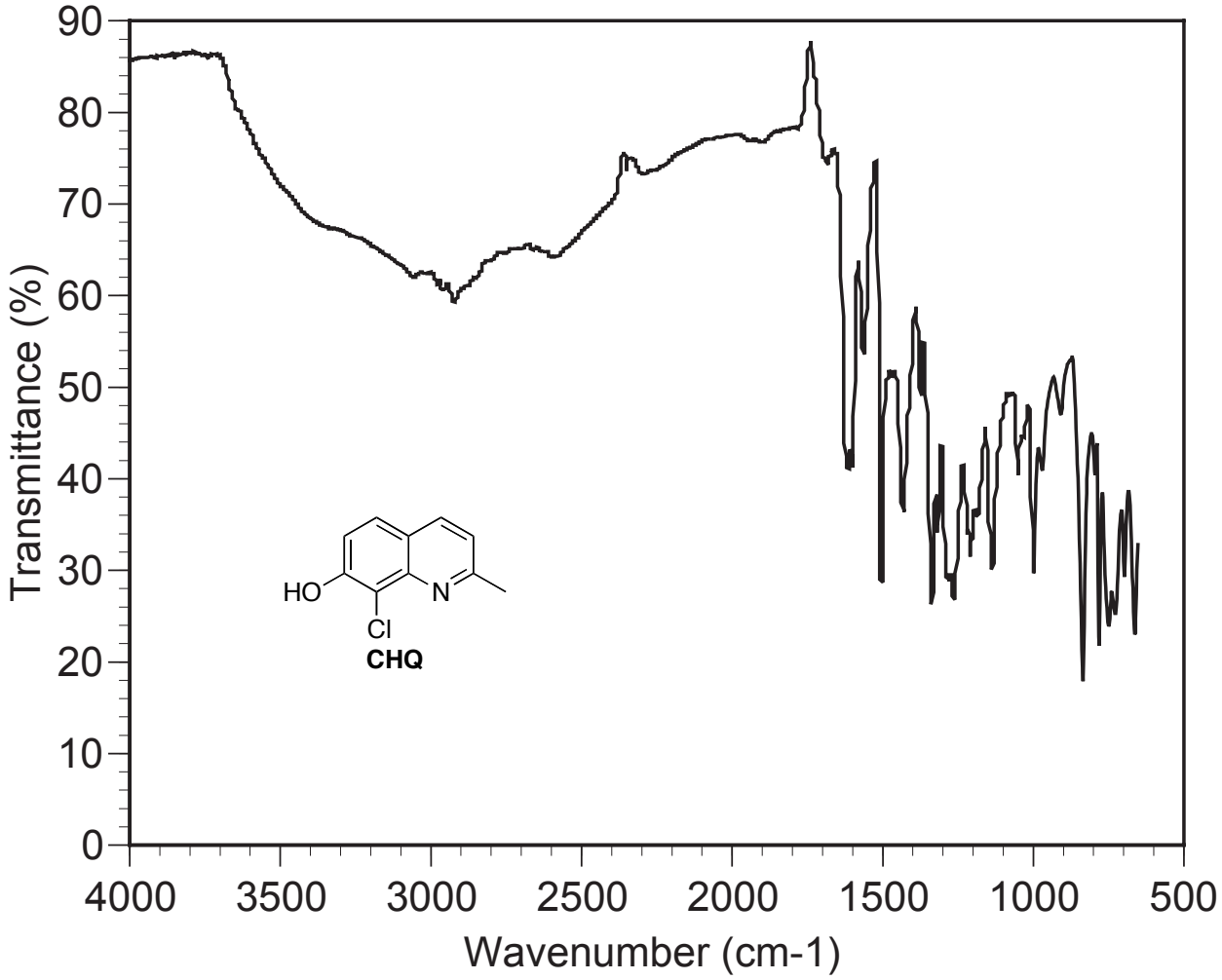
^{1}H NMR ($CDCl_3$) δ 8.00 (1H, d, J = 8.0 Hz), 7.64 (1H, d, J = 9.2 Hz), 7.28 (1H, d, J = 8.0 Hz), 7.23, (1H, d, J = 8.4 Hz), 2.80 (3H, s).

^{13}C NMR ($CDCl_3$) δ 160.6, 152.8, 144.9, 136.6, 127.5, 122.7, 120.7, 117.0, 114.6, 25.9.

FTIR (neat) 2926, 1619, 1509, 1437, 1343, 1267, 1215, 1141, 1002, 835, 750 cm^{-1}.

MS (ESI) *m/z* calcd for $(C_{10}H_8ClNO + H)^+$ 194 (^{35}Cl) and 196 (^{37}Cl), found 194 (^{35}Cl) and 196 (^{37}Cl).

HRMS (ESI) *m/z* calcd for $(C_{10}H_8ClNO + H)^+$ 194.0373 (^{35}Cl) and 196.0343 (^{37}Cl), found 194.0362 (^{35}Cl) and 196.0333 (^{37}Cl).


Transmittance (%)
90
80
70
60
50
40
30
20
10
0
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)
HO
N
Cl
CHQ

INDEX	FREQUENCY	PPM	HEIGHT
1	3203.729	8.006	18.7
2	3195.523	7.986	19.7
3	3061.105	7.650	18.9
4	3052.118	7.627	21.8
5	2916.918	7.290	21.4
6	2909.103	7.270	82.9
7	2897.771	7.242	20.6
8	2889.565	7.221	18.3
9	1120.640	2.801	135.2
10	2.704	0.007	66.4

File: Wilson57-chlorideF37_Proton_2008Feb09_01

Pulse Sequence: s2pul

Solvent: cdcl3
Ambient temperature
Sample #39, Operator: hunter
File: Wilson57-chlorideF37_Proton_2008Feb09_01
INOVA-500 "LinuxWS1"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.998 sec
Width 6402.0 Hz
8 repetitions
OBSERVE H1, 400.1493213 MHz
DATA PROCESSING
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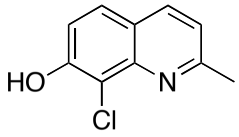
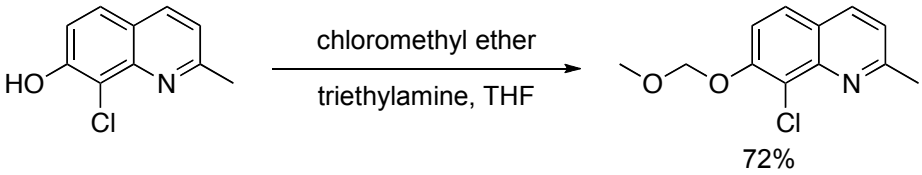
Total time 0 min, 24 sec



CHQ

8-Chloro-7-methoxymethylquinoline (3).



Triethylamine (1.879 mL, 13.49 mmol) was added to a solution of **2** (0.2612 g, 1.349 mmol) in THF (20 mL) under a nitrogen atmosphere. Chloromethyl methyl ether (0.241 mL, 3.17 mmol) was added to the mixture dropwise. After stirring for 2 h, the solution was diluted with EtOAc and washed successively with water (×2) and brine. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The resulting residue was purified by column chromatography (3:7 EtOAc/hexane) to afford **3** (0.2298g, 0.9668 mmol, 72%) as a yellow solid (mp 78–79 °C).

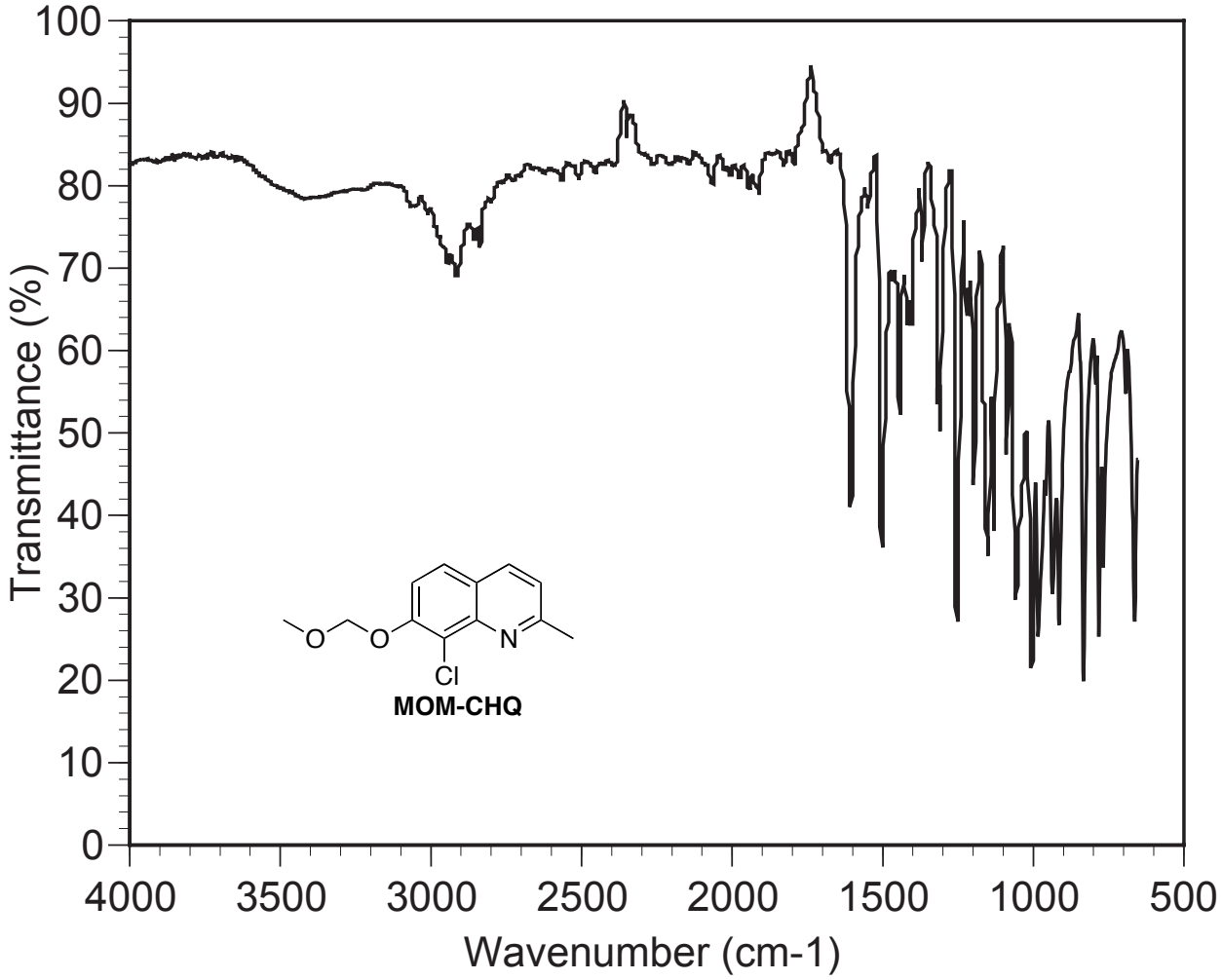
^{1}H NMR ($CDCl_3$) δ 8.01 (1H, d, J = 8.8 Hz), 7.67 (1H, d, J = 8.8 Hz), 7.47 (1H, d, J = 9.2 Hz), 7.26 (1H, d, J = 8.8 Hz), 5.40 (2H, s), 3.58 (3H, s), 2.82 (3H, s).

^{13}C NMR ($CDCl_3$) δ 160.9, 153.8, 145.4, 136.6, 126.9, 123.5, 121.4, 116.7, 105.0, 95.7, 56.8, 26.0.

FTIR (neat) 2919, 1611, 1508, 1259, 1011, 833, 783 cm^{-1}.

MS (ESI) *m/z* calcd for $(C_{12}H_{12}ClNO_2 + H)^+$ 238 (^{35}Cl) and 240 (^{37}Cl), found 238 (^{35}Cl) and 240 (^{37}Cl).

HRMS (ESI) *m/z* calcd for $(C_{12}H_{12}ClNO_2 + H)^+$ 238.0635 (^{35}Cl) and 240.0605 (^{37}Cl), found 238.0623 (^{35}Cl) and 240.0593 (^{37}Cl).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20
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Transmittance (%)
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2500
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Wavenumber (cm-1)
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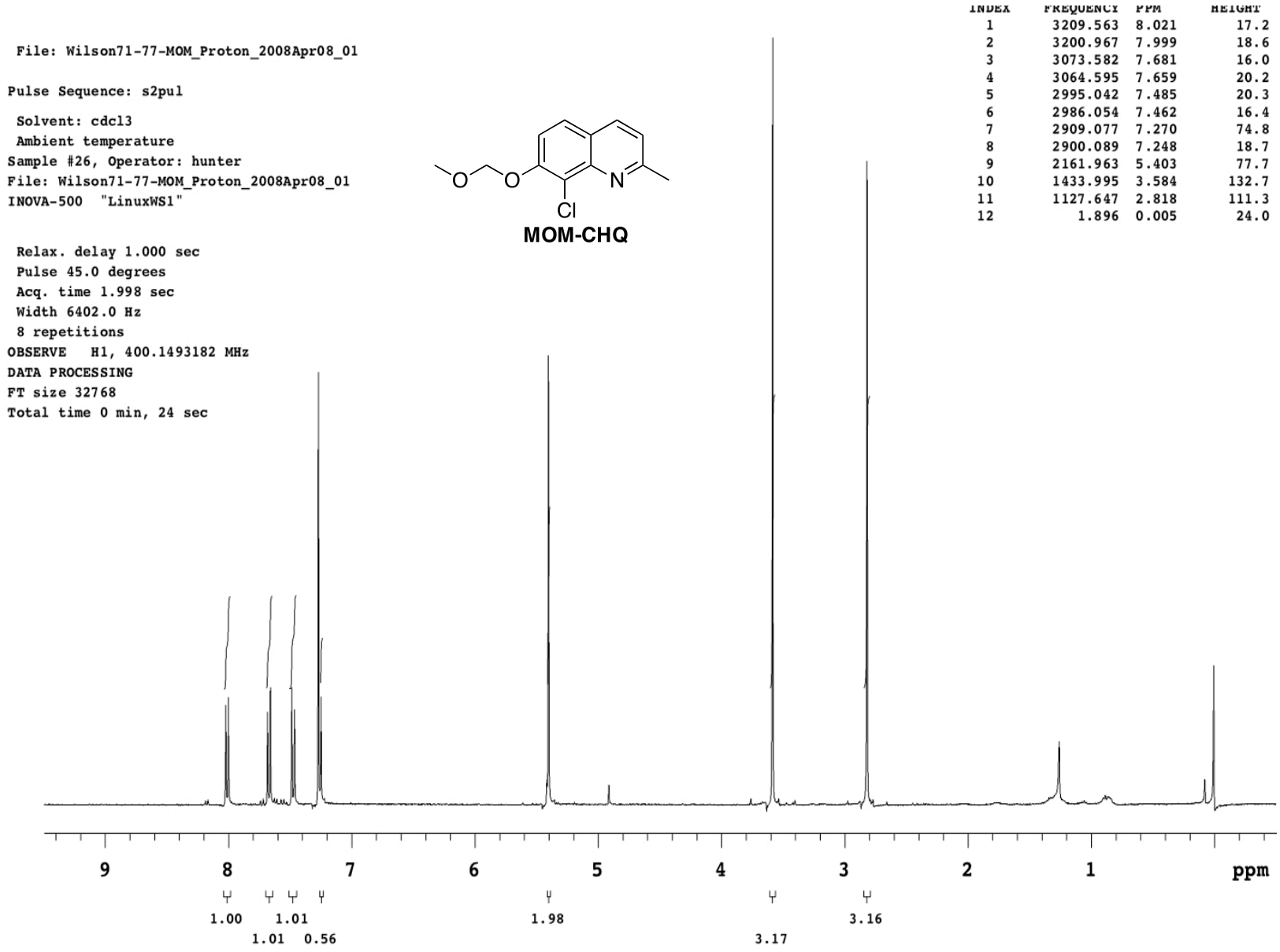
O
N
Cl
MOM-CHQ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Solvent: cdcl3
Ambient temperature
Sample #26, Operator: hunter
File: Wilson71-77-MOM_Proton_2008Apr08_01
INOVA-500 "LinuxWS1"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.998 sec
Width 6402.0 Hz
8 repetitions
OBSERVE H1, 400.1493182 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 24 sec
MOM-CHQ
INDEX FREQUENCY PPM HEIGHT
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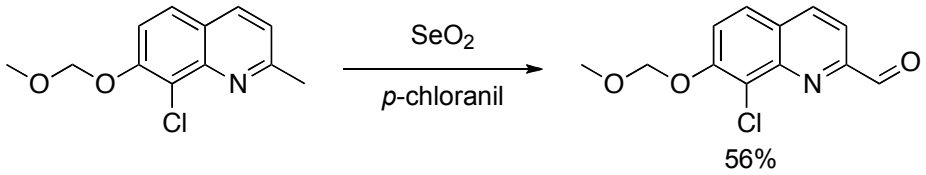
2 3200.967 7.999 18.6
3 3073.582 7.681 16.0
4 3064.595 7.659 20.2
5 2995.042 7.485 20.3
6 2986.054 7.462 16.4
7 2909.077 7.270 74.8
8 2900.089 7.248 18.7
9 2161.963 5.403 77.7
10 1433.995 3.584 132.7
11 1127.647 2.818 111.3
12 1.896 0.005 24.0
9
8
7
6
5
4
3
2
1
ppm
1.00
1.01
1.01
0.56
1.98
3.17
3.16

8-Chloro-2-formyl-7-methoxymethylquinoline (4).



56%

Under a nitrogen atmosphere, selenium dioxide (0.0604 g, 0.544 mmol) was added to a solution of **3** (0.1232 g, 0.5183 mmol) in *p*-dioxane (3 mL). The resulting orange solution was stirred in a sealed flask for 2 h at 85 °C. The black precipitate was removed by vacuum filtration and the filtrand was evaporated, leaving a residue, which was purified by column chromatography (2:8 EtOAc/hexane) to afford **4** (0.0724 g, 0.288 mmol, 56%) as a pale yellow solid (mp 118–121 °C).

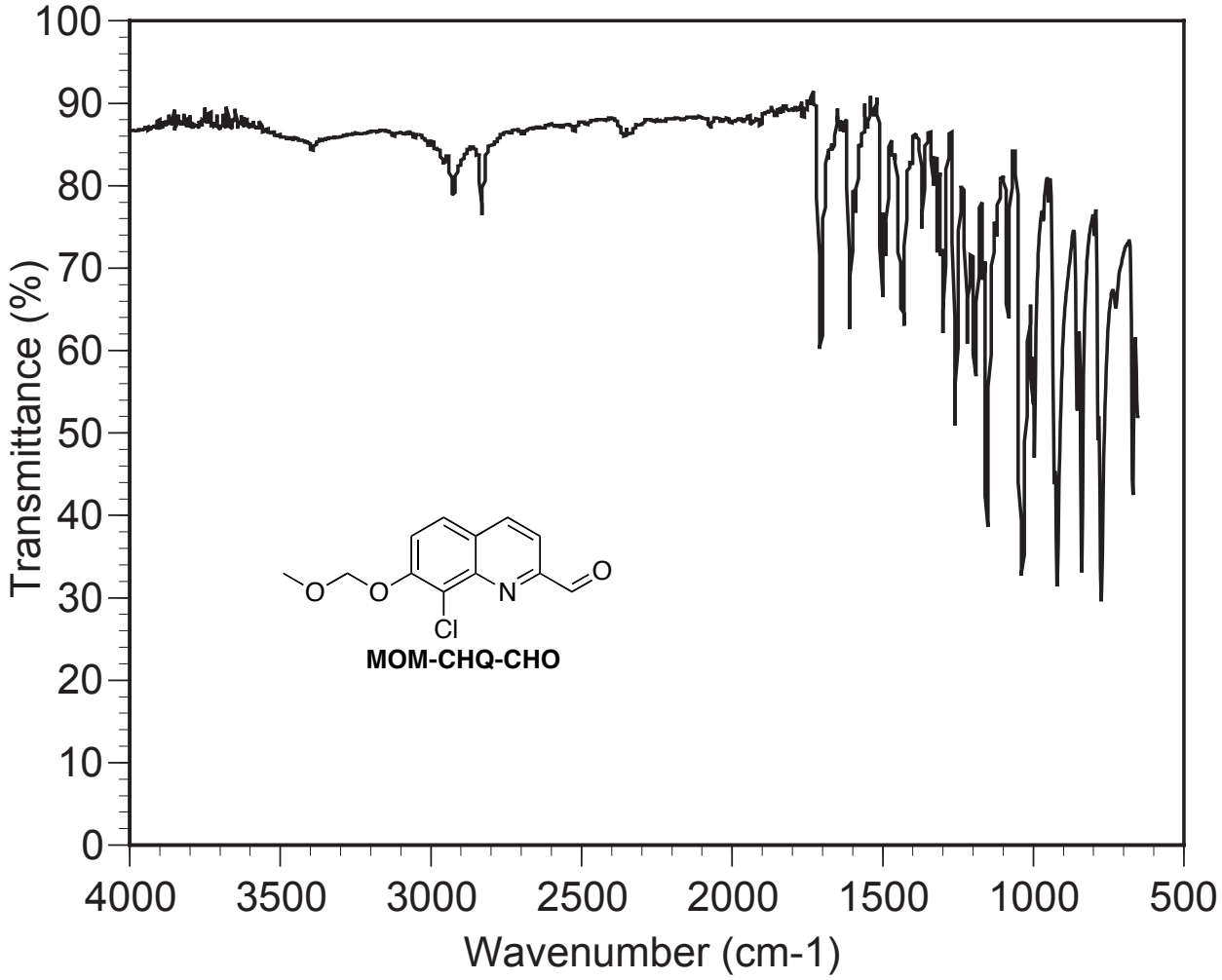
^{1}H NMR ($CDCl_3$) δ 10.30 (1H, s), 8.30 (1H, d, J = 8.0 Hz), 7.99 (1H, d, J = 8.0 Hz), 7.81 (1H, d, J = 9.6 Hz), 7.70 (1H, d, J = 8.8 Hz), 5.45 (2H, s), 3.60 (3H, s).

^{13}C NMR ($CDCl_3$) δ 193.9, 154.7, 153.4, 145.6, 138.0, 127.2, 127.0, 121.3, 120.2, 116.5, 95.7, 57.0.

FTIR (neat) 2931, 2835, 1713, 1615, 1303, 1159, 1043, 920, 775 cm^{-1}.

MS (ESI) *m*/*z* calcd for $(C_{12}H_{10}ClNO_3 + H)^+$ 252 (^{35}Cl) and 254 (^{37}Cl), found 252 (^{35}Cl) and 254 (^{37}Cl).

HRMS (ESI) *m*/*z* calcd for $(C_{12}H_{10}ClNO_3 + H)^+$ 252.0427 (^{35}Cl) and 254.0398 (^{37}Cl), found 252.0418 (^{35}Cl) and 254.0390 (^{37}Cl).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20
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Transmittance (%)
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3000
2500
2000
1500
1000
500
Wavenumber (cm-1)
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O
N
O
Cl
MOM-CHQ-CHO

INDEX	FREQUENCY	PPM	HEIGHT
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2	3323.285	8.305	14.5
3	3315.079	8.285	16.4
4	3202.543	8.003	21.4
5	3194.337	7.983	19.3
6	3129.473	7.821	15.3
7	3120.095	7.797	23.0
8	3084.927	7.709	23.6
9	3075.940	7.687	16.2
10	2179.950	5.448	81.3
11	1439.869	3.598	128.7

File: Wilson97-aldehyde_Proton_20080421_01

Pulse Sequence: s2pul

Solvent: cdcl3
Ambient temperature
Operator: hunter
File: Wilson97-aldehyde_Proton_20080421_01
INOVA-500 "LinuxWS1"

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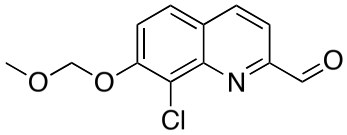
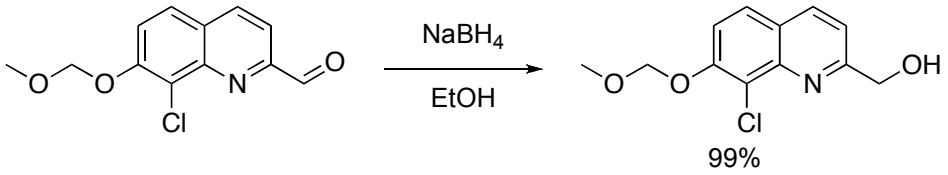
Pulse 45.0 degrees
Acq. time 1.998 sec
Width 6402.0 Hz
8 repetitions
OBSERVE H1, 400.1493068 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 24 sec



MOM-CHQ-CHO

8-Chloro-2-hydroxymethyl-7-methoxymethylquinoline (5).



$NaBH_4$ (0.0028 g, 0.074 mmol) was added to a solution of **4** (0.0397 g, 0.158 mmol) in ethanol (4 mL). The mixture was stirred for 20 min. The solvent was evaporated, and the resulting solid residue was dissolved in chloroform, then washed with water (×2) followed by brine, dried over $MgSO_4$, and concentrated to afford **5** (0.0396 g, 0.156 mmol, 99%) as a white, crystalline solid (mp 84–87 °C).

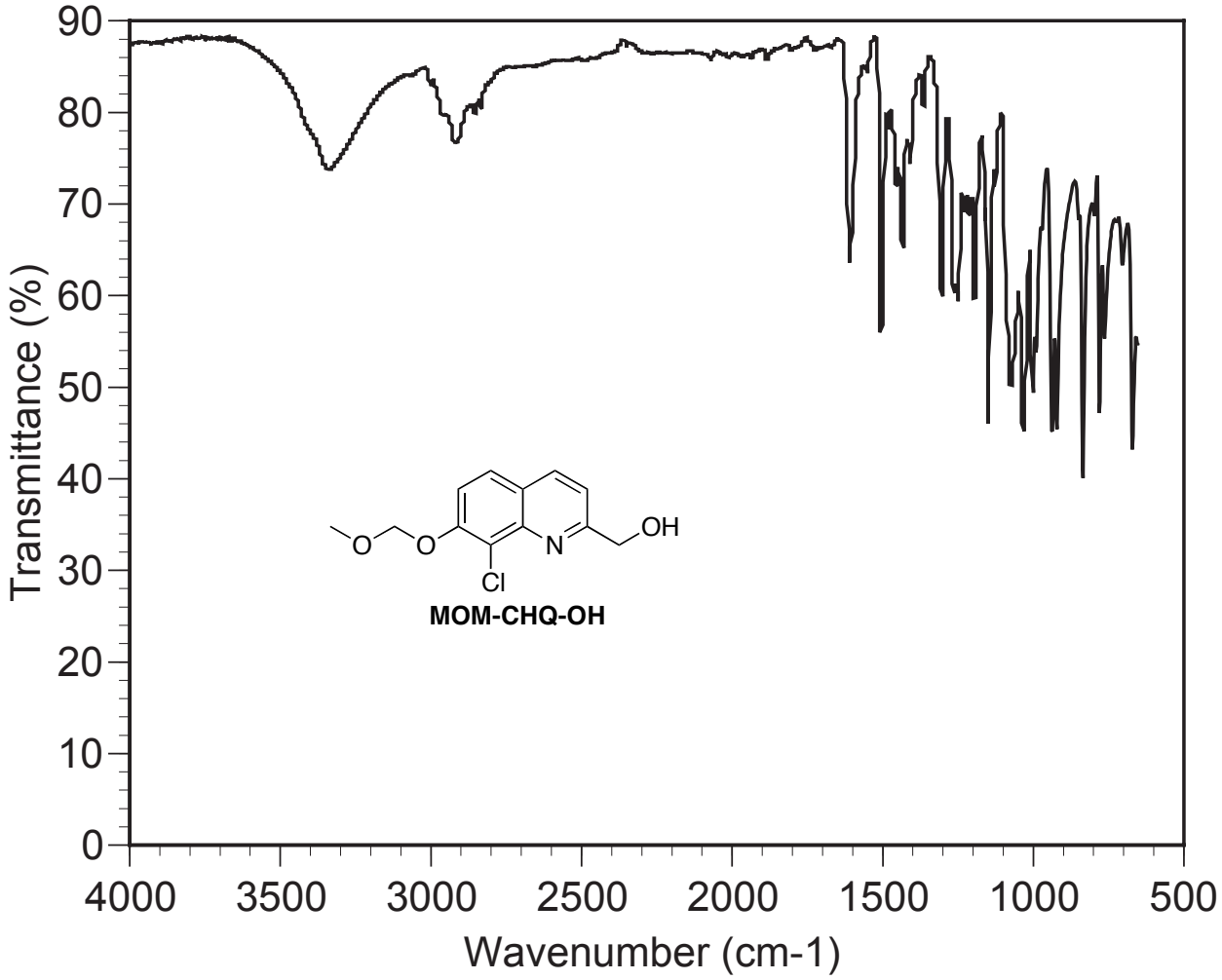
^{1}H NMR ($CDCl_3$) δ 8.09 (1H, d, J = 8.4 Hz), 7.71 (1H, d, J = 8.8 Hz), 7.52 (1H, d, J = 9.2 Hz), 7.24 (1H, d, J = 8.4 Hz), 5.41 (2H, s), 4.94 (2H, s), 3.58 (3H, s).

^{13}C NMR ($CDCl_3$) δ 160.3, 154.2, 144.1, 137.2, 127.0, 124.4, 120.0, 117.4, 117.2, 95.7, 64.4, 56.8.

FTIR (neat) 3342, 2925, 1615, 1155, 1005, 835, 781 cm^{-1}.

MS (ESI) *m/z* calcd for $(C_{12}H_{12}ClNO_3 + H)^+$ 254 (^{35}Cl) and 256 (^{37}Cl), found 254 (^{35}Cl) and 256 (^{37}Cl).

HRMS (ESI) *m/z* calcd for $(C_{12}H_{12}ClNO_3 + H)^+$ 254.0584 (^{35}Cl) and 256.0554 (^{37}Cl), found 254.0577 (^{35}Cl) and 256.0546 (^{37}Cl).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2000
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1000
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Wavenumber (cm-1)
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O
N
OH
Cl
MOM-CHQ-OH

File: Wilson105-alcohol_Proton_20080430_03

Pulse Sequence: s2pul

Solvent: cdcl3
Ambient temperature
Operator: hunter
File: Wilson105-alcohol_Proton_20080430_03
INOVA-500 "LinuxWS1"

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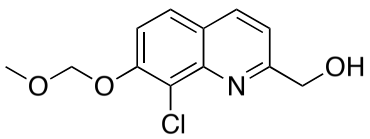
Pulse 45.0 degrees
Acq. time 1.998 sec
Width 6402.0 Hz
8 repetitions
OBSERVE H1, 400.1493134 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 24 sec



MOM-CHQ-OH

INDEX	FREQUENCY	PPM	HEIGHT
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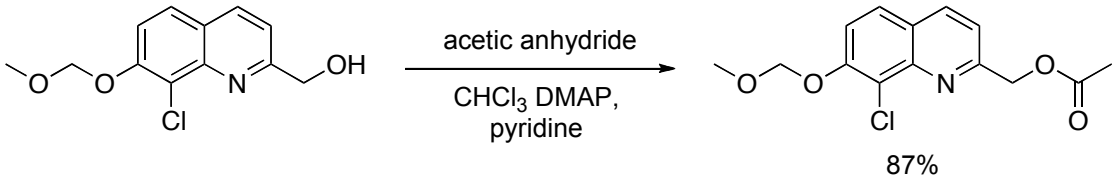
3	3089.632	7.721	17.0
4	3080.645	7.699	21.4
5	3014.999	7.535	21.1
6	3006.011	7.512	16.8
7	2908.714	7.269	9.4
8	2900.899	7.250	18.0
9	2892.694	7.229	17.7
10	2165.899	5.413	82.5
11	1977.557	4.942	64.8
12	1434.024	3.584	127.4
13	-0.029	-0.000	9.6

(8-Chloro-7-(methoxymethoxy)quinolin-2-yl)methyl acetate (6).



Under a nitrogen atmosphere, DMAP (0.0079 g), pyridine (1.0 mL), and acetic anhydride (0.121 mL, 1.28 mmol) were added to a solution of **5** (0.0630 g, 0.2483 mmol) in chloroform (4 mL). After stirring for 2 h, the solvents were evaporated and the remaining residue was purified by column chromatography (1:3 EtOAc/hexane) to afford **6** (0.0640g, 0.216 mmol, 87%) as a white solid (mp 114–117 °C).

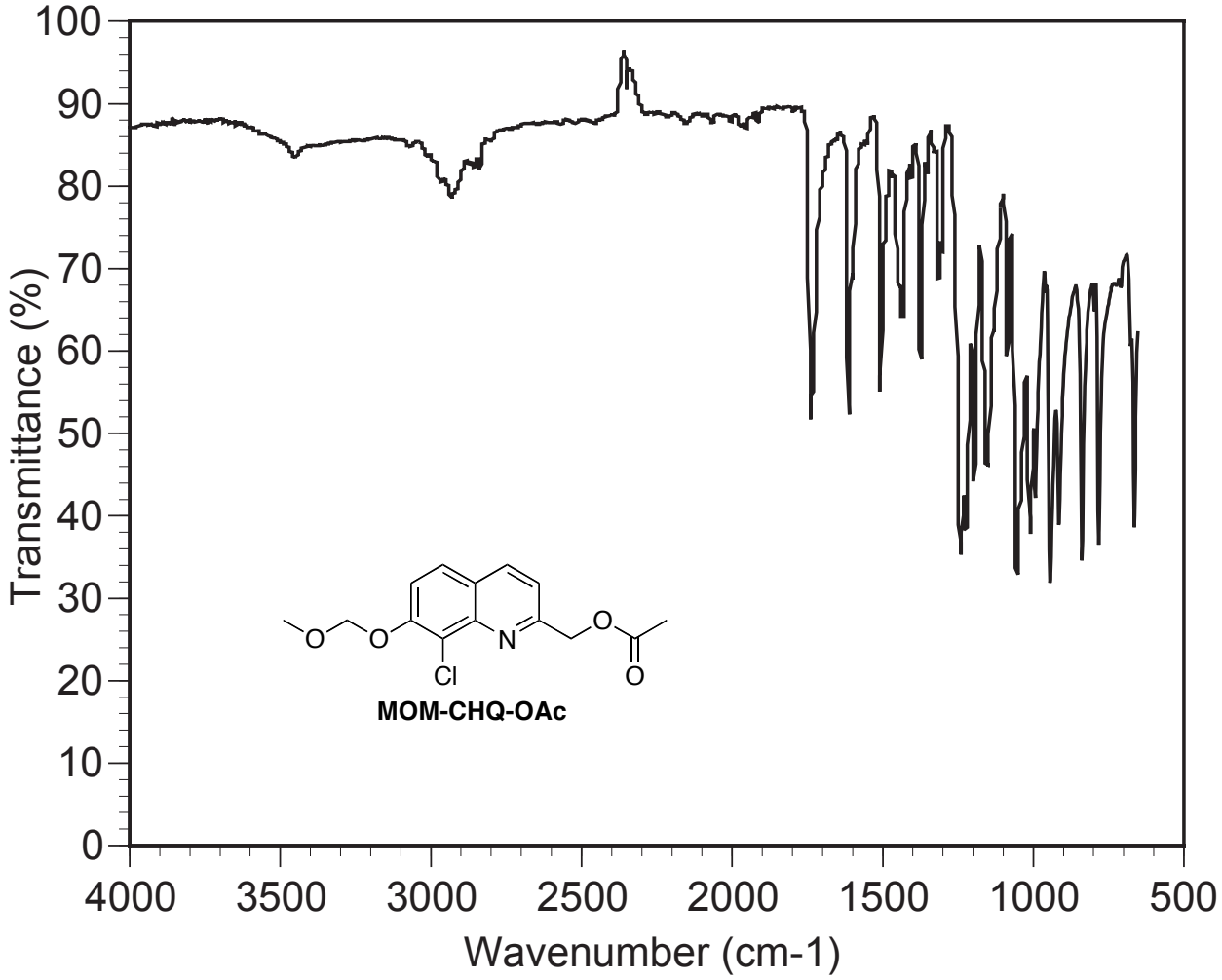
^{1}H NMR ($CDCl_3$) δ 8.14 (1H, d, J = 8.8 Hz), 7.71 (1H, d, J = 9.2 Hz), 7.54 (1H, d, J = 8.8 Hz), 7.42 (1H, d, J = 8.0), 5.47 (2H, s), 5.41 (2H, s), 3.58 (3H, s), 2.23 (3H, s).

^{13}C NMR ($CDCl_3$) δ 170.9, 157.8, 154.2, 145.2, 137.3, 127.0, 124.5, 120.4, 118.4, 117.8, 95.8, 67.6, 56.9, 21.2.

FTIR (neat) 2937, 1745, 1619, 1514, 1379, 1245, 1058, 839, 783 cm^{-1}.

MS (ESI*) m/z* calcd for $(C_{12}H_{12}ClNO_3 + H)^+$ 254 (^{35}Cl) and 256 (^{37}Cl), found 254 (^{35}Cl) and 256 (^{37}Cl).

HRMS (ESI) *m/z* calcd for $(C_{12}H_{12}ClNO_3 + H)^+$ 254.0584 (^{35}Cl) and 256.0554 (^{37}Cl), found 254.0577 (^{35}Cl) and 256.0546 (^{37}Cl).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Wavenumber (cm-1)
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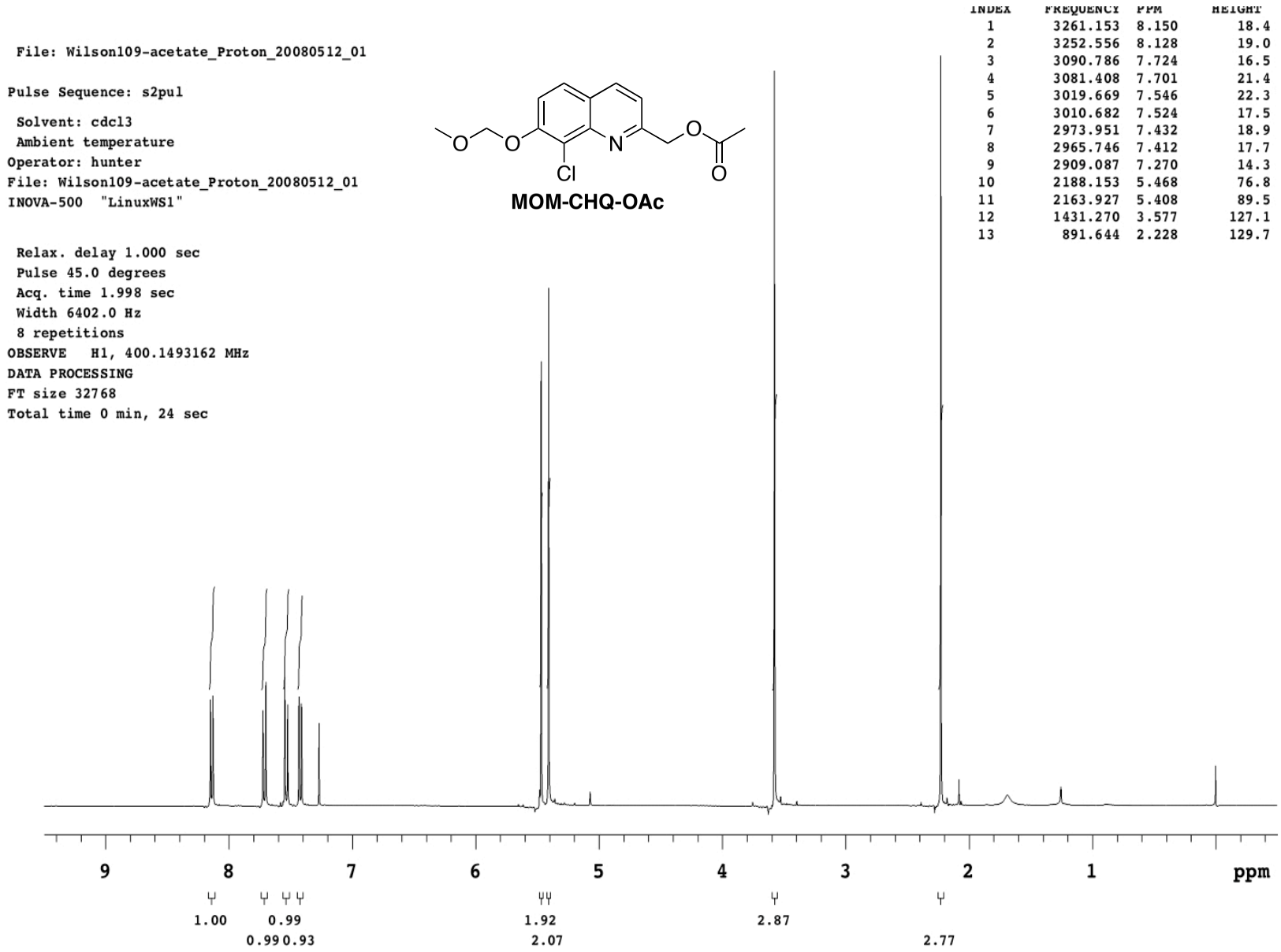
N
O
Cl
O
MOM-CHQ-OAc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Operator: hunter
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Pulse 45.0 degrees
Acq. time 1.998 sec
Width 6402.0 Hz
8 repetitions
OBSERVE H1, 400.1493162 MHz
DATA PROCESSING
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Total time 0 min, 24 sec
MOM-CHQ-OAc
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5 3019.669 7.546 22.3
6 3010.682 7.524 17.5
7 2973.951 7.432 18.9
8 2965.746 7.412 17.7
9 2909.087 7.270 14.3
10 2188.153 5.468 76.8
11 2163.927 5.408 89.5
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7
6
5
4
3
2
1
ppm
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0.99
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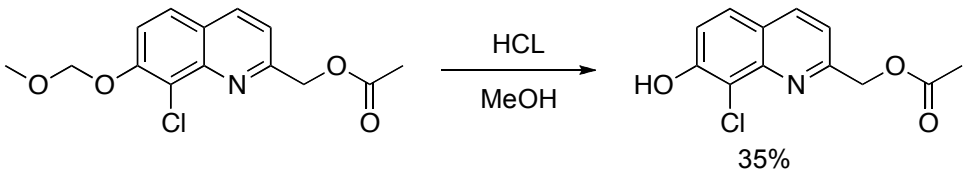
2.07
2.87
2.77

(8-Chloro-7-hydroxyquinolin-2-yl)methyl acetate (7, CHQ-OAc).



12N HCl (1 drop) was added to a solution of **6** (0.0140 g, 0.0473 mmol) in methanol (3 mL), and the mixture was stirred for 10 min. The solvent was evaporated, and the residue was purified by column chromatography (3:7 EtOAc/hexane) to afford **7, CHQ-OAc** (0.0042g, 0.017 mmol, 35%) as a white solid (mp 126–127 °C).

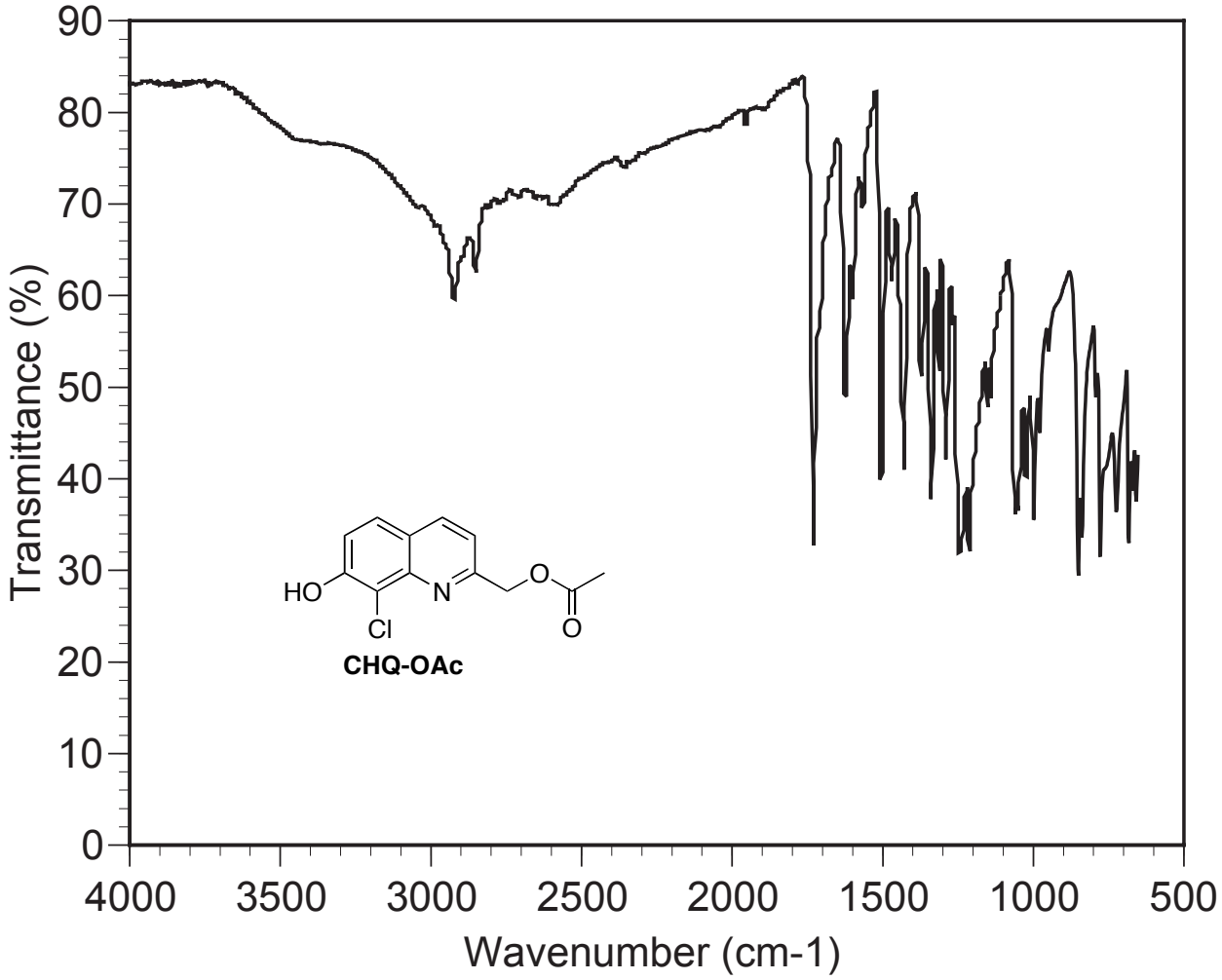
^{1}H NMR ($CDCl_3$) δ 8.13 (1H, d, J = 8.5 Hz), 7.68 (1H, d, J = 8.5 Hz), 7.40 (1H, d, J = 8.5 Hz), 7.34 (1H, d, J = 9.0 Hz), 5.46 (2H, s), 2.23 (3H, s).

^{13}C NMR ($CDCl_3$) δ 171.0, 157.7, 153.2, 144.7, 137.4 127.6 123.8, 118.2, 117.8, 115.1, 67.5, 21.2.

FTIR (neat) 2928, 1737, 1629, 1511, 1343, 1251, 850, 777 cm^{-1}.

MS (ESI) *m/z* calcd for $(C_{12}H_{10}ClNO_3 + H)^+$ 252 (^{35}Cl) and 254 (^{37}Cl), found 252 (^{35}Cl) and 254 (^{37}Cl).

HRMS (ESI) *m/z* calcd for $(C_{12}H_{10}ClNO_3 + H)^+$ 252.0427 (^{35}Cl) and 254.0398 (^{37}Cl), found 252.0420 (^{35}Cl) and 254.0390 (^{37}Cl).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Wavenumber (cm-1)
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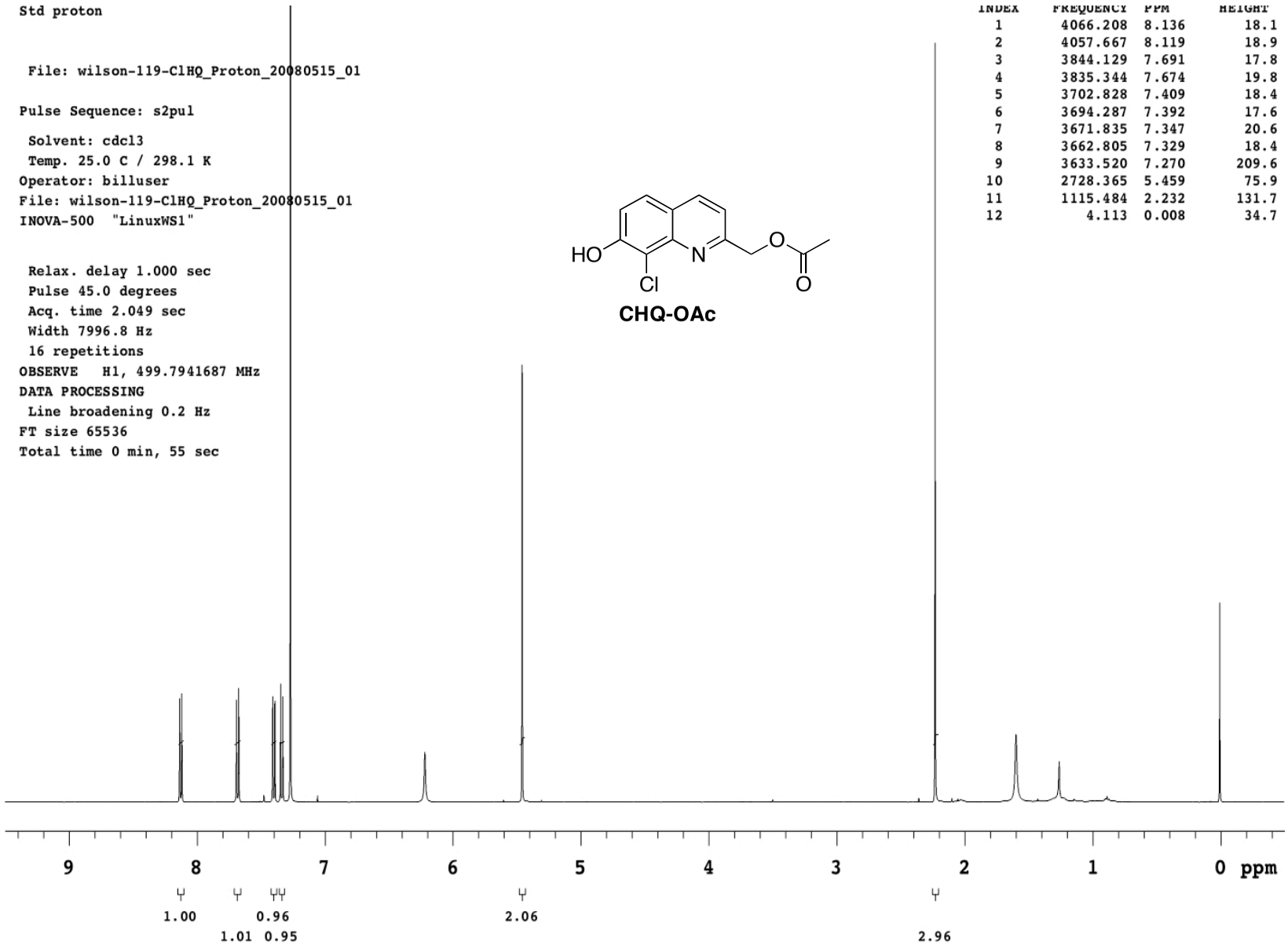
O
O
Cl
CHQ-OAc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Solvent: cdcl3
Temp. 25.0 C / 298.1 K
Operator: billuser
File: wilson-119-ClHQ_Proton_20080515_01
INOVA-500 "LinuxWS1"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.049 sec
Width 7996.8 Hz
16 repetitions
OBSERVE H1, 499.7941687 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 65536
Total time 0 min, 55 sec
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2 4057.667 8.119 18.9
3 3844.129 7.691 17.8
4 3835.344 7.674 19.8
5 3702.828 7.409 18.4
6 3694.287 7.392 17.6
7 3671.835 7.347 20.6
8 3662.805 7.329 18.4
9 3633.520 7.270 209.6
10 2728.365 5.459 75.9
11 1115.484 2.232 131.7
12 4.113 0.008 34.7
HO
N
O
Cl
O
CHQ-OAc
9
8
7
6
5
4
3
2
1
0 ppm
1.00
1.01
0.96
0.95
2.06
2.96

Determination of the Molar Extinction Coefficient (ε)

A weighed portion of CHQ-OAc was dissolved in methanol. A measured aliquot of this solution was withdrawn and placed in KMOPS buffer (3.0 mL) and mixed thoroughly to generate a 100 μM solution of CHQ-OAc. The absorbance A of this solution at λ_{max} = 370 nm was measured. This method was repeated twice with different masses of CHQ-OAc. The three absorbencies were averaged and the molar extinction coefficient at λ_{max}= 370 nm was calculated to be 2,800 $M^{-1} \cdot cm^{-1}$ using the equation A = εlc, where A is the absorbance, l is the pathlength of the cuvette, and c is the concentration of the solution.

Determination of the Uncaging Quantum Efficiency (Q_u)

The quantum efficiency was calculated using the equation $Q_u = (I \cdot \sigma t_{90\%})^{-1}$, where I is the irradiation intensity in $ein \cdot cm^{-2}$, σ is the decadic extinction coefficient (1,000ε) and $t_{90\%}$ is the time in seconds required for the conversion of 90% of the starting material to product.[46] To find $t_{90\%}$, a solution of CHQ-OAc in KMOPS was prepared and placed in a cuvette along with a small stir bar. While stirring, the solution was irradiated with UV light from a mercury lamp (Spectroline SB-100P, Spectronics Corporation) equipped with two glass filters (CS0-52, CS7-60, Ace Glass) so that the wavelength was restricted to 365 ± 15 nm. Periodically, 20 μL aliquots were removed and analyzed by HPLC; the time points collected were as follows: 0, 5, 10, 20, 30, 60, 90, and 120 s. The concentrations of the solution at the time of removal for each of the samples were calculated by correlating HPLC peak area to an external standard. Percent CHQ-OAc remaining was plotted verses time of photolysis (Figure 8). A simple single exponential decay curve provided the best fit for the data and was used to extrapolate $t_{90\%}$. The lamp's UV intensity I was measured using potassium ferrioxalate actinometry. Initially, 6 mM potassium ferrioxalate solution (3 mL) was irradiated with the mercury lamp for 60 s. A portion

of this solution (2mL) was combined with aqueous buffer (3 mL), 0.1% phenanthroline solution (3 mL), and 2M KF solution (1 mL) in a 25 mL volumetric flask. Deionized water was added to generate a 25 mL solution. A blank solution was also prepared using the same method, but the potassium ferrioxalate used in the blank was not irradiated. Both solutions were allowed to sit for one hour and the blank was then used as a baseline against which the absorbance of the irradiated solution was measured at 510 nm. The following equation was used to calculate lamp intensity:

$$I = \frac{V_3 \Delta D_{510}}{1000 \varepsilon_{510} V_2 \phi_{Fe} t}$$

where V_3 is the volume of dilution (25 mL), V_2 is the volume of irradiated potassium ferrioxalate solution taken for analysis (2 mL), ΔD_{510} is the absorption of the solution at 510 nm, ε_{510} is the actinometry extinction coefficient ($1.11 \cdot 10^4$), ϕ_{Fe}^{2+} is the quantum yield for production of ferrous ions from potassium ferrioxalate at 365 nm, and t represents the time of irradiation. The ΔD_{510} value used for calculations is the average of two measurements taken before and after irradiation of CHQ-OAc. Compilation of the measurements yielded an uncaging quantum efficiency Q_u of 0.10.

Determination of Two-photon Action Cross-Sections (δ_u)

A portion of CHQ-OAc was dissolved in KMOPS buffer and the concentration of the solution was found using UV-Vis absorption in conjunction with Beer's law. 25 μL aliquots of this solution were placed in a microcuvette (10×1×1 mm illuminated dimensions, 25 μL) and irradiated with a fs-pulsed and mode-locked Ti:Sapphire laser (Chameleon Ultra II, Coherent) with 740 nm light at an average power of 350 mW. Three samples were irradiated for each of

the following time periods: 0, 5, 10, 20, and 40 min. The samples were compiled and analyzed by HPLC. Standard curves were used to find concentrations of CHQ-OAc and a simple decay curve was applied to the data. A solution of fluorescein at pH 9.0 was prepared to act as a standard for CHQ-OAc because of its well-characterized 2PE cross-section (δ_{aF} = 30 GM at 740 nm) and quantum yield (Q_{F2} = 0.9). UV-Vis absorption spectroscopy was used to correlate absorption at 488 nm to precise concentration. 25 μL aliquots of fluorescein solution were placed in the microcuvette and irradiated by the laser under the same conditions used for the CHQ-OAc solution. The fluorescence of the solution was measured with a radiometer before and after the CHQ-OAc samples were irradiated and the two values were averaged. The following equation was used to calculate the two-photon action cross-section for CHQ-OAc:

$$\delta_u = \frac{N_p \phi Q_{F2} \delta_{aF} C_F}{< F(t) > C_s}$$

where N_p is the number of product molecules formed per second (determined by HPLC), ϕ is the collection efficiency of the detector (SED033 on an IL-1700, International Light) used to measure the fluorescence of fluorescein passing through the cuvette window and through a 535/545 nm bandpass filter at a right angle to the laser's beam, C_F is the concentration of fluorescein (M), $<F(t)>$ is the time averaged fluorescent photon flux (photons/s) of fluorescein and C_s is the initial concentration of the caged compound (M).

This method was repeated for determination of the two-photon action cross-sections of 2-XAA and 4-XAA with the following exceptions: (1) the wavelength of irradiation was set at 700 nm; notably, at 700 nm, the 2PE cross-section for fluorescein δ_{aF} = 19 GM; (2) the power was

set at 250 mW; and (3) the 2-XAA and 4-XAA samples were irradiated in 100 mM phosphate buffer calibrated to pH 7.4 rather than KMOPS.

Creation of Standard Curves

A 110 μM solution of 2-XAA in phosphate buffer was created. Serial dilutions were performed and three aliquots of each concentration were analyzed on the HPLC. A line of best fit was applied to correlate peak area to concentration. The process was repeated for 4-XAA, 2-MeX and 4-MeX. For 2-MeX and 4-MeX, ethanol was added to the phosphate buffer solution to alleviate solubility issues. Concentrations above 20 μM deviated from linearity and were not used for 2-MeX and 4-MeX standard curves.

A standard curve for CHQ-OAc was made in KMOPS buffer using the same method.

Determination of the Dark Hydrolysis Rate (τ)

Three 100 μM solutions of CHQ-OAc in KMOPS were created and stored in the dark. 20 μL aliquots were removed periodically from each solution and analyzed by HPLC. An external standard was used to convert peak area to solution concentrations. The percents remaining for each time point for each solution were averaged and plotted versus time. A simple single exponential decay curve provided the best fit. The time constant for dark hydrolysis τ was determined to be 49 h.

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