Experimental part

General remarks

Unless otherwise stated, all chemicals were of reagent grade and purchased from Sigma, Aldrich, TCI or Merck. Chemical manipulations were carried out under argon in oven-dried glass equipment. For reactions, solvents were dried or freshly distilled by common practice. Organic extracts were dried over $MgSO_4$ or Na_2SO_4 and then evaporated under reduced pressure. Microbes were obtained from KCTC (Korean Collection for Type Cultures). Chromatography: Merck silica gel 60 (40-63 mm). NMR spectra were recorded on $CDCl_3$ using Varian 300 MHz FT-NMR (Chapter I), 300 MHz JEOL JNM AL 300 (Chapter II) or 500 MHz Jeol JNM-a 500 spectrometer (Chapter II). Chemical shifts are reported relative to TMS (δ 0.00) with $CHCl_3$ as internal standard, coupling constants (*J*) are given in Hz. TLC plates were run on silica gel Merck 60 F254 and compounds were visualized by dipping with Mo-reagent [$H_3(PMo_{12}O_{40})nH_2O$ in EtOH (10%)].

Analytical Methods

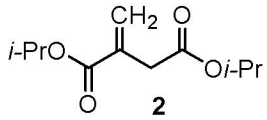
The bioconversion of **5** and **6** was determined with Shimadzu GC10 gas chromatography on a column code CBP1-M25-025 (Shimadzu). Method: column oven temperature 70°C → 120°C, 3°C /min; inj. port temp 150°C ; detector temp. 280°C ; carrier gas N_2 ; column flow 6.10 ml/min. Optical rotation values were measured on a Jasco p-1020 polarimeter in a 10 ml cuvette (Chapter I) and Jasco DIP-1000 plarimeter in a 2 ml cuvette (Chapter II).

The optical purity of δ-nonalactone **22** was determined with Agilent 6890N gas chromatography on a column code CHIRAMIXTM (0.25mmI.D×30mL. df = 0.25 μm). Method: column oven temperature 40°C → 180°C, 0.7°C /min; inj. port temp 230°C ; detector temp. 250°C ; carrier gas N_2, or He ; column flow 0.7 ml/min.

Chapter I-1

I-1-1. Synthesis of 4-acetoxy-2-methylene-butanol (5).

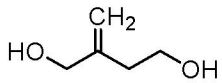
2-methylenesuccinic acid, diisopropyl ester (2).



2

To a stirred solution of itaconic acid (**1**, 130 g, 1.00 mol) and isopropanol (100 ml, 1.60 mol) in benzene (1 L), conc. H_2SO_4 (300 μl) was added. After stirring for 48 h at 70°C, the solution was cooled at room temperature, and extracted with EtOAc. Combined organic phases were washed with aq. $NaHCO_3$, dried over $MgSO_4$ and evaporated. The crude product was purified by distillation to give the diisopropyl ester **2** (210 g, 98%). ; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.237 (s, 1H), 5.117 (s, 1H), 4.542 (s, 2H), 2.627-2.474 (m, 2H), 1.207 (d, J = 11.1, 6H), 1.183 (d, J = 10.8, 6H).

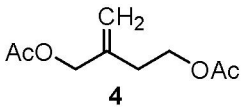
2-Methylene-1,4-butanediol (3).



3

To a stirred solution of $AlCl_3$ (13.4 g, 0.10 mol) in dried Et_2O (300 ml), LAH (11.4 g , 0.30 mol) in Et_2O (200 ml) was added at 0°C. After 30 min, ester **2** (32.1 g, 0.15 mol) in Et_2O (100 ml) was slowly added at -30°C. The reaction was immediately stopped after the addition of **2**, by adding 10% of aq. H_2SO_4. The reaction mixture was extracted with EtOAc, and the combined organic phases were washed with aq. $NaHCO_3$, dried over $MgSO_4$ and evaporated to give diol **3** (15.3g, 98%). ; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.069 (s, 1H), 4.923 (s, 1H), 4.029 (s, 2H), 3.690 (t, J = 6.0 Hz, 2H), 2.313 (t, J = 6.0 Hz, 2H).

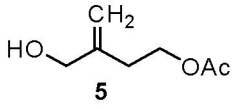
2-Methylene-1,4-butanediol, diacetate (4).



4

To a stirring solution of diol **3** (20.4 g, 0.20 mol) in pyridine (200 ml), Ac_2O (25.0 g) was slowly added at 0°C and stirred for 5 h spontaneously reached at room temperature. The solution was evaporated with MeOH to remove pyridine and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum hexane/EtOAc 4:1) to give diacetate **4** (36.5 g, 2 steps, 98%). ; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.096 (s, 1H), 4.979 (s, 1H), 4.506 (s, 2H), 4.160 (t, J = 6.6 Hz, 2H), 2.364 (t, J = 6.6 Hz, 2H), 2.056 (s, 3H), 2.007 (s, 3H).

4-Acetoxy-2-methylene-1-butanol (5).



5

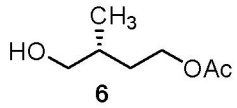
To a stirring solution of LPS (Amano, 200 mg) in sodium phosphate buffer (pH 7.0, 10 ml), the diacetate **4** (200 mg, 1.10 mmol) was added. After stirring was continued for 5min at room temperature, the solution was celite filtrated to remove enzyme. The monoacetate was extracted with EtOAc, the organic phase was washed with aq. $NaHCO_3$, dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum hexane/EtOAc 4:1) to give monoacetate **5** (113 mg, 71.4%). ; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 5.049 (s, 1H), 4.862 (s, 1H), 4.146 (t, J = 6.6 Hz, 2H), 4.023 (s, 2H), 2.342 (t, J = 6.6 Hz, 2H), 1.983 (s, 3H).

I-1-1. Synthesis of 4-Acetoxy-(*R*)-2-methyl-1-butanol (6).

Determination of the bioreduction activity of microbes.

6 Microbes, *Candida rugosa*, *Pseudomonas putida*, *Alcaligenes* sp., *Achromobacter* sp., *Chrombacterium* sp. and *Kleuveromyces fragilis* were grown up in culture tube containing 5 ml of their proper medium at 27-30°C, 180 rpm. After 24 h, substrate **5** (10 μl, 0.07 mmol) was added and kept incubating for another 24 h. And then, each cultured mediums (100 μl) were collected and extracted with EtOAc (200 μl) to check by G.C.

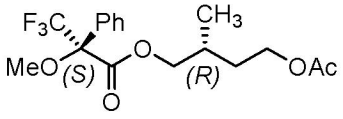
Synthesis of 4-Acetoxy-(*R*)-2-methyl-1-butanol (6).



6

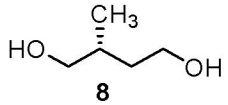
To YPD broth (50 ml) in 500 ml of Erlenmeyer flask, the seed cultured medium (1.5 ml) which was grown up in YPD at 30°C, 180 rpm, for 20 h, was added. At the same time, the substrate **5** (100 mg, 0.7 mmol) was also added and cultured at 30 °C, 180 rpm, for 18 h. The reaction progress was checked every 5 h by G.C. After about 20 h, the cultured medium was extracted with EtOAc the organic phase was washed with water, dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum hexane/EtOAc 4:1) to give asymmetric alcohol **6** (66 mg, 65%).; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 4.032-4.133 (m, 2H), 3.397 (d, J = 5.6 Hz, 2H), 2.002 (s, 3H), 1.649-1.783 (m, 2H), 1.328-1.412 (m, 1H), 0.871 (d, J = 6.8 Hz, 3H).

Synthesis of (*S*)-MTPA ester of 6.



The absolute configuration of **6** was assigned. To the stirred solution of **6** (20 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (1 ml) and Et_3N (0.5 ml), (*R*)-MTPA chloride (40μl) was added dropwise. H_2O was added to the reaction mixture and it was extracted with EtOAc. The organic phase was washed with 1N HCl, dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum hexane/EtOAc 7:1) to give (*S*)-MTPA ester of **6** (33 mg, 69.2%). By ^1H NMR analysis, compound **6** was supposed to be high optical purity.

Assignment of the absolute configuration: preparation and comparison of specific rotation value of 8.



8

Specific rotation value of **6** was measured on a Jasco p-1020 polarimeter in a 10 ml cuvette. To compare the value of **6** with commercially available compound (*R*)-2-methyl-butane-1,4-diol (>98.0%, G.C.), it was deacylated.

To the stirred solution of **6** (60 mg, 0.4 mmol) in MeOH (1 ml), K_2CO_3 (30 mg) was added. After stirring 1 h, aq. NH_4Cl was added and the reaction mixture was evaporated to remove MeOH and extracted with EtOAc. Combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum hexane/EtOAc 2:1) to give diol **8** (35 mg, 84%). ; ^1H NMR

(300 MHz, $CDCl_3$) δ = 3.632 (t, 2H, *J* = 5.4 Hz), 3.663 (m, 1H), 3.568 (dd, 1 H, *J* = 10.8, 4.8),1.560-1.636 (m, 2H), 0.932 (d, *J* = 6.9, 3H)

; (*R*)-2-methyl-butane-1,4-diol, made from **6**: $[\alpha]_D^{27}$ = +13.1 (*c* 0.5, MeOH),

(*R*)-2-methyl-butane-1,4-diol, purchased from TCI: $[\alpha]_D^{27}$ = +13.2° (*c* 1.0, MeOH)/

$[\alpha]_D^{27}$ = +13.6° (c 3.3, MeOH)/

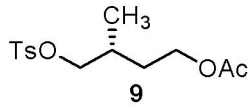
Ref. 12: $[\alpha]_D^{20}$ = +12.5° (neat)

So, I could find that the bioconversion product **6** has (*R*)-configuration with high optical purity (≥98%).

Chapter I-2

I-2-1. Synthesis of methyl-(*S*)-3-methyl octanoate (12).

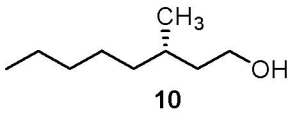
Synthesis of (*R*)-4-acetoxy-2-methylbutyl, *p*-toluene sulfonate (9).



9

A solution of **6** (6.00 g, 41.1 mmol) in CH_2Cl_2 (40.0 ml) containing pyridine (6.40 g, 80.0 mmol) was cooled with ice-bath. *p*-TsCl (9.00 g, 47.2 mmol) was slowly added and the reaction mixture was allowed to warm to r.t. After stirring for 10 h cold water was added, and the mixture was extracted with EtOAc. After washing with aq. $CuSO_4$, water, brine and $MgSO_4$ drying, the solvent was evaporated. The crude product was purified by flash chromatography (petroleum hexane/EtOAc 10:1) to give tosylate **9** (12.4 g, 99%). ; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.767 (d, J = 8.4 Hz, 2H), 7.329 (d, J = 8.4 Hz, 2H), 4.106-3.957 (m, 2H), 3.901-3.816 (m, 2H), 2.433 (s, 3H), 1.977 (s, 3H), 1.917 (m, 1H), 1.703 (m, 1H), 1.431 (m, 1H), 0.917 (d, J = 6.9 Hz, 3H); $[\alpha]_D^{26}$ = -3.0° (*c* 2.0, Et_2O).

Synthesis of (*S*)-3-methyloctan-1-ol (10).

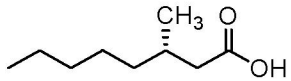


10

To a suspension of CuBr (450 mg, 12.0 mmol) in dried THF (12.0 ml) 1M Grignard reagent (30.0 ml, 30.0 mmol) which was prepared from 1-bromobutane was added dropwise at -30°C. After 1 h, the mixture was cooled to -70°C and tosylate (1.00 g, 3.30 mmol) in THF (5.00 ml) was added. Then, the reaction mixture was allowed to r.t. and

stirred for 3h. After which time sat. NH_4Cl was poured into the solution and the mixture was extracted with Et_2O. Combined organic phases were washed with NH_4Cl, brine and dried over $MgSO_4$ and evaporated. The crude residue was purified by flash chromatography (petroleum hexane/EtOAc 10:1) to give alcohol **10** (0.38 g, 80%). ; ^1H NMR (300 MHz, $CDCl_3$) δ = 3.711-3.6400 (m, 2H), 1.621-1.557 (m, 2H), 1.405-1.363 (m, 1H), 1.343-1.231 (m, 8H), 0.891 (d, *J* = 6.5 Hz, 3H), 0.880 (t, *J* = 6.5 Hz, 3H); $[\alpha]_D^{27}$ = -4.4° (*c* 0.5, Et_2O).

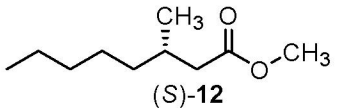
Synthesis of (*S*)-3-methyloctanoic acid (11).



11

To a stirred solution of CrO_3 (1.70 g, 17.0 mmol) in water (8.50 ml), H_2SO_4 (2.75 g, 28.1 mmol) was added dropwise. And this mixture was added to a solution of octanol **10** (2.00 g, 13.8 mmol) in acetone (15.0 ml) under cooling with ice-bath, and the reaction mixture was allowed to warm up to r.t. After stirring for 15 h, the acetone was removed by evaporation, and the aqueous phase was extrated with Et_2O, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude compound was diluted again with Et_2O, washed with $NaHCO_3$ several times. The combined aqueous phases were acidified with HCl to pH = 3 and extracted with Et_2O. The combined organic phases were dried over $MgSO_4$, and evaporated. The crude product was purified by flash chromatography (petroleum hexane/ Et_2O 1:1) to give acid **11** (1.71, 98%). ; ^1H NMR (300 MHz, $CDCl_3$) δ = 2.372 (dd, *J* = 6.0, 14 Hz, 1H), 2.135 (dd, *J* = 8.4, 14 Hz, 1H), 1.954 (m, 1H), 1.380-1.170 (m, 8H), 0.958 (d, *J* = 6.6 Hz, 3H), 0.876 (t, *J* = 6.6 Hz, 3H); $[\alpha]_D^{19}$ = -6.0° (*c* 1.2, Et_2O), Ref. 28: $[\alpha]_D^{19}$ = -6.1° (*c* 1.2, Et_2O).

Synthesis of methyl (*S*)-3-methyloctanoate (12).

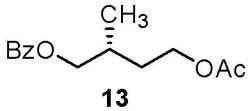


(*S*)-**12**

To a stirred solution of acid **11** (1.40 g, 8.80 mmol) in MeOH (30 ml), conc. H_2SO_4 (300 μl) was added and refluxed at 80°C. After which time, the solution was cooled to room temperature and extracted with Et_2O. The combined organic phases were washed with aq. $NaHCO_3$, brine, dried over $MgSO_4$ and evaporated. The crude product was purified by by flash chromatography (petroleum hexane/ Et_2O 2:1) to give methyl ester (*S*)-**12** (1.71 g, 77%). ; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 3.670 (s, 3H), 2.309 (dd, *J* = 5.7 Hz, *J* = 14 Hz, 1H), 2.134 (dd, *J* = 14, 6.6 Hz, 1H), 2.011 (m, 1H), 1.360-1.985 (m, 8H), 0.092 (d, *J* = 6.6 Hz, 3H), 0.884 (t, *J* = 6.6 Hz, 3H); $[\alpha]_D^{24}$ = -6.0° (*c* 2.0, Et_2O), Ref. 28: $[\alpha]_D^{20}$ = -5.48° (*c* 1.2, Et_2O).

I-2-2. Synthesis of methyl-(*R*)-3-methyl octanoate (12).

Synthesis of (*R*)-4-acetoxy-2-methylbutyl benzoate (13).

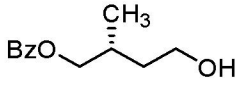


13

A solution of **6** (3.45 g 23.6 mmol) in CH_2Cl_2 (20.0 ml) containing pyridine (2.08 g, 26.0 mmol) was cooled with ice-bath. Benzoyl chloride (14.6 g, 26.0 mmol) was slowly added and the reaction mixture was allowed to warm to r.t. After stirring for 3 h, cold

water was added, and the mixture was extracted with Et_2O. After washing with aq. $CuSO_4$, $NaHCO_3$, brine and $MgSO_4$ drying, the solvent was evaporated to give the crude diester **13** which was used in the next reaction without purification. ; ^1H NMR (300 MHz, $CDCl_3$) δ = 8.052 (d, J = 4.2 Hz, 2H), 7.682 (t, J = 4.2 Hz, 1H), 7.457 (t, J = 4.2 Hz, 2H), 4.232-4.177 (m, 2H), 4.180-4.075 (m, 2H), 2.041 (s, 3H), 1.420-1.200 (m, 3H), 1.398 (t, J = 4.2 Hz, 2H), 1.078 (d, J = 4.2 Hz, 3H); $[\alpha]_D^{19}$ = -43° (*c* 0.5, Et_2O).

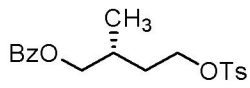
Synthesis of (*R*)-4-hydroxy- 2-methylbutyl benzate (14).



14

To a stirred solution of diester **13** in 30 ml of MeOH, of K_2CO_3 (2.00 g, 14.5 mmol) in of H_2O (5 ml) was added. After stirring for 24 h, the reaction mixture was evaporated and extracted with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by flash chromatography (petroleum hexane/Et2O 4:1) to give benzoate **14** (4.24 g, 86% in 2 steps). ; ^1H NMR (300 MHz, $CDCl_3$) δ = 8.048 (d, J = 7.2 Hz, 2H), 7.565 (t, J = 7.2 Hz, 1H), 7.445 (t, J = 7.2 Hz, 2H), 4.273-4.155 (m, 2H), 3.831-3.722 (m, 2H), 2.191 (m, 1H), 1.844-1.731 (m, 2H), 1.607-1.492 (m, 2H), 1.074 (d, J = 6.6 Hz, 3H); $[\alpha]_D^{20}$ = -4.8° (*c* 1.3, Et_2O).

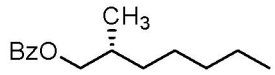
Synthesis of (*R*)-2-methyl-4-(*p*-toluenesulfonylxy)butyl benzoate (15).



15

A solution of **14** (0.78 g, 3.75 mmol) in CH_2Cl_2 (5.0 ml) containing pyridine (0.36 g, 4.5 mmol) was cooled with ice-bath. *p*-TsCl (1.50 g, 7.80 mmol) was slowly added and the reaction mixture was allowed to warm to r.t.. After stirring for 5 h, cold water was added, and the mixture was extracted with Et_2O. After washing with aq. $CuSO_4$, water, brine and $MgSO_4$ drying, the solvent was evaporated. The crude product was purified by flash chromatography (petroleum hexane/EtOAc 10:1) to give 1.21 g (88%) of tosylate **15**. ; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.005 (d, J = 7.5 Hz, 2H), 7.771 (d, J = 8.1 Hz, 2H), 7.570 (t, J = 7.5 Hz, 1H), 7.442 (t, J = 7.5 Hz, 2H), 7.321 (d, J = 8.1 Hz, 2H), 4.181-4.100 (m, 4H), 2.428 (s, 3H), 2.067 (m, 1H), 1.889 (m, 1H), 1.594 (m, 1H), 0.991 (d, J = 6.9 Hz, 3H); $[\alpha]_D^{18}$ = -6.2° (*c* 0.8, Et_2O).

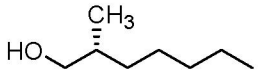
Synthesis of (*R*)-2-methylheptyl benzoate (16).



16

To a suspension of CuBr (0.20 g, 14.0mmol) in dried THF (15.0 ml), 1M Grignard reagent (30.0 ml, 30.0 mmol) which was prepared from 1-bromopropane was added dropwise at -30°C. After 1 h, the mixture was cooled to -70°C and tosylate **15** (1.81 g, 5.00 mmol) in THF (10.0 ml) was added. Then, the reaction mixture was allowed to r.t. and stirred for 3h. After which time sat. NH_4Cl was poured into the solution and the mixture was extracted with Et_2O. Combined organic phases were washed with sat. NH_4Cl and brine and dried over $MgSO_4$ and evaporated. The crude product was used in next step without purification. ; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.052 (d, J = 7.2 Hz, 2H), 7.557 (t, J = 7.5 Hz, 1H), 7.442 (t, J = 7.5 Hz, 2H), 4.214 (m, 1H), 4.117 (m, 1H), 1.934 (m, 1H), 1.505-1.382 (m, 2H), 1.325-1.233 (m, 6H), 1.020 (d, J = 6.6 Hz, 3H), 0.910-0.852 (m, 3H); $[\alpha]_D^{22}$ = -5.9° (*c* 0.8, Et_2O).

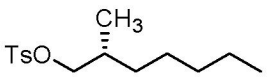
Synthesis of (*R*)-2-methylheptan-1-ol (17).



17

To hydrolyze the remained benzoyl group of **16**, the crude mixture was dissolved in MeOH (150 ml) and NaOH (3.0 g, 75 mmol) in H_2O (1 ml) was added. After stirring for 10 h, the MeOH was removed by evaporation and the aqueous phase was extrated with Et_2O, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude compound was diluted again with Et_2O, washed with $NaHCO_3$ several times. The crude product was purified by flash chromatography (petroleum hexane/ Et_2O 5:1) to give alcohol **17** (0.48 g, 2 steps 74%). ; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 3.511 (dd, J = 10.5, 6.3 Hz, 1H), 3.393 (dd, J = 10.5, J = 6.3 Hz, 1H), 1.618 (m, 1H), 1.324 (m, 7H), 1.569 (m, 1H), 0.917 (d, J = 6.9 Hz, 3H), 0.888 (t, J = 7.2 Hz, 3H); $[\alpha]_D^{22}$ = +14 (*c* 1.0, MeOH), $[\alpha]_D^{25}$ = +13° (*c* 0.5, Et_2O), Ref. 28: $[\alpha]_D^{22}$ = +14.3° (*c* 1.2, MeOH).

Synthesis of (*R*)-2-methylheptyl *p*-toluenesulfonate (18).

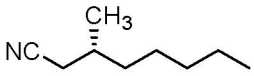


18

A solution of **17** (0.84 g 6.46 mmol) in CH_2Cl_2 (10.0 ml) containing pyridine (1.60 g, 20.0 mmol) was cooled with ice-bath. *p*-TsCl (1.55 g, 8.15 mmol) was slowly added and the reaction mixture was allowed to warm to r.t.. After stirring for 10 h, cold water was added and the mixture was extracted with Et_2O. After washing with aq. $CuSO_4$, water,

brine and $MgSO_4$ drying, the solvent was evaporated. The crude product was purified by flash chromatography (petroleum hexane/Et_2O 8:1) to give tosylate **18** (1.12 g, 61%). ; ^1H NMR (300 MHz, $CDCl_3$) δ = 7.790 (d, J = 8.4 Hz, 2H), 7.345 (d, J = 8.4 Hz, 2H), 3.882 (dd, J = 9.6, 5.7 Hz, 1H), 3.800 (dd, J = 9.6, 6.3 Hz, 1H), 2.452 (s, 3H), 1.795-1.731 (m, 1H), 1.317-1.063 (m, 8H), 0.873 (d, J = 6.9 Hz, 3H), 0.812 (t, J = 6.6 Hz, 3H); $[\alpha]_D^{25}$ = -2.9° (*c* 1.0, Et_2O) (It was not dissolved in MeOH), Ref. 28: $[\alpha]_D^{20}$ = -0.94° (*c* 1.4, MeOH).

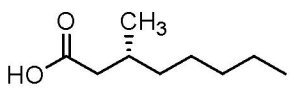
Synthesis of (*R*)-3-Methyloctanenitrile (19).



19

To a mixture of DMSO (2 ml) and NaCN (0.12 g, 2.40 mmol) was added and heated at 60°C. After dissolve, tosylate **18** (0.28 g, 1.00 mmol) was added and stirred at 55°C for 10 h. The mixture was cooled to r.t., diluted with Et_2O and washed with water and brine, and after $MgSO_4$ drying, the solvent was evaporated. The crude product was purified by flash chromatography (petroleum hexane/Et_2O 9:1) to give nitrile **19** (0.06 g, 75%). ; ^1H NMR (300 MHz, $CDCl_3$) δ = 2.370-2.188 (m, 2H), 1.873-1.790 (m, 1H), 1.389-1.170 (m, 8H), 1.064 (d, J = 6.6 Hz, 3H), 0.919-0.862 (m, 3H).

Synthesis of (*R*)-3-Methyl-octanoic acid 20.

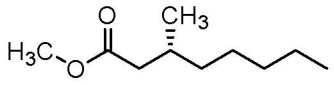


20

To a stirred solution of nitrile **19** (0.77 g 5.50 mmol) in EtOH (17 ml, 95%), aq. NaOH

(6.5 ml, 42%) was added and refluxed. After 20 h, the mixture was cooled to r.t. and acidified with HCl to pH 3. And then it was extracted with Et_2O, washed with $NaHCO_3$, dried over $MgSO_4$, filtered, evaporated and purified by flash chromatography (petroleum hexane/Et_2O 4:1) to give acid **20** (0.50 g, 57%). ; ^1H NMR (300 MHz, $CDCl_3$) δ = 2.372 (dd, J = 6.0, 14 Hz, 1H), 2.135 (dd, J = 8.4, 14 Hz, 1H), 1.954 (m, 1H), 1.380-1.170 (m, 8H), 0.958 (d, J = 6.6 Hz, 3H), 0.876 (t, J = 6.6 Hz, 3H) ; $[\alpha]_D^{24}$ = +7.0° (*c* 0.4, Et_2O), Ref. 28: $[\alpha]_D^{21}$ = +5.95° (*c* 1.2, Et_2O).

Synthesis of Methyl (*R*)-3-methyl octanoate (12).



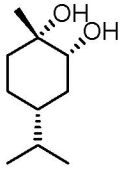
(*R*)-**12**

(Same procedure as above (*S*)-**12**)

To a stirred solution of acid **20** (1.40 g, 8.80 mmol) in MeOH (30 ml), conc. H_2SO_4 (300 μl) was added and refluxed at 80°C. After which time, the solution was cooled to room temperature and extracted with Et_2O. The combined organic phases were washed with aq. $NaHCO_3$, brine, dried over $MgSO_4$ and evaporated. The crude product was purified by by flash chromatography (petroleum hexane/ Et_2O 2:1) to give methyl ester (*R*)-**12** (1.71 g, 77%). ; ^1H NMR (300 MHz, $CDCl_3$) δ = 3.670 (s, 3H), 2.309 (dd, J = 5.7 Hz, J = 14 Hz, 1H), 2.134 (dd, J = 14, 6.6 Hz, 1H), 2.011 (m, 1H), 1.360-1.985 (m, 8H), 0.092 (d, J = 6.6 Hz, 3H), 0.884 (t, J = 6.6 Hz, 3H); $[\alpha]_D^{25}$ = +3.3° (*c* 0.4, Et_2O), Ref. 28: $[\alpha]_D^{22}$ = +5.13° (*c* 1.1, Et_2O).

Chapter II

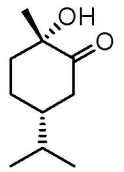
(1*S*,2*R*,4*R*)-4-isopropyl-1-methylcyclohexane-1,2-diol (26).



26

Synthesis of **26** from (*R*)-(+)-limonene was performed according to the referenced paper (Ref. 35: Mori, K. *Tetrahedorn: Asymmetry* **2006**, *17*, 2133).

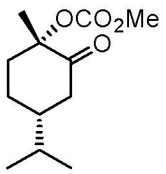
Synthesis of (2*S*,5*R*)-2-hydroxy-5-isopropyl-2-methylcyclohexanone (27).



27

To a stirred solution of oxalylchloride (17 ml, 0.2 mol) in of CH_2Cl_2 (250 ml), DMSO (28 ml, 0.4 mol) was added dropwise at -78°C. After 5 min, diol **26** (17.2 g, 0.1 mol) in CH_2Cl_2 (50 ml) was slowly added at -78°C and stirred for 30 min. After adding Et_3N (89 ml, 0.64 mol), the mixture was warmed to r.t. Water was added to the reaction mixture, and it was extracted with EtOAc. Combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by flash chromatography (petroleum hexane/ EtOAc 10:1) to give hydroxyl ketone **27** (12.7 g, 75%). ; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 2.622 (dd, J = 6.3, 13.2, 1H), 2.466 (dd, J = 6.3, 13.2, 1H), 1.640-1.917 (m, 4H), 1.395-1.512 (m, 2H), 0.900 (d, J = 8.1 Hz, 3H), 1.352 (s, 3H), 0.878 (d, J = 8.1 Hz, 3H); $[\alpha]_D^{25}$ = -29° (c = 1.0, $CHCl_3$), Ref. 35: $[\alpha]_D^{20}$ = -25° (c = 2.0, $CHCl_3$).

(1*S*,4*R*)-(4-isopropyl-1-methyl-2-oxocyclohexyl)methylcarbonate (28).



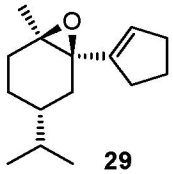
28

To a stirred solution of **27** (3.7 g, 21.8 mmol) in THF (220 ml), of LiHMDS(1 M solution in THF, 22.2 ml, 22.2 mmol) was added dropwise at -78°C. After 20 min, of MeOCOCl (3.5 ml, 45.5 mmol) was added and stirred for 20 h. The reaction mixture was spontaneously warmed to room temperature. The reaction was stopped by adding water and extracted with Et_2O. The organic phase was washed with $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (petroleum hexane/Et_2O 10:1) to give carbonate **28** (3.65 g, 73%). ; ^1H NMR (300 MHz, C_6D_6) δ = 3.272 (s, 3H), 2.343-2.414 (m, 2H), 2.014 (m, 1H), 1.504 (s, 3H), 1.504 (m, 1H), 1.053-1.282 (m, 4H), 0.627 (d, J = 6.6 Hz, 3H), 0.617 (d, J = 6.6 Hz, 3H); $[\alpha]_D^{25}$ = +20.5° (c = 1.5, $CHCl_3$).

Synthesis of (1*R*, 3*R*, 6*R*)-1-cyclopentenyl-3-isopropyl-6-methyl-7-oxa-bicyclo[4.1.0]heptane (29) and (3a*S*, 5*R*, 7a*S*)-3a-cyclopentenyl-hexahydro-5-isopropyl-7a-methylbenzo[d][1,3]dioxol-2-one (30).

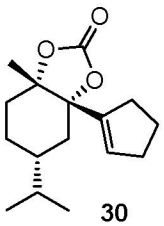
The cyclopentenyl iodide was prepared by refered paper. Cyclopentenyl iodide (1.07 g, 5.5 mmol) was dissolved in THF (20 ml), and *t*-BuLi (1.58 M in pentane, 7.0 ml, 11.0 mmol) was added dropwise at -78°C. After 30 min, it was added to the stirred solution of carbonate **28** (1.07 g, 4.7 mmol) in THF (30 ml), at -78°C. After stirred for 30 min, the reaction mixture was allowed to warm spontaneously to room temperature. The reaction was stopped by adding aq. NH_4Cl, and the mixture was extracted with Et_2O. Combined organic phases were washed with water and brine, dried over $MgSO_4$ and

evaporated. The crude product was purified by flash chromatography (petroleum hexane/ Et_2O 10:1) to give epoxide **29** (0.42 g, 38%) and cyclic carbonate **30** (0.6 g, 46%).



29

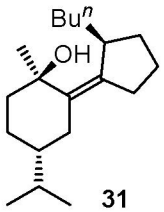
Epoxide **29** ; ^1H NMR (300 MHz, $CDCl_3$) δ = 5.550 (dt, J = 2.1, 2.1, 1H), 2.319-2.437 (m, 3H), 2.272 (m, 1H), 1.828-1.962 (m, 5H), 1.451-1.584 (m, 2H), 1.268-1.429 (m, 2H), 1.160 (s, 3H), 0.994 (m, 1 H), 0.850 (d, J = 6.3, 3H), 0.840 (d, J = 6.3, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ = 143.77, 126.46, 66.37, 66.13, 36.42, 33.60, 33.37, 32.53, 31.95, 31.81, 30.57, 25.57, 23.85, 21.07, 19.89; MS: *m/z*: 220 (M+); $[\alpha]_D^{27}$ = +28.4° (*c* = 1.0, $CHCl_3$).



30

Cyclic carbonate **30**; IR (film) *v*: 1795 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ = 5.812 (t, J = 1.8, 1H), 2.233-2.490 (m, 5H), 2.117 (dd, J = 2.7 Hz, 12, 1H), 1.803-2.020 (m, 2H), 1.388-1.658 (m, 4H), 1.240 (s, 3H), 1.193-1.365 (m, 2 H), 0.878 (d, J = 6.6 Hz, 3H), 0.873 (d, J = 6.6 Hz, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ = 154.06, 139.55, 127.21, 87.68, 84.63, 39.54, 38.56, 33.54, 33.43, 32.04, 31.88, 23.44, 23.29, 19.32; MS: *m/z*: 264 (M+); $[\alpha]_D^{26}$ = +126.4° (*c* = 1.2, $CHCl_3$).

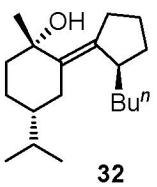
Synthesis of (*Z*, 1*R*, 4*R*)-2-((*S*)-2-butylcyclopentylidene)-4-isopropyl-1-methylcyclohexanol (31).



31

To a stirred suspension of dried (120°C, for 20 h) LiI (1.5 g, 11.5 mmol) and CuI (2.2 g, 11.5 mmol) in Et_2O (100 ml), *n*-BuLi (1.59 M in hexane, 14 ml, 22.2 mmol) was added dropwise at -30°C. The color of reaction mixture turned gradually to white, yellow, dark yellow, grey violet and dark violet finally. After 30 min, 0.46 g (2.1 mmol) of epoxide **29** in Et_2O (50 ml) was slowly added at -30°C and stirred -30°C to r.t., for overnight. The reaction was stopped by adding aq. NH_4Cl, and the mixture was extracted with Et_2O. Combined organic phases were washed with aq. NH_4Cl and brine, dried over $MgSO_4$ and evaporated. The crude product was purified by flash chromatography (petroleum hexane/Et_2O 20:1) to give **31** (0.39 g, 67%); ^1H NMR (300 MHz, C_6D_6) δ = 3.450 (m, 1H), 2.329-2.474 (m, 2H), 2.240 (m, 1H), 1.750 (m, 1H), 1.494-1.677 (m, 7H), 1.310-1.466 (m, 6H), 1.286 (s, 3H), 1.041-1.106 (m, 3H), 0.966 (t, J = 6.9, 3H), 0.869 (d, J = 6.6 Hz, 3H), 0.847 (d, J = 6.6 Hz, 3H); $[\alpha]_D^{24}$ = +61.7° (c = 1.1, $CHCl_3$).

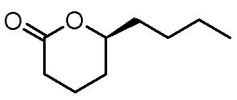
Synthesis of (*E*, 1*S*, 4*R*)-2-((*R*)-2-butylcyclopentylidene)-4-isopropyl-1-methylcyclohexanol (32).



32

To a stirred suspension of CuCN (0.49 g, 5.5 mmol) in THF (55 ml), Grignard reagent (1 M in THF, 5.5 ml, 5.5 mmol) which was prepared from 1-bromobutane was added dropwise at -78°C. After 30 min, cyclic carbonate (0.3 g, 1.1 mmol) in THF(1 ml) was slowly added at -78°C and stirred -30°C for 3 h. The reaction was stopped by adding aq. NH_4Cl, and the mixture was extracted with Et_2O. Combined organic phases were washed with aq. NH_4Cl and brine, dried over $MgSO_4$ and evaporated. The crude product was purified by flash chromatography (petroleum hexane/Et_2O 20:1) to give **33** (0.23 g, 75%). ; ^1H NMR (300 MHz, C_6D_6) δ = 2.691 (m, 1H), 2.414-2.593 (m, 2H), 2.217-2.336 (m, 2H), 1.565-1.741 (m, 3H), 1.431-1.548 (m, 5H), 1.411 (s, 3H), 1.265-1.390 (m, 3H), 1.086-1.227 (m, 5H), 0.967 (d, J = 6.6 Hz, 3H), 0.912 (t, J = 6.6 Hz, 3H), 0.912 (d, J = 6.6 Hz, 3H); $[\alpha]_D^{22}$ = -11.6 (c = 0.5, $CHCl_3$).

Synthesis (*S*)-δ-nonalactone 22 from 31.



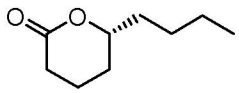
(*S*)-δ-nonalactone **22**

The S_N2' product **31** (0.1 g, 0.4 mmol) was dissolved in MeOH (6 ml), the solution was cooled to -78°C, and O_3 was bubbled through. When the solution remained light blue, the ozone flow was stopped. After Ar bubbled, Me_2S (excess) was added to the reaction mixture. The mixture was stirred at r.t. for 1 h, and evaporated to remove MeOH and Me_2S. The residue was diluted with Et_2O, washed with brine, dried over $MgSO_4$, and evaporated. The crude mixture was used for the next step without purification.

To a stirred solution of crude cyclopentanone in CH_2Cl_2 (2 ml), mcpba (total 0.33 g, 1.8 mmol) was added. The reaction mixture was stirred for 2 days. Et_2O diluted reaction mixture was washed with Na_2SO_3, 1 N NaOH and brine and dried (Na_2SO_4) and evaporated. The crude mixture was purified by flash chromatography (petroleum

hexane/Et_2O 4:1) to give purposed compound (*S*)-δ-nonalactone **21** (45 mg, 2 steps 73%). ; IR (film) *v*: 1735 cm^{-1}. ^1H NMR (300 MHz, C_6D_6) δ = 4.262 (m, 1H), 2.551 (m, 1H), 2.459 (m, 1H) 1.844-1.937 (m, 3H), 1.671 (m, 1H), 1.443-1.613 (m, 3H), 1.243-1.389 (m, 3H), 0.900 (t, *J* = 6.9 Hz, 3H); $[\alpha]_D^{24}$ = -39. 6° (*c* = 0.85, $CHCl_3$), Ref. 32: $[\alpha]_D^{24}$ = -48° (*c* = 0.83, $CHCl_3$), Ref. 33: $[\alpha]_D^{24}$ = -52.5° (*c* = 1.0, $CHCl_3$).

Synthesis (*R*)-δ-nonalactone 22 from 32.



(*R*)-δ-nonalactone **22**

The S_N2' product **32** (0.04 g, 0.18 mmol) was dissolved in MeOH (2 ml). The solution was cooled to -78°C, and O_3 was bubbled through. When the solution remained light blue, the ozone flow was stopped. After Ar bubbled, the reaction mixture was evaporated to remove MeOH. The crude mixture was diluted in CH_2Cl_2 (2 ml), mcpba (total 0.12 g, 0.72 mmol) was added. The reaction mixture was stirred for 2 days.

The residue was diluted with Et_2O, washed with brine, dried over $MgSO_4$, and evaporated. The crude mixture was purified by flash chromatography (petroleum hexane/Et_2O 4:1) to give purposed compound (*R*)-δ-nonalactone **22** (34.4 mg, 2 steps 37.5%); ^1H NMR was identical to (*S*)-δ-nonalactone, $[\alpha]_D^{18}$ = +49.7° (*c* = 0.5, $CHCl_3$), Ref. 33: $[\alpha]_D^{24}$ = +50.6° (*c* = 1.0, $CHCl_3$).

References

1. Leitereg T. J., Guadagni D. G., Harris J., Mon T. R., Teranishi R., *Nature*, **1971**, *230*, 455-456.
2. J. Tramper, H. C. van der Pas, P. Linko, *Biocatalyst in Organic Syntheses*, Elsevier Science Publishers B. N., Amsterdam, 1985.
3. ILB Inc., *Opportunites for Bioconversion and Biocatalysis in Speciality and Fine Chemicals,* **1992**, *2,* 16-24.
4. P. Ferraboschi, P. Grisenti, R. Casati, A. Fiecchi, E. Santaniello, *J. Chem. Soc. Perkin Trans. I.,* **1987**, 1743-1748.
5. G. Kirchner, M. P. Scollar, A. M. Klibanov, *J. Am. Chem. Soc.,* **1985**, *107*, 7072-7076.
6. Wieland L. C., Deng, H., Snapper M. L., Hoveyda A. H., *J. Am. Chem. Soc.*, **2005**, *127*, 15453-15456.
7. Kacprzynski M. A., Hoveyda A. H., *J. Am. Chem. Soc.,* **2004**, *126*, 10676-10681.
8. Marshall J. A., *Chem. Rev.*, **1989**, *89*, 1503-1511.
9. Alexakis A., Croset K., *Org. Lett.* **2002**, *4*, 4147-4149.
10. Drauz K., *Enzyme Catalysis in Organic Synthesis*, VCH, Germany, **1995**.
11. Nakamura K., Kaway Y., Nakajima N., Ohno A., *J. Org. Chem.,* **1991**, *56*, 4778-4783
12. TCI(Tokyo Chemical Industry Co., Ltd.) *Laboratory Chemicals*, **2008-2009**, *39*, 1230.
13. Kaiser R., *The scent of orchids: Olfactory and chemical investigations,* Elsevier, Amsterdam, **1993**.
14. Kitahara T., Kang S H., Tamogami S., Kaiser R., *Natural Product Letters,* **1994**, *5*, 157-164.
15. Mori K., Kitahara T., Japanese patent, **2005**, No. JP7233119.
16. Mori K., *Tetrahedron* **1989**, *45*, 3233-3298.
17. Ramachandran P. V, Krzeminsk M. P., Reddy M. V. R., Brown H. C., *Tetrahedron:*

Asymmetry, **1999**, *10*, 11-15.

18. (*R*)-(+)-Tuberolactone, *Polyanthes tuberose*: L. a) Bourdineaud, J.-P.; Ehret, C., Petrzilka M., Optically Active Lactones. World Patent, WO/94107887, April 19, **1994**, b) Kaiser, R.; Lamparsky, D. *Tetrahedron Lett.* **1976**, 1659-1660.
19. (*S*)-(+)-Jasmine lactone, *Jasminum grandiflorum*, L., powerful, creamy, jasmine, peachy, coconut: Winter, M.; Malet, G.; Pfeiffner, M.; Demole, E. *Helv. Chim. Acta* **1962**, *45*, 250–1255.
20. δ-Nonalactone, *Piper nigrum*, L, creamy, coconut, sweet and dairy like with milky flavor: Baker, Richard R.; Pereira da Silva, Jose R.; Smith, Graham., *Food and Chemical Toxicol*, **2004**, *42*, 3-37.
21. *cis*-Oak lactone, wood of *Platycarya strobilacea*, coconut, vanilla, or dark chocolate: Hayasaka Y., Wilkinson K. L., Elsey G. M., l Raunkjær M., Sefton M. A., *J. Agric. Food Chem.*, **2007**, 55, 9195-9201.
22. Dihydroactinidiolide, queen-produced queen recognition pheromone of the red imported fire ant, Soknnopsis inicta, sweet, tea-like odor: Mori, K. and Nakazono, Y., *Tetrahedron*, **1986**, *42*, 6459.
23. Epianastrephin, male-produced sex pheromone of the Caribbean fruit fly, *Anastrepha suspensa*: Strekowski, L., Visnick M., Battiste M. A., *J. Org. Chem.* **1986**, *51*, 4836-4839.
24. Nepetalactone, female-produced sex pheromone of the vetch aphid, *Megoura viciae*: Dawson G. W., Griffiths D. C., Janes N. F., Mudd A., Pickett J. A., Wadhams L. J., Woodcock C. M., *Nature*, **1987**, *325*, 614-616.
25. Queen-produced pheromone of the oriental hornet, *Vespa orientalis*: Mori K., *Tetrahedron*, **1989**, *45*, 3233-3298.
26. Male-produced pheromone of the carpenter bee, *Xylocopa hirutissima*: Mori K. Senda, S. *Tetrahedron* **1985**, *41*, 541.
27. Ferrulactone II, female-produced pheromone of the rusty grain beetle, *Cryptolestes ferrugineus*: Oehlschlager A. C., King G. G. S., Pierce H. D. Jr., Pierce A. M., Slessor K. N., Millar J. G., Borden J. H., *J. Chem. Ecol*, **1987**, *13*, 1543-1554.
28. Elephantopin, *Elephantopus scaber L.*, cytotoxic antitumor agents: Haruna M., Rice

G., Lee K. H., *J. Nat. Prod.* **1985**, *48*, 93-96.

29. Artemisinin, *Artemisia annua*, antimalarial drug: Acton, N., Roth R. J., *J. Org. Chem.*, **1992**, *57*, 3610-3614.
30. Antheculariη, *Anthemis auriculata* , antiprotozoal Activity: Karioti A., Skaltsa H., Linden A., Perozzo R., Brun R., Tasdemir D., *J. Org. Chem.* **2007**, *72*, 8103.
31. Nemoto H., Zhong W., Kawamura T., Kamiya M., Nakano Y., Sakamoto K., *Synlett*, **2007**, *15*, 2243-2346.
32. Kayser M. M., Chen G., Stewart J. D., *J. Org. Chem.*, **1998**, *63*, 7103-7106.
33. Haase B., Schneider M. P., *Tetrahedron: Asymmetry*, **1993**, *4*, 1017-1026.
34. Spino C., Beaulieu C., Lafreniere J., *J. Org. Chem.*, **2000**, *55*, 7091-7097.
35. Mori, K. *Tetrahedorn: Asymmetry*, **2006**, *17*, 2133-2142
36. Bettadaiah B. K., Gurudutt K. N., Srinivas P., *J. Org. Chem.*, **2002**, *68*, 2460-2462.

Acknowledgements

It is my pleasure to thank the many people who made this thesis possible.

Foremost, I would like to thank my supervisor Professor Watanabe Hidenori, who has a passion for organic chemistry, keen research instinct and great understanding about students. He always motivated me to be an active Ph D. student and this paper would not have been possible without his unceasing efforts and counsels on my work.

It is difficult to overstate my appreciation for Professor Seo Young-Bae, who first brought me into the academic world to let me discover the worth of scientific research. He has been like a father to me and I hope that he continues to be so.

I am deeply grateful to Professor Mori Kenji and Professor Kitahara Takeshi. They showed me excellent models of a research scientist. Their heartfelt advices have always helped me to be confident and ambitious.

I want to thank Associate Professor Ishigami Ken, who has provided assistance in numerous ways. Especially, his ability to analyze materials at a glance was invaluable to my research.

This work would not have been possible without the support and encouragement of my colleagues. In spite of the fact that I was an overseas student; they did not show any bias towards me but rather always cared and tried to understand how hard things have been for me. Their intelligence and diligence was admirable and has made a big inspiration on me for which I am grateful.

I am grateful to Doctor Tamogami Shigeyuki for his rapid and accurate G.C analysis and Mass measurement.

I wish to thank everybody with whom I have shared experiences in Japan and Korea in my life.

My deepest gratitude goes to the Hasegawa International Scholarship Foundation, which has supported me during my first two years of research. They made possible lots of wonderful experiences such as regular international exchange parties and study trips. They supported me not only financially but also mentally.

I also thank the Japan Student Services Organization for the Honors Scholarship and The University of Tokyo Foundation for the International student Scholarship, which supported me for one year.

I cannot finish without saying how grateful I am to my family. I want to express my gratitude to my sisters, who always cheered me up and blessed me with happiness. They are wonderful presents for me.

Lastly, and most importantly, I wish to thank my parents, the most special persons in my life. They have always supported and encouraged me to do my best in all matters. Their advice to take the sweet with the sour and to have a sense of purpose in life has helped me to get over many difficulties. Without them, this work would not have even been possible.

I dedicate this thesis to my beloved parents.