CHAPTER III
EXPERIMENTAL SECTION

3.1 Materials

Chitosan samples with a degree of deacetylation of 75.8 % and 85.9 % were provided from the Asian Institute of Technology, Bangkok, and Wako Chemicals, Japan, respectively. *N,N'*-carbonyldiimidazole was produced by TCI, Japan. Chloroform, methanol and hydrochloric acid were purchased from J.T. Baker, USA. Acetic acid, *N,N*-dimethylformamide, benzene, sodium hydroxide and di-potassium hydrogen orthophosphate were supplied from UNIVAR, Australia. Potassium dihydrogenphosphate, sodium iodide and sodium hydride were purchased from Fluka Chemika, Switzerland. All these chemicals were used without further purification. A commercial grade of 1-naphthyl methylcarbamate or carbaryl 85 % WP was supplied from AG-GRO (THAILAND) Co., Ltd. and recrystallized in acetone before use.

3.2 Instruments and Equipment

3.2.1 Fourier Transform Infrared Spectrophotometer (FT-IR)

Qualitative and quantitative FT-IR spectra were obtained from a VECTOR 3.0 BRUKER Spectrometer with 16 scans at a resolution of 4 cm^{-1}. A frequency range of 4000-400 cm^{-1} was observed using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D^*, of $1x10^9$ cm. $Hz^{1/2}W^{-1}$.

3.2.2 Elemental Analysis (EA)

The percent elements were obtained from PE 2400 Series II CHNS/O Analyzer with combustion temperature at 975 °C, reduction temperature at 500 °C and vial receptacle for 1000 runs. The sample was put in tin foil for 1-2 mg and analyzed under air (flowing rate 60 psi) with O_2 as a combustion gas (flowing rate 15 psi) using He gas as a carrier gas (flowing rate 20 psi).

3.2.3 Nuclear Magnetic Resonance Spectrometer (NMR)

Solid state ^{13}C-NMR spectra were analyzed by a DPX-300 Avance 300 MHz Digital NMR Spectrometer of Bruker, Switzerland by courtesy of the National Metal and Materials Technology Center (MTEC), Bangkok, Thailand.

3.2.4 Thermal Gravimetric Analysis (TGA)

Perkin -Elmer Thermogravimetric analyzer was used for TGA study. Samples (approximately 5 mg) were loaded in a platinum pan and heated under a N_2 flowing rate of 20 ml/min. The heating rate was 10°C/min from room temperature to 700 °C.

3.2.5 Ultraviolet-Visible Spectrophotometer (UV-VIS)

A Lamda-10 UV-VIS spectrophotometer from Perkin-Elmer was used for qualitative and quantitative analysis. The concentration of carbaryl in the buffer solution in the release study was determined at λ_{max} 279 and 220 nm.

3.2.6 X-ray Diffraction (XRD)

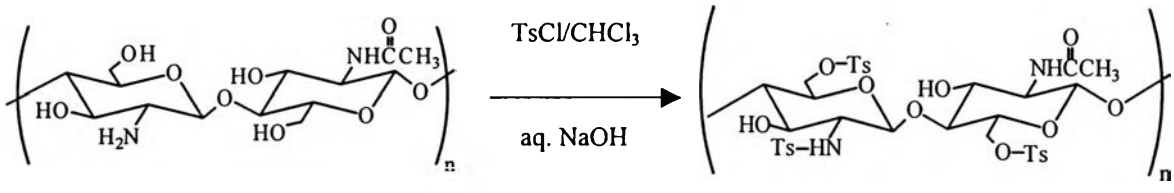
X-ray diffraction patterns were obtained from a RIGAKU RINT2000. Cu Kα (λ=0.154 nm) was used as x-ray source and operated at 40 kV, 30mA with Ni filter. Sample (0.1-0.2 g) was ground with agate mortar and spread on a glass slide specimen holder to examine at 2θ of 5-90°.

3.3 Experimental Procedure

3.3.1 Preparation of Chitosan Precursors

3.3.1.1 Preparation of Tosylchitosan

Tosylchitosan was prepared as reported by Tachaboonyakiat, *et al.* (1998) as shown in Scheme 3.1. The product was characterized by FT-IR, solid state ^{13}C-NMR, EA, TGA and XRD.

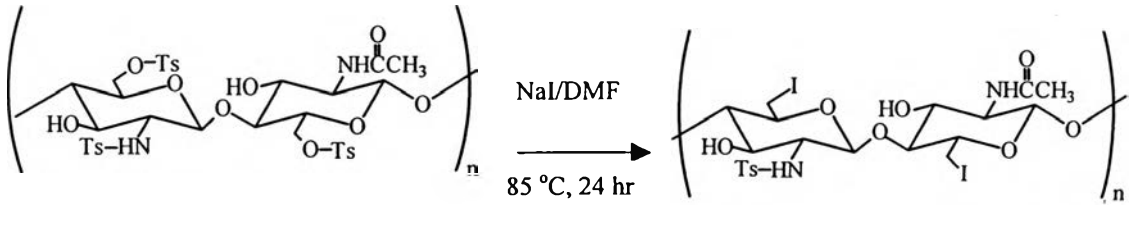


Chitosan Tosylchitosan

Scheme 3.1 Preparation of tosylchitosan

3.3.1.2 Preparation of Iodochitosan

One gram of tosylchitosan was dispersed in 28 mL of *N,N*-dimethylformamide and stirred in nitrogen atmosphere for 15 minutes. A solution of 3.3 g (10 mole equivalent to pyranose rings) of sodium iodide in 50 mL of DMF was added, and the mixture was stirred under nitrogen at 85 °C for 24 hours (Scheme 3.2). The precipitate was collected and washed with acetone and ether. The obtained product was dried *in vacuo* to give a tan powder. The obtained product was characterized by FT-IR, EA, TGA and XRD.



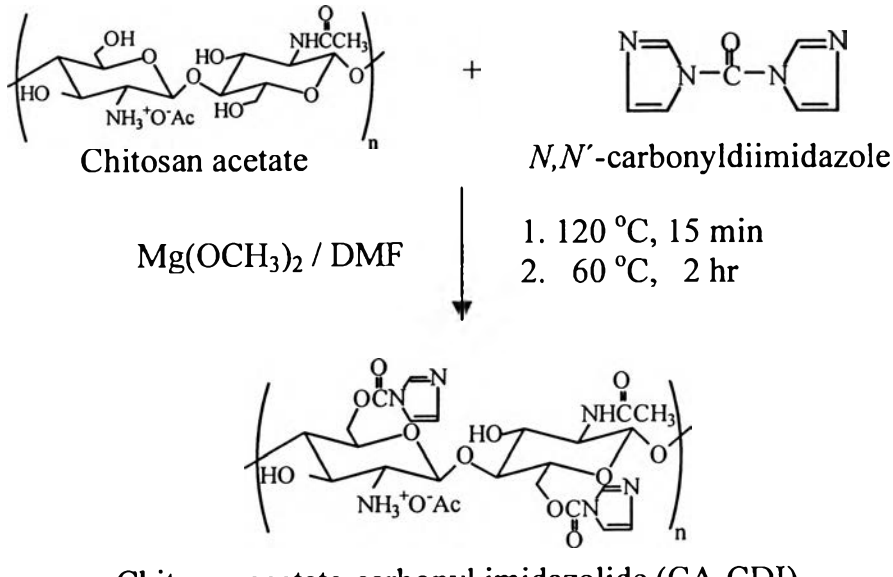
Scheme 3.2 Preparation of iodochitosan

3.3.1.3 Preparation of Chitosan acetate

Chitosan with a degree of deacetylation 75.8% was used as a starting material. The chitosan acetate was prepared by the same procedure as reported by Chunharotrit *et al.* (1998). The product was characterized by FT-IR, EA, TGA and XRD.

3.3.1.4 Preparation of Chitosan acetate-Carbonyl imidazolide (CA-CDI)

Chitosan acetate (1 g) was dispersed in *N,N*-dimethyl formamide (100 mL) under vacuum conditions and heated to 120 °C. A catalytic amount of magnesium methoxide was added to the solution. After 15 minutes, *N,N′*-carbonyldiimidazole (3.0 g, 4 mole equivalent to pyranose rings) was added and reacted at 120 °C for 15 minutes. The temperature was then reduced to 60 °C and stirring was continued for 2 hours (Scheme 3.3). After the reaction, the precipitate was washed thoroughly with methanol and chloroform and dried *in vacuo* to give a pale yellow powder. The obtained product was characterized by FT-IR, ^{13}C-NMR, EA, TGA and XRD.

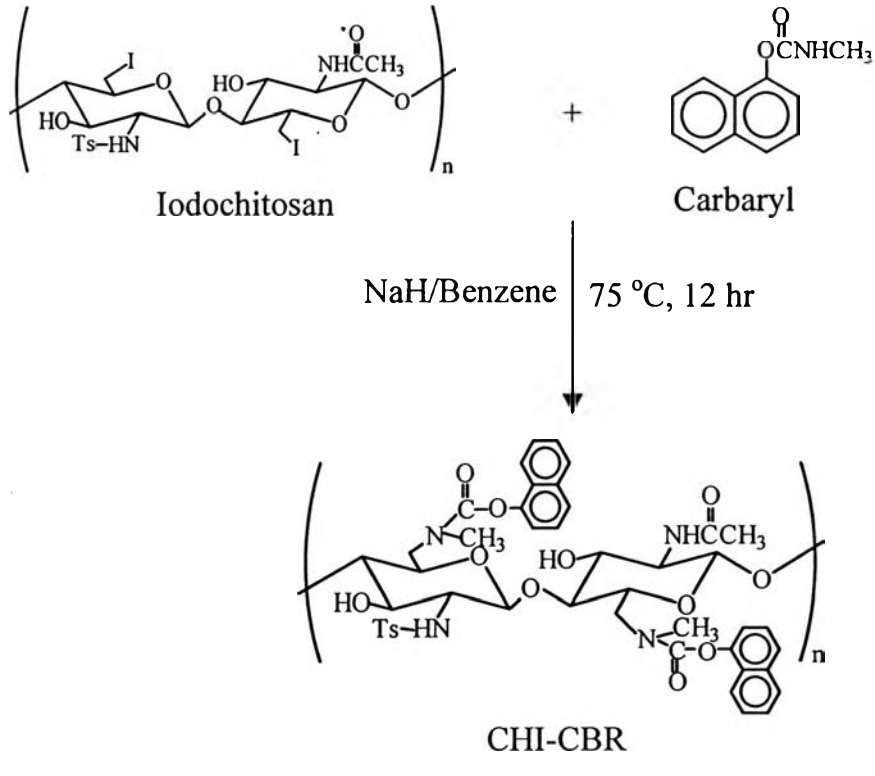


Scheme 3.3 Preparation of CA-CDI

3.3.2 Preparation of Chitosan Conjugated Drug

3.3.2.1 Synthesis of Chitosan Conjugated Drug Type 1: Chitosan-Carbaryl (CHI-CBR)

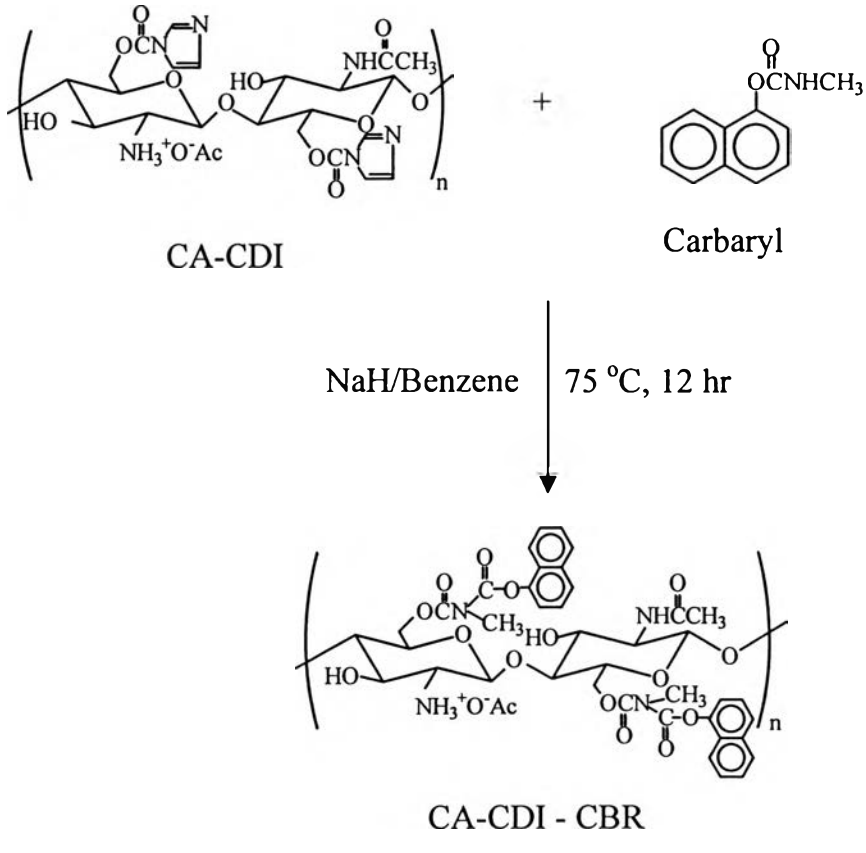
A solution of carbaryl 0.7 g (3 mole equivalent to pyranose rings) in absolute benzene 50 mL was cooled in an ice bath and stirred under vacuum for 15 minutes. Sodium hydride, 0.15 g (5 moles equivalent to pyranose rings), was added in the system and the reaction was proceeded under nitrogen atmosphere for 15 minutes. Iodochitosan 0.5 g was added and the ice bath was replaced by an oil bath. The mixture was reacted at 75 °C and refluxed under nitrogen atmosphere for 12 hours (Scheme 3.4). A brown precipitate was collected and washed thoroughly with ethanol and acetone. The product was characterized by FT-IR, TGA and XRD.



Scheme 3.4 Preparation of CHI-CBR (Type1)

3.3.2.2 Synthesis of Chitosan Conjugated Drug Type2 : Chitosan acetate-Carbonyl imidazolide-Carbaryl (CA-CDI-CBR)

A solution of carbaryl 0.6 g (3 mole equivalent to pyranose rings) in absolute benzene 25 mL was cooled in an ice bath and stirred under vacuum for 15 minutes. Sodium hydride, 0.12 g (5 moles equivalent to pyranose rings), was added and the reaction proceeded under nitrogen atmosphere for 15 minutes. CA-CDI 0.3 g was added after 15 minutes and the ice bath was replaced by an oil bath. The mixture was reacted at 75°C and refluxed under nitrogen atmosphere for 12 hours (Scheme 3.5). A brown precipitate was collected and washed thoroughly with ethanol and acetone. The product was characterized by FT-IR, TGA and XRD.



Scheme 3.5 Preparation of CA-CDI-CBR (Type 2)

3.3.3 Stability Study of Chitosan-Carbaryl (CHI-CBR) (Type 1)

Chitosan-carbaryl (0.005 g) was immersed in aq. NaOH 0.01 N (10 mL) for 2 hours at 60 °C. The solution was examined for trace degraded carbaryl by UV spectrophotometer, using carbaryl as a control.

3.3.4 Release Study of Chitosan acetate-Carbonyl-imidazolide-Carbaryl (CA-CDI-CBR) (Type 2)

Chitosan acetate-carbonyl imidazolide-carbaryl (0.005 g) was immersed in aq. NaOH 0.01 N (10 mL) for 0.5 hour at 60 °C. The release of the chitosan-conjugated drug was qualitatively analyzed by the UV-VIS spectrophotometer.