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"The Story of Immune System"Captain-Nitrogen. 18 February 2012. 2 August 2013 <http://captain-nitrogen.tumblr.com/post/17850004239/the-story-of-the-immune-system-warfare-at-the>

APPENDICES

Appendix A Supporting Synthesis for Chapter III

Synthesis of mPEG-COOH

The reaction was carried out as reported by Yoksan et al.(Yoksan *et al.*, 2003) In brief, mPEG (M_n = 5000 Da, 20 g, 4.0 mmol, 1.0 eq.) was reacted with succinic anhydride (0.48 g, 4.8 mmol, 1.2 eq.) overnight at 60°C in the presence of a catalytic amount of pyridine. The solution obtained was concentrated and precipitated in diethyl ether before drying *in vacuo* to obtain mPEG-COOH (20.15 g, 3.95 mmol, 98%).

mPEG-COOH: FT-IR (ZnSe, cm^{-1}); 3503 (OH), 2864 (C-H stretching), 1733 (C=O), and 1112 (C-O-C). ^{1}H NMR (δ, ppm); 2.56 ($COCH_2CH_2CO$) and 3.28 ppm for (CH_3).

In vitro cytotoxicity by MTT assay

After the cells were incubated with the products. 20 µL of MTT-solution (5 mg MTT/mL phosphate-buffered saline) (Invitrogen), was added to each well, followed by 4 h incubation in darkness. All wells were then aspirated, and 100 µL acid isopropanol (4% 1 mol/L HCl in 2-isopropanol) was added. The spectrophotometric absorbance at 570 nm was then measured using an ELx808 absorbance microplate reader (BioTek Instruments). The mean absorbance values corrected for a blank (media only) were calculated as percentages of control. The same procedures were repeated with the samples without cells as a blank. The experiments were run in triplicate (n=3).

Allergen detection by Elisa

The entrapped- and released- allergen contents were measured by collecting supernatant. 96-well plates were coated with the supernatant (50 µL/well) and incubated overnight at 4°C. Plates were blocked with 2% BSA in washing buffer and incubated 2 h at 37 °C. Duck polyclonal IgY was diluted (1:200), placed in wells, and incubated 2 h at 37 °C. (HRP)-conjugated duck polyclonal IgY was placed in wells, and incubated 2 h at 37 °C. Every time after incubating the mixture, the wells were decanted and washed with 400 µL of washing buffer, in total 4 times. To

observe the color of mixture, a 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution was added and incubated 1 h at 37°C before finishing the reaction with 50 µL 1 M H_2SO_4. The optical density at 450 nm was determined by Thermo Scientific Multiskan FC Microplate reader visible spectroscopy.

In vitro release

The *in vitro* releases of allergen were studied in model media, i.e. phosphate buffer saline (PBS, pH 7.4), citric acid/trisodium citate buffer (pH 5.2), and tris buffer (pH 8). The allergen-entrapped CS-Phe1.0-mPEG0.3 (1 mg) and media (1 mL) were placed in a microtube and incubated at 37°C. The incubated mixture was centrifuged before collecting the supernatant (200 µL) to determine the released allergen content. An equal volume of fresh media was added to the mixture, and the whole procedure was repeated for the subsequent sampling. The released allergen content was evaluated by the enzyme-linked immunosorbent assay or ELISA (see supporting experiment S3).

Appendix B Supporting Structural Characterization for Chapter III

Scheme B1. (A) water soluble chitosan coming from complexation between -NH_2 of chitosan and HOBt, (B) mechanism of ester linkage by using EDC conjugating agent.

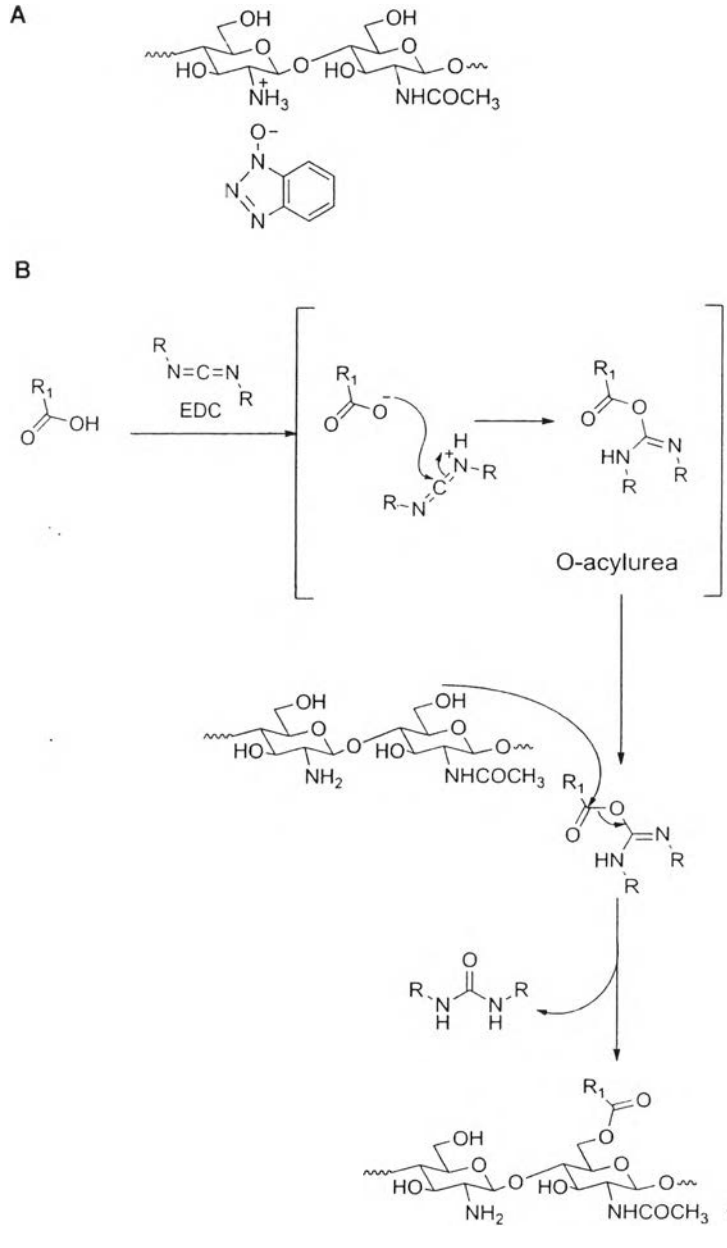
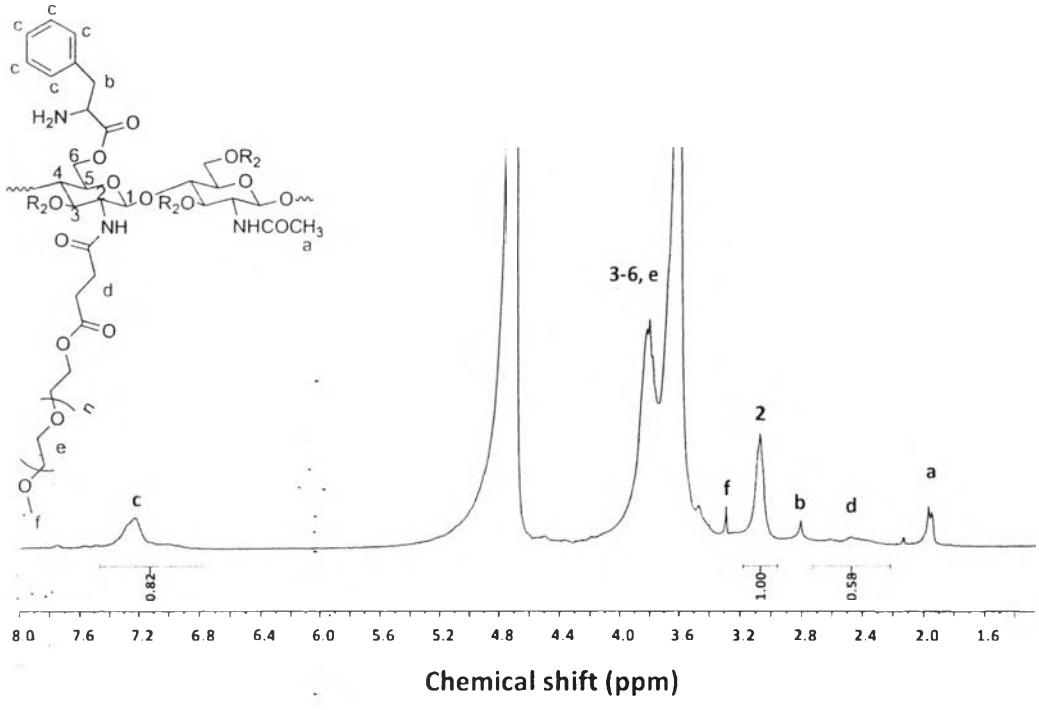


Table B1 Integral ratio of chitosan and chitosan derivatives by FT-IR curve fitting

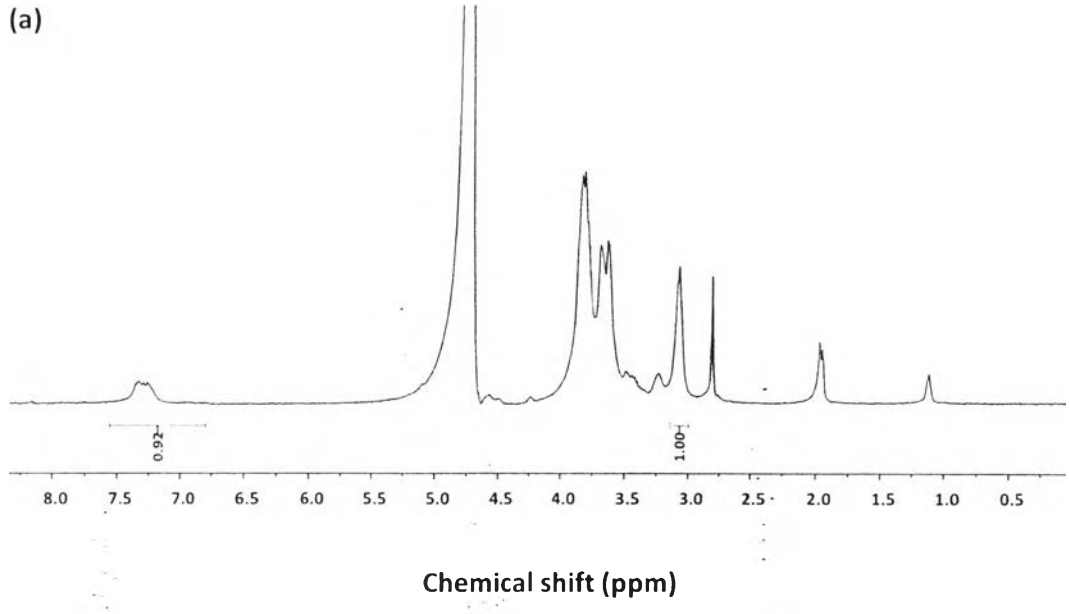
Sample	Integral ratio		
	Amine/std	AmideII/std	Ester/std
CS	12.2	0.0	0.0
CS-Phe1.0	3.8	3.4	0.7
CS-Phe3.0	3.5	1.2	0.2
1S-CS-Phe0.5-mPEG0.3	1.4	7.9	3.8
1S-CS-Phe1.0-mPEG0.3	1.4	7.4	3.2
1S-CS-Phe1.5-mPEG0.3	2.9	6.7	1.5
1S-CS-Phe2.0-mPEG0.3	5.2	5.1	1.4
1S-CS-Phe3.0-mPEG0.3	6.7	5.0	1.3
2S-CS-Phe1.0-mPEG0.3	1.6	7.2	2.9
2S-CS-Phe3.0-mPEG0.3	2.3	6.6	1.4
CS-mPEG0.3	1.6	4.6	2.2

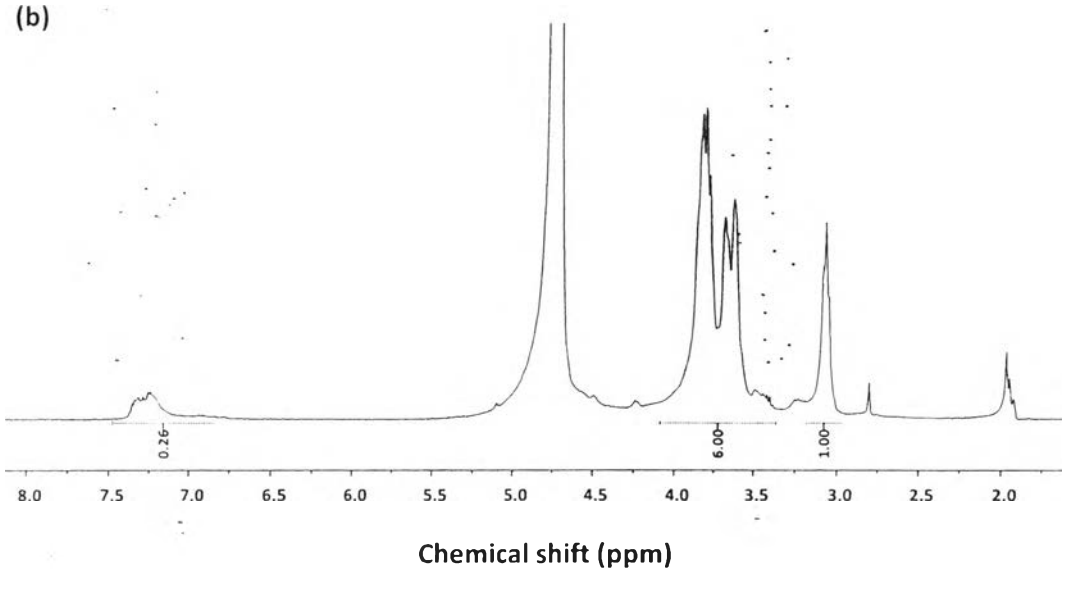


$$\%DS \text{ of Phe} = \frac{\text{Integration of c/5}}{\text{Integration of 2}} \times 100$$

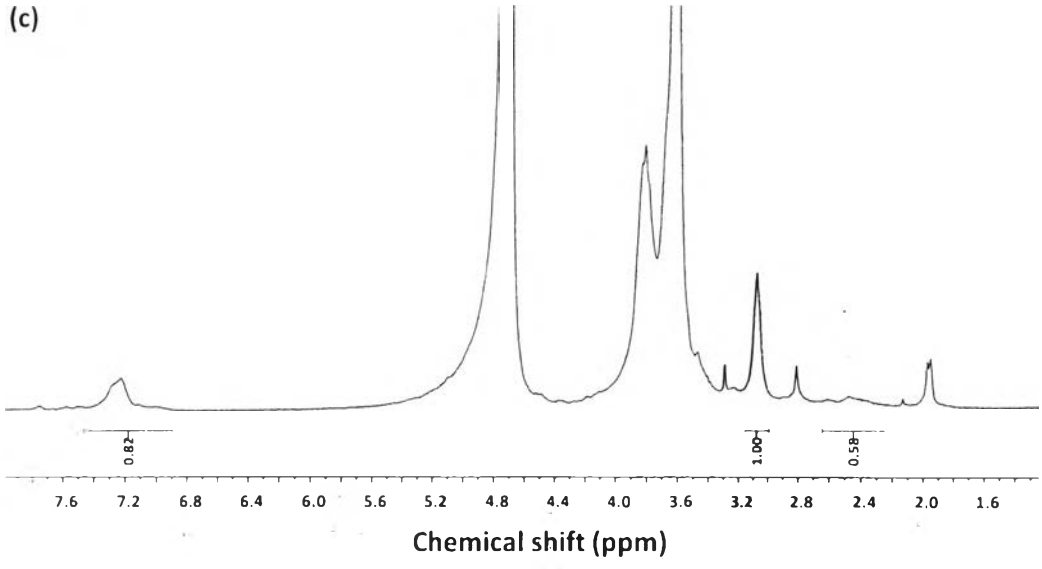
$$\%DS \text{ of mPEG} = \frac{\text{Integration of d/4}}{\text{Integration of 2}} \times 100$$

Figure B1. Determination of degree of substitution (%DS) of phenylalanine (Phe) and poly(ethylene glycol)methyl ether (mPEG) by ^{1}H NMR.

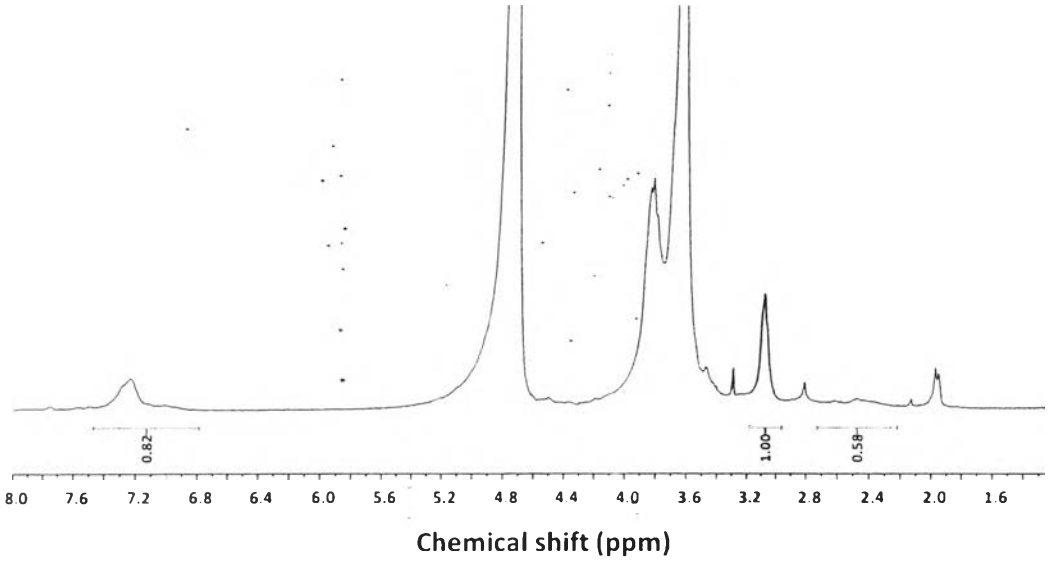

(a)
0.92
1.00
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
Chemical shift (ppm)


(b)
0.26
6.00
1.00
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0
Chemical shift (ppm)

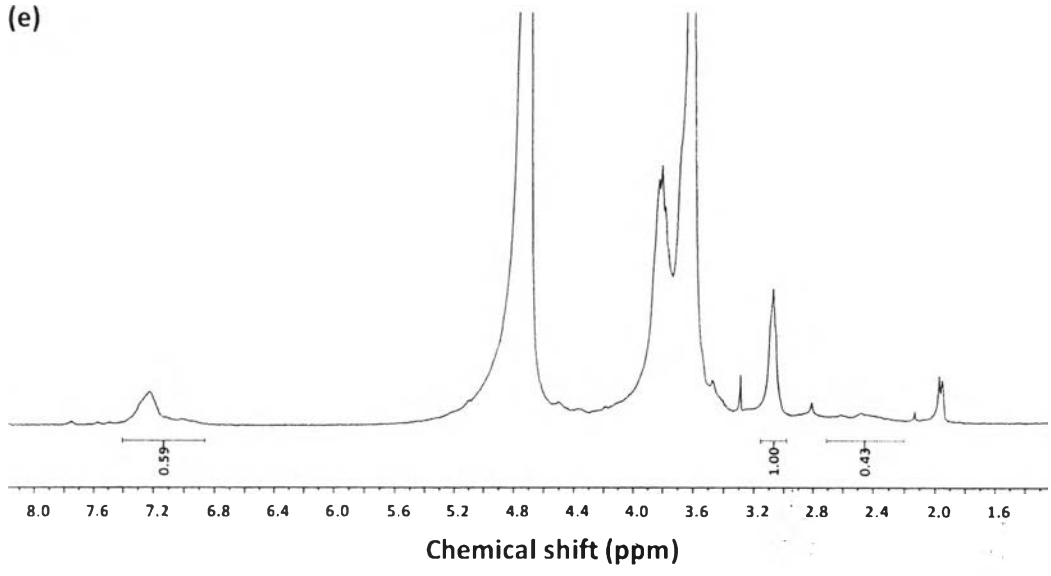
(c)


0.82
1.00
0.58
7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6
Chemical shift (ppm)

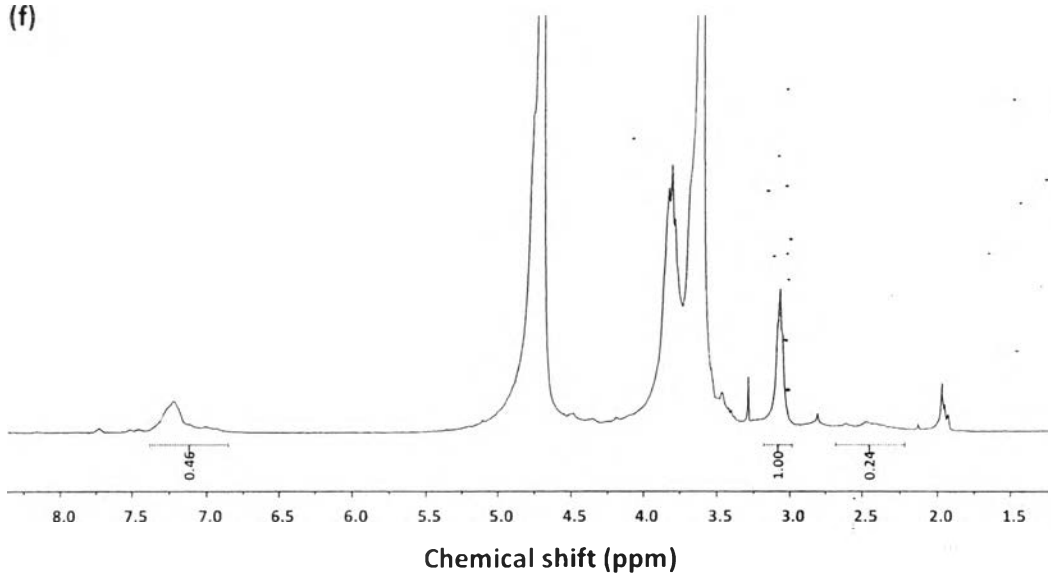
(d)


0.82
1.00
0.58
8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6
Chemical shift (ppm)

(e)


0.59
1.00
0.43
8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6
Chemical shift (ppm)

(f)


0.46
1.00
0.24
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
Chemical shift (ppm)

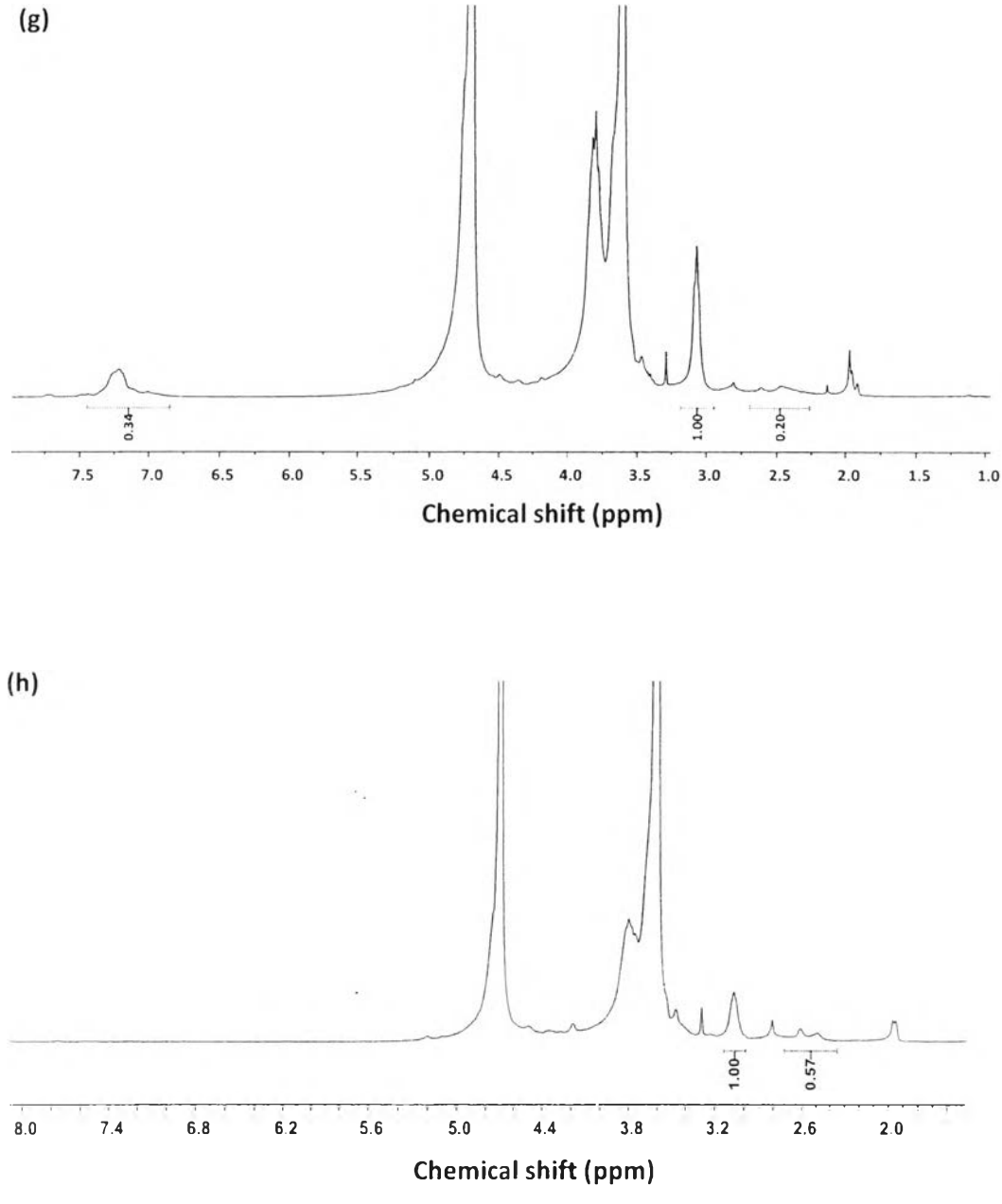


Figure B2. ^{1}H-NMR spectra of (a) CS-Phe1.0, (b) CS-Phe3.0, (c) 1S-CS-Phe0.5-mPEG0.3, (d) 1S-CS-Phe1.0-mPEG0.3, (e) 1S-CS-Phe1.5-mPEG0.3, (f) 1S-CS-Phe2.0-mPEG0.3, (g) 1S-CS-Phe3.0-mPEG0.3, (h) CS-mPEG0.3 in 2% CD_3COOD/D_2O.

Table B2 Degree of substitution (%DS) of Phe and mPEG-COOH of chitosan derivatives as identified by ^{1}H-NMR

Samples	%DS	
	Phe	mPEG
CS	-	-
CS-Phe1.0	17.9 ± 0.5	-
CS-Phe3.0	5.5 ± 0.4	-
1S-CS-Phe0.5-mPEG0.3	15.7 ± 1.1	13.8 ± 0.7
1S-CS-Phe1.0-mPEG0.3	15.2 ± 1.1	13.4 ± 0.7
1S-CS-Phe1.5-mPEG0.3	12.0 ± 0.7	9.1 ± 0.9
1S-CS-Phe2.0-mPEG0.3	9.0 ± 0.2	5.6 ± 0.9
1S-CS-Phe3.0-mPEG0.3	6.2 ± 0.5	4.7 ± 0.7
2S-CS-Phe1.0-mPEG0.3	16.9 ± 0.6	11.3 ± 0.3
2S-CS-Phe3.0-mPEG0.3	5.1 ± 0.1	13.2 ± 0.9
CS-mPEG0.3	-	14.9 ± 1.3

CS: Anal. Calcd for $(C_6H_{11}O_4N)_{0.92}(C_8H_{13}O_5N)_{0.08}$ (%): C, 44.97; H, 6.79; and N, 8.51. Found (%): C, 38.85; H, 67.73; and N, 7.35.

CS-Phe1.0: Anal. Calcd for $(C_6H_{11}O_4N)_{0.85}(C_8H_{13}O_5N)_{0.08}(C_{15}H_{20}O_5N_2)_{0.07}$ (%): C, 46.65; H, 7.26; and N, 8.58. Found (%): C, 41.95; H, 7.08; and N, 8.36.

CS-Phe3.0: Anal. Calcd for $(C_6H_{11}O_4N)_{0.90}(C_8H_{13}O_5N)_{0.08}(C_{15}H_{20}O_5N_2)_{0.02}$ (%): C, 45.48; H, 6.78; and N, 8.54. Found (%): C, 45.06; H, 6.74; and N, 8.46.

1S-CS-Phe0.5-mPEG0.3: Anal. Calcd for $(C_6H_{11}O_4N)_{0.86}(C_8H_{13}O_5N)_{0.05}(C_{15}H_{20}O_5N_2)_{0.06}$ $(C_{239}H_{471}O_{121}N_1)_{0.03}$ (%): C, 50.07; H, 7.77; and N, 4.70. Found (%): C, 44.50; H, 8.66; and N, 5.25.

1S-CS-Phe1.0-mPEG0.3: Anal. Calcd for $(C_6H_{11}O_4N)_{0.85}(C_8H_{13}O_5N)_{0.07}(C_{15}H_{20}O_5N_2)_{0.05}$ $(C_{239}H_{471}O_{121}N_1)_{0.03}$ (%): C, 59.89; H, 7.76; and N, 4.76. Found (%): C, 44.29; H, 8.78; and N, 5.38.

1S-CS-Phe1.5-mPEG0.3: Anal. Calcd for $(C_6H_{11}O_4N)_{0.88}(C_8H_{13}O_5N)_{0.07}(C_{15}H_{20}O_5N_2)_{0.03}(C_{239}H_{471}O_{121}N_1)_{0.02}$ (%): C, 49.40; H, 7.74; and N, 4.85. Found (%): C, 44.78; H, 8.02; and N, 5.03.

1S-CS-Phe2.0-mPEG0.3: Anal. Calcd for $(C_6H_{11}O_4N)_{0.89}(C_8H_{13}O_5N)_{0.07}(C_{15}H_{20}O_5N_2)_{0.02}(C_{239}H_{471}O_{121}N_1)_{0.02}$ (%): C, 49.07; H, 7.67; and N, 5.11. Found (%): C, 49.01; H, 7.66; and N, 5.09.

1S-CS-Phe3.0-mPEG0.3: Anal. Calcd for $(C_6H_{11}O_4N)_{0.90}(C_8H_{13}O_5N)_{0.07}(C_{15}H_{20}O_5N_2)_{0.01}(C_{239}H_{471}O_{121}N_1)_{0.02}$ (%): C, 48.65; H, 7.59; and N, 5.41. Found (%): C, 48.49; H, 7.54; and N, 5.41.

2S-CS-Phe1.0-mPEG0.3: Anal. Calcd for $(C_6H_{11}O_4N)_{0.83}(C_8H_{13}O_5N)_{0.08}(C_{15}H_{20}O_5N_2)_{0.06}$ $(C_{239}H_{471}O_{121}N_1)_{0.03}$ (%): C, 49.92; H, 7.73; and N, 4.86. Found (%): C, 49.92; H, 7.72; and N, 4.82.

2S-CS-Phe3.0-mPEG0.3: Anal. Calcd for $(C_6H_{11}O_4N)_{0.88}(C_8H_{13}O_5N)_{0.08}(C_{15}H_{20}O_5N_2)_{0.01}$ $(C_{239}H_{471}O_{121}N_1)_{0.03}$ (%): C, 49.67; H, 7.85; and N, 4.44. Found (%): C, 49.70; H, 7.84; and N, 4.46.

CS-mPEG0.05: Anal. Calcd for $(C_6H_{11}O_4N)_{0.91}(C_8H_{13}O_5N)_{0.08}(C_{239}H_{471}O_{121}N_1)_{0.01}$ (%): C, 47.23; H, 7.31; and N, 6.50. Found (%): C, 40.57; H, 7.69; and N, 6.94.

CS-mPEG0.3: Anal. Calcd for $(C_6H_{11}O_4N)_{0.88}(C_8H_{13}O_5N)_{0.08}$ $(C_{239}H_{471}O_{121}N_1)_{0.04}$ (%): C, 50.71; H, 8.14; and N, 3.34. Found (%): C, 46.61; H, 9.22; and N, 3.81.

Figure B3. Elemental Analysis of various types of chitosans.

Appendix C Supporting Evaluation of Morphology, Size, and ζ-potential

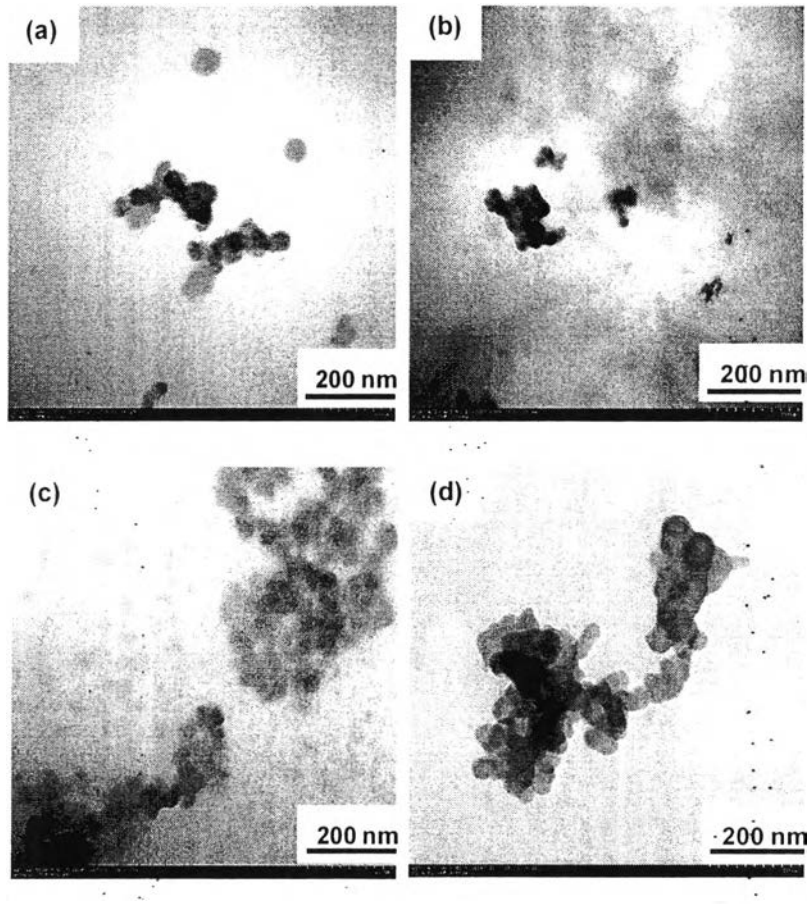


Figure C1. TEM micrographs of (a) 1S-CS-Phe0.5-mPEG0.3, (b) 1S-CS-Phe1.5-mPEG0.3, (c) 1S-CS-Phe2.0-mPEG0.3, and (d) 1S-CS-Phe3.0-mPEG0.3.

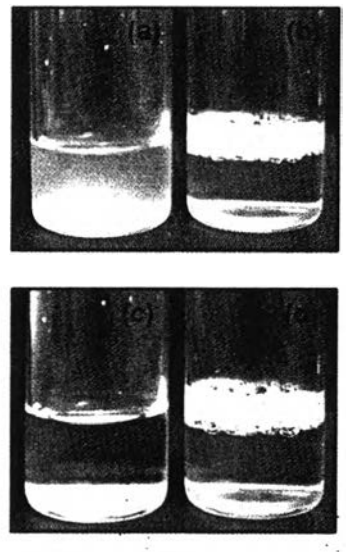
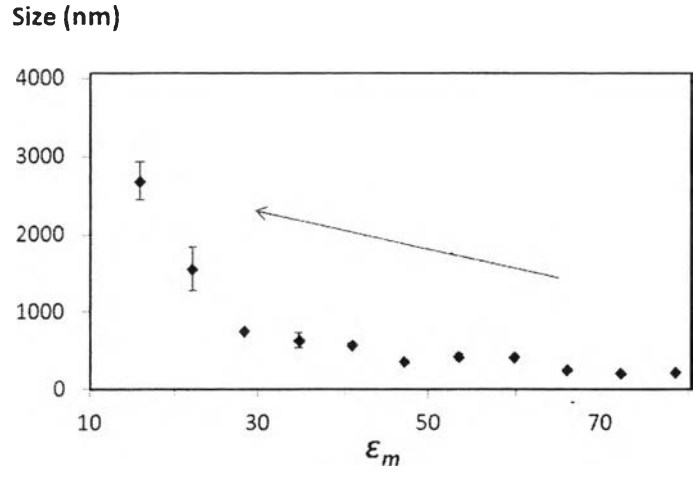


Figure C2. Appearances of CS after stirring (a) 0 day, (c) 1 day, and 1S-CS-Phe1.0-mPEG0.3 after stirring (b) 0 day, (d) 1 day. The samples were dispersed in deionized water with the concentration 1 mg/mL.

A



B

$$\varepsilon_m = \emptyset_1\varepsilon_1 + \emptyset_2\varepsilon_2$$

ε_m = dielectric constants of the binary mixture of solvents

ε_1 = dielectric constants of 2-propanol

ε_1 = dielectric constants water

$\emptyset_1$ = volume fraction of 2-propanol

$\emptyset_2$ = volume fraction of water

Figure C3. (A) size (determined by DLS) of IS-CS-Phe1.0-mPEG0.3 (concentration 1 mg/mL) in solvents with different dielectric constants, (B) determination of dielectric constants of the binary mixture of solvents (Jouyban *et al.*, 2004).

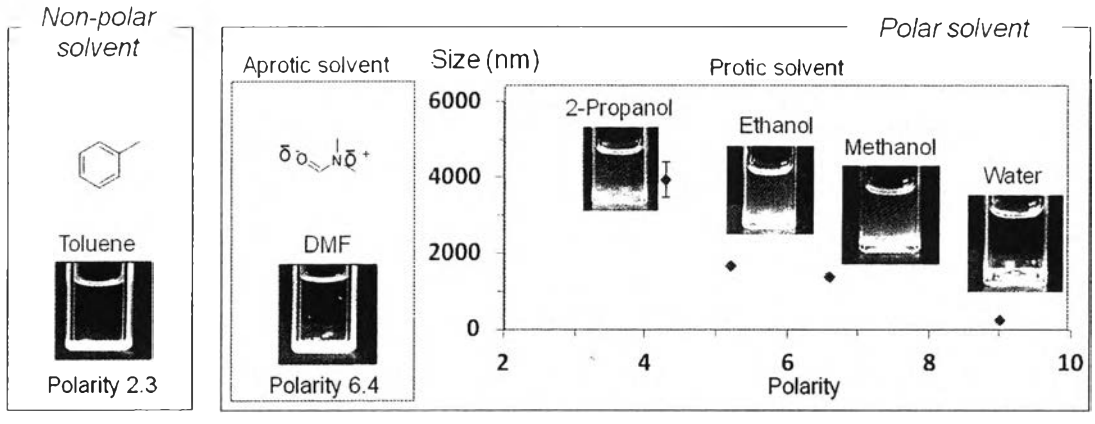


Figure C4. Appearance and average diameter size (determined by DLS) of 1S-CS-Phe1.0-mPEG0.3 in different types of solvents.

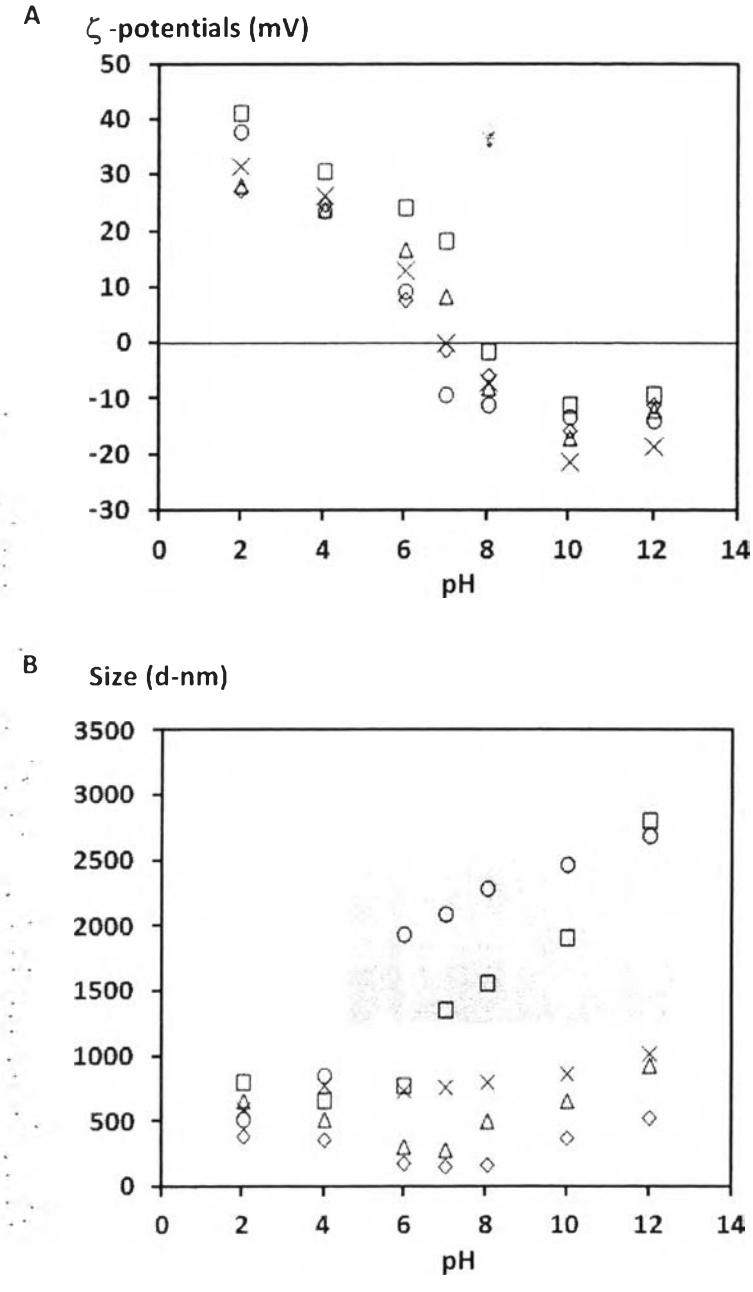


Figure C5. (A) ζ -potentials and (B) size of (○) CS, (□) CS-Phe1.0, (◇) CS-mPEG0.3, (Δ) 1S-CS-Phe1.0-mPEG0.3, and (×) 1S-CS-Phe3.0-mPEG0.3 at pH 2-12 (concentration of products: 1 mg/mL) in HCl/NaOH solution. Results are means ± SD (n=3).

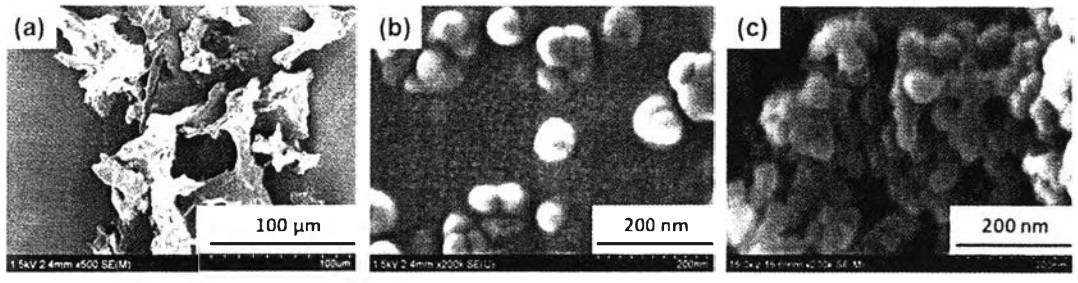


Figure C6. SEM micrographs of (a) CS, (b) 1S-CS-Phe1.0-mPEG0.3, and (c) 2S-CS-Phe1.0-mPEG0.3.

Appendix D Supporting Determination of Allergen Content

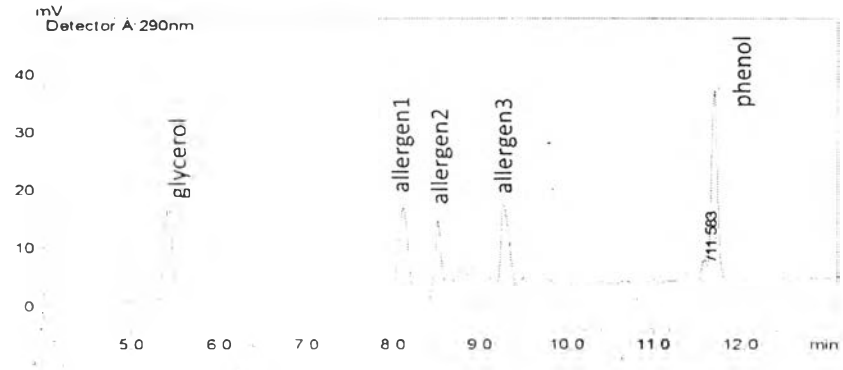


Figure D1. HPLC spectrum of allergen supernatant.

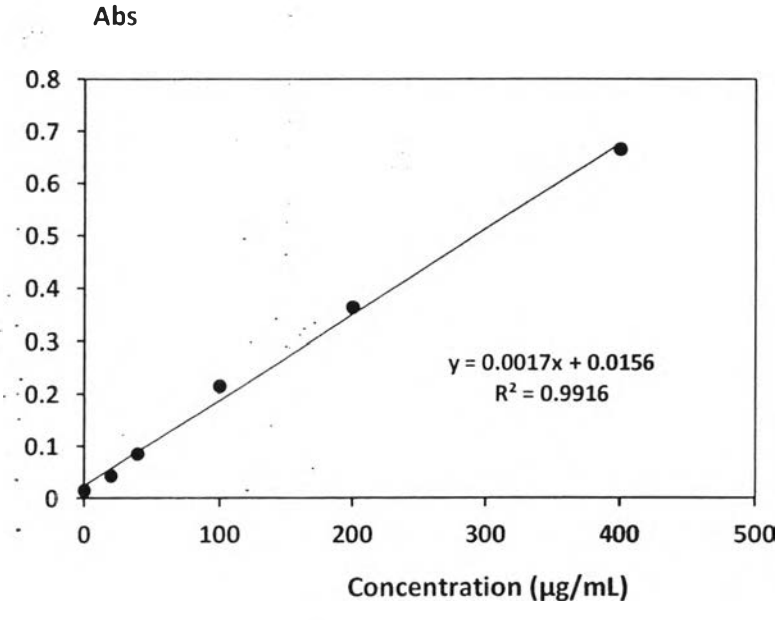


Figure D2. Standard curve of Bovine serum albumin (BSA) by Bradford Assay in order to determine the crude allergen concentration.

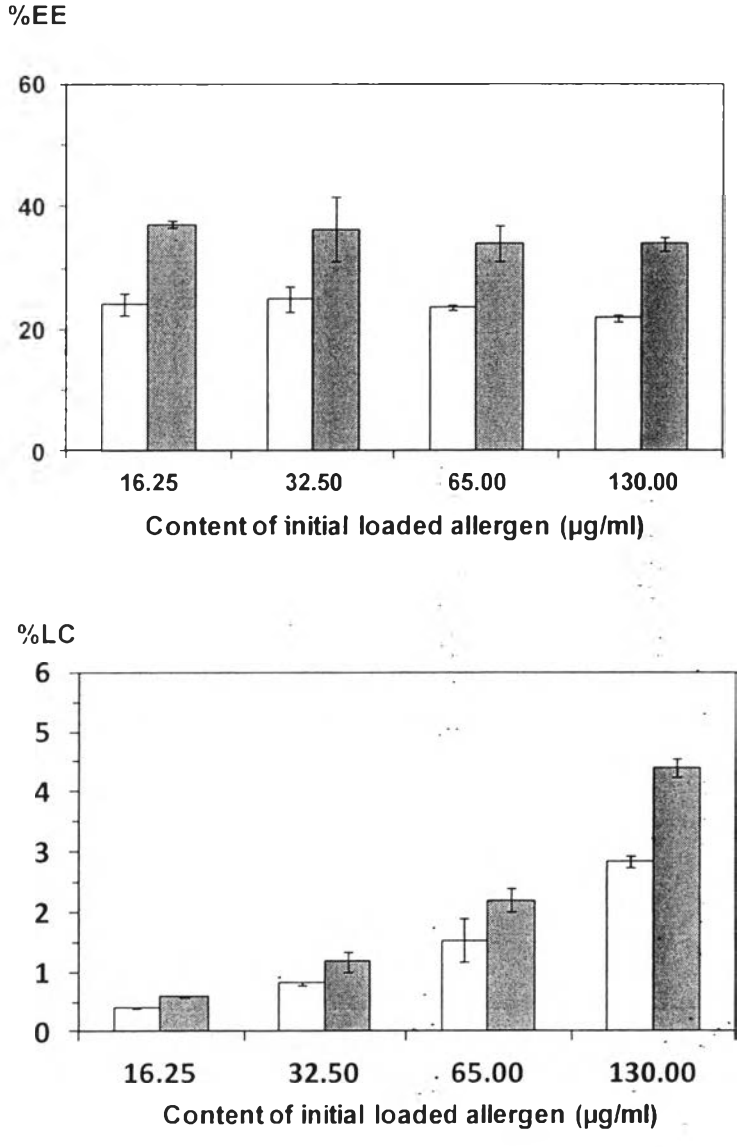


Figure D3. %EE and %LC of (▭) CS and (▭) 1S-CS-Phe1.0-mPEG0.3 (concentration 1 mg/mL) at different contents of initial loaded allergen in DI water adjusted to pH 6.0 by HCl/NaOH. The contents were determined by Elisa. Results are means ± SD (n=3).

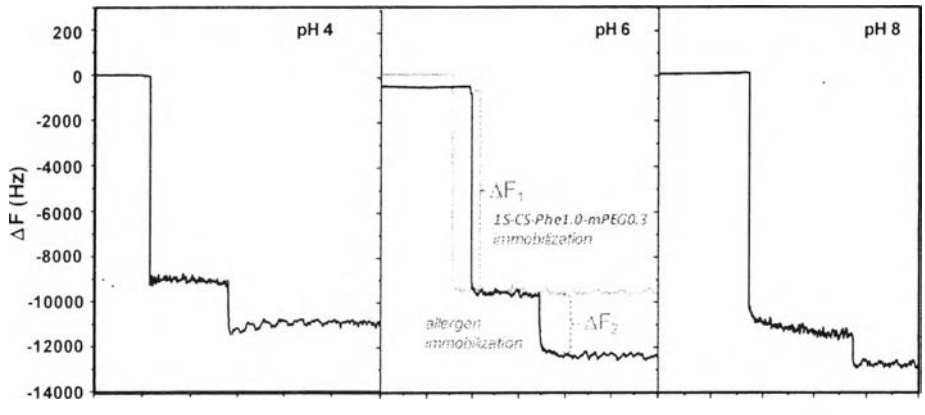
Scheme D1 (A) Sauebrey equation, eq. 3, (Huang *et al.*, 2000; Nishino *et al.*, 2004) for analysis of immobilized mass per surface area (ΔM), and (B) QCM spectra and calculation of immobilized 1S-CS-Phe1.0-mPEG0.3, and immobilized allergen including allergen immobilization equation.

A

$$\Delta F = \frac{-2 F_0^2}{A(\rho_q \mu_q)^{1/2}} \Delta M \qquad \text{----- (3)}$$

ΔF = Frequency change (Hz)
F_0 = Fundamental frequency (Hz)
A = Surface area of the gold disk
μ_q = Shear modulus of quartz
ρ_q = Density of quartz
ΔM = Immobilized mass per surface area

B



At pH 6

$$\Delta F_1 = \frac{-2 F_0^2}{A(\rho_q \mu_q)^{1/2}} \Delta M_1$$

$$-9115 = \frac{-2 (27^2)}{0.071(2.648)^{1/2} (2.947 \times 10^{11})^{1/2}} \Delta M_1$$

$$\Delta M_1 = 0.394 \ \mu g$$

$$\Delta F_2 = \frac{-2 F_0^2}{A(\rho_q \mu_q)^{1/2}} \Delta M_2$$

$$-2837 = \frac{-2 (27^2)}{0.071(2.648)^{1/2} (2.947 \times 10^{11})^{1/2}} \Delta M_2$$

$$\Delta M_2 = 0.122 \ \mu g$$

$$\text{Allergen immobilization efficiency (\%)} = \frac{\Delta M_2}{\Delta M_1} \times 100 \qquad \text{---- (4)}$$

$$= \frac{0.122}{0.394} \times 100$$

$$= 30.99$$

Appendix E Supporting Evaluation of In Vitro Cytotoxicity

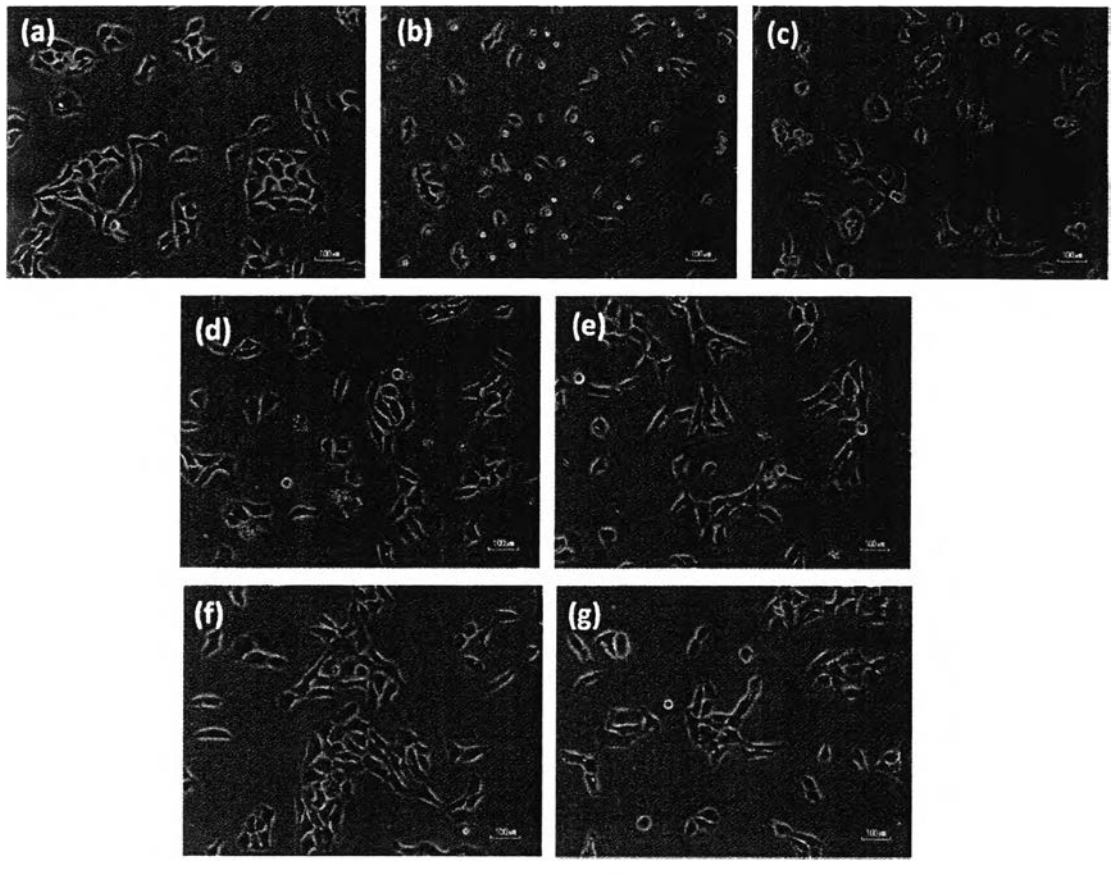


Figure E1. Optical micrographs of HaCaT cells after incubation for 24 h at 37 °C with different samples; (a) control, (b) DMSO, (c) HDM-allergen, (d) CS, (e) HDM-allergen-entrapped CS, (f) CS-Phe-mPEG, HDM-allergen-entrapped CS-Phe-mPEG.

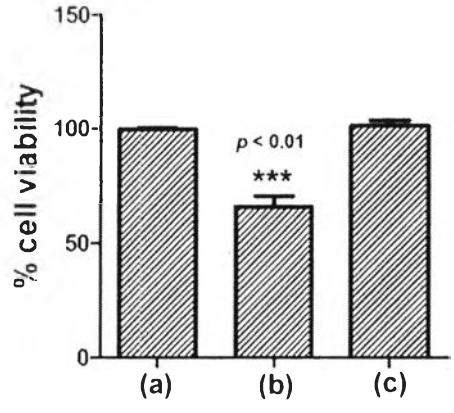


Figure E2. Cell viability of PBMC determined by Alarmar blue in the presence of (a) no treatment, (b) HDM-allergen, and (c) HDM-allergen-entrapped CS-Phe-mPEG incubated for 5 day.

Appendix F Supporting Evaluation of Allergen Release Profile

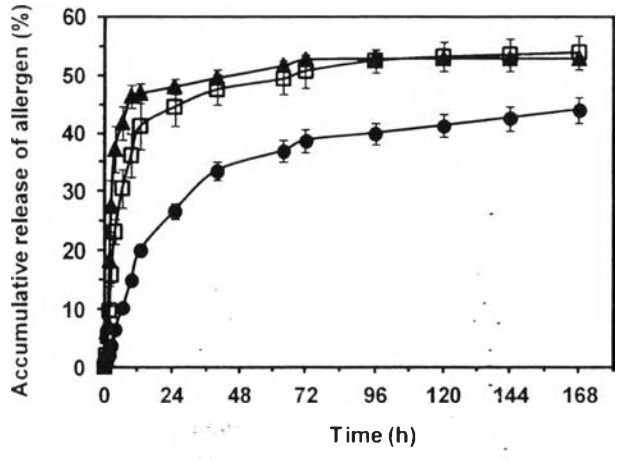


Figure F1. Release profiles of HDM-allergen-entrapped CS-Phe-mPEG in (●) Citric buffer (pH 5.2), (□) PBS buffer (pH 7.4), and (▲) Tris buffer (pH 8.1). The allergen content was determined by Elisa. Results are means ± SD (n=3).

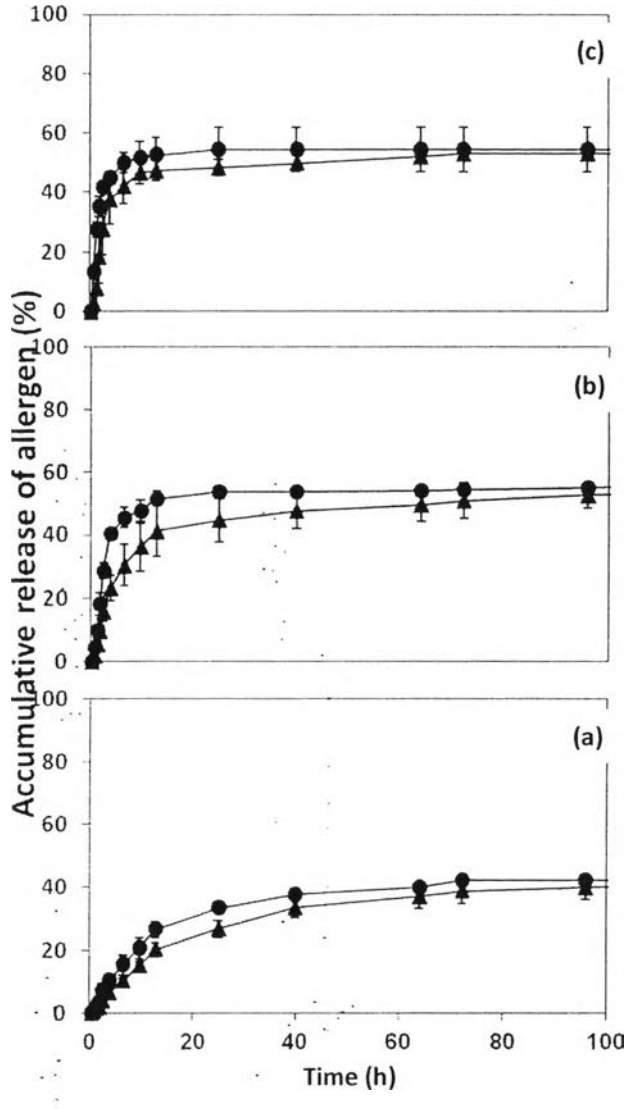


Figure F2. Release profile of (●) allergen-entrapped CS and (▲) allergen-entrapped 1S-CS-Phe1.0-mPEG0.3 in (a) Citric buffer pH 5.2, (b) PBS buffer pH 7.4, (c) Tris buffer pH 8.1. The allergen content was determined by Elisa. Results are means ± SD (n=3).

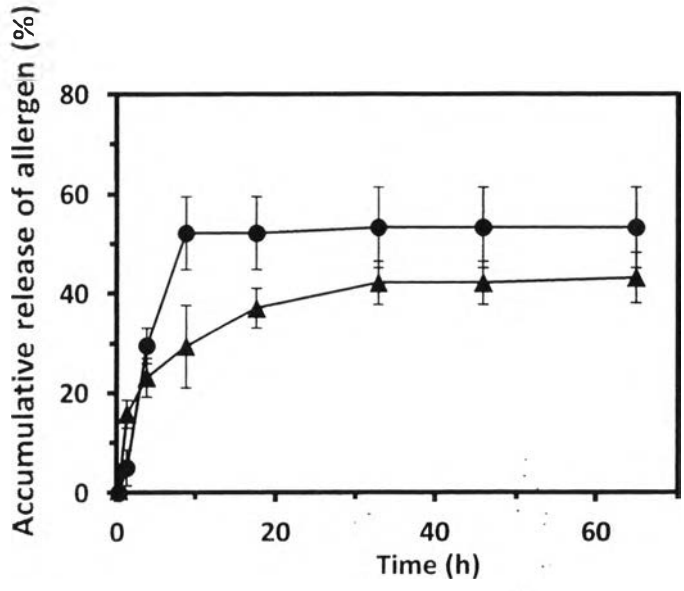
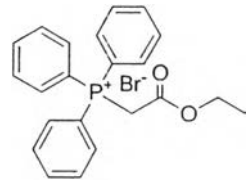


Figure F3. Accumulative release allergen of (●) HDM-allergen-entrapped CS, (▲) HDM-allergen-entrapped CS-Phe-mPEG in DI water. The allergen content was determined by HPLC. Results are means ± SD (n=3).

Appendix G Supporting Synthesis for Chapter IV

Oxanorbornadiene derivatives (1, 2, 3) were synthesized based on the protocols of Rutjes et al(van Berkel et al., 2007; van Berkel et al., 2008) and Dräger et al(Su *et al.*, 2010), briefly

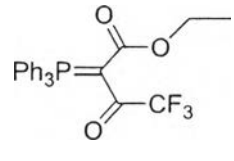
(2-Ethoxy-2-oxoethyl)triphenylphosphonium bromide, a



A mixture of ethyl 2-bromoacetate 30.2 mL (272.9 mmol, 1.1 eq.), triphenylphosphine (65 g, 248.1 mmol, 1 eq.), and catalytic amount of KI in anhydrous toluene (500 mL) was stirred at room temperature for 2 days. After filtration, the solid was washed with toluene until colorless solid was observed. The desired product a was obtained after the solid was dried in vacuo (111.6 g, 261.3 mmol, in quantitative yield).

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.72-7.67 (m, 15H, Ar-H), 3.98-3.92 (q, 2H, CH_2), 0.90-0.87 (t, 3H, CH_3) ppm.

Ethyl 4,4,4-trifluoro-3-oxo-2-(triphenylphosphoranylidene)butanoate, b

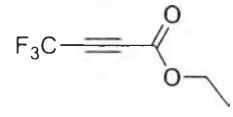


a (100 g, 234.2 mmol, 1 eq.) was dispersed in 250 mL anhydrous THF. The slurry was cooled to 0 °C, 71.3 mL triethylamine was added and stirred for 20 min. Then, trifluoroacetic anhydride 35.8 mL (257.6 mmol, 1.1 eq.) was added dropwise to the reaction mixture and continually stirred for 1 h. The mixture was filtrated and the precipitate was washed with cold THF (three times). The filtrate was

concentrated in vacuo to obtain a yellow viscous liquid. Then, cold water was added and strongly shaken to provide a light yellow crystalline compound b. After filtration, compound b was dried in vacuo (99.6 g, 224.4 mmol, 99.8 %).

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.79-7.68 (m, 15H, Ar-H), 3.82-3.76 (q, 2H, CH_2), 0.9-0.87 (t, 3H, CH_3) ppm.

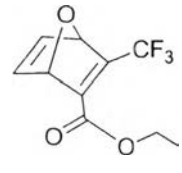
Ethyl 4,4,4-trifluorobut-2-ynoate, c



b (99.6 g, 224.3 mmol, 1 eq.) was heated to 180-230 °C at low pressure (~ 15 mbar) for 1 h. c product was collected at -70 °C as a dark yellow liquid (18.5 g, 111.1 mmol, 49.6 %).

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 4.32-4.26 (q, 2H, CH_2), 1.42-1.35 (t, 3H, CH_3) ppm.

(1S,4R)-Ethyl 3-(trifluoromethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2-carboxylate, d

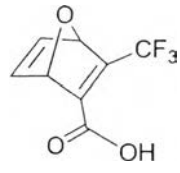


c (8.2 g, 49.5 mmol, 1 eq.) was mixed with furan 4.3 mL (4.0 g, 59.3 mmol, 1.2 eq.). The mixture was heated under microwave irradiation at 60 °C for 30 min. After the compound was concentrated in vacuo, the crude was purified by column chromatography (mobile phase: petroleum ether (PE) and ethylacetate (EtOAc) (10:1)). The resulted compound was concentrated in vacuo to provide the desirable product d as dark red viscous liquid.

R_F: 0.60

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.34-7.30 (q, 1H), 7.25-7.21 (q, 1H), 5.75-5.71 (m, 1H), 5.70-5.68 (t, 1H), 4.34-4.27 (m, 2H), 1.33-1.31 (t, 3H) ppm.

3-Trifluoromethyl-7-oxa-bicyclo[2.2.1] hepta-2, 5-diene-2-carboxylic acid, 1

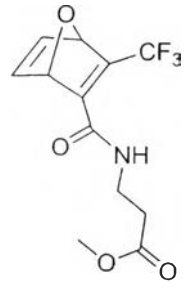


d (1.9 g, 8.1 mmol, 1 eq.) was dissolved in solvent mixture of THF (25 mL) and water (75 mL). After reaction was cooled to 0 °C, LiOH solution (20 mL, 1M) was added to obtain a pH of 8-9 and stirred for 1 h. Then, the reaction was stirred at room temperature for 2 h. The formation of 1 was traced by TLC (petroleum ether (PE)/ethylacetate (EtOAc) 10:1). To extract unreacted d, EtOAc was added, the aqueous layer was separated and acidified to pH 2-3 by HCl solution (1 M). The acidified solution was extracted by EtOAc (3×). The organic phase was collected and dried over $MgSO_4$. After drying in vacuo, 1 was obtained as a white solid (1.3 mg, 6.2 mmol, 77%).

R_F: 0.00

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.37-7.33 (q, 1H), 7.28-7.24 (q, 1H), 5.80-5.76 (m, 1H), 5.75-5.73 (t, 1H) ppm.

Methyl 3-((1S,4R)-3-(trifluoromethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2-carboxamido)propanoate, 1a

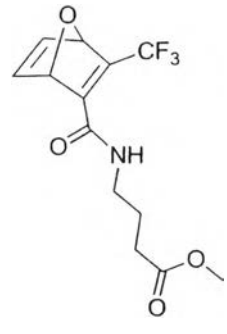


1 (1.0 g, 4.9 mmol, 1 eq.) was dissolved in dichloromethane (10 mL) and cooled to 0 °C. Then β-alanine methyl ester hydrochloride (0.8 g, 5.8 mmol, 1.2 eq.), DMAP (0.9 g, 7.2 mmol, 1.5 eq.), and EDC·HCl (1.1 g, 5.8 mmol, 1.2 eq.) were added and stirred for 1 h at 0 °C and additionally at room temperature overnight. The reaction progress was monitored via TLC. After the reaction finished, saturated NaCl solution was added, the aqueous layer was separated, extracted by dichloromethane (3×), and dried over $MgSO_4$. The crude product 1a was purified by TLC (PE:EE 3:2). Finally, the extracted product was dried in vacuo to obtain 1a (0.7 mg, 2.5 mmol, 52%) as a green viscous liquid.

R_F: 0.55

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.33-7.29 (q, 1H), 7.24-7.20 (q, 1H), 5.67-5.65 (t, 1H), 5.56-5.53 (m, 1H), 3.67 (s, 3H), 3.32-3.29 (m, 2H), 2.60-2.54 (t, 2H) ppm.

Methyl4-((1S,4R)-3-(trifluoromethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2-carboxamido)butanoate, 1b

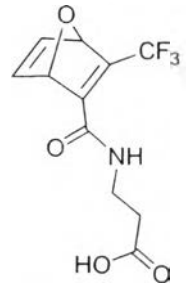


1b was obtained from oxanorbornadienyl carboxylic acid 1 and 4-aminobutanoate in 60% yield according to the procedure as described in 1a.

R_F: 0.55

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.33-7.29 (q, 1H), 7.24-7.20 (q, 1H), 5.67-5.65 (t, 1H), 5.56-5.53 (m, 1H), 3.67 (s, 3H), 3.32-3.29 (m, 2H), 2.60-2.54 (t, 2H), 2.08-2.04 (m, 2H) ppm.

3-((1S,4R)-3-(Trifluoromethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2-carboxamido)propanoic acid, 2

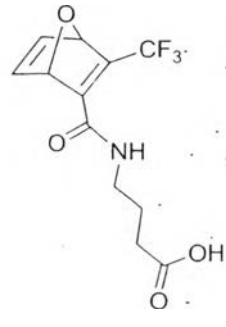


2 was obtained from acidification of 1a with a 78% yield according to the procedure as described in 1.

R_f: 0.00

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.33-7.29 (q, 1H), 7.24-7.20 (q, 1H), 5.67-5.65 (t, 1H), 5.56-5.53 (m, 1H), 3.32-3.29 (m, 2H), 2.60-2.54 (t, 2H) ppm.

4-((1S,4R)-3-(trifluoromethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2-carboxamido)butanoic acid, 2b

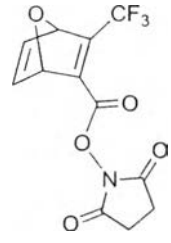


2a was obtained from acidification of 1b in 75 % yield according to the procedure as described in 1.

R_F: 0.00

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.33-7.29 (q, 1H), 7.24-7.20 (q, 1H), 5.67-5.65 (t, 1H), 5.56-5.53 (m, 1H), 3.32-3.29 (m, 2H), 2.60-2.54 (t, 2H), 2.08-2.04 (m, 2H) ppm.

1-((1S,4R)-3-Trifluoromethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2-carbonyl)pyrrolidine-2,5-dione, 3a

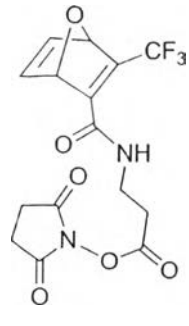


1 (0.1 g, 0.48 mmol, 1 eq.) was dissolved in dichloromethane and cooled to 0 °C. DCC (0.12 g, 0.58 mmol, 1.2 eq.) and NHS (0.67 g, 0.58 mmol, 1.2 eq.) were added to and stirred for 1 h. The reaction was continually stirred at room temperature overnight. The reaction progress was monitored via TLC. After finishing the reaction, the precipitate was filtered. The solvent of filtrate was removed under vacuo and purified by column chromatography (PE:EE 2:3). After drying in vacuo, 3a was obtained (0.89 g, 0.29 mmol, 60%).

R_F: 0.80

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.36-7.35 (q, 1H), 7.27-7.26 (q, 1H), 5.90-5.88 (m, 1H), 5.76-5.75 (m, 1H), 2.90-2.87 (m, 2×2H) ppm.

2,5-Dioxopyrrolidin-1-yl 3-((1S,4R)-3-(trifluoromethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2-carboxamido)propanoate, 3b

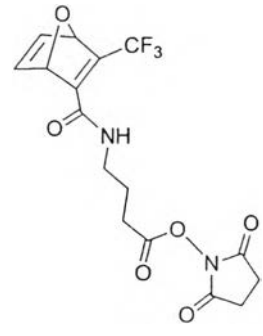


3b was obtained from oxanorbornadienyl propanoic acid 1a and NHS in 95% yield according to the procedure as described in 3a.

R_F: 0.80

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.33-7.29 (q, 1H), 7.24-7.20 (q, 1H), 5.67-5.65 (t, 1H), 5.56-5.53 (m, 1H), 3.32-3.29 (m, 2H), 2.60-2.54 (t, 2H), 2.90-2.87 (m, 2×2H) ppm.

2,5-Dioxopyrrolidin-1-yl 4-((1S,4R)-3-(trifluoromethyl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2-carboxamido)butanoate, 3

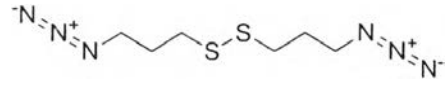


3 was obtained from oxanorbornadienyl propanoic acid 1b and NHS in 60% yield according to the procedure as described in 3a.

R_F: 0.80

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.33-7.29 (q, 1H), 7.24-7.20 (q, 1H), 5.67-5.65 (t, 1H), 5.56-5.53 (m, 1H), 3.32-3.29 (m, 2H), 2.60-2.54 (t, 2H), 2.08-2.04 (m, 2H), 2.90-2.87 (m, 2×2H) ppm.

1,2-bis(3-Azidopropyl)disulfane, 5



3-Chloropropane-1-thiol (0.5 g, 4.5 mmol, 1 eq.) and sodium azide (0.4 g, 5.5 mmol, 1.2 eq.) were dissolved in DMF 20 mL, and stirred overnight at 110 °C. The color of solution was changed to yellow. After cooling down, water was added to dissolve excess of sodium azide. The crude product was extracted with diethyl ether (3×) and alternately washed by adding water. Then the organic phase was

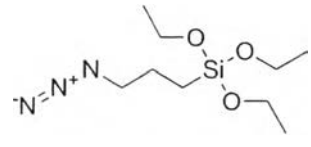
collected and dried over $MgSO_4$, and the solvent was removed at 40 °C 700-800 mbar to obtain 5.

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 3.45-3.40 (t, 2×2H), 2.78-2.70 (t, 2×2H), 2.03-1.91 (m, 2×2H) ppm.

FT-IR: (KBr, cm^{-1}): 2915 (C-H, antisym stretching), 2095 ($N=N^+=N^-$, antisym stretching).

ESI : Calcd $C_6H_{12}N_6S_2$: 232.06; found 232.00

(3-Azidopropyl)triethoxysilane, 6



(3-Chloropropyl)triethoxysilane (0.4 g, 1.7 mmol, 1 eq.) and sodium azide (1.3 g, 2.0 mmol, 1.2 eq.) were dissolved in DMF 20 mL, and stirred overnight at 80 °C. After cooling down, the precipitate was filtered. Then, the filtrate was washed with water and extracted by diethyl ether. The organic phase of diethyl ether was collected and dried over $MgSO_4$, and the solvent was removed at 40 °C 700-800 mbar to obtain 6.

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 3.85-3.80 (q, 3×2H), 3.28-3.25 (t, 2H), 1.75-1.68 (m, 2H), 1.24-1.22 (t, 3×3H), 0.69-0.66 (t, 2H) ppm.

FT-IR: (KBr, cm^{-1}): 2974-2884 (C-H, antisym and sym stretching), 2093 ($N=N^+=N^-$, antisym stretching), 1077 (Si-O, antisym strething).

Chitosan functionalized 1 (CS-1)

Chitosan CS (10.0 mg, 0.06 mmol, 1 eq., MW 15 kDa) and HOBt (9.8 mg, 0.07 mmol, 1.2 eq.) were dissolved in water 10 mL. Then, CS-HOBt solution was added to 1 (37.5 mg, 0.18 mmol, 3 eq.) which was dissolved in the mixture of THF (2.5 mL) and water (7.5 mL). Then, EDC·HCl (34.9 mg, 0.18 mmol, 3 eq.) and DMAP (22.2 mg, 0.18 mmol, 3 eq.) were added to the mixture. The reaction was cooled to 0 °C and stirred overnight at room temperature. The mixture was purified

by dialysis against NaCl solution followed by DI water for 3 days. After lyophilisation. CS-1 was obtained.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37-7.33 (m, 2H), 5.79-5.77(m, 1H), 5.72-5.71 (m, 1H), 3.91-3.50(m, 5H from pyranose ring), 3.16-3.12(m, 1H from pyranose ring), 2.07-2.05 (m, 3H) ppm.

Chitosan functionalized 2 (CS-2)

CS-2 was obtained from 2 and CS according to the procedure as described in *CS-1*.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37-7.33 (m, 2H), 5.85-5.84(m, 1H), 5.72-5.71 (m, 1H), 3.95-3.68(m, 5H from pyranose ring), 3.51-3.48 (m, 2H), 3.20-3.17(m, 1H from pyranose ring), 2.59-2.54 (m, 2H), 2.06-2.04 (m, 3H) ppm.

Chitosan functionalized 3 (CS-3)

CS (10.0 mg, 0.06 mmol, 1 eq., MW 15 kDa) and HOBt (9.8 mg, 0.07 mmol, 1.2 eq.) were dissolved in water 10 mL. Then, CS-HOBt solution was added in to the 3 (70.6 mg, 0.18 mmol, 3 eq.) which was dissolved in the mixture of THF (2.5 mL) and water (7.5 mL). Additionally, diisopropylethylamine (DIPEA) (23.5 mg, 0.18 mmol, 3 eq.) were added to the mixture. The reaction was cooled to 0 °C and stirred overnight at room temperature. The mixture was purified by dialysis against NaCl solution followed by DI water for 3 days. After lyophilisation, CS-3 was obtained.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37-7.33 (m, 2H), 5.85-5.84(m, 1H), 5.72-5.71 (m, 1H), 3.89-3.62(m, 5H from pyranose ring), 3.31-3.29 (m, 2H), 3.17-3.13(m, 1H from pyranose ring), 2.34-2.31(m, 2H), 2.06-2.04 (m, 3H), 1.85-1.82 (m, 2H) ppm.

Chitosan functionalized A (CS-4)

CS-3 (2 mg, 0.0045 mmol, 1 eq.) was dispersed in 2 % CD_3COOD/D_2O (1 mL). Then 4 0.65 µl (0.65 mg, 0.0045 mmol, 1 eq.) was added in to the mixture. The ligation progress over time was monitored by ^{1}H-NMR.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.42-7.40 (m, 2H), 6.36-6.34 (m, 2H), 3.86-3.55 (m, 5H from pyranose ring), 3.23-3.18 (m, 2H), 2.30-2.26(m, 2H), 1.94-1.91 (m, 2H), 1.53-1.50 (m, 2H) ppm.

Chitosan functionalized 5 (CS-5)

CS-3 (5 mg, 0.01 mmol, 1 eq.) was dispersed in 2 % CD_3COOD/D_2O (1 mL). Then 5 1.8 µl (1.3 mg, 0.01 mmol, 1 eq.) was added in to the mixture. The ligation progress over time was monitored by ^{1}H-NMR.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.46-7.44 (m, 2H), 6.40-6.38 (m, 2H), 3.88-3.57 (m, 5H from pyranose ring), 3.40-3.32 (m, 2H), 3.17-3.13(m, 1H from pyranose ring), 2.75-2.68(m, 2H), 1.96-1.92 (m, 3H) ppm.

Chitosan functionalized 4 (CS-6)

CS-3 (2 mg, 0.0045 mmol, 1 eq.) was dispersed in 2 % CD_3COOD/D_2O (1 mL). Then 6 (1.12 mg, 0.0045 mmol, 1 eq.) was added in to the mixture. The ligation progress over time was monitored by ^{1}H-NMR.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.44-7.42 (m, 2H), 6.38-6.36 (m, 2H), 3.87-3.58 (m, 5H from pyranose ring), 3.54-3.51 (m, 3×2H), 3.23-3.17 (m, 2H), 1.60-1.55 (m, 2H), 1.07-1.04 (m, 3×3H), 0.60-0.58 (m, 2H) ppm.

Appendix H Supporting Structural Characterization for Chapter IV

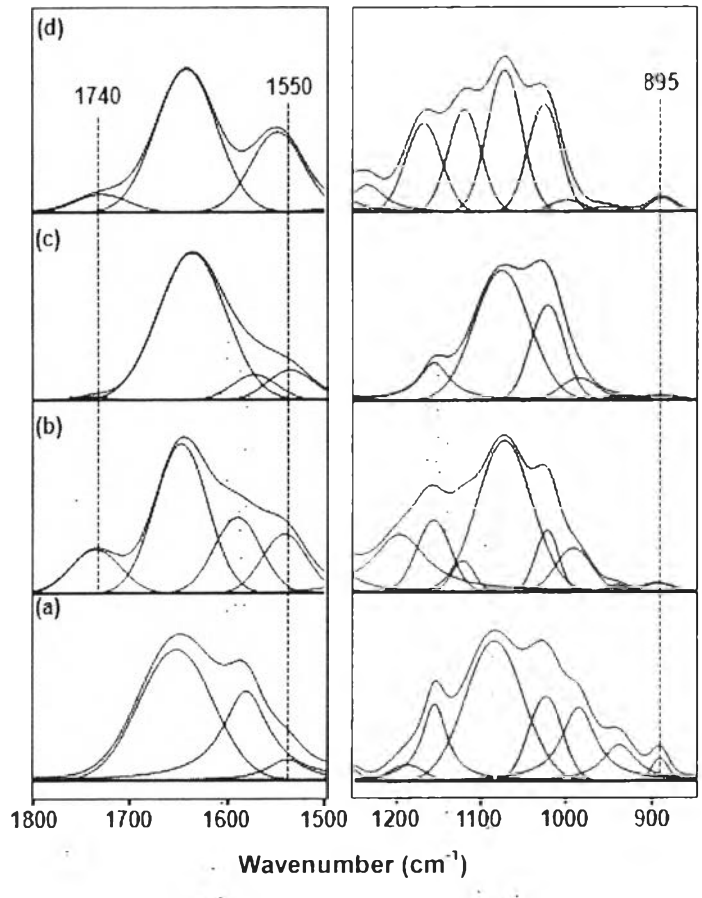


Figure H1. Curve fitting FT-IR spectra of (a) CS (b) CS-1, (c) CS-2, and (d) CS-3.

Table H1. Curve fitting FT-IR integral ratio of CS, CS-1, CS-2, and CS-3

Sample	Integral ratio Ester/std (at position 1740/895 cm^{-1})	Amide2/std (at position 1550/895 cm^{-1})
CS	0	1.5
CS-1	5.8	5.6
CS-2	0.5	6.2
CS-3	3.5	15.3

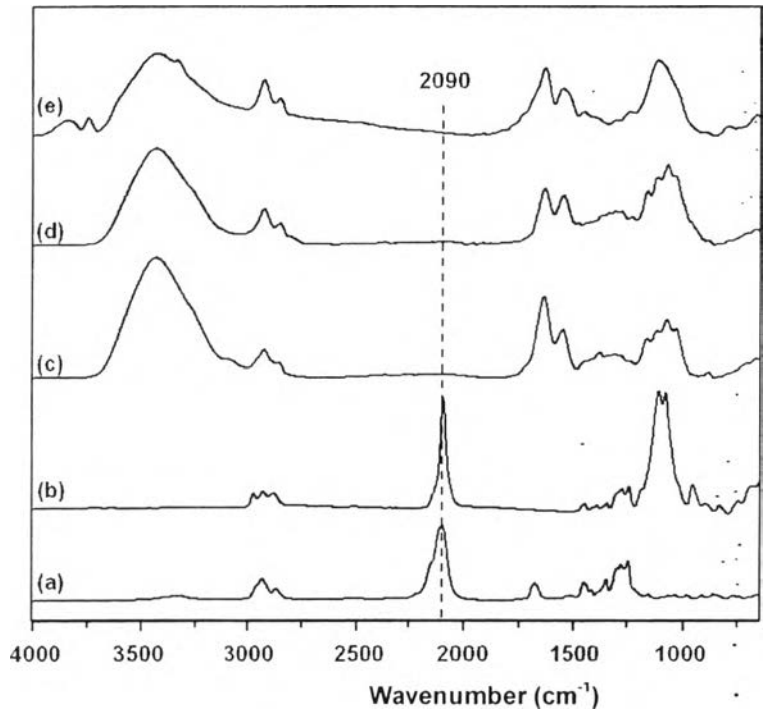
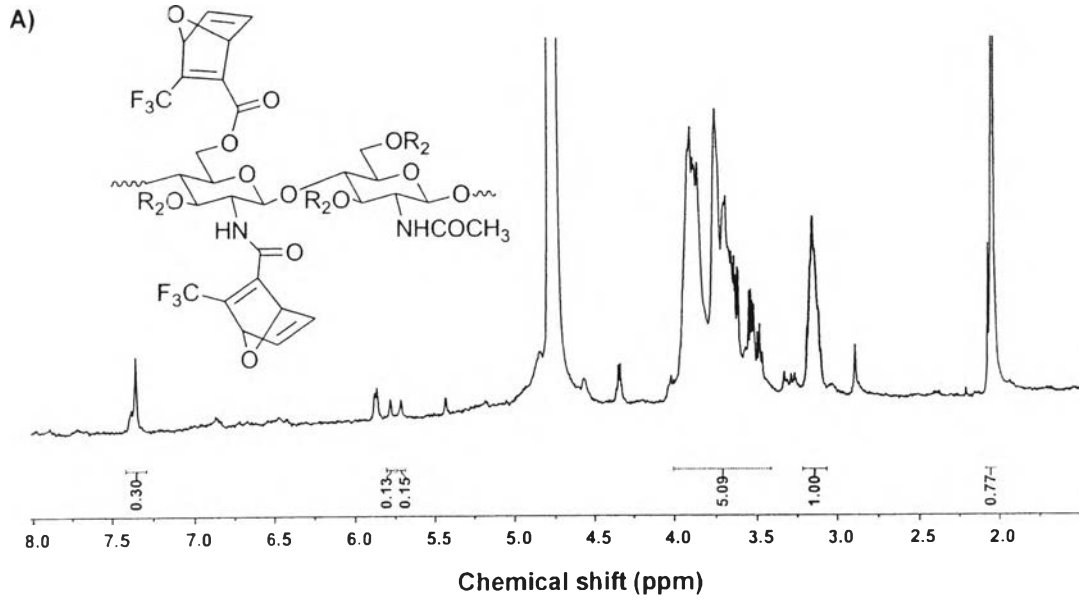
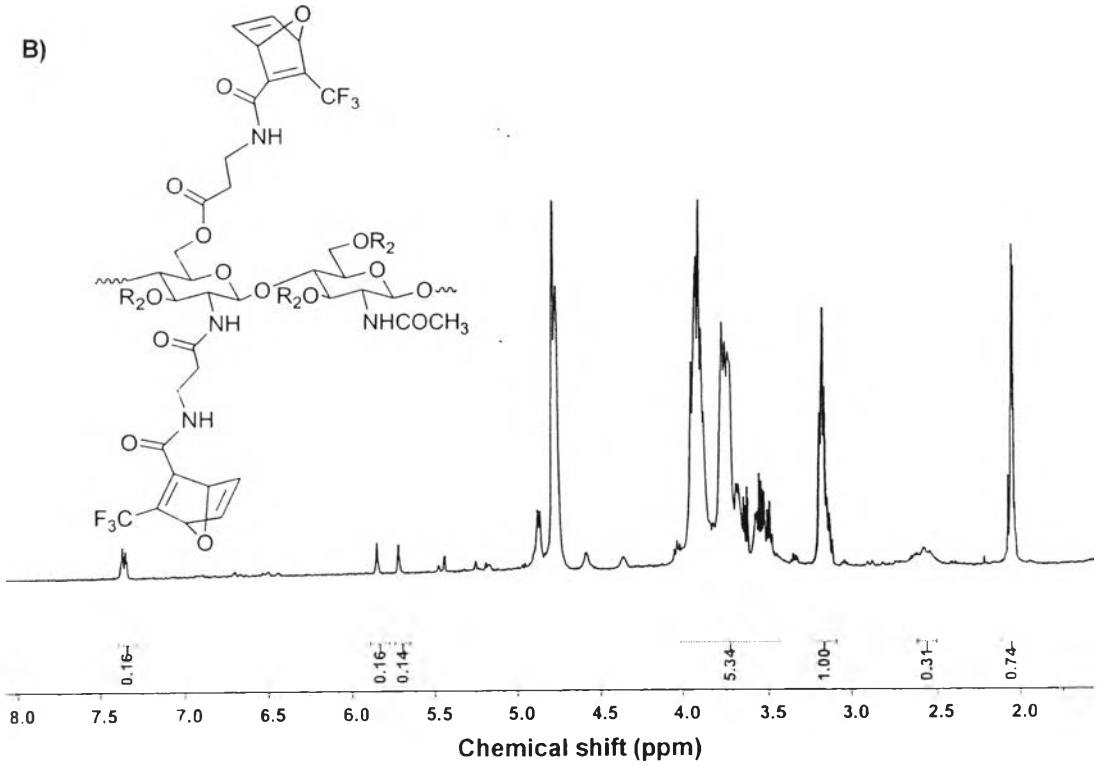
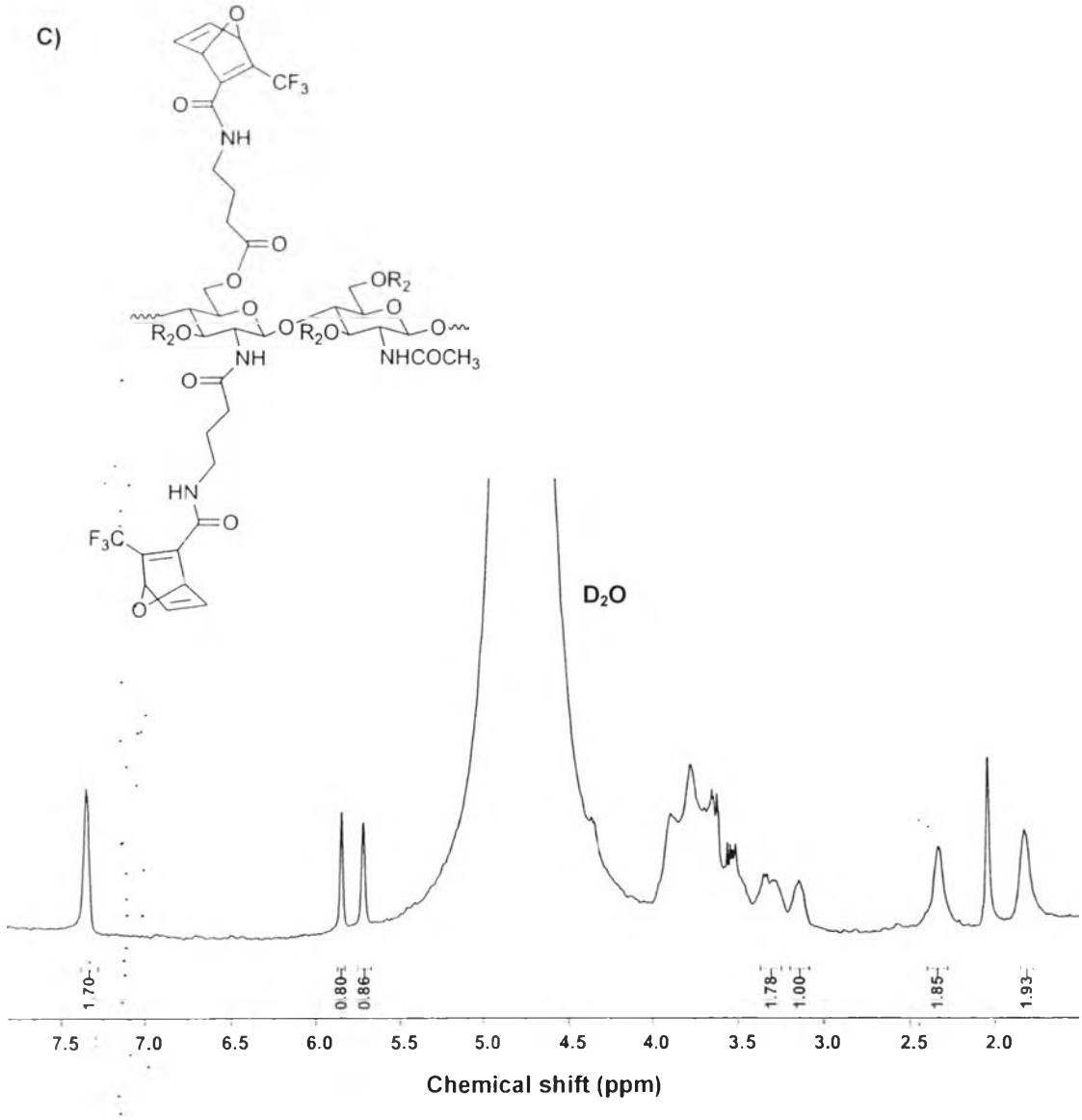


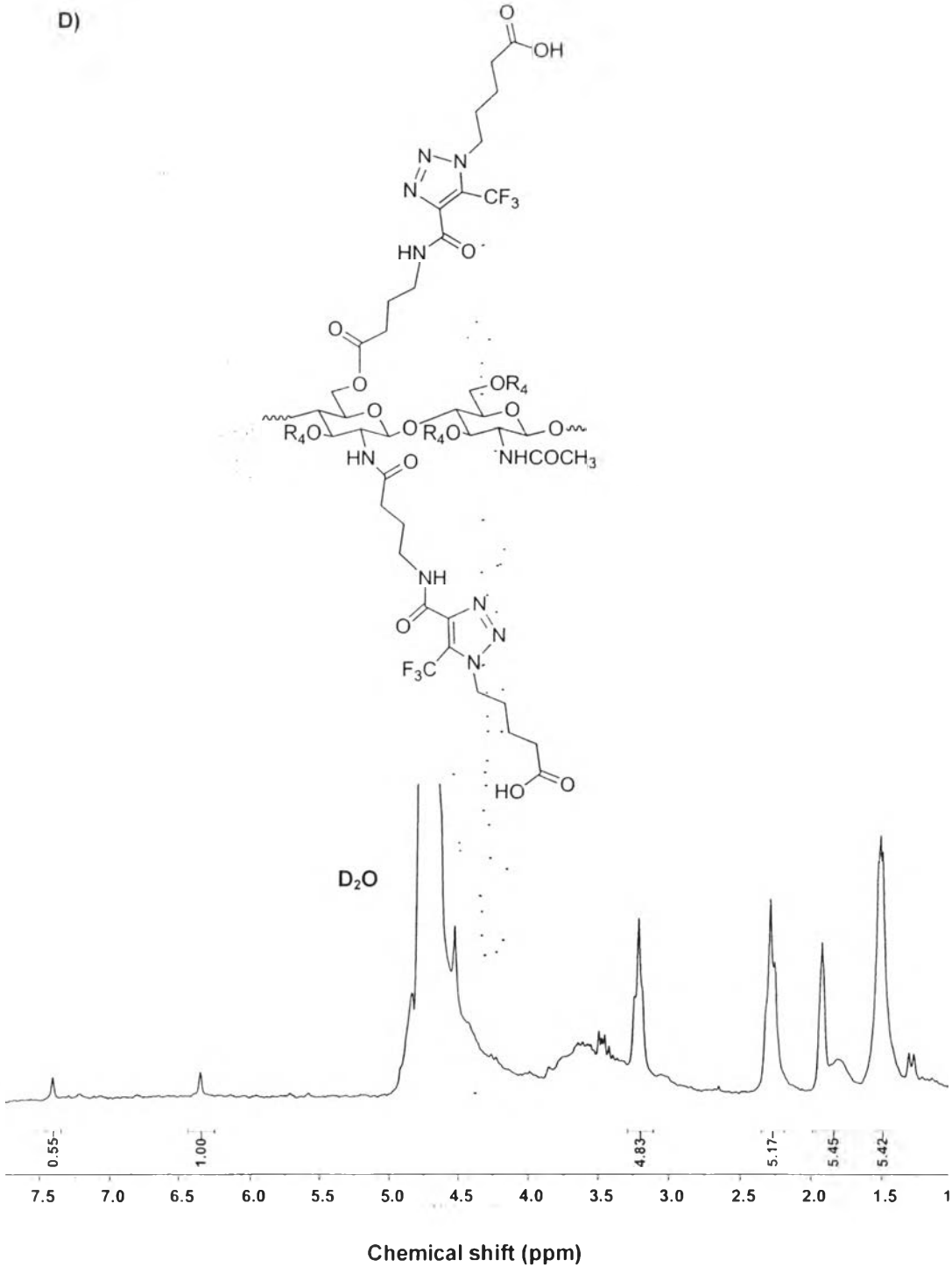
Figure H2. FT-IR spectra of (a) 5, (b) 6, (c) CS-3, (d) CS-5, and (e) CS-6.

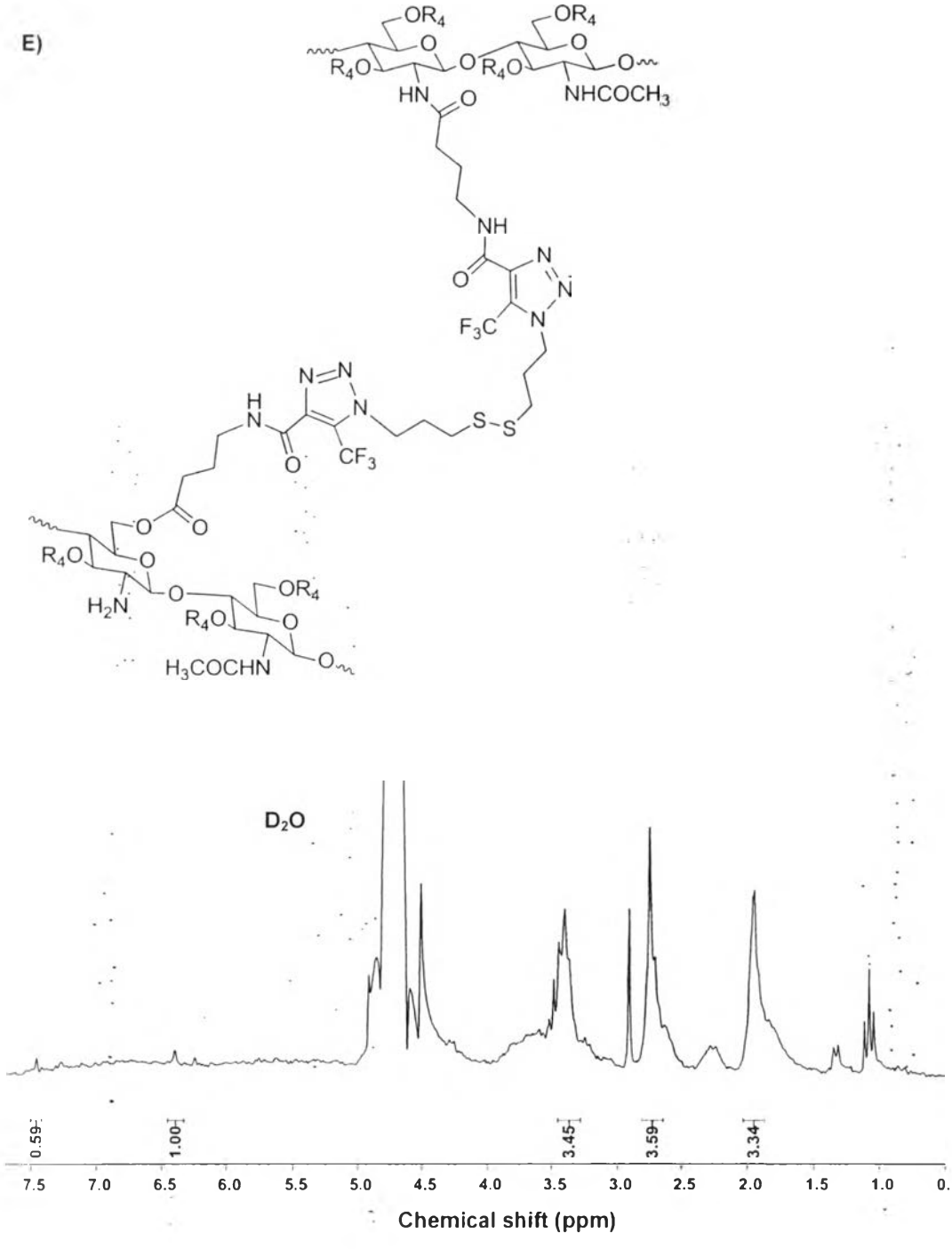

A)
F3C
O
OR2
R2O
NHCOCH3
HN
0.30
0.13
0.15
5.09
1.00
0.77
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chemical shift (ppm)


B)
CF3
NH
OR2
R2O
NHCOCH3
F3C
0.16
0.16
0.14
5.34
1.00
0.31
0.74
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chemical shift (ppm)

C)


D2O
1.70
0.80
0.86
1.78
1.00
1.85
1.93
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chemical shift (ppm)


D)
OH
CF3
HN
OR4
R4O
NHCOCH3
NH
F3C
HO
D2O
0.55
1.00
4.83
5.17
5.45
5.42
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
Chemical shift (ppm)


E)
D2O
0.59
1.00
3.45
3.59
3.34
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Chemical shift (ppm)

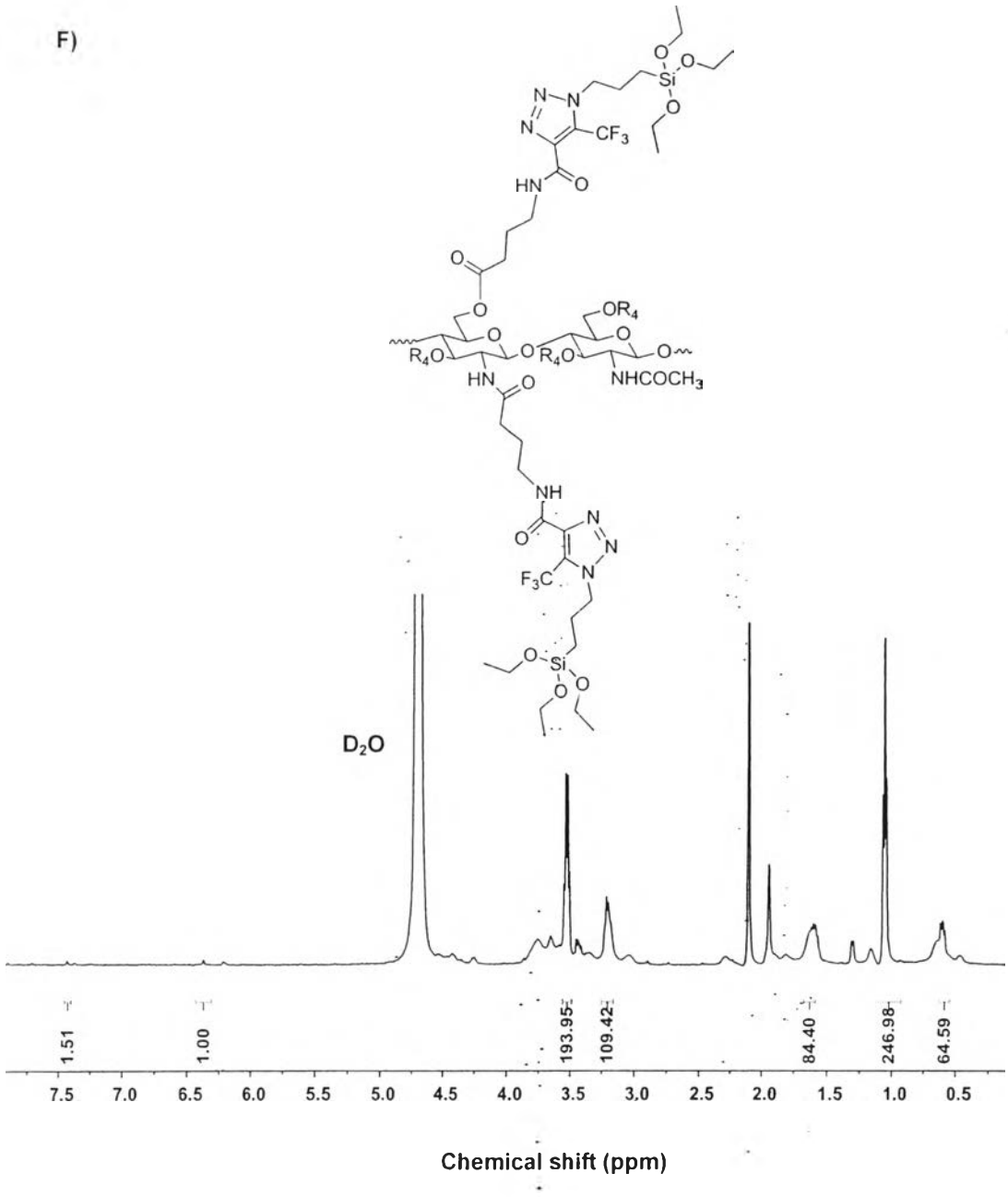


Figure H3. ^{1}H-NMR spectra of A) CS-1, B) CS-2, C) CS-3, D) CS-4, E) CS-5, and F) CS-6.

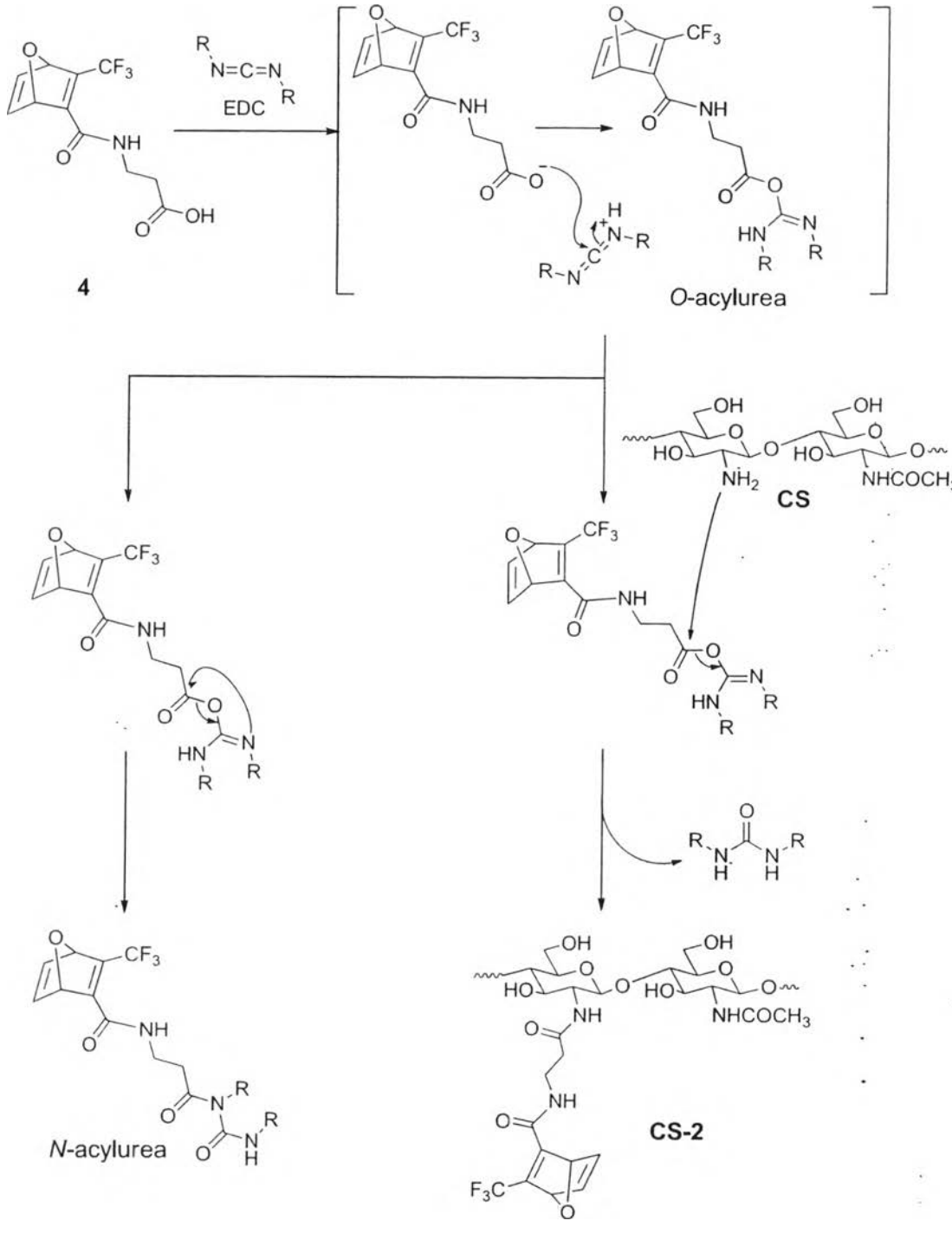
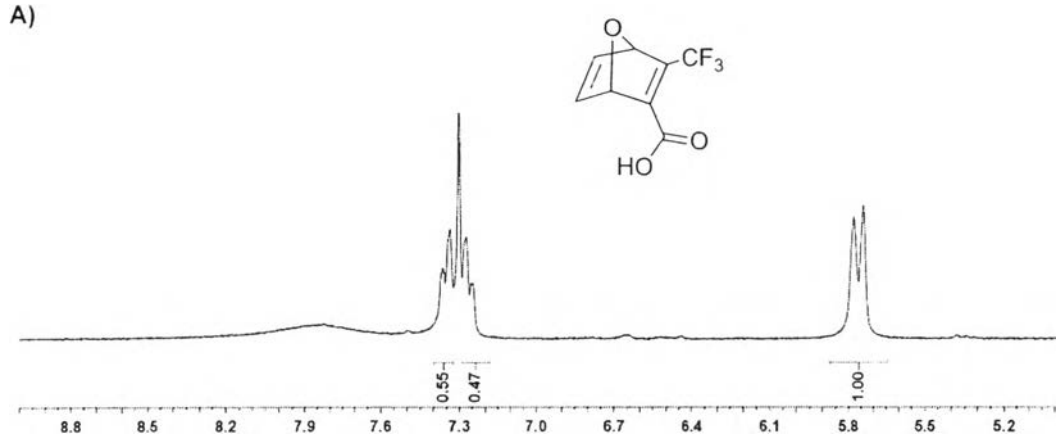


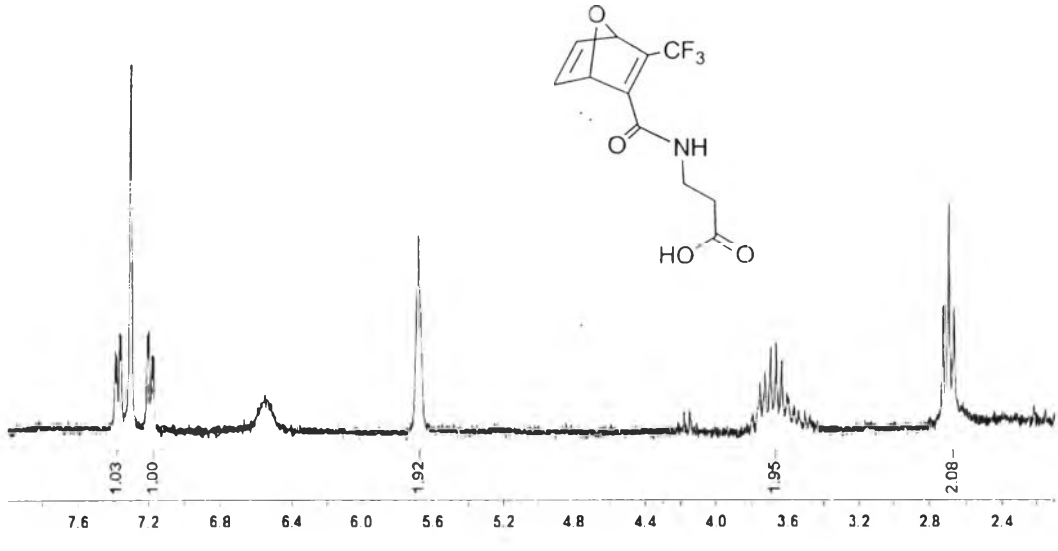
Figure H4. Mechanism of CS-2 and *N*-acylurea by using EDC.

A)

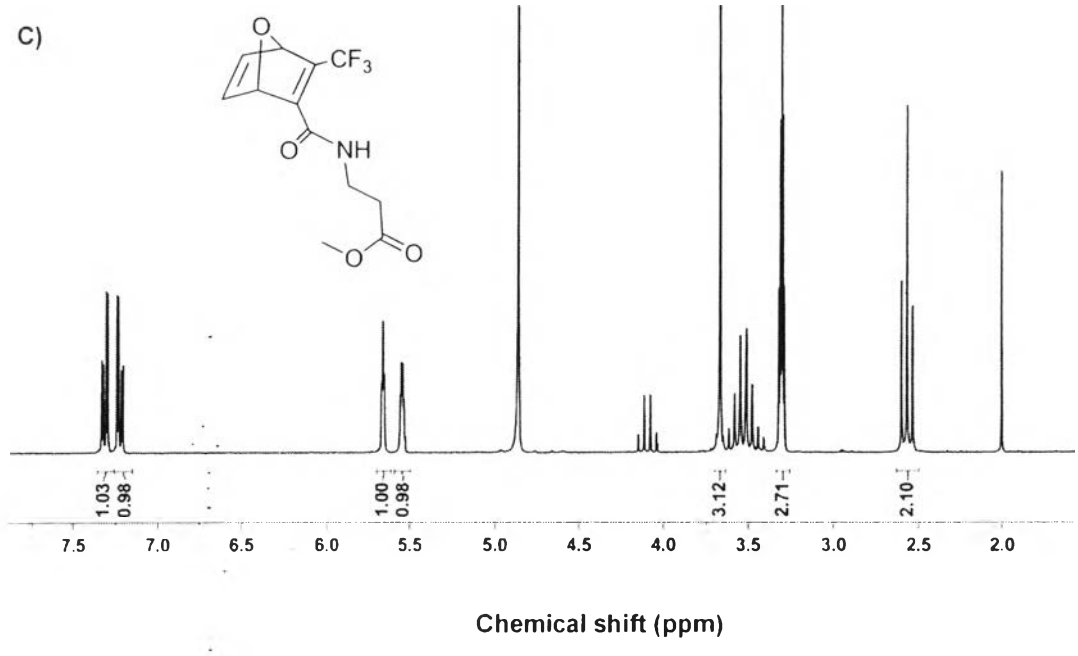


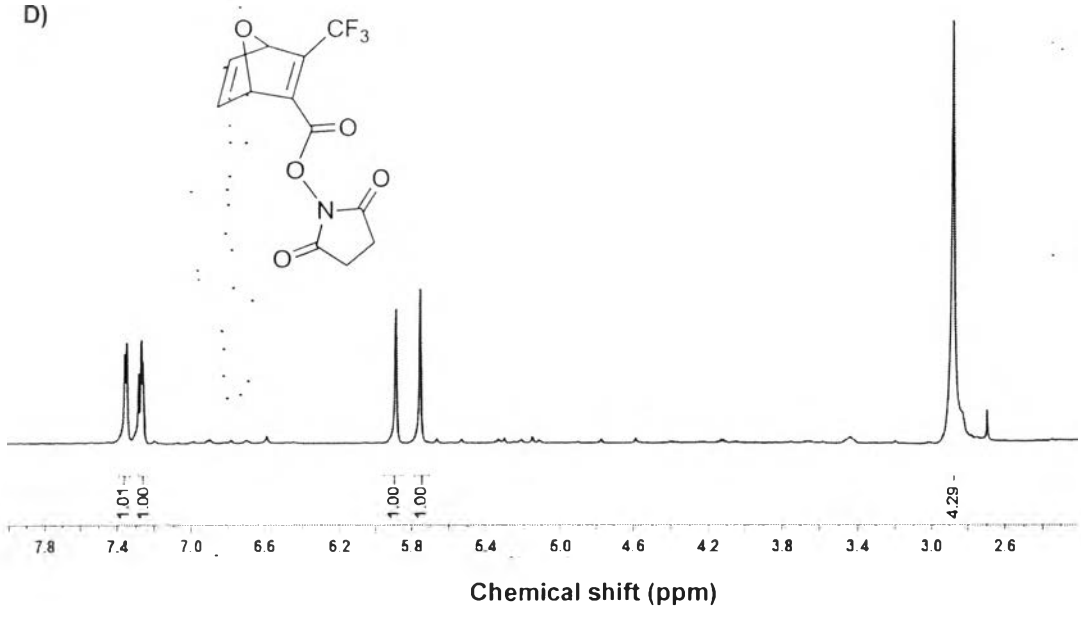
Chemical shift (ppm)

B)

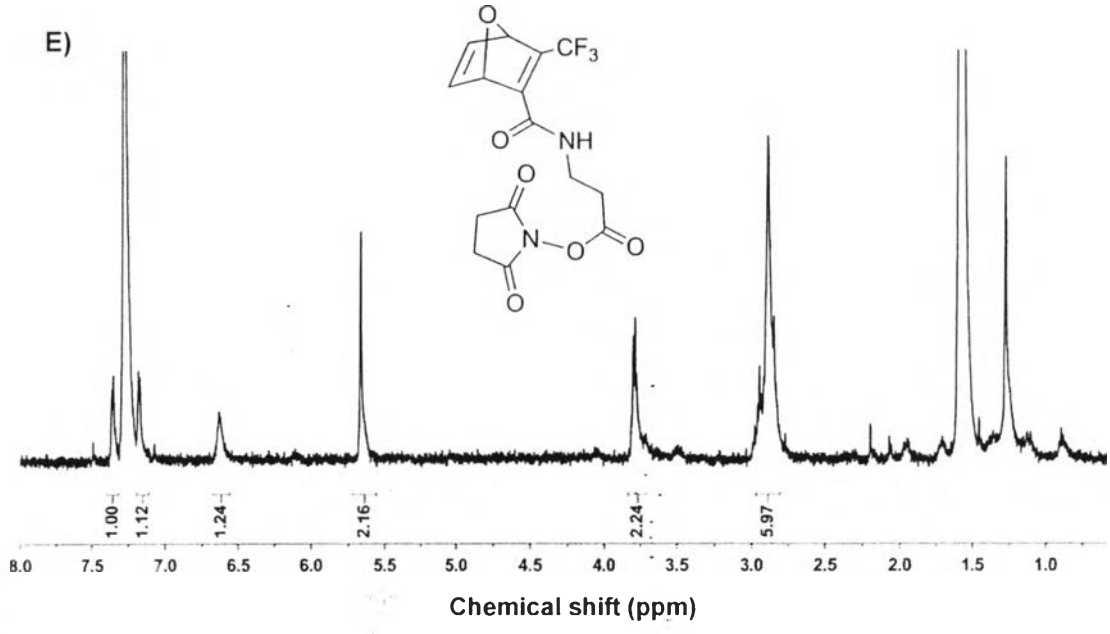


Chemical shift (ppm)

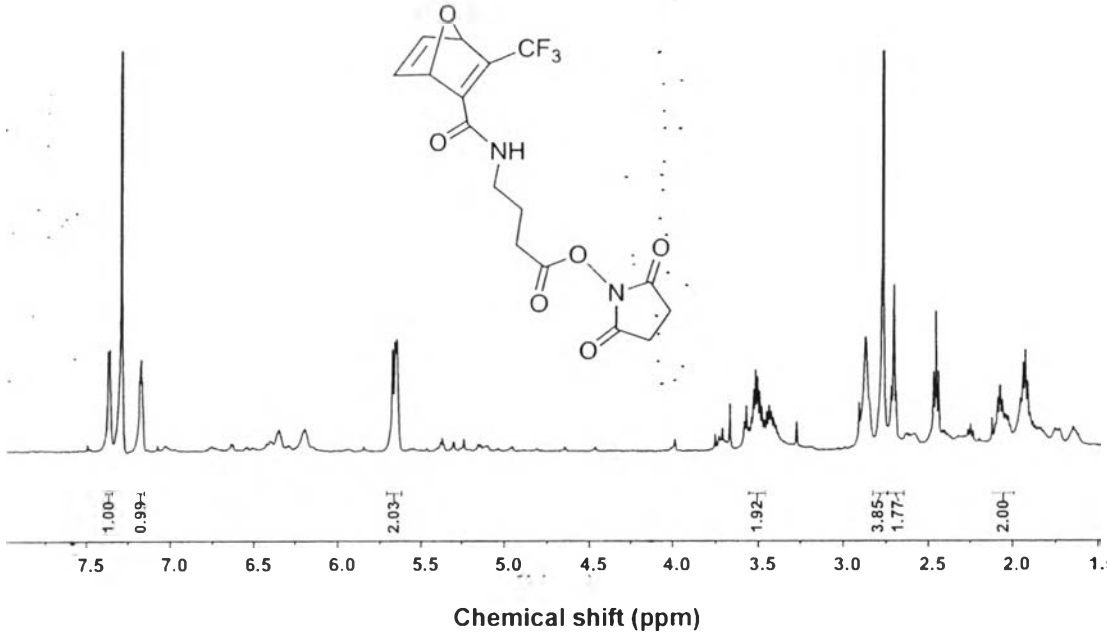

C)
O
CF3
O
NH
O
O
1.03
0.98
1.00
0.98
3.12
2.71
2.10
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chemical shift (ppm)


D)
O
CF3
O
O
N
O
O
1.01
1.00
1.00
1.00
4.29
7.8
7.4
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
Chemical shift (ppm)

E)


O
CF3
NH
O
N
O
O
O
O
1.00
1.12
1.24
2.16
2.24
5.97
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Chemical shift (ppm)

F)


O
CF3
O
NH
O
O
N
O
O
1.00
0.99
2.03
1.92
3.85
1.77
2.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chemical shift (ppm)

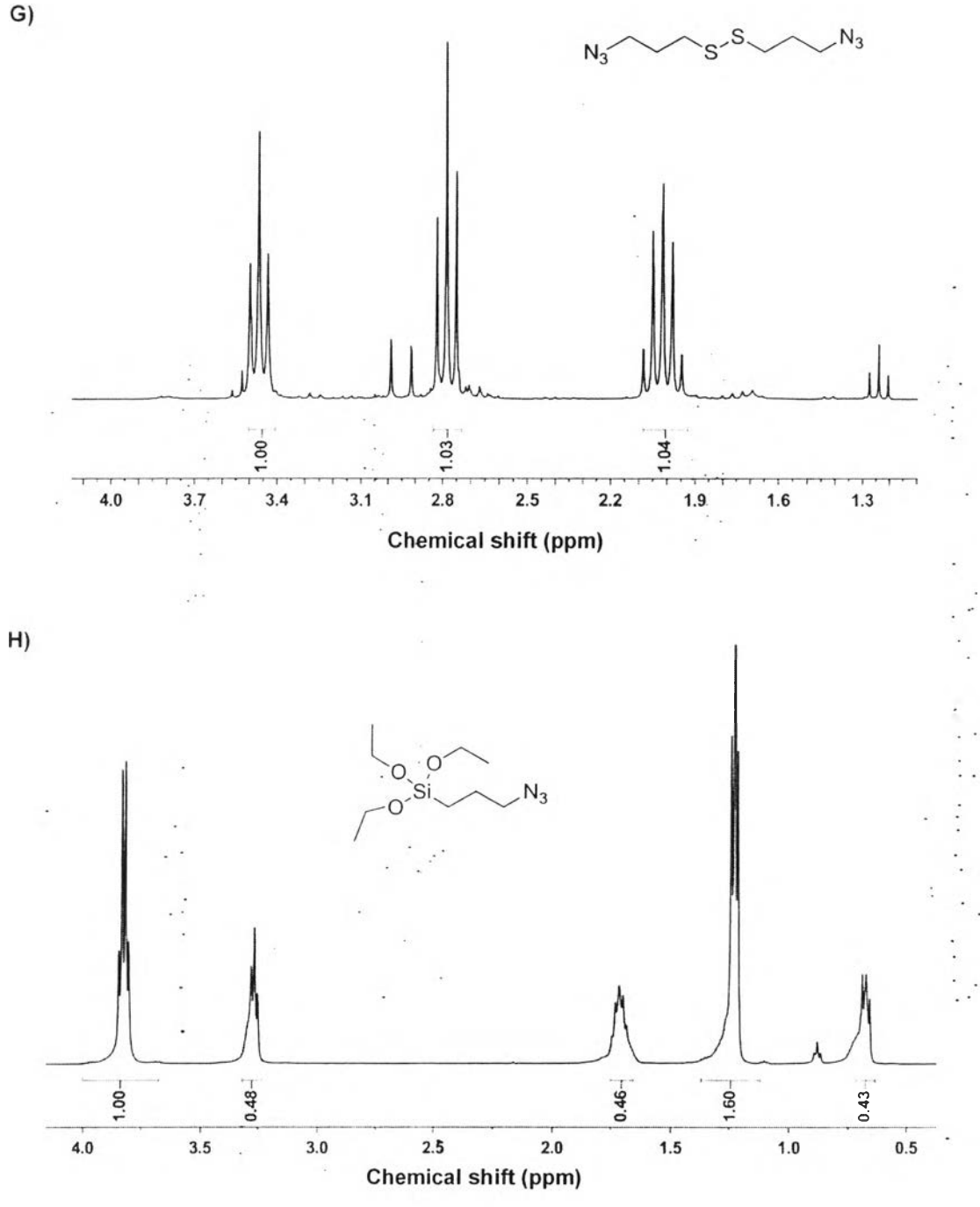
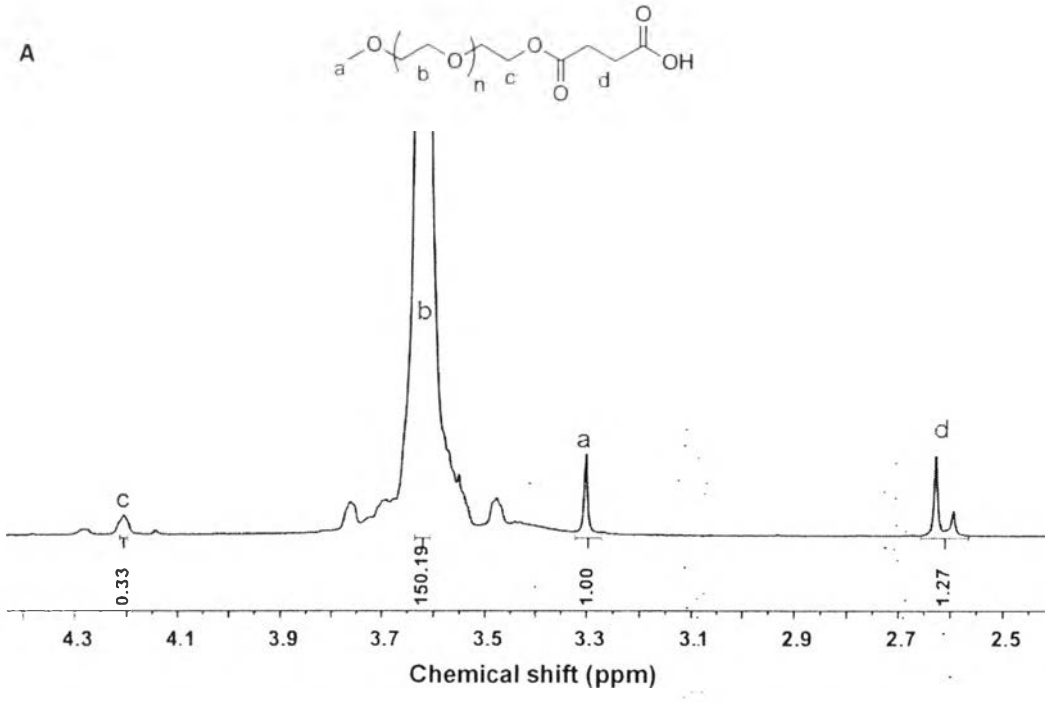


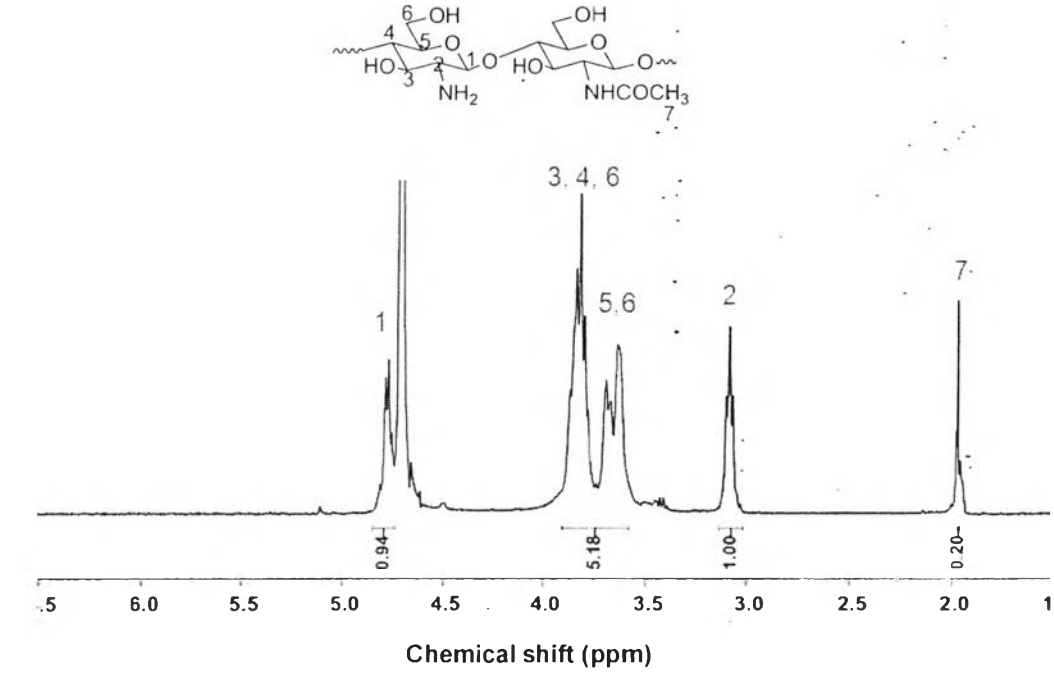
Figure H5. ^{1}H-NMR spectra of A) 1, B) 2, C) 1a, D) 3a, E) 3b, F) , G) 5, and H) 6.

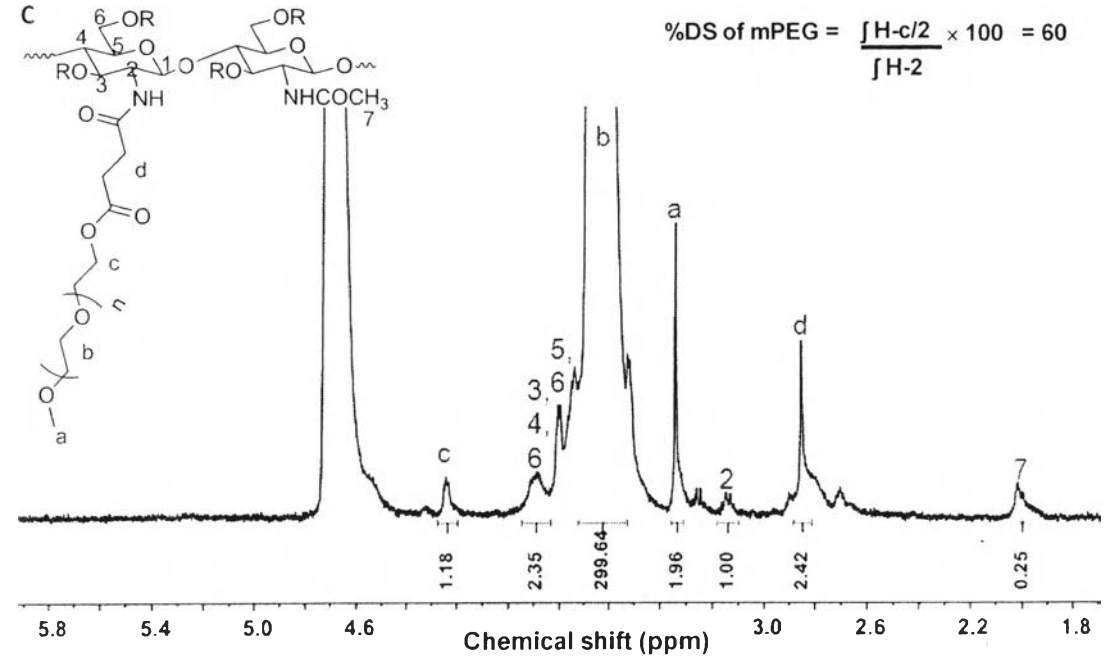
Appendix I Supporting Structural Characterization for Chapter V

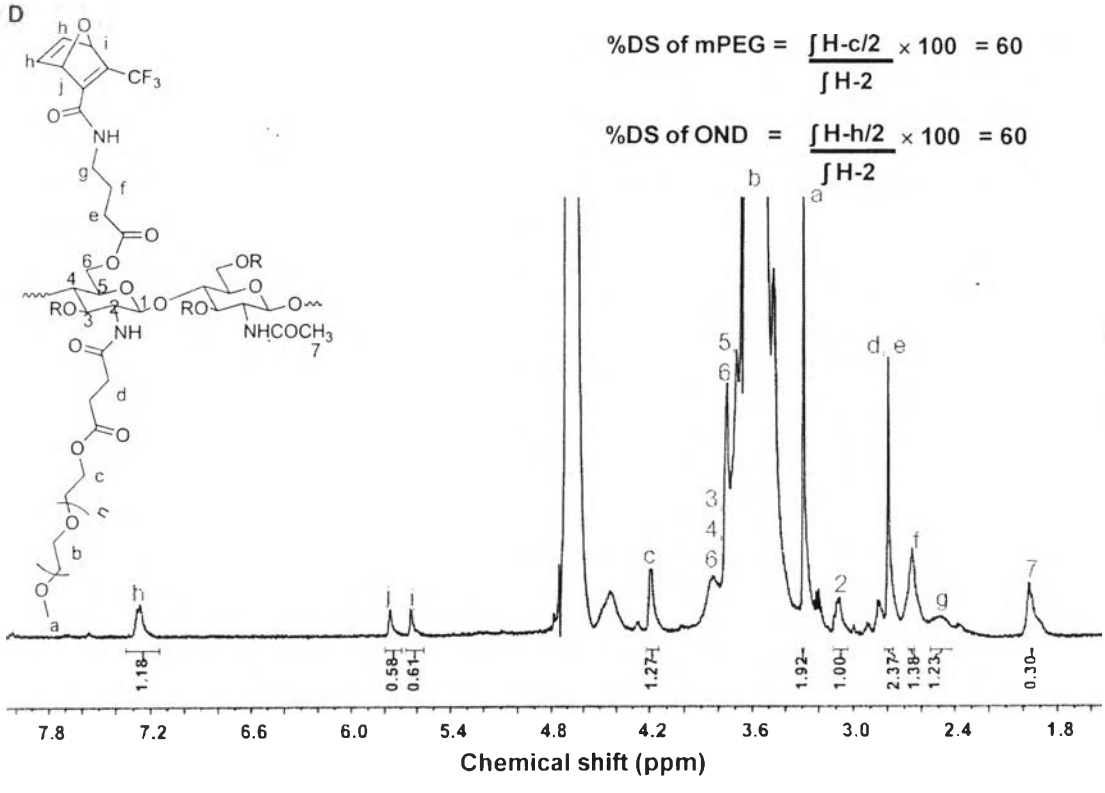
A



B




C
%DS of mPEG = ∫H-c/2 × 100 = 60
∫H-2
Chemical shift (ppm)


D
%DS of mPEG = ∫H-c/2 × 100 = 60
∫H-2
%DS of OND = ∫H-h/2 × 100 = 60
∫H-2
Chemical shift (ppm)

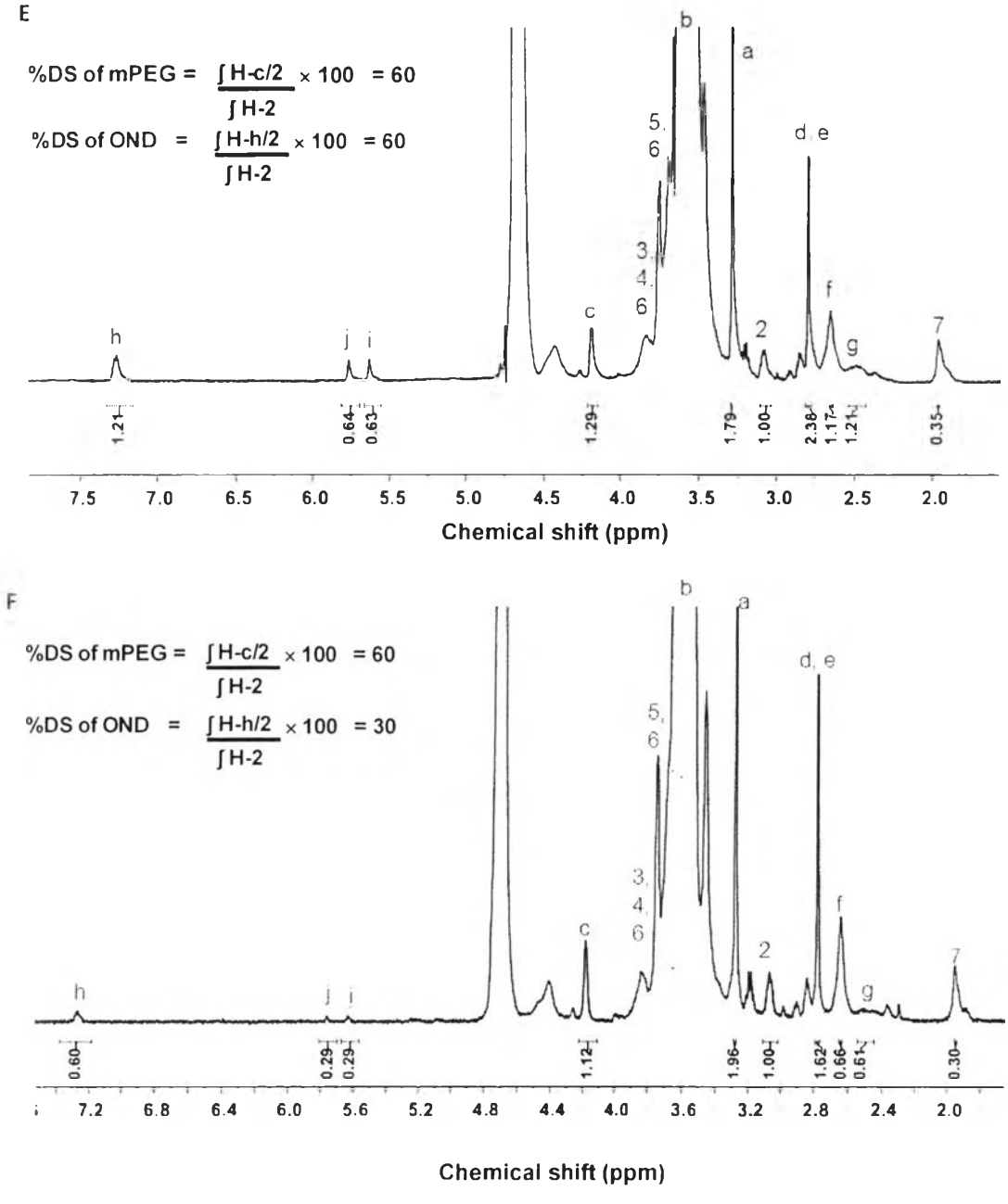


Figure I1. ^{1}H-NMR spectra of A) mPEG-COOH, B) CS, C) CS-mPEG, CS-mPEG-OND from the initial content of OND; D) 1.5, E) 1.0, F) 0.5 equivalent to CS.

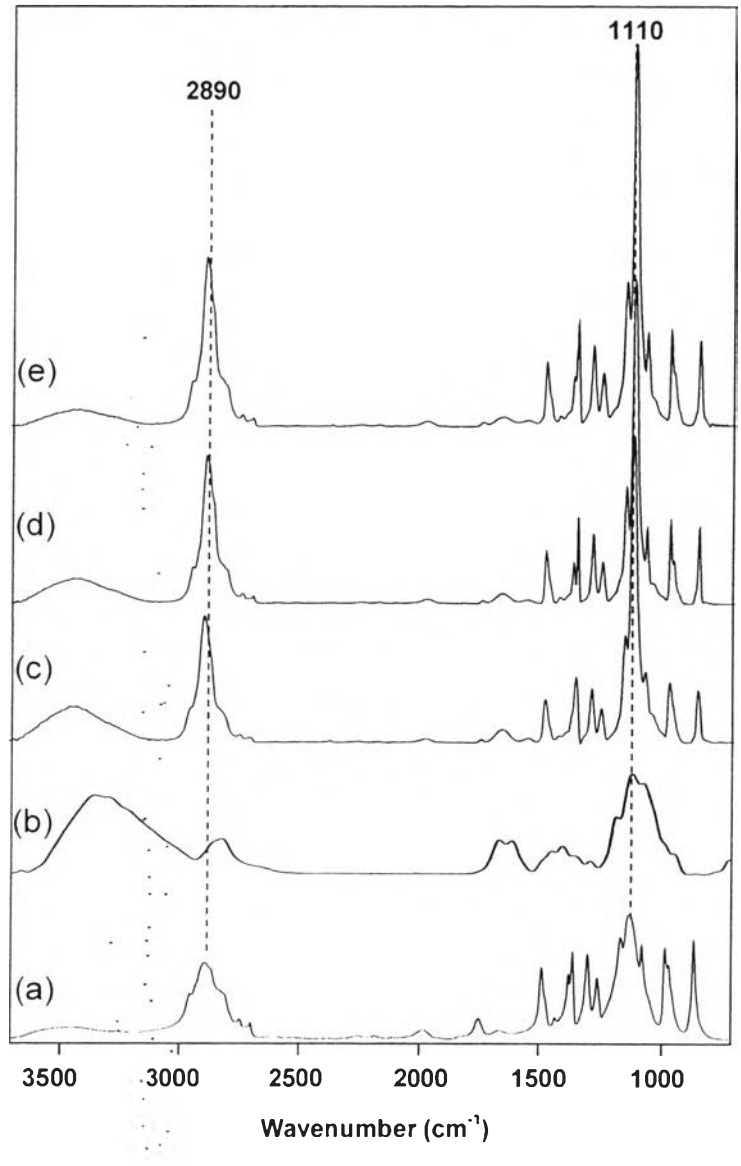


Figure I2. FTIR spectra of (a) mPEG-COOH, (b) CS (c) CS-mPEG, (d) CS-mPEG-OND with 30 %DS of OND, and (e) CS-mPEG-OND-Ab with 30 %DS of OND.

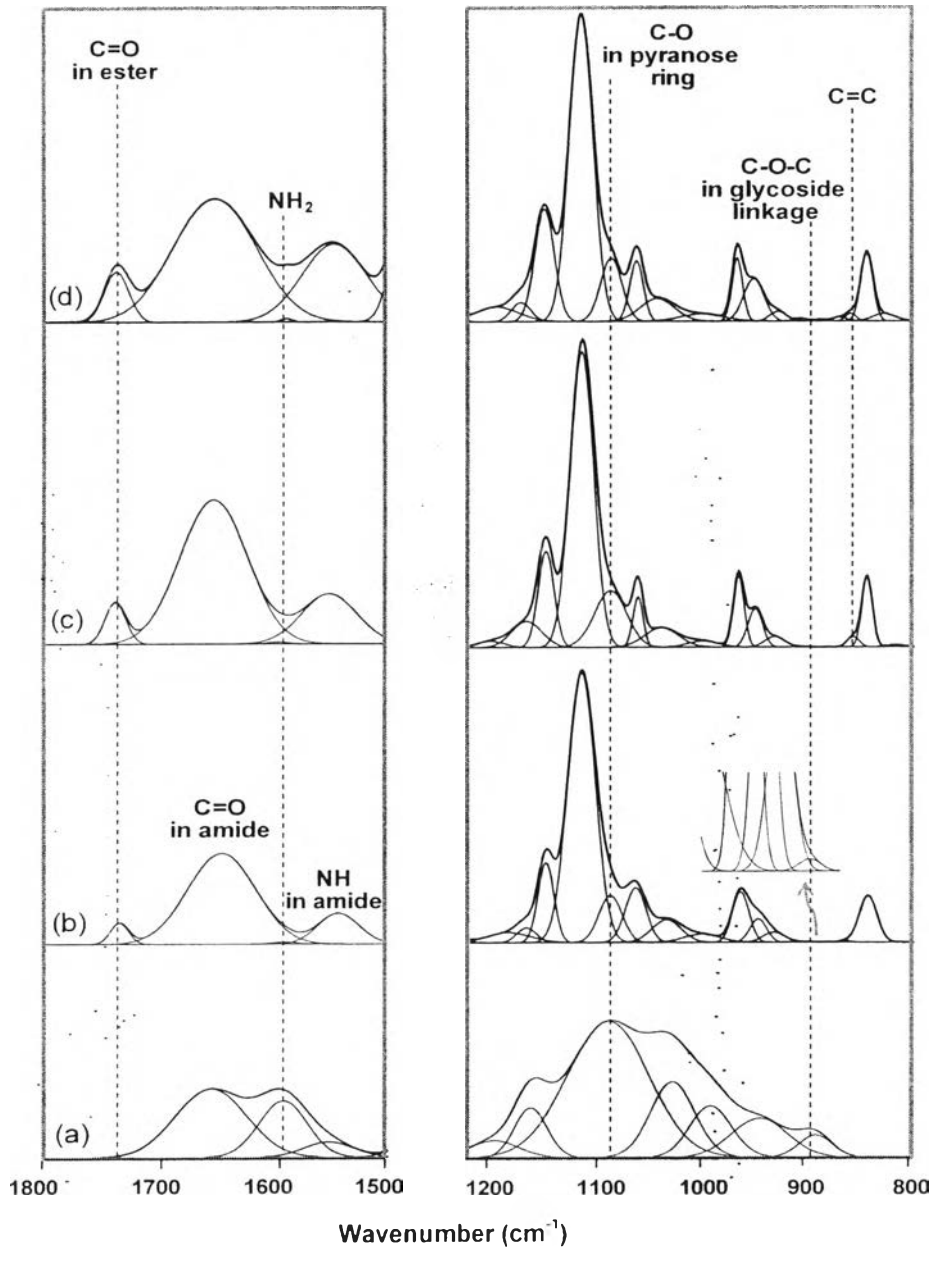


Figure I3. Curve fitting FTIR spectra of (a) CS, (b) CS-mPEG, (c) CS-mPEG-OND with 30 % DS of OND, and (d) CS-mPEG-OND-Ab with 30 %DS of OND.

Table I1. Curve fitting FTIR integral ratio of CS, CS-mPEG, CS-mPEG-OND with 30 %DS of OND, and CS-mPEG-OND-Ab with 30%DS of OND

Samples	Integral ratio at peak positions (cm^{-1}/cm^{-1})			
	1730/895	1650/895	1595/895	1550/895
CS	0	4.2	2.4	0.7
CS-mPEG	0.2	57.4	0.5	12.1
CS-mPEG-OND	5.6	64.5	0.3	17.5
CS-mPEG-OND-Ab	5.7	68.7	0.2	19

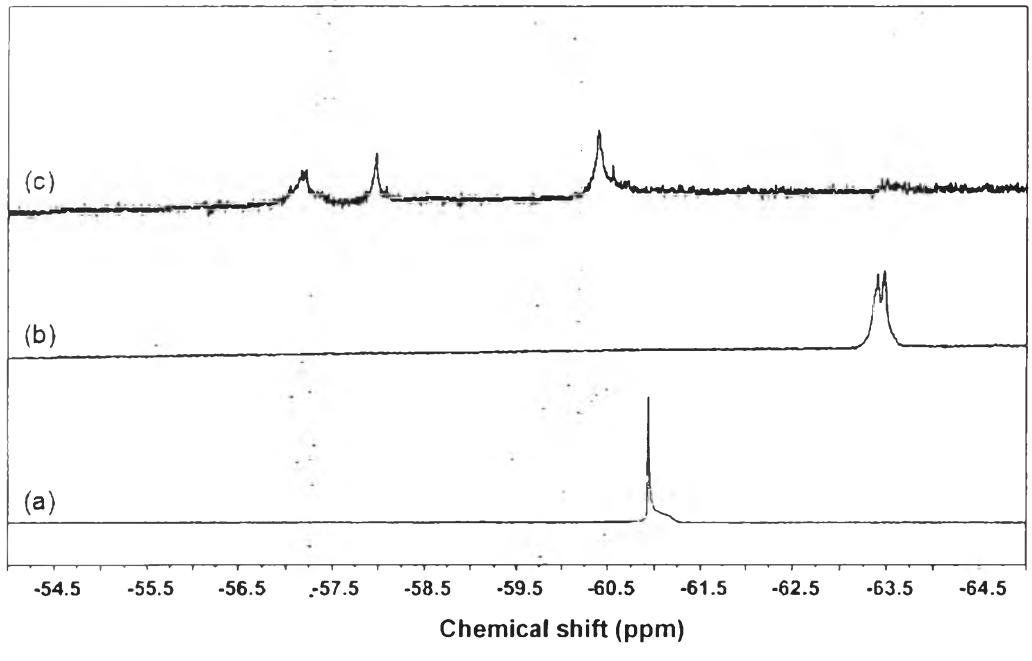


Figure I4. ^{19}F-NMR spectra of (a) oxanorbornadiene derivative (OND), (b) CS-mPEG-OND-Ab, and (c) CS-mPEG-Ab-click-disulfide.

Appendix J Observation of Chitosan Solution Appearances

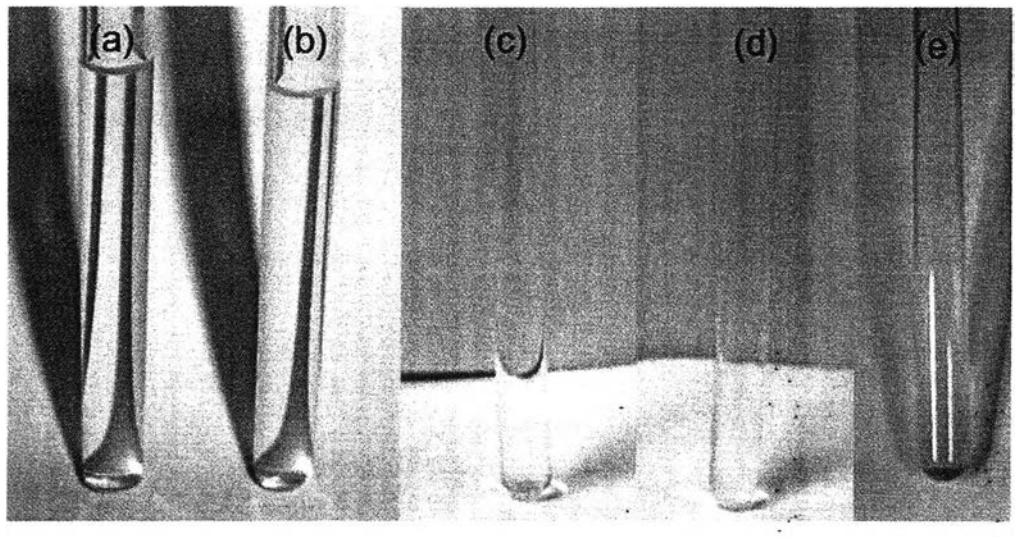


Figure J1. Appearances of (a) CS-mPEG, (b) CS-mPEG-OND with 60 % and 60 %DS of mPEG and OND, respectively, (c) CS-mPEG-OND-Ab with 30 %DS of OND, (d) CS-mPEG-OND-Ab with 60 %DS of OND, and (e) CS-mPEG-OND-Ab with 30 %DS of OND after adding azido-disulfide for 11 d.

Appendix K Evaluation of Antibody Immobilization

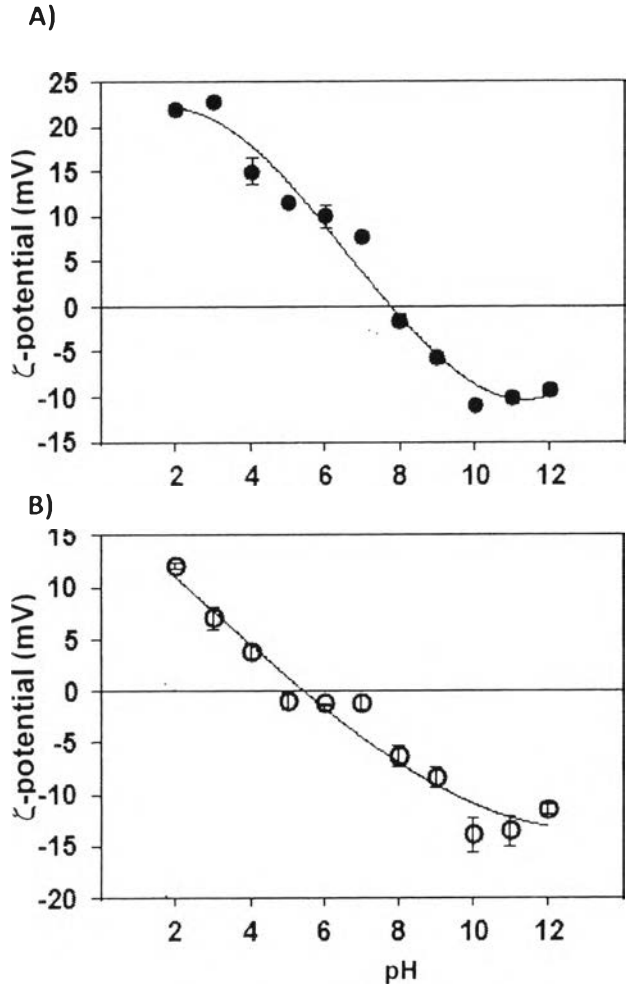


Figure K1. ζ-potentials of (A) WSC-OND and (B) WSC-OND-Ab at pH 2-12 adjusted by 0.1 M NaOH/0.1 M NaCl.

Appendix L Evaluation of Azido-disulfide Content

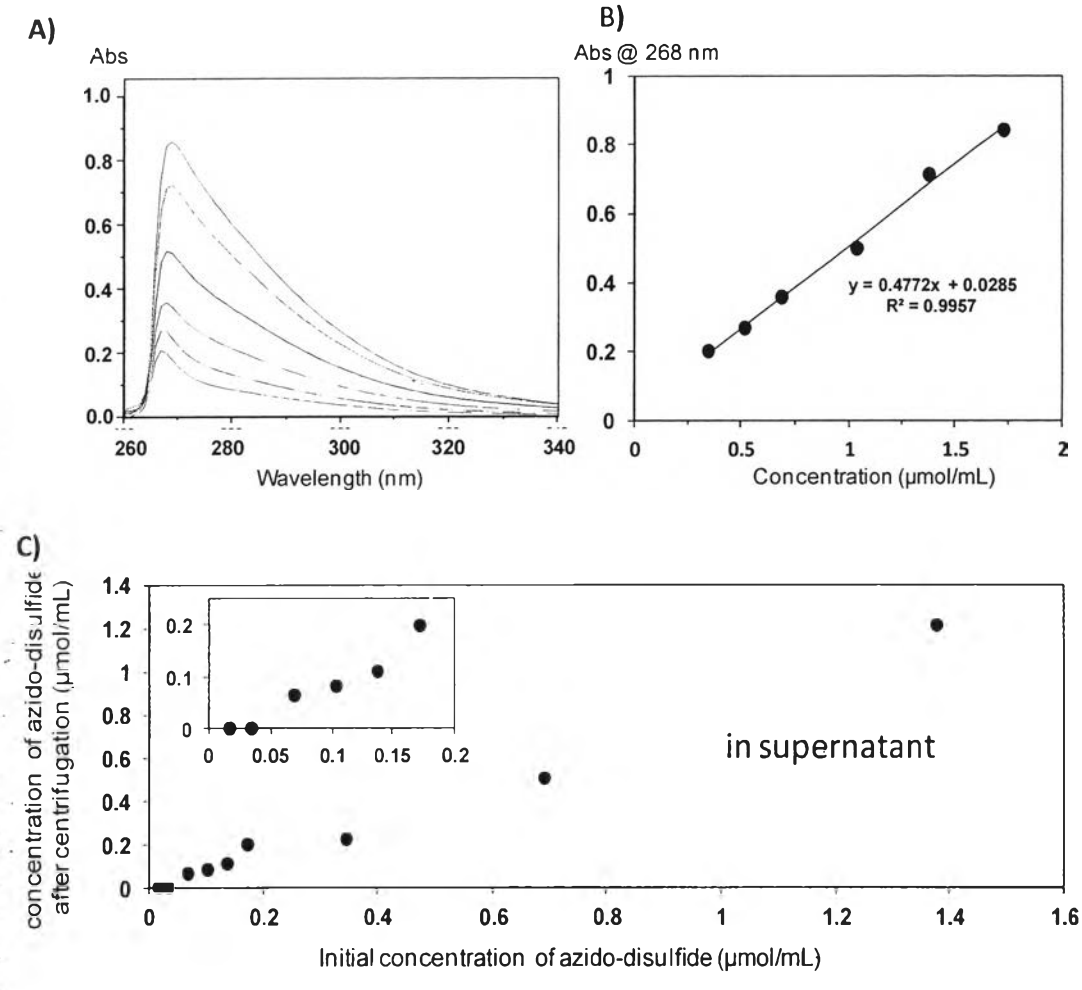


Figure L1. (A) UV-Vis spectra of azido-disulfide in DMSO (B) Standard Curve plotting between concentration of azido-disulfide and absorbance. (C) Concentration of supernatant (after centrifugation with 21,000 rpm to separate AuNPs at the bottom part) measured by UV-VIS spectrophotometer with a variation of initial concentration of azido-disulfide.

Appendix M Mechanism of Disulfide Cleavage and Azido-Gold Nanoparticle Forming

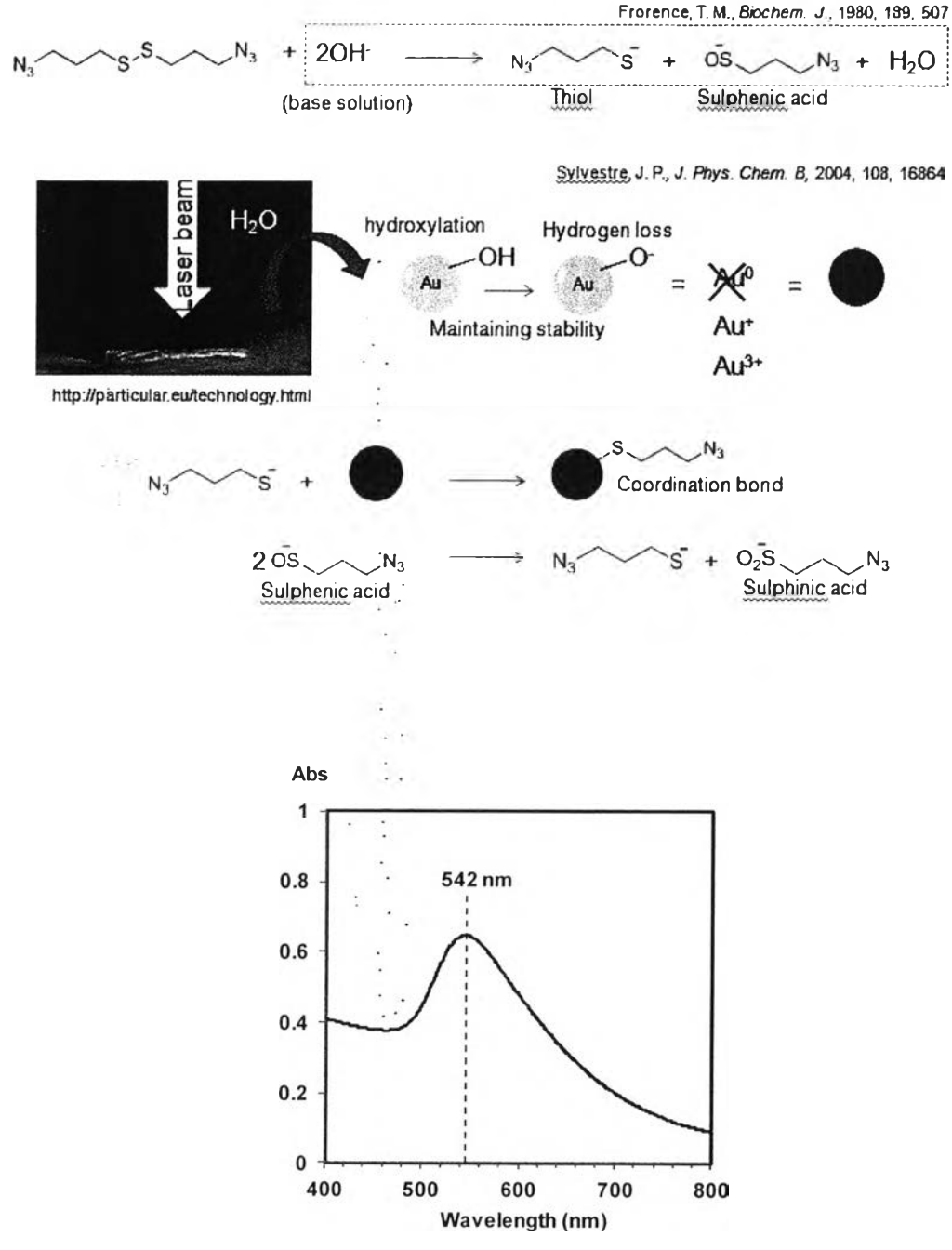


Figure M1. UV-Vis spectra of azido-AuNPs.

Appendix N Appearance of Gold Nanoparticle Solution Mixed with Chitosan Solutions

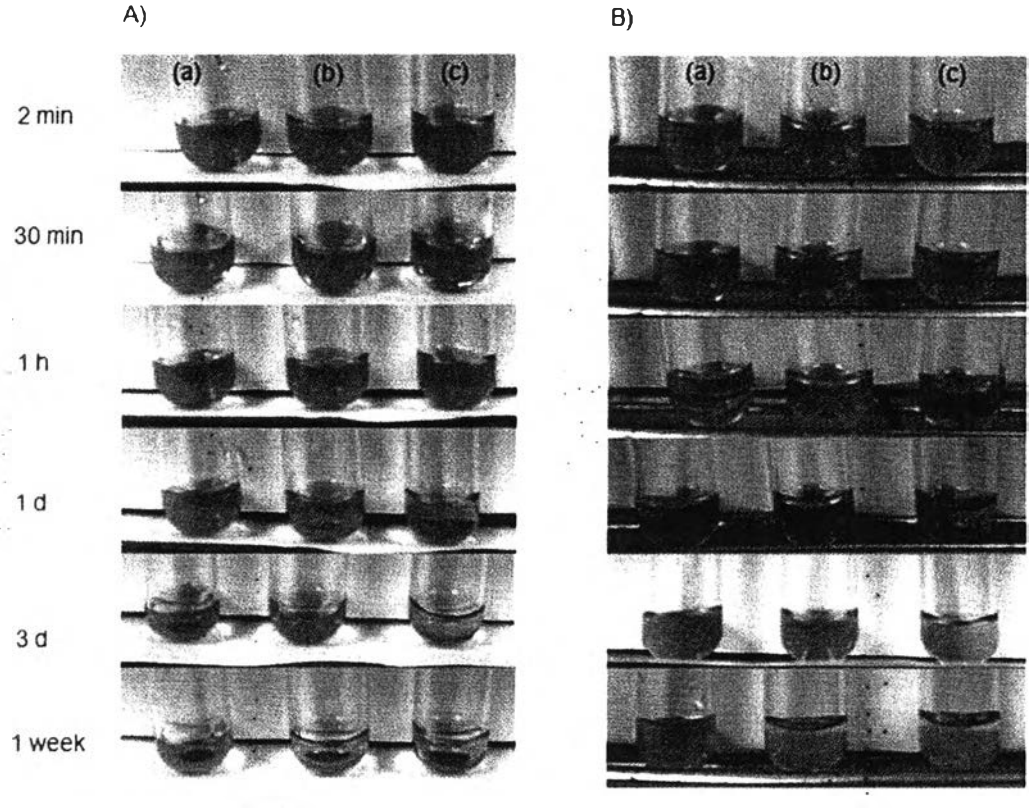


Figure N1. Appearances of mixture between azido-AuNP solution and (a) water (b) CS-mPEG-Ab solution, and (c) CS-mPEG-OND-Ab solution with A) 30% and B) 60% OND substitution and over the time at room temperature.

Appendix O Cycloaddition Time of Mixture between WSC-OND-Ab and Azido-disulfide

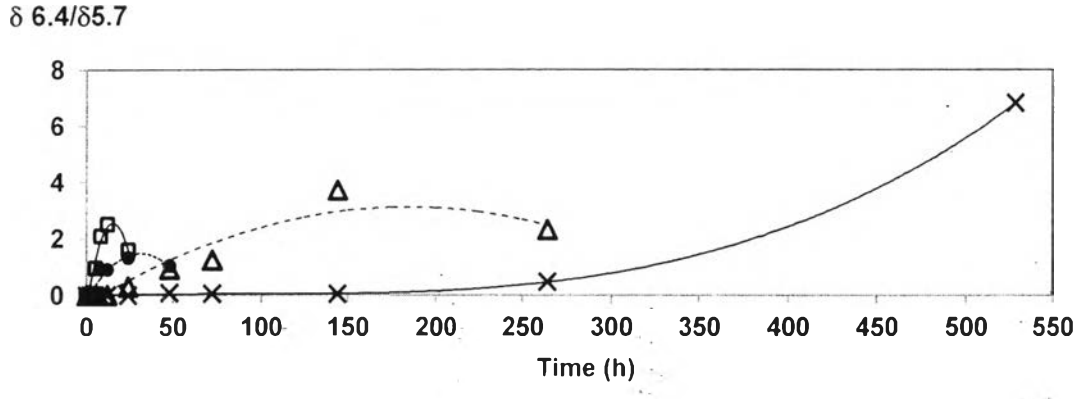


Figure O1. Ratio of furan and oxanorbornadiene (integral ratio of δ 6.4/δ5.7) of cycloaddition between CS-mPEG-OND-Ab and azido-disulfide over the reaction time detected by ^{1}H-NMR based on the integration at; (□) 60 °C , (Δ) 25 °C, (●) 40 °C, and (×) 4 °C.

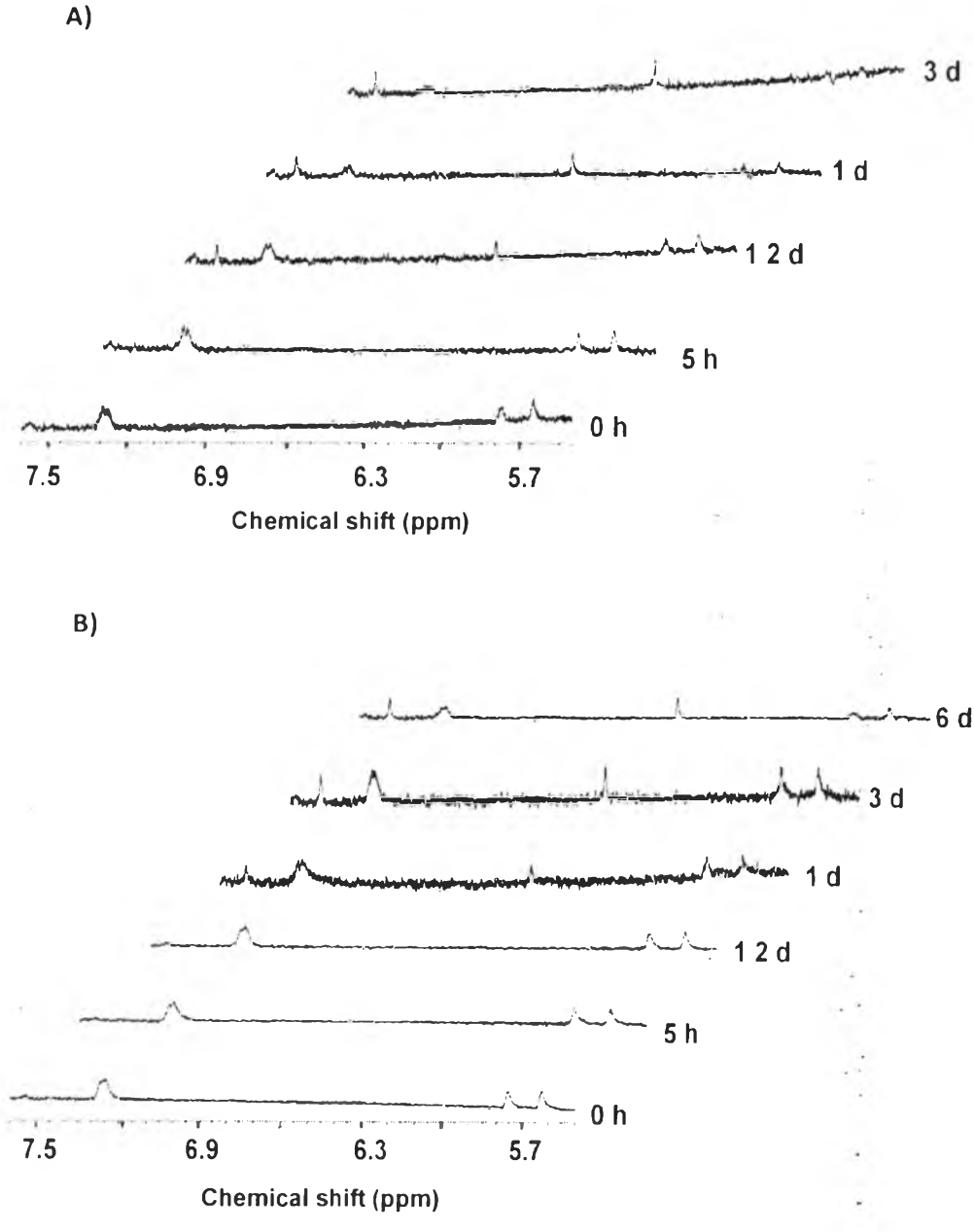


Figure O2. ^{1}H-NMR spectra of ligation progress in the presence of A) phosphate buffer and B) 10% DMSO over time between CS-mPEG-OND-Ab and azido-disulfide.

Appendix P Synthesis of 1,2-bis (3-azidopropyl) disulfane Immobilized Gold Nanoparticle, Azido-AuNPs

1,2-bis (3-azidopropyl) disulfane or azido-disulfide (2 µL, 8.6 µmol/mL in phosphate buffer pH 9.4) was added into AuNPs aqueous solution (498 µL, 1.0 µmol/mL in 10% DMSO/DI water V/V) to obtain azido-AuNPs. The mixture was incubated for 1 h at room temperature before use.

Appendix Q Antigen Detection by Naked Eyes

This technique was adapted from Dot-blot Elisa. In brief, 2 µl of antigen (Ag, 0.44 mg/mL) mixed with coating buffer, was coated on a nitrocellulose membrane (Millipore, Ireland). After drying, 2 µl of CS-mPEG-OND-Ab solution (4 mg/mL in PBS buffer, pH 7.4) was dropped at the center of membrane and allowed to dry. Then, the membrane was washed with PBS-Tween 20. After drying, the membrane was soaked in 600 µl of azido-AuNP solution for 10 min and was dried by air dryer. The soaking with azido-AuNP solution and washing steps were done for four times before observing the appearance of the color. For comparative studies, a series of compounds, CS-mPEG, CS-mPEG-Ab, CS-mPEG-OND, Ab, with and without Ag were also followed from above steps.

CURRICULUM VITAE

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2004-2007	Bachelor's Degree of science (Physico-Chemical Processing Technology), Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand

Work Experience:

2007	Position:	Internship-training
	Company name:	Giffarine Skyline Laboratory and Health Care Co., Ltd.

Publications:

1. Jirawutthiwongchai, J.; Draeger, G.; and Chirachanchai, S. (2014) Rapid Hybridization of Chitosan-Gold-Antibodies via Metal-free Click in Water-based Systems: A Model Approach for Naked-eye Detectable Antigen Sensors. Macromolecular Rapid Communications, 35, 1204-1210.
2. Jirawutthiwongchai, J.; Krause, A.; Draeger, G.; and Chirachanchai, S. (2013) Chitosan-oxanorbornadiene: a convenient chitosan derivative for Click Chemistry without metal catalyst problem. ACS Macro Letters, 2, 177-180.
3. Yoksan, R.; Jirawutthiwongchai, J.; and Arpo, K. (2010) Encapsulation of ascorbyl palmitate in chitosan nanoparticles by oil-in-water emulsion and ionic gelation processes. Colloids and Surfaces B: Biointerfaces, 76, 292–297.

4. Jirawutthiwongchai, J.; Klaharn, I.; Hobang, N.; Mai-Ngam, K.; Sereemaspun, A.; and Chirachanchai, S. Chitosan-Phenylalanine-mPEG Nanoparticles: A Single Step Water-based Conjugation and their Synergistic Effect of Structural Composition and Nano-size on House Dust Mite Allergen Delivery. Biomaterials (submitted).
5. Jirawutthiwongchai, J.; and Chirachanchai, S. *N*-Phthaloylchitosan for Stabilization of Multi-walled Carbon Nanotube by Noncovalent Functionalization. Macromolecular Rapid Communications (in preparation).

Proceedings:

1. Jirawutthiwongchai, J.; Draeger, G.; and Chirachanchai, S. (2014, July 6- 11) Rapid Hybridization of Chitosan-Gold Nanoparticles via Metal-free Click in Water-based System for Naked-eye Antigen Detection. Proceeding of The 2014 IUPAC World Polymer Congress (MACRO 2014), Chiang Mai, Thailand.
2. Jirawutthiwongchai, J.; Draeger, G.; and Chirachanchai, S. (2014, March 20- 21) Hybridization of Water-based Chitosan-Gold via Copper-free Click for Naked-eye Antigen Detection. Proceeding of the Fourth Polymer Conference of Thailand (PCT-4), Bangkok, Thailand.
3. Jirawutthiwongchai, J.; Mai-Ngam, K.; and Chirachanchai, S. (2011, May 29- June 3) Nanoparticulate low molecular weight chitosan-g-phenylalanine-g-mPEG and its potential application of allergen delivery system. Proceeding of the Europolymer Conference 2011 - Biobased Polymer and Related Biomaterials (EUPOC 2011), Gargnano, Italy.
4. Jirawutthiwongchai, J.; and Chirachanchai, S. (2011, March 25-26) Water-based nanoparticulate chitosan: molecular design, synthesis, and potential application in allergen delivery system. Proceeding of the 3rd PKU-CU Nano Bilateral seminar, Bangkok, Thailand.
5. Jirawutthiwongchai, J.; Mai-ngam, K.; and Chirachanchai, S. (2010, August 26-27) Chitosan nanospheres for allergen delivery system: molecular design, synthesis, and allergen incorporation. Proceeding of the Sixth National Chitin-Chitosan Conference (6th NCCC), Bangkok, Thailand.
6. Jirawutthiwongchai, J.; Mai-ngam, K.; and Chirachanchai, S. (2010, October 7-8) Chitosan nanoparticles by self-assembly with biomolcules for allergen delivery

system. Proceeding of the First Polymer Conference of Thailand (PCT-1), Bangkok, Thailand.

7. Jirawutthiwongchai, J.; and Chirachanchai, S. (2010, July 9-10) Chitosan nanospheres for delivery system. Proceeding of Joint Symposium on Advanced Polymers And Nanomaterials (Chula - Inha Joint Symposium), Bangkok, Thailand.
8. Jirawutthiwongchai, J. and Chirachanchai, S., (2010, April 22). Nano-structured Chitosan for Allergen Delivery System. Proceeding of the 16th PPC Symposium on Petroleum, Petrochemicals, and Polymers 2010, Bangkok, Thailand.
9. Jirawutthiwongchai, J. and Chirachanchai, S., (2009, August 23-25). Molecular Design and Synthesis of Nanoparticles Chitosan. Proceeding of the International Symposium in Science and Technology, Kansai, Japan.

Presentations:

1. Jirawutthiwongchai, J.; Draeger, G.; and Chirachanchai, S. (2014, July 8) Rapid Hybridization of Chitosan-Gold Nanoparticles via Metal-free Click in Water-based System for Naked-eye Antigen Detection. Paper presented at The 2014 IUPAC World Polymer Congress (MACRO 2014), Chiang Mai, Thailand. (Oral presentation)
2. Jirawutthiwongchai, J.; Draeger, G.; and Chirachanchai, S. (2014, March 20- 21) Hybridization of Water-based Chitosan-Gold via Copper-free Click for Naked-eye Antigen Detection. Paper presented at The Fourth Polymer Conference of Thailand (PCT-4), Bangkok, Thailand. (Oral presentation)
3. Jirawutthiwongchai, J; and Chirachanchai, S. (2012, July 17) Modification of chitosan: molecular design, synthesis, and.potential application. Paper presented at Organic Chemistry Lecture Hall, Leibniz University of Hannover, Hannover, Germany. (Oral presentation)
4. Jirawutthiwongchai, J.; Mai-Ngam, K.; and Chirachanchai, S. (2011, May 29-June 3) Nanoparticulate low molecular weight chitosan-g-phenylalanine-g-mPEG and its potential application of allergen delivery system. Paper presented at Europolymer Conference 2011 - Biobased Polymer and Related Biomaterials (EUPOC2011), Gargnano, Italy. (Oral presentation)

5. Jirawutthiwongchai, J.; and Chirachanchai, S. (2011, March 25-26) Water-based nanoparticulate chitosan: molecular design, synthesis, and potential application in allergen delivery system. Paper presented at The 3rd PKU-CU Nano Bilateral seminar, Bangkok, Thailand. (Poster presentation)
6. Jirawutthiwongchai, J.; Mai-ngam, K.; and Chirachanchai, S. (2010, August 26-27) Chitosan nanospheres for allergen delivery system: molecular design, synthesis, and allergen incorporation. Paper presented at The Sixth National Chitin-Chitosan Conference (6^{th} NCCC), Bangkok, Thailand. (Poster presentation)
7. Jirawutthiwongchai, J.; Mai-ngam, K.; and Chirachanchai, S. (2010, October 7-8) Chitosan nanoparticles by self-assembly with biomolcules for allergen delivery system. Paper presented at The First Polymer Conference of Thailand (PCT-1), Bangkok, Thailand. (Poster presentation)
8. Jirawutthiwongchai, J.; and Chirachanchai, S. (2010, July 9-10) Chitosan nanospheres for delivery system. Paper presented at Joint Symposium on Advanced Polymers And Nanomaterials (Chula - Inha Joint Symposium), Bangkok, Thailand. (Poster presentation)
9. Jirawutthiwongchai, J. and Chirachanchai, S., (2010, April 22). Nano-structured Chitosan for Allergen Delivery System. Paper presented at the 16^{th} PPC Symposium on Petroleum, Petrochemicals, and Polymers 2010, Bangkok, Thailand. (Poster presentation)
10. Jirawutthiwongchai, J. and Chirachanchai, S., (2009, August 23-25). Molecular Design and Synthesis of Nanoparticles Chitosan. Paper presented at International Symposium in Science and Technology, Kansai, Japan. (Poster presentation)