Autopoietic Self-Reproduction of Chiral Fatty Acid Vesicles

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Abstract: The self-reproduction of vesicles formed by (*S*)- and (*R*)-2-methyldodecanoic acid **(4)** was investigated in order to relate the autocatalytic increase of the vesicle concentration with enantioselectivity. **4(R)** and **4(S)** were synthesized with an enantiomeric excess greater than 98%. **4** forms vesicles in aqueous solution in the pH region between 8.8 and 7.5. Chiral properties of the vesicles were studied by differential scanning calorimetry (DSC) and circular dichroism (CD). For self-reproduction studies, the hydrolysis of the water-insoluble 2-methyldodecanoic anhydride (**8**) was investigated in a biphasic system consisting of an aqueous solution and **8**. The reaction rates of **8(RR)** and **8(SS)** catalyzed by **4(R)** or **4(S)** vesicles were the same within experimental errors, indicating that the chiral vesicles cannot induce significant enantioselectivity. However, a clear effect was observed at 10 °C: racemic vesicles destabilized during hydrolysis, causing phase separation, whereas homochiral vesicles remained stable and continued to self-reproduce.

Introduction

In the last couple of years vesicular systems have been described which are able to self-reproduce, i.e. able to increase their population number owing to an autocatalytic process which takes place within their own boundary.[1,2] The surfactant was a fatty acid (e.g. caprylic or oleic acid).[3] The reaction was the vesicle-catalyzed hydrolysis of the corresponding water-insoluble anhydride which takes place within the boundary of the vesicles, yielding the same fatty acid surfactant which then leads to the spontaneous increase of the vesicle number.[1] Self-reproduction is a key process in the mechanism of life, and in fact this particular vesicular reaction has been viewed as a simple model for the transition to autopoiesis and for mimicking cellular life.[4,5] The present work focusses on the self-reproduction of chiral vesicles (i.e. vesicles made of chiral surfactants), with the aim of establishing a connection between self-reproduction and chirality. More specifically, we have addressed the question whether and to what extent the exponential autocatalytic process leading to self-reproduction may also lead to an increase of the population of one enantiomer over the other.

For this study a surfactant system based on the chemistry developed earlier with linear fatty acids has been chosen,[1] preparing fatty acids and fatty anhydrides bearing an asymmetric carbon atom in the α-position to the carboxyl group.

In the following, we are reporting in the first part on the chemical synthesis of 2-methyldodecanoic acid (**4**) and on the preparation and physicochemical characterization of vesicles of **4**. In the second part, the self-reproduction of these supramolecular aggregates will be described. The hydrolysis of 2-methyldodecanoic anhydride (**8**) was studied by comparing first the behaviors of homochiral and racemic vesicles.[6] Secondly, we will investigate whether homochiral vesicles induce selection of one enantiomer over the other in the autocatalytic self-reproduction process.

Results and Discussion

Synthesis of 4 and 8. (a) Synthesis of the Enantiomers of 2-Methyldodecanoic Acid (4). The title compound was prepared according to Evans et al. (Scheme 1).[7] Reaction of lithiated (*R*)-(+)-4-benzyl-2-oxazolidinone (**1**) with dodecanoyl chloride in anhydrous THF afforded (4*R*)-3-(dodecanoyl)-4-benzyl-2-oxazolidinone (**2(R)**) in 91% yield, $[\alpha]_D$ −71.6° (*c* 1, CH_3COCH_3). This compound was added to a −78 °C solution of sodium hexamethyldisilylamide in tetrahydrofuran to give the corresponding enolate, which was alkylated with methyl iodide affording (4*R*)-3-((2′*R*)-2′-methyldodecanoyl)-4-benzyl-2-oxazolidinone (**3(RR)**) in 61% yield, $[\alpha]_D$ −88.7° (*c* 1, CH_3COCH_3). The deacylation of oxazolidinone **3(RR)** was carried out with lithium hydroperoxide at 0 °C according to the literature,[8] giving (*R*)-(−)-2-methyldodecanoic acid (**4(R)**) in 90% yield, $[\alpha]_D$ −15.6° (*c* 1, CH_3COCH_3). The (*R*) absolute configuration of compound **4** has been made by comparison of

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(1) (a) Walde, P.; Wick, R.; Fresta, M.; Mangone, A.; Luisi, P. L. *J. Am. Chem. Soc.* **1994**, *116*, 11649−11654. (b) Wick, R.; Walde, P.; Luisi, P. L. *J. Am. Chem. Soc.* **1995**, *117*, 1435−1436. (c) Bachmann, P. A.; Luisi, P. L.; Lang, J. *Nature* **1992**, *357*, 57−59.

(2) We use in this paper the terms *self-replication* and *self-reproduction* as described previously.[1a] The term *self-replication* is limited to linear structures whose replication is based on the template chemistry of nucleic acids; the term *self-reproduction* is a more general one, valid for all structures including micelles and vesicles; and the term *autopoietic self-reproduction* is for the particular case in which reactions leading to self-reproduction take place within the boundary of the reproductive unit (e.g., within the boundary of a vesicle).

(3) In analogy to the previous work,[1a] we use for simplicity the term fatty acid independent of the protonation degree. Therefore, a "fatty acid vesicle" is composed of a mixture of fatty acid molecules and soap molecules.

(4) Maturana, H. R.; Varela, F. J. *Autopoiesis and Cognition: The Realization of the Living*; D. Reidel Publishing Co.: Boston, MA, 1980.

(5) (a) Walde, P.; Goto, A.; Monnard, P.-A.; Wessicken, M.; Luisi, P. L. *J. Am. Chem. Soc.* **1994**, *116*, 7541−7547. (b) Oberholzer, T.; Wick, R.; Luisi, P. L.; Biebricher, C. K. *Biochem. Biophys. Res. Commun.* **1995**, *207*, 250−257. (c) Oberholzer, T.; Albrizio, M.; Luisi, P. L. *Chem. Biol.* **1995**, *2*, 677−682.

(6) With *homochiral vesicles* we mean vesicles formed by one pure enantiomer; with *racemic vesicles* we mean vesicles composed of the racemic mixture of the two enantiomers.

(7) Gage, J. R.; Evans, D. A. *Org. Synth.* **1990**, *68*, 83−91.

(8) Evans, D. A.; Britton, T. C.; Eliman, J. A. *Tetrahedron Lett.* **1987**, *28*, 6141−6144.

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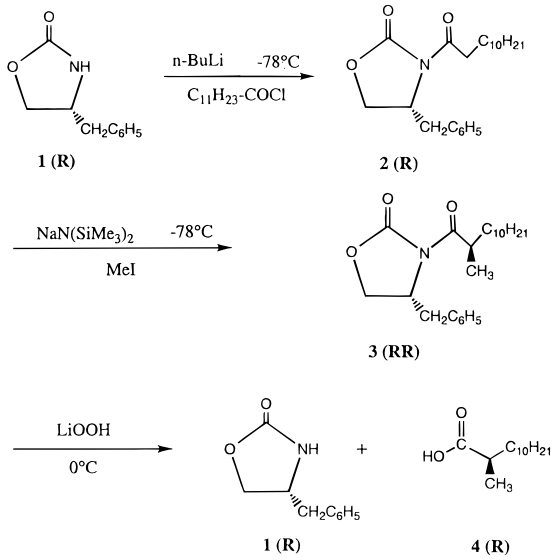
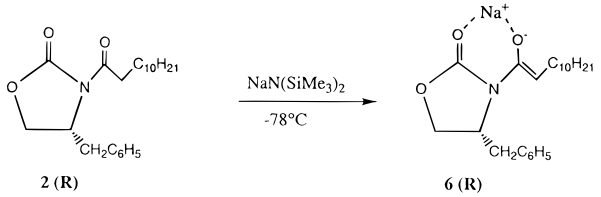
Scheme 1



Scheme 2



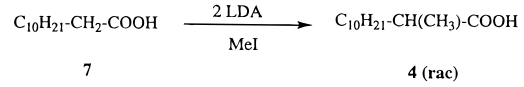
the sign of its optical rotation with that reported in the literature.[9] The enantiomeric excess of (*R*)-(−)-2-methyldodecanoic acid (**4(R)**) (99% e.e.) was determined via ^{1}H-NMR spectroscopy in the presence of Eu(hfc)$_3$ as chiral shift reagent, and confirmed through the conversion of compound **4(R)** into the corresponding diastereoisomeric (*RR*) and (*RS*) amides **5** via reaction of its acyl chloride with enantiomerically pure α-phenylethylamine (see Experimental Section).[10] The overall process is highly stereoselective and its stereochemical course is in line with the formation of chelated (*Z*)-enolate such as **6** (Scheme 2), in the creation of a diastereofacial bias in the acylation reaction.[11] It is worth mentioning that the stereoselectivity of the acylation is very high in spite of the small steric requirement of methyl iodide.

In agreement with Evans et al.[11] one must employ alkylating agents reacting at convenient rate at temperature <20 °C in order to avoid the decomposition of sodium enolate **6**. Satisfactory chemical yields are obtained by using allyl bromide and benzyl bromide as alkylating agents whereas with ethyl iodide no alkylation occurs (unpublished result). A possible explanation is that nucleophiles may attack hindered acyloxazolidinones more rapidly at the ring carbonyl than at the exocyclic *N*-acyl group, as recently found by Fleming and co-workers.[12]

The (*S*)-2-methyldodecanoic acid **4(S)** (99% e.e.), $[\alpha]_D$ +15.6° (*c* 1, CH_3COCH_3), has been obtained by the same procedure starting from (*S*)-(−)-4-benzyl-2-oxazolidinone.

(b) Synthesis of Racemic 2-Methyldodecanoic Acid (4(rac)). Dodecanoic acid **7** reacted with 2 molar equiv of LDA in anhydrous THF at 0 °C to give the corresponding dianion;

Scheme 3



DMPU was added as a cosolvent and the reaction mixture was treated with 1.1 molar equiv of methyl iodide, to give the title compound in 90% yield[13] (Scheme 3).

(c) DSC Analysis. The melting points of *n*-dodecanoic acid **7** and of all 2-methyldodecanoic acids **4(R)**, **4(S)**, and **4(rac)** as determined by DSC measurements are 42.7 (for **7**), 19.9 (for **4(rac)**), 20.7 (for **4(R)**), and 21.3 °C (for **4(S)**). Although the melting points of the two enantiomers **4(R)** and **4(S)** were very similar to the melting point of the racemate **4(rac)**, differences were seen in the DSC traces. The DSC (heating) trace of **4(R)** or **4(S)** showed a small exothermal peak at about 2 °C, indicating a transition between two different types of crystals. This is probably due to polymorphism and it indicates that **4(R)** or **4-(S)** can form two types of crystals depending on the temperature. In contrast, **4(rac)** forms only one type of crystal in the temperature range measured. We have also measured the melting points of various compositions of the two opposite enantiomers **4(R)** and **4(S)** (data not shown). Analysis of the corresponding DSC traces indicated that the crystalline structure of the racemic mixture is a racemic compound.[14] In other words, two enantiomers are coexisting in the same unit cell and they do not separate into domains of enantiomeric crystals spontaneously.

(d) Synthesis of 2-Methyldodecanoic Anhydride (8). One equivalent of **4** and anhydrous pyridine were mixed in anhydrous Et_2O and 0.5 equiv of thionyl chloride was added at −10 °C. The title compound was obtained in 80% yield after purification. **8(RR)**, **8(SS)**, and **8(mix)** were obtained starting from **4(R)**, **4(S)**, and **4(rac)**, respectively. For **8(RR)** and **8(SS)** DSC heating measurements gave almost identical endothermic peaks at around 12 °C.

Preparation and Characterization of Vesicles of 4. (a) Spontaneous Formation of Vesicles. After having described the chemical synthesis work, we will now report on the aggregation properties of **4**, in particular on the formation of vesicles of **4**.

In aqueous medium, dispersed fatty acids form different types of aggregates, depending on the ionization degree of the carboxyl group:[15−18] while at alkaline pH micelles are formed,[19] the fatty acid molecules assemble into bilayers (vesicles) at conditions where about half of the molecules are protonated. In analogy to the previous work on linear fatty acids,[16,17] we have studied the aggregation behavior of **4** at various degrees of ionization by a controlled addition of HCl to a micellar

(9) Meyers, A. I.; Poindexter, G. S.; Brich, Z. J. *Org. Chem.* **1978**, *43*, 892−898.
(10) Sonnet, P. E.; Baillangeon, M. W. *Lipids* **1991**, *26*, 295−300.
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(16) (a) Cistola, D. P.; Atkinson, D.; Hamilton, J. A.; Small, D. M. *Biochemistry* **1986**, *25*, 2804−2812. (b) Cistola, D. P.; Hamilton, J. A.; Jackson, D.; Small, D. M. *Biochemistry* **1988**, *27*, 1881−1888. (c) Cistola, D. P.; Small, D. M. *J. Am. Chem. Soc.* **1990**, *112*, 3214−3215.
(17) Rosano, H. L.; Chistodoulou, A. P.; Feinstein, M. E. *J. Colloid Interface Sci.* **1969**, *29*, 335−344.
(18) (a) Haines, T. H. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 160−164. (b) Li, W.; Haines, T. H. *Biochemistry* **1986**, *25*, 7477−7483.
(19) The cmc values for **7**, **4(R)**, and **4(rac)** are listed in Table 1. The cmc values of **4** are lower than the cmc value of the linear *n*-dodecanoic acid **7**. This can be understood on the basis of the fact that 2-methyldodecanoic acid has a larger hydrophobic part than *n*-dodecanoic acid. The cmc value was the same for **4(R)**, **4(S)**, and **4(rac)**.

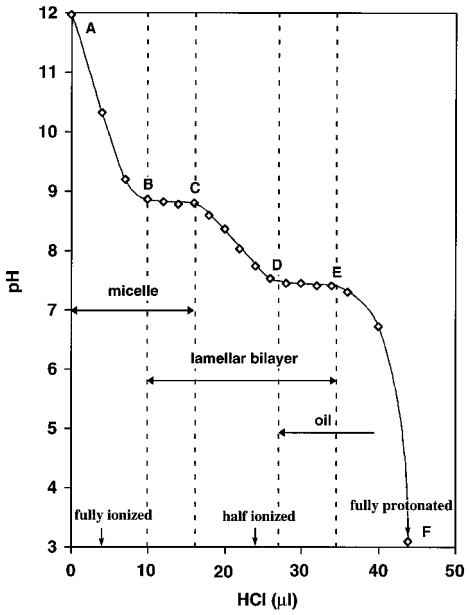


Figure 1. Equilibrium titration curve of **4(R)**. Various amounts of 1 M HCl were added to samples of 0.5 mL of 80 mM **4(R)**, 88 mM NaOH, and the pH was measured at 25 °C 6 days after preparation. The horizontal arrows indicate the region where micelles, vesicles (lamellar bilayer), and/or oil emulsion are present.

Table 1. Chain-Melting Transition Temperature (T_c) of Hydrated Bilayers (50% Ionized) of *n*-Dodecanoic Acid (**7**) and 2-Methyldodecanoic Acid (**4**) and cmc Values of the Corresponding Sodium Salts (T = 25 °C)

		cmc (mM)	
fatty acid	T_c (°C)[a]	conductometry	spectrometry
7[b]	30.0	25	22
4(rac)	12.2	21	16
4(R)	7.6	21	19
4(S)	7.4	21	n.d.[c]

[a] T_c were determined by extrapolating the slope of the onset of the endothermal peak to the base line. [b] Literature cmc values: 24.4 mM;[30b] 23 mM;[30c] 22.5 mM.[30d] [c] n.d.: not determined.

solution of fully ionized **4**. Figure 1 shows the room temperature titration curve for **4(R)** which will now be discussed on the basis of the Gibbs phase rule.[16b,20]

Before the titration was started, **4(R)** was dissolved at a concentration of 0.08 M (>cmc, Table 1) in water by adding a 10 mol % excess of NaOH. This solution was optically clear, containing micelles in equilibrium with monomers (Figure 1, point A). Addition of HCl induced slight turbidity at point B. Between B and C the pH remained constant at 8.8 and the turbidity of the system increased, indicating the coexistence of micelles, vesicles, and monomers. Upon further addition of HCl, the pH value decreased again from C to D (vesicles and monomers are coexisting). At point D the system became milky white and between D and E the pH remained constant at 7.5 (vesicles, oil emulsion, and monomers are coexisting) and then dropped abruptly at point E and the sample contained a separate oil phase floating on top of a clear aqueous phase (point F).

Half of the carboxylate groups were protonated at pH 7.7,[21] a value which is much higher than the pK_a values of the monomeric fatty acids in aqueous solution (typically 4.8). This bilayer effect is known from literature for the case of linear carboxylic acids.[15−18] The same results as in Figure 1 were obtained with **4(rac)**.

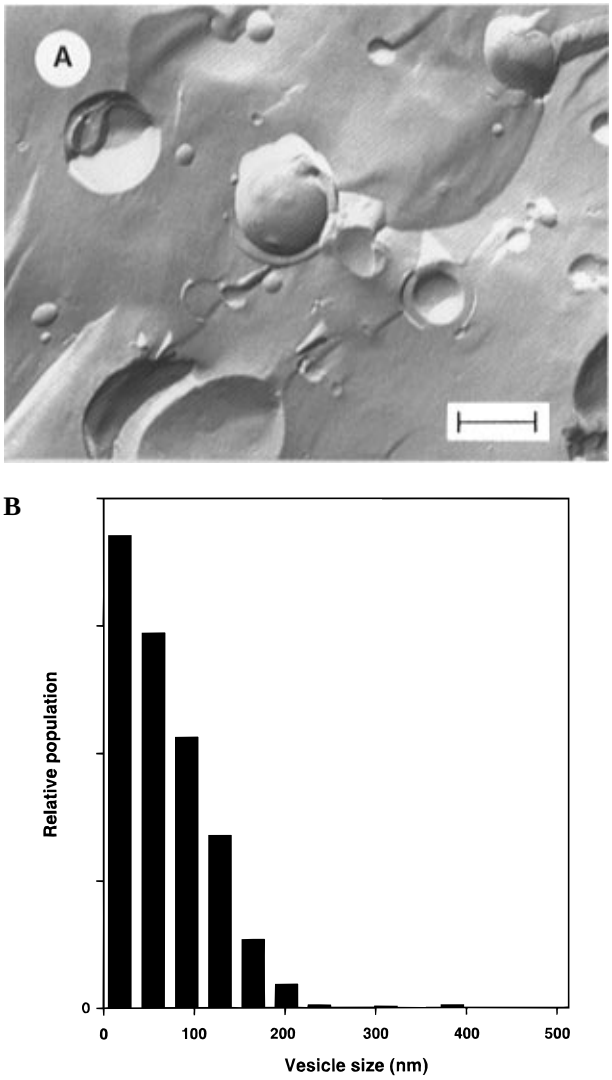


Figure 2. (A) Freeze fracture electron micrograph of spontaneously formed vesicles of **4(rac)** in 0.05 M Tris/HCl buffer solution at pH 8.0. The length of the bar corresponds to 200 nm. (B) Size distribution of the vesicles formed upon hydrolysis of **8(SS)** in a two-phase system consisting initially of an aqueous phase (10 mL of 0.05 M bicine buffer, pH 8.2) and 0.15 mmol of **8(SS)** at 25 °C. Freeze fracture electron micrographs were analyzed after the reaction had finished.

The observation described above shows that vesicles of **4** are formed spontaneously by adjusting pH. We could also obtain vesicles in the same pH region starting from acidic solutions by adding the appropriate amount of NaOH.

Figure 2A shows a freeze fracture electron micrograph of **4(rac)** suspension which was spontaneously formed (0.05 M Tris/HCl, pH 8.0; pH region between point C and point D in Figure 1): polydisperse multilamellar vesicles are present with

(20) Since the chain-melting phase transition temperature (T_c) of anhydrous and hydrated samples of **4** (acid−soap 1:1 complexes) are both below room temperature (Table 1), all lipids during titration were in the fluid-analogue state (above T_c). It is in clear contrast with lauric acid (**7**) whose T_c is higher than room temperature. It has been generally noted that chain substitution of lipids changes their thermal properties. (Menger, F. M.; Wood, M. G., Jr.; Zhou, Q. Z.; Hopkins, H. P.; Fumero, J. *J. Am. Chem. Soc.* **1988**, *110*, 6804−6810).

(21) The degree of ionization has been calculated from the amount of HCl added and the amount of HCl needed for full protonation of the carboxyl group, and the aggregation morphology could be correlated to the degree of ionization from the titration curve. Point B represents the minimum ionization degree for micelles (ca. 0.8). Oil emulsions were formed at a very low degree of ionization (<0.25). Vesicles were present at intermediate ionization degrees (0.4−0.7, point C−point D). It should be noted that the ionization degree is not restricted to 0.5 for the formation of lamellar phase above T_c. It is in sharp contrast to the crystalline phases of 1:1 acid−soaps (below T_c) which require a fixed 1:1 stoichiometry of fatty acid to soap (e.g., the case of *n*-dodecanoic acid at room temperature).[22b]

vesicle sizes ranging from 10 nm to at least 1 μm. The presence of giant vesicles (with diameters greater than 1 μm) has been confirmed by light microscopy (data not shown). After extrusion through membranes with 50-nm pores large vesicles disappeared and the size became more homogeneous. Spontaneously formed and extruded vesicle suspensions were fairly stable, as estimated by monitoring the optical density (turbidity) at 450 nm as a function of time, which remained constant over 1 week at room temperature (data not shown).

The internal volume of spontaneously formed **4(rac)** vesicles was in the range of 2.5−4.0 μL/μmol of lipid, somewhat higher than in the case of spontaneously formed oleic acid vesicles (1.0−1.6 μL/μmol of lipid).[1a] The larger internal volume of **4(rac)** vesicles is presumably due to the presence of a larger fraction of bigger vesicles.

As in the case of vesicles obtained from linear fatty acids,[1,15−18] several observations indicate that we are dealing with equilibrium systems. First of all, these vesicles are formed spontaneously by simply mixing the surfactant in water at appropriate pH and concentration—see also the examples in the literature.[22] Once formed, they are very sensitive to changes of the external medium, e.g. changes by dilution or changes in pH.

It is worthwhile recalling that a high monomer concentration and the rapid equilibrium between monomers and aggregates are characteristic features of the fatty acid vesicles, clearly different from the case of the double chain phospholipids. The concentration of **4(rac)** free monomer in equilibrium with the vesicles at various pH was determined by ultrafiltration. The monomer concentration obtained by this means cannot be strictly considered as the cac (the critical concentration for aggregate formation), but the fact that it is sensitive to pH certainly reflects the dynamic character of the vesicles. At pH 7.7, this concentration was 2.7 mM, at pH 8.0 it was 4.4 mM, at pH 8.2 it was 6.2 mM, and at pH 8.5 it was 9.1 mM. These values are considerably lower than the cmc at alkaline pH (Table 1: 21 mM). In other words, the lower the pH, the lower is the monomer concentration, reflecting the lower mole fraction of ionized **4** in the bilayer.[23]

(b) Thermal Analysis of the Vesicles. We have measured thermal phase transitions of vesicles of **4** as a function of the composition of the (*R*) and (*S*) isomers, and a binary phase diagram has been constructed (Figure 3). Enantiomeric **4(R)** or **4(S)** bilayers show a transition peak at 9.5 °C, whereas the endothermic peak of the **4(rac)** bilayer is at 15 °C. There were two minimal temperatures at the **4(R)** mole fractions of ca. 0.2 and 0.8. These temperatures correspond to the two eutectic points of enantiomeric and racemic crystals. The shape of the binary phase diagram indicates that the crystal of the racemate is a racemic compound, as in the case of the bulk compound (see above). The racemic bilayer has a higher T_c than the enantiomeric bilayer which is in agreement with the macroscopic observation that **4(rac)** vesicles crystallize more easily than enantiomeric vesicles at low temperature (see below).

In the second series of measurements, the kinetics of fatty acid exchange between vesicles was followed qualitatively by DSC. Figure 4 shows the DSC traces after mixing separately prepared vesicle suspensions of **4(R)** and **4(S)**. Both of the enantiomeric vesicles initially showed an endothermic peak at ca. 9.5 °C (trace (a) in Figure 4 for **4(R)** vesicles). The DSC measurements carried out 3 min after mixing (trace (b) in Figure 4) showed that a large portion of the molecules have been exchanged between vesicles and an endothermic peak has appeared at a temperature which has been obtained for racemic vesicles. The measurement which was carried out 30 min after mixing (trace (c) in Figure 4) revealed that the molecules have been almost completely exchanged and the DSC trace is superimposable to that of **4(rac)** vesicles (trace (d) in Figure 4). Although precise kinetic data are not available from these measurements, they indicate that the molecular exchange between vesicles is rapid.

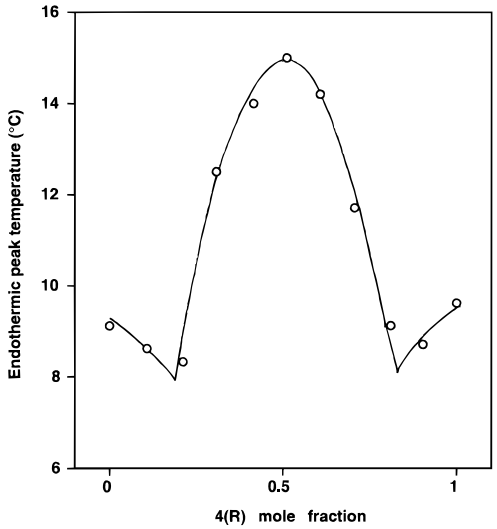


Figure 3. Dependence of the phase transition temperature (T_c) of vesicles on the compositions of **4(R)** and **4(S)** (binary phase diagram). Total concentration of **4** was 100 mM. Half of the carboxyl groups were ionized.

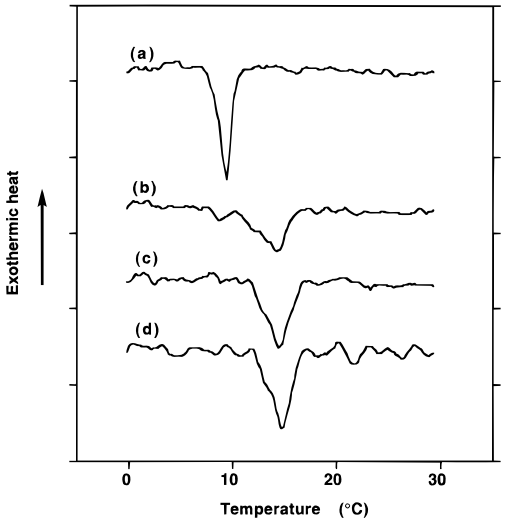


Figure 4. Change in the DSC heating traces upon mixing equal amounts of **4(R)** vesicles and **4(S)** vesicles. Concentration of **4** was 100 mM. (a) **4(R)** vesicles, before mixing; (b) 3 min after mixing; (c) 30 min after mixing; (d) **4(rac)** vesicles (equilibrated for several days).

(c) Optical Activity of the Vesicles. Figure 5A compares the CD spectrum of **4(R)** dissolved in methanol with the CD spectrum of vesicles from **4(R)**. In both cases a negative peak was observed at about 210 nm. The chromophore is the carboxyl group ($\lambda_{max} \sim 210$ nm, n $\rightarrow \pi^*$ transition) next to the

(22) (a) Talmon, Y.; Evans, D. F.; Ninham, B. W. *Science* **1983**, *221*, 1047−1048. (b) Hauser, H.; Gains, N.; Müller, M. *Biochemistry* **1983**, *22*, 4775−4781. (c) Kaler, E. W.; Murthy, A. K.; Rodriguez, B. E.; Zasadzinski, J. A. N. *Science* **1989**, *245*, 1371−1374. (d) Safran, S. A.; Pincus, P.; Andelman, D. *Science* **1990**, *248*, 354−356. (e) Cantu, L.; Corti, M.; Musolino, M.; Salina, P. *Prog. Colloid Polym. Sci.* **1991**, *84*, 21−23.

(23) The carboxyl group of monomers in the aqueous phase is thought to be mostly ionized, since the solubility of protonated fatty acid in water is much lower than ionized fatty acid. For example, the solubility of *n*-dodecanoic acid in water is 12 μM (Bell, G. H. *Chem. Phys. Lipids* **1973**, *10*, 1−10), whereas the monomer solubility of sodium *n*-dodecanoate is approximately 25 mM (cmc, Table 1).

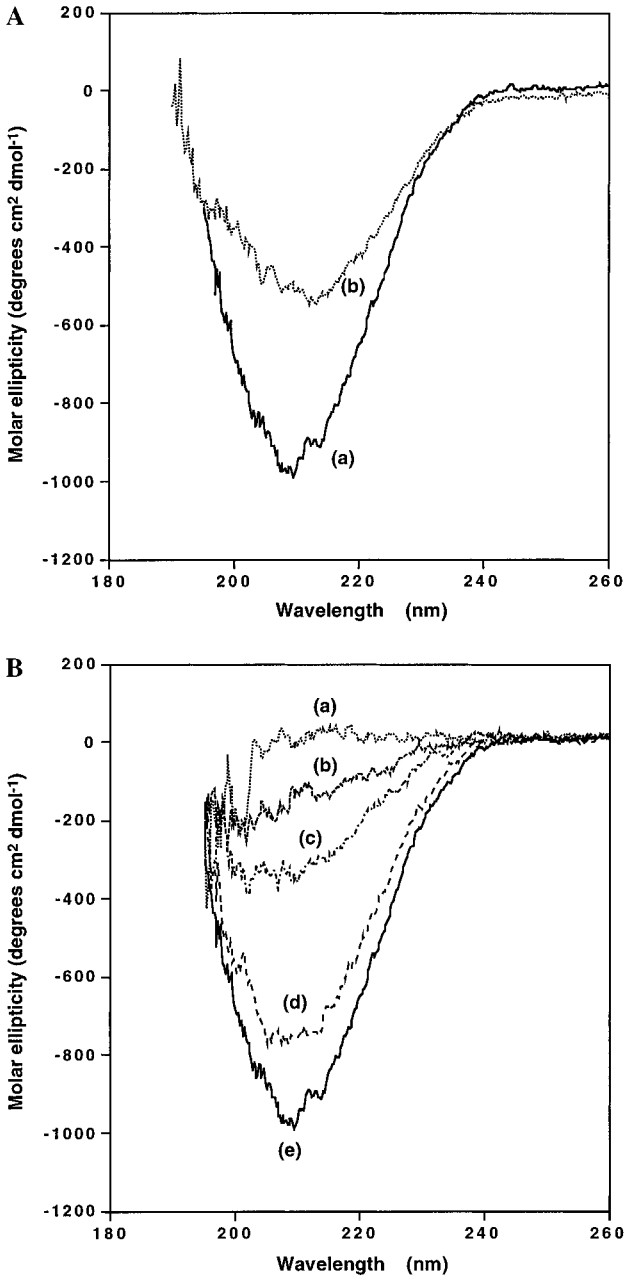


Figure 5. CD spectra of **4(R)**. (A) Comparison of (a) an aqueous solution (vesicles, concentration of **4(R):** 80 mM, pH 7.8) and (b) a methanol solution (monomers, concentration of **4(R)**: 88 mM). (B) pH dependence of the CD spectrum in water. Concentration of **4(R)** was 80 mM: (a) pH 12.1; (b) pH 9.1; (c) pH 8.8; (d) pH 8.6; (e) pH 7.8. All spectra were measured at 25 °C. We have measured CD spectra at various temperatures and observed that they are identical, if the bilayers are in the fluid analogue state.

chiral α-carbon. The CD signal of methanolic **4(R)** arises from the molecular optical activity. The vesicles of **4(R)** (80 mM, pH 7.8), on the other hand, showed a significantly more intense negative CD band at 210 nm, being about 1.8 times larger than that of the methanolic solution. Since, as we shall discuss later, only protonated species show a CD signal, and since half of the molecules are protonated in the bilayer, the normalized CD signal of the protonated species in the bilayer is about 3.5 times stronger than the signal observed in methanol solution.

Since the CD signal arises from the carboxyl group which is localized on the surface of the bilayer, it is expected to be sensitive to protonation and ionization of the carboxyl group.[24] We have therefore studied the pH dependence of the CD signal of aggregated **4(R)**, and Figure 5B shows the corresponding

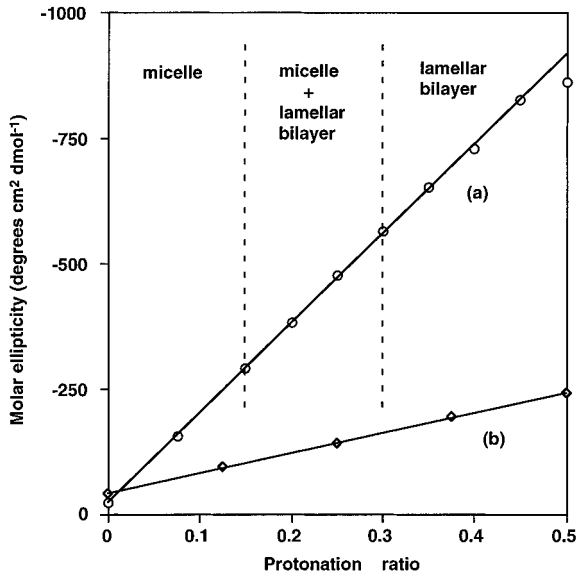


Figure 6. Dependence of the CD signal intensity at 220 nm on the degree of protonation of the carboxyl group in **4(R)**. (a) Vesicle suspensions in water and (b) methanol solution. Concentration of **4-(R)** was 80 mM. The vertical lines indicate the boundaries between regions where micelles and/or vesicles (lamellar bilayer) are present in the aqueous solution.

CD spectra. The CD signal intensity decreases as the pH is raised, and practically no CD signal is observed in a micellar solution at pH 12.1. A plot of the CD signal intensity at 220 nm versus the mole fraction of the protonated species in solution is shown in Figure 6. The signal intensity is proportional to the mole fraction of protonated molecules up to half-protonation. Above that ratio, measurements became difficult due to increased turbidity. The linearity in Figure 6 is a clear indication that only protonated molecules are contributing to the CD signal around 210 nm; the type of aggregates (micelles or vesicles) did not affect the intensity.

However, as shown by Figure 6, a linear behavior is also observed for the methanol solution, and in this case the intensity of the dichroic signal is a factor 3.5 lower than that of the water solution. In other words, the aggregation formed in water brings about an enhancement of the dichroic signal.[25] It is due to a restriction of conformational equilibrium in the aggregated form. A similar effect has been reported in the case of lecithin reverse micelles,[26] and actually also in the case of phosphatidylcholine liposomes (unpublished result). Furthermore, we would like to draw attention to the fact that monomers coexisting with the vesicles are mostly ionized and therefore the CD signal of vesicular solution comes exclusively from the protonated species in vesicles. Formation of the aggregates is accompanied by protonation of the carboxyl groups due to their higher pK_a and induces the specific CD signal of the aggregates.

Self-Reproduction of the Vesicles. (a) Catalytic Properties of the Vesicles. Basically two set of hydrolysis experiments were performed (Figure 7A).

(i) In the first case, the hydrolysis of anhydride **8** was studied in a two-phase system, the aqueous phase being a buffer solution

(24) Similar dependence of optical activity on the protonation of carboxyl groups has been studied for amino acids and hydroxy acids. (a) Jirgensons, B. *Optical Activity of Proteins and Other Macromolecules*; Springer-Verlag: Berlin, 1973. (b) Katzin, L. I.; Gulyas, E. *J. Am. Chem. Soc.* **1968**, *90*, 247−251.

(25) It is important to notice that this effect is not due to solvent perturbation effects on the chromophore, as the UV absorption spectrum in methanol (data not shown) is the same as that in water solution.

(26) Colombo, L. M.; Nastruzzi, C.; Luisi, P. L.; Thomas, R. M. *Chirality* **1991**, *3*, 495−502.

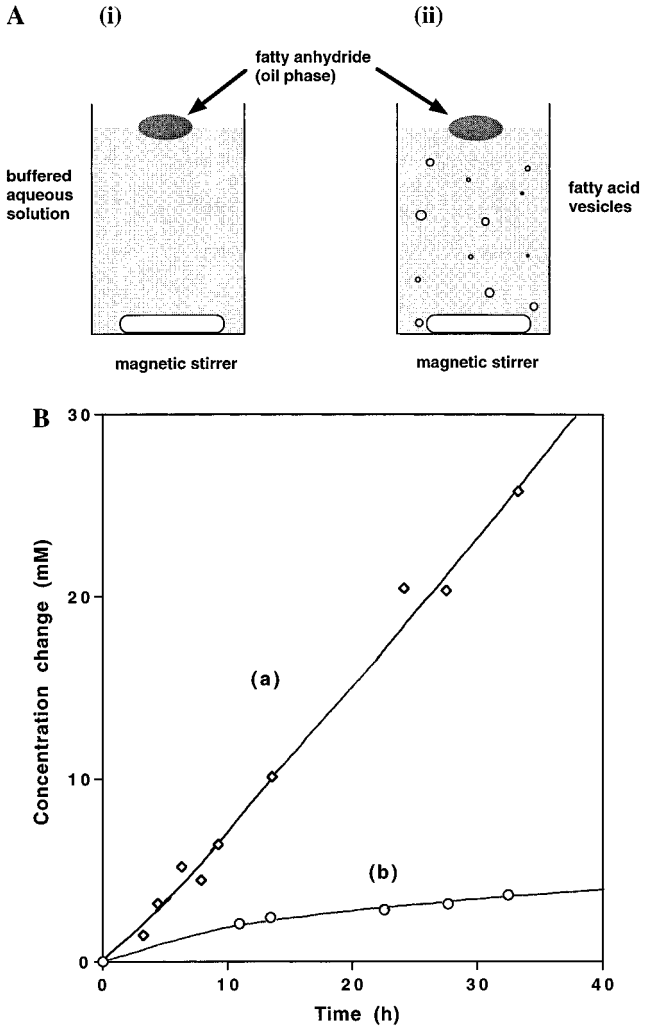


Figure 7. (A) Schematic illustration of the hydrolysis experiments, (i) starting from a buffer solution without vesicles and (ii) with preformed vesicles. (B) Hydrolysis of **8(mix)** (0.2 mmol) at 25 °C in a two-phase system containing an aqueous phase (10 mL, 0.05 M bicine buffer, pH 8.7) initially either (a) with vesicles of 20 mM **4(rac)** or (b) without vesicles. The increase in concentration of **4** in the aqueous phase was determined as a function of time. In the case of (a), slight decrease of the concentration was observed for the initial 2 h, due to the uptake of **4** molecules from the vesicles into the oil phase. We have plotted in the graph the concentration increase that followed this initial process to make the true comparison of hydrolysis rates.

at pH ~8.5 and the oil phase of the anhydride on the surface of the aqueous phase. In this case the anhydride is hydrolyzed and fatty acids are formed which partition into the aqueous phase where aggregates (vesicles) are formed as soon as the critical concentration for aggregate formation is reached. The hydrolysis of the anhydride is accelerated autocatalytically at the onset of the newly formed vesicles.

(ii) In the second series of experiments, the rate of anhydride hydrolysis was studied in the presence of vesicles from the beginning. In other words, the two-phase system was composed of an aqueous buffer system containing preformed vesicles and a supernatant anhydride phase. In this case the rate of hydrolysis is accelerated by the presence of vesicles from the very beginning.

The concentration increase of **4** in the aqueous phase due to the hydrolysis is shown in Figure 7B. Without vesicles, the reaction initially—before vesicles are formed—is slow, while in contrast, the presence of vesicles catalyzes the hydrolysis of **8(mix)**. After 30 h, for example, about 3 mM **4(rac)** was formed in the absence of vesicles, while in the presence of the

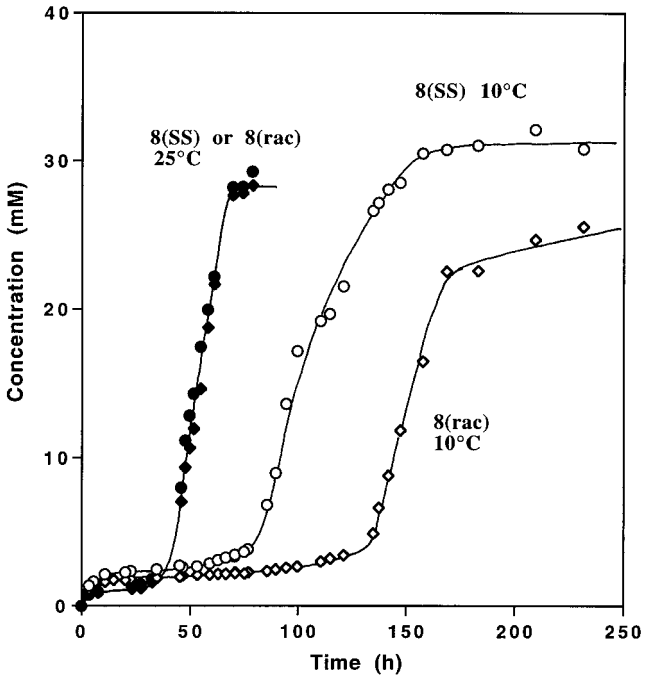


Figure 8. Hydrolysis of **8(SS)** (circles) of **8(rac)** (diamonds) in a two-phase system consisting initially of an aqueous phase (10 mL of 0.05 M bicine buffer, pH 8.2) and 0.15 mmol of **8** (corresponding to 30 mM of **4** in the aqueous phase after hydrolysis). The concentration of **4** in the aqueous phase was determined as a function of time at a reaction temperature of 25 (filled symbols) and 10 °C (open symbols).

vesicles (initially 20 mM **4(rac)**) about 23 mM **4(rac)** was newly formed in the same period of time. The result clearly shows that vesicles acted as catalyst, in analogy to the systems based on normal fatty acids described before.[1]

(b) Self-Reproduction of the Homochiral and Racemic Vesicles. We have studied the autocatalytic hydrolysis in the presence of homochiral and racemic vesicles. The hydrolyses of **8(SS)** and **8(rac)** (the 1:1 mixture of **8(RR)** and **8(SS)**) were compared starting from the aqueous/anhydride two-phase systems ((i) in Figure 7A). The results are shown in Figure 8. In all cases, the reaction initially was rather slow until the rate of hydrolysis drastically increased due to the formation of aggregates (vesicles). At 25 °C we obtained the same behavior for the hydrolysis of **8(SS)** and **8(rac)**. The length of the slow initial phase necessary for the formation of aggregates was the same for **8(SS)** and **8(rac)**. In both cases, formation of aggregates led to the enhancement of hydrolysis rates. The autocatalytic hydrolysis rates of the enantiomer and the racemate were also the same. It indicates that the enantiomeric composition of the bilayer does not affect the aggregation property of **4** and the catalytic ability of the vesicles at this temperature.

The mean size and size distribution of the vesicles formed during the hydrolysis of **8(SS)** has been determined by analyzing freeze fracture electron micrographs. Figure 2B shows the results analyzed after 8 days reaction at 25 °C. Most of the vesicles formed during the reaction were unilamellar and smaller than 200 nm with a mean diameter of 60 nm.[27]

Let us compare now the hydrolysis of **8(SS)** and **8(rac)** at 10 °C. This temperature is higher than the T_c of vesicles of **4(S)**, but lower than the T_c of vesicles of **4(rac)**. We have observed quite different behaviors in the two cases. First, the lag time before the onset of the autocatalytic hydrolysis was considerably shorter for the hydrolysis of **8(SS)** compared with that of **8(rac)**. Secondly, the hydrolysis of **8(rac)** gave rise to

(27) Some giant vesicles with diameters considerably larger than 1 μm were also present after the reaction (data not shown).

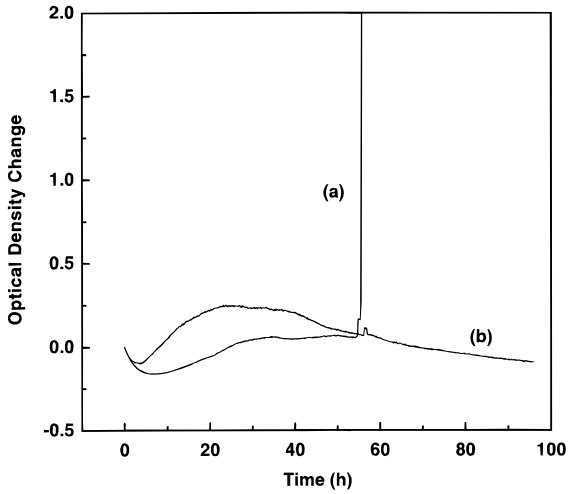


Figure 9. Optical density (turbidity) change at 800 nm during the hydrolysis of (a) **8(SS)** and (b) **8(RR)** in the presence of **4(R)** vesicles. Either **8(RR)** or **8(SS)** (0.03 mmol; corresponding to 20 mM of **4(R)** or **4(S)** after hydrolysis, respectively) was overlaid on the aqueous phase (3 mL) containing the vesicle suspension of 40 mM **4(R)** and was hydrolyzed at 10 °C.

phase separation: a white, gel-like material was formed after ca. 150 h (concentration of ca. 20 mM) and precipitated out. At this point, the hydrolysis rate was considerably slowed down. This precipitate is due to the fact that at the given temperature (10 °C) the racemic vesicles are below their T_c and are not stable. On the other hand, the aggregates formed by **4(S)**—which were regular vesicles as confirmed by electron micrographs—are at this temperature in a fluid−liquid analogue state and stable. The hydrolysis reaction continued until **8(SS)** was exhausted.

In order to evidence even more clearly this effect, the experiment illustrated in Figure 9 was carried out, in which we started from already formed vesicles of **4(R)**. The process was monitored by following the optical density (turbidity) of the vesicle dispersion at the arbitrary chosen wavelength of 800 nm. Hydrolysis of **8(RR)** in the presence of **4(RR)** vesicles changed the turbidity of vesicles only slightly and gradually.[28] In contrast, in the case of **8(SS)** hydrolysis, an abrupt increase of the turbidity due to the phase separation was observed, a process which can easily be seen by the eyes. The macroscopic feature of the condensed phase was a heterogeneous mixture of white granulates and a gel-like substance swollen with the aqueous solution. The aqueous phase was separated from the precipitated phase by centrifugation at 4 °C. The supernatant aqueous phase was turbid, indicating the presence of vesicles. To obtain the enantiomeric composition of the separated phases, **4** was extracted from each phase into isooctane and the concentration and optical activity were determined by FT-IR and CD measurements, respectively. The precipitates contained 58 ± 2% **4(R)** and 42 ± 2% **4(S)**, whereas the aqueous phase contained 78 ± 2% **4(R)** and 22 ± 2% **4(S)**. In other words, the enantiomeric excess in the aqueous phase is considerably higher than that in the precipitated phase, and the latter has an enantiomeric composition that is close to that of the racemate. Considering the quantitative separation of the two phases by centrifugation was not very efficient and that the precipitates still contained a considerable amount of aqueous solution, the real composition of the solid phase is most likely very close to that of the racemate.

The observation just described can be interpreted as follows. As **8(SS)** is hydrolyzed at 10 °C in the presence of **4(R)** vesicles, the enantiomeric composition of the bilayer changes continuously toward the racemate. T_c of the bilayers changes accordingly, since the racemic bilayer of **4** has a higher T_c than the enantiomers. When the amount of **4(S)** in the bilayer exceeds a critical value, T_c becomes higher than the reaction temperature (10 °C) and formation of the precipitates starts. The solid phase is the racemate that has the highest T_c value. Formation of the racemic precipitates leaves vesicles in the aqueous phase with a higher enantiomeric excess of **4(R)**. All this is in sharp contrast to the hydrolysis of **8(RR)** in the presence of **4(R)** vesicles, as in this case the enantiomeric composition of bilayers does not change and the vesicle suspension remains stable.

(c) Stereoselectivity of the Vesicle Catalysis. One interesting question is whether the self-reproduction process occurs with some degree of enantioselectivity. In order to answer the question, we compared the hydrolysis rates of **8(RR)** and **8(SS)** in the presence of enantiomerically pure vesicles (either **4(R)** or **4(S)**). Typically 0.1 mmol of **8(RR)** or **8(SS)** (corresponding to 20 mM of **4(R)** or **4(S)** in the aqueous phase after hydrolysis) was overlaid on 10 mL vesicle suspensions of **4(R)** or **4(S)** (40 mM, pH 8.3) and the hydrolysis reaction was followed by FT-IR and CD spectroscopies. The reactions were conducted at both 25 and 10 °C repeatedly. In the limit of error (which in this type of experiment is relatively large, ca. 10%), anhydrides with the same chirality as the vesicles were hydrolyzed at the same rate as anhydrides with the opposite chirality with respect to the vesicles, indicating that the chiral vesicles of **4** cannot induce significant enantioselectivity.

Conversely, a significant difference was observed when we compared the hydrolysis of **8(RR)** and the diastereomeric mixture **8(mix)**. The appropriate amount of **8** was overlaid on vesicle suspensions of **4(R)** and the concentration increase of **4** during the hydrolysis reaction was monitored. Figure 10 shows a typical result. The release of **4** was slightly faster during the hydrolysis of **8(RR)** compared with **8(mix)**. We observed the same trend also in the presence of **4(rac)** vesicles **(8(RR)** was hydrolyzed faster than **8(mix)**). The difference of hydrolysis rates between **8(RR)** and **8(mix)** is due to differences in the reactivity of the mesoform **8(RS)** in comparison with the two enantiomers **8(RR)** and **8(SS)**. This observation demonstrates diastereomeric effects in catalytic vesicles.

Concluding Remarks

The vesicles from 2-methyldodecanoic acid (**4**) share with the previously described normal fatty acids[1] two important features: the spontaneous vesiculation, namely the capability of building vesicles without additional energy input, and the capability of undergoing an autocatalytic self-reproduction process. The main idea was to possibly combine exponential autocatalysis and enantioselectivity, i.e., to use the power of an exponential growth process to kinetically discriminate between two stereoisomers. It did not work the way we wanted at room temperature. However, a marked difference in the behaviors of homochiral and racemic vesicles was observed at 10 °C. The fact that "racemic vesicles" separate out in a gel-like form potentially provides a way to enrich the enantiomeric purity of the starting mixture in vesicles.

One may ask why the difference between homochiral and racemic vesicles observed at 10 °C is not reflected in an observable difference in the self-reproduction rate at room

(28) The gradual change of the absorbance in Figure 9 is presumably due to the constraints of experimental setup. We conducted the experiment in the spectrophotometer using a round glass tube in order to make efficient mixing of the aqueous phase possible. Consequently, we could obtain only poor transmission of light through the cell. Furthermore, the rather vigorous stirring of the aqueous phase during the reaction might have caused formation of oil emulsions of **8** in the aqueous phase during the reaction, which would have influenced the turbidity.

(29) Gerrand, W.; Thrush, A. M. *J. Chem. Soc.* **1953**, 2117−2120.

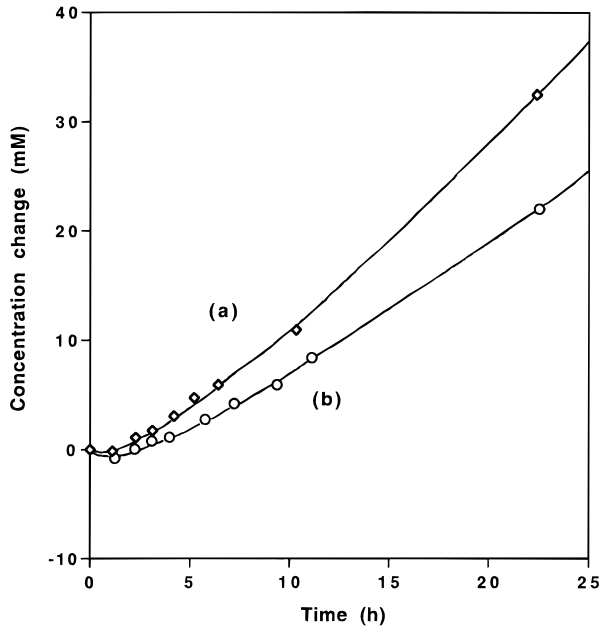


Figure 10. Hydrolysis of 0.2 mmol of **8(RR)** (diamonds) or 0.2 mmol of **8(mix)** (circles) (corresponding to 40 mM of **4(R)** and **4(rac)** after hydrolysis, respectively) in a two-phase system containing an aqueous phase (10 mL, 50 mM bicine buffer, pH 8.7) initially with vesicles of 20 mM **4(R)**. The increase in concentration of **4** in the aqueous phase was determined as a function of time at a reaction temperature of 25 °C.

temperature where vesicles are stable and do not precipitate. Most likely, above T_c the bilayer are too fluid to allow for a discrimination of the pair interactions $R-R$ with respect to $R-S$. This points out an interesting difficulty for further studies on this field: above T_c the interaction difference may be too small to give a rate effect, however below T_c it may be so large that phase separation with destruction of the vesicles may occur.

Materials and Methods

Reagents. All the following reagents used were purchased from Fluka (Buchs, Switzerland): (*R*)-4-benzyl-2-oxazolidinone (**1(R)**), (*S*)-4-benzyl-2-oxazolidinone (**1(S)**), *n*-butyllithium (*n*-BuLi), methyl iodide (MeI), dodecanoyl chloride ($C_{11}H_{23}COCl$), dichloromethane, tetrahydrofuran (THF), sodium hexamethyldisilylamide ($NaN(SiMe_3)_2$), europium(III) tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorat] ($Eu(hfc)_3$), (*R*)-(+)-α-phenylethylamine, thionyl chloride ($SOCl_2$), *N*,*N*-dimethylformamide (DMF), diisopropylamine, pyridine (C_5H_5N), 1,3-dimethyl-2-oxohexahydropyrimidine (DMPU), triethylamine, *n*-dodecanoic acid (**7**) (≥99%), oleic acid (≥99%), and arsenazo III. Pinacyanol chloride was from Sigma (St. Louis, MO, USA), and sepharose 4B from Pharmacia (Uppsala, Sweden). Standard 1 M solutions of NaOH and HCl were purchased from Merck (Darmstadt, Germany). Lithium hydroperoxide (LiOOH) was generated *in situ* from H_2O_2 and LiOH.

When necessary solvents and reagents were distilled under nitrogen prior to use. Dichloromethane and diisopropylamine were distilled from calcium hydride. THF, diethyl ether, and triethylamine were distilled from sodium metal/benzophenone ketyl.

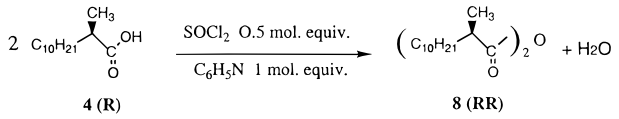
General Methods for Synthesis and Product Characterization. Infrared spectra were recorded on a Perkin Elmer FT-IR 16PC spectrometer. ^{1}H-NMR spectra were recorded on a Bruker AC300 spectrometer at ambient temperature. Chemical shifts are reported in ppm from tetramethylsilane on the δ scale, with the solvent resonance employed as the internal standard (deuteriochloroform at 7.24 ppm). Optical rotations were measured on a Jasco DIP-181 digital polarimeter. Melting points are uncorrected. Analytical thin-layer chromatography was performed on EM Reagent 0.25 mm silica gel 60-F plates. Purification of reaction products was carried out by liquid chromatography using a forced flow (flash chromatography) of the indicated solvent system on EM Reagents silica gel 60 (230−400 mesh). All reactions were carried out under an atmosphere of nitrogen in glassware that had been flame dried under a stream of nitrogen.

Synthesis of (4*R*)-3-(Dodecanoyl)-4-benzyl-2-oxazolidinone (2(R)). To a magnetically stirred solution of 3.5 g (20.0 mmol) of (*R*)-4-benzyl-2-oxazolidinone (**1**) in 60 mL of anhydrous THF under nitrogen at −78 °C was added dropwise 12.6 mL (1.6 M in hexane, 20.2 mmol) of *n*-butyllithium over a 15-min period. Freshly distilled dodecanoyl chloride (5.08 mL, 22.0 mmol) was added in one portion after completion of the addition of butyllithium. The resulting clear, yellow solution was warmed to 0 °C over a 1-h period and then stirred for an additional hour. Excess dodecanoyl chloride was quenched by the addition of 20 mL of saturated aqueous ammonium chloride. Volatiles were removed in vacuo and the product was extracted with three 20-mL portions of dichloromethane. The combined organic extracts were successively washed with 10 mL of 1 M aqueous potassium carbonate and 10 mL of brine (saturated solution of NaCl), dried over anhydrous sodium sulfate, and filtered. The solvent was removed in vacuo to leave 10.0 g of a yellow solid, which was purified by flash chromatography (9:1 hexane/ethyl acetate as eluant) to afford 6.54 g (91%) of (4*R*)-3-(dodecanoyl)-4-benzyl-2-oxazolidinone (**2(R)**) as a white crystalline solid: mp 46−47 °C; ^{1}H-NMR (300MHz, $CDCl_3$) δ ppm 0.85 (t, 3H, CH_3, J = 7.2 Hz), 1.1−1.5 (m 18H, CH_2), 2.8 (dd, 1H, J = 13.3, 9.6 Hz, $CH_2C_6H_5$), 2.9 (m, 2H, CH_2CO), 3.3 (dd, 1H, J = 13.4, 3.3 Hz, $CH_2C_6H_5$), 4.1 (m, 2H, CH_2O), 4.65 (m, 1H, CHN), 7.1−7.4 (m, 5H, ArH); $[\alpha]_D$ −71.6° (*c* 1, CH_3COCH_3). Elemental Anal. Calcd for $C_{22}H_{33}NO_3$: C, 73.49; H, 9.25; N, 3.90. Found: C, 73.50; H, 9.23; N, 3.80.

Synthesis of (4*R*)-3-((2′*R*)-2′-Methyldodecanoyl)-4-benzyl-2-oxazolidinone (3(RR)). To a −78 °C solution of 14.7 mL (14.7 mmol, 1.1 equiv, 1.0 M in tetrahydrofuran) of sodium hexamethyldisilylamide was added dropwise a 0 °C solution of 4.8 g (13.4 mmol, 1 equiv) of (4*R*)-3-(dodecanoyl)-4-benzyl-2-oxazolidinone (**2(R)**) in 14.4 mL of THF. After the reaction mixture was stirred at −78 °C for 1 h, 9.5 g (4.2 mL, 67.0 mmol, 5 equiv) of iodomethane in 1.4 mL of THF at −78 °C was added. The solution was stirred 4 h at −78 °C and then quenched by the addition of 20 mL of aqueous saturated ammonium chloride solution. Volatiles were removed by rotary evaporation, and the resultant slurry was extracted with three 20-mL portions of methylene chloride. The combined organic fractions were washed with 100 mL of aqueous 1 M sodium sulfite solution, dried over anhydrous sodium sulfate, and concentrated in vacuo to give 5.0 g of a yellow oil. Purification by flash chromatography (9:1 hexane/ethyl acetate as eluant) gave 3.05 g (61%) of the title compound as a clear oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 0.85 (t, 3H, CH_3), 1.1−1.45 (d + m, 19H, CH_3, CH_2), 1.3−1.45 (m, 1H, CHH), 1.65−1.8 (m, 1H, CHH), 2.7 (dd, 1H, J = 13.3, 9.6 Hz, $CH_2C_6H_5$), 3.25 (dd, 1H, J = 13.4, 3.3 Hz, $CH_2C_6H_5$), 3.65−3.75 (m, 1H, CHCO), 4.1 (m, 2H, CH_2O), 4.65 (m, 1H, CHN), 7.1−7.4 (m, 5H, ArH), $[\alpha]_D$ −88.7 (*c* 1, CH_3COCH_3). Elemental Anal. Calcd for $C_{23}H_{35}NO_3$: C, 73.95; H, 9.44; N, 3.75. Found: C, 73.90; H, 9.40; N, 3.73.

Synthesis of (*R*)-2-Methyldodecanoic Acid (4(R)). A solution of 3.0 g (8 mmol) of (4*R*)-3-((2′*R*)-2′-methyldodecanoyl)-4-benzyl-2-oxazolidinone (**3(RR)**) in 160 mL of 3:1 THF/distilled water solution (0.05 M) was treated at 0 °C with 6.5 mL of 30% H_2O_2 (8 equiv) followed by 680 mg of 98% $LiOH\cdot H_2O$ (2 equiv). After the solution was stirred at 0 °C for 3 h, the excess peroxide was quenched at 0 °C with a 10% excess of 1.5 N aqueous sodium sulfite. The bulk of the THF was removed in vacuo and the resulting mixture was acidified to pH 1 by the addition of an aqueous 10% hydrochloric acid solution. The aqueous layer was extracted with three 20-mL portions of methylene chloride. The combined organic extracts, containing a mixture of the title compound **4(R)** and of the recovered oxazolidinone **1**, were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to give 2.8 g of a yellow solid. This slurry was chromatographed (flash silica gel, gradient from 8:2 hexane/ethyl acetate to 100% ethyl acetate) to give 1.3 g of the recovered (*R*)-4-benzyl-2-oxazolidinone (**1**) as a white crystalline solid and 1.5 g (90%) of pure (*R*)-2-methyldodecanoic acid (**4(R)**) as a colorless liquid: bp 120 °C (5×10^{-3} mmHg); ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 0.85 (t, 3H, CH_3), 1.17 (d, 3H), 1.18−1.35 (m, 16H, CH_2), 1.35−1.45 (m, 1H, CHH), 1.55−1.75 (m, 1H, CHH), 2.45 (m, 1H, CHCO); $[\alpha]_D$ −15.6° (*c* 1,

Scheme 4



4 (R) 8 (RR)

CH_3COCH_3). Elemental Anal. Calcd for $C_{13}H_{26}O_2$: C, 72.84; H, 12.23. Found: C, 72.85; H, 12.20.

(*R*)-2-methyldodecanoic acid (**4(R)**) was quantitatively converted into the corresponding methyl ester by reaction with an ethereal solution of diazomethane. Chiral shift studies on this methyl ester using Eu(hfc)$_3$ indicated that the product **4(R)** had an enantiomeric excess ≥98%. The same value (d.e. ≥98%) was obtained by GLC analysis (Carbowax 20 M/230 °C) on the diastereomeric mixture of amides **5a** and **5b** obtained by reaction of the acid **4(R)** with (*R*)-(+)-α-phenylethylamine.

Synthesis of the Diastereomeric Amides 5a and 5b. To a magnetically stirred solution of 140 mg (0.65 mmol) of 2-methyldodecanoic acid (**4**) in anhydrous diethyl ether were added dropwise 71.4 μL (0.98 mmol) of thionyl chloride and 4.6 μL of DMF. After standing at 25 °C for 2 h, the mixture was decanted, concentrated, dissolved in 2 mL of CH_2Cl_2, and added to a CH_2Cl_2 solution of (*R*)-(+)-α-phenylethylamine (126.3 mL, 0.98 mmol) and triethylamine (136 mL, 0.98 mmol). The mixture was stirred for 30 min at 25 °C, then washed with 2 N HCl and water, dried over anhydrous Na_2SO_4, and filtered. The solvent was removed in vacuo to give 186 mg (90%) of the corresponding (*RR*)- and (*RS*)-α-phenylethylamides as white solid: mp 60−62 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.85 (t, 3H, CH_3), 1.15 (d, 3H, CH_3), 1.15−1.4 (m, 17H, CH_2CHH), 1.5 (d, 3H, CH_3), 1.5−1.7 (m, 1H, CHH), 2.15 (m, 1H, CHCO), 5.15 (m, 1H, CHNH), 5.65 (d, 1H, NH), 7.2−7.4 (m, 5H, C_6H_5). GLC analysis (Carbowax 20m, 230 °C) of the amides gave the relative amounts of the diastereomers.

Synthesis of Racemic 2-Methyldodecanoic Acid (4(rac)). A solution of diisopropylamine (4.12 mL, 29 mmol) in anhydrous THF (25 mL) was charged in a flask under nitrogen atmosphere. After cooling at 0 °C, 18.1 mL (29 mmol) of *n*-butyllithium (1.6 M in hexane) was slowly added in order to keep the temperature at 0 °C. After standing at this temperature for an additional 20 min, *n*-dodecanoic acid (**7**) (2.8 g, 14 mmol) was added dropwise while maintaining the reaction temperature below 0 °C. A milky white solution formed and after 30 min DMPU (1.7 mL, 14 mmol) was added and the mixture was stirred at room temperature for 1 h. Then it was cooled again at 0 °C and CH_3I (0.9 mL, 15 mmol) was rapidly added. The reaction was complete by stirring the mixture at room temperature overnight. The product was recovered by neutralization with ice cooled 10% HCl, followed by extraction with Et_2O (3 × 30 mL). The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to obtain 2.9 g of a yellow liquid. Pure **4(rac)** (2.7 g, 90% yield) was obtained by distillation. Bp 120 °C (5 × 10^{-3} mmHg); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.85 (t, 3H, CH_3), 1.17 (d, 3H); 1.18−1.35 (m, 16H, CH_2), 1.35−1.45 (m, 1H, CHH), 1.55−1.75 (m, 1H, CHH), 2.45 (m, 1H, CHCO).

Synthesis of (*RR*)-(−)-2-Methyldodecanoic Anhydride (8(RR)) (Scheme 4). A solution of 2 g (9.3 mmol) of **4(R)** and 0.75 mL (9.3 mmol) of anhydrous pyridine in 9.5 mL of anhydrous Et_2O was cooled at −10 °C. Dropwise addition of 0.34 mL (4.65 mmol) of thionyl chloride afforded immediately a white precipitate. The mixture was stirred at −10 °C for 15 min, then filtered, and the residue was rapidly washed with anhydrous Et_2O. The solvent was removed in vacuo to leave 1.9 g of yellow oil. Purification by distillation (bp 165 °C, 5 × 10^{-3} mmHg) gave 1.5 g (80%) of the title compound as a colorless oil. ^{1}H-NMR (300MHz, $CDCl_3$) δ 0.85 (t, 6H, CH_3), 1.17 (d 6H, CH_3), 1.18−1.35 (m, 32H, CH_2), 1.35−1.45 (m, 2H, CHH), 1.55−1.75 (m, 2H, CHH), 2.51 (m, 2H, CHCO). IR: 1748 and 1815 cm^{-1} (C═O). Elemental Anal. Calcd for $C_{26}H_{50}O_5$: C, 69.04; H, 13.37. Found: C, 69.00; H, 13.37. $[\alpha]_D$ −17.2° (*c* 1, CH_3COCH_3).[29] DSC heating measurements gave an endothermic peak at around 12 °C.

Synthesis of (*SS*)-(+)-2-Methyldodecanoic Anhydride (8(SS)). 8-(SS) was synthesized in the same manner starting from **4(S)**. DSC heating measurements gave an endothermic peak around 12 °C, almost identical to **8(RR)**.

Synthesis of a Mixture of (*RR*)-, (*SS*)- and (*RS*)-(−)-2-Methyldodecanoic Anhydride (8(mix)). The diastereomeric mixture of the three anhydrides was prepared by the same procedure used for the synthesis of **8(RR)**, starting from racemic 2-methyldodecanoic acid (**4-(rac)**).

Differential Scanning Calorimetry Measurements. The thermal phase transitions of anhydrous and hydrated fatty acid samples of **4**, **7**, and **8** were determined by differential scanning calorimetry (DSC) using a TA-4000 system from Mettler (Nänikon-Uster, Switzerland). For anhydrous samples, a scan rate of 1 K/min was used. In the case of hydrated fatty acids, the samples were first prepared in glass tubes by dispersing appropriate amounts of lipid in water. NaOH corresponding to half the equivalent of the lipid was added to the water in advance to form a 1:1 acid−soap mixture. Equilibration was ensured by leaving the sealed tubes at room temperature for 5 days. The samples were cooled slowly (typical rate: 0.3−1 K/min) down to a temperature substantially below the phase transition temperature (T_c), kept at that temperature for 1 or 2 h for equilibration, and then heated (5 K/min) to get the DSC traces. We used a relatively high scan rate to obtain a reasonable signal-to-noise ratio from a limited amount of sample. Cooling and heating processes were repeated for the same sample and it was confirmed that DSC traces are reproducible.

In order to get a qualitative idea on the kinetics of exchange of molecules between vesicles, DSC measurements were carried out after mixing equal amounts of **4(R)** and **4(S)** vesicle suspensions. The change in the thermal phase transition temperature (T_c) was observed as a function of time after mixing.

Cmc Determinations. The critical concentration for micelle formation (cmc) of alkaline fatty acid solutions was determined either by conductivity measurements using a CDM 83 conductometer (Radiometer, Copenhagen, Denmark) or spectrophotometrically using pinacyanol chloride.[30,31] In the latter method, 8 μL of a 1.3 mM solution of pinacyanol chloride in methanol was added to 1 mL of the aqueous soap solution and the absorbance was measured at 605 nm with an Uvikon 820 spectrometer (Kontron, Zürich, Switzerland). Concentrations where abrupt changes in the concentration dependence of either conductivity or absorption of pinacyanol chloride occurred were used as cmc values.

Titration of Alkaline Fatty Acid Solutions with HCl. Ten milliliters of a micellar fatty acid solution were first prepared by dissolving 0.08 M fatty acid in 0.088 M NaOH (10 mol % excess with respect to the fatty acid). Aliquots of 0.5 mL of this solution were pipeted into small glass vials and various amounts of 1 M HCl were added to each vial. The glass vials were sealed and the samples were equilibrated at room temperature for at least 5 days. Then pH measurements were conducted using a PHM 82 pH meter (Radiometer, Copenhagen, Denmark) with a micro pH-electrode (Ingold, Steinbach, Germany).

Preparation of Fatty Acid Vesicles. The fatty acid was weighed into a glass flask and an appropriate amount of buffer solution was added. NaOH was then added to readjust the pH. This procedure led to the formation of a turbid suspension containing spontaneously formed vesicles. Homogenization of the suspension was achieved by vortexing. The samples were left for a few days to equilibrate. In certain cases, the mean size of the vesicles was decreased by extrusion through Nucleopore polycarbonate membranes filters with pores of defined size,[32] using "The Extruder" from Lipex Biomembranes Inc. (Vancouver, Canada). This process reduced the maximum size of the vesicles to approximately the size of the pores and made the suspensions more homogeneous.

The formation of vesicles was confirmed mainly by microscopic methods. Electron micrographs were taken by using the freeze-fracture technique as described before.[1a] The size of vesicles was determined from the electron micrographs by taking into account nonequatorial fracturing.[33] Vesicle dispersions were also analyzed by light microscopy

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for the presence of giant vesicles, using an Axioplan light microscope from Zeiss (Germany), connected to a Sony XC-75CE video camera.

Determination of the Internal Volume of the Vesicles. The internal aqueous volume of **4(rac)** vesicles was determined with arsenazo III[34] as water-soluble dye marker. The vesicle suspension was first prepared in the presence of arsenazo III (4.9 mM), and vesicles with entrapped dye molecules were separated from non-entrapped arsenazo III by size exclusion chromatography (sepharose 4B, length 5 cm, diameter 0.6 cm). The internal aqueous volume of the vesicles was determined by assuming equal dye concentration both inside and outside of the vesicles. The elution was performed with an eluent saturated with monomeric **4(rac)** (3 mM in 50 mM Tris/HCl, pH 7.7) to avoid dissolution of vesicles during the filtration. The concentration of the fatty acid was determined by FT-IR spectroscopy (see below). For the calculation of the internal volume, the free monomer concentration—as estimated by ultrafiltration experiments (see below)—has been taken into account: $[\text{fatty acid}]_{\text{in vesicles}} = [\text{fatty acid}]_{\text{total}} - [\text{fatty acid}]_{\text{monomer}}$.

Ultrafiltration of Fatty Acid Vesicle Samples. The experiments for the determination of the amount of non-aggregated monomeric fatty acid at a particular pH value were carried out in the following way: 80 mM **4(rac)** vesicles were first prepared in 50 mM Tris/HCl buffer (pH ranging from 7.7 to 8.5) and equilibrated for 3 days at room temperature. The vesicle sample was then subjected to ultrafiltration by using a Centricon-30 membrane filter device (molecular weight cutoff 30 000, Amicon Inc.: Beverly, MA) at a centrifugation speed of 2000 rpm for 90 min. The concentration of fatty acids appearing in the ultrafiltrate was determined by FT-IR spectroscopy.

Circular Dichroism Measurements. The optical activity of vesicles of **4** was determined by circular dichroism (CD) measurements using a Jasco J-600 instrument. The cells used for the measurement had path lengths of either 0.02 or 0.05 cm depending on the turbidity of the samples.

Infrared Spectroscopy (FT-IR). The concentrations of **4** in vesicle suspensions were determined through the intensity of C═O(st) at 1711 cm^{-1} by FT-IR. The typical procedure was as follows: 50 μL of the vesicle suspension was acidified by adding 100 μL of 1 N HCl. The protonated fatty acid was then extracted into 200 μL of isooctane and FT-IR was measured by a Nicolet 5SXC FT-IR spectrometer using a CaF_2 cell with a 0.2 mm optical path length.

Kinetic Measurements. Typical experimental conditions were as follows:[1a]

(a) Hydrolysis of 8 in the Absence of Vesicles. Inside a flat-bottom test tube (diameter 2.5 cm) 0.15 mmol (6.15 mg) of **8** were added onto 10 mL of bicine buffer (0.05 M, pH 8.3), containing a magnetic stirrer bar (length 2.4 cm, thickness 0.5 cm; 100 rpm), at 25 °C. From time to time 50 μL of the aqueous phase were withdrawn and the concentration of **4** was determined by FT-IR.

(b) Hydrolysis of 8 in the Presence of Vesicles of 4. All the conditions were the same as those just described, with the only exception that the aqueous phase contained spontaneously formed vesicles of **4**, typically at a concentration of 40 mM.

Some of the hydrolysis experiments were performed in a diode array spectrophotometer (Hewlett Packard, Model 8452A). In these experiments, the turbidity of the reaction solution was monitored continuously under the following experimental configuration. A flat-bottom test tube (diameter 1.0 cm) was filled with 3 mL of **4** vesicle solution (40 mM of **4** in 0.05 M bicine buffer at pH 8.3). The temperature of the cell was controlled by a thermostat. The solution was stirred constantly using a magnetic stirrer. The appropriate amount of **8** (typically 0.03 mmol, corresponding to 20 mM of **4** after hydrolysis) was put onto the surface of the solution and the hydrolysis reaction was started.

Centrifugation. Hydrolysis experiments which were carried out at 10 °C using vesicles and anhydride molecules of opposite chirality led to the formation of a gel-like solid phase (see below). This solid phase was separated from the aqueous phase by centrifugation at 4 °C ($6.7 \times 10^3 \times$ g, 70 min) using a ALC micro CENTRIFUGETTE 4214 centrifugator.

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