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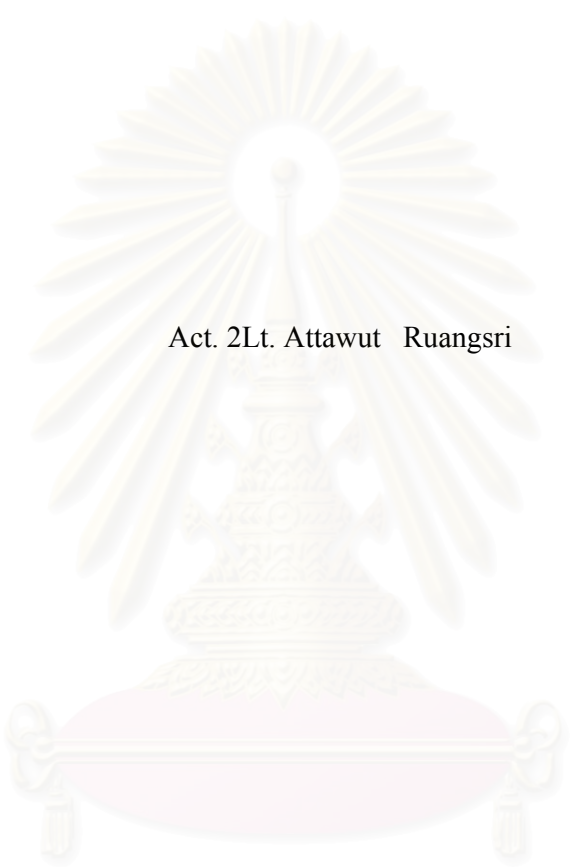
SYNTHETIC METHODOLOGY OF ISOXAZOLE DERIVATIVES

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วิทยานิพนธ์นี้เป็นการศึกษาวิธีการสังเคราะห์อนุพันธ์ไอซอกซาโซล   ซึ่งสามารถสังเคราะห์เอทิล 3-เอริล-5-เมทิลไอซอกซาโซล-4-คาร์บอกซีเลตนี้ได้จากเบนซาลดีไฮด์โดยผ่าน 3 ขั้นตอน โดยสังเคราะห์ผ่านเบนซาลดอกซีม และได้เปอร์เซ็นต์ผลิตภัณฑ์เท่ากับ 37 % นอกจากนี้ ยังได้ทำการศึกษาวิธีการสังเคราะห์อนุพันธ์ชนิดนี้ด้วยวิธีการอื่นๆ เช่น การใช้เบนซิลโบรไมด์ทำปฏิกิริยากับโซเดียมไนไตรต์ เกิดเป็นอินเทอร์มีเดียทไนไตรต์ออกไซด์และทำปฏิกิริยากับเอทิลบีต้าพิโรลลิดิโนโครโตเนต   และปฏิกิริยาระหว่างเบนโซอิลคลอไรด์กับไฮดรอกซิลแอมีนไฮโดรคลอไรด์เพื่อเกิดเป็นเบนโซไฮดรอกซามิกแอซิด   จากนั้นทำปฏิกิริยากับเอทิล อะซีโตอะซีเตต อย่างไรก็ตามวิธีการทั้งสองที่กล่าวมา ไม่สามารถให้ผลิตภัณฑ์ที่ต้องการได้คือ เอทิล 3-เอริล-5-เมทิลไอซอกซาโซล-4-คาร์บอกซีเลต

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This thesis deals with the study of the synthetic method of isoxazole derivatives. Ethyl 3-aryl-5-methylisoxazole-4-carboxylate was synthesized from benzaldehyde in three steps via benzaldoxime with the yield of 37%. Other methods have also been screened, including the reaction of benzyl bromide and sodium nitrite to generate the reactive intermediate nitrile oxide, which then reacted with ethyl β-pyrrolidinocrotonate; and the reaction of benzoyl chloride with hydroxylamine hydrochloride to yield benzohydroxamic acid, which then reacted with ethyl acetoacetate. However, the desired product, ethyl 3-aryl-5-methylisoxazole-4-carboxylate, could not be obtained from the latter two methods.

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List of Abbreviations

br	broad (NMR)
°C	degree Celsius
DMSO	dimethylsulfoxide
EBC	ethyl β-pyrrolidinocrotonate
eq	equivalent
g	gram
h	hour(s)
Hz	hertz
min	minute(s)
mL	milliliter
mmol	millimole
MS4A	Molecular sieves 4A
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
ppm	part per million
py	pyridine
RT	room temperature
TEA	triethylamine
δ	chemical shift

CHAPTER I

INTRODUCTION

Antibiotics [1-2], sometimes known as antibacterials, are groups of drugs used to kill or harm specific bacteria. Since the discovery of certain antibiotics in the 1930s, they have made it possible to cure diseases caused by bacteria such as pneumonia, tuberculosis, and meningitis - saving lives of millions of people around the world. Our immune systems, with antibodies and special white blood cells, can usually kill harmful bacteria before they multiply enough to cause symptoms. And even when symptoms do occur, the body can often fight off the infection. But sometimes the body is overwhelmed by a bacterial infection and needs help to get rid of it. This is where antibiotics come in. The very first antibiotic was penicillin and along with a family of related antibiotics (such as ampicillin, amoxicillin and benzylpenicillin). They are still widely used to treat many common infections today.

Similar to other developing countries, Thailand's basic need on pharmaceutical supplies is heavily dependent on imports. Antibiotics in penicillin family are a group of drugs that are widely used and imported at high cost and quantity (Table 1.1). Together with the fact that most of the patents that protected against the production of the generic forms of these drugs have been expired, these penicillin antibiotics have become attractive among the targets to be developed locally. The research following the production of the drugs would trigger further growth of pharmaceutical industry in Thailand.

Table 1.1 The value of antibiotics in Thailand, from 1990 – 1998 (million baht) [3]

year	production	imported	total
1990	1870.944	434.680	2305.624
1991	2157.242	549.555	2706.624
1992	2191.891	623.495	2815.386
1993	2390.763	647.855	3038.618
1994	2869.177	825.673	3694.850
1995	4112.671	1548.898	5661.569
1996	4701.024	1606.196	6307.220
1997	4833.090	1979.875	6812.965
1998	4225.796	1259.839	5485.635

Antibacterial drugs have three primary targets: cell-wall biosynthesis, protein biosynthesis, and DNA replication and repair (Figure 1.1). [4]
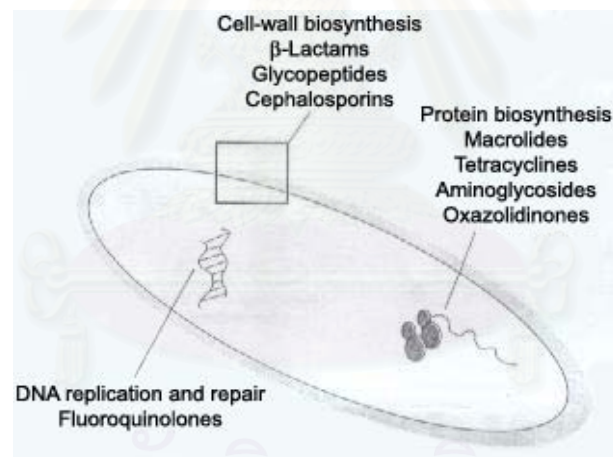
Figure 1.1 Bacterial targets of antibiotics [5]

There are now so many different antibiotics on the market. For example, penicillins that was discovered by Alexander Fleming, kill bacteria by interfering the wall-building system. The bacterial cell has a double layer on its outside. The outermost layer, the "cell wall", is similar to the outer layer of plant cells, but is missing in human and animal cells. This wall must grow along with the cell, or the growing cell will eventually become too big for the wall and burst then die. Since we

don't have cell walls, and plants have a different wall-building system, neither we, nor animals, nor plants are affected by the medicine.

Penicillins are classified as beta-lactam antibacterial drugs because of their unique four-membered lactam rings that inhibit bacterial growth by interfering with a specific step in bacterial cell wall synthesis. The cell wall is a rigid outer layer that completely surrounds the cytoplasmic membrane. It maintains the shape of the cell preventing it from cell lysis that would occur as a result of the high osmotic pressure within the cell compared to its external environment.

Cell wall is composed of a complex cross-linked polymer, peptidoglycan (murein, nucleopeptide), consisting of polysaccharides and polypeptides. The polysaccharide contains alternating amino sugars, *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) (Figure 1.2).

N-acetyl glucosamine
(NAG)

N-acetylmuramic acid
(NAM)
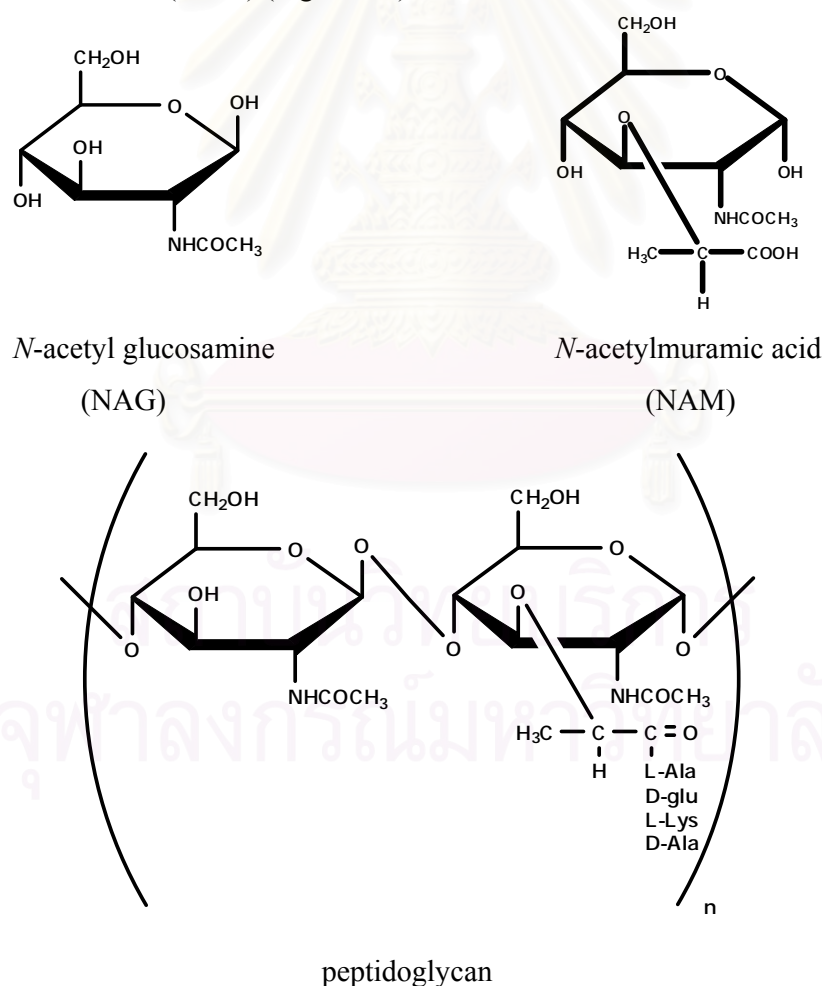
peptidoglycan

Figure 1.2 Structures of *N*-acetyl glucosamine, *N*-acetylmuramic acid, and peptidoglycan

A five-amino-acid peptide is linked to the *N*-acetylmuramic acid sugar. This peptide terminates in D-alanyl-D-alanine residues. Penicillin-binding proteins (PBPs) catalyze the transpeptidase reaction that removes the terminal alanine to form a crosslink with a nearby peptide, which gives cell wall its structural rigidity.

β-Lactam antibiotics are structural analogs of the natural D-Ala-D-Ala substrate and they can be bound by PBPs at the active site. After a β-lactam antibiotic has attached to the PBP, the transpeptidation reaction is inhibited (Figure 1.3), peptidoglycan synthesis is blocked, and the cell dies by lysis. [6]
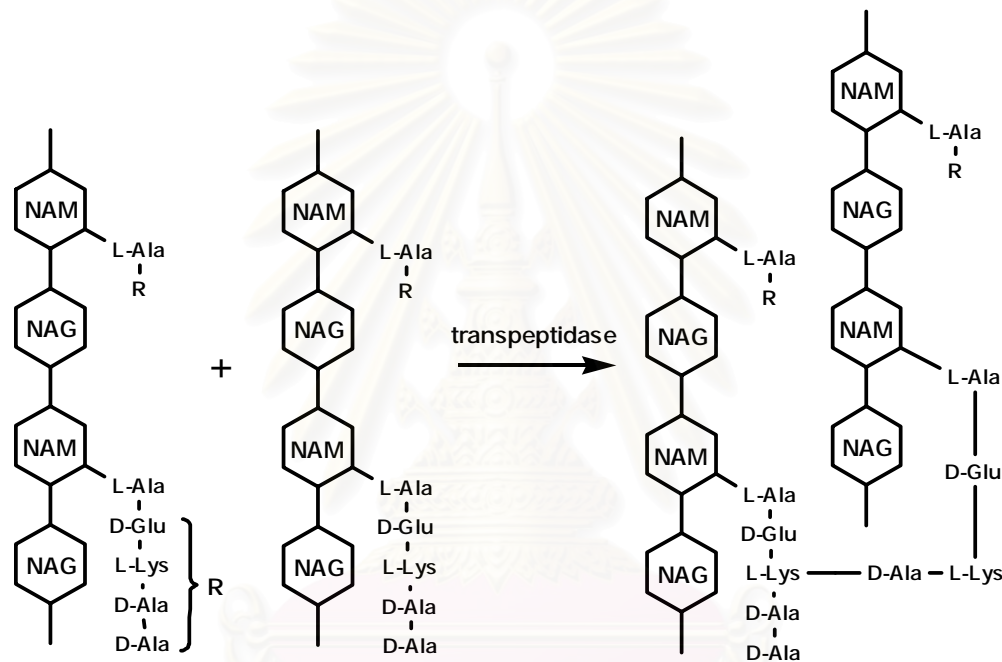
Figure 1.3 The transpeptidation

Some antibiotics can be used to treat a wide range of infections and are known as 'broad-spectrum' antibiotics. Others are only effective against a few types of bacteria and are called 'narrow-spectrum' antibiotics. Some antibiotics work against aerobic bacteria, that are organisms that need oxygen to live, while others work against anaerobic bacteria, organisms that don't need oxygen. Sometimes antibiotics are given to prevent an infection occurring, for example, before certain operations. This is known as prophylactic use of antibiotics and is common before orthopaedic and bowel surgery.

But antibiotics must be used wisely. Because bacteria are living organisms, they are always changing in an effort to resist the drugs that can kill them. When

antibiotics are used incorrectly, bacteria can adapt and become resistant. Antibiotics are then no longer useful in fighting them. Antibiotic resistance is now a major public health issue. The correct use of these drugs is the best way to ensure that antibiotics remain useful in treating infections.

There are a very few bacteria that don't have cell walls, in which they are immune to penicillins. Most bacteria do have cell walls, but many have changed their wall-building systems so that penicillins can't interfere, or have come up with ways to break down the medicines before the medicines can work. Now, because we have used penicillins too often, there are many bacteria that can't be killed by plain penicillin or its later generations.

Resistance to penicillins and other β-lactams is due to one of three general mechanisms: [5, 7-9]

1) Inactivation of antibiotic by β-lactamase

More than 100 different β-lactamases or penicillase have been identified. Some, such as those produced by *Staphylococcus aureus*, haemophilus species, and *E. coli*, are relatively narrow in substrate specificity and will hydrolyze penicillins. Because the four-membered, strained lactam ring is the chemically activated functionality in the drugs that acylate and irreversibly modify the cell wall-crosslinking PBPs, the hydrolysed, ring-opened penicilloic acid product is now deactivated and nonfunctional as a PBP pseudosubstrate and useless as an antibiotic (Figure 1.4). β-Lactamase production is the most common mechanism of resistance.

Penicilloic acid
(inactive)
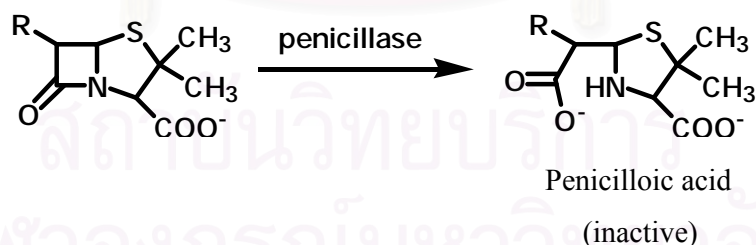
Figure 1.4 The antibiotic is destroyed by chemical modification by penicillase.

2) Modification of target PBPs

A second resistance strategy focuses on a reprogramming or camouflaging of the target in the now resistant bacteria. An example of the erythromycin-resistance manifolds, resistant bacteria have emerged that have leaned to mono- or dimethylate a specific adenine residue, A2058, in the peptidyl transferase loop of the 23S RNA

component of the ribosome. This modification is carried out by a methyl transferase enzyme that does not impair protein biosynthesis but does lower the affinity of all the members of the erythromycin class of drugs for the RNA, as well as for the pristinamycin class.

      3) The presence of an efflux pump

For antibiotics to be effective they must reach their specific bacterial targets and accumulate at concentrations that can act in some reasonable time frame. For example, the protein-synthesis machinery is located in the cytoplasm so antibacterials that are inhibitors of protein synthesis must pass through the cell membranes (outer and inner permeability barriers for Gram-negative bacteria; inner membrane barriers for Gram-positive bacteria) and then accumulate to a high enough concentration to block the particular susceptibility step of protein assembly. Both Gram-positive and Gram-negative bacteria that become resistant to tetracyclines commonly overproduce related membrane proteins (with relative molecular masses of 42,000) that act as an export or efflux pump for the drug. As schematized in Figure 1.5, the drug is pumped out faster than it can diffuse in, so intrabacterial concentrations are kept low and ineffectual; bacterial protein synthesis proceeds at largely unimpeded rates. The pumps are variants of membrane pumps possessed by all bacteria to move lipophilic or amphipathic molecules in and out of the cells. Some are used by antibiotic producers to pump antibiotics out of the cells as fast as they are made and so constitute an immunity or protective mechanism for the bacteria to prevent being killed by their own chemical weapons. Equivalent drug efflux pumps have been observed in several bacteria, including staphylococci, which become resistant to the erythromycin class of macrolide antibiotics (Figure 1.5).
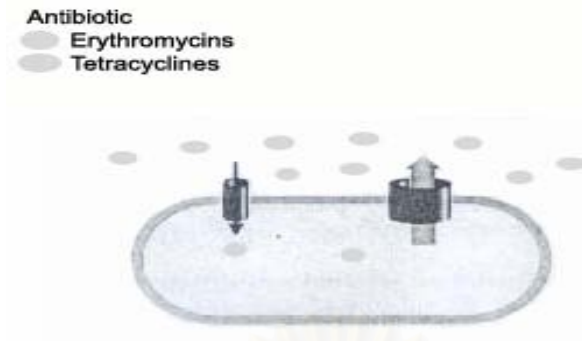
Figure 1.5 Drugs such as tetracyclines or erythromycins are pumped back out of bacterial cells through efflux pump proteins to keep intracellular drug concentrations below therapeutic level. [5]

Oxacillin and derivatives are a group of semisynthetic drugs in penicillin family that hold a promised potential to be locally prepared replacing the elevated cost of the import of the drugs themselves or their pre-synthesized starting materials. These drugs can be produced from two main subunits (Figure 1.6): the antibiotic-active β-lactam moiety (A), derived from fermentation process, and the isoxazole nucleus (B) from laboratory synthesis which will add the stability to the drugs against the penicillase produced from the penicillin-resistant bacteria to destroy the drugs. Two common derivatives of oxacillin commercially available are cloxacillin and dicloxacillin, which carry different aryl groups on isoxazole nucleus that further enhance the stability of the drugs against the penicillase. However, the more favorable property increases the complexity of the structures and their syntheses, hence heightens the final cost of the drugs. [10-11]
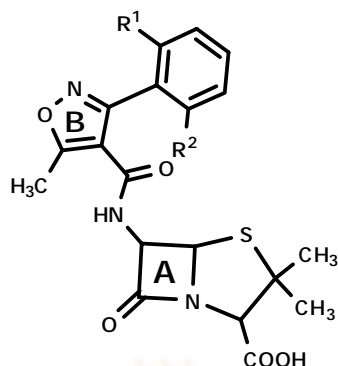
Figure 1.6 The structure of oxacillin (R^1=H, R^2=H), cloxacillin (R^1=Cl, R^2=H), and dicloxacillin (R^1=R^2=Cl)

Hydroxylamine hydrochloride, ethyl acetoacetate and benzaldehyde or its derivatives were used to produce the isoxazole nuclei of oxacillin, cloxacillin and dicloxacillin (Figure 1.7). [12] Benzaldehyde reacted with hydroxylamine to form benzaldoxime. Then, chlorination of benzaldoxime generated chloro benzaldoxime which reacted with ethyl acetoacetate and sodium methoxide to generate isoxazole nuclei of oxacillin and derivatives.
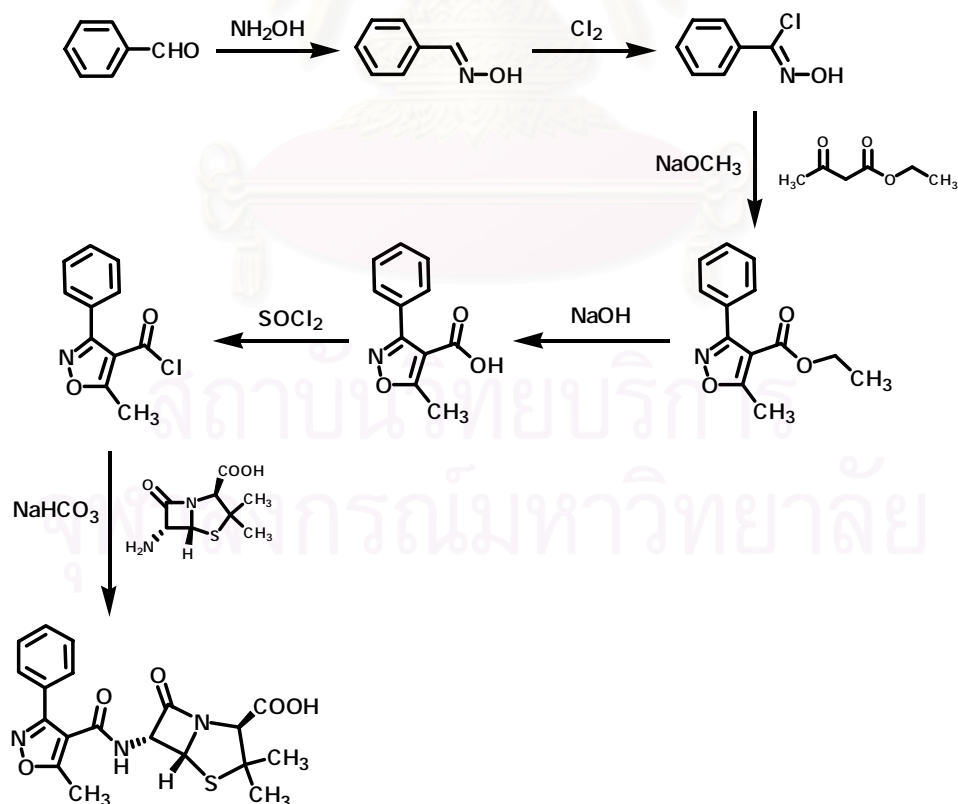
Figure 1.7 The industrial synthesis of oxacillin

Isoxazole nucleus can be obtained by 3 pathways : [13-14]

First, the condensation of hydroxylamine with α,β-acetylenic carbonyl compound or with an α,β-olefinic carbonyl compound. Second, 1,3-dipolar addition of nitrile oxide and the last are other methods.

1. By the condensation of hydroxylamine with an α,β-acetylenic carbonyl compound or with an α,β-olefinic carbonyl component substituted at either the α- or β-carbon with a readily displace group. This rather useful preparative method likewise suffers from the occurrence of mixture of the two possible tautomers in some instances. There exists a direct rate competition between the process which involves initial oxime formation followed by cyclization and the process in which preliminary Michael-type addition of hydroxylamine to the electron deficient unsaturated linkage occurs. The examples of synthesis of isoxazole nucleus by this pathway are:

In 1996, Dominguez and coworkers have reported the convenient one-pot preparative method for 4,5-diarylisoxazoles which involved amine exchange reactions (Figure 1.8). [15] Enamino ketones were submitted to reaction with hydroxylamine hydrochloride under standard oximation conditions, yielding 4,5-diarylisoxazoles in a one-pot reaction. The proposed mechanism of the formation for the target isoxazoles was in which the first step was an amine exchange reaction (Figure 1.9). Subsequent nucleophilic attack of the so-obtained hydroxylamine derivative to the carbonyl group, followed by elimination, yielded the target isoxazole compounds.

(88-99 %)
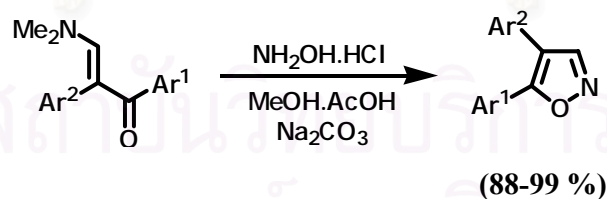
Figure 1.8 Dominguez's synthesis of isoxazole derivatives
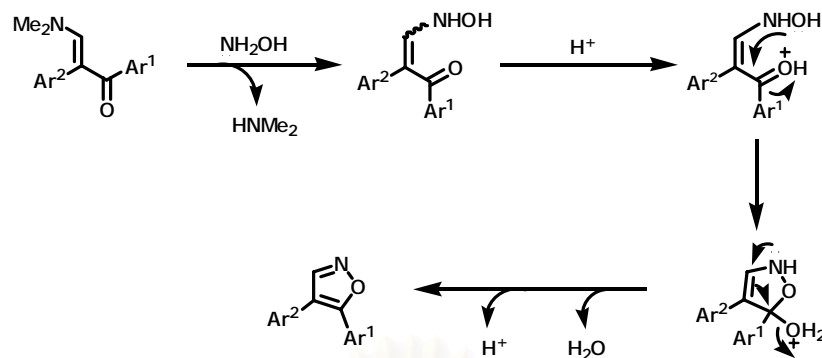
Figure 1.9 The mechanism of Dominguez's synthesis of isoxazole derivatives

Lautens and coworkers have reported the formation of isoxazoles from *N*-acetoacetyl derivatives reacted with hydroxylamine hydrochloride (Figure 1.10, Table 1.1). [16] Upon treatment with $NH_2OH \cdot HCl$, the corresponding isoxazoles were isolated in good yields when aliphatic R groups were present (entries 1 – 3 and 7). Substrate with aromatic substituents was proven to be substantially less reactive (entries 4 – 6). The yields were lower, starting material was recovered, and in one case, epimerization at C2 was observed (entry 6). Two factors may be responsible for this outcome. The carbonyl group undergoing reaction (C3) may be less electrophilic due to conjugation with the aromatic ring. Second, the aromatic ring may disfavor the trans oxime, which is necessary for the reaction to occur (Figure 1.11)
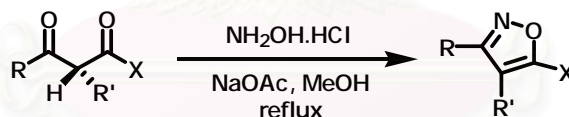
Figure 1.10 Lautens' synthesis of isoxazole derivatives
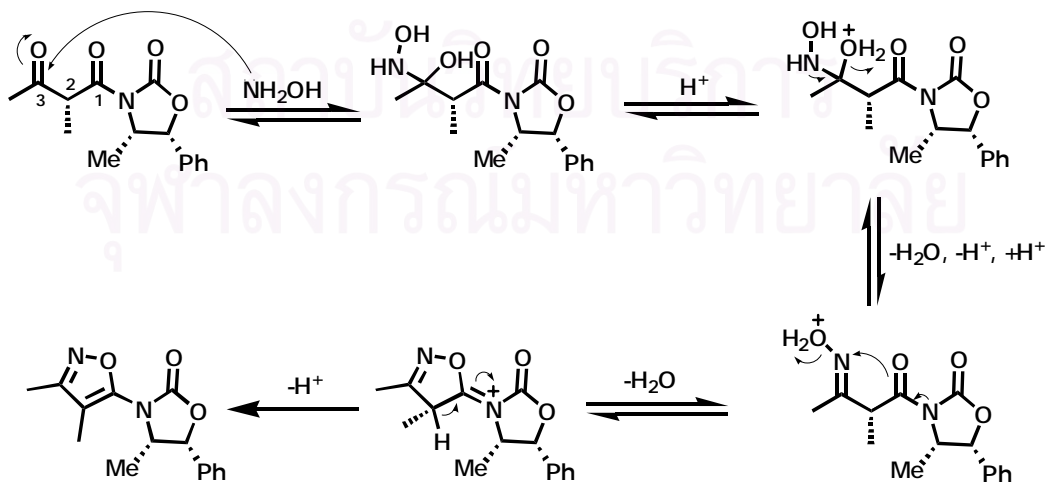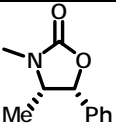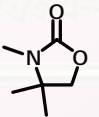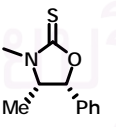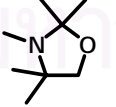
Figure 1.11 The mechanism of Lauten's synthesis of isoxazole derivatives

Table 1.2 Yield of isoxazole compound by Lautens' method

entry	R	R'	X	Yield (%)
1	CH$_3$	CH$_3$	(oxazolidinone, Me/Ph)	80
2	CH$_3$CH$_2$	CH$_3$	"	84
3	PhCH$_3$CH$_2$	CH$_3$	"	67
4	Ph	CH$_3$	"	45 (15)[a]
5	p-F-Ph	CH$_3$	"	30 (25)[a]
6	p-OMe-Ph	CH$_3$	"	6 (36)[a] (6)[b]
7	CH$_3$	H	"	82
8	CH$_3$	CH$_3$	N(CH$_3$)$_2$	degradation
9	CH$_3$	CH$_3$	N(Et)$_2$	degradation
10	CH$_3$	CH$_3$	N(i-Pr)$_2$	60
11	CH$_3$	CH$_3$	(oxazolidinone)	95
12	CH$_3$CH$_2$	CH$_3$	"	72
13	PhCH$_2$CH$_2$	CH$_3$	"	53
14	CH$_3$	CH$_3$	(4,4-dimethyl oxazolidinone)	85
15	CH$_3$CH$_2$	CH$_3$	"	no reaction
16	CH$_3$CH$_2$	H	"	92
17	CH$_3$CH$_2$	CH$_3$	(thiazolidinone, Me/Ph)	auxiliary recovered
18	CH$_3$	H	(dimethyl oxazolidine)	degradation

[a] Recovered starting material. [b] Epimerization at C2.

Lin and coworker have reported the reaction of 1-aryl-3-(dimethylamino)-2-propen-1-ones (enaminones) with hydroxylamine-O-sulfonic acid (HSA) that gave isoxazoles in 76-84 % yield (Figure 1.12). [17] Enaminones were prepared in 87 – 93

% yields by the reaction of acetophenones with N,N-dimethylalkanamide diethyl acetal or dimethyl acetal. The reaction of the enaminones with HSA gave isoxazoles in 76 – 84 % yields.
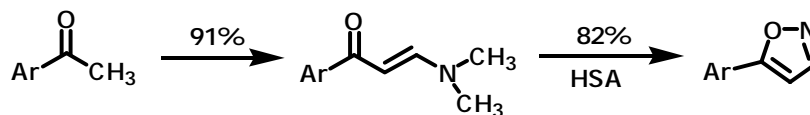
Figure 1.12 Synthesis of isoxazole derivatives by Lin's method

Katrizky and coworkers have reported the reaction of α-benzotriazolyl-α,β-unsaturated ketones with hydroxylamine to give 3,5-disubstituted isoxazoles regioselectively (Figure 1.13). [18] A number of disubstituted isoxazoles were regioselectively prepared in 55 – 81 % yields by treatment of α-benzotriazolyl-α,β-unsaturated ketones with hydroxylamine in tetrahydrofuran.
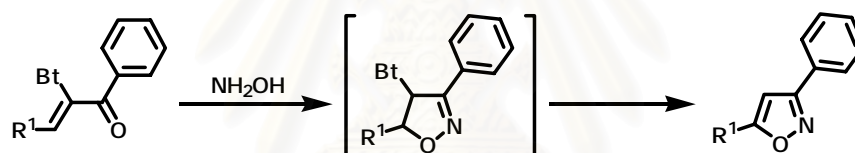
Figure 1.13 Synthesis of isoxazole derivatives by Katrizky's method

Martins and coworkers have reported the synthesis of 3-aryl-5-trihalomethylisoxazoles and 3-aryl-5-hydroxy-5-trihalomethyl-4,5-dihydroisoxazoles in good yield (Figure 1.14). [19]
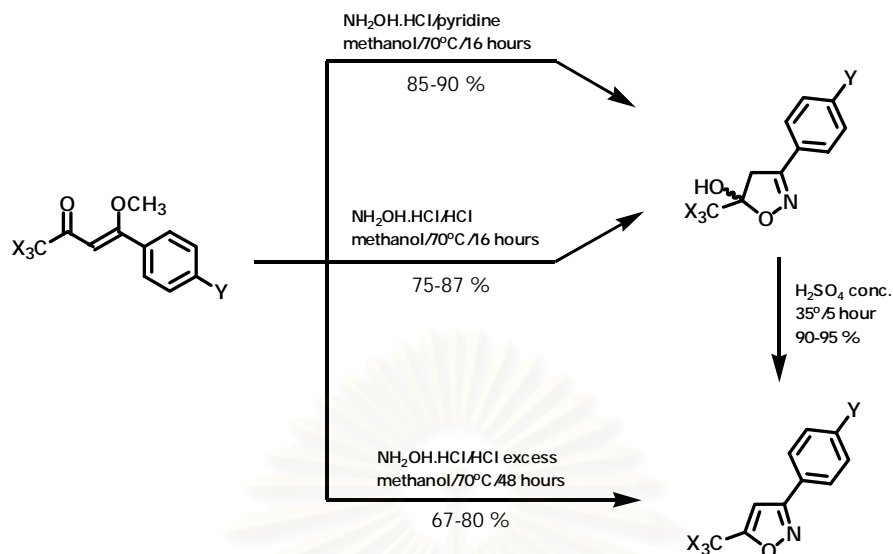
Figure 1.14 Synthesis of isoxazole derivatives by Martins' method

Moreover, Manning and coworker have reported the reaction of hydroxylamine with 3,3-disubstituted 2,4-pentanediones to generate novel isoxazole derivatives 77 % yield. [20] Barluenga and coworkers have reported the regiospecific synthesis of isoxazoles by reaction of 1-azabutadiene derivatives with hydroxylamine hydrochloride giving 63-96% yield. [21]

2. By 1,3-dipolar addition of a nitrile oxide (often generated *in situ* by the dehydrohalogenation of the corresponding hydroxamic acid chloride) or a diazoalkane to an acetylene, the triple bond of which frequently is activated by an electron-withdrawing substituent. In brief, such condensation reactions proceed by virtue of the fact that nitrile oxide and diazoalkanes may be considered as ambivalent compounds which display electrophilic and nucleophilic reactivity at the 1- and 3-positions. The examples of synthesis of isoxazole nucleus by this pathway are:

Brüning and coworkers have reported the use of *N*-phenylhydroxylamine, benzaldehyde and styrene to give 2,3,5-triphenylisoxazolidine with its yield in the range of 76-82 % (Figure 1.15). [22]
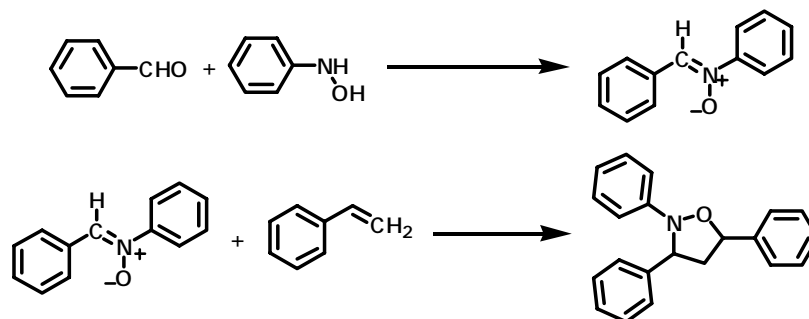
Figure 1.15 Brüning's synthesis of isoxazolidine derivatives

The next method to synthesize isoxazole derivatives is McMurry's method using ethyl acetoacetate, pyrrolidine and nitro compound (Figure 1.16). [23] It has been shown that the reaction of a primary nitro compound with a dehydrating agent such as phosphorus oxychloride produces an intermediate nitrile oxide which then undergoes a 1,3-dipolar cycloaddition to the enamine (Figure 1.17).
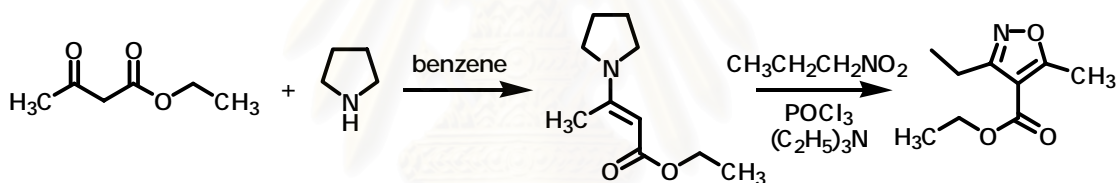
Figure 1.16 McMurry's synthesis of isoxazole derivatives
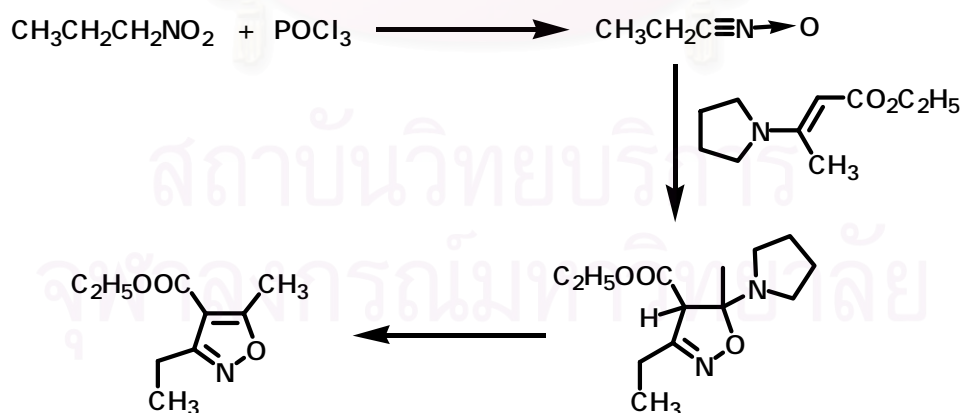
Figure 1.17 The mechanism of McMurry's synthesis of isoxazole derivatives

In 1997, Mioskowski and coworkers have reported the syntheses of isoxazole compound by transformation of nitro compound to reactive intermediate nitrile oxide and then intercepted with 1-hexene to yield 72 % of isoxazole adduct (Figure 1.18). [24]
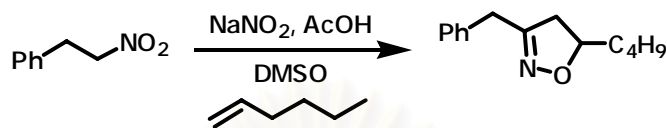
Figure 1.18 Mioskowski's synthesis of isoxazole derivatives

Dornow and Wiehler have reported the synthesis of isoxazoles from condensation of aryl aldehydes and α-nitro-esters. Interestingly, the nitrogen atom at the isoxazole ring came from the nitro group (Figure 1.19). [25]
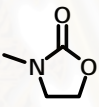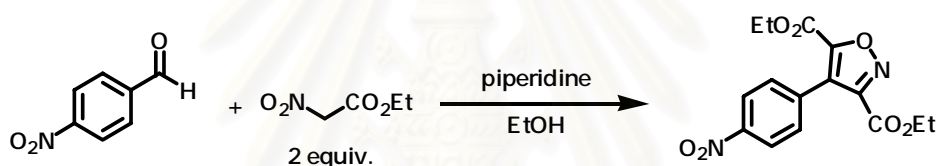

Figure 1.19 Dornow's synthesis of isoxazole derivatives

In 2004, Weidner-Wells and coworkers have reported the synthesis of isoxazole compounds by the conversion of aldehyde to the oxime followed by chlorination with *N*-chlorosuccinimide produced the nitrile oxide precursor. Dehydrohalogenation of chlorooxime with base in the presence of an alkenyl dipolarophile afforded the isoxazole cycloadducts (Figure 1.20). [26]

R =
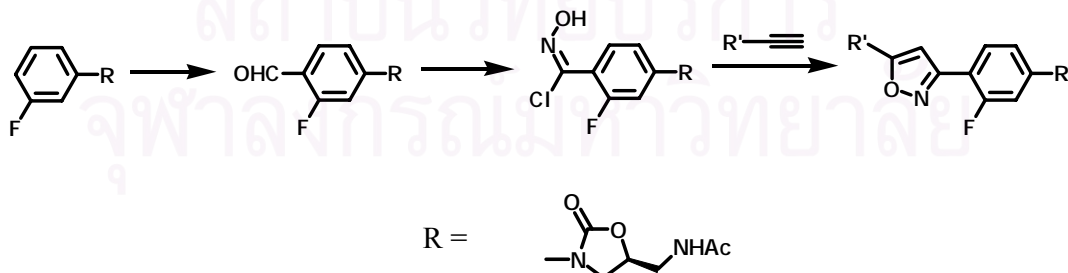
Figure 1.20 Weidner-Wells' synthesis of isoxazole derivatives

In 2004, Saad and coworkers have reported a simple one-pot procedure for the preparation of 5-aminoisoxazoles in toluene using 1,3-dipolar cycloaddition reaction between nitrile oxides and captodative α-cyanoenamines (Figure 1.21). [27]
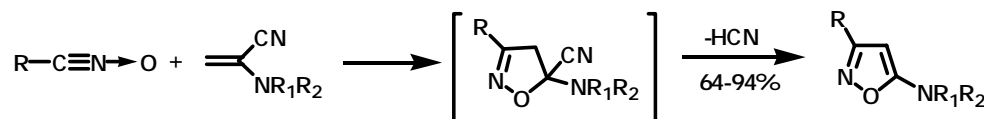
Figure 1.21 Saad's synthesis of isoxazole derivatives

Giacomelli and coworkers have reported the use of solid-phase 1,3-dipolar cycloaddition to generate isoxazoles by anchoring the acetylenic compounds on the resin and generating the nitrile oxide in situ from suitable carbonyl compounds (Figure 1.22). [28] Alkenyl alcohol was anchored on trityl chloride resin (Figure 1.23) and treated with aldoxime and *N*-chlorosuccinimide (NCS) in methylene chloride in a one-pot three-component reaction. Then, triethylamine was added slowly and dropwise to generate the nitrile oxide. The isoxazole was cleaved off the resin under standard conditions (5 % trifluoroacetic acid in CH_2Cl_2).

X, Y = CH$_2$OH, CHO, COOH
n = 0, 1, 2, ...
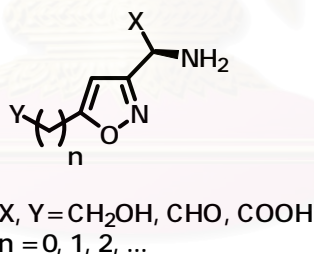
Figure 1.22 Giacomelli's synthesis of isoxazole derivatives
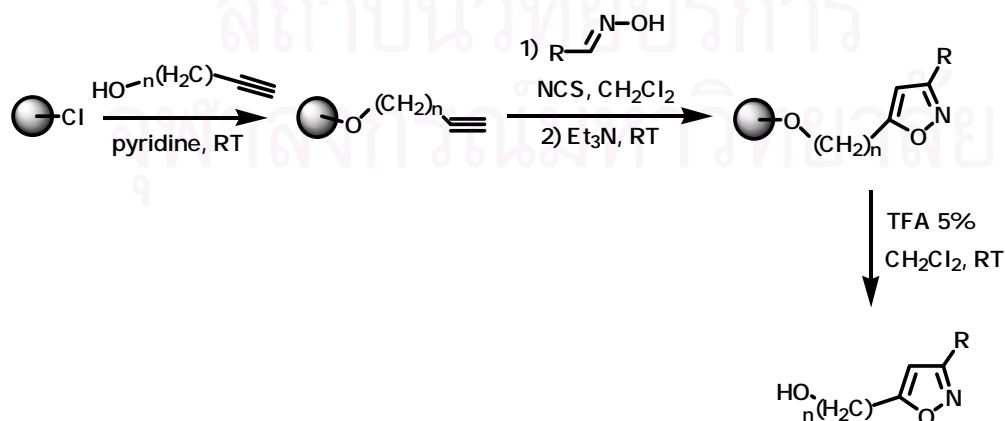
Figure 1.23 The mechanism of Giacomelli's synthesis of isoxazole derivatives

Napoletano and coworkers have reported the one-pot synthesis of 3-chloro-5-substituted isoxazoles by 1,3-dipolar cycloaddition giving 35-52% yield (Figure 1.24). [29]
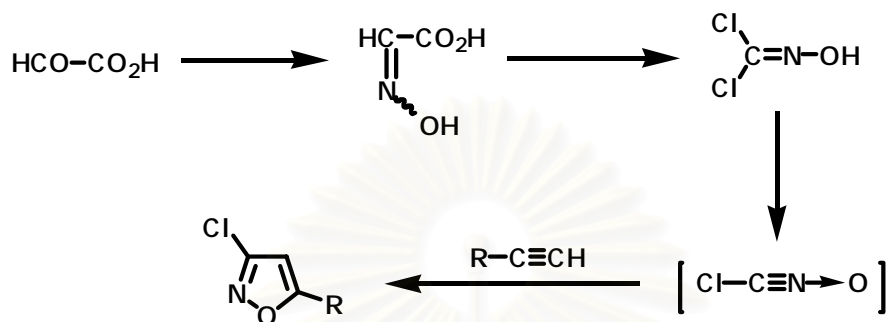
Figure 1.24 Napoletano's synthesis of isoxazole derivatives

Howe and coworkers have reported the reaction of nitrile oxides with 3-benzylidenephthalides to generate isoxazole compounds (Figure 1.25). [30]

X = Ph (31%)
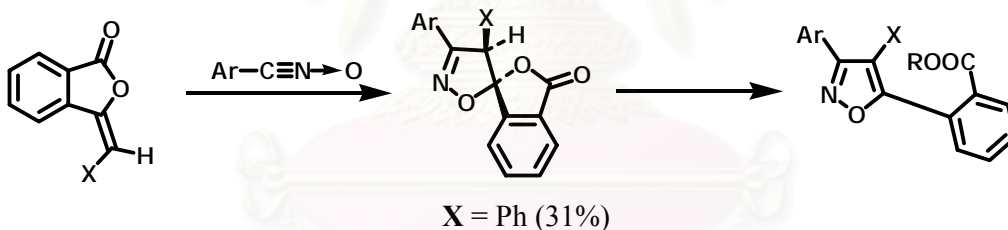
Figure 1.25 Howe's synthesis of isoxazole derivatives

Ariga and coworkers have reported the one pot synthesis of polyfunctionalized isoxazol(in)es. Pyridinium salt of 4-nitroisoxazolin-5(2*H*)-one was converted to cyano-*aci*nitroacetate which condensed with ketones or aldehydes to furnish dinitronates (Figure 1.26). [31]
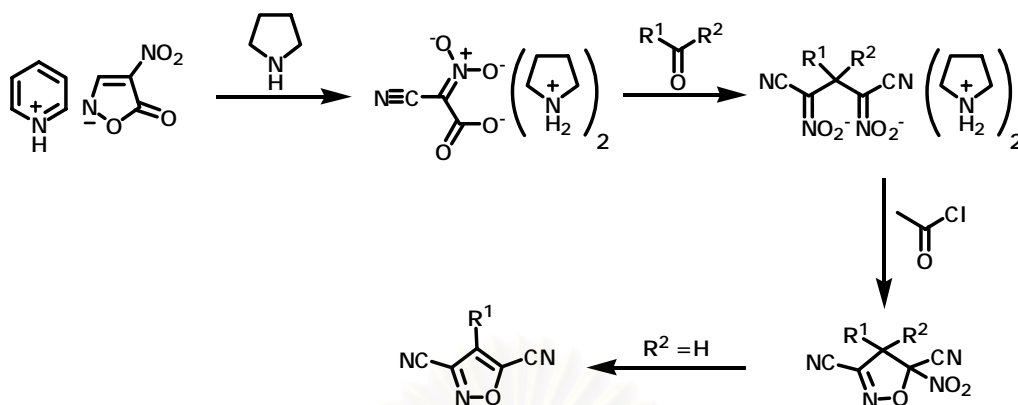
Figure 1.26 Ariga's synthesis of isoxazole derivatives

Kurth and coworkers have reported the use of diisocyanates for in situ preparation of nitrile oxides to generate isoxazoles which yielded 34-72 %. [32]

Stork and coworker have reported the synthesis of ethyl 3-methyl-4-isoxazolecarboxylate in 85% yield by using ethyl β-pyrrolidinoacrylate, nitroethane and phenyl isocyanate. [33]

3. Other pathways to synthesis of isoxazole compounds are:

Renfrow and coworkers have reported the reactions of O-benzoyl oximes with sodium hydride to give substituted isoxazoles. [34]

Olofson and coworkers have reported the regiospecific synthesis of 3-substituted 5-alkylisoxazoles by modification of the dilithiooxime route to isoxazoles yielding 17-18%. [35]

In the current research, we designed new synthetic methods for isoxazole compound that is the core nucleus of oxacillin drug

1. Benzaldehyde was used to generate benzaldoxime and then bromination of benzaldoxime to form bromobenzaldoxime. The final step, we used ethyl acetoacetate to react with bromo benzaldoxime to generate isoxazole nucleus (Figure 1.27).
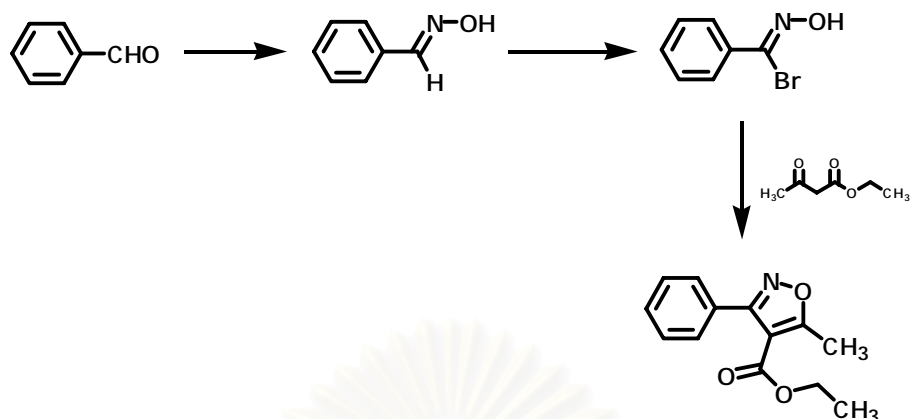
Figure 1.27 The synthetic method of isoxazole derivatives via bromobenzaldoxime

2. Utilizing the nucleophilic substitution condensation strategy, Benzoyl chloride was reacted with hydroxylamine hydrochloride to form benzohydroxamic acid, then condensed with ethyl acetoacetate to form isoxazole derivatives (Figure 1.28).
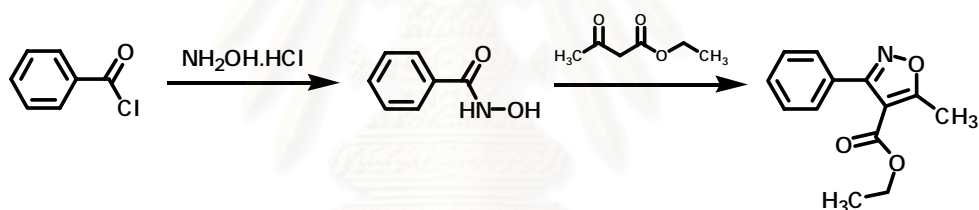
Figure 1.28 The synthetic method of isoxazole derivatives via benzohydroxamic acid

Hydroxamic acid, the intermediate from this method, is widespread in plant tissues and metabolites of bacteria and fungi. Hydroxamic acid and their derivatives fulfill a variety of important roles in biology and medicine. For example, they act variously as growth factors, food additives, tumor inhibitors, antimicrobial agents, antituberculous, antileukemic agents, key pharmacophore in many important chemotherapeutic agents, pigments and cell-division factors. Several of them have been advanced into human clinical trials as pharmaceutical drugs, for the treatment of several diseases. [37]

Moreover, Chakrabarti and coworkers have studied and found that compounds containing a 2,3- or 3,4-dihydroxyphenyl group as well as benzohydroxamate appeared to be effective inhibitors of the malaria parasite. [38]

3. Followed McMurry's method, the condensation reaction of ethyl acetoacetate with pyrrolidine to form enamine, was carried out then reacted the enamine intermediate with a nitro compound to yield isoxazole nucleus (Figure 1.29). [23]
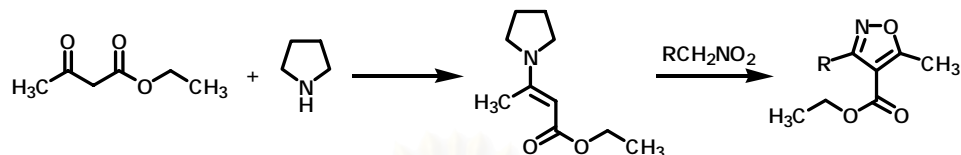
Figure 1.29 The synthesis of isoxazole derivatives using McMurry's method

4. Adopt from Mioskowski's method, Substitution of nitrite anion onto benzyl bromide generated nitrile oxide. Before this reactive intermediate would continue the reaction to benzoic acid, it could react through dipolar [3+2] cycloaddition with alkenes or alkynes to yield isoxazole compounds (Figure 1.30). [24]
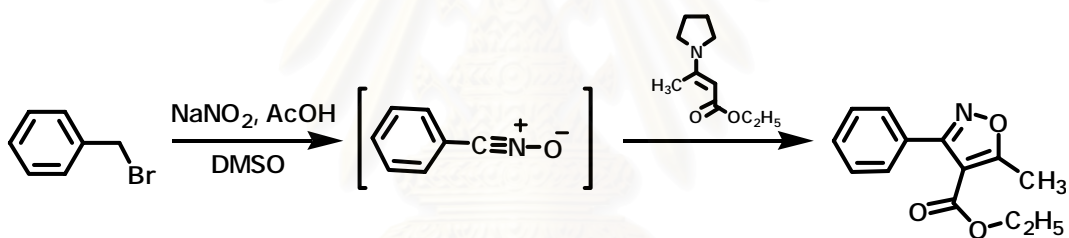
Figure 1.30 The synthesis of isoxazole derivatives via nitrile oxide

CHAPTER II

EXPERIMENTAL

2.1 General procedures and materials

All reagents were purchased as analytical grade from commercial sources and used without further purification. Acetone, toluene, hexane, dichloromethane and ethyl acetate were purchased as commercial grade and were distilled prior to use. The melting points were determined using an Electrothermal 900 melting point apparatus (Electrothermal Engineering, Essex, UK). Infrared spectrophotometric experiments were done on a Nicolet Impact 410 FT-IR (Thermo Nicolet, Wisconsin, USA) using thin film of neat samples cast from solutions in methylene chloride on NaCl windows. Mass analyses were carried out on a Varian Saturn 2200 GC/MS. The NMR spectra were acquired on a Bruker ACF 200 NMR (Bruker, Fällanden, Switzerland) or Varian YH 400 (Varian, California, USA), using $CDCl_3$ as a solvent unless specified otherwise. All column chromatography were operated using silica gel 60, Scharlau.

2.2 Synthesis of ethyl 3-aryl-5-methylisoxazole-4-carboxylate

2.2.1 Pathway I
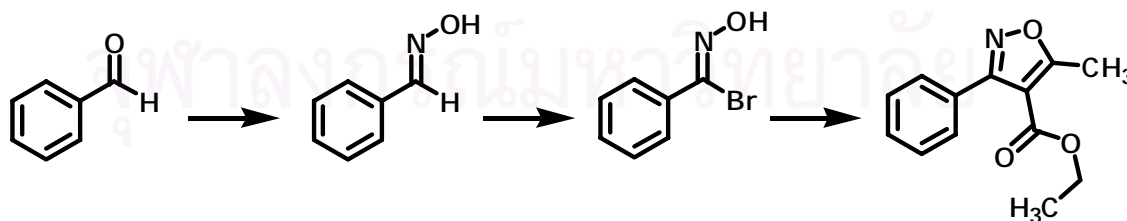
2.2.1.1 Preparation of benzaldoxime
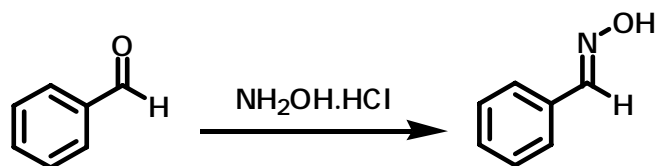
In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, hydroxylamine hydrochloride (6.949 g, 0.1000 mole) in water 15 mL was stirred and cooled down in ice bath. Sodium hydroxide (3.03 g, 0.0757 mole) in water 15 mL was added dropwise to the solution. After that, benzaldehyde (5.0 mL, 0.0492 mole) was added dropwise followed by ethanol until homogeneous. The reaction was refluxed for 30 and 60 minutes. Then, it was cooled down to room temperature. Ether was added and acidified the solution by acetic acid. The organic layer was separated and washed by water and then brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed, yielding the product as yellow oil (67% when reaction time was 30 minutes and 74 % when reaction time was 60 minutes) , which was used in next step without further distillation; ^{1}H-NMR (CDCl$_3$) δ (ppm) = 8.2 (s, C$\underline{H}$), 7.6 (o-Ar), 7.4 (m-, p-Ar), 4.8-5.0 (OH).

Alternatively, the above procedure was repeated by using sodium hydrogen carbonate and conditions as follow, in a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, hydroxylamine hydrochloride (0.2516 g, 0.0037 mole) in water 15 mL. The temperature was adjusted to 0 °C. Sodium hydrogen carbonate (0.4726 g, 0.0056 mole) was added in 2-3 portions. The mixture was stirred for 30 minutes at room temperature. Then, benzaldehyde (0.31 mL, 0.0031 mole) in methanol 5 mL was added to the mixture. The mixture was stirred for 6 hour. After that, methanol was removed and ether was added and extracted with water and then brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to yield benzaldoxime as a yellow oil (67 %).

2.2.1.2 Synthesis of ethyl 3-aryl-5-methylisoxazole-4-carboxylate
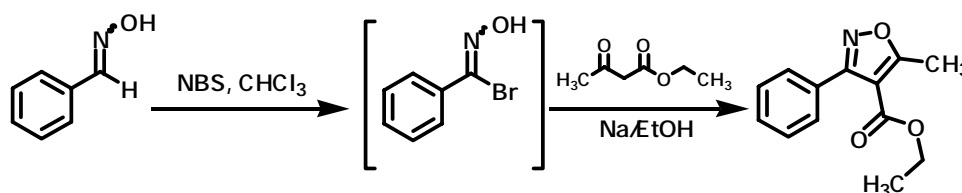
In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar a benzaldoxime (0.7018 g, 0.0058 mole) in chloroform 5 mL was stirred at -10 °C – 0 °C. *N*-bromosuccinimide (NBS) (1.13 g, 0.0064 mole) in chloroform 10 mL was added dropwise. The reaction was stirred at room temperature for 24 hour. After that, the solvent was removed, this crude bromo benzaldoxime was used without further purification.

Bromo benzaldoxime (0.43 g, 0.0021 mole) in ethanol 5 mL was cooled down to -10 °C – 0 °C. Mixture of sodium (0.06 g, 0.0021 mole), ethanol 5 mL and ethyl acetoacetate (0.30 mL, 0.0024 mole) was added into the bromo benzaldoxime solution dropwise. The reaction was stirred at room temperature for 48 hour. Then, ether was added and the solution was extracted with 5% sodium hydroxide solution and then water. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed. The crude product was chromatographed (hexane:ethyl acetate, 100:0 – 100:3) to give the desired product with 37% yield as a colorless oil; ^{1}H-NMR (CDCl$_3$) δ = 7.6 (d, *o*-Ar), 7.4 (m, m-, *p*-Ar), 4.2 (q, 2H, COOC$\underline{H}_2$CH$_3$), 2.8 (s, 3H, C-C$\underline{H}_3$) and 1.2 (t, 3H, COOCH$_2$C$\underline{H}_3$); ^{13}C-NMR (CDCl$_3$) δ = 175 (C=O), 162 ($\underline{C}$-CH$_3$), 161 (C-Ar), 130 (*p*-Ar), 129 (*o*-Ar), 128 (C-isoxazole), 127 (*m*-Ar), 108 ($\underline{C}$COOCH$_2$CH$_3$), 60 (CCOO$\underline{C}$H$_2$CH$_3$), 14 (C-$\underline{C}$H$_3$), 13 (CCOOCH$_2$$\underline{C}H_3$); MS (GC) : *m/z* 231 (M$^+$), 216 (M$^+$ – CH$_3$ at isoxazole ring) and 144 (M$^+$ – COOCH$_2$CH$_3$); Anal. Calcd for C$_{13}$H$_{13}$NO$_3$: C, 67.52; H, 5.67; N, 6.06. Found : C, 67.16; H, 5.39; N, 6.03.

The above procedure was repeated by varying reagents and conditions as follow, the mixture of benzaldoxime (3.4532 g, 0.0285 mole) NBS (8.92 g, 0.05 mole) was stirred at room temperature for 48 hour. Bromo benzaldoxime (4.6456 g, 0.023 mole) in ethanol 5 mL was cooled down to -10 °C – 0 °C. Sodium (0.62 g, 0.027 mole) in ethanol

15 mL was added to ethyl acetoacetate (3.31 mL, 0.026 mole) dropwise. After that, this solution was added to bromo benzaldoxime dropwise. The reaction was stirred at room temperature for 48 hour. Ether was added and the organic layer was separated and washed by 5% sodium hydroxide solution and then water. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed. The crude product was chromatographed (hexane:ethyl acetate, 100:0 – 100:3) to give the desired product with 30% yield as a colorless oil.

Other conditions used as follow: the mixture of benzaldoxime (0.16 g, 0.0013 mole) NBS (0.2317 g, 0.0013 mole) was dissolved in chloroform 15 mL. 2 Drops of Pyridine was added and the mixture was stirred at 50 ° - 60 °C for 60 minute. After that, the reaction was cooled down to room temperature and ethyl acetoacetate (0.17 mL, 0.0014 mole) was added and triethylamine (0.27 mL, 0.0019 mole) in chloroform 5 mL was added to the mixture. The reaction was stirred at 25 °C for 20 minutes. Then, water was added and the organic phase was extracted with 10% hydrochloric acid, water and dried over anhydrous sodium sulfate. The solvent was removed to yield yellow oil. The benzaldoxime was detected on ^{1}H-NMR instead of desired product in this crude mixture.

2.2.2 Pathway II
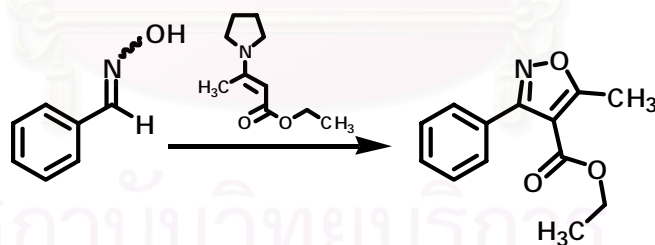
2.2.2.1 Preparation of ethyl β-pyrrolidinocrotonate (EBC) [23]
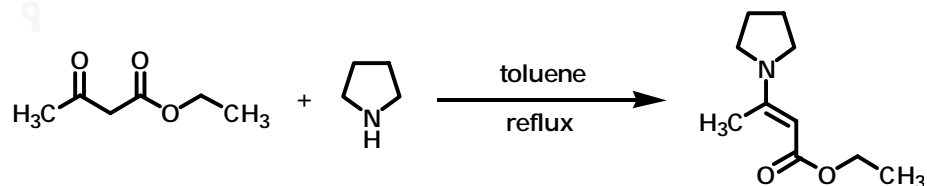
In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, ethyl acetoacetate (0.13 g, 1.00 mmol) and pyrrolidine (0.071 g, 1.00 mmol) were dissolved in 4 mL of toluene. The reaction mixture was refluxed under a nitrogen atmosphere, maintained at a vigorous reflux for 60 minutes. Toluene was removed with a rotary evaporator, yielding 0.18 g (98 %) of highly pure ethyl ß-pyrrolidinocrotonate, which was used without distillation; ^{1}H-NMR (CDCl$_3$) δ (ppm) = 4.4 (s, 1H), 4.1 (m, 2H), 3.2 – 3.4 (br, α-H of pyrrole), 2.5 (s, 3H), 1.9 (m, β-H of pyrrole) and 1.2 (t, 3H).

2.2.2.2 Synthesis of ethyl 3-aryl-5-methylisoxazole-4-carboxylate
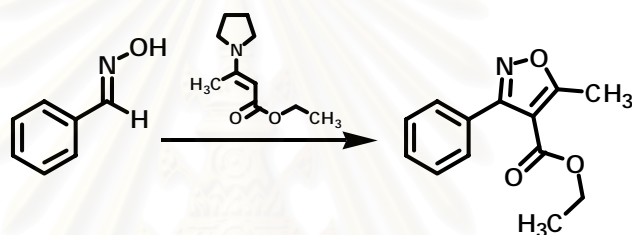
In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, mixture of benzaldoxime (0.5226 g, 0.0043 mole) in toluene 15 mL and EBC (0.7861 g, 0.0043 mole) was refluxed for 7.5 hour under drying tube. After that, the reaction was cooled down to room temperature and extracted with 10% hydrochloric acid and then brine. The organic layer was dried over anhydrous sodium sulfate and the solvent was removed to give crude product as brown oil which was not the desired product but benzaldoxime instead, according to TLC and ^{1}H-NMR analysis.

The above procedure was repeated by varying reagents and conditions as follow,

A. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, mixture of benzaldoxime in toluene 15 mL, EBC was stirred and triethylamine (1.20 mL, 0.0086 mole) was added to the mixture dropwise. The reaction was refluxed for 6 hour under drying tube. After that, the reaction was extracted like the above procedure and gave crude product as brown oil which was not the

desired product but benzaldoxime instead, according to TLC and ^{1}H-NMR analysis.

B. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, mixture of benzaldoxime in toluene 15 mL, EBC was stirred and *p*-toluenesulfonic acid (0.83 g, 0.0043 mole) was added to the mixture portionwise. The reaction was stirred at room temperature for 50 hour and then refluxed for 29 hour under drying tube. After that, the reaction was extracted like the above procedure and gave crude product as brown oil which was not the desired product but benzaldoxime and benzoic acid instead, according to TLC and ^{1}H-NMR analysis.

C. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, mixture of benzaldoxime in toluene 15 mL, EBC was stirred and iron(III) chloride (0.6975 g, 0.0043 mole) was added to the mixture portionwise. The reaction was refluxed for 48 hour under drying tube. After that, the reaction was extracted like the above procedure and gave crude product as yellow brown oil which was the desired product as analyzed by TLC and ^{1}H-NMR but it is too little to separate from crude product.

D. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, mixture of benzaldoxime in toluene 15 mL, ethyl acetoacetate (0.54 mL, 0.0043 mole) and triethylamine (1.20 mL, 0.0086 mole) was refluxed for 3 hour under drying tube. After that, the reaction was extracted like the above procedure and gave crude product as brown oil which was not the desired product but benzaldoxime instead, according to TLC and ^{1}H-NMR analysis.

2.2.3 Pathway III

2.2.3.1 Method 1

In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, sodium nitrite (0.24 g, 0.0036 mole) was dissolved in dimethylsulfoxide 4 mL. Benzyl bromide (0.20 mL, 0.0018 mole) was added and the reaction was stirred at room temperature for 20 minutes. After that, EBC (0.39 g, 0.0022 mole) was added to the reaction. The temperature of the mixture was adjusted to 60 °C and then acetic acid (0.4 mL, 0.0072 mole) was added to the reaction dropwise. The reaction was stirred at 60 °C for 5 hour. Then, it was cooled down to room temperature, ether was added and extracted with sodium hydrogen carbonate solution, 10 % hydrochloric acid and water, respectively. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to give crude product as brown solid which was chromatographed (hexane:ethyl acetate, 100:0 – 90:10) to give product which was not the desired product but benzyl bromide and benzoic acid instead, according to TLC and ^{1}H-NMR analysis.

The above procedure was repeated by varying reagents and conditions as follow,

A. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, sodium nitrite (0.5934 g, 0.0086 mole) was dissolved in dimethylsulfoxide 4 mL. Benzyl bromide (0.51 mL, 0.0043 mole) was added and the reaction was stirred at room temperature for 4 hours. After that, EBC (0.95 g, 0.0052 mole) was added to

the reaction. The mixture was refluxed for 4 days. Then, it was cooled down and extracted similar to the above procedure. The crude product as strong yellow oil which was chromatographed (hexane:dichloromethane, 100:0 – 60:40) to give product which was not the desired product but benzoic acid instead, according to TLC and ^{1}H-NMR analysis.

B. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, sodium nitrite (0.24 g, 0.0036 mole) was dissolved in dimethylsulfoxide 4 mL. Benzyl bromide (0.21 mL, 0.0018 mole) was added and the reaction was stirred at room temperature for 45 minutes. After that, EBC (0.39 g, 0.0022 mole) and acetic acid (1.0 mL, 0.036 mole) was added. The mixture was stirred at room temperature for 26.5 hours. Then, it was cooled down and extracted similar to the above procedure. The crude product as a yellow oil which was chromatographed (hexane:ethyl acetate, 100:0 – 80:20) to give product which was not the desired product but benzoic acid instead, according to TLC and ^{1}H-NMR analysis.

2.2.3.2 Method 2
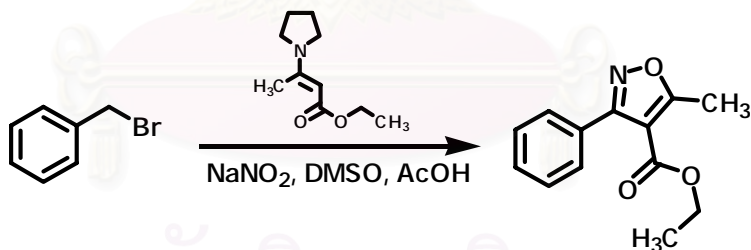
In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, sodium nitrite (0.24 g, 0.0036 mole) was dissolved in dimethylsulfoxide 4 mL. Benzyl bromide (0.20 mL, 0.0018 mole) was added, acetic acid (1.0 mL, 0.018 mole) was added dropwise, the reaction was stirred at 0 °C for 8 hours. After that, EBC (2.4 g, 0.0126 mole) was added. The mixture was stirred at room temperature for 24 hours. Then, ether was added and extracted with sodium hydrogen carbonate solution, 10 % hydrochloric acid and water respectively. The organic phase was dried over anhydrous sodium sulfate

and the solvent was removed to give crude product as a strong yellow oil. However, the product was not characterized as the desired product on TLC and ^{1}H-NMR .
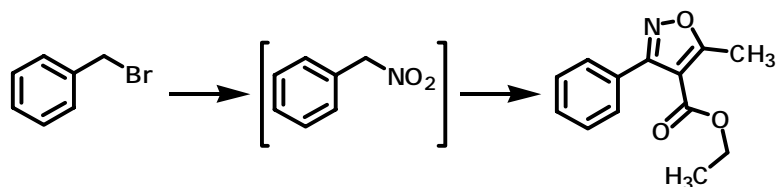
The above procedure was repeated by varying reagents and conditions as follow,

A. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, sodium nitrite was dissolved in dimethylsulfoxide. The reaction was adjusted the temperature to 15 °C – 20 °C and then acetic acid was added dropwise. Benzyl bromide was added, the reaction was stirred for 50 minutes. After that, EBC was added. The mixture was stirred at room temperature for 8 hours. Then, extraction was similar to the above procedure. Crude product was strong yellow oil which was not the desired product, according to TLC and ^{1}H-NMR analysis.
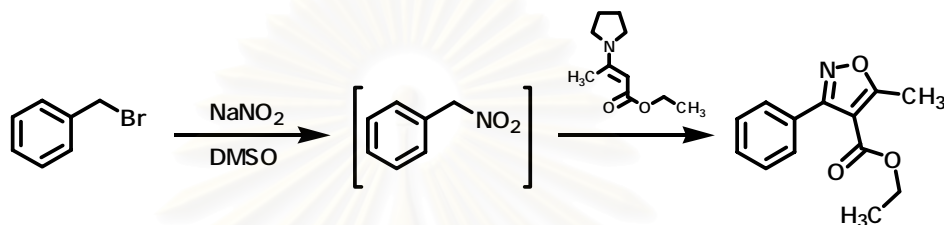
B. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, sodium nitrite was dissolved in dimethylsulfoxide. The reaction was adjusted the temperature to 15 °C – 20 °C and then acetic acid was added dropwise. Benzyl bromide was added, the reaction was stirred for 90 minutes. After that, EBC (0.1647 g, 0.0009 mole) was added to the reaction. The mixture was stirred at room temperature for 24 hours. Then, extraction was similar to the above procedure. Crude product was strong yellow oil which was not the desired product but nitro-methylbenzene instead, according to TLC and ^{1}H-NMR analysis.

C. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, sodium nitrite was dissolved in dimethylsulfoxide. The reaction was adjusted the temperature to 15 °C – 20 °C and then acetic acid was added dropwise. Benzyl bromide was added, the reaction was stirred at 2 °C for 8 hours and at room temperature for 16 hours. After that, EBC was added to the reaction. The mixture was stirred at room temperature for 5 hours. Then, extraction was similar to the above procedure. Crude product was strong yellow oil which was not the desired product, according to TLC and ^{1}H-NMR analysis.

D. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, sodium nitrite was dissolved in dimethylsulfoxide. The reaction was adjusted the temperature to 15 °C – 20 °C and then acetic acid was added dropwise. Benzyl bromide was added, the reaction was stirred for 8 hours. After that, EBC was added to the reaction. The mixture was stirred at room temperature for 24 hours. Then, extraction was similar to the above procedure. Crude product was strong yellow oil which was not the desired product, according to TLC and ^{1}H-NMR analysis.

2.2.4 Pathway IV
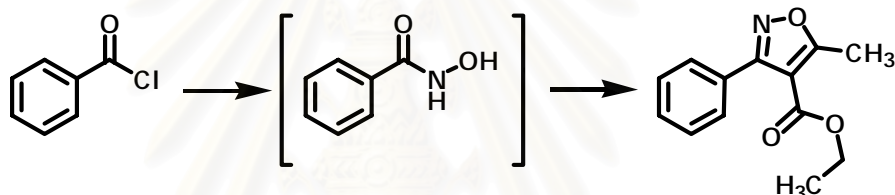
2.2.4.1 Method 1
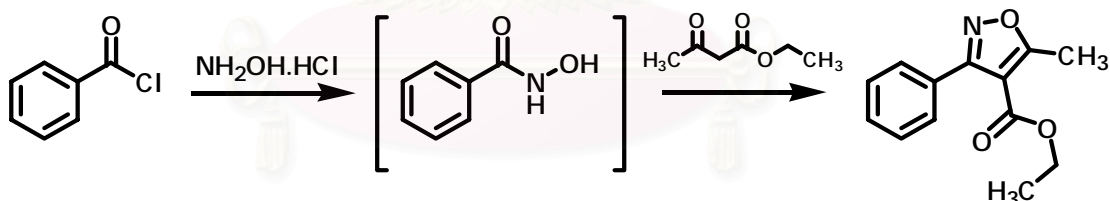
In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, mixture of hydroxylamine hydrochloride (0.5077 g, 0.0073 mole), benzoyl chloride (0.85 mL, 0.0073 mole), triethylamine (10 mL) as a solvent was stirred at room temperature for 2 hours. Ethyl acetoacetate (0.80 mL, 0.0063 mole) was added, the mixture was refluxed for 5 hours. After that, ether was added and extracted with 10 % hydrochloric acid and then brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to give the crude product, which was purified by column chromatography (hexane:ethyl acetate, 100:0 – 60:40). Unfortunately, the ethyl acetoacetate and benzoic acid were obtained instead.

The above procedure was repeated by varying reagents and conditions as follow:

A. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, hydroxylamine hydrochloride (0.0073 mole) was dissolved in acetonitrile, triethylamine (4.0 mL, 0.0029 mole) and benzoyl chloride (0.0073 mole) was added. The mixture was stirred at room temperature for 2 hours. Then, trietylamine (2.0 mL, 0.0015 mole) and ethyl acetoacetate (0.93 mL, 0.0073 mole) was added, respectively. The mixture was refluxed for 5 hours. After that, acetonitrile was removed, ether was added to the mixture and extracted with 10 % hydrochloric acid, sodium hydrogen carbonate solution and then brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to give the crude product, which was found to be ethyl acetoacetate and benzoic acid, not the desired product.

B. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, hydroxylamine hydrochloride (0.0073 mole) was dissolved in acetonitrile, triethylamine (4.1 mL, 0.0029 mole) and benzoyl chloride (0.0073 mole) was added. The mixture was stirred at room temperature for 2 hours. Then, ethyl acetoacetate (0.0073 mole) was added, the mixture was refluxed for 4 hours. After that, acetonitrile was removed and ether was added, extracted with 10 % hydrochloric acid and then brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to give the brown oil as a crude product, which was not the desired product but benzohydroxamic acid instead.

C. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, hydroxylamine hydrochloride (1.5051 g, 0.0216 mole) was dissolved in toluene, triethylamine (12.1 mL, 0.0864 mole) and benzoyl chloride (2.6 mL, 0.0216 mole) was added. The mixture was stirred at room temperature for 1.5 hours. Then, ethyl acetoacetate (2.75 mL, 0.0216 mole) was added, the mixture was refluxed for 1.5 hours. After that, it was extracted with 10 % hydrochloric acid and then brine. The organic phase was dried over anhydrous sodium sulfate and

the solvent was removed to give the brown oil as a crude product, which was not the desired product but benzohydroxamic acid instead.

D. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, hydroxylamine hydrochloride (0.6175 g, 0.0089 mole) was dissolved in toluene, triethylamine (2.48 mL, 0.0178 mole) and benzoyl chloride (0.0073 mole) was added. The mixture was stirred at room temperature for 1.5 hours. Then, ethyl acetoacetate (1.13 ml, 0.0089 mole) was added, the mixture was refluxed for 3 hours. After that, it was extracted with 10 % hydrochloric acid and then brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to give the brown oil as a crude product. After purification by column chromatography (hexane:ethyl acetate, 100:0-60:40), benzohydroxamic acid was recovered instead of the desired product.

E. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, hydroxylamine hydrochloride (1.5002 g, 0.0216 mole) was dissolved in toluene, triethylamine (12.1 mL, 0.0864 mole) and benzoyl chloride (2.6 mL, 0.0216 mole) was added. The mixture was stirred at room temperature for 1.5 hours. Then, ethyl acetoacetate (2.75 mL, 0.0216 mole) was added, it was refluxed for 1.5 hours. After that, the mixture was extracted with 10% hydrochloric acid and then brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to give the brown oil as a crude product. After purification by column chromatography (hexane:ethyl acetate, 100:0-50:50), benzohydroxamic acid was recovered instead of the desired product.

F. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, hydroxylamine hydrochloride (0.6175 g, 0.0089 mole) was dissolved in toluene, triethylamine (2.48 mL, 0.0178 mole) and benzoyl chloride (0.0073 mole) was added. The mixture was stirred at room temperature for 1.5 hours. Then, ethyl acetoacetate (1.13 mL, 0.0089 mole) was added, the mixture was refluxed for 3 hours. After that, it was extracted with 10 % hydrochloric acid and then brine.

The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to give the brown oil as a crude product. After purification by column chromatography (hexane:ethyl acetate, 100:0-60:40), benzohydroxamic acid was recovered instead of the desired product.

G. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, hydroxylamine hydrochloride (0.6016 g, 0.0086 mole) was dissolved in toluene, triethylamine (2.4 mL, 0.0172 mole) and benzoyl chloride (1.0 mL, 0.0086 mole) was added. The mixture was stirred at room temperature for 2 hours. Then, ethyl acetoacetate (0.89 mL, 0.0069 mole) and p-toluenesulfonic acid (1.64 g, 0.0086 mole) was added, the mixture was connected to dean-stark and refluxed for 5 hours. After that, the mixture was extracted with 10 % hydrochloric acid, sodium hydrogen carbonate solution and then brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to give the brown oil as a crude product, which was purified by column chromatography (hexane:ethyl acetate, 100:0-60:40). The desired product was not detected from TLC and ^{1}H-NMR.

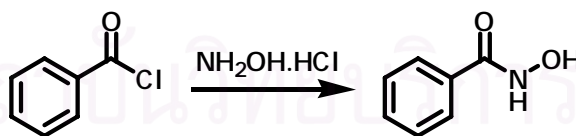2.2.4.2 Method 2

2.2.4.2.1 Preparation of benzohydroxamic acid

In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, hydroxylamine hydrochloride (2.5373 g, 0.0365 mole) was dissolved in acetonitrile, triethylamine (15.3 mL, 0.1095 mole) and benzoyl chloride (4.24 mL, 0.0365 mole) was added. The mixture was stirred at room temperature for 2 hours. Then, ether was added and the mixture was extracted with 10 % hydrochloric acid and then brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to give the

softly yellow solid as a crude product, which was crystallized by ethyl acetate to yield the desired product with 68 % yield; ^{1}H-NMR : δ = 7.7 (m, 2H), 7.5 (m, 3H); melting point 126°-128°C.

The above procedure was repeated by varying reagents and conditions as shown in Table 2.1

Table 2.1 Varying reagents and conditions of preparing benzohydroxamic acid

solvent	base	eq. base	time	%yield
toluene	pyridine	1	24 hour	trace
toluene	triethylamine	4	5 hour	trace
toluene	sodium carbonate	4	2.5 hour	trace
toluene	triethylamine	4	4.5 hour	trace
toluene	triethylamine	4	6.5 hour	no desired product
toluene	triethylamine	2	2 hour	no desired product
toluene	triethylamine	3	4 hour	trace
acetonitrile	triethylamine	4	1 hour	52%
acetonitrile	triethylamine	2	2 hour	55%
acetonitrile	triethylamine	4	2 hour	58%
acetonitrile	triethylamine	2	4.5 hour	22%
acetonitrile	triethylamine	3	5 minutes	24%

2.2.4.2.2 Synthesis of ethyl 3-aryl-5-methylisoxazole-4-carboxylate

In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, benzohydroxamic acid (0.5949 g, 0.0043 mole) was dissolved in toluene, triethylamine (1.2 mL, 0.0086 mole), ethyl acetoacetate (0.50 mL, 0.0043 mole) and molecular sieve 4 Å (1.0 g). The mixture was stirred at room temperature for 4 hours and then refluxed for 4 hours. After that, the mixture was extracted with 10 % hydrochloric acid and then brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to give the yellow oil as a crude product, which was purified by column chromatography (hexane:ethyl acetate:dichloromethane, 100:0:0-60:10:30). The desired product was not detected from TLC and ^{1}H-NMR but benzoic acid and benzohydroxamic acid was found.

The above procedure was repeated by varying reagents and conditions as follow:

A. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, benzohydroxamic acid (0.0043 mole) was dissolved in toluene, triethylamine (0.0086 mole), ethyl acetoacetate (0.0043 mole) and p-toluenesulfonic acid (1.64 g, 0.0043 mole) were added. The mixture was stirred at room temperature for 1 hour and then refluxed for 8 hours. After that, the mixture was extracted with 10 % hydrochloric acid and then brine. The organic phase was dried over anhydrous sodium sulfate and the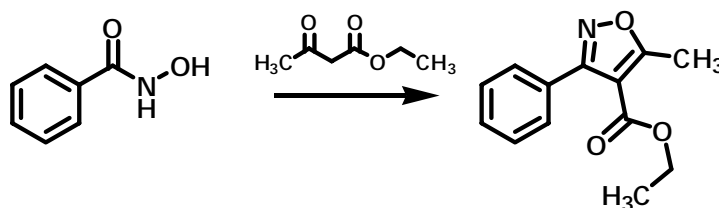 solvent was removed to give the yellow oil as a crude product, which was purified by column chromatography (hexane:ethyl acetate, 100:0-60:40). The desired product was not detected from TLC and ^{1}H-NMR but benzoic acid, benzohydroxamic acid and ethyl acetoacetate were found.

B. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, benzohydroxamic acid (0.6178 g, 0.0045 mole) was dissolved in toluene, ethyl acetoacetate (0.0045 mole), molecular sieve 4 Å (1.0 g) and *p*-toluenesulfonic acid (0.0045 mole) were added. The mixture was stirred at room temperature for 30 minutes and then refluxed for 8 hours. After that, the mixture was extracted with 10 % hydrochloric acid and then brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to give the yellow oil as a crude product, which was purified by column chromatography (hexane:ethyl acetate, 100:0-50:50). The desired product was not detected from TLC and ^{1}H-NMR but benzoic acid was found.

C. In a 50 mL, two-necked round-bottom flask equipped with a magnetic stirring bar, benzohydroxamic acid (0.0043 mole) was dissolved in dichloromethane, ethyl acetoacetate (0.32 mL, 0.0026 mole) and aluminum (III) chloride (0.69 g, 0.0052 mole). The mixture was stirred at room temperature for 26 hours. After that, ethyl acetate was added and the mixture was extracted with 10 % hydrochloric acid and then brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed to give the orange solid as a crude product, which was purified by column chromatography (hexane:ethyl acetate, 100:0-60:40). The desired product was not detected from TLC and ^{1}H-NMR but benzoic acid and benzohydroxamic acid was found.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Synthesis of ethyl 3-aryl-5-methylisoxazole-4-carboxylate *from benzaldehyde*

3.1.1 Preparation of benzaldoxime (1)

The benzaldoxime (**1**) was prepared from 2 conditions. First, the mixture of benzaldehyde, hydroxylamine hydrochloride and sodium hydroxide were put under reflux for half an hour to an hour to give benzaldoxime (**1**). The other condition, sodium hydrogen carbonate was used instead of sodium hydroxide and the reaction was prolonged at RT for 6 hours to generate benzaldoxime (Scheme 3.1). The benzaldoxime was obtained from the first reaction as a colourless solid up to 74% yield while the second condition gave almost comparable result (70%) but with longer reaction time at RT (Table 3.1).
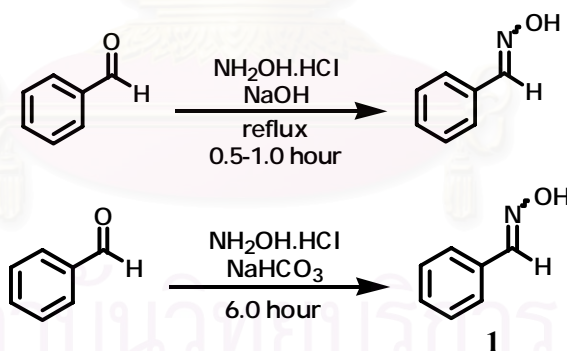
Scheme 3.1 Synthetic pathway of benzaldoxime (**1**)

Table 3.1 Percent yield of benzaldoxime (**1**) in various conditions*

Entry	Condition	%yield
1	NH₂OH.HCl(2.0 eq), NaOH(1.75 eq), EtOH, H₂O, reflux, 30 min	67
2	NH₂OH.HCl(2.0 eq), NaOH(1.75 eq), EtOH, H₂O, reflux, 60 min	74
3	NH₂OH.HCl(1.2 eq), NaHCO₃(1.80 eq), MeOH, H₂O, RT, 6 h	67

* benzaldehyde (1eq)

3.1.2 Synthesis of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (2)

The first reaction, benzaldoxime (1 eq), *N*-bromosuccinimide (1.12 eq) were mixed in chloroform. The reaction was stirred at room temperature for 24 hours. The bromobenzaldoxime (**3**) was obtained and used in the next reaction without further purification. Then, bromobenzaldoxime was reacted with ethyl acetoacetate (1.14 eq) according to the condition in Table 3.2 entry 1. The final product, ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) was obtained in 37% yield. All the characterization data corresponded well with the structure of the product.
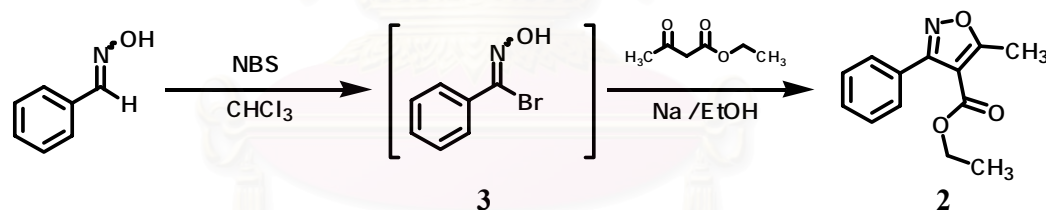
Scheme 3.2 Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**)

An attempt with higher equivalence of reagent was made with NBS 1.75 eq to benzaldoxime and run the reaction in the same condition as above to generate the desired product (Table 3.2, entry 2). After purification, the product was obtained in 30% yield. The slightly lower yield may be resulted from the excess of leftover NBS that could lead to overbromination and the decomposition of bromobenzaldoxime, affecting the yield of the final product to be less than the first condition.

The other condition of this type, we used NBS 1.00 equivalent to benzaldoxime in chloroform, and 2 drops of pyridine were added and stirred at 50°-60°C for 1 hour. Then, ethyl acetoacetate 1.08 equivalent, triethylamine 1.5 equivalent were added and the reaction mixture was stirred at 25 °C for 20 minutes. (Table 3.2, entry 3) We recovered only the substrates, ethyl acetoacetate and benzaldoxime while the desired product was not found.
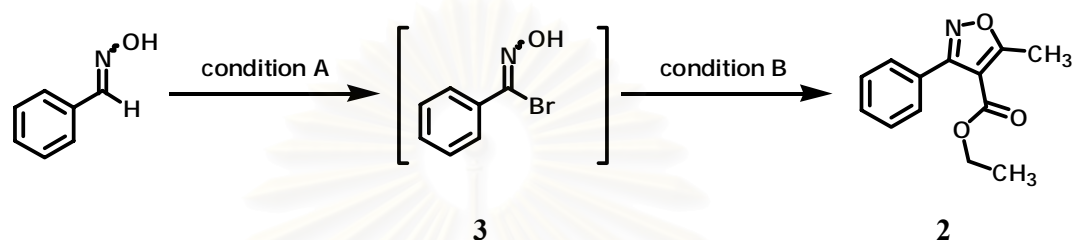
Scheme 3.3 Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) via bromobenzaldoxime

Table 3.2 Conditions and results for synthesis ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) via bromobenzaldoxime

| entry | condition A[a] | condition B | | | | result |
		ethyl acetoacetate (eq)	base	temp	time	
1	NBS (1.12eq), room temperature 24 hours	1.14	Na/EtOH	RT	48 h	product **2** 37%
2	NBS (1.75eq), room temperature 48 hours	1.14	Na/EtOH	RT	48 h	product **2** 30%
3	NBS (1.00eq), py[b] 50 °-60 °C 1 hour	1.08	TEA (1.5eq)	RT	20 min	recovered starting materials

[a] solvent = CHCl$_3$ [b] py = pyridine 2 drops

3.2 Synthesis of ethyl 3-aryl-5-methylisoxazole-4-carboxylate *from benzaldoxime and ethyl β-pyrrolidinocrotonate*

3.2.1 Preparation of ethyl β-pyrrolidinocrotonate (4)

The ethyl β-pyrrolidinocrotonate (EBC, **4**) used in this work was synthesized by the reaction of ethyl acetoacetate (1.0 eq) and pyrrolidine (1.0 eq) in benzene or toluene (Scheme 3.4). The product was obtained as yellow oil in 98% yield, which was used without further purification.
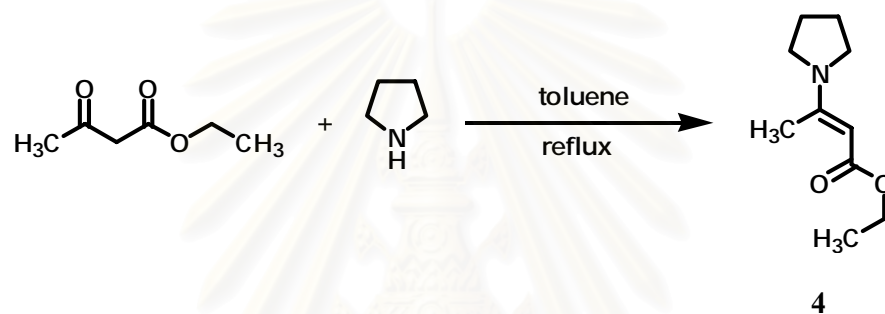
Scheme 3.4 Synthetic pathway of ethyl β-pyrrolidinocrotonate (EBC, **4**)

3.2.2 Synthesis of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (2)

We attempted to synthesize the desired product, ethyl 3-aryl-5-methylisoxazole-4-carboxylate, by using benzaldoxime (1 eq) and ethyl β-pyrrolidinocrotonate (1 eq) in toluene (Scheme 3.4). The reaction yielded benzoic acid and benzaldoxime instead (Table 3.3, entry 1). Replacing with triethylamine (2 eq) as the additive base still did not give the desired product (entry 2). Switching to acidic condition by using *p*-toluenesulfonic acid (1 eq) (entry 3) or iron (III) chloride (1 eq) (entry 4) did not give an appreciable amount of product. The latter case gave some indications that corresponded to the desired product in ^{1}H-NMR and on TLC plate but it's too small comparing to other major components which was not worth the separation.

The other condition using benzaldoxime (1 eq), triethylamine (2 eq) and ethyl acetoacetate (1 eq) instead of ethyl β-pyrrolidinocrotonate detected only the starting ethyl acetoacetate while the desired product was not found.
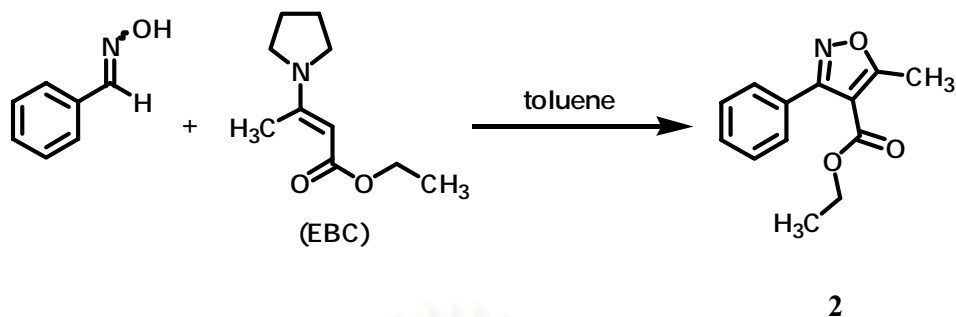
Scheme 3.5 Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) from benzaldoxime

Table 3.3 Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) via benzaldoxime and EBC[a]

entry	additive	temp	time (h)	result
1	-	reflux	7.5	benzoic acid benzaldoxime
2	triethylamine (2 equivalent)	reflux	6.0	benzaldoxime
3	*p*-toluene sulfonic acid	room temp / reflux	50 / 29	benzoic acid
4	FeCl₃	reflux	48	trace
5[b]	triethylamine (2 equivalent)	reflux	3.0	ethyl acetoacetate

[a] toluene, benzaldoxime (1 eq), EBC (1 eq)

[b] ethyl acetoacetate (1 eq) was used instead of EBC.

3.3 Synthesis of ethyl 3-aryl-5-methylisoxazole-4-carboxylate *from benzyl bromide*

3.3.1 Method 1
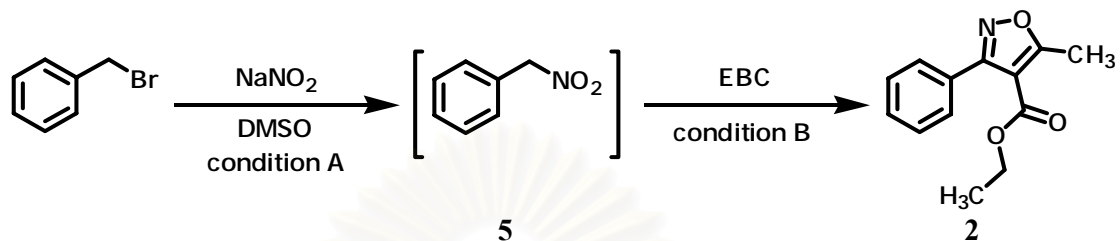
Scheme 3.6   Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) from benzyl bromide, method 1

The nucleophilic substitution of benzyl bromide with sodium nitrite in DMSO under room temperature gave α-nitromethylbenzene. In the case of entry 1, Table 3.4, the starting material, benzyl bromide and the undesired overoxidized product, benzoic acid were obtained. To complete the reaction of the remaining benzyl bromide, the reaction was stirred at room temperature for 4 hours and then only ethyl β-pyrrolidinocrotonate (1.2 eq) was added. The reaction was refluxed for 4 days. Only benzoic acid was found in this reaction (entry 2). The last attempt shortened the stirring at room temperature for 45 minutes in the first step. After that, ethyl β-pyrrolidinocrotonate (1.2 eq) and acetic acid (10 eq) were added to the reaction which was stirred for 26.5 hours at room temperature. Similarly, benzoic acid was still obtained instead of the desired product (entry 3).

Table 3.4 Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) from benzyl bromide, method 1

| entry | condition A[a] | condition B[b] | | | result |
	time	additive	temp	time	
1	20 min	AcOH (4 eq)	60°C	5 h	benzyl bromide and benzoic acid
2	4 h	-	reflux	4 days	benzoic acid
3	45 min	AcOH (10 eq)	RT	26.5 h	benzoic acid

[a] NaNO$_2$ (2 eq), benzyl bromide (1 eq), solvent = DMSO

[b] ethyl β-pyrrolidinocrotonate (1.2 eq), solvent = DMSO

3.3.2 Method 2

Scheme 3.7 Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) from benzyl bromide, method 2

Next, we tried to change the conditions as shown in Table 3.5. Unfortunately, we obtained only benzoic acid while the desired product was not found in all conditions.

The reactions of both pathways started with benzyl bromide and sodium nitrite in DMSO to generate α-nitromethylbenzene and then used ethyl β-pyrrolidinocrotonate to capture the nitrile oxide, the reactive intermediate before it changed to benzoic acid, to form isoxazole or isoxazoline (Figure 1.18) [24] products. The mechanisms are shown in Scheme 3.8 and 3.9. It seemed that the nitrile oxide intermediate from these conditions were too labile and only benzoic acid was obtained instead of the desired product.
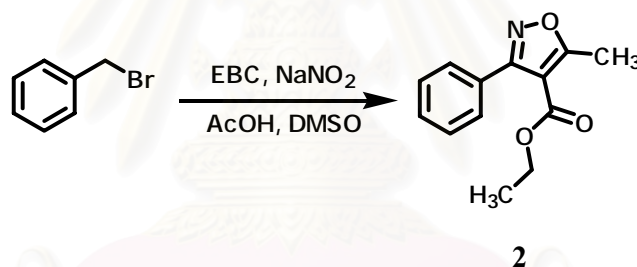
Table 3.5 Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) from benzyl bromide, method 2*

entry	eq. of EBC	time	result
1	7.0	27 h	benzoic acid
2	7.0	8 h	benzoic acid
3	0.5	24 h	benzoic acid
4	7.0	29 h	benzoic acid
5	7.0	32 h	benzoic acid

* benzyl bromide (1 eq), NaNO$_2$ (2 eq), AcOH (10 eq) and
  all reactions were run at room temperature
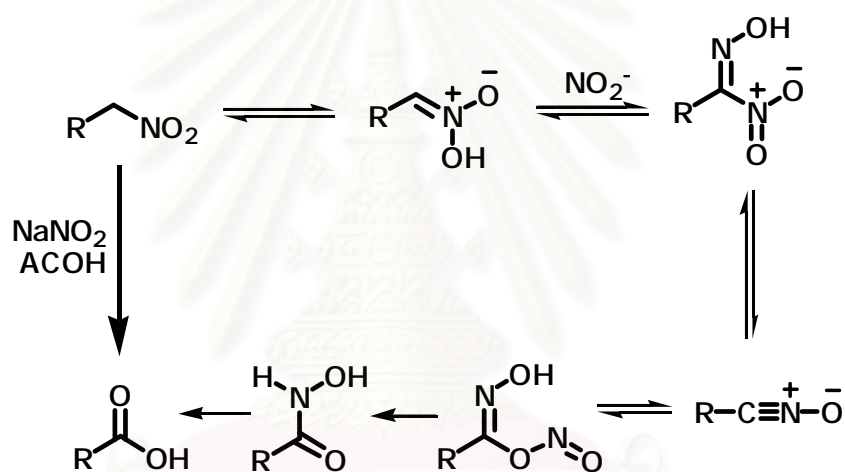
Scheme 3.8 Mechanism of the conversion of α-nitromethyl benzene to benzoic acid (R = Phenyl)
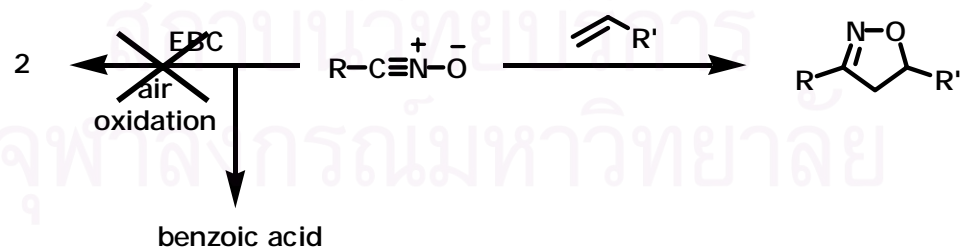
Scheme 3.9 Trapping of nitrile oxide intermediate

3.4 Synthesis of ethyl 3-aryl-5-methylisoxazole-4-carboxylate *from benzoyl chloride*

3.4.1 Method 1

3.4.1.1 Preparation of benzohydroxamic acid

The benzohydroxamic acid (**6**) was prepared from the reaction of benzoyl chloride, hydroxylamine hydrochloride and base (Scheme 3.10). We varied conditions as shown in Table 3.6 and found the optimized reaction that used acetonitrile as the solvent, triethylamine (3 eq) as the base and the reaction was stirred for 2 hours which gave benzohydroxamic acid as a colourless solid in 68% yield (entry 12). The ^{1}H-NMR showed a doublet at δ 7.7 – 7.8 (2H, ArH) and a multiplet at δ 7.4 – 7.6 (3H, ArH).
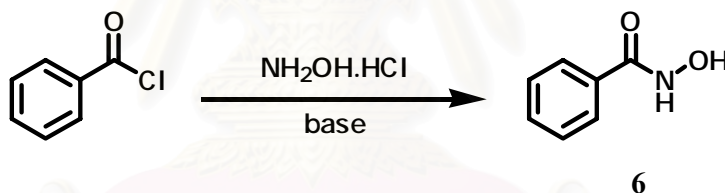
Scheme 3.10 Synthetic pathway of benzohydroxamic acid (**6**)

Because solubility of hydroxylamine hydrochloride in toluene was poor in toluene, benzohydroxamic acid was obtained in trace amount. In the case of acetonitrile, hydroxylamine hydrochloride could dissolved more than in toluene so yields of benzohydroxamic acid was higher.

Table 3.6 Synthetic pathway of benzohydroxamic acid (**6**) in various conditions

| entry	solvent	base	eq.base	time	result
1	toluene	pyridine	1	24 h	trace
2	toluene	triethylamine	4	5 h	trace
3	toluene	Na$_2$CO$_3$	4	2.5 h	trace
4	toluene	triethylamine	4	4.5 h	trace
5	toluene	triethylamine	4	6.5 h	NDP[a]
6	toluene	triethylamine	2	2 h	NDP[a]
7	toluene	triethylamine	3	4 h	trace
8	acetonitrile	triethylamine	4	1 h	52%
9	acetonitrile	triethylamine	2	2 h	55%
10	acetonitrile	triethylamine	4	2 h	58%
11	acetonitrile	triethylamine	2	4.5 h	22%
12	acetonitrile	triethylamine	3	2 h	68%
13	acetonitrile	triethylamine	3	5 min	24%

[a] NDP = no detectable product

3.4.1.2 Synthesis of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**)
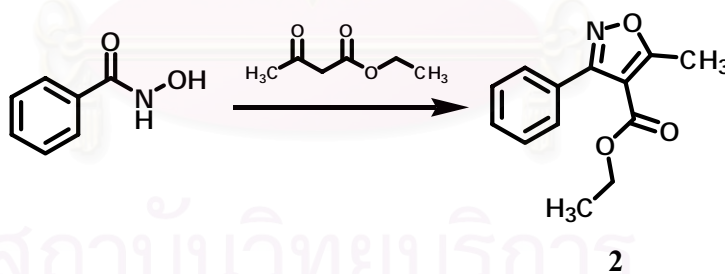
Scheme 3.11 Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) from benzohydroxamic acid

We tried to synthesize ethyl 3-aryl-5-methylisoxazole-4-carboxylate by using benzohydroxamic acid and ethyl acetoacetate while the conditions were varied as shown in Table 3.7. However, only the starting materials, benzohydroxamic acid and ethyl acetoacetate were recovered instead.

Table 3.7 Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) from benzohydroxamic acid

entry	base	eq of ethyl acetoacetate	solvent	MS4A[a]	Time (RT)	Time (reflux)	additive	result
1	TEA (2eq)	1	toluene	✓	4 hour	4 h	-	NDP[c]
2	TEA (2eq)	1	toluene	✓	1 hour	8 h	acid[b] (1eq)	NDP
3	-	1	toluene	✓	30 min	8 h	acid[b] (1eq)	NDP
4	-	0.5	CH_2Cl_2	✗	26 hour	-	$AlCl_3$ (1eq)	NDP

[a] MS4A = molecular sieve 4A; ✓ = used ✗ = not used

[b] acid = *p*-toluenesulfonic acid, [c] NDP = no detectable product

3.4.2 Method 2

3.4.2.1 Synthesis of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**)
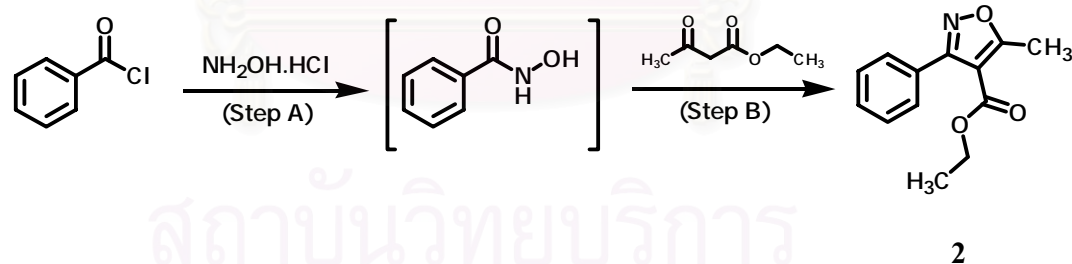
Scheme 3.12 Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) from benzoyl chloride

Next, we decided to synthesize ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) by using the same substrates to generate benzohydroxamic acid (Table 3.8A for the first step and Table 3.8B for the second step). Similar to the previous method, all conditions did not give the desired product but gave the benzohydroxamic acid or

ethyl acetoacetate instead. It seemed that the benzohydroxamic acid or its salt were too inert toward condensation reaction under all conditions attempted.

Table 3.8A Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) from benzoyl chloride in various conditions (STEP A)[a]

entry	eq.NH$_2$OH.HCl	eq. base	solvent	time
1	1.0	solvent	triethylamine	2 h
2	1.0	4.0	acetonitrile	2 h
3	1.2	4.0	acetonitrile	2 h
4	1.0	4.0	toluene	1.5 h
5	1.2	2.0	toluene	1.5 h
6	1.2	2.4	toluene	1.5 h
7	1.0	4.0	toluene	1.5 h
8	1.0	2.0	toluene	2 h

[a] all reaction was stirred at room temperature, triethylamine was used as base.
benzoyl chloride (1eq)

Table 3.8B Synthetic pathway of ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) from benzoyl chloride in various conditions (STEP B)[a]

entry	eq.ethyl acetoacetate	additive	solvent	time	result
1	0.8	-	triethylamine	2 h	NDP[c]
2	1.0	-	acetonitrile	5 h	NDP
3	1.2	-	acetonitrile	4 h	NDP
4	1.0	-	toluene	1.5 h	NDP
5	1.2	-	toluene	3 h	NDP
6	1.2	-	toluene	1.5 h	NDP
7	1.0	-	toluene	1.5 h	NDP
8	1.0	acid[b]	toluene	5 h	NDP

[a] all reaction was refluxed, [b] acid = *p*-toluenesulfonic acid (1 eq),

[c] NDP = no detectable product

CHAPTER IV

CONCLUSION

Ethyl 3-aryl-5-methylisoxazole-4-carboxylate (**2**) has been synthesized starting from benzaldehyde in three steps. First, benzaldoxime (**1**) was generated from benzaldehyde with 67 – 74% yield using hydroxylamine hydrochloride and sodium hydroxide in ethanol. Second, benzaldoxime was reacted with NBS in chloroform to form bromobenzaldoxime (**3**). Finally, the reaction between bromo benzaldoxime and ethyl acetoacetate afforded the desired isoxazole in moderate yields (30-37%).

The reaction of benzaldoxime and ethyl β-pyrrolidinocrotonate (**4**) in the presence of $FeCl_3$ showed some indications of the isoxazole product, detected by TLC and ^{1}H-NMR. However, the quantity obtained was too small to be seperated from the crude mixture.

Other conditions including the reaction of benzaldoxime and ethyl β-pyrrolidinocrotonate with triethylamine or *p*-toluenesulfonic acid at room temperature or reflux, and in all conditions tested, failed to yield the desired product. The reaction of benzyl bromide with sodium nitrite in DMSO to generate the reactive intermediate, the nitrile oxide, could not be trapped by either ethyl β-pyrrolidinocrotonate or ethyl acetoacetate in any conditions used in this work. Similarly, the reaction of benzoyl chloride with hydroxylamine hydrochloride to yield benzohydroxamic acid (**6**) and then reacted with ethyl acetoacetate did not give the desired product, ethyl 3-aryl-5-methylisoxazole-4-carboxylate. Only the starting materials were recovered in most cases.

Suggestions and future work

1. Try to optimize the reaction of benzaldehyde with hydroxylamine hydrochloride to generate benzaldoxime and then reacted with NBS and ethyl acetoacetate to form isoxazole product.

2. Try to optimize the reaction of benzaldoxime and EBC when $FeCl_3$ was used and reaction was refluxed for 48 hours.

3. Change the substrate from benzaldehyde to chlorobenzaldehyde, dichlorobenzaldehyde and m-terphenyl to generate the isoxazole derivatives.

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APPENDICES
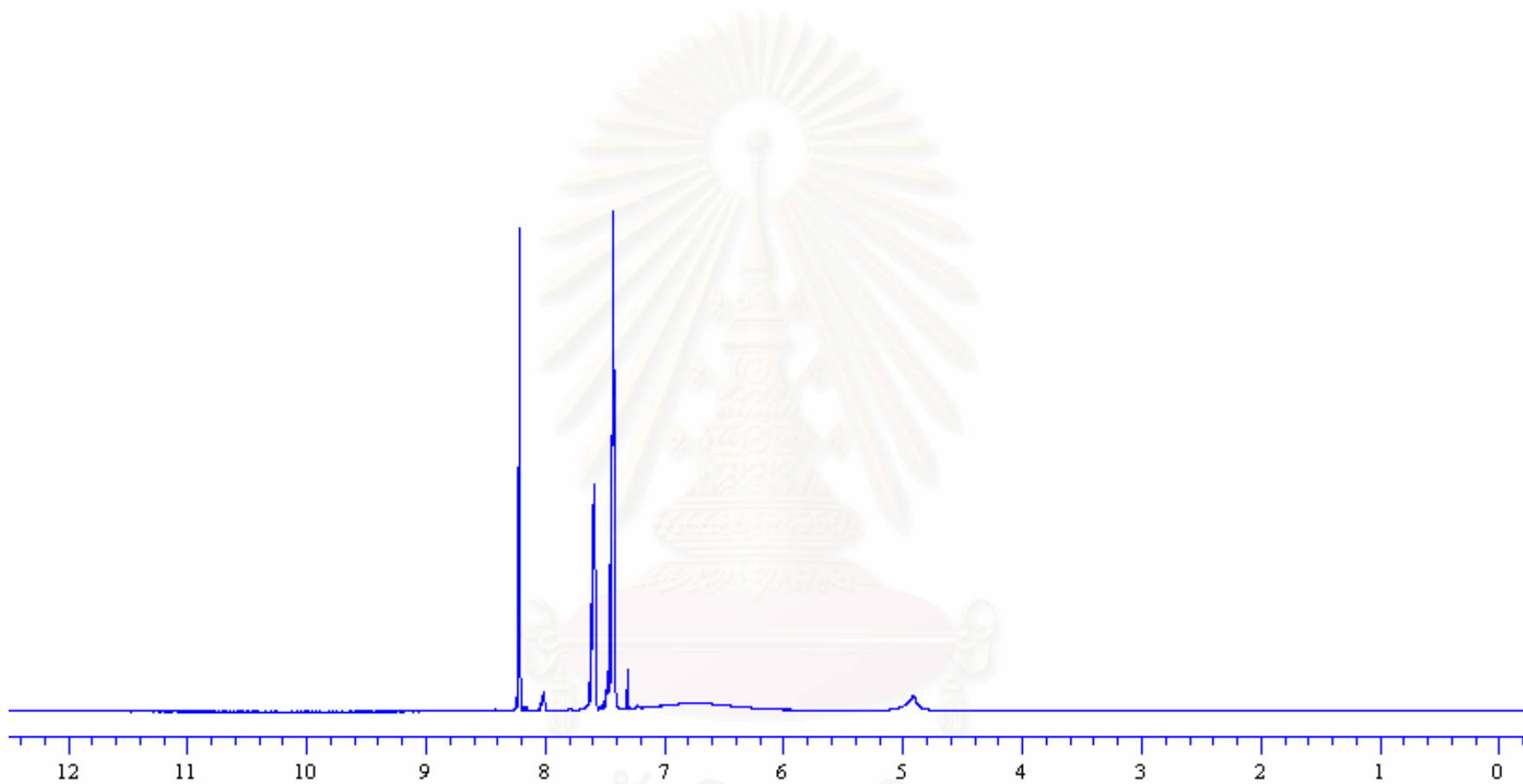
Figure A-1 ^{1}H-NMR spectrum of benzaldoxime, **1**
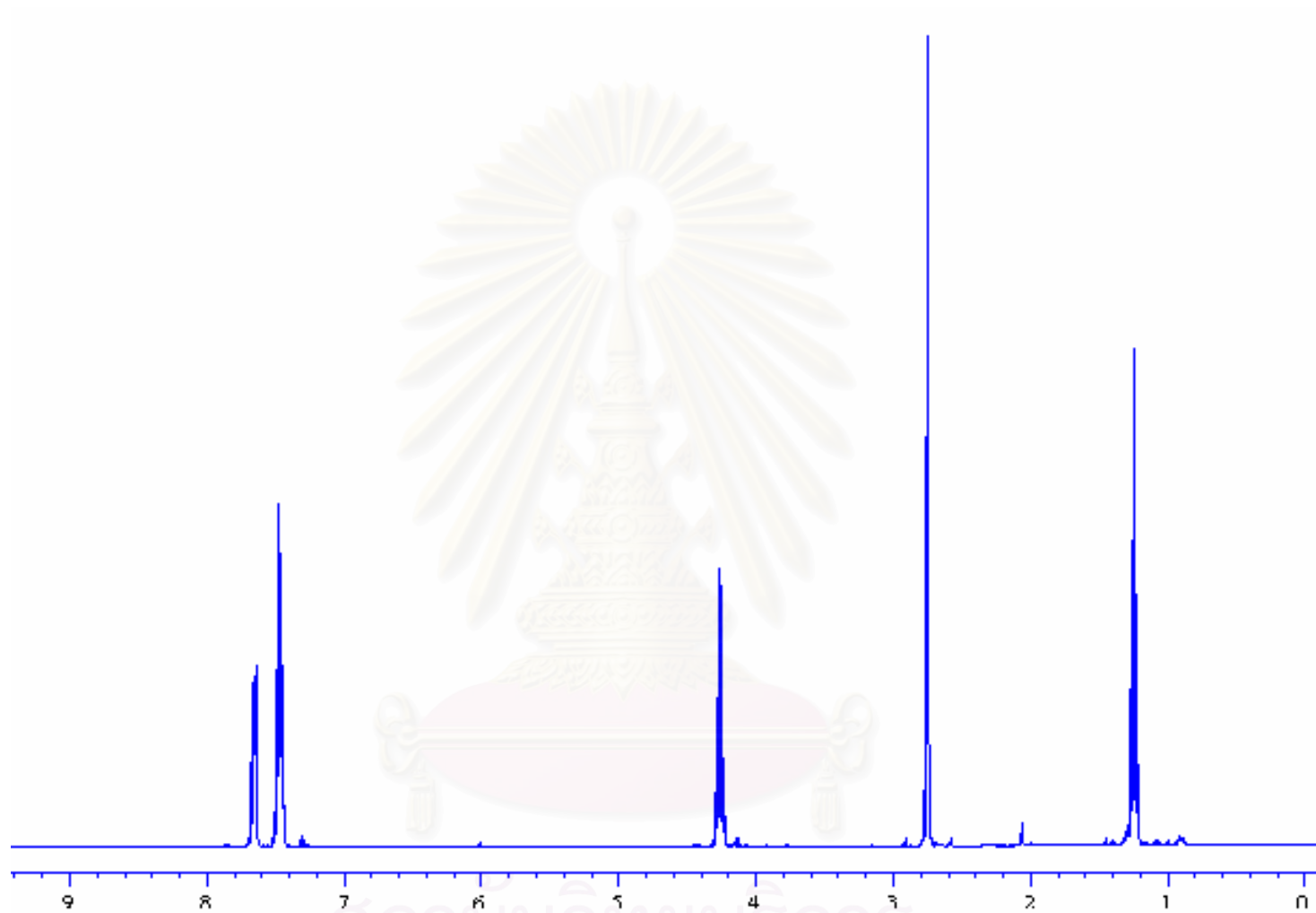
Figure A-2 ^{1}H-NMR spectrum of ethyl 3-aryl-5-methylisoxazole-4-carboxylate, **2**
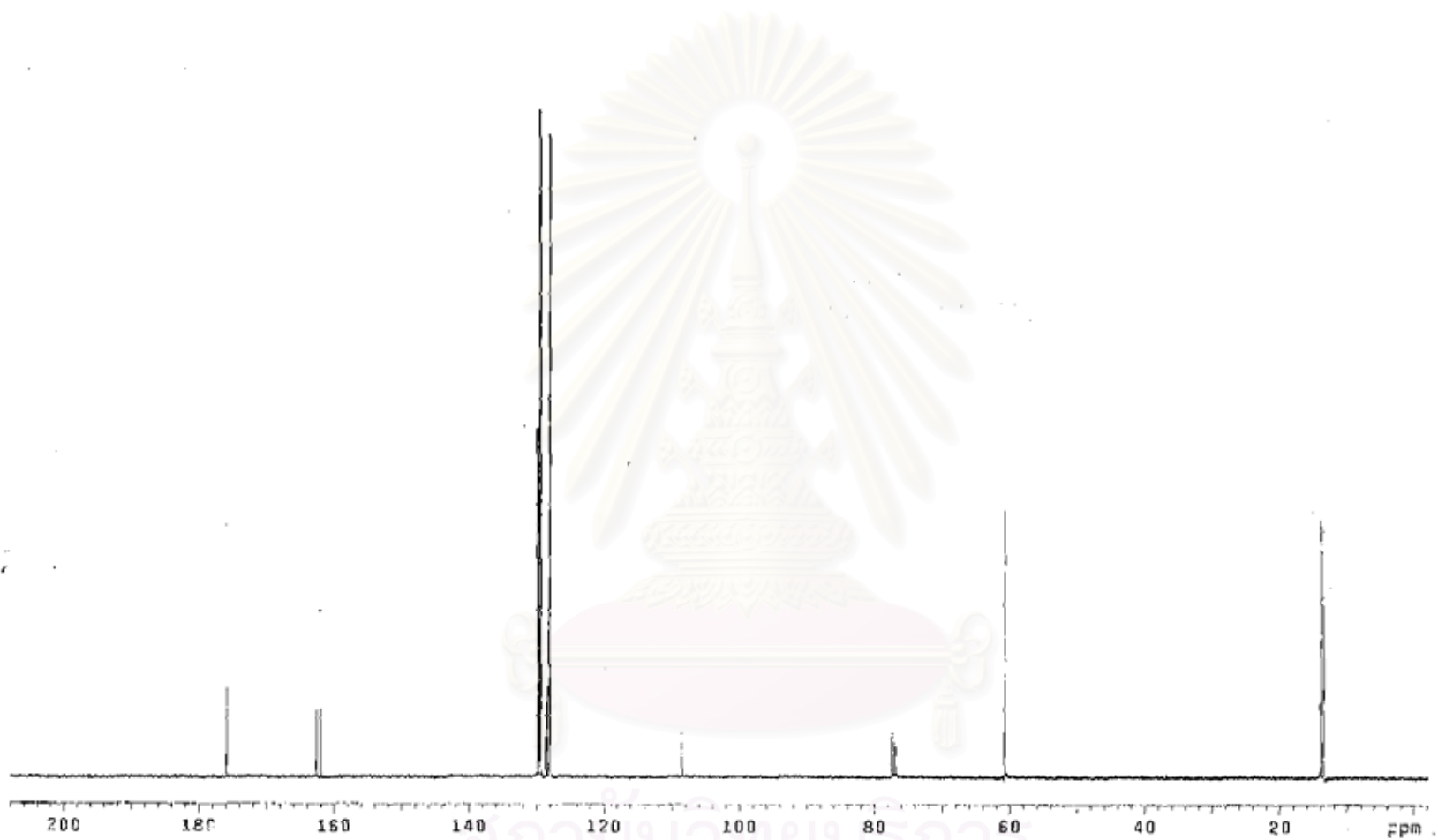
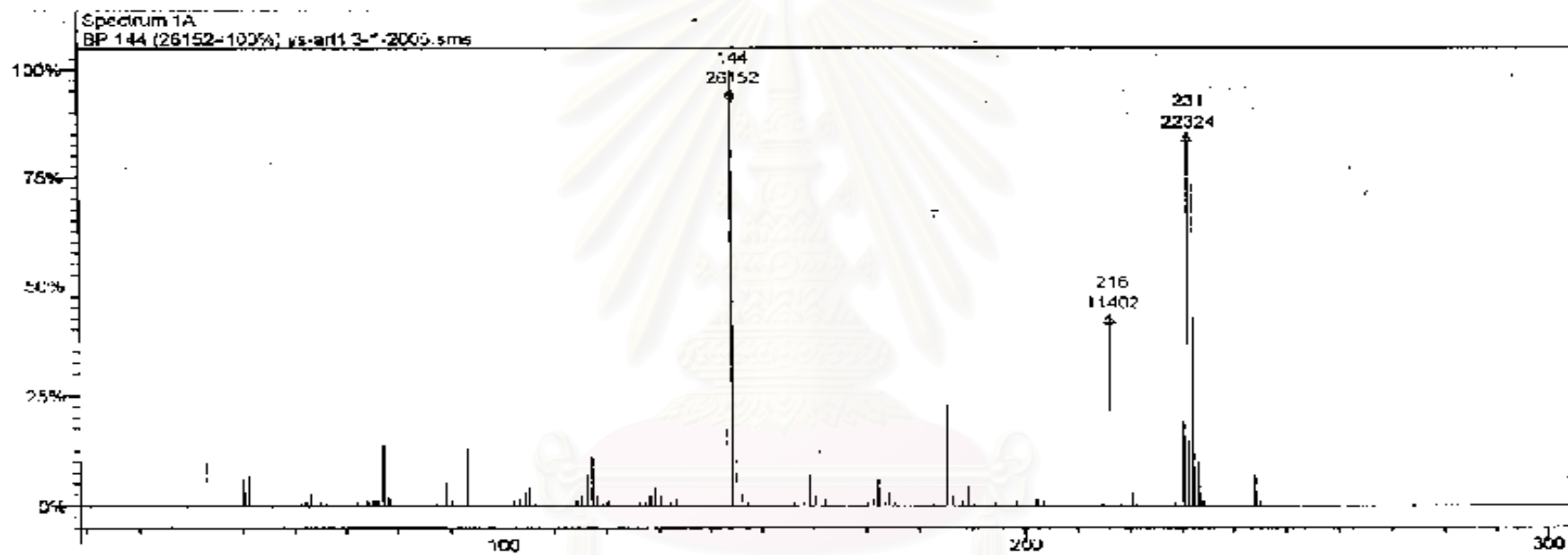**Figure A-3** ^{13}C-NMR spectrum of ethyl 3-aryl-5-methylisoxazole-4-carboxylate, **2**

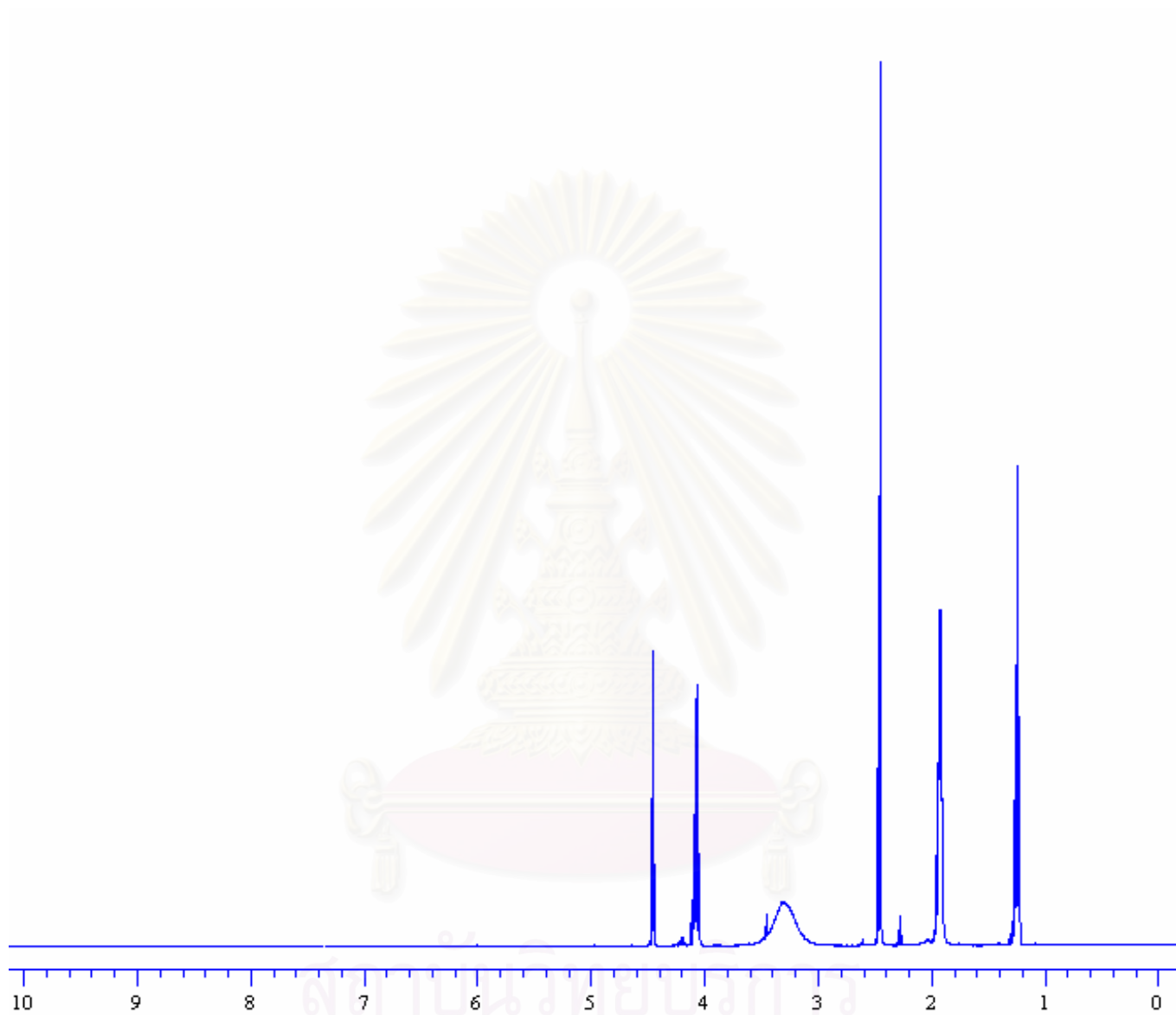**Figure A-4** Mass spectrum of ethyl 3-aryl-5-methylisoxazole-4-carboxylate, **2**

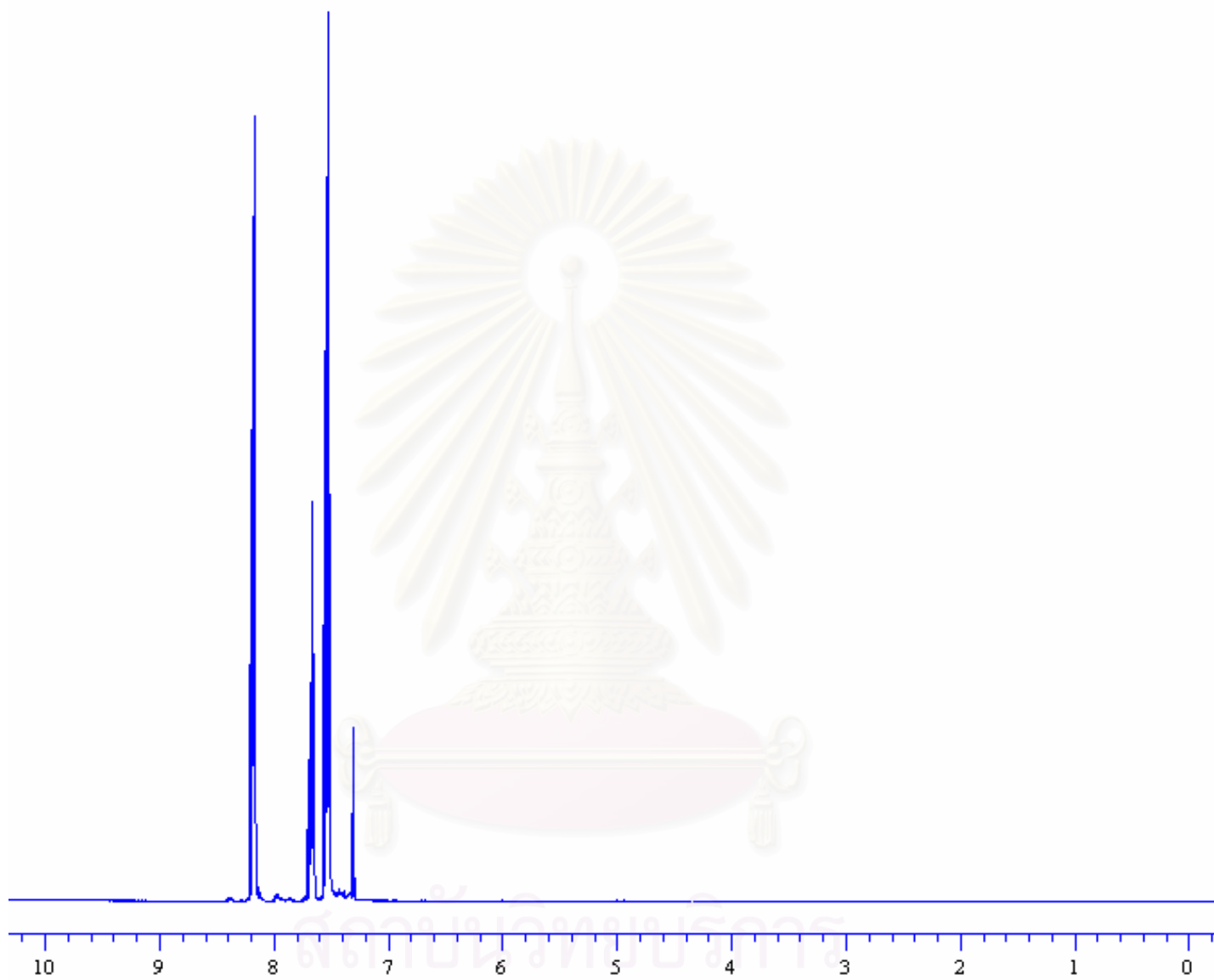**Figure A-5** ^{1}H-NMR spectrum of ethyl β-pyrrolidinocrotonate (EBC), **4**

Figure A-6 ^{1}H-NMR spectrum of benzohydroxamic acid, **6**

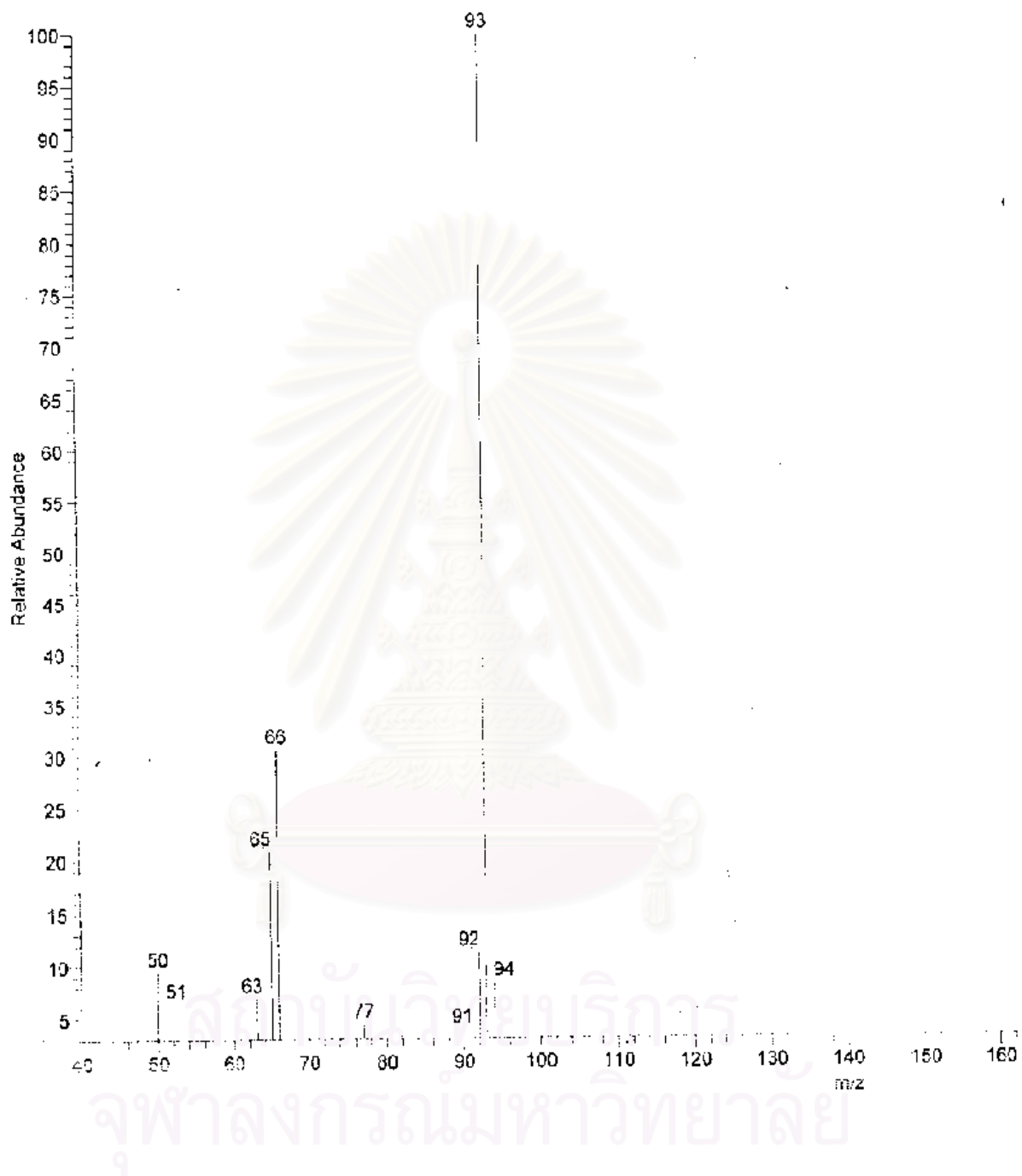**Figure A-7** Mass spectrum of benzohydroxamic acid, **6**

VITAE

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