สารประกอบไอโซไนทริลชนิดใหม่ที่มีฤทธิ์ต้านมาลาเรีย

นางสาวนันทน์ นนทปัทมะดุลย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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NEW ISONITRILE ANTI-MALARIAL COMPOUNDS

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การสังเคราะห์อนุพันธ์พื้นฐานของแอกไอโซไนทริล-3 (1-ไอโซไซยาโน-2, 10-ไดเมทิล-7-(1-เมทิลเอทิล)-สไปโร [4,5] เดค-6-อีน) ซึ่งเป็นสารผลิตภัณฑ์ธรรมชาติจากทะเลในกลุ่มไอโซไนทริลสามารถสังเคราะห์จากเมนทอล โดยทำปฏิกิริยามิทซูโนบุกับสารประกอบทัลลิไมด์ซึ่งนับว่าเป็นขั้นตอนสำคัญและอนุพันธ์ดังกล่าวมีฤทธิ์ต้านเชื้อมาลาเรีย

สำหรับการสังเคราะห์อนุพันธ์ของแอกไอโซไนทริล-3 ที่มีโครงสร้างที่ซับซ้อนขึ้นนั้นใช้ปฏิกิริยาฟอร์มิลเลชันและแอลลิลเลชันของเมนโทน จากนั้นทำปฏิกิริยาการแทนที่จากการทดลองพบว่าความเกะกะของโครงสร้างมีผลต่อสภาวะที่ใช้ในการทำปฏิกิริยา

ในทำนองเดียวกันเมื่อทำปฏิกิริยาโดยใช้ 4-tert-butylcyclohexanone จากการทดลองพบว่าปฏิกิริยาแอลลิเลชัน โดยใช้แพลเลเดียมเป็นตัวเร่งปฏิกิริยาจะถูกควบคุม โดยผลทางสเตริโออิเลคโทรนิค

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....

ภาควิชา.....เคมี.....ลายมืออาจารย์ที่ปรึกษา.....

ปีการศึกษา....2543.....ลายมืออาจารย์ที่ปรึกษาร่วม.....

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Simple analogs of axisonitrile-3 (1-isocyano-2,10-dimethyl-7-(1-methylethyl)-spiro[4,5]dec-6-ene), a marine isonitrile natural product, were prepared from menthol employing a Mitsunobu reaction with phthalimide as the key step. The analogs showed moderate activity.

The synthesis of more complex analogs was undertaken by formylation and allylation of menthone, followed by a series of reductions and substitution protocols. The effect of steric hindrance of the system on the reaction condition was noted.

Parallel chemistry using 4-*tert*-butyl cyclohexanone was carried out. It was shown that palladium catalyzed allylation is under stereoelectronic control.

Department……**……**……**..**Student’s signature**……**

Field of study **……**Advisor's signature**……**

Academic year……**……**Co-Advisor's signature**……**

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List of Abbreviations

Br	Broad	IR	Infrared
CDI	Carbonyl diimidazole	m	Multiplet (NMR)
D	Doublet (NMR)	m	Medium (IR)
Dd	Double doublet (NMR)	m.p.	Melting point
Dba	Dibenzylidene acetone	MS	Mass spectrometry
DEAD	Diethyl azodicarboxylate	*m/z*	mass per charge
DMSO	Dimethylsulfoxide	NMR	nuclear magnetic resonance
Dt	Double triplet (NMR)	ppm	part per million
HRMS	High Resolution Mass	q	quartet (NMR)
	Spectrometry	s	strong (IR)
Hz	Hertz	s	singlet (NMR)
IC_{50}	Concentration that causes 50%	t	triplet (NMR)
	Inhibition	w	weak (IR)

CHAPTER I

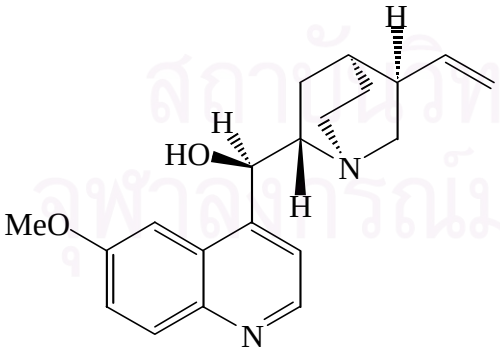
INTRODUCTION

Malaria is responsible for the deaths of about 2 million people each year. According to the World Health Organization (WHO), it is estimated that about 40% of the world's population is at risk from this disease.[1]

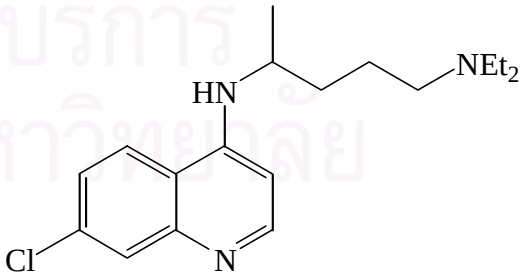
Malaria is an infectious disease carried by the mosquito, which affects the tropical and subtropical regions. Most climate models suggest that world temperatures will increase in the future, putting more people at risk.[2]

The parasites that cause malaria are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. *Plasmodium falciparum* is the most dangerous.

Quinine (**1**) was the first drug used against malaria. It is a natural product isolated from the bark of the South American cincona tree. Use of the powdered bark of the tree was the first reported by Jesuits in the sixteenth century. Pure quinine was isolated in 1820. Although quinine is now of little use against malaria, it is still produced as a flavouring agent for tonic water.[3] Analogs of quinine such as chloroquine (**2**) and mefloquine (**3**), have been synthesised but now some strains of parasites are resistant to these analogs.



(**1**)



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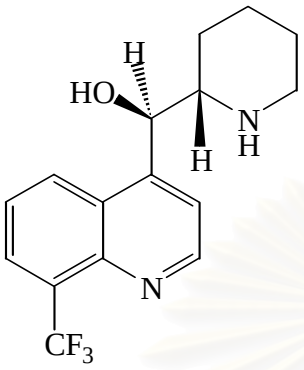
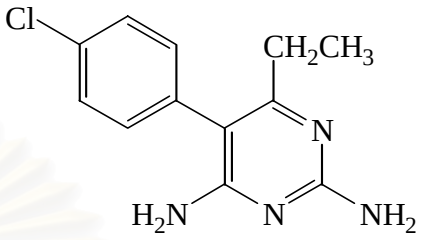


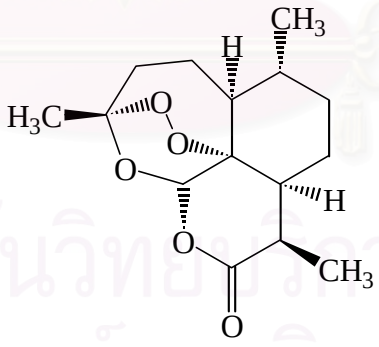
(**3**)



(**4**)

Pyrimethamine (**4**) is a member of a group of drugs known as antifolates. Pyrimethamine has unpleasant side effects but it is useful in treating resistant *P. falciparum* strains.

Artemisinin (**5**) has good anti-malarial activity and it is a promising drug. It is still undergoing trials. Artemisinin is isolated from the Chinese plant *Artemisia annua* and contains a cyclic peroxide that is responsible for its activity.[4, 5]



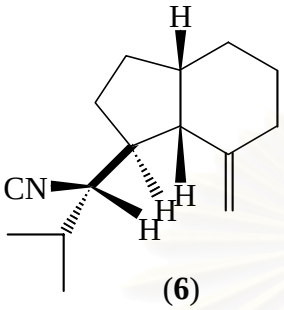
(**5**)

Research for new anti-malarial drugs, especially with new modes of action, is necessary. It is a research area largely neglected by the pharmaceutical industry.

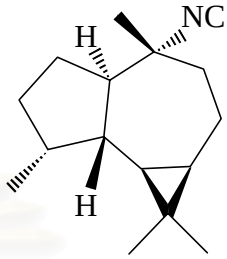
Many substances, often with unusual structures, have been isolated from sponges. Many have shown potent biological activity, such as bryostatin, an NCI priority anti-tumor compound.

Axisonitrile-1 (**6**) was isolated from the sponge *Axinella cannabina* in the Bay of Taranto, Italy in 1973. The absolute configuration was determined by X-ray analysis of a derivative.[6,7]

The total syntheses of axisonitrile-1 and axamide-1 have been described.[8, 9]



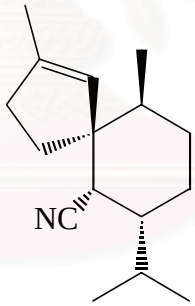
(**6**)



(**7**)

Axisonitrile-2 (**7**) was isolated from this sponge in the same place. The structure and stereochemistry were determined by NMR experiments and by chemical interconversion.[10, 11,12]

Axisonitrile-3 (**8**) was later isolated from this same sponge.[13]



(**8**)

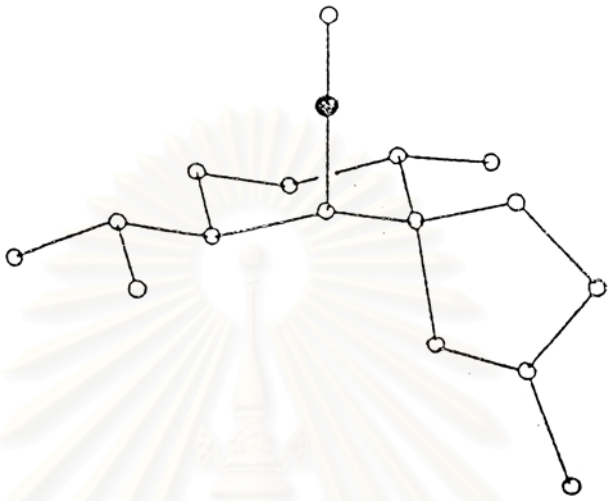
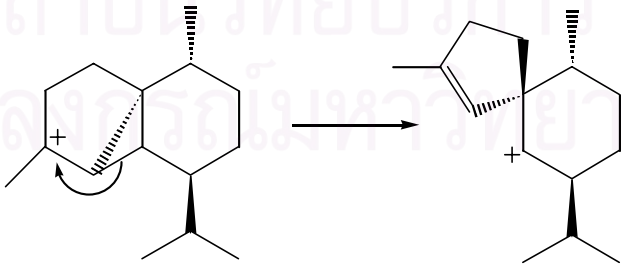


Figure 1 X-Ray structure of Axisonitrile-3

While spectroscopic techniques revealed some features of the structure, such as the isonitrile group, complete structure determination required X-Ray crystallography. (**Figure 1**) This showed that axisonitrile-3 possesses a cyclohexane in a chair conformation, spiro-fused to a cyclopentene ring. This new structure type was termed a "spiroaxane" and it was postulated that its biosynthesis was from the previously proposed carbonium ion (**9**).[13]



(**9**)

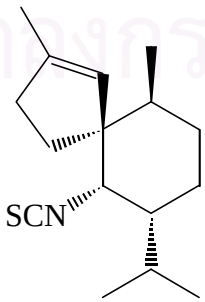
Further biosynthetic studies have been hindered by the shortage of the natural material and by the difficulty of manipulating the hindered nitrogen atom for the introduction of ^{13}C labels.[14]

Sica 's determination of the structure left one question unanswered: the absolute stereochemistry. This was later determined by total synthesis and found to be opposite to that depicted by Sica.[15] Surprisingly, all of the subsequent natural product papers continued to use Sica 's structure.

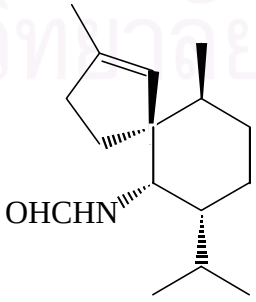
Subsequently, Axisonitrile-3 was isolated from a marine sponge of Topsentia sp. (order Halichondrida) in Thailand,[16] sponge *Acanthela kletha* in Pelorus Island, Queensland, Australia,[17] an unidentified sponge in Pohnpei,[18] sponge *Acanthella cavernosa* in Heron Island, at the southern end of the Great Barrier Reef[14] and nudibranches of the family Phyllididae, *Phylilida ocelata*, *Phylilida varicosa* and *Phyllidiopsis krempfi* in Koshiki-jima Island and *P. pustulosa* in Yakushima Island, Japan.[19]

Various compounds related to Axisonitrile-3 have also been isolated, including the thiocyanate, the formamide and 10-epi-axisonitrile-3.

Axisothiocyanate-3 (**10**) was isolated from the sponge *Axinella cannabina* in Taranto, Italy, as were Axisonitrile-1, Axisonitrile-2, Axisonitrile-3 and Axamide-3 (**11**).[13] Axisothiocyanate-3 was also isolated from other sponges; *Acanthella klethra* in Pelous, Island, Queensland,[17] *Acanthella sp*. and *Cadlina luteomarginata* in Conehead Point Renell, Grahan Island, British Columbia[20] and *Acanthela cavernosa* in Heron Island, Great Barrier Reef.[14] Axisonitrile-3 could be converted to Axisothiocyanate-3 by treatment with sulfur. Axamide-3 could also be produced by hydration of Axisonitrile-3 with aqueous acetic acid.[13]

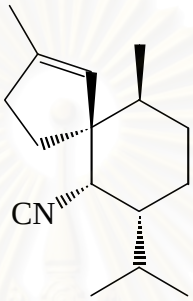


(**10**)



(**11**)

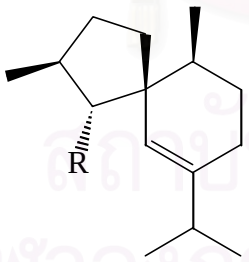
10-*epi*-Axisonitrile-3 (**12**) was isolated from nudibranches of the family *Phyllididae*, *Phylilida ocelata*, *Phylilida varicosa* and *Phyllidiopsis krempfi* in Koshiki-jima Island and *P. pustulosa* in Yakushima Island, Japan. The planar structure of 10-epi-axisonitrile-3 was determined by COSY, HMQC and HMBC and compared to axisonitrile-3.[19]



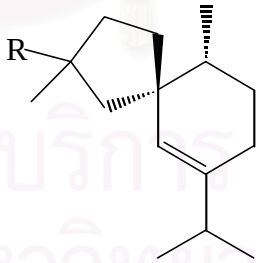
(**12**)

A wide range of other isonitriles has also been isolated from marine sources, some of them share the spiroaxane skeleton, but differ from axisonitrile-3, particularly in the location of the isonitrile group.

In compounds (**13**)[21] and (**14**)[18] the position of the alkene and the heteroatom substituents are different to Axisonitrile-3. They were isolated from *Acanthella acuta* in the Bay of Napoli, Italy.



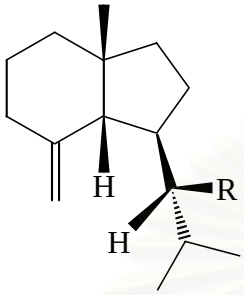
(**13**) R = NC
(**14**) R = NCS



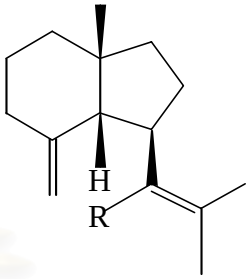
(**15**) R = NCS
(**16**) R = NHCHO

Two closely related natural products (**14**) and (**15**) had also exchanged the alkene and isonitrile group positions. They were isolated from a specimen of *Axinyssa aplysinoides* in Palau.[18]

Various other bicyclic ring systems with isonitrile and related groups have also been found, including decalins and perhydroazulenes. They are also believed to be biosynthesized from carbonium ion (**9**).



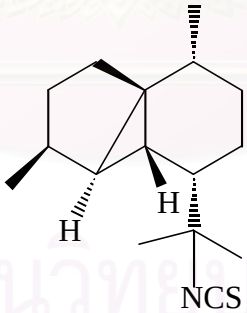
(**17**) R = NCS
(**18**) R = NHCHO



(**19**) R = NC
(**20**) R = NCS
(**21**) R = NHCHO

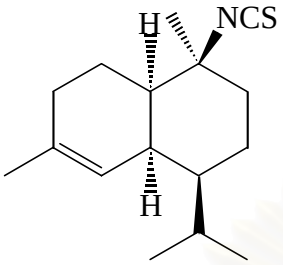
Axisothiocyanate-1 (**17**), axamide-1 (**18**), axisonitrile-4 (**19**), axisothiocyanate-4 (**20**) and axamide-4 (**21**) were isolated from the sponge *Axinella cannabina*.[7]

Isothiocyanate (**22**) was isolated from *Axinyssa aplysiniodes* at Pohnpei. It appears to have the same carbon skeleton as carbonium ion (**9**).[18]

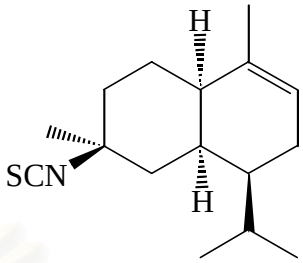


(**22**)

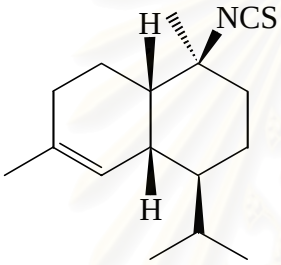
Isothiocyanates (**23**) and (**24**) which are structurally related have been isolated from the Fijian sponge *Axinyssa ferestratus.*[16]



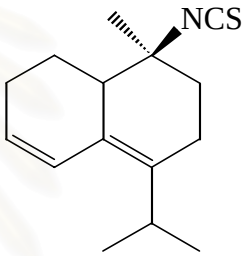
(**23**)



(**27**)

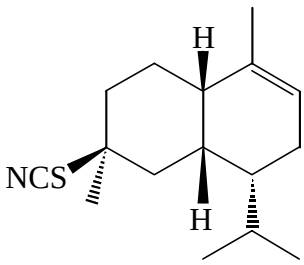


(**24**) R = NCS
(**25**) R = NC



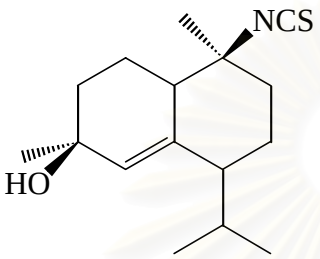
(**26**)

Compounds (**25**) and (**26**) were isolated from *Phyllidia pustulosa* from Koshiki-jima. Compound (**26**) was isolated from *Phylidia ocelate* at Kamikoshiki-jima, specimen of *Phillidiosis krempfi* from Shimokoshiki-jima.[19] Compound (**27**) is a dehydrogenated form of compound (**23**). Compound (**24**) is related to compound (**23**) by exchange of the alkene and isothiocyanate positions. Compound (**24**) is also similar to compound (**28**). The difference is that one is an isothiocyanate and the other is a thiocyanate. Compounds (**24**) and (**28**) were both isolated from *Axinyssa fenestratus*.[18] Compound (**28**) was also isolated from *Axinyssa aplyspoids*.[16] The absolute stereochemistry of (**24**) and (**28**) have not been determined.

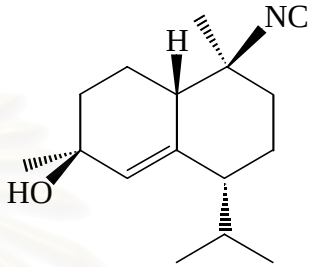


(**28**)

Compound (**29**) was isolated from *Axinyssa fenestratus*.[16] The isonitrile analog (**30**) was isolated from nudibranch *Phyllidia pustulosa* at Katsuura on the Kii Peninsula in western Japan.[22] They are the hydroxylated versions of compounds (**25**) and (**26**). Compound (**30**) is identical to compound (**29**) except that it has an isonitrile in place of the thiocyanate.

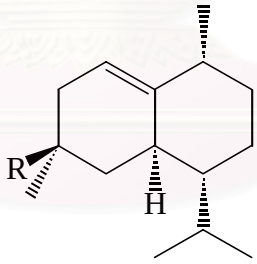


(**29**)



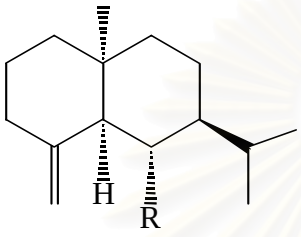
(**30**)

Once again, the isonitrile (**31**), isothiocyanate (**32**) and formamide (**33**) were obtained. Isonitrile (**31**) and formamide (**33**) were isolated from sponge *Axinyssa aplysinoids* at Palau while isothiocyanate (**32**) was isolated from specimen of *Halichondria panicea* from Okinawa.[23]

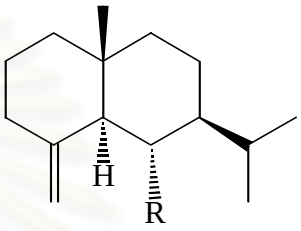


(**31**) R = NC
(**32**) R = NCS
(**33**) R = NHCHO

Isonitrile (**34**), isothiocyanate (**35**) and formamide (**36**) were isolated from sponge *Acanthela acuta* and sponge *Axinella cannabina* in the bay of Napoli, Italy.[24] Acanthene B (**37**) was isolated from *Acanthella sp* at Queen Charlotte Island.[20] The dorid nudibranch *Cadlina luteomarginta* contained a new formamide, Acanthene C (**38**).[20] Isothiocyanate (**35**) is diastereoisomeric with Acanthene B (**37**), differing in the ring junction stereochemistry.

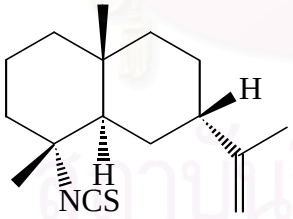


(**34**) R = NC
(**35**) R = NCS
(**36**) R = NHCHO

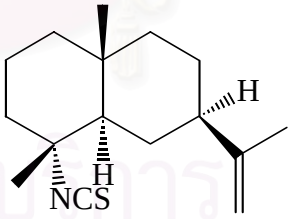


(**37**) R = NCS
(**38**) R = NHCHO

New isothiocyanates (**39**) and (**40**) were isolated from *Acanthela klethra* (Queensland, Australia).[17] Compound (**39**) was also isolated from *Acanthella sp* at Queen Charlotte Island and dorid nudibrance *Cadlina meomarginatu.*[20] Compound (**39**) is a diastereoisomer of (**40**) differing in the stereochemistry of the isopropenyl group.

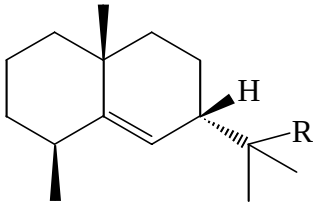


(**39**)



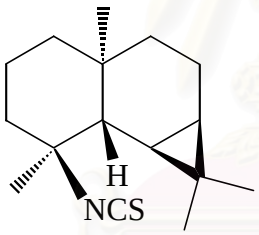
(**40**)

Compounds (**41**), (**42**) and (**43**) were isolated from the sponge *Axinella cannabina*.[12] Compounds (**41**) and (**42**) were also isolated from the sponge *Acanthella sp.* at Queen Charlotte Island and from dorid nudibranch *Cadlina meomarginata*.[20] Compound (**42**) was also isolated from *Acanthella klethra*[17]from Queensland and *Acanthella carvernosa* from Heron Island.[14] The structural relationship of compounds (**41**)-(**43**) to compounds (**34**)-(**38**) is striking.

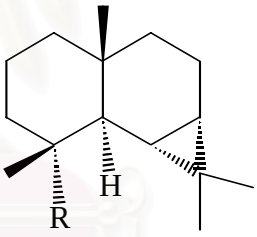


(**41**) R = NC
(**42**) R = NCS
(**43**) R = NHCHO

(-) Epipolasin-A (**44**) was isolated from a sponge of the *Axinyssa sp*. and the nudibranch predator *P. pustulosa* at One Tree Island on the Great Barrier Reef[25] and from the sponge *Acanthella cavernosa* from Heron Island.[14] Compounds (**45**), (**46**) and (**47**) were isolated from the sponge *Acanthella sp* at Queen Charlotte Island and compounds (**45**) and (**47**) were isolated from a dorid nudibranch *Cadlina meomarginata* at the same place.[20,26] The absolute stereochemistry of (**44**) - (**47**) have not been determined.

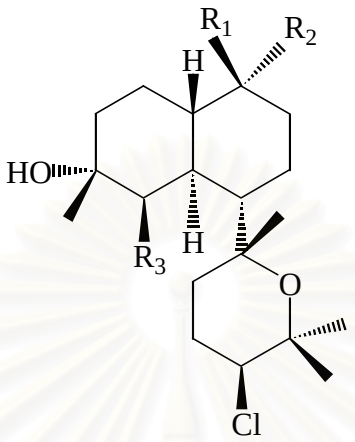


(**44**)



(**45**) R = NC
(**46**) R = NCS
(**47**) R = NHCHO

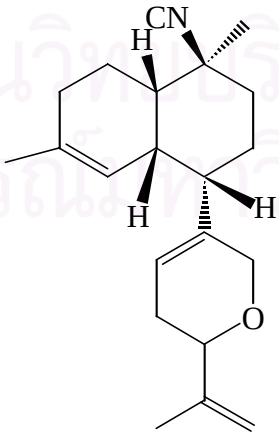
Compounds (**48**), (**49**), (**50**) and (**51**) which possess a tetrahydropyran ring in addition to the decalin were obtained from a Thailand collection of *Acanthella cavernosa* and other species.[16, 27, 28]



	R_1	R_2	R_3
(**48**)	Me	NCS	NC
(**49**)	Me	NCS	NHCHO
(**50**)	$=CH_2$		NC
(**51**)	Me	NCS	NCS

Once again the isonitrile, isothiocyanate and formamide compounds were found. The presence of the halogen atom is not uncommon in marine natural products,[29] but quite rare in terrestrial natural products.

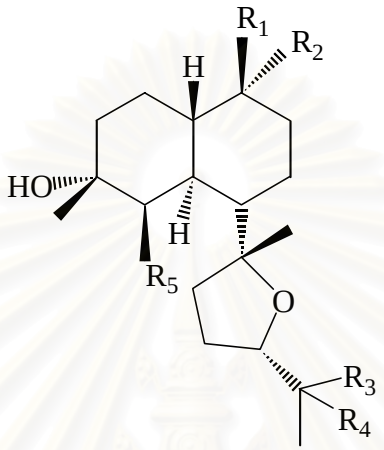
Kalihipyran (**52**) was isolated from *Acanthella cavernosa* at Beau Vallon Beach Mahe′, Seychelles.[30] It is a member of the same structural group. The relative stereochemistry of the isopropenyl group has not been determined.



(**52**)

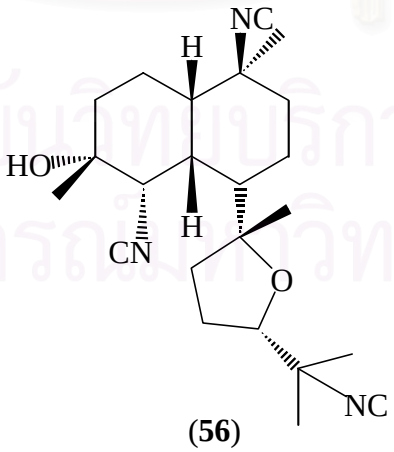
A number of related compounds having a tetrahydrofuran ring have also been isolated.

Kalihinol B (**53**) was isolated from the sponge *Phakellia pulcherrima* in the Philippines[27] and sponge *Acanthella sp*. at Guam which contained kalihinol C (**54**) and D (**55**).[, 28, 30, 31]



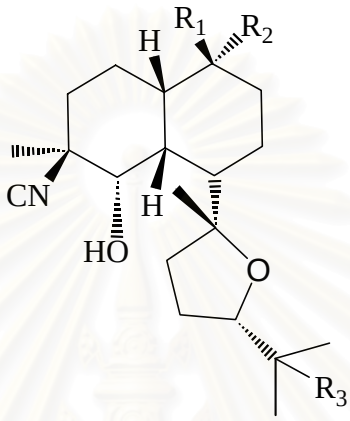
	R_1	R_2	R_3	R_4	R_5
(**53**)	NC	Me	Me	Cl	NC
(**54**)	NC	Me	Me	NCS	NC
(**55**)	NC	Me	Me	NC	NC

The diterpenoid trisisonitrile kalhinol F (**56**) was isolated from sponge *Acanthella sp.* at Guam.[30]



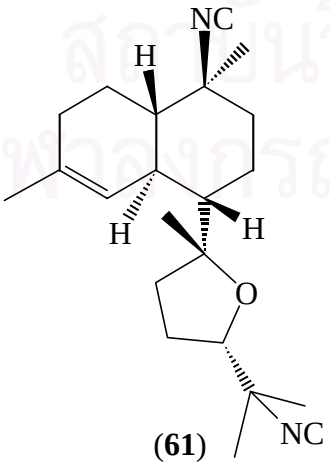
(**56**)

Compounds (**57**), (**58**), (**59**) and (**60**) were isolated from sponge *Acanthela carvernosa* at Beau Vallon Beach in the Seychelles.[30] Although they have the same structure, they possess a range of heteroatom substituents. Their principle difference, relative to Kalhinol F, is the exchange of position of the left hand cyclohexane heterosubstituents.

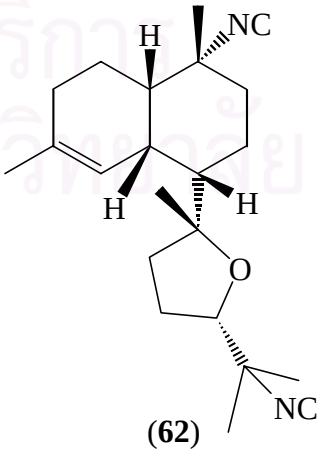


	R_1	R_2	R_3
(**57**)	CH_3	NC	NC
(**58**)	CH_3	NCS	NC
(**59**)	NC	CH_3	Cl
(**60**)	NC	CH_3	CN

Compounds (**61**) and (**62**) was isolated from *Acanthella cavernosa* at Beau Vallon Beach.[30] Kalihinene (**62**) was isolated from *Phakella pulcherryma* in the Philippines.[27] 6-

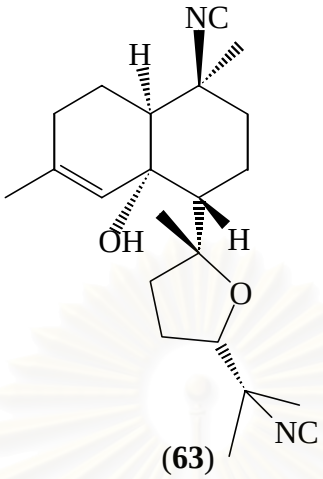


(**61**)



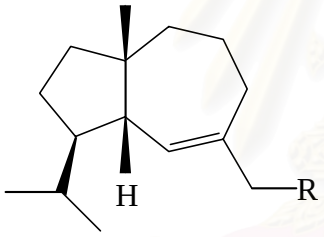
(**62**)

Hydroxykallihinene (**63**) was isolated from *Acanthella sp*. at Okinawa, Japan.[31]

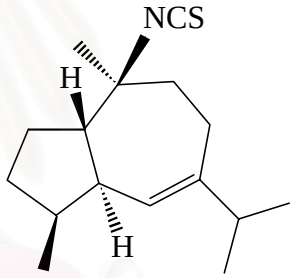


(**63**)

Examples with a guaiane skeleton rather than a decalin or a spiroaxane have also been found.

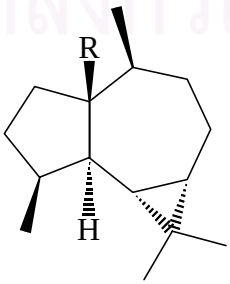


(**64**) R = NC
(**65**) R = NCS

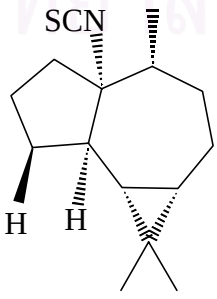


(**66**)

Compounds (**64**), (**65**), (**67**) and (**68**) were isolated from the sponge *Acanthella acuta* from the Bay of Napoli, Italy.[21] Sesquiterpenes (**66**) and (**69**) were isolated from Palauan specimen of *Axinyssa aplysinoid.*[18] Compound (**68**) is diastereoisomer of compound (**69**).

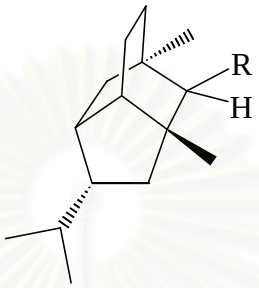


(**67**) R = NC
(**68**) R = NCS



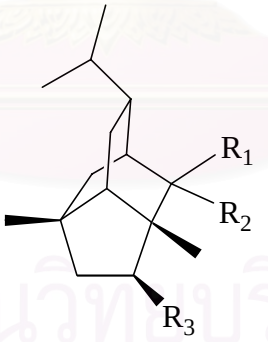
(**69**)

More complex polycyclic isonitriles and isothiocyanates have also been isolated, 2-isocyanopupukeanane (**70**) and 2-thiocyanatopupukeanne (**71**) were isolated from *Axinyssa aplysinoides* at Palau.[18]



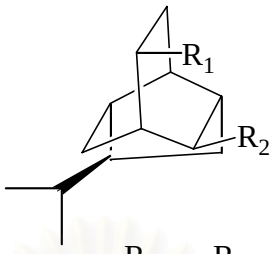
(**70**) R = CN
(**71**) R = SCN

2-Thiocyanateopupukaenane (**72**) was isolated from an unidentified sponge from Pohnpei, 4-thiocyanatoneopupukaenane (**73**) was isolated from *Phycopsis terpnis* at Okinawa and 2-thiocyanatoneopupukaenane (**74**) was isolated from specimen of *Axinyssa aplysinoides* at Pohnpei.[18]



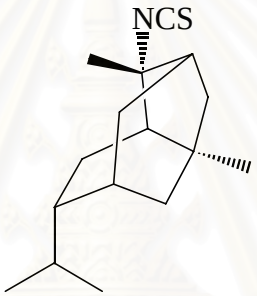
	R_1	R_2	R_3
(**72**)	SCN	H	H
(**73**)	H	H	SCN
(**74**)	H	SCN	H

Compounds (**75**) and (**76**) were isolated from sponge *Hymeniacidon sp.*[26]



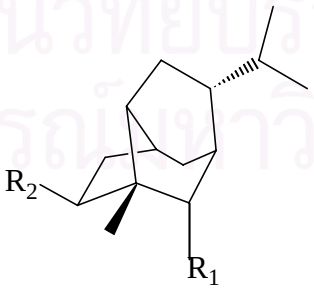
	R_1	R_2
(**75**)	NC	H
(**76**)	NC	H

Compound (**77**) was isolated from *Axinyssa aplysiniode*.[18]



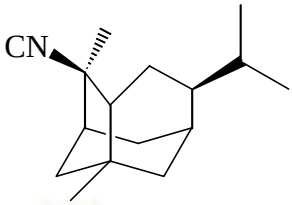
(**77**)

2-Thiocyanatoneopupukeanane (**78**) and 4-thiocyanatoneopupukeanane (**79**) were isolated from nudibranchs *Phyllidia pustulosa* at Koshiki-jima Islands,[19] from sponge *Phycopsis ternis* at Okinawa and from an unidentified sponge from Pohnpei.[32]



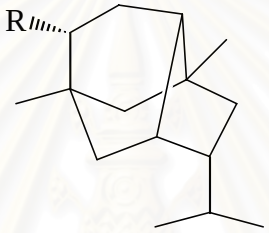
	R_1	R_2
(**78**)	SCN	H
(**79**)	H	SCN

The new sesquiterpene isocyanide 2-isocyanotrachyopsane (**80**) was isolated from Family *Phllidiidae* and *Trachyopsis aplysinoides*.[19]



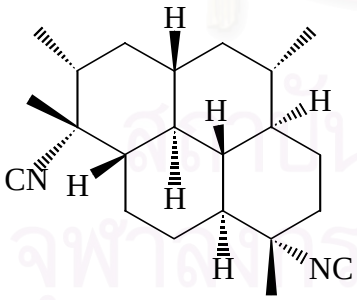
(**80**)

(-)-9-Isocyanopupukeanane (**81**) and isothiocyanatopupukeanane (**82**) were isolated from *Axinyssa sp*.[25]

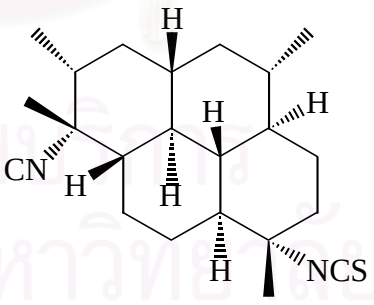


(**81**) R = NC
(**82**) R = NCS

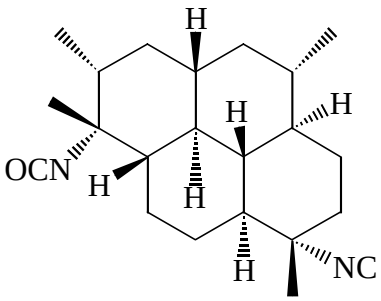
Compound (**83**), (**84**), (**85**), and (**86**) were isolated from *Cymbastela hooperi*.[33]These molecules contain a network of cyclohexanes in *trans* fused relationships with various substituents.



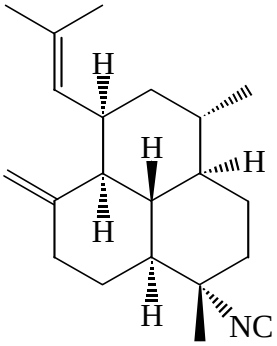
(**83**)



(**84**)



(**85**)



(**86**)

Wright *et al* have reported anti-malarial activity for a number of marine isonitriles.[33] A number of conclusions can be drawn from this limited data. Firstly compounds possessing an isocyanate, isothiocyanate or formamide in place of the isonitrile are significantly less active. Steric hindrance around the isonitrile does not seem to be very significant as both secondary and tertiary compounds show good activity.

Axisonitrile-3 has a special position in the group. While it is not the most active, it is still a good candidate and it is structurally the simplest. This makes it amenable to synthesis. Importantly no cytotoxicity was found for this compound.

Activity for axisonitrile-3 (**8**) has been reported. Axisonitrile-3, (**83**), (**84**), (**85**) and (**86**) have antimalarial activity against cultured *Plasmodium falciparum* (chloroquine-sensitive D6 and chloroquine resistant W2 strains) (**Table 1**).

Table 1 Evaluation of the cytotoxic and Antimalarial Activity of Selected Marine Isonitriles and Current Antimalarial Drugs.

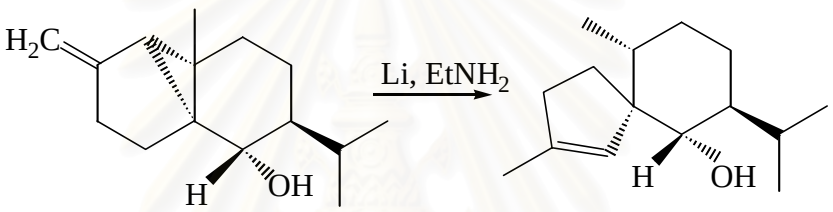
Compound	**KB Cell**	***Plasmodium falciparum* strain**	
		D6	**W2**
	IC_{50} ng/ml	**IC_{50}** ng/ml	**IC_{50}** ng/ml
Chloroquine[34]	17,400	1.95	22.8
Quinine[34]	>20,000	10.2	22.3
Mefloquine[34]	3,500	8.2	0.49
Arthemisinin[34]	>20,000	4.1	0.71
Axisonitrile-3[34]	>20,000	142	16.5
83[33]	4,700	4.7	4.3
84[33]	1,600	45.1	28.5
85[33]	4,300	3.2	2.5
86[33]	3,200	14.1	9.3

Axisonitrile-3 has been evaluated as an antimicrobial against *N. brasiliensis* with modest activity, *Staphylococcus aureus*, *Candida albicans* and trichophyton mentagroghytes with medium activity.[16] Axisonitrile-3 and 10-epi-axisonitrile-3 inhibited settlement and metamorphosis of cyprid larvae of *Balanus amphitrile* by 10-epi-axisonitrile-3 had antifouling activity at about 10 μg/mL, while axisonitrile-3 was active at about 3.2 μg/mL.[19] Axisonitrile-3 has been evaluated against *Bacillus subtilis* and *Candida albicans* and was active at 2 mg/mL.[14]

Why do the sponges contain these compounds? As sponges have no physical or biochemical defence, it would seem likely that they are part of a chemical defense system. Some observations have been made with nudibranches that appear to support this idea. Nudibrances are sponge predators that often contain the same natural products as the sponges. It is highly likely that the nudibranches acquire the compounds during feeding and do not biosynthesize them. Faulkner *et al* found that the nudibranch *Cadlina*

luteomarginata concentrated the compounds in the dosum, rather than the guts or feet; a clear indication of a defensive role. They further found that many of the compounds were icthyotoxic at 100 μg/mg in fish food pellets, while at a lower dose (10 μg/mg) they acted as antifeedants.[26]

The antifouling activity reported indicates that they may have a wider defensive role too, perhaps protecting the sponges against a range of parasites. Caine and Deutsch achieved a total synthesis of axisonitrile-3 which showed that the absolute configuration of the natural product is opposite to that shown in the literature. Axisonitrile-3 was synthesised from (+) dihydrocarvone. A key step was the conversion of the tricyclodecanone (**87**) into the spiroaxane (**88**).[15]



(**87**) (**88**)

The marine isonitriles display a wide diversity of structures. The various forms of biological activity that have been found indicate that they need further study as drug candidates. The low natural abundance and the possibility of analog synthesis means that much of this must be through synthetic chemistry.

Axisonitrile-3, as a structurally simple member of the group, is, therefore, a suitable starting point.

CHAPTER II

EXPERIMENTAL

General NMR spectra were obtained in $CDCl_3$ at 200 MHz (^1H) or 50 MHz (^{13}C). Chemical Shift (δ) are in ppm and coupling constants (J) are in Hz. All reactions were followed by Thin layer Chromatography (TLC): glass or plastic sheets coated with silica gel F_{254} (Merck) and visualized using uv light (254 nm), iodine, molybdate, $KMnO_4$ or $Co(SCN)_2$. Flash chromatography was carried out on silica gel: 230-400 Mesh. Evaporation refers to the rotary evaporation of solvent under aspirator pressure. Mass Spectrometry and High Resolution Mass Spectrometry were determine at the Chulabhorn Research Institute, using a GCQ Mass Spectrometer from Finigan and a Mat 90 from Finigan. Elemental analysis were determined at the Instrument Centre of Chulalongkorn University. Anti-malarial activity was determined at NSTDA according to the method of Desjardins, R. G., et al, *Antimicrob Agents Chemother*, **1979**, *16*, 710.

(1R, 2R, 5S)-1-phthalimido-2-isopropyl-5-methylcyclohexane (90) A solution of DEAD (2.42 ml, 15.36 mmole) in THF (20 ml) was added dropwise to a solution of (1S, 2R, 5S) menthol (2 g, 12.82 mmole), phthalimide (2.26 g, 15.36 mmole) and triphenylphosphine (3.69 g, 14.07 mmole) in THF (20 ml) at room temperature with a water bath under N_2. The orange colour faded to yellow and the mixture was stirred overnight. The THF was removed *in vacuo*, and the solid residue was taken up in ethyl acetate and filtered. Silica gel was added and the ethyl acetate was evaporated. The residue was applied to the top of a silica gel column (60 g) and it was eluted by flash chromatography with 5% EtOAc:Hexane to give the product as a white solid (2.37 g, 65 %). ^1H NMR δ 0.70-1.05 (10H, m, CH_3, CH), 1.20-1.40 (3H, m, CH_3), 1.65-2.05 (4H, m, CH), 2.05-2.30 (1H, m, CH), 4.75 (1H, m, H1), 7.55-7.65 (2H, m, Ar), 7.70-7.80 (2H, m, Ar); ^{13}C NMR δ 20.9, 21.5, 22.6, 26.0, 26.7, 29.8, 34.9, 41.3, 45.3, 49.4, 122.9, 131.9, 133.8, 169.6; IR (ν, cm^{-1}) 3455, 3064, 2921, 2860, 1776, 1714(s), 1608, 1472, 1398, 1323, 1069; MS (m/z) 285 (100) (M^+), 200 (85) (M^+-C_6H_{13}), 160 (48) ($PhthNCH_2^+$), 148 (59) ($PhthNH_2^+$); Found C 75.77 %, H 8.20 %, N 4.91 %, $C_{18}H_{23}NO_2$ requires C 75.76 %, H 8.12 %, N 4.91 %, mp 70-71 °C

(1S, 2S, 5R)-1-phthalimido-2-isopropyl-5-methylcyclohexane The same as above was used but (1R, 2S, 5R) menthol was used instead of (1S, 2R, 5S) menthol to give the product as a white solid (2.36 g, 65%).

(1R, 2R, 5R)-1-phthalimido-2-isopropyl-5-methylcyclohexane (92e) The same as above but (1S, 2R, 5R) isomenthol was used instead of (1R, 2S, 5R) menthol to give product as an oil (0.32 g, 9%). ^{1}H NMR δ 0.70 (3H, d, J = 6 Hz, CH_3), 0.90 (7H, t, J = 4, 6 Hz, CH_3), 1.35-2.15 (7H, m, CH), 2.48 (1H, app.q. , J = 13, 14 Hz, H6-ax), 4.30 (1H, dt, J = 8, 4 Hz, H1), 7.65 (2H, m, Ar), 7.75 (2H, m, Ar);^{13}C NMR δ 21.4, 22.4, 24.3, 25.2, 27.2, 30.3, 33.3, 33.2, 42.6, 56.1, 122.9, 131.8, 133.7, 169.0; IR (ν, cm^{-1}) 3451, 2967, 2865, 1761, 1713, 1611, 1465, 1326, 1191, 1358, 1110, 1067; MS (m/z) 285 (74) (M^+), 200 (100) (M^+-C_6H_{13}), 160 (72) ($PhthNCH_2^+$), 148 (71) ($PhthNH_2^+$), 138 (64) (M+-$PhthNH^+$); HRMS calcd for $C_{18}H_{23}NO_2$ (M^+) [286.18070], found [286.17944]

(1R, 2R, 5S)-1-formamido-2-isopropyl-5-methylcyclohexane (98) Sodium borohydride (1.89 g, 49.92 mmole) was added to a solution of (1R, 2R, 5S) -1-phthalimido-2-isopropyl-5-methylcyclohexane (**90**) (2.30 g, 8.07 mmole) in isopropanol (76 ml) and water (12 ml). The reaction was stirred until TLC showed disappearance of the phthalimido compound (4 hrs). Trifluoroacetic acid (13.5 ml, 166.4 mmole) was added and the mixture was heated at reflux for 3 hrs, cooled and treated with aqueous NaOH (88 ml of a 2M solution). The mixture was extracted with CH_2Cl_2. The organic extract was washed (brine), dried (Na_2SO_4) and evaporated. The residue was taken up in chloroform, filtered and evaporated to give a mixture of the amine (**95**) and the lactone (**96**). The crude mixture was taken up in CH_2Cl_2 (16 ml) and added by cannula to a mixture of CDI (1.73 g, 11.56 mmole) and formic acid (442 μl, 11.56 mmole) in CH_2Cl_2 (16 ml) at room temperature under N_2. The mixture was stirred overnight, then washed with aq. HCl (2M). The organic layer was washed (brine), dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (30 g) eluting with 10% EtOAc:Hexane to give the formamide (**98**) a mixture of rotamers, as a white solid (0.67 g, 63%). ^{1}H NMR δ 0.75-1.15 (11H, m, CH), 1.15-1.55 (3H, m, CH), 3.82 (1H, dd, J = 3, 10 Hz, CH_{cis}),

4.45 (1H, dd, J = 3, 10 Hz, $H1_{trans}$), 5.62 (1H, bs, -NH), 8.05 (1H, s, OHC-NH), 8.13 (1H, s, OHC-NH), 8.19 (1H, s, OHC-NH). ^{13}C NMR showed a mixture of formamide rotamers; ^{13}C NMR 20.4, 20.7, 21.0, 22.2, 24.6, 25.1, 26.2, 26.6, 28.8, 29.4, 34.5, 34.7, 40.2, 42.6, 44.9, 46.1, 46.2, 49.6, 160.8, 164.4; IR (ν, cm^{-1}) 3293, 1685(s), 1651(s); MS (m/z) 183 (62) (M^+), 138 (46) (M^+-C_3H_9), 123 (35) (M+- C_4H_{12}), 98 (100) (M^+-C_6H_{13}), 81 (43) (M^+-$C_5H_{12}NO$); HRMS calcd for $C_{11}H_{21}NO$ (M^+) [184.17014], found [184.16953]; mp 116.5-118.5 °C

(1S, 2S, 5R)-formamide-2-isopropyl-5-methylcyclohexane The same as above but (1S, 2S, 5R)-1-phthalimido-2-isopropyl-5-methylcyclohexane was used instead of (1R, 2R, 5S)-1-phthalimido-2-isopropyl-5-methylcyclohexane to give product as a white solid (0.67 g, 63%).

(1R, 2R, 5S)-1-isocyano-2-isopropyl-5-methylcyclohexane (89) A solution of the formamide (**98**) (0.2 g, 1.09 mmole) in CH_2Cl_2 (2 ml) was added to a solution of tosyl chloride (0.24 g, 1.15 mmole) and pyridine (176 μl, 2.18 mmole) in CH_2Cl_2 (2ml) at room temperature under N_2. The mixture was stirred for 6 hrs. N-pentylamine (75 μl, 0.65 mmole) was added and the mixture was stirred for a further 1 hr to destroy excess TsCl. The mixture was diluted with CH_2Cl_2 and washed with aq. NH_4Cl, aq. NaOH (2M) and brine. The organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (6 g) eluting with n-pentane and 3% EtO_2:n-pentane to give the isonitrile (**89**) as an oil (0.05 g, 30%), (Green TLC spot with $Co(SCN)_2$ reagent). ^1H NMR δ 0.70-1.85 (17H, m, CH), 1.95 (1H, m, CH), 3.95 (1H, br, H1); ^{13}C NMR δ 20.2, 20.6, 21.6, 24.6, 26.1, 29.5, 34.3, 39.7, 46.2, 53.4 (t, J = 4.5 Hz, CHNC), 155.6 (t, J = 4.8 Hz, NC). IR (ν, cm^{-1}) 2951, 2917, 2859, 2133(s), 1454, 1367, 1321; MS (m/z) 164 (15) (M^+-H), 150 (100) (M^+-CH_3), 122 (22) (M^+-iPr), 108 (19) (M^+-C_4H_9), 95 (35) (M^+-C_5H_{10})

(1S, 2S, 5R)-1-isocyano-2-isopropyl-5-methylcyclohexane The same as above but (1S, 2S, 5R)-formamide-2-isopropyl-5-methylcyclohexane was used instead of (1R, 2R, 5S)-formamide-2-isopropyl-5-methylcyclohexane to give the product as an oil (0.05 g, 30%).

(3S, 6S)-2-(hydroxymethylene)-3-methyl-6-isopropylcyclohexanone (**102**) A solution of (2R, 5S) menthone (**101**) (4.22 g, 27.23 mmole) in toluene (20 ml) was added to a solution of sodium methoxide (4.41 g, 81.68 mmole) in toluene (19 ml) by cannula. Ethyl formate (3.28 ml, 81.68 mmole) was added to the solution at room temperature under N_2. The solution was stirred overnight, forming a brown precipitate. The mixture was acidified with aq. HCl (41 ml of a 2M solution). The mixture was extracted with CH_2Cl_2. The organic layer was washed (brine), dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (90 g) eluting with 5% EtOAc:Hexane to give formyl menthone (**102**) as an oil (3.67 g, 74%). ^{1}H NMR δ 0.65-1.10 (9H, m, CH_3), 1.50 (3H, m, CH), 0.65-2.10 (1H, m, CH), 2.10-2.70 (3H, m, CH), 8.65 (1H, s, =C<u>H</u>OH), 14.75 (1H, s, =CHO<u>H</u>); ^{13}C NMR δ 13.9, 16.2, 17.1, 20.0, 22.5, 27.4, 28.4, 31.4, 46.0, 114.9, 188.5

(3R, 6R)-2-(hydroxymethylene)-3-methyl-6-isopropylcyclohexanone The same procedure as above was used but (2R, 5R) menthone was used instead of (2R, 5S) menthone to give the product (**102**) as an oil (3.50 g, 70%). ^{1}H NMR δ 0.70-1.35 (9H, m, CH_3), 0.50 (1H, s, CH), 0.65-2.10 (3H, m, CH), 2.15-2.70 (3H, m, CH), 8.65 (1H, s, =C<u>H</u>-OH) ,14.75 (1H, s, =CH-O<u>H</u>); ^{13}C NMR δ 14.0, 16.2, 17.5, 18.5, 20.2, 21.3, 22.3, 22.8, 25.7, 27.4, 27.5, 27.7, 28.4, 33.8, 35.3, 46.0, 50.7, 55.7, 60.2, 115.0, 181.5, 188.5, 200.7.

(3S, 6R)-2-allyl-2-formyl-3-methyl-6-isopropylcyclohexanone (108)

Method A : Anhydrous potassium carbonate (0.08 g, 0.55 mmole) and benzyltriethyl ammonium chloride were added to the solution of formylmenthone (**102**) (0.10 g, 0.55 mmole) in methyl *tert*-butyl ether (1 ml), Allyl bromide (57 μl, 0.66 mmole) was then added to the mixture under N_2 at room temperature. The solution was stirred overnight, acidified with ammonium chloride and extracted with methyl *tert*- butyl ether. The organic layer was washed (brine), dried (Na_2SO_4) and evaporated to give a mixture of the C-alkylation product (**108**) [(3S, 6R)-2-allyl-2-formyl-3-methyl-6-isopropylcyclohexanone] and the O-alkylation product (**107**) [(3S, 6R)-2-allyloxymethylene-3-methyl-6-isopropylcyclohexanone]. ^{1}H NMR for (**108**) (C-alkylation) δ 0.70-1.20 (9H, m, CH_3), 1.45-1.80 (2H, m, CH), 1.80-2.30 (5H, m, CH), 2.65 (1H, dd, J = 7, 8 Hz, C<u>H</u>-CH=CH_2), 2.75 (1H, dd, J = 7, 8 Hz, C<u>H</u>-

$CH=CH_2$), 4.90 (2H, bd, J = 9.5 Hz, -CH=C$\underline{H}_2$), 5.40 (1H, bd, J = 9.5 Hz, C$\underline{H}$=CH_2), 10.00 (1H, s, -CHO); ^{13}C NMR δ 15.7, 16.0, 19.0, 22.0, 26.5, 28.0, 37.5, 39.0, 54.0, 65.5, 119.0, 132.50, 205.5, 213.5; IR (ν, cm^{-1}) 2953, 2876.04, 1724, 1720, 1629 MS (m/z) 223 (1.4) (M^+-H), 139 (43) (M^+-C_5H_9+H), 111 (60) (M^+-C_8H_{16}+H), 69 (100) ($C_5H_9^+$), 55 (89) ($C_4H_8^+$), 41 (63) ($C_3H_5^+$); HRMS calcd for $C_{14}H_{22}O_2$ (M^+) [223.16981], found [223.16933]; ^1H NMR for (**107**) (O-alkylation) δ 0.70-3.20 (16H, m, CH), 4.60 (2H, dd, J = 6, 19 Hz, O-C$\underline{H}_2$-CH=CH_2), 5.00-5.50 (2H, m, CH=C$\underline{H}_2$), 5.80-6.15 (1H, bd, C$\underline{H}$=CH_2), 10.15 (1H, s, CHO)

Method B : The same as method A, but using CH_3CN instead of MTBE to give a mixture of C-alkylation product and O-alkylation product with ratio 1:1.

Method C : The same as method B, but with added lithium chloride (0.02 g, 0.55 mmole) to give a mixture of the C-alkylation product and O-alkylation product.

Method D : A solution of formyl menthone (**102**) (2.03 g, 11.09 mmole) in THF (20 ml) was added to a mixture of anhydrous potassium carbonate (1.53 g, 11.09 mmole), triphenylphosphine (0.15 g, 0.55 mmole) and $Pd(dba)_2$ (0.16 g, 0.28 mmole) in THF (20 ml) at room temperature under N_2. Then allyl acetate was added to the solution mixture. The mixture was stirred overnight, then filtered though celite. The THF was removed *in vacuo*. The residue was purified by flash chromatography on silica gel (60 g) eluting with 4% EtOAc : Hexane to give only the C-allylated compound (**108**) as an oil (2.09 g, 85%).

2-formyl-4-*tert*-butylcyclohexanone (110) : The same procedure as for (**102**) was used but 4-*tert*-butylcyclohexanone (**109**) was used instead of menthone to give the product as a solid (4.78 g, 96%), which was used without further purification. The product decomposed on standing. ^1H NMR δ 0.75-0.95 (9H, m, CH_3), 1.15-1.35 (2H, m, CH_2), 1.75-1.90 (1H, m, CH), 1.95-2.10 (1H, m, CH), 2.30-2.45 (3H, m, CH), 8.60 (1H, s, -C$\underline{H}$=OH), 14.3 (1H, s, CH=CH-O$\underline{H}$); ^{13}C NMR δ 22.4, 24.4, 27.2, 32.0, 44.4, 108.6, 184.2, 188.1, 199.5; IR (ν, cm^{-1}) 3329, 2955, 2879, 1716, 1608, 1470, 1363, 1173

2-allyl-4-*tert*-butyl-1-oxocyclohexane-2-carboxaldehyde (111) The same procedure as for (**108**) (Method D) was used but formyl-4-*tert*-butylcyclohexanone (**110**) was

used instead of formylmenthone (**102**) to give a separable mixture of two isomers. Minor isomer (0.01 g, 10 %) ^{1}H NMR δ 0.80-1.05 (9H, m, CH_3), 1.05-1.70 (3H, m, CH), 1.80-2.20 (3H, m, CH), 2.20-2.60 (4H, m, CH), 4.90-5.10 (2H, m, $CH=CH_2$), 5.55-5.85 (1H, m, $CH=CH_2$), 9.35 (1H, s, CHO) ^{13}C NMR δ 27.9, 28.3, 32.1, 33.1, 34.2, 41.2, 46.7, 48.9, 115.8, 136.3, 200.3, 212.0; Major isomer (0.09 g, 71%) ^{1}H NMR δ 0.80-0.90 (9H, m, CH_3), 1.35-1.90 (5H, m, CH), 2.30-2.75 (4H, m, CH), 4.90-5.20 (2H, m, $CH=CH_2$), 9.75 (1H, s, CHO); ^{13}C NMR δ 25.5, 26.9, 27.6, 30.7, 32.4, 36.3, 39.1, 40.9, 62.0, 67.9, 119.2, 131.4, 202.0, 211.8; IR (ν, cm^{-1}) 3411, 3068, 2960, 2863, 1716, 1634, 1486, 1368; MS (m/z) 225 (2) (M^+-H), 191 (15) (M^+-H_3O_2), 135 (9) (M^+-$C_5H_{13}O_2$), 121 (15) (M^+-$C_7H_{15}O$), 81 (24) (M^+-$C_8H_{16}O_2$)

2-allyl-4-*tert*-butyl-2-hydroxylmethyl-cyclohexanol (112) The same procedure as for (**121**) was used but ketoaldehyde (**111**) was used instead of (**108**) they crude product was purified by flash chromatrography with 10 % and 50% EtOAc:Hexane to give diol (**112**) (0.09 g, 62 %). ^{1}H NMR δ 0.45 (1H, t, J = 12.5 Hz, CH), 0.65-0.90 (10H, m, CH_3, CH), 0.90-1.80 (5H, m, CH), 2.10-2.25 (1H, m, C<u>H</u>$_2$-CH-CH_2), 2.35-2.60 (1H, m, C<u>H</u>$_2$-CH=CH_2), 2.90-3.10 (2H, br, CH_2), 3.35 (2H, d, J = 8 Hz, OH), 3.34-3.80 (2H, m, OH, CH), 4.90-5.20 (2H, m, CH=C<u>H</u>$_2$), 5.65-5.90 (1H, m, C<u>H</u>=CH_2); ^{13}C NMR δ 28.9, 31.1, 32.8, 33.6, 35.9, 44.2, 44.5, 65.3, 75.1, 65.3, 75.1, 82.2, 122.5, 138.0 MS (m/z) 224 (10) (M^+-2H), 193 (25) (M^+-CH_2OH-OH-2H), 123 (46) (M^+-$C_6H_{13}O$), 107 (69) (M^+-$C_6H_{15}O_2$), 95 (100) (M^+-$C_8H_{19}O$); Found C 72.97 %, H 10.49 %, $C_{14}H_{26}O_2$ requires C 74.29 %, H 11.58 %; mp 64-67 °C

Benzylidene acetal (**113**) Benzaldehyde dimethylacetal (66 μl, 0.44 mmole) was added to a solution of diol (**112**) (0.10 g, 0.44 mmole) in CH_2Cl_2 (2 ml) and the mixture was stirred at room temperature overnight. $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and the solvent was removed to give the benzylidene acetal as an oil (0.11 g, 79 %), sufficiently pure for NMR analysis. ^{1}H NMR δ 0.70-2.00 (15H, m, CH), 2.35 (1H, dd, J = 6, 11 Hz, CH), 2.80 (1H, dd, J = 6 ,11 Hz, CH), 3.35 (1H, d, J = 11 Hz, CH), 3.60 (1H, dd, J = 10 , 6 Hz, CH), 4.0 (1H, d, J = 11 Hz, CH), 5.15 (2H, m, CH=C<u>H</u>$_2$), 5.55 (1H, s, CHPh), 5.85 (1H, m, C<u>H</u>=CH_2), 7.20-7.60 (5H, m, Ph)

Alkene (114) Carbomethoxy methylene triphenyl phosphorane (0.18 g, 0.54 mmole) was added to the β-ketone aldehyde (**108**) (0.10 g, 0.45 mmole) in CH_3CN (0.5 ml) under nitrogen. The solution was heated at 100°C overnight until ^1H NMR showed the disappearance of the aldehyde peak. Dichloromethane and silica gel were added and the solvent was removed in vacuo. The residue was applied to the top of a silica gel coloumn (3g) and it was eluted by flash chromatography with 5% EtOAc:Hexane to give the mixture of the alkene (**114**) (*cis*: *trans*) as an oil (0.09 g, 75%). ^1H NMR δ 0.50-2.35 (17H, m-CH), 2.73 (1H, dt, J = 5, 1.6 Hz, CH), 3.70 (3H, s, $-OCH_3$), 4.80-5.10 (2H, m, $CH=CH_2$), 5.85 (2H, d, J = 16 Hz, $CH=CH_2$), 7.20 (1H, dd, J = 16, 20 Hz, CH). ^{13}C NMR δ16.4,18.3, 21.8, 26.2, 27.2, 30.1, 37.5, 38.3, 51.5, 53.7, 64.3, 117.5, 122.9, 134.5, 147.5, 166.4, 209.5; IR (ν, cm^{-1}) 1296, 1383, 1465,1644(s), 1721(s), 2873, 2950, 3078; MS (m/z) 279 (19) (M^++H^+), 263 (70) (M^+-H-O), 247 (58) (M^+-OCH_3), 203 (59) (M^+-O-CO_2Me), 107 (27) (M^+-$C_{10}H_{15}O_2$); minor isomer ^1H NMR δ 0.75-1.00 (1H, m, $-CH_3$), 1.30 (1H, t, J = 6 Hz, CH), 1.55-1.85 (2H, m, CH_2), 1.85-2.40 (6H, m, CH), 2.60-2.85 (2H, m, CH_2), 3.80 (3H, s, OCH_3), 5.00-5.20 (2H, m, $CH=C\underline{H}_2$), 5.40-5.70 (1H, m, $C\underline{H}=CH_2$), 5.60 (1H, d, J = 16 Hz, CH), 7.15 (1H, d, J = 16 Hz, CH); ^{13}C NMR δ 16.0, 18.9, 21.3, 23.6, 26.5, 27.3, 39.0, 40.6, 51.7, 52.8, 58.2, 118.5, 121.6, 132.8, 148.7, 167.0, 211.7;

Saturated ester (115) Palladium on carbon (0.09 g, 10%) was added to a solution of the alkene (**114**) (0.93 g, 3.33 mmole) in MeOH (3 ml). The solution was flushed with N_2, then stirred under hydrogen gas at 90 psi overnight. The mixture was flushed with N_2, then filtered through celite and evaporated to give the saturated ester (**115**) (0.85 g, 90%). ^1H NMR δ 0.70-2.35 (27H, m, CH), 3.60 (3H, s, $-OCH_3$); ^{13}C NMR δ 15.1, 15.2, 17.5, 18.6, 21.1, 26.1, 27.5, 27.9, 28.2, 29.2, 34.3, 37.8, 51.6, 52.6, 54.3, 174.0, 214.2; IR (ν, cm^{-1}) 2963, 1748, 1702, 1460, 1431, 1373, 1293, 1201, 1183; MS (m/z) 283 (100) (M^++H^+), 240 (31) (M^++H^+-C_3H_7), 225 (20) (M^++H^+-C_4H_{10}), 208 (26) (M^++H^+-$C_4H_{11}O$), 193 (31) (M^++H^+-$C_5H_{14}O$)

Diol (116) A solution of the ester (**115**) (0.15 g, 0.53 mmole) in THF (1 ml) was added to the solution of lithium aluminium hydride (0.14 g, 3.69 mmole) in THF (1ml) by cannula under nitrogen at 0°C. The solution was stirred until TLC showed

disappearence of the ester (**115**). Water was added carefully until a white solid formed. The mixture was filtered through celite washing with EtOAc. The organic solvent was removed *in vacuo* to give a mixture of the two isomers of the diol [OH axial as minor isomer (0.01 g, 10 %) and OH equatorial as major isomer (0.07 g, 46 %)] which was purified by flash chromatrography with 5% EtOAc:Hexane as eluent. Major isomer; ^{1}H NMR δ 0.70-1.05 (12H, m, CH), 1.05-1.85 (16H, m, CH), 3.30 (1H, d, J = 9 Hz, H1), 3.57 (2H, t, J = 6 Hz, CH_2-OH) ^{13}C NMR δ 15.1, 15.3, 15.4, 15.9, 20.9, 23.1, 25.3, 26.4, 28.3, 29.1, 34.3, 36.1, 42.3, 42.6, 64.3, 75.6; IR (ν, cm^{-1}) 3278, 2920, 1454(s), 1373(s), 1025; MS (m/z) 256 (10) (M^+), 239 (100) (M^+-OH), 238 (33) (M^+-H_2O), 196 (11) (M^+-C_3H_8O), 177 (11) (M^+-C_3H_8O-H_2O-H); Found C 75.29 %, H 12.25 %, $C_{16}H_{31}O_2$ requires C 75.24 %, H 12.23 %; mp 100-103 °C; Minor isomer ^{1}H NMR δ 0.70-1.00 (12H, m, CH), 1.00-1.75 (17H, m, CH), 3.80 (2H, t, J = 6 Hz, -OH), 3.70 (1H, s, H1) ^{13}C NMR δ 15.3, 16.3, 17.6, 20.7, 21.1, 23.8, 26.7, 27.7, 29.3, 30.6, 34.4, 39.5, 41.1, 43.1, 64.1, 73.1 IR (ν, cm^{-1}) 3319 (br) , 2940, 2847, 1460(s), 1378, 1045

Hydroxy ether (117) Sodium hydride (0.07 g, 1.78 mmole) was added to a solution of diol (**116**) (0.10g, 0.36 mmole) in THF (2 ml) under nitrogen at 0°C. Benzyl bromide (43 μl, 0.36 mmole) was added to the solution and the mixture was stirred overnight. TLC showed that the diol had not disappeared, and additional benzyl bromide (43 μl, 0.36 mmloe) was added to the mixture and stirring was continued for 9 hours. Ammonium chloride and water were added to the mixture. The mixture was extracted with ethyl acetate, washed (brine) and dried (Na_2SO_4). Ethyl acetate and THF were removed *in vacuo*. The residue was purified by flash chromatography on silica gel (3g) eluting with 4% EtOAc:Hexane to give hydroxy ether (**117**) (0.11 g, 87%). ^{1}H NMR δ 0.70-1.10 (12H, m, CH_3), 1.15-2.15 (16H, m, CH), 3.30 (1H, dd, J = 5, 5 Hz, H1), 3.45 (2H, t, J = 6 Hz, C<u>H</u>OH), 4.50 (2H, s, CH_2-O-R), 7.30 (5H, bs, Ph); ^{13} C NMR δ 15.0, 15.2, 15.3, 15.9, 20.9, 23.1, 25.2, 25.5, 26.2, 29.2, 33.8, 34.3, 42.4, 42.6, 71.9, 72.7, 75.5, 127.4, 127.5, 128.2 IR (ν, cm^{-1}) 3476 (br), 2957, 2860, 1454, 1368, 1264, 1206, 1097, 1039 MS (m/z) 346 (4) (M^+), 237 (30) (M^+-OBn-2H), 219 (100) (H^+-HOBn-H_2O-H); HRMS calcd for $C_{23}H_{36}O_2$ (M^+) [347.29501], found [347.29564]

Triflate (118) Sodium hydride (0.01 g, 0.24 mmole) was added to a solution of alcohol (**117**) (0.04 g, 0.21 mmole) in dry CH_2Cl_2 (1 ml) under nitrogen at –10 °C and the mixture was stirred for 10 min. A solution of trifluoromethane sulfonic anhydride (37 μl, 0.22 mmole) in dry CH_2Cl_2 (1 ml) was added and the solution was stirred overnight. Ammonium chloride and water were added and the solution was extracted with CH_2Cl_2. The organic layer was dried (brine) and evaporated to give the trifate (0.03 g, 56%) which was used directly in the next step. ^{1}H NMR δ 0.50-1.05 (11H, m, CH), 1.05-2.70 (16H, m, CH), 3.20-3.60 (1H, m, CH), 3.60-4.15 (2H, m, CH_2), 4.40-4.60 (1H, m, CHPh), 6.70-7.50 (5H, m, Ph)

Attempted Synthesis of Azide ether (120)

Method A : Tetrabutyl ammonium hydrogen sulfate (0.04 g, 0.12 mmole) and sodium azide (0.03 g, 0.39 mmole) were added to a solution of trifate (**118**) (0.03 g, 0.06 mmole) in toluene (0.6 ml) under nitrogen at room temperature. The solution was stirred for 2 days. Sodium hydrogen carbonate and water were added to the solution. The solution was extracted with EtOAc. The organic layer was washed (brine), dried (Na_2SO_4) and evaporated. ^{1}H NMR showed no reaction.

Method B : The same as above but the mixture was heated at 80°C overnight. ^{1}H NMR showed no reaction.

Method C :Sodium azide (0.02 g, 0.24 mmole) was added to the solution of trifated (**118**) (0.04 g, 0.08 mmole) in DMF (1 ml) under N_2 at room temperature and the mixture was stirred overnight. TLC showed only trifate (**118**) ether compound. The solution was heated at 90°C overnight. Water was added and extracted with EtOAc, washed (brine), dried (Na_2SO_4) and evaporated. ^{1}H NMR showed trifate (**118**).

Benzyl ether (128) The same procedure as for (**117**) was used, except that diol (**112**) was used in place of (**116**) to give benzyl ether (**128**) (0.10 g, 73%) after purification by flash chromatography with 2 % EtOAc:Hexane as eluent. ^{1}H NMR δ 0.30-0.60 (1H, m, CH), 0.65-1.00 (9H, m, CH), 1.0-1.5 (3H, m, CH), 1.60-1.80 (2H, m, CH), 2.05-2.25 (1H, m, CH), 2.40-2.60 (1H, m, CH), 3.15 (1H, d, J= 5.7 Hz, CH), 3.40 (2H, d, J = 6 Hz, CH_2), 3.60 (2H, dd, J = 2.9, 8.6 Hz, CH), 4.4 (2H, s, CH_2Ph), 4.90-5.10 (2H, m, CH=CH_2), 5.50-5.58 (1H, m, CH=CH_2), 7.10-7.35 (4H, m, Ph) ^{13} C

NMR δ 27.4, 29.3, 30.1, 30.4, 32.1, 40.6, 40.9, 73.6, 77.6, 79.4, 85.5, 117.8, 127.8, 128.4, 134.3, 137.6; IR (ν, cm^{-1}) 3516, 2951, 2865, 1632, 1449, 1395, 1374, 1245,1202, 1089

Attempted synthesis of Phthalimide (129) Benzyl ether (**128**) (0.05 g, 0.16 mmole) was treated with PPh_3 (0.05 g, 0.176 mmole), phthalimide (0.03 g, 0.19 mmole) and DEAD (30 μl, 0.19 mmole) according to the procedure for (**90**). It was purified by flash chromatography with 5 % EtOAc:Hexane as an eluent gave alkene (**130**) an oil (0.01 g, 30%). ^{1}H NMR δ 0.70-0.90 (9H, s, CH_3, CH), 1.30-2.30 (7H, m, CH), 3.12 (1H, d, J = 11 Hz, C<u>H</u>HO), 3.21 (1H, d, J = 11 Hz, C<u>H</u>HO), 4.45 (2H, s, CH_2Ph), 4.89 (1H, m, =CH); 4.97 (1H, m, =CH), 5.40-5.85 (3H, m, C<u>H</u>=CH_2, CH_2-CH=C<u>H</u>$_2$); IR (ν, cm^{-1}) 3459, 3008, 2940, 2866, 2399, 1213 (s); MS (m/z) 299 (1) (M^++H^+), 283 (5) (M^+-CH_3), 271 (7) (M^+-CH_2=CH), 191 (8) (M^+-OBn), 149 (13) (M^+-OBn-C_3H_6)

Diol (121) A solution of the β-keto aldehyde (**108**) (0.08 g, 0.35 mmole) in THF (1 ml) was added to a solution of lithium aluminium hydride (0.03 g, 0.70 mmole) in THF (1 ml) by cannula under nitrogen at 0°C. The solution was stirred until TLC showed disappearence of the β-keto aldehyde. Water was added carefully until a white solid formed. The mixture was filtered through celite washed with EtOAc. The organic solvent was removed *in vacuo* .The residue was purified by flash chromatography on silica gel (3 g) eluting with 3% EtOAc:Hexane to give diol (**121**) with OH axial (0.02 g, 35%) and OH equatorial (0.04 g, 51%). For OH equatorial. ^{1}H NMR δ 0.70-1.05 (8H, m, CH), 1.05-1.45 (2H, m, CH), 1.45-1.95 (4H, m, CH), 1.95-2.35 (2H, m, CH), 2.45-2.75 (2H, m, C<u>H</u>$_2$-CH=CH_2), 3.55 (1H, bd, J = 11 Hz, C<u>H</u>OH), 3.80 (2H, dd, J = 11 Hz, C<u>H</u>OH), 4.95-5.20 (2H, m, CH=C<u>H</u>$_2$), 5.70-6.00 (1H, m, C<u>H</u>=CH_2); ^{13} C NMR δ 14.1, 15.4, 17.2, 21.0, 27.7, 30.3, 31.4, 42.0, 43.6, 69.8, 75.7, 117.4, 135.4 IR (ν, cm^{-1}) 3436 (br), 3071, 2940, 2253(w), 1838(w), 1701(s), 1639(s), 1434, 1385, 1292, 1054; MS (m/z) 224 (10) (M^+-2H), 193 (25) (M^+-CH_2OH-2H), 123 (46) (M^+-$C_6H_{13}O$), 107 (69) (M^+-$C_6H_{15}O_2$), 95 (100) (M^+-$C_8H_{19}O$); HRMS calcd for $C_{14}H_{26}O_2$ (M^+) [225.18546], found [225.18542]; For OH at axial ^{1}H NMR δ 0.70-1.25 (8H, m, CH), 1.25-1.85 (10H, m, CH), 2.00-2.30 (2H, m, C<u>H</u>$_2$-

$CH=CH_2$), 3.63 (1H, d, J = 11 Hz, C<u>H</u>OH), 3.85 (2H, t, J = 11 Hz, C<u>H</u>OH), 5.05 (2H, m, CH=C<u>H</u>$_2$), 5.75-5.95 (1H, m, C<u>H</u>=CH_2)

β-keto alcohol (122) Sodium borohydride (0.32 g, 8.72 mmole) was added to a solution of β-keto aldehyde (**108**) (3.89 g, 17.44 mmole) in methanol (38 ml) under N_2 at -78°C. The mixture was stirred 10 minutes, then allowed to warm to room temperature. Ammonium chloride and water were added to the mixture. It was extracted with EtOAc. The organic layer was washed (brine), dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography as silica gel (90 g) eluting with 3% EtOAc:Hexane to give the β-keto alcohol (**122**) as a white solid (2.54 g, 65 %) . ^{1}H NMR δ 0.65-0.95 (9H, m, CH_3), 1.35-1.60 (3H, m, CH), 1.70-2.65 (7H, m, CH), 3.35 (1H, d, J = 12 Hz, C<u>H</u>$_2$OH), 3.83 (1H, d, J = 12 Hz, C<u>H</u>$_2$OH), 4.95-5.15 (2H, m, CH=C<u>H</u>$_2$), 5.55-5.75 (1H, m, C<u>H</u>=CH_2); ^{13}C NMR δ 13.3,15.0, 18.0, 20.8, 23.3, 25.4, 27.4, 35.3, 36.7, 51.7, 55.7, 62.8, 118.1, 132.76 ; IR (ν, cm^{-1}) 3299(s), 2919, 1690(s), 1464, 1370; MS (m/z) 225 (18) (M^++H^+), 224 (12) (M+), 191 (10) (M^+-CH_3-H_2O), 153 (21) (M^++H^+-C_4H_8O), 107 (76) (M^++H^+-$C_6H_{14}O_2$); Found C 74.98 %, H 10.70 %, $C_{14}H_{24}O_2$ requires C 74.95 %, H 10.78 %; mp 72-75 °C

Benzoyl isocyanate (123) Oxalyl chloride (864 μl, 9.91 mmole) was added to a solution of benzamide (1.0 g, 8.25 mmole) in CH_2Cl_2 (1.65 ml) at 2°C under a calcium chloride tube. The mixture was stirred at room temperature for 1 hour, then it was heated at reflux for 5 hours (check IR ; N=C=O at 2240 cm^{-1}) to give a solution of benzoyl-isocyanate in CH_2Cl_2 (5M).

N-Acylcarbamate (124) A solution of benzoyl isocyanate in CH_2Cl_2 (88 μl, of a 5M solution, 0.44 mmole) was added to a solution of diol (**121**) (0.10 g, 0.44 mmole) in CH_2Cl_2 (4 ml) under N_2 at –78°C. It was stirred at –78°C for 0.5 hour, then additional portion of isocyanate were addition until TLC showed that the diol (**121**) was consumed. The mixture was filtered through celite. Silica gel was added and the dichloromethane was evaporated. The residue was applied to the top of a silica gel column (3 g) and it was eluted by flash chromatography with 15% EtOAc:Hexane to give the mixture of diol (2 mg, 2%), N-acylcarbamate (**124**) (0.06 g, 38 %) and the bis- N-acylcarbamate (**125**) (0.4 g, 19%) ^{1}H NMR δ N-acylcarbamate (**124**) 0.80-

1.10 (8H, m, CH), 1.10-1.75 (5H, m, CH), 1.75-2.15 (3H, m, CH), 2.30-2.50 (2H, m, CH), 2.65-2.80 (1H, m, CH), 3.90 (1H, d, J = 11 Hz, C<u>H</u>OR), 5.00-5.20 (2H, m, CH=C<u>H</u>$_2$), 5.75-5.95 (1H, m, CH=C<u>H</u>$_2$), 7.45-7.70 (3H, m, Ph), 8.35 (1H, bs, NH) ^{1}H NMR δ bis N-acylcarbamate **(125)**; 0.75-1.00 (5H, m, CH), 1.20-2.10 (6H, m, CH), 2.40-2.60 (1H, m, C<u>H</u>$_2$-CH=CH$_2$), 2.90 (1H, dd, J = 7, 15 Hz, C<u>H</u>-CH=CH$_2$), 4.10-4.30 (2H, m, CH), 5.00-5.20 (2H, m, CH=C<u>H</u>$_2$), 5.30 (1H, d, J = 12 Hz, CH), 5.60-5.90 (1H, m, C<u>H</u>=CH$_2$), 7.15-8.15 (10H, m, Ph), 8.80 (2H, bd, NH)

N-acylcarbamate (126) A solution of benzoyl isocyanate in CH_2Cl_2 (88 μl, of a 5M solution, 0.44 mmole) was added to a solution of alcohol (**119**) (0.10 g, 0.44 mmole) in CH_2Cl_2 (4 ml) under N_2 at –78°C. It was stirred at –78°C for 0.5 hour, then filtered through celite. Silica gel was added and the dichloromethane was evaporated. The residue was applied to the top of a silica gel column (3 g) and it was eluted by flash chromatography with 15% EtOAc:Hexane to give the N-acylcarbamate (**126**) as a white solid (0.15g, 91%). ^{1}H NMR δ 0.80-1.00 (9H, m, CH_3), 1.45-1.70 (2H, m, CH_2), 1.95-2.60 (6H, m, CH), 2.80 (1H, m, CH), 4.34 (1H, d, J = 11 Hz, C<u>H</u>HO), 4.47 (1H, d, J = 11 Hz, C<u>H</u>HO), 5.00-5.15 (2H, m, CH=C<u>H</u>$_2$), 5.40-5.70 (1H, m, C<u>H</u>=CH$_2$), 7.35-7.60 (3H, m, Ph), 7.85 (2H, d, J = 7 Hz, Ph), 9.00 (1H, s, NH); ^{13}C NMR δ 15.2,18.6, 21.3, 25.0, 26.1, 27.9, 36.6, 37.5, 52.5, 55.2, 65.6, 118.9, 127.8, 128.6, 132.1, 152.3, 165.0, 213.9; IR (ν, cm^{-1}) 3428, 3278, 3186, 3073, 2966, 2873, 2249, 1762, 13,690, 1609, 1527, 1387, 1296; Found C 70.19 %, H 7.82 %, N 3.75 %, $C_{22}H_{25}O_4N$ requires C 71.13 %, H 7.87 %, N 3.77 %; mp 58-62 °C

O-alkyl carbamate (127) Anhydrous potassium carbonate (0.22 g, 1.71 mmole) was added to a solution of N-acylcarbamate (**126**) (0.64 g, 1.71 mmole) in methanol (3.5 ml) at room temperature, then the mixture was stirred for 4 hours. Ammonium chloride and water were added. The mixture was extracted with CH_2Cl_2. The organic layer was washed (brine), dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (30 g) eluting with 15%, 20% EtOAc : Hexane to give major isomer (**127t**) (0.32 g, 70%) and another minor isomer (**127c**) (0.10 g, 21%). ^{1}H NMR for major isomer (**127t**) δ 0.70-1.00 (9H, m, CH_3), 1.15-1.40 (1H, m, CH), 1.65-2.15 (6H, m, CH), 2.28 (1H, m, C<u>H</u>$_2$-CH=CH$_2$), 2.80 (1H, dd, J = 5, 14 Hz,

CH_2-CH=CH_2), 4.00 (1H, d, J = 11 Hz, C<u>H</u>OH), 4.55-4.80 (3H, m, C<u>H</u>OH, -NH_2), 4.95-5.10 (2H, m, CH=CH_2), 5.70-5.95 (1H, m, C<u>H</u>=CH_2). ^{13}C NMR δ 15.6, 19.1, 21.6, 26.6, 29.1, 30.5, 37.2, 39.0, 53.6, 55.7, 67.9, 117.3, 135.6, 156.8, 212.7 IR (ν, cm^{-1}) 3432, 3334, 3268, 3201, 2925, 2853, 1737, 1706, 1609, 1460, 1388, 1321, 1066 Another minor isomer (**127c**) ; ^{1}H NMR δ 0.65-0.90 (9H, m, CH_3), 1.50 (2H, bd, J = 10 Hz, CH_2), 1.80-2.40 (5H, m, CH), 2.70 (1H, dd, J = 3, 12 Hz, C<u>H</u>$_2$-CH=CH_2), 4.10 (1H, d, J = 0.6, 12 Hz, C<u>H</u>HO), 4.25 (1H, d, J = 12 Hz, C<u>H</u>OH), 4.85-5.25 (4H, m, CH=C<u>H</u>$_2$, NH_2), 5.35-5.60 (1H, m, C<u>H</u>=CH_2) ^{13}C NMR δ 15.2, 18.7, 21.4, 24.9, 25.9, 27.8, 37.1, 37.6, 52.5, 55.2, 64.0, 118.6, 132.4, 157.0, 213.8; IR (ν, cm^{-1}) 3452, 3329, 3262, 3170, 2929, 1731, 1690, 1608, 1455, 1378, 1337, 1060; MS (m/z) 268 (100) (M^++H^+), 206 (22) (M^+-CH_3NO_2), 191 (51) (M^+-CH_2NO_3), 163 (20) (M^+-CH_2NO_3-C_3H_8), 135 (19) (M^+-CH_2NO_2-C_5H_{12}); Found C 67.39 %, H 9.44 %, N 5.31 %, $C_{15}H_{25}NO_3$ requires C 67.38 %, H 9.42 %, N 5.24 %; mp 125-127 °C

CHAPTER III

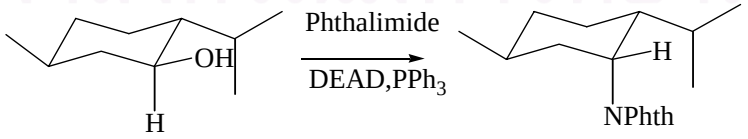
RESULTS AND DISCUSSION

The aims of this project were to develop methods for the preparation of axisonitril-3 analogs and to prepare analogs for screening. The simplest analogs of axisonitrile–3 would be those without any substituents in the 2-position. We anticipated that these could be prepared from menthol by nucleophilic substitution (**Scheme 1**). As both enantiomers of menthol are commercially available, then both enantiomers of the isonitrile could be prepared.

(**89**)

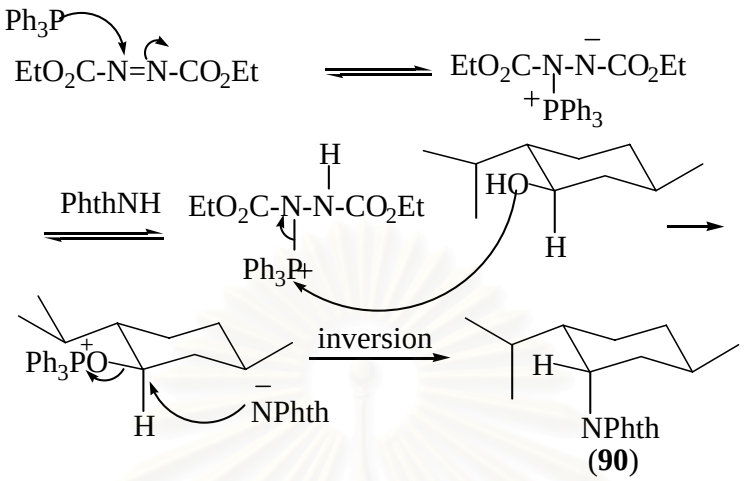
Scheme 1 Synthesis of analog isonitrile from menthol

To effect the nucleophilic substitution, we chose to use the Mitsunobu reaction which almost always proceeds with 100% inversion.[35] The mechanism is believed to involve activation of the alcohol as a leaving group by phosphorus, followed by S_N2 displacement by a nucleophile. A limitation of the reaction is that the conjugate acid of the nucleophile must be sufficiently acidic to protonate the azo dicarboxylate. Ragnarsson *et al* have correlated the pK_a with Mitsunobu reaction yields for a series of nitrogen pro-nucleophiles.[36] One of the most effective and most widely used is phthalimide and this is the one that we selected (**Scheme 2, 3**).



(**90**)

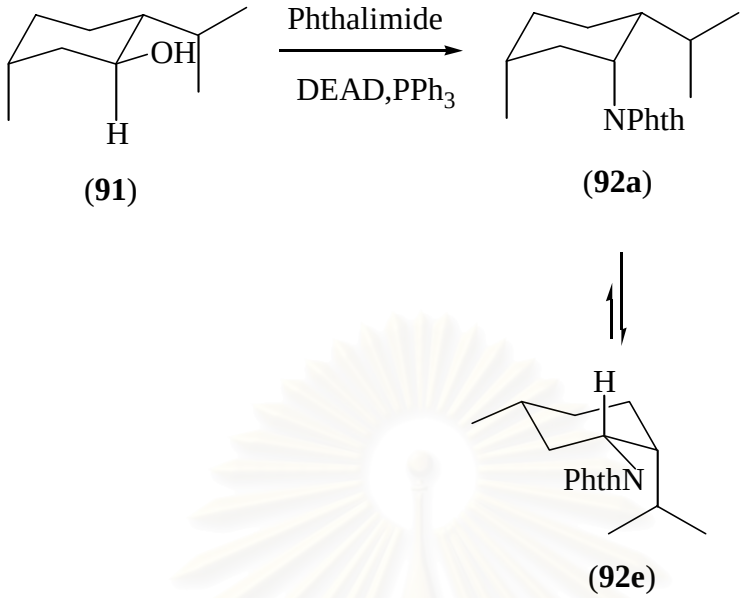
Scheme 2 The Mitsunobu Reaction of menthol with phthalimide



Scheme 3 The Mechanism of The Mitsunobu Reaction of menthol with phthalimide

Treatment of menthol with triphenylphosphine, DEAD and phthalimide gave the expected compound (**90**) in 65 % yield. The ^{1}H NMR spectrum clearly showed that the phthalimide group was axial. The proton α to nitrogen showed no large couplings which would be characteristic of a proton with a dihedral angle of 180°.

We thought that an isomeric series of analogs might be produced from commercially avialable isomenthol (**91**). However, under the same Mitsunobu conditions, the product (**92e**) was formed in only 9% yield (**Scheme 4**). This low yield can be attributed to the steric hindrance of the axial methyl substituent.

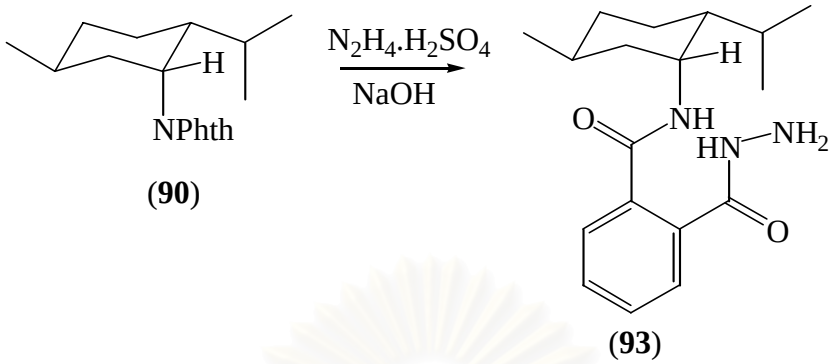


(**91**) (**92a**) (**92e**)

Scheme 4 The Mitsunobu Reaction of isomenthol

Curiously, the ^{1}H NMR spectrum showed that the phthalimide substituent was equatorial, because it did not display the typical equatorial /equatorial coupling constant of about 2-4 Hz, but it showed two typical / axial coupling constants of about 14 Hz. As the Mitsunobu reaction almost always proceeds with inversion, except in the cases where neighbouring group participation is possible,[37] we attributed this to an inversion of conformation or ring flip. The product would seem to be more stable in conformation (**92e**) rather than (**92a**), indicating that the sum of the A-values of methyl and phthalimido is greater than the A-valve of *i*-propyl. As the reaction was low yielding, this line was not pursued.[38]

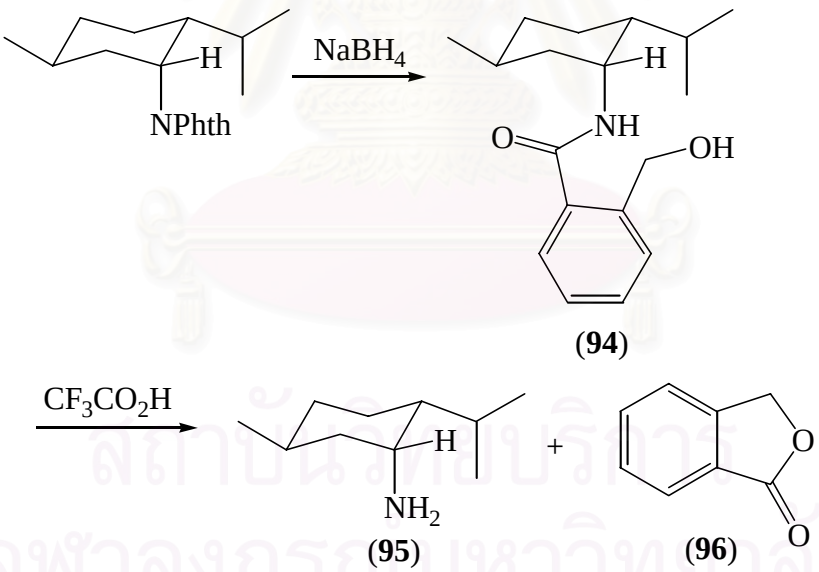
Removal of the phthaloyl moiety from phthalimide compound (**90**) was required. It is known that the classical Ing-Manske procedure[39] is not effective for sterically hindered phthalimides. Indeed, treatment of phthalimide compound (**90**) with hot hydrazine resulted in ring-opening to a hydrazide (**93**) but not complete deprotection (**Scheme 5**).



(**90**) (**93**)

Scheme 5 Ing-Manske's conditions

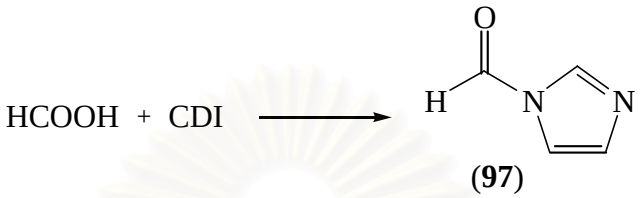
On the other hand, Ganem's procedure (**Scheme 6**) worked very smoothly: reduction of (**90**) with sodium borohydride gave the amido alcohol (**94**), which on treatment with trifluoroacetic acid, yielded a mixture of the amine (**95**) and the lactone (**96**).[40]



(**94**) (**95**) (**96**)

Scheme 6 Ganem's conditions for deprotection

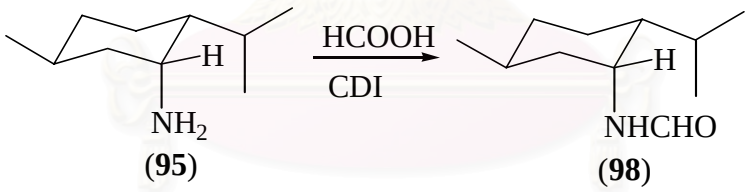
This mixture was not separated, but converted to the formamide by treatment with a formic acid–carbonyl diimidazole mixture.[41] This mixture generates N-formyl imidazole (**97**) *in situ* and is an alternative to the more usual acetic anhydride/formic acid procedure (**Scheme 7, 8, 10**).[42]



(**97**)

Scheme 7 Generation of Formyl Imidazole

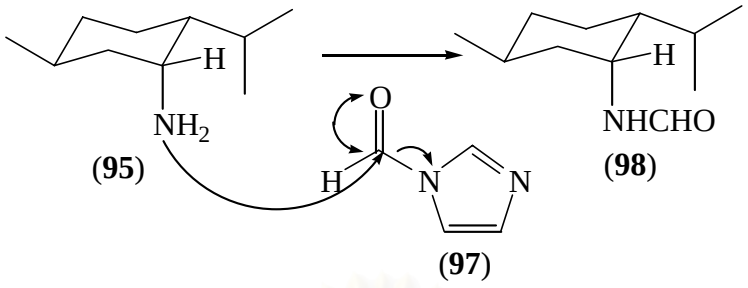
The formamide (**97**) was found to exist as a 5:1 ratio of rotamers. The ^{1}H NMR spectrum clearly showed the presence of two formyl protons in the range of 8-8.2 ppm. This phenomenum is well known in amides and can be attributed to the fact that the C-N bond has partial double bond character due to donation of the N lone pair into the carbonyl group (**Scheme 9**).[43]



(**95**) (**98**)

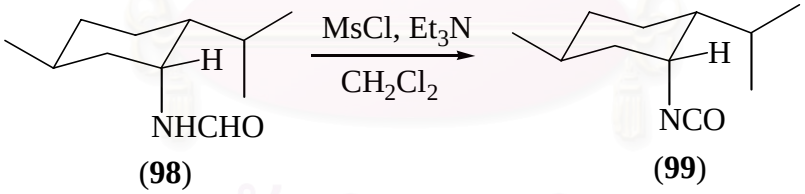
Scheme 8 Amine formylation

Scheme 9 Restricted rotation in amides



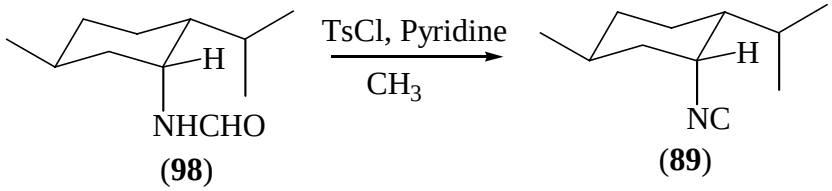
Scheme 10 The mechanism of formylation

Dehydration of formamides to isonitriles can be achieved with a number of reagents including tosyl chloride[44] and trific anhydride[45] under basic conditions. We elected to use mesyl chloride on the grounds that it is cheaper than trific anhydride and work-up should be easier than for tosyl chloride. To our surprise, the product was the isocyanate (**99**) (**Scheme 11**). This was identified on the basis of a number of spectroscopic observations. The carbon of the isocyanate appeared in the ^{13}C spectrum at 125 ppm and the NCO stretching frequency in the IR spectrum was found at 2265 cm^{-1}. Both are in the known ranges for isocyanates.[46]



Scheme 11 The Attempted dehydration of formamide with MsCl

In contrast, when formamide (**98**) was dehydrated with tosyl chloride, a different compound (**100**) was produced (**Scheme 12**). The carbon of the functional group was found at 155.6 ppm and the stretching frequency at 2133 cm^{-1}



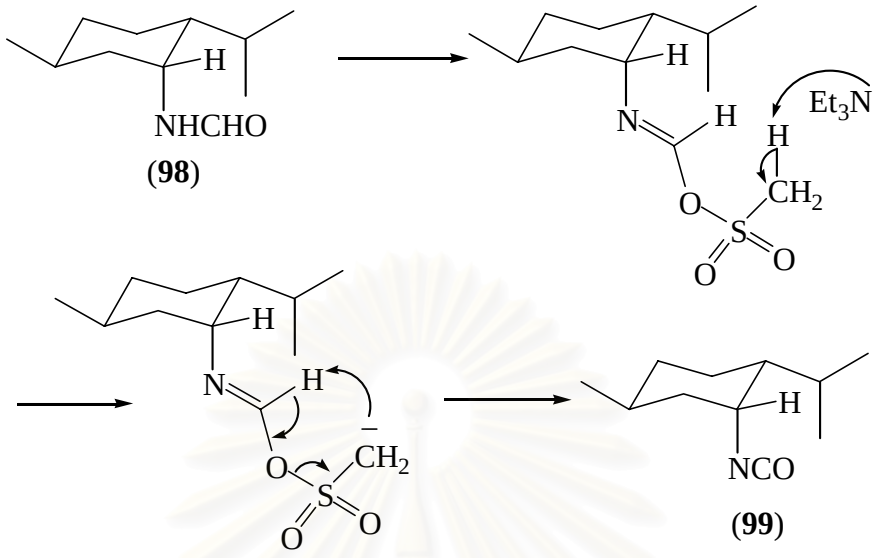
(**98**) (**89**)

Scheme 12 The dehydration of formamide with TsCl

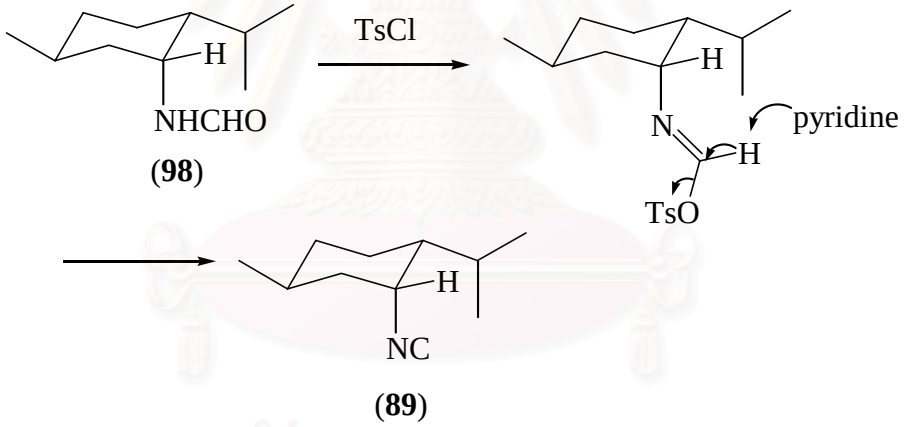
Both of these are consistant with an isonitrile group. In addition the C appeared as a 1:1:1 triplet (J = 4.8 Hz) due to coupling between the ^{13}C and the ^{14}N. Isonitriles are one of very few groups that display ^{13}C-^{14}N coupling.[11] Finally, an interesting TLC observation was made: when reaction TLC plates of the dehydration were visualized with cobalt thiocyanate dip, which typically gives blue spots with amines, an intense green spot was observed perhaps due to coordination of the isonitrile to cobalt. This observation should prove useful for detection of isocyanides.

A disadvantage of the tosyl chloride procedure is the purification, especially the separation of the product from traces of tosyl chloride. We eventually developed a chemical separation. Addition of n-pentyl amine converted the residual tosyl chloride to a sulfonamide. Washing with aqueous sodium hydroxide removed the sulfonamide and washing with aqueous ammonium chloride removed the excess pentylamine.

The different behavior of the two sulfonyl chlorides can be rationalized (**Scheme 13,14**). In both cases, initial sulfonylation must be at the formamide oxygen. In the case of the tosyl group this is followed by α-elimination to give the isonitrile (**89**). On the other hand, in the case of the mesyl group, the methyl group can be deprotected. A [2,3]-sigmatropic shift, similar to that involved in the Swern oxidation, then yields the isocyanate (**99**).



Scheme 13 The mechanism of the reaction of the formamide with MsCl



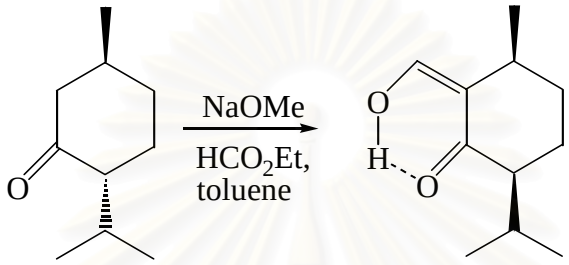
Scheme 14 The mechanism of the reaction of the formamide with TsCl

With both enatiomers of isonitrile (**89**) in hand, they were screened against *plasmodium falciparum*. Both were active in range of 3-6 μM. This is moderate activity, somewhat less than that reported for axisonitrile-3.[34]

In order to introduce substituents at the 2-position, menthol was oxidised to menthone (**101**) according to the literature procedure.[47] An electron withdrawing group

was then introduced to facilitate alkylation. The formyl group was chosen for this as it is readily introduced and sufficiently reactive for further manipulation.[48]

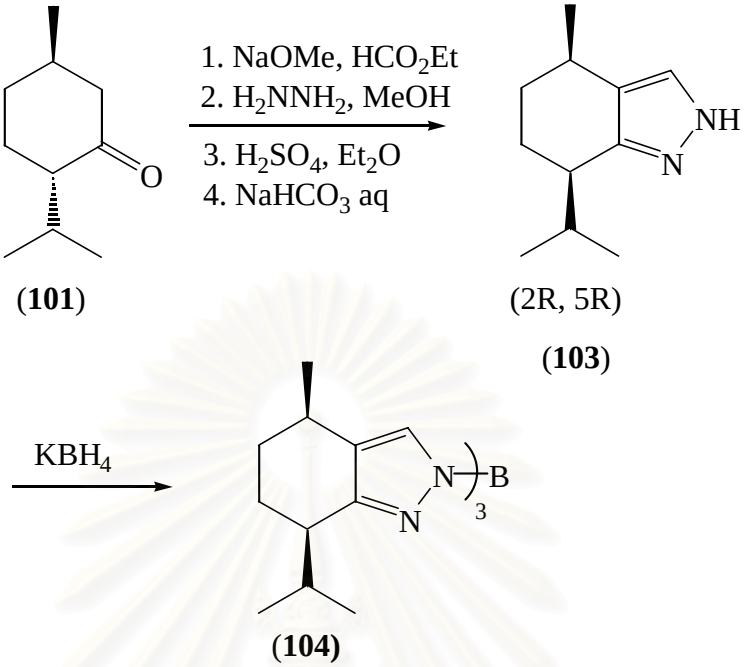
Formylation was carried out according to the literature procedure using ethyl formate and sodium methoxide in toluene (**Scheme 15, 17**).[48] This yields the sodium salt of formyl menthone. The parent neutral compound (**102**) is obtained on acidification with aqueous HCl.



(**101**) (**102**)

Scheme 15 Formylation of menthone

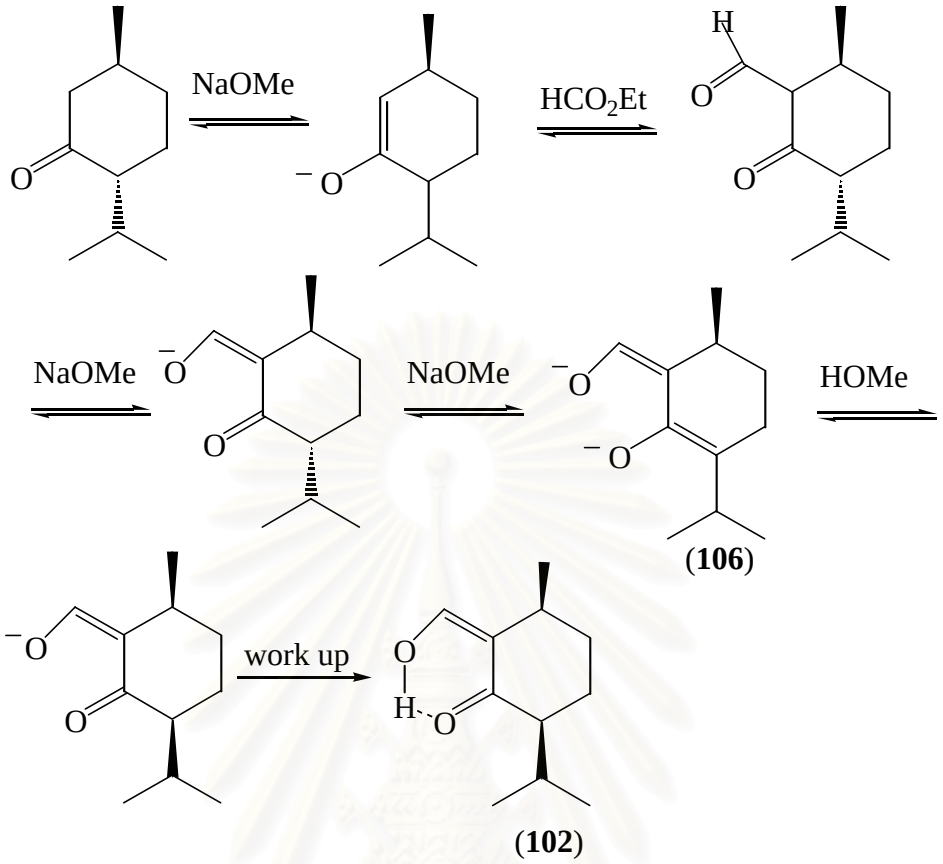
Based on the literature report, we expected the product to be formyl menthone. The gross structure was comfirmed by NMR and, as expected, the compound existed as its enol tautomer. This tautomer is stabilized by an intramolecular hydrogen bond. The existence of the hydrogen bond is indicated both by the O-H stretching band in the IR spectrum at 3550-3450 cm^{-1} (br) and by the high downfield shift of the hydroxy proton in the ^{1}H NMR spectrum at 14.75 ppm. During the course of this work, however, we became aware of a footnote to a paper claiming that formylation of menthone results in epimerization of the isopropyl group (**Scheme 16**).[49] The claim was supported by an X-ray structure of the zinc complex of compound (**104**) derived from formylmenthone.



(**101**) (2R, 5R) (**103**)

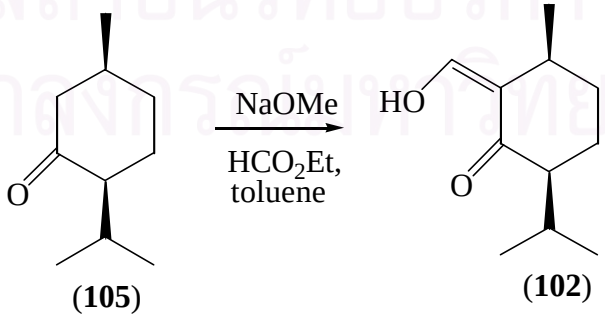
(**104**)

Scheme 16 Professor Tolman's menthone chemistry



Scheme 17 The mechanism of formylation and epimerization

To eliminate the possibility that the reported isomerization occurred during a step subsequent to formylation, we carried out the formylation of isomenthone (**105**) (**Scheme 18**). To our disappointment, the formylated compounds had identical ^{1}H and ^{13}C NMR spectra.



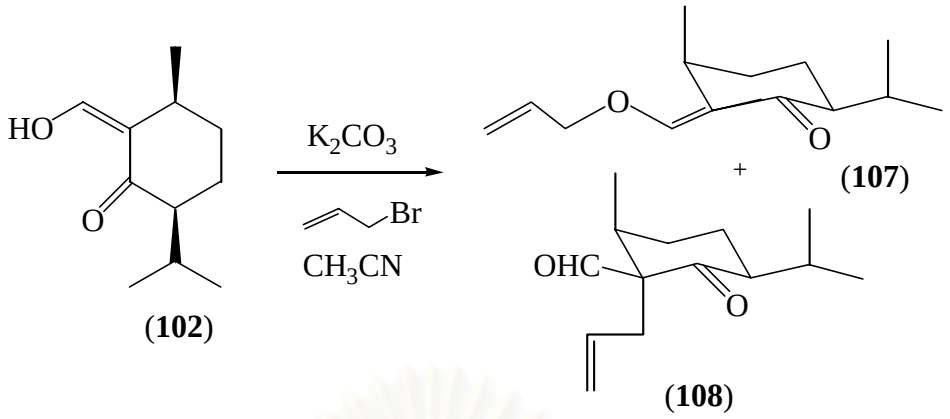
Scheme 18 The formylation of isomenthone

The epimerization may be due to deprotonation of formyl menthone (**102b**) by excess sodium methoxide to generate a dianion (**106**). The greater stability of the epimerized compound is likely to be due to the elimination of the 1,3- interaction between the vinyl hydrogen and the methyl group (**Scheme17, Figure 2**).

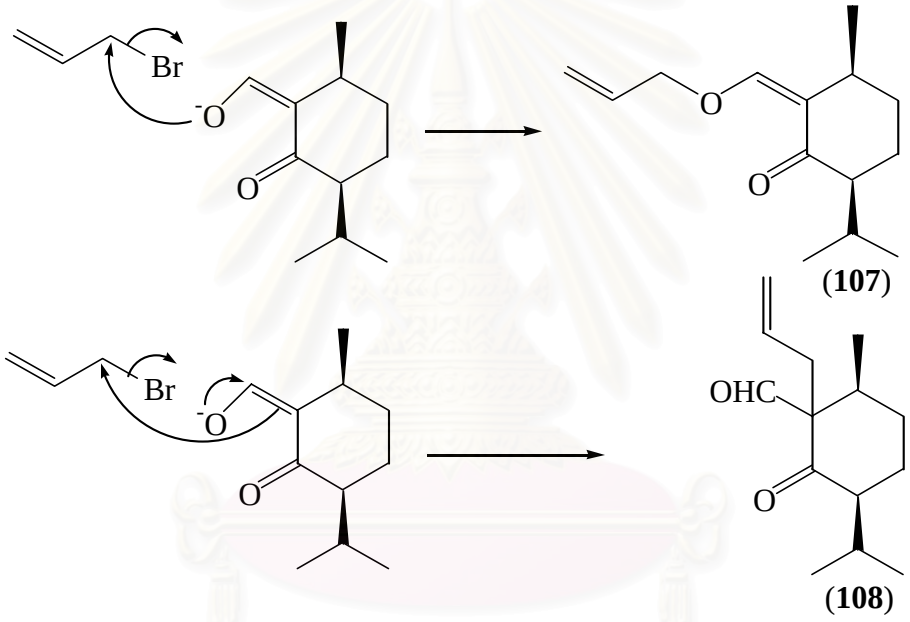
expected compound obtained compound

Figure 2 Structure of isomeric formylmenthones

Despite this setback, it was decided to use the formyl compound (**102**) for further studies. Allylation was attempted in order to introduce an additional substituent. Using simple classical conditions of potassium carbonate and allyl bromide in acetonitrile, a mixture of the desired C- allylation (**108**) and the undesired O-allylation products (**107**) was obtained (**Scheme 19, 20**). Interestingly, the C-allylated compound (**108**) appeared to be a single diastereomer. Separation of the two isomeric products was impossible and their identity was deduced from the crude ^{1}H NMR spectrum. The C-allylation product (**108**) clearly shows an aldehyde signal at 10.1 ppm, while the O-allylation product (**107**) shows an enol ether proton at 6.90 ppm. Both compounds showed signals for the allyl group. For the O-allylated compound (**107**), the allylic methylene proton were at 4.45-4.70 ppm., while for the C-allylated compound (**108**) they were at 2.65 and 2.75 ppm.



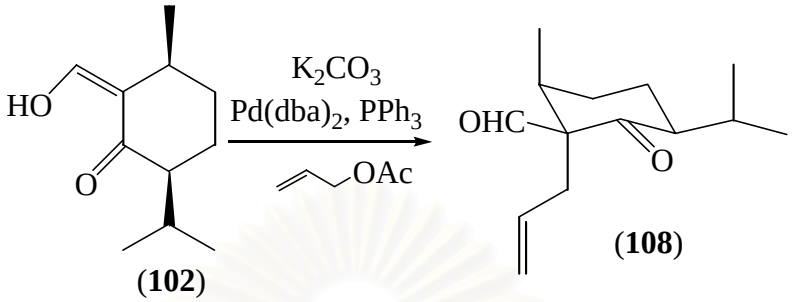
Scheme 19 Allylation of formylmenthone



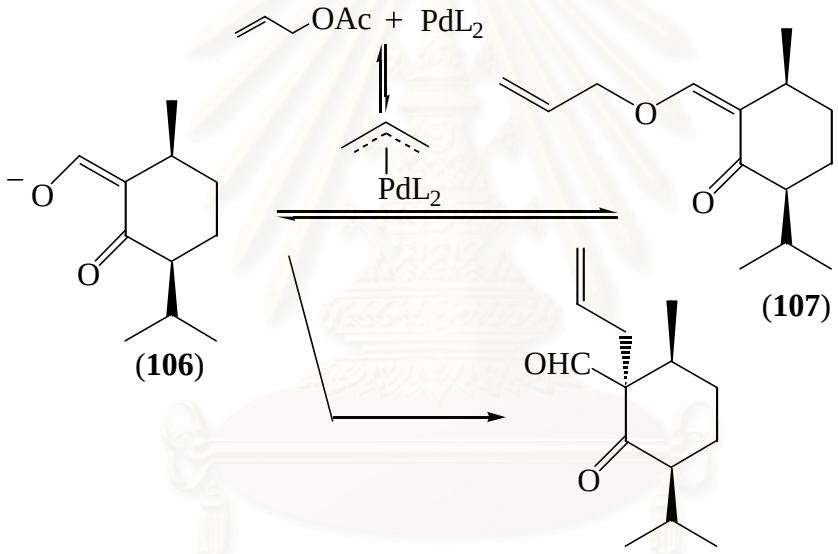
Scheme 20 The mechanism of the O- and C-allylation

The question of C-versus O-alkylation has been throughly studied.[50] The proportion of products can be influenced by the choice of couter-ion, leaving group and solvent. In general, C-alkylation is favoured by hard counter-ions, soft leaving groups and protic solvents. In our hands, however, mixtures were always obtained. The problem was solved by turning to organopalladium chemistry. Allylation under Trost's conditions[51] using allyl acetate and a $Pd(dba)_2$/ PPh_3 catalyst system gave exclusively the C-allylated product (**108**), once again as a single diastereomer. The C-selectivity can be

attributed to the reversibility of O-allylation (**107**) under the conditions of palladium catalysis, a point that has been demonstrated several times.[52]



Scheme 21 Allylation of formylmenthone under Trost'scondition

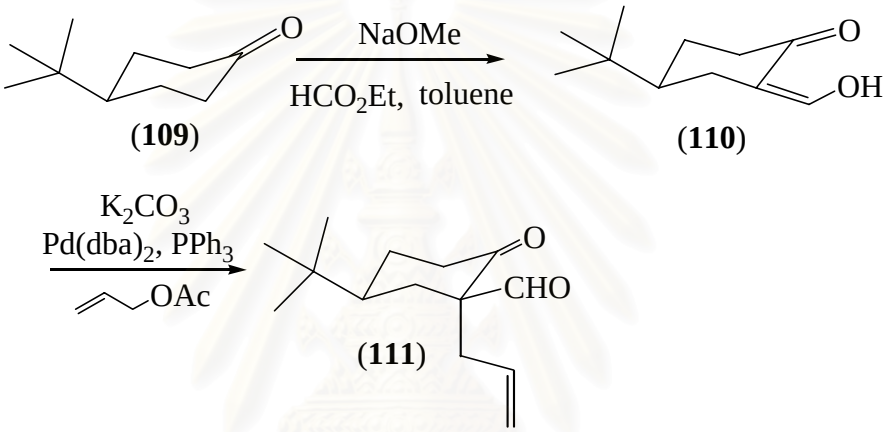


Scheme 22 The mechanism of allylation under Trost's condition

Why was only one stereoisomer of the C-allylated product formed ? An obvious reason is that the upper face of the molecule is shielded by the pseudo-axial methyl group. An additional possibility is that stereoelectronic effects also influence the outcome of the reaction. It is known that cyclohexenolates and related species preferentially react with electrophiles to give the axial product.[53] This can be attributed to the fact that the axial reaction proceeds via a chair transition state, while the equatorial reaction is constrained to proceed via a higher-energy boat transition state. Although there have been

numerous studies of alkylation and protonation reactions, this phenomenon has not, to our knowledge, been reported for palladium catalyzed reactions. Stereochemical studies on palladium catalyzed allylation reaction have focused on the acetate[54] and on the palladium ligands.[55]

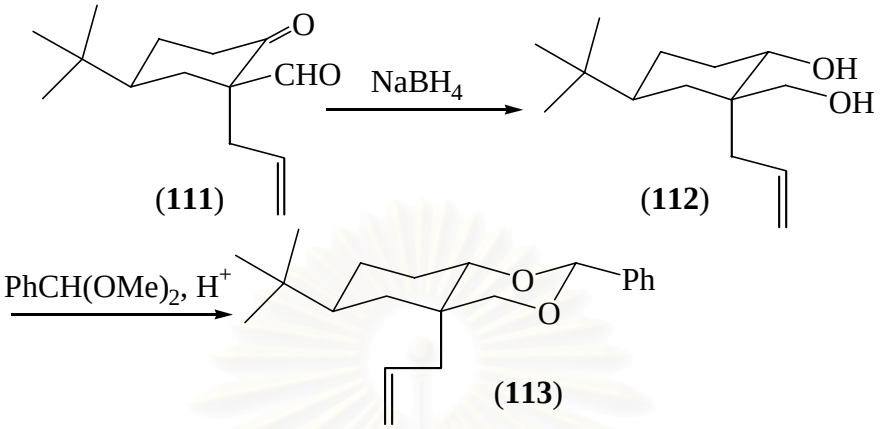
In order to test whether stereochemical control is influencing the reaction, 4-*t*-butylcyclohexanone (**109**) was selected. This compound has no steric bias, but it is locked in a single conformation by the *tert*-butyl group. It was formylated under the usual conditions, and then subjected to palladium catalyzed allylation (**Scheme 23**).



Scheme 23 Allylation with no steric bias

Once again, exclusive C-allylation was observed. On this occasion, however, two diastereoisomers were formed in a ratio of 7:1. The major diastereoisomer (**111**) was isolated by flash chromatography. The position of the allyl group was determined by, first, reduction to a diol (**112**) with sodium borohydride (**Scheme 24**). As expected, a single isomer was obtained. This has the secondary alcohol in an equatorial location.[56] This was determined by ^{1}H NMR. The methyne proton α to the hydroxy group was found to be a double doublet with one coupling constant equal to 11 Hz, typical for an axial-axial proton relationship.

Treatment of the diol with benzaldehyde dimethyl acetate and a catalytic amount of PPTS, yielded the benzylidene acetal (**113**).



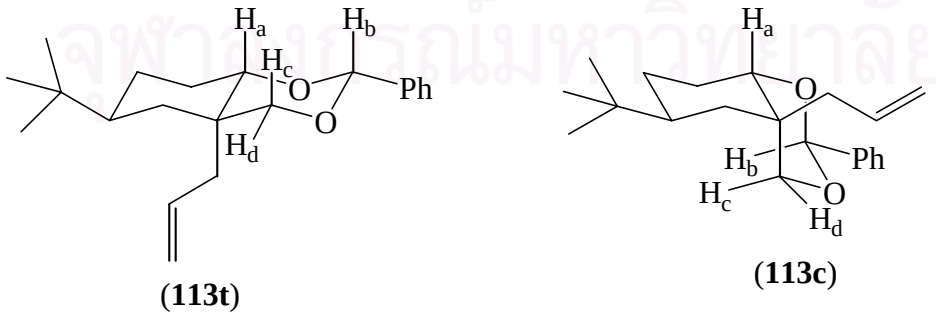
(**111**) (**112**) (**113**)

Scheme 24 Synthesis of a benzylidene acetal

Once again a single stereoisomer was obtained. The acetalization was very clean and the crude product was analyzed by NMR. A NOESY experiment revealed cross peaks between H_a, H_b and H_c as well as between the H_c-H_d pair. No cross peaks were observed between H_a, H_b or H_c and the protons of the allyl moiety (**Figure 3, 4**).

This is consistent with axial allylation, as the acetal would assume a *trans*-decalin structure (**113t**) with the phenyl group equatorial. H_a, H_b and H_c would then be axial and in close proximity on the dioxane ring.

If allylation had been equatorial, then the result would have been a *cis*-decalin (**113c**) structure, once again with the phenyl group equatorial. This structure places H_b and H_c in proximity, but far separated from H_a. If this has been produced the cross peaks between H_a and H_b or H_c would not have been observed.



(**113t**) (**113c**)

Figure 3 Possible benzylidene acetal structures

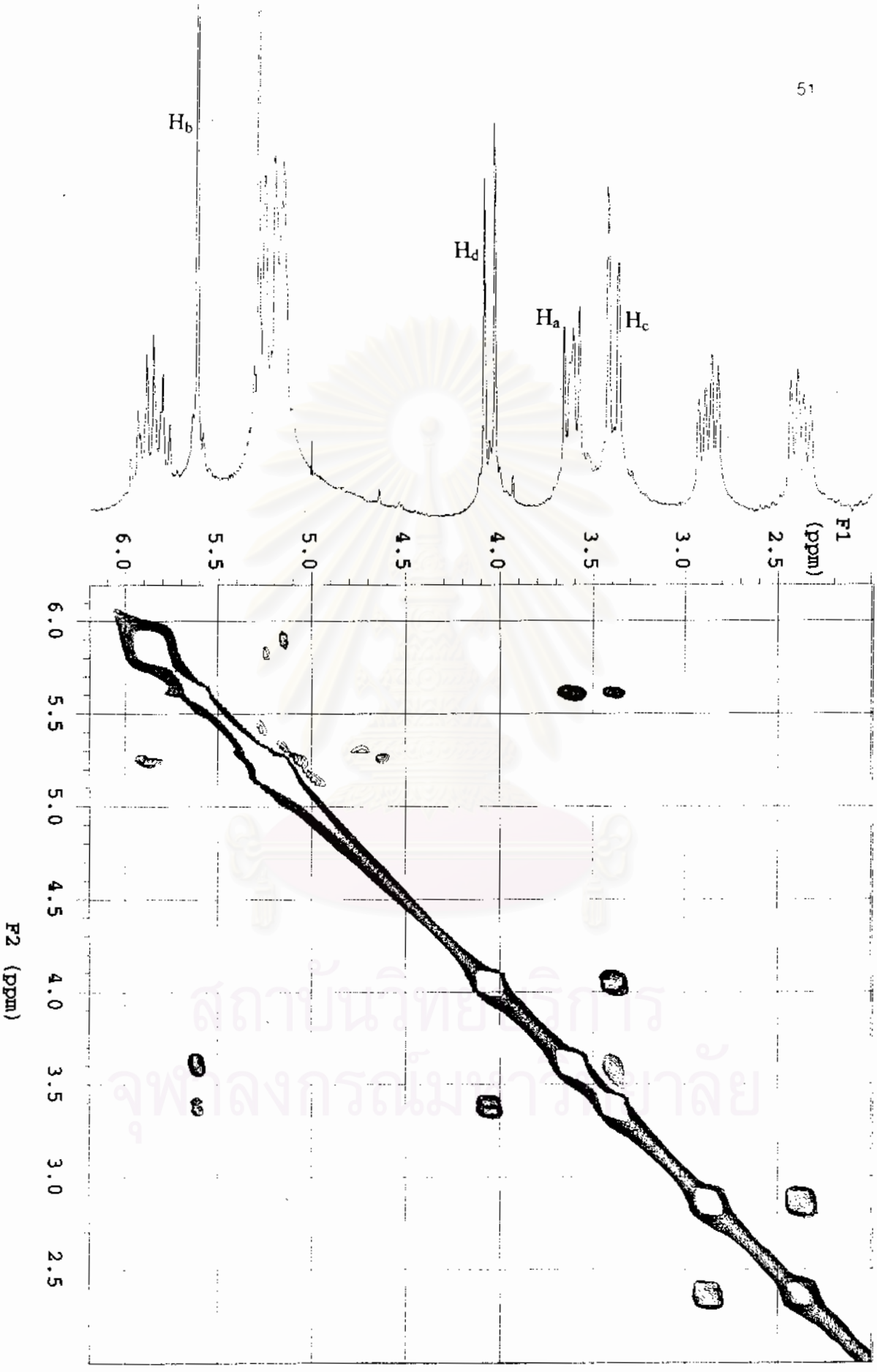
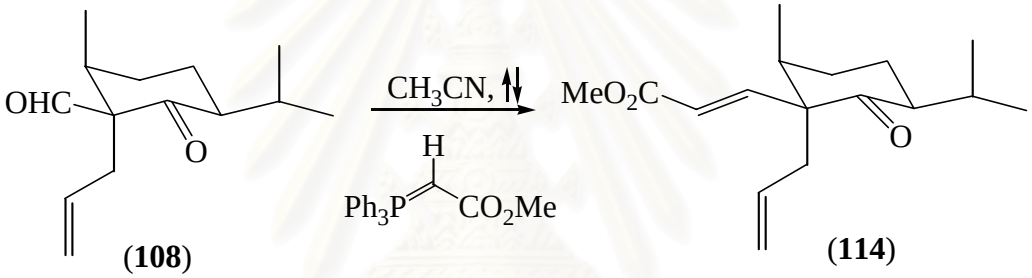


Figure 4 The NOESY spectum of benzylidene acetal (**113**)

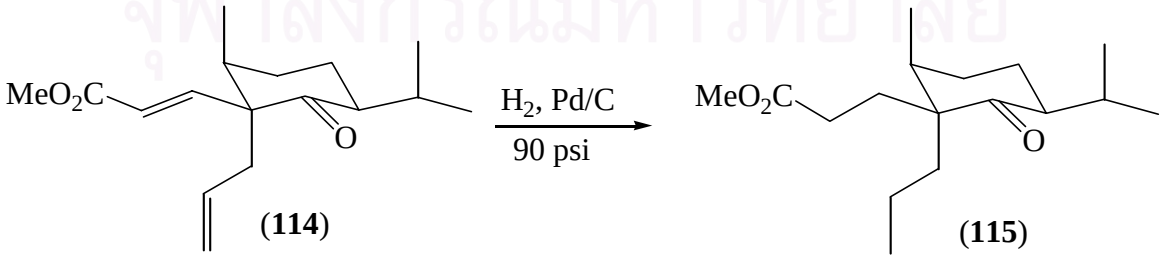
It can, therefore, be concluded that stereoelectronic effects are significant in palladium catalyzed allylation. We are not aware of any previous demonstration of this.

The aldehyde group of compound (**108**) was functionalized by a Wittig reaction using a stabilized ylid. With this reagent, aldehydes usually react at room temperature (**Scheme 25**).[57] Under these conditions, however, the aldehyde compound (**108**) failed to react. It was necessary to heat it with the ylid in acetonitrile to obtain the product. This is likely to be due to steric hindrance around the aldehyde. It has previously been reported that pivaldehyde does not react with this kind of ylid.[58] No reaction was observed at the ketone group. The alkene (**114**) was obtained principally as the *trans*-isomer, as indicated by the coupling constant of 16 Hz. The product was contaminated by a small amount of the *cis* isomer.



Scheme 25 The Wittig Reaction

Reduction of the double bonds was achieved by catalytic hydrogenation. At atmospheric pressure, only the mono-substituted alkene was reduced. The low reactivity of the other alkene must again be due to steric hindrance. In order to reduce the disubstitued alkene, it was necessary to increase the pressure to 100 psi. Under these conditions, both alkenes could be reduced in one night (**Scheme 26**).



Scheme 26 The reduction at high pressure

Reduction at 100 psi can be conveniently carried out using a Fisher- Porter tube (**Figure 5**).[59]

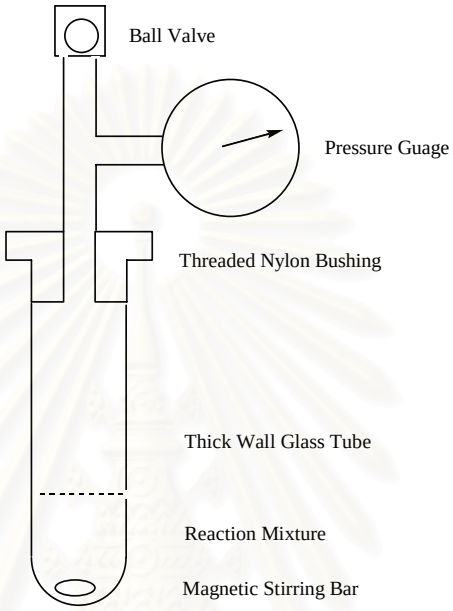
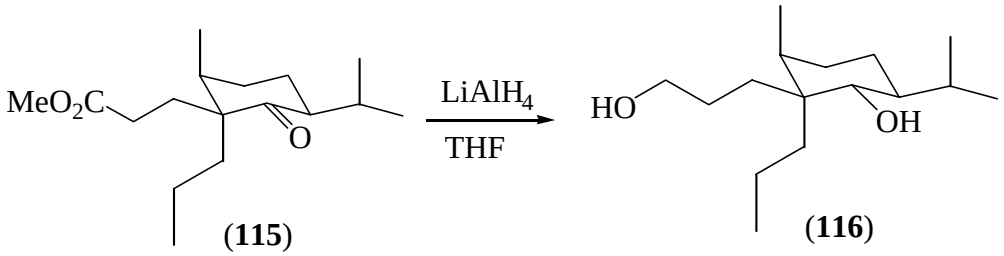


Figure 5 Fisher-Porter tube

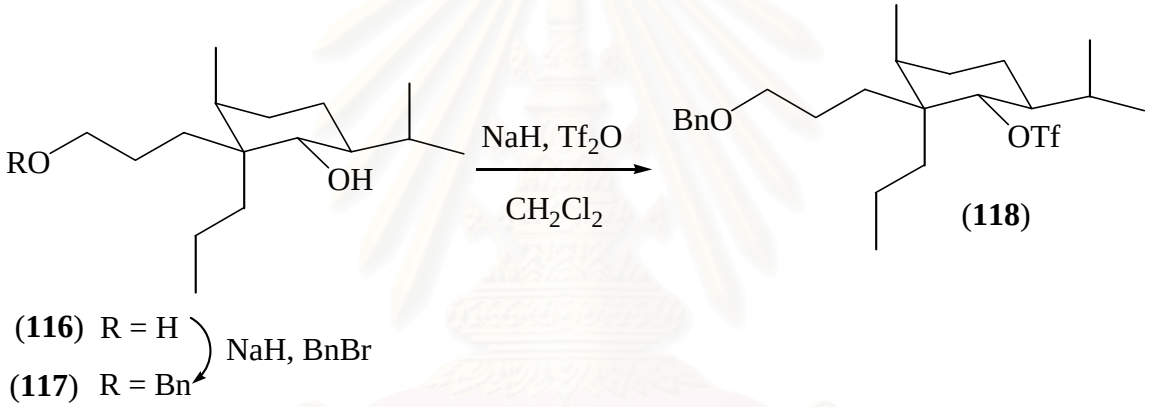
Treatment of ester (**115**) with lithium aluminium hydride in THF reduced both the ester and the ketone groups (**Scheme 27**). The major isomer from the reduction was found, by analysis of coupling constants, to be the equatorial secondary alcohol (**116**). A small amount of the axial alcohol was also obtained. Simple cyclohexanones typically give quite high equatorial : axial ratios in $LiAlH_4$ reductions.[57] The relatively low 3:1 ratio in this case is due to the steric hindrance of the upper face by the axial methyl group.



(**115**) (**116**)

Scheme 27 The reduction of the keto ester

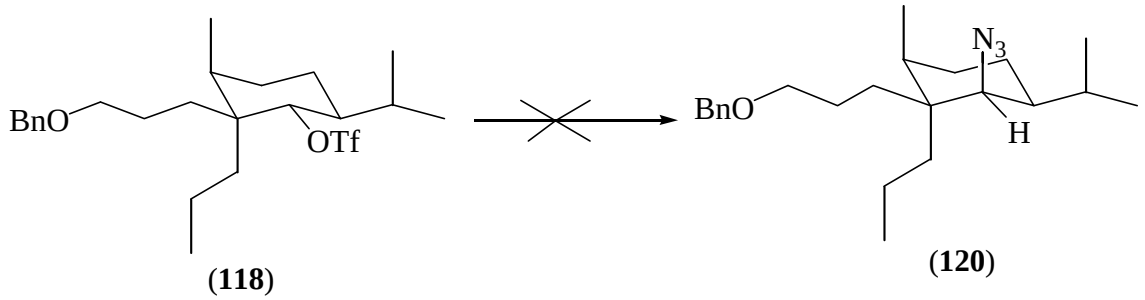
The primary alcohol of the diol (**116**) was protected as a benzyl ether (**117**) by a Williamson reaction. The remaining secondary alcohol was then converted to a leaving group; the triflate (**118**) (**Scheme 28**).



(**118**)

Scheme 28 The protection and activation of diol (**116**)

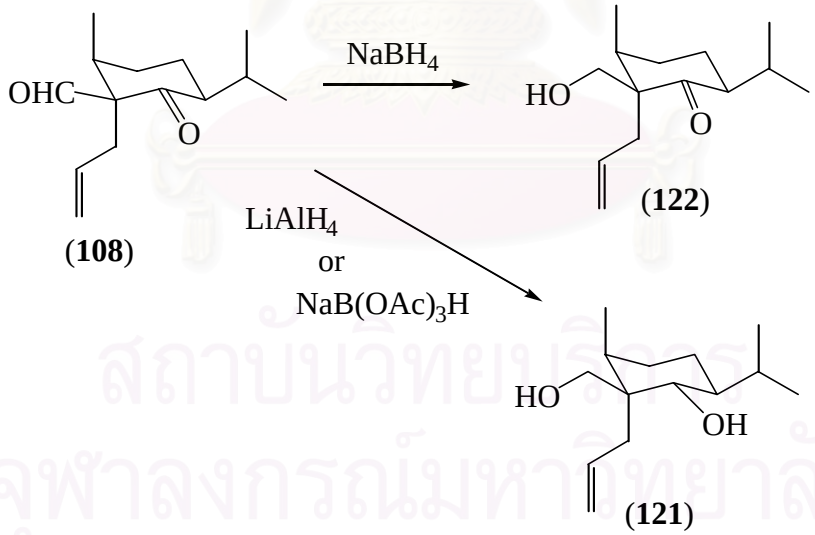
On treatment with azide (**Scheme 29**), however, none of the desired product (**120**) could be detected. This low reactivity, is once again due to steric hindrance on the upper face. This failure was despite using fairly vigorous phase-transfer conditions that have been successful with other difficult substrates.



(**118**) (**120**)

Scheme 29 Attempted displaceent by azide

In order to favour the difficult substitution reaction, it was thought that an intramolecular process would offer advantages.[60] To this end, keto aldehyde (**108**) was reduced with $LiAlH_4$ to the diol (**121**). $NaBH_4$ was found to be inefficient for complete reduction to the diol, however, under controlled conditions it could give good yields of the corresponding keto alcohol (**122**). Reduction to the diol (**121**) could also be achieved using sodium triacetoxyborohydride,[61] although reliable results with this reagent could not be achieved (**Scheme 30**).

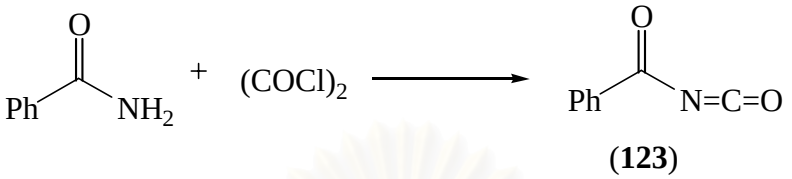


(**108**) (**122**) (**121**)

Scheme 30 The reduction of a keto aldehyde with selective reagents

The strategy was to use the primary alcohol group to attach a nitrogen nucleophile, and then to use this to displace the secondary alcohol. A mild method for

attaching a nitrogen is the reaction with an isocyanate. We selected, initially, benzoyl isocyanate (**123**) as the benzoyl group should be easily removable. Knapp has extensively used benzoyl isocyanate in order to generate tethered nitrogen moieties.[60]

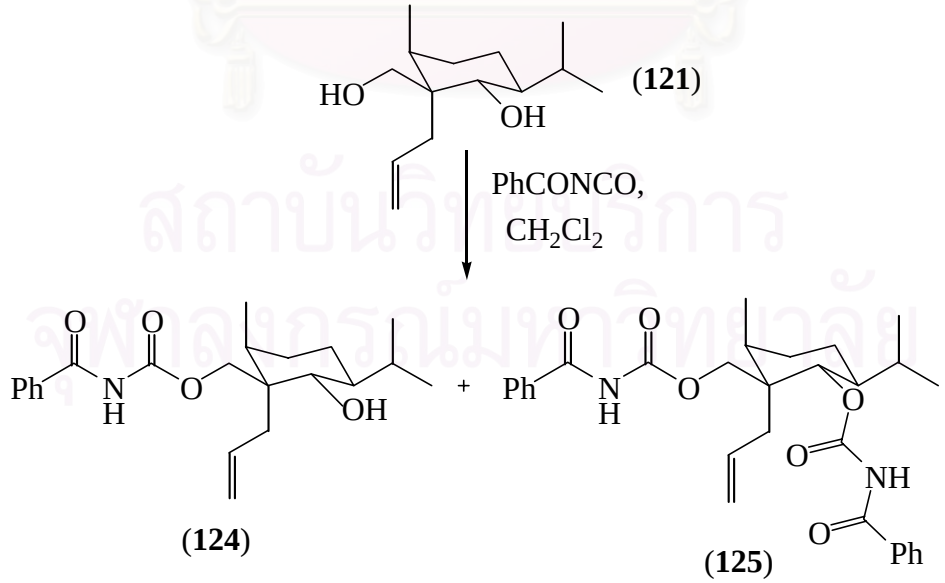


(**123**)

Scheme 31 Synthesis of benzoyl isocyanate

Considerable effort was put into finding a convenient method for preparing benzoyl isocyanate which is a highly water sensitive compoud. Monitoring by IR spectroscopy at 2240 cm^{-1} indicated that the reaction of benzoyl chloride with either sodium[62] or silver isocyanate[63] gave incomplete conversion. Eventually, we found that the best method is the treatment of benzamide with oxalyl chloride (**Scheme 31**).[64] In this way stock solutions of benzoyl isocyanate (**123**) could easily be generated.

Surprisingly, the reaction between the diol (**121**) and benzoyl isocyanate (**123**) was not selective for the primary alcohol group, even at –78 ^{o}C. Despite many attempts, it was impossible to obtain the mono-acylated product (**124**) in any more than trace amounts (**Scheme 32**).

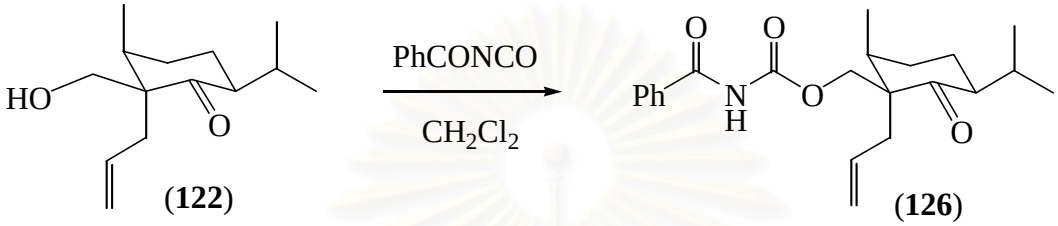


(**124**) (**125**)

.

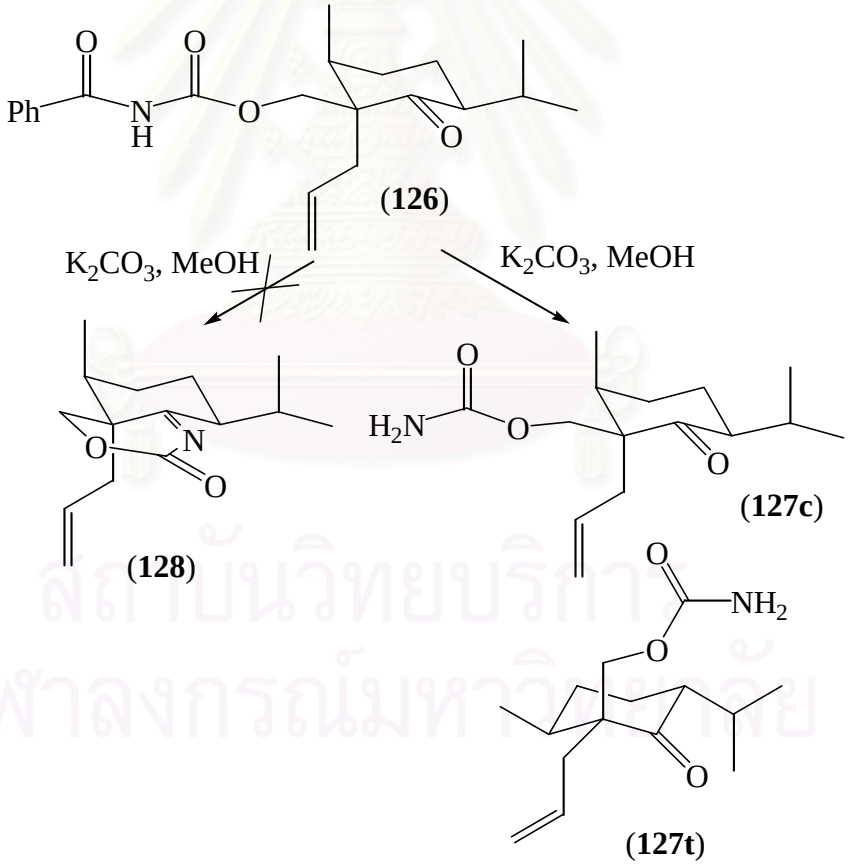
Scheme 32 Reaction of a diol with benzoyl isocyanate

Treatment of the keto alcohol (**122**) with benzoyl isocyanate (**123**), however, cleanly gave the expected derivative (**126**) (**Scheme 33**), which could be debenzoylated on treatment with methanolic potassium carbonate (**Scheme 34, 35**). Two compounds were obtained on methanolysis. The ^{1}H, ^{13}C NMR and IR spectra indicated that they were stereoisomers. The puzzle was solved by X-Ray crystallography.



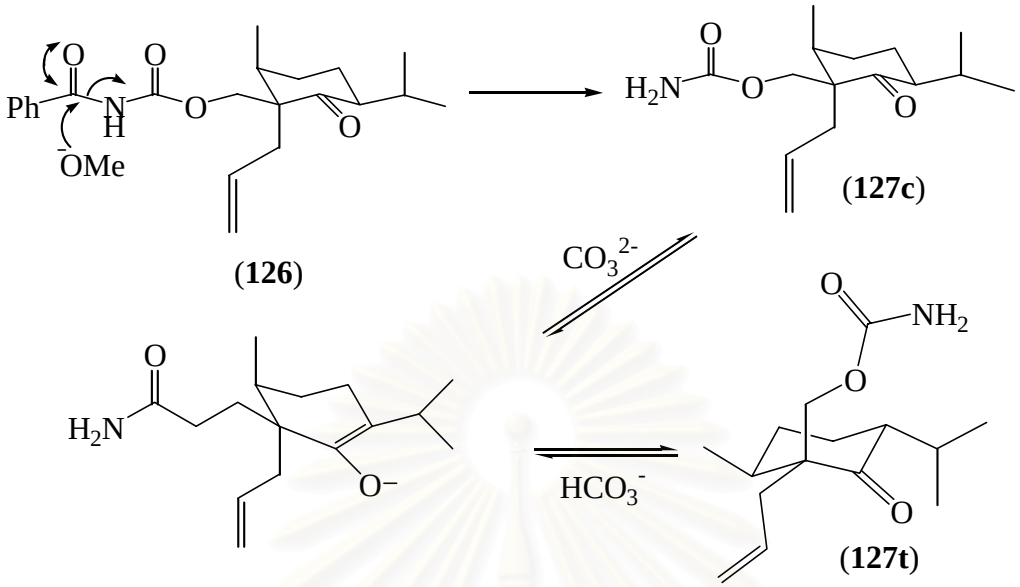
(**122**) (**126**)

Scheme 33 Reaction of a keto alcohol with benzoyl isocyanate



(**126**) (**128**) (**127c**) (**127t**)

Scheme 34 Methanolysis of (**126**)



Scheme 35 The mechanism of the Methanolysis and epimerization of (**126**)

Suitable crystals of one were prepared and it was found that the methyl and isopropyl groups were *trans*. Clearly the basic conditions of the methanolysis had reversed the epimerization observed during formylation. The serendipitous discovery that this is possible is an important observation because it means that this scheme may be employed both to prepare analogs of axisonitrile-3 and to synthesize the natural product itself. It is clear, however, that after the second epimerization it is necessary to use the allyl group as the source of the tether for introduction of an axial nitrogen. There, the two products of methanolysis are the two epimeric O-carbamate (**127c**), differing in the stereochemistry at the isopropyl group. One isomer ((**127t**) has the methyl and isopropyl groups *trans*, as in menthol; the other has them *cis*.

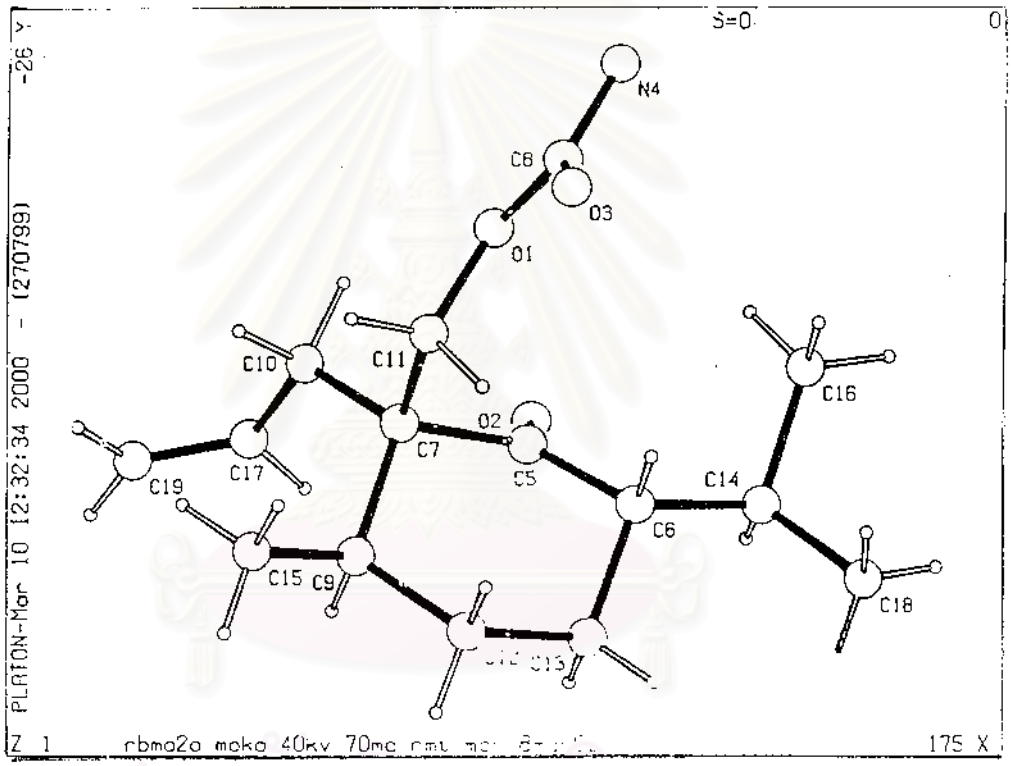
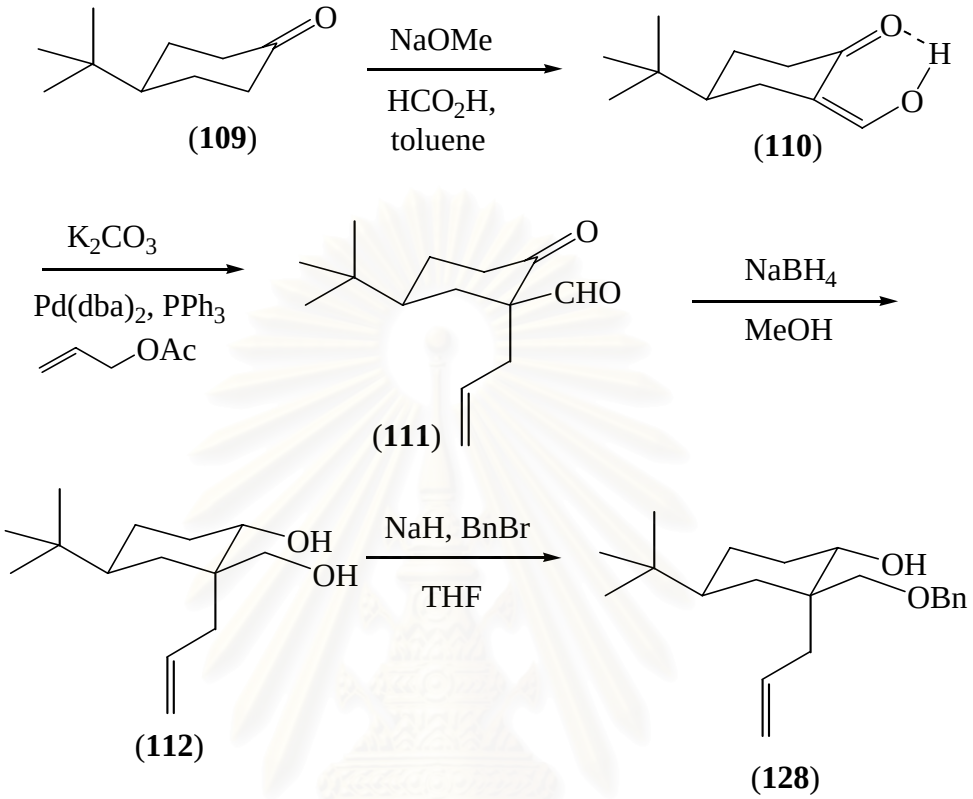


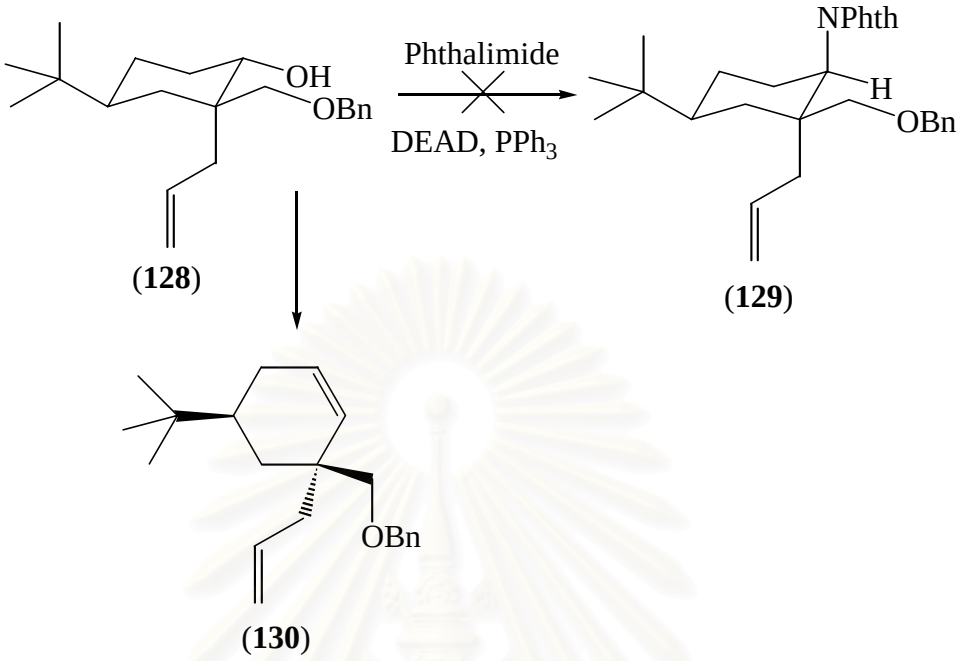
Figure 6 X-Ray Structure of carbamate **(127t)**

We also decided to study the reaction of a stereochemical more reliable system : the 4-*tert*-butylcyclohexanone system already described (**Scheme 36**).



Scheme 36 The reaction scheme for 4-*tert*-butylcyclohexane

Benzylation of the diol (**112**) could be achieved with modest selectivity for the primary alcohol over the secondary. However, under the usual Mitsunobu conditions, none of the expected substitution product (**129**) was obtained. The only identifiable product (**130**) was the alkene identified on the basis of the five vinyl protons and four vinyl carbons in the NMR spectra (**Scheme 37**). Obtaining an elimination product was very surprising as the cyclohexane is locked so that the leaving group cannot became axial. Previously it has been suggested that such eliminations take place via a skew-boat conformation, rather than a chair.[65]



Scheme 37 The Mitsunobu Reaction of a secondary alcohol

CHAPTER IV

CONCLUSION

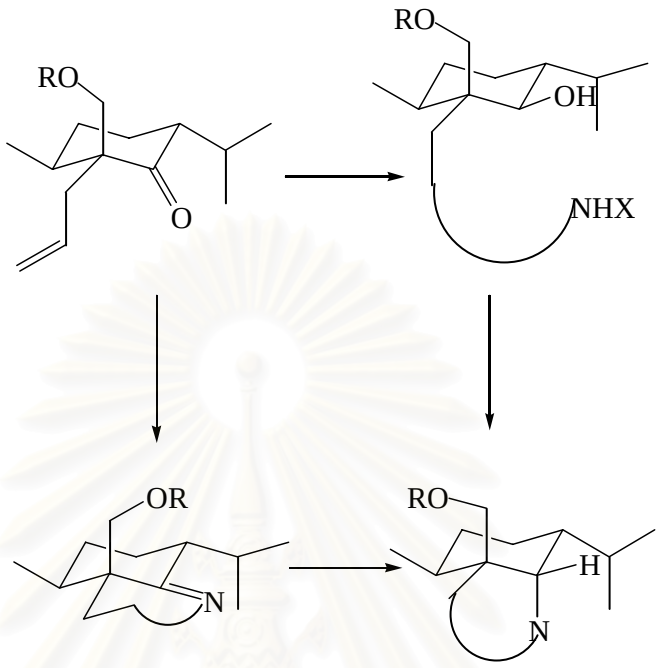
The results obtained with the substituted menthol systems clearly demonstrate how the steric hindrance around the reactive centre creates enormous difficulties for these transformations. It is clear that an intramolecular reaction is necessary to form the desired C-N bond.

Three important results have came from this work so far. Firstly, the synthetic isonitriles do have moderate antimalarial activity (3-6 μM), but a higher degree of substitution is required.

Secondly, we have shown that palladium catalyzed allylation is under stereoelectronic control. This reaction should be generally useful in organic synthesis.

Thirdly, we have shown that, although epimerization occurs during formylation, it can subsequently be reversed.

The immediated consequence of the third conclusion is that the allyl group must provide the tether for the nitrogen nucleophile in order to obtain an axial nitrogen (**Scheme 38**). Development of these reactions will allow the synthesis of additional analogs of axisonitrile-3 with higher degrees of substitution.



Scheme 38 Proposed solutions

REFERENCES

1. Lee, M. *Malaria in Search of Solutions*; *Chemistry in Britain*, **1996**, 28.
2. Martenes, P. *How Will Climate Change Affect Human Health?*; *American Scientist,* **1999**, *87*, 534.
3. Mann, J. *Murder, Magic and Medicine*, Oxford ; Oxford University, Press, **1992**, 202-207.
4. Klayman, D. I. *Qinghaosu (Artemisinin): An Antimalarial Drug from China*; *Science*, **1985**, *228*, 1049.
5. Haynes, R. K., and Vonwiller, S. C. *From Qinghao, Marvelous Herb of Antiquity, to the Antimalarial Trioxane Qinghaosu and some Remarkable New Chemistry; Acc. Chem. Res*., **1997**, *30*, 73.
6. Cafieri, F., Fattorusso, E., Magno, S., Santacroce C., and Sica D. *Isolation and Structure Axisonitrile-1 and Axisothiocyanate-1 two Unusual Sesquiterpenoids from the Marine* Sponge *Axinella cannabina*; *Tetrahedron*, **1973**, *29*, 2459.
7. Adinolfi, M., Napoli, L. De Blasio, B. D., Iengo, A., Pedone, C., and Santacroce, C. *The Absolute Configuration of the Axane Sesquiterpenes from the Sponge Axinella cannabina*; *Tetrahedron Lett.*, **1977**, *32*, 2815.
8. Piers, E., and Yeung, B. W. A. *Total Syntheses of the Sesquiterpenoids (±)-Axamide-1, Axisonitrile-1, and the Corresponding C-10 epimers*; *Can. J. Chem*., **1986**, *64*, 2475.
9. Piers, E., Yeung, B. W. A., and Rittig, S. J. *Methylenecyclohexane annulation. Total Syntheses of (±)-Axamide-1, (±)-Axisonitrile-1, and the corresponding C-10 epimers*; *Tetrahedron*, **1987**, *43*, 5521.
10. Fattorusso, E., Mango, S., Mayol, L., Santacroce, C., and Sica, D. *Isolation and Structure of Axisonitrile-2*; *Tetrahedron*, **1974**, *30*, 3911.
11. Fattorusso, E., Magno, S., Mayol, L., Santacroce, C., and Sica, D. *New Sesquiterpenoids from the Sponge Axinella cannabina*; *Tetrahedron*, **1975**, *31*, 269.
12. Ciminiello, P., Fattorusso, E., Magno, S., and Mayol, L. *New Nitrogenous Sesquiterpenes based on Alloaromadendrane and epi-Eudesmane skeletons from the Marine Sponge Axinella cannabina*; *Can. J. Chem*., **1987**, *65*, 518.
13. De Blasio, B., Fattorusso, E., Magno, S., Mayol, L., Redone, C., Santacroce, C.,

and Sica, D. *Axisonitrile-3, Axisothiocyanate-3 and Axamide-3. Sesquiterpenes with a novel Spiro [4,5] decane Skeleton from the Sponge Axinella cannabina; Tetrahedron,* **1976**, *32*, 473.

14. Dumdei, E. J., Flowers, A. E., Garson, M. J., and Moore, C. J. *The Biosynthesis of Sesquiterpene Isocyanates in the Marine Sponge Acanthella cavernosa (Dendy); Evidence for Dietary Transfer to the Dorid Nudibranch Phyllidiella pustulosa*; *Comp. Biochem. Physiol*., **1997**, *118A*, 1385.; Garson, M. J., private communication.
15. Caine, D., and Deutsch, H. *Total synthesis of (-)-Axisonitrile-3. An Application of the Reductive Ring Opening of the Vinylcyclopropenes*; *J. Am. Chem. Soc.*, **1978**, *100*, 8030.
16. Alvi, K. A., Tenenbaum, L., and Crews, P. *Anthelmintic Polyfunctional Nitrogen-Containing Terpenoids from Marine Sponges; J. Nat. Prod*., **1991**, *54*, 71.
17. Konig, G. M., Wright, A. D., and Sticher, O. *Two new Sesquiterpene Isothiocyanates from the Marine Sponge Acanthella klethra*; *J. Nat. Prod.*, **1992**, *55*, 633.
18. He, H-Y., Salva, J., Catalos, R. F., and Faulkner, D. J. *Sesquiterpene Thiocyanates from Axinyssa aplysinoides*; *J. Org. Chem*., **1992**, *57*, 3191.
19. Okino, T., Yoshimura, E., Hirota, H., and Fusetani, N. *New Antifouling Sesquiterpenes from Four Nudibranchs of the Family Phyllidiidae*; *Tetrahedron,* **1996**, *52*, 9447.
20. Burgoyne, D. L., Dumdei, E. J., and Andersen, R. J. *Acanthenes A to C; A Chloro, Isothiocyanate, Formamide, sesquiterpene Triad Isolated from the Northestern Pacific marine sponge Acanthella sp. and the dorid Nudibranch Cadlina luteomarginata*; *Tetrahedron,* **1993**, *49*, 4503.
21. Mayol, L., Piccialli, V., and Sica, D. *Nitrogen Sesquiterpenes from the marine sponge Acanthella acuta: Three new Isocyanide-isocyanide-isothiocyanate pairs*; *Tetrahedron*, **1987**, *43*, 5381.
22. Hirota, H., Okino, T., Yoshimura, E., Fusetani, N. *Five New Antifouling Sesquiterpenes from Two Marine Sponges of the Genus Axinyssa and the Nudibranch Phyllidia pustulosa*; *Tetrahedron,* **1998**, *54*, 13971.
23. Compagnone, R. S., Faulkner, D. J. *Metabolites of the Palauan Sponge Axinyssa aplysiniodes*; *J. Nat. Prod.*, **1995**, *58*, 145.
24. Ciminiello, P., Magno, S., Mayol, L. *Cis- Eudesmane Nitrogenous Metabolites*

from the Marine Sponges Axinella cannabina and acanthella acuta; *J. Nat. Prod*., **1987**, *50*, 217.

25. Simpson, J. S., Garson, M. J., Hooper, J. N. A., Cline, E. I., Angerhofer, C. K. *Terpene Metabolites from the Tropical Marine Sponge Axinyssa sp. nov.; Aust. J. Chem.,* **1997**, *50*, 1123.
26. Thompson, J. E., Walker, R. P., Wraltten, S. J., Faulkner, D. J. *A Chemical Defense Mechanism for the Nudibranch Cadlina luteomarginata; Tetrahedron*, **1982**, *38*, 1865.
27. Wolf, D., Schmitz, F. J. *New Diterpene Isonitriles from the sponge Phakellia pulcherrima; J. Nat. Prod.*, **1998**, *61*, 1524.
28. Chang, C. W. J., Patra, A., Backer, J. A., Scheuer, P. J. *Kalihinols, Multifunctional Diterpenoid Antibiotics from Marine Sponges Acanthella sp.; J. Am. Chem. Soc.*, **1987**, *109*, 6119.
29. Gribble, G. W. *Naturally Occuring Organohalogen Compounds; Acc. Chem. Res*., **1988**, *31*, 141*;* Gribble, G. W.*The diversity of Organochlorine in the living organism*; *Pure& Appl. Chem.*, **1996**, *68*, 1699.
30. Trimurtulu, G., Faulkner, D. J. *Six New Terpene Isonitriles from the Sponge Acanthella cavernosa*; *J. Nat. Prod*., **1994**, *57*, 501.
31. Miyaoka, H., Shimomura, M., Kimura, H., Yamada, Y. *Antimalarial Activity of Kalihinol A and New Relative Diterpenoids from the Okinawan Sponge, Acanthella sp.; Tetrahedron*, **1998**, *54*, 13467.
32. Pham, A. T., Ichiba, T., Yoshida, W. Y., Scheuer, P. J. *Two Marine Sesquiterpene Thiocyanates; Tetrahedron Lett*., **1991**, *32*, 4843..
33. Wright, A. D., Konig, G. M. *Antimalarial Activity: The search for Marine-Derived Natural Products with Selective Antimalarial Activity; J. Nat. Prod*., **1996**, *59*, 710.
34. Angerhofer, C. K., Pezzuto, J. M. *Antimalarial Activity of Sesquiterpens from the Marine Sponge Acanthella klethra; J. Nat. Prod.*, **1992**, *55*, 1787.
35. Mitsonubu, O., *The used of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products; Synthesis*, **1981**, 1; Hughes D. L. *Progress in the Mitsunobu Reaction. A review*; *Org Prep& Proc Int*., **1996**, *28*, 129; Simon, C., Hosztafi, S., and Makleit, S., *Application of the Misunobu Reaction in the field of Alkaloids*; *J. Het. Chem.*, **1997**, *34*, 349.
36. Koppel, I., Koppel, J., Degerbeck, F., Grehn, L., and Ragnarsson, U. *Acidity of*

Imidodicarbonates and Tosylcarbamates in Dimethyl Sulfoxide. *Correlation with Yields in the Misunobu Reaction; J. Org. Chem*., **1991**, *56*, 7172.

37. Van, Maarseveen, J. H., Oborye, E. H. H., Bolster, M. B., Scheeren, H. W., and Kruse C. G. *Transformations of the tetracyclic skeleton of Eudistomins by neighboring-group participation in the Mitsunobu Reaction; Red. Trav. Chim. Pays- bas*, **1995**, *114*, 27.
38. To our knowledge, the A-value of the phthalimido group is not known.
39. Ing, H. R., and Manske, R. H. F. *A Modification of the Gabriel Synthesis of Amines; J. Chem. Soc*., **1926**, 2348; Bates, R. W., Boonsombat, J., and Kaewpoonsri, N. unpublished results.
40. Ganem, B., Obsy, J. O., and Martin, M. G. *An Exceptionally mild Deprotection of Phthalimides; Tetrahedron Lett*., **1984**, *25*, 2093.
41. Staab, H. A. *New Methods of Preparative Organic Chemistry IV; Syntheses using Heterocyclic Amides (Azolides); Angew. Chem. Int. Ed.*, **1962**, *1*, 351; Staab, H. A., and Mannschreck A. *Synthesis von Carbosaureestern nach der Imidazolidmethode*; *Chem. Ber*., **1962**, *95*, 1284.
42. Strazzolini, P., Giumanini, A. G., and Cauci, S.; *Acetic formic anhydride*; *Tetrahedron*, **1990**, *46*, 1081.
43. Challis, B.C., and Challis, J.A. *The Chemistry of Amides.* Zabicky, J., ed., New York; Interscience, **1970**, 731.
44. Stephen, C. R., Bianco, E. J., and Pilgrim, F. J. *A New Reagent for Dehydrating Primary Amides under Mild Conditions; J. Am.Chem. Soc*., **1955**, *77*, 1701.
45. Baldwin, J. E., and O'Neil, I. A. *Trifluoromethanesulfonic Anhydride, a superior Reagent for the Conversion of Formamides to Isonitriles; Synlett*., **1990**, 603.
46. Beimann, K., Clerc, P., and Simon, S. *Table of Spectral Data for Structure Determination of Organic Compound*. nd., Berlin; Springer-Verlag, **1989** , c 200.
47. Richard, B., Brown, H. C., and Garc, C. P. *A Simple Procedure for the Chromic acid;* Sandborn, L. T. *l-menthone; Org. Syn*. Coll. Vol. I, **1941**, 340.
48. Tanaka, A., Tanaka, R., Uda, H., and Yoshikoshi, A. *Synthesis of Cubene –type Sesquiterpenenoids and the Stereochemistry of Cubebol; J. Chem. Soc. Perkin. I.*, **1972**, 1721.
49. Le Clour, D. D., Tolman, W. B. *Synthesis and Transition Metal Complexation of enantiomerically Pure Tris (pyrazolyl) hydroborate Ligand;*

J. Am. Chem. Soc., **1993**, *115*, 1153.

50. Jackmann, L. M., Lange, B. C. *Structure and Reactivity of Alkali Metal Enolates; Tetrahedron*, **1977**, *33*, 2737.
51. Bates, R., *Organic Synthesis using Transition Metals*, Sheffield; Sheffield Academic Press, **2000**, 131.
52. Trost, B. M., Runge, Juagheim, L. M. *A 1, 3-O to C-alkyl Shift Catalyzed by Palladium; J. Am. Chem. Soc*., **1980**, *102*, 2840;
Trost, B.M., Runge, T. A. *Palladium-catalyst. 1,3-oxygen to carbon alkyl shift basic studied*; *J. Am. Chem. Soc*. **1981**, *103*, 7550; Watsa, S. P., Knox, G. R., Heron, N. M. *A Novel palladium (0) catalysed tandem 1,3-allyl shift and Heck arylation*; *Tetrahedron Lett*., **1994**, *35*, 9763.
53. Deslongchamps, P. *Steroelectronic Effects in Organic Chemistry*. Oxford; Pergamon Press, **1983**, 280-284.
54. Heumann, A., Righer, M. *The Stereochemistry of the Palladium-catalysed Cyclisation Reaction Part B: Addition to π-allyl intermediates*; *Tetrahedron*, **1995**, *51*, 975.
55. Trost B. M., Van Vranken D. L. *Asymmetric Transition Metal-Catalyzed; Allylic Alkylation; Chem Rev*., **1996**, *96*, 395.
56. Brown, C.A. *The Rapid quantitative Reaction of Potassium Hydride with Weak Lewis acids. A Highly Convenient New Route to Hindered Complex Borohydrides*; *J. Am. Chem. Soc*., **1972**, *94*, 7159; Newman, H. *The Rapid Quantitative Reaction of Potassium Hydride with Weak Lewis Acids. A Highly Convenient New Route to Hindered Complex Borohydrides; J. Am. Chem. Soc*., **1973**, *95*, 4100.
57. Lawrence, N. J. *Preparation of Alkenes*. Williams J. M. J.ed. Oxford; OUP, **1996**, 19.
58. Loh T. P., private communication.
59. Modified from a design kindly provided by Professor L. S. Hegedus, Colorado State University
60. Knapp, S. *The Tethered Nitrogen in Natural Products Synthesis*; *Chem. Soc. Rev.*, **1999**, *28*, 61-72.
61. Gribble, G. W. *Chem. Soc. Rev*., **1998**, *27*, 395.
62. Kim, T. H., Rapoport, H. *2-Imidazolidinones from 1,2-amino alcohols;*

Application to the synthesis of a 2-Imidazolidinone Analogue of Pilocarpine; *J. Org. Chem*., **1990**, *55*, 3699; Pfister, J. R., Wymann, W. E. *A Useful Variant of the Curtius Reaction; Synthesis*, **1983**, 38; Deng M.-z., Caubere P. *Condensation of acyl chlorides on sodium cyanate: Preparation of acyl isocyanates*; *Tetrahedron*, **1988**, *44*, 6079.

63. Hunth, H., *Oxecyanogen II. The free radical; J. Am. Chem. Soc*., **1932**, *54*, 907; Shaw-Ponter, S., Mills, G., Robertson, M.,Bostwick, R. D., Hardy, G. W., Young, R. J. *Acyl carbamate Directing Group in Nucleoside synthesis Applications in the Synthesis of 2'-Deoxy-5-Ethyl-4'-Thiouridine*; *Tetrahedron Lett*., **1996**, *37*, 1867.
64. Speziate, A. J., Smith, L. R. *The reaction of Oxalyl Chloride with Amide, Part A. II.oxazolidinediones and Acyl Isocyanates, ; J. Org. Chem.,* **1963**,1805.
65. Limori, T. Ohtsuka, Y., and Oishi, T. *Regioselective dehydration in cyclic system with triphenylphosphine-azodicarboxylate; Tetrahedron Lett.,* **1991**, *32*, 1209.

APPENDIX

Table 2 GEOMETRY TABLES

FRACTIONAL ATOMIC COORDINATES & U(iso)

Atom	x/a	y/b	z/c	U(iso)
O(1)	0.3396(7)	0.0331(8)	0.9265(2)	0.058(2)
O(2)	0.5651(9)	0.1866(8)	0.8692(2)	0.077(3)
O(3)	0.2750(9)	0.0158(8)	0.9893(2)	0.074(3)
N(4)	0.152(1)	0.137(1)	0.9452(3)	0.070(3)
C(5)	0.581(1)	0.129(1)	0.9020(3)	0.053(3)
C(6)	0.633(1)	0.202(1)	0.9367(3)	0.062(4)
C(7)	0.561(1)	-0.025(1)	0.9036(3)	0.052(3)
C(8)	0.255(1)	0.059(1)	0.9561(3)	0.053(3)
C(9)	0.702(1)	-0.087(1)	0.9151(3)	0.071(4)
C(10)	0.510(1)	-0.081(1)	0.8656(4)	0.079(4)
C(11)	0.453(1)	-0.051(1)	0.9356(3)	0.059(3)
C(12)	0.770(1)	-0.007(1)	0.9497(3)	0.070(4)
C(13)	0.776(1)	0.143(1)	0.9441(3)	0.065(4)
C(14)	0.632(1)	0.357(1)	0.9330(3)	0.080(4)
C(15)	0.696(1)	-0.234(2)	0.9264(4)	0.086(4)
C(16)	0.483(2)	0.403(2)	0.9314(4)	0.112(6)
C(17)	0.603(2)	-0.086(2)	0.8303(4)	0.104(5)
C(18)	0.699(2)	0.425(2)	0.9697(4)	0.113(5)
C(19)	0.635(2)	-0.196(2)	0.8126(5)	0.142(7)
H(6)	0.574470	0.181060	0.958600	0.050000
H(9)	0.768210	-0.075270	0.892860	0.050000
H(11A)	0.438220	-0.148560	0.937920	0.050000
H(11B)	0.501680	-0.028290	0.962180	0.050000
H(12A)	0.859300	-0.042790	0.952790	0.050000
H(12B)	0.715540	-0.026490	0.972330	0.050000
H(13A)	0.816600	0.187850	0.966240	0.050000
H(13B)	0.832290	0.164670	0.920560	0.050000
H(14)	0.676740	0.383770	0.907790	0.050000
H(15A)	0.653410	-0.288590	0.906590	0.050000
H(15B)	0.638430	-0.246890	0.951160	0.050000
H(15C)	0.783200	-0.272490	0.933150	0.050000
H(16A)	0.431550	0.373400	0.906650	0.050000
H(16B)	0.470150	0.508180	0.928780	0.050000
H(16C)	0.427510	0.379000	0.953010	0.050000
H(17)	0.657810	0.001090	0.821360	0.050000
H(18A)	0.784400	0.389420	0.974020	0.050000
H(18B)	0.641420	0.383580	0.994520	0.050000
H(18C)	0.684060	0.512760	0.970290	0.050000
H(19A)	0.701490	-0.204610	0.789630	0.050000
H(19B)	0.597580	-0.280230	0.821640	0.050000
H(10A)	0.423400	-0.028630	0.858920	0.050000
H(10B)	0.474410	-0.173850	0.871110	0.050000

Temperature factor of the form: exp[-2pi^2U], U=U(iso)
or 1/3 SUM(i)SUM(j){U(ij)*astar(i).astar(j).a(i).a(j).cos(ij)}

Table 3

INTRAMOLECULAR BOND LENGTHS (H omitted)

Bond length limits based on covalent radii

O(1) - C(8)	1.33(1)	O(1) - C(11)	1.42(1)
O(2) - C(5)	1.25(1)	O(3) - C(8)	1.22(1)
N(4) - C(8)	1.32(2)	C(5) - C(6)	1.47(2)
C(7) - C(10)	1.48(2)	C(9) - C(15)	1.49(2)
C(12) - C(13)	1.49(2)	C(17) - C(19)	1.27(2)

Table 4

INTRAMOLECULAR BOND ANGLES (H omitted)

Bond length limits based on covalent radii

C(8) - O(1) - C(11)	116.2(8)	O(2) - C(5) - C(6)	121.7(11)
O(1) - C(8) - O(3)	121.6(11)	O(1) - C(8) - N(4)	112.5(10)
O(3) - C(8) - N(4)	25.9(11)		

Table 5

INTRAMOLECULAR TORSION ANGLES

Bond length limits based on covalent radii

C(11) - O(1) - C(8) - O(3)	1.3(10)	C(11) - O(1) - C(8) - N(4)	-179.9(14)
C(8) - O(1) - C(11) - H(11A)	77.5(12)	O(2) - C(5) - C(6) - H(6)	132.3(15)
H(12A)- C(12) - C(13) - H(13A)	-60.2(13)	H(12B)- C(12) - C(13) - H(13A)	61.5(13)

Table 6

INTERMOLECULAR NON-BONDED DISTANCES (H omitted)

Non-bonded distance limits based on Van der Waal's radii

Atom(1)		Atom(2)	dist	e.s.d.	ns	np	Ta	Tb	Tc	x(2)	y(2)	z(2)
O(2)	-	N(4)	2.91	0.01	3	1	0	0	1	0.65211	0.36252	0.80479
O(3)	-	N(4)	2.92	0.01	6	1	0	0	2	0.13748	0.15211	1.05479
O(3)	-	C(13)	3.51	0.01	6	1	0	-1	2	0.14321	-0.22423	1.05586
C(8)	-	C(19)	3.81	0.02	3	1	-1	-1	1	0.13464	-0.30410	0.93745
C(10)	-	C(15)	3.85	0.02	3	1	-1	-1	1	0.19636	-0.26631	0.82364
C(15)	-	C(18)	3.66	0.02	1	1	0	-1	0	0.69863	-0.57530	0.96968
O(2)	-	N(4)	2.91	0.01	3	1	0	0	1	0.65211	0.36252	0.80479
O(3)	-	N(4)	2.92	0.01	6	1	0	0	2	0.13748	0.15211	1.05479
O(3)	-	C(13)	3.51	0.01	6	1	0	-1	2	0.14321	-0.22423	1.05586
C(8)	-	C(19)	3.81	0.02	3	1	-1	-1	1	0.13464	-0.30410	0.93745
C(10)	-	C(15)	3.85	0.02	3	1	-1	-1	1	0.19636	-0.26631	0.82364
C(15)	-	C(18)	3.66	0.02	1	1	0	-1	0	0.69863	-0.57530	0.96968

---

ns is the symmetry operator number - (* denotes inversion indicator)
np is the lattice point number
Ta,Tb & Tc are unit cell translations. The symmetry operations are:

1 + X, + Y, + Z
2 - X, - Y, 1/2 + Z
3 1/2 + X, 1/2 - Y, 3/4 - Z
4 1/2 - X, 1/2 + Y, 1/4 - Z
5 - Y, - X, 1/2 - Z
6 + Y, + X, - Z
7 1/2 + Y, 1/2 - X, 3/4 + Z
8 1/2 - Y, 1/2 + X, 1/4 + Z

VITA

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