

- Uehara, Y.; Hori, M.; Umezawa, H. *Biochim. Biophys. Acta* **1974**, 374, 82. (d) Hall, C.; Bertasso, A.; Watkins, J.; Georgopapadakou, N. *J. Antibiot.* **1992**, 45, 1697.
3. (a) Streicher, W.; Reinshagen, H.; Turnowsky, F. *J. Antibiotics*. **1978**, 31, 725. (b) Pierdet, A.; Nedelec, L.; Delaroff, V.; Allais, A. *Tetrahedron* **1980**, 36, 1736. (c) Pasquet, G.; Boucherot, D.; Pilgrim, W.; Wright, B. *Tetrahedron Lett.* **1980**, 21, 931. (d) Kasahara, K.; Iida, H.; Kibayashi, C. *J. Org. Chem.* **1989**, 54, 2225.
4. (a) Wang, Y.; Izawa, T.; Kobayashi, S.; Ohno, M. *J. Am. Chem. Soc.* **1982**, 104, 6465. (b) Tanner, D.; Somfai, P. *Tetrahedron Lett.* **1988**, 29, 2373. (c) Hashiguchi, S.; Kawada, A.; Natsugari, H. *J. Chem. Soc., Perkin Trans. I* **1991**, 2435. (d) Schmidt, U.; Staebler, F.; Lieberknecht, A. *Synthesis* **1992**, 482. (e) Maycock, C.; Barros, M.; Santos, A.; Godinho, L. *Tetrahedron Lett.* **1992**, 33, 4633. (f) Masters, J.; Hegedus, L. *J. Org. Chem.* **1993**, 58, 4547. (g) Socha, D.; Jurczak, M.; Chmielewski, M. *Tetrahedron Lett.* **1995**, 36, 135.
5. Shibahara, S.; Kondo, S.; Maeda, K.; Umezawa, H.; Ohno, M. *J. Am. Chem. Soc.* **1972**, 94, 4353.
6. Ohno, M.; Kobayashi, S.; Iimori, t.; Wang, Y.; Izawa, T. *J. Am. Chem. Soc.* **1981**, 103, 2405.
7. Borch, R.; Bernstein, M.; Durst, H. *J. Am. Chem. Soc.* **1971**, 93, 2897.
8. (a) Ognyanov, V.; Datcheva, V.; Kyler, K. *J. Am. Chem. Soc.* **1991**, 113, 6992. (b) Huuhtanen, T.; Kanerva, L. *Tetrahedron: Asymmetry* **1992**, 3, 1223.
9. Parker, D. *J. Chem. Soc., Perkin Trans. II* **1983**, 83.
10. ¹H NMR (200 MHz, CDCl₃) δ: 3: 2.73 (m, 4H), 3.67 (s, 3H), 4.4 (m, 1H), 5.9 (s, 2H), 5.62 (bd, *J*=9.1 Hz, 1H), 7.35 (s, 5H). 4: 1.41 (s, 9H), 2.5 (dq, *J*=5.1 Hz, 15.7 Hz, 2H), 3.2 (bs, 1H), 3.62 (m, 2H), 3.95 (m, 1H), 5.1 (s, 2H), 5.7 (bd, *J*=9.1 Hz, 1H), 7.35 (s, 5H). 5: 1.4 (s, 9H), 2.5 (d, *J*=5.86 Hz, 2H), 2.76 (t, *J*=5.86 Hz, 2H), 4.4 (m, 1H), 5.1 (s, 2H), 5.9 (bd, *J*=8.0 Hz, 1H), 7.3 (s, 5H), 9.72 (s, 1H). 6: 1.45 (s, 9H), 2.0 (m, 2H), 2.55 (dq, *J*=4.9 Hz, 16.3 Hz, 2H), 4.19 (m, 1H), 4.5 (m, 1H), 4.67 (bs, 1H), 5.12 (s, 2H), 5.95 (bd, *J*=8.4 Hz, 1H) 7.35 (s, 5H). 7: 1.4 (s, 9H), 2.0 (m, 2H), 2.2 (s, 3H), 2.7 (m, 2H), 4.2 (m, 1H), 5.12 (s, 2H), 5.46 (t, *J*=7.8 Hz, 1H), 5.85 (s, 1H), 7.3-7.45 (m, 10H). ¹H NMR (500 MHz, D₂O) δ: 8: 1.8 (m, 1H), 2.1 (m, 1H), 2.7 (m, 2H), 2.87 (dd *J*=4.51 Hz, 17.95 Hz, 1H), 3.2 (d, *J*=12.83 Hz, 1H), 3.37 (dd, *J*=4.04 Hz, 12.80 Hz, 1H), 4.1 (m, 1H). ES-MS (negative) 8: *m/z* 161 [M-H].

Itaconate Copolymer Bearing the Second-Order Nonlinear Optical Chromophores in Both Side Chains

Sangyup Song, Dong Hoon Choi*, Sun Jin Lim, Woong Sang Jahng, and Nakjoong Kim

Division of Polymer Research, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang 130-650, Korea

*Department of Textile Engineering, Kyung Hee University, Yongin-shi, Kyungki-Do 449-701, Korea

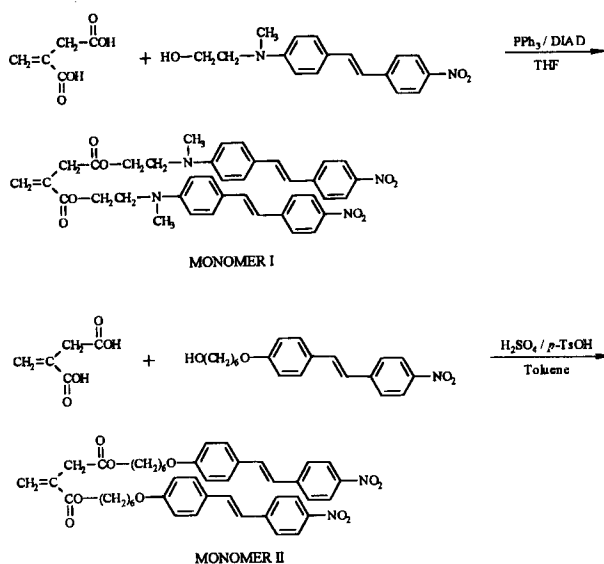
Received November 1, 1996

The nonlinear optical (NLO) properties of polymeric materials have been highlighted as a subject of numerous investigations for application in electro-optic (EO) and photonic devices over a decade.¹⁻³ Particularly, side chain polymeric structures drew much interest owing to their ease of synthesis and processibility. We also could find a lot of new features about the nonlinear optical active copolymers recently.¹⁻³ Resulting from our synthetic strategy, we could optimize our polymeric structure to maximize the second-order NLO effect employing a new monomer unit such as itaconate. Itaconic ester is a very promising monomer that can contain two NLO chromophores in one repeating unit. It was found that the itaconate is capable of building the special copolymer with commonly used comonomers such as methylmethacrylate, styrene, etc.⁴ Assuming that the non-centrosymmetry could be induced under poling process practically, the second-order nonlinear optical coefficient was known to be directly proportional to the concentration of the active chromophore. In this respect, we conclude our choice of itaconate as the most favorable NLO monomer. The purpose of this work is how much improvement of NLO effect we can achieve with the two fold increase of

the chromophore density in the copolymer. Therefore, we introduced our used chromophore of nitrostilbene in these structures by way of direct esterification and Mitsunobu reaction easily. Even though we employed several kinds of comonomers, we only reported here about the synthesis and NLO properties of the itaconate copolymers with methyl methacrylate.

In this study, we prepared itaconate monomers bearing second-order NLO-active dyes as shown in Scheme 1. 2-Methylene-succinic acid bis-[2-(methyl-{4-[2-(4-nitrophenyl)-vinyl]-phenyl}-amino)-ethyl] ester (monomer I) was synthesized by Mitsunobu reaction using diisopropylazodicarboxylate (DIAD) and triphenylphosphine in THF (49.9% yield).⁵ We also obtained 2-methylene-succinic acid bis-(2-{4-[2-(4-nitrophenyl)-vinyl]-phenoxy}-hexyl) ester (monomer II) by simple direct esterification using a Dean-stark apparatus under refluxing the reaction mixture in toluene with H₂SO₄/p-toluensulfonic acid as acid catalysts (81.7% yield).⁶

We carried out radical copolymerization of methylmethacrylate (MMA) and itaconate monomer in freshly distilled N-methyl-2-pyrrolidinone (NMP) (Figure 1). A mixture of itaconate monomer I (1.46 g, 2.11 mmole), MMA



Scheme 1.

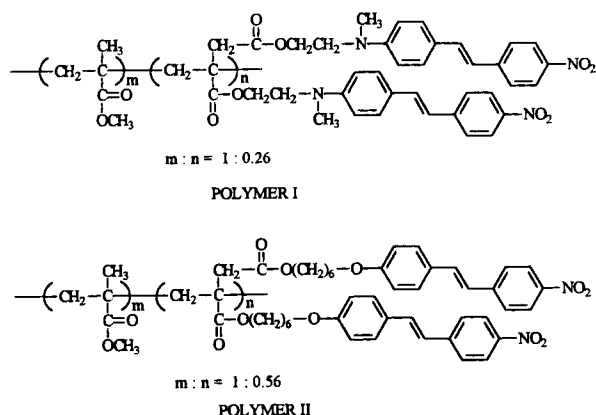


Figure 1. Structures of new itaconate-based copolymers.

(212 mg, 2.11 mmol) and AIBN (35.0 mg) in NMP (10.0 mL) were charged into a vacuum sealable tube. After freeze-vacuum-thaw cycle, the tube was sealed. The reaction mixture was stored at 65 °C for 48 hours. The resulting polymer was isolated by precipitation in hot ethanol and purified by reprecipitation from THF into methanol twice (Yield 38.0%). The resultant molar compositions of the copolymers were determined using UV-Visible absorption spectroscopy.

In the case of polymer I, the monomer feeding ratio (itaconate : MMA = 1 : 1) resulted in the composition of 0.26 : 1 in the copolymer. The molar composition of polymer II was figured out (itaconate : MMA = 0.56 : 1) with monomer feeding ratio (1 : 1). The resultant mole ratios of copolymers were hard to separate the characteristic chemical shift for precise integration. Therefore, we recorded the UV-VIS spectra of two copolymers to calculate the mole ratio even with some error. The film of itaconate polymer I exhibited absorption maximum around 431 nm and polymer II, around 376 nm. The melting temperature of monomer I was recorded at 155 °C in DSC thermogram. It was, however, peculiar that the monomer II might showed the liquid crystalline temperature ranging from 70 °C to 100 °C,

Table 1. Physical Properties and Measured d_{33} Values of the Polymers

Polymer	$\lambda_{\max}$ (nm)	$\lambda_{\text{cut-off}}$ (nm)	T_g (°C)	η_{inh}^a (dL/g)	Film thickness (μm)	d_{33} , d_{31} (pm/V)
Polymer I	431	534	114	0.06	0.86	78.1, 18.8,
Polymer II	376	455	90	0.05	0.91	29.2, 6.50

^a In DMF solution at 30 °C (0.5 g/dL).

which was confirmed under polarized optical microscope with hot stage. This will be discussed elsewhere. We also could observe the clear glass transition behavior in DSC thermograms of two copolymers. As we expected, the T_g was determined relatively lower than the polymethylmethacrylate. The homopolymer of itaconic ester showed relatively lower T_g than those of the other amorphous polymers such as poly(methyl methacrylate), polystyrene, polycarbonate, etc.⁴ The measured values of T_g were shown in Table 1.

Polymeric thin films were elaborated for nonlinear optical study. New copolymers showed good solubility in common organic solvent such as THF, TCE, chloroform, DMF, etc. All the films were spin coated on slide glass from THF/cyclohexanone solution filtered through microsyringe filter (pore size : 0.2 μm) and dried in vacuum oven at 70 °C overnight.

The second-order NLO properties of the films were characterized by second-harmonic generation (SHG). The films were poled using a corona poling technique in a wire-plane geometry.^{7,8} The poling voltage of polymer I was set around 7 kV at 105 °C for 15 min and in the case of polymer II was 6 kV at 80 °C for 15 min. The film thickness was measured using Tencor stylus P10 (resolution 5 Å) (Table 1). We followed the standard Maker fringe technique that was already well understood.⁹ A Q-switched Nd³⁺:YAG laser ($\lambda=1.064 \mu\text{m}$) operating in the TEM₀₀ mode was used as the fundamental beam and a calibrated quartz crystal was used as a reference ($d_{11}=0.5 \text{ pm/V}$).

Regarding the second-order nonlinear optical coefficient, d_{33} , two copolymers showed outstanding NLO effect after poling. We conclude that the soft segment of itaconate part can provide greater improvement of the molecular alignment to the poling direction compared to the other methylmethacrylate, styrene etc. Therefore, the degree of side chain orientation was achieved much higher than the others. Under the moderate degree of alignment, we can employ the Kleinman's symmetry rule to calculate the second-order NLO coefficient, d_{33} and d_{31} . It is, however, evidenced that the Kleinman's symmetry rule ($d_{33}=3d_{31}$) is not applicable to d_{33} and d_{31} of these copolymers. The factor of the ratio between d_{33} and d_{31} is about 4.1-4.5 which is larger than three.

Resulting from above outstanding features, we can claim the itaconate to be a good monomer unit for great improvement of NLO activity owing to higher degree of molecular alignment in the copolymer and their high density of the chromophore.

Shortly, we have synthesized a new class of NLO active copolymer using itaconate NLO monomers and studied their NLO properties using SHG measurement. The second-

order NLO coefficients, d_{33} values of these copolymers can be largely enhanced by our synthetic strategy. We are pursuing our endeavor to prepare new copolymers bearing this itaconate for exhibiting the high glass transition temperature (~ 130 – 150 °C). Additionally, thermal and photo-crosslinkable polymers will be designed and synthesized in a heterogeneous or spontaneous way for improving the temporal stability at a high temperature.

Acknowledgment. This work was supported by Ministry of Science and Technology. Authors thank Prof. Sin Doo Lee and Mr. Moo Jong Lim for helping the SHG measurement.

References

- Choi, D. H.; Song, S.; Lee, T. S.; Park, S. Y.; Kim, N. J. *Appl. Polym. Sci.* **1996**, 59, 9.
- Park, K. H.; Jahng, W. S.; Lim, S. J.; Kim, N. *Mol. Cryst. Liq. Cryst.* **1996**, 280, 27.
- Robello, D. R.; Dao, P. T.; Schildkraut, J. S.; Scozzafava, M.; Urankar, E. J.; Willand, C. S. *Chem. Mater.* **1995**, 7, 284.
- Sato, T.; Hirose, Y.; Seno, M.; Tanaka, H. J. *Polym. Sci., Polym. Chem. Ed.* **1995**, 33, 797; Cowie, J. M. G.; McEwen, I. J. *Polymer* **1975**, 16, 869.
- Monomer I mp 155.5 °C (by DSC); ^1H NMR (200 MHz, DMSO- d_6): δ 8.18 (d, 4H), 7.79 (d, 4H), 7.51 (d, 4H), 7.40 (d, 2H), 7.10 (d, 2H), 6.72 (m, 4H), 6.12 (s, 1H), 5.72 (s, 1H), 4.20 (t, 4H), 3.61 (t, 4H), 3.22 (s, 2H), 2.98 (d, 6H); Anal.: $\text{C}_{39}\text{H}_{38}\text{N}_4\text{O}_8$ (690.75) Calcd. C 67.8, H 5.54, N 8.11; Found C 67.5, H 5.61, N 8.09.
- Monomer II mp 143.6 °C (by DSC); ^1H NMR (200 MHz, DMSO- d_6): δ 8.15 (d, 4H), 7.74 (d, 4H), 7.53 (d, 4H), 7.40 (d, 2H), 7.20 (d, 2H), 6.90 (d, 4H), 6.19 (s, 1H), 5.81 (s, 1H), 3.89–4.09 (m, 8H), 3.34 (s, 2H), 1.35–1.67 (m, 16H); Anal.: $\text{C}_{45}\text{H}_{48}\text{N}_2\text{O}_{10}$ (776.33) Calcd. C 69.5, H 6.23, N 3.61; Found C 68.9, H 6.80, N 3.58.
- Mortazavi, M. A.; Knoesen, A.; Kowel, S. J. *J. Opt. Soc. Am. B* **1989**, 6, 733.
- Wijekoon, W. M. K. P.; Zhang, Y.; Karna, S. P.; Prasad, P. N.; Griffin, A. C.; Bhatti, A. M. *J. Opt. Soc. Am. B* **1992**, 9, 1832.
- Singer, K. D.; Sohn, J. E.; Lalama, S. J. *Appl. Phys. Lett.* **1986**, 49, 248.

Interactions of Mastoparan B with Phospholipid Vesicles: Relationships with Fusion and Leakage Actions (I)

Jang-Su Park*, Kyoung Ok Jung, Jong Cheul Kim, Kwang Ho Kim,
Nam Gyu Park[†], Hong Suk Suh, and Shin Won Kang

Department of Chemistry, Pusan National University, Pusan 609-735, Korea

[†]Department of Biotechnology and Bioengineering, Pukyong National University, Pusan 608-737, Korea

Received November 9, 1996

Mastoparan B (MP-B) is a tetradecapeptide toxin isolated from the venom of the hornet (*Vespa basalins*), and its primary structure has been determined as H-LKLKLSIVSWA-KKVL-NH₂.¹ The peptide belongs to the mastoparan homologs of vespid venoms. Mastoparan is known to modulate several kinds of enzymes, including calmodulin sensitive phosphodiesterase,² phospholipase A₂,³ and some kinds of GTP-binding regulatory proteins.⁴ Furthermore, mastoparan was found to interact with phospholipid membrane and to perturb the ion permeability of black lipid membrane.⁵

In this study, we analyzed the interactions of MP-B with unilamellar vesicles. Such interaction induces the release of aqueous content of liposomes alone or leakage followed of vesicle fusion, depending upon charges of vesicles and peptides employed, respectively. Finally, a model showing the relationship between peptide interactions, aggregation, leakage and fusion effects is proposed.

MP-B and [Ala¹²]MP-B (the 12st Lys residue was replaced by Ala) were synthesized by Fmoc-method in order to attain further information of the membrane-binding characteristics. Crude peptides, separated from resin, were

partially purified by HPLC, using C₁₈-ODS column (4.6 × 250 mm). The peptides were confirmed by amino acid analyzer and tandem mass spectrometer. Egg yolk phosphatidylcholine (EYPC) was isolated with silica gel column chromatography. Egg yolk phosphatidylglycerol (EYPG) was obtained by the hydrolysis of EYPC with phospholipase D, and was purified by passing through a silica gel column. The phospholipase D was partially purified from the inner yellowish-white leaves of Savoy cabbage by heat treatment and acetone precipitation. Small unilamellar vesicles (SUVs) were prepared with two lipids composed of EYPC and EYPG (3:1) as neutral and acidic vesicles, respectively. Phospholipid (20 mg, about 25 μmol) was dissolved in chloroform (1 mL) and dried by breathing of nitrogen in a conical glass tube. The dried lipid was hydrated in 2 mL of 5 mM Tris buffer (pH 7.4) with repeated vortexed-mixing at 50 °C for 30 min using an ultrasonic disrupter and diluted to 25 mL with the same buffer (lipid concentration, about 1.0 mM).

We have studied the interaction between peptide and membrane by monitoring the CD spectra which were ob-