Preparation of Stable Single-Compartment Vesicles with Cationic and Zwitterionic Amphiphiles Involving Amino Acid Residues[1]

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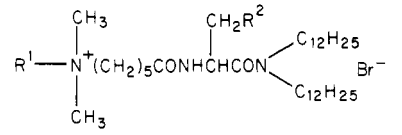
Received September 9, 1981

Cationic and zwitterionic amphiphiles involving amino acid residues were synthesized as modifications of the phospholipids. Their aggregated structures in both dilute aqueous dispersion and solution were investigated by electron microscopy. Amphiphiles involving an alanine residue or a sarcosine residue, *N,N*-didodecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{12}$), *N,N*-didodecyl-N^{α}-[6-[dimethyl(2-carboxyethyl)ammonio]hexanoyl]-L-alaninamide bromide ($CAC_2N^+C_5Ala2C_{12}$), *N,N*-didodecyl-N^{α}-[6-(trimethylammonio)hexanoyl]sarcosinamide bromide ($N^+C_5Sar2C_{12}$), and *N,N*-didodecyl-N^{α}-[6-[dimethyl(2-carboxyethyl)ammonio]hexanoyl]sarcosinamide bromide ($CAC_2N^+C_5Sar2C_{12}$), readily form well-developed multiwalled bilayer assemblies (lamellae and vesicles) and stable single-walled vesicles without any additives in dilute aqueous dispersion and sonicated aqueous solution, respectively. On the other hand, those involving one or three leucine residues, *N,N*-didodecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-leucinamide bromide ($N^+C_5Leu2C_{12}$), *N,N*-didodecyl-N^{α}-[6-[dimethyl(2-carboxyethyl)ammonio]hexanoyl]-L-leucinamide bromide ($CAC_2N^+C_5Leu2C_{12}$), and *N,N*-didodecyl-N^{α}-[$N^{\alpha'}$-[$N^{\alpha''}$-[6-(trimethylammonio)hexanoyl]-L-leucyl]-L-leucyl]-L-leucinamide bromide ($N^+C_5Leu_32C_{12}$), form partly developed multiwalled bilayer assemblies in aqueous dispersion while stable single- or double-walled vesicles are observable in sonicated aqueous solution. The significance of the tripartite structure in amphiphile assemblies for the formation of stable single-walled bilayer vesicles has been discussed.

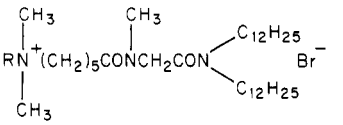
The major component molecules of cell membranes are lipids and phospholipids with some specific proteins. Membrane proteins are not well characterized nor is their molecular structure precisely known. It is true that the orientational structure of such proteins in relation to the molecular structure of the lipids in a membrane needs to be clarified for understanding how they function. As the initial step, however, various structural aspects of the membrane-forming lipids must be investigated. Phospholipids swell in water, form many spherical bodies composed of concentric layers, lamellae, with water trapped between them, and are transformed into vesicles by sonication, referred to as liposomes.[2,3] Although the structure of the phospholipid bilayers has been extensively investigated in connection with their biological functions,[4] their complexity and chemical instability have necessitated development of more stable membrane-forming amphiphiles. Although such efforts have been exerted in recent years,[5] stable single-compartment vesicles have not been obtained with a single species of amphiphiles.

The phospholipids are known to have a tripartite structure:[6] a hydrophobic aliphatic double chain, a hydrophilic head group of phosphate ester, and the region where these two moieties are linked. Brockerhoff named the interface between hydrophobic and polar layers the "hydrogen belt".[6] We intend to improve the stability of bilayer assemblies by modifying the "hydrogen belt", in reference to our results that an amino acid residue placed in the hydrophobic region acts to tighten the micellar structure,[7] and to evaluate the significance of the tripartite concept for the formation of stable bilayer assemblies. The structural resemblance between our synthetic amphiphiles and phospholipids, as they form molecular assemblies, is illustrated in Figure 1. We report here syntheses of four cationic ($N^+C_5Ala2C_{12}$, $N^+C_5Sar2C_{12}$, $N^+C_5Leu2C_{12}$, and $N^+C_5Leu_32C_{12}$) and three zwitterionic amphiphiles ($CAC_2N^+C_5Ala2C_{12}$, $CAC_2N^+C_5Sar2C_{12}$, and $CAC_2N^+C_5Leu2C_{12}$; see Chart I), physical properties of these amphiphiles, and the structures of their aggregates in aqueous media.

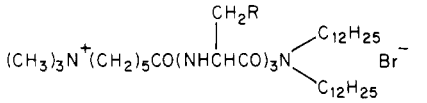
Chart I



$N^+C_5Ala2C_{12}$, $R^1 = CH_3$; $R^2 = H$
$CAC_2N^+C_5Ala2C_{12}$, $R^1 = CH_2CH_2COOH$; $R^2 = H$
$N^+C_5Leu2C_{12}$, $R^1 = CH_3$; $R^2 = CH(CH_3)_2$
$CAC_2N^+C_5Leu2C_{12}$, $R^1 = CH_2CH_2COOH$; $R^2 = CH(CH_3)_2$



$N^+C_5Sar2C_{12}$, R = CH_3
$CAC_2N^+C_5Sar2C_{12}$, R = CH_2CH_2COOH



$N^+C_5Leu_32C_{12}$, R = $CH(CH_3)_2$

Results and Discussion

Preparation and Properties. Cationic and zwitterionic amphiphiles involving a leucine residue, $N^+C_5Leu2C_{12}$ and $CAC_2N^+C_5Leu2C_{12}$, were prepared according to Scheme IA. *N,N*-Didodecyl-N^{α}-(*tert*-butoxycarbonyl)-L-leucine (1) was obtained by condensation of didodecylamine and (*tert*-butoxycarbonyl)-L-leucine in the presence of dicyclohexylcarbodiimide. The *tert*-butoxycarbonyl group of 1 was removed by treatment with excess trifluoroacetic acid, and the elimination was confirmed by NMR spectroscopy. The amine component (2) was cou-

(1) Preliminary account: Murakami, Y.; Nakano, A.; Fukuya, K. *J. Am. Chem. Soc.* **1980**, *102*, 4253.
(2) Bangham, A. D.; Standish, M. M.; Watkins, J. C. *J. Mol. Biol.* **1965**, *13*, 238.
(3) Huang, C. *Biochemistry* **1969**, *8*, 344.
(4) For example: Bangham, A. D. In "Liposomes in Biological Systems"; Gregoriadis, G., Allison, A. C., Eds.; Wiley: New York, 1978.
(5) (a) Kunitake, T.; Okahata, Y. *J. Am. Chem. Soc.* **1980**, *102*, 549. (b) Deguchi, K.; Mino, J. *J. Colloid Interface Sci.* **1978**, *65*, 155. (c) Mortara, R. A.; Quina, F. H.; Chaimovich, H. *Biochem. Biophys. Res. Commun.* **1978**, *81*, 1080. (d) Sudhölter, E. J. R.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **1980**, *102*, 2467.
(6) Brockerhoff, H. In "Bioorganic Chemistry"; van Tamelen, E. E., Ed.; Academic Press: New York, 1977; Vol. 3, Chapter 1.
(7) Murakami, Y.; Nakano, A.; Iwamoto, K.; Yoshimatsu, A. *J. Chem. Soc., Perkin Trans. 2* **1980**, 1809.

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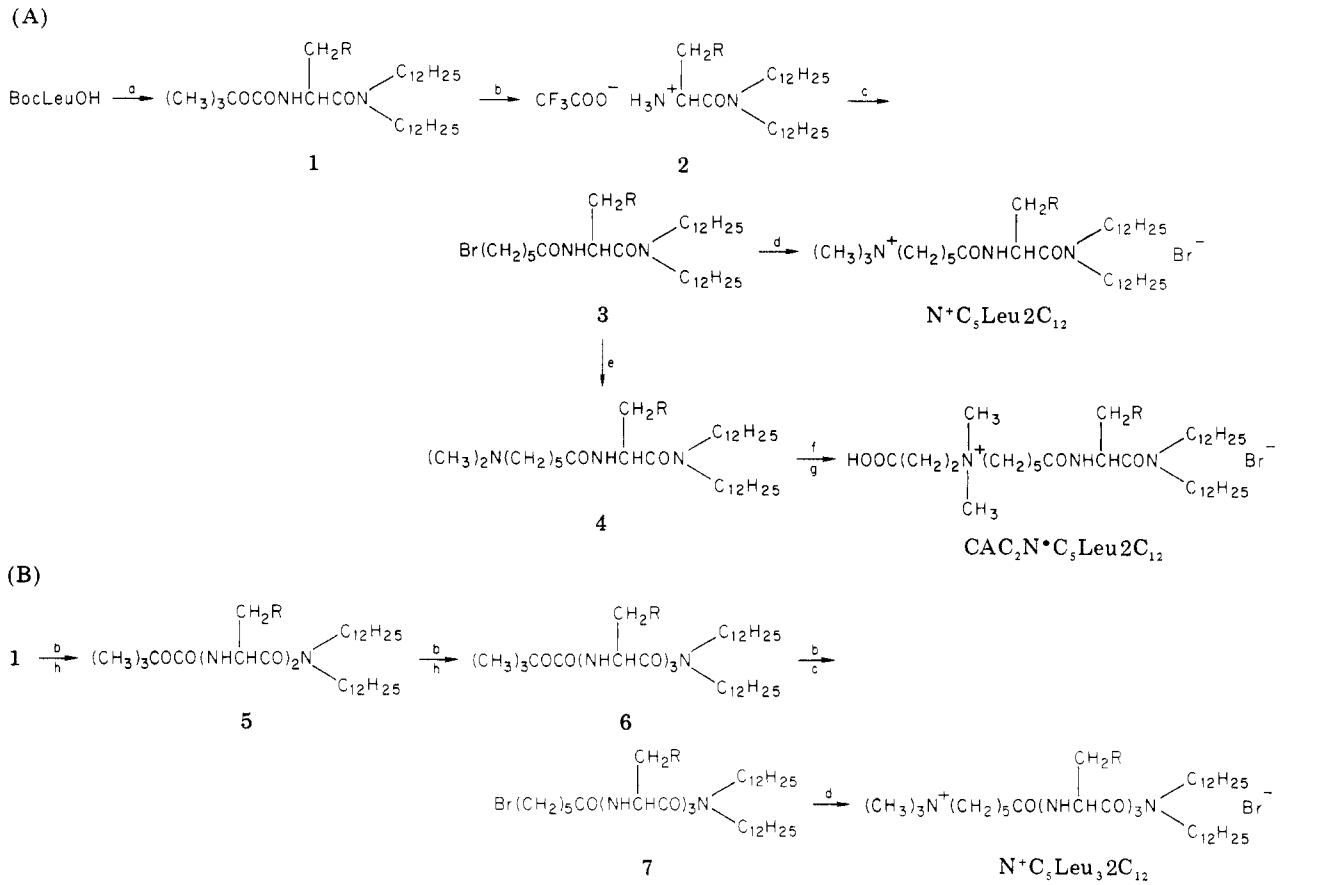
Scheme I[a]



[a] R = $CH(CH_3)_2$. Reagents: a, $(C_{12}H_{25})_2NH$ and DCC; b, CF_3COOH; c, $Br(CH_2)_5COCl$; d, $(CH_3)_3N$; e, $(CH_3)_2NH$; f, $BrCH_2CH_2COOH$ and NaOH; g, HBr; h, BocLeuOH and DCC.

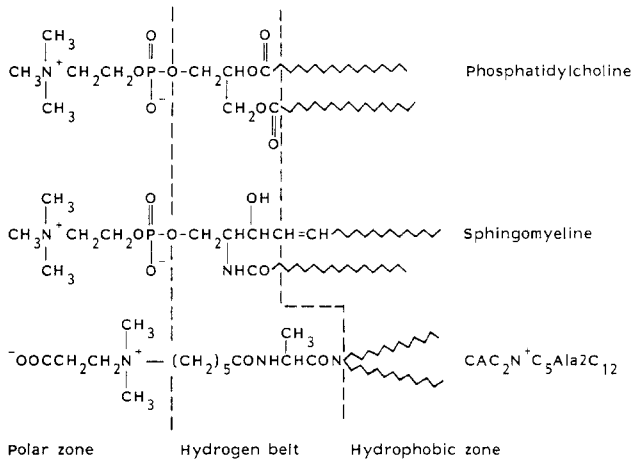


Figure 1. Structural resemblance among molecular assemblies formed with phospholipids and synthetic amphiphile.

pled with 6-bromohexanoyl chloride to give *N,N*-didodecyl-N^{α}-(6-bromohexanoyl)-L-leucinamide (**3**) in a good yield. Dry trimethylamine and dimethylamine gases were introduced into a benzene solution of **3** to afford *N,N*-didodecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-leucinamide bromide ($N^+C_5Leu2C_{12}$) and *N,N*-didodecyl-N^{α}-[6-(dimethylamino)hexanoyl]-L-leucinamide hydrobromide (**4**·HBr), respectively. The latter was isolated as a free base form, *N,N*-didodecyl-N^{α}-[6-(dimethylamino)hexanoyl]-L-leucinamide (**4**), by treatment of the hydrobromide salt with aqueous sodium hydrogen carbonate. The corresponding zwitterionic amphiphile was prepared from **4** by reaction with 3-bromopropionic acid in the presence of an equimolar amount of sodium hydroxide in aqueous acetone and isolated as a hydrobromide salt, *N,N*-didodecyl-N^{α}-[6-[dimethyl(2-carboxyethyl)ammonio]hexanoyl]-L-leucinamide bromide ($CAC_2N^+C_5Leu2C_{12}$). The other amphiphiles involving a L-alanine and a sarcosine residue, $N^+C_5Ala2C_{12}$, $N^+C_5Sar2C_{12}$, $CAC_2N^+C_5Ala2C_{12}$, and $CAC_2N^+C_5Sar2C_{12}$, were prepared in similar experimental procedures. An amphiphile involving three L-leucine residues, $N^+C_5Leu_32C_{12}$, was prepared according to Scheme IB. *N,N*-Didodecyl-N^{α}-[$N^{\alpha'}$-[$N^{\alpha''}$-(*tert*-butoxycarbonyl)-L-leucyl]-L-leucyl]-L-leucinamide (**6**) was obtained by condensation of (*tert*-butoxycarbonyl)-L-leucine with didodecylamine, followed by successive condensation with (*tert*-butoxycarbonyl)-L-leucine in a step-by-step manner.

The physical and analytical data for amphiphiles prepared in this work are summarized in Table I. All these amphiphiles are liquid crystals and only final melting points to the liquid state are listed. It is of interest to note here that the nature of a polar head group primarily controls the melting point as suggested by Chapman for phospholipids.[8] Melting points of the zwitterionic amphiphiles are lower than those of the corresponding cationic ones, possibly due to the fact that the zwitterionic ones are not actually in zwitterionic state as isolated. An ionic network must exist in the crystalline state of $N^+C_5Ala2C_{12}$ and $N^+C_5Sar2C_{12}$ with the bromide ion as a counteranion. On the other hand, such an ionic network would be disturbed by the neutral bulky 2-carboxyethyl group placed in a spatial region of the charged ammonium head group, as expected for $CAC_2N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Sar2C_{12}$. Steric hindrance exercised by the

(8) Chapman, D. In "Form and Function of Phospholipids"; Ansell, G. B., Dawson, R. M. C., Hawthorne, J. N., Eds.; Elsevier: Amsterdam, 1973; Chapter 6.

Table I. Physical and Analytical Data of Amphiphiles

amphiphile	formula	elemental analysis,[a] % C	H	N	mp,[b] °C	$[\alpha]^{20}_D$,[c] deg	CAC, M	ST,[d] dyne cm^{-1}
$N^+C_5Ala2C_{12}$	$C_{36}H_{74}N_3O_2Br$	65.07 (65.42)	11.27 (11.28)	6.09 (6.36)	195	−18.5 (0.91)	1.5×10^{-5}	26
$CAC_2N^+C_5Ala2C_{12}$	$C_{38}H_{76}N_3O_4Br$	64.07 (63.48)	11.07 (10.65)	6.15 (5.84)	98	−18.7 (1.23)	2.1×10^{-5}	25
$N^+C_5Sar2C_{12}$	$C_{36}H_{74}N_3O_2Br$	65.42 (65.43)	11.21 (11.29)	6.22 (6.36)	198		1.0×10^{-5}	26
$CAC_2N^+C_5Sar2C_{12}$	$C_{38}H_{76}N_3O_4Br$	63.73 (63.48)	10.96 (10.65)	5.97 (5.84)	110		3.5×10^{-6}	20
$N^+C_5Leu2C_{12}$	$C_{39}H_{80}N_3O_2Br + {}^3/_2H_2O$	64.44 (64.17)	11.23 (11.05)	5.68 (5.76)	80	−10.5 (1.05)	1.1×10^{-5}	22
$CAC_2N^+C_5Leu2C_{12}$	$C_{41}H_{82}N_3O_4Br + 0.5H_2O$	64.05 (63.95)	11.12 (10.86)	5.39 (5.45)	73	−19.9 (1.06)	1.2×10^{-5}	23
$N^+C_5Leu_32C_{12}$	$C_{51}H_{102}N_5O_4Br + {}^2/_3H_2O$	65.07 (65.03)	11.14 (11.06)	7.01 (7.34)	130	−48.3 (1.04)	8.2×10^{-6}	22

[a] Calculated values are given in parentheses. [b] Final melting point to liquid state. [c] Amphiphile concentration in ethanol (g/100 mL) is given in parentheses. [d] Minimal value of surface tension for sonicated solution.

Table II. Structures of Molecular Assemblies

amphiphile	A (dispersion)[a,b]	B (sonicated)[a,c]	particle size,[e] Å
$N^+C_5Ala2C_{12}$	vesicle (Figure 3A) (lamella)	single (Figure 3B)	160–600
$CAC_2N^+C_5Ala2C_{12}$	vesicle (lamella)[d]	single[d]	125–600
$N^+C_5Sar2C_{12}$	vesicle, lamella	single	125–600
$CAC_2N^+C_5Sar2C_{12}$	lamella (Figure 4A) (vesicle)	single (Figure 4B)	125–800
$N^+C_5Leu2C_{12}$	vesicle (Figure 5A) (lamella)	double (single) (Figure 5B)	125–600
$CAC_2N^+C_5Leu2C_{12}$	lamella (Figure 6A) (vesicle)	double (single) (Figure 6B)	200–1200
$N^+C_5Leu_32C_{12}$	vesicle, lamella (Figure 7A)	double (single) (Figure 7B)	125–1200

[a] Vesicle, multiwalled vesicle; lamella, multiwalled lamella; single, single-walled vesicle; double, double-walled vesicle. [b] Structure in parentheses partly observed. [c] Sonicated for 2 min at a 30-W power level; structure in parentheses partly observed. [d] Reference 13a. [e] For sample B.

bulky hydrophobic side chain of a leucine residue seems to perturb the ionic network and to reduce the ionic lattice interaction for the amphiphiles involving a leucine residue or residues, resulting in relatively lower melting points.

The critical aggregate concentrations (CAC) were determined from the break points of plots of amphiphile concentration vs. surface tension. The CAC values obtained for the present amphiphiles ($2 \times 10^{-5} - 3 \times 10^{-6}$ M) are in a range observed for peptide surfactants *N*-dodecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-*S*-benzyl-L-cysteinamide bromide and *S*,*S*′-bis[*N*-dodecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-hemicysteinamide] bromide,[7] which may form tight and large cylindrical micelles. The CAC values for the present amphiphiles are also comparable to those for ammonium amphiphiles bearing either a single or a double chain ($<6 \times 10^{-5}$ M) which yield lamellae and/or vesicles in aqueous solution.[5a,9] On the other hand, ammonium amphiphiles bearing a single hydrophobic chain such as *N*-dodecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-aspartamide bromide[7] and octadecyltrimethylammonium chloride,[10] which do not form stable bilayer assemblies in dilute aqueous solution, have comparatively higher CAC values. These experimental facts apparently indicate that an ammonium amphiphile must have a lower CAC value ($<6 \times 10^{-5}$ M)[5a] in order to afford a bilayer assembly even though double-headed ammonium amphiphiles forming monolayer lamella-type aggregates are an exception.[11] The minimal surface tension values for the present amphiphiles are listed in Table I. The lower the surface tension of an amphiphile solution is, the lower the CAC value for the corresponding aggregate observed, in general. As for micelle-forming amphiphiles, the aggregation tendency increases as the surface activity of an amphiphilic monomer becomes larger, and the CAC value of the micelle is consequently lowered.[12] Thus, no matter what kind of molecular assembly an amphiphile produces, such an aggregation tendency is enhanced as its surface activity increases. In turn, such macroscopic CAC observations reflect the structural effects of hydrophobic segments involved in amphiphiles for the formation of molecular assemblies. Apparently, a significantly bulky hydrophobic segment favors the formation of bilayer assemblies, lamellae, or vesicles.

The transition between crystalline and liquid crystalline states for vesicles has been examined by ESR and fluorescence techniques; phase-transition occurs below ca. 20 °C for double-chain amphiphiles involving an amino acid residue.[13] Fluorescence spectroscopy was adopted in this work, and the phase transition for all the amphiphiles was seen to occur below 25 °C regardless of the nature of an amino acid residue (Figure 2, as an example).

Morphology of Aggregates. The structure of molecular assemblies can be elucidated most directly by electron microscopy. All the present amphiphiles were dispersed in water at concentrations of 5 mM and at room temperature (≥25 °C) with occasional shaking, which resulted in a turbid state. Both multiwalled vesicles and lamellae were generally observed in electron micrographs of these aqueous dispersions as summarized in Table II, in which parenthesized structures were partly observed. Sonication

(9) Kunitake, T.; Okahata, Y.; Ando, R.; Shinkai, S.; Hirakawa, S. *J. Am. Chem. Soc.* **1980**, *102*, 7877.
(10) Grieger, P. F.; Kraus, C. A. *J. Am. Chem. Soc.* **1948**, *70*, 3803.
(11) Okahata, Y.; Kunitake, T. *J. Am. Chem. Soc.* **1979**, *101*, 5231.
(12) Fendler, J. H.; Fendler, E. J. "Catalysis in Micellar and Macromolecular Systems"; Academic Press: New York, 1975; p 33.
(13) (a) Murakami, Y.; Nakano, A.; Fukuya, K. *J. Am. Chem. Soc.* **1980**, *102*, 4253. (b) Murakami, Y.; Nakano, A.; Yoshimatsu, A.; Fukuya, K. *Ibid.* **1981**, *103*, 728. (c) Murakami, Y.; Aoyama, Y.; Nakano, A.; Tada, T.; Fukuya, K. *Ibid.* **1981**, *103*, 3951.

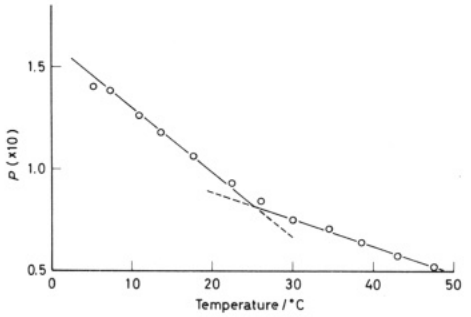


Figure 2. Correlation between fluorescence polarization (P) of DSTA (5.3×10^{-6} M) and temperature in single-walled vesicles of $N^+C_5Leu2C_{12}$ (7.62×10^{-4} M) dispersed in aqueous phosphate buffer [pH 6.94 and $\mu = 0.10$ (KCl)].

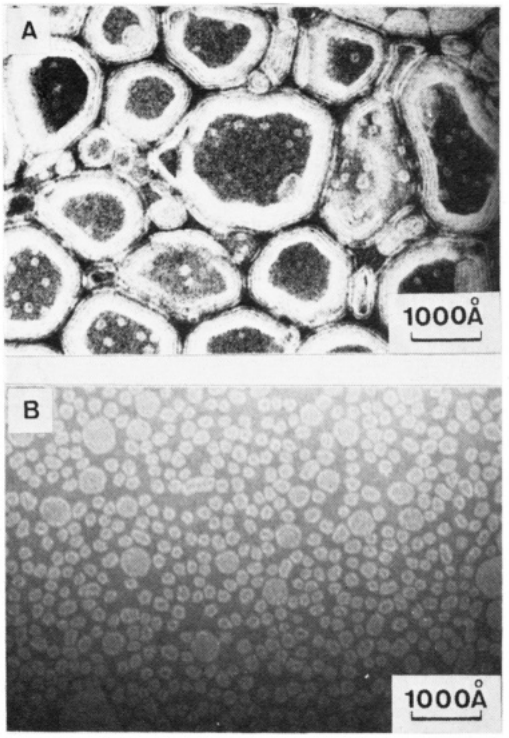


Figure 3. Electron micrographs negatively stained with uranyl acetate: A, 5 mM aqueous dispersion of $N^+C_5Ala2C_{12}$ (magnification, ×94 000); B, 5 mM aqueous solution of $N^+C_5Ala2C_{12}$ sonicated for 2 min at a 30-W power level and allowed to stand for 10 min at 5 °C (magnification, ×94 000).

of aqueous dispersions with a probe-type sonicator for 2 min at a 30-W power level gave clear solutions, and the electron micrographs showed the presence of small particles. These particles observed for the amphiphiles involving an alanine or a sarcosine residue are apparently single-walled vesicles as shown in Figures 3B and 4B. On the other hand, double-walled vesicles are mainly observed for the aqueous solutions of the amphiphiles involving a leucine residue or residues though clear single-walled vesicles are partly detected (Figures 5B, 6B, and 7B). An aqueous dispersion of phosphatidylcholine–phosphatidylethanolamine (1:1) in the presence of Cytochrome c gave an electron micrograph showing a large number of small particles in the range 200–1000 Å, mainly multiwalled vesicles and a few single-walled ones.[14a] The general appearance of each of these different vesicles bears a close resemblance to the corresponding electron micrographs

(14) (a) Papahadjopoulos, D.; Miller, N. *Biochim. Biophys. Acta* **1967**, *135*, 624. (b) Bangham, A. D.; Horne, R. W. *J. Mol. Biol.* **1964**, *8*, 660.

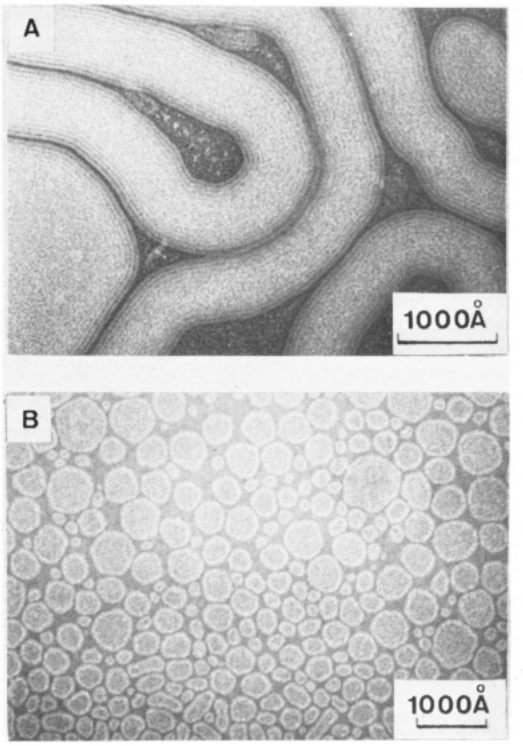


Figure 4. Electron micrographs negatively stained with uranyl acetate: A, 5 mM aqueous dispersion of $CAC_2N^+C_5Sar2C_{12}$ (magnification, ×132 000); B, 5 mM aqueous solution of $CAC_2N^+C_5Sar2C_{12}$ sonicated for 2 min at a 30-W power level and allowed to stand for 10 min at 5 °C (magnification, ×94 000).

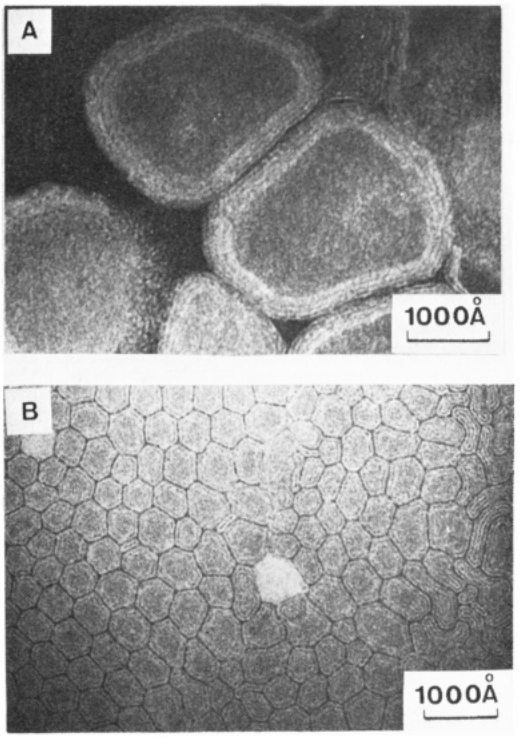


Figure 5. Electron micrographs negatively stained with uranyl acetate: A, 5 mM aqueous dispersion of $N^+C_5Leu2C_{12}$ (magnification, ×109 000); B, 5 mM aqueous solution of $N^+C_5Leu2C_{12}$ sonicated for 2 min at a 30-W power level and allowed to stand for 10 min at 5 °C (magnification, ×97 000).

observed in this work. The double-walled vesicles of $N^+C_5Leu2C_{12}$ were transformed into single-walled ones under stronger sonication conditions (for 6 min at 40 W with a probe-type sonicator), though the aggregate structures for $CAC_2N^+C_5Leu2C_{12}$ and $N^+C_5Leu_32C_{12}$ remained

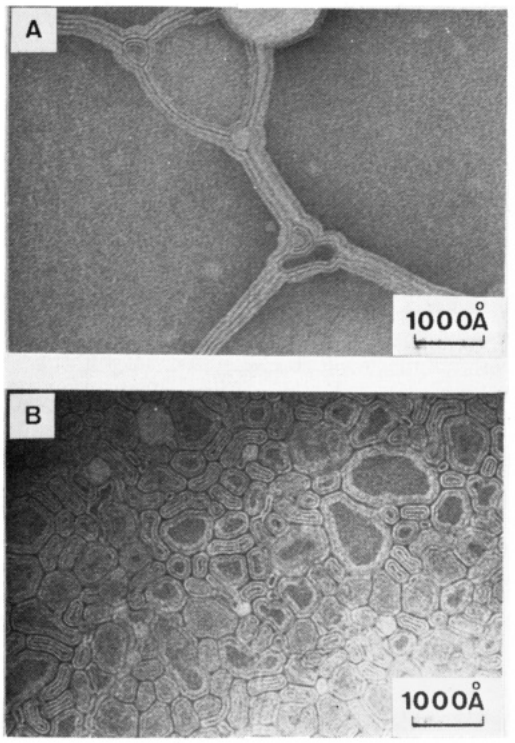


Figure 6. Electron micrographs negatively stained with uranyl acetate: A, 5 mM aqueous dispersion of $CAC_2N^+C_5Leu2C_{12}$ (magnification, ×94 000); B, 5 mM aqueous solution of $CAC_2N^+C_5Leu2C_{12}$ sonicated for 2 min at a 30-W power level and allowed to stand for 10 min at 5 °C (magnification, ×94 000).

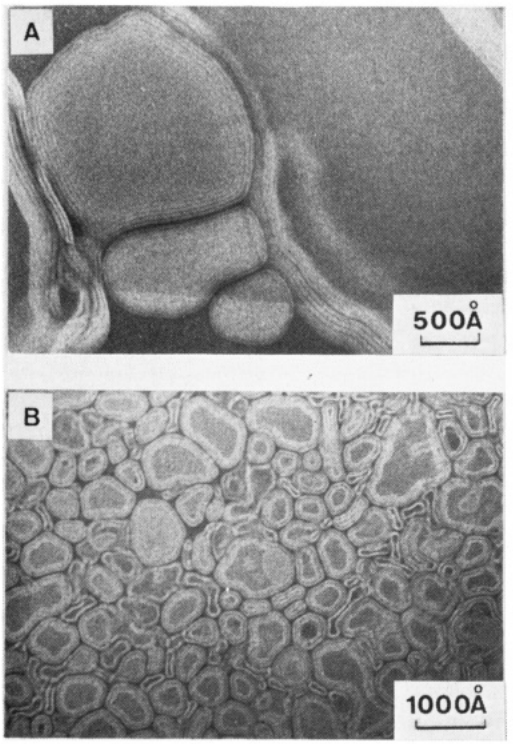


Figure 7. Electron micrographs negatively stained with uranyl acetate: A, 5 mM aqueous dispersion of $N^+C_5Leu_32C_{12}$ (magnification, ×156 000); B, 5 mM aqueous solution of $N^+C_5Leu_32C_{12}$ sonicated for 2 min at a 30-W power level and allowed to stand for 10 min at 5 °C (magnification, ×94 000).

the same under the identical sonication conditions. The thickness of each layer, regardless of multiwalled and single-walled vesicles, is estimated to be 40–60 Å, approximately twice the length of the hydrophobic segment of the amphiphiles. The observed layer thickness is consistent with the formation of bilayer membrane, as is the case for phospholipids.[14] All the sonicated solutions of the present amphiphiles remained in a clear state over at least several weeks without any additives, and their electron micrographs taken after such a prolonged period of time were identical with those taken with fresh solutions. Although small particles, which are composed of a single species of synthetic amphiphiles, have been claimed to be single-walled, such vesicles can hardly be identified from their electron micrographs,[5b-d,15,16] with the exception of ours.[13] Therefore, the present amphiphiles are exclusive ones which afford multiwalled bilayer assemblies (lamellae and vesicles) and single-walled vesicles with a single species of amphiphiles in turbid dispersion and upon sonication, respectively, as observed clearly by electron microscopy. Electron microscopic observations show that these amphiphiles, except for the ones bearing a leucine residue or residues, readily form stable single-walled vesicles regardless of the nature of the hydrophilic head group (cationic or zwitterionic) and of the amino acid residue (alanine or sarcosine). Under stronger sonication conditions, however, $N^+C_5Leu2C_{12}$ can form single-walled vesicles as mentioned above. The bulky hydrophobic side chain of a leucine residue seems to disturb the formation of intermolecular hydrogen bonds in the hydrogen belt region of molecular assemblies and prevents the amphiphiles from forming single-walled vesicles due to thermodynamic reasons. As for the amphiphiles forming single-walled vesicles, $N^+C_5Ala2C_{12}$ and $CAC_2N^+C_5Ala2C_{12}$ can provide efficient hydrogen donors and acceptors without a bulky side chain for the construction of a stable hydrogen belt region. On the other hand, $N^+C_5Sar2C_{12}$ and $CAC_2N^+C_5Sar2C_{12}$ may afford only hydrogen acceptors. Since a microenvironment where an amino acid residue is placed in a bilayer assembly is as polar as methanol,[13c] a few water molecules must be incorporated into this range. Thus, such water molecules must take part in the formation of a stable hydrogen belt domain.

In conclusion, our present amphiphiles may form bilayer aggregates in a manner similar to that observed for the naturally occurring phospholipids. Single-compartment vesicles are quite stable in aqueous media at room temperature above 25 °C owing to the so-called tripartite structure of assemblies[6] for which the hydrogen belt effect provided by an amino acid residue seems to be important. Since the present vesicles involve asymmetric centers in the hydrophobic region, they may provide asymmetric recognition sites for various chiral guest molecules. Furthermore, various amino acid residues, not only the present ones but also others, except those having a bulky hydrophobic side chain, can be utilized as efficient molecular components for the hydrogen belt formation. Preparation of various amphiphiles eligible for affording *functionalized membranes* is now in progress in our laboratories.

Experimental Section

IR spectra were measured with a JASCO DS-403G grating spectrophotometer. ^1H NMR spectra were taken on either a Hitachi R-24B or a Hitachi Perkin-Elmer R-20 spectrometer with tetramethylsilane in deuteriochloroform as an internal reference. ESR spectra were recorded at room temperature (22 °C) on a JEOL JES-ME-3 X-band spectrometer equipped with a 100-kHz field-modulation unit; a standard MgO/Mn(II) sample calibrated with a NMR magnetometer was employed for calibration of the magnetic field. Optical rotations were measured with a Union

(15) Kunitake, T.; Okahata, Y. *J. Am. Chem. Soc.* **1977**, *99*, 3860.
(16) Kano, K.; Romero, A.; Bjermouni, B.; Ache, H. J.; Fendler, J. H. *J. Am. Chem. Soc.* **1979**, *101*, 4030.

Giken PM-71 high-sensitivity polarimeter. Melting points were measured with a Yanaco MP-S1 melting point apparatus (hot-plate type) with a filter for polarized light.

Fluorescence Polarization. Phase-transition temperatures for the amphiphiles were determined from the break points of plots of fluorescence polarization of *N*-dansyltetradecylamine (DSTA), incorporated into the bilayer assemblies, vs. temperature according to the procedure reported by Iwamoto and Sunamoto.[17] All the fluorescence polarization measurements with DSTA as a probe were made on a Hitachi 650-60 spectrofluorometer equipped with polarizers and a thermostatic cell holder for control of temperature within ±0.1 °C over the range of 5–48 °C. With excitation at 340 nm, the emission maximum shifted to longer wavelength as the temperature was raised (e.g., with DSTA in single-walled vesicles of $N^+C_5Leu2C_{12}$, from 508 to 517 nm as the temperature increased from 5.1 to 47.4 °C). Such emission maxima were used for the measurements with a slit width of 5 nm for both excitation and emission sides. The fluorescence polarization (P) was calculated by eq 1, where I is the fluorescence

$$P = \frac{I_{vv} - C_f I_{vh}}{I_{vv} + C_f I_{vh}} \quad (1)$$

intensity, and the subscripts v and h refer to the orientation, vertical and horizontal, respectively, for the excitation and analyzer polarizers in this sequence; e.g., I_{vh} indicates the fluorescence intensity measured with a vertical excitation polarizer and a horizontal analyzer polarizer.[18] C_f is the grating correction factor, given by I_{hv}/I_{hh}.

The sample solutions were prepared as follows. An amphiphile (7–9 mg) was suspended in 20 mL of aqueous phosphate buffer (5×10^{-4} M) at pH 6.94 and $\mu = 0.10$ (KCl). The dispersion was sonicated for 2 min with a probe-type sonicator at a 30-W power level (W-220F, Heat Systems-Ultrasonics), allowed to stand for 10 min at 5 °C, and left at room temperature. The clear sonicated solution (3.0 mL) was pipetted into a thermostated cell set in the spectrofluorometer, and a methanol solution of DSTA (30 μL, 5.30×10^{-4} M) was injected into the solution, resulting in a molar ratio of 100:1 amphiphile/DSTA.

Surface Tension. The surface tension measurements were performed at room temperature with a Shimadzu ST-1 surface tension apparatus assembled by the Wilhelmy principle. An aqueous dispersion of an amphiphile was sonicated for 5 min at 30 W with a probe-type sonicator and allowed to stand for 10 min at 5 °C to give a clear solution. This was appropriately diluted with pure water for the measurements.

Electron Microscopy. Aqueous dispersions (A) and ultrasonicated aqueous solutions (B) of amphiphiles were employed. For sample A, 7–9 mg of an amphiphile was suspended in 2 mL of deionized and distilled water containing 2% (w/w) uranyl acetate, and the dispersion was shaked occasionally and heated until the glassy solid disappeared completely to give a turbid dispersion. For sample B, an aqueous dispersion of an amphiphile, as mentioned above for preparation of sample A, was sonicated for 2–6 min with a probe-type sonicator at 30–40 W (W-220F, Heat Systems-Ultrasonics) and allowed to stand for 10 min at 5 °C. A clear or slightly turbid solution was then obtained. Samples A and B were applied on carbon grids and dried in a vacuum desiccator. A JEOL JEM-200B electron microscope, installed at the Research Laboratory of High Voltage Electron Microscopy of our university, was used for the measurements.

Materials. (*tert*-Butoxycarbonyl)-L-alanine, (*tert*-butoxycarbonyl)-L-leucine, and (*tert*-butoxycarbonyl)sarcosine were prepared from the corresponding amino acids and *tert*-butyl *S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate by the methods of Nagasawa et al.[19] 6-Bromohexanoic acid was synthesized from 1,6-hexanediol via 6-bromo-1-hexanol by the method of Degering and Boatright;[20] bp 134–136 °C (7 mmHg) [lit.[20] bp 129–130 °C (5 mmHg)]. Didodecylamine was prepared by reaction of dodecylamine with dodecyl bromide in the presence of sodium carbonate: bp 194–196 °C (0.15 mmHg); mp 45–47 °C. *N*-Dansyltetradecylamine was prepared in a manner similar to that reported for preparation of *N*-dansylhexadecylamine[17] and isolated as pale yellow crystalline mass, mp 62–63 °C [recrystallized from hexane–ether (9:1 v/v)]. Anal. Calcd for $C_{26}H_{42}N_2O_2S$: C, 69.90; H, 9.48; N, 6.27. Found: C, 69.47; H, 9.54; N, 6.26.

The synthetic procedures for *N,N*-didodecyl-N^α-[6-(trimethylammonio)hexanoyl]-L-leucinamide bromide ($N^+C_5Leu2C_{12}$) and *N,N*-didodecyl-N^α-[6-[dimethyl(2-carboxyethyl)ammonio]-hexanoyl]-L-leucinamide bromide ($CAC_2N^+C_5Leu2C_{12}$) are outlined in Scheme IA.

***N,N*-Didodecyl-N^α-(*tert*-butoxycarbonyl)-L-leucine (1).** To a solution of *N*-(*tert*-butoxycarbonyl)-L-leucine (4.1 g, 17.7 mmol) in dry dichloromethane (23 mL) was added dicyclohexylcarbodiimide (4.3 g, 20.9 mmol) with stirring at 0 °C. After 10 min, *N,N*-didodecylamine (6.35 g, 18.0 mmol) was added to the solution, and the mixture was stirred for 3 h at 0 °C and for a further 12 h at room temperature. Precipitates (*N,N'*-dicyclohexylurea) were removed by filtration. The solvent was evaporated off in vacuo, and the residual oil was dissolved in ethyl acetate (200 mL). The solution was then washed sequentially with 10% aqueous citric acid, saturated aqueous sodium chloride, 4% aqueous sodium hydrogen carbonate, and saturated aqueous sodium chloride. After being dried (Na_2SO_4), the mixture was evaporated in vacuo to give an oil which was subsequently purified on a column of activated alumina (chromatography grade, Ishizu Pharmaceutical Co., 300 mesh) with dioxane–methanol (4:1 v/v) as an eluant: yield 3.44 g (34%); IR (neat) ν_{max} 3284 (NH), 2934 and 2864 (CH), 1710 and 1640 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.70–1.10 [12 H, m, $(CH_2)_{11}CH_3$ and $CH_2CH(CH_3)_2$], 1.25 [40 H, s, $CH_2(CH_2)_{10}CH_3$], 1.42 [9 H, s, $(CH_3)_3COCO$], ~2.05 [3 H, m, $CHCH_2CH(CH_3)_2$], 3.21 [4 H, m, $NCH_2(CH_2)_{10}CH_3$], 4.40 [1 H, br t, $CH(CH_2CH(CH_3)_2)$].

***N,N*-Didodecyl-N^α-(6-bromohexanoyl)-L-leucinamide (3).** Trifluoroacetic acid (25.0 g, 219 mmol) was added to a dichloromethane solution (30 mL) of **1** (3.21 g, 5.67 mmol), and the mixture was stirred for 1.5 h at room temperature. Evaporation of an excess amount of trifluoroacetic acid in vacuo below 40 °C gave a colorless oil (**2**). Elimination of the *tert*-butoxycarbonyl group was confirmed by NMR spectroscopy. The amine **2** and triethylamine (5.23 g, 28 mmol) were dissolved in dry dichloromethane (15 mL), and the solution was cooled to 0 °C. 6-Bromohexanoyl chloride (1.45 g, 6.73 mmol) dissolved in dry dichloromethane (15 mL) was added dropwise to the solution at 0 °C with stirring. The mixture was further stirred for 2 h at room temperature and then washed with 5% aqueous sodium hydrogen carbonate (2 × 50 mL), saturated aqueous sodium chloride (50 mL), 5% aqueous citric acid (2 × 50 mL), and saturated sodium chloride (50 mL) in this sequence. After being dried (Na_2SO_4), the mixture was evaporated in vacuo at 40 °C to give a pale yellow oil which was used for the subsequent reaction without further purification: yield 2.70 g (74%); IR (neat) ν_{max} 3274 (NH), 2924 and 2874 (CH), 1711 and 1627 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.70–1.10 [12 H, m, $(CH_2)_{11}CH_3$ and $CH_2CH(CH_3)_2$], 1.26 [40 H, s, $CH_2(CH_2)_{10}CH_3$], ~2.05 [9 H, m, $CHCH_2CH(CH_3)_2$ and $BrCH_2(CH_2)_3CH_2CO$], 2.20 [2 H, br t, $Br(CH_2)_4CH_2CO$], 2.89–3.50 [6 H, m, $BrCH_2(CH_2)_4CO$ and $NCH_2(CH_2)_{10}CH_3$], 4.85 [1 H, br t, $CH(CH_2CH(CH_3)_2)$], 6.45 (1 H, d, NH).

***N,N*-Didodecyl-N^α-[6-(trimethylammonio)hexanoyl]-L-leucinamide Bromide ($N^+C_5Leu2C_{12}$).** Dry trimethylamine gas was introduced into a benzene solution (60 mL) of **3** (2.70 g, 4.20 mmol) over a period of 3 h at room temperature, and the solution was stirred at the same temperature for 15–20 h. After benzene was evaporated off in vacuo, the crude product was purified twice by gel filtration chromatography (Sephadex LH-20, methanol as an eluant): yield 0.8 g (27%); IR (Nujol) ν_{max} 3200–3400 (NH and OH), 1630 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.70–1.10 [12 H, m, $CH(CH_3)_2$ and $N(CH_2)_{11}CH_3$], 1.25 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], ~2.10 [9 H, m, $N^+CH_2(CH_2)_3CH_2CO$ and $CHCH_2CH(CH_3)_2$], 2.24 [2 H, br t, $N^+(CH_2)_4CH_2CO$], 2.80–3.75 [6 H, m, $NCH_2(CH_2)_{10}CH_3$ and $N^+CH_2(CH_2)_4CO$], 3.39 [9 H, s, $N^+(CH_3)_3$], 4.88 [1 H, br t, $CH(CH_2CH(CH_3)_2)$], 6.90 (1 H, d, NH).

***N,N*-Didodecyl-N^α-[6-(dimethylamino)hexanoyl]-L-leucinamide (4).** Dry dimethylamine gas was introduced into a benzene solution (60 mL) of **3** (3.40 g, 5.29 mmol) over a period of 3 h at room temperature. The solution was stirred at the same

(17) Iwamoto, K.; Sunamoto, J. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 399.
(18) Azumi, T.; McGlynn, S. P. *J. Chem. Phys.* **1962**, *37*, 2413.
(19) Nagasawa, T.; Kuroiwa, K.; Narita, K.; Isowa, Y. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 1269.
(20) Degering E. F.; Boatright, L. G. *J. Am. Chem. Soc.* **1950**, *72*, 5137.

temperature for 15–20 h and washed with 5% aqueous sodium hydrogen carbonate (50 mL). After being dried (Na_2SO_4), the mixture was evaporated in vacuo to afford a pale yellow oil which was purified by gel filtration chromatography (Sephadex LH-20, methanol as an eluant): yield 0.52 g (16%); liquid crystal; final mp 80 °C; IR (neat) ν_{max} 3260 (NH), 2930, 2860, and 2800 (CH), 1625 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.70–1.10 [12 H, m, $N(C-H_2)_{11}CH_3$ and $CH(CH_3)_2$], 1.25 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], ~2.05 [9 H, m, $(CH_3)_2NCH_2(CH_2)_3CH_2CO$ and $CHCH_2CH(CH_3)_2$], 2.03–2.36 [2 H, m, $(CH_3)_2N(CH_2)_4CH_2CO$], 2.19 [6 H, s, $(CH_3)_2N$], 3.03–3.43 [6 H, m, $(CH_3)_2NCH_2(CH_2)_4CO$ and $NCH_2(CH_2)_{10}CH_3$], 4.93 [1 H, br t, $CH(CH_2CH(CH_3)_2)$], 6.41 (1 H, d, NH).

N,N-Didodecyl-N$^\alpha$-[6-[dimethyl(2-carboxyethyl)ammonio]hexanoyl]-L-leucinamide Bromide ($CAC_2N^+C_5Leu2C_{12}$). To an aqueous solution (10.8 mL) of 3-bromopropionic acid (0.13 g, 0.86 mmol) containing sodium hydroxide (0.86 mmol) was added **4** (0.52 g, 0.86 mmol). Acetone (20 mL) was added to the mixture to yield a homogeneous solution which was subsequently refluxed for 72 h. Acetone was evaporated off in vacuo, water (5 mL) and 47% hydrobromic acid (0.11 mL, 0.96 mmol) were added to the residue, and the mixture was stirred for 1 h at room temperature. After removal of the solvent, the residual oil was purified by gel filtration chromatography on Sephadex LH-20 and on Toyopearl HW-40F in this sequence (ethanol as an eluant): yield 0.178 g (27%); liquid crystal; final mp 73 °C; IR (Nujol) ν_{max} 3350 (OH), 1730 and 1630 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75–1.10 [12 H, m, $CH_2CH(CH_3)_2$ and $N(CH_2)_{11}CH_3$], 1.26 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], ~2.05 [9 H, m, $N^+CH_2(CH_2)_3CH_2CO$ and $CHCH_2CH(CH_3)_2$], 2.05–2.43 [4 H, m, $HOOCCH_2CH_2N^+$ and $N^+(CH_2)_4CH_2CO$], 2.83 [6 H, s, $N^+(CH_3)_2$], 2.99–3.56 [8 H, m, $CH_2N^+CH_2(CH_2)_4CO$ and $NCH_2(CH_2)_{10}CH_3$], 4.81 [1 H, br t, $CH(CH_2CH(CH_3)_2)$], 6.60 (1 H, d, NH).

Other amphiphiles, *N,N*-didodecyl-N$^\alpha$-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{12}$), *N,N*-didodecyl-N$^\alpha$-[6-[dimethyl(2-carboxyethyl)ammonio]hexanoyl]-L-alaninamide bromide ($CAC_2N^+C_5Ala2C_{12}$), *N,N*-didodecyl-N$^\alpha$-[6-(trimethylammonio)hexanoyl]sarcosinamide bromide ($N^+C_5Sar2C_{12}$), and *N,N*-didodecyl-N$^\alpha$-[6-[dimethyl(2-carboxyethyl)ammonio]hexanoyl]sarcosinamide bromide ($CAC_2N^+C_5Sar2C_{12}$), were prepared from (*tert*-butoxycarbonyl)-L-alanine and (*tert*-butoxycarbonyl)sarcosine in manners similar to those described for $N^+C_5Leu2C_{12}$ and $CAC_2N^+C_5Leu2C_{12}$.

N,N-Didodecyl-N$^\alpha$-(*tert*-butoxycarbonyl)-L-alanine: yield 36%, colorless oil; IR (neat) ν_{max} 3285 (NH), 2900 and 2825 (CH), 1715 and 1640 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 [6 H, br t, $N(CH_2)_{11}CH_3$], 1.28 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], 1.44 [9 H, s, $(CH_3)_3COCO$], 1.34 [3 H, s, sh, $CH(CH_3)$], 3.25 [4 H, br t, $NCH_2(CH_2)_{10}CH_3$], 4.50 [1 H, br q, $CH(CH_3)$], 5.45 (1 H, d, NH).

N,N-Didodecyl-N$^\alpha$-(6-bromohexanoyl)-L-alanine: yield 90%; colorless oil, solidifies in a refrigerator; IR (neat) ν_{max} 3200 (NH), 2830 (CH), 1730 and 1630 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 [6 H, br t, $N(CH_2)_{11}CH_3$], 1.26 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], 1.35 [3 H, s, sh, $CH(CH_3)$], ~2.00 [6 H, m, $BrCH_2(CH_2)_3CH_2CO$], 2.21 [2 H, br t, $Br(CH_2)_4CH_2CO$], 3.05–ca. 3.5 [4 H, m, $NCH_2(CH_2)_{10}CH_3$], 2.40 [2 H, t, $BrCH_2(CH_2)_4CO$], 4.91 [1 H, q, $CH(CH_3)$], 6.61 (1 H, d, NH).

N,N-Didodecyl-N$^\alpha$-[6-(trimethylammonio)hexanoyl]-L-alaninamide Bromide ($N^+C_5Ala2C_{12}$): yield 59%; liquid crystal; final mp 195 °C; IR (neat) ν_{max} 3400 (NH), 2920 and 2830 (CH), 1630 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 [6 H, br t, $(CH_2)_{11}CH_3$], 1.25 [40 H, s, $CH_2(CH_2)_{10}CH_3$], 1.35 [3 H, s, sh, $CH(CH_3)$], ~2.00 [6 H, m, $NCH_2(CH_2)_3CH_2CO$], 2.26 [2 H, br t, $N(CH_2)_4CH_2CO$], 3.42 [9 H, s, $N^+(CH_3)_3$], 3.04–3.79 [6 H, m, $CH_2N^+(CH_3)_3$ and $NCH_2(CH_2)_{10}CH_3$], 4.78 [1 H, br q, $CH(CH_3)$], 7.04 (1 H, br d, NH).

N,N-Didodecyl-N$^\alpha$-[6-(dimethylamino)hexanoyl]-L-alaninamide: yield 84%; prisms; mp 44.0–44.6 °C; IR (KBr) ν_{max} 3240 (NH), 2925 and 2820 (CH), 1635 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 [6 H, br t, $(CH_2)_{11}CH_3$], 1.24 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], 1.34 [3 H, s, sh, $CH(CH_3)$], ~2.00 [6 H, m, $NCH_2(CH_2)_3CH_2CO$], 2.20 [6 H, s, $(CH_3)_2N$], 1.97–2.50 [4 H, m, $NCH_2(CH_2)_3CH_2CO$], 3.30 [4 H, m, $NCH_2(CH_2)_{10}CH_3$], 4.78 [1 H, q, $CH(CH_3)$], 6.48 (1 H, d, NH).

N,N-Didodecyl-N$^\alpha$-[6-[dimethyl(2-carboxyethyl)ammonio]hexanoyl]-L-alaninamide Bromide ($CAC_2N^+C_5Ala2C_{12}$): yield 37%; liquid crystal; final mp 98 °C; IR (neat) ν_{max} 3430 (OH), 3240 (NH), 2924 and 2820 (CH), 1725 and 1640 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 [6 H, br t, $(CH_2)_{11}CH_3$], 1.25 [40 H, s, $CH_2(CH_2)_{10}CH_3$], 1.36 [3 H, s, sh, $CH(CH_3)$], ~2.00 [6 H, m, $N^+CH_2(CH_2)_3CH_2CO$], 2.21 [4 H, br t, $N^+(CH_2)_4CH_2CO$ and $HOOCCH_2CH_2N^+$], 2.89 [6 H, s, $N^+(CH_3)_2(CH_2)_5CO$], 2.75–3.71 [8 H, m, $HOOCCH_2CH_2N^+CH_2$ and $NCH_2(CH_2)_{10}CH_3$], 6.74 (1 H, d, NH),8.18 (1 H, br s, COOH).

N,N-Didodecyl-N$^\alpha$-(*tert*-butoxycarbonyl)sarcosine: yield 64%; colorless oil; IR (neat) ν_{max} 2925 and 2875 (CH), 1710 and 1650 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 [6 H, br t, $N(CH_2)_{11}CH_3$], 1.25 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], 1.45 [9 H, s, $(CH_3)_3COCO$], 2.89 [3 H, s, $CON(CH_3)CH_2$], 2.95–3.40 [4 H, m, $NCH_2(CH_2)_{10}CH_3$], 3.95 [2 H, s, $N(CH_3)CH_2CO$].

N,N-Didodecyl-N$^\alpha$-(6-bromohexanoyl)sarcosine: yield 71%; pale yellow oil; IR (neat) ν_{max} 2925 and 2880 (CH), 1705 and 1650 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 [6 H, br t, $NCH_2(CH_2)_{10}CH_3$], 1.25 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], ~2.00 [6 H, m, $NCH_2(CH_2)_3CH_2CO$], 2.35 [2 H, t, $Br(CH_2)_4CH_2CO$], 3.04 [3 H, s, $CON(CH_3)CH_2$], 2.88–ca. 3.5 [4 H, m, $NCH_2(CH_2)_{10}CH_3$], 3.36 [2 H, t, $BrCH_2(CH_2)_4CO$], 4.10 [2 H, s, $N(CH_3)CH_2CO$].

N,N-Didodecyl-N$^\alpha$-[6-(trimethylammonio)hexanoyl]sarcosinamide Bromide ($N^+C_5Sar2C_{12}$): yield 23%; liquid crystal; final mp 198 °C; IR (Nujol) ν_{max} 1630 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 [6 H, br t, $N(CH_2)_{11}CH_3$], 1.25 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], ~2.00 [6 H, m, $N^+CH_2(CH_2)_3CH_2CO$], 2.45 [2 H, br t, $N^+(CH_2)_4CH_2CO$], 3.04 [3 H, s, $CON(CH_3)CH_2$], 2.90–3.74 [6 H, m, $N^+CH_2(CH_2)_4CO$ and $NCH_2(CH_2)_{10}CH_3$], 3.39 [9 H, s, $(CH_3)_3N^+$], 4.10 [2 H, s, $CON(CH_3)CH_2CO$].

N,N-Didodecyl-N$^\alpha$-[6-(dimethylamino)hexanoyl]sarcosinamide: yield, quantitative; colorless oil; IR (neat) ν_{max} 2910 and 2840 (CH), 1740 and 1650 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 [6 H, br t, $N(CH_2)_{11}CH_3$], 1.25 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], ~2.00 [6 H, m, $NCH_2(CH_2)_3CH_2CO$], 2.24 [6 H, s, $(CH_3)_2N$], 2.20–ca.2.5 [4 H, m, $NCH_2(CH_2)_3CH_2CO$], 3.06 [3 H, s, $CON(CH_3)CH_2$], ca. 2.9–3.56 [4 H, m, $NCH_2(CH_2)_{10}CH_3$], 4.14 [2 H, s, $N(CH_3)CH_2CO$].

N,N-Didodecyl-N$^\alpha$-[6-[dimethyl(2-carboxyethyl)ammonio]hexanoyl]sarcosinamide Bromide ($CAC_2N^+C_5Sar2C_{12}$): yield 11%; liquid crystal; final mp 110 °C; IR (Nujol) ν_{max} 1650 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 [6 H, br t, $N(CH_2)_{11}CH_3$], 1.25 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], ~2.00 [6 H, m, $N^+CH_2(CH_2)_3CH_2CO$], 2.12–2.64 [4 H, m, $HOOCCH_2CH_2N^+$ and $N^+(CH_2)_4CH_2CO$], 2.83 [6 H, s, $N^+(CH_3)_2$], 3.04 [2 H, s, $CON(CH_3)CH_2$], ca. 2.7–3.42 [8 H, m, $CH_2N^+CH_2(CH_2)_4CO$ and $NCH_2(CH_2)_{10}CH_3$], 4.10 [2 H, s, $N(CH_3)CH_2CO$].

N,N-Didodecyl-N$^\alpha$-[N$^{\alpha'}$-(*tert*-butoxycarbonyl)-L-leucyl]-L-leucinamide (5): yield 52%; colorless oil; IR (neat) ν_{max} 3250 (NH), 2930 and 2825 (CH), 1710 and 1640 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84–1.08 [18 H, m, $CH_2CH(CH_3)_2$ and $NCH_2(CH_2)_{10}CH_3$], 1.26 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], 1.43 [9 H, s, $(CH_3)_3CO$], ~2.10 [6 H, m, $CHCH_2CH(CH_3)_2$], 3.30 [4 H, br t, $NCH_2(CH_2)_{10}CH_3$], 4.98 [2 H, t, $CH(CH_2CH(CH_3)_2)$].

N,N-Didodecyl-N$^\alpha$-[N$^{\alpha'}$-[N$^{\alpha''}$-(*tert*-butoxycarbonyl)-L-leucyl]-L-leucyl]-L-leucinamide (6): yield 58%; colorless oil; IR (neat) ν_{max} 3280 (NH), 2950 and 2900 (CH), 1710 and 1620 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.74–1.08 [24 H, m, $CHCH_2CH(CH_3)_2$ and $N(CH_2)_{11}CH_3$], 1.26 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], ~2.05 [9 H, m, $CH(CH_2CH(CH_3)_2)$], 1.43 [9 H, s, $(CH_3)_3CO$], 3.30 [4 H, br t, $NCH_2(CH_2)_{10}CH_3$], ca. 4.7–5.2 [3 H, m, $CH(CH_2CH(CH_3)_2)$], 6.60–7.10 (3 H, m, NH).

N,N-Didodecyl-N$^\alpha$-[N$^{\alpha'}$-[N$^{\alpha''}$-(6-bromohexanoyl)-L-leucyl]-L-leucyl]-L-leucinamide (7): yield 75%; colorless oil; IR (neat) ν_{max} 3265 (NH), 2940 and 2870 (CH), 1730, 1700, and 1620 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.78–1.08 [24 H, m, $CH_2CH(CH_3)_2$ and $N(CH_2)_{11}CH_3$], 1.25 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], ~2.14 [15 H, m, $CH(CH_2CH(CH_3)_2)$ and $BrCH_2(CH_2)_3CH_2CO$], 2.30 [2 H, t, $Br(CH_2)_4CH_2CO$], 3.36 [2 H, t, $BrCH_2(CH_2)_4CO$], 2.97 [4 H, m, $NCH_2(CH_2)_{10}CH_3$], ca. 4.4–5.2 [3 H, m, $CH(CH_2CH(CH_3)_2)$], 6.10–7.00 (3 H, m, NH).

N,N-Didodecyl-N$^\alpha$-[N$^{\alpha'}$-[N$^{\alpha''}$-[6-(trimethylammonio)hexanoyl]-L-leucyl]-L-leucyl]-L-leucinamide Bromide ($N^+C_5Leu_32C_{12}$): yield 14%; liquid crystal; final mp 130 °C; IR (Nujol) ν_{max} 3400 (OH), 3250 (NH), 1630 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75–1.10 [24 H, m, $CHCH_2CH(CH_3)_2$ and $N(CH_2)_{11}CH_3$], 1.25 [40 H, s, $NCH_2(CH_2)_{10}CH_3$], ~2.20 [15 H, m,

$CH(CH_2CH(CH_3)_2)$ and $N^+CH_2(CH_2)_3CH_2CO$], 2.30 [2 H, t, $N^+(CH_2)_4CH_2CO$], 3.39 [9 H, s, $(CH_3)_3N^+$], 2.95–ca. 4.0 [6 H, m, $N^+CH_2(CH_2)_4CO$ and $NCH_2(CH_2)_{10}CH_3$], 4.20–5.15 [3 H, m, $CH(CH_2CH(CH_3)_2)$], 6.78–7.18 (3 H, m, NH).

Registry No. 1, 80997-12-2; 2, 80997-13-3; 3, 80975-61-7; 4, 80975-62-8; 5, 80975-63-9; 6, 80975-64-0; 7, 80975-65-1; $N^+C_5Leu2C_{12}$, 80975-66-2; $CAC_2N^+C_5Leu2C_{12}$, 80975-67-3; $N^+C_5Ala2C_{12}$, 78761-16-7; $CAC_2N^+C_5Ala2C_{12}$, 80975-68-4; $N^+C_5Sar2C_{12}$, 80975-69-5; $CAC_2N^+C_5Sar2C_{12}$, 80975-70-8; $N^+C_5Leu_32C_{12}$, 80975-71-9; *N*-(*tert*-butoxycarbonyl)-L-leucine, 13139-15-6; *N,N*-didodecylamine, 3007-31-6; 6-bromohexanoyl chloride, 22809-37-6; *N*-(*tert*-butoxycarbonyl)-L-alanine, 15761-38-3; *N*-(*tert*-butoxycarbonyl)sarcosine, 13734-36-6; *N,N*-didodecyl-N^α-(*tert*-butoxycarbonyl)-L-alanine, 80975-72-0; *N,N*-didodecyl-N^α-(6-bromohexanoyl)-L-alanine, 80975-73-1; *N,N*-didodecyl-N^α-[6-(dimethylamino)hexanoyl]-L-alaninamide, 80975-74-2; *N,N*-didodecyl-N^α-(*tert*-butoxycarbonyl)sarcosine, 80975-75-3; *N,N*-didodecyl-N^α-(6-bromohexanoyl)sarcosine, 80975-76-4; *N,N*-didodecyl-N^α-[6-(dimethylamino)hexanoyl]sarcosinamide, 80975-77-5.

Syntheses of Enzyme-Inhibitory Phospholipid Analogues. Stereospecific Synthesis of 2-Amidophosphatidylcholines and Related Derivatives

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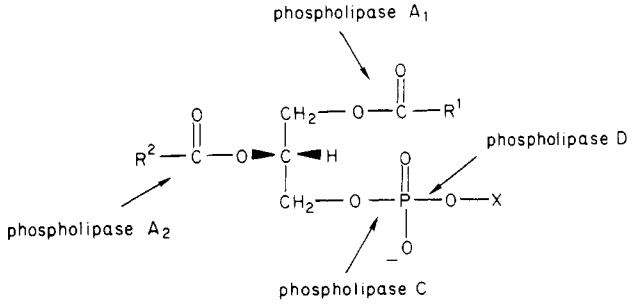
Received October 27, 1981

A novel stereospecific synthesis of the enzyme-inhibitory 2-*sn*-deoxy-2-amidophosphatidylcholine is reported. The synthesis is based on (1) utilizing the chirality of the α-carbon of the starting amino acid serine, (2) protecting the asymmetric center via formation of an oxazoline ring, and (3) introducing the phosphorylcholine moiety through the 2-chloro-2-oxo-1,3,2-dioxaphospholane–trimethylamine sequence. The compound has been shown to be a specific and potent phospholipase A_2 inhibitor, exhibiting higher affinity to the enzyme under the reaction conditions than the natural substrate. The synthetic method used for the preparation of the inhibitor provides a general route to a wide range of other phospholipid analogues as well. Along these lines 2-deoxy-2-aminolysolecithin has been shown to react with octyl chloroformate and stearoyl isocyanate to form the corresponding 2-*sn*-carbamoyl and 2-*sn*-alkylureido derivatives. The scope of the synthesis is being investigated for the preparation of reversible as well as irreversible phospholipase inhibitors.

The synthesis of enzyme-inhibitory phospholipid analogues is one of the most timely problems in membrane biochemistry today.[1,2] Nonhydrolyzable isosteric phospholipid derivatives are required for structural as well as dynamic studies of biomembranes and membrane-bound enzymes, with particular emphasis on investigation of phospholipid–phospholipid and phospholipid–protein interactions.[3] As part of our ongoing research aimed at the elucidation of the mechanism of action of phospholipase A_2,[4,5] we have recently began focusing our efforts on the development of new synthetic methods for the preparation of specific and potent inhibitory phospholipid analogues.[6]

Specifically, phospholipase A_2 is one of the four phospholipid hydrolyzing enzymes. Its unique biological importance arises from the fact that it also participates in a number of physiologically vital regulatory processes,[7-11] including platelet aggregation, cardiac contraction and excitation, prostaglandin biosynthesis, and aldosterone-dependent sodium transport. Availability of specific and potent phospholipase A_2 inhibitors, therefore, should be valuable for delineation of the precise function of the enzyme in vivo and for kinetic studies aimed at the elucidation of its catalytic mechanism in vitro.



1

X = choline, serine, ethanolamine

Utilizing a new approach to the problem, we have recently accomplished the stereospecific synthesis of the first series of inhibitory 2-deoxyamidophosphatidylcholines.[6] In the present article we describe the synthesis of compound **2** in detail and demonstrate the applicability of the method for the preparation of a series of other related phospholipid analogues as well.

Results and Discussion

The structural design of **2** as our target compound has originated from early studies by de Haas and van Deenen, who have demonstrated, using short-chain lecithin analogues, that replacement of the ester moiety by the corresponding amide function at the catalytic 2-position abolishes the catalytic hydrolysis by the enzyme.[13]

Our synthetic approach to the preparation of **2** is based on the recognition that the chirality of the optically active α-carbon in L-serine (**3**) is identical with that of the asymmetric center in the desired phospholipid analogue **2**. Consequently, our strategy for the preparation of **2** is based on construction of the chiral amidophospholipid

(1) Gupta, C. M.; Radhakrishan, R.; Khorana, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4315.
(2) Brockerhoff, H.; Jensen, R. G. "Lipolytic Enzymes"; Academic Press: New York, 1974; pp 194–243.
(3) Verger, R.; de Haas, G. H. *Ann. Rev. Biophys. Bioeng.* **1976**, *5*, 77.
(4) Dinur, D.; Kantrowitz, E. R.; Hajdu, J. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 785–792.
(5) Hajdu, J.; Smith, G. M. *J. Am. Chem. Soc.* **1981**, *103*, 6192–6197.
(6) Chandrakumar, N. S.; Hajdu, J. *Tetrahedron Lett.* **1981**, *22*, 2949–2952.
(7) Pickett, W. C.; Jesse, R. L.; Cohen, P. *Biochem. J.* **1976**, *160*, 405.
(8) Geisler, C.; Mentz, P.; Forrester, W. *Pharmacol. Res. Commun.* **1977**, *9*, 117.
(9) Flower, R. J.; Blackwell, G. J. *Biochem. Pharmacol.* **1976**, *258* 285.
(10) Vogt, W. *Adv. Prostaglandin Tromboxane Res.* **1978**, *3*, 89.
(11) Yorio, T.; Bentley, P. L. *Nature (London)* **1978**, *79*, 271.
(12) Selinger, Z.; Lapidot, Y. *J. Lipid Res.* **1966**, *7*, 174–175.
(13) Bonsen, P. P. M.; de Haas, G. H.; Pieterson, W. A.; van Deenen, L. L. M. *Biochem. Biophys. Acta* **1972**, *270*, 364.

0022-3263/82/1947-2144$01.25/0