Studies on Antifouling Substances against Cypris Larvae of the Barnacle *Balanus amphitrite*

タテジマフジツボキプリス幼生に対する

付着阻害物質に関する研究

2004

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Contents

Abstract

Macrofouling on ship hulls and man-made surfaces submerged in seawaters causes technical and economic problem worldwide. To prevent settlement of sessile organisms, paints containing organotin compounds, such as TBT and TBTO, and cuprous oxide compounds have been commonly used. However, the use of these metal-based compounds has been brought to public attention by many reports of environmental contamination. Therefore, antifouling agents that are not only effective and but also environmentally benign are urgently needed.

In order to apply not only effective but also "environmentally benign" antifouling agent, attempts were made to search novel antifouling substances from marine invertebrates, and to synthesize variety isocyano and related compounds based on 3-isocyanotheonellin, reported as a potent antifouling compound.

1. Antifouling activity of marine invertebrates and isolation of antifouling sesquiterpenes from the marine sponge *Acanthella cavernosa*

A total of 118 Japanese marine invertebrates were extracted with methanol, and their chloroform-soluble materials were tested for antifouling activity as well as toxicity against cyprids of the barnacle *Balanus amphitrite*. Among 86 species that showed more than 80 % inhibition of larval settlement, 13 species, including 8 sponges were weakly toxic to cyprids larvae.

The marine sponge *Acanthella cavernosa* which showed most promising activity was extracted with ethanol, whose extract was fractionated by solvent partitioning, silica gel column chromatography, gel filtration, and ODS HPLC to afford two active compounds. They were identified as 10-formamido-4-cadinene and T-cadinol, on the basis of spectral data and chemical transformation. They inhibited larval settlement with EC_{50} values of 0.50 and 0.53 μg/mL, respectively.

2. Synthesis and antifouling activity of isocyano compounds

3-Isocyanotheonellin, isolated from the nudibranch *P. pustulosa*, is a sesquiterpene of the bisabolone class with an isocyano functional group at the C-3 position. Despite of its simple structure, 3-isocyanotheonellin showed potent settlement inhibition activity against barnacle larvae without toxicity to larvae.

To develop efficient and environmentally benign antifouling agents, an attempt has been made to synthesize isocyano compounds based on structure of 3-isocyanotheonellin, and to evaluate their antifouling activity and toxicity against cyprids larvae of the barnacle, *Balanus amphitrite*. A total of 59 derivatives were

synthesized: 20 isocyanocyclohexane compounds, including 3-isocyanotheonellin and its analogues; 27 isocyanobenzene compounds and related compounds, and 12 simple isocyanides, such as linear alkyl-chain isocyanides. They were examined for their settlement inhibition activity against cyprids larvae.

Many synthesized isocyano compounds inhibited larval settlement with EC_{50} values of 0.01-20.0 □g/mL, while their toxicity to cyprids larvae were much less than that of $CuSO_4$. Some isocyanides and acetamide showed better of antifouling against barnacle larvae compared to antifouling active natural isocyano compounds, despite their simple structures. These results suggest that simple isocyanides would be promising non-toxic antifouling agents.

3. Field experiment of antifouling test paints using synthesized compounds

As described in the previous chapter, some synthesized compound showed promising activity. In order to examine whether these compounds prevent the settlement of fouling organisms in the field, promising compounds *N*-(4-hexylphenyl) acetamide (paint A) and 1,1-dimethyl-10-undecyl isocyanide (paint B) were incorporated in paints and evaluated their antifouling activity in the fields. Field experiments were carried out at two field sites; Shizugawa bay, Miyagi and Tokyo bay, Tokyo for 3 and 2 months, respectively.

In both field experiments, test paint A and B showed promising performance as antifouling paint. These paints surface were settled by diatoms and hydrozoas, however the macrofouling organisms, such as barnacles, ascidians, and bryozoans, were not observed on their surfaces during the test period. These results suggest that isocyanides are very useful model compounds for the development of environmentally benign antifouling paints.

As described above, some isocyanides showed a good ability of antifouling against barnacle larvae compared to antifouling active natural isocyano compounds, despite their simple structures. These results suggested that simple isocyanides would be promising non-toxic antifouling agents.

INTRODUCTION

1. Background of this research

The term "fouling" is commonly employed to distinguish the assemblages of animals and plants, which grow on artificial structures from those occurring on rocks, stones, and other natural objects. Frequently, its use is limited to situations of the excessive growth may be considered harmful to the human communities. Fouling results from the growth of marine sessile organisms, such as barnacles, mussels, tubeworms, and algae, on the surface of submerged objects, and causes technical and economic problems worldwide.[1-3] The fouling on ships'hulls results in a reduction of speed, an increased cost in fuel, and losses in time and money in applying the necessary remedial ships. Fouling gives serious trouble when it occurs in pipes and conduits used for cooling water of power plants on shore. Flow is interfered with due to the decreased size of the channel and increased roughness of the surface. Marine fouling on aquaculture nets cause death of fish from oxygen deficiency. The costs of antifouling measures are estimated to be at least US $1-4 billions/year in the world.[4]

In order to prevent marine organisms from fouling on surface, paints containing metal-based compounds have been widely used. Using of TBT-based antifouling paints is estimated to save the shipping industry at least US $5.7 billions/year.[5] Although organotin compounds, such as TBT (tri-*n*-butyltin) and TBTO (tri-*n*-butyltin oxide), are very effective, they are found to be toxic to most marine organisms,[6-9] which led to regulations and bans of their use in various countries.[10,11] Since 1992 the use of organotin compounds has been banned in Japan. The Marine Environment Protection Committee of the International Maritime Organization is currently proposing a total ban by the year 2008.[12] Therefore, antifouling agents that are not only effective but also environmentally benign are urgently needed.

2. Tin-Free Antifouling Paints[13]

2-1. Copper paints

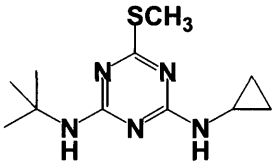
Copper has been employed for bacteriocide, molluscicide, and fungicide agents for a long time. Copper is used as the most important agent for antifouling paints beside organotin compounds. The alternative and/or new tin-free antifouling coatings usually contain copper-based compounds, such as cuprous oxide, which act mainly as cupric ion in seawater.[14] The use of cuprous oxide paints that are effective for preventing settlement of sessile organisms has been brought to public concern due to their adverse effects on environment.[15,16]

2-2. Organic Booster Biocides

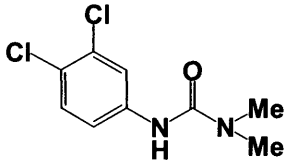
The alternative antifouling paints are usually containing booster biocides,[17,18] which show broad-spectrum of toxicity to organisms. The biocides used most recently are as follows:[18]

1) Irgarol 1051: 2-methylthio-4-*tert*-butylamino-6-cyclo-propylamino-*s*-triazine.
2) Diuron: 3,(3,4-dichlorophenyl)-1,1-dimethylurea.
3) Zinc pyrithione: 2-mercaptopyridine N-oxide zinc salt.
4) KH101: pyridine triphenyl borane.
5) Sea-Nine 211: 4,5-dichloro-2-*n*-octyl-2(2H)isothiazolone.
6) Copper pyrithione: 2-mercaptopyridine N-oxide copper salt.

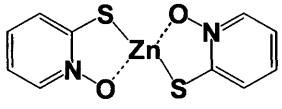
These booster biocides affect development of sea urchin embryos at low concentrations. In addition, Irgarol 1051 is highly toxic to mostly marine algae, with the growth inhibition at concentrations as low as 50ng/L. Some reports suggest that such antifouling biocides were positively detected above the concentrations of inhibition of algal growth.[10,17,19]



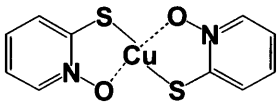
Irgarol 1051



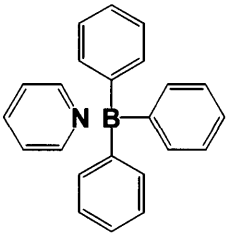
Diuron



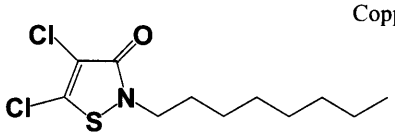
Zinc pyrithione



Copper pyrithione



KH101



Sea-Nine 211

2-3. Fouling-Release Coatings

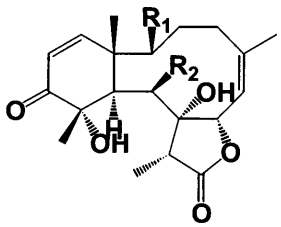
Silicon coatings are called "fouling-release coatings" to differentiate them from the self-polishing antifouling paints containing biocides. Ideally, these "nonstick" coatings should completely prevent from the fouling. Actually, the silicon coatings get fouled only weakly. Even though once a fouling organism settled to the surface, the fouling was removed easily.[20] However silicon coatings are very expensive, and show short durability performance.

3. Marine natural products as antifoulants[10, 13, 21, 22]

Many marine benthic invertebrates, such as ascidians, sponges, and coelenterates are generally free from epibionts, which is believed to be due to their chemical defenses. Therefore, secondary metabolites of such organisms are thought to be potential antifouling agents.[21-26] A variety of natural products with antifouling activity have been isolated from marine organisms, some of which are promising as non-toxic antifouling agents. Important antifouling substances derived from marine organisms are as follows.

3-1. Terpenoids

Renillafoulins **A-C** (**1-3**), isolated from the sea pansy *Renilla reniformis*, inhibited the settlement of barnacle larvae with EC_{50} values ranging from 0.02 to 0.2 μg/mL.[27, 28]

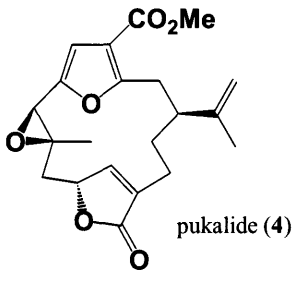


renillafoulin A (**1**) R_1=R_2= **Ac**

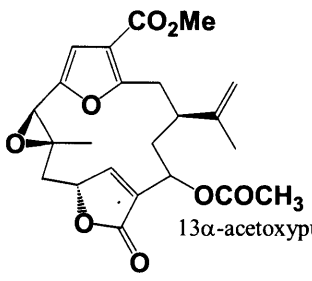
renillafoulin B (**2**) R_1= **Ac**, R_2= C_2H_5CO

renillafoulin C (**3**) R_1= **Ac**, R_2= C_3H_7CO

Pukalide (**4**),[29] obtained from the gorgonian *Leptogorgia virgulata*, showed potent antifouling activity against cypris larvae with an EC_{50} value of 19 ng/mL.[30] A related compound, 13α-acetoxypukalide (**5**),[31] isolated from a soft coral *Sinularia* sp. collected off the Palau Islands, showed a potent inhibitory activity against the settlement of cypris larvae with a MIC value of 0.1 μg/mL, while it showed only a slight effect against the blue mussel settlement.[32]

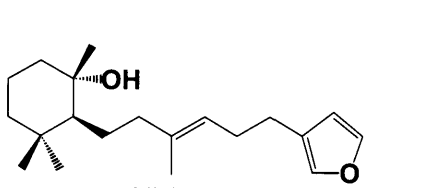


pukalide (**4**)

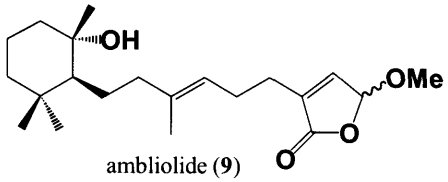


13α-acetoxypukalide (**5**)

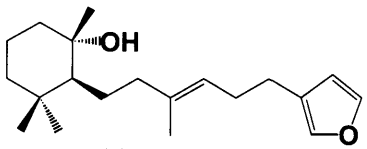
Six furanoterpenes, ambliol A (**6**),[33] ambliol C (**7**),[34] ambliofuran (**8**),[33] ambliolide (**9**),[33] dehydroambliol A (**10**),[33] and Pallescensin A (**11**)[34] were isolated from the marine sponge *Dysidea amblia*. Among them, compounds **6** and **11** exhibited settlement inhibition activity against the polychaete *Salmacina tribranchiata* at 10 μg/mL.[35] Idiadione (**12**) from the marine sponge *Leiosella idia* showed settlement inhibition activity against the polychaete *S. tribranchiata* at 10 μg/mL.[35]



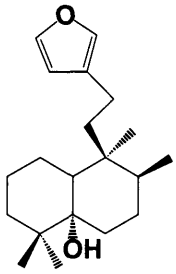
ambliol A (**6**)



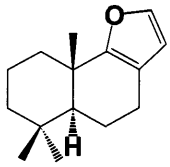
ambliolide (**9**)



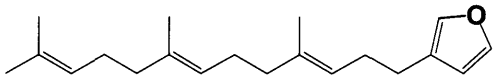
dehydroambliol A (**10**)



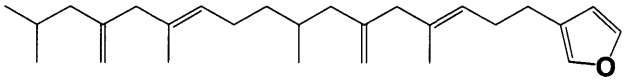
ambliol C (**7**)



pallescensin A (**11**)



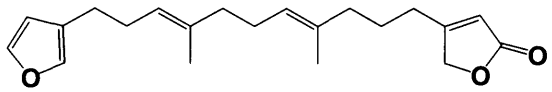
ambliofuran (**8**)



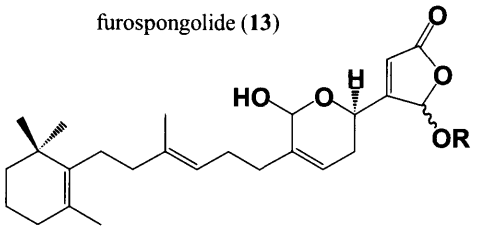
Idiadione (**12**)

A linear furanoterpene, furospongolide (**13**) isolated from the marine sponge *Phyllospongia papyracea*, inhibited barnacle settlement at 20 μg/cm^2.[36]

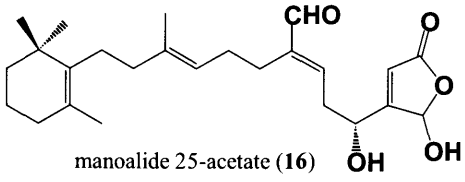
Four manoalides, manoalide (**14**),[37] *seco*-manoalide (**15**),[38] manoalide 25-acetate (**16**),[39] and (4*E*, 6*E*)-dehydromanoalide (**17**),[40] isolated from a marine sponge *Smenospongia* sp., showed antifouling activity against the barnacle *B. amphitrite* larvae with EC_{50} values of 0.24, 0.80, 0.53, and 2.7 μg/mL, respectively.[41]



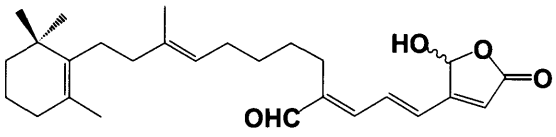
furospongolide (**13**)



manoalide (**14**) R= H
seco-manoalide (**15**) R= Ac

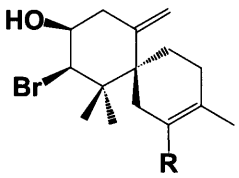


manoalide 25-acetate (**16**)

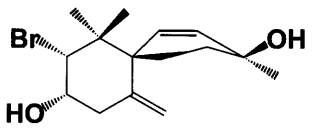


(4*E*, 6*E*)-dehydromanoalide (**17**)

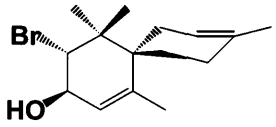
Elatol (**18**)[42] and deschloroelatol (**19**)[43] are popular metabolites of red algae of the genus *Laurencia*. Compound **18** inhibited larval settlement of the barnacle *B. amphitrite* and the bryozoan *Bugula neritina* at 0.01, and 0.01 μg/cm^2, respectively; compound **19** also inhibited at 0.1 and 0.01 μg/cm^2, respectively.[44] Two sesquiterpenes, rigidol (**20**) and (-)-10α-bromo-9β-hydroxy-α-chamigrene (**21**) were isolated from the red alga *L. rigida* along with compounds **18** and **19**.[45] Compounds **18-21** showed antifouling activity against the alga *Chirella fusca*.[45]



elatol (**18**) R = Cl
deschloroelatol (**19**) R = H



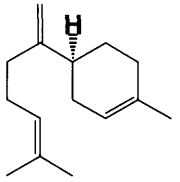
rigidol (**20**)



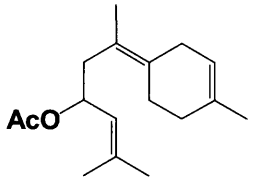
(-)-10α-bromo-9β-hydroxy-α-chamigrene (**21**)

(-)-β-Bisabolene (**22**)[46] from a Japanese soft coral *Sinularia* sp. was antifouling against blue mussel *M. edulis*,[47] while 3-acetoxy-*E*-γ-bisabolene (**23**) isolated from the red alga *L. rigida*, showed antialgal activity against *C. fusca*.[45]

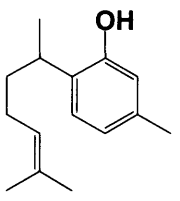
Aromatic sesquiterpene, (+)-curcuphenol (**24**)[48,49] and (+)-curcudiol (**25**)[48] were reisolated from the marine sponge *Myrmekioderma dendyi* as settlement inhibition compounds against barnacle cyprids with EC_{50} values of 2.5 and 2.8 μg/mL, respectively.[41]



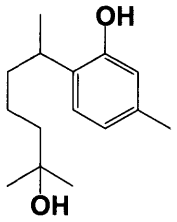
bisabolene (**22**)



3-acetoxy-*E*-γ-bisabolene (**23**)

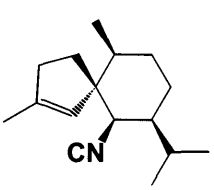


(+)-curcuphenol (**24**)

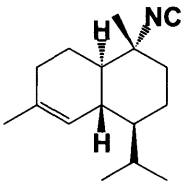


(+)-curcudiol (**25**)

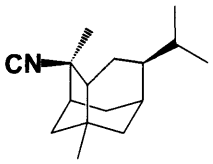
A total of 10 antifouling terpenoids was isolated from four nudibranchs, *Phyllidia pustulosa*, *P. ocelata*, *P. varicose*, and *Phillidiopsis krempfi*,[50,51] that is 4 novel compounds, 10-epiaxisonitrile-3 (**26**),[50] 10-isocyano-4-cadinene (**27**),[50] 2-isocyanotrachyopsane (**28**),[50] and 1,7-epidioxy-5-cadinene (**29**),[50] along with known axisonitrile-3 (**30**),[52] 10-isothiocyanato-4-amorphene (**31**),[53] 10-isocyano-4-amorphene (**32**),[53] 2-thiocyanatoneo-pupukeanane (**33**),[54] 4-thiocyanatoneo-pupukeanane (**34**),[54] and 3-isocyanotheonellin (**35**).[55,56] Antifouling activity of these compounds (**26** - **35**) are summarized in Table 1.[50,51]



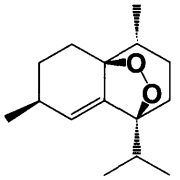
10-epi-axisonitrile (**26**)



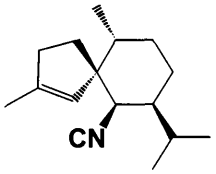
10-isocyano-4-cadinene (**27**)



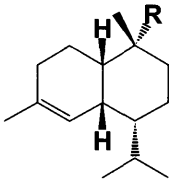
2-isocyanotrachyopsane (**28**)



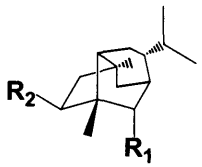
1,7-epidioxy-5-cadinene (**29**)



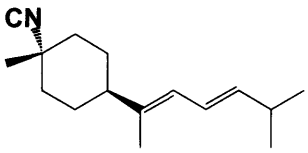
axisonitrile-3 (**30**)



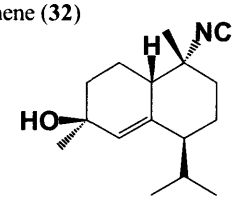
(-)-10-isothiocyanato-4-amorphene (**31**)
R= **NCS**
10-isocyano-4-amorphene (**32**)
R= **NC**



2-thiocyanato*neo*pupukeanane (**33**)
R_1= **SCN** R_2= **H**
4-thiocyanato*neo*pupukeanane (**34**)
R_1=**H** R_2= **SCN**



3-isocyanotheonellin (**35**)

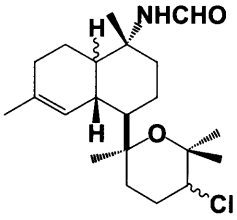


10-isocyano-5-cadinene-4-ol (**36**)

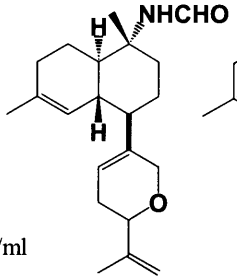
Table1. Antifouling Activities of Sesquiterpenes from Nudibranchs of the Family Phyllidiidae[51,57]

Compounds No.	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
Activity [EC_{50} (μg/mL)]	10	0.14	0.33	>50	3.2	7.2	0.70	4.6	2.3	0.13	0.17

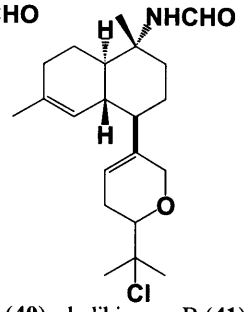
Isocyanosesquiterpene alcohol, (1S, 4S, 7R, 10S)-10-isocyano-5-cadinene-4-ol (**36**)[57] was also isolated from the nudibranch *P. pustulosa* collected from Katsuura on the Kii Peninsula. Compound **36** inhibited barnacle settlement of *B. amphitrite* with the EC_{50} values of 0.17 μg/mL.[57]



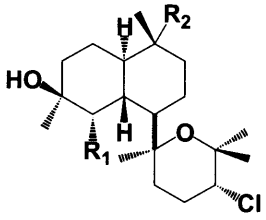
kalihinene X (**37**) EC_{50}=0.49μg/ml
1β-**H**, 14α-**Cl**
kalihinene Y (**38**) EC_{50}=0.45μg/ml
1α-**H**, 14α-**Cl**
kalihinene Z (**39**) EC_{50}=1.1μg/ml
1β-**H**, 14β-**Cl**



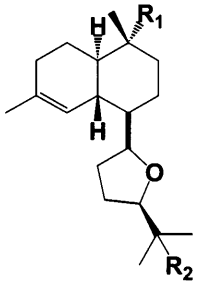
kalihipyran A (**40**)
EC_{50}=1.3μg/ml



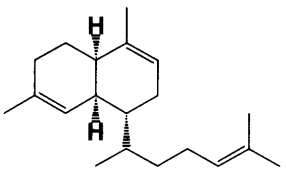
kalihipyran B (**41**)
EC_{50}=0.85μg/ml



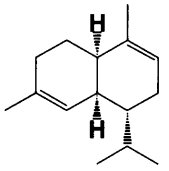
10β-formamidokalihinol-A (**42**)
EC_{50}=0.12μg/ml
R_1= **NC** R_2= **NHCHO**
10β-formamidokalihinol-E (**43**)
EC_{50}=0.12μg/ml
R_1= **NC** R_2= **NHCHO** 14-*epi*
10β-formamido-5β-isocyan
atokalihinol-A(**44**)
EC_{50}=0.74μg/ml
R_1= **NCO** R_2= **NHCHO**
10β-formamido-5β-isothiocyana
tokalihinol-A (**45**)
EC_{50}=0.088μg/ml
R_1= **NCS** R_2= **NHCHO**
kalihinol-A (**51**)
EC_{50}=0.087μg/ml
R_1= **NC** R_2= **NC**
kalihinol-E (**52**)
EC_{50}=0.088μg/ml
R_1= **NC** R_2= **NC** 14-*epi*



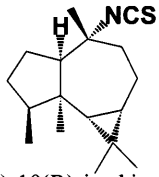
15-formamidokalihinene (**49**)
EC_{50}=0.14
R_1= **NC** R_2= **NHCHO**
10-formamidokalihinene (**50**)
EC_{50}=0.095
R_1= **NHCHO** R_2= **NC**



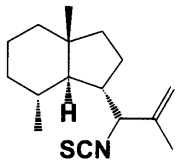
biflora-4,9,15-triene (**46**)



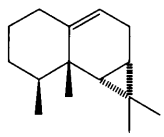
α-muurolene (**54**)



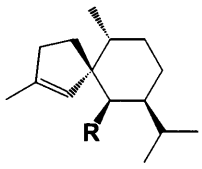
(+)-10(R)-isothiocyanato
alloaromadendrane (**47**)



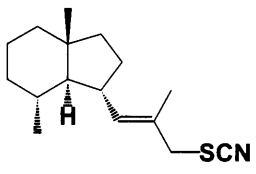
10-isothiocyanato-11-axene (**48**)



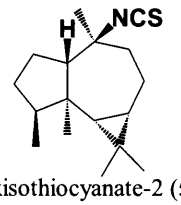
9-aristolene (**53**)



axisothiocyanate-3 (**55**)
R=**NCS**
axamide-3 (**56**)
R=**NHCHO**



cavernothiocyanate (**57**)



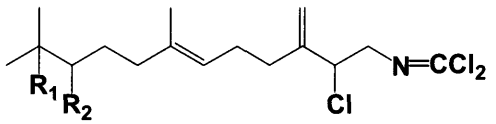
axisothiocyanate-2 (**58**)

Many isocyanoterpenoids and related compounds were also reported from the marine sponge *Acanthella cavernosa* as antifouling compounds. A total of 13 novel compound including 9 kalihinane type compounds kalihinene X(**37**), kalihinene Y (**38**), kalihinene Z(**39**), kalihipyran A (**40**), kalihipyran B (**41**), 10β-formamido-kalihinol-A (**42**), 10β-formamido-kalihinol-E (**43**), 10β-formamido-5β-isocyanatokalihinol-A (**44**), and 10β-formamido-5β-isothiocyanatokalihinol-A (**45**) together with 3 terpene biflora-4,9,15-triene (**46**), (+)-10(R)-isothiocyanatoalloaromadendrane (**47**), 10-isothiocyanato-11-axene (**48**) were obtained.[51,58-60] Moreover, 10 known compounds, 15-formamidokalihinene (**49**),[61] 10-formamidokalihinene (**50**),[61] kalihinol A (**51**),[62] kalihinol E (**52**),[63] 9-aristolene (**53**),[64] α-muurolene (**54**),[65] axisothiocyanate-3 (**55**),[52] axamide-3 (**56**),[52] cavernothiocyanate (**57**), and axisothiocyanate-2 (**58**),[66-68] were isolated as antifouling compounds.[51,58-60] Antifouling activity of kalihinane-type compounds is shown with structures. Table 2 summarizes percentages of metamorphosed and dead larvae when exposed to some terpenoids at 5.0 μg/mL for 48h.[60]

Table 2. Percentages of Metamorphosed and Dead Larvae

	Compounds								
	46	**47**	**48**	**53**	**54**	**55**	**56**	**57**	**58**
Metamorphosed	29	4	38	8	37	8	0	42	33
Dead	0	0	0	0	13	0	0	0	0

Three novel sesquiterpene axinyssimides A-C (**59-61**) were obtained from a marine sponge *Axinyssa* sp. collected off Hachijo-jima Island. They showed anti-barnacle activity; **59**: EC_{50} = 1.2 μg/mL, **60**: 70% settlement inhibition at 0.5 μg/mL, **61**: more than 90% settlement inhibition at 0.5 μg/mL, respectively.[57]

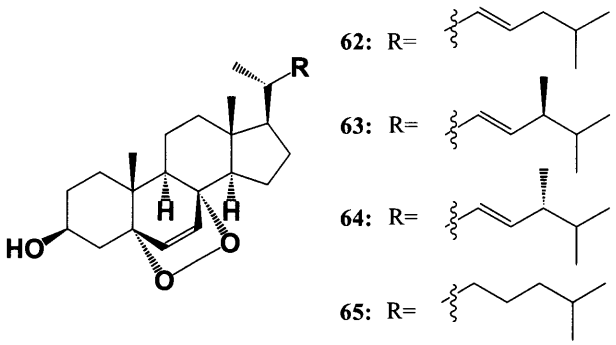


59: R_1, R_2 = **O** (epoxide)

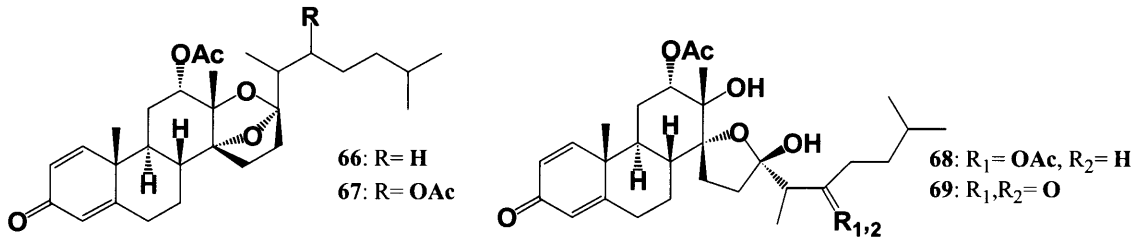
60, 61: R_1=R_2= **OH**

3-2. Steroids

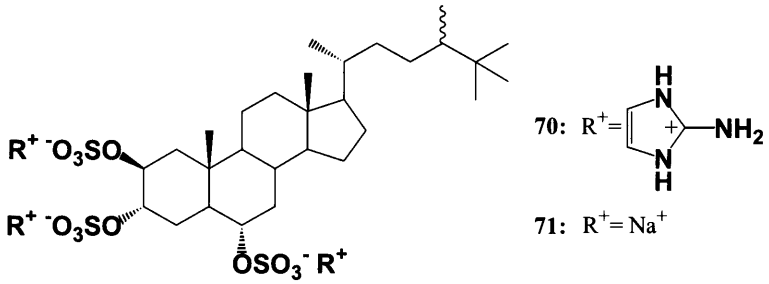
Four epidioxy steroids (**62-65**),[69] isolated from the sponge *A. cavernosa*, prolonged the larval swimming period from 1-2 days to 3-4 days at a concentration of 10 μg/mL, while ergosterol did not show this activity.[70]



Novel D-*seco*steroids isogosterone A-D (**66-69**)[71] from a soft coral of the genus *Dendronephthya* inhibited the larval settlement of *B. amphitrite* with an EC_{50} value of 2.2 μg/mL.[71]

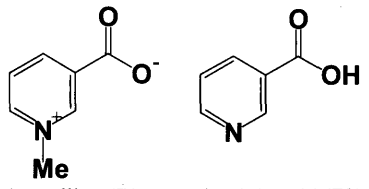


Halistanol sulfate salts, tri-2-aminoimidazolium halistanol sulfate (**70**)[72] and halistanol sulfate (**71**)[72] isolated from a marine sponge *Tposentia* sp., showed antifouling activity against cypris larvae with the EC_{50} values of 4.0 and 2.9 μg/mL, respectively.[41]



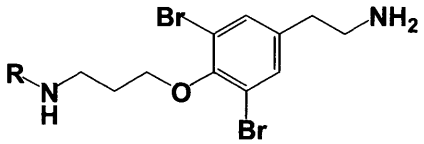
3-3. Amino acids and related compounds

Trigonelline (**72**), isolated from a soft coral *Dendronephthya* sp., showed antifouling activity against barnacle with an MIC value of 2.0 μg/mL, whereas nicotinic acid (**73**) was weakly active.[73]

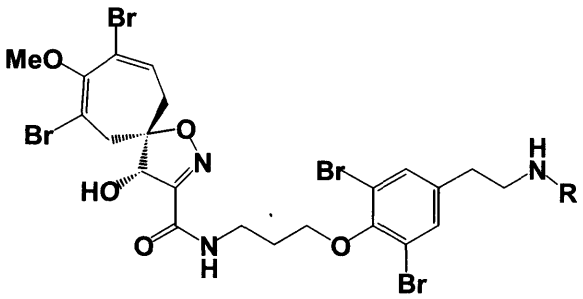


trigonelline (**72**) nicotinic acid (**73**)

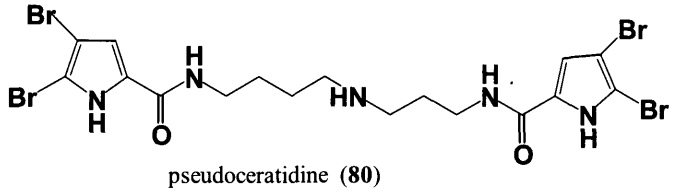
Antifouling bromotyrosine derivatives, ceratinamide A (**74**),[74] ceratinamide B (**75**),[74] ceratinamine (**76**),[75] moloka'iamine (**77**),[76] psammaplysin A (**78**),[77] psammaplysin E (**79**),[78] and pseudoceratidine (**80**)[79] were isolated from the Japanese marine sponge *Pseudoceratina purpurea*. The antifouling activity and other biological activities of these compounds are shown in Table 3.[74]



ceratinamine (**76**) R= **COCN**
moloka'iamine (**77**) R= **H**



ceratinamide A (**74**) R= **CHO**
ceratinamide B (**75**) R= **$CO(CH_2)_{11}CHMe_2$**
psammaplysin A (**78**) R= **H**
psammaplysin E (**79**) R=



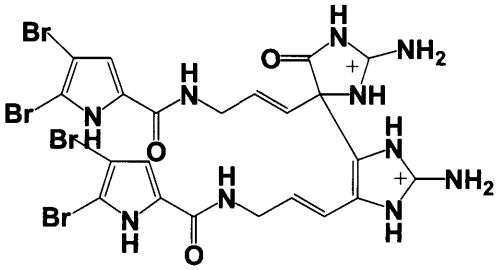
pseudoceratidine (**80**)

Table3. Biological Activities of Compounds **74-80**[74]

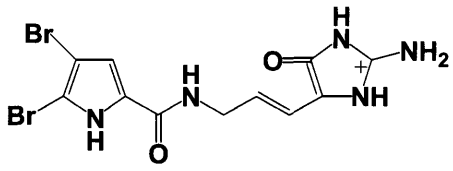
Compounds	Metamorphose inducing activity ED_{100}(μg/mL)	Settlement inhibition activity ED_{50}(μg/mL)	Antimicrobial activity *F.marinotypicum* halo (mm)*	Cytotoxic activity P388 IC_{50}(μg/mL)
Ceratinamide A (**74**)		0.10		>10
Ceratinamide B (**75**)		2.4		>10
Ceratinamine (**76**)		5.0		3.4
Moloka'iamine (**77**)		4.3		2.1
Psammaplysin A (**78**)	1.2	0.27	10	>10
Psammaplysin E (**79**)		4.8		2.1
Pseudoceratidine (**80**)		8.0	15	>10

*Each 10μg of sample was put on a disk

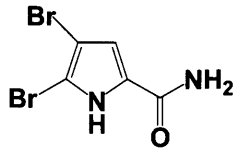
An oroidin dimer, mauritiamine (**81**)[80] was isolated from the marine sponge *Agelas mauritiana* along with the known oroidin (**82**)[81,82] and 4,5-dibromopyrrole-2-carbamide (**83**).[81] Compounds **81** and **82** inhibited larval settlement of the barnacle *B. amphitrite* with EC_{50} values of 15 μg/mL and 19 μg/mL, respectively, while **83** promoted larval metamorphosis of the ascidian *Ciona savignyi* at a concentration of 2.5 μg/mL.[80]



mauritiamine (**81**)



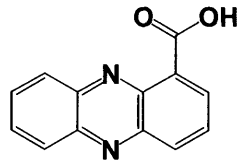
oroidin (**82**)



4,5-dibromopyrrole-2-carbamide (**83**)

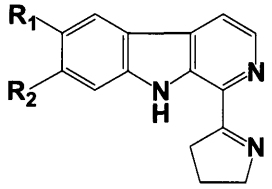
3-4. Other compounds

Phenazine-1-carboxylic acid (**84**) and related compounds were isolated from a culture of the nudibranch-associated *Pseudomonas* sp. Test paints containing crude extract of **84** showed antifouling activity against barnacle and algae.[83]



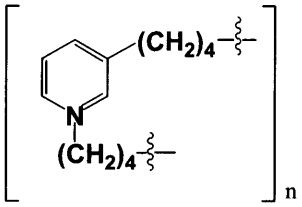
phenazine-1-carboxylic acid (**84**)

Eudistomins G (**85**) and H (**86**)[84] were isolated from the ascidian *Eudistoma olivaceum* as inhibitors of larval settlement against the bryozoan *B. neritina* at as low as 0.5% of natural concentrations in the living ascidian.[85]



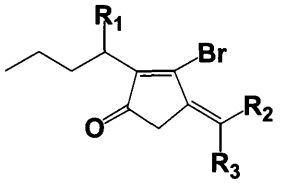
eudistomin G (**85**) R_1 = H, R_2 = Br
eudistomin H (**86**)R_1 = Br, R_2 = H

3-Alkylpyridinium salts (**87**),[86] isolated from the marine sponge *Reniera sarai,* showed moderate antifouling activity but very low toxicity against cypris larvae in comparison with booster biocides, copper pyrithione or zinc pyrithione.[87]

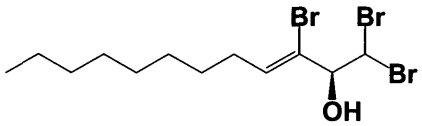


1,3-alkylpyridine salts (**87**)

Five halogenated furanones **88-92** and a (2*S*, 3*Z*)-1,1,3-tribromododec-3-en-2-ol (**93**), isolated from the Australian red alga *Delisea pulchra* showed promising antifouling activity against the barnacle *B. amphitrite* with the EC_{50} values of 20, 20, 60, 510, 320, and 170 μg/mL, respectively.[88]

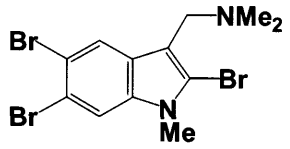


88: R_1 = H, R_2 = R_3 = Br
89: R_1 = R_2 = H, R_3 = Br
90: R_1 = OAc, R_2 = H, R_3 = Br
91: R_1 = OAc, R_2 = H, R_3 = I
92: R_1 = H, R_2 = H, R_3 = Br



(2S, 3Z)-1,1,3-tribromododec-3-en-2-ol (**93**)

2,5,6-Tribromo-1-methylgramine (**94**),[89] isolated from the marine bryozoan *Zoobotryon pellucidum*, showed promising antifouling activity against barnacle with an MIC value of 0.03 μg/mL.[89]

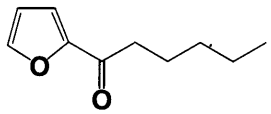


2,5,6-tri-bromo-1-methylgramine (**94**)

3-5. Analogue Development

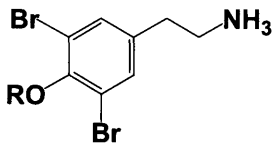
Although some promising natural antifoulants were discovered, no natural antifouling compounds have been commercialized. Perhaps the poor yields of natural compounds have hampered further development as antifouling agents. The most potent natural products are often too structurally complex to be commercially synthesized.[10,21,22,51] Possible ways to overcome this issue are aquaculture of antifoulants producers, analogue development, and chemical synthesis. In order to supply sufficient amounts of materials for further trial, chemical synthesis would be the most reasonable methods for comparatively simple compounds. Several promising antifouling compounds and their analogues were synthesized and evaluated for antifouling activity against cyprids larvae as well as for paints trial, *e.g.* 2,5,6-tribromo-1-methylgramine [89,90] and 3-alkylpridinium salts.[87]

Furans, as well as γ-lactones, have been tested as analogues of the renillafoulins (**1-3**) and the pukalides (**4**, **5**). A total of 19 analogues, based on the functional groups of lactone and furan rings in the parent molecules, was evaluated for antifouling activity against cyprids larvae and for toxicity to nauplii of *B. amphitrite*.[91] 2-Furyl-*n*-pentyl ketone (**95**) was the most potent of the analogues against larval settlement with a therapeutic ratio (LC_{50} for nauplii/EC_{50} for settlement = 186.7 μM/0.002 μM) of 87,243.[91]

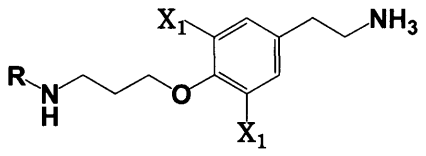


2-furyl-*n*-pentyl ketone (**95**)

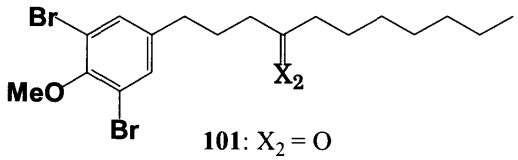
Eight bromotyrosine derivatives (**96-102**), including moloka'iamine (**77**), were synthesized and evaluated for antifouling activity as well as for toxicity against the barnacle *B. amphitrite* cyprids larvae.[92] The antifouling activity of these compounds is summarized in Table 4. Investigation into the use of **102** as an antifouling paint additive is being pursued.[92]



96: R = H
97: R = $CH_2CH_2NH_3$
98: R = $(CH_2)_4NH_3$



99: X_1 = H
100: X_1 = Cl

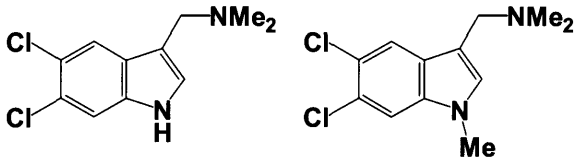


101: X_2 = O
102: X_2 = H_2

Table 1-4. Antifouling Activity and Toxicity of Synthesized Bromotyrosine Derivatives **77** and **96-102**

Compound	EC_{50}(μg/mL)	LD_{50} (μg/mL)
77 (synthesized)	5.0	nt
96	0.07	0.2
97	0.8	nt
98	6.0	nt
99	> 50	nt
100	33	nt
101	0.2	1.0
102	0.008	0.03

A total of 111 indole derivatives of **94** was synthesized and evaluated for antifouling activity, which led to discovery of 5,6-dichrologramine (**103**) and 5,6-dichrolo-1-methylgramine (**104**). Compounds **104** showed very high potential in the silicon-based paints in the field tests.[90] The antifouling activity of these compounds is shown along with **94** and TBTO in Table 5.



5,6-dichrologramine (**103**) 5,6-dichrolo-1-methylgramine (**104**)

Table 1-5. Antifouling Activity and Toxicity of Compounds **94**, **103**, **104**, and TBTO

Compound	MIC[1] (ppm)	LC_{30} (ppm)
94	**0.03**	**0.60**
103	**0.008**	**> 1.0**
104	**0.063**	**0.60**
TBTO	**0.20**	**0.06**

1)minimum inhibitory concentration.

The objectives of the present study are to discover promising antifouling substances against barnacle larvae. This thesis consists of three chapters.

Chapter 1 describes the antifouling activity of the extracts of a total of 118 Japanese marine invertebrates. Their chloroform-soluble extracts were evaluated for antifouling activity as well as for toxicity against the barnacle *B. amphitrite* cyprids larvae. Among 86 species that showed more than 80 % larval settlement inhibition, 13 species, including 8 sponges were weakly toxic to the barnacle larvae.

The marine sponge *Acanthella cavernosa* which showed most promising activity was extracted and fractionated by solvent partitioning, column chromatography, and HPLC to afford two active compounds; a new compound, 10-formamido-4-cadinene and a known compound, T-cadinol. They inhibited larval settlement with EC_{50} values of 0.50 and 0.53 μg/mL, respectively.

In Chapter 2, a total of 59 isocyano compounds was synthesized and evaluated for antifouling activity as well as for toxicity of cyprids, in order to obtain the structure-activity relationships of a model compound, 3-isocyanotheonellin, that was reported to be promising antifoulant. The synthesized compounds are: 20 isocyanocyclohexanes, including 3-isocyanotheonellin and its analogues, 27 isocyanobenzenes and related compounds, 12 simple isocyanides, such as linear

alkyl-chain isocyanides. Evaluation of their antifouling activity and toxicity against *B. amphitrite* cyprids disclosed that isocyano functional group would be very important for potent antifouling activity with non-toxicity and that simple isocyanides would be promising non-toxic antifouling agents.

Chapter 3 deals with field experiments of *N*-(4-hexylphenyl) acetamide (**131**) and 1,1-dimethyl-10-undecyl isocyanide (**153**) which were considered promising compounds and synthesized comparatively easily. In order to examine whether these compounds prevent the settlement of fouling organisms in the field, test paints using **131** and **153** were prepared by combining with acrylic copolymer containing carboxylic acid in a solution of 2-acetoxy-1-methoxy propane. Field experiments were carried out at Shizugawa bay and Tokyo bay for 3 and 2 months, respectively. Both field experiments showed two compounds are promising as antifouling agents.

Parts of the present work have been published as below.

Y. Kitano, Y. Nogata, K. Shinshima, E. Yoshimura, K. Chiba, M. Tada, and I. Sakaguchi, Synthesis and Anti-Barnacle Activities of Novel Isocyanocyclohexane Compounds Containing an Ester or an Ether Functional Group. *Biofouling*, (in press).

Y. Nogata, Y. Kitano, E. Yoshimura, K. Shinshima, and I. Sakaguchi, Antifouling Activity of Synthetic Simple Isocyanides against the Barnacle *Balanus amphitrite* Larvae. *Biofouling*, (in press).

Y. Kitano, A. Yokoyama, Y. Nogata, K. Shinshima, E. Yoshimura, K. Chiba, M. Tada, I. Sakaguchi (2003) Synthesis and Anti-Barnacle Activity of Novel 3-isocyanotheonellin analogues. *Biofouling*, **19** (supplement), 187-192.

Y. Nogata, E. Yoshimura, K. Shinshima, Y. Kitano, and I. Sakaguchi (2003) Antifouling Substances against Larvae of the Barnacle *Balanus amphitrite* from the Marine Sponge, *Acanthella cavernosa*. *Biofouling*, **19** (supplement), 193-196.

Y. Kitano, T. Ito, T. Suzuki, Y. Nogata, K. Shinshima, E. Yoshimura, K. Chiba, M. Tada, and I. Sakaguchi (2002) Synthesis and antifouling activity of 3-isocyanotheonellin and its analogues. *J. Chem. Soc., Perkin Trans. 1*, 2251-2255.

CHAPTER 1

ANTIFOULING ACTIVITY OF MARINE INVERTEBRATES AND ISOLATION OF ANTIFOULING SESQUITERPENES FROM THE MARINE SPONGE *ACANTHELLA CAVERNOSA*

A total of 118 Japanese marine invertebrates were extracted with methanol, and their chloroform-soluble materials were tested for antifouling activity as well as toxicity against cyprids of the barnacle *Balanus amphitrite*. Among 86 species that showed more than 80 % inhibition of larval settlement, 13 species, including 8 sponges were weakly toxic to cyprids.

The marine sponge *Acanthella cavernosa* which showed most promising activity was extracted with ethanol, whose extract was fractionated by solvent partitioning, silica gel column chromatography, gel filtration, and ODS HPLC to afford two active compounds. They were identified as 10-formamido-4-cadinene and T-cadinol on the basis of spectral data and chemical transformation. They inhibited larval settlement with EC_{50} values of 0.50 and 0.53 μg/mL, respectively.

Results and Discussion

1. Screening of Marine Invertebrates for Antifouling Activity

The chloroform-soluble materials of the methanol extracts of 118 species of Japanese marine invertebrates (Table 1-1) were tested for antifouling activity against *B. amphitrite* larvae. As shown in Table 1-2, 86 samples inhibited more than 80% of larval

Table 1-1. Number of Collection Invertebrates and Collection Sites

Collection Site	Sponges	Coelenterates	Bryzoans	Tunicates
Amami Island	7	5	0	4
Uke Island	7	0	0	0
Kakeroma Island	9	0	0	0
Nakanoshima Island	17	0	0	0
Sata Cope	0	0	5	1
Gokasyo Bay	2	3	0	1
Shikine Island	53	1	0	1
Atami	1	0	0	0
Total	96	9	5	8

settlement at a concentration of 10 μg/mL, but many active samples were also toxic. Thirteen samples, including 8 sponges, were found to be promising (Table 1-3), since their lethality to barnacle larvae was low (<15%) at the same concentration. Based on these results, the marine sponge, *Acanthella cavernosa* (100% settlement inhibititon and 0% lethality) collected off Atami, Shizuoka prefecture was chosen for further study.

Table 1-2. Antifouling Activity of Japanese Marine Invertebrates

Animal Class	No. of sample	No. of active sample[1)]	No. of promising Samples[2)]
Sponges	96	68	8
Coelenterates	9	9	2
Bryozoans	5	3	1
Tunicates	8	6	2
TOTAL	118	86	13

1)Those which showed 80% inhibition of larval settlement at 10μg/ml.
2)Those which showed 80% inhibiton and low toxicity (<15% lethality).

Table 1-3. Percentages of Settlement Inhibition and Lethality of Promising Samples at 10 μg/mL

Animal Class	Sample No.	Settlement (%)	Lethality (%)
Sponges	*A. cavernosa*	0	0
	S97-309	17	15
	S97-314	13	6
	S97-316	0	15
	S97-329	10	5
	S97-337	12	15
	S97-348	6	6
	S97-351	12	15
Coelenterates	C97-004	0	11
	C97-006	0	6
Bryozoans	B97-002	8	15
Tunicates	T97-008	20	6
	T97-201	5	10
Control[1]		93	0

1)control (no samples)

2. Isolation and Structure Elucidation of Antifouling Compounds from the Marine Sponge *Acanthella cavernosa* [93]

The frozen sponge was extracted with ethanol, and the extract was subsequently fractionated by solvent partitionings, silica gel chromatography and ODS HPLC to isolate two antifouling substances (Sheme 1-1).

Acanthella cavernosa (150g, wet wt.)

↓ extracted with EtOH (250mL x 3)
partitioned with EtOAc and H_2O

EtOAc layer

↓ partitioned with *n*-hexane and 90% MeOH

n-hexane layer

↓ Silica gel column chromatography
eluted with $CHCl_3$: MeOH = 98:2

↓ gel-filtered on Toyopeal HW40
eluted with $CHCl_3$: MeOH = 1:1

↓ ODS HPLC
(75% MeOH-100% MeOH linear gradient)

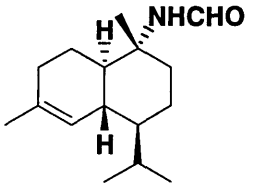
↓ ODS HPLC
(90% MeOH)

10-formamido-4-cadinene (**105**: 3.9 mg)
T-cadinol (**106**: 0.7 mg)

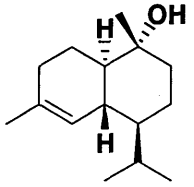
Scheme 1-1. Purification procedure of compounds **105** and **106.**

10-formamido-4-cadinene (**105**)[93] had a molecular formula of $C_{16}H_{27}NO$, which was established by NMR and FABMS [*m/z* 250 $(M+H)^+$] data (Chart 1-1). The ^1H NMR spectrum contained two methyl singlets [δ 1.23 and 1.67 (minor: 1.26 and 1.66)], two methyl doublets [δ 0.78 and 0.92 (minor: 0.77 and 0.91)], one olefin singlet [δ 5.49, s (minor: 5.51, s)] and an amide proton signal [δ 5.58 (d, *J* = 12.1 Hz) (minor: 5.12 br s)] (Chart 1-2). In addition, the presence of a formamide group was inferred from ^1H NMR [mixture of δ 8.08 (d, *J* = 2.1 Hz) and 8.29 (d, *J* = 12.5 Hz)] and ^{13}C

NMR signals (δ 160.4 and 162.8) (Chart 1-3). This was supported by ^{1}H and ^{13}C NMR signals were observed as an equilibrium mixture (1:2) of s-*cis* and s-*trans* rotational isomers. The ^{1}H-^{1}H COSY (Chart 1-4) and HMQC spectra revealed two partial structures, (CH_2-3)-(CH_2-2)-(CH-1)-(CH-6)-(CH-7)-(CH_2-8)-(CH_2-9) and (CH-7)-(CH-11)-2(CH_3-12, 13). The gross structure of **105** was determined as 10-formamido-4-cadinene by interpretation of HMBC data. In order to determine the complete structure, **105** was converted to the known 10-isocyano-4-cadinene (**27**) with TsCl in pyridine. The ^{1}H and ^{13}C NMR spectra of the synthesized 10-isocyano-4-cadinene (**27**) were identical with those reported in the literature[51] (Chart 1-5). As a result of database search, **105** was identified to be a new compound; 10-formamido-4-cadinene. The structure of T-cadinol (**106**) was readily identified by comparison of spectral data in the literature[94] (Chart 1-6).



105



106

3. Antifouling activity[93]

Two sesquiterpenes, **105** and **106** thus obtained were assessed for antifouling activity against cyprids of *B. amphitrite*; The known **106** inhibited larval settlement with an EC_{50} value of 0.53 μg/mL, while its toxicity to larvae was much less than that of $CuSO_4$ (Figure 1-1, Table 1-4). The new compound **105** showed similar antifouling activity (EC_{50} = 0.50 μg/mL), but its toxicity was much higher than that of **106**. Both compounds completely inhibited larval settlement at a concentration of 3.0 μg/mL without killing any larvae. **106** was not lethal to cypris larvae at 30 μg/mL, while **105** exhibited toxicity at 10 μg/mL (LD_{100}). Although **106** is a well known compound contained in wood oil, this is the first report of its antifouling activity. Related compounds containing isocyano and isothiocyanato functionalities, *e.g.*, 10-isocyano-4-cadinene (**27**), 10-isocyano-4-amorphene (**32**), 10-isothiocyanato-4-amorphene (**31**), were isolated as antifouling substances from the same sponge and four species of nudibranchs of the family Phyllidiidae.[50,51,60] These compounds were highly antifouling against cyprid larvae, but weakly toxic. The poor yields of these compounds hampered further development as antifouling agents. Most potent natural antifouling compounds are often too structurally complex to be commercially synthesized.[10,26,51] In contrast, T-cadinol is contained in relatively large

amounts in the essential oil of a Japanese cypress.[95,96]

Table 1-4. Antifouling Activities against Cyprids (EC_{50} in μg/mL)

10-formamido-4-cadinene (**105**)	0.50
T-cadinol (**106**)	0.53
10-isocyano-4-cadinene (**27**)	0.14
10-isocyano-4-amorphene (**32**)	0.70
10-isothiocyano-4-amorphene (**31**)	7.2

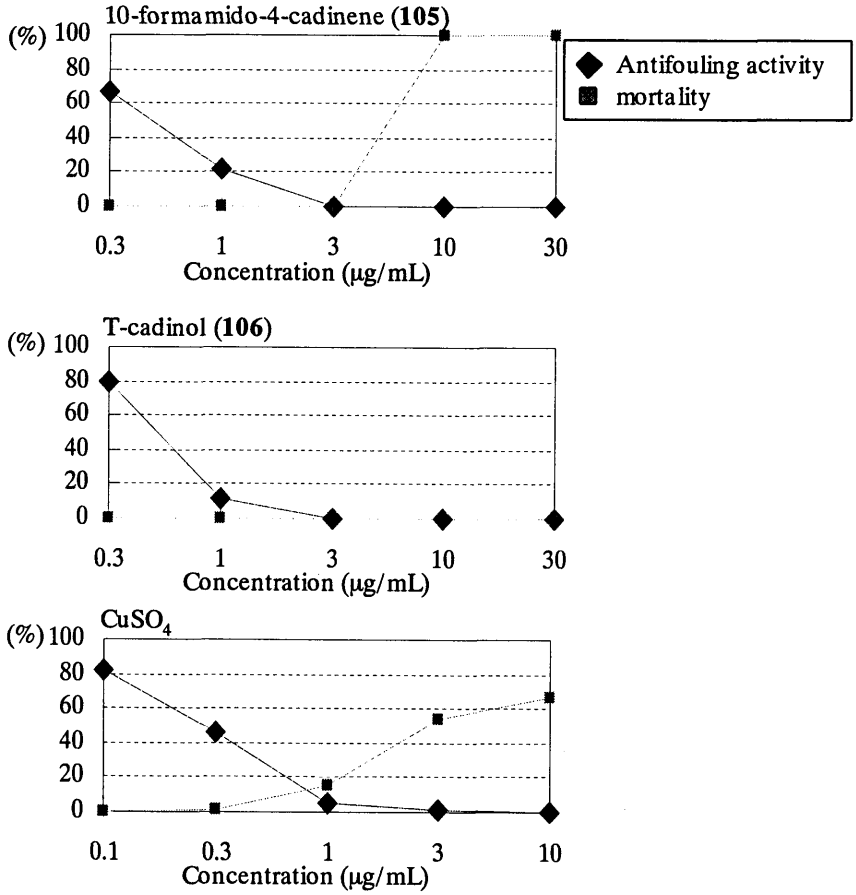


Figure 1-1. Antifouling activity and mortality of 10-formamido-4-cadinene (**105**), T-cadinol (**106**), and $CuSO_4$.

Experimental Section

General experimental procedure

NMR spectra were recorded on a JEOL A600 NMR spectrometer operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C. ^{1}H and ^{13}C NMR chemical shifts were referenced to solvent peaks: δ_H 7.24 and δ_C 77.0 for $CDCl_3$. FAB mass spectra were measured with a JEOL JMX-SX102/SX102 tandem mass spectrometer using 3-nitrobenzyl alcohol as matrix. Optical rotation was determined on a JASCO DIP-1000 digital polarimeter in $CHCl_3$.

Larval culture

Adult barnacles, *Balanus amphitrite*, attached to bamboo poles were procured from oyster farms in Lake Hamana, Shizuoka, and maintained in an aquarium at 20 ±1 °C by feeding with the brine shrimp *Artemia sarina* nauplii. Broods released I-II stage nauplii upon immersion in seawater after being dried for 1 day. Nauplii thus obtained were cultured in 2-L glass beakers at an initial density of 3 larvae/mL by feeding with the diatom *Chaetoceros gracilis* at a concentration of 2.5×10^5 cells/mL. Cultures were maintained in filtered seawater diluted to 80 % by deionized water (80 % filtered seawater) at 25 °C with mild aeration. From day 1 to day 4, larvae were collected on 100 μm nylon nets, washed with 80 % filtered seawater, and transferred to newly prepared algal diet suspensions. Larvae reached the cyprid stage in 5 days. The cyprids were stored in the dark at 5 °C until used. The day of collecting newly transformed cyprids was designated as Day 0.[97]

Antifouling assay

Test samples were dissolved in MeOH; aliquots of the solution were pipetted into wells of 24-well polystyrene tissue culture plates and air-dried. Two milliliters of filtered seawater diluted to 80 % by deionized water (80 % filtered seawater) and 6 cyprids (2- or 3-day-old) were added to each well. Each level of the experiments was carried out with three wells. The plates were kept in the dark at 25 °C, and numbers of larvae, which attached, metamorphosed, died, or did not settle, were counted under a microscope after 120 h.[98] Cyprids that did not move, had extended appendages and did not respond after a light touch by a metal probe were regarded as dead.

The antifouling activity was expressed as an EC_{50} value with a 95% confidence interval, which indicated the concentration that reduces the larval settlement to 50% of the control. The EC_{50} values were estimated by straight-line graphical interpolation. The toxicity was expressed as an LD_{50} value, which is defined as the concentration that

results in 50% mortality estimated by straight-line graphical interpolation. A non-toxic solution was defined as one where cyprids did not settle but remained alive after 120 h.

Marine organisms and preparation of test solutions

A total of 118 marine invertebrates, including sponges, coelenterates, bryozoans, and tunicates were collected at several locations on the Japanese coasts by hands using SCUBA or snorkeling. The MeOH extract of each organism (10 g) was separated into organic and aqueous fractions by partition between water and $CHCl_3$. The organic soluble materials were dissolved in MeOH and tested for antifouling activity at 20 μg/well.

Isolation of antifouling substances

The sponge, *Acanthella cavernosa* was collected off Atami, Shizuoka prefecture, 90 km west of Tokyo, immediately frozen, and kept at –20 °C until processed. Frozen sample (150 g) was extracted with EtOH (250 mL, 3 times). The extract was concentrated and partitioned between water and ethyl acetate. The ethyl acetate layer was further partitioned between n-hexane and 90% MeOH. The antifouling n-hexane portion was successively fractionated by silica gel column chromatography with $CHCl_3$, 2 % MeOH/$CHCl_3$, 5 % MeOH/$CHCl_3$, 10 % MeOH/$CHCl_3$, 20 % MeOH/$CHCl_3$, and $CHCl_3$/MeOH/H_2O (7:3:0.5). The active fraction eluted with 2 % MeOH/$CHCl_3$ was gel-filtered on Toyopeal HW40 with $CHCl_3$/MeOH (1:1), and followed by ODS HPLC on Capcell Pak using linear gradient elution from 75 % MeOH to 100 % MeOH in 90 min. Final purification was carried out by ODS HPLC on Capcell Pak with 90 % MeOH to afford 10-formamido-4-cadinene (**105**: 3.9 mg) and T-cadinol (**106**: 0.7 mg).

10-formamido-4-cadinene (105): $[\alpha]_D^{22}$ = +8.32° (*c* 0.001, $CHCl_3$). ^1H NMR of major *s-trans* isomer (600 MHz, $CDCl_3$): δ 8.29 (1H, d, *J* = 12.5 Hz, CHO), 5.58 (1H, br d, *J* = 12.1 Hz, NH), 5.49 (1H, br s, H-5), 2.18 (1H, m, H-11), 2.00 (1H, m, H-3), 1.99 (1H, m, H-3), 1.91 (1H, td, *J* = 3.3, 12.8 Hz, H-9), 1.87 (1H, m, H-6), 1.82 (1H, m, H-2), 1.67 (3H, s, H-14), 1.62 (1H, qd, *J* = 3.3, 13.6 Hz, H-8), 1.55 (1H, m, H-9), 1.28 (1H, m, H-2), 1.24 (1H, dt, *J* = 3.6, 13.6 Hz, H-8), 1.23 (3H, s, H-15), 1.20 (1H, m, H-1), 1.05 (1H, tt, *J* = 3.3, 12.1 Hz, H-7), 0.92 (3H, d, *J* = 7.0 Hz, H-13), 0.78 (3H, d, *J* = 7.0 Hz, H-12). minor *s-cis* isomer (600 MHz, $CDCl_3$): δ 8.08 (1H, d, *J* = 2.1 Hz, CHO), 5.51 (1H, br s, H-5), 5.12 (1H, br s, NH), 2.17 (1H, m, H-11), 2.11 (1H, m, H-9), 2.01 (1H, m, H-3), 2.00 (1H, H-9), 1.94 (1H, m, H-3), 1.85 (1H, m, H-6), 1.80 (1H, m, H-2), 1.66 (3H, s, H-14), 1.58 (1H, m, H-8), 1.29 (1H, m, H-2), 1.26 (3H, s, H-15), 1.21 (1H,

m, H-1), 1.18 (1H, m, H-8), 1.11 (1H, tt, J = 3.3, 12.1 Hz, H-7), 0.91 (3H, d, J = 7.3 Hz, H-13), 0.77 (3H, d, J = 8.0 Hz, H-12). ^{13}C NMR of major *s-trans* isomer (150 MHz, $CDCl_3$): δ 162.8 (CHO), 135.0 (C-4), 121.9 (C-5), 55.6 (C-10), 49.1 (C-1), 46.4 (C-7), 41.9 (C-9), 38.7 (C-6), 30.9 (C-3), 26.0 (C-11), 23.7 (C-14), 23.4 (C-2), 21.4 (C-13), 20.7 (C-8), 18.9 (C-15), 15.1 (C-12). minor *s-cis* isomer (150 MHz, $CDCl_3$): δ 160.4 (CHO), 135.0 (C-4), 122.3 (C-5), 57.4 (C-10), 46.2 (C-7), 45.7 (C-1), 38.5 (C-6), 37.4 (C-9), 30.9 (C-3), 25.9 (C-11), 23.7 (C-14), 23.7 (C-2), 21.4 (C-13), 20.7 (C-8), 18.8 (C-15), 15.1 (C-12).FABMS (3-nitrobenzyl alcohol, positive) *m/z* 250 $(M + H)^+$.

T-cadinol (106): ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.50 (1H, H-5), 2.15 (1H, H-11), 2.03 (1H, H-6), 1.90~2.02 (2H, H-3), 1.80 (2H, H-9), 1.78 (1H, H-2), 1.65 (3H, H-14), 1.52 (1H, H-8), 1.25 (2H, H-2,8), 1.18 (3H, H-15), 1.13 (1H, H-1), 1.06 (1H, H-7), 0.91 (3H, H-13), 0.77 (3H, H-14).

Preparation of 10-isocyano-4-cadinene(27) from 10-formamido-4-cadinene (105)

To a solution of **105** (1.5 mg in 1 mL of pyridine) was added 5 mg of TsCl, and the mixture was stirred at room temperature for 24h. After addition of 1 mL of brine, the mixture was extracted with n-hexane, and the extract was washed with 1N HCl, 1N $NaHCO_3$, and brine. The reaction product was filtered and dried to yield **27** (1.1 mg).

10-isocyano-4-cadinene **27**: ^{13}C NMR (150 MHz, $CDCl_3$): δ 135.3 (C-4), 121.4 (C-5), 48.0 (C-1), 46.2 (C-7), 40.6 (C-9), 37.9 (C-6), 30.8 (C-3), 25.9 (C-11), 23.7 (C-2), 23.6 (C-14), 21.3 (C-13), 20.2 (C-8), 20.0 (C-15), 15.0 (C-12). Two carbon signals of C-10 and C-16 were not observed.

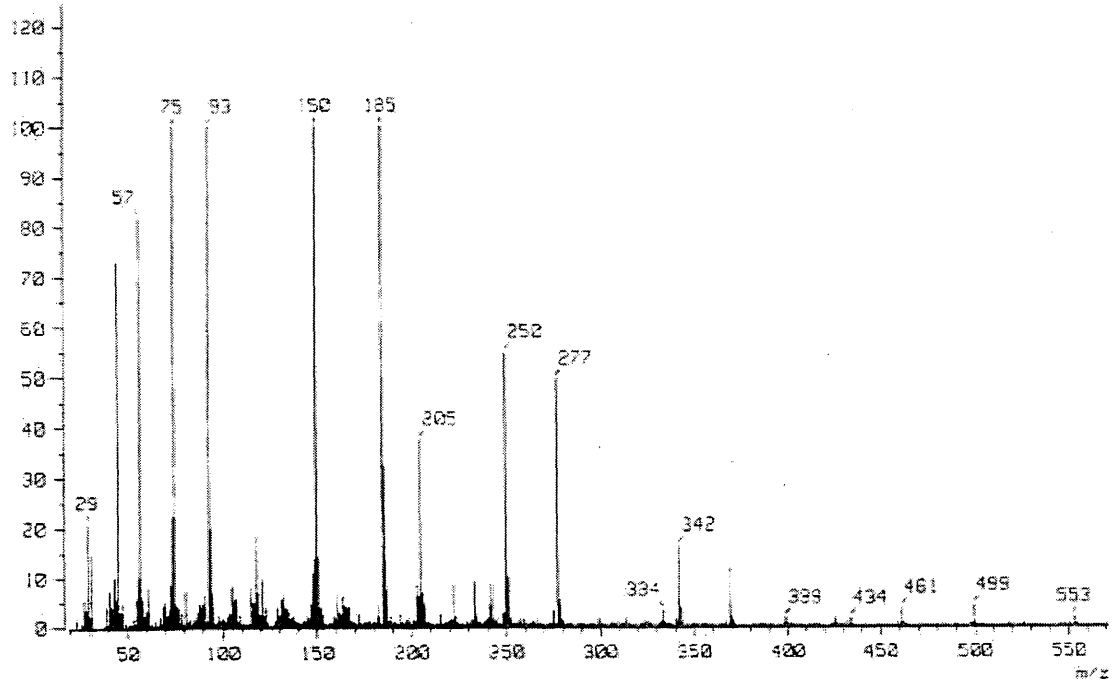


Chart 1-1. FABMS spectrum of 10-formamido-4-cadinene (**105**) (positive mode, 3-nitrobenzyl alcohol).

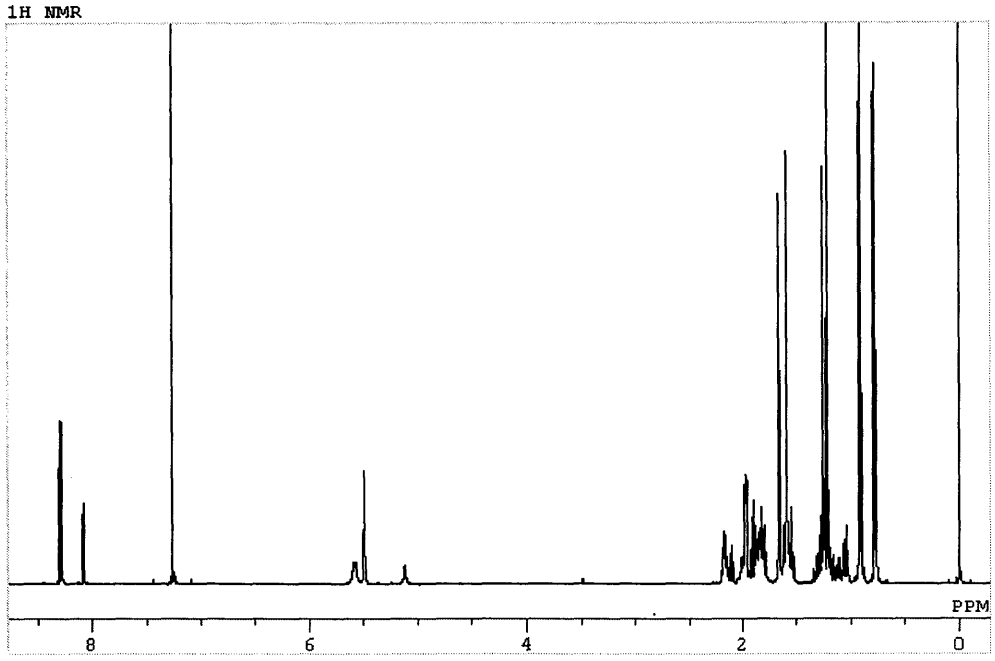


Chart 1-2. ^{1}H NMR spectrum of 10-formaido-4-cadinene (**105**) in $CDCl_3$.

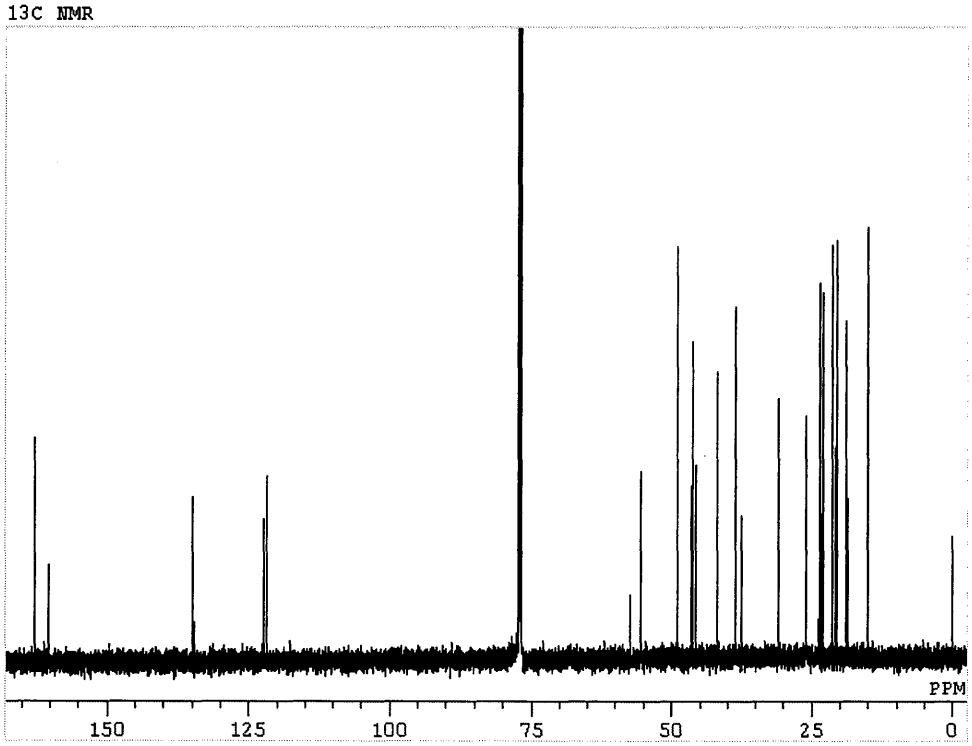


Chart 1-3. ^{13}C NMR spectrum of 10-formaido-4-cadinene (**105**) in $CDCl_3$.

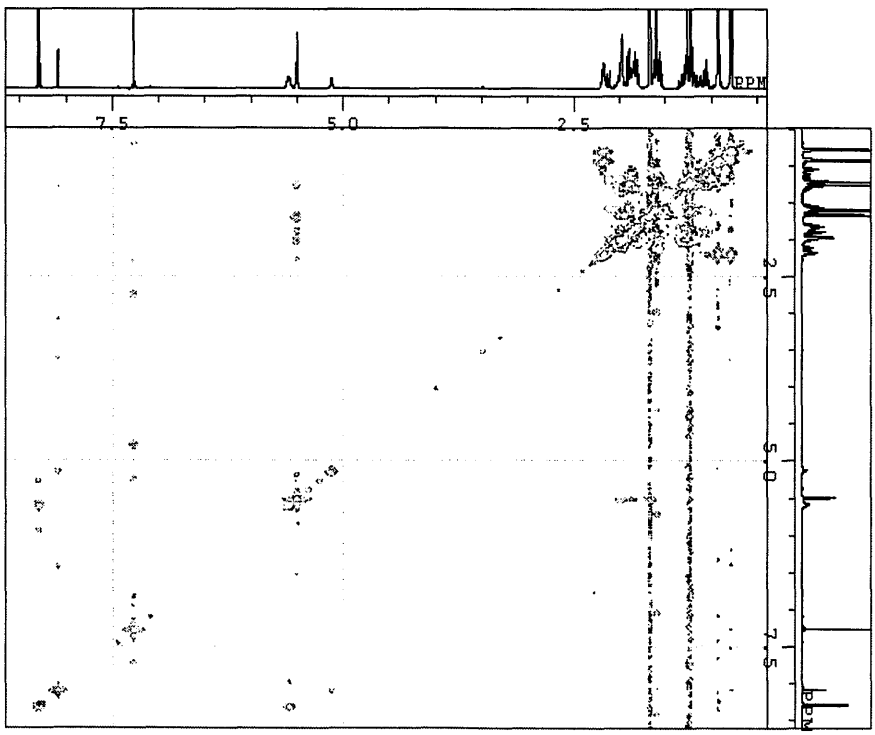


Chart 1-4. ^{1}H-^{1}H COSY spectrum of 10-formaido-4-cadinene (**105**) in $CDCl_3$.

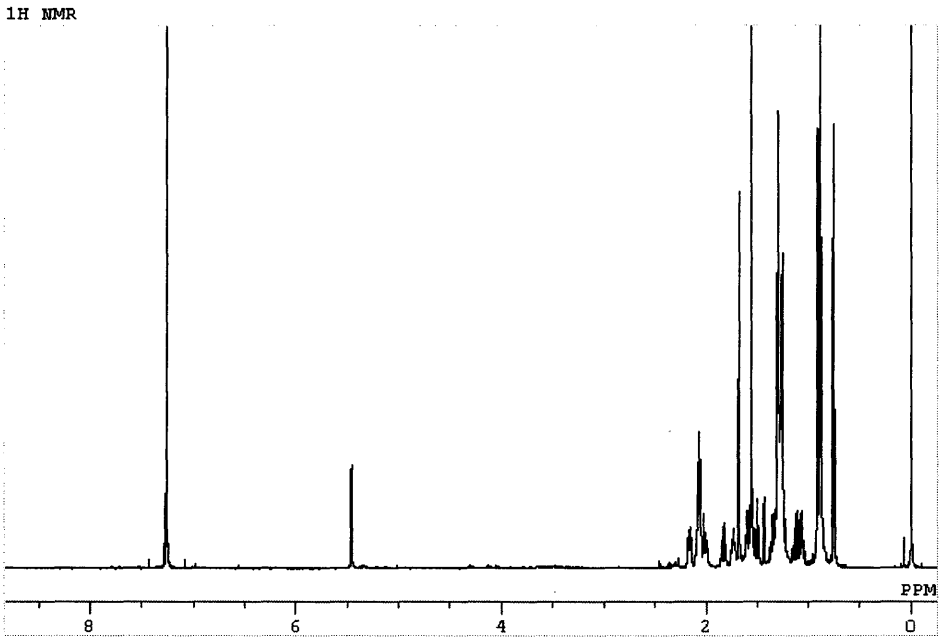


Chart 1-5. ^{1}H NMR spectrum of synthetic 10-isocyano-4-cadinene (**27**) in $CDCl_3$.

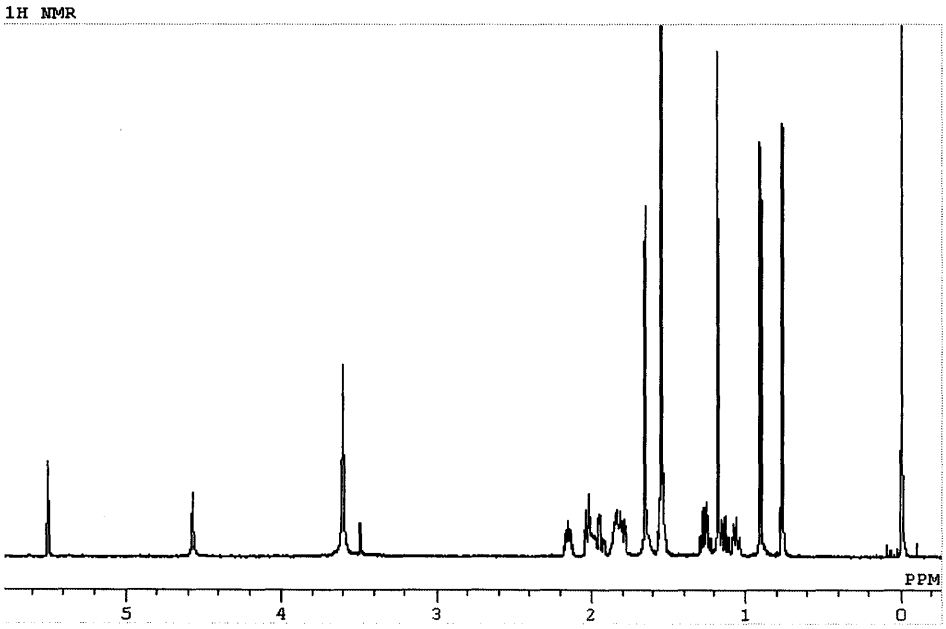


Chart 1-6. ^{1}H NMR spectrum of T-cadinol (**106**) in $CDCl_3$.

CHAPTER 2

SYNTHESIS AND ANTIFOULING ACTIVITY OF ISOCYANO COMPOUNDS

A variety of natural products with antifouling activity has been isolated from marine organisms,[10,13,21,26] and some of which are expected to be promising compounds in non-toxic antifouling agents. 3-Isocyanotheonellin (**35**), isolated from the nudibranch *P. pustulosa*, is a sesquiterpene of the bisabolone class with an isocyano functional group at the C-3 position.[55,56] Despite of its simple structure, **35** showed potent settlement inhibition activity against barnacle larvae without toxicity to larvae.[50,51]

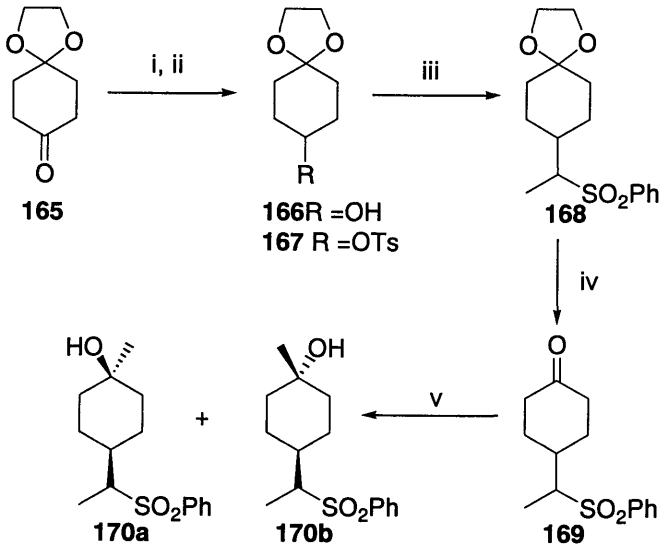
To develop efficient and environmentally benign antifouling agents, attempt has been made to synthesize isocyano compounds based on structure of 3-isocyanotheonellin, and to evaluate their antifouling activity and toxicity against cyprids larvae of the barnacle, *Balanus amphitrite*. A total of 59 derivatives were synthesized: 20 isocyanocyclohexane compounds, including 3-isocyanotheonellin and its analogues; 27 isocyanobenzene compounds and related compounds, and 12 simple isocyanides, such as linear alkyl-chain isocyanides. They were examined their settlement inhibition activity against cyprids larvae.

Many synthesized isocyano compounds inhibited larval settlement with EC_{50} values of 0.01-20.0 μg/mL, while their toxicity to cyprids larvae were much less than that of $CuSO_4$. Some isocyanides and acetamide showed better of antifouling against barnacle larvae compared to antifouling active natural isocyano compounds, despite their simple structures. These results suggested that simple isocyanides would be promising non-toxic antifouling agents.

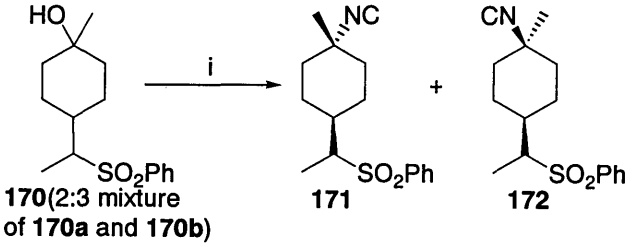
Results and Discussion

1. Antifouling Activity of 3-Isocyanotheonellin (35) and Its Analogues 107-113[99,100]

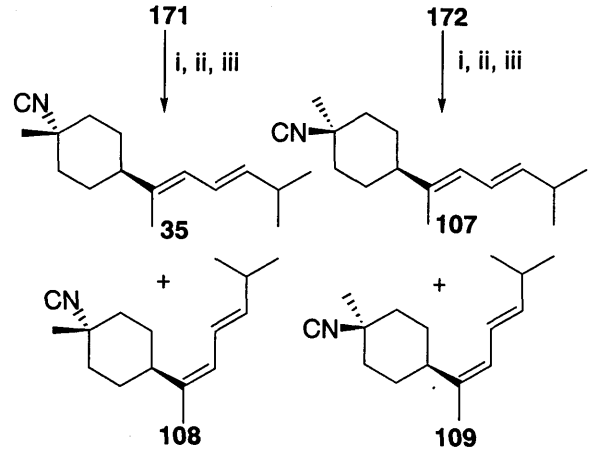
3-Isocyanotheonellin (**35**) and its analogs **107-109** were synthesized from 1,4-cyclohexanedione mono-ethylene ketal **165** by the synthetic routes as shown in Scheme 2-1, 2-2, and 2-3.[99] Compounds **110-113** were obtained from isocyano-sulfone **174** as depicted in Scheme 2-4 (synthesis of isocyano-sulfone **174**) and 2-5 (synthesis of compound **110-113**).[100]



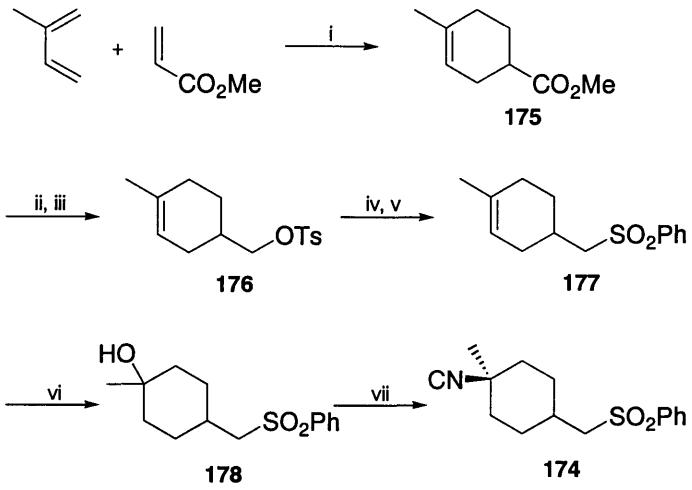
Scheme 2-1. Reagents and conditions: (i) $NaBH_4$, MeOH, 0°C, quant.; (ii)TsCl, Pyr, rt; (iii) *n*-BuLi, $EtSO_2Ph$, 0 °C→rt; (iv) *p*-TsOH, 10%aq. acetone; (v) MeLi, THF, 0 °C (mixture of **170a** : **170b** = 2 : 3).



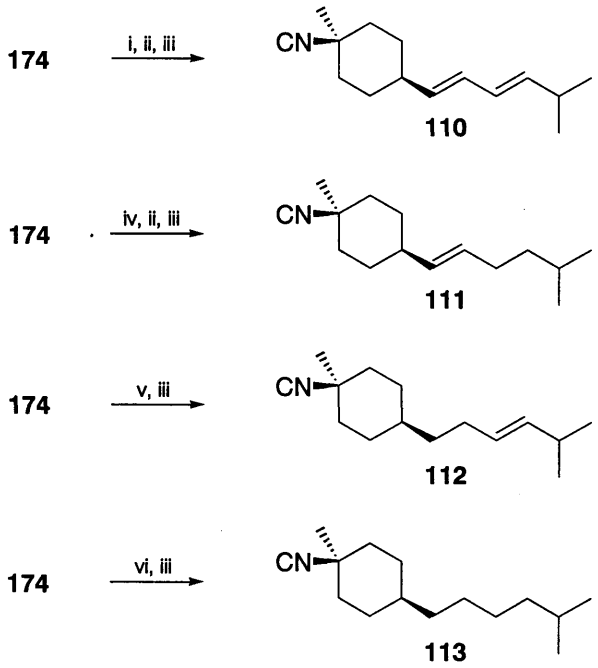
Scheme 2-2. Reagents and conditions: (i) TMSCN, $AgBF_4$, (mixture of **171** : **172** = 3 : 7).



Scheme 2-3. Reagents and conditions: (i) LDA, THF, then 4-methylpent-2-enal, -78°C; (ii) Ac_2O, Pyr, rt; (iii) 5% Na-Hg, Na_2HPO_4, EtOAc-MeOH, -10 °C, (**35** : **108**, 2 : 1, 50%; **107** : **109**, 2 : 1, 55%).



Scheme 2-4. Synthesis of isocyano-sulfone **174**. Reagents and conditions: (i) Et_2AlCl, -78°C; (ii) $LiAlH_4$, THF, 0°C; (iii) TsCl, pyridine, rt, quant; (iv) NaI, CH_3CN, reflux, (v) $PhSO_2Na$, DMF, rt; (vi) 35% H_2SO_4, rt; (vii) TMSCN, $AgClO_4$, $MeNO_2$, rt.



Scheme 2-5. Synthesis of isocyano compounds **110**, **111**, **112**, and **113**. Reagents and conditions: (i) BuLi, THF, then 4-methyl-2-pentenal, -78°C; (ii), Ac_2O, pyridine, rt; (iii) 5% Na-Hg, Na_2HPO_4, EtOAc-MeOH, -10°C; (iv) BuLi, THF, then 4-methylpentanal, -78°C; (v) BuLi, THF, then 1-bromo-4-methyl-2-pentene, 0°C; (vi) BuLi, THF, then p-toluensulfonyl 4-methylpentanoate, 0°C; (**110**, 53%; **111**, 51%; **112**, 43%; **113**, 49%).

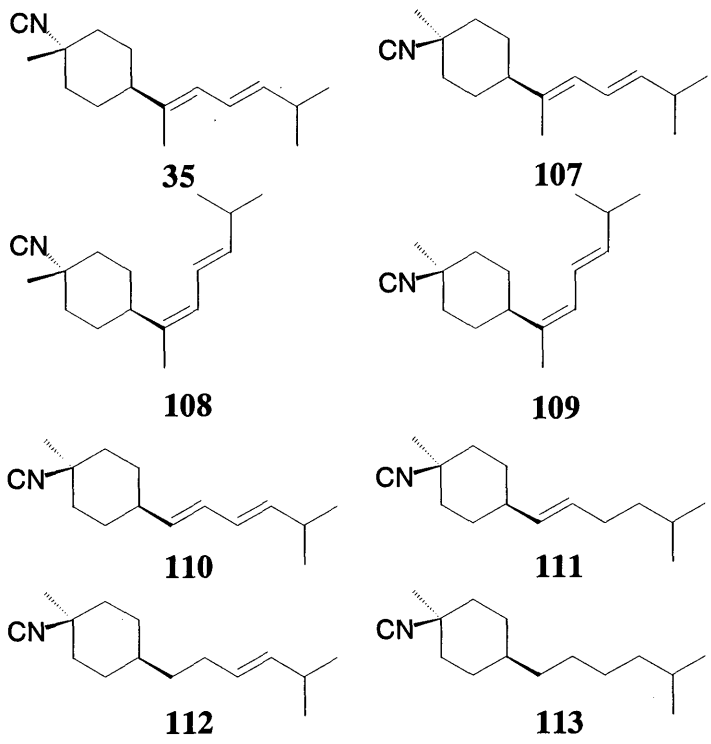


Figure 2-1. Structures of compounds **35** and **107-113.**

Structures of 3-isocyanotheonellin (**35**) and its analogs **107-113** are illustrated in Figure 2-1, and their antifouling activity and toxicity are summarized in Table 2-1. $CuSO_4$ was used as a positive control; its EC_{50} and LD_{50} are 0.30 μg/mL and 2.95 μg/mL, respectively as shown in Figure 2-2. Compounds **35** and **107-113** were active with EC_{50} values ranging from 0.19 to 7.20 μg/mL, while their toxicity to cyprids larvae was much less than $CuSO_4$. The synthesized 3-isocyanotheonellin (**35**) showed similar antifouling activity to that of the natural product (EC_{50} = 0.13 μg/mL).[50,51] Compounds **107-109**, either stereoisomers or geometrical isomers of **35**, were as active as the parent compound, thus suggesting that the stereochemistry at C-3 and the geometry of the butadiene do not affect the activity. Compounds **110, 111, 112,** and **113** were moderately antifouling with EC_{50} values of 1.80, 7.20, 1.80, and 3.80 μg/mL, respectively. Since antifouling activity of compound **110,** which lacked the C-14 methyl group in **107,** exhibited 10-fold less active than that of **107,** thus suggesting that the methyl group at C-14 position may influence activity. Since the saturated alkane **113** was as active as compounds **110-112,** the presence of double bonds seems to be not essential for antifouling activity.

Table 2-1. Antifouling Activity and Toxicity of 3-Isocyanotheonellin and Its Analogues

Compound No.	EC_{50} (μg/mL)	LD_{50} (μg/mL)
35	0.19	>100
107	0.18	>100
108	0.29	>100
109	0.41	>100
110	1.80	>100
111	7.20	>100
112	1.80	>100
113	3.80	>100

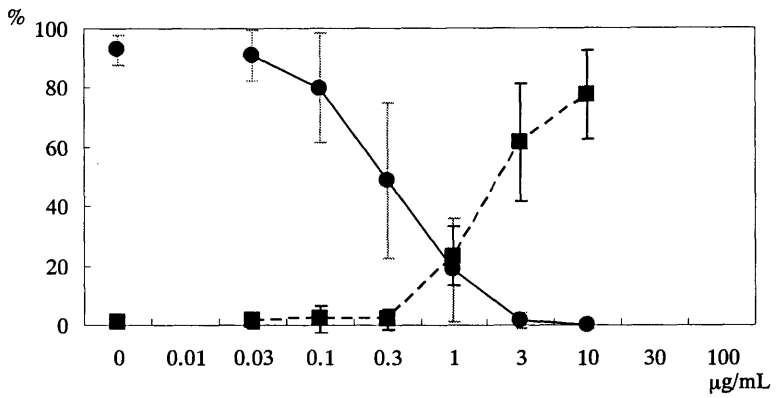


Figure 2-2. Antifouling activity (closed circle) and mortality (closed square) of $CuSO_4$.

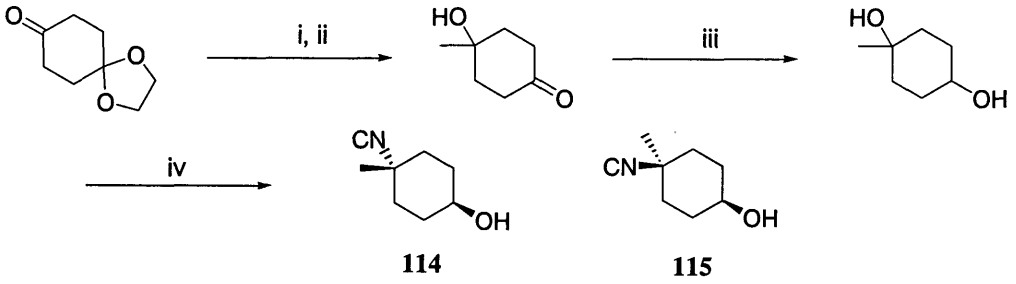
Antifouling Activity of Isocyanocyclohexanes 114-125[101]

Isocyanocyclohexanols (**114** and **115**), their esters (**116-121**), and ethers (**122** and **123**) were synthesized and evaluated for their antifouling activity (Figure 2-3). Isocyanocyclohexanols **114** and **115** were synthesized according to Scheme 2-6. These stereoisomers were slight lower active than isocyanotheonellin (**35**), whereas their toxicity was low (LD_{50} > 30 μg/mL) (Figure 2-2). Again, stereochemistry of C-4 did not affect the activity.

As shown in Table 2-2, the esters and ethers were highly antifouling except for the ether **123**, but not toxic to cyprids. Particularly active were the acetate **116**, the benzoate **119** and the pivaloate **120**; interestingly their stereoisomeric counterparts were much less active. It is thus puzzling why they showed different activity, since the stereochemistry of C-4 did not affect the activity in the cyclohexanols (**114** and **115**) and

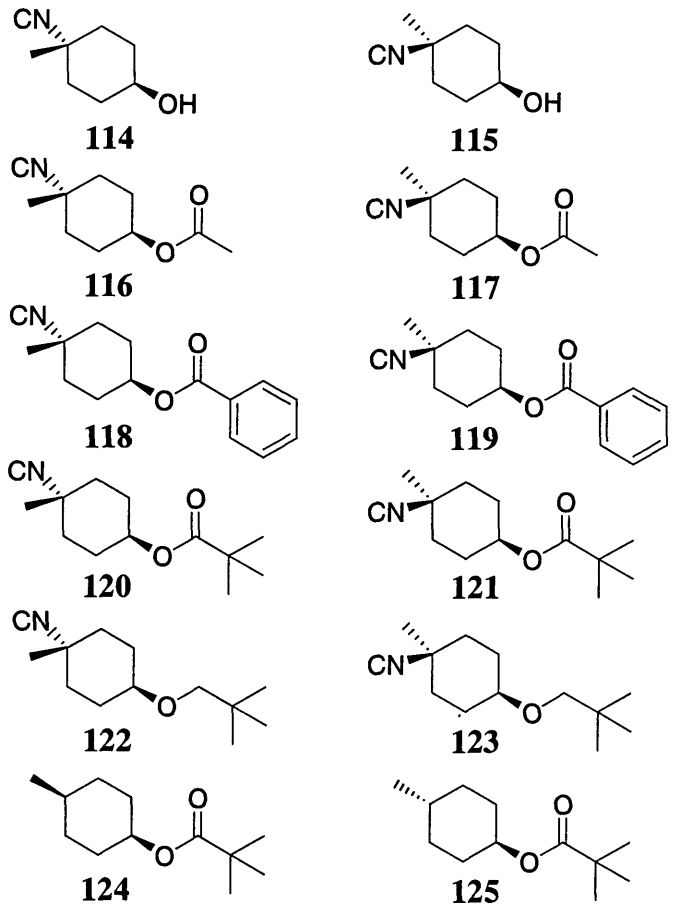
isocyanotheonellin related compounds. Although significant toxicity was found for the benzoate **118**, it was considerably less toxic than $CuSO_4$; its mortality at 3.0 μg/mL was 13.6 % (the mortality of $CuSO_4$ was 68.8 % at the same concentration). The weak activity of the neopentyl ether **123** is also not understandable.

The inactivity of the des-isocyano derivatives **124** and **125** clearly indicates the importance of isocyano group for antifouling activity.



114 **115**

Scheme 2-6. Synthesis of isocyanocyclohexanol **114** and **115**. Reagents and conditions: (i) MeLi, THF, 0°C; (ii) HCl, rt; (iii) $NaBH_4$, MeOH, 0°C; (iv) TMSCN, $AgClO_4$, $MeNO_2$, rt.(mixture of **114** : **115** = 75 : 25)



114 **115**

116 **117**

118 **119**

120 **121**

122 **123**

124 **125**

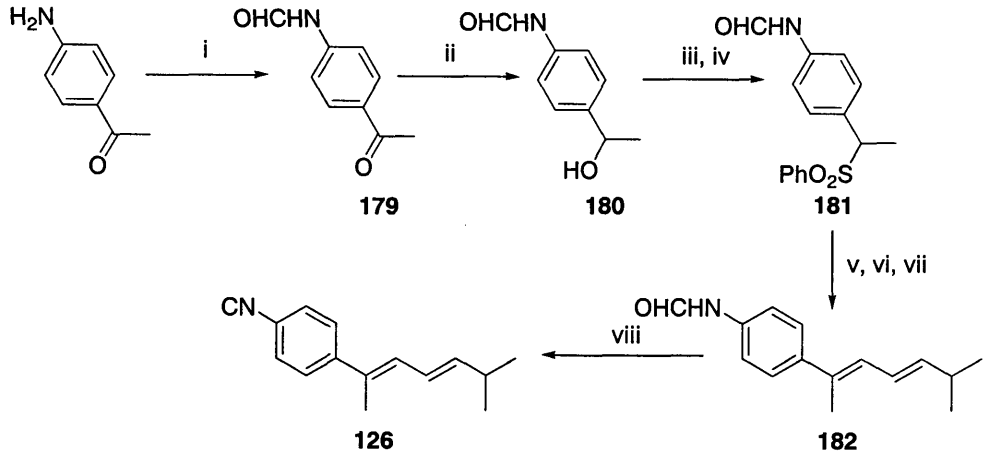
Figure 2-3. Structures of isocyanocyclohexnes.

Table 2-2. Antifouling Activity and Toxicity of Isocyanocyclohexanes.

Compound No.	EC_{50} (μg/mL)	LD_{50} (μg/mL)
114	0.48	> 100
115	0.98	> 30
116	0.0096	> 30
117	0.12	> 30
118	0.11	> 3
119	0.049	> 30
120	0.019	> 30
121	0.54	> 100
122	0.18	> 100
123	17.0	> 30
124	> 30	> 30
125	> 30	> 30

2. Antifouling Activity of Isocyanobenzenes 126-152

Isocyanobenzenes and related compounds (Figures 2-4 and 2-5) were synthesized (**126**: Scheme 2-7) and evaluated for their antifouling activities. Antifouling activity of compounds **126-152** is summarized along with their LD_{50} values in Tables 2-3 and 2-4.



Scheme 2-7. Synthesis of isocyanobenzene **126**. Reagents and conditions: (i) *p*-TsOH, EtOCHO, reflux; (ii) $NaBH_4$, MeOH, 0°C, quant.; (iii)ZnI_2, PhSH, CH_2Cl_2, rt; (iv) *m*CPBA, CH_2Cl_2, rt; (v) LDA, THF, then 4-methylpent-2-enal, -78°C ; (vi) Ac_2O, Pyr, rt; (vii) 5% Na-Hg, Na_2HPO_4, EtOAc-MeOH, -10°C; (viii) Tf_2O, DIPEA, CH_2Cl_2, -78°C.

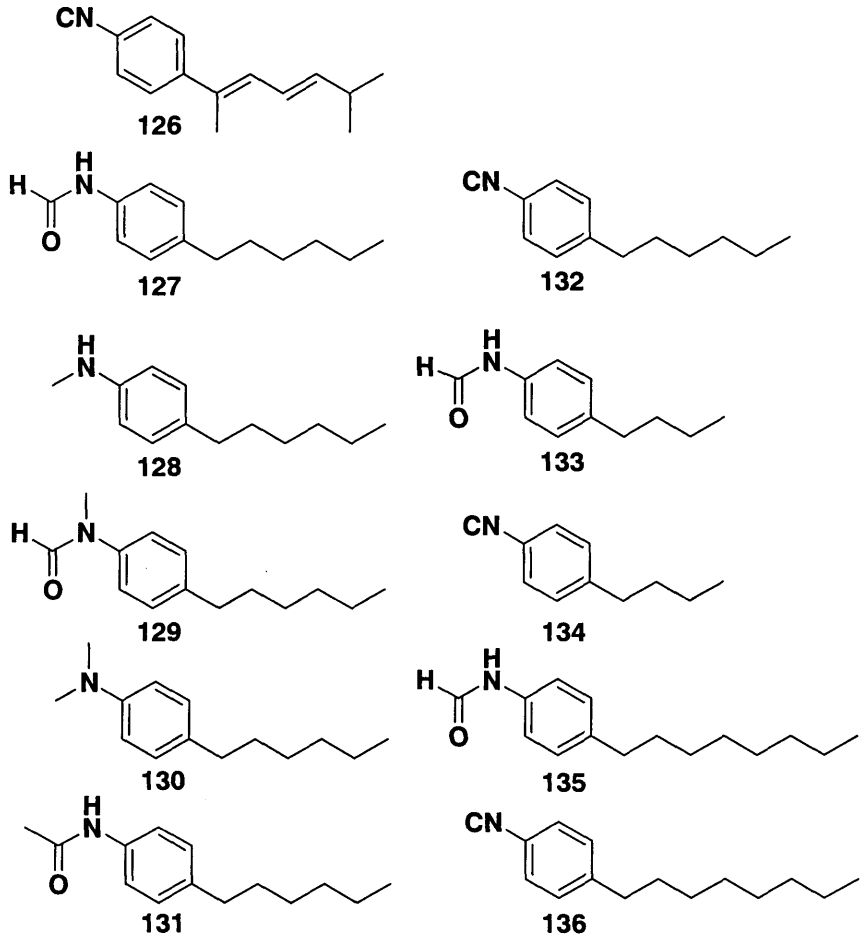


Figure 2-4. Structures of isocyanobenzenes and its related compounds.

Compound **126**, in which the cyclohexane moiety of **35** was replaced by a phenyl group, showed potent antifouling activity (EC_{50} 0.078 μg/mL) without significant toxicity.

Among alkylbenzene-type compounds **127-136**, only 4-hexylphenyl acetamide **131** showed promising antifouling activity (EC_{50} = 0.20 μg/mL and LD_{50} > 100 μg/mL). Isocyanobenzenes **132**, **134**, and **136,** which have different length of alkyl chains, were moderately active with EC_{50} values of 0.64, 1.03, and 0.58 μg/mL, respectively. However, **132** was considerably toxic to cyprids with an LD_{50} value of 14.2 μg/mL. Formamide **127**, *N*-methyl aniline **128**, and *N*-methyl formamide **129** were also active with EC_{50} values ranging from 0.51 to 3.95 μg/mL. Formamide **127** was as toxic as $CuSO_4$ (LD_{50} = 3.65 μg/mL). Dimethylaminobenzene **130** and octylphenyl formamide **135** were clearly less active with EC_{50} values of 38.0 and 36.0 μg/mL, respectively.

Table 2-3. Antifouling Activity and Toxicity of Isocyanobenzenes and Its Related Compounds **126-136**

Compound No.	EC_{50} (μg/ml)	LD_{50} (μg/ml)
126	0.078	> 100
127	1.48	3.65
128	3.95	88.2
129	0.51	14.0
130	38.0	> 100
131	0.20	> 100
132	0.64	14.2
133	2.25	34.8
134	1.03	> 3.0
135	36.0	> 100
136	0.58	> 100

As shown in Figures 2-5 and Table 2-4, some benzyloxy derivatives **143**, **144**, **145**, **146**, and **148** were neither antifouling nor toxic. Isocyanobenzene **138** was more active than $CuSO_4$ (EC_{50} = 0.054 μg/mL), but also as toxic as $CuSO_4$ (LD_{50} = 3.0 μg/mL). Compounds **137**, and **139-142** showed antifouling activity with EC_{50} values ranging from 0.22 to 4.3 μg/mL, but they were also lethal to cyprids with LD_{50} values ranging from 4.2 to 28.2 μg/mL. Cyanide **149** slightly inhibited larval settlement with an EC_{50} value of 1.3μg/mL. Compounds **150-152** were weakly active with EC_{50} values ranging from 7.0 to 12.6 μg/mL.

Compounds **126** and **131** showed promising activity, which is equivalent to that of **35**. Although some benzyloxy compounds showed potent antifouling activity, they were also toxic. In particular, benzyloxy isocyanobenzene **138** showed not only strong antifouling activity but also high toxicity comparable to $CuSO_4$.

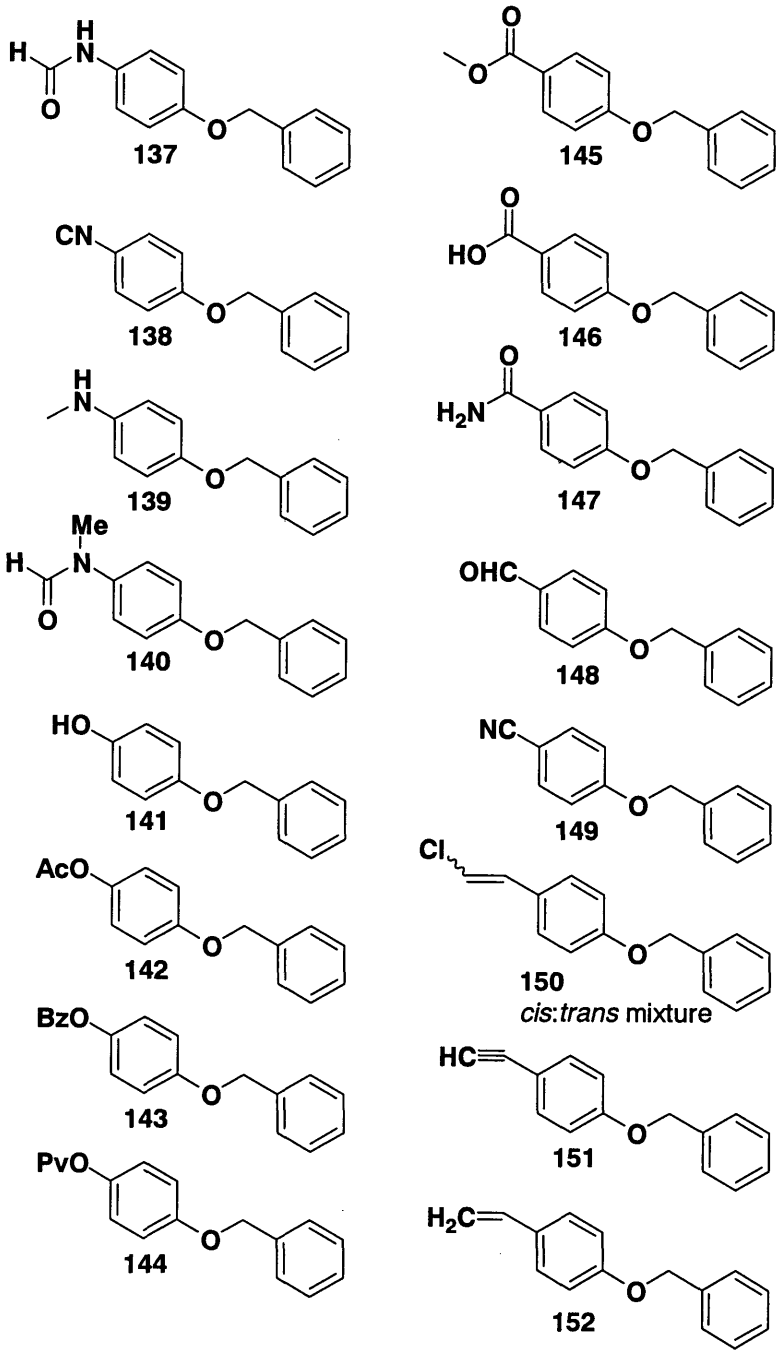


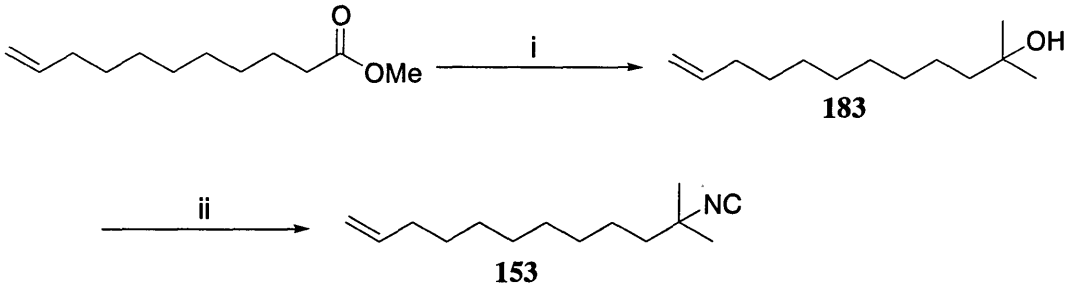
Figure 2-5. Structures of benzyloxy isocyano benzene and its related compounds (2).

Table 2-4. Antifouling Activity and Toxicity of Benzyloxy Isocyano Benzenes and Its Related Compounds **137-152**

Compound No.	EC_{50} (μg/ml)	LD_{50} (μg/ml)
137	4.25	20.4
138	0.054	3.0
139	0.22	28.2
140	0.34	24.5
141	1.7	4.2
142	4.3	7.5
143	> 30	> 30
144	> 30	> 30
145	> 30	> 30
146	> 30	> 30
147	4.3	> 30
148	> 30	> 30
149	1.3	> 30
150	7.0	> 30
151	10.4	> 30
152	12.6	> 30

Antifouling Activity of Simple Linear Isocyanides 153-164[102]

Twelve simple linear isocyanides were synthesized (**153**: Scheme 2-8) and examined for antifouling activity and toxicity. Structures and antifouling activity of compounds **153-164** are shown in Figure 2-6 and their EC_{50} values are summarized along with their LD_{50} values in Table 2-5.



Scheme 2-8. Synthesis of compound **153**. Reagents and conditions: (i) MeLi, THF, 0°C; (ii) TMSCN, $AgClO_4$, $MeNO_2$, rt.

As shown in Table 2-5, compounds **153-164** showed considerable antifouling activity; compounds **153**, **156**, **161**, and **162** were more active than $CuSO_4$ (EC_{50} < 0.11 μg/mL), while **154**, **155**, **157**, **163**, and **164** were as active as $CuSO_4$. Benzoate **158**, **159**, and **160** were moderately active with EC_{50} values ranging from 1.09 to 1.90 μg/mL, but they were not lethal to cyprids even at 300 μg/mL. On the other hand, phenylsulfide **161** not only showed potent antifouling activity but also was non-toxic, though it did not inhibit larval settlement completely even at high concentrations. Compounds **154**, **155**, **156**, and **164** were moderately toxic with LD_{50} values ranging from 21.3 to 70.0 μg/mL, which were much higher than that of $CuSO_4$. The amino and acetamide derivatives **155** and **156** were 7-fold less toxic than $CuSO_4$, while their antifouling activity was much stronger than that of $CuSO_4$. Compounds **153**, **163**, and **164**, which have similar structures with the different number of methyl groups, showed different antifouling activity and toxicity.

It is apparent that isocyano group is essential for antifouling activity. Toxicity seems to be due to the presence of a hydrogen bonding donor, because compounds **154**, **155**, and **156** possess a hydrogen bonding donor moiety and show slight toxicity. Toxicity of alkyl amines against the brine shrimp *Artemia salina* increased with increase in chain length up to tridecylamine (C_{13}).[103] Since compounds **154**, **155**, and **156** have similar alkyl chain lengths (C_{11}), their active mechanisms, which are probably similar to primary alkyl amines, might be attributed to interactions with cell membranes resulting in increased fluidity and impaired function.[103] Among three compounds **153**, **163**, and

164, which have similar structures with the different number of methyl groups, only primary isocyanide **164** showed weak toxicity (LD_{50} = 70.0 μg/mL), perhaps the lipophilicity of isocyano moiety may influence larval settlement and toxicity. Benzoates **158**, **159**, and **160** showed moderate antifouling activity, which is perhaps due to their poor solubility.

Table 2-5. Antifouling activity and toxicity of 12 linear isocyanides

Compound	EC_{50} (μg/ml)	LD_{50} (μg/ml)
153	0.046	>30
154	0.31	>10
155	0.16	21.28
156	0.10	22.25
157	0.21	>100
158	1.09	>300
159	1.90	>300
160	1.66	>300
161	0.056	>30
162	0.11	>100
163	0.48	>100
164	0.14	70.0

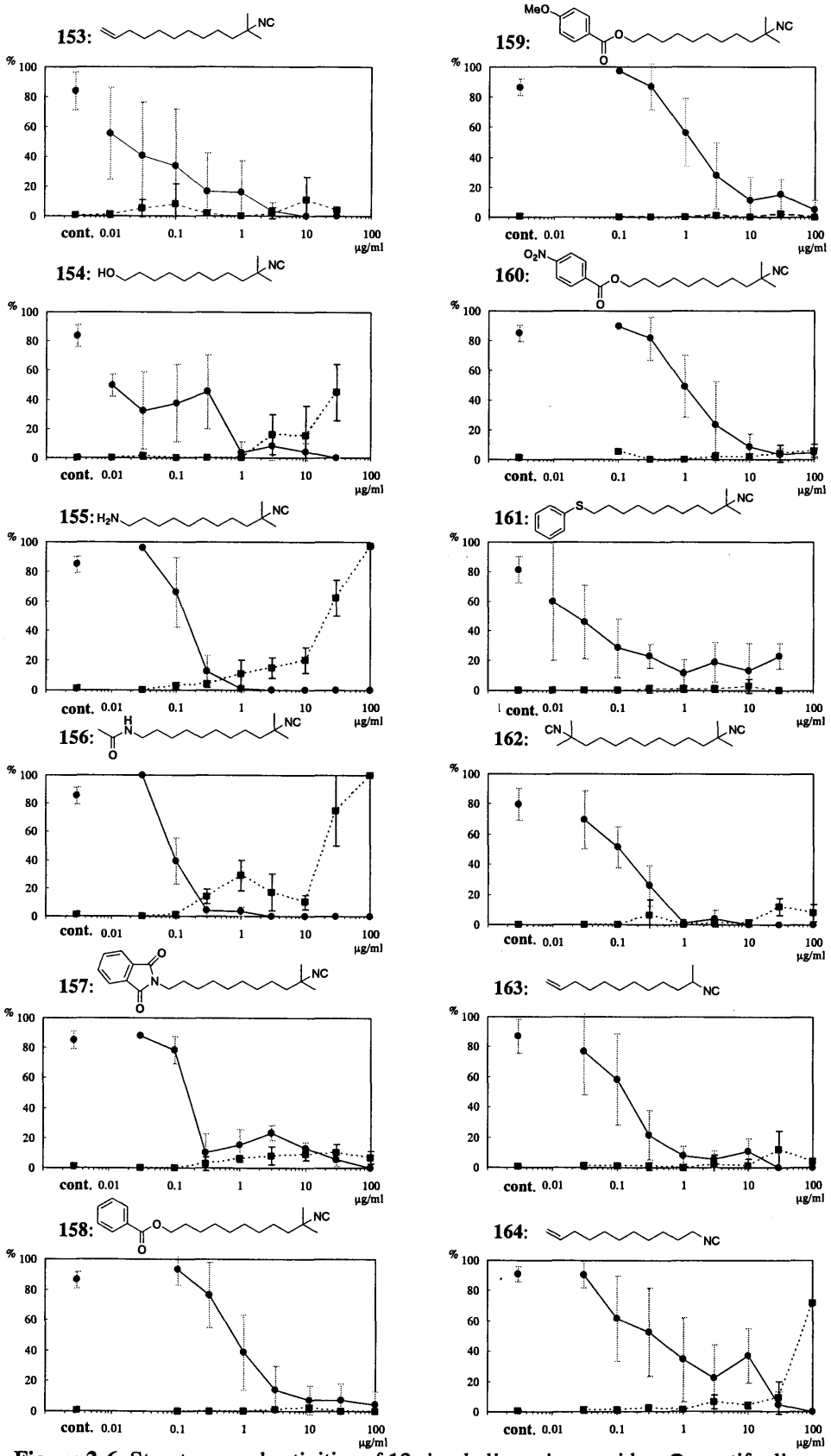


Figure 2-6. Structures and activities of 12 simple linear isocyanides. ● = antifouling activity, ■ = mortality.

Experimental Section

General experimental procedures

NMR spectra were recorded on a JEOL A600 NMR or JEOL A400 NMR spectrometer operating at 600 MHz or 400MHz for ^{1}H and 150 MHz for ^{13}C. ^{1}H NMR chemical shifts were referenced to TMS, and ^{13}C NMR chemical shifts were referenced to solvent peaks: δ_C 77.0 ($CDCl_3$). Low- and high-resolution mass spectra were recorded on a JEOL SX-102A spectrometer. Chromatographic separations were carried out on a silica gel column (Fuji Silysia Chemical BW-127ZH; 100-270 mesh).

Antifouling assay

Antifouling assay was performed essentially as described in Chapter 1 with some modification as below.

Each level of experiments was carried out with four wells (18-30 cyprids). The assay of compounds and the controls was repeated 5 times with different batches of larvae. The repetitions of each concentration were 3-5 times.

The antifouling activity of isocyanides and $CuSO_4$ was expressed as an EC_{50} value, which indicated the concentration that reduces the larval settlement to 50% of the control. The EC_{50} values were calculated by a probit analysis. If data were not appropriate for probit analysis, EC_{50} values were estimated by straight-line graphical interpolation. The toxicity of compounds was expressed as a LD_{50} value, which indicates the concentration that results in 50% mortality estimated by a probit analysis. If data were not appropriate for probit analysis, LD_{50} values were estimated by straight-line graphical interpolation.

Synthesis of 3-isocyanotheonellin (35) and its analogues 107-113[99]

3-Isocyanotheonellin (**35**).

cis-4-[(*E,E*)-1,5-Dimethylhexan-1,3-dienyl]-1-methylcyclohexyl isocyanide (**107**).

trans-4-[(*Z,E*)-1,5-Dimethylhexan-1,3-dienyl]-1-methylcyclohexyl isocyanide (**108**).

cis-4-[(*Z,E*)-1,5-Dimethylhexan-1,3-dienyl]-1-methylcyclohexyl isocyanide (**109**).

These compounds were synthesized starting with 1,4-cyclohexanedione monoethylene ketal (**165**) as follows: Ketone **165** was reduced to alcohol 4,4-ethylendioxycyclohexane-1-ol (**166**) (5.06 g, 32.0 mmol) with $NaBH_4$ (1.46 g, 38.4 mmol), which was then treated with *p*-toluenesulfonyl chloride (TsCl, 7.24 g, 38 mmol) and pyridine (30 mL) to give 4,4-ethylendioxycyclohexyl toluene-*p*-sulfonate (**167**) in 98% yield over two steps. The nucleophilic substitution of tosylate **167** with ethyl phenyl sulfone (5.31 g, 31.2 mmol), followed by lithiated with *n*-BuLi (excess) yielded

4-[(1-phenylsulfonyl)ethyl]cyclohexane-1-one ethylene ketal (**168**) (4.5 g, 70%). Sulfone **168** was converted to 4-[(1-phenylsulfonyl)ethyl]cyclohexane-1-one (**169**) by deprotection of the acetal (4.4 g, 14.17 mmol) with *p*-TsOH (266 mg, 1.4 mmol) in 82% yield. Methylation of ketone **169** (2.48 g, 9.31mmol) with MeLi (1.14M in Et_2O, 25mL) led to the diastereomers of *trans*-1-methyl-4-[(1-phenylsulfonyl)ethyl]cyclohexane-1-ol (**170a**) and *cis*-1-methyl-4-[(1-phenylsulfonyl)ethyl]cyclohexane-1-ol (**170b**) (*trans*:*cis* = 2:3 mixture) in 85% yield (Scheme 2-1).

trans-1-methyl-4-[(1-phenylsulfonyl)ethyl]cyclohexyl isocyanide (**171**) and *cis*-1-methyl-4-[(1-phenylsulfonyl)ethyl]cyclohexyl isocyanide (**172**) were prepared from *tert*-alcohol **170** (2:3 mixture of **170a** and **170b**, 141 mg, 0.5 mmol) by treatment with trimethylsilyl cyanide (TMSCN, 135 μL, 1.0 mmol) and $AgBF_4$ (194 mg, 0.5 mmol) in CH_2Cl_2. Isocyanides **171** and **172** were obtained as a 3:7 mixture (70 mg, 48%). (Scheme 2-2)

Compounds **35**, and **107-109** were obtained by the Julia olefination as the final stage of the reaction (Scheme 2-3). Treatment of isocyanosulfone **171** (115 mg, 0.395 mmol) with LDA [prepared from diisopropylamine (0.12 ml, 0.84 mmol) and *n*-BuLi (1.6 M in hexane, 0.5 mL, 0.8 mmol)] followed by entrapment of the generated anion with 4-methylpent-2-enal (10 μL, 0.86 mmol) provided the coupling product, which was converted to the corresponding β-acetoxysulfone (135 mg) with acetic anhydride (2 mL) and pyridine (2 mL). Reductive elimination of the β-acetoxysulfone with a 5% sodium amalgam gave the 2 : 1 geometrical mixture (45 mg) of **35** and (*Z, E*)-diene **108** in 50% yield from **171**. A part of the mixture was purified by ODS HPLC to give pure **35** (24 mg) and **108** (12 mg). The analogues **107** and **109** were obtained from isocyanosulfone **172** (314 mg, 1.07 mmol) by the same method to give a mixture of **107** and **109** (2:1, 146 mg, 59%). A part of the mixture was purified by HPLC to give pure **107** (40 mg) and **109** (20 mg). (Scheme 2-3)

35; Colorless oil; ^{1}H NMR d 6.21 (1H, ddd, J = 15, 10.5 and 1 Hz), 5.80 (1H, dd, J =10.5 and 1 Hz), 5.59 (1H, dd, J = 15 and 7 Hz), 2.39-2.31 (1H, m), 2.00-1.88 (3H, m), 1.86-1.78 (2H, m), 1.75-1.67 (5H, m) including 1.72 (3H, br, s), 1.47-1.37 (5H, m) including 1.44 (3H, t, J = 2Hz), 1.01 (6H, d, J = 6.5Hz); ^{13}C NMR d 152.14 (t, J = 5 Hz), 140.75, 140.75, 138.70, 123.87, 123.42, 56.77(t, J = 5.0 Hz), 44.81, 38.27, 31.42, 26.48, 25.12, 22.54, 15.24; LR-EIMS: *m/z* (%) 231 (M^+, 46), 216 (8), 204 (21), 189 (25), 188 (14), 161 (54), 121 (49), 105 (88), 95 (73), 93 (100); HR-EIMS: calcd. for $C_{16}H_{25}N$ 231.1987, found 231.2004.

107; Colorless oil; ^{1}H NMR d 6.22 (1H, ddd, J = 15, 10.5 and 1 Hz), 5.84 (1H, d, J =10.5), 5.60 (1H, dd, J = 15 and 7 Hz), 2.40-2.31 (1H, m), 1.98-1.93 (2H, m), 1.88-1.81

(1H, m), 1.75 (3H, br, s), 1.74-1.61 (4H, m), 1.43 (3H, t, J = 2Hz), 1.41-1.33 (2H, m), 1.02 (6H, d, J = 6.5Hz); ^{13}C NMR d 153.93 (t, J = 4 Hz), 140.49, 139.60, 123.84, 123.44, 57.75 (t, J = 5.0 Hz), 46.02, 38.31, 31.40, 30.06, 26.71, 22.54, 14.77; LR-EIMS: *m/z* (%) 231 (M^+, 73), 216 (33), 204 (35), 189 (100), 188 (78), 136 (63), 121 (61), 95 (89), 93 (83); HR-EIMS: calcd. for $C_{16}H_{25}N$ 231.1987, found 231.1975.

108; Colorless oil; ^{1}H NMR d 6.19 (1H, dd, J = 15 and 11 Hz), 5.76 (1H, d, J =11 Hz), 5.56 (1H, dd, J = 15 and 7.5 Hz), 2.61 (1H, tt, J = 12 and 4 Hz), 2.39-2.30 (1H, m), 2.03-1.97 (2H, m), 1.95-1.88 (2H, m), 1.67 (3H, br, s), 1.54-1.40 (6H, s) including 1.47 (3H, t, J = 2Hz), 1.33-1.25 (1H, m), 1.01 (6H, d, J = 7 Hz); ^{13}C NMR d 152.00 (t, J = 5 Hz), 140.79, 140.75, 138.60, 126.10, 122.23, 56.33(t, J = 5.0 Hz), 38.80, 31.47, 26.21, 24.36, 22.62, 19.73, 14.14; LR-EIMS: *m/z* (%) 231 (M^+, 60), 216 (11), 204 (26), 189 (35), 188 (19), 161 (48), 121 (65), 105 (89), 95 (84), 93 (100); HR-EIMS: calcd. for $C_{16}H_{25}N$ 231.1987, found 231.1981.

109; Colorless oil; ^{1}H NMR d 6.19 (1H, dd, J = 15and 11 Hz), 5.77 (1H, d, J =11 Hz), 5.55 (1H, dd, J = 15 and 7.5 Hz), 2.53 (1H, tt, J = 12 and 3 Hz), 2.38-2.28 (1H, m), 1.99-1.92 (2H, m), 1.84-1.75 (2H, m), 1.74 (3H, br, s), 1.54-1.39 (7H, s) including 1.45 (3H, t, J = 2Hz), 1.00 (6H, d, J = 7 Hz); ^{13}C NMR d 154.00 (t, J = 5 Hz), 140.36, 139.61, 125.61, 122.35, 57.67 (t, J = 5.0 Hz), 38.53, 38.18, 31.47, 26.01, 22.64, 19.81; LR-EIMS: *m/z* (%) 231 (M^+, 78), 216 (43), 204 (19), 189 (88), 188 (100), 136 (75), 121 (66), 121 (66), 95 (93), 93 (83); HR-EIMS: calcd. for $C_{16}H_{25}N$ 231.1987, found 231.1968.

cis-4-[(*E,E*)-5-Methylhexa-1,3-dienyl]-1-methylcyclohexyl isocyanide (**110**).[100]

Compound **110** was obtained from *cis*-4-benzenesulfonylmethyl-1-methylcyclohexyl iscyanide (**174**) (277 mg, 1.0 mmol) as follows (Scheme 2-4): A Diels-Alder reaction of isoprene and methyl acrylate with diethylaluminium chloride (1.4 equiv) yielded methyl 4-methylcyclohexa-3-enecarboxylate (**175**) in an 85%. This was reduced to alcohol with lithium aluminium hydride (2.2 equiv) in dry ether followed by tosylation of the resulting alcohol with TsCl (1.5 equiv) and pyridine (exess) to give 4-methylcyclohexa-3-enylmethyl *p*-toluenesulfonate (**176**) in a 98% yield. Tosylate **176** (11.0 g, 39.2 mmol) was converted to alkyl iodide with sodium iodide (2.0 equiv), which was then treated with phenylsulfinic acid, sodium salt (1.5equiv) to give (4-methylcyclohexa-3-enylmethyl) phenyl sulfone (**177**) in an 80% yield. Hydration of alkene moiety in sulfone **177** with 35% H_2SO_4 (excess) led to 4-benzenesulfonylmethyl-1-methyl-cyclohexanol (**178**) in a 65% yield. *tert*-alcohol **178** was converted to isocyanosulfone **174** directly with trimethylsilyl cyanide and silver

perchlorate in a 70% yield.[100]

Isocyano compound **110** was prepared from isocyano-sulfone **174** (277 mg, 1.0 mmol) according to standard Julia olefination described for compound **35** with 4-methyl-2-pentenal (3.0 equiv) in a 53% yield (Scheme 2-5).

110; colorless oil; ^{1}H NMR d 6.04-5.93 (2H, m), 5.59 (1H, dd, J = 14.3 and 6.6 Hz), 5.54 (1H, dd, J = 14.3 and 7.0 Hz), 2.37-2.28 (1H, m), 1.96-1.88 (3H, m), 1.71-1.66 (2H, m), 1.59-1.50 (4H, m), 1.42 (3H, t, J = 1.8Hz), 1.40-1.33 (2H, m), 1.31-1.25 (2H, m), 1.00 (6H, d, J = 6.6 Hz); ^{13}C NMR d 154.03 (t, J = 5 Hz), 140.42, 136.15, 129.06, 127.26, 57.61 (t, J = 5.0 Hz), 39.53, 37.96, 31.05, 30.11, 28.08, 22.37.

cis-4-[(*E*)-5-Methylhexa-1-enyl]-1-methylcyclohexyl isocyanide (**111**).[100]

Compound **111** was synthesized from **174** (277 mg, 1.0 mmol) and 4-methylpentanal (3.0 equiv) by the standard Julia olefination described for compound **27** (51% yield) (Scheme 2-5).

111; colorless oil; ^{1}H NMR d 5.44-5.33 (2H, m), 2.00-1.95 (2H, m), 1.94-1.88 (2H, m), 1.86-1.80 (2H, m), 1.68-1.62 (2H, m), 1.57-1.47 (2H, m), 1.42 (3H, t, J = 1.8Hz), 1.39-1.31 (2H, m), 1.25-1.20 (2H, m), 0.88 (6H, d, J = 6.6 Hz); ^{13}C NMR d 153.85 (t, J = 5 Hz), 134.30, 129.25, 57.68 (t, J = 5.0 Hz), 39.50, 38.80, 38.05, 30.44, 30.13, 28.35, 27.49, 22.53.

cis-4-[(*E*)-5-Methylhexa-3-enyl]-1-methyl-cyclohexyl isocyanide (**112**).[100]

Compound **112** was prepared from isocyano-sulfone **174** (277 mg, 1.0 mmol) by treatment with 1-bromo-4-methyl-2-pentene (2.0 equiv) followed by the reductive elimination of sulfone with 5% sodium-amalgam (43% yield) (Scheme 2-5).

112; colorless oil; ^{1}H NMR d 5.40-5.30 (2H, m), 2.27-2.18 (1H, m), 2.03-1.96 (2H, m), 1.92-1.85 (2H, m), 1.70-1.64 (2H, m), 1.41 (3H, t, J = 1.8Hz), 1.36-1.25 (6H, m), 1.22-1.13 (1H, m), 0.96 (6H, d, J = 6.6 Hz); ^{13}C NMR d 153.64 (t, J = 5 Hz), 137.74, 126.95, 58.17 (t, J = 5.0 Hz), 38.28, 36.55, 35.87, 31.00, 30.12, 29.80, 28.25, 22.69.

cis-4-(5-Methylhexyl)-1- methylcyclohexyl isocyanide (**113**).[100]

Compound **113** was prepared from isocyano-sulfone **174** (277 mg, 1.0 mmol) by treatment with *p*-toluenesulfonyl 4-methylpentanoate (2.0 equiv) followed by the reductive elimination of sulfone with 5% sodium-amalgam (49% yield) (Scheme 2-5).

113; colorless oil; ^{1}H NMR d 1.90-1.86 (2H, m), 1.69-1.64 (2H, m), 1.55-1.48 (1H, m), 1.41 (3H, t, J = 1.8Hz), 1.37-1.22 (10H, m), 1.18-1.12 (3H, m), 0.86 (6H, d, J = 6.6 Hz); ^{13}C NMR d 153.63 (t, J = 5 Hz), 58.20 (t, J = 5.0 Hz), 39.03, 38.35, 36.75, 36.50,

30.14, 28.40, 27.97, 27.63, 27.14, 22.66.

Synthesis of isocyanocyclohexanes 114-125[101]

trans-4-Hydroxy-1-methylcyclohexyl isocyanide (**114**).

cis-4-Hydroxy-1-methylcy-clohexyl isocyanide (**115**).

Compound **114** and **115** were prepared from 1,4-cyclohexanedione *mono*-ethylene ketal (6.0 g, 38.4 mmol) as follows. 4-Hydroxy-4-methylcyclohexanone was synthesized according to methylation with MeLi (1.5 equiv) followed by hydrolysis of acetal with aqueous HCl. After converting the ketone to the primary-alcohol by treatment with $NaBH_4$ (1.5 equiv), compounds **114** and **115** were obtained from 1-methylcyclohexane-1,4-diol (*cis*:*trans* mixture) by treatment with trimethylsilyl cyanide and silver perchlorate in an 38% yield (**114**:**115** = 75:25) (Scheme 2-6.). [103]

114: white solid; ^{1}H NMR d 4.08 (1H, m), 1.91 (2H, m), 1.81 (2H, m), 1.72-1.66 (4H, m), 1.44 (3H, t, J = 1.8 Hz), 1.28 (1H, br s); ^{13}C NMR d 153.80 (t, J = 5 Hz), 64.67, 57.85 (t, J = 5.0 Hz), 32.41, 29.54, 28.77.

115: white solid; ^{1}H NMR d 3.57 (1H, m), 1.99-1.88 (4H, m), 1.70 (2H, m) , 1.49 (1H, br s), 1.43 (3H, t, J = 1.8 Hz), 1.42 (2H, m); ^{13}C NMR d 154.61 (t, J = 5 Hz), 69.29, 57.01 (t, J = 5.0 Hz), 36.68, 30.98, 29.25.

trans-4-Isocyano-4-methylcyclohexyl acetate (**116**).[104]

cis-4-Isocyano-4-methylcyclohexyl acetate (**117**).[104]

Compounds **116** and **117** were prepared from a mixture of **114** and **115** (170 mg, 1.30 mmol) according to acetylation with acetic anhydride and pyridine (excess) by treatment with trimethylsilyl cyanide and silver perchlorate in an 86% yield (**116**:**117** = 75:25).

116: colorless oil; ^{1}H NMR d 5.05 (1H, m), 2.05 (3H, s), 1.92 (2H, m), 1.84 (2H, m) , 1.76 (2H, m), 1.67 (2H, m), 1.47 (3H, t, J = 1.8 Hz); ^{13}C NMR d 170.27, 154.47 (t, J = 5 Hz), 67.72, 57.49 (t, J = 5.0 Hz), 32.95, 29.64, 25.82, 21.32.

117: colorless oil; ^{1}H NMR d 4.65 (1H, m), 2.05 (3H, s), 1.98 (2H, m), 1.92 (2H, m) , 1.80 (2H, m), 1.47 (2H, m), 1.45 (3H, t, J = 1.8 Hz); ^{13}C NMR d 170.63, 154.93 (t, J = 5 Hz), 71.12, 56.96 (t, J = 5.0 Hz), 36.41, 29.15, 27.03, 21.31.

trans-4-Isocyano-4-methylcyclohexyl benzoate (**118**).[101]

Compound **118** was prepared from alcohol **114** (57 mg, 0.41 mmol) in a 95% yield according to a standard esterification reaction with benzoyl chloride (1.5equiv) and pyridine (excess).

118; white solid; ^{1}H NMR d 8.08-8.01 (2H, m), 7.60-7.53 (1H, m), 7.48-7.41 (2H, m), 4.97-4.88 (1H, m), 2.11-1.89 (6H, m), 1.62-1.50 (2H, m), 1.49 (3H, t, J = 1.8 Hz); ^{13}C NMR d 165.81, 154.67 (t, J = 4.1 Hz), 132.83, 130.18, 129.44, 128.21, 71.50, 56.94 (t, J = 5.0 Hz), 36.39, 29.06, 27.11.

cis-4-Isocyano-4-methylcyclohexyl benzoate (**119**).[101]

Compound **119** was prepared from alcohol **115** (18 mg, 0.13 mmol) in an 89% yield according to a standard esterification reaction with benzoyl chloride (1.5 equiv) and pyridine (excess).

119; white solid; ^{1}H NMR d 8.03-7.98 (2H, m), 7.60-7.54 (1H, m), 7.48-7.42 (2H, m), 5.33-5.28 (1H, m), 2.10-1.95 (2H, m), 1.90-1.73 (4H, m), 1.51 (3H, t, J = 1.8 Hz); ^{13}C NMR d 165.48, 154.45 (t, J = 4.5 Hz), 132.91, 130.48, 129.37, 128.34, 68.33, 57.61 (t, J = 5.0 Hz), 33.20, 29.84, 26.00.

trans-4-Isocyano-4-methylcyclohexyl pivaloate (**120**).[101]

Compound **120** was prepared from alcohol **114** (57 mg, 0.41 mmol) in a 92% yield according to a standard esterification reaction with pivaloyl chliride (1.5 equiv) and pyridine (excess).

120; white solid; ^{1}H NMR d 5.06-5.00 (1H, m), 1.98-1.74 (6H, m), 1.70-1.58 (2H, m), 1.47 (3H, t, J = 1.8 Hz), 1.20 (9H, s); ^{13}C NMR d 177.33, 154.25 (t, J = 4.2 Hz), 67.11, 57.49 (t, J = 5.0 Hz), 38.94, 33.02, 29.85, 27.21, 25.76.

cis-4-Isocyano-4-methylcyclohexyl pivaloate (**121**).[101]

Compound **121** was prepared from alcohol **115** (18 mg, 0.13 mmol) in an 89% yield according to a standard esterification reaction with pivaloyl chliride (1.5 equiv) and pyridine (excess).

121; white solid; ^{1}H NMR d 4.72-4.55 (1H, m), 2.04-1.72 (6H, m), 1.54-1.41 (5H, m) including 1.45 (3H, t, J = 1.8 Hz), 1.19 (9H, s); ^{13}C NMR d 177.88, 154.30 (t, J = 4.2 Hz), 70.49, 56.91 (t, J = 5.0 Hz), 38.63, 36.26, 28.98, 27.07, 26.83.

trans-4-(2,2-Dimethylpropoxy)-1-methylcyclohexyl isocyanide (**122**).[101]

Compound **122** was synthesized from alcohol **114** (30 mg, 0.22 mmol) in 40% yield with sodium hydride (3 equiv) and 1-bromo-2,2-dimethylpropane (3 equiv).

122; white solid; ^{1}H NMR d 3.49-3.46 (1H, m), 3.00 (2H, S), 1.85-1.79 (2H, m), 1.78-1.66 (4H, m), 1.66-1.61 (2H, m), 1.41 (3H, t, J = 1.8 Hz), 0.89 (9H, s); ^{13}C NMR d, 153.47 (t, J = 4.3 Hz), 78.43, 71.67, 58.11 (t, J = 5.0 Hz), 32.87, 31.98, 29.88, 26.72,

25.74.

cis-4- (2,2-Dimethylpropoxy)-1-methylcyclohexyl isocyanide (**123**).[101]

Compound **123** was synthesized from alcohol **115** (30 mg, 0.22 mmol) in 37% yield with sodium hydride (3 equiv) and 1-bromo-2,2-dimethylpropane (3 equiv).

123; white solid; ^{1}H NMR d 3.15-3.09 (1H, m), 3.08 (2H, S), 1.99-1.93 (2H, m), 1.92-1.86 (2H, m), 1.71-1.63 (2H, m), 1.42 (3H, t, J = 1.8 Hz), 1.42-1.34 (2H, m), 0.89 (9H, s); ^{13}C NMR d, 154.22 (t, J = 4.3 Hz), 78.72, 76.62, 57.31 (t, J = 5.0 Hz), 36.59, 32.01, 29.10, 27.59, 25.74.

cis-4-Methylcyclohexyl pivaloate (**124**).[101]

Compound **124** was prepared from *cis*-4-methylcyclohexanol (570 mg, 5.0 mmol) in a 99% yield according to an esterification reaction with pivaloyl chloride (1.1 equiv) and pyridine (excess).

124; colorless oil; ^{1}H NMR d 4.97-4.94 (1H, m), 1.85-1.78 (2H, m), 1.54-1.39 (5H, m), 1.28-1.22 (2H, m), 1.21 (9H, s), 0.92 (3H, d, J = 6.6 Hz); ^{13}C NMR d 177.93, 69.14, 38.94, 31.57, 29.66, 29.59, 27.25, 22.28.

trans-4- Methylcyclohexyl pivaloate (**125**).[101]

Compound **125** was prepared from *trans*-4-methylcyclohexanol (570 mg, 5.0 mmol) in a 99% yield according to an esterification reaction with pivaloyl chloride (1.1 equiv) and pyridine (excess).

125; colorless oil; ^{1}H NMR d 4.66-4.59 (1H, m), 1.95-1.86 (2H, m), 1.76-1.68 (2H, m), 1.42-1.26 (3H, m), 1.17 (9H, s), 1.08-0.99 (2H, m), 0.89 (3H, d, J = 6.6 Hz); ^{13}C NMR d 178.16, 72.95, 38.64, 32.99, 31.73, 31.46, 27.17, 21.83.

Synthesis of benzene derivatives 126-152

4-[(*E,E*)-1,5-Dimethylhexa-1,3-dienyl] isonitrile (**126**).

Compound **126** was synthesized starting with *p*-acetylanilline as follows. *p*-Acetylaniline (2.0g, 14.8mmol) in ethyl formate (EtOCHO, 30 mL) was converted to *N*-(4-acethylphenyl)-formamide (**179**) with *p*-TsOH (220 mg, 1.4 mmol) in an 81% yield. Reduction of **179** (1.1 g, 6.6 mmol) with $NaBH_4$ (1.5 equiv) led to alcohol *N*-[4-(1-hydroxyethyl)-phenyl]-formamide (**180**) in a 69% yield. Alcohol **180** (100 mg, 0.61 mmol) was converted to sulfide with thiophenol (1.2 equiv) and zinc iodide (0.5 equiv) in CH_2Cl_2, followed by oxidation of resulting compound with *m*-chloroperbenzoic acid (mCPBA, 5.0 equiv) in CH_2Cl_2 to give

N-[4-(1-benzenesulfonylethyl)phenyl]formamide (**181**) in a 62% yield. *N*-[4-(1,5-Dimehylhexa-1,3-dienyl)phenyl]formamide (**182**) was synthesized from **181** (362.5 mg, 1.26 mmol) by the Julia olefination described for compound **35** with 4-methyl-2-pentenal (3 equiv) in a 37% yield. Dehydration of **182** (107 mg, 0.46 mmol) with diidopropyl ethyl amine (DIPEA, excess) and trifluoromethane sulfonic anhydride (Tf_2O, 3 equiv) in CH_2Cl_2 led to compound **126** in a 4% yield (4.9 mg, 0.018 mmol) (Scheme 2-7.).

126; brown oil; ^{1}H NMR d 7.43 (2H, d, *J* = 8.8 Hz), 7.31 (2H, d, *J* = 8.8 Hz), 6.44 (1H, d, *J* = 11 Hz), 6.38 (1H, dd, *J* = 11, 14 Hz), 5.86 (1H, dd, *J* = 6.9, 14 Hz), 2.49-2.39 (1H, m), 2.13 (3H, s), 1.06 (6H, d, *J* = 6.6 Hz); ^{13}C NMR d 168.05, 144.77, 144.54, 132.03, 129.03, 126.27, 126.24, 124.70, 123.78, 31.70, 22.40, 15.72; LR-EIMS: *m/z* (%) 211 (M^+, 80), 196 (64), 182 (22), 168 (100), 154 (39), 116 (42), 77 (15); HR-EIMS: calcd.for $C_{15}H_{17}N$ 211.1361, found 211.1366.

N-(4-Hexylphenyl) formamide (**127**).

Compound **127** was synthesized from *p*-hexylaniline (2.0 g, 11.6 mmol) with *p*-TsOH (0.1 equiv) in ethyl formate (EtOCHO, 30mL) in an 80% yield.

127; white crystal; ^{1}H NMR d 8.83 (1H, d, *J* = 11.0 Hz), 8.64 (1H, d, *J* = 11.0 Hz), 8.32 (1H, d, *J* = 1.5 Hz), 7.96 (1H, s), 7.45 (1H, d, *J* = 8.4 Hz), 7.15 (1H, d, *J* = 8.4Hz), 7.12 (1H, d, *J* = 8.4 Hz), 7.01 (1H, d, *J* = 8.0 Hz), 2.62-2.51 (2H, m), 1.64-1.51 (1H, m), 1.37-1.22 (6H, m), 0.92-0.84 (3H, m); ^{13}C NMR d 163.09, 159.27, 140.14, 139.50, 134.53, 134.30, 129.52, 128.85, 120.06, 119.00, 35.33, 31.63, 31.41, 31.40, 28.86, 28.83, 22.55, 14.05; LR-EIMS: *m/z* (%) 205 (M^+, 74), 134 (100), 106 (64), 91 (4), 77 (8); HR-EIMS: calcd.for $C_{13}H_{19}NO$ 205.1467, found 205.1458.

N-Methyl-4-hexylaniline (**128**).

Compound **128** was prepared from **127** (490 mg, 2.39 mmol) by reduction with lithium aluminium hydride (LAH, 2 equiv) in a 59% yield.

128; colorless oil; ^{1}H-NMR (400 MHz) d 7.02-6.98 (2H, m), 6.57-6.53 (2H, m), 3.56 (1H, br s), 2.81 (3H, s), 2.49 (2H, t, *J* = 7.7 Hz), 1.61-1.50 (2H, m), 1.37-1.21 (6H, m), 0.88 (3H, t, *J* = 6.8 Hz); ^{13}C-NMR (100.4 MHz) d 147.16, 131.74, 128.97, 112.44, 35.08, 31.92, 31.83, 31.09, 29.05, 22.71, 14.20.

N-(4-Hexylphenyl)-*N*-methylformamide (**129**).

Compound **129** was prepared from **128** (170 mg, 0.89 mmol) by treatment with *p*-TsOH (0.1 equiv) in ethyl formate (excess) in a 98% yield.

129; ^{1}H-NMR (400 MHz) d 8.43 (1H, s), 7.23-7.18 (2H, m), 7.10-7.15 (2H, m), 3.30 (3H, s), 2.61 (2H, t, J = 7.7 Hz), 1.66-1.55 (2H, m), 1.39-1.23 (6H, m), 0.89 (3H, t, J = 6.8 Hz); ^{13}C-NMR (100.4 MHz) d 162.26, 141.33, 139.70, 129.38, 122.41, 35.36, 32.23, 31.71, 31.45, 28.95, 22.63, 14.14.

N,*N*-Dimethyl-4-hexylaniline (**130**).

Compound **130** was prepared from *p*-hexylaniline (1.0 g, 5.2 mmol) by alkylation with MeI (2.1 equiv) and Na_2CO_3 (2.5 equiv) in THF in a 14% yield.

130; ^{1}H-NMR (400 MHz) d 7.08-7.03 (2H, m), 6.72-6.66 (2H, m), 2.90 (6H, s), 2.50 (2H, t, J = 7.7 Hz), 1.62-1.50 (2H, m), 1.38-1.23 (6H, m), 0.88 (3H, t, J = 6.8 Hz); ^{13}C-NMR d 148.82, 131.24, 128.85, 112.97, 41.02, 34.97, 31.85 (x 2), 29.10, 22.71, 14.20.

N-(4-Hexylphenyl) acetamide (**131**).

Compound **131** was synthesized from *p*-hexylaniline (580 mg, 3.27 mmol) with acetic anhydride (Ac_2O, equiv) in pyridine (excess) in a 70% yield.

131; ^{1}H-NMR (270 MHz) d 7.43-7.35 (2H, m), 7.15-7.08 (2H, m), 2.56 (2H, t, J = 7.7 Hz), 2.16 (3H, s), 1.65-1.50 (2H, m), 1.38-1.22 (6H, m), 0.88 (3H, t, J = 6.8 Hz); ^{13}C-NMR (67.8 MHz) d 168.04, 138.97, 135.28, 128.73, 119.87, 35.40, 31.76, 31.53, 28.97, 24.61, 22.68, 14.19.

4-Hexylphenyl isocyanide (**132**).

Compound **132** was prepared from formamide **127** (170 mg, 0.89 mmol) by the dehydration reaction described for compound **126** (98% yield).

132; yellow oil; ^{1}H NMR d 7.27 (2H, d, J = 7.7 Hz), 7.17 (2H, d, J = 7.7 Hz), 2.61 (2H, t, J = 7.7 Hz), 1.64-1.55 (2H, m), 1.35-1.25 (6H, m), 0.88 (3H, m); ^{13}C NMR d163.03 (br s), 144.52, 129.15, 126.07, 124.11 (t , J = 13 Hz), 35.64, 31.59, 31.08, 28.78, 22.53, 14.03; LR-EIMS: *m/z* (%) 187 (M^+, 69), 117 (100), 116 (97), 90 (33), 89 (22), 71 (7), 77 (15); HR-EIMS: calcd.for $C_{13}H_{17}N$ 187.1361, found 187.1352.

N-(4-Butylphenyl) formamide (**133**).

Compound **133** was synthesized from *p*-butylaniline (2.98 g, 20 mmol) with *p*-TsOH (0.1 equiv) in ethyl formate (excess) in a 73% yield.

133: E : Z = 52 : 48

E; ^{1}H-NMR d 8.88 (1H, d, J = 11.0 Hz), 8.64 (1H, br d, J = 11.0 Hz), 7.14 (2H, d, J = 8.1 Hz), 7.00 (2H, d, J = 8.1 Hz), 2.58 (2H, t, J = 7.3 Hz), 1.61-1.54 (2H, m), 1.38-1.29

(2H, m), 0.92 (3H, t, J = 7.3 Hz); *Z*; ^{1}H-NMR d 8.31 (1H, d, J = 1.8 Hz), 8.09 (1H, br s), 7.44 (2H, d, J = 8.1 Hz), 7.11 (2H, d, J = 8.1 Hz), 2.56 (2H, t, J = 7.3 Hz), 1.59-1.52 (2H, m), 1.38-1.29 (2H, m), 0.91 (3H, t, J = 7.3 Hz); ^{13}C-NMR d 163.04 and 159.30, 140.03 and 139.36, 134.56 and 134.31, 129.46 and 128.78, 120.07 and 118.96, 34.95 and 34.84, 33.50 and 33.48, 22.15, 13.79.

4-Butylphenyl isocyanide (**134**).

Compound **134** was prepared from formamide **133** (443 mg, 2.5 mmol) by the dehydration reaction described for compound **126** (10% yield).

134: ^{1}H-NMR d 7.26 (2H, d, J = 8.1 Hz), 7.17 (d, J = 8.1 Hz), 2.61 (2H, t, J = 7.3 Hz), 1.61-1.54 (2H, m), 1.37-1.30 (2H, m), 0.92 (3H, t, J = 7.3 Hz); ^{13}C-NMR d 163.28 (m), 144.56, 129.22, 126.10, 124.15 (t , J = 13 Hz), 35.25, 33.17, 22.11, 13.75.

N-(4-Octylphenyl) formamide (**135**).

Compound **135** was synthesized from *p*-octylaniline (2.05 g, 10 mmol) with *p*-TsOH (0.1 equiv) in ethyl formate (excess) in a 64% yield.

135: *E* : *Z* = 55 : 45

E-**135**; ^{1}H-NMR d 8.64 (1H, d, J = 11.4 Hz), 8.64 (1H, br d, J = 11.4 Hz), 7.15 (2H, d, J = 8.1 Hz), 7.00 (2H, d, J = 8.1 Hz), 2.58 (2H, t, J = 7.3 Hz), 1.62-1.55 (2H, m), 1.35-1.21 (10H, m), 0.88 (3H, t, J = 7.3 Hz); *Z*-**135**; ^{1}H-NMR d 8.33 (1H, d, J = 1.8 Hz), 7.67 (1H, br s), 7.44 (2H, d, J = 8.1 Hz), 7.12 (2H, d, J = 8.1 Hz), 2.56 (2H, t, J = 7.3 Hz), 1.60-1.54 (2H, m), 1.35-1.21 (2H, m), 0.88 (3H, t, J = 7.3 Hz); ^{13}C-NMR d 162.89 and 159.04, 140.18 and 139.54, 134.49 and 134.28, 129.53 and 128.86, 120.03 and 119.05, 35.33 and 35.22, 31.80, 31.40, 29.40 and 29.38, 29.19, 29.16, 22.60, 14.02.

4-Octylphenyl isocyanide (**136**).

Compound **136** was prepared from formamide **135** (467 mg, 2.0 mmol) by the dehydration reaction described for compound **126** (13% yield).

136; ^{1}H-NMR d 7.30-7.26 (2H, m), 7.20-7.16 (2H, m), 2.61 (2H, t, J = 7.7 Hz), 1.62-1.56 (2H, m), 1.34-1.21 (8H, m), 0.88 (3H, t, J = 7.0 Hz); ^{13}C-NMR d 163.35 (br s), 144.72, 129.31, 126.22, 124.27 (t , J = 12 Hz), 35.69, 31.85, 31.16, 29.40, 29.21, 29.17, 22.66, 14.09.

N-(4-Benzyloxyphenyl) formamide (**137**).

Compound **137** was synthesized from 4-benzyloxyaniline hydrochloride (4.71 g, 20 mmol) with *p*-TsOH (0.1 equiv), TEA (1.1 equiv), and ethyl formate (excess) in a 91%

yield.

137: *E* : *Z* = 47 : 53

E-**137**; ^{1}H-NMR d 8.50 (1H, d, *J* = 11.4 Hz), 7.58 (1H, br d, *J* = 11.4 Hz), 7.44-7.30 (5H, m), 7.03-7.00 (2H, m), 6.95-6.92 (2H, m), 5.06 (2H, s); *Z*-**137**; ^{1}H-NMR d 8.32 (1H, d, *J* = 1.8 Hz), 7.45-7.30 (7H, m), 7.10 (1H, br s), 6.98-6.95 (2H, m), 5.05 (2H, s); ^{13}C-NMR d162.75 and 158.69, 156.85 and 155.96, 136.88 and 136.65, 130.12 and 129.68, 128.66and 128.61, 128.13 and 128.01, 127.46 and 127.44, 121.74, 116.00 and 115.35, 70.41 and 70.33.

4-Benzyloxyphenyl isocyanide (**138**).

Compound **138** was prepared from formamide **137** (455 mg, 2.0 mmol) by the dehydration reaction described for compound **126** (22% yield).

138; ^{1}H-NMR d 7.45-7.26 (7H, m), 6.96-6.90 (2H, m), 5.07 (2H, s); ^{13}C-NMR d 162.62 (m), 158.87, 135.93, 128.61, 128.19, 127.68, 127.32, 119.63 (t , *J* = 13 Hz), 115.42, 70.32.

N-Methyl-4-benzyloxyaniline (**139**).

Compound **139** was prepared from **137** (590 mg, 2.59 mmol) by treatment with lithium aluminium hydride (LAH, 2 equiv) in an 81% yield.

139; ^{1}H-NMR d 7.44-7.26 (5H, m), 6.90-6.80 (2H, m), 6.6-6.51 (2H, m), 4.98 (2H, s), 2.79 (3H, s); ^{13}C-NMR d151.15, 143.80, 137.54, 128.39, 127.66, 127.40, 116.11, 113.50, 70.91, 31.59.

N-(4-Benzyloxyphenyl)-*N*-methylformamide (**140**).

Compound **140** was synthesized from **139** (420 mg, 1.97 mmol) by treatment with *p*-TsOH (0.1 equiv) in ethyl formate (excess) in an 84% yield.

140: ^{1}H-NMR d 8.34 (1H, br s), 7.44-7.31 (5H, m), 7.10-7.06 (2H, m), 7.00-6.97 (2H, m), 5.07 (2H, s), 3.26 (3H, s); ^{13}C-NMR d 162.33, 157.39, 136.55, 135.46, 128.62, 128.08, 127.37, 124.55, 115.73, 70.30, 32.57.

4-(Benzyloxy)-phenol (**141**).

Compound **141** was purchased from the Wako Pure Chemicals Company.

4-Benzyloxyphenyl acetate (**142**).

Compound **142** was prepared from alcohol **141** (135 mg, 0.7 mmol) by treatment with acetic anhydride (1 mL) and pyridine (excess) in a 36% yield.

142; white crystals; ^{1}H NMR d 7.43 (2H, d, J = 7.3 Hz), 7.39 (2H, t, J = 7.3 Hz), 7.34 (1H, t, J = 7.3 Hz), 7.01 (2H, d, J = 8.8 Hz), 6.97 (2H, d, J = 8.8 Hz), 5.04 (2H, s), 2.28 (3H, s); ^{13}C NMR d 169.8, 156.5, 144.4, 136.8, 128.6, 128.0, 127.4, 122.3, 115.4, 70.3, 21.0; LR-EIMS: *m/z* (%) 242 (M^+, 20), 200 (60), 91 (100); HR-EIMS: calcd.for $C_{15}H_{14}O_3$ 242.0943, found 242.0930.

4-Benzyloxyphenyl benzoate (**143**).

Compound **143** was prepared from alcohol **141** (205 mg, 1.0 mmol) by treatment with benzyl chloride (1.2 equiv) and pyridine (excess) in a 61% yield.

143; white crystals; ^{1}H NMR d 8.19 (2H, d, J = 7.3 Hz), 7.62 (1H, t, J = 7.3 Hz), 7.50 (2H, t, J = 7.3 Hz), 7.44 (2H, d, J = 7.3 Hz), 7.39 (2H, t, J = 7.3 Hz), 7.33 (1H, t, J = 7.3 Hz), 7.13 (2H, d, J = 9.2 Hz), 7.01 (2H, d, J = 9.2 Hz), 5.07 (2H, s); ^{13}C NMR d 165.5, 156.5, 144.6, 136.8, 133.5, 130.1, 129.6, 128.6, 128.5, 128.0, 127.5, 122.5, 115.5, 70.4; LR-EIMS: *m/z* (%) 304 (M^+, 33), 105 (100), 91(64), 77(25); HR-EIMS: calcd. for $C_{20}H_{16}O_3$ 304.1099, found 304.1098.

4-Benzyloxyphenyl pivaloate (**144**).

Compound **144** was prepared from alcohol **141** (202 mg, 1.0 mmol) by treatment with pivaloyl chloride (1.2 equiv) and pyridine (excess) in a 67% yield.

144; ^{1}H NMR d 7.42 (2H, d, J = 7.3 Hz), 7.38 (2H, t, J = 7.3 Hz), 7.32 (1H, t, J = 7.3 Hz), 6.96 (4H, d, J = 1.1 Hz), 5.04 (2H, s),1.34 (9H, s); ^{13}C NMR d 177.4, 156.3, 144.8, 136.8, 128.5, 128.0, 127.4, 122.2, 115.4, 70.4, 39.0, 27.1; LR-EIMS: *m/z* (%) 284 (M^+, 49), 200 (41), 91(100), 57(31); HR-EIMS: calcd. for $C_{18}H_{20}O_3$ 284.1412, found 284.1411.

4-Benzyloxybenzoic acid methyl ester (**145**).

Compound **145** was prepared from 4-hydroxybenzoic acid methyl ester (13.2 mmol in acetone, 20 mL) in a 75% yield by Williamson reaction with benzyl bromide (1.5 equiv) and K_2CO_3 (5.0 equiv).

145; white powder; ^{1}H-NMR d 7.99 (2H, d, J = 8.8 Hz), 7.43 (2H, d, J = 7.3 Hz), 7.40 (2H, t, J = 7.3 Hz), 7.34 (1H, t, J = 7.3 Hz), 6.99 (2H, d, J = 8.8 Hz), 5.12 (2H, s), 3.88 (3H, s); ^{13}C-NMR d 166.8, 162.5, 136.3, 131.6, 128.7, 128.2, 127.5, 122.9, 114.5, 70.1, 51.9.

4-Benzyloxybenzoic acid (**146**).

Compound **146** was prepared from **145** (2.02 g, 8.34 mmol) by treatment with NaOH

(excess) in a 35% yield.

146; white powder; ^{1}H NMR d 8.06 (2H, d, J = 8.8 Hz), 7.44 (2H, d, J = 7.3 Hz), 7.41 (2H, t, J = 7.3 Hz), 7.35 (1H, t, J = 7.3 Hz), 5.14 (2H, s); ^{13}C NMR d 170.8, 163.2, 136.1, 132.4, 128.7, 128.3, 127.5, 121.8, 114.6, 70.2.

4-Benzyloxybenzamide (**147**).

Treatment of acid **146** (477 mg, 2.0 mmol) with thionyl chloride (10 mL), followed by a standared reaction with NH_3 (10 mL) to give compound **147** in a 78% yield.

147; white powder; ^{1}H NMR d 7.78 (2H, d, J = 9.2 Hz), 7.43 (2H, d, J = 7.3 Hz), 7.40 (2H, t, J = 7.3 Hz), 7.34 (1H, t, J = 7.3 Hz), 7.01 (2H, d, J = 9.2 Hz), 5.12 (2H, s); ^{13}C NMR d 168.7, 161.8, 136.3, 129.3, 128.7, 128.2, 127.5, 125.8, 114.7, 70.2; LR-EIMS: *m/z* (%) 227 (M^+, 91), 91(100), 65(25); HR-EIMS: calcd. for $C_{14}H_{13}NO_2$ 227.0946, found 227.0948.

4-Benzyloxybenzaldehyde (**148**).

Aldehyde **148** was synthesized from *p*-hydroxybenzaldehyde (24.6 mmol in DMF 20 mL) by Williamson reaction described for compound **145** in a 75% yield.

148; yellow solid; ^{1}H-NMR d 9.89 (1H, s), 7.84 (2H, d, J = 8.4 Hz), 7.43 (2H, d, J = 7.3 Hz), 7.41 (2H, t, J = 7.3 Hz), 7.35 (1H, t, J = 7.3 Hz), 7.08 (2H, d, J = 8.4 Hz), 5.16 (2H, s); ^{13}C NMR d 190.8, 163.7, 136.0, 132.0, 130.2, 128.7, 128.3, 127.5, 115.2, 70.3.

4-Benzyloxybenzonitrile (**149**).

Nitrile **149** was synthesized from 4-cyanophenol (1.7 mmol in DMF 20mL) by Williamson reaction described for compound **145** in a 73% yield.

149; white crystal; ^{1}H NMR d 7.59 (2H, d, J = 8.8 Hz), 7.43－7.34 (5H, m), 7.02 (2H, t, J = 8.8 Hz), 5.12 (2H, s); ^{13}C NMR d 161.9, 135.7, 134.0, 128.7, 128.4, 127.4, 119.1, 115.6, 104.2, 70.2.

4-Benzyloxy-(2-chlorovinyl) benzene (**150**).

Compound **150** was synthesized from aldehyde **148** by Wittig reaction as follows. To THF (30mL) was added chloromethyl triphenyl phosphonium chloride (2.04 g, 5.76 mmol) and *n*-BuLi (1.6 M in hexane, 3.8 mL, 5.8 mmol) and stirred. Aldehyde **148** (614 mg, 2.9 mmol) was added dropwise to the mixture led to compound **150** in a 97% yield (*cis*: *trans* = 10:7).

150; yellow solid;

cis-**150**; ^{1}H NMR d 7.64 (2H, d, J = 8.7 Hz), 7.49-7.28 (5H, m), 6.97 (2H, d, J = 8.7 Hz),

6.55 (1H, d, J = 8.1 Hz), 6.15 (1H, d, J = 8.1 Hz), 5.09 (2H, s); ^{13}C NMR d 158.5, 136.75, 132.6, 130.7, 128.6, 128.0, 127.4, 127.1, 115.5, 114.6, 69.9;
trans-**150**; ^{1}H NMR d7.22 (2H, d, J = 8.7 Hz), 7.49-7.28 (5H, m), 6.92 (2H, d, J = 8.7 Hz), 6.76 (1H, d, J = 13.5 Hz), 6.50 (1H, d, J = 13.5 Hz), 5.06 (2H, s); ^{13}C NMR d 158.7, 136.70, 132.6, 130.7, 128.6, 127.9, 127.3, 127.1, 116.5, 115.1, 70.0;
LR-EIMS: *m/z* (%) 246 (M^++2, 27), 244 (M^+, 86), 183(5), 91(100), 65(32); HR-EIMS: calcd. for $C_{15}H_{13}OCl$ 244.0655, found 244.0654.

4-Benzyloxyphenylacetylene (**151**).

Compound **151** was prepared from **150** (100 mg, 0.4 mmol) by treatment with n-BuLi (1.6 M in hexane, 0.75 mL, 1.2 mmol) followed with H_2O (60% yield).

151; white solid, ^{1}H NMR d 7.42 (2H, d, J = 8.8 Hz), 7.41－7.40 (2H, m), 7.38 (2H, t, J = 7.3 Hz), 7.33 (1H, t, J = 7.3 Hz), 6.91(2H, d, J = 8.8 Hz), 5.06 (2H, s), 2.99 (1H, s); [ref.[4]; ^{1}H NMR d 7.38 (2H, d, J = 10 Hz), 7.36 (5H, br.s), 6.84 (2H, d, J = 10 Hz), 5.02 (2H, s), 2.94 (1H, s)]; ^{13}C NMR d159.1, 136.5, 133.6, 128.6, 128.1, 127.4, 114.8, 114.4, 83.6, 75.8, 70.0.

4-Benzyloxyvinyl benzene (**152**).

Compound **152** was synthesized from aldehyde **148** (695 mg, 3.30 mmol) and methyl triphenyl phosphonium bromide (2.0 equiv) according to the general Wittig method in an 83% yield.

152; white crystal; ^{1}H NMR d 7.43 (2H, d, J = 7.1 Hz), 7.38 (2H, t, J = 7.1 Hz), 7.34 (2H, d, J = 8.7 Hz), 7.35-7.28 (1H, m), 6.93 (2H, d, J = 8.7 Hz), 6.65 (1H, dd, J = 17.6, 11.0 Hz), 5.60 (1H, d, J = 17.6 Hz), 5.12 (1H, d, J = 11.0 Hz), 5.07 (2H, s); [ref.5; (100 MHz, $CDCl_3$) d 7.4-7.1 (7H, m), 7.0-6.8 (2H, m), 6.8-6.5 (1H, m), 5.7-5.0 (2H, m), 5.0 (2H, s)]; ^{13}C NMR d 158.6, 136.9, 136.2, 130.7, 128.6, 128.0, 127.43, 127.38, 114.9, 111.7, 70.0.

Synthesis of simple linear isocyanides 153-164

1,1-Dimethyl-10-undecyl isocyanide (**153**).[105]

Compound **153** was prepared from 10-undecenoic acid methyl ester as follows. First, *tert*-alcohol **183** was synthesized from 10-undecenoic acid methyl ester (8.1 g, 40.8 mmol) by treatment with MeLi (2 equiv) in a 99% yield. *Tert*-alcohol **183** (990 mg, 5 mmol) was converted to compound **153** directly with trimethylsilyl cyanide (1.2 equiv) and silver perchlorate (1.2 equiv) in a 92% yield.

10-Hydroxy-1,1-dimethyldecyl isocyanide (**154**).[104]

Compound **154** was prepared from **183** as follows. First, 10-methylundecan-1,10-diol (**184**) was synthesized from **183** (5.0 g, 25.2 mmol) according to ozonolysis followed by reduction with $NaBH_4$ (2.0 equiv) in an 83% yield. Compound **154** was obtained from alcohol **184** in a 91% yield by treatment with trimethylsilyl cyanide (1.2 equiv) and silver perchlorate (1.2 equiv).

154; colorless oil; ^{1}H NMR d 3.64 (2H, t, J = 6.7 Hz), 1.63 (1H, br s), 1.60-1.52 (4H, m), 1.48-1.41 (2H, m), 1.40 (6H, t, J = 1.8 Hz), 1.38-1.27 (10H, m); ^{13}C NMR d 152.57 (t, J = 4.3 Hz), 63.04, 57.43 (t, J = 5.0 Hz), 42.45, 32.76, 29.45, 29.45, 29.36, 29.36, 28.97, 25.69, 24.10; LR-EIMS: *m/z* (%) = 211 (M^+, 1.3), 196 (25), 185 (16), 69 (100).

10-Amino-1,1-dimethyldecyl isocyanide (**155**).[104]

Compound **155** was prepared from **185** as follows. First, 11-amino-2-methylundecan-2-ol (**186**) was synthesized from **185** (1.16 g, 3.5 mmol) by treatment with hydrazine monohydrate (2.0 equiv) in a 78% yield. Compound **155** was obtained from compound **186** in a 70% yield by treatment with trimethylsilyl cyanide (1.2 equiv) and silver perchlorate (1.2 equiv).

155; colorless oil; ^{1}H NMR d 2.68 (2H, t, J = 6.9 Hz), 1.58-1.52 (2H, m), 1.48-1.40 (4H, m), 1.39 (6H, t, J = 1.8 Hz), 1.35-1.23 (10H, m); ^{13}C NMR d 151.96 (t, J = 5.0 Hz), 56.69 (t, J = 5.0 Hz), 41.79, 41.54, 33.15, 28.82, 28.81, 28.77, 28.72, 28.31, 26.18, 23.45; LR-EIMS: *m/z* (%) = 210 (M^+, 15), 195 (24), 183 (100), 142(24), 69 (86).

N-(10-Isocyano-10-methylundecyl) acetamide (**156**).[104]

Compound **156** was prepared from **186** as follows. First, *N*-(10-hydroxy-10-methylundecyl)acetamide was synthesized from **186** (263 mg, 1.31 mmol) according to acetylation with acetic anhydride and pyridine (excess) in an 84% yield. Compound **156** was obtained from the acetamide in a 91% yield by treatment with trimethylsilyl cyanide (1.2 equiv) and silver perchlorate (1.2 equiv).

156; colorless oil; ^{1}H NMR d 5.49 (1H, br s), 3.23 (2H, q, J = 6.7 Hz), 1.97 (3H, s), 1.58-1.40 (6H, m), 1.39 (6H, t, J = 1.8 Hz), 1.35-1.23 (10H, m); ^{13}C NMR d 169.86, 152.66 (t, J = 5.0 Hz), 57.46 (t, J = 5.0 Hz), 42.51, 39.73, 29.66, 29.47, 29.41, 29.37, 29.26, 29.06, 26.93, 24.15, 23.44; LR-EIMS: *m/z* (%) = 252 (M^+, 1), 225 (100), 210 (9), 182 (23), 69 (34).

2-(10-Isocyano-10-methylundecyl) isoindoline-1,3- dione (**157**).[104]

Compound **157** was prepared from **184** as follows. First,

2-(10-hydroxy-10-methylundecyl)isoindoline-1,3-dione (**185**) was synthesized from **184** (971 mg, 4.8 mmol) according to the Mitsunobu reaction with phthalimide (1.5 equiv), Ph_3P (1.2 equiv), and DEAD (1.2 equiv) in a 94% yield. Compound **157** was obtained from the **185** in a 96% yield by treatment with trimethylsilyl cyanide (1.2 equiv) and silver perchlorate (1.2 equiv).

157; colorless oil; ^{1}H NMR d 7.84 (2H, dd, J = 5.5, 3.1), 7.70 (2H, dd, J = 5.5, 3.1), 3.68 (2H, t, J = 7.4 Hz), 1.67 (2H, m), 1.54 (2H, m), 1.39 (6H, t, J = 1.7 Hz), 1.49-1.23 (12H, m); ^{13}C NMR d 168.48, 152.85 (t, J = 5.0 Hz), 133.85, 132.22, 123.16, 57.41 (t, J = 5.0 Hz), 42.50, 38.06, 29.47, 29.38, 29.36, 29.12, 28.99, 28.59, 26.82, 24.12; LR-EIMS: *m/z* (%) = 340 (M^+, 19), 313 (46), 258 (23), 160 (100), 69 (9).

10-Isocyano-10-methylundecyl benzoate (**158**).[102]

Compound **158** was prepared from alcohol **154** (54 mg, 0.255 mmol) in a 92% yield by treatment with benzoyl chloride (1.5 equiv) and pyridine (excess).

158; colorless oil; ^{1}H NMR d 8.07-8.03 (2H, m), 7.57-7.53 (1H, m), 7.46-7.42 (2H, m), 4.32 (2H, t, J = 6.7 Hz), 1.80-1.74 (2H, m), 1.57-1.52 (2H, m), 1.49-1.41 (4H, m), 1.39 (6H, t, J = 1.8 Hz), 1.38-1.29 (8H, m); ^{13}C NMR d 166.71, 152.86 (t, J = 5.0 Hz), 132.80, 130.56, 129.54, 128.33, 65.10, 57.41 (t, J = 5.0 Hz), 42.50, 29.48, 29.43, 29.39, 29.24, 29.00, 28.74, 26.02, 24.14.

10-Isocyano-10-methylundecyl 4-methoxybenzoate (**159**).[102]

Compound **159** was prepared from alcohol **154** (54 mg, 0.255 mmol) in a 99% yield by treatment with 4-methoxybenzoyl chloride (1.5 equiv) and pyridine (excess).

159; colorless crystal; ^{1}H NMR d 8.01-7.98 (2H, m), 6.93-6.90 (2H, m), 4.28 (2H, t, J = 6.7 Hz), 3.86 (3H, s), 1.78-1.72 (2H, m), 1.57-1.52 (2H, m), 1.48-1.41 (4H, m), 1.39 (6H, t, J = 1.8 Hz), 1.39-1.29 (8H, m); ^{13}C NMR d 166.47, 163.28, 152.87 (t, J = 5.0 Hz), 131.55, 123.01, 113.58, 64.80, 57.42 (t, J = 5.0 Hz), 55.43, 42.50, 29.48, 29.43, 29.39, 29.25, 29.00, 28.79, 26.04, 24.14.

10-Isocyano-10-methylundecyl 4-nitrobenzoate (**160**).[102]

Compound **160** was prepared from alcohol **154** (54 mg, 0.255 mmol) in an 87% yield by treatment with 4-nitorobenzoyl chloride (1.5 equiv) and pyridine (excess).

160; colorless oil; ^{1}H NMR d 8.31-8.27 (2H, m), 8.23-8.19 (2H, m), 4.37 (2H, t, J = 6.7 Hz), 1.83-1.76 (2H, m), 1.57-1.52 (2H, m), 1.49-1.41 (4H, m), 1.39 (6H, t, J = 1.8 Hz), 1.39-1.29 (8H, m); ^{13}C NMR d 164.78, 152.91 (t, J = 5.0 Hz), 150.54, 135.91, 130.68, 123.55, 66.10, 57.42 (t, J = 5.0 Hz), 42.50, 29.48, 29.41, 29.40, 29.23, 29.02,

28.63, 25.97, 24.14.

1,1-Dimethyl-10-phenyltioldecyl isocyanide (**161**).[104]

Compound **161** was prepared from **184** as follows. 2-Methyl-11-phenylthioundecane-2-ol was synthesized from **184** (610 mg, 3.0 mmol) according to the Mitsunobu reaction with thiophenol (1.7 equiv), Ph3P (1.3 equiv), and DEAD (1.3 equiv) in a 34% yield. The alcohol was converted to compound **161** directly with trimethylsilyl cyanide (1.2 equiv) and silver perchlorate (1.2 equiv) in a 94% yield.

161; colorless oil; ^{1}H NMR d 7.33-7.24 (4H, m), 7.15 (1H, m), 2.91 (2H, t, J = 7.4 Hz), 1.65 (2H, m), 1.58-1.39 (4H, m), 1.39 (6H, t, J = 1.7 Hz), 1.33-1.26 (10H, m); ^{13}C NMR d 152.76 (t, J = 4.2 Hz), 136.97, 128.82, 128.75, 125.59, 57.45 (t, J = 5.0 Hz), 42.55, 33.67, 29.55, 29.46, 29.45, 29.21, 29.19, 29.07, 28.86, 24.21; LR-EIMS: *m/z* (%) = 276 (M^{+}-HCN, 69), 123 (50), 110 (100), 69 (32).

2,12-Diisocyano-2,12-dimethyltridecane (**162**).[102]

Compound **162** was prepared from 10-undecenoic acid methyl ester as follows. First, 11-methyl-10-dodecenoic acid methyl ester was synthesized from 10-undecenoic acid methyl ester (1.5 g, 7.56 mmol) in a 44% yield by ozonolysis followed by a standard Wittig reaction with isopropyltriphenylphosphonium iodide (1.2 equiv) and butyllithium (1.2 equiv). After converting the ester (750 mg, 3.3 mmol) to the corresponding *tert*-alcohol with methyllithium (2.2 equiv) in a 99 % yield, **162** was obtained from the alcohol (68 mg, 0.3 mmol) in a 70% yield by treatment with trimethylsilyl cyanide (3.0 equiv) and silver perchlorate (3.0 equiv).

162; colorless oil; ^{1}H NMR d 1.58-1.52 (4H, m), 1.49-1.42 (4H, m), 1.40 (12H, t, J = 1.8 Hz), 1.35-1.27 (10H, m); ^{13}C NMR d 152.86 (t, J = 4.2 Hz), 57.41 (t, J = 5.0 Hz), 42.48, 29.45, 29.39, 28.99, 24.12.

11-Isocyano-1-dodecene (**163**).[102]

Compound **163** was prepared from isocyanide **164** (570 mg, 3.18 mmol) in a 32% yield by methylation with lithium diisopropylamide (5.0 equiv) and methyl iodide (4.5 equiv).

163; colorless oil; ^{1}H NMR d 5.85-5.77 (1H, m), 5.02-4.91 (2H, m), 3.64-3.57 (1H, m), 2.07-2.01 (2H, m), 1.65-1.45 (2H, m), 1.43-1.24 (13H, m); ^{13}C NMR d 154.25 (t, J = 5.0 Hz), 139.18, 114.19, 50.31 (t, J = 5.5 Hz), 37.69, 33.79, 29.35, 29.35, 29.07, 28.96, 28.90, 25.71, 21.72.

11-Isocyano-1-undecene (**164**).[104]

Compound **164** was prepared from 10-undecene-1-ol as follows. 10-unedecene-1-amine was synthesized from 10-undecene-1-ol (2.0 g, 11.7 mmol) in a 71% yield according to a standard Mitsunobu reaction with phtalimide (1.5 equiv), triphenylphosphine (1.2 equiv), and diethyl azodicarboxylate (1.2 equiv) followed by a reaction with hydrazine monohydate (2.2 equiv). Treatment of the amine (1.0 g, 5.9 mmol) with ethyl formate (excess) to give the formamide which was then dehydrated with *p*-toluenesulfonyl chloride (2.0 equiv) and pyridine (excess) to furnish **164** in a 65% yield.

164; colorless oil; ^{1}H NMR d 5.90-5.72 (1H, m), 5.05-4.87 (2H, m), 3.38 (2H, tt, J = 6.8, 2.0 Hz), 2.10-1.98 (2H, m), 1.75-1.60 (2H, m), 1.50-1.21 (12H, m); ^{13}C NMR d 155.68 (t, J = 5.5 Hz), 139.16, 114.19, 41.58 (t, J = 6.0 Hz), 33.78, 29.34, 29.32, 29.14, 29.06, 28.90, 28.70, 26.34.

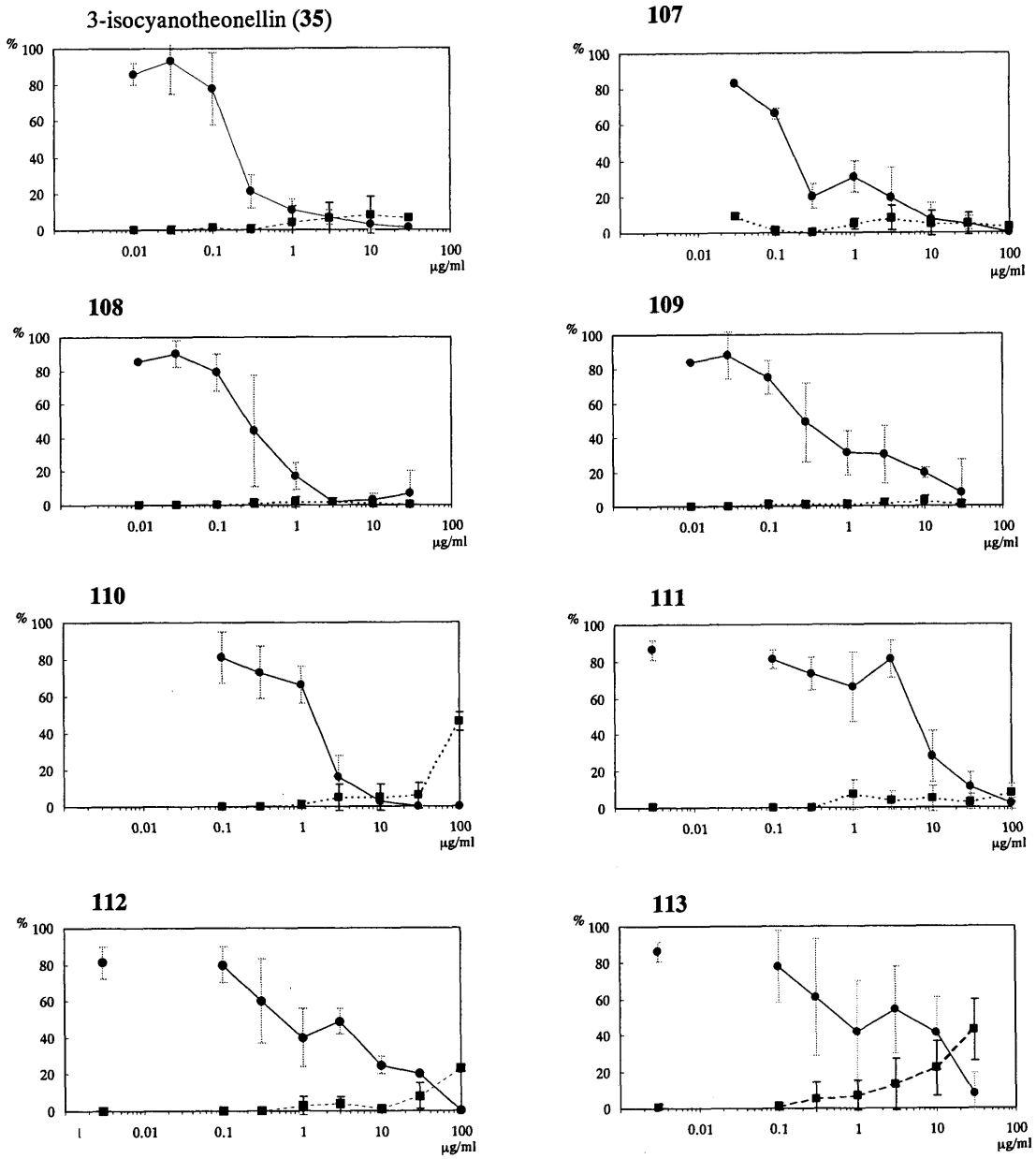


Chart 2-1. Antifouling activity (closed circle) and mortality (closed square) of compounds **35** and **107-113**.

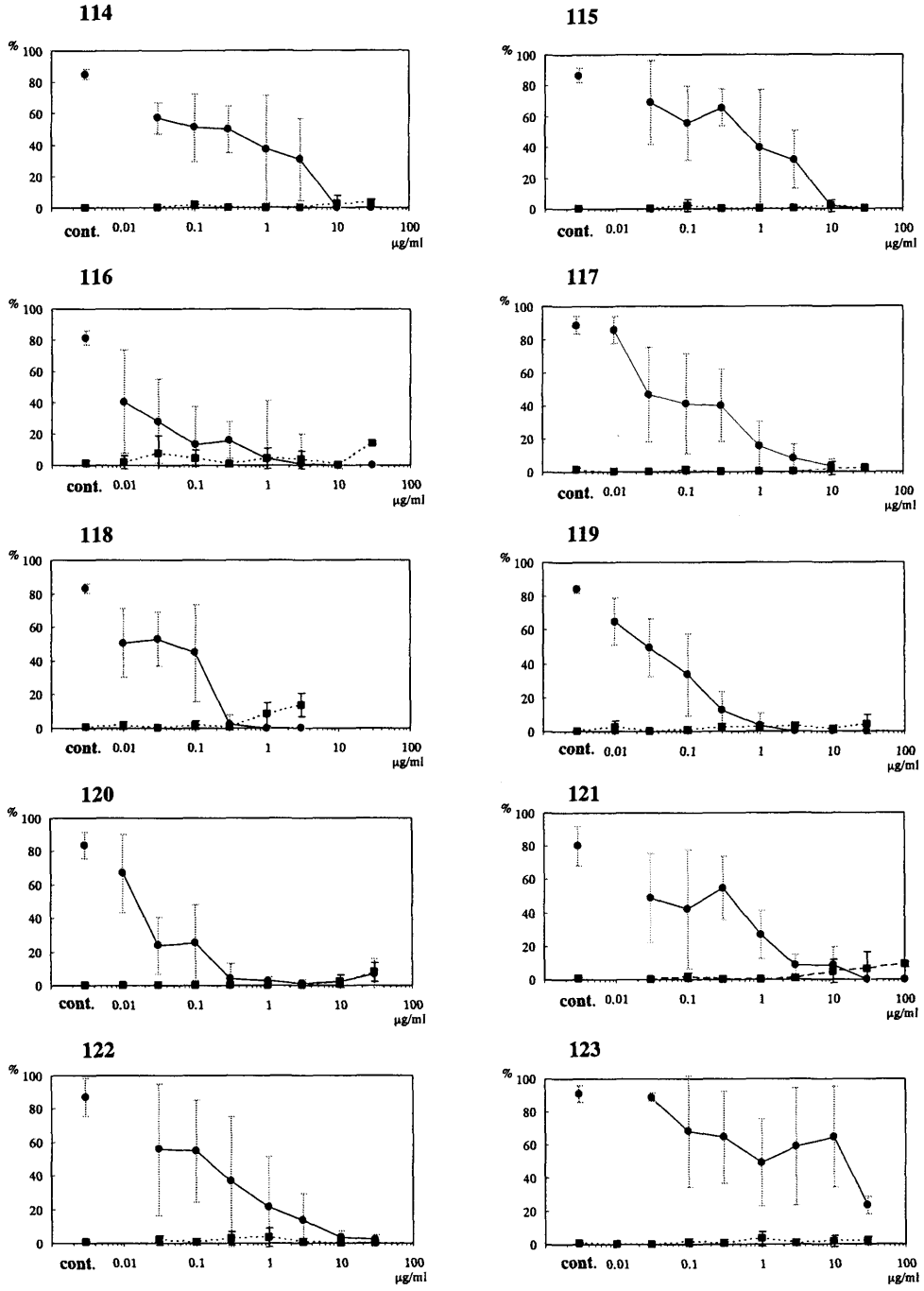


Chart 2-2. Antifouling activity (closed circle) and mortality (closed square) of compounds **114 – 123**.

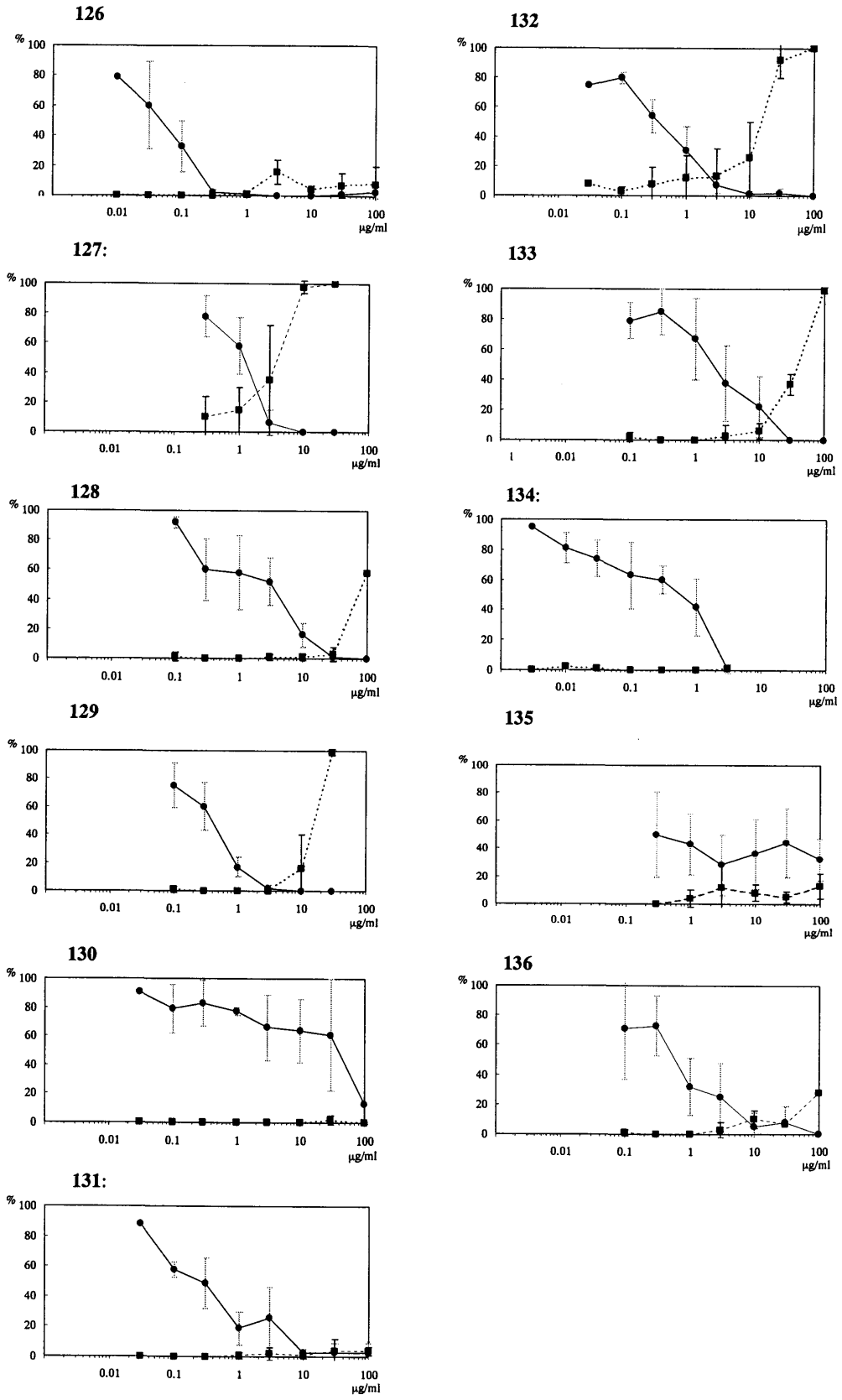


Chart 2-3. Antifouling activity (closed circle) and mortality (closed square) of compounds **126-136**.

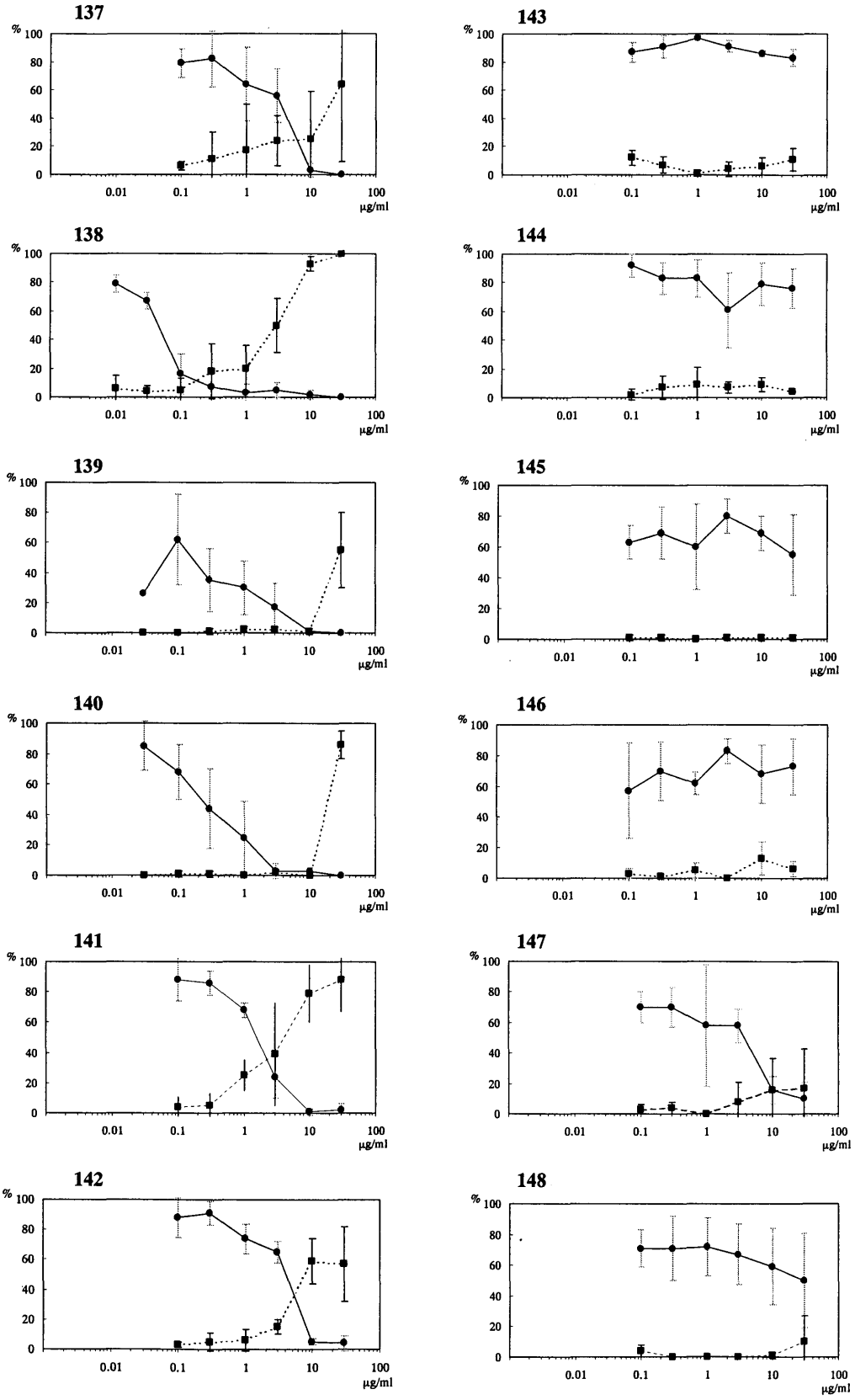


Chart 2-4. antifouling activity (closed circle) and mortality (closed square) of compounds **137-148**.

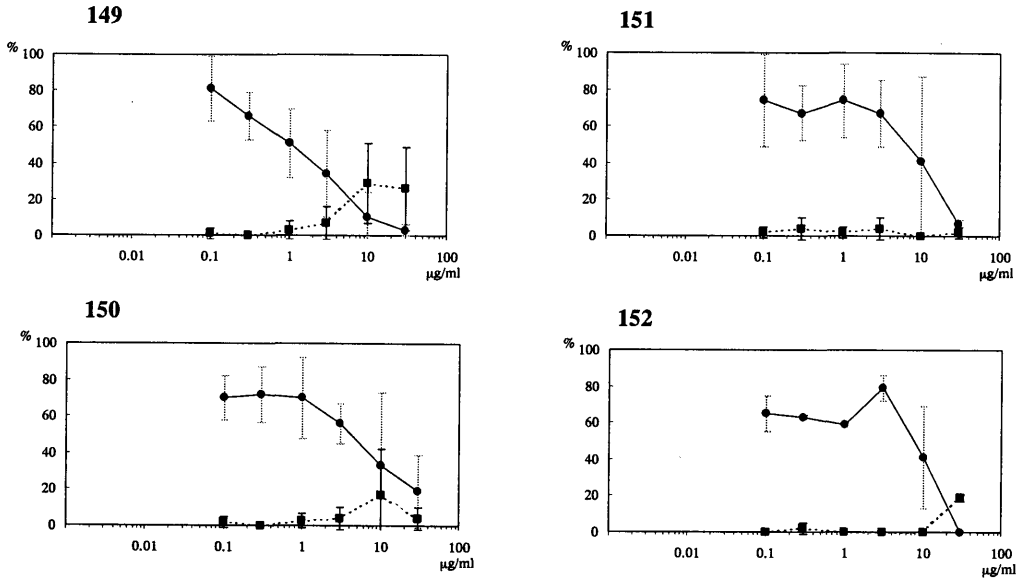


Chart 2-5. Antifouling activity (closed circle) and mortality (closed square) of compounds **149-152**.