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.

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5. Monomer I mp 155.5 °C (by DSC); ^{1}H 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.: $C_{39}H_{38}N_4O_8$ (690.75) Calcd. C 67.8, H 5.54, N 8.11; Found C 67.5, H 5.61, N 8.09.
6. Monomer II mp 143.6 °C (by DSC); ^{1}H 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.: $C_{45}H_{48}N_2O_{10}$ (776.33) Calcd. C 69.5, H 6.23, N 3.61; Found C 68.9, H 6.80, N 3.58.
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Interactions of Mastoparan B with Phospholipid Vesicles: Relationships with Fusion and Leakage Actions (I)

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Mastoparan B (MP-B) is a tetradecapeptide toxin isolated from the venom of the hornest (*Vespa basalins*), and its primary structure has been determined as H-LKLKSIVSWA-KKVL-NH_2.[1] The peptide belongs to the mastoparan homologes of vespid venoms. Mastoparan is known to modulate several kinds of enzymes, including calmodulin sensitive phosphodiesterase,[2] phospholipase A_2[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 [Ala^{12}]MP-B (the 12st Lys residue was replaced by Ala) were synthesized by Fomoc-method in order to attain further information of the membrane-binding characteristics. Crude peptides, separated from resin, were partially purified by HPLC, using C_{18}-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 phosphatidyglycerol (EYPG) was obtained by the hydrolysis of EYPC with phopholipase D, and was purified by passing through a silica gel column. The phosholipase 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 EYP-C-EYPG (3 : 1) as neutral and acidic vesicles, respectively. Phospholipid (20 mg, about 25 mmol) 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 a 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 monitring the CD spectra which were ob-

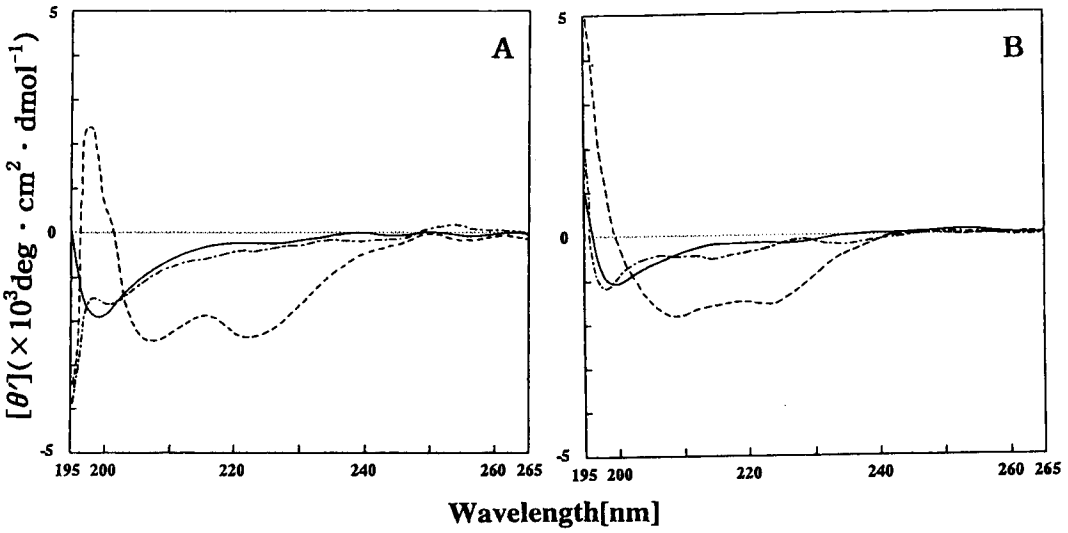

Figure 1. The CD spectra of MP-B(A) and [Ala[12]]MP-B (B) in Tes buffer (———), in the presence of EYPC-EYPG (3 : 1) (---) and EYPC (- • -) liposomes. The concentrations of peptide and liposomes are 0.25 mM and 1 mM, respectively.

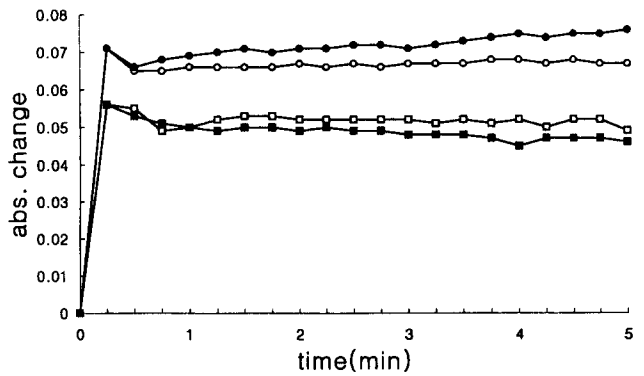

Figure 2. The light scattering of MP-Bs-induced aggregation of EYPC[MP-B (□), [Ala[12]]MP-B (■)] and EYPC/EYPG (3 : 1) [MP-B (○), [Ala[12]]MP-B (●)]. To obtain a light scattering due to aggregation of liposomes, A_{450} of liposomes was substracted from that of the liposomes in the presence of peptide.

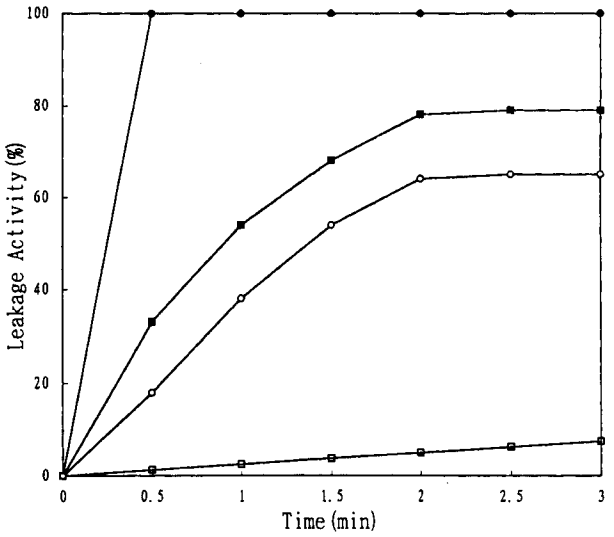

Figure 3. Time dependence of aqueous content leakage from EYPC[MP-B (□), [Ala[12]]MP-B (■)] and EYPC/EYPG (3 : 1) [MP-B (○), [Ala[12]]MP-B (●)] induced by MP-Bs. Preparation of liposomes containing 100 mM carboxyfluorescence and percentage of dye-release were performed according to the reported procedure.[6]

tained on Jasco J-715 spectropolarimeter at 25 °C (Figure 1). In buffer solution and in the presence of EYPC liposomes, MP-B and [Ala[12]]MP-B adopted a mainly random structure. In acidic liposome(EYPC-EYPG, 3 : 1), MP-Bs(MP-B and [Ala[12]]MP-B) had double minimum peaks at 205 and 222 nm region responsible for α-helical structure. Accordingly, the conformational change of MP-B on binding with membrane is probably due to the electrostatic interaction between the positively charged groups of MP-B and the negatively charged phosphate groups of phospholipid membrane, rather than hydrophobic interactions.

Aggregation was followed measuring light scattering with the wavelength set up at 450 nm and 25 °C (Figure 2). The presence of 1×10^{-4} M MP-B increased the light scattering of a sample of EYPC/EYPG liposomes, as compared with the scattering level of EYPC liposomes. [Ala[12]]MP-B showed similar scattering levels to MP-B in both EYPC and EYPC/EYPG liposomes. These results suggested that aggregation of liposomes induced by MP-B is strongly dependent on the negative charges of liposomes rather than the postive charge effect of MP-B.

Since the association of peptides to lipid bilayers frequently alters the membrane integrity and changes the permeability, we examined the release of encapsulated carboxyfluorescein from liposome induced by MP-B association. Figure 3 shows a typical time-course of aqueous content leakage from EYPC and EYPC/EYPG (3 : 1) vesicles induced by MP-Bs at pH 7.6 and 25 °C. Leakage of liposome contents was determined according to the reported fluorescence dye-release experiment.[6]

The result shows that the membrane perturbation effect of [Ala[12]]MP-B(substitution of one positively charged Lys residue by a neutral Ala at MP-B molecular) is larger than that of MP-B in both EYPC and EYPC/EYPG liposomes. Less

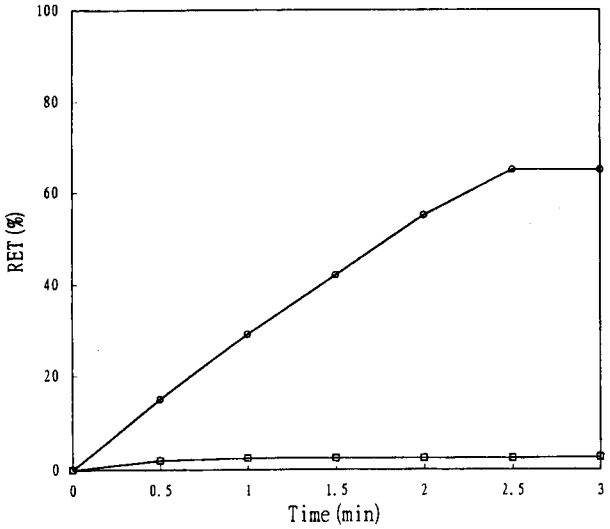

Figure 4. Time dependence of lipid intermixing from EYPC (□) and EYPC/EYPG (○) induced by 1×10^{-4} M MP-B at 25 °C. The RET (resonance energy transfer) assays monitor the energy transfer between the donor (cholesteryl anthracene 9-carboxylate) and acceptor(N-(7-nitrobenz-2-oxa-1,3-diazol)phosphatidylethanolamine) which were incorporated in separate vesicle populations. When both fluorescent lipids were in separate vesicles no energy transfer occurs. Fusion of vesicles results in intermixing of lipids and increase of RET. The percentage of RET (% RET) was calculated according to the following: % RET=(Fo-F)/(Fo-Fs)× 100 Where Fo and F are the donor fluorescence in the absence and in the presence of MP-B respectively, and Fs the fluorescence of donor when both donor and acceptor are in the same vesicle population.

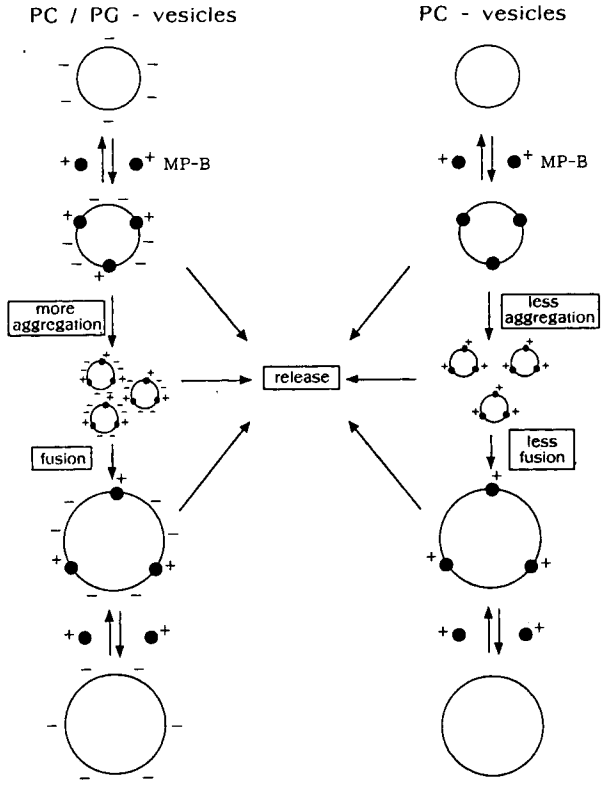

Figure 5. Schematic drawing showing the molecular interaction of MP-B with unchaged (EYPC) and negatively charged (EYPC/EYPG, 3 : 1) phospholipid vesicles. Relationships with fusion and leakage of aqueous content.

positive charge [Ala[12]]MP-B might lead to such difference. It should be noted that the ability of peptides induced dye release is much smaller in neutral liposomes than in the acidic liposomes. We also measured the time-course of EYPC and EYPC/EYPG vesicles fusion induced by 1×10^{-4} M MP-B (Figure 4).

Comparisons of kinetic release of aqueous content and fusion clearly indicate that release occurs before vesicle fusion is completed. Thus, leakage and fusion actions of MP-B appear to be not necessarily linked, and may be uncoupled. From our results, we can explain the different molecular interaction models of MP-B between neutral and acidic liposomes (Figure 5).

Phospholipid unilamella vesicles bearing negative charges (EYPC/EYPG, 3 : 1) are so stable that they do not aggregate and fuse spontaneously, because of electrostatic repulsion (Figure 5). But MP-B is a basic peptide, when MP-B interacts with negatively charged vesicles, both negative charges of the phospholipid head group and positive charges of peptide would facilitate vesicle cross-linking and subsquently aggregation and fusion (see Figure 5).

Since fusion of phospholipid vesicles consists of two distinct coupled steps, formation of vesicle aggregates and the fusion reaction itself, the association of MP-B with neutral vesicles will induce less fusion because less aggregation of vesicles occurs (see Figure 2). Association with negatively charged vesicles facilitates vesicle aggregation and appearance of defects, due to the presence of the peptide immersed into the lipid bilayer. The defects are brought *opposed* to each other because of vesicle aggregation. This configuration would render a structure which is energetically unfavorable, which will finally conduce to fusion. The membrane fusion activity of polycationic proteins was investigated in several system that use negatively charged membranes. Cytochrome *c*[7], myelin basic protein[8], as well as synthetic polylysine[9] and polyhistidine[10], among others, have fusion activity accompanied by an increase of membrane permeability. The interaction of myelin basic protein with unilamellar vesicles and subsequent aggregation, release and fusion, was extensively examined by Terbeest and Hoekstra.[11]

Our results obtained with MP-Bs show a similarity to those found with myelin basic protein, since both BP-Bs and myelin interact with liposomes induce leakage and aggregation in separate process.

The results presented here do not allow us to establish the molecular mechanism of fusion induced by MP-Bs. Futher studies are in progress in our laboratory.

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Versatile Synthetic Routes to Enantiomeric Dihydroxyethylene Dipeptide Isosteres *via* Intramolecular Amidation

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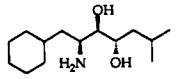
Since the aspartic protease renin catalyzes the hydrolysis of angiotensinogen to angiotensin I,[1] its inhibitors are expected to be of potential use in the treatment of hypertension and congestive heart failure.[1,2] Based on the transition state mimic of the scissile Leu-Val amide bond in angiotensinogen, the dihydroxyethylene dipeptide (DHED) isostere **1** was designed as a prospective C-terminal component for the development of renin inhibitors.[3] Several synthetic approaches to **1** have been described by employing stereoselective alkylation of imines,[4] one-pot reductive amination of epoxy ketone,[5] ring-opening of epoxides with sodium azide,[6] diastereoselective dihydroxylation of allylic amines[7] and enzymatic resolution.[8] Recently we have reinvestigated the electrophile promoted cyclization of trichloroacetimidates from allylic and homoallylic alcohols to attain a highly stereoselective amidation.[9] In this paper we report a divergent synthetic route to **1** and its enantiomer *ent*-**1** by extending the cyclization protocol to the stereocontrolled intramolecular amidation of trichloroacetimidates from (3R, 4S)-3,4-dihydroxy-6-methyl-1-heptene **4** and (2R,3S)-1,2,3-trihydroxy-5-pentene **9**.



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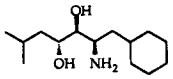
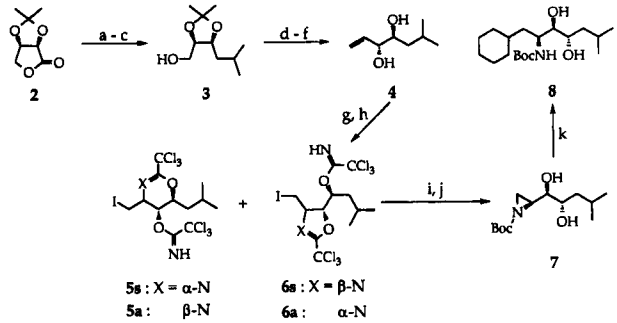


ent - **1**

The synthesis of DHED isostere **1** was initiated with DIBAL reduction of the known lactone **2**[10] followed by Wittig isopropylenation and hydrogenation to give alcohol **3** in 87% overall yield (Scheme 1). Swern oxidation[11] of **3** and the subsequent methylenation provided the volatile methylenic acetonide in 69% yield. Its acidic hydrolysis afforded (3*R*, 4*S*)-3,4-dihydroxy-6-methyl-1-heptene **4**, mp 55-56 °C, $[\alpha]_D^{15}$ −13.5 ($CHCl_3$, *c* 1.00) in 88% yield, of which the physical constants were appreciably higher than the reported values.[4,12] For the intended functionalization of the olefinic double bond in **4**, it reacted with trichloroacetonitrile and DBU, and the generated bis(trichloroacetimidate) was cyclized using iodine in the presence of sodium bicarbonate in acetonitrile at 0 °C to furnish a 3.7 : 1 mixture of dihydro-1, 3-oxazines **5** and oxazolines **6** in 89% combined yield. While the isomeric ratio of **5** turned out to be 28 : 1 in favor of **5s**, mp 93-95 °C, $[\alpha]_D^{10}$ +22.5 ($CHCl_3$, *c* 1.10), only *trans* isomer **6s**, mp 81-82 °C, $[\alpha]_D^{18}$ −87.4 ($CHCl_3$, *c* 1.04) was found in the case of **6**. The structures of **5s** and **6s** were corroborated from the following C=N stretching band frequencies[13] and proton-proton coupling constants : for **5s** : 1672 cm^{-1}, $J_{H4,H5}$=3.1 Hz and $J_{H5,H6}$=0 Hz. For **6s** : 1666 cm^{-1} and $J_{H4,H5}$=5.9 Hz. The assignments were supported by the derivatization of **5s** and **6s** into the identical Boc-aziridine **7** (*vide infra*).



Scheme 1. [a] DIBAL/CH_2Cl_2/−78 °C. [b] $Me_2CH^+PPh_3I^-$/*n*-BuLi/HMPA/THF/0 °C→rt. [c] H_2/10% Pd-C/$NaHCO_3$/MeOH/rt. [d] Swern ox. [e] $Me^+PPh_3I^-$/*n*-BuLi/HMPA/THF/0 °C. [f] AcOH/H_2O/45 °C. [g] Cl_3CCN/DBU/MeCN/0 °C. [h] I_2/$NaHCO_3$/MeCN/0 °C. [i] 6 N HCl/MeOH/rt. [j] $NaHCO_3$/MeOH/rt; Boc_2O/rt. [k] TMSOTf/HMDS/THF/−40 °C; *c*-HxMgCl/Li_2CuCl_4/−30 °C; acidic work-up (pH=2-3).