CHAPTER II

EXPERIMENTAL

2.1 Instruments and experiments

Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel (Merck Kieselgel 60 F_{254}) (Merck KgaA, Darmstadt, Germany). Melting points were determined with a Stuart Scientific Melting Point SMP 1 (Bibby Sterilin, Ltd., Staffordshire, UK.). For UV irradiation, broad band UVA (320-400 nm) was generated by F24T12/BL/HO (PUVA) lamp (National Biological Corporation, Twinsburg, Ohio, USA) and broad band UVB (280-320 nm) was generated by FSX24T12/UVB/HO lamp (National Biological Corporation, Twinsburg, Ohio, USA). UV Irradiance was measured using UVA-400C and UVB-500C power meter (National Biological Corporation, Twinsburg, Ohio, USA).

The ^{1}H- and ^{13}C-NMR spectra were obtained in deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) with tetramethylsilane (TMS) as an internal reference using Varian Mercury spectrometer which operated at 400.00 MHz for ^{1}H and 100.00 MHz for ^{13}C nuclei (Varian Company, U.S.A.). The FT-IR spectra were recorded on a Nicolet Fourier Transform Infrared spectrophotometer: Impact 410 (Nicolet Instrument Technologies, Inc. WI, U.S.A.). Molecular weight were determined by gel permeation chromatography: Waters 600E Multisolvent Delivery System (Waters, MA U.S.A.). UV Spectra were obtained with the aid of HP 8453 UV/VIS spectrophotometer (Agilent Technologies, CA U.S.A.). The UV absorbances were recorded using a quartz cell with 1 cm pathlength. The mass spectra were recorded on Mass Spectrometer: Waters Micromass Quatto micro API ESCi (Waters, MA U.S.A.)

2.2 Chemicals

Solvents used in syntheses and spectroscopic techniques were reagent or analytical grades purchased from Labscan (Bangkok, Thailand). *p*-Hydroxycinnamic acid, 2-bromoethanol, 3-bromo-1-propanol, 6-bromo-1-hexanol, 11-bromo-1-undecalnol, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), and 1-Hydroxy-benzotriazole (HOBt) were purchased from Acros (New Jersey, U.S.A.). Potassium carbonate, *p*-toluene sulfonic acid, Amberlyst-15 and

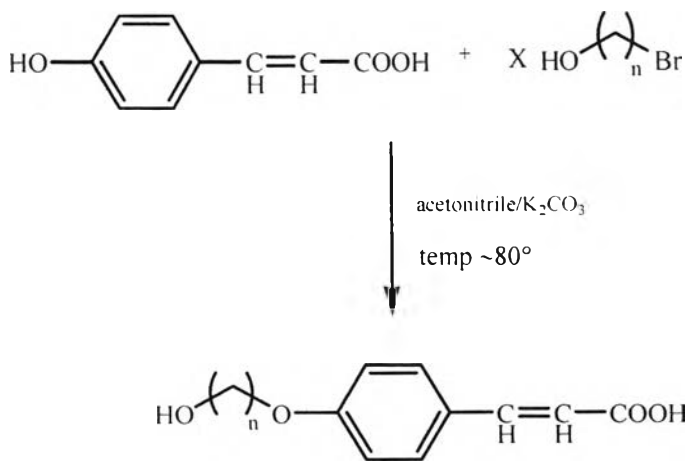
pentaethylene glycol ditosylate were purchased from Fluka Chemical Company (Buchs, Switzerland). N,N'-Dicyclohexylcarbodiimide (DCC) was purchased from Merck (Darmstad, Germany). Standard OMC was a gift from Merck Co., Ltd. (Bangkok, Thailand).

2.3 Syntheses of monomers (M-2, M-3, M-6 and M-11)

In a 250 mL 2 necks round bottom flask, *p*-hydroxycinnamic acid (1.64 g, 0.01 mol) was dissolved in 50 mL of acetonitrile. Potassium carbonate (13.82 g, 0.10 mol) and bromo alkyl alcohol were added (Table 2.1) and the mixture was then refluxed at 81-82°C until no *p*-hydroxycinnamic acid could be detected (by TLC). The reaction mixture was filtered through filter paper and washed with acetonitrile.

For reaction using 2-bromoethanol, 3-bromo-1-propanol and 6-bromo-1-hexanol, the filtrants were extracted with 30 mL of saturated sodium bicarbonate and 20 mL of dichloromethane 2 times to remove bromo alkyl alcohol. The aqueous layer was poured slowly into a beaker containing 20 mL of cold 40% hydrochloric acid. The white solid was separated by suction filtration, washed with cold water and dichloromethane.

For reaction using 11-bromo-1-undecanol, the filtrant was extracted with 30 mL of saturated sodium bicarbonate and 30 mL of hexane 3 times to remove 11-bromo-1-undecanol. The aqueous layer was acidified by slowly adding with 40% hydrochloric acid. The white solid was separated by suction filtration, washed with cold water and hexane.



Cpd M-n

n = 2, 3, 6, 11

Table 2.1 Amount of bromo-alkyl alcohol used in the reactions

Compounds	n	Mole equivalent of bromo-alkyl alcohol (X)
M-2	2	10
M-3	3	5
M-6	6	3
M-11	11	3

Owing to an excessive addition of bromo-alkyl alcohol, it was essential to find a way to recover the excess bromo alkyl alcohol. In this research, acid-base technique was done using basic aqueous and different organic solvents; dichloromethane was used to extract 2-bromoethanol, 3-bromo-1-propanol and 6-bromo-1-hexanol while hexane was used to extract 11-bromo-1-undecanol.

p-(2-Hydroxy ethoxy) cinnamic acid **(M-2)** : white solid (73%), m.p. 193.5-195.5°C, R_f 0.31 (70% EtOAc/Hex), IR (KBr, cm^{-1}) 3387-2580, 1676, 1599 and 1245; ^{1}H-NMR ($CDCl_3$) δ (ppm) : 7.70 (d, J = 16.6 Hz, 1H, Ar-C**H**=), 7.50 (d, J = 8.6 Hz, 2H, Ar-**H**), 6.94 (d, J = 8.5 Hz, 2H, Ar-**H**), 6.32 (d, J = 15.3 Hz, 1H, =C**H**-COOH), 4.13 (t, J = 4.1 Hz, 2H, -C**H**$_2$-O-Ar), 3.99 (t, J = 4.9 Hz, 2H, HO-C**H**$_2$); ^{1}H-NMR (DMSO-d_6) δ (ppm) : 12.2 (br, 1H, -COO**H**), 7.61 (d, J = 8.0 Hz, 2H, Ar-**H**), 7.52 (d, J = 15.5 Hz, 1H, Ar-C**H**=), 6.95 (d, J = 8.5 Hz, 2H, Ar-**H**), 6.35 (d, J = 16.0 Hz, 1H, =C**H**-COOH), 4.01(t, J = 11.9 Hz, 2H, -O-C**H**$_2$), 3.70 (t, J = 12.7 Hz, 2H, HO-C**H**$_2$); ^{13}C-NMR (DMSO-d_6) δ (ppm) : 168.4 (-**C**OOH), 144.3 (Ar-**C**H=), 160.9 (-O-Ar), 127.2 (Ar-CH), 130.4, 115.3 (aromatic carbons), 116.9 (=**C**H-COOH) , 70.1 (–**C**H$_2$-O-Ar), 60.1 (HO-**C**H$_2$-); MS-ES negative: m/z = 206.87 **(Figure B.5)**.

p-(3-Hydroxy propoxy) cinnamic acid **(M-3)** : white solid (97%), m.p. 152.0-154.0°C, R_f 0.31 (70% EtOAc/Hex), IR (KBr, cm^{-1}) 3420-2362, 1676, 1603 and 1247; ^{1}H-NMR ($CDCl_3$) δ (ppm) : 7.71 (d, J = 16.6 Hz, 1H, Ar-C**H**=), 7.49 (d, J = 8.6 Hz, 2H, Ar-**H**), 6.92 (d, J = 8.6 Hz, 2H, Ar-**H**), 6.31 (d, J = 16.5 Hz, 1H, =C**H**-COOH), 4.16 (t, J = 6.3 Hz, 2H, -C**H**$_2$-O-Ar), 3.88 (t, J = 5.3 Hz, 2H, HO-C**H**$_2$); ^{1}H-NMR (DMSO-d_6) δ (ppm) : 10.02 (br, 1H, -COO**H**), 7.59 (d, J = 8.5 Hz, 2H, Ar-**H**), 7.52 (d, J =16.4 Hz, 1H, Ar-C**H**=), 6.93 (d, J = 8.1 Hz, 2H, Ar-**H**),

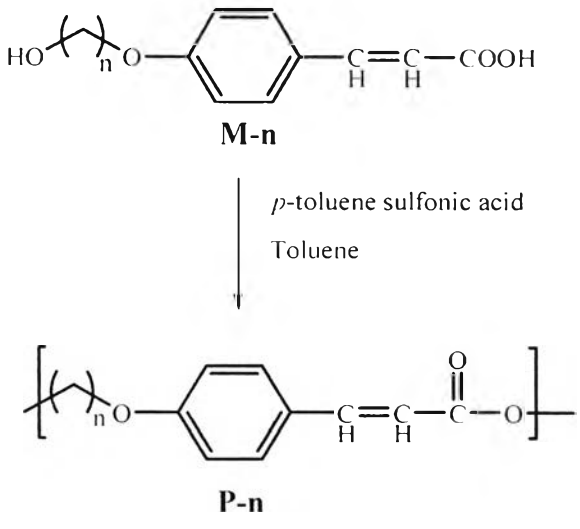
6.34 (d, J = 15.9 Hz, 1H, =C**H**-COOH), 4.04 (t, J = 5.6 Hz, 2H, -C**H**$_2$-O-Ar), 3.53 (t, J = 4.7 Hz, 2H, HO-C**H**$_2$); ^{13}C-NMR (DMSO-d_6) δ (ppm) : 168.3 (-**C**OOH), 144.2 (Ar-**C**H=), 160.8 (-O-Ar), 127.1 (Ar-CH), 130.4, 115.2 (aromatic carbons), 116.8 (=**C**H-COOH) , 65.2 (–**C**H$_2$-O-Ar), 57.6 (HO-**C**H$_2$-) and 32.4 (-**C**H$_2$-); MS-ES negative: m/z = 221.09 (**Figure B.10**).

p-(6-Hydroxy hexyloxy) cinnamic acid **(M-6)** : white solid (69%), m.p. 162-165°C, R$_f$ 0.36 (70% EtOAc/Hex), IR (KBr, cm^{-1}) 3250-2547, 1668, 1603 and 1247; ^{1}H-NMR ($CDCl_3$) δ (ppm) : 7.70 (d, J = 15.5 Hz, 1H, Ar-C**H**=), 7.48 (d, J = 7.9 Hz, 2H, Ar-**H**), 6.90 (d, J = 7.8 Hz, 2H, Ar-**H**), 6.31 (d, J = 17.4 Hz, 1H, =C**H**-COOH), 3.99 (t, J = 6.3 Hz, 2H, -C**H**$_2$-O-Ar), 3.66 (t, J = 3.6 Hz, 2H, HO-C**H**$_2$); ^{1}H-NMR (DMSO-d_6) δ (ppm) : 12.20 (br, 1H, -COO**H**), 7.59 (d, J = 7.3 Hz, 2H, Ar-**H**), 7.50 (d, J = 16.1 Hz, 1H, Ar-C**H**=), 6.92 (d, J = 7.9 Hz, 2H, Ar-**H**), 6.33 (d, J = 16.5 Hz, 1H, =C**H**-COOH), 3.97 (t, J = 7.0 Hz, 2H, -O-C**H**$_2$), 3.30 (t, J = 7.9 Hz, 2H, HO-C**H**$_2$), 1.68, 1.55-1.09 (br, -C**H**$_2$-); ^{13}C-NMR (DMSO-d_6) δ (ppm) : 168.0 (-**C**OOH), 144.0 (Ar-**C**H=), 160.8 (-O-Ar), 127.1 (Ar-CH), 130.4, 115.2 (aromatic carbons), 116.8 (=**C**H-COOH) , 68.0 (-**C**H$_2$-O-Ar), 61.1 (HO-**C**H$_2$-), 32.90, 29.08 and 25.82 (alkyl carbons); MS-ES negative: m/z = 263.20 (**Figure B.15**).

p-(11-Hydroxy undecyloxy) cinnamic acid **(M-11)** : white solid (89%), m.p. 128.0-130.0°C, R$_f$ 0.39 (70% EtOAc/Hex), IR (KBr, cm^{-1}) 3396-2567, 1683, 1606 and 1280; ^{1}H-NMR ($CDCl_3$) δ (ppm) : 7.71 (d, J = 15.8 Hz, 1H, Ar-C**H**=), 7.48 (d, J = 8.46 Hz, 2H, Ar-**H**), 6.90 (d, J = 8.56 Hz, 2H, Ar-**H**), 6.31 (d, J = 15.71 Hz, 1H, =C**H**-COOH), 3.99 (t, J = 6.0 Hz, 2H, -C**H**$_2$-O-Ar), 3.64 (t, J = 6.5 Hz, 2H, HO-C**H**$_2$); ^{1}H-NMR (DMSO-d_6) δ (ppm) : 7.58 (d, J = 9.0 Hz, 2H, Ar-**H**), 7.51 (d, J = 16.6 Hz, 1H, Ar-C**H**=), 6.91 (d, J = 9.0 Hz, 2H, Ar-**H**), 6.33 (d, J = 16.8 Hz, 1H, =C**H**-COOH), 3.95 (t, J = 7.2 Hz, 2H, -O-C**H**$_2$), 3.34 (t, J = 7.4 Hz, 2H, HO-C**H**$_2$), 1.67,1.37 (br, -C**H**$_2$-); ^{13}C-NMR (DMSO-d_6) δ (ppm) : 168.3 (-**C**OOH), 144.2 (Ar-**C**H=), 160.8 (-O-Ar), 127.1 (Ar-CH), 130.3, 115.2 (aromatic carbons), 116.8 (=CH-COOH), 68.0 (-**C**H$_2$-O-Ar), 61.2 (HO-**C**H$_2$-), 33.0, 29.4 and 26.0 (alkyl carbons); MS-ES negative: m/z = 333.32 (**Figure B.20**).

2.4 Syntheses of polymers

2.4.1 Homopolymer



2.4.1.1 Polymerization by *p*-toluene sulfonic acid

Monomer (0.50 g) and *p*-toluene sulfonic acid (0.10 g, 20% w/w) were refluxed in 30 mL of toluene at 110°C. TLC (using 70%ethyl acetate/hexane as mobile phase) was used to monitor the progress of the reaction; disappearing of monomer's spot together with the appearance of polymer tail indicated the complete of the reaction. The reaction mixture was then cooled to room temperature and filtered through No.1 Whatman filter paper. Since some of the polymeric products were in the filtrate and some were solid precipitate, both filtrate and filtrant were worked out.

The filtrate was evaporated, and then dissolved with 25 mL of dichloromethane. This solution was then neutralized with 3x20 mL of saturated sodium bicarbonate solution. The organic layer was dried with anhydrous sodium sulfate, and solvent was removed by rotary evaporator. Solid product obtained was then subjected to spectroscopic analyses.

The insoluble part from the reaction mixture was washed with 20 mL of dichloromethane and 3x20 mL of water to remove *p*-toluene sulfonic acid. Insoluble product was then dried and subjected to spectroscopic analyses.

Poly(*p*-propoxy cinnamate) **(P-3)** : brown solid , IR (KBr, cm^{-1}) 3427, 2958, 1708, 1604 and 1251; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.80-6.15 (Ar-**H** , Ar-C**H**=, =C**H**-COO-), 4.46-3.50 (-C**H**$_2$-O-Ar, -COO-C**H**$_2$-) and 2.48-1.86 (-C**H**$_2$-).

Poly(*p*-hexyloxy cinnamate) **(P-6)** : pale brown solid, IR (KBr, cm^{-1}) 3403, 2939, 1709 1605 and 1290; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.69-6.10 (Ar-**H** , Ar-C**H**=, =C**H**-COO-), 4.24-3.29 (-C**H**$_2$-O-Ar, -COO-C**H**$_2$-) and 2.44-1.12 (-C**H**$_2$-).

Poly(*p*-undecyloxy cinnamate) **(P-11)** : pale brown solid IR (KBr, cm^{-1}) 3423, 2977, 1709, 1604 and 1252; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.67-6.25 (Ar-**H**, Ar-C**H**=, =C**H**-COO-), 4.24-3.60 (-C**H**$_2$-O-Ar, -COO-C**H**$_2$-) and 1.83-1.15 (-C**H**$_2$-).

Dilution system

To enhance cyclization, dilution of the reaction mixture was introduced after an appropriate time. Monomer M-11 (0.20 g) and *p*-toluene sulfonic acid (0.04 g, 20% w/w) were refluxed in 20 mL of toluene at 110°C. TLC (using 70% ethyl acetate/hexane as mobile phase) was used to monitor the progress of the reaction. After 24 hour 80 mL toluene was added to the reaction mixture and the reaction was refluxed for another 48 hours. The reaction mixture was cooled to room temperature and filtered through No.1 Whatman filter paper. Since some of the product had precipitated out during the cooling of the reaction mixture, both filtrate and filtrant were then worked out.

The filtrate was evaporated, then dissolved with 25 mL of dichloromethane and neutralized with 3x20 mL of saturated sodium bicarbonate solution. Product was dried with anhydrous sodium sulfate, and solvent was removed by rotary evaporator.

The insoluble part from the reaction mixture was dissolved with dichloromethane and washed with 3x20 mL of water to remove *p*-toluene sulfonic acid. Insoluble product was dried.

Poly(*p*-undecyloxy cinnamate) **(P-11dil)** : pale yellow solid IR (KBr, cm^{-1}) 3431, 2926, 1708, 1605 and 1297; ^{1}H-NMR ($CDCl_3$) δ (ppm) : 7.56 (d, J = 15.6 Hz, 1H, Ar-C**H**=), 7.38 (Ar-**H**), 6.80 (Ar-**H**), 6.23 (d, J = 16.3 Hz, 1H, =C**H**-COOH), 4.12, 3.86 and 3.56 (t, 2H, -C**H**$_2$-O-Ar, -COO-C**H**$_2$-).

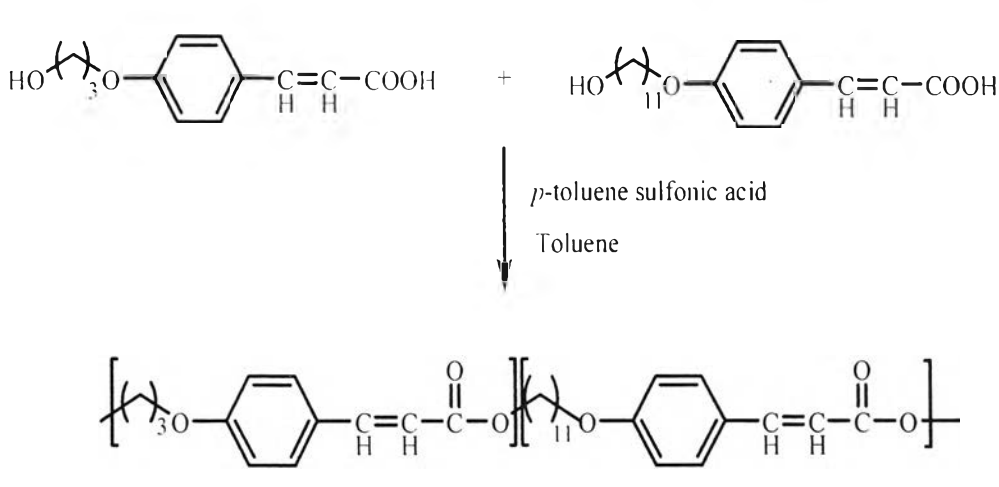
2.4.1.2 Polymerization by coupling agent

Monomer M-2 (0.20 g, 1×10^{-3} mole) and N,N′-dicyclohexylcarbodiimide, DCC (0.20 g, 1×10^{-3} mole) were refluxed in 15 mL of acetone at 54-56°C. After 2 hours at room temperature, white precipitate was observed, but the reaction was still stirred. After 24 hours, white solid N,N′-dicyclohexylurea (DCU) was filtered off and the filtrate was evaporated, then dissolved with 30 mL of dichloromethane and washed with 25 mL of water 2 times. Product was dehydrated with anhydrous sodium sulfate and solvent was removed by rotary evaporator.

Poly(*p*-ethoxy cinnamate) **(P-2)** : orange solid IR (KBr, cm^{-1}) 3000-3300, 2932, 1704, 1647 and 1211-1130; ^{1}H-NMR ($CDCl_3$) δ(ppm): 7.89-6.40 (Ar-**H**, Ar-C**H**=, =C**H**-COO-), 4.13, 4.00 and 3.78 (-O-C**H**$_2$-, -C**H**$_2$-O-Ar, -COO-C**H**$_2$-).

2.4.2 Copolymer

2.4.2.1 Poly(*p*-propoxy cinnamate)-*co*-(*p*-undecyloxy cinnamate)



Monomer M-3 (0.25 g), monomer M-11 (0.09 g) and *p*-toluene sulfonic acid (0.10 g, 20% w/w) were refluxed in 30mL toluene at 110°C. During 72 hours of the reflux, two batches of 0.08 g M-11 were added to the reaction mixture at 12 and 24 hours of the reaction. The reaction was quenched by removing from heat and then filtered through Whatman No.1 paper. Both insoluble (filtrant) and soluble product (filtrate) were worked out.

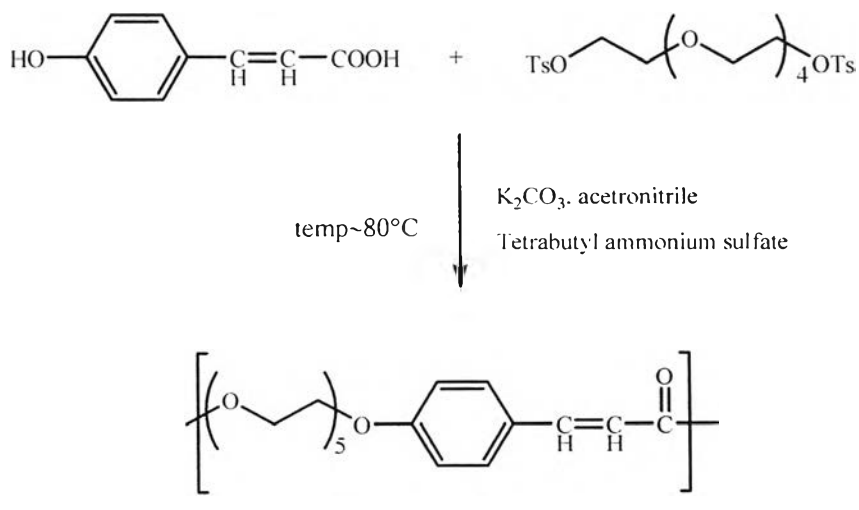
The filtrate was evaporated, then dissolved in 30 mL of dichloromethane and washed with 25 mL of saturated sodium bicarbonate solution 3 times. The solution was then dried with anhydrous sodium sulfate before subjected to rotary evaporator to remove the solvent.

The insoluble part from reaction mixture was washed with water to remove *p*-toluene sulfonic acid.

Poly(*p*-propoxy cinnamate)-*co*-(*p*-undecyloxy cinnamate) **(P-3/11)**: pale brown solid IR (KBr, cm^{-1}) 3409, 2927, 1688, 1605 and 1252; ^{1}H-NMR ($CDCl_3$) δ (ppm) : 7.75-6.09 (Ar-**H** , Ar-C**H**=, =C**H**-COO-), 4.40-3.44 (-C**H**$_2$-O-Ar, -COO-C**H**$_2$-) and 2.17-1.10 (-C**H**$_2$-).

2.4.2.2 Poly(penta ethylene glycol cinnamate)

In the 50 ml 2 necks round bottom flask, *p*-hydroxycinnamic acid (0.16 g, 1×10^{-3} mole) was dissolved in 10 mL of acetonitrile until clear (a little heat was used). Potassium carbonate (1.38 g, 1×10^{-2} mole), tetrabutyl ammonium sulfate (0.04 g, 20% w/w) and pentaethylene glycol ditosylate (1.09 g, 2×10^{-3} mole) were added. The mixture was refluxed at 78-80°C. TLC (using 70%ethyl acetate/hexane as mobile phase) was used to monitor the progress of the reaction; disappearing of *p*-hydroxycinnamic acid spot together with the appearance of polymer tail indicated the complete of the reaction. The mixture was cooled to room temperature, then evaporated and redissolved in 25 mL ethyl acetate before it was washed with (3x20mL) water. Waters was removed from the organic solution using anhydrous sodium sulfate. The solvent was then removed by rotary evaporator.



Poly(pentaethylene glycol cinnamate) **(PPGC)** : yellowish oil, IR (Nujol, cm^{-1}) 2955, 2856, 1707, 1603 and 1258; ^{1}H-NMR ($CDCl_3$) δ (ppm) : 7.62 (d, J = 16.1 Hz, 1H, Ar-C**H**=), 7.43 (d, J = 8.16 Hz, 2H, Ar-**H**), 6.88 (d, J = 7.8 Hz, 2H, Ar-**H**), 6.31 (d, J = 15.5 Hz, 1H, =C**H**-COOH), 4.32 (br, -COO-C**H**$_2$-CH$_2$-O), 4.25 (br, -CH$_2$-C**H**$_2$-O-), 4.12 (br, -CH$_2$-C**H**$_2$-O-Ar), 3.86-3.54 (br, -C**H**$_2$-O-Ar); ^{13}C-NMR ($CDCl_3$) δ (ppm) : 167.2 (-COO-), 144.7 (Ar-**C**H=), 160.6 (-O-Ar), 127.2 (Ar-CH), 129.7, 114.9 (aromatic carbons), 115.3 (=**C**H-COO) and 72.7-61.6 (–O-**C**H$_2$-).

2.5 General procedure for molar absorptivity measurements[38,39]

Tested compounds were dissolved in dichloromethane or methanol to the concentration of about 1 g/L. The resulting stock solution was then diluted to appropriate concentrations using corresponding solvents. The UV absorbance of each final dilution was recorded by scanning wavelengths between 200 and 800 nm. The molar absorptivity (ε) at the wavelength of maximum absorbance (λ_{max}) was calculated using Beer's law:

$$A = \varepsilon bc$$

Where A is absorbance

b is the cell path length (1 cm)

c is the concentration of the absorbing species in mole per litre

2.6 General procedure for photostability test[38]

The photostability tests for the UV filters were performed in dichloromethane and methanol. Stock solution of each compound was prepared in a 100 mL volumetric flask. The resulting solutions were divided into two parts. One part was kept away from light (covered with foil) at room temperature (dark sample) while at the same temperature the other part was irradiated by artificial UV lamp (irradiated sample) at 5.8 mW/cm^2 UVA and 0.47 mW/cm^2 UVB. Then UV absorption profile of each sample was acquired using UV/VIS spectrometer. The absorbance of irradiated sample at various irradiant times were compared to those of dark samples.

The calculation of percent relative absorbance of each irradiated sample was done using the following equation.

$$\text{Percent of relative absorbance} = \left[\frac{\text{Absorbance of irradiated sample at time X}}{\text{Absorbance of dark sample (starting time)}} \right] \times 100$$