NEW CHIRAL PHOSPHONATES FOR ORGANOCATALYSIS

AND

ISOLATION AND CHEMICAL-BIOLOGY OF NATURAL PRODUCTS FROM ONTARIO PLANTS

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By

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NMR 8 ^{1}H NMR for the cis-dihydronarciclasine

ABBREVIATIONS

DMF	*N,N*-dimethylformaamide
$CHCl_3$	Chloroform
RT	Room temperature
DMSO	Dimethyl sulfoxide
Et	Ethyl
Bu	Butyl
Ph	Phenyl
DCM	Dichloromethane
TEA	Triethylamine
$(Boc)_2O$	Di-*tert*-butyl dicarbonate
LAH	Lithium Aluminium hydride
THF	Tetrahydrofuran
MsCl	Methanesulfonyl chloride
DIPEA	N,N-Diisopropylethylamine
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography
n-BuLi	*n*-butyllithium
DBU	1,8-Diazabicycloundec-7-ene
Boc	Benzyloxy-carbonyl
NMR	Nuclear magnetic resonance
Ac	Acetyl
SAMP	(*S*)-1-amino-2-methoxymethylpyrrolidine
$CDCl_3$	Deuterated chloroform
EtOH	Ethanol
NaH	Sodium hydride
Nu	Nucleophile
Na	Sodium
H_2O	Water
$Pd(OAc)_2$	Palladium(II) acetate
PPh_3	Triphenyl phosphine
CO_2	Carbon dioxide
PhCl	Chlorobenzene
Na_2SO_4	Sodium sulphate
KOH	Potassium hydroxide
MeOH	Methanol
n-BuOH	*n*-butanol
Pd/C	Palladium on carbon
Ac_2O	Acetic anhydride
NaOMe	Sodium methoxide
HRMS	High resolution mass spectrometry
EtOAc	Ethyl acetate

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ABSTRACT

The catalytic asymmetric transformation of carbonyl compounds via iminium and enamine intermediates using chiral amines as organocatalyst has grown remarkably over last decade. Various "metal-free" reactions including aldol, Mannich, Michael, alkylation and Knoevenagel types have now been reported efficiently using simple amino acids as the sole organocatalysts. Amongst these, proline has proven to be particularly useful as a general catalyst in such catalytic asymmetric syntheses due to its unique properties. Nonetheless, proline suffers inherent fundamental disadvantages as an organocatalyst in terms of its solubility in organic media, limiting reactions to polar aprotic solvents such as DMF or DMSO. We have been interested in the synthesis of derivatives of proline incorporating functional groups that would render the chiral secondary amine fully soluble in organic solvents such as THF or dichloromethane and others that could be employed in aqueous media. The synthesis of these catalysts and their application towards the asymmetric synthesis will be presented.

The thesis also describes isolation and identification of natural products and synthesis of their semi-stabilised derivatives. Sakuranetin and dihydrowagonine were isolated from *Prunus avium* plant parts, menisadurilide was isolated from *Dicentra spectabilis* extracts while narciclasine was isolated from *Narcissus pseudonarcissus* plant parts. Semi-synthetic derivative, *trans*-dihydronarciclasine was prepared from natural product narciclasine. Both the derivatives were subjected to antifungal and human cytochrome inhibition activity and their correlation is explained. The Amaryllidaceae family alkaloids and their semi-synthetic derivatives were subjected to biological testing and an important structural property is correlated to their activities.

CHAPTER 1.

New Chiral Phosphonates for Organocatalysis

1.1 Introduction:

One of the major objectives in the science of chemical synthesis is the identification of new strategies or methodologies that will allow access to moieties of increased structural complexity. Such research has applications in many fields including the fine chemicals, pharmaceuticals and materials areas. The synthesis of biologically active molecules has always been a challenge. Structural complexity in conjunction with valuable biological activity has provided the challenge and impetus for synthetic organic chemists to investigate new methods and to tackle challenging molecules, particularly employing methods of asymmetric synthesis. Many bioactive molecules, including both natural products and synthetic pharmaceuticals, contain one or more stereogenic centres and exist as single enantiomers. The development of methodology which would lead to asymmetric synthesis is a major area of research interest. Nature's catalysts for asymmetric synthesis – the enzymes - are well known for their efficiency and enantioselectivity; however, their limitations in terms of substrate specificity and applicability to only one enantiomeric series have always reduced their scope. Chiral-pool methods for asymmetric synthesis are likewise limited in scope. Until very recently, most general methods for asymmetric synthesis employed either chiral auxiliaries, or were based on the use of chiral transition metal catalysts, for example the Noyori catalysts for asymmetric hydrogenation, or chiral metal-containing Lewis acid catalysts. Since the year 2001, the use of a growing number of small organic molecules as efficient and enantioselective catalysts has been reported based on chiral secondary amines, such as (*S*)-proline. In recent years "asymmetric organocatalysis" has emerged as an important and forefront area for asymmetric synthesis, now complementing the use of auxiliaries and metal based catalysis.

Asymmetric catalysis of carbonyl transformations via iminium ion and enamine intermediates using chiral amines as organocatalysts has grown remarkably over the last decade. Proline-catalyzed intramolecular aldol reactions were discovered in the early

1970s[1]. The reaction was not completely explored and its applications were limited to a particular area at that time[2]. Lately a renaissance in the area has resulted in an explosive growth in chiral amine catalysis[3]. As an example, while the (*S*)-proline catalyzed asymmetric intramolecular aldol reaction was discovered in 1974, it took until the year 2000 for the discovery of the first intermolecular asymmetric aldol reaction [4].

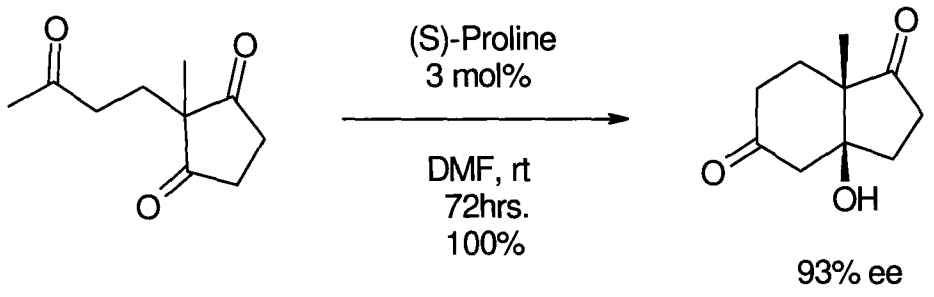
Although several strategies are available for enantioselective organocatalysis, the iminium and enamine activation remains the most popular. Several small molecules have been suggested for carrying out asymmetric organocatalytic reactions but proline plays a crucial role in the development of these reactions. It has been used as an effective asymmetric organocatalyst in various important transformations such as aldol, Michael and Mannich reactions. There are number of advantages of proline being used as organocatalyst including its stability in air, relatively inexpensive, readily available, containing a bifunctional acid/base nature and also the availability of both the enantiomers. While all amino acids have the bifunctionality advantage, the unique cyclic secondary amine of proline exhibits an increased *pKa* value compared to primary amino acids. It also exhibits an increased level of Lewis basicity because of the cyclic structure[5]. Although proline possesses these advantages as an organocatalyst, it also carries a few drawbacks such as, its negligible solubility in non polar organic solvents thus requiring the use of polar reaction solvents and often the need of high catalyst loading. Thus, development of new organocatalysts possessing advantages and eliminating disadvantages that proline carries would be one greater step in the field of organocatalysis.

1.2 History:

1.2.1 Enamine activation:

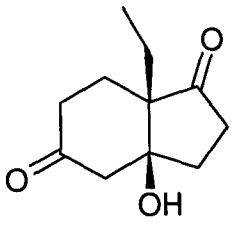
Electrophilic substitution reactions at the α– position of carbonyl compounds using primary or secondary amines as organocatalysts proceeds via enamine

intermediates and is called enamine catalysis. In the early 1970's Hajos & Parrish reported a proline catalyzed intramolecular aldol reaction[6] (Scheme 1).



Scheme 1: Proline catalyzed intramolecular aldol reactions

This type of proline catalyzed asymmetric intramolecular aldol, now known as the Hajos- Parrish- Eder- Sauer- Wiechert reaction was applied to a number of commercial transformations[7]. Shortly after its discovery, the reaction was extensively utilised for the synthesis of steroids and natural products. A few molecules synthesized by organocatalyzed intramolecular aldol reactions are shown below (Figure 1).



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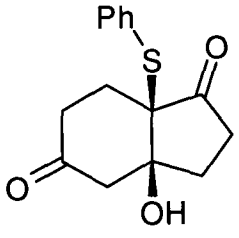

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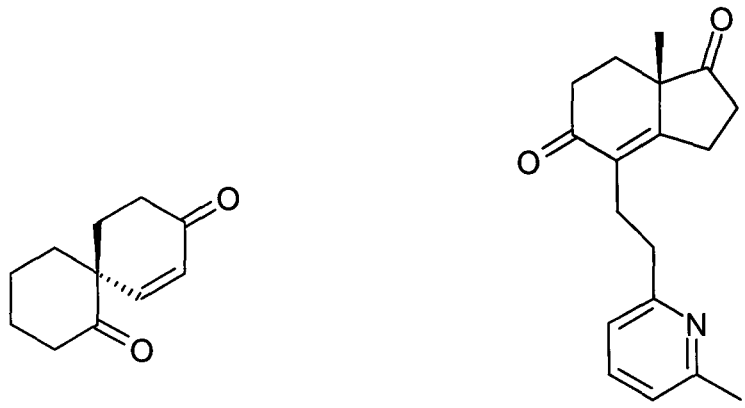

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Figure 1: Molecules synthesized by organocatalyzed intramolecular aldol reactions[7]

Various possible reaction mechanisms for the proline catalyzed intramolecular aldol reaction have been proposed. According to Hajos and Parrish the carbonyl group is activated as a carbinolamine which undergoes nucleophilic substitution by side chain enol[1, 7] (Figure 2, intermediate I). Later, in 1984, Agami *et al.* proposed a two-proline molecule model[8] (Figure 2, intermediate II) where one proline activates the nucleophilic carbonyl component by forming an enamine while the other proline increases electrophilicity of another carbonyl group by hydrogen bonding. On the basis of the insolubility of proline in organic solvents a heterogeneous catalysis having concerted acid – base mechanism was proposed by Rajagopal and workers[9] (Figure 2, intermediate III). Although all these mechanisms explain the mechanistic behind formation of product, none of them explained the stereochemical outcome. Recently, Houk *et al.* proposed a new mechanism (Figure 2, intermediate IV), which also explains the enantioselectivity due to hydrogen bonding between the acidic proton and oxygen of the electrophilic carbonyl group[10].

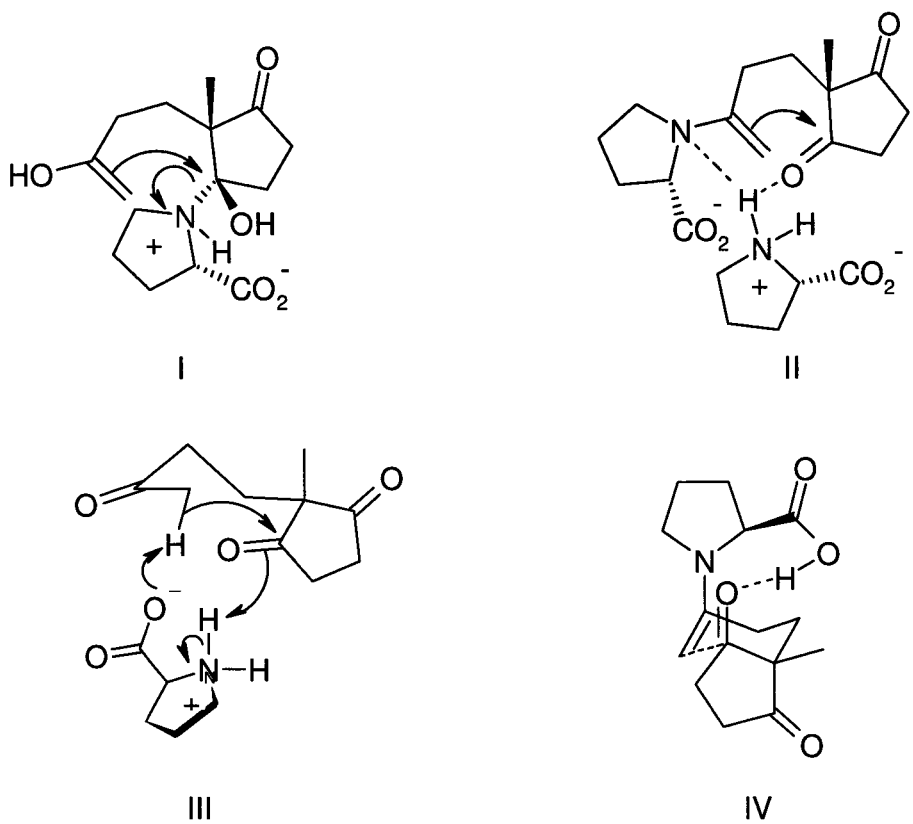


Figure 2: Mechanism proposed for proline catalysis[10]

Primary amine-containing amino acids like phenylalanine work well for a few organocatalytic intramolecular aldol reactions but prove to be poor catalysts for intermolecular organocatalytic aldol reactions[11]. Researchers also tried other amino acids having primary and cyclic or acyclic secondary amines as organocatalysts; however they found proline more efficient[12]. Although the results showed the necessity of a secondary amine group, acyclic, secondary amino acids like N-methylvaline were not catalytic and a six-membered ring amine, as in pipecolic acid, was inactive. From these studies it was clear that pyrrolidines readily forms catalytic amounts of enamines with carbonyl compounds compared to piperidines[13]. This work singled out proline as an important organocatalyst readily proceeding via enamine activation.

1.2.2 Iminium activation:

Condensation of primary amines with aldehydes or ketones was discovered by Schiff[14] in 1864. This reaction gives rise to an imine, which is basic in nature[15] and also known as a Schiff base. Under acidic conditions it exists as the corresponding protonated iminium ion which is a stronger electrophile compared to the parent carbonyl group. Even though both primary and secondary amines can form iminium ions, secondary amines have dominated the field of iminium ion catalysis.

A specific date of introduction of iminium catalysis is not clear. There are discoveries and theoretical advances which developed the field over a long period of time, prior to asymmetric catalysis. The earliest example is perhaps the Knoevenagel condensation using primary and secondary amines[16]. Blanchard in 1931 suggested the possibility of the involvement of positive ions in the catalysis of the Knoevenagel condensation[17] while Crowell and Peck showed kinetic evidence for the presence of imine or iminium intermediates in the Knoevenagel condensation[18]. A few more well known examples involving iminium catalysis include the proline-catalyzed partial deracemization of an intermediate in Woodward's synthesis of erythromicin[19] and the discovery of iminium-catalyzed transimination reactions by Cords and Jencks[20]. In the 1970s research interest was shifted to deprotonation of the iminium intermediate, which relates back to enamine formation.

1.3 Background:

1.3.1 Enamine catalytic cycle:

Under dehydrating conditions, an amine condenses with a carbonyl compound giving an iminium ion, which after deprotonation at the α-position, forms an enamine. This reaction can proceed via either nucleophilic addition or substitution depending upon the electrophile and results in the iminium ion. Hydrolysis of the iminium ion reproduces the carbonyl group as shown in the following Figure[4] (Figure 3).

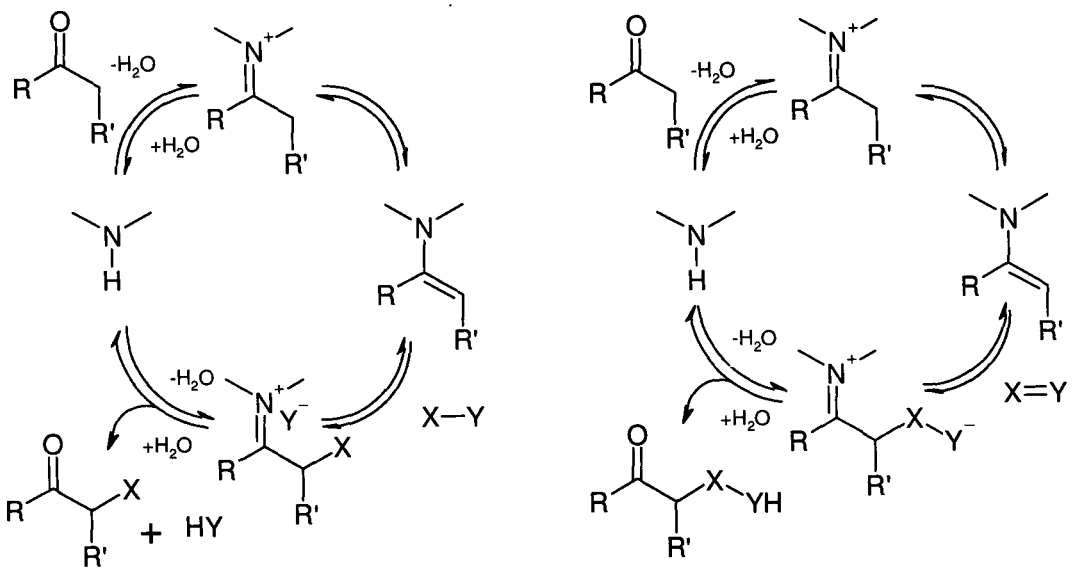


Figure 3: Nucleophilic substitution and addition by enamine catalysis

1.3.2 Iminium catalytic cycle:

The secondary amine condenses with a carbonyl compound giving an iminium ion which is more electrophilic than the parent carbonyl compound. A nucleophile attacks the electrophilic carbon to give an enamine, which on protonation at the α-position gives back the iminium ion, which upon hydrolysis regenerates the carbonyl group (Figure 4).

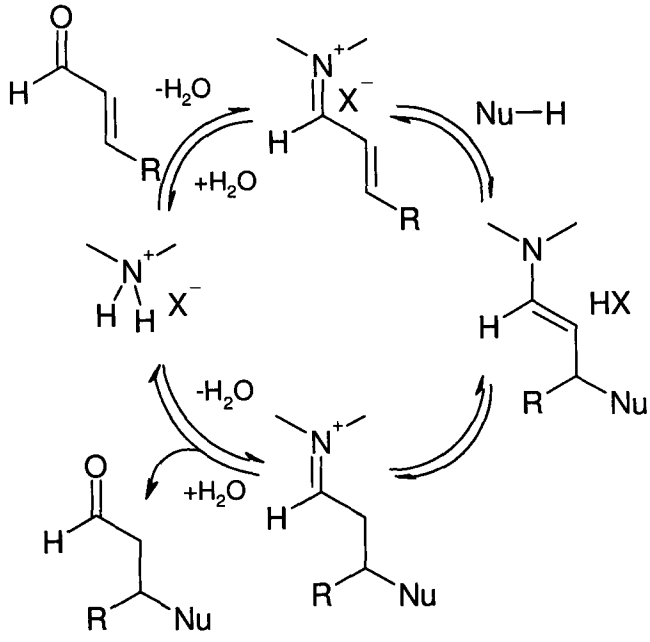
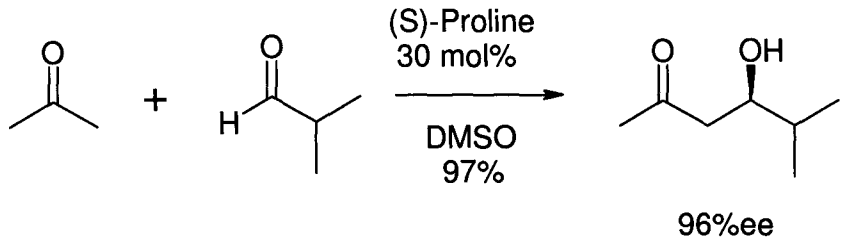


Figure 4: Iminium activation catalytic cycle

For many intermolecular and intramolecular nucleophilic addition and substitution reactions, proline has been used as an asymmetric catalyst[4]. Proline activates nucleophilic carbonyl groups (>C=O) in the aldol and Mannich reactions[11] and electrophilic α, β unsaturated carbon-carbon double bonds (>C=C<)[21] in the Michael reaction.

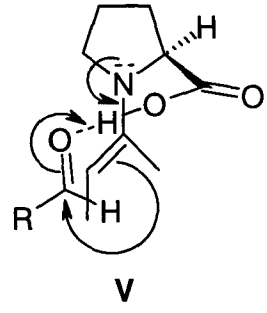
1.3.3 Aldol reaction:

The intermolecular aldol reactions between two carbonyl compounds can be catalyzed through enamine intermediate[22]. Earlier it was established that proline did not catalyze a stoichiometric reaction between a ketone and an aldehyde, but the reaction is possible if large excess of ketone was used. For example, 20 equivalents of acetone reacted with isobutyraldehyde in DMSO giving a very good yield and ee[4] of the aldol product (Scheme 2).



Scheme 2: Proline catalyzed intermolecular aldol reaction

List advanced a mechanistic model for the proline-catalyzed intermolecular aldol reaction (Figure 5, intermediate V)[5]. The model explains the stereoselective outcome by virtue of hydrogen bonding between the acidic proton of proline and the carbonyl oxygen of the electrophile. This model is nothing but an extension to the Houk's model (Figure 2, intermediate IV)[10] for intramolecular aldol reactions and highlights the dual functionality of proline mentioned previously.



V

Figure 5: Mechanism proposed for proline catalyzed intermolecular aldol reaction[5]

Different α-branched aldehydes gave the product with good yields and selectivity in DMSO, along with significant amounts of side product from aldol condensation unlike the standard reaction conditions for the aldol reaction without any condensation product[4]. Solvent change from DMSO to acetone/ $CHCl_3$ gave moderate yields (22-35%) with good enantioselectivity[23] (36-73%). Highly complex sugar molecules have been synthesized recently using this proline-catalyzed intermolecular aldol reaction[24] (Scheme 3).

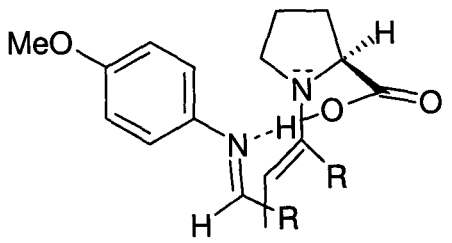
Scheme 3: Complex sugar molecules using proline-catalyzed intramolecular aldol reactions

An important limitation of this reaction would be its applicability to more complex and hence expensive ketones (than acetone) due to the required number of equivalents.

1.3.4 Mannich reaction:

Proline catalyzes the Mannich reaction to give β-amino ketones from the corresponding aldehyde, ketone and amine with high yields and enantioselectivity[25]. Unlike the aldol reaction discussed earlier, a broad range of aldehydes work well with the Mannich reaction including α-unbranched aldehydes. Although both aliphatic and aromatic aldehydes gave Mannich products, aromatic aldehydes work better, giving modest yields (50-96%) and high enantioselectivity (94-99%)[26].

One interesting difference between the products of the proline catalyzed intermolecular Mannich and aldol reactions was their opposite stereoselectivity. List proposed a transition state model for the proline catalyzed Mannich reaction (Figure 6, intermediate VI). The opposite stereoselectivity was explained and was due to the oppositely placed imine in the Mannich reaction compared to the position of the aldehyde in the aldol reaction (V). List further explained that the formation of the transition state that avoids steric interaction between the pyrrolidine and the aromatic ring was preferred (Figure 6, intermediate VI)[4].

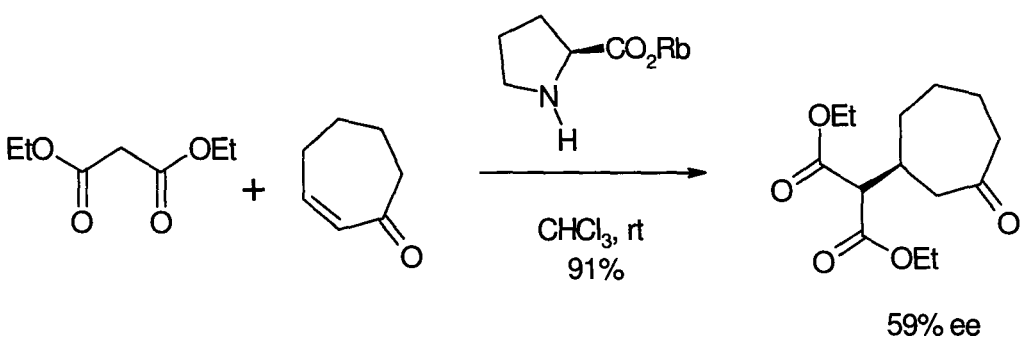


VI

Figure 6: Mechanism proposed for proline-catalyzed Mannich reaction[4]

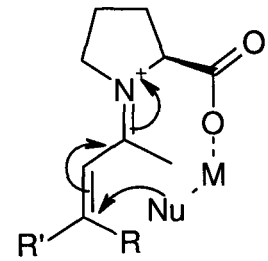
1.3.5 Michael reaction:

Proline catalyzed Michael reactions proceed through iminium catalysis. A few secondary amines were tried by Yamaguchi for the Michael reactions between dimethyl malonate and hex-2-enal in methanol[27]. Although the lithium salt of proline gave better yields compared to the yields with proline, the products were found to be racemic. α, β unsaturated aldehydes showed no change in enantioselectivity with lithiated proline. Change of reaction solvent from methanol to chloroform showed induction of enantioselectivity. When proline salts of different metals were tried, the rubidium salt gave optimal yield (58-91%) and enantioselectivity (41-76%)[28] (Scheme 4). Interestingly, lithium and alkaline metal salts of proline showed reversed absolute configuration.



Scheme 4: Iminium activated Michael addition

Later, Yamaguchi reported that better enantioselectivty can be achieved by adding cesium fluoride as cocatalyst[29]. List proposed a mechanism for the metallated proline catalyzed Michael reaction (Figure 7, intermediate VII)[4].



VII

Figure 7: Mechanism proposed for proline-catalyzed Michael addition[4]

1.4 Discussion:

Over the years from several scientific reports and literature reviews, it was clear that secondary amines play important role in organocatalyzed reactions and offer much potential in organic chemistry. Asymmetric organocatalysis is making major advances in academic as well as in industrial fields, and thus is becoming a fascinating field in chemical science. In this field, particularly proline catalysis is fetching most of the interest. Being easily available, stable at ambient conditions, easy to handle and cheap, proline has several advantages over other organocatalysts.

We decided to synthesize different derivatives of proline which could potentially be used as organocatalysts and which would allow modulation of the solubility problems and drawbacks intrinsic to proline. A major disadvantage and limitation of proline is its solubility in most organic solvents. Reactions are often performed in polar solvents such as DMSO, DMF, water or salt water containing other additives such as carboxylic acids. Proline, being a polar molecule and an amino acid, does not provide a homogeneous reaction mixture in the non-polar organic solvents which are generally used for most organic reactions. The partial solubility of proline would affect reaction scope, yields and

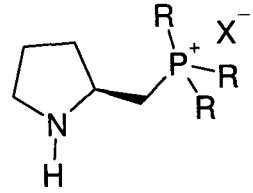
selectivity of the product to a considerable extent. Also the use of polar solvents like DMSO, to compensate for the solubility issue of proline, limits reactant possibilities to using non polar reactants and/or requires excess of reactants. Being high boiling, removal of DMSO during the work-up is problematic.

For intermolecular organocatalytic aldol reactions, α-branched aldehydes work well in DMSO, but α-unbranched aldehydes do not yield the expected aldol products. The reaction works in acetone/ chloroform as solvent but only with moderate yields (22-35%). A large number of equivalents of ketone is required for intermolecular organocatalyzed aldol reactions, which would be an important limitation if an expensive ketone is one of the starting materials for the aldol[4]. The opposite stereochemistry of the aldol and Mannich reaction catalyzed by proline was explained by List. He proposed a new transition state for proline catalyzed Mannich reaction. The transition state explained stereoselectivity in the Mannich reaction for aromatic amines but failed to explain it for aliphatic amines; thus there was created a doubt about the proposed mechanism for both the organocatalyzed aldol and Mannich reactions.

The Michael reaction did not work well with proline alone, but required metal salts of proline[28]. It also required caesium fluoride as a cocatalyst[29].

Thus to get rid of some of the above mentioned drawbacks and in pursuit of better yields and selectivity, we decided to synthesize derivatives of proline and test them as organocatalysts.

To resolve the solubility issue and to have better co-ordination in organocatalyzed reaction transition states, we decided to synthesize the phosphonium salt derivative of (*S*)-proline (Figure 8, **A**).

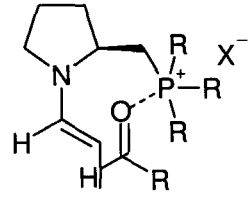


R=Et, Bu, Ph

A

Figure 8: Proposed phosphonium salt catalyst

Phosphonium salts are well known for their solubility in many non polar organic solvents such as halogenated solvents (dichloromethane), and common solvents such as toluene, THF, diethyl ether etc. They are highly tunable through selection of the aryl or alkyl chain on phosphorus. Hence the proposed organocatalyst (Figure 8, **A**), would not require the use of highly polar solvents like DMSO, but should work in non polar solvents. Also the positively charged phosphorus cation would be put to use in coordinating to the oxygen of the electrophilic carbonyl component, resulting in better stereoselectivity in the aldol as well as Mannich reactions (Figure 9, intermediate VIII). Hence, these species could also work as dual functional catalysts.

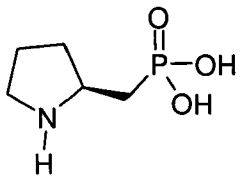


VIII

Figure 9: Proposed mechanism for the phosphonium salt organocatalyst

The phosphonic acid derivatives of (S)-proline (Figure 10, **B**) would be one more choice as organocatalysts. As these derivatives are water soluble, it could be possible to use water as reaction medium. Various pH conditions would protonate the phosphonic acid to a different extent making it possible to coordinate with carbonyl oxygen atoms at

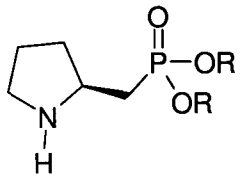
a particular pH. Thus, hypothetically, at a particular pH condition, the phosphonic acid derivative should work most efficiently to give maximum selectivity.



B

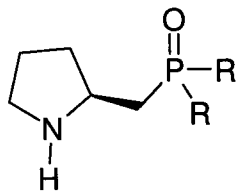
Figure 10: Proposed phosphonic acid organocatalyst

The phosphonates (Figure 11, **C**) and the phosphine oxides (Figure 11, **D**) offer a few more possibilities as organocatalysts. Bulky R groups will help to provide steric strain, forcing nucleophile/ electrophile to attack from one particular plane and thus improving the selectivity.



R=Bu, Ph

C



R=Bu, Ph

D

Figure 11: Proposed phosphonate and phosphine oxide organocatalyst

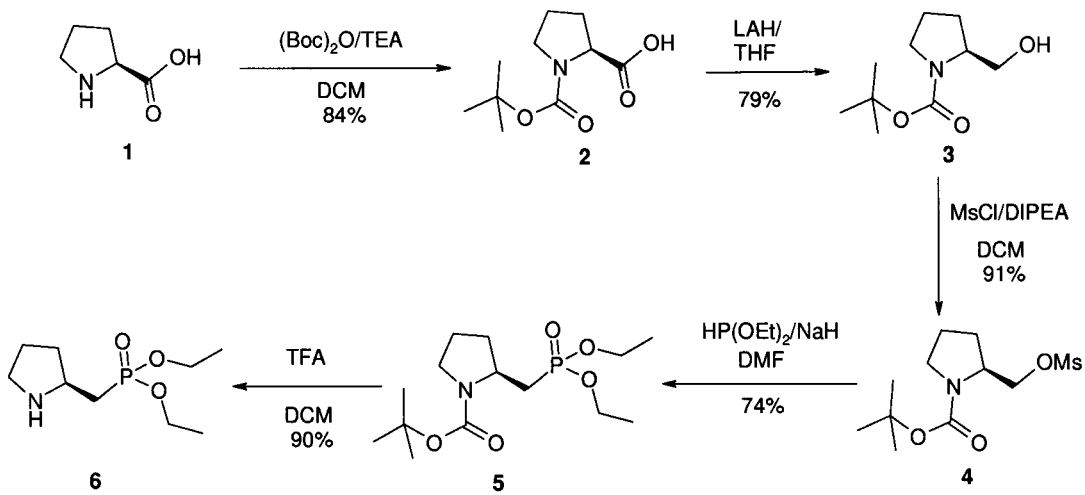
1.5 Synthesis:

Synthesis of the first target was started from the natural abundant chiral amino acid (*S*)-proline **1**. The secondary amine of (*S*)-proline was protected using di-tertiary-butyl dicarbonate under basic conditions giving N-Boc-(*S*)-proline **2** in quantitative yield. The carboxylic group of N-Boc-(*S*)-proline **2** was then reduced using lithium aluminium

hydride to accomplish compound **3**. The reaction was carried out at room temperature for longer duration to avoid reduction of the tertiary butyloxy carbonyl group. The reduction at room temperature worked well giving primary alcohol compound **3** in 79% yield. Even at room temperature the tertiary butyloxy carbonyl group of **2** was partly reduced giving corresponding N-methyl product and thus preventing quantitative yield of the desired product for the reaction.

In an attempt to prepare a derivative of the primary alcohol, containing a good leaving group, various methodologies were tried. Bromination was overlooked because of the unstable nature of the bromo derivative, which was found easily susceptible to hydrolysis. Conversion of the alcohol **3** to either a mesylate or a tosylate worked well. Under basic conditions the primary alcohol group of **3** was converted to mesylate **4** in 91% yield. The mesylate intermediate **4** is stable and can be stored at room temperature for several months. The intermediate **4** can be used to synthesize phosphonium salts and different phosphonate derivatives.

Diethyl phosphite was used to introduce the phosphonate group in the proposed organocatalyst. The sodium salt of diethyl phosphite was prepared using sodium hydride in DMF. The nucleophilic substitution of the mesylate group by the diethyl phosphite anion yielded the corresponding phosphonate derivative **5**.

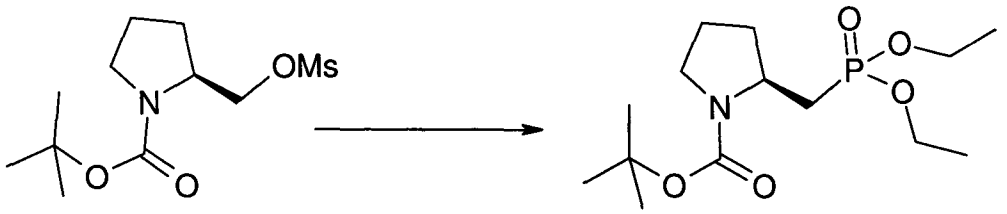


Scheme 5: Synthetic scheme for the phosphonate catalyst **6**

The nucleophilic substitution reaction using diethyl phosphite was carried out using different conditions (Table 1). Different bases, solvents and quantities of reagents were tried. The reaction was tried using 10 equivalents of sodium hydride and diethyl phosphite (Table 1, entry 1), following a reported protocol[30], which gave 87% yield. However, determination of the specific rotation showed racemisation of the product. This behaviour was suspected due to the excess amount of the strong hydride base used in the reaction. Hence we carried out the same reaction using 5 equivalents of sodium hydride and diethyl phosphite (Table 1, entry 2), which gave 74% yield with excellent specific rotation (-43°). We also attempted the same reaction using a few mild, non-hydride bases, which either produced no product or impurities. Hence the method using 5 equivalents of sodium hydride and diethyl phosphite (Table 1, entry 2) was used to achieve the crucial conversion. The deprotection of the intermediate **5** was carried out using TFA in DCM, thus accomplishing first of the proposed catalysts **6**.

Various efforts towards synthesizing other proposed organocatalysts were unsuccessful. A number of attempts was made to prepare the phosphonium salt derivative (Figure 8). Initially introduction of a phosphine group into the molecule proved unexpectedly troublesome, but this was achieved after a number of trials. Isolation of the

final molecule has been a bigger problem. Similar difficulties were faced for the phosphonic acid derivative (Figure 10) preparation. Being a zwitterionic species, the phosphonic acid derivative was difficult to isolate and purify. Even after several attempts, isolation of the phosphonium salt (Figure 8) and the phosphonic acid (Figure 8) derivatives were not achieved.



4 **5**

No.	Condition	Yields (%)	Sp. Rotation (degree)
1.	10 eq. $HPO(OEt)_2$/10 eq. NaH in DMF	87	-2.5
2.	5 eq. $HPO(OEt)_2$/5 eq. NaH in DMF	74	-43
3.	1 eq. $HPO(OEt)_2$/1 eq. NaH in DMF	No reaction/ SM as it is	---
4.	2 eq. $HPO(OEt)_2$/2 eq. NaH in DMF	Very faint spot of product on TLC/ not isolated.	---
5.	3 eq. $HPO(OEt)_2$/3 eq. NaH in DMF	20	-46
6.	10 eq. $HPO(OEt)_2$/10 eq. Na in DMF	22 (with minor impurities)	-51
7.	5 eq. $HPO(OEt)_2$/5 eq. Na in DMF	Very faint spot of product on TLC/ not isolated.	---
8.	10 eq. $HPO(OEt)_2$/10 eq. NaH in THF	No reaction/ SM as it is	---
9.	10 eq. $HPO(OEt)_2$/10 eq. n-BuLi in DMF	No product formation/ SM consumed	---

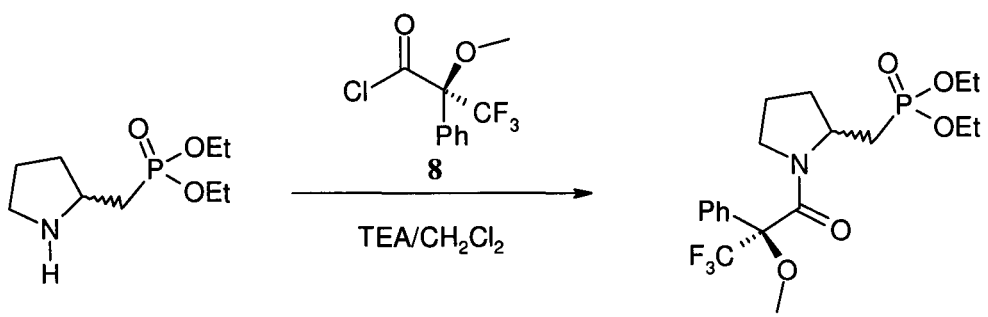
- No reaction with 1, 5, 10 eq. of other bases such as DIPEA, DBU.
- Specific rotation of (*S*)-Proline was –96°, N-boc (*S*)-prolinol was –51.5° and N-boc (*S*)-prolinol methanesulphonate was –64.5°.

Table 1: Various conditions used for the nucleophilic substitution reaction

Being a chiral organocatalyst, enantiopurity of the molecule is its most important feature. Although the specific rotation suggested that the product is not racemic, it cannot

express the extent of the chiral purity. As the final phosphonate compound **6** and its specific rotation have not been reported, we needed some other strategy to determine extent of chiral purity of the compound **6**.

We decided to make diastereomeric Mosher's amides from the phosphonate derivative **6**. The Mosher's amide forms diastereomers from starting pair of enantiomers. NMR spectroscopy of the diasteriotopic protons should show different chemical shifts. Hence if the catalyst prepared was completely enantiopure, the Mosher's amide should show one singlet for the $-OCH_3$ group in the ^1H NMR and also singlets for the $-CF_3$ and the $-P(O)(OEt)_2$ groups in ^{19}F and ^{31}P NMR spectra respectively.



6 **7**

Scheme 6: Preparation of Mosher's amide (racemic)

Using Mosher's acid chloride we synthesized the corresponding amide of the phosphonate derivative made by a reported protocol (Table 1, entry 1), from **6** that had a specific rotation of -2.5°. The reaction was carried out in a basic medium using DCM as a solvent yielding two diastereomeric Mosher's amide quantitatively (Scheme 6). Proton and ^{31}P NMR spectra of the Mosher's amides were recorded (Figure 12, 13).

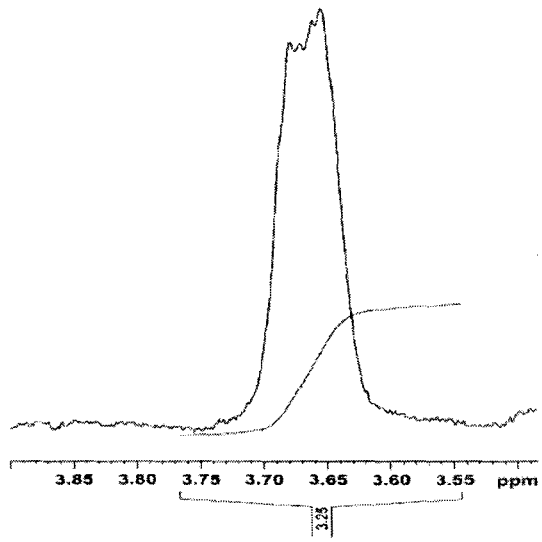


Figure 12: ^{1}H NMR peak for the $-OCH_3$ group of Mosher's amides **7** showing two peaks for the two diastereomers

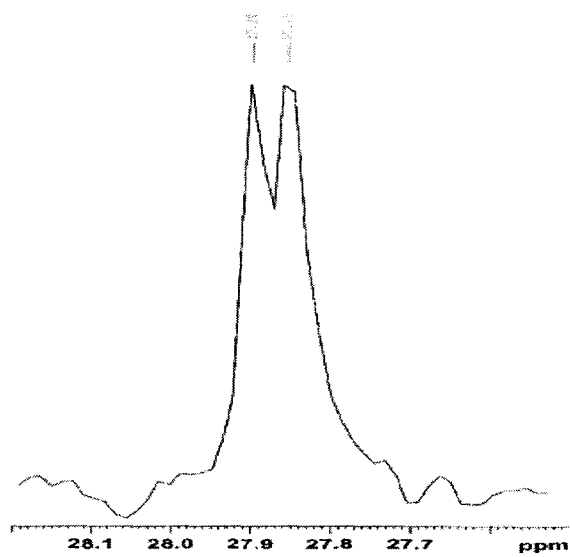
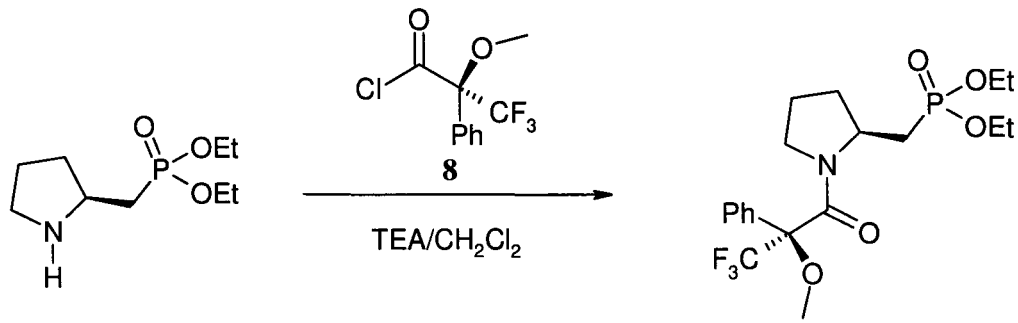


Figure 13: ^{31}P NMR peak for $-P(O)(OEt)_2$ group of Mosher's amides **7** showing two peaks for the two diastereomers

The Mosher's amide ^{1}H and ^{31}P NMR spectra showed two peaks for the $-OCH_3$ and $-P(O)(OEt)_2$ of the two diastereomers. Thus, from the NMR spectra, racemisation was evident. The corresponding Mosher's amide of the phosphonate organocatalyst **6** made by the new protocol (Table 1, entry 2) was prepared (Scheme 7) and subjected to similar NMR analysis.



Scheme 7: Preparation of Mosher's amide from enantiopure **6**

The ^{1}H and ^{31}P NMR spectra of the new Mosher's amide were different from the earlier spectra. These NMR spectra showed a sharp singlet for the $-OCH_3$ group in the ^{1}H NMR and also a singlet for the $-P(O)(OEt)_2$ group in the ^{31}P NMR spectrum. Thus the spectra were supporting enantiopurity of the phosphonate organocatalyst **6** made by the new protocol (Table 1 entry 2). Fluorine NMR of the new Mosher's amide sample also proved the chiral nature of the organocatalyst **6**. Specific rotations for the intermediate **5** made by different methods were recorded (Table 1). From the comparison of this data, it was clear that the phosphonate organocatalyst made using 5 equivalents sodium hydride and diethyl phosphite (Table 1, entry 2) was enantiopure.

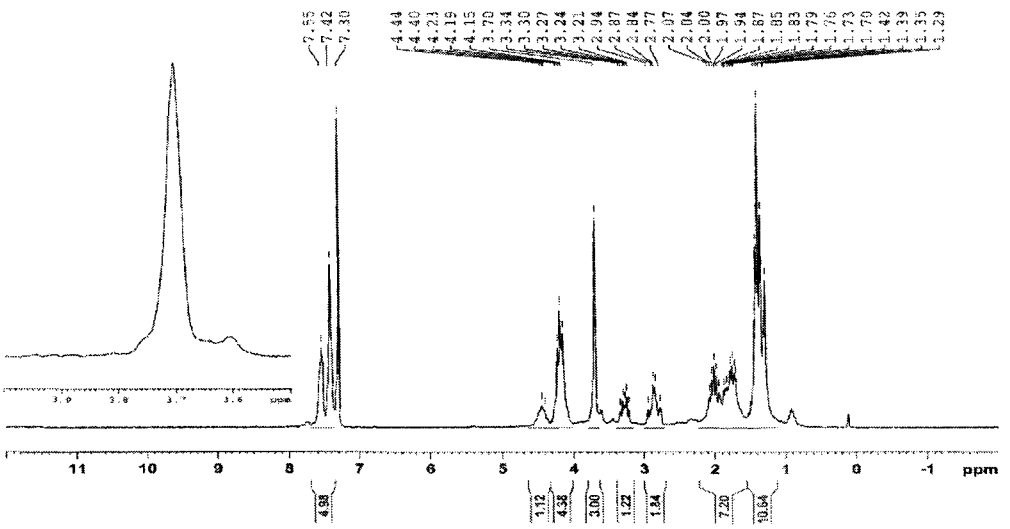


Figure 14: ^{1}H NMR of Mosher's amide showing a single peak for the $-OCH_3$ group in blown up region for compound **9**

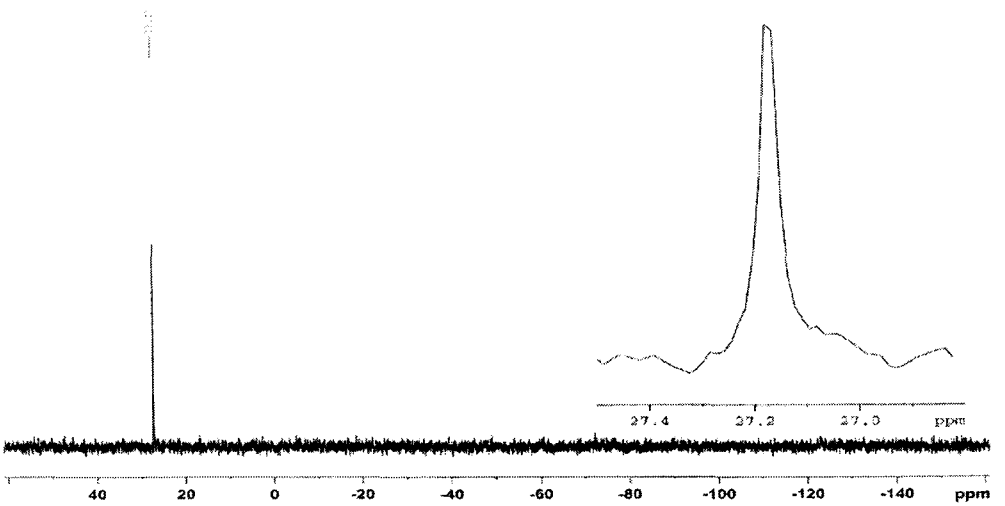


Figure 15: ^{31}P NMR of Mosher's amide showing a single peak for the $-P(O)(OEt)_2$ group for compound **9**

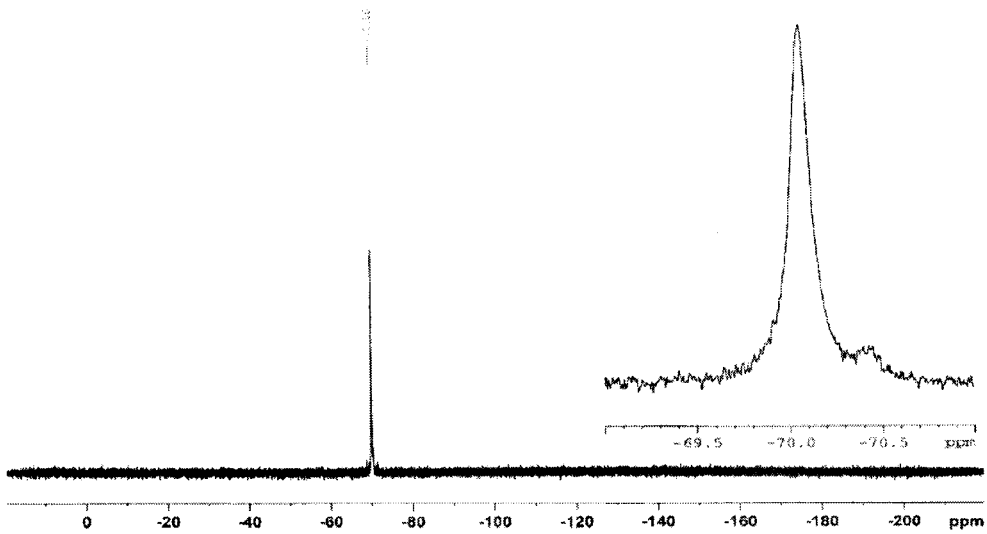


Figure 16: ^{19}F NMR of Mosher's amide showing a single peak for the $-CF_3$ group for compound **9**

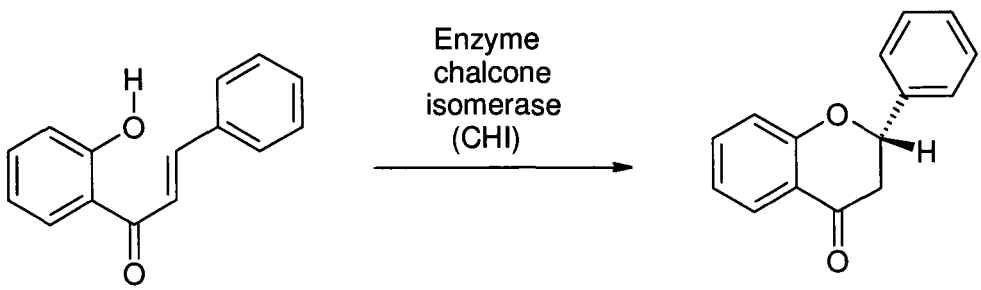
As all the above results proved that the organocatalyst we prepared was chiral and essentially a single enantiomer, we next proceeded to investigate its application to enamine-iminium organocatalysis of different reactions. As expanding the scope of reactions that are amenable to organocatalysis is also very important, we chose to investigate novel transformations that could potentially proceed through enamine or iminium catalysis. These reactions involve chalcone cyclization via an expected iminium ion, a Carroll rearrangement via an enamine intermediate and a Diels-Alder reaction expected to proceed via iminium ion activation.

1.6 Applications:

1.6.1 Flavanones:

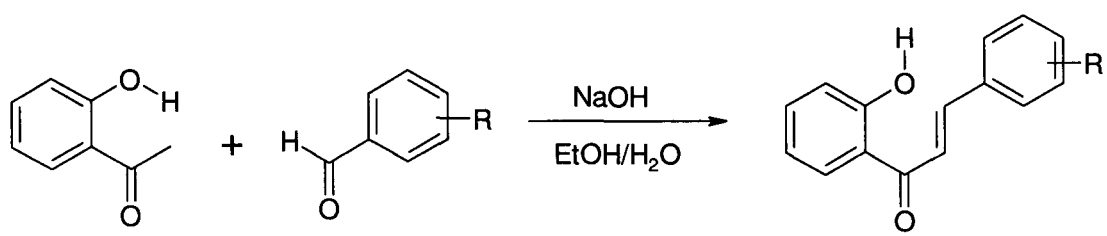
Flavanones constitute a family of well known natural products found in several plants. Many derivatives of flavanones are known to be biologically active. Antifungal, anticancerous and antioxidant activities of different derivatives of flavanones are well-

known[31]. It has been proved that chalcone isomerase (CHI) catalyzes the intramolecular cyclization of 2'-hydroxy chalcones into flavanones[32] in natural systems (Scheme 8).



Scheme 8: Chalcone isomerase catalyzed flavanone synthesis

Assuming that the enzyme catalyzes the cyclization via enamine-iminium activation, we decided to check our catalysis hypothesis for a chiral flavanone synthesis reaction. The reaction has not been reported using enamine-iminium activation. We decided to synthesize a few simple derivatives of flavanones from the corresponding 2'-hydroxy chalcones. The chalcones were synthesized using the aldol condensation reaction of 2-hydroxy acetophenone and different aromatic aldehydes using sodium hydroxide as a base (Scheme 9). Some chalcones were obtained directly from undergraduate student David McLeod.

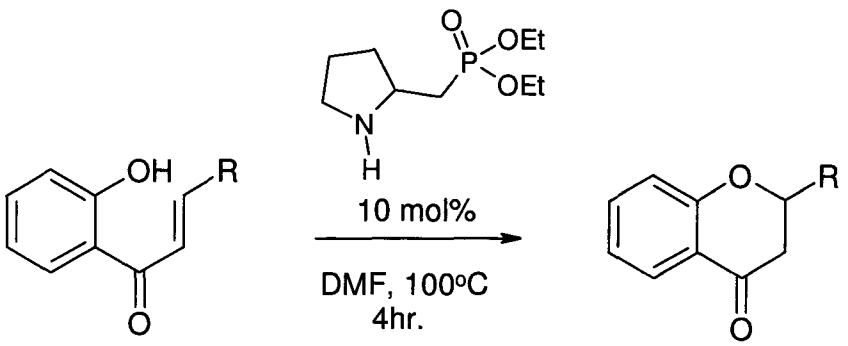


Scheme 9: Synthesis of 2'-hydroxy chalcone

After trying various conditions with respect to solvents, temperature and catalyst loading, we found out that 2'-hydroxy chalcones can be cyclized using microwave heating

in the presence of organocatalyst **6** as catalyst. When 2'-hydroxy chalcones and 0.1 equivalents of organocatalyst **6** in DMF were subjected to microwave heating to 100°C for 20 min., the chalcones were cyclised to flavanones. When the same reaction was tried using conventional heating, the conversion was more efficient. Catalyst loading was varied which showed that 0.1 equivalent catalyst loading was most efficient. Using the set protocol, several flavanones were prepared from the corresponding 2'-hydroxy chalcones (Table 2).

Interesting fact about the reaction was that none of the reaction went to completion. This behaviour could be due to the equilibrium between chalcone and flavanone in the reaction mixture. To prove this equilibrium we heated pure unsubstituted flavanone in DMF adding 10% of the organocatalyst **6** for 1hr. TLC of the reaction showed formation of 2'-hydroxy chalcone. When the same reaction was carried out without adding the organocatalyst **6**, the flavanone remained unchanged. This explains the equilibrium between starting material and product of the reaction, which never shifted completely to the product side and thus resulting in reasonable yields.



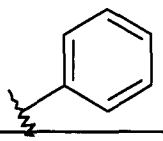
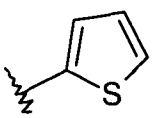
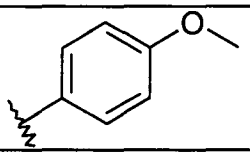
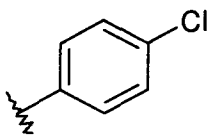
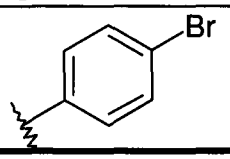
Compound no.	R	Yield %
10		79
11		76
12		73
13		75
14		76

Table 2: Synthesis of flavanone derivatives

To support the chalcone cyclization, we proposed a catalytic cycle using an iminium intermediate. In the first step, the catalyst condenses with the carbonyl group of chalcone to give the iminium ion, making it more electrophilic compared to the parent carbonyl group. This increase in electrophilicity facilitates attack of the phenolic oxygen

resulting in cyclization with formation of the six membered ring. The iminium ion which was converted to an enamine in this sequence abstracts a proton from water molecule to give another iminium ion, which after hydrolysis gives the carbonyl compound i.e. the flavanone. The catalyst is generated and again takes part in the catalytic cycle (Figure 17).

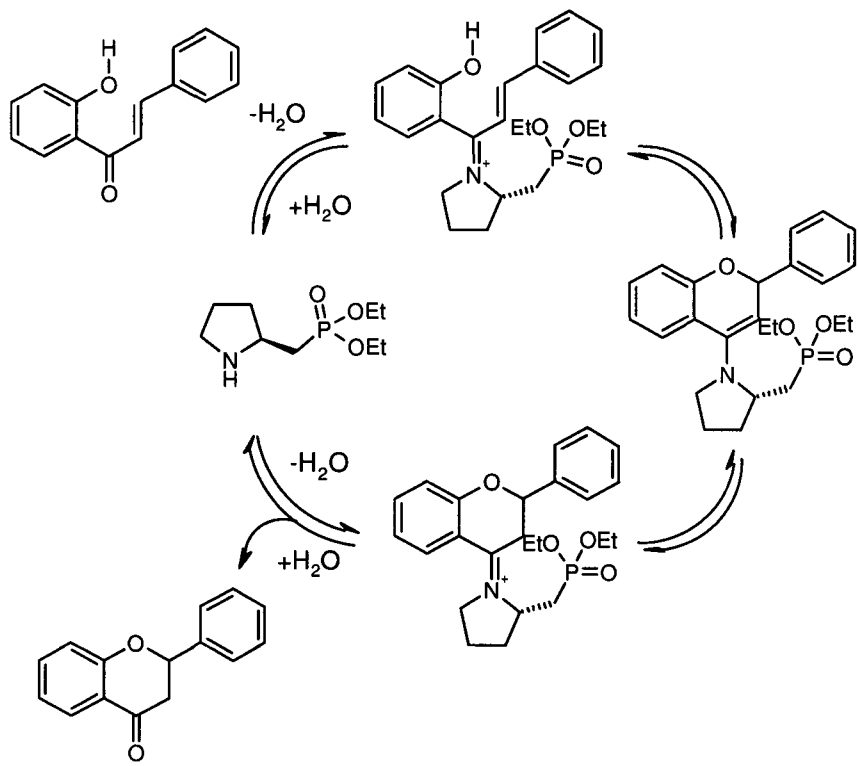
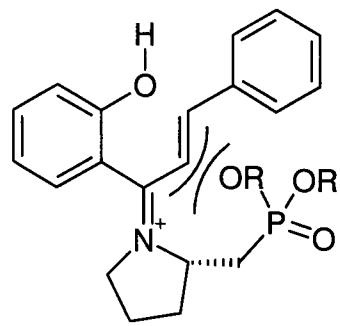


Figure 17: Catalytic cycle for flavanone synthesis using organocatalyst **6**

The main objective was now to find out whether the organocatalyst **6** forms an iminium intermediate and whether it induces chirality in the flavanone. To check our hypothesis, we tried few test reactions. Firstly the cyclization was tried without adding the organocatalyst **6.** Two more reactions were also tried adding acetic acid or diisopropylethylamine instead of the organocatalyst **6.** None of these reactions produced flavanone, supporting our hypothesis of iminium ion formation. The chirality was anticipated based on following transition state (Figure 18).



IX

Figure 18: Proposed intermediate for the flavanone synthesis

The steric strain created using bulky R groups was expected to force the hydroxyl group to attack from one side of the olefin and thus create a chiral centre at the carbon that is forming a new bond with hydroxyl oxygen.

Flavanones synthesized using the set protocol were analyzed using chiral HPLC and specific rotation showed no induction of asymmetry. The lack of stereochemical control in the reaction could be because of one or more of the following reasons.

i) High reaction temperature providing sufficient energy for the reaction to work through a path which gives the racemic product.

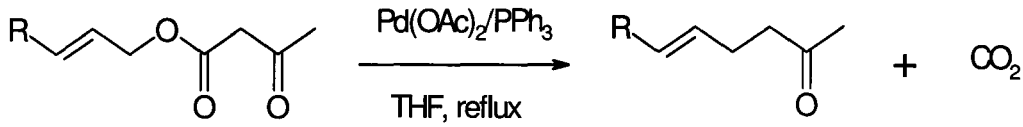
ii) Small ethoxy groups in the catalyst, which are not bulky enough to create sufficient steric strain to induce asymmetry.

iii) The equilibrium condition between 2'-hydroxy chalcone and flavanone.

Various reactions were tried to get the desired conversion at lower temperature. The reaction worked at room temperature in ethanol giving flavanone after several (7) days, but the product, when tested using chiral HPLC, did not show induction of chirality.

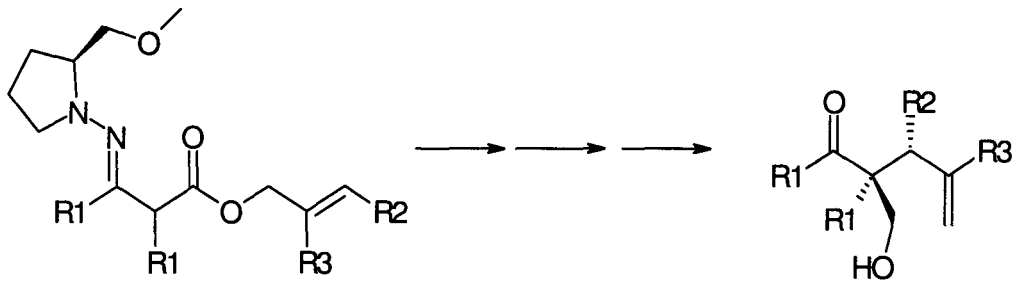
1.6.2 Carroll rearrangement:

The Carroll rearrangement[33] is an important conversion of β-keto esters into γ,δ unsaturated ketones under thermal conditions. The reaction is a [3,3] sigmatropic rearrangement similar to the Claisen rearrangement[34]. Although the reaction has been applied to synthesize geranyl acetone & β-ionones[35] on a commercial scale, it was not explored extensively[36] probably because of its reaction conditions, which require high temperature. But in 1980, Jiro Tsuji reported milder conditions for the conversion where he used palladium acetate & triphenyl phosphine[37] (Scheme 10).



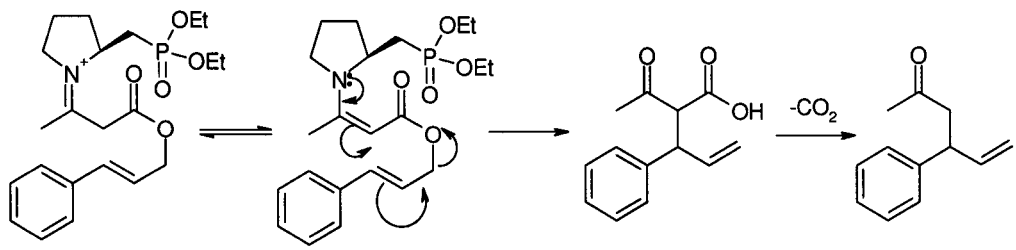
Scheme 10: Carroll rearrangement using palladium acetate & triphenyl phosphine

Although the methodology described only racemic conversions, later, various attempts were made to facilitate enantioselective reaction. Enders attempted it first using SAMP ((*S*)-1-amino-2-methoxymethylpyrrolidine) as a chiral auxilliary[38] (Scheme 11). Different Palladium and Rhuthenium catalysts were also tried in combination with different ligands[39] containing phosphorus, but the Trost ligand worked efficiently in the presence of a palladium complex as a catalyst and an alcohol as additive giving up to 99% yield and as high as 98% ee[40].



Scheme 11: Carroll rearrangement using SAMP as chiral auxilliary

No reports on the Carroll rearrangement using enamine based organocatalysts have appeared in the literature to our knowledge. The substrate being a β-dicarbonyl compound, we considered this approach to have good potential with an enamine donor having allylic ester as acceptor as outlined in Scheme 12. Hence, we decided to test our organocatalyst under various conditions to explore this possibility for the Carroll rearrangement reaction.



Scheme 12: Proposed mechanism for Carroll rearrangement using the phosphonate catalyst

In an attempt to prepare a substrate for the reaction, we used the simple trans-esterification reaction between ethyl acetoacetate & cinnamyl alcohol at higher temperature[41]. The reaction worked well in toluene with iodine as additive giving the cinnamyl acetoacetate as a substrate for the Carroll rearrangement. To ensure higher enantioselectivity for Carroll rearrangement, initial attempts were made to carry out the reaction at ambient temperature and with varied number of equivalents of the organocatalyst **6** in different solvents. Various attempts were made to carry out the reaction with or without additives like TFA and various bases, but they did not facilitate the reaction (Table 3). Hence the reaction was tried at higher temperatures. After prolonged heating of cinnamyl acetoacetate in the presence of the organocatalyst **6** a polar product appeared on TLC. Eventually it was found out that it was nothing but the cinnamyl alcohol, which was obviously the product of hydrolysis of cinnamyl acetoacetate. Even under anhydrous conditions, small amounts of hydrolysis took place.

Besides the hydrolysis reaction, starting material was found to be unreacted and recovered in quantitative yields.

No.	Solvent	Temperature	Additive	Result
1.	THF	22 °C	None	SM unreactive
2.	THF	70 °C	None	SM unreactive
3.	THF	22 °C	TFA/TEA	SM unreactive
4.	THF	70 °C	TFA/TEA	SM hydrolysed
5.	THF	22 °C	$Pd(OAc)_2$	SM unreactive
6.	Toluene	22 °C	None	SM unreactive
7.	Toluene	110 °C	None	SM hydrolysed
8.	Toluene	22 °C	TFA/DIPEA	SM unreactive
9.	DMF	22 °C	None	SM unreactive
10.	DMF	22 °C	TFA/DIPEA	SM unreactive
11.	PhCl	22 °C	None	SM unreactive
12.	Brine	22 °C	None	SM unreactive

Table 3: Reaction conditions for the Carroll rearrangement

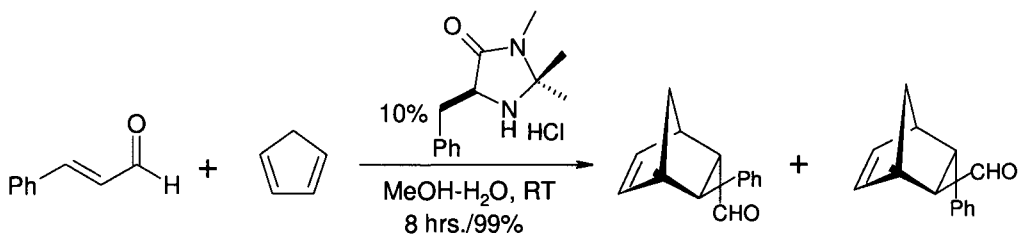
Possible reasons for the failure of the reaction could be the trouble in forming the iminium-enamine intermediate at lower temperature whereas starting material was hydrolysed at higher temperature.

1.6.3 Diels-Alder reaction:

Diels-Alder reactions have been one of the most useful chemical transformations during the last 80 years[42]. The reaction has been extensively used in laboratories by chemists for long time[43]. Evidence showed that the reaction also exists in biological systems through Diels-Alderase enzymes[44]. The enzymatic D.A. reaction supports the idea of the organocatalysed D.A. reaction which would take place through iminium activation.

MacMillan and group reported the first iminium ion catalysed Diels-Alder reaction in the year 2000, using an imidazolidinone as an organocatalyst[45]. The iminium ion catalyzed Diels-Alder reaction saw significant increase in the number of examples reported over the last decade. The organocatalysed D.A. reaction got attention because of its ability to provide complex enantiorich products obtained from simple substrates.

The first report of iminium activated organocatalysed D.A. reaction was from the MacMillan group. The reaction includes pentadiene as a four electron system- the diene, while an α,β unsaturated aldehyde acts as a two electron system- the dienophile. Different secondary amines were tried as organocatalysts but the imidazolidinone prepared by MacMillan worked excellently giving 99% yield with 1.3 :1 exo-endo ratio and as high as 93% ee for both exo and endo isomers[45] (Scheme 13).



Scheme 13: Organocatalyzed Diels-Alder reaction by MacMillan

The reaction was investigated using different dienes and dienophiles and various derivatives of the imidazolidinone catalyst. MacMillan's group has reported numerous organocatalyzed enantioselective D.A. reactions[46]. Besides these, few other reports have appeared for iminium ion activation of dienophiles in the organocatalyzed D.A. reaction[47].

From all these examples we hypothesised that the secondary amine of the phosphonate derivative we synthesized would be able to condenes with a carbonyl group of a dienophile giving an iminium ion as an intermediate. Being a better electrophile than the parent carbonyl compound, the iminium ion would be in a position to react instantaneously with a diene present in its vicinity, to accomplish a D.A. cycloaddition. Eventual hydrolysis of the iminium ion should yield the D.A. reaction product (Figure 19). To check this proposition we examined the D.A. cycloaddition reaction catalyzed by the organocatalyst **6** we synthesized.

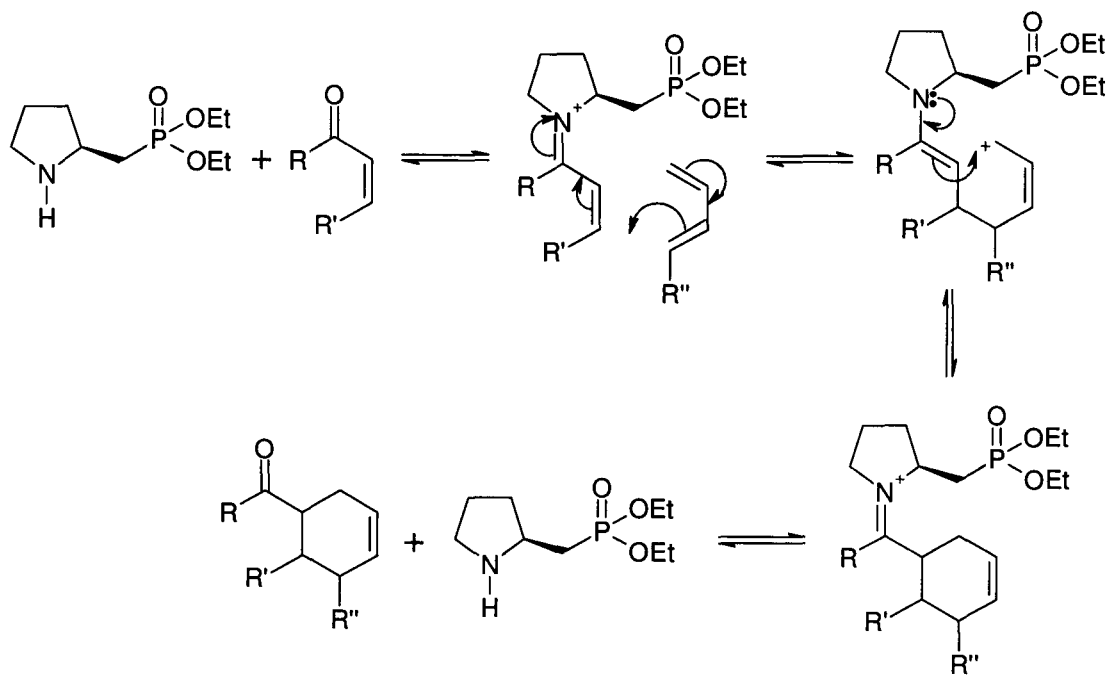
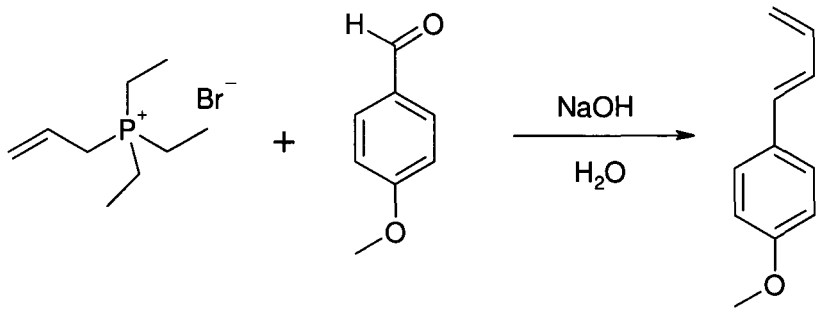


Figure 19: Iminium-enamine activation for the proposed Diels-Alder reaction

A starting compound for the proposed D.A. reaction was prepared using a simple method devised by our group for the Wittig reaction[48]. Reaction between allyl triethylphosphonium bromide **15** and p-methoxy benzaldehyde **16** accomplished 4-(p-methoxyphenyl) 1,3-butadiene **17** (Scheme 14) which was used as a diene for the D.A. reaction.

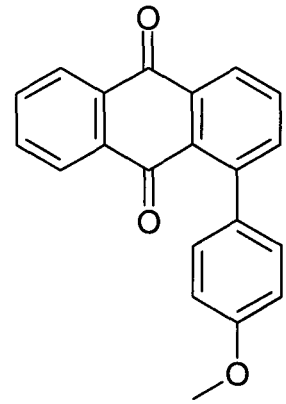


15 **16** **17**

Scheme 14: Synthesis of 4-(*p*-methoxyphenyl) 1,3-butadiene[48]

For the initial test reactions, temperature and catalyst loading was kept constant varying solvent type. With 20% catalyst loading, different solvents were tried for the reaction between 1,4-naphthoquinone and 4-(p-methoxyphenyl) 1,3-butadiene at room temperature. A number of reactions in DCM, toluene, THF, DMF, brine with or without addition of an acid such as TFA, acetic acid and benzoic acid were tried. None of these showed consumption of starting dienophile. But notably, the diene was disappearing on TLC with formation of a polar spot. It was found that the 4-(p-methoxyphenyl)-1, 3-butadiene is prone to decomposition when exposed to air. Hence similar reactions were tried under inert atmosphere, where the decomposition of the butadiene was restricted to much lesser extent but still the dienophile remained unreactive.

When a similar reaction was tried at higher temperature in toluene, a polar spot was observed on TLC with consumption of the dienophile. The polar spot was isolated and analysed using ^{1}H NMR which showed formation of an aromatised analogue, anthraquinone derivative (Figure 20), instead of the expected cycloaddition product. There have been several reports of D.A. reactions at higher temperatures, yielding aromatised product along with a D.A. cycloaddition product[49]. Two more high boiling solvents, PhCl and DMF, were tried for the similar high temperature D.A. reaction. Both the reactions accomplished aromatised analogues instead of the expected D.A. product.



18

Figure 20: Anthraquinone derivative from Diels-Alder reaction

1.7 Conclusion:

After a number of attempts, we were able to synthesize one of the proposed catalysts. The catalyst worked for 2'-hydroxy-chalcone cyclization through iminium activation, giving different derivatives of flavanones. Thus we showed that the catalyst we made is capable of forming iminium ions. But the catalyst failed to induce chirality into the flavanones.

The catalyst **6** was also tried for a very interesting reaction, the Carroll rearrangement. The reaction was attempted using cinnamyl acetoacetate, which failed to provide desired the product of the Carroll rearrangement under various reaction conditions. Decomposition of starting material was one of the major problems to carry out the conversion.

The organocatalysed Diels-Alder reaction was attempted using the organocatalyst **6**. We did not observe the expected D.A. cycloaddition product with different reaction protocols; instead an aromatised analogue of the desired product was obtained. Decomposition of starting diene was the major problem during the reaction.

Many attempts were made to synthesize the other proposed organocatalysts. All the attempts failed mainly at the final isolation step of the desired compounds.

1.8 Future work:

As remaining proposed organocatalysts are yet to be prepared, future work should be concentrated on synthesis of these molecules. Final isolation of these molecules was a crucial step and hence more emphasis should be given to it.

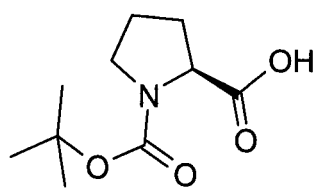
A new phosphonate organocatalyst with bulkier substituents should provide another opportunity to examine asymmetric flavanone synthesis.

These organocatalysts should be tested for various reactions which work through the iminium-enamine activation cycle.

1.9 Experimental:

General information: Reactions were carried out under an argon atmosphere in oven-dried glassware. All fine chemicals were obtained from Aldrich. Toluene, diethyl ether and THF were distilled from sodium metal with benzophenone indicator. Dichloromethane and ethyl acetate were distilled over calcium hydride. Melting points (uncorrected) were measured on a Gallenkamp melting point apparatus. Optical rotations were determined on a Perkin-Elmer 241 polarimeter installed with a λ_{589} sodium lamp. CIMS were run on a Micromass Quattro Ultima spectrometer fitted with a direct injection probe (DIP) with ionization energy set at 70 eV and HRMS (CI) were performed with a Micromass Q-Tof Ultima spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 200, 600 or AV 700 spectrometer in $CDCl_3$ with TMS as internal standard, chemical shifts (δ) are reported in ppm downfield of TMS and coupling constants (J) are expressed in Hz.

Synthesis of 1,2-Pyrrolidinedicarboxylic acid, 1-(1,1-dimethylethyl) ester, (*S*)-2[50]:



To a solution of (*S*)-proline (1.00 g, 8.69 mmol) and triethylamine (2.22 mL, 17.37 mmol) in 15.0 mL of CH_2Cl_2 at 0 °C was added Di-*tert*-butyl dicarbonate (1.99 g, 9.12 mmol). The resulting mixture was stirred overnight at 22 °C, quenched with 5.0 mL of 5% citric acid aqueous solution, and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and evaporated to yield the crude product as a thick oil. Hexane (20.0 mL) was added to the product, stirred for 30

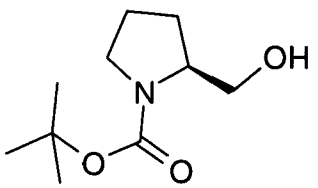
min, chilled in an ice bath and filtered to give 1,2-pyrrolidinedicarboxylic acid,1-(1,1-dimethylethyl)ester, (*S*)- **2** (1.57 g, 84%) as a white solid.

M.P. 130-134 °C, Lit. M.P. 134-137 °C

$[\alpha]_D$ −54.1° (*c* = 1, glacial acetic acid), Lit. $[\alpha]_D$ −59.84° to −61.6° (*c* = 1, glacial acetic acid)

^{1}H-NMR (200MHz, DMSO): δ 1.4(9H, s), 1.9(4H, m), 3.35(2H, t), 4.1(1H, t), 12.3 (1H, s)

Synthesis of (*S*)-2-hydroxymethyl-pyrrolidine-1-carboxylic acid tert-butyl ester 3[51]:



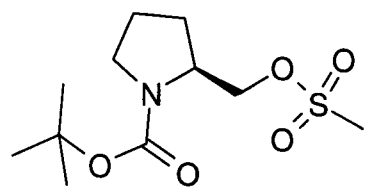
To a suspension of $LiAlH_4$ (0.31 g, 8.14 mmol) in dry THF (10 mL) was added a solution of (*S*)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester **2** (1.49 g, 6.51 mmol) in THF (7 mL). The mixture was heated at reflux for 120 min. The mixture was cooled to 0 °C, excess of $LiAlH_4$ was decomposed by adding solution of 0.1 g of KOH in 3 mL of water. The resulting reaction mixture was filtered through a bed of celite which was washed with 2x 5 mL warm (40 °C) THF; the collected filtrate was concentrated under reduced pressure. The resulting residue was redissolved in 15 mL ethyl acetate and washed with 2x5 mL 5% citric acid solution and 5 mL brine. The separated organic layer was dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a thick oil. 10 mL hexane was added to the oil, the precipitated solid was filtered through a Buchner funnel and washed with 2x5 mL hexane. The solid was dried well to give (*S*)-2-hydroxymethyl-pyrrolidine-1-carboxylic acid tert-butyl ester **3** (1.03 g, 79%) as a crystalline, white solid.

M.P. 54-59 °C, Lit. M.P. 56-58 °C

$[\alpha]_D$ −51.5° (*c* = 1, MeOH), Lit. $[\alpha]_D$ −54.5° to −59.6° (*c* = 1, MeOH)

^{1}H-NMR (200MHz, $CDCl_3$): δ 1.4(9H, s), 1.6-2.0(4H, m), 3.2-3.5(2H, m), 3.6(2H, dd), 3.9(1H, m), 4.8(1H, bs).

Synthesis of (*S*)-2-methanesulfonyloxymethyl-pyrrolidine-1-carboxylic acid tert-butyl ester 4[52]:

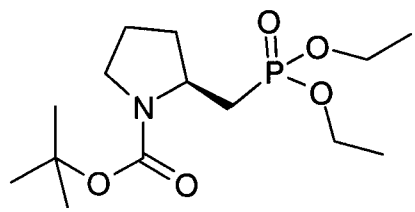


To a solution of (*S*)-2-hydroxymethyl-pyrrolidine-1-carboxylic acid tert-butyl ester **3** (1.25 g, 6.21 mmol) in CH_2Cl_2 (15 mL) at 0 °C was added diisopropylethylamine (2.35 mL, 13.66 mmol). Resulting reaction mixture was cooled to 0 °C and methanesulfonyl chloride (0.58 mL, 7.45 mmol) was added. After stirring for 3 hrs at 22 °C, the mixture was quenched with 5 mL 5% citric acid solution and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated to yield crude product as a yellow oil. The crude product was purified using column chromatography on silica gel using isocratic elution using dichloromethane as eluent, to give pure (*S*)-2-methanesulfonyloxymethyl-pyrrolidine-1-carboxylic acid tert-butyl ester **4** (1.82 g, 91%) as pale yellow oil.

$[\alpha]_D$ –64.5° (*c* = 1, DCM), Lit. $[\alpha]_D$ –60.5° to –62.6° (*c* = 1, DCM)

^{1}H-NMR (200MHz, $CDCl_3$): δ 1.5(9H, s), 1.8-2.1(4H, m), 3.0(3H, s), 3.3-3.5(2H, m), 4.0-4.2(2H, m), 4.3(1H, m)

Synthesis of (*S*)-2-(diethoxy-phosphorylmethyl)-pyrrolidine-1-carboxylic acid tert-butyl ester 5:



To a solution of diethyl phosphite (3.45 mL, 26.85 mmol) in DMF (10 mL) was added 60% suspension of sodium hydride (1.09 g, 26.85 mmol) slowly at 0 °C. RM was stirred for 1 hr at 22 °C, cooled to 0 °C and solution of (*S*)-2-methanesulfonyloxymethyl-pyrrolidine-1-carboxylic acid tert-butyl ester **4** (1.5 g, 5.35 mmol) in DMF (5 mL) was added. Resulting suspension was stirred at 55 °C for 6 hrs, cooled to 0 °C and quenched with 5 mL saturated NH_4Cl solution. The solvent evaporated under reduced pressure, and the resulting residue was redissolved in ethyl acetate (20 mL) and washed with water and brine. The separated organic layer was dried over sodium sulphate and concentrated to give the crude product as a pale yellow oil, which was purified through column chromatography on silica gel using gradient elution of ethyl acetate/ hexane to afford (*S*)-2-(diethoxy-phosphorylmethyl)-pyrrolidine-1-carboxylic acid tert-butyl ester **5** (1.07 g, 74%) as a colourless oil.

$[\alpha]_D$ –43.0° (*c* = 1, MeOH)

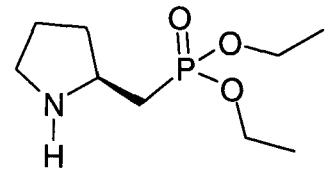
^{1}H-NMR (200MHz, $CDCl_3$): δ 1.3(6H, t), 1.4(9H, s), 1.6-1.9(3H, m), 1.9-2.1(2H, q), 2.2-2.5(1H, m), 3.2-3.4(2H, m), 4.0-4.3(5H, m)

^{13}C-NMR (50MHz, DMSO): δ 16.4 (d, J_{PC}=6), 23.7, 29.0, 29.5, 31.2, 46.2(d, J_{PC}=19.5), 52.6, 61.5 (d, J_{PC}=6), 79.6, 154.1

^{31}P-NMR (80 MHz, DMSO): δ 27.8

HRMS (M^+): 322.1782 calcd for $C_{14}H_{29}NO_5P$ 322.1783

Synthesis of (*S*)-2-(diethoxy-phosphorylmethyl)-pyrrolidine 6:



To a solution of (*S*)-2-(diethoxy-phosphorylmethyl)-pyrrolidine-1-carboxylic acid tert-butyl ester **5** (1.15 g, 3.58 mmol) in DCM (20 mL) was added TFA (1.35 mL, 17.89 mmol) and the mixture was heated at reflux for 8 hrs. The resulting RM was neutralised to pH=7 using triethylamine and concentrated under reduced pressure. The residue was directly subjected to column chromatography on silica gel using gradient elution of methanol / DCM to afford pure (*S*)-2-(diethoxy-phosphorylmethyl)-pyrrolidine **6** (0.71 g, 90%) as a pale yellow oil.

^{1}H-NMR (200MHz, DMSO): δ 1.2(6H, t), 1.6-2.0(3H, m), 2.1-2.5(3H, m), 3.1-3.3(2H, m), 3.5-3.7(1H, q), 4.0-4.2(4H, m)

^{13}C-NMR (50MHz, DMSO): δ 16.7 (d, J_{PC}=6), 23.5, 29.5, 31.2 (d, J_{PC}=8), 44.9, 55.1, 62.1 (d, J_{PC}=6)

^{31}P-NMR (80 MHz, DMSO): δ 25.9

HRMS (M^+): 222.1257 calcd for $C_9H_{21}NO_3P$ 222.1259

General procedure to synthesize 2'-hydroxy chalcones:

To a solution of 2'-hydroxyacetophenone (18.40 mmol) in ethanol (65 mL) was added 4M aqueous sodium hydroxide (90 mL) at 0 °C. After ½ hr of stirring at 0 °C, neat benzaldehyde (18.40 mmol) was added drop-wise. The reaction mixture was stirred vigorously at the same temperature for 2-3 hr before being placed in a refrigerator overnight. The reaction was diluted with ice-cold water, and neutralized with 10%

aqueous hydrochloric acid. The aqueous layer was extracted with ethyl acetate (3 x 20.0 mL), dried over sodium sulphate and concentrated under reduced pressure to give the crude product as thick oil. The crude product was then recrystallized from aqueous ethanol to afford the pure product as a yellow crystalline solid.

General procedure to synthesize flavanones:

Solution of 2'-OH chalcone (0.044 mmol) and (S)-2-(Diethoxy-phosphorylmethyl)-pyrrolidine **6** (4 mmol) and DMF (0.5 mL) was heated to 100 °C for 4 hrs. Resulting reaction mixture was concentrated under reduced pressure. The crude residue was purified by column chromatography using gradient elution of ethyl acetate and hexane to afford flavanone (73-79%) as white solid.

2,3-dihydro-2-phenyl-4H-1-Benzopyran-4-one[53] **(Table 2, entry 1):** ^{1}H-NMR (200MHz, $CDCl_3$): δ 2.8-3.2(2H, m), 5.4-5.6(1H, dd), 6.9-7.1(2H, m), 7.3-7.6(6H, m), 7.9-8.0(1H, dd)

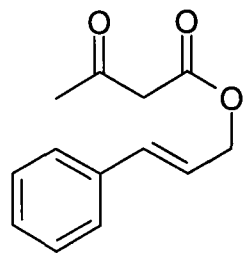
2,3-dihydro-2-(2-thiophene)-4H-1-Benzopyran-4-one[54] **(Table 2, entry 2):** ^{1}H-NMR (200MHz, $CDCl_3$): δ 3.0-3.3(2H, m), 5.6-5.8(1H, dd), 6.9-7.2(4H, m), 7.4(1H, d), 7.5(1H, t), 7.9(1H, d)

2,3-dihydro-2-(4'-methoxyphenyl-4H-1-Benzopyran-4-one)[53] **(Table 2, entry 3):** ^{1}H-NMR (200MHz, $CDCl_3$): δ 2.8-3.0(1H, dd), 3.1-3.3(1H, dd), 3.9(3H, s), 4.4-4.6(1H, dd), 6.9-7.2(4H, m), 7.4-7.6(3h, m), 8.0(1H, dd)

2,3-dihydro-2-(4'-chlorophenyl-4H-1-Benzopyran-4-one)[53] **(Table 2, entry 4):** ^{1}H-NMR (200MHz, $CDCl_3$): δ 2.8-3.2(2H, m), 5.4-5.6(1H, dd), 7.0-7.2(2H, t), 7.4-7.7(5H, m), 8.0(1H, dd)

2,3-dihydro-2-(4'-bromophenyl-4H-1-Benzopyran-4-one)[55] **(Table 2, entry 5):** ^{1}H-NMR (200MHz, $CDCl_3$): δ 2.8-3.2(2H, m), 5.4-5.6(1H, dd), 7.4(2H, d), 7.5-7.7(3H, m), 8.0(1H, dd)

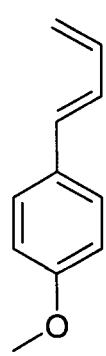
Synthesis of cinnamyl acetoacetate[41]:



To a solution of cinnamyl alcohol (0.258 g, 1.92 mmol) and ethyl acetoacetate (0.250 g, 1.92 mmol) in toluene (3 mL) was added iodine (0.100 g, 0.384 mmol) and the mixture was heated at reflux for 8 hrs. The resulting RM was concentrated under reduced pressure. The residue was directly subjected to column chromatography on silica gel using gradient elution of ethyl acetate / hexane to afford pure cinnamyl acetoacetate (0.320 g, 76%) as a colourless oil.

^{1}H-NMR (200MHz, $CDCl_3$): δ 2.3(3H, s), 3.5(2H, s), 4.8(2H, d), 6.2-6.3(1H, m), 6.6-6.7(1H, d), 7.3-7.4(5H, m)

Synthesis of 4-(p-methoxyphenyl) 1,3-butadiene 17[48]:



To a solution of allyl-triethylphosphonium bromide (0.16 g, 0.667 mmol) and distilled water (0.3 mL) was added powdered NaOH (0.08 g, 2.001 mmol) was added slowly. After 2 min 4-methoxybenzaldehyde (0.10 g, 0.734 mmol) was added slowly to

the reaction flask. Resulting reaction mixture was stirred vigorously at 70 °C for 1 hr. Reaction mixture was cooled to RT and water (5.0 mL) was added to the reaction mixture, stirred for 10 min. and extracted with dichloromethane (3 X 15.0 mL). The combined organic layers were dried over sodium sulphate, filtered, and concentrated. The product was purified over a short silica gel column (35% ethyl acetate in hexane) to yield 4-(p-methoxyphenyl) 1,3-butadiene **17** (0.09 g, 76%) as yellow semi-solid.

^{1}H-NMR (200 MHz, CDCl3): δ 3.8 (3H, s), 5.1-5.4 (2H, m), 6.4-6.7 (3H, m), 6.8-6.9 (2H, d), 7.3 (2H, d)

CHAPTER 2.

Isolation and Chemical-Biology of Natural Products from Ontario Plants

2.1 Introduction:

A natural product is a chemical compound, produced as a secondary metabolite by living organisms, which generally demonstrates biological activity. Human interest in the biologically active natural products can be traced back for centuries. Since long past, natural remedies are serving an important class of medicines in many cultures of the world. The plant parts utilised in ancient Asian and Chinese medicines contain biologically active ingredients. Among micro-organisms, animals and plants, plants are believed to be the most important source of biologically active compounds. Some of these natural products act as drugs, while some provide substrates to prepare synthetic drugs.

In this part of the thesis, the purification and identification of natural products from three different plants will be explained. All these isolated compounds were subjected to biological testing, while a few were extended to semi-synthetic derivatives and then tested. The first extracted plant was *Prunus avium* which is the sweet cherry plant. It is known for a wide range of anti-oxidants known as flavanoids. Flavanoids are also known for many other activities besides anti-oxidants like anti-cancer and anti-fungal activities[56].

The second plant extracted was *Dicentra spectabilis* which is commonly known as bleeding heart or Venus's car. This plant is used as an ornamental garden plant. Our group has been engaged in extraction of *D. spectabilis* since few years. This thesis explains the isolation of compounds from an already extracted fraction prepared two years ago. The third plant we used for extraction was *Narcissus pseudonarcissus* commonly known as wild daffodil. It is a garden plant with bulbs as its root system. The plant belongs to the Amaryllidaceae family which is known for producing biologically active alkaloids. A biologically active compound was isolated from bulbs of *N. pseudonarcissus* and was subjected to a number of reactions to synthesize a semi-synthetic analogue of the natural product. Synthesis and biological activity of the semi-synthetic derivative is also explained herein.

2.2 Isolation of Flavanoids:

2.2.1 Introduction:

Flavanoids are one of the most abundant natural products available in nature, and show a broad array of biological activities[56]. Fruits, vegetables and many herbal medicines contain flavanoid compounds. Epidemiological evidence showed that flavanoids are important cancer chemopreventative agents because of their ani-oxidant properties[57]. These compounds act as scavengers against activated carcinogens and mutagens[58]. Studies also showed that flavanoids are absorbed by the body in appreciable amounts[59] and remain stable in luminal fluid[60]. Even though the reports suggest so much healing potential for the flavanoids, only a few have been widely studied. As the biological activity depends on the individual flavanoid compound, systematic study of each compound is necessary.

2.2.2 Background:

Over the last six years the McNulty group has been engaged in isolation of flavanoids and their biological testing. Flavanoids from the bark and heartwood of sweet cherry, *Prunus avium*, have been isolated and identified[61]. The resin exuded by the plant has attracted attention in the study. Interestingly, the plant gives out the resin in response to the bracket fungi on the bark. This behaviour was hypothesized to be the plant's response to attack by producing some compounds that are active against the fungus. Hence the natural products from the bark resin and the heartwood were isolated and investigated for biological activity.

Various samples of heartwood were collected from a live, fully grown specimen of *P. avium* in St. Catharines, Ontario, Canada. The sample collection for previous extractions and isolations was mainly performed in the spring. The samples were collected from four to eight year old branches of a fully grown tree. Only the central yellow-brown part, which was distinct in colour from the sapwood, was removed. The

heartwood was cut into small pieces and then extracted in chloroform first and then in methanol. Both the extracted residues were separately applied to partition and gel chromatography yielding different compounds as described in Figure 21[61]. Purity of the compounds was validated by the TLC technique.

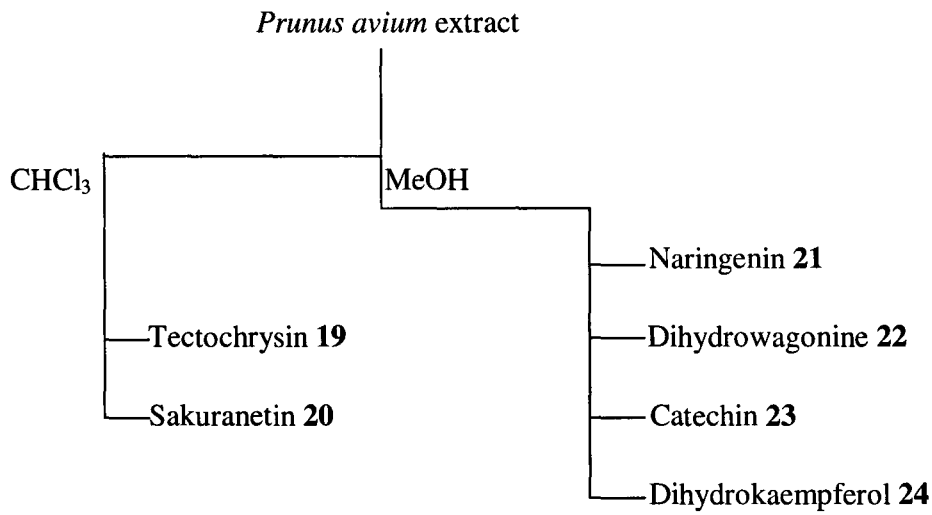
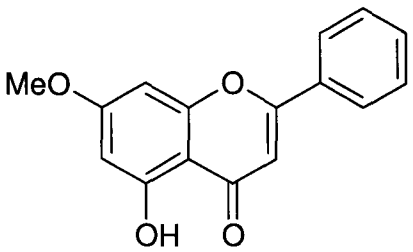


Figure 21: Isolation of different compounds from *P. avium* plant parts[61]

The compounds shown in Figure 21 have been isolated before from different plant sources. Table 4 below shows the known biological activity demonstrated by a few compounds in Figure 21.



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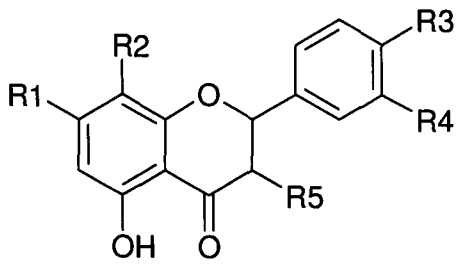


20-23

No.	Compound	R1	R2	R3	R4	R5	Biological Activity
19	Tectochrysin						Anti oxidant
20	Sakuranetin	OCH_3	H	OH	H	H	Anti microbial
21	Naringenin	OH	H	OH	H	H	Anti inflammatory
22	Dihydrowagonin	OH	OCH_3	H	H	H	Anti fungal
23	Catechin	OH	H	OH	OH	OH	Anti tumour
24	Dihydrokaempferol	OH	H	OH	H	OH	---

Table 4: Biological activity of the isolated flavanoids[61]

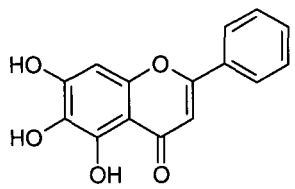
2.2.3 Results and discussion:

P. avium bark and resin were extracted to isolate sakuranetin **20** for biological testing and to study its differential occurrence with respect to time. Accordingly, the samples of the plant parts were collected in the autumn of 2008. The dried and chopped bark pieces along with resin were stirred in DCM at room temperature overnight. Solid residue was removed by filtration through a celite bed. The collected filtrate was analysed using the TLC technique which showed the presence of two major components along with several minor constituents. The filtrate was concentrated and subjected to silica gel chromatography. Both the major compounds were isolated and purified by repeated silica gel chromatography. The purified compounds were compared with previously isolated sakuranetin **20**, using TLC. The TLC analysis as well as NMR data revealed that one of

the isolated compounds was sakuranetin **20** while the other was dihydrowagonine **22**. The interesting fact was revealed that the amount of sakuranetine **20** isolated was much greater in the harvested plant parts from the summer compared to the harvested plant parts from autumn. The sample collected during the summer was found to contain 12 to 15% w/w of sakuranetin of the organic soluble plant parts while the sample collected during autumn yielded only about 4% w/w. The amount of dihydrowagonin **22** isolated was 11% w/w of the resin while previously it comprised only 2 to 4%, establishing some correlation between the two compounds with respect to the time that the sample was collected.

To study occurrence of these compounds with respect to the fungal pathogen, the compounds were tested for antifungal activity as well as for cytochrome P_{450} inhibitory activity. Human cytochrome inhibition by flavanoid compounds has been extensively studied[62]. These compounds inhibit human cytochrome P_{450}[57]. To correlate the antifungal and the cytochrome P_{450} activity possessed by the flavanoid compounds present in the resin, we isolated the flavanoids and subjected them to the antifungal as well as human cytochrome inhibition activity. Because of their anti-oxidant properties, flavanoids and their derivatives are also important anticancer agents[62f].

A group of flavanoid derivatives was screened against hCYP1A1, hCYP3A4 and hCYP19 enzymes at a concentration of 10μM[63]. Sakuranetin **20** inhibited all three enzymes to 70-90%. Dihydrokaempferol **24** showed inhibition of only hCYP1A1 while tectochrysin **19**, baicalein **25** and galangin **26** selectively inhibited hCYP1A1 to 100% at 10 μM. Apigenin **27** inhibited both hCYP1A1 and hCYP3A4 to 100% while partially inhibiting hCYP19 to 76%. Thus the data confirms the biological activity of the aromatic flavones over the flavanones except sakuranetin. Important results for the screening of these compounds against the three cytochromes are summarised in Table 5.



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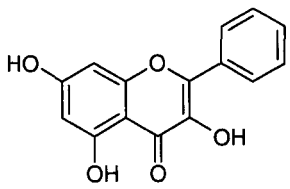

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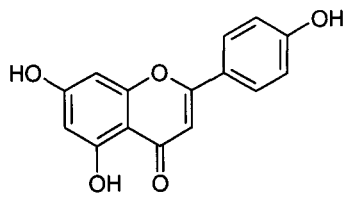


27

Flavanoids isolated	hCYP1A1		hCYP3A4		hCYP19	
	Ki (μM)	pKi (M)	Ki (μM)	pKi (M)	Ki (μM)	pKi (M)
Sakuranetin **20**	0.3	6.5±0.1	2.6	5.59±0.04	1.2	6.0±0.2
Tectochrysin **19**	0.2	7.0±0.3	7.4	5.13±0.0	2.1	5.8±0.2
Baicalein **25**	0.4	6.6±0.4	>7.5	<5	5.1	5.3±0.1
Galangin **26**	0.03	7.9±0.4	4.3	5.36±0.02	>5.1	<5
Apigenin **27**	0.08	7.1±0.1	0.2	6.79±0.06	1.0	6.0±0.1

Table 5: Inhibition activity of different flavanoids against human cytochrome enzyme[63]

After obtaining the human cytochrome inhibitory results, it was important to compare these results with antifungal activity. Hence, these compounds were subjected to an antifungal activity assay at initial concentrations of 10 to 28 μg/mL. Surprisingly, sakuranetin **20**, which inhibited the human cytochromes up to 90%, failed to show any significant activity against a panel of pathogenic fungi. Even at higher concentrations, sakuranetin **20** remained inactive. The two flavones, tectochrysin **19** and apigenin **27** showed little activity while baicalein **25** showed potent antifungal activity and galangin **25** showed selective activity (Table 6).

Pathogenic fungi	**20**	**25**	**26**	Amphotericin B
Candida albicans	>128	2	>128	≤0.25
Candida parapsilosis	>128	2	>128	≤0.25
Cryptococcus gatti	>128	0.5	4	≤0.25
Cryptococcus neoformans	>128	1	>128	≤0.25
Saccharomyces cerevisiae	>128	4	8	≤0.25

IC_{50} values (μg/mL) of flavanoid derivatives to select fungi

Table 6: Antifungal activity of selected flavanoids[63]

From the biological testing, it was clear that only the antifungal agents exhibit potent activity against the human cytochrome P_{450}. These results revealed strong evidence that the antifungal activity is the result of cytochrome inhibition.

2.2.4 Conclusion:

The isolation of flavanoids from the bark and raisin of *Prunus avium* was undertaken. A small library of flavanoids and flavones was made and subjected to human cytochrome inhibition and antifungal activity. The results of both the tests showed that the cytochrome inhibition and antifungal activity overlap with each other, thus proving that the cytochrome target was involved in the antifungal activity. The isolated contents of the resin were flavanones while only sakuranetin **20** amongst those flavanones showed human cytochrome P_{450} inhibitory activity; however **20** was found to be inactive in the antifungal testing.

2.3 Isolation of Menisdaurilide:

2.3.1 Introduction:

Dicentra spectabilis is a perennial herbaceous plant which is popular as an ornamental garden plant. Previous studies on *Dicentra* plants showed that these plants are sources of alkaloids which have potent antifungal[64] and antibacterial activities[65]. Previous protocols to isolate these compounds generally used acidic extraction using dilute or concentrated hydrochloric acid followed by basification with aqueous ammonia[65d, 66].

2.3.1 Background:

In the year 2007, our group extracted natural products from *Dicentra spectabilis*. Considering the possibilities of undesirable reactions of sensitive functionalities during the extraction process, the process was changed from that of the earlier. The extraction was carried out under acid and base free conditions[67]. Figure 22 explains the isolation protocol.

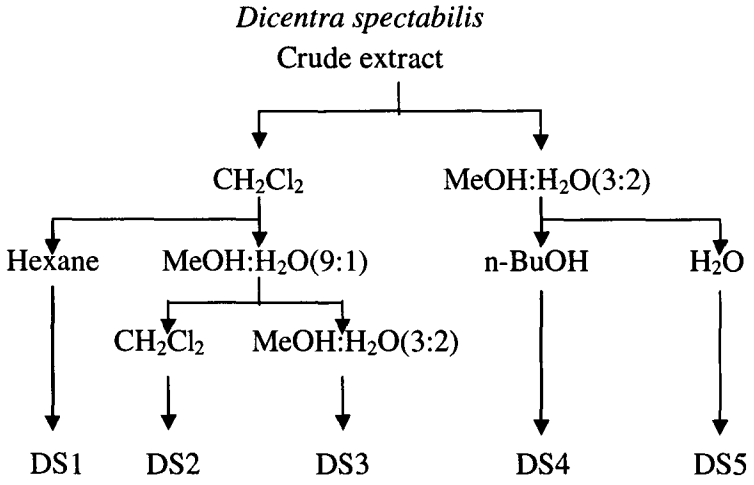
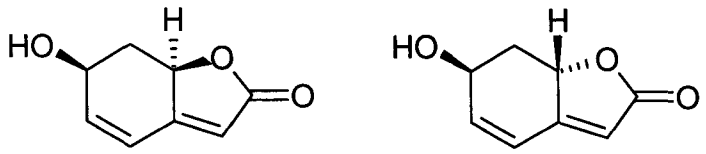


Figure 22: Isolation protocol for *Dicentra spectabilis*

Further purification of the fraction DS3 through silica gel column chromatography yielded menisdaurilide **28** and aquilegiolide **29** in 80: 20 ratio (Figure 23).



28 **29**

Figure 23: Menisdaurilide **28** and aquilegiolide **29**

Compounds **28** and **29** in 80:20 ratio were subjected to biological testing against cancer cells of Jurkat (Human T-cell lymphoma) and HT-29 (Human colon carcinoma) at various time-points and concentrations. At 10μM concentration, these compounds induced 33% apoptosis in Jurkat cells after 72 hours.

2.3.2 Results and discussion:

The crude n-butanol extract from *Dicentra spectabilis* (DS4) was found to exhibit antibacterial activity in conjuction with a screening program carried out in collaboration with Professor Gerry Wright at McMaster. TLC analysis of DS4 showed that it contains 2 highly polar constituents along with 2 moderately polar compounds. Purification of the DS4 fraction through sephadex column chromatography separated three fractions, DS 41, DS42 and DS43. As DS42 and DS43 were not completely purified, these fractions were subjected to silica gel chromatography to yield pure fractions DS421, DS422 and DS431 as shown in Figure 24.

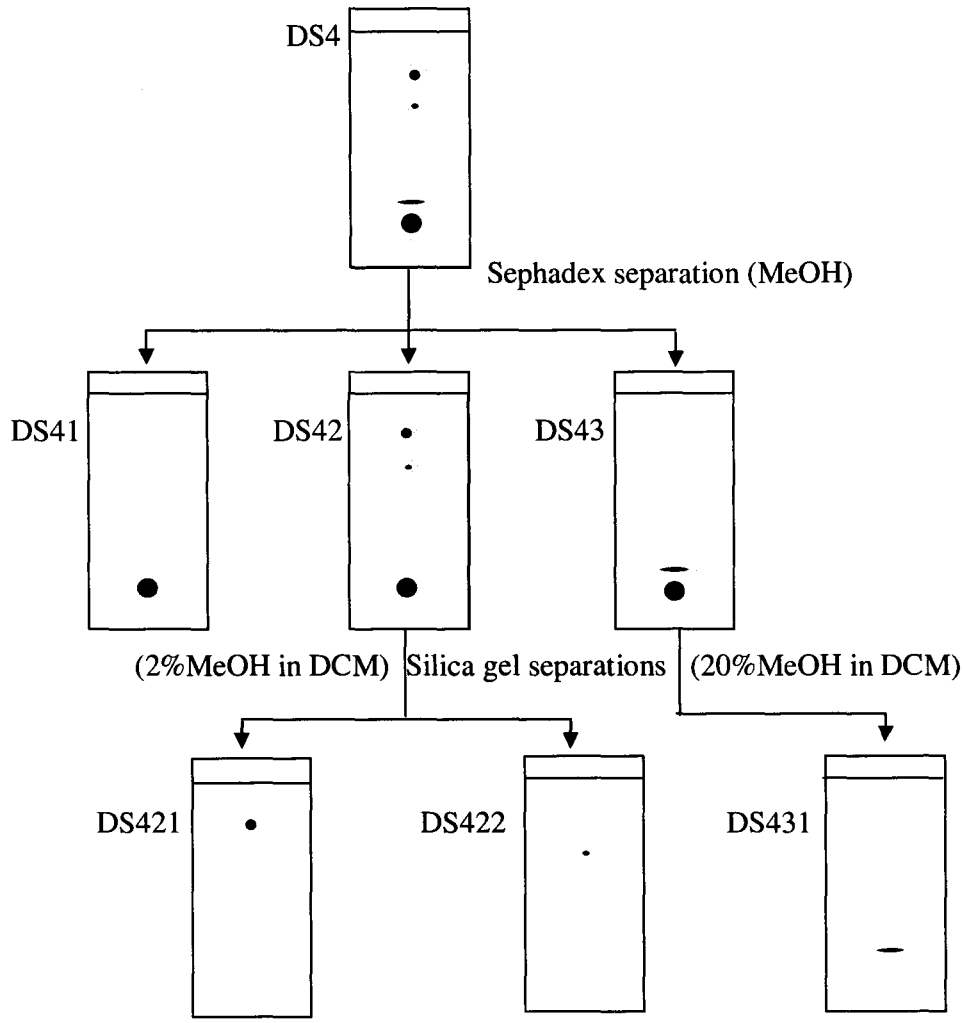


Figure 24: Isolation protocol for DS4 using TLC pictures

NMR analysis of DS41 and DS431 did not show any proton in ^{1}H NMR. Moreover acetylation of DS 41 yielded a non-polar product which showed typical acetate group spectra for ^{1}H and ^{13}C NMR, while DS431 remained unreactive under the acetylation conditions. Thus, the NMR analysis performed was inconclusive towards

structure determination of these compounds. Further study is necessary to elucidate structures of DS41 and DS431.

Both the compounds DS421 and DS 422 showed a characteristic ^{1}H NMR for an organic compound containing aromatic groups. Further studies revealed that the DS 421 is menisdaurilide **28**. To our delight it was next determined that the purified compound menisdaurilide exhibited potent antibacterial activity and was active in a *Pseudomonas syringae* biofilm forming essay. This compound appears to be largely responsible for the initial activity found in the crude extract. Further biochemical studies to determine the biological target, mechanism of action and overall scope are currently under investigation.

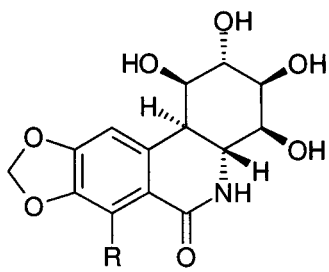
2.4 Isolation of Narciclasine and synthesis of *trans*-dihydronarciclasine:

2.4.1 Introduction:

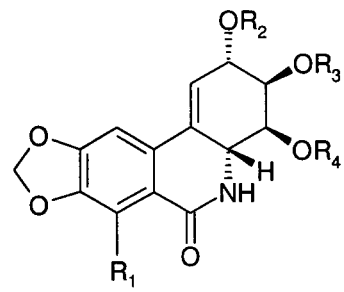
Various alkaloids from the Amaryllidaceae family are attracting tremendous interest because of their structural differentiation and the biological activities[68]. Pancratistatin **30a** is one of these alkaloids which, because of its potent anticancer activity, attracted considerable interest[69, 70]. Pancratistatin **30a** is also known for its selectivity towards cancer cells. It induces apoptosis in cancer cells selectively without affecting normal cells. It has also been reported that the compound attacks mitochondria in the cancerous cells[69, 70d]. The exact mechanism of the biological action of the compound is still unknown.

Three major drawbacks have troubled the development of a pancratistatin **30a** or its derivatives as a medicinal candidate. Low aqueous solubility (53μg/mL) is the first of those; this was solved by synthesis its phosphate derivative[71]. The low natural abundance

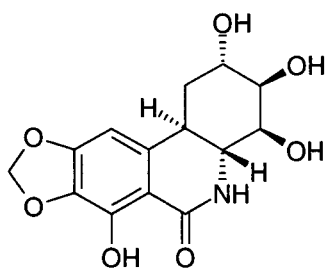
of the molecule is the second drawback, which was tackled by synthesizing semi-synthetic derivatives. Conversion of narciclasine **31a** to pancratistatin **30a** has been reported with 3.6% overall yield over 10 steps[72]. However, a commercially viable synthetic approach for preparation of pancratistatin **30a** is not available. Hence other alkaloid derivatives found in the Amaryllidacea family came under focus. 7-Deoxypancratistatin **30b**, narciclasine **31a**, 7-deoxynarciclasine **31b** and *trans*-dihydronarciclasine **32** demonstrate potent anticancer activity, while *cis*-dihydronarciclasine **33** was found to be inactive[72].



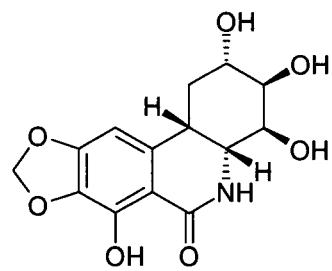
30a, R=OH, Pancratistatin
30b, R=H, 7-deoxypancratistatin



31a, R_1=OH; R_2,R_3,R_4=H, Narciclasine
31b, R_1,R_2,R_3,R_4=H, 7-deoxynarciclasin



32 *Trans*-dihydronarciclasine



33 *Cis*-dihydronarciclasine

Figure 25: Some alkaloids from the Amaryllidacea family

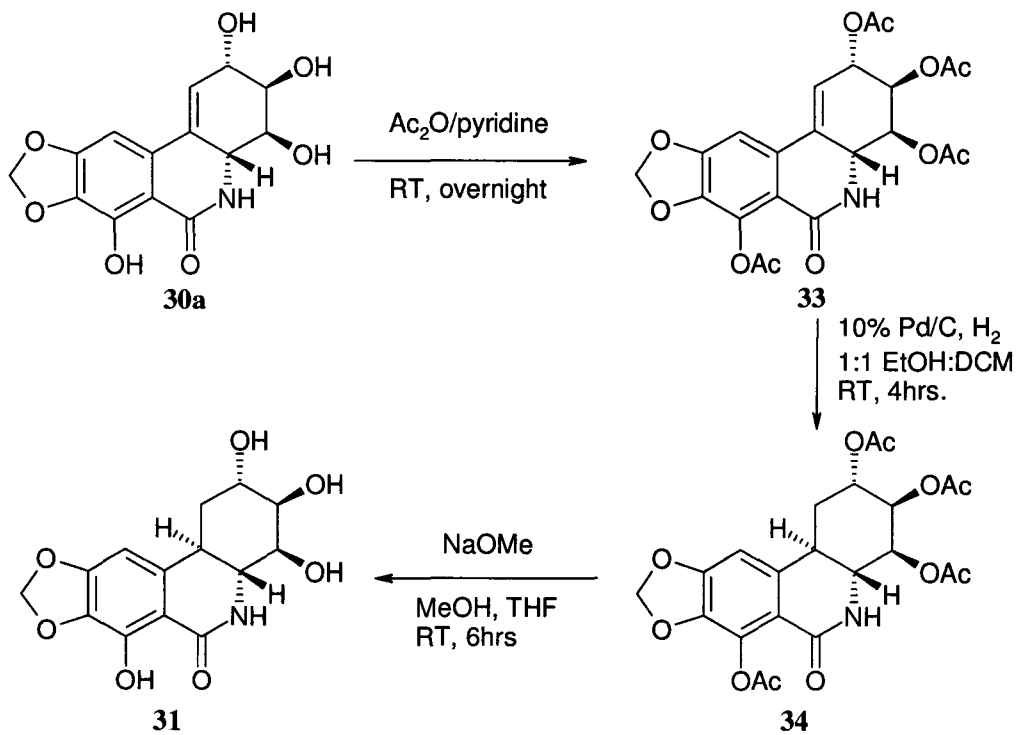
Our group recently disclosed the third drawback. Several natural and semi-synthetic analogues of Amaryllidacea family metabolites were screened for inhibition of human cytochrome 3A4 (CYP3A4)[73]. The study showed that, along with anticancer

activity, these compounds also interact with the most prominent enzyme 3A4. Reports have suggested that many drug development programs have failed because of these interactions[74]. This inhibition of CYP3A4 can create adverse effects which may lead to toxicity. While pancratistatin **30a** was selective towards cancer cells and importantly inactive to CYP3A4, our study showed that narciclasine **31a** inhibits CYP3A4. This was a major setback in considering narciclasine **31a** and its derivatives as anticancer agents. The comparison between pancratistatin **30a** and narciclasine **31a** structures and their CYP3A4 inhibition results drew our attention. The results showed that the C_1-C_{10b} double bond present in narciclasine **31a** was likely to be the site of interaction for the CYP3A4. As the *trans*-dihydronarciclasine **32** derivative has been reported as a potent anticancer agent, we studied its interaction with CYP3A4. As the *trans*-dihydronarciclasine **32** is not abundantly present in nature, we prepared it from narciclasine **31a**. Hydrogenation of the C_1-C_{10b} double bond present in narciclasine **31a** yielded *trans*-dihydronarciclasine **32**. Interactions of narciclasine **31a** and *trans*-dihydronarciclasine **32** with CYP3A4 were studied.

2.4.2 Results and discussion:

Freshly harvested bulbs of *Narcissus pseudonarcissus* were washed with plenty of water. They were then chopped into small pieces and soaked into 95% EtOH for one week. The EtOH with suspended plant parts in it was filtered through a celite bed and concentrated under reduced pressure to give an aqueous residue. The temperature throughout the concentration was maintained below 40 °C. The residue obtained was extracted with DCM first and then with EtOAc. The EtOAc layer was concentrated and purified through a number of silica gel columns to accomplish pure narciclasine **31a**. The purified narciclasine **31a** was analysed using ^1H and ^{13}C NMR as well as using HRMS data. Also, the tetra-acetate of narciclasine **31a** was prepared and analysed using NMR to validate the isolated compound as narciclasine **31a**. All these pieces of evidence supported that the isolated natural product was narciclasine **31a**.

Synthesis of the *trans*-dihydronarciclasine **32** derivative required protection of four hydroxy groups in the first step. As the earlier synthesis of *trans*-dihydronarciclasine **32** suggested[75], we protected the hydroxyl groups by making the corresponding acetates. The reaction was carried out in pyridine using acetic anhydride as the protecting reagent. In the next crucial step, the protected narciclasine **31a** was subjected to hydrogenation. Interesting results were reported by Pettit for the hydrogenation reaction. The ratio of *trans* to *cis* product during the hydrogenation reaction was crucially dependent upon the solvent used for the reaction[75]. A maximum 7:3, *trans*:*cis* ratio has been reported with 1:1, EtOH:DCM solvent system and 10 mol% loading of 10%Pd/C. We performed the hydrogenation reaction strictly following reported protocol. As expected and reported, TLC showed reaction giving one major along with two minor products. The major product was isolated and analysed using ^{1}H NMR analysis. As the NMR spectrum matched the reported data, product was subjected to deprotection of the acetate groups. After deprotection using sodium methoxide in MeOH and THF, the final product was purified and the ^{1}H NMR was checked. Although most of the recorded NMR matched the reported data for *trans*-dihydronarciclasine **32**, a peak at 2.19 ppm was missing with an extra peak appearing at 1.65 ppm, thus creating doubt about the structure of the synthesized product.



Scheme 15: Synthesis of *trans*-dihydronarciclasine

As the literature does not describe the detailed NMR spectrum of *cis*-dihydronarciclasine NMR report, some other technique was needed to validate the product structure. Hence, a single crystal structure of the product was recorded, which revealed a surprising result. From the single crystal structure it was clear that the product we presumed to be the *trans* isomer, was actually the *cis* isomer (Figure 26). Thus it was clear that the hydrogenation reaction accomplished *cis* isomer as major product instead of the *trans* isomer.

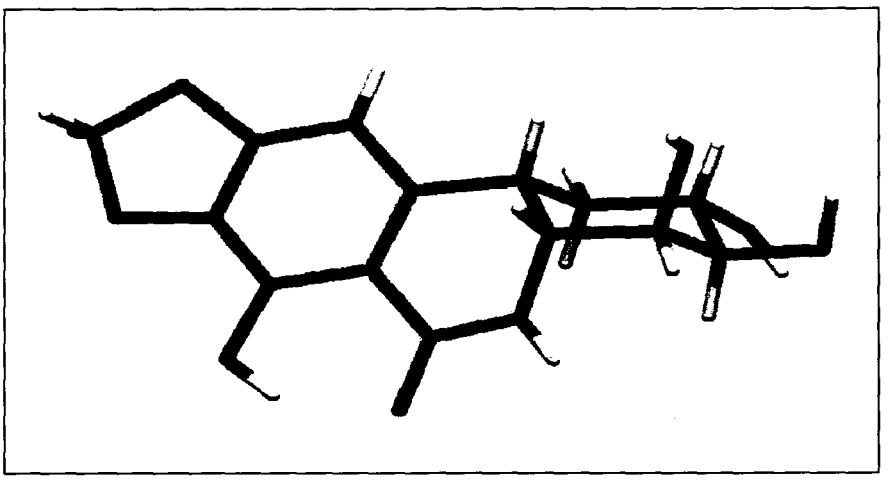


Figure 26: Single crystal structure of *cis*-dihydronarciclasine

The strange behaviour of the hydrogenation reaction was suspected to be due to the incorrect solvent ratio during the reaction. To maintain a complete hydrogen atmosphere in the reaction flask, during the reaction, some of the hydrogen gas was released from the flask using a syringe. It was predicted that, being low boiling, some DCM might have evaporated leaving behind a lesser amount of DCM than the required ratio. Hence the major *cis* isomer formation was taking place. The hydrogenation reaction was performed again making a slight change in the procedure. Instead of making a solution of the reactant and then purging with the hydrogen, only reactant and 10%Pd/C were taken into the flask. Hydrogen was purged and then 1:1 EtOH:DCM solvent was added to the reaction flask. The reaction worked well in this way, producing two *trans* isomer as the major product with two minor products. The deprotection reaction using the earlier protocol yielded *trans*-dihydronarciclasine. ^{1}H NMR and HRMS data matched that of the reported *trans*-dihydronarciclasine.

Pancratistatin **30a** and narciclasine **31a** were screened for CYP3A4 inhibitory activity using Ketoconazole as a control[76]. Narciclasine showed potent inhibitory activity against CYP3A4 while pancratistatin **30a** was inactive up to >10μM as explained

earlier[73]. When *trans*-dihydronarciclasine **32** was subjected to the testing[77], it showed lack of inhibition of CYP3A4 as expected, thus supporting our hypothesis that the C_1-C_{10b} double bond present in narciclasine **31a** is likely to be the site of interaction for CYP3A4.

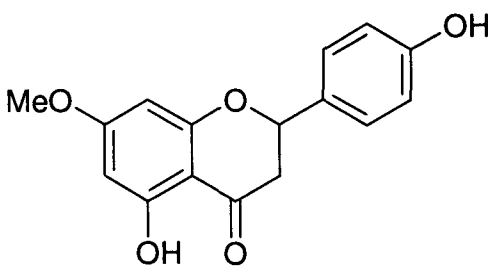
2.4.3 Conclusion:

Work herein described isolation, purification and identification of a natural product extracted from *P. avium* bulbs. A semi-synthetic analogue, *trans*-dihydronarciclasine was made and tested for CYP3A4 inhibitory activity. Comparison between the human cytochrome inhibitory activities of narciclasine **31a** and *trans*-dihydronarciclasine **32** showed that the C_1-C_{10b} double bond in narciclasine **31a** is necessary for the exhibition of the activity. Further investigation is under progress.

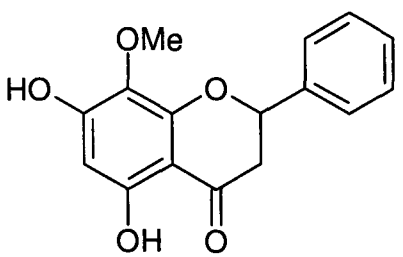
2.4.4 Experimental:

General information: Reactions were carried out under an Argon atmosphere in oven-dried glassware. All fine chemicals were obtained from Aldrich. Toluene, diethyl ether and THF were distilled from sodium metal with benzophenone indicator. Dichloromethane and ethyl acetate were distilled over calcium hydride. Melting points (uncorrected) were measured on a Gallenkamp melting point apparatus. Optical rotations were determined on a Perkin-Elmer 241 polarimeter installed with a λ_{589} sodium lamp. CIMS were run on a Micromass Quattro Ultima spectrometer fitted with a direct injection probe (DIP) with ionization energy set at 70 eV and HRMS (CI) were performed with a Micromass Q-Tof Ultima spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 200, 600 or AV 700 spectrometer in $CDCl_3$ with TMS as internal standard, chemical shifts (δ) are reported in ppm downfield of TMS and coupling constants (J) are expressed in Hz.

Extraction and purification of sakuranetin 20 and dihydrowagonin 22 from *Prunus avium* plant parts[63]:



Sakuranetin



Dihydrowaginin

The resin and bark wood (50.0 g) of four to eight year old tree of a *Prunus avium* were stirred in DCM (300.0 mL) for 48hrs. Resulting solution with suspended plant parts was filtered through celite bed, washed with 2 x 50.0 mL DCM. The filtrate was

concentrated under reduced pressure to give crude residue (0.70 g) which was subjected to column chromatography on silica gel (column: 35.0 X 2.0 cm) using gradient elution of methanol / DCM (1% MeOH in DCM to 5% MeOH in DCM). The collected fractions were concentrated under reduced pressure and repurified using column chromatography on silica gel (column: 25.0 X 2.0 cm) using gradient elution of ethyl acetate / hexane (10% ethyl acetate in hexane to 55% ethyl acetate in hexane). The fractions showing dark spots in TLC technique under UV light (no. 12-18 for sakuranetin **20** and no. 24-33 for dihydrowagonin **22**) were concentrated under reduced pressure to give sakuranetin **20** (0.026 g, 3.71%) and dihydrowagonin **22** (0.076 g, 10.85%) as pale yellow crystalline solid.

Sakuranetin **20**[78]**:**

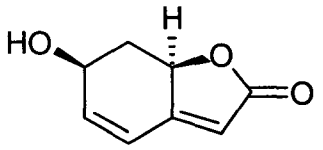
M.P. 154-157 °C, Lit. M.P. 152-154 °C

^{1}H-NMR (200MHz, $CDCl_3$): δ 2.7-2.9(1H, dd), 3.0-3.2(1H, dd), 3.8(3H, s), 5.1(1H, bs), 5.3-5.5(1H, dd), 6.1(2H, d), 6.9(2H, d), 7.4(2H, d), 12(1H, s)

Dihydrowagonin **22**[79]**:**

^{1}H-NMR (200MHz, $CDCl_3$): δ 2.8-2.9(1H, dd), 3.0-3.1(1H, dd), 3.9(3H, s), 5.4-5.5(1H, dd), 6.2 (1H, s), 6.6(1H, bs), 7.3(1H, m), 7.4-7.5(4H, m), 11.9(1H, s)

Isolation and purification of menisdaurilide from *Dicentra spectabilis* plant parts extract[80]**:**

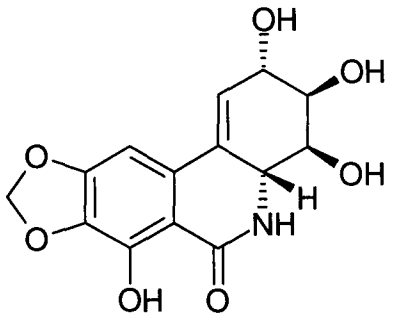


Crude n-butanol extract of *Dicentra spectabilis* (DS4, 0.09 g) was subjected to sephadex column chromatography and eluted using MeOH. Collected fractions were concentrated under reduced pressure and subjected to silica gel chromatography and eluted using gradient elution with MeOH / DCM. Collected fractions were concentrated under reduced pressure to give menisdaurilide (0.004 g, 4.55%) as a pale yellow semi-solid.

M.P. 90-95 °C, Lit. M.P. 96-98 °C

^{1}H-NMR (700MHz, $CDCl_3$): δ 1.6 (1H, ddd), 3.0 (1H, m), 4.6 (1H, d), 4.9 (1H, dd), 5.8 (1H, s), 6.3 (1H, d), 6.6 (1H, dd)

Extraction and purification of narciclasine from *Narcissus pseudonarcissus* plant parts[81]:



Finely chopped bulbs of *Narcissus pseudonarcissus* (550 g) were soaked in 95% EtOH (1.50 L) for a week. The resulting solution with suspended plant parts was filtered through a celite bed, which was then washed with 3 x 75.0 mL 95% EtOH. The filtrate was concentrated under reduced pressure to give an aqueous residue. The temperature throughout the concentration was maintained below 40 °C. The residue thus obtained was extracted with DCM first and then with EtOAc. The EtOAc layer was concentrated and purified through a silica gel column using gradient elution of MeOH / DCM to give crude narciclasine, which was subjected to silica gel column chromatography using gradient

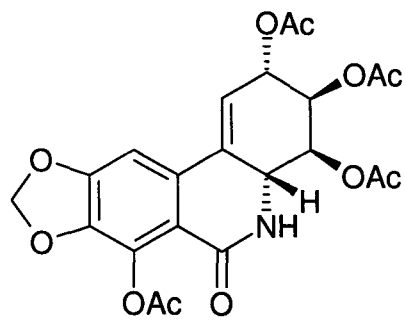
elution of EtOAc / hexane to accomplish pure narciclasine **31a** (0.037 g, 0.006%) as an off-white solid.

M.P. 242-247 °C, Lit. M.P. 251-251 °C

^{1}H-NMR (700MHz, DMSO): δ 3.7 (1H, bs), 3.8 (1H, m), 4.0 (1H, m), 4.2 (1H, d), 5.0 (1H, d), 5.2 (1H, dd), 6.1 (2H, d), 6.2 (1H, s), 6.9 (1H, s), 7.9 (1H, s)

HRMS (M^+): 308.0771 calcd for $C_{14}H_{13}NO_7$ 308.0770

Synthesis of 2,3,4,7-O-Tetracetoxynarciclasine 34[75]:

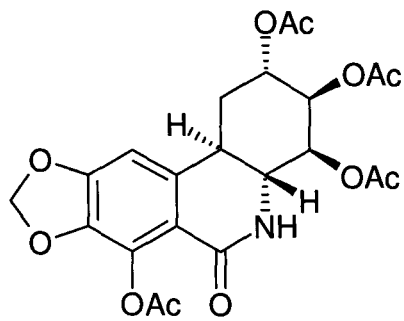


To a stirred solution of narciclasine (0.008 g, 0.0026 mmol) in pyridine (0.10 mL) was added acetic anhydride (0.20 mL). After stirring for 16 hrs at 23 °C, solvent was evaporated under reduced pressure. The resulting residue was purified by column chromatography on silica gel using gradient elution of ethyl acetate/ hexane to afford 2,3,4,7-O-Tetracetoxynarciclasine **34** (0.011 g, 89%) as white solid.

M.P. 226-230 °C, Lit. M.P. 230-231 °C

^{1}H-NMR (600MHz, $CDCl_3$): 2.1 (3H, s), 2.2 (3H, s), 2.3 (3H, s), 2.4 (3H, s), 4.6 (1H, d), 5.2 (1H, dd), 5.3 (1H, m), 5.4 (1H, m), 6.0 (1H, d), 6.1 (1H, d), 6.2 (1H, m), 6.9 (1H, s)

Synthesis of 2,3,4,7-O-Tetracetoxy-*trans*-dihydronarciclasine 35[81]:



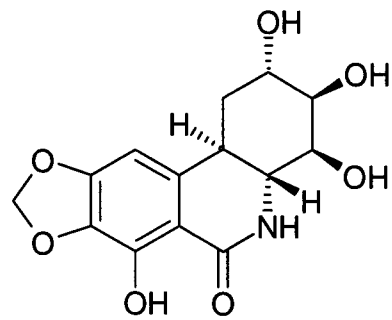
A mixture of narciclasine tetracetate (0.010 g, 0.0021 mmol) and 10% Pd/C catalyst (0.0002 g, 0.0002 mmol) in a RBF was purged with hydrogen gas for 1 min. 1:1 CH_2Cl_2-EtOH (0.50 mL) was added and the mixture was stirred under 1 atm hydrogen at 23 °C for 4 hrs. The reaction mixture was filtered through a pad of celite, and solvent was evaporated under reduced pressure. Resulting residue was purified through column chromatography on silica gel using gradient elution of ethyl acetate/ hexane to afford 2,3,4,7-O-Tetracetoxy-*trans*-dihydronarciclasine **35** (0.005 g, 50%) as white solid.

M.P. 180-186 °C, Lit. M.P. 181-182 °C

^{1}H-NMR (600MHz, $CDCl_3$): δ 1.9 (1H, ddd), 2.0 (3H, s), 2.1 (3H, s), 2.2 (3H, s), 2.4 (3H, s), 2.5 (1H, ddd), 3.1 (1H, dd), 3.8 (1H, dd), 5.1 (1H, ddd), 5.3 (1H, m), 5.4 (1H, dd), 6.0 (1H, d), 6.1 (1H, d), 6.6 (1H, s)

HRMS (M^+): 478.1339 calcd for $C_{22}H_{16}NO_{11}$ 478.1271

Synthesis of *trans*-dihydronarciclasine 32[75]:



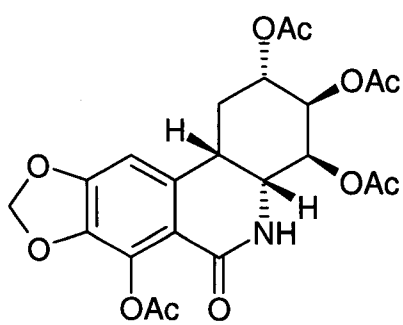
To a stirred solution of 2,3,4,7-O-tetracetoxy-*trans*-dihydronarciclasine (0.004 g, 0.00084 mmol) in THF (0.20 mL) was added a solution of NaOMe (0.045 g, 0.0084 mmol) in MeOH (0.10 mL). After stirring for 3 hrs at 23 °C, the mixture was quenched with 2.0 mL saturated NH_4Cl solution, and solvent was evaporated under reduced pressure. The resulting residue was purified through column chromatography on silica gel using gradient elution of MeOH/ DCM to afford *trans*-dihydronarciclasine **32** (0.0015 g, 58%) as a white solid.

M.P. 282-287 °C, Lit. M.P. 290-291 °C

^{1}H-NMR (600MHz, CD_3OD): δ 1.8–1.9 (1H, m), 2.2-2.3 (1H, m), 3.0-3.1 (1H, m), 3.5 (1H, dd), 3.8 (1H, dd), 3.9 (1H, t), 4.1 (1H, dd), 6.0 (1H, d), 6.1 (1H, d), 6.5 (1H, d)

HRMS (M^+): 310.0916 calcd for $C_{14}H_{16}NO_7$ 310.0927

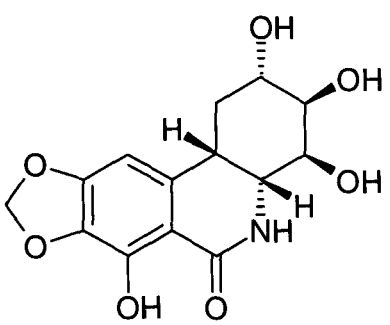
Synthesis of 2,3,4,7-O-Tetracetoxy-*cis*-dihydronarciclasine 35[81]:



A mixture of narciclasine tetracetate (0.010 g, 0.0021 mmol) and 10% Pd/C catalyst (0.0002 g, 0.0002 mmol) in 1:1 CH_2Cl_2-EtOH (0.50 mL) in a RBF was purged with hydrogen gas for 1 min. The mixture was stirred under 1 atm hydrogen at 23 °C for 4 hrs. The reaction mixture was filtered through a pad of celite, and solvent was evaporated under reduced pressure. The resulting residue was purified through column chromatography on silica gel using gradient elution of ethyl acetate/ hexane to afford 2,3,4,7-O-Tetracetoxy-*cis*-dihydronarciclasine **35** (0.006 g, 60%) as a white solid.

^{1}H-NMR (600MHz, $CDCl_3$): δ 1.9 (1H, m), 2.0 (6H, 2 s), 2.1 (1H, m), 2.2 (3H, s), 2.4 (3H, s), 3.2 (1H, dd), 3.9 (1H, dd), 5.1-5.2 (2H, m), 5.4 (1H, m), 5.7 (1H, s), 6.0 (1H, d), 6.1 (1H, d), 6.6 (1H, s)

Synthesis of *cis*-dihydronarciclasine 32[75]:



To a stirred solution of 2,3,4,7-O-tetracetoxy-*cis*-dihydronarciclasine (0.004 g, 0.00084 mmol) in THF (0.20 mL) was added a solution of NaOMe (0.045 g, 0.0084 mmol) in MeOH (0.10 mL). After stirring for 3 hrs at 23 °C, the mixture was quenched with 2.0 mL saturated NH_4Cl solution, and solvent was evaporated under reduced pressure. The resulting residue was purified through column chromatography on silica gel using gradient elution of MeOH/ DCM to afford *cis*-dihydronarciclasine **32** (0.002 g, 70%) as a white solid.

^{1}H-NMR (600MHz, CD_3OD): δ 1.5–1.6 (1H, m), 1.8-1.9 (1H, m), 3.1-3.2 (1H, m), 3.5 (1H, dd), 3.8 (1H, dd), 3.9 (1H, m), 4.1 (1H, dd), 6.0 (1H, d), 6.1 (1H, d), 6.4 (1H, d)

HRMS (M^+): 308.0773 calcd for $C_{14}H_{14}NO_7$ 308.0770

Single crystal structure also supported the product structure.

CHAPTER 3. References

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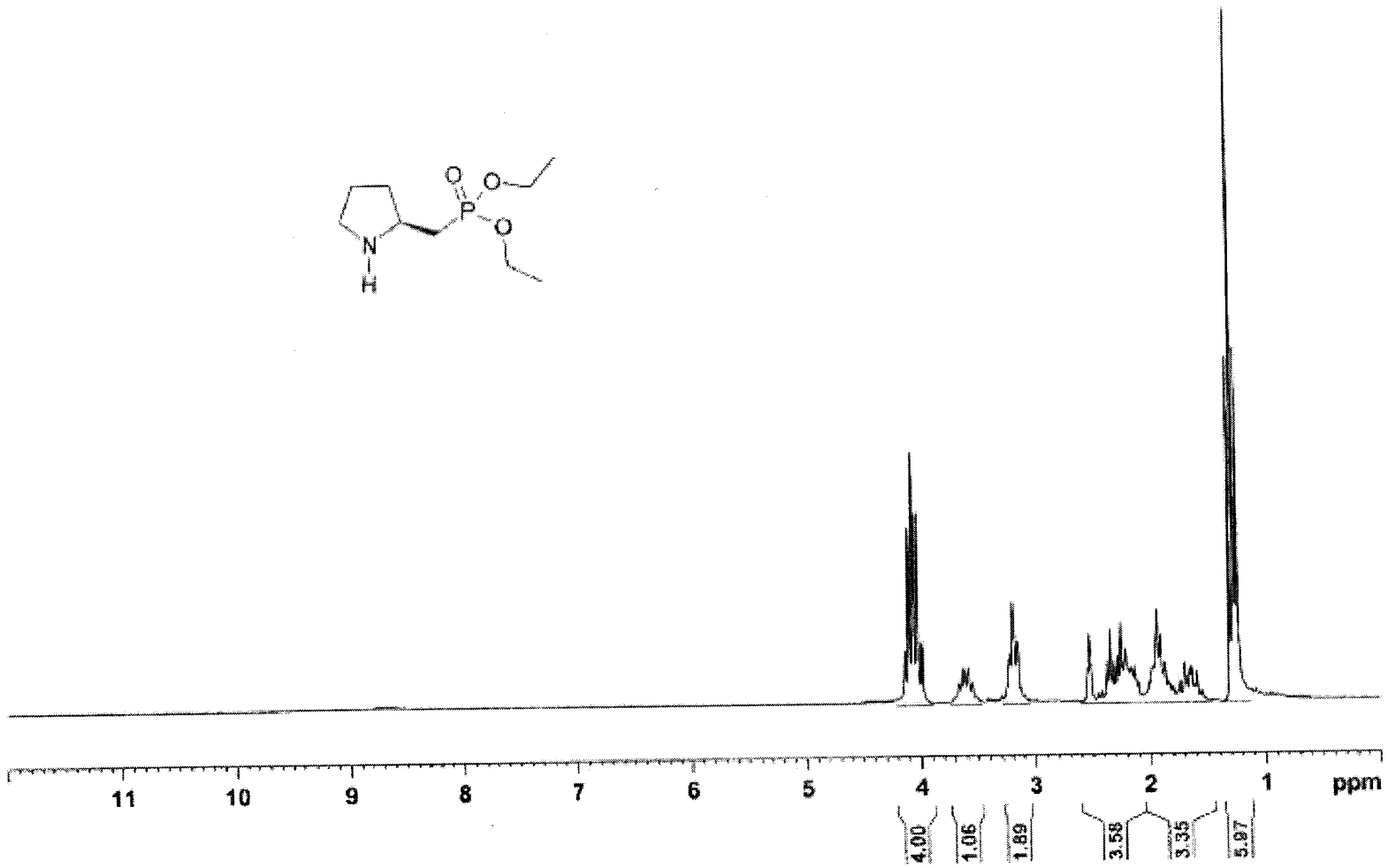
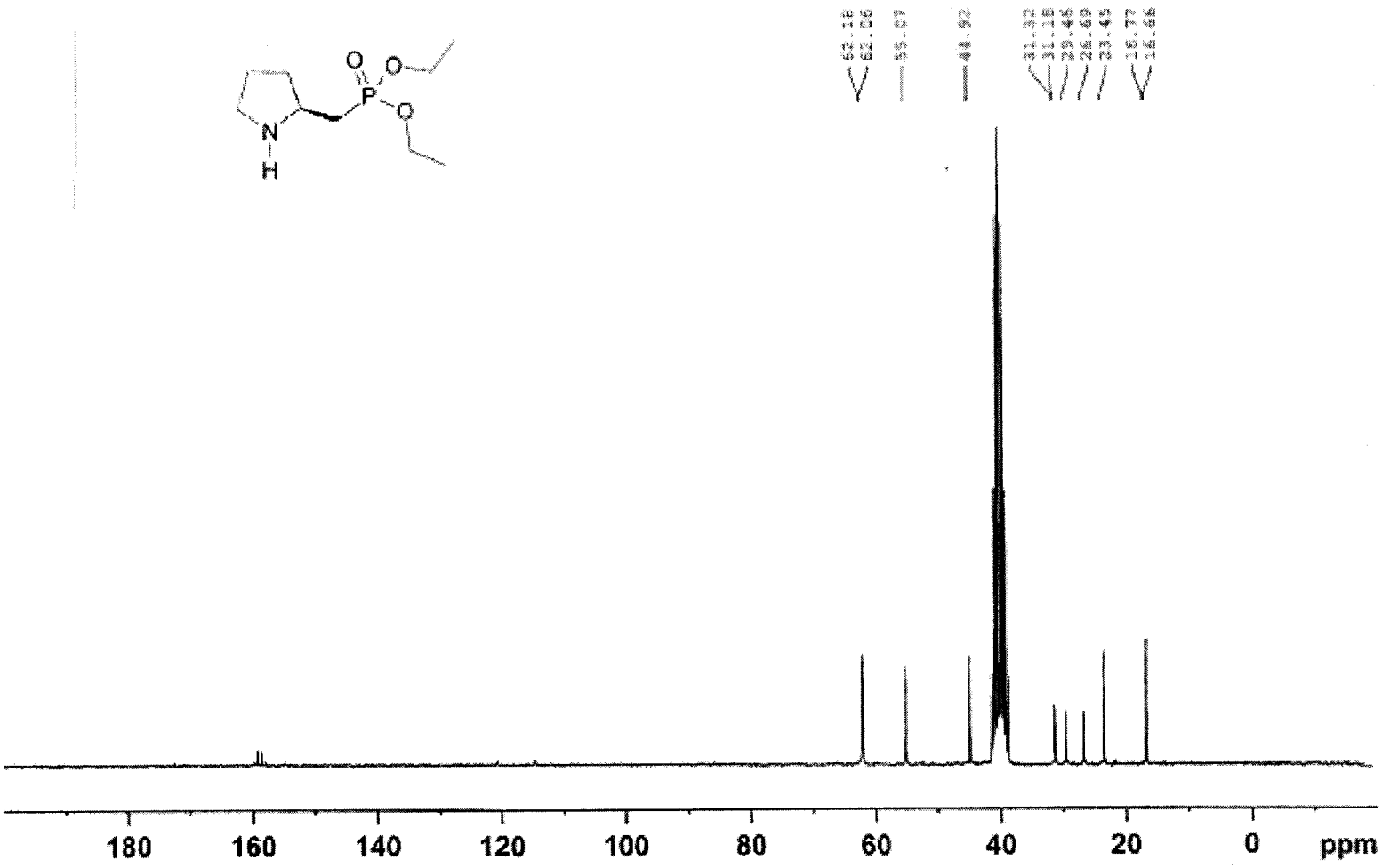
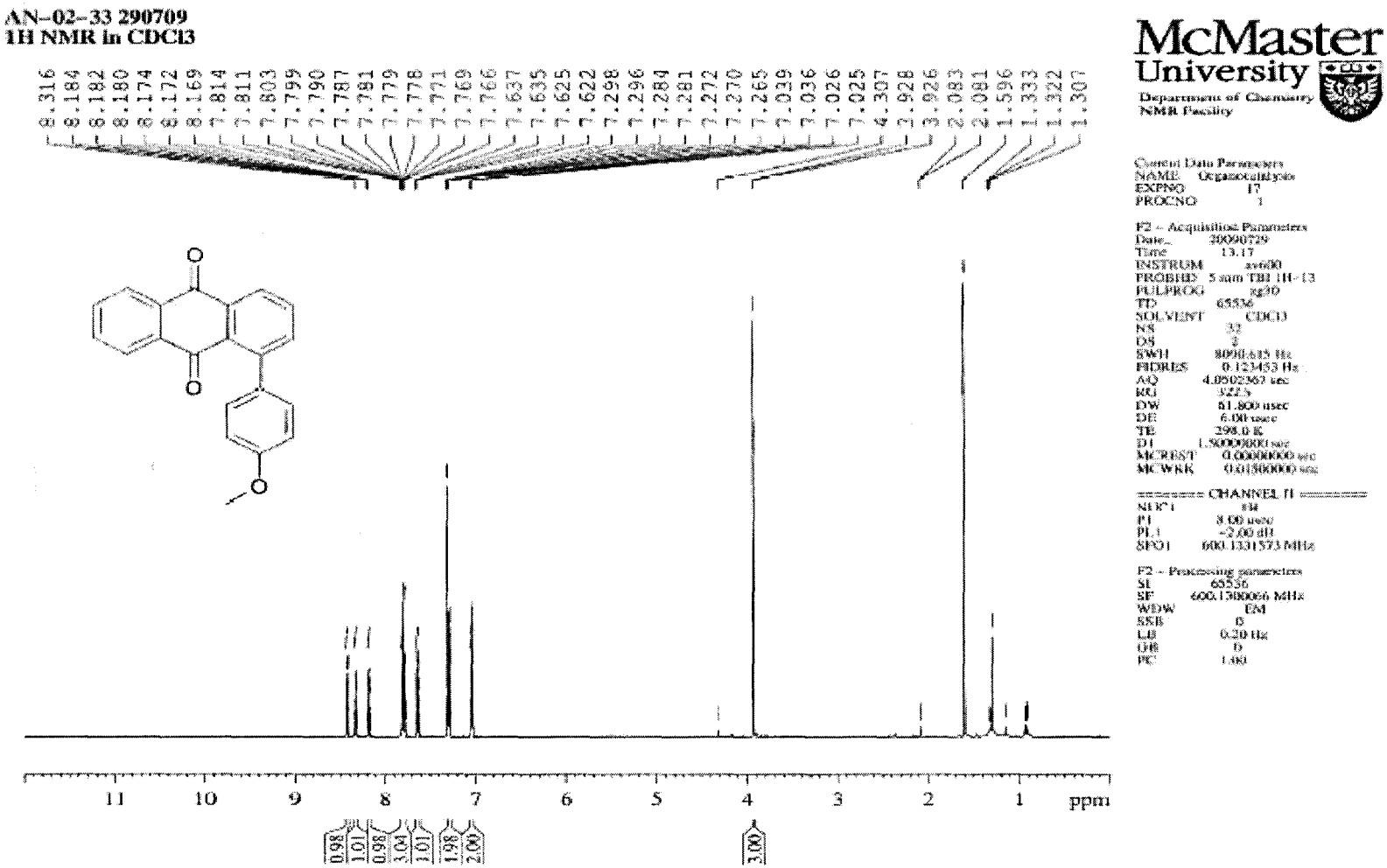
NMR spectra



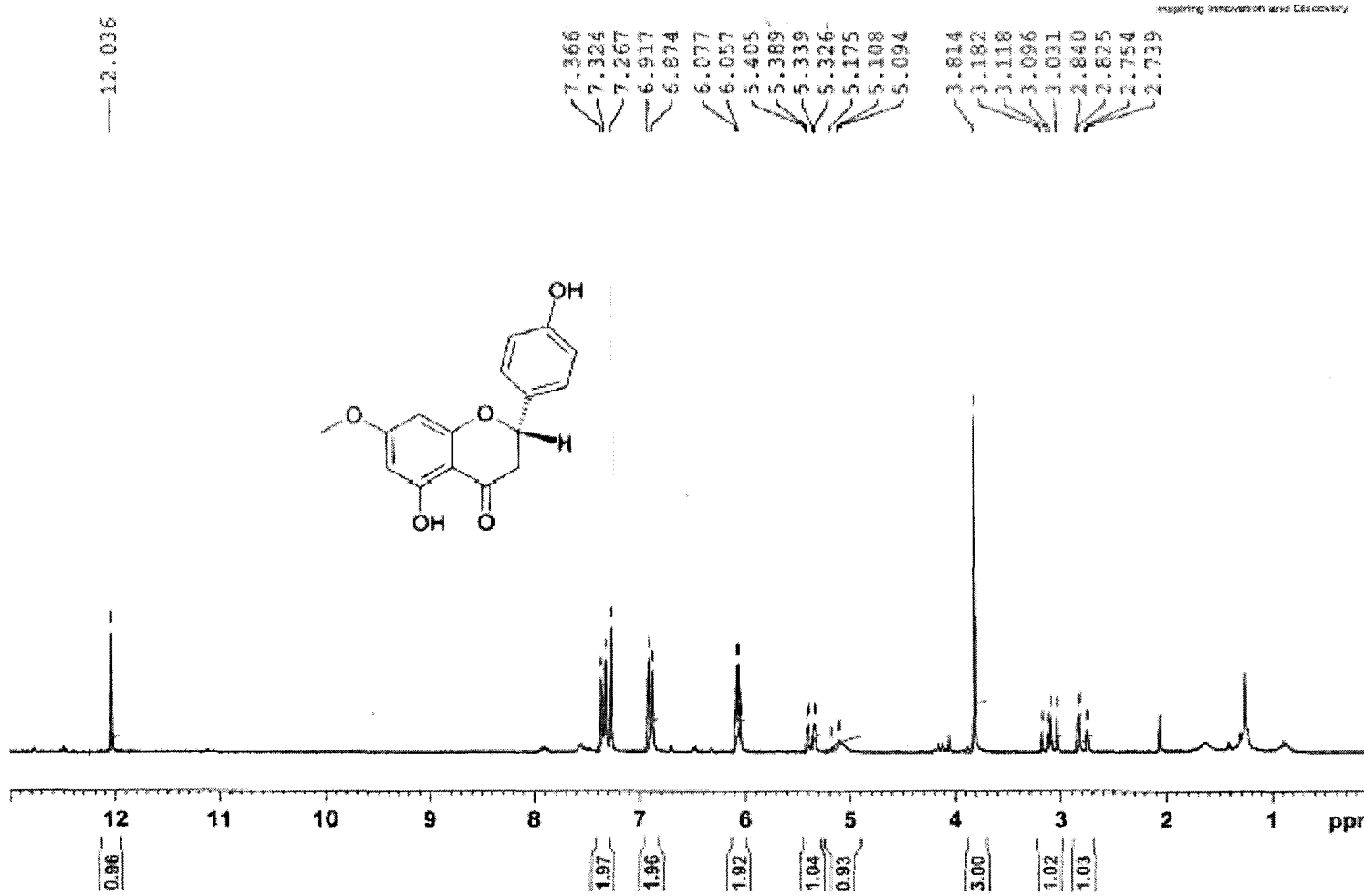
NMR 1: ^{1}H NMR for the diethyl phosphonate catalyst



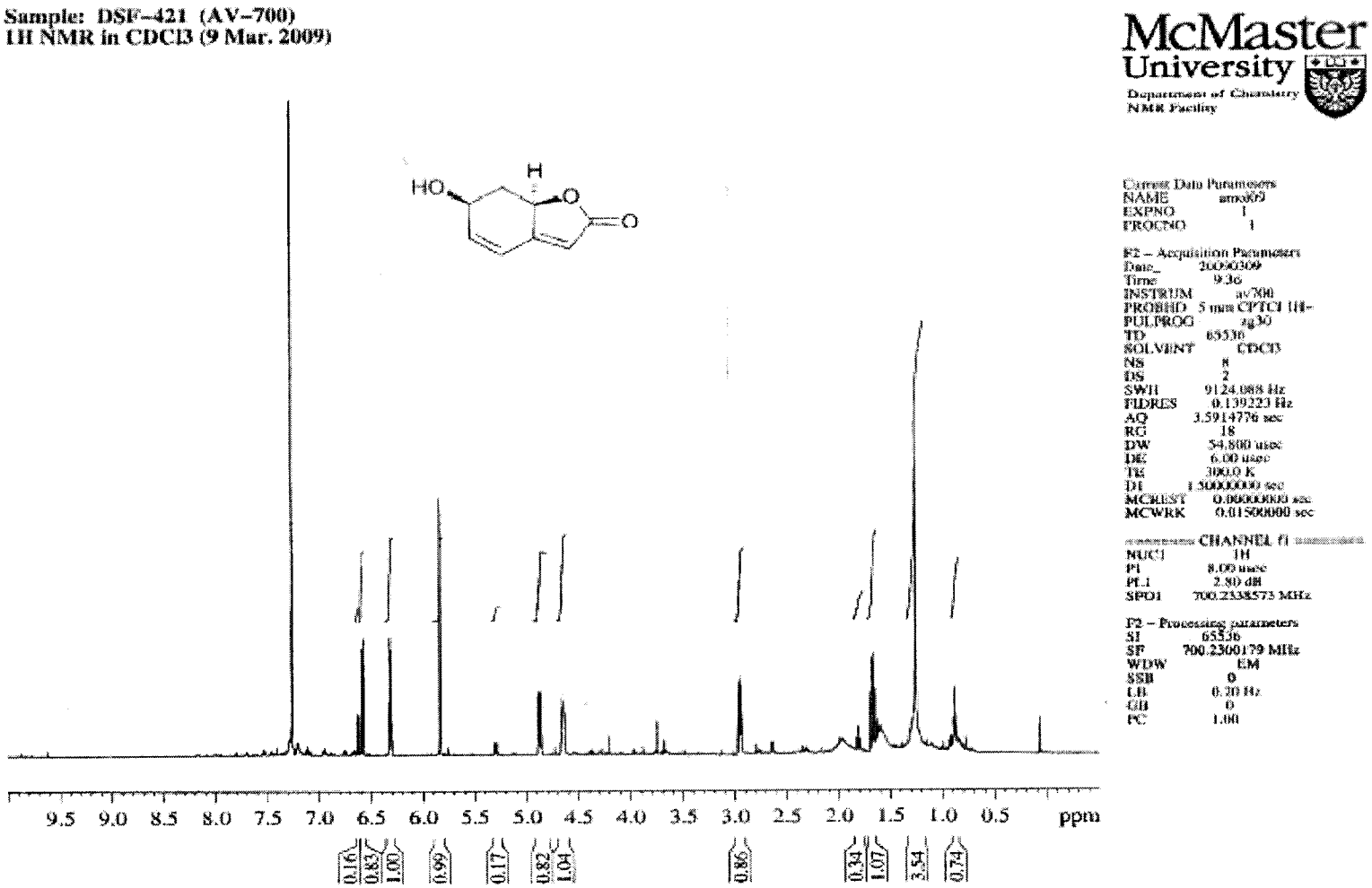
NMR 2: ^{13}C NMR for the diethyl phosphonate catalyst



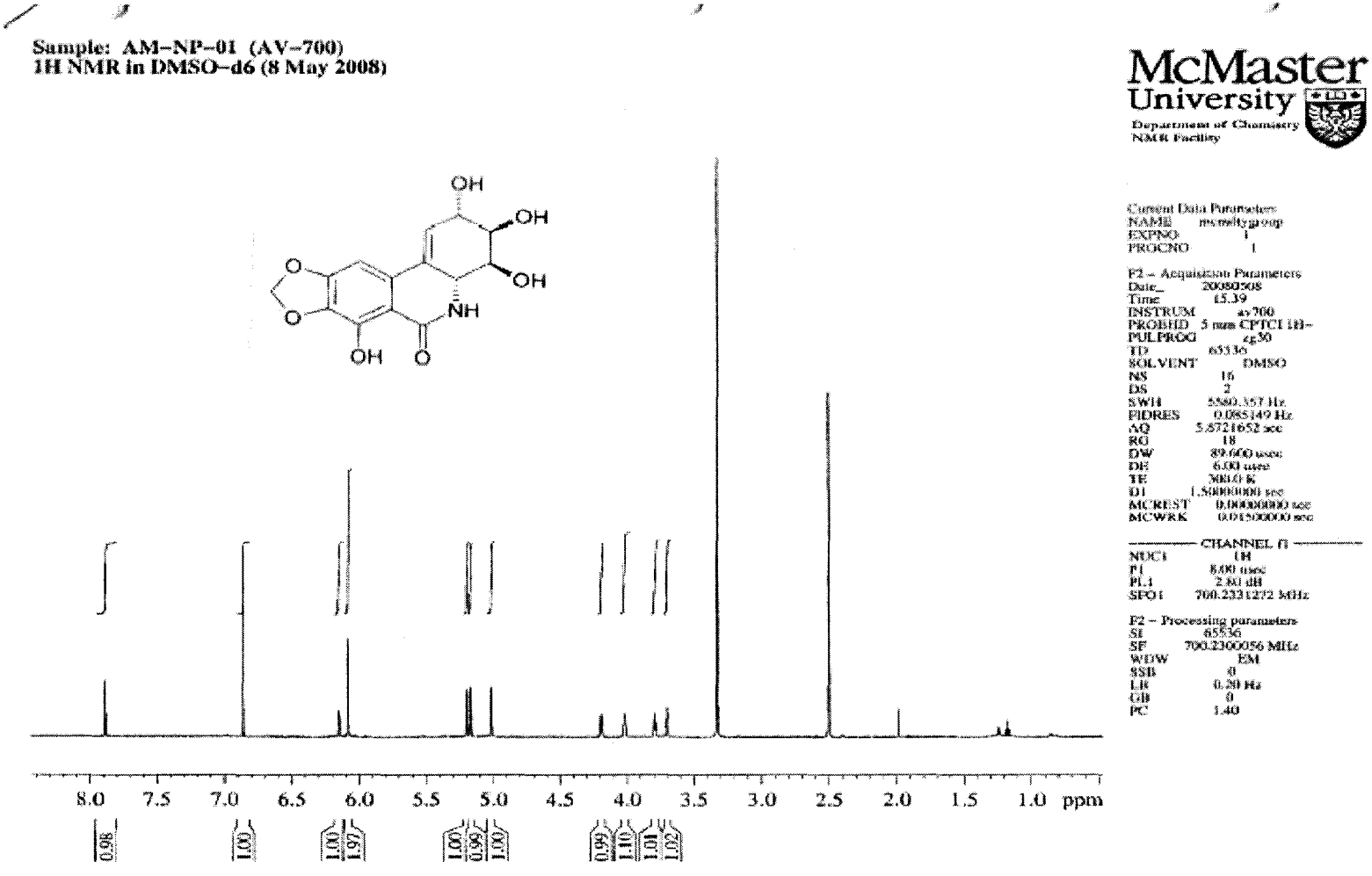
NMR 3: ^{1}H NMR for the anthraquinone derivative made by D.A. reaction



NMR 4: ^{1}H NMR for the sakuranetin



NMR 5: ^{1}H NMR for the menisdaurilide



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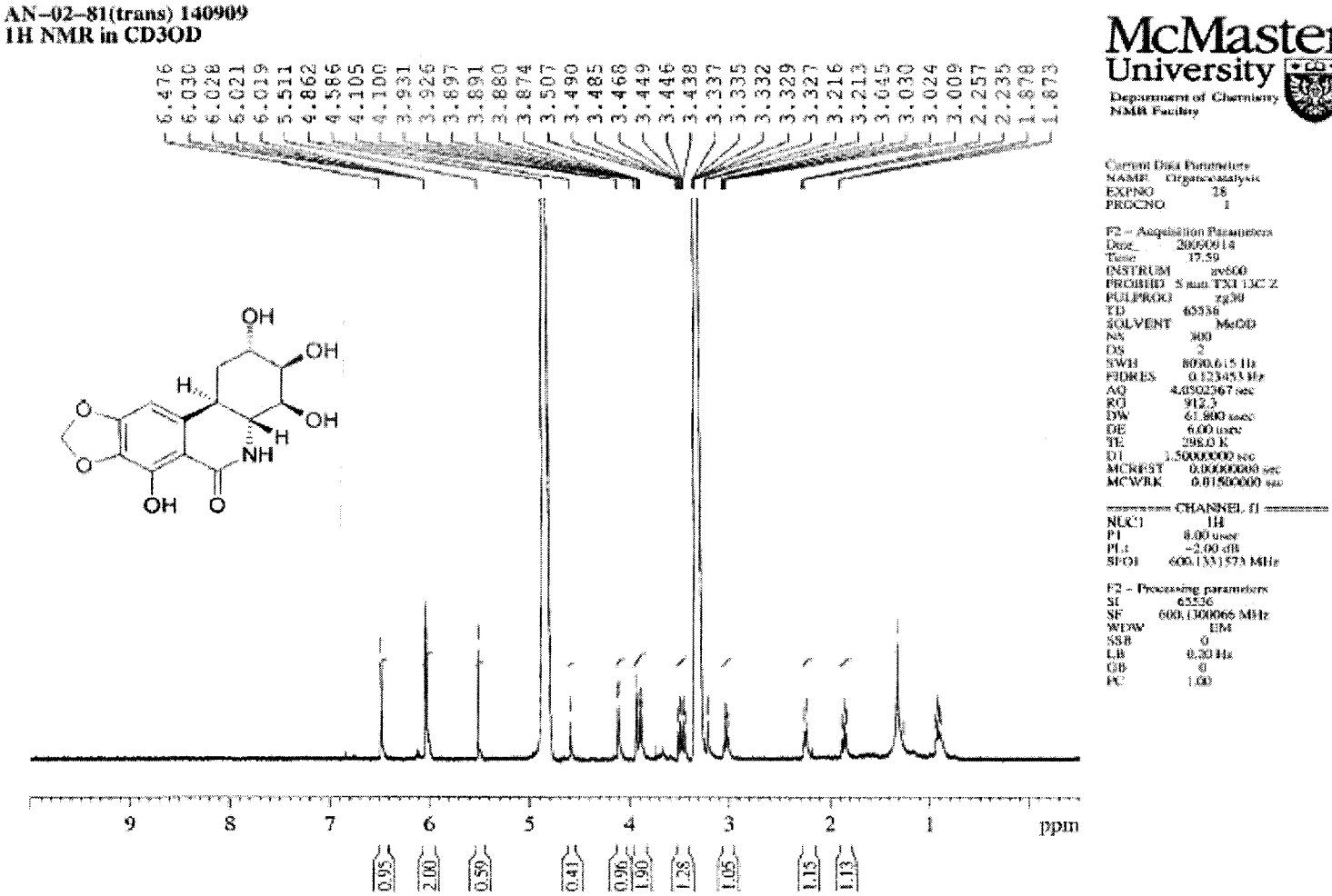
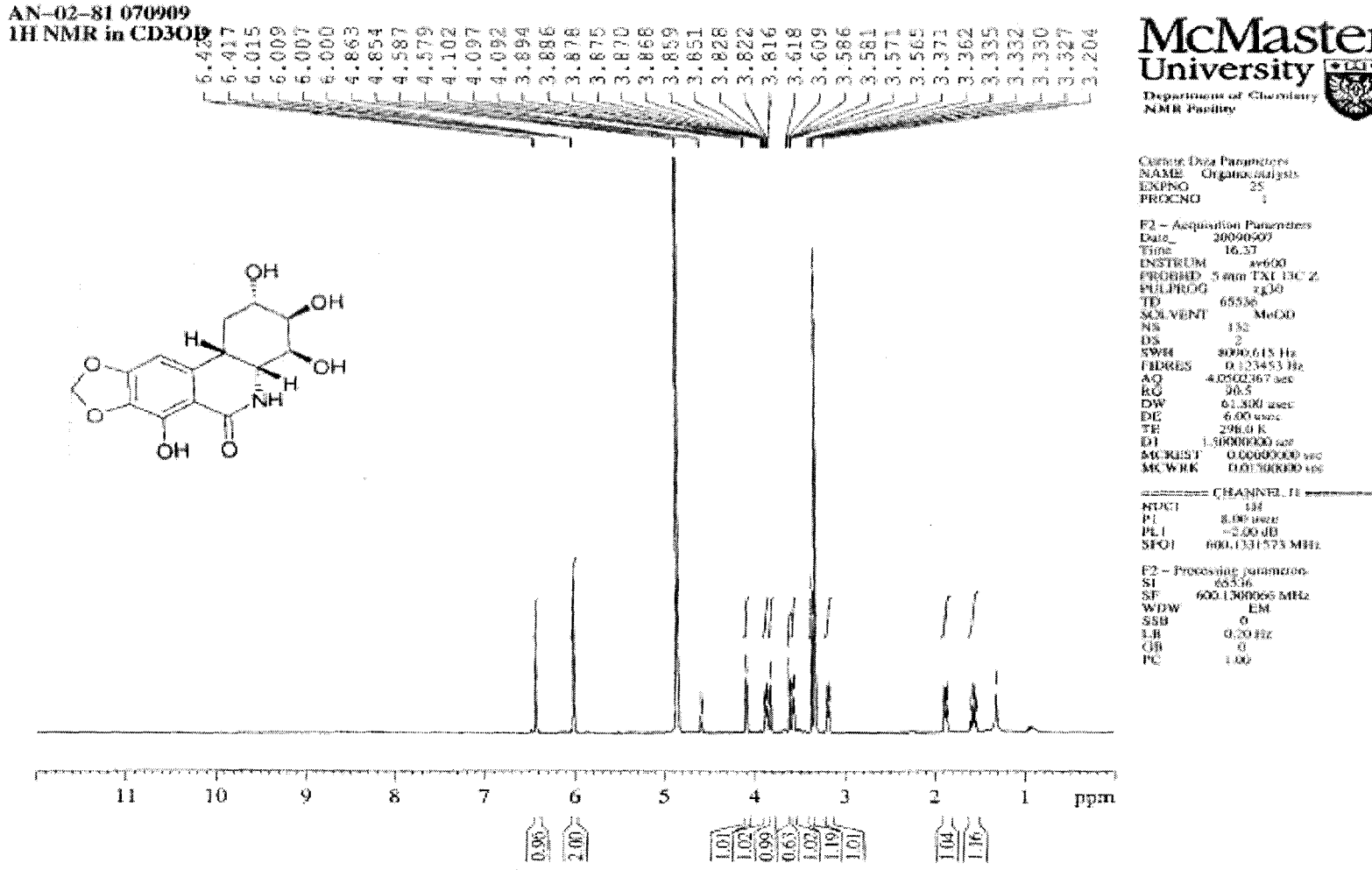


NMR 7: ^{1}H NMR for the *trans*-dihydronarciclasine



NMR 8: ^{1}H NMR for the *cis*-dihydronarciclasine