Uncaging of Volatile Honey Bee Pheromones from Water-soluble Precursors

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List of Abbreviations

Boc	*tert*-Butyloxycarbonyl
NPPOC	2-(2-nitrophenyl)propyloxycarbonyl
Nvoc	6-Nitroveratryloxycarbonyl
PPG	Photo protecting group
DCM	Dichloromethane
THF	Tetrahydrofuran
TMS	Trimethylsilyl, tetramethylsilane
TFE	2,2,2-Trifluoroethanol
PPh_3	Triphenylphosphine
DEAD	Diethyl azodiarboxylate
DMAP	4-Dimethylaminopyridine
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
Et_3N	Triethylamine
EtOH	Ethanol
MeOH	Methanol
IPNBSH	*N*-isopropylidene-*N'*-2-nitrobenzenesulfonyl hydrazine
NB	o-Nitrobenzyl
NBSH	2-Nitrobenzenesulfonyl hydrazide
S_N2	Bimolecular nucleophilic substitution
IR	Infrared
MS	Mass spectrometry
TLC	Thin layer chromatography
UV	Ultraviolet
NMR	Nuclear magnetic resonance
DEPT	Distortionless enhancement by polarisation transfer
COSY	Correlation spectroscopy
HMQC	Heteronuclear multiple quantum coherance
HMBC	Heteronuclear multiple bond

	coherance
s	Singlet
d	Doublet
dd	Doublet of doublets
t	Triplet
q	Quartet
m	Multiplet
bs	Broad singlet

Abstract

The potential of using hydrazones as water-labile protecting groups on sulfonyl hydrazides has been explored. These sulfonyl hydrazides will eventually become water-soluble precursors of lipids and small hydrophobic molecules. Several hydrazinoacetates were synthesized by transesterification of commercial ethyl hydrazinoacetate. Ethyl and *n*-butyl hydrazinoacetate were then reacted with the photo protecting group NPPOC to form 'caged' molecules, followed by the reaction with a sulfonyl chloride such as *para*-nitrobenzenesulfonyl chloride to form the sulfonyl hydrazides. The synthesis of sulfonyl hydrazides was monitored by NMR spectroscopy, and then the products were irradiated with light to trigger the cleavage of the caging groups, releasing nitrogen and a sulfinate ion. The uncaging process was monitor by NMR spectroscopy, but the product mixtures were too complex to show the formation of the desired acetate esters.

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Declaration

No portion of the work referred to in the dissertation has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright statement

Acknowledgements

At this very point of finishing my thesis, I would like to express my sincere thanks to all those who have helped me in my project.

First of all, I would like to extend my sincere gratitude to my supervisor -Dr. Simon Webb, for his instructive advice and always providing me enlightenment.

I'd like to express my appreciation to Rebecca Booth and Wen Li, for kindly lending me hands in my laboratory works. I really feel delight working with them.

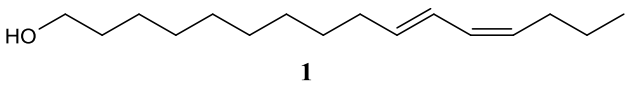
I would also like to thank all my friends in the Webb Group and in the chemistry building, for their patience in answering my queries and supporting me in my work and life. I'll always cherish the memory with them.

1. Introduction

1.1 Introduction of insect pheromones

Pheromones are signal-carrying chemicals, some of which are used during insect intraspecies' communication. They can be divided into two types by their effect either upon self-behavior or upon other insects, as prime pheromones or releaser pheromones. There are three types of releaser pheromones: sex pheromones, alarm pheromones and recruiting pheromones. These pheromones directly influence the living of insects.

The complex pheromone system has been studied for a long peried of time, and the structure of pheromone compounds has been analyzed by many scientists over the years. In 1959, an active pheromone named bombykol was extracted from the silkworm moth, *Bombyx mori*, by Butenandt and co-workers. [1] Bombykol was proved to consist of *trans*-10-*cis*-12-hexadecadien-1-ol **1** by a series of assays (Fig. 1.1). [2,3,4,5] Bossert and co-workers also reported that the synthetic bombykol could conduct a high positive effect to attract female insects in low concentration, which showed that cheomoreception of sexual odorants by male insects is highly selective and efficient.[1]



1

Fig. 1.1 *Trans*-10-*cis*-12-hexadecadien-1-ol.

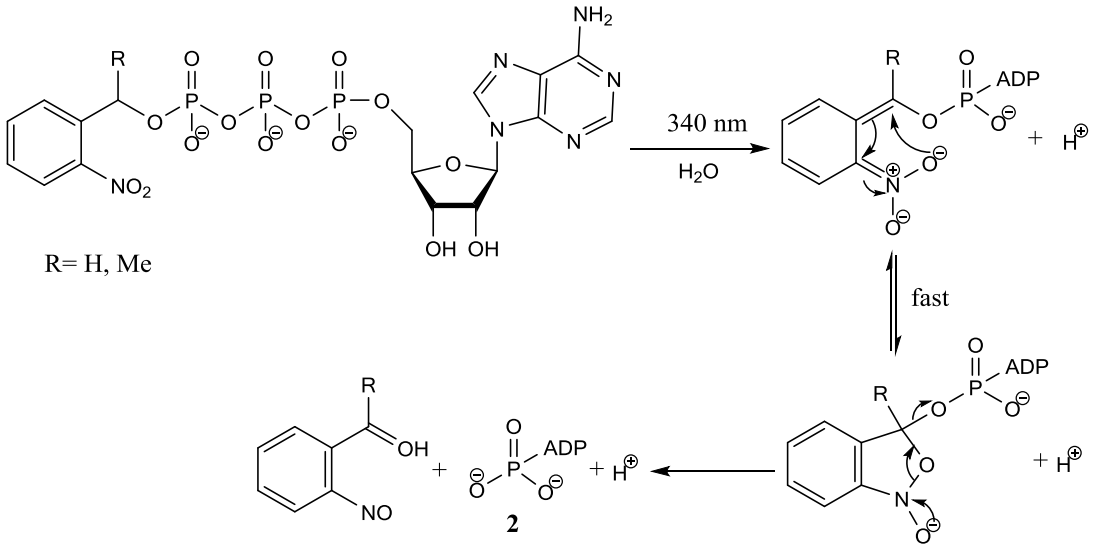
Esters are also known to be potent insect pheromones. Butyl acetate, *iso*-pentyl acetate and *n*-pentyl acetate are alarm pheromones for the honey bee. [6] Acetates are of particular interest because the acetate group can be 'caged' by a photocleavable group. The implication of this caging would be the ability to release a pheromone using light

to cleave a labile bond, leaving an unactivated C-H bond behind. Such functionalised acetates are available through transesterification. In this project, the synthesis of these honey bee pheromones using the photo protecting group will be discussed.

1.2 A backgrounds to 'caged' compounds

1.2.1 The concept of 'caged' compounds

In 1978, Kaplan and his colleagues utilized a derivative of adenosine triphosphate (ATP), 'caged' ATP, to achieve the control of metabolism of ATP **2** by ATPases in a living environment (Scheme 1.1). [7]



Scheme 1.1 The first caged molecule made by Kaplan and co-workers and the mechanism for removing the photo-protecting group. [7-11]

Since then, the term 'caged compounds' is used to describe synthetic molecules of which the biological activities are masked by the covalent attachment of photo-removable protecting groups, and can be unmarked by photolytic reactions. [8-12] 'Caged' compounds are important in the study of intracellular biological processes

because their reactivity can be controlled inside cells by 'uncaging' process which is triggered by light. Since most of the cells and organisms are transparent and light accessible, the uncaging process can be straight forwardly achieved.

1.2.2 The principles of selecting the caging groups

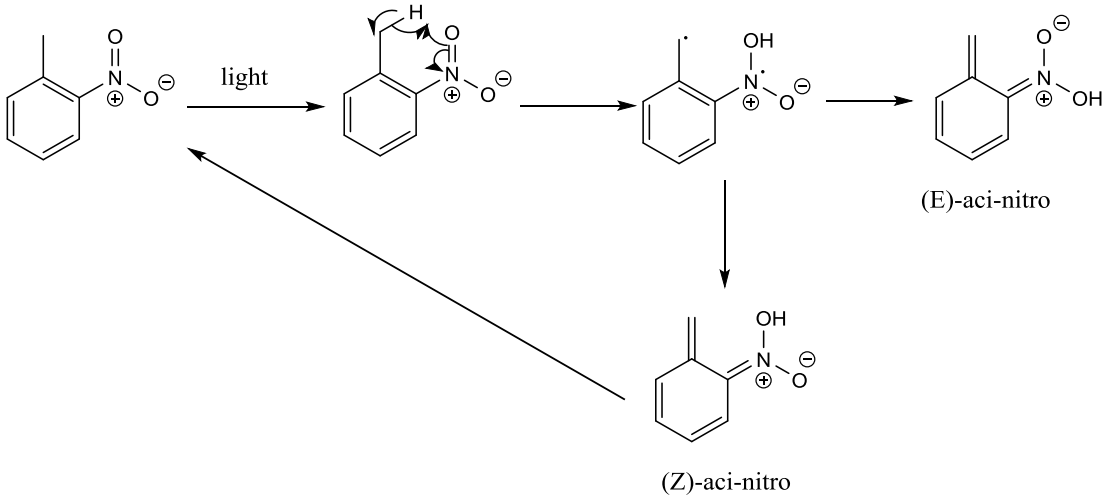
Generally speaking, good caging groups should satisfy the following requirements: [13] (1) The caging groups are soluble and stable to physiological conditions. (2) The uncaging process should be fast and follow an expected reaction pathway, and can be triggered by wavelengths where the sample is transparent and other unintended reactions are avoided. (3) The caging groups should be compatible with other leaving groups, and the by-product from the uncaging process should be non-toxic. (4) The caging groups should have a high molar extinction coefficient ε to allow efficient photoactivation. (5) If possible the process of uncaging can be monitored.

1.3 Previous studies on nitrophenethyl and nitrobenzyl groups

1.3.1 Introduction of nitrophenethyl and nitrobenzyl groups

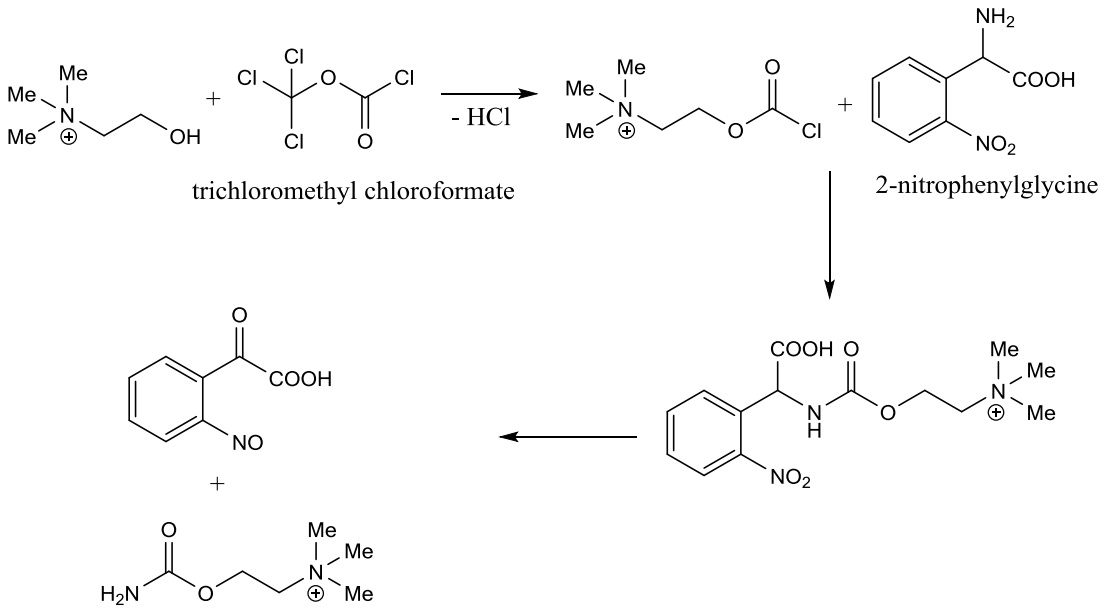
By far the most commonly used caging groups are nitrophenethyl, nitrobenzyl compounds along with their dimethoxy derivatives. [14] Before proposed by Patchomik and his co-workers in 1970 as photo-protecting groups, [15] *o*-nitrobenzyl photochemistry had been explored. [16] The primary photoreaction of *o*-alkylnitroarenes is hydrogen transfer from the *o*-alkyl substituent to the nitro group. The derivatives of nitrophenethyl and nitrobenzyl compounds with a leaving group at the benzylic position can be uncaged upon irradiation, thus different substituents at the benzylic position were studied to increase their performance as caging groups. Gilch and co-workers summarized the phototautomerization of *o*-nitrobenzyl in THF

in their work using femtosecond transient stimulated Raman spectroscopy (Scheme 1.2). [17]



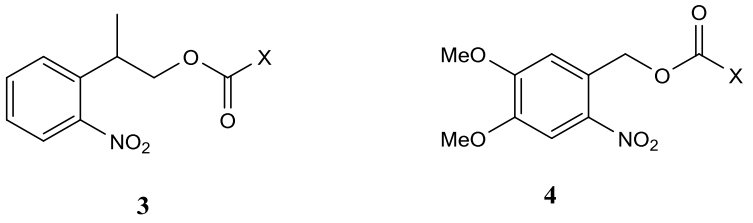
Scheme 1.2 The mechanism for the phototautomerization of o-nitrobenzyl in THF which was reported by Gilch and co-workers. [17]

In 1989, Hess and his co-workers noted that the uncaging rate can be increased by adding a carboxylate to the carbon in benzylic carbon of *o*-nitrobenzyl (Scheme 1.3).[18] In their work, *N*-(*α*-carboxy-2-nitrobenzyl)carbamoylcholine was synthesized from (2-nitrophenyl)glycine, and the photolysis rates for the two compounds were compared. As a result, they found *N*-(*α*-carboxy-2-nitrobenzyl)carbamoylcholine had faster photolysis rate. Thus the substituents of *o*-nitrobenzyl have the potential to influence the photolysis rate.



Scheme 1.3 Adding a carboxylate to the carbon in benzylic group of o-nitrobenzyl will increase the uncaging rate. [18]

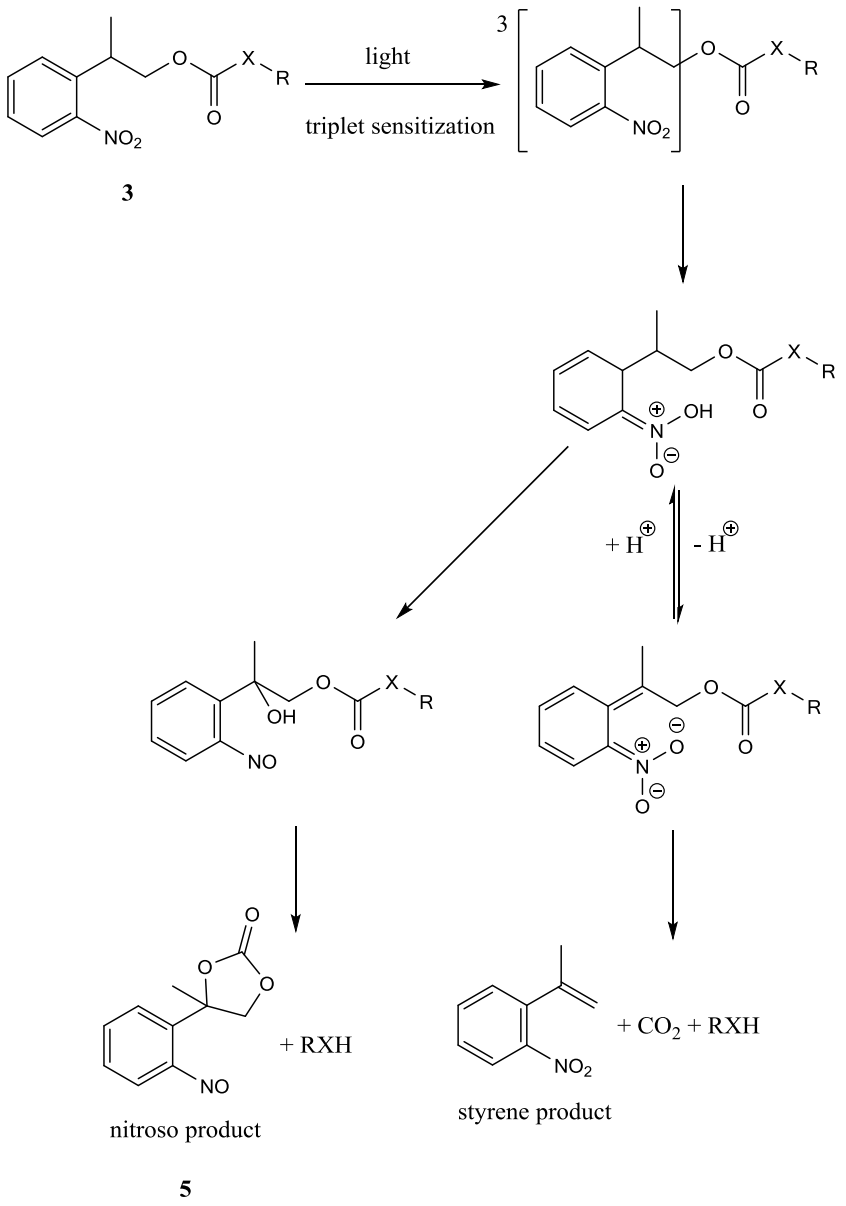
In the 1990s, Hasan and co-workers reported an α-methylated analogue of 1-(2-nitrophenyl)ethyloxycarbonyl as NPPOC (Figure 1.2, **3**), [19] which is currently widely utilized in synthetic chemistry as a caging group. Despite 6-nitroveratryloxycarbonyl (NVOC) (Figure 1.2, **4**) being a superior protecting group to protect the amino function, it is not ideal because of low photosensitivity and deprotecting rate. [20]



3 **4**

Fig. 1.2 Structures of NPPOC and NVOC protecting groups, which are widely used as caging groups.

NPPOC has a different uncaging mechanism to NVOC in which an alkene byproduct is produced. The photocleavage mechanism of NPPOC has been discussed by Wöll and co-workers, [21] and they proposed that there was β-elimination and formation of nitroso byproduct **5**. Nitroso product is highly reactive but can be suppressed using suitable reaction conditions (Scheme 1.4).[22] Since NPPOC has better quantitative yields and photolytic efficiency, thus NPPOC will be selected as caging group instead of NVOC or Boc in this project.



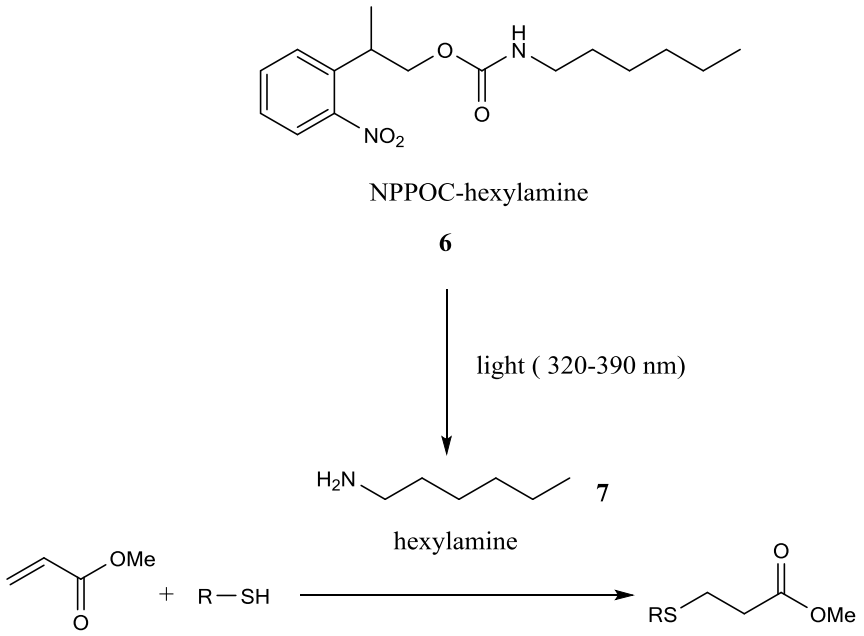
Scheme 1.4 The photocleavage mechanism of NPPOC which contains *β*-elimination and formation of nitroso byproduct.[22]

1.3.2 Utilization of 'caged' compounds with NPPOC

As discussed above, NPPOC has attracted the interest of many groups of researchers, especially in protecting with amines. By using NPPOC as caging group to block and

deprotect the key functional groups of target compounds under chemically selective condition, some special designs of control mechanism can be realized.

In 2013, Xi and co-workers proposed a new strategy to trigger the thiol-Michael 'click' by light with the utilization of NPPOC as photo-protecting group to cage a primary amine. The NPPOC-hexylamine **6** was formed by amidation and the decomposition was conducted under 320-390nm UV light irradiation. By triggering the cleavage of NPPOC-hexylamine with UV light, the release of hexylamine **7** can be turned-on, which catalyses a thiol-Michael addition reaction (Scheme 1.5). [23]



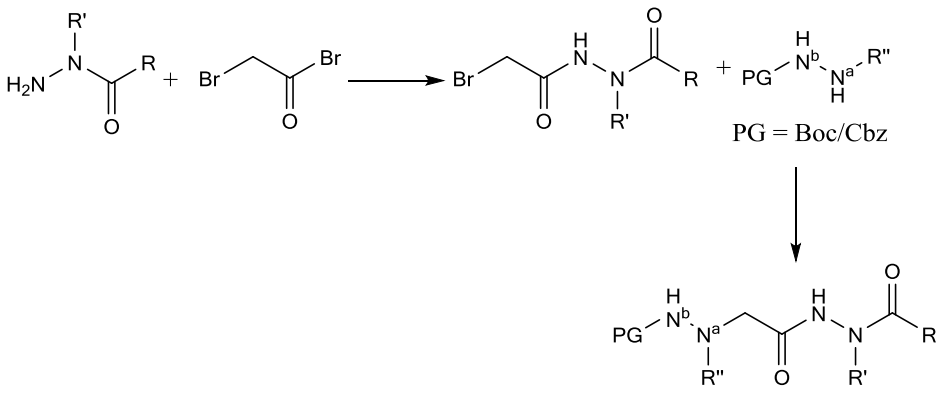
Scheme 1.5 The design of photo-triggered thiol-Michael addtion reaction controlled with NPPOC-hexylamine. [23]

1.4 Hydrazines

Hydrazines have been widely exploited in the pharmaceutical and organic chemistry fields as precursors in organic synthesis because of their significant biological

activities. Depending on the substituent, the hydrazine derivatives may be active against diseases such as Parkinson's disease, tuberculosis and hypertension,[24,25] or may be used in peptidomimetics for treating hepatitis, AIDS and SARS.[26,27,28] In recent years, various synthetic routes for substituted hydrazines have been developed in order to meet the demand for hydrazine derivatives by the pharmaceutical and agrochemical industries.

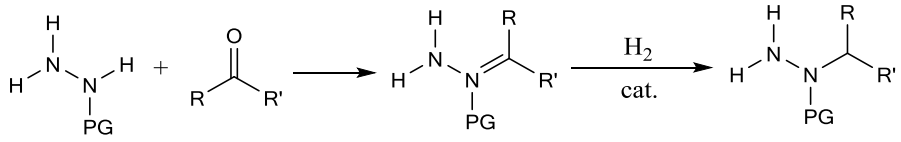
In 1999, Cheguillaume and co-workers described a synthesis of aza-peptide mimetics in which hydrazinoglycines were used as monomers in the aza-peptide chain. They pointed out that the two nitrogens of monosubstituted hydrazines have balanced nucleophilicities because of electronic effects of the substituent as well as steric effects. The N^b of the hydrazines was protected to avoid alkylation there, leading to the N^a-substituted product (Scheme 1.6). Then by caging and uncaging the N^a of hydrazines, the expected elongation could be achieved on the C or N terminus. [29]



Scheme 1.6 Alkylation of hydrazides used in the synthesis of aza-peptide mimetics.[29]

Another popular synthetic route for hydrazines is from the amination product of ketones or aldehydes. [30-35] The first step was the condensation of an aldehyde or ketone with hydrazine to form the hydrazone, and which was then reduced with H_2

and a catalyst such as palladium to produce the hydrazine (Scheme 1.7). [31] In the condensation step, the yields of the products depended on the length of the aldehydes or ketones due to the influence of sterics and solubility. [31]



Scheme 1.7 Condensation of aldehydes/ketones and hydrazides to form the hydrazones which are then reduced to put out hydrazines.

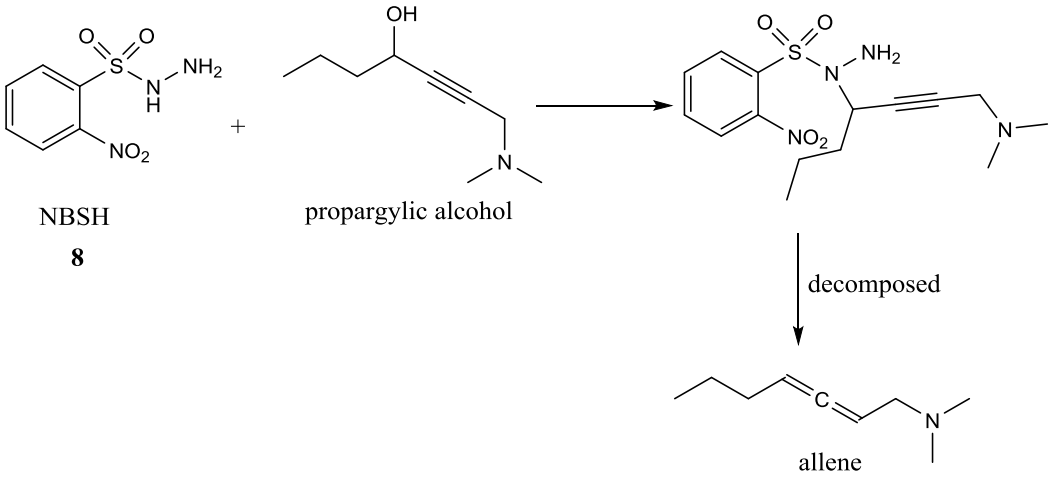
1.5 Previous studies for diazene chemistry

1.5.1 Previous Synthesis of NBSH and IPNBSH

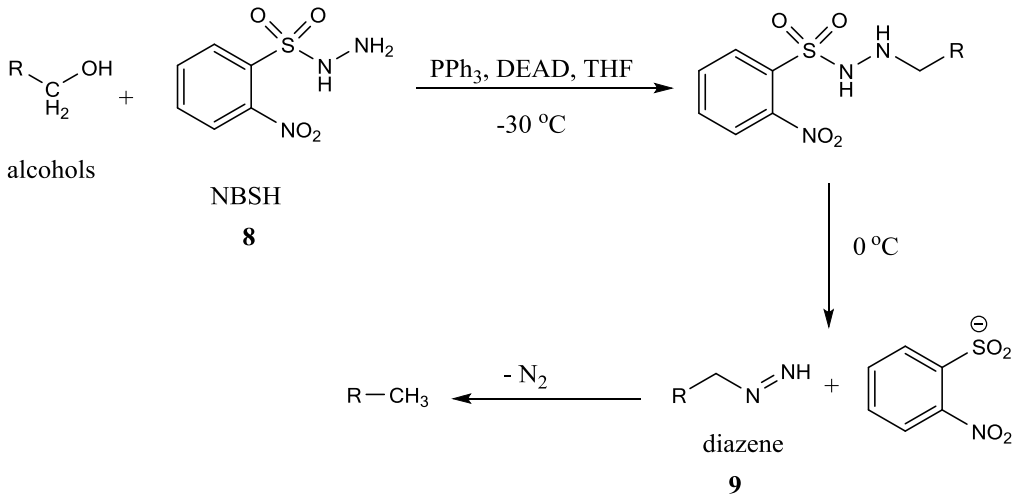
Diazenes, which can be created from hydrazines, is widely used to remove hydrophilic functional groups from organic molecules by releasing nitrogen. Compared to other methods for the removal of polar groups, diazene transformation has been identified as the most remarkable and effective method because the loss of nitrogen has a faster rate than the loss of carbon dioxide in protodecarboxylation. Such elimination can be concerted, in contrast with decarboxylation and diazotisation whose intermediates could generate unexpected polar groups and byproducts.

In 1996, Myers and co-workers reported a synthesis of allenes from propargylic alcohols with the reagent of *o*-nitrobenzenesulfonylhydrazide (NBSH), which involved Mitsunobu displacement of an alcohol with NBSH (Scheme 1.8) and formation of a monoalkyl diazene **9** as intermediate (Scheme 1.9). [36] They found that the decomposition rate of NBSH was influenced by the temperature as well as the polarity of the solvent, i.e. the rate of decomposition was faster in polar solvents such

as water and methanol, than in nonpolar organic solvents like tetrahydrofuran (THF) at low temperature. Thus, in order to avoid the decomposition of NBSH, the synthesis process was in THF as solvent and ethyl acetate for the workup. In the elimination process, *o*-nitrobenzenesulfinic acid was produced *via* a free-radical mechanism or a sigmatropic rearrangement. [37]

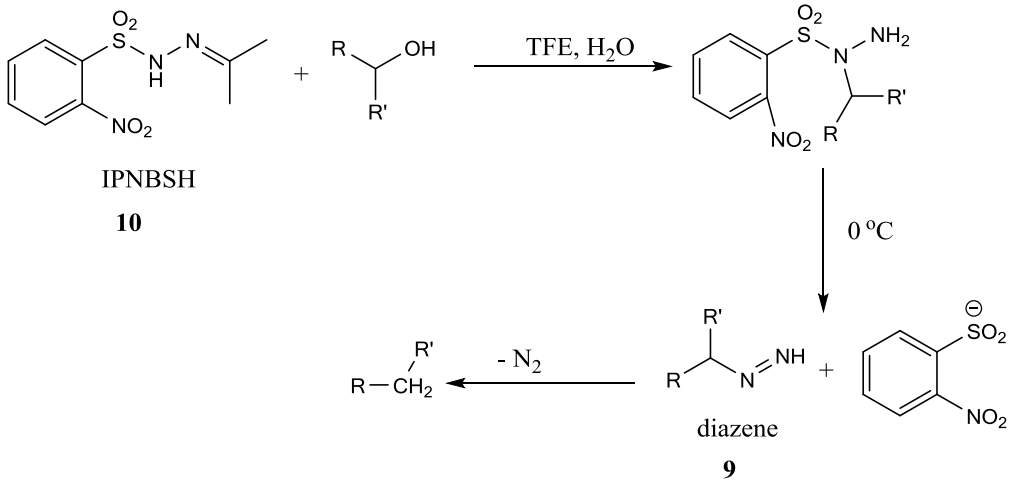


Scheme 1.8 The synthesis of allenes from propargylic alcohols via a Mitsunobu reaction. [36]



Scheme 1.9 General process of decomposition of NBSH. [36]

By dissolving NBSH in acetone, Myers and co-workers obtained another reagent to be used in the Mitsunobu displacement of alcohols, *N*-isopropylidene-*N'*-2-nitrobenzenesulfonyl hydrazine (IPNBSH) **10** (Scheme 1.10). Comparing to NBSH, IPNBSH is more stable in greater range of temperatures, and gives access to wider selection of reaction conditions such as concentration of reactants. [38] IPNBSH is also an important intermediate in Mitsunobu reactions, allylation, asymmetric hydrocyanation and Mannich reactions as both a nucleophilic and electrophilic reactant. [39-43]



Scheme 1.10 General process of decomposition of IPNBSH.[39]

1.5.2 General study of sulfonyl hydrazides

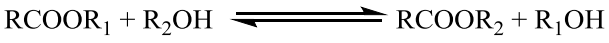
This decomposition of the sulfonyl hydrazides is of interest to the Webb group due to its potential to be applied in biological studies. Substituted hydrazides can have high polarity and be unable to cross the cell membrane, while the hydrophobic decomposition product would successfully enter the cell.

1.6 Transesterification

1.6.1 Concept of transesterification

As one of the important organic reactions, transesterification has been widely used as a convenient method to produce esters. Transesterification has plenty of advantages over esterification. First of all, since some of the carboxylic acids are hardly dissolved in non-polar solvents, in contrast with most of the esters, many reactions can not be carried out by general esterification procedures. Furthermore, transesterification allows commercially available esters such as methyl esters and ethyl esters to be transformed into a variety of esters with convenient processes. Thus, transesterification has a wide utilization not only in a laboratory setting but also in industry.[44]

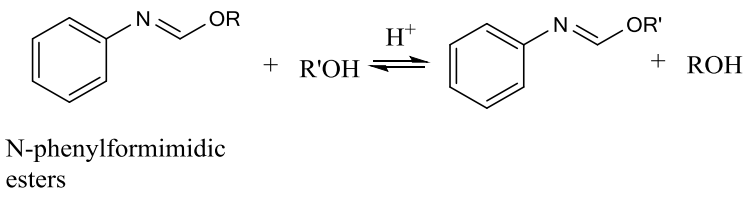
The mechanism of transesterification is the interchange of the alkoxy moiety between the parent ester and the product ester. Since esterification is an equilibrium process, transesterification can be carried out by simply mixing the alcohol and the ester (Scheme 1.11).



Scheme 1.11 Transesterification process.

1.6.2 Catalysts for transesterification

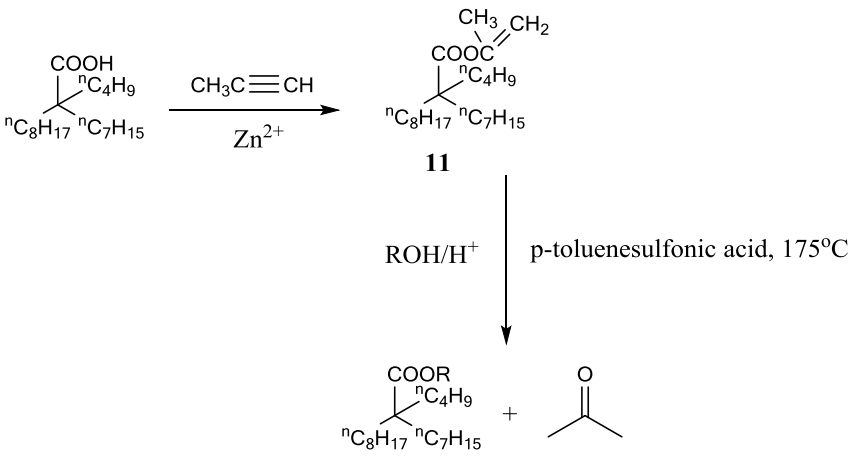
Catalysts for transesterification have been designed to drive the reaction with high selectivity and efficiency since 1950s. In 1955, Royston and co-workers reported an efficient synthesis of N-phenylformimidic esters by transesterification (Scheme 1.12).[45]



N-phenylformimidic esters

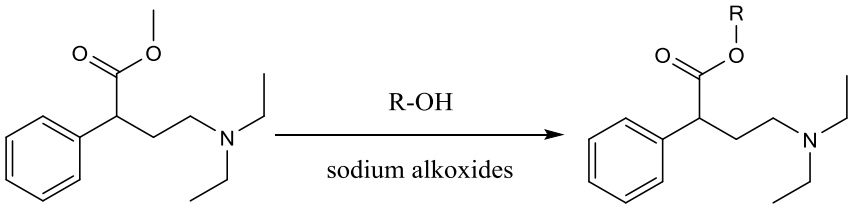
Scheme 1.12 The synthesis of *N*-phenylformimidic esters through transesterification.[45]

They noted that the best procedure to obtain complete transformation of cyclohexyl alcohol and other high-boiling secondary alcohols was adding acid catalysts and removing the formed alcohol. The acid catalysts, including sulfuric, sulfonic, hydrochloric, and phosphoric acids, are also considered to be advantageous for transesterification with primary and the low-boiling secondary alcohols. High steric demand alcohols such as *sec*-butyl alcohol can also be transeterified in the presence of acid catalysts. In 1972, Rothman and co-workers carried out a successful transesterification of isopropenyl 2-butyl-2-heptyldecanoate **11**, which was highly steric hindered, catalyzed by *p*-toluenesulfonic acid. The reaction resulted in 75-95% yields (Scheme 1.13). [46]



Scheme 1.13 The transesterification of isopropenyl 2-butyl-2-heptyldecanoate with acid catalyst.[46]

Besides acid catalysts, base catalysts are also efficient in transesterification. The most widely used base catalysts are sodium and potassium alkoxides. Billman noted in his work that sodium alkoxides performed the best for the transesterification from the methyl ester to γ-(diethylamino)-α-phenylbutyric acids (Scheme1.14). [47] Other common base-catalysts including DBU are also widely used in transesterification.



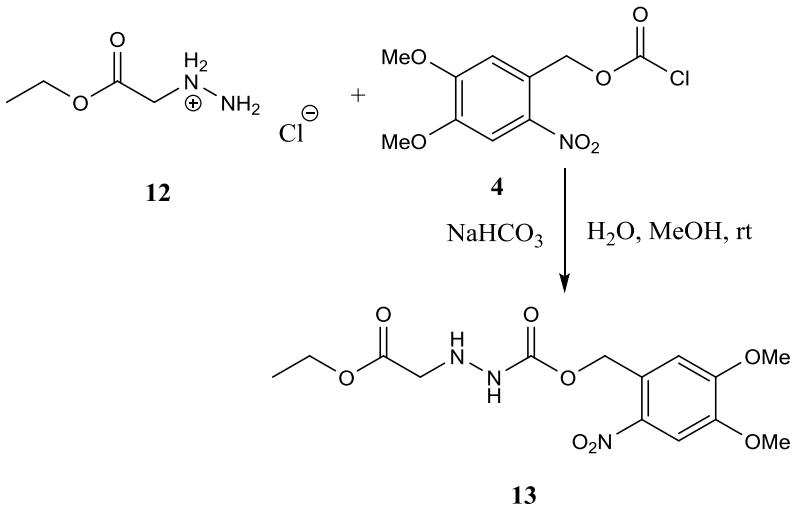
Scheme 1.14 Synthesis of γ-(diethylamino)-α-phenylbutyrate by transesterification. [47]

1.7 Previous Work in the Webb Research Group

1.7.1 Synthesis of NVOC-protected hydrazine

The Webb group has been interested in combining 'caging' chemistry and diazene chemistry to allow photo-release of hydrophobes. As part of these studies they have looked at generating ethyl acetate from commercially available ethyl hydrazinoacetate.

Commercially available ethyl hydrazinoacetate hydrochloride **12** was used to react with NVOC-chloride **4** in sodium bicarbonate at room temperature (Scheme 1.15). [48]



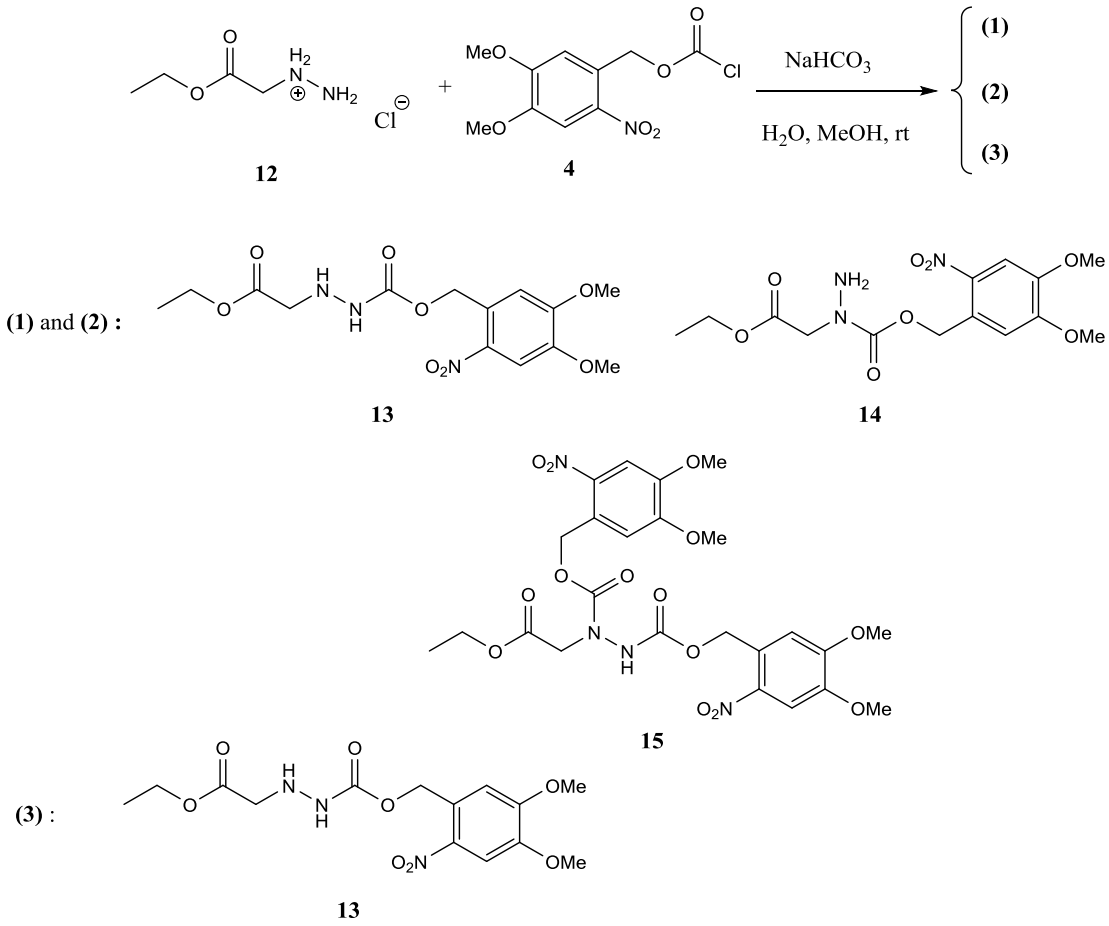
Scheme 1.15 Synthesis of NVOC-protected ethyl hydrazide 13.[48]

Because of the very different polarity of the two reactants, methanol was added to increase the solubility of NVOC-chloride in water. Since two reactive sites exist in ethyl hydrazinoacetate hydrochloride, three possible products were formed in different ratio of reactants (Scheme 1.16):

(1) Five equivalents of hydrazine was added into the reaction with NVOC chloride at room temperature for two hours, and the product was the mixture of di-protected hydrazine **15** and two isomers **13** and **14**.

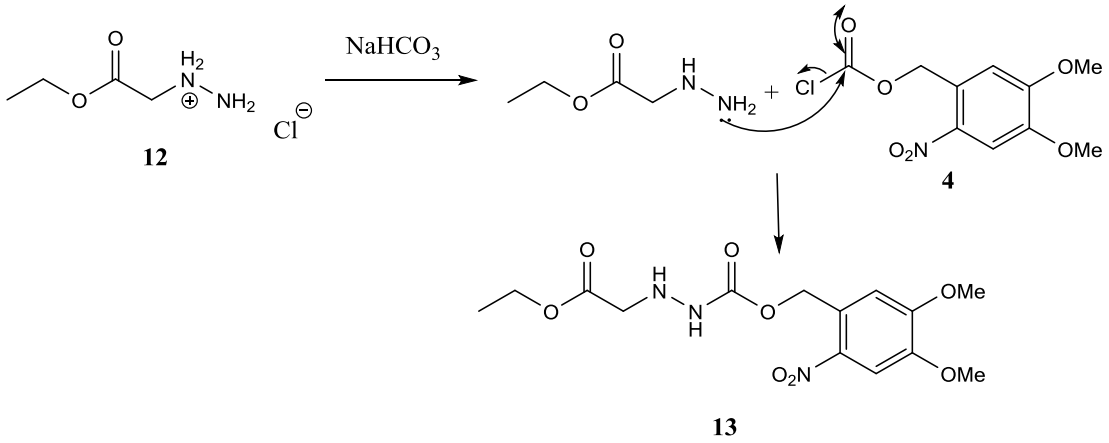
(2) Five equivalents of hydrazine was added into the reaction with NVOC chloride at room temperature for 30 min, this again produced the mixture of di-protected hydrazine and two mono-protected hydrazine.

(3) Ten equivalents of hydrazine was added into the reaction with NVOC chloride at room temperature for 30 min, only the desired product was obtained and confirmed by mass and ^{1}H NMR spectra.[49]



Scheme 1.16 Three possible products were formed in different ratio of reactants due to two reactive sites on ethyl hydrazinoacetate.[49]

The reaction follows an acyl substitution mechanism (Scheme 1.17). Firstly, the lone pair of electrons on the terminal nitrogen conducts a nucleophilic attack to the carbonyl carbon of ethyl hydrazinoacetate **12**, which then leads to a tetrahedral intermediate, which releases the chloride as leaving group and form the NVOC-protected hydrazide.

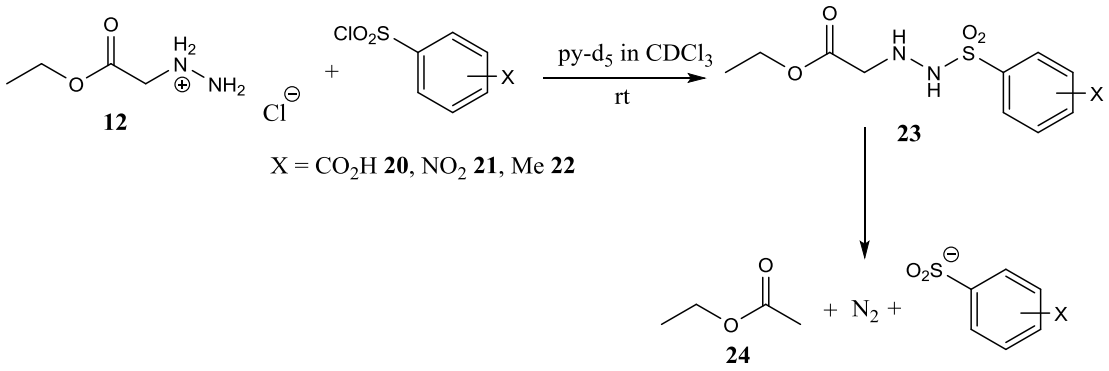


Scheme 1.17 The addition of NVOC protecting group to ethyl hydrazinoacetate.

1.7.2 Decomposition Studies

In order to determine the best sulfonyl compound for both synthesis and subsequent elimination, the decomposition of several sulfonyl hydrazides was studied.

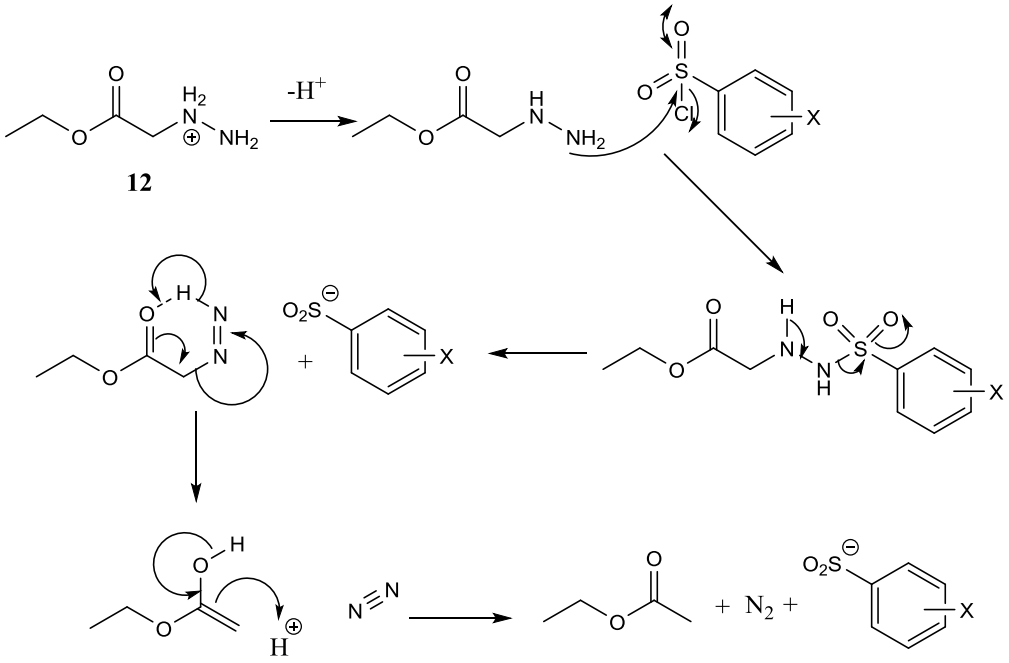
The spontaneous decomposition was expected when sulphonyl hydrazide was formed from ethyl hydrazinoacetate **12** and the corresponding sulfonyl chloride or anhydride, the process was monitored by NMR analysis in each case (Scheme 1.18). [51]



Scheme 1.18 The decomposition process which consequently conducted the release of nitrogen and ethyl acetate **24**.

The reaction proceeded by the deprotonation of the amine group on ethyl hydrazinoacetate **12**, followed by the nucleophilic attack by the lone electron pair at

the sulfur of the sulfonyl compound, which then lost chloride ion. The sulfonyl hydrazide **23** was deprotonated in the presence of base to undergo the loss of the sulfinate. Finally, the sulfonyl group was cleaved to generate acetate **24** and eliminate of N_2 in a sigmatropic rearrangement (Scheme 1.19).



Scheme 1.19 The mechanism for the decomposition of sulfonyl hydrazide.

The reaction was carried out in the solution of 0.3M d_5-pyridine in $CDCl_3$ in an NMR tube. In consideration of the solubility and decomposition rate of the sulfonyl hydrazide, the 4-nitrobenzene sulfonyl chloride was chosen to be used in the formation of hydrazides. The NMR spectrum showed the peak at 1.96ppm, which was the ethyl acetate **24**, grew over time (Figure **1.3**).

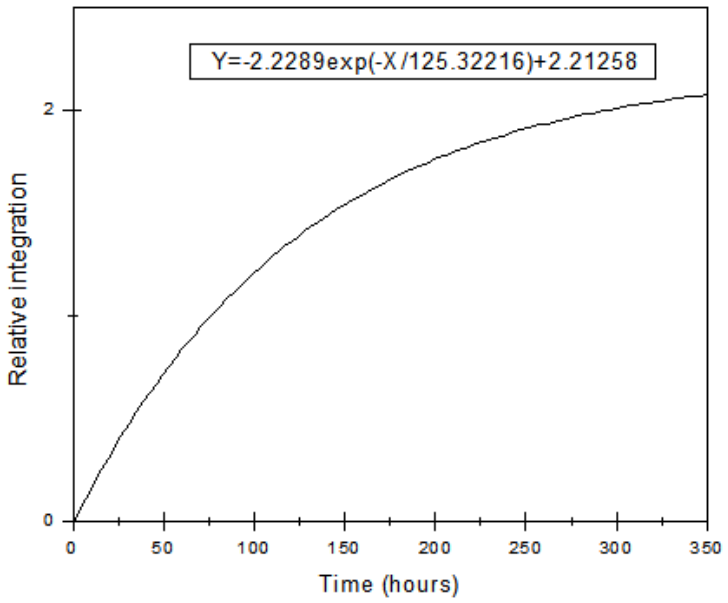
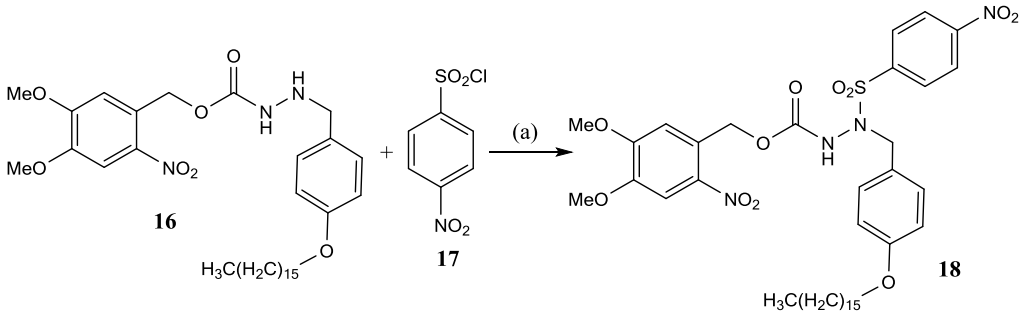


Figure 1.3 Rate of generating of ethyl acetate **24** in the decomposition of sulfonyl hydrazine formed by ethyl hydrazinoacetate **12** and 4-nitrobenzene sulfonyl chloride. [51]

1.7.3 Synthesis of sulfonyl hydrazide 18 from NVOC-protected hydrazine.

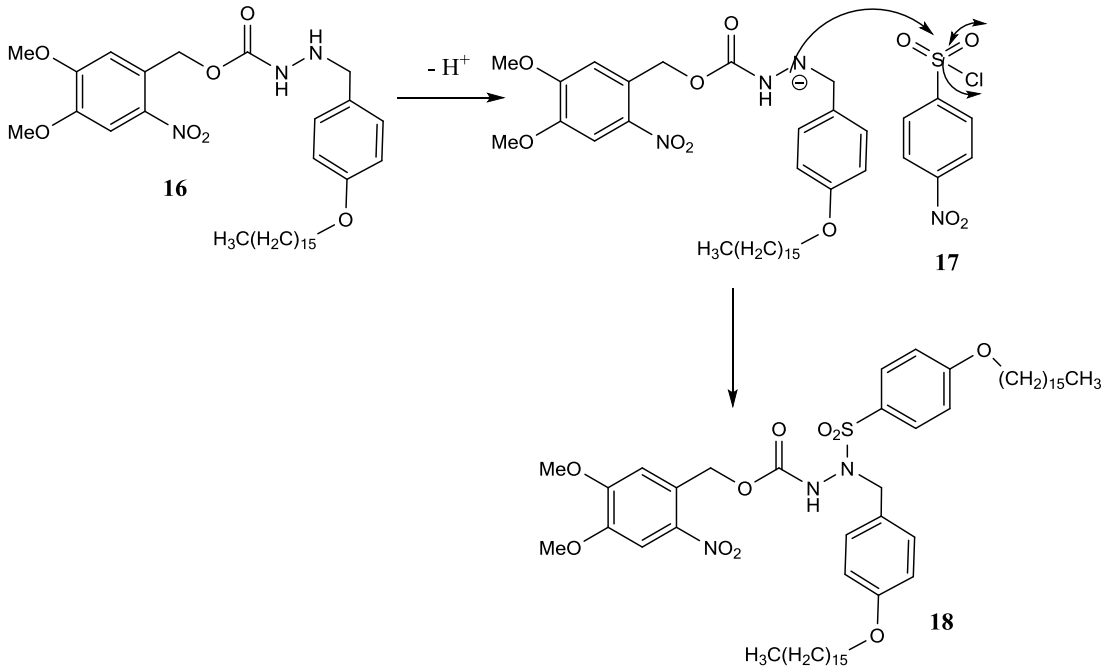
In order to explore the photo-uncaging process of sulfonyl hydrazide, the NVOC-protected sulfonyl hydrazide was synthesized.

NVOC-protected hydrazine **16** and *para*-nitrobenzene sulfonyl chloride **17** were dissolved in 0.3M d_5-pyridine in $CDCl_3$ and stirred at room temperature for 18 hours (Scheme 1.20). [50]



Scheme 1.20 The synthesis of sulfonyl hydrazide **18**. (a): d_5-pyridine in $CDCl_3$, rt, 18h. [50]

The progress of the reaction was monitored by ^{1}H NMR. The pyridine provided a basic environment to initiate the reaction to initiate the deprotonation of the hydrazine, which then attacked the sulfur of sulfonyl group and release the chloride (Scheme 1.21).



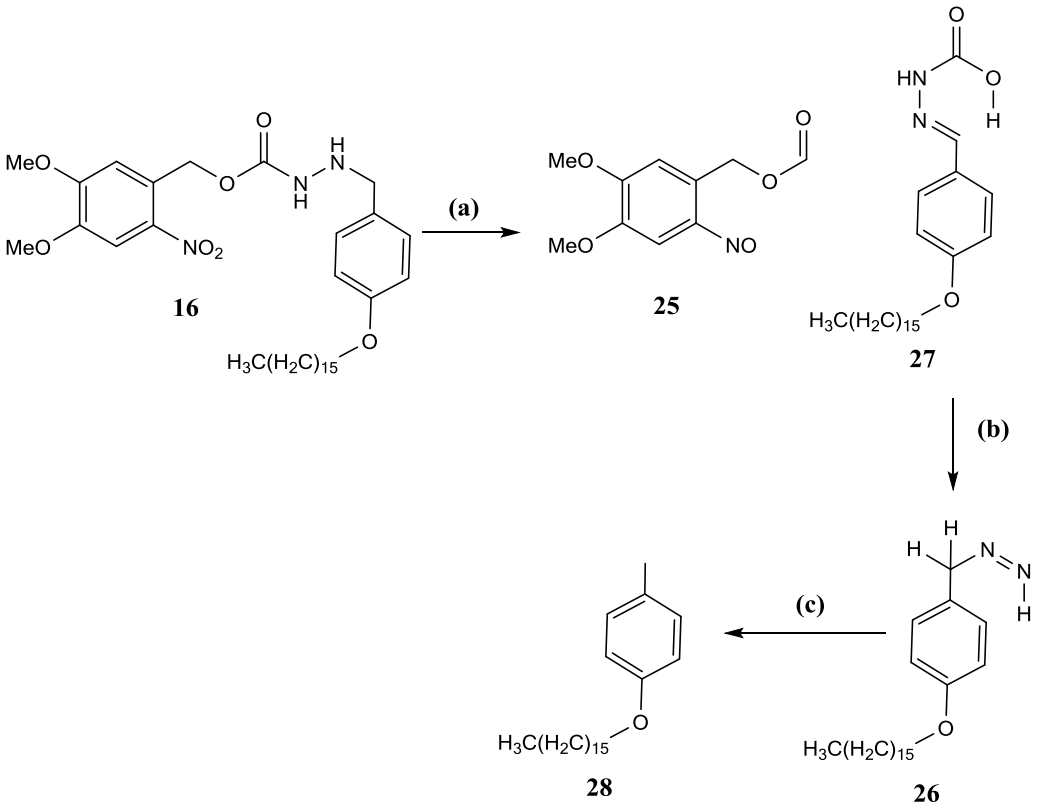
Scheme 1.21 The mechanism for the addition of 4-nitrobenzene sulfonyl group.

The product was decomposed when purified by column chromatography. However it showed that the only impurity of the reaction before purification was only the starting material. Thus, in order to avoid the possible decomposition during purification, the methods should be tried to drive the reaction to complete transformation.

1.7.4 Photolysis of NVOC-protected hydrazone 16

After irradiating with light from an LED, NVOC-protected hydrazone was expected to undergo series of transformations. At first, the NVOC-protected group would be cleaved from hydrazone **16** to form the carboxylic acid derivative **27**. In this step, 4,5-dimethoxy-2-nitrosobenzyl formate **25** would be formed as a byproduct. Then the

amide bond would be cleaved as decarboxylation to form a diazene **26**, which would generate the elimination of nitrogen *via* a sigmatropic rearrangement. The last step involves the proton transfer from terminal nitrogen to benzylic carbon to form an ether **28**, which was similar to the proton transfer in the Wolff-Kishner reaction, and was proved to be the rate-determining step (Scheme 1.22). [51]



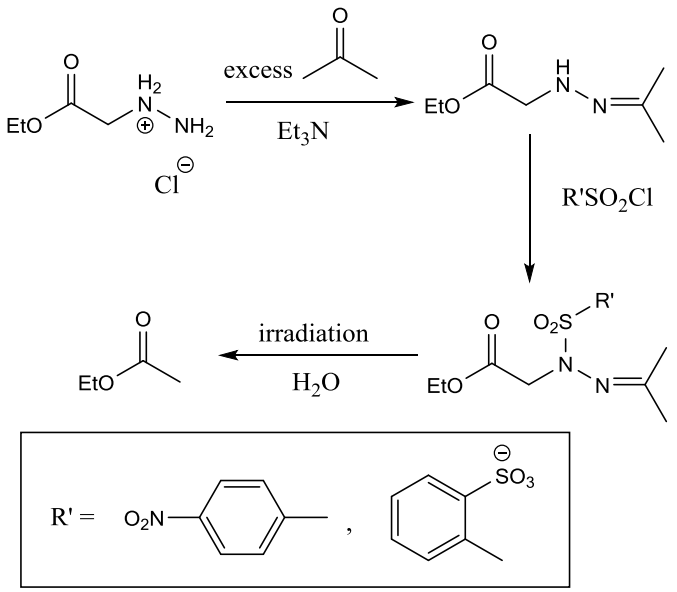
Scheme 1.22 Photolysis of NVOC-protected hydrazone **16**. **(a)** Irradiation; THF, MeOH; **(b)** irradiation; THF, MeOH,-CO_2; **(c)** irradiation; THF, MeOH.

The NVOC-protected hydrazone **16** was dissolved in a solution of 10% methanol in THF and the solution of 3 mg/mL was irradiated by light of wavelength 330 nm in a quartz cuvette. [51] The photolysis process was monitored by ^{1}H NMR spectroscopy. Although the photolysis was proceeding very slowly, the procedure was followed through the NMR spectra. After two hours' irradiation, what could have been the

reduction product appeared in the NMR spectrum as the peaks at 6.99 ppm and 6.91 ppm had increased. The reaction was finished after six hours, indicated by that no more change on the NMR spectrum. However, the reaction was proved to be inconclusive since the COSY didn't show a coupling between the signals at 6.99 ppm and 6.91 ppm. The influence by the byproduct, 4,5-dimethoxy-2-nitrosobenzaldehyde **25**, was also a concern as it could react with newly generated free hydrazine function.[52]

1.8 Project targets

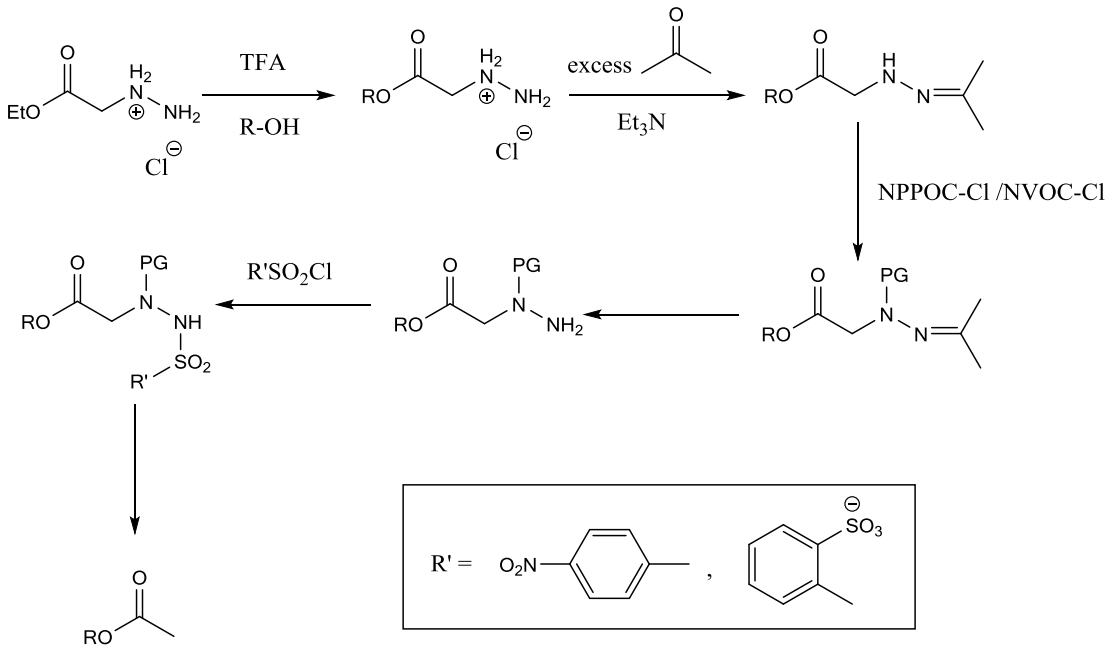
This project is initially aimed to explore the potential of using hydrazones as water-labile protecting groups on sulfonyl hydrazides, which would be water-soluble precursors of lipids and small hydrophobic molecules. Since the synthesis of sulfonyl hydrazides from mono-substituted hydrazines can give multiple products due to multiple reactive sites, the protection of hydrazines by acetone to form the hydrazones will be considered in order to shut down the reactivity. Then the hydrazone will be reacted with a sulfonyl compound such as *para*-nitrobenzene sulfonyl chloride to form the sulfonyl hydrazide, which will then be irradiated with light to trigger the cleavage of caging groups and release the nitrogen and sulfinate ion. The uncaging process will be monitor by NMR spectroscopy (Scheme 1.23).



Scheme 1.23 Project aim for synthesis of the ethyl sulfonyl hydrazide.

Ethyl hydrazinoacetate will be the first choice to carry on the above reactions and the expected product of the corresponding sulfonyl hydrazide will be ethyl acetate, which is easy to be identified by NMR spectroscopy.

Then different hydrazines will be made from ethyl hydrazinoacetate by transesterification, which will then undergo the protection from acetone. The hydrazone will then be reacted with light-sensitive caging groups such as NPPOC **3** and NVOC **4**, and then the acetone group will be eliminated to reveal the reactive site, which will be attached to a sulfonyl group to form the sulfonyl hydrazide. Then the decomposition of the sulfonyl hydrazide will be monitored by NMR spectroscopy (Scheme 1.24).



Scheme 1.24 Project aim for synthesis of NPPOC-protected sulfonyl hydrazide.

2. Results and Discussion

2.1 Synthesis of hydrazinoacetates

In order to prepare different hydrazinoacetates for the following reactions, transesterification is the best way to convert the commercially available ethyl hydrazinoacetate into other hydrazinoacetates. Transesterification is a process which interchanges the alkoxy moiety of an ester and an alcohol (Scheme 2.1). Since esterification is an equilibrium process, an excess of alcohol in the reaction can lead to full conversion into the target product. [53] Thus by changing the alcoholic solvent for ethyl hydrazinoacetate **12**, hydrazinoacetates with various alkyl groups can be obtained. According to a preliminary successful transesterification experiment in 2013 with ethyl hydrazinoacetate **12**, the Webb group decided to run the experiment at 80~90 °C and use TFA as the catalyst.

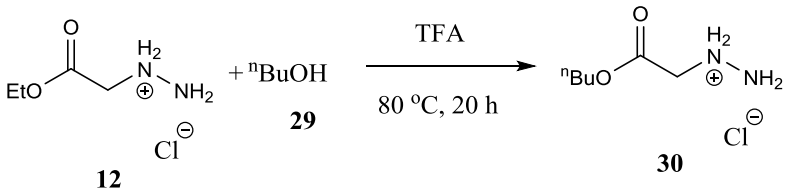
$$RCOOR_1 + R_2OH \rightleftharpoons RCOOR_2 + R_1OH$$

$$RCOOR_1 + R_2OH(excess) \rightleftharpoons RCOOR_2 + R_1OH$$

Scheme 2.1 General process of transesterification.

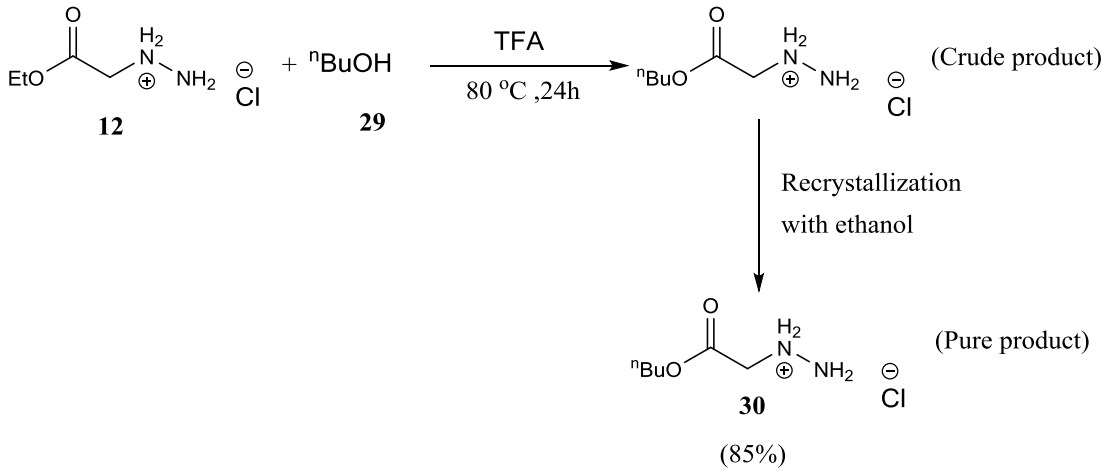
2.1.1 Synthesis of hydrazinoacetate with low-boiling-point alcohols

The Webb group successfully carried out the transesterification of ethyl hydrazinoacetate **12** with n-butanol **29** in 2013. [54] However the yield was low because the transformation of the starting material was incomplete and the purification by recrystallization led to significant lost of product (Scheme 2.2).



Scheme 2.2 Previous synthesis of *n*-butyl hydrazinoacetate through transesterification. [54]

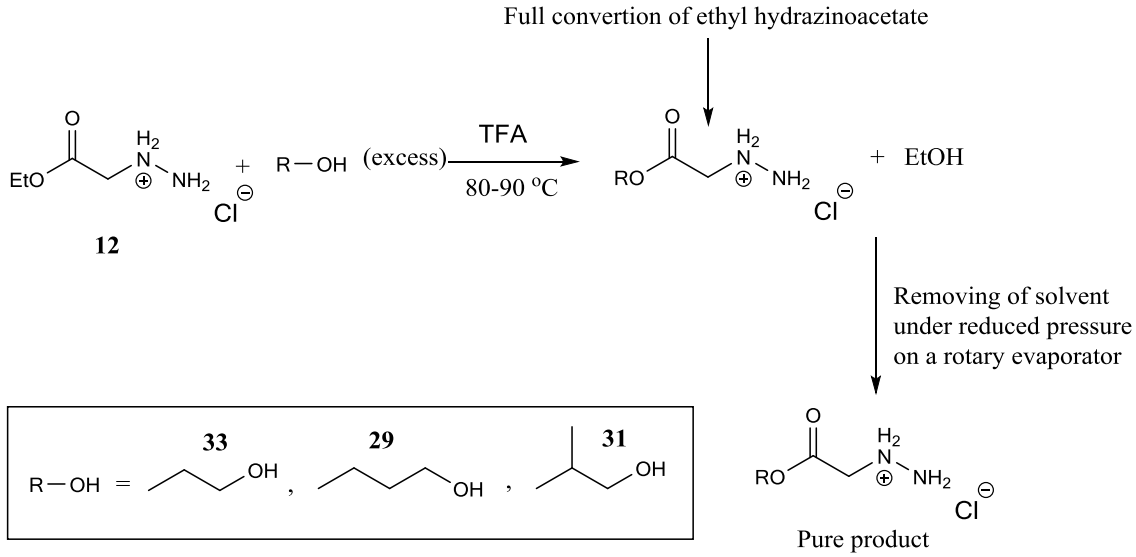
By increasing the quantity of ethyl hydrazinoacetate **12** from 100 mg to 1.54 g and *n*-butanol **29** from 5 mL to 10 mL, and extend the reaction period from 20h to 24h, however, the equilibrium became highly product-favored and led to the complete reaction of starting material (Scheme 2.3). The remaining solvent was removed under reduced pressure from the pure *n*-butyl hydrazinoacetate **30** without needing any other purification. As the consequence, the yield of the product has increased from 22% to 85%. Since the ^{13}C NMR spectrum did not show a peak for TFA byproduct, it indicated that the product was with chloride ion instead of trifluoroacetate.



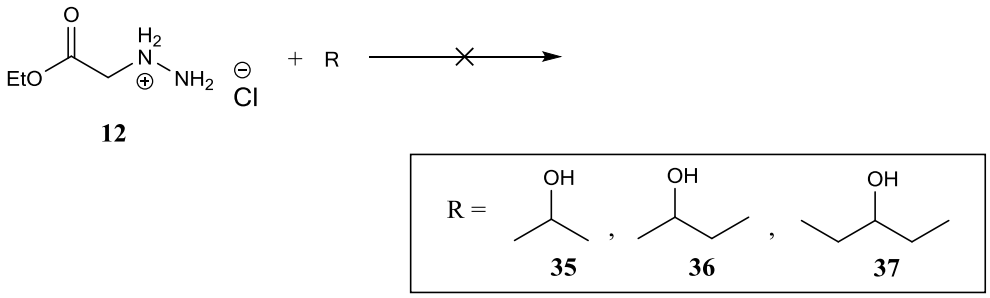
Scheme 2.3 The synthesis of *n*-butyl hydrazinoacetate **30**.

Other low-boiling-point alcohols such as *iso*-butanol **31** and *n*-propanol **33** can also produce the corresponding hydrazinoacetates **32** and **34** by simply refluxing an acidic alcoholic solution of ethyl hydrazinoacetate **12**, then removing the solvent under reduced pressure （Scheme 2.4）. Notably, their ^{13}C spectra usually miss the signal for carbonyl carbon and the adjacent carbon. The reason may be that the signal for carbonyl carbon is very weak, while the signal for the adjacent carbon is overlap with the peaks for MeOD at 47 ppm.

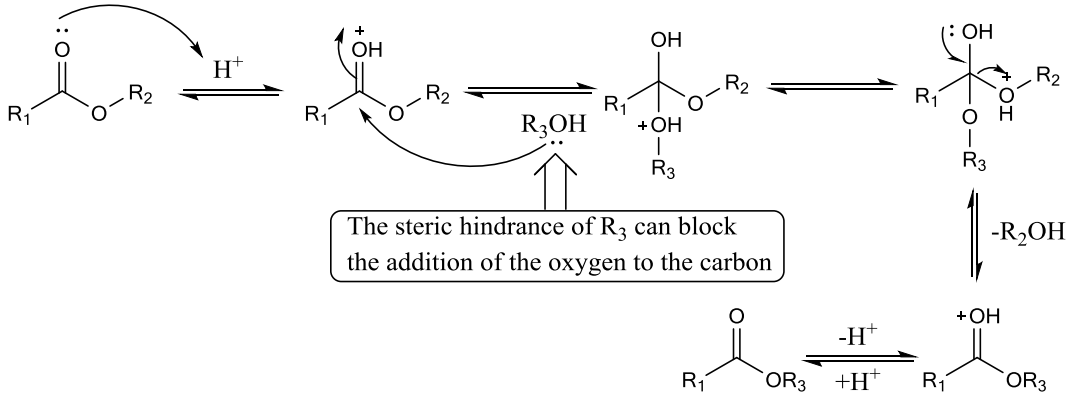
Nevertheless, not all low-boiling-point alcohols can convert to the expected product under these conditions. In the case of 2-propanol **35**, for instance, the two symmetric methyl groups form a steric barrier to slow down the esterification. Thus, the reaction mixture tends to remain as the alcohol and ethyl ester instead of forming the 2-propyl ester. Similarly, 2-butanol **36** and 3-pentanol **37** also have steric hindrance which obstructs the transesterification (Scheme 2.5). Thus, other syntheses for hydrazinoacetates of similar structure need to be further explored.



Scheme 2.4 Synthesis of hydrazinoacetate esters from low-boiling-point alcohols.



The mechanism of transesterification:



Scheme 2.5 The alcohols which do not participate in transesterification because of steric hindrance: 2-propanol **35**, 2-butanol **36**, 3-pentanol **37**. The steric hindrance of the alkoxy moiety can block the addition of the alcohol to form the intermediate.

2.1.2 Synthesis of hydrazinoacetate with high-boiling-point alcohols

Theoretically, linear alcohols with more than 4 carbons can form the corresponding hydrazinoacetate by transesterification, but they need longer times to finish the reaction and are harder to react completely due to steric hindrance. But since these alcohols usually have high boiling points, the removal of the solvents is a challenge. Two ways of getting rid of excess solvent were compared. Initially, the ethyl hydrazinoacetate **12** was reacted with *iso*-amyl alcohol **38** for 3 days and the solvent was removed using a high vacuum rotary evaporator to form the crude product as

brown oil, which was then be recrystallized with ethanol. The product was a mixture of *iso*-amyl hydrazinoacetate, starting material and impurities (Fig. 2.1 **(b)**).

In the other repeated reaction, the solution was put in the freezer for one night to form crystals, which were filtered and then washed with petroleum ether. The product was pure and the yield was 22% (Fig. 2.1, **(a)**).

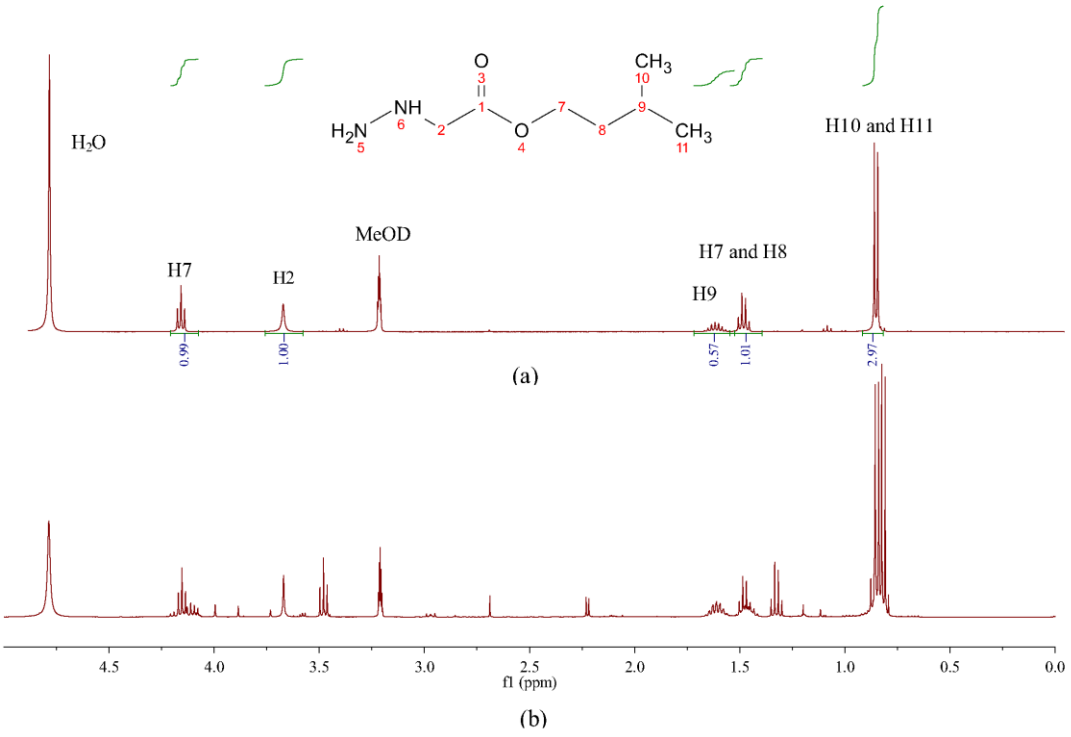
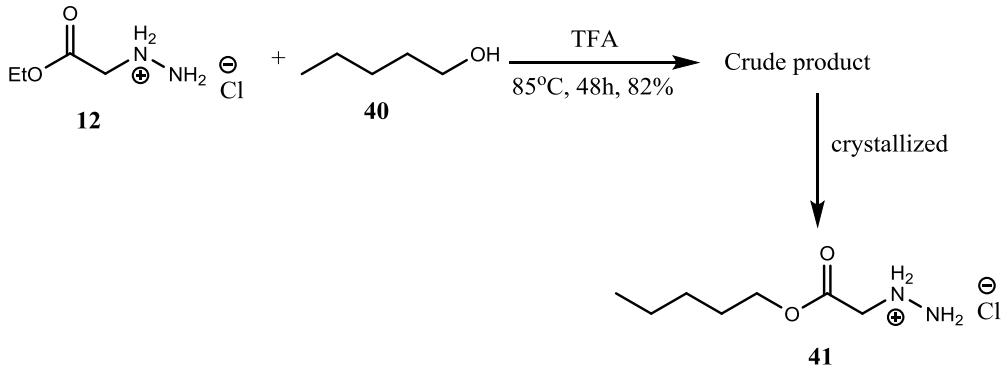


Fig. 2.1 NMR spectra for *iso*-amyl hydrazinoacetate **39**; **(a)** The product obtained by crystallization from *iso*-amyl alcohol was pure *iso*-amyl hydrazinoacetate **39**, the yield was 22%; **(b)** The product obtained by removing the solvent was a mixture of the starting material and other impurities, but further purification could be undertaken.

By comparing the results obtained in the two experiments, crystallization from the mother liquor was considered to be the better way to condense the product because it generated pure product and the process was simple and safe.

The advantage of crystallization was significant when using 1-pentanol **40** as the reactant. After 2 days reflux, the reaction of 1-pentanol **40** and ethyl hydrazinoacetate **12** was crystallized in the freezer overnight to form a large amount of product, which was washed by petroleum ether and resulted in 82% yield of 1-pentyl hydrazinoacetate **41**. The yield was comparatively high and no other purification was needed (Scheme 2.6).

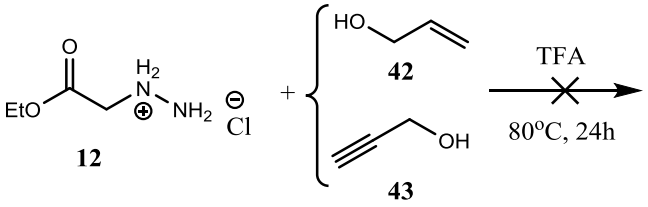


Scheme 2.6 Synthesis of 1-pentyl hydrazinoacetate **41**.

2.1.3 Synthesis of hydrazinoacetate with unsaturated alcohols

Unsaturated alcohols such as allyl alcohol **42** and propargyl alcohol **43** have additional reactivity because of their double or triple carbon-carbon bonds. Thus, when reacted with ethyl hydrazinoacetate **12**, the allyl alcohol **42** and propargyl alcohol **43** tended to generate more complicated reaction mixtures. Consequently, the ^{1}H NMR spectra of the two reactions had messy signals which could not be assigned.

So the TFA catalysed method to catalyse transesterification can not apply to unsaturated alcohols (Scheme 2.7).



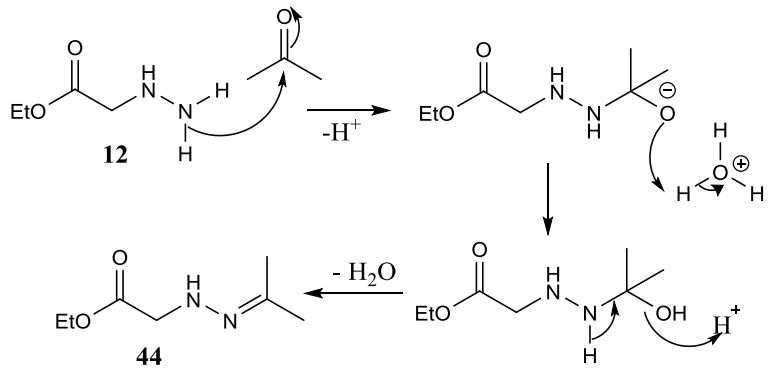
Scheme 2.7 Transesterification of allyl alcohol **42** and propargyl alcohol **43** failed using TFA catalysis.

2.2 Synthesis of NPPOC-protected hydrazine

The aim of the project is to synthesize sulphonyl hydrazides which can then undergo elimination to release N_2 and a sulfinate ion, revealing an unfunctionalised C-H bond. However, the synthesis of these sulphonyl hydrazides can be difficult because they have multiple reactive sites and can degrade spontaneously. Thus it was decided to try attaching protecting groups on hydrazinoacetate, using acetone to form the hydrazone in order to shut down reactivity.

2.2.1 Synthesis of hydrazone 44

In the reaction, the terminal amine of ethyl hydrazinoacetate **12** was reacted with acetone to form an N=C bond. The nitrogen on ethyl hydrazinoacetate **12** initially generated a nucleophilic attacked on the carbonyl carbon of acetone, followed by the release of H^+ to form alkoxide, which was then protonated by a hydronium ion to form hydrazone **44** (Scheme 2.8).

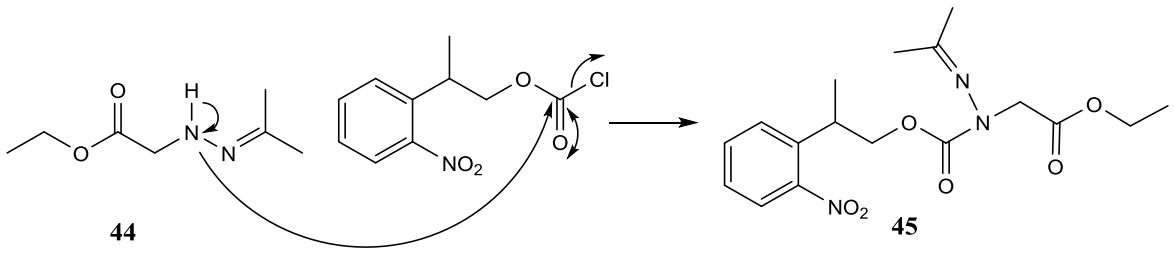


Scheme 2.8 Mechanism for acetone forming the hydrazone **44**.

After this reaction, the nitrogen atom in the hydrazone was unable to react with electrophilic groups, so that the light-sensitive protect group would be directed to the secondary amine in the next step.

2.2.2 Addition of NPPOC 3 to the hydrazone 44

In this project, NPPOC was chosen as the photo-sensitive caging group in the synthesis of caged hydrazide. The addition is initiated by the nucleophilic attack from ethyl hydrazone **44** to the carbonyl carbon of NPPOC-Cl, and consequently releases chloride as leaving group (Scheme 2.9).



Scheme 2.9 Mechanism of the synthesis of NPPOC-protected hydrazone **45**.

NPPOC was added to the hydrazone in DCM at 0°C for 0.5h (NPPOC: hydrazone = 1:1). [55] The crude product was purified by column chromatography and three fractions were collected as shown on the TLC, named as “Spot 1”, “Spot 2” and

"Spot 3" (Fig. 2.2). At this stage, "Spot 1" can not be identified yet; "Spot 2" was the hydrolysis product of NPPOC **46 (a)**; "Spot 3" was impurity. The expected product was not collected in this reaction, so an adjustment of reaction conditions was needed.

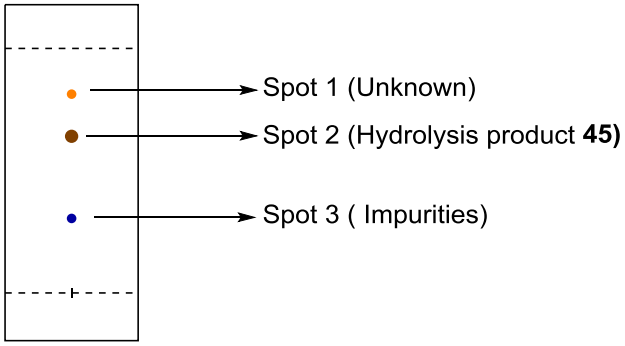
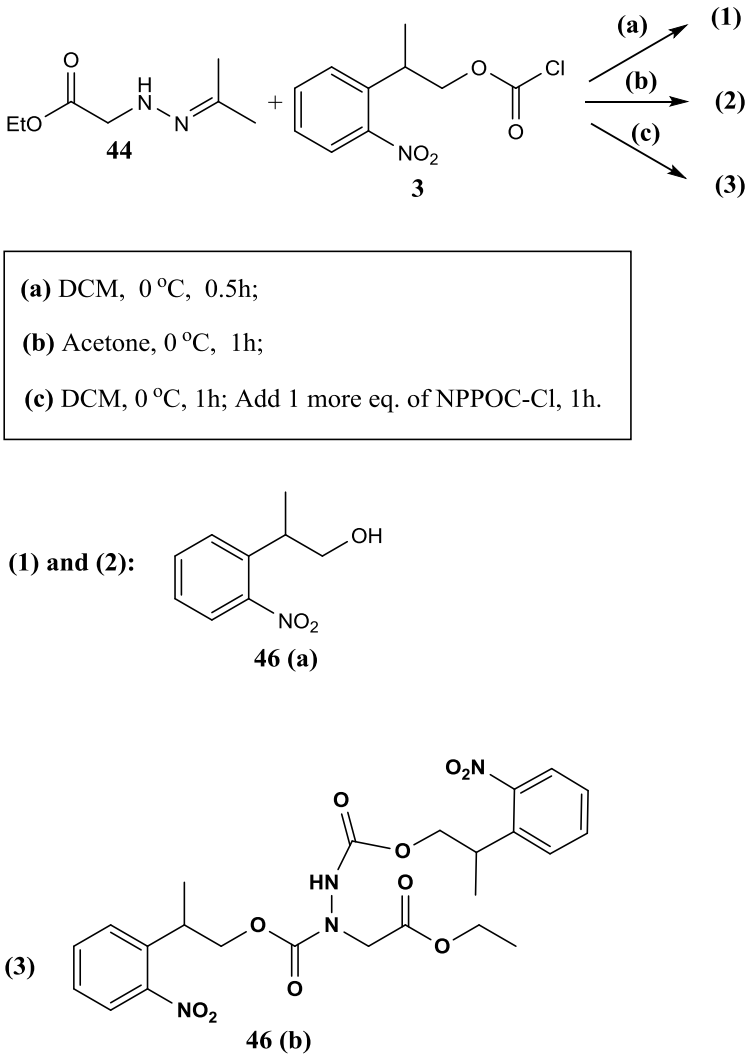


Figure 2.2 The thin layer chromatography of the first attempted reaction between hydrazone **44** and NPPOC-Cl **3**.

In the second attempt, 1 equivalent of NPPOC was reacted with hydrazone in DCM at 0 °C. As monitored by TLC, the starting material was not used up after 1 hour so then one more equivalent of NPPOC was added into the solution and left to react for one more hour. The crude product was purified by the column chromatography to give a product as brown oil. A $[M+Na]^+$ peak at m/z = 532.18 was found in the mass spectrum which was identified as the product **46 (b)**. The protected group had been cleaved and two molecules of NPPOC were attached on both amine groups of the hydrazine.

It was hypothesized that the problem may lie in the selection of solvent. To avoid the decomposition of the hydrazone, acetone was used as solvent in the third attempt instead of DCM. NPPOC was reacted with ethyl hydrazinoacetate **12** in acetone at 0 °C for 1 hour to form the crude product which was purified by the column chromatography. The result, however, was the same as the first attempt. The main

product obtained was from the hydrolysis of the NPPOC (Scheme 2.10). The reason why the NPPOC –protected hydrazone would be hydrolyzed in the reaction was still unclear.

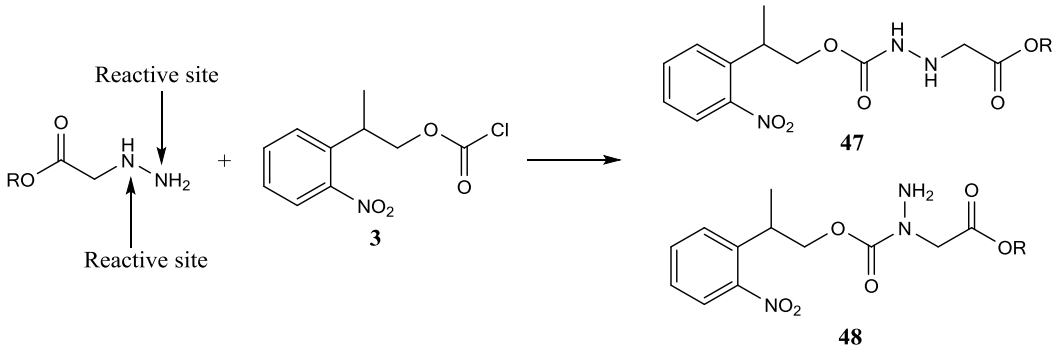


Scheme 2.10 Three different conditions used during the addition to NPPOC on hydrazone **44**.

As a result of these findings, the synthesis of hydrazone **44** from acetone was stopped.

2.3 Synthesis of NPPOC-protected hydrazide

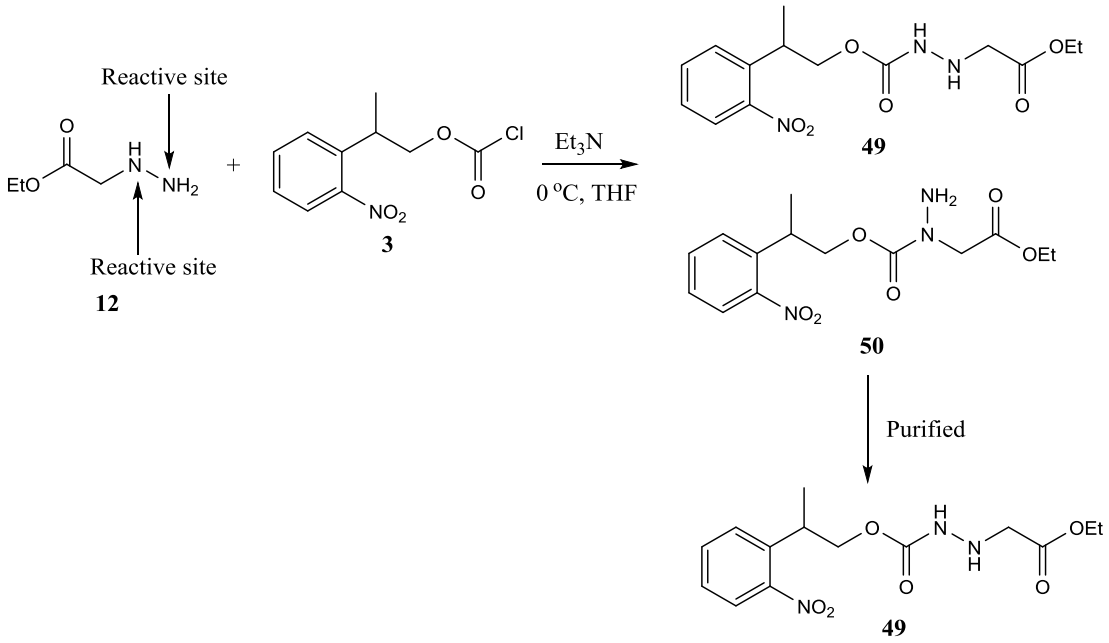
After the failure of the reaction between NPPOC-Cl and hydrazone **44**, it was decided to attach the NPPOC directly on the hydrazine using the same conditions used for the hydrazone reaction. The two amine groups of the hydrazine have similar chemical environments, thus they had approximately equal possibilities to react with the NPPOC. Thus, the reaction will form two isomers **47** and **48**（Scheme 2.11）. However, to avoid the attachment of two NPPOC molecules, the ratio of the two reactants was adjusted to NPPOC: hydrazine = 1:2.



Scheme 2.11 Addition of NPPOC to ethyl hydrazinoacetate **12** to get isomers **47** and **48**.

2.3.1 Addition of NPPOC onto ethyl hydrazinoacetate 12

The reaction of ethyl hydrazinoacetate **12** and NPPOC-chloride **3** was allowed to stir for 30 min at 0 °C with triethylamine to form the crude product which contained two isomers. The mixture can be separated by the column chromatography, however, due to the similarity of isomers in chemical property, the separation was very difficult. Column chromatography was employed twice with the solvent gradually from low-polarity to high-polarity (ethyl acetate: petroleum ether = 8:1 to 2:1) and the first fraction to come out was the pure product **49** (Scheme 2.12).



Scheme 2.12 Synthesis of NPPOC-protected ethyl hydrazide **49.**

The fraction collected later was compound **49** with about 30% compound **50**. After removing the solvent, the pure product **49** was obtained, which could be used in the next reactions. Theoretically, compound **50** can also be separated from the mixture of isomers if further purified by column chromatography. But since the overlap of the two compounds was large on TLC, the predicted yield of **50** would be too low to be used. Thus this compound **50** was not further purified.

Notably, the ^{1}H NMR spectrum for **49** showed diastereotopic protons H_a and H_b splitting the signals for proton c, which showed a quartet of doublet instead of normal sextet (Fig. 2.3).

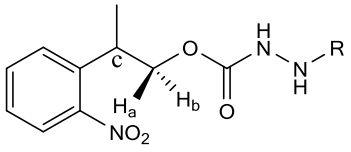
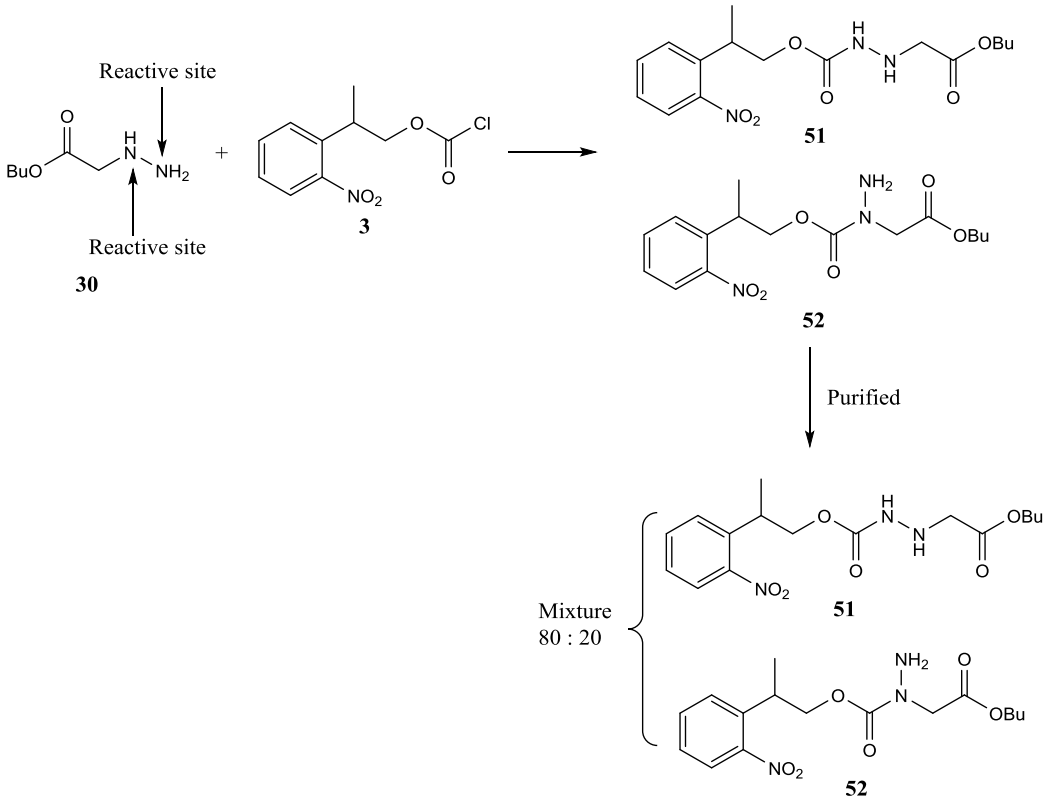


Fig. 2.3 Diastereotopic protons in NPPOC-protected hydrazides.

2.3.2 Addition of NPPOC onto *n*-butyl hydrazinoacetate 30

N-butyl hydrazinoacetate **30** was reacted with NPPOC-Cl using the same conditions as for ethyl hydrazinoacetate **12**, and produced a crude product containing two isomers. The crude product was purified by column chromatography for twice to form the product **51** containing 20% **52**. Since no better purification method was found, the product **51** was used in the next reaction with this slight contamination by the isomer (Scheme 2.13).

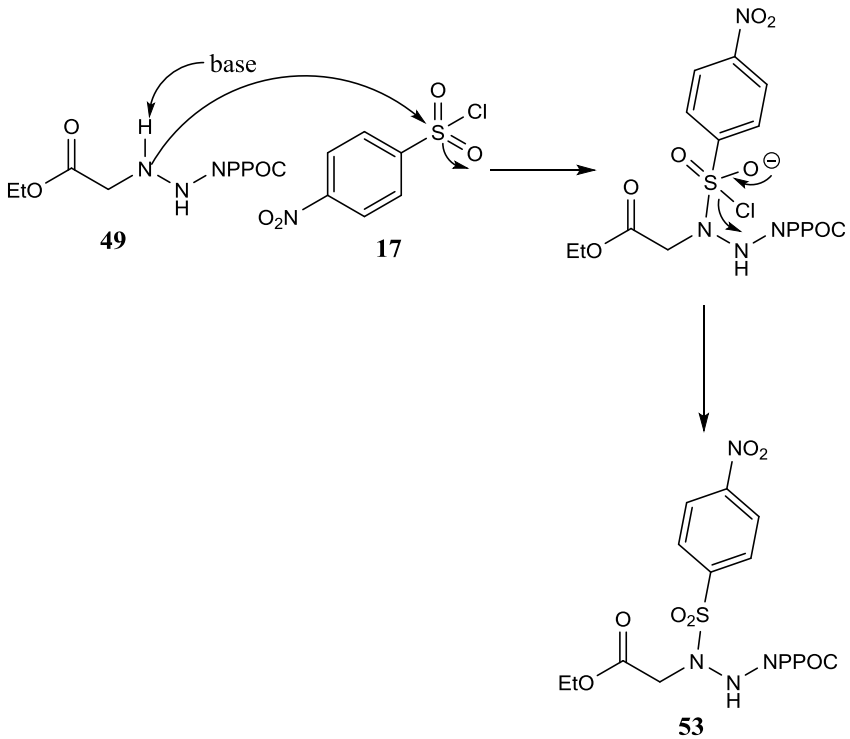


Scheme 2.13 Synthesis of NPPOC-protected *n*-butyl hydrazides **51** and **52**.

2.4 Synthesis of sulfonyl hydrazide

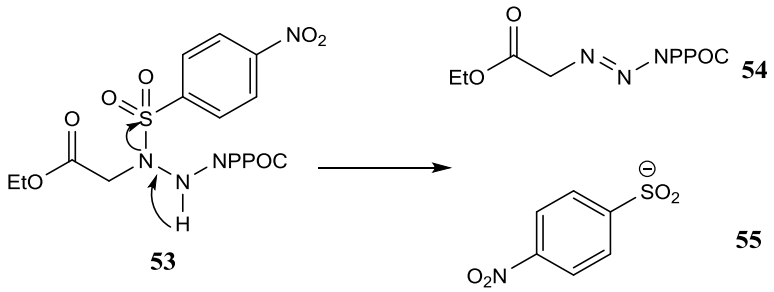
2.4.1 Addition of 4-nitrobenzene sulfonyl chloride 17 to NPPOC-protected ethyl hydrazide 49

As shown during previous decomposition studies of the Webb group, [56] 4-nitrobenzene sulfonyl chloride **17** is the most appropriate reactant for the formation and subsequent reactivity of the sulfonyl hydrazide, and the best solvent for the reaction is 0.2M d_5-pyridine in $CDCl_3$ (Scheme 2.14). The reason for using the d_5-pyridine is to provide a basic environment which could catalyse the reaction. In the previous work, 0.3M d_5-pyridine in $CDCl_3$ was used as solvent. But in this project, the concentration of d_5-pyridine will be reduced to 0.2M to slow down the decomposition rate.



Scheme 2.14 Mechanism for the addition of 4-nitrobenzene sulfonyl group to **49**.

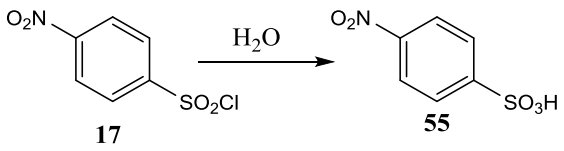
The NPPOC-protected ethyl hydrazine **49** and 4-nitrobenzene sulfonyl chloride **17** were dissolved in 0.2M d_5-pyridine in $CDCl_3$ at the ratio of 1:1. Five equivalents of triethylamine was added to increase the basicity of the solvent. The reaction was allowed to stir for 4 days and the solvent was removed to give the crude product as brown oil, which was then purified by column chromatography with hexane: ethyl acetate= 8:1 to 2:1 to give 5 mg product. While the ^{1}H NMR spectrum was messy, a $[M+Na]^+$ peak at m/z = 533.2, corresponding to the sulfonyl hydrazide **53**, was apparent in on the mass spectrum. The crude product also contained the diazene product **54**, which was formed by the decomposition of sulfonyl hydrazide **53**, with a $[M+Na]^+$ at 346.1 in the mass spectrum. The reaction was repeated to explore the reason for the low yield. However, the expected product **53** was not obtained after purification. The NMR and mass spectra had similar results, suggesting that the sulfonyl hydrazide **53** had been decomposed to form the diazene **54**. The implication is that the sulfonyl hydrazide **53** is not stable on silica, which corresponded to a previous experiment using NVOC instead of NPPOC (Scheme 2.15). [57]



Scheme 2.15 Decomposition of NPPOC-protected sulfonyl hydrazide **53**.

In order to analyze the decomposition rate of sulfonyl hydrazide **53**, the reaction was repeated and monitored by NMR spectra. Since the mechanism for the decomposition could be deprotonation of the sulfonyl hydrazide **53**, which was initiated by base, the

triethylamine was eliminated in order to decrease the decomposition rate. The peaks at 8.4 ppm and 8.2 ppm in the NMR spectra were the protons on the aromatic ring of 4-nitrobenzene sulfonyl chloride **17**. After 30 minutes, two new doublets at 8.2 ppm and 8.0 ppm formed while the peaks at 8.4ppm and 8.2ppm reduced slightly, which indicated attachment of the sulfonyl group to the hydrazide **49**. In contrary to expectations, the broad peak at 3.6 ppm, which represent the proton on the NH adjacent to the nitrogen attached with sulfonyl group, didn't undergo a strong change except for slightly shift downfield. Thus, the shift for the signals of the NPPOC-Cl would be used to monitor the reaction. The reaction carried on at a relatively low rate. After 4 days, the peaks at 8.4 ppm and 8.2 ppm vanished, which indicated the addition of the sulfonyl group has been complete. Notably, after the addition of sulfonyl group, new peaks at 8.3 ppm and 7.9 ppm were formed which might be due to the hydrolysis of para-nitrobenzenesulfonyl chloride (Scheme 2.16). Despite the addition of sulfonyl group was initiated quickly, it was proceeding at relatively slow rate as about 40% of the NPPOC-protected hydrazide **49** transformed to the product **53** after 8 days (Figure 2.4).



17 **55**

Scheme 2.16 Hydrolysis of para-nitrobenzenesulfonyl chloride **17**.

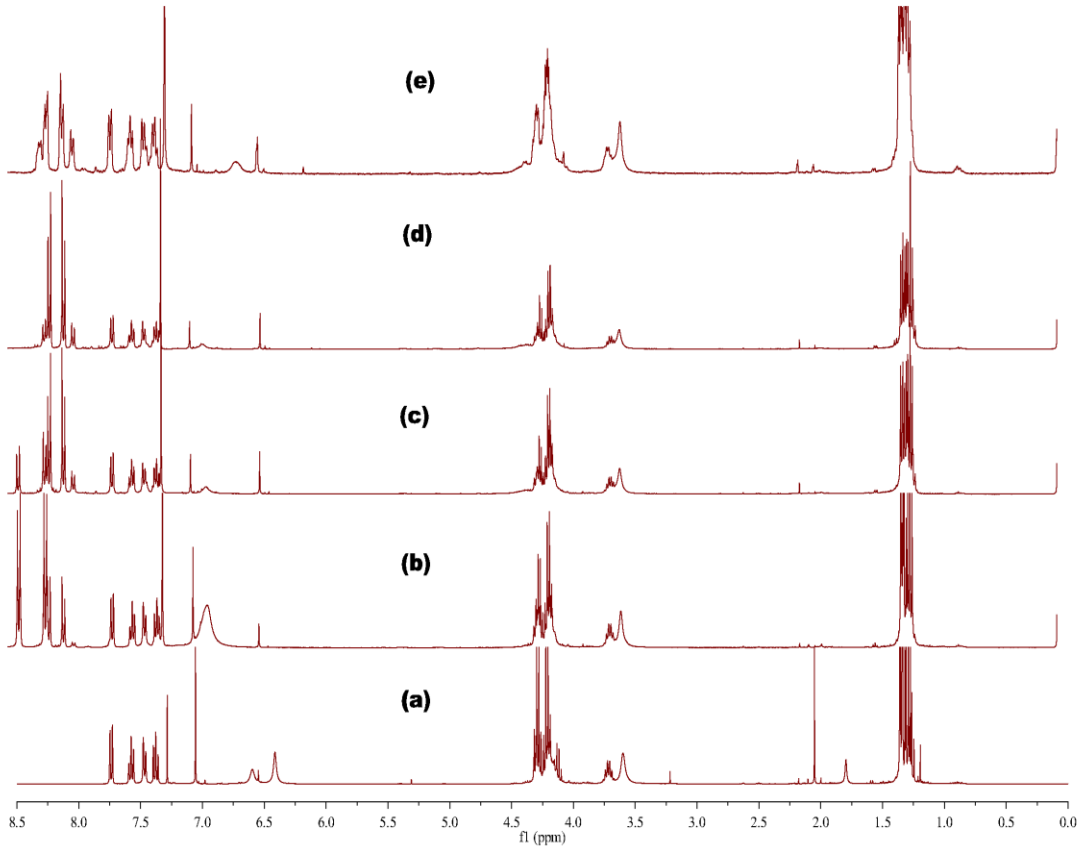


Fig. 2.4 NMR spectra for: (a) The NPPOC-protected ethyl hydrazide **46**; (b) The reaction with **17** after 30 min: The peaks at 8.4 ppm and 8.2 ppm were reduced while the new doublets formed at 8.2 ppm and 8.0 ppm. The singlet at 3.5 ppm which represented the -NH- of hydrazide shift slightly to downfield. (c) The reaction after 48 h: The peaks at 8.4 ppm and 8.2 ppm were continuingly decreased as the peaks at 8.2 ppm and 8.0 ppm grew over time. Notably, though not obvious, a new peak near 8.0 ppm had been form, which indicating the hydrolysis of para-nitrobenzenesulfonyl chloride **17**; (d) The reaction after 4 days: The peaks of 4-nitrobenzenesulfonyl hydrazide **17** had been vanished, this indicated the para-nitrobenzenesulfonyl chloride **17** was fully transformed to the product **53** or photolysis product **55**. (e) The reaction after one week: New peaks at 8.3 ppm and 7.9 ppm was increasing.

In order to accelerate the reaction, the reaction was repeated again using the mixture of 40% **49** and 60% **50** with the presence of triethylamine and was monitored by

NMR spectra. The reaction was much faster with base present. The shift for the doublets from 8.4 ppm and 8.2 ppm to 8.3 ppm and 8.0 ppm was not observed, indicating that the hydrolysis of para-nitrobenzenesulfonyl chloride **17** was avoided. After 7 days, the NMR spectrum showed about 80% of the starting material **49** had been reacted into the sulfonyl hydrazide **53**, and the result obtained at the 8th day showed no further reaction. The mass spectrum which had a $[M+Na]^+$ peak at m/z = 533.2 also testified to the existence of the right product. Then the solvent was removed to give the crude product as brown oil. As the product would be decomposed in column chromatography, no further purification was taken. Thus the product would still be together with the byproduct and triethylamine in the following reaction.

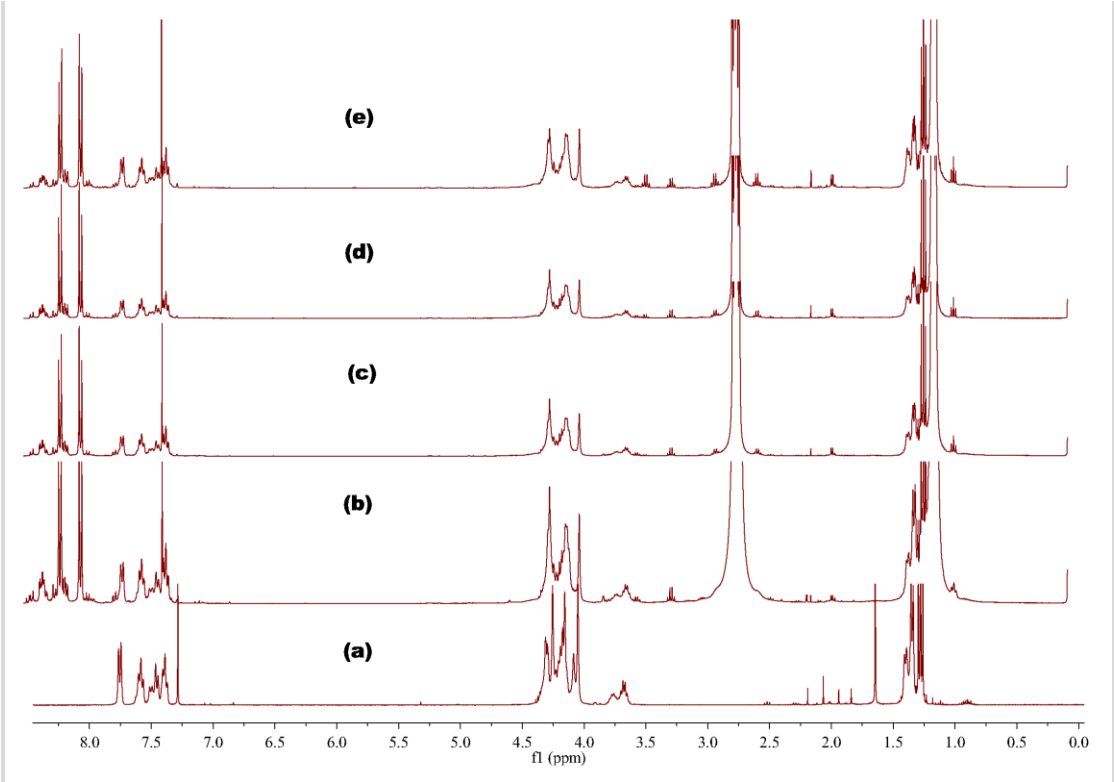
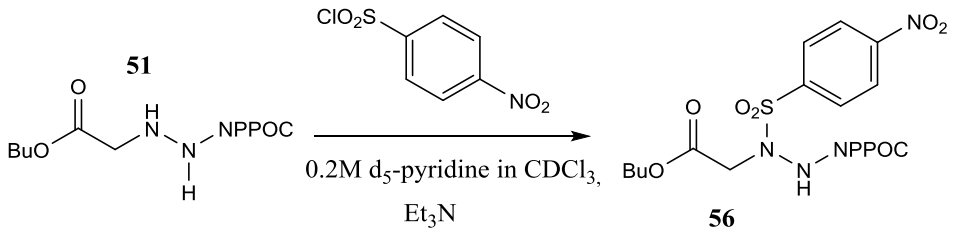


Fig. 2.5 NMR spectra for: (a) The NPPOC-protected ethyl hydrazide **49** (40%) and **50** (60%) ; (b) The reaction after 30 min; The peaks at 8.4 ppm and 8.2 ppm were reduced a lot while the new doublets

formed at 8.3 ppm and 7.9 ppm. The formation of the product **53** was accelerated by the presence of triethylamine; (c) The reaction after 48 h: The peaks at 8.4 ppm and 8.2 ppm were continuingly decreased as the peaks at 8.3 ppm and 7.9 ppm grew over time. (d) The reaction after 7 days: About 80% of the starting material **46** and **50** had been reacted in to the sulfonyl hydrazide **53**. (e) The reaction after 8 days: The reaction had been complete due to no more product **53** was formed. No spontaneous decomposition was observed among these 8 days.

2.4.2 Addition of 4-nitrobenzene sulfonyl chloride on NPPOC-protected n-butyl hydrazide mixture of 51 and 52

Since *n*-butyl hydrazide **51** has a similar structure to ethyl hydrazide **49**, the reaction took the same condition as described in the previous part. The reaction was employed with 5 equivalents of triethylamine in order to provide basic conditions. The reaction was monitored by ^{1}H NMR spectroscopy (Scheme 2.17). In contrary with the slow rate for ethyl hydrazide **49**, the addition of the sulfonyl group to *n*-butyl hydrazide **51** and **52** was relatively fast. The shift of the doublets at 8.4 ppm and 8.2 ppm indicated reaction, and after 24 hours, all spectral changes had stopped, which showed the reaction was complete (Fig. 5).



Scheme 2.17 Synthesis of NPPOC-protected sulfonyl hydrazide **56**.

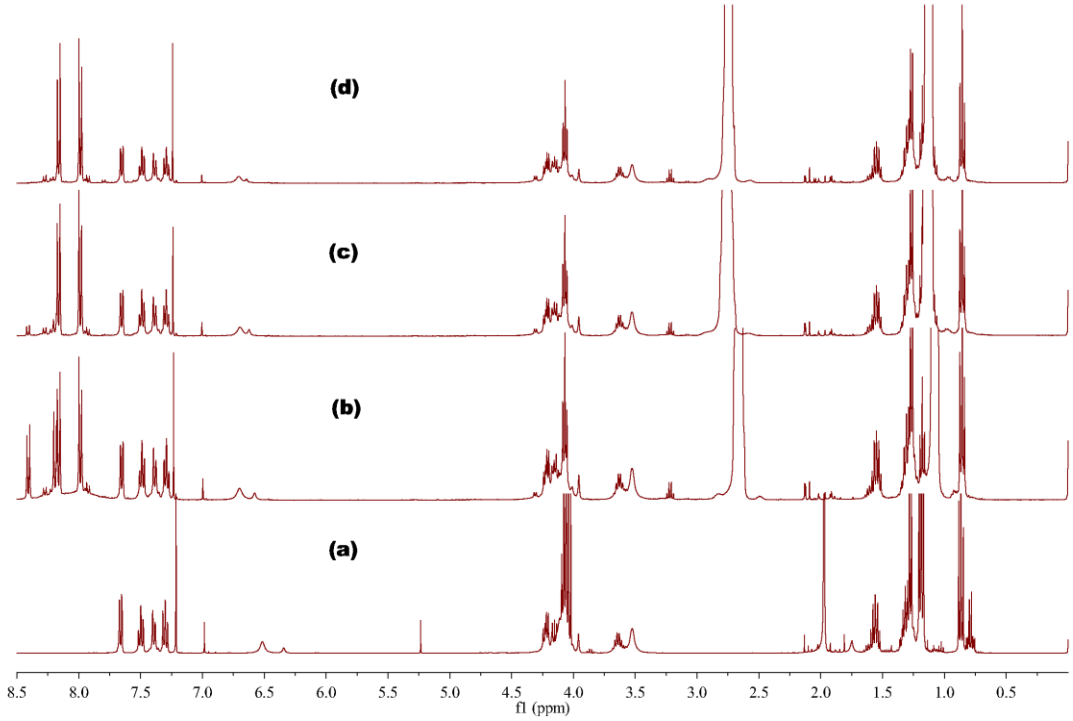
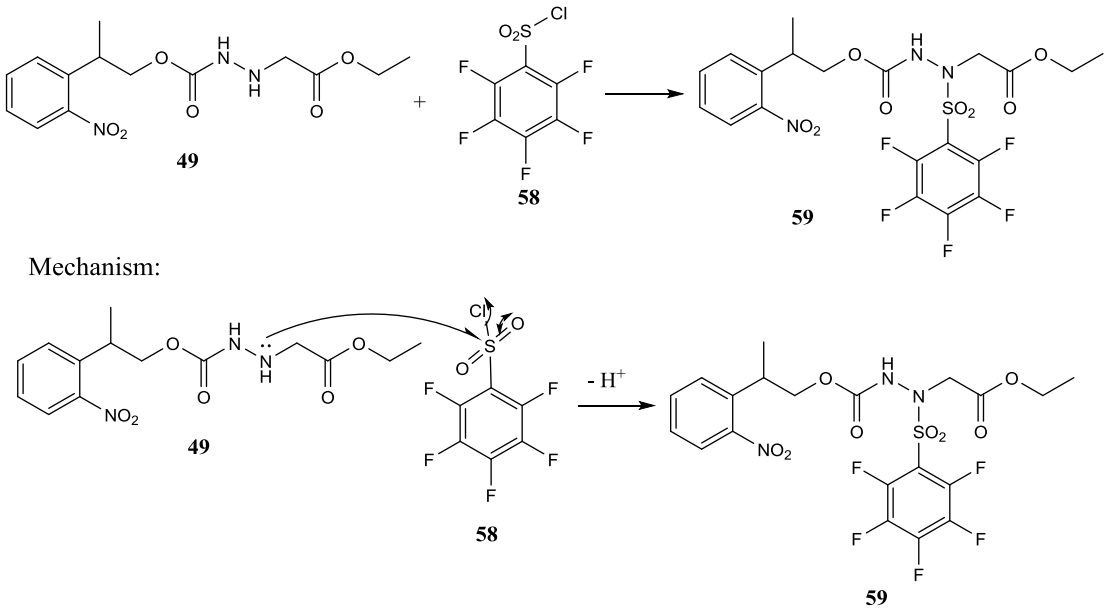


Fig. 2.6 NMR spectra for: (a) The NPPOC-protected *n*-butyl hydrazide **51** (90%) and **52** (10%); (b) The reaction with **17** after 30 min: The peaks at 8.4 ppm and 8.2 ppm decreased and new doublets at 8.3 ppm and 7.9 ppm were formed. (c) The reaction after 6 h: The peaks at 8.4 ppm and 8.2 ppm decreased as the peaks at 8.3 ppm and 7.9 ppm grew over time. (d) The reaction after 24 h; The peaks at 8.4 ppm and 8.2 ppm vanished which indicated the complete transformation of NPPOC-protected *n*-butyl hydrazide **51** to sulfonyl hydrazide **56.**

Despite indications the product might not be decomposed by column chromatography, which was proved by 2D TLC, the output of the crude product was 13 mg and too little for the next reaction. Thus, no further purification was taken. In order to improve the result of photolysis, pure reactant was needed to avoid the influence by other byproducts. So this reaction can be repeated to collect more crude product which can be purified by column chromatography to produce relative pure product for the photolysis.

2.4.3 Addition of pentafluorobenzene sulfonyl chloride 58 on NPPOC-protected ethyl hydrazide 49

Since the addition product of 4-nitrobenzenesulfonyl chloride **17** and NPPOC-protected ethyl hydrazide **49** appeared to be decomposed by column chromatography, an alternative sulfonyl compound, pentafluorobenzenesulfonyl chloride **58** was considered. Because pentafluorobenzene sulfonyl chloride **58** has no protons, it will not appear in the ^{1}H NMR spectrum, which will make the spectra clearer. The NPPOC-protected ethyl hydrazide **49** and pentafluorobenzenesulfonyl chloride **58** was dissolved in 0.2M d_5-pyridine in $CDCl_3$ at the ratio of 1:1 with 5 equivalents of triethylamine (Scheme 2.18).



Scheme 2.18 Addition of pentafluorobenzenesulfonyl chloride **58** onto ethyl hydrazide **49**.

As the reaction was monitored by NMR spectra, little change was observed on the spectra, indicating the reaction was rather slow. After 48 hours, messy signals appeared at the area of 6.7~7.0 ppm and 3~3.5 ppm (Fig. 2.7). But the changes of the spectra were slight over time. The mass spectrum showed no peak for the addition

product **59**, which also suggested the reaction had not been carried out successfully. To solve this problem, more starting material could be added to the reaction to give a high ratio of pentafluorobenzene sulfonyl chloride **58**: NPPOC-protected ethyl hydrazide **49**.

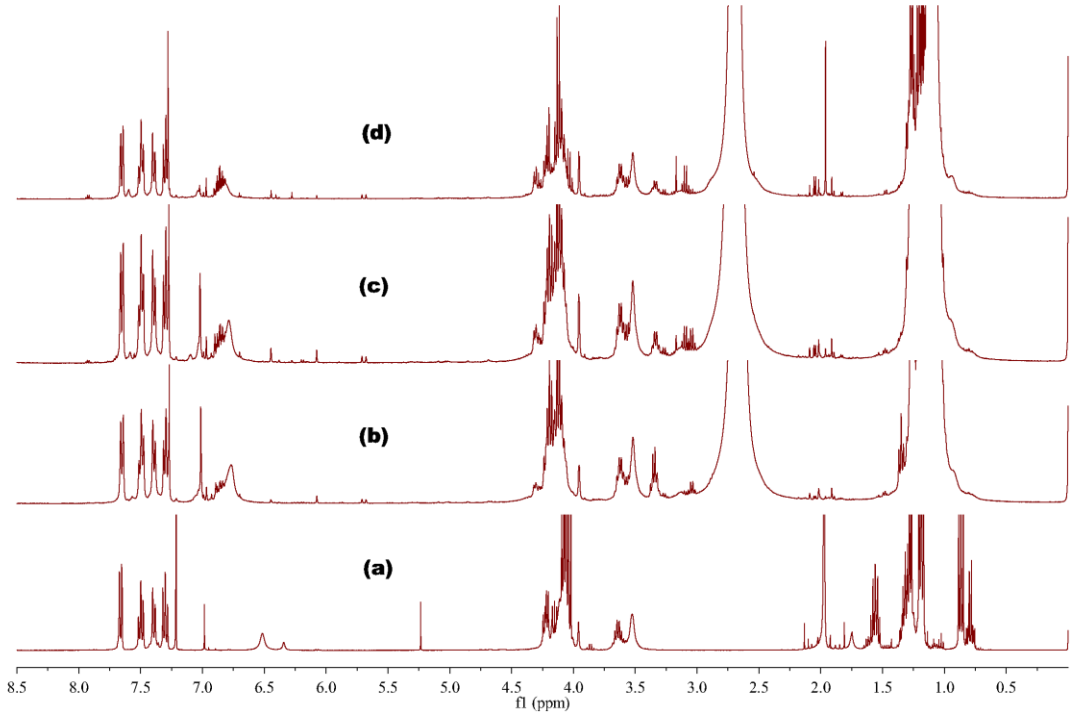
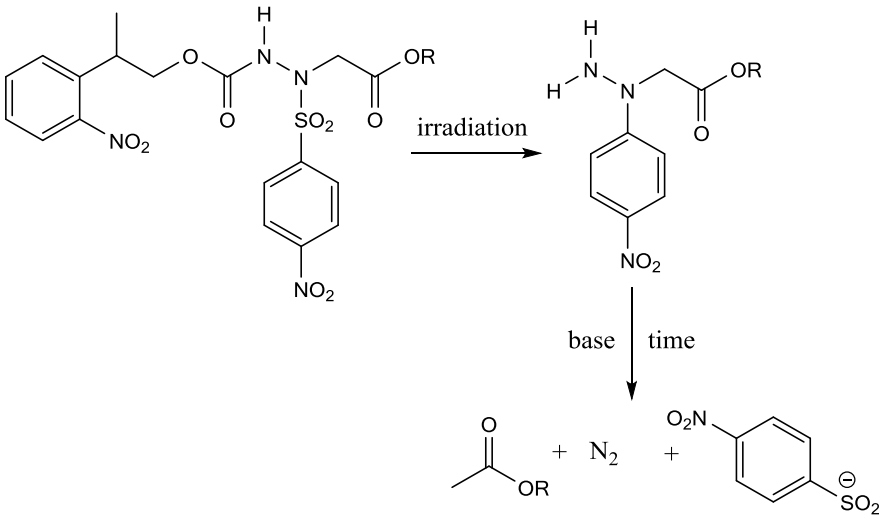


Fig. 2.7 NMR spectra for: (a) The NPPOC-protected ethyl hydrazide **49**; (b) The reaction with **58** after 30 min; Messy signals appeared at the area of 6.7~7.0 ppm and 3~3.5 ppm but can not be identidied. (c) The reaction after 24h. (d) The reaction after 48 hours; No product of **59** was observed.

2.5 Photolysis of NPPOC-protected sulfonyl hydrazide

Theoretically, after irradiating with light, NPPOC-protected sulfonyl hydrazide would undergo the cleavage of NPPOC-protected group to form the carboxylic acid, which would then form a diazene by decarboxylation and generate the elimination of N_2 and sulfinate ion to form the pheromone molecules (Scheme 2.19).



Scheme 2.19 Photolysis of NPPOC-protected sulfonyl hydrazide.

The photolysis conditions were the same as previous experiments of NVOC-protected hydrazones. [58] The crude NPPOC-protected sulfonyl hydrazide was divided into two equal samples, one was dissolved in a solution of $CDCl_3$ at concentration of 3 mg/mL, and the other sample was dissolved in a solution of 0.2M d_5-pyridine in $CDCl_3$ at concentration of 3 mg/mL. The reason to add d_5-pyridine was to accelerate the reaction by adding base. Each solution was transferred in a quartz cuvette and then irradiated by light of wavelength 330 nm for 15 minutes. Since the expected product was an ester, a signal at around 2.0 ppm for the methyl group was significant.

The NMR spectra for the photolysis products were obtained. For ethyl hydrazide, about 41% of the doublets at 8.2 ppm and 8.0 ppm decreased as the doublet at 7.9 ppm grew, which might indicate the sulfonyl group was transformed into sulfinate ion. The signals at 7.4~7.6 ppm which showed the phenyl part of NPPOC group were messier due to the chemical change caused by decomposition. In the area of 4.5~6.2 ppm, some new signals were observed. These signals also showed up in other photolysis experiments for NPPOC-protected hydrazide by the Webb group, but they

have not yet been identified. Despite the NMR spectrum turned to be messier, the most notable thing was that a new signal appeared at 2.1 ppm, which could possibly be ethyl acetate. In order to testify this, pure ethyl acetate was 'spiked' into the NMR tube to see if the singlet at 2.1 ppm would increase. A new singlet at 2.0 ppm showed up, indicating the singlet at 2.1 ppm was not ethyl acetate (Fig. 2.8). The mass spectrum didn't show a peak for ethyl acetate. Thus, for the future work, the reaction time of photolysis should be extended. Notably, the result for the sample in 0.2M d_5-pyridine in $CDCl_3$ was the same as in $CDCl_3$, indicating that the pyridine did not influence the reaction outcome.

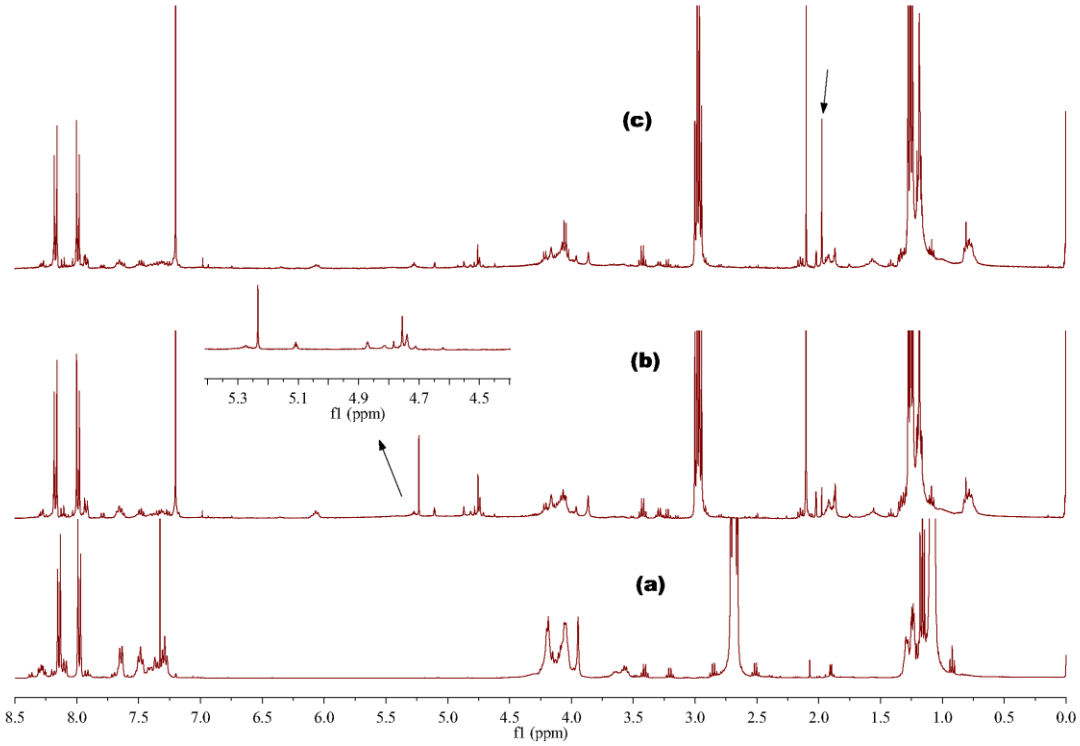


Fig. 2.8 The NMR spectra for: **(a)** NPPOC-protected ethyl sulfonyl hydrazide **53; (b)** The product obtained after irradiation for 15 min; New peaks was observed at 4.5~5.3 ppm, which could be byproducts from photolysis. A new singlet at 2.1 ppm was formed. (c) After adding drops of ethyl

acetate in (b), a new peak, indicated by the arrow, appeared. Therefore the peak at 2.1 ppm was not representing the formation of ethyl acetate.

The NPPOC-protected butyl sulfonyl hydrazide **56** also underwent the irradiation with light, which gave the similar result as ethyl sulfonyl hydrazide 53 (Fig. 2.9).

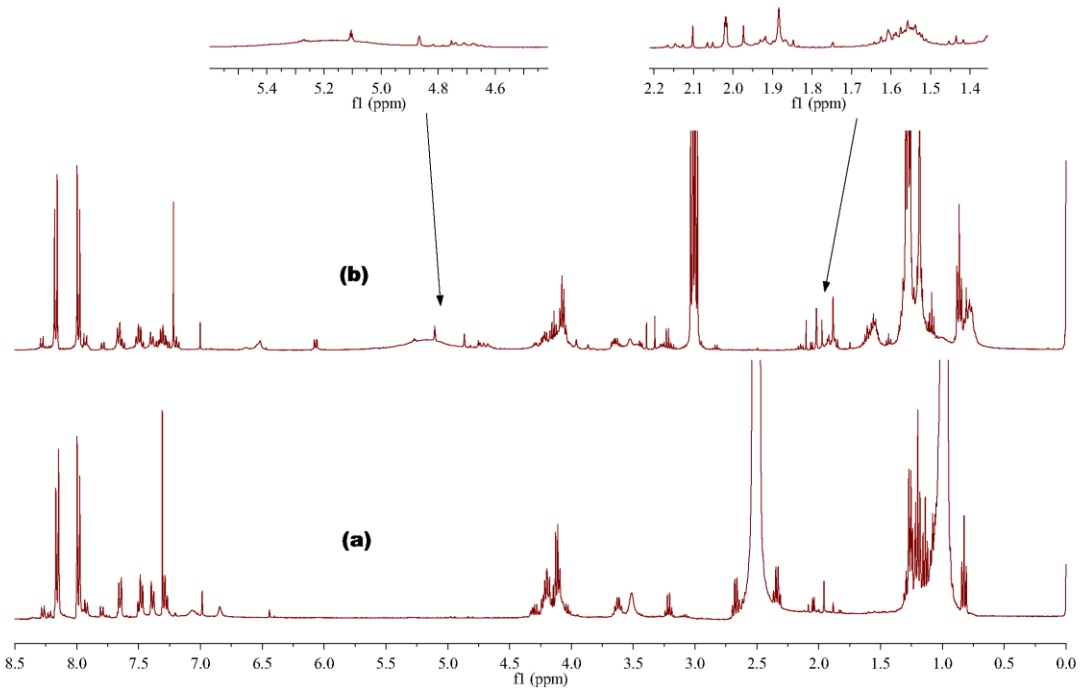
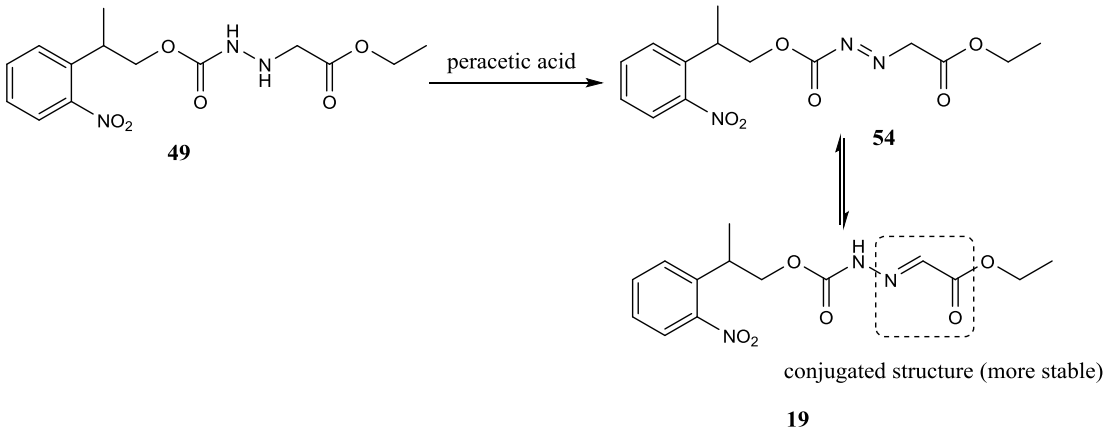


Fig. 2.9 The NMR spectrum for: **(a)** NPPOC-protected butyl sulfonyl hydrazide **56**; **(b)** The product obtained after 15 min's irradiation. New peaks appeared at the area of 4.5~5.0 ppm and 1.4~2.1 ppm, but no signals for the expected product was observed.

2.6 The synthesis of diazene

The N-N bond of hydrazides can be oxidized to N=N bond by peracetic acid to form the diazene (Scheme 2.20). The condition for the oxidation was the same as the reaction conditions described by Gilbert and Borden.[59] The NPPOC-protected ethyl

hydrazide **49** was dissolved in DCM at the concentration of 80 mL/g, and 1.1 equivalent of peracetic acid was added into the solution. The reaction was allowed to be stirring at room temperature for 50 min, and then the solvent was removed and the NMR spectrum of the crude product was obtained. Since some starting material **49** was remained unchanged, peracetic acid was again added and the reaction was allowed to stir for 30 more minutes. Since diazene **54** was highly likely to be tautomerised over time to form hydrazone **19** due to the stable conjugated structure in hydrazone, the reaction was stopped and the NMR spectrum for the sample was obtained as soon as possible. The ^{1}H NMR and mass spectrum both testified the successful synthesis of diazene **54**. For further studies, the diazene **54** can be irradiated with light to uncage the NPPOC group and release nitrogen. The photolysis rate of the diazene **54** could also be analyzed.

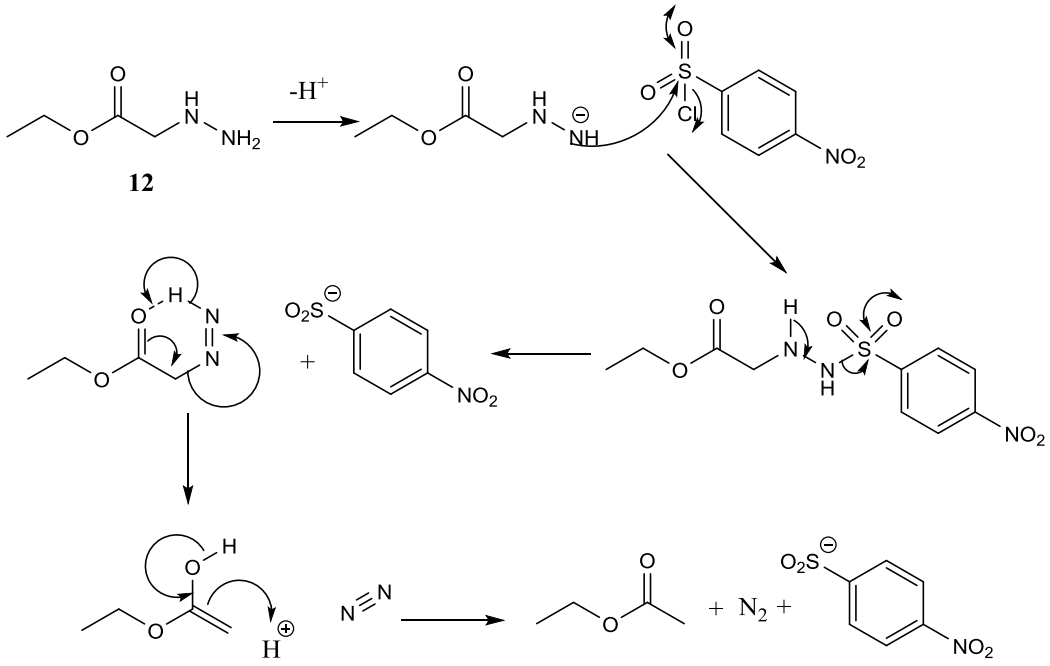


Scheme 2.20 Synthesis of diazene **54**.

2.7 The decomposition of the adduct of $EtOC(=O)CH_2NH_2NH_2^+Cl^-$ and 17

Sulfonyl hydrazides with nitrobenzenesulfonyl groups can undergo elimination spontaneously to release nitrogen. In the previous decomposition studies by the Webb group, ethyl hydrazinoacetate was chosen to be the reactant since the product, ethyl

acetate, was easy to be identified on the NMR spectra (Scheme 2.21). However, due to the low-boiling point of ethyl acetate, the product was difficult to be isolated for further characterization. Thus, 1-pentyl hydrazinoacetate was selected as it can produce high-boiling-point ester which can be isolated with non-polar solvent.



Scheme 2.21 Previous decomposition study with ethyl hydrazinoacetate **12**.

The 1-pentyl hydrazinoacetate **41** and 4-nitrobenzenesulfonyl chloride **17** one equivalent were dissolved in the solvent of 0.2M d_5-pyridine in $CDCl_3$ and the reaction was monitored by NMR spectroscopy. As the reaction was carrying on, the peaks at 8.4 ppm and 8.2 ppm were shifting to 8.1 ppm and 8.0 ppm indicating the sulfonyl group was transforming to sulfinate ion. A new singlet at 2.0 ppm was growing over time, which suggestes the formation of 1-pentyl acetate. After 5 days, the singlet at 2.0 ppm had reacted its maximum intensity and the product was concentrated and purified by column chromatography in a Pasteur pipette with d_6-benzene (Fig. 2.10). Benzene-d_6 was used due to its high non-polarity, which could

wash the non-polar acetate out and leave the other compounds. Benzene-d_6 was employed so the collected fragment could be characterized directly. However, the 1-pentyl acetate was failed to be collected after the column chromatography. The reason may be that the Benzene-d_6 was not a good solvent for wash out the 1-pentyl acetate. For further study, other solvent should be tried to collect the product.

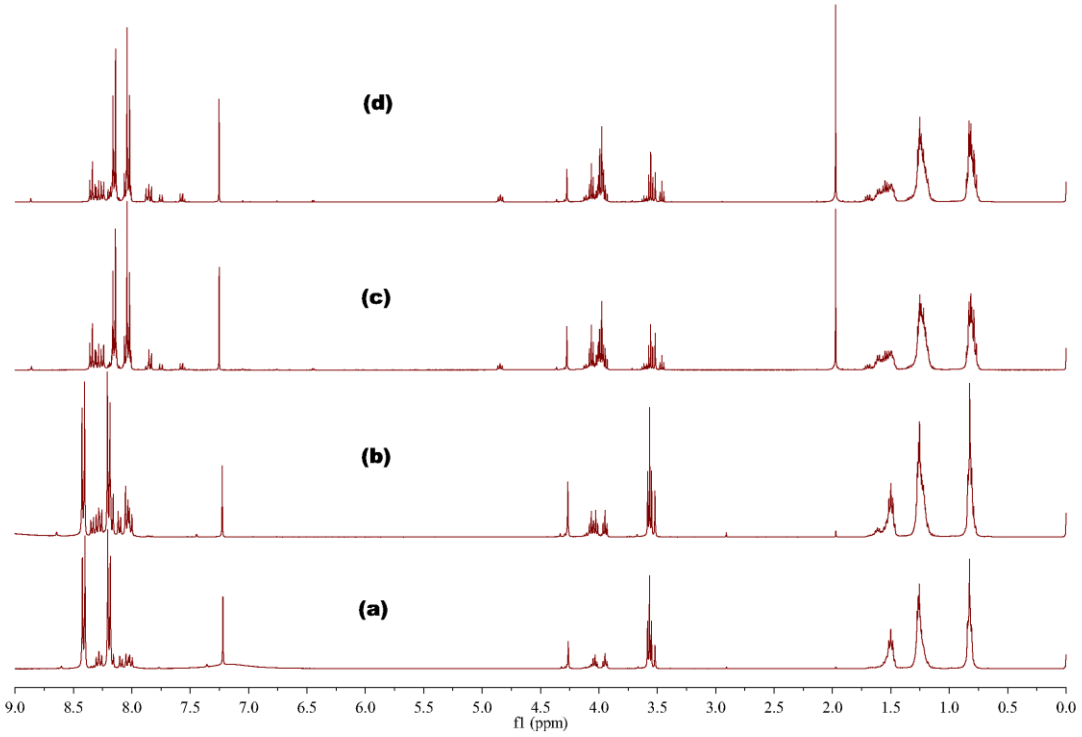


Fig. 2.10 The NMR spectra for: (a) The beginning of the reaction between **12** and **17**; (b) The reaction after 6 h, the peaks at 8.4 ppm and 8.2 ppm started to reduce as new peaks at 8.1 ppm and 8.0 ppm grew, indicating the decomposition of sulfonyl hydrazide. A new singlet at 2.0 ppm was formed. (b) The reaction after 48 hours, the doublets at 8.4 ppm and 8.2 ppm continued shifting to 8.1 ppm and 8.0 ppm, as the singlet at 2.0 ppm was increased. (d) The reaction after 5 d, the singlet at 2.0 ppm stopped growing, which indicated the decomposition was complete.

3. Conclusion

N-butyl hydrazinoacetate **30**, *iso*-butyl hydrazinoacetate **32**, *n*-propyl hydrazinoacetate **34**, 1-pentyl hydrazinoacetate **41** and *iso*-amyl hydrazinoacetate **39** was synthesized by TFA catalysed transesterification from ethyl hydrazinoacetate **12** and corresponding alcohols. *Iso*-propanol **35**, 2-butanol **36** and 3-pentanol **37** did not react with ethyl hydrazinoacetate **12** because of their steric hindrance. Allyl alcohol **42** and propargyl alcohol **43** gave multiple products during transesterification, presumably due to their unsaturated functionality. A good method for removing the high-boiling-point solvent and obtaining pure products was crystallizing the crude product in the freezer.

The acetone hydrazone **44** was synthesized in the reaction of ethyl hydrazinoacetate **12** and acetone, which aimed to shut down one reactive site on hydrazine. However, the addition of NPPOC-Cl **3** to the product hydrazone **44** was difficult due to the hydrolysis of the hydrazone group. Thus the protecting process was eliminated and NPPOC-Cl **3** was reacted with hydrazines to form the di-product **46 (b)** and isomers. The desired product **45** was isolated by column chromatography.

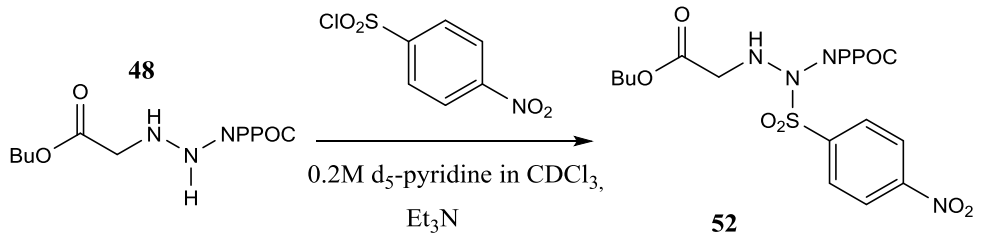
The NPPOC-protected hydrazines were then reacted with 4-nitrobenzenesulfonyl chloride **17** in an NMR tube. The processes of the reaction were monitored by NMR spectra. The sulponylation of the free amine was observed, but the transformation to the product was at a slow rate when triethylamine was not added. NPPOC-protected sulfonyl hydrazides were synthesized in the presence of triethylamine and no spontaneous decomposition was observed. Nevertheless, the purification of NPPOC-protected sulfonyl hydrazides encountered with difficulty, since they appeared to decompose on silica. Thus the crude products were used for the photolysis directly.

The photolysis of NPPOC-protected sulfonyl hydrazides didn't produce the expected product and the reaction products observed are yet remained to be characterised and explained.

Finally, the synthesis of diazene from NPPOC-protected ethyl hydrazide was carried out successfully, and the spontaneous decomposition of the para-nitrophenyl sulfonyl hydrazide obtained from 1-pentyl hydrazinoacetate was observed by ^{1}H NMR spectrocopy.

4. Future Work

4.1 Studies of sulfonyl hydrazides



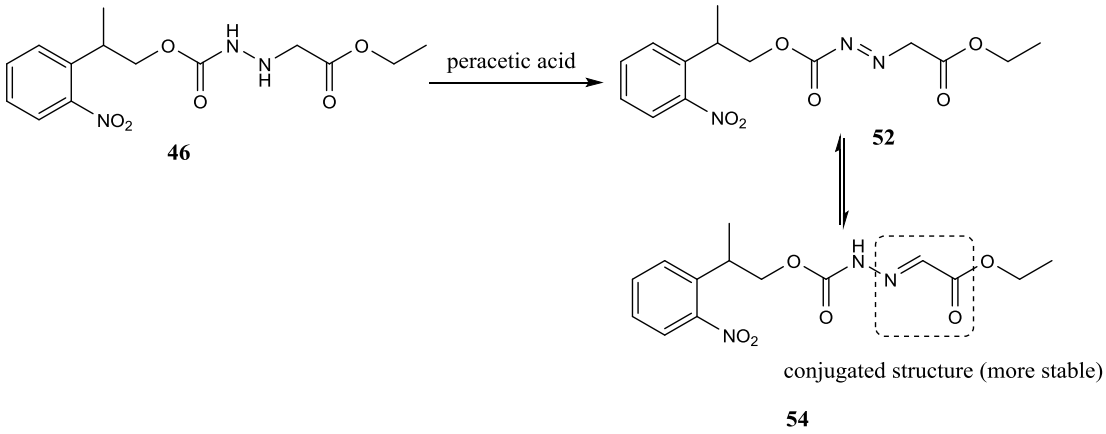
Scheme 4.1 Synthesis of NPPOC-protected butyl sulfonyl hydrazide **52**.

The synthesis of NPPOC-protected butyl sulfonyl hydrazide will be repeated as the above scheme to obtain enough product **52**, which can be purified by column chromatography. Then the pure product can be irradiated to cleave the photo protected group, which is expected to produce fewer impurities.

Except ethyl and n-butyl hydrazinoacetates, other hydrazines will react with NPPOC-Cl to be attached with the photo protect group and then synthesize the sulfonyl hydrazide with the same condition as ethyl or n-butyl hydrazides.

4.2 Photolysis of NPPOC-protected sulfonyl hydrazides

Since the ^{1}H NMR spectra of photolyzed products from NPPOC-protected sulfonyl hydrazides didn't show the expected cleavage of photo protected group, which may due to the short reaction time, the NPPOC-protected sulfonyl hydrazides will be irradiated for longer time and the photolysis process will be monitored by NMR spectra.



Scheme 4.2 Synthesis of diazene **52**.

The diazene **52** will be synthesized with the above synthesis and then be irradiated with light, which will be monitored. Theoretically, the cleavage of the NPPOC group will be shown on the ^{1}H NMR spectra.

4.3 Decomposition studies

Although the decomposition of the 1-pentyl sulfonyl hydrazide was shown on the ^{1}H NMR spectra, the attempt for collecting the product, 1-pentyl acetate by the column chromatography using d_6-benzene was failed. Other non-polar solvent such as DCM will be used in the column chromatography to wash the product out, which will then be characterized.

5 Experimental Section

5.1 General methods

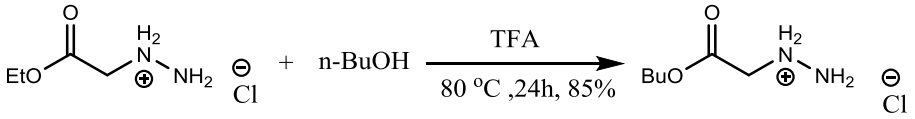
All reactions were carried out in dried glassware under a nitrogen atmosphere. When dry solvents were necessary, they were obtained after distillation. Otherwise, all chemicals and solvents were from commercial suppliers (Sigma-Aldrich, Fisher Scientific) and were of analytical grade. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{245} pre-coated plates and visualized by (1) UV light with fluorescent indicator 254 nm, (2) dipping the plates into staining solution (phosphomolybdic acid in ethanol). Column chromatography was carried out using silica gel.

All NMR spectra including ^{1}H, ^{13}C, COSY and HMQC were measured on Bruker 400 MHz Fourier transform spectrometers with an internal deuterium lock. Chemical shifts are quoted in parts per million (ppm) and coupling constants (*J*) are quoted in Hertz (Hz). TMS was defined as 0 ppm for ^{1}H NMR. Abbreviations will be used when reporting the multiplicities as following: singlet (s), doublet (d), triplet(t), quartet (q), doublet of doublets (dd), multiplet (m) and broad singlet(br.s).

Low resolution mass spectrometry was carried out by staff in the Mass Spectroscopy Laboratory, the School of Chemistry using electrospray ionization (ES) and methanol as solvent. Infra-red (IR) spectra were performed using a Perkin Elmer FT-IR instrument. Absorption (υ_{max}) was recorded in wavenumbers (cm^{-1}).

5.2 Synthetic Methods

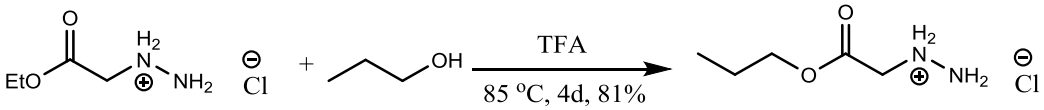
Butyl hydrazinoacetate chloride 30



A mixture of ethyl hydrazinoacetate chloride **12** (1.54 g, 9.96 mmol), trifluoroacetic acid (1.05 mL, 13.63 mmol), and n-BuOH **29** (10 mL) was stirred at 80°C for 24h. The solvent was removed *in vacuo* to give the pure product as a colorless solid (1.24 g, 8.48 mmol, 85%).

^{1}**H NMR** (400 MHz, MeOD): δ_H 4.21 (2H, t, ^{3}J = 7.1 Hz, CH_2-*C*H_2-O), 3.79 (2H, s, CO-*C*H_2-NH), 1.67 (2H, m, ^{3}J = 8.0 Hz, *C*H_2-CH_2-O), 1.43 (2H, m, ^{3}J =8.0 Hz, CH_3-*C*H_2-CH_2), 0.98 (3H, t, ^{3}J = 8.0 Hz, *C*H_3-CH_2-CH_2); ^{13}**C NMR** (100 MHz, MeOD): δ_c=69.0, 52.1, 34.2, 22.6, 16.5 (missing 1 signal for C=O) ; **IR** (cm^{-1}): 3279.87 (N-H), 1727.25 (C=O), 1590.60, 1518.70, 1404.85, 1355.25, 1235.51, 1209.52, 1093.47; ***m/z* (ES$^+$)** 147.1 ([M+H]$^+$, 100%); **HRMS (ES$^+$)**: calcd for [M+H]$^+$: 147.1133, found: 147.1129 (100%).

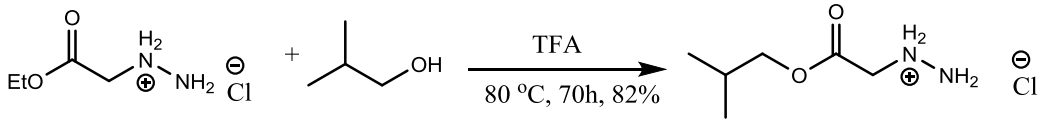
1-Propyl hydrazinoacetate chloride 34



A mixture of ethyl hydrazinoacetate chloride **12** (103.0 mg, 0.64 mmol), trifluoroacetic acid (0.067 mL, 0.87 mmol), and 1-propanol (5 mL) was stirred at 85°C for 4d. The solvent was removed *in vacuo* to give the pure product as colorless solid (68.5 mg, 0.51 mmol, 81%).

^{1}H NMR (400 MHz, MeOD): δ_H 4.19 (2H, t, ^3J =8.19 Hz, CH_2-*CH_2*-O), 3.84 (2H, s, CO-*CH_2*-NH), 1.71 (2H, m, ^3J =8.0 Hz, CH_3-*CH_2*-CH_2), 0.99 (3H, t, ^3J =8.0 Hz, *CH_3*-CH_2-CH_2); **^{13}C NMR** (100 MHz, MeOD): δ_c=70.8, 51.8, 25.5, 13.1 (missing 1 signal for C=O); **IR** (cm^{-1}) : 3373.38 (N-H), 1724.78 (C=O), 1402.96, 1358.06, 1220.73, 1157.81; ***m/z* (ES$^+$)** 133.0 ($[M+H]^+$, 100%); **HRMS (ES$^+$)**: calcd for $[M+H]^+$: 133.0977, found: 133.0969 (100%).

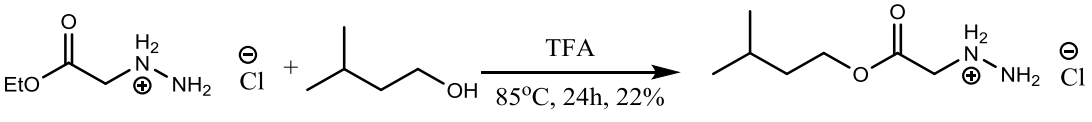
Iso-butyl hydrazinoacetate chloride 32



A mixture of ethyl hydrazinoacetate chloride **12** (105.0 mg, 0.68 mmol), trifluoroacetic acid (0.067 mL, 0.87 mmol), and *iso*-butanol (5 mL) was stirred at 80°C for 70h. The solvent was removed *in vacuo* to give the pure product as colorless solid (81.5 mg, 0.57 mmol, 82%).

^{1}H NMR (400 MHz, MeOD): δ_H 4.02 (2H, d, ^3J =8.0 Hz, CH-*CH_2*-O), 3.81 (2H, s, CO-*CH_2*-NH), 1.99 (1H, m, ^3J =8.0 Hz, CH_3-*CH*-CH_3), 0.98 (6H, d, ^3J =8.0 Hz, *$(CH_3)_2$*-CH); **^{13}C NMR** (100MHz, MeOD): δ_c=81.7, 75.3, 52.0, 31.4, 22.0 (missing 1 signal for C=O); **IR** (cm^{-1}) : 3280.62 (N-H), 1728.88 (C=O), 1590.72, 1473.22, 1400.88, 1383.24, 1228.30, 1094.11; ***m/z* (ES$^+$)** 147.2 ($[M+H]^+$, 100%); **HRMS (ES$^+$)**: calcd for $[M+H]^+$: 147.1133, found: 147.1130 (100%).

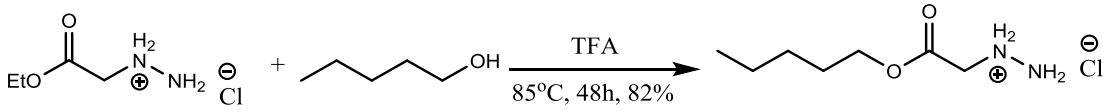
Iso-amyl hydrazinoacetate chloride 39



A mixture of ethyl hydrazinoacetate chloride **12** (500.00 mg, 3.24 mmol), trifluoroacetic acid (0.335 mL, 4.35 mmol), and *iso*-amyl alcohol (3 mL) was stirred at 85°C for 24h. The solvent was removed to give the crude product as orange oil, which was washed by diethyl ether and crystallized in the freezer. The crystals were filtered and washed with petroleum ether to give the pure product as an orange solid (114mg, 0.71 mmol, 22%).

^{1}**H NMR** (400 MHz, MeOD): δ_H 4.27 (2H, t, ^{3}J =8.0 Hz, CH_2-*CH_2*-O), 3.79 (2H, s, CO-*CH_2*-NH), 1.78 (1H, m, ^{3}J =8.0 Hz, $(CH_3)_2$-*CH*), 1.60 (2H, q, ^{3}J =8.0 Hz, CH-*CH_2*-CH_2), 0.97 (6H, d, ^{3}J =8.0 Hz, *$(CH_3)_2$*-CH); ^{13}**C NMR** (100 MHz, MeOD): δ_c=169.9, 63.9, 49.4, 36.9, 24.7, 21.4; **IR** (cm^{-1}): 3282.88 (N-H), 1728.94 (C=O), 1401.75, 1353.83, 1220.54; ***m/z* (ES$^+$)** 161.2 ($[M+H]^+$, 34%); **HRMS (ES$^+$)**: calcd for $[M+H]^+$: 161.1290, found: 161.1289 (100%).

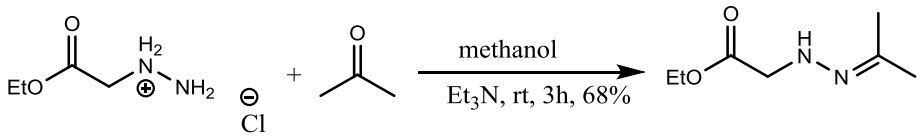
1-Pentyl hydrazinoacetate chloride 41



A mixture of ethyl hydrazinoacetate chloride **12** (500 mg, 3.24 mmol), trifluoroacetic acid (1.05 mL, 4.35 mmol), and 1-pentanol (3 mL) was stirred at 85°C for 48h. The mixture was crystallized in the freezer to give the crude product as yellow solid, and the filtrate was washed by petroleum ether to give the pure product as a colorless solid (425.92 mg, 2.66 mmol, 82%).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 4.23(2H, t, ^{3}J =8.0 Hz, CH_2-*CH_2*-O), 3.80 (2H, s, CO-*CH_2*-NH), 3.34 (2H, m, ^{3}J =4.0 Hz, CH_2-*CH_2*-CH_2-O), 1.72 (2H, m, ^{3}J =4.0 Hz, CH_2-CH_2-*CH_2*-CH_2), 1.40 (2H, m, ^{3}J =8.0 Hz, CH_2-*CH_2*-CH_2-CH_2), 1.39 (2H, m, ^{3}J =4.0 Hz, *CH_2*-CH_2-CH_2-CH_2), 0.95 (3H, t, ^{3}J =8.0 Hz, *CH_3*-CH_2-CH_2); **^{13}C NMR** (100 MHz, $CDCl_3$): δ_c=65.4, 61.6, 49.4, 32.0, 27.8, 22.2, 13.0; **IR** (cm^{-1}) : 3274.97 (N-H), 1727.56 (C=O), 1579.31, 1205.29; ***m/z* (ES$^+$)** 161.1 ($[M+H]^+$, 100%); **HRMS (ES$^+$)**: calcd for $[M+H]^+$: 161.1290, found: 161.1288 (100%).

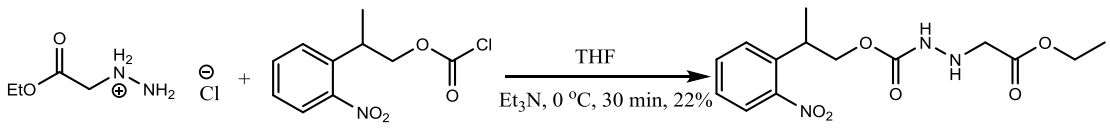
Ethyl (propan-2-ylideneamino)glycinate 44



Acetone (4 mL, excess) was added into a solution of ethyl hydrazinoacetate chloride **12** (618.4 mg, 4 mmol) and triethylamine (2.8 mL) in methanol (5 mL) and the reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was diluted with de-ionized water (15 mL) and DCM (15 mL). The resulting aqueous layer was extracted with DCM (3 × 10 mL) and the combined organic layers were washed with de-ionized water, dried over magnesium sulfate, filtered and concentrated *in vacuo* to give the pure product as a colorless solid (430.3 mg, 2.72 mol, 68%)

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 4.12 (2H, q, ^{3}J = 7.15 Hz, CH_3-*CH_2*-O), 3.83 (2H, s, CO-*CH_2*-NH), 1.87 (3H, s, N=C-(*CH_3*)$_2$) ,1.76 (3H, s, N=C-(*CH_3*)$_2$), 1.21 (3H, t, *CH_3*-CH_2-O) ; **^{13}C NMR** (100MHz, $CDCl_3$): δ_c 172.3, 60.9, 52.1, 31.0, 25.2, 15.9, 14.2; ***m/z* (ES$^+$)** 159.16 ($[M+H]^+$, 70%).

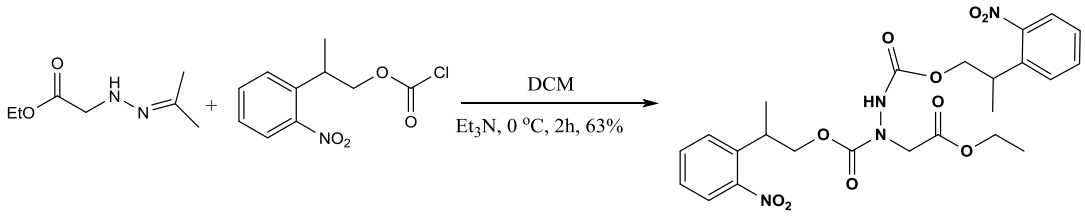
NPPOC-protected ethyl hydrazinoacetate 49 [55]



A solution of ethyl hydrazinoacetate chloride **12** (309.2 mg, 2 mmol), triethylamine (0.7 mL, 5 mmol) in dry THF was stirred in an ice water bath, and NPPOC-Cl (0.183 mL, 1 mmol) was added into the reaction dropwise. The reaction was left stirring at 0°C for 30 min. The solvent was removed *in vacuo* to give the crude produce as brown oil, which was purified by column chromatography (4:1-2:1 petroleum ether: ethyl acetate) to form the pure product as yellowish oil (70.7 mg, 0.22 mmol, 22%).

^{1}**H NMR** (400 MHz, $CDCl_3$): δ_H 7.73 (1H, d, ^3J = 8.0 Hz, NO_2-Ar (*CH*)), 7.56 (1H, t, ^3J = 8.0 Hz, NO_2-Ar(*CH*)), 7.38 (1H, d, ^3J = 8.0 Hz, NO_2-Ar(*CH*)), 7.38 (1H, t, ^3J = 8.0 Hz, NO_2-Ar(*CH*)), 3.71 (1H, m, ^3J = 4.0 Hz, CH_3-*CH*-CH_2), 3.60 (2H, s, NH-*CH_2*-CO), 4.21 (2H, m, ^3J = 8.0 Hz, CH-*CH_2*-O), 1.35 (3H, d, ^3J = 8.0 Hz, *CH_3*-CH-CH_2), 1.29 (3H, t, ^3J = 8.0 Hz, *CH_3*-CH_2-O); **IR** (cm^{-1}) : 2980.38 (N-N), 1743.75, 1704.43 (C=O), 1523.36 (C=C), 1351.56 (NO_2), 1119.18, 1025.08; ***m/z* (ES$^+$)** 326.1 ($[M+H]^+$, 40%), 348.1 ($[M+Na]^+$, 100%); **HRMS (ES$^+$)**: calcd for $[M+Na]^+$: 348.1172, found: 348.1183 (100%).

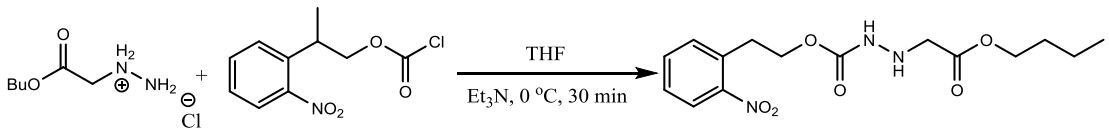
Di-NPPOC-protected ethyl hydrazide 46 (b)



NPPOC-Cl **3** (0.36 ml, 2 mmol) was added dropwise in the solution of hydrazone **44** (316.4 mg, 2 mmol) in DCM at 0 °C. The reaction was allowed to be stirred for 1 hour and then NPPOC-Cl **3** (0.36 ml, 2 mmol) was added into the solution to react for one more hour. The crude product was purified by column chromatography to give the product as brown oil (671.0 mg, 1.26 mmol, 63%). The product was shown to be highly impure by ^{1}H NMR, thus no further characterisation was carried out.

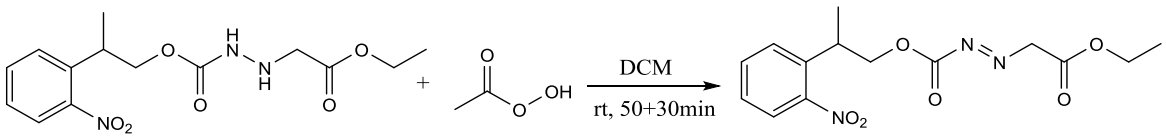
***m/z* (ES$^+$)** 555.3 ([M+Na]$^+$, 50%); **HRMS (ES$^+$)**: calcd for [M+Na]$^+$: 555.1703, found: 555.1703 (100%).

NPPOC-protected butyl hydrazinoacetate 51



A solution of ethyl hydrazinoacetate chloride (365.3 mg, 2 mmol), triethylamine (0.7 mL, 5 mmol) in dry THF was stirred in an ice water bath, and NPPOC-Cl **3** (0.183 mL, 1mmol) was added into the reaction dropwise. The reaction was left stirring at 0°C for 30 min. The solvent was removed *in vacuo* to give the crude produce as brown oil, which was purified by column chromatography (4:1-2:1 petroleum ether: ethyl acetate) to give the product as yellowish oil (66.0 mg, 0.19 mmol). The product was shown to be highly impure by ^{1}H NMR, thus no further characterisation was carried out.

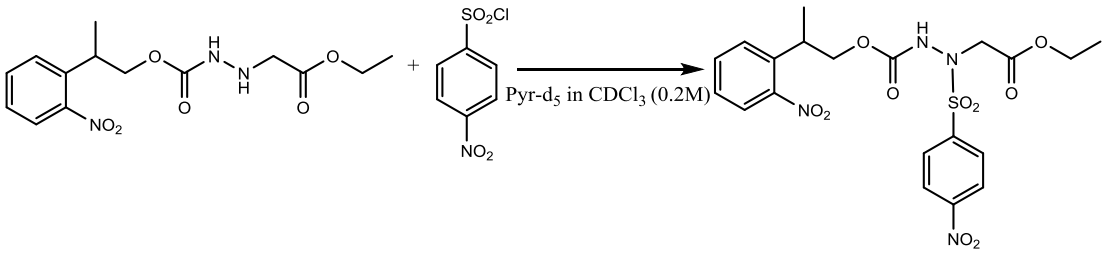
2-(2-nitrophenyl)propyl (E)-2-(2-ethoxy-2-oxoethyl)diazene-1-carboxylate 54 [59]



The mixture of NPPOC-protected ethyl hydrazide **49** (40%) and **50** (60%) (42.3 mg, 0.13 mmol) was dissolved in DCM (3.4 mL) at the concentration of 80 mL/g, and peracetic acid (0.024 mL, 0.14 mmol) was added in the solution. The reaction was stirred at room temperature for 50 min. The reaction mixture was then diluted with a 2% solution of sodium bicarbonate (15 mL) and DCM (15 mL). The resulting aqueous layer was extracted with DCM (3 × 10 mL) and the combined organic layers were washed with a 2% solution of sodium bicarbonate, dried over magnesium sulfate, filtered and concentrated *in vacuo* to form the crude product as yellowish oil. The ^{1}H NMR spectrum of the crude product was obtained, and then DCM (1.596 mL) and peracetic acid (0.010 mL, 0.07 mmol) were added in the product and the reaction was stirred again for 30 min. The reaction mixture was again diluted with a 2% solution of sodium bicarbonate (15 mL) and DCM (15 mL). The resulting aqueous layer was extracted with DCM (3 × 10 mL) and the combined organic layers were washed with 2% solution of sodium bicarbonate, dried over magnesium sulfate, filtered and concentrated *in vacuo* to form the crude product as yellowish oil. The product was shown to be highly impure by ^{1}H NMR, thus no further characterisation was carried out.

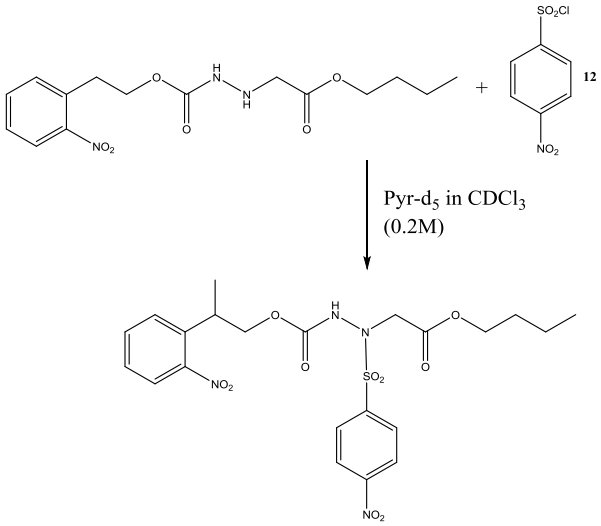
5.3 ^{1}H NMR spectra monitored reactions

Addition of 4-nitrobenzene sulfonyl chloride 17 on NPPOC-protected ethyl hydrazide 49



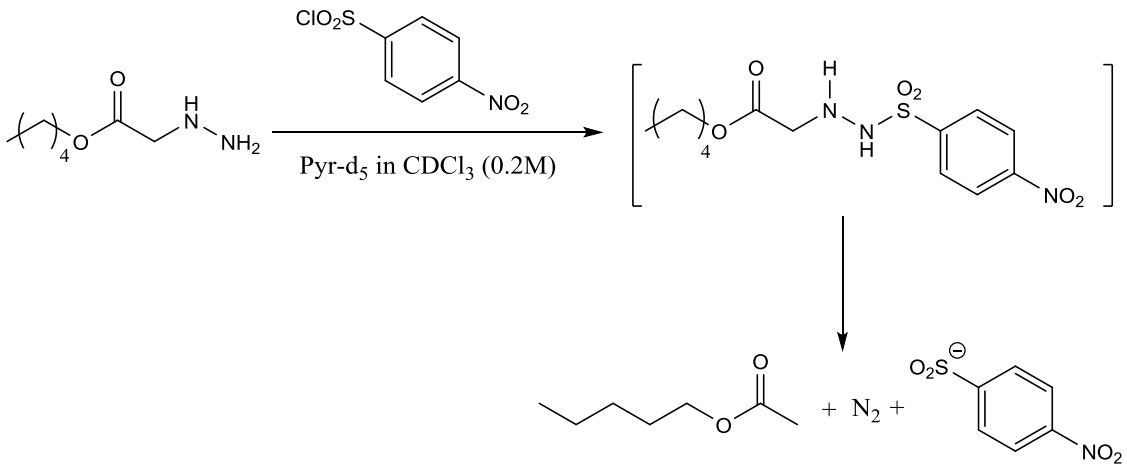
NPPOC-protected ethyl hydrazinoacetate chloride (44.8 mg, 0.14 mmol), triethylamine (0.09 mL, 0.07 mmol) and *para*-nitrobenzenesulfonyl chloride **17** (30.4 mg, 0.14 mmol) were dissolved in the solvent of 0.2M d_5-pyridine in $CDCl_3$ (0.8 mL) and the reaction was monitored by ^{1}H NMR spectroscopy.

Addition of 4-nitrobenzene sulfonyl chloride 17 on NPPOC-protected *n*-butyl hydrazide 51 and 52



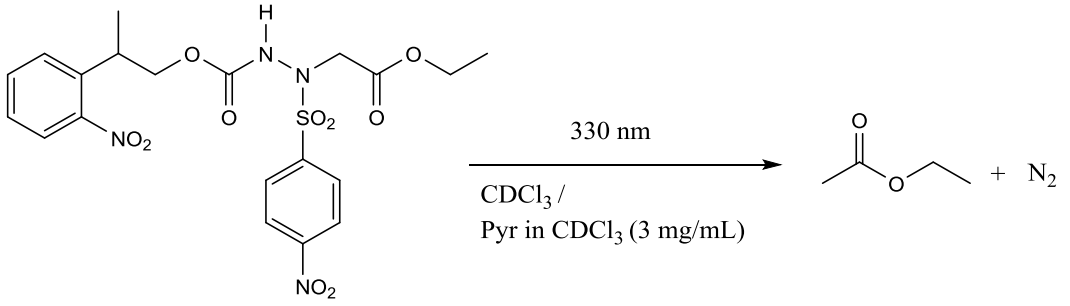
A mixture of NPPOC-protected ethyl hydrazinoacetate chloride **51** (90%) and **52** (10%) (16.0 mg, 0.10 mmol), triethylamine (0.09 mL, 0.07 mmol) and *para*-nitrobenzenesulfonyl chloride **17** (22.1 mg, 0.10 mmol) were dissolved in the solvent of 0.2M d_5-pyridine in $CDCl_3$ (0.8 mL) and the reaction was monitored by ^1H NMR spectroscopy.

The decomposition study of sulfonyl hydrazide

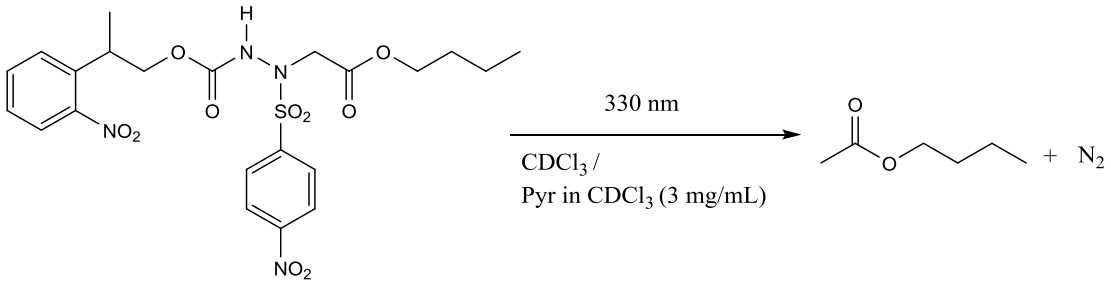


The 1-pentyl hydrazinoacetate **40** (24.5 mg, 0.08 mmol), 4-nitrobenzenesulfonyl chloride **17** (20.1 mg, 0.08 mmol) and triethylamine (0.05 mL, 0.04 mmol) were dissolved in 0.2M d_5-pyridine in $CDCl_3$ (0.8mL) and the reaction was monitored by ^1H NMR spectroscopy.

5.4 Photolysis of NPPOC-protected sulfonyl hydrazides 53 and 56



Crude NPPOC-protected ethyl sulfonyl hydrazide (15 mg, 0.03 mmol) was divided into two equal samples, one was dissolved in a solution of $CDCl_3$ at concentration of 3 mg/mL, and the other sample was dissolved in a solution of 0.2M d_5-pyridine in $CDCl_3$ at concentration of 3 mg/mL. Each solution was transferred in a quartz cuvette and then irradiated by light of wavelength 330 nm for 15 minutes. The solvent was then removed and the ^{1}H NMR spectrum of the reaction mixture was obtained.

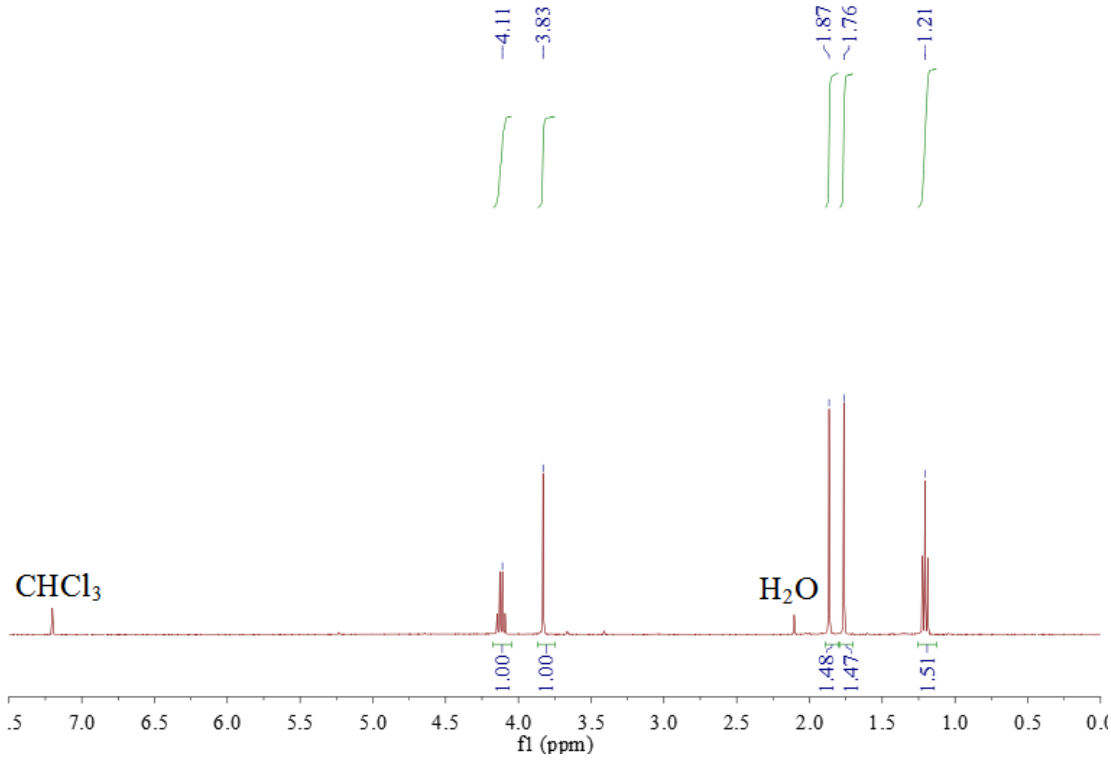


Crude NPPOC-protected butyl sulfonyl hydrazide (15 mg, 0.03 mmol) was divided into two equal samples, one was dissolved in a solution of $CDCl_3$ at concentration of 3 mg/mL, and the other sample was dissolved in a solution of 0.2M d_5-pyridine in $CDCl_3$ at concentration of 3 mg/mL. Each solution was transferred in a quartz cuvette and then irradiated by light of wavelength 330 nm for 15 minutes. The solvent was then removed and the ^{1}H NMR spectrum of the reaction mixture was obtained.

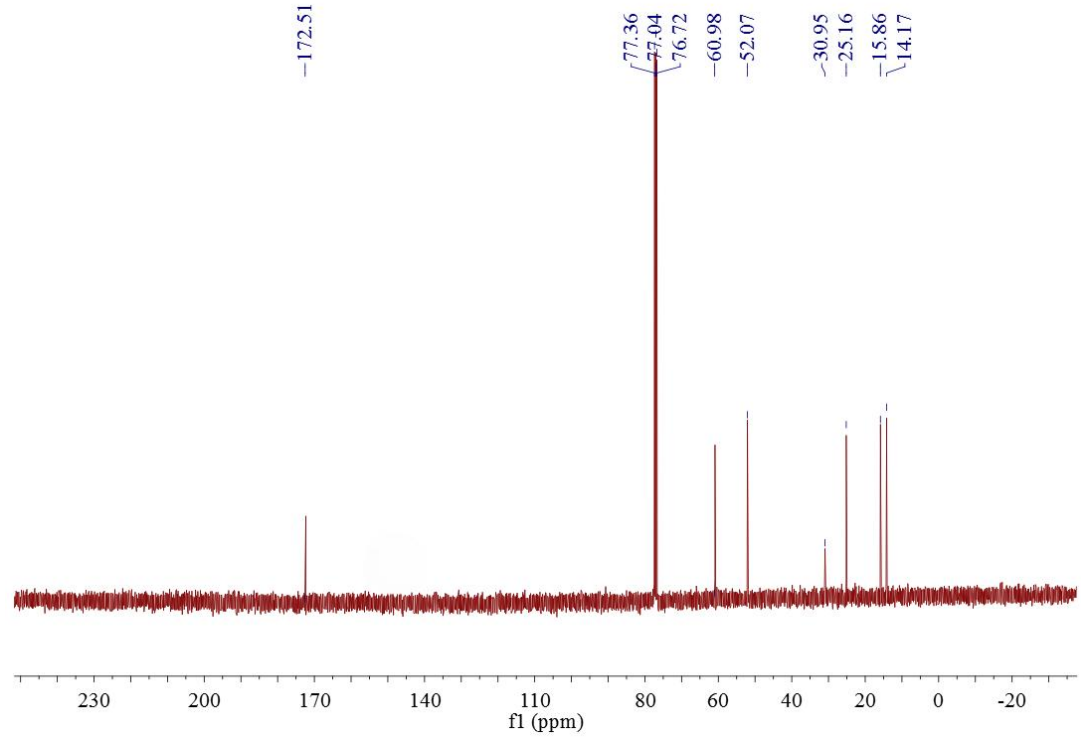
Appendices

A1. Ethyl (propan-2-ylideneamino)glycinate 44

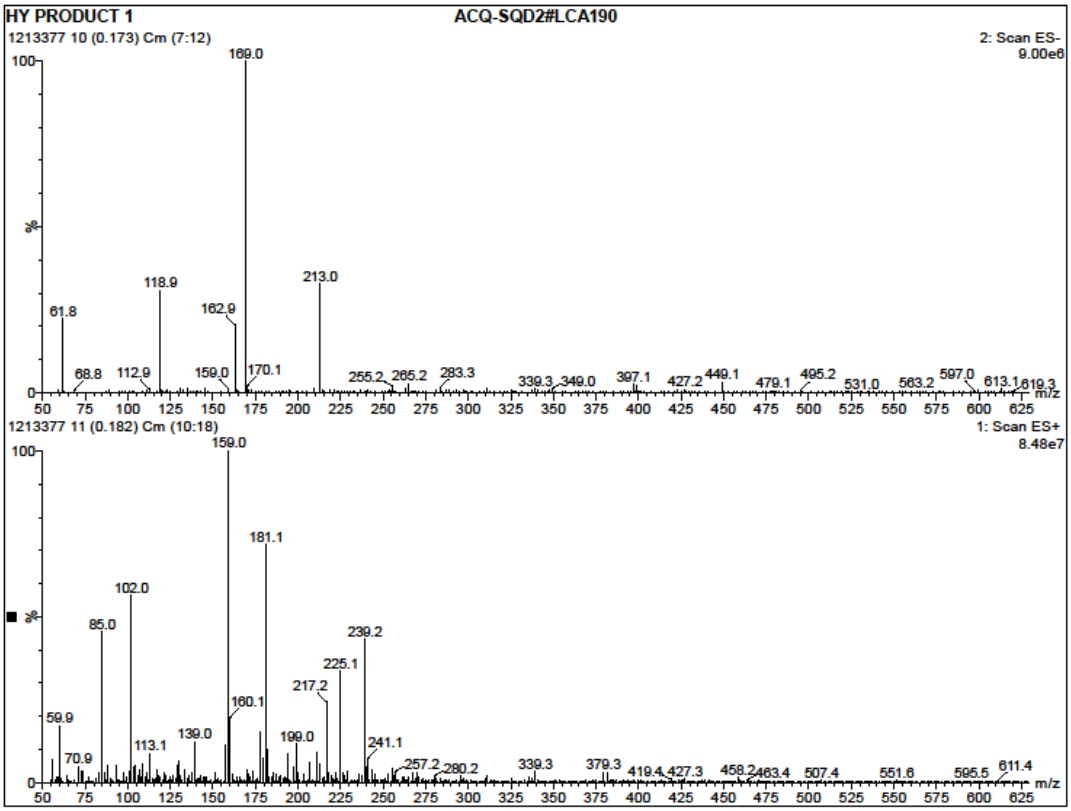
^{1}H NMR (400 MHz, in $CDCl_3$)



^{13}C NMR (400 MHz, in $CDCl_3$)

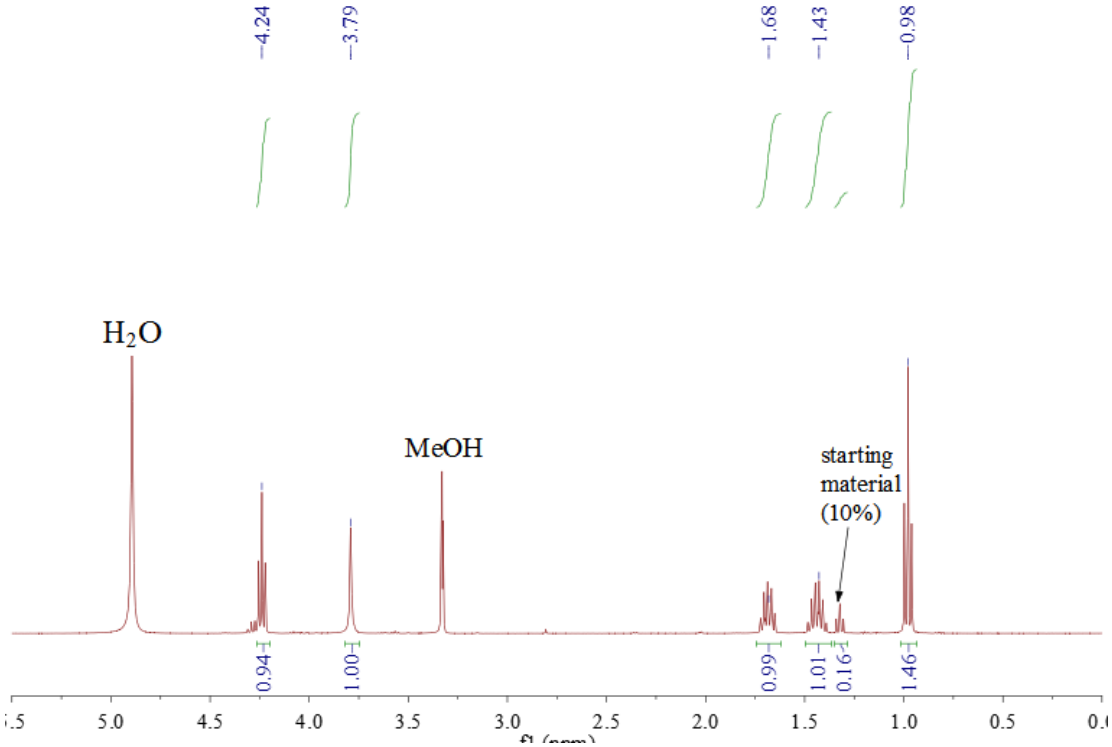


MS

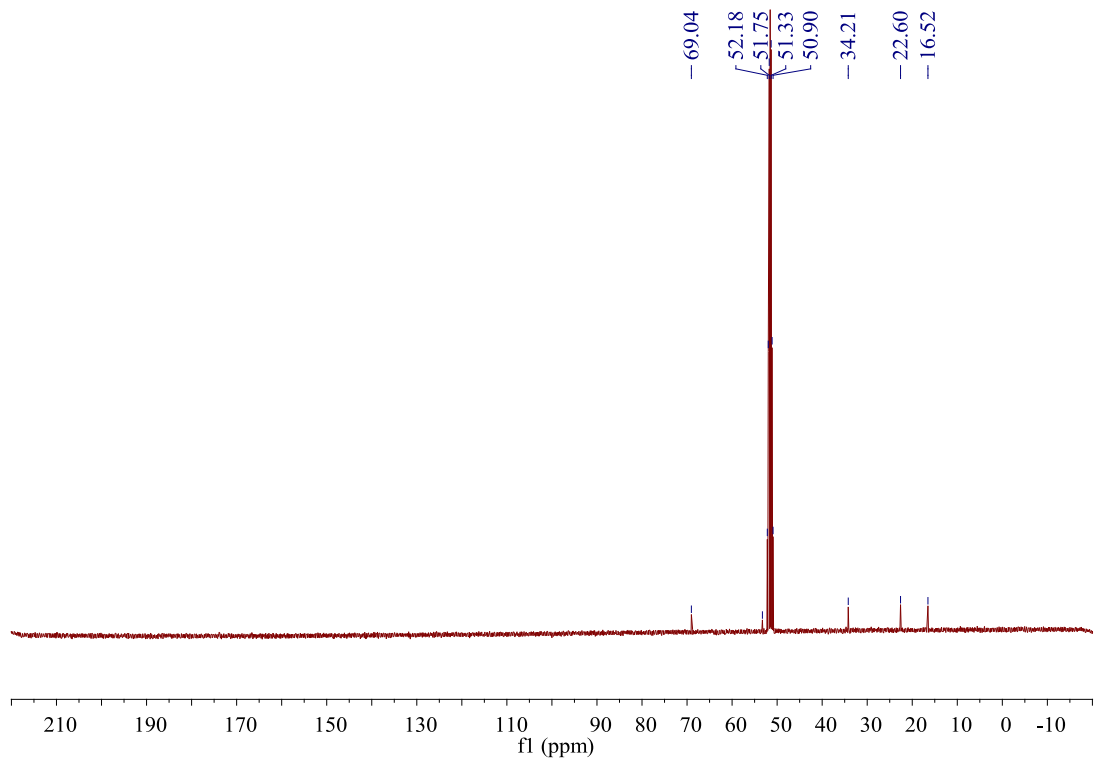

HY PRODUCT 1
ACQ-SQD2#LCA190
1213377 10 (0.173) Cm (7:12)
2: Scan ES-
9.00e6
169.0
213.0
118.9
162.9
61.8
68.8
112.9
159.0
170.1
255.2
265.2
283.3
339.3
349.0
397.1
427.2
449.1
479.1
495.2
531.0
563.2
597.0
613.1
619.3
m/z
1213377 11 (0.182) Cm (10:18)
1: Scan ES+
8.48e7
159.0
181.1
102.0
85.0
239.2
225.1
217.2
160.1
59.9
70.9
113.1
139.0
199.0
241.1
257.2
280.2
339.3
379.3
419.4
427.3
458.2
463.4
507.4
551.6
595.5
611.4
m/z

A2. *N*-butyl hydrazinoacetate chloride 30

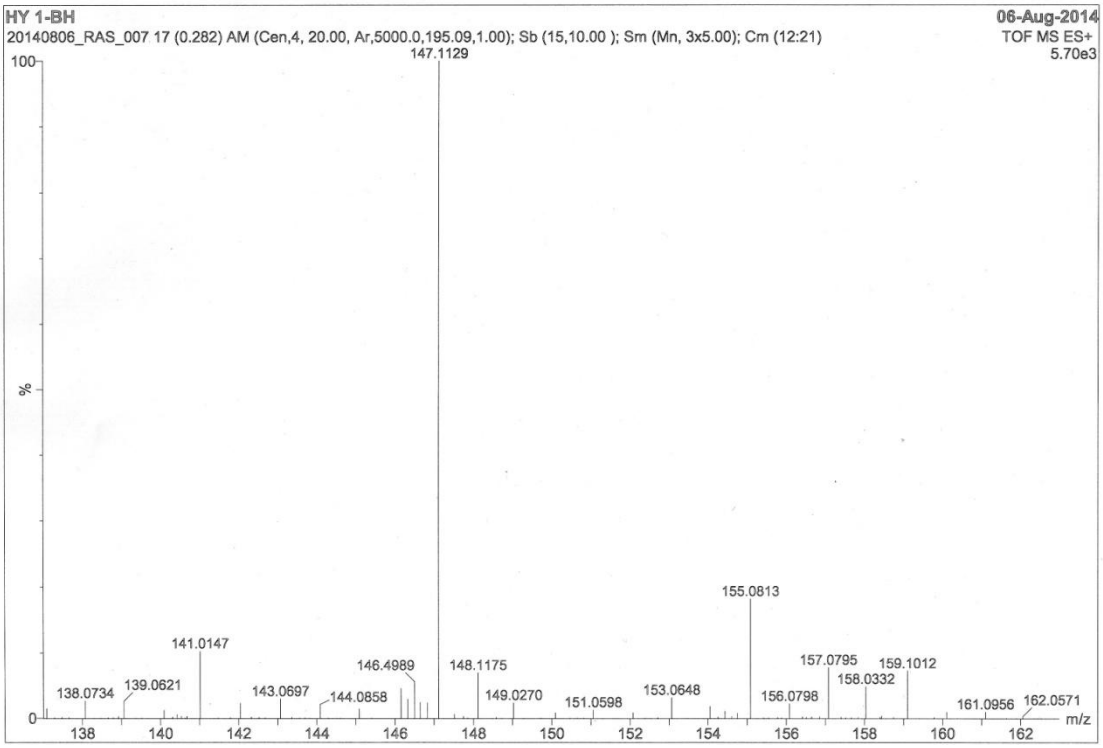
^{1}H NMR (400 MHz, in MeOD)



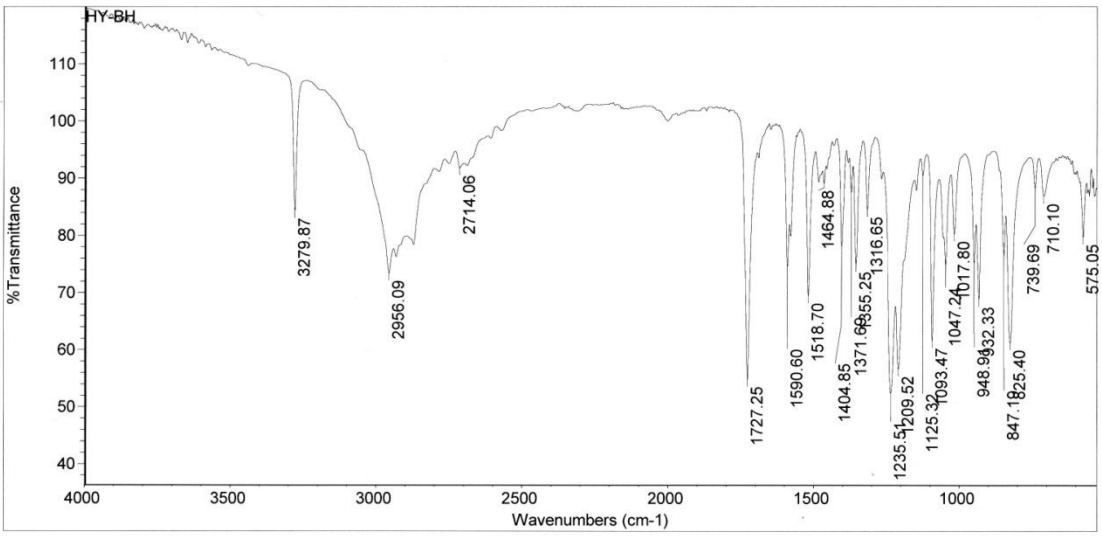
^{13}C NMR (400 MHz, in MeOD)



HRMS



IR



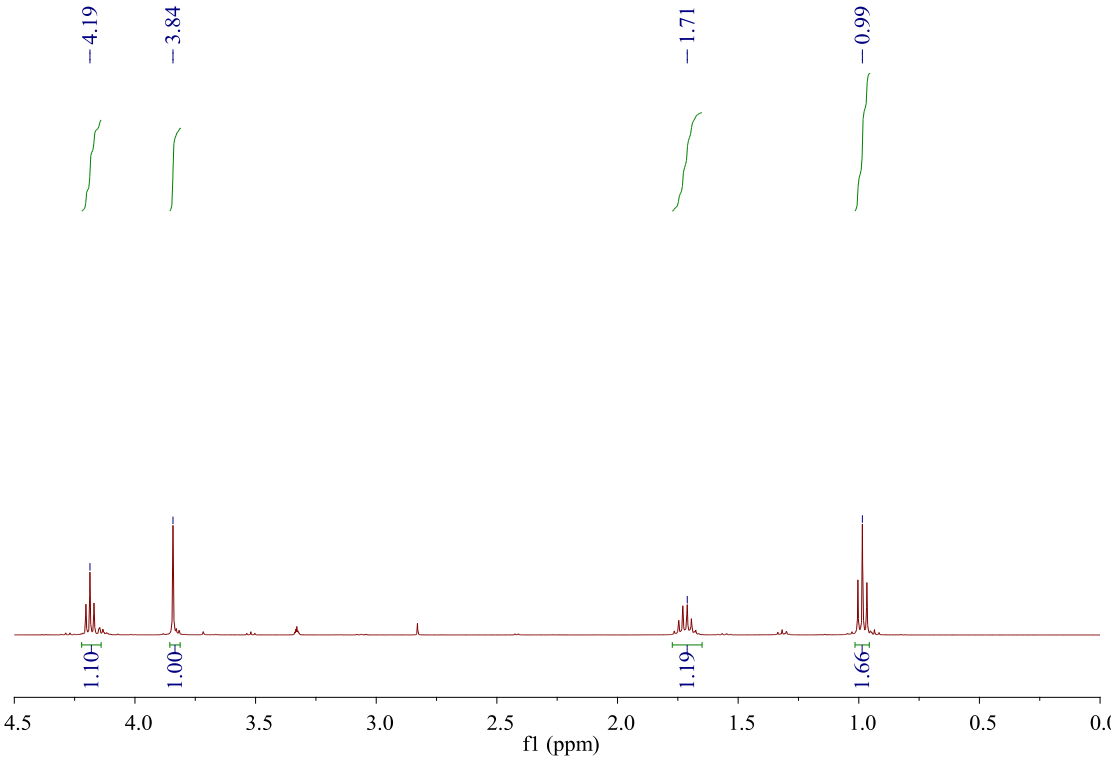
Fri Jul 25 15:44:11 2014 (GMT+01:00)
FIND PEAKS:

Spectrum:	HY-BH		
Region:	4000.12	529.85	
Absolute threshold:	93.177		
Sensitivity:	50		
Peak list:			

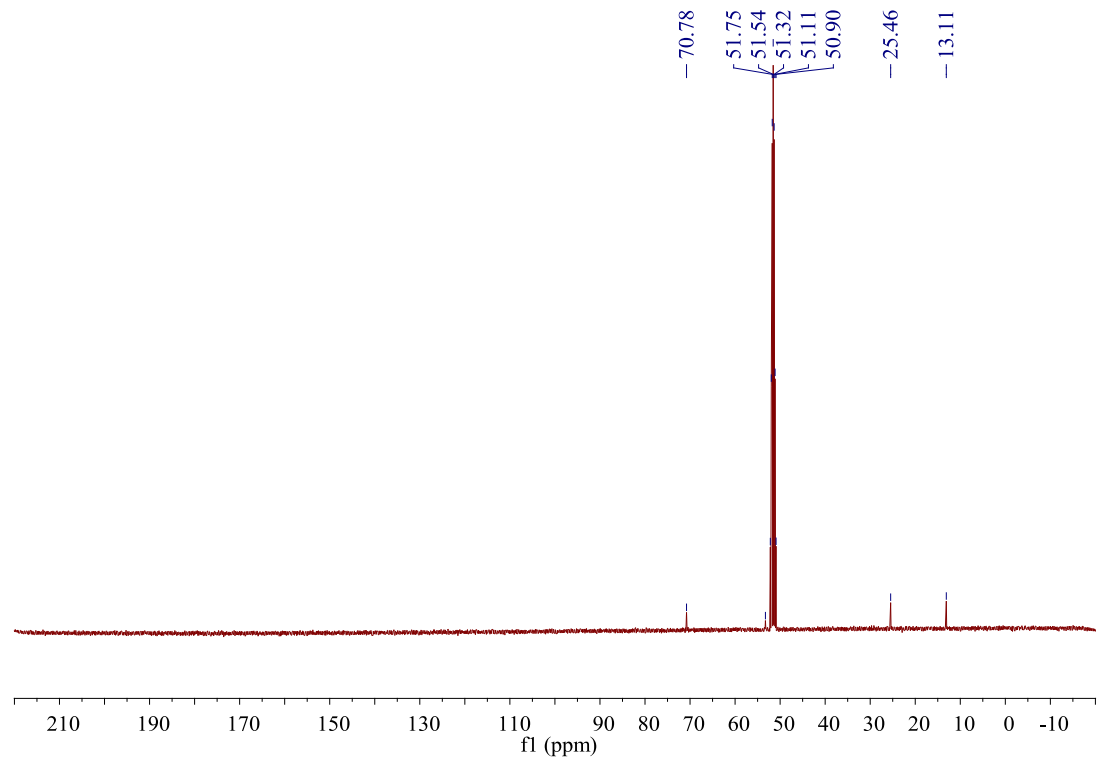
Position:	575.05	Intensity:	79.690
Position:	710.10	Intensity:	86.888
Position:	739.69	Intensity:	88.243
Position:	825.40	Intensity:	61.194
Position:	847.19	Intensity:	76.518
Position:	932.33	Intensity:	68.767
Position:	948.91	Intensity:	75.075
Position:	1017.80	Intensity:	80.292

A3. 1-Propyl hydrazinoacetate chloride 34

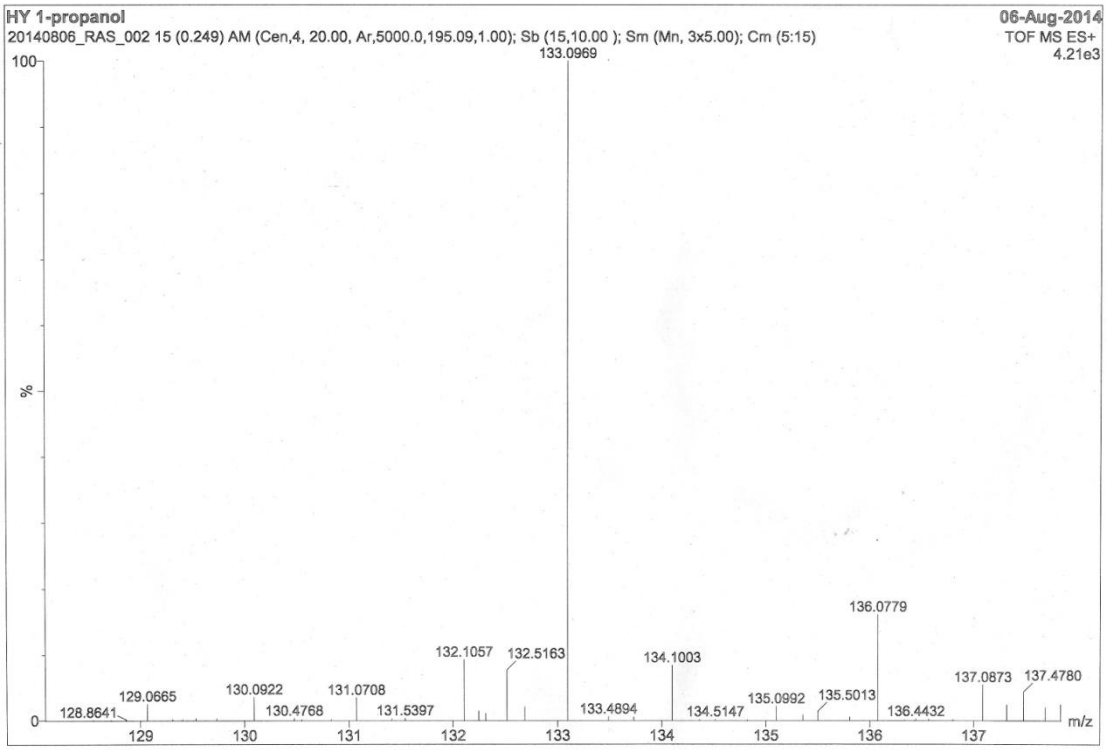
^{1}H NMR (400 MHz, in MeOD)



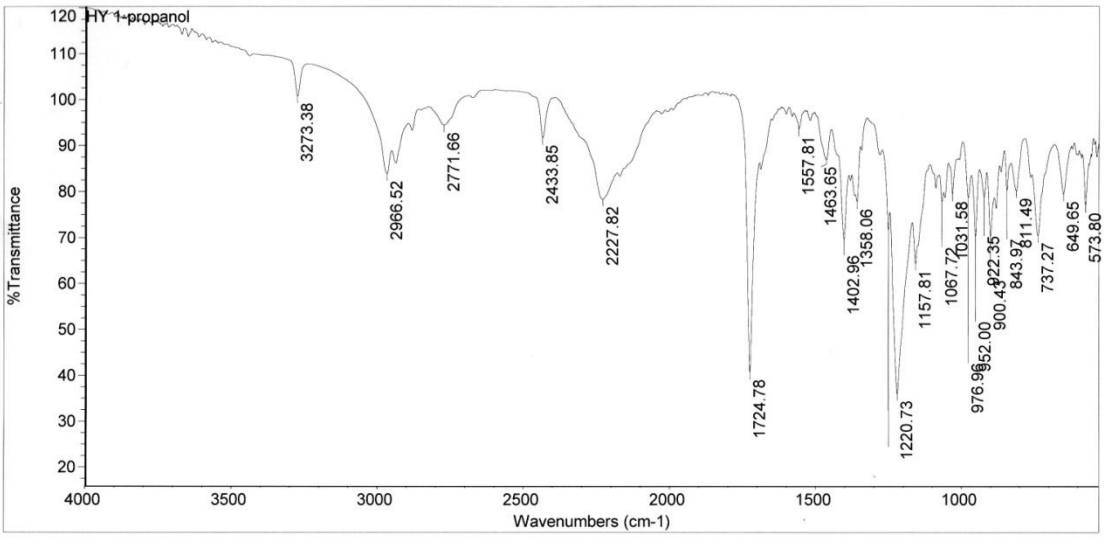
^{13}C NMR (400 MHz, in MeOD)



HRMS



IR

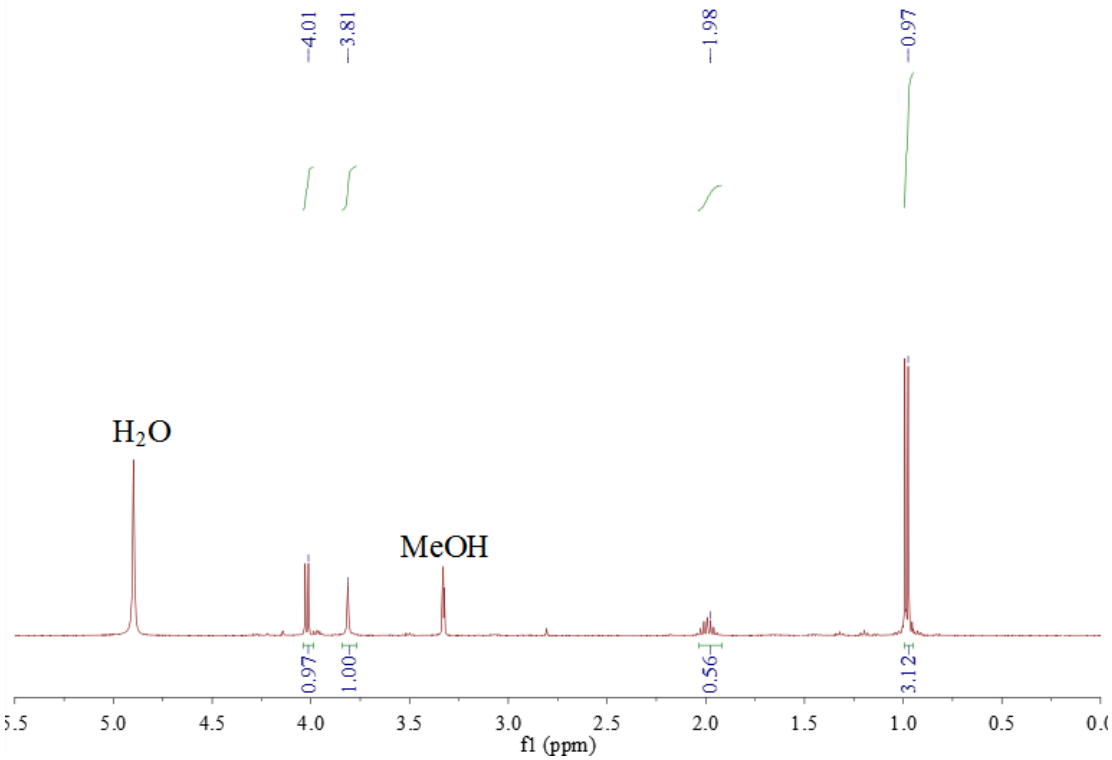


Fri Jul 25 16:04:23 2014 (GMT+01:00)
FIND PEAKS:

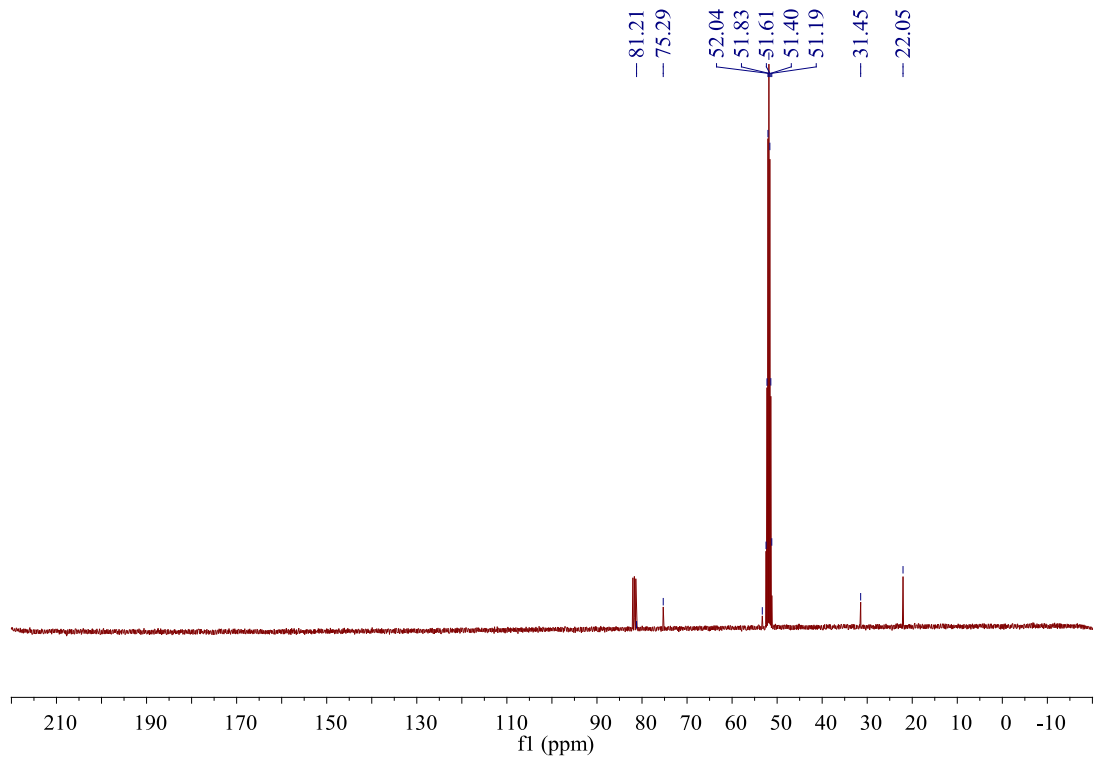
Spectrum:	HY 1-propanol			
Region:	4000.12	529.85		
Absolute threshold:	103.837			
Sensitivity:	50			
Peak list:				
	Position:	573.80	Intensity:	76.925
	Position:	649.65	Intensity:	79.456
	Position:	737.27	Intensity:	70.395
	Position:	811.49	Intensity:	80.049
	Position:	843.97	Intensity:	80.153
	Position:	900.43	Intensity:	70.314
	Position:	922.35	Intensity:	78.837
	Position:	952.00	Intensity:	70.173

A4. *Iso*-butyl hydrazinoacetate chloride 32

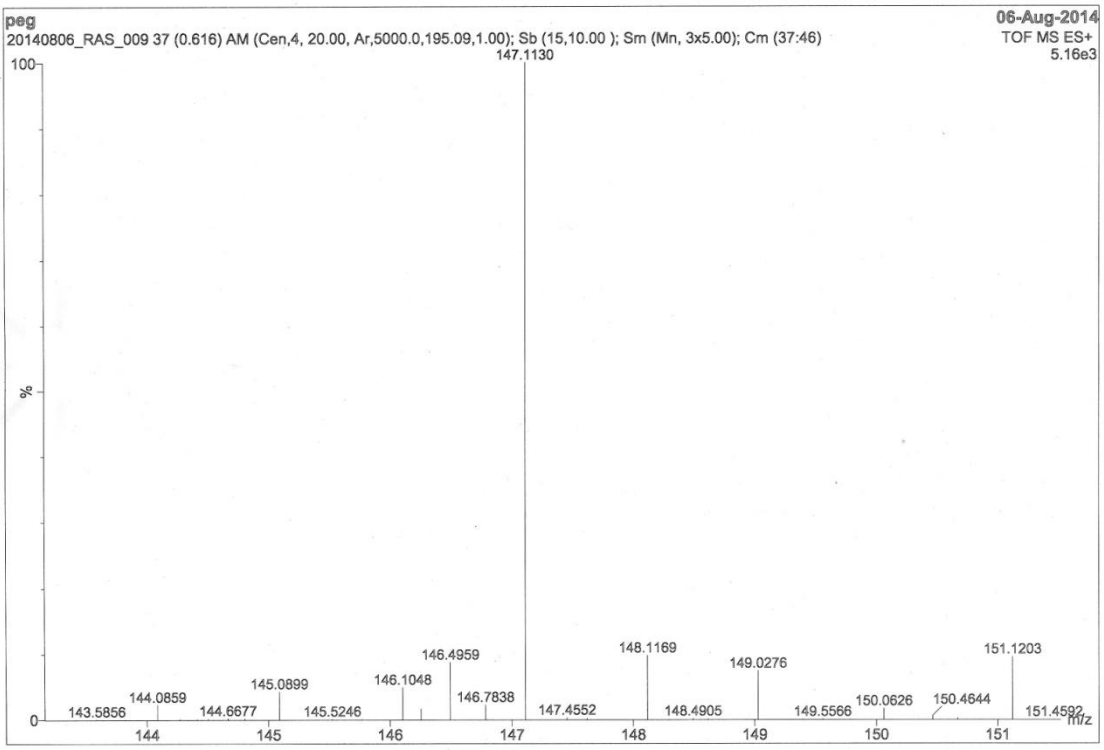
^{1}H NMR (400 MHz, in MeOD)



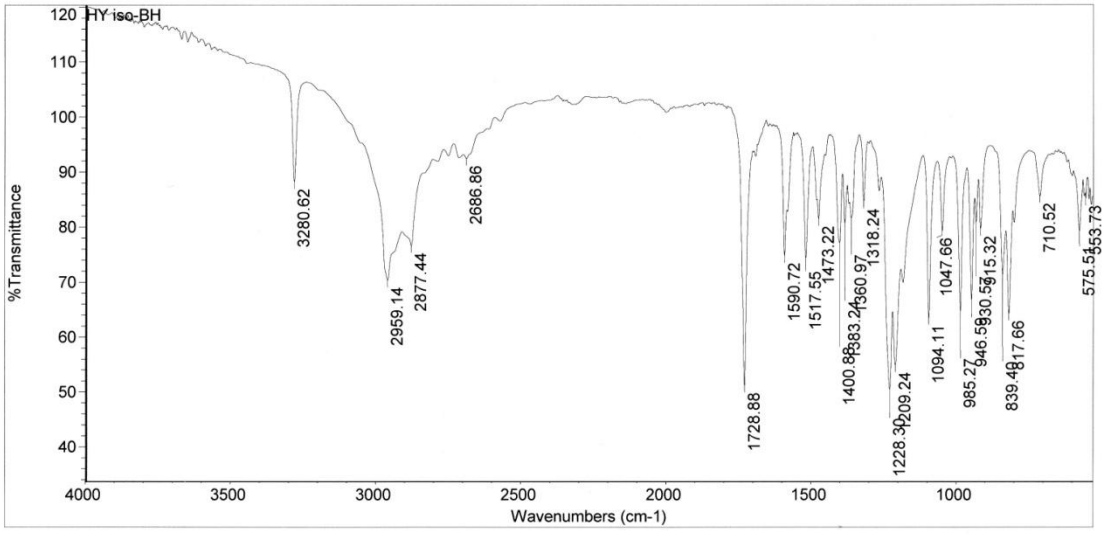
^{13}C NMR (400 MHz, in MeOD)



HRMS



IR

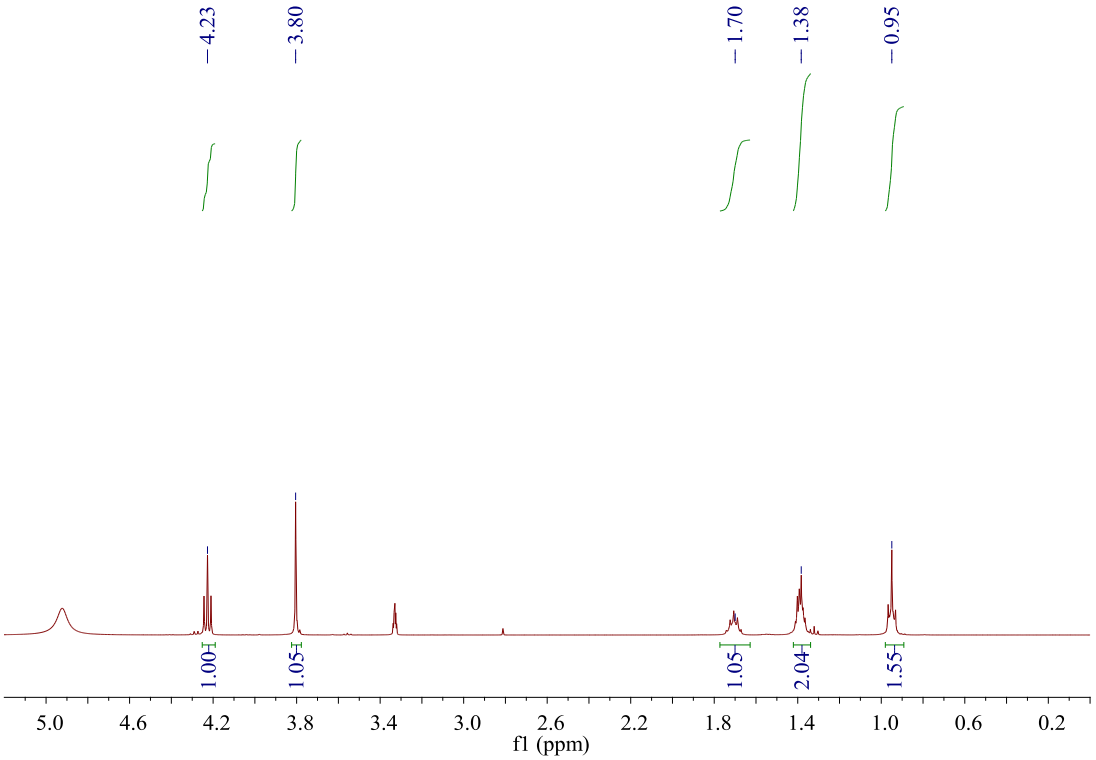


Fri Jul 25 16:07:21 2014 (GMT+01:00)
FIND PEAKS:
Spectrum: HY iso-BH
Region: 4000.12 529.85
Absolute threshold: 95.387
Sensitivity: 50
Peak list:

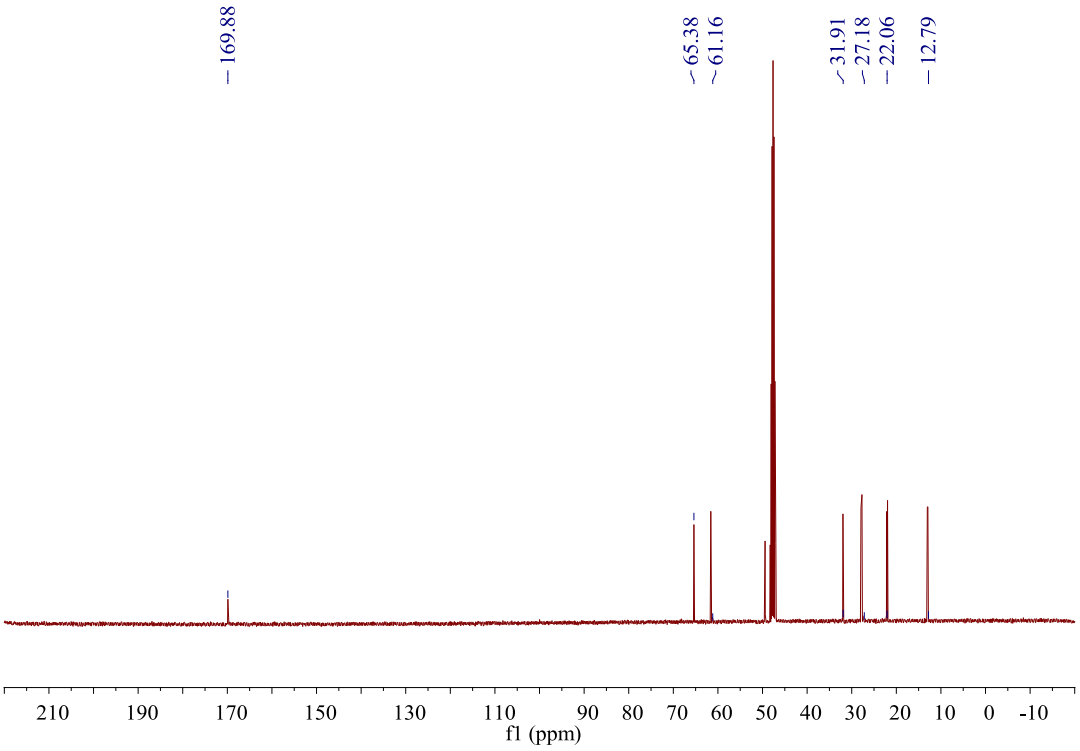
Position:	553.73	Intensity:	85.192
Position:	575.51	Intensity:	79.299
Position:	710.52	Intensity:	85.606
Position:	817.66	Intensity:	64.271
Position:	839.40	Intensity:	71.383
Position:	915.32	Intensity:	79.619
Position:	930.57	Intensity:	80.804
Position:	946.59	Intensity:	66.805

A5. 1-Pentyl hydrazinoacetate chloride 41

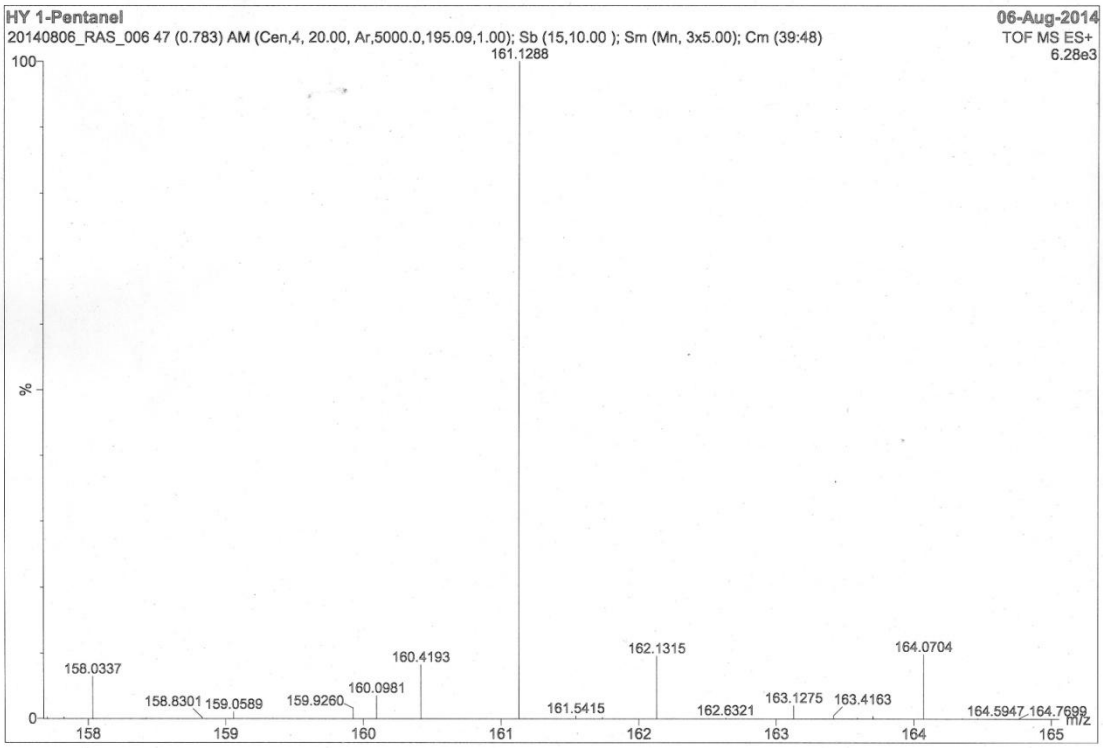
^{1}H NMR (400 MHz, in MeOD)



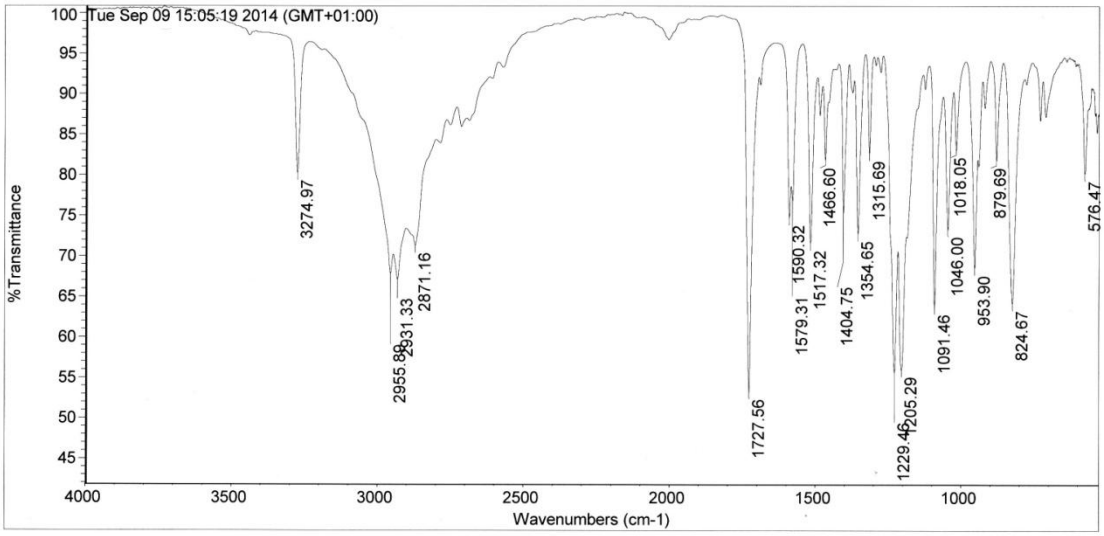
^{13}C NMR (400 MHz, in MeOD)



HRMS



IR

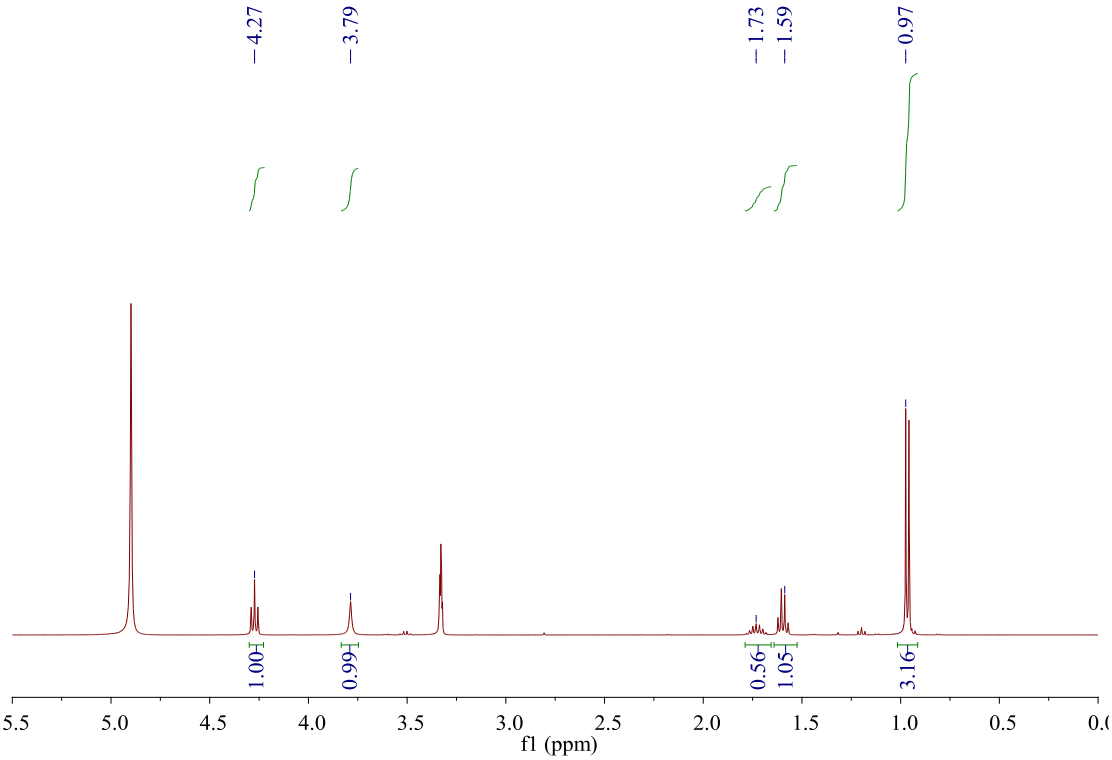


Tue Sep 09 15:08:31 2014 (GMT+01:00)
FIND PEAKS:
Spectrum: Tue Sep 09 15:05:19 2014 (GMT+01:00)
Region: 4000.12 529.85
Absolute threshold: 84.937
Sensitivity: 50
Peak list:

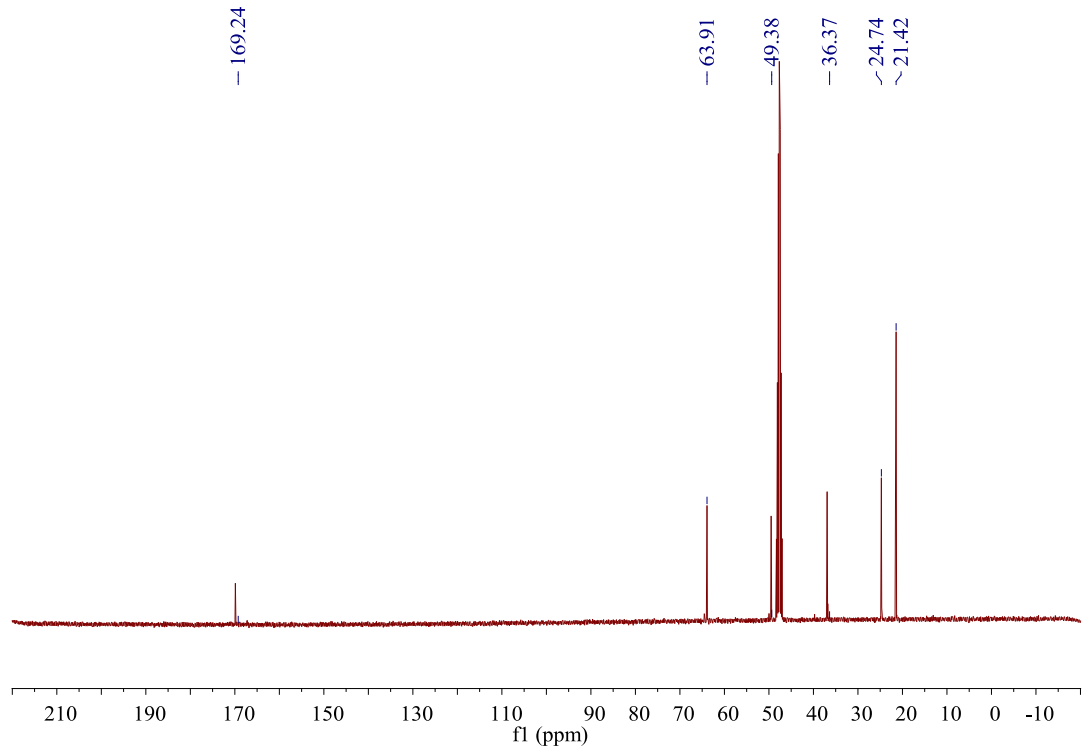
Position:	576.47	Intensity:	80.176
Position:	824.67	Intensity:	64.178
Position:	879.69	Intensity:	81.765
Position:	953.90	Intensity:	68.461
Position:	1018.05	Intensity:	83.073
Position:	1046.00	Intensity:	73.267
Position:	1091.46	Intensity:	63.684
Position:	1205.29	Intensity:	55.960

A6. *Iso*-pentyl hydrazinoacetate chloride 39

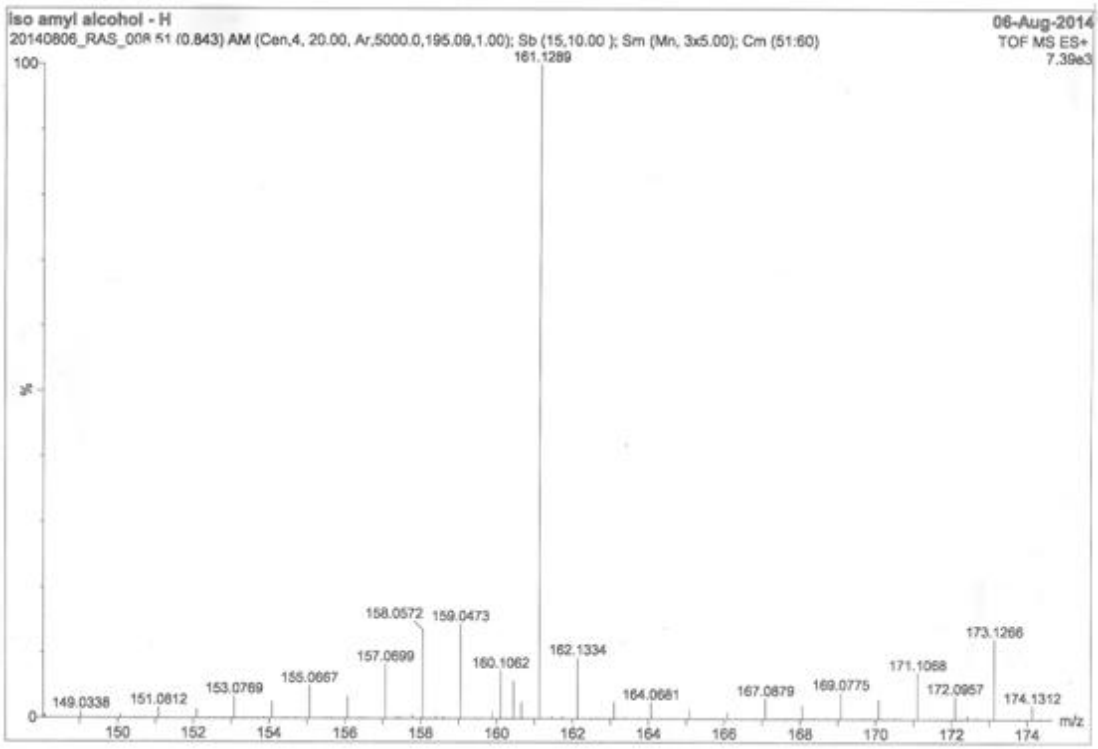
^{1}H NMR (400 MHz, in MeOD)



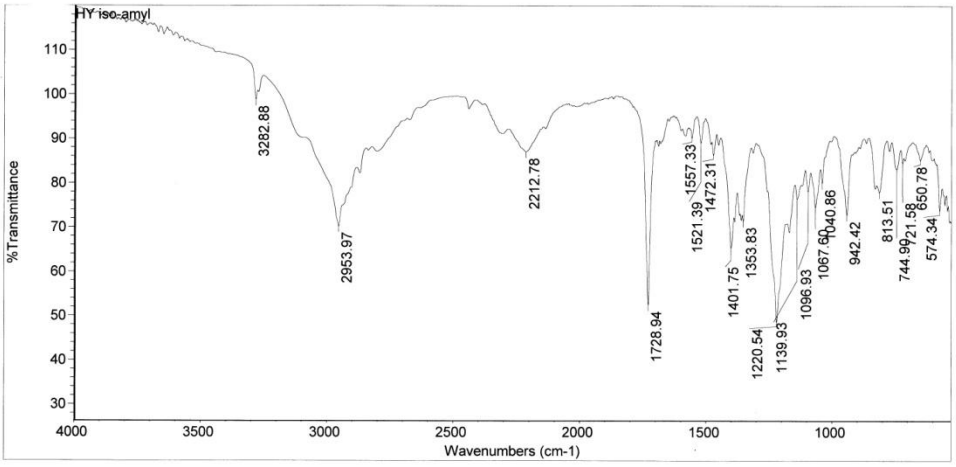
^{13}C NMR (400 MHz, in MeOD)



HRMS



IR

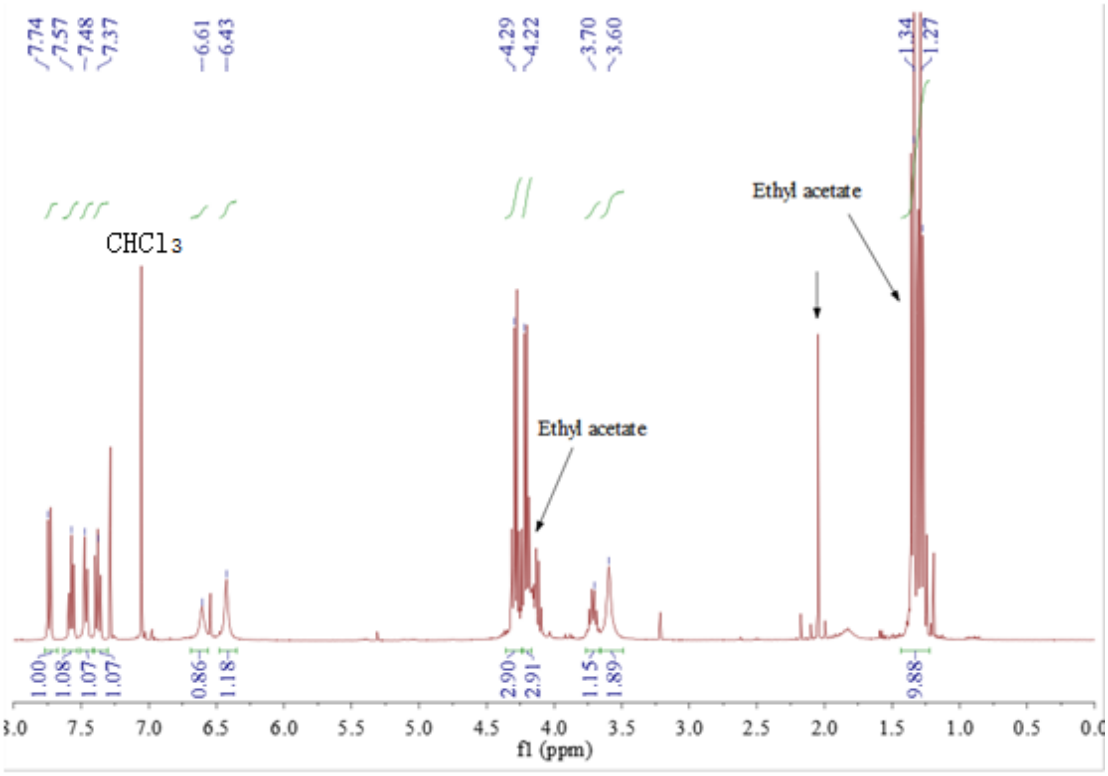


Fri Jul 25 16:01:18 2014 (GMT+01:00)
FIND PEAKS:

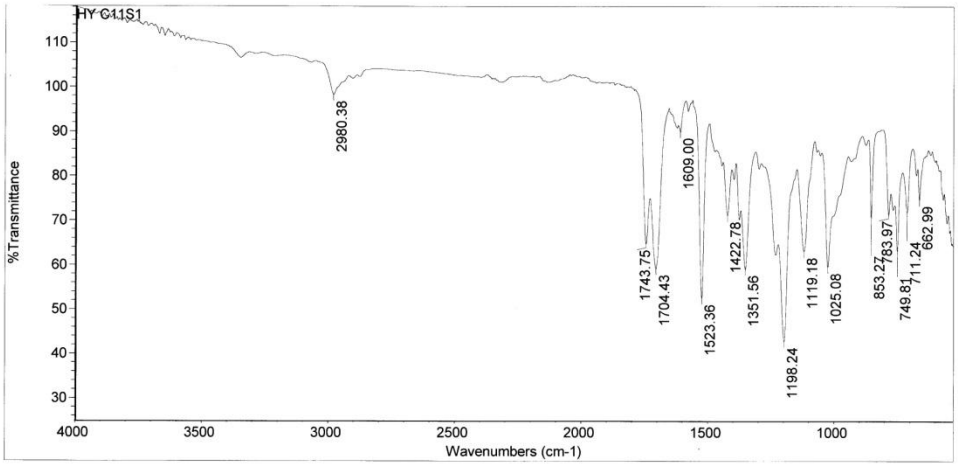
Spectrum:	HY iso-amyl			
Region:	4000.12	529.85		
Absolute threshold:	100.964			
Sensitivity:	50			
Peak list:				
	Position:	574.34	Intensity:	73.750
	Position:	650.78	Intensity:	85.177
	Position:	721.58	Intensity:	84.448
	Position:	744.90	Intensity:	83.129
	Position:	813.51	Intensity:	77.900
	Position:	942.42	Intensity:	72.814
	Position:	1040.86	Intensity:	79.996
	Position:	1067.60	Intensity:	74.570

A7. NPPOC-protected ethyl hydrazinoacetate 49

^{1}H NMR (400 MHz, in $CDCl_3$)



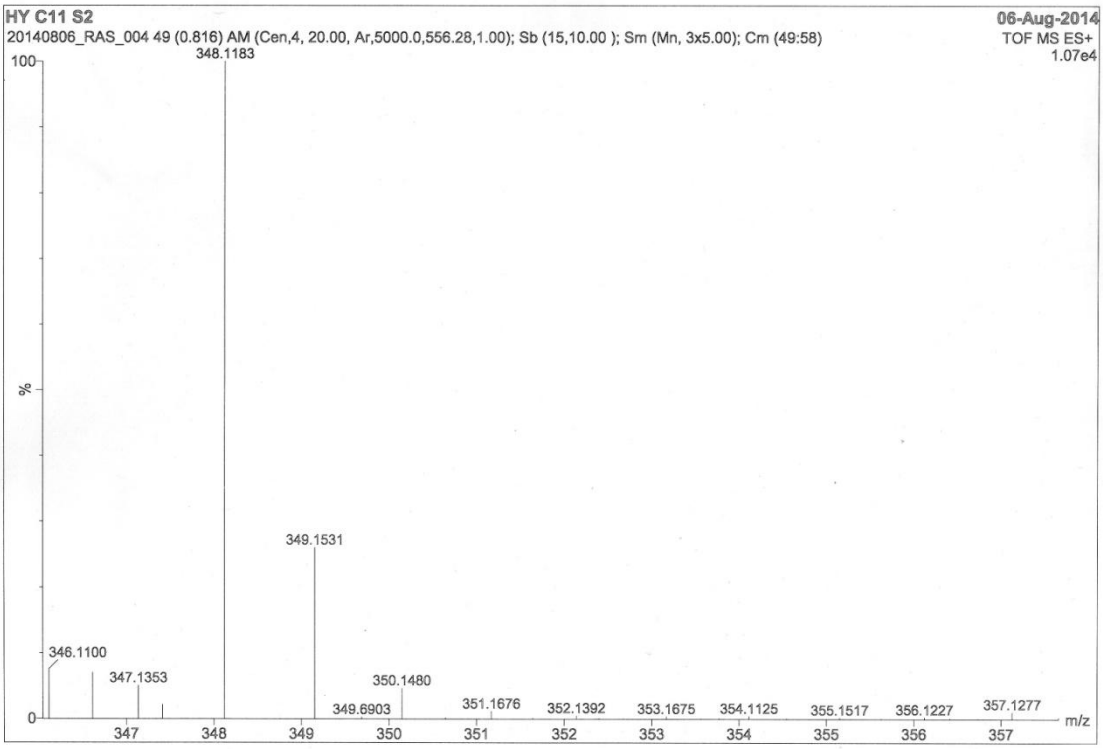
IR



Fri Jul 25 15:53:58 2014 (GMT+01:00)
FIND PEAKS:

Spectrum:	HY C11S1			
Region:	4000.12	529.85		
Absolute threshold:	99.679			
Sensitivity:	50			
Peak list:				
	Position:	662.99	Intensity:	74.696
	Position:	711.24	Intensity:	71.891
	Position:	749.81	Intensity:	63.110
	Position:	783.97	Intensity:	71.350
	Position:	853.27	Intensity:	70.609
	Position:	1025.08	Intensity:	59.564
	Position:	1119.18	Intensity:	63.149
	Position:	1198.24	Intensity:	42.838

HRMS


HY C11 S2
06-Aug-2014
20140806_RAS_004 49 (0.816) AM (Cen,4, 20.00, Ar,5000.0,556.28,1.00); Sb (15,10.00); Sm (Mn, 3x5.00); Cm (49:58)
TOF MS ES+
1.07e4
100
%
0
348.1183
349.1531
346.1100
347.1353
350.1480
349.6903
351.1676
352.1392
353.1675
354.1125
355.1517
356.1227
357.1277
347
348
349
350
351
352
353
354
355
356
357
m/z

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